PRODRUGS OF CYTOTOXIC ACTIVE AGENTS HAVING ENZYMATICALLY CLEAVABLE GROUPS

The invention relates to novel prodrugs in which cytotoxic drugs, for example kinesin spindle protein inhibitors, are conjugated to groups which are selectively cleaved by tumour-associated proteases and hence release the drug, and to the use of these prodrugs or conjugates for treatment and/or prevention of diseases, and to the use of these prodrugs for production of medicaments for treatment and/or prevention of diseases, especially of hyperproliferative and/or angiogenic disorders, for example cancers. Such treatments can be effected as monotherapy or else in combination with other medicaments or further therapeutic measures.

Cancer cells frequently express particular proteases to a higher degree than normal cells. This has led to approaches for increasing the selectivity of cytotoxic drugs for cancer cells, in which the drugs are bonded to groups that are eliminated by such proteases, as a result of which the active ingredient is released.

Such a tumour-associated protease is legumain. Legumain is an asparaginyl endopeptidase (S. Ishii, Methods Enzymol. 1994, 244, 604; J. M. Chen et al. J. Biol. Chem. 1997, 272, 8090) and has been utilized for processing of prodrugs of small cytotoxic molecules, for example of doxorubicin and etoposide derivatives among others (W. Wu et al. Cancer Res. 2006, 66, 970; L.Stern et al; Bioconjugate Chem. 2009, 20, 500; K.M. Bajjuri et al. ChemMedChem 2011, 6, 54).

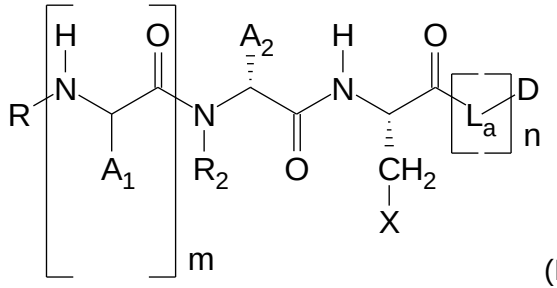
US 2015/0343083 A1 describes legumain-cleavable peptide-active ingredient conjugates of the formula R-Y-Z-Asn-linker-D where linker represents p-aminobenzylcarbamoyl or p-aminobenzylcarbonate, R is a residue selected from different chemical groups, and D is a cytotoxic drug, Asn represents the amino acid asparagine, and Y represents an amino acid selected from Ala, Thr, Ser, Leu, Arg, Pro, Val, Tyr and Phe, Z represents an amino acid selected from Ala, Thr, Asn and Pro, where these amino acids are always in the natural L configuration.

Summary of the invention

The invention relates to the problem of improving the tumour selectivity of cytotoxic drugs. To solve this problem, the invention provides prodrugs of cytotoxic drug molecules. In this context, the active ingredient molecule is conjugated to a group cleavable by the enzyme legumain, with the active ingredient and the legumain-cleavable group joined either directly via a covalent bond or via a self-immolative linker. These prodrugs preferably contain a binder which, after binding to a target molecule of a tumour cell, is internalized by the tumour cell and processed intracellularly (preferably lysosomally). This binder may either be bonded to the active ingredient molecule, optionally via a linker, such that both groups (legumain-cleavable group and binder) have to be processed independently for formation of an active metabolite, or the binder may be bonded to the group cleavable by the enzyme legumain, optionally via a linker (such that, after cleavage of the legumain-cleavable group, the active ingredient is present separately from the binder or a derivative thereof). A preferred active ingredient molecule is a kinesin spindle protein inhibitor (KSP inhibitor). A preferred binder which is internalized after binding to a target molecule on a tumour cell and is processed intracellularly (preferably lysosomally) is an antibody. Particular preference is given to antibody-active ingredient conjugates (ADCs), wherein antibody and active ingredient are joined to one another via a linker having a legumain-cleavable group. In addition, preference is given to conjugates of prodrugs with antibodies (APDCs), wherein the antibody is bonded to a prodrug of the antibody via a linker, wherein the action of the active ingredient is masked by a legumain-cleavable group. The legumain-cleavable group used in accordance with the invention has the structure X-L-Asn or X-L-Asp, where X represents D-Ala, D-Pro, D-Val, D-Nva, D-Leu, D-Ile, D-Met, D-Phe, D-Tyr, D-Trp, D-Ser, D-Thr, D-Cys, D-Asn, D-Gln, D-Asp, D-Glu, D-Lys, D-Arg, D-citrulline or D-His or the corresponding N-alkylated amino acid (C1-3 alkylated, preferably methylated), and where up to 2 further amino acids may be present in N-bonded form to X. In this context, the introduction of the D-amino acid in the legumain-cleavable linker brings about an increase in the stability in the lysosomes of healthy organs (shown in chapter C-1c). As has been shown by representative comparisons with suitable reference examples (chapter C-1a), the inventive ADCs and APDCs having a D-amino acid in the linker have high anti-tumour action which is surprisingly barely inferior, if at all, to the

epimers having all-L configuration in the linker (reference example series R3, 4, 5 and 9) (see chapter C-1a).

The inventive prodrugs of a drug D have the following general formula Ia:



(Ia)

in which

m is 0 to 2;

n is 0 or 1;

X is –C(=O)-NH_2 or –COOH,

L_a is a self-immolative linker,

A_1 is a radical which derives from one of the amino acids Gly, Pro, Ala, Val, Nva, Leu, Ile, Met, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Asp, Glu, Lys, Arg, citrulline and His, or one of the respective N-alkyl amino acids,

A_2 is a radical which derives from one of the amino acids D-Ala, D-Pro, D- Val, D-Nva, D-Leu, D-Ile, D-Met, D-Phe, D-Tyr, D-Trp, D-Ser, D-Thr, D-Cys, D-Asn, D-Gln, D-Asp, D-Glu, D-Lys, D-Arg, D-citrulline or D-His, or one of the respective N-alkyl amino acids,

R_2 is –H- or C_1-C_3-alkyl,

or

R_2 is the bond to the methylene group of the proline ring if A_2 is a radical which derives from D-Pro,

D is $-D_1-(L_b)_o-(LIG)_p$,

D_1 is a cytotoxic drug,

LIG is a binder which, after binding to a target molecule of a tumour cell, is internalized by the tumour cell and processed intracellularly, preferably lysosomally,

L_b is a linker,

o and p are each independently 0 or 1,

R is $Z_1-(C=O)q-$,

q is 0 or 1,

Z_1 is a C_{1-10}-alkyl, C_{5-10}-aryl or C_{6-10}-aralkyl, C_{5-10}-heteroalkyl, C_{1-10}-alkyl-O-C_{6-10}-aryl, C_{5-10}-heterocycloalkyl, heteroaryl, heteroarylalkyl, C_{5-10}-heteroarylalkoxy, C_{1-10}-alkoxy, C_{6-10}-aryloxy, C_{6-10}-aryl-C_{1-10}-alkyloxy or C_{6-10}-aralkoxy, C_{5-10}-heteroalkoxy, C_{1-10}-alkyl-O-C_{6-10}-aryloxy- or C_{5-10}-heterocycloalkoxy group which may be mono- or polysubstituted by $-NH_2$, -C(=O)-, -NH-alkyl, $-N(alkyl)_2$, -NH-C(=O)-alkyl, -N(alkyl)-C(=O)-alkyl, $-S(=O)_3-H$, $-S(=O)_2-NH_2$, $-S(=O)_2-N(alkyl)_2$, -COOH, $-C(=O)NH_2$, $-C(=O)-N(alkyl)_2$ or –OH,

or is -H or a $-(CH_2)_{0-1}-O_x-(CH_2CH_2O)_v-R^1$ group,

x is 0 or 1,

v is a number from 1 to 20,

R^1 is –H, –alkyl, $-CH_2-COOH$, $-CH_2-CH_2-COOH$, or $-CH_2-CH_2-NH_2$,

or

R is $LIG-(L_c)_r-$,

LIG	is a binder which, after binding to a target molecule on a tumour cell, is internalized by the tumour cell and processed intracellularly, preferably lysosomally,
L_c	is a linker and
r	is 0 or 1.

In this context, R^1 when defined as alkyl is preferably C_{1-12}-alkyl.

Preferably, the radical mentioned in A_1 and A_2 which derives from one of the particular N-alkyl amino acids is a C_1-C_3-alkylated amino acid, more preferably a methylated amino acid.

Preferably, the binder (LIG) is an antibody or an antigen-binding antibody. The antibody is preferably a human, humanized or chimeric monoclonal antibody or an antigen-binding fragment thereof, especially an anti-TWEAKR antibody, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment thereof. Particular preference is given to the anti-TWEAKR antibodies TPP-7006, TPP-7007, TPP-10336 and TPP-10337, the anti-B7H3 antibodies TPP-8382 and TPP-8567, the anti-EGFR-antibody cetuximab (TPP-981) and the anti-HER2-antibodies trastuzumab and TPP-1015, or an antigen-binding fragment of these.

Description of the figures

Fig. 1 shows the strategy of the transglutaminase-catalysed conjugation site-specific functionalization of aglycosylated antibodies.

Fig. 2 shows examples of successive enzymatic steps for drug release, for example by means of histone deacetylase and cathepsin L according to Nat. Commun., 2013, 4, 2735.

Fig. 3 shows annotated sequences of preferred antibodies for binder-drug conjugates. What are shown are the protein sequences of the heavy and light chains of the IgGs, and the VH and VL regions of these antibodies. Below the sequences,

important regions are annotated (VH and VL regions in IgGs, and the CDR regions (H-CDR1, H-CDR2, H-CDR3, L-CDR1, L-CDR2, L-CDR3)).

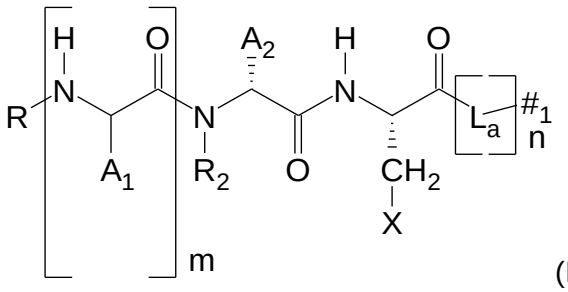
Fig. 4 shows the sequence listing of sequences of the preferred antibodies for binder-drug conjugates and of sequences of the target proteins.

Detailed description of the invention

First of all, there follows a description of the legumain-cleavable groups usable in accordance with the invention and of the cytotoxic drugs D that are optionally joined to one another via a self-immolative linker. This is followed by a description of the binder LIG preferred in accordance with the invention, which, after binding to a target molecule of a tumour cell, is internalized by the tumour cell and processed intracellularly (preferably lysosomally). The various elements of the compounds according to the invention can be used in any desired combination without restriction. In particular, the drugs D described in each case as preferred or particularly preferred can be used in combination with the binders LIG described in each case as preferred or particularly preferred, optionally in combination with the linkers described in each case as preferred or particularly preferred.

Legumain-cleavable group

The inventive compounds of the general formula (Ia) have a legumain-cleavable group of the formula (Ia')



(Ia')

in which

m is a number from 0 to 2,

n is 0 or 1,

X is –C(=O)-NH_2 or –COOH,

L_a is a self-immolative linker,

A_1 is a radical which derives from one of the amino acids Gly, Pro, Ala, Val, Nva, Leu, Ile, Met, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Asp, Glu, Lys, Arg, citrulline and His, or one of the respective N-alkyl amino acids;

A_2 is a radical which derives from one of the amino acids D-Ala, D-Pro, D- Val, D-Nva, D-Leu, D-Ile, D-Met, D-Phe, D-Tyr, D-Trp, D-Ser, D-Thr, D-Cys, D-Asn, D-Gln, D-Asp, D-Glu, D-Lys, D-Arg, D-citrulline or D-His, or one of the respective N-alkyl amino acids;

R_2 is –H- or C_1-C_3-alkyl,

or

R_2 is the bond to the methylene group of the proline ring if A_2 is a radical which derives from D-Pro,

R is Z_1-(C=O)q-,

q is 0 or 1,

Z_1 is a C_{1-10}-alkyl, C_{5-10}-aryl or C_{6-10}-aralkyl, C_{5-10}-heteroalkyl, C_{1-10}-alkyl-O-C_{6-10}-aryl, C_{5-10}-heterocycloalkyl, heteroaryl, heteroarylalkyl, C_{5-10}-heteroarylalkoxy, C_{1-10}-alkoxy, C_{6-10}-aryloxy, C_{6-10}-aryl-C_{1-10}-alkyloxy or C_{6-10}-aralkoxy, C_{5-10}-heteroalkoxy, C_{1-10}-alkyl-O-C_{6-10}-aryloxy- or C_{5-10}-heterocycloalkoxy group which may be mono- or polysubstituted by -NH_2, -C(=O)-, -NH-alkyl, -N(alkyl)$_2$, -NH-C(=O)-alkyl, -N(alkyl)-C(=O)-alkyl, -$S(=O)_3$-H, -$S(=O)_2$-NH_2, -$S(=O)_2$-N(alkyl)$_2$, -COOH, -C(=O)-NH_2, -C(=O)-N(alkyl)$_2$ or –OH,

or is -H or a -$(CH_2)_{0-1}$-O_x-$(CH_2CH_2O)_v$-R^1 group,

x is 0 or 1,

v is a number from 1 to 20,

R^1 is –H, –alkyl, -CH_2-COOH, -CH_2-CH_2-COOH, or -CH_2-CH_2-NH_2,

or

R is LIG-$(L_c)_r$-,

LIG is a binder which, after binding to a target molecule of a tumour cell, is internalized by the tumour cell and processed intracellularly, preferably lysosomally,

L_c is a linker,

r is 0 or 1 and

#1 represents the bond to the cytotoxic drug.

In this context, R^1 when defined as alkyl is preferably C_{1-12}-alkyl and m is preferably 1.

When R is Z_1-(C(=O))q-, the legumain-cleavable group of the formula Ia' is also referred to as legumain-cleavable head group.

When R is LIG-$(L_c)_r$-, the legumain-cleavable group of the formula Ia' is also referred to as legumain-cleavable linker.

When the legumain-cleavable group of the formula Ia' refers to a legumain-cleavable head group, q is preferably 1.

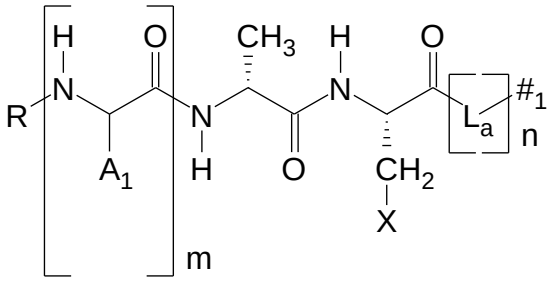
A legumain-cleavable head group is understood to mean a group which bioreversibly blocks an amino group of the drug (preferably a KSP inhibitor) which is important for the action.

X is preferably –C(=O)-NH_2.

A_2 is preferably a radical which derives from one of the amino acids D-Ala and D-Pro.

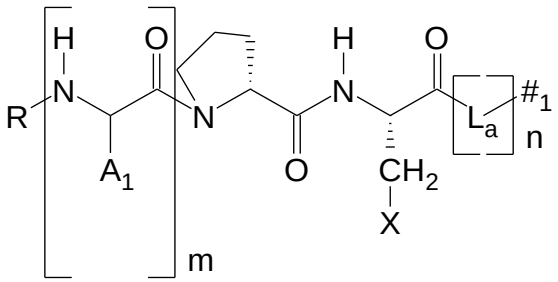
Preference is further given to radicals which derive from D-Asp, D-Asn, D-His and D-Ser.

When A_2 is a radical which derives from one of the amino acids D-Ala and D-Pro, the legumain-cleavable group of the general formula (Ia') has the following general formulae (Ia'') and (Ia'''):



(Ia'')

and



(Ia''')

When A_2 in the general formula (Ia') is not D-proline, R_2 is preferably –H or a methyl group, more preferably –H.

In the legumain-cleavable group of the formula Ia', the amino acid residues A_1 may be either in the L configuration or in the D configuration. A_1 preferably derives from Ala or Val. Preference is given to L-Ala, D-Ala or L-Val.
Particular preference is given to L-Ala.

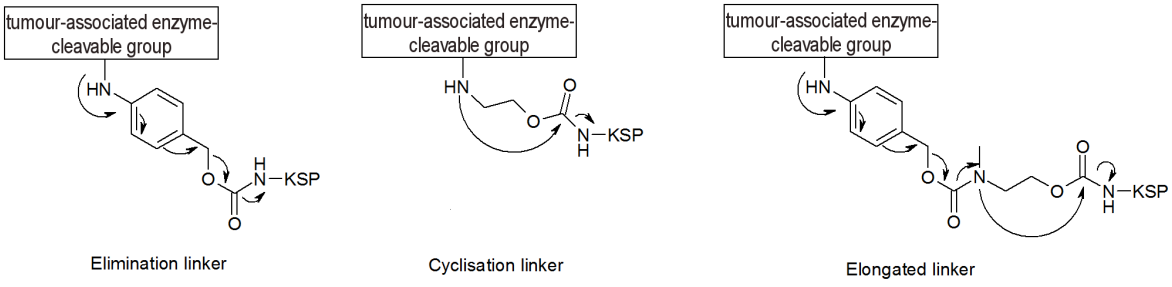
In the legumain-cleavable protecting group, q is preferably 1.

Z_1 is preferably a C_{1-10}-alkyl, C_{6-10}-aralkyl, C_{5-10}-heteroarylalkyl, C_{6-10}-aralkoxy, C_{6-10}-aryl-C_{1-10}-alkyloxy or C_{5-10}-heteroarylalkoxy group or a $-(CH_2)_{0-1}$-Ox-(CH_2CH_2O)v-R^1 group, in which x, v and R^1 have the definitions given above.

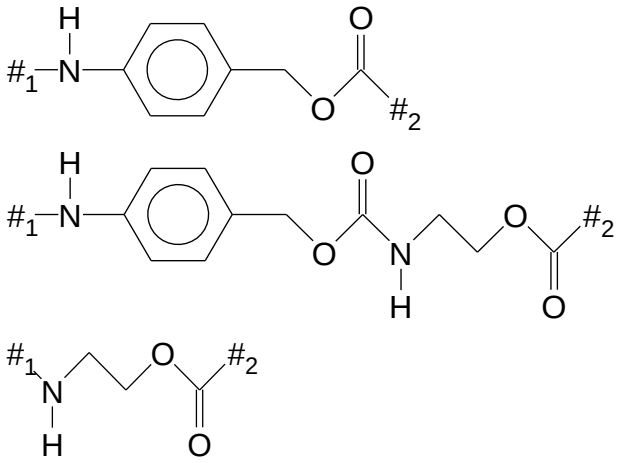
"Radical which derives from one of the amino acids" refers, as usual in chemistry, to the side groups of the amino acids. In other words, $–CH_3$ in the case of alanine, and so forth.

Self-immolative linker L_a

In order to assure efficient release of the free drug, it is optionally also possible to incorporate what are called self-immolative linker elements (L_a) between the enzymatic cleavage site and drug (Anticancer Agents in Medicinal Chemistry, 2008, 8, 618-637). The drug can be released by various mechanisms, for example after initial enzymatic release of a nucleophilic group by subsequent elimination via an electronic cascade (Bioorg. Med. Chem., 1999, 7, 1597; J. Med. Chem., 2002, 45, 937; Bioorg. Med. Chem., 2002, 10, 71) or by cyclization of the corresponding linker element (Bioorg. Med. Chem., 2003, 11, 2277; Bioorg. Med. Chem., 2007, 15, 4973; Bioorg. Med. Chem. Lett., 2007, 17, 2241) or by a combination of the two (Angew. Chem. Inter. Ed., 2005, 44, 4378). Examples of such linker elements are shown in the figure:



Preference is given in accordance with the invention to one of the following groups as L_a:



where $\#_1$ represents the bond to the carbonyl group and $\#_2$ the bond to the hydroxyl or amino group of D1.

Cytotoxic drugs

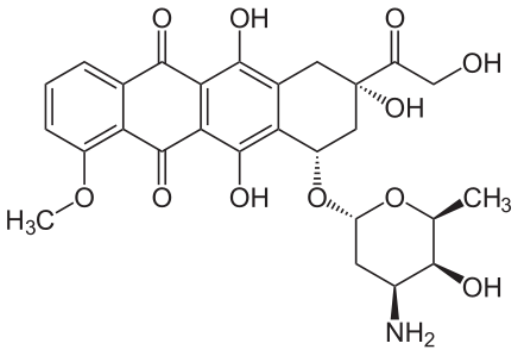
In the inventive compounds of the formula Ia, D is the $-D_1-(L_b)_o-(LIG)_p$ group where

D_1	is a cytotoxic drug,
LIG	represents a binder which, after binding to a target molecule of a tumour cell, is internalized by the tumour cell and processed intracellularly and preferably lysosomally,
L_b	represents a linker and
o and p	are independently 0 or 1.

The cytotoxic drug used is preferably mitomycin, doxorubicin, aminopterin, actinomycin, bleomycin, 9-aminocamptothecin, n8-acetylspermidine, 1-(2-chloroethyl)-1,2-dimethanesulphonyl hydrazide, tallysomycin, cytarabin, etoposid, camptothecin, taxol, esperamicin, podophyllotoxin, anguidin, vincristin, vinblastin, morpholine-doxorubicin, n-(5,5-diacetoxypentyl)doxorubicin, duocarmycin, auristatin, pyrrolobenzodiazepine derivatives, indolinobenzodiazepine (IGN) derivatives and mono-imine-IGN derivatives,

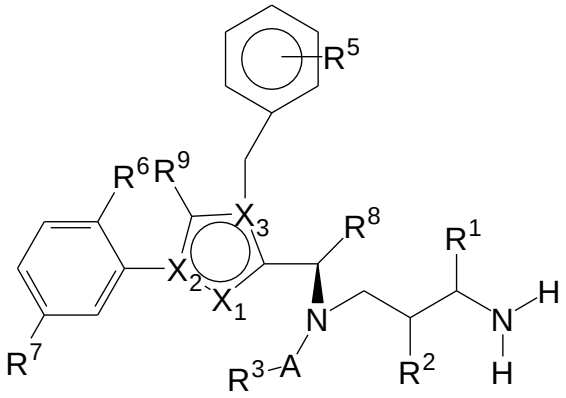
calicheamicin, daunorubicin, camptophecin DX8951 (exatecan) or a kinesin spindle protein inhibitor (KSP inhibitor), the drug being bonded via its hydroxyl or amino group to L_a (when n = 1) or the carbonyl group (when n = 0) according to the general formula (Ia) A corresponding derivatization of these drugs may be based on known methods (see, for example, Synthesis, 1999, 1505 with regard to duocarmycin, Nat. Struct. Biol., 2002, 9, 337, Journal of Med. Chem., 2010, 53(3), 1043 with regard to camptothecin, ChemMedChem,. 2011, 6(1), 54 with regard to auristatin, Mol. Cancer. Ther., 2005, 4, 751 with regard to doxorubicin, and J. Biol. Chem, 2008, 283, 9318 with regard to pyrrolobenzodiazepine derivatives (PBD derivatives); see also J. Med. Chem 2013, 56, 7564 and further references in the introduction, J. Med. Chem. 2001, 44, 1341, Oncology Reports 2011, 26, 629)).

Particular preference is given to those cytotoxic drugs having a free hydroxyl or amino group that is essential to their efficacy, especially those having a free amino group that is essential to their efficacy. The coupling of the legumain-cleavable group to such a group can mask the efficacy of the cytotoxic drug. This group of drugs includes, for example, doxorubicin having the following formula:



By conjugation of the legumain-cleavable group to the free amino group of doxorubicin, the efficacy thereof can be masked.

Further cytotoxic drugs preferred in accordance with the invention are kinesin spindle protein inhibitors, as disclosed in WO2015/096982. Especially preferred are those kinesin spindle protein inhibitors of the following formula II:



(II)

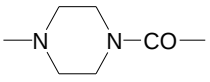
in which

X_1		is N,
X_2		is N and
X_3		is C;
	or	
X_1		is N,
X_2		is C and
X_3		is N;
	or	
X_1		is CH or CF,
X_2		is C and
X_3		is N;
	or	
X_1		is NH,
X_2		is C and
X_3		is C;
	or	
X_1		is CH,
X_2		is N and
X_3		is C.

R^1	is -H, –L-#1, –MOD or $-(CH_2)_{0-3}Z$,
Z	is -H, $-NHY^3$, $-OY^3$, $-SY^3$, halogen, $-C(=O)-NY^1Y^2$, or $–C(=O)-OY^3$,
Y^1 and Y^2	are independently -H, $-NH_2-(CH_2CH_2O)_{0-3}-(CH_2)_{0-3}Z'$ or $-CH(CH_2W)Z'$,

Y^3	is -H or -$(CH_2)_{0-3}$Z',
Z'	is -H, -NH_2, -$S(=O)_3H$, -COOH, -NH-C(=O)-CH_2-CH_2-CH(NH_2)-COOH or -(C(=O)-NH-CHY^4)$_{1-3}$-COOH;
W	is -H or -OH,
Y^4	is linear or branched C_{1-6} alkyl- optionally substituted by -NH-C(=O)-NH_2, or is aryl or benzyl which may be optionally be substituted by –NH_2,
R^2	is –L-#1, -H, -MOD, -C(=O)-CHY^4-NHY^5 or -$(CH_2)_{0-3}$Z,
Z	is -H, halogen, -OY^3, -SY^3, -NHY^3, -C(=O)-NY^1Y^2, or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}$Z',
Y^3	is -H or -$(CH_2)_{0-3}$Z',
Z'	is -H, -$S(=O)_3H$, -NH_2 or –COOH;
Y^4	is linear or branched C_{1-6} alkyl- optionally substituted by -NH-C(=O)-NH_2, or is aryl or benzyl optionally substituted by -NH_2,
Y^5	is -H or –C(=O)-CHY^6-NH_2,
Y^6	is linear or branched C_{1-6}-alkyl;
A	is –C(=O)-, -S(=O)-, -$S(=O)_2$-, or -$S(=O)_2$-NH-,
R^3	is –L-#1, -MOD, or an optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, heteroalkyl, heterocycloalkyl group which may be substituted by one to three OH groups, one to three halogen atoms, one to three mono-, di- or trihalogenated alkyl groups, one to three -O-alkyl groups, one to three -SH groups, one to three -S-alkyl groups, one to three -O-C(=O)-alkyl groups, one to three -O-C(=O)-NH-alkyl groups, one to three -NH-C(=O)-alkyl groups, one to three -NH-C(=O)-NH-alkyl groups, one to three -S(=O)$_n$-alkyl groups, one to three –$S(=O)_2$-NH-alkyl groups, 1-3 -NH-alkyl groups, one to three -N(alkyl)$_2$ groups, one to three NH_2 groups or one to three -$(CH_2)_{0-3}$Z groups,
n	is 0, 1 or 2,

Z	is -H, halogen, -OY^3, -SY^3, -NHY^3, -C(=O)-NY^1Y^2, or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}$Z',
Y^3	is -H, -$(CH_2)_{0-3}$-CH-(NHC(=OCH_3)Z', -$(CH_2)_{0-3}$-CH(NH_2)Z' or -$(CH_2)_{0-3}$Z',
Z'	is -H, -S(=O)$_3$H, -NH_2 or –COOH,
R^5	is -H, -NH_2, -NO_2, halogen, -CN, -CF_3, -OCF_3, -CH_2F, -CH_2F, -SH or -$(CH_2)_{0-3}$Z,
Z	is -H, -OY^3, -SY^3, halogen, -NHY^3, -C(=O)-NY^1Y^2 or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}$Z',
Y^3	is -H or -$(CH_2)_{0-3}$Z',
Z'	is -H, -S(=O)$_3$H, -NH_2 or –COOH,
R^6 and R^7	are independently -H, -CN, C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, hydroxyl, -NO_2, -NH_2, -COOH or halogen,
R^8	is C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, C_{4-10}-cycloalkyl, fluoro-C_{4-10}-cycloalkyl or -$(CH_2)_{0-2}$-(HZ^2),
HZ^2	is a 4- to 7-membered heterocycle having up to two heteroatoms selected from N, O and S, which may be substituted by –OH, –COOH, -NH_2 or –L-#1,
R^9	is -H, -F, -CH_3, -CF_3, -CH_2F or -CHF_2,
–L-#1	is -$(L_b)_o$-$(LIG)_p$,
LIG	is a binder which, after binding to a target molecule of a tumour cell, is internalized by the tumour cell and processed intracellularly and preferably lysosomally,
L_b	is a linker,
o and p	are independently 0 or 1,
–MOD	is -$(NR^{10})_n$-$(G1)_o$-G2-G3,
R^{10}	is -H or C_1-C_3-alkyl,

G1	is –NH-C(=O)- , -C(=O)-NH- or  ,
n	is 0 or 1,
o	is 0 or 1,
G2	is a straight-chain or branched hydrocarbon chain which has 1 to 20 carbon atoms and may be interrupted once or more than once by one or more of the groups -O-, -S-, -S(=O)-, -$S(=O)_2$-, -NR^y-, -$NR^yC(=O)$-, -$C(=O)$-NR^y-, -NR^yNR^y-, -$S(=O)_2$-NR^yNR^y-, -$C(=O)$-NR^yNR^y-$C(=O)$-, -CR^x=N-O, where the straight-chain or branched hydrocarbon chain may be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid,
G3	is -H or –COOH,
R^y	is -H, phenyl, C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl, or C_2-C_{10}-alkynyl, each of which may be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid,
R^x	is -H, C_1-C_3-alkyl or phenyl,

and the salts, solvates and salts of the solvates thereof.

Preference is given here to those compounds in which

R^3	is –L-#1, or a C_{1-10}-alkyl, C_{6-10}-aryl or C_{6-10}-aralkyl, C_{5-10}-heteroalkyl, C_{1-10}-alkyl-O-C_{6-10}-aryl or C_{5-10}-heterocycloalkyl group which may be substituted by one to three OH groups, one to three halogen atoms, one to three mono-, di- or trihalogenated alkyl groups, one to three –O-alkyl groups, one to three -SH groups, one to three -S-alkyl groups, one to three -O-C(=O)-alkyl groups, one to three -O-C(=O)-NH-alkyl groups, one to three -NH-C(=O)-alkyl groups, one to three -NH-C(=O)-NH-alkyl groups, one to three -$S(=O)_n$-alkyl groups, one to three –$S(=O)_2$-NH-alkyl groups, 1-3 -NH-alkyl groups, one to three -N(alkyl)$_2$ groups, one to three NH_2 groups or one to three -$(CH_2)_{0-3}$Z groups, and

–MOD has at least one –COOH group.

Preference is given to those compounds in which

X_1 is CH,

X_2 is C and

X_3 is N.

By conjugation of the legumain-cleavable group to the free amino group of the compounds of the formula II, the efficacy thereof can be masked.

These kinesin spindle protein inhibitors used in accordance with the invention have an amino group which is essential to the effect. By modification of this amino group with peptide derivatives, the effect with respect to the kinesin spindle protein is blocked and hence the development of a cytotoxic effect is also inhibited. If this peptide residue, however, can be released by tumour-associated enzymes such as legumain, the effect can be re-established in a controlled manner in the tumour tissue.

Definitions

The term "substituted" means that one or more hydrogens on the designated atom or the designated group has/have been replaced by a selection from the group specified, with the proviso that the normal valency of the designated atom is not exceeded under the circumstances in question. Combinations of substituents and/or variables are permissible.

The term "optionally substituted" means that the number of substituents can be equal to or different from zero. Unless stated otherwise, optionally substituted groups may be substituted by as many optional substituents as can be accommodated by replacement of a hydrogen atom by a non-hydrogen substituent on any available carbon or nitrogen or sulphur atom. Normally, the number of optional substituents (if present) may be 1, 2, 3, 4 or 5, especially 1, 2 or 3.

As used here, the expression "mono- or poly-", for example in the definition of the substituents of the compounds of the general formulae of the present invention, means "1, 2, 3, 4 or 5, preferably 1, 2, 3 or 4, more preferably 1, 2 or 3, most preferably 1 or 2".

If radicals in the compounds according to the invention are substituted, the radicals may be mono- or polysubstituted, unless stated otherwise. Within the scope of protection of the present invention, the definitions of all radicals which occur more than once are independent of one another. Substitution by one, two or three identical or different substituents is preferred. Substitution by one substituent is particularly preferred.

Alkyl

Alkyl is a linear or branched saturated monovalent hydrocarbon radical having 1 to 10 carbon atoms (C_1-C_{10}-alkyl), generally 1 to 6 (C_1-C_6-alkyl), preferably 1 to 4 (C_1-C_4-alkyl) and more preferably 1 to 3 carbon atoms (C_1-C_3-alkyl).
Preferred examples include:
methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, tert-butyl, isopentyl, 2-methylbutyl, 1-methylbutyl, 1-ethylpropyl, 1,2-dimethylpropyl, neopentyl, 1,1-dimethylpropyl, 4-methylpentyl, 3-methylpentyl, 2-methylpentyl, 1-methylpentyl, 2-ethylbutyl, 1-ethylbutyl, 3,3-dimethylbutyl, 2,2-dimethylbutyl, 1,1-dimethylbutyl, 2,3-dimethylbutyl, 1,3-dimethylbutyl, 1,2-dimethylbutyl.

Particular preference is given to a methyl, ethyl, propyl, isopropyl or tert-butyl radical.

Heteroalkyl

Heteroalkyl is a straight-chain and/or branched hydrocarbon chain which has 1 to 10 carbon atoms and may be interrupted once or more than once by one or more of the groups -O-, -S-,
-C(=O)-, -S(=O)-, -$S(=O)_2$-, -NR^y-, -$NR^yC(=O)$-, -$C(=O)$-NR^y-, -NR^yNR^y-, -$S(=O)_2$-NR^yNR^y-, -$C(=O)$-NR^yNR^y-, -CR^x=N-O-, and where the hydrocarbon chain including the side chains, if present, may be substituted by –NH-$C(=O)$-NH_2, -COOH, -OH, -NH_2, -NH-$C(=NNH_2)$-, sulphonamide, sulphone, sulphoxide, or sulphonic acid.

In this context, R^y in each case is -H, phenyl, C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl or C_2-C_{10}-alkynyl, which may in turn be substituted in each case by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, -NH-C(=NNH_2)-, sulphonamide, sulphone, sulphoxide, or sulphonic acid.
In this context, R^x is -H, C_1-C_3-alkyl or phenyl.

Alkenyl

Alkenyl is a straight-chain or branched monovalent hydrocarbon chain having one or two double bonds and 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms (C_2-C_{10}-alkenyl), especially 2 or 3 carbon atoms (C_2-C_3-alkenyl), where, as will be apparent, when the alkenyl group contains more than one double bond, the double bonds may be isolated from one another or conjugated to one another. The alkenyl group is, for example, an ethenyl (or vinyl), prop-2-en-1-yl (or "allyl"), prop-1-en-1-yl, but-3-enyl, but-2-enyl, but-1-enyl, pent-4-enyl, pent-3-enyl, pent-2-enyl, pent-1-enyl, hex-5-enyl, hex-4-enyl, hex-3-enyl, hex-2-enyl, hex-1-enyl, prop-1-en-2-yl (or "isopropenyl"), 2-methylprop-2-enyl, 1-methylprop-2-enyl, 2-methylprop-1-enyl, 1-methylprop-1-enyl, 3-methylbut-3-enyl, 2-methylbut-3-enyl, 1-methylbut-3-enyl, 3-methylbut-2-enyl, 2-methylbut-2-enyl, 1-methylbut-2-enyl, 3-methylbut-1-enyl, 2-methylbut-1-enyl, 1-methylbut-1-enyl, 1,1-dimethylprop-2-enyl, 1-ethylprop-1-enyl, 1-propylvinyl, 1-Isopropylvinyl, 4-methylpent-4-enyl, 3-methylpent-4-enyl, 2-methylpent-4-enyl, 1-methylpent-4-enyl, 4-methylpent-3-enyl, 3-methylpent-3-enyl, 2-methylpent-3-enyl, 1-methylpent-3-enyl, 4-methylpent-2-enyl, 3-methylpent-2-enyl, 2-methylpent-2-enyl, 1-methylpent-2-enyl, 4-methylpent-1-enyl, 3-methylpent-1-enyl, 2-methylpent-1-enyl, 1-methylpent-1-enyl, 3-ethylbut-3-enyl, 2-ethylbut-3-enyl, 1-ethylbut-3-enyl, 3-ethylbut-2-enyl, 2-ethylbut-2-enyl, 1-ethylbut-2-enyl, 3-ethylbut-1-enyl, 2-ethylbut-1-enyl, 1-ethylbut-1-enyl, 2-propylprop-2-enyl, 1-propylprop-2-enyl, 2-isopropylprop-2-enyl, 1-isopropylprop-2-enyl, 2-propylprop-1-enyl, 1-propylprop-1-enyl, 2-isopropylprop-1-enyl, 1-isopropylprop-1-enyl, 3,3-dimethylprop-1-enyl, 1-(1,1-dimethylethyl)ethenyl, buta-1,3-dienyl, penta-1,4-dienyl or hexa-1,5-dienyl group. More particularly, the group is vinyl or allyl.

Alkynyl

Alkynyl is a straight-chain or branched monovalent hydrocarbon chain having one triple bond and having 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms (C_2-C_{10}-alkynyl), especially 2 or 3

carbon atoms (C_2-C_3-alkynyl). The C_2-C_6-alkynyl group is, for example, an ethynyl, prop-1-ynyl, prop-2-ynyl (or propargyl), but-1-ynyl, but-2-ynyl, but-3-ynyl, pent-1-ynyl, pent-2-ynyl, pent-3-ynyl, pent-4-ynyl, hex-1-ynyl, hex-2-ynyl, hex-3-ynyl, hex-4-ynyl, hex-5-ynyl, 1-methylprop-2-ynyl, 2-methylbut-3-ynyl, 1-methylbut-3-ynyl, 1-methylbut-2-ynyl, 3-methylbut-1-ynyl, 1-ethylprop-2-ynyl, 3-methylpent-4-ynyl, 2-methylpent-4-ynyl, 1-methylpent-4-ynyl, 2-methylpent-3-ynyl, 1-methylpent-3-ynyl, 4-methylpent-2-ynyl, 1-methylpent-2-ynyl, 4-methylpent-1-ynyl, 3-methylpent-1-ynyl, 2-ethylbut-3-ynyl, 1-ethylbut-3-ynyl, 1-ethylbut-2-ynyl, 1-propylprop-2-ynyl, 1-isopropylprop-2-ynyl, 2,2-dimethylbut-3-ynyl, 1,1-dimethylbut-3-ynyl, 1,1-dimethylbut-2-ynyl or 3,3-dimethylbut-1-ynyl group. More particularly, the alkyl group is ethynyl, prop-1-ynyl or prop-2-ynyl.

Cycloalkyl

Cycloalkyl is a saturated monovalent mono- or bicyclic hydrocarbyl radical having 3-12 carbon atoms (C_3-C_{12}-cycloalkyl).

In this context, a monocyclic hydrocarbyl radical is a monovalent hydrocarbyl radical having generally 3 to 10 (C_3-C_{10}-cycloalkyl), preferably 3 to 8 (C_3-C_8-cycloalkyl) and more preferably 3 to 7 (C_3-C_7-cycloalkyl) carbon atoms.

Preferred examples of monocyclic hydrocarbyl radicals include:
cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

Particular preference is given to a cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

In this context, a bicyclic hydrocarbyl radical is a hydrocarbyl radical having generally 3 to 12 carbon atoms (C_3-C_{12}-cycloalkyl), which should be understood here to mean a fusion of two saturated ring systems which together share two directly adjacent atoms. Preferred examples of bicyclic hydrocarbyl radicals include: bicyclo[2.2.0]hexyl, bicyclo[3.3.0]octyl, bicyclo[4.4.0]decyl, bicyclo[5.4.0]undecyl, bicyclo[3.2.0]heptyl, bicyclo[4.2.0]octyl, bicyclo[5.2.0]nonyl, bicyclo[6.2.0]decyl, bicyclo[4.3.0]nonyl, bicyclo[5.3.0]decyl, bicyclo[6.3.0]undecyl and bicyclo[5.4.0]undecyl.

Heterocycloalkyl

Heterocycloalkyl is a nonaromatic mono- or bicyclic ring system having one, two, three or four heteroatoms which may be the same or different. The heteroatoms may be nitrogen atoms, oxygen atoms or sulphur atoms.

A monocyclic ring system according to the present invention may have 3 to 8, preferably 4 to 7 and more preferably 5 or 6 ring atoms.

Preferred examples of a heterocycloalkyl having 3 ring atoms include:
aziridinyl.

Preferred examples of a heterocycloalkyl having 4 ring atoms include:
azetidinyl, oxetanyl.

Preferred examples of a heterocycloalkyl having 5 ring atoms include:
pyrrolidinyl, imidazolidinyl, pyrazolidinyl, pyrrolinyl, dioxolanyl and tetrahydrofuranyl.

Preferred examples of a heterocycloalkyl having 6 ring atoms include:
piperidinyl, piperazinyl, morpholinyl, dioxanyl, tetrahydropyranyl and thiomorpholinyl.

Preferred examples of a heterocycloalkyl having 7 ring atoms include:
azepanyl, oxepanyl, 1,3-diazepanyl, 1,4-diazepanyl.

Preferred examples of a heterocycloalkyl having 8 ring atoms include:
oxocanyl, azocanyl.

Among monocyclic heterocycloalkyl, preference is given to 4- to 7-membered saturated heterocyclyl radicals having up to two heteroatoms from the group of O, N and S. Particular preference is given to morpholinyl, piperidinyl, pyrrolidinyl and tetrahydrofuranyl.

A bicyclic ring system having one, two, three or four heteroatoms which may be the same or different may, according to the present invention, have 6 to 12 and preferably 6 to 10 ring atoms, where one, two, three or four carbon atoms may be exchanged for identical or different heteroatoms from the group of O, N and S.

Preferred examples include: azabicyclo[3.3.0]octyl, azabicyclo[4.3.0]nonyl, diazabicyclo[4.3.0]nonyl, oxazabicyclo[4.3.0]nonyl, thiazabicyclo[4.3.0]nonyl or azabicyclo[4.4.0]decyl, and radicals derived from further possible combinations as per the definition.

Particular preference is given to perhydrocyclopenta[c]pyrrolyl, perhydrofuro[3,2-c]pyridinyl, perhydropyrrolo[1,2-a]pyrazinyl, perhydropyrrolo[3,4-c]pyrrolyl and 3,4-methylenedioxyphenyl.

Heterocycloalkoxy

Heterocycloalkoxy is heterocycloalkyl bonded via an -O- group to the rest of the molecule.

Alkoxy

Alkoxy is a linear or branched saturated alkyl ether radical of the formula –O-alkyl having generally 1 to 6 (C_1-C_6-alkoxy), preferably 1 to 4 (C_1-C_4-alkoxy) and more preferably 1 to 3 (C_1-C_3-alkoxy) carbon atoms.

Preferred examples include:
methoxy, ethoxy, n-propoxy, isopropoxy, *tert*-butoxy, n-pentyloxy and n-hexyloxy.

Aryl

Aryl is a monovalent mono- or bicyclic aromatic ring system consisting of carbon atoms. Examples are naphthyl and phenyl; preference is given to phenyl or a phenyl radical.

C_6-C_{10}-Aralkyl

C_{6-10}-Aralkyl in the context of the invention is a monocyclic aromatic aryl, phenyl by way of example, to which a C_1-C_4-alkyl group is bonded.

An illustrative C_{6-10}-aralkyl group is benzyl.

Heteroaryl

Heteroaryl is a monovalent monocyclic, bicyclic or tricyclic aromatic ring system which has 5, 6, 8, 9, 10, 11, 12, 13 or 14 ring atoms (a "5- to 14-membered heteroaryl" group), especially 5, 6, 9 or 10 ring atoms, and contains at least one ring heteroatom and optionally one, two or three further ring heteroatoms from the group of N, O and S, and is bonded via a ring carbon atom or optionally (when permitted by the valency) via a ring nitrogen atom.

The heteroaryl group may be a 5-membered heteroaryl group, for example thienyl, furyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl or tetrazolyl; or a 6-membered heteroaryl group, for example pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl or triazinyl; or a tricyclic heteroaryl group, for example carbazolyl, acridinyl or phenazinyl; or a 9-membered heteroaryl group, for example benzofuranyl, benzothienyl, benzoxazolyl, benzisoxazolyl, benzimidazolyl, benzothiazolyl, benzotriazolyl, indazolyl, indolyl, isoindolyl, indolizinyl or purinyl; or a 10-membered heteroaryl group, for example quinolinyl, quinazolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinoxalinyl or pteridinyl.

In general, and unless stated otherwise, the heteroaryl radicals include all possible isomeric forms, for example tautomers and positional isomers in relation to the attachment point to the rest of the molecule. Thus, as an illustrative, non-exclusive example, the term pyridinyl includes pyridin-2-yl, pyridin-3-yl and pyridin-4-yl; or the term thienyl includes thien-2-yl and thien-3-yl.

C_5-C_{10}-Heteroaryl

C_{5-10}-Heteroaryl in the context of the invention is a mono- or bicyclic aromatic ring system having one, two, three or four heteroatoms which may be the same or different. The heteroatoms that can occur are: N, O, S, S(=O) and/or $S(=O)_2$. The bonding valence may be at any aromatic carbon atom or at a nitrogen atom.

A monocyclic heteroaryl radical according to the present invention has 5 or 6 ring atoms. Preference is given to heteroaryl radicals having one or two heteroatoms. Particular preference is given here to one or two nitrogen atoms.

Heteroaryl radicals having 5 ring atoms include, for example, the following rings:

thienyl, thiazolyl, furyl, pyrrolyl, oxazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, tetrazolyl and thiadiazolyl.

Heteroaryl radicals having 6 ring atoms include, for example, the following rings: pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl and triazinyl.

A bicyclic heteroaryl radical in accordance with the present invention has 9 or 10 ring atoms.

Heteroaryl radicals having 9 ring atoms include, for example, the following rings: phthalidyl, thiophthalidyl, indolyl, isoindolyl, indazolyl, benzothiazolyl, benzofuryl, benzothienyl, benzimidazolyl, benzoxazolyl, azocinyl, indolizinyl, purinyl, indolinyl.

Heteroaryl radicals having 10 ring atoms include, for example, the following rings: isoquinolinyl, quinolinyl, quinolizinyl, quinazolinyl, quinoxalinyl, cinnolinyl, phthalazinyl, 1,7- and 1,8-naphthyridinyl, pteridinyl, chromanyl.

Aryloxy

Aryloxy is an aryl radical of the formula aryl-O-.

Preferred examples include: phenoxy and naphthyloxy.

Heteroalkoxy

Heteroalkoxy is a straight-chain and/or branched hydrocarbyl chain which has 1 to 10 carbon atoms and is bonded via -O- to the rest of the molecule and may additionally be interrupted once or more than once by one or more of the groups -O-, -S-, -C(=O)-, -S(=O)-, $-S(=O)_2-$, $-NR^y-$,

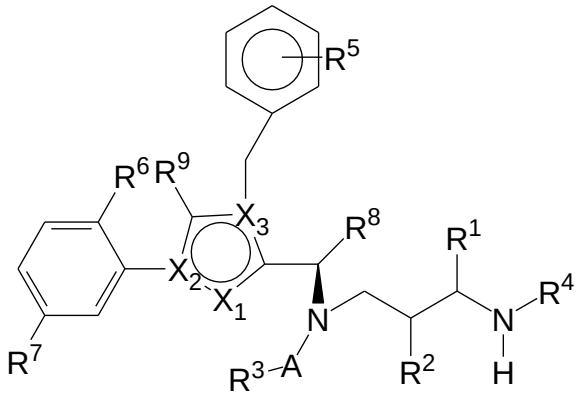
$-NR^yC(=O)-$, $-C(=O)-NR^y-$, $-NR^yNR^y-$, $-S(=O)_2-NR^yNR^y-$, $-C(=O)-NR^yNR^y-$, $-CR^x=N-O-$, and where the hydrocarbon chain, including the side chains, if present, may be substituted by $–NH-C(=O)-NH_2$, -COOH, -OH, $-NH_2$, $-NH-C(=NNH_2)-$, sulphonamide, sulphone, sulphoxide, or sulphonic acid.

In this context, R^y in each case is -H, phenyl, C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl or C_2-C_{10}-alkynyl, which may in turn be substituted in each case by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, -NH-C(=NNH_2)-, sulphonamide, sulphone, sulphoxide, or sulphonic acid. In this context, R^x is -H, C_1-C_3-alkyl or phenyl.

Halogen or halogen atom in the context of the invention is fluorine (-F), chlorine (-Cl), bromine (-Br), or iodine (-I).

Preference is given to fluorine (-F), chlorine (-Cl) and bromine (-Br).

The kinesin spindle protein inhibitor prodrugs according to the invention preferably have the following formula (IIa):



(IIa)

in which

X_1	is N,
X_2	is N and
X_3	is C,
or	
X_1	is N,
X_2	is C and
X_3	is N,
or	
X_1	is CH or CF,
X_2	is C and

X_3	is N,
or	
X_1	is NH,
X_2	is C and
X_3	is C,
or	
X_1	is CH,
X_2	is N and
X_3	is C.
A	is –C(=O)-, -S(=O)-, -S(=O)$_2$-, or -S(=O)$_2$-NH-,
R^1	is -H, –L-#1, –MOD or -$(CH_2)_{0-3}$Z,
Z	is -H, -NHY^3, -OY^3, -SY^3, halogen, -C(=O)-NY^1Y^2, or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2-$(CH_2CH_2O)_{0-3}$-$(CH_2)_{0-3}$Z' or -$CH(CH_2W)$Z',
Y^3	is -H or -$(CH_2)_{0-3}$Z',
Z'	is -H, -NH_2, -S(=O)$_3$H, -COOH, -NH-C(=O)-CH_2-CH_2-$CH(NH_2)$C(=O)- or -$(C(=O)$-NH-$CHY^4)_{1-3}$-COOH,
W	is -H or -OH,
Y^4	is linear or branched C_{1-6} alkyl optionally substituted by -NH-C(=O)-NH_2, or is aryl or benzyl optionally substituted by -NH_2,
R^2	is -H, –L-#1 , -MOD, -C(=O)-CHY^4-NHY^5 or -$(CH_2)_{0-3}$Z,
Z	is -H, halogen, -OY^3, -SY^3, -NHY^3, -C(=O)-NY^1Y^2, or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}$Z',
Y^3	is -H or -$(CH_2)_{0-3}$Z',
Z'	is -H, -S(=O)$_3$H, -NH_2 or –COOH,
Y^4	is linear or branched C_{1-6} alkyl optionally substituted by -NH-C(=O)-NH_2, or is aryl or benzyl optionally substituted by -NH_2,
Y^5	is -H or –C(=O)-CHY^6-NH_2,
Y^6	is linear or branched C_{1-6}-alkyl,
R^3	is -MOD, –L-#1, or an optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, heteroalkyl, heterocycloalkyl group which may be substituted by one to three OH groups, one to three halogen atoms, one to three mono-,

	di- or trihalogenated alkyl groups, one to three –O-alkyl groups, one to three -SH groups, one to three -S-alkyl groups, one to three -O-C(=O)-alkyl groups, one to three -O-C(=O)-NH-alkyl groups, one to three -NH-C(=O)-alkyl groups, one to three -NH-C(=O)-NH-alkyl groups, one to three -$S(=O)_n$-alkyl groups, one to three –$S(=O)_2$-NH-alkyl groups, 1-3 -NH-alkyl groups, one to three -$N(alkyl)_2$ groups, one to three NH_2 groups or one to three -$(CH_2)_{0-3}Z$ groups,
n	is 0, 1 or 2,
Z	is -H, halogen, -OY^3, -SY^3, -NHY^3, -C(=O)-NY^1Y^2 or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}Z'$,
Y^3	is -H, -$(CH_2)_{0-3}$-CH(NHC(=O)CH_3)Z',-$(CH_2)_{0-3}$-CH(NH_2)Z' or -$(CH_2)_{0-3}Z'$,
Z'	is -H, -$S(=O)_3H$, -NH_2 or –COOH,
R^4	is the legumain-cleavable group of the formulae Ia', Ia'' and Ia''',
R^5	is -H, -NH_2, -NO_2, halogen, -CN, CF_3, -OCF_3, -CH_2F, -CH_2F, -SH or -$(CH_2)_{0-3}Z$,
Z	is -H, -OY^3, -SY^3, halogen, -NHY^3, -C(=O)-NY^1Y^2, or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}Z'$,
Y^3	is -H or -$(CH_2)_{0-3}Z'$,
Z'	is -H, -$S(=O)_3H$, -NH_2 or –COOH,
R^6 and R^7	are independently -H, -CN, C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, hydroxyl, -NO_2, -NH_2, -COOH or halogen,
R^8	is C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, C_{4-10}-cycloalkyl, fluoro-C_{4-10}-cycloalkyl or -$(CH_2)_{0-2}$-(HZ^2),
HZ^2	is a 4- to 7-membered heterocycle having up to two heteroatoms selected from N, O and S, which may be substituted by –OH, -COOH, -NH_2 or –L-#1,

R^9 is -H, -F, $-CH_3$, $-CF_3$, $-CH_2F$ or $-CHF_2$,

–L-#1 is $-(L_b)_o-(LIG)_p$,

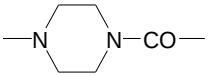
LIG is a binder which, after binding to a target molecule of a tumour cell, is internalized by the tumour cell and processed intracellularly and preferably lysosomally,

L_b is a linker,

o and p are independently 0 or 1,

–MOD is $–(NR^{10})_n-(G1)_o-G2-G3$,

R^{10} is -H or C_1-C_3-alkyl,

G1 is –NH-C(=O)- , -C(=O)-NH- or 

,

n is 0 or 1;

o is 0 or 1 and

G2 is a straight-chain or branched hydrocarbon chain which has 1 to 20 carbon atoms and may be interrupted once or more than once by -O-, -S-, -S(=O)-, $-S(=O)_2-$, $-NR^y-$, $-NR^yC(=O)-$, $-C(=O)NR^y-$, $-NR^yNR^y-$, $-S(=O)_2-NR^yNR^y-$, $-C(=O)-NR^yNR^y-$, -C(=O)-, $-CR^x=N-O$ and the straight-chain or branched hydrocarbon chain may be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid,

R^y is -H, phenyl, C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl or C_2-C_{10}-alkynyl, each of which may be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid,

Rx is -H, C_1-C_3-alkyl or phenyl,

G3 is –H or –COOH and

–MOD has at least one –COOH group,

and the salts, solvates and salts of the solvates thereof.

Preference is given here to those compounds in which

R^3 is a C_{1-10}-alkyl, C_{6-10}-aryl or C_{6-10}-aralkyl, C_{5-10}-heteroalkyl, C_{1-10}-alkyl-O-C_{6-10}-aryl or C_{5-10}-heterocycloalkyl group which may be substituted by one to

three OH groups, one to three halogen atoms, one to three mono-, di- or trihalogenated alkyl groups, one to three -O-alkyl groups, one to three -SH groups, one to three -S-alkyl groups, one to three -O-C(=O)-alkyl groups, one to three -O-C(=O)-NH-alkyl groups, one to three -NH-C(=O)-alkyl groups, one to three -NH-C(=O)-NH-alkyl groups, one to three -$S(=O)_n$-alkyl groups, one to three –$S(=O)_2$-NH-alkyl groups, 1-3 -NH-alkyl groups, one to three -$N(alkyl)_2$ groups, one to three NH_2 groups or one to three -$(CH_2)_{0-3}Z$ groups, where n and Z have the definitions given above.

The -$(C(=O)\text{-}NH\text{-}CHY^4)_{1-3}$-COOH radical means that one -C(=O)-NH-CHY^4-COOH radical is present, or two -(C(=O)-NH-CHY^4) radicals may be joined to one another, according to

-C(=O)-NH-CHY^4-C(=O)-NH-CHY^4-COOH,

or three radicals may be joined to one another, according to

-C(=O)-NH-CHY^4-C(=O)-NH-CHY^4-C(=O)-NH-CHY^4-COOH.

Particular preference is given to the compounds of the general formula (IIa) in which

X^1 is N,
X_2 is N and
X_3 is C,
or
X_1 is CH or CF,
X_2 is C and
X_3 is N,
or
X_1 is NH,
X_2 is C and
X_3 is C,
or
X_1 is H,
X_2 is N and
X_3 is C.

Especially preferred are those compounds of the general formula (IIa) in which

X_1 is N,
X_2 is N and
X_3 is C,
or
X_1 is CH,
X_2 is C and
X_3 is N.

Very particular preference is given to those compounds of the general formula (IIa) in which
X_1 is CH,
X_2 is C and
X_3 is N.

Preference is given to those compounds of the general formula (IIa) in which A is –C(=O)-.

Additionally preferred are those compounds of the general formula (IIa) in which
R^1 is –L-#1, -MOD, -H, -COOH, -C(=O)-NH-NH_2, -$(CH_2)_{1-3}NH_2$, -C(=O)-NZ''$(CH_2)_{1-3}NH_2$ and –C(=O)-NZ''CH_2COOH and
Z'' is -H or -NH_2.

If, in the general formula (IIa), R^4 is –L-#1, R^1 is preferably –MOD.

More particularly, in the general formula (IIa), R^4 is –L-#1 and R^1 is –MOD if R^3 is not -MOD.

In the general formula (IIa), R^2 is preferably -H.

In the general formula (IIa), R^3 is preferably –L-#1 or -MOD, or is C_{1-10}-alkyl which may optionally be substituted by –OH, -O-alkyl, -SH, -S-alkyl, -O-C(=O)-alkyl, -O-C(=O)-NH-alkyl, -NH-C(=O)-alkyl, -NH-C(=O)-NH-alkyl, -$S(=O)_n$-alkyl, -$S(=O)_2$-NH-alkyl, -NH-alkyl, -$N(alkyl)_2$, or -NH_2.
Alkyl here is preferably C_{1-3}alkyl-.

In the general formula (IIa), R^5 is preferably -H or -F.

In the general formula (IIa), R^6 and R^7 are preferably independently -H, C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, hydroxyl or halogen.

In the general formula (IIa), R^8 is preferably a branched C_{1-5}-alkyl group, especially a $-C(CH_3)_2-(CH_2)_{0-2}–R_y$ group, where R_y is –H, –OH, $-C(=O)_2H$, or $-NH_2$.

More preferably, R^8 is the $–C(CH_3)_2-(CH_2)–R_y$ group where R_y is –H.

In the general formula (IIa), R^9 is preferably -H or -F.

In the general formula (IIa), –MOD is preferably the group

$HOOC-(CHX)_x-AM-CH_2-CH_2-NH-C(=O)-$,
where
x is a number from 2 to 6,
X is -H, $-NH_2$ or -COOH, and
AM is -C(=O)-NH- or -NH-C(=O)-.

In the general formula (IIa), –MOD is more preferably the group

$HOOC-CH_2-CH_2-CH(COOH)-NH-C(=O)-CH_2-CH_2-NH-C(=O)-$,
$HOOC-CH(NH_2)-CH_2-CH_2-C(=O)-NH-CH_2-CH_2-NH-C(=O)-$
and
$HOOC-CH(NH_2)-(CH_2)_4-NH-C(=O)-CH_2-CH_2-NH-C(=O)-$.

Especially preferred are compounds of the general formula (IIa) in which none or one of the substituents R^1 and R^3 is –L-#1, and
in which
X_1 is N,
X_2 is N and
X_3 is C,

or

X_1 is CH or CF,

X_2 is C and

X_3 is N,

or

X_1 is NH,

X_2 is C and

X_3 is C,

or

X_1 is CH,

X_2 is N and

X_3 is C

and

A is –C(=O)-,

R^1 is -H, -COOH, -C(=O)-NH-NH_2, -$(CH_2)_{1-3}NH_2$, -C(=O)-NZ''$(CH_2)_{1-3}NH_2$ and –C(=O)-NZ''CH_2-COOH,

Z'' is -H or -NH_2,

R^2 is -H,

R^3 is a phenyl group which may be mono- or polysubstituted by halogen, C_{1-3}-alkyl or fluoro-C_{1-3}-alkyl, or is a C_{1-10}-alkyl group which may optionally be substituted by fluorine, –OY^4, -SY^4, -O-C(=O)-Y^4, -O-C(=O)-NH-Y^4, -NH-C(=O)-Y^4, -NH-C(=O)-NH-Y^4, -$S(=O)_n$-Y^4, -$S(=O)_2$-NH-Y^4, -NH-Y^4 or -$N(Y^4)_2$,

n is 1 or 2 and

Y^4 is –H or a phenyl group which may optionally be mono- or polysubstituted by halogen, C_{1-3}-alkyl or fluoro-C_{1-3}-alkyl, or is alkyl- which may be substituted by -OH, -COOH, and/or –NH-C(=O)-C_{1-3}-alkyl.

In this case, R^3 and Y^4 are preferably a phenyl group which may be mono- or polysubstituted by fluorine.

In this case, Y^4 is preferably C_{1-3}-alkyl.

Also especially preferred are those compounds of the general formula (IIa) in which

R^3	is a phenyl group which may be mono- or polysubstituted by –OH, -O-alkyl, -SH, -S-alkyl, -O-C(=O)-alkyl, -O-C(=O)-NH-alkyl, -NH-C(=O)-alkyl, -NH-C(=O)-NH-alkyl, $-S(=O)_n$-alkyl, $-S(=O)_2$-NH-alkyl, -NH-alkyl, $-N(alkyl)_2$, or $-NH_2$,
n	is 1 or 2,
R^5	is -H or -F,
R^6 and R^7	are independently -H, C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, hydroxyl or halogen,
R^8	is a branched C_{1-5}-alkyl group and
R^9	is -H or -F.

In this case, C_{1-3}-alkyl is particularly preferred.

Additionally preferred are the compounds of the general formula (IIa) in which

R^1	is –H, –L-#1, -COOH, HOOC-CH_2-CH_2-CH(COOH)-NH-C(=O)-CH_2-CH_2-NH-C(=O)-; HOOC-CH(NH_2)-CH_2-CH_2-C(=O)-NH-CH_2-CH_2-NH-C(=O)- or HOOC-CH(NH_2)-$(CH_2)_4$-NH-C(=O)-CH_2-CH_2-NH-C(=O)-,
R^2	is -H,
A	is –C(=O)-,
R^3	is -(CH_2)OH, -CH(CH_3)OH, -CH_2-S-CH_2CH-(COOH)-NH-C(=O)-CH_3,

		-$CH(CH_3)OCH_3$, a phenyl group which may be substituted by one to three halogen atoms, one to three amino groups, one to three alkyl groups or one to three haloalkyl groups,
		$HOOC-CH_2-CH_2-CH(COOH)-NH-C(=O)-CH_2-CH_2-NH-C(=O)-$;
		$HOOC-CH(NH_2)-CH_2-CH_2-C(=O)-NH-CH_2-CH_2-NH-C(=O)-$;
		$HOOC-CH(NH_2)-(CH_2)_4-NH-C(=O)-CH_2-CH_2-NH-C(=O)-$ or
		$–CH_2-S_x-(CH_2)_{0-4}-CHY^5-COOH$,
	x	is 0 or 1,
	Y^5	is -H or $-NHY^6$,
	Y^6	is –H, $–C(=O)-CH_3$ or –L-#1,
	R^5	is -H,
	R^6 and R^7	are independently -H, C_{1-3}-alkyl or halogen,
	R^8	is C_{1-4}-alkyl and
	R^9	is –H.

Preference is given here especially to those compounds in which

	R^6 and R^7	are independently hydrogen or fluorine and
	R^8	is tert-butyl.

Additionally preferred are those compounds in which

	R^1	is –H, -COOH,
		$HOOC-CH_2-CH_2-CH(COOH)-NH-C(=O)-CH_2-CH_2-NH-C(=O)-$,
		$HOOC-CH(NH_2)-CH_2-CH_2-C(=O)-NH-CH_2-CH_2-NH-C(=O)-$ or
		$HOOC-CH(NH_2)-(CH_2)_4-NH-C(=O)-CH_2-CH_2-NH-C(=O)-$,
	R^2	is -H,
	A	is $–C(=O)-$,

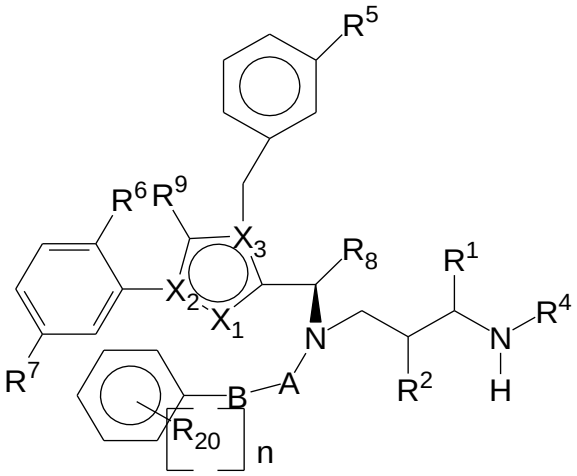
R^3	is -(CH_2)OH, -$CH(CH_3)$OH, -CH_2-S-CH_2CH(COOH)NH-C(=O)-CH_3, -$CH(CH_3)OCH_3$, HOOC-CH_2-CH_2-CH(COOH)-NH-C(=O)-CH_2-CH_2-NH-C(=O)-, HOOC-$CH(NH_2)$-CH_2-CH_2-C(=O)-NH-CH_2-CH_2-NH-C(=O)-, HOOC-$CH(NH_2)$-$(CH_2)_4$-NH-C(=O)-CH_2-CH_2-NH-C(=O)-, –CH_2-S_x-$(CH_2)_{0-4}$-CHY^5-COOH or a phenyl group which may be substituted by 1-3 halogen atoms, one to three amino groups, one to three alkyl groups or one to three haloalkyl groups,
x	is 0 or 1,
Y^5	is -H or -NHY^6,
Y^6	is –H, –C(=O)-CH_3 or –L-#1,
R^5	is -H,
R^6 and R^7	are independently -H, C_{1-3}-alkyl or halogen,
R^8	is C_{1-4}-alkyl and
R^9	is -H,

where one of the substituents R^1 and R^3 is –L-#1.

Preference is given here especially to those compounds in which

R^6 and R^7	are -F and
R^8	is tert-butyl.

Additionally preferred are compounds of the general formula (IIb)



(IIb)

in which

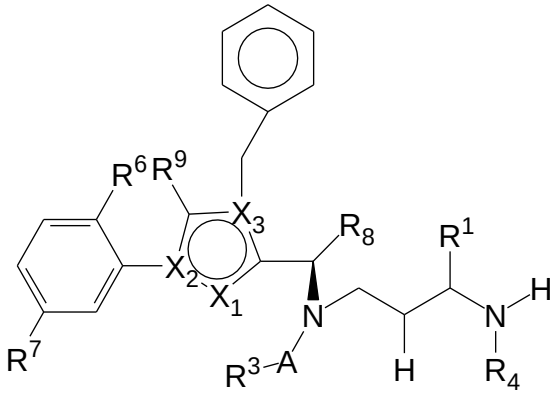
X_1, X_2,X_3, R^1,
R^2, R^4, R^5, R^6,
R^7, R^8 and R^9 have the definitions given in the general formula (IIa) and

A	is –C(=O)-,
B	is a single bond, –O-CH_2– or –CH_2-O-,
R^{20}	is –NH_2, -F, -CF_3, or -CH_3 and
n	is 0, 1 or 2.

Preference is given here to those compounds of the general formula (IIb) in which

X_1	is CH,
X_2	is C and
X_3	is N.

Preference is also given to those compounds of the general formula (IIc)



(IIc)

in which

X_1, X_2, X_3 A, R^1, R^3, R^4, R^6, R^7, R^8 and R^9 have the definitions given in the general formula (IIa).

Preference is given here to those compounds of the general formula (IIc) in which

X_1 is CH,

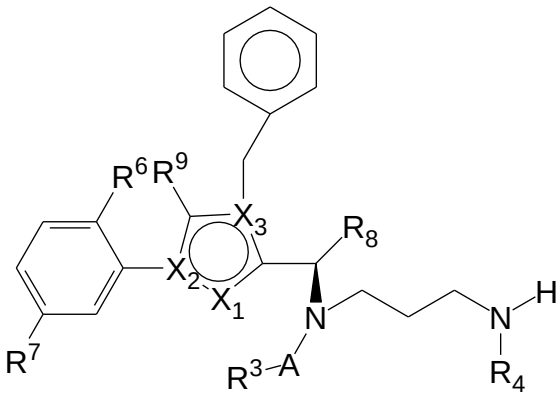
X_2 is C,

X_3 is N,

A is –C(=O)- and

R^3 is $–CH_2OH$, $-CH_2OCH_3$, $-CH(CH_3)OH$ or $-CH(CH_3)OCH_3$.

Preference is further also given to those compounds of the general formula (IId)



(IId)

in which

X_1, X_2, X_3, A, R^3, R^4, R^6, R^7, R^8 and R^9 have the definitions given in the general formula (IIa).

Preference is given here to those compounds of the general formula (IId) in which

X_1 is CH,

X_2 is C,

X_3 is N,

A is –C(=O)-,

R^3 is $–CH_2-S_x-(CH_2)_{0-4}-CHY^5-COOH$,

x is 0 or 1,

Y^5 is -H or $-NHY^6$ and

Y^6 is -H or $–C(=O)CH_3$.

Additionally preferred are those compounds of the general formulae (IIa), (IIb), (IIc) and (IId) in which

- Z is -Cl or -Br;

- R^1 is $-(CH_2)_{0-3}Z$,
  Z is $–C(=O)-NY^1Y^2$,
  Y^1 is -H, $-NH_2$, or $-(CH_2CH_2O)_{0-3}-(CH_2)_{0-3}Z'$;
  Y^2 is $-(CH_2CH_2O)_{0-3}-(CH_2)_{0-3}Z'$ and
  Z' is –COOH;

- Y^1 is -H,
  Y^2 is $-(CH_2CH_2O)_3-CH_2CH_2Z'$ and
  Z' is –COOH;

- Y^1 is -H,
  Y^2 is $-CH_2CH_2Z'$,
  Z' is $-(C(=O)NHCHY^4)_2-COOH$ and
  Y^4 has the definition given in the general formula (IIa);

- Y^1 is -H,
  Y^2 is $-CH_2CH_2Z'$,
  Z' is $-(C(=O)-NHCHY^4)_2-COOH$ and
  Y^4 is *i*-propyl or $–(CH_2)_3-NH-C(=O)-NH_2$;

- Y^1 is -H,
  Y^2 is $-CH_2CH_2Z'$,
  Z' is $-(C(=O)-NHCHY^4)_2-COOH$ and
  Y^4 is $–CH_3$ or $–(CH_2)_3-NH-C(=O)-NH_2$;

- Y^4 is linear or branched C_{1-6} alkyl optionally substituted by $–NH-C(=O)-NH_2$;

- Y^4 is *i*-propyl or $–CH_3$;

- Y^1 is -H,
  Y^2 is $-CH_2CH_2Z'$,
  Z' is $–C(=O)-NHCHY^4-COOH$ and
  Y^4 is optionally $–NH_2$-substituted aryl or benzyl;

- Y^4 is aminobenzyl;

- R^2 is $–(CH_2)_{0-3}Z$,
  Z is $–SY^3$ and
  Y^3 has the definition given above;

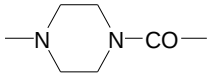
- R^4 is $–C(=O)-CHY^4-NHY^5$,
  Y4 has the definition given above and
  Y^5 is -H;

- R^4 is $–C(=O)-CHY^4-NHY^5$,
  Y^5 is $-C(=O)-CHY^6-NH_2$ and
  Y^4 and Y^6 have the definitions given above;

- Y^4 is linear or branched C_{1-6} alkyl which may optionally be substituted by $–NH-C(=O)-NH_2$.

Additionally preferred are those compounds of the general formula (IIa) in which R^1, R^2 or R^3 is -MOD.

Particular preference is given to those compounds in which R^3 is -MOD and R^1 is –L-#1, where

–MOD	is $-(NR^{10})_n-(G1)_o-G2-G3$,
R^{10}	is -H or C_1-C_3-alkyl;
G1	is –NH-C(=O)-, -C(=O)-NH- or  ,
n	is 0 or 1,
o	is 0 or 1,
G2	is a straight-chain or branched hydrocarbon chain which has 1 to 20 carbon atoms and may be interrupted once or more than once, identically or differently, by -O-, -S-, -S(=O)-, $-S(=O)_2$, $-NR^y$-, $-NR^yC(=O)$-, $-C(=O)-NR^y$, $-NR^yNR^y$-, $-S(=O)_2-NR^yNR^y$-, $-C(=O)-NR^yNR^y$-, -C(=O)-, $-CR^x$=N-O-, where the straight-chain or branched hydrocarbon chain may be substituted by –NH-C(=O)-NH_2, -COOH, -OH, $-NH_2$, sulphonamide, sulphone, sulphoxide, or sulphonic acid,
R^y	is -H, phenyl, C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl or C_2-C_{10}-alkynyl, each of which may be substituted by –NH-C(=O)-NH_2, -COOH, -OH, $-NH_2$, sulphonamide, sulphone, sulphoxide, or sulphonic acid,
Rx	is -H, C_1-C_3-alkyl or phenyl,
G3	is -H or -COOH and

where the –MOD group preferably has at least one –COOH group.

More preferably, the group –MOD has a COOH group, for example in a betaine group.

Preferably, this COOH group is in a terminal position.

Additionally more preferably, the –MOD group is the group

$-CH_2-S_x-(CH_2)_{0-4}-CHY^5-COOH$

in which

x is 0 or 1,

Y^5 is -H or -NHY^6 and

Y6 is -H or –$C(=O)CH_3$.

Additionally preferred are the compounds of the general formulae (IIa), (IIb), (IIc) and (IId) in which

X_1 is N,

X_2 is N and

X_3 is C,

or

X_1 is N,

X_2 is C and

X_3 is N,

or

X_1 is CH or CF,

X_2 is C and

X_3 is N,

or

X_1 is NH,

X_2 is C and

X_3 is C,

or

X_1 is CH or CF,

X_2 is N and

X_3 is C,

R^1 is -H, –L-#1, –MOD or -$(CH_2)_{0-3}$Z,

Z is -H, -NHY^3, -OY^3, -SY^3, halogen, -C(=O)-NY^1Y^2 or –C(=O)-OY^3,

Y^1 and Y^2 are independently -H, -NH_2, -$(CH_2CH_2O)_{0-3}$-$(CH_2)_{0-3}$Z'
or -$CH(CH_2W)$Z',

Y^3 is -H or -$(CH_2)_{0-3}$Z',

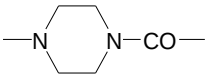
Z' is -H, -NH_2, -$S(=O)_3H$, -COOH, -NH-C(=O)-CH_2-CH_2-$CH(NH_2)$-COOH
or -(C(=O)-NH-CHY^4)$_{1-3}$COOH,

W is -H or -OH,

Y^4 is linear or branched C_{1-6} alkyl optionally substituted by

-NHC(=O)-NH_2, or is aryl or benzyl optionally substituted by –NH_2,

R^2	is -H, -C(=O)-CHY^4-NHY^5 or -$(CH_2)_{0-3}$Z,
Z	is -H, halogen, -OY^3, -SY^3, -NHY^3, -C(=O)-NY^1Y^2, or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}$Z',
Y^3	is -H or -$(CH_2)_{0-3}$Z',
Z'	is -H, -$S(=O)_3H$, -NH_2 or –COOH;
Y^4	is linear or branched C_{1-6} alkyl optionally substituted by –NH-C(=O)-NH_2 or is aryl or benzyl optionally substituted by –NH_2,
Y^5	is -H or –C(=O)-CHY^6-NH_2,
Y^6	is linear or branched C_{1-6}-alkyl,
A	is –C(=O)-, -S(=O)-, -$S(=O)_2$- or -$S(=O)_2$-NH-,
R^3	is –L-#1, -MOD, or an alkyl, cycloalkyl, aryl, heteroaryl, heteroalkyl, heterocycloalkyl group which may optionally be substituted by one to three OH groups, one to three halogen atoms, one to three mono-, di- or trihalogenated alkyl groups, one to three –O-alkyl groups, one to three -SH groups, one to three -S-alkyl groups, one to three -O-C(=O)-alkyl groups, one to three -O-C(=O)-NH-alkyl groups, one to three -NH-C(=O)-alkyl groups, one to three -NH-C(=O)-NH-alkyl groups, one to three -$S(=O)_n$-alkyl groups, one to three –$S(=O)_2$-NH-alkyl groups, one to three -NH-alkyl groups, one to three -$N(alkyl)_2$ groups, one to three -$NH((CH_2CH_2O)_{1-20}H)$- groups, one to three NH_2 groups or one to three -$(CH_2)_{0-3}$Z- groups,
Z	is -H, halogen, -OY^3, -SY^3, -NHY^3, -C(=O)-NY^1Y^2 or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}$Z',
Y^3	is -H, -$(CH_2)_{0-3}$-$CH(NH\text{-}C(=O)CH_3)$Z',-$(CH_2)_{0-3}$-$CH(NH_2)$Z' or -$(CH_2)_{0-3}$Z',
Z'	is -H, -$S(=O)_3H$, -NH_2 or –COOH,
R^5	is -H, -MOD, -NH_2, -NO_2, halogen, -CN, -CF_3, -OCF_3, -CH_2F, -CH_2F, -SH or -$(CH_2)_{0-3}$Z,
Z	is -H, -OY^3, -SY^3, halogen, -NHY^3, -C(=O)-NY^1Y^2, or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}$Z',
Y^3	is -H or -$(CH_2)_{0-3}$Z' and

Z'	is -H, -$S(=O)_3H$, -NH_2 or –C(=O)-OH,
R^6 and R^7	are independently -H, -CN, C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, hydroxyl, -NO_2, -NH_2, -COOH or halogen,
R^8	is C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, C_{4-10}-cycloalkyl or fluoro-C_{4-10}-cycloalkyl,
R^9	is -H, -F, -CH_3, -CF_3, -CH_2F or -CHF_2,
–MOD	is the -$(NR^{10})_n$-$(G1)_o$-G2-G3 group,
R^{10}	is -H or C_1-C_3-alkyl,
G1	is –NH-C(=O)- , -C(=O)-NH- or 
n	is 0 or 1,
o	is 0 or 1,
G2	is a straight-chain or branched hydrocarbon chain which has 1 to 20 carbon atoms and may be interrupted once or more than once by -O-, -S-, -S(=O)-, -$S(=O)_2$-, -NR^y-, -$NR^yC(=O)$-, -$C(=O)$-NR^y-, -NR^yNR^y-, -$S(=O)_2$-NR^yNR^y-, -$C(=O)$-NR^yNR^y-, where the straight-chain or branched hydrocarbon chain may be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid,
R^y	is –H, -C(=O)-, -CR^x=N-O- or is optionally NH-C(=O)-NH_2-, -COOH-, -OH-, -NH_2-, sulphonamide-, sulphone-, sulphoxide- or sulphonic acid-substituted phenyl, C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl or C_2-C_{10}-alkynyl,
R^x	is -H, C_1-C_3-alkyl or phenyl,
G3	is -H or -COOH and

where the –MOD group preferably has at least one –COOH group and

where R^1 and R^3 are not both –L-#1,

and the salts, solvates and salts of the solvates thereof.

Particular preference is given here to the compounds of the general formulae (IIa), (IIb), (IIc) and (IId) in which

X_1 is CH,
X_2 is C and
X_3 is N.

Additionally particularly preferred here are the compounds of the general formulae (IIa), (IIb), (IIc) and (IId) in which

R3	is a C_{1-10}-alkyl, C_{6-10}-aryl, C_{6-10}-aralkyl, C_{5-10}-heteroalkyl, C_{1-10}-alkyl-O-C_{6-10}-aryl or C5-10-heterocycloalkyl group which may optionally be substituted by one to three OH groups, one to three halogen atoms, one to three mono-, di- or trihalogenated alkyl groups, one to three –O-alkyl groups, one to three -SH groups, one to three -S-alkyl groups, one to three -O-C(=O)-alkyl groups, one to three -O-C(=O)-NH-alkyl groups, one to three -NH-C(=O)-alkyl groups, one to three -NH-C(=O)-NH-alkyl groups, one to three -S(=O)$_n$-alkyl groups, one to three –S(=O)$_2$-NH-alkyl groups, one to three -NH-alkyl groups, one to three -N(alkyl)$_2$ groups, one to three -NH((CH$_2$CH$_2$O)$_{1-20}$H) groups, one to three NH_2 groups or one to three -(CH$_2$)$_{0-3}$Z groups, and
Z	is -H, halogen, -OY^3, -SY^3, -NHY^3, -C(=O)-NY^{1}Y^2 or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -(CH$_2$)$_{0-3}$Z',
Y^3	is -H, -(CH$_2$)$_{0-3}$-CH(NH-C(=O)CH$_3$)Z',-(CH$_2$)$_{0-3}$-CH(NH_2)Z' or -(CH$_2$)$_{0-3}$Z' and
Z'	is -H, -S(=O)$_3$H, -NH_2 or –COOH.

Additionally preferred compounds of the general formulae (IIa), (IIb), (IIc) and (IId) are those in which

X_1 is N,
X_2 is N and
X_3 is C,
or
X_1 is N,
X_2 is C and
X_3 is N,
or
X_1 is CH or CF,

X_2 is C and
X_3 is N,
or
X_1 is NH,
X_2 is C and
X_3 is C,
or
X_1 is CH or CF,
X_2 is N and
X_3 is C,

R^1 is -H, –L-#1, –MOD or $-(CH_2)_{0-3}Z$,
Z is -H, $-NHY^3$, $-OY^3$, $-SY^3$, halogen, $-C(=O)-NY^1Y^2$ or $–C(=O)-OY^3$,
Y^1 and Y^2 are independently -H, $-NH_2$, $-(CH_2CH_2O)_{0-3}-(CH_2)_{0-3}Z'$ or $-CH(CH_2W)Z'$,
Y^3 is -H or $-(CH_2)_{0-3}Z'$,
Z' is -H, $-NH_2$, $-S(=O)_3H$, -COOH, $-NH-C(=O)-CH_2-CH_2-CH(NH_2)-COOH$ or $-(C(=O)-NH-CHY^4)_{1-3}COOH$,
W is -H or -OH,
Y^4 is linear or branched, optionally $–NH-C(=O)-NH_2$-substituted C_{1-6} alkyl or optionally $–NH_2$-substituted aryl or benzyl,

R^2 is -H, $-C(=O)-CHY^4-NHY^5$ or $-(CH_2)_{0-3}Z$,
Z is -H, halogen, $-OY^3$, $-SY^3$, $-NHY^3$, $-C(=O)-NY^1Y^2$ or $–C(=O)-OY^3$,
Y^1 and Y^2 are independently -H, $-NH_2$, or $-(CH_2)_{0-3}Z'$,
Y^3 is -H or $-(CH_2)_{0-3}Z'$,
Z' is -H, $-S(=O)_3H$, $-NH_2$ or –COOH,
Y^4 is linear or branched, optionally $–NH-C(=O)-NH_2$-substituted C_{1-6} alkyl or optionally $–NH_2$-substituted aryl or benzyl,
Y^5 is -H or $–C(=O)-CHY^6-NH_2$,
Y^6 is linear or branched C_{1-6}-alkyl,

A is –C(=O)-, -S(=O)-, $-S(=O)_2$- or $-S(=O)_2-NH-$,

R^3 is –L-#1, -MOD or an alkyl, cycloalkyl, aryl, heteroaryl, heteroalkyl, heterocycloalkyl group which may optionally be substituted by one to three

	OH groups, one to three halogen atoms, one to three mono-, di- or trihalogenated alkyl groups, one to three -O-alkyl groups, one to three -SH groups, one to three -S-alkyl groups, one to three -O-C(=O)-alkyl groups, one to three -O-C(=O)-NH-alkyl groups, one to three -NH-C(=O)-alkyl groups, one to three -NH-C(=O)-NH-alkyl groups, one to three $-S(=O)_n$-alkyl groups, one to three $–S(=O)_2$-NH-alkyl groups, one to three -NH-alkyl groups, one to three $-N(alkyl)_2$ groups, one to three $-NH((CH_2CH_2O)_{1-20}H)$- groups, one to three NH_2 groups or one to three $-(CH_2)_{0-3}Z$- groups,
Z	is -H, halogen, $-OY^3$, $-SY^3$, $-NHY^3$, $-C(=O)-NY^1Y^2$ or $–C(=O)-OY^3$,
Y^1 and Y^2	are independently -H, $-NH_2$, or $-(CH_2)_{0-3}Z'$,
Y^3	is -H, $-(CH_2)_{0-3}$-CH(NH-C(=O)CH_3)Z',$-(CH_2)_{0-3}$-CH(NH_2)Z' or $-(CH_2)_{0-3}Z'$,
Z'	is -H, $-S(=O)_3H$, $-NH_2$ or –COOH,
R^5	is -H, -MOD, $-NH_2$, $-NO_2$, halogen, -CN, $-CF_3$, $-OCF_3$, $-CH_2F$, $-CH_2F$, SH or $-(CH_2)_{0-3}Z$,
Z	is -H, $-OY^3$, $-SY^3$, halogen, $-NHY^3$, $-C(=O)-NY^1Y^2$, or $–C(=O)-OY^3$,
Y^1 and Y^2	are independently -H, $-NH_2$, or $-(CH_2)_{0-3}Z'$,
Y^3	is -H or $-(CH_2)_{0-3}Z'$,
Z'	is -H, $-S(=O)_3H$, $-NH_2$ or –COOH,
R^6 and R^7	are independently -H or halogen,
R^8	is C_{1-10}-alkyl or fluoro-C_{1-10}-alkyl,
R^9	is -H, -F, $-CH_3$, $-CF_3$, $-CH_2F$ or $-CHF_2$,
–L-#1	is the $-(L_b)_o-(LIG)_p$ group,
LIG	is a binder which, after binding to a target molecule of a tumour cell, is internalized by the tumour cell and processed intracellularly, preferably lysosomally,
L_b	is a linker,
o and p	are each independently 0 or 1,

–MOD is $–CH_2-S_x-(CH_2)_{0-4}-CHY^5-COOH$,

x is 0 or 1,

Y^5 is -H or $-NHY^6$,

Y^6 is -H or $–C(=O)CH_3$ and

where R^1 and R^3 are not both –L-#1,

and the salts, solvates and salts of the solvates thereof.

Preference is given here to those compounds of the general formulae (IIa), (IIb), (IIc) and (IId) in which

X_1 is CH,

X_2 is C and

X_3 is N.

Additionally preferred here are those compounds of the general formulae (IIa), (IIb), (IIc) and (IId) in which

R^3 is a C_{1-10}-alkyl, C_{6-10}-aryl or C_{6-10}-aralkyl, C_{5-10}-heteroalkyl,

C_{1-10}-alkyl-O-C_{6-10}-aryl or C_{5-10}-heterocycloalkyl group which may be substituted by one to three OH groups, one to three halogen atoms, one to three mono-, di- or trihalogenated alkyl groups, one to three –O-alkyl groups, one to three -SH groups, one to three -S-alkyl groups, one to three -O-C(=O)-alkyl groups, one to three -O-C(=O)-NH-alkyl groups, one to three -NH-C(=O)-alkyl groups, one to three -NH-C(=O)-NH-alkyl groups, one to three $-S(=O)_n$-alkyl groups, one to three $–S(=O)_2$-NH-alkyl groups, 1-3 -NH-alkyl groups, one to three $-N(alkyl)_2$ groups, one to three NH_2 groups or one to three $-(CH_2)_{0-3}Z$ groups,

Z is -H, halogen, $-OY^3$, $-SY^3$, $-NHY^3$, $-C(=O)-NY^1Y^2$ or $–C(=O)-OY^3$,

Y^1 and Y^2 are independently -H, $-NH_2$, or $-(CH_2)_{0-3}Z'$,

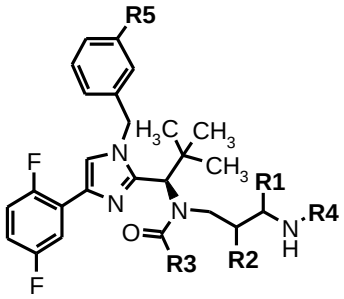
Y^3 is -H, $-(CH_2)_{0-3}-CH(NH-C(=O)CH_3)Z'$,$-(CH_2)_{0-3}-CH(NH_2)Z'$ or $-(CH_2)_{0-3}Z'$ and

Z' is - H, $-S(=O)_3H$, $-NH_2$ or –COOH.

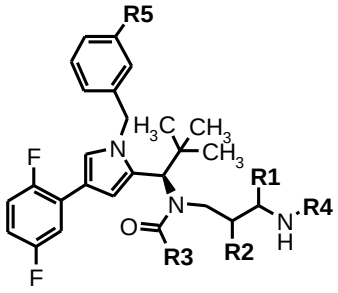
If the term "alkyl" is otherwise undefined, alkyl is preferably C_1-C_{10}-alkyl.

If the term "halogen" is otherwise undefined, halogen is fluorine (-F), chlorine (-Cl) and bromine (-Br).

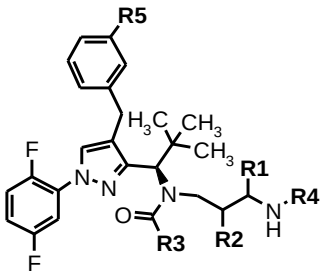
Particular preference is given to the following compounds of the general formulae (V), (VI) and (VII) in which R^1, R^2, R^3, R^4 and R^5 have the definitions given in the general formula (IIa):



Formula (V),



Formula (VI) and



Formula (VII)

Particular preference is given to the compounds of the general formulae (V), (VI) and (VII) in which
R^1, R^2 and R^5 are –H and R^4 has the definitions given in the general formula (IIa).

Especial preference is given here to the compounds of the general formula (VI).

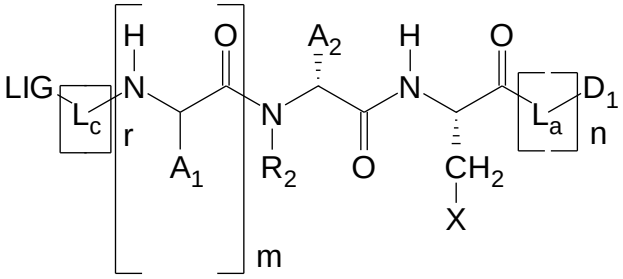
Binder which binds to a target molecule of a tumour cell

In the broadest sense, the term "binder" is understood to mean a molecule which binds to a target molecule present at a certain target cell population to be addressed by the binder-drug conjugate. The term binder is to be understood in its broadest meaning and also comprises, for example, lectins, proteins capable of binding to certain sugar chains, and phospholipid-binding proteins. Such binders include, for example, high molecular weight proteins (binding proteins), polypeptides or peptides (binding peptides), non-peptidic (e.g. aptamers (US5,270,163) review by Keefe AD., et al., Nat. Rev. Drug Discov. 2010; 9:537-550), or vitamins) and all other cell-binding molecules or substances. Binding proteins are, for example, antibodies and antibody fragments or antibody mimetics, for example affibodies, adnectins, anticalins, DARPins, avimers, nanobodies (review by Gebauer M. et al., Curr. Opinion in Chem. Biol. 2009; 13:245-255; Nuttall S.D. et al., Curr. Opinion in Pharmacology 2008; 8:608-617). Binding peptides are, for example, ligands of a ligand/receptor pair such as, for example, VEGF of the ligand/receptor pair VEGF/KDR, such as transferrin of the ligand/receptor pair transferrin/transferrin receptor or cytokine/cytokine receptor, such as TNFalpha of the ligand/receptor pair TNFalpha/TNFalpha receptor.

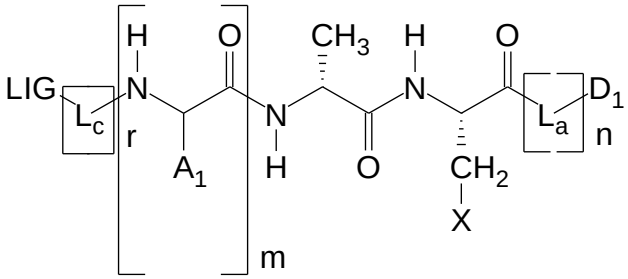
The prodrugs according to the invention preferably contain a binder which can bind to a target molecule of a tumour cell and is generally, after binding to the target molecule, internalized by the tumour cell and processed intracellularly, preferably lysosomally. One way in which this binder can be joined is by the group cleavable by the enzyme legumain, optionally via a linker, such that, after cleavage of the legumain-cleavable group, the active ingredient is present separately from the binder or a derivative thereof. In this case, -D in the general formula (Ia) represents $-D_1$ and -R in the general formula (Ia) represents $(L_c)_r$-LIG (embodiment A). In addition, the binder can be joined to the drug molecule, optionally via a linker, such that, after cleavage of the legumain-cleavable group, the active ingredient is present together with the binder or a derivative thereof. In this case, -D

in the general formula (Ia) represents $–D_1-(L_b)_o$-LIG and R- in the general formula (Ia) represents Z_1-(C(=O))q- (embodiment B).

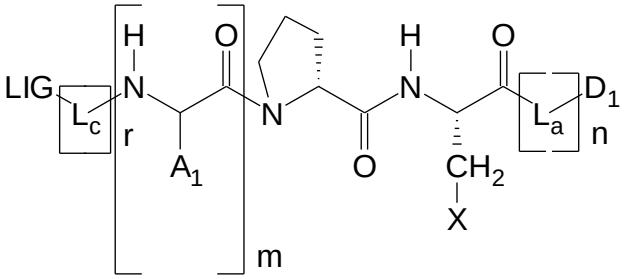
The compounds of embodiment A preferably have the following general formula III', more preferably the following general formulae III'' and III''':



(III'),



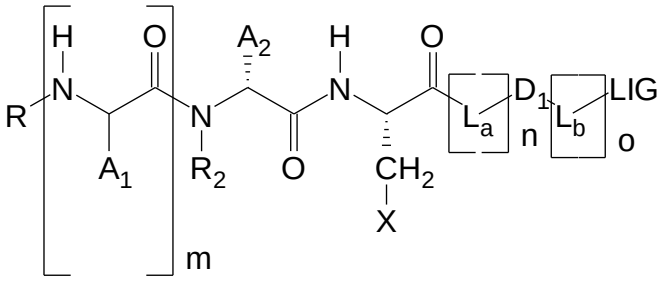
(III'') and



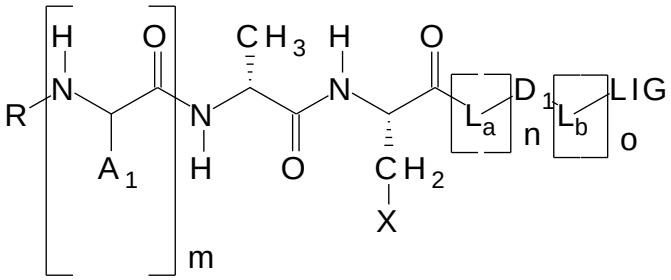
(III'''),

where m, n, r, LIG, L_a, L_c, D_1, X, A_1 , A_2 and R_2 have the definitions given in the general formula (Ia).

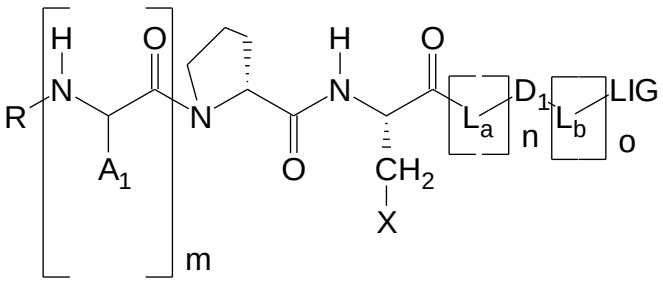
The compounds of embodiment B preferably have the following general formula (IV'), more preferably the following general formulae (IV'') and (IV'''):



(IV'),



(IV'') and



(IV'''),

where m, n, o, R, LIG, L_a, L_b, D_1, X, A_1, A_2 and R_2 have the definitions given in the general formula (Ia).

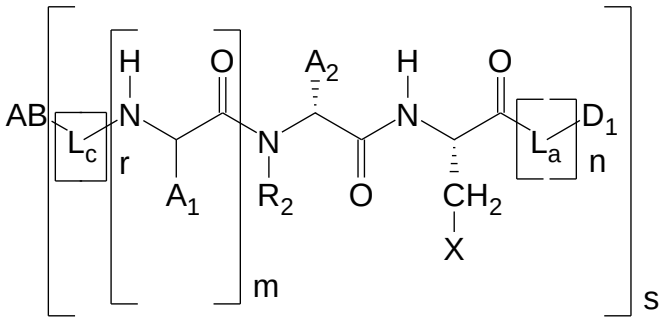
The binder LIG is generally a peptide, protein (e.g. an antibody) or a derivative thereof. Corresponding peptides are known from the literature (a review is given by D. Böhme and A. Beck-Sickinger, J.Pept.Sci. 2015-21.186; see also B. Forner et al., Specialty Chemicals Magazine, May 2012; V. Ahrens et al., Future Med. Chem. 2012, 4, 1567;

W. Tai et al., Mol. Pharmaceutics 2011, 8, 901; R. Soudy et al., J. Med. Chem. 2013, 56, 7564 and further references in the introduction by R. Soudy et al., M. Langer et al., J. Med. Chem. 2001, 44, 1341; C. Gruendker et al., Oncology Reports 2011, 26, 629). The peptide or derivative thereof is preferably selected from octreotide, GnRH-III, [D-Tyr^{6}, β-Ala^{11}, Phe^{13}, Nle^{14}]BN(6-14), NT(8-13), c(RGDfK), HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO: 161), NAPamide, [Phe^{7}, Pro^{34}]NPY, HER2-targeting peptide, ATEPRKQYATPRVFWTDAPG (SEQ ID NO: 162) or LQWRRDDNVHNFGVWARYRL (SEQ ID NO: 163) [the peptide sequences are stated here in the standard 1-letter code for amino acids]. It is possible to ascertain further peptide sequences with the aid of a screening method, as described in Umlauf et al, Bioconj.Chem. 2014, Oct. 15; 25(10): 1829-37.

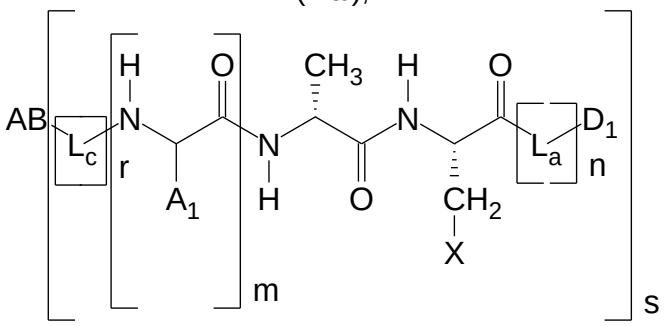
In the case of embodiment A, the peptide can be bonded directly (for example by its C terminus) to the N terminus of the legumain-cleavable group by a peptide bond. It is also possible for the peptide to be bonded to the N terminus of the legumain-cleavable group via a linker L_c, in which case the linker is preferably bonded to the C or N terminus of the peptide or to a lysine or cysteine side chain of the peptide.

In the case of embodiment B, the peptide can be bonded directly to the drug molecule. However, it is preferable for the peptide to be bonded to the drug molecule via a linker Lb, in which case the linker is preferably bonded to the C or N terminus of the peptide or to a lysine or cysteine side chain of the peptide. The binding of Lb or of the peptide is generally effected by substitution of a hydrogen atom in the drug molecule.

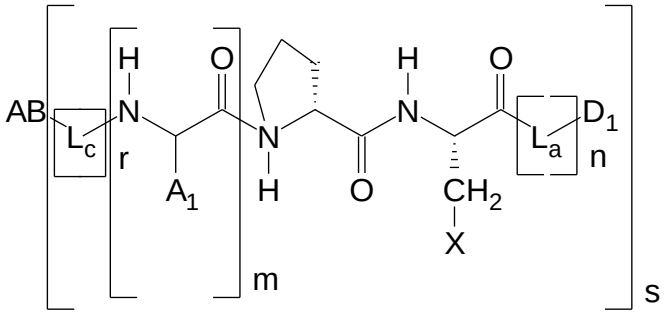
For instance, in the case of the compounds of the general formulae (IIa), (IIb), (IIc), (IId), (V), (VI) or (VII), it is possible to obtain conjugates by substitution of a hydrogen atom in R^1, R^2, R^3, R^5 or R^8, in a manner known to the person of average skill in the art, where one of the substituents R^1, R^2, R^3, R^5 or R^8 represents -(Lb)o-LIG.
A particularly preferred binder LIG is an antibody or an antigen-binding fragment or derivative thereof, which binds to an extracellular target molecule of a tumour cell. More preferably, LIG is an antibody or a fragment thereof to which one or more cytotoxic drug molecules are bound. In the case of embodiment A, the compounds according to the invention are thus antibody-drug conjugates (ADCs) of the following general formulae (IIIa') or (IIIa'') or (IIIa'''):



(IIIa'),



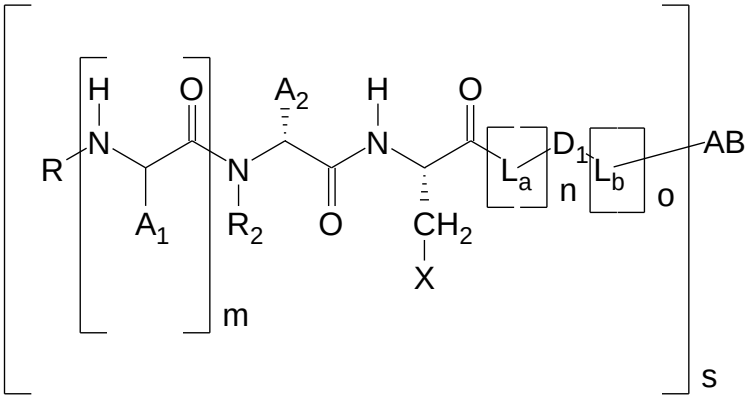
(IIIa'') and



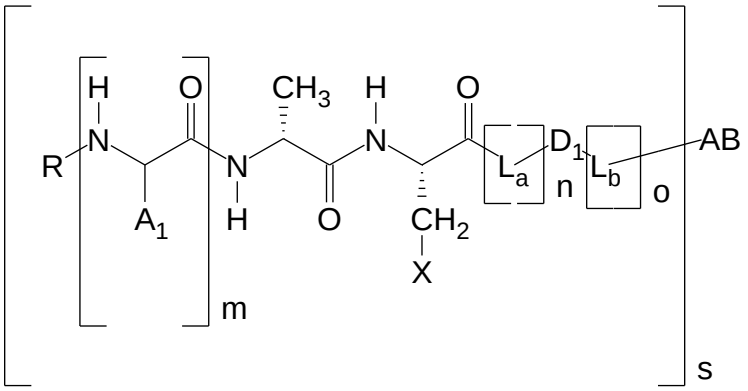
(IIIa''')

where m, n, r, L_a, L_c, D_1, X, A_1 , A_2 and R_2 have the definitions given in the general formula (Ia), AB represents an antibody, and s represents a number from 1 to 20, preferably 2 to 8, more preferably 3 to 5, for example 4. In this context, D_1 is preferably a compound of the general formula (IIa), (IIb), (IIc), (IId), (V), (VI) or (VII), where one substituent selected from R^1, R^2, R^3, R^4, R^8 does not have the definition given above under the general formulae (IIa), (IIb), (IIc), (IId), (V), (VI) and (VII), but represents the bond to L_a or a carbonyl group.

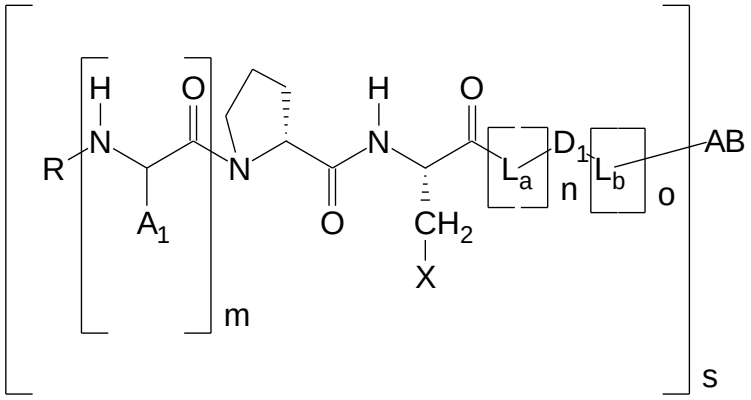
In the case of embodiment B, the compounds according to the invention are antibody-prodrug conjugates (APDCs) of the following general formulae (IVa') or (IVa'') or (IVa'''):



(IVa')



(IVa'') and



(IVa'''),

in which m, n, o, R, L_a, L_b, D_1, X, A_1 , A_2 and R_2 have the definitions given in the general formula (Ia) and AB represents an antibody, and s represents a number from 1 to 20, preferably 2 to 8, more preferably 3 to 5, for example 4. In this context, D_1 is preferably a compound of the general formulae (IIa), (IIb), (IIc), (IId), (V), (VI) or (VII), where one substituent R^4 does not have the definition given above under the general formulae (IIa), (IIb), (IIc), (IId), (V), (VI) or (VII), but represents the bond to L_a or a carbonyl group.

The antibody (for example AB in the above general formulae (IIIa) and (IVa) is preferably a human, humanized or chimeric monoclonal antibody or antigen-binding fragment thereof, especially an anti-TWEAKR antibody, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment of these. Particular preference is given to the anti-TWEAKR antibodies TPP-7006, TPP-7007, TPP-10336 and TPP-10337, the anti-B7H3 antibodies TPP-8382 and TPP-8567, the anti-EGFR-antibody cetuximab (TPP-981) and the anti-HER2-antibodies trastuzumab and TPP-1015, or an antigen-binding fragment of these.

The literature also discloses various options of covalent coupling (conjugation) of organic molecules to antibodies. Preference according to the invention is given to conjugation to the antibody via one or more sulphur atoms of cysteine residues of the antibody and/or via one or more NH groups of lysine residues of the antibody. However, it is also possible to bind the organic molecule to the antibody via free carboxyl groups or via sugar residues of the antibody.

The antibody binds to an extracellular target molecule of the tumour cell. A "target molecule" in the broadest sense is understood to mean a molecule which is present in the target cell population and which may be a protein (for example a receptor of a growth factor) or a non-peptidic molecule (for example a sugar or phospholipid). It is preferably a receptor or an antigen.

The term "extracellular" target molecule describes a target molecule, bound to the cell, which is on the outside of a cell, or the part of a target molecule which is on the outside of a cell, i.e. an antibody can bind to its extracellular target molecule in an intact cell. An extracellular target molecule may be anchored in the cell membrane or be a component of the cell membrane. The person skilled in the art is aware of methods for identifying

extracellular target molecules. For proteins, this may be by determining the transmembrane domain(s) and the orientation of the protein in the membrane. These data are usually deposited in protein databases (e.g. SwissProt).

The term “cancer target molecule” describes a target molecule which is more abundantly present on one or more cancer cell species than on non-cancer cells of the same tissue type. Preferably, the cancer target molecule is selectively present on one or more cancer cell species compared with non-cancer cells of the same tissue type, where selectively describes an at least two-fold enrichment on cancer cells compared to non-cancer cells of the same tissue type (a “selective cancer target molecule”). The use of cancer target molecules allows the selective therapy of cancer cells using the conjugates according to the invention.

The term "binder" according to the present invention is understood to mean a binder peptide, a derivative of a binder peptide, a binder protein or a derivative of a binder protein. The binder is linked to the linker via a bond. The binder can be linked by means of a heteroatom of the binder. Inventive heteroatoms of the binder which can be used for linkage are:

sulphur, via a sulphhydryl group of the binder,

oxygen, via a carboxylic group or hydroxyl group of the binder, and

nitrogen, via a primary or secondary amine group.

More particularly, according to the present invention, the term "binder" is understood to mean an antibody.

The above-listed heteroatoms may be present in the natural antibody or are introduced by chemical methods or methods of molecular biology. According to the invention, the attachment of the antibody to the organic radical in formula (I) has only a minor effect on the binding activity of the antibody with respect to the target molecule.

In a preferred embodiment, the linkage has no effect on the binding activity of the binder with respect to the target molecule.

In accordance with the present invention, the term "antibody" is to be understood in its broadest meaning and comprises immunoglobulin molecules, for example intact or modified monoclonal antibodies, polyclonal antibodies or multispecific antibodies (e.g. bispecific antibodies). An immunoglobulin molecule preferably comprises a molecule having four polypeptide chains, two heavy chains (H chains) and two light chains (L chains) which are typically linked by disulphide bridges. Each heavy chain comprises a variable domain of the heavy chain (abbreviated VH) and a constant domain of the heavy chain. The constant domain of the heavy chain may, for example, comprise three domains CH1, CH2 and CH3. Each light chain comprises a variable domain (abbreviated VL) and a constant domain. The constant domain of the light chain comprises a domain (abbreviated CL). The VH and VL domains may be subdivided further into regions having hypervariability, also referred to as complementarity determining regions (abbreviated CDR) and regions having low sequence variability (framework region, abbreviated FR). Typically, each VH and VL region is composed of three CDRs and up to four FRs. For example from the amino terminus to the carboxy terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. An antibody may be obtained from any suitable species, e.g. rabbit, llama, camel, mouse or rat. In one embodiment, the antibody is of human or murine origin. An antibody may, for example, be human, humanized or chimeric.

The term “monoclonal” antibody refers to antibodies obtained from a population of substantially homogeneous antibodies, i.e. individual antibodies of the population are identical except for naturally occurring mutations, of which there may be a small number. Monoclonal antibodies recognize a single antigenic binding site with high specificity. The term monoclonal antibody does not refer to a particular preparation process.

The term “intact” antibody refers to antibodies comprising both an antigen-binding domain and the constant domain of the light and heavy chain. The constant domain may be a naturally occurring domain or a variant thereof having a number of modified amino acid positions.

The term “modified intact” antibody refers to intact antibodies fused via their amino terminus or carboxy terminus by means of a covalent bond (e.g. a peptide bond) with a further polypeptide or protein not originating from an antibody. Furthermore, antibodies may be modified such that, at defined positions, reactive cysteines are introduced to facilitate coupling to a toxophor (see Junutula et al. Nat Biotechnol. 2008, 26(8)925-32).

The term “human” antibody refers to antibodies which can be obtained from a human or which are synthetic human antibodies. A “synthetic” human antibody is an antibody which is partially or entirely obtainable in silico from synthetic sequences based on the analysis of human antibody sequences. A human antibody can be encoded, for example, by a nucleic acid isolated from a library of antibody sequences of human origin. An example of such an antibody can be found in Söderlind et al., Nature Biotech. 2000, 18:853-856.

The term “humanized” or “chimeric” antibody describes antibodies consisting of a non-human and a human portion of the sequence. In these antibodies, part of the sequences of the human immunoglobulin (recipient) is replaced by sequence portions of a non-human immunoglobulin (donor). In many cases, the donor is a murine immunoglobulin. In the case of humanized antibodies, amino acids of the CDR of the recipient are replaced by amino acids of the donor. Sometimes, amino acids of the framework, too, are replaced by corresponding amino acids of the donor. In some cases the humanized antibody contains amino acids present neither in the recepient nor in the donor, which were introduced during the optimization of the antibody. In the case of chimeric antibodies, the variable domains of the donor immunoglobulin are fused with the constant regions of a human antibody.

The term complementarity determining region (CDR) as used herein refers to those amino acids of a variable antibody domain which are required for binding to the antigen. Typically, each variable region has three CDR regions referred to as CDR1, CDR2 and CDR3. Each CDR region may embrace amino acids according to the definition of Kabat and/or amino acids of a hypervariable loop defined according to Chotia. The definition according to Kabat comprises, for example, the region from about amino acid position 24 – 34 (CDR1), 50 – 56 (CDR2) and 89 – 97 (CDR3) of the variable light chain and 31 – 35 (CDR1), 50 – 65 (CDR2) and 95 – 102 (CDR3) of the variable heavy chain (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The definition according to Chotia comprises, for example, the region from about amino acid position 26 – 32 (CDR1), 50 – 52 (CDR2) and 91 –96 (CDR3) of the variable light chain and 26 – 32 (CDR1), 53 – 55 (CDR2) and 96 – 101 (CDR3) of the variable heavy chain (Chothia and Lesk; J Mol Biol 196 901-917 (1987)). In some cases, a CDR may comprise amino acids from a CDR region defined according to Kabat and Chotia.

Depending on the amino acid sequence of the constant domain of the heavy chain, antibodies may be categorized into different classes. There are five main classes of intact antibodies: IgA, IgD, IgE, IgG and IgM, and several of these can be divided into further subclasses. (Isotypes), e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The constant domains of the heavy chain, which correspond to the different classes, are referred to as [alpha/α], [delta/δ], [epsilon/ε], [gamma/γ] and [my/μ]. Both the three-dimensional structure and the subunit structure of antibodies are known.

The term “functional fragment” or “antigen-binding antibody fragment” of an antibody/immunoglobulin is defined as a fragment of an antibody/immunoglobulin (e.g. the variable domains of an IgG) which still comprise the antigen binding domains of the antibody/immunoglobulin. The “antigen binding domain” of an antibody typically comprises one or more hypervariable regions of an antibody, for example the CDR, CDR2 and/or CDR3 region. However, the “framework” or “skeleton” region of an antibody may also play a role during binding of the antibody to the antigen. The framework region forms the skeleton of the CDRs. Preferably, the antigen binding domain comprises at least amino acids 4 to 103 of the variable light chain and amino acids 5 to 109 of the variable heavy chain, more preferably amino acids 3 to 107 of the variable light chain and 4 to 111 of the variable heavy chain, especially preferably the complete variable light and heavy chains, i.e. amino acids 1 – 109 of the VL and 1 to 113 of the VH (numbering according to WO97/08320).

“Functional fragments” or “antigen-binding antibody fragments” of the invention encompass, non-conclusively, Fab, Fab’, F(ab’)2 and Fv fragments, diabodies, Single Domain Antibodies (DAbs), linear antibodies, individual chains of antibodies (single-chain Fv, abbreviated to scFv); and multispecific antibodies, such as bi and tri-specific antibodies, for example, formed from antibody fragments C. A. K Borrebaeck, editor (1995) Antibody Engineering (Breakthroughs in Molecular Biology), Oxford University Press; R. Kontermann & S. Duebel, editors (2001) Antibody Engineering (Springer Laboratory Manual), Springer Verlag. Antibodies other than “multispecific” or “multifunctional” antibodies are those having identical binding sites. Multispecific antibodies may be specific for different epitopes of an antigen or may be specific for epitopes of more than one antigen (see, for example, WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., 1991, J. Immunol. 14760 69; U. S. Pat. Nos. 4,474,893; 4,7 14,68 1 ; 4,925,648; 5,573,920; 5,601,8 19; or Kostelny et al., 1992, J. Immunol. 148 1547 1553). An $F(ab')_2$ or Fab molecule may be constructed such that the

number of intermolecular disulphide interactions occurring between the Ch1 and the CL domains can be reduced or else completely prevented.

“Epitopes” refer to protein determinants capable of binding specifically to an immunoglobulin or T cell receptors. Epitopic determinants usually consist of chemically active surface groups of molecules such as amino acids or sugar side chains or combinations thereof, and usually have specific 3-dimensional structural properties and also specific charge properties.

“Functional fragments” or “antigen-binding antibody fragments” may be fused with another polypeptide or protein, not originating from an antibody, via the amino terminus or carboxyl terminus thereof, by means of a covalent bond (e.g. a peptide linkage). Furthermore, antibodies and antigen-binding fragments may be modified by introducing reactive cysteines at defined locations, in order to facilitate coupling to a toxophor (see Junutula et al. Nat Biotechnol. 2008 Aug; 26(8)925-32).

Polyclonal antibodies can be prepared by methods known to a person of ordinary skill in the art. Monoclonal antibodies may be prepared by methods known to a person of ordinary skill in the art (Köhler and Milstein, Nature, 256, 495-497, 1975). Human and humanized monoclonal antibodies may be prepared by methods known to a person of ordinary skill in the art (Olsson et al., Meth Enzymol. 92, 3-16 or Cabilly et al US 4,816,567 or Boss et al US 4,816,397).

A person of ordinary skill in the art is aware of diverse methods for preparing human antibodies and fragments thereof, such as, for example, by means of transgenic mice (N Lonberg and D Huszar, Int Rev Immunol. 1995; 13(1)65-93) or phage display technologies (Clackson et al., Nature. 1991 Aug 15;352(6336)624-8). Antibodies of the invention may be obtained from recombinant antibody libraries consisting for example of the amino acid sequences of a multiplicity of antibodies compiled from a large number of healthy volunteers. Antibodies may also be produced by means of known recombinant DNA technologies. The nucleic acid sequence of an antibody can be obtained by routine sequencing or is available from publically accessible databases.

An “isolated” antibody or binder has been purified to remove other constituents of the cell. Contaminating constituents of a cell which may interfere with a diagnostic or therapeutic use are, for example, enzymes, hormones, or other peptidic or non-peptidic constituents of a cell. A preferred antibody or binder is one which has been purified to an extent of

more than 95% by weight, relative to the antibody or binder (determined for example by Lowry method, UV-Vis spectroscopy or by SDS capillary gel electrophoresis). Moreover an antibody which has been purified to such an extent that it is possible to determine at least 15 amino acids of the amino terminus or of an internal amino acid sequence, or which has been purified to homogeneity, the homogeneity being determined by SDS-PAGE under reducing or non-reducing conditions (detection may be determined by means of Coomassie Blau staining or preferably by silver coloration). However, an antibody is normally prepared by one or more purification steps.

The term “specific binding” or “binds specifically” refers to an antibody or binder which binds to a predetermined antigen/target molecule. Specific binding of an antibody or binder typically describes an antibody or binder having an affinity of at least 10^{-7} M (as Kd value; i.e. preferably those with Kd values smaller than 10^{-7} M), with the antibody or binder having an at least two times higher affinity for the predetermined antigen/target molecule than for a non-specific antigen/target molecule (e.g. bovine serum albumin, or casein) which is not the predetermined antigen/target molecule or a closely related antigen/target molecule. The antibodies preferably have an affinity of at least 10^{-7} M (as Kd value; in other words preferably those with smaller Kd values than 10^{-7} M), preferably of at least 10^{-8} M, more preferably in the range from 10^{-9} M to 10^{-11} M. The Kd values may be determined, for example, by means of surface plasmon resonance spectroscopy.

The antibody-drug conjugates of the invention likewise exhibit affinities in these ranges. The affinity is preferably not substantially affected by the conjugation of the drugs (in general, the affinity is reduced by less than one order of magnitude, in other words, for example, at most from 10^{-8} M to 10^{-7} M).

The antibodies used in accordance with the invention are also notable preferably for a high selectivity. A high selectivity exists when the antibody of the invention exhibits an affinity for the target protein which is better by a factor of at least 2, preferably by a factor of 5 or more preferably by a factor of 10, than for an independent other antigen, e.g. human serum albumin (the affinity may be determined, for example, by means of surface plasmon resonance spectroscopy).

Furthermore, the antibodies of the invention that are used are preferably cross-reactive. In order to be able to facilitate and better interpret preclinical studies, for example toxicological or activity studies (e.g. in xenograft mice), it is advantageous if the antibody used in accordance with the invention not only binds the human target protein but also

binds the species target protein in the species used for the studies. In one embodiment the antibody used in accordance with the invention, in addition to the human target protein, is cross-reactive to the target protein of at least one further species. For toxicological and activity studies it is preferred to use species of the families of rodents, dogs and non-human primates. Preferred rodent species are mouse and rat. Preferred non-human primates are rhesus monkeys, chimpanzees and long-tailed macaques.

In one embodiment, the antibody used in accordance with the invention, in addition to the human target protein, is cross-reactive to the target protein of at least one further species selected from the group of species consisting of mouse, rat and long-tailed macaque (Macaca fascicularis). Especially preferred are antibodies used in accordance with the invention which in addition to the human target protein are at least cross-reactive to the mouse target protein. Preference is given to cross-reactive antibodies whose affinity for the target protein of the further non-human species differs by a factor of not more than 50, more particularly by a factor of not more than ten, from the affinity for the human target protein.

Antibodies directed against a cancer target molecule

The target molecule towards which the binder, for example an antibody or an antigen-binding fragment thereof, is directed is preferably a cancer target molecule. The term “cancer target molecule” describes a target molecule which is more abundantly present on one or more cancer cell species than on non-cancer cells of the same tissue type. Preferably, the cancer target molecule is selectively present on one or more cancer cell species compared with non-cancer cells of the same tissue type, where selectively describes an at least two-fold enrichment on cancer cells compared to non-cancer cells of the same tissue type (a “selective cancer target molecule”). The use of cancer target molecules allows the selective therapy of cancer cells using the conjugates according to the invention.

Antibodies which are specific against an antigen, for example cancer cell antigen, can be prepared by a person of ordinary skill in the art by means of methods with which he or she is familiar (such as recombinant expression, for example) or may be acquired commercially (as for example from Merck KGaA, Germany). Examples of known commercially available antibodies in cancer therapy are Erbitux® (cetuximab, Merck KGaA), Avastin® (bevacizumab, Roche) and Herceptin® (trastuzumab, Genentech). Trastuzumab is a recombinant humanized monoclonal antibody of the IgG1kappa type

which in a cell-based assay (Kd = 5 nM) binds the extracellular domains of the human epidermal growth receptor with high affinity. The antibody is produced recombinantly in CHO cells.

In a preferred embodiment, the target molecule is a selective cancer target molecule.

In a particularly preferred embodiment, the target molecule is a protein.

In one embodiment, the target molecule is an extracellular target molecule. In a preferred embodiment, the extracellular target molecule is a protein.

Cancer target molecules are known to those skilled in the art. Examples of these are listed below.

Examples of cancer target molecules are:

(1) EGFR (EGF receptor, NCBI Reference Sequence NP_005219.2, NCBI Gene ID: 1956)

(2) mesothelin (SwissProt Reference Q13421-3), mesothelin being encoded by amino acids 296-598. Amino acids 37-286 code for megakaryocyte-potentiating factor. Mesothelin is anchored in the cell membrane by a GPI anchor and is localized extracellularly.

(3) Carboanhydrase IX (CA9, SwissProt Reference Q16790), NCBI Gene ID: 768)

(4) C4.4a (NCBI Reference Sequence NP_055215.2; synonym LYPD3, NCBI Gene ID: 27076)

(5) CD52 (NCBI Reference Sequence NP_001794.2)

(6) HER2 (ERBB2; NCBI Reference Sequence NP_004439.2; NCBI Gene ID: 2064)

(7) CD20 (NCBI Reference Sequence NP_068769.2)

(8) the lymphocyte activation antigen CD30 (SwissProt ID P28908)

(9) the lymphocyte adhesion molecule CD22 (SwissProt ID P20273; NCBI Gene ID: 933)

(10) the myloid cell surface antigen CD33 (SwissProt ID P20138; NCBI Gene ID: 945)

(11) the transmembrane glycoprotein NMB (GPNMB, SwissProt ID Q14956, NCBI Gene ID: 10457)

(12) the adhesion molecule CD56 (SwissProt ID P13591)

(13) the surface molecule CD70 (SwissProt ID P32970, NCBI Gene ID: 970)

(14) the surface molecule CD74 (SwissProt ID P04233, NCBI Gene ID: 972)

(15) the B-lymphocyte antigen CD19 (SwissProt ID P15391, NCBI Gene ID: 930)

(16) the surface protein Mucin-1 (MUC1, SwissProt ID P15941, NCBI Gene ID: 4582)

(17) the surface protein CD138 (SwissProt ID P18827)

(18) the integrin alphaV (NCBI Reference Sequence: NP_002201.1, NCBI Gene ID: 3685)

(19) the teratocarcinoma-derived growth factor 1 protein TDGF1 (NCBI Reference Sequence: NP_003203.1, NCBI Gene ID: 6997)

(20) the prostate-specific membrane antigen PSMA (Swiss Prot ID: Q04609; NCBI Gene ID: 2346)

(21) the tyrosine protein kinase EPHA2 (Swiss Prot ID: P29317, NCBI Gene ID: 1969)

(22) the surface protein SLC44A4 (NCBI Reference Sequence: NP_001171515.1, NCBI Gene ID: 80736)

(23) the surface protein BMPR1B (SwissProt: O00238)

(24) the transport protein SLC7A5 (SwissProt: Q01650)

(25) the epithelial prostate antigen STEAP1 (SwissProt: Q9UHE8, Gene ID: 26872)

(26) the ovarian carcinoma antigen MUC16 (SwissProt: Q8WXI7, Gene ID: 94025)

(27) the transport protein SLC34A2 (SwissProt: O95436, Gene ID: 10568)

(28) the surface protein SEMA5b (SwissProt: Q9P283)

(29) the surface protein LYPD1 (SwissProt: Q8N2G4)

(30) the endothelin receptor type B EDNRB (SwissProt: P24530, NCBI Gene ID: 1910)

(31) the ring finger protein RNF43 (SwissProt: Q68DV7)

(32) the prostate carcinoma-associated protein STEAP2 (SwissProt: Q8NFT2)

(33) the cation channel TRPM4 (SwissProt: Q8TD43)

(34) the complement receptor CD21 (SwissProt: P20023)

(35) the B-cell antigen receptor complex-associated protein CD79b (SwissProt: P40259, NCBI Gene ID: 974)

(36) the cell adhesion antigen CEACAM6 (SwissProt: P40199)

(37) the dipeptidase DPEP1 (SwissProt: P16444)

(38) the interleukin receptor IL20Ralpha (SwissProt: Q9UHF4, NCBI Gene ID: 3559)

(39) the proteoglycan BCAN (SwissProt: Q96GW7)

(40) the ephrin receptor EPHB2 (SwissProt: P29323)

(41) the prostate stem cell-associated protein PSCA (NCBI Reference Sequence: NP_005663.2)

(42) the surface protein LHFPL3 (SwissProt: Q86UP9)

(43) the receptor protein TNFRSF13C (SwissProt: Q96RJ3)

(44) the B-cell antigen receptor complex-associated protein CD79a (SwissProt: P11912)

(45) the receptor protein CXCR5 (CD185; SwissProt: P32302; NCBI Gene ID 643, NCBI Reference Sequence: NP_001707.1)

(46) the ion channel P2X5 (SwissProt: Q93086)

(47) the lymphocyte antigen CD180 (SwissProt: Q99467)

(48) the receptor protein FCRL1 (SwissProt: Q96LA6)

(49) the receptor protein FCRL5 (SwissProt: Q96RD9)

(50) the MHC class II molecule Ia antigen HLA-DOB (NCBI Reference Sequence: NP_002111.1)

(51) the T-cell protein VTCN1 (SwissProt: Q7Z7D3)

(52) TWEAKR (FN14, TNFRSF12A, NCBI Reference Sequence: NP_057723.1, NCBI Gene ID: 51330)

(53) the lymphocyte antigen CD37 (Swiss Prot: P11049, NCBI Gene ID: 951)

(54) the FGF receptor 2; FGFR2 (NCBI Gene ID: 2263; Official Symbol: FGFR2). FGFR2 receptor occurs in different splice variants (alpha, beta, IIIb, IIIc). All splice variants can act as target molecule.

(55) the transmembrane glycoprotein B7H3 (CD276; NCBI Gene ID: 80381 NCBI Reference Sequence: NP_001019907.1, Swiss Prot: Q5ZPR3-1)

(56) the B cell receptor BAFFR (CD268; NCBI Gene ID:115650)

(57) the receptor protein ROR 1 (NCBI Gene ID: 4919)

(58) the surface receptor CD123 (IL3RA; NCBI Gene ID: 3563; NCBI Reference Sequence: NP_002174.1; Swiss-Prot: P26951)

(59) the receptor protein syncytin (NCBI Gene ID 30816)

(60) aspartate beta-hydroxylase (ASPH; NCBI Gene ID 444)

(61) the cell surface glycoprotein CD44 (NCBI Gene ID: 960)

(62) CDH15 (Cadherin 15, NCBI Gene ID: 1013)

(63) the cell surface glycoprotein CEACAM5 (NCBI Gene ID: 1048)

(64) the cell adhesion molecule L1-like (CHL1, NCBI Gene ID: 10752)

(65) the receptor tyrosine kinase c-Met (NCBI Gene ID: 4233)

(66) the notch ligand DLL3 (NCBI Gene ID: 10683)

(67) the ephrin A4 (EFNA4, NCBI Gene ID: 1945)

(68) ectonucleotide pyrophosphatase/phosphodiesterase 3 (ENPP3, NCBI Gene ID: 5169)

(69) coagulation factor III (F3, NCBI Gene ID: 2152)

(70) FGF receptor 3 (FGFR3, NCBI Gene ID: 2261)

(71) the folate hydrolase FOLH1 (NCBI Gene ID: 2346)

(72) the folate receptor 1 (FOLR1; NCBI Gene ID: 2348)

(73) the guanylate cyclase 2C (GUCY2C, NCBI Gene ID: 2984)

(74) the KIT proto-oncogen receptor tyrosine kinase (NCBI Gene ID: 3815)

(75) lysosomal-associated membrane protein 1 (LAMP1, NCBI Gene ID: 3916)

(76) lymphocyte antigen 6 complex, locus E (LY6E, NCBI Gene ID: 4061)

(77) the protein NOTCH3 (NCBI Gene ID: 4854)

(78) protein tyrosine kinase 7 (PTK7, NCBI Gene ID: 5754)

(79) nectin cell adhesion molecule 4 (PVRL4, NECTIN4, NCBI Gene ID: 81607)

(80) the transmembrane protein syndecan 1 (SDC1, NCBI Gene ID: 6382)

(81) SLAM family member 7 (SLAMF7, NCBI Gene ID: 57823)

(82) the transport protein SLC39A6 (NCBI Gene ID: 25800)

(83) SLIT- and NTRK-like family member 6 (SLITRK6, NCBI Gene ID: 84189)

(84) the cell surface receptor TACSTD2 (NCBI Gene ID: 4070)

(85) the receptor protein TNFRSF8 (NCBI Gene ID: 943)

(86) the receptor protein TNFSF13B (NCBI Gene ID: 10673)

(87) the glycoprotein TPBG (NCBI Gene ID: 7162)

(88) the cell surface receptor TROP2 (TACSTD2, NCBI Gene ID: 4070)

(89) the galanin-like G protein-coupled receptor KISS1R (GPR54, NCBI Gene ID: 84634)

(90) the transport protein SLAMF6 (NCBI Gene ID: 114836)

In a preferred subject of the invention, the cancer target molecule is selected from the group consisting of the cancer target molecules (1) – (90), especially TWEAKR, B7H3, EGFR and HER2.

In a further particularly preferred subject of the invention, the binder binds to an extracellular cancer target molecule which is selected from the group consisting of the cancer target molecules (1) – (90), especially TWEAKR, B7H3, EGFR and HER2.

In a further particularly preferred subject of the invention, the binder binds specifically to an extracellular cancer target molecule which is selected from the group consisting of the cancer target molecules (1) – (90), especially TWEAKR, B7H3, EGFR and HER2. In a preferred embodiment, the binder, after binding to its extracellular target molecule on the target cell, is internalized by the target cell through the binding. This causes the binder-drug conjugate, which may be an immunoconjugate or an ADC, to be taken up by the target cell. The binder is then processed, preferably intracellularly, with preference lysosomally.

In one embodiment the binder is a binding protein. In a preferred embodiment the binder is an antibody, an antigen-binding antibody fragment, a multispecific antibody or an antibody mimetic.

Preferred antibody mimetics are affibodies, adnectins, anticalins, DARPins, avimers, or nanobodies. Preferred multispecific antibodies are bispecific and trispecific antibodies.

In a preferred embodiment the binder is an antibody or an antigen-binding antibody fragment, more preferably an isolated antibody or an isolated antigen-binding antibody fragment.

Preferred antigen-binding antibody fragments are Fab, Fab', F(ab')2 and Fv fragments, diabodies, DAbs, linear antibodies and scFv. Particularly preferred are Fab, diabodies and scFv.

In a particularly preferred embodiment the binder is an antibody. Particularly preferred are monoclonal antibodies or antigen-binding antibody fragments thereof. Further particularly preferred are human, humanized or chimeric antibodies or antigen-binding antibody fragments thereof.

Antibodies or antigen-binding antibody fragments which bind cancer target molecules may be prepared by a person of ordinary skill in the art using known processes, such as, for example, chemical synthesis or recombinant expression. Binders for cancer target molecules may be acquired commercially or may be prepared by a person of ordinary skill in the art using known processes, such as, for example, chemical synthesis or recombinant expression. Further processes for preparing antibodies or antigen-binding antibody fragments are described in WO 2007/070538 (see page 22 “Antibodies”). The person skilled in the art knows how processes such as phage display libraries (e.g. Morphosys HuCAL Gold) can be compiled and used for discovering antibodies or antigen-binding antibody fragments (see WO 2007/070538, page 24 ff and AK Example 1 on page 70, AK Example 2 on page 72). Further processes for preparing antibodies that use DNA libraries from B cells are described for example on page 26 (WO 2007/070538). Processes for humanizing antibodies are described on page 30-32 of WO2007070538 and in detail in Queen, et al., Pros. Natl. Acad. Sci. USA 8610029-10033,1989 or in WO 90/0786. Furthermore, processes for recombinant expression of proteins in general and of antibodies in particular are known to the person skilled in the art (see, for example, in Berger and Kimrnel (Guide to Molecular Cloning Techniques, Methods in Enzymology, Vo1. 152, Academic Press, Inc.); Sambrook, et al., (Molecular Cloning A Laboratory Manual, (Second Edition, Cold Spring Harbor Laboratory Press; Cold Spring Harbor, N.Y.; 1989) Vol. 1-3); Current Protocols in Molecular Biology, (F. M. Ausabel et al. [Eds.], Current Protocols, Green Publishing Associates, Inc. / John Wiley & Sons, Inc.); Harlow et al., (Monoclonal Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory Press

(19881, Paul [Ed.]); Fundamental Immunology, (Lippincott Williams & Wilkins (1998)); and Harlow, et al., (Using Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory Press (1998)). The person skilled in the art knows the corresponding vectors, promoters and signal peptides which are necessary for the expression of a protein/antibody. Commonplace processes are also described in WO 2007/070538 on pages 41–45. Processes for preparing an IgG1 antibody are described for example in WO 2007/070538 in Example 6 on page 74 ff. Processes which allow the determination of the internalization of an antibody after binding to its antigen are known to the skilled person and are described for example in WO 2007/070538 on page 80. The person skilled in the art is able to use the processes described in WO 2007/070538 that have been used for preparing carboanhydrase IX (Mn) antibodies in analogy for the preparation of antibodies with different target molecule specificity.

Bacterial expression

The person skilled in the art is aware of the way in which antibodies, antigen-binding fragments thereof or variants thereof can be produced with the aid of bacterial expression.

Suitable expression vectors for bacterial expression of desired proteins are constructed by insertion of a DNA sequence which encodes the desired protein within the functional reading frame together with suitable translation initiation and translation termination signals and with a functional promoter. The vector comprises one or more phenotypically selectable markers and a replication origin in order to enable the retention of the vector and, if desired, the amplification thereof within the host. Suitable prokaryotic hosts for transformation include but are not limited to *E. coli, Bacillus subtilis, Salmonella typhimurium* and various species from the genus *Pseudomonas, Streptomyces*, and *Staphylococcus*. Bacterial vectors may be based, for example, on bacteriophages, plasmids, or phagemids. These vectors may contain selectable markers and a bacterial replication origin, which are derived from commercially available plasmids. Many commercially available plasmids typically contain elements of the well-known cloning vector pBR322 (ATCC 37017). In bacterial systems, a number of advantageous expression vectors can be selected on the basis of the intended use of the protein to be expressed.

After transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is de-reprimed/induced by suitable means (for example a change in temperature or chemical induction), and the cells are cultivated for an additional period. The cells are typically harvested by centrifugation and if necessary digested in a physical manner or by chemical means, and the resulting raw extract is retained for further purification.

Therefore, a further embodiment of the present invention is an expression vector comprising a nucleic acid which encodes a novel antibody of the present invention.

Antibodies of the present invention or antigen-binding fragments thereof include naturally purified products, products which originate from chemical syntheses, and products which are produced by recombinant technologies in prokaryotic hosts, for example *E. coli*, *Bacillus subtilis, Salmonella typhimurium* and various species from the genus *Pseudomonas, Streptomyces*, and *Staphylococcus*, preferably *E. coli.*

Mammalian cell expression

The person skilled in the art is aware of the way in which antibodies, antigen-binding fragments thereof or variants thereof can be produced with the aid of mammalian cell expression.

Preferred regulatory sequences for expression in mammalian cell hosts include viral elements which lead to high expression in mammalian cells, such as promoters and/or expression amplifiers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), simian virus 40 (SV40) (such as the SV40 promoter/enhancer), from adenovirus, (for example the adenovirus major late promoter (AdMLP)) and from polyoma. The expression of the antibodies may be constitutive or regulated (for example induced by addition or removal of small molecule inductors such as tetracycline in combination with the Tet system).

For further description of viral regulatory elements and sequences thereof, reference is made, for example, to U.S. 5,168,062 by Stinski, U.S. 4,510,245 by Bell et al. and U.S. 4,968,615 by Schaffner et al. The recombinant expression vectors may likewise include a replication origin and selectable markers (see, for example, U.S. 4,399,216, 4,634,665 and U.S. 5,179,017). Suitable selectable markers include genes which impart resistance

to substances such as G418, puromycin, hygromycin, blasticidin, zeocin/bleomycin, or methotrexate, or selectable markers which lead to auxotrophy of a host cell, such as glutamine synthetase (Bebbington et al., Biotechnology (N Y). 1992 Feb;10(2):169-75), when the vector has been introduced into the cell.

For example, the dihydrofolate reductase (DHFR) gene imparts resistance to methotrexate, the neo gene imparts resistance to G418, the bsd gene from *Aspergillus terreus* imparts resistance to blasticidin, puromycin N-acetyltransferase imparts resistance to puromycin, the Sh ble gene product imparts resistance to zeocin, and resistance to hygromycin is imparted by the *E. coli* hygromycin resistance gene (hyg or hph). Selectable markers such as DHFR or glutamine synthetase are also helpful for amplification techniques in conjunction with MTX and MSX.

The transfection of an expression vector into a host cell can be executed with the aid of standard techniques, including by electroporation, nucleofection, calcium phosphate precipitation, lipofection, polycation-based transfection such as polyethyleneimine (PEI)-based transfection and DEAE-dextran transfection.

Suitable mammalian host cells for the expression of antibodies, antigen-binding fragments thereof, or variants thereof include Chinese hamster ovary (CHO) cells such as CHO-K1, CHO-S, CHO-K1SV [including DHFR-CHO cells, described in Urlaub and Chasin, (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220 and Urlaub et al., Cell. 1983 Jun;33(2):405-12, used with a DHFR-selectable marker, as described in R. J. Kaufman and P. A. Sharp (1982) Mol. Biol. 159:601-621, and other knockout cells, as detailed in Fan et al., Biotechnol Bioeng. 2012 Apr;109(4):1007-15), NS0 myeloma cells, COS cells, HEK293 cells, HKB11 cells, BHK21 cells, CAP cells, EB66 cells, and SP2 cells.

The expression of antibodies, antigen-binding fragments thereof, or variants thereof can also be effected in a transient or semi-stable manner in expression systems such as HEK293, HEK293T, HEK293-EBNA, HEK293E, HEK293-6E, HEK293 Freestyle, HKB11, Expi293F, 293EBNALT75, CHO Freestyle, CHO-S, CHO-K1, CHO-K1SV, CHOEBNALT85, CHOS-XE, CHO-3E7 or CAP-T cells (for example like Durocher et al., Nucleic Acids Res. 2002 Jan 15;30(2):E9)

In some embodiments, the expression vector is constructed in such a way that the protein to be expressed is secreted into the cell culture medium in which the host cells are

growing. The antibodies, the antigen-binding fragments thereof, or the variants thereof can be obtained from the cell culture medium with the aid of protein purification methods known to those skilled in the art.

Purification

The antibodies, the antigen-binding fragments thereof, or the variants thereof can be obtained and purified from recombinant cell cultures with the aid of well-known methods, examples of which include ammonium sulphate or ethanol precipitation, acid extraction, protein A chromatography, protein G chromatography, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography (HIC), affinity chromatography, hydroxyapatite chromatography and lectin chromatography. High-performance liquid chromatography ("HPLC") can likewise be employed for purification. See, for example, Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, N.Y., (1997-2001), e.g., Chapters 1, 4, 6, 8, 9, 10.

Antibodies of the present invention or antigen-binding fragments thereof, or variants thereof include naturally purified products, products from chemical synthesis methods and products which are produced with the aid of recombinant techniques in prokaryotic or eukaryotic host cells. Eukaryotic hosts include, for example, yeast cells, higher plant cells, insect cells and mammalian cells. Depending on the host cell chosen for the recombinant expression, the protein expressed may be in glycosylated or non-glycosylated form.

In a preferred embodiment, the antibody is purified (1) to an extent of more than 95% by weight, measured, for example, by the Lowry method, by UV-vis spectroscopy or by SDS capillary gel electrophoresis (for example with a Caliper LabChip GXII, GX 90 or Biorad Bioanalyzer instrument), and in more preferred embodiments more than 99% by weight, (2) to a degree suitable for determination of at least 15 residues of the N-terminal or internal amino acid sequence, or (3) to homogeneity determined by SDS-PAGE under reducing or non-reducing conditions with the aid of Coomassie blue or preferably silver staining.

Usually, an isolated antibody is obtained with the aid of at least one protein purification step.

The antigen-binding fragment according to any of the preceding embodiments or an antigen-binding fragment of an antibody according to any of the preceding embodiments which is an scFv, Fab, Fab fragment or a F(ab)2 fragment.

The antibody or the antigen-binding fragment according to any of the preceding embodiments which is a monoclonal antibody or an antigen-binding fragment thereof.

The antibody or the antigen-binding fragment according to any of the preceding embodiments which is a human, humanized or chimeric antibody or an antigen-binding fragment.

Anti-TWEAKR antibodies

According to the invention, it is possible to use anti-TWEAKR antibodies.

The expression “anti-TWEAKR antibody” or “an antibody which binds to TWEAKR” relates to an antibody which specifically binds the cancer target molecule TWEAKR (NCBI Reference Sequence: NP_057723.1, SEQ ID NO: 164), preferably having an affinity sufficient for a diagnostic and/or therapeutic application. In particular embodiments, the antibody binds TWEAKR with a dissociation constant (K_D) of ≤ 1µM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM.

Examples of antibodies which bind to TWEAKR are disclosed, for example, in WO2009/020933(A2), WO2009/140177 (A2), WO 2014/198817 (A1) and WO 2015/189143 (A1). These antibodies and antigen-binding fragments can be used in the context of this invention.

ITEM-4 is an anti-TWEAKR antibody which was described by Nakayama et al. (Nakayama, et al., 2003, Biochem Biophy Res Comm, 306:819-825). Humanized variants of this antibody based on CDR grafting are described by Zhou et al. (Zhou et al., 2013, J Invest Dermatol. 133(4):1052-62) and in WO 2009/020933. Humanized variants of ITEM-4 are TPP-7006, TPP-7007, TPP-10334, TPP-10335, TPP-10336 and TPP-10337. These antibodies and antigen-binding fragments can be used in the context of this invention.

Preference is given in the context of this invention to the anti-TWEAKR antibodies TPP-2090, TPP-2658, TPP-5442, TPP-8825, TPP-7006, TPP-7007, TPP-10334, TPP-10335, TPP-10336 and TPP-10337. More preferred are the anti-TWEAKR antibodies TPP-7006,

TPP-7007, TPP-10334, TPP-10335, TPP-10336 and TPP-10337. Particular preference is given to the anti-TWEAKR antibodies TPP-7006, TPP-7007, TPP-10336 and TPP-10337.

Anti-B7H3 antibodies

According to the invention, it is possible to use anti-B7H3 antibodies.

The expression “anti-B7H3 antibody” or “an antibody which binds to B7H3” relates to an antibody which specifically binds the cancer target molecule B7H3 (NCBI Reference Sequence: NP_001019907.1, SEQ ID NO: 165), preferably having an affinity sufficient for a diagnostic and/or therapeutic application. In particular embodiments, the antibody binds B7H3 with a dissociation constant (K_D) of ≤ 1µM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM.

Examples of antibodies and antigen-binding fragments which bind to B7H3 are known to those skilled in the art and are described, for example, in WO201109400, EP1773884 and WO2014061277. EP2121008 describes the anti-B7H3 antibody 8H9 and the CDR sequences thereof.

These antibodies and antigen-binding fragments can be used in the context of this invention.

A preferred embodiment of the anti-B7H3 antibodies was obtained by screening an antibody phage display library for cells that express recombinant mouse B7H3 (mouse CD276; Gene ID: 102657) and human B7H3 (human CD276; Gene ID: 80381). The antibodies obtained were transformed to the human IgG1 format. The anti-B7H3 antibody TPP-8382 is a preferred example.

Preference is given in the context of this invention to the anti-B7H3 antibodies TPP-8382 and TPP-8567.

Anti-HER2 antibodies:

According to the invention, it is possible to use anti-HER2 antibodies.

The expression “anti-HER2 antibody” or “an antibody which binds to HER2” relates to an antibody which specifically binds the cancer target molecule HER2 (NCBI Reference Sequence: NP_004439.2, SEQ ID NO: 166), preferably having an affinity sufficient for a

diagnostic and/or therapeutic application. In particular embodiments, the antibody binds HER2 with a dissociation constant (K_D) of ≤ 1µM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM.

An example of an antibody that binds to the cancer target molecule HER2 is trastuzumab (Genentech). Trastuzumab is a humanized antibody used inter alia for the treatment of breast cancer. In a particularly preferred embodiment, the anti-HER2 antibody is TPP-1015 (trastuzumab analogue).

Further examples of antibodies that bind to HER2 are, in addition to trastuzumab (INN 7637, CAS No: RN: 180288-69-1) and pertuzumab (CAS No: 380610-27-5), also antibodies as disclosed in WO 2009/123894-A2, WO 200/8140603-A2, or in WO 2011/044368-A2. An example of an anti-HER2 conjugate is trastuzumab-emtansine (INN-No. 9295). These antibodies and antigen-binding fragments can be used in the context of this invention.

Particular preference is given in the context of this invention to the anti-HER2 antibodies trastuzumab and TPP-1015.

Anti-EGFR antibodies

According to the invention, it is possible to use anti-EGFR antibodies.

The expression “anti-EGFR antibody” or “an antibody which binds to EGFR” relates to an antibody which specifically binds the cancer target molecule EGFR (NCBI Reference Sequence: NP_005219.2, SEQ ID NO: 167), preferably having an affinity sufficient for a diagnostic and/or therapeutic application. In particular embodiments, the antibody binds EGFR with a dissociation constant (K_D) of ≤ 1µM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM.

In a preferred embodiment, the anti-EGFR antibodies are selected from the group consisting of TPP-981 (Cetuximab), panitumumab, nimotuzumab. In a particularly preferred embodiment, the anti-EGFR antibody is TPP-981 (cetuximab).

Further embodiments of EGFR antibodies are as follows:

- zalutumumab / 2F8 / HuMax-EGFr, from Genmab A/S (WO 02/100348, WO 2004/056847, INN number 8605)
- necitumumab / 11F8, ImClone / IMC-11F8, from ImClone Systems Inc. [Eli Lilly & Co] (WO 2005/090407 (EP 01735348-A1, US 2007/0264253-A1, US 7,598,350, WO 2005/090407-A1), INN number 9083)
- matuzumab / anti-EGFR MAb, Merck KGaA / anti-EGFR MAb, Takeda / EMD 72000 / EMD-6200 / EMD-72000 and EMD-55900 / MAb 425 / monoclonal antibody 425, from Merck KGaA / Takeda (WO 92/15683, INN number 8103 (Matuzumab))
- RG-7160 / GA-201 / GA201 / R-7160 / R7160 / RG7160 / RO-4858696 / RO-5083945 / RO4858696 / RO5083945, from Glycart Biotechnology AG (Roche Holding AG) (WO 2010/112413-A1, WO 2010/115554)
- GT-MAB 5.2-GEX / CetuGEX, from Glycotope GmbH (WO 2008/028686-A2 (EP 01900750-A1, EP 01911766-A1, EP 02073842-A2, US 2010/0028947-A1)
- ISU-101, from Isu Abxis Inc (ISU Chemical Co Ltd) / Scancell (WO 2008/004834-A1)
- ABT-806 / mAb-806 / ch-806 / anti-EGFR monoclonal antibody 806, from Ludwig Institute for Cancer Research / Abbott / Life Science Pharmaceuticals (WO 02/092771, WO 2005/081854 and WO 2009/023265)
- SYM-004 (consists of two chimeric IgG1 antibodies (992 and 1024)), from Symphogen A/S (WO 2010/022736-A2)
- MR1-1 /MR1-1KDEL, from IVAX Corp (Teva Pharmaceutical Industries Ltd) (Duke University), (patent: WO2001/062931-A2)
- Antibody against the deletion mutant, EGFRvIII, from Amgen/Abgenix (WO 2005/010151, US 7,628,986)
- SC-100, from Scancell Ltd (WO 01/088138-A1)

- MDX-447 / EMD 82633 / BAB-447 / H 447 / MAb, EGFR, Medarex/Merck KgaA, from Bristol-Myers Squibb (US) / Merck KGaA (DE) / Takeda (JP), (WO 91/05871, WO 92/15683)
- anti-EGFR-Mab, from Xencor (WO 2005/056606)
- DXL-1218 / anti-EGFR monoclonal antibody (cancer), InNexus, from InNexus Biotechnology Inc, Pharmaprojects PH048638

Anti-carboanhydrase IX antibodies

Examples of antibodies which bind the cancer target molecule carboanhydrase IX are described in WO 2007/070538-A2 (e.g. Claims 1 – 16).

Anti-CD123 antibodies

The expression “anti-CD123 antibody” or “an antibody which binds to CD123” relates to an antibody which specifically binds the cancer target molecule CD123 (NCBI Reference Sequence: NP_002174.1; Swiss-Prot: P26951), preferably having an affinity sufficient for a diagnostic and/or therapeutic application. In particular embodiments, the antibody binds CD123 with a dissociation constant (K_D) of ≤ 1µM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM.

Sun et al. (Sun et al., 1996, Blood 87(1)83-92) describe the generation and properties of the monoclonal antibody 7G3, which binds the N-terminal domain of IL-3Rα, CD123. US Patent Number 6,177,078 (Lopez) relates to the anti-CD123 antibody 7G3. A chimeric variant of this antibody (CSL360) is described in WO 2009/070844, and a humanized version (CSL362) in WO 2012/021934. The sequence of the 7G3 antibody is disclosed in EP2426148. This sequence constitutes the starting point for humanized antibodies which are obtained by CDR grafting.

An antibody which, after cell surface antigen binding, is internalized particularly well is the anti-CD123 antibody 12F1 disclosed by Kuo et al. (Kuo et al., 2009, Bioconjug Chem. 20(10):1975-82). The antibody 12F1 binds with higher affinity to CD123 than the antibody

7G3 and, after cell surface antigen binding, is internalized markedly faster than 7G3. Bispecific scFv immunofusion proteins based on 12F1 are disclosed in WO 2013/173820.

Humanized variants of the murine 7G3 and 12F1 antibodies are generated on the basis of CDR grafting in germline sequences and subsequent optimization.

Anti-CXCR5 antibodies

The expression “anti-CXCR5 antibody” or “an antibody which binds to CXCR5” relates to an antibody which specifically binds the cancer target molecule CXCR5 (NCBI Reference Sequence: NP_001707.1), preferably having an affinity sufficient for a diagnostic and/or therapeutic application. In particular embodiments, the antibody binds CXCR5 with a dissociation constant (K_D) of ≤ 1µM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM.

Examples of antibodies and antigen-binding fragments which bind to CXCR5 are known to those skilled in the art and are described, for example, in EP2195023.

The hybridoma cells for the rat antibody RF8B2 (ACC2153) were purchased from DSMZ and the sequence of the antibody was identified by standard methods. This sequence constitutes the starting point for the humanized antibodies which are obtained by CDR grafting.

Humanized variants of this antibody are generated on the basis of CDR grafting in germline sequences.

Anti-C4.4a antibodies:

Examples of C4.4a antibodies and antigen-binding fragments are described in WO 2012/143499 A2. The sequences of the antibodies are given in Table 1 of WO 2012/143499 A2, where each row shows the respective CDR amino acid sequences of the variable light chain or the variable heavy chain of the antibody listed in column 1.

Anti-CD20 antibodies:

An example of an antibody that binds the cancer target molecule CD20 is rituximab (Genentech). Rituximab (CAS Number: 174722-31-7) is a chimeric antibody used for the treatment of non-Hodgkin's lymphoma. These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-CD52 antibodies:

An example of an antibody that binds the cancer target molecule CD52 is alemtuzumab (Genzyme). Alemtuzumab (CAS Number: 216503-57-0) is a humanized antibody used for the treatment of chronic lymphocytic leukaemia. These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-mesothelin antibodies:

Examples of anti-mesothelin antibodies are described, for example, in WO2009/068204. All antibodies and antigen-binding fragments disclosed in WO2009/068204 can be used in the context of the invention disclosed herein. More preferably, the antibody disclosed in WO2009/068204 is MF-T.

Anti-CD30 antibodies

Examples of antibodies which bind the cancer target molecule CD30 and can be used for treatment of cancer, for example Hodgkin's lymphoma, are brentuximab, iratumumab and antibodies disclosed in WO 2008/092117, WO 2008/036688 or WO 2006/089232. An example of an anti-CD30 conjugate is brentuximab vedotin (INN No. 9144). These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-CD22 antibodies

Examples of antibodies which bind the cancer target molecule CD22 and can be used for treatment of cancer, for example lymphoma, are inotuzumab and epratuzumab. Examples of anti-CD22 conjugates are inotuzumab ozagamycin (INN No. 8574) or anti-CD22-MMAE and anti-CD22-MC-MMAE (CAS RN: 139504-50-0 and 474645-27-7, respectively). These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-CD33 antibodies

Examples of antibodies which bind the cancer target molecule CD33 and can be used for treatment of cancer, for example leukaemia, are gemtuzumab and lintuzumab (INN 7580). An example of an anti-CD33 conjugate is gemtuzumab-ozagamycin. These antibodies and antigen-binding fragments can be used in the context of this invention.

Anti-NMB antibodies

An example of an antibody which binds the cancer target molecule NMB and can be used for treatment of cancer, for example melanoma or breast cancer, is glembatumumab (INN 9199). An example of an anti-NMB conjugate is glembatumumab vedotin (CAS RN: 474645-27-7). These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-CD56 antibodies

An example of an antibody which binds the cancer target molecule CD56 and can be used for treatment of cancer, for example multiple myeloma, small-cell lung carcinoma, MCC or ovarial carcinoma is lorvotuzumab. An example of an anti-CD57 conjugate is lorvotuzumab mertansine (CAS RN: 139504-50-0). These antibodies and antigen-binding fragments can be used in the context of this invention.

Anti-CD70 antibodies

Examples of antibodies which bind the cancer target molecule CD70 and can be used for treatment of cancer, for example non-Hodgkin's lymphoma or renal cell cancer, are disclosed in WO 2007/038637-A2 and WO 2008/070593-A2. An example of an anti-CD70 conjugate is SGN-75 (CD70 MMAF). These antibodies and antigen-binding fragments can be used in the context of this invention.

Anti-CD74 antibodies

An example of an antibody which binds the cancer target molecule CD74 and can be used for treatment of cancer, for example multiple myeloma, is milatuzumab. An example of an anti-CD74 conjugate is milatuzumab-doxorubicin (CAS RN: 23214-92-8). These antibodies and antigen-binding fragments can be used in the context of this invention.

Anti-CD19 antibodies

An example of an antibody which binds the cancer target molecule CD19 and can be used for treatment of cancer, for example non-Hodgkin's lymphoma, is disclosed in WO 2008/031056-A2. Further antibodies and examples of an anti-CD19 conjugate (SAR3419) are disclosed in WO 2008/047242-A2. These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-mucin antibodies

Examples of antibodies which bind the cancer target molecule mucin-1 and can be used for treatment of cancer, for example non-Hodgkin's lymphoma, are clivatuzumab and the antibodies disclosed in WO 2003/106495-A2, WO 2008/028686-A2. Examples of anti-mucin conjugates are disclosed in WO 2005/009369-A2. These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-CD138 antibodies

Examples of antibodies which bind the cancer target molecule CD138 and conjugates thereof, which can be used for treatment of cancer, for example multiple myeloma, are disclosed in WO 2009/080829-A1, WO 2009/080830-A1. These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-integrin-alphaV antibodies

Examples of antibodies which bind the cancer target molecule integrin alphaV and can be used for treatment of cancer, for example melanoma, sarcoma or carcinoma, are intetumumab (CAS RN: 725735-28-4), abciximab (CAS RN: 143653-53-6), etaracizumab (CAS RN: 892553-42-3) and the antibodies disclosed in US 7,465,449, EP 719859-A1, WO 2002/012501-A1 and WO2006/062779-A2. Examples of anti-integrin AlphaV conjugates are intetumumab-DM4 and other ADCs disclosed in WO 2007/024536-A2. These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-TDGF1 antibodies

Examples of antibodies which bind the cancer target molecule TDGF1 and can be used for treatment of cancer are the antibodies disclosed in WO 02/077033-A1, US 7,318,924, WO 2003/083041-A2 and WO 2002/088170-A2. Examples of anti-TDGF1 conjugates are

disclosed in WO 2002/088170-A2. These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-PSMA antibodies

Examples of antibodies which bind the cancer target molecule PSMA and can be used for treatment of cancer, for example prostate carcinoma, are the antibodies disclosed in WO 97/35616-A1, WO 99/47554-A1, WO 01/009192-A1 and WO2003/034903. Examples of anti-PSMA conjugates are disclosed in WO 2009/026274-A1 and WO 2007/002222. These antibodies and antigen-binding fragments can be used in the context of this invention.

Anti-EPHA2 antibodies

Examples of antibodies which bind the cancer target molecule EPHA2 and can be used for preparation of a conjugate and for treatment of cancer are disclosed in WO 2004/091375-A2. These antibodies and antigen-binding fragments can be used in the context of this invention.

Anti-SLC44A4 antibodies

Examples of antibodies which bind the cancer target molecule SLC44A4 and can be used for preparation of a conjugate and for treatment of cancer, for example pancreas or prostate carcinoma, are disclosed in WO2009/033094-A2 and US2009/0175796-A1. These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-HLA-DOB antibodies

An example of an antibody that binds the cancer target molecule HLA-DOB is the antibody Lym-1 (CAS RN: 301344-99-0) which can be used for treatment of cancer, for example non-Hodgkin's lymphoma. Examples of anti-HLA-DOB conjugates are disclosed, for example, in WO 2005/081711-A2. These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-VTCN1 antibodies

Examples of antibodies which bind the cancer target molecule VTCN1 and can be used for preparation of a conjugate and for treatment of cancer, for example ovarial carcinoma, pancreas, lung or breast cancer, are disclosed in WO 2006/074418-A2. These antibodies and antigen-binding fragments thereof can be used in the context of this invention.

Anti-FGFR2 antibodies

Examples of anti-FGFR2 antibodies and antigen-binding fragments are described in WO2013076186. The sequences of the antibodies are shown in Table 9 and Table 10 of WO2013076186. Preference is given to antibodies, antigen-binding fragments and variants of the antibodies which derive from the antibodies referred to as M048-D01 and M047-D08.

Preferred antibodies and antigen-binding antibody fragments for binder-drug conjugates according to the invention

In this application, in the context of the binder-drug conjugate, reference is made to the following preferred antibodies as shown in the following table: TPP-2090, TPP-2658, TPP-5442, TPP-8825, TPP-7006, TPP-7007, TPP-10334, TPP-10335, TPP-10336, TPP-10337, TPP-1015, TPP-7510, TPP-7511, TPP-8382 and TPP-8567.

Table: Protein sequences of the antibodies:

Antibody TPP-XXX	Antigen	SEQ ID NO: VH	SEQ ID NO: H-CDR1	SEQ ID NO: H-CDR2	SEQ ID NO: H-CDR3	SEQ ID NO: VL	SEQ ID NO: L-CDR1	SEQ ID NO: L-CDR2	SEQ ID NO: L-CDR3	SEQ ID NO: IgG heavy chain	SEQ ID NO: IgG light chain
TPP-981	EGFR	1	2	3	4	5	6	7	8	9	10
TPP-1015	HER2	11	12	13	14	15	16	17	18	19	20
TPP-2090	TWEAKR	21	22	23	24	25	26	27	28	29	30
TPP-2658	TWEAKR	31	32	33	34	35	36	37	38	39	40
TPP-5442	TWEAKR	41	42	43	44	45	46	47	48	49	50
TPP-7006	TWEAKR	51	52	53	54	55	56	57	58	59	60
TPP-7007	TWEAKR	61	62	63	64	65	66	67	68	69	70

Antibody TPP-XXX	Antigen	SEQ ID NO: VH	SEQ ID NO: H-CDR1	SEQ ID NO: H-CDR2	SEQ ID NO: H-CDR3	SEQ ID NO: VL	SEQ ID NO: L-CDR1	SEQ ID NO: L-CDR2	SEQ ID NO: L-CDR3	SEQ ID NO: IgG heavy chain	SEQ ID NO: IgG light chain
TPP-7510	HER2	71	72	73	74	75	76	77	78	79	80
TPP-7511	HER2	81	82	83	84	85	86	87	88	89	90
TPP-8382	B7H3	91	92	93	94	95	96	97	98	99	100
TPP-8567	B7H3	101	102	103	104	105	106	107	108	109	110
TPP-8825	TWEAKR	111	112	113	114	115	116	117	118	119	120
TPP-10334	TWEAKR	121	122	123	124	125	126	127	128	129	130
TPP-10335	TWEAKR	131	132	133	134	135	136	137	138	139	140
TPP-10336	TWEAKR	141	142	143	144	145	146	147	148	149	150
TPP-10337	TWEAKR	151	152	153	154	155	156	157	158	159	160

TPP-2090, TPP-2658, TPP-5442, TPP-8825, TPP-7006, TPP-7007, TPP-10334, TPP-10335, TPP-10336, TPP-10337, TPP-1015, TPP-7510, TPP-7511, TPP-8382 and TPP-8567 are antibodies comprising one or more of the CDR sequences specified in the above table (H-CDR1, H-CDR2, H-CDR3, L-CDR1, L-CDR2, L-CDR3) in the variable region of the heavy chain (VH) or the variable region of the light chain (VL). Preferably, the antibodies comprise the specified variable region of the heavy chain (VH) and/or the variable region of the light chain (VL). Preferably, the antibodies comprise the specified region of the heavy chain (IgG heavy chain) and/or the specified region of the light chain (IgG light chain).

TPP-981 is an anti-EGFR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ

ID NO: 2, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 3 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 4, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 6, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 7 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 8.

TPP-1015 is an anti-HER2 antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 12, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 13 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 14, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 16, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 17 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 18.

TPP-2090 is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 22, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 23 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 24, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 26, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 27 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 28.

TPP-2658 is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 32, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 33 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 34, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 36, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 37 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 38.

TPP-5442 is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 42, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 43 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 44, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 46, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 47 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 48.

TPP-7006 is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 52, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 53 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 54, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 56, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 57 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 58.

TPP-7007 is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 62, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 63 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 64, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 66, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 67 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 68.

TPP-7510 is an anti-HER2 antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 72, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 73 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 74, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 76, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 77 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 78.

TPP-7511 is an anti-HER2 antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 82, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 83 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 84, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 86, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 87 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 88.

TPP-8382 is an anti-B7H3 antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 92, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 93 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 94, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 96, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 97 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 98.

TPP-8567 is an anti-B7H3 antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 102, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 103 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 104, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 106, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 107 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 108.

TPP-8825 is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 112, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 113 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 114, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 116, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 117 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 118.

TPP-10334 is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 122, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 123 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 124, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 126, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 127 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 128.

TPP-10335 is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 132, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 133 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 134, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 136, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 137 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 138.

TPP-10336 is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 142, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 143 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 144, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 146, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 147 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 148.

TPP-10337 is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 152, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 153 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 154, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 156, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 157 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 158.

TPP-981 is an anti-EGFR antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 1 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 5.

TPP-1015 is an anti-HER2 antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 11 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 15.

TPP-2090 is an anti-TWEAKR antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 21 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 25.

TPP-2658 is an anti-TWEAKR antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 31 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 35.

TPP-5442 is an anti-TWEAKR antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 41 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 45.

TPP-7006 is an anti-TWEAKR antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 51 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 55.

TPP-7007 is an anti-TWEAKR antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 61 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 65.

TPP-7510 is an anti-HER2 antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 71 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 75.

TPP-7511 is an anti-HER2 antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 81 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 85.

TPP-8382 is an anti-B7H3 antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 91 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 95.

TPP-8567 is an anti-B7H3 antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 101 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 105.

TPP-8825 is an anti-TWEAKR antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 111 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 115.

TPP-10334 is an anti-TWEAKR antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 121 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 125.

TPP-10335 is an anti-TWEAKR antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 131 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 135.

TPP-10336 is an anti-TWEAKR antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 141 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 145.

TPP-10337 is an anti-TWEAKR antibody comprising preferably a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 151 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 155.

TPP-981 is an anti-EGFR antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 9 and a region of the light chain corresponding to SEQ ID NO: 10.

TPP-1015 is an anti-HER2 antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 19 and a region of the light chain corresponding to SEQ ID NO: 20.

TPP-2090 is an anti-TWEAKR antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 29 and a region of the light chain corresponding to SEQ ID NO: 30.

TPP-2658 is an anti-TWEAKR antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 39 and a region of the light chain corresponding to SEQ ID NO: 40.

TPP-5442 is an anti-TWEAKR antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 49 and a region of the light chain corresponding to SEQ ID NO: 50.

TPP-7006 is an anti-TWEAKR antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 59 and a region of the light chain corresponding to SEQ ID NO: 60.

TPP-7007 is an anti-TWEAKR antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 69 and a region of the light chain corresponding to SEQ ID NO: 70.

TPP-7510 is an anti-HER2 antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 79 and a region of the light chain corresponding to SEQ ID NO: 80.

TPP-7511 is an anti-HER2 antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 89 and a region of the light chain corresponding to SEQ ID NO: 90.

TPP-8382 is an anti-B7H3 antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 99 and a region of the light chain corresponding to SEQ ID NO: 100.

TPP-8567 is an anti-B7H3 antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 109 and a region of the light chain corresponding to SEQ ID NO: 110.

TPP-8825 is an anti-TWEAKR antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 119 and a region of the light chain corresponding to SEQ ID NO: 120.

TPP-10334 is an anti-TWEAKR antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 129 and a region of the light chain corresponding to SEQ ID NO: 130.

TPP-10335 is an anti-TWEAKR antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 139 and a region of the light chain corresponding to SEQ ID NO: 140.

TPP-10336 is an anti-TWEAKR antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 149 and a region of the light chain corresponding to SEQ ID NO: 150.

TPP-10337 is an anti-TWEAKR antibody comprising preferably a region of the heavy chain corresponding to SEQ ID NO: 159 and a region of the light chain corresponding to SEQ ID NO: 160.

Linkers for the LIG binder (L_b and L_c)

The literature discloses various options for covalent coupling (conjugation) of organic molecules to peptides or proteins such as antibodies (see, for example, K. Lang and J. W. Chin. *Chem. Rev.* **2014**, *114*, 4764-4806, M. Rashidian et al. *Bioconjugate Chem.* **2013**, *24*, 1277-1294). Preference is given in accordance with the invention to conjugation of the organic radical to an antibody via one or more sulphur atoms of cysteine residues of the antibody which are either already present as free thiols or are generated by reduction of disulphide bridges, and/or via one or more NH groups of lysine residues of the antibody. However, it is also possible to bind the KSP inhibitor or prodrug to the antibody via tyrosine residues, via glutamine residues, via residues of unnatural amino acids, via free carboxyl groups or via sugar residues of the antibody.

It is also possible in accordance with the invention to conjugate the drug molecules to specific conjugation sites of the binder, which improves product homogeneity. The literature describes various methods of conjugation site-specific conjugation (Agarwal et al., *Bioconjug. Chem.* 26, 176-192 (2015); Cal et al., *Angew. Chem. Int. Ed. Engl.*53, 10585-10587 (2014); Behrens et al., *MAbs* 6, 46-53 (2014); Panowski et al., *MAbs* 6, 34-

45 (2014)). These methods also include, in particular, enzymatic conjugation methods which use, for example, transglutaminases (TGases), glycosyltransferases or the formylglycine-generating enzyme ((Sochaj et al., *Biotechnology Advances* 33, 775-784, (2015)).

According to the invention, it is possible to provide conjugation site-specific binder conjugates of the kinesin spindle protein inhibitor, in which the kinesin spindle protein inhibitors are conjugated to glutamine side chains of the binders.

When the binder is an antibody, it contains an acceptor glutamine, preferably in the constant region. Such acceptor glutamines can be introduced via mutation of suitable positions to glutamine (for example the mutation N297Q of the heavy chain, Kabat EU numbering) or via generation of deglycosylated or aglycosylated antibodies (for example via enzymatic deglycosylation by means of PNGaseF or via mutation N297X of the heavy chain, Kabat EU numbering (X here may be any amino acid except N)). In the latter case of a deglycosylated or aglycosylated antibody, the glutamine residue Q295 (Kabat EU numbering) of the heavy chain becomes an acceptor glutamine. Particular preference is given to an antibody containing the N297A or N297Q mutation (Kabat EU numbering). Therefore, all the antibodies described in this invention likewise include aglycosylated variants of these antibodies, which are produced either via deglycosylation by means of PNGaseF or by mutation of N297 (Kabat EU numbering) (Kabat numbering system of antibodies, see Kabat et al., Sequences of Proteins of Immulological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) of the heavy chain to any other amino acid except N. In addition, all the antibodies described here likewise contain variants of the antibodies described which, by virtue of engineering, contain one or more acceptor glutamine residues for transglutaminase-catalysed reactions.

One method for such conjugation site specific-conjugations is approaches described in the literature which are concerned with conjugation site-specific conjugation of binders by means of transglutaminase. Transglutaminases (TGases) which also include bacterial transglutaminase (BTG) (EC 2.3.2.13) are a family of enzymes which catalyse the formation of a covalent bond between the γ-carbonyl-amide group of glutamines and the primary amine group of lysines. Since such transglutaminases also accept substrates other than lysine as amine donor, they have been used in order to modify proteins

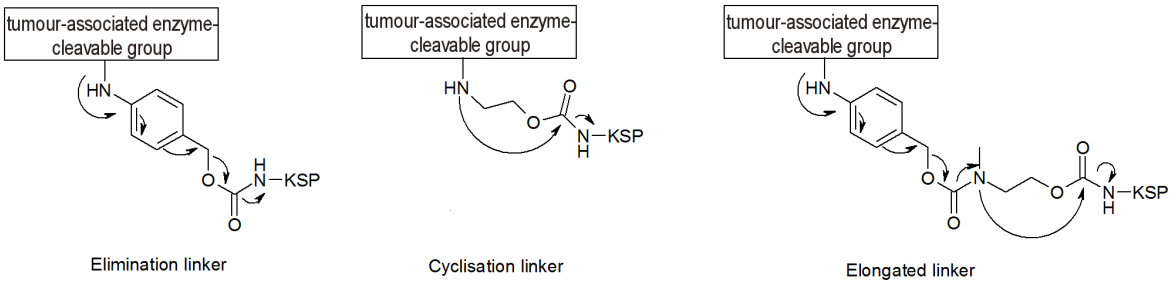
including antibodies at suitable acceptor glutamines (Jeger et al., *Angewandte Chemie Int. Ed. Engl* 49, 9995-9997 (2010); Josten et al., *J. Immunol. Methods* 240, 47-54 (2000); Mindt et al., *Bioconjugate Chem.* 19, 271-278 (2008); Dennler et al., in *Antibody Drug Conjuagtes* (Ducry, L., Ed.), pp 205-215, Humana Press. (2013)). On the one hand, transglutaminases have been used for the conjugation of drugs to antibodies containing artificial glutamine tags which are acceptor glutamine residues which have been introduced into the antibody by genetic engineering (Strop et al., *Chem. Biol.* 20, 161-167 (2013)). On the other hand, it has been stated that the conserved glutamine residue Q295 (Kabat EU numbering) of the constant region of the heavy chain of antibodies is the only γ-carbonyl-amide donor for the bacterial transglutaminase (EC 2.3.2.13) in the backbone of aglycosylated IgG1 molecules, and is thus an acceptor glutamine, whereas no acceptor glutamine is present in the backbone of IgG1 when the antibody has been glycosylated at position N297 (Kabat EU numbering) of the heavy chain (Jeger et al., *Angewandte Chemie Int. Ed. Engl* 49, 9995-9997 (2010)). In summary, bacterial transglutaminase can be used for the conjugation of an amine-donor substrate, for example a drug-linker construct, at an acceptor glutamine residue of an antibody. Such acceptor glutamines can be introduced by engineering of the antibody by mutations or by the generation of aglycosylated antibodies. Such aglycosylated antibodies can be introduced by deglycosylation using N-glycosidase F (PNGase F) or by mutation of N297 of the glycosylation site of the heavy chain (Kabat EU numbering) to any other amino acid except N. The enzymatic conjugation of such aglycosylated antibodies using bacterial transglutaminase has been described for aglycosylated antibody variants containing the mutations N297D, N297Q (Jeger et al., *Angewandte Chemie Int. Ed. Engl* 49, 9995-9997 (2010)) or N297S (see patent applications WO2013092998A1 and WO2013092983A2). The enzymatic conjugation of such aglycosylated antibodies by means of transglutaminase generally affords ADCs having a DAR of 2, in which both heavy chains are specifically functionalized at position Q295 (Kabat EU numbering). Only mutation N297Q of the heavy chain affords an additional conjugation site per heavy chain. The conjugation of such variants leads to ADCs having a DAR of 4, in which both heavy chains are specifically functionalized at positions Q295 and Q297. Antibody variants in which the heavy chains bear the mutations Q295N and N297Q have only one acceptor glutamine residue at position Q297 (Kabat numbering) per heavy chain (Simone Jeger, Site specific conjugation of tumour targeting antibodies using transglutaminase, Thesis at ETH Zurich (2009)). There exist several examples in the literature which describe the

conjugation site-specific conjugation of aglycosylated antibodies using bacterial transglutaminase (for example Dennler et al., *Bioconjugate Chemistry* 19, 569-578 (2014); Lhospice et al., *Molecular Pharmaceutics* 12, 1863-1871 (2015)). The strategy of transglutaminase-catalysed conjugation site-specific functionalization of aglycosylated antibodies is summarized in Figure 1.

Coupling – both in a conjugation site-specific and in a conjugation site-nonspecific manner – is accomplished using what are called linkers. Linkers can be categorized into the group of the linkers which can be cleaved *in vivo* and the group of the linkers which are stable *in vivo* (see L. Ducry and B. Stump, *Bioconjugate Chem.* 21, 5-13 (2010)). The linkers which can be cleaved *in vivo* have a group which can be cleaved *in vivo*, where, in turn, a distinction may be made between groups which are chemically cleavable *in vivo* and groups which are enzymatically cleavable *in vivo*. "Chemically cleavable *in vivo*" and "enzymatically cleavable *in vivo*" means that the linkers or groups are stable in circulation and are cleaved only at or in the target cell by the chemically or enzymatically different environment therein (lower pH; elevated glutathione concentration; presence of lysosomal enzymes such as cathepsin or plasmin, or glyosidases such as, for example, β-glucuronidases), thus releasing the low-molecular weight KSP inhibitor or a derivative thereof. Groups which can be cleaved chemically *in vivo* are in particular disulphide, hydrazone, acetal and aminal; groups which can be cleaved enzymatically *in vivo* are in particular the 2-8-oligopeptide group, especially a dipeptide group or glycoside. Peptide cleaving sites are disclosed in *Bioconjugate Chem.* **2002,** *13,* 855-869 and *Bioorganic & Medicinal Chemistry Letters* 8 (**1998**) 3341-3346 and also *Bioconjugate Chem.* **1998,** *9,* 618-626. These include, for example, alanine-alanine-asparagine, valine-alanine, valine-lysine, valine-citrulline, alanine-lysine and phenylalanine-lysine (optionally with additional amide group).

In order to assure efficient release of the free drug, it is optionally also possible to incorporate what are called self-immolative linker elements (SIG) between the enzymatic cleavage site and drug (Anticancer Agents in Medicinal Chemistry, 2008, 8, 618-637). The drug can be released by various mechanisms, for example after initial enzymatic release of a nucleophilic group by subsequent elimination via an electronic cascade (Bioorg. Med. Chem., 1999, 7, 1597; J. Med. Chem., 2002, 45, 937; Bioorg. Med. Chem., 2002, 10, 71) or by cyclization of the corresponding linker element (Bioorg. Med. Chem., 2003, 11, 2277; Bioorg. Med. Chem., 2007, 15, 4973; Bioorg. Med. Chem. Lett., 2007, 17, 2241) or

by a combination of the two (Angew. Chem. Inter. Ed., 2005, 44, 4378). Examples of such linker elements are shown in the figure:



Examples of successive enzymatic steps for drug release, for example by means of histone deacetylase and cathepsin L, are described in Nat. Commun., 2013, 4, 2735 and are illustrated in Figure 2.

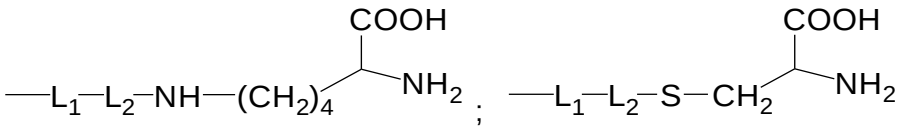
Linkers which are stable *in vivo* are distinguished by a high stability (less than 5% metabolites after 24 hours in plasma) and do not have the chemically or enzymatically *in vivo* cleavable groups mentioned above.

The linker $–L_b-$ or $–L_c-$ preferably has one of the following base structures (i) to (iv):

(i) $–(C=O)_m–$SG1-L1-L2-
(ii) $–(C=O)_m$ –L1-SG-L1-L2-
(iii) $–(C=O)_m$ –L1-L2-
(iv) $–(C=O)_m$ –L1-SG-L2

where m is 0 or 1; SG is a (chemically or enzymatically) *in vivo* cleavable group (in particular disulphide, hydrazone, acetal and aminal; or a 2-8-oligopeptide group which can be cleaved by legumain, cathepsin or plasmin), SG1 is an oligopeptide group or preferably a dipeptide group, L1 independently of one another represent *in vivo* stable organic groups, and L2 represents a coupling group to the binder or a single bond. Here, coupling is preferably to a cysteine residue or a lysine residue of the antibody. Alternatively, coupling can be to a tyrosine residue, glutamine residue or to an unnatural amino acid of the antibody. The unnatural amino acids may contain, for example, aldehyde or keto groups (such as, for example, formylglycine) or azide or alkyne groups (see Lan & Chin, Cellular Incorporation of Unnatural Amino Acids and Bioorthogonal Labeling of Proteins, Chem. Rev. 2014, 114, 4764-4806).

Particular preference according to the invention is given to the basic linker structure (iii). Via metabolization, the administration of a conjugate according to the invention having a basic linker structure (iii) and coupling of the linker to a cysteine or lysine residue of the antibody leads to cysteine or lysine derivatives of the following formulae:



where L1 is in each case joined to the cytotoxic drug, for example the low molecular weight KSP inhibitor, for example a compound of the formula (IIa), (IIb), (IIc), (IId), (V), (VI) or (VII).

Preference is also given in accordance with the invention to the linker base structures (ii) and (iv), especially in the case of binding to position R1 in a compound of the formula (IIa), (IIb), (IIc), (IId), or (V), especially when the L_1 group has one of the following structures:

(a) $–NH-(CH_2)_{0-4}-(CHCH_3)_{0-4}-CHY^5-C(=O)-Y^7$ in which

Y^5 is -H or $-NHY^6$,

Y^6 is -H or $–C(=O)-CH_3$ and

Y^7 is a single bond or $–NH-(CH_2)_{0-4}–CHNH_2-C(=O)-$,

such that, after cleavage, the corresponding structure

$–NH-(CH_2)_{0-4}-(CHCH_3)_{0-4}-CHY^5-COOH$ or

$–NH-(CH_2)_{0-4}-(CHCH_3)_{0-4}-CHY^5-C(=O)-NH-(CH_2)_{0-4}-CHNH_2-COOH$ is obtained.

(b) $–CH_2-S_x-(CH_2)_{0-4}-CHY^5-C(=O)-$ in which

x is 0 or 1,

Y^5 is -H or $-NHY^6$ and

Y^6 is -H or $–C(=O)-CH_3$,

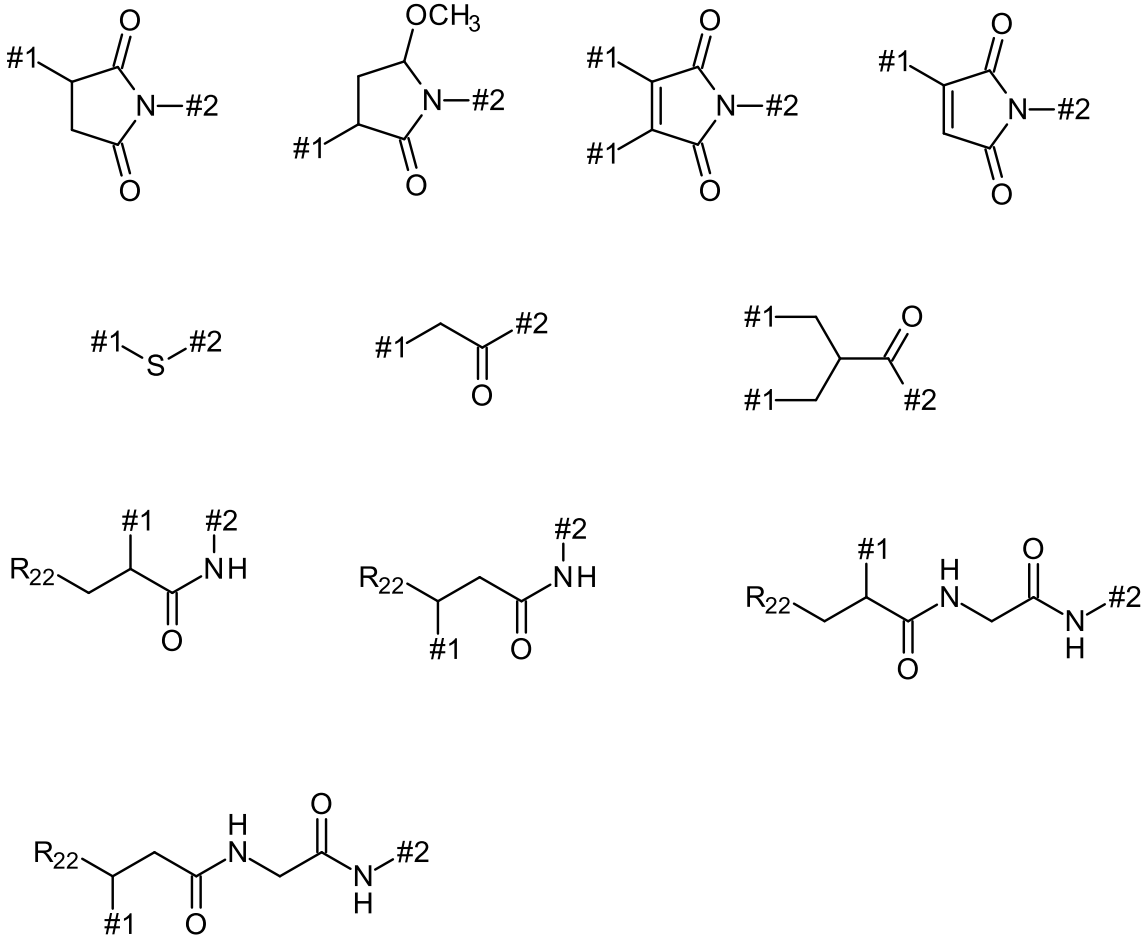
such that, after cleavage, the corresponding structure

$–CH_2-S_x-(CH_2)_{0-4}-CHY^5-COOH$

is obtained.

When the linker is joined to a cysteine side chain or a cysteine residue, L2 preferably derives from a group which reacts with the sulphhydryl group of the cysteine. These include haloacetyls, maleimides, aziridines, acryloyls, arylating compounds, vinylsulphones, pyridyl disulphides, TNB thiols and disulphide-reducing agents. These groups generally react in an electrophilic manner with the sulphhydryl bond, forming a sulphide (e.g. thioether) or disulphide bridge. Preference is given to stable sulphide bridges.

L2 preferably has the following structures:



in which

$\#^1$ is the linkage site to the sulphur atom of the antibody,

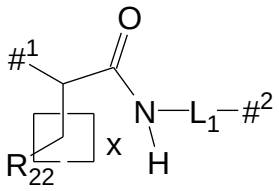
$\#^2$ is the linkage site to the L_1 group, and

R_{22} is -COOH, -C(=O)-OR, -C(=O)R, -C(=O)-NHR or -C(=O)N$(R)_2$ and

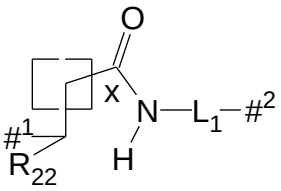
R is $C_{1\text{-}3}$-alkyl, -C(=O)-NH_2 or –COOH.

It is preferable here when R is –COOH.

Particular preference is given to the compounds of the present invention in which L2 has the following formulae A3 and A4:



Formula A3,



Formula A4,

in which

#1 is the linkage site to the sulphur atom of the antibody,

#2 is the linkage site to the drug molecule,

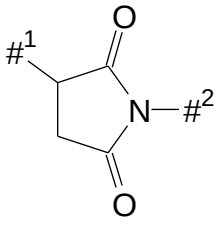
x is 1 or 2 and

R^{22} is –COOH, -C(=O)-OR, -C(=O)-R, -C(=O)-NR_2, -C(=O)-NHR or -C(=O)-NH_2 and

R is $C_{1\text{-}3}$-alkyl.

Preferably in this context, R^{22} is –COOH and, in particular, in this context, R^{22} is –COOH if x is 1.

In a conjugate according to the invention or in a mixture of the conjugates according to the invention, the bonds to a cysteine residue of the antibody are present to an extent of preferably more than 80%, more preferably more than 90% (based in each case on the total number of bonds of the linker to the antibody), more preferably as one of the two structures of the formula A3 or A4. Here, the structures of the formula A3 or A4 are generally present together, preferably in a ratio of from 60:40 to 40:60, based on the number of bonds to the antibody. The remaining bonds are then present as the structure



in which

$\#^1$ is the linkage site to the sulphur atom of the antibody and

$\#^2$ is the linkage site to the drug molecule,

According to the invention, L1 is preferably represented by the formula

$$\#^1\text{–}(NR^{10})_n\text{-}(G1)_o\text{-}G2\text{-}\#^2$$

in which

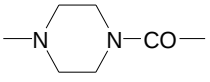
$\#^1$ is the linkage site to the sulphur atom of the antibody and

$\#^2$ is the linkage site to the drug molecule,

R^{10} is -H, $-NH_2$ or C_1-C_3-alkyl,

n is 0 or 1,

o is 0 or 1,

G1 is –NH-C(=O)- , -C(=O)-NH- or 

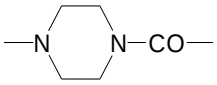
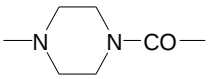
and

G2 is a straight-chain or branched hydrocarbyl chain having 1 to 100 carbon atoms consisting of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups, which may be interrupted once or more than once by one or more of the groups -O-, -S-, -S(=O)-, $-S(=O)_2$, $-NR^y$-, $-NR^yC(=O)$-, $-C(NH)NR^y$-, $-C(=O)-NR^y$-, $-NR^yNR^y$-, $-S(=O)_2NR^yNR^y$-,

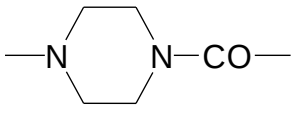
-C(=O)-NR^yNR^y-, -C(=O)-, -CR^x=N-O- and/or a 3- to 10-membered, aromatic or nonaromatic heterocycle having up to 4 heteroatoms selected from N, O and S, -S(=O)- or –$S(=O)_2$-, and where the straight-chain or branched hydrocarbon chain may additionally be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid,

R^y is -H, phenyl, C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl or C_2-C_{10}-alkynyl, each of which may be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid, and

Rx is -H, C_1-C_3-alkyl or phenyl.

In this context, G1 is preferably  and R^{10} is preferably not -NH_2 if G1 is -NH-C(=O)- or .

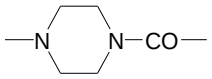
Preferably, G2 is a straight-chain or branched hydrocarbyl chain having 1 to 100 carbon atoms composed of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups, which may be interrupted once or more than once by one or more of the groups -O-, -S-, -S(=O)-, -$S(=O)_2$, -NH-, -C(=O)-, -NH-C(=O)-, -C(=O)-NH-, -NMe-, -NHNH-, -$S(=O)_2$-NHNH-, -C(=O)-NHNH- and a 5- to 10-membered aromatic or nonaromatic heterocycle having up to 4 heteroatoms selected from N, O and S, or -S(=O)-.

More preferably, G2 is , and the straight-chain or branched hydrocarbon chain may additionally be substituted by –NH-C(=O)-NH_2.

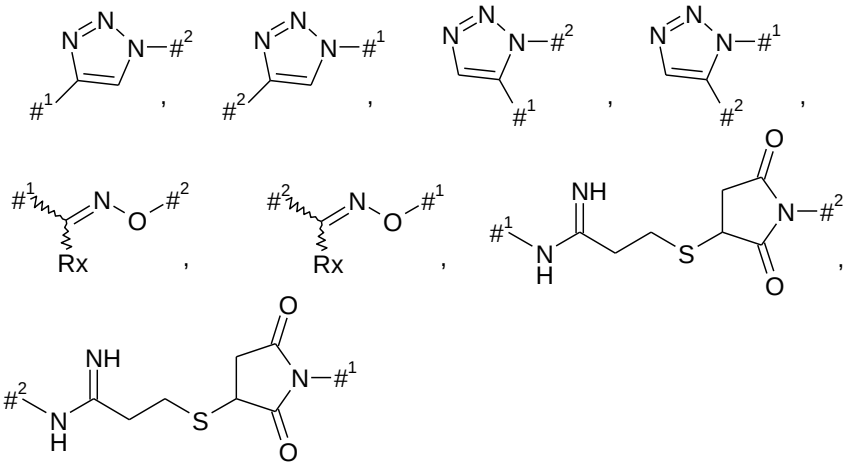
G2 is further preferably a straight-chain or branched hydrocarbyl chain having 1 to 100 carbon atoms composed of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups, which may be interrupted once or more than once by one or more of the groups -O-, -S-, -S(=O)-, -$S(=O)_2$, -NH-, -C(=O)-, -NH-

C(=O)-, -C(=O)-NH-, -NMe-, -NHNH- -S(=O)$_2$-NHNH-, -C(=O)-NHNH-, -CR^x=N-O- and/or a 3- to 10-membered, aromatic or nonaromatic heterocycle having up to 4 heteroatoms selected from N, O and S, -S(=O)- or –S(=O)$_2$-, where the straight-chain or branched hydrocarbon chain may additionally be substituted by –NH-C(=O)-NH$_2$, -COOH, -OH, -NH$_2$, sulphonamide, sulphone, sulphoxide, or sulphonic acid and

Rx is -H, C$_1$-C$_3$-alkyl or phenyl.

In this context, G2 is preferably .

Preferably, G2 represents the interrupting groups of the structures



in which

Rx is -H, C$_1$-C$_3$-alkyl or phenyl,

#1 is the bond to the KSP inhibitor or prodrug and

#2 is the bond to the coupling group to the antibody (e.g. L2).

A straight-chain or branched hydrocarbon chain of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups generally comprises a α,ω-divalent alkyl radical having the respective number of carbon atoms stated. Preferred examples include: methylene, ethane-1,2-diyl (1,2-ethylene), propane-1,3-diyl (1,3-propylene), butane-1,4-

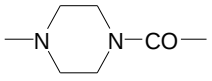
diyl (1,4-butylene), pentane-1,5-diyl (1,5-pentylene), hexane-1,6-diyl (1,6-hexylene), heptane-1,7-diyl (1,7-hexylene), octane-1,8-diyl (1,8-octylene), nonane-1,9-diyl (1,9-nonylene), decane-1,10-diyl (1,10-decylene).

A branched hydrocarbon chain means that one or more hydrogen atoms in the straight hydrocarbon chain or the straight alkylene groups are substituted by C_{1-10}-alkyl groups, thus forming branched hydrocarbon or side chains.

The hydrocarbon chain may additionally contain cyclic alkylene groups (cycloalkanediyl), for example 1,4-cyclohexanediyl or 1,3-cyclopentanediyl. These cyclic groups may be unsaturated. In particular, aromatic groups (arylene groups), for example phenylene, may be present in the hydrocarbon chain. It is also possible in turn for one or more hydrogen atoms in the cyclic alkylene groups and the arylene groups to be optionally substituted by C_{1-10}-alkyl groups. In this way, an optionally branched hydrocarbon chain is formed. This hydrocarbon chain has a total of 0 to 100 carbon atoms, preferably 1 to 50, particularly preferably 2 to 25 carbon atoms.

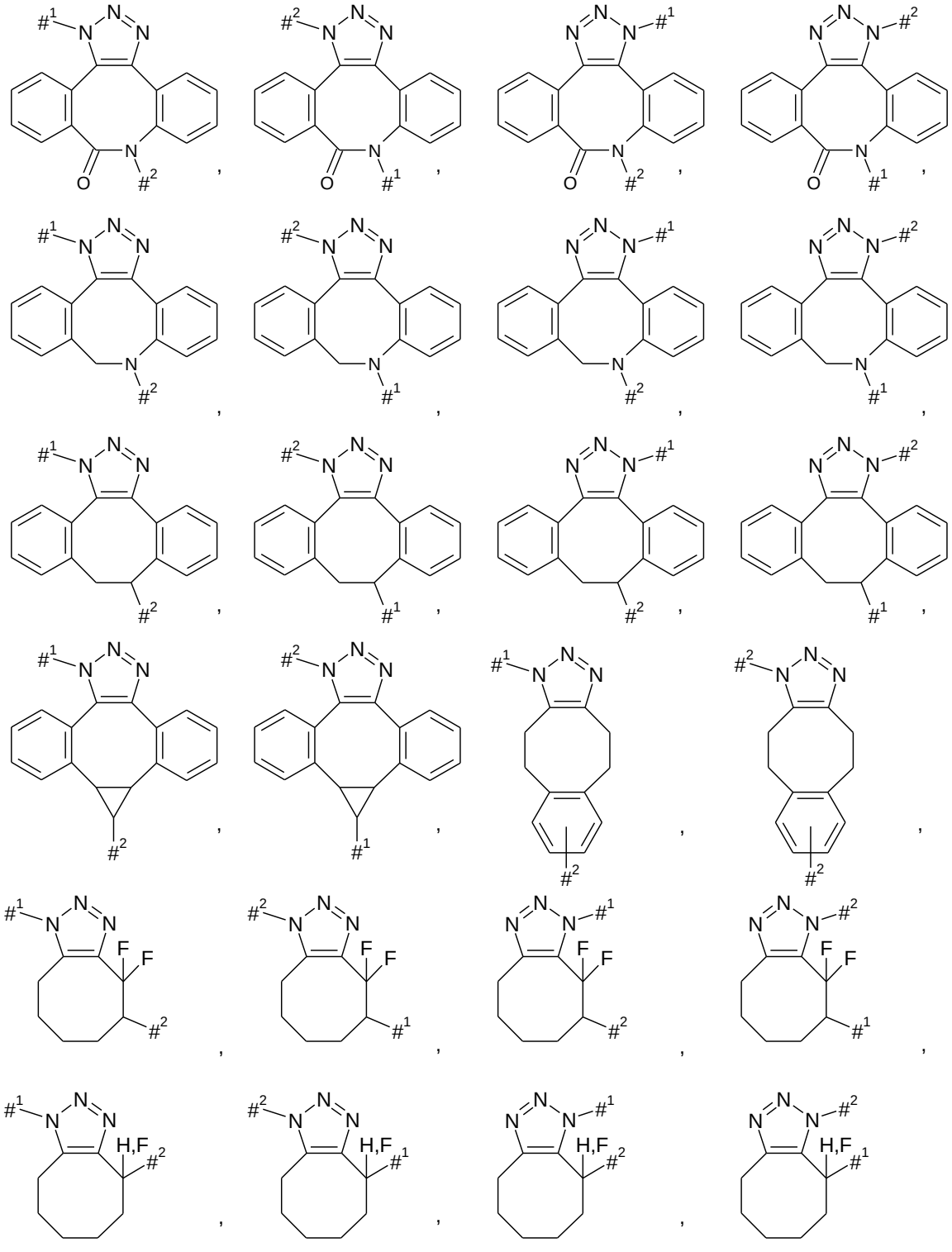
The branched hydrocarbon or side chains may be substituted by –NH-C(=O)-NH2, -COOH,
-OH, $-NH_2$, sulphonamide, sulphone, sulphoxide, or sulphonic acid.

The hydrocarbon chains may be interrupted once or more than once by one or more of the groups
-O-, -S-, -S(=O)-, $-S(=O)_2$-, -NH-, -C(=O)-, -NH-C(=O)-, -C(=O)-NH-, -NMe-, -NHNH-, $-S(=O)_2$-NHNH-, -C(=O)-NHNH- and a 5- to 10-membered aromatic or nonaromatic heterocycle having up to 4 heteroatoms selected from =N-, -O- and –S-, -S(=O)- or $–S(=O)_2$-.

Preference is given here to a group 

.

Further interrupting groups in G2 are preferably



Preferably, the linker L corresponds to the following formula:

§-(C(=O))m-L1-L2-§§,

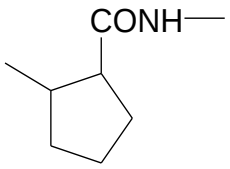
in which

m	is 0 or 1,
§	is the bond to the drug molecule or prodrug,
§§	is the bond to the binder peptide or protein, and
L1 and L2	have the definitions given above.

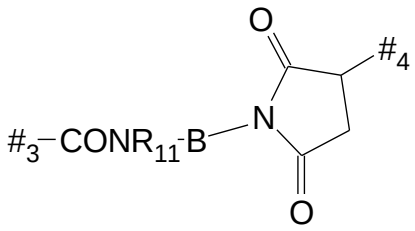
More preferably, and with reference to the above definitions, L1 corresponds to the following simplified formula:

$$–NR^{11}B-$$

in which

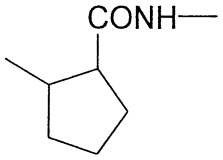
R^{11}	is -H or $-NH_2$,
B	is the $–[(CH_2)_x-(X^4)_y]_w-(CH_2)_z-$ group,
w	is 0 to 20,
x	is 0 to 5,
y	is 0 or 1,
z	is 0 to 5 and
X^4	is –O-, -C(=O)-NH-,–NH-C(=O)- or  .

Preferably, the linker L has the formula



in which

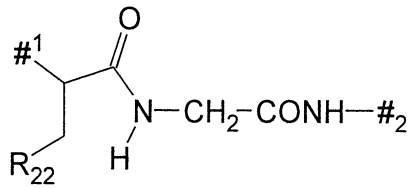
#3	is the bond to the drug molecule or prodrug,

#4	is the bond to the binder peptide or protein,
R^{11}	is -H or $-NH_2$,
B	is the $-[(CH_2)_x-(X^4)_y]_w-(CH_2)_z-$ group,
w	is 0 to 20,
x	is 0 to 5,
y	is 0 or 1,
z	is 1 to 5 and
X^4	is –O-, -C(=O)-NH-, –NH-C(=O)- or  .

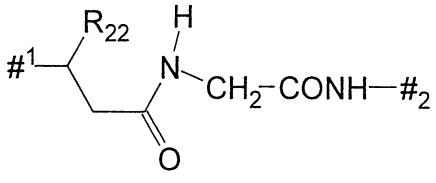
The abovementioned linkers are especially preferred in conjugates of the formula (IIa) in which the linker couples to R^1 by substitution of a hydrogen atom or to R^4 in conjunction with a cleavable linker SG1, i.e. R^1 is -L-#1 or R^4 is -SG1-L-#1 where #1 is the bond to the antibody.

In a conjugate according to the invention or in a mixture of the conjugates according to the invention, the bonds to a cysteine residue of the antibody are present to an extent of preferably more than 80%, more preferably more than 90% (based in each case on the total number of bonds of the linker to the antibody).

Particular preference is given here to the two structures of the general formulae (A5) and (A6)



(A5) and



(A6),

in which

$\#^1$ is the linkage site to the sulphur atom of the antibody,

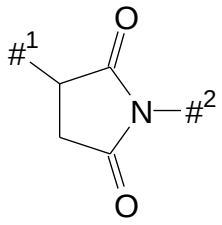
$\#^2$ is the linkage site to the L^1 group,

R^{22} is –COOH, -C(=O)-OR, -C(=O)-R, -C(=O)-NH_2, -C(=O)-NR_2 or -C(=O)-NHR and

R is C_{1-3}-alkyl.

More preferably, R^{22} is –COOH.

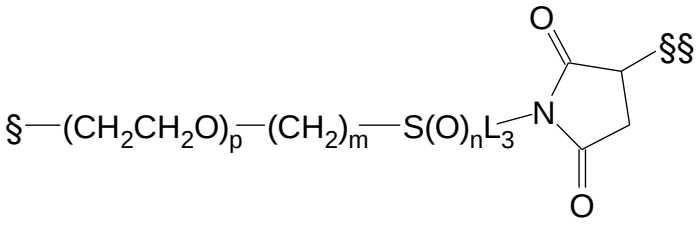
The structures of the general formulae A5 or A6 are generally present here together, preferably in a ratio of from 60:40 to 40:60, based on the number of bonds to the antibody. The remaining bonds are then present in the structure



in which

#1 and #2 have the definitions given above.

The linkers $–L_b-$ and $-L_c-$ bonded to a cysteine side chain or a cysteine residue have the general formula



in which

§ is the bond to the drug molecule or prodrug,

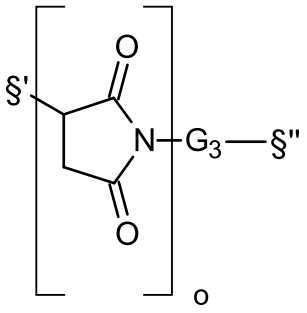
§§ is the bond to the binder peptide or protein,

m is 0, 1, 2, or 3,

n is 0, 1 or 2,

p is 0 to 20,

L3 is the group



in which

o is 0 or 1,

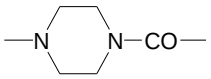
G3 is a straight-chain or branched hydrocarbyl chain having 1 to 100 carbon atoms composed of arylene groups and/or straight-chain and/or cyclic alkylene groups, which may be interrupted once or more than once by -O-, -S-, -S(=O)-, -$S(=O)_2$, -NH-, -C(=O)-, -NHC(=O)-, -C(=O)-NH-, -NMe-, -NHNH-, -$S(=O)_2$-NHNH-, -C(=O)-NHNH- and a 3- to 10-membered aromatic or nonaromatic heterocycle having up to 4 heteroatoms selected from =N-, -O- and –S-, -S(=O)- or –$S(=O)_2$-, where the straight-chain or branched hydrocarbon chain may additionally be

substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide or sulphonic acid,

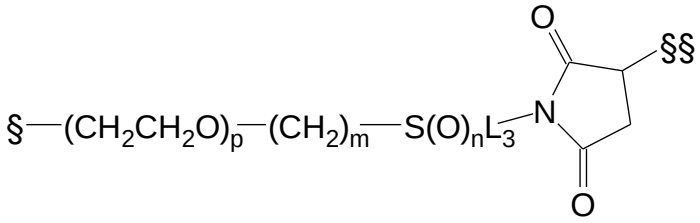
§' is the bond to the –S(O)n group and

§'' is the bond to the nitrogen atom in the ring.

Preferably, the aromatic or nonaromatic heterocycle is 5- to 10-membered.

Preferably, G_3 is .

Preference is given to those compounds of the formula



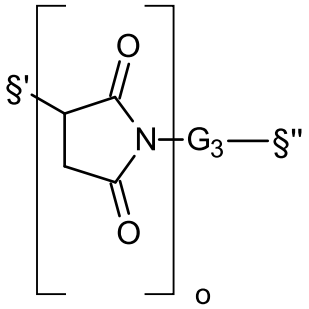
in which

m is 1,

p is 0,

n is 0,

L3 is the group

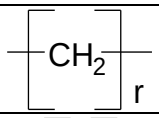
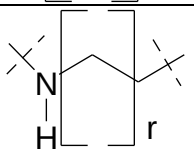
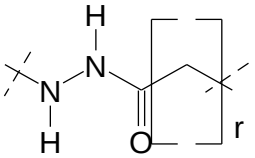
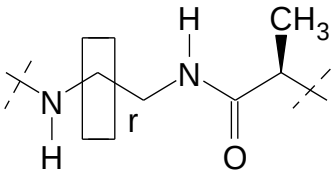
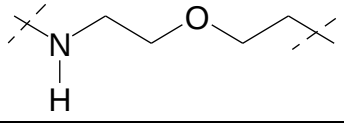


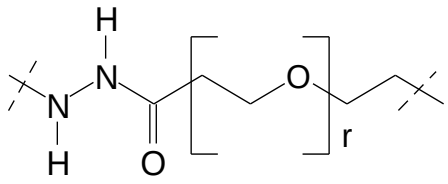
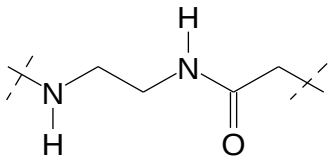
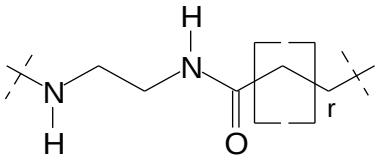
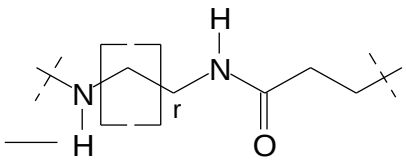
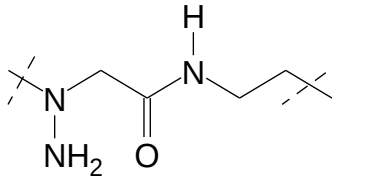
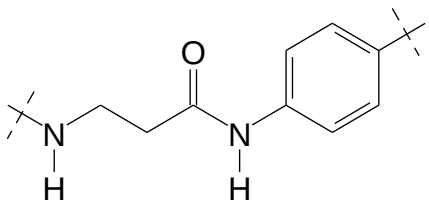
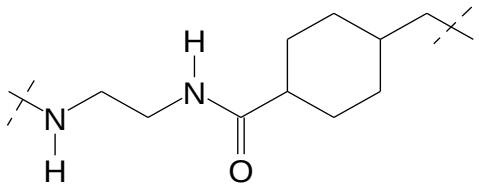
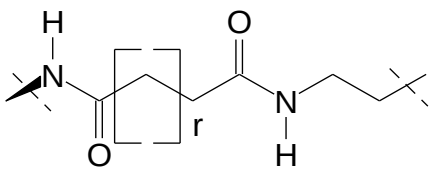
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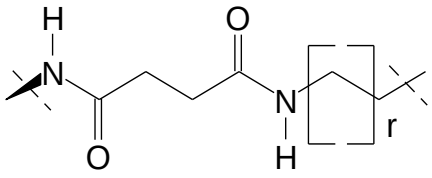
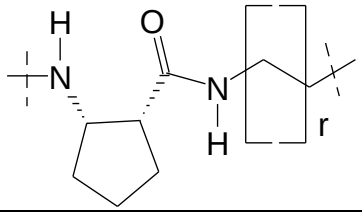
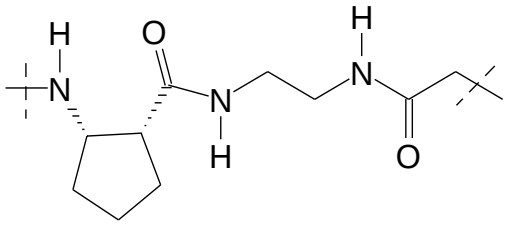
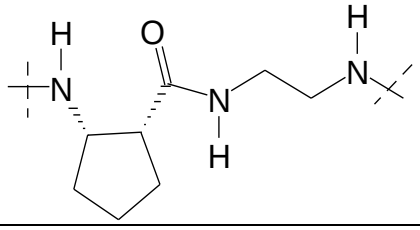
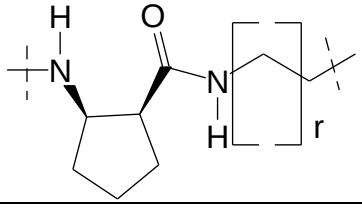
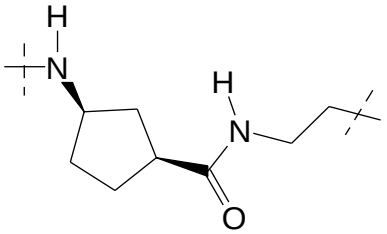
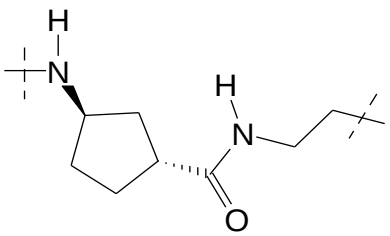
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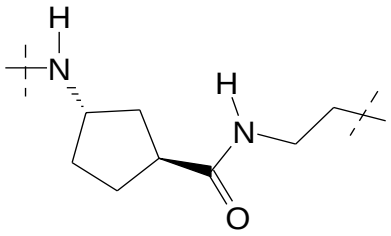
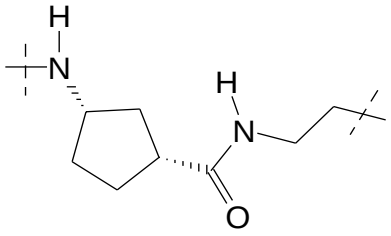
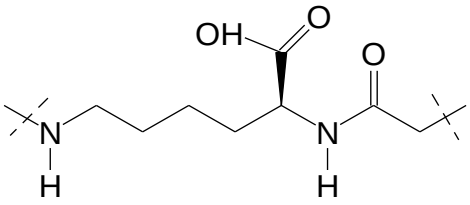
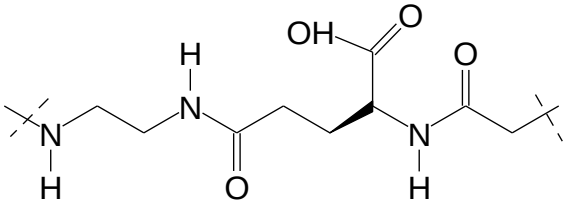
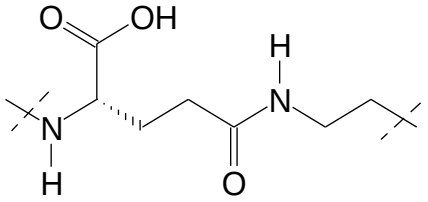
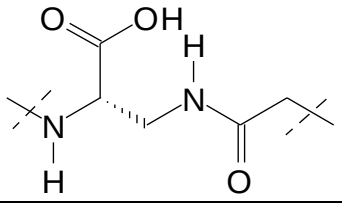
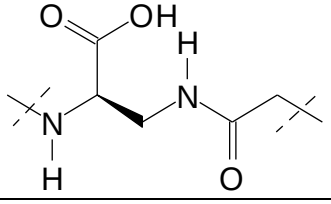
G_3	is $–(CH_2CH_2O)_s - (CH_2)_t - (C(=O)-NH)_u - CH_2CH_2O)_v - (CH_2)_w$-,
s, t, v and w	are independently 0 to 20,
u	is 0 or 1,
§'	is the bond to the –S(O)n group and
§''	is the bond to the nitrogen atom in the ring.

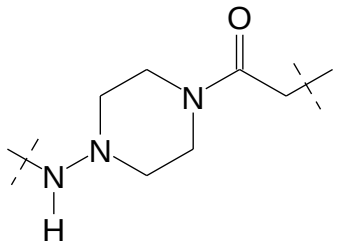
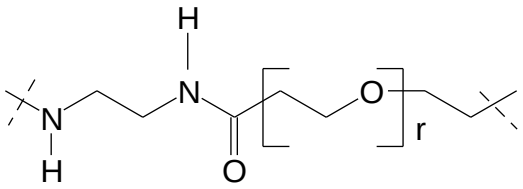
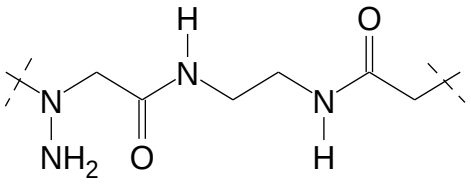
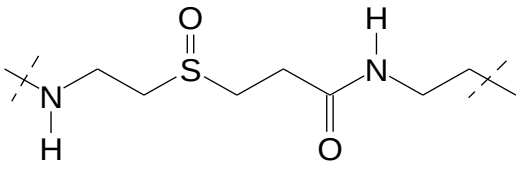
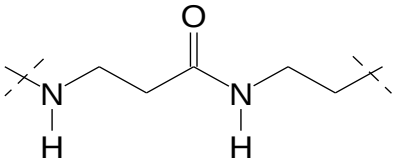
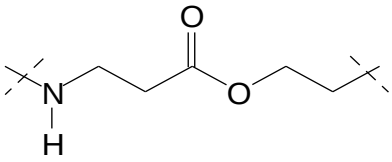
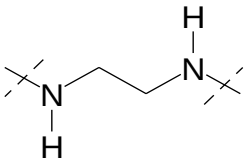
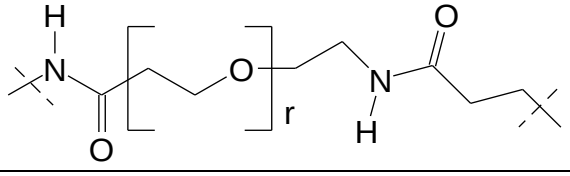
Preferred groups L1 in the above formula §-(C(=O))m-L1-L2-§§ are those listed in the table which follows, where r is a number from 0 to 20, preferably from 0 to 15, more preferably from 1 to 20, especially preferably from 2 to 10:

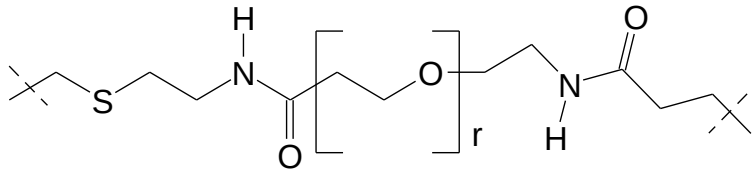
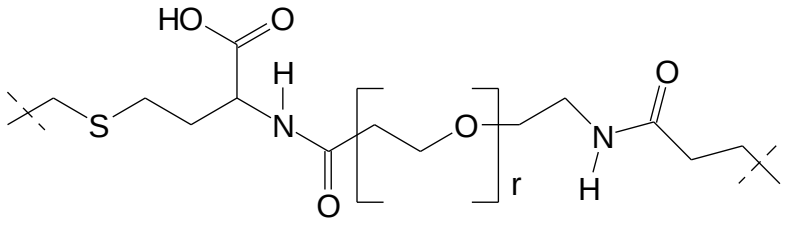
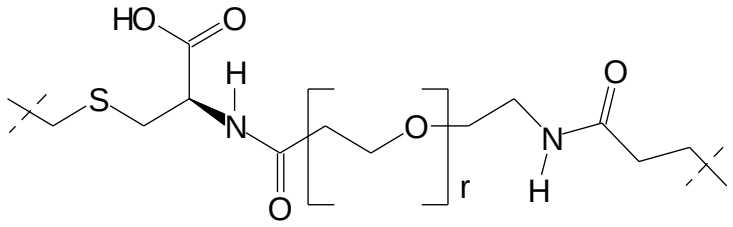
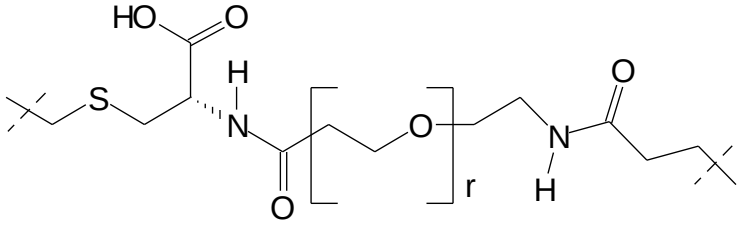
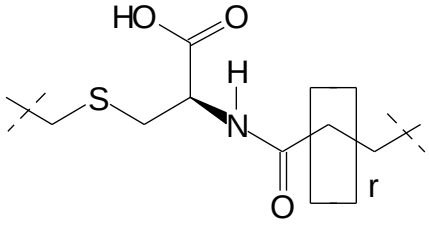
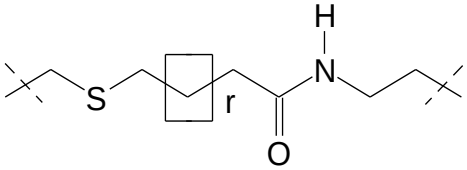
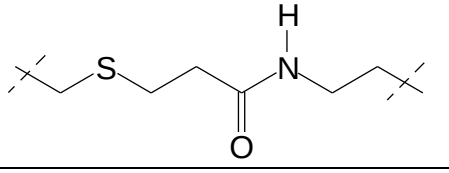
L1






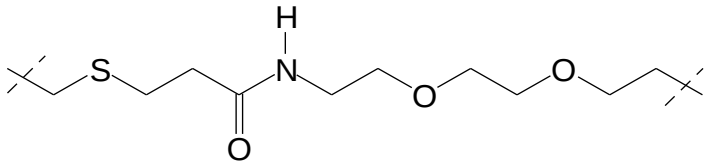
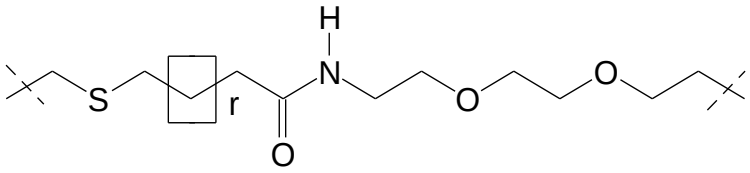
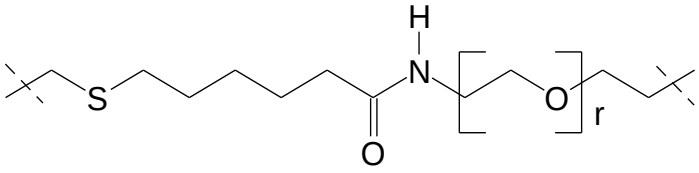
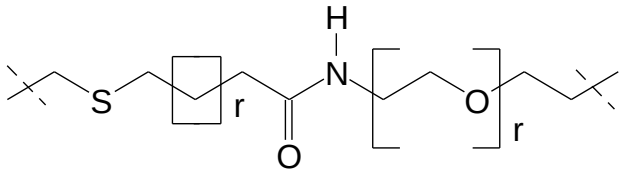
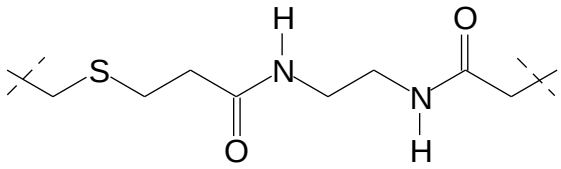
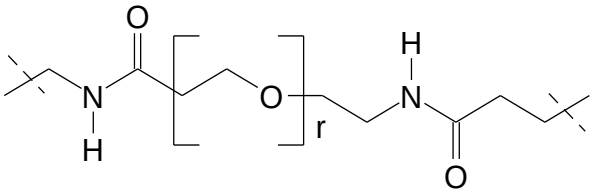
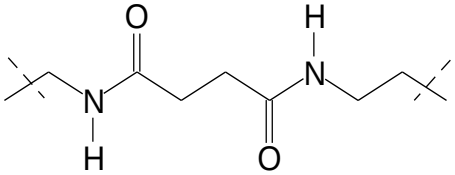
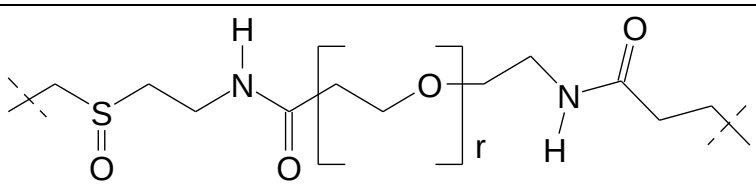
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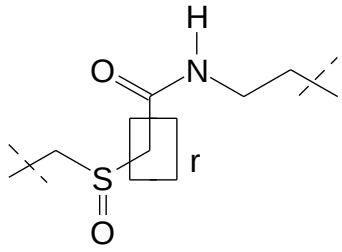
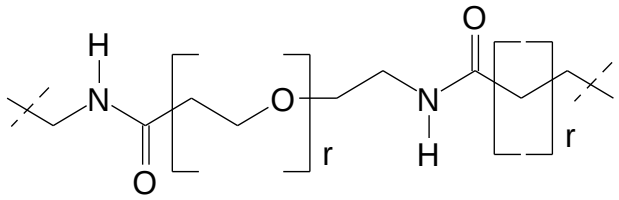
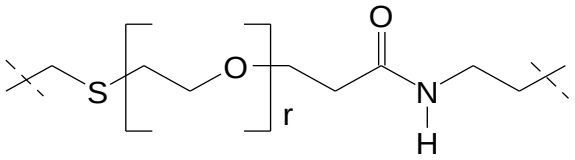
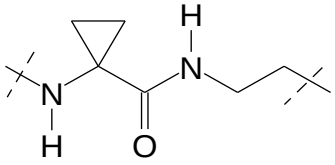
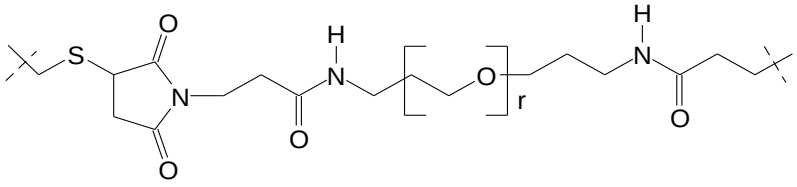
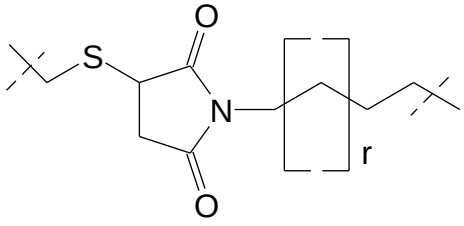
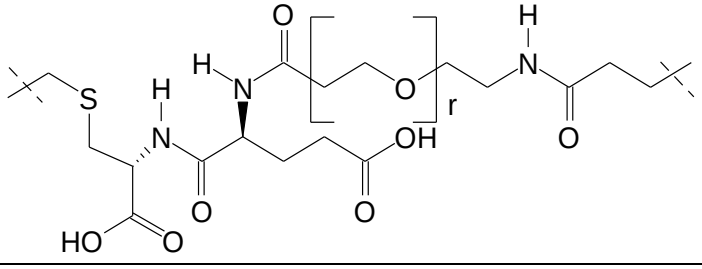
L1








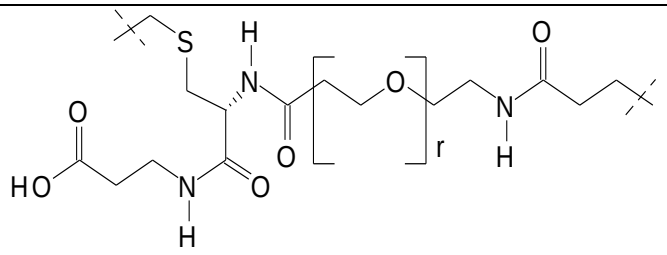
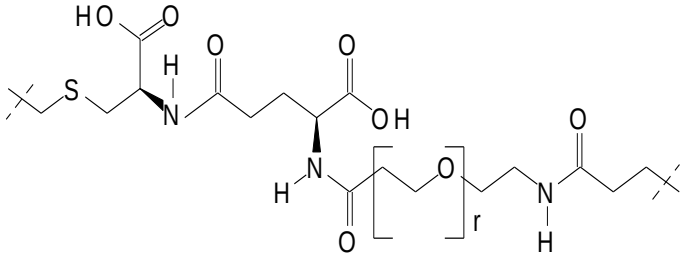
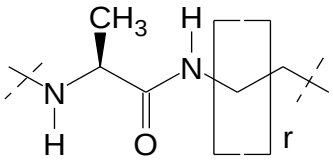
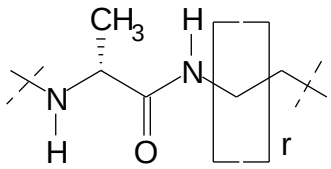
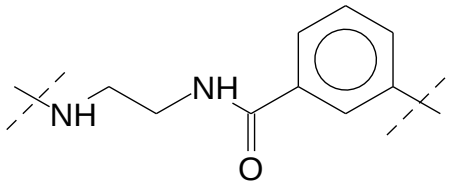
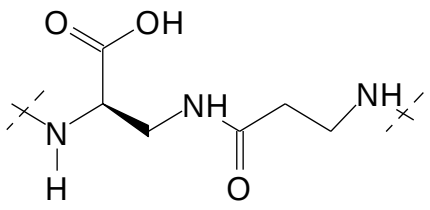
L1








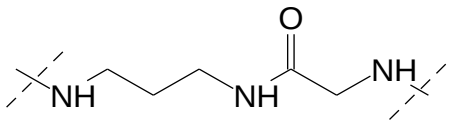
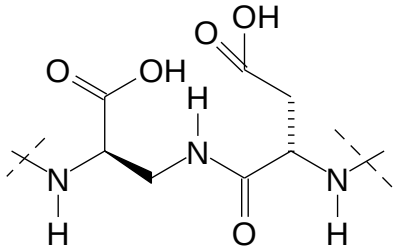
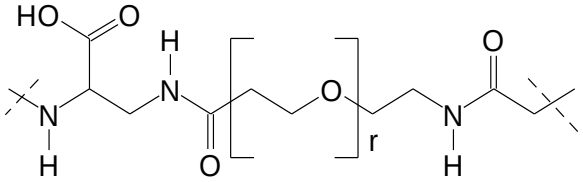
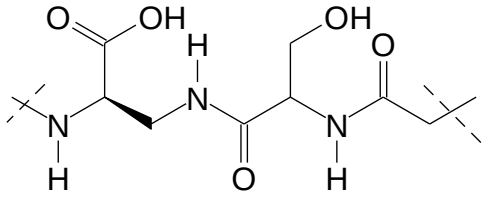
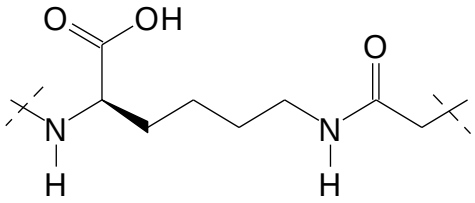
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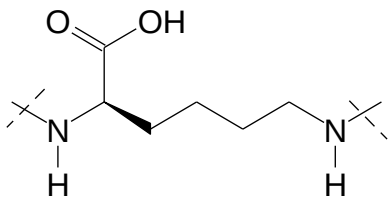
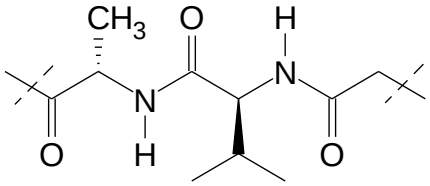
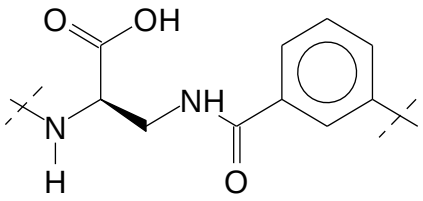
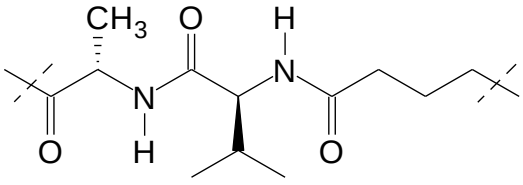
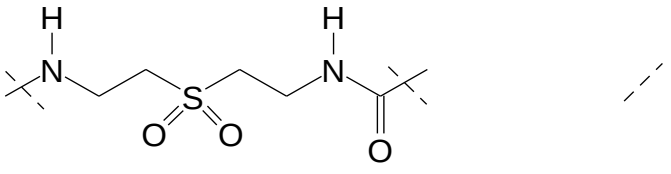
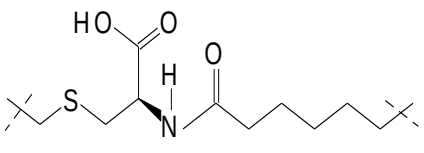
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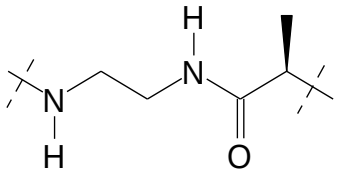
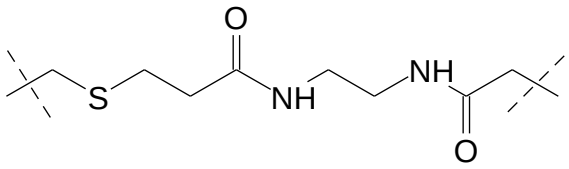
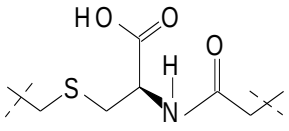
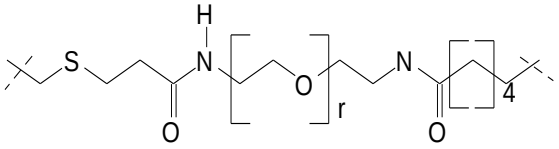
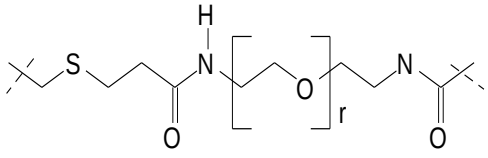
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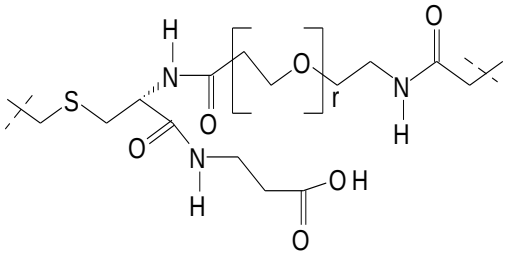
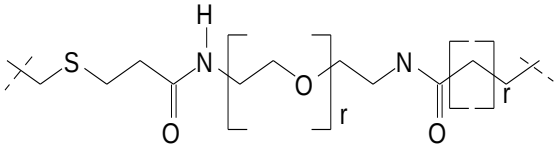
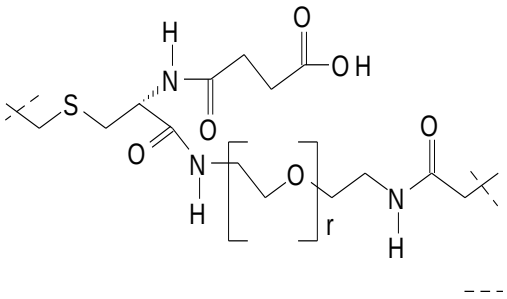
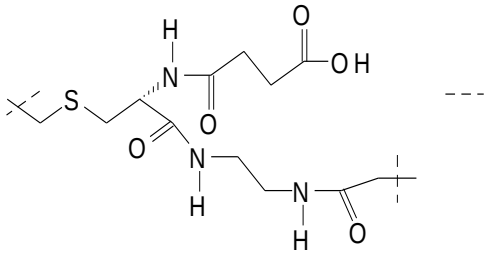
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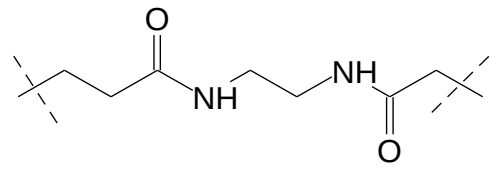
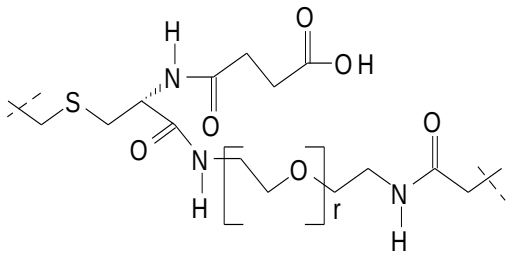
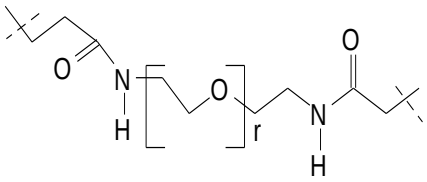
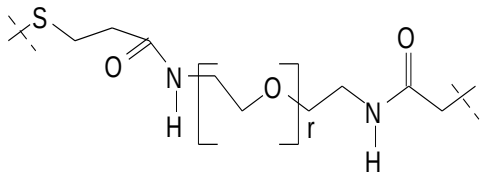
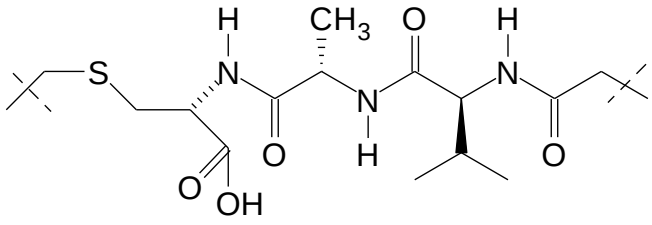
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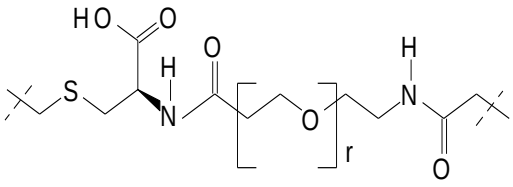
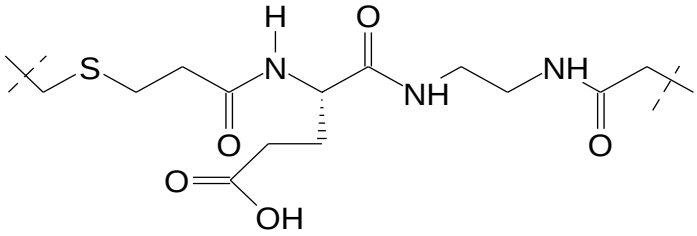
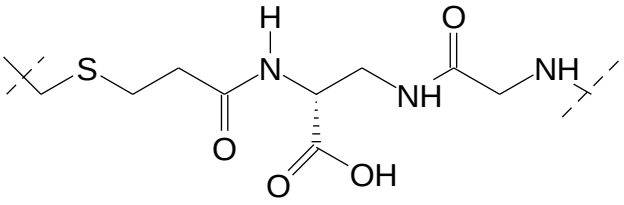
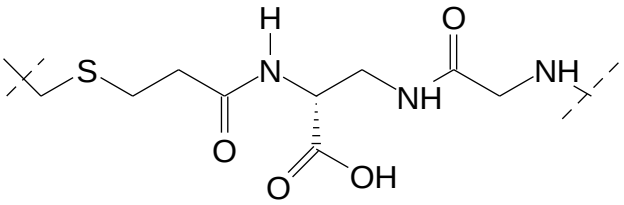
L1






L1







L1






L1





L1






L1





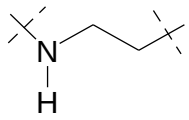
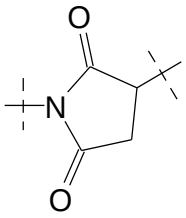
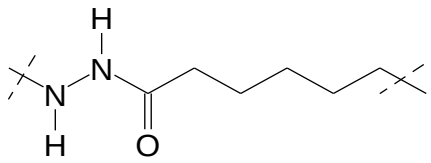
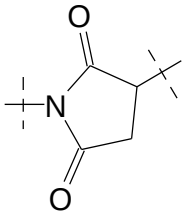
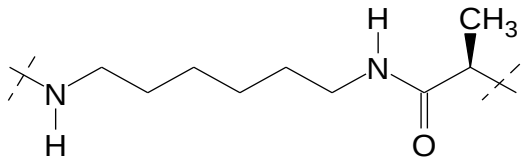
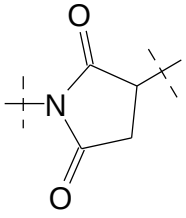
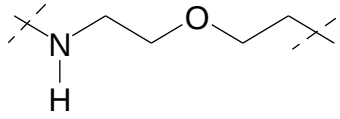
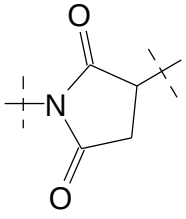
Further examples of L1 are given in Table C, in which this group is highlighted in a box.

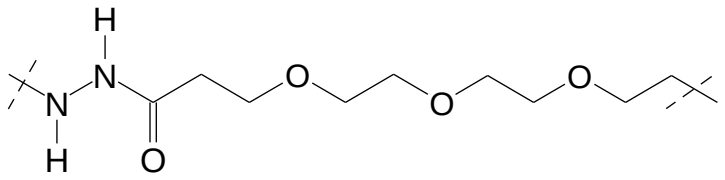
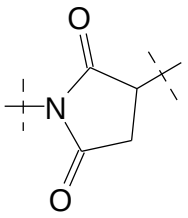
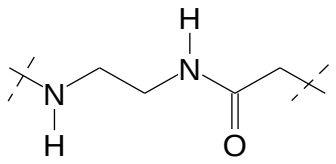
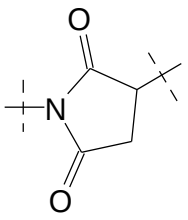
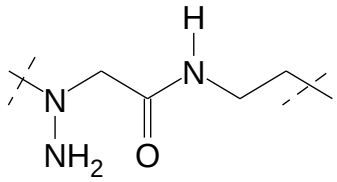
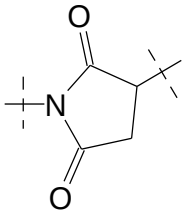
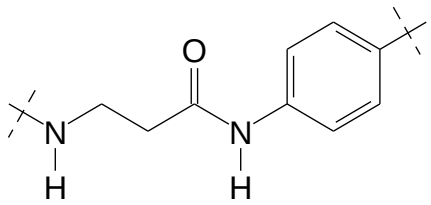
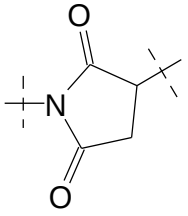
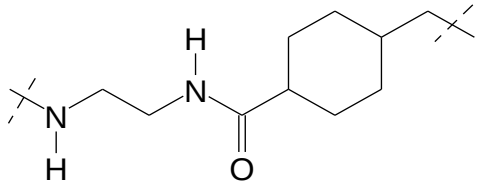
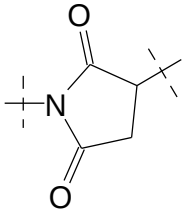
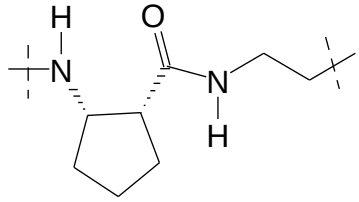
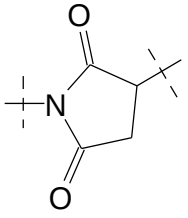
Tables A and A' below give examples of a linker moiety L1. The tables also state the L2 group with which these examples of L1 are preferably combined, and also the preferred coupling site (R^1 or R^3 in a compounds of the formulae (IIa), (IIb), (IIc), (IId), (V) and (VI)) and the preferred value of m, i.e. whether or not there is a carbonyl group before L1 (cf. §-

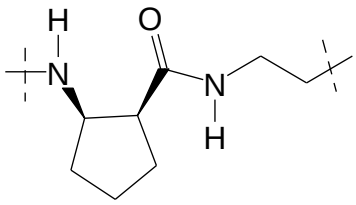
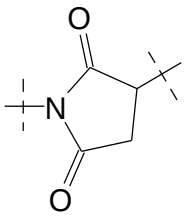
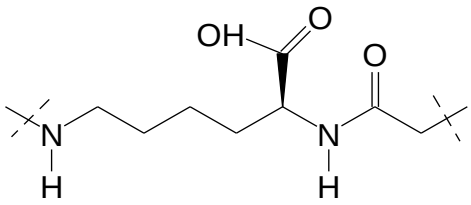
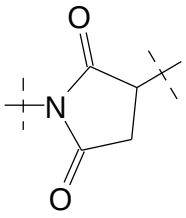
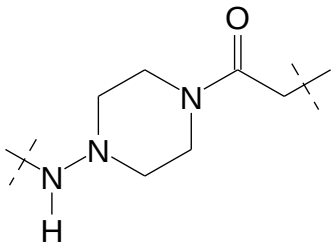
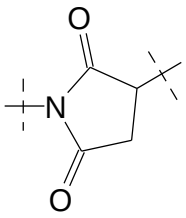
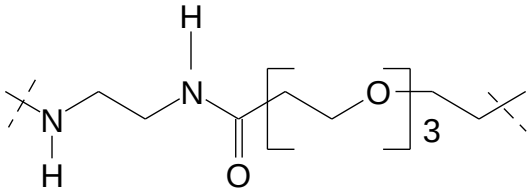
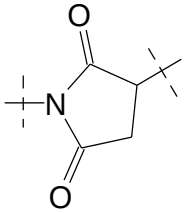
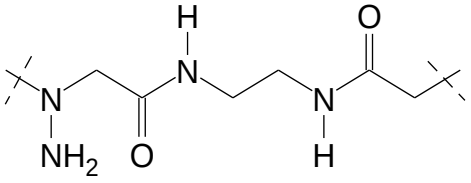
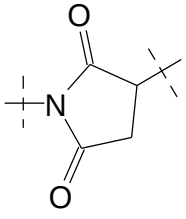
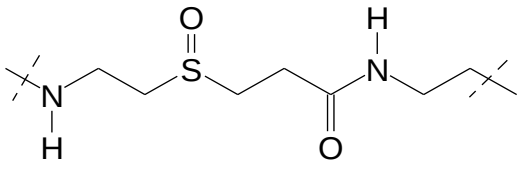
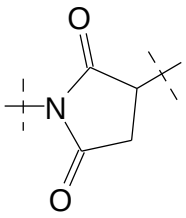
(C(=O))m-L1-L2-§§). These linkers are preferably coupled to a cysteine residue. If L2 is a succinimide or derived therefrom, this imide may also be fully or partly in the form of the hydrolysed open-chain succinamide, as described above. Depending on L1, the extent of this hydrolysis to open-chain succinamides may be more or less significant or even absent.

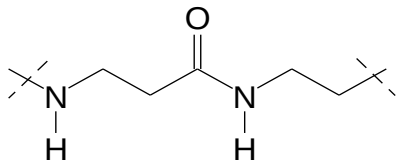
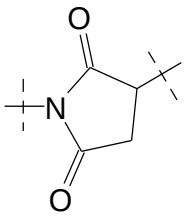
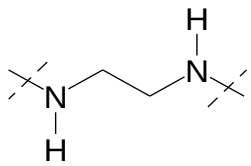
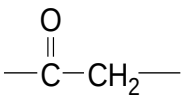
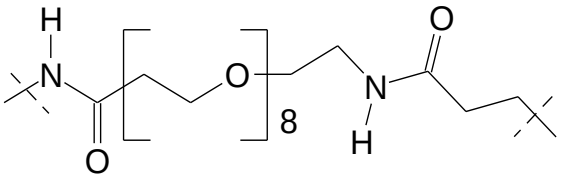
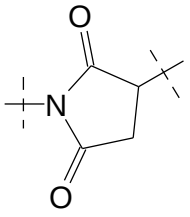
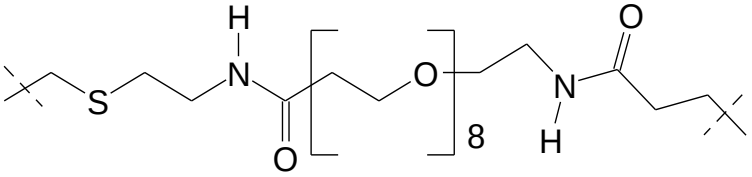
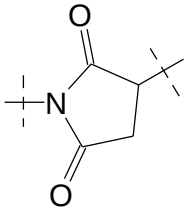
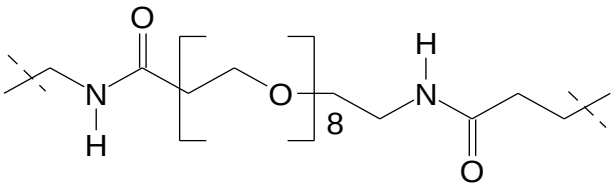
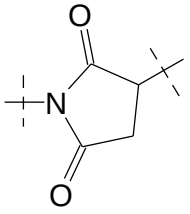
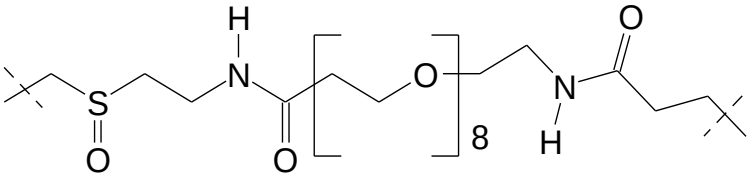
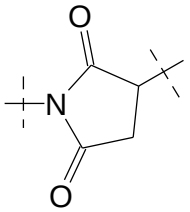
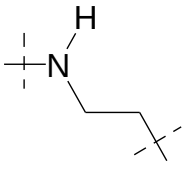
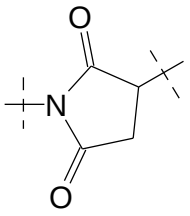
Table A

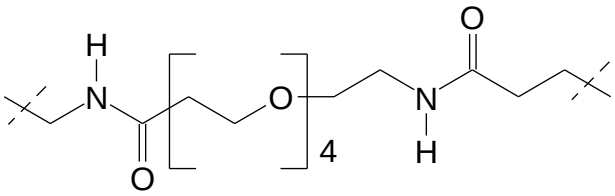
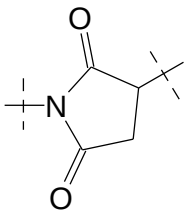
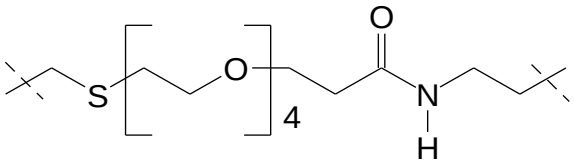
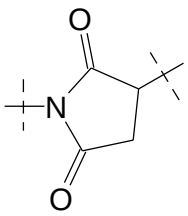
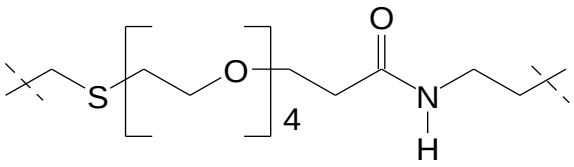
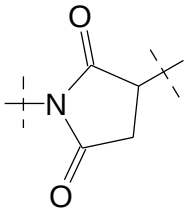
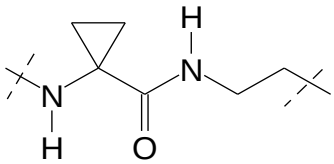
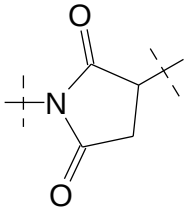
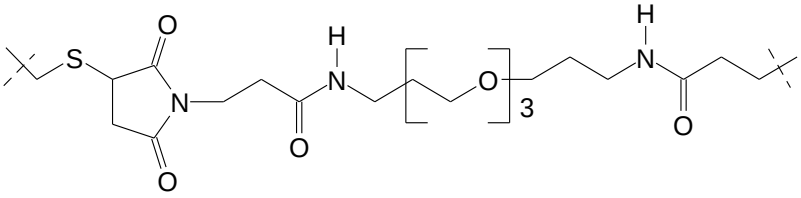
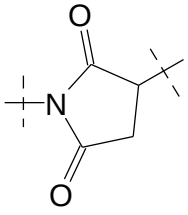
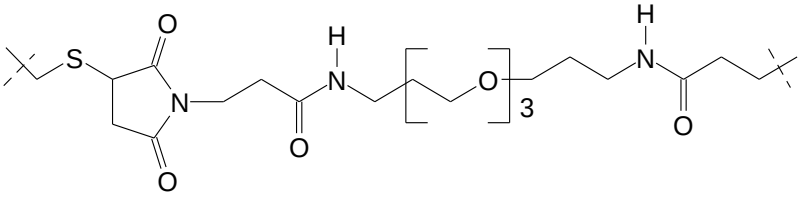
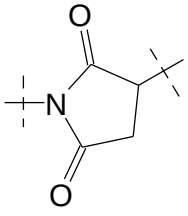
(Subst. = substituent)

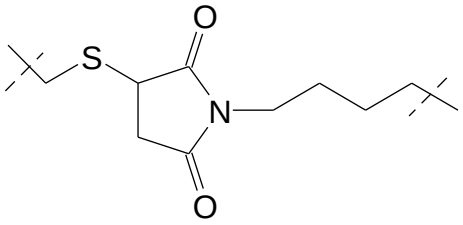
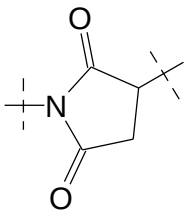
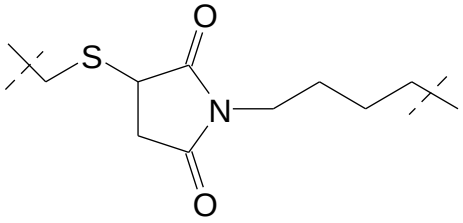
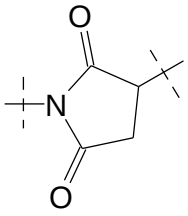
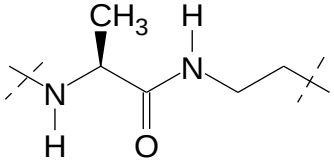
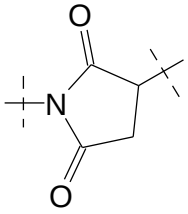
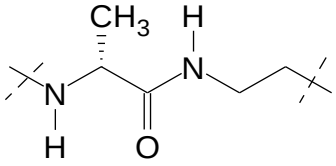
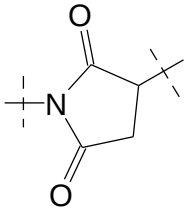
Subst.	**m**	**L1**	**L2**
R^1	1		
R^1	1		
R^1	1		
R^1	1		

Subst.	m	L1	L2
R^1	1		
R^1	1		 See note **
R^1	1		
R^1	1		
R^1	1		
R^1	1		

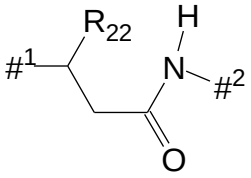
Subst.	m	L1	L2
R^1	1		
R^1	1		 See note **
R^1	1		 See note **
R^1	1		
R^1	1		 See note **
R^1	1		

Subst.	m	L1	L2
R^1	1		
R^1	1		
R^1	1		
R^3	0		
R^1	1		
R^3	0		
R^1	1		

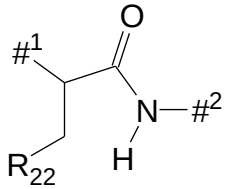
Subst.	m	L1	L2
R^1	0		
R^3	0		
R^3	0		
R^1	1		
R^3	0		
R^3	0		

Subst.	m	L1	L2
R^3	0		
R^3	0		
R^1	1		
R^1	1		

**More preferably, the linkers L1 specified in these lines are joined to a linker L2 selected from the general formulae (A7) and (A8):



(A7),



(A8)

in which

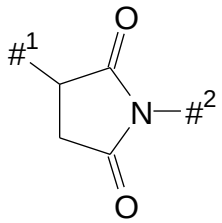
$\#^1$ is the linkage site to the sulphur atom of the binder,

$\#^2$ is the linkage site to the L^1 group, and

R^{22} is preferably –COOH.

In a conjugate according to the invention or in a mixture of the conjugates according to the invention, the bonds to a cysteine residue of the binder are present to an extent of preferably more than 80%, more preferably more than 90%, based in each case on the total number of bonds of the linker to the binder, and more preferably in one of the two structures of the formulae (A7) and (A8).

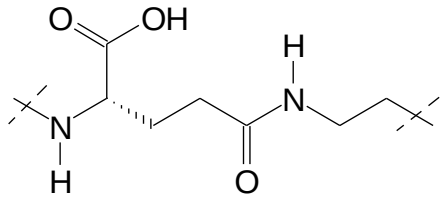
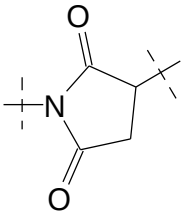
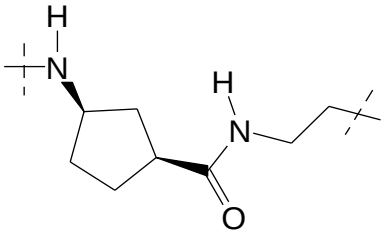
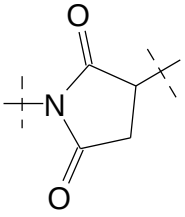
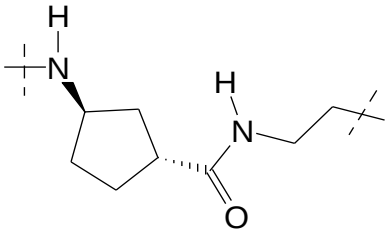
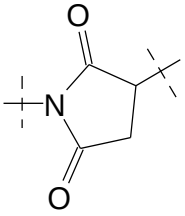
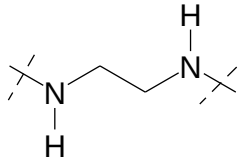
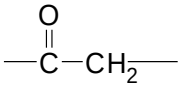
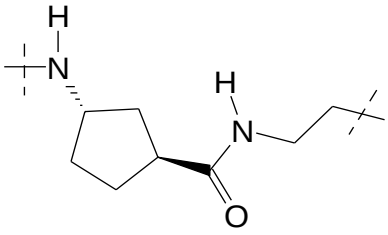
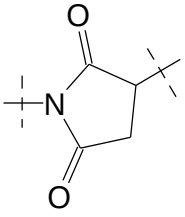
In this context, the structures of the formulae (A7) and (A8) are generally present together, preferably in a ratio of from 60:40 to 40:60, based on the number of bonds to the binder. The remaining bonds are then present in the structure

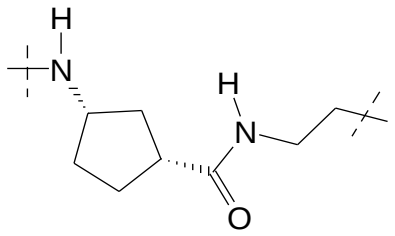
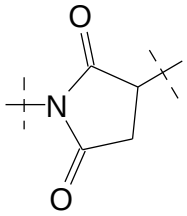
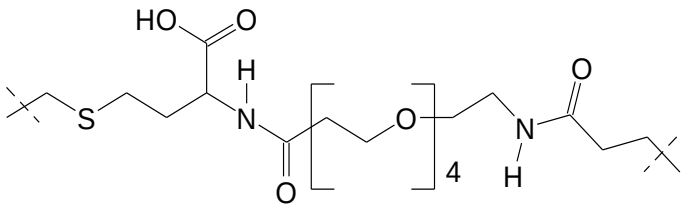
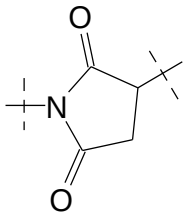
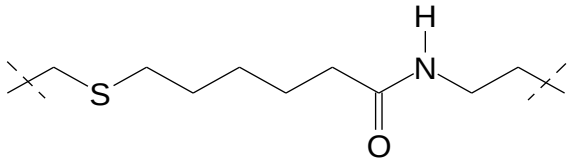
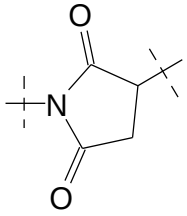
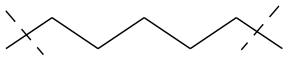
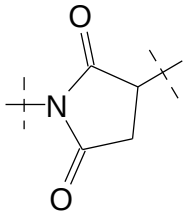
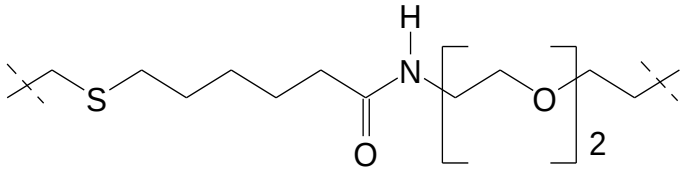
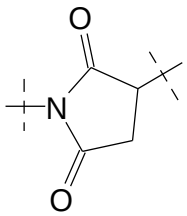


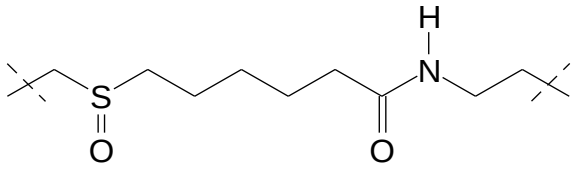
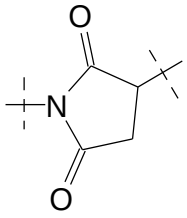
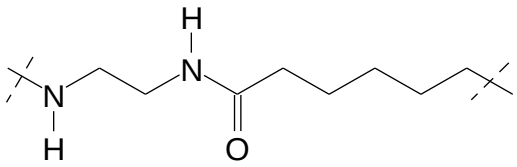
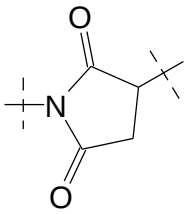
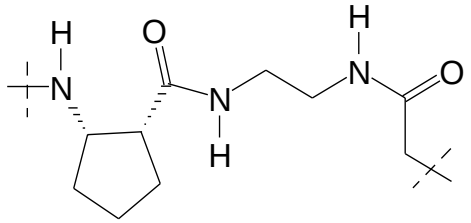
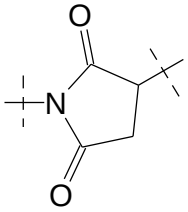
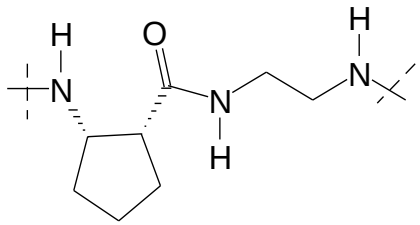
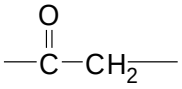
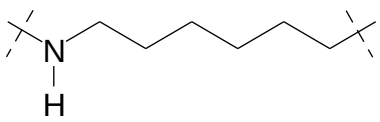
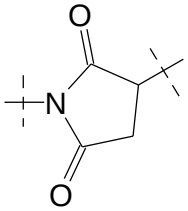
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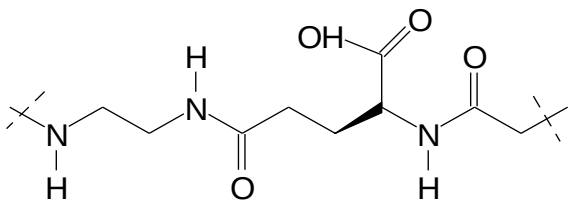
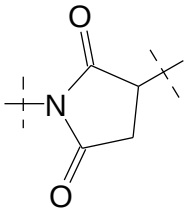
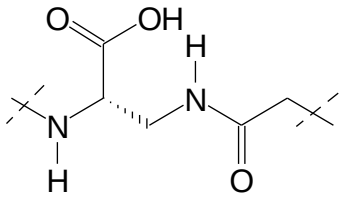
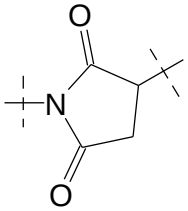
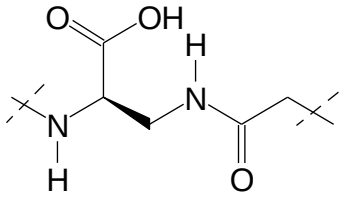
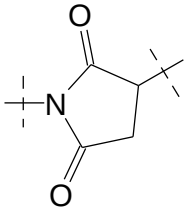
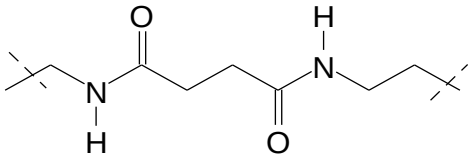
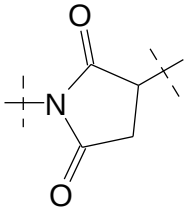
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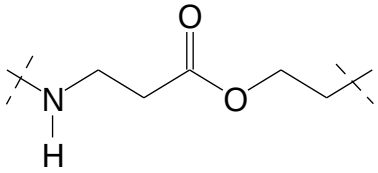
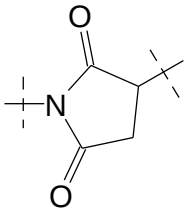
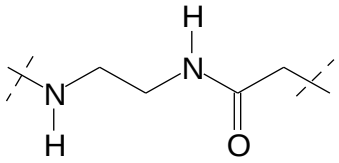
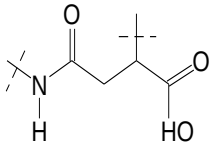
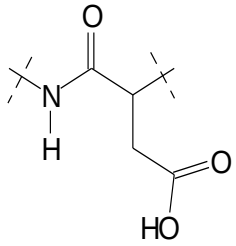
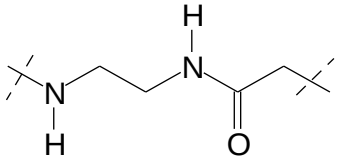
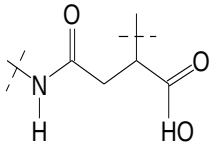
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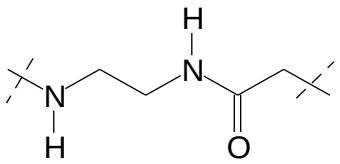
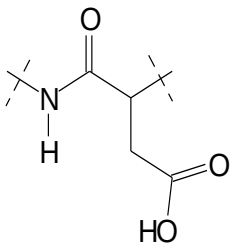
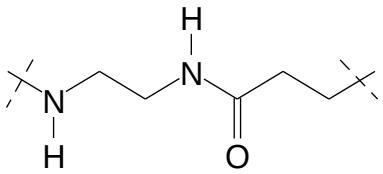
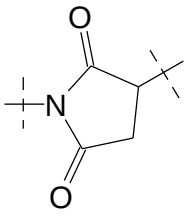
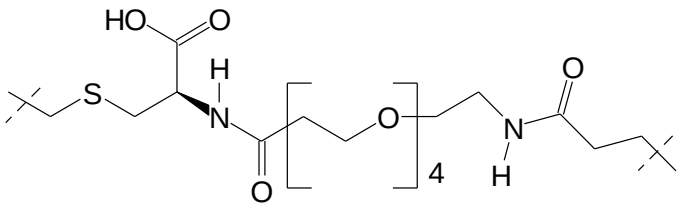
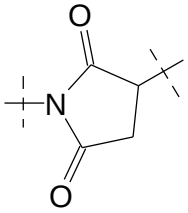
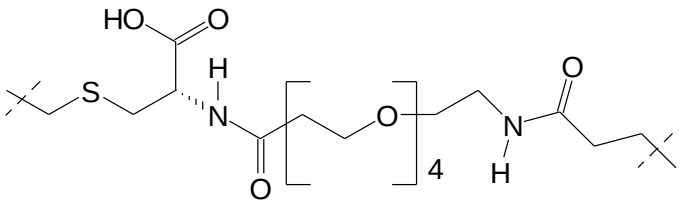
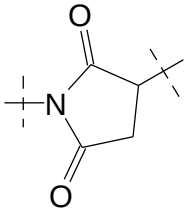
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R^1	1		
R^1	1		
R^1	1		
R^1	1		

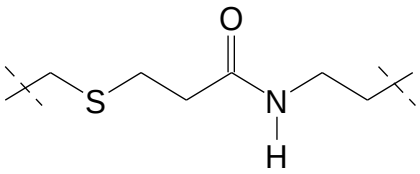
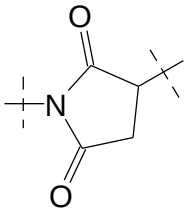
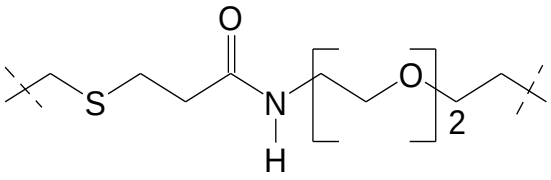
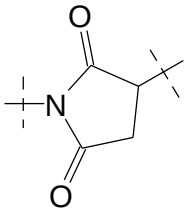
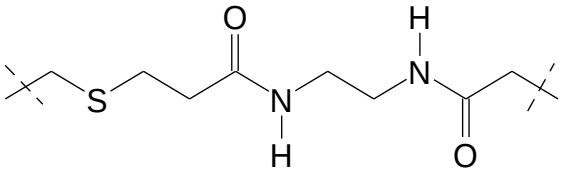
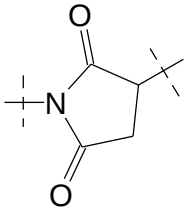
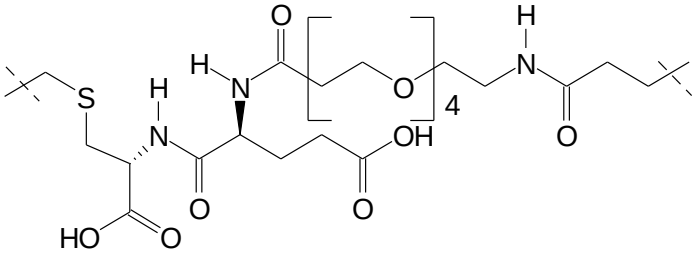
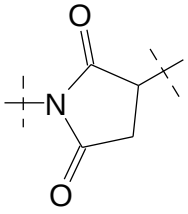
Subst.	m	L1	L2
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R^3	0		
R^3	0		
R^3	0		
R^3	0		

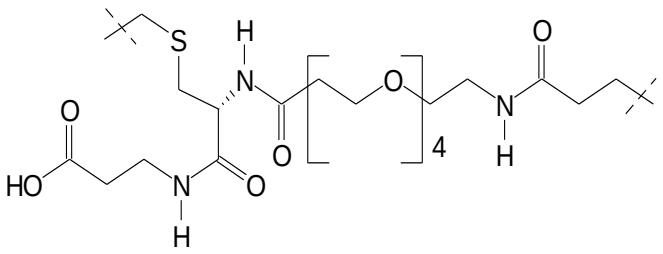
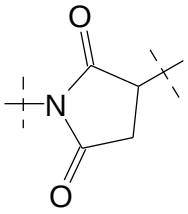
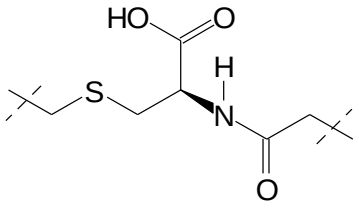
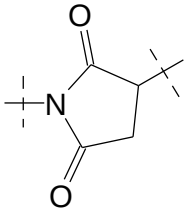
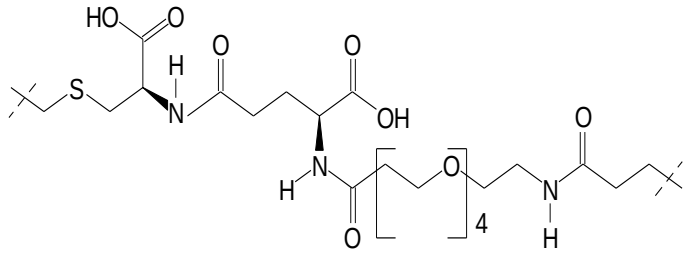
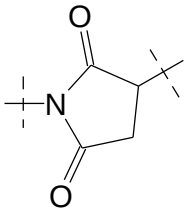
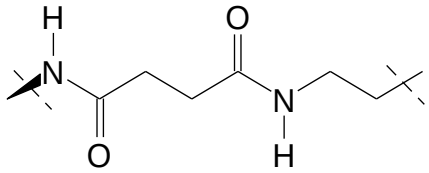
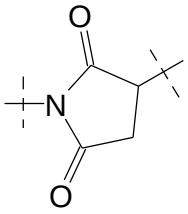
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R^1	1		
R^1	1		 See note **
R^1	1		
R^1	1		

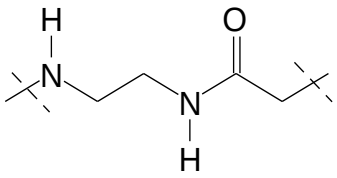
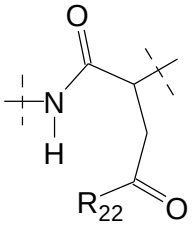
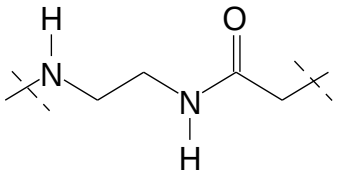
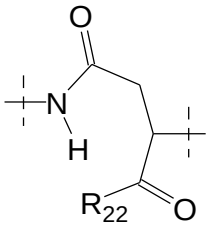
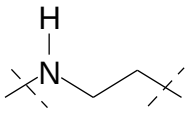
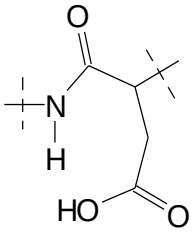
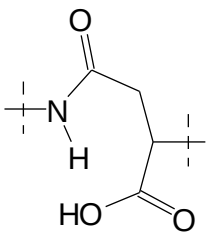
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R^1	1		 See note **
R^1	1		 See note **
R^1	0		

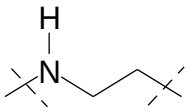
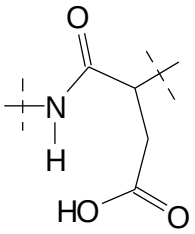
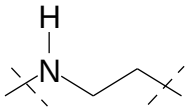
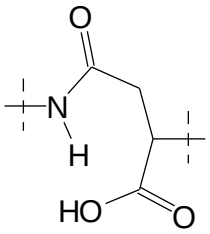
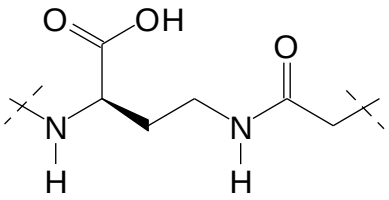
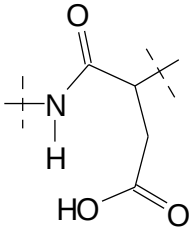
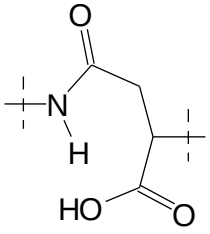
Subst.	m	L1	L2
R^1	1		
R^1	1		 and  See note ***
R^1	1		

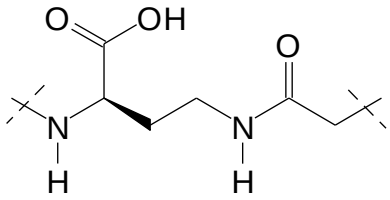
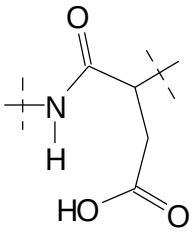
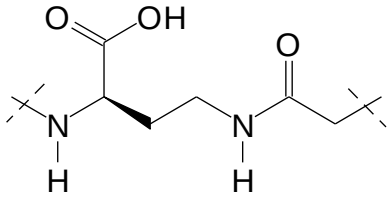
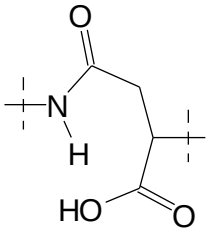
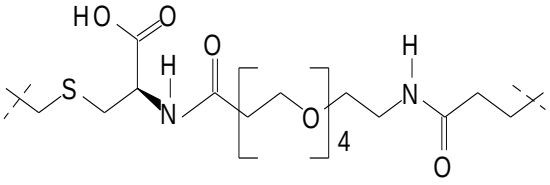
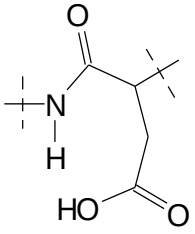
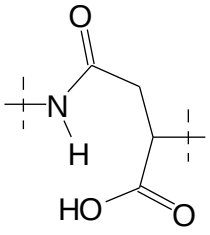
Subst.	m	L1	L2
R^1	1		 Identical to the two above
R^1	1		
R^3	0		
R^3	0		

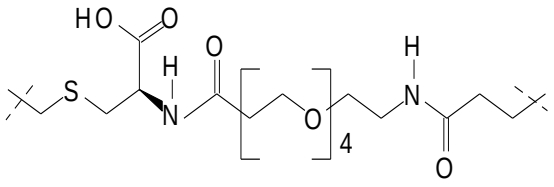
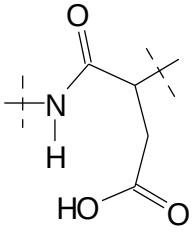
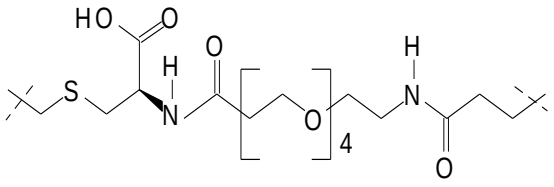
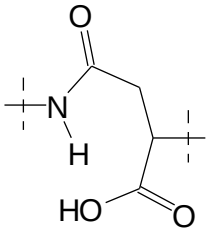
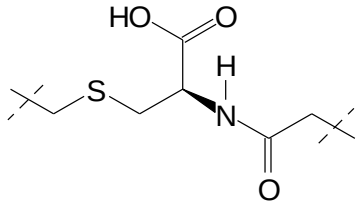
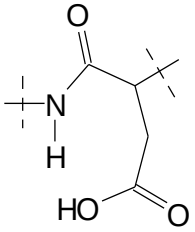
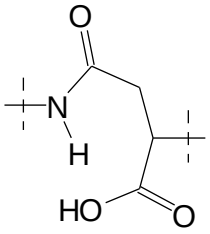
Subst.	m	L1	L2
R^3	0		
R^3	0		
R^3	0		 See note **
R^3	0		

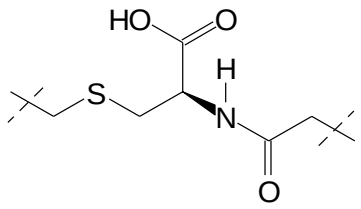
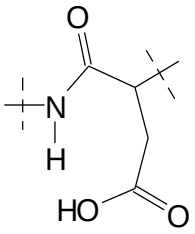
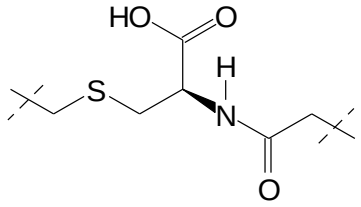
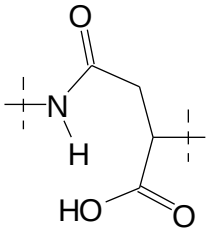
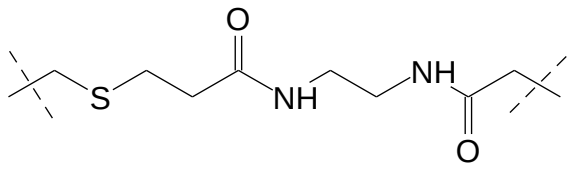
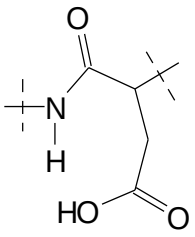
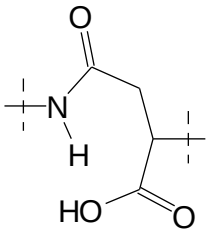
Subst.	m	L1	L2
R^3	0		
R^3	0		 See note **
R^3	0		
R^2	0		

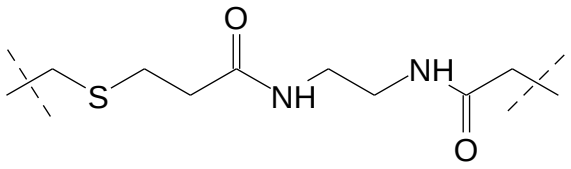
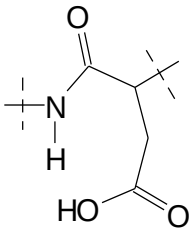
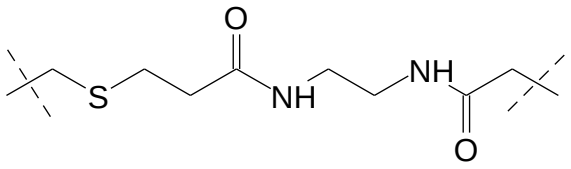
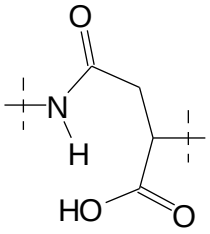
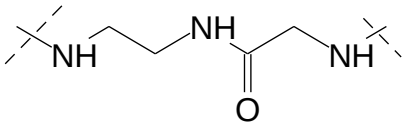
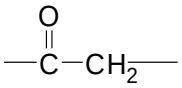
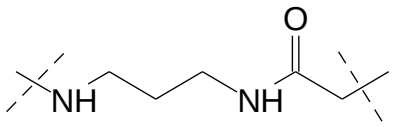
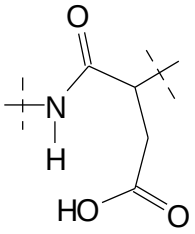
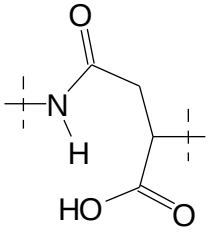
Subst.	m	L1	L2
R^1	1		 where R_{22} = -OH or -NH_2
R^1	1		 where R_{22} = -OH or -NH_2
R^1	1		 and  See

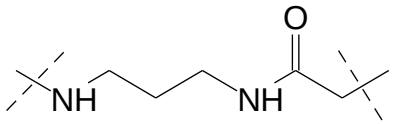
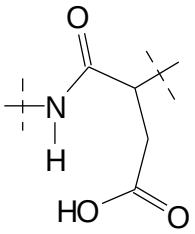
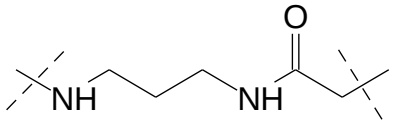
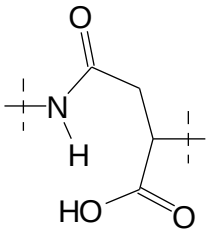
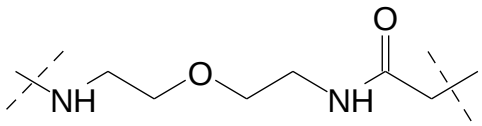
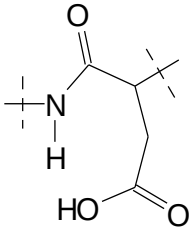
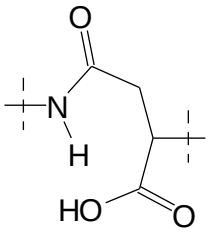
Subst.	m	L1	L2
			note ***
R^1	1		
R^1	1		
R^1	1		 and  See note ***

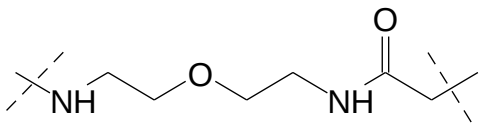
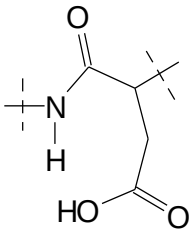
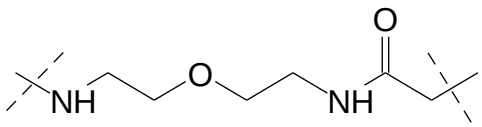
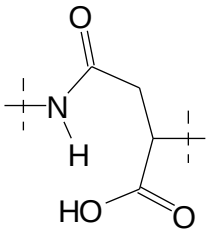
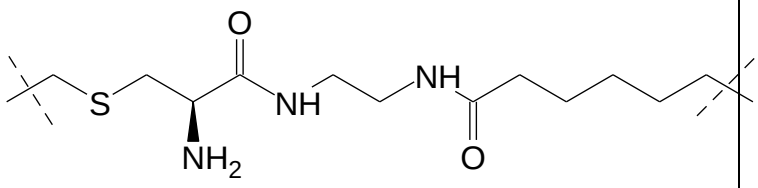
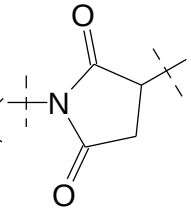
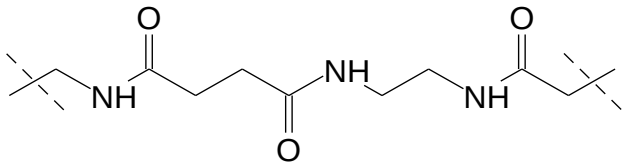
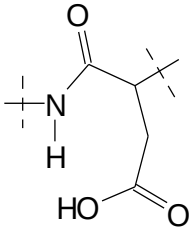
Subst.	m	L1	L2
R^1	1		
R^1	1		
R^3	0		 and  See note ***

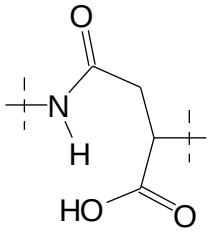
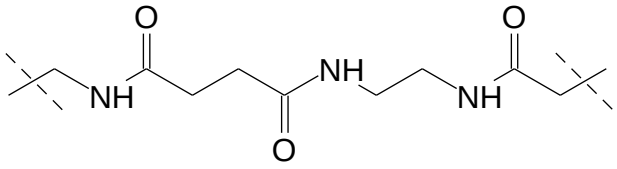
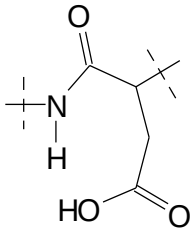
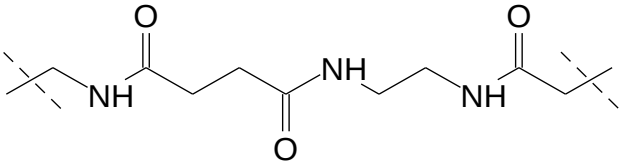
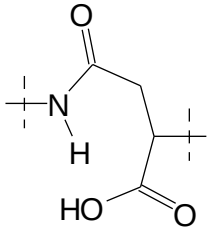
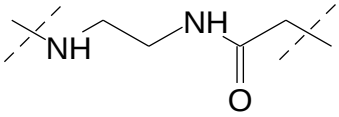
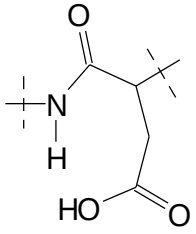
Subst.	m	L1	L2
R^3	0		
R^3	0		
R^3	0		 and  See note ***

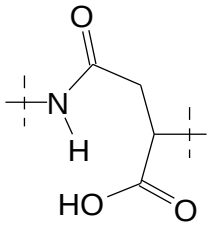
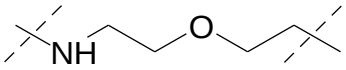
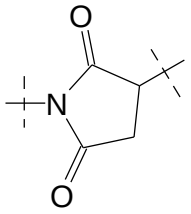
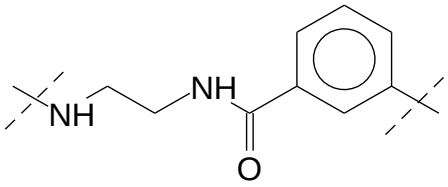
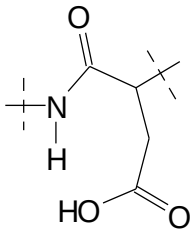
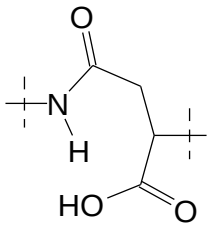
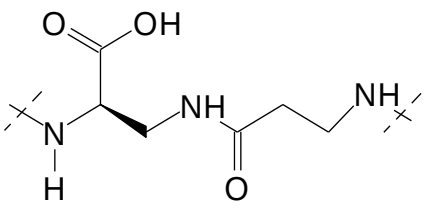
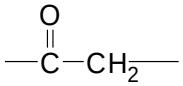
Subst.	m	L1	L2
R^3	0		
R^3	0		
R^3	0		 and  See note ***

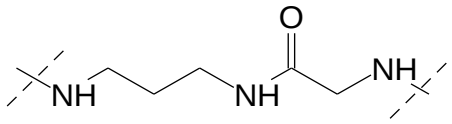
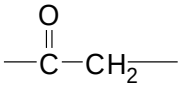
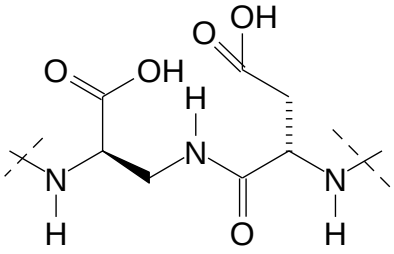
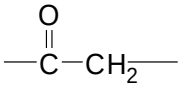
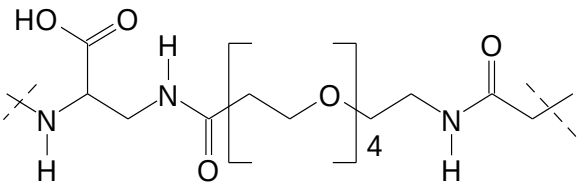
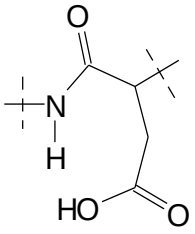
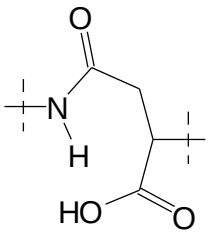
Subst.	m	L1	L2
R^3	0		
R^3	0		
R^1	1		
R^1	1		 and  See

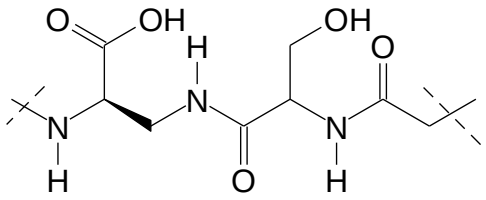
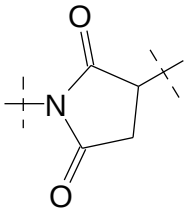
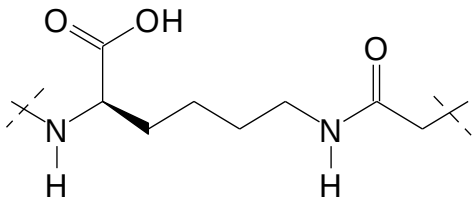
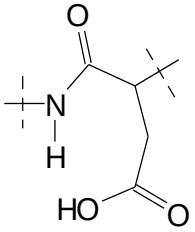
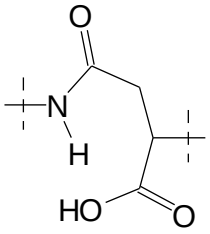
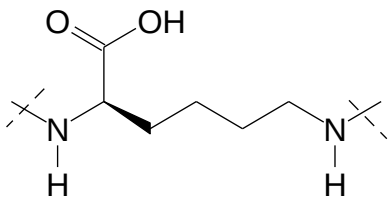
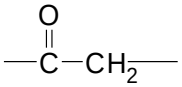
Subst.	m	L1	L2
			note ***
R^1	1		
R^1	1		
R^1	1		 and  See

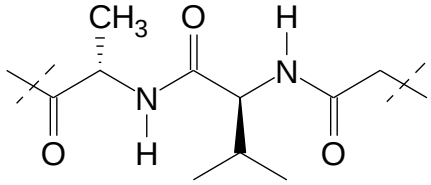
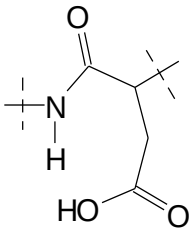
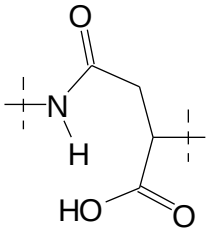
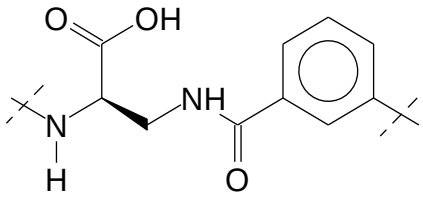
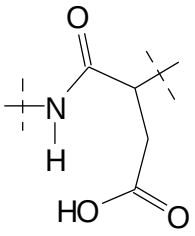
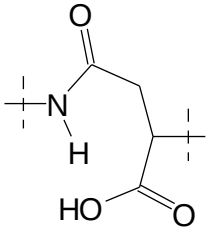
Subst.	m	L1	L2
			note ***
R^1	1		
R^1	1		
R3	0		
R1	0		 and

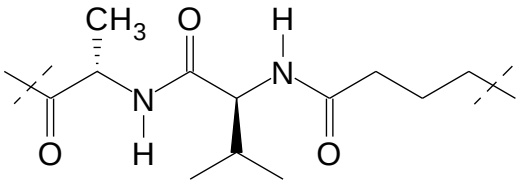
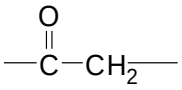
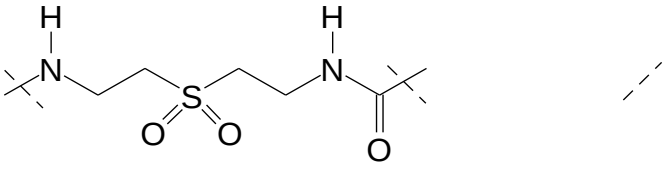
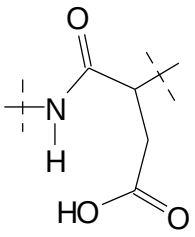
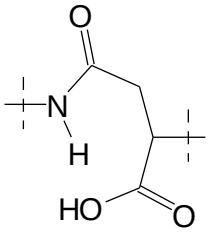
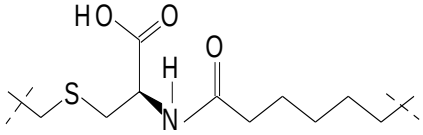
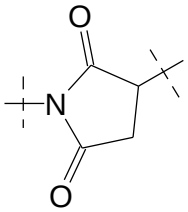
Subst.	m	L1	L2
			 See note ***
R1	0		
R1	0		
R1	1		 and

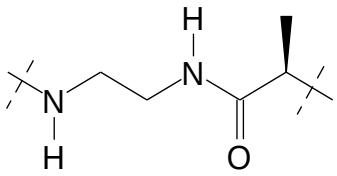
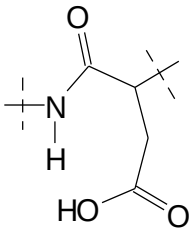
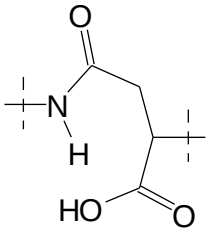
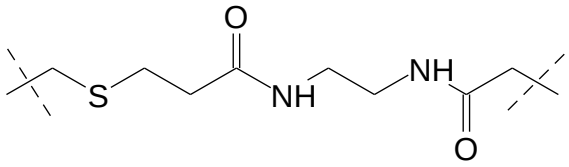
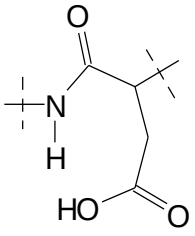
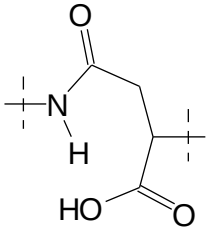
Subst.	m	L1	L2
			
R1	1		
R1	1		 and 
R^1	1		

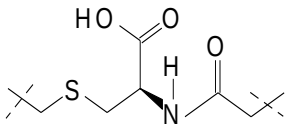
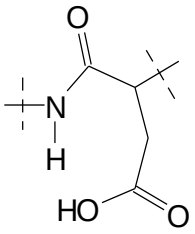
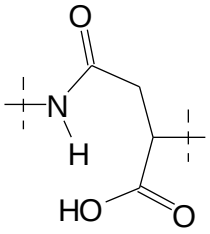
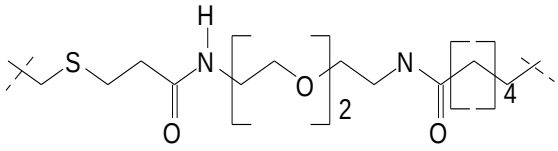
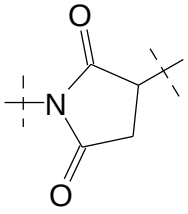
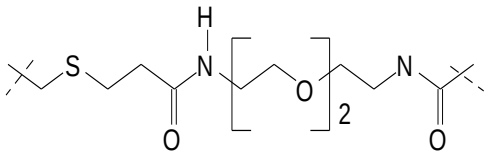
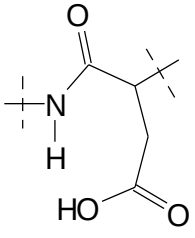
Subst.	m	L1	L2
R^1	1		
R^1	1		
R^1	1		 and  See note ***

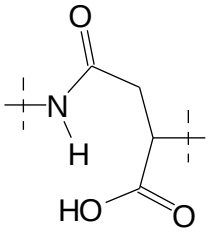
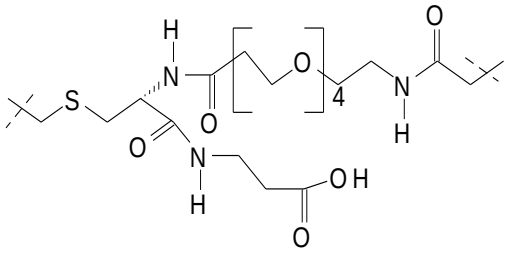
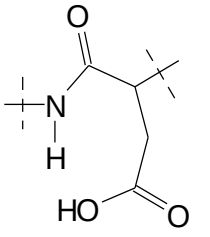
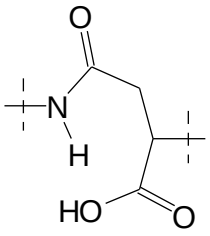
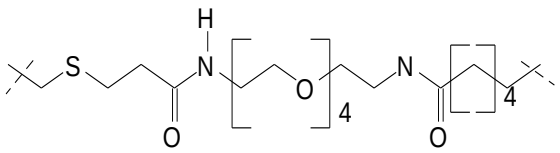
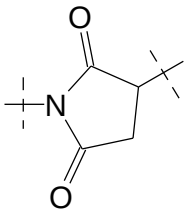
Subst.	m	L1	L2
R^1	1		 See note **
R^1	1		 and  See note ***
R^1	1		

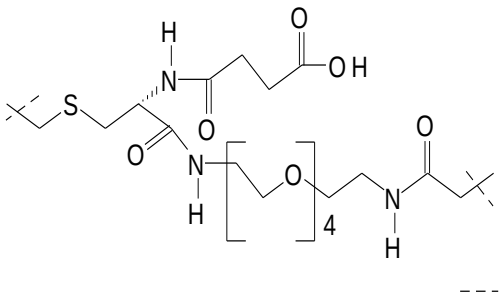
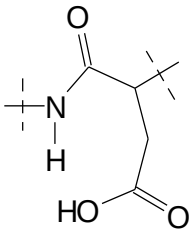
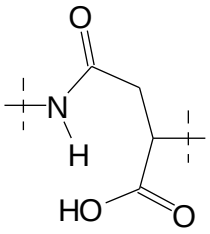
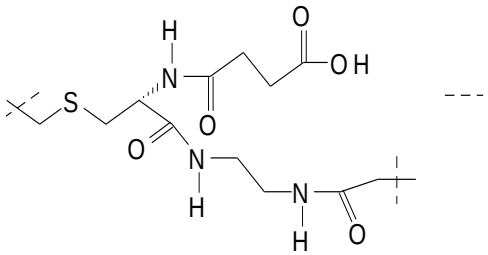
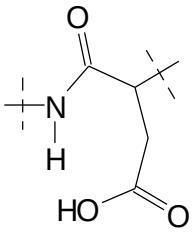
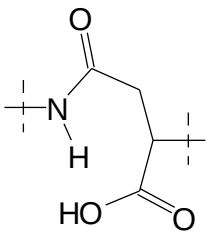
Subst.	m	L1	L2
R^4	0		 and  See note ***
R^1	1		 and  See

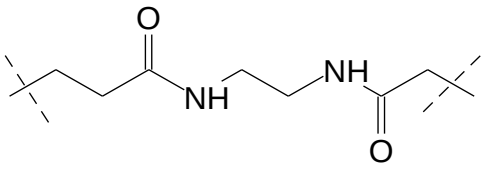
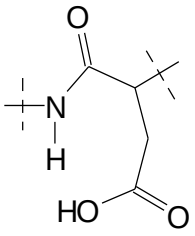
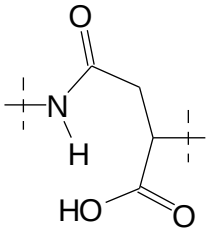
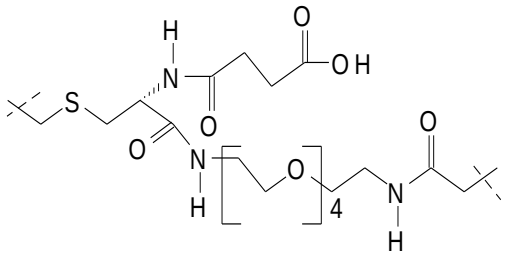
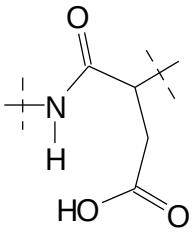
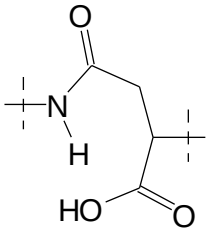
Subst.	m	L1	L2
			note ***
R^4	0		
R^1	1		 and  See note **
R^3	0		 See note **

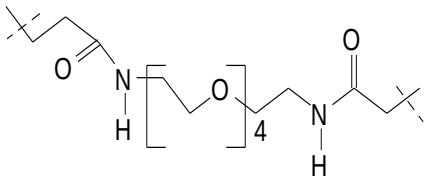
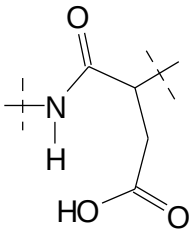
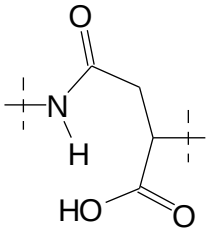
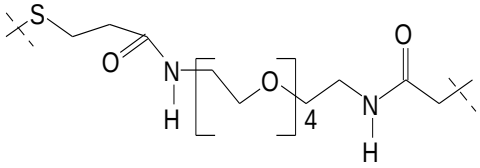
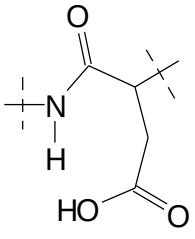
Subst.	m	L1	L2
R^1	1		 and  See note **
R^3	0		 and  See note ***

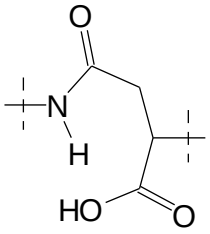
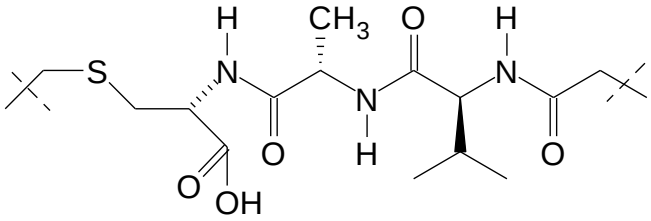
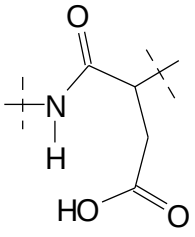
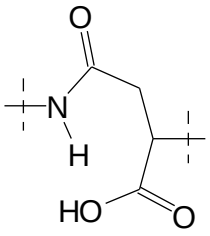
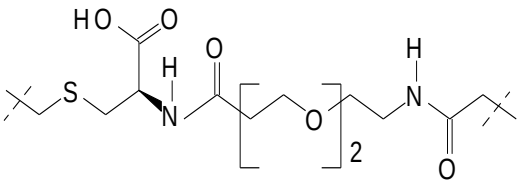
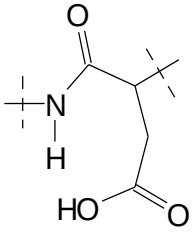
Subst.	m	L1	L2
R^3	0		 and  See note ***
R^3	0		 See note **
R^3	0		 and

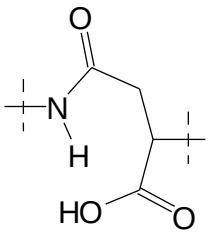
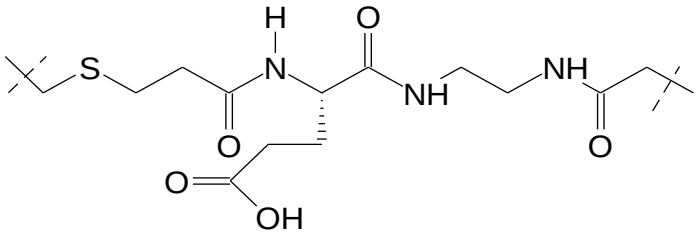
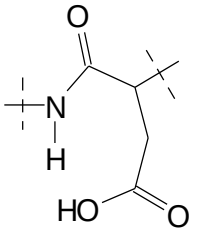
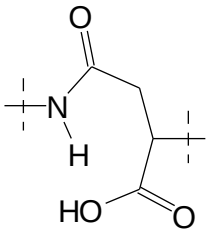
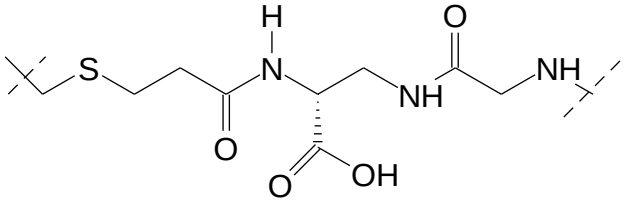
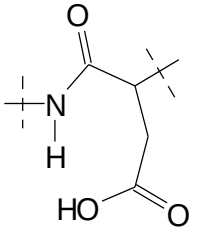
Subst.	m	L1	L2
			 See note ***
R^3	0		 and  See note ***
R^3	0		

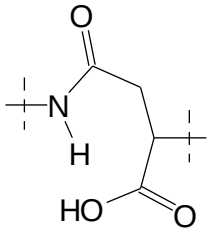
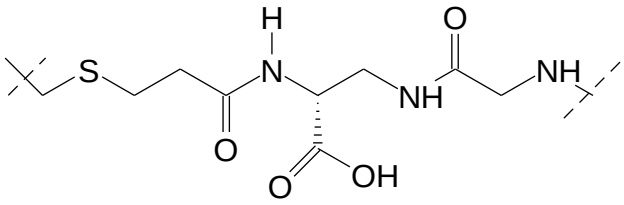
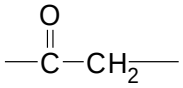
Subst.	m	L1	L2
			See note **
R^3	0		 and  See note ***
R^3	0		 and 

Subst.	m	L1	L2
			See note ***
R^3	0		 and  See note ***
R^3	0		 and 

Subst.	m	L1	L2
			See note ***
R^3	0		 and  See note ***
R^3	0		 and

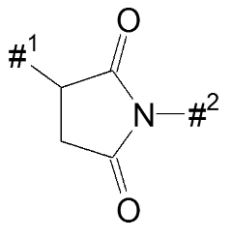
Subst.	m	L1	L2
			 See note ***
R^3	0		 and  See note ***
R^3	0		

Subst.	m	L1	L2
			and  See note ***
R^3	0		 and  See note ***
R^3	0		 and

Subst.	m	L1	L2
			 See note ***
R^3	0		

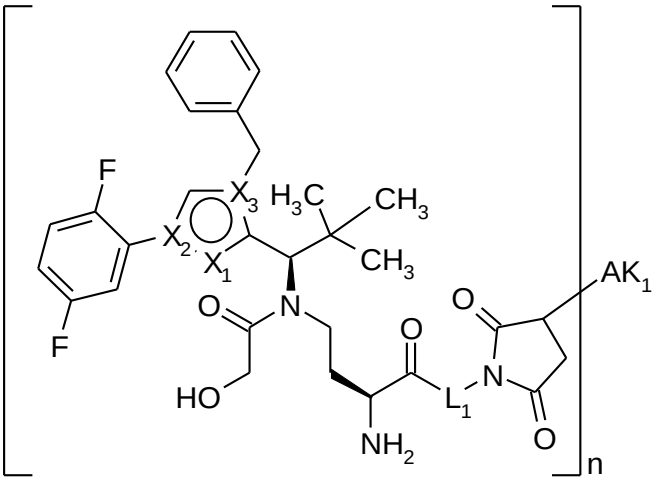
**: See note ** for Table A.

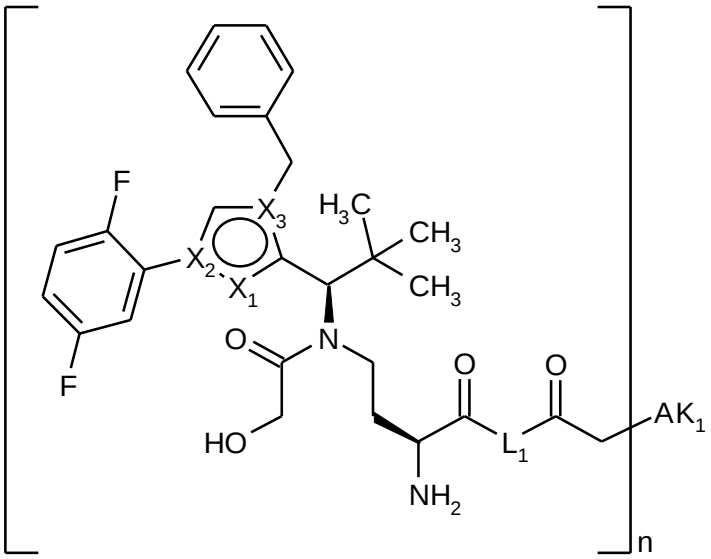
***: When this structure L2 is present, there may simultaneously be a structure L2 of the following formula:

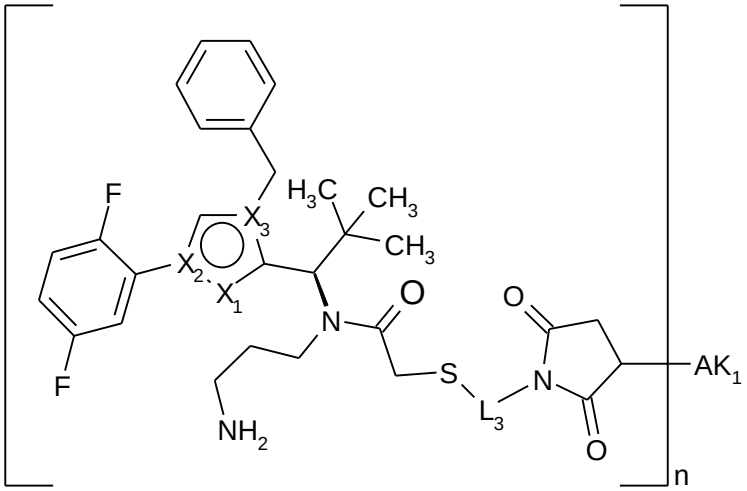


Examples of conjugates with corresponding linkers have the following structures, where X1 represents CH, X2 represents C and X3 represents N and L1 has the definition given above, L2 and L3 have the same definition as L1, AK1 is an antibody bonded via a cysteine residue and n is a number from 1 to 10.

More preferably, AK1 is an antibody or an antigen-binding antibody. The antibody is preferably a human, humanized or chimeric monoclonal antibody or an antigen-binding fragment thereof, especially an anti-TWEAKR antibody, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment thereof. Particular preference is given to the anti-TWEAKR antibodies TPP-7006, TPP-7007, TPP-10336 and TPP-10337, the anti-B7H3 antibodies TPP-8382 and TPP-8567, the anti-EGFR-antibody cetuximab (TPP-981) and the anti-HER2-antibodies trastuzumab and TPP-1015, or an antigen-binding fragment of these.


F
F
X3
X2
X1
H3C
CH3
CH3
O
N
HO
NH2
O
L1
N
O
O
AK1
n


F
F
X3
X2
X1
H3C
CH3
CH3
O
N
HO
NH2
O
L1
O
O
AK1
n


F
F
X3
X2
X1
H3C
CH3
CH3
O
N
NH2
S
L3
N
O
O
AK1
n

5

In this context,

AK1 is an anti-TWEAKR antibody, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment of these and

n is a number from 1 to 20.

Additionally preferred is the base structure (i), (ii) and (iv) of the linkers

(i) $–(C=O)_m–SG1-L1-L2-$

(ii) $–(C=O)_m –L1-SG-L1-L2-$

(iii) $–(C=O)_m –L1-SG-L2,$

where SG1 or SG represents a cathepsin-cleavable group, and L1 and L2 have the definitions listed in Table A'. Particular preference is given to the following groups:

-Val-Ala-C(=O)-NH- (resulting in cleavage of the amide bond at the C-terminal amide of alanine)

-NH-Val-Lys-C(=O)-NH- (cleavage of the amide bond at the C-terminal amide of lysine)

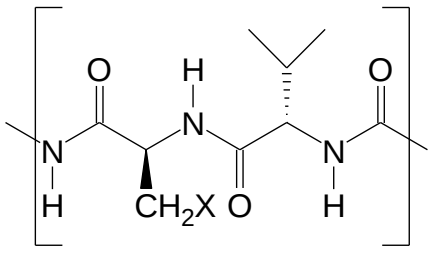
-NH-Val-Cit-C(=O)-NH- (cleavage of the amide bond at the C-terminal amide of citrulline)

-NH-Phe-Lys-C(=O)-NH- (cleavage of the amide bond at the C-terminal amide of lysine)

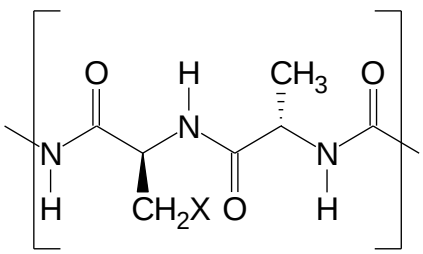
-NH-Ala-Lys-C(=O)-NH- (cleavage of the amide bond at the C-terminal amide of lysine)

-NH-Ala-Cit-C(=O)-NH- (cleavage of the amide bond at the C-terminal amide of citrulline)

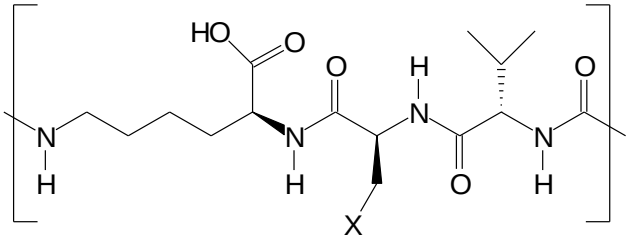
In this context, particular preference is given to SG1 or SG according to the general formulae



,



and



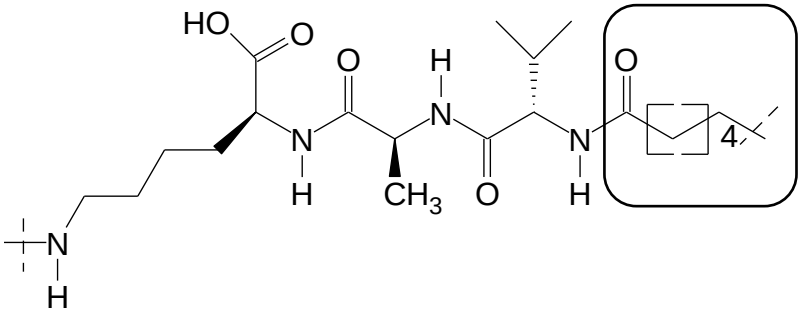
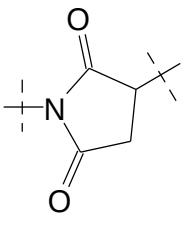
in which

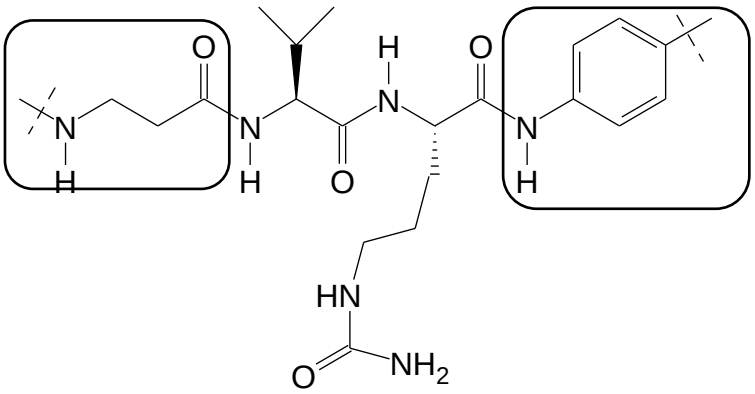
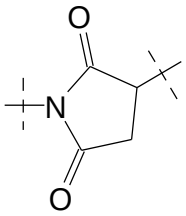
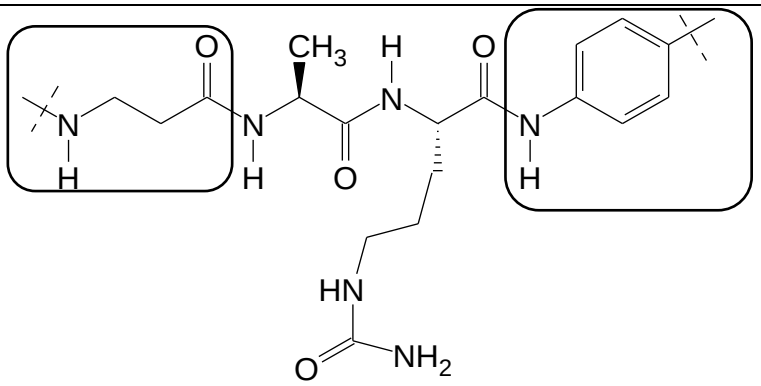
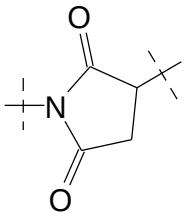
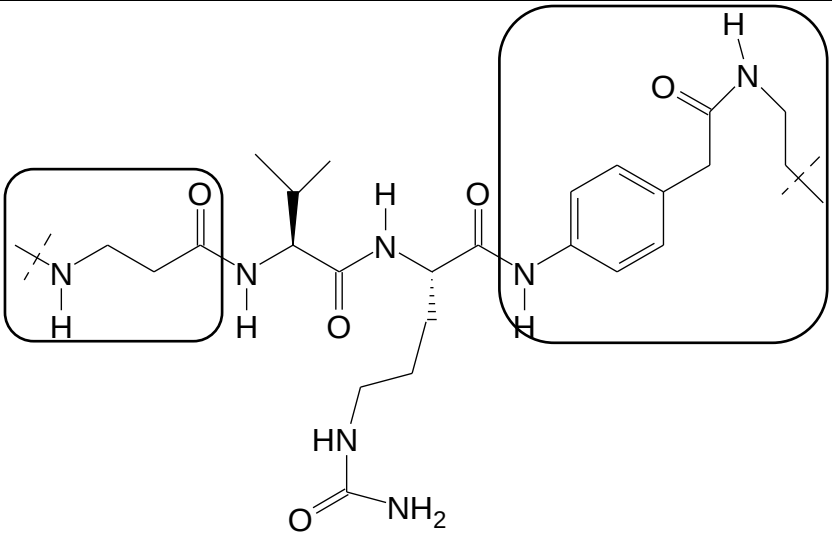
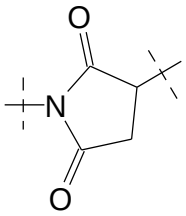
X is -H or a C_{1-10}-alkyl group which may optionally be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, or sulphonic acid.

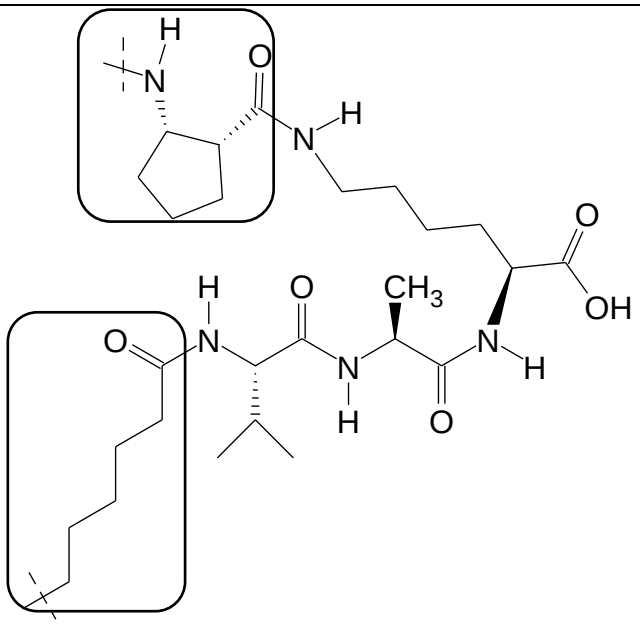
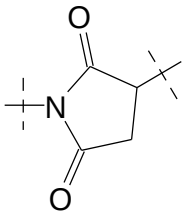
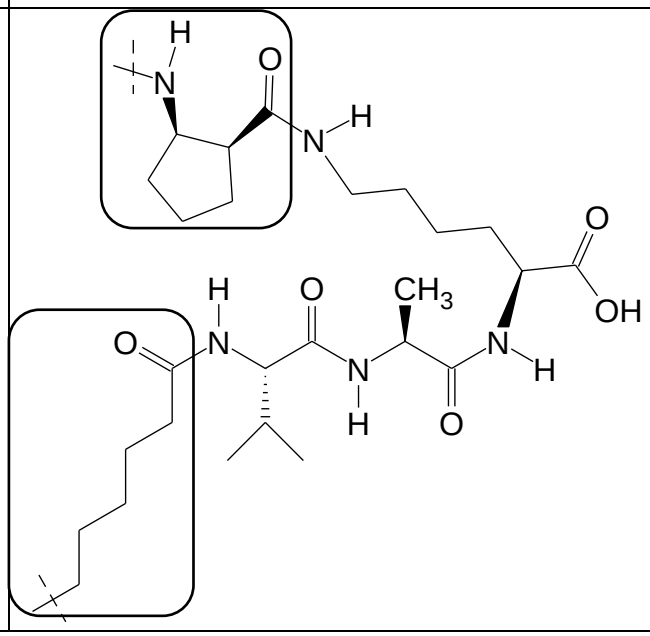
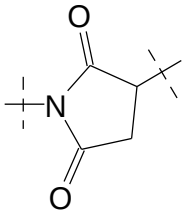
Table C below gives examples of a linker moiety –SG1-L1- or –L1-SG-L1-, where SG1/SG is a cathepsin-cleavable group. Table C also states the L2 group with which these examples of –SG1-L1- and –L1-SG-L1- are preferably combined, and also the preferred coupling site (R^1-R^5) and the preferred value for m, i.e. whether or not there is a carbonyl group before L1 (cf. §-(C(=O))m-L1-L2-§§). These linkers are preferably coupled to a cysteine residue. The L1 group is highlighted in a box. However, these L1 groups may be replaced by one of the L1 groups specified for the above formula §-(C(=O))m-L1-L2-§§. If L2 is a succinamide or derived therefrom, this amide may also be fully or partly in the form of the hydrolysed open-chain succinamide, as described above.

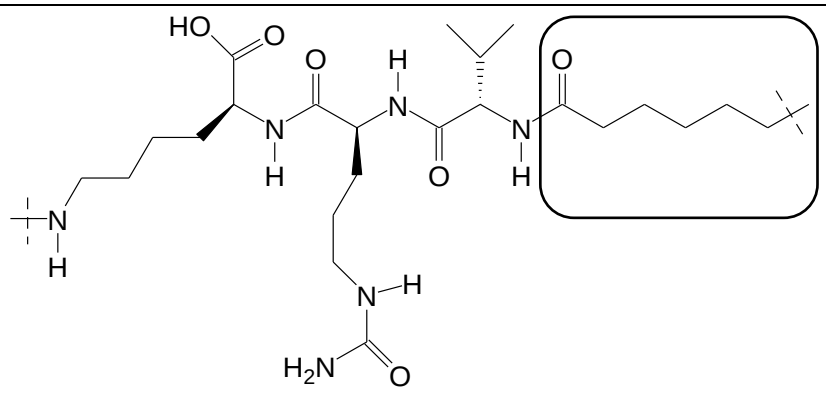
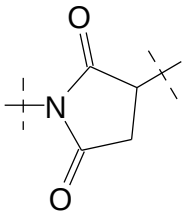
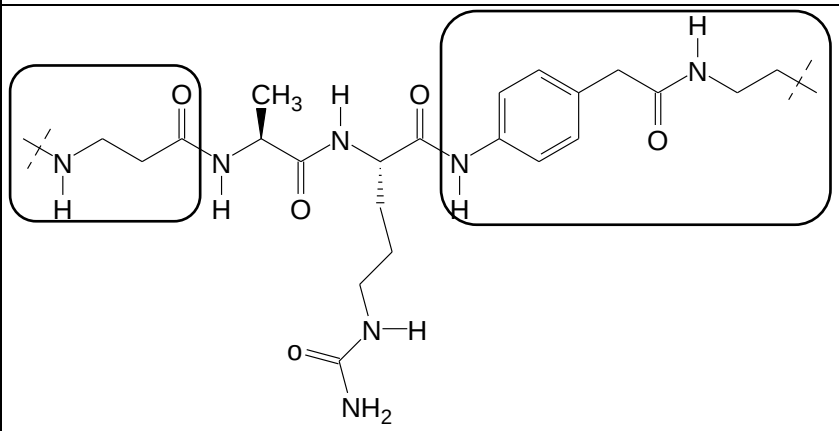
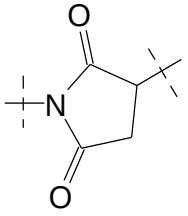
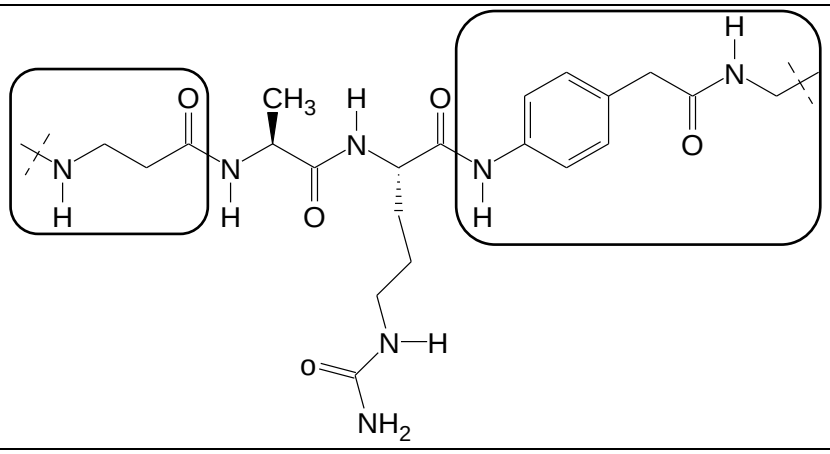
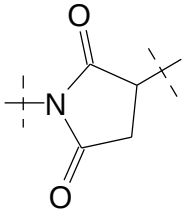
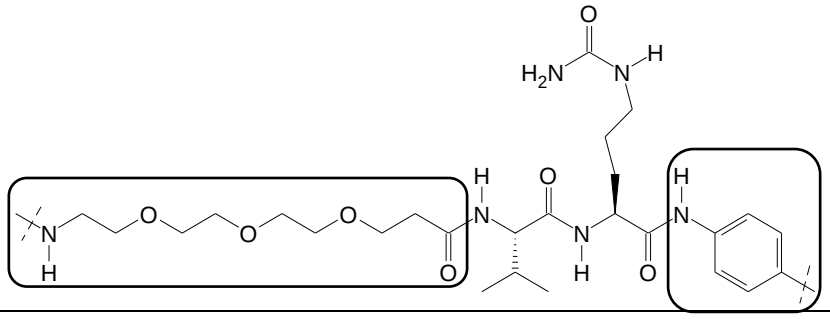
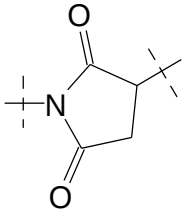
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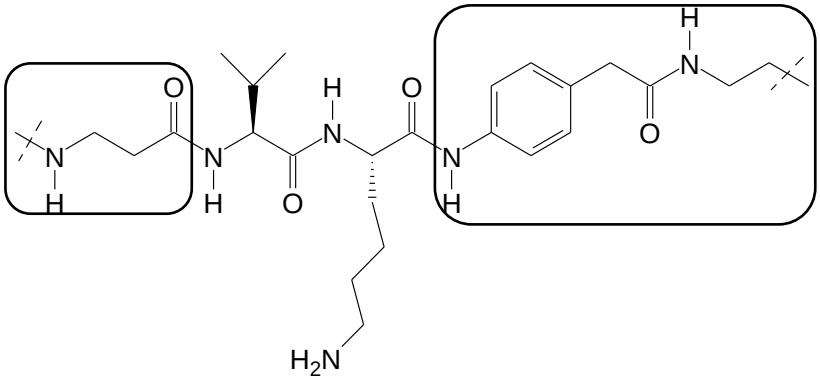
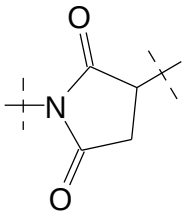
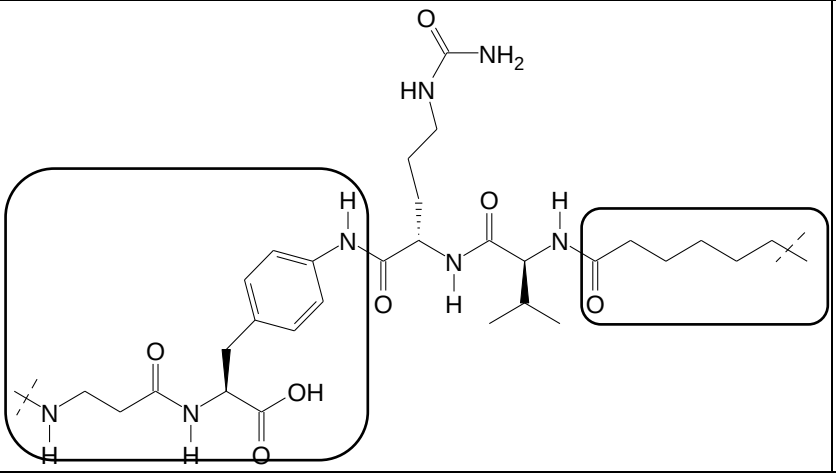
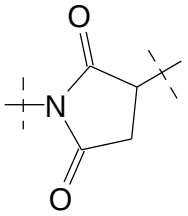
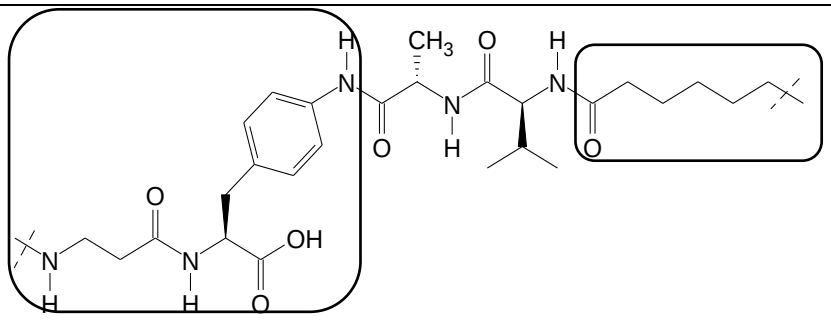
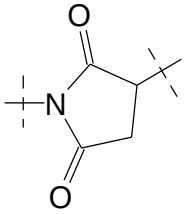
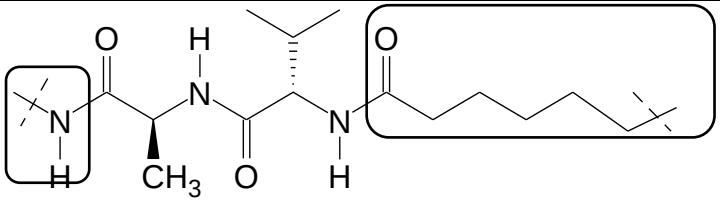
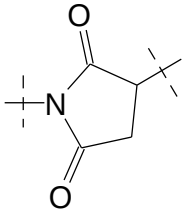
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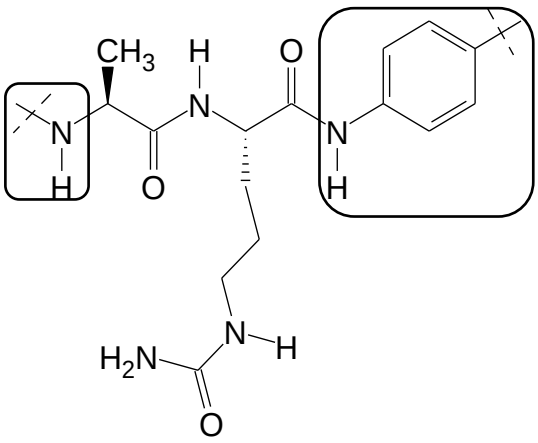
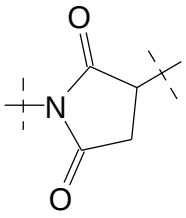
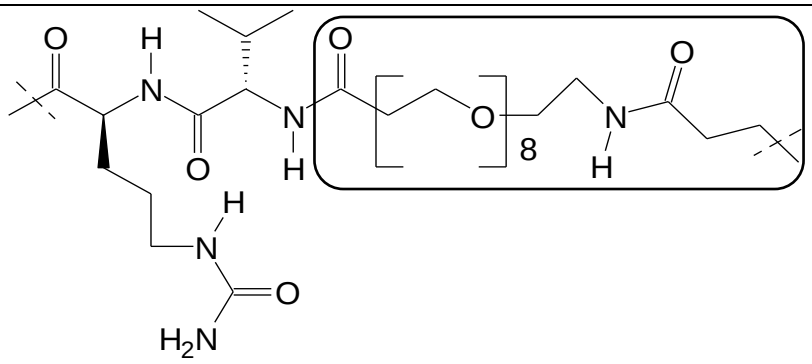
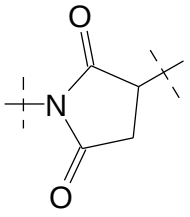
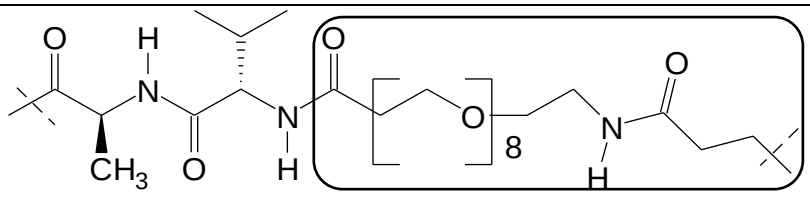
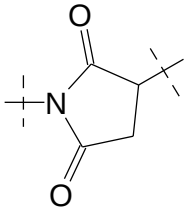
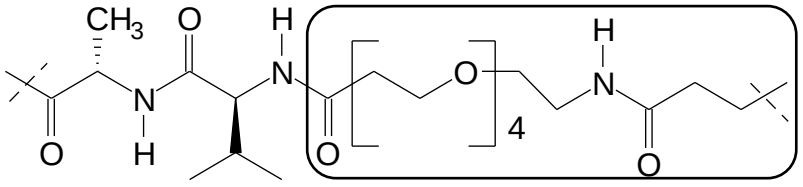
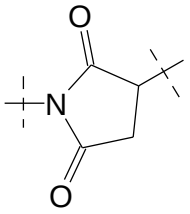
Subst.	**m**	**-SG1-L1- or –L1-SG-L1-**	**L2**
R^1	1		

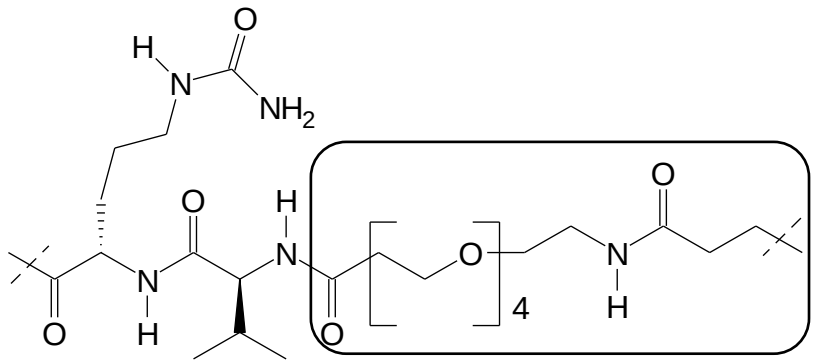
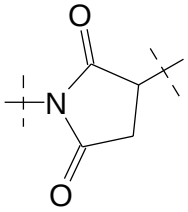
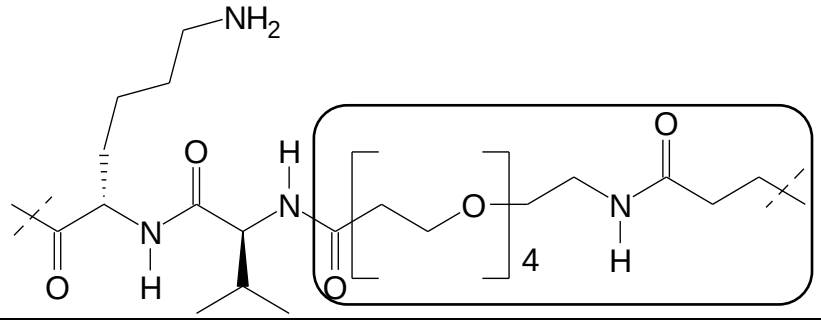
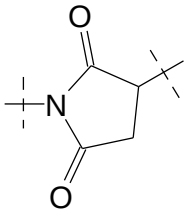
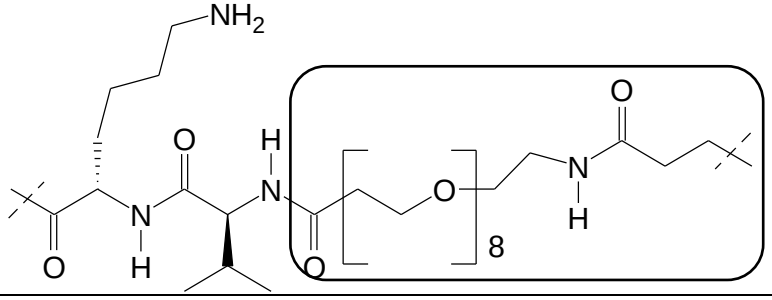
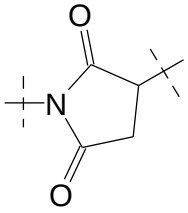
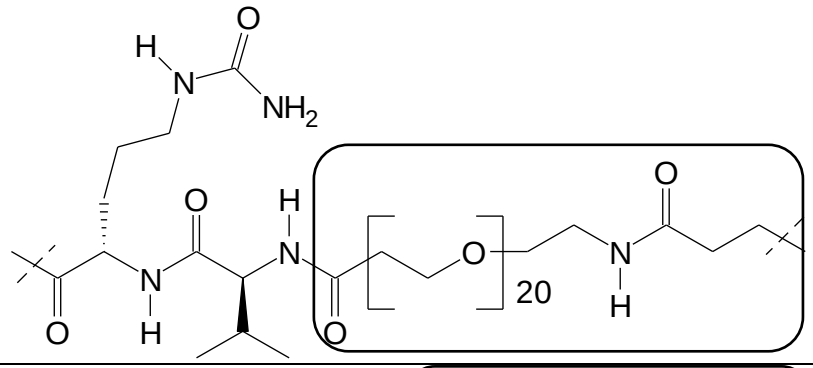
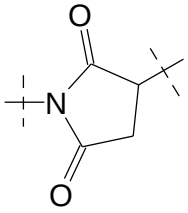
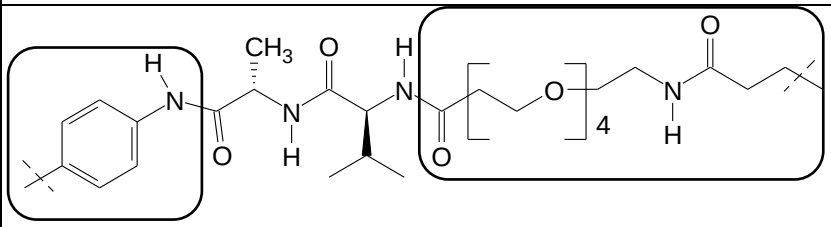
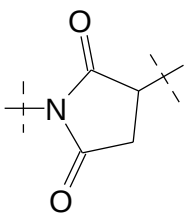
Subst.	m	-SG1-L1- or –L1-SG-L1-	L2
R^1	1		
R^1	1		
R^1	1		

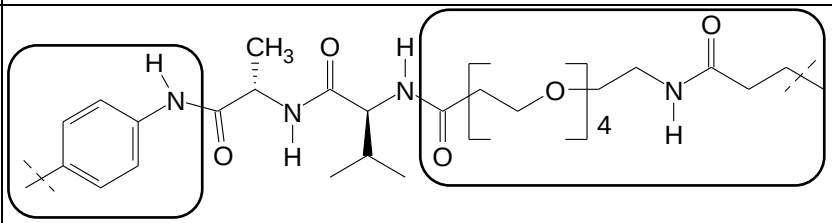
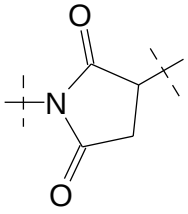
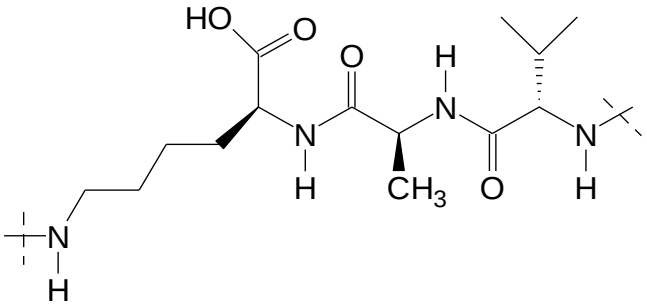
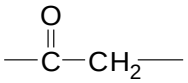
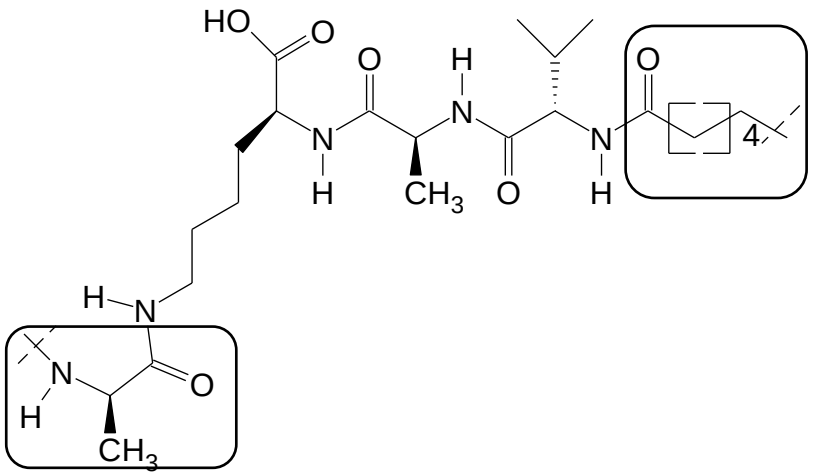
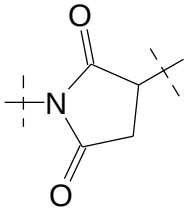
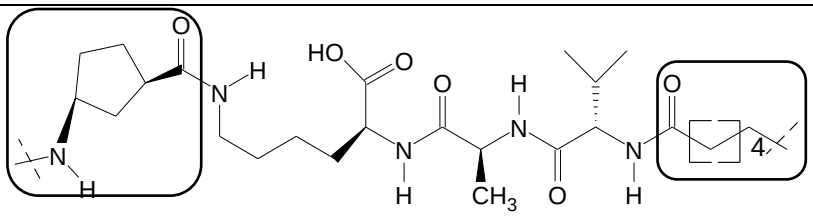
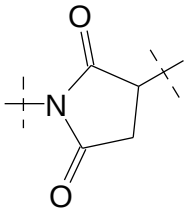
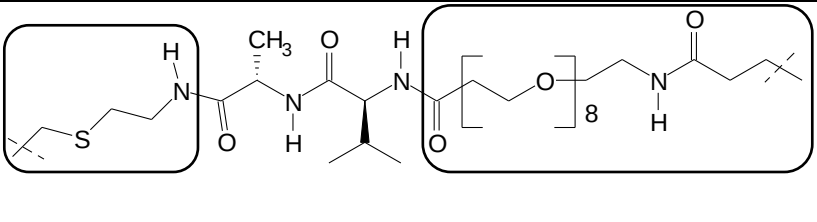
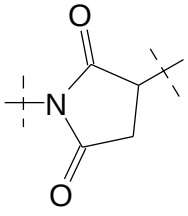
Subst.	m	-SG1-L1- or –L1-SG-L1-	L2
R^1	1		
R^1	1		

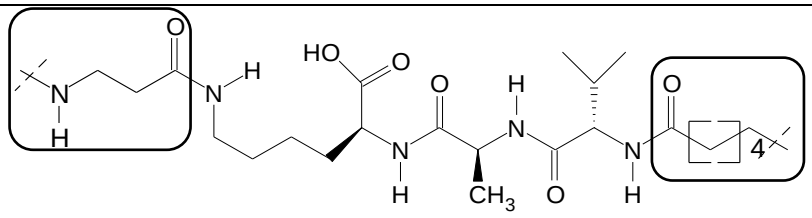
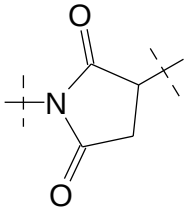
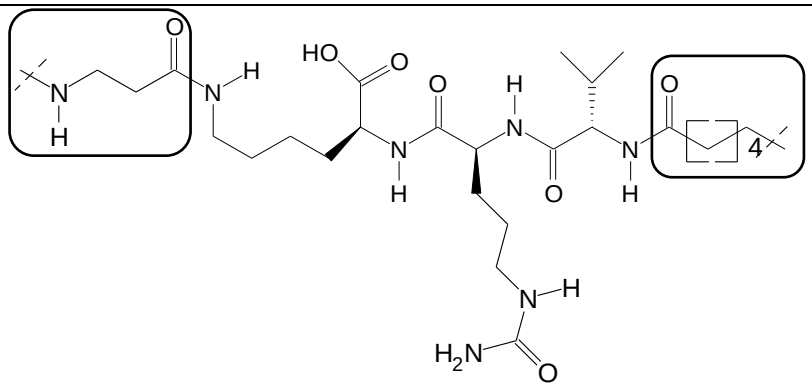
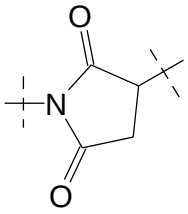
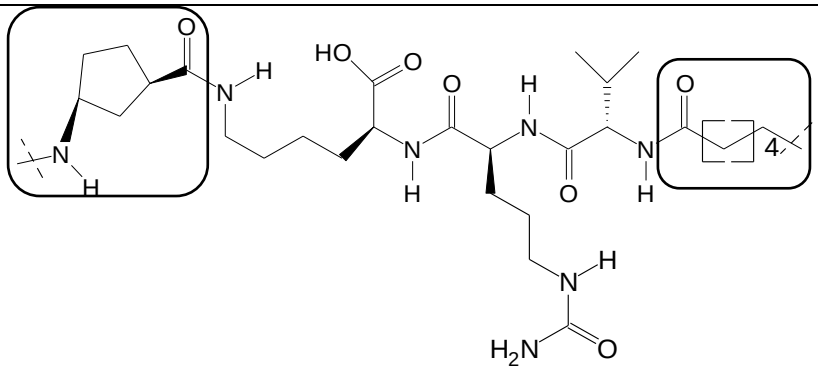
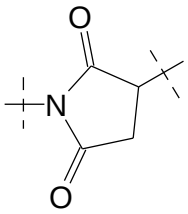
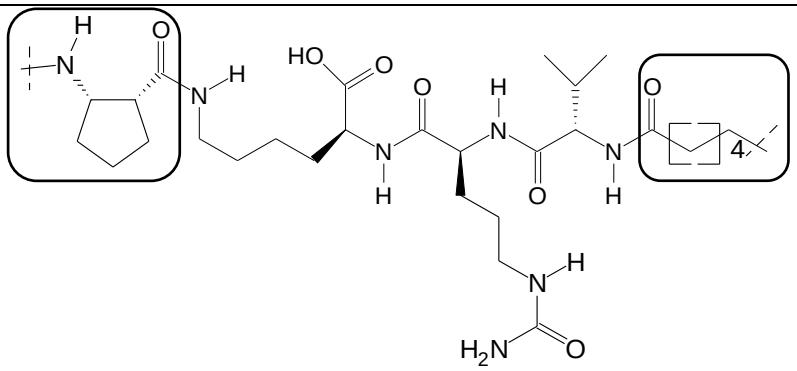
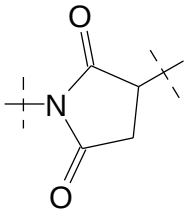
Subst.	m	-SG1-L1- or –L1-SG-L1-	L2
R^1	1		
R^1	1		
R^1	1		
R^1	1		

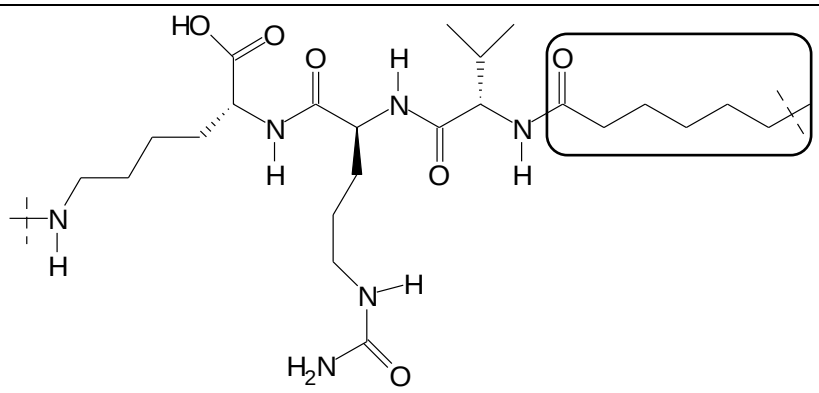
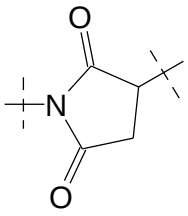
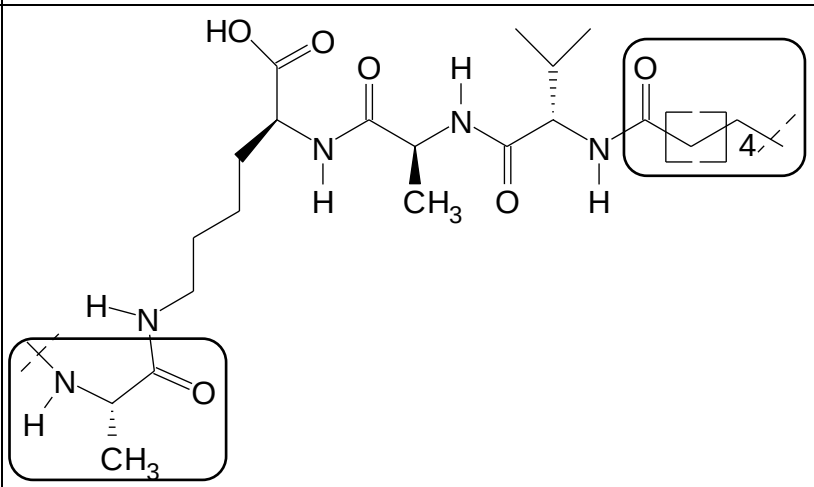
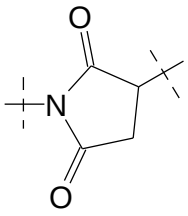
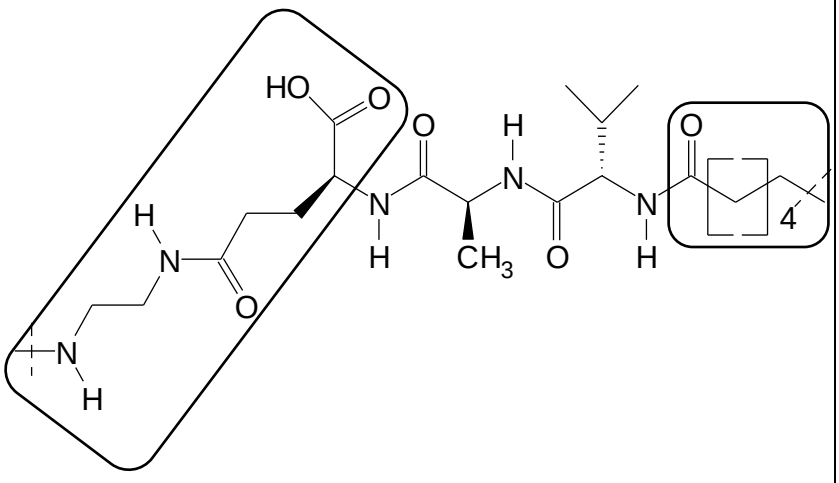
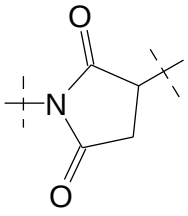
Subst.	m	-SG1-L1- or –L1-SG-L1-	L2
R^1	1		
R^1	1		
R^1	1		
R^1	0		

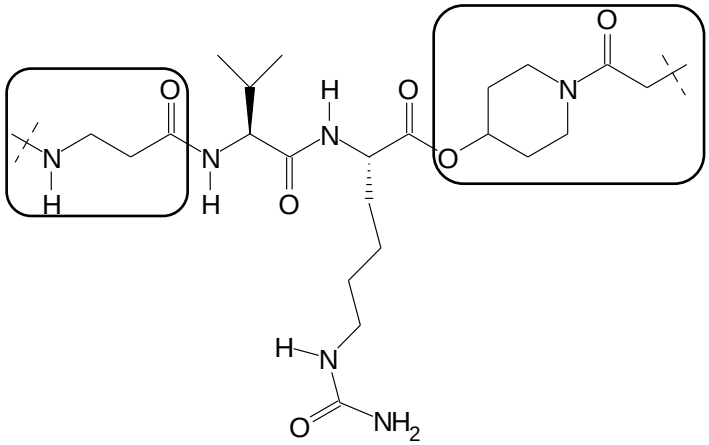
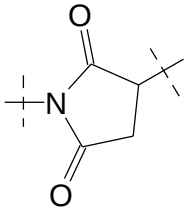
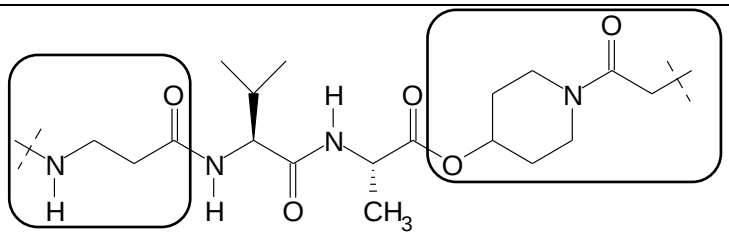
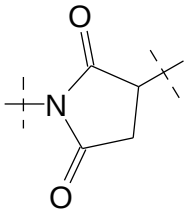
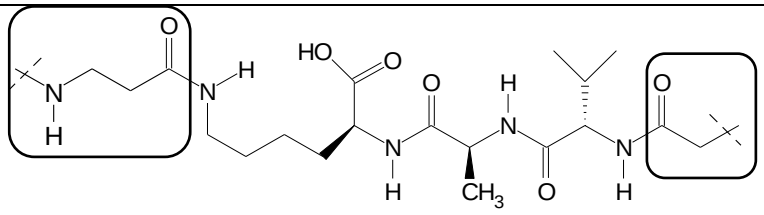
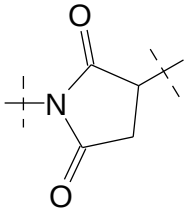
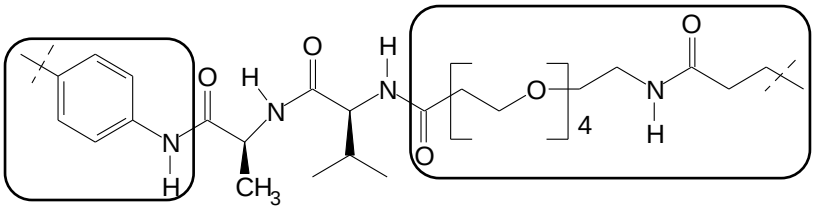
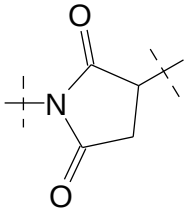
Subst.	m	-SG1-L1- or –L1-SG-L1-	L2
R^1	1		
R^1	0		
R^1	0		
R^1	0		

Subst.	m	-SG1-L1- or –L1-SG-L1-	L2
R^1	0		
R^1	0		
R^1	0		
R^1	0		
R^3	0		

Subst.	m	-SG1-L1- or –L1-SG-L1-	L2
R^3	0		
R^1	1		
R^1	1		
R^1	1		
R^3	0		

Subst.	m	-SG1-L1- or –L1-SG-L1-	L2
R^1	1		
R^1	1		
R^1	1		
R^1	1		

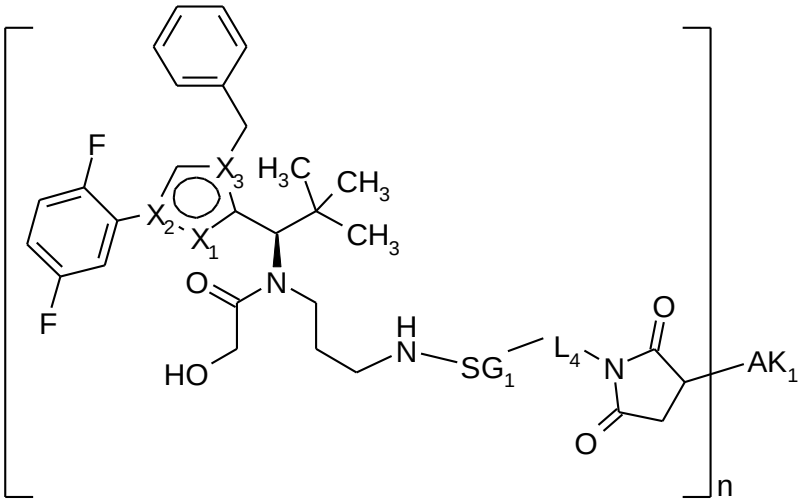
Subst.	m	-SG1-L1- or –L1-SG-L1-	L2
R^1	1		
R^1	1		
R^1	1		

Subst.	m	-SG1-L1- or –L1-SG-L1-	L2
R^1	1		
R^1	1		
R^1	1		
R^3	0		

Examples of conjugates having the base structure (i) of the linker

(i) $–(C=O)_m–$SG1-L1-L2-

in which m, SG1, L1 and L2 have the definitions given in table C have one of the following structures:



in which

X1 is CH,

X2 is C,

X3 is N,

L4 has the same definition as specified above in table C for L1,

AK1 is an anti-TWEAKR antibody, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment of these which is bonded via a cysteine residue,

and n is a number from 1 to 20, and the hydrogen atom in position R4 according to formula IIa (i.e. in the $–NH_2$ group) is replaced by the legumain-cleavable group of the formula Ia used in accordance with the invention.

More preferably, AK1 is an antibody or an antigen-binding antibody. The antibody is preferably a human, humanized or chimeric monoclonal antibody or an antigen-binding fragment thereof, especially an anti-TWEAKR antibody, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment thereof. Particular preference is given to the anti-TWEAKR antibodies TPP-7006, TPP-7007,TPP-10336 and TPP-10337, the anti-B7H3 antibodies TPP-8382 and TPP-8567, the anti-EGFR-antibody cetuximab (TPP-981) and the anti-HER2-antibodies trastuzumab and TPP-1015, or an antigen-binding fragment of these.

In the case of transglutaminase-catalysed conjugation, the literature discloses various options for the covalent coupling (conjugation) of organic molecules to binders, for

example antibodies, in a conjugation site-specific manner (see, for example Sochaj et al., *Biotechnology Advances,* 33, 775-784, (2015), Panowski et al., *MAbs* 6, 34-45 (2014)). Preference is given in accordance with the invention to the conjugation of the KSP inhibitors or prodrugs to an antibody via acceptor glutamine residues of the antibody using transglutaminase. Such acceptor glutamine residues can be generated by engineering of the antibody or by mutations which create aglycosylated antibodies. The number of these acceptor glutamines in the antibody is preferably 2 or 4. Suitable linkers are used for the coupling (conjugation). Suitable linker structures are those which possess a free amine donor functionality which constitutes a suitable substrate for the transglutaminase. The linker can be joined to the antibody in various ways.

Preferably, in the case of transglutaminase-catalysed conjugation, the linker has one of the base structures (i) to (iv) already mentioned above

(i) $–(C=O)_m–SG1-L1-L2-$

(ii) $–(C=O)_m –L1-SG-L1-L2-$

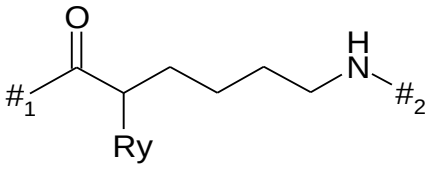
(iii) $–(C=O)_m –L1-L2-$

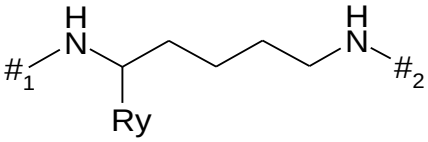
(iv) $–(C=O)_m –L1-SG-L2$

in which

L1, SG, SG1 and m have the definitions given above,

L2 preferably, however, represents one of the following groups:





in which

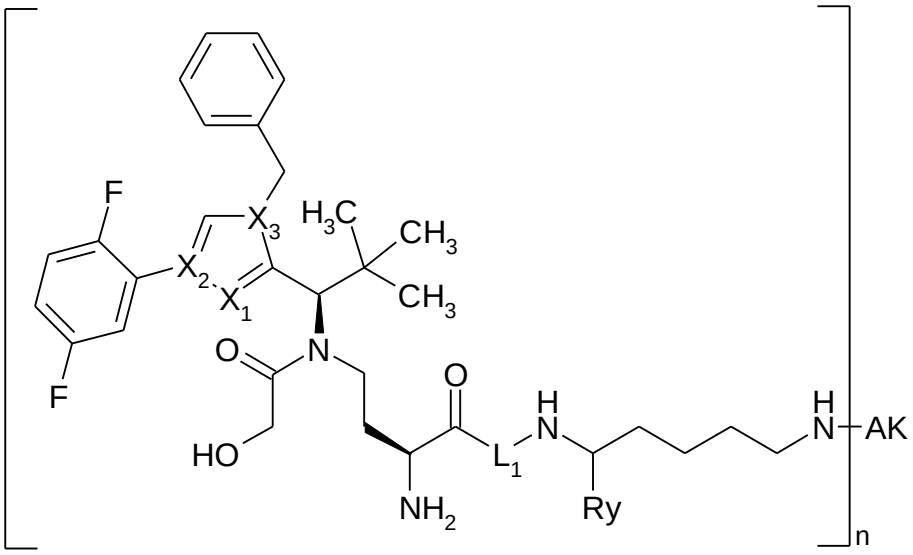
Ry is -H, -C(=O)-NH-alkyl, -NH-C(=O)-alkyl, $-C(=O)-NH_2$ or $-NH_2$,

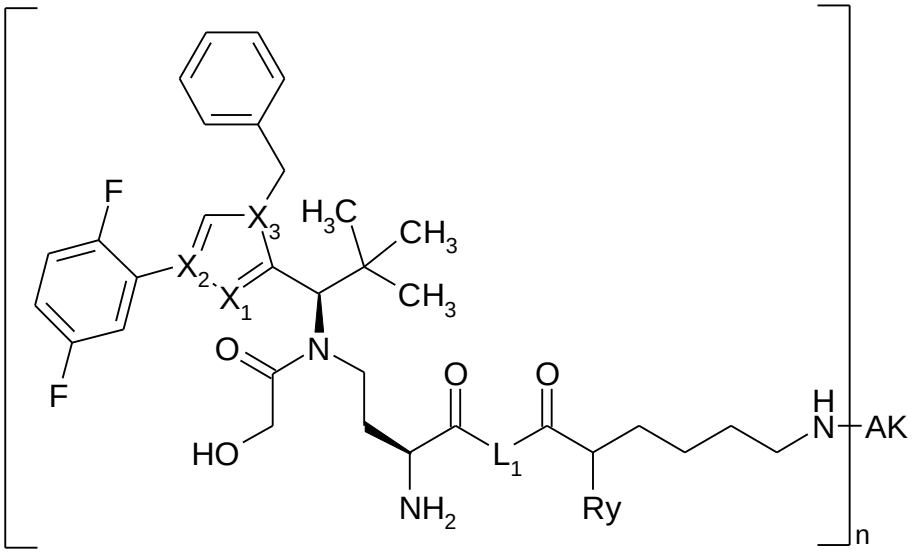
$\#^1$ is the linkage point to L^1 and

$\#^2$ is the linkage point to the glutamine residue of the binder.

Preferably in this context, Ry is -H or $–NH-C(=O)-CH_3$.

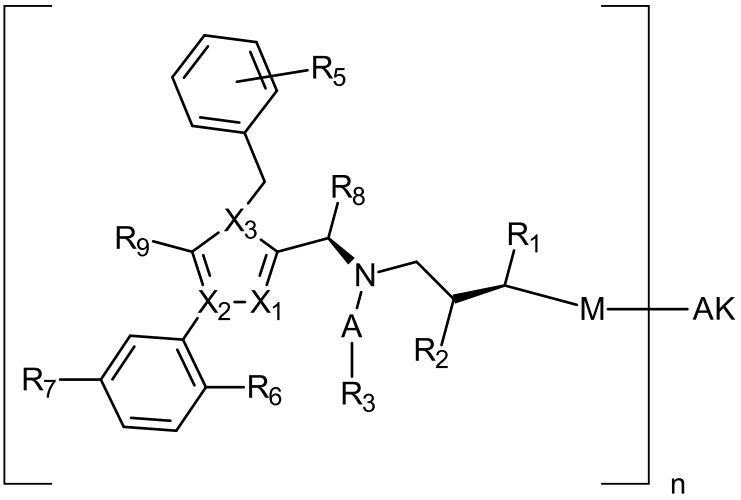
Examples of corresponding conjugates have the following structures, where X1, X2, X3, Ry and L1 have the definitions given above, AK is a binder which is preferably an antibody, and n is preferably 2 or 4.





Particularly preferred KSP inhibitor conjugates

Preference is also given to conjugates composed of a binder or a derivative thereof (preferably an antibody) with a compound of the formula (X)



(X)

in which

AK		is the binder (preferably the antibody) AK_1, AK_2, AK_3, or a derivative thereof,
n		is a number from 1 to 50, preferably 1 to 20, more preferably 2 to 8 and especially 2 to 6,
X_1		is N,
X_2		is N and
X_3		is C;
or		
X_1		is N,
X_2		is C and
X_3		is N;
or		
X_1		is CH or CF,
X_2		is C and
X_3		is N;
or		
X_1		is NH,
X_2		is C and
X_3		is C;

or

X_1 is CH,

X_2 is N and

X_3 is C.

A is –C(=O)-, -S(=O)-, -S(=O)$_2$- or -S(=O)$_2$-NH-,

M is the group

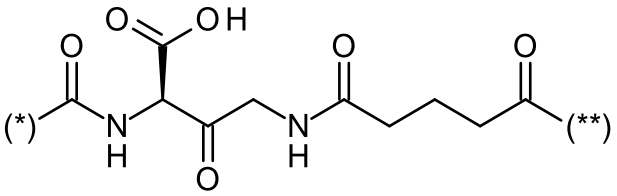
(*)-C(=O)-NH-(CH$_2$)$_3$-C(=O)-(**),

(*)-C(=O)-NH-(CH$_2$)$_2$-NH-C(=O)-(CH$_2$)$_3$-C(=O)-(**),

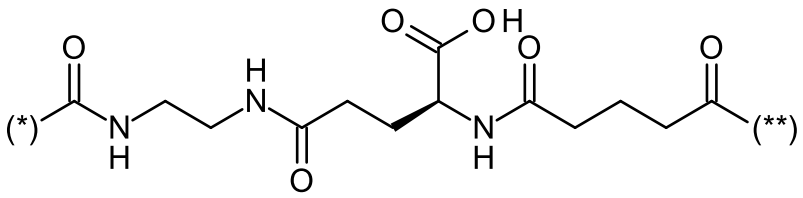
(*)-C(=O)-NH-(CH$_2$)$_2$-NH-C(=O)-CH$_2$-NH-C(=O)-(CH$_2$)$_3$-C(=O)-(**),

(*)-NH-C(=O)-CH(CH$_2$-CH$_2$-COOH)-NH-C(=O)-CH(CH$_3$)-NH-C(=O)-CH(CH$_3$)-NH-C(=O)-(CH$_2$)$_3$-C(=O)-(**),

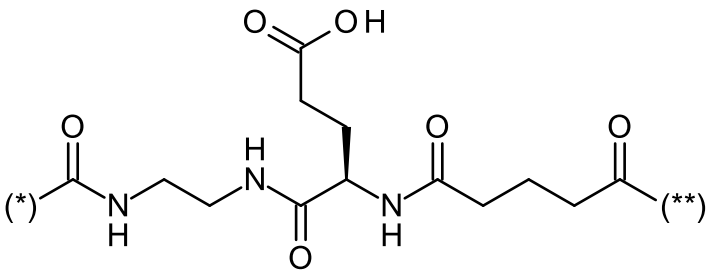
(*)-NH-C(=O)-CH(CH$_2$-C(=O)-NH$_2$)-NH-C(=O)-CH(CH$_3$)-NH-C(=O)-CH(CH$_3$)-NH-C(=O)-(**),



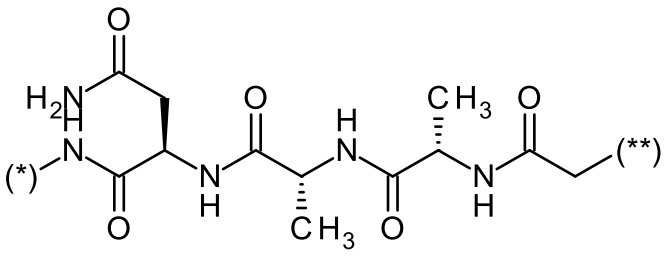
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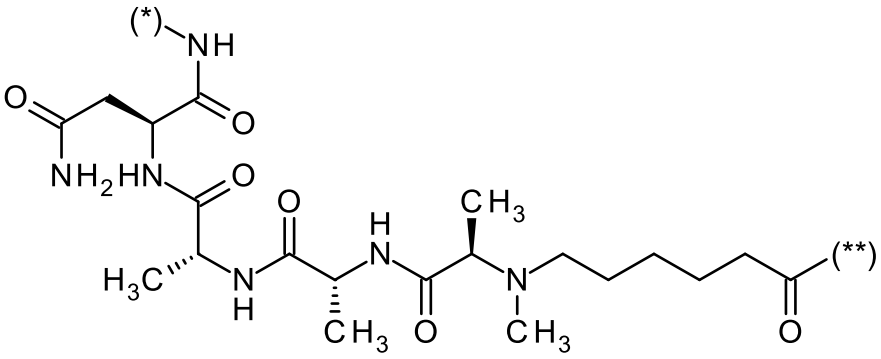
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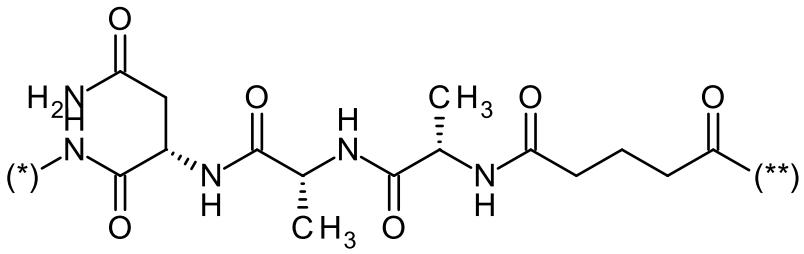
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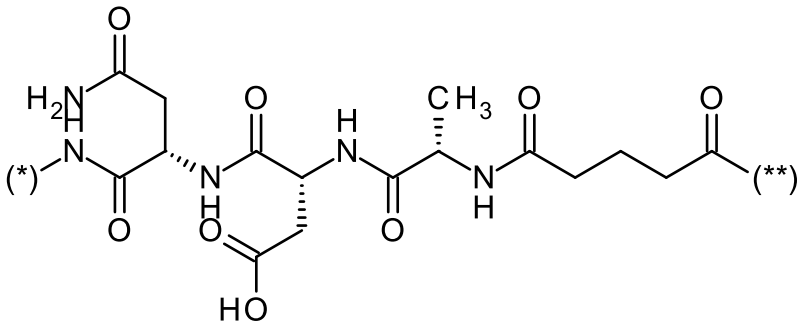
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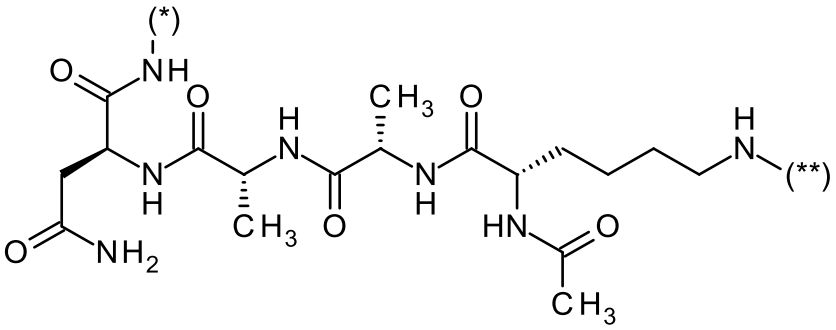
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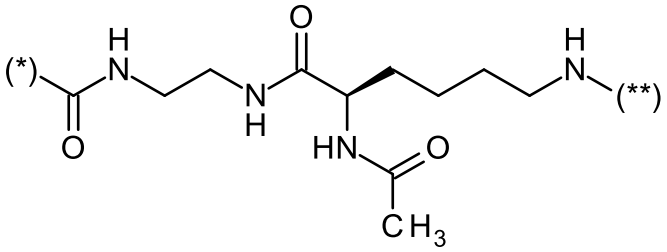
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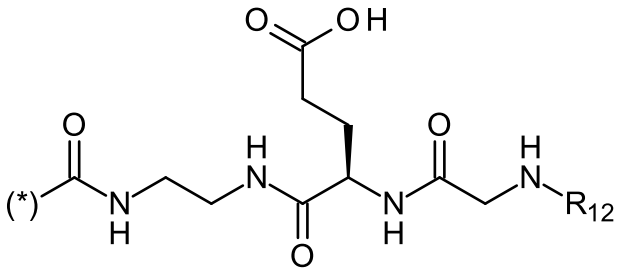


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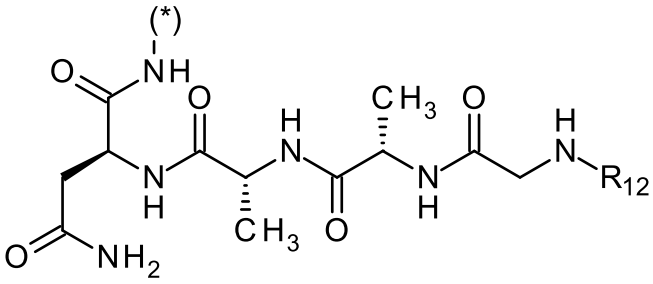
(*)-C(=O)-NH-$(CH_2)_2$-NH-C(=O)-CH_2-NH-R^{12},

(*)-NH-C(=O)-$CH(CH_3)$-NH-C(=O)-$CH(CH_3)$-NH-C(=O)-CH_2-NH-R^{12},

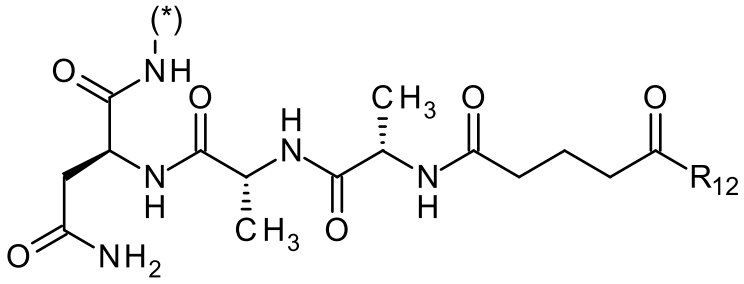
(*)-NH-C(=O)-$CH(CH_2$-C(=O)-$NH_2)$-NH-C(=O)-$CH(CH_3)$-NH-C(=O)-$CH(CH_3)$-NH-C(=O)-CH_2-C(=O)-NH-R^{12},



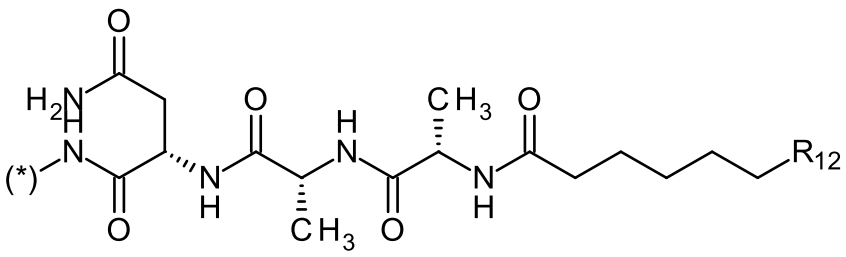
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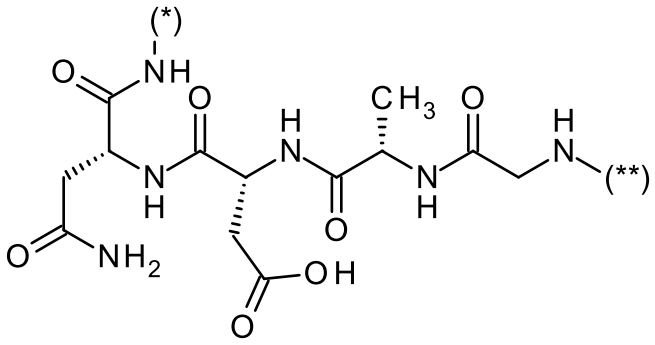
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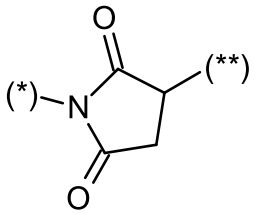


and

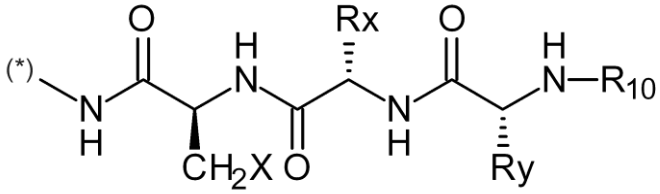


R^{12} is the group

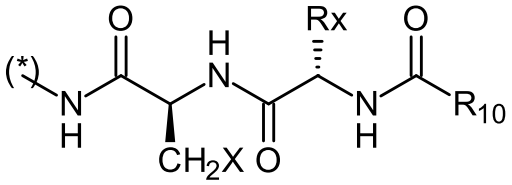
(*)-C(=O)-CH(**)-CH_2-COOH, (*)-C(=O)-CH_2-CH(**)-COOH or

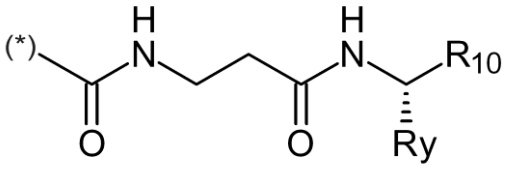


R^1 is hydrogen or the group

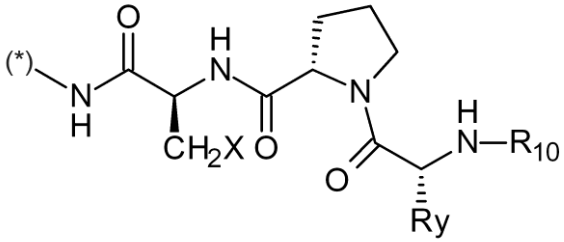


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	X	is –C(=O)-NH_2,
	Rx and Ry	are independently –CH_3, -CH_2-COOH, -$(CH_2)_2$-COOH, -CH_2-C(=O)-NH_2, -CH_2OH or –CH_2R^{11},
	R^{10}	is methyl, –$(C(=O))_q$-O-R^{11}, –C(=O)-$(CH_2)_m$-R^{11},
	q	is 0 or 1,
	m	is 0, 1 or 2,
	R^{11}	is hydrogen, methyl, benzyl, pyridyl, imidazolyl or the group
		–$(O\text{-}CH_2\text{-}CH_2)_p$-O-$CH_3$,
	p	is 1 to 11,
	R^2	is -H,

R^3	is an optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, heteroalkyl, heterocycloalkyl group which may be substituted by one to three OH groups, one to three halogen atoms, one to three mono-, di- or trihalogenated alkyl groups, one to three –O-alkyl groups, one to three -SH groups, one to three -S-alkyl groups, one to three -O-C(=O)-alkyl groups, one to three -O-C(=O)-NH-alkyl groups, one to three -NH-C(=O)-alkyl groups, one to three -NH-C(=O)-NH-alkyl groups, one to three -$S(=O)_n$-alkyl groups, one to three –$S(=O)_2$-NH-alkyl groups, 1-3 -NH-alkyl groups, one to three -$N(alkyl)_2$ groups, one to three NH_2 groups or one to three -$(CH_2)_{0-3}Z$ groups,
n	is 0, 1 or 2,
Z	is -H, halogen, -OY^3, -SY^3, -NHY^3, -C(=O)-NY^1Y^2 or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}Z'$,
Y^3	is -H, -$(CH_2)_{0-3}$-CH-($NHC(=OCH_3)Z'$, -$(CH_2)_{0-3}$-$CH(NH_2)Z'$ or -$(CH_2)_{0-3}Z'$,
Z'	is -H, -$S(=O)_3H$, -NH_2 or –COOH,
R^5	is -H, -NH_2, -NO_2, halogen, -CN, -CF_3, -OCF_3, -CH_2F, -CH_2F, -SH or -$(CH_2)_{0-3}Z$,
Z	is -H, -OY^3, -SY^3, halogen, -NHY^3, -C(=O)-NY^1Y^2, or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}Z'$,
Y^3	is -H or -$(CH_2)_{0-3}Z'$,
Z'	is -H, -$S(=O)_3H$, -NH_2 or –COOH,
R^6 and R^7	are independently -H, -CN, C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, hydroxyl, -NO_2, -NH_2, -COOH or halogen,
R^8	is C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, C_{4-10}-cycloalkyl, fluoro-C_{4-10}-cycloalkyl or -$(CH_2)_{0-2}$-(HZ^2),
HZ^2	is a 4- to 7-membered heterocycle having up to two heteroatoms selected from N, O and S, which may be substituted by –OH, –COOH or -NH_2,

R^9	is -H, -F, $-CH_3$, $-CF_3$, $-CH_2F$ or $-CHF_2$,
(*)	is the bond to the drug molecule or the legumain-cleavable group,
(**)	is the bond to the binder,

and the salts, solvates and salts of the solvates thereof.

Of interest among these are those conjugates of the formula (X) in which

AK	is the binder, preferably an antibody or antigen-binding fragment,
n	is a number from 1 to 50, preferably 1 to 20, more preferably 2 to 8 and especially 2 to 6,
X_1	is CH,
X_2	is C and
X_3	is N;
A	is –C(=O)-,
M	is the group

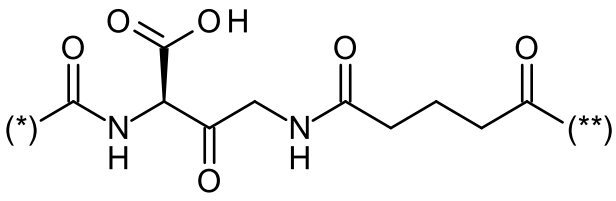
(*)-C(=O)-NH-$(CH_2)_3$-C(=O)-(**),

(*)-C(=O)-NH-$(CH_2)_2$-NH-C(=O)-$(CH_2)_3$-C(=O)-(**),

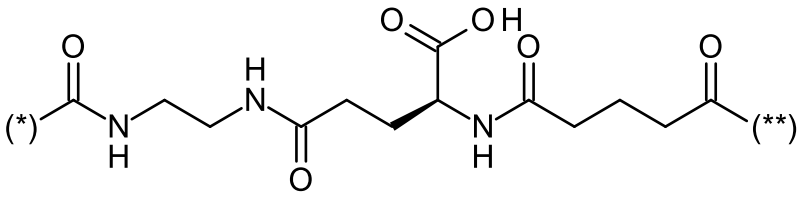
(*)-C(=O)-NH-$(CH_2)_2$-NH-C(=O)-CH_2-NH-C(=O)-$(CH_2)_3$-C(=O)-(**),

(*)-NH-C(=O)-CH(CH_2-CH_2-COOH)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-$(CH_2)_3$-C(=O)-(**),

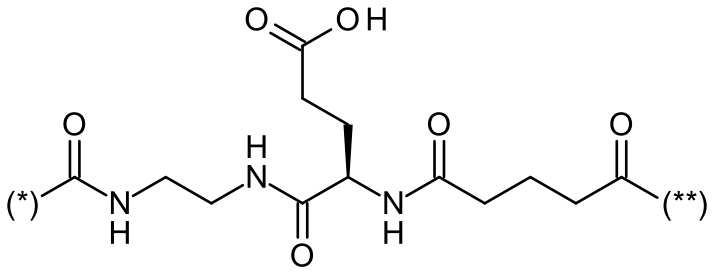
(*)-NH-C(=O)-CH(CH_2-C(=O)-NH_2)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-(**),



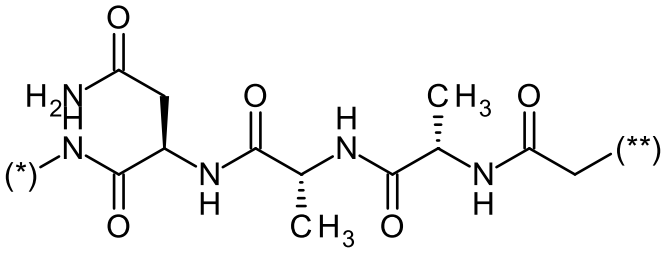
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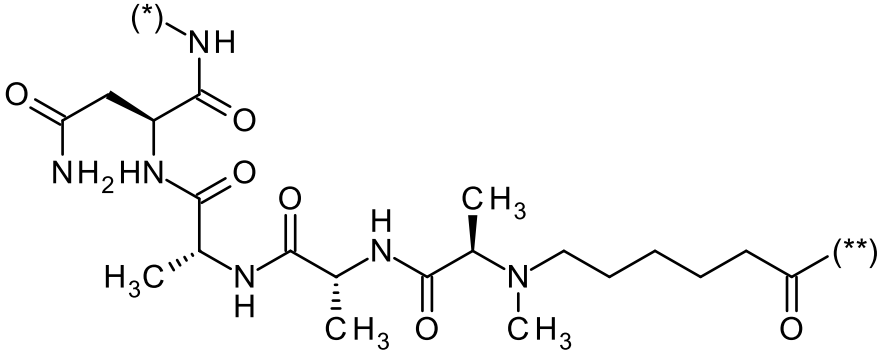
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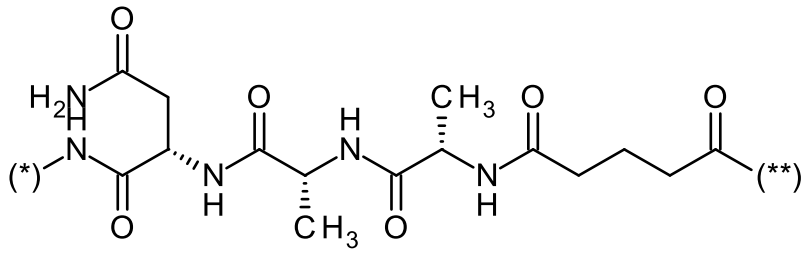
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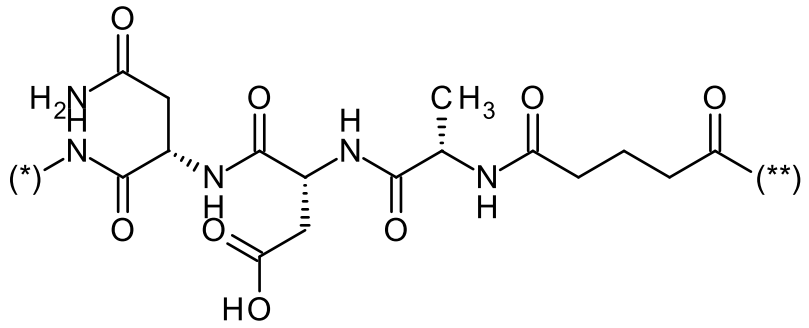
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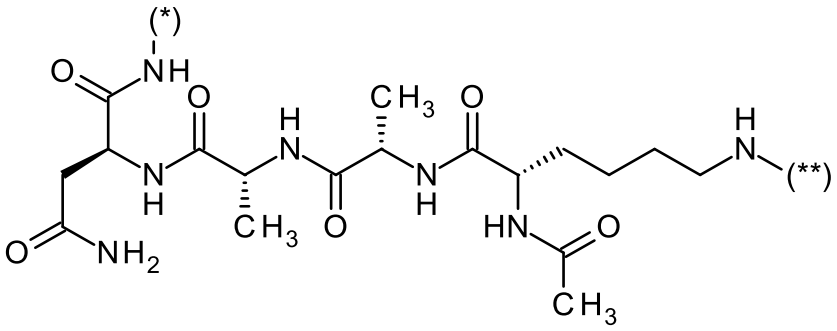
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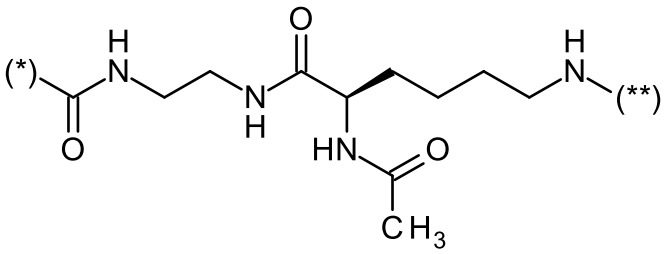
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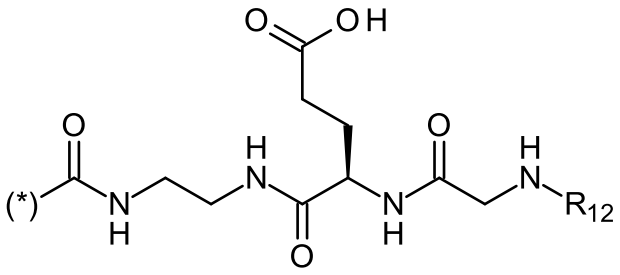


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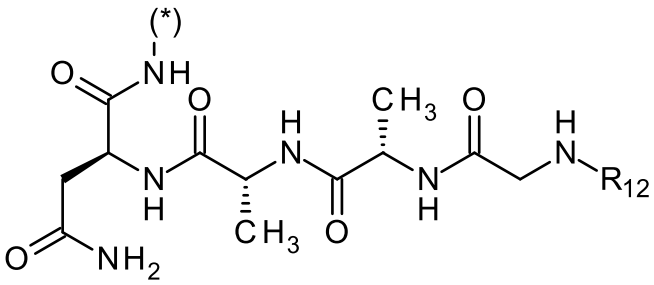
(*)-C(=O)-NH-$(CH_2)_2$-NH-C(=O)-CH_2-NH-R^{12},

(*)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH_2-NH-R^{12},

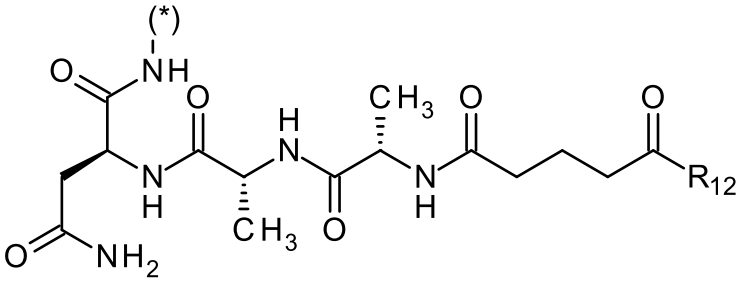
(*)-NH-C(=O)-CH(CH_2-C(=O)-NH_2)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH_2-C(=O)-NH-R^{12},



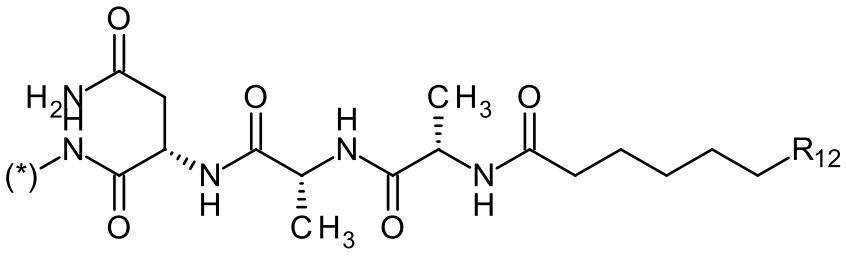
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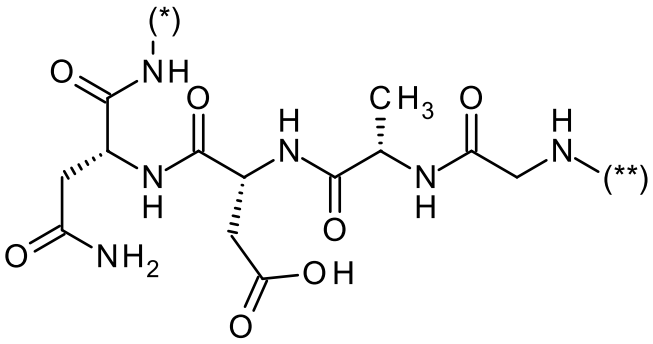
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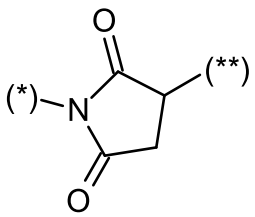
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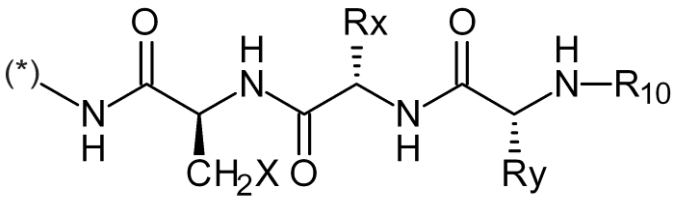
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R^{12} is the group

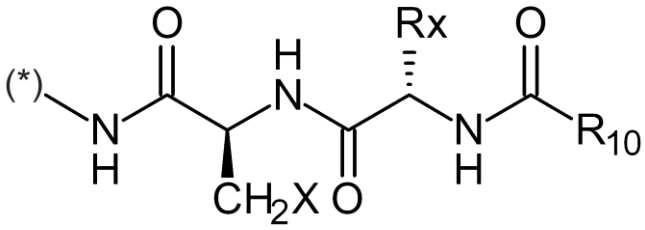
(*)-C(=O)-CH(**)-CH_2-COOH, (*)-C(=O)-CH_2-CH(**)-COOH or



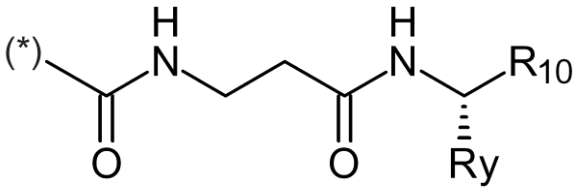
R^1 is hydrogen or the group



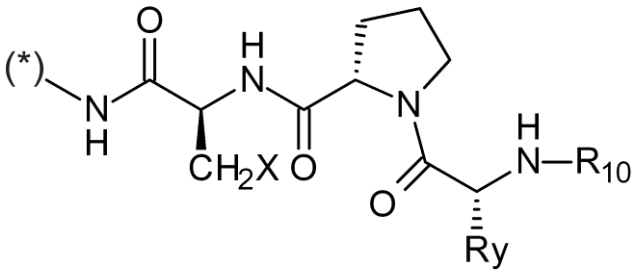
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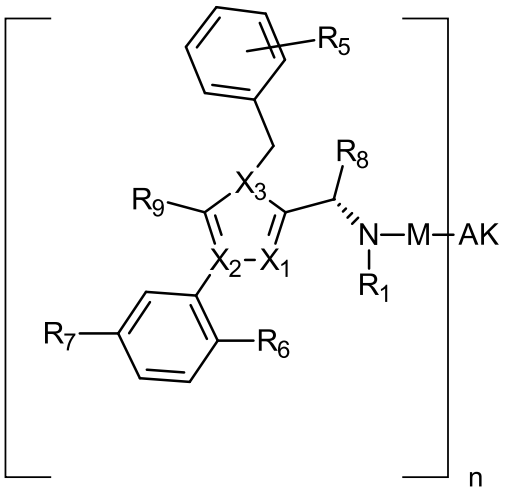


X is $–C(=O)-NH_2$,

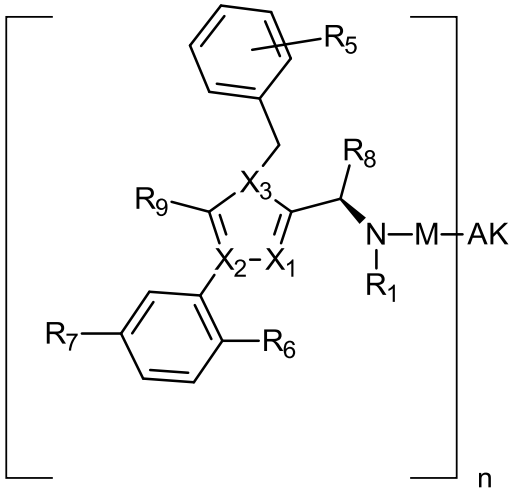
Rx and Ry	are independently $–CH_3$, $-CH_2-COOH$, $-(CH_2)_2-COOH$, $-CH_2-C(=O)-NH_2$, $-CH_2OH$ or $–CH_2R^{11}$,
R^{10}	is methyl, $–(C(=O))_q-O-R^{11}$, $–C(=O)-(CH_2)_m-R^{11}$,
q	is 0 or 1,
m	is 0, 1 or 2,
R^{11}	is hydrogen, methyl, benzyl, pyridyl, imidazolyl or the group $–(O-CH_2-CH_2)_p-O-CH_3$,
p	is 1 to 11,
R^2	is -H,
R^3	is a $–CH_2-OH$ group,
R^5	is –H,
R^6 and R^7	are independently fluorine,
R^8	is t-butyl,
R^9	is -H,
(*)	is the bond to the drug molecule or the legumain-cleavable group,
(**)	is the bond to the binder,

and the salts, solvates and salts of the solvates thereof.

Also of interest are those conjugates composed of a binder or a derivative thereof (preferably an antibody) with a compound of the formulae (XI) and (XI')



(XI)



(XI'')

in which

AK		is the binder, preferably an antibody or antigen-binding fragment, and
n		is a number from 1 to 50, preferably 1 to 20, more preferably 2 to 8 and especially 2 to 6,
X_1		is N,
X_2		is N and
X_3		is C;
or		
X_1		is N,
X_2		is C and
X_3		is N;
or		
X_1		is CH or CF,
X_2		is C and
X_3		is N;
or		
X_1		is NH,
X_2		is C and
X_3		is C;

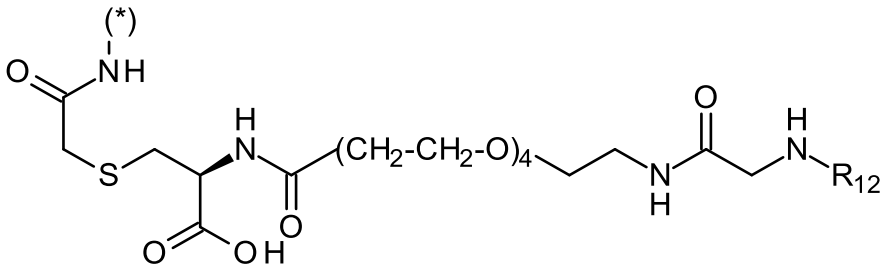
or

X_1 is CH,

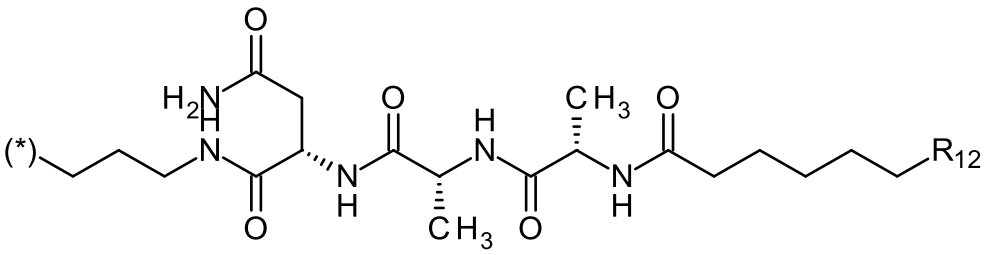
X_2 is N and

X_3 is C.

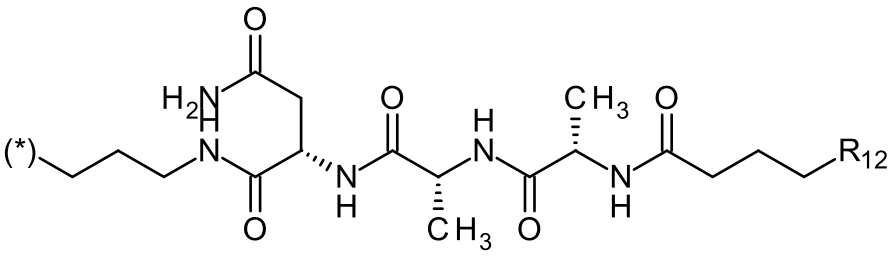
M is the group



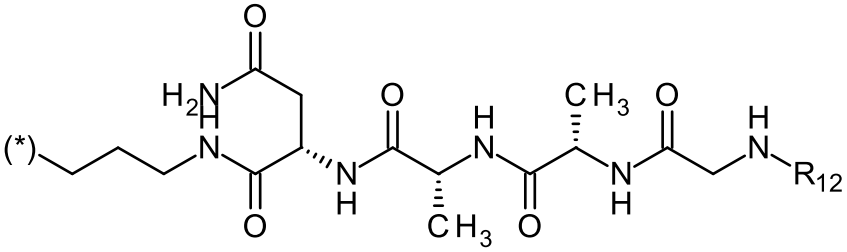
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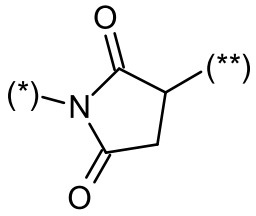


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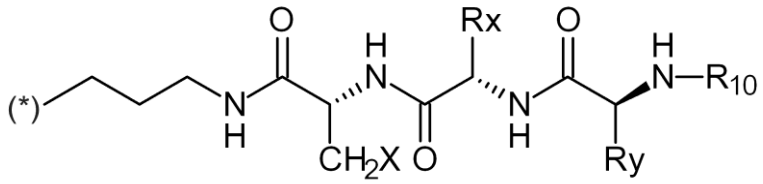
$-(CH_2)_3-NH-C(=O)-CH(CH_2-C(=O)-NH_2)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-(CH_2)_3-R^{12}$

R^{12} is the group

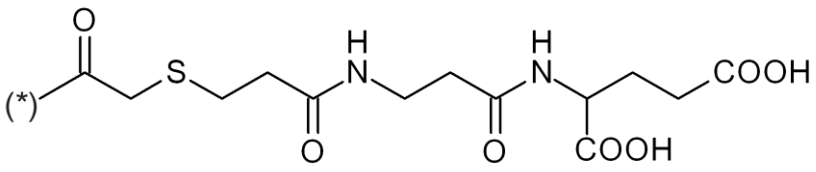
(*)-C(=O)-(**), (*)-C(=O)-CH(**)-CH_2-COOH, (*)-C(=O)-CH_2-CH(**)-COOH



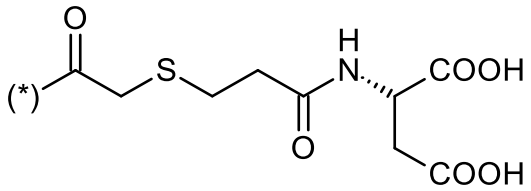
R^1 is the group



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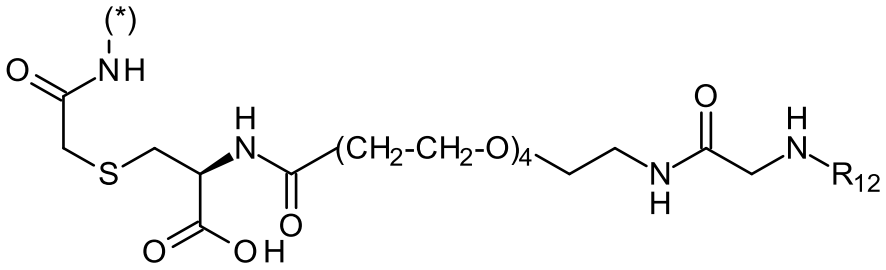


	X	is $–CH_2-C(=O)-NH_2$, $–C(=O)-NH_2$,
	Rx and Ry	are $–CH_3$, propyl,
	R^{10}	is $–C(=O)-(CH_2)-R^{11}$, $–C(=O)-(CH_2-CH_2-O)_p-CH_3$,
	R^{11}	is hydrogen, pyridyl,
	p	is 8 to 12,
	R^5	is –H,
	R^6 and R^7	are independently fluorine,
	R^8	is t-butyl,
	R^9	is -H,
	(*)	is the bond to the drug molecule or the legumain-cleavable group,
	(**)	is the bond to the binder,

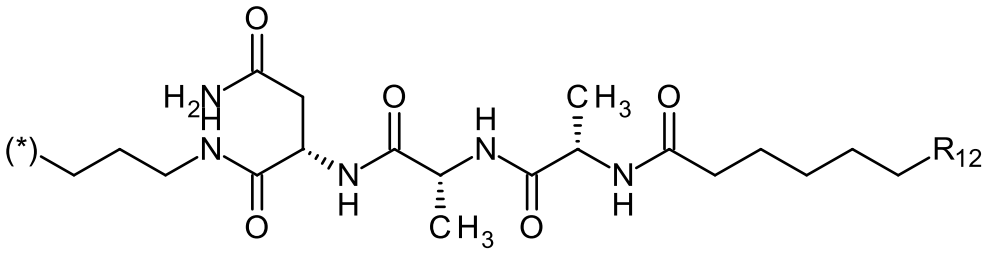
and the salts, solvates and salts of the solvates thereof.

Of interest among these are those conjugates of the formulae (XI) and (XI') in which

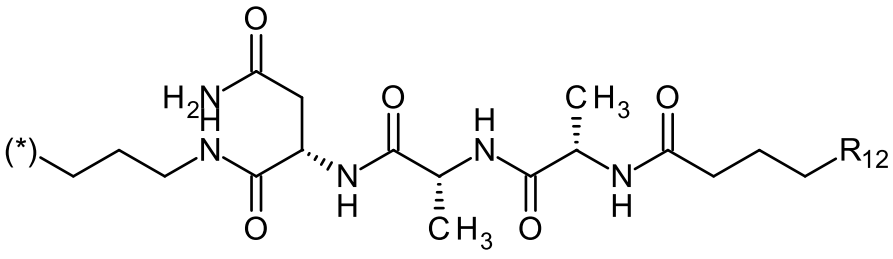
	AK	is the binder, preferably an antibody or antigen-binding fragment,
	n	is a number from 1 to 50, preferably 1 to 20, more preferably 2 to 8 and especially 2 to 6,
	X_1	is CH,
	X_2	is C and
	X_3	is N;
	M	is the group



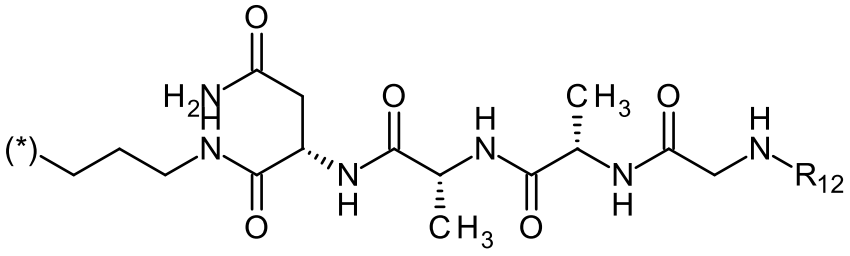
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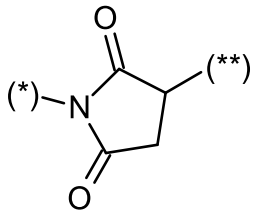


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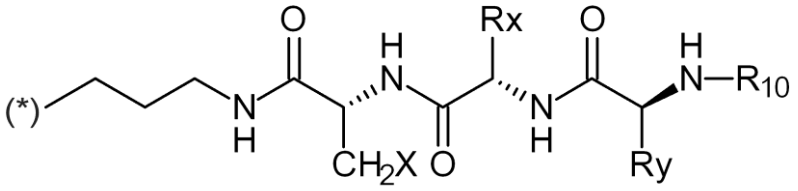
$-(CH_2)_3-NH-C(=O)-CH(CH_2-C(=O)-NH_2)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-(CH_2)_3-R^{12}$

R^{12} is the group

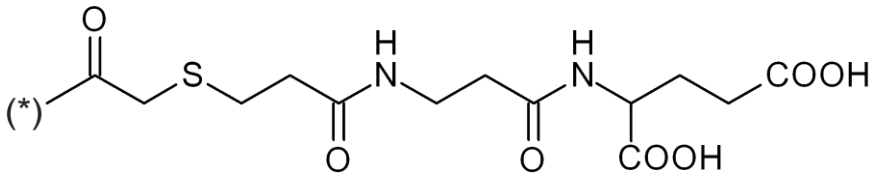
(*)-C(=O)-(**), (*)-C(=O)-CH(**)-CH_2-COOH,
(*)-C(=O)-CH_2-CH(**)-COOH



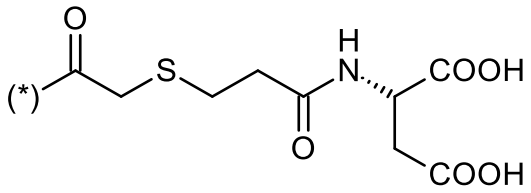
R^1 is the group



,



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	X	is $–CH_2$-C(=O)-NH_2, –C(=O)-NH_2,
	Rx and Ry	are $–CH_3$, propyl,
	R^{10}	is –C(=O)-(CH_2)-R^{11}, –C(=O)-$(CH_2$-CH_2-$O)_p$-CH_3,
	R^{11}	is hydrogen, pyridyl,
	p	is 8 to 12,
	R^5	is –H,
	R^6 and R^7	are independently fluorine,
	R^8	is t-butyl,
	R^9	is -H,
	(*)	is the bond to the drug molecule or the legumain-cleavable group,
	(**)	is the bond to the binder,

and the salts, solvates and salts of the solvates thereof.

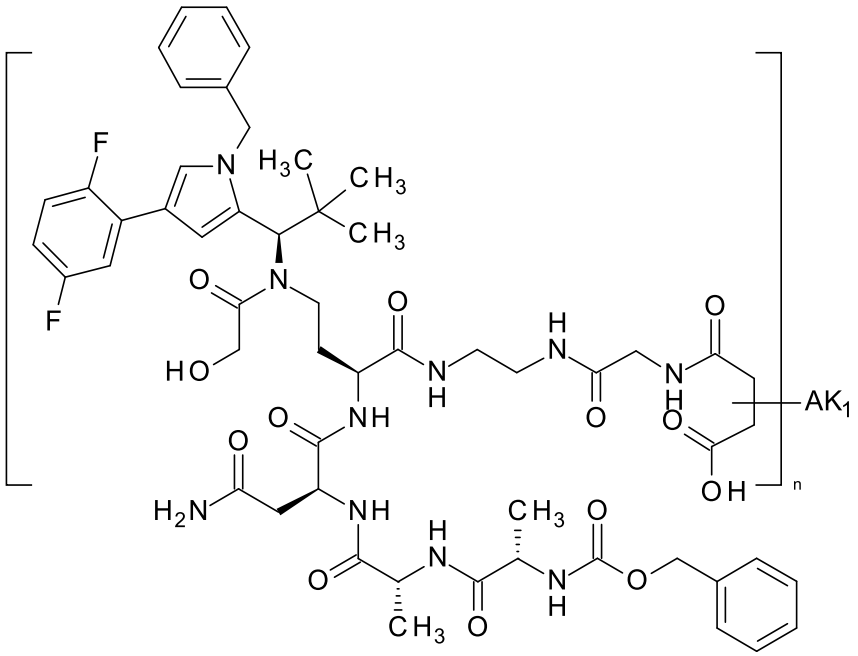
Particular preference is given in accordance with the invention to the following KSP-inhibitor conjugates in which

AK (AK_1; AK_2; AK_3)		is a binder, preferably an antibody or antigen-binding fragment, and
n		is a number from 1 to 50, preferably 1 to 20, more preferably 2 to 8 and especially 2 to 6.
AK_1		is preferably an antibody bonded to the KSP inhibitor via a cysteine residue.
AK_2		is preferably an antibody bonded to the KSP inhibitor via a lysine residue.
AK_3		is preferably an antibody bonded to the KSP inhibitor via a glutamine residue.

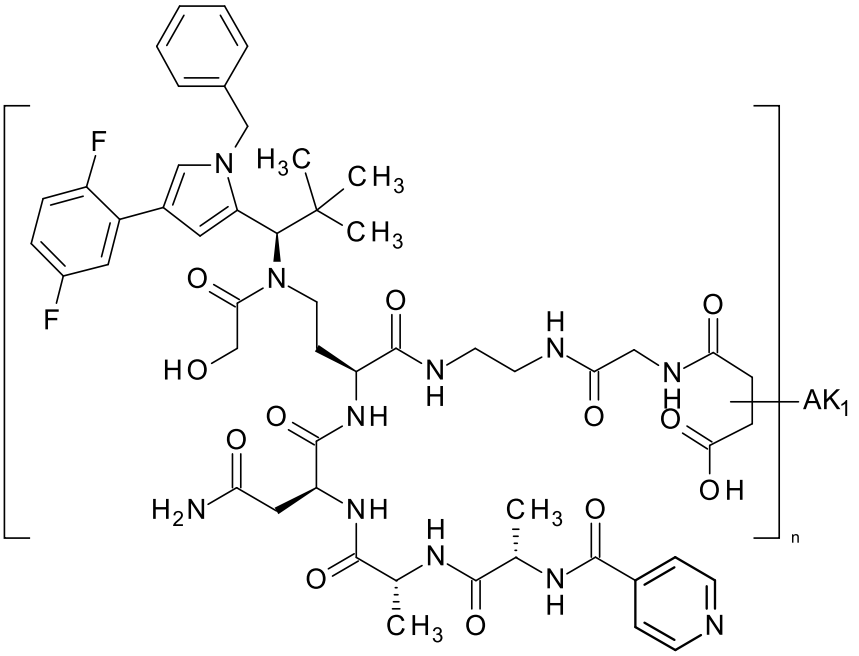
The binders or antibodies used here are preferably the binders and antibodies described as preferred in the description.

Especially preferred are the anti-TWEAKR antibodies TPP-7006, TPP-7007, TPP-10336 and TPP-10337, the anti-B7H3 antibodies TPP-8382 and TPP-8567, the anti-EGFR-antibody cetuximab (TPP-981) and the anti-HER2-antibodies trastuzumab and TPP-1015, or an antigen-binding fragment of these.

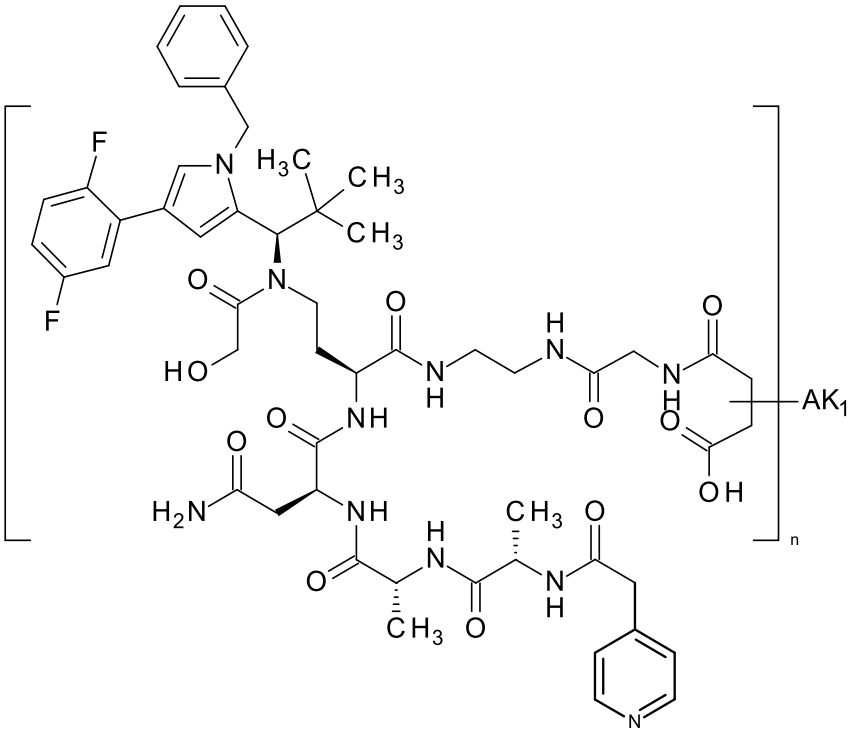
Particularly preferred conjugates are:



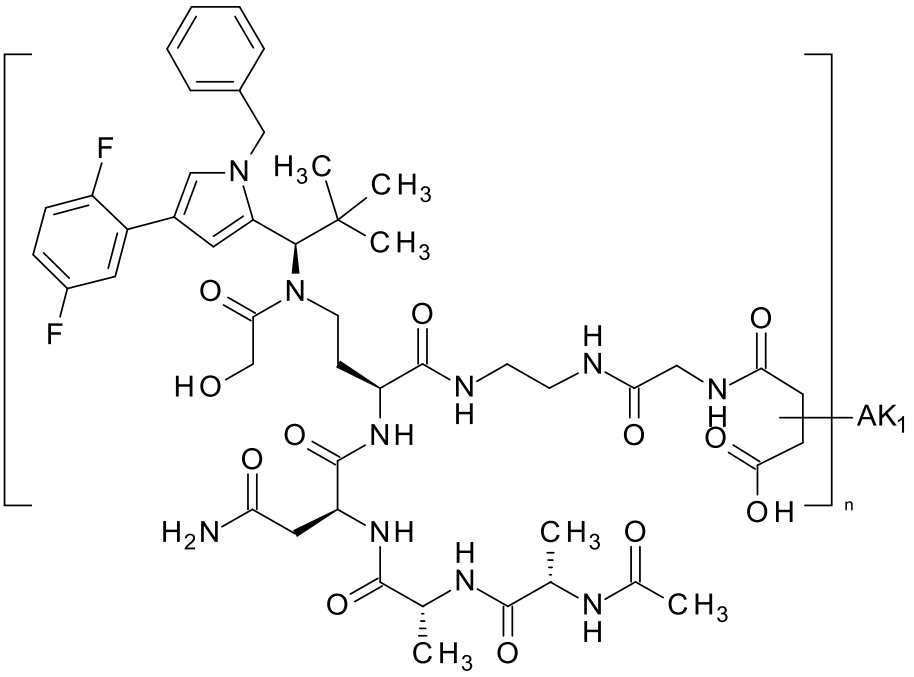
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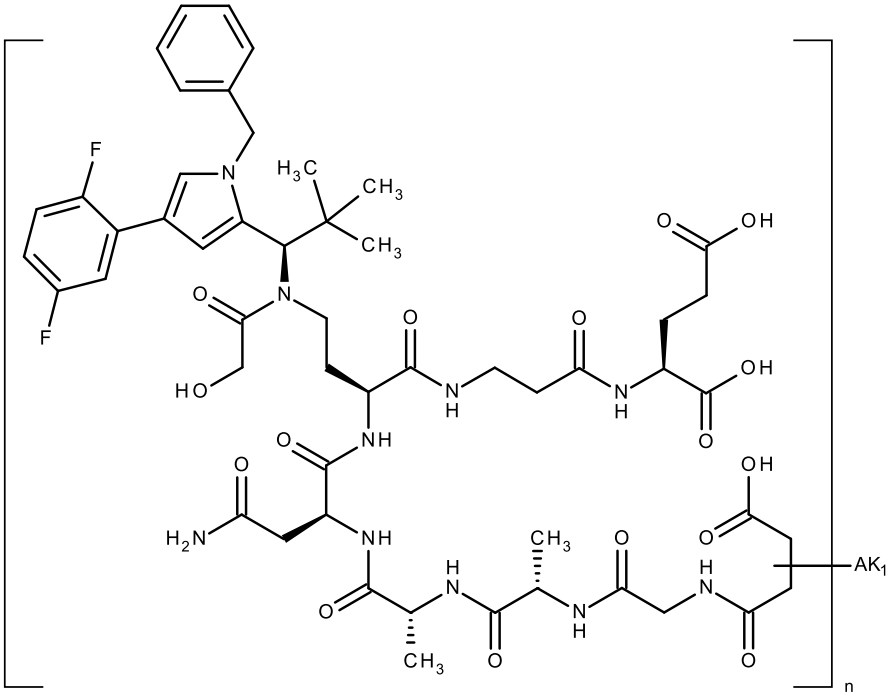
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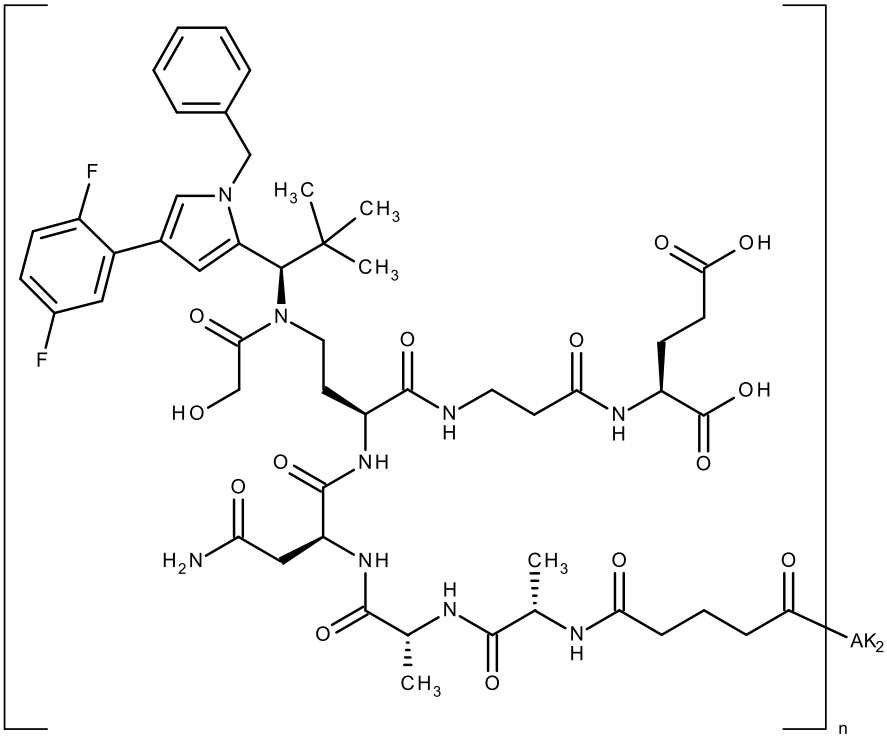
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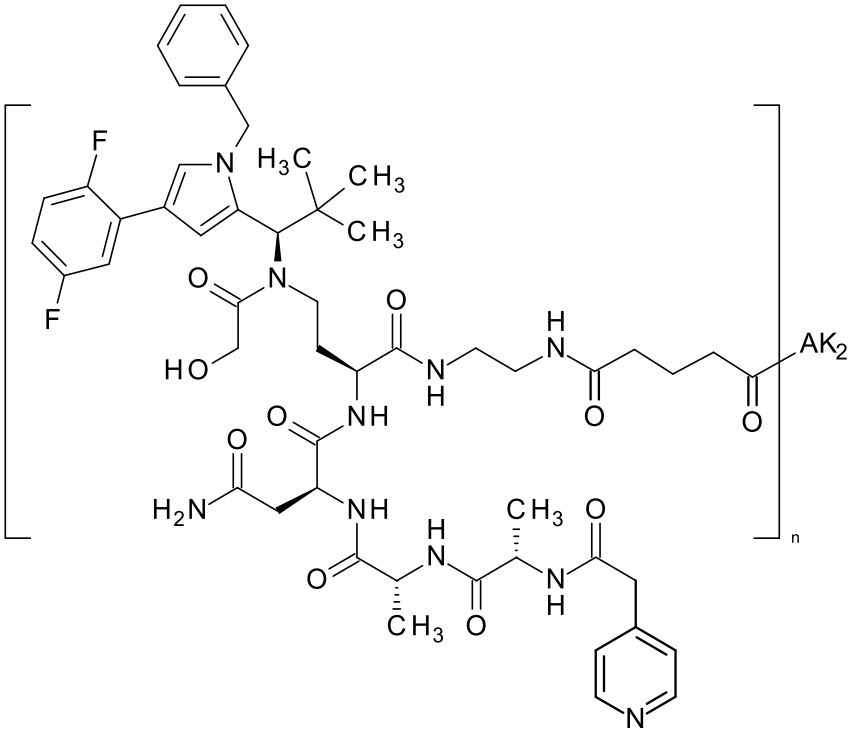
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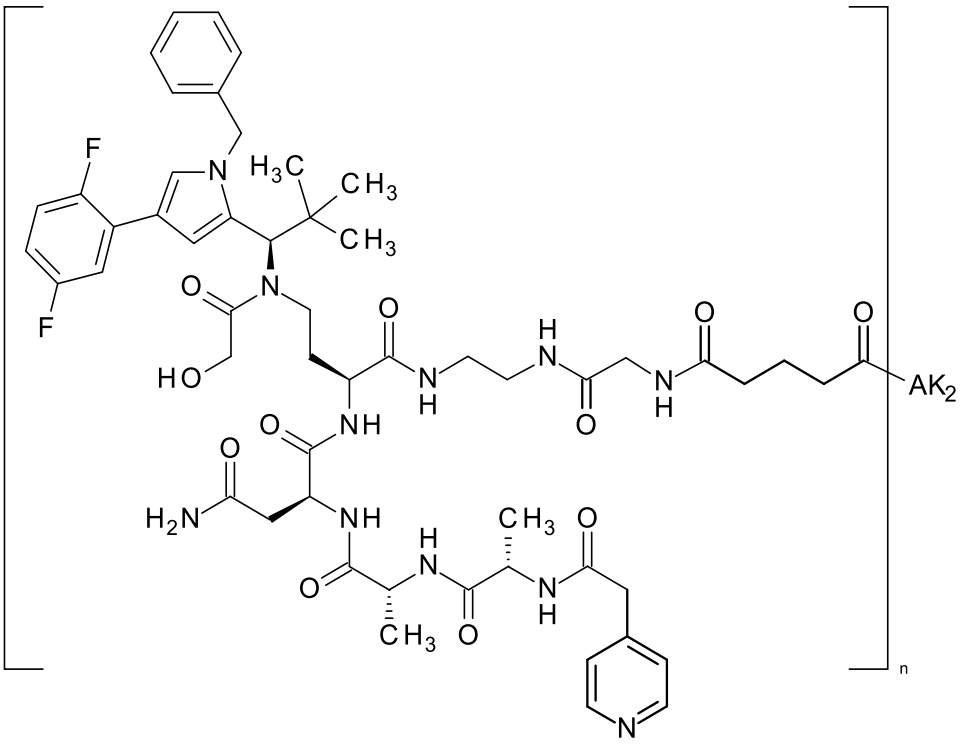
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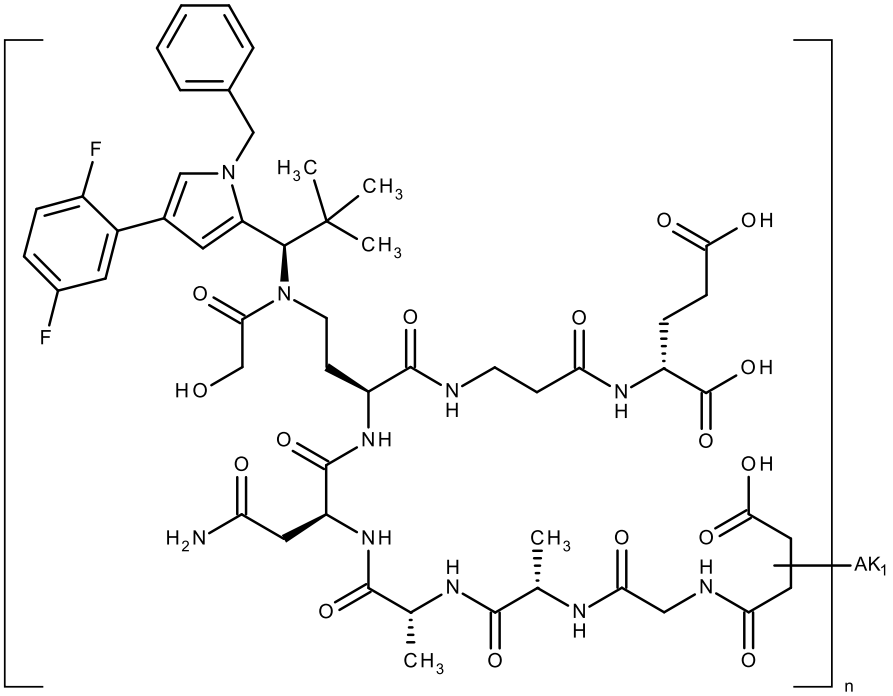
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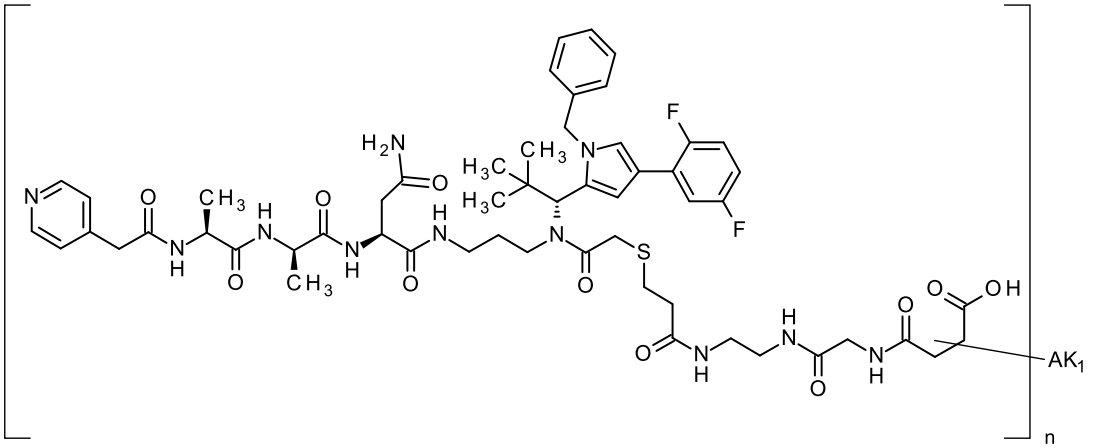
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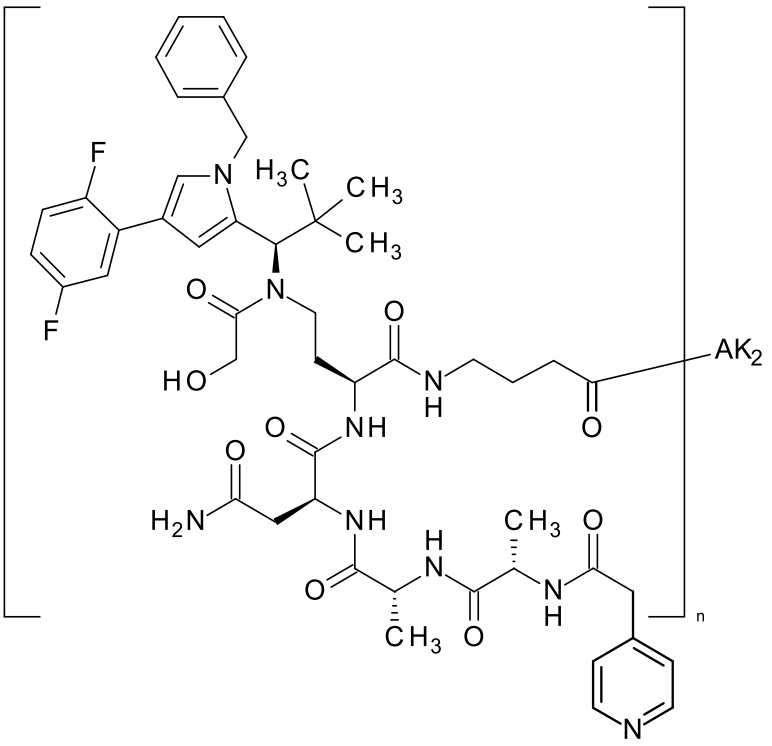
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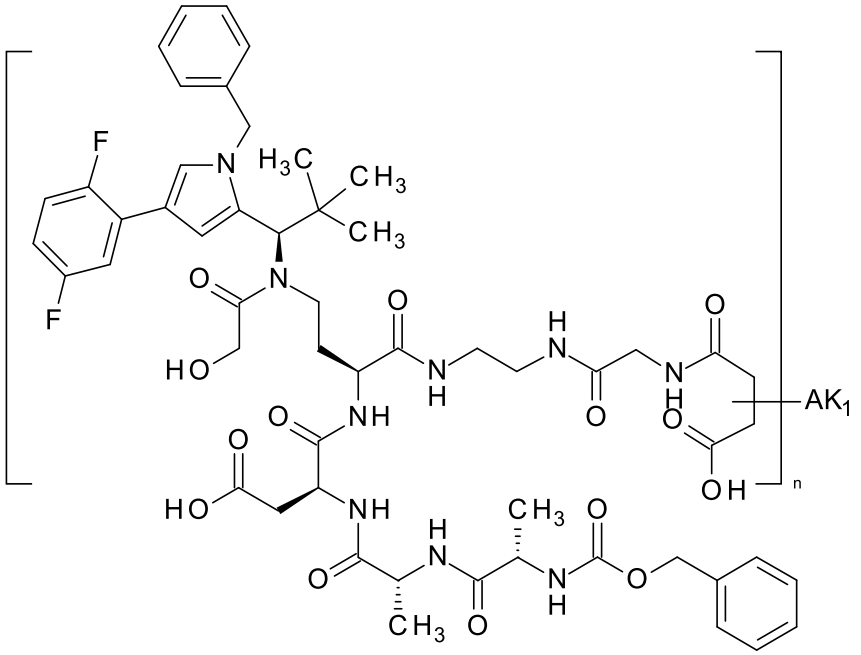
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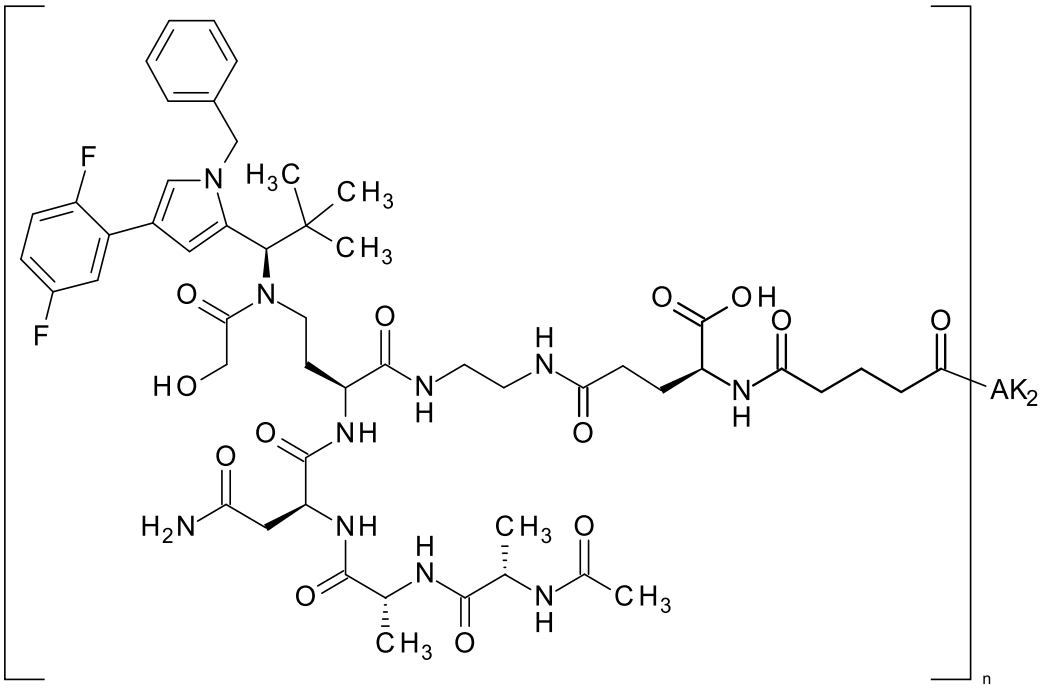
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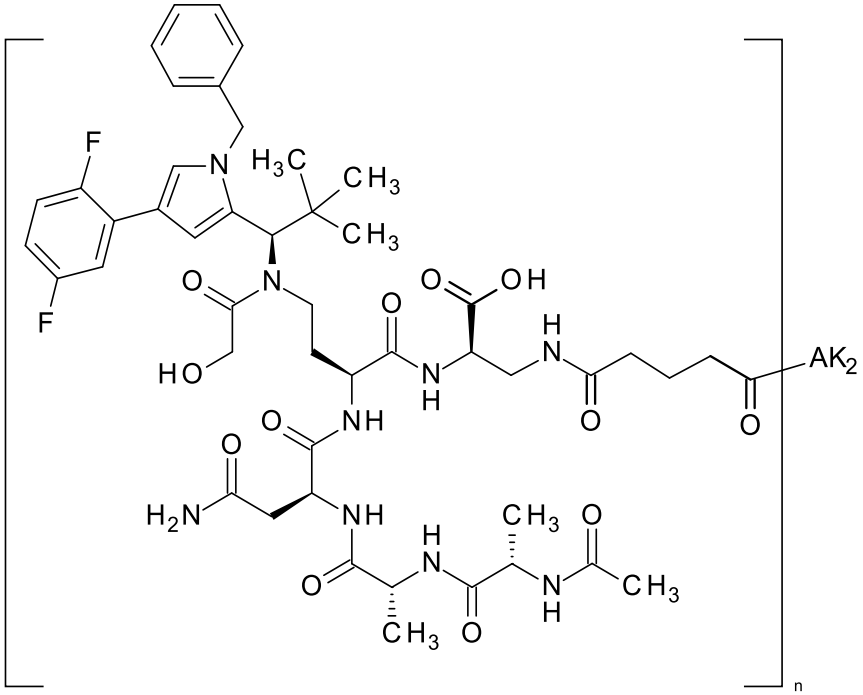
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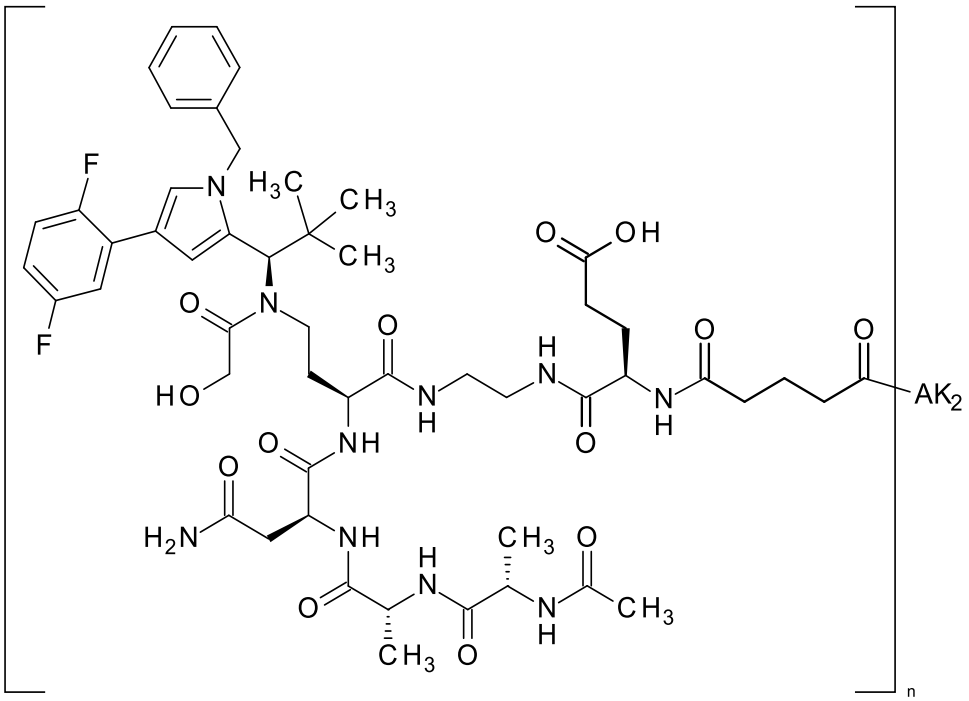
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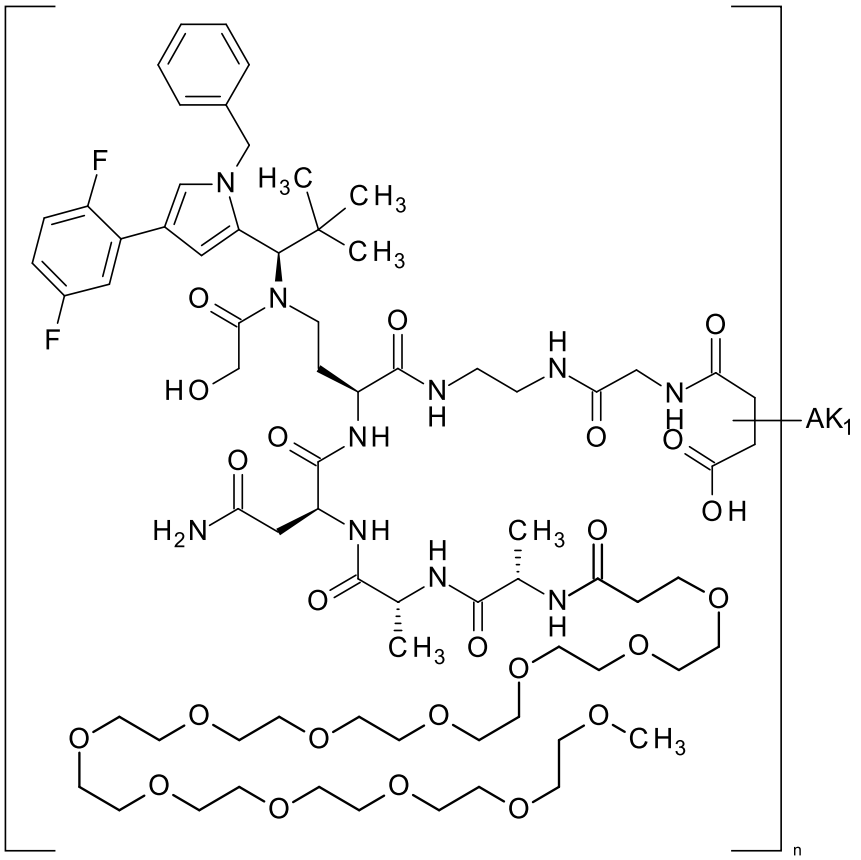
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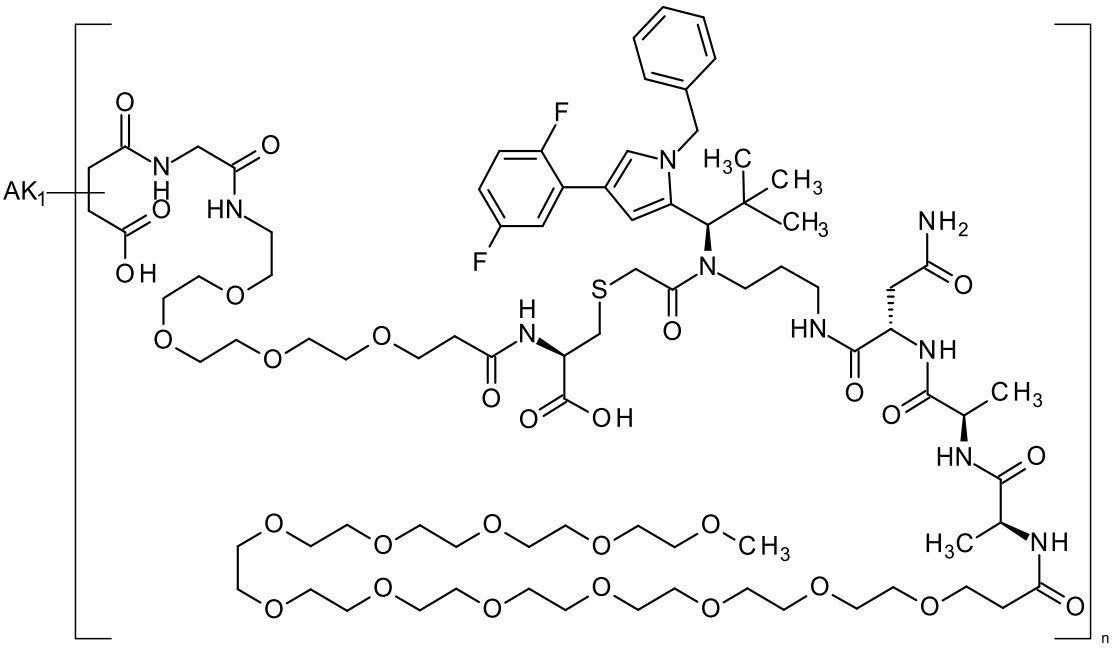
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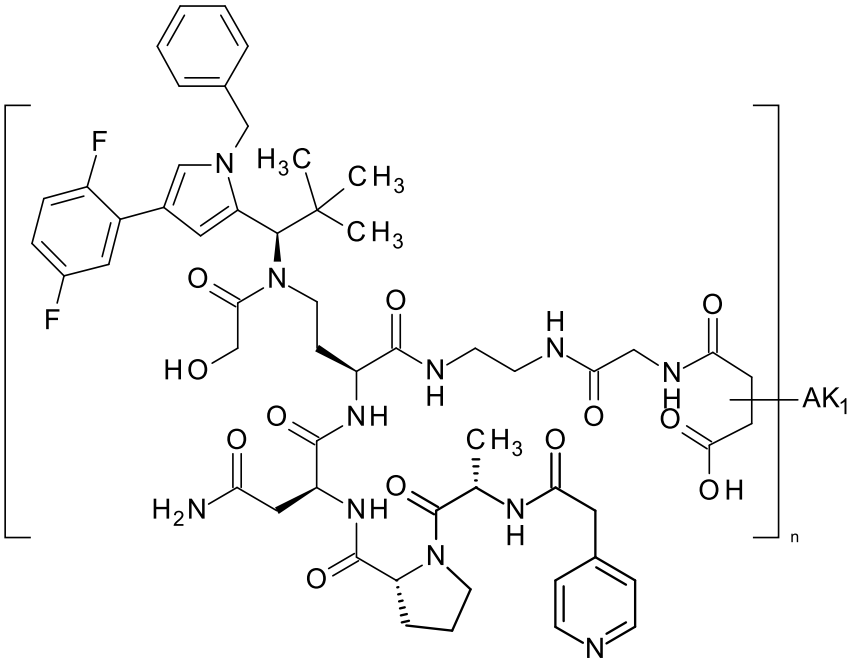
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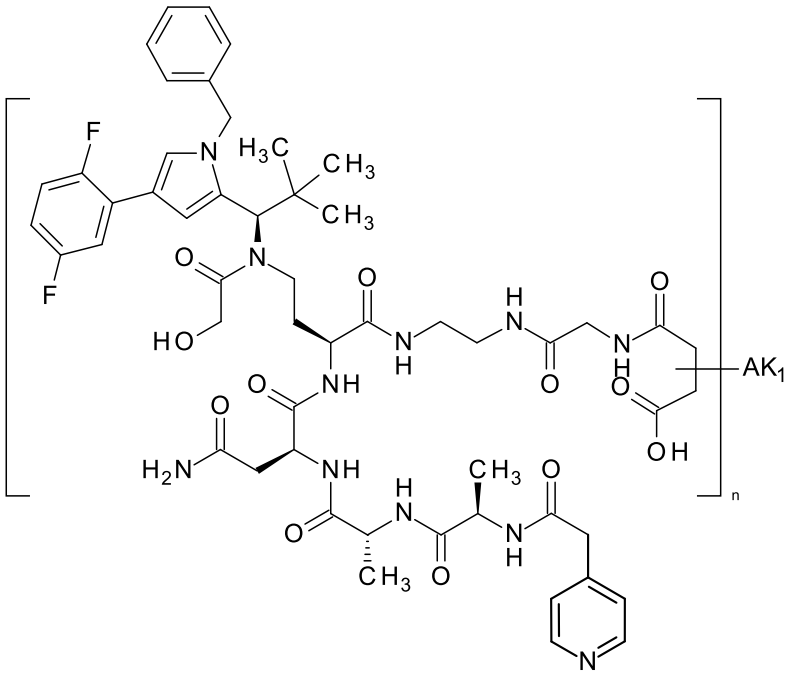
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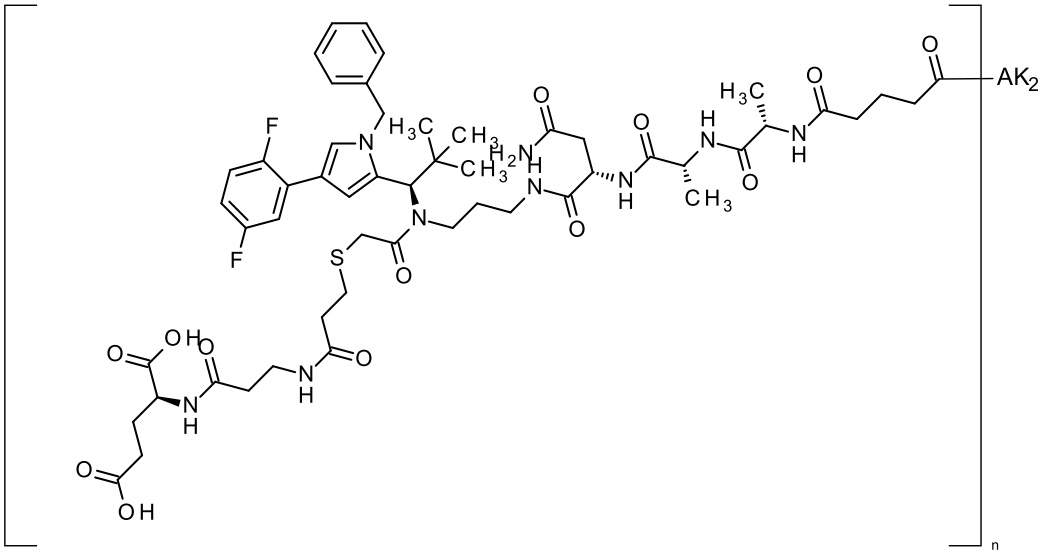
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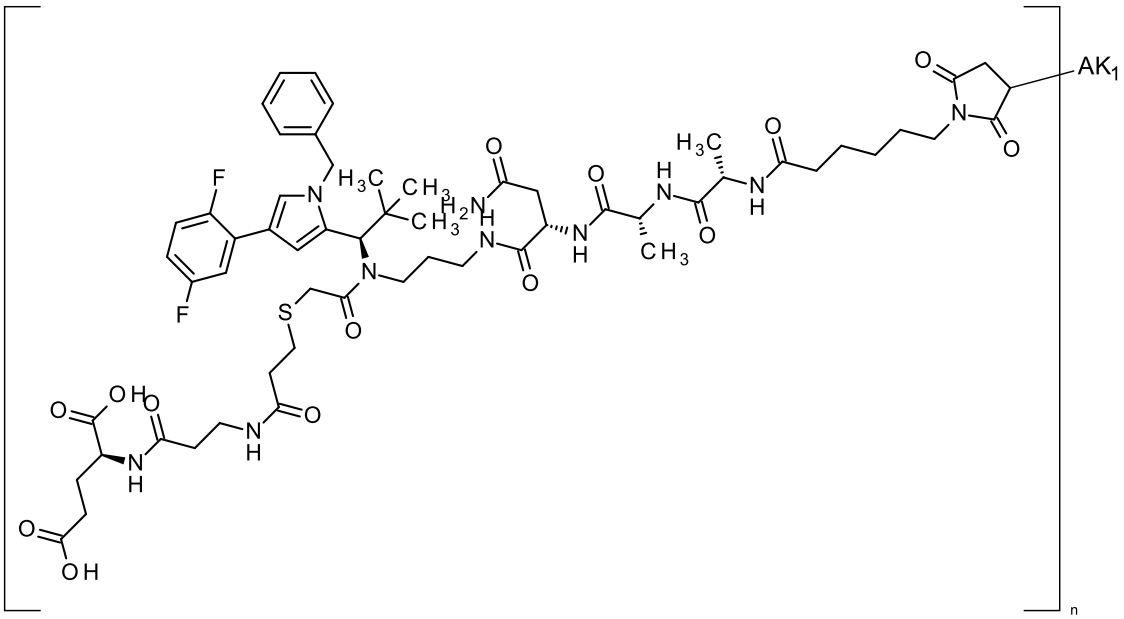
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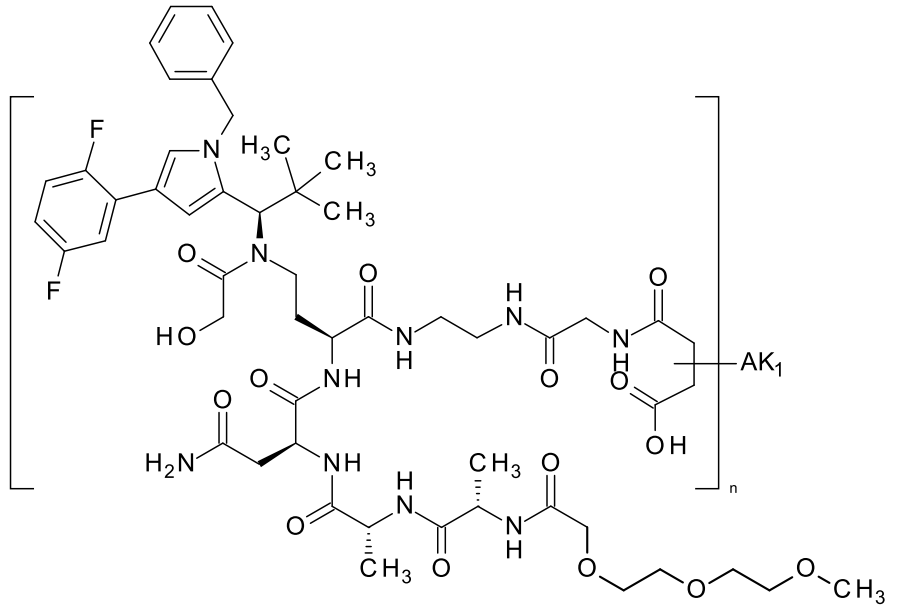
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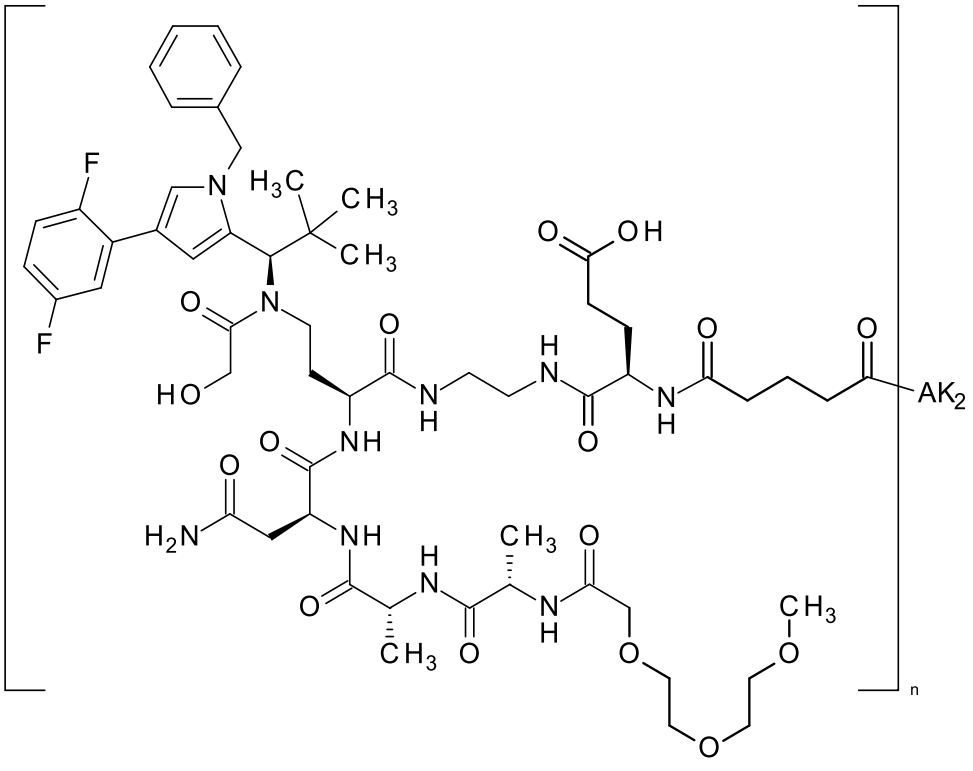
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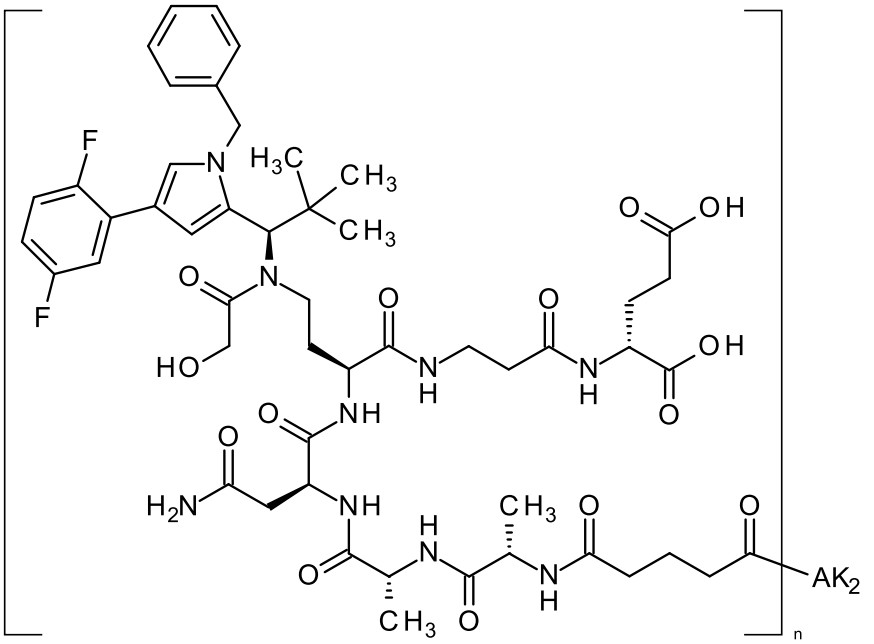
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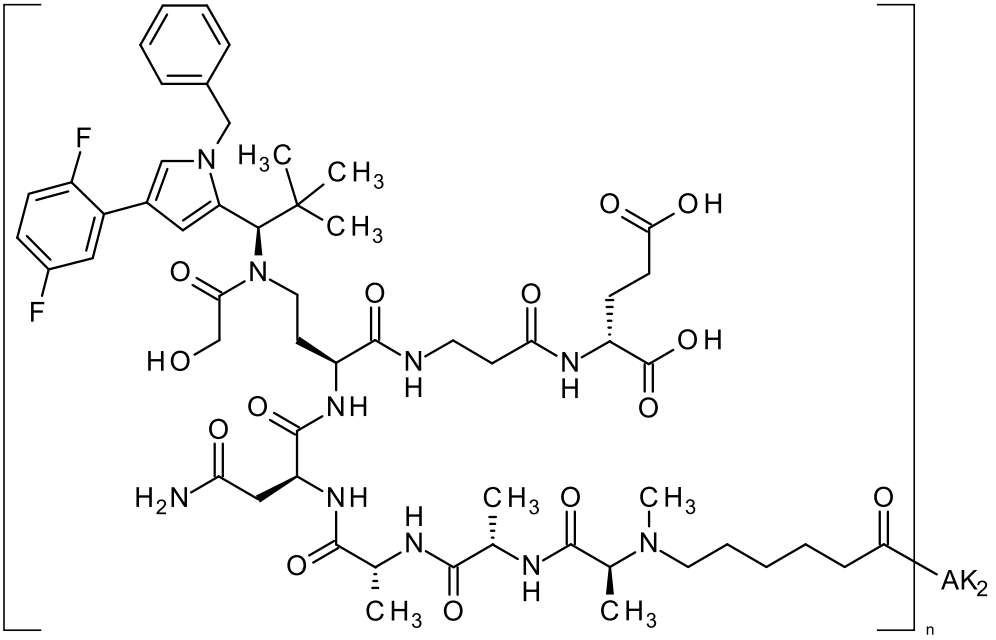
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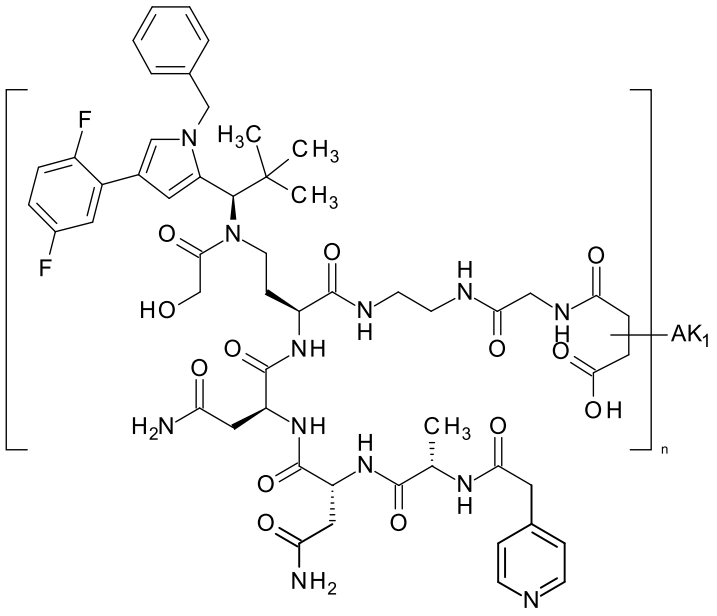
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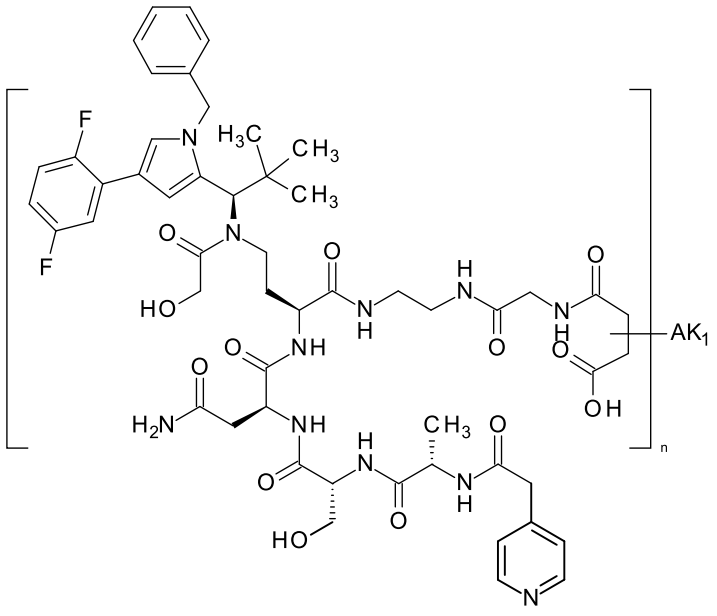
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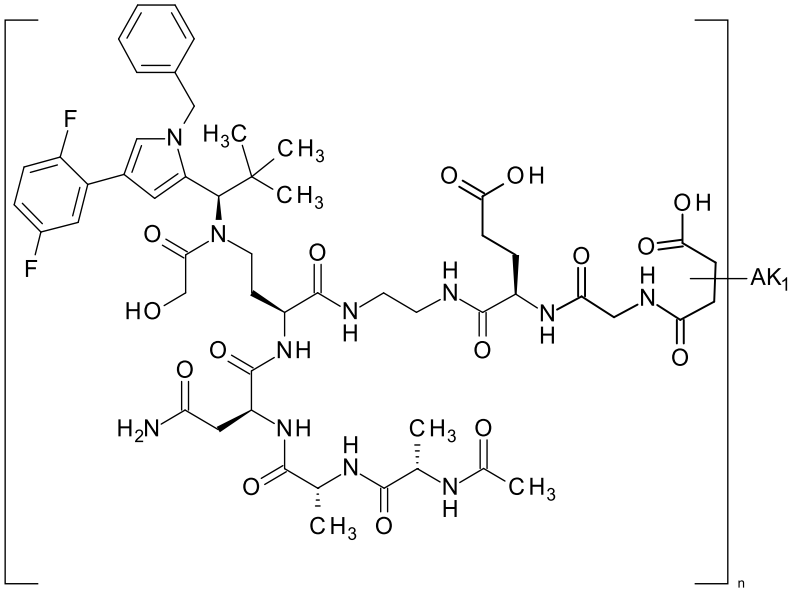
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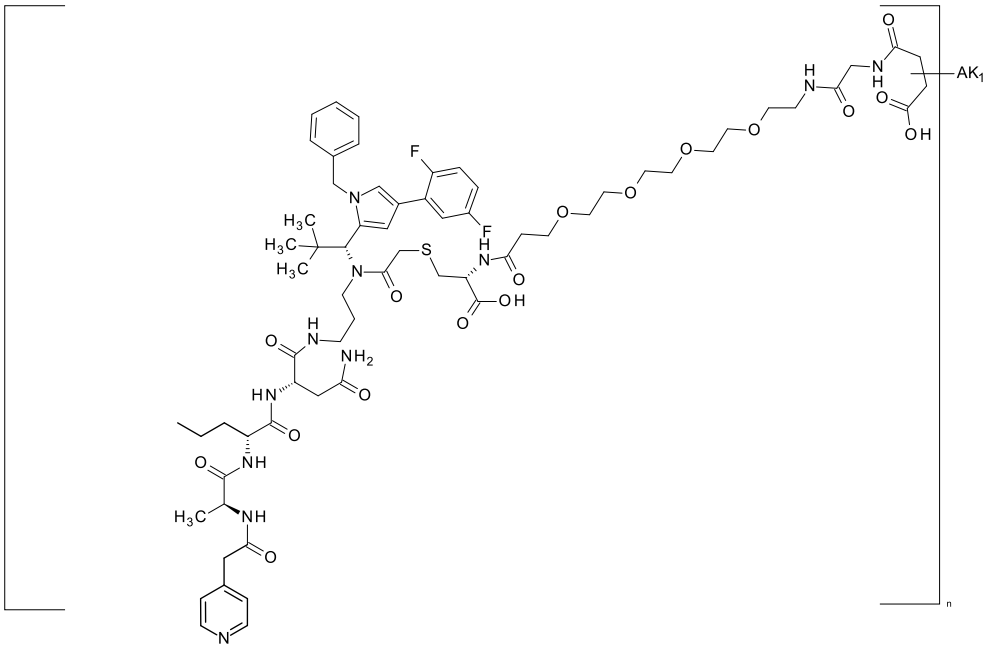
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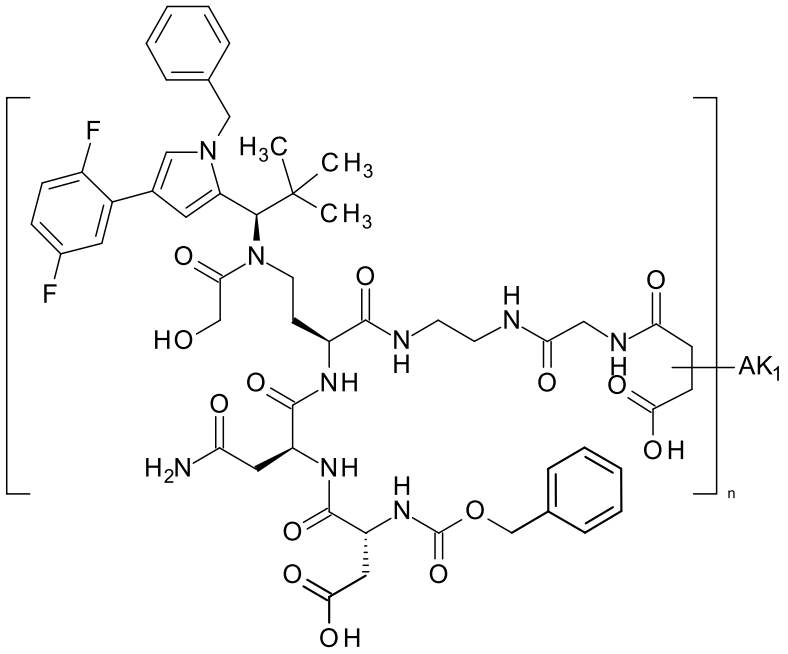
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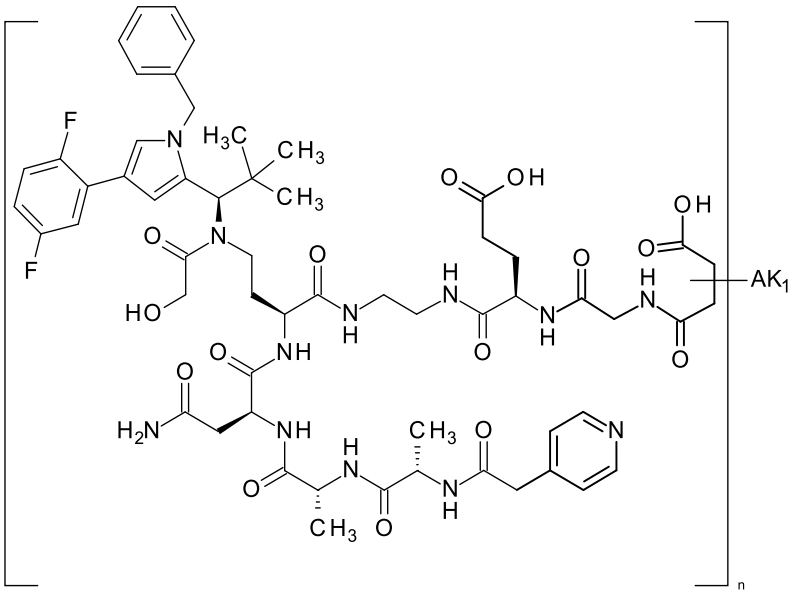
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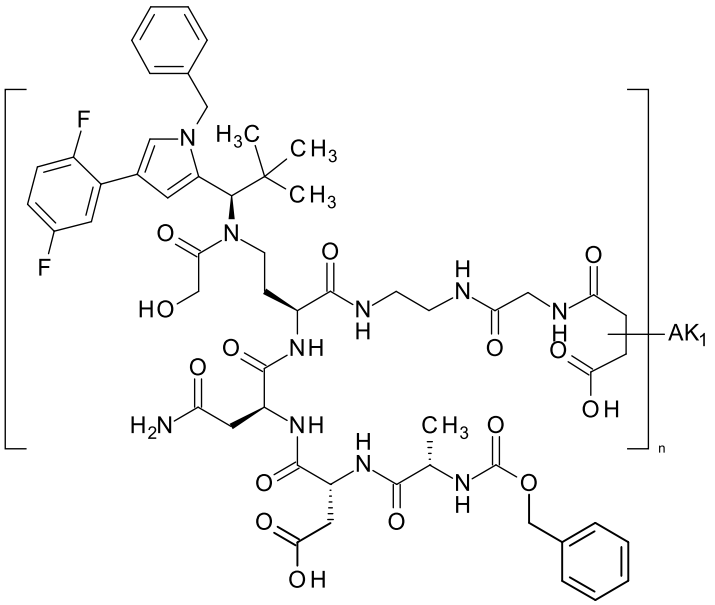
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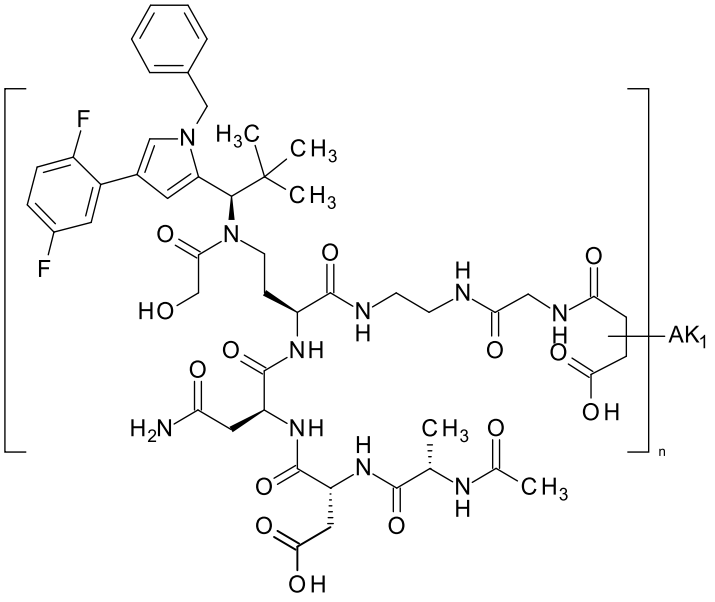
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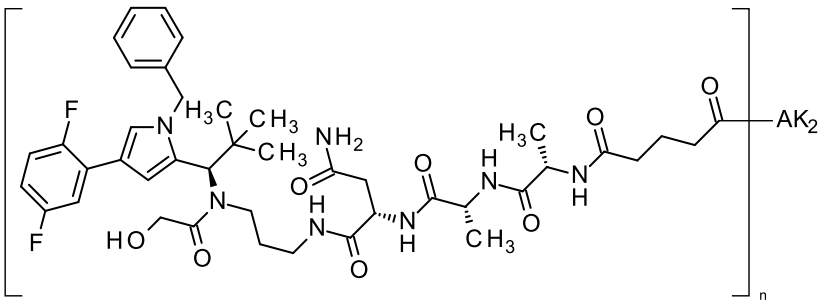
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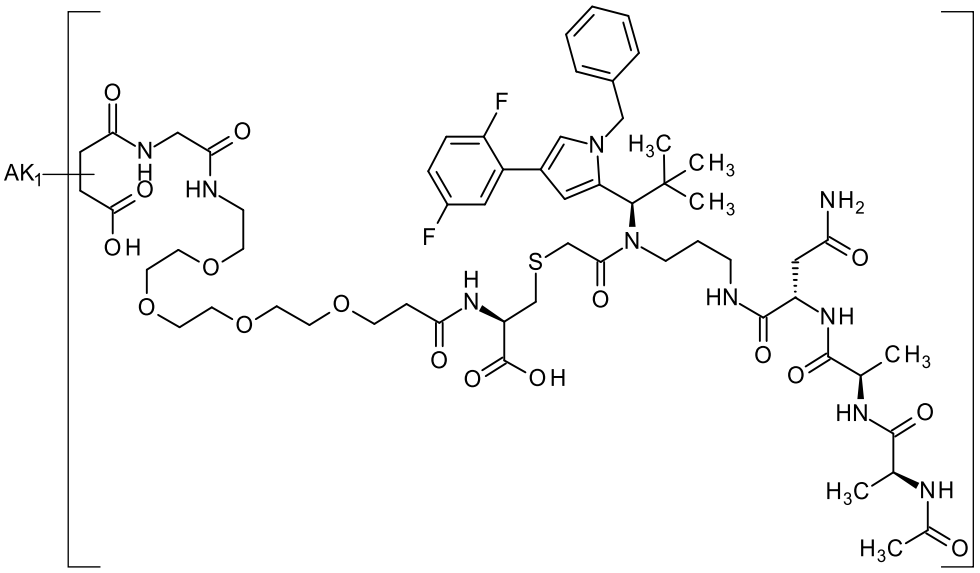
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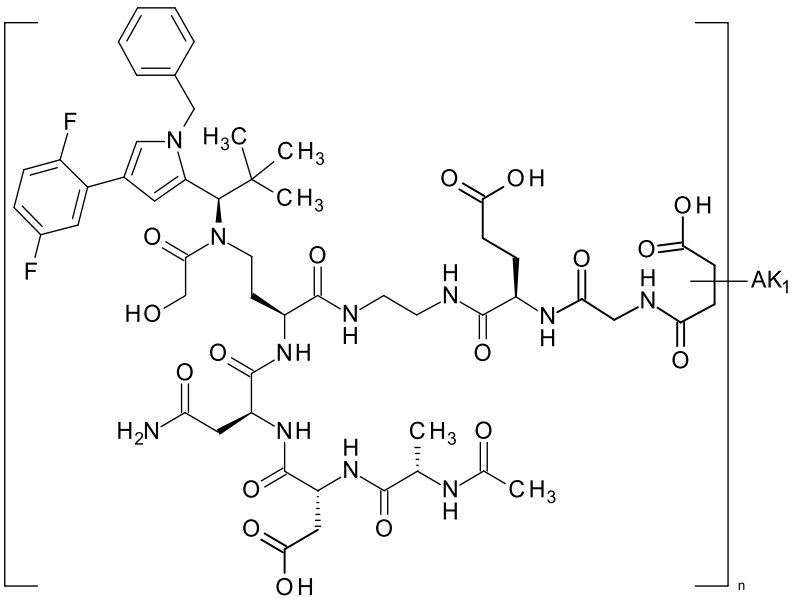
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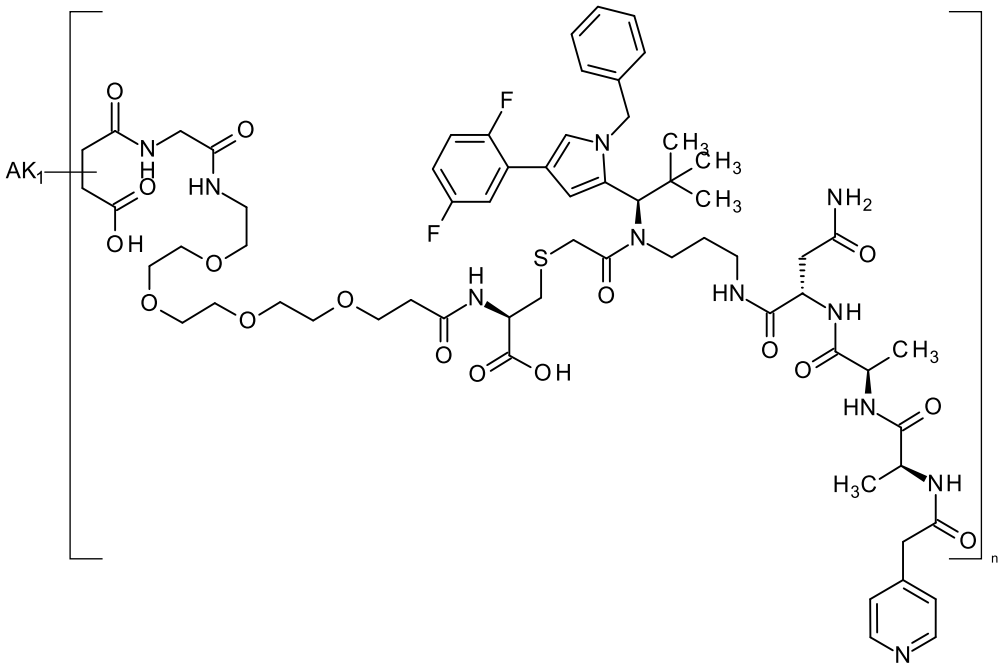
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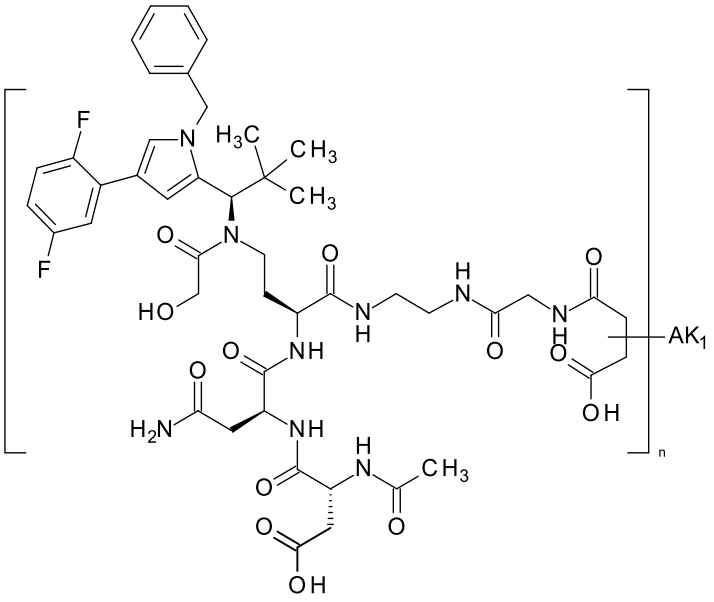
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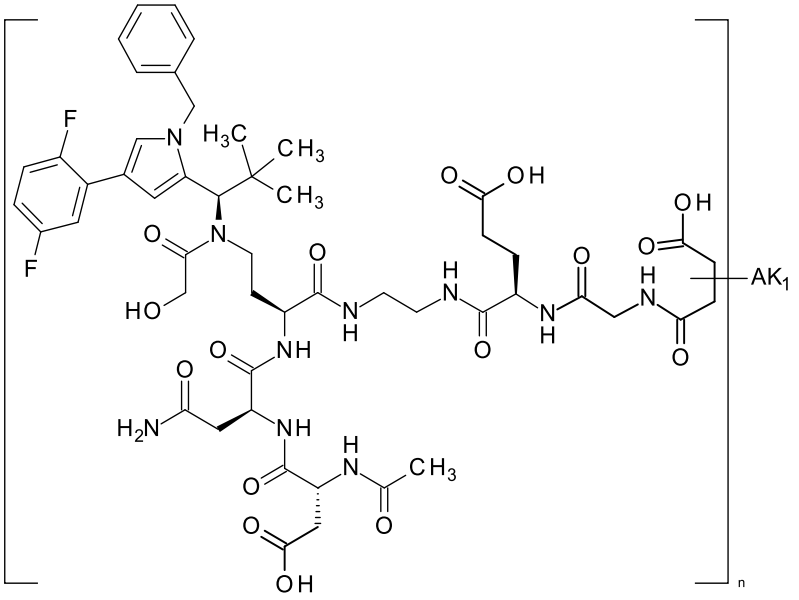
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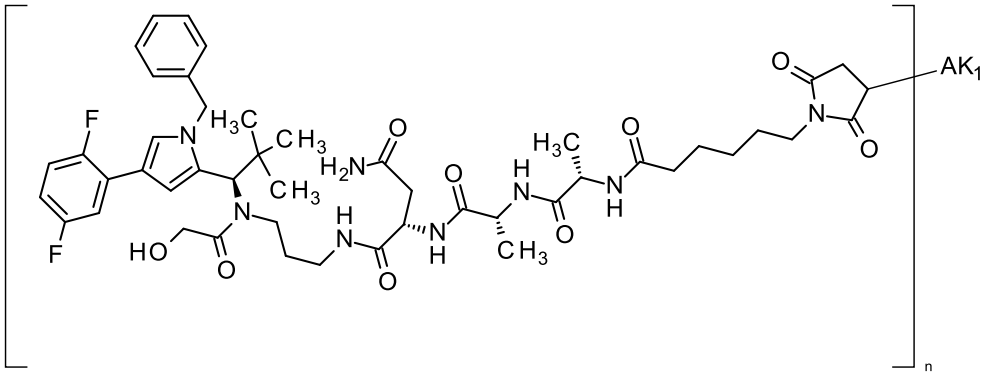
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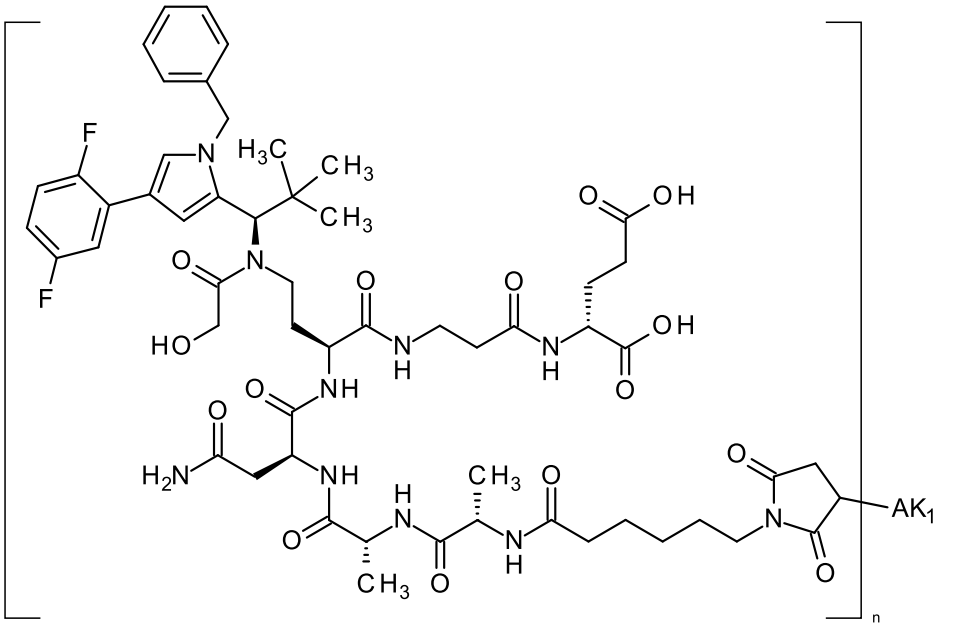
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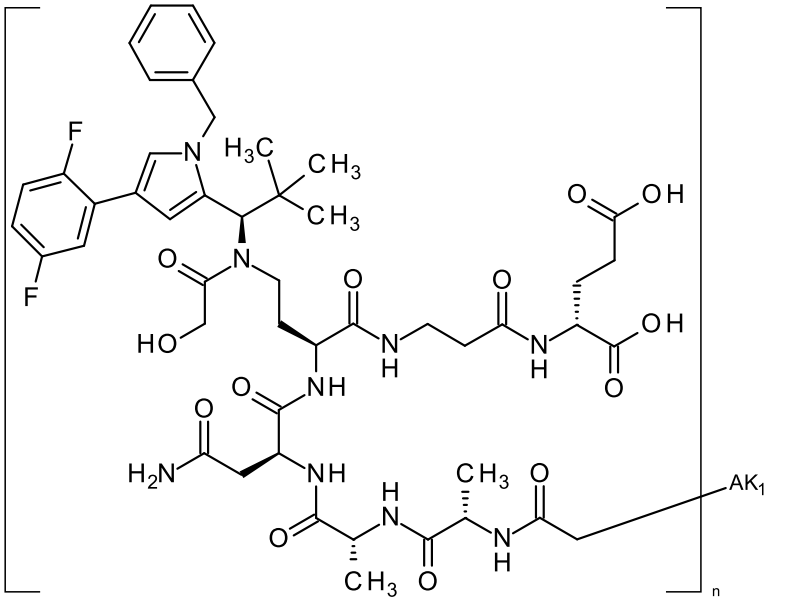
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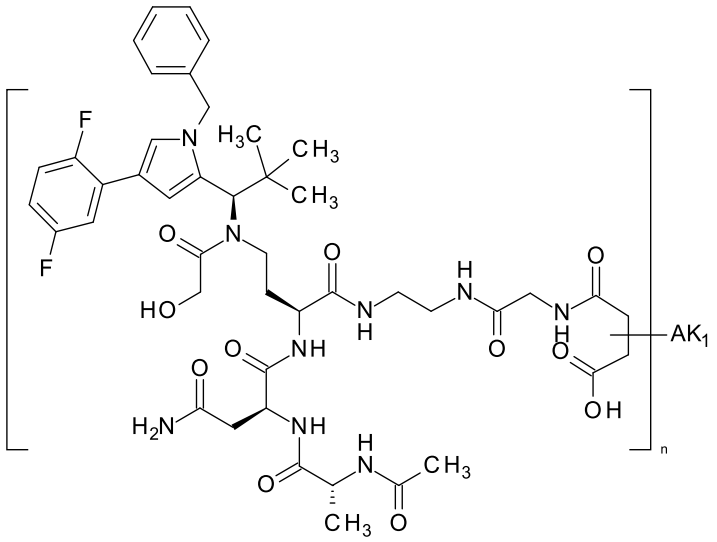
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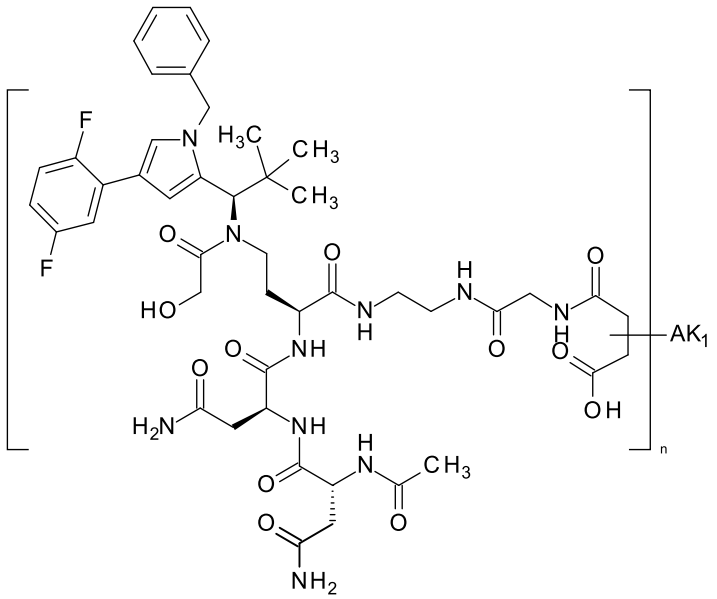
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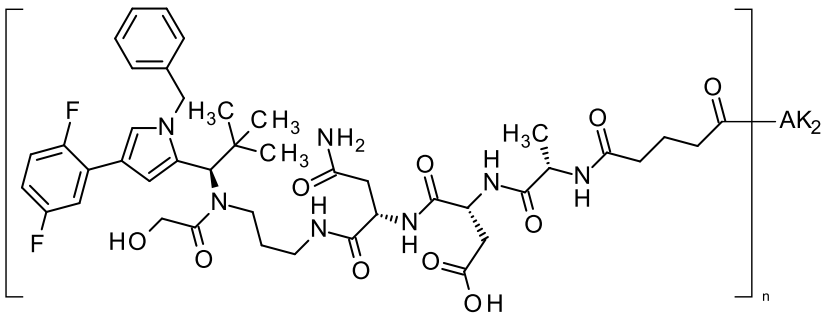
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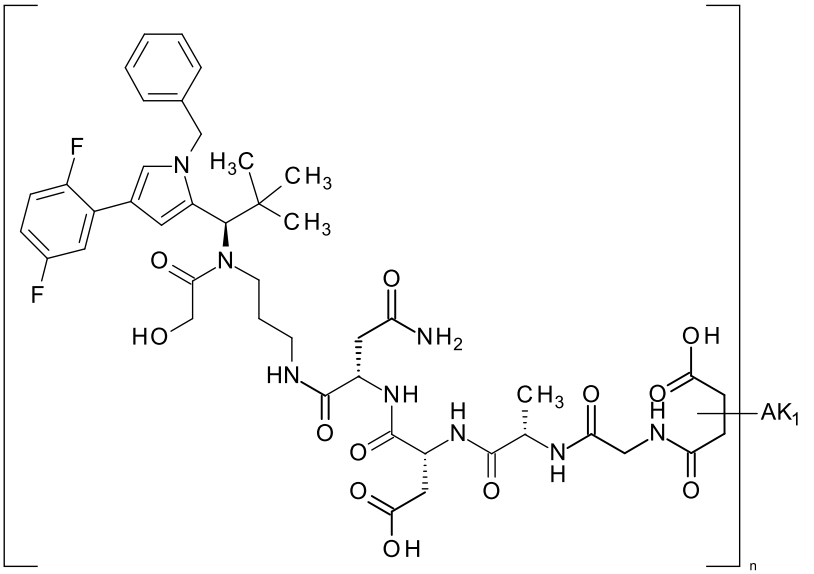
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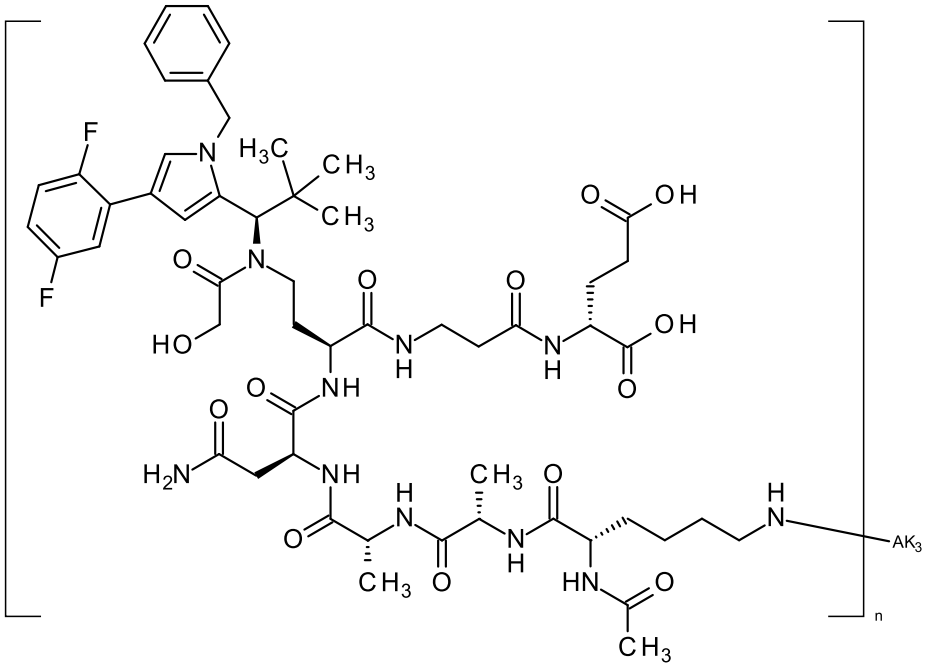
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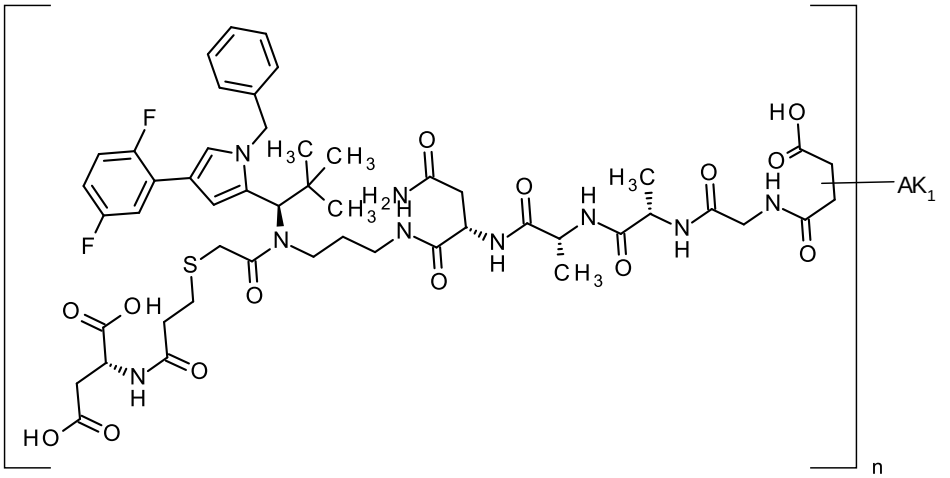
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F
F
N
H3C
CH3
CH3
O
N
HO
O
NH2
HN
NH
O
O
H
N
CH3
O
O
H
N
O
O
H
N
O
OH
O
OH
AK1
n

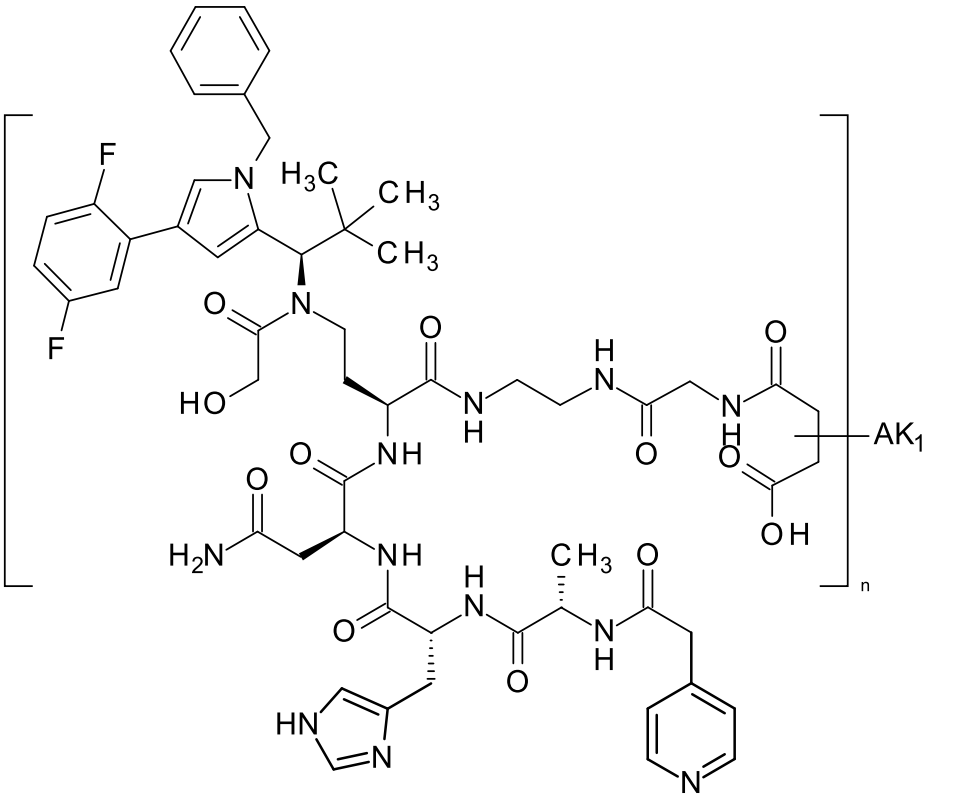
,


F
F
N
H3C
CH3
CH3
O
N
HO
O
O
OH
O
N
H
O
N
H
OH
O
O
NH
O
H2N
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HN
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CH3
H
N
AK3
n

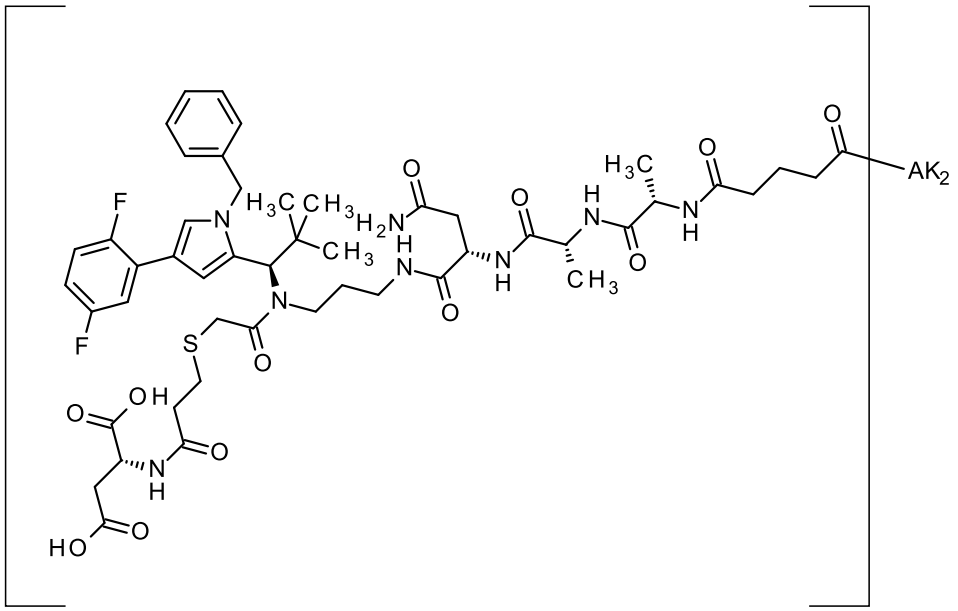
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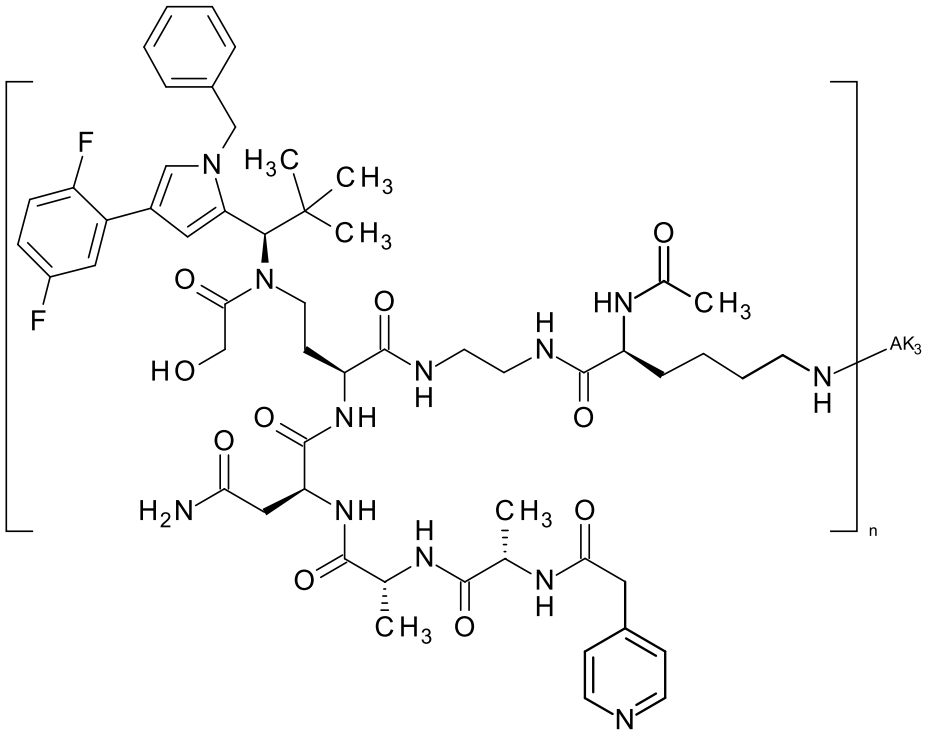
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and



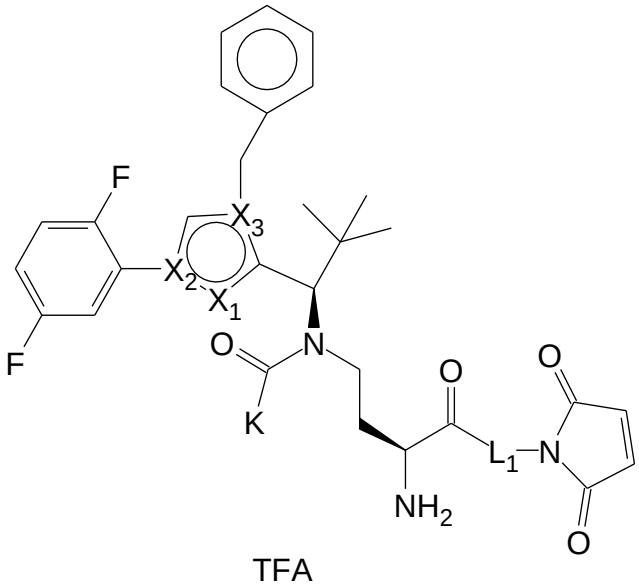
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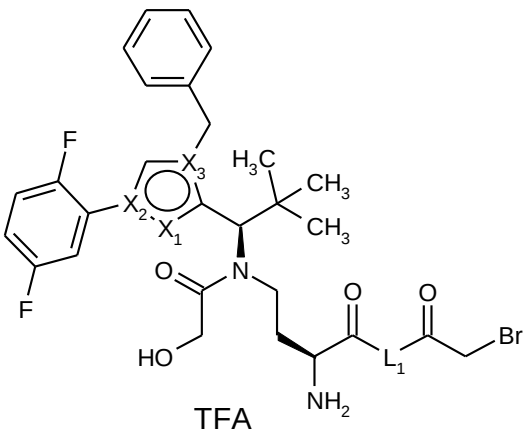
KSP inhibitor-linker intermediates or prodrug-linker intermediates and preparation of the conjugates

The conjugates according to the invention are prepared by initially providing the low-molecular weight KSP inhibitor or the prodrug thereof with a linker. The intermediate obtained in this manner is then reacted with the binder (preferably antibody).

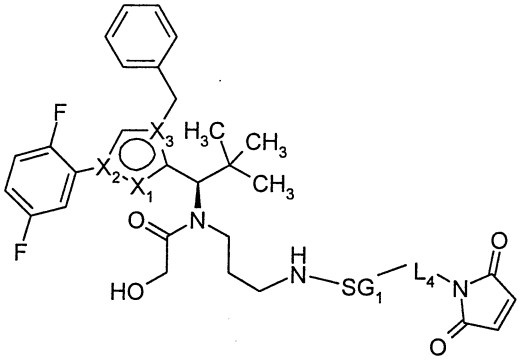
Preferably, for coupling to a cysteine residue, one of the compounds below is reacted with the cysteine-containing binder such as an antibody, which is optionally partially reduced for this purpose:

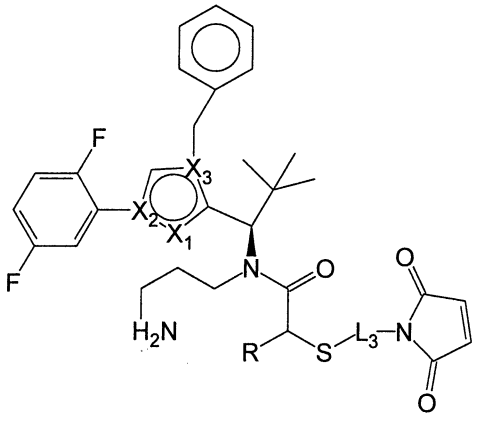


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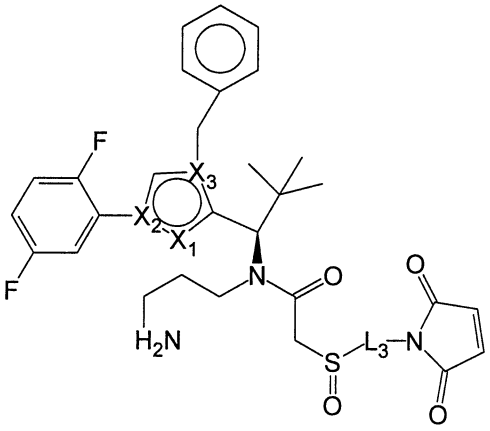


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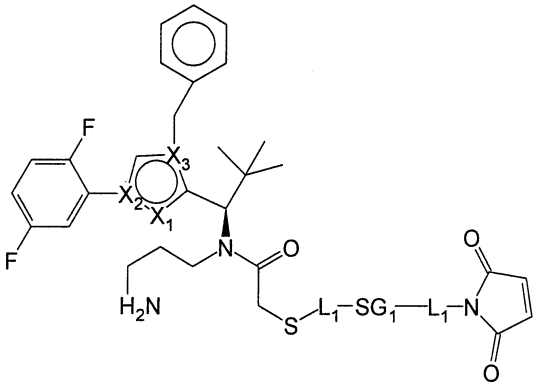




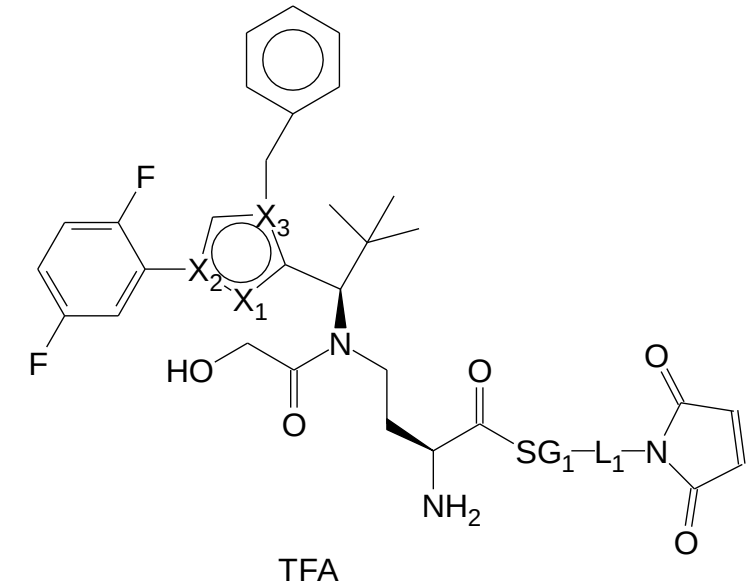
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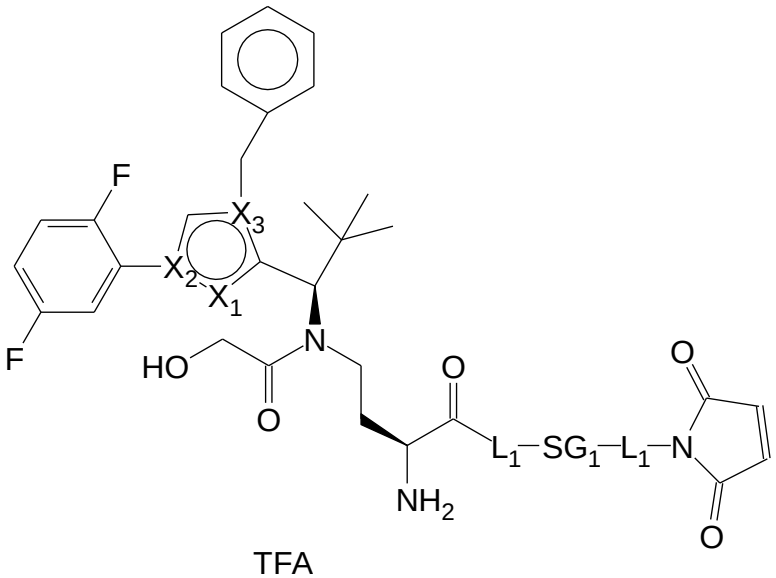
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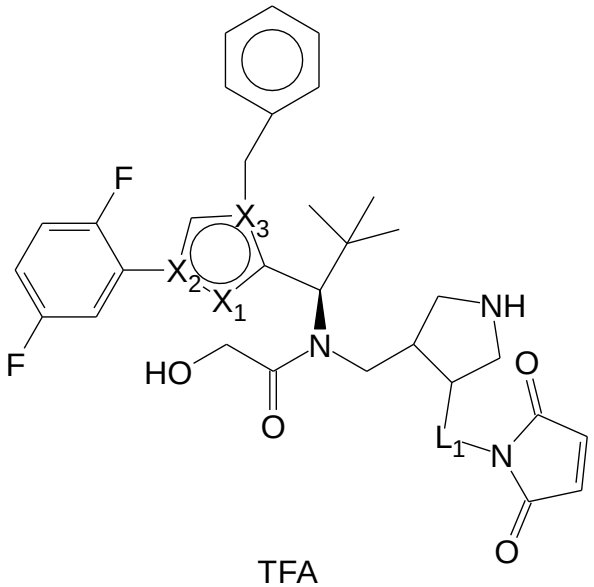
TFA


F
X3
X2
X1
F
HO
N
O
O
O
SG1—L1—N
O
NH2
O

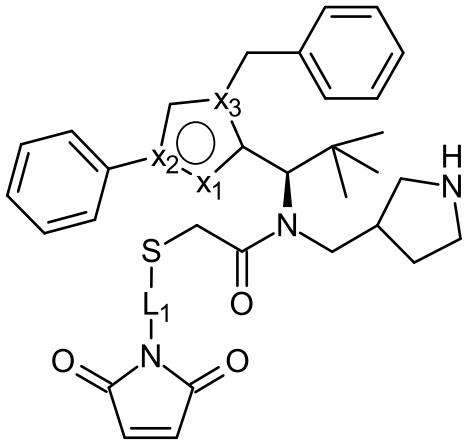
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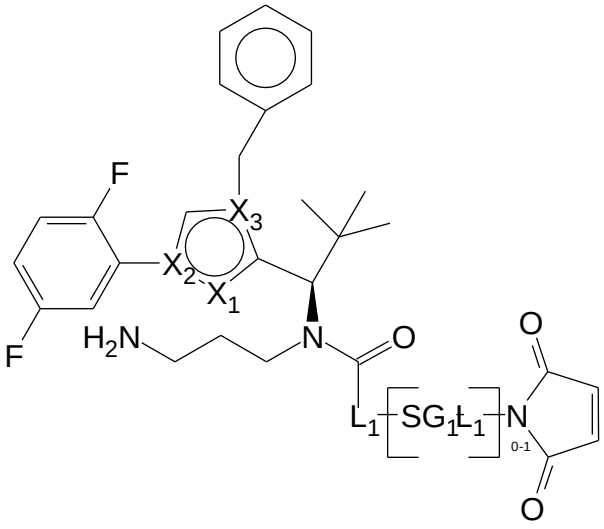

F
X3
X2
X1
F
HO
N
O
O
L1—SG1—L1—N
O
NH2
O

TFA


F
X3
X2
X1
F
HO
N
NH
O
O
L1
N
O

TFA


x3
x2
x1
H
N
N
S
L1
O
O
N
O


F
X3
X2
X1
F
H2N
N
O
L1
SG1
L1
N
0-1
O
O

TFA

in which

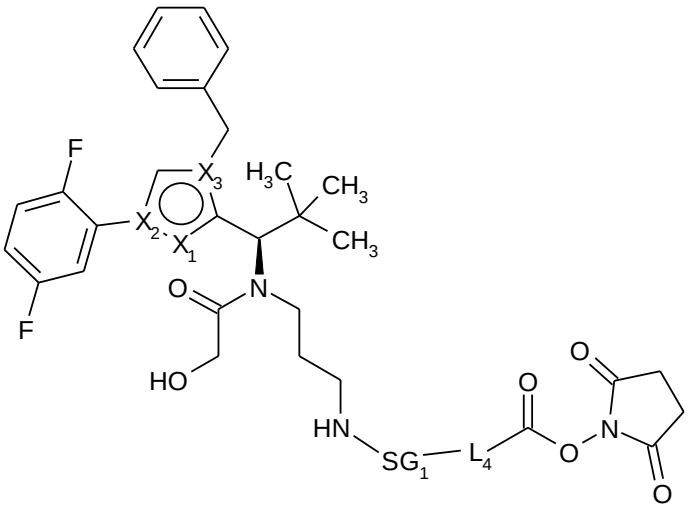
X_1	is CH,
X_2	is C,
X_3	is N,
R	is -H or –COOH,
K	is linear or branched, optionally C_1-C_6 alkoxy- or –OH-substituted C_1-C_6 alkyl and
SG_1, L_1, L_2, L_3 and L_4	have the definitions given above.

In the above-described formulae, and also in the reaction schemes and structural formulae which follow, the hydrogen atom in position R^4 according to formula IIa, i.e. in the $–NH_2$ group, may be replaced by the legumain-cleavable group of the formula Ia used in accordance with the invention.

In each of the above compounds and in the compounds below, the tert-butyl group may be replaced by cyclohexyl.

The compound can be used, for example, in the form of its trifluoroacetic acid salt. For reaction with the binder, for example with the antibody, the compound is preferably used in a 2- to 12-fold molar excess with respect to the binder.

Preferably, for coupling to a lysine residue, one of the compounds below is reacted with the lysine-containing binder such as an antibody:



In the formula,

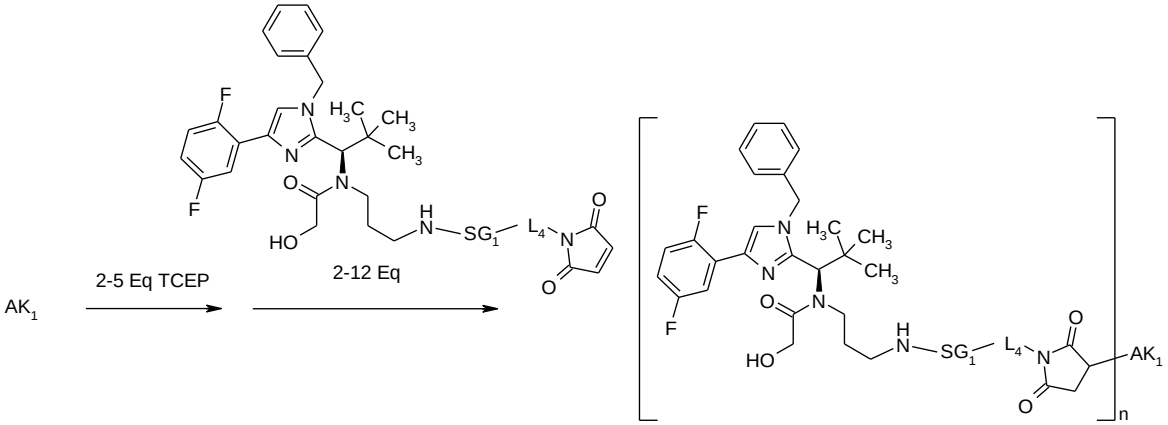
X_1 is CH,

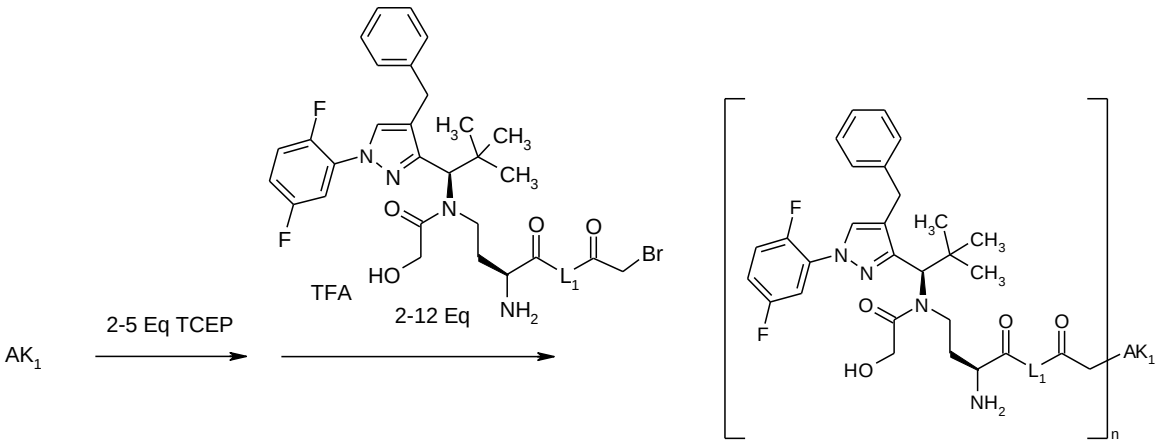
X_2 is C,

X_3 is N and

L_4 has the same definition as L_1, where L_1 has the same definition as described above.

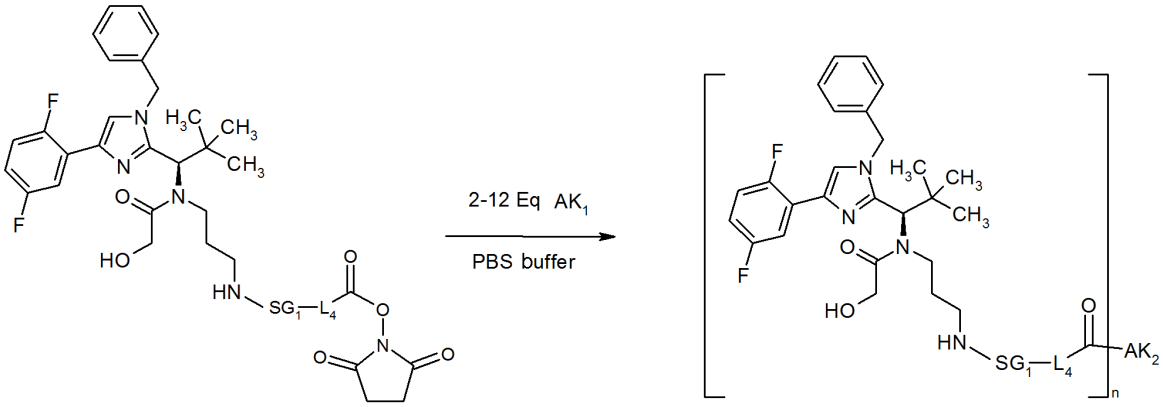
For an intermediate that couples to a cysteine residue, the reactions can be illustrated as follows:



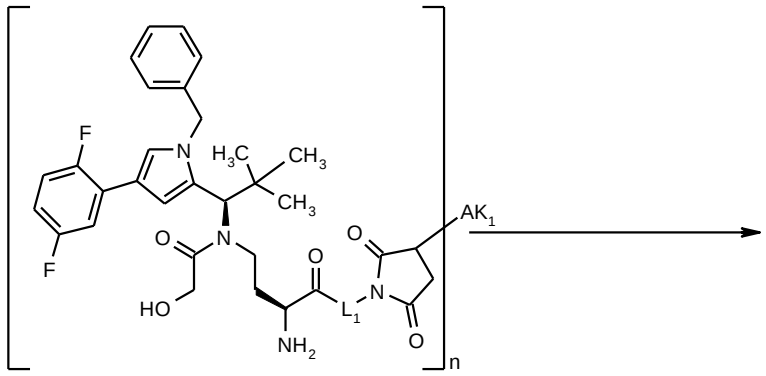


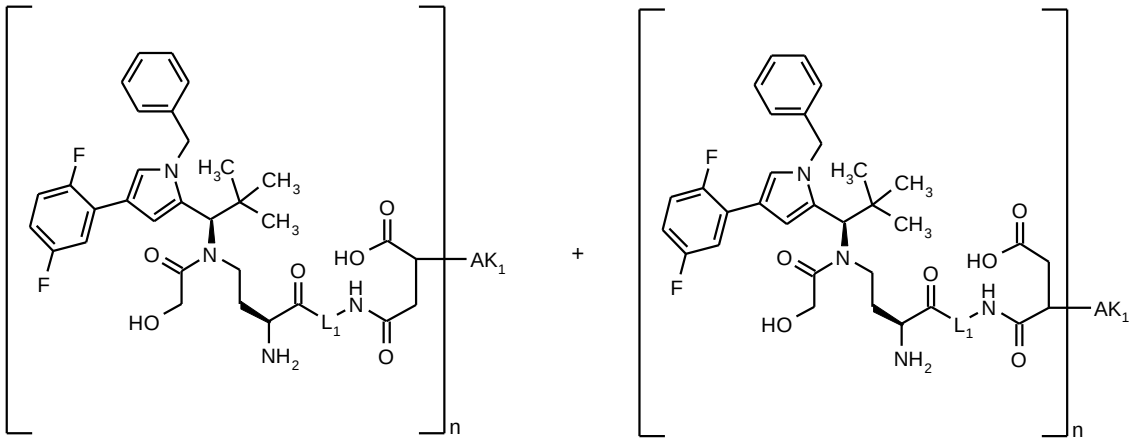
The other intermediates and other antibodies can be reacted correspondingly.

For an intermediate that couples to a lysine residue, the reaction can be illustrated as follows:



In accordance with the invention, this gives the following conjugates:





This reaction (ring opening) can be effected at pH 7.5 to 9, preferably at pH 8, at a temperature of 25°C to 37°C, for example by stirring. The preferred stirring time is 8 to 30 hours.

In the above formulae, X1 represents CH, X2 represents C and X3 represents N, SG1 and L1 have the same definition as described above, and L2, L3 and L4 have the same definition as L1; and R and K have the same definition as described above.

AK1 is an anti-TWEAKR antibody coupled via a cysteine residue, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment of these, and AK2 is an anti-TWEAKR antibody coupled via a lysine residue, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment of these. More preferably, AK1 and AK2 are the anti-TWEAKR antibodies TPP-7006, TPP-7007, TPP-10336 and TPP-10337, the anti-B7H3 antibodies TPP-8382 and TPP-8567, the anti-EGFR-antibody cetuximab (TPP-981) and the anti-HER2-antibodies trastuzumab and TPP-1015, or an antigen-binding fragment of these.

Further definitions

The expression "transglutaminase", also used interchangeably as "TGase" or "TG", is understood to mean an enzyme having the ability to join proteins via an acyl transfer reaction between the γ-carboxamide group of peptide-bound glutamine and the ε-amino group of lysine or a structurally related primary amine, for example an aminopentyl group or, for example, a peptide-bound lysine, which results in an 8-(γ-glutamyl)-lysine isopeptide bond. TGases include bacterial transglutaminase (BTG), for example the enzyme having EC reference number 2.3.2.13 (protein-glutamine γ-glutamyltransferase).

The expression "acceptor glutamine" means, when referring to an amino acid residue of an antibody, a glutamine residue which, under suitable conditions, is recognized by a transglutaminase and can be joined therewith under transglutaminase catalysis by a reaction between this specific glutamine and a lysine or a structurally related primary amine, for example an aminopentyl group. The acceptor glutamine may be a surface-exposed glutamine.

"Amino acid modification" or "mutation" here means an amino acid substitution, insertion and/or deletion in a polypeptide sequence. The preferred amino acid modification here is a substitution. "Amino acid substitution" or "substitution" here means an exchange of an amino acid at a given position in a protein sequence for another amino acid. For example, the substitution Y50W describes a variant of a parent polypeptide in which the tyrosine at

position 50 has been exchanged for a tryptophan. A "variant" of a polypeptide describes a polypeptide having an amino acid sequence substantially identical to a reference polypeptide, typically a native or "parent" polypeptide. The polypeptide variant may have one or more amino acid exchanges, deletions and/or insertions at particular positions in the native amino acid sequence.

The expression "conjugation site-specific conjugate" describes a conjugate of a binder, preferably an antibody, and a residue, preferably a linker-drug residue, where the binder is functionalized at one or more defined positions, preferably glutamine residues. Transglutaminases (TGases), including bacterial transglutaminase (BTG) (EC 2.3.2.13), show strong specificity in the recognition of glutamine-protein substrates and can catalyse "conjugation site-specific conjugation".

The expression "homogeneous conjugate" or "homogeneous ADC" describes a mixture of conjugation site-specific conjugates wherein at least 60%, 70%, 80% or 90% of the binders have the same number of conjugated residues per binder. In the case of an antibody, this number should be an even number, preferably 2 or 4.

Isotopes, salts, solvates, isotopic variants

The present invention also encompasses all suitable isotopic variants of the compounds according to the invention. An isotopic variant of a compound of the invention is understood here to mean a compound in which at least one atom within the compound of the invention has been exchanged for another atom of the same atomic number, but with a different atomic mass from the atomic mass which usually or predominantly occurs in nature. Examples of isotopes which can be incorporated into a compound of the invention are those of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulphur, fluorine, chlorine, bromine and iodine, such as ^{2}H (deuterium), ^{3}H (tritium), ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{32}P, ^{33}P, ^{33}S, ^{34}S, ^{35}S, ^{36}S, ^{18}F, ^{36}Cl, ^{82}Br, ^{123}I, ^{124}I, ^{129}I and ^{131}I. Particular isotopic variants of a compound according to the invention, especially those in which one or more radioactive isotopes have been incorporated, may be beneficial, for example, for the examination of the mechanism of action or of the active ingredient distribution in the body; due to the comparatively easy preparability and detectability, especially compounds labelled with ^{3}H or ^{14}C isotopes are suitable for this purpose. In addition, the incorporation of isotopes, for example of deuterium, may lead to particular therapeutic benefits as a consequence of greater metabolic stability of the compound, for example an extension of the half-life in the

body or a reduction in the active dose required; such modifications of the compounds according to the invention may therefore in some cases also constitute a preferred embodiment of the present invention. Isotopic variants of the compounds according to the invention can be prepared by the processes known to those skilled in the art, for example by the methods described further down and the procedures described in the working examples, by using corresponding isotopic modifications of the respective reagents and/or starting compounds.

Preferred salts in the context of the present invention are physiologically acceptable salts of the compounds according to the invention. Also encompassed are salts which are not themselves suitable for pharmaceutical applications but can be used, for example, for isolation or purification of the compounds according to the invention.

Physiologically acceptable salts of the compounds according to the invention include acid addition salts of mineral acids, carboxylic acids and sulphonic acids, for example salts of hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methanesulphonic acid, ethanesulphonic acid, benzenesulphonic acid, toluenesulphonic acid, naphthalenedisulphonic acid, acetic acid, trifluoroacetic acid, propionic acid, lactic acid, tartaric acid, malic acid, citric acid, fumaric acid, maleic acid and benzoic acid.

Physiologically acceptable salts of the compounds according to the invention also include salts of conventional bases, by way of example and with preference alkali metal salts (e.g. sodium and potassium salts), alkaline earth metal salts (e.g. calcium and magnesium salts) and ammonium salts derived from ammonia or organic amines having 1 to 16 carbon atoms, by way of example and with preference ethylamine, diethylamine, triethylamine, ethyldiisopropylamine, monoethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, dimethylaminoethanol, procaine, dibenzylamine, N-methylpiperidine, N-methylmorpholine, arginine, lysine and 1,2-ethylenediamine.

Solvates in the context of the invention are described as those forms of the compounds according to the invention which form a complex in the solid or liquid state by coordination with solvent molecules. Hydrates are a specific form of the solvates in which the coordination is with water. Solvates preferred in the context of the present invention are hydrates.

The present invention additionally also encompasses prodrugs of the compounds according to the invention. The term “prodrugs” in this context refers to compounds which

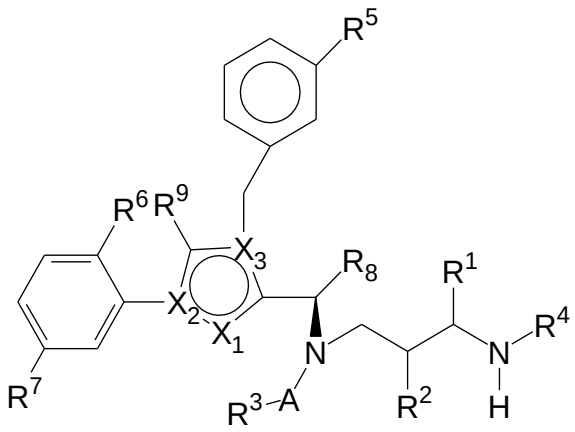
may themselves be biologically active or inactive but are reacted (for example metabolically or hydrolytically) to give compounds according to the invention during their residence time in the body.

Particular embodiments

The following embodiments are particularly preferred:

Embodiment A:

An APDC of the formulae IVa' or IVa'' or IVa''', where D_1 in the formulae IVa' or IVa'' or IVa''' is a compound of the formula (IIe)



(IIe)

in which

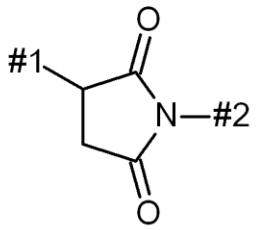
X_1		is N,
X_2		is N and
X_3		is C
	or	
X_1		is CH,
X_2		is C and
X_3		is N
	or	
X_1		is NH,
X_2		is C and
X_3		is C
	or	
X_1		is CH,

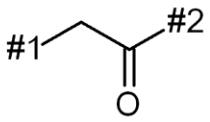
X_2	is N and
X_3	is C,
A	is –C(=O)-,
R^1	is –L-#1, -H, -COOH, -C(=O)-$NHNH_2$, -$(CH_2)_{1-3}NH_2$, -C(=O)-NZ''$(CH_2)_{1-3}NH_2$ and –C(=O)-NZ‘‘CH_2-COOH,
Z‘‘	is -H or -NH_2,
R^2	is -H,
R^4	is a group of the formula (Ia)
R^3	is –L-#1 or a C_{1-10}-alkyl group which may optionally be substituted by –OH, -O-alkyl, -SH, -S-alkyl, -O-C(=O)-alkyl, -O-C(=O)-NH-alkyl, -NH-C(=O)-alkyl, -NH-C(=O)-NH-alkyl, -$S(=O)_n$-alkyl, -$S(=O)_2$-NH-alkyl, -NH-alkyl, -$N(alkyl)_2$, or -NH_2,
R^5	is -H or -F,
R^6 and R^7	are independently -H, C_{1-3}-alkyl, fluoro-C_{1-3}-alkyl, C_{2-4}-alkenyl, fluoro-C_{2-4}-alkenyl, C_{2-4}-alkynyl, fluoro-C_{2-4}-alkynyl, hydroxyl or halogen,
R^8	is a branched C_{1-5}-alkyl group or cyclohexyl group,
R^9	is -H or -F,
–L-#1	is the linker group

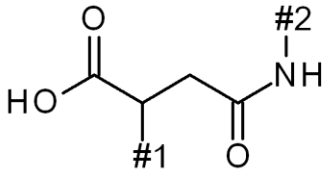
§-$(C(=O))_m$-L1-L2-§§

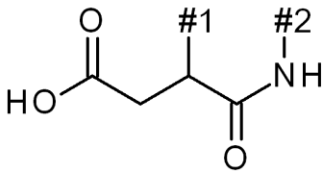
in which

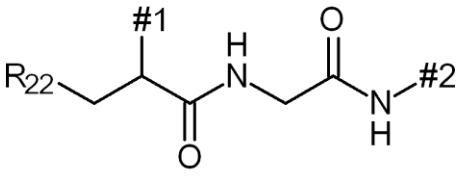
m	is 0 or 1,
§	represents the bond to the KSP inhibitor,
§§	represents the bond to the antibody,
L2	is one of the groups

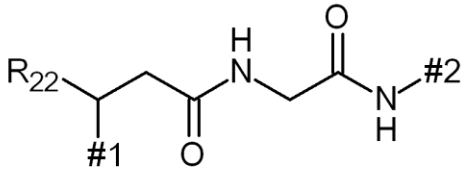


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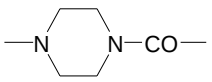
,


or


$\#^1$		is the linkage site to the sulphur atom of the antibody,
$\#^2$		is the linkage site to the L1 group,
L1		is the group

$$\#^1\text{–}(NR^{10})_n\text{-}(G1)_o\text{-}G2\text{-}\#^2$$

in which

R^{10}		is -H, $-NH_2$ or $C_{1\text{-}3}$-alkyl,
G1		is –NHC(=O)- or  ,
n		is 0 or 1,

o is 0 or 1 and

G2 is a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms, composed of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups, which may be interrupted once or more than once, identically or differently, by -O-, -S-, -S(=O)-, -$S(=O)_2$, -NH-, -C(=O)-, -NH-C(=O)-, -C(=O)-NH-, -NMe-, -NHNH-, -$S(=O)_2$-NHNH-, -C(=O)-NHNH- and a 3- to 10-membered aromatic or nonaromatic heterocycle having up to 4 heteroatoms selected from =N-, -O- and –S-, or –S(=O)-, where the straight-chain or branched hydrocarbon chain may be substituted by –NH-C(=O)-NH2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid,

$\#^1$ is the bond to the KSP inhibitor,

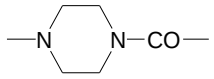
$\#^2$ is the bond to L2 to the antibody,

where one of the substituents R^1 and R^3 is the linker group –L-#1, and the salts, solvates and salts of the solvates thereof, and where the antibodies mentioned in the formulae IVa' or IVa'' or IVa''' are human, humanized or chimeric monoclonal antibodies or an antigen-binding fragment thereof, and n in the formulae IVa' or IVa'' or IVa''' is a number from 1 to 10.

Preferably, the antibody here is an anti-TWEAKR antibody, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment of these.

Particular preference is given to the anti-TWEAKR antibodies TPP-7006, TPP-7007, TPP-10336 and TPP-10337, the anti-B7H3 antibodies TPP-8382 and TPP-8567, the anti-EGFR-antibody cetuximab (TPP-981) and the anti-HER2-antibodies trastuzumab and TPP-1015, or an antigen-binding fragment of these.

Preference is given here to those compounds of the formula (IIe) in which R_3 is defined as alkyl, preferably as C_{1-3} alkyl.

In this context, G2 is preferably  .

Alternatively, the linker –L-#1 may be bonded to a lysine side chain or a lysine residue. In that case, it preferably has the following formula:

-§-$(SG)_x$-L4-C(=O)-§§

in which

§ represents the bond to the KSP inhibitor,

§§ represents the bond to the antibody,

x is 0 or 1,

SG is a cleavable group,

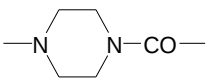
L4 is a single bond or a group

$–(C(=O))_y$-G4-

y is 0 or 1 and

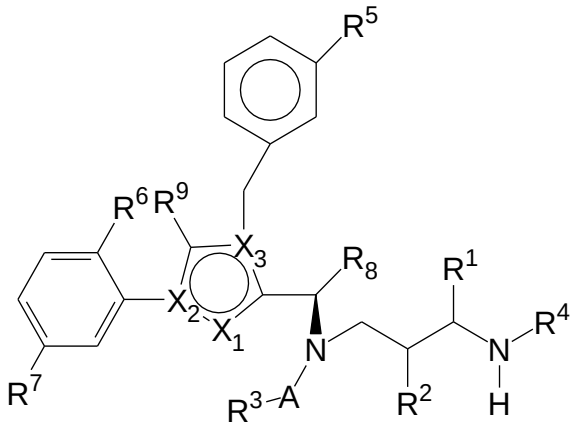
G4 is a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms, composed of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups, which may be interrupted once or more than once, identically or differently, by -O-, -S-, -S(=O)-, $-S(=O)_2$, -NH-, -C(=O)-, -NH-C(=O)-, -C(=O)-NH-, -NMe-, -NHNH-, $-S(=O)_2$-NHNH-, -C(=O)-NHNH- and a 5- to 10-membered aromatic or nonaromatic heterocycle having up to 4 heteroatoms selected from =N-, -O- and –S-, -S(=O)- or $–S(=O)_2$-, where the straight-chain or branched hydrocarbon chain may be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide or sulphonic acid.

In this context, SG is preferably a 2-8 oligopeptide, more preferably a dipeptide.

Preferably, the straight-chain or branched hydrocarbon chain of G4 may be interrupted by 

Embodiment B:

An APDC of the formulae IVa' or IVa'' or IVa''', where D_1 in the formulae IVa' or IVa'' or IVa''' is a compound of the formula (IIf)



(IIf)

in which

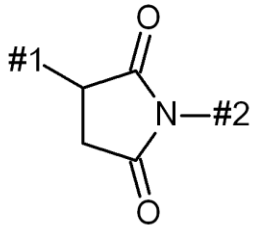
X_1		is N,
X_2		is N and
X_3		is C,
	or	
X_1		is CH,
X_2		is C and
X_3		is N,
	or	
X_1		is NH,
X_2		is C and
X_3		is C,
	or	
X_1		is CH,
X_2		is N and
X_3		is C,
A		is –C(=O)-,
R^1		is –L-#1, -H, -COOH, -C(=O)-NHNH$_2$, -(CH$_2$)$_{1-3}$NH$_2$, -C(=O)-NZ“(CH$_2$)$_{1-3}$NH$_2$ and –C(=O)-NZ“CH$_2$C(=O)-OH,
Z“		is -H or -NH$_2$,

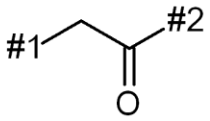
R^2	is -H,
R^4	is a group of the formula (Ia),
R^3	is –L-#1 or a C_{1-10}-alkyl group which may optionally be substituted by –OH, -O-alkyl, -SH, -S-alkyl, -O-C(=O)-alkyl, -O-C(=O)-NH-alkyl, -NH-C(=O)-alkyl, -NH-C(=O)-NH-alkyl, $-S(=O)_n$-alkyl, $-S(=O)_2$-NH-alkyl, -NH-alkyl, $-N(alkyl)_2$ and $-NH_2$,
R^5	is -H or -F,
R^6 and R^7	are independently -H, C_{1-3}-alkyl, fluoro-C_{1-3}-alkyl, C_{2-4}-alkenyl, fluoro-C_{2-4}-alkenyl, C_{2-4}-alkynyl, fluoro-C_{2-4}-alkynyl, hydroxyl or halogen,
R^8	is a branched C_{1-5}-alkyl group,
R^9	is -H or -F,
–L-#1	is the group

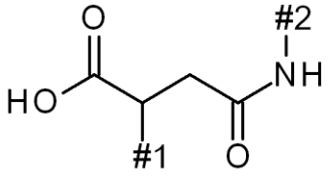
$$\S\text{-}(C(=O))_m\text{-}L1\text{-}L2\text{-}\S\S$$

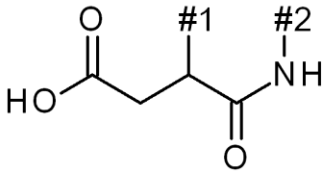
in which

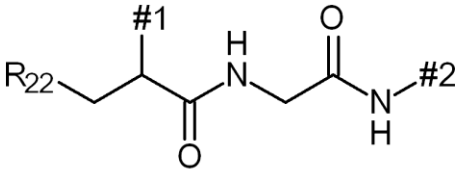
m	is 0 or 1;
§	represents the bond to the KSP inhibitor,
§§	represents the bond to the antibody,
L2	is the group

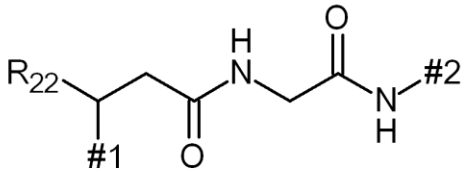


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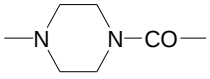
,


,


or


	$\#^1$	is the linkage site to the sulphur atom of the antibody,
	$\#^2$	is the linkage site to the L1 group,
	L1	is the group

$$\#^1\text{-}(NR^{10})_n\text{-}(G1)_o\text{-}G2\text{-}\#^2$$

		in which
	R^{10}	is -H, $-NH_2$ or $C_{1\text{-}C3}$-alkyl,
	G1	is –NH-C(=O)- or  ,
	n	is 0 or 1,

o is 0 or 1,

G2 is a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms, composed of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups, which may be interrupted once or more than once, identically or differently, by -O-, -S-, -S(=O)-, -$S(=O)_2$.-, -NH-, -C(=O)-, -NH-C(=O)-, -C(=O)-NH-, -NMe-, -NHNH-, -$S(=O)_2$-NHNH-, -C(=O)-NHNH- and a 3- to 10-membered, aromatic or nonaromatic heterocycle having up to 4 heteroatoms, selected from =N-, -O- and –S-, or –S(=O)-, where the straight-chain or branched hydrocarbon chain may be substituted by –NH-C(=O)-NH2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid,

$\#^1$ is the bond to the KSP inhibitor,

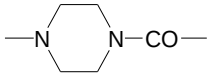
$\#^2$ is the bond to L2 to the antibody,

where one of the substituents R^1 and R^3 is the linker group –L-#1, and the salts, solvates and salts of the solvates thereof, and where the antibodies mentioned in the formulae IVa' or IVa'' or IVa''' are human, humanized or chimeric monoclonal antibodies or an antigen-binding fragment thereof, and n in the formulae IVa' or IVa'' or IVa''' is a number from 1 to 10.

Preferably, the antibody here is an anti-TWEAKR antibody, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment of these.

Particular preference is given to the anti-TWEAKR antibodies TPP-7006, TPP-7007, TPP-10336 and TPP-10337, the anti-B7H3 antibodies TPP-8382 and TPP-8567, the anti-EGFR-antibody cetuximab (TPP-981) and the anti-HER2-antibodies trastuzumab and TPP-1015, or an antigen-binding fragment of these.

Preference is given here to those compounds of the formula (IIf) in which R_3 is defined as alkyl, preferably as C_{1-3} alkyl.

In this context, G2 is preferably 

.

Alternatively, the linker –L-#1 may be bonded to a lysine side chain or a lysine residue. In that case, it preferably has the following formula:

$$-\S-(SG)_x-L4-C(=O)-\S\S$$

in which

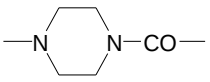
§	represents the bond to the KSP inhibitor,
§§	represents the bond to the antibody,
x	is 0 or 1,
SG	is a cleavable group,
L4	is a single bond or a group

$$–(C=O)_y-G4-$$

y	is 0 or 1 and
G4	is a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms, composed of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups, which may be interrupted once or more than once, identically or differently, by -O-, -S-, -S(=O)-, -S(=O)$_2$, -NH-, -C(=O)-, -NH-C(=O)-, -C(=O)-NH-, -NMe-, -NHNH-, -S(=O)$_2$-NHNH-, -C(=O)-NHNH- and a 5- to 10-membered, aromatic or nonaromatic heterocycle having up to 4 heteroatoms, selected from =N-, -O- and –S-, -S(=O)- or –S(=O)$_2$-, where the straight-chain or branched hydrocarbon chain may be substituted by –NH-C(=O)-NH$_2$, -COOH, -OH, -NH$_2$, sulphonamide, sulphone, sulphoxide, or sulphonic acid.

In this context, SG is preferably a 2-8 oligopeptide, more preferably a dipeptide.

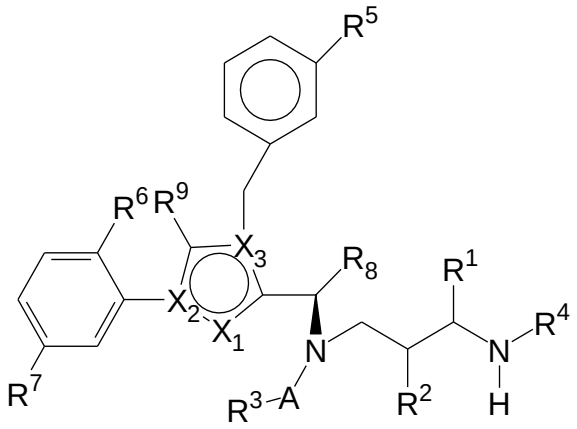
Preferably, the straight-chain or branched hydrocarbon chain of G4 may be interrupted by



.

Embodiment C:

An APDC of the formula IVa' or IVa'' or IVa''', where D_1 in the formulae IVa' or IVa'' or IVa''' is a compound of the formula (IIg)



(IIg)

in which

X_1		is N,
X_2		is N and
X_3		is C
	or	
X_1		is CH,
X_2		is C and
X_3		is N
	or	
X_1		is NH,
X_2		is C and
X_3		is C
	or	
X_1		is CH,
X_2		is N and
X_3		is C,
A		is –C(=O)-,
R^1		is –L-#1,
R^2		is -H,

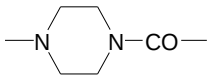
R^4 is a group of the formula (Ia),

R^3 is a C_{1-10}-alkyl group which may optionally be substituted by –OH, -O-alkyl, -SH, -S-alkyl, -O-C(=O)-alkyl, -O-C(=O)-NH-alkyl, -NH-C(=O)-alkyl, -NH-C(=O)-NH-alkyl, $-S(=O)_n$-alkyl, $-S(=O)_2$-NH-alkyl, -NH-alkyl, $-N(alkyl)_2$ or $-NH_2$, or is –MOD,

-MOD is the group

$$–(NR^{10})_n-(G1)_o-G2-H$$

R^{10} is -H or C_1-C_3-alkyl;

G1 is –NH-C(=O)-, -C(=O)-NH- or ,

n is 0 or 1,

o is 0 or 1,

G2 is a straight-chain and/or branched hydrocarbon chain which has 1 to 20 carbon atoms and may be interrupted once or more than once, identically or differently, by -O-, -S-, -SO-, $-S(=O)_2$, $-NR^y$-, $-NR^yC(=O)$-, $-C(=O)NR^y$-, $-NR^yNR^y$-, $-S(=O)_2-NR^yNR^y$-, $-C(=O)-NR^yNR^y-C(=O)$- or $-CR^x=N-O$-, where the straight-chain or branched hydrocarbon chain may be substituted by $–NH-C(=O)-NH_2$, -COOH, -OH, $-NH_2$, sulphonamide, sulphone, sulphoxide, or sulphonic acid,

Rx is -H, C1-C3-alkyl or phenyl,

R^y is -H, phenyl, C1-C10-alkyl, C2-C10-alkenyl or C2-C10-alkynyl, each of which may be substituted by –NH-C(=O)-NH2, -COOH, -OH, $-NH_2$, sulphonamide, sulphone, sulphoxide, or sulphonic acid,

R^5 is -H or -F,

R^6 and R^7 are independently -H, C_{1-3}-alkyl, fluoro-C_{1-3}-alkyl, C_{2-4}-alkenyl, fluoro-C_{2-4}-alkenyl, C_{2-4}-alkynyl, fluoro-C_{2-4}-alkynyl, hydroxyl or halogen,

R^8 is a branched C_{1-5}-alkyl group,

R^9 is -H or -F,

–L-#1 is the linker group

$$§-(C(=O))_m-L1-L2-§§$$

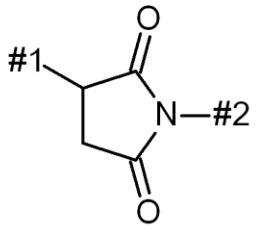
in which

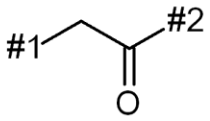
m is 0 or 1,

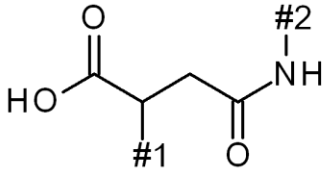
§ represents the bond to the KSP inhibitor,

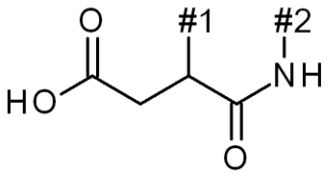
§§ represents the bond to the antibody,

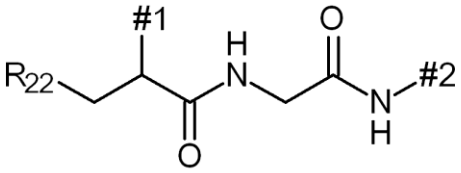
L2 is one of the groups



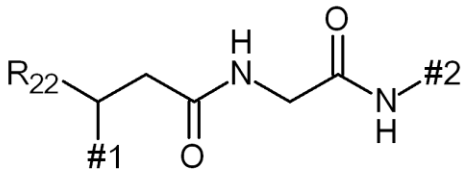
,


,


,


,


or



$#^1$ is the linkage site to the sulphur atom of the antibody,

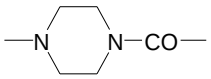
$#^2$ is the linkage site to the L1 group,

L1 is the group

$$#^1–(NR^{10})_n\text{-}(G1)_o\text{-}G2\text{-}#^2$$

in which

R^{10} is -H, $-NH_2$ or C_{1-3}-alkyl,

G1 is –NHC(=O)- or ,

n is 0 or 1,

o is 0 or 1 and

G2 is a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms, composed of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups, which may be interrupted once or more than once, identically or differently, by -O-, -S-, -S(=O)-, $-S(=O)_2$, -NH-, -C(=O)-, -NH-C(=O)-, -C(=O)-NH-, -NMe-, -NHNH-, $-S(=O)_2$-NHNH-, -C(=O)-NHNH- and a 3- to 10-membered aromatic or nonaromatic heterocycle having up to 4 heteroatoms selected from =N-, -O- and –S-, or –S(=O)-, where the straight-chain or branched hydrocarbon chain may be substituted by –NH-C(=O)-NH2, -COOH, -OH, $-NH_2$, sulphonamide, sulphone, sulphoxide, or sulphonic acid,

$\#^1$ is the bond to the KSP inhibitor,

$\#^2$ is the bond to L2 to the antibody,

and the salts, solvates and salts of the solvates thereof, and where the antibodies mentioned in the formulae IVa' or IVa'' or IVa''' are human, humanized or chimeric monoclonal antibodies or an antigen-binding fragment thereof, and n in the formulae IVa' or IVa'' or IVa''' is a number from 1 to 10.

Preferably, the antibody here is an anti-TWEAKR antibody, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment of these.

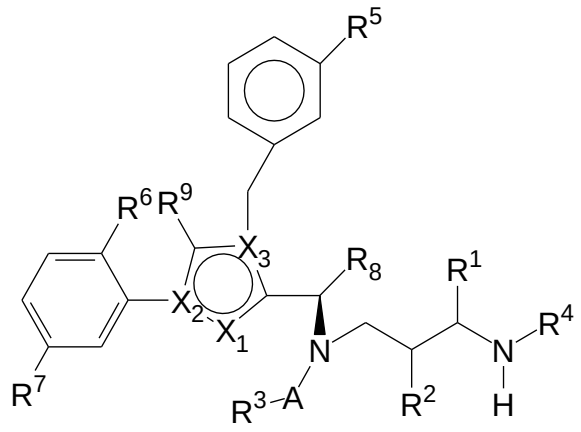
Particular preference is given to the anti-TWEAKR antibodies TPP-7006, TPP-7007, TPP-10336 and TPP-10337, the anti-B7H3 antibodies TPP-8382 and TPP-8567, the anti-EGFR-antibody cetuximab (TPP-981) and the anti-HER2-antibodies trastuzumab and TPP-1015, or an antigen-binding fragment of these.

Preference is given here to those compounds of the formula (IIg) in which R^3 is defined as alkyl, preferably as C_{1-3} alkyl.

In this case, –MOD preferably has at least one COOH- group.

Embodiment D:

An APDC of the formulae IVa' or IVa'' or IVa''', where D_1 in the formulae IVa' or IVa'' or IVa''' is a compound of the formula (IIh)



(IIh)

in which

X_1 is N,
X_2 is N and
X_3 is C

or

X_1 is CH,
X_2 is C and
X_3 is N

or

X_1 is NH,
X_2 is C and
X_3 is C

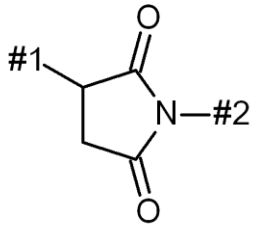
or

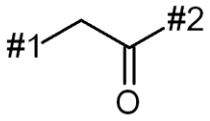
X_1 is CH,

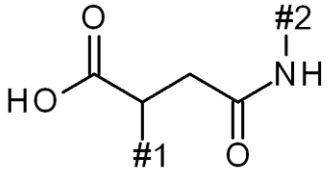
X_2	is N and
X_3	is C,
A	is –C(=O)-,
R^1	is -H or –COOH,
R^2	is -H,
R^4	is a group of the formula Ia,
R^3	is –L-#1,
R^5	is -H or -F,
R^6 and R^7	are independently -H, C_{1-3}-alkyl, fluoro-C_{1-3}-alkyl, C_{2-4}-alkenyl, fluoro-C_{2-4}-alkenyl, C_{2-4}-alkynyl, fluoro-C_{2-4}-alkynyl, hydroxyl or halogen,
R^8	is a branched C_{1-5}-alkyl group,
R^9	is -H or -F,
–L-#1	is the linker group

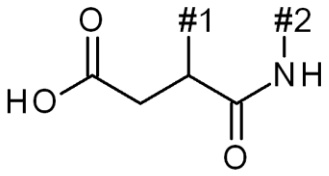
§-$(C(=O))_m$-L1-L2-§§

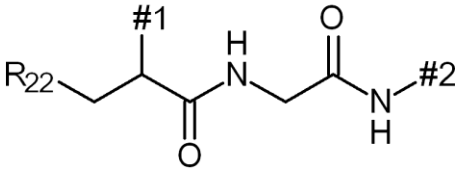
	in which
m	is 0 or 1,
§	represents the bond to the KSP inhibitor,
§§	represents the bond to the antibody,
L2	is one of the groups

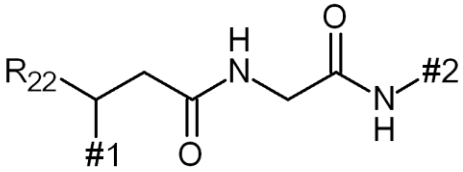


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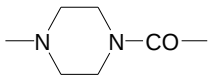
,


or


	$\#^1$	is the linkage site to the sulphur atom of the antibody,
	$\#^2$	is the linkage site to the L1 group,
	L1	is the group

$$\#^1\text{–}(NR^{10})_n\text{-}(G1)_o\text{-}G2\text{-}\#^2$$

in which

	R^{10}	is -H, $-NH_2$ or $C_{1\text{-}3}$-alkyl,
	G1	is –NHC(=O)- or  ,
	n	is 0 or 1,
	o	is 0 or 1 and

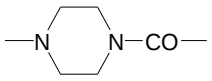
G2 is a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms, composed of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups, which may be interrupted once or more than once, identically or differently, by -O-, -S-, -S(=O)-, -$S(=O)_2$-, -NH-, -C(=O)-, -NH-C(=O)-, -C(=O)-NH-, -NMe-, -NHNH-, -$S(=O)_2$-NHNH-, -C(=O)-NHNH- and a 3- to 10-membered aromatic or nonaromatic heterocycle having up to 4 heteroatoms selected from =N-, -O- and –S-, or –S(=O)-, where the straight-chain or branched hydrocarbon chain may be substituted by –NH-C(=O)-NH2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid,

$\#^1$ is the bond to the KSP inhibitor,

$\#^2$ is the bond to L2 to the antibody, and the salts, solvates and salts of the solvates thereof, and where the antibodies mentioned in the formulae IVa' or IVa'' or IVa''' are human, humanized or chimeric monoclonal antibodies or an antigen-binding fragment thereof, and n in the formulae IVa' or IVa'' or IVa''' is a number from 1 to 10.

Preferably, the antibody here is an anti-TWEAKR antibody, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment of these.

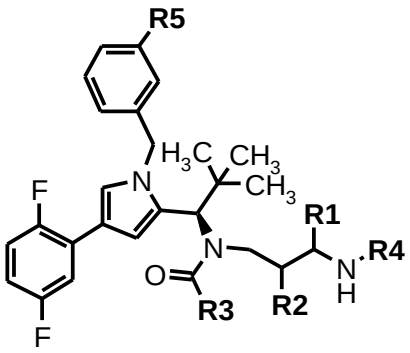
Particular preference is given to the anti-TWEAKR antibodies TPP-7006, TPP-7007, TPP-10336 and TPP-10337, the anti-B7H3 antibodies TPP-8382 and TPP-8567, the anti-EGFR-antibody cetuximab (TPP-981) and the anti-HER2-antibodies trastuzumab and TPP-1015, or an antigen-binding fragment of these.

In this context, G2 is preferably 

.

Embodiment E:

An APDC of the formulae IVa' or IVa'' or IVa''', where D_1 in the formulae IVa' or IVa'' or IVa''' is a compound of the formula (IIi)



(IIi)

in which

R^1 is –L-#1,

–L-#1 is the linker group

§-(C(=O))$_m$-L1-L2-§§

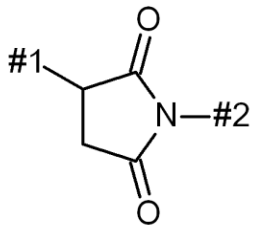
in which

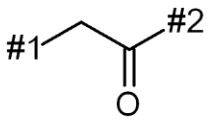
m is 0 or 1,

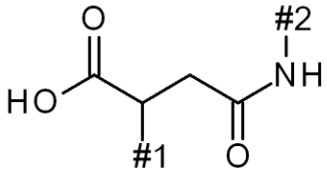
§ represents the bond to the KSP inhibitor,

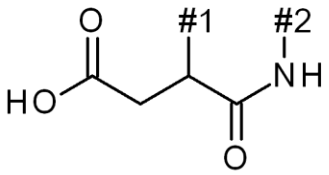
§§ represents the bond to the antibody,

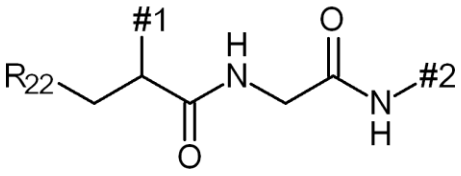
L2 is the group

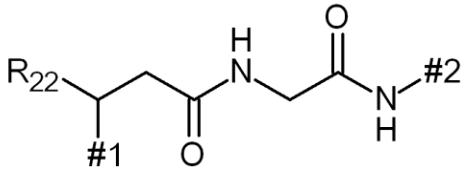


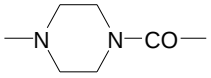
,


,


,


,


or


R^{22}	is –COOH, -C(=O)-O-C_{1-3}-alkyl, -C(=O)-C_{1-3}-alkyl, -C(=O)-NH-C_{1-3}-alkyl or -C(=O)-NH_2,	
$\#^1$	is the linkage site to the sulphur atom of the antibody,	
$\#^2$	is the bond to L1,	
L1	is the group	
		$\#^1$–$(NR^{10})_n$-$(G1)_o$-G2-$\#^2$
	in which	
R^{10}	is –H,	
G1	is –NHC(=O)- or  ,	
n	is 0 or 1,	
o	is 0 or 1,	

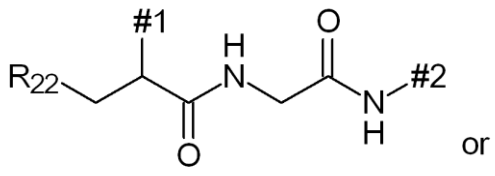
G2	is C_{1-3}-alkyl,
$\#^1$	is the bond to the KSP inhibitor,
$\#^2$	is the bond to L2 to the antibody,
R^2 and R^5	are -H,
R^3	is $-CH_2OH$ and
R^4	is a group of the formula (Ia),

and the salts, solvates and salts of the solvates thereof, and where the antibodies mentioned in the formulae IVa' or IVa'' or IVa''' are human, humanized or chimeric monoclonal antibodies or an antigen-binding fragment thereof, and n in the formulae IVa' or IVa'' or IVa''' is a number from 1 to 10.

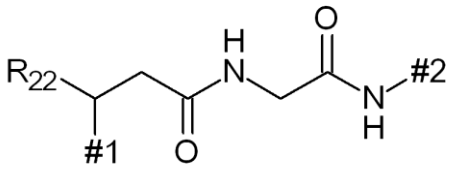
Preference is given to those compounds of the formula (IIi) in which R^{22} is –COOH.

In a conjugate according to the invention or in a mixture of the conjugates according to the invention, the bonds to a cysteine residue of the antibody, based in each case on the total number of bonds of the linker to the antibody, are preferably present to an extent of more than 80%, more preferably to an extent of more than 90%.

Particular preference is given here in accordance with the invention to conjugates having, as L2, the group



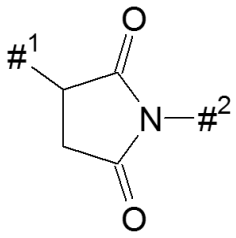
or



in which R^{22} has the definitions given above.

In general, conjugates having both kinds of L2 group are present, preferably in a ratio of from 60:40 to 40:60, based on the number of bonds to the antibody.

The remaining bonds are then present with the structure



in which #1 and #2 have the definitions given above.

Preferably, the antibody here is an anti-TWEAKR antibody, an anti-EGFR antibody, an anti-B7H3 antibody or an anti-HER2 antibody or an antigen-binding fragment of these

Particular preference is given to the anti-TWEAKR antibodies TPP-7006, TPP-7007, TPP-10336 and TPP-10337, the anti-B7H3 antibodies TPP-8382 and TPP-8567, the anti-EGFR-antibody cetuximab (TPP-981) and the anti-HER2-antibodies trastuzumab and TPP-1015, or an antigen-binding fragment of these.

Therapeutic uses

The hyperproliferative diseases, for the treatment of which the compounds according to the invention may be employed, include in particular the group of cancer and tumour diseases. In the context of the present invention, these are understood to mean especially the following diseases, but without any limitation thereto: mammary carcinomas and mammary tumours (mammary carcinomas including ductal and lobular forms, also *in situ*), tumours of the respiratory tract (small-cell and non-small cell carcinoma, bronchial carcinoma), cerebral tumours (e.g. of the brain stem and of the hypothalamus, astrocytoma, ependymoma, glioblastoma, glioma, medulloblastoma, meningioma and neuro-ectodermal and pineal tumours), tumours of the digestive organs (carcinomas of the oesophagus, stomach, gall bladder, small intestine, large intestine, rectum and anal carcinomas), liver tumours (inter alia hepatocellular carcinoma, cholangiocarcinoma and mixed hepatocellular cholangiocarcinoma), tumours of the head and neck region (larynx, hypopharynx, nasopharynx, oropharynx, lips and oral cavity carcinomas, oral melanomas), skin tumours (basaliomas, spinaliomas, squamous cell carcinomas, Kaposi's sarcoma, malignant melanoma, non-melanomatous skin cancer, Merkel cell skin cancer, mast cell tumours), tumours of soft tissue (inter alia soft tissue sarcomas, osteosarcomas, malignant fibrous histiocytomas, chondrosarcomas, fibrosarcomas, hemangiosarcomas,

leiomyosarcomas, liposarcomas, lymphosarcomas and rhabdomyosarcomas), tumours of the eyes (inter alia intraocular melanoma and retinoblastoma), tumours of the endocrine and exocrine glands (e.g. of the thyroid and parathyroid glands, pancreas and salivary gland carcinomas, adenocarcinomas), tumours of the urinary tract (tumours of the bladder, penis, kidney, renal pelvis and ureter) and tumours of the reproductive organs (carcinomas of the endometrium, cervix, ovary, vagina, vulva and uterus in women and carcinomas of the prostate and testes in men). These also include proliferative diseases of the blood, the lymph system and the spinal cord, in solid form and as circulating cells, such as leukaemias, lymphomas and myeloproliferative diseases, for example acute myeloid, acute lymphoblastic, chronic lymphocytic, chronic myelogenous and hairy cell leukaemia, and AIDS-correlated lymphomas, Hodgkin's lymphomas, non-Hodgkin's lymphomas, cutaneous T cell lymphomas, Burkitt's lymphomas and lymphomas in the central nervous system.

These well-characterized diseases in humans can also occur with a comparable aetiology in other mammals and can likewise be treated there with the compounds of the present invention.

The treatment of the cancer diseases mentioned above with the compounds according to the invention comprises both a treatment of the solid tumours and a treatment of metastasizing or circulating forms thereof.

In the context of this invention, the term "treatment" or "treat" is used in the conventional sense and means attending to, caring for and nursing a patient with the aim of combating, reducing, attenuating or alleviating a disease or health abnormality, and improving the living conditions impaired by this disease, as, for example, in the event of a cancer.

The present invention thus further provides for the use of the compounds of the invention for treatment and/or prevention of disorders, especially of the aforementioned disorders.

The present invention further provides for the use of the compounds of the invention for production of a medicament for treatment and/or prevention of disorders, especially of the aforementioned disorders.

The present invention further provides for the use of the compounds of the invention in a method for treatment and/or prevention of disorders, especially of the aforementioned disorders.

The present invention further provides a process for treatment and/or prevention of disorders, especially of the aforementioned disorders, using an effective amount of at least one of the compounds of the invention.

The compounds of the invention can be used alone or, if required, in combination with one or more other pharmacologically active substances, provided that this combination does not lead to undesirable and unacceptable side effects. The present invention therefore further provides medicaments comprising at least one of the compounds of the invention and one or more further drugs, especially for treatment and/or prevention of the aforementioned disorders.

For example, the compounds of the present invention can be combined with known anti-hyper-proliferative, cytostatic or cytotoxic substances for the treatment of cancer diseases. Examples of suitable combination drugs include:

131I-chTNT, abarelix, abiraterone, aclarubicin, adalimumab, ado-trastuzumab emtansin, afatinib, aflibercept, aldesleukin, alemtuzumab, alendronic acid, alitretinoin, altretamine, amifostine, aminoglutethimide, hexyl 5-aminolevulinate, amrubicin, amsacrine, anastrozole, ancestim, anethole dithiolethione, anetumab ravtansin, angiotensin II, antithrombin III, aprepitant, arcitumomab, arglabin, arsenic trioxide, asparaginase, atezolizumab, axitinib, azacitidine, belotecan, bendamustine, besilesomab, belinostat, bevacizumab, bexaroten, bicalutamide, bisantrene, bleomycin, blinatumomab, bortezomib, buserelin, bosutinib, brentuximab vedotin, busulfan, cabazitaxel, cabozantinib, calcitonin, calcium folinate, calcium levofolinate, capecitabine, capromab, carbamazepine, carboplatin, carboquone, carfilzomib, carmofur, carmustine, catumaxomab, celecoxib, celmoleukin, ceritinib, cetuximab, chlorambucil, chlormadinone, chlormethine, cidofovir, cinacalcet, cisplatin, cladribine, clodronic acid, clofarabine, cobimetinib, copanlisib(BAY 80-6946), crisantaspase, crizotinib, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daratumumab, dabrafenib, dasatinib, daunorubicin, decitabine, degarelix, denileukin diftitox, denosumab, depreotide, deslorelin, dexrazoxane, dibrospidium chloride, dianhydrogalactitol, diclofenac, docetaxel, dolasetron, doxifluridine, doxorubicin, doxorubicin + estrone, dronabinol, edrecolomab, elliptinium acetate, endostatin, enocitabine, enzalutamide, epirubicin, epitiostanol, epoetin-alfa, epoetin-beta, epoetin-zeta, eptaplatin, eribulin, erlotinib, esomeprazole, estramustine, etoposide, ethinylestradiol, everolimus, exemestane, fadrozole, fentanyl, fluoxymesterone, floxuridine, fludarabine, fluoruracil, flutamide, folic acid, formestan,

fosaprepitant, fotemustine, fulvestrant, gadobutrol, gadoteridol, gadoteric acid meglumine salt, gadoversetamide, gadoxetic acid disodium salt (Gd-EOB-DTPA disodium salt), gallium nitrate, ganirelix, gefitinib, gemcitabine, gemtuzumab, glucarpidase, glutoxim, goserelin, granisetron, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), histamine dihydrochloride, histrelin, hydroxycarbamide, I-125 seeds, ibandronic acid, ibritumomab tiuxetan, ibrutinib, idarubicin, ifosfamide, imatinib, imiquimod, improsulfan, indisetron, incadronic acid, ingenol mebutate, interferon alfa, interferon beta, interferon-gamma, iobitridol, iobenguane (123I), iomeprol, ipilimumab, irinotecan, itraconazole, ixabepilone, ixazomib, lanreotide, lansoprazole, lansoprazole, lapatinib, lasocholine, lenalidomide, lenvatinib, lenograstim, lentinan, letrozole, leuprorelin, levamisole, levonorgestrel, levothyroxin-sodium, lipegfilgrastim, lisurid, lobaplatin, lomustin, lonidamin, masoprocol, medroxyprogesterone, megestrol, melarsoprol, melphalan, mepitiostane, mercaptopurine, mesna, methadone, methotrexate, methoxsalen, methyl aminolevulinate, methylprednisolone, methyltestosterone, metirosine, mifamurtide, miltefosine, miriplatin, mitobronitol, mitoguazone, mitolactol, mitomycin, mitotane, mitoxantrone, mogamulizumab, molgramostim, mopidamol, morphine hydrochloride, morphine sulfate, nabilone, nabiximols, nafarelin, naloxone + pentazocine, naltrexone, nartograstim, necitumumab, nedaplatin, nelarabine, neridronic acid, netupitant/palonosetron, nivolumab pentetreotide, nilotinib, nilutamide, nimorazole, nimotuzumab, nimustine, nintedanib, nitracrin, nivolumab, obinutuzumab, octreotide, ofatumumab, olaparib, olaratumab, omacetaxin mepesuccinate, omeprazole, ondansetron, orgotein, orilotimod, osimertinib, oxaliplatin, oxycodone, oxymetholone, ozogamicin, p53 gene therapy, paclitaxel, palbociclib, palifermin, palladium-103 seed, palonosetron, pamidronic acid, panitumumab, panobinostat, pantoprazole, pazopanib, pegaspargase, pembrolizumab, peg interferon alfa-2b, pembrolizumab, pemetrexed, pentostatin, peplomycin, perflubutane, perfosfamide, pertuzumab, picibanil, pilocarpine, pirarubicin, pixantrone, plerixafor, plicamycin, poliglusam, polyestradiol phosphate, polyvinylpyrrolidone + sodium hyaluronate, polysaccharide-K, pomalidomide, ponatinib, porfimer-sodium, pralatrexate, prednimustine, prednisone, procarbazine, procodazole, propranolol, quinagolide, rabeprazole, racotumomab, radium-223 chloride, radotinib, raloxifen, raltitrexed, ramosetron, ramucirumab, ranimustine, rasburicase, razoxane, refametinib, regorafenib, risedronic acid, rhenium-186 etidronate, rituximab, rolapitant, romidepsin, romurtide, roniciclib, samarium (153Sm) lexidronam, satumomab, secretin, siltuximab, sipuleucel-t, sizofiran, sobuzoxane, sodium glycididazole, sonidegib, sorafenib, stanozolol,

streptozocin, sunitinib, talaporfin, talimogen laherparepvec, tamibarotene, tamoxifen, tapentadol, tasonermin, teceleukin, technetium (99mTc) nofetumomab merpentan, 99mTc-HYNIC-[Tyr3]-octreotide, tegafur, tegafur + gimeracil + oteracil, temoporfin, temozolomide, temsirolimus, teniposide, testosterone, tetrofosmin, thalidomide, thiotepa, thymalfasin, thyrotropin alfa, tioguanine, tocilizumab, topotecan, toremifene, tositumomab, trabectedin, trametinib, tramadol, trastuzumab, treosulfan, tretinoin, trifluridine + tipiracil, trametinib, trilostane, triptorelin, trofosfamide, thrombopoietin, ubenimex, valrubicin, vandetanib, vapreotide, valatinib, vemurafenib, vinblastine, vincristine, vindesine, vinflunine, vinorelbine, vismodegib, vorinostat, yttrium-90 glass microbeads, zinostatin, zinostatin-stimalamer, zoledronic acid, zorubicin.

In addition, the antibodies may be selected from the class of the MPS1 inhibitors or antibodies against the targets OX-40, CD137 / 4-1BB, DR3, IDO1 / IDO2, LAG-3 and CD40.

In addition, the compounds according to the invention can also be used in combination with radiotherapy and/or surgical intervention.

Generally, the following aims can be pursued with the combination of compounds of the present invention with other cytostatically or cytotoxically active agents:

- improved efficacy in slowing the growth of a tumour, in reducing its size or even in completely eliminating it, compared with treatment with an individual active ingredient;
- the possibility of using the chemotherapeutics used in a lower dosage than in the case of monotherapy;
- the possibility of a more tolerable therapy with fewer side effects compared with individual administration;
- the possibility of treatment of a broader spectrum of neoplastic disorders;
- the achievement of a higher rate of response to the therapy;
- a longer survival time of the patient compared with present-day standard therapy.

In addition, the compounds according to the invention can also be used in combination with radiotherapy and/or surgical intervention.

The present invention further provides medicaments which comprise at least one compound of the invention, typically together with one or more inert, non-toxic, pharmaceutically suitable excipients, and for the use thereof for the aforementioned purposes.

The compounds according to the invention can act systemically and/or locally. For this purpose, they can be administered in a suitable manner, for example parenterally, possibly inhalatively or as implants or stents.

The compounds according to the invention can act systemically and/or locally. For this purpose, they can be administered in a suitable manner, for example by the oral, parenteral, pulmonal, nasal, sublingual, lingual, buccal, rectal, vaginal, dermal, transdermal, conjunctival or otic route, or as an implant or stent.

The compounds according to the invention can be administered in administration forms suitable for these administration routes.

Suitable administration forms for oral administration are those which function according to the prior art and deliver the inventive compounds rapidly and/or in modified fashion, and which contain the inventive compounds in crystalline and/or amorphized and/or dissolved form, for example tablets (uncoated or coated tablets, for example having enteric coatings or coatings which are insoluble or dissolve with a delay, which control the release of the compound according to the invention), tablets which disintegrate rapidly in the mouth, or films/wafers, films/lyophilizates, capsules (for example hard or soft gelatin capsules), sugar-coated tablets, granules, pellets, powders, emulsions, suspensions, aerosols or solutions.

Parenteral administration can bypass an absorption step (for example intravenously, intraarterially, intracardially, intraspinally or intralumbally) or include an absorption (for example intramuscularly, subcutaneously, intracutaneously, percutaneously or intraperitoneally). Administration forms suitable for parenteral administration include

preparations for injection and infusion in the form of solutions, suspensions, emulsions, lyophilizates or sterile powders.

Suitable administration forms for the other administration routes are, for example, pharmaceutical forms for inhalation (including powder inhalers, nebulizers), nasal drops, solutions or sprays; tablets for lingual, sublingual or buccal administration, films/wafers or capsules, suppositories, eye drops, eye ointments, eyewashes, ocular inserts, ear drops, sprays, powders, washes or tampons, vaginal capsules, aqueous suspensions (lotions, shaking mixtures), lipophilic suspensions, emulsions, microemulsions, ointments, creams, transdermal therapeutic systems (for example patches), milk, pastes, foams, dusting powders, implants or stents.

Preference is given to parenteral administration, especially intravenous administration.

The compounds according to the invention can be converted to the administration forms mentioned. This can be accomplished in a manner known per se by mixing with pharmaceutically suitable excipients. These excipients include

- fillers and carriers (for example cellulose, microcrystalline cellulose, for example Avicel®, lactose, mannitol, starch, calcium phosphates, for example Di-Cafos®),
- ointment bases (for example vaseline, paraffins, triglycerides, waxes, wool wax, wool wax alcohols, lanolin, hydrophilic ointment, polyethylene glycols),
- suppository bases (for example polyethylene glycols, cocoa butter, hard fat),
- solvents (e.g. water, ethanol, isopropanol, glycerol, propylene glycol, mid-chain triglycerides fatty oils, liquid polyethylene glycols, paraffins),
- surfactants, emulsifiers, dispersants or wetting agents (for example sodium dodecylsulphate, lecithin, phospholipids, fatty alcohols, for example Lanette®, sorbitan fatty acid esters, for example Span®, polyoxyethylene sorbitan fatty acid esters, for example Tween®, polyoxyethylene fatty acid glycerides, for example Cremophor®, polyoxyethylene fatty acid esters, polyoxyethylene fatty alcohol ethers, glycerol fatty acid esters, poloxamers, for example Pluronic®),
- buffer substances, and also acids and bases (for example phosphates, carbonates, citric acid, acetic acid, hydrochloric acid, sodium hydroxide, ammonium carbonate, trometamol, triethanolamine),

- isotonizing agents (for example glucose, sodium chloride),
- adsorbents (for example finely divided silicas),
- viscosity-increasing agents, gel formers, thickeners or binders (for example polyvinylpyrrolidone, methyl cellulose, hydroxypropyl methyl cellulose, hydroxypropyl cellulose, carboxymethyl cellulose-sodium, starch, carbomers, polyacrylic acids, for example Carbopol®, alginates, gelatins),
- disintegrants (for example modified starch, carboxymethyl cellulose-sodium, sodium starch glycolate, for example Explotab®, crosslinked polyvinylpyrrolidone, croscarmellose-sodium, for example AcDiSol®),
- flow regulators, lubricants, glidants and mould release agents (for example magnesium stearate, stearic acid, talc, finely divided silicas, for example Aerosil®),
- coating agents (for example sugar, shellac) and film formers for films or diffusion membranes with fast or modified dissolution (for example by polyvinylpyrrolidones, for example Kollidon®, polyvinyl alcohol, hydroxypropyl methyl cellulose, hydroxypropyl cellulose, ethyl cellulose, hydroxypropyl methyl cellulose phthalate, cellulose acetate, cellulose acetate phthtalate, polyacrylates, polymethacrylates, for example Eudragit®),
- capsule materials (e.g. gelatins, hydroxypropyl methyl cellulose),
- synthetic polymers (for example polylactides, polyglycolides, polyacrylates, polymethacrylates, for example Eudragit®, polyvinylpyrrolidones, for example Kollidon®, polyvinyl alcohols, polyvinyl acetate, polyethylene oxides, polyethylene glycols and the copolymers and block copolymers thereof),
- plasticizers (for example polyethylene glycols, propylene glycol, glycerol, triacetin, triacetyl citrate, dibutyl phthalate),
- penetration enhancers,
- stabilizers (e.g. antioxidants, for example ascorbic acid, ascorbyl palmitate, sodium ascorbate, butylhydroxyanisole, butylhydroxytoluene, propyl gallate),
- preservatives (for example parabens, sorbic acid, thiomersal, benzalkonium chloride, chlorhexidine acetate, sodium benzoate),
- dyes (e.g. inorganic pigments, for example iron oxides, titanium dioxide),
- aromas, sweeteners, flavour and/or odour correctors.

The present invention further provides pharmaceutical compositions comprising at least one compound according to the invention, typically together with one or more pharmaceutically suitable excipients, and the use thereof for the aforementioned purposes.

In general, it has been found to be advantageous in the case of parenteral administration to administer amounts of about 0.1 to 20 mg/kg, preferably about 0.3 to 7 mg/kg, of body weight to achieve effective results.

It may nevertheless be necessary in some cases to deviate from the stated amounts, specifically as a function of body weight, route of administration, individual response to the active ingredient, nature of the preparation and time or interval over which administration takes place. Thus in some cases it may be sufficient to manage with less than the abovementioned minimum amount, while in other cases the upper limit mentioned must be exceeded. In the case of administration of greater amounts, it may be advisable to divide them into several individual doses over the day.

The compounds according to the invention may also take the form of isotopic variants. The invention therefore encompasses one or more isotopic variants of the compounds according to the invention, especially deuterium-containing compounds.

The term "isotopic variant" of a compound or reagent is defined as a compound with an unnatural fraction of one or more isotopes from which such a compound is constituted.

The term "isotopic variant of the compounds according to the invention" is defined as a compound according to the invention with an unnatural fraction of one or more isotopes from which such a compound is constituted.

The expression "unnatural fraction" is understood to mean a fraction of such an isotope higher than its natural frequency. The natural frequencies of isotopes to be employed in this connection can be found in "Isotopic Compositions of the Elements 1997", Pure Appl. Chem., 70(1), 217-235, 1998.

Examples of such isotopes are stable and radioactive isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulphur, fluorine, chlorine, bromine and iodine, such as ^{2}H

(deuterium), ^{3}H (tritium), ^{11}C, ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{32}P, ^{33}P, ^{33}S, ^{34}S, ^{35}S, ^{36}S, ^{18}F, ^{36}Cl, ^{82}Br, ^{123}I, ^{124}I, ^{125}I, ^{129}I and ^{131}I.

With regard to the treatment and/or prophylaxis of the disorders specified here, the isotopic variant(s) of the compounds according to the invention preferably contain deuterium ("deuterium-containing compounds according to the invention"). Isotopic variants of the compounds according to the invention into which one or more radioactive isotopes such as ^{3}H or ^{14}C have been incorporated are beneficial, for example, in medicament and/or substrate tissue distribution studies. Because of their easy incorporability and detectability, these isotopes are particularly preferred. It is possible to incorporate positron-emitting isotopes such as ^{18}F or ^{11}C into a compound according to the invention. These isotopic variants of the compounds according to the invention are suitable for use in in vivo imaging applications. Deuterium-containing and ^{13}C-containing compounds according to the invention can be used within preclinical or clinical studies in mass spectrometry analyses.

Isotopic variants of the compounds according to the invention can generally be prepared by processes known to those skilled in the art as described in the schemes and/or examples described here, by replacing a reagent with an isotopic variant of the reagent, preferably a deuterium-containing reagent. According to the desired deuteration sites, in some cases, deuterium from D_2O can either be incorporated directly into the compounds or into reagents which can be used for the synthesis of such compounds. Another useful reagent for incorporation of deuterium into molecules is deuterium gas. A rapid route to the incorporation of deuterium is the catalytic deuteration of olefinic bonds and acetylenic bonds. For direct exchange of hydrogen for deuterium in hydrocarbons containing functional groups, it is also possible to use metal catalysts (i.e. Pd, Pt and Rh) in the presence of deuterium gas. Various deuterated reagents and synthesis units are commercially available from companies like, for example, C/D/N Isotopes, Quebec, Canada; Cambridge Isotope Laboratories Inc., Andover, MA, USA; and CombiPhos Catalysts, Inc., Princeton, NJ, USA.

The term "deuterium-containing compound" is defined as a compound according to the invention in which one or more hydrogen atoms have been replaced by one or more deuterium atoms and in which the frequency of deuterium in every deuterated position in the compound of the general formula (I) is higher than the natural frequency of deuterium, which is about 0.015%. More particularly, in a deuterium-containing compound according to the invention, the frequency of deuterium in every deuterated position in the compound

of the general formula (I) is higher than 10%, 20%, 30%, 40%, 50%, 60%, 70% or 80%, preferably higher than 90%, 95%, 96% or 97%, even further preferably higher than 98% or 99%, in this position or these positions. It will be apparent that the frequency of deuterium in every deuterated position is independent of the frequency of deuterium in other deuterated positions.

Through the selective incorporation of one or more deuterium atoms into a compound according to the invention, it is possible to alter the physicochemical properties (for example acidity [C. L. Perrin, et al., *J. Am. Chem. Soc.*, 2007, 129, 4490], basicity [C. L. Perrin et al., J. Am. Chem. Soc., 2005, 127, 9641], lipophilicity [B. Testa et al., Int. J. Pharm., 1984, 19(3), 271]) and/or the metabolic profile of the molecule and cause changes in the ratio of parent compound to metabolites or the amounts of metabolites formed. Such changes may lead to particular therapeutic benefits and therefore be preferable under particular circumstances. Reduced rates of metabolism and metabolic switching, where the ratio of metabolites is changed, have been reported (A. E. Mutlib et al., Toxicol. Appl. Pharmacol., 2000, 169, 102). These changes in the exposure to parent drug and metabolites can have important consequences with respect to the pharmacodynamics, tolerability and efficacy of a deuterium-containing compound according to the invention. In some cases deuterium substitution reduces or eliminates the formation of an undesired or toxic metabolite and enhances the formation of a desired metabolite (e.g. Nevirapine: A. M. Sharma et al., Chem. Res. Toxicol., 2013, 26, 410; Efavirenz: A. E. Mutlib et al., Toxicol. Appl. Pharmacol., 2000, 169, 102). In other cases the major effect of deuteration is to reduce the rate of systemic clearance. As a result, the biological half-life of the compound is increased. The potential clinical benefits would include the ability to maintain similar systemic exposure with decreased peak levels and increased trough levels. This could result in lower side effects and enhanced efficacy, depending on the particular compound's pharmacokinetic/pharmacodynamic relationship. Examples of this deuterium effect are ML-337 (C. J. Wenthur et al., J. Med. Chem., 2013, 56, 5208) and odanacatib (K. Kassahun et al., WO2012/112363). Still other cases have been reported in which reduced rates of metabolism result in an increase in exposure of the drug without changing the rate of systemic clearance (e.g. Rofecoxib: F. Schneider et al., Arzneim. Forsch. Drug. Res., 2006, 56, 295; Telaprevir: F. Maltais et al., J. Med. Chem., 2009, 52, 7993). Deuterated drugs showing this effect may have reduced dosing requirements (e.g. lower number of doses or lower dosage to achieve the desired effect) and/or may produce lower metabolite loads.

A compound according to the invention may have two or more potential attack sites for metabolization. To optimize the above-described effects on physicochemical properties and metabolic profile, deuterium-containing compounds according to the invention having a certain pattern of one or more deuterium-hydrogen exchange(s) can be selected. More particularly, the deuterium atom(s) of deuterium-containing compound(s) according to the invention is/are bonded to a carbon atom and/or is/are at those positions in the compounds according to the invention that are attack sites for metabolizing enzymes, for example cytochrome P_{450}.

Examples

The examples which follow illustrate the executability of the present invention, the invention is not restricted solely to these examples.

Unless stated otherwise, the percentages in the tests and examples which follow are percentages by weight; parts are parts by weight. Solvent ratios, dilution ratios and concentration data for the liquid/liquid solutions are based in each case on volume.

Synthesis routes:

By way of example for the working examples, the schemes which follow show illustrative synthesis routes.

In these schemes, according to formula IIa, the R^4 substituent on the amino group $-NHR^4$ may be the Z_1-(C=O)$_{(0-1)}$-(P3)$_{(0-2)}$-P2-NH-CH(CH_2C(=O)NH_2)-C(=O)- group.

In this context,

P2 is a D-amino acid selected from the group of D-Gly, D-Pro, D-Ala, D-Val, D-Nva, D-Leu, D-Ile, D-Met, D-Phe, D-Tyr, D-Trp, D-Ser, D-Thr, D-Cys, D-Asn, D-Gln, D-Asp, D-Glu, D-Lys, D-Arg, D-citrulline and D-His,

P3 is an L- or D-amino acid selected from the group Gly, Pro, Ala, Val, Nva, Leu, Ile, Met, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Asp, Glu, Lys, Arg, citrulline and His,

Z_1 is a C_{1-10}-alkyl, C_{5-10}-aryl or C_{6-10}-aralkyl, C_{5-10}-heteroalkyl, C_{1-10}-alkyl-O-C_{6-10}-aryl, C_{5-10}-heterocycloalkyl, heteroaryl, heteroarylalkyl, C_{5-10}-

heteroarylalkoxy, C_{1-10}-alkoxy, C_{6-10}-aryloxy, C_{6-10}-aryl-C_{1-10}-alkyloxy or C_{6-10}-aralkoxy, C_{5-10}-heteroalkoxy, C_{1-10}-alkyl-O-C_{6-10}-aryloxy- or C_{5-10}-heterocycloalkoxy group which may be mono- or polysubstituted by $-NH_2$, -C(=O)-, -NH-alkyl, $-N(alkyl)_2$, -NH-C(=O)-alkyl, -N(alkyl)-C(=O)-alkyl, $-S(=O)_3$-H, $-S(=O)_2-NH_2$, $-S(=O)_2-N(alkyl)_2$, -COOH, $-C(=O)NH_2$, -C(=O)-$N(alkyl)_2$ or –OH,

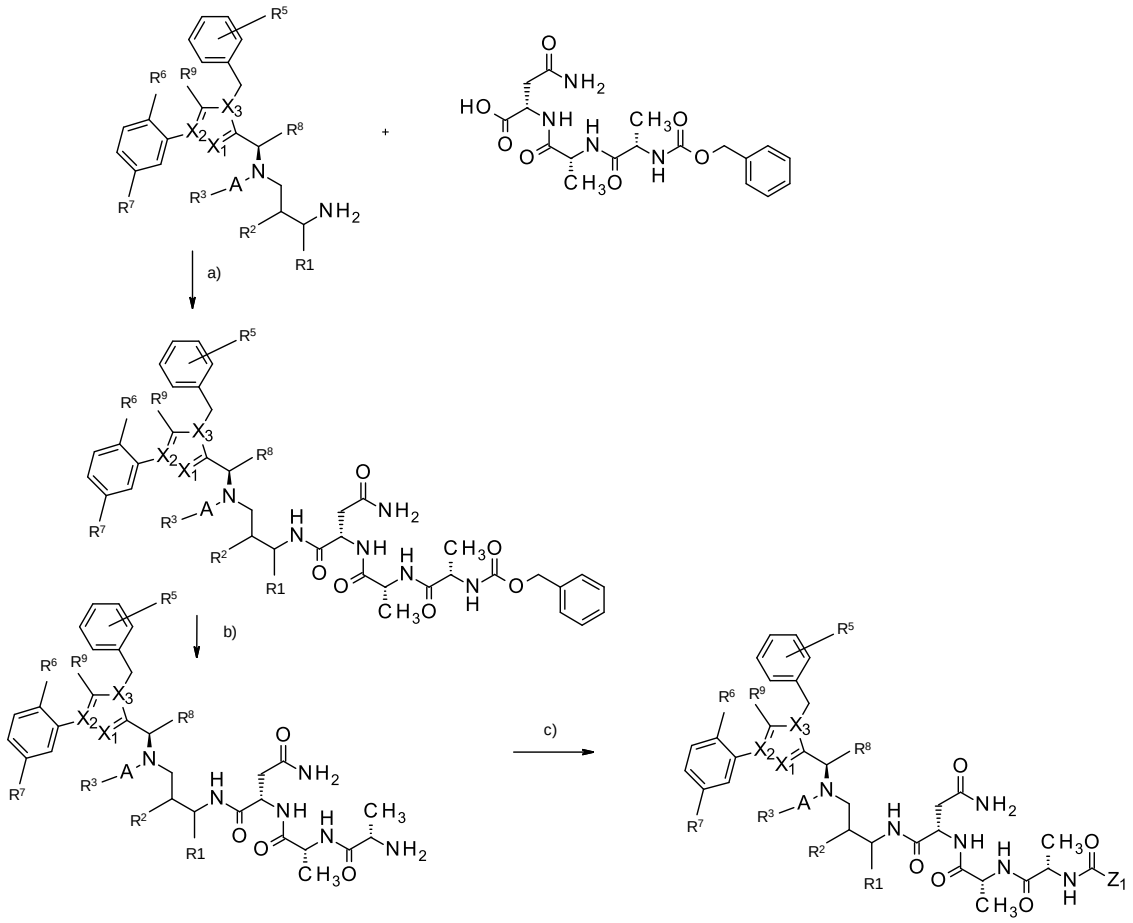
or is -H or a $-(CH_2)_{0-1}$-Ox-(CH_2CH_2O)v-R^1 group,

x is 0 or 1,

v is a number from 1 to 20 and

R^1 has the definitions given in the formula (II).

Scheme 1:

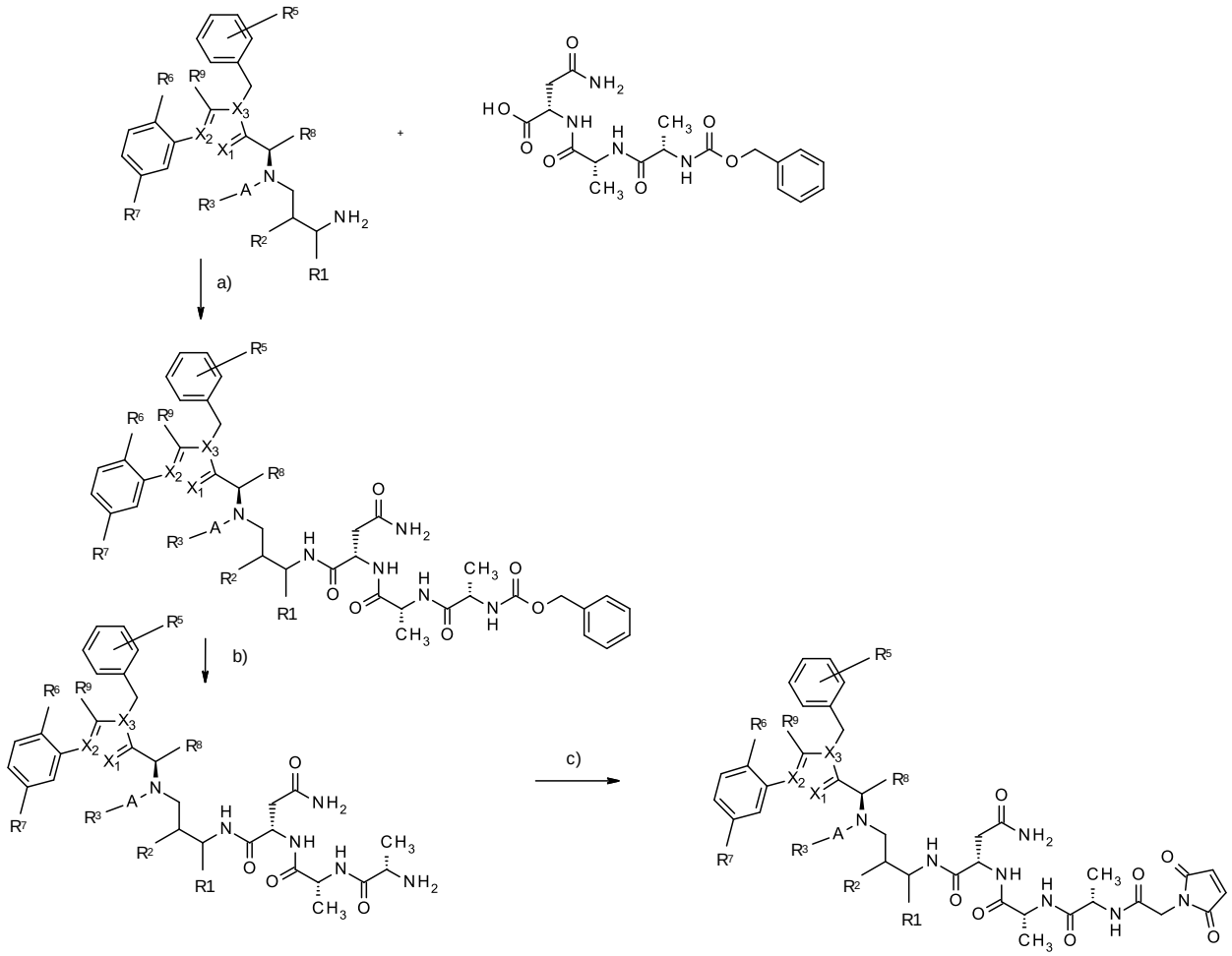


[a): HATU, DMF, N,N-diisopropylethylamine, RT or EDCI, HOBT, N,N-diisopropylethylamine, DMF, RT b) H_2, 10% Pd-C, MeOH, RT; c) Z_1-COOH, EDCI, HOBT,

N,N-diisopropylethylamine, DMF, RT or Z_1-COOH, HATU, N,N-diisopropylethylamine, DMF, RT or Z_1-COOSu, N,N-diisopropylethylamine, DMF, RT]

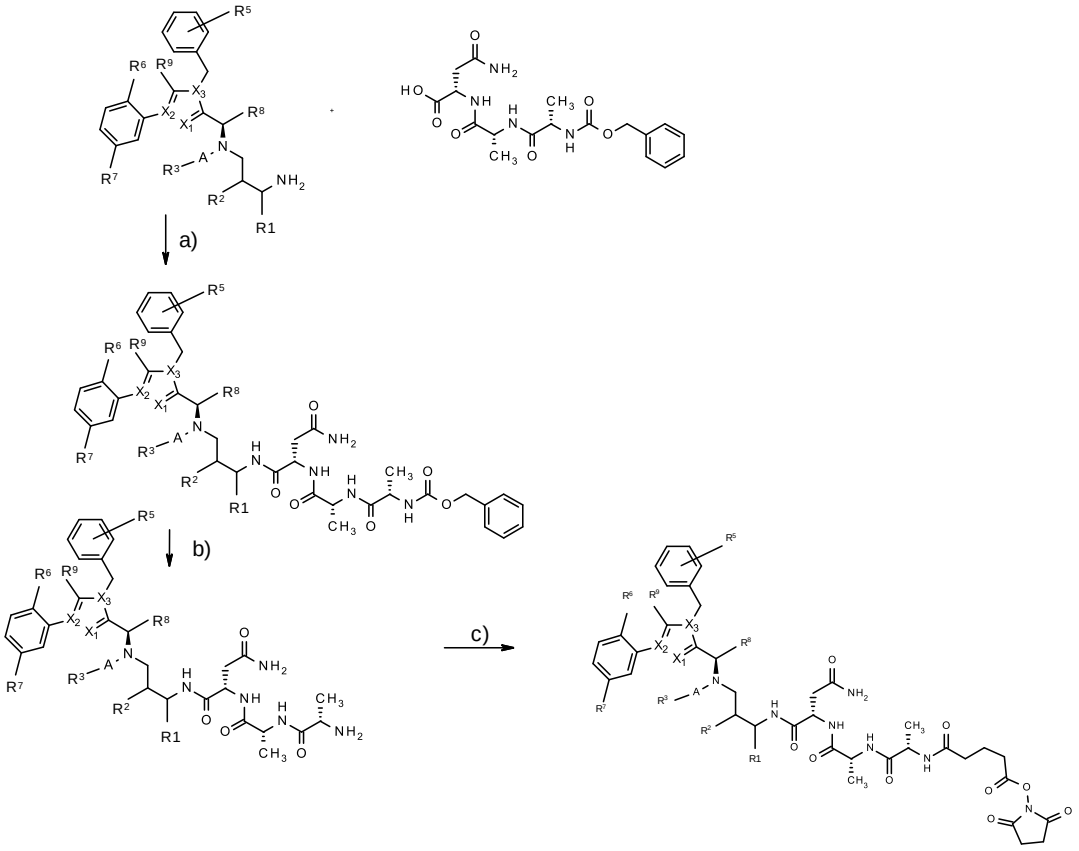
In addition, other intermediates according to Schemes 2 and 3 can be converted to legumain-cleavable ADC precursors:

Scheme 2:



[a): HATU, DMF, N,N-diisopropylethylamine, RT or EDCI, HOBT, N,N-diisopropylethylamine, DMF, RT b) H_2, 10% Pd-C, MeOH, RT; c) 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, N,N-diisopropylethylamine, DMF, RT]

Scheme 3:

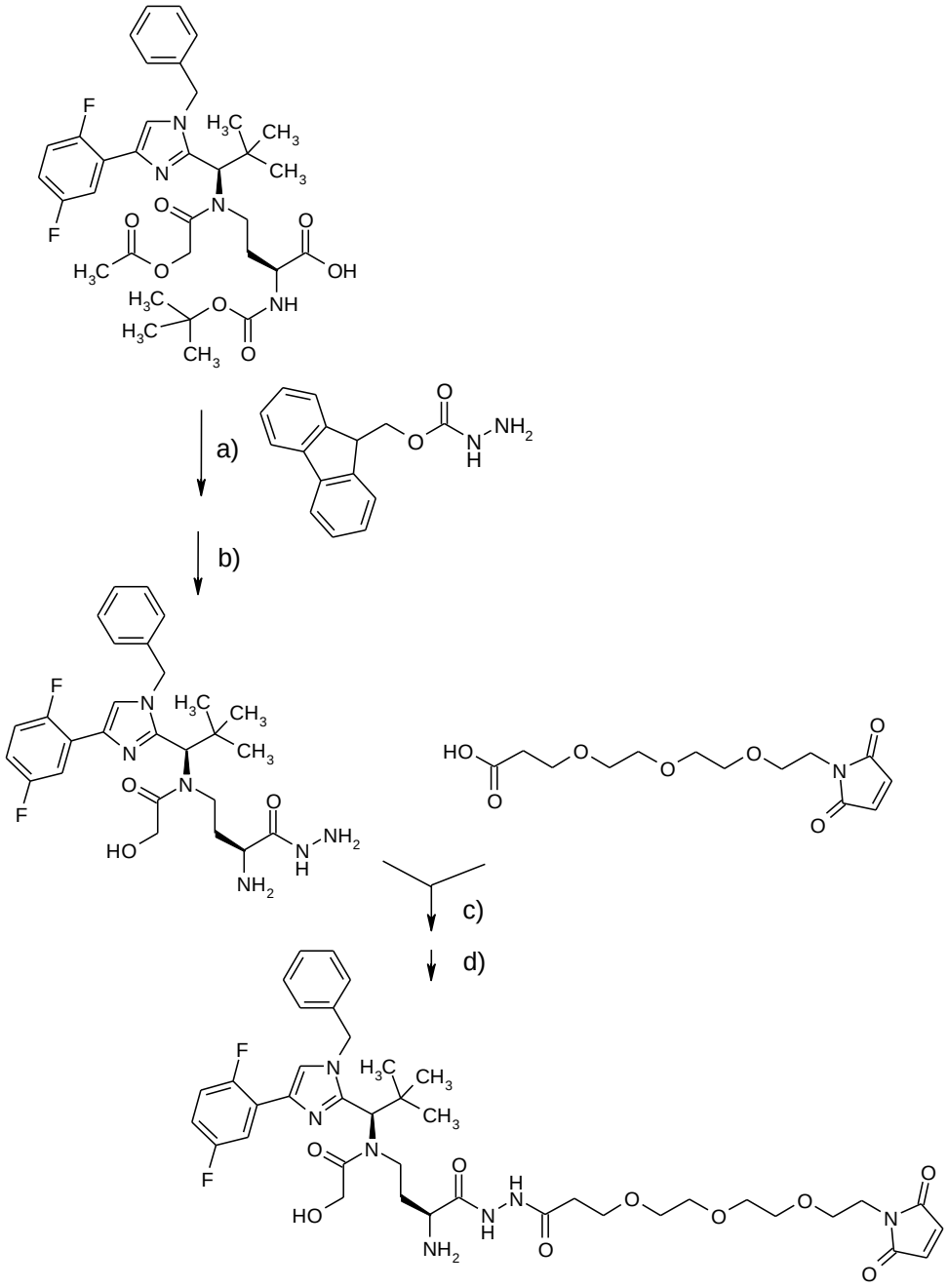


[a): HATU, DMF, N,N-diisopropylethylamine, RT or EDCI, HOBT, N,N-diisopropylethylamine, DMF, RT b) H_2, 10% Pd-C, MeOH, RT; c) 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, N,N-diisopropylethylamine, DMF, RT]

As an alternative to the benzyloxycarbonyl group shown in Schemes 1-3, it is possible to use other protecting groups established in peptide chemistry and detach them by corresponding methods that are likewise known. The selection of the protecting group strategy is made according to requirements known to those skilled in the art relating to compatibility with other structural elements that occur in the molecule. If they are still present, further protecting groups in the molecule may be removed in a last step. The syntheses may also optionally be rearranged in terms of their sequence.

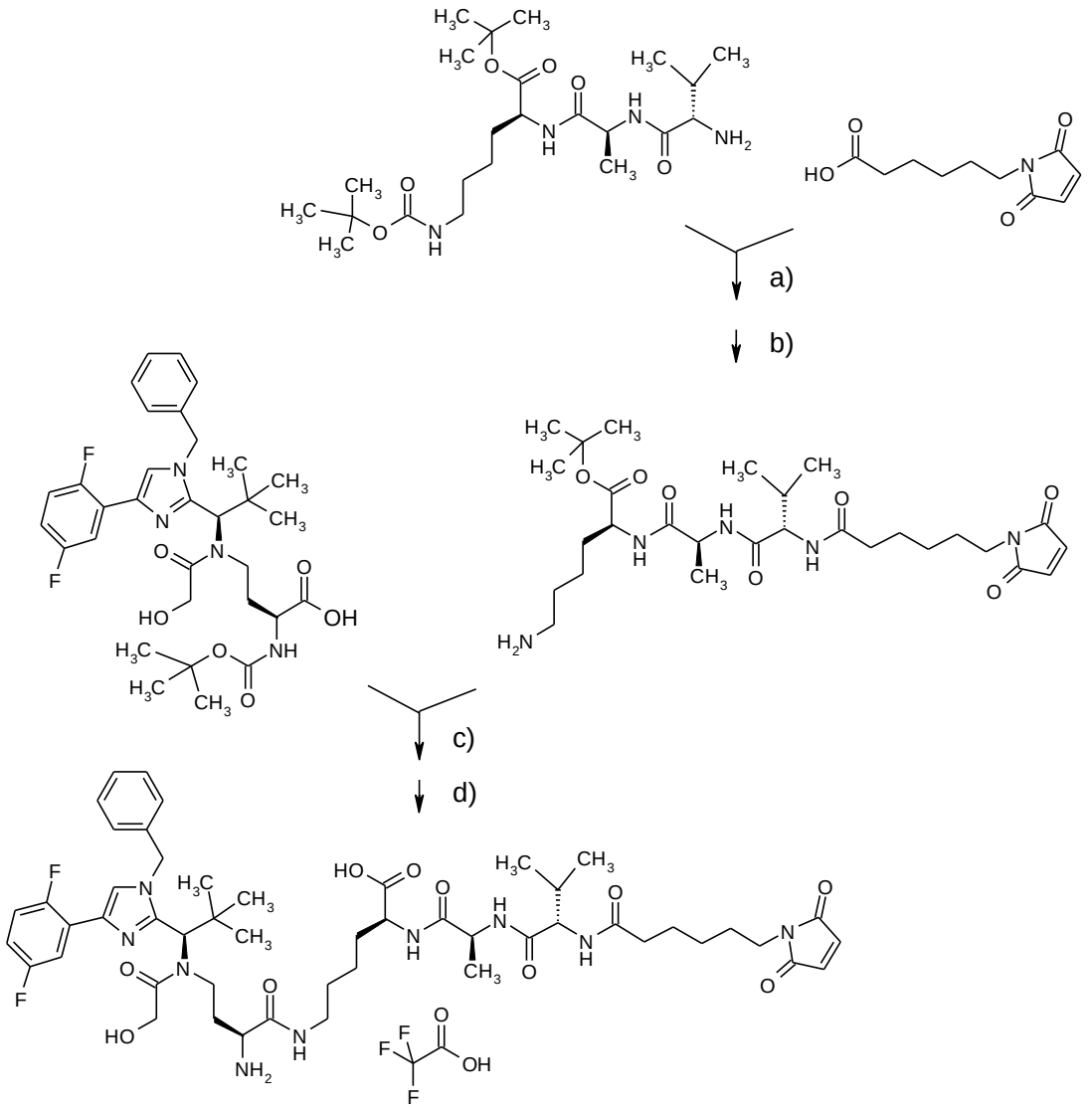
In addition, the protein-reactive group in the context of the linker structures L1-L2 may be varied within the scope of the claims.

Scheme 4: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



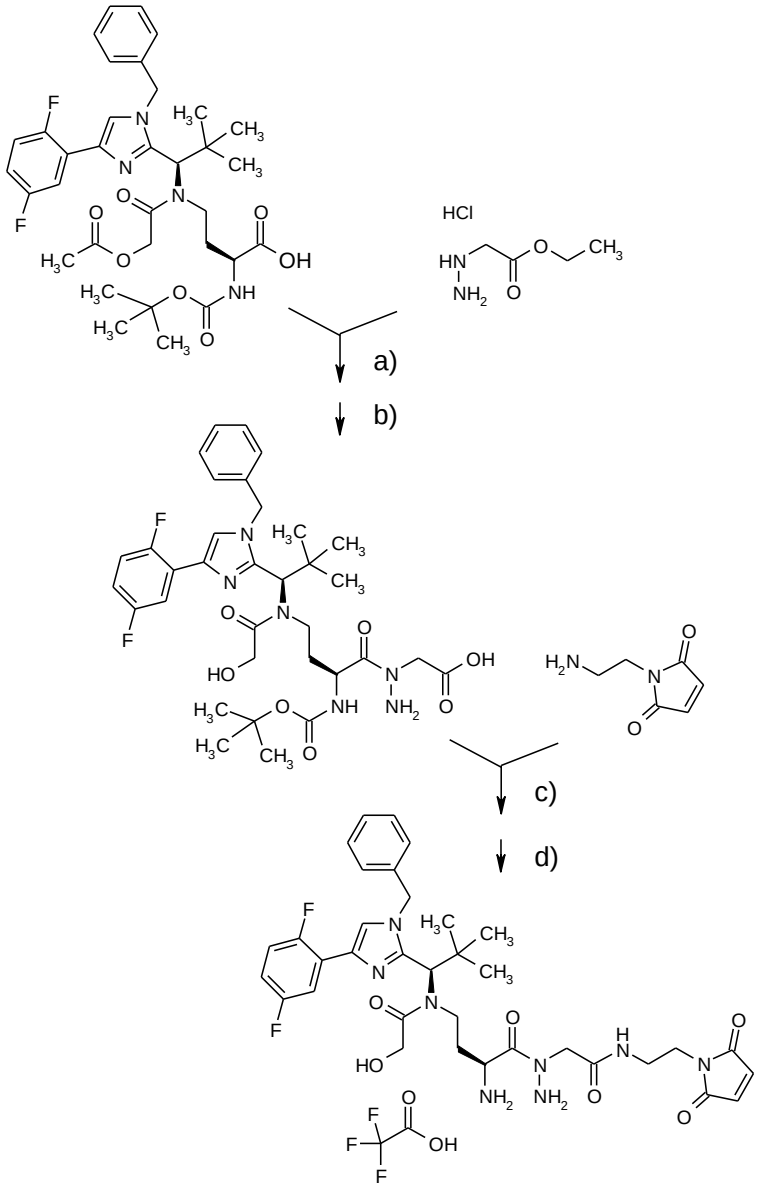
[a): EDCI, HOBT, diisopropylethylamine, RT; b) ethanol, piperidine, methylamine, water, RT; c) HATU, diisopropylethylamine, RT; d) TFA, DCM, RT]

Scheme 5: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



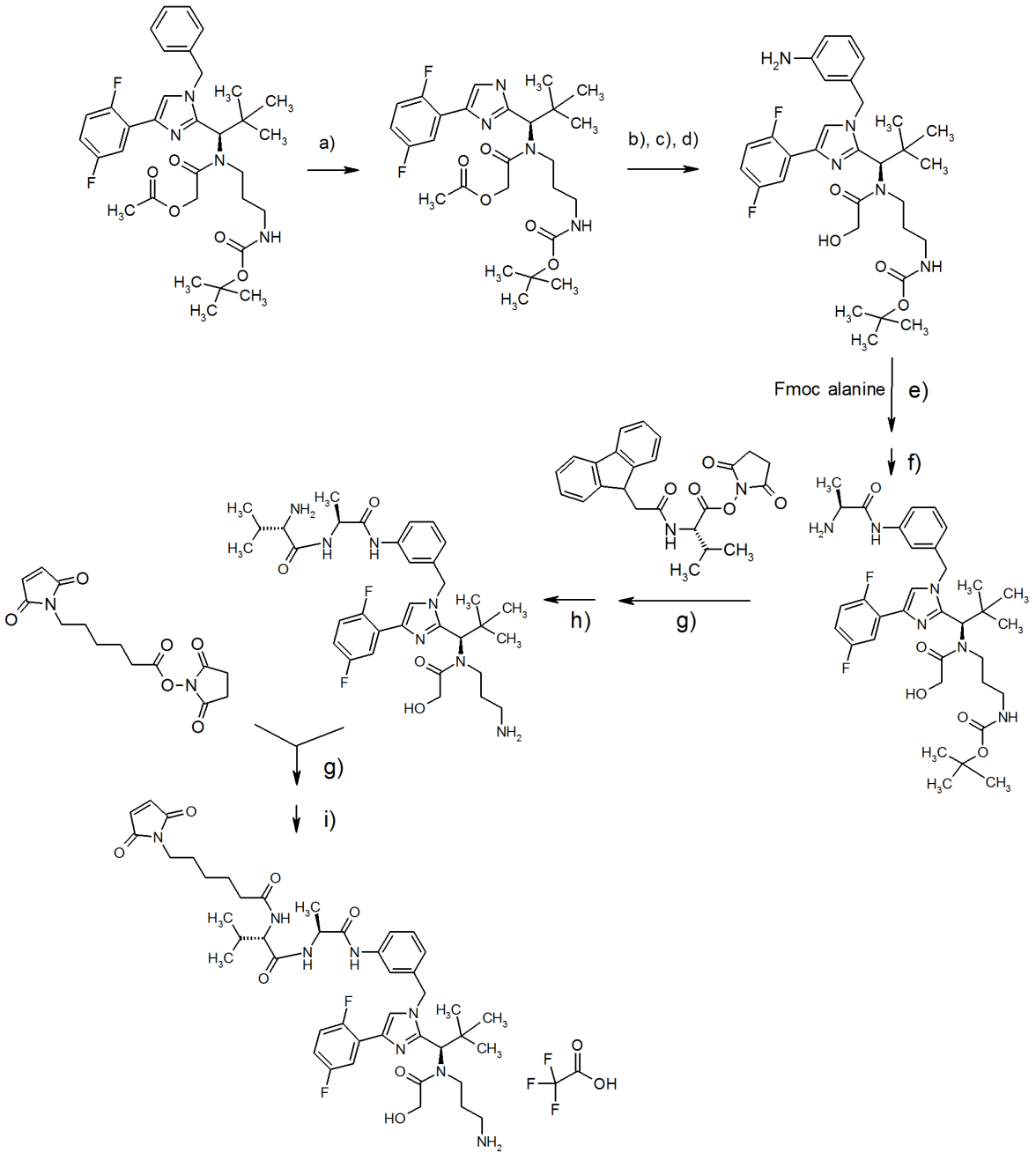
[a): for example EDCI, HOBT, diisopropylethylamine, DMF, RT; b) for example DCM/TFA 20:1, RT; c) for example HATU, diisopropylethylamine, DMF, RT; d) for example TFA, DCM, RT]

Scheme 6: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



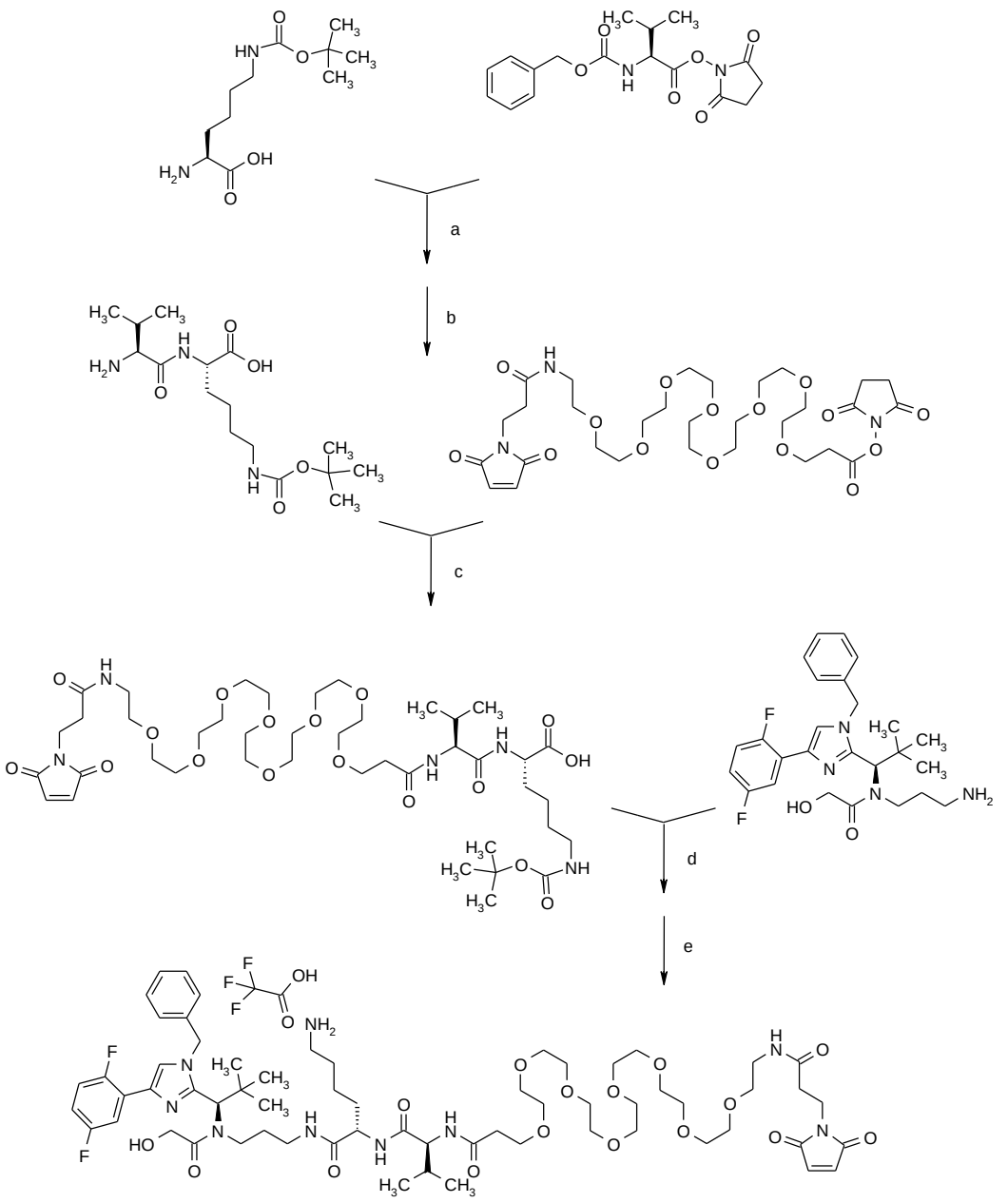
[a): for example 2-bromo-1-ethylpyridinium tetrafluoroborate, diisopropylethylamine, DCM, RT; b) for example 2M LiOH solution, THF, water, RT, HPLC separation of the regiosisomers; c) for example EDCI, HOBT, diisopropylethylamine, DMF, RT; d) for example TFA, DCM, RT]

Scheme 7: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



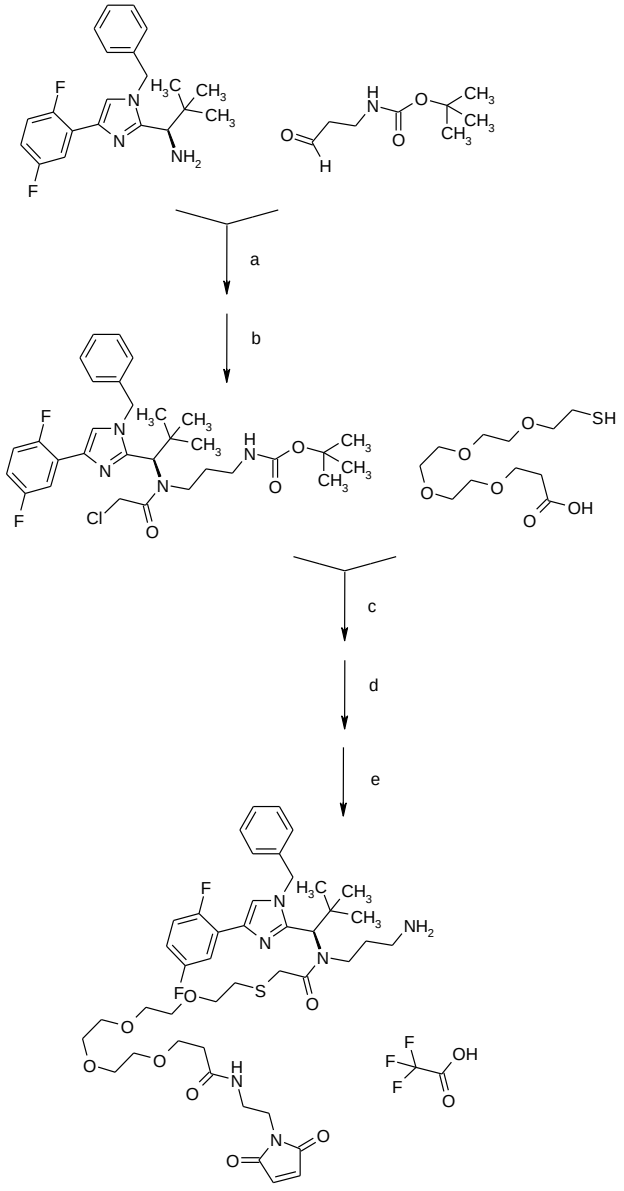
[a): for example H_2, Pd-C, EtOH, RT; b) for example p-nitrobenzyl bromide, K_2CO_3, DMF; c) for example ethanol, 40% methylamine solution in water, 50°C; d) for example disodium dithionite, THF, water, 50°C; e) for example HATU, diisopropylethylamine, DMF, RT; f) for example piperidine, 40% methylamine solution in water, ethanol, 50°C; g) for example diisopropylethylamine, DMF, RT; h) for example piperidine, DMF, RT; i) TFA, DCM, RT]

Scheme 8: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



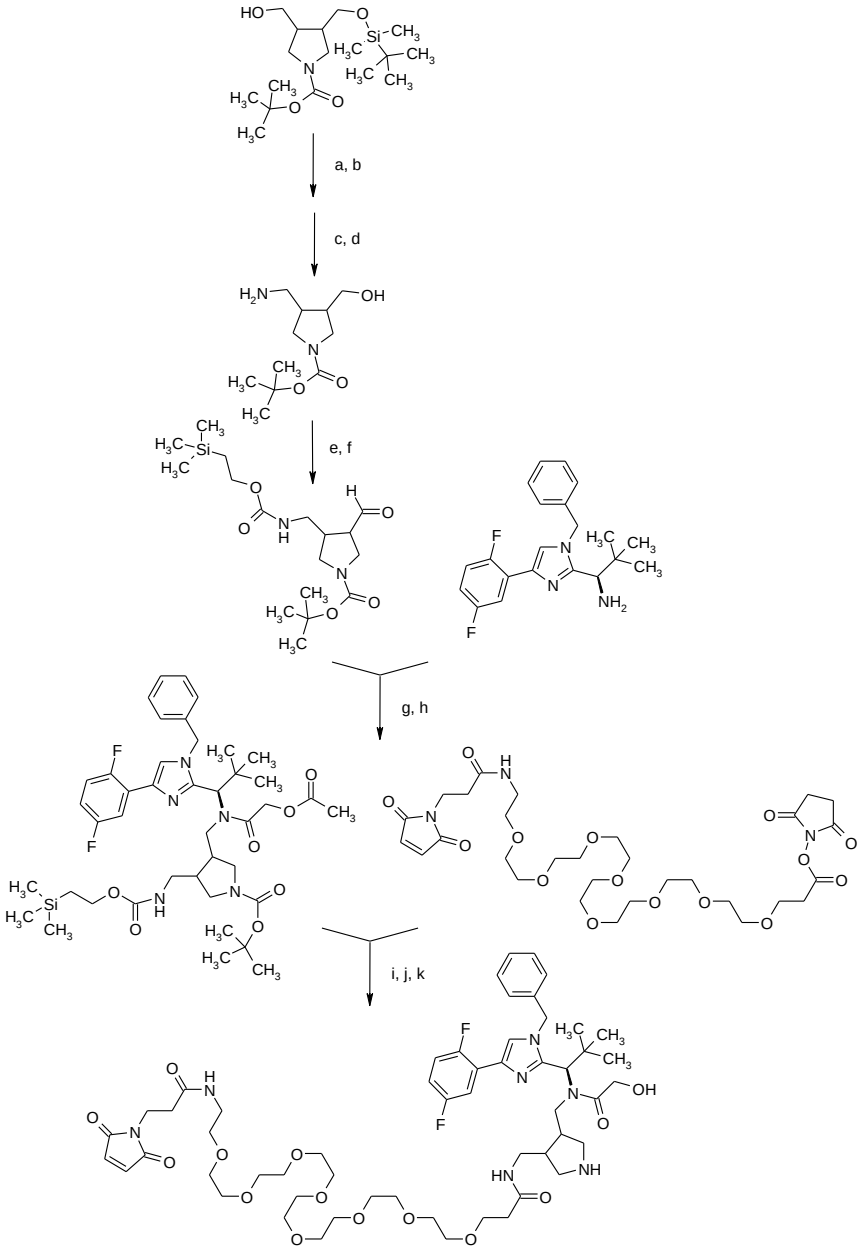
[a): for example Et_3N, DMF, RT; b) for example H_2, Pd-C, EtOH/ethyl acetate/THF (1:1:1), RT; c) for example 4-methylmorpholine, DMF, RT; d) for example HATU, HOAt, diisopropylethylamine, DMF, RT; e) for example TFA, RT]

Scheme 9: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



[a): for example $NaBH(OAc)_3$, HOAc, dichloromethane, RT; b) for example chloroacetyl chloride, NEt_3, DCM, RT; c) for example Cs_2CO_3, DMF, 50 °C; d) for example 1-(2-aminoethyl)-1H-pyrrole-2,5-dione hydrochloride (1:1), T3P$^{(R)}$, diisopropylethylamine, MeCN, RT; e) for example TFA, RT]

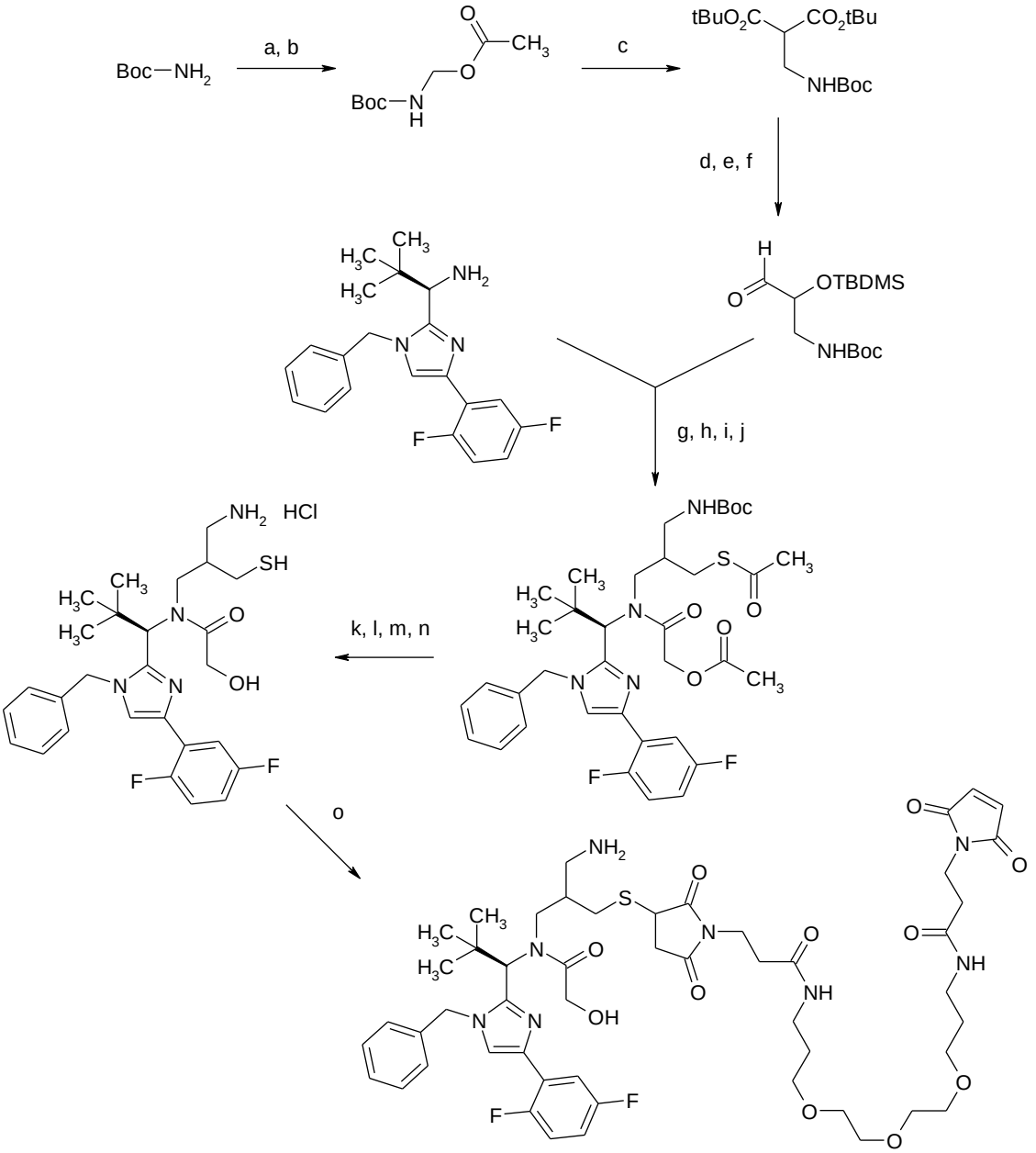
Scheme 10: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



[a): for example methanesulphonyl chloride, NEt_3, dichloromethane, 0°C; b) for example NaN_3, DMF, 40°C; c) for example H_2, Pd-C, EtOH/ethyl acetate (1:1), RT; d) for example TBAF, THF, RT; e) for example 1-({[2-(trimethylsilyl)ethoxy]carbonyl}oxy)pyrrolidine-2,5-dione, NEt_3, $CaCO_3$, 1,4-dioxane, RT; f) for example N-chlorosuccinimide, TEMPO, tetra-n-butylammonium chloride, chloroform, 0.05 N potassium carbonate/0.05 N sodium hydrogencarbonate solution (1:1); g) for example $NaBH(OAc)_3$, HOAc, dichloromethane,

RT; h) for example chloroacetyl chloride, NEt_3, DCM, RT; i) for example TBAF, THF, water, RT; j) for example 4-methylmorpholine, DMF, RT; k) for example TFA, RT]

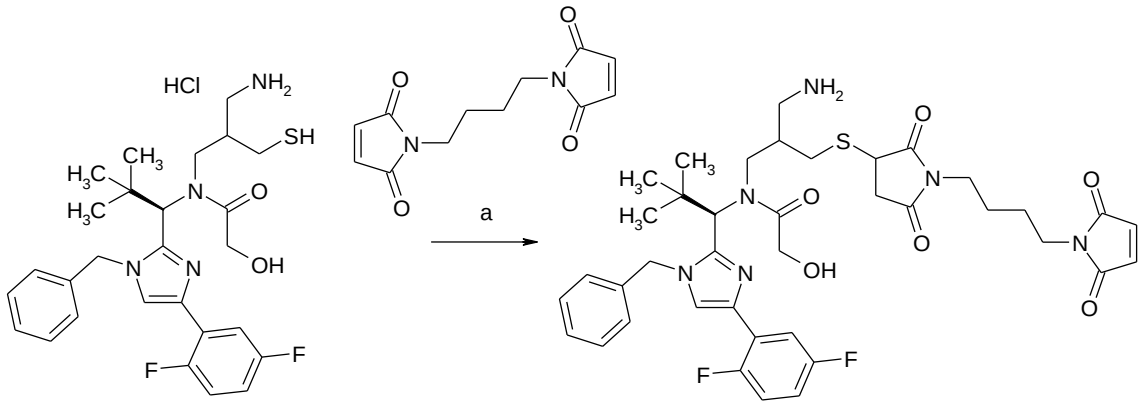
Scheme 11: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



[a): for example formaldehyde, Na_2CO_3, water, RT; b) for example Ac_2O, pyridine, THF, RT; c) for example di-tert-butyl malonate, KOtBu, THF, RT; d) for example $LiBH_4$, THF,

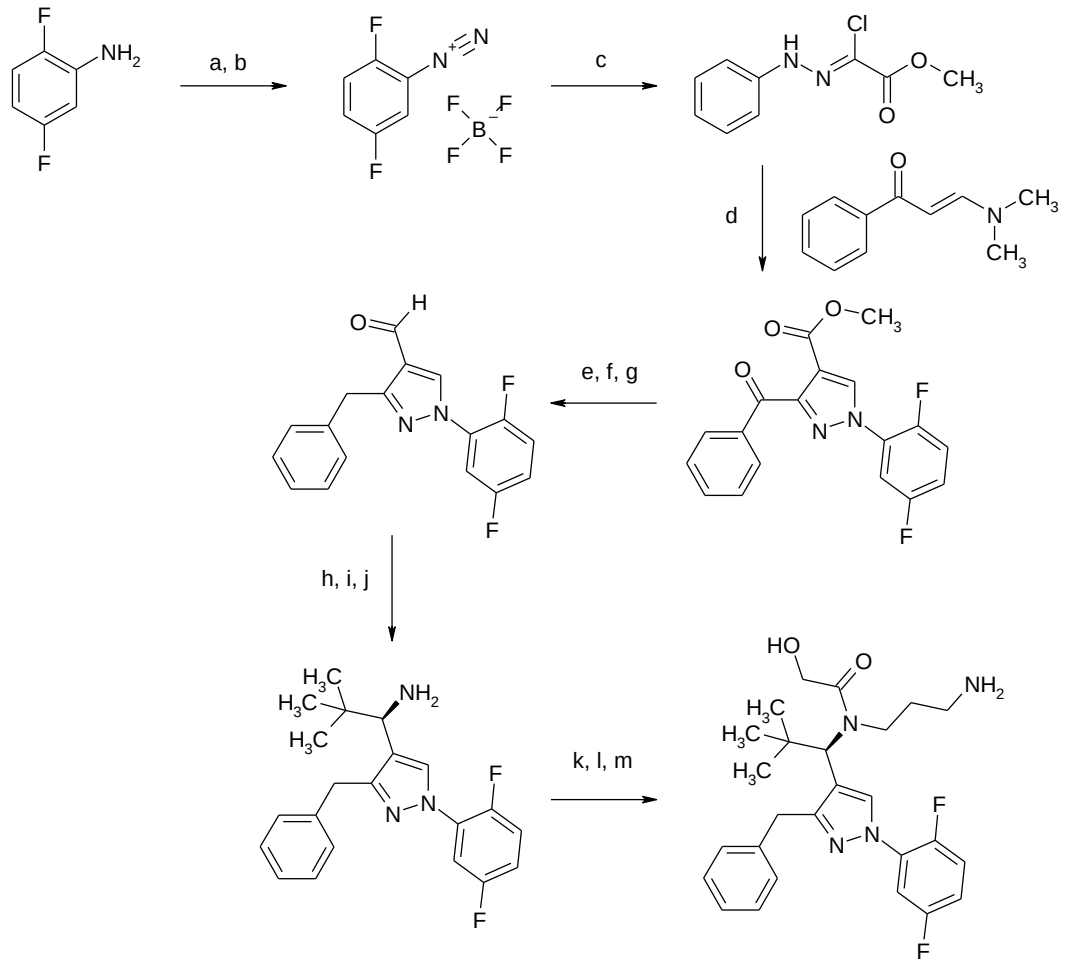
RT; e) for example TBDMSCl, imidazole, DCM, RT; f) for example Dess-Martin periodinane, DCM; g) for example sodium triacetoxyborohydride, AcOH, DCM, RT; h) for example nBu_4NF, THF, RT; i) for example $SOCl_2$, THF, RT; j) for example AcSK, nBu_4NI, DMF, 90°C; k) for example NaOH, MeOH, THF, RT; l) for example TCEP, dioxane, RT; m) for example separation of the epimers; n) for example 6N hydrochloric acid, THF, RT o) for example Mal-dPEG(3)-Mal, PBS buffer, ACN, RT]

Scheme 12: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



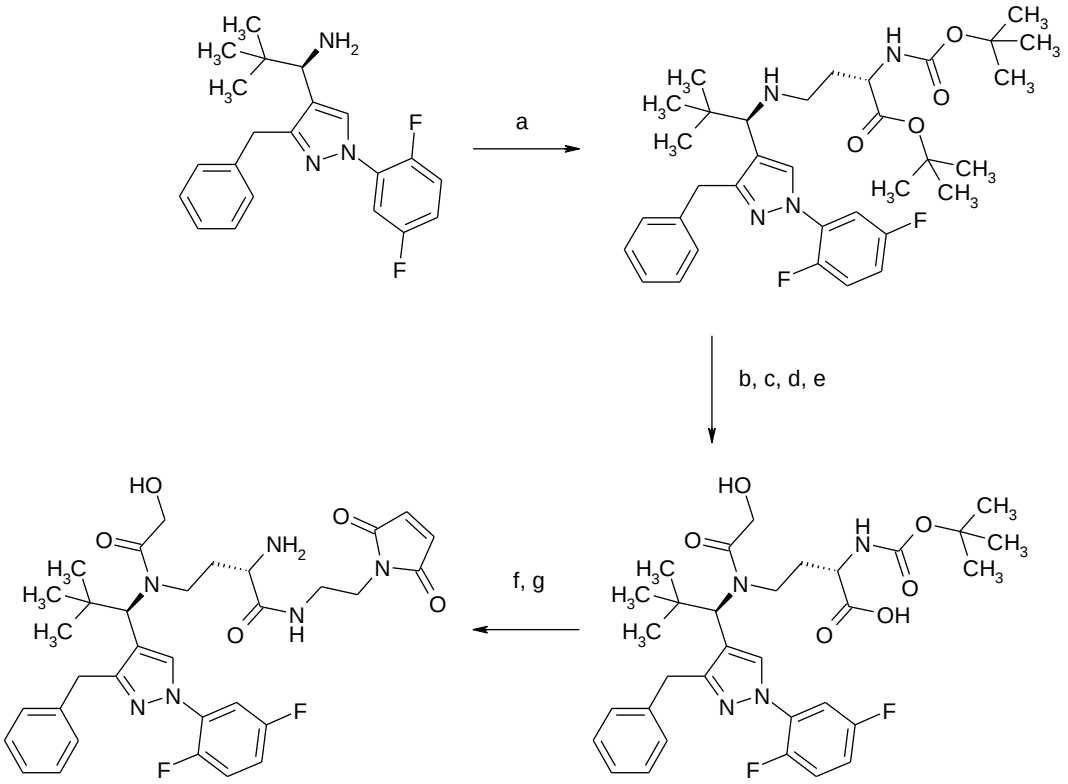
[a): for example Mal-dPEG(3)-Mal, PBS buffer, ACN, RT]

Scheme 13: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



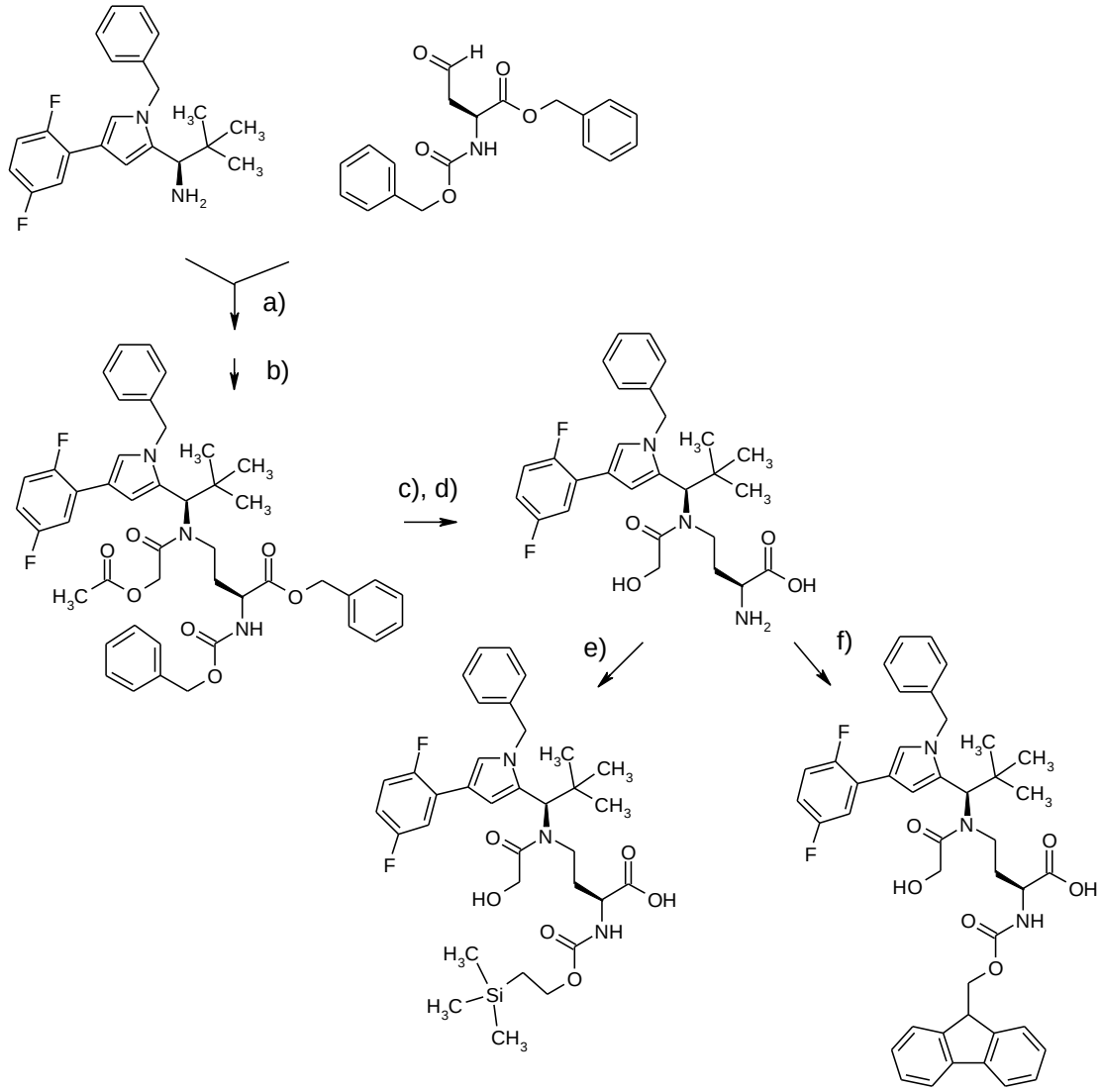
[a): for example BF_3OEt_2, THF, 0°C; b): for example isoamyl nitrite, -10°C, 0.5 h; c): for example methyl 2-chloro-3-oxobutanoate, pyridine, water, -5°C; d): for example NEt_3, toluene, RT; e): for example Et_3SiH, TFA, RT; f): for example $LiBH_4$, THF, 60°C; g): for example Dess-Martin periodinane, DCM, RT; h): for example (R)-(+)-methyl-2-propanesulphinamide, titanium(IV) isopropoxide, THF, RT; i): for example tert-BuLi, pentane, THF, -78°C; j): for example HCl in dioxane, THF, MeOH, RT; k): for example 3-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propanal, $NaB(OAc)_3H$, AcOH, DCM, RT; l): for example 2-chloro-2-oxoethyl acetate, NEt_3, DCM, RT; m): for example methylamine, water, EtOH, 50°C]

Scheme 14: Synthesis of precursors of ADC precursor molecules



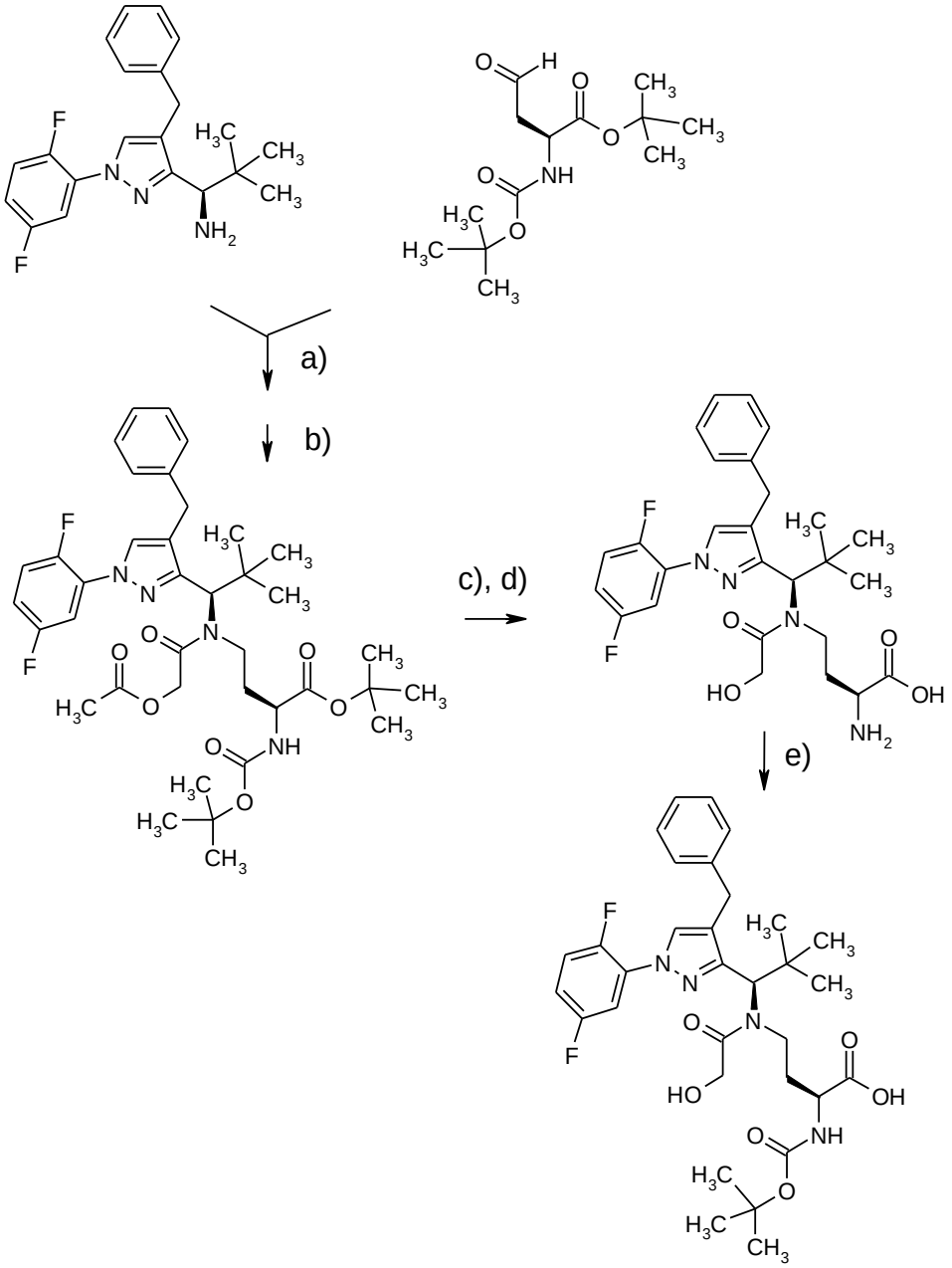
[a): for example tert-butyl N-(tert-butoxycarbonyl)-5-oxo-L-norvalinate, $NaB(OAc)_3H$, AcOH, DCM, RT; b): for example 2-chloro-2-oxoethyl acetate, NEt_3, DCM, RT; c): for example methylamine, water, EtOH, 60°C; d): for example THF, DCM, 50°C; e): for example Boc_2O, NEt_3, DCM, RT; f): for example trifluoroacetic acid / 1-(2-aminoethyl)-1H-pyrrole-2,5-dione (1:1), HATU, diisopropylethylamine, DMF, RT; f): for example TFA, DCM, RT]

Scheme 15: Synthesis of intermediates



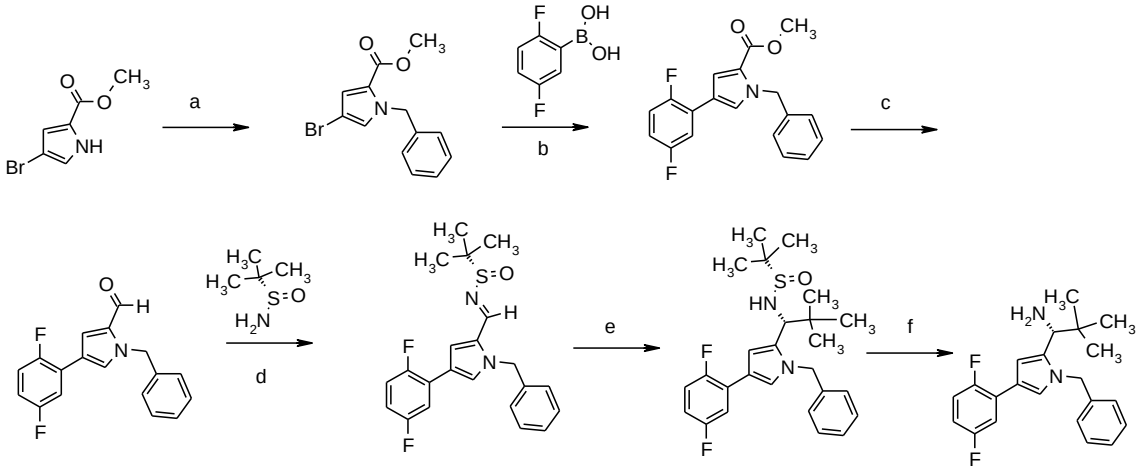
[a): for example sodium triacetoxyborohydride, acetic acid, DCM, RT; b) for example acetoxyacetyl chloride, NEt3, DCM, RT; c) for example LiOH, THF/water, RT; d) for example H_2, Pd-C, EtOH, RT; e) for example Teoc-OSu, NEt3, dioxane, RT; f) for example Fmoc-Cl, diisopropylethylamine, dioxane/water 2:1, RT]

Scheme 16: Synthesis of intermediates



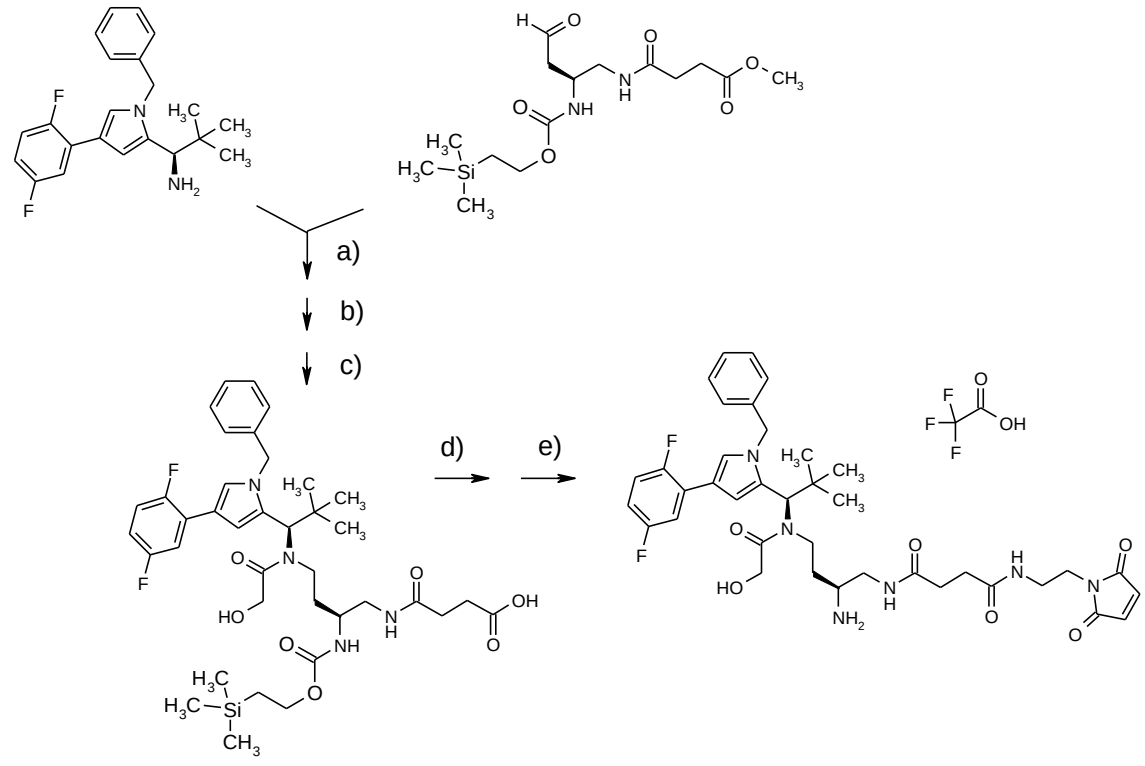
[a): for example sodium triacetoxyborohydride, acetic acid, DCM, RT; b) for example acetoxyacetyl chloride, NEt3, DCM, RT; c) for example LiOH, methanol, RT; d) for example TFA, DCM, RT; e) for example Boc_2O, diisopropylethylamine, DCM, RT]

Scheme 17: Synthesis of intermediates



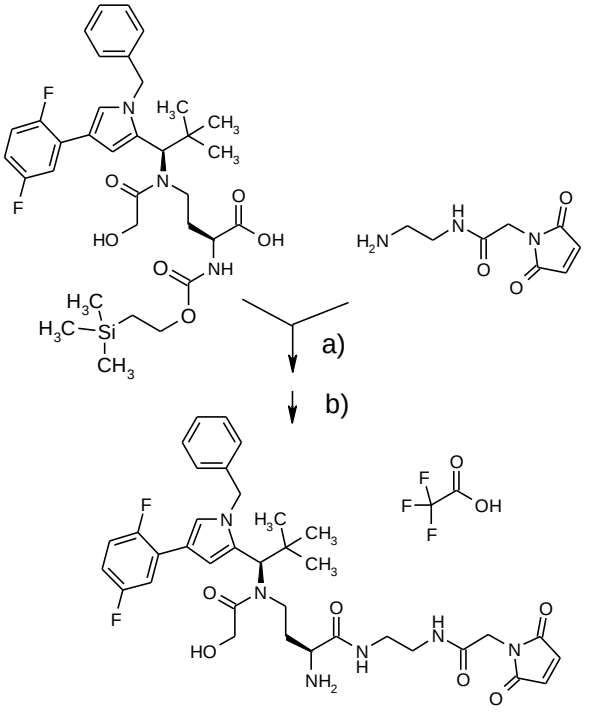
[a): for example benzyl bromide, Cs_2CO_3, DMF, RT; b) for example $Pd(dppf)_2Cl_2$, DMF, Na_2CO_3, 85°C; c) for example $LiAlH_4$, THF, 0°C; MnO_2, DCM, RT; d) for example $Ti(iOPr)_4$, THF, RT; e) for example tBuLi, THF, -78°C; MeOH, NH_4Cl; f) for example HCl/1,4-dioxane]

Scheme 18: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



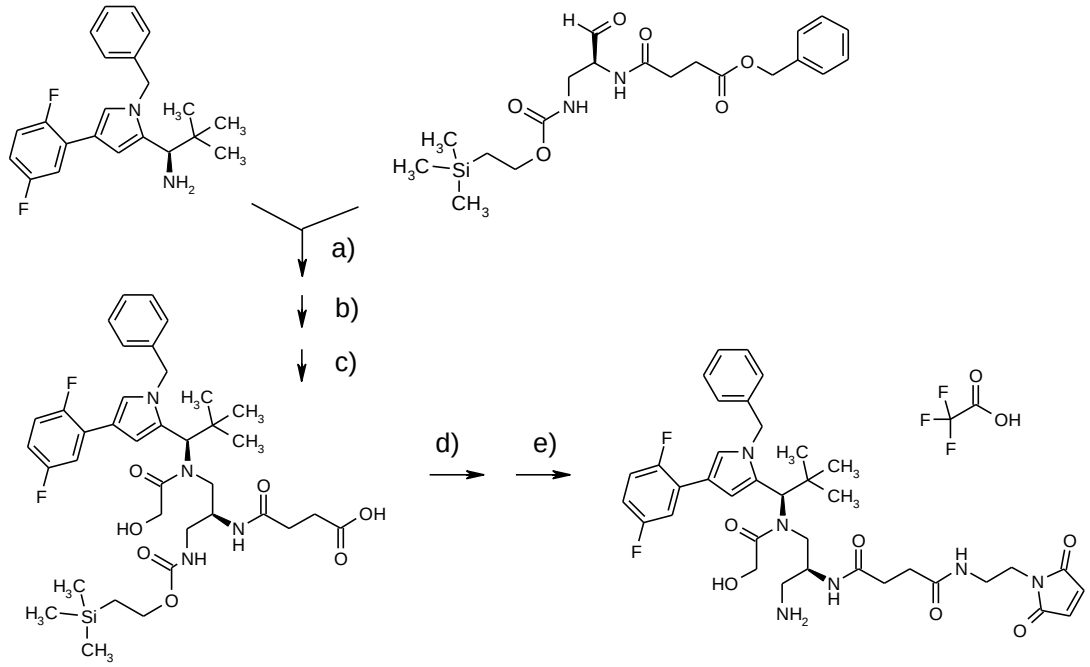
[a): sodium triacetoxyborohydride, acetic acid, DCM, RT; b) acetoxyacetyl chloride, diisopropylethylamine, DCM, RT; c) LiOH, MeOH, RT; d) trifluoroacetic acid / 1-(2-aminoethyl)-1H-pyrrole-2,5-dione (1:1) HATU, DMF, diisopropylethylamine, RT; e) zinc chloride, trifluoroethanol, 50°C, EDTA.]

Scheme 19: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



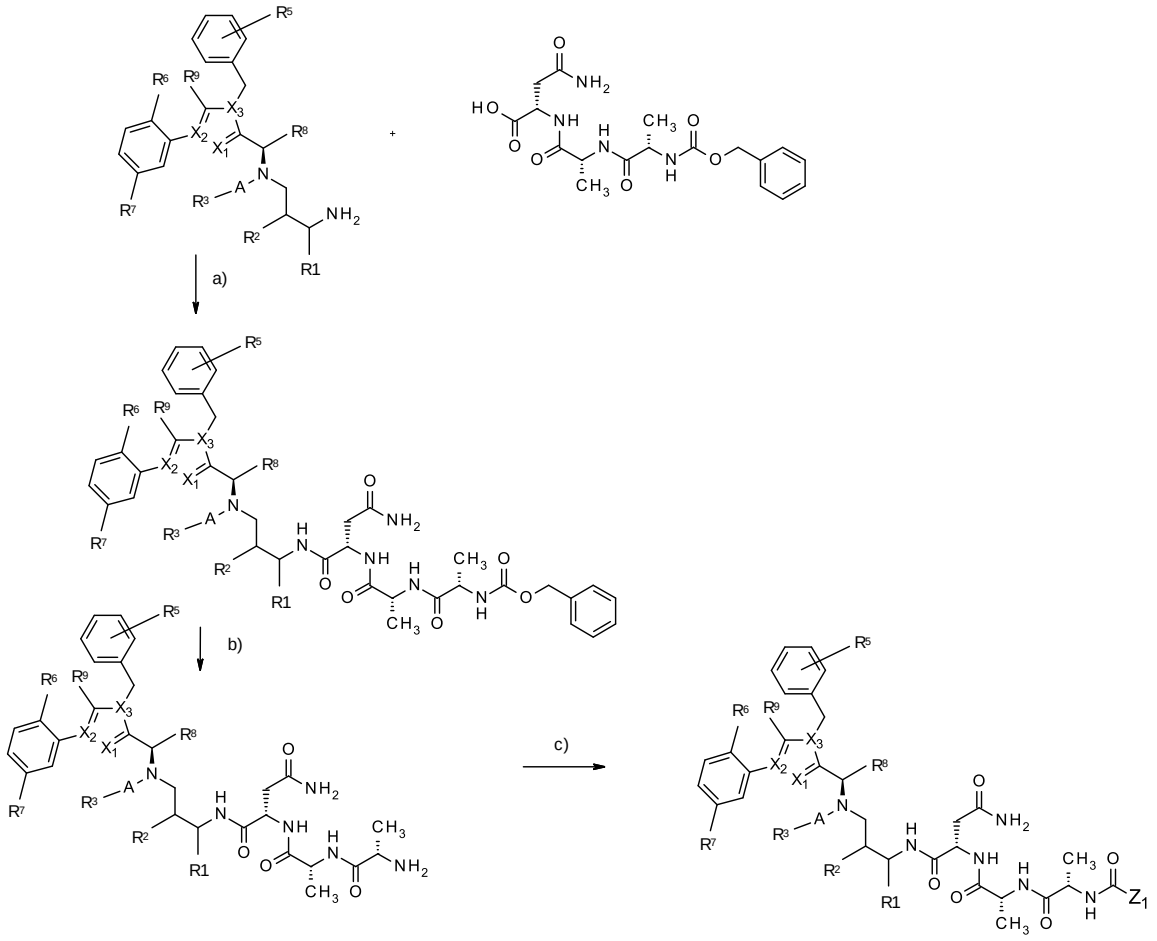
[a): HATU, DMF, diisopropylethylamine, RT; b) zinc chloride, trifluoroethanol, 50°C, EDTA.]

Scheme 20: Synthesis of ADC precursor molecules of the intermediate series F which can be converted to APDCs according to Scheme 1



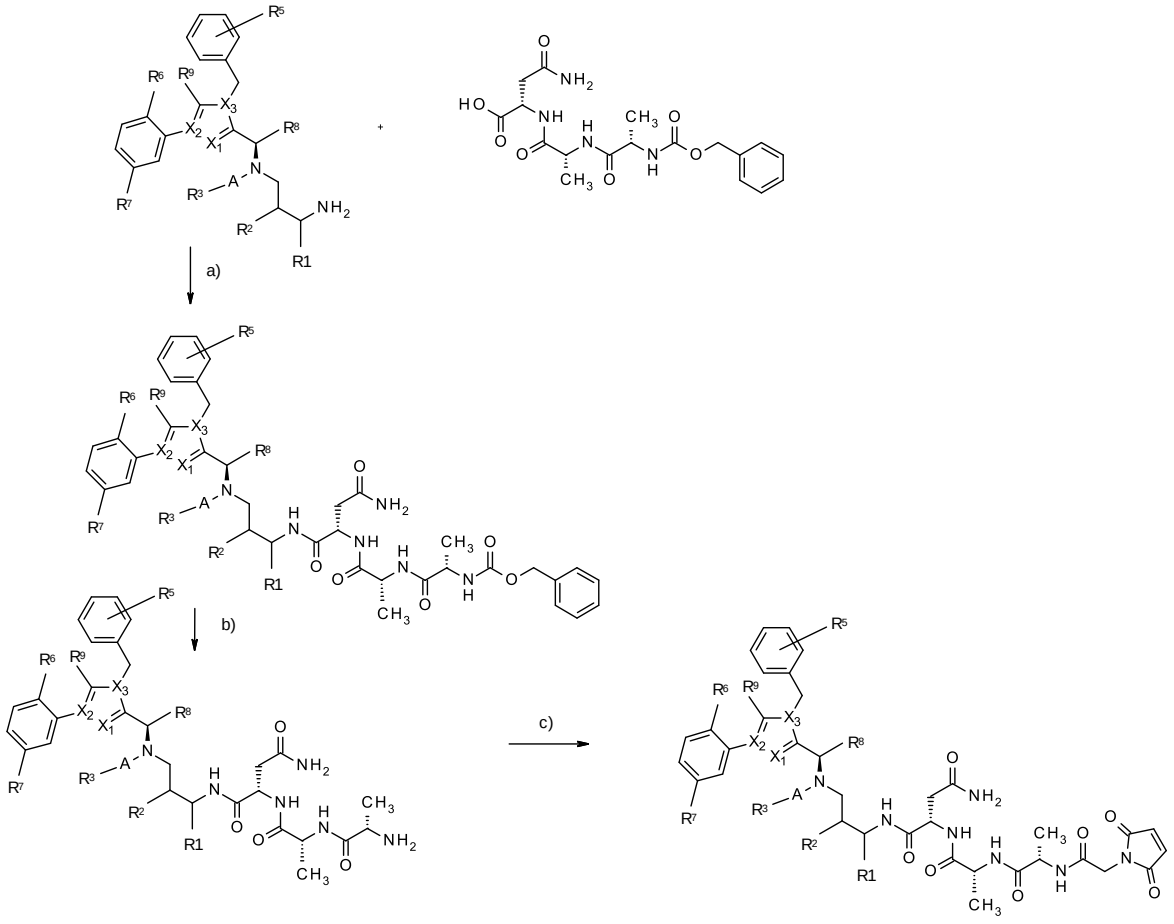
[a): sodium triacetoxyborohydride, acetic acid, DCM, RT; b) acetoxyacetyl chloride, triethylamine, DCM, RT; c) LiOH, MeOH, RT; d) trifluoroacetic acid / 1-(2-aminoethyl)-1H-pyrrole-2,5-dione (1:1) HATU, DMF, diisopropylethylamine, RT; e) zinc chloride, trifluoroethanol, 50°C, EDTA.]

Scheme 21: General method for synthesis of intermediates and ADC precursors



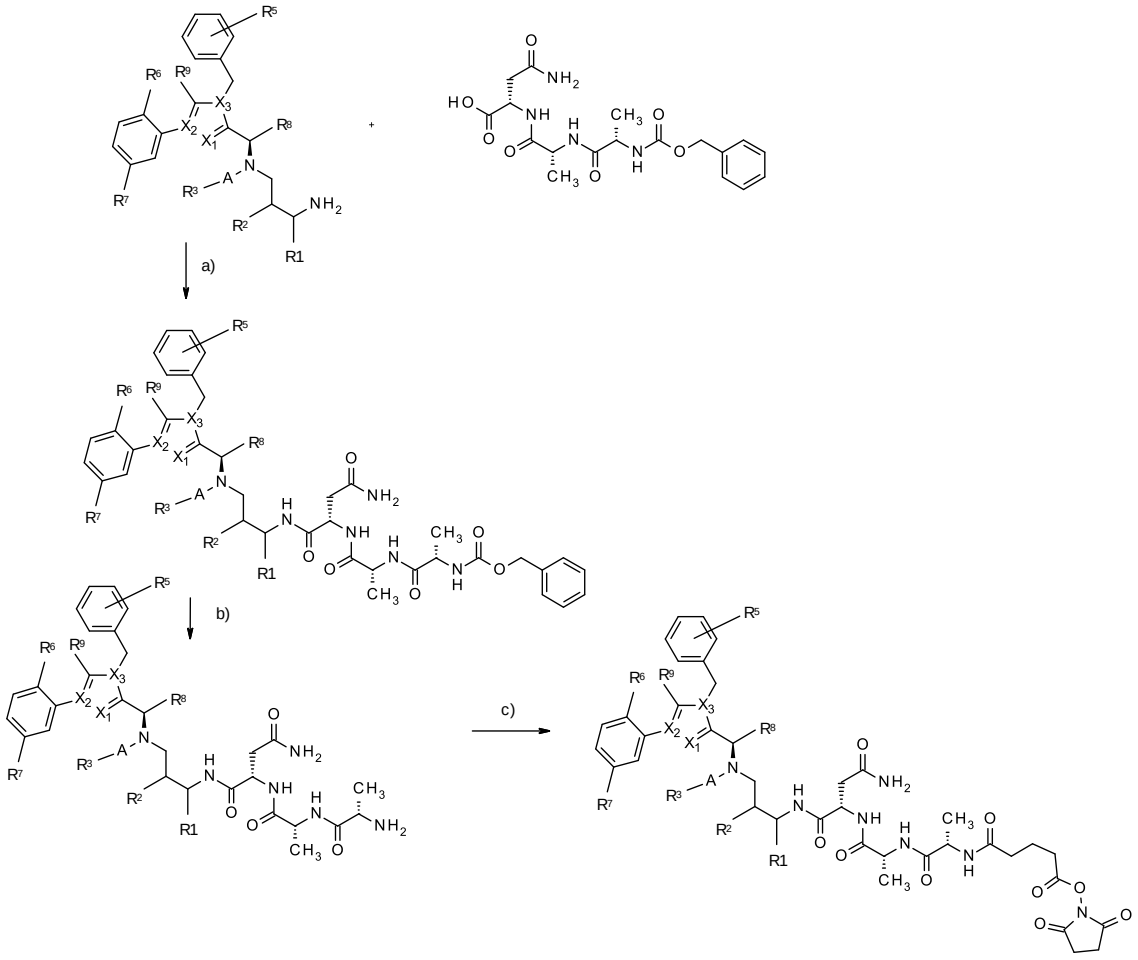
[a): HATU, DMF, N,N-diisopropylethylamine, RT or EDCI, HOBT, N,N-diisopropylethylamine, DMF, RT b) H_2, 10% Pd-C, MeOH, RT; c) Z_1-COOH, EDCI, HOBT, N,N-diisopropylethylamine, DMF, RT or Z_1-COOH, HATU, N,N-diisopropylethylamine, DMF, RT or Z^1-COOSu, N,N-diisopropylethylamine, DMF, RT]

Scheme 22: Synthesis of ADC precursor molecules having legumain-cleavable linkers



[a): HATU, DMF, N,N-diisopropylethylamine, RT or EDCI, HOBT, N,N-diisopropylethylamine, DMF, RT b) H_2, 10% Pd-C, MeOH, RT; c) 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, N,N-diisopropylethylamine, DMF, RT]

Scheme 23: Synthesis of ADC precursor molecules having legumain-cleavable linkers



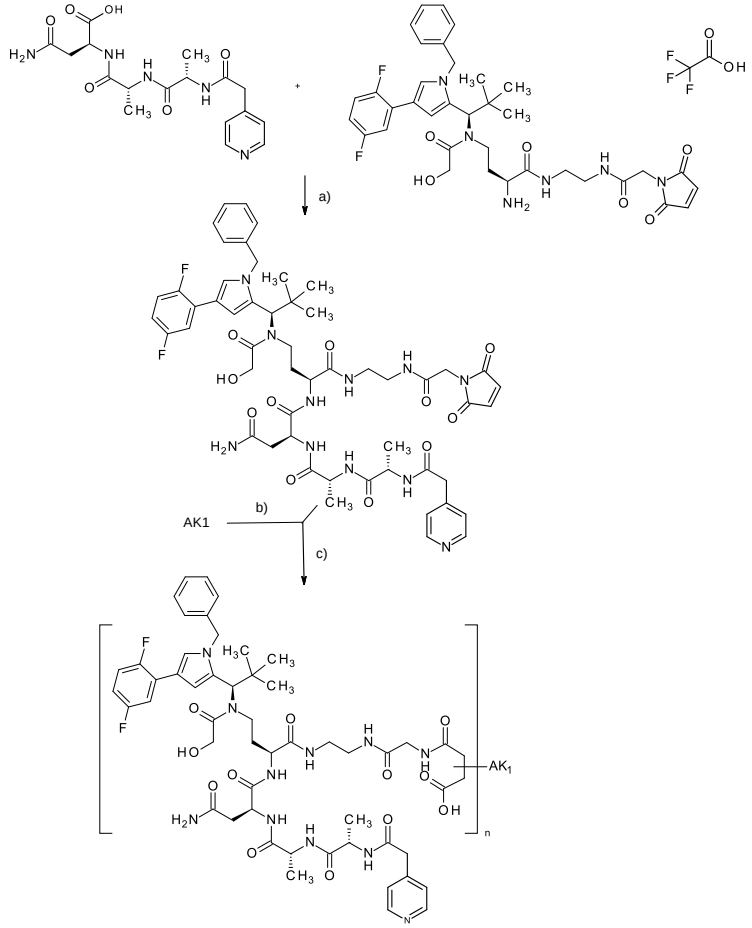
[a): HATU, DMF, N,N-diisopropylethylamine, RT or EDCI, HOBT, N,N-diisopropylethylamine, DMF, RT b) H_2, 10% Pd-C, MeOH, RT; c) 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, N,N-diisopropylethylamine, DMF, RT]

In addition, other intermediates according to Schemes 21, 22 and 23 can be converted to legumain-cleavable ADC and APDC precursors.

As an alternative to the benzyloxycarbonyl group shown in Schemes 21-23, it is possible to use other protecting groups established in peptide chemistry and detach them by corresponding methods that are likewise known. The selection of the protecting group strategy is made according to requirements known to those skilled in the art relating to

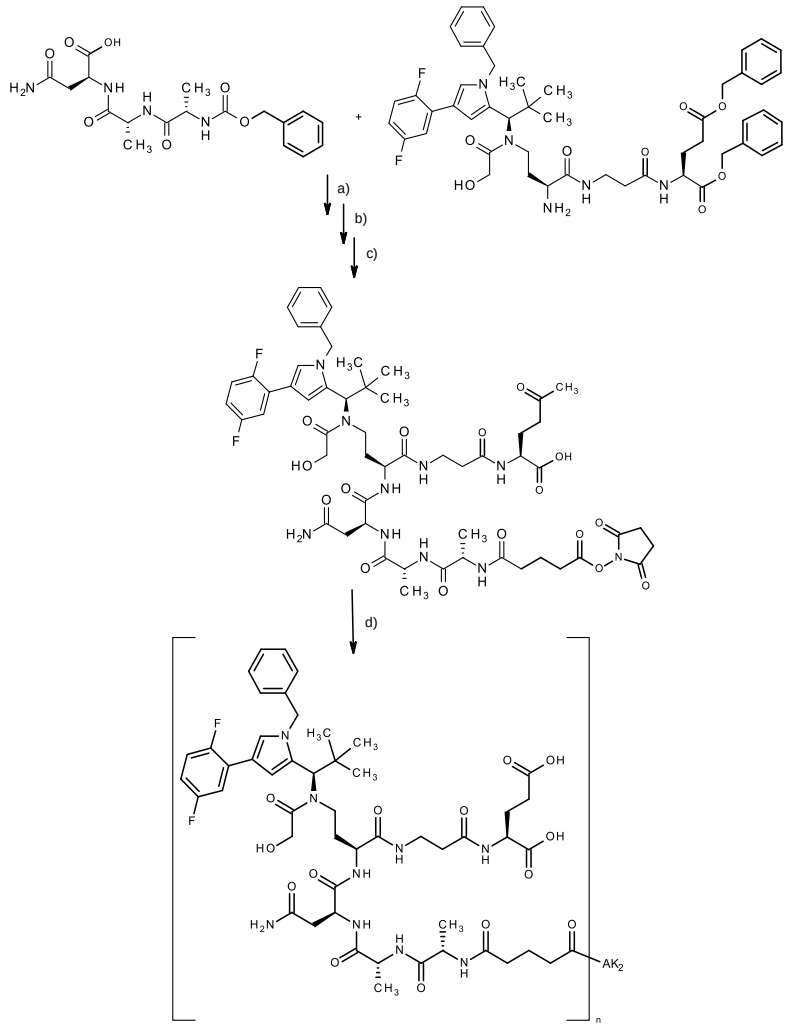
compatibility with other structural elements that occur in the molecule. If they are still present, further protecting groups in the molecule may be removed in a last step. The syntheses may also optionally be rearranged in terms of their sequence.

Scheme 24: Synthesis of cysteine-bonded ADCs with legumain-cleavable head group



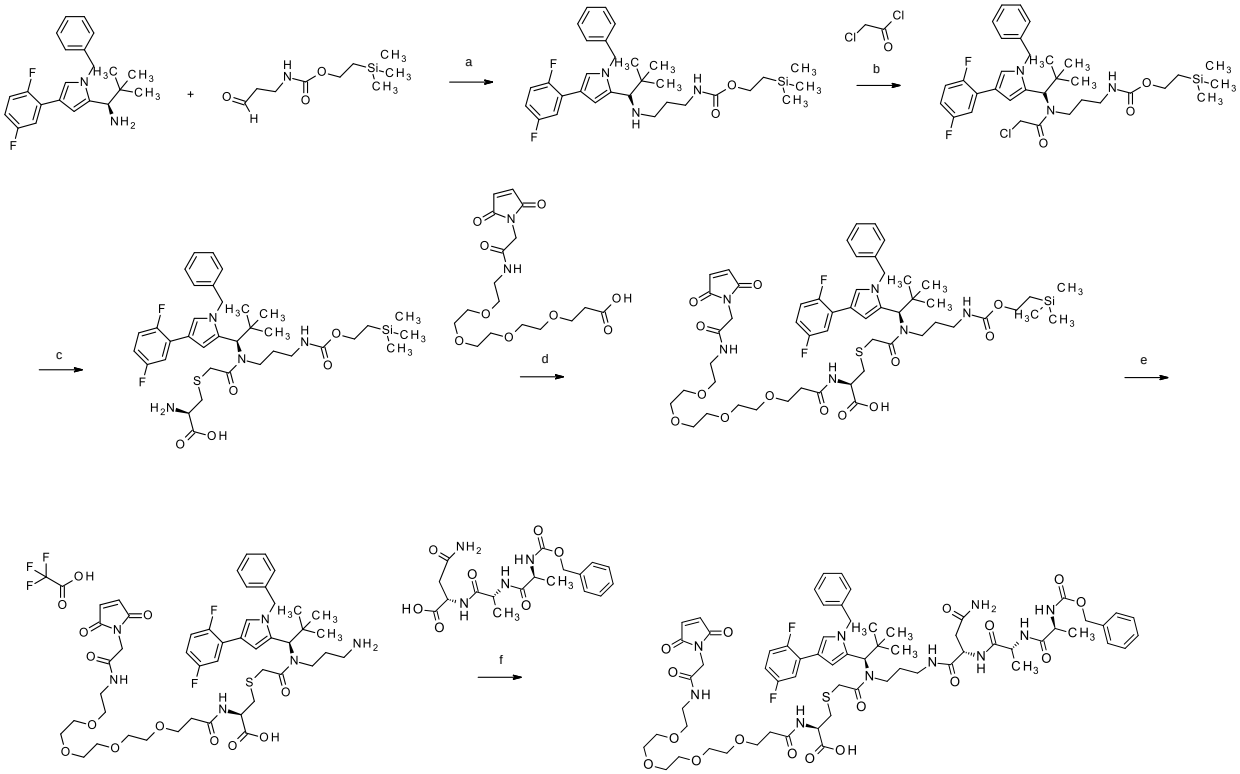
[a): HATU, DMF, N,N-diisopropylethylamine, RT; b) 2-5 eq TCEP, PBS pH7.2, stirring at RT for 30 min; c) stirring at RT under argon for 90 min, then rebuffering to pH 8 by means of PD 10 columns (Sephadex® G-25, GE Healthcare) and stirring under argon at RT overnight and subsequent concentration by means of ultracentrifugation and setting of the concentration desired with PBS at pH 7.2)]

Scheme 25: Synthesis of lysine-bonded ADCs with legumain-cleavable linker



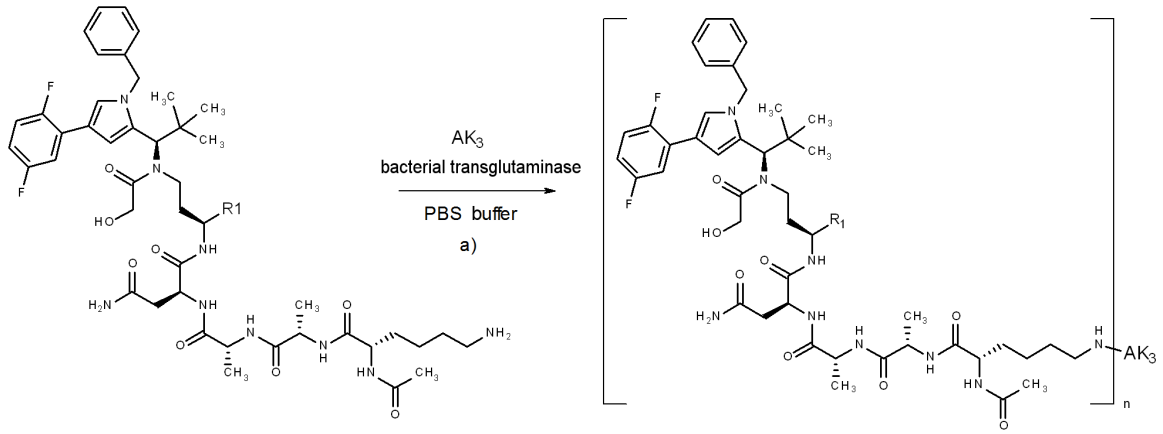
[a): HATU, DMF, N,N-diisopropylethylamine, RT; b) H_2, 10% Pd-C, methanol 1.5 h, RT; c) 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, N,N-diisopropylethylamine, DMF, stirring at RT overnight; d) AK2 in PBS, addition of 5 equiv. of active ester dissolved in DMSO, stirring at RT under argon for 60 min, addition of another 5 equiv. of active ester dissolved in DMSO, stirring at RT under argon for 60 min, then purification by means of PD 10 columns equilibrated with PBS buffer (pH 7.2) (Sephadex® G-25, GE Healthcare) and subsequent concentration by means of ultracentrifugation and setting of the concentration desired with PBS buffer (pH 7.2)]

Scheme 26: Synthesis of ADC precursors with legumain-cleavable head group



[a): $NaBH(OAc)_3$, HOAc, dichloromethane, RT; b) chloroacetyl chloride, NEt_3, DCM, RT; c) L-cysteine, $NaHCO_3$, DBU, isopropanol/water, 50°C; d) HATU, DMF, diisopropylethylamine, RT; e) zinc chloride, trifluoroethanol, 50°C; f) d) HATU, DMF, diisopropylethylamine, RT]

Scheme 27: Synthesis of ADCs via transglutaminase coupling



[a: 5 mg AK3 in DPBS pH 7.4 (c~10 mg/ml), 6 equivalents of a toxophor-linker precursor (e.g. Intermediate Q31-Q34), add 50 µl of a solution of 12.5 µl (1.25 U) of recombinant bacterial transglutaminase solution in water (100 U/ml) and 37.5 µl of DPBS pH 7.4, incubate at 37°C for 24 h]

A. Examples

Abbreviations and acronyms:

A498	human tumour cell line
ABCB1	ATP-binding cassette sub-family B member 1 (synonym for P-gp and MDR1)
abs.	absolute
Ac	acetyl
ACN	acetonitrile
aq.	aqueous, aqueous solution
ATP	adenosine triphosphate
BCRP	breast cancer resistance protein, an efflux transporter
BEP	2-bromo-1-ethylpyridinium tetrafluoroborate
Boc	*tert*-butoxycarbonyl
br.	broad (in NMR)
Ex.	Example
BxPC3	human tumour cell line
ca.	*circa*, about
CI	chemical ionization (in MS)
D	doublet (in NMR)
D	day(s)
TLC	thin-layer chromatography
DCI	direct chemical ionization (in MS)
DCM	dichloromethane
Dd	doublet of doublets (in NMR)
DMAP	4-*N,N*-dimethylaminopyridine

DME	1,2-dimethoxyethane
DMEM	Dulbecco's Modified Eagle Medium (standardized nutrient medium for cell culture)
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulphoxide
D/P	dye (fluorescent dye)/protein ratio
DPBS, D-PBS, PBS	Dulbecco's phosphate-buffered salt solution PBS = DPBS = D-PBS, pH 7.4, from Sigma, No D8537 Composition: 0.2 g KCl 0.2 g KH_2PO_4 (anhyd) 8.0 g NaCl 1.15 g Na_2HPO_4 (anhyd) made up ad 1 l with H_2O
Dt	doublet of triplets (in NMR)
DTT	DL-dithiothreitol
EDC	*N'*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride
EGFR	epidermal growth factor receptor
EI	electron impact ionization (in MS)
ELISA	enzyme-linked immunosorbent assay
eq.	equivalent(s)
ESI	electrospray ionization (in MS)
ESI-MicroTofq	ESI- MicroTofq (name of the mass spectrometer with Tof = time of flight and q = quadrupole)
FCS	foetal calf serum
Fmoc	(9*H*-fluoren-9-ylmethoxy)carbonyl
sat.	saturated
GTP	guanosine-5'-triphosphate
H	hour(s)
HATU	*O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulphonic acid
HOAc	acetic acid
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxy-1*H*-benzotriazole hydrate

HOSu	*N*-hydroxysuccinimide
HPLC	high-pressure, high-performance liquid chromatography
IC_{50}	half-maximal inhibitory concentration
i.m.	intramuscularly, administration into the muscle
i.v.	intravenously, administration into the vein
conc.	concentrated
KPL-4	human tumour cell lines
KU-19-19	human tumour cell line
LC-MS	liquid chromatography-coupled mass spectrometry
LLC-PK1 cells	Lewis lung carcinoma pork kidney cell line
L-MDR	human MDR1 transfected LLC-PK1 cells
LoVo	human tumour cell line
m	multiplet (in NMR)
MDR1	Multidrug resistance protein 1
MeCN	acetonitrile
min	minute(s)
MS	mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
NCI-H292	human tumour cell line
-Nme-	a methyl group bonded to the nitrogen atom
NMM	*N*-methylmorpholine
NMP	*N*-methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance spectrometry
NMRI	mouse strain originating from the Naval Medical Research Institute (NMRI)
Nude mice	experimental animals
NSCLC	non small cell lung cancer
PBS	phosphate-buffered salt solution
Pd/C	palladium on activated carbon
P-gp	P-glycoprotein, a transporter protein
PNGaseF	enzyme for cleaving sugar
quant.	quantitative (in yield)

Quart	quartet (in NMR)
Quint	quintet (in NMR)
R_f	retention index (in TLC)
RT	room temperature
R_t	retention time (in HPLC)
S	singlet (in NMR)
s.c.	subcutaneously, administration under the skin
SCC-4	human tumour cell line
SCID mice	test mice with severe combined immunodeficiency
SK-HEP-1	human tumour cell line
t	triplet (in NMR)
TBAF	tetra-n-butylammonium fluoride
TCEP	tris(2-carboxyethyl)phosphine
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl
Teoc	trimethylsilylethoxycarbonyl
tert	tertiary
TFA	trifluoroacetic acid
THF	tetrahydrofuran
T3P®	2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphinane 2,4,6-trioxide
U251	human tumour cell line
UV	ultraviolet spectrometry
v/v	volume to volume ratio (of a solution)
Z	benzyloxycarbonyl

Amino acid abbreviations

Ala = alanine
Arg = arginine
Asn = asparagine
Asp = aspartic acid
Cys = cysteine
Glu = glutamic acid
Gln = glutamine
Gly = glycine

His = histidine
Ile = isoleucine
Leu = leucine
Lys = lysine
Met = methionine
Nva = norvaline
Phe = phenylalanine
Pro = proline
Ser = serine
Thr = threonine
Trp = tryptophan
Tyr = tyrosine
Val = valine

HPLC and LC-MS methods:

Method 1 (LC-MS):

Instrument: Waters ACQUITY SQD UPLC System; column: Waters Acquity UPLC HSS T3 1.8 µ 50 x 1 mm; eluent A: 1 l water + 0.25 ml 99% formic acid, eluent B: 1 l acetonitrile + 0.25 ml 99% formic acid; gradient: 0.0 min 90% A → 1.2 min 5% A → 2.0 min 5% A; oven: 50°C; flow rate: 0.40 ml/min; UV detection: 208-400 nm.

Method 2 (LC-MS):

MS instrument type: Waters Synapt G2S; UPLC instrument type: Waters Acquity I-CLASS; column: Waters, BEH300, 2.1 x 150 mm, C18 1.7 µm; eluent A: 1 l water + 0.01% formic acid; eluent B: 1 l acetonitrile + 0.01% formic acid; gradient: 0.0 min 2% B → 1.5 min 2% B → 8.5 min 95% B → 10.0 min 95% B; oven: 50°C; flow rate: 0.50 ml/min; UV detection: 220 nm

Method 3 (LC-MS):

MS instrument: Waters (Micromass) QM; HPLC instrument: Agilent 1100 Series; column: Agilent ZORBAX Extend-C18 3.0x50mm 3.5-micron; eluent A: 1 l water + 0.01 mol ammonium carbonate, eluent B: 1 l acetonitrile; gradient: 0.0 min 98% A → 0.2min 98% A → 3.0 min 5% A→ 4.5 min 5% A ; oven: 40°C; flow rate: 1.75 ml/min; UV detection: 210 nm

Method 4 (LC-MS):

MS instrument type: Waters Synapt G2S; UPLC instrument type: Waters Acquity I-CLASS; column: Waters, HSST3, 2.1 x 50 mm, C18 1.8 µm; eluent A: 1 l water + 0.01% formic acid; eluent B: 1 l acetonitrile + 0.01% formic acid; gradient: 0.0 min 10% B → 0.3 min 10% B → 1.7 min 95% B → 2.5 min 95% B; oven: 50°C; flow rate: 1.20 ml/min; UV detection: 210 nm.

Method 5 (LC-MS):

Instrument: Waters ACQUITY SQD UPLC System; column: Waters Acquity UPLC HSS T3 1.8 µ 50 x 1 mm; eluent A: 1 l water + 0.25 ml 99% formic acid, eluent B: 1 l acetonitrile + 0.25 ml 99% formic acid; gradient: 0.0 min 95% A → 6.0 min 5% A → 7.5 min 5% A; oven: 50°C; flow rate: 0.35 ml/min; UV detection: 210-400 nm.

Method 6 (LC-MS):

Instrument: Micromass Quattro Premier with Waters UPLC Acquity; column: Thermo Hypersil GOLD 1.9 µ 50 x 1 mm; eluent A: 1 l water + 0.5 ml 50% formic acid, eluent B: 1 l acetonitrile + 0.5 ml 50% formic acid; gradient: 0.0 min 97% A → 0.5 min 97% A → 3.2 min 5% A → 4.0 min 5% A; oven: 50°C; flow rate: 0.3 ml/min; UV detection: 210 nm.

Method 7 (LC-MS):

Instrument: Agilent MS Quad 6150; HPLC: Agilent 1290; column: Waters Acquity UPLC HSS T3 1.8 µ 50 x 2.1 mm; eluent A: 1 l water + 0.25 ml 99% formic acid, eluent B: 1 l acetonitrile + 0.25 ml 99% formic acid; gradient: 0.0 min 90% A → 0.3 min 90% A → 1.7 min 5% A → 3.0 min 5% A; oven: 50°C; flow rate: 1.20 ml/min; UV detection: 205-305 nm.

Method 8 (LC-MS):

MS instrument type: Waters Synapt G2S; UPLC instrument type: Waters Acquity I-CLASS; column: Waters, HSST3, 2.1 x 50 mm, C18 1.8 µm; eluent A: 1 l water + 0.01% formic acid; eluent B: 1 l acetonitrile + 0.01% formic acid; gradient: 0.0 min 2% B → 2.0 min 2% B → 13.0 min 90% B → 15.0 min 90% B; oven: 50°C; flow rate: 1.20 ml/min; UV detection: 210 nm.

Method 9: LC-MS-Prep purification method for Examples 181-191 (Method LIND-LC-MS-Prep)

MS instrument: Waters; HPLC instrument: Waters; Waters X-Bridge C18 column, 19 mm x 50 mm, 5 µm, eluent A: water + 0.05% ammonia, eluent B: acetonitrile (ULC), with gradient; flow rate: 40 ml/min; UV detection: DAD; 210-400 nm.

or

MS instrument: Waters; HPLC instrument: Waters; Phenomenex Luna 5µ C18 100A column, AXIA Tech. 50 x 21.2 mm, eluent A: water + 0.05% formic acid, eluent B: acetonitrile (ULC) with gradient; flow rate: 40 ml/min; UV detection: DAD; 210-400 nm.

Method 10: LC-MS analysis method for Examples 181-191 (LIND_SQD_SB_AQ)

Instrument MS: Waters SQD; Instrument HPLC: Waters UPLC; column: Zorbax SB-Aq (Agilent), 50 mm x 2.1 mm, 1.8 µm; eluent A: water + 0.025% formic acid, eluent B: acetonitrile (ULC) + 0.025% formic acid; gradient: 0.0 min 98%A - 0.9 min 25%A – 1.0 min 5%A - 1.4 min 5%A – 1.41 min 98%A – 1.5 min 98%A; oven: 40°C; flow rate: 0.600 ml/min; UV detection: DAD; 210 nm.

Method 11 (HPLC):

Instrument:	HP1100 Series
Column:	Merck Chromolith SpeedROD RP-18e, 50-4.6mm, Cat. No. 1.51450.0001, Chromolith Guard Cartridge Kit precolumn, RP-18e, 5-4.6mm, Cat. No. 1.51470.0001
Gradient:	flow rate 5 ml/min injection volume 5 µl Solvent A: $HClO_4$ (70%) in water (4 ml/l) Solvent B: acetonitrile Start 20% B 0.50 Min 20% B

3.00 Min 90% B
3.50 Min 90% B
3.51 Min 20% B
4.00 Min 20% B

Column temperature: 40°C

Wavelength: 210 nm

Method 12: (LC-MS):

MS instrument type Thermo Scientific FT-MS; UHPLC+ instrument type Thermo Scientific UltiMate 3000; column Waters, HSST3, 2.1 x 75 mm, C18 1.8 µm; eluent A 1 l of water + 0.01% formic acid; eluent B 1 l of acetonitrile + 0.01% formic acid; gradient 0.0 min 10% B → 2.5 min 95% B → 3.5 min 95% B; oven 50°C; flow rate 0.90 ml/min; UV detection 210 nm/optimum integration path 210-300 nm

Method 13: (LC-MS):

MS instrument: Waters (Micromass) Quattro Micro; Instrument Waters UPLC Acquity; column: Waters BEH C18 1.7 µ 50 x 2.1 mm; eluent A: 1 l water + 0.01 mol ammonium formate, eluent B: 1 of acetonitrile; gradient: 0.0 min 95% A → 0.1 min 95% A → 2.0 min 15% A → 2.5 min 15% A→ 2.51 min 10% A → 3.0 min 10% A; oven: 40°C; flow rate: 0.5 ml/min; UV detection: 210 nm

All reactants or reagents whose preparation is not described explicitly hereinafter were purchased commercially from generally accessible sources. For all other reactants or reagents whose preparation likewise is not described hereinafter and which were not commercially obtainable or were obtained from sources which are not generally accessible, a reference is given to the published literature in which their preparation is described.

Method 14: (LC-MS) (MCW-LTQ-POROSHELL-TFA98-10min)

MS instrument type: ThermoFisher Scientific LTQ-Orbitrap-XL; HPLC instrument type: Agilent 1200SL; column: Agilent, POROSHELL 120, 3 x 150 mm, SB – C18 2.7 µm; eluent A: 1 l water + 0.1% trifluoroacetic acid; eluent B: 1 l acetonitrile + 0.1%

trifluoroacetic acid; gradient: 0.0 min 2% B → 0.3 min 2% B → 5.0 min 95% B → 10.0 min 95% B; oven: 40°C; flow rate: 0.75 ml/min; UV detection: 210 nm

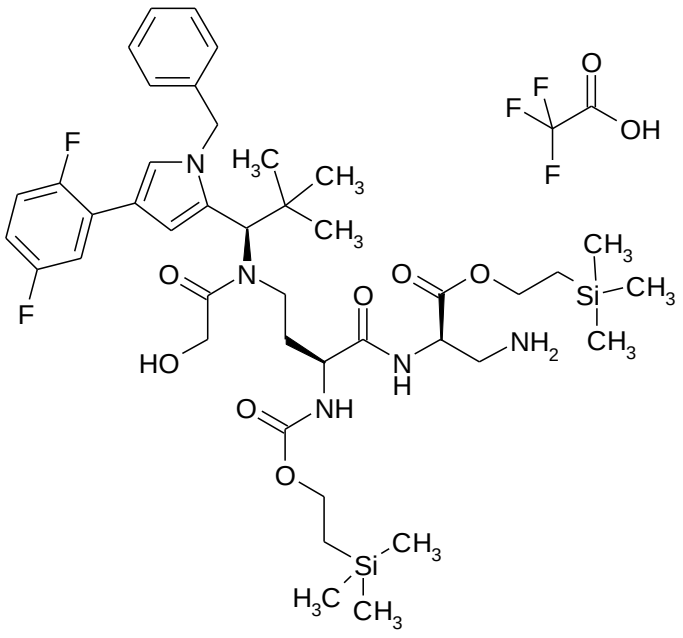
Starting compounds and intermediates:

Starting compounds suitable for the preparation of the compounds according to the invention and the preparation of suitable intermediates have already been described in WO2015/96982 A1.

The intermediates C1 to C73, L1 to L73, F1 to F58 and F82 to F91, F103 to F129, F142 to F156, F163 to F180, F192 to F196, F204 to F207, F209 to F218, F235, F236, F238, F241 to F245, F247, F248 and F254 according to WO2015/96982 A1 form part of the disclosure of the present application. Where reference is made hereinafter to compounds having particular numbers (e.g. Intermediate C1, L1 or F1), this means the compounds having these numbers according to WO2015/96982 A1. Further starting compounds and intermediates are described hereinafter.

Intermediate C74

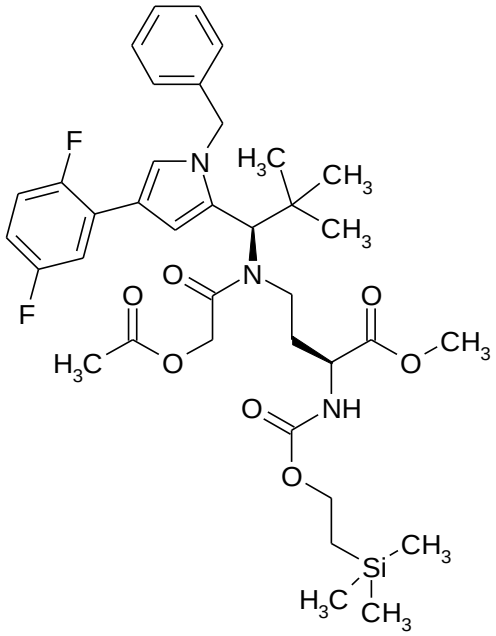
Trifluoroacetic acid 2-(trimethylsilyl)ethyl 3-amino-N-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)butanoyl]-D-alaninate (1:1)



75 mg (0.114 mmol) of Intermediate C58 were taken up in 12.5 ml of DMF and coupled to 78 mg (0.171 mmol) of Intermediate L75 in the presence of 65 mg (0.11 mmol) of HATU and 79 µl of N,N-diisopropylethylamine. After purification by preparative HPLC, the intermediate was taken up in 20 ml of ethanol and hydrogenated over 10% palladium on activated carbon at RT under hydrogen standard pressure for 1 h. The catalyst was then filtered off, the solvent was removed under reduced pressure and the product was purified by preparative HPLC. Lyophilization from acetonitrile/water 1:1 gave 63 mg (64% of theory over 2 steps) of the title compound.
LC-MS (Method 1): R_t = 1.16 min; MS (EIpos): m/z = 844 $[M+H]^+$.

Intermediate C75

Methyl (2S)-4-[(acetoxyacetyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)butanoate

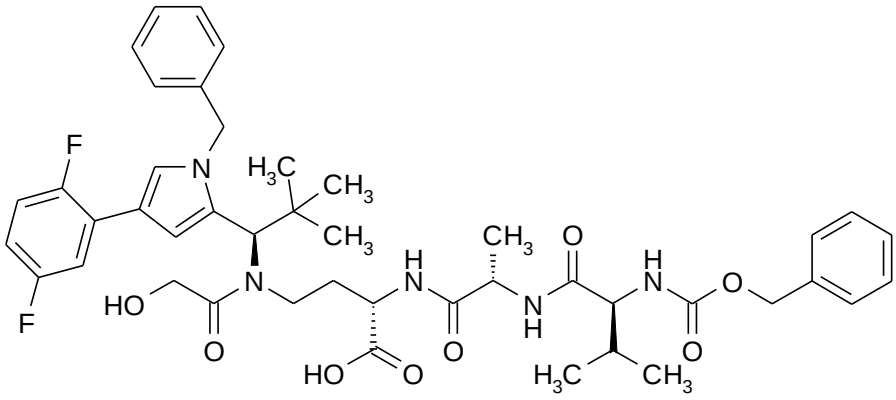


4.3 g (12.2 mmol) of Intermediate C52 were dissolved in 525 ml of DCM, and 3.63 g (17.12 mmol) of sodium triacetoxyborohydride and 8.4 ml of acetic acid were added. After stirring at RT for 5 min, 3.23 g (11.85 mmol) of methyl (2S)-4-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)butanoate (prepared from (3S)-3-amino-4-methoxy-4-oxobutanoic acid by conventional methods) dissolved in 175 ml of DCM were added,

and the mixture was stirred at RT for a further 45 min. The mixture was then diluted with DCM and extracted twice with 100 ml of saturated sodium hydrogencarbonate solution and then with saturated sodium chloride solution. The organic phase was dried over magnesium sulphate, filtered and then concentrated. The residue was purified by means of preparative HPLC. Combination of the appropriate fractions, concentration and drying of the residue under high vacuum gave 4.6 g (61% of theory) of the intermediate.
LC-MS (Method 12): R_t = 1.97 min; MS (ESIpos): m/z = 614.32 $(M+H)^+$.
200 mg (0.33 mmol) of this intermediate were dissolved in 10 ml of DCM, and 105 µl of triethylamine and 77 µl (0.717 mmol) of acetoxyacetyl chloride were then added. The mixture was stirred at RT overnight and then concentrated under reduced pressure. The residue was taken up in ethyl acetate and extracted twice with saturated sodium hydrogencarbonate solution and then with saturated sodium chloride solution. The organic phase was dried over magnesium sulphate and then concentrated. This gave 213 mg (75%) of the title compound as a beige foam.
LC-MS (Method 1): R_t = 1.46 min; MS (ESIpos): m/z = 714 $(M+H)^+$.

Intermediate C76

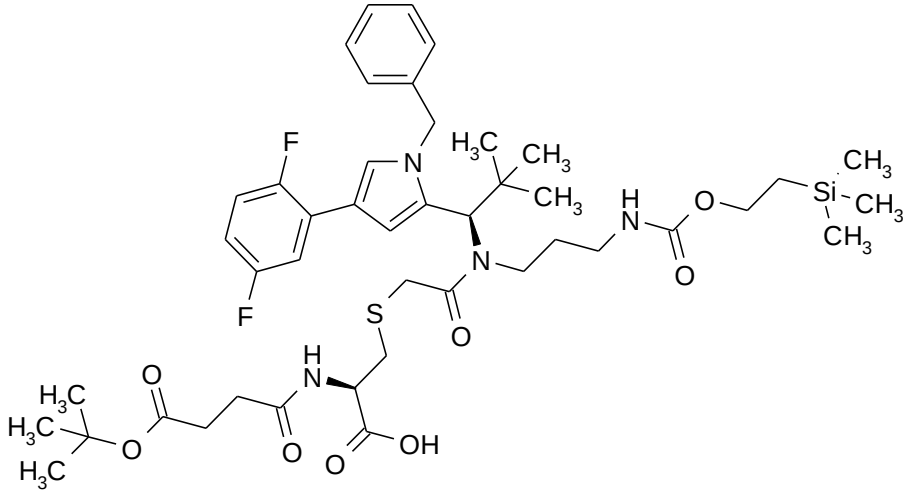
N-[(Benzyloxy)carbonyl]-L-valyl-N-{(1S)-3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-carboxypropyl}-L-alaninamide



The title compound was prepared from Intermediate C75 by conventional methods of peptide chemistry (removal of the Teoc protecting group with zinc chloride, acylation with *N*-[(benzyloxy)carbonyl]-L-valyl-L-alanine in the presence of HATU and ester cleavage with lithium hydroxide in THF/water).
LC-MS (Method 1): R_t = 1.23 min; MS (ESIpos): m/z = 818 $(M+H)^+$.

Intermediate C77

S-(11-{(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-(4-tert-butoxy-4-oxobutanoyl)-L-cysteine

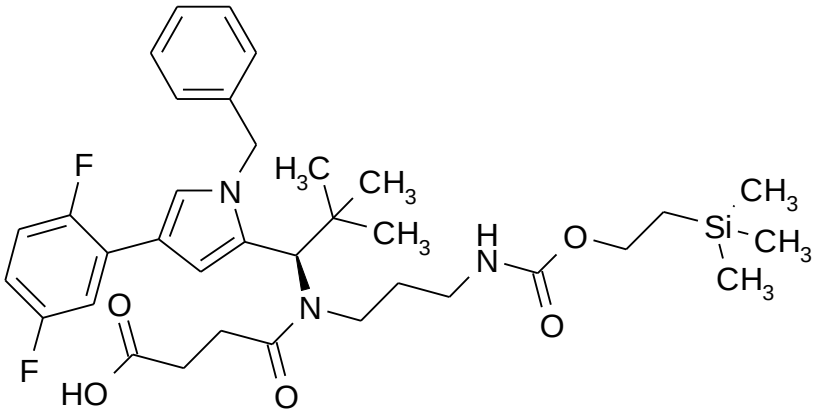


4-tert-Butoxy-4-oxobutanoic acid (8.39 mg, 48.1 µmol) was initially charged in 1.0 ml of DMF, 7.37 mg (48.1 µmol) of 1-hydroxy-1H-benzotriazole hydrate, 15.5 mg ((48.1 µmol) of (benzotriazol-1-yloxy)bisdimethylaminomethylium fluoroborate and 8.60 µl (48.1 µmol) of N,N-diisopropylethylamine were added and the mixture was stirred at RT for 10 minutes. 40.0 mg (0.048 mmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine trifluoroacetic acid (1:1) (Intermediate C71) were initially charged in 1.0 ml of DMF, 25.4 µl (141.9 µmol) of N,N-diisopropylethylamine were added, the mixture was added to the reaction and the reaction mixture was stirred at RT for 4 h. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 35.0 mg (83% of theory) of the title compound.

LC-MS (Method 12): R_t = 2.76 min; MS (ESIpos): m/z = 873 $[M+H]^+$

Intermediate C78

11-{(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silapentadecan-15-oic acid



197 mg (0.354 mmol) of 2-(trimethylsilyl)ethyl [3-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino)propyl]carbamate (see synthesis of Intermediate C11) were initially charged in 5.0 ml of dichloromethane, and the mixture was heated to 40°C. At this temperature, 240 µl (3.0 mmol) of pyridine and 220 µl (1.8 mmol) of methyl 4-chloro-4-oxobutanoate were added, and the mixture was stirred at RT for 1 h. 240 µl (3.0 mmol) of pyridine and 220 µl (1.8 mmol) of methyl 4-chloro-4-oxobutanoate were then added, and the mixture was stirred at RT for 1 h. 240 µl (3.0 mmol) of pyridine and 220 µl (1.8 mmol) of methyl 4-chloro-4-oxobutanoate were then added, and the mixture was stirred at RT for 1 h. The reaction mixture was diluted with ethyl acetate and the organic phase was extracted in each case three times with 5% $KHSO_4$ solution. The organic phase was washed with saturated NaCl solution and dried over magnesium sulphate. The solvents were evaporated under reduced pressure. The residue was purified by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 74.1 mg (31% of theory) of methyl 11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silapentadecan-15-oate.

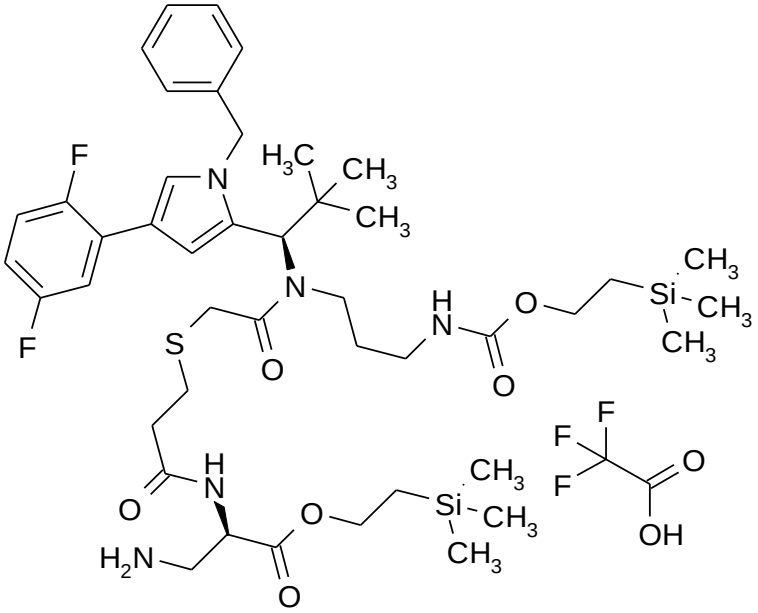
LC-MS (Method 1): R_t = 1.49 min; MS (ESIpos): m/z = 670 $[M+H]^+$

78.3 mg (117 µmol) of methyl 11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silapentadecan-15-oate were initially charged in 4.0 ml of THF, and 800 µl of methanol, 160 µl of water and 230 µl (230 µmol) of aqueous LiOH solution (1M) were added. The reaction mixture was stirred at RT for 3 h, quenched with acetic acid and purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The

solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 64.8 mg (85% of theory) of the title compound.
LC-MS (Method 12): R_t = 2.61 min; MS (ESIneg): m/z = 654 $[M-H]^-$

Intermediate C79

Trifluoroacetic acid / 2-(trimethylsilyl)ethyl 3-amino-N-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-D-alaninate (1:1)



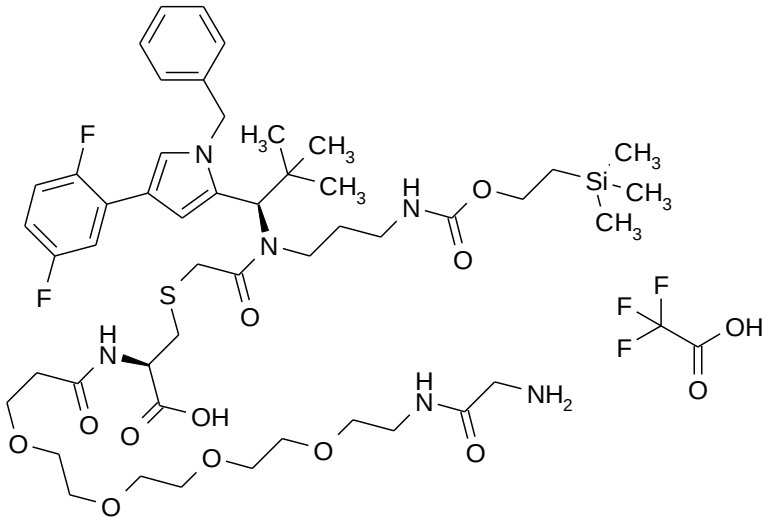
57.4 mg (81.8 µmol) of 11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-oic acid (Intermediate C69) were initially charged in 5.7 ml of DMF, 74.0 mg (164 µmol) of trifluoroacetic acid 2-(trimethylsilyl)ethyl 3-{[(benzyloxy)carbonyl]amino}-D-alaninate (1:1) (Intermediate L75), 43 µl (250 µmol) of N,N-diisopropylethylamine and 62.2 mg (164 µmol) of HATU were added and the mixture was stirred at RT for 1 h. The reaction mixture was stirred at RT for 1 h, quenched with acetic acid and purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 52.4 mg (63% of theory) of the compound 2-(trimethylsilyl)ethyl N-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-3-{[(benzyloxy)carbonyl]amino}-D-alaninate.

LC-MS (Method 1): R_t = 1.64 min; MS (ESIpos): m/z = 1022 $[M]^+$

Under argon, 6.23 mg (27.7 µmol) of palladium(II) acetate were initially charged in 3.0 ml of dichloromethane, 12 µl (83 µmol) of triethylamine and 89 µl (550 µmol) of triethylsilane were added and the mixture was stirred for 5 minutes. 56.7 mg (55.5 µmol) of 2-(trimethylsilyl)ethyl N-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-3-{[(benzyloxy)carbonyl]amino}-D-alaninate in 3.0 ml of dichloromethane were then added, and the mixture was stirred at RT overnight. The mixture was concentrated almost to dryness, acetonitrile/water was added, and the mixture was filtered and purified by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 37.4 mg (67% of theory) of the title compound.
LC-MS (Method 12):): R_t = 2.15 min; MS (ESIpos): m/z = 888 $[M+H]^+$

Intermediate C80

S-(11-{(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[15-(glycylamino)-4,7,10,13-tetraoxapentadecan-1-oyl]-L-cysteine trifluoroacetic acid (1:1)



Under argon, 43.4 mg (95.1 µmol) of 1-({N-[(benzyloxy)carbonyl]glycyl}amino)-3,6,9,12-tetraoxapentadecan-15-oic acid (Intermediate L90) were initially charged in 2.5 ml of DMF, 14.6 mg (95.1 µmol) of 1-hydroxy-1H-benzotriazole hydrate, 30.5 mg (95.1 µmol) of (benzotriazol-1-yloxy)bisdimethylaminomethylium fluoroborate and 16.5 µl (95.1 µmol) of

N,N-diisopropylethylamine were added and the mixture was stirred for 10 min. 79.0 mg (95.1 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine trifluoroacetic acid (1:1) (Intermediate C71) were dissolved in 2.5 ml of DMF, 49.5 µl (285.3 µmol) of N,N-diisopropylethylamine were added and the mixture was added to the reaction. The reaction mixture was stirred at RT for 2 h and purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 44.2 mg (40% of theory) of the compound S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[15-({N-[(benzyloxy)carbonyl]glycyl}amino)-4,7,10,13-tetraoxapentadecan-1-oyl]-L-cysteine.

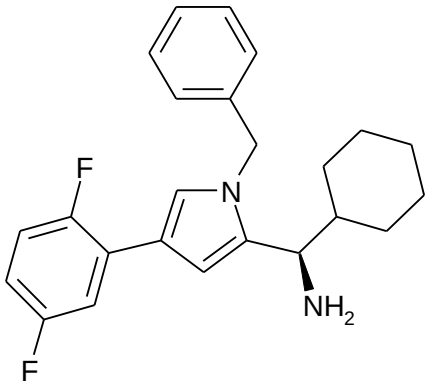
LC-MS (Method 12): R_t = 2.57 min; MS (ESIpos): m/z = 1156 $[M+H]^+$

60.2 mg (52.1 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[15-({N-[(benzyloxy)carbonyl]glycyl}amino)-4,7,10,13-tetraoxapentadecan-1-oyl]-L-cysteine were suspended in 3.0 ml of ethanol, 6.0 mg of palladium on activated carbon (10%) were added and the mixture was hydrogenated with hydrogen at RT and standard pressure for 1 h. Twice, 6.0 mg of palladium on activated carbon (10%) were added and the mixture was hydrogenated with hydrogen at RT and standard pressure for 1 h. The catalyst was filtered off and the reaction mixture was freed from the solvent under reduced pressure and dried under high vacuum. The residue was purified by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 29.4 mg (50% of theory) of the title compound.

LC-MS (Method 5): R_t = 3.77 min; MS (ESIpos): m/z = 1021 $[M+H]^+$

Intermediate C81

(R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-1-cyclohexylmethanamine



Under argon and at -78°C, 18.7 ml (37.45 mmol) of cyclohexylmagnesium chloride in diethyl ether (2M) were added to a solution of 3.12 ml (6.24 mmol) of dimethylzinc in toluene (2.0 M), and the mixture was stirred at -78°C for 30 minutes. A solution of 5.0 g (12.48 mmol) of (R)-N-{(E/Z)-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]methylene}-2-methylpropane-2-sulphinamide in THF was then added at -78°C, and the reaction mixture was stirred at this temperature for 1 h and then at RT for 4 h. At -78°C, ml of saturated ammonium chloride solution were then added and the reaction mixture was allowed to warm to RT. The mixture was diluted with ethyl acetate and washed with water. The organic phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was purified using Biotage Isolera (silica gel, ethyl acetate/cyclohexane 25:75). This gave 1.59 g (26% of theory) of the intermediate.

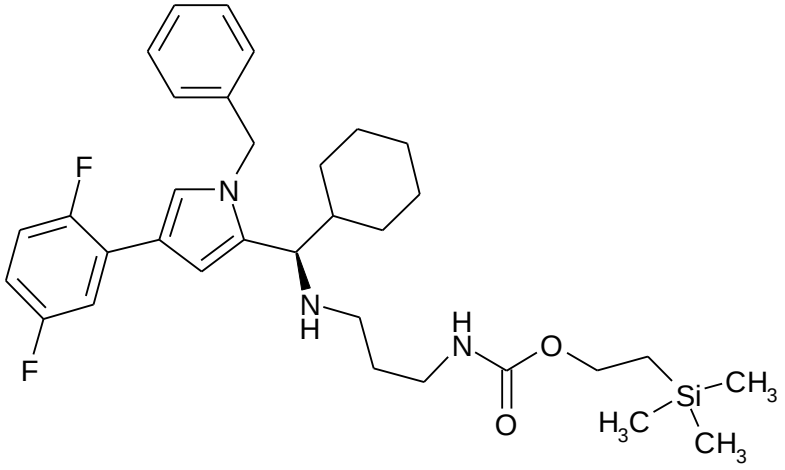
LC-MS (Method 12): R_t = 2.76 min; MS (ESIneg): m/z = 483 $[M-H]^-$

Under argon, 264.0 mg (0.54 mmol) of this intermediate were initially charged in 0.5 ml of 1,4-dioxane, and 1.36 ml of HCl in 1,4-dioxane solution (4.0 M) were then added. The reaction mixture was stirred at RT for 1 h. Dichloromethane was added, and the reaction mixture was washed with an aqueous 1M sodium hydroxide solution. The organic phase was dried with magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was purified using Biotage Isolera (silica gel, methanol/dichloromethane 98:2). The solvent was evaporated under reduced pressure and the residue was dissolved in dichloromethane, washed with a sodium hydrogencarbonate solution and dried over sodium sulphate. The solvent was evaporated under reduced pressure and the residue was dried under high vacuum. This gave 148 mg (72% of theory) of the title compound.

LC-MS (Method 13): R_t = 2.07 min; MS (ESIpos): m/z = 364 $[M-NH_2]^+$

Intermediate C82

2-(Trimethylsilyl)ethyl (3-{[(R)-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl]amino}propyl)carbamate

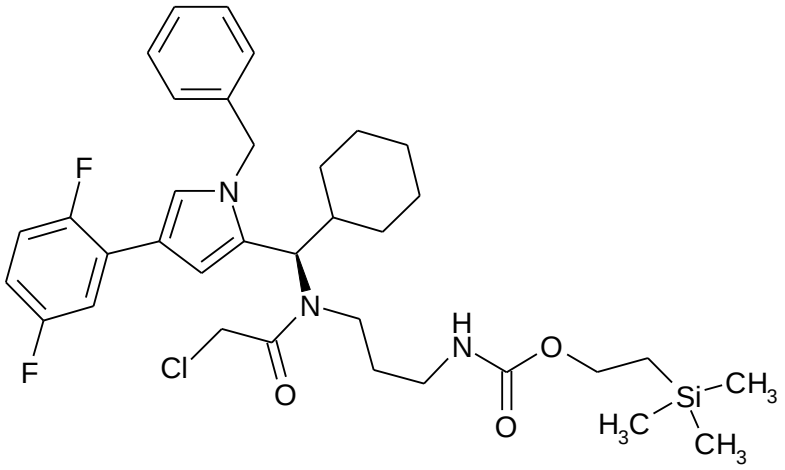


Under argon, 392.2 mg (1.85 mmol) of sodium triacetoxyborohydride and 91.29 mg (1.52 mmol) of acetic acid were added to a solution of 503.0 mg (1.32 mmol) of 1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-1-cyclohexylmethanamine (Intermediate C81) in 1.4 ml of dichloromethane, and the reaction mixture was stirred at RT for 10 minutes. A solution of 574.6 (2.38 mmol) of 2-(trimethylsilyl)ethyl (3-oxopropyl)carbamate in dichloromethane was then added, and the mixture was stirred at RT overnight. After addition of 143 mg (0.66 mmol) of 2-(trimethylsilyl)ethyl (3-oxopropyl)carbamate, the mixture was stirred for a further 2 h. The reaction mixture was diluted with dichloromethane and the organic phase was washed in each case twice with saturated sodium carbonate solution and with saturated NaCl solution, dried over sodium sulphate and concentrated. The residue was purified by preparative HPLC. The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave g (50% of theory) of the title compound. This gave 488 g (63% of theory) of the title compound.

LC-MS (Method 12): R_t = 1.89 min; MS (ESIpos): m/z = 582 $(M+H)^+$.

Intermediate C83

2-(Trimethylsilyl)ethyl (3-{[(R)-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl](chloroacetyl)amino}propyl)carbamate

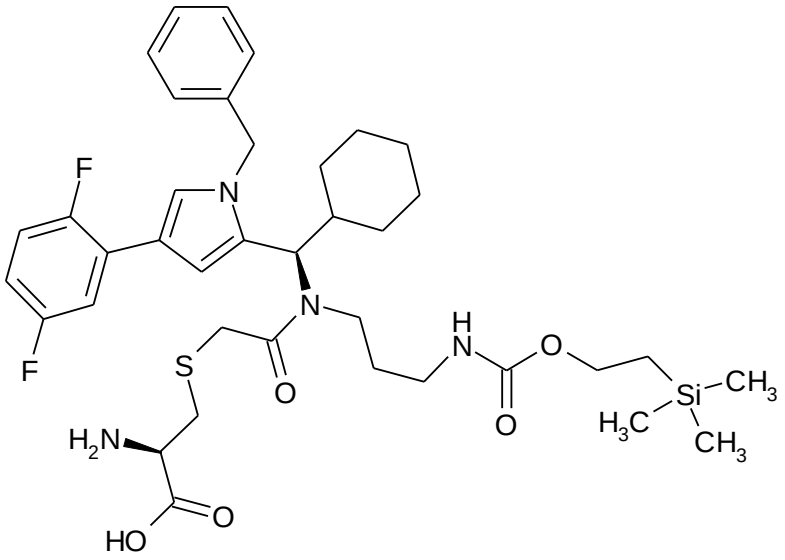


280.0 mg (2.77 mmol) of triethylamine and 397.8 mg (3.52 mmol) of chloroacetyl chloride were added to a solution of 487.9 mg (0.84 mmol) of 2-(trimethylsilyl)ethyl (3-{[(R)-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl]amino}propyl)carbamate (Intermediate C82) in 8.40 ml of dichloromethane with 4 Å molecular sieve, and the reaction mixture was stirred at RT for 6 h. The reaction mixture was diluted with dichloromethane and the organic phase was washed with saturated sodium hydrogencarbonate solution and saturated ammonium chloride solution. The organic phase was dried over sodium sulphate and concentrated. The residue was used further without purification. This gave 470 mg (85% of theory) of the title compound.

LC-MS (Method 12): R_t = 2.88 min; MS (ESIpos): m/z = 680 $(M+Na)^+$.

Intermediate C84

S-{11-[(R)-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl]-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl}-L-cysteine

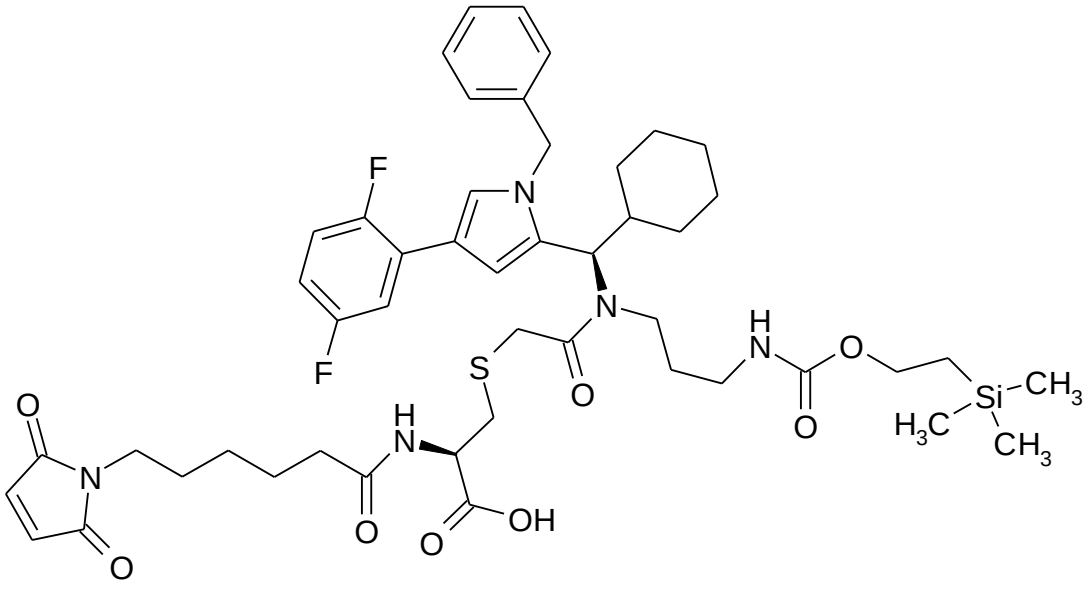


322.1 mg (2.66 mmol) of L-cysteine were suspended in 0.19 ml of water together with 319.0 mg (3.80 mmol) of sodium hydrogencarbonate. 250.0 mg (0.38 mmol) of 2-(trimethylsilyl)ethyl (3-{[(R)-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl](chloroacetyl)amino}propyl)carbamate (Intermediate C83) dissolved in 1.90 ml of *iso*-propanol and 693.8 g (4.56 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene were added. The reaction mixture was stirred at 50°C for 3.5 h. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with saturated sodium hydrogencarbonate solution and with saturated NaCl solution. The organic phase was dried over sodium sulphate and the solvent was evaporated under reduced pressure. The residue was used further without further purification. This gave 276 mg (97% of theory) of the title compound.

LC-MS (Method 12): R_t = 2.34 min; MS (ESIpos): m/z = 744 $(M+H)^+$.

Intermediate C85

S-{11-[(R)-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl]-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl}-N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-cysteine

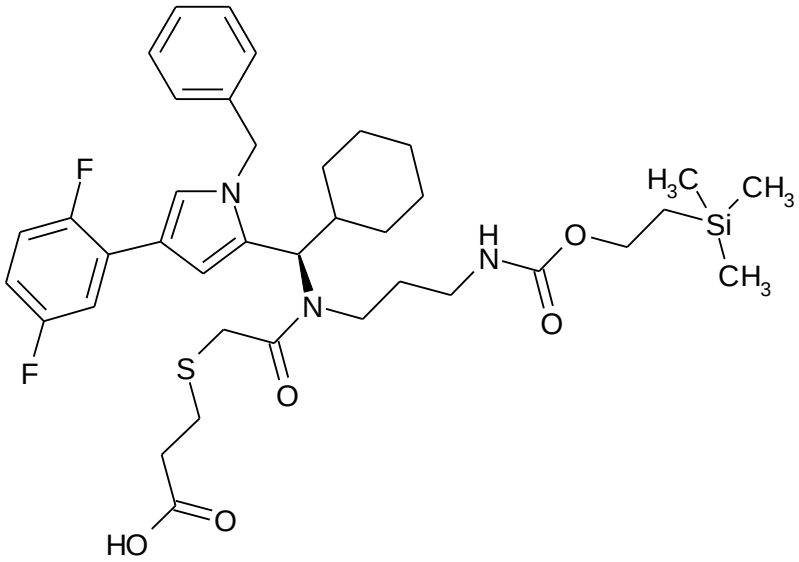


34.8 mg (0.27 mmol) of N,N-diisopropylethylamine were added to a mixture of 100 mg (0.13 mmol) of S-{11-[(R)-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl]-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl}-L-cysteine (1:1) (Intermediate C84) and 41.5 mg (0.13 mmol) of 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione in 4.0 ml of DMF, and the reaction mixture was stirred at RT for 3 h. Without workup, the mixture was purified by preparative HPLC. This gave 88 mg (70% of theory) of the title compound.

LC-MS (Method 12): R_t = 2.71 min; MS (ESIpos): m/z = 936 $(M+H)^+$.

Intermediate C86

11-[(R)-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl]-2,2-dimethyl-6,12-dioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-oic acid

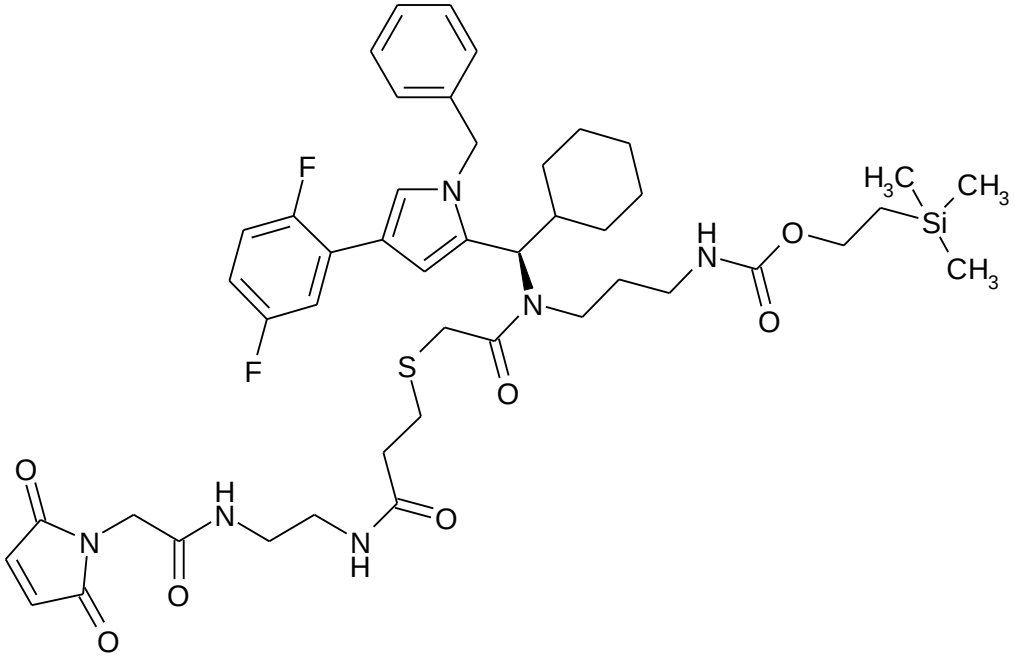


161.65 mg (1.17 mmol) of potassium carbonate were added to a mixture of 220.0 mg (0.33 mmol) of 2-(trimethylsilyl)ethyl (3-{[(R)-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl](chloroacetyl)amino}propyl)carbamate (Intermediate C83) and 39.02 mg (0.37 mmol) of 3-sulphanylpropanoic acid in 7.45 ml of methanol and a few drops of water. The reaction mixture was stirred at 50°C for 4 h. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with water and with saturated NaCl solution. The organic phase was dried over sodium sulphate and the solvent was evaporated under reduced pressure. The residue was used further without workup. This gave 201 mg (83% of theory) of the title compound.

LC-MS (Method 12): R_t = 2.72 min; MS (ESIneg): m/z = 726 $(M-H)^-$.

Intermediate C87

2-(Trimethylsilyl)ethyl {13-[(R)-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl]-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,7,12-trioxo-10-thia-3,6,13-triazahexadecan-16-yl}carbamate



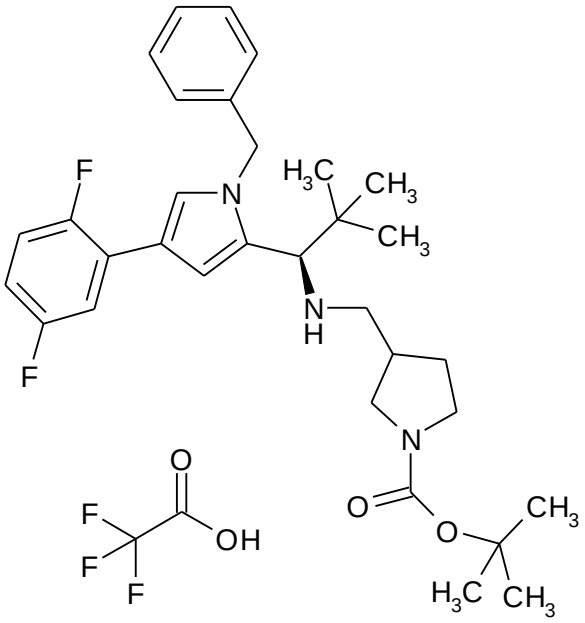
54.18 mg (0.28 mmol) of N-(2-aminoethyl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (Intermediate L1), 71.01 mg (0.50 mmol) of N,N-diisopropylethylamine, 104.46 mg (0.27 mmol) of HATU and 0.23 ml (0.14 mmol) of 1-hydroxy-7-azabenzotriazole 0.5 M in DMF were added to a solution of 100 mg (0.14 mmol) of 11-[(R)-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl]-2,2-dimethyl-6,12-dioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-oic acid (Intermediate C86) in 1.37 ml of DMF. The reaction mixture was stirred at RT for 5 h. Without further workup, the mixture was purified by preparative HPLC. This gave 41 mg (33% of theory) of the title compound.

LC-MS (Method 12): R_t = 2.61 min; MS (ESIpos): m/z = 907 $(M+H)^+$.

Intermediate C88

tert-Butyl 3-[({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino)methyl]pyrrolidine-1-carboxylate trifluoroacetic acid (1:1)

Mixture of stereoisomers

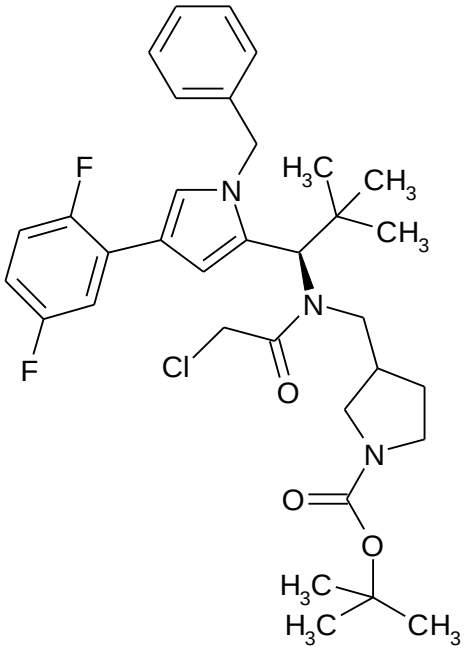


1.71 g (8.05 mmol) of sodium triacetoxyborohydride and 0.40 g (6.61 mmol) of acetic acid were added to a solution of 2.04 g (5.75 mmol) of (1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropane-1-amine (Intermediate C52) in 51 ml of dichloromethane, and the reaction mixture was stirred at RT for 5 minutes. A solution of 1.32 g (6.61 mmol) of tert-butyl 3-formylpyrrolidine-1-carboxylate in 20 ml of dichloromethane was then added, and the mixture was stirred at RT overnight. The reaction mixture was diluted with ethyl acetate and the organic phase was washed in each case twice with saturated sodium carbonate solution and with saturated NaCl solution, dried over magnesium sulphate and concentrated. The residue was purified by preparative HPLC. The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 1.86 g (50% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.99 min; MS (ESIpos): m/z = 538 $(M+H-CF_3CO_2H)^+$.

Intermediate C89

tert-Butyl 3-{[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(chloroacetyl)amino]methyl}pyrrolidine-1-carboxylate



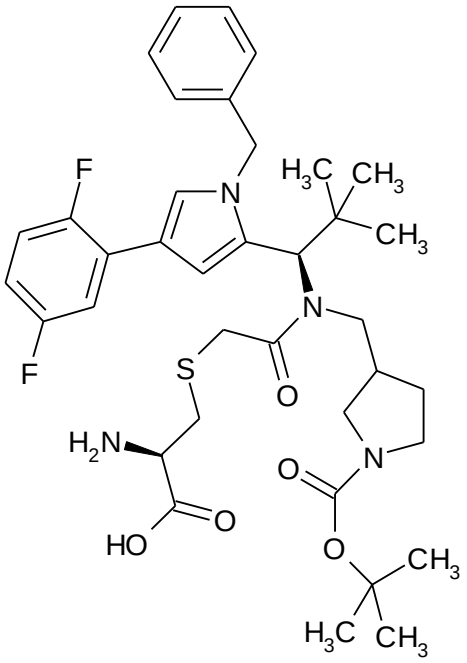
1.36 g (13.42 mmol) of triethylamine and 2.13 g (18.87 mmol) of chloroacetyl chloride were added to a solution of 2.89 g (4.19 mmol, 80% pure) of tert-butyl 3-[({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino)methyl]pyrrolidine-1-carboxylate (Intermediate C88) in 42 ml of dichloromethane with 4 Å molecular sieve. The reaction mixture was stirred at RT for 5 h. The mixture was concentrated by rotary evaporation and the residue was purified by preparative HPLC. This gave 449 mg (17% of theory) of Isomer 1 and 442 mg (17% of theory) of Isomer 2 of the title compound.

Isomer 1 LC-MS (Method 1): R_t = 2.74 min; MS (ESIpos): m/z = 614 $(M+H)^+$.

Isomer 2 LC-MS (Method 1): R_t = 2.78 min; MS (ESIpos): m/z = 614 $(M+H)^+$.

Intermediate C90

S-[2-({(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-L-cysteine (Isomer 1)

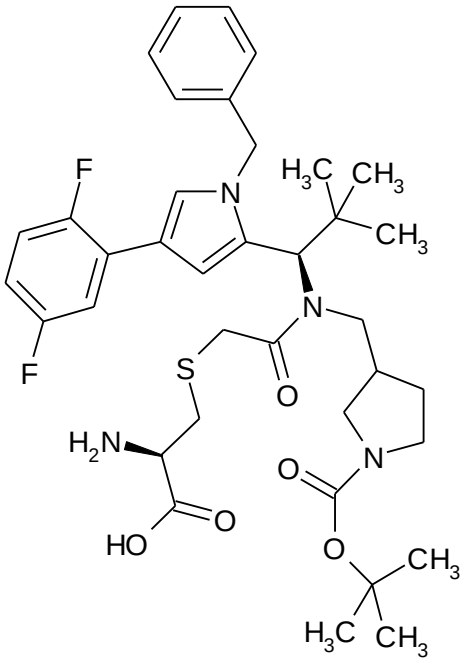


357.3 mg (0.58 mmol) of L-cysteine were suspended in 2.3 ml of water together with 488.7 mg (4.07 mmol) of sodium hydrogencarbonate. 357.0 mg (0.58 mmol) of tert-butyl 3-{[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(chloroacetyl)amino]methyl}pyrrolidine-1-carboxylate (Intermediate C89, Isomer 1) dissolved in 23.0 ml of *iso*-propanol and 1.06 g (6.98 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene were added. The reaction mixture was stirred at 50°C for 3 h. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with saturated sodium hydrogencarbonate solution and once with saturated NaCl solution. The organic phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was used further without purification. This gave 255.0 mg (62% of theory) of the title compound.

LC-MS (Method 1): R_t = 1.09 min; MS (ESIpos): m/z = 699 $(M+H)^+$.

Intermediate C91

S-[2-({(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-L-cysteine (Isomer 2)

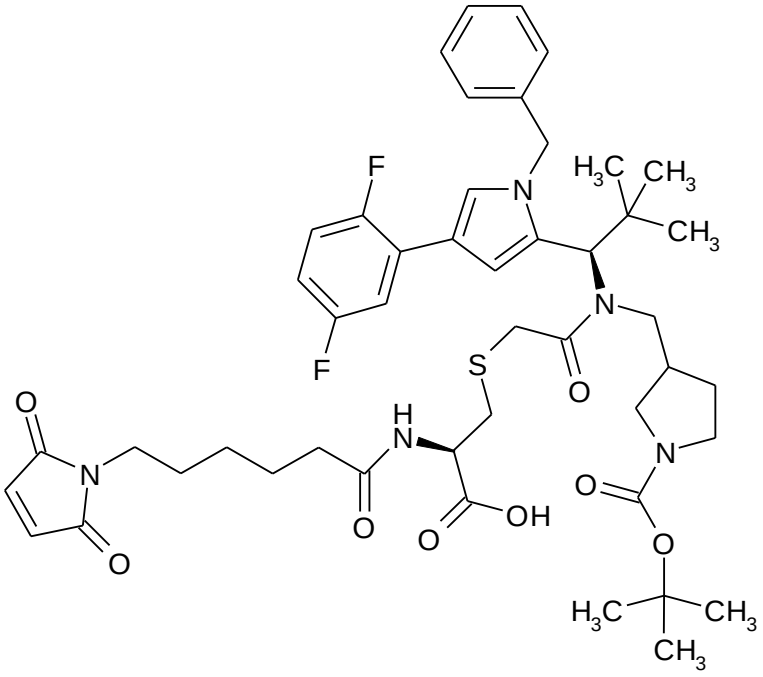


453.5 mg (3.74 mmol) of L-cysteine were suspended in 2.1 ml of water together with 449.2 mg (5.35 mmol) of sodium hydrogencarbonate. 3287.4 mg (0.54 mmol) of tert-butyl 3-{[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(chloroacetyl)amino]methyl}pyrrolidine-1-carboxylate (Intermediate C89, Isomer 2) dissolved in 21.1 ml of *iso*-propanol and 0.98 g (6.42 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene were added. The reaction mixture was stirred at 50°C for 3 h. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with saturated sodium hydrogencarbonate solution and once with saturated NaCl solution. The organic phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was used further without purification. This gave 221.0 mg (59% of theory) of the title compound.

LC-MS (Method 1): R_t = 1.12 min; MS (ESIpos): m/z = 699 $(M+H)^+$.

Intermediate C92

S-[2-({(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-cysteine (Isomer 1)

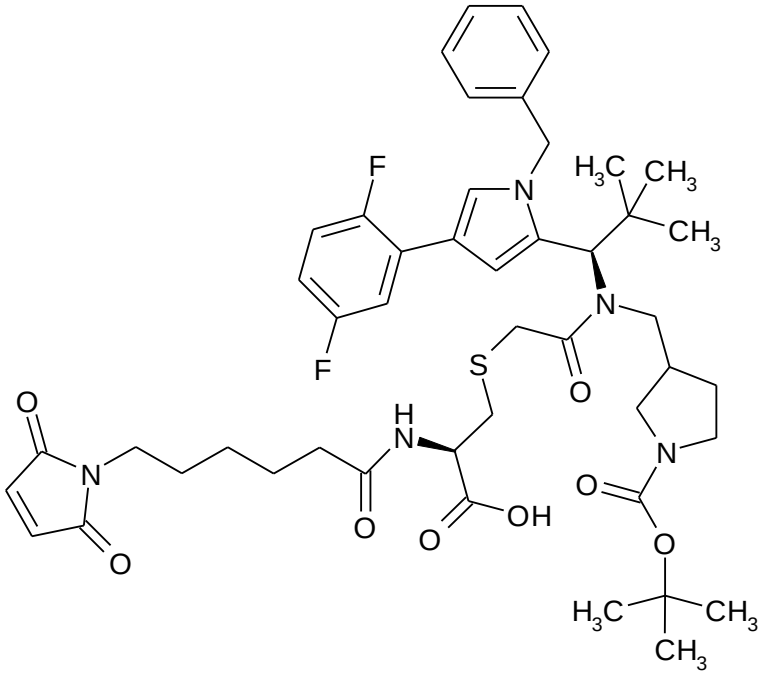


18.49 mg (0.14 mmol) of N,N-diisopropylethylamine were added to a mixture of 50 mg (0.07 mmol) of S-[2-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-L-cysteine (Intermediate C90) and 22.06 mg (0.07 mmol) of 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione in 3.3 ml of DMF, and the reaction mixture was stirred at RT for 45 minutes. Without workup, the mixture was purified by preparative HPLC. This gave 65 mg (100% of theory, 71% pure) of the title compound.

LC-MS (Method 1): R_t = 1.31 min; MS (ESIpos): m/z = 892 $(M+H)^+$.

Intermediate C93

S-[2-({(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-cysteine (Isomer 2)

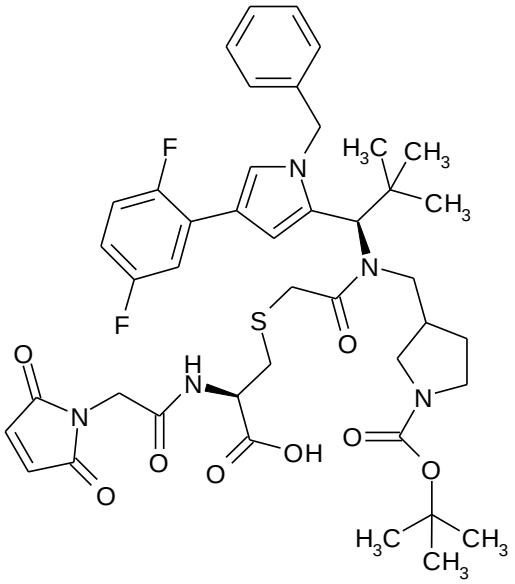


18.49 mg (0.14 mmol) of N,N-diisopropylethylamine were added to a mixture of 50.0 mg (0.07 mmol) of S-[2-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-L-cysteine (Intermediate C91) and 22.06 mg (0.07 mmol) of 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione in 3.0 ml of DMF, and the reaction mixture was stirred at RT for 90 minutes. Without workup, the mixture was purified by preparative HPLC. This gave 63 mg (98% of theory, 73% pure) of the title compound.

LC-MS (Method 1): R_t = 1.34 min; MS (ESIpos): m/z = 892 $(M+H)^+$.

Intermediate C94

S-[2-({(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-cysteine (Isomer 1)

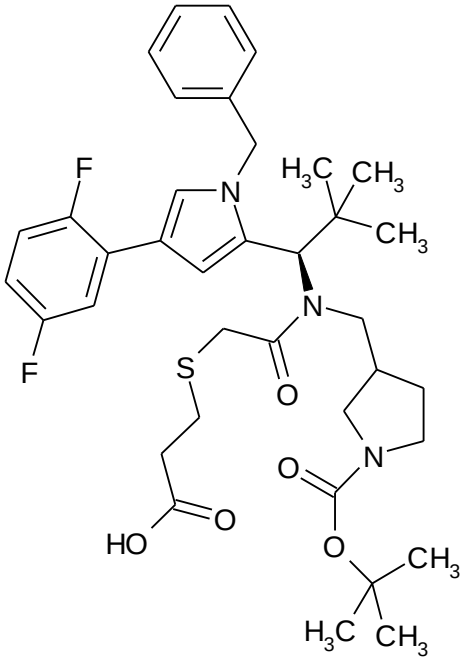


18.5 mg (0.14 mmol) of N,N-diisopropylethylamine were added to a mixture of 50.0 mg (0.07 mmol) of S-[2-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[-1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-L-cysteine (Intermediate C90) and 18.0 mg (0.07 mmol) of -{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione in 3.3 ml of DMF, and the reaction mixture was stirred at RT for 30 minutes. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with saturated NH_4Cl solution and once with saturated NaCl solution. The organic phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was used without further purification. This gave 57 mg (81% of theory, 85% pure) of the title compound.

LC-MS (Method 1): R_t = 0.96 min; MS (ESIpos): m/z = 836 $(M+H)^+$.

Intermediate C95

3-{[2-({(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]sulphanyl}propanoic acid (Isomer 1)

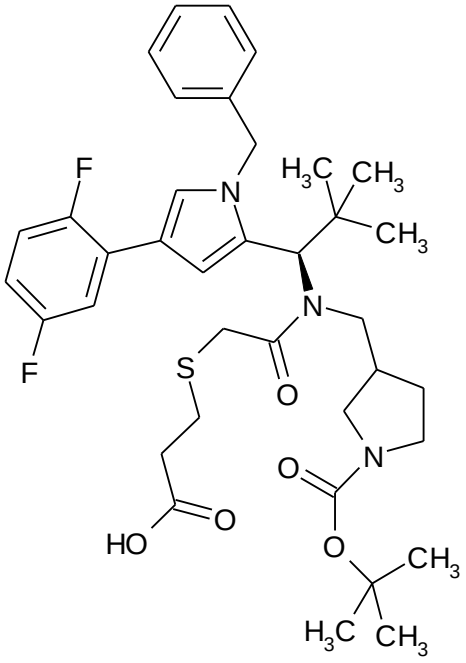


302.5 mg (2.19 mmol) of potassium carbonate were added to a mixture of 384.0 mg (0.62 mmol) of tert-butyl 3-{[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(chloroacetyl)amino]methyl}pyrrolidine-1-carboxylate (Intermediate C89, Isomer 1) and 73.0 mg (0.69 mmol) of 3-sulphanylpropanoic acid in 14 ml of methanol and a few drops of water. The reaction mixture was stirred at 50°C for 2.5 h. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with water and with saturated NaCl solution. The organic phase was dried over magnesium sulphate, the solvent was evaporated under reduced pressure and the residue was dried under high vacuum. The residue was used further without workup. This gave 358.0 mg (84% of theory) of the title compound.

LC-MS (Method 1): R_t = 1.33 min; MS (ESIpos): m/z = 684 $(M+H)^+$.

Intermediate C96

3-{[2-({(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]sulphanyl}propanoic acid (Isomer 2)

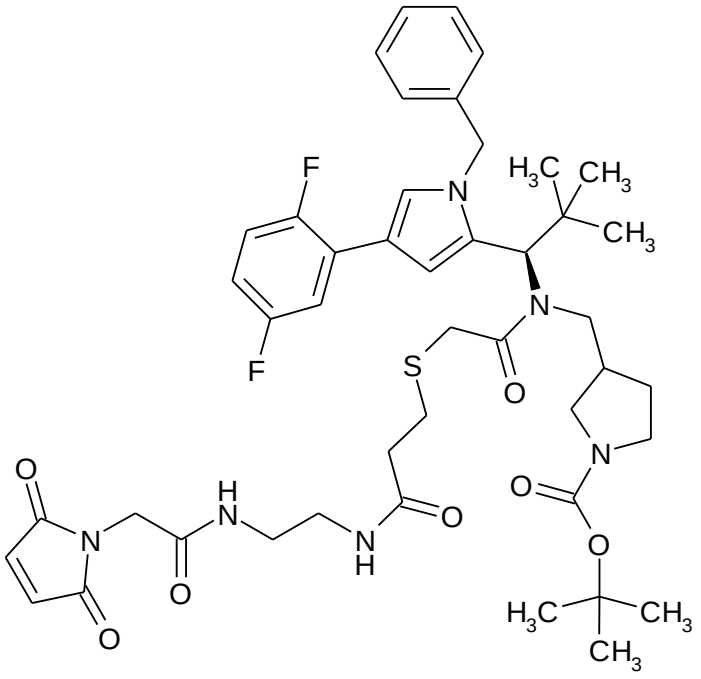


226.0 mg (1.64 mmol) of potassium carbonate were added to a mixture of 287.0 mg (0.45 mmol) of tert-butyl 3-{[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(chloroacetyl)amino]methyl}pyrrolidine-1-carboxylate (Intermediate C89, Isomer 2) and 54.6 mg (0.51 mmol) of 3-sulphanylpropanoic acid in 14 ml of methanol and a few drops of water. The reaction mixture was stirred at 50°C for 2.5 h. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with water and with saturated NaCl solution. The organic phase was dried over magnesium sulphate, the solvent was evaporated under reduced pressure and the residue was dried under high vacuum. The residue was used further without workup. This gave 318.7 mg (88% of theory, 88% pure) of the title compound.

LC-MS (Method 1): R_t = 1.36 min; MS (ESIpos): m/z = 684 $(M+H)^+$.

Intermediate C97

tert-Butyl 3-[2-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-14-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,8,13-trioxo-5-thia-2,9,12-triazatetradec-1-yl]pyrrolidine-1-carboxylate (Isomer 2)

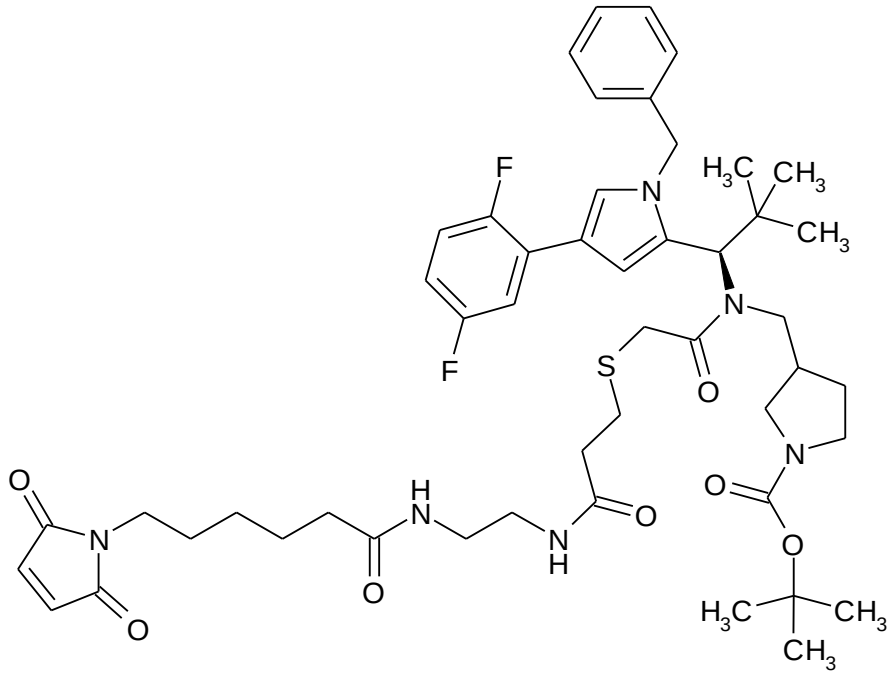


Under argon, 14.17 mg (0.11 mmol) of N,N-diisopropylethylamine and 27.80 mg (0.07 mmol) of HATU were added to a solution of 25.0 mg (0.04 mmol) of 3-{[2-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]sulphanyl}propanoic acid (Intermediate C96) in 2.81 ml of DMF. The reaction mixture was stirred at RT for 10 minutes. A solution of 22.75 mg (0.07 mmol) of N-(2-aminoethyl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide-ethane (1:1) trifluoroacetic acid (Intermediate L1) in 1.4 ml of DMF and 5 mg (0.04 mmol) of N,N-diisopropylethylamine was then added, and the mixture was stirred at RT overnight. The mixture was admixed with water and extracted with dichloromethane. The organic phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was used further without workup. This gave 26 mg (84% of theory) of the title compound.

LC-MS (Method 5): R_t = 4.39 min; MS (ESIpos): m/z = 863 $(M+H)^+$.

Intermediate C98

tert-Butyl 3-[2-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-18-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,8,13-trioxo-5-thia-2,9,12-triazaoctadec-1-yl]pyrrolidine-1-carboxylate (Isomer 2)

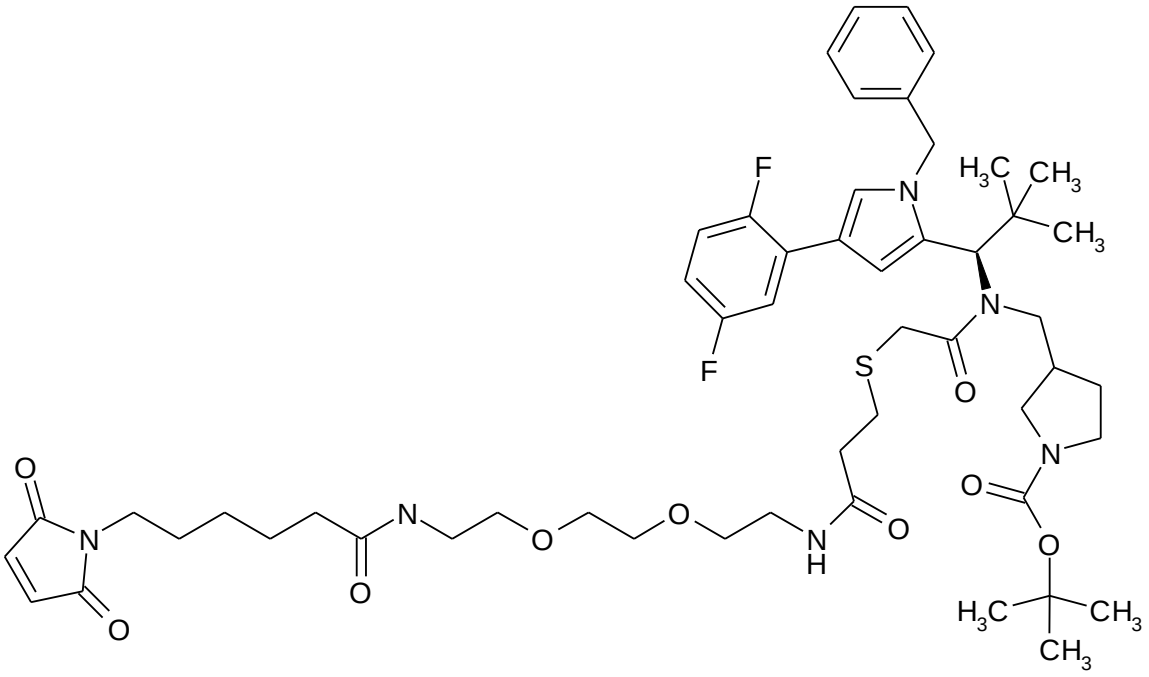


Under argon, 14.17 mg (0.11 mmol) of N,N-diisopropylethylamine and 27.80 mg (0.07 mmol) of HATU were added to a solution of 25.0 mg (0.04 mmol) of 3-{[2-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]sulphanyl}propanoic acid (Intermediate C96) in 2.81 ml of DMF. The reaction mixture was stirred at RT for 10 minutes. A solution of 37.30 mg (0.07 mmol) of N-(2-aminoethyl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide-ethane (1:1) trifluoroacetic acid in 1.4 ml of DMF and 5 mg (0.04 mmol) of N,N-diisopropylethylamine was then added, and the mixture was stirred at RT overnight. Water was added and the mixture was extracted with dichloromethane. The organic phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was used without further purification. This gave 22 mg (63% of theory) of the title compound.

LC-MS (Method 5): R_t = 4.54 min; MS (ESIpos): m/z = 919 $(M+H)^+$.

Intermediate C99

tert-Butyl 3-[2-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,8,19-trioxo-12,15-dioxa-5-thia-2,9,18-triazatetracos-1-yl]pyrrolidine-1-carboxylate (Isomer 2)

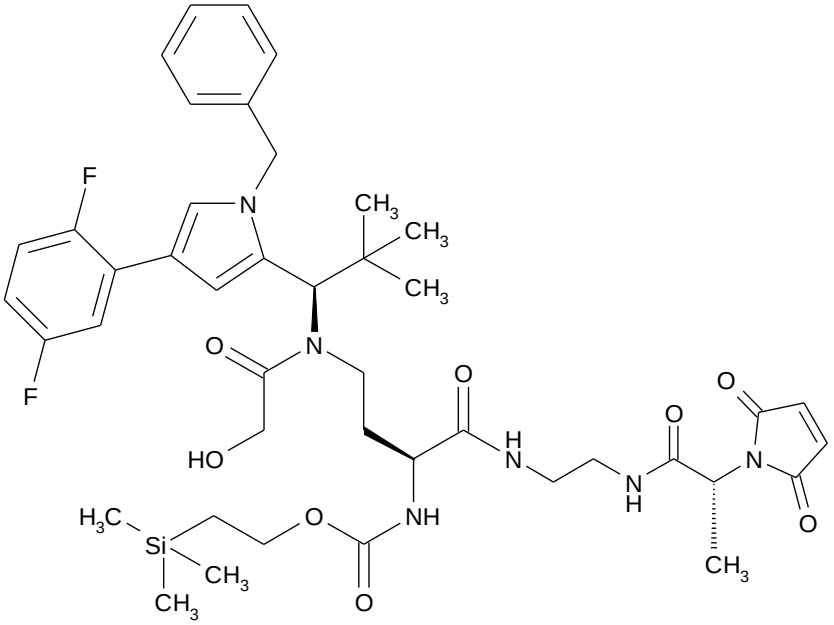


Under argon, 14.17 mg (0.11 mmol) of N,N-diisopropylethylamine and 27.80 mg (0.07 mmol) of HATU were added to a solution of 25.0 mg (0.04 mmol) of 3-{[2-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]sulphanyl}propanoic acid (Intermediate C96) in 2.81 ml of DMF. The reaction mixture was stirred at RT for 10 minutes. A solution of 35.05 mg (0.07 mmol) of N-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide-ethane (1:1) trifluoroacetic acid (Intermediate L82) in 1.4 ml of DMF and 5 mg (0.04 mmol) of N,N-diisopropylethylamine was then added, and the mixture was stirred at RT overnight. Water was added and the mixture was extracted with dichloromethane. The organic phase was dried over magnesium sulphate, the solvent was evaporated under reduced pressure and the residue was dried under high vacuum. The residue was purified by prep. HPLC. This gave 25 mg (60% of theory) of the title compound.

LC-MS (Method 1): R_t = 4.52 min; MS (ESIpos): m/z = 1007 $(M+H)^+$.

Intermediate C100

2-(Trimethylsilyl)ethyl {(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2R)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethyl)amino]-1-oxobutan-2-yl}carbamate

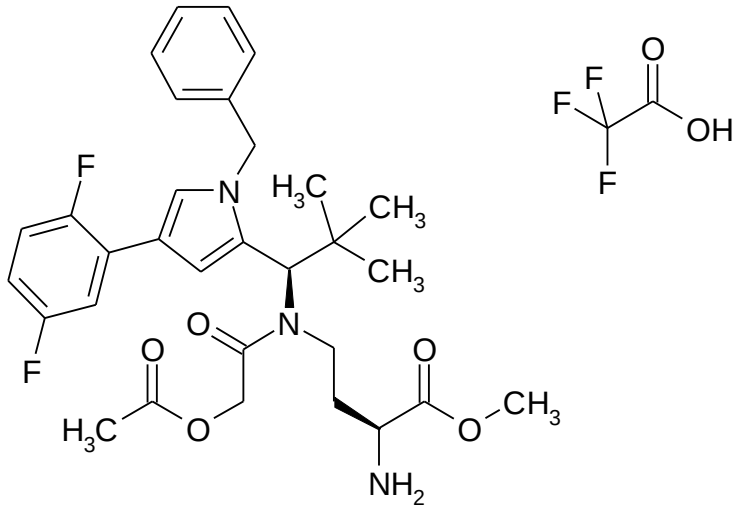


22.2 mg (0.068 mmol) of (2R)-N-(2-aminoethyl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide (1:1) trifluoroacetic acid were added to a solution of 45 mg (0.068 mmol) of (2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)butanoic acid (Intermediate C58) in 5.8 ml of DMF. After stirring at RT for 30 minutes, 39 mg (0.10 mmol) of HATU and 36 mg (0.27 mmol) of N,N-diisopropylethylamine were added to the mixture. The reaction mixture was stirred at RT for 1 h. Without workup, the mixture was purified by preparative HPLC. This gave 7 mg (12% of theory) of the title compound.

LC-MS (Method 1): R_t = 1.41 min; MS (ESIpos): m/z 851 $(M+H)^+$.

Intermediate C101

Trifluoroacetic acid / methyl (2S)-4-[(acetoxyacetyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-aminobutanoate (1:1)



4.3 g (12.2 mmol) of Intermediate C52 were dissolved in 525 ml of DCM, and 3.63 g (17.12 mmol) of sodium triacetoxyborohydride and 8.4 ml of acetic acid were added. After stirring at RT for 5 min, 3.23 g (11.85 mmol) of methyl (2S)-4-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)butanoate (prepared from (3S)-3-amino-4-methoxy-4-oxobutanoic acid by conventional methods) dissolved in 175 ml of DCM were added, and the mixture was stirred at RT for a further 45 min. The mixture was then diluted with DCM and extracted twice with 100 ml of saturated sodium hydrogencarbonate solution and then with saturated sodium chloride solution. The organic phase was dried over magnesium sulphate, filtered and then concentrated. The residue was purified by means of preparative HPLC. Combination of the appropriate fractions, concentration and drying of the residue under high vacuum gave 4.6 g (61% of theory) of the intermediate.
LC-MS (Method 12): R_t = 1.97 min; MS (ESIpos): m/z = 614.32 $(M+H)^+$.

2.06 g (3.36 mmol) of this intermediate were initially charged in 76 ml of DCM and acylated with 0.81 ml (7.17 mmol) of 2-chloro-2-oxoethyl acetate in the presence of 2.1 ml of triethylamine. After stirring at RT for 20 h, a further 0.36 ml of 2-chloro-2-oxoethyl acetate and 0.94 ml of triethylamine were added and the mixture was stirred at RT for a further 15 min. The mixture was then diluted with 500 ml of ethyl acetate and extracted successively twice with 300 ml of 5% citric acid, twice with 300 ml of saturated sodium hydrogencarbonate solution and once with 100 ml of saturated sodium chloride solution and then dried over magnesium sulphate and concentrated. Drying under high vacuum gave 2.17 g (79% of theory) of the protected intermediate.

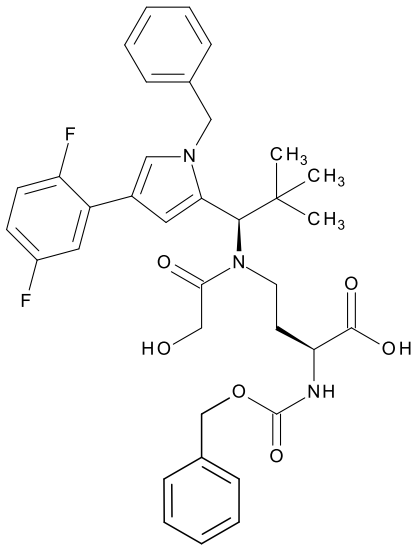
LC-MS (Method 1): R_t = 1.48 min; MS (ESIpos): m/z = 714 $(M+H)^+$.

321 mg (0.342 mmol) of this intermediate were dissolved in 7 ml of 2,2,2-trifluoroethanol. 279.5 mg (2.05 mmol) of zinc chloride were added, and the reaction mixture was stirred at 50°C for 2 h. 599 mg (2.05 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid and 2 ml of a 0.1% trifluoroacetic acid solution in water were then added, and the mixture was then concentrated under reduced pressure. The residue was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 60 mg (26% of theory) of the title compound, which still contained a portion of the deacetylated compound.

LC-MS (Method 1): R_t = 0.91 min and 0.95 min; MS (ESIpos): m/z = 528 and 570 $(M+H)^+$.

Intermediate C102

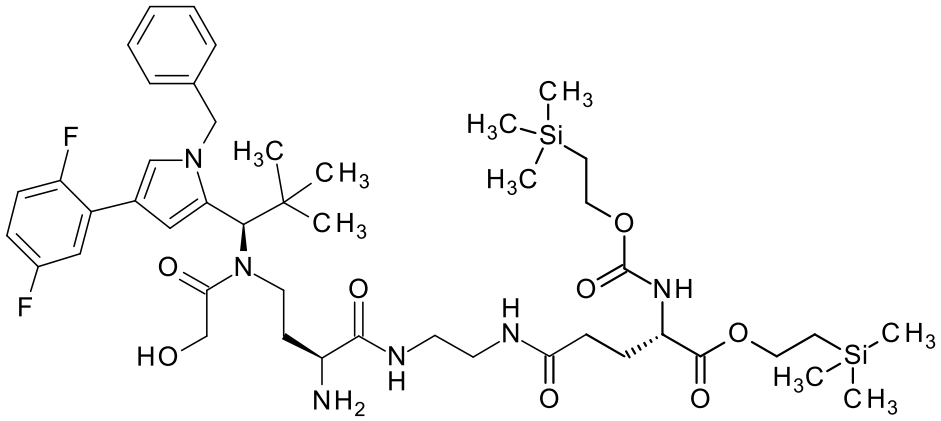
(2S)-4-[{(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-2-{[(benzyloxy)carbonyl]amino}butanoic acid



First, intermediate C52 was reductively alkylated with benzyl (2S)-2-{[(benzyloxy)carbonyl]amino}-4-oxobutanoate analogously to intermediate C2. The secondary amino group was then acylated with 2-chloro-2-oxoethyl acetate, and the two ester groups were then hydrolysed with 2M lithium hydroxide solution in methanol.
LC-MS (Method 1): R_t = 1.31 min; MS (ESIpos): m/z = 646 $(M-H)^-$.

Intermediate C103

2-(Trimethylsilyl)ethyl N-[2-({(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]-N2-{[2-(trimethylsilyl) ethoxy]carbonyl}-L-glutaminate

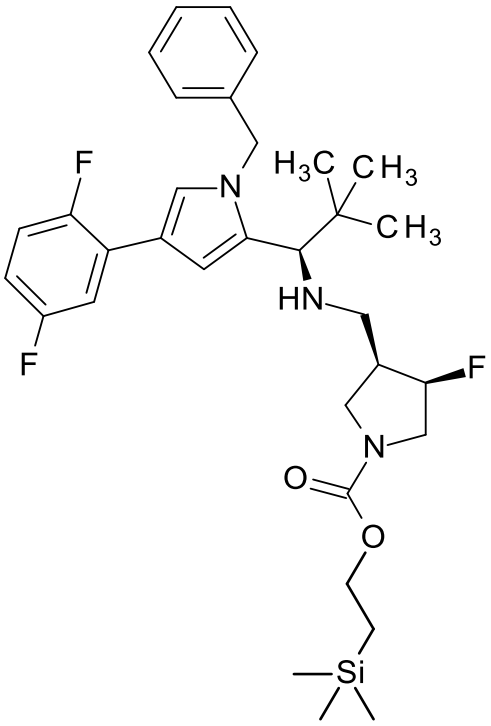


The title compound was first prepared by coupling 151 mg (0.23 mmol) of Intermediate C102 with 128 mg (0.234 mmol) of Intermediate L98 in DMF in the presence of HATU and N,N-diisopropylethylamine. Subsequently, the Z protecting group was removed by hydrogenation over 10% palladium on activated carbon at RT under standard hydrogen pressure for 30 minutes, giving the title compound.
Yield: 30% of theory over 2 stages

LC-MS (Method 1): R_t = 1.14 min; MS (ESIpos): m/z = 929 $(M+H)^+$.

Intermediate C104

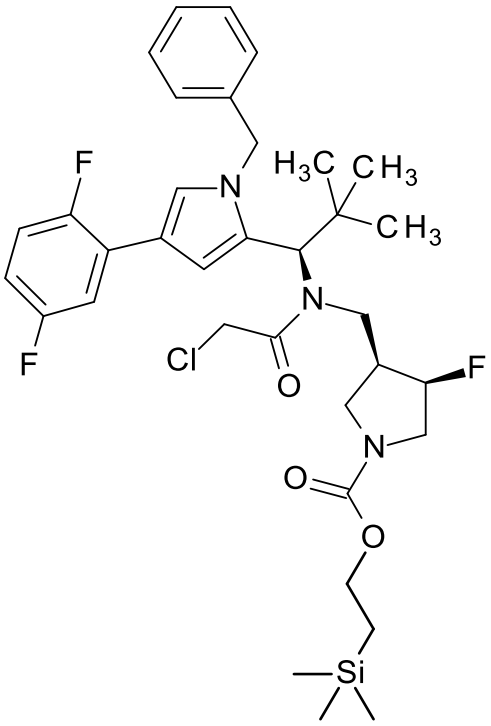
2-(Trimethylsilyl)ethyl (3R,4R)-3-[({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino)methyl]-4-fluoropyrrolidine-1-carboxylate



To a solution of 2.24 g (6.31 mmol) of (1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropan-1-amine in 56.0 ml of dichloromethane together with 4 Å molecular sieve were added 1.87 g (8.84 mmol) of sodium triacetoxyborohydride, and the mixture was stirred at room temperature for 15 minutes. Subsequently, 2.20 g (7.58 mmol) of 2-(trimethylsilyl)ethyl (3R,4S)-3-fluoro-4-formylpyrrolidine-1-carboxylate (WO 2014/151030A1) were added, and the reaction mixture was stirred at room temperature for 3.5 h. The mixture was diluted with dichloromethane and the organic phase was washed with saturated sodium hydrogencarbonate solution and water. The organic phase was dried over sodium sulphate and concentrated. The residue was purified by means of preparative HPLC. This gave 1.39 g (24% of theory) of the title compound.
LC-MS (Method 1): R_t = 1.15 min; MS (ESIpos): m/z = 600 $(M+H)^+$.

Intermediate C105

2-(Trimethylsilyl)ethyl (3R,4R)-3-{[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(chloroacetyl)amino]methyl}-4-fluoropyrrolidine-1-carboxylate

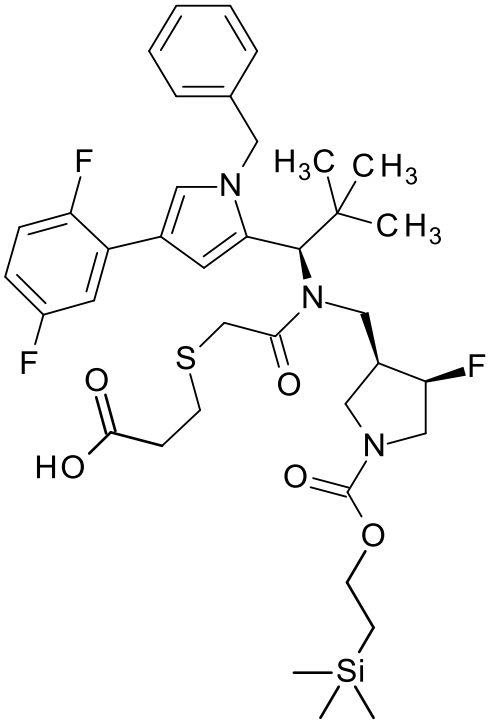


To a solution of 692.8 mg (0.88 mmol) of 2-(trimethylsilyl)ethyl (3R,4R)-3-[({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino)methyl]-4-fluoropyrrolidine-1-carboxylate (Intermediate C104) in 8.7 ml of dichloromethane together with 4 Å molecular sieve were added 295.0 mg (2.91 mmol) of triethylamine and 418.9 mg (3.71 mmol) of chloroacetyl chloride, and the reaction mixture was stirred at RT for 2.5 h. The reaction mixture was diluted with dichloromethane and the organic phase was washed with saturated sodium hydrogencarbonate solution and saturated ammonium chloride solution. The organic phase was dried over sodium sulphate and concentrated. The residue was once again dissolved in 8.7 ml of dichloromethane together with 4 Å molecular sieve and 295.0 mg (2.91 mmol) of triethylamine and 418.9 mg (3.71 mmol) of chloroacetyl chloride were added and the reaction mixture was stirred at RT for 3 h. The reaction mixture was diluted with dichloromethane and the organic phase was washed with saturated sodium hydrogencarbonate solution and saturated ammonium chloride solution. The organic phase was dried over sodium sulphate and concentrated. The organic phase was dried over sodium sulphate, concentrated and used further without purification. This gave 691 mg (74% of theory, 64% pure) of the title compound.

LC-MS (Method 1): R_t = 1.78 min; MS (ESIpos): m/z = 676 $(M+H)^+$.

Intermediate C106

3-{[2-({(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[(3R,4R)-4-fluoro-1-{[2-(trimethylsilyl)ethoxy]carbonyl}pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]sulphanyl}propanoic acid

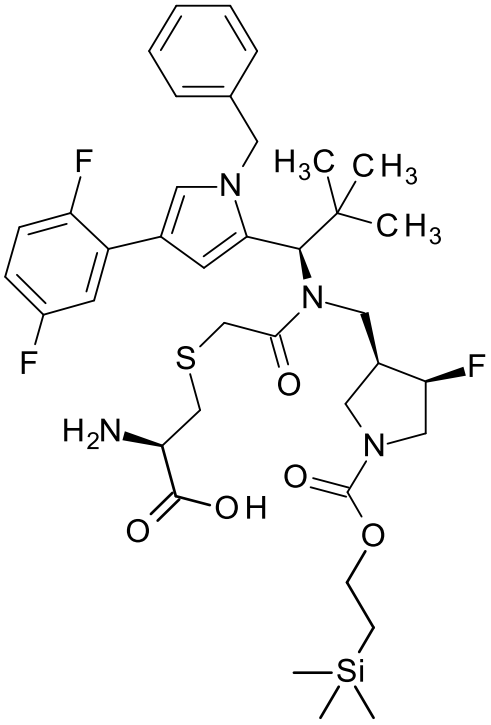


To a mixture of 691.0 mg (0.65 mmol) of 2-(trimethylsilyl)ethyl (3R,4R)-3-{[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(chloroacetyl)amino]-methyl}-4-fluoropyrrolidine-1-carboxylate (Intermediate C105) and 76.3 mg (0.72 mmol) of 3-sulphanylpropanoic acid in 15 ml of methanol and a few drops of water were added 316 mg (2.29 mmol) of potassium carbonate. The reaction mixture was stirred at 50°C for 1.5 h. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with water and with saturated NaCl solution. The organic phase was dried over magnesium sulphate, the solvent was evaporated under reduced pressure and the residue was dried under high vacuum. The residue was used further without workup. This gave 502 mg (67% of theory, 65% pure) of the title compound.

LC-MS (Method 1): R_t = 1.48 min; MS (ESIneg): m/z = 744 $(M-H)^-$.

Intermediate C107

S-{[2-({(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[(3R,4R)-4-fluoro-1-{[2-(trimethylsilyl)ethoxy]carbonyl}pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-L-cysteine

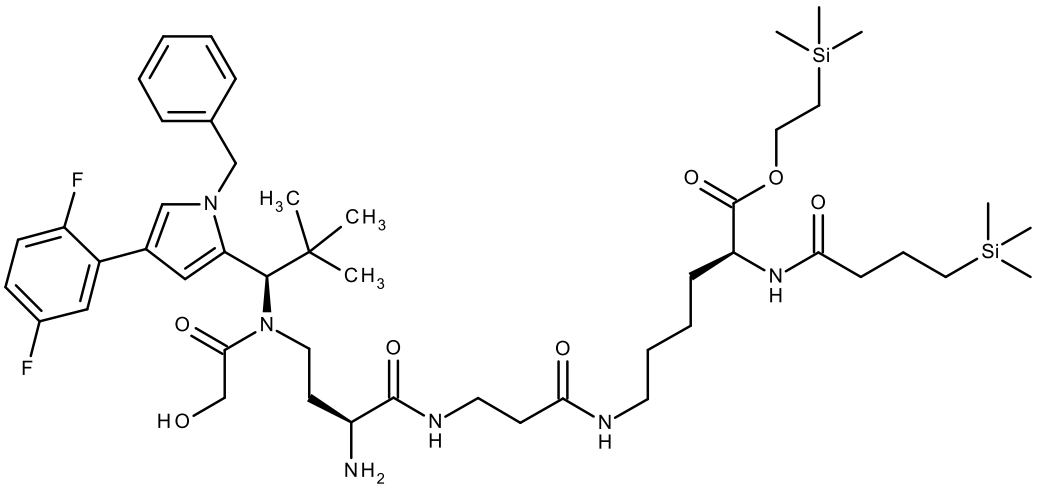


203.6 mg (1.68 mmol) of L-cysteine were suspended in 0.95 ml of water together with 201.7 mg (2.40 mmol) of sodium hydrogencarbonate. To this were added 170.0 mg (0.24 mmol) of 2-(trimethylsilyl)ethyl (3R,4R)-3-{[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(chloroacetyl)amino]methyl}-4-fluoropyrrolidine-1-carboxylate (Intermediate 105) dissolved in 9.5 ml of *iso*-propanol and 438.5 g (2.40 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene. The reaction mixture was stirred at 50°C for 3 h. Ethyl acetate was added to the mixture and the organic phase was washed repeatedly with saturated sodium hydrogencarbonate solution and with saturated NaCl solution. The organic phase was dried over sodium sulphate and the solvent was evaporated under reduced pressure. The residue was used further without further purification. This gave 152 mg (83% of theory) of the title compound.

LC-MS (Method 1): R_t = 1.26 min; MS (ESIpos): m/z = 762 $(M+H)^+$.

Intermediate C108

2-(Trimethylsilyl)ethyl N^6-(N-{(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-beta-alanyl)-N^2-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-lysinate



The title compound was prepared by coupling 103 mg (0.16 mmol) of Intermediate C102 with 110 mg (0.175 mmol) of 2-(trimethylsilyl)ethyl N^6-beta-alanyl-N^2-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-lysinate in DMF in the presence of EDCI, HOBT and N,N-diisopropylethylamine. Subsequently, the Z protecting group was removed by hydrogenation over 10% palladium on activated carbon in dichloromethane/methanol 1:1 at RT under standard hydrogen pressure for 1 hour, giving the title compound in a yield of 113 mg (75% of theory over 2 stages).

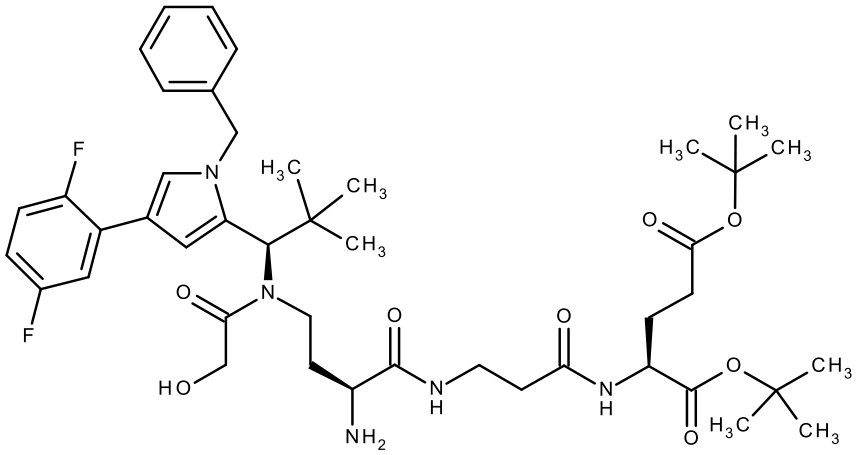
LC-MS (Method 1): R_t = 1.17 min; MS (ESIpos): m/z = 957 $(M+H)^+$.

The intermediate used here was prepared by conventional methods of peptide chemistry by coupling of commercially available N-(tert-butoxycarbonyl)-beta-alanine and 2-(trimethylsilyl)ethyl N^2-[(benzyloxy)carbonyl]-L-lysinate in the presence of HATU, hydrogenolytic detachment of the Z protecting group, introduction of the trimethylsilylethyloxycarbonyl (Teoc) protecting group with 1-({[2-(trimethylsilyl)ethoxy]carbonyl}oxy)pyrrolidine-2,5-dione and final gentle detachment of the Boc protecting group by stirring in a 7.5% trifluoroacetic acid solution in dichloromethane for 45 minutes.

LC-MS (Method 1): R_t = 0.83 min; MS (ESIpos): m/z = 462 $(M+H)^+$.

Intermediate C109

Di-tert-butyl N-{(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-beta-alanyl-L-glutamate

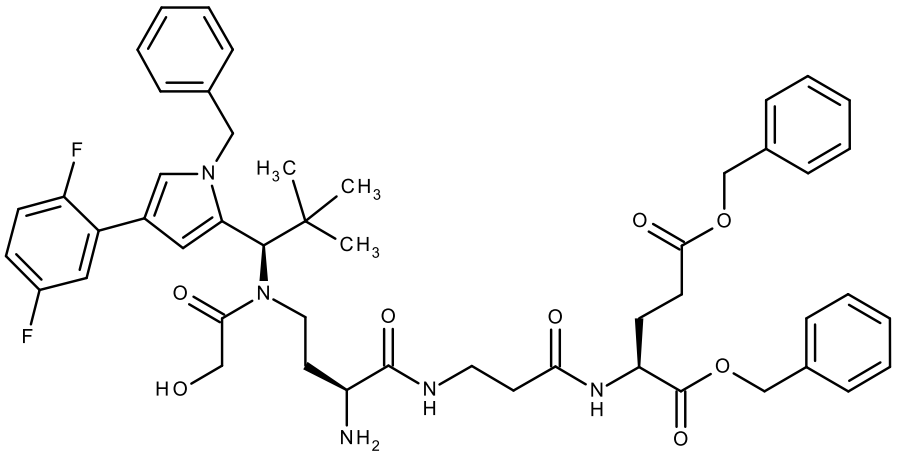


First of all, the dipeptide derivative di-tert-butyl beta-alanyl-L-glutamate was prepared by conventional methods of peptide chemistry by coupling of commercially available N-[(benzyloxy)carbonyl]-beta-alanine and di-tert-butyl L-glutamate hydrochloride (1:1) in the presence of HATU and subsequent hydrogenolytic detachment of the Z protecting group. The title compound was then prepared by coupling this intermediate with Intermediate C102 in the presence of HATU and N,N-diisopropylethylamine and subsequent detachment of the Z protecting group by hydrogenation over 10% palladium on activated carbon in methanol at RT under standard hydrogen pressure for 45 minutes.

LC-MS (Method 1): R_t = 0.99 min; MS (ESIpos): m/z = 826 $[M+H]^+$.

Intermediate C110

Dibenzyl N-{(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-beta-alanyl-L-glutamate

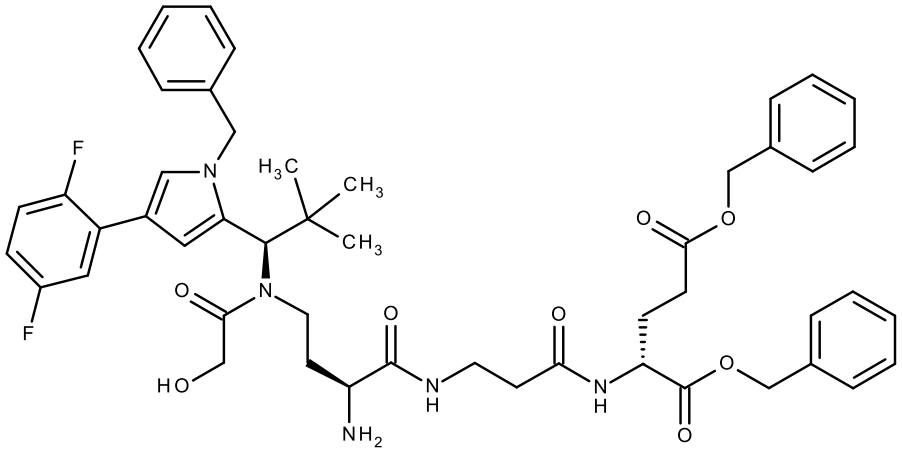


The title compound was prepared by coupling dibenzyl L-glutamate, which had been released beforehand from its p-toluenesulphonic acid salt by partitioning between ethyl acetate and 5% sodium hydrogencarbonate solution, with Intermediate C61 in the presence of HATU and N,N-diisopropylethylamine and subsequent detachment of the Teoc protecting group with zinc chloride in trifluoroethanol.

LC-MS (Method 1): R_t = 1.09 min; MS (ESIpos): m/z = 894 $[M+H]^+$.

Intermediate C110(D)

Dibenzyl N-{(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-beta-alanyl-D-glutamate

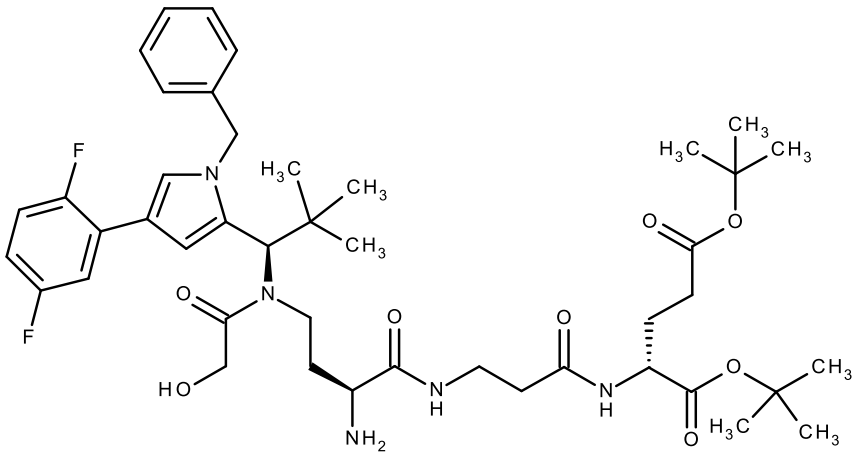


The title compound was prepared by coupling dibenzyl D-glutamate, which had been released beforehand from its p-toluenesulphonic acid salt by partitioning between ethyl acetate and 5% sodium hydrogencarbonate solution, with Intermediate C61 in the presence of HATU and N,N-diisopropylethylamine and subsequent detachment of the Teoc protecting group with zinc chloride in trifluoroethanol.

LC-MS (Method 1): R_t = 1.08 min; MS (ESIpos): m/z = 894 $[M+H]^+$.

Intermediate C111

Di-tert-butyl N-{(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-beta-alanyl-D-glutamate

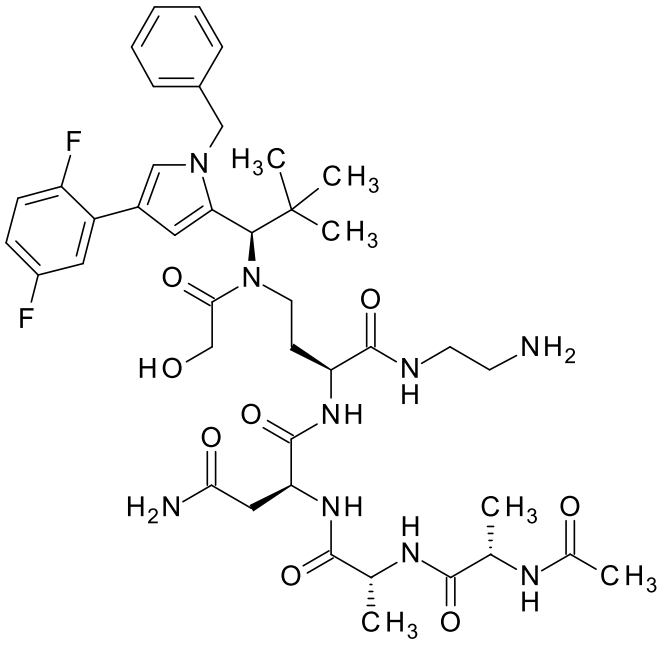


The title compound was synthesized analogously to Intermediate C109.

LC-MS (Method 1): R_t = 1.06 min; MS (ESIpos): m/z = 826 $[M+H]^+$.

Intermediate C112

N-Acetyl-L-alanyl-D-alanyl-N^1-{(2S)-1-[(2-aminoethyl)amino]-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-oxobutan-2-yl}-L-aspartamide

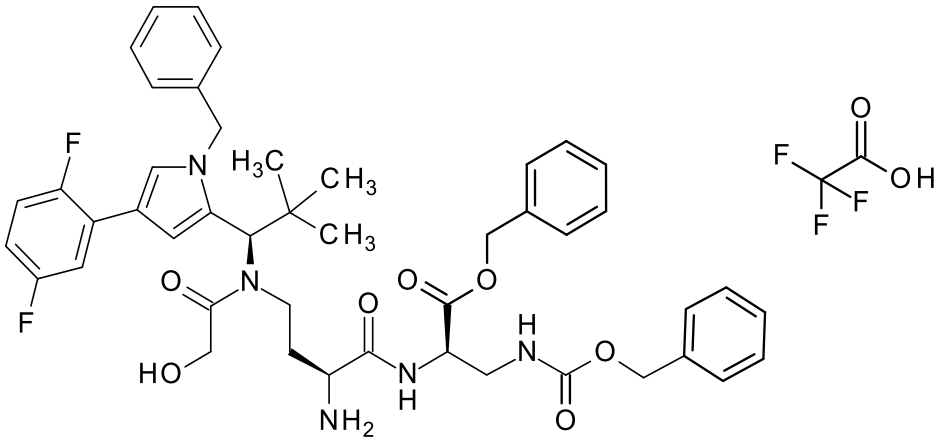


First of all, (2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethyl propyl}(glycoloyl)amino]-2-[(tert-butoxycarbonyl)amino]butanoic acid was coupled to benzyl (2-aminoethyl)carbamate hydrochloride (1:1) in the presence of HATU and N,N-diisopropylethylamine. Alternatively, it is also possible to use Intermediate C58 as a reactant. This was followed by the detachment of the Boc protecting group by stirring with 4 equivalents of zinc chloride in trifluoroethanol at 50°C for 5 hours. The intermediate obtained was then coupled to Intermediate L111 in the presence of HATU and N,N-diisopropylethylamine. In the last step, the title compound was obtained by hydrogenation over 10% palladium on activated carbon in DCM/methanol 1:1 at RT under hydrogen standard pressure for 1 hour.

LC-MS (Method 1): R_t = 0.8 min; MS (ESIpos): m/z = 854 $[M+H]^+$.

Intermediate C113

Trifluoroacetic acid / benzyl N-{(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-3-{[(benzyloxy)carbonyl]amino}-D-alaninate (1:1)

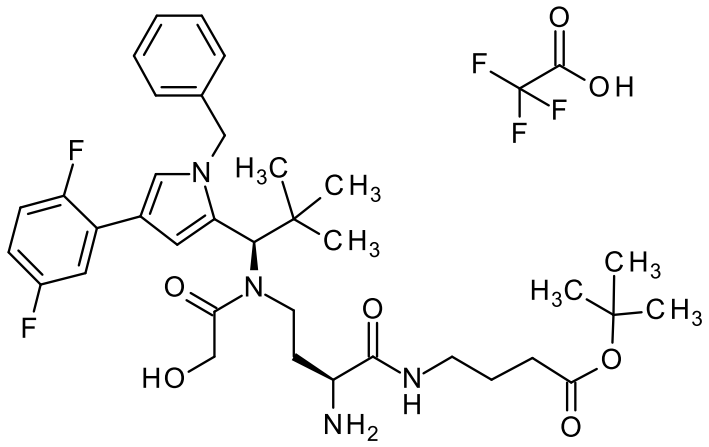


First of all, trifluoroacetic acid / benzyl 3-{[(benzyloxy)carbonyl]amino}-D-alaninate (1:1) was prepared proceeding from commercially available 3-{[(benzyloxy)carbonyl]amino}-N-(tert-butoxycarbonyl)-D-alanine by esterification with benzyl alcohol in the presence of EDC/DMAP and subsequent detachment of the Boc protecting group with trifluoroacetic acid. This amino acid unit was then coupled to Intermediate C58 in the presence of HATU and N,N-diisopropylethylamine in DMF. In the last step, the title compound was obtained by stirring with 6 equivalents of zinc chloride in trifluoroethanol at 50°C for 2 hours and purification by preparative HPLC.

LC-MS (Method 1): R_t = 1.05 min; MS (ESIpos): m/z = 824 $[M+H]^+$.

Intermediate C114

Trifluoroacetic acid / tert-butyl 4-({(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)butanoate (1:1)

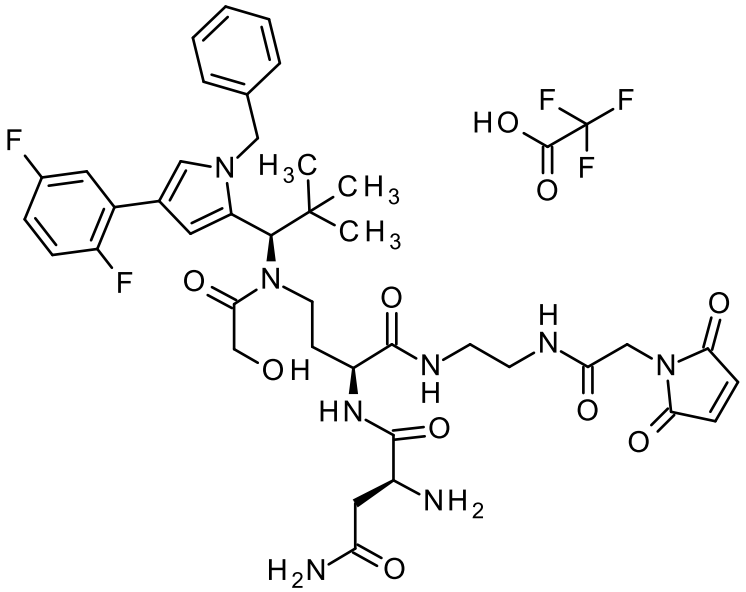


First of all, Intermediate C102 was coupled to tert-butyl 4-aminobutanoate hydrochloride (1:1) in the presence of HATU and N,N-diisopropylethylamine. Subsequently, the title compound was obtained by hydrogenation over 10% palladium on activated carbon in DCM/methanol 1:1 at RT under hydrogen standard pressure for 1 hour.

LC-MS (Method 1): R_t = 1.0 min; MS (ESIpos): m/z = 655 $[M+H]^+$.

Intermediate C116

Trifluoroacetic acid / N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide (1:1)



Trifluoroacetic acid / (2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-N-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)butanamide (1:1) (81.0 mg, 100 µmol) (Intermediate F104) and 2,5-dioxopyrrolidin-1-yl N^2-(tert-butoxycarbonyl)-L-asparaginate (43.0 mg, 131 µmol) were dissolved in 5.0 ml of DMF. The reaction mixture was stirred with N,N-diisopropylethylamine (61 µl, 350 µmol) at RT for a further 1 h, and then purified directly by preparative RP-HPLC (column: Chromatorex 125x30; 10µ, flow rate: 75 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was lyophilized. This gave 84 mg (88% of theory) of the compound tert-butyl [(2S)-4-amino-1-({(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}amino)-1,4-dioxobutan-2-yl]carbamate.

LC-MS (Method 1): R_t = 1.09 min; MS (ESIpos): m/z = 907 $[M+H]^+$

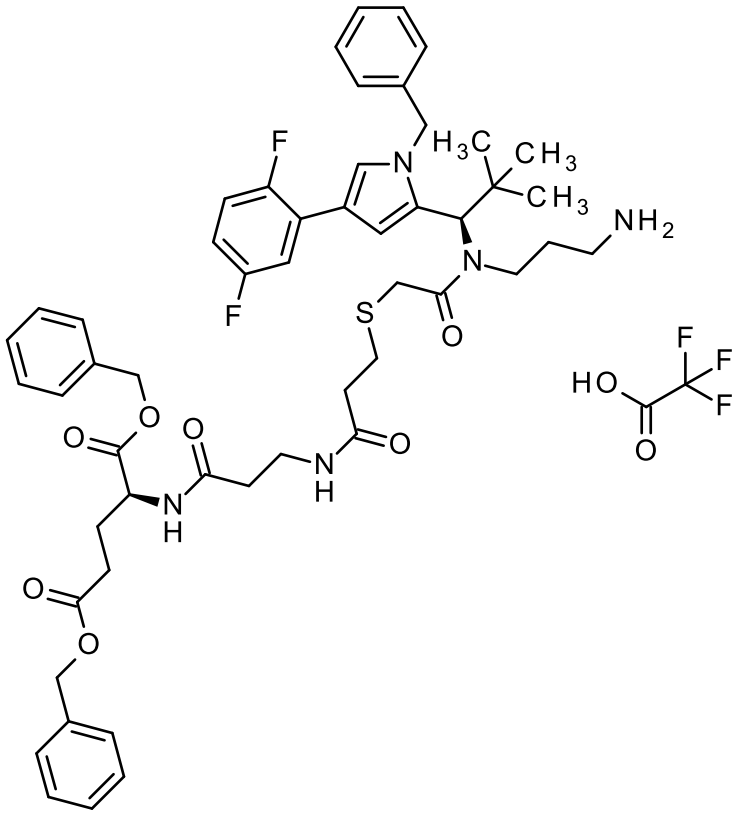
tert-Butyl [(2S)-4-amino-1-({(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}amino)-1,4-dioxobutan-2-yl]carbamate (83.0 mg, 91.5 µmol) was dissolved in 5.0 ml of trifluoroethanol. The reaction mixture was admixed with zinc chloride (74.8 mg, 549 µmol) and stirred at 50°C for 15 min. The mixture was admixed with ethylenediamine-N,N,N',N'-tetraacetic acid (160 mg, 549 µmol) and diluted with 5.0 ml of acetonitrile/water, TFA (20 µl) was added and the mixture was

stirred for a further 10 min. The mixture was filtered through a syringe filter and purified by preparative RP-HPLC (column: Chromatorex 125x30; 10µ, flow rate: 75 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 50 mg (58% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.81 min; MS (ESIpos): m/z = 807 $[M+H]^+$

Intermediate C117

Trifluoroacetic acid / dibenzyl N-[3-({2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)propanoyl]-beta-alanyl-L-glutamate (1:1)

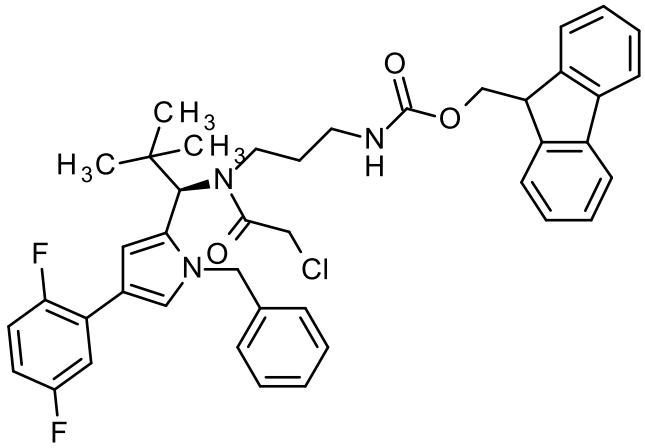


The title compound was prepared by coupling trifluoroacetic acid / dibenzyl beta-alanyl-L-glutamate (1:1) (Intermediate L127) to 11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-oic acid in the presence of HATU and N,N-diisopropylethylamine and subsequent detachment of the Teoc protecting group by means of zinc chloride in trifluoroethanol.

LC-MS (Method 1): R_t = 1.07 min; MS (ESIpos): m/z = 938 $[M+H]^+$.

Intermediate C118

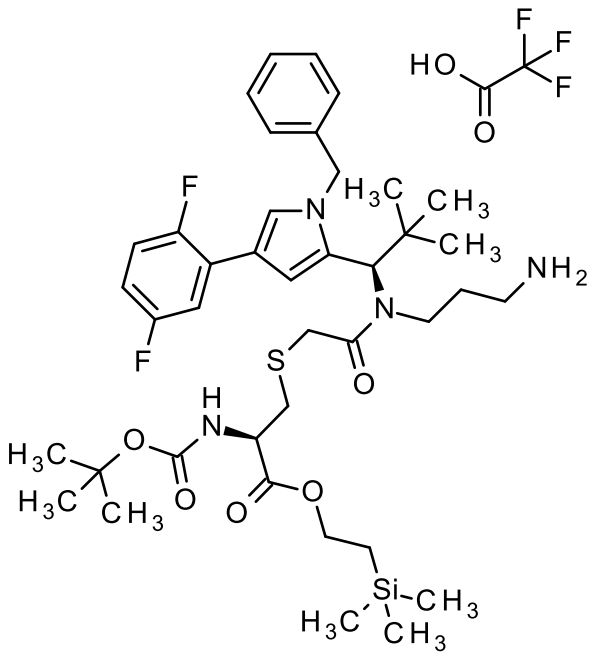
9H-Fluoren-9-ylmethyl {3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(chloroacetyl)amino]propyl}carbamate



9H-Fluoren-9-ylmethyl [3-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino)propyl]carbamate (2.50 g, 3.94 mmol) and triethylamine (1.6 ml, 12 mmol) were initially charged in 200 ml of dichloromethane and cooled to 0°C. At this temperature, chloroacetyl chloride (2.23 g, 19.7 mmol) was added. The reaction mixture was stirred at RT for 5 h and diluted with ethyl acetate. The organic phase was washed three times with saturated sodium hydrogencarbonate solution and saturated ammonium chloride solution. The organic phase was washed with saturated sodium chloride solution and dried over magnesium sulphate. The residue was used without further purification in the next stage of the synthesis. This gave 1.7 g (63% of theory) of the title compound.
LC-MS (Method 1): R_t = 1.52 min; MS (ESIpos): m/z = 710 $(M+H^+)^+$.

Intermediate C119

Trifluoroacetic acid / 2-(trimethylsilyl)ethyl S-{2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-N-(tert-butoxycarbonyl)-L-cysteinate (1:1)



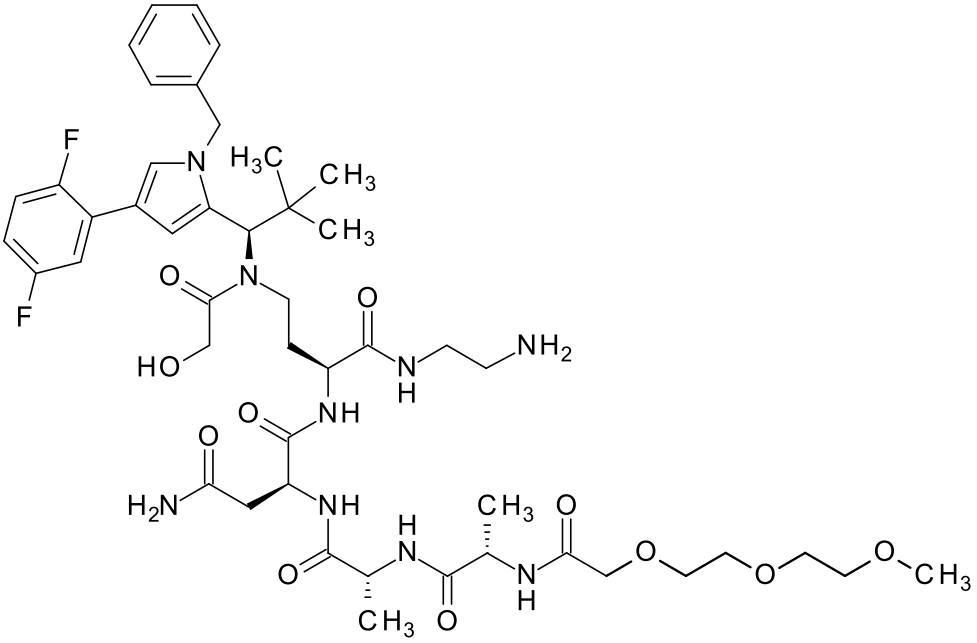
9H-Fluoren-9-ylmethyl {3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(chloroacetyl)amino]propyl}carbamate (Intermediate C118) (208 mg, 294 µmol) and 2-(trimethylsilyl)ethyl N-(tert-butoxycarbonyl)-L-cysteinate (99.3 mg, 309 µmol) (Intermediate L128) were initially charged in 5.0 ml of DMF, 1 drop of triethylamine was added and the mixture was stirred at RT overnight. The reaction mixture was admixed with 1.0 ml of water (0.1% TFA) and purified by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 252 mg (86% of theory) of 2-(trimethylsilyl)ethyl S-{2-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(3-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}propyl)amino]-2-oxoethyl}-N-(tert-butoxycarbonyl)-L-cysteinate.
LC-MS (Method 1): Rt = 1.76 min; MS (ESIpos): m/z = 995 $[M+H]^+$

2-(Trimethylsilyl)ethyl S-{2-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(3-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}propyl)amino]-2-oxoethyl}-N-(tert-butoxycarbonyl)-L-cysteinate (63.1 mg, 63.4 µmol) was initially charged in 2.0 ml of DMF, 200 µl of morpholine were added and the mixture was stirred at RT for 2h30. The reaction mixture was admixed with 1.0 ml of water (0.1% TFA) and purified by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA).

The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 51 mg (91% of theory) of the title compound.
LC-MS (Method 1): R_t = 1.36 min; MS (ESIpos): m/z = 773 $[M+H]^+$

Intermediate C120

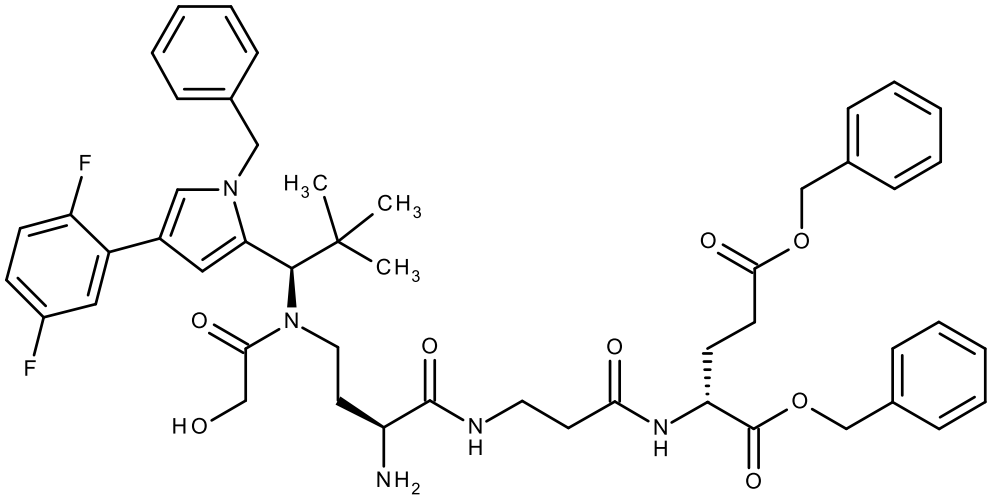
N-{[2-(2-Methoxyethoxy)ethoxy]acetyl}-L-alanyl-D-alanyl-N1-{(2S)-1-[(2-aminoethyl)amino]-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-oxobutan-2-yl}-L-aspartamide



This Intermediate was prepared in analogy to Intermediate C112, using Intermediate L129 for the coupling.
LC-MS (Method 1): R_t = 0.77 min; MS (ESIpos): m/z = 972 $[M+H]^+$

Intermediate C121

Dibenzyl N-{(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-beta-alanyl-D-glutamate

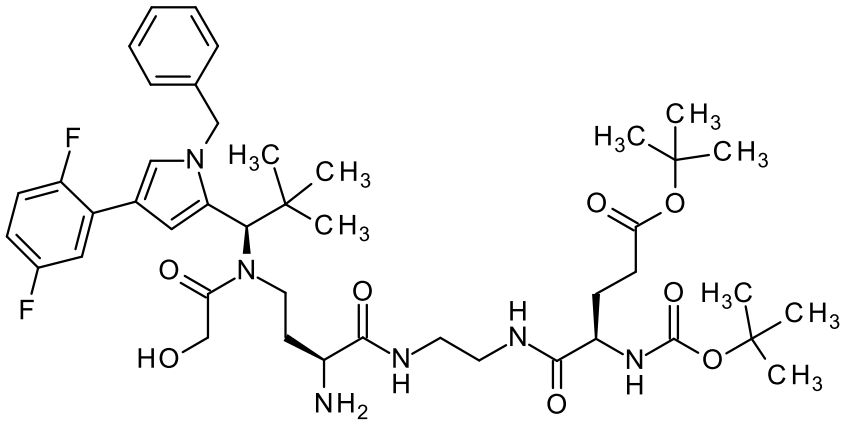


The title compound was prepared by coupling dibenzyl D-glutamate, which had been released beforehand from its p-toluenesulphonic acid salt by partitioning between ethyl acetate and 5% sodium hydrogencarbonate solution, with Intermediate C61 in the presence of HATU and N,N-diisopropylethylamine and subsequent detachment of the Teoc protecting group with zinc chloride in trifluoroethanol.

LC-MS (Method 1): R_t = 1.05 min; MS (ESIpos): m/z = 894 $[M+H]^+$.

Intermediate C122

tert-Butyl N-[2-({(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]-N^2-(tert-butoxycarbonyl)-D-alpha-glutaminate

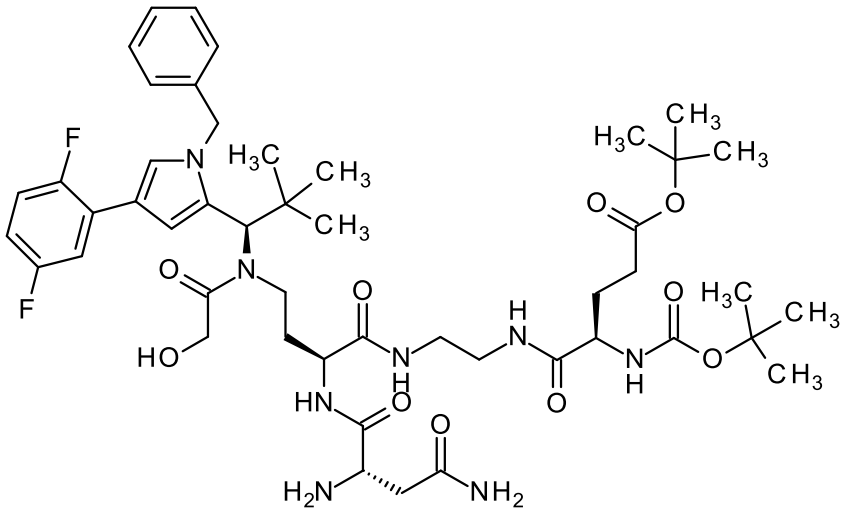


The title compound was prepared by coupling dibenzyl D-glutamate, which had been released beforehand from its p-toluenesulphonic acid salt by partitioning between ethyl acetate and 5% sodium hydrogencarbonate solution, with Intermediate C61 in the presence of HATU and N,N-diisopropylethylamine and subsequent detachment of the Teoc protecting group with zinc chloride in trifluoroethanol.

LC-MS (Method 1): R_t = 1.05 min; MS (ESIpos): m/z = 894 $[M+H]^+$.

Intermediate C123

tert-Butyl N-[2-({(2S)-2-(L-asparaginylamino)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]-N^2-(tert-butoxycarbonyl)-D-alpha-glutaminate

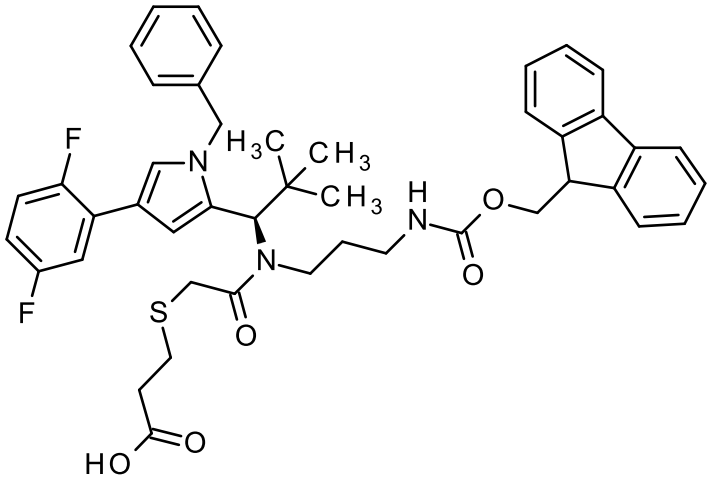


The title compound was prepared by coupling of 4-nitrophenyl N^2-[(benzyloxy)carbonyl]-L-asparaginate to Intermediate C122 in DMF in the presence of N,N-diisopropylethylamine and subsequent detachment of the Z protecting group by hydrogenation over 10% palladium on activated carbon in DCM/methanol 1:1 under standard hydrogen pressure at RT.

LC-MS (Method 1): R_t = 0.98 min; MS (ESIpos): m/z = 955 $[M+H]^+$.

Intermediate C124

8-{(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(9H-fluoren-9-yl)-3,9-dioxo-2-oxa-11-thia-4,8-diazatetradecan-14-oic acid



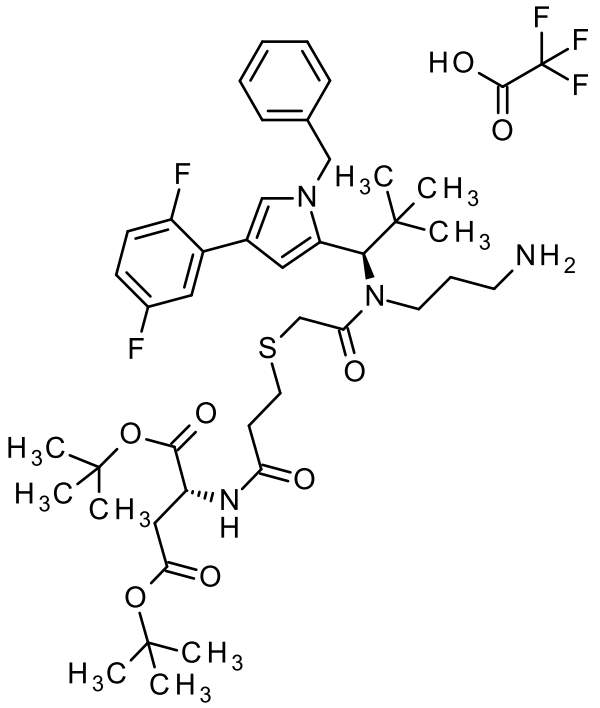
9H-Fluoren-9-ylmethyl [3-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino)propyl]carbamate (3.50 g, 5.52 mmol) (Intermediate C67) and triethylamine (2.3 ml, 17 mmol) were initially charged in dichloromethane (700 ml, 11 mol). Chloroacetyl chloride (3.12 g, 27.6 mmol) was added and the mixture was stirred at RT for 5 h. The reaction mixture was diluted with ethyl acetate and the organic phase was washed first with a 10% citric acid solution and then with saturated sodium hydrogencarbonate solution and saturated sodium chloride solution. The organic phase was washed with saturated NaCl solution and dried over magnesium sulphate. The solvents were evaporated under reduced pressure.

The 9H-fluoren-9-ylmethyl {3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(chloroacetyl)amino]propyl}carbamate (350 mg, 493 µmol) intermediate thus obtained was dissolved together with 3-sulphanylpropanoic acid (93 µl, 990 µmol) in DMF (7.0 ml), and triethylamine was added. The mixture was stirred at room temperature for 5 h, then the reaction was stopped by addition of 3 ml of water + 0.1% TFA. The mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC. 307 mg (80%) of the title compound were obtained.

LC-MS (Method 1): R_t = 1.40 min; MS (ESIpos): m/z = 780 $[M+H]^+$

Intermediate C125

Di-tert-butyl N-[3-({2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)propanoyl]-D-aspartate trifluoroacetic acid salt



To a mixture of 8-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(9H-fluoren-9-yl)-3,9-dioxo-2-oxa-11-thia-4,8-diazatetradecan-14-oic acid (200 mg, 256 µmol, Intermediate C124) and di-tert-butyl-D-aspartate hydrochloride salt (86.7 mg, 308 µmol) in DMF (3.0 ml) were added HATU (117 mg, 308 µmol) and N,N-diisopropylethylamine (130 µl, 770 µmol), and the reaction was stirred at room temperature for 10 min. The reaction mixture was quenched with water + 0.1% TFA and purified directly by preparative HPLC. The solvents were evaporated under reduced pressure and the residue was dried under high vacuum.

LC-MS (Method 1): R_t = 1.61 min; MS (ESIpos): m/z = 1007 $[M+H]^+$

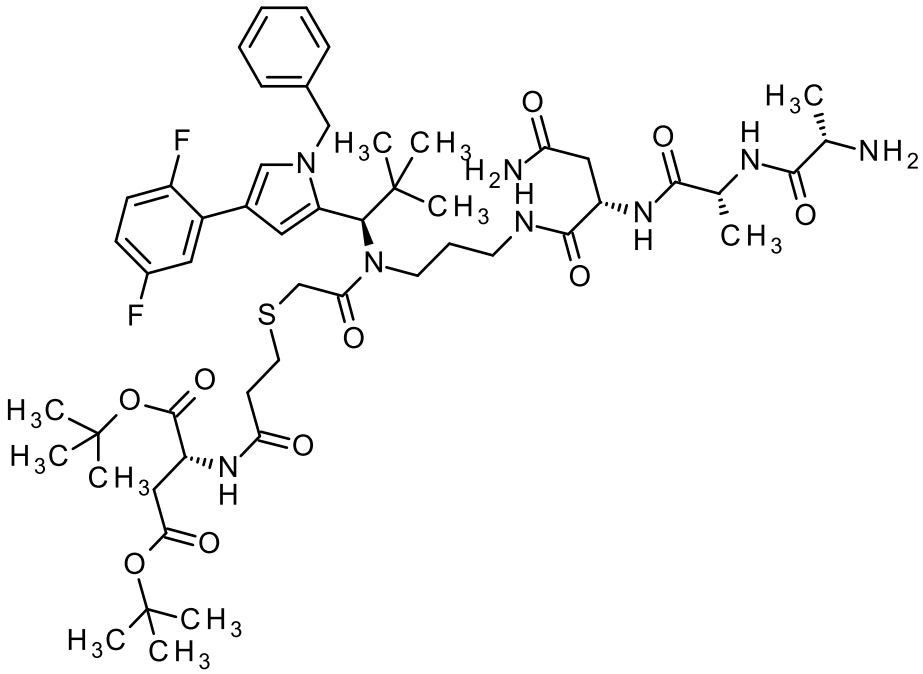
The intermediate obtained was dissolved in DMF (3.0 ml) and admixed with morpholine (200 µl, 2.3 mmol). The mixture was stirred at room temperature for 5 h and then quenched with water + 0.1% TFA and purified directly by preparative HPLC. The solvents

were evaporated under reduced pressure and the residue was dried under high vacuum. 182 mg of the title compound were obtained.

LC-MS (Method 5): R_t = 3.84 min; MS (ESIpos): m/z = 785 $[M+H]^+$

Intermediate C126

Di-tert-butyl (2R)-2-{[(2S,5R,8S)-2-amino-8-(2-amino-2-oxoethyl)-14-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-5-methyl-3,6,9,15,20-pentaoxo-17-thia-4,7,10,14-tetraazaicosan-20-yl]amino}succinate

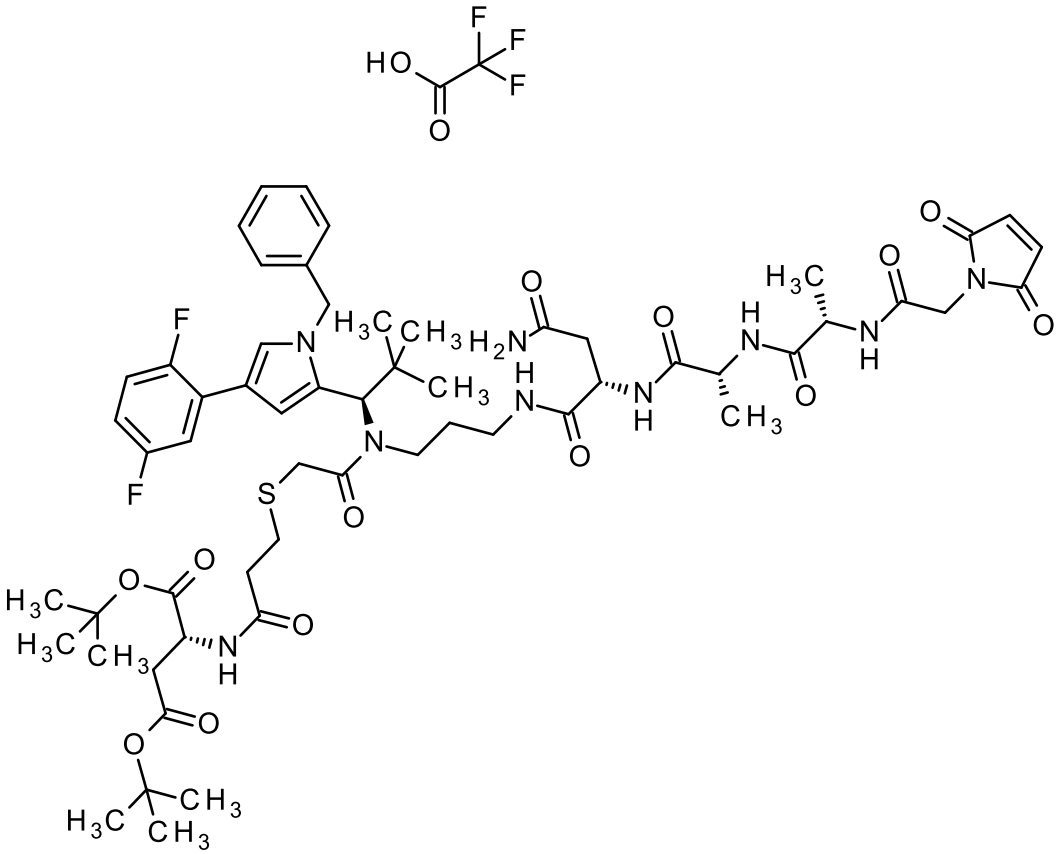


The title compound was prepared by coupling of N-[(benzyloxy)carbonyl]-L-alanyl-D-alanyl-L-asparagine (Intermediate L108) to Intermediate C125 in DMF in the presence of N,N-diisopropylethylamine and HATU and subsequent detachment of the Z protecting group by hydrogenation over 10% palladium on activated carbon in ethyl acetate/ethanol 1:1 under standard hydrogen pressure at RT.

LC-MS (Method 1): R_t = 1.02 min; MS (ESIpos): m/z = 1041 $[M+H]^+$

Intermediate C127

Trifluoroacetic acid di-tert-butyl-(2R)-2-{[(4S,7R,10S)-10-(2-amino-2-oxoethyl)-16-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-4,7-dimethyl-2,5,8,11,17,22-hexaoxo-19-thia-3,6,9,12,16-pentaazadocosan-22-yl]amino}succinate salt

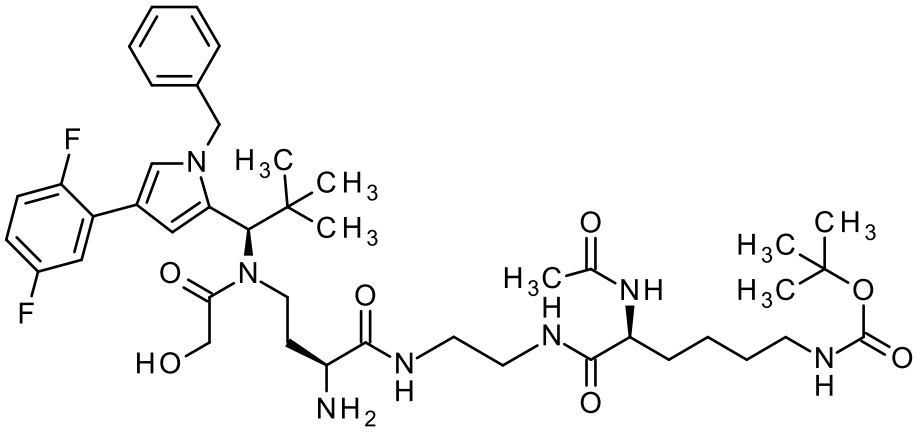


Di-tert-butyl (2R)-2-{[(2S,5R,8S)-2-amino-8-(2-amino-2-oxoethyl)-14-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-5-methyl-3,6,9,15,20-pentaoxo-17-thia-4,7,10,14-tetraazaicosan-20-yl]amino}succinate (12.0 mg, 11.5 µmol, Intermediate C126) and 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione (3.20 mg, 12.7 µmol) were dissolved in DMF (1.0 ml), and N,N-diisopropylethylamine (4.0 µl, 23 µmol) was added. The mixture was stirred at room temperature for 1 h. This was followed by quenching with water + 0.1% TFA, and direct purification of the mixture by means of preparative RP-HPLC. The solvents were evaporated under reduced pressure and the residue was dried under high vacuum.

LC-MS (Method 1): R_t = 1.25 min; MS (ESIpos): m/z = 1178 $[M+H]^+$

Intermediate C128

N^2-Acetyl-N-[2-({(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]-N^6-(tert-butoxycarbonyl)-L-lysinamide



To a solution of (2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-2-{[(benzyloxy)carbonyl]amino}butanoic acid (17.0 mg, 26.2 µmol, Intermediate C102) in DMF (2.8 ml) were added N2-acetyl-N-(2-aminoethyl)-N6-(tert-butoxycarbonyl)-L-lysinamide (9.54 mg, 28.9 µmol, Intermediate L135), HATU (18.0 mg, 47.2 µmol) and N,N-diisopropylethylamine (9.1 µl, 52 µmol). The reaction mixture was stirred at room temperature for 1 h. Subsequently, the solvent was removed under high vacuum and the residue was purified by preparative HPLC.

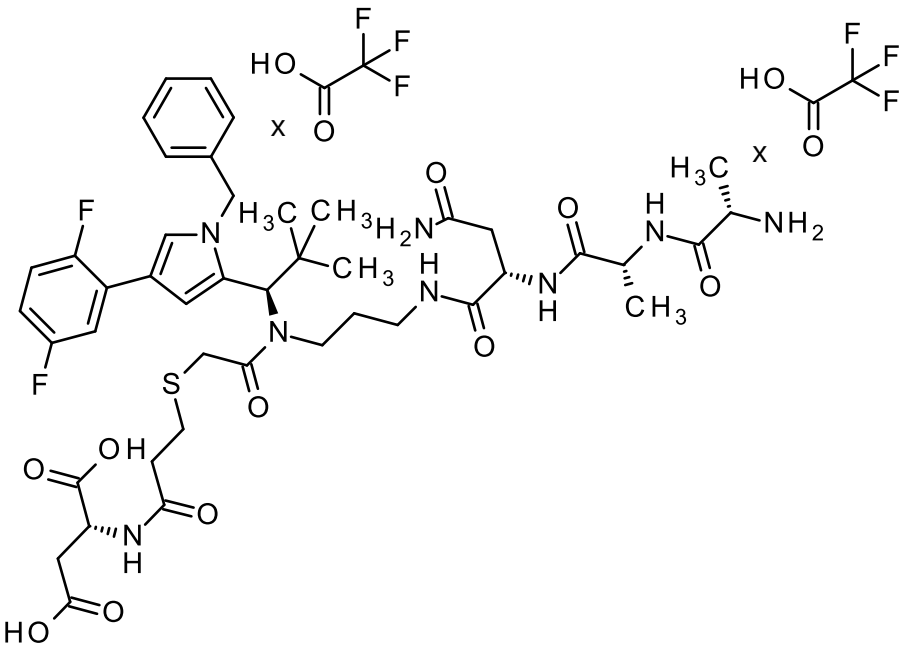
LC-MS (Method 1): R_t = 1.27 min; MS (ESIpos): m/z = 960 $[M+H]^+$

The was dissolved in DCM/EtOH and the Z protecting group was detached by hydrogenation over 10% palladium on activated carbon under standard hydrogen pressure at RT.

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 826 $[M+H]^+$

Intermediate C129

L-Alanyl-D-alanyl-N^1-[3-({(1R)-1-[1-benzyl-4-(2,5-difluorphenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}[(3-{[(1R)-1,2-dicarboxyethyl]amino}-3-oxopropyl)sulphanyl]acetyl}amino)propyl]-L-aspartamide trifluoroacetic acid salt

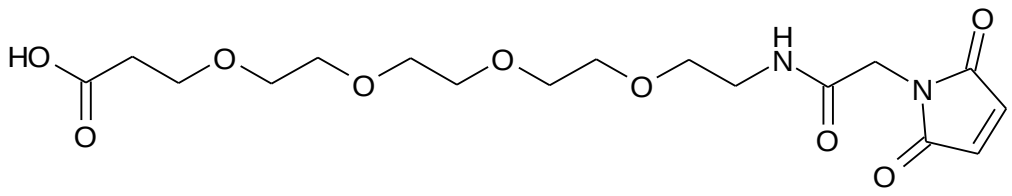


Intermediate C126 was dissolved in trifluoroethanol (2.0 ml), and zinc dichloride (9.19 mg, 67.4 µmol) was added. After stirring at 50°C for one hour, zinc chloride (9.19 mg, 67.4 µmol) was added and the reaction mixture was stirred at 50°C for a further hour. Ethylenediamine-N,N,N',N'-tetraacetic acid (19.7 mg, 67.4 µmol) was added to the reaction mixture, which was stirred briefly, and then water (0.1% TFA) was added. Purification was effected directly by means of preparative RP-HPLC. The solvents were evaporated under reduced pressure and the residue was lyophilized. This gave 7.6 mg (52% of theory) of the title compound.

LC-MS (Method 3): R_t = 0.84 min; MS (ESIneg): m/z = 927 $[M-H]^-$

Intermediate L74

3-[2-[2-[2-[2-[[2-(2,5-Dioxopyrrol-1-yl)acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid



107 mg (0.335 mmol) of *tert*-butyl 3-[2-[2-[2-(2-aminoethoxy)ethoxy]ethoxy]ethoxy]propanoate and 93 mg (0.369 mmol) of (2,5-dioxopyrrolidin-1-yl) 2-(2,5-dioxopyrrol-1-yl)acetate were dissolved in 5 ml of dimethylformamide, and 0.074 ml (0.671 mmol) of N-methylmorpholine was added. The reaction mixture was stirred at RT overnight. 0.048 ml (0.838 mmol) of acetic acid was added and the reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water/0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 133 mg (86%, 100% purity) of *tert*-butyl 3-[2-[2-[2-[2-[[2-(2,5-dioxopyrrol-1-yl)acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]propanoate.

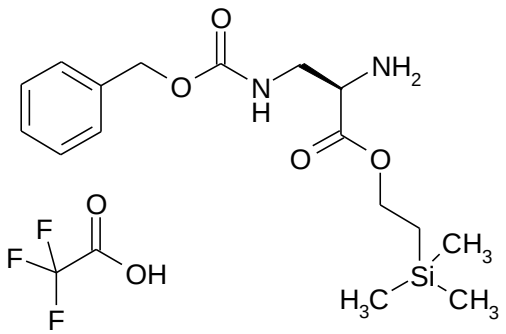
LC-MS (Method 1): R_t = 0.82 min; MS (ESIpos): m/z = 459 $(M+H)^+$.

0.5 ml of TFA was added to a solution of *tert*-butyl 3-[2-[2-[2-[2-[[2-(2,5-dioxopyrrol-1-yl)acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]propanoate (130 mg, 0.284 mmol) in 5 ml of dichloromethane. The reaction mixture was stirred at RT overnight. The reaction mixture was concentrated under reduced pressure and the residue was taken up in water and lyophilized. The residue was used further without further purification. This gave 102 mg (90%, purity 100%) of the title compound.

LC-MS (Method 1): R_t = 0.52 min; MS (ESIpos): m/z = 402 $(M+H)^+$.

Intermediate L75

Trifluoroacetic acid / 2-(trimethylsilyl)ethyl 3-{[(benzyloxy)carbonyl]amino}-D-alaninate (1:1)



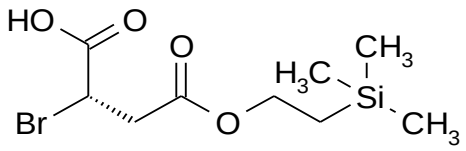
The title compound was prepared from 3-{[(benzyloxy)carbonyl]amino}-N-(tert-butoxycarbonyl)-D-alanine by conventional methods of peptide chemistry (esterification

with 2-(trimethylsilylethanol using EDCI/DMAP and removal of the Boc protecting group with trifluoroacetic acid. This gave 405 mg (58% of theory over 2 steps) of the title compound.

LC-MS (Method 1): R_t = 0.75 min; MS (ESIpos): m/z = 339 $(M+H)^+$.

Intermediate L76

(2S)-2-Bromo-4-oxo-4-[2-(trimethylsilyl)ethoxy]butanoic acid



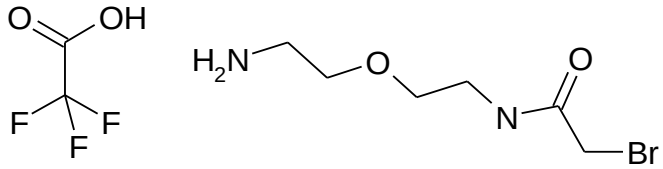
First, a suitably protected aspartic acid derivative was prepared from (3S)-4-(benzyloxy)-3-{[(benzyloxy)carbonyl]amino}-4-oxobutanoic acid by conventional methods of peptide chemistry (esterification with 2-(trimethylsilyl)ethanol using EDCI/DMAP and hydrogenolytic removal of the Z protecting group and the benzyl ester.
470 mg (1.8 mmol) of the (2S)-2-amino-4-oxo-4-[2-(trimethylsilyl)ethoxy]butanoic acid obtained in this manner were suspended in 10 ml of water, and 1.8 ml of a 1 molar hydrochloric acid and 0.5 ml of concentrated sulphuric acid were added, followed by 863 mg (7.25 mmol) of potassium bromide. At 10°C, a solution of 150 mg (2.175 mmol) of sodium nitrite in 1 ml of water was then added dropwise over a period of 30 min, and the mixture was stirred at 10-15°C for 2 h. The mixture was then extracted with 50 ml of ethyl acetate. The organic phase was washed with saturated sodium chloride solution and dried over magnesium sulphate. Evaporation of the solvent and purification of the product by preparative HPLC gave 260 mg (48% of theory) of the title compound.

LC-MS (Method 1): R_t = 1.03 min; MS (ESIneg): m/z = 295 and 297 $(M-H)^-$.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.03 (s, 9H), 0.95 (t, 2H), 2.94 and 3.2 (2dd, 2H), 4.18 (t, 2H), 4.57 (t, 1H).

Intermediate L77

Trifluoroacetic acid / N-[2-(2-aminoethoxy)ethyl]-2-bromoacetamide (1:1)

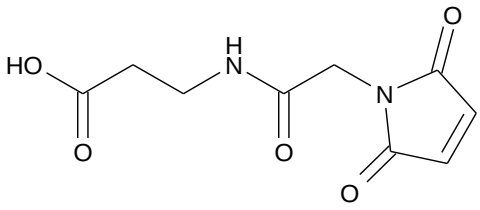


418 mg (2.05 mmol) of tert-butyl [2-(2-aminoethoxy)ethyl]carbamate were first reacted with 638 mg (2.46 mmol) of bromoacetic anhydride, and then the Boc protecting group was removed with trifluoroacetic acid. This gave 551 mg (63% of theory over 2 steps) of the title compound.

LC-MS (Method): R_t = 0.32 min; MS (ESIpos): m/z = 227 and 225 $(M+H)^+$.

Intermediate L78

N-[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-beta-alanine

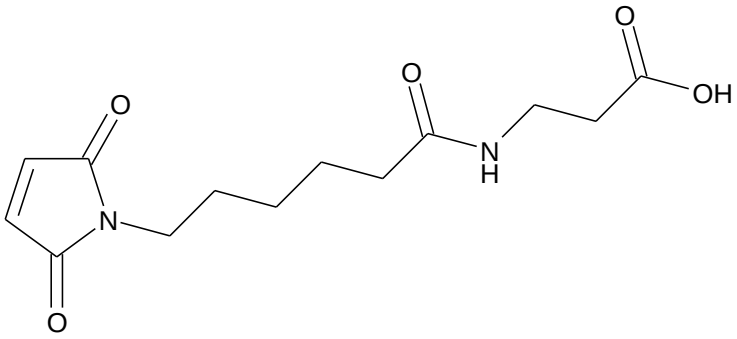


The title compound was prepared from commercially available (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid by coupling to *tert*-butyl beta-alaninate hydrochloride (1:1) in the presence of EDCI/HOBt and *N,N*-diisopropylethylamine and subsequent deprotection with trifluoroacetic acid.

LC-MS (Method 1): R_t = 0.32 min; MS (ESIpos): m/z = 227 $(M+H)^+$.

Intermediate L79

N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanine



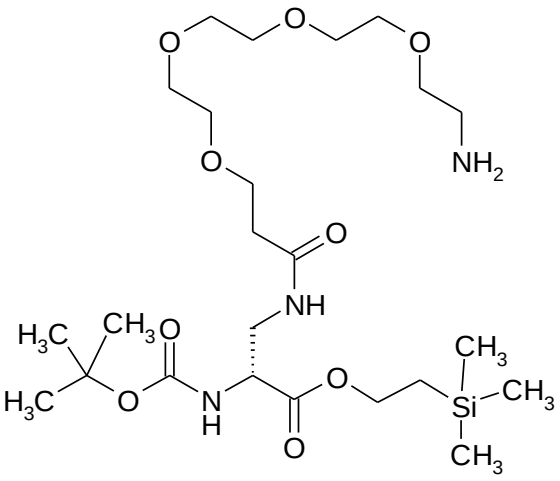
64.8 mg (0.357 mmol) of *tert*-butyl beta-alaninate hydrochloride (1:1) and 100 mg (0.324 mmol) of 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione were dissolved in 4 ml of dimethylformamide, and 65.6 mg (0.649 mmol) of N-methylmorpholine were added. The reaction mixture was stirred at RT overnight. 0.048 ml (0.838 mmol) of acetic acid was added and the reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water/0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 84.5 mg (77%, purity 100%) of *tert*-butyl N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alaninate.

LC-MS (Method 1): R_t = 0.78 min; MS (ESIpos): m/z = 339 $(M+H)^+$.

1.62 ml of TFA were added to a solution of *tert*-butyl N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alaninate (82.8 mg, 0.244 mmol) in 8 ml of dichloromethane. The reaction mixture was stirred at RT for 2 hours. The reaction mixture was concentrated under reduced pressure and the residue was taken up in water and lyophilized. The residue was used further without further purification. This gave 62.7 mg (87%, purity 95%) of the title compound.
LC-MS (Method 1): R_t = 0.75 min; MS (ESIpos): m/z = 283 $(M+H)^+$.

Intermediate L80

2-(Trimethylsilyl)ethyl 3-[(15-amino-4,7,10,13-tetraoxapentadecan-1-oyl)amino]-N-(tert-butoxycarbonyl)-D-alaninate

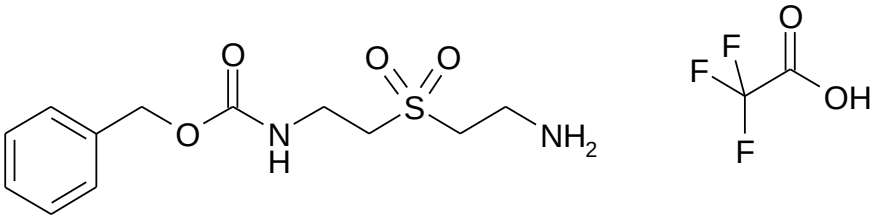


The title compound was prepared from commercially available 3-{[(benzyloxy)carbonyl] amino}-N-(tert-butoxycarbonyl)-D-alanine / N-cyclohexylcyclohexanamine (1:1) by conventional methods of peptide chemistry (release from the salt and esterification with 2-(trimethylsilyl)ethanol using EDCI/DMAP, hydrogenolytic removal of the Z protecting group, coupling to commercially available 3-oxo-1-phenyl-2,7,10,13,16-pentaoxa-4-azanonadecan-19-oic acid in the presence of HATU and N,N-diisopropylethylamine and another hydrogenolytic removal of the Z protecting group).

LC-MS (Method 1): R_t = 0.70 min; MS (ESIpos): m/z = 552 $(M+H)^+$.

Intermediate L81

Trifluoroacetic acid / benzyl {2-[(2-aminoethyl)sulphonyl]ethyl}carbamate (1:1)

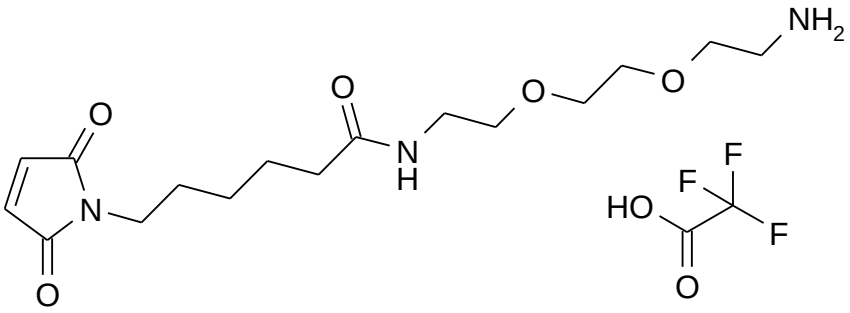


250 mg (1.11 mmol) of 2,2'-sulphonyldiethanamine were coupled to 92.3 mg (0.37 mmol) of 1-{[(benzyloxy)carbonyl] oxy}pyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine in DMF. Subsequent purification by HPLC gave 70 mg (47% of theory) of the title compound.

C-MS (Method 12): R_t = 0.64 min; MS (ESIpos): m/z = 257.11 $(M+H)^+$.

Intermediate L82

Trifluoroacetic acid / N-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide (1:1)



88.6 mg (0.357 mmol) of N-Boc-2,2'-(ethylenedioxy)diethylamine and 100 mg (0.324 mmol) of N-succinimidyl 6-maleimidohexanoate were dissolved in 4.0 ml of dimethylformamide, and 0.071 ml (0.650 mmol) of N-methylmorpholine were added. The reaction mixture was stirred at RT overnight. 0.048 ml (0.838 mmol) of acetic acid was added and the reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 75 ml/min, MeCN/water/0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 127 mg (81% of theory) of tert-butyl {2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]ethyl}carbamate.

LC-MS (Method 1): R_t = 0.78 min; MS (ESIpos): m/z = 442 $(M+H)^+$.

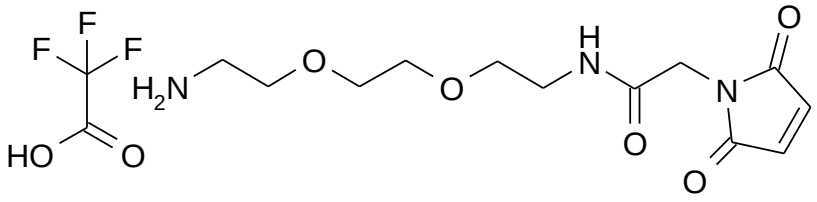
2.0 ml of TFA were added to a solution of 123 mg (225 µmol) of tert-butyl {2-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]ethyl}carbamate in 7.5 ml of dichloromethane. The reaction mixture was stirred at RT for 2 h. The reaction mixture was concentrated under reduced pressure and the residue was taken up in water and lyophilized. The residue was used further without further purification. This gave 111 mg (100% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.31 min; MS (ESIpos): m/z = 342 $(M+H)^+$.

^{1}H-NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.17 (m, 2H), 1.47 (m, 4H), 2.04 (m, 2H), 2.98 (m, 2H), 3.19 (m, 2H), 3.39 (m, 4H), 3.56 (m, 6H), 7.01 (s, 2H), 7.72 (bs, 3H), 7.80 (m, 1H).

Intermediate L83

Trifluoroacetic acid / N-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (1:1)



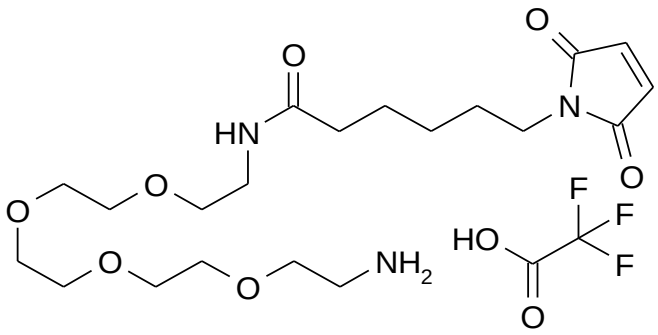
200 mg (0.805 mmol) of tert-butyl {2-[2-(2-aminoethoxy)ethoxy]ethyl}carbamate, 150 mg (0.966 mmol) of (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid and 560 µl (3.2 mmol) of N,N-diisopropylethylamine were dissolved in 10 ml of dimethylformamide, and 459 mg (1.21 mmol) of HATU were added. The reaction mixture was stirred at RT for 30 minutes. The solvents were evaporated under reduced pressure and the residue was dissolved in dichloromethane. The organic phase was washed twice with 5% citric acid solution and dried over magnesium sulphate, and the solvent was evaporated under reduced pressure. The residue was purified using Biotage Isolera (silica gel, column 25 g SNAP, dichloromethane:methanol 98:2). This gave 276 mg (89% of theory) of tert-butyl {2-[2-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethoxy]ethyl}carbamate.

LC-MS (Method 1): R_t = 0.67 min; MS (ESIpos): m/z = 386 $(M+H)^+$.

4 ml of TFA were added to a solution of tert-butyl {2-[2-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethoxy]ethyl}carbamate (275 mg, 714 µmol) in 15 ml of dichloromethane. The reaction mixture was stirred at RT for 30 minutes. The reaction mixture was concentrated under reduced pressure and the residue was taken up in water and lyophilized. This gave 281 mg (99% of theory) of the title compound.
LC-MS (Method 1): R_t = 0.17 min; MS (ESIpos): m/z = 286 $(M+H)^+$.

Intermediate L84

Trifluoroacetic acid / N-(14-amino-3,6,9,12-tetraoxatetradec-1-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide (1:1)



200 mg (0.594 mmol) of tert-butyl (14-amino-3,6,9,12-tetraoxatetradec-1-yl)carbamate and 202 mg (0.654 mmol) of 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione were dissolved in 4.0 ml of dimethylformamide, and 0.130 ml (1.2 mmol) of N-methylmorpholine were added. The reaction mixture was stirred at RT overnight. 0.085 ml (1.5 mmol) of acetic acid was added and the reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water/0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 275 mg (73% of theory) of tert-butyl [21-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-16-oxo-3,6,9,12-tetraoxa-15-azahenicos-1-yl]carbamate.

LC-MS (Method 1): R_t = 0.81 min; MS (ESIpos): m/z = 530 $(M+H)^+$.

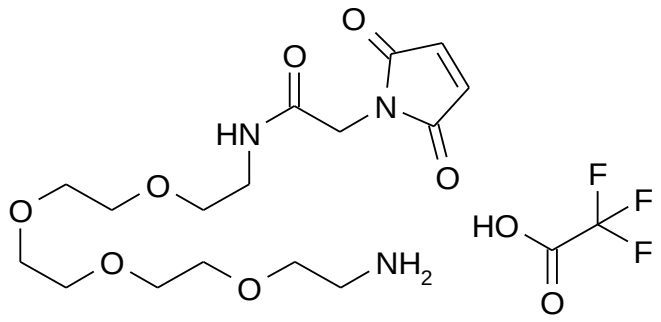
780 µl (10 mmol) of TFA were added to a solution of tert-butyl [21-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-16-oxo-3,6,9,12-tetraoxa-15-azahenicos-1-yl]carbamate (268 mg, 505 µmol) in 5.0 ml of dichloromethane. The reaction mixture was stirred at RT overnight. The reaction mixture was concentrated under reduced pressure and the residue was taken up in water and lyophilized. The residue was used further without further purification. This gave 266 mg (97% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.46 min; MS (ESIpos): m/z = 430 $(M+H)^+$.

^{1}H-NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.17 (m, 2H), 1.47 (m, 4H), 2.03 (m, 2H), 2.99 (m, 2H), 3.18 (m, 2H), 3.38 (m, 4H), 3.52 (m, 8H), 3.58 (m, 6H), 7.01 (s, 2H), 7.73 (bs, 3H), 7.80 (m, 1H).

Intermediate L85

Trifluoroacetic acid / N-(14-amino-3,6,9,12-tetraoxatetradec-1-yl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (1:1)



200 mg (0.594 mmol) of tert-butyl (14-amino-3,6,9,12-tetraoxatetradec-1-yl)carbamate, 111 mg (0.713 mmol) of (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid and 410 µl (2.4 mmol) of N,N-diisopropylethylamine were dissolved in 6 ml of dimethylformamide, and 339 mg (0.892 mmol) of HATU were added. The reaction mixture was stirred at RT for 1 h and purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 130 mg (43% of theory) of tert-butyl [17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-16-oxo-3,6,9,12-tetraoxa-15-azaheptadec-1-yl]carbamate.

LC-MS (Method 1): R_t = 0.71 min; MS (ESIpos): m/z = 474 $(M+H)^+$.

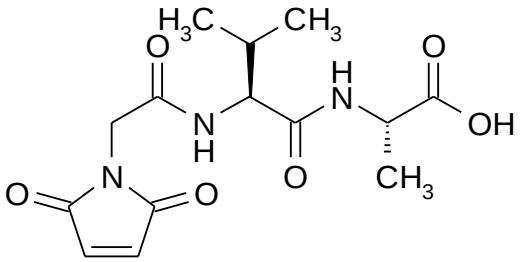
410 µl (5.3 mmol) of TFA were added to a solution of tert-butyl [17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-16-oxo-3,6,9,12-tetraoxa-15-azaheptadec-1-yl]carbamate (126 mg, 267 µmol) in 4.0 ml of dichloromethane. The reaction mixture was stirred at RT overnight. The reaction mixture was concentrated under reduced pressure and the residue was dried under high vacuum. This gave 124 mg (95% of theory) of the title compound.

LC-MS (Method 13): R_t = 0.74 min; MS (ESIpos): m/z = 374 $(M+H)^+$.

^{1}H-NMR (400 MHz, DMSO-d_6): δ [ppm] = 2.99 (m, 2H), 3.22 (m, 2H), 3.41 (m, 2H), 3.53 (m, 8H), 3.58 (m, 6H), 4.02 (s, 2H), 7.09 (s, 2H), 7.73 (bs, 3H), 8.21 (m, 1H).

Intermediate L86

N-[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-L-alanine

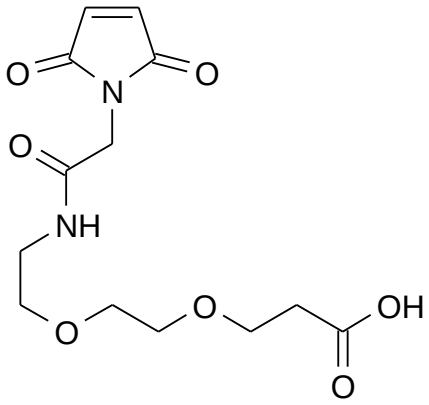


100 mg (0.531 mmol) of L-valyl-L-alanine and 134 mg (0.531 mmol) of 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione were dissolved in 3 ml of dimethylformamide, and 0.150 ml (1.1 mmol) of triethylamine were added. The reaction mixture was stirred at RT for 8 h. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate; 50 ml/min, MeCN/water). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 71.5 mg (41% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.42 min; MS (ESIpos): m/z = 326 $(M+H)^+$.

Intermediate L87

3-[2-(2-{[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethoxy]propanoic acid



250 mg (1.07 mmol) of tert-butyl 3-[2-(2-aminoethoxy)ethoxy]propanoate, 151 mg (0.974 mmol) of 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid, 224 mg (1.46 mmol) of 1-hydroxy-1H-benzotriazole hydrate and 224 mg (1.17 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were dissolved in 5.0 ml of dimethylformamide. The reaction mixture was stirred at RT for 1 h. Ethyl acetate was added and the mixture was extracted twice with 5% citric acid solution and with saturated sodium hydrogencarbonate solution. The organic phase was washed twice with saturated sodium chloride solution and dried over magnesium sulphate, and the solvent was evaporated off under reduced

pressure. The residue was purified by preparative RP-HPLC (column: Reprosil 250x40; 10µ, flow rate: 50 ml/min, MeCN/water/0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 267 mg (64% of theory) of tert-butyl 3-[2-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethoxy]propanoate.

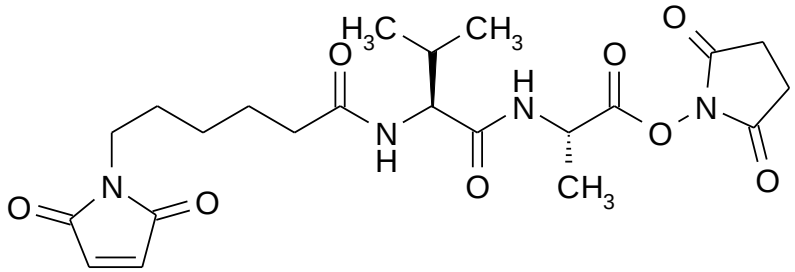
LC-MS (Method 1): Rt = 0.73 min; MS (ESIpos): m/z = 371 $(M+H)^+$.

1.1 ml (14 mmol) of TFA were added to a solution of tert-butyl 3-[2-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethoxy]propanoate (263 mg, 710 µmol) in 10 ml of dichloromethane. The reaction mixture was stirred at RT overnight. The reaction mixture was concentrated under reduced pressure and the residue was dried under high vacuum. This gave 240 mg (94% of theory) of the title compound.

LC-MS (Method 12): R_t = 0.57 min; MS (ESIpos): m/z = 315 $(M+H)^+$.

Intermediate L88

2,5-Dioxopyrrolidin-1-yl N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alaninate



150 mg (0.797 mmol) of L-valyl-L-alanine and 246 mg (0.797 mmol) of 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione were dissolved in 4.0 ml of dimethylformamide, and 0.220 ml (1.6 mmol) of triethylamine was added. The reaction mixture was stirred at RT overnight. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate; 50 ml/min, MeCN/water). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 302 mg (97% of theory) of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanine.

LC-MS (Method 12): R_t = 1.02 min; MS (ESIpos): m/z = 382 $(M+H)^+$.

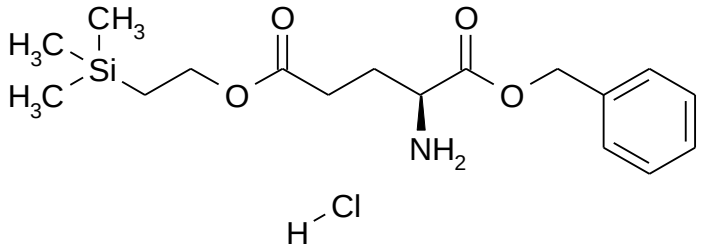
^{1}H-NMR (400 MHz, DMSO-d_6): δ [ppm] = 0.82 (dd, 6H), 1.17 (m, 2H), 1.27 (d, 3H), 1.48 (m, 4H), 1.94 (m, 1H), 2.13 (m, 2H), 3.38 (t, 2H), 4.17 (m, 2H), 7.00 (s, 2H), 7.75 (d, 1H), 8.19 (d, 1H).

130 mg (0.531 mmol) of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alanine were dissolved in 6.5 ml of dichloromethane, and 58.8 mg (0.511 mmol) of 1-hydroxypyrrolidine-2,5-dione and 78.4 mg (0.409 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were added. Another 58.8 mg (0.511 mmol) of 1-hydroxypyrrolidine-2,5-dione and 78.4 mg (0.409 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were added. Dichloromethane was added and the mixture was washed three times with water. The organic phase was dried over magnesium sulphate, the solvent was evaporated under reduced pressure and the residue was dried under high vacuum. This gave 172 mg (87% of theory) of the title compound.

LC-MS (Method 12): R_t = 1.28 min; MS (ESIpos): m/z = 479 $(M+H)^+$.

Intermediate L89

1-Benzyl-5-[2-(trimethylsilyl)ethyl]-L-glutamate hydrochloride (1:1)



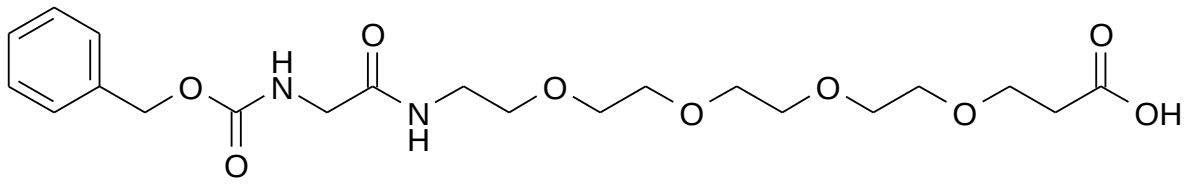
1.00 g (2.96 mmol) of (4S)-5-(benzyloxy)-4-[(tert-butoxycarbonyl)amino]-5-oxopentanoic acid was initially charged in 13.0 ml of THF, and 510 µl (3.6 mmol) of 2-(trimethylsilyl)ethanol and 109 mg (889 µmol) of 4-dimethylaminopyridine were added. The reaction mixture was cooled to 0°C, and 682 mg (3.56 mmol) of N-ethyl-N'-3-(dimethylaminopropyl)carbodiimide hydrochloride were added. The reaction mixture was stirred at RT overnight. The solvents were evaporated under reduced pressure and the residue was dissolved in ethyl acetate. The organic phase was washed twice with 0.1 N HCl solution and saturated sodium chloride solution and dried over magnesium sulphate, and the solvent was evaporated under reduced pressure. The residue was purified using Biotage Isolera (silica gel, column: 25 g SNAP, cyclohexane:ethyl acetate 80:20). This gave 649 mg (50% of theory) of the compound 1-benzyl-5-[2-(trimethylsilyl)ethyl]-N-(tert-butoxycarbonyl)-L-glutamate.

LC-MS (Method 1): R_t = 4.6 min; MS (ESIpos): m/z = 438 $(M+H)^+$.

649 mg (1.48 mmol) of 1-benzyl-5-[2-(trimethylsilyl)ethyl]-N-(tert-butoxycarbonyl)-L-glutamate were dissolved in 7.0 ml of dioxane and, with ice bath cooling, 14 ml (59 mmol) of 4N HCl in dioxane were added. The reaction mixture was stirred at RT overnight. The reaction mixture was concentrated under reduced pressure and the residue was dried under high vacuum and purified by Biotage Isolera (silica gel, column 25 g SNAP, dichloromethane:methanol 90:10). This gave 320 mg (57% of theory) of the title compound.
LC-MS (Method 1): R_t = 0.79 min; MS (ESIpos): m/z = 338 $(M+H)^+$.

Intermediate L90

1-({N-[(Benzyloxy)carbonyl]glycyl}amino)-3,6,9,12-tetraoxapentadecan-15-oic acid

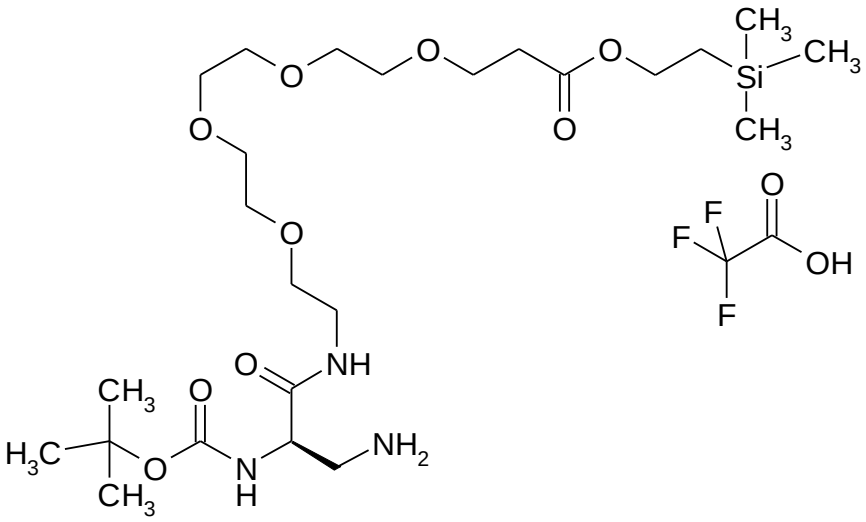


118 mg (566 µmol) of N-[(benzyloxy)carbonyl]glycine were initially charged in 5.0 ml of DMF, 200 mg (622 µmol) of tert-butyl 1-amino-3,6,9,12-tetraoxapentadecan-15-oate, 130 mg (849 µmol) of 1-hydroxy-1H-benzotriazole hydrate and 130 mg (679 µmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were added and the mixture was stirred at RT for 1 h. Ethyl acetate was added and the mixture was extracted twice with 5% citric acid solution and with saturated sodium hydrogencarbonate solution. The organic phase was washed twice with saturated sodium chloride solution and dried over magnesium sulphate. The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 274 mg (95% of theory) of tert-butyl 1-({N-[(benzyloxy)carbonyl]glycyl}amino)-3,6,9,12-tetraoxapentadecan-15-oate.
LC-MS (Method 12): Rt = 1.69 min; MS (ESIpos): m/z = 513 $(M+H)^+$.

820 µl (11 mmol) of TFA were added to a solution of 274 mg (535 µmol) of tert-butyl 1-({N-[(benzyloxy)carbonyl]glycyl}amino)-3,6,9,12-tetraoxapentadecan-15-oate in 5.0 ml of dichloromethane. The reaction mixture was stirred at RT for 3 h. The reaction mixture was concentrated under reduced pressure and the residue was taken up in water and lyophilized. This gave 262 mg (100% of theory) of the title compound.
LC-MS (Method 12): R_t = 1.12 min; MS (ESIpos): m/z = 457 $(M+H)^+$.

Intermediate L91

Trifluoroacetic acid / 2-(trimethylsilyl)ethyl 1-{[3-amino-N-(tert-butoxycarbonyl)-D-alanyl]amino}-3,6,9,12-tetraoxapentadecan-15-oate (1:1)

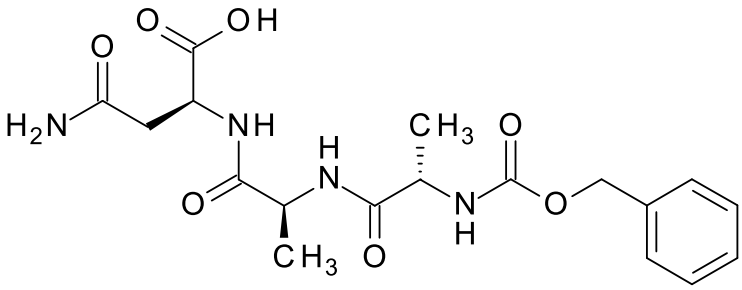


The title compound was prepared from commercially available 3-oxo-1-phenyl-2,7,10,13,16-pentaoxa-4-azanonadecan-19-oic acid by conventional methods of peptide chemistry (esterification with 2-trimethylsilylethanol using EDCI/DMAP, hydrogenolytic removal of the Z protecting group, coupling to commercially available N-(tert-butoxycarbonyl)-3-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-D-alanine and removal of the Fmoc protecting group).

LC-MS (Method 1): R_t = 0.74 min; MS (ESIpos): m/z = 552 $(M+H)^+$.

Intermediate L92

N-[(Benzyloxy)carbonyl]-L-alanyl-L-alanyl-L-asparagine

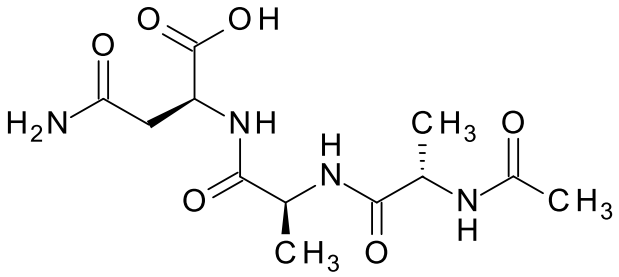


The title compound was prepared by conventional methods of peptide chemistry by HATU coupling, in the presence of N,N-diisopropylethylamine, of commercially available N-[(benzyloxy)carbonyl]-L-alanyl-L-alanine with tert-butyl L-asparaginate and subsequent deprotection of the carboxyl group with trifluoroacetic acid.

LC-MS (Method 1): R_t = 0.5 min; MS (ESIpos): m/z = 409 $(M+H)^+$.

Intermediate L93

N-Acetyl-L-alanyl-L-alanyl-L-asparagine



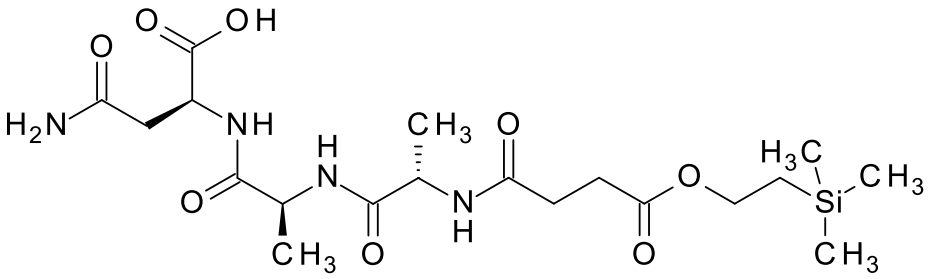
The title compound was prepared by conventional methods of peptide chemistry by HATU coupling, in the presence of N,N-diisopropylethylamine, of commercially available N-[(benzyloxy)carbonyl]-L-alanyl-L-alanine with tert-butyl L-asparaginate, subsequent deprotection of the Z protecting group by hydrogenation in DCM/methanol over 10% palladium on activated carbon, followed by acetylation with acetic acid in DMF in the presence of HATU and N,N-diisopropylethylamine and finally deprotection of the carboxyl group with trifluoroacetic acid.

LC-MS (Method 1): R_t = 0.16 min; MS (ESIpos): m/z = 317 $(M+H)^+$.

^{1}H-NMR (400 MHz, DMSO-d_6): δ [ppm] = 1.19 (2d, 6H), 1.82 (s, 3H), 2.5 (m, 2H), 4.26 (m, 2H), 4.48 (q, 1H), 6.9 (s, 1H), 7.36 (s, 1H), 8.0 (m, 3H), 12.54 (s, 1H).

Intermediate L94

N-{4-Oxo-4-[2-(trimethylsilyl)ethoxy]butanoyl}-L-alanyl-L-alanyl-L-asparagine



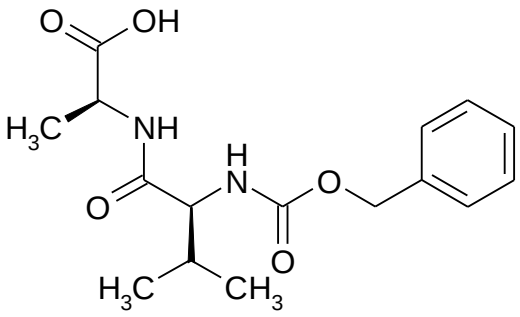
First of all, 4-oxo-4-[2-(trimethylsilyl)ethoxy]butanoic acid was prepared by reaction of 4-(benzyloxy)-4-oxobutanoic acid with 2-(trimethylsilyl)ethanol in the presence of EDCI/DMAP in DCM and subsequent hydrogenolytic cleavage of the benzyl ester.
LC-MS (Method 1): R_t = 0.89 min; MS (ESIpos): m/z = 217 $(M-H)^-$.

In addition, trifluoroacetic acid / 4-nitrobenzyl-L-alanyl-L-alanyl-L-asparaginate (1:1) was prepared by coupling N-(tert-butoxycarbonyl)-L-alanyl-L-alanine with 4-nitrobenzyl L-asparaginate hydrobromide (1:1) in DMF in the presence of HATU and N,N-diisopropylethylamine and then deprotecting the amino group with trifluoroacetic acid in DCM.
LC-MS (Method 1): R_t = 0.43 min; MS (ESIpos): m/z = 410 $(M+H)^+$.

The title compound was then prepared by coupling these two intermediates in DMF in the presence of HATU and N,N-diisopropylethylamine and then detaching the p-nitrobenzyl ester by hydrogenation in DCM-methanol 1:9 over 10% palladium on activated carbon.
LC-MS (Method 1): R_t = 0.79 min; MS (ESIpos): m/z = 475 $(M+H)^+$.

Intermediate L95

N-[(Benzyloxy)carbonyl]-L-valyl-L-alanine

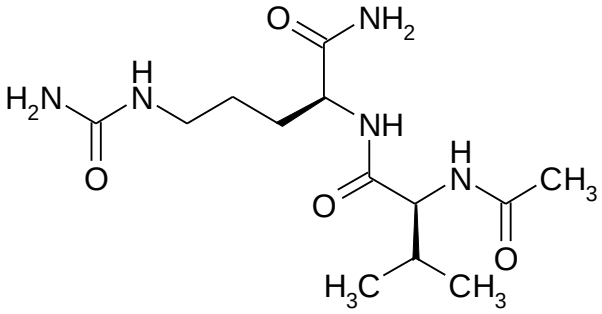


This intermediate was prepared proceeding from N-[(benzyloxy)carbonyl]-L-valine and tert-butyl L-alaninate hydrochloride (1:1) by conventional methods of peptide chemistry.

LC-MS (Method 12): R_t = 1.34 min; MS (ESIpos): m/z = 323.16 $(M+H)^+$.

Intermediate L96

N-Acetyl-L-valyl-N^5-carbamoyl-L-ornithinamide

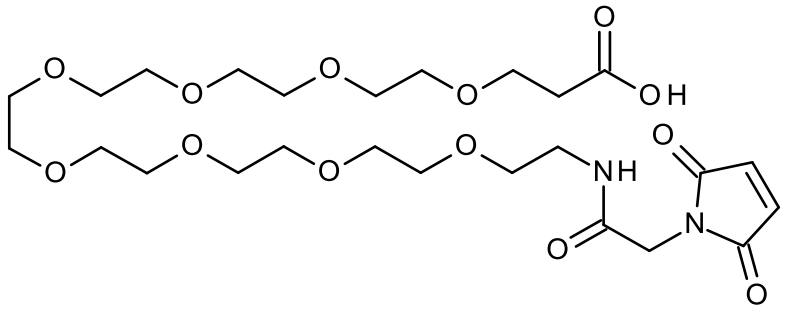


This intermediate was prepared by conventional methods of peptide chemistry commencing with the coupling of 2,5-dioxopyrrolidin-1-yl-N-[(benzyloxy)carbonyl]-L-valinate with N^5-carbamoyl-L-ornithine, followed by hydrogenolytic cleavage of the Z protecting group over 10% palladium/activated carbon in ethanol and finally by reaction of the dipeptide obtained with 1-acetoxypyrrolidine-2,5-dione.

LC-MS (Method 1): R_t = 0.25 min; MS (ESIpos): m/z = 317 $(M+H)^+$.

Intermediate L97

1-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-6,9,12,15,18,21,24,27-octaoxa-3-azatriacontan-30-oic acid

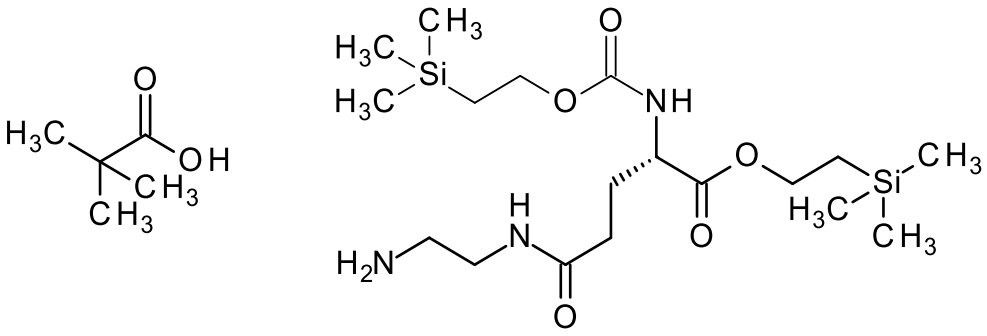


tert-Butyl 1-amino-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oate (100 mg, 201 µmol) was initially charged in 1.0 ml of DMF, and (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid (46.8 mg, 301 µmol), 1-hydroxy-1H-benzotriazole hydrate (76.9 mg, 502 µmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (77.0 mg, 402 µmol) were added. The reaction mixture was stirred at RT overnight, and ethyl acetate was then added. The organic phase was washed twice with 5% citric acid solution, and with saturated sodium hydrogencarbonate solution and then with saturated sodium chloride solution. The organic phase was dried over magnesium sulphate. The solvents were evaporated under reduced pressure and the residue was purified by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water/0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 19.1 mg (13% of theory) of tert-butyl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-6,9,12,15,18,21,24,27-octaoxa-3-azatriacontan-30-oate.
LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 635 $[M+H]^+$

To a solution of tert-butyl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-6,9,12,15,18,21,24,27-octaoxa-3-azatriacontan-30-oate (19.1 mg, 30.1 µmol) in 1.0 ml of DCM was added TFA (62 µl, 600 µmol). The reaction mixture was stirred at RT for 3 h. The reaction mixture was concentrated under reduced pressure and the residue was taken up in water and lyophilized. The residue was used further without further purification. This gave 10.8 mg (46% of theory) of the title compound.
LC-MS (Method 1): R_t = 0.55 min; MS (ESIneg): m/z = 577 $[M-H]^-$.

Intermediate L98

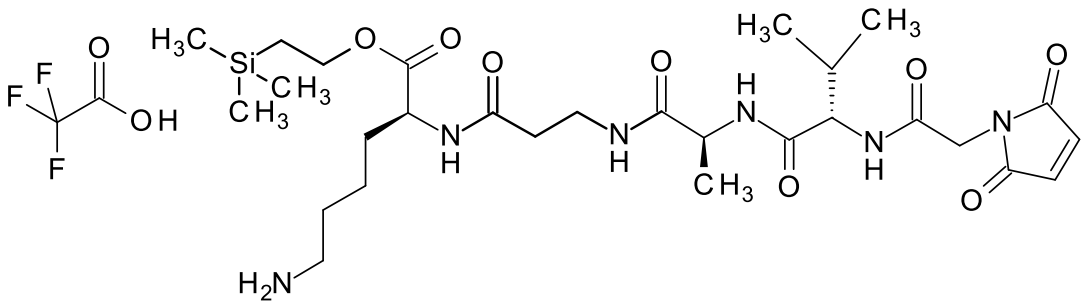
2,2-Dimethylpropanoic acid / 2-(trimethylsilyl)ethyl N-(2-aminoethyl)-N^2-{[2-(trimethylsilyl) ethoxy]carbonyl}-L-glutaminate (1:1)



First of all, (4S)-5-tert-butoxy-4-[(tert-butoxycarbonyl)amino]-5-oxopentanoic acid was coupled in the presence of HATU and *N,N*-diisopropylethylamine with benzyl (2-aminoethyl)carbamate. Subsequently, by means of trifluoroacetic acid in DCM, the Boc protecting group and the tert-butyl ester were detached. Then, first the amino group was reprotected by reaction with 1-({[2-(trimethylsilyl)ethoxy]carbonyl}oxy)pyrrolidine-2,5-dione in DMF/water in the presence of *N,N*-diisopropylethylamine, and then the carboxyl group by reaction with 2-(trimethylsilyl)ethanol in DCM in the presence of EDCI/DMAP. In the last step, the terminal amino group was deprotected by means of hydrogenolysis over 10% palladium on activated carbon in ethanol under standard pressure. After removal of the catalyst by filtration, concentration, purification by preparative HPLC and freeze-drying of the residue from acetonitrile/water, the title compound was obtained.
LC-MS (Method 1): R_t = 0.82 min; MS (ESIpos): m/z = 434 $(M+H)^+$.

Intermediate L99

Trifluoroacetic acid / 2-(trimethylsilyl)ethyl N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-L-alanyl-beta-alanyl-L-lysinate (1:1)

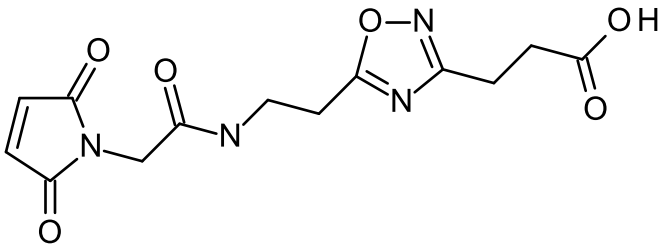


First of all, 2-(trimethylsilyl)ethyl N6-(tert-butoxycarbonyl)-L-lysinate was prepared proceeding from N2-[(benzyloxy)carbonyl]-N6-(tert-butoxycarbonyl)-L-lysine by conventional methods of peptide chemistry. This intermediate was then coupled in the presence of HATU and *N,N*-diisopropylethylamine with the tripeptide unit N-[(benzyloxy) carbonyl]-L-valyl-L-alanyl-beta-alanine prepared by standard methods. The Z protecting group was then removed by hydrogenolysis in methanol and the intermediate obtained was coupled to (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid in the presence of HATU and N,N diisopropylethylamine. In the last step, the side-chain amino group was deprotected under gentle conditions by stirring in 10% trifluoroacetic acid in DMF at RT for 1 h. After concentration and freeze-drying from acetonitrile/water, the title compound was obtained.

LC-MS (Method 1): R_t = 0.64 min; MS (ESIpos): m/z = 625 $(M+H)^+$

Intermediate L100

3-[5-(2-{[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)-1,2,4-oxadiazol-3-yl]propanoic acid



To a solution of methyl 3-cyanopropanoate (4 g, 35.4 mmol) in 120 ml of ethanol were added 3.69 g (53 mmol) of hydroxylamine hydrochloride and 15 ml (110 mmol) of triethylamine. The reaction mixture was stirred at 50°C for 3 h. The mixture was concentrated and the residue was dissolved in ethyl acetate and then washed with water and brine. The organic phase was dried over magnesium sulphate and concentrated. The residue was used without further purification. This gave 5 g (97% of theory) of methyl (4Z)-4-amino-4-(hydroxyimino)butanoate.

To a solution of methyl (4Z)-4-amino-4-(hydroxyimino)butanoate (4.85 g, 33.19 mmol) in 120.0 ml of dioxane were added 6.91 g (36.50 mmol) of N-(tert-butoxycarbonyl)-beta-alanine and 8.22 g (39.82 mmol) of 1,3-dicyclohexylcarbodiimide. The reaction mixture was stirred at room temperature for 3 h. The mixture was concentrated and the residue

was dissolved in water and extracted with ethyl acetate. The organic phase was dried over sodium sulphate and concentrated. The residue was purified by means of flash chromatography. This gave 6.0 g (57% of theory) of methyl (4E)-4-{[N-(tert-butoxycarbonyl)-beta-alanyl]amino}-4-(hydroxyimino)butanoate.

A solution of methyl (4E)-4-{[N-(tert-butoxycarbonyl)-beta-alanyl]amino}-4-(hydroxyimino)butanoate (6.0 g, 18.9 mmol) in 100 ml of DMF was stirred at 120°C for 5 h. Water was added and the mixture was extracted with ethyl acetate. The organic phase was dried over sodium sulphate and concentrated. The residue was purified by preparative HPLC. This gave 4 g (71% of theory) of methyl 3-(5-{2-[(tert-butoxycarbonyl)amino]ethyl}-1,2,4-oxadiazol-3-yl)propanoate.

To a solution of methyl (4E)-4-{[N-(tert-butoxycarbonyl)-beta-alanyl]amino}-4-(hydroxyimino)butanoate (4.00 g, 13.4 mmol) in 60 ml of THF was added a solution of LiOH (1.60 g, 66.8 mmol) in 10 ml of water. The reaction mixture was stirred at 60°C overnight. Water was added and the mixture was extracted with ethyl acetate. The organic phase was dried over sodium sulphate and concentrated. The residue was used without further purification. This gave 3.60 g (87% of theory) of 3-(5-{2-[(tert-butoxycarbonyl)amino]ethyl}-1,2,4-oxadiazol-3-yl)propanoic acid.

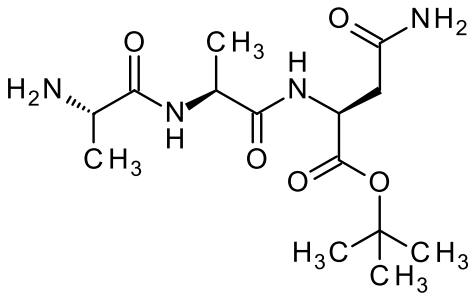
To a solution of 3-(5-{2-[(tert-butoxycarbonyl)amino]ethyl}-1,2,4-oxadiazol-3-yl)propanoic acid (2.0 g, 7.01 mmol) in 30 ml of dichloromethane were added 2.0 ml (26 mmol) of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 1 h. The mixture was admixed with water and extracted with dichloromethane. The organic phase was dried over sodium sulphate and concentrated. The residue was used without further purification. This gave 1.50 g (72% of theory) of 3-[5-(2-aminoethyl)-1,2,4-oxadiazol-3-yl]propanoic acid/trifluoroacetic acid (1:1).

To a solution of 3-[5-(2-aminoethyl)-1,2,4-oxadiazol-3-yl]propanoic acid (1.5 g, 5.01 mmol) in 25 ml of DMF were added 1.30 g (5.52 mmol) of 1-[2-(2,5-dioxopyrrolidin-1-yl)-2-oxoethyl]-1H-pyrrole-2,5-dione and 1.52 g (15.04 mmol) of triethylamine. The reaction mixture was stirred at room temperature for 1 h. The mixture was admixed with water and extracted with dichloromethane. The organic phase was dried over sodium sulphate and concentrated. The residue was purified by preparative HPLC. This gave 774 mg (47% of theory) of the title compound.

^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm] = 2.67 (t, 2H), 2.91 (t, 2H), 3.03 (t, 2H), 3.46 (q, 2H), 4.28 (s, 2H), 7.01 (s, 2H), 8.37 (t, 1H), 12.28 (bs, 1H).

Intermediate L101

tert-Butyl L-alanyl-L-alanyl-L-asparaginate

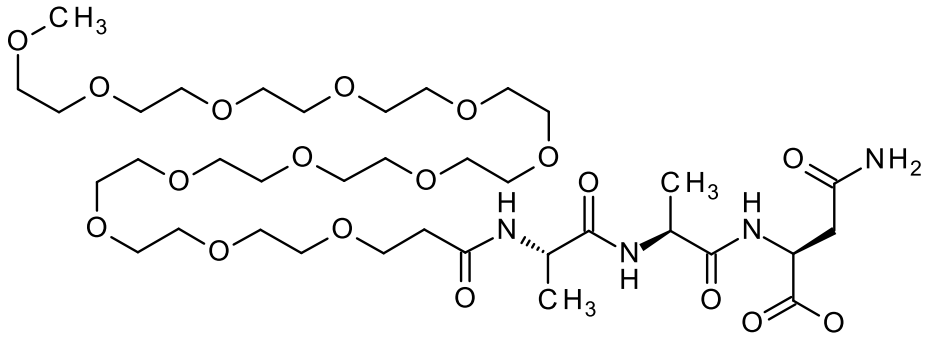


The title compound was prepared by conventional methods of peptide chemistry by HATU coupling, in the presence of N,N-diisopropylethylamine, of commercially available N-[(benzyloxy)carbonyl]-L-alanyl-L-alanine with tert-butyl L-asparaginate hydrochloride, followed by hydrogenolytic detachment of the Z protecting group over 10% palladium/activated carbon in methanol.

LC-MS (Method 7): R_t = 0.23 min; MS (ESIneg): m/z = 329 $(M-H)^-$.

Intermediate L102

N-(38-Oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaoctatriacontan-38-yl)-L-alanyl-L-alanyl-L-asparagine

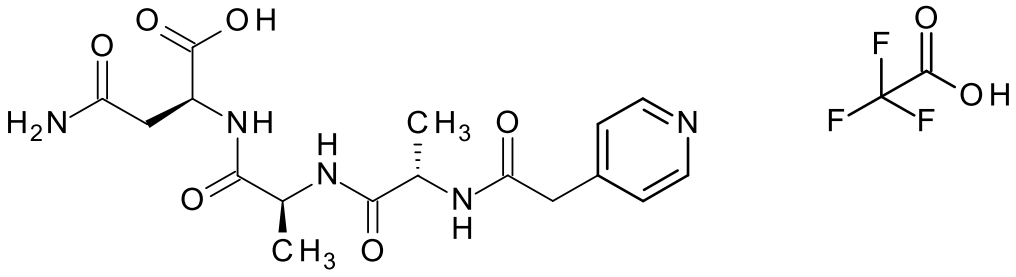


215 mg of 2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaoctatriacontan-38-oic acid (365 µmol) and 133 mg of Intermediate L101 (402 µmol) were initially charged in 1.4 ml of DMF, 146 mg of HATU (384 µmol) and 160 µl of *N,N*-diisopropylethylamine (910 µmol) were added and the mixture was stirred at RT for 3 h. Water (1.5 ml) and ACN (0.5 ml) were added. The reaction solution was purified by preparative HPLC (eluent: ACN/water + 0.1% TFA, gradient = 1:9 → 3:2) and subsequent detachment of the butoxycarbonyl protecting group with 2 ml of TFA in 2 ml of DCM (stirred at RT for 3 h).

LC-MS (Method 1): R_t = 0.56 min; MS (ESIneg): m/z = 844.5 $(M+H)^+$.

Intermediate L103

N-(Pyridin-4-ylacetyl)-L-alanyl-L-alanyl-L-asparagine trifluoroacetate (1:1)

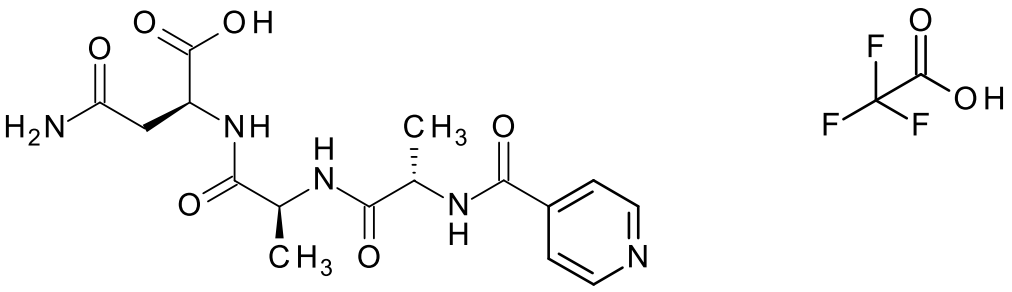


The title compound was prepared by conventional methods of peptide chemistry commencing with the coupling of 4-pyridineacetic acid with commercially available tert-butyl L-alanyl-L-alaninate in the presence of HATU and N,N-diisopropylethylamine, followed by deprotection with trifluoroacetic acid, coupling to tert-butyl L-asparaginate and subsequent deprotection of the carboxyl group with trifluoroacetic acid.

LC-MS (Method 1): R_t = 0.15 min; MS (ESIpos): m/z = 394 $(M+H)^+$.

Intermediate L104

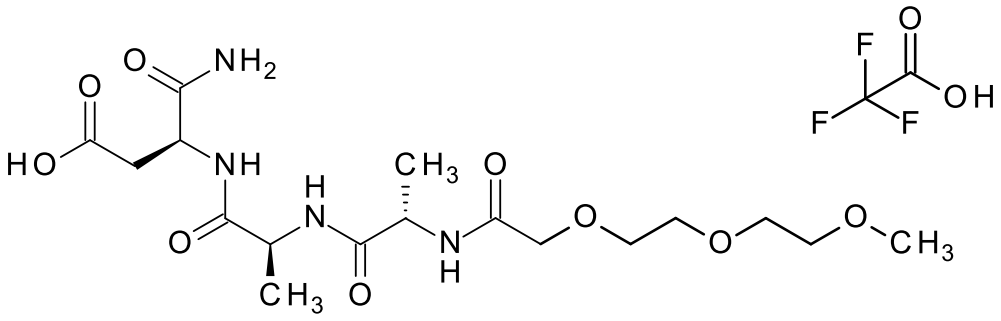
N-Isonicotinoyl-L-alanyl-L-alanyl-L-asparagine trifluoroacetate (1:1)



The title compound was prepared in analogy to intermediate L103 commencing with the coupling of isonicotinic acid with commercially available tert-butyl L-alanyl-L-alaninate.
LC-MS (Method 1): R_t = 0.17 min; MS (ESIpos): m/z = 380 $(M+H)^+$.

Intermediate L105

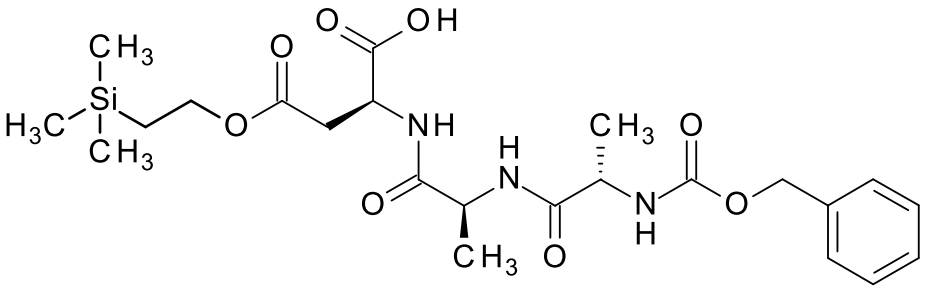
tert-Butyl N-{[2-(2-methoxyethoxy)ethoxy]acetyl}-L-alanyl-L-alanyl-L-asparaginate trifluoroacetate (1:1)



The title compound was prepared in analogy to intermediate L103 commencing with the coupling of [2-(2-methoxyethoxy)ethoxy]acetic acid with commercially available tert-butyl L-alanyl-L-alaninate.
LC-MS (Method 1): R_t = 0.17 min; MS (ESIpos): m/z = 380 $(M+H)^+$.

Intermediate L106

N-[(Benzyloxy)carbonyl]-L-alanyl-L-alanyl-L-asparagine

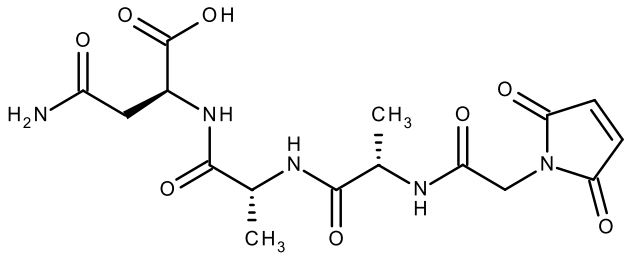


The title compound was prepared by conventional methods of peptide chemistry by coupling of commercially available N-[(benzyloxy)carbonyl]-L-alanyl-L-alanine with 1-tert-butyl 4-[2-(trimethylsilyl)ethyl]-L-aspartate in the presence of HATU and N,N-diisopropylethylamine. This amino acid unit was prepared from (3S)-4-tert-butoxy-3-[(tert-butoxycarbonyl)amino]-4-oxobutanoic acid by esterification with 2-(trimethylsilyl)ethanol in the presence of EDCI and DMAP and subsequent gentle removal of the tert-butoxycarbonyl protecting group by means of 5% trifluoroacetic acid in DCM. Subsequently, 745 mg (1.317 mmol) of the fully protected intermediate were dissolved in 43.5 ml of DCM and the tert-butyl ester was gently hydrolysed by adding 3.5 ml of trifluoroacetic acid and stirring at RT for 5 hours. 168 mg (25% of theory) of the title compound were isolated from the resultant product mixture after purification by preparative HPLC.

LC-MS (Method 1): R_t = 0.95 min; MS (ESIpos): m/z = 510 $(M+H)^+$.

Intermediate L107

N-[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-alanyl-D-alanyl-L-asparagine



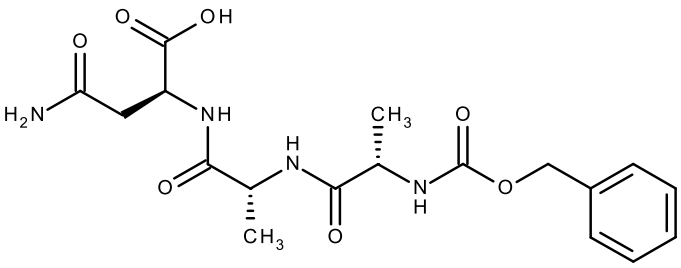
The title compound was prepared by conventional methods of peptide chemistry by HATU coupling of N-[(benzyloxy)carbonyl]-L-alanyl-D-alanine to tert-butyl L-asparaginate, in the

presence of N,N-diisopropylethylamine, subsequent deprotection of the Z protecting group by hydrogenation in methanol over 10% palladium on activated carbon, followed by acylation with 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione in DMF in the presence of N,N-diisopropylethylamine and finally deprotection of the carboxyl group by means of trifluoroacetic acid.

LC-MS (Method 1): R_t = 0.18 min; MS (ESIpos): m/z = 412 $(M+H)^+$.

Intermediate L108

N-[(Benzyloxy)carbonyl]-L-alanyl-D-alanyl-L-asparagine

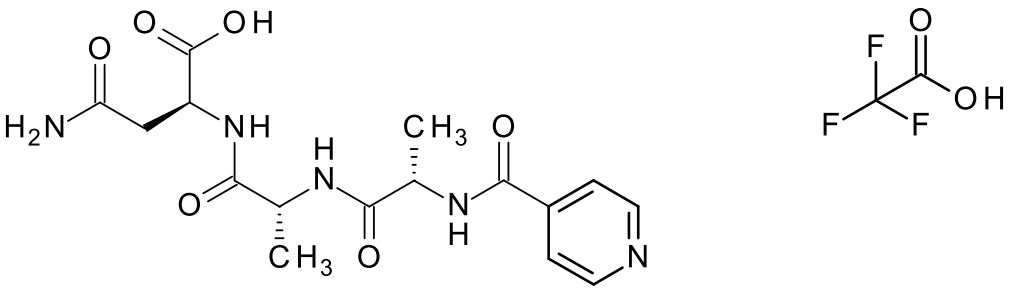


The title compound was prepared in analogy to Example L92 by conventional methods of peptide chemistry by HATU coupling of N-[(benzyloxy) carbonyl]-L-alanyl-D-alanine to tert-butyl L-asparaginate in the presence of N,N-diisopropylethylamine and subsequent deprotection of the carboxyl group with trifluoroacetic acid.

LC-MS (Method 12): R_t = 0.88 min; MS (ESIpos): m/z = 409 $(M+H)^+$.

Intermediate L109

N-Isonicotinoyl-L-alanyl-D-alanyl-L-asparagine trifluoroacetate (1:1)

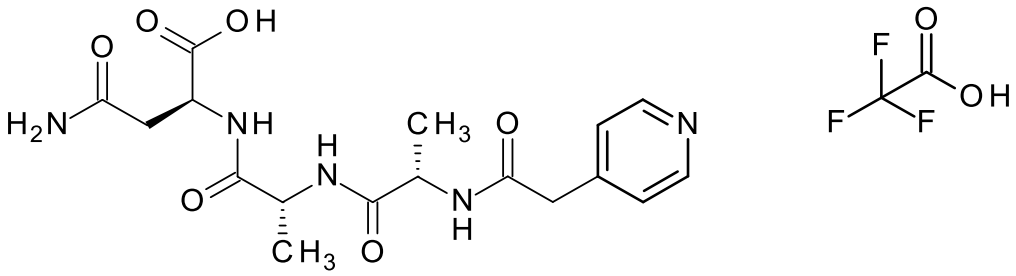


The title compound was prepared by conventional methods of peptide chemistry by HATU coupling of N-[(benzyloxy) carbonyl]-L-alanyl-D-alanine to tert-butyl L-asparaginate in the presence of N,N-diisopropylethylamine, subsequent hydrogenolytic detachment of the Z protecting group, coupling to isonicotinic acid in the presence of HATU and N,N-diisopropylethylamine, and finally deprotection of the carboxyl group with trifluoroacetic acid.

LC-MS (Method 13): R_t = 0.54 min; MS (ESIpos): m/z = 380 $(M+H)^+$.

Intermediate L110

N-(Pyridin-4-ylacetyl)-L-alanyl-D-alanyl-L-asparagine trifluoroacetate (1:1)

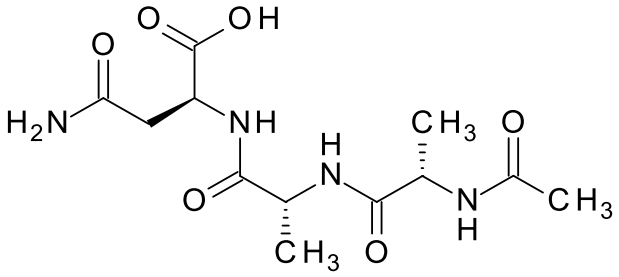


The title compound was prepared by conventional methods of peptide chemistry by HATU coupling of N-[(benzyloxy) carbonyl]-L-alanyl-D-alanine to tert-butyl L-asparaginate in the presence of N,N-diisopropylethylamine, subsequent hydrogenolytic detachment of the Z protecting group, coupling to pyridin-4-ylacetic acid hydrochloride (1:1) in the presence of HATU and N,N-diisopropylethylamine, and finally deprotection of the carboxyl group with trifluoroacetic acid.

LC-MS (Method 13): R_t = 0.5 min; MS (ESIpos): m/z = 394 $(M+H)^+$.

Intermediate L111

N-Acetyl-L-alanyl-D-alanyl-L-asparagine

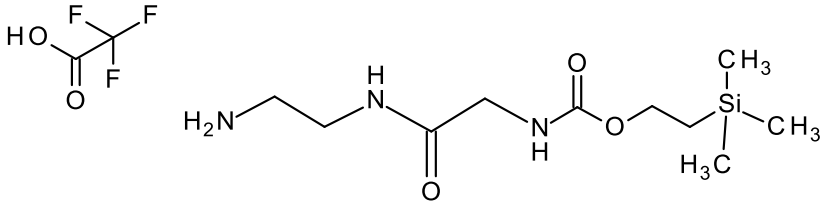


The title compound was prepared by conventional methods of peptide chemistry by HATU coupling of N-[(benzyloxy)carbonyl]-L-alanyl-D-alanine to tert-butyl L-asparaginate in the presence of N,N-diisopropylethylamine, subsequent deprotection of the carboxyl group with trifluoroacetic acid, followed by hydrogenolytic detachment of the Z protecting group and finally coupling to 1-acetoxypyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 0.17 min; MS (ESIpos): m/z = 317 $(M+H)^+$.

Intermediate L112

Trifluoroacetic acid N-(2-aminoethyl)-N^2-{[2-(trimethylsilyl)ethoxy]carbonyl}glycinamide (1:1)



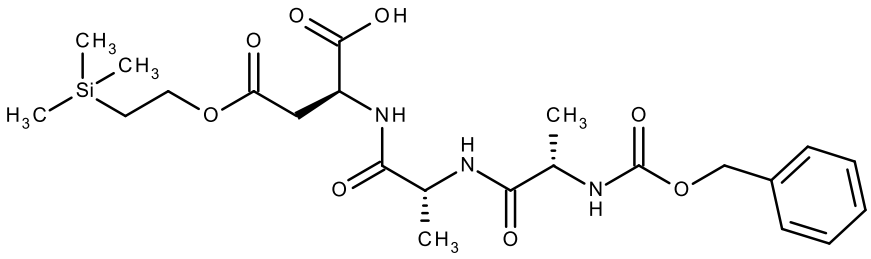
The title compound was prepared by conventional methods of peptide chemistry by reaction of glycine with 1-({[2-(trimethylsilyl)ethoxy]carbonyl}oxy)pyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine, subsequent HATU coupling to benzyl (2-

aminoethyl)carbamate hydrochloride (1:1) in the presence of N,N-diisopropylethylamine and finally by hydrogenolytic detachment of the Z group.

LC-MS (Method 1): R_t = 0.46 min; MS (ESIpos): m/z = 262 $(M+H)^+$.

Intermediate L113

(5S,8R,11S)-5,8-Dimethyl-3,6,9-trioxo-11-{2-oxo-2-[2-(trimethylsilyl)ethoxy]ethyl}-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oic acid

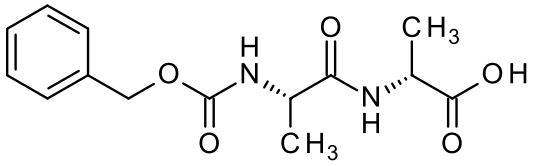


The title compound was prepared by conventional methods of peptide chemistry by HATU coupling of N-[(benzyloxy)carbonyl]-L-alanyl-D-alanine to 1-tert-butyl-4-[2-(trimethylsilyl)ethyl]-L-aspartate in DMF in the presence of N,N-diisopropylethylamine and subsequent gentle detachment of the tert-butyl ester by stirring in 7.5% trifluoroacetic acid in DCM. The title compound was isolated from the product mixture obtained by preparative HPLC.

LC-MS (Method 12): R_t = 1.77 min; MS (ESI neg): m/z = 508 $(M-H)^-$.

Intermediate L114

N-[(Benzyloxy)carbonyl]-L-alanyl-D-alanine



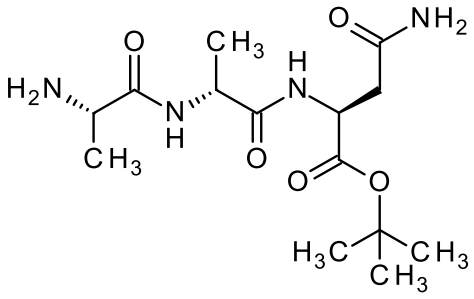
The title compound was prepared by conventional methods of peptide chemistry by HATU coupling of N-carbobenzyloxy-L-alanine to D-alanine tert-butyl ester hydrochloride in the

presence of N,N-diisopropylethylamine, and finally detachment of the butoxycarbonyl protecting group with TFA and DCM.

LC-MS (Method 1): R_t = 0.61 min; MS (ESIpos): m/z = 295 $(M+H)^+$.

Intermediate L115

tert-Butyl L-alanyl-D-alanyl-L-asparaginate

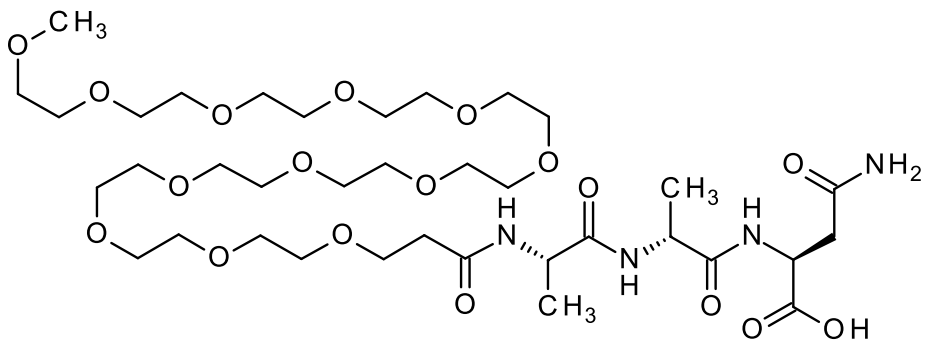


The title compound was prepared by conventional methods of peptide chemistry by HATU coupling of tert-butyl-L-asparaginate hydrochloride to N-[(benzyloxy)carbonyl]-L-alanyl-D-alanine (Intermediate L114) in the presence of N,N-diisopropylethylamine, and finally detachment of the Z protecting group.

LC-MS (Method 3): R_t = 0.91 min; MS (ESIpos): m/z = 331 $(M+H)^+$.

Intermediate L116

N-(38-Oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaoctatriacontan-38-yl)-L-alanyl-D-alanyl-L-asparagine

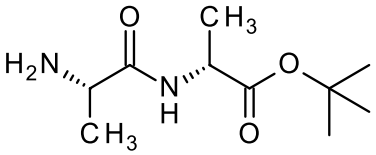


500 mg of 2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaoctatriacontan-38-oic acid (849 µmol) and 309 mg of tert-butyl L-alanyl-D-alanyl-L-asparaginate (Intermediate L115, 934 µmol) were initially charged in 5.8 ml of DMF, 420 mg of HATU (1.104 mmol) and 518 µl of *N,N*-diisopropylethylamine (2.97 mmol) were added and the mixture was stirred at 0°C for 40 min. Water (1 ml) and ACN (2 ml) were added. The reaction solution was purified by preparative HPLC (eluent: ACN/water + 0.1% TFA, gradient = 1:9 → 3:2) and subsequent detachment of the butoxycarbonyl protecting group with 3 ml of TFA in 3 ml of DCM (stirred at RT for 3 h).

LC-MS (Method 1): R_t = 0.56 min; MS (ESIneg): m/z = 843 $(M-H)^-$.

Intermediate L117

tert-Butyl L-alanyl-D-alaninate

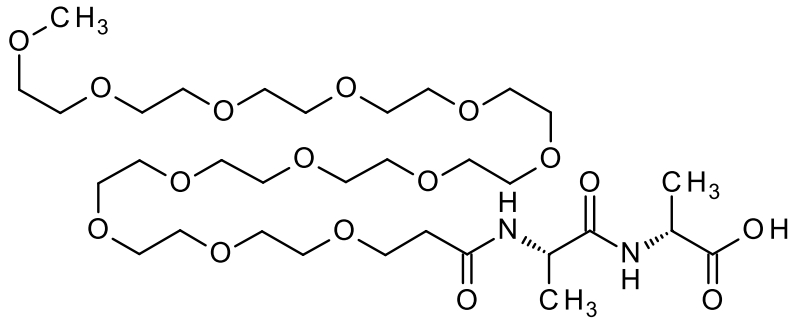


The title compound was prepared by conventional methods of peptide chemistry by HATU coupling of N-carbobenzyloxy-L-alanine to D-alanine tert-butyl ester hydrochloride in the presence of N,N-diisopropylethylamine, and finally detachment of the Z protecting group.

LC-MS (Method 7): R_t = 0.29 min; MS (ESIpos): m/z = 217 $(M+H)^+$.

Intermediate L118

N-(38-Oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaoctatriacontan-38-yl)-L-alanyl-D-alanine

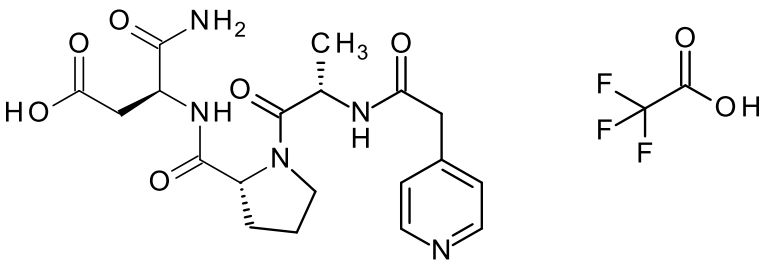


223 mg of 2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaoctatriacontan-38-oic acid (379 µmol) and 90.4 mg of tert-butyl L-alanyl-D-alaninate (Intermediate L117, 417 µmol) were initially charged in 1.2 ml of DMF, 144 mg of HATU (379 mmol) and 198 µl of *N,N*-diisopropylethylamine (1.14 mmol) were added and the mixture was stirred at 0°C for 30 min. Water (1 ml) and ACN (2 ml) were added. The reaction solution was purified by preparative HPLC (eluent: ACN/water + 0.1% TFA, gradient = 1:9 → 3:2) and subsequent detachment of the butoxycarbonyl protecting group with 1.5 ml of TFA in 1.5 ml of DCM (stirred at RT for 90 min).

LC-MS (Method 1): R_t = 0.59 min; MS (ESIneg): m/z = 729 $(M-H)^-$.

Intermediate L119

N-(Pyridin-4-ylacetyl)-L-alanyl-D-prolyl-L-alpha-asparagine / trifluoroacetic acid (1:1)



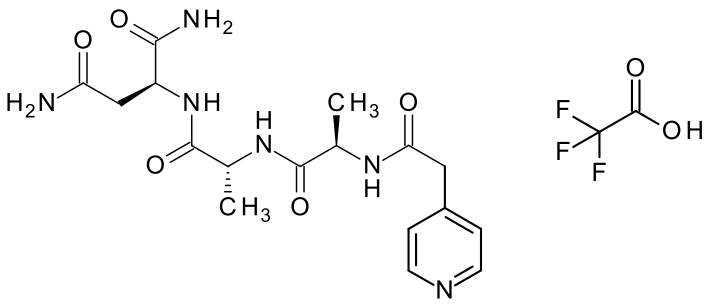
The title compound was synthesized by conventional methods of peptide chemistry starting with the HATU coupling of N-[(benzyloxy)carbonyl]-L-alanine to tert-butyl D-prolinate in the presence of N,N-diisopropylethylamine and subsequent deprotection of the carboxyl group with trifluoroacetic acid in DCM. This was followed by coupling to tert-butyl L-asparaginate in the presence of HATU and N,N-diisopropylethylamine and then

hydrogenolytic detachment of the Z protecting group in DCM/methanol 1:1 over 10% palladium on activated carbon at RT under standard hydrogen pressure. Finally, the intermediate obtained was converted to the title compound by coupling to 4-pyridineacetic acid in the presence of N,N-diisopropylethylamine and subsequent deprotection of the carboxyl group with trifluoroacetic acid in DCM.

LC-MS (Method 1): R_t = 0.16 min; MS (ESIpos): m/z = 420 $(M+H)^+$.

Intermediate L120

Trifluoroacetic acid / N-(pyridin-4-ylacetyl)-D-alanyl-D-alanyl-L-aspartamide (1:1)

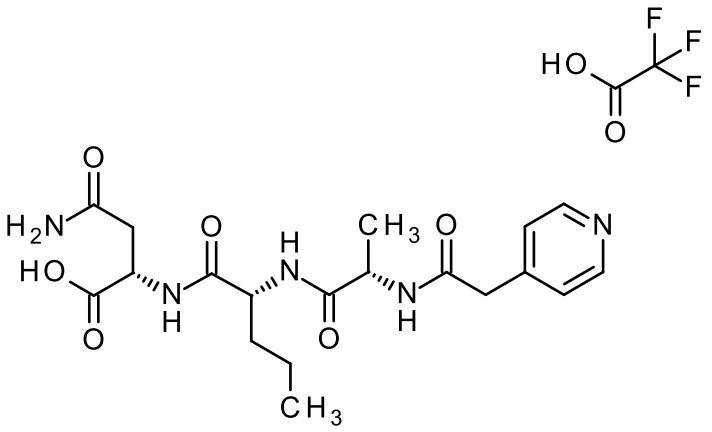


The title compound was synthesized by conventional methods of peptide chemistry starting with the HATU coupling of N-[(benzyloxy)carbonyl]-L-alanine to tert-butyl D-alaninate hydrochloride (1:1) in the presence of N,N-diisopropylethylamine and subsequent deprotection of the carboxyl group with trifluoroacetic acid in DCM. This was followed by coupling to tert-butyl L-asparaginate in the presence of HATU and N,N-diisopropylethylamine and then hydrogenolytic detachment of the Z protecting group in DCM/methanol 1:1 over 10% palladium on activated carbon at RT under standard hydrogen pressure. Finally, the intermediate obtained was converted to the title compound by coupling to 4-pyridineacetic acid in the presence of HATU and N,N-diisopropylethylamine and subsequent deprotection of the carboxyl group with trifluoroacetic acid in DCM.

LC-MS (Method 1): R_t = 0.16 min; MS (ESIpos): m/z = 394 $(M+H)^+$.

Intermediate L126

N-(Pyridin-4-ylacetyl)-L-alanyl-D-norvalyl-L-asparagine trifluoroacetic acid salt

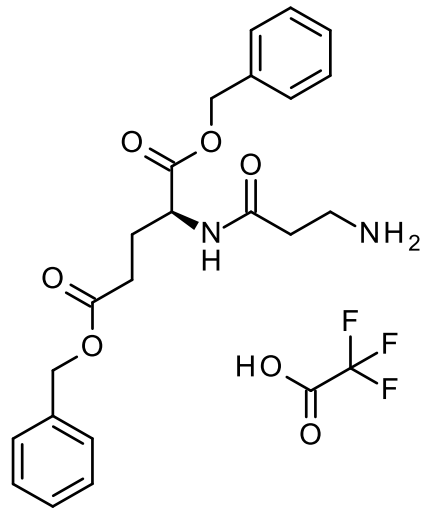


The title compound was synthesized by conventional methods of peptide chemistry starting with the HATU coupling of pyridin-4-ylacetic acid hydrochloride to tert-butyl L-alaninate hydrochloride (1:1) in the presence of N,N-diisopropylethylamine and subsequent deprotection of the carboxyl group with trifluoroacetic acid in DCM. This was followed by coupling to 4-methylbenzenesulphonic acid-benzyl-D-norvalinate in the presence of HATU and N,N-diisopropylethylamine and then the detachment of the benzyl protecting group with lithium hydroxide monohydrate in THF/water at RT under standard hydrogen pressure. Finally, the intermediate obtained was converted to the title compound by coupling to tert-butyl L-asparaginate in the presence of HATU and N,N-diisopropylethylamine and subsequent deprotection of the carboxyl group with trifluoroacetic acid in DCM.

LC-MS (Method 1): R_t = 0.18 min; MS (ESIpos): m/z = 422 $[M+H]^+$.

Intermediate L127

Trifluoroacetic acid dibenzyl-beta-alanyl-L-glutamate salt

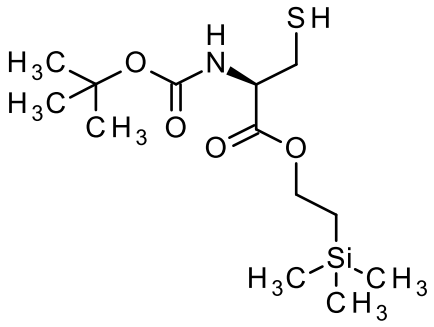


The title compound was prepared by coupling of 4-methylbenzenesulphonic acid / dibenzyl-L-glutamate (1:1) to N-(tert-butoxycarbonyl)-beta-alanine in the presence of HATU and N,N-diisopropylethylamine and subsequent detachment of the *t*-butyl protecting group by means of trifluoroacetic acid in dichloromethane.

LC-MS (Method 1): R_t = 0.72 min; MS (ESIpos): m/z = 399 $[M+H]^+$

Intermediate L128

2-(Trimethylsilyl)ethyl N-(tert-butoxycarbonyl)-L-cysteinate



N,N'-Bis(tert-butoxycarbonyl)-L-cystine (7.00 g, 15.9 mmol) was dissolved in 80 ml of acetonitrile and cooled to 0°C. At this temperature, pyridine (2.6 ml, 32 mmol), 2-(trimethylsilyl)ethanol (2.5 ml, 17 mmol) and 1,3-dicyclohexylcarbodiimide (3.61 g, 17.5 mmol) were added. The reaction mixture was stirred at 0°C for 1 h and then at RT overnight. The reaction mixture was filtered and the filtercake was washed with

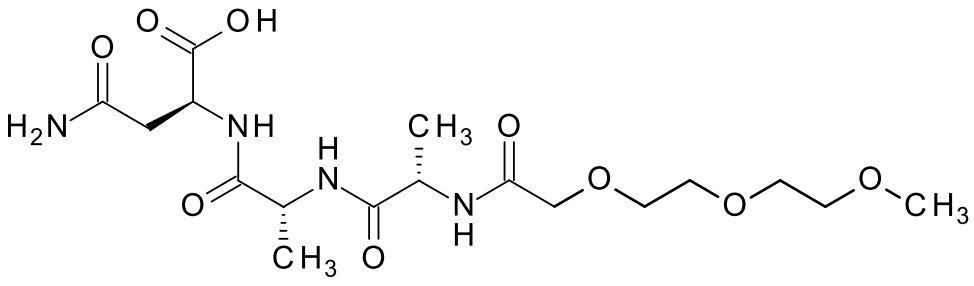
acetonitrile. The solvent was evaporated under reduced pressure. The residue was purified using Biotage Isolera (silica gel, ethyl acetate/cyclohexane 1:2). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 4.14 g (41% of theory) of the compound bis[2-(trimethylsilyl)ethyl]-N,N'-bis(tert-butoxycarbonyl)-L-cystinate.

Under argon, bis[2-(trimethylsilyl)ethyl]-N,N'-bis(tert-butoxycarbonyl)-L-cystinate (300 mg, 468 µmol) was initially charged in 1.0 ml of water and 3.0 ml of DMF. The reaction mixture was admixed with TCEP (335 mg, 1.17 mmol) and stirred at RT for a further 1 h. The mixture was diluted with ethyl acetate and washed repeatedly with water and with saturated sodium chloride solution. The organic phase was dried over magnesium sulphate and then concentrated. The residue was purified by column chromatography on Biotage/Isolera (SNAP 25 g) using cyclohexane/ethyl acetate 9:1 as eluent. The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 106 mg (70% of theory) of the title compound.

LC-MS (Method 1): R_t = 1.28 min; MS (ESIneg): m/z = 320 $[M-H]^-$

Intermediate L129

N-{[2-(2-Methoxyethoxy)ethoxy]acetyl}-L-alanyl-D-alanyl-L-asparagine

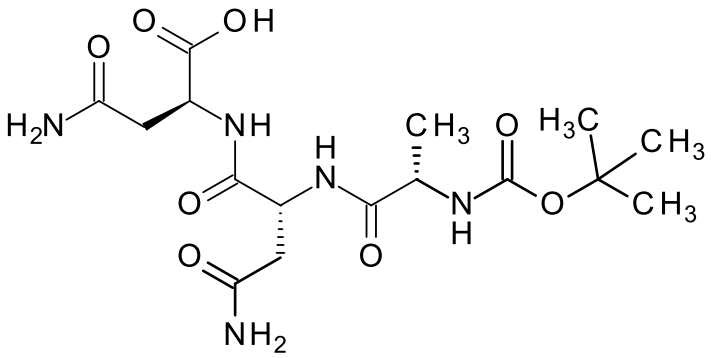


The title compound was prepared by conventional methods of peptide chemistry by HATU coupling, in the presence of N,N-diisopropylethylamine and of N-[(benzyloxy)carbonyl]-L-alanyl-D-alanine, to tert-butyl L-asparaginate hydrochloride, followed by hydrogenolytic detachment of the Z protecting group over 10% palladium/activated carbon in methanol, then another HATU coupling to [2-(2-methoxyethoxy)ethoxy]acetic acid and subsequent cleavage of the tert-butyl ester with TFA.

LC-MS (Method 1): R_t = 0.6 min; MS (ESIpos): m/z = 491 $(M+H)^+$.

Intermediate L130

N-(tert-Butoxycarbonyl)-L-alanyl-D-asparaginyl-L-asparagine

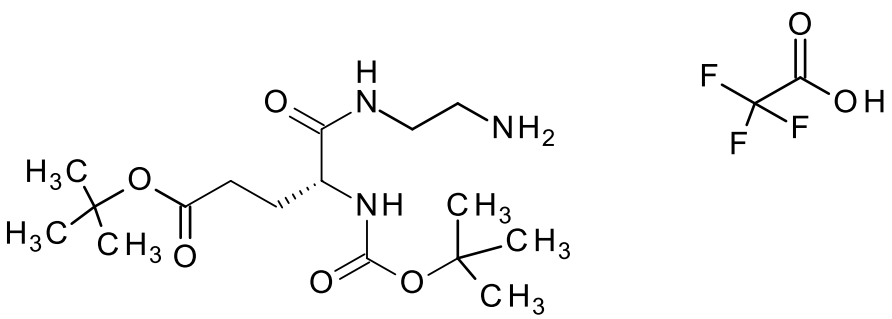


The title compound was prepared by conventional methods of peptide chemistry by HATU coupling in the presence of N,N-diisopropylethylamine and of N^2-(tert-butoxycarbonyl)-D-asparagine to 4-nitrobenzyl-L-asparaginate hydrobromide (1:1), followed by cleavage of the Boc protecting group with TFA, subsequent coupling of tert-butoxycarbonyl-L-alanine, likewise by means of HATU, and finally hydrogenolytic detachment of the p-nitrobenzyl ester over 10% palladium/activated carbon in dichloromethane/methanol 1:1.

LC-MS (Method 1): R_t = 0.38 min; MS (ESIpos): m/z = 418 $(M+H)^+$.

Intermediate L131

Trifluoroacetic acid / tert-butyl-N-(2-aminoethyl)-N^2-(tert-butoxycarbonyl)-D-alpha-glutaminate (1:1)



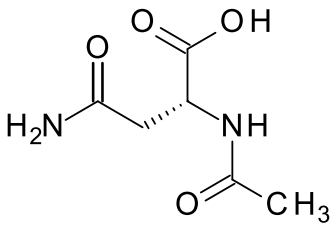
The title compound was prepared by conventional methods of peptide chemistry by HATU coupling in the presence of N,N-diisopropylethylamine and of (2R)-5-tert-butoxy-2-[(tert-

butoxycarbonyl)amino]-5-oxopentanoic acid to benzyl (2-aminoethyl)carbamate hydrochloride (1:1), followed by hydrogenolytic detachment of the benzyl ester over 10% palladium/activated carbon in ethanol.

LC-MS (Method 1): R_t = 0.6 min; MS (ESIpos): m/z = 346 $(M+H)^+$.

Intermediate L132

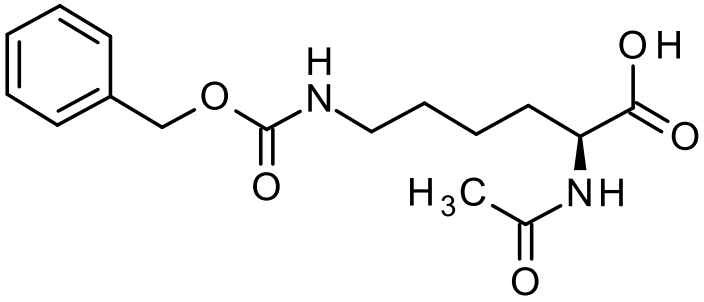
N^2-Acetyl-D-asparagine



The title compound was prepared by conventional methods of peptide chemistry, at first by esterification of N^2-[(benzyloxy)carbonyl]-D-asparagine with (2-trimethylsilyl)ethanol with EDCI/DMAP in DCM, followed by hydrogenolytic detachment of the benzyl ester over 10% palladium/activated carbon in DCM/methanol 1:1, then by reaction with 1-acetoxypyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine and finally by detachment of the Teoc protecting group by stirring at 50°C with 6 equivalents of zinc chloride in trifluoroethanol for 1 h.
LC-MS (Method 1): R_t = 0.15 min; MS (ESIneg): m/z = 173 $(M-H)^-$.

Intermediate L133

N^2-Acetyl-N^6-[(benzyloxy)carbonyl]-L-lysine

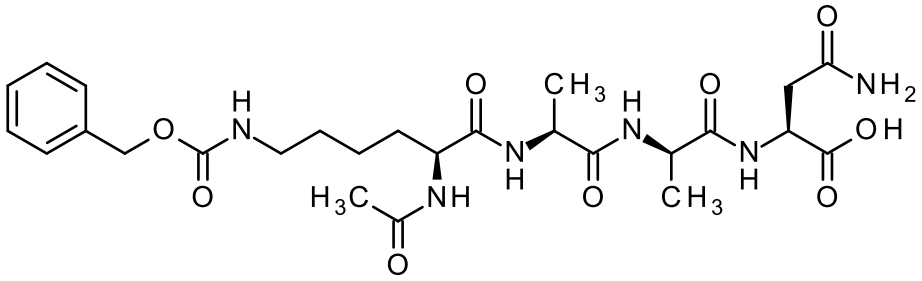


To a solution of N2-acetyl-L-lysine (947 mg, 5.03 mmol) in THF/water/NaHCO3 solution (15 ml/6 ml/12 ml) was added benzyl carbonochloridate (944 mg, 5.53 mmol) dissolved in 5 ml of THF. The mixture was stirred at RT for 2 h and then diluted with water and ethyl acetate. A pH of 4 was established with dilute HCl. The organic phase was dried over MgSO4, filtered off with suction and concentrated. The residue and the freeze-dried aqueous phase were purified together via preparative HPLC.

LC-MS (Method 1): R_t = 0.65 min; MS (ESIpos): m/z = 323 $[M+H]^+$

Intermediate L134

N^2-Acetyl-N^6-[(benzyloxy)carbonyl]-L-lysyl-L-alanyl-D-alanyl-L-asparagine

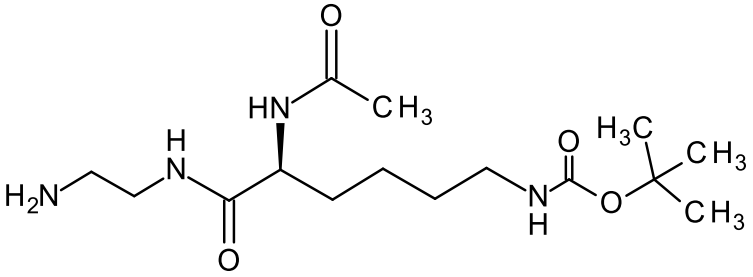


The title compound was synthesized by conventional methods of peptide chemistry starting with the HATU coupling of N2-acetyl-N6-[(benzyloxy)carbonyl]-L-lysine (97.6 mg, 303 µmol) (Intermediate L133) to tert-butyl L-alanyl-D-alanyl-L-asparaginate (Intermediate L115) (100 mg, 303 µmol) in the presence of N,N-diisopropylethylamine and subsequent deprotection of the carboxyl group with trifluoroacetic acid in DCM.

LC-MS (Method 1): R_t = 0.58 min; MS (ESIpos): m/z = 579 $[M+H]^+$

Intermediate L135

N^2-Acetyl-N-(2-aminoethyl)-N^6-(tert-butoxycarbonyl)-L-lysinamide

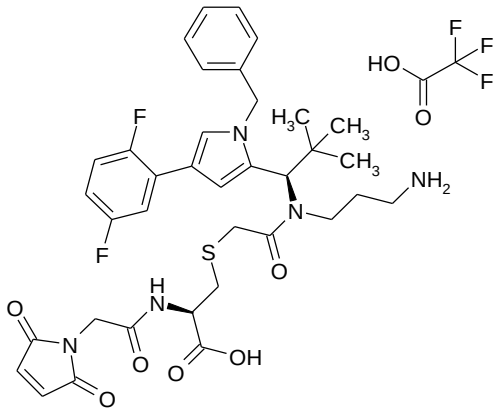


The title compound was prepared by conventional methods of peptide chemistry by HATU coupling of commercially available N^2-acetyl-N^6-(tert-butoxycarbonyl)-L-lysine with benzyl (2-aminoethyl)carbamate hydrochloride (1:1) in the presence of N,N-diisopropylethylamine and subsequent detachment of the Z protecting group by hydrogenation in DCM/methanol 1:1 over 10% palladium on activated carbon.

LC-MS (Method 1): R_t = 0.43 min; MS (ESIpos): m/z = 331 $(M+H)^+$.

Intermediate F239

S-{2-[(3-Aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-cysteine / trifluoroacetic acid (1:1)



Under argon, 7.5 mg (0.05 mmol) of (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid were initially charged in 1.5 ml of DMF, and 7.5 mg (0.05 mmol) of HOBt, 15.5 mg (0.05 mmol)

of TBTU and 6.2 mg (0.05 mmol) of N,N-diisopropylethylamine were added. The reaction mixture was stirred at RT for 10 min. 40.0 mg (0.05 mmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine / trifluoroacetic acid (1:1) (Intermediate C71), dissolved in 1.5 ml of DMF, and 18.7 mg (0.14 mmol) of N,N-diisopropylethylamine were then added, and the reaction mixture was stirred at RT overnight. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 11.2 mg (25% of theory) of the compound S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-cysteine.

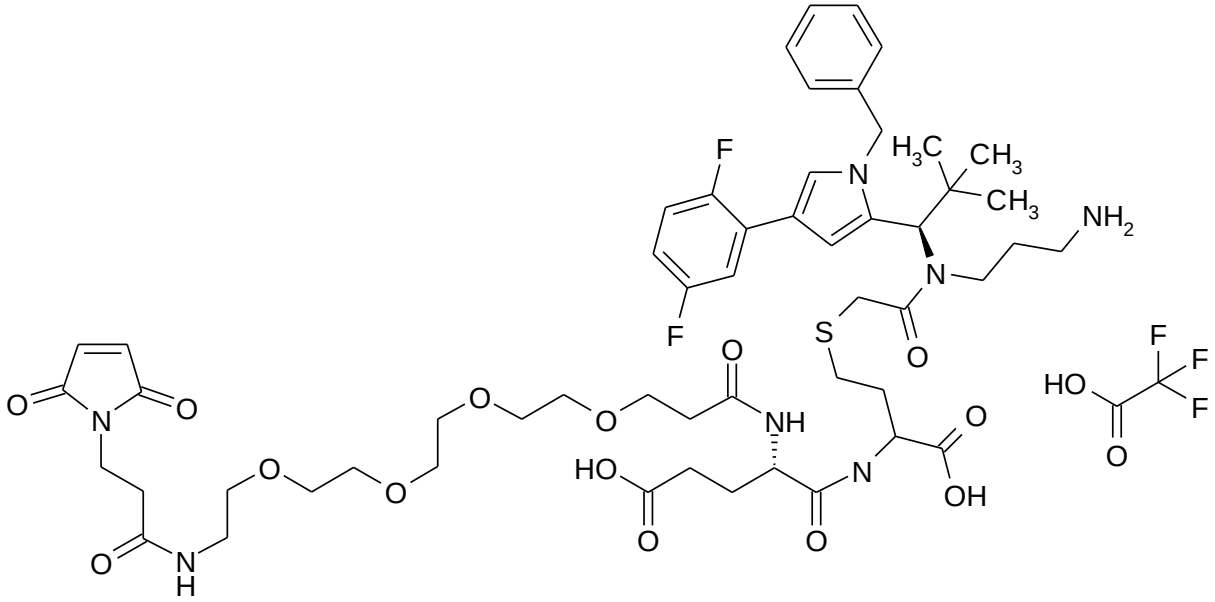
LC-MS (Method 1): R_t = 1.37 min; MS (ESIpos): m/z = 854 $(M+H)^+$.

10.9 mg (12.8 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-cysteine were dissolved in 2.0 ml of trifluoroethanol, and 10.4 mg (76.6 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 4 h. 22.4 mg (0.08 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was lyophilized. This gave 7.5 mg (65% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 710 $(M+H)^+$.

Intermediate F255

R/S-(N-[19-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-alpha-glutamyl-S-{2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl})homocysteine / trifluoroacetic acid (1:1)



13.1 mg (0.04 mmol) of (2S)-5-(benzyloxy)-2-{[(benzyloxy)carbonyl]amino}-5-oxopentanoic acid were initially charged in 1.0 ml of DMF, and 5.4 mg (0.04 mmol) of HOBt, 11.4 mg (0.04 mmol) of TBTU and 4.6 mg (0.04 mmol) of N,N-diisopropylethylamine were added. The reaction mixture was stirred at RT for 10 min. 30.0 mg (0.04 mmol) of R/S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)homocysteine / trifluoroacetic acid (1:1) (Intermediate C11) dissolved in 12.9 mg (0.1 mmol) of N,N-diisopropylethylamine and 1 ml of DMF were then added. The reaction mixture was stirred at RT overnight. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 32 mg (73 %) of the compound 4-[2-[[(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)pyrrol-2-yl]-2,2-dimethylpropyl]-[3-(2-trimethylsilylethoxycarbonylamino)propyl]amino]-2-oxoethyl]sulphanyl-2-[[(2S)-5-benzyloxy-2-(benzyloxycarbonylamino)-5-oxo-pentanoyl]amino]butanoic acid.
LC-MS (Method 1): R_t = 1.53 min; MS (ESIpos): m/z = 1084 $(M+H)^+$.

41.4 mg (0.038 mmol) of 4-[2-[[(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)pyrrol-2-yl]-2,2-dimethylpropyl]-[3-(2-trimethylsilylethoxycarbonylamino)propyl]amino]-2-oxoethyl]sulphanyl-2-[[(2S)-5-benzyloxy-2-(benzyloxycarbonylamino)-5-oxo-pentanoyl]amino]butanoic acid were dissolved in 10 ml of ethanol, 4.2 mg of Pd/C were added and the mixture was hydrogenated under standard pressure. The reaction mixture

was filtered through a cardboard filter and the filtercake was washed with ethanol. The solvent was evaporated under reduced pressure without heating. The residue was purified by preparative RP-HPLC (column: Reprosil 250x40; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 21.1 mg (56%) of the compound R/S-(L-alpha-glutamyl-S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl))homocysteine / trifluoroacetic acid (1:1).

LC-MS (Method 1): R_t = 1.11 min; MS (ESIpos): m/z = 860 $(M+H)^+$.

20.4 mg (20.94 µmol) of R/S-(L-alpha-glutamyl-S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl))homocysteine / trifluoroacetic acid (1:1) were initially charged together with 11.8 mg (23.04 µmol) of 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-{15-[(2,5-dioxopyrrolidin-1-yl)oxy]-15-oxo-3,6,9,12-tetraoxapentadec-1-yl}propanamide in 1.0 ml of DMF, and 4.2 mg (41.88 µmol) of 4-methylmorpholine were added. The reaction mixture was stirred at RT overnight, and 3.1 mg (0.05 mmol) of acetic acid were then added. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 9.5 mg (36%) of the compound R/S-(N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-alpha-glutamyl-S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl))homocysteine.

LC-MS (Method 1): R_t = 1.66 min; MS (ESIpos): m/z = 1259 $(M+H)^+$.

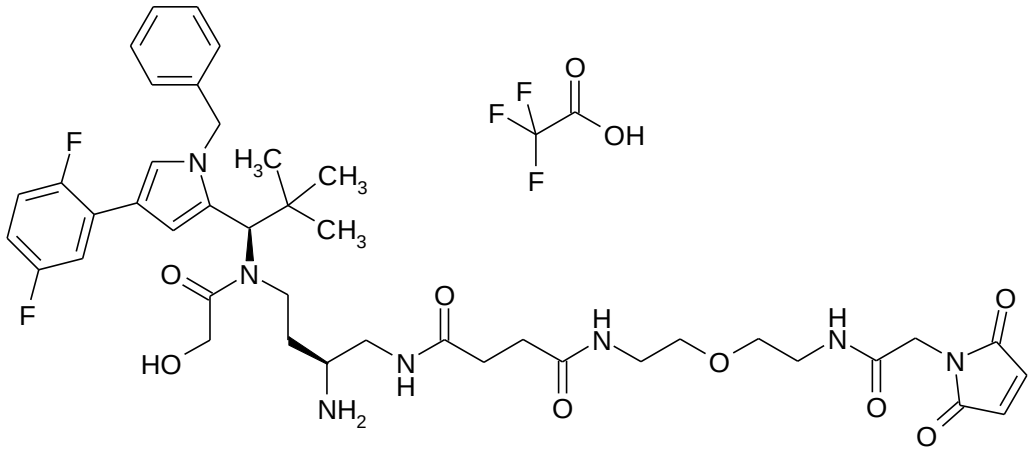
9.4 mg (7.47 µmol) of R/S-(N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-alpha-glutamyl-S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl))homocysteine were dissolved in 1.5 ml of trifluoroethanol, and 6.1 mg (44.81 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 3 h. 13.1 mg (0.05 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under

reduced pressure and the residue was dried under high vacuum. This gave 6.9 mg (75%) of the title compound.

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 1114 $(M+H)^+$.

Intermediate F256

Trifluoroacetic acid / N-{(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butyl}-N'-[2-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethyl]succinamide (1:1)



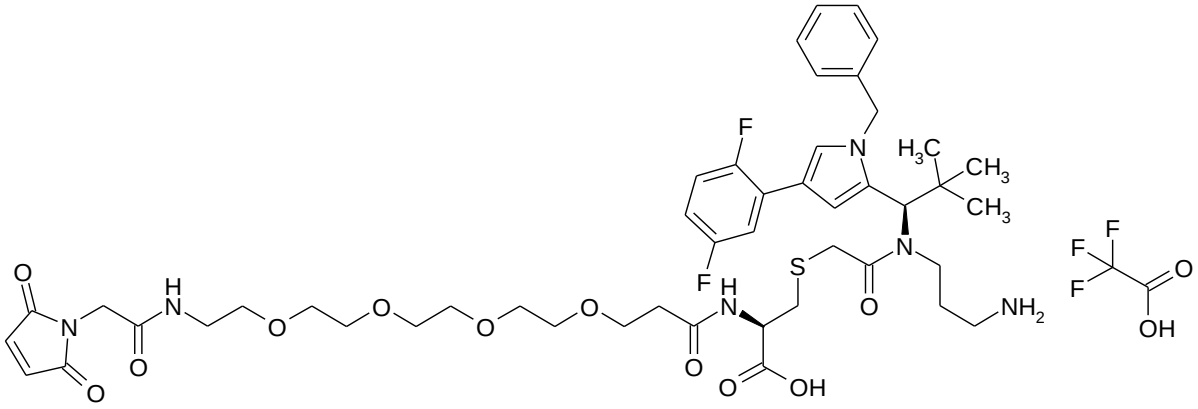
The title compound was prepared by coupling of 10 mg (0.014 mmol) of Intermediate C65 and

9.6 mg (0.027 mmol) of trifluoroacetic acid / N-[2-(2-aminoethoxy)ethyl]-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (1:1) in the presence of HATU and N,N-diisopropylethylamine and subsequent deprotection with zinc chloride in trifluoroethanol as described for Intermediate F119. Purification by preparative HPLC gave 8 mg (64% of theory over 2 steps) of the title compound.

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 822 $(M+H)^+$.

Intermediate F257

R-{2-[(3-Aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-N-[18-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azaoctadecan-1-oyl]-L-cysteine / trifluoroacetic acid (1:1)



50.0 mg (0.06 mmol) of R-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine / trifluoroacetic acid (1:1) (Intermediate C71) and 29 mg (0.07 mmol) of 3-[2-[2-[2-[2-[[2-(2,5-dioxopyrrol-1-yl)acetyl]amino]ethoxy]ethoxy]ethoxy]ethoxy]propanoic acid (Intermediate L74) were dissolved in 3.0 ml of DMF, and 27.3 mg (0.07 mmol) of HATU and 23.3 mg (0.18 mmol) of N,N-diisopropylethylamine were added. The reaction mixture was stirred at RT for 2 hours. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 17.4 mg (26%) of the compound R-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[18-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azaoctadecan-1-oyl]-L-cysteine.

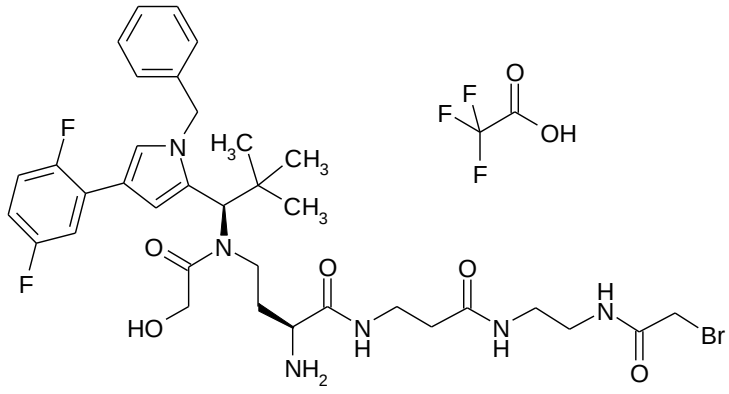
LC-MS (Method 6): R_t = 1.34 min; MS (ESIpos): m/z = 1101 $(M+H)^+$.

17 mg (0.02 mmol) of R-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[18-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azaoctadecan-1-oyl]-L-cysteine were dissolved in 1.0 ml of trifluoroethanol, and 6.3 mg (0.05 mmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C overnight. 13.5 mg (0.05 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 7.6 mg (46%) of the title compound.

LC-MS (Method 1): R_t = 0.91 min; MS (ESIpos): m/z = 957 $(M+H)^+$.

Intermediate F258

Trifluoroacetic acid / (2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-N-[3-{2-[(bromoacetyl)amino]ethyl}amino)-3-oxopropyl]butanamide (1:1)

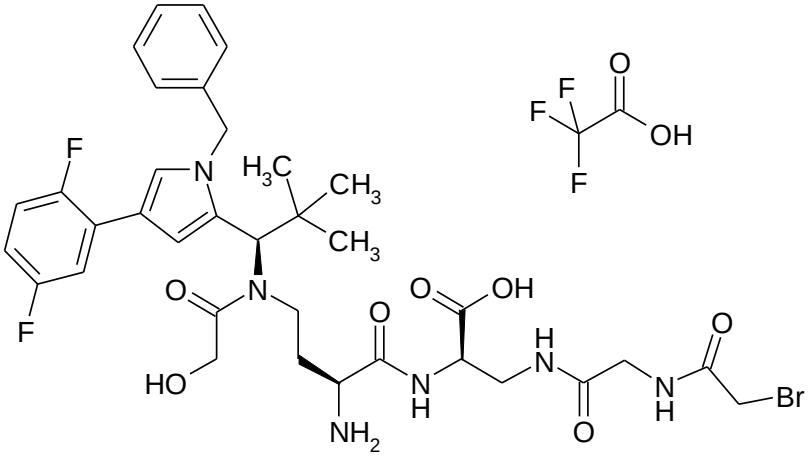


The title compound was prepared by coupling of Intermediate C58 with trifluoroacetic acid / benzyl [2-(beta-alanylamino)ethyl]carbamate (1:1) using HATU, subsequent hydrogenolysis, followed by coupling to 1-(2-bromoacetoxy)pyrrolidine-2,5-dione and finally by deprotection with zinc chloride.

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 747 and 749$(M+H)^+$.

Intermediate F259

N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethyl propyl}(glycoloyl)amino]butanoyl}-3-{[N-(bromoacetyl)glycyl]amino}-D-alanine / trifluoroacetic acid (1:1)



75 mg (0.114 mmol) of Intermediate C58 were taken up in 12.5 ml of DMF and coupled to 78 mg (0.171 mmol) of Intermediate L75 in the presence of 65 mg (0.11 mmol) of HATU and 79 µl of N,N-diisopropylethylamine. After purification by preparative HPLC, the intermediate was taken up in 20 ml of ethanol and hydrogenated over 10% palladium on activated carbon at RT under hydrogen standard pressure for 1 h. The catalyst was then filtered off, the solvent was removed under reduced pressure and the product was purified by preparative HPLC. Lyophilization from acetonitrile/water 1:1 gave 63 mg (64% of theory over 2 steps) of 2-(trimethylsilyl)ethyl 3-amino-N-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)butanoyl]-D-alaninate.
LC-MS (Method 1): R_t = 1.16 min; MS (EIpos): m/z = 844 $[M+H]^+$.

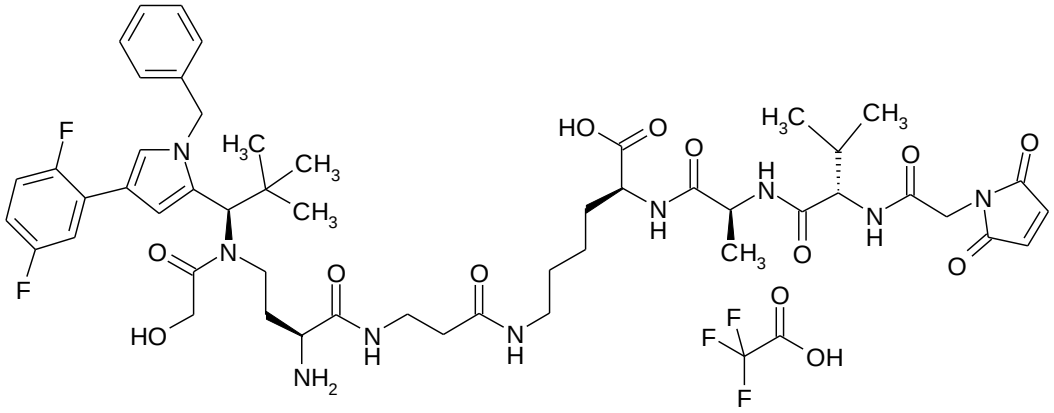
40 mg (0.047 mmol) of this intermediate were then coupled as described above with N-[(benzyloxy)carbonyl]glycine in the presence of HATU and then once more hydrogenolytically deprotected.

The title compound was then prepared by coupling of 10 mg (0.012 mmol) of this intermediate with 7.7 mg (0.032 mmol) of commercially available 1-(2-bromoacetoxy)pyrrolidine-2,5-dione in the presence of 4 µl of N,N-diisopropylethylamine and subsequent deprotection with zinc chloride in trifluoroethanol as described for Intermediate F119. Purification by preparative HPLC gave 1.3 mg of the title compound.

LC-MS (Method 1): R_t = 0.83 min; MS (ESIpos): m/z = 777 and 779 $(M+H)^+$.

Intermediate F260

N^6-(N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-beta-alanyl)-N^2-{N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-L-alanyl}-L-lysine / trifluoroacetic acid (1:1)

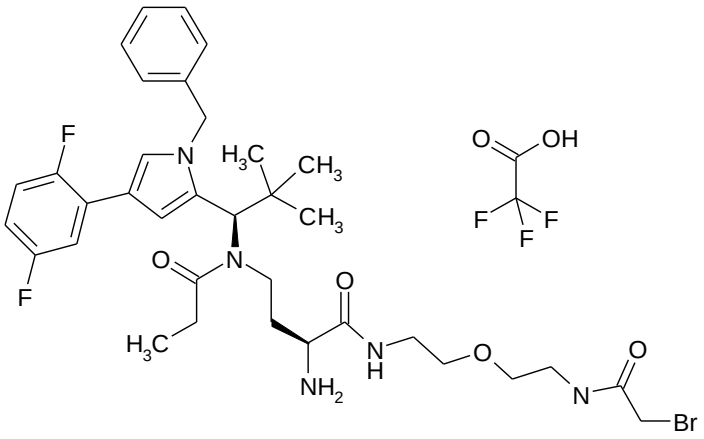


The title compound was prepared analogously to Intermediate F155.

LC-MS (Method 1): R_t = 0.81 min; MS (ESIpos): m/z = 1020 $(M+H)^+$.

Intermediate F261

Trifluoroacetic acid / (2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-N-(2-{2-[(bromoacetyl)amino]ethoxy}ethyl)-butanamide (1:1)

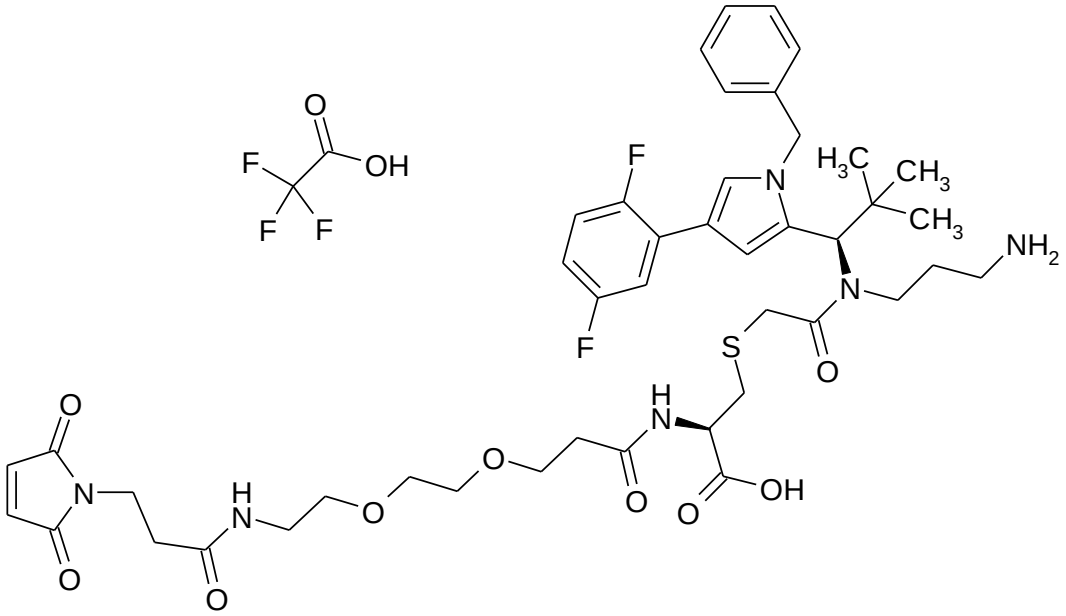


The title compound was prepared by coupling of 20 mg (0.03 mmol) of Intermediate C58 with 25.8 mg (0.061 mmol) of Intermediate L77 in the presence of HATU and subsequent deprotection with zinc chloride. This gave 11.9 mg (47% of theory over 2 steps) of the title compound.

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 722 and 720 $(M+H)^+$.

Intermediate F262

S-{2-[(3-Aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-N-{3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]propanoyl}-L-cysteine / trifluoroacetic acid (1:1)



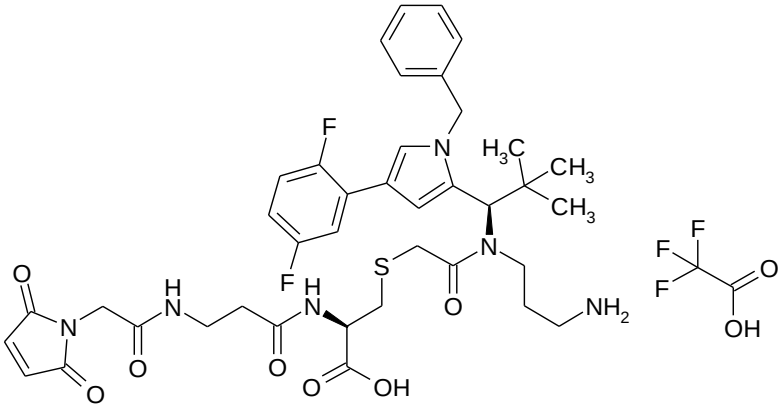
30 mg (36 µmol) of S-{2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-L-cysteine / trifluoroacetic acid (1:1) (Intermediate C71) together with 16.9 mg (40 µmol) of 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-[2-(2-{3-[(2,5-dioxopyrrolidin-1-yl)oxy]-3-oxopropoxy}ethoxy)ethyl]propanamide were initially charged in 1.5 ml of DMF, and 10.9 mg (108 µmol) of 4-methylmorpholine were added. The reaction mixture was stirred at RT overnight, and 7.58 mg (0.13 mmol) of acetic acid were then added. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 33.4 mg (80% of theory) of the compound S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-{3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]propanoyl}-L-cysteine.

LC-MS (Method 1): R_t = 1.34 min; MS (ESIpos): m/z = 1027 $(M+H)^+$.

32.8 mg (32 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-{3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]propanoyl}-L-cysteine were dissolved in 3.0 ml of trifluoroethanol, and 26.1 mg (192 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 2 h. 56.0 mg (0.192 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was lyophilized. This gave 22.9 mg (71% of theory) of the title compound.
LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 883 $(M+H)^+$.

Intermediate F263

N-[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-beta-alanyl-S-{2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-L-cysteine / trifluoroacetic acid (1:1)



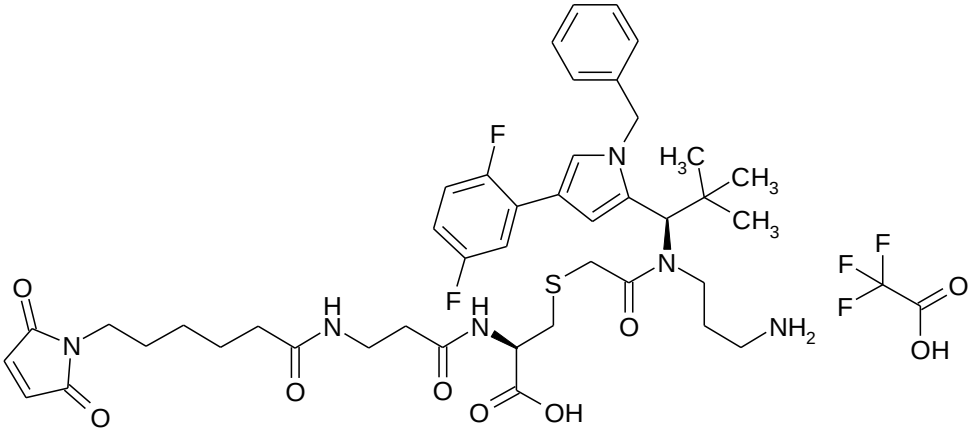
30.0 mg (0.036 mmol) of R-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine / trifluoroacetic acid (1:1) (Intermediate C71) and 9.8 mg (0.04 mmol) of N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-beta-alanine (Intermediate L78) were dissolved in 1.0 ml of DMF, and 16.4 mg (0.04 mmol) of HATU and 14.0 mg (0.11 mmol) of N,N-diisopropylethylamine were added. The reaction mixture was stirred at RT for 2 hours. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This

gave 4.2 mg (13%) of the compound N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-beta-alanyl-S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine.
LC-MS (Method 6): R_t = 1.31 min; MS (ESIpos): m/z = 925 $(M+H)^+$.

11.3 mg (0.011 mmol) of N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-beta-alanyl-S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine were dissolved in 2.0 ml of trifluoroethanol, and 5.0 mg (0.04 mmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 2 hours. 10.7 mg (0.04 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 4.4 mg (40%) of the title compound.
LC-MS (Method 1): R_t = 0.91 min; MS (ESIpos): m/z = 781 $(M+H)^+$.

Intermediate F264

N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl-S-{2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-L-cysteine / trifluoroacetic acid (1:1)



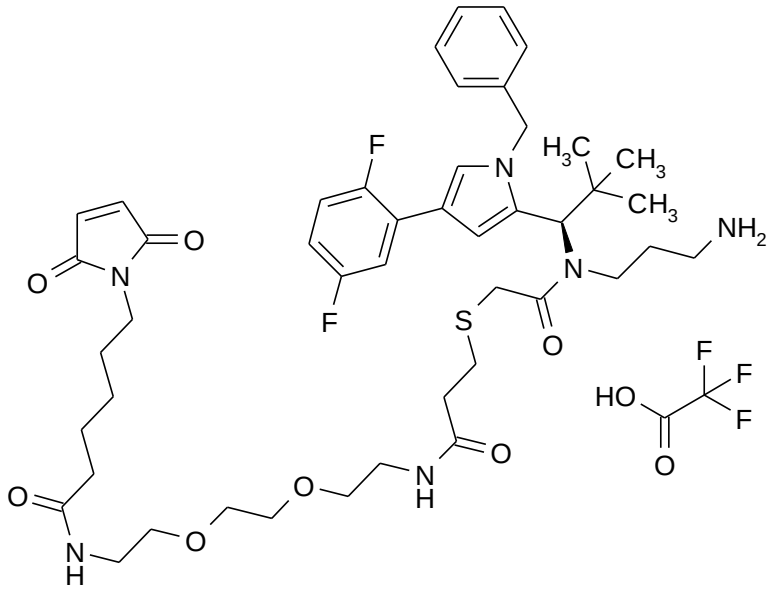
30.0 mg (0.036 mmol) of R-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine / trifluoroacetic acid (1:1) (Intermediate C71) and 12.2 mg (0.04 mmol) of N-[6-

(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanine (Intermediate L79) were dissolved in 1.0 ml of DMF, and 16.4 mg (0.04 mmol) of HATU and 14.0 mg (0.11 mmol) of N,N-diisopropylethylamine were added. The reaction mixture was stirred at RT for 2 hours. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 8.9 mg (24%) of the compound N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl-S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine.
LC-MS (Method 6): R_t = 1.38 min; MS (ESIpos): m/z = 981 $(M+H)^+$.

15.3 mg (0.015 mmol) of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl-S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine were dissolved in 2.0 ml of trifluoroethanol, and 6.3 mg (0.045 mmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 2 hours. 13.5 mg (0.045 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 9.1 mg (62%) of the title compound.
LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 837 $(M+H)^+$.

Intermediate F265

Trifluoroacetic acid / N-(3-aminopropyl)-N-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-22-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-6,17-dioxo-10,13-dioxa-3-thia-7,16-diazadocosane-1-amide (1:1)



30.0 mg (42.7 µmol) of 11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-oic acid (Intermediate C69) and 25.3 mg (55.6 µmol) of trifluoroacetic acid / N-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide (1:1) (Intermediate L82) were initially charged in 1.9 ml of acetonitrile, and 60 µl (340 µmol) of N,N-diisopropylethylamine and 33 µl (56 µmol) of 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphinane 2,4,6-trioxide 50% in ethyl acetate were added. The reaction mixture was stirred at RT overnight. Water (2.0 ml) was added and purification was effected directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 26.7 mg (60% of theory) of the compound 2-(trimethylsilyl)ethyl [4-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-26-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,10,21-trioxo-14,17-dioxa-7-thia-4,11,20-triazahexacos-1-yl]carbamate.

LC-MS (Method 1): R_t = 1.40 min; MS (ESIpos): m/z = 1025 $(M+H)^+$.

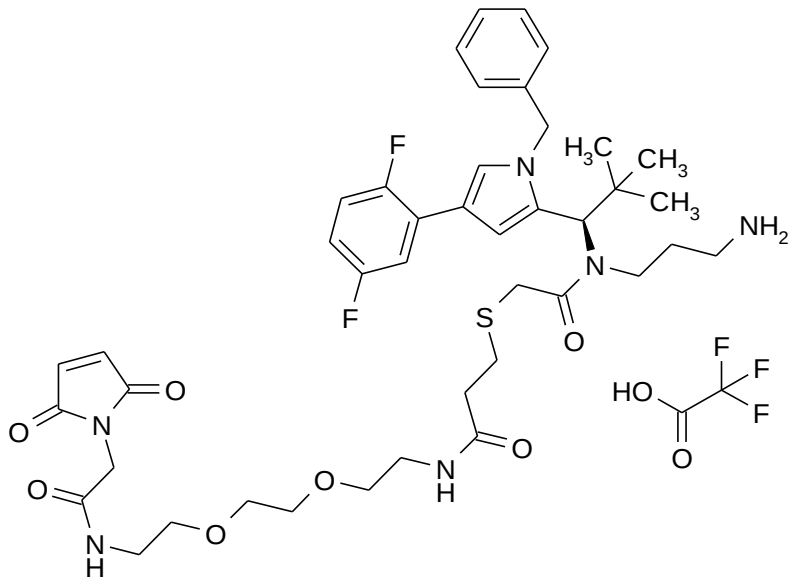
25.3 mg (24.7 µmol) of 2-(trimethylsilyl)ethyl [4-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-26-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,10,21-trioxo-14,17-dioxa-7-thia-4,11,20-triazahexacos-1-yl]carbamate were dissolved in 2.0 ml of trifluoroethanol, and 20.2 mg (148 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 1 h. 43.3 mg (148 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1%

TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 23.4 mg (95% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.89 min; MS (ESIpos): m/z = 881 $(M+H)^+$.

Intermediate F266

Trifluoroacetic acid / N-(3-aminopropyl)-N-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,13-dioxo-6,9-dioxa-16-thia-3,12-diazaoctadecan-18-amide (1:1)



30.0 mg (0.043 mmol) of 11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-oic acid (Intermediate C69) were initially charged together with 22.2 mg (0.056 mmol) of trifluoroacetic acid / N-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (1:1) (Intermediate L83) in 1.9 ml of acetonitrile. 60 µl (0.34 mmol) of N,N-diisopropylethylamine were then added, and 33 µl (0.056 mmol) of T3P (50% in ethyl acetate) were added dropwise. The reaction mixture was stirred at RT overnight. Water (2.0 ml) was added. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 20.5 mg (49% of theory) of the compound 2-(trimethylsilyl)ethyl [19-

{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,13,18-trioxo-6,9-dioxa-16-thia-3,12,19-triazadocosan-22-yl]carbamate.

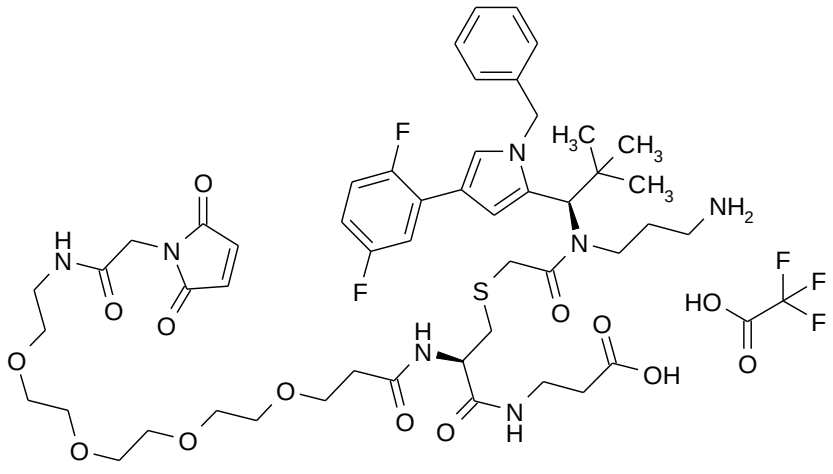
LC-MS (Method 1): R_t = 1.38 min; MS (ESIpos): m/z = 969 $(M+H)^+$.

19.1 mg (19.7 µmol) of 2-(trimethylsilyl)ethyl [19-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,13,18-trioxo-6,9-dioxa-16-thia-3,12,19-triazadocosan-22-yl]carbamate were dissolved in 2.0 ml of trifluoroethanol, and 16.1 mg (118 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 1 h. 34.6 mg (118 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 13.9 mg (75% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 825 $(M+H)^+$.

Intermediate F267

S-{2-[(3-Aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-N-[1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,18-dioxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-yl]-L-cysteinyl-beta-alanine / trifluoroacetic acid (1:1)



Under argon, 13.4 mg (33.3 µmol) of 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-oic acid (Intermediate L74) were initially charged in 1.0 ml of DMF, and 9.3 µl (54.4 µmol) of N,N-diisopropylethylamine and 12.6 mg (33.3 µmol) of HATU were added. The reaction mixture was stirred at RT for 10 min. 25.0 mg (27.7 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteinyl-beta-alanine / trifluoroacetic acid (1:1) (see synthesis of Intermediate F216) dissolved in 4.7 µl (27.7 µmol) of N,N-diisopropylethylamine and 1.0 ml of DMF were then added. The reaction mixture was stirred at RT for 90 minutes. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 6.90 mg (19% of theory) of the compound S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,18-dioxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-yl]-L-cysteinyl-beta-alanine.

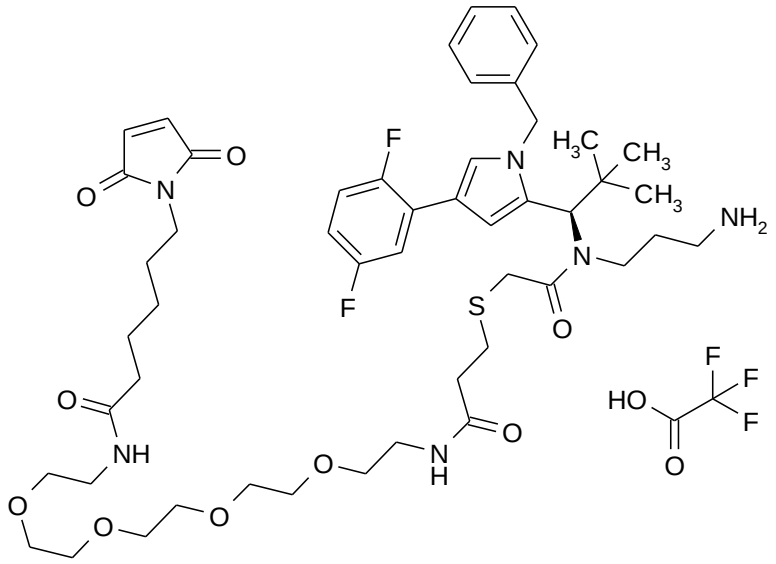
LC-MS (Method 5): R_t = 4.44 min; MS (ESIpos): m/z = 1172 $(M+H)^+$.

6.70 mg (5.71 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,18-dioxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-yl]-L-cysteinyl-beta-alanine were dissolved in 1.0 ml of trifluoroethanol, and 4.67 mg (34.3 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 1 h. 10 mg (34.3 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 4.4 mg (67% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 1028 $(M+H)^+$.

Intermediate F268

Trifluoroacetic acid / N-(3-aminopropyl)-N-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-28-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-6,23-dioxo-10,13,16,19-tetraoxa-3-thia-7,22-diazaoctacosane-1-amide (1:1)



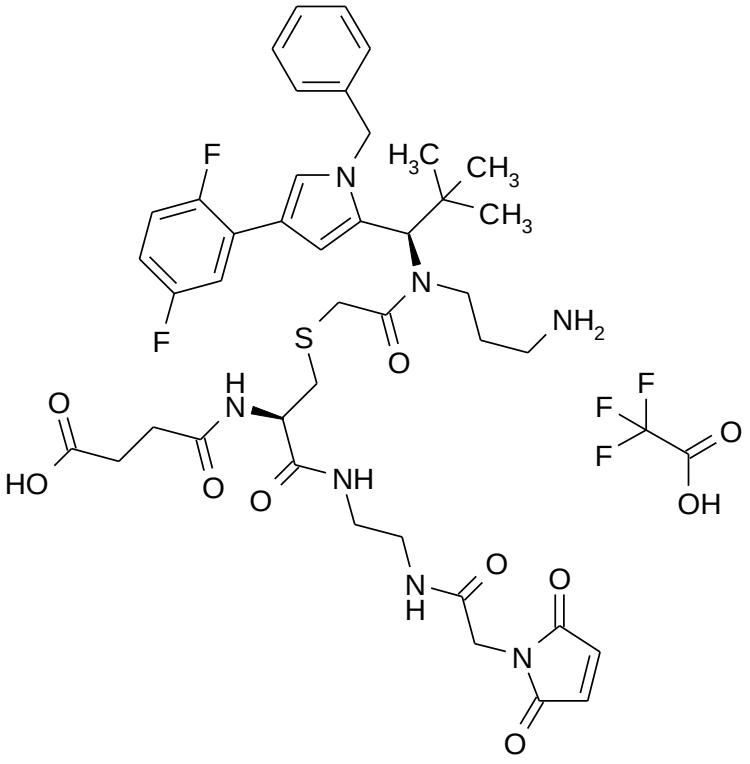
30.0 mg (0.043 mmol) of 11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-oic acid (Intermediate C69) were initially charged together with 30.2 mg (0.056 mmol) of trifluoroacetic acid / N-(14-amino-3,6,9,12-tetraoxatetradec-1-yl)-6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamide (1:1) (Intermediate L84) in 2.0 ml of acetonitrile. 60 µl (0.34 mmol) of N,N-diisopropylethylamine were then added, and 33 µl (0.056 mmol) of T3P (50% in ethyl acetate) were added dropwise. The reaction mixture was stirred at RT overnight. Water (2.0 ml) was added. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 27.9 mg (59% of theory) of the compound 2-(trimethylsilyl)ethyl [4-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-32-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,10,27-trioxo-14,17,20,23-tetraoxa-7-thia-4,11,26-triazadotriacont-1-yl]carbamate.

LC-MS (Method 1): R_t = 1.41 min; MS (ESIpos): m/z = 1114 $(M+H)^+$.

25.6 mg (23.0 µmol) of 2-(trimethylsilyl)ethyl [4-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-32-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,10,27-trioxotrioxo-14,17,20,23-tetraoxa-7-thia-4,11,26-triazadotriacont-1-yl]carbamate were dissolved in 2.5 ml of trifluoroethanol, and 18.8 mg (138 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 1 h. 40.3 mg (138 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 22.2 mg (88% of theory) of the title compound.
LC-MS (Method 1): R_t = 0.94 min; MS (ESIpos): m/z = 969 $(M+H)^+$.

Intermediate F269

4-{[(8R,14R)-13-(3-Aminopropyl)-14-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-15,15-dimethyl-2,7,12-trioxo-10-thia-3,6,13-triazahexadecan-8-yl]amino}-4-oxobutanoic acid / trifluoroacetic acid (1:1)



17.0 mg (0.0195 mmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-(4-tert-butoxy-4-oxobutanoyl)-L-cysteine (Intermediate C77) were initially charged together with 4.99 mg (0.0253 mmol) of N-(2-aminoethyl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (Intermediate L1) in 1.0 ml of acetonitrile. 27 µl (0.16 mmol) of N,N-diisopropylethylamine were then added, and 15 µl (0.025 mmol) of T3P (50% in ethyl acetate) were added dropwise. The reaction mixture was stirred at RT overnight. Water (2.0 ml) was added. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 9.5 mg (46% of theory) of the compound tert-butyl 4-{[(16R)-11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-23-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,2-dimethyl-6,12,17,22-tetraoxo-5-oxa-14-thia-7,11,18,21-tetraaza-2-silatricosan-16-yl]amino}-4-oxobutanoate.

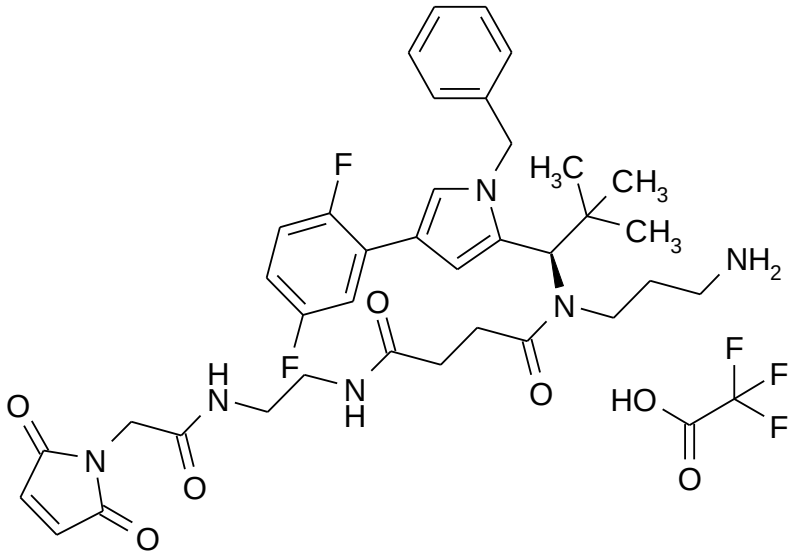
LC-MS (Method 1): R_t = 1.47 min; MS (ESIpos): m/z = 1052 $(M+H)^+$.

8.3 mg (7.89 µmol) of tert-butyl 4-{[(16R)-11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-23-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,2-dimethyl-6,12,17,22-tetraoxo-5-oxa-14-thia-7,11,18,21-tetraaza-2-silatricosan-16-yl]amino}-4-oxobutanoate were dissolved in 1.0 ml of trifluoroethanol, and 6.45 mg (47.3 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 6 h. 6.45 mg (47.3 µmol) of zinc dichloride were added and the reaction mixture was stirred at 50°C overnight. 27.7 mg (94.6 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added and the reaction mixture was stirred for 10 min, and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 1.10 mg (14% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.89 min; MS (ESIpos): m/z = 852 $(M+H)^+$.

Intermediate F270

Trifluoroacetic acid / N-(3-aminopropyl)-N-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-N'-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)succinamide (1:1)



Under argon, 15.0 mg (22.9 µmol) of 11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silapentadecan-15-oic acid (Intermediate C78) were initially charged in 1.0 ml of DMF, and 8.0 µl (45.8 µmol) of N,N-diisopropylethylamine and 10.4 mg (27.4 µmol) of HATU were added. The reaction mixture was stirred at RT for 10 min. 8.54 mg (27.4 µmol) of trifluoroacetic acid / N-(2-aminoethyl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (1:1) (Intermediate L1) dissolved in 4.0 µl (22.9 µmol) of N,N-diisopropylethylamine and 1.0 ml of DMF were then added. The reaction mixture was stirred at RT for 1 h. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 14.7 mg (77% of theory) of the compound 2-(trimethylsilyl)ethyl [3-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{4-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-4-oxobutanoyl}amino)propyl]carbamate.
LC-MS (Method 5): R_t = 1.33 min; MS (ESIpos): m/z = 835 $(M+H)^+$.

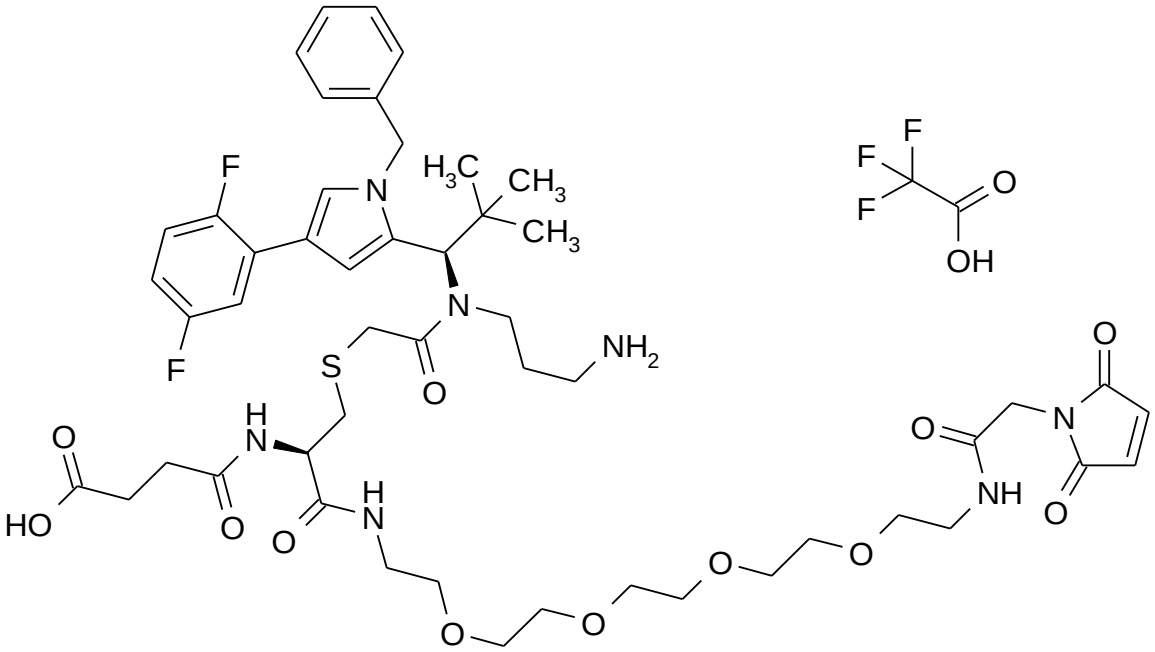
13.2 mg (15.8 µmol) of 2-(trimethylsilyl)ethyl [3-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{4-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-4-oxobutanoyl}amino)propyl]carbamate were dissolved in 2.0 ml of trifluoroethanol, and 12.9 mg (94.8 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 1 h. 27.7 mg (94.6 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1%

TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 10.9 mg (83% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.83 min; MS (ESIpos): m/z = 691 $(M+H)^+$.

Intermediate F271

4-{[(20R,26R)-25-(3-Aminopropyl)-26-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-27,27-dimethyl-2,19,24-trioxo-6,9,12,15-tetraoxa-22-thia-3,18,25-triazaoctacosan-20-yl]amino}-4-oxobutanoic acid / trifluoroacetic acid (1:1)



Under argon, 19.4 mg (22.2 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-(4-tert-butoxy-4-oxobutanoyl)-L-cysteine (Intermediate C77) were initially charged in 2.0 ml of DMF, and 21.7 mg (44.4 µmol) of trifluoroacetic acid / N-(14-amino-3,6,9,12-tetraoxatetradec-1-yl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (1:1) (Intermediate L74), 12 µl (67 µmol) of N,N-diisopropylethylamine and 16.9 mg (44.4 µmol)

of HATU were added. The reaction mixture was stirred at RT for 1 h. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 18.1 mg (66% of theory) of the compound tert-butyl 4-{[(16R)-11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-35-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,2-dimethyl-6,12,17,34-tetraoxo-5,21,24,27,30-pentaoxa-14-thia-7,11,18,33-tetraaza-2-silapentatriacontan-16-yl]amino}-4-oxobutanoate.

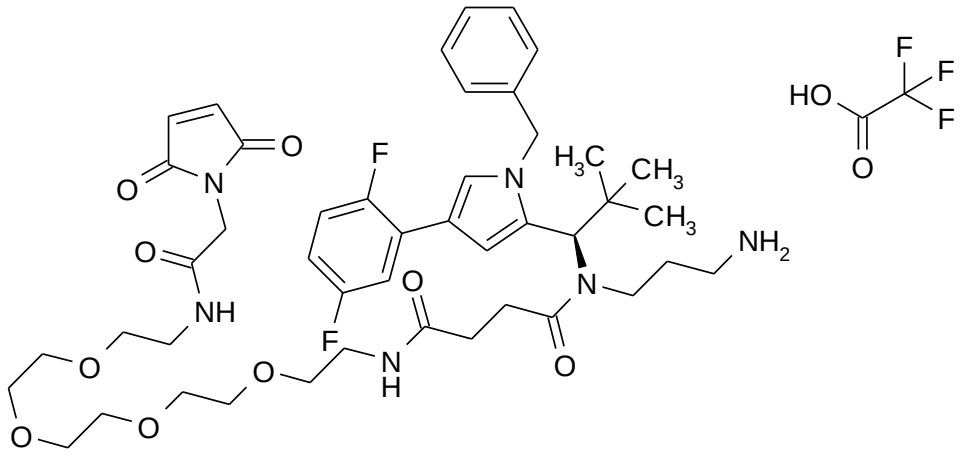
LC-MS (Method 4): R_t = 1.79 min; MS (ESIpos): m/z = 1250 $(M+Na)^+$.

18.1 mg (14.7 µmol) of tert-butyl 4-{[(16R)-11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-35-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,2-dimethyl-6,12,17,34-tetraoxo-5,21,24,27,30-pentaoxa-14-thia-7,11,18,33-tetraaza-2-silapentatriacontan-16-yl]amino}-4-oxobutanoate were dissolved in 2.0 ml of trifluoroethanol, and 12.0 mg (88.4 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 4 h. 25.8 mg (88.4 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 12.3 mg (73% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 1028 $(M+H)^+$.

Intermediate F272

Trifluoroacetic acid / N-(3-aminopropyl)-N-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-N'-[17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-16-oxo-3,6,9,12-tetraoxa-15-azaheptadec-1-yl]succinamide (1:1)



Under argon, 15.0 mg (22.9 µmol) of 11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silapentadecan-15-oic acid (Intermediate C78) were initially charged in 1.0 ml of DMF, and 8.0 µl (45.8 µmol) of N,N-diisopropylethylamine and 10.4 mg (27.4 µmol) of HATU were added. The reaction mixture was stirred at RT for 10 min. 13.4 mg (27.4 µmol) of trifluoroacetic acid / N-(14-amino-3,6,9,12-tetraoxatetradec-1-yl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (1:1) (Intermediate L85) dissolved in 4.0 µl (22.9 µmol) of N,N-diisopropylethylamine and 1.0 ml of DMF were then added. The reaction mixture was stirred at RT for 1 h. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 15.8 mg (68% of theory) of the compound 2-(trimethylsilyl)ethyl [23-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,19,22-trioxo-6,9,12,15-tetraoxa-3,18,23-triazahexacosan-26-yl]carbamate.

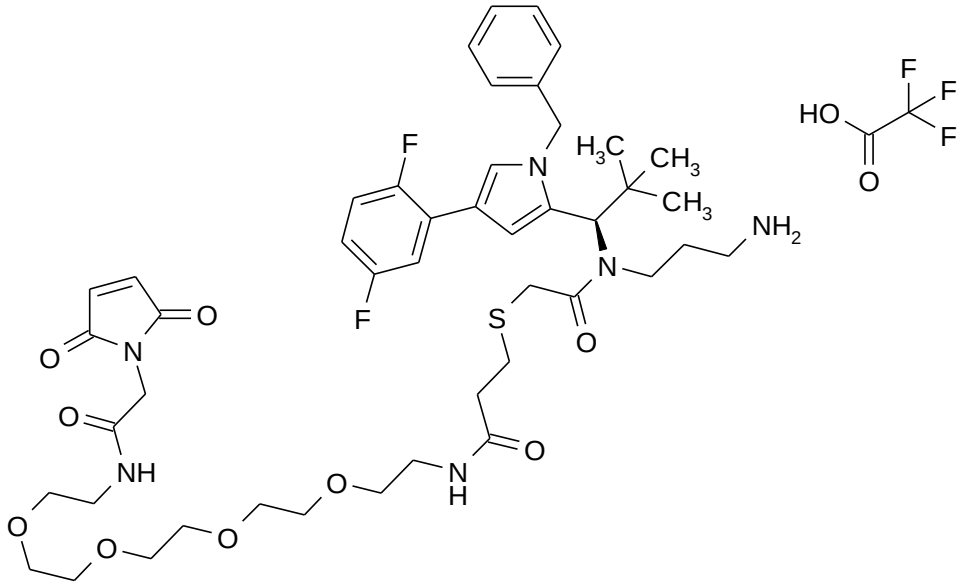
LC-MS (Method 1): R_t = 1.35 min; MS (ESIpos): m/z = 1011 $(M+H)^+$.

15.1 mg (14.9 µmol) of 2-(trimethylsilyl)ethyl [23-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,19,22-trioxotrioxo-6,9,12,15-tetraoxa-3,18,23-triazahexacosan-26-yl]carbamate were dissolved in 2.0 ml of trifluoroethanol, and 12.2 mg (89.6 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 1 h. 26.2 mg (89.6 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-

HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 10.3 mg (70% of theory) of the title compound.
LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 867 $(M+H)^+$.

Intermediate F273

Trifluoroacetic acid / N-(3-aminopropyl)-N-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,19-dioxo-6,9,12,15-tetraoxa-22-thia-3,18-diazatetracosane-24-amide (1:1)



Under argon, 20.0 mg (28.5 µmol) of 11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silapentadecan-17-oic acid (Intermediate C69) were initially charged in 1.0 ml of DMF, and 10.0 µl (57.0 µmol) of N,N-diisopropylethylamine and 13.0 mg (34.2 µmol) of HATU were added. The reaction mixture was stirred at RT for 10 min. 16.7 mg (34.2 µmol) of trifluoroacetic acid / N-(14-amino-3,6,9,12-tetraoxatetradec-1-yl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide (1:1) (Intermediate L85) dissolved in 5.0 µl (28.5 µmol) of N,N-diisopropylethylamine and 1.0 ml of DMF were then added. The reaction mixture was stirred at RT for 1 h. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high

vacuum. This gave 18.6 mg (62% of theory) of the compound 2-(trimethylsilyl)ethyl [25-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,19,24-trioxo-6,9,12,15-tetraoxa-22-thia-3,18,25-triazaoctacosan-28-yl]carbamate.

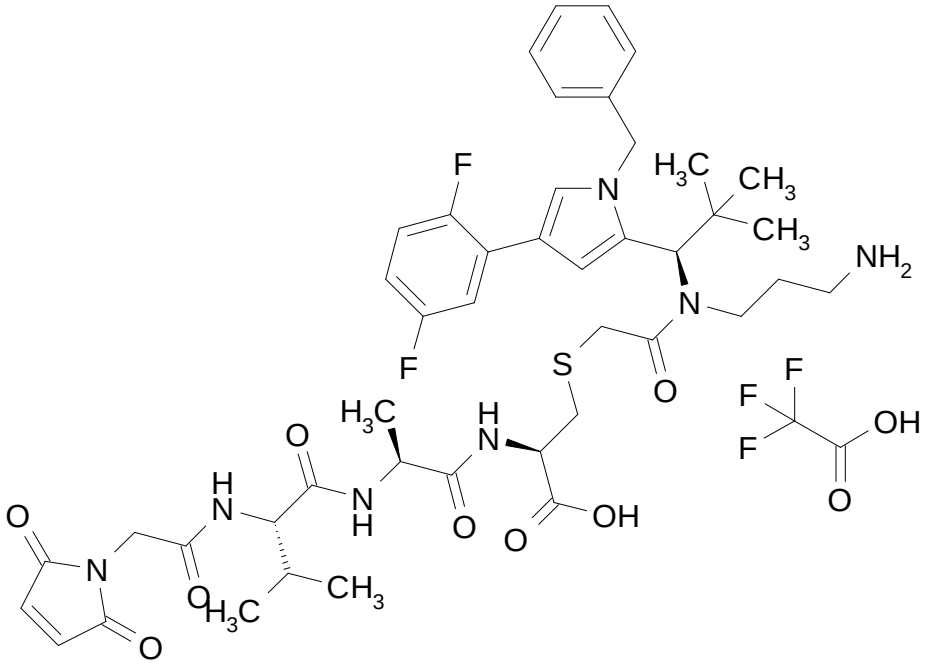
LC-MS (Method 1): R_t = 1.37 min; MS (ESIpos): m/z = 1057 $(M+H)^+$.

17.1 mg (16.2 µmol) of 2-(trimethylsilyl)ethyl [25-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,19,24-trioxo-6,9,12,15-tetraoxa-22-thia-3,18,25-triazaoctacosan-28-yl]carbamate were dissolved in 2.0 ml of trifluoroethanol, and 13.2 mg (97.0 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 1 h. 28.4 mg (97.0 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 9.80 mg (59% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 913 $(M+H)^+$.

Intermediate F274

N-[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-L-alanyl-S-{2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-L-cysteine / trifluoroacetic acid (1:1)



13.9 mg (0.0167 mmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine / trifluoroacetic acid (1:1) (Intermediate C71) were initially charged together with 7.07 mg (0.0217 mmol) of N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-L-alanine (Intermediate L86) in 2.0 ml of acetonitrile. 23 µl (0.13 mmol) of N,N-diisopropylethylamine were then added, and 13 µl (0.022 mmol) of T3P (50% in ethyl acetate) were added dropwise. The reaction mixture was stirred at RT overnight. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 3.70 mg (19% of theory) of the compound N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-L-alanyl-S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine.
LC-MS (Method 1): R_t = 1.34 min; MS (ESIpos): m/z = 1024 $(M+H)^+$.

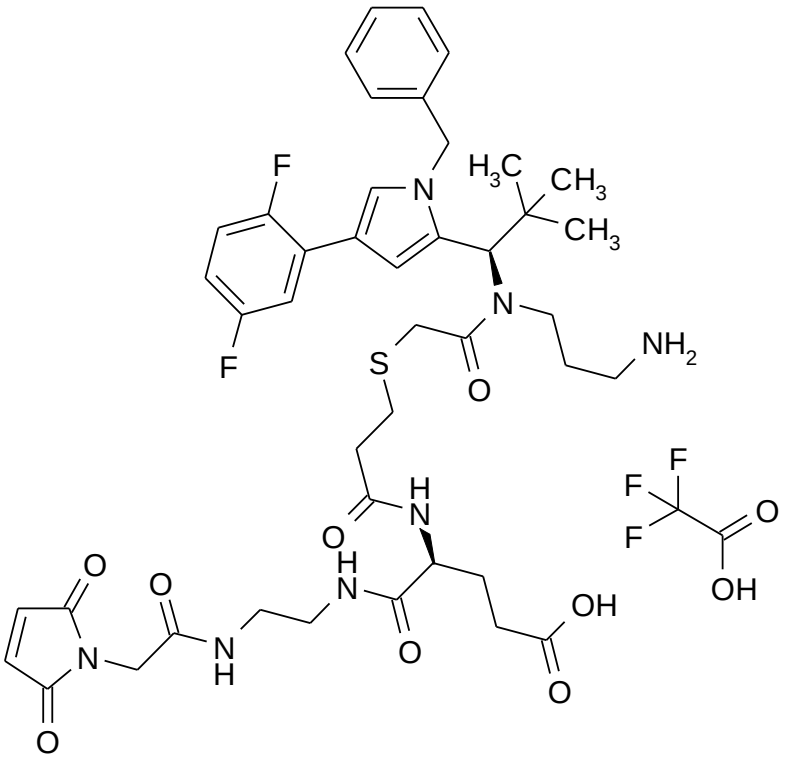
10.6 mg (10.3 µmol) of N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-L-alanyl-S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine were dissolved in 2.0 ml of trifluoroethanol, and 8.46 mg (62.1 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 1 h. 18.1 mg (62.1 µmol) of ethylenediamine-

N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 5.60 mg (54% of theory) of the title compound.

LC-MS (Method 12): R_t = 1.69 min; MS (ESIpos): m/z = 880 $(M+H)^+$.

Intermediate F275

N-[3-({2-[(3-Aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)propanoyl]-N-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)-L-alpha-glutamine / trifluoroacetic acid (1:1)



39.0 mg (55.6 µmol) of 11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-oic acid (Intermediate C69) were initially charged in 4.0 ml of DMF, 41.6 mg (111 µmol) of 1-benzyl-5-[2-(trimethylsilyl)ethyl]-L-glutamate hydrochloride (1:1) (Intermediate L89), 29 µl (170 µmol) of N,N-diisopropylethylamine and 42.3 mg (111 µmol) of HATU were added and the mixture was stirred at RT for 1 hour. The reaction mixture was stirred at RT for 1

hour, quenched with acetic acid and purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 53.1 mg (93% of theory) of the compound 1-benzyl-5-[2-(trimethylsilyl)ethyl]-N-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-L-glutamate.
LC-MS (Method 1): R_t = 1.71 min; MS (ESIpos): m/z = 1021 $[M+H]^+$

Under argon, 7.60 mg (33.9 µmol) of palladium(II) acetate were initially charged in 3.0 ml of dichloromethane, and 14 µl (100 µmol) of triethylamine and 110 µl (680 µmol) of triethylsilane were added. The reaction mixture was stirred at RT for 5 min, and 69.2 mg (67.7 µmol) of 1-benzyl-5-[2-(trimethylsilyl)ethyl]-N-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-L-glutamate dissolved in 3.0 ml of dichloromethane were added. The reaction mixture was stirred at RT overnight. The reaction mixture was filtered through a cardboard filter and the filter cake was washed with dichloromethane. The solvent was evaporated under reduced pressure. The residue was purified by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 38.4 mg (61% of theory) of the compound (19S)-11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-19-{3-oxo-3-[2-(trimethylsilyl)ethoxy]propyl}-5-oxa-14-thia-7,11,18-triaza-2-silaicosan-20-oic acid.
LC-MS (Method 1): R_t = 1.53 min; MS (ESIpos): m/z = 931 $(M+H)^+$.

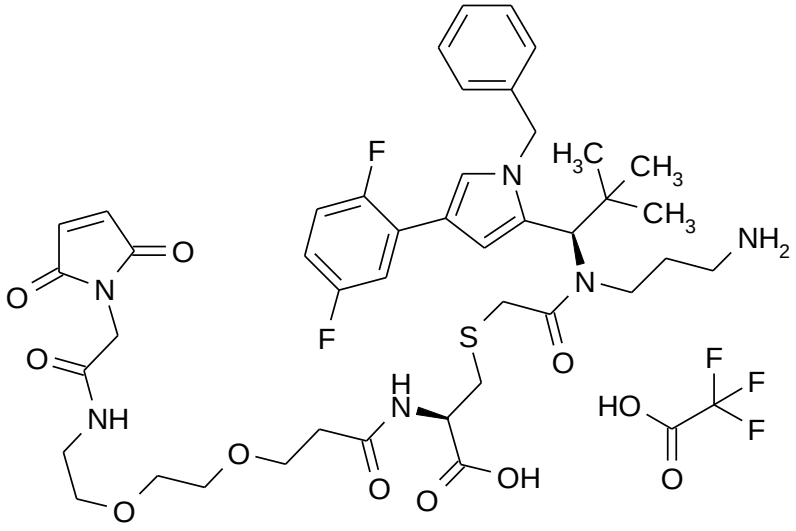
10.0 mg (10.7 µmol) of (19S)-11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-19-{3-oxo-3-[2-(trimethylsilyl)ethoxy]propyl}-5-oxa-14-thia-7,11,18-triaza-2-silaicosan-20-oic acid were initially charged in 1.0 ml of DMF, 6.73 mg (21.5 µmol) of N-(2-aminoethyl)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide / 2,2,2-trifluoroethane-1,1-diol (1:1) (Intermediate L1), 5.6 µl (32 µmol) of N,N-diisopropylethylamine and 8.17 mg (21.5 µmol) of HATU were added and the mixture was stirred at RT for 1 hour. The reaction mixture was stirred at RT for 3 hour, quenched with acetic acid and purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high

vacuum. This gave 6.90 mg (58% of theory) of the compound 2-(trimethylsilyl)ethyl N2-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-N-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)-L-alpha-glutaminate.
LC-MS (Method 1): R_t = 1.57 min; MS (ESIpos): m/z = 1110 $[M+H]^+$

6.90 mg (6.21 µmol) of 2-(trimethylsilyl)ethyl N^2-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-N-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)-L-alpha-glutaminate were dissolved in 2.0 ml of trifluoroethanol, and 5.1 mg (37.2 µmol) zinc dichloride were added. The reaction mixture was stirred at 50°C for 3 h. 5.1 mg (37.2 µmol) of zinc dichloride were added and the reaction mixture was stirred at 50°C for 3 h. 5.1 mg (37.2 µmol) of zinc dichloride were added and the reaction mixture was stirred at 50°C for 3 h. 10.1 mg (74.4 µmol) of zinc dichloride were added and the reaction mixture was stirred at 50°C overnight and at RT for 72 h. 54.5 mg (186 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 2.4 mg (39% of theory) of the title compound.
LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 866 $(M+H)^+$.

Intermediate F276

S-{2-[(3-Aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-N-{3-[2-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethoxy]propanoyl}-L-cysteine / trifluoroacetic acid (1:1)



Under argon, 9.08 mg (28.9 µmol) of 3-[2-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethoxy]propanoic acid (Intermediate L87) were initially charged in 1.0 ml of DMF, and 8.33 µl (48.2 µmol) of N,N-diisopropylethylamine and 11.0 mg (28.9 µmol) of HATU were added. The reaction mixture was stirred at RT for 10 min. 20.0 mg (27.7 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine / trifluoroacetic acid (1:1) (Intermediate C71) dissolved in 4.67 µl (24.1 µmol) of N,N-diisopropylethylamine and 1.0 ml of DMF were then added. The reaction mixture was stirred at RT for 1 h. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 4.70 mg (19% of theory) of the compound S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-{3-[2-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethoxy]propanoyl}-L-cysteine.

LC-MS (Method 12): R_t = 2.47 min; MS (ESIpos): m/z = 1013 $(M+H)^+$.

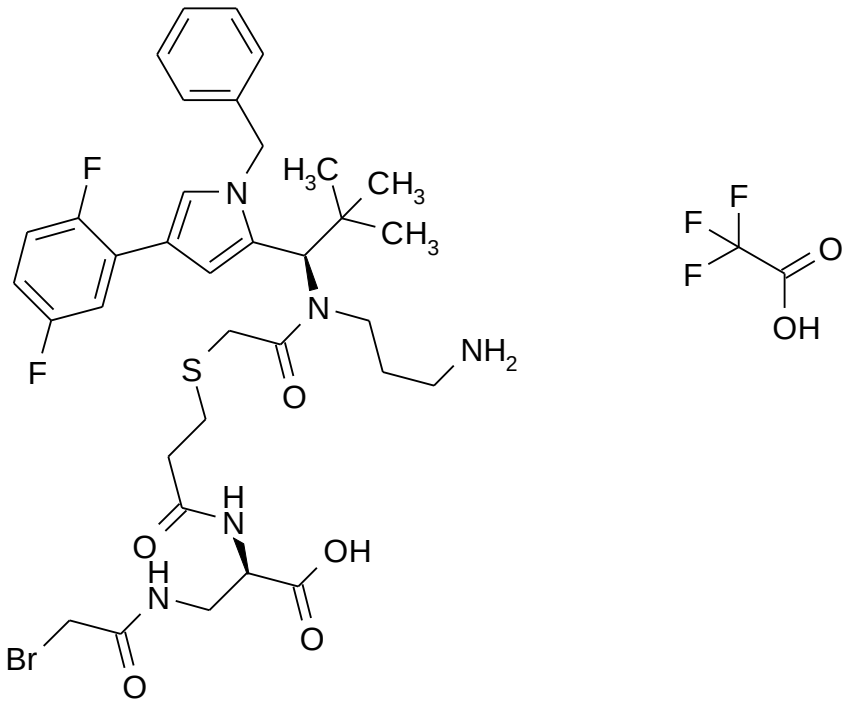
13.9 mg (13.7 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-{3-[2-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethoxy]propanoyl}-L-cysteine were dissolved in 2.0 ml of trifluoroethanol, and 5.6 mg (41.2 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 1 h. 5.6 mg (41.2 µmol) of zinc dichloride were added and the reaction mixture was stirred at 50°C for 30 minutes. 24.1

mg (82.4 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added and the reaction mixture was stirred for 10 min, and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 10.8 mg (80% of theory) of the title compound.

LC-MS (Method 12): R_t = 1.58 min; MS (ESIpos): m/z = 869 $(M+H)^+$.

Intermediate F277

N-[3-({2-[(3-Aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)propanoyl]-3-[(bromoacetyl)amino]-D-alanine / trifluoroacetic acid (11)



8.90 mg (8.88 µmol) of trifluoroacetic acid / -2-(trimethylsilyl)ethyl 3-amino-N-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-D-alaninate (1:1) (Intermediate C80) and 2.31 mg (9.77 µmol) of 1-(2-bromoacetoxy)pyrrolidine-2,5-dione were dissolved in 1 ml of dimethylformamide, and 2.9 µl (27 µmol) of N-methylmorpholine were added.

The reaction mixture was stirred at RT for 1 h. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 5.80 mg (65% of theory) of the compound 2-(trimethylsilyl)ethyl N-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-3-[(bromoacetyl)amino]-D-alaninate.

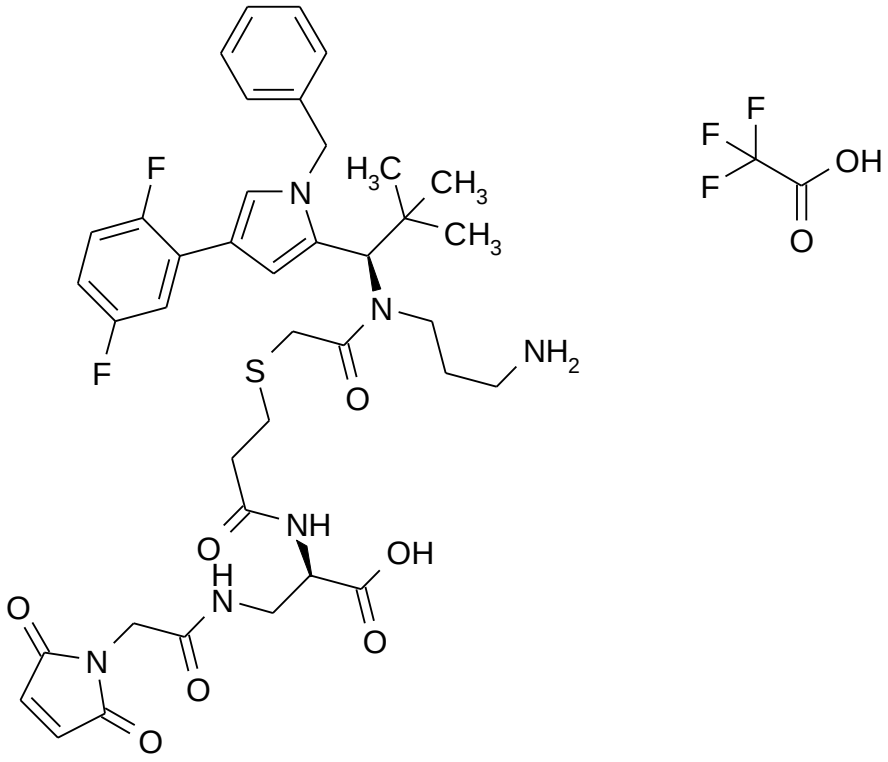
LC-MS (Method 1): R_t = 1.57 min; MS (ESIpos): m/z = 1008 $(M+H)^+$.

5.80 mg (5.75 µmol) of 2-(trimethylsilyl)ethyl N-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-3-[(bromoacetyl)amino]-D-alaninate were dissolved in 2.0 ml of trifluoroethanol, and 4.70 mg (34.5 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 3 h. 4.70 mg (34.5 µmol) of zinc dichloride were added and the reaction mixture was stirred at 50°C for 5 h. 20.2 mg (69.0 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added and the reaction mixture was stirred for 10 min, and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 1.70 mg (34% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.90 min; MS (ESIpos): m/z = 764 $(M+H)^+$.

Intermediate F278

N-[3-({2-[(3-Aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)propanoyl]-3-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}-D-alanine / trifluoroacetic acid (1:1)



10.0 mg (9.98 µmol) of trifluoroacetic acid / -2-(trimethylsilyl)ethyl 3-amino-N-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-D-alaninate (1:1) (Intermediate C80) and 2.77 mg (11.0 µmol) of 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione were dissolved in 1 ml of dimethylformamide, and 3.3 µl (30 µmol) of N-methylmorpholine were added. The reaction mixture was stirred at RT overnight. 2.0 µl (35 µmol) of acetic acid were added to the reaction mixture, which was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water/0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 5.50 mg (54% of theory) of the compound 2-(trimethylsilyl)ethyl N-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-3-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}-D-alaninate. LC-MS (Method 1): R_t = 1.51 min; MS (ESIpos): m/z = 1024 $(M+H)^+$.

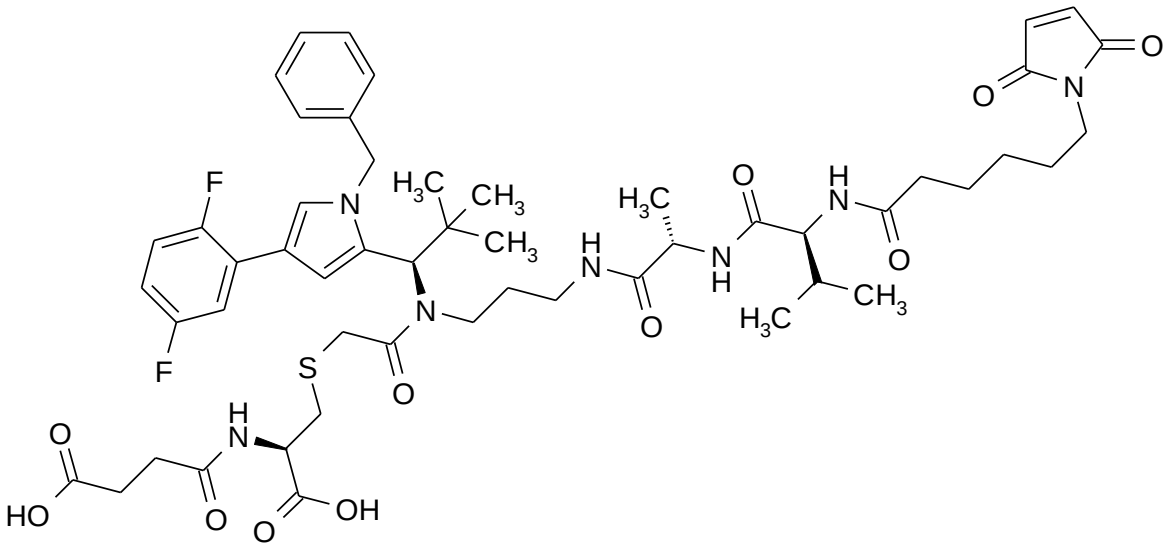
5.50 mg (5.36 µmol) of 2-(trimethylsilyl)ethyl N-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12,17-trioxo-5-oxa-14-thia-7,11-diaza-2-silaheptadecan-17-yl)-3-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}-D-alaninate were dissolved in 1.0 ml of trifluoroethanol, and 4.39 mg

(32.2 µmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 1 h. 4.39 mg (32.2 µmol) of zinc dichloride were added and the reaction mixture was stirred at 50°C for 1 h. 4.39 mg (32.2 µmol) of zinc dichloride were added and the reaction mixture was stirred at 50°C for 4 h. 28.2 mg (96.5 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added and the reaction mixture was stirred for 10 min, and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 2.70 mg (56% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.89 min; MS (ESIpos): m/z = 781 $(M+H)^+$.

Intermediate F279

N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-[3-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}[({(2R)-2-carboxy-2-[(3-carboxypropanoyl)amino]ethyl}sulphanyl)acetyl]amino)propyl]-L-alaninamide



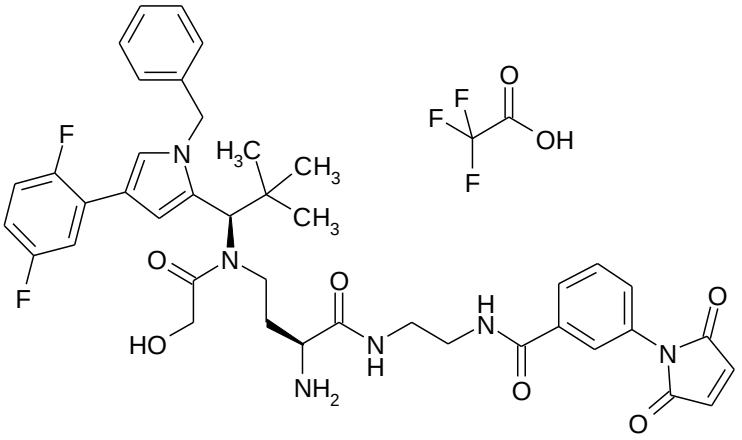
12.2 mg (14 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-(4-tert-butoxy-4-oxobutanoyl)-L-cysteine (Intermediate C77) were dissolved in 2.0 ml of trifluoroethanol, and 11.4 mg (83.8 µmol) of zinc dichloride were added. The reaction

mixture was stirred at 50°C for 3 h. 24.5 mg (83.8 µmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 4.60 mg (42% of theory) of the compound 4-{[(1R)-2-({2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)-1-carboxyethyl]amino}-4-oxobutanoic acid / trifluoroacetic acid (1:1).
LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 673 $(M+H)^+$.

10.0 mg (12.7 µmol) of 4-{[(1R)-2-({2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)-1-carboxyethyl]amino}-4-oxobutanoic acid / trifluoroacetic acid (1:1) and 7.41 mg (12.7 µmol) of 2,5-dioxopyrrolidin-1-yl N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-L-alaninate (Intermediate L88) were dissolved in 1.5 ml of dimethylformamide, and 4.4 µl (25 µmol) of N,N-diisopropylethylamine were added. The reaction mixture was stirred at RT for 2 h. 2.0 µl (35 µmol) of acetic acid were added to the reaction mixture, which was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water/0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 5.20 mg (39% of theory) of the title compound.
LC-MS (Method 1): R_t = 1.11 min; MS (ESIpos): m/z = 1036 $(M+H)^+$.

Intermediate F280

Trifluoroacetic acid / N-[2-({(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)benzamide (1:1)

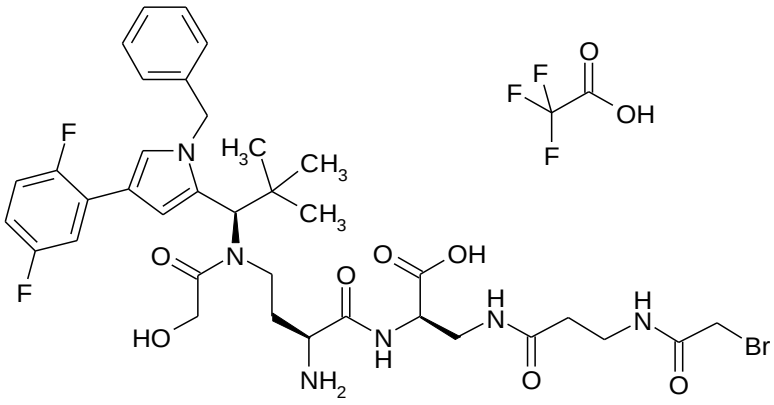


The title compound was prepared from Intermediate C64 by coupling to commercially available 1-(3-{[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}phenyl)-1H-pyrrole-2,5-dione and subsequent deprotection with zinc chloride.

LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 755 $(M+H)^+$.

Intermediate F281

N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-3-{[N-(bromoacetyl)-beta-alanyl]amino}-D-alanine / trifluoroacetic acid (1:1)



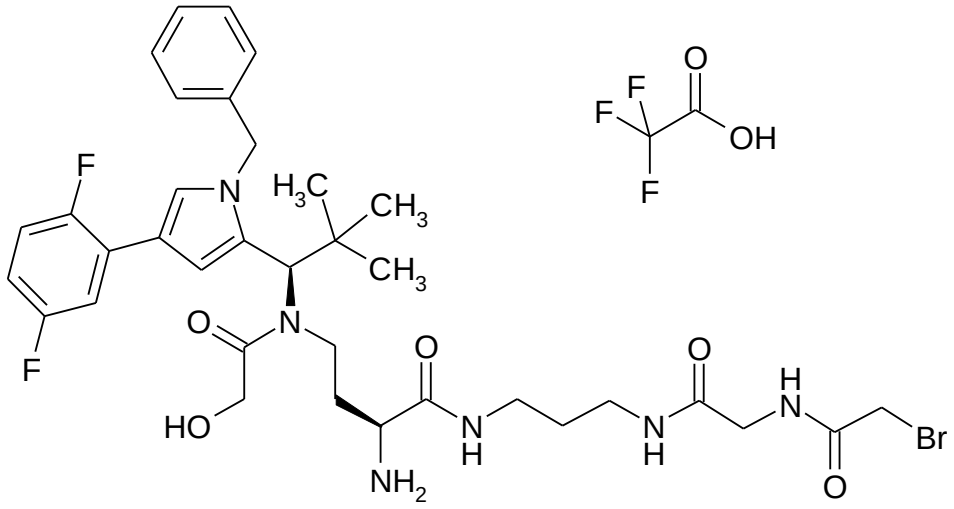
First of all, the modified amino acid units N-(bromoacetyl)-beta-alanine and 2-(trimethylsilyl)ethyl-3-amino-N-(tert-butoxycarbonyl)-D-alaninate were prepared by conventional methods of peptide chemistry. These were then coupled to one another in the presence of HATU and morpholine. The tert-butoxycarbonyl protecting group was then removed using 10% trifluoroacetic acid in dichloromethane, giving the 2-(trimethylsilyl)ethyl 3-{[N-(bromoacetyl)-beta-alanyl]amino}-D-alaninate intermediate.

Finally, the title compound was prepared by coupling this intermediate to intermediate C58 in the presence of HATU and 4-methylmorpholine, followed by deprotection with zinc chloride.

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 791 and 793 $(M+H)^+$.

Intermediate F282

Trifluoroacetic acid / (2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-N-(3-{[N-(bromoacetyl)glycyl]amino}propyl)-butanamide (1:1)

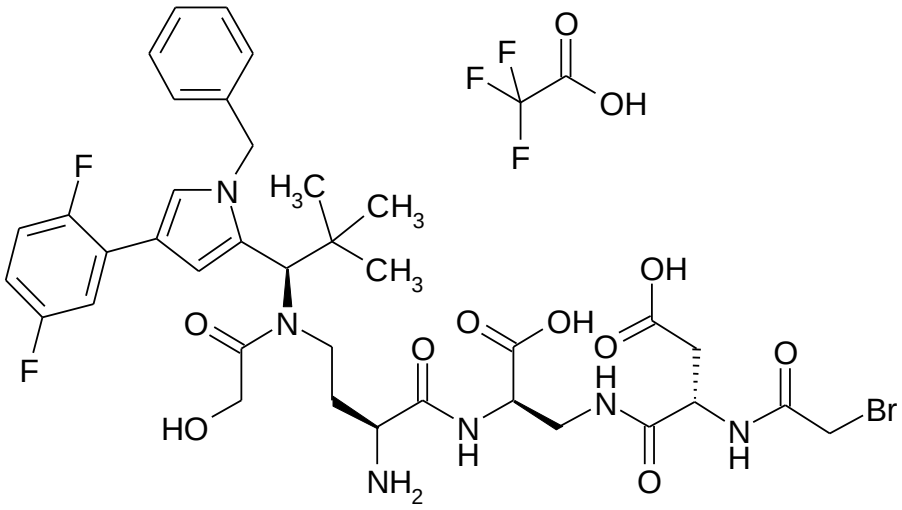


First of all, the intermediate trifluoroacetic acid / N-(3-aminopropyl)-N2-(bromoacetyl)glycinamide (1:1) was prepared from tert-butyl glycinate and bromoacetic anhydride by conventional methods of peptide chemistry.
Finally, the title compound was prepared by coupling this intermediate to intermediate C58 in the presence of HATU and 4-methylmorpholine, followed by deprotection with zinc chloride.

LC-MS (Method 1): R_t = 0.83 min; MS (ESIpos): m/z = 747 and 749 $(M+H)^+$.

Intermediate F283

N-[(2R)-2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)-2-carboxyethyl]-N^2-(bromoacetyl)-L-alpha-asparagine / trifluoroacetic acid (1:1)



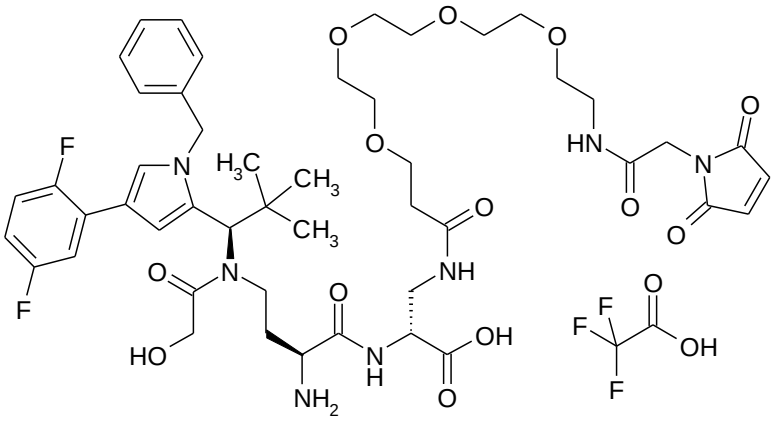
First of all, the modified amino acid unit (2S)-2-[(bromoacetyl)amino]-4-oxo-4-[2-(trimethylsilyl)ethoxy]butanoic acid was prepared proceeding from (2S)-2-amino-4-oxo-4-[2-(trimethylsilyl)ethoxy]butanoic acid and bromoacetic anhydride, and the amino acid unit 2-(trimethylsilyl)ethyl-3-amino-N-(tert-butoxycarbonyl)-D-alaninate proceeding from commercially available 3-{[(benzyloxy)carbonyl]amino}-N-(tert-butoxycarbonyl)-D-alanine / N-cyclohexylcyclohexanamine (1:1). The two units were coupled to one another in the presence of HATU and morpholine, and then the tert-butoxycarbonyl protecting group was removed using 5% trifluoroacetic acid in dichloromethane, giving the silylethyl ester protecting groups and thus the trifluoroacetic acid / 2-(trimethylsilyl)ethyl-N-{(2R)-2-amino-3-oxo-3-[2-(trimethylsilyl)ethoxy] propyl}-N2-(bromoacetyl)-L-alpha-asparaginate (1:1) intermediate was obtained.

Finally, the title compound was prepared by coupling this intermediate to intermediate C58 in the presence of HATU and 4-methylmorpholine, followed by deprotection with zinc chloride.

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 835 and 837 $(M+H)^+$.

Intermediate F284

N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-3-{[1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,18-dioxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-yl]amino}-D-alanine / trifluoroacetic acid (1:1)



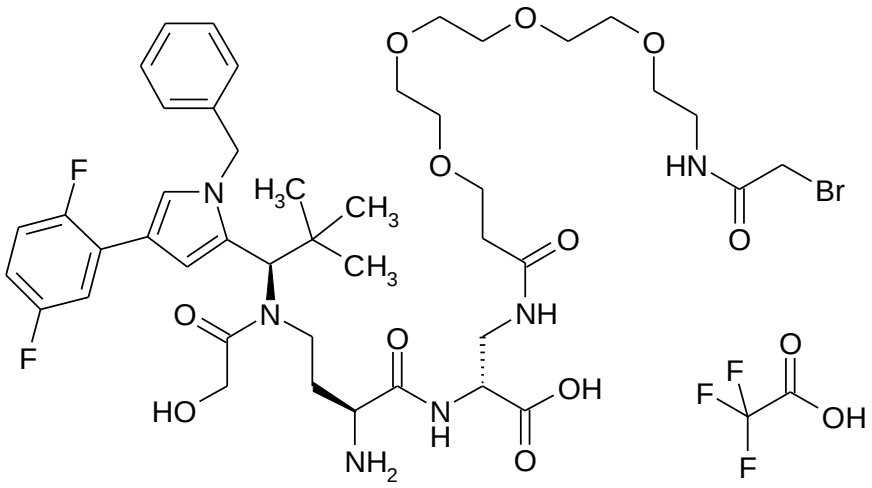
First of all, Intermediate L80 was coupled to commercially available (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid in the presence of HATU and N,N-diisopropylethylamine, and then the tert-butoxycarbonyl protective group was removed using 16% trifluoroacetic acid in dichloromethane, giving the silylethyl ester protective group.

Finally, the title compound was prepared by coupling this intermediate to Intermediate C58 in the presence of HATU and N,N-diisopropylethylamine, followed by deprotection with zinc chloride.

LC-MS (Method 12): R_t = 1.46 min; MS (ESIpos): m/z = 984.45 $(M+H)^+$.

Intermediate F285

N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-3-[(18-bromo-17-oxo-4,7,10,13-tetraoxa-16-azaoctadecan-1-oyl)amino]-D-alanine / trifluoroacetic acid (1:1)

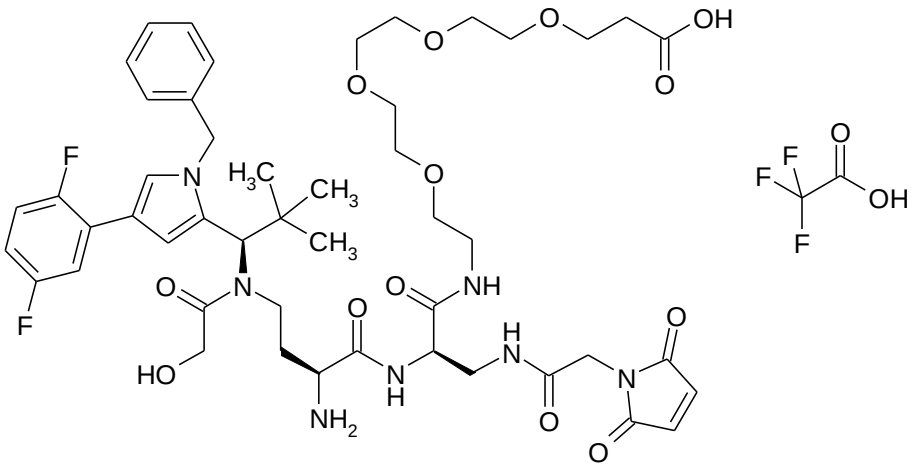


First of all, Intermediate L80 was acylated with commercially available bromoacetic anhydride, and the tert-butoxycarbonyl protective group was then removed using 20% trifluoroacetic acid in dichloromethane, giving the silylethyl ester protective group. Finally, the title compound was prepared by coupling this intermediate to Intermediate C58 in the presence of HATU and N,N-diisopropylethylamine, followed by deprotection with zinc chloride.

LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 967 and 969 $(M+H)^+$.

Intermediate F286

1-[(N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-3-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) acetyl]amino}-D-alanyl)amino]-3,6,9,12-tetraoxapentadecan-15-oic acid / trifluoroacetic acid (1:1)

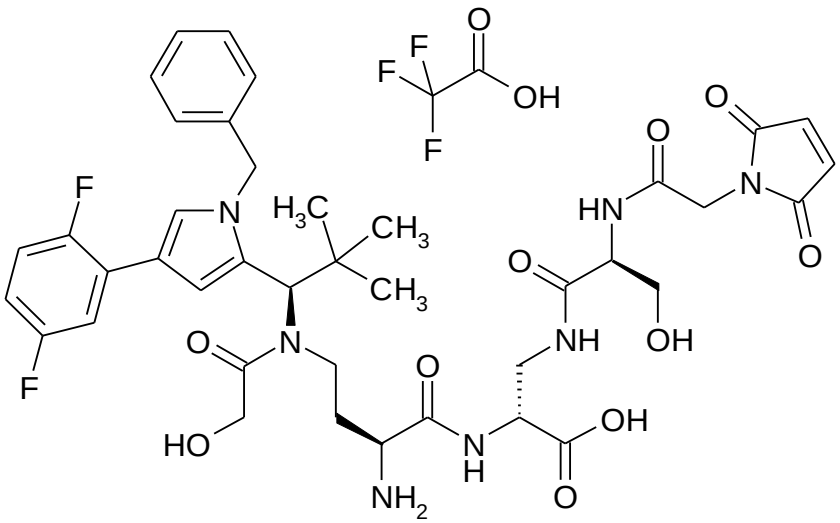


First of all, Intermediate L91 was coupled to (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid in the presence of HATU and N,N-diisopropylethylamine, and then the Boc protecting group was removed with 12.5% TFA in DCM. The resulting intermediate was coupled to intermediate C58 in the presence of HATU and N,N-diisopropylethylamine and then converted into the title compound by deprotection with zinc chloride.

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 984 $(M+H)^+$.

Intermediate F288

N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-3-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-seryl}amino)-D-alanine / trifluoroacetic acid (1:1)

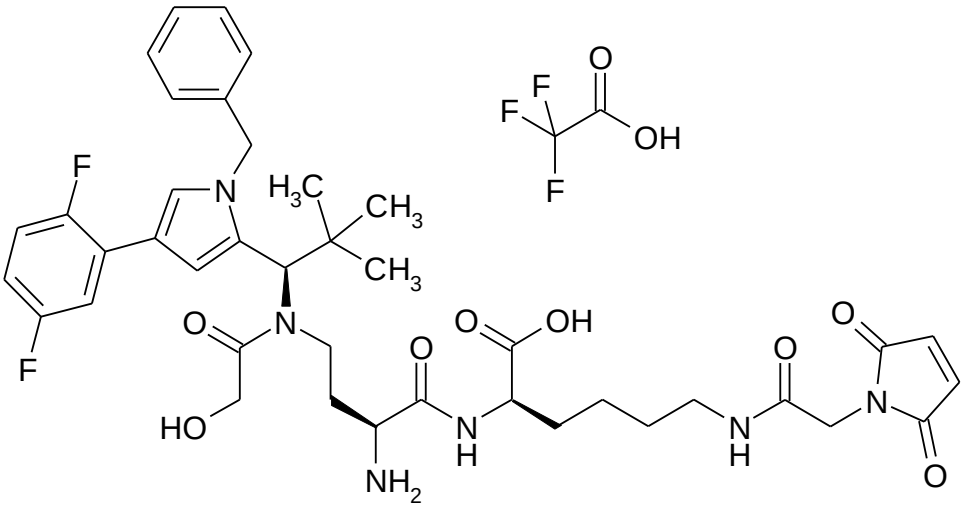


35 mg (39 µmol) of intermediate C74 were coupled in the presence of HATU and N,N-diisopropyethylamine with N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-serine which had been prepared beforehand proceeding from tert-butyl O-tert-butyl-L-serinate and (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid. Deprotection with zinc chloride and purification by HPLC gave 14 mg (38% of theory) of the title compound.

LC-MS (Method 12): R_t = 1.43 min; MS (ESIpos): m/z = 824.34 $(M+H)^+$.

Intermediate F289

N^2-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-N^6-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-D-lysine / trifluoroacetic acid (1:1)



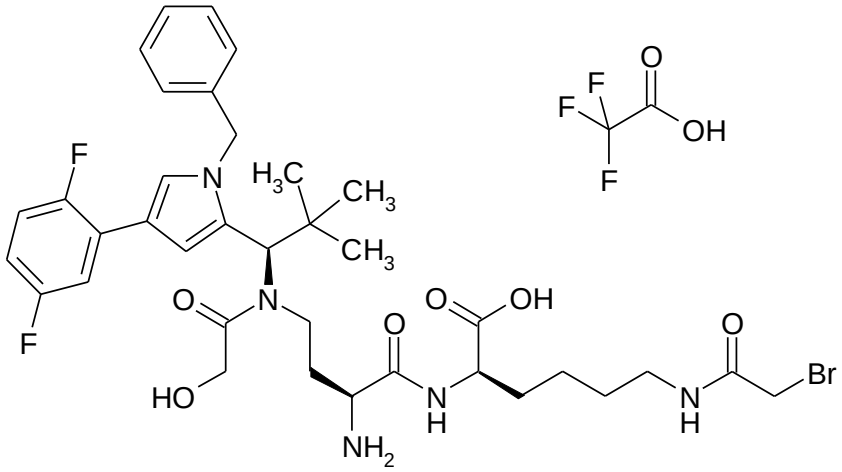
First of all, trifluoroacetic acid / 2-(trimethylsilyl)ethyl-N^6-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-D-lysinate (1:1) was prepared by conventional methods of peptide chemistry proceeding from N^6-[(benzyloxy)carbonyl]-N^2-(tert-butoxycarbonyl)-D-lysine.

12.5 mg (25 µmol) of this intermediate were then coupled in the presence of HATU and 4-methylmorpholine with 15 mg (23 µmol) of Intermediate C58. Deprotection with zinc chloride and purification by HPLC gave 14 mg (53% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.83 min; MS (ESIpos): m/z = 779 $(M+H)^+$.

Intermediate F290

N^2-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-N^6-(bromoacetyl)-D-lysine / trifluoroacetic acid (1:1)



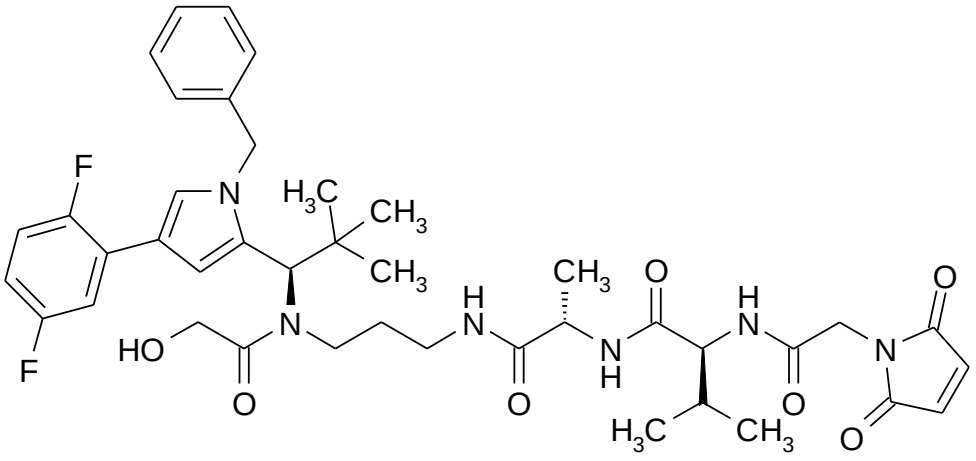
First of all, trifluoroacetic acid / 2-(trimethylsilyl)ethyl-N6-(bromoacetyl)-D-lysinate (1:1) was prepared by conventional methods of peptide chemistry proceeding from N^6-[(benzyloxy)carbonyl]-N^2-(tert-butoxycarbonyl)-D-lysine.

12 mg (25 µmol) of this intermediate were then coupled in the presence of HATU and 4-methylmorpholine with 15 mg (23 µmol) of Intermediate C58. Deprotection with zinc chloride and purification by HPLC gave 7 mg (36% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 762 and 764 $(M+H)^+$.

Intermediate F291

N-[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-N-{3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]propyl}-L-alaninamide

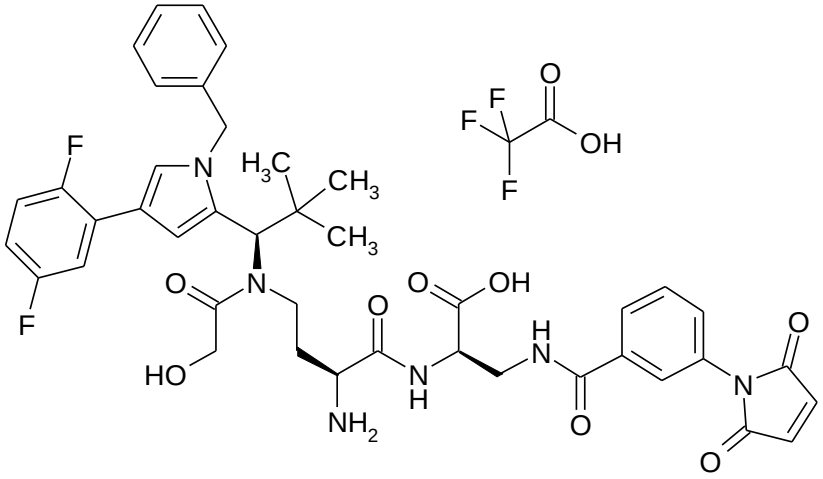


The title compound was prepared from Example M9 first by coupling to N-[(benzyloxy)carbonyl]-L-valyl-L-alanine in the presence of HATU and N,N-diisopropylethylamine. In the next step, the Z protecting group was removed by hydrogenating over 10% palladium on activated carbon at RT under hydrogen standard pressure for 1 hour and then converting the deprotected intermediate to the title compound by coupling to (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid in the presence of HATU and N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 1.21 min; MS (ESIpos): m/z = 777 $(M+H)^+$.

Intermediate F293

N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-3-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)benzoyl]amino}-D-alanine / trifluoroacetic acid (1:1)

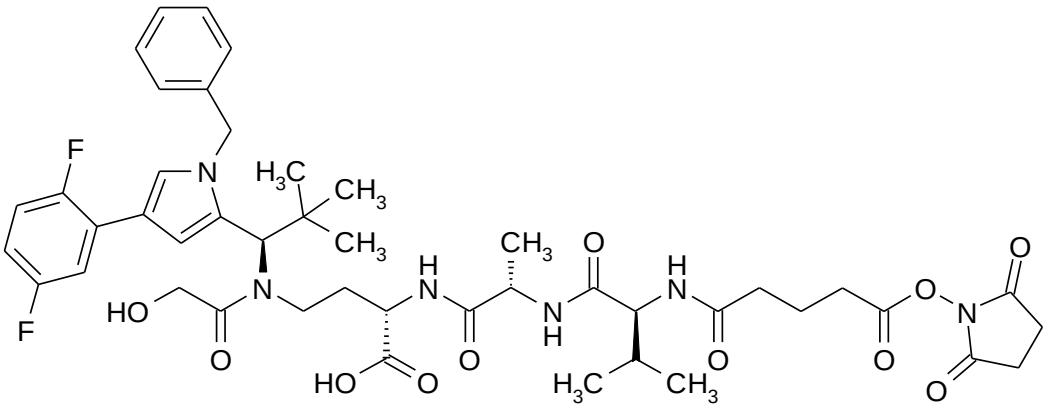


35 mg (39 µmol) of Intermediate C74 were dissolved in 4 ml of DMF and, in the presence of N,N-diisopropylethylamine, coupled to 13.5 mg (43 µmol) of commercially available 1-(3-{[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl}phenyl)-1H-pyrrole-2,5-dione. Deprotection with zinc chloride and purification by HPLC gave 12 mg (34% of theory) of the title compound.

LC-MS (Method 12): R_t = 0.93 min; MS (ESIpos): m/z = 799 $(M+H)^+$.

Intermediate F294

N-{5-[(2,5-Dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}-L-valyl-N-{(1S)-3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-carboxypropyl}-L-alaninamide



41 mg (0.05 mmol) of Intermediate C76 dissolved in 12 ml of methanol were hydrogenated over 10 mg of 10% palladium on activated carbon at RT for 1 h under

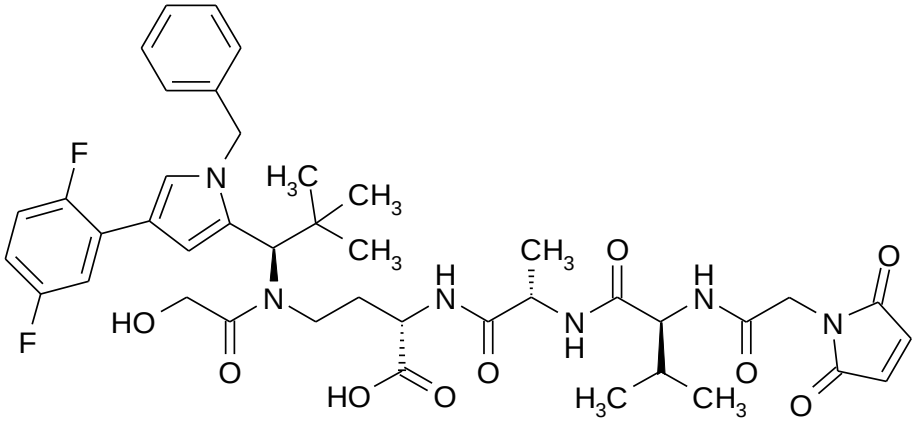
hydrogen standard pressure. The catalyst was then filtered off and the solvent was removed under reduced pressure. This gave 32 mg (92% of theory) of the deprotected intermediate.

15 mg (0.022 mmol) of this intermediate were dissolved in DMF, and 13 mg (0.039 mmol) of 1,1'-[(1,5-dioxopentan-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione and 7 µl of N,N-diisopropylethylamine were added. After stirring at RT for 1 h, the reaction mixture was concentrated and the residue was purified by HPLC. This gave 9 mg (45% of theory) of the title compound.

LC-MS (Method 1): R_t = 1.08 min; MS (ESIpos): m/z = 895 $(M+H)^+$.

Intermediate F295

N-[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-N-{(1S)-3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-carboxypropyl}-L-alaninamide



41 mg (0.05 mmol) of Intermediate C76 dissolved in 12 ml of methanol were hydrogenated over 10 mg of 10% palladium on activated carbon at RT for 1 h under hydrogen standard pressure. The catalyst was then filtered off and the solvent was removed under reduced pressure. This gave 32 mg (92% of theory) of the deprotected intermediate.

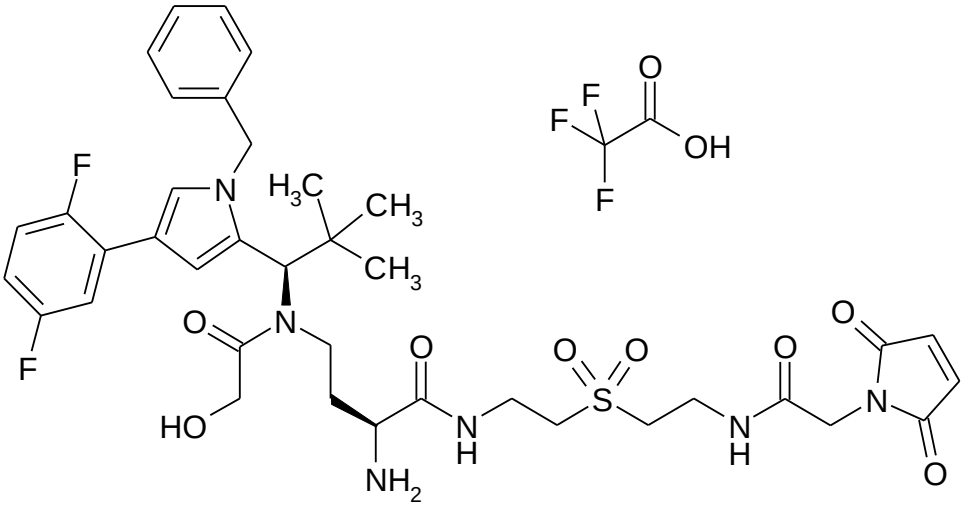
15 mg (0.022 mmol) of this intermediate were dissolved in 4 ml of DMF, and 10 mg (0.039 mmol) of 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione and 7 µl of N,N-diisopropylethylamine were added. After stirring at RT for 2 h, the reaction mixture

was concentrated and the residue was purified by HPLC. This gave 10 mg (56% of theory) of the title compound.

LC-MS (Method 1): R_t = 1.08 min; MS (ESIpos): m/z = 821 $(M+H)^+$.

Intermediate F296

Trifluoroacetic acid / (2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-N-{2-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)sulphonyl]ethyl}butanamide (1:1)

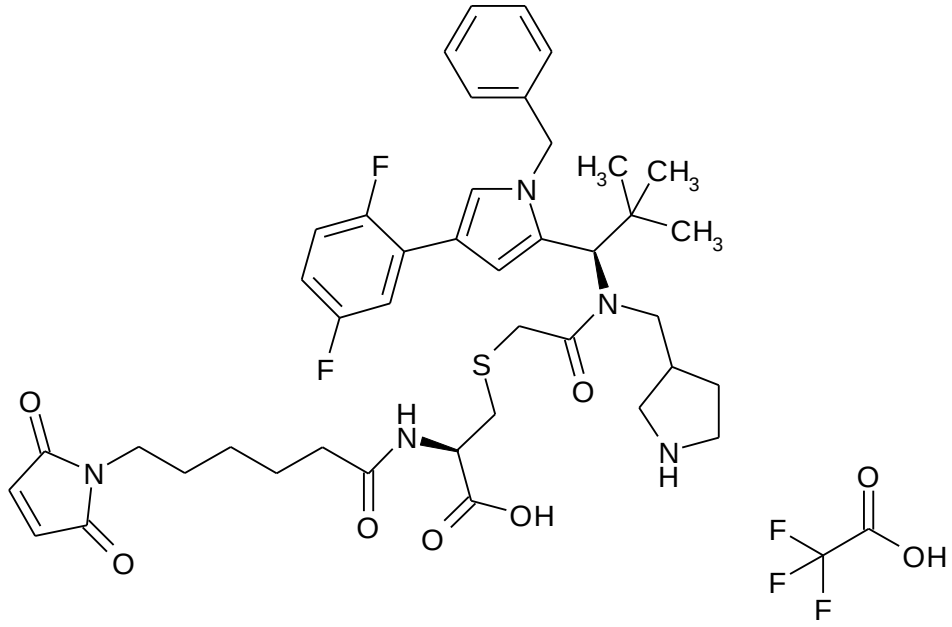


The title compound was prepared proceeding from Intermediate L81 by coupling to Intermediate C58 in the presence of HATU and N,N-diisopropylethylamine. In the next step, the Z protecting group was removed by hydrogenation over 10% palladium on activated carbon in DCM/methanol 1:1 at RT under hydrogen standard pressure for 30 min. The deprotected intermediate was then converted to the title compound by coupling to (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid in the presence of HATU and N,N-diisopropylethylamine and finally by deprotection with zinc chloride.

LC-MS (Method 1): R_t = 0.83 min; MS (ESIpos): m/z = 785 $(M+H)^+$.

Intermediate F297

S-{2-[{(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(pyrrolidin-3-ylmethyl)amino]-2-oxoethyl}-N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-cysteine / trifluoroacetic acid (1:1) (Isomer 1)

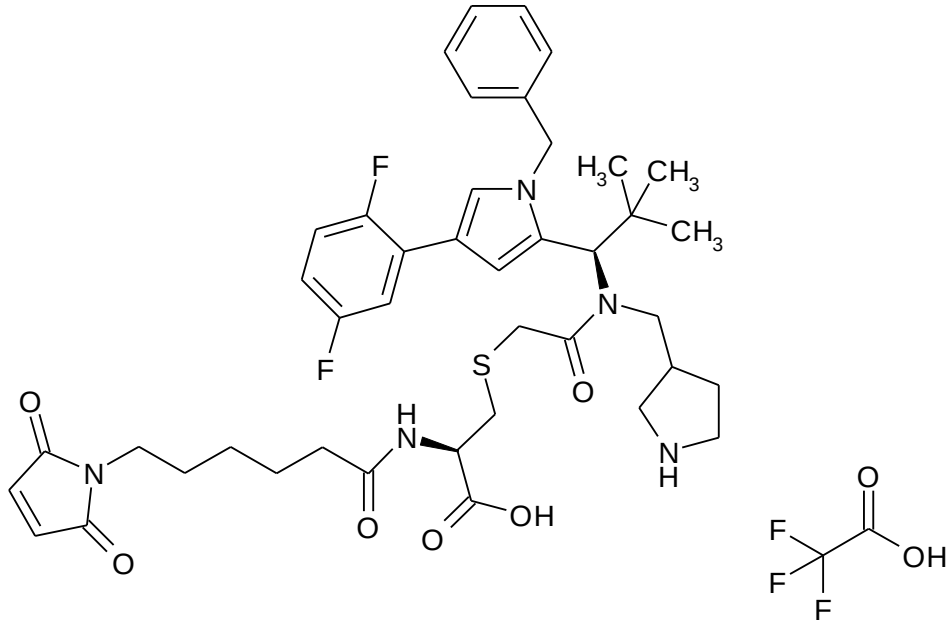


Under argon, 15 mg (0.11 mmol) of zinc chloride were added to a solution of 36 mg (0.03 mmol, 68% purity) of S-[2-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-cysteine (Intermediate C92) in 0.74 ml of 2,2,2-trifluoroethanol, and the reaction mixture was stirred at 50°C for 7 h. 32 mg (0.11 mmol) of EDTA were then added and the mixture was stirred for 15 minutes. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with water and with saturated NaCl solution. The organic phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC. This gave 6.4 mg (25% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.95 min; MS (ESIpos): m/z = 792 $(M+H-CF_3CO_2H)^+$.

Intermediate F298

S-{2-[{(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(pyrrolidin-3-ylmethyl)amino]-2-oxoethyl}-N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-cysteine / trifluoroacetic acid (1:1) (Isomer 2)

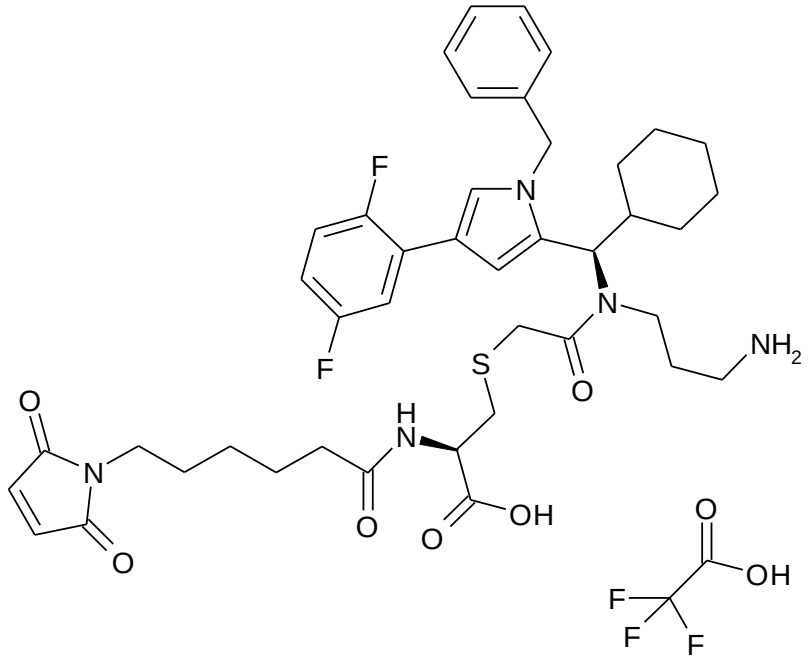


Under argon, 19 mg (0.14 mmol) of zinc chloride were added to a solution of 45 mg (0.04 mmol, 71% purity) of S-[2-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-cysteine (Intermediate C91) in 0.94 ml of 2,2,2-trifluoroethanol, and the reaction mixture was stirred at 50°C for 3 h. 42 mg (0.14 mmol) of EDTA were then added and the mixture was stirred for 15 minutes. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with water and with saturated NaCl solution. The organic phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC. This gave 5.7 mg (18% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.96 min; MS (ESIpos): m/z = 791 $(M+H-CF_3CO_2H)^+$.

Intermediate F299

S-(2-{(3-Aminopropyl)[(R)-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl]amino}-2-oxoethyl)-N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-cysteine / trifluoroacetic acid (1:1)

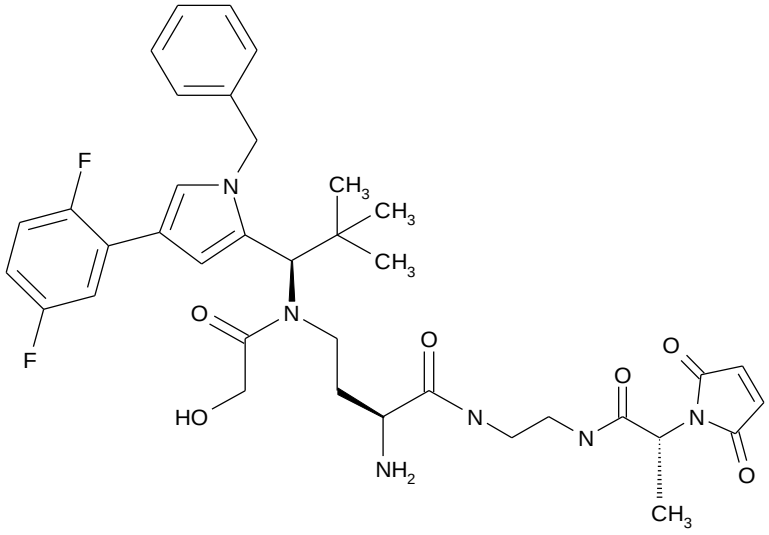


To a solution of 88.0 mg (0.09 mmol) of S-{11-[(R)-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl](cyclohexyl)methyl]-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl}-N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-cysteine (Intermediate C84) in 1.88 ml of 2,2,2-trifluoroethanol was added 76.8 mg (0.57 mmol) of zinc chloride and the reaction mixture was stirred at 50°C for 3 h. 164.6 mg (0.57 mmol) of EDTA were then added and the mixture was stirred for 15 minutes. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with water and with saturated NaCl solution. The organic phase was dried over sodium sulphate and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC. This gave 31 mg (35% of theory) of the title compound.

LC-MS (Method 12): R_t = 1.82 min; MS (ESIpos): m/z = 792 $(M+H)^+$.

Intermediate F300

(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-N-(2-{[(2R)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethyl)butanamide

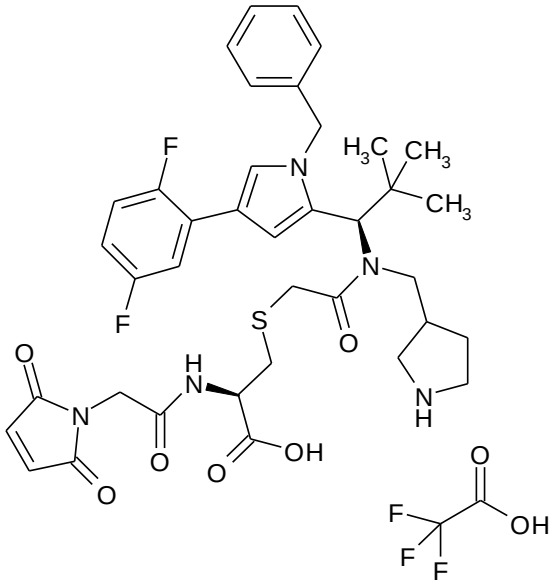


To a solution of 7 mg (0.08 mmol) of 2-(trimethylsilyl)ethyl {(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2R)-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethyl)amino]-1-oxobutan-2-yl}carbamate (Intermediate C100) in 0.2 ml of 2,2,2-trifluoroethanol under argon were added 11 mg (0.08 mmol) of zinc chloride, and the reaction mixture was stirred at 50°C for 8 h. 14 mg (0.05 mmol) of EDTA were then added and the mixture was stirred for 15 minutes. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with water and with saturated NaCl solution. The organic phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC. This gave 1.6 mg (27% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 707 $(M+H-CF_3CO_2H)^+$.

Intermediate F302

S-{2-[{(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(pyrrolidin-3-ylmethyl)amino]-2-oxoethyl}-N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-cysteine trifluoroacetate (1:1) (Isomer 1)

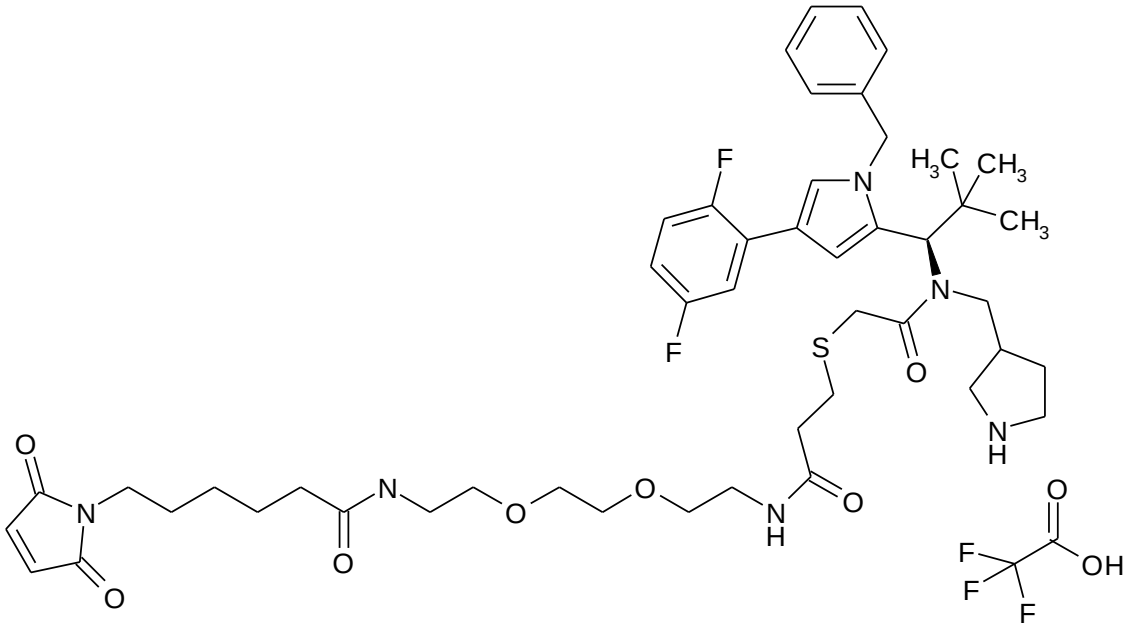


To a mixture of 56.9 mg (58.2 mmol, 85% purity) of S-[2-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[(1-(tert-butoxycarbonyl)pyrrolidin-3-yl]methyl}amino)-2-oxoethyl]-N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-cysteine (Intermediate C94) in 1.4 ml of 2,2,2-trifluoroethanol under argon were added 31.7 mg (0.23 mmol) of zinc chloride and the reaction mixture was stirred at 50°C for 3 h. 68.0 mg (0.23 mmol) of EDTA were then added and the mixture was stirred for 15 minutes. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with water and with saturated NaCl solution. The organic phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC. This gave 7 mg (13% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.91 min; MS (ESIpos): m/z = 736 $(M+H-CF_3CO_2H)^+$.

Intermediate F305

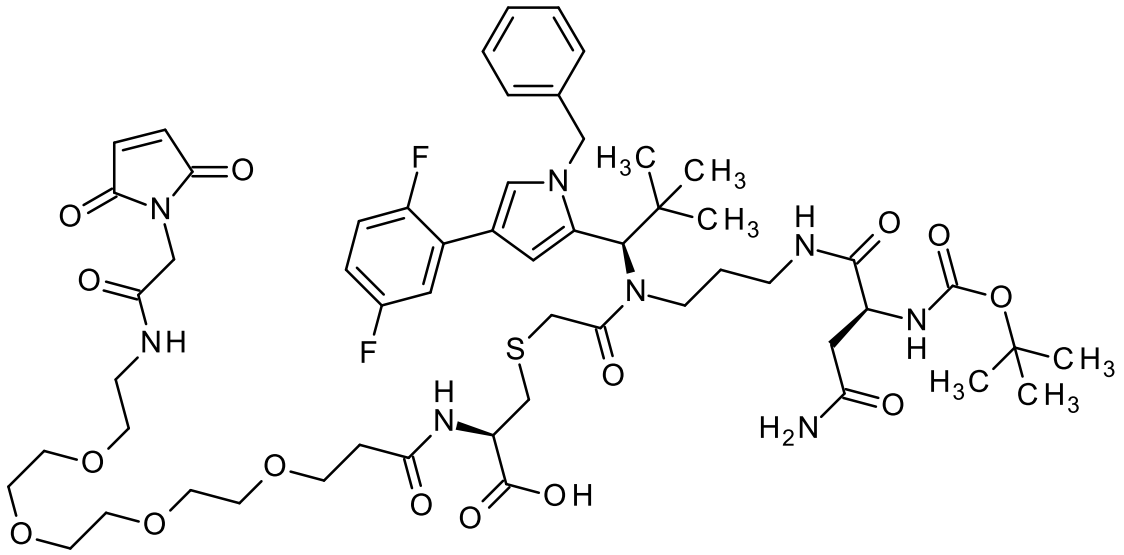
N-{(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-22-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-6,17-dioxo-N-(pyrrolidin-3-ylmethyl)-10,13-dioxa-3-thia-7,16-diazadocosan-1-amide / trifluoroacetic acid (1:1) (Isomer 2)



To a solution of 24.80 mg (0.02 mmol) of tert-butyl 3-[2-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-24-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3,8,19-trioxo-12,15-dioxa-5-thia-2,9,18-triazatetracos-1-yl]pyrrolidine-1-carboxylate (Intermediate C99) in 0.65 ml of 2,2,2-trifluoroethanol were added 13.42 mg (0.10 mmol) of zinc chloride and the reaction mixture was stirred at 50°C for 8 h. 28.78 mg (0.10 mmol) of EDTA were then added and the mixture was stirred for 15 minutes. Ethyl acetate was added to the reaction mixture and the organic phase was washed repeatedly with water and with saturated NaCl solution. The organic phase was dried over magnesium sulphate and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC. This gave 10 mg (44% of theory) of the title compound.
LC-MS (Method 5): R_t = 3.11 min; MS (ESIpos): m/z = 907 $(M+H-CF_3CO_2H)^+$.

Intermediate F306

S-{2-[{(1R)-1-[1-Benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(3-{[N2-(tert-butoxycarbonyl)-L-asparaginyl]amino}propyl)amino]-2-oxoethyl}-N-[1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,18-dioxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-yl]-L-cysteine

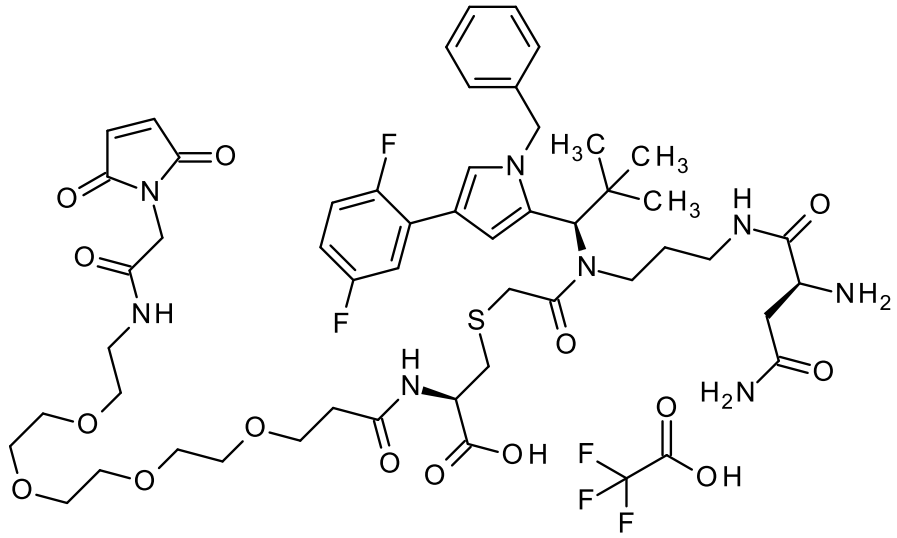


To a solution of 22 mg of Intermediate F257 (0.02 mmol) in 0.22 ml of DMF at RT were added 10.7 µl of N,N-diisopropylethylamine (0.062 mmol) and 10 mg of N-alpha-Boc-L-asparagine N-hydroxysuccinimide ester. The mixture was stirred for 15 minutes. Water (2 ml) and ACN (4 ml) were added. The reaction solution was purified by preparative HPLC (eluent: ACN/water + 0.1% TFA, gradient = 1:9 → 3:2). This gave 21 mg (87% of theory) of the title compound.

LC-MS (Method 1): R_t = 1.07 min; MS (ESIpos): m/z = 1171 $(M+H)^+$.

Intermediate F307

S-[2-([3-(L-Asparaginylamino)propyl]{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino)-2-oxoethyl]-N-[1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,18-dioxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-yl]-L-cysteine / trifluoroacetic acid (1:1)



To a solution of 21 mg of Intermediate F306 (0.017 mmol) in 1.5 ml of 2,2,2-trifluoroethanol were added 24.3 mg (0.178 mmol) of zinc chloride, and the reaction mixture was stirred at 60°C for 60 min. 52.1 mg (0.178 mmol) of EDTA were then added and the mixture was stirred for 15 minutes. Water (2 ml) and ACN (4 ml) were added. The reaction solution was filtered and purified by preparative HPLC (eluent: ACN/water + 0.1% TFA, gradient = 1:9 → 3:2). This gave 18 mg (85% of theory) of the title compound.
LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 1071 $(M+H)^+$.

General method for synthesis of the APDC or ADC precursors (intermediate series Q)

APDC precursors:

The above-described intermediates of the F series (F1-F305) or optionally protected precursors can be converted to the APDC precursor Q according to Scheme 1 optionally even after final deprotection. After release of the N-terminal amino group of the legumain-cleavable head group and subsequent modification of the APDC precursor molecules with substituents Z_1 of various structures (Scheme 1), an improvement in the profile of properties of the APDCs can also be achieved. The protein-reactive group for attachment of the APDC precursor molecule to the antibody may be in the position R1, R2 or R3.

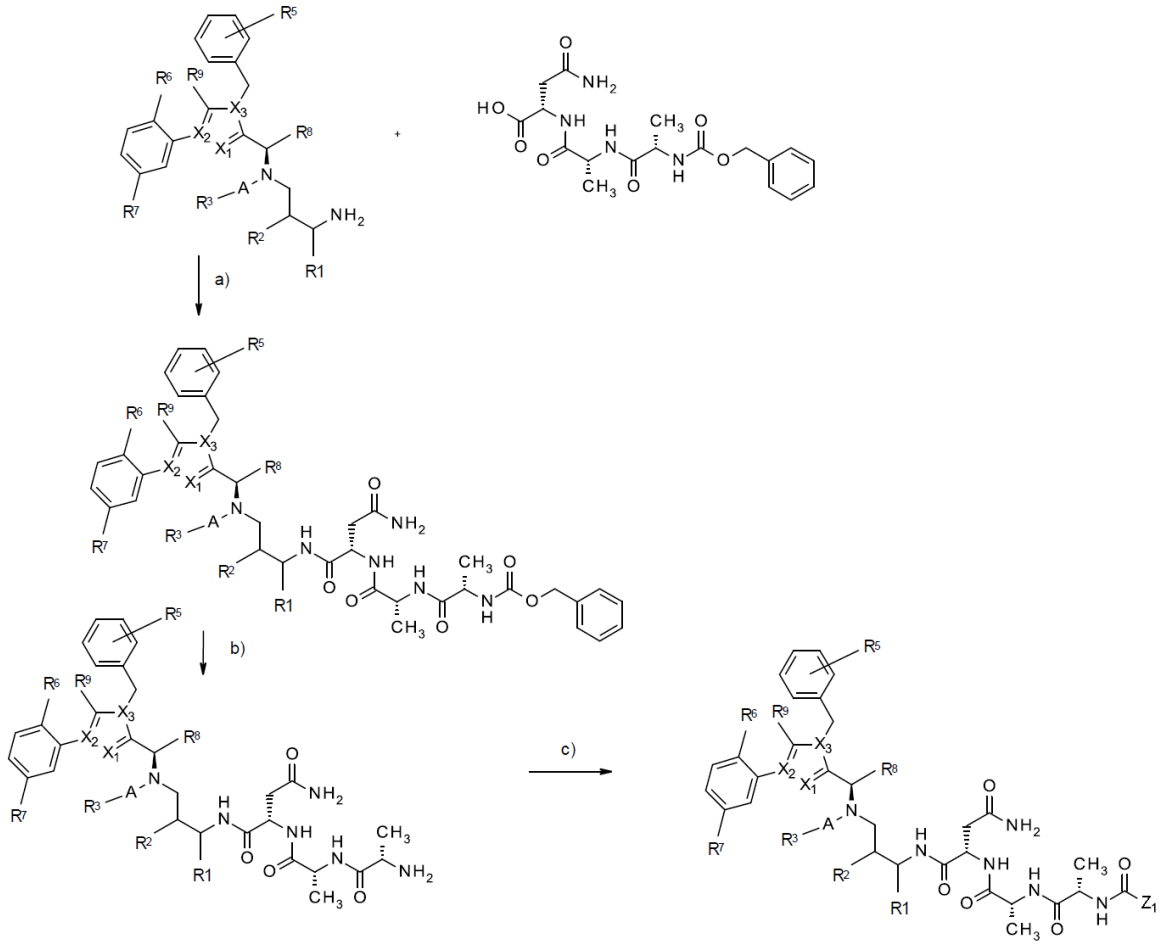
An illustrative method is described here:

0.037 mmol of an intermediate F1-Fx is taken up in 1-20 ml, preferably 5-10 ml, of a suitable solvent, for example DMF, DMSO, DCM, chloroform, toluene, THF, methanol or a mixture thereof, and 0.039 mmol of an N-terminally modified tripeptide derivative, for example Intermediate L92, is added, as are 0.041 mmol of a standard coupling reagent, for example HATU, EDCI/HOBT, BEP etc., and 0.11 mmol of a standard base, for example N,N-diisopropylethylamine, triethylamine, 4-methylmorpholine etc. After stirring at RT for 5 min, the mixture is acidified with 2 drops of trifluoroacetic acid and concentrated. The residue is purified by preparative HPLC. The appropriate fractions are concentrated under reduced pressure and the residue is lyophilized from acetonitrile/water.

When said N-terminal modification of the attached tripeptide derivative is a protecting group, this can subsequently be detached by known methods, for example a Z protecting group preferably by means of hydrogenolysis, a Boc protecting group by means of acid hydrolysis or by means of zinc chloride, an Fmoc protecting group by base hydrolysis or a Teoc group by means of fluorides or with zinc chloride.

Finally, the amino group thus released can be acylated or alkylated to improve the profile of properties, for example with amine-reactive groups such as active esters, acid chlorides, isocyanates, etc., or by coupling to carboxylic acid derivatives in the presence of a standard coupling reagent, for example HATU, EDCI/HOBT, BEP etc., and of a standard base, for example N,N-diisopropylethylamine, triethylamine, 4-methylmorpholine etc. If they are still present, further protecting groups in the molecule may be removed in a last step.

Scheme 1:

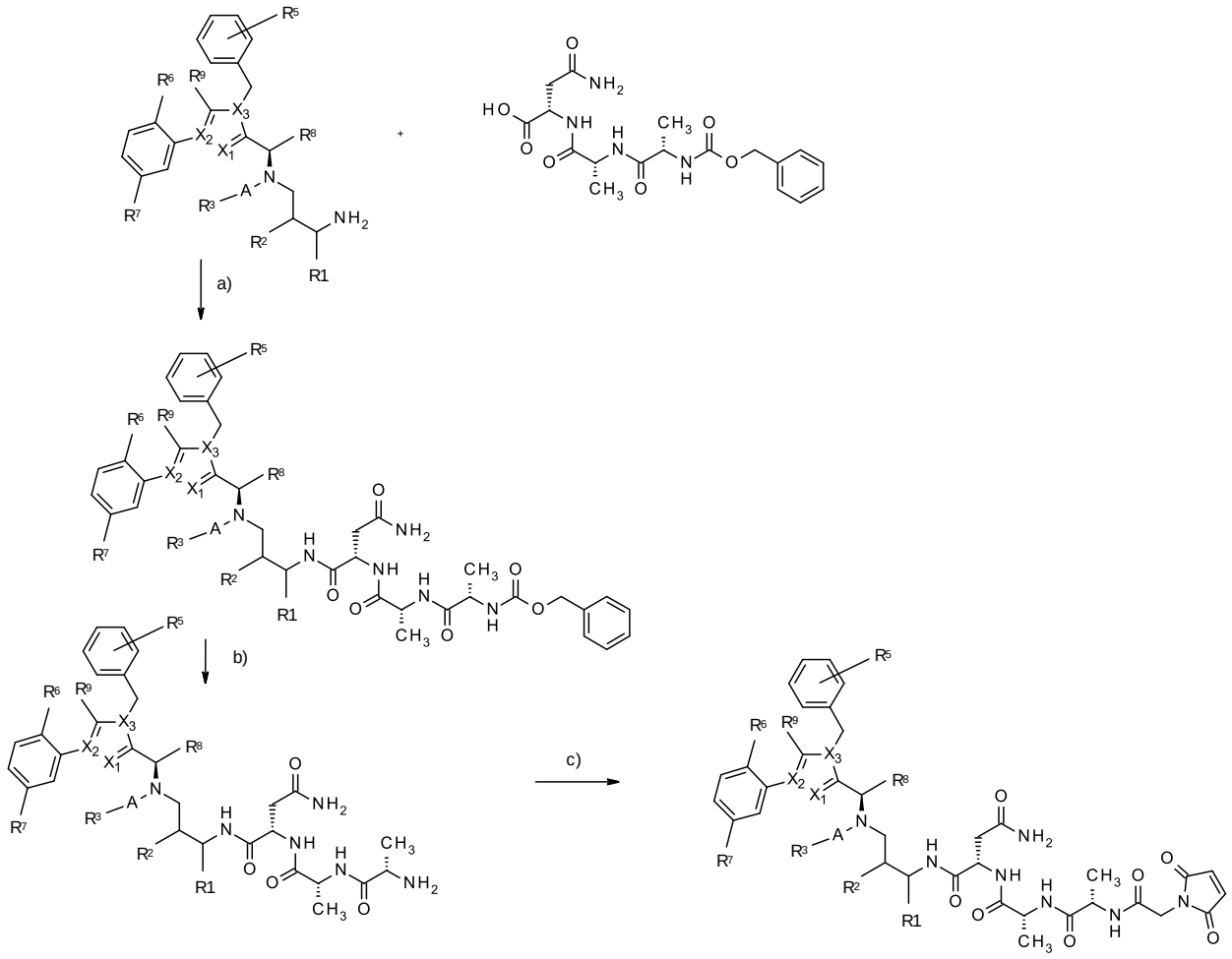


[a): HATU, DMF, N,N-diisopropylethylamine, RT or EDCI, HOBT, N,N-diisopropylethylamine, DMF, RT b) H_2, 10% Pd-C, MeOH, RT; c) Z_1-COOH, EDCI, HOBT, N,N-diisopropylethylamine, DMF, RT or Z_1-COOH, HATU, N,N-diisopropylethylamine, DMF, RT or Z_1-COOSu, N,N-diisopropylethylamine, DMF, RT]

ADC precursors:

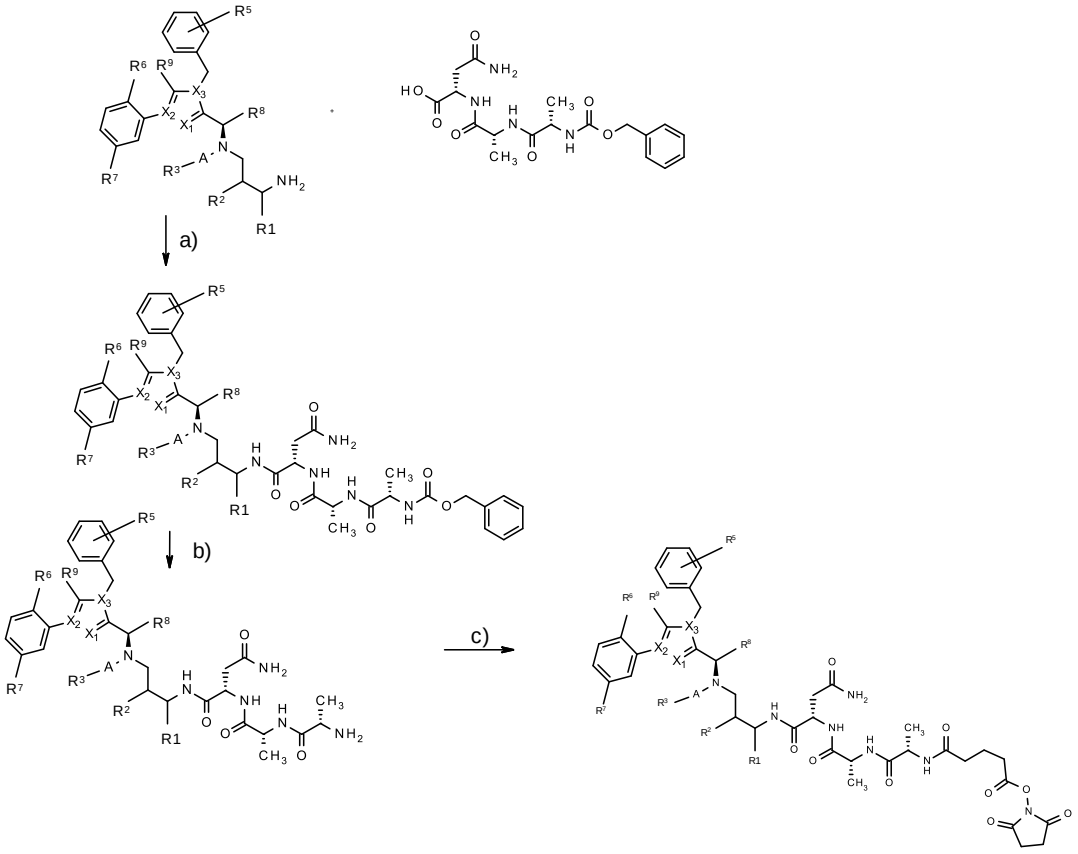
In addition, other intermediates which do not yet contain protein-reactive groups can be converted to legumain-cleavable ADC precursors according to Schemes 2 and 3:

Scheme 2:



[a): HATU, DMF, N,N-diisopropylethylamine, RT or EDCI, HOBT, N,N-diisopropylethylamine, DMF, RT b) H_2, 10% Pd-C, MeOH, RT; c) 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, N,N-diisopropylethylamine, DMF, RT]

Scheme 3:



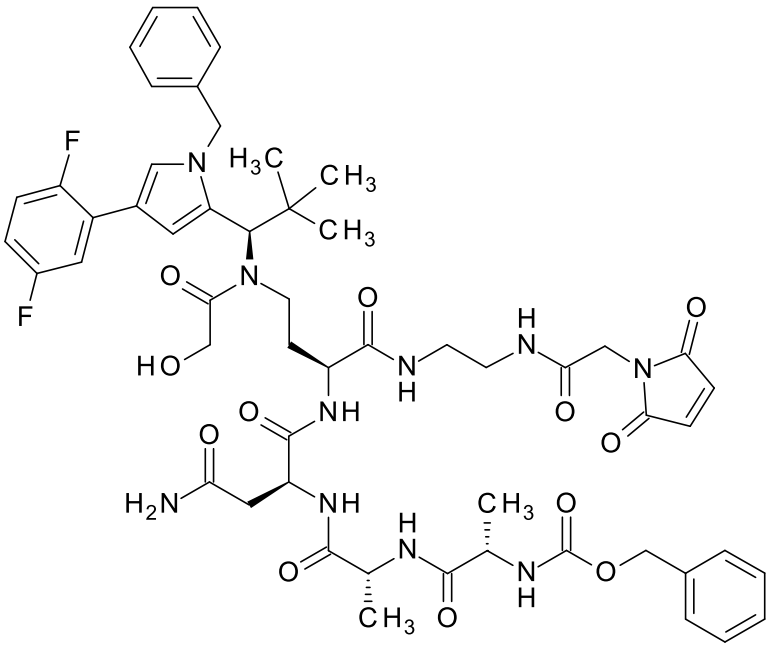
[a): HATU, DMF, N,N-diisopropylethylamine, RT or EDCI, HOBT, N,N-diisopropylethylamine, DMF, RT b) H_2, 10% Pd-C, MeOH, RT; c) 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, N,N-diisopropylethylamine, DMF, RT]

As an alternative to the benzyloxycarbonyl group shown in Schemes 1-3, it is possible to use other protecting groups established in peptide chemistry and detach them by corresponding methods that are likewise known. The selection of the protecting group strategy is made according to requirements known to those skilled in the art relating to compatibility with other structural elements that occur in the molecule. If they are still present, further protecting groups in the molecule may be removed in a last step. The syntheses may also optionally be rearranged in terms of their sequence.

In addition, the protein-reactive group in the context of the linker structures L1-L2 may be varied within the scope of the claims.

Intermediate R1

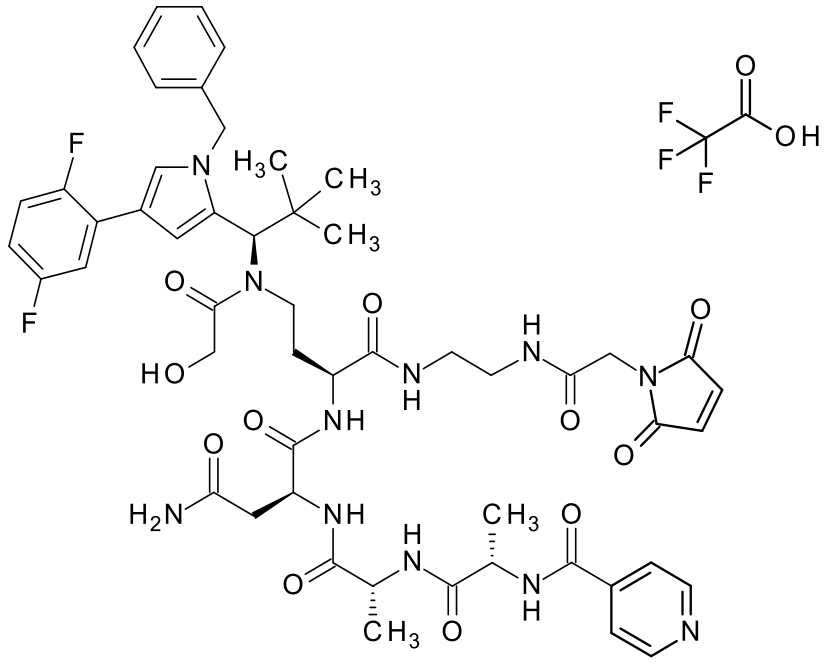
N-[(Benzyloxy)carbonyl]-L-alanyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide



7.5 mg (0.009 mmol) of Intermediate F104 were dissolved in 2 ml of DMF and, after addition of 5.3 mg (0.014 mmol) of HATU, 5 µl of N,N-diisopropylethylamine and 5.8 mg (0.011 mmol) of Intermediate L108, stirred at RT for 15 min. Subsequently, the mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC. 3.2 mg (31% of theory) of the title compound were obtained.
LC-MS (Method 1): R_t = 1.10 min; MS (ESIpos): m/z = 1083 $(M+H)^+$.

Intermediate R2

N-Isonicotinyl-L-alanyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycolyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide trifluoroacetate (1:1)

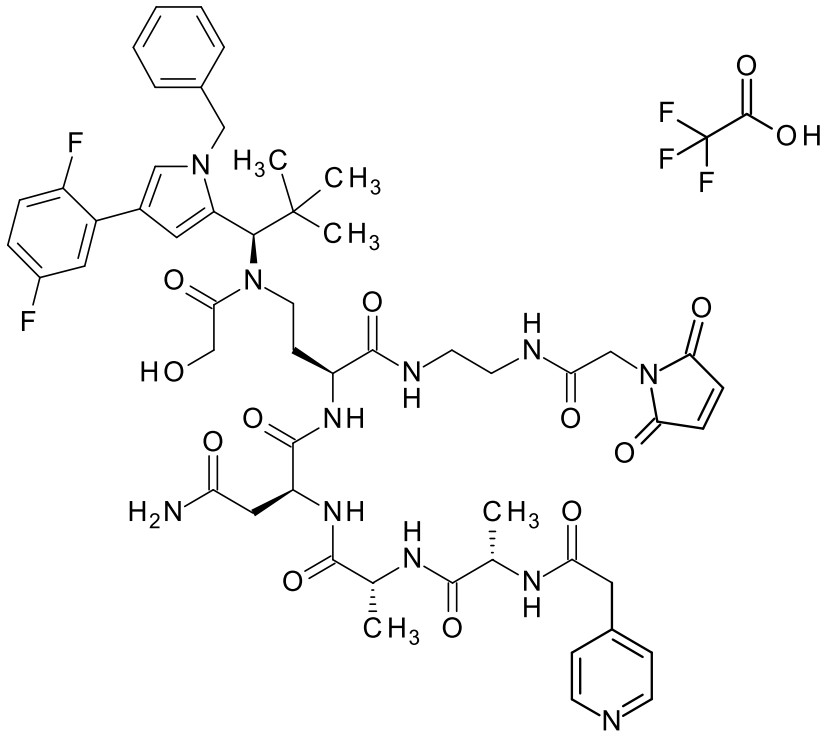


12.7 mg (0.016 mmol) of Intermediate F104 were coupled to 7.8 mg (0.016 mmol) of Intermediate L109 in analogy to Intermediate R1. 4.5 mg (24% of theory) of the title compound were obtained.

LC-MS (Method 12): R_t = 1.74 min; MS (ESIpos): m/z = 1054 $(M+H)^+$.

Intermediate R3

N-(Pyridin-4-ylacetyl)-L-alanyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide trifluoroacetate (1:1)

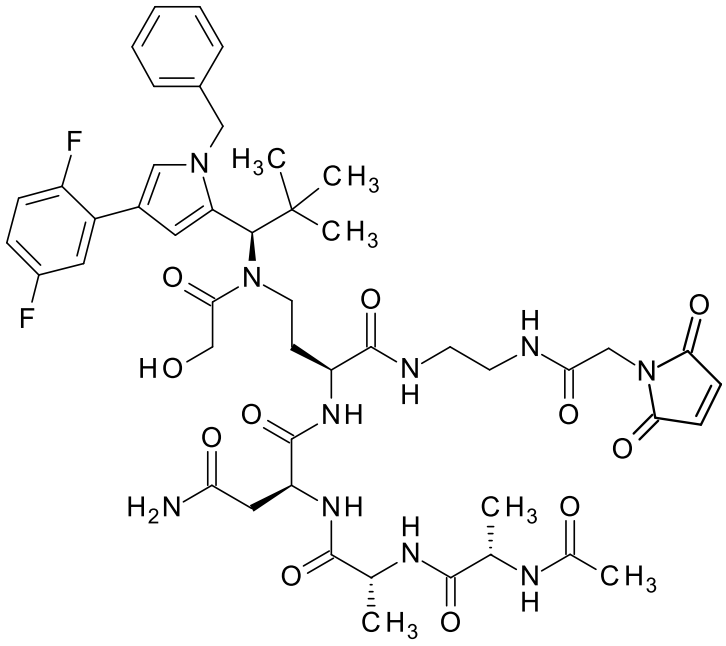


100 mg (0.124 mmol) of Intermediate F104 were coupled to 75 mg (0.15 mmol) of Intermediate L110 in analogy to Intermediate R1. Purification by preparative HPLC gave 58 mg (39% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 1068 $(M+H)^+$.

Intermediate R4

N-Acetyl-L-alanyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide

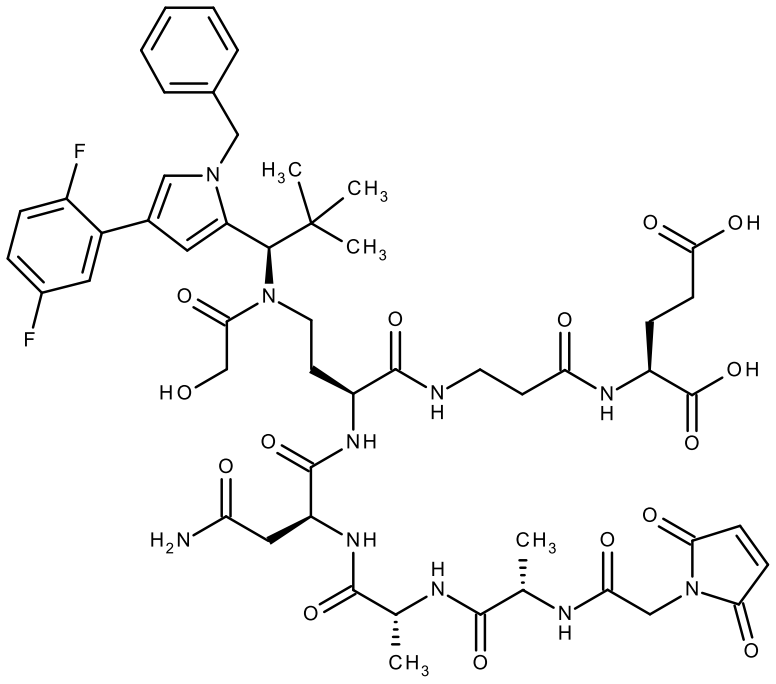


20 mg (0.025 mmol) of Intermediate F104 were coupled to 30.6 mg (0.062 mmol) of Intermediate L111 in analogy to Intermediate R1. 2.3 mg (9% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 991 $(M+H)^+$.

Intermediate R5

N-[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-alanyl-D-alanyl-N1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(3-{[(1S)-1,3-dicarboxypropyl]amino}-3-oxopropyl)amino]-1-oxobutan-2-yl}-L-aspartamide

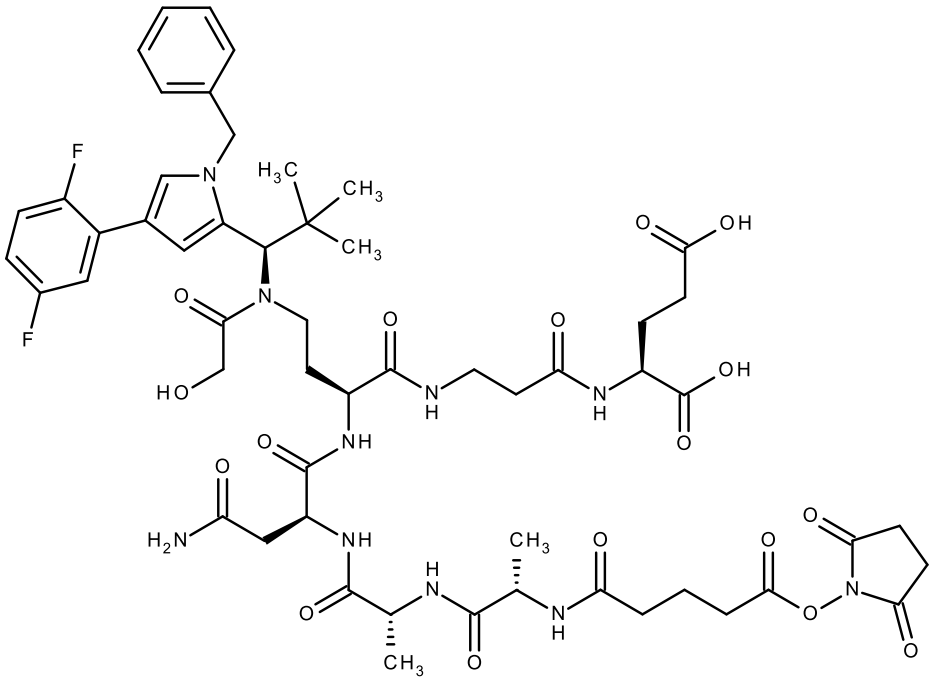


The title compound was prepared proceeding from compound C110, first by coupling to Intermediate L108 in the presence of HATU and N,N-diisopropylethylamine. In the next step, all protecting groups were removed by hydrogenation over 10% palladium on activated carbon in methanol under standard hydrogen pressure at RT for 1 hour and the deprotected intermediate was then converted to the title compound by reaction with 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione in the presence of N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 0.94 min; MS (ESIpos): m/z = 1107 $[M+H]^+$.

Intermediate R6

N-{5-[(2,5-Dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}-L-alanyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(3-{[(1S)-1,3-dicarboxypropyl]amino}-3-oxopropyl)amino]-1-oxobutan-2-yl}-L-aspartamide

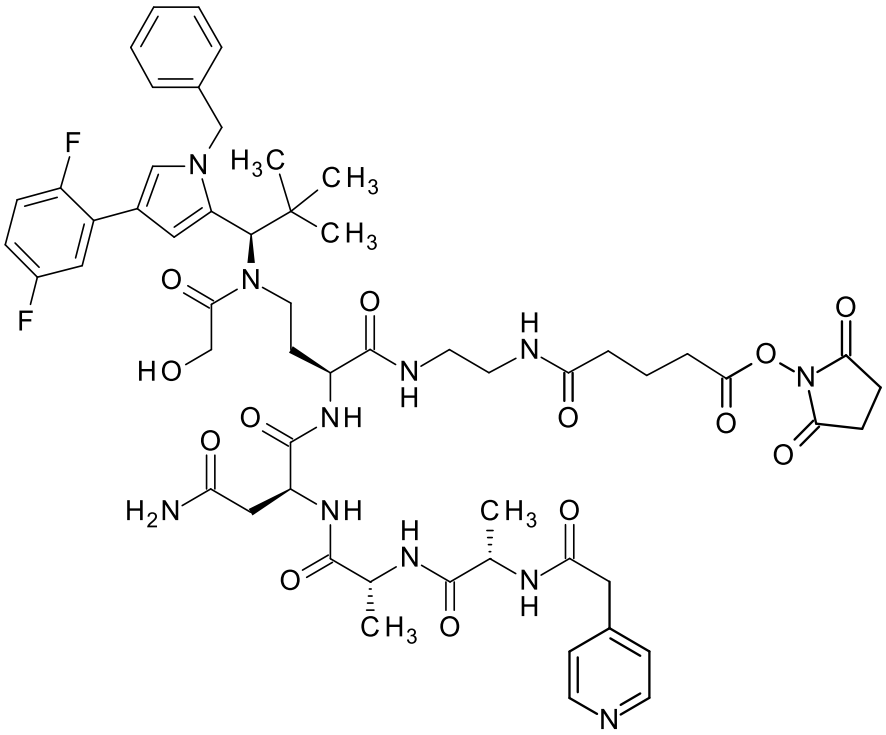


The title compound was prepared proceeding from compound C110, first by coupling to Intermediate L108 in the presence of HATU and N,N-diisopropylethylamine. In the next step, all protecting groups were removed by hydrogenation over 10% palladium on activated carbon in methanol under standard hydrogen pressure at RT for 1.5 hours and the deprotected intermediate was then converted to the title compound by reaction with 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine in DMF.

LC-MS (Method 1): R_t = 0.95 min; MS (ESIpos): m/z = 1181 $[M+H]^+$.

Intermediate R7

N-(Pyridin-4-ylacetyl)-L-alanyl-D-alanyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-{[2-({5-[(2,5-dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}amino)ethyl]amino}-1-oxobutan-2-yl]-L-aspartamide



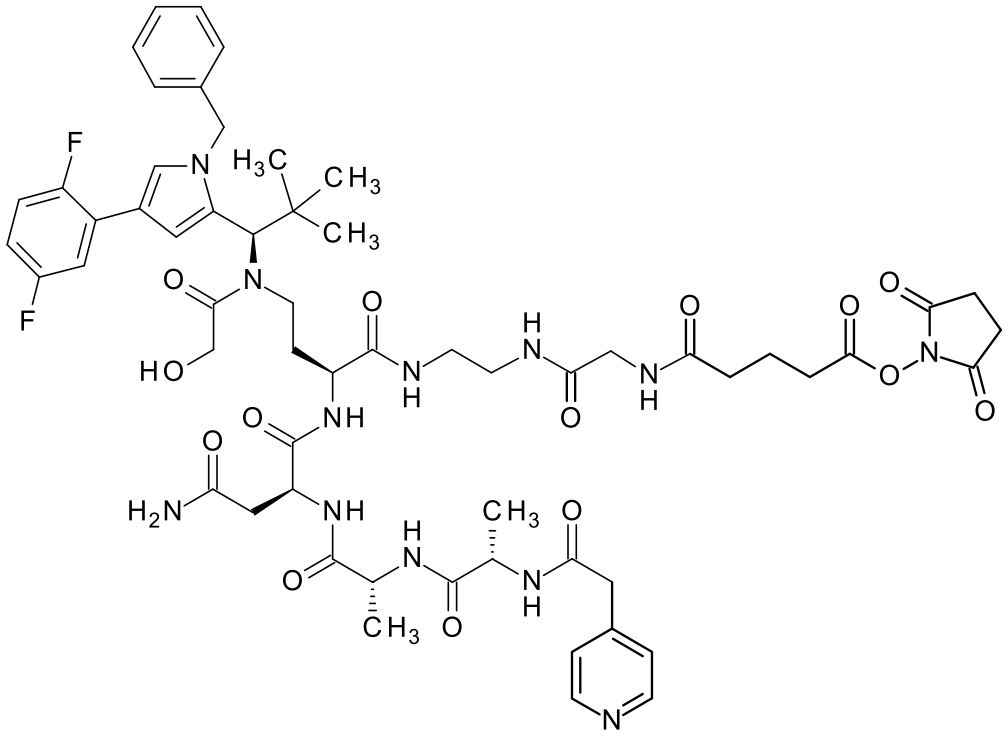
The title compound was prepared by coupling of Intermediate C102 to tert-butyl (2-aminoethyl)carbamate in the presence of HATU and N,N-diisopropylethylamine, followed by detachment of the Z protecting group by hydrogenation over 10% palladium on activated carbon in DCM-methanol 1:1 at RT under standard hydrogen pressure for 1 hour, then coupling to Intermediate L110 in the presence of HATU and N,N-diisopropylethylamine and subsequent detachment of the Boc group by stirring at 50°C in trifluoroethanol with 6 equiv. of zinc chloride for 2 hours. In the last step, the intermediate obtained was taken up in DMF, and converted to the title compound with 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 1142 $[M+H]^+$.

Intermediate R8

N-(Pyridin-4-ylacetyl)-L-alanyl-D-alanyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-({2-[(N-{5-[(2,5-

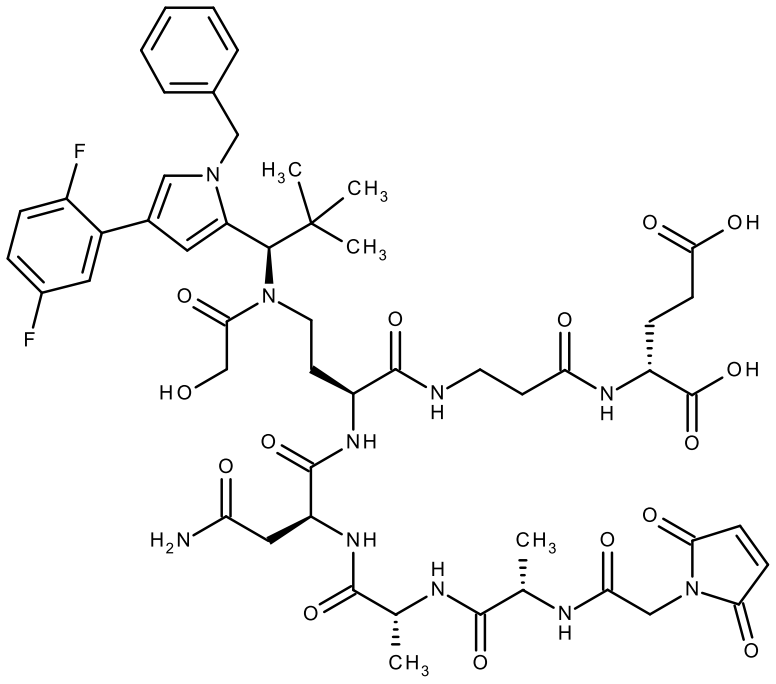
dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}glycyl)amino]ethyl}amino)-1-oxobutan-2-yl]-L-aspartamide



The title compound was prepared in analogy to Intermediate R7 proceeding from Intermediate C102 and Intermediate L112.
LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 1199 $[M+H]^+$.

Intermediate R9

N-[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-alanyl-D-alanyl-N1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(3-{[(1R)-1,3-dicarboxypropyl]amino}-3-oxopropyl)amino]-1-oxobutan-2-yl}-L-aspartamide

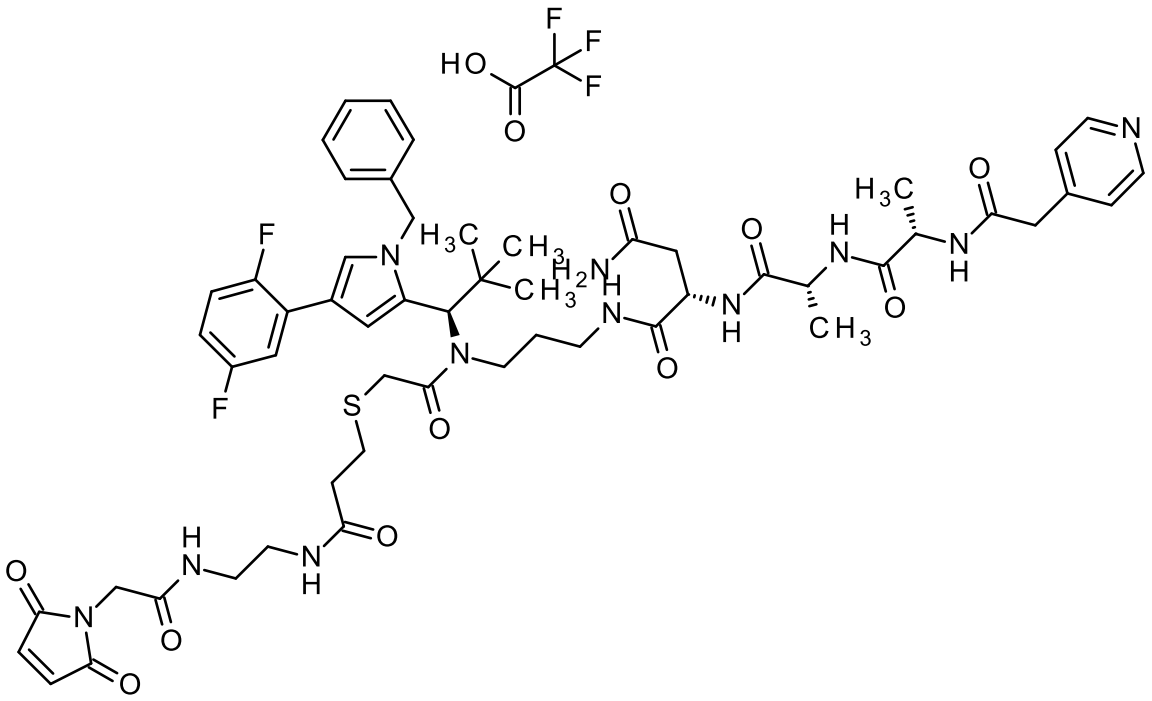


The title compound was prepared proceeding from compound C111, first by coupling to Intermediate L107 in the presence of HATU and N,N-diisopropylethylamine, followed by deprotection by means of zinc chloride.

LC-MS (Method 1): R_t = 0.9 min; MS (ESIpos): m/z = 1107 $[M+H]^+$.

Intermediate R10

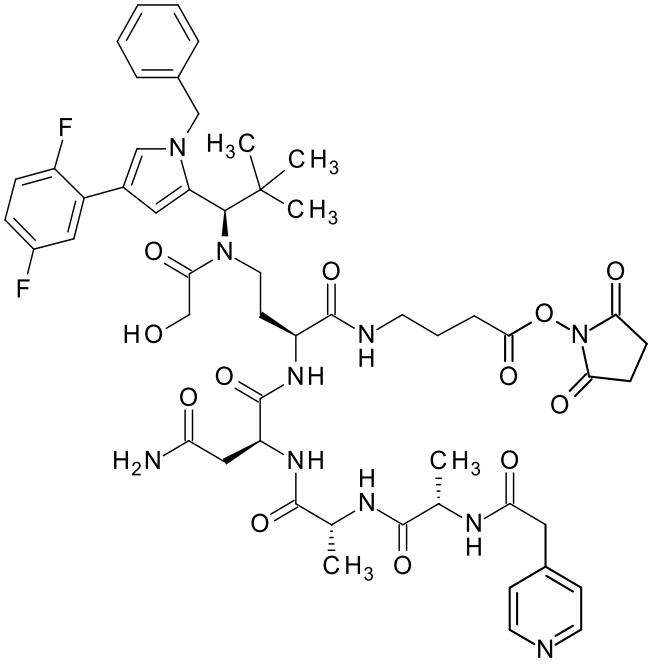
Trifluoroacetic acid / N-(pyridin-4-ylacetyl)-L-alanyl-D-alanyl-N1-[13-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,7,12-trioxo-10-thia-3,6,13-triazahexadecan-16-yl]-L-aspartamide (1:1)



15.0 mg (17.6 µmol) of trifluoroacetic acid / 3-({2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)-N-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)propanamide (1:1) (Intermediate F240) were initially charged together with 11.6 mg (22.9 µmol) of N-(pyridin-4-ylacetyl)-L-alanyl-D-alanyl-L-asparagine / trifluoroacetic acid (1:1) (Intermediate L110) in 2.0 ml of acetonitrile. Then 25 µl (0.14 mmol) of N,N-diisopropylethylamine were added and 14 µl (23 µmol) of T3P (50% in ethyl acetate) were added dropwise. The reaction mixture was stirred at RT for 30 minutes. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 5.70 mg (26% of theory) of the title compound.
LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1112 $(M+H)^+$.

Intermediate R11

N-(Pyridin-4-ylacetyl)-L-alanyl-D-alanyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-({4-[(2,5-dioxopyrrolidin-1-yl)oxy]-4-oxo butyl}amino)-1-oxobutan-2-yl]-L-aspartamide

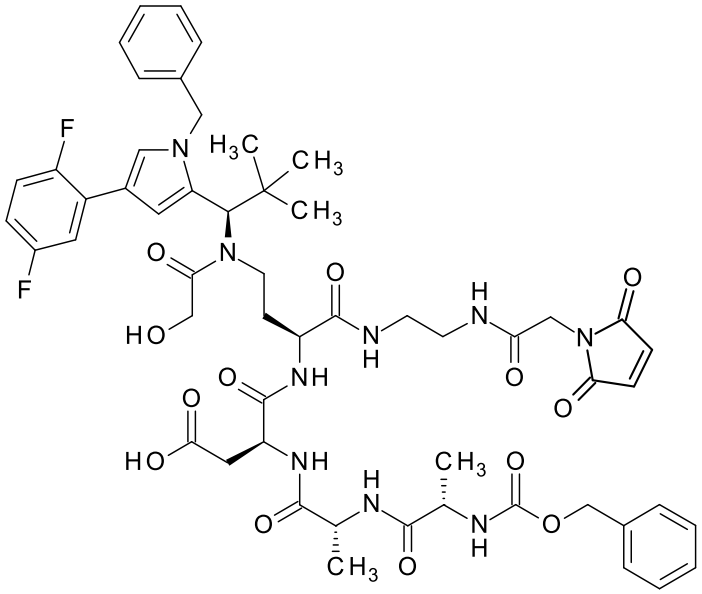


20 mg (31 µmol) of Intermediate C114 were initially charged together with 11.6 mg (22.9 µmol) of N-(pyridin-4-ylacetyl)-L-alanyl-D-alanyl-L-asparagine / trifluoroacetic acid (1:1) (Intermediate L110) in 5.0 ml of DMF. Then 11 µl of N,N-diisopropylethylamine and 21 mg (55 µmol) of HATU were added. The reaction mixture was stirred at RT for 60 minutes. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 250x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This was followed by the detachment of the tert-butyl ester group by stirring with 6 equiv. of zinc chloride in trifluoroethanol at 50°C for 2 hours. Addition of 6 equiv. of EDTA was followed by purification by preparative HPLC. In the last step, the intermediate obtained was taken up in DMF, and converted to the title compound with 15 equivalents of 1-hydroxypyrrolidine-2,5-dione and by stirring in the presence of 5 equiv. of HATU and 5 equiv. of N,N-diisopropylethylamine for 60 minutes. The latter was purified by preparative HPLC.

LC-MS (Method 1): R_t = 0.87 min; MS (ESIpos): m/z = 1071 $(M+H)^+$.

Intermediate R12

N-[(Benzyloxy)carbonyl]-L-alanyl-D-alanyl-N1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-alpha-asparagine

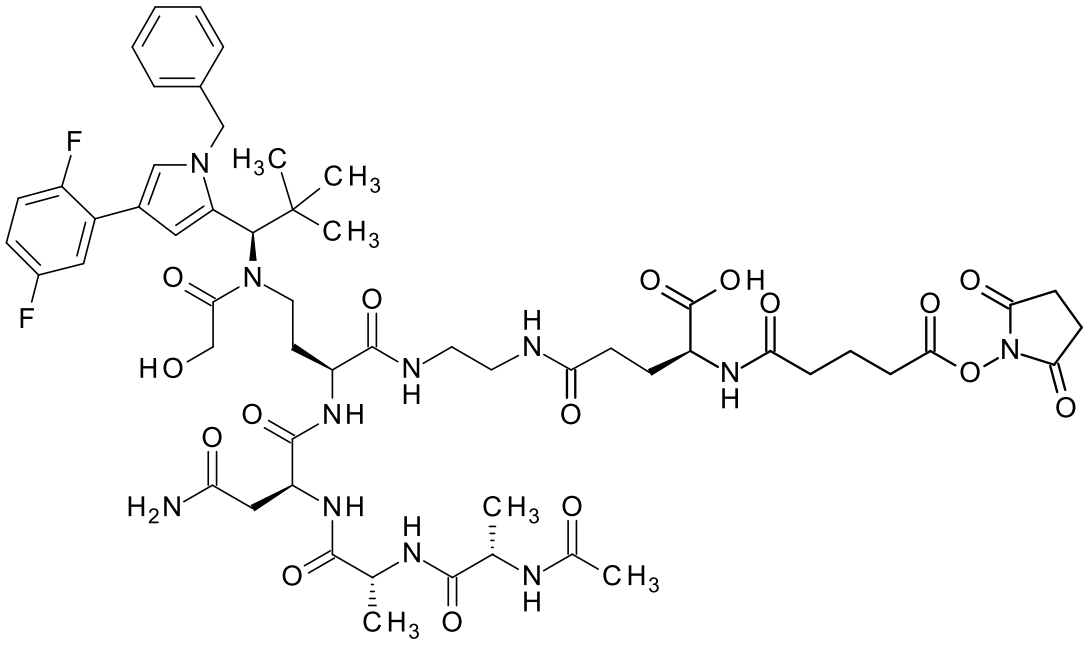


The title compound was prepared proceeding from compound F104, first by coupling to Intermediate L113 in DMF in the presence of HATU and N,N-diisopropylethylamine, followed by deprotection by means of zinc chloride.

LC-MS (Method 1): R_t = 1.1 min; MS (ESIpos): m/z = 1084 $[M+H]^+$.

Intermediate R13

N-Acetyl-L-alanyl-D-alanyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-({2-[(N-{5-[(2,5-dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}-L-gamma-glutamyl)amino]ethyl}amino)-1-oxobutan-2-yl]-L-aspartamide

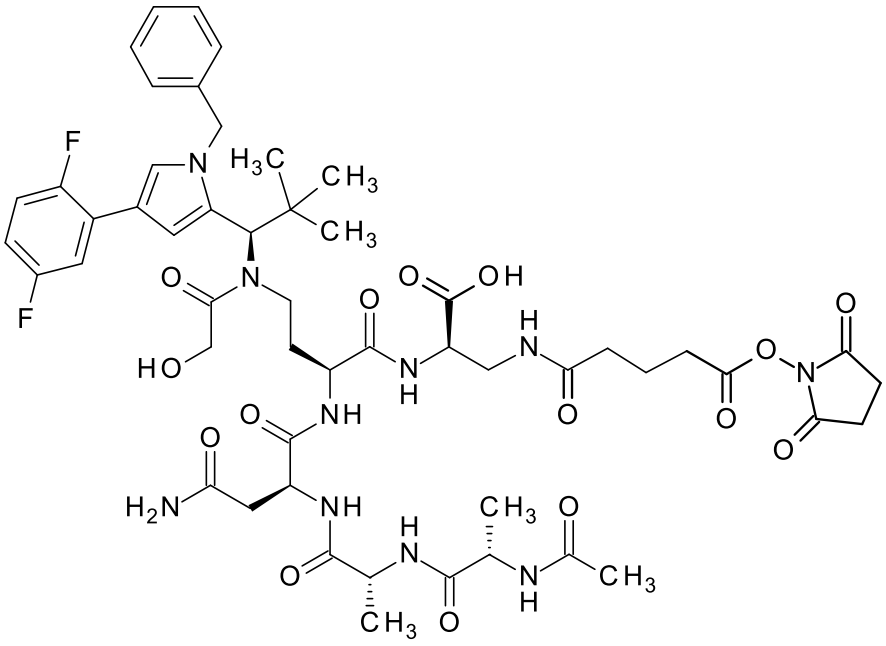


First of all, Intermediate C112 was coupled to (4S)-5-(benzyloxy)-4-{[(benzyloxy)carbonyl]amino}-5-oxopentanoic acid in the presence of HATU and N,N-diisopropylethylamine. This was followed by complete deprotection by hydrogenation over 10% palladium on activated carbon in DCM/methanol 1:1 at RT under hydrogen standard pressure for 1 hour. Finally, the title compound was obtained by reaction with 5 equivalents of 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione in DMF in the presence of 3 equivalents of N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 0.91 min; MS (ESIpos): m/z = 1194 $[M+H]^+$.

Intermediate R14

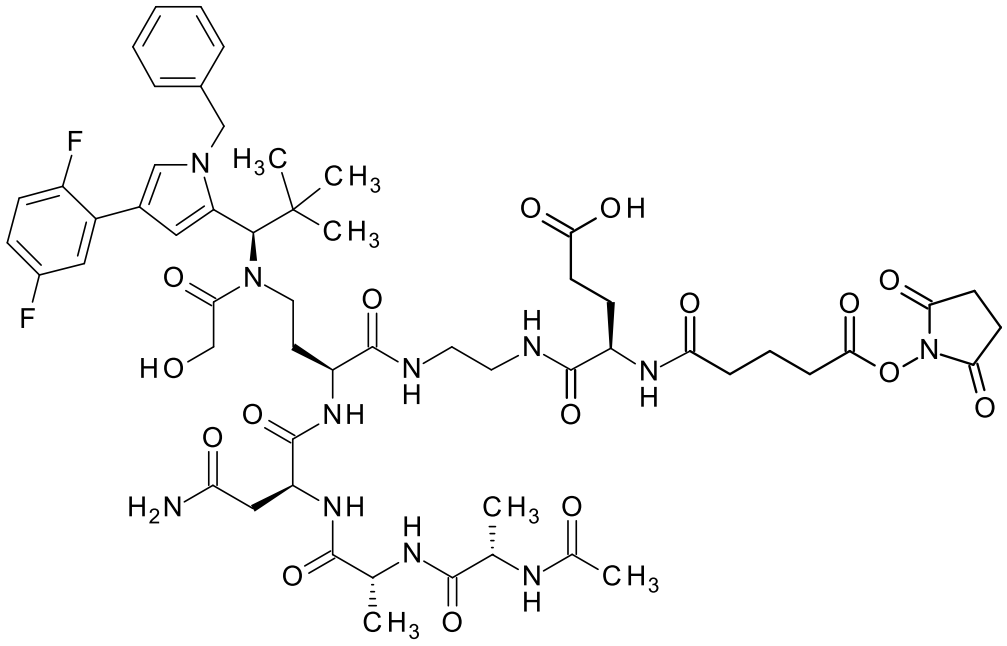
N-Acetyl-L-alanyl-D-alanyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-{[(1R)-1-carboxy-2-({5-[(2,5-dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}amino)ethyl]amino}-1-oxobutan-2-yl]-L-aspartamide



First of all, Intermediate C113 was coupled to Intermediate L111 in the presence of HATU and N,N-diisopropylethylamine. This was followed by complete deprotection by hydrogenation over 10% palladium on activated carbon in DCM/methanol 1:1 at RT under hydrogen standard pressure for 1 hour. Finally, the title compound was obtained by reaction with 2.5 equivalents of 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione in DMF in the presence of 3.5 equivalents of N,N-diisopropylethylamine.
LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 1109 $[M+H]^+$.

Intermediate R15

N-Acetyl-L-alanyl-D-alanyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-({2-[(N-{5-[(2,5-dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}-D-alpha-glutamyl)amino]ethyl}amino)-1-oxobutan-2-yl]-L-aspartamide

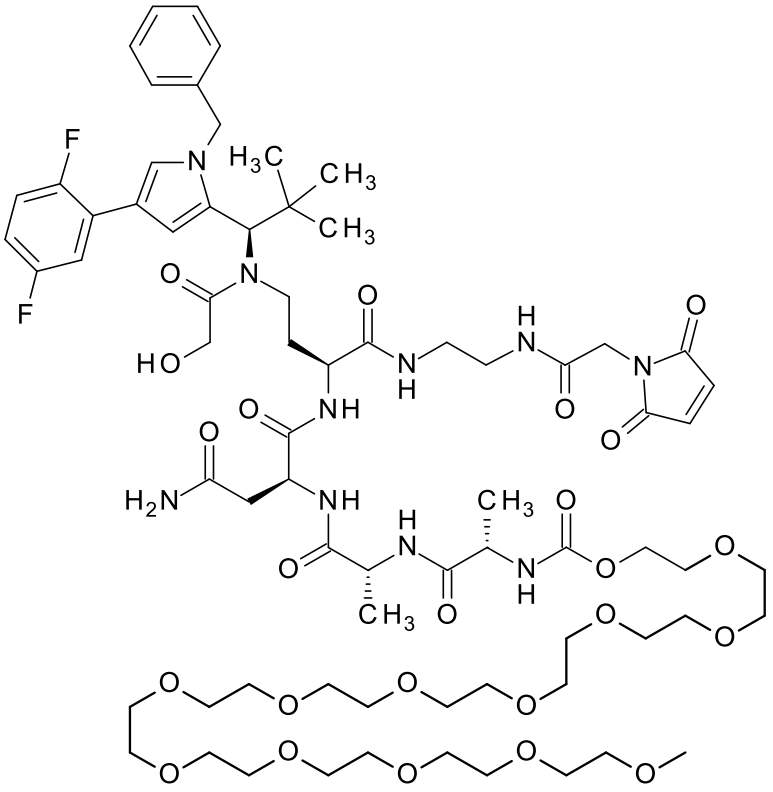


First of all, Intermediate C112 in DMF was coupled to commercially available (2R)-5-(benzyloxy)-2-{[(benzyloxy)carbonyl]amino}-5-oxopentanoic acid in the presence of HATU and N,N-diisopropylethylamine. This was followed by complete deprotection by hydrogenation over 10% palladium on activated carbon in methanol at RT under hydrogen standard pressure for 1 hour. Finally, the title compound was obtained by reaction with 2.5 equivalents of 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione in DMF in the presence of 3 equivalents of N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 1194 $[M+H]^+$.

Intermediate R16

N-(38-Oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaoctatriacontan-38-yl)-L-alanyl-D-alanyl-N1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide

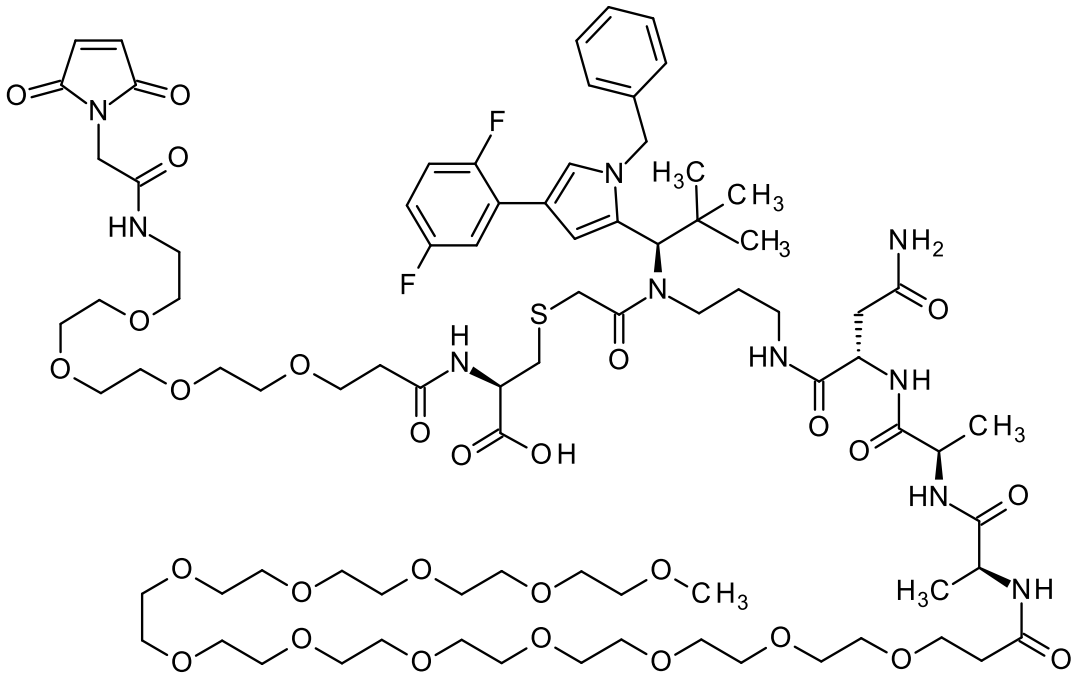


20 mg (0.025 mmol) of Intermediate F104 and 23 mg of Intermediate L116 (0.027 mmol) were dissolved in 0.2 ml of DMF and, after addition of 11.3 mg (0.029 mmol) of HATU and 13 µl of N,N-diisopropylethylamine (0.074 mmol), stirred at RT for 45 min. Water (1 ml) and ACN (1 ml) were added. The reaction solution was purified by preparative HPLC (eluent: ACN/water + 0.1% TFA, gradient = 3:7 → 7:3). 19 mg (50% of theory) of the title compound were obtained.

LC-MS (Method 4): R_t = 1.19 min; MS (ESIpos): m/z = 1519.8055 $(M+H)^+$.

Intermediate R17

N-(38-Oxo-2,5,8,11,14,17,20,23,26,29,32,35-dodecaoxaoctatriacontan-38-yl)-L-alanyl-D-alanyl-N1-[(20R)-25-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-20-carboxy-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,18,24-trioxo-6,9,12,15-tetraoxa-22-thia-3,19,25-triazaoctacosan-28-yl]-L-aspartamide

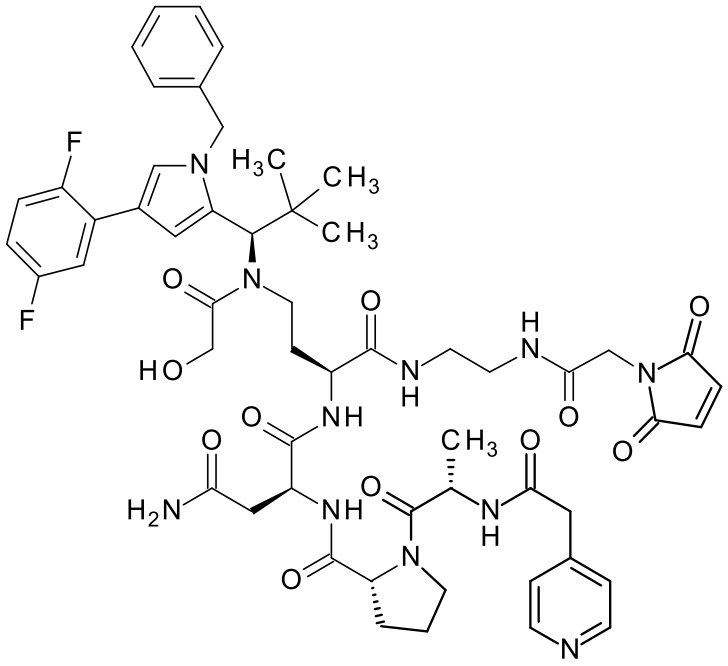


To a solution of 14.4 mg of Intermediate L118 (0.020 mmol) in 0.25 ml of DMF were added 9 µl of 4-ethylmorpholine (0.08 mmol) and 5.78 mg of HATU (0.015 mmol), and, after addition of 18 mg of Intermediate F307 (0.015 mmol), the mixture was stirred at RT for 30 minutes. Water (1.5 ml) and ACN (1.5 ml) were added. The reaction solution was purified by preparative HPLC (eluent: ACN/water + 0.1% TFA, gradient = 35% → 65%). 10 mg (36% of theory) of the title compound were obtained.

LC-MS (Method 14): R_t = 5.44 min; MS (ESIpos): m/z = 892.4320 $(M+2H)^{2+}$.

Intermediate R18

N-(Pyridin-4-ylacetyl)-L-alanyl-D-prolyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide

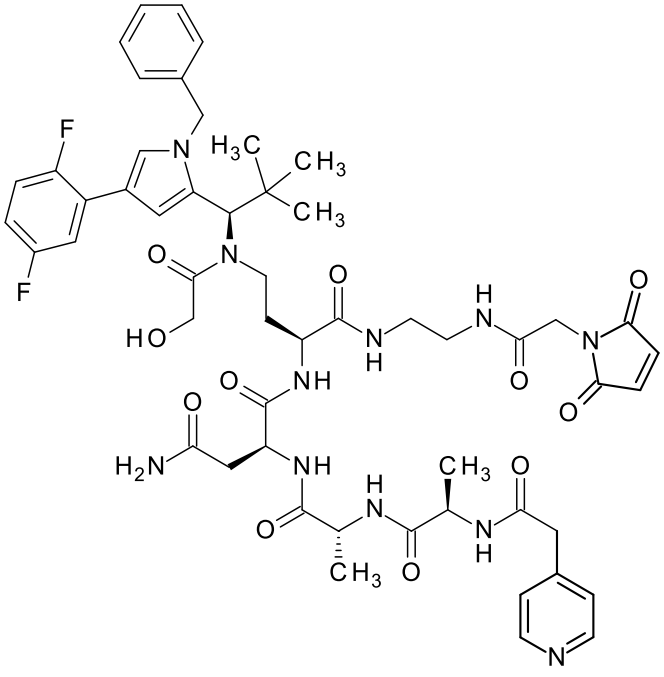


15 mg (0.019 mmol) of Intermediate F104 were coupled to 12 mg (0.022 mmol) of Intermediate L119 in analogy to Intermediate R1. Purification by preparative HPLC gave 4.7 mg (23% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 1094 $(M+H)^+$.

Intermediate R19

N-(Pyridin-4-ylacetyl)-D-alanyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide

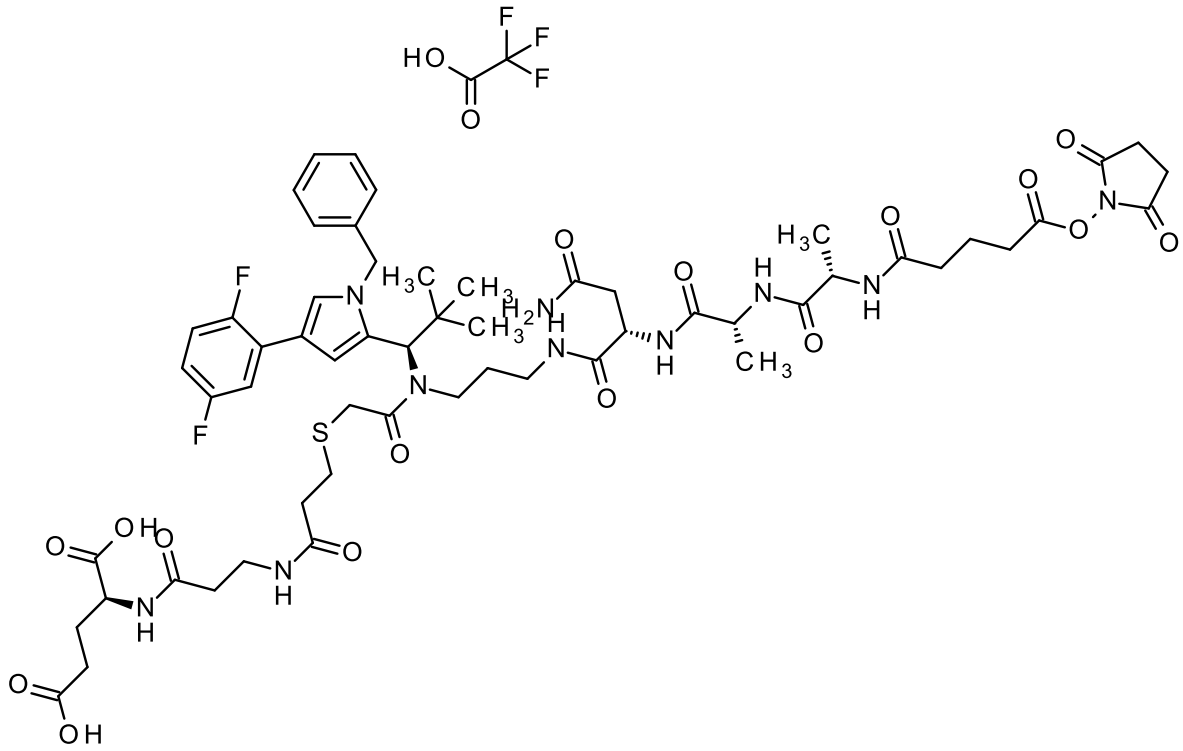


15 mg (0.019 mmol) of Intermediate F104 were coupled to 10.4 mg (0.02 mmol) of Intermediate L120 in analogy to Intermediate R1. Purification by preparative HPLC gave 5.7 mg (29% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.83 min; MS (ESIpos): m/z = 1068 $(M+H)^+$.

Intermediate R20

N-{5-[(2,5-Dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}-L-alanyl-D-alanyl-N^1-[(16S)-4-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-16,18-dicarboxy-5,10,14-trioxo-7-thia-4,11,15-triazaoctadec-1-yl]-L-aspartamide / trifluoroacetic acid salt

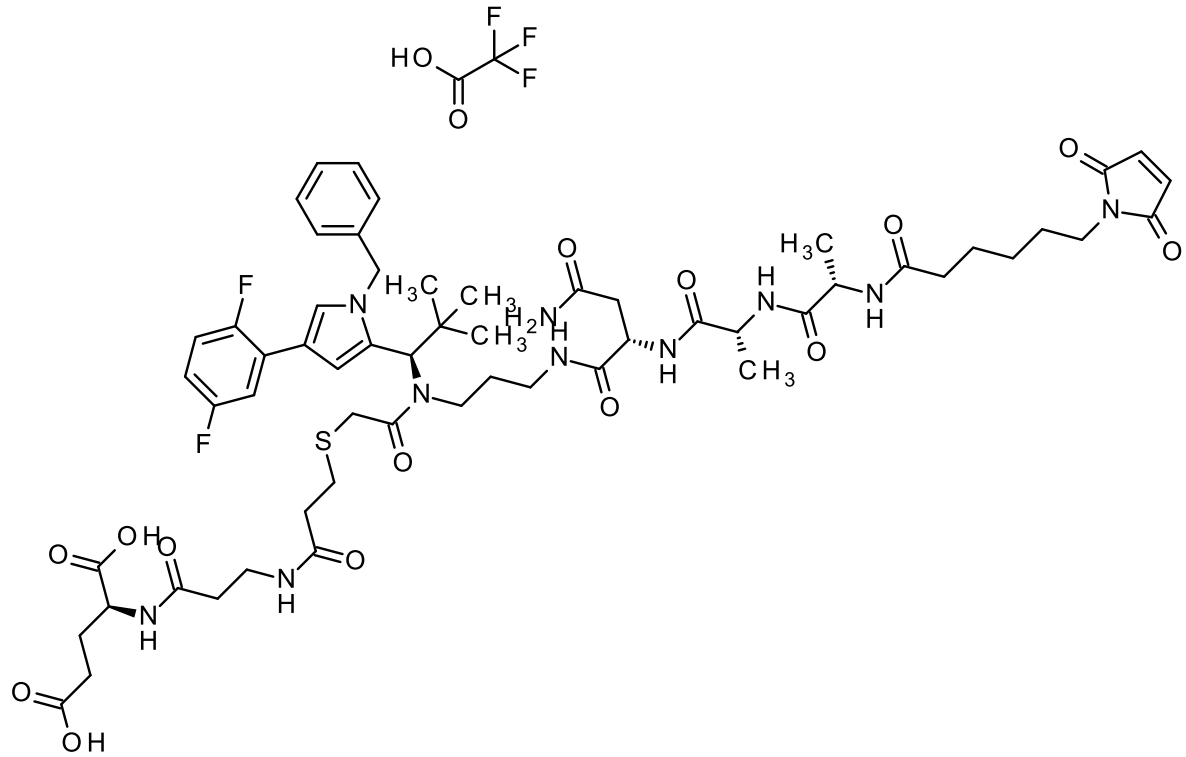


The title compound was prepared proceeding from compound C117, first by coupling to Intermediate L108 in the presence of HATU and N,N-diisopropylethylamine. In the next step, all protecting groups were removed by hydrogenation over 10% palladium on activated carbon in ethanol:ethyl acetate under standard hydrogen pressure at RT overnight and the deprotected intermediate was then converted to the title compound by reaction with 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine in DMF.

LC-MS (Method 1): R_t = 0.94 min; MS (ESIneg): m/z = 1223 $[M-H]^-$.

Intermediate R21

N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-alanyl-D-alanyl-N^1-[(16S)-4-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-16,18-dicarboxy-5,10,14-trioxo-7-thia-4,11,15-triazaoctadec-1-yl]-L-aspartamide / trifluoroacetic acid (1:1)

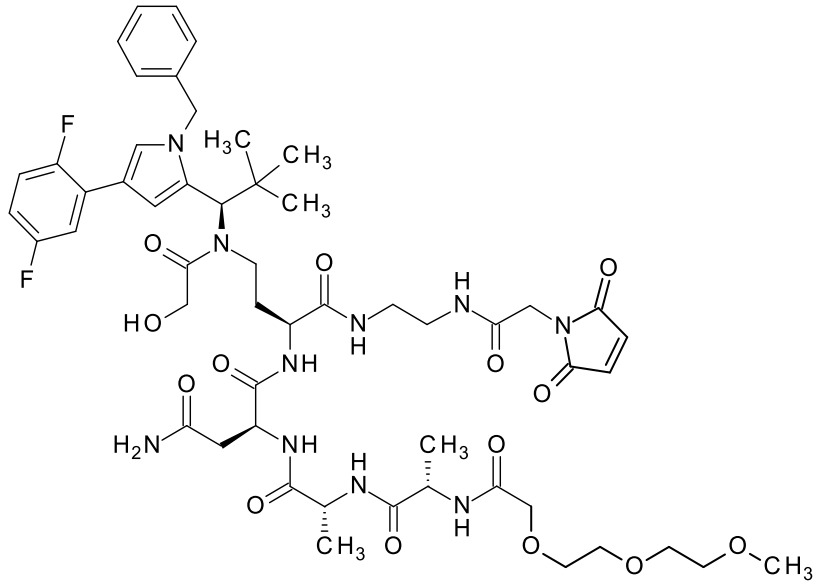


The title compound was prepared proceeding from compound C117, first by coupling to Intermediate L108 in the presence of HATU and N,N-diisopropylethylamine. In the next step, all protecting groups were removed by hydrogenation over 10% palladium on activated carbon in ethanol:ethyl acetate 1:1 under standard hydrogen pressure at RT overnight and the deprotected intermediate was then converted to the title compound by reaction with 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione in the presence of N,N-diisopropylethylamine in DMF.

LC-MS (Method 1): R_t = R_t = 0.97 min; MS (ESIneg): m/z = 1205 $[M-H]^-$.

Intermediate R22

N-{[2-(2-Methoxyethoxy)ethoxy]acetyl}-L-alanyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide

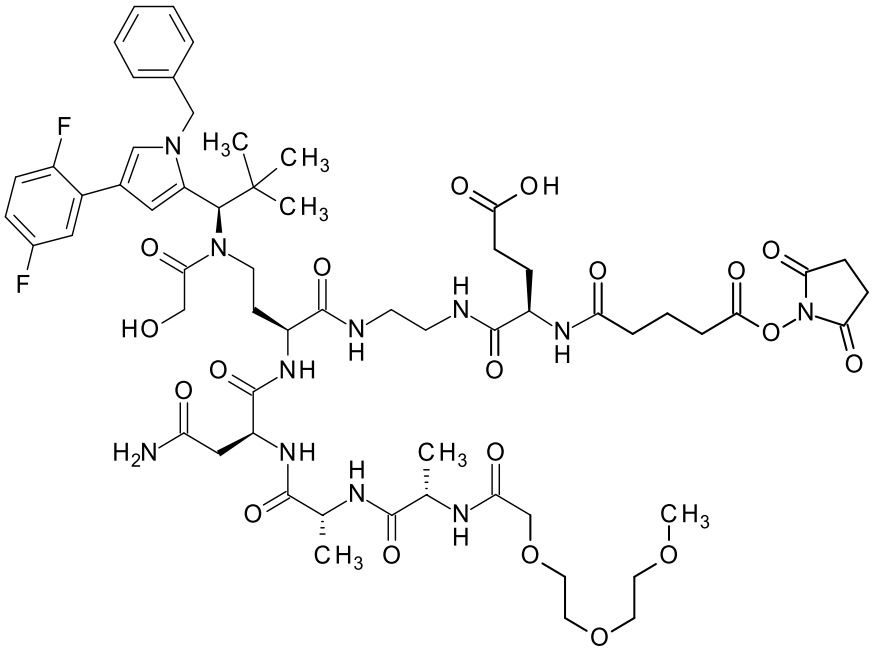


20 mg (0.025 mmol) of Intermediate F104 were coupled to 11.9 mg (0.027 mmol) of Intermediate L129 in analogy to Intermediate R1. Purification by preparative HPLC gave 13.4 mg (49% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.98 min; MS (ESIpos): m/z = 1109 $(M+H)^+$.

Intermediate R23

N-{[2-(2-Methoxyethoxy)ethoxy]acetyl}-L-alanyl-D-alanyl-N1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-({2-[(N-{5-[(2,5-dioxo pyrrolidin-1-yl)oxy]-5-oxopentanoyl}-D-alpha-glutamyl)amino]ethyl}amino)-1-oxobutan-2-yl]-L-aspartamide

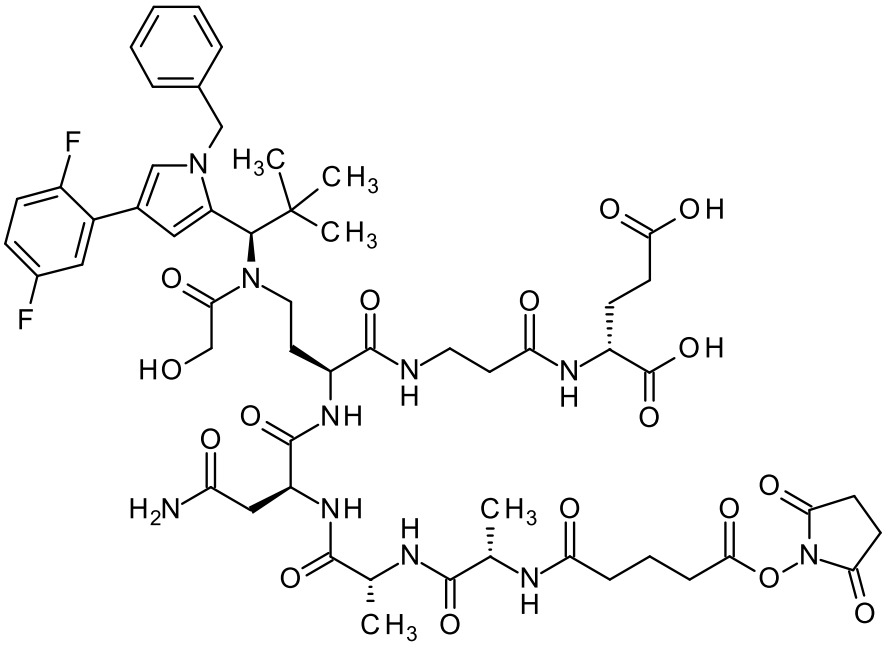


First of all, Intermediate C120 was coupled to (2R)-5-(benzyloxy)-2-{[(benzyloxy)carbonyl]amino}-5-oxopentanoic acid in the presence of HATU. Then the benzyloxycarbonyl protecting group and the benzyl ester were removed by hydrogenolysis over 10% palladium/activated carbon. The intermediate obtained was converted to the title compound with 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)] dipyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine in DMF.

LC-MS (Method 1): R_t = R_t = 0.94 min; MS (ESIpos): m/z = 1312 $[M+H]^+$.

Intermediate R24

N-{5-[(2,5-Dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}-L-alanyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(3-{[(1R)-1,3-dicarboxypropyl]amino}-3-oxopropyl)amino]-1-oxobutan-2-yl}-L-aspartamide

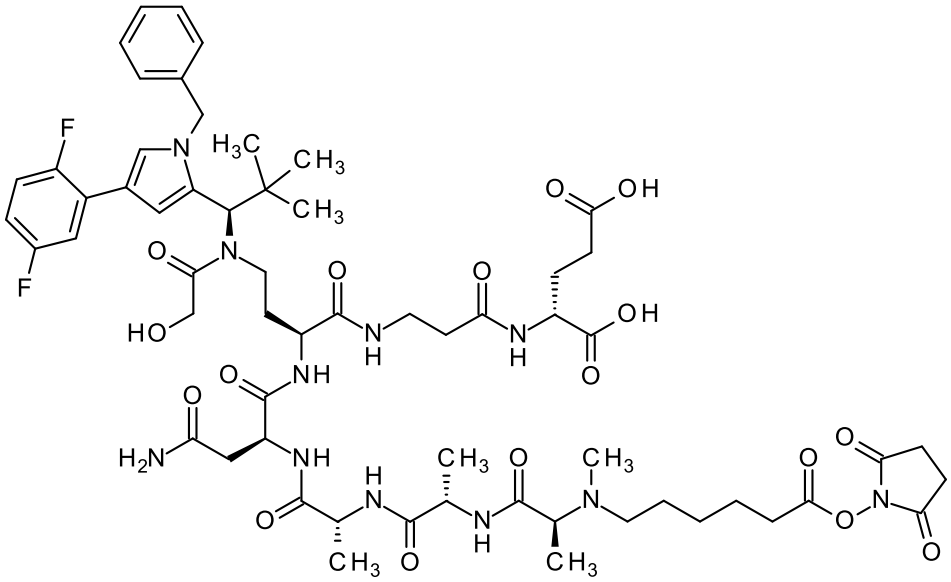


The title compound was prepared proceeding from compound C121, first by coupling to Intermediate L108 in the presence of HATU and N,N-diisopropylethylamine. In the next step, all protecting groups were removed by hydrogenation over 10% palladium on activated carbon in ethanol under standard hydrogen pressure at RT and the deprotected intermediate was then converted to the title compound by reaction with 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine in DMF.

LC-MS (Method 1): R_t = R_t = 0.92 min; MS (ESIpos): m/z = 1181 $[M+H]^+$.

Intermediate R25

N-{6-[(2,5-Dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-N-methyl-L-alanyl-L-alanyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(3-{[(1R)-1,3-dicarboxypropyl]amino}-3-oxopropyl)amino]-1-oxobutan-2-yl}-L-aspartamide



First, trifluoroacetic acid / 4-nitrobenzyl-L-alanyl-D-alanyl-L-asparaginate (1:1) was prepared by coupling N-(tert-butoxycarbonyl)-L-alanyl-D-alanine with 4-nitrobenzyl L-asparaginate hydrobromide (1:1) in DMF in the presence of HATU and N,N-diisopropylethylamine and then deprotecting the amino group with trifluoroacetic acid in DCM.

This intermediate was coupled to N-(tert-butoxycarbonyl)-N-methyl-L-alanine in DMF in the presence of HATU and N,N-diisopropylethylamine. Subsequently, the p-nitrobenzyl ester was detached by hydrogenation in DCM-methanol 1:1 over 10% palladium on activated carbon.

The intermediate thus obtained was coupled to Intermediate C121 in DMF in the presence of HATU and N,N-diisopropylethylamine. Subsequently, the Boc protecting group was detached by stirring with 4 equivalents of zinc chloride in trifluoroethanol at 50°C for 1 h. LC-MS (Method 1): R_t = 0.99 min; MS (ESIpos): m/z = 1235 $(M+H)^+$.

45 mg (33 µmol) of this intermediate were combined with 26 mg (200 µmol) of 6-oxohexanoic acid, which had been prepared beforehand by a literature method (J. Org. Chem. 1993, 58, 2196), in 20.5 ml of methanol, and 7.6 µl of acetic acid and 30 mg (320 µmol) of borane-pyridine complex were added. The mixture was stirred at RT for 4.5 h and then concentrated under reduced pressure and purified by preparative HPLC. 38 mg (84% of theory) of the intermediate were obtained.

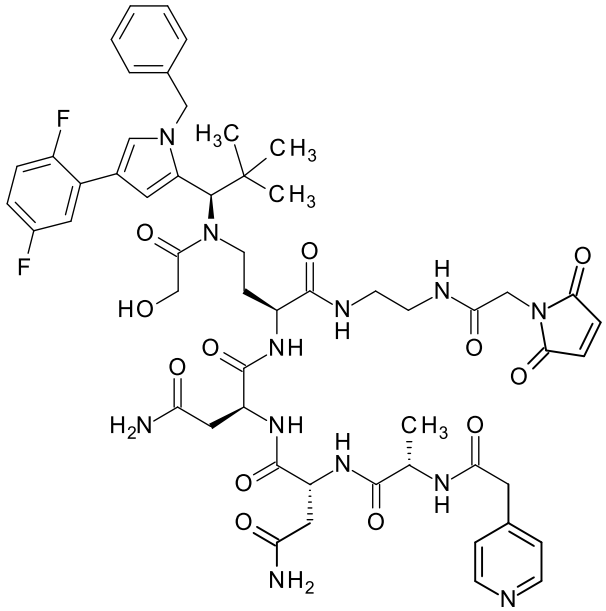
38 mg (0.028 mmol) of this intermediate were dissolved in 10 ml of DMF, and 53 mg (0.42 mmol) of 1-hydroxypyrrolidine-2,5-dione, 24.5 µl of *N,N*-diisopropylethylamine and, in

portions, a total of 77 mg (0.2 mmol) of HATU were added. After stirring at RT for 2 h, the reaction solution was adjusted to pH of 3-4 with TFA and then concentrated and purified by preparative HPLC. 39 mg (96%) of the protected intermediate were obtained, which were then taken up in 15 ml of ethanol. After 10% palladium on activated carbon had been added, the benzyl ester groups were removed by hydrogenolysis under standard hydrogen pressure and, after the catalyst had been filtered off, the remaining solution had been concentrated and then the residue had been lyophilized from acetonitrile/water 9:1, 34 mg (94% of theory) of the title compound were obtained.

LC-MS (Method 1): R_t = 0.8 min; MS (ESIpos): m/z = 1266 $(M+H)^+$.

Intermediate R26

N-(Pyridin-4-ylacetyl)-L-alanyl-D-asparaginyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide

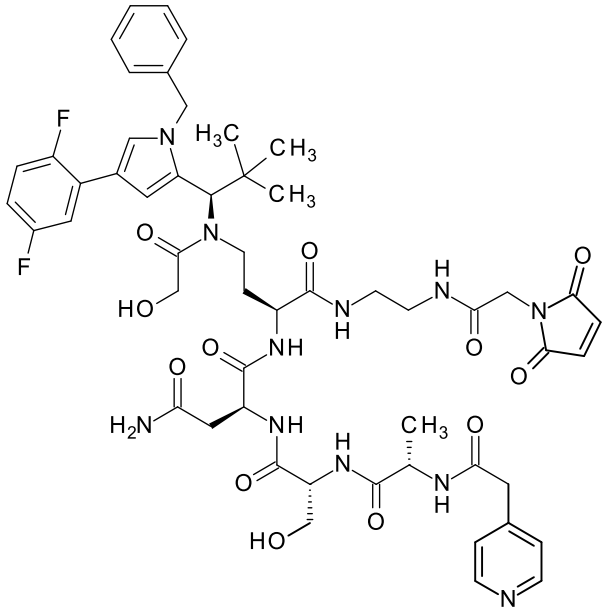


15 mg (0.019 mmol) of Intermediate F104 were coupled to 10.3 mg (0.022 mmol) of Intermediate L130 in analogy to Intermediate R1. Subsequently, the Boc protecting group was detached by stirring with 6 equivalents of zinc chloride in trifluoroethanol at 50°C for 2 h. In the last step, the intermediate was coupled to 4-pyridineacetic acid.

LC-MS (Method 1): R_t = 0.84 min; MS (ESIpos): m/z = 1111 $(M+H)^+$.

Intermediate R27

N-(Pyridin-4-ylacetyl)-L-alanyl-D-seryl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide



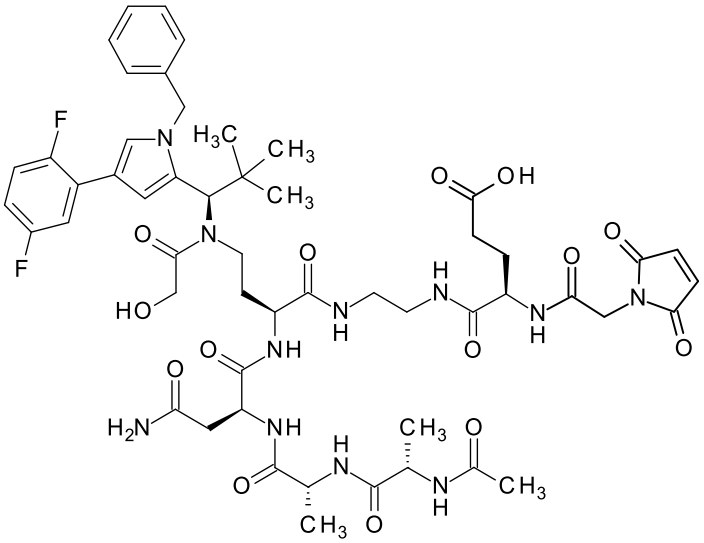
First of all, N-(pyridin-4-ylacetyl)-L-alanyl-D-serine was prepared by coupling of the pyridin-4-ylacetic acid hydrochloride (1:1) and tert-butyl L-alaninate hydrochloride (1:1) units by means of HATU, followed by tert-butyl ester cleavage with TFA in DCM, coupling to benzyl D-serinate hydrochloride (1:1) and finally by hydrogenolytic cleavage of the benzyl ester over 10% palladium/activated carbon.

8.4 mg (25 µmol) of this intermediate were then converted to the title compound by coupling to 20 mg (21.2 µmol) of Intermediate C116 in DMF in the presence of 9.7 mg (25.5 µmol) of HATU and 18.5 µl of *N,N*-diisopropylethylamine.

LC-MS (Method 5): R_t = 2.71 min; MS (ESIpos): m/z = 1084 $(M+H)^+$.

Intermediate R28

N-Acetyl-L-alanyl-D-alanyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-{[2-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-D-alpha-glutamyl}amino)ethyl]amino}-1-oxobutan-2-yl]-L-aspartamide

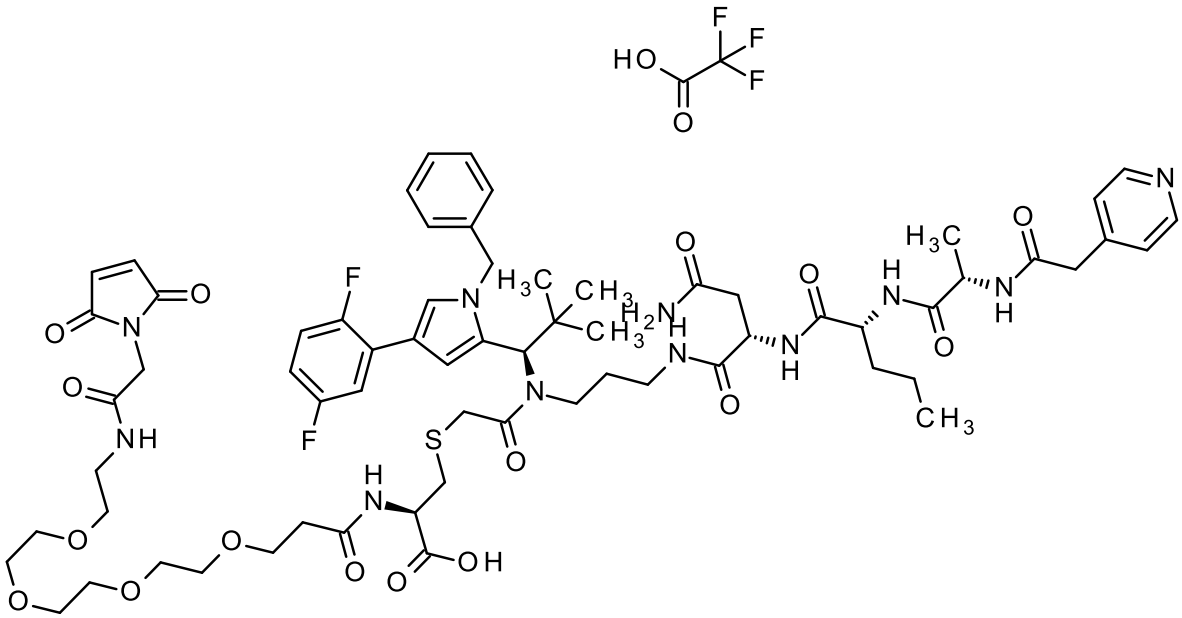


The title compound was prepared analogously to Intermediate R15. In the last step, instead of 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione, the maleimide derivative 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione was used in the coupling.

LC-MS (Method 1): R_t = 0.91 min; MS (ESIpos): m/z = 1121 $(M+H)^+$.

Intermediate R29

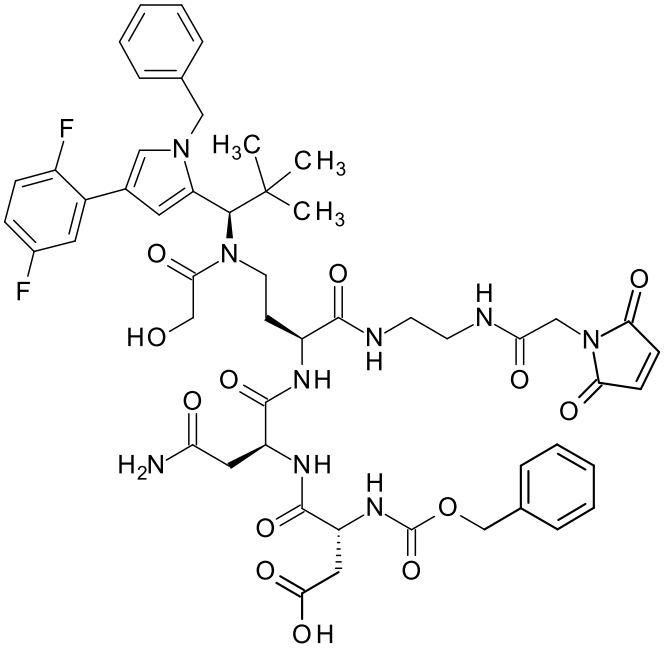
N-(Pyridin-4-ylacetyl)-L-alanyl-D-norvalyl-N^1-[(20R)-25-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-20-carboxy-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,18,24-trioxo-6,9,12,15-tetraoxa-22-thia-3,19,25-triazaoctacosan-28-yl]-L-aspartamide/ / trifluoroacetic acid (1:1)



The title compound was prepared proceeding from compound C119, first by coupling to Intermediate L126 in the presence of HATU and N,N-diisopropylethylamine. In the next step, the Teoc protecting group was removed by means of zinc chloride in trifluoroethanol and the deprotected intermediate was then converted to the title compound by reaction with 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-oic acid in the presence of HATU and N,N-diisopropylethylamine.
LC-MS (Method 14): R_t = 5.28 min; MS (ESIpos): m/z = 1360 $[M+H]^+$.

Intermediate R30

N-[(Benzyloxy)carbonyl]-D-alpha-asparagyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide

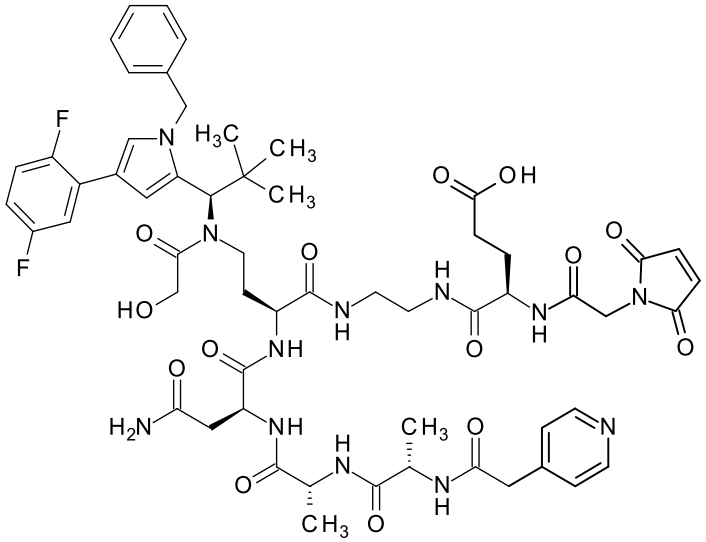


The title compound was prepared from compound C116 first by coupling to (2R)-2-{[(benzyloxy)carbonyl]amino}-4-tert-butoxy-4-oxobutanoic acid in the presence of HATU and N,N-diisopropylethylamine. Subsequently, the tert-butyl ester was detached by stirring at 50°C with 6 equivalents of zinc chloride in trifluoroethanol for 2 h and, after purification by preparative HPLC, the title compound was obtained.

LC-MS (Method 1): $R_t = R_t = 1.06$ min; MS (ESIpos): m/z = 1056 $[M+H]^+$.

Intermediate R31

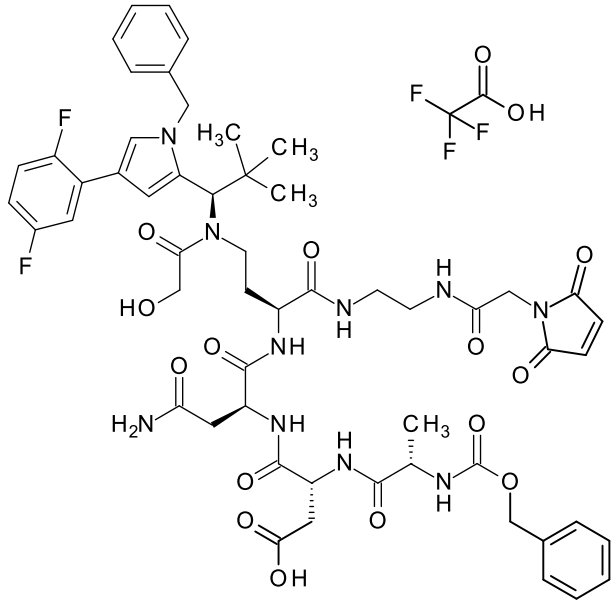
N-(Pyridin-4-ylacetyl)-L-alanyl-D-alanyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-{[2-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-D-alpha-glutamyl}amino)ethyl]amino}-1-oxobutan-2-yl]-L-aspartamide



The synthesis of the title compound commenced with the coupling of Intermediate C102 to Intermediate L131 in the presence of HATU and N,N-diisopropylethylamine. This was followed by hydrogenolytic detachment of the Z group with hydrogen under standard pressure over 10% palladium/activated carbon in ethanol. This was followed by coupling to Intermediate L110 in DMF in the presence of HATU and N,N-diisopropylethylamine. Then the Boc protecting group and the tert-butyl ester were detached by stirring with 6 equivalents of zinc chloride in trifluoroethanol at 50°C for 6 h. In the last step, the intermediate obtained was coupled to 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione in DMF in the presence of N,N-diisopropylethylamine and, after purification by preparative HPLC, the title compound was obtained.
LC-MS (Method 12): $R_t = R_t = 1.49$ min; MS (ESIpos): m/z = 1197 $[M+H]^+$.

Intermediate R32

N-[(Benzyloxy)carbonyl]-L-alanyl-D-alpha-asparagyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide / trifluoroacetic acid (1:1)

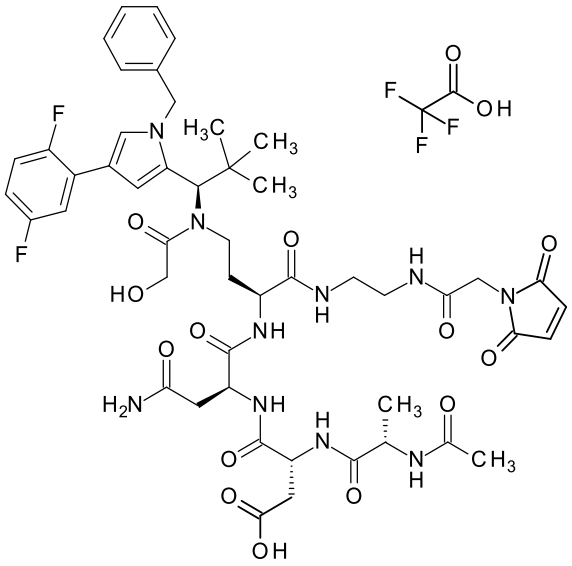


The synthesis of the title compound commenced with the HATU coupling, in the presence of N,N-diisopropylethylamine, of 2,5-dioxopyrrolidin-1-yl N-[(benzyloxy)carbonyl]-L-alaninate to (2R)-2-amino-4-tert-butoxy-4-oxobutanoic acid. The intermediate obtained was then reacted with Intermediate C116, likewise by means of HATU coupling in the presence of N,N-diisopropylethylamine. In the last step, the tert-butyl ester was detached by stirring at 50°C with 6 equivalents of zinc chloride in trifluoroethanol for 2 h.

LC-MS (Method 1): R_t = 1.05 min; MS (ESIpos): m/z = 1127 $(M+H)^+$.

Intermediate R33

N-Acetyl-L-alanyl-D-alpha-asparagyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide / trifluoroacetic acid (1:1)

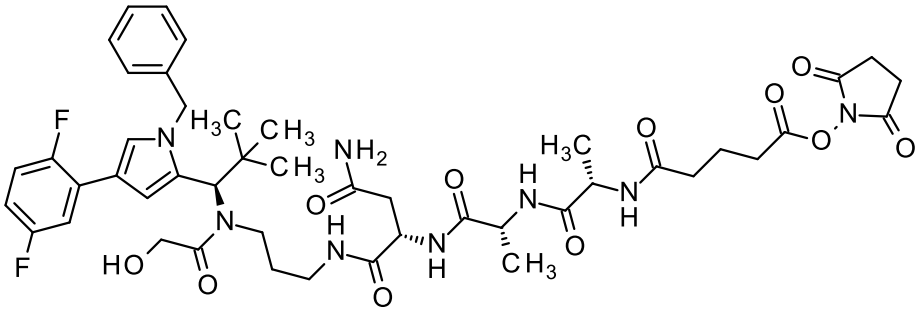


The synthesis of the title compound commenced with the HATU coupling, in the presence of N,N-diisopropylethylamine, of 2,5-dioxopyrrolidin-1-yl N-[(benzyloxy)carbonyl]-L-alaninate to (2R)-2-amino-4-tert-butoxy-4-oxobutanoic acid. This was followed by hydrogenolytic detachment of the Z group with hydrogen under standard pressure over 10% palladium/activated carbon in ethanol. Then reaction was effected with 1-acetoxypyrrolidine-2,5-dione in DMF in the presence of N,N-diisopropylethylamine.The intermediate obtained was then reacted with Intermediate C116 by means of HATU coupling in the presence of N,N-diisopropylethylamine. In the last step, the tert-butyl ester was detached by stirring at 50°C with 6 equivalents of zinc chloride in trifluoroethanol for 2 h.

LC-MS (Method 1): R_t = 0.95 min; MS (ESIpos): m/z = 1035 $(M+H)^+$.

Intermediate R34

N-{5-[(2,5-Dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}-L-alanyl-D-alanyl-N^1-{3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]propyl}-L-aspartamide

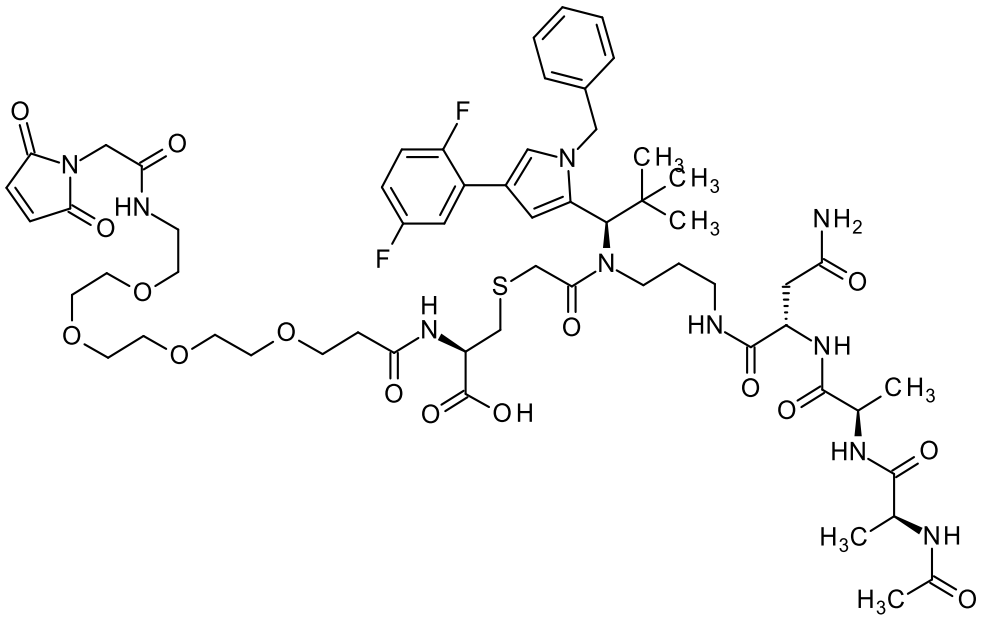


The title compound was prepared proceeding from compound M9, first by coupling to Intermediate L108 in the presence of HATU and N,N-diisopropylethylamine. In the next step, the Z protecting group was removed by hydrogenation over 10% palladium on activated carbon in DCM/methanol under standard hydrogen pressure at RT and the deprotected intermediate was then converted to the title compound by reaction with 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine in DMF.

LC-MS (Method 1): R_t = R_t = 1.01 min; MS (ESIpos): m/z = 937 $[M+H]^+$.

Intermediate R35

N-Acetyl-L-alanyl-D-alanyl-N^1-[(20R)-25-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-20-carboxy-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,18,24-trioxo-6,9,12,15-tetraoxa-22-thia-3,19,25-triazaoctacosan-28-yl]-L-aspartamide

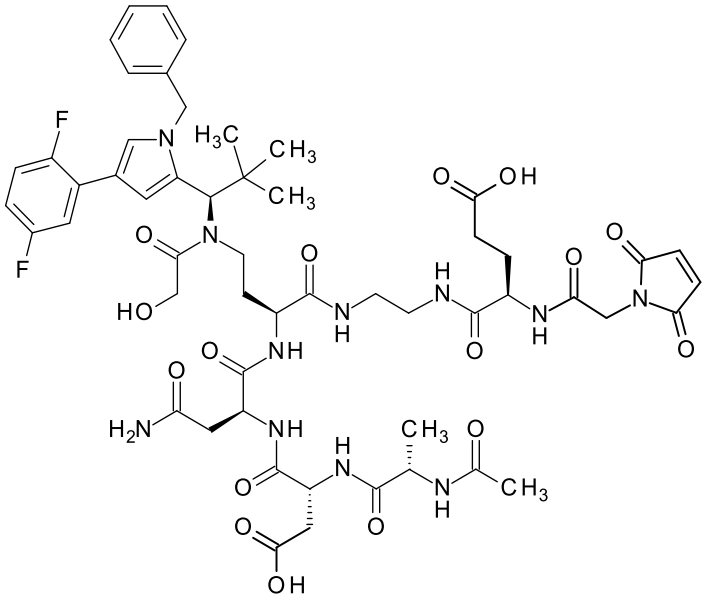


The title compound was prepared proceeding from compound C119, first by coupling to Intermediate L111 in the presence of HATU and N,N-diisopropylethylamine. In the next step, the Boc protecting group and the trimethylsilylethyl ester were removed by means of zinc chloride in trifluoroethanol and the deprotected intermediate was then converted to the title compound by reaction with 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-oic acid in the presence of HATU and N,N-diisopropylethylamine.

LC-MS (Method 12): R_t = 1.78 min; MS (ESIneg): m/z = 1253 $[M-H]^-$.

Intermediate R36

N-Acetyl-L-alanyl-D-alpha-asparagyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-{[2-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-D-alpha-glutamyl}amino)ethyl]amino}-1-oxobutan-2-yl]-L-aspartamide

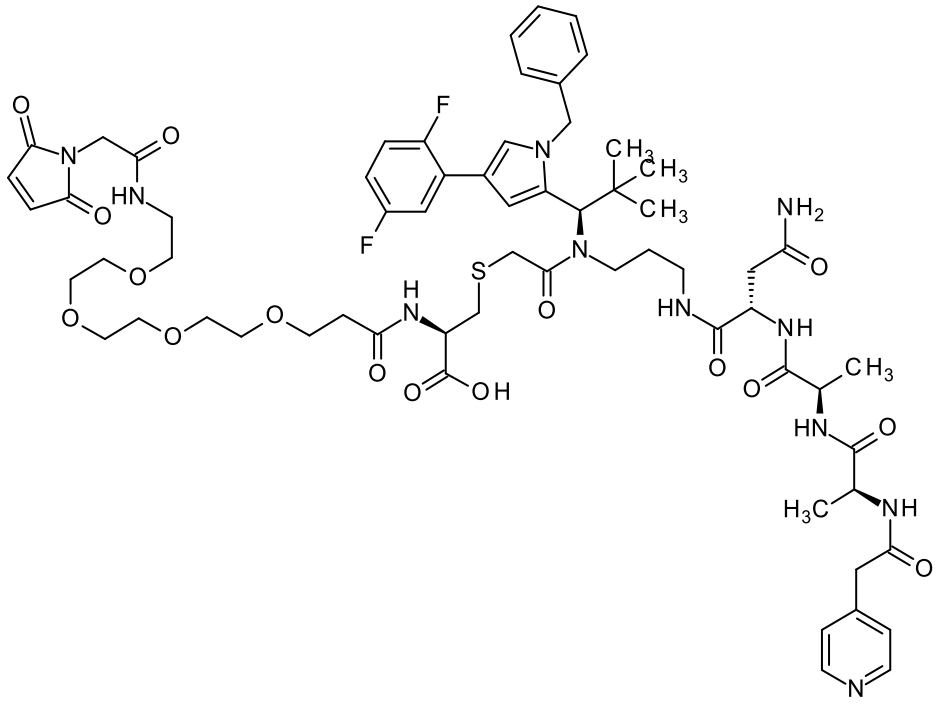


The synthesis of the title compound commenced with the HATU coupling, in the presence of N,N-diisopropylethylamine, of 2,5-dioxopyrrolidin-1-yl N-[(benzyloxy)carbonyl]-L-alaninate to (2R)-2-amino-4-tert-butoxy-4-oxobutanoic acid. This was followed by hydrogenolytic detachment of the Z group with hydrogen under standard pressure over 10% palladium/activated carbon in ethanol. Then reaction was effected with 1-acetoxypyrrolidine-2,5-dione in DMF in the presence of N,N-diisopropylethylamine.The intermediate obtained was then reacted with Intermediate C123 by means of HATU coupling in DMF in the presence of N,N-diisopropylethylamine. Then the tert-butyl ester and the Boc protecting group were detached by stirring at 50°C with 6 equivalents of zinc chloride in trifluoroethanol for 2 h. In the last step, the title compound was prepared by coupling to 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione in DMF in the presence of N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 0.89 min; MS (ESIpos): m/z = 1164 $(M+H)^+$.

Intermediate R37

N-(Pyridin-4-ylacetyl)-L-alanyl-D-alanyl-N^1-[(20R)-25-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-20-carboxy-1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,18,24-trioxo-6,9,12,15-tetraoxa-22-thia-3,19,25-triazaoctacosan-28-yl]-L-aspartamide

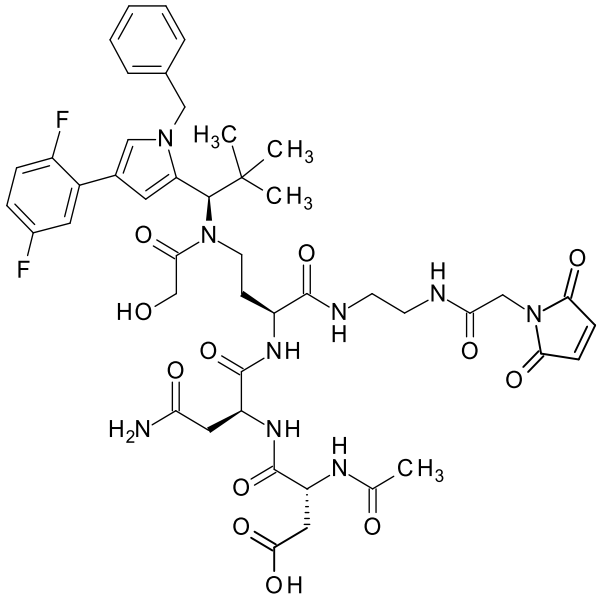


The title compound was prepared proceeding from compound C119, first by coupling to Intermediate L110 in the presence of HATU and N,N-diisopropylethylamine. In the next step, the Boc protecting group and the trimethylsilylethyl ester were removed by means of zinc chloride in trifluoroethanol and the deprotected intermediate was then converted to the title compound by reaction with 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-oic acid in the presence of HATU and N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1332 $[M+H]^+$.

Intermediate R38

N-Acetyl-D-alpha-asparagyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide

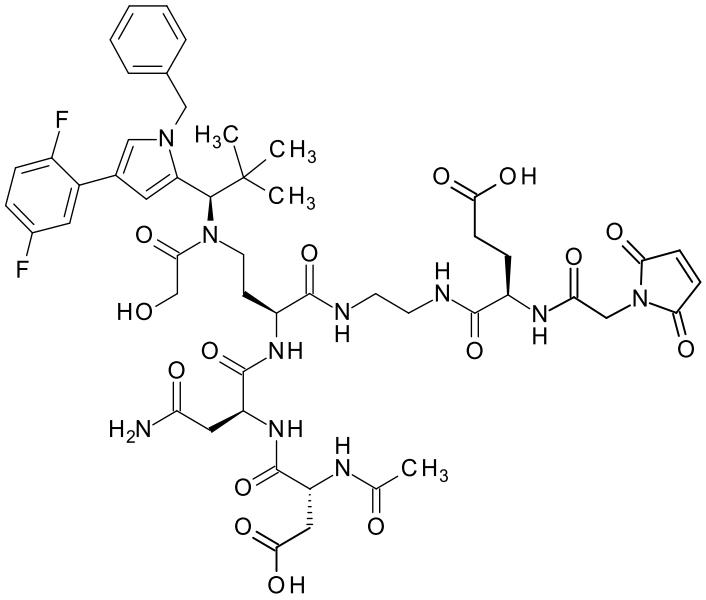


The synthesis of the title compound commenced with the reaction of 1-acetoxypyrrolidine-2,5-dione in DMF with (2R)-2-amino-4-tert-butoxy-4-oxobutanoic acid in the presence of N,N-diisopropylethylamine. The intermediate obtained was then reacted in DMF with Intermediate C116 by means of HATU coupling in the presence of N,N-diisopropylethylamine. In the last step, the tert-butyl ester was detached by stirring at 50°C with 6 equivalents of zinc chloride in trifluoroethanol for 40 min.

LC-MS (Method 1): R_t = 0.92 min; MS (ESIpos): m/z = 964 $(M+H)^+$.

Intermediate R39

N-Acetyl-D-alpha-asparagyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-{[2-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-D-alpha-glutamyl}amino)ethyl]amino}-1-oxobutan-2-yl]-L-aspartamide

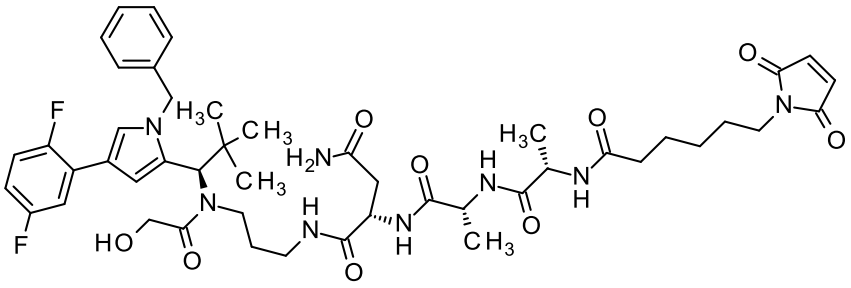


The synthesis of the title compound commenced with the reaction of 1-acetoxypyrrolidine-2,5-dione in DMF with (2R)-2-amino-4-tert-butoxy-4-oxobutanoic acid in the presence of N,N-diisopropylethylamine. The intermediate obtained was then reacted in DMF with Intermediate C123 by means of HATU coupling in the presence of N,N-diisopropylethylamine. Then the tert-butyl ester and the Boc protecting group were detached by stirring at 50°C with 8 equivalents of zinc chloride in trifluoroethanol for 8 h. In the last step, the title compound was prepared by coupling to 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione in DMF in the presence of N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 0.89 min; MS (ESIpos): m/z = 1093 $(M+H)^+$.

Intermediate R40

N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-alanyl-D-alanyl-N^1-{3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]propyl}-L-aspartamide

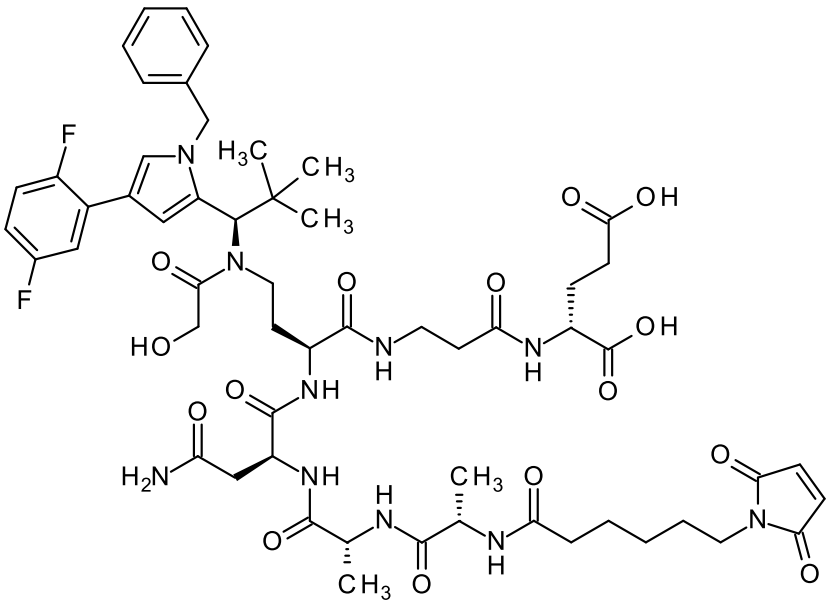


The title compound was prepared proceeding from compound M9, first by coupling to Intermediate L108 in the presence of HATU and N,N-diisopropylethylamine. In the next step, the Z protecting group was removed by hydrogenation over 10% palladium on activated carbon in DCM/methanol under standard hydrogen pressure at RT and the deprotected intermediate was then converted to the title compound by reaction with 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione in the presence of N,N-diisopropylethylamine in DMF.

LC-MS (Method 1): R_t = R_t = 1.05 min; MS (ESIpos): m/z = 919 $[M+H]^+$.

Intermediate R41

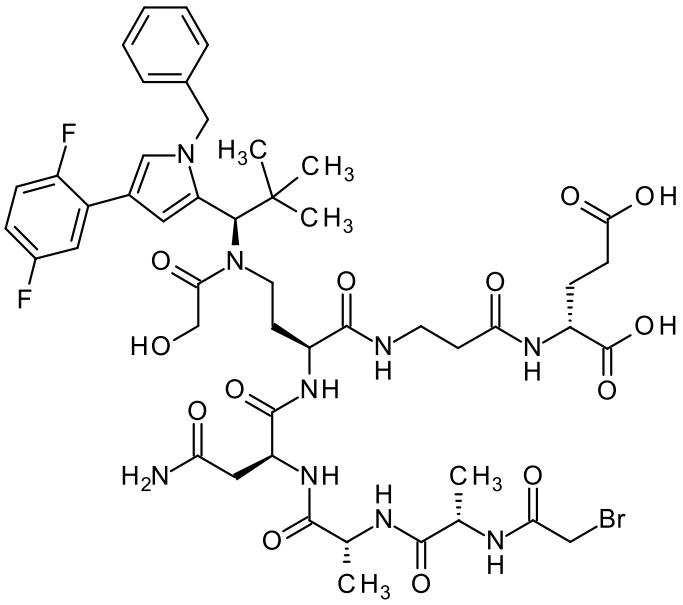
N-[6-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-alanyl-D-alanyl-N^1-{3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]propyl}-L-aspartamide



The title compound was prepared proceeding from compound C121, first by coupling to Intermediate L108 in the presence of HATU and N,N-diisopropylethylamine. In the next step, all protecting groups were removed by hydrogenation over 10% palladium on activated carbon in ethanol under standard hydrogen pressure at RT and the deprotected intermediate was then converted to the title compound by reaction with 1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl}-1H-pyrrole-2,5-dione in the presence of N,N-diisopropylethylamine in DMF.
LC-MS (Method 1): R_t = R_t = 0.96 min; MS (ESIpos): m/z = 1163 $[M+H]^+$.

Intermediate R42

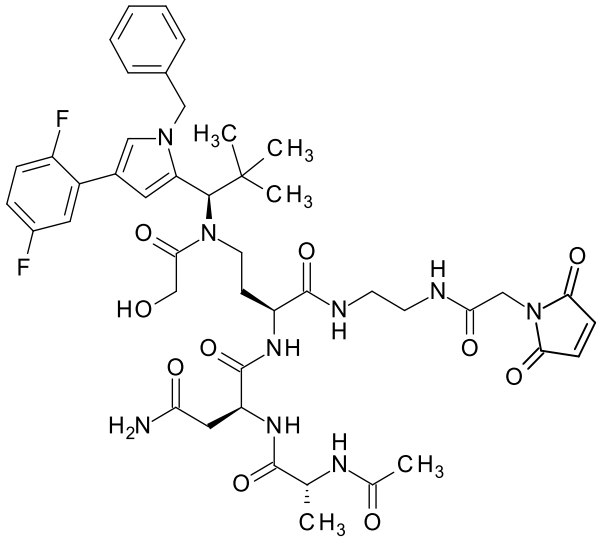
N-(Bromoacetyl)-L-alanyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(3-{[(1R)-1,3-dicarboxypropyl]amino}-3-oxopropyl)amino]-1-oxobutan-2-yl}-L-aspartamide



The title compound was prepared proceeding from compound C121, first by coupling to Intermediate L108 in the presence of HATU and N,N-diisopropylethylamine. In the next step, all protecting groups were removed by hydrogenation over 10% palladium on activated carbon in ethanol under standard hydrogen pressure at RT and the deprotected intermediate was then converted to the title compound by reaction with 1-(2-bromoacetoxy)pyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine in DMF.
LC-MS (Method 1): R_t = R_t = 0.93 min; MS (ESIpos): m/z = 1090 and 1092 $[M+H]^+$.

Intermediate R43

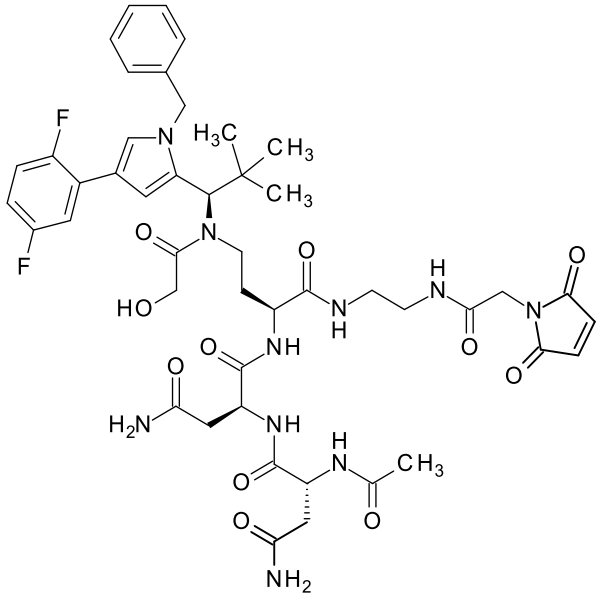
N-Acetyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-amino} ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide



81 mg (0.1 mmol) of Intermediate F104 were coupled to 43 mg (0.13 mmol) of 2,5-dioxopyrrolidin-1-yl N^2-(tert-butoxycarbonyl)-L-asparaginate in analogy to Intermediate R1. Then the Boc protecting group was detached by stirring at 50°C with 6 equivalents of zinc chloride in trifluoroethanol for 20 min. In the last step, the title compound was prepared by coupling to N-acetyl-D-alanine in DMF by means of HATU in the presence of N,N-diisopropylethylamine.
LC-MS (Method 12): R_t = 1.77 min; MS (ESIpos): m/z = 920 $(M+H)^+$.

Intermediate R44

N-Acetyl-D-asparaginyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-amino} ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide

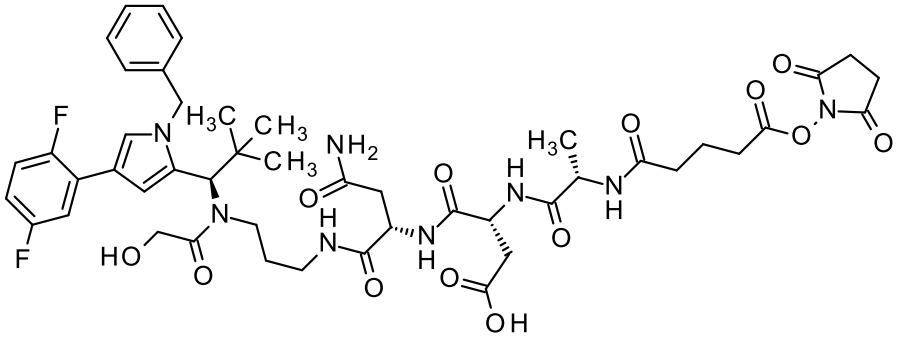


The title compound was prepared proceeding from compound C116 by coupling to Intermediate L132 in the presence of HATU and N,N-diisopropylethylamine.

LC-MS (Method 1): $R_t = R_t = 0.91$ min; MS (ESIpos): m/z = 963 $[M+H]^+$.

Intermediate R45

N-{5-[(2,5-Dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}-L-alanyl-D-alpha-asparagyl-N^1-{3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)-amino]propyl}-L-aspartamide

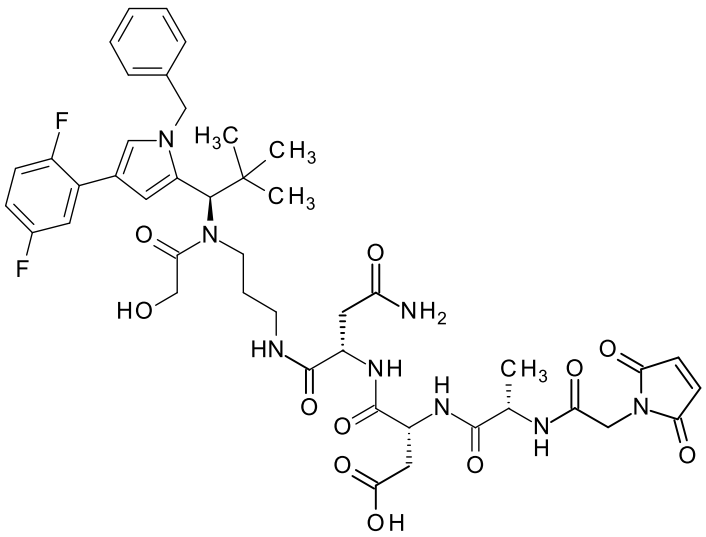


The title compound was prepared over several stages proceeding from compound M9 by methods of peptide chemistry known to those skilled in the art:

first by coupling of compound M9 to 4-nitrophenyl N^2-[(benzyloxy)carbonyl]-L-asparaginate in DMF in the presence of N,N-diisopropylethylamine; then subsequent detachment of the Z protecting group by hydrogenation over 10% palladium on activated carbon in ethanol under standard hydrogen pressure at RT; then subsequent coupling to (2R)-2-{[(benzyloxy)carbonyl]amino}-4-tert-butoxy-4-oxobutanoic acid in the presence of HATU and N,N-diisopropylethylamine; then subsequent detachment of the Z protecting group by hydrogenation over 10% palladium on activated carbon in DCM/methanol 1:1 under standard hydrogen pressure at RT; then subsequent coupling to N-[(benzyloxy)carbonyl]-L-alanine in the presence of HATU and N,N-diisopropylethylamine and another hydrogenolytic detachment of the Z protecting group; subsequent cleavage of the tert-butyl ester by stirring at 50°C with 6 equivalents of zinc chloride in trifluoroethanol for 3 hours and finally by reaction with 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine in DMF. LC-MS (Method 1): R_t = 0.98 min; MS (ESIpos): m/z = 981 $[M+H]^+$.

Intermediate R46

N-[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-alanyl-D-alpha-asparagyl-N^1-{3-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-propyl}-L-aspartamide

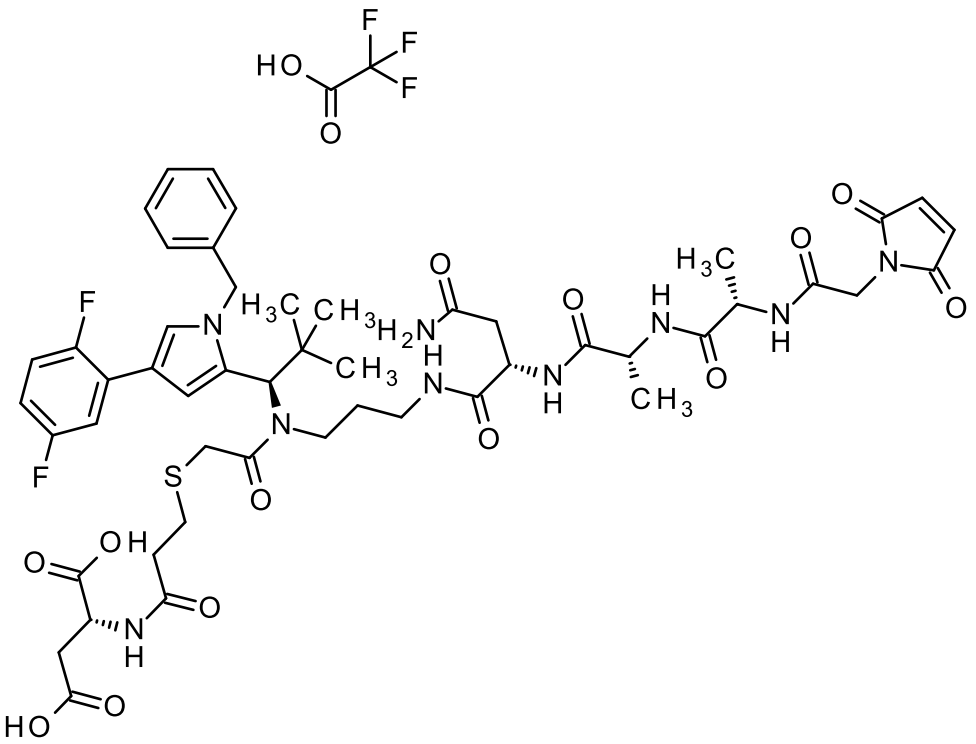


The title compound was prepared analogously to Intermediate R45. In the last step, however, the place of 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione in

the coupling was taken by 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione.
LC-MS (Method 1): R_t = R_t = 0.99 min; MS (ESIpos): m/z = 907 $[M+H]^+$

Intermediate R47

N-[(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-alanyl-D-alanyl-N^1-[3-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}[(3-{[(1R)-1,2-dicarboxyethyl]-amino}-3-oxopropyl)sulphanyl]acetyl}amino)propyl]-L-aspartamide trifluoroacetic acid salt

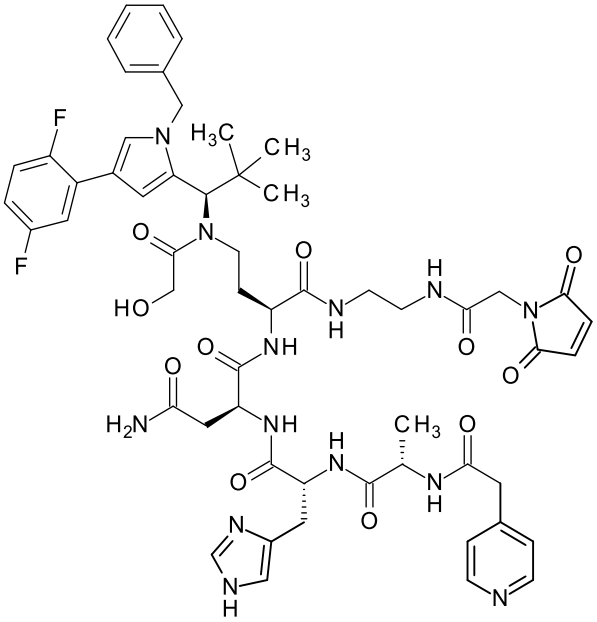


Intermediate C127 was dissolved in trifluoroethanol (2.0 ml), and zinc dichloride (4.18 mg, 30.6 µmol) was added. After stirring at 50°C for one hour, zinc chloride (4.18 mg, 30.6 µmol) was added and the reaction mixture was stirred at 50°C for a further hour. Zinc dichloride (4.18 mg, 30.6 µmol) was added again and the reaction mixture was stirred at 50°C for 1 h. Ethylenediamine-N,N,N',N'-tetraacetic acid (8.95 mg, 30.6 µmol) was added to the reaction mixture, which was stirred for 10 min, and then water (0.1% TFA) was added. Purification was effected directly by preparative RP-HPLC. The solvents were evaporated under reduced pressure and the residue was lyophilized. This gave 4.3 mg (71% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.95 min; MS (ESIneg): m/z = 1064 $[M-H]^-$

Intermediate R48

N-(Pyridin-4-ylacetyl)-L-alanyl-D-histidyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl)amino]-1-oxobutan-2-yl}-L-aspartamide



The title compound was prepared by coupling Intermediate C102 to tert-butyl (2-aminoethyl)carbamate in the presence of HATU and N,N-diisopropylethylamine, subsequent detachment of the Z protecting group by hydrogenation over 10% palladium on activated carbon in DCM-methanol 1:1 under standard hydrogen pressure at RT for 1 hour, followed by coupling to 4-nitrophenyl N^2-[(benzyloxy)carbonyl]-L-asparaginate in DMF and subsequent detachment of the Z protecting group by hydrogenation over 10% palladium on activated carbon in ethanol under standard hydrogen pressure at RT for 1 hour.

This intermediate was subsequently coupled to N-[(9H-fluoren-9-ylmethoxy)carbonyl]-1-{[2-(trimethylsilyl)ethoxy]carbonyl}-D-histidine, which had been prepared beforehand by

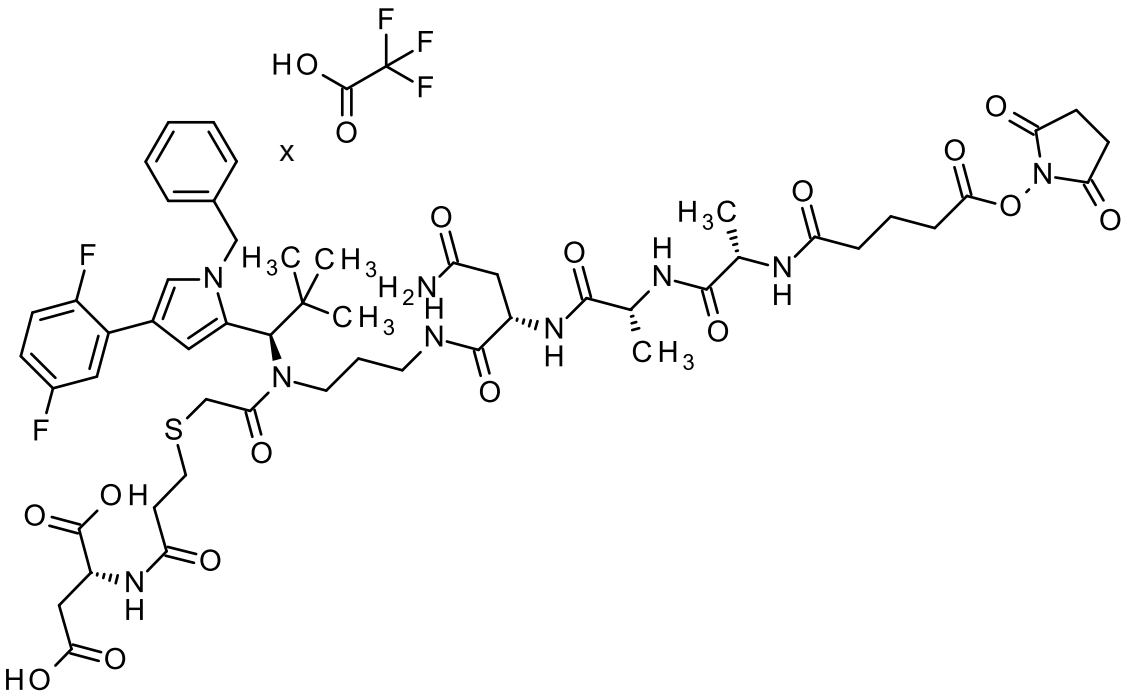
reaction of N-[(9H-fluoren-9-ylmethoxy)carbonyl]-D-histidine with 1-({[2-(trimethylsilyl)ethoxy]carbonyl}-oxy)pyrrolidine-2,5-dione in the presence of N,N-diisopropylethylamine in DMF, in the presence of HATU and N,N-diisopropylethylamine in DMF. Then the Fmoc protecting group was detached with piperidine in DMF. The deprotected compound was coupled in the presence of HATU and N,N-diisopropylethylamine to N-(pyridin-4-ylacetyl)-L-alanine hydrochloride (1:1) which had been prepared beforehand by reaction of pyridin-4-ylacetic acid hydrochloride (1:1) with tert-butyl L-alaninate hydrochloride (1:1) by means of HATU, and subsequent tert-butyl ester hydrolysis with TFA in DCM.

The Boc group was detached from the intermediate obtained by stirring at 50°C in trifluoroethanol with 6 equiv. of zinc chloride for one hour. In the last step, the title compound was obtained by reaction with 1-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1H-pyrrole-2,5-dione in DMF in the presence of N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 0.75 min; MS (ESIpos): m/z = 1134 $[M+H]^+$.

Intermediate R49

N-{5-[(2,5-Dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}-L-alanyl-D-alanyl-N^1-[3-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}[(3-{[(1R)-1,2-dicarboxyethyl]amino}-3-oxopropyl)sulphanyl]acetyl}amino)propyl]-L-aspartamide trifluoroacetic acid salt

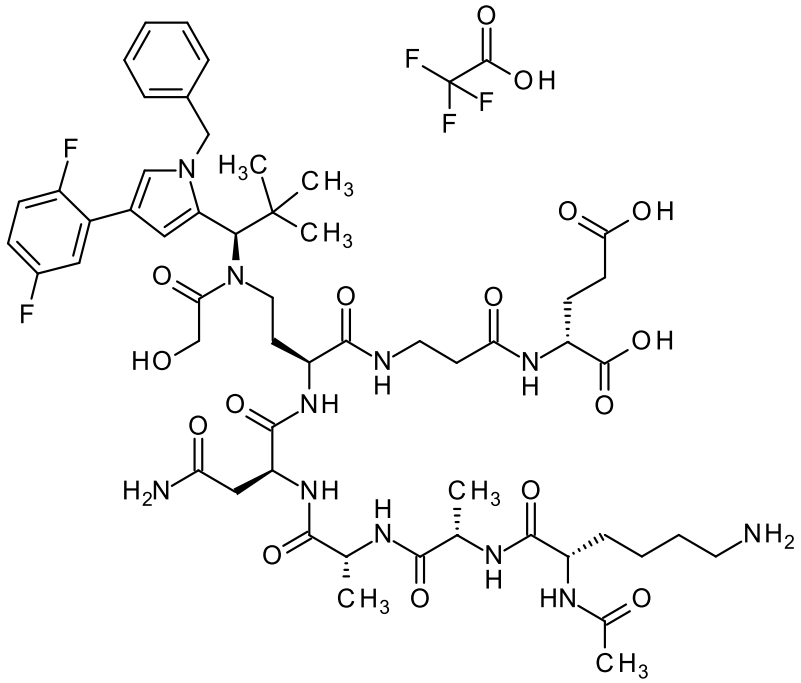


L-Alanyl-D-alanyl-N1-[3-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}{[(3-{[(1R)-1,2-dicarboxyethyl]amino}-3-oxopropyl)sulphanyl]acetyl}amino)-propyl]-L-aspartamide trifluoroacetic acid salt (7.60 mg, 6.57 µmol, Intermediate C129) and 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione (5.36 mg, 16.4 µmol) were dissolved in DMF (1.0 ml), and N,N-diisopropylethylamine (4.6 µl, 26 µmol) was added. The mixture was stirred at room temperature for 3.5 h. This was followed by quenching with water + 01% TFA, and the mixture was purified directly by means of preparative RP-HPLC. The solvents were evaporated under reduced pressure and the residue was lyophilized.

LC-MS (Method 1): R_t = 0.97 min; MS (ESIneg): m/z = 1138 $[M-H]^-$

Intermediate R50

N^2-Acetyl-L-lysyl-L-alanyl-D-alanyl-N^1-{(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-[(3-{[(1R)-1,3-dicarboxypropyl]amino}-3-oxopropyl)amino]-1-oxobutan-2-yl}-L-aspartamide trifluoroacetic acid salt



The title compound was prepared over several stages proceeding from compound C110D by methods of peptide chemistry known to those skilled in the art:

First of all, Intermediate C110D was coupled to Intermediate L134 in DMF in the presence of N,N-diisopropylethylamine and HATU.

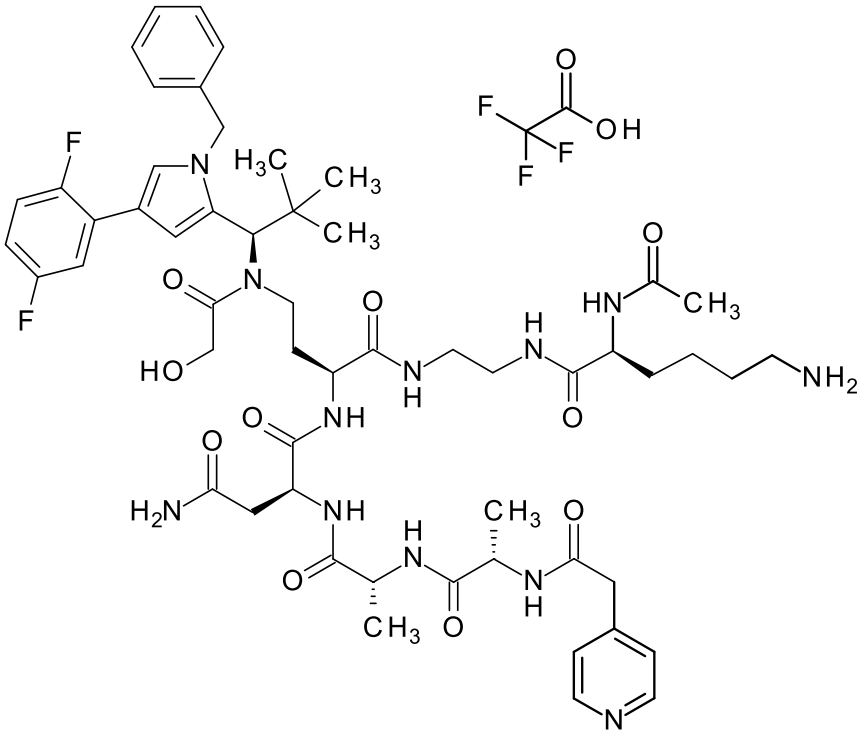
LC-MS (Method 1): R_t = 1.27 min; MS (ESIpos): m/z = 1454 $[M+H]^+$

Subsequently, the Z protective group was detached by hydrogenation over 10% palladium on activated carbon in dichloromethane/methanol 1:1 under hydrogen standard pressure at RT.

LC-MS (Method 1): R_t = 0.76 min; MS (ESIpos): m/z = 1140 $[M+H]^+$

Intermediate R51

Trifluoroacetic acid N-(pyridin-4-ylacetyl)-L-alanyl-D-alanyl-N^1-{(2S)-1-({2-[(N^2-acetyl-L-lysyl)amino]ethyl}amino)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-oxobutan-2-yl}-L-aspartamide salt



The title compound was prepared over several stages proceeding from compound C128 by methods of peptide chemistry known to those skilled in the art:

First of all, Intermediate C128 was coupled to Intermediate L110 in DMF in the presence of N,N-diisopropylethylamine and HATU.

LC-MS (Method 1): R_t = 0.97 min; MS (ESIpos): m/z = 1201 $[M+H]^+$

Subsequent cleavage of the tert- butyl ester by stirring at 50°C with 6 equivalents of zinc chloride in trifluoroethanol for 30 min gave the title compound.

LC-MS (Method 1): R_t = 0.72 min; MS (ESIpos): m/z = 1101 $[M+H]^+$

B: Preparation of antibody-drug conjugates (ADC)

B-1. General method for generation of antibodies

The protein sequence (amino acid sequence) of the antibodies used, for example TPP-2090, TPP-2658, TPP-5442, TPP-8825, TPP-7006, TPP-7007, TPP-10334, TPP-10335, TPP-10336, TPP-10337, TPP-1015, TPP-7510, TPP-7511, TPP-8382 and TPP-8567, was transformed into a DNA sequence that encodes the protein by a method well known to those skilled in the art and inserted into an expression vector suitable for transient mammalian cell culture (as described by Tom et al., Chapter 12 in Methods Express: Expression Systems, edited by Michael R. Dyson and Yves Durocher, Scion Publishing Ltd, 2007).

The commercially available antibody cetuximab (TPP-981; trade name: Erbitux) was used for the working examples described here.

B-2. General method for expression of antibodies in mammalian cells

The antibodies, for example TPP-2090, TPP-2658, TPP-5442, TPP-8825, TPP-7006, TPP-7007, TPP-10334, TPP-10335, TPP-10336, TPP-10337, TPP-1015, TPP-7510, TPP-7511, TPP-8382 and TPP-8567, were produced in transient mammalian cell cultures, as described by Tom et al., Chapter 12 in Methods Express: Expression Systems, edited by Michael R. Dyson and Yves Durocher, Scion Publishing Ltd, 2007.

B-3. General method for purification of antibodies from cell supernatants

The antibodies, for example TPP-2090, TPP-2658, TPP-5442, TPP-8825, TPP-7006, TPP-7007, TPP-10334, TPP-10335, TPP-10336, TPP-10337, TPP-1015, TPP-7510, TPP-7511, TPP-8382 and TPP-8567, were obtained from the cell culture supernatants. The cell supernatants were clarified by centrifugation of cells. The cell supernatant was then purified by affinity chromatography on a MabSelect Sure (GE Healthcare) chromatography column. To this end, the column was equilibrated in DPBS pH 7.4 (Sigma/Aldrich), the cell supernatant was applied and the column was washed with about 10 column volumes of DPBS pH 7.4 + 500 mM sodium chloride. The antibodies were eluted in 50 mM sodium acetate pH 3.5 + 500 mM sodium chloride and then purified further by gel filtration chromatography on a Superdex 200 column (GE Healthcare) in DPBS pH 7.4.

The commercially available antibodies were purified by standard chromatography methods (protein A chromatography, preparative gel filtration chromatography (SEC – size exclusion chromatography)).

B-4. General method for coupling to cysteine side chains

The following antibodies were used in the coupling reactions:

Examples a: cetuximab (anti-EGFR AK)

Examples e: TPP-1015 (anti-Her2 AK)

Examples h1: anti-B7H3 AK (TPP-8382)

Examples h2: anti-B7H3 AK (TPP-8567)

Examples k: anti-TWEAKR AK (TPP-2658)

Examples l1: anti-TWEAKR AK (TPP-7007)

Examples l2: anti-TWEAKR AK (TPP-7006)

Examples l3: anti-TWEAKR AK (TPP-10336)

Examples l4: anti-TWEAKR AK (TPP-10337)

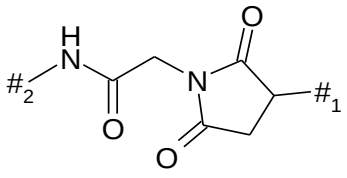
The coupling reactions were usually carried out under argon.

Between 2 and 5 equivalents of tris(2-carboxyethyl)phosphine hydrochloride (TCEP), dissolved in PBS buffer, were added to a solution of the appropriate antibody in PBS buffer in the concentration range between 1 mg/ml and 20 mg/ml, preferably in the range of about 10 mg/ml to 15 mg/ml, and the mixture was stirred at RT for 30 min to 1 h. For this purpose, the solution of the respective antibody used can be employed at the concentrations stated in the working examples, or it may optionally also be diluted with PBS buffer to about half of the stated starting concentrations in order to get into the preferred concentration range. Subsequently, depending on the intended loading, from 2 to 12 equivalents, preferably about 5-10 equivalents of the maleimide precursor compound or halide precursor compound to be coupled were added as a solution in

DMSO. Here, the amount of DMSO should not exceed 10% of the total volume. The mixture was stirred in the case of maleimide precursors for 60-240 min at RT and in the case of halide precursors between 8 and 24 h at RT and then applied to PBS-equilibrated PD 10 columns (Sephadex® G-25, GE Healthcare) and eluted with PBS buffer. Generally, unless indicated otherwise, 5 mg of the antibody in question in PBS buffer were used for the reduction and the subsequent coupling. Purification on the PD10 column thus in each case afforded solutions of the respective ADCs in 3.5 ml PBS buffer. The sample was then concentrated by ultracentrifugation and optionally rediluted with PBS buffer. If required, for better removal of low-molecular weight components, concentration by ultrafiltration was repeated after redilution with PBS buffer. For biological tests, if required, the concentrations of the final ADC samples were optionally adjusted to the range of 0.5-15 mg/ml by redilution. The respective protein concentrations, stated in the working examples, of the ADC solutions were determined. Furthermore, antibody loading (drug/mAb ratio) was determined using the methods described under B-7.

Depending on the linker, the ADCs shown in the examples may also be present to a lesser or higher degree in the form of the hydrolysed open-chain succinamides linked to the antibodies.

Particularly the KSP-I-ADCs linked via the linker substructure



to thiol groups of the antibodies can optionally also be prepared selectively by rebuffering after the coupling and stirring at pH 8 for about 20-24 h according to Scheme 28 via the ADCs linked via open-chain succinamides.

#1 represents the sulphur bridge to the antibody, and #2 the point of attachment to the modified KSP inhibitor

Such ADCs where the linker is attached to the antibodies through hydrolysed open-chain succinamides can optionally also be prepared selectively by an illustrative method as follows:

Small-scale coupling:

Between 2 and 5 equivalents of tris(2-carboxyethyl)phosphine hydrochloride (TCEP), dissolved in PBS buffer, were added to a solution of 2-5 mg of the appropriate antibody in PBS buffer in the concentration range between 1 mg/ml and 20 mg/ml, preferably in the range of about 5 mg/ml to 15 mg/ml, and the mixture was stirred at RT for 30 min to 1 h. Subsequently, depending on the intended loading, from 2 to 12 equivalents, preferably about 5-10 equivalents of the maleimide precursor compound to be coupled were added as a solution in DMSO. Here, the amount of DMSO should not exceed 10% of the total volume. The mixture was stirred at RT for 60-240 min and then diluted to a volume of 2.5-7.5 ml with PBS buffer which had been adjusted to pH 8 beforehand and then passed through a PD 10 column (Sephadex® G-25, GE Healthcare) equilibrated with PBS buffer pH 8, and eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight. Subsequently, the solution was concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2).

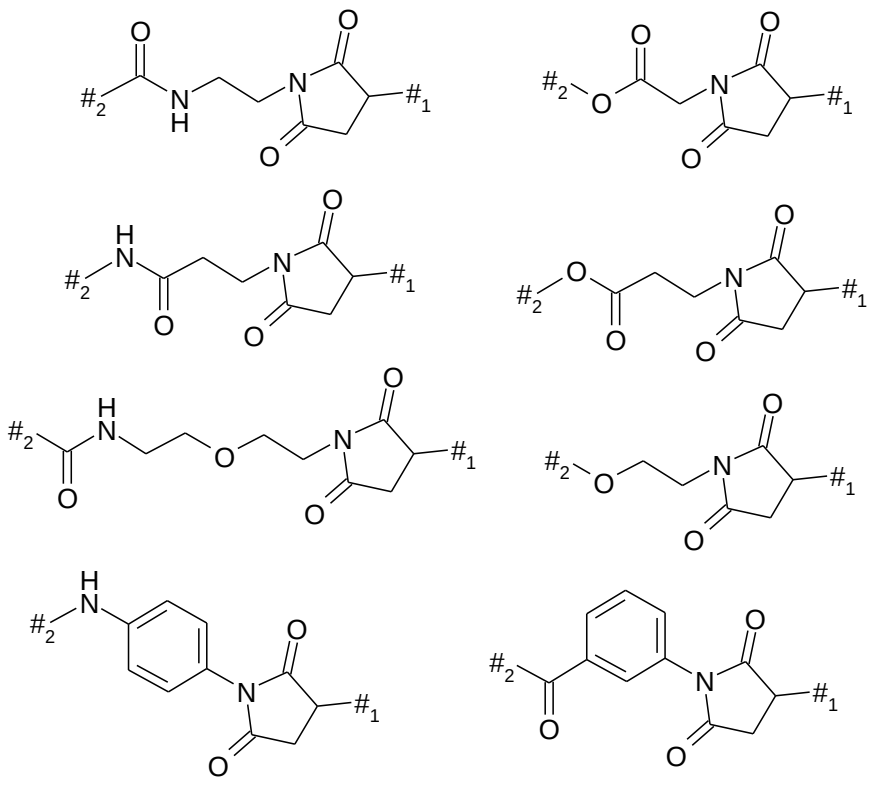
Medium-scale coupling:

Under argon, a solution of 2-5 equivalents, preferably 3 equivalents, of TCEP in PBS buffer (c ~ 0.2-0.8 mg/ml, preferably 0.5 mg/ml) were added to 20-200 mg of the antibody in question in PBS buffer (c ~ 5-15 mg/ml). The mixture was stirred at RT for 30 min, and then 2-12, preferably 5-10, equivalents of a maleimide precursor compound dissolved in DMSO were added. After stirring at RT for a further 1.5 h-2 h, the mixture was diluted with PBS buffer which had been adjusted to pH 8 beforehand.

This solution was then applied to PD 10 columns (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was diluted with PBS buffer pH 8 to a concentration of 1-7 mg/ml. This solution was stirred at RT under argon overnight. If required, the solution was then rebuffered to pH 7.2. The ADC solution was concentrated by ultracentrifugation, rediluted with PBS

buffer (pH 7.2) and then optionally concentrated again to a concentration of about 10 mg/ml.

Other potentially hydrolysis-sensitive thianylsuccinimide bridges to the antibody in the working examples contain the following linker substructures, where #1 represents the thioether linkage to the antibody and #1 the linkage site to the modified KSP inhibitor:



These linker substructures represent the linking unit to the antibody and have (in addition to the further linker composition) a significant effect on the structure and the profile of the metabolites formed in the tumour cells.

In the structural formulae shown, AK_1 has the meaning

Examples a: cetuximab (partially reduced)- S§[1]

Examples e: TPP-1015 (partially reduced)- S§[1]

Examples h1: anti-B7H3 AK (TPP-8382 partially reduced) - S§[1]

Examples h2: anti-B7H3 AK (TPP-8567 partially reduced) - S§[1]

Examples k: anti-TWEAKR AK (TPP-2658 partially reduced) - S§[1]

Examples l1: anti-TWEAKR AK (TPP-7007 partially reduced) - S§[1]

Examples l2: anti-TWEAKR AK (TPP-7006 partially reduced) - S§[1]

Examples l3: anti-TWEAKR AK (TPP-10336 partially reduced) - S§[1]

Examples l4: anti-TWEAKR AK (TPP-10337 partially reduced) - S§[1]

where

§[1] represents the linkage to the succinimide group or to any isomeric hydrolysed open-chain succinamides or the alkylene radical resulting therefrom,

and

S represents the sulphur atom of a cysteine residue of the partially reduced antibody.

B-5. General process for coupling to lysine side chains

The following antibodies were used for the coupling reactions:

Examples a: cetuximab (anti-EGFR AK)

Examples e: TPP-1015 (anti-Her2 AK)

Examples h1: anti-B7H3 AK (TPP-8382)

Examples h2: anti-B7H3 AK (TPP-8567)

Examples k: anti-TWEAKR antibody (TPP-2658)

Examples l1: anti-TWEAKR AK (TPP-7007)

Examples l2: anti-TWEAKR AK (TPP-7006)

Examples l3: anti-TWEAKR AK (TPP-10336)

Examples l4: anti-TWEAKR AK (TPP-10337)

The coupling reactions were usually carried out under argon.

From 2 to 8 equivalents of the precursor compound to be coupled were added as a solution in DMSO to a solution of the antibody in question in PBS buffer in a concentration range between 1 mg/ml and 20 mg/ml, preferably about 10 mg/ml, depending on the intended loading. After stirring at RT for 30 min to 6 h, the same amount of precursor compound in DMSO was added again. Here, the amount of DMSO should not exceed 10% of the total volume. After stirring at RT for a further 30 min to 6 h, the mixture was applied to PD 10 columns (Sephadex® G-25, GE Healthcare) equilibrated with PBS and eluted with PBS buffer. Generally, unless indicated otherwise, 5 mg of the antibody in question in PBS buffer were used for the coupling. Purification on the PD10 column thus in each case afforded solutions of the respective ADCs in 3.5 ml PBS buffer. The sample was then concentrated by ultracentrifugation and optionally rediluted with PBS buffer. If required, for better removal of low-molecular weight components, concentration by ultrafiltration was repeated after redilution with PBS buffer. For biological tests, if required, the concentrations of the final ADC samples were optionally adjusted to the range of 0.5-15 mg/ml by redilution.

The respective protein concentrations, stated in the working examples, of the ADC solutions were determined. Furthermore, antibody loading (drug/mAb ratio) was determined using the methods described under B-7.

In the structural formulae shown, AK_2 has the meaning

Examples a: cetuximab - NH§^{2}

Examples e: TPP-1015 - NH§^{2}

Examples h1: anti-B7H3 AK (TPP-8382) - NH§^{2}

Examples h2: anti-B7H3 AK (TPP-8567) - NH§^{2}

Examples k: anti-TWEAKR antibody (TPP-2658) - NH§^{2}

Examples l1: anti-TWEAKR AK (TPP-7007) - NH§^{2}

Examples l2: anti-TWEAKR AK (TPP-7006) - NH§^{2}

Examples l3: anti-TWEAKR AK (TPP-10336) - NH§^{2}

Examples l4: anti-TWEAKR AK (TPP-10337) - NH§^{2}

where

§^{2} represents the linkage to the carbonyl group

and

NH represents the side-chain amino group of a lysine residue of the antibody.

B-5a. General method for ADC synthesis by means of bacterial transglutaminase

In the coupling reactions in example series t, the antibodies which follow were used (the antibody-HC-N297Z nomenclature which follows means the antibody where the amino acid N297 (Kabat numbering) has been exchanged for the amino acid Z in both heavy chains, the TPP-xxxx-HC-Q295N-HC-N297Q nomenclature means the antibody with the TPP-XXXX where the amino acid Q295 (Kabat numbering) has been exchanged for the amino acid N and the amino acid N297 (Kabat numbering) has been exchanged for the amino acid Q in both heavy chains. The antibody name of the original antibody may either be reported as the name (for example trastuzumab) or as TPP-XXXX (antibody with the TPP number XXXX)):

AK_{3a}: anti-TWEAKR antibody (TPP-2658) (corresponding to TPP-2090-HC-N297A)

AK_{3b}: anti-TWEAKR antibody (TPP-5442) (corresponding to TPP-2090-HC-N297Q)

AK_{3c}: anti-TWEAKR antibody (TPP-8225) (corresponding to TPP-2090-HC-Q295N-HC-N297Q)

AK_{3d}: anti-HER2 antibody (TPP-7510) (corresponding to TPP-1015-HC-N297A)

AK_{3e}: anti-HER2 antibody (TPP-7511) (corresponding to TPP-1015-HC-N297Q)

General procedure to achieve a maximum DAR of 2:

To a solution of 5 mg of the corresponding aglyco antibody variant (HC-N297A) in DPBS pH 7.4 (c ~ 5-15 mg/ml) were added 20 µl (6 equivalents) of a solution of a suitable toxophor linker precursor (e.g. Intermediates R50 and R51; 10 mM solution in DMSO). After incubation at 37°C for 5 min, 50 µl of a solution of recombinant bacterial transglutaminase solution in water (product number T001 from Zedira GmbH, Darmstadt, Germany) (25 U/ml) were added and incubation was continued at 37°C for a further 24 h. Then the reaction mixture was diluted with DPBS pH 7.4 to a total volume of 2.5 ml and passed by gel filtration through DPBS-equilibrated PD 10 columns (Sephadex® G-25, GE Healthcare) and eluted with DPBS buffer at pH 7.4. Subsequently, the ADC solution was concentrated by means of Amicon Ultracel-30K centrifugation (Millipore), and it was rediluted again with DPBS to a volume of about 2.5 ml. Finally, 0.00625 µmol of the b-transglutaminase blocker Zedira C100 in 12.5 µl of DPBS was added to the solution. The respective protein concentrations, stated in the working examples, of the ADC solutions were determined. Furthermore, antibody loading (drug/mAb ratio) was determined using the methods described under B-7.

General procedure to achieve a maximum DAR of 4:

To a solution of 5 mg of the corresponding aglyco antibody variant (HC-N297Q) in DPBS pH 7.4 (c ~ 5-15 mg/ml) were added 16-24 equivalents of a solution of a suitable toxophor linker precursor (e.g. Intermediate R50 and R51; 10 mM solution in DMSO). After incubation at 37°C for 5 min, 400 µl (10 U) of a solution of recombinant bacterial transglutaminase solution in water (product number T001 from Zedira GmbH, Darmstadt, Germany) (25 U/ml) were added and incubation was continued at 37°C for a further 24 h. Then the reaction mixture was diluted with DPBS pH 7.4 to a total volume of 2.5 ml and passed by gel filtration through DPBS-equilibrated PD 10 columns (Sephadex® G-25, GE Healthcare) and eluted with DPBS buffer at pH 7.4. Subsequently, the ADC solution was concentrated by means of Amicon Ultracel-30K centrifugation (Millipore), and it was rediluted again with DPBS to a volume of about 2.5 ml. Finally, 0.1 µmol of the b-transglutaminase blocker Zedira C100 in 200 µl of DPBS was added to the solution. The respective protein concentrations, stated in the working examples, of the ADC solutions were determined. Furthermore, antibody loading (drug/mAb ratio) was determined using the methods described under B-7.

General procedure for transglutaminase-mediated coupling on a larger scale to obtain a maximum DAR of 2:

To a solution of 30 mg of the aglycosylated variant (HC-N297A) of the particular antibody in DPBS pH 7.4 (c ~ 5-15 mg/ml) were added 6 equivalents of a solution of the appropriate toxophor linker precursor (10 mM in DMSO). After incubation at 37°C for 5 min, 200 µl (7.5 U) of a solution of recombinant bacterial transglutaminase in water (product number T001 from Zedira GmbH, Darmstadt, Germany) (25 U/ml) were added and incubation was continued at 37°C for a further 24 h. The reaction mixture was purified via gel filtration chromatography on a Superdex 200 column (GE Healthcare) in DPBS pH 7.4, in order to separate small molecules and the transglutaminase from the ADC. Subsequently, the ADC solution was concentrated to final concentrations of 5-25 mg/ml using Amicon Ultracel-30K centrifugation tubes (Millipore). The solution was then sterile-filtered.

The respective concentrations of the ADC solutions reported in the working examples were determined. The loading was determined by the methods described in Chapter B7. The ADC batches were characterized as indicated in the working examples.

General procedure for transglutaminase-mediated coupling on a larger scale to obtain a maximum DAR of 4:

To a solution of 30 mg of the aglycosylated variant (HC-N297Q) of the particular antibody in DPBS pH 7.4 (c ~ 5-15 mg/ml) were added 16-24 equivalents of a solution of the appropriate toxophor linker precursor (10 mM in DMSO). After incubation at 37°C for 5 min, 2400 µl (60 U) of a solution of recombinant bacterial transglutaminase in water (product number T001 from Zedira GmbH, Darmstadt, Germany) (25 U/ml) were added and incubation was continued at 37°C for a further 24 h. The reaction mixture was purified via gel filtration chromatography on a Superdex 200 column (GE Healthcare) in DPBS pH 7.4, in order to separate small molecules and the transglutaminase from the ADC. Subsequently, the ADC solution was concentrated to final concentrations of 5-25 mg/ml using Amicon Ultracel-30K centrifugation tubes (Millipore). The solution was then sterile-filtered.

The respective concentrations of the ADC solutions reported in the working examples were determined. The loading was determined by the methods described in Chapter B7. The ADC batches were characterized as indicated in the working examples.

In the structural formulae shown for example series t, AK_3 in each case has the following meaning:

AK_{3a}: anti-TWEAKR antibody (TPP-2658) (corresponding to TPP-2090-HC-N297A) – CO-§2

AK_{3b}: anti-TWEAKR antibody (TPP-5442) (corresponding to TPP-2090-HC-N297Q) – CO-§2

AK_{3c}: anti-TWEAKR antibody (TPP-8825) (corresponding toTPP-2090-HC-Q295N-HC-N297Q) – CO-$§_2$

AK_{3d}: anti-HER2 antibody (TPP-7510) (corresponding to TPP-1015-HC-N297A) – CO-$§_2$

AK_{3e}: anti-HER2 antibody (TPP-7511) (corresponding to TPP-1015-HC-N297Q) – CO-$§_2$

where

$§^2$ denotes the linkage to the amino group of a toxophor linker precursor,

and

CO represents the side-chain carbonyl group of a glutamine residue of the antibody.

B-6a. General method for preparation of closed succinimide-cysteine adducts:

In an illustrative embodiment, 10 µmol of the above-described maleimide precursor compounds were taken up in 3-5 ml of DMF, and 2.1 mg (20 µmol) of L-cysteine were added. The reaction mixture was stirred at RT for 2 h to 24 h, then concentrated under reduced pressure and then purified by preparative HPLC.

B-6aa. General method for preparation of isomeric open succinamide-cysteine adducts:

In an illustrative embodiment, 68 µmol of the maleimide precursor compounds described above were taken up in 15 ml of DMF, and 36 mg (136 µmol) of N-{[2-

(trimethylsilyl)ethoxy]carbonyl}-L-cysteine were added. The reaction mixture was stirred at RT for ~20 h, then concentrated under reduced pressure and then purified by preparative HPLC. The appropriate fractions were combined and the solvents were evaporated under reduced pressure, and the residue was then dissolved in 15 ml of THF/water 1:1. 131 µl of a 2M aqueous lithium hydroxide solution were added and the mixture was stirred at RT for 1 h. The reaction was then neutralized with a 1M hydrochloric acid, the solvent was evaporated under reduced pressure and the residue was purified by preparative HPLC. This gave ~50% of theory of the regioisomeric protected intermediates as a colourless foam.

In the last step, 0.023 mmol of these regioisomeric hydrolysis products were dissolved in 3 ml of 2,2,2-trifluoroethanol. 12.5 mg (0.092 mmol) of zinc chloride were added, and the reaction mixture was stirred at 50°C for 4 h. 27 mg (0.092 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were then added, and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave the hydrolysed open sulphanylsuccinamides as a regioisomer mixture.

Further purification and characterization of the conjugates according to the invention

After the reaction, in some instances the reaction mixture was concentrated, for example by ultrafiltration, and then desalted and purified by chromatography, for example using a Sephadex® G-25 column. Elution was carried out, for example, with phosphate-buffered saline (PBS). The solution was then sterile filtered and frozen. Alternatively, the conjugate can be lyophilized.

B-7. Determination of the antibody, the toxophor loading and the proportion of open cysteine adducts

For protein identification in addition to molecular weight determination after deglycosylation and/or denaturing, a tryptic digestion was carried out which, after denaturing, reduction and derivatization, confirms the identity of the protein via the tryptic peptides found.

The toxophor loading of the PBS buffer solutions obtained of the conjugates described in the working example was determined as follows:

Determination of toxophor loading of lysine-linked ADCs was carried out by mass spectrometry determination of the molecular weights of the individual conjugate species. Here, the antibody conjugates were first deglycosylated with PNGaseF, and the sample was acidified and, after HPLC separation/desalting, analysed by mass spectrometry using ESI-MicroTof$_Q$ (Bruker Daltonik). All spectra over the signal in the TIC (Total Ion Chromatogram) were added and the molecular weight of the different conjugate species was calculated based on MaxEnt deconvolution. The DAR (= drug/antibody ratio) was then calculated after signal integration of the different species. For this purpose, the sum total of the integration results for all species weighted by the toxophor count was divided by the sum total of the simply weighted integration results for all species.

The toxophor loading of cysteine-linked conjugates was determined by reversed-phase chromatography of the reduced and denatured ADCs. Guanidinium hydrochloride (GuHCl) (28.6 mg) and a solution of DL-dithiothreitol (DTT) (500 mM, 3 µl) were added to the ADC solution (1 mg/ml, 50 µl). The mixture was incubated at 55°C for one hour and analysed by HPLC.

HPLC analysis was carried out on an Agilent 1260 HPLC system with detection at 220 nm. A Polymer Laboratories PLRP-S polymeric reversed-phase column (catalogue number PL1912-3802) (2.1 x150 mm, 8 µm particle size, 1000 Å) was used at a flow rate of 1 ml/min with the following gradient: 0 min, 25%B; 3 min, 25%B; 28 min, 50%B. Eluent A consisted of 0.05% trifluoroacetic acid (TFA) in water, eluent B of 0.05% trifluoroacetic acid in acetonitrile.

The detected peaks were assigned by retention time comparison with the light chain (L0) and the heavy chain (H0) of the non-conjugated antibody. Peaks detected exclusively in the conjugated sample were assigned to the light chain with one toxophor (L1) and the heavy chains with one, two and three toxophors (H1, H2, H3).

Average loading of the antibody with toxophors was calculated from the peak areas determined by integration as double the sum of HC load and LC load, where LC load is calculated from the sum of the toxophor number-average weighed integration results of all LC peaks divided by the sum of the singly weighed integration results of all LC peaks, and where the HC load is calculated from the sum of the toxophor number-average weighed

integration results of all HC peaks divided by the sum of the singly weighed integration results of all HC peaks. In individual cases, it was be possible that, owing to co-elution of some peaks, it was not possible to determine toxophor loading accurately.

In the cases where light and heavy chains could not be separated sufficiently by HPLC, determination of toxophor loading of cysteine-linked conjugates was carried out by mass spectrometry determination of the molecular weights of the individual conjugate species at light and heavy chain.

For this purpose, guanidinium hydrochloride (GuHCl) (28.6 mg) and a solution of DL-dithiothreitol (DTT) (500 mM, 3 µl) were added to the ADC solution (1 mg/ml, 50 µl). The mixture was incubated for one hour at 55°C and analysed by mass spectrometry after online desalting using ESI-MicroTofQ (Bruker Daltonik).

For the DAR determination, all spectra were added over the signal in the TIC (Total Ion Chromatogram), and the molecular weight of the different conjugate species at light and heavy chain was calculated based on MaxEnt deconvolution. The average loading of the antibody with toxophors was determined from the peak areas determined by integration as twice the sum total of the HC loading and the LC loading. In this context, the LC loading is calculated from the sum total of the integration results for all LC peaks weighted by the toxophor count, divided by the sum total of the simply weighted integration results for all LC peaks, and the HC loading from the sum total of the integration results for all HC peaks weighted by the toxophor count, divided by the sum total of the simply weighted integration results for all HC peaks.

In the case of the open constructs, to determine the proportion of the open cysteine adduct, the molecular weight area ratio of closed to open cysteine adduct (molecular weight delta 18 daltons) of all singly conjugated light and heavy chain variants was determined. The mean of all variants yielded the proportion of the open cysteine adduct.

The toxophor loading of glutamine-linked conjugates was determined by reversed-phase chromatography of the reduced and denatured ADCs. Guanidinium hydrochloride (GuHCl) (28.6 mg) and a solution of DL-dithiothreitol (DTT) (500 mM, 3 µl) were added to the ADC solution (1 mg/ml, 50 µl). The mixture was incubated at 55°C for one hour and analysed by HPLC.

HPLC analysis was carried out on an Agilent 1260 HPLC system with detection at 220 nm. A Polymer Laboratories PLRP-S polymeric reversed-phase column (catalogue number PL1912-3802) (2.1 x150 mm, 8 µm particle size, 1000 Å) was used at a flow rate of 1 ml/min with the following gradient: 0 min, 31% B; 1 min, 31% B; 14 min, 38% B, 16 min, 95% B. Eluent A consisted of 0.05% trifluoroacetic acid (TFA) in water, eluent B of 0.05% trifluoroacetic acid in acetonitrile.

The detected peaks were assigned by retention time comparison with the light chain (L0) and the heavy chain (H0) of the non-conjugated antibody. Peaks detected exclusively in the conjugated sample were assigned to the heavy chains with one and two toxophors (H1, H2).

Average loading of the antibody with toxophors was calculated from the peak areas determined by integration as double the sum of HC load and LC load, where LC load is calculated from the sum of the toxophor number-average weighed integration results of all LC peaks divided by the sum of the singly weighed integration results of all LC peaks, and where the HC load is calculated from the sum of the toxophor number-average weighed integration results of all HC peaks divided by the sum of the singly weighed integration results of all HC peaks.

Alternatively, the toxophor loading of glutamine-linked ADCs was determined by mass spectrometry determination of the molecular weights of the individual conjugate species. In this case, the sample was acidified and, after HPLC separation/desalting, analysed by mass spectrometry using ESI-MicroTof$_Q$ (Bruker Daltonik). All spectra over the signal in the TIC (Total Ion Chromatogram) were added and the molecular weight of the different conjugate species was calculated based on MaxEnt deconvolution. The DAR (= drug/antibody ratio) was then calculated after signal integration of the different species. For this purpose, the sum total of the integration results for all species weighted by the toxophor count was divided by the sum total of the simply weighted integration results for all species.

B-8. Verification of the antigen binding of the ADCs

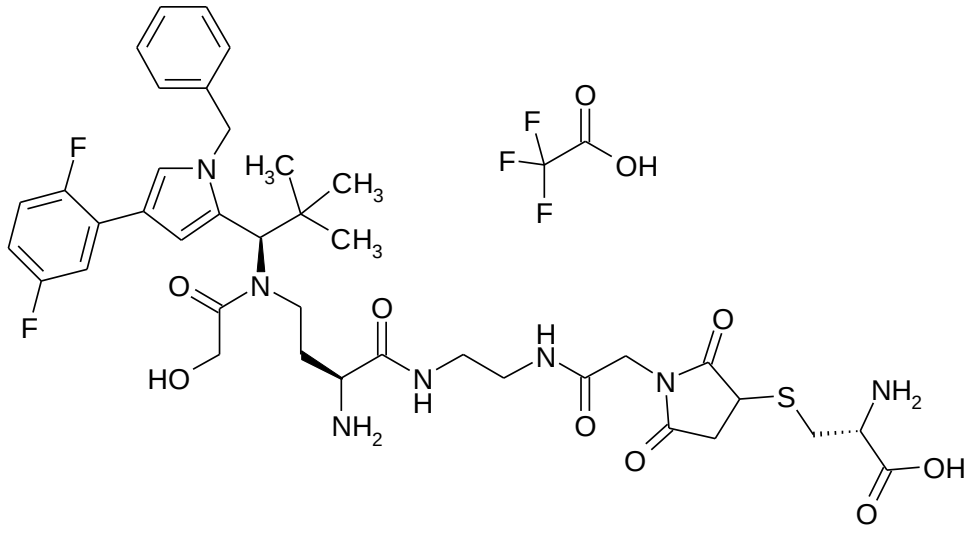
The capability of the binder of binding to the target molecule was checked after coupling had taken place. The person skilled in the art is familiar with various methods which can

be used for this purpose; for example, the affinity of the conjugate can be checked using ELISA technology or surface plasmon resonance analysis (BIAcore™ measurement). The conjugate concentration can be measured by the person skilled in the art using customary methods, for example for antibody conjugates by protein determination. (see also Doronina et al.; Nature Biotechnol. 2003; 21:778-784 and Polson et al., Blood 2007; 1102:616-623).

Working examples of metabolites

Example M1

S-[1-(2-{[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]amino}-2-oxoethyl)-2,5-dioxopyrrolidin-3-yl]-L-cysteine / trifluoroacetic acid (1:1)

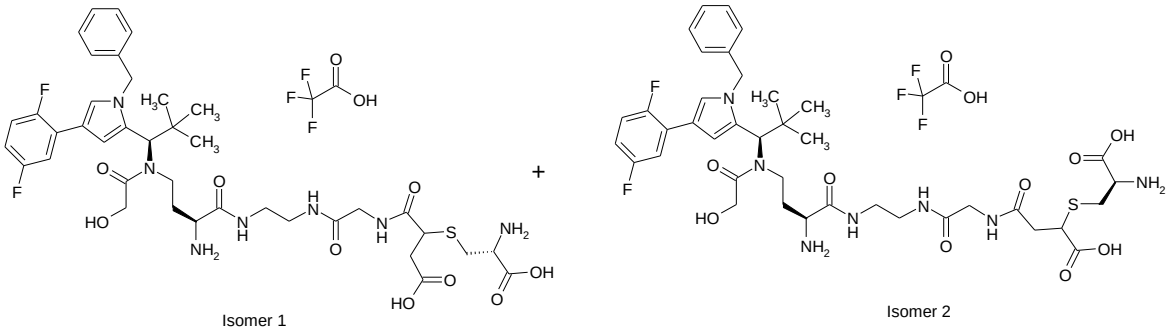


1.8 mg (2 µmol) of Intermediate F104 were taken up in 1 ml of DMF, and 2.7 mg (22 µmol) of L-cysteine were added. The reaction mixture was stirred at RT for 20 h, then concentrated under reduced pressure and then purified by preparative HPLC. 0.6 mg (26% of theory) of the title compound remained as a colourless foam.

LC-MS (Method 1): R_t = 0.80 min; MS (EIpos): m/z = 814 $[M+H]^+$.

Example M2

4-[(2-{[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]amino}-2-oxoethyl)amino]-3-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-4-oxobutanoic acid / trifluoroacetic acid (1:1)
and
4-[(2-{[2-({(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]amino}-2-oxoethyl)amino]-2-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-4-oxobutanoic acid / trifluoroacetic acid (1:1)



LC-MS (Method 1): R_t = 0.80 min; MS (EIpos): m/z = 814 $[M+H]^+$.

First, L-cysteine was converted with 1-({[2-(trimethylsilyl)ethoxy]carbonyl}oxy)pyrrolidine-2,5-dione in DMF in the presence of *N,N*-diisopropylethylamine into N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteine.

406 mg (1.53 mmol) of N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteine were dissolved in 10 ml of DMF, 157.5 mg (1.606 mmol) of maleic anhydride were added and the mixture was stirred at RT for 1 hour. 7.5 mg (0.01 mmol) of intermediate C66 were added to 130 µl of this solution, and the mixture was stirred at RT for 5 min. The mixture was then concentrated under reduced pressure, and the residue was purified by preparative HPLC. The solvent was evaporated under reduced pressure and the residue was dried under high vacuum. This gave 10 mg (89%) of the protected intermediate; it was not possible to separate the regioisomers neither by HPLC nor by LC-MS.

LC-MS (Method 1): R_t = 1.38 min; MS (EIpos): m/z = 1120 $[M+H]^+$.

In the last step, the 10 mg of this intermediate were dissolved in 2 ml of 2,2,2-trifluoroethanol. 12 mg (0.088 mmol) of zinc chloride were added, and the mixture was stirred at 50°C for 30 min. 26 mg (0.088 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were then added, and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 8.3 mg (99% of theory) of the title compound as a regioisomer mixture in a ratio of 87:13.

LC-MS (Method 5): R_t = 2.3 min and 2.43 min; MS (ESIpos): m/z = 832 $(M+H)^+$.

^{1}H-NMR main regioisomer: (500 MHz, DMSO-d_6): δ = 8.7 (m, 1H), 8.5 (m, 2H), 8.1 (m, 1H), 7.6 (m, 1H), 7.5 (s, 1H) 7.4-7.15 (m, 6H), 6.9-7.0 (m, 1H), 6.85 (s, 1H), 5.61 (s, 1H), 4.9 and 5.2 (2d, 2H), 4.26 and 4.06 (2d, 2H), 3.5-3.8 (m, 5H), 3.0-3.4 (m, 5H), 2.75-3.0 (m, 3H), 2.58 and 2.57 (dd, 1H), 0.77 and 1.5 (2m, 2H), 0.81 (s, 9H).

Alternatively, the regioisomeric title compounds were prepared as follows:

First of all, L-cysteine was converted with 1-({[2-(trimethylsilyl)ethoxy]carbonyl}oxy)pyrrolidine-2,5-dione in DMF in the presence of *N,N*-diisopropylethylamine to N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteine.

55 mg (0.068 mmol) of Intermediate F104 and 36 mg (0.136 mmol) of N-{[2-(trimethylsilyl) ethoxy]carbonyl}-L-cysteine were dissolved in 15 ml of DMF, and the mixture was stirred at RT for 20 h. The mixture was then concentrated and the residue was purified by preparative HPLC. The appropriate fractions were combined and the solvents were evaporated under reduced pressure, and the residue was then dissolved in 15 ml of THF/water 1:1. 131 µl of a 2M aqueous lithium hydroxide solution were added and the mixture was stirred at RT for 1 h. The mixture was then neutralized with a 1M hydrochloric acid, the solvent was evaporated under reduced pressure and the residue was purified by preparative HPLC. This gave 37 mg (50% of theory) of the regioisomeric protected intermediates as a colourless foam.

LC-MS (Method 5): R_t = 3.33 min and 3.36 min; MS (ESIpos): m/z = 976 $(M+H)^+$.

In the last step, 25 mg (0.023 mmol) of this intermediate were dissolved in 3 ml of 2,2,2-trifluoroethanol. 12.5 mg (0.092 mmol) of zinc chloride were added, and the reaction mixture was stirred at 50°C for 4 h. 27 mg (0.092 mmol) of ethylenediamine-N,N,N',N'-

tetraacetic acid were then added, and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 18.5 mg (85% of theory) of the title compound as a regioisomer mixture in a ratio of 21:79.

LC-MS (Method 5): R_t = 2.37 min and 3.44 min; MS (ESIpos): m/z = 832 $(M+H)^+$.

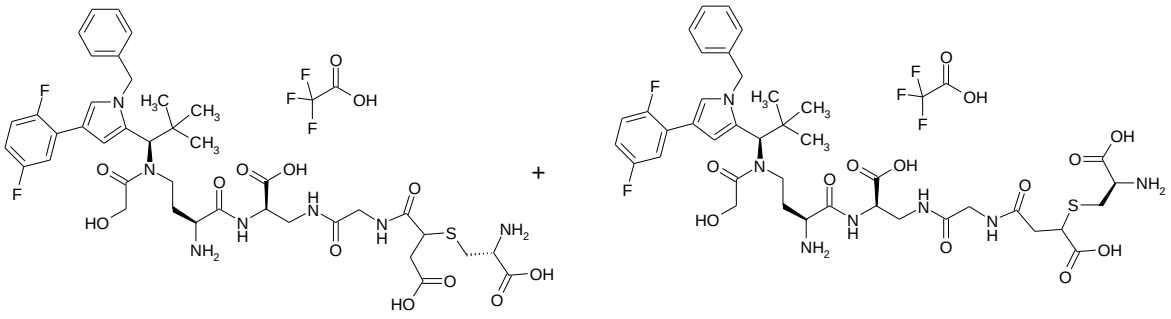
The targeted preparation of the individual regioisomers of the title compounds was carried out as follows:

Example M3

4-[(2-{[(2R)-2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)-2-carboxyethyl]amino}-2-oxoethyl)amino]-3-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-4-oxobutanoic acid / trifluoroacetic acid (1:1)

and

4-[(2-{[(2R)-2-({(2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)-2-carboxyethyl]amino}-2-oxoethyl)amino]-2-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-4-oxobutanoic acid / trifluoroacetic acid (1:1)



First of all, L-cysteine was converted with 1-({[2-(trimethylsilyl)ethoxy]carbonyl}oxy)pyrrolidine-2,5-dione in DMF in the presence of *N,N*-diisopropylethylamine to N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteine.

11 mg (0.013 mmol) of Intermediate F193 and 8 mg (0.016 mmol) of N-{[2-(trimethylsilyl) ethoxy]carbonyl}-L-cysteine were dissolved in 3 ml of DMF, and the mixture was stirred at RT for 20 h. The mixture was then concentrated and the residue was purified by preparative HPLC.

The appropriate fractions were combined and the solvents were evaporated under reduced pressure, and the residue was then dissolved in 2 ml of THF/water 1:1. 19 µl of a 2M aqueous lithium hydroxide solution were added and the mixture was stirred at RT for 1 h. Another 19 µl of the 2M aqueous lithium hydroxide solution were then added and the mixture was stirred at RT overnight. The mixture was then neutralized with a 1M hydrochloric acid, the solvent was evaporated under reduced pressure and the residue was purified by preparative HPLC. This gave 4.1 mg (38% of theory) of the regioisomeric protected intermediates as a colourless foam.

LC-MS (Method 1): Rt = 1.03 min (broad); MS (ESIpos): m/z = 1020 $(M+H)^+$.

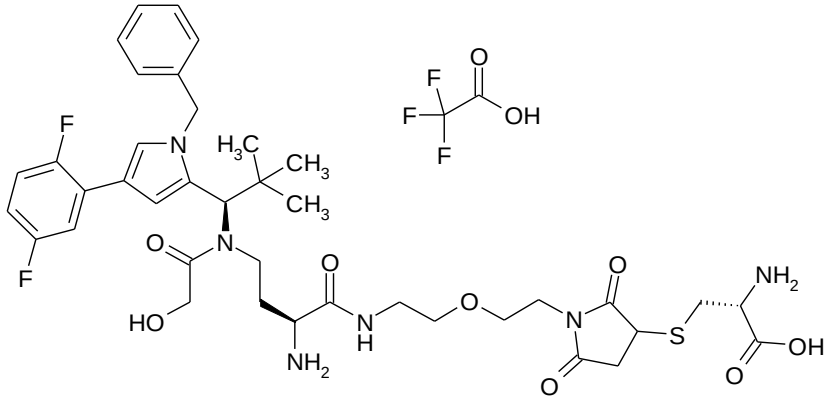
In the last step, 4.1 mg (0.004 mmol) of this intermediate were dissolved in 3 ml of 2,2,2-trifluoroethanol. 3 mg (0.022 mmol) of zinc chloride were added, and the reaction mixture was stirred at 50°C for 1 h. 6 mg (0.022 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid and 2 ml of a 0.1% aqueous trifluoroacetic acid were then added, and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 5 mg (quant.) of the title compound as a regioisomer mixture in a ratio of 20:80.

LC-MS (Method 1): R_t = 0.78 min (broad); MS (ESIpos): m/z = 876 $(M+H)^+$.

LC-MS (Method 5): R_t = 2.36 min and 2.39 min; MS (ESIpos): m/z = 876 $(M+H)^+$.

Example M4

S-(1-{2-[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethoxy]ethyl}-2,5-dioxopyrrolidin-3-yl)-L-cysteine / trifluoroacetic acid (1:1)

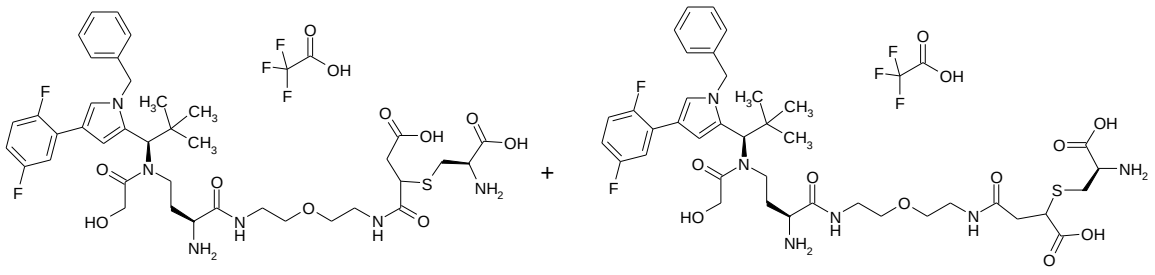


3 mg (4 µmol) of Intermediate F248 were taken up in 2 ml of DMF, and 0.9 mg (8 µmol) of L-cysteine was added. The reaction mixture was stirred at RT for 18 h and then concentrated under reduced pressure. The residue was purified by preparative HPLC. The appropriate fractions were concentrated, giving, after lyophilization of the residue from acetonitrile/water, 1.1 mg (32% of theory) of the title compound as a white solid.

LC-MS (Method 1): R_t = 0.78 min; MS (EIpos): m/z = 801 $[M+H]^+$.

Example M5

(3R,7S)-7-Amino-17-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-3-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-4-glycoloyl-2,2-dimethyl-8,16-dioxo-12-oxa-4,9,15-triazanonadecan-19-oic acid / trifluoroacetic acid (1:1)
and
(3R,7S)-7-amino-18-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-3-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-4-glycoloyl-2,2-dimethyl-8,16-dioxo-12-oxa-4,9,15-triazanonadecan-19-oic acid / trifluoroacetic acid (1:1)



8 mg (0.010 mmol) of the protected intermediate of Intermediate F248 and 5.1 mg (0.02 mmol) of N-{[2-(trimethylsilyl) ethoxy]carbonyl}-L-cysteine were dissolved in 3 ml of DMF, and the mixture was stirred at RT for 18 h and then treated in an ultrasonic bath for 2 h. The mixture was then concentrated and the residue was purified by preparative HPLC. The appropriate fractions were combined and the solvents were evaporated under reduced pressure, and the residue was then dissolved in 2 ml of THF/water 1:1. 15 µl of a 2M aqueous lithium hydroxide solution were added and the mixture was stirred at RT for 15 min. The mixture was then adjusted to a pH of ~3 with a 1M hydrochloric acid, diluted with 20 ml of sodium chloride solution and extracted twice with 20 ml of ethyl acetate. The organic phase was dried over magnesium sulphate and concentrated, and the residue was lyophilized from acetonitrile/water. This gave 8.4 mg (78% of theory over 2 steps) of the regioisomeric protected intermediates as a colourless foam.

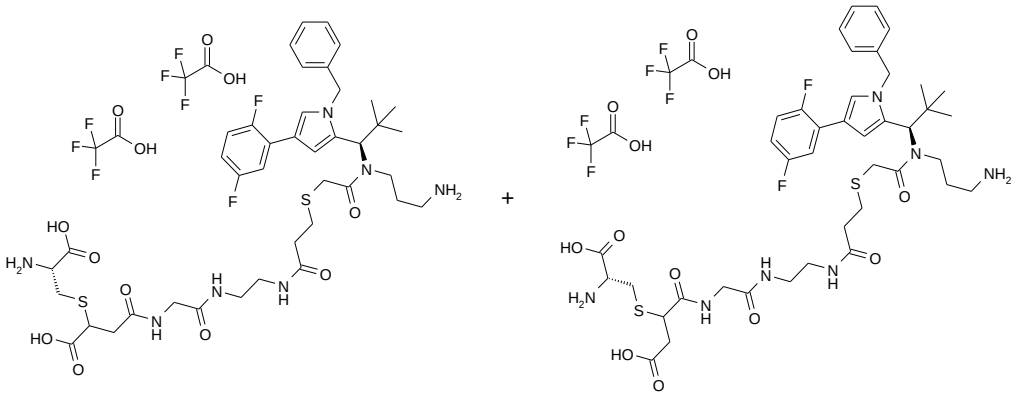
LC-MS (Method 1): R_t = 1.44 min and 3.43 min; MS (ESIpos): m/z = 1107 $(M+H)^+$.

In the last step, 8 mg (0.007 mmol) of this intermediate were dissolved in 5 ml of 2,2,2-trifluoroethanol. 9.8 mg (0.072 mmol) of zinc chloride were added, and the reaction mixture was stirred at 50°C for 1.5 h. Ethylenediamine-N,N,N',N'-tetraacetic acid were then added, and the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 4 mg (59% of theory) of the title compound as a regioisomer mixture in a ratio of 31:67.

LC-MS (Method 1): R_t = 0.79 min and 0.81 min; MS (ESIpos): m/z = 819 $(M+H)^+$.

Example M6

2-{[(2R)-2-Amino-2-carboxyethyl]sulphanyl}-4-({(14R)-13-(3-aminopropyl)-14-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-15,15-dimethyl-2,7,12-trioxo-10-thia-3,6,13-triazahexadec-1-yl}amino)-4-oxobutanoic acid / trifluoroacetic acid (1:2) and 3-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-4-({(14R)-13-(3-aminopropyl)-14-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-15,15-dimethyl-2,7,12-trioxo-10-thia-3,6,13-triazahexadec-1-yl}amino)-4-oxobutanoic acid / trifluoroacetic acid (1:2)



18 mg (0.021 mmol) of Intermediate F213 and 11.2 mg (0.04 mmol) of N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteine were dissolved in 2 ml of DMF, and the mixture was stirred at RT for 18 h. The reaction mixture was concentrated under reduced pressure. The residue (21.2 mg) was dissolved in 3 ml of THF/water 1:1. 0.04 ml of a 2M aqueous lithium hydroxide solution was added and the mixture was stirred at RT for 3 hours. 0.02 ml of a 2M aqueous lithium hydroxide solution was added and the mixture was stirred at RT for 1 hour. The mixture was then adjusted to a pH of ~7 using 7.2 mg (0.12 mmol) of acetic acid. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 13 mg (57% over 2 steps) of the regioisomeric protected intermediates.

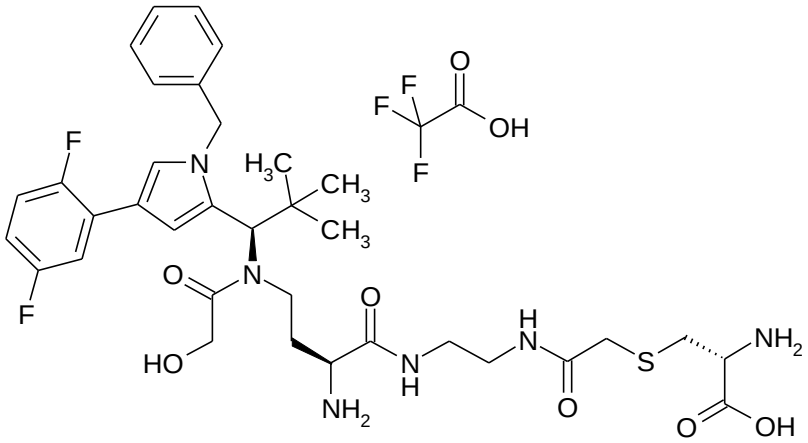
LC-MS (Method 1): R_t = 1.03 min; MS (ESIpos): m/z = 1020 $(M+H)^+$.

In the last step, 13 mg (0.01 mmol) of this intermediate were dissolved in 2 ml of 2,2,2-trifluoroethanol. 6.2 mg (0.05 mmol) of zinc chloride were added, and the reaction mixture was stirred at 50°C for 7 h. 13.3 mg (0.05 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were then added, and the product was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 10.3 mg (81.4%) of the title compound as a regioisomer mixture.

LC-MS (Method 1): R_t = 1.03 min; MS (ESIpos): m/z = 875 $(M+H)^+$.

Example M7

S-(2-{[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]amino}-2-oxoethyl)-L-cysteine/trifluoroacetic acid (1:1)

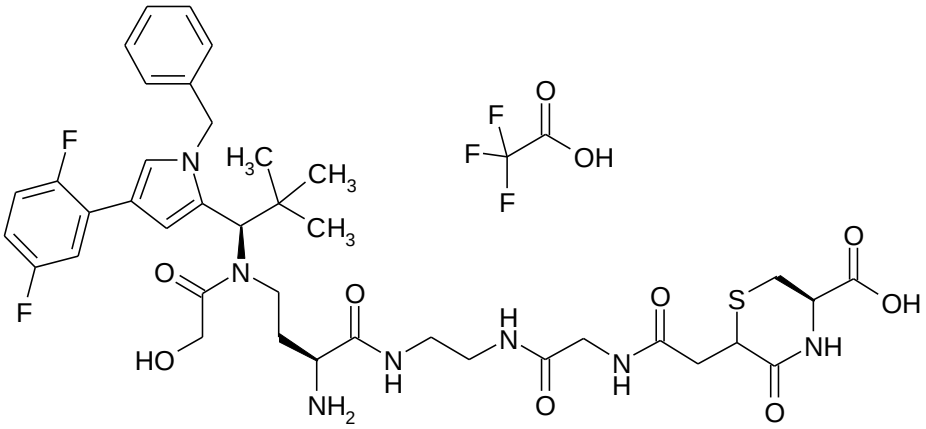


6 mg (8 µmol) of Intermediate F119 were taken up in 3 ml of DMF, and 1.8 mg (15 µmol) of L-cysteine were added. The reaction mixture was stirred at RT for 6 h and then allowed to stand at RT for 3 days. The reaction was then concentrated under reduced pressure, and the product was purified by preparative HPLC.

LC-MS (Method 1): R_t = 0.81 min; MS (ESIpos): m/z = 717 $(M+H)^+$.

Example M8

(3R)-6-{(11S,15R)-11-Amino-15-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-14-glycoloyl-16,16-dimethyl-2,5,10-trioxo-3,6,9,14-tetraazaheptadec-1-yl}-5-oxothiomorpholine-3-carboxylic acid / trifluoroacetic acid (1:1)

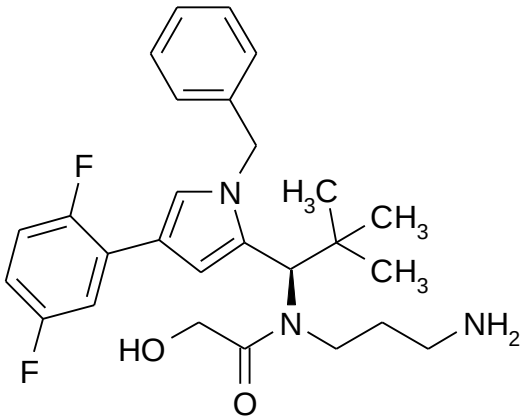


4 mg (0.004 mmol) of the compound from Example 135 were dissolved in 4 ml of THF/water, and 48 µl of a 2-molar aqueous lithium hydroxide solution were added. The mixture was stirred at RT for 1 h and then concentrated and purified by preparative HPLC. Combination, concentration and lyophilization of the appropriate fractions from acetonitrile/water gave 2.4 mg (60% of theory) of the title compound.

LC-MS (Method 1): R_t = 0.86 min; MS (EIpos): m/z = 814 $[M+H]^+$.

Example M9

N-(3-Aminopropyl)-N-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2-hydroxyacetamide



150.0 mg (0.42 mmol) of (1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropan-1-amine (Intermediate C52) were initially charged in 2.0 ml of dichloromethane, and 29.2 mg (0.49 mmol) of HOAc and 125.6 mg (0.59 mmol) of sodium triacetoxyborohydride were added and the mixture was stirred at RT for 5 min. 98.9 mg (0.49 mmol) of 3-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propanal were added. The reaction mixture was stirred at RT overnight. The reaction mixture was diluted with ethyl acetate and the organic phase was washed twice with saturated sodium carbonate solution and once with saturated NaCl solution. After drying over magnesium sulphate, the solvent was evaporated under reduced pressure and the residue was purified on silica gel (eluent: dichloromethane/methanol 100:1). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 188.6 mg (74%) of the compound 2-[3-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino)propyl]-1H-isoindole-1,3(2H)-dione.

LC-MS (Method 1): R_t = 1.00 min; MS (ESIpos): m/z = 541 $[M+H]^+$.

171.2 mg (0.32 mmol) of 2-[3-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino)propyl]-1H-isoindole-1,3(2H)-dione were initially charged in 5.0 ml of dichloromethane, and 73.6 mg (0.73 mmol) of triethylamine were added. At 0°C, 94.9 mg (0.70 mmol) of acetoxyacetyl chloride were added, and the reaction mixture was stirred at RT overnight. The reaction mixture was diluted with ethyl acetate and the organic phase was washed twice with saturated sodium hydrogencarbonate solution and once with saturated NaCl solution. After drying over magnesium sulphate, the solvent was evaporated under reduced pressure and the residue was purified using Biotage Isolera (silica gel, column 10 g SNAP, flow rate 12 ml/min, ethyl acetate/cyclohexane 1:3). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 159.0 mg (77%) of the compound 2-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}[3-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propyl]amino)-2-oxoethyl acetate.

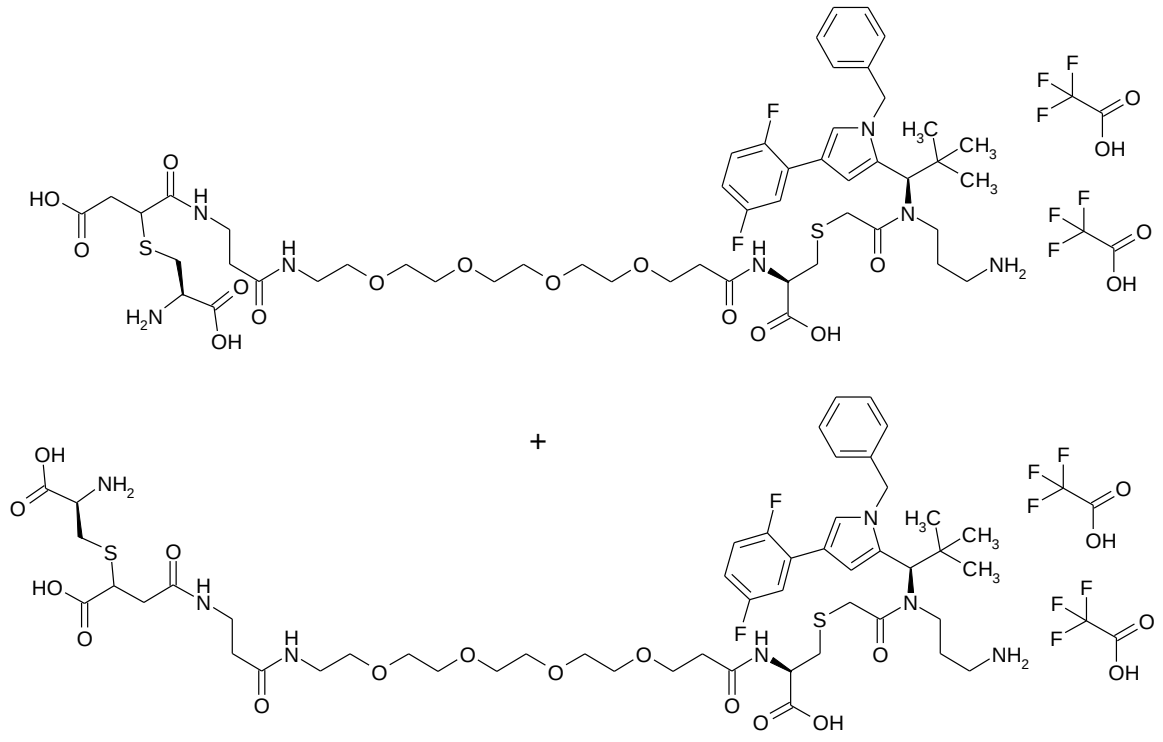
LC-MS (Method 1): R_t = 1.35 min; MS (ESIpos): m/z = 642 $[M+H]^+$.

147.2 mg (0.23 mmol) of 2-({(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}[3-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propyl]amino)-2-oxoethyl acetate were initially charged in 4.0 ml of ethanol, and 356.2 mg (4.59 mmol) of methanamine (40% in water) were added. The reaction mixture was stirred at 50°C overnight. The solvent was evaporated under reduced pressure and the residue co-distilled three times

with toluene. The residue was purified on silica gel (eluent: dichloromethane/methanol = 10:1). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 67.4 mg (63%) of the title compound.
LC-MS (Method 1): R_t = 0.91 min; MS (ESIpos): m/z = 470 $[M+H]^+$.

Example M10

(2R,28R)-28-Amino-2-[({2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)methyl]-25-(carboxymethyl)-4,20,24-trioxo-7,10,13,16-tetraoxa-26-thia-3,19,23-triazanonacosan-1,29-dioic acid / trifluoroacetic acid (1:2) and
(1R,28R,34R)-1-amino-33-(3-aminopropyl)-34-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-35,35-dimethyl-6,10,26,32-tetraoxo-14,17,20,23-tetraoxa-3,30-dithia-7,11,27,33-tetraazahexatriacontan-1,4,28-tricarboxylic acid / trifluoroacetic acid (1:2)



20 mg (0.018 mmol) of R-{2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-L-cysteine / trifluoroacetic acid (1:1) (Intermediate F209) and 9.78 mg (0.036 mmol) of N-{[2-(trimethylsilyl)

ethoxy]carbonyl}-L-cysteine were dissolved in 2 ml of DMF, and the mixture was stirred at RT for 18 h. The reaction mixture was concentrated under reduced pressure. The residue (47.7 mg) was dissolved in 3 ml of THF/water 1:1. 0.08 ml of a 2M aqueous lithium hydroxide solution was added and the mixture was stirred at RT for 1 hour. The reaction was then adjusted to a pH of ~7 using 9.26 mg (0.15 mmol) of acetic acid. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 15.3 mg (29% over 2 steps) of the regioisomeric protected intermediates.

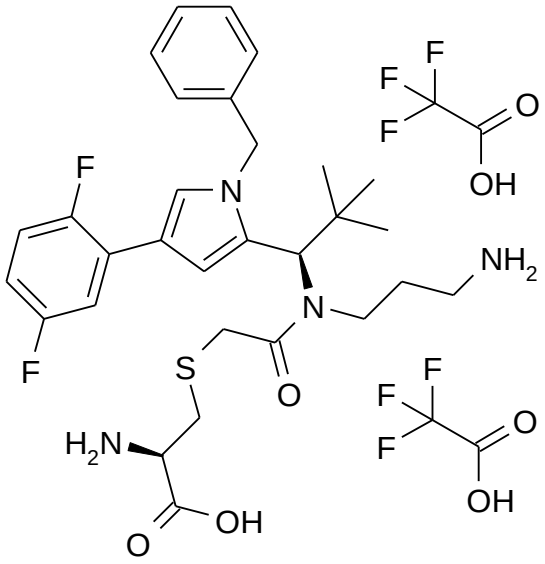
LC-MS (Method 6): R_t = 12.26 min and 12.30 min; MS (ESIpos): m/z = 1254 $(M+H)^+$.

In the last step, 15.3 mg (0.01 mmol) of this intermediate were dissolved in 2 ml of 2,2,2-trifluoroethanol. 6.1 mg (0.05 mmol) of zinc chloride were added, and the reaction mixture was stirred at 50°C for 2 h. 13.1 mg (0.05 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were then added, and the product was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 11.9 mg (79.5%) of the title compound as a regioisomer mixture.

LC-MS (Method 1): R_t = 0.85 min; MS (ESIpos): m/z = 1110 $(M+H)^+$.

Example M11

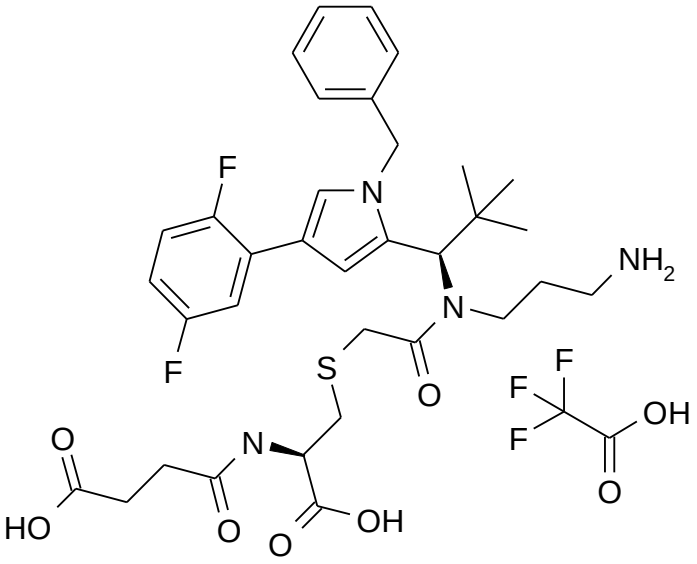
S-{2-[(3-Aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-L-cysteine / trifluoroacetic acid (1:2)



15.0 mg (0.018 mmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-L-cysteine / trifluoroacetic acid (1:1) (Intermediate C71) were dissolved in 1.0 ml of trifluoroethanol, and 7.4 mg (0.054 mmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C overnight. 15.8 mg (0.054 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 11.1 mg (77%) of the title compound.
LC-MS (Method 1): R_t = 0.83 min; MS (ESIpos): m/z = 573 $(M+H)^+$.

Example M12

4-{[(1R)-2-({2-[(3-Aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)-1-carboxyethyl]amino}-4-oxobutanoic acid / trifluoroacetic acid (1:1)

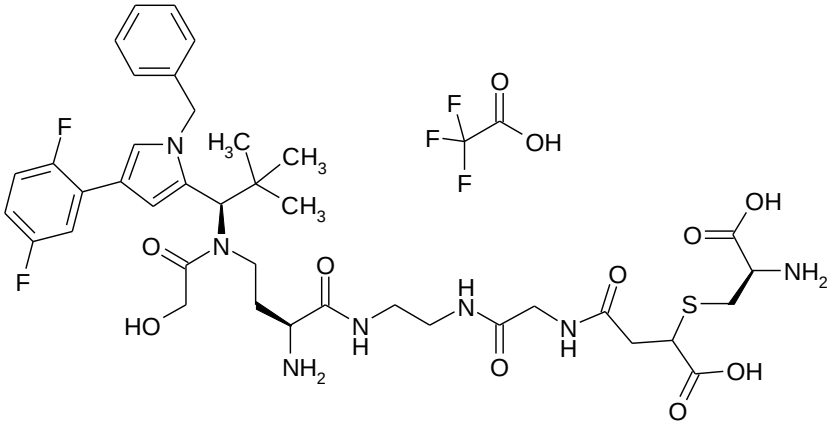


12.2 mg (0.014 mmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-(4-tert-butoxy-4-oxobutanoyl)-L-cysteine (Intermediate C77) were dissolved in 2.0 ml of trifluoroethanol, and 11.4 mg (0.084 mmol) of zinc dichloride were added. The reaction mixture was stirred at 50°C for 3 h. 24.5 mg (0.084 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were added, the reaction mixture was stirred for 10 min and water (0.1% TFA) was then added. Purification was effected directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 4.6 mg (42%) of the title compound.

LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 673 $(M+H)^+$.

Example M13

4-[(2-{[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]amino}-2-oxoethyl)amino]-2-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-4-oxobutanoic acid / trifluoroacetic acid (1:1)
Regioisomer 1, Epimer 1 (2R) or (2S)



LC-MS (Method 5): R_t = 2.44 min; MS (ESIpos): m/z = 832 $[M+H]^+$.

First, methyl L-cysteinate hydrochloride (1:1) was converted with 1-({[2-(trimethylsilyl)ethoxy]carbonyl}oxy)pyrrolidine-2,5-dione in DMF in the presence of *N,N*-diisopropylethylamine into methyl N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteinate.

408 mg (1.93 mmol) of commercially available 3-bromo-4-methoxy-4-oxobutanoic acid and 180 mg (0.644 mmol) of methyl N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteinate were dissolved in 8 ml of DMF, and 147 mg (0.97 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene were added. After stirring at RT for 18 h, another 136 mg (0.64 mmol) of 3-bromo-4-methoxy-4-oxobutanoic acid and 147 mg (0.97 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene were added, and the mixture was stirred at RT for a further 12 h and then concentrated under reduced pressure. The residue was purified by preparative HPLC. Combination of the appropriate fractions and evaporation of the solvents under reduced pressure gave 151 mg (57% of theory) of 4-methoxy-3-{[(2R)-3-methoxy-3-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)propyl]sulphanyl}-4-oxobutanoic acid.

LC-MS (Method 12): R_t = 1.74 min; MS (ESIneg): m/z = 408 $(M-H)^-$.

Of this intermediate, 145 mg were separated by supercritical fluid chromatography via chiral columns into the individual diastereomers (SFC; column DAICEL, AD-H 5u 250x20 mm; flow rate 80 ml/min; method AD-25%ETOH-80 ml; pressure 100 bar; wavelength 210 nM), giving 63 mg (43%) of Epimer 1 and 58 mg (40%) of Epimer 2.

Epimer 1 was characterized as follows:

LC-MS (Method 5): R_t = 2.94 min; MS (ESIneg): m/z = 408 $(M-H)^-$.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ = 7.57 (d, 1H), 4.24 (m, 1H), 4.05 (t, 2H), 3.67 (t, 1H), 3.65 (s, 3H), 3.62 (s, 3H), 3.05 (dd, 1H), 2.70-2.88 (m, 2H), 2.59 (dd, 1H), 0.93 (t, 2H), 0.02 (s, 9H).

Epimer 2 was characterized as follows:

LC-MS (Method 5): R_t = 2.95 min; MS (ESIneg): m/z = 408 $(M-H)^-$.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ = 7.58 (d, 1H), 4.16-4.23 (m, 1H), 4.05 (t, 2H), 3.67 (dd, 1H), 3.65 (s, 3H), 3.64 (s, 3H), 3.04 (dd, 1H), 2.88 (dd, 1H), 2.77 (dd, 1H), 2.61 (dd, 1H), 0.92 (t, 2H), 0.02 (s, 9H).

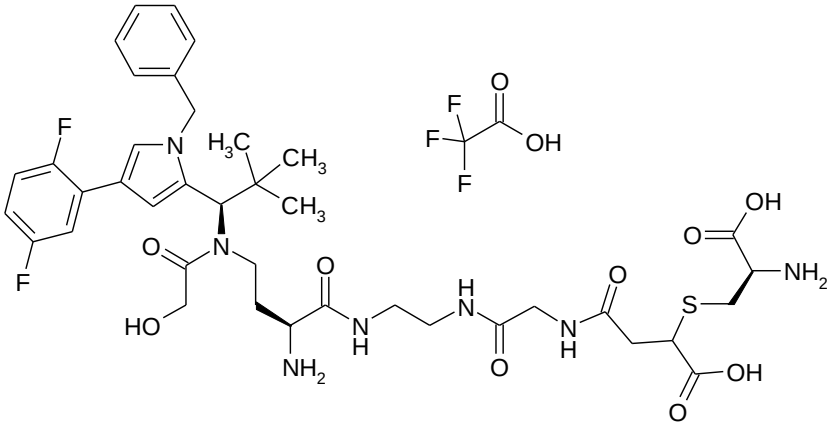
32.5 mg (0.079 mmol) of Epimer 1 were coupled in the presence of 30 mg (0.079 mmol) of HATU and 13.4 mg (0.132 mmol) of 4-methylmorpholine with 50 mg (0.066 mmol) of Intermediate C66, giving, after HPLC purification, 43 mg (57% of theory) of the fully protected intermediate methyl 4-{[(8S)-8-{2-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]ethyl}-2,2-dimethyl-6,9,14-trioxo-5-oxa-7,10,13-triaza-2-silapentadecan-15-yl]amino}-2-{[(2R)-3-methoxy-3-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)propyl]sulphanyl}-4-oxobutanoate.
40 mg (0.035 mmol) of this intermediate were then stirred at RT with 0.9 ml of a 2-molar lithium hydroxide solution in 11 ml of methanol for 20 min, resulting in the cleavage of both methyl ester groups. Purification by HPLC gave 12 mg (31% of theory) of the dicarboxylic acid derivative.
LC-MS (Method 5): R_t = 4.74 min; MS (ESIpos): m/z = 1120 $[M+H]^+$.

Finally, 10 mg (0.009 mmol) of this intermediate were completely deprotected with zinc chloride in trifluoroethanol as described above. The residue was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 2.6 mg (30% of theory) of the title compound.

LC-MS (Method 5): R_t = 2.44 min; MS (ESIpos): m/z = 832 $[M+H]^+$.

Example M14

4-[(2-{[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]amino}-2-oxoethyl)amino]-2-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-4-oxobutanoic acid / trifluoroacetic acid (1:1)
Regioisomer 1, Epimer 2 (2R or 2S)



LC-MS (Method 5): R_t = 2.44 min; MS (EIpos): m/z = 832 $[M+H]^+$.

The intermediate Epimer 2 described in Example M13 was reacted analogously to the description in Example M13:
32.5 mg (0.079 mmol) of Epimer 2 were coupled in the presence of 30 mg (0.079 mmol) of HATU and 13.4 mg (0.132 mmol) of 4-methylmorpholine with 50 mg (0.066 mmol) of Intermediate C66, giving, after HPLC purification, 43 mg (57% of theory) of the fully protected intermediate methyl 4-{[(8S)-8-{2-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]ethyl}-2,2-dimethyl-6,9,14-trioxo-5-oxa-7,10,13-triaza-2-silapentadecan-15-yl]amino}-2-{[(2R)-3-methoxy-3-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)propyl]sulphanyl}-4-oxobutanoate.
40 mg (0.035 mmol) of this intermediate were then stirred at RT with 0.9 ml of a 2-molar lithium hydroxide solution in 11 ml of methanol for 20 min, resulting in the cleavage of both methyl ester groups. Purification by HPLC gave 11 mg (28% of theory) of the dicarboxylic acid derivative.
LC-MS (Method 5): R_t = 4.74 min; MS (ESIpos): m/z = 1120 $[M+H]^+$.

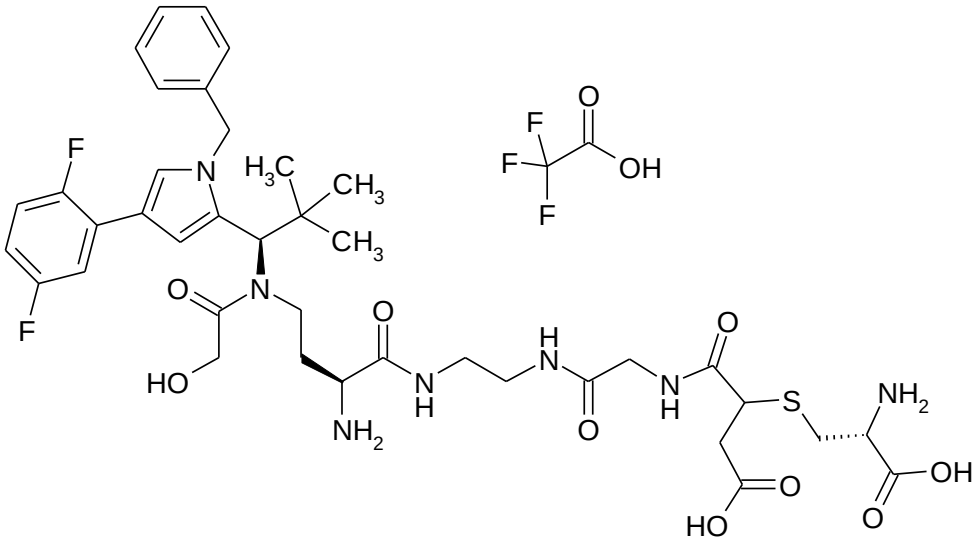
Finally, 10 mg (0.009 mmol) of this intermediate were completely deprotected with zinc chloride in trifluoroethanol as described above. The residue was purified by preparative

HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 4.4 mg (52% of theory) of the title compound.

LC-MS (Method 5): R_t = 2.44 min; MS (ESIpos): m/z = 832 $[M+H]^+$.

Example M15

4-[(2-{[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]amino}-2-oxoethyl)amino]-3-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-4-oxobutanoic acid / trifluoroacetic acid (1:1)
Regioisomer 2, epimer 1 (3R or 3S)



LC-MS (Method 5): R_t = 2.45 min; MS (EIpos): m/z = 832 $[M+H]^+$.

742.8 mg (3.3 mmol) of commercially available 2-bromo-4-ethoxy-4-oxobutanoic acid and 802 mg (2.87 mmol) of methyl N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteinate were dissolved in 32 ml of DMF, and 655.4 mg (4.31 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene were added. After stirring at RT for 20 h, the mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC. Combination of the appropriate fractions and evaporation of the solvents under reduced pressure gave 521 mg (43% of theory) of 4-ethoxy-2-{[(2R)-3-methoxy-3-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)propyl]sulphanyl}-4-oxobutanoic acid.

LC-MS (Method 5): R_t = 3.13 min; MS (ESIpos): m/z = 424 $(M+H)^+$.

Of this intermediate, 510 mg were separated by supercritical fluid chromatography via chiral columns into the individual diastereomers (SFC; column DAICEL, AD-H 5u 250x20 mm; flow rate 80 ml/min; method AD-10%ETOH-80 ml; pressure 100 bar; wavelength 210 nM), giving 100 mg (20%) of Epimer 1 and 141 mg (28%) of Epimer 2.

Epimer 1 was characterized as follows:

LC-MS (Method 1): R_t = 0.99 min; MS (ESIneg): m/z = 422 $(M-H)^-$.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ = 7.60 (d, 1H), 4.18-4.26 (m, 1H), 4.01-4.08 (m, 4H), 3.63 (s, 3H), 3.59 (dd, 1H), 3.04 (dd, 1H), 2.92 (dd, 1H), 2.80 (dd, 1H), 2.63 (dd, 1H), 1.17 (t, 3H), 0.92 (t, 2H), 0.02 (s, 9H).

Epimer 2 was characterized as follows:

LC-MS (Method 5): R_t = 2.95 min; MS (ESIneg): m/z = 408 $(M-H)^-$.

^{1}H-NMR: (400 MHz, DMSO-d_6): δ = 7.56 (d, 1H), 4.21-4.29 (m, 1H), 4.01-4.1 (m, 4H), 3.64 (s, 3H), 3.58 (dd, 1H), 3.08 (dd, 1H), 2.85 (dd, 1H), 2.78 (dd, 1H), 2.60 (dd, 1H), 1.17 (t, 3H), 0.93 (t, 2H), 0.02 (s, 9H).

33.6 mg (0.079 mmol) of Epimer 1 were coupled in the presence of 30 mg (0.079 mmol) of HATU and 13.4 mg (0.132 mmol) of 4-methylmorpholine with 50 mg (0.066 mmol) of Intermediate C66, giving, after HPLC purification, 51 mg (63% of theory) of the fully protected intermediate
ethyl 4-{[(8S)-8-{2-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]ethyl}-2,2-dimethyl-6,9,14-trioxo-5-oxa-7,10,13-triaza-2-silapentadecan-15-yl]amino}-3-{[(2R)-3-methoxy-3-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)propyl]sulphanyl}-4-oxobutanoate.
49 mg (0.042 mmol) of this intermediate were then stirred at RT with 0.5 ml of a 2-molar lithium hydroxide solution in 12 ml of THF/water 1:1 for 30 min, resulting in the cleavage of both methyl ester groups. Acidification and purification by HPLC gave 11 mg (24% of theory) of the dicarboxylic acid derivative.
LC-MS (Method 5): R_t = 4.68 min; MS (ESIpos): m/z = 1120 $[M+H]^+$.

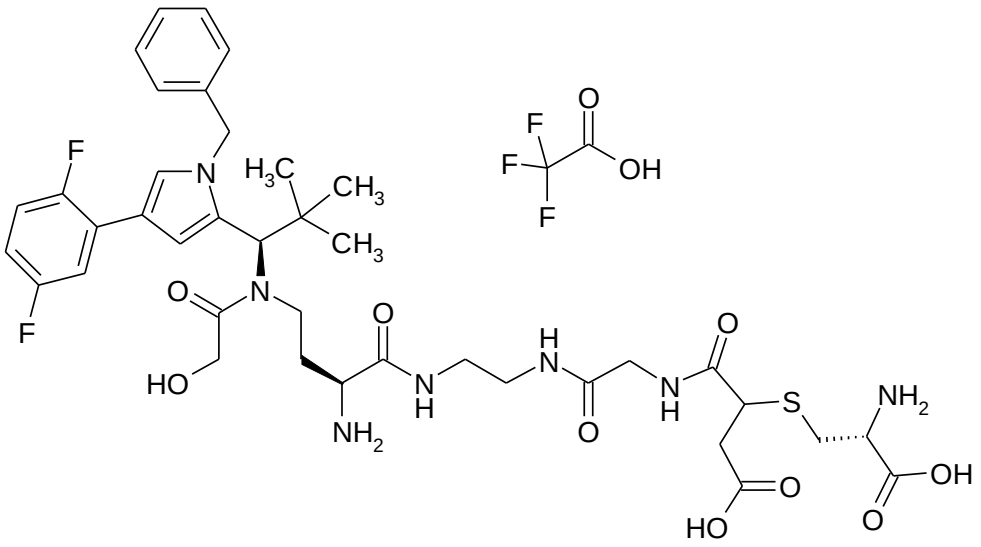
Finally, 11 mg (0.01 mmol) of this intermediate were completely deprotected with zinc chloride in trifluoroethanol as described above. The residue was purified by preparative

HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 3.7 mg (39% of theory) of the title compound.

LC-MS (Method 5): R_t = 2.45 min; MS (ESIpos): m/z = 832 $[M+H]^+$.

Example M16

4-[(2-{[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]amino}-2-oxoethyl)amino]-3-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-4-oxobutanoic acid / trifluoroacetic acid (1:1)
Regioisomer 2, epimer 2 (3R or 3S)



LC-MS (Method 5): R_t = 2.44 min; MS (EIpos): m/z = 832 $[M+H]^+$.

The Epimer 2 intermediate described in Example M15 was converted analogously to the description in Example M15:

33.6 mg (0.079 mmol) of Epimer 2 were coupled in the presence of 30 mg (0.079 mmol) of HATU and 13.4 mg (0.132 mmol) of 4-methylmorpholine with 50 mg (0.066 mmol) of Intermediate C66, giving, after HPLC purification, 51 mg (63% of theory) of the fully protected intermediate
ethyl 4-{[(8S)-8-{2-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]ethyl}-2,2-dimethyl-6,9,14-trioxo-5-oxa-7,10,13-triaza-2-

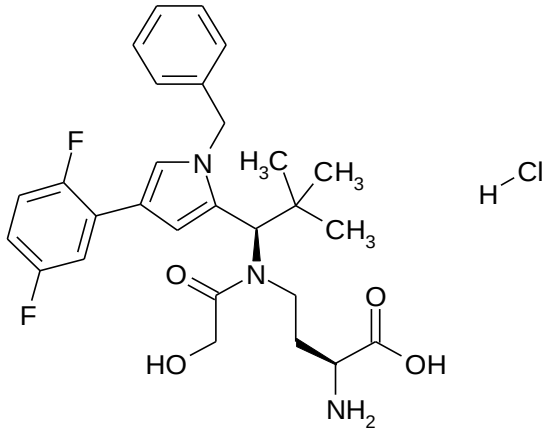
silapentadecan-15-yl]amino}-3-{[(2R)-3-methoxy-3-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)propyl]sulphanyl}-4-oxobutanoate.
49 mg (0.042 mmol) of this intermediate were then stirred at RT with 0.5 ml of a 2-molar lithium hydroxide solution in 12 ml of THF/water 1:1 for 30 min, resulting in the cleavage of both methyl ester groups. Acidification and purification by HPLC gave 13.4 mg (28% of theory) of the dicarboxylic acid derivative.
LC-MS (Method 5): R_t = 4.66 min; MS (ESIpos): m/z = 1120 $[M+H]^+$.

Finally, 13.4 mg (0.012 mmol) of this intermediate were completely deprotected with zinc chloride in trifluoroethanol as described above. The residue was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 7.5 mg (66% of theory) of the title compound.

LC-MS (Method 5): R_t = 2.44 min; MS (ESIpos): m/z = 832 $[M+H]^+$.

Example M17

(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoic acid hydrochloride (1:1)



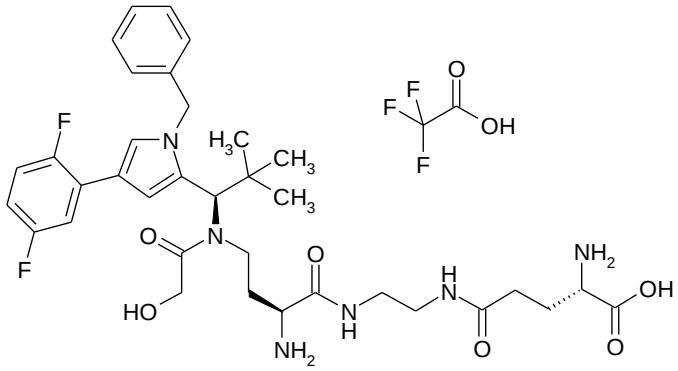
150 mg (0.2 mmol) of Intermediate C53 were dissolved in 15 ml of DMF, and 2.29 g (20.39 mmol) of DABCO were added. The reaction mixture was treated in an ultrasonic bath for 30 min. Addition of 1.17 ml of acetic acid then brought the mixture to pH 3-4, and it was concentrated under reduced pressure. The residue was purified by preparative HPLC and the appropriate fractions were concentrated at RT under reduced pressure. The residue was taken up in acetonitrile/water 1:1, 5 ml of a 4N hydrochloric acid were

added and the mixture was then lyophilized. This gave 81 mg (68% of theory) of the title compound.

LC-MS (Method 5): R_t = 2.69 min; MS (EIpos): m/z = 514 $[M+H]^+$.

Example M18

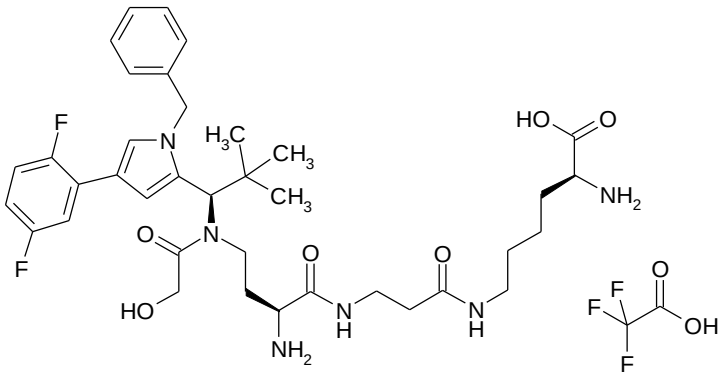
N-[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]-L-glutamine / trifluoroacetic acid (1:1)



First of all, trifluoroacetic acid / benzyl N-(2-aminoethyl)-N^2-[(benzyloxy)carbonyl]-L-glutaminate (1:1) was prepared by methods that are common knowledge to the person skilled in the art. In the presence of HATU, this intermediate was then coupled to Intermediate C58. Subsequently, first the benzyloxycarbonyl protecting group and the benzyl ester were removed by hydrogenolytic cleavage, and then the 2-(trimethylsilyl)ethoxycarbonyl protecting group was removed using zinc chloride.
LC-MS (Method 6): R_t = 1.91 min; MS (EIpos): m/z = 685 $[M+H]^+$.

Example M19

N^6-(N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-beta-alanyl)-L-lysine / trifluoroacetic acid (1:1)

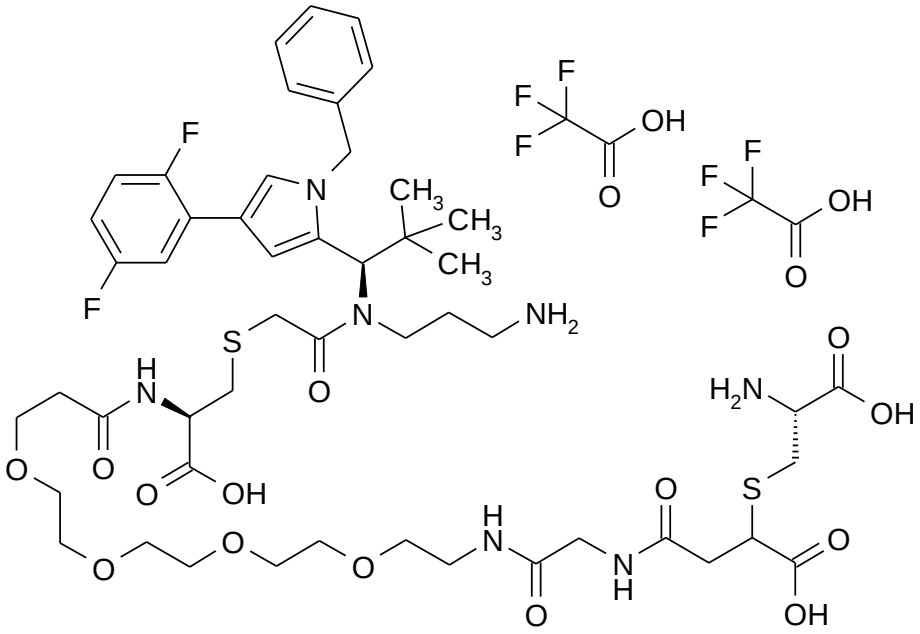


First of all, trifluoroacetic acid / 2-(trimethylsilyl)ethyl-N2-[(benzyloxy)carbonyl]-L-lysinate (1:1) was prepared using conventional protecting group operations known in peptide chemistry. In the presence of HATU, this intermediate was then coupled to Intermediate C61. Subsequently, first the 2-(trimethylsilyl)ethoxycarbonyl protecting group and the 2-(trimethylsilyl)ethyl ester were cleaved using zinc chloride. Finally, the title compound was obtained by hydrogenolytic cleavage of the benzyloxycarbonyl protecting group and purification by preparative HPLC.

HPLC (Method 11): R_t = 1.65 min;

Example M20

(1R,4R,27R,33R)-1-Amino-32-(3-aminopropyl)-33-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-34,34-dimethyl-6,9,25,31-tetraoxo-13,16,19,22-tetraoxa-3,29-dithia-7,10,26,32-tetraazapentatriacontane-1,4,27-tricarboxylic acid / trifluoroacetic acid (1:2)



First, methyl L-cysteinate hydrochloride (1:1) was converted with 1-({[2-(trimethylsilyl)ethoxy]carbonyl}oxy)pyrrolidine-2,5-dione in DMF in the presence of *N,N*-diisopropylethylamine into methyl N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteinate.

408 mg (1.93 mmol) of commercially available 3-bromo-4-methoxy-4-oxobutanoic acid and 180 mg (0.644 mmol) of methyl N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteinate were dissolved in 8 ml of DMF, and 147 mg (0.97 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene were added. After stirring at RT for 18 h, another 136 mg (0.64 mmol) of 3-bromo-4-methoxy-4-oxobutanoic acid and 147 mg (0.97 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene were added, and the mixture was stirred at RT for a further 12 h and then concentrated under reduced pressure. The residue was purified by preparative HPLC. Combination of the appropriate fractions and evaporation of the solvents under reduced pressure gave 151 mg (57% of theory) of 4-methoxy-3-{[(2R)-3-methoxy-3-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)propyl]sulphanyl}-4-oxobutanoic acid.

LC-MS (Method 12): R_t = 1.74 min; MS (ESIneg): m/z = 408 $(M-H)^-$.

3.66 mg (8.93 µmol) of 4-methoxy-3-{[(2R)-3-methoxy-3-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)propyl]sulphanyl}-4-oxobutanoic acid were coupled in the presence of 3.66 mg (8.93 µmol) of HATU and 1.6 µl (15 µmol) of 4-methylmorpholine with 13.0 mg (7.44 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-

diaza-2-silatridecan-13-yl)-N-[15-(glycylamino)-4,7,10,13-tetraoxapentadecan-1-oyl]-L-cysteine / trifluoroacetic acid (1:1) (Intermediate C80), giving, after HPLC purification, 3.9 mg (37% of theory) of the fully protected intermediate S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[15-({N-[(8R,11R)-8,11-bis(methoxycarbonyl)-2,2-dimethyl-6,13-dioxo-5-oxa-10-thia-7-aza-2-silatridecan-13-yl]glycyl}amino)-4,7,10,13-tetraoxapentadecan-1-oyl]-L-cysteine.

3.90 mg (2.76 µmol) of this intermediate were then stirred at RT with 35 µl of a 2-molar lithium hydroxide solution in 1.0 ml of THF/water 3:1 for 15 min, resulting in the cleavage of both methyl ester groups. Purification by HPLC gave 3.60 mg (94% of theory) of the dicarboxylic acid derivative.

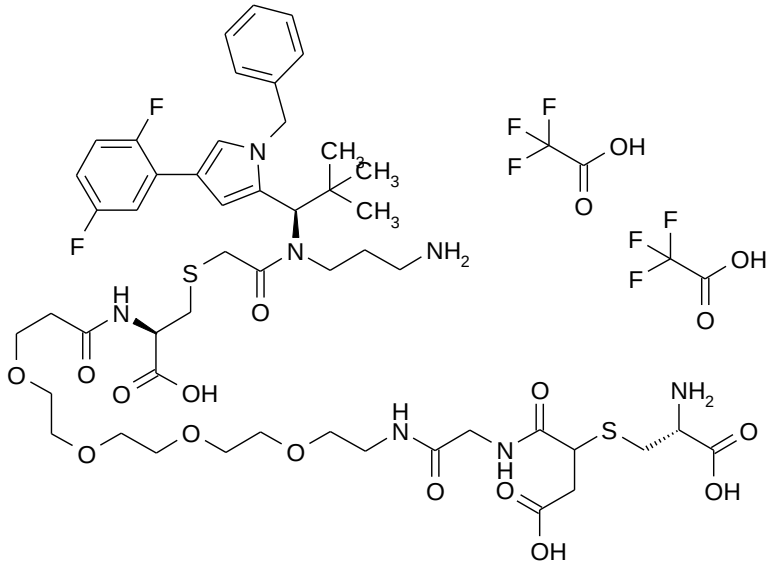
LC-MS (Method 5): R_t = 4.83 min; MS (ESIpos): m/z = 1385 $[M+H]^+$.

Finally, 3.6 mg (2.6 µmol) of this intermediate were completely deprotected with zinc chloride in trifluoroethanol as described above. The residue was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 1.92 mg (55% of theory) of the title compound.

LC-MS (Method 5): R_t = 2.72 min; MS (ESIneg): m/z = 1094 $[M-H]^-$.

Example M21

(2R,24S,27R)-27-Amino-2-[({2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)methyl]-24-(carboxymethyl)-4,20,23-trioxo-7,10,13,16-tetraoxa-25-thia-3,19,22-triazaoctacosane-1,28-dioic acid / trifluoroacetic acid (1:2)



742.8 mg (3.3 mmol) of commercially available 2-bromo-4-ethoxy-4-oxobutanoic acid and 802 mg (2.87 mmol) of methyl N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteinate were dissolved in 32 ml of DMF, and 655.4 mg (4.31 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene were added. After stirring at RT for 20 h, the mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC. Combination of the appropriate fractions and evaporation of the solvents under reduced pressure gave 521 mg (43% of theory) of 4-ethoxy-2-{[(2R)-3-methoxy-3-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)propyl]sulphanyl}-4-oxobutanoic acid.

LC-MS (Method 5): R_t = 3.13 min; MS (ESIpos): m/z = 424 $(M+H)^+$.

4.36 mg (10.3 µmol) of 4-ethoxy-2-{[(2R)-3-methoxy-3-oxo-2-({[2-(trimethylsilyl)ethoxy]carbonyl}amino)propyl]sulphanyl}-4-oxobutanoic acid were coupled in the presence of 3.92 mg (10.3 µmol) of HATU and 1.9 µl (17 µmol) of 4-methylmorpholine with 15.0 mg (8.59 µmol) of S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[15-(glycylamino)-4,7,10,13-tetraoxapentadecan-1-oyl]-L-cysteine / trifluoroacetic acid (1:1) (Intermediate C80), giving, after HPLC purification, 3.6 mg (26% of theory) of the fully protected intermediate S-(11-{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}-2,2-dimethyl-6,12-dioxo-5-oxa-7,11-diaza-2-silatridecan-13-yl)-N-[15-({N-[(8R,11S)-11-(2-ethoxy-2-oxoethyl)-8-(methoxycarbonyl)-2,2-dimethyl-6,12-dioxo-5-oxa-10-thia-7-aza-2-siladodecan-12-yl]glycyl}amino)-4,7,10,13-tetraoxapentadecan-1-oyl]-L-cysteine.

6.20 mg (2.82 µmol) of this intermediate were then stirred at RT with 35 µl of a 2-molar lithium hydroxide solution in 1.0 ml of THF/water 1:1 for 15 min, resulting in the cleavage of both ester groups. Acidification and purification by HPLC gave 3.60 mg (92% of theory) of the dicarboxylic acid derivative.

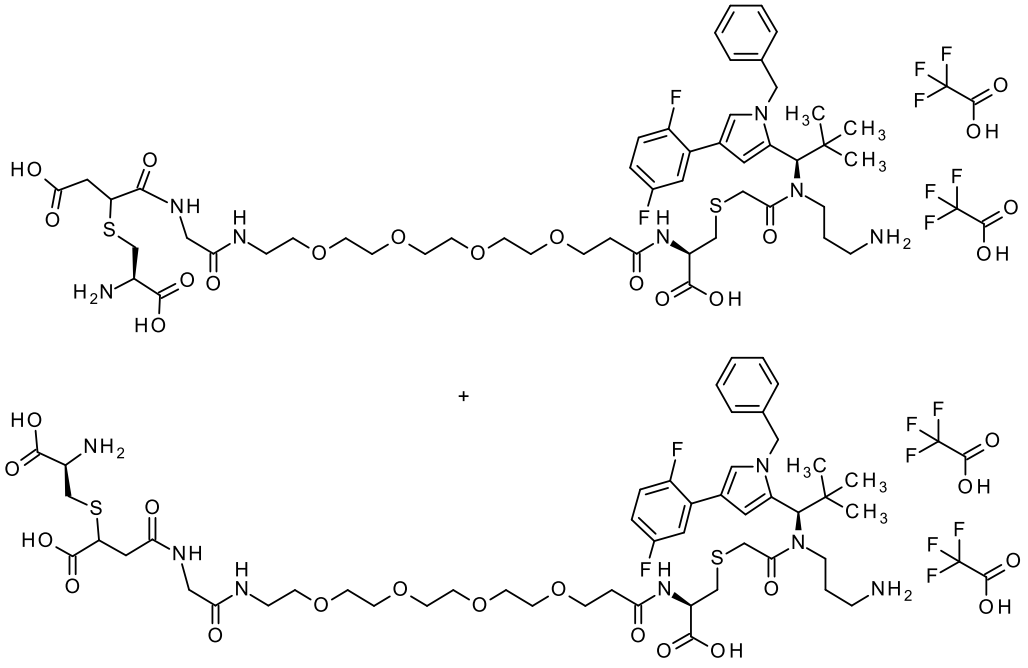
LC-MS (Method 5): R_t = 4.71 min; MS (ESIpos): m/z = 1385 $[M+H]^+$.

Finally, 3.60 mg (1.69 µmol) of this intermediate were completely deprotected with zinc chloride in trifluoroethanol as described above. The residue was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 0.88 mg (39% of theory) of the title compound.

LC-MS (Method 5): R_t = 2.72 min; MS (ESIneg): m/z = 1094 $[M-H]^-$.

Example M22

(2R,27R)-27-Amino-2-[({2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}sulphanyl)methyl]-24-(carboxymethyl)-4,20,23-trioxo-7,10,13,16-tetraoxa-25-thia-3,19,22-triazaoctacosane-1,28-dioic acid / trifluoroacetic acid (1:2) and
(1R,27R,33R)-1-amino-32-(3-aminopropyl)-33-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-34,34-dimethyl-6,9,25,31-tetraoxo-13,16,19,22-tetraoxa-3,29-dithia-7,10,26,32-tetraazapentatriacontane-1,4,27-tricarboxylic acid / trifluoroacetic acid (1:2)



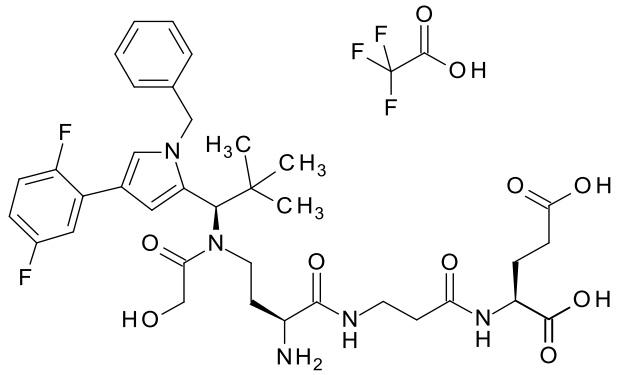
16.5 mg (0.015 mmol) of S-{2-[(3-aminopropyl){(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}amino]-2-oxoethyl}-N-[1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2,18-dioxo-6,9,12,15-tetraoxa-3-azaoctadecan-18-yl]-L-cysteine / trifluoroacetic acid (1:1) (Intermediate F257) and 8.18 mg (0.031 mmol) of N-{[2-(trimethylsilyl)ethoxy]carbonyl}-L-cysteine were dissolved in 2 ml of DMF, and the mixture was stirred at RT for 18 h. The reaction mixture was concentrated under reduced pressure. The residue (28.9 mg) was dissolved in 3 ml of THF/water 1:1. 0.046 ml of a 2M aqueous lithium hydroxide solution was added and the mixture was stirred at RT for 3 hour. The reaction mixture was then adjusted to a pH of ~7 using 5.2 µl (0.092 mmol) of acetic acid. The reaction mixture was purified directly by preparative RP-HPLC (column: Reprosil 125x30; 10µ, flow rate: 50 ml/min, MeCN/water, 0.1% TFA). The solvents were evaporated under reduced pressure and the residue was dried under high vacuum. This gave 12.1 mg (58% over 2 steps) of the regioisomeric protected intermediates.
LC-MS (Method 12): R_t = 1.82 min; MS (ESIpos): m/z = 1240 $(M+H)^+$.

In the last step, 12.1 mg (0.009 mmol) of this intermediate were dissolved in 2 ml of 2,2,2-trifluoroethanol. 7.3 mg (0.054 mmol) of zinc chloride were added, and the reaction mixture was stirred at 50°C for 2 h. 15.7 mg (0.054 mmol) of ethylenediamine-N,N,N',N'-tetraacetic acid were then added, and the product was purified by preparative HPLC. Concentration of the appropriate fractions and lyophilization of the residue from acetonitrile/water gave 6.4 mg (59%) of the title compound as a regioisomer mixture.

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 1096 $(M+H)^+$.

Example M23

N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-beta-alanyl-L-glutamic acid / trifluoroacetic acid (1:1)

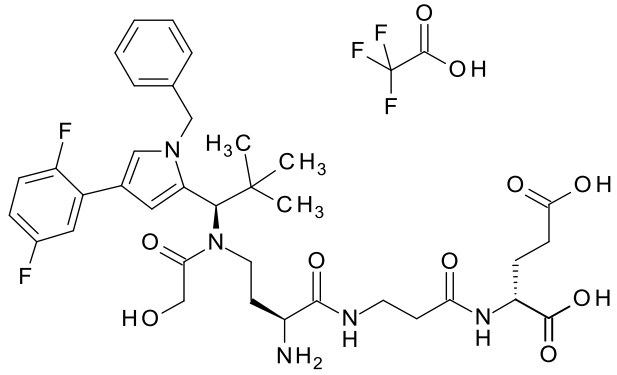


First of all, di-tert-butyl L-glutamate hydrochloride (1:1) was coupled with Intermediate C61 in the presence of HATU and *N,N*-diisopropylethylamine. Then the protected intermediate was taken up in trifluoroethanol and fully deprotected by stirring at 50°C in the presence of zinc chloride overnight. After addition of EDTA, the workup was effected by purification by means of preparative HPLC.

LC-MS (Method 12): R_t = 1.45 min; MS (ESIpos): m/z = 714 $[M+H]^+$.

Example M24

N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-beta-alanyl-D-glutamic acid / trifluoroacetic acid (1:1)



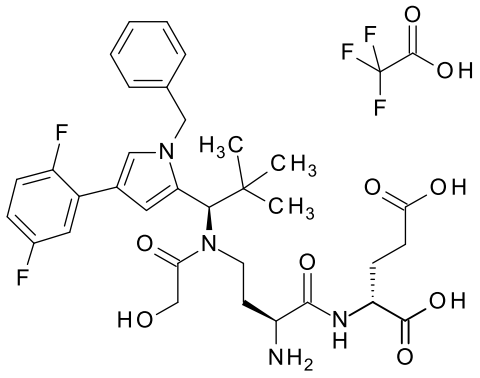
First of all, di-tert-butyl L-glutamate hydrochloride (1:1) was coupled to Intermediate C61 in the presence of HATU and *N,N*-diisopropylethylamine. Then the protected intermediate was taken up in trifluoroethanol and fully deprotected by stirring at 50°C in the presence of

zinc chloride. After addition of EDTA, the workup was effected by purification by means of preparative HPLC.

LC-MS (Method 12): R_t = 1.41 min; MS (ESIpos): m/z = 714 $[M+H]^+$.

Example M25

N-{(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}-L-glutamic acid / trifluoroacetic acid (1:1)

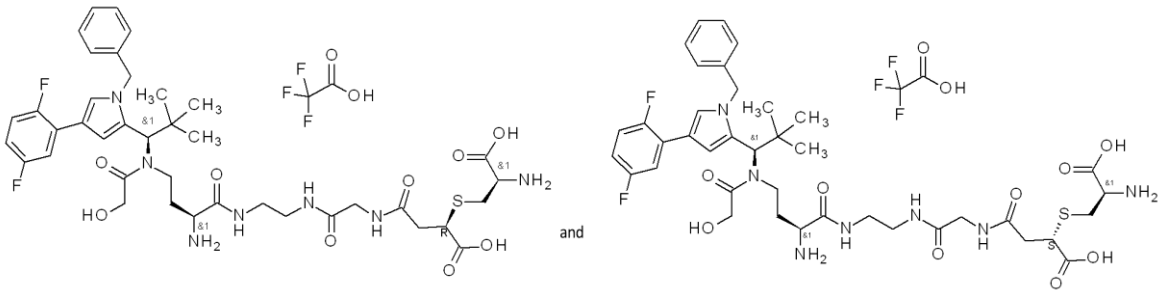


First of all, di-tert-butyl L-glutamate hydrochloride (1:1) was coupled with Intermediate C61 in the presence of HATU and *N,N*-diisopropylethylamine. In the next step, the Z protecting group was removed by hydrogenation over 10% palladium on activated carbon in methanol at RT under hydrogen standard pressure for 45 min. Then the partially protected intermediate was taken up in trifluoroethanol and fully deprotected by stirring at 50°C in the presence of zinc chloride for 7 hours. After addition of EDTA, the workup was effected by purification by means of preparative HPLC.

LC-MS (Method 12): R_t = 1.44 min; MS (ESIpos): m/z = 643 $[M+H]^+$.

Example M26

4-[(2-{[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]amino}-2-oxoethyl)amino]-2-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-4-oxobutanoic acid / trifluoroacetic acid (1:1)
Regioisomer 1, epimer mixture

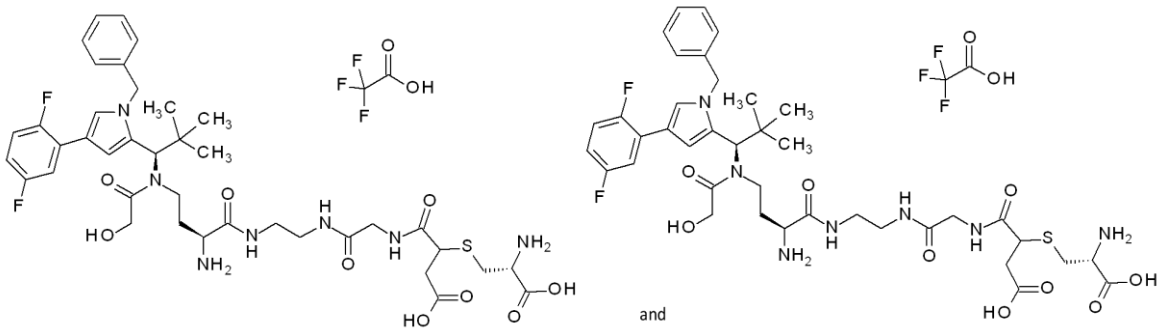


This example describes the epimer mixture of the compounds from Example 13 and Example 14. The synthesis was effected in analogy to Example 13, dispensing with the separation of the two epimers by supercritical fluid chromatography and preparing the title compound as an epimer mixture.

LC-MS (Method 5): R_t = 2.43 min; MS (ESIpos): m/z = 832 $[M+H]^+$.

Example M27

4-[(2-{[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]butanoyl}amino)ethyl]amino}-2-oxoethyl)amino]-3-{[(2R)-2-amino-2-carboxyethyl]sulphanyl}-4-oxobutanoic acid / trifluoroacetic acid (1:1)
Regioisomer 2, epimer mixture

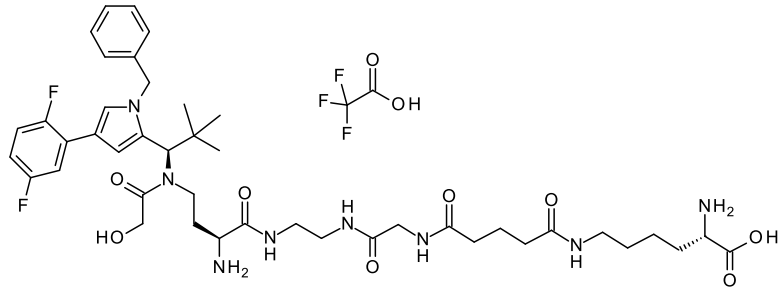


This example describes the epimer mixture of the compounds from Example 15 and Example 16. The synthesis was effected in analogy to Example 15, dispensing with the separation of the two epimers by supercritical fluid chromatography and preparing the title compound as an epimer mixture.

LC-MS (Method 5): R_t = 2.45 min; MS (EIpos): m/z = 832 $[M+H]^+$.

Example M28

N^6-{(3R,7S)-7-Amino-3-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-4-glycoloyl-2,2-dimethyl-8,13,16,20-tetraoxo-4,9,12,15-tetraazaicosan-20-yl}-L-lysine / trifluoroacetic acid (1:1)

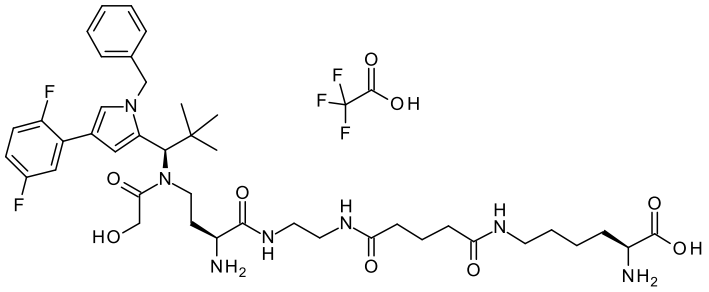


The title compound was prepared proceeding from Intermediate C66 by conventional methods of peptide chemistry, first by coupling to 1,1'-[(1,5-dioxopentan-1,5-diyl)bis(oxy)]-dipyrrolidine-2,5-dione to give 2-(trimethylsilyl)ethyl [(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-({2-[(N-{5-[(2,5-dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl}glycyl)amino]ethyl}amino)-1-oxobutan-2-yl]carbamate in DMF in the presence of N,N-diisopropylethylamine. In the next step, likewise in DMF in the presence of N,N-diisopropylethylamine, coupling was effected to tert-butyl N^2-(tert-butoxycarbonyl)-L-lysinate hydrochloride (1:1), before, in the last step, all protecting groups were then detached with 6 equivalents of zinc chloride in trifluoroethanol by stirring at 50°C for 3 hours.

LC-MS (Method 1): R_t = 0.74 min; MS (ESIpos): m/z = 855 $[M+H]^+$.

Example M29

N^6-(5-{[2-({(2S)-2-Amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl} (glycoloyl)amino]butanoyl}amino)ethyl]amino}-5-oxopentanoyl)-L-lysine / trifluoroacetic acid (1:1)



First of all, (2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethyl propyl}(glycoloyl)amino]-2-[(tert-butoxycarbonyl)amino]butanoic acid was coupled to benzyl (2-aminoethyl)carbamate hydrochloride (1:1) in the presence of HATU and N,N-diisopropylethylamine. This was followed by detachment of the Z protecting group by hydrogenation over 10% palladium on activated carbon in methanol at RT under hydrogen standard pressure for 45 minutes. Then, in analogy to Example M28, coupling was effected to 1,1'-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione. In the next step, the intermediate was coupled to tert-butyl N^2-(tert-butoxycarbonyl)-L-lysinate hydrochloride (1:1) in DMF in the presence of N,N-diisopropylethylamine, before, in the last step, all protecting groups were then detached with 8 equivalents of zinc chloride in trifluoroethanol by stirring at 50°C for 5.5 hours. Purification by preparative HPLC gave the title compound.

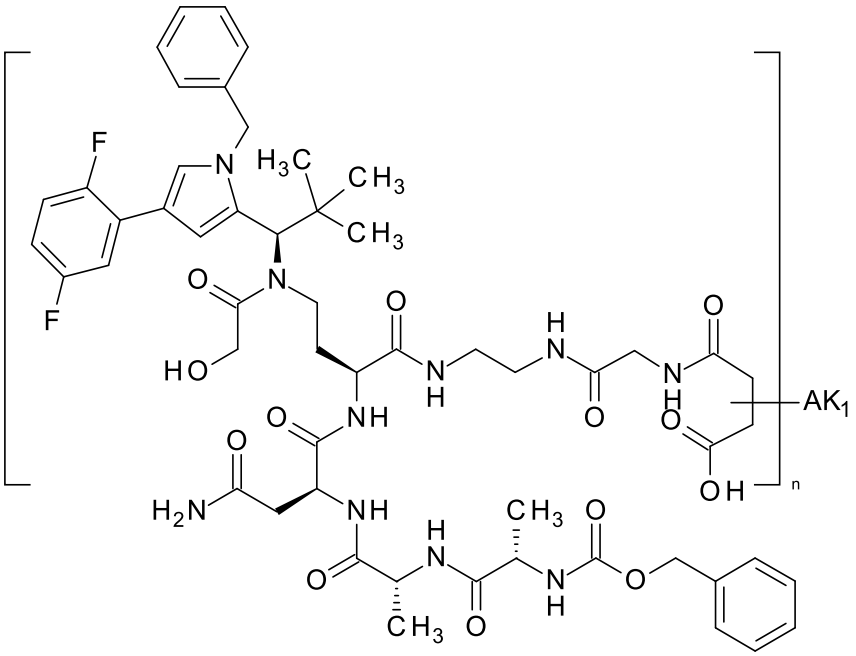
LC-MS (Method 12): R_t = 1.28 min; MS (ESIneg): m/z = 796 $[M-H]^+$.

Working examples of APDCs and ADCs

The APDCs and ADCs shown in the structural formulae of the Working examples, which were coupled to the cysteine side chains of the antibodies via maleimide radicals, are, depending on the linker and the coupling procedure, mainly present in the ring-opened or ring-closed forms shown in each case. However, the preparation may comprise a small proportion of the respective other form.

The coupling reactions were carried out under argon.

Example 1a



Under argon, a solution of 0.028 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.25 mg (0.00023 mmol) of Intermediate R1 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand and then passed through a PD 10 column (Sephadex® G-25, GE Healthcare) equilibrated with PBS buffer pH 8, and eluted with PBS buffer pH 8. The eluate was then stirred at RT under argon overnight.

This was followed by concentration by ultracentrifugation and redilution with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.85 mg/ml
Drug/mAb ratio: 2.6

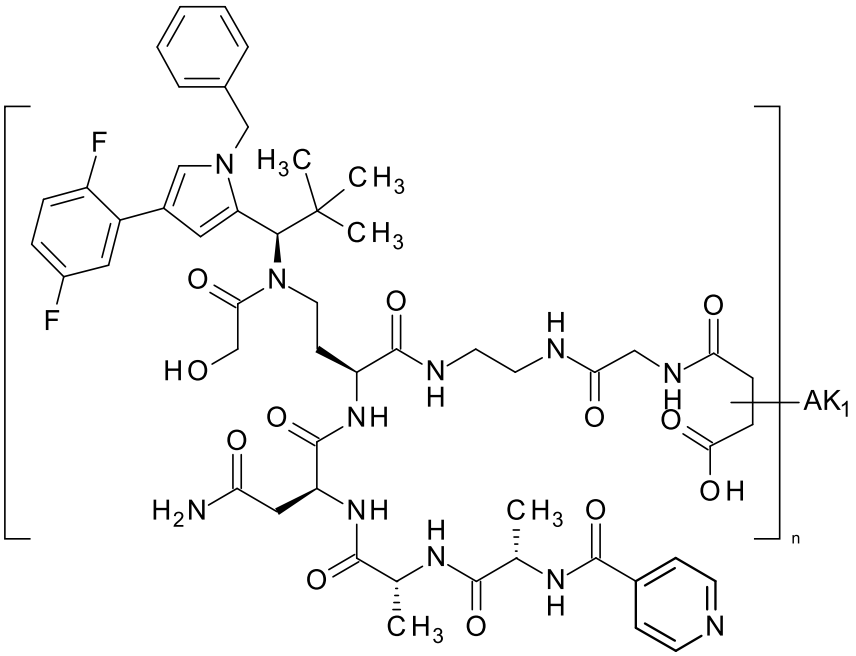
Example 1e

In an analogous manner, Intermediate R1 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.85 mg/ml
Drug/mAb ratio: 3.4

Example 1k

In an analogous manner, Intermediate R1 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.42 mg/ml
Drug/mAb ratio: 2.9

Example 2a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.458 ml of PBS (c = 10.92 mg/ml). The mixture was stirred at RT for 30 min, and then 0.27 mg (0.00023 mmol) of Intermediate R2 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted with 1.942 ml of PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight. This was followed by concentration by ultracentrifugation and redilution with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.99 mg/ml
Drug/mAb ratio: 2.6

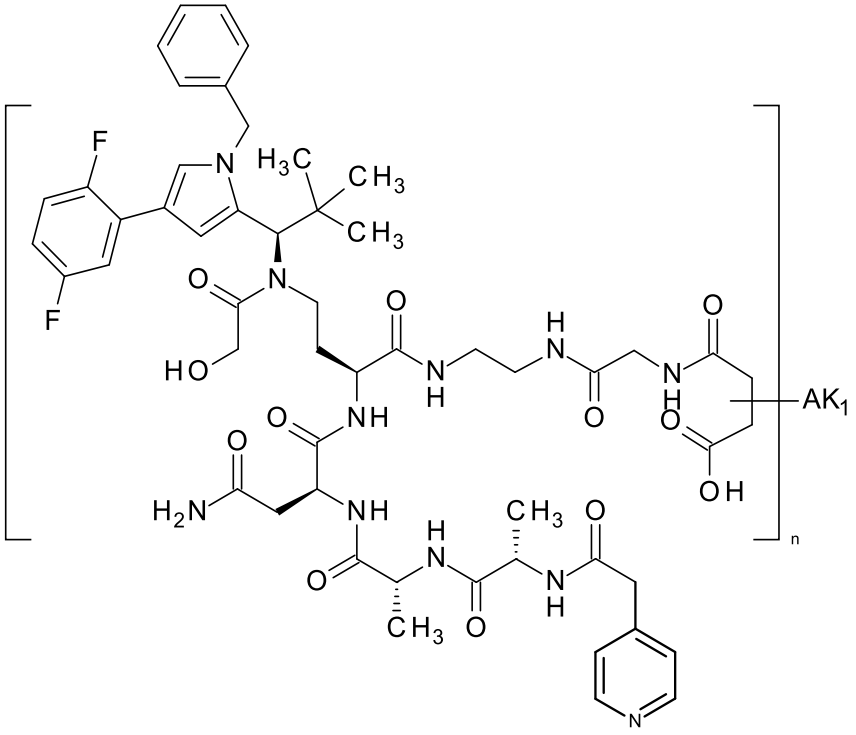
Example 2e

In an analogous manner, Intermediate R2 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.6 mg/ml
Drug/mAb ratio: 3.5

Example 2k

In an analogous manner, Intermediate R2 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 2.05 mg/ml
Drug/mAb ratio: 3.4

Example 3a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.28 mg (0.00023 mmol) of Intermediate R3 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction was diluted with 1.942 ml of PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 2.07 mg/ml
Drug/mAb ratio: 2.2

Example 3e

In an analogous manner, Intermediate R3 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.85 mg/ml

Drug/mAb ratio: 3.6

Example 3h1

Under argon, a solution of 0.46 mg of TCEP in 0.75 ml of PBS buffer (pH 7.2) was added to 80 mg of the anti-B7H3 antibody TPP-8382 in 5.1 ml of PBS (c = 15.7 mg/ml). The mixture was stirred at RT for 30 min, and then 4.41 mg (0.0037 mmol) of Intermediate R3 dissolved in 585 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was diluted to 7.5 ml with PBS buffer which had been adjusted to pH 8 beforehand and then passed in portions through a PD 10 column (Sephadex® G-25, GE Healthcare) equilibrated with PBS buffer pH 8, and eluted with PBS buffer pH 8. The eluates were combined, diluted to 12.5 ml with PBS buffer pH 8 and stirred under argon at RT overnight. This solution was then applied to PD 10 columns (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 7.2 and was eluted with PBS buffer pH 7.2. This was followed by crossflow concentration. The ADC batch obtained was characterized as follows:
Protein concentration: 13.86 mg/ml
Drug/mAb ratio: 4.8

Example 3h2

In an analogous manner, Intermediate R3 was coupled to 50 mg of anti-B7H3 antibody TPP-8567. The ADC batch obtained was characterized as follows:
Protein concentration: 9.64 mg/ml
Drug/mAb ratio: 4.3

Example 3k

In an analogous manner, Intermediate R3 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.47 mg/ml
Drug/mAb ratio: 3.4

Example 3l1

Under argon, a solution of 0.172 mg of TCEP in 0.3 ml of PBS buffer (pH 7.2) was added to 30 mg of the anti-TWEAKR antibody TPP-7007 in 3.4 ml of PBS (c = 8.8 mg/ml). The mixture was stirred at RT for 30 min, and then 1.66 mg (0.0014 mmol) of Intermediate R3 dissolved in 300 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was diluted to 5 ml with PBS buffer which had been adjusted to pH 8 beforehand and then passed in portions through a PD 10 column (Sephadex® G-25, GE Healthcare) equilibrated with PBS buffer pH 8, and eluted with PBS buffer pH 8. The eluates were combined, diluted to 7.5 ml with PBS buffer pH 8 and stirred under argon at RT overnight. This solution was then applied to PD 10 columns (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 7.2 and was eluted with PBS buffer pH 7.2. The eluate was then concentrated by ultracentrifugation, rediluted with PBS buffer (pH 7.2) and reconcentrated and sterile-filtered again. The ADC batch obtained was characterized as follows:
Protein concentration: 8.86 mg/ml
Drug/mAb ratio: 4.4

Example 3l2

In an analogous manner, Intermediate R3 was coupled to 48.5 mg of anti-TWEAKR antibody TPP-7006 in 4.16 ml of PBS (c = 11.6 mg/ml) The ADC batch obtained was characterized as follows:
Protein concentration: 11.4 mg/ml
Drug/mAb ratio: 2.5

Example 3l3

In an analogous manner, Intermediate R3 was coupled to 30 mg of anti-TWEAKR antibody TPP-10336 in 2.61 ml of PBS (c = 11.5 mg/ml). The ADC batch obtained was characterized as follows:
Protein concentration: 7.72 mg/ml
Drug/mAb ratio: 2.5

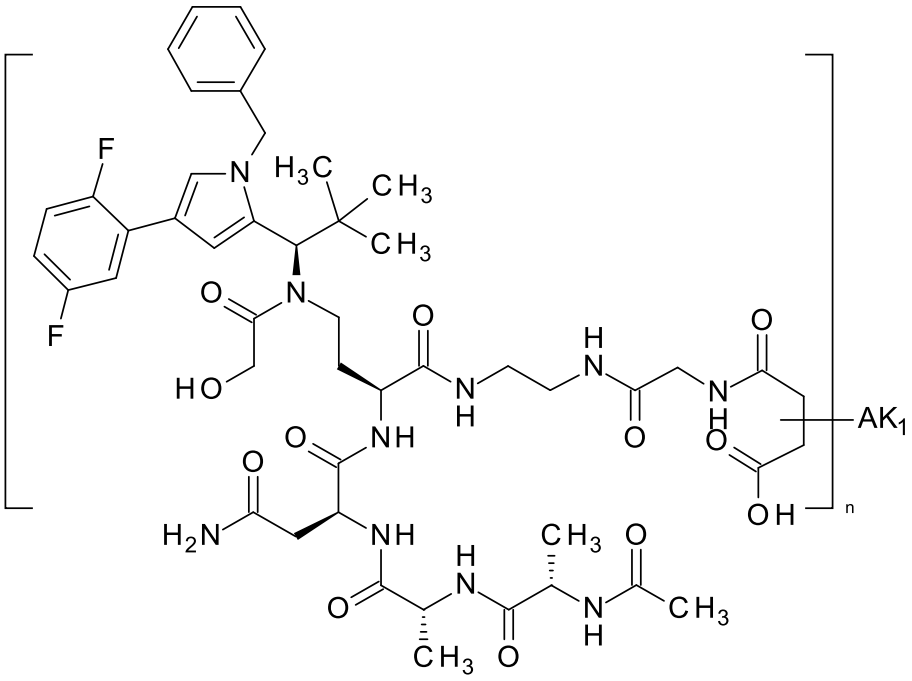
Example 3l4

In an analogous manner, Intermediate R3 was coupled to 30 mg of anti-TWEAKR antibody TPP-10337 in 2.78 ml of PBS (c = 10.8 mg/ml). The ADC batch obtained was characterized as follows:

Protein concentration: 9.33 mg/ml

Drug/mAb ratio: 2.7

Example 4a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.23 mg (0.00023 mmol) of Intermediate R4 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was diluted to a volume of 2.5 ml with 1.9 ml of PBS buffer which had been adjusted to pH 8 beforehand and then passed through a PD 10 column (Sephadex® G-25, GE Healthcare) equilibrated with PBS buffer pH 8, and eluted with PBS buffer pH 8. The eluate was then stirred at RT under argon overnight.This was followed by concentration by ultracentrifugation and redilution with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:

Protein concentration: 2.29 mg/ml

Drug/mAb ratio: 2.5

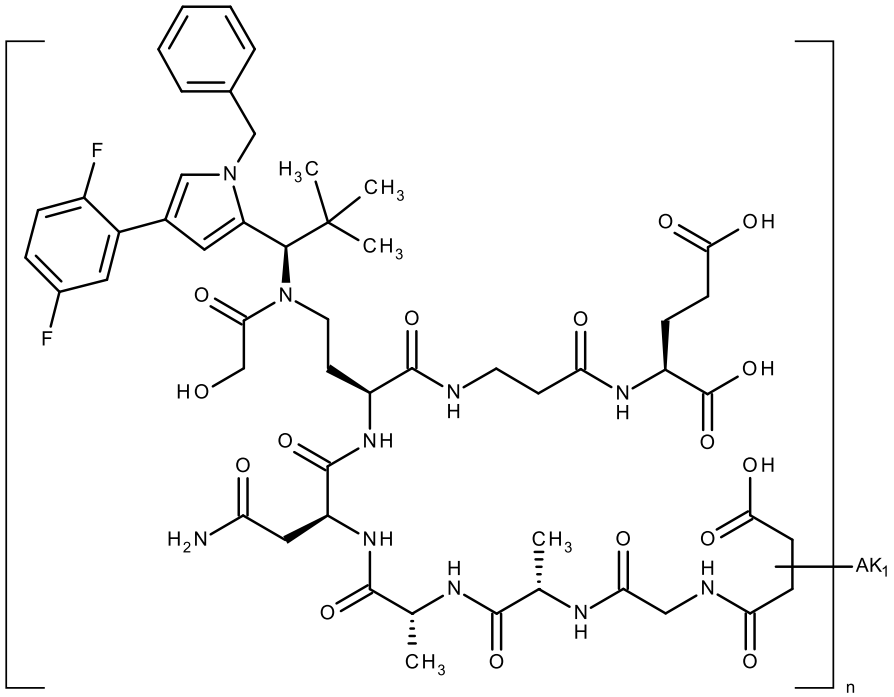
Example 4e

In an analogous manner, Intermediate R4 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.75 mg/ml
Drug/mAb ratio: 3.2

Example 4k

In an analogous manner, Intermediate R4 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.73 mg/ml
Drug/mAb ratio: 3.3

Example 5a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.458 ml of PBS (c = 10.92 mg/ml). The mixture was stirred at RT for 30 min, and then 0.26 mg (0.00023 mmol) of Intermediate R5 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted with

1.942 ml of PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred under argon at RT overnight. This was followed by concentration by ultracentrifugation and redilution with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.91 mg/ml
Drug/mAb ratio: 2.7

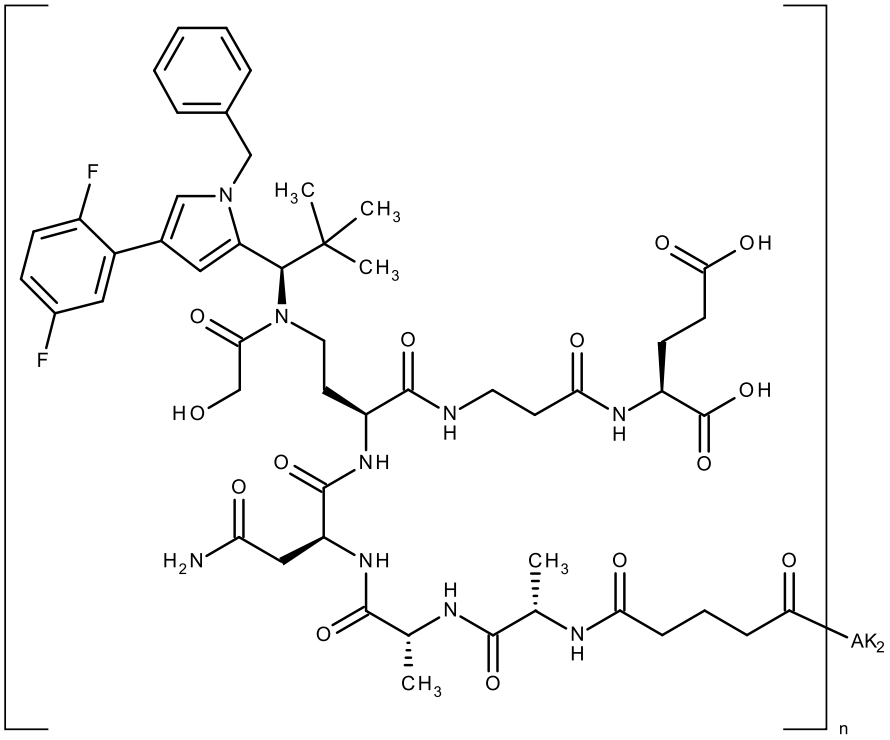
Example 5e

In an analogous manner, Intermediate R5 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.40 mg/ml
Drug/mAb ratio: 3.6

Example 5k

In an analogous manner, Intermediate R5 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.85 mg/ml
Drug/mAb ratio: 3.0

Example 6a



5 mg of cetuximab in 459 µl of PBS (c = 10.92 mg/ml) were used here for coupling to Intermediate R6. First of all, 5 eq (0.2 mg) of Intermediate R6 dissolved in 50 µl of DMSO were added, and after stirring at RT for 1 h the same amount was added again and the mixture was stirred at RT for a further hour. The reaction mixture was subsequently diluted to 2.5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).

Protein concentration: 2.04 mg/ml

Drug/mAb ratio: 2.1

Example 6e

In an analogous manner, Intermediate R6 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:

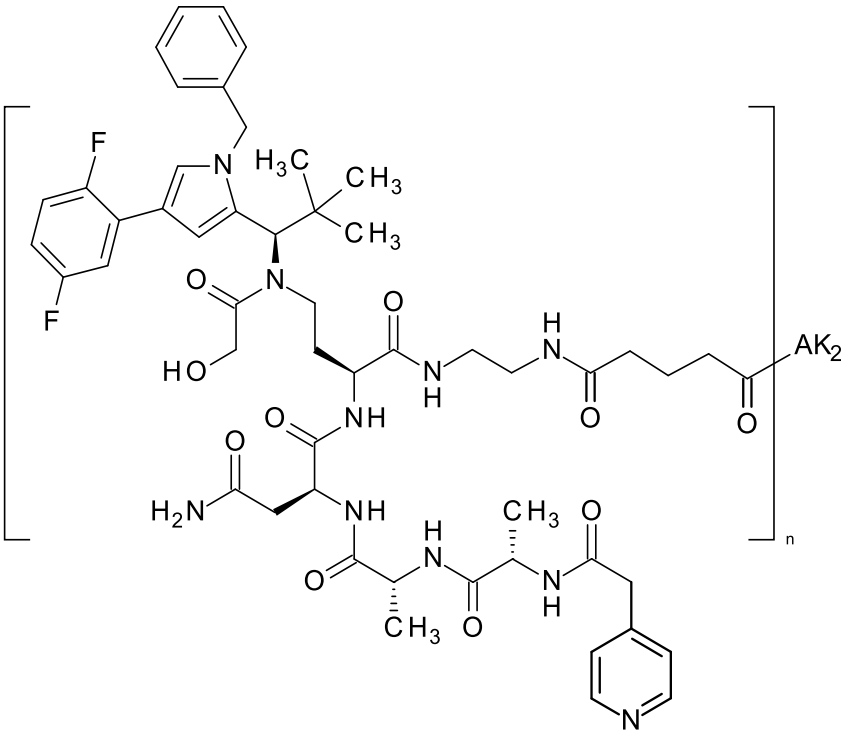
Protein concentration: 1.74 mg/ml

Drug/mAb ratio: 2.0

Example 6k

In an analogous manner, Intermediate R6 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 2.0 mg/ml
Drug/mAb ratio: 2.4

Example 7a



To 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml) under argon was added a solution of 5 eq (0.19 mg) of Intermediate R7 dissolved in 50 µl of DMSO, and after stirring at RT for 1 h the same amount again was added and the mixture was stirred at RT for a further hour. The reaction mixture was subsequently diluted to 2.5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).
Protein concentration: 2.29 mg/ml
Drug/mAb ratio: 2.9

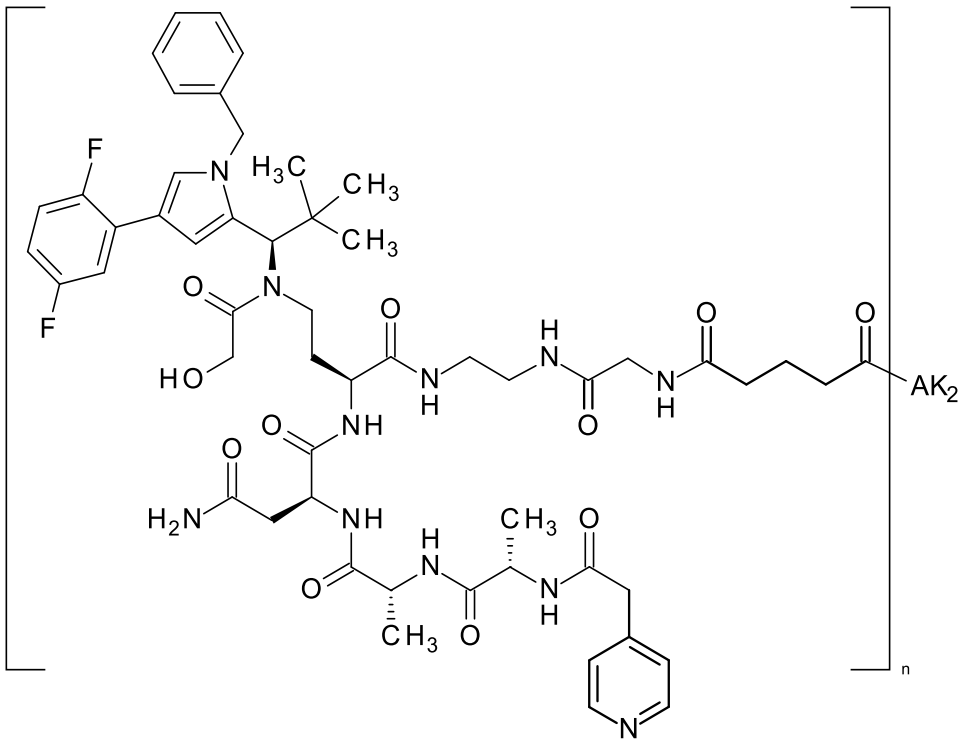
Example 7e

In an analogous manner, Intermediate R7 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.95 mg/ml
Drug/mAb ratio: 3.5

Example 7k

In an analogous manner, Intermediate R7 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.60 mg/ml
Drug/mAb ratio: 5.3

Example 8a



To 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml) under argon was added a solution of 5 eq (0.2 mg) of Intermediate R8 dissolved in 50 µl of DMSO, and after stirring at RT for 1 h the same amount again was added and the mixture was stirred at RT for a further hour. The reaction was subsequently diluted to 2.5 ml with PBS buffer (pH 7.2), purified

on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).
Protein concentration: 1.57 mg/ml
Drug/mAb ratio: 3.6

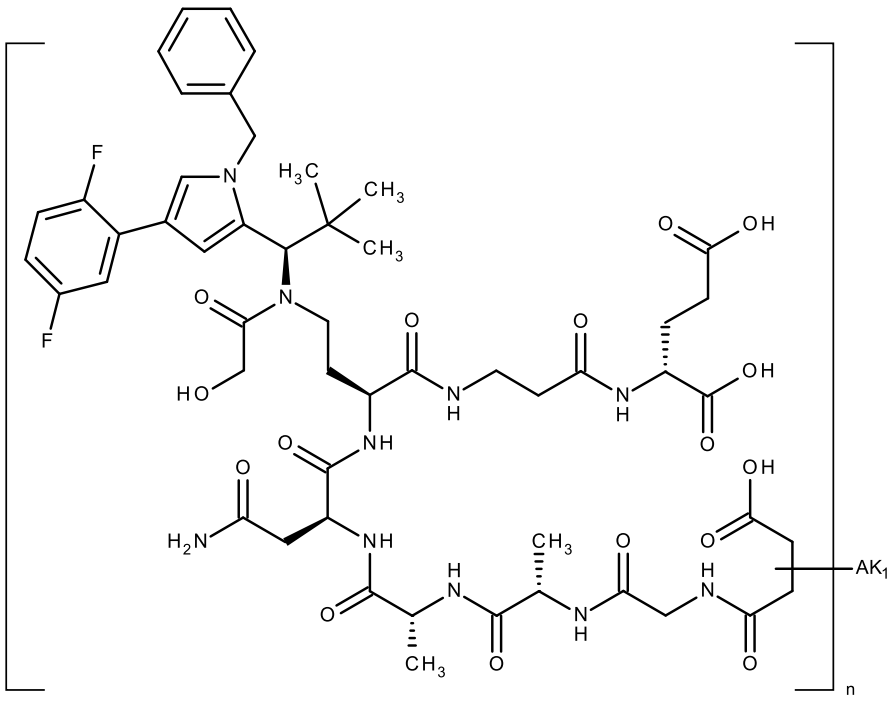
Example 8e

In an analogous manner, Intermediate R8 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.25 mg/ml
Drug/mAb ratio: 4.8

Example 8k

In an analogous manner, Intermediate R8 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.15 mg/ml
Drug/mAb ratio: 7.0

Example 9a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.458 ml of PBS (c = 10.92 mg/ml). The mixture was stirred at RT for 30 min, and then 0.26 mg (0.00023 mmol) of Intermediate R9 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted with 1.9 ml of PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred under argon at RT overnight. This was followed by concentration by ultracentrifugation and redilution with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.95 mg/ml
Drug/mAb ratio: 2.7

Example 9e

In an analogous manner, Intermediate R9 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.88 mg/ml
Drug/mAb ratio: 3.2

Example 9h1

Under argon, a solution of 0.23 mg of TCEP in 0.5 ml of PBS buffer (pH 7.2) was added to 30 mg of the anti-B7H3 antibody TPP-8382 in 2.55 ml of PBS (c = 15.69 mg/ml). The mixture was stirred at RT for 30 min, and then 2.07 mg (0.00187 mmol) of Intermediate R9 dissolved in 250 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was diluted to 5 ml with PBS buffer which had been adjusted to pH 8 beforehand, divided and then each portion was passed through a PD 10 column (Sephadex® G-25, GE Healthcare) equilibrated with PBS buffer pH 8, and eluted with PBS buffer pH 8. The eluates were combined, diluted to 7.5 ml with PBS buffer pH 8 and stirred under argon at RT overnight. This solution was then applied to PD 10 columns (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 7.2 and was eluted with PBS buffer pH 7.2. The eluate was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2) and reconcentrated and sterile-filtered again. The ADC batch obtained was characterized as follows:
Protein concentration: 8.46 mg/ml
Drug/mAb ratio: 2.8

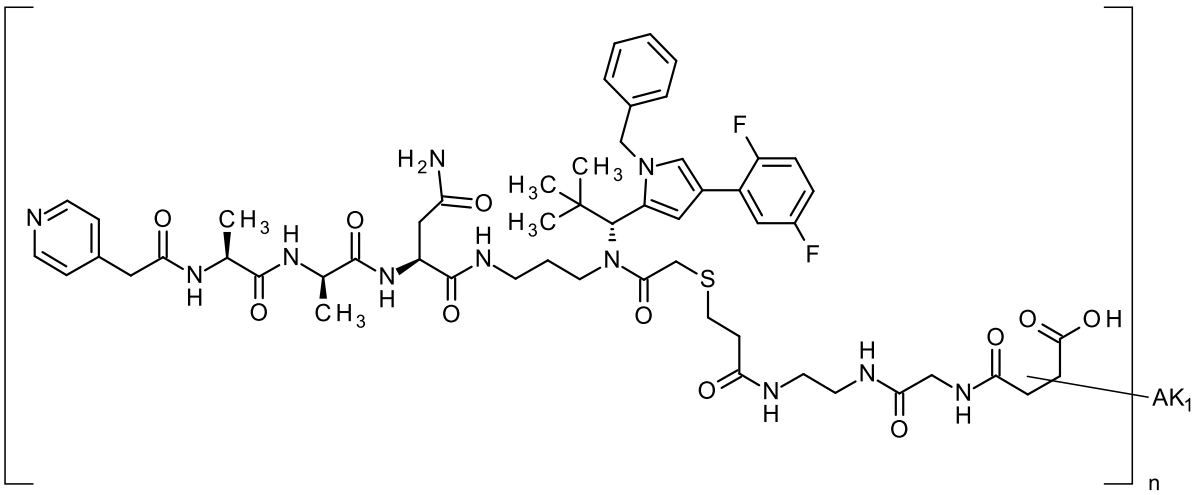
Example 9h2

In an analogous manner, Intermediate R9 was coupled to 30 mg of anti-B7H3 antibody TPP-8567 in 3 ml of PBS (c = 10 mg/ml) The ADC batch obtained was characterized as follows:
Protein concentration: 8.08 mg/ml
Drug/mAb ratio: 3.9

Example 9k

In an analogous manner, Intermediate R9 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.81 mg/ml
Drug/mAb ratio: 3.2

Example 10k



Under argon, a solution of 0.028 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of anti-TWEAKR antibody TPP-2658 in 0.24 ml of PBS (c = 20.9 mg/ml). The mixture was diluted with 2.11 ml of PBS buffer and stirred at RT for 30 min. Then 0.25 mg (0.00023 mmol) of Intermediate R10 dissolved in 100 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight. This was followed by

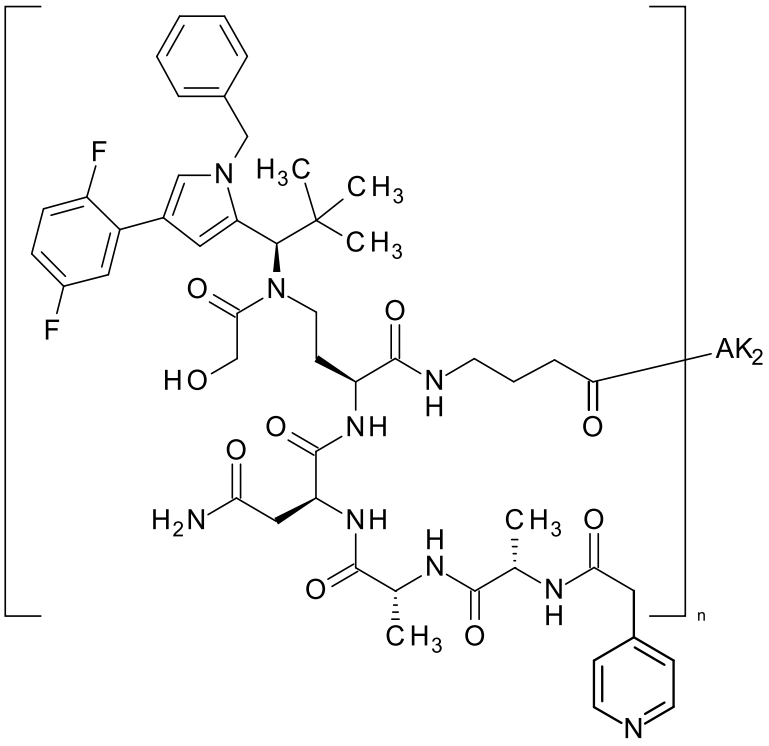
concentration by ultracentrifugation and redilution with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:

Protein concentration: 1.0
Drug/mAb ratio: 0

Example 10h1

In an analogous manner to Example 9h1, Intermediate R10 was coupled to 30 mg of anti-B7H3 antibody TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 10.95 mg/ml
Drug/mAb ratio: 4.4

Example 11a



To 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml) under argon was added a solution of 5 eq (0.2 mg) of Intermediate R11 dissolved in 50 µl of DMSO, and after stirring at RT for 1 h the same amount again was added and the mixture was stirred at RT for a further hour. The reaction mixture was subsequently diluted to 2.5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).

Protein concentration: 2.2 mg/ml
Drug/mAb ratio: 4.0

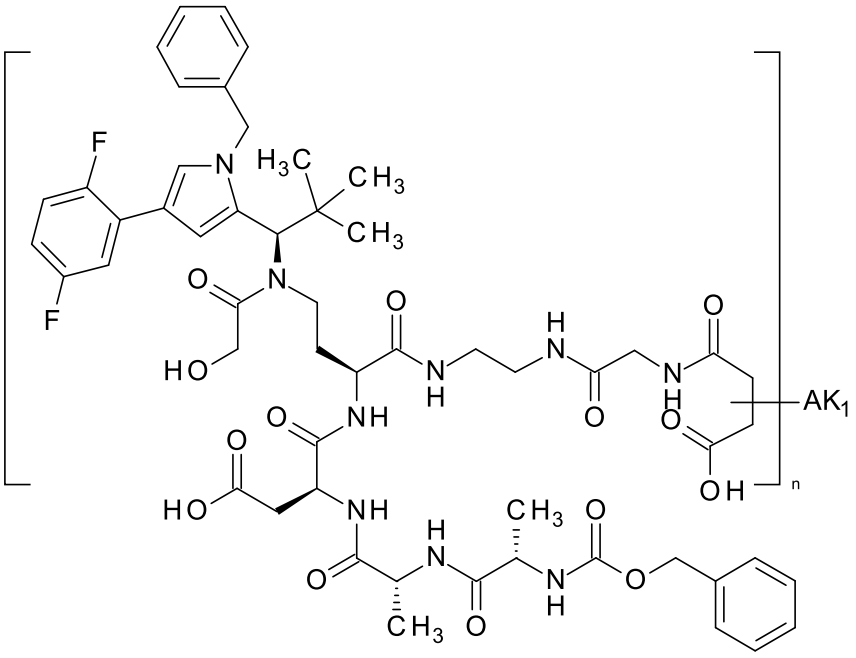
Example 11e

In an analogous manner, Intermediate R11 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.13 mg/ml
Drug/mAb ratio: 4.4

Example 11k

In an analogous manner, Intermediate R11 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 0.98 mg/ml
Drug/mAb ratio: 6.0

Example 12a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.458 ml of PBS (c = 10.92 mg/ml). The mixture was stirred at RT for 30 min, and then 0.25 mg (0.00023 mmol) of Intermediate R12 dissolved in 50 µl of DMSO

was added. After stirring at RT for a further 90 min, the reaction was diluted with 1.9 ml of PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight. This was followed by concentration by ultracentrifugation and redilution with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 2.0 mg/ml
Drug/mAb ratio: 3.3

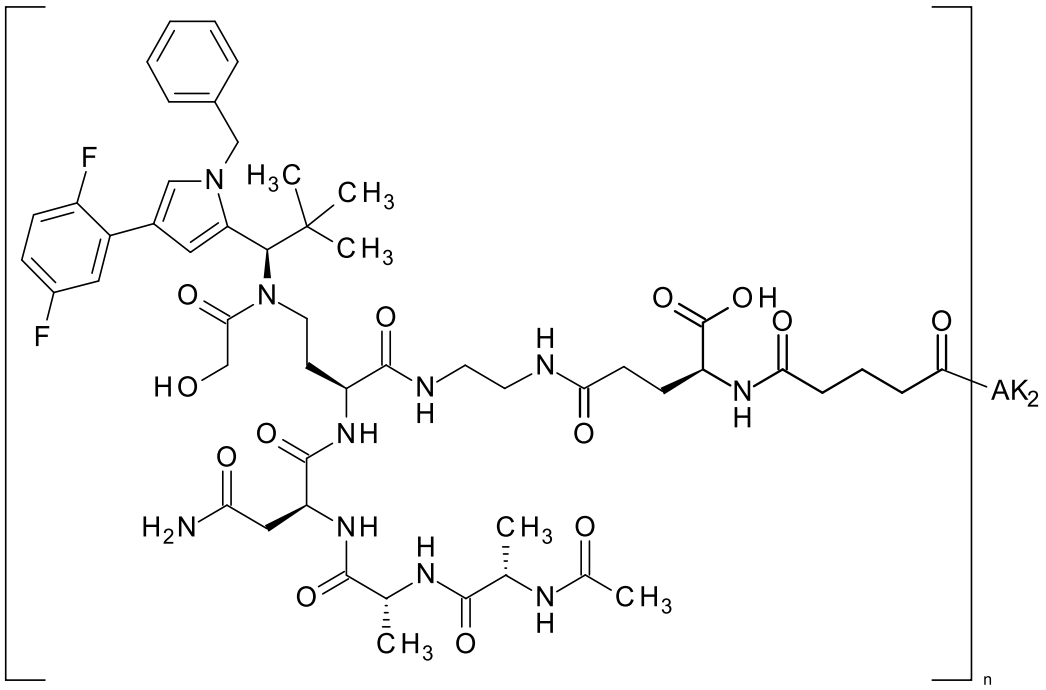
Example 12e

In an analogous manner, Intermediate R12 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.88 mg/ml
Drug/mAb ratio: 3.2

Example 12k

In an analogous manner, Intermediate R12 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.98 mg/ml
Drug/mAb ratio: 4.2

Example 13a



To 5 mg of cetuximab in 0.55 ml of PBS (c = 9 mg/ml) under argon was added a solution of 5 eq (0.21 mg) of Intermediate R13 dissolved in 50 µl of DMSO, and after stirring at RT for 1 h the same amount again was added and the mixture was stirred at RT for a further hour. The reaction mixture was subsequently diluted to 2.5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).

Protein concentration: 2.1 mg/ml

Drug/mAb ratio: 3.5

Example 13e

In an analogous manner, Intermediate R13 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:

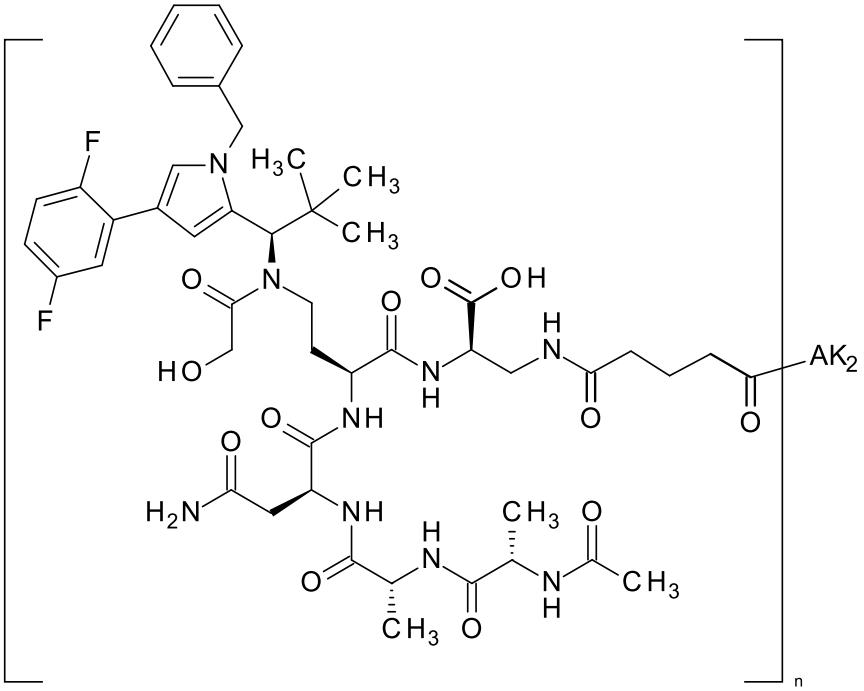
Protein concentration: 2.01 mg/ml

Drug/mAb ratio: 4.1

Example 13k

In an analogous manner, Intermediate R13 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 2.07 mg/ml
Drug/mAb ratio: 4.5

Example 14a



To 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml) under argon was added a solution of 5 eq (0.19 mg) of Intermediate R14 dissolved in 50 µl of DMSO, and after stirring at RT for 1 h the same amount again was added and the mixture was stirred at RT for a further hour. The reaction mixture was subsequently diluted to 2.5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).
Protein concentration: 2.04 mg/ml
Drug/mAb ratio: 3.9

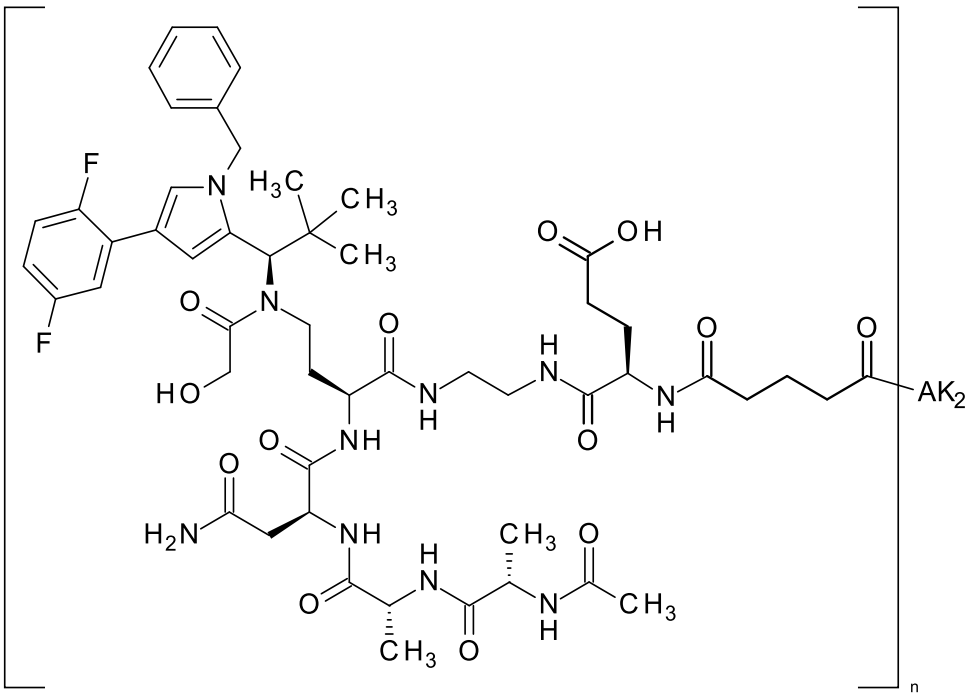
Example 14e

In an analogous manner, Intermediate R14 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.79 mg/ml
Drug/mAb ratio: 5.9

Example 14k

In an analogous manner, Intermediate R14 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 2.07 mg/ml
Drug/mAb ratio: 5.4

Example 15a



5 mg of cetuximab in 0.458 ml of PBS (c = 10.9 mg/ml) were admixed under argon with a solution of 5 eq (0.2 mg) of Intermediate R15 dissolved in 50 µl of DMSO, and after stirring at RT for 1 h the same amount again was added and the mixture was stirred at RT for a further hour. The reaction mixture was subsequently diluted to 2.5 ml with PBS buffer

(pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).
Protein concentration: 2.31 mg/ml
Drug/mAb ratio: 5.5

Example 15e

In an analogous manner, Intermediate R15 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.89 mg/ml
Drug/mAb ratio: 6.8

Example 15h1

30 mg of the B7H3 antibody TPP-8382 in 1.91 ml of PBS (c=15.7 mg/ml) were admixed under argon with a solution of 5 eq (1.2 mg) of Intermediate R15 dissolved in 200 µl of DMSO, and after stirring at RT for 1 h the same amount again was added and the mixture was stirred at RT for a further hour. Subsequently, the mixture was diluted to 2.5 ml with PBS buffer (pH7.2) and purified using a Sephadex column. The eluate was then concentrated by ultracentrifugation, rediluted with PBS (pH 7.2) and reconcentrated and sterile-filtered again.
Protein concentration: 10.39 mg/ml
Drug/mAb ratio: 5.6

Example 15k

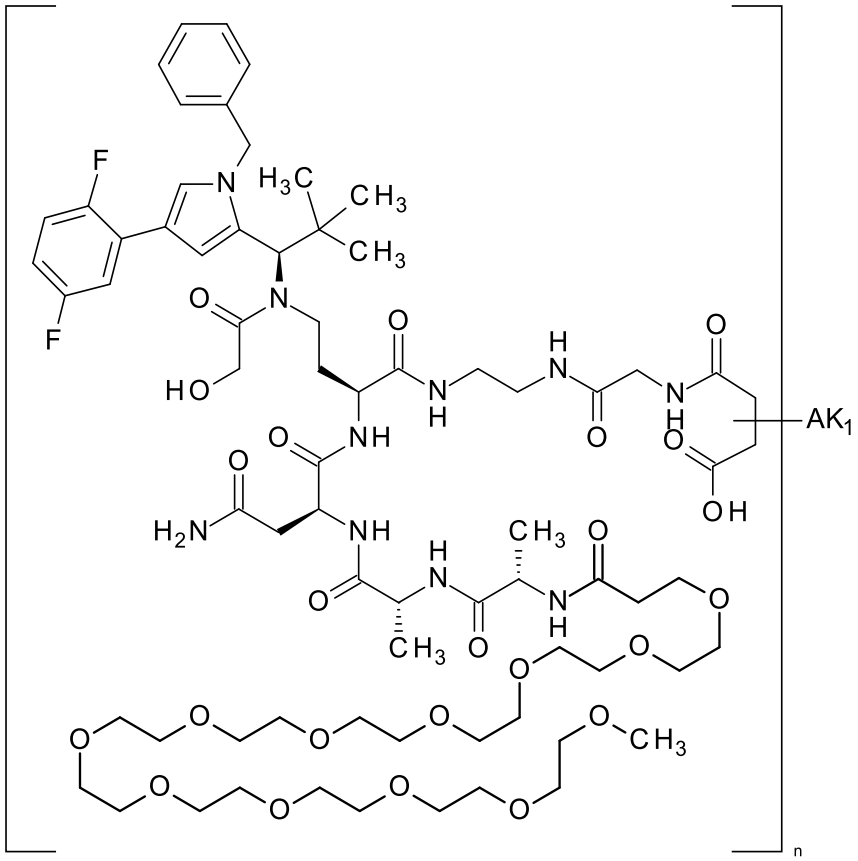
In an analogous manner, Intermediate R15 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.92 mg/ml
Drug/mAb ratio: 8.1

Example 15l1

In an analogous manner, Intermediate R15 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The excess of R15 was reduced here from 10 equivalents to 5 equivalents. The ADC batch obtained was characterized as follows:
Protein concentration: 1.64 mg/ml

Drug/mAb ratio: 2.2

Example 16a



Under argon, a solution of 0.076 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.458 ml of PBS (c = 10.92 mg/ml). The mixture was stirred at RT for 150 min, and then 0.811 mg (0.000533 mmol) of Intermediate R16 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 120 min, the reaction mixture was diluted with 1.95 ml of PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight. This was followed by concentration by ultracentrifugation and redilution with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:

Protein concentration: 2.20 mg/ml

Drug/mAb ratio: 7.3

Example 16e

In an analogous manner, Intermediate R16 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 2.25 mg/ml
Drug/mAb ratio: 7.3

Example 16h1

Under argon, a solution of 0.77 mg of TCEP in 0.4 ml of PBS buffer was added to 50 mg of the B7H3 antibody TPP-8382 in 3.19 ml of PBS (c = 15.7 mg/ml). The mixture was stirred at RT for 150 min. Then 16 eq (8.1 mg) of Intermediate R16 dissolved in 400 µl of DMSO were added and the mixture was stirred at RT for 2 h. Then the mixture was diluted to 5 ml with PBS buffer (pH8). This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was diluted with 0.5 ml of PBS buffer pH 8 and stirred under argon at RT overnight. This was followed by concentration by ultracentrifugation and redilution with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 9.56 mg/ml
Drug/mAb ratio: 7.2

Example 16h2

40 mg of the B7H3 antibody TPP-8567 in 2.79 ml of PBS (c = 14.4 mg/ml) were coupled in an analogous manner to Intermediate R16.
Protein concentration: 9.93 mg/ml
Drug/mAb ratio: 7.9

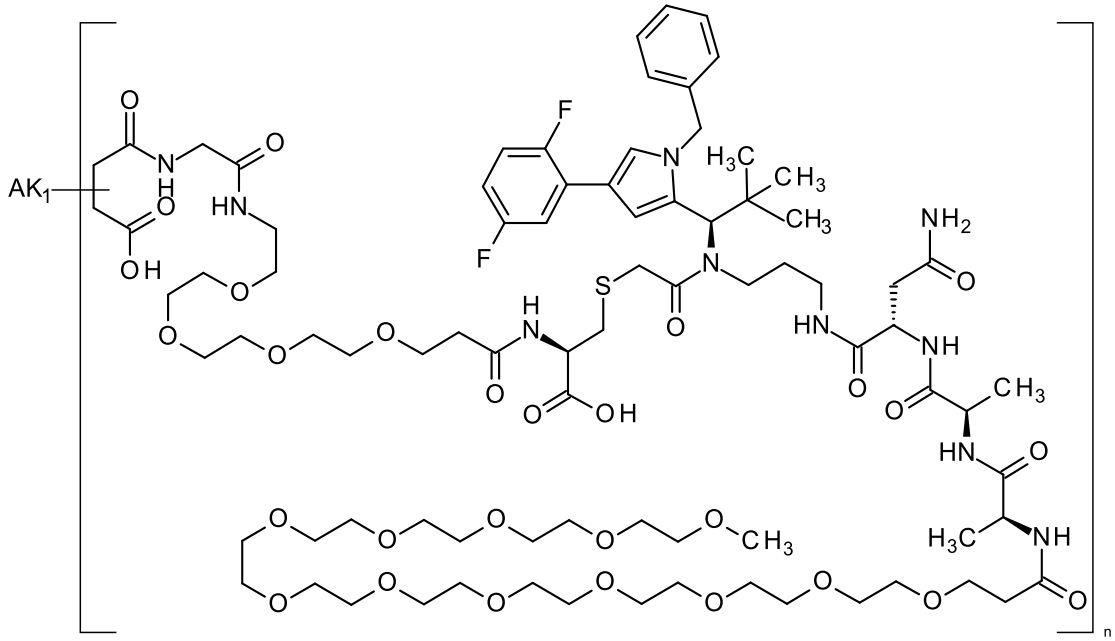
Example 16k

In an analogous manner, Intermediate R16 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 2.36 mg/ml
Drug/mAb ratio: 7.4

Example 16l2

25 mg of the anti-TWEAKR antibody TPP-7006 in 1.514 ml of PBS (c = 16.5 mg/ml) were coupled as described under 16h1 to Intermediate R16.
Protein concentration: 11.54 mg/ml
Drug/mAb ratio: 8.0

Example 17a



Under argon, a solution of 0.086 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.458 ml of PBS (c = 10.92 mg/ml). The mixture was stirred at RT for 150 min, and then 1.07 mg (0.00060 mmol) of Intermediate R17 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 120 min, the reaction mixture was diluted with 1.95 ml of PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight. This was followed by concentration by ultracentrifugation and redilution with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 2.2 mg/ml
Drug/mAb ratio: 5.5

Example 17e

In an analogous manner, Intermediate R17 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.71 mg/ml
Drug/mAb ratio: 4.3

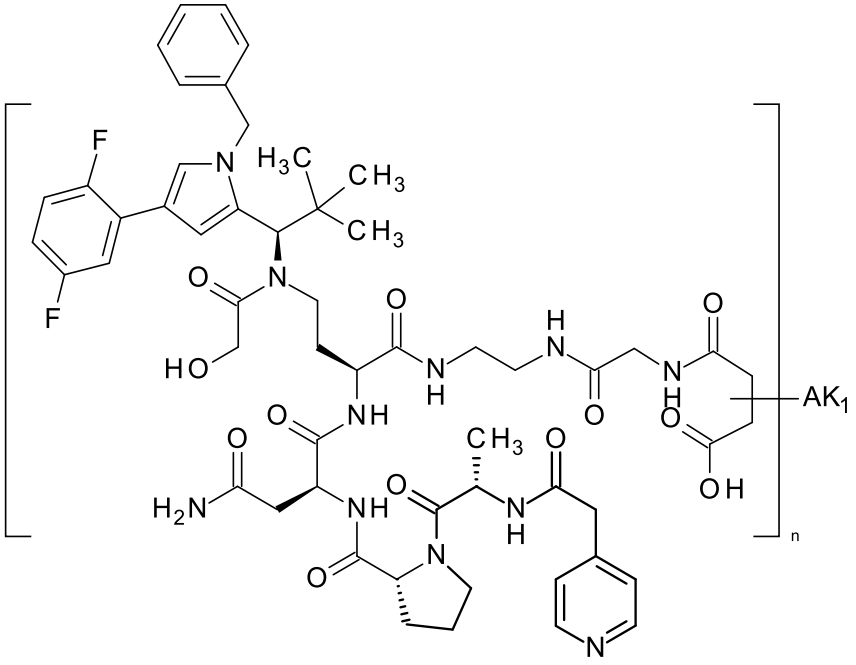
Example 17h1

In an analogous manner, Intermediate R17 was coupled to 5 mg of anti-B7H3 antibody TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 1.52 mg/ml
Drug/mAb ratio: 5.1

Example 17k

In an analogous manner, Intermediate R17 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.65 mg/ml
Drug/mAb ratio: 5.4

Example 18a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.26 mg (0.00023 mmol) of Intermediate R18 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.82 mg/ml
Drug/mAb ratio: 3.0

Example 18e

In an analogous manner, Intermediate R18 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:

Protein concentration: 1.7 mg/ml
Drug/mAb ratio: 4.1

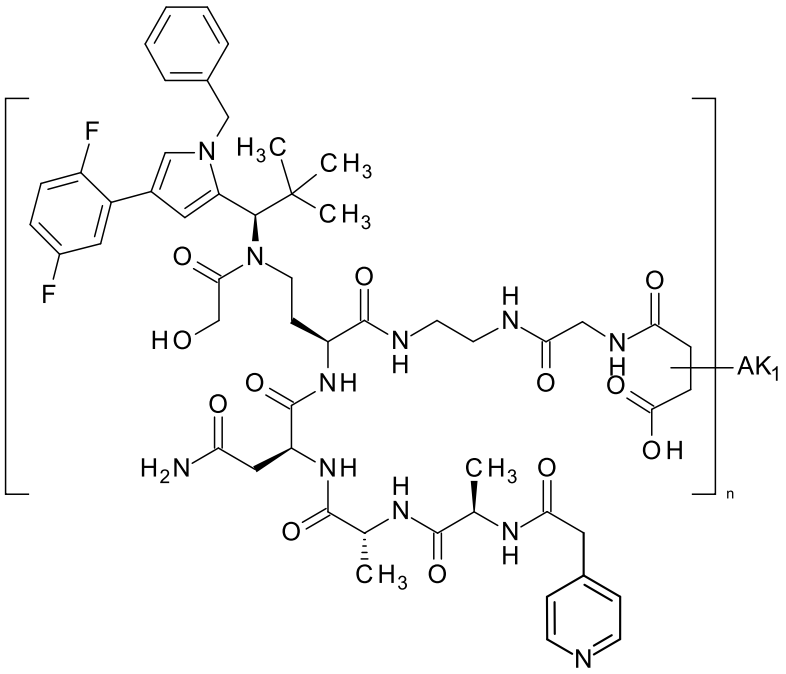
Example 18h1

In an analogous manner, Intermediate R18 was coupled to 5 mg of anti-B7H3 TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 1.71 mg/ml
Drug/mAb ratio: 4.5

Example 18k

In an analogous manner, Intermediate R18 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.69 mg/ml
Drug/mAb ratio: 3.6

Example 19a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.25 mg (0.00023 mmol) of Intermediate R19 dissolved in 50 µl of DMSO was

added. After stirring at RT for a further 90 min, the reaction mixture was diluted to 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.88 mg/ml
Drug/mAb ratio: 2.8

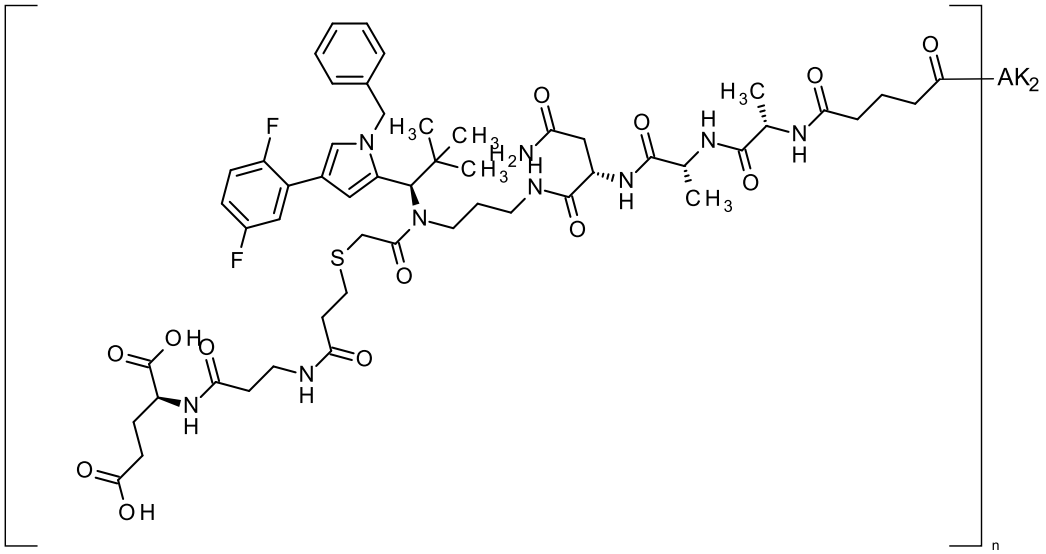
Example 19e

In an analogous manner, Intermediate R19 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.7 mg/ml
Drug/mAb ratio: 3.3

Example 19k

In an analogous manner, Intermediate R19 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 2.35 mg/ml
Drug/mAb ratio: 3.2

Example 20a



3 mg of cetuximab in 0.3 ml of PBS (c = 10 mg/ml) were admixed under argon with a solution of 5 eq (0.268 mg) of Intermediate R20 dissolved in 30 µl of DMSO, and after stirring at RT for 1 h the same amount again was added and the mixture was stirred at RT for a further hour. Then the mixture was diluted to 2.5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).

Protein concentration: 2.35 mg/ml

Drug/mAb ratio: 5.3

Example 20e

In an analogous manner, Intermediate R20 was coupled to 3 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:

Protein concentration: 1.94 mg/ml

Drug/mAb ratio: 7.1

Example 20h1

In an analogous manner, Intermediate R20 was coupled to 3 mg of anti-B7H3 antibody TPP-8382. The ADC batch obtained was characterized as follows:

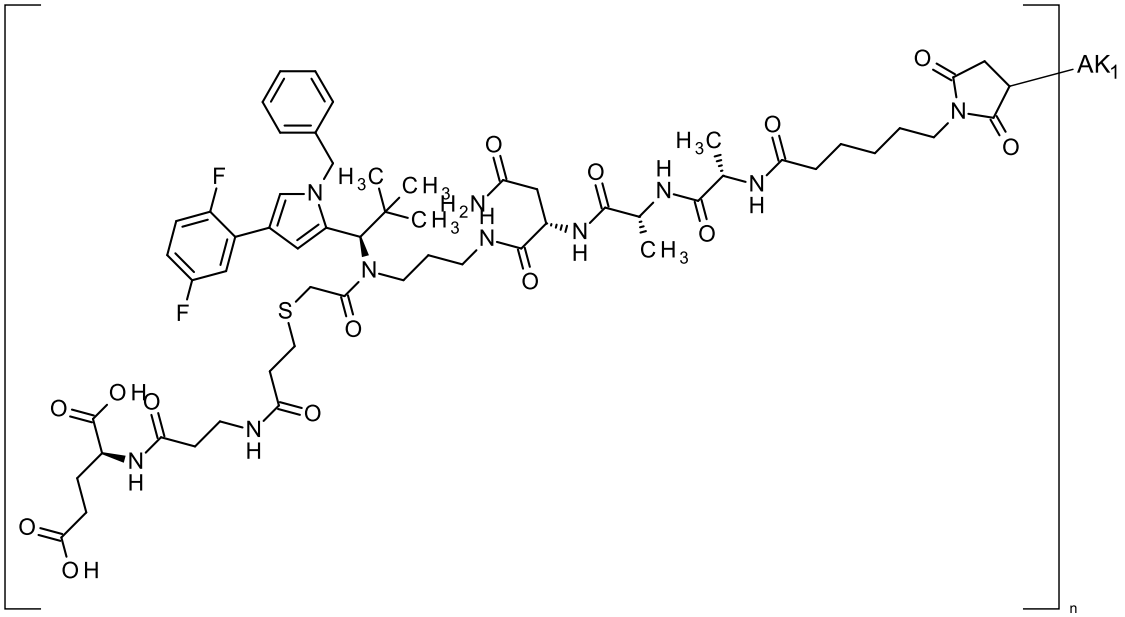
Protein concentration: 2.12 mg/ml

Drug/mAb ratio: 5.9

Example 20k

In an analogous manner, Intermediate R20 was coupled to 3 mg of anti-B7H3 TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 2.21 mg/ml
Drug/mAb ratio: 6.8

Example 21a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.31 mg (0.00023 mmol) of Intermediate R21 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was diluted to a volume of 2.5 ml with PBS buffer, purified on a Sephadex column and then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 2.17 mg/ml
Drug/mAb ratio: 5.6

Example 21e

In an analogous manner, Intermediate R21 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.99 mg/ml
Drug/mAb ratio: 3.8

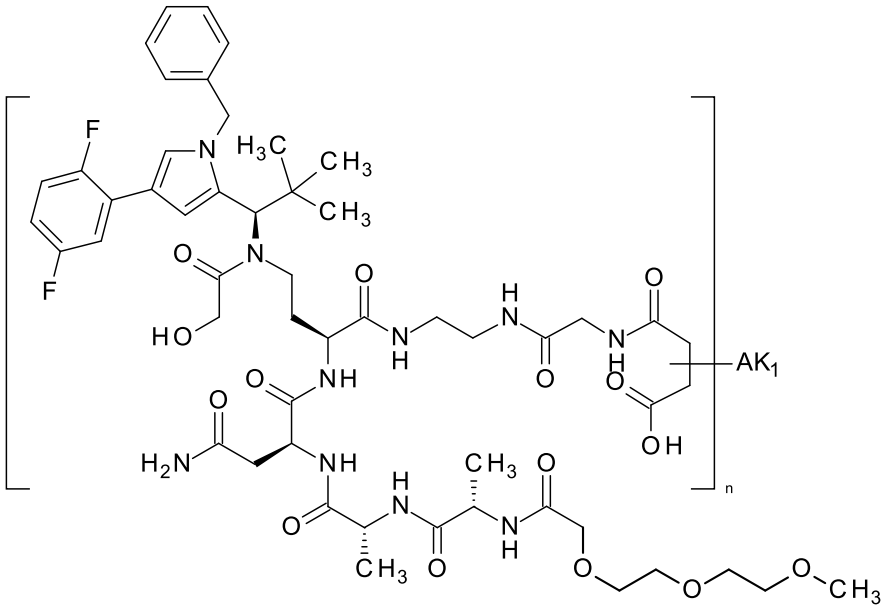
Example 21h1

In an analogous manner, Intermediate R21 was coupled to 5 mg of anti-B7H3 TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 2.08 mg/ml
Drug/mAb ratio: 4.6

Example 21k

In an analogous manner, Intermediate R21 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 2.13 mg/ml
Drug/mAb ratio: 4.4

Example 22a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.26 mg (0.00023 mmol) of Intermediate R22 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.99 mg/ml
Drug/mAb ratio: 2.8

Example 22e

In an analogous manner, Intermediate R22 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.9 mg/ml
Drug/mAb ratio: 3.3

Example 22h1

In an analogous manner to Example 9h1, Intermediate R22 was coupled to 30 mg of anti-B7H3 antibody TPP-8382 in 3 ml of PBS. The ADC batch obtained was characterized as follows:
Protein concentration: 8.74 mg/ml
Drug/mAb ratio: 3.9

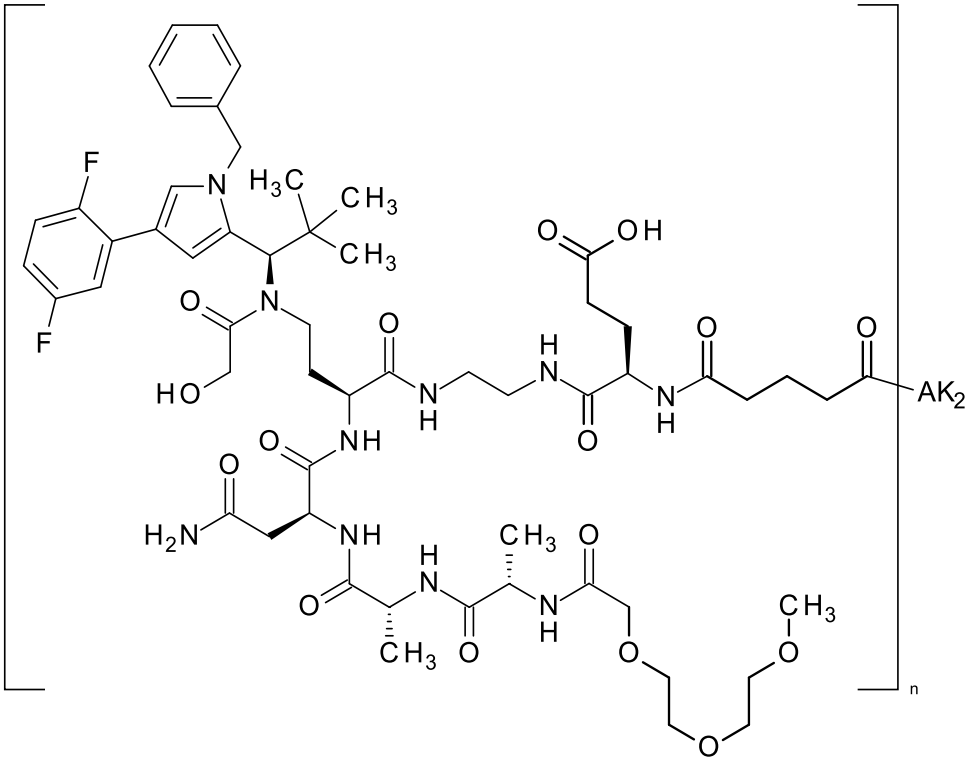
Example 22h2

In an analogous manner, Intermediate R22 was coupled to 5 mg of anti-B7H3 antibody TPP-8567. The ADC batch obtained was characterized as follows:
Protein concentration: 2.11 mg/ml
Drug/mAb ratio: 4.7

Example 22k

In an analogous manner, Intermediate R22 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.79 mg/ml
Drug/mAb ratio: 3.3

Example 23a



5 mg of cetuximab in 0.55 ml of PBS (c = 9.1 mg/ml) were admixed under argon with a solution of 5 eq (0.24 mg) of Intermediate R23 dissolved in 50 µl of DMSO. After stirring at RT for 1 h, the same amount again was added and the mixture was stirred at RT for a further hour. The reaction mixture was subsequently diluted to 2.5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).
Protein concentration: 2.13 mg/ml
Drug/mAb ratio: 6.1

Example 23e

In an analogous manner, Intermediate R23 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 2.02 mg/ml
Drug/mAb ratio: 7.6

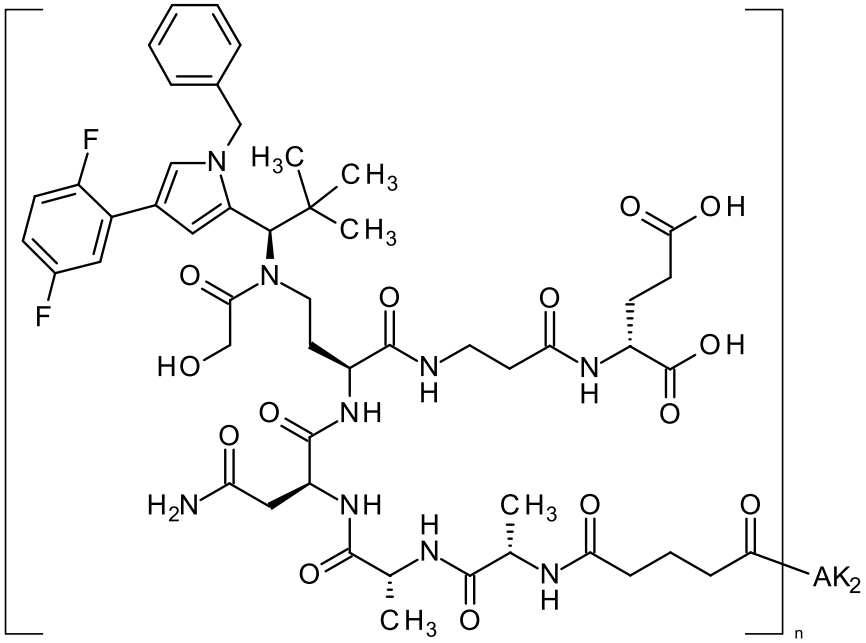
Example 23h1

In an analogous manner to Example 15h1, Intermediate R23 was coupled to 30 mg of anti-B7H3 antibody TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 9.97 mg/ml
Drug/mAb ratio: 6.4

Example 23k

In an analogous manner, Intermediate R23 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 2.24 mg/ml
Drug/mAb ratio: 7.3

Example 24a



5 mg of cetuximab in 0.458 ml of PBS (c=10.9 mg/ml) were admixed under argon with a solution of 5 eq (0.24 mg) of Intermediate R24 dissolved in 50 µl of DMSO. After stirring at RT for 1 h, the same amount again was added and the mixture was stirred at RT for a further hour. The reaction mixture was subsequently diluted to 2.5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).

Protein concentration: 2.2 mg/ml

Drug/mAb ratio: 4.7

Example 24e

In an analogous manner, Intermediate R24 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:

Protein concentration: 1.89 mg/ml

Drug/mAb ratio: 5.8

Example 24k

In an analogous manner, Intermediate R24 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 2.21 mg/ml
Drug/mAb ratio: 5.7

Example 24l1

To a solution of 30 mg of the anti-TWEAKR antibody TPP-7007 in 2.52 ml of PBS (c = 11.9 mg/ml) were added, under argon, 1.2 mg (0.001 mmol) of Intermediate R24 dissolved in 100 µl of DMSO. After stirring at RT for 60 min, the same amount again was added and the mixture was stirred at RT for a further hour. Then the mixture was diluted to 5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation, rediluted with PBS (pH 7.2) and reconcentrated and sterile-filtered again. The ADC batch obtained was characterized as follows:
Protein concentration: 10.5 mg/ml
Drug/mAb ratio: 3.3

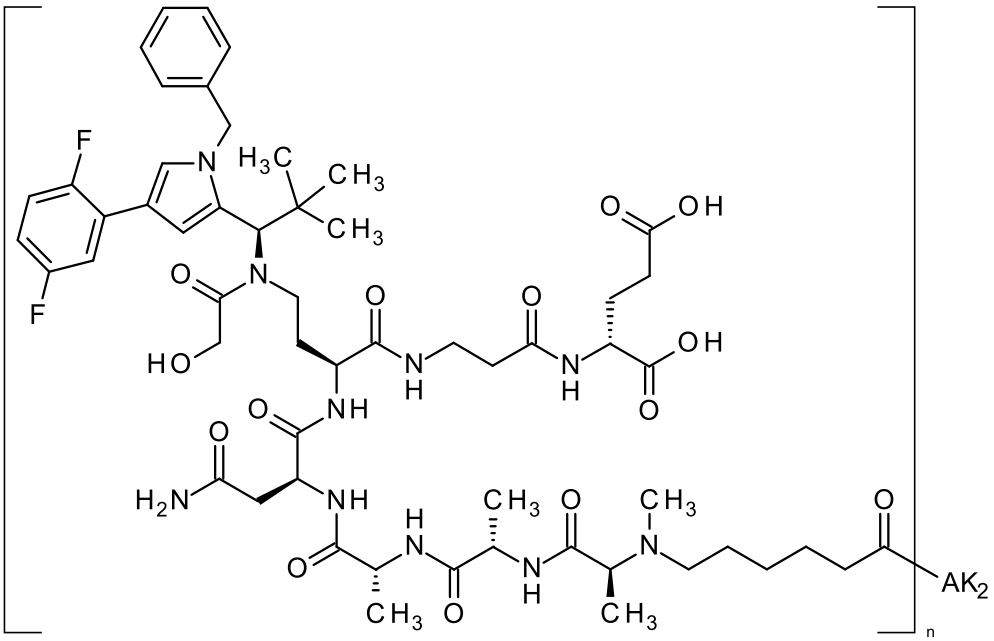
Example 24l3

In an analogous manner, Intermediate R24 was coupled to 30 mg of anti-TWEAKR antibody TPP-10336 in 2.61 ml of PBS (c = 11.5 mg/ml). The ADC batch obtained was characterized as follows:
Protein concentration: 10.46 mg/ml
Drug/mAb ratio: 5.0

Example 24l4

In an analogous manner, Intermediate R24 was coupled to 30 mg of anti-TWEAKR antibody TPP-10337 in 2.78 ml of PBS (c = 10.8 mg/ml). The ADC batch obtained was characterized as follows:
Protein concentration: 7.85 mg/ml
Drug/mAb ratio: 3.7

Example 25a



5 mg of cetuximab in 0.55 ml of PBS (c = 9.1 mg/ml) were admixed under argon with a solution of 5 eq (0.23 mg) of Intermediate R25 dissolved in 50 µl of DMSO, and after stirring at RT for 1 h the same amount again was added and the mixture was stirred at RT for a further hour. The reaction mixture was subsequently diluted to 2.5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).

Protein concentration: 2.07 mg/ml

Drug/mAb ratio: 4.5

Example 25e

In an analogous manner, Intermediate R25 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:

Protein concentration: 1.42 mg/ml

Drug/mAb ratio: 5.5

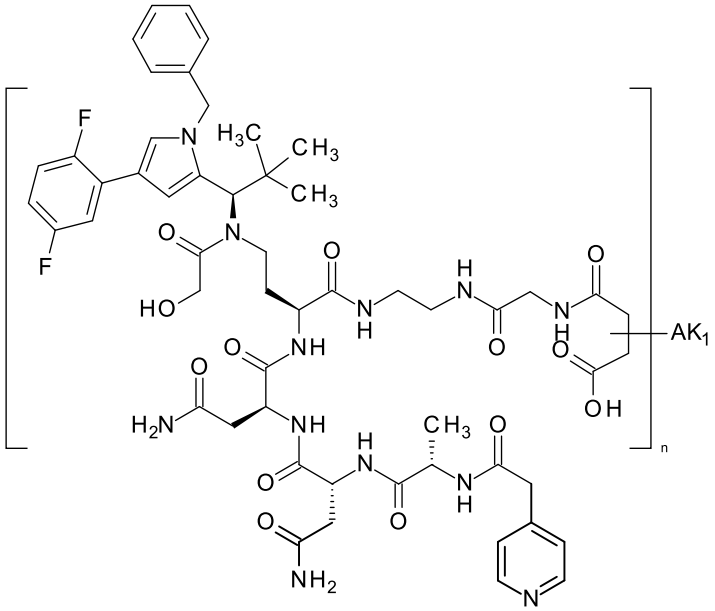
Example 25h1

In an analogous manner, Intermediate R25 was coupled to 5 mg of anti-B7H3 antibody TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 1.82 mg/ml
Drug/mAb ratio: 5.7

Example 25k

In an analogous manner, Intermediate R25 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 2.16 mg/ml
Drug/mAb ratio: 5.6

Example 26a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.26 mg (0.00023 mmol) of Intermediate R26 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been

equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.94 mg/ml
Drug/mAb ratio: 2.8

Example 26e

In an analogous manner, Intermediate R26 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.86 mg/ml
Drug/mAb ratio: 3.5

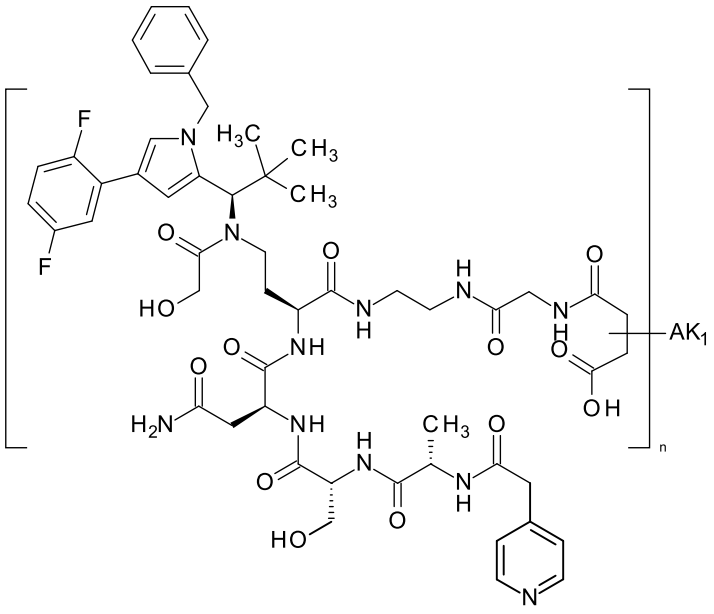
Example 26h1

In an analogous manner, Intermediate R26 was coupled to 5 mg of anti-B7H3 antibody TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 2.0 mg/ml
Drug/mAb ratio: 4.0

Example 26k

In an analogous manner, Intermediate R26 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.75 mg/ml
Drug/mAb ratio: 3.7

Example 27a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.23 mg (0.00023 mmol) of Intermediate R27 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.65 mg/ml
Drug/mAb ratio: 3.1

Example 27e

In an analogous manner, Intermediate R27 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.67 mg/ml
Drug/mAb ratio: 4.0

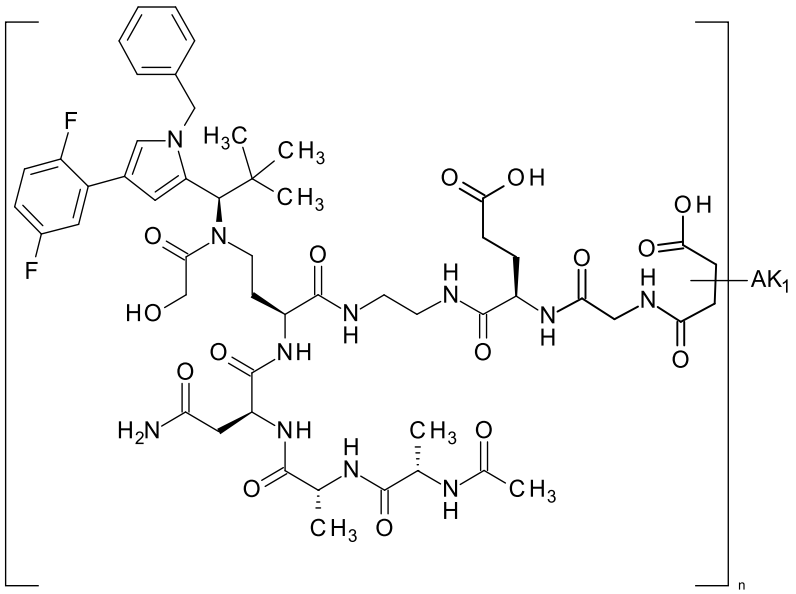
Example 27h1

In an analogous manner, Intermediate R27 was coupled to 5 mg of anti-B7H3 antibody TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 1.86 mg/ml
Drug/mAb ratio: 3.9

Example 27k

In an analogous manner, Intermediate R27 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.8 mg/ml
Drug/mAb ratio: 4.4

Example 28a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.26 mg (0.00023 mmol) of Intermediate R28 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which

had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.68 mg/ml
Drug/mAb ratio: 3.0

Example 28e

In an analogous manner, Intermediate R28 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.55 mg/ml
Drug/mAb ratio: 3.6

Example 28h1

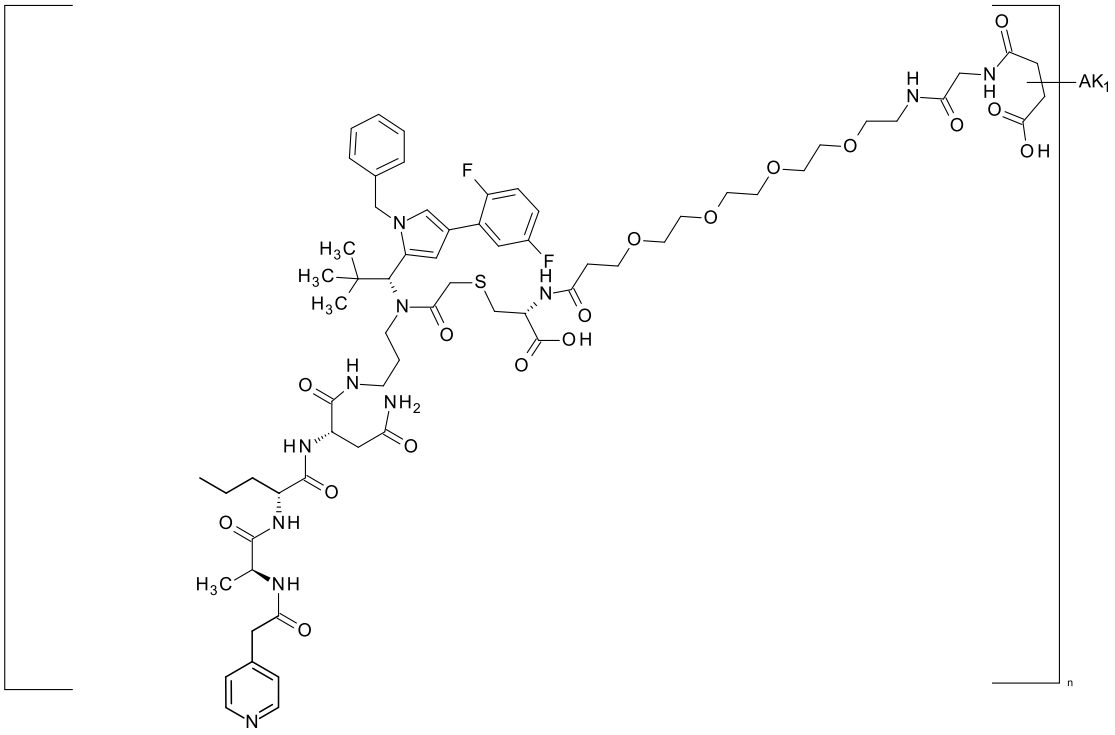
In an analogous manner, Intermediate R28 was coupled to 5 mg of anti-B7H3 antibody TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 1.95 mg/ml
Drug/mAb ratio: 3.4

Example 28l1

Under argon, a solution of 0.17 mg of TCEP in 0.25 ml of PBS buffer (pH 7.2) was added to 30 mg of the anti-TWEAKR antibody TPP-7007 in 2.5 ml of PBS (c = 12 mg/ml). The mixture was stirred at RT for 30 min, and then 1.57 mg (0.0014 mmol) of Intermediate R28 dissolved in 250 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was diluted to 5 ml with PBS buffer which had been adjusted to pH 8 beforehand, divided and then each portion was passed through a PD 10 column (Sephadex® G-25, GE Healthcare) equilibrated with PBS buffer pH 8, and eluted with PBS buffer pH 8. The eluates were combined, diluted to 7.5 ml with PBS buffer pH 8 and stirred under argon at RT overnight. This solution was then applied to PD 10 columns (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 7.2 and was eluted with PBS buffer pH 7.2. The eluate was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2) and reconcentrated and sterile-filtered again. The ADC batch obtained was characterized as follows:
Protein concentration: 8.2 mg/ml

Drug/mAb ratio: 3.1

Example 29a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.34 mg (0.00023 mmol) of Intermediate R29 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction was diluted to 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:

Protein concentration: 1.66 mg/ml

Drug/mAb ratio: 3.6

Example 29e

In an analogous manner, Intermediate R29 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.24 mg/ml
Drug/mAb ratio: 3.8

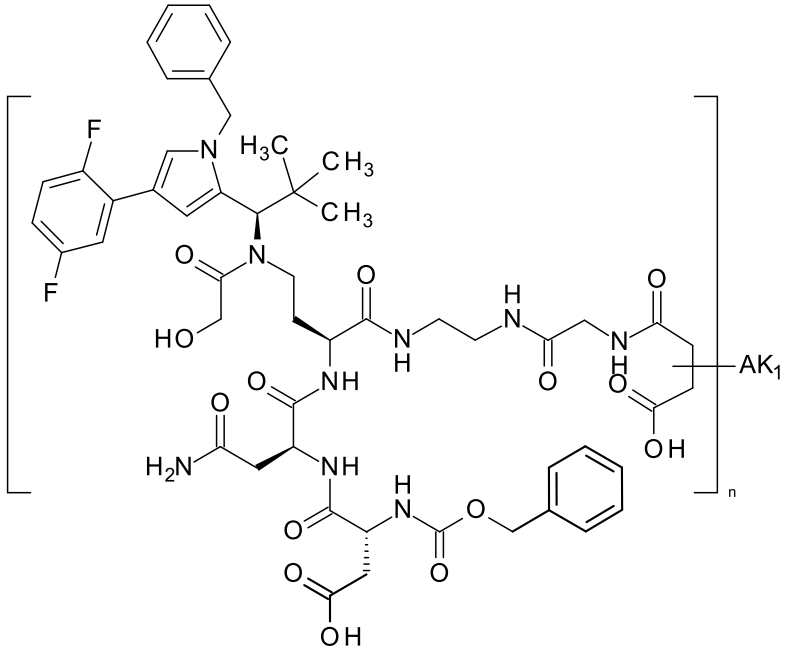
Example 29h1

In an analogous manner, Intermediate R29 was coupled to 5 mg of anti-B7H3 antibody TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 1.54 mg/ml
Drug/mAb ratio: 2.6

Example 29k

In an analogous manner, Intermediate R29 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.63 mg/ml
Drug/mAb ratio: 3.8

Example 30a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.26 mg (0.00023 mmol) of Intermediate R30 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction was diluted to 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:

Protein concentration: 1.74 mg/ml

Drug/mAb ratio: 3.0

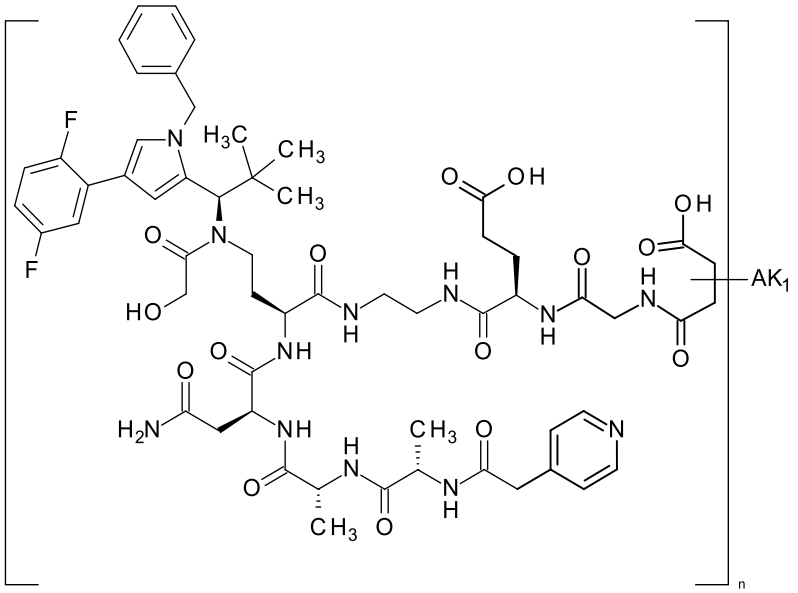
Example 30e

In an analogous manner, Intermediate R30 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.82 mg/ml
Drug/mAb ratio: 3.3

Example 30k

In an analogous manner, Intermediate R30 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.7 mg/ml
Drug/mAb ratio: 3.0

Example 31a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.26 mg (0.00023 mmol) of Intermediate R31 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been

equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 2.4 mg/ml
Drug/mAb ratio: 2.8

Example 31e

In an analogous manner, Intermediate R31 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.76 mg/ml
Drug/mAb ratio: 3.1

Example 31h1

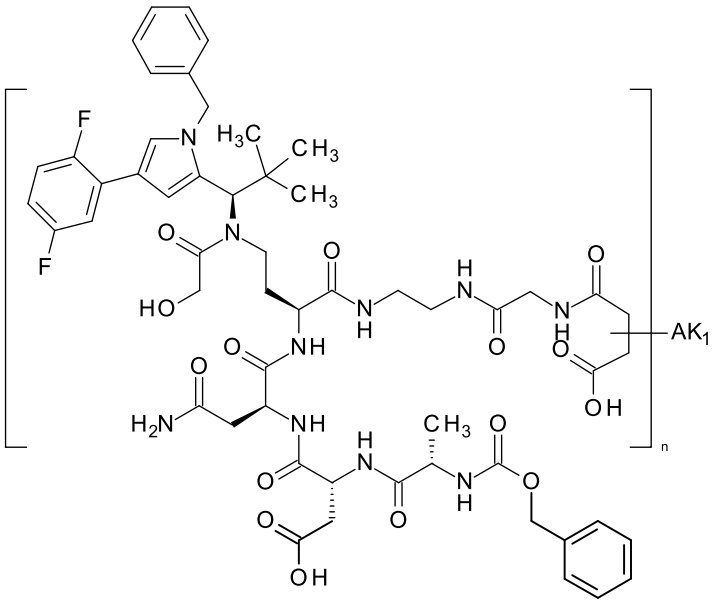
In an analogous manner to Example 9h1, Intermediate R31 was coupled to 20 mg of anti-B7H3 antibody TPP-8382 in 2 ml of PBS. The ADC batch obtained was characterized as follows:
Protein concentration: 9.45 mg/ml
Drug/mAb ratio: 3.3

Example 31l1

Under argon, a solution of 0.17 mg of TCEP in 0.3 ml of PBS buffer (pH 7.2) was added to 30 mg of the anti-TWEAKR antibody TPP-7007 in 3.4 ml of PBS (c = 8.8 mg/ml). The mixture was stirred at RT for 30 min, and then 1.68 mg (0.0014 mmol) of Intermediate R31 dissolved in 250 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was diluted to 5 ml with PBS buffer which had been adjusted to pH 8 beforehand, divided and then each portion was passed through a PD 10 column (Sephadex® G-25, GE Healthcare) equilibrated with PBS buffer pH 8, and eluted with PBS buffer pH 8. The eluates were combined again, diluted to 7.5 ml with PBS buffer pH 8 and stirred under argon at RT overnight. This solution was then applied to PD 10 columns (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 7.2 and was eluted with PBS buffer pH 7.2. The eluate was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2) and reconcentrated and sterile-filtered again. The ADC batch obtained was characterized as follows:

Protein concentration: 8.06 mg/ml
Drug/mAb ratio: 4.1

Example 32a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.29 mg (0.00023 mmol) of Intermediate R32 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.84 mg/ml
Drug/mAb ratio: 3.5

Example 32e

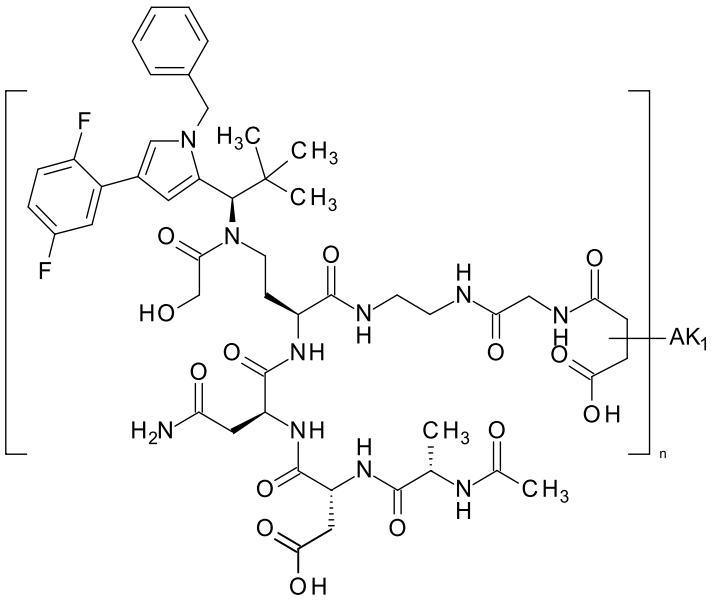
In an analogous manner, Intermediate R32 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:

Protein concentration: 1.82 mg/ml
Drug/mAb ratio: 3.9

Example 32k

In an analogous manner, Intermediate R32 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.88 mg/ml
Drug/mAb ratio: 3.7

Example 33a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.27 mg (0.00023 mmol) of Intermediate R33 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:

Protein concentration: 2.13 mg/ml
Drug/mAb ratio: 3.6

Example 33e

In an analogous manner, Intermediate R33 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.93 mg/ml
Drug/mAb ratio: 3.5

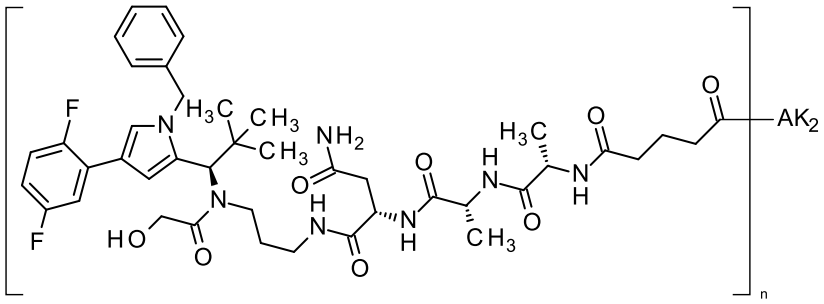
Example 33h1

In an analogous manner, Intermediate R33 was coupled to 5 mg of anti-B7H3 antibody TPP-8382. The ADC batch obtained was characterized as follows:
Protein concentration: 1.81 mg/ml
Drug/mAb ratio: 4.5

Example 33l1

In an analogous manner, Intermediate R33 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.86 mg/ml
Drug/mAb ratio: 3.8

Example 34a



5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml) were admixed under argon with a solution of 5 eq (0.15 mg) of Intermediate R34 dissolved in 50 µl of DMSO, and after

stirring at RT for 1 h the same amount again was added and the mixture was stirred at RT for a further hour. Then the mixture was diluted to 2.5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).

Protein concentration: 1.84 mg/ml
Drug/mAb ratio: 3.4

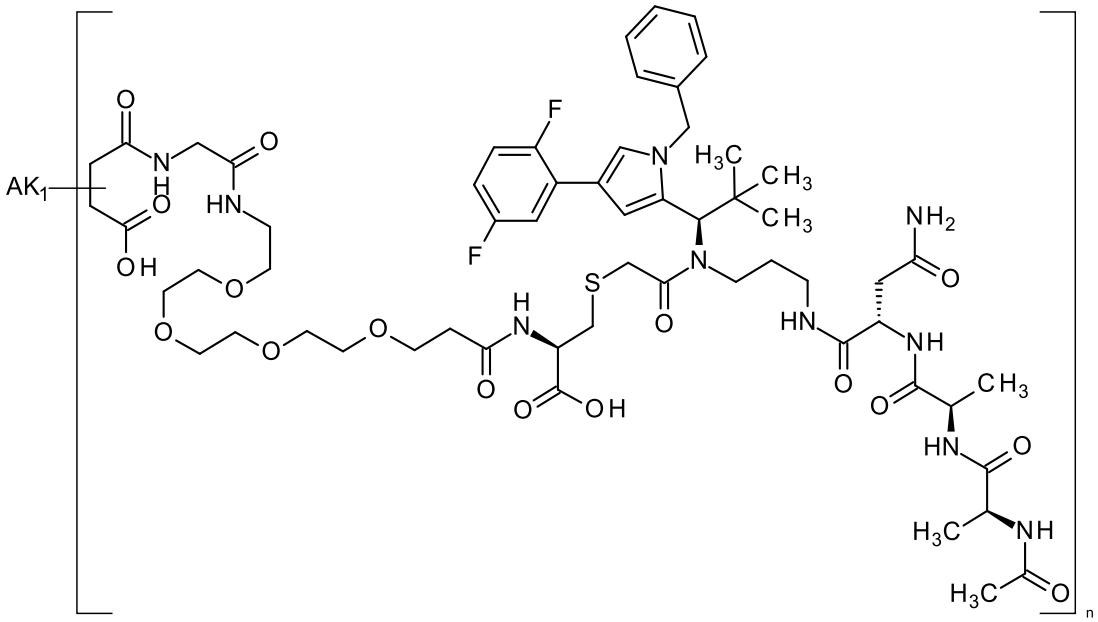
Example 34e

In an analogous manner, Intermediate R34 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.75 mg/ml
Drug/mAb ratio: 4.3

Example 34l1

In an analogous manner, Intermediate R34 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.76 mg/ml
Drug/mAb ratio: 3.3

Example 35a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.32 mg (0.00023 mmol) of Intermediate R35 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:

Protein concentration: 1.72 mg/ml

Drug/mAb ratio: 4.3

Example 35e

In an analogous manner, Intermediate R35 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:

Protein concentration: 1.57 mg/ml

Drug/mAb ratio: 4.4

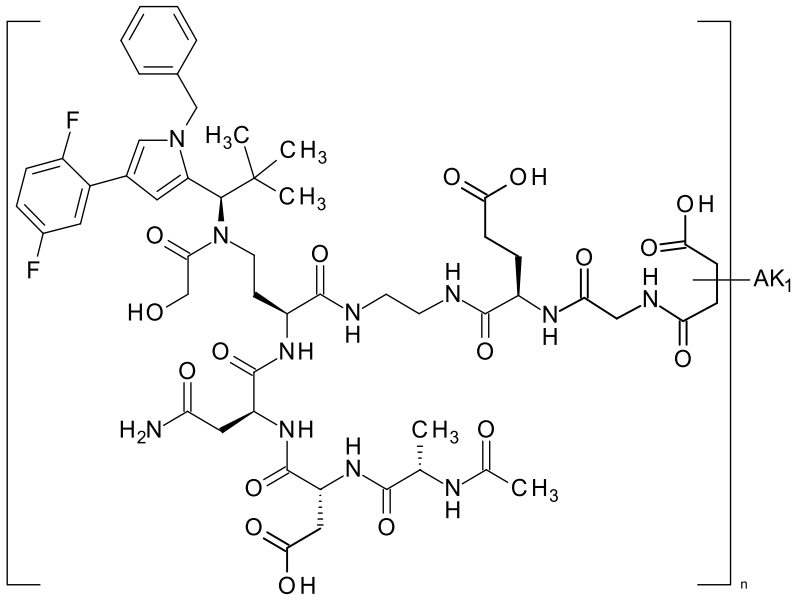
Example 35k

In an analogous manner, Intermediate R35 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.73 mg/ml
Drug/mAb ratio: 4.3

Example 35l1

Intermediate R35 was coupled to 25 mg of anti-TWEAKR antibody TPP-7007 in 2.5 ml of PBS in analogy to Example 31l1. The ADC batch obtained was characterized as follows:
Protein concentration: 11.55 mg/ml
Drug/mAb ratio: 3.5

Example 36a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.3 mg (0.00023 mmol) of Intermediate R36 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a

volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.95 mg/ml
Drug/mAb ratio: 2.8

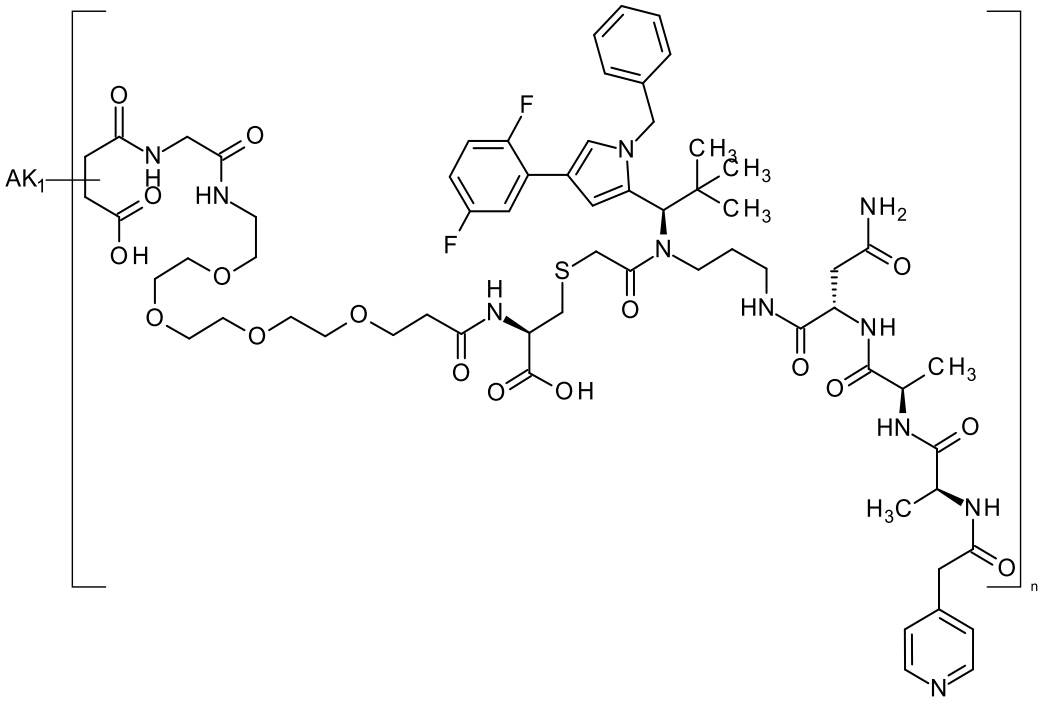
Example 36e

In an analogous manner, Intermediate R36 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.42 mg/ml
Drug/mAb ratio: 3.2

Example 36l1

In an analogous manner, Intermediate R36 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.94 mg/ml
Drug/mAb ratio: 2.7

Example 37a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.32 mg (0.00023 mmol) of Intermediate R37 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.77 mg/ml
Drug/mAb ratio: 4.2

Example 37e

In an analogous manner, Intermediate R37 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.44 mg/ml

Drug/mAb ratio: 4.6

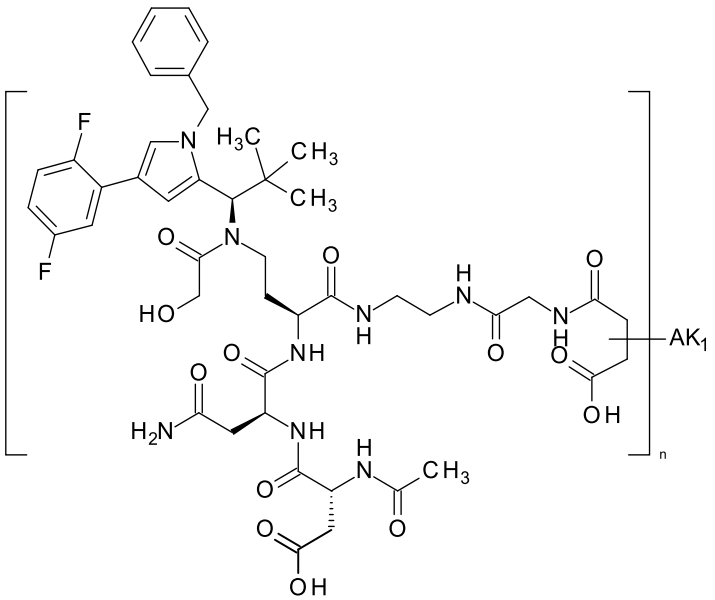
Example 37k

In an analogous manner, Intermediate R37 was coupled to 5 mg of anti-TWEAKR antibody TPP-2658. The ADC batch obtained was characterized as follows:
Protein concentration: 1.53 mg/ml
Drug/mAb ratio: 4.0

Example 37l1

Intermediate R37 was coupled to 25 mg of anti-TWEAKR antibody TPP-7007 in 2.5 ml of PBS in analogy to Example 31l1. The ADC batch obtained was characterized as follows:
Protein concentration: 11.46 mg/ml
Drug/mAb ratio: 3.3

Example 38a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.4 ml of PBS (c = 12.5 mg/ml). The mixture was stirred at RT for 30 min, and then 0.23 mg (0.00023 mmol) of Intermediate R38 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This

solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 2.02 mg/ml
Drug/mAb ratio: 3.0

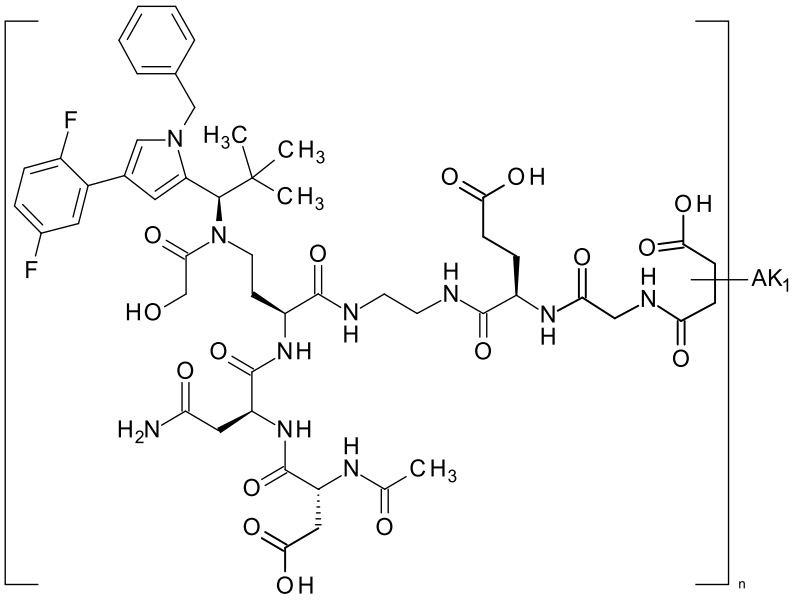
Example 38e

In an analogous manner, Intermediate R38 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.77 mg/ml
Drug/mAb ratio: 3.3

Example 38l1

In an analogous manner, Intermediate R38 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.06 mg/ml
Drug/mAb ratio: 4.0

Example 39a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.3 mg (0.00023 mmol) of Intermediate R39 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 2.01 mg/ml
Drug/mAb ratio: 3.6

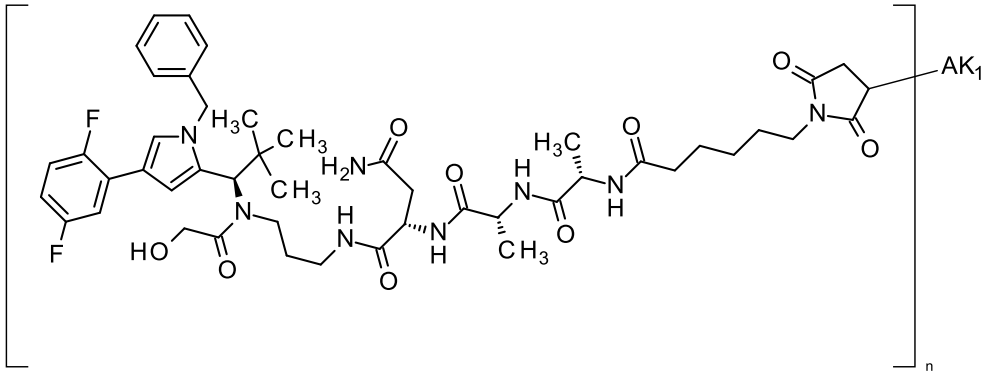
Example 39e

In an analogous manner, Intermediate R39 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.85 mg/ml
Drug/mAb ratio: 3.5

Example 39l1

In an analogous manner, Intermediate R39 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.84 mg/ml
Drug/mAb ratio: 3.4

Example 40a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.31 mg (0.00023 mmol) of Intermediate R40 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was diluted to a volume of 2.5 ml with PBS buffer, purified on a Sephadex column and then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.85 mg/ml
Drug/mAb ratio: 3.3

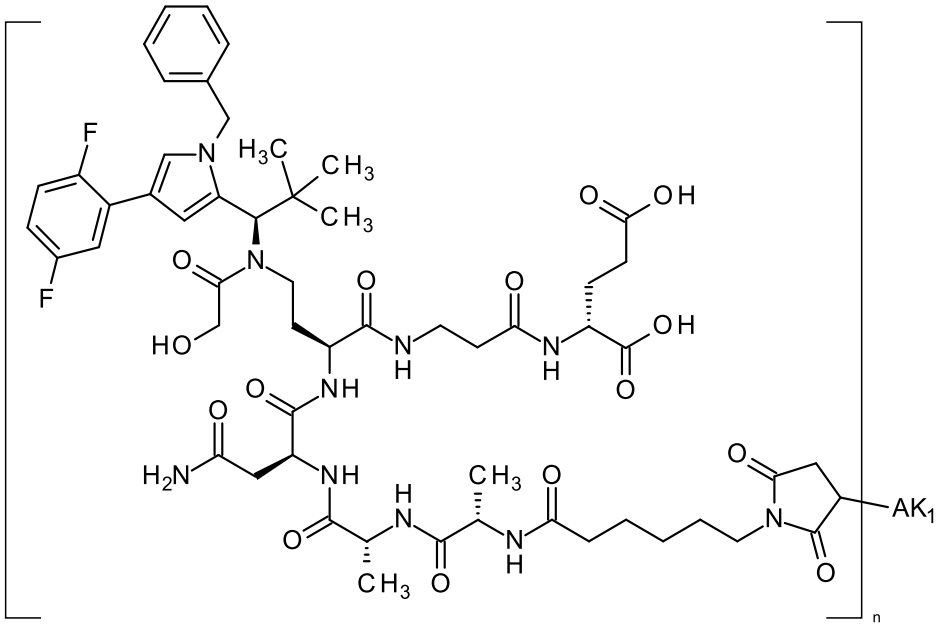
Example 40e

In an analogous manner, Intermediate R40 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 2.05 mg/ml
Drug/mAb ratio: 3.9

Example 40l1

In an analogous manner, Intermediate R40 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.98 mg/ml
Drug/mAb ratio: 3.9

Example 41a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.27 mg (0.00023 mmol) of Intermediate R41 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was diluted to a volume of 2.5 ml with PBS buffer, purified on a Sephadex column and then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 2.02 mg/ml
Drug/mAb ratio: 3.5

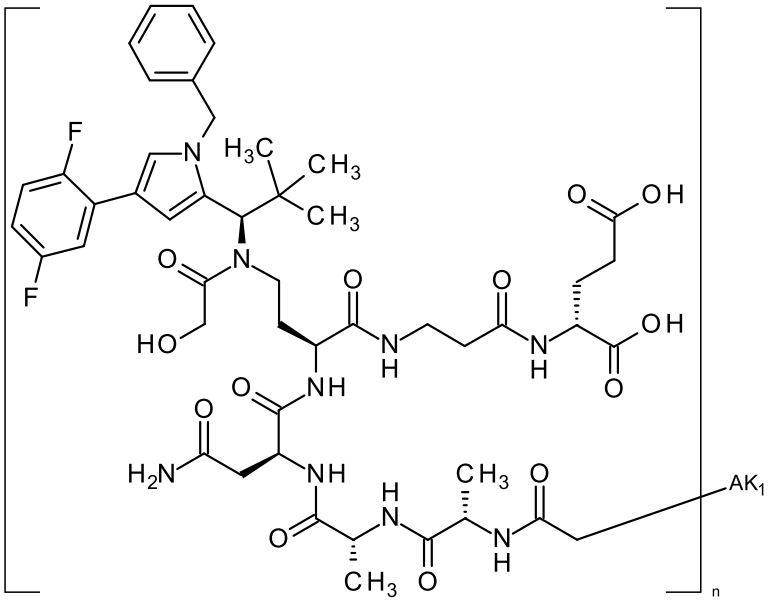
Example 41e

In an analogous manner, Intermediate R41 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 2.08 mg/ml
Drug/mAb ratio: 3.5

Example 41l1

In an analogous manner, Intermediate R41 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.94 mg/ml
Drug/mAb ratio: 3.9

Example 42a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.29 mg (0.00027 mmol) of Intermediate R42 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 4 h, the mixture was diluted to a volume of 2.5 ml with PBS buffer, purified on a Sephadex column and then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.68 mg/ml
Drug/mAb ratio: 2.6

Example 42e

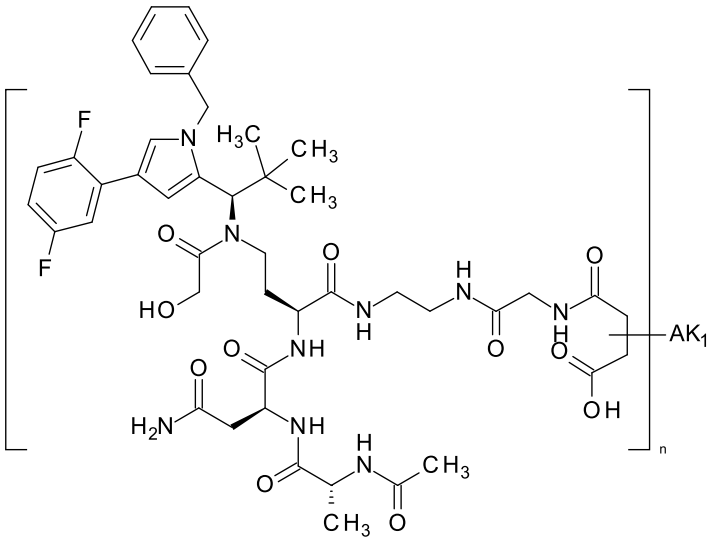
In an analogous manner, Intermediate R42 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:

Protein concentration: 2.09 mg/ml
Drug/mAb ratio: 3.0

Example 42l1

In an analogous manner, Intermediate R42 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.7 mg/ml
Drug/mAb ratio: 3.4

Example 43a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.4 ml of PBS (c = 12.5 mg/ml). The mixture was stirred at RT for 30 min, and then 0.22 mg (0.00023 mmol) of Intermediate R43 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 2.08 mg/ml
Drug/mAb ratio: 3.5

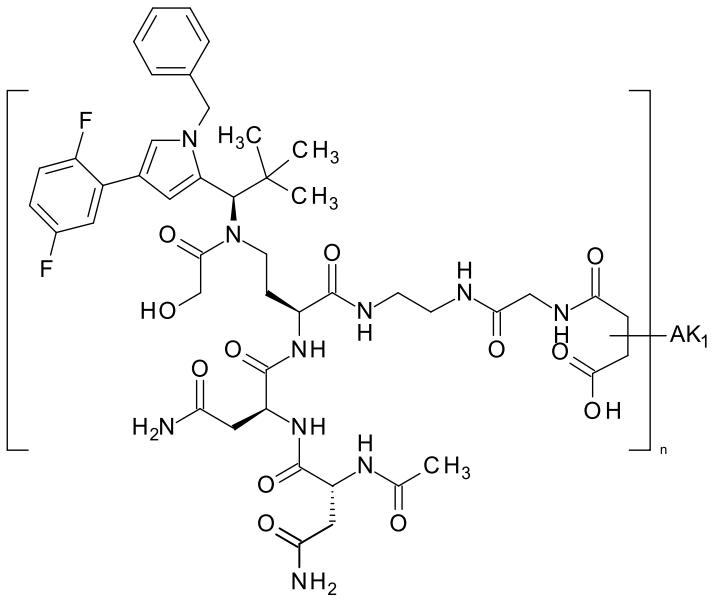
Example 43e

In an analogous manner, Intermediate R43 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.9 mg/ml
Drug/mAb ratio: 3.6

Example 43l1

In an analogous manner, Intermediate R38 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.57 mg/ml
Drug/mAb ratio: 3.1

Example 44e



Under argon, a solution of 0.023 mg of TCEP in 0.05 ml of PBS buffer was added to 4 mg of anti-HER2 antibody TPP-1015 in 0.4 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.18 mg (0.00019 mmol) of Intermediate R44 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8

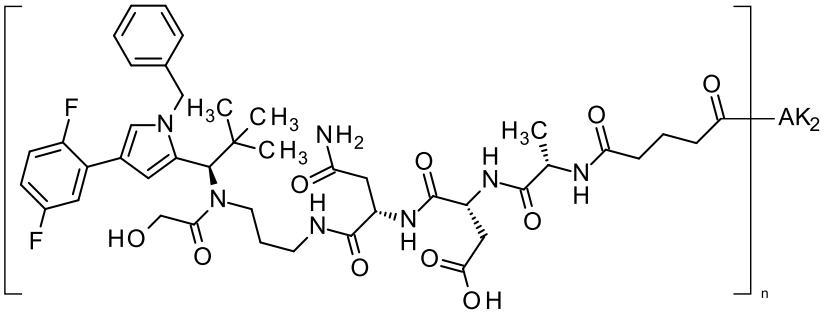
beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.59 mg/ml
Drug/mAb ratio: 3.4

Example 44l1

In an analogous manner, Intermediate R45 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.57 mg/ml
Drug/mAb ratio: 3.9

Example 45a



5 mg of cetuximab in 0.5 ml of PBS (c=10 mg/ml) were admixed under argon with a solution of 5 eq (0.16 mg) of Intermediate R45 dissolved in 50 µl of DMSO. After stirring at RT for 1 h, the same amount again was added and the mixture was stirred at RT for a further hour. Then the mixture was diluted to 2.5 ml with PBS buffer (pH 7.2), purified on a Sephadex column, then concentrated by ultracentrifugation and rediluted with PBS (pH 7.2).
Protein concentration: 2.14 mg/ml
Drug/mAb ratio: 4.6

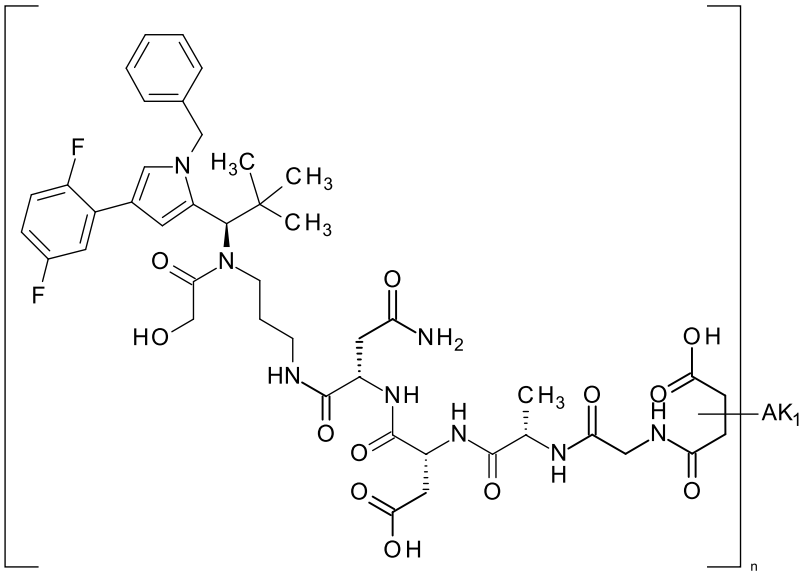
Example 45e

In an analogous manner, Intermediate R45 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.84 mg/ml
Drug/mAb ratio: 4.9

Example 45l1

In an analogous manner, Intermediate R45 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 2.01 mg/ml
Drug/mAb ratio: 4.3

Example 46a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml). The mixture was stirred at RT for 30 min, and then 0.21 mg (0.00023 mmol) of Intermediate R46 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which

had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.83 mg/ml
Drug/mAb ratio: 3.1

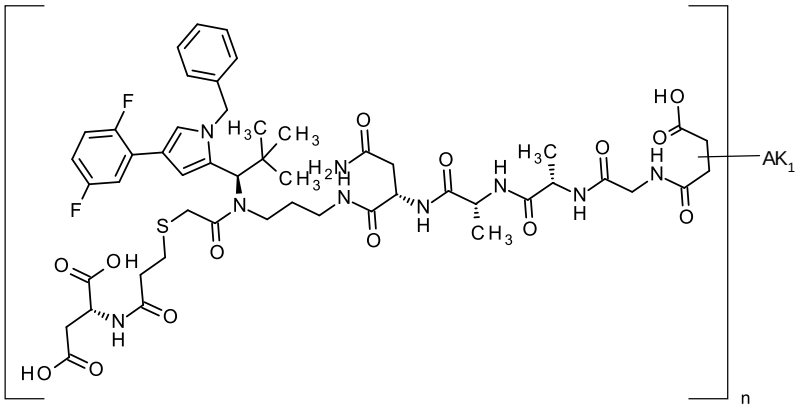
Example 46e

In an analogous manner, Intermediate R46 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.71 mg/ml
Drug/mAb ratio: 2.9

Example 46l1

In an analogous manner, Intermediate R46 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.78 mg/ml
Drug/mAb ratio: 3.4

Example 47a



Under argon, a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer was added to 5 mg of cetuximab in 0.45 ml of PBS (c = 11 mg/ml). The mixture was stirred at RT for 30 min, and then 0.275 mg (0.00023 mmol) of Intermediate R47 dissolved in 50 µl of DMSO was

added. After stirring at RT for a further 90 min, the reaction mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was stirred at RT under argon overnight.
This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.77 mg/ml
Drug/mAb ratio: 3.9

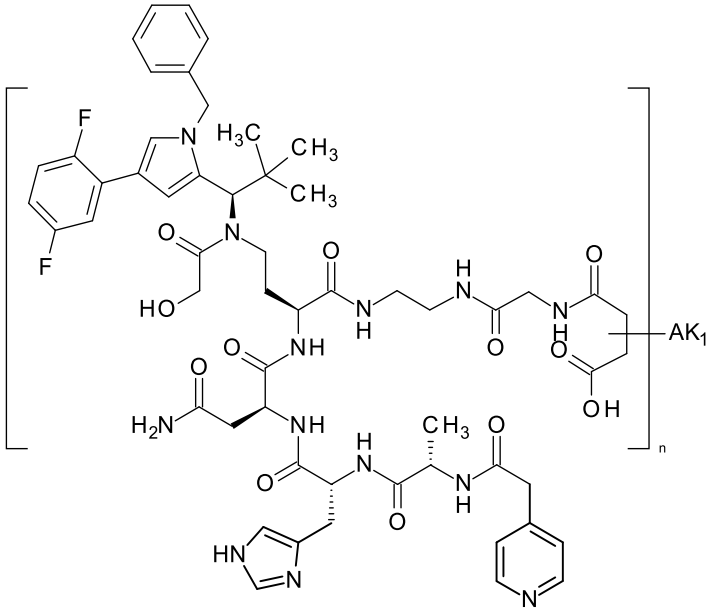
Example 47e

In an analogous manner, Intermediate R47 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.43 mg/ml
Drug/mAb ratio: 4.0

Example 47l1

In an analogous manner, Intermediate R47 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.60 mg/ml
Drug/mAb ratio: 4.1

Example 48a



To 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml) under argon was added a solution of 0.029 mg of TCEP in 0.05 ml of PBS buffer. The mixture was stirred at RT for 30 min and then 0.29 mg (0.00023 mmol) of Intermediate R48 dissolved in 50 µl of DMSO was added. After stirring at RT for a further 90 min, the mixture was diluted to a volume of 2.5 ml with PBS buffer which had been adjusted to pH 8 beforehand. This solution was then applied to a PD 10 column (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and eluted with PBS buffer pH8. The eluate was stirred at RT under argon overnight.

This solution was then concentrated by ultracentrifugation and rediluted with PBS buffer (pH 7.2). The ADC batch obtained was characterized as follows:
Protein concentration: 1.75 mg/ml
Drug/mAb ratio: 4.1

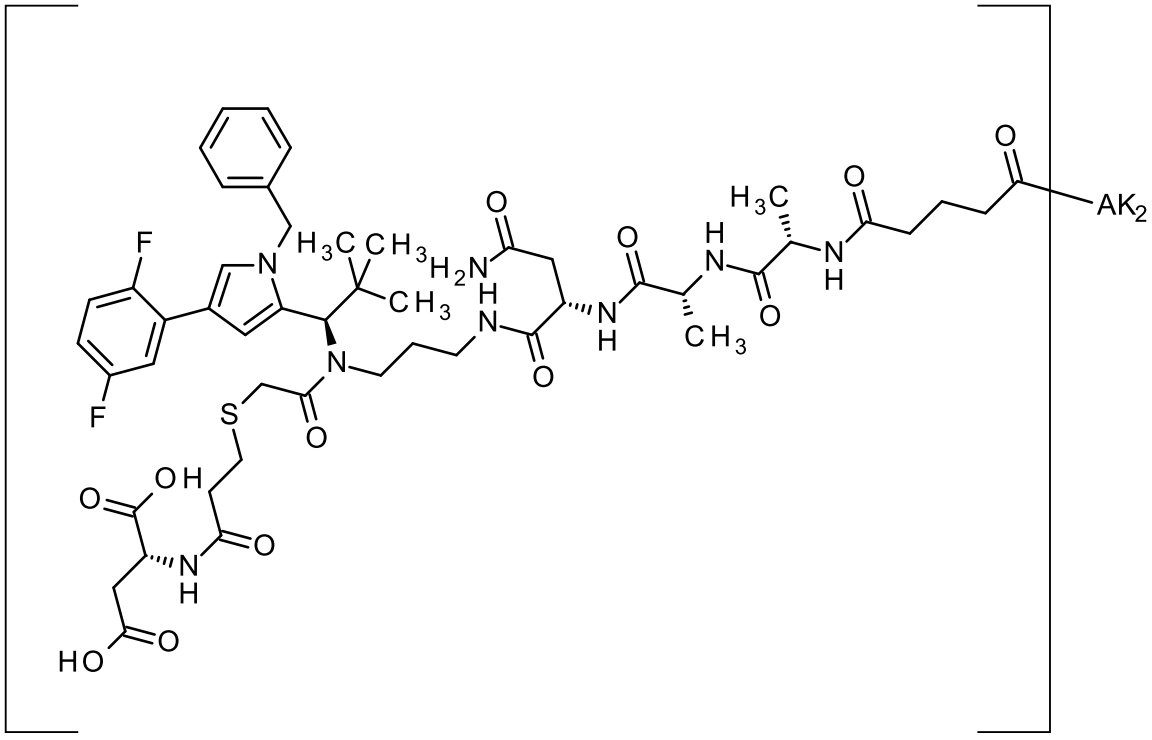
Example 48e

In an analogous manner, Intermediate R48 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:
Protein concentration: 1.72 mg/ml
Drug/mAb ratio: 4.7

Example 48l1

In an analogous manner, Intermediate R48 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:
Protein concentration: 1.71 mg/ml
Drug/mAb ratio: 2.4

Example 49a



To 5 mg of cetuximab in 0.5 ml of PBS (c = 10 mg/ml) under argon was added a solution of 5 eq (0.47 mg) of Intermediate R34 dissolved in 50 µl of DMSO and, after stirring at RT for 1 h, the same amount again was added and the mixture was stirred at RT for a further hour. Subsequently, the mixture was diluted to 2.5 ml with PBS buffer (pH 7.2), purified by means of a Sephadex column and then concentrated by ultracentrifugation and rediluted to 2.5 ml with PBS (pH 7.2).

The ADC batch obtained was characterized as follows:

Protein concentration: 2.31 mg/ml

Drug/mAb ratio: 5.6

Example 49e

In an analogous manner, Intermediate R34 was coupled to 5 mg of anti-HER2 antibody TPP-1015. The ADC batch obtained was characterized as follows:

Protein concentration: 2.12 mg/ml

Drug/mAb ratio: 7.1

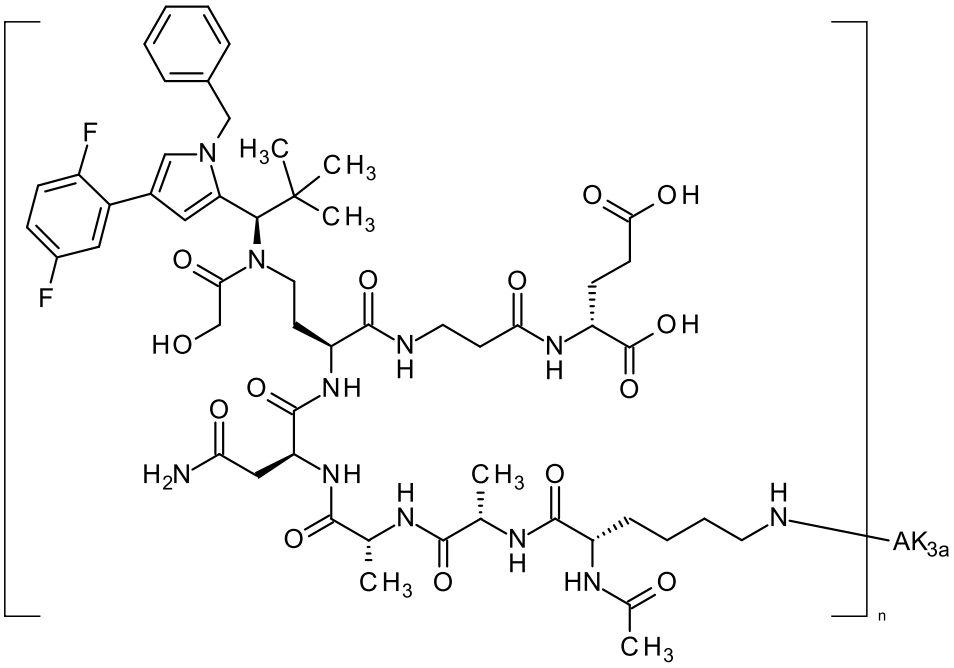
Example 49l1

In an analogous manner, Intermediate R34 was coupled to 5 mg of anti-TWEAKR antibody TPP-7007. The ADC batch obtained was characterized as follows:

Protein concentration: 2.25 mg/ml

Drug/mAb ratio: 5.8

Example 50dt-2

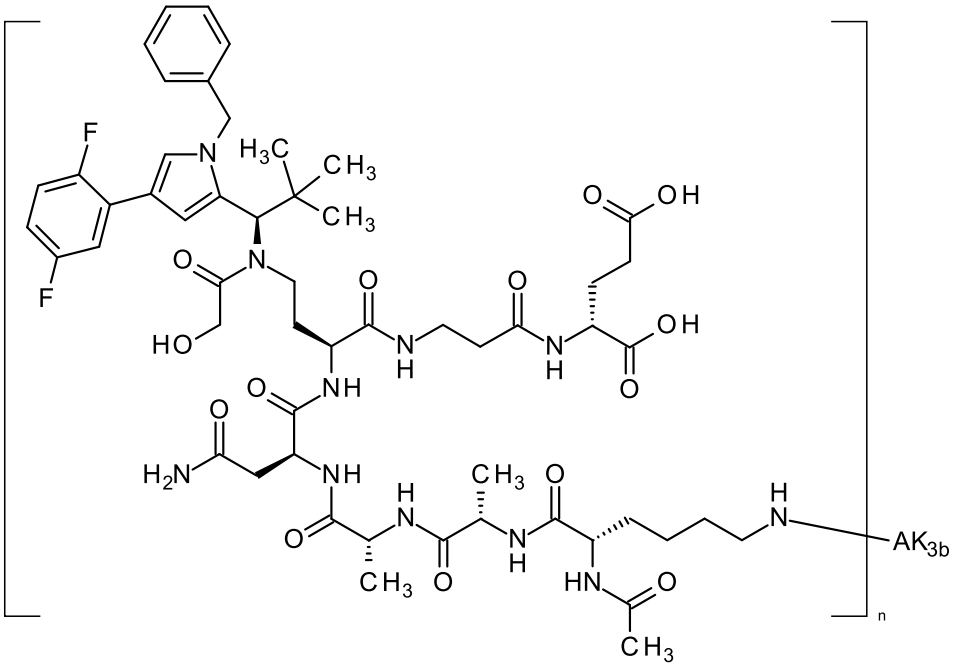


To a solution of 5 mg of the anti-TWEAKR antibody TPP-2658 (corresponding to TPP-2090-HC-N297A) in 540 µl of DPBS pH 7.2 (c ~ 10mg/ml) were added 20 µl of a 10 mmol solution of Intermediate R50 in DMSO. After incubation at 37°C for 5 min, 40 µl of a solution of recombinant bacterial transglutaminase solution in water (product number T001 from Zedira GmbH, Darmstadt, Germany) (25 U/ml) were added and incubation was continued at 37°C for a further 24 h. Then the reaction mixture was diluted with DPBS pH 7.2 to a volume of 2.5 ml and passed by gel filtration through DPBS-equilibrated PD 10 columns (Sephadex® G-25, GE Healthcare) and eluted with DPBS buffer at pH 7.4. Subsequently, the ADC solution was concentrated by means of Amicon Ultracel-30K centrifugation (Millipore), and it was rediluted again with DPBS. Finally, 0.005 µmol of the b-transglutaminase blocker Zedira C100 in 12.5 µl of DPBS was added to the solution. The ADC solution obtained was characterized as follows:

Protein concentration: 1.71 mg/ml

Drug/mAb ratio: 1.9

Example 50dt-4

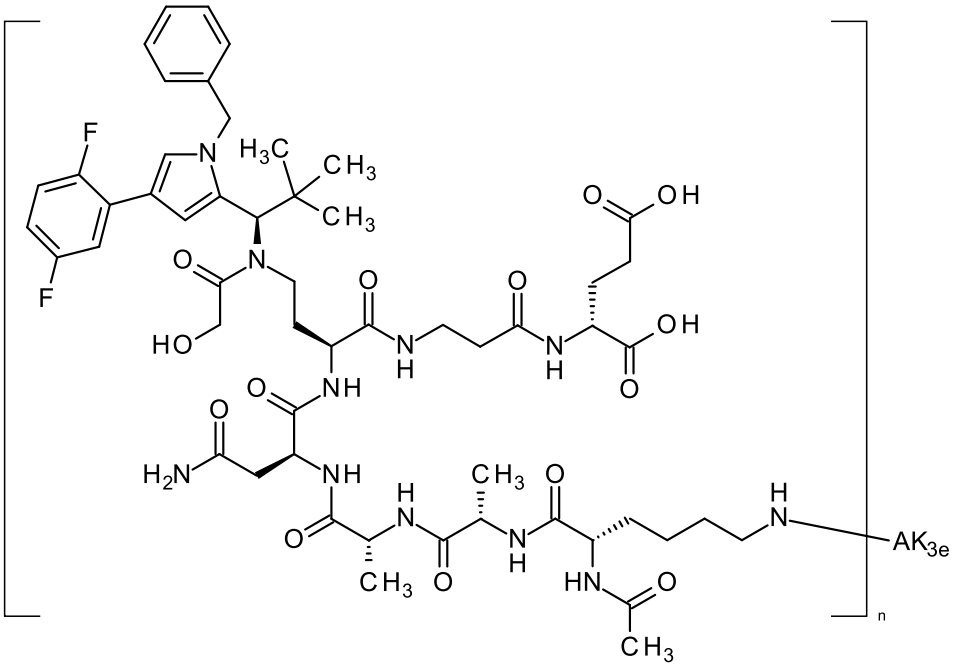


To a solution of 5 mg of the anti-TWEAKR antibody TPP-5442 (corresponding to TPP-2090-HC-N297Q) in DPBS pH 7.2 (c = 7.4 mg/ml) were added 80 µl of a 10 mmol solution of Intermediate R50 in DMSO. After incubation at 37°C for 5 min, 400 µl of a solution of recombinant bacterial transglutaminase solution in water (product number T001 from Zedira GmbH, Darmstadt, Germany) (25 U/ml) were added and incubation was continued at 37°C for 24 h. Then the reaction mixture was diluted with DPBS pH 7.2 to a total volume of 2.5 ml and passed by gel filtration through DPBS-equilibrated PD 10 columns (Sephadex® G-25, GE Healthcare) and eluted with DPBS buffer at pH 7.4. Subsequently, the ADC solution was concentrated by means of Amicon Ultracel-30K centrifugation (Millipore), and it was rediluted again with DPBS to a volume of about 2.5 ml. Finally, 0.1 µmol of the b-transglutaminase blocker Zedira C100 in 200 µl of DPBS was added to the solution. The ADC solution obtained was characterized as follows:

Protein concentration: 1.69 mg/ml

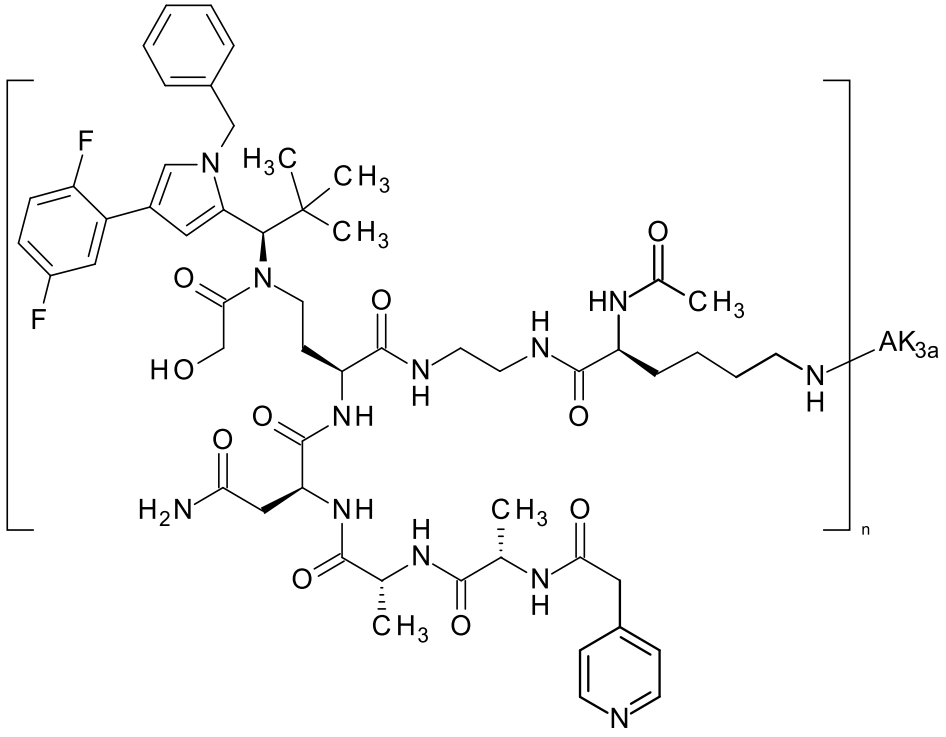
Drug/mAb ratio: 3.7

Example 50et-4



To a solution of 5 mg of the anti-HER2 antibody TPP-7511 (corresponding to anti-HER2 antibody -HC-N297Q) in DPBS pH 7.2 (c = 10 mg/ml) were added 80 µl of a 10 mmol solution of Intermediate R50 in DMSO. After incubation at 37°C for 5 min, 400 µl of a solution of recombinant bacterial transglutaminase solution in water (product number T001 from Zedira GmbH, Darmstadt, Germany) (25 U/ml) were added and incubation was continued at 37°C for 24 h. Then the reaction mixture was diluted with DPBS pH 7.2 to a total volume of 2.5 ml and passed by gel filtration through DPBS-equilibrated PD 10 columns (Sephadex® G-25, GE Healthcare) and eluted with DPBS buffer at pH 7.4. Subsequently, the ADC solution was concentrated by means of Amicon Ultracel-30K centrifugation (Millipore), and it was rediluted again with DPBS to a volume of about 2.5 ml. Finally, 0.1 µmol of the b-transglutaminase blocker Zedira C100 in 200 µl of DPBS was added to the solution. The ADC solution obtained was characterized as follows:
Protein concentration: 1.64 mg/ml
Drug/mAb ratio: 3.8

Example 51dt-2

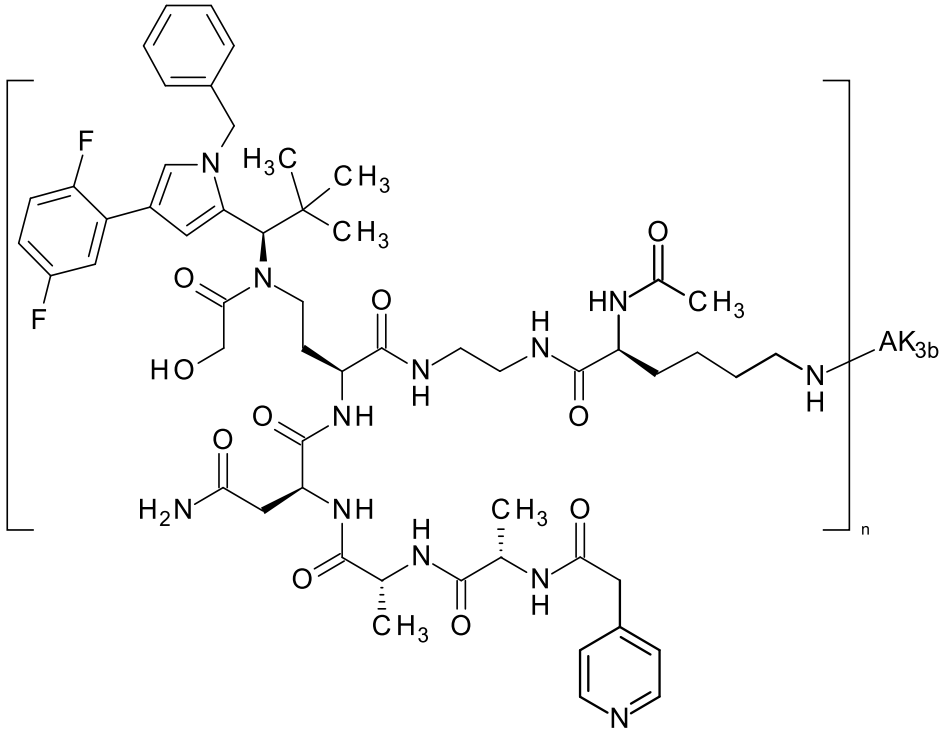


To a solution of 5 mg of the anti-TWEAKR antibody TPP-2658 (corresponding to TPP-2090-HC-N297A) in 530 µl of DPBS pH 7.2 (c ~ 10mg/ml) were added 20 µl of a 10 mmol solution of Intermediate R51 in DMSO. After incubation at 37°C for 5 min, 50 µl of a solution of recombinant bacterial transglutaminase solution in water (product number T001 from Zedira GmbH, Darmstadt, Germany) (25 U/ml) were added and incubation was continued at 37°C for a further 24 h. Then the reaction mixture was diluted with DPBS pH 7.2 to a volume of 2.5 ml and passed by gel filtration through DPBS-equilibrated PD 10 columns (Sephadex® G-25, GE Healthcare) and eluted with DPBS buffer at pH 7.4. Subsequently, the ADC solution was concentrated by means of Amicon Ultracel-30K centrifugation (Millipore), and it was rediluted again with DPBS. Finally, 0.005 µmol of the b-transglutaminase blocker Zedira C100 in 12.5 µl of DPBS was added to the solution. The ADC solution obtained was characterized as follows:

Protein concentration: 2.08 mg/ml

Drug/mAb ratio: 1.9

Example 51dt-4



To a solution of 5 mg of the anti-TWEAKR antibody TPP-5442 (corresponding to TPP-2090-HC-N297Q) in DPBS pH 7.2 (c = 10 mg/ml) were added 53 µl of a 10 mmol solution of Intermediate R51 in DMSO. After incubation at 37°C for 5 min, 400 µl of a solution of recombinant bacterial transglutaminase solution in water (product number T001 from Zedira GmbH, Darmstadt, Germany) (25 U/ml) were added and incubation was continued at 37°C for 24 h. Then the reaction mixture was diluted with DPBS pH 7.2 to a total volume of 2.5 ml and passed by gel filtration through DPBS-equilibrated PD 10 columns (Sephadex® G-25, GE Healthcare) and eluted with DPBS buffer at pH 7.4. Subsequently, the ADC solution was concentrated by means of Amicon Ultracel-30K centrifugation (Millipore), and it was rediluted again with DPBS to a volume of about 2.5 ml. Finally, 0.05 µmol of the b-transglutaminase blocker Zedira C100 in 200 µl of DPBS was added to the solution. The ADC solution obtained was characterized as follows:

Protein concentration: 1.56 mg/ml

Drug/mAb ratio: 3.3

Reference examples: Small molecules, APDCs and ADCs

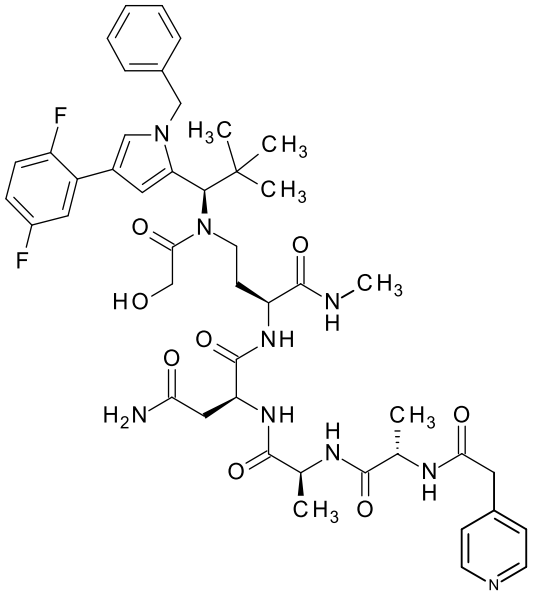
First of all, to examine the legumain-mediated cleavage and stability under various conditions, the legumain-cleavable prodrugs R3r-LLL and R3r-LDL were prepared.

In addition, as representative reference compounds, the epimers of the legumain-cleavable APDCs and ADCs according to the invention were prepared, wherein all the amino acids in the legumain-cleavable linker are in the natural L configuration (Reference Examples R3a,e,k; R4a,e,k; R5a,e,k and R9a,e,k)

Finally, to determine the concentrations of the active metabolites in tumour and other tissues (-> Chapter C5b), for comparison with Example 3k (with legumain-cleavable group), the reference ADCs R10k and R10h1 (without a legumain-cleavable group) was prepared. Since both ADCs form the same active metabolite, it is thus possible to examine the effect of the legumain-cleavable group according to the invention on the organ distribution thereof.

Reference Example R3r-LLL

N-(Pyridin-4-ylacetyl)-L-alanyl-L-alanyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-(methylamino)-1-oxobutan-2-yl]-L-aspartamide

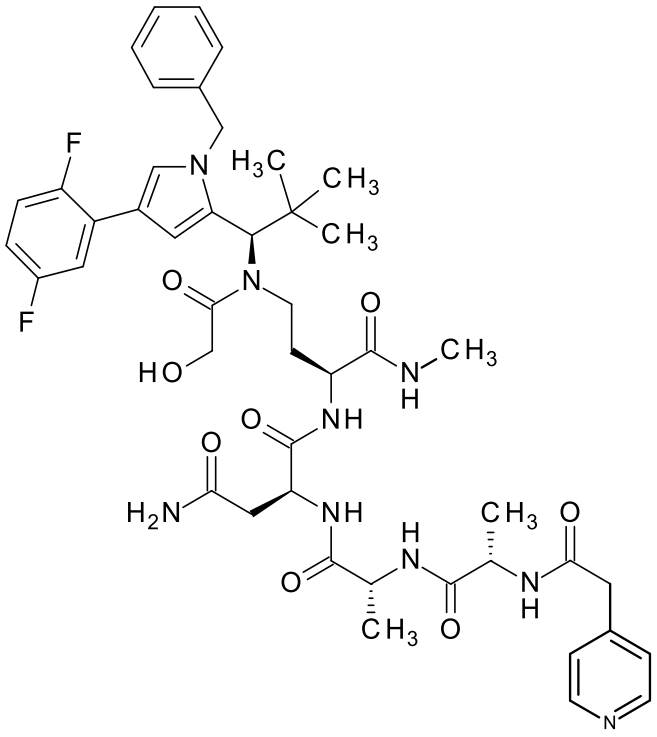


First of all, trifluoroacetic acid / (2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-N-methylbutanamide (1:1) was prepared as described in WO 2015096982 A1. Subsequently, this intermediate was used to prepare the title compound by coupling to Intermediate L103 in DMF in the presence of HATU and of N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 0.86 min; MS (ESIpos): m/z = 902 $[M+H]^+$.

Reference Example R3r-LDL

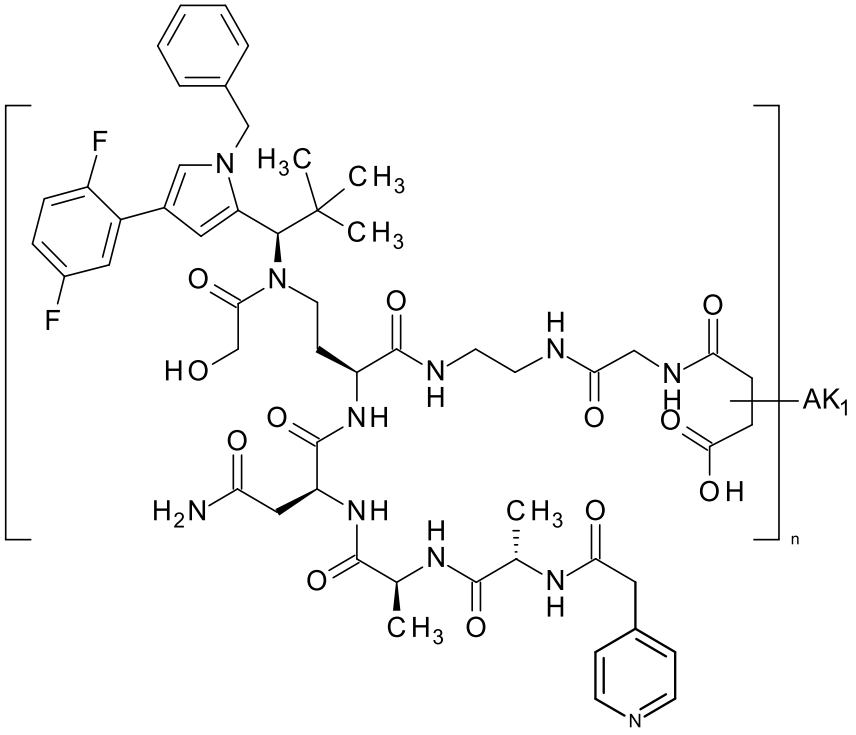
N-(Pyridin-4-ylacetyl)-L-alanyl-D-alanyl-N^1-[(2S)-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-1-(methylamino)-1-oxobutan-2-yl]-L-aspartamide



First of all, trifluoroacetic acid / (2S)-2-amino-4-[{(1R)-1-[1-benzyl-4-(2,5-difluorophenyl)-1H-pyrrol-2-yl]-2,2-dimethylpropyl}(glycoloyl)amino]-N-methylbutanamide (1:1) was prepared as described in WO 2015096982 A1. Subsequently, this intermediate was used to prepare the title compound by coupling to Intermediate L110 in DMF in the presence of HATU and of N,N-diisopropylethylamine.

LC-MS (Method 1): R_t = 0.88 min; MS (ESIpos): m/z = 902 $[M+H]^+$.

Reference Example R3a



The preparation was effected analogously to Example 3a.
Protein concentration: 1.99 mg/ml
Drug/mAb ratio: 2.6

Likewise, in an analogous manner, Reference Example R3e was prepared with the anti-HER2 antibody TPP-1015, and Reference Example R3k with the anti-TWEAKR antibody TPP-2658:

Reference Example R3e

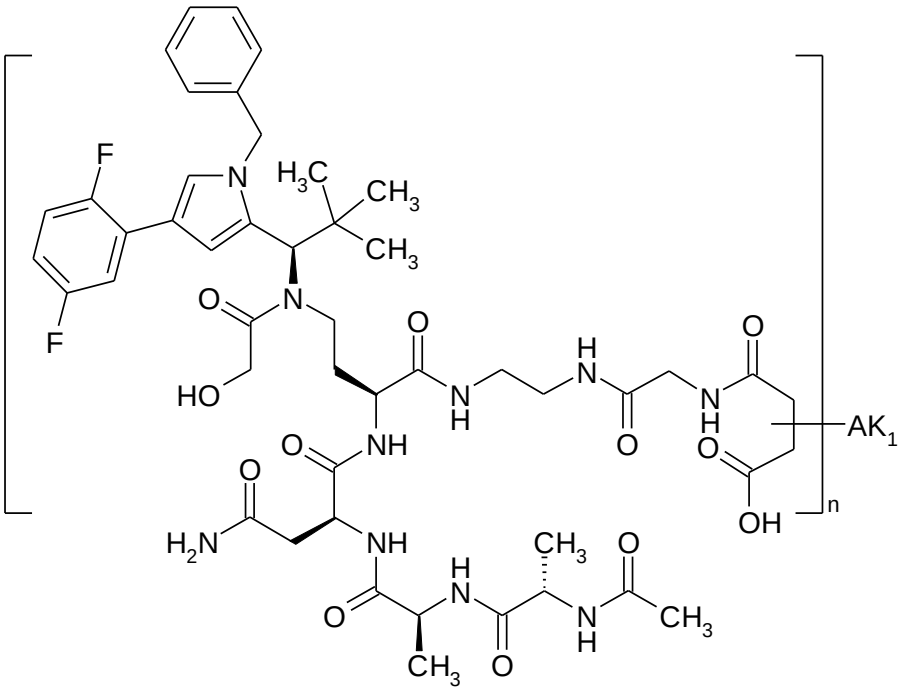
Protein concentration: 1.70 mg/ml
Drug/mAb ratio: 3.3

Reference Example R3k

Protein concentration: 1.71 mg/ml

Drug/mAb ratio: 3.1

Reference Example R4a



The preparation was effected analogously to Example 4a.
Protein concentration: 1.87 mg/ml
Drug/mAb ratio: 3.2

Likewise, in an analogous manner, Reference Example R4e was prepared with the anti-HER2 antibody TPP-1015, and Reference Example R4k with the anti-TWEAKR antibody TPP-2658:

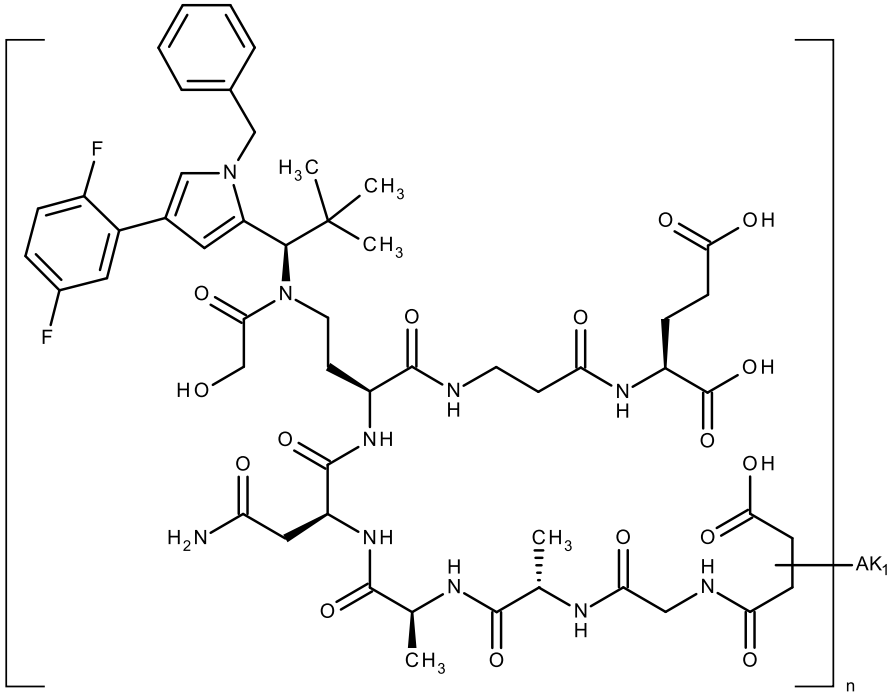
Reference Example R4e

Protein concentration: 1.70 mg/ml
Drug/mAb ratio: 3.3

Reference Example R4k

Protein concentration: 1.79 mg/ml
Drug/mAb ratio: 3.3

Reference Example R5a



The preparation was effected analogously to Example 5a.
Protein concentration: 2.02 mg/ml
Drug/mAb ratio: 3.5

Likewise, in an analogous manner, Reference Example R5e was prepared with the anti-HER2 antibody TPP-1015, and Reference Example R5k with the anti-TWEAKR antibody TPP-2658:

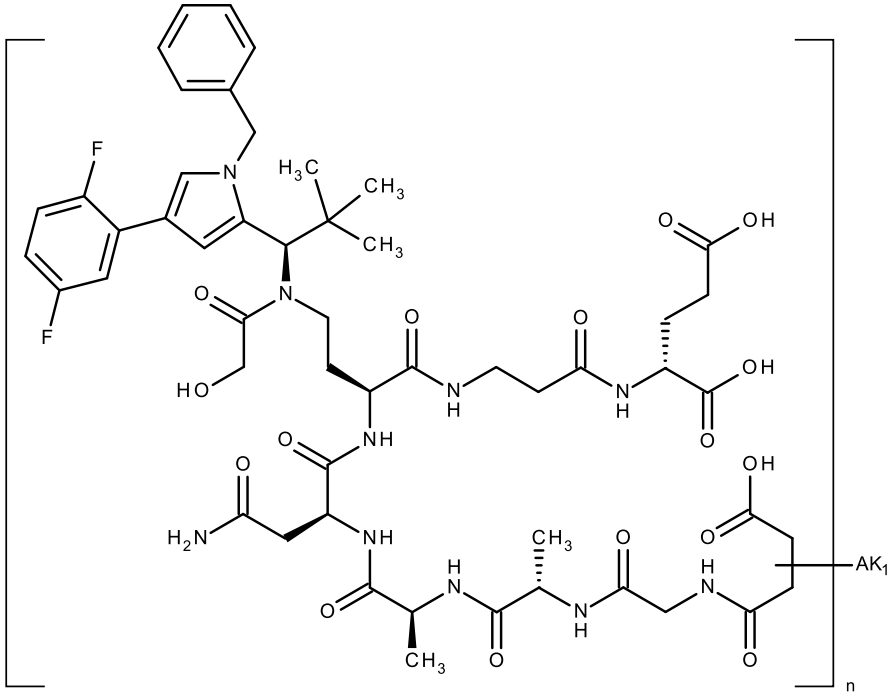
Reference Example R5e

Protein concentration: 1.72 mg/ml
Drug/mAb ratio: 4.2

Reference Example R5k

Protein concentration: 1.79 mg/ml
Drug/mAb ratio: 3.1

Reference Example R9a



The preparation was effected analogously to Example 9a.

Protein concentration: 1.57 mg/ml

Drug/mAb ratio: 2.4

Likewise, in an analogous manner, Reference Example R9e was prepared with the anti-HER2 antibody TPP-1015, and Reference Example R9k with the anti-TWEAKR antibody TPP-2658:

Reference Example R9e

Protein concentration: 1.79 mg/ml

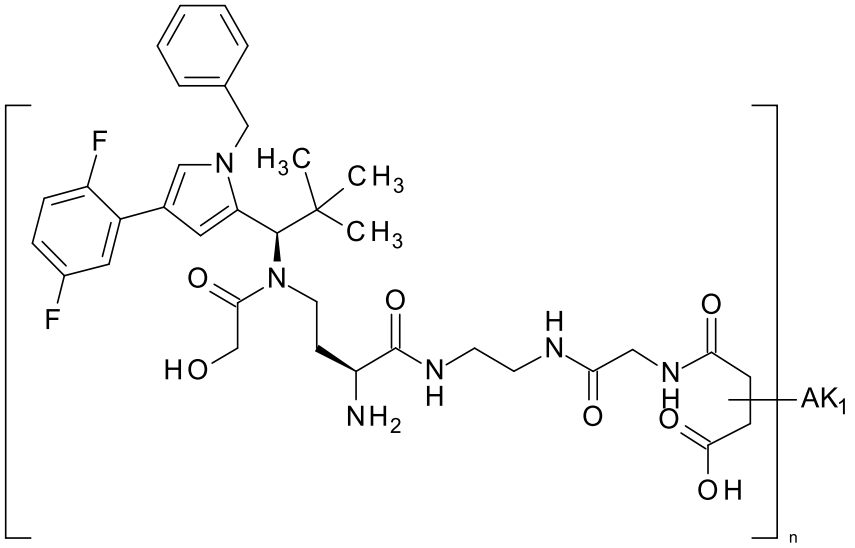
Drug/mAb ratio: 3.1

Reference Example R9k

Protein concentration: 0.68 mg/ml

Drug/mAb ratio: 2.8

Reference Example R10k



Under argon, a solution of 0.86 mg of TCEP in 2 ml of PBS buffer was added to 150 mg of anti-TWEAKR antibody TPP-2658 in 10.5 ml of PBS (c = 14.28 mg/ml). The mixture was stirred at RT for 30 min, and then 6.63 mg (0.008 mmol) of Intermediate F104 dissolved in 1250 µl of DMSO were added. After stirring at RT for a further 90 min, the reaction was diluted with 1250 µl of PBS buffer which had been adjusted to pH 8 beforehand.

This solution was then applied to PD 10 columns (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was diluted with PBS buffer pH 8 to a total volume of 22.5 ml. This solution was stirred under argon at RT overnight and then re-buffered to pH 7.2 using PD-10 columns. The eluate was then concentrated by ultracentrifugation, rediluted with PBS buffer (pH 7.2) and reconcentrated again. The ADC batch obtained was characterized as follows

Protein concentration: 14.06 mg/ml
Drug/mAb ratio: 3.4

Reference Example R10h1

In an analogous manner, Intermediate F104 was coupled to 100 mg of anti-B7H3 antibody TPP-8382. The ADC batch obtained was characterized as follows
Protein concentration: 14.12 mg/ml
Drug/mAb ratio: 3.2

C: Assessment of biological efficacy

The biological activity of the compounds according to the invention can be shown in the assays described below:

C-1a Determination of the cytotoxic effect of the ADCs

The analysis of the cytotoxic effects of the ADCs was carried out with various cell lines:

NCI-H292: human mucoepidermoid lung carcinoma cells, ATCC-CRL-1848, standard medium: RPMI 1640 (Biochrom; #FG1215, stab. glutamine) + 10% FCS (Sigma; #F2442), TWEAKR-positive; EGFR-positive.

BxPC3: human pancreas carcinoma cells, ATCC-CRL-1687, standard medium: RPMI 1640 (Biochrom; #FG1215, stab. glutamine) + 10% FCS (Sigma; #F2442), TWEAKR-positive.

LoVo human colorectal cancer cells, ATCC No. CCL-229, cultivation for MTT assay: standard medium: Kaighn's + L-glutamine (Invitrogen 21127) + 10% heat inactivated FCS (from Gibco, No. 10500-064). Cultivation for CTG assay: RPMI 1640 (Biochrom; #FG1215, stab. glutamine) + 10% FCS (Sigma #F2442). TWEAKR-positive.

KPL4: human breast cancer cell line, Bayer Pharma AG (identity checked and confirmed on 19.7.2012 at DSMZ), standard medium: RPMI 1640 (from Gibco; #21875- 059, stab. L-Glutamin) + 10% heat inactivated FCS (from Gibco, No. 10500-064); HER2-positive.

SK-HEP-1: human liver cell cancer line, ATCC No. HTB-52, standard medium: MEM with Earle's salts + Glutamax I (Invitrogen 41090) + 10% heat inactivated FCS (from Gibco, No. 10500-064); EGFR-positive, TWEAKR-positive

KU-19-19: human bladder carcinoma cells, DMSZ, standard medium: RPMI 1640 (Biochrom; #FG1215, stab. glutamine) + 10% FCS (Sigma; #F2442), TWEAKR-positive.

SCC4: human tongue base carcinoma, standard medium: DMEM / Ham's F12; (Biochrom; # FG 4815, with stable glutamine), ATCC-CRL-1624+ 10% FCS (Sigma #F2442), TWEAKR-positive

The cells were cultivated by the standard method as stated by the American Tissue Culture Collection (ATCC) or the Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) for the cell lines in question.

CTG assay

The cells were cultivated by the standard method, with the growth media specified under C-1a. The test was carried out by detaching the cells with a solution of trypsin (0.05%) and EDTA (0.02%) in PBS (Biochrom AG #L2143), pelleting, resuspending in culture medium, counting and sowing into a 96-well culture plate with white bottom (Costar #3610) (at 75 µl/well, the following cell numbers per well are: NCI-H292: 2500 cells/well, BxPC3 2500 cells/well, LoVo 3000 cells/well) and incubating in an incubator at 37°C and 5% carbon dioxide. After 24 h, the antibody drug conjugates were added in 25 µl of culture medium (concentrated four-fold) to the cells to give final antibody drug conjugate concentrations of 3 x 10^{-7} M to 3 x 10^{-11} M on the cells (triplicates). The cells were then incubated in an incubator at 37°C and 5% carbon dioxide. On a parallel plate, the cell activity at the start of the drug treatment (day 0) was determined using the Cell Titer Glow (CTG) luminescent cell viability assay (Promega #G7573 and #G7571). To this end, per cell batch 100 µl of the substrate were added, the plates were then covered with aluminium foil, shaken on the plate shaker at 180 rpm for 2 minutes, allowed to stand on the laboratory bench for 8 minutes and then measured using a luminometer (Victor X2, Perkin Elmer). The substrate detects the ATP content in the living cells generating a luminescence signal whose intensity is directly proportional to the viability of the cells. After incubation with the antibody drug conjugates for 72 h, the viability of these cells was then also determined using the Cell Titer Glow luminescent cell viability assay as described above. From the data measured, the IC_{50} of the growth inhibition was calculated in comparison to day 0 using the DRC (Dose Response Curve) analysis spreadsheets and a 4-parameter fit. The DRC analysis spreadsheet is a biobook spreadsheet developed by Bayer Pharma AG and Bayer Business Services on the IDBS E-WorkBook Suite platform (IDBS: ID Business Solutions Ltd., Guildford, UK).

Table 1a below lists the IC_{50} values for representative working examples from this assay:

Table 1a

Example	BxPC3 IC_{50} [M] CTG	NCI-H292 IC_{50} [M] CTG	LoVo IC_{50} [M] CTG
1k	1.76E-09	1.13E-09	4.36E-10
2k	1.68E-09	1.58E-09	8.70E-10
3k	1.42E-09	1.34E-09	7.90E-10
3l1	3.00E-10	1.59E-10	3.10E-11
3l2	6.68E-10	4.27E-10	1.84E-10
3l3	1.22E-09	6.45E-10	5.34E-11
3l4	8.91E-10	4.15E-10	3.97E-11
4k	2.66E-09	1.39E-09	7.89E-10
5k	7.24E-10	1.05E-09	3.80E-10
6k	1.34E-09	2.04E-09	8.95E-10
7k	2.14E-09	3.09E-09	>6.00E-07
8k	1.48E-09	2.11E-09	4.62E-08
9k	6.25E-10	7.39E-10	3.24E-10
11k	1.14E-08	4.00E-08	>6.00E-07
12k	5.96E-09	>6.00E-07	>6.00E-07
13k	4.02E-09	6.77E-09	2.16E-09
14k	4.19E-08	1.86E-07	>6.00E-07
15k	1.35E-09	2.22E-09	4.49E-10
15l1	1.71E-09	8.61E-10	7.76E-10
16k	1.16E-09	1.45E-09	3.35E-10
16l2	1.95E-10	1.70E-10	5.58E-11
17k	5.53E-10	5.36E-10	9.49E-11
18k	4.34E-08	1.53E-08	1.31E-09
19k	1.05E-08	9.02E-09	1.39E-09
20k	8.39E-10	2.94E-09	7.51E-10
21k	1.81E-09	2.74E-09	4.52E-09
22k	1.74E-09	4.96E-09	5.74E-10
23k	1.90E-09	3.01E-09	6.11E-10
24k	8.38E-08	1.09E-09	4.81E-10
24l1	1.70E-10	1.80E-10	1.50E-11

Example	BxPC3 IC_{50} [M] CTG	NCI-H292 IC_{50} [M] CTG	LoVo IC_{50} [M] CTG
24l3	7.26E-11	5.83E-11	3.00E-11
24l4	1.44E-10	1.02E-10	3.00E-11
25k	7.23E-10	1.59E-09	4.41E-10
26k	2.02E-09	2.17E-09	8.13E-10
27k	1.01E-09	8.72E-10	1.95E-10
28l1	3.64E-10	1.42E-10	1.50E-11
29k	9.46E-10	1.16E-09	8.86E-11
30k	8.73E-10	1.09E-09	1.50E-10
31l1	3.87E-10	2.05E-10	1.50E-11
32k	6.19E-10	7.65E-10	1.13E-10
33l1	2.96E-10	1.36E-10	1.50E-11
34l1	5.22E-10	3.98E-10	6.99E-11
35k	2.59E-10	3.15E-10	1.93E-10
35l1	2.00E-10	2.08E-10	9.23E-11
36l1	2.80E-10	1.68E-10	3.44E-11
37k	3.77E-10	6.11E-10	2.98E-10
37l1	2.12E-10	2.31E-10	9.21E-11
38l1	1.75E-10	1.29E-10	1.50E-11
39l1	1.36E-10	9.60E-11	1.15E-11
40l1	1.42E-09	5.00E-09	6.00E-07
41l1	1.77E-10	1.33E-10	1.50E-11
42l1	1.98E-10	2.00E-10	1.50E-11
43l1	3.66E-10	1.60E-10	1.50E-11
44l1	3.15E-10	9.57E-11	3.15E-11
45l1	3.00E-10	1.81E-10	6.96E-08
46l1	5.54E-10	2.72E-10	6.00E-07
47l1	3.44E-10	1.67E-10	5.79E-11
48l1	3.83E-10	3.54E-10	1.50E-11
49l1	2.06E-10	1.50E-10	1.50E-11

Table 1b below lists comparative data for representative examples of the ADCs and APDCs according to the invention with the corresponding epimeric ADCs and APDCs in which all the amino acids of the legumain-cleavable group are in the L configuration (reference example series R). No significant differences are apparent:

Table 1b)

Example	BxPC3 IC_{50} [M] CTG	NCI-H292 IC_{50} [M] CTG	LoVo IC_{50} [M] CTG
3k	1.42E-09	1.34E-09	7.90E-10
R3k (L-L-L)	1.36E-09	8.51E-10	2.28E-10
4k	2.66E-09	1.39E-09	7.89E-10
R4k (L-L-L)	2.94E-09	5.84E-10	2.04E-10
5k	7.24E-10	1.05E-09	3.80E-10
R5k (L-L-L)	5.34E-10	8.61E-10	3.460E-10
9k	6.25E-10	7.39E-10	3.24E-10
R9k (L-L-L)	2.56E-10	3.91E-10	1.09E-10

The activity data reported relate to the working examples described in the present experimental section, with the drug/mAB ratios indicated. The values may possibly deviate for different drug/mAB ratios. The IC50 values are means of several independent experiments or individual values. The action of the antibody drug conjugates was selective for the respective isotype control comprising the respective linker and toxophor.

MTT assay

The cells were cultivated by the standard method, with the growth media specified under C-1a. The test was carried out by detaching the cells with a solution of Accutase in PBS

(from Biochrom AG #L2143), pelletizing, resuspending in culture medium, counting and sowing into a 96-well culture plate with white bottom (from Costar #3610) (NCI H292: 2500 cells/well; SK-HEP-1: 1000 cells/well; KPL4: 1200 cells/well; in total volume 100 µl). The cells were then incubated in an incubator at 37°C and 5% carbon dioxide. After 48 h, the medium was replaced. The antibody drug conjugates in 10 µl of culture medium in concentrations from 10^{-5}M to 10^{-13}M were then pipetted to the cells (in triplicate), and the assay was then incubated in an incubator at 37°C and 5% carbon dioxide. After 96 h, the cell proliferation was detected using the MTT assay (ATCC, Manassas, Virginia, USA, catalogue No. 30-1010K). To this end, the MTT reagent was incubated with the cells for 4 h, followed by lysis of the cells overnight by addition of the detergent. The dye formed was detected at 570 nm (Infinite M1000 pro, Tecan). The measured data were used to calculate the IC_{50} of the growth inhibition using the DRC (dose response curve). The proliferation of cells which were not treated with test substance but were otherwise identically treated was defined as the 100% figure.

Tables 1c-f below list the IC_{50} values for representative working examples from this assay:

Table 1c

Example	NCI-H292 IC_{50} [M] MTT Assay	SK-HEP-1 IC_{50} [M] MTT Assay
1a	6.87E-11	4.96E-08
2a	1.62E-10	8.31E-08
3a	5.06E-10	1.13E-07
4a	1.82E-10	9.52E-08
5a	2.55E-11	3.30E-08
6a	3.45E-12	9.93E-08
7a	5.33E-10	7.52E-08
8a	3.01E-11	6.83E-10
9a	2.53E-11	2.47E-07
11a	4.05E-10	4.35E-07
12a	1.86E-10	5.00E.07
13a	6.04E-10	8.27E-08
14a	1.30E-10	1.13E-07

Example	NCI-H292 IC_{50} [M] MTT Assay	SK-HEP-1 IC_{50} [M] MTT Assay
15a	1.99E-10	1.03E-08
16a	1.67E-11	1.33E-11
17a	2.65E-12	2.94E-11
18a	7.18E-11	6.62E-08
19a	7.63E-11	7.14E-08
20a	1.00E-12	1.57E-11
21a	1.20E-11	2.59E-07
22a	1.89E-10	7.32E-08
23a	1.00E-12	1.00E-11
24a	1.00E-12	1.04E-10
25a	2.92E-12	2.16E-08
26a	3.02E-09	6.01E-10
27a	2.55E-12	6.02E-09
28a	5.00E-07	5.00E-07
29a	3.59E-11	1.04E-07
30a	5.72E-11	6.03E-08
31a	2.65E-10	2.35E-08
32a	3.00E-11	6.39E-09
33a	3.44E-12	1.49E-09
34a	8.96E-10	1.71E-09
35a	1.23E-12	2.89E-10
36a	9.98E-12	1.02E-07
37a	1.90E-12	3.16E-10
38a	7.62E-12	4.28E-09
39a	8.68E-12	1.35E-08
40a	5.93E-10	5.00E-07
41a	2.46E-12	5.00E-07
42a	3.28E-10	5.00E-07
43a	4.58E-12	9.18E-08
45a	7.63E-12	5.40E-09
46a	5.37E-11	9.15E-08
47a	8.41E-12	4.50E-08

Example	NCI-H292 IC_{50} [M] MTT Assay	SK-HEP-1 IC_{50} [M] MTT Assay
48a	2.15E-12	2.07E-10
49a	3.15E-13	2.51E-12
50dt-2	4.29E-10	3.10E-10
50dt-4	1.49E-10	2.77E-10
51dt-2	1.67E-08	8.79E-08
51dt-4	2.84E-09	1.21E-07

Table 1d below lists comparative data for representative examples comprising the ADCs according to the invention and corresponding APDCs in which all the amino acids of the legumain-cleavable group are in the L configuration (reference example series R). No distinctly altered IC_{50} values were measured on the NCI-H292 cell line.

Table 1d:

Example	NCI-H292 IC_{50} [M] MTT Assay	SK-HEP-1 IC_{50} [M] MTT Assay
3a	5.06E-10	1.13E-07
R3a (L-L-L)	2.80E-12	3.86E-10
4a	1.82E-10	9.52E-08
R4a (L-L-L)	4.65E-11	1.25E-10
5a	2.55E-11	3.30E-08
R5a (L-L-L)	3.17E-12	4.83E-09
9a	2.53E-11	2.47E-07
R9a (L-L-L)	1.69E-11	2.56E-09

Table 1e

Example	KPL4 IC_{50} [M] MTT Assay
1e	8.00E-10
2e	2.58E-10
3e	1.47E-10
4e	2.10E-10
5e	1.43E-10
6e	1.16E-10
7e	1.17E-10
8e	2.42E-11
9e	8.45E-11
12e	4.53E-08
13e	1.16E-10
14e	2.01E-09
15e	4.37E-11
16e	1.91E-11
17e	1.16E-11
18e	1.83E-10
19e	1.25E-10
20e	2.77E-12
21e	1.56E-11
22e	7.11E-11
23e	2.30E-11
24e	9.73E-11
25e	1.30E-10
28e	1.48E-10
29e	2.82E-10
30e	1.77E-10
31e	3.77E-10
32e	1.75E-10
33e	6.10E-12
34e	6.44E-10
35e	4.06E-12
36e	4.31E-11

Example	KPL4 IC_{50} [M] MTT Assay
37e	7.31E-12
38e	6.22E-12
39e	3.75E-11
40e	5.95E-08
41e	2.69E-10
42e	3.89E-10
43e	1.19E-10
44e	6.79E-11
45e	7.36E-10
46e	5.37E-09
47e	1.17E-10
48e	7.50E-11
49e	3.00E-11
50et-4	3.77E-10

Table 1f below lists comparative data for representative examples comprising the ADCs according to the invention and corresponding APDCs in which all the amino acids of the legumain-cleavable group are in the L configuration (reference example series R). No significantly altered IC_{50} values were measured.

Table 1f:

Example	KPL4 IC_{50} [M] MTT Assay
3e	1.47E-10
R3e (L-L-L)	9.37E-11
4e	2.10E-10
R4e (L-L-L)	4.15E-09
5e	1.43E-10

Example	KPL4 IC_{50} [M] MTT Assay
R5e (L-L-L)	4.81E-10
9e	8.45E-11
R9e (L-L-L)	8.25E-11

The activity data reported relate to the working examples described in the present experimental section, with the drug/mAB ratios indicated. The values may possibly deviate for different drug/mAB ratios. The IC50 values are means of several independent experiments or individual values. The action of the antibody drug conjugates was selective for the respective isotype control comprising the respective linker and toxophor.

C-1b Determination of the inhibition of the kinesin spindle protein KSP/ Eg5 by selected examples

The motor domain of the human kinesin spindle protein KSP/Eg5 (tebu-bio/ Cytoskeleton Inc, No. 027EG01-XL) was incubated in a concentration of 10 nM with microtubuli (bovine or porcine, tebu-bio/ Cytoskeleton Inc) stabilized with 50 µg/ml taxol (Sigma No. T7191-5MG) for 5 min at RT in 15 mM PIPES, pH 6.8 (5 mM $MgCl_2$ and 10 mM DTT, Sigma). The freshly prepared mixture was aliquoted into a 384 MTP (from Corning). The inhibitors to be examined at concentrations of 1.0 x 10-6 M to 1.0 x 10-13 M and ATP (final concentration 500 µM, Sigma) were then added. Incubation was at RT for 2 h. ATPase activity was detected by detecting the inorganic phosphate formed using malachite green (Biomol). After addition of the reagent, the assay was incubated at RT for 50 min prior to detection of the absorption at a wavelength of 620 nm. The positive controls used were monastrol (Sigma, M8515-1mg) and ispinesib (AdooQ Bioscience A10486). The individual data of the dose-activity curve are eight-fold determinations. The IC_{50} values are means of two independent experiments. The 100% control was the sample which had not been treated with inhibitors.

Table 2 below lists the IC_{50} values of representative working examples from the assay described and summarizes the corresponding cytotoxicity data (MTT assay):

Table 2

Examples	KSP assay IC_{50} [M]	NCI-H292 IC_{50} [M] MTT Assay	SK-HEP-1 IC_{50} [M] MTT Assay	KPL4 IC_{50} [M] MTT Assay
M01	2.01E-09	5.00E-07		5.00E-07
M02	2.45E-09	1.62E-07	1.40E-07	1.63E-07
M03	1.52E-09	2.34E-08	1.18E-07	9.00E-08
M04	2.71E-10	4.43E-08	3.45E-08	1.76E-07
M05	4.57E-10	7.94E-08	2.19E-07	2.22E-07
M06	1.78E-09	4.63E-08	1.77E-07	1.93E-07
M07	6.21E-10	2.12E-08		9.23E-08
M08	1.64E-08			4.87E-07
M09	1.09E-09	2.70E-10	7.62E-09	5.14E-10
M10	4.70E-10	3.03E-07	1.37E-07	2.26E-07
M11	1.11E-09	4.32E-11	1.89E-10	
M12	4.46E-10	3.3E-08	2.11E-08	
M13	1.50E-09	1.52E-07	6.81E-08	1.69E-07
M14	2.16E-09	1.74E-07	9.43E-08	1.77E-07
M15	9.64E-10	1.33E-07	4.98E-08	1.69E-07
M16	1.48E-09	1.43E-07	4.40E-08	1.95E-07
M17	4.17E-09	7.35E-09		
M18	5.17E-09	3.55E-08		
M19	2.58E-09	1.21E-07		
M20	1.50E-09	1.49E-07	1.81E-07	2.13E-07
M21	2.31E-09			
M22	8.27E-10	2.89E-08	2.03E-08	1.82E-07
M23	1.26E-09	5.00E-07	5.00E-07	5.00E-07
M24	2.57E-09	1.67E-07	5.73E-08	5.00E-07
M25	2.91E-09	5.00E-07	5.00E-07	5.00E-07
M26	9.441E-10	6.38E-08	1.90E-08	
M27	2.03E-09	2.76E-07	8.90E-08	
M28	1.41E-09	1.55E-07	1.87E-07	2.10E-07
M29	2.03E-09	1.87E-07	2.07E-07	
R3r-LDL	2.64E-07	1.90E-07	2.83E-07	

Examples	KSP assay IC_{50} [M]	NCI-H292 IC_{50} [M] MTT Assay	SK-HEP-1 IC_{50} [M] MTT Assay	KPL4 IC_{50} [M] MTT Assay
R3r- LLL	1.62E-07	2.04E-07	2.32E-07	

The activity data reported relate to the working examples described in the present experimental section.

C-1c Enzymatic assays and stability studies

a: Cathepsin B assay

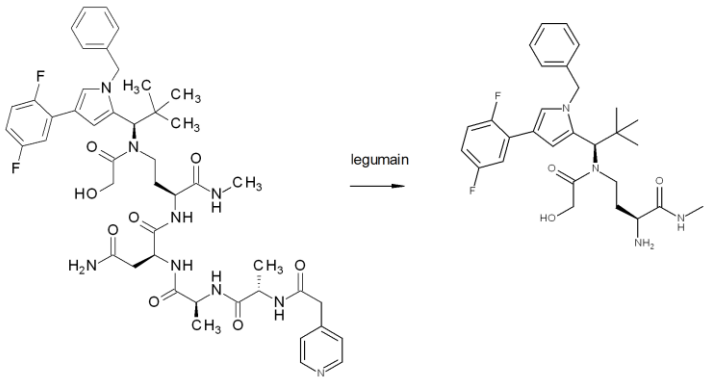
For every cathepsin B-cleavable prodrug to be examined, a mixture was made up in a micro reaction vessel (0.5 ml, from Eppendorf). The enzyme used here was obtained from human liver tissue. 2 µg of cathepsin B (Sigma C8571 25 µg) were initially charged and made up to a total volume of 200 µl with 50mM Na phosphate buffer, pH6.0, 2 mM DTT. Then 50 µl of the substrate solution to be examined were pipetted in. The mixture was incubated in a thermoblock (from Thermo Fisher Scientific) at 40°C under constant agitation at 300 rpm. The enzymatic reaction was controlled kinetically. For this purpose, a 10 µl sample was taken at different times. The sample taken was admixed immediately with 20 µl of ice-cold methanol in order to stop the enzymatic reaction and then frozen at -20°C. The times selected for sampling were after 10 min, 2 h, 4 h and 24 h. The samples were then analysed by RP-HPLC analysis (reverse phase HPLC, Agilent Technologies 1200 Series). The determination of the toxophor released enabled the determination of the half-life $t_{1/2}$ of the enzymatic reaction.

b: Legumain Assay

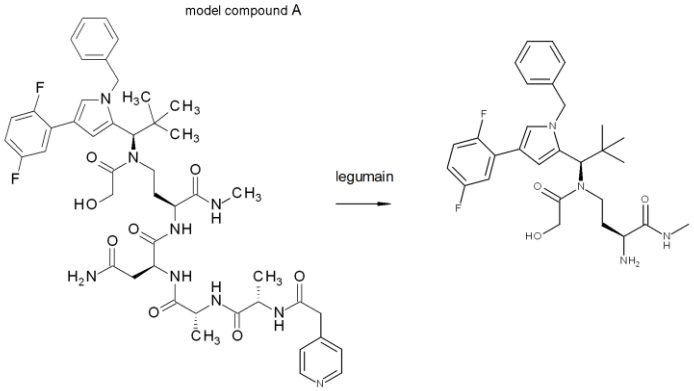
The legumain assay was conducted with recombinant human enzyme. The rh legumain enzyme solution (catalogue # 2199-CY, R&D Systems) was diluted to the desired concentration in 50mM Na acetate buffer/ 100mM NaCl, pH4.0 and preincubated at 37°C for 2 h. rh legumain was then adjusted to a final concentration of 1 ng/µl in 50 mM MES buffer, 250mM NaCl, pH 5.0. For every legumain-cleavable prodrug to be examined, a mixture was made up in a micro reaction vessel (0.5 ml, from Eppendorf). For this purpose, the substrate solution was adjusted to the desired concentration (double concentration) with 50 mM MES buffer, 250 mM NaCl, pH 5.0. For the kinetic

measurement of the enzymatic reaction, 250 µl of the legumain solution were first initially charged and the enzyme reaction was started by adding 250 µl of the substrate solution (final concentration: single concentration). At different times, 50 µl samples were taken. This sample was admixed immediately with 100 µl of ice-cold methanol in order to stop the enzymatic reaction and then frozen at -20°C. The times selected for sampling were after 0.5 h, 1 h, 3 h and 24 h. The samples were then analysed by means of RP-HPLC analysis and by LC-MS analysis. The determination of the toxophor released enabled the determination of the half-life $t_{1/2}$ of the enzymatic reaction (Figure 1).

In order to show the legumain-mediated cleavage with representative examples, substrates prepared in the legumain assay were the stereoisomeric model compounds A (= Reference Example R3r-LLL) and B (= Reference Example R3r-LDL) with respective S and R configuration on the central alanine unit. Compound A was cleaved under the conditions described above to the target compound with a half-life of 1.1 h. Compound B was cleaved to the target compound to an extent of about 20% within 24 h.



model compound A



model compound B

Figure 1: Legumain-mediated cleavage of the model compounds A (Reference Example R3r-LLL) and B (Reference Example R3r-LDL)

c: Assay for determination of stability in rat plasma

To examine the stability of compounds A and B, 1 ml of rat plasma in a 1.5 ml Eppendorf tube in each case was equilibrated to 37°C on an Eppendorf shaker. A stock solution (9 acetonitrile/1 DMSO) having a concentration of 100 µg/ml for compound A and compound B was prepared. 10 µl in each case of the stock solution were pipetted into 1 ml of equilibrated rat plasma, so as to give a concentration of 1 µg/ml.

The samples were kept at 450 rpm and 37°C for 24 h. At each of the sampling times of 0, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h and 24 h, 50 µl were taken and pipetted into 150 µl of methanol. Internal standard was included in the initial charge of methanol at a concentration of 0.05 µg/ml. After brief vortexing, 300 µl of 10 mM ammonium acetate buffer (pH 6.8) were added and centrifuged at 1881 g for 10 min. The samples were then analysed by means of RP-HPLC analysis and by LC-MS analysis.

In this assay, both compound A (Reference Example R3r-LLL) and compound B (Reference Example R3r-LDL) were stable over 24 h.

d: Assay for determination of stability in rat liver lysosomes

To determine the lysosomal stability of compounds A and B, lysosomal enzymes were isolated from rat liver cells. Compounds A and B were each added to this lysosomal extract in order to examine stability under lysosomal conditions. The proteolytic enzyme legumain is expressed only in a very small amount, if any, in rat liver lysosomes (Chen, J-M. *et al* 1997). To monitor the enzymatic activity of the lysosomal enzymes, a cathepsin-specific substrate was added.

First of all, a fresh rat liver was removed, weighed and immediately placed on ice in homogenization medium (0.25 M sucrose, 1 mM EDTA, 10 mM HEPES, pH7). The liver was comminuted and a change of medium was undertaken. The rat livers were homogenized with 4 times the amount of the rat liver weight at 750 rpm in a Potter (B. Braun). The homogenate was centrifuged at 1000 g for 10 min and the supernatant was filtered. In the next step, with the aid of an ultracentrifuge, the "light mitochondrial fraction" (LMF) was centrifuged out of the supernatant at 26 500 g over 20 min. Also present in the pellet apart from mitochondria are the lysosomes. The supernatant was discarded and the pellet was resuspended with 0.8 ml/g of homogenization medium.

In order to separate the lysosomal fraction from the other cell constituents of the LMF, 6 Optiprep density gradients were prepared with a sucrose content of 8%, 12%, 16%, 19%, 22.5% and 27% in the Optiprep buffer (100 mM MOPS, 20mM EDTA, 0.5% EtOH, pH 7.6). The sucrose was added from a 2.3 M stock solution of the particular percentage. 2.5 ml of isolated LMF were additionally added to the density stage with a sucrose content of 19%. Subsequently, the density stages were layered one on top of another in 10 ml centrifuge tubes and centrifuged at 48 500 g for 17 h. Fractions 1-8 are in the upper 5.6 ml of the gradient and were discarded. Fractions 9 and 10 are in the 1.6 ml beneath and were removed from the gradient and lysed with 1.6 ml of lysis buffer (25 mM HEPES, 150 mM NaCl, 0.1% Triton X100, pH 5) on ice for 5 min. The lysosomes are present in fractions 9 and 10. To monitor the lysis, the protein content of the lysed lysosomal fraction was monitored with the aid of a BCA assays (Pierce BCA protein assay kit).
To examine the lysosomal stability of compounds A and B, 6 µl of a 100 µg/ml stock solution (9 acetonitrile / 1 DMSO) were added to 290 µl of 90 mM citrate buffer and 300 µl of lysosomal extract and incubated at 37°C on an Eppendorf shaker. 50 µl each time were taken from the incubation solution after 0 h, 1 h, 2 h, 6 h, 24 h and 48 h and pipetted into 150 µl of MeOH. Internal standard was included in the initial charge at 0.05 µg/ml. For the RP-HPLC LCMS analysis, the samples were diluted with 300 µl of 10 mM ammonium acetate buffer (pH 6.8) and analysed.

Under the conditions of the lysosomal stability assay, compound A (Reference Example R3r-LLL) was cleaved to an extent of about 80% with a half-life of about 6 h within 24 h, while compound B (Reference Example R3r-LDL) was stable over the same period.

C-2 Internalization assay

Internalization is a key process which enables specific and efficient provision of the cytotoxic payload in antigen-expressing cancer cells via antibody drug conjugates (ADC). This process is monitored via fluorescent labelling of specific antibodies and an isotype control antibody. First, the fluorescent dye was conjugated to lysines of the antibody. Conjugation was carried out using a two-fold molar excess of CypHer 5E mono NHS ester (Batch 357392, GE Healthcare) at pH 8.3. After the coupling, the reaction mixture was purified by gel chromatography (Zeba Spin Desalting Columns, 40K, Thermo Scientific, No. 87768; elution buffer: DULBECCO´S PBS, Sigma-Aldrich, No. D8537), to eliminate excess dye and to adjust the pH. The protein solution was concentrated using VIVASPIN

500 columns (Sartorius stedim biotec). The dye load of the antibody was determined by spectrophotometric analysis (NanoDrop) and subsequent calculation

(D: $P = A_{dye}\,\varepsilon_{protein}:(A_{280}-0.16A_{dye})\varepsilon_{dye}$).

The dye load of the antibodies examined here and the isotype control were of a comparable order of magnitude. In cell binding assays, it was confirmed that the coupling did not lead to any change in the affinity of the antibodies.

The labelled antibodies were used for the internalization assay. Prior to the start of the treatment, cells (2 x 10^4/well) were sown in 100 µl medium in a 96-well MTP (fat, black, clear bottom No 4308776, from Applied Biosystems). After 18 h of incubation at 37°C/5%CO_2, the medium was replaced and labelled antibodies were added in different concentrations (10, 5, 2.5, 1, 0.1 µg/ml). The same treatment protocol was applied to the labelled isotype control (negative control). The chosen incubation times were 0 h, 0.25 h, 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 6 h and 24 h. The fluorescence measurement was carried out using the InCellAnalyzer 1000 (from GE Healthcare). This was followed by kinetic evaluation via measurement of the parameters granule counts/cell and total granule intensity/cell.

Following binding to the receptor, antibodies were examined for their internalization capacity. For this purpose, cells with different receptor expression levels were chosen. A target-mediated specific internalization was observed with the antibodies utilized in the context of the invention, whereas the isotype control showed no internalization.

C-3 In vitro tests for determining cell permeability

The cell permeability of a substance can be investigated by means of in vitro testing in a flux assay using Caco-2 cells [M.D. Troutman and D.R. Thakker, *Pharm. Res.* 20 *(8)*, 1210-1224 (2003)]. For this purpose, the cells were cultured for 15-16 days on 24-well filter plates. For the determination of permeation, the respective test substance was applied in a HEPES buffer to the cells either apically (A) or basally (B) and incubated for 2 hours. After 0 hours and after 2 hours, samples were taken from the cis and trans compartments. The samples were separated by HPLC (Agilent 1200, Böblingen, Germany) using reverse phase columns. The HPLC system was coupled via a Turbo Ion

Spray Interface to a Triple Quadropol mass spectrometer API 4000 (AB SCIEX Deutschland GmbH, Darmstadt, Germany). The permeability was evaluated on the basis of a P_{app} value, which was calculated using the formula published by Schwab et al. [D. Schwab *et al*., *J. Med. Chem.* 46, 1716-1725 (2003)]. A substance was classified as actively transported when the ratio of P_{app} (B-A) to P_{app} (A-B) (efflux ratio) was >2 or <0.5.

Of critical importance for toxophors which are released intracellularly is the permeability from B to A [P_{app} (B-A)] and the ratio of P_{app} (B-A) to P_{app} (A-B) (efflux ratio): the lower this permeability, the slower the active and passive transport processes of the substance through the monolayer of Caco-2 cells. If additionally the efflux ratio does not indicate any active transport, the substance may, following intracellular release, remain longer in the cell. Hence, there is also more time available for interaction with the biochemical target (in this case: kinesin spindle protein, KSP / Eg5).

Table 3 below sets out permeability data for representative working examples from this assay:

Table 3

Working Example	P_{app} (B-A) [nm/s]	Efflux ratio
M1	7.8	4
M2	4.8	6.4
M3	1.4	1.3
M4	21.3	18.7
M5	20.3	26.5
M6	1.7	0.7
M7	5.6	2.2
M9	213	16
M10	2.0	0.4
M11	24.3	27.7
M12	3.3	1.8
M13	7.1	3.6

Working Example	P_{app} (B-A) [nm/s]	Efflux ratio
M14	12.7	6.6
M15	6.4	4.4
M16	9.0	7.0
M17	93.6	81.5
M18	1.6	2.9
M19	1.9	2.9
M20	0.9	0.2
M21	0.5	1.5
M22	0.9	0.9
M23	2.8	2.0
M24	3.9	1.0
M25	8.1	3.6
M26	13.0	9.6
M27	13.2	11.9
M28	1.9	1.0
M29	4.1	5.3

C-4 In vitro tests for determining the substrate properties for P-glycoprotein (P-gp)

Many tumour cells express transporter proteins for drugs, and this frequently accompanies the development of resistance towards cytostatics. Substances which are not substrates of such transporter proteins, such as P-glycoprotein (P-gp) or BCRP, for example, could therefore exhibit an improved activity profile.

The substrate properties of a substance for P-gp (ABCB1) were determined by means of a flux assay using LLC-PK1 cells which overexpress P-gp (L-MDR1 cells) [A.H. Schinkel *et al*., *J. Clin. Invest.* 96, 1698-1705 (1995)]. For this purpose, the LLC-PK1 cells or L-MDR1 cells were cultured on 96-well filter plates for 3-4 days. For determination of the permeation, the respective test substance, alone or in the presence of an inhibitor (such as ivermectin or verapamil, for example), was applied in a HEPES buffer to the cells either

apically (A) or basally (B) and incubated for 2 hours. After 0 hours and after 2 hours, samples were taken from the cis and trans compartments. The samples were separated by HPLC using reverse phase columns. The HPLC system was coupled via a Turbo Ion Spray Interface to an API 3000 triple quadropole mass spectrometer (Applied Biosystems Applera, Darmstadt, Germany). The permeability was evaluated on the basis of a P_{app} value, which was calculated using the formula published by Schwab et al. [D. Schwab *et al*., *J. Med. Chem.* 46, 1716-1725 (2003)]. A substance was classified as P-gp substrate when the efflux ratio of P_{app} (B-A) to P_{app} (A-B) was >2.

As further criteria for the evaluation of the P-gp substrate properties, the efflux ratios in L-MDR1 and LLC-PK1 cells or the efflux ratio in the presence or absence of an inhibitor may be compared. If these values differ by a factor of more than 2, the substance in question is a P-gp substrate.

C-5 Pharmacokinetics

C5a: Identification of the ADC metabolites after internalization in vitro

Description of the method:

Internalization studies with immunoconjugates are carried out to analyse metabolites formed intracellularly. To this end, human lung tumour cells NCI H292 ($3x10^5$/well) are sown in 6-well plates and incubated overnight (37°C, 5% CO_2). The cells are treated with 10 µg/ml (66 nM) of the ADC to be examined. Internalization was carried out at 37°C and 5% CO_2. Cell samples are taken for further analysis at various times (0, 4, 24, 48, 72 h). First of all, the supernatants (about 5 ml) are harvested and, after centrifugation (2 min, RT, 1000 rpm Heraeus Variofuge 3.0R), stored at -80°C. The cells are washed with PBS and detached with Accutase, and the cell number is determined. After another washing, a defined number of cells (2×10^5) is treated with 100 ml of lysis buffer (Mammalian Cell Lysis Kit (Sigma MCL1) and incubated with continuous shaking (Thermomixer, 15 min, 4°C, 650 rpm) in Protein LoBind tubes (Eppendorf Cat. No. 0030 108.116). After the incubation, the lysate is centrifuged (10 min, 4°C, 12000 g, eppendorf 5415R) and the supernatant is harvested. The supernatant obtained is stored at –80°C. All samples are then analysed as follows.

Measurement of the compounds in the culture supernatant or cell lysate is carried out after precipitation of the proteins with methanol or acetonitrile by high-pressure liquid chromatography (HPLC) coupled to a triple-quadrupole mass spectrometer (MS).

For workup of 50 µl of culture supernatant/cell lysate, 150 µl of precipitation reagent (methanol) are added and the mixture is shaken for 10 seconds. The precipitation reagent contains an internal standard (ISTD) in a suitable concentration (generally in the range of 20-100 µg/l). After centrifugation at 1881 g for 10 minutes, the supernatant is transferred into an autosampler vial, made up with 300 µl of a buffer matched to the eluent and shaken again and centrifuged at 1881 g for 10 min.
The cell lysate and supernatant samples are finally analysed using the HPLC-coupled API6500 triple-quadrupole mass spectrometer from AB SCIEX Deutschland GmbH.

For calibration, blank lysate or blank supernatant is admixed with appropriate concentrations (0.1-1000 µg/l). The detection limit (LLOQ) is about 0.2 µg/l.

Quality controls for testing validity contain 4 and 40 µg/l.

C5b: Identification of the ADC metabolites in vivo

After i.v. administration of 10 mg/kg of various conjugates according to the invention in xenograft mice, 24 h after administration of these conjugates, it is possible to measure the plasma, tumour, liver and kidney concentrations of the antibody and potential metabolites. Under C-6 there is a more specific description of the method with regard to the xenograft model. All that are to be addressed here are the metabolite concentrations of the conjugates according to the invention. The measurements for the metabolites in the matrices mentioned additionally give information as to the extent of the metabolite load in the plasma, kidney and liver compared to the load in the tumour.

Analysis for quantification of the potential metabolites

The analysis of the compounds in the plasma, tumour, liver and kidney follows after precipitation of the proteins with generally methanol by high-pressure liquid chromatography (HPLC) coupled to a triple-quadrupole mass spectrometer (MS).

For workup of 50 µl of plasma, 150 µl of precipitation reagent (generally methanol) are added and the mixture is shaken for 10 sec. The precipitation reagent contains an internal standard (ISTD) in a suitable concentration (generally in the range of 20-100 µg/l). After centrifugation at 1881 g for 10 minutes, the supernatant is transferred into an autosampler vial, made up with 300 µl of a buffer matched to the eluent and shaken again.

In the workup of tumour or organ material, the particular material is admixed with 3-20 times the amount of extraction buffer. The extraction buffer contains 50 ml of Tissue Protein Extraction Reagent (Pierce, Rockford, IL), two pellets of Complete-Protease-Inhibitor-Cocktail (Roche Diagnostics GmbH, Mannheim, Germany) and phenylmethylsulphonyl fluoride (Sigma, St. Louis, MO) in a final concentration of 1 mM. According to the tissue type (hard: tumour; soft: liver, kidney), the lysis and homogenization programme of the Prescellys 24 lysis and homogenization system (Bertin Technologies) is selected (www.prescellys.com). The homogenized samples are left to stand at 4°C overnight. 50 µl of the homogenizate are transferred into an autosampler vial and made up with 150 µl of methanol including ISTD, agitated for 10 sec and then left to stand for 5 min. After adding 300 µl of ammonium acetate buffer (pH 6.8) and agitating briefly, the sample is centrifuged at 1881 g for 10 minutes.

For calibration, plasma for plasma samples and corresponding blank matrix for tissue samples is admixed with concentrations of 0.6-1000 µg/l. According to the sample type or tissue type, the detection limit (LOQ) is between 1 and 20 µg/l.

The plasma and matrix samples are finally analysed using the HPLC-coupled API6500 triple-quadrupole mass spectrometer from AB SCIEX Deutschland GmbH.

Quality controls for testing validity contain 4, 40 and 400 µg/l.

Table 4: Metabolite concentrations (MW: mean, SD: standard deviation) in the tumour, plasma, liver and kidney of xenograft mice (n = 3) 24 h after administration of conjugates according to the invention in Ku-19-19 xenograft mice. LLOQ tumour: 3-20 µg/l; LLOQ plasma: 1 µg/l; LLOQ liver and kidney: 5 µg/l.

Table 4:

		M26	

		MW (µg/L)	SD (µg/L)
Tumour	R10k	151.7	11.8
	Example 3k	76.3	2.7
Plasma	R10k	3.2	0.05
	Example 3k	<LLOQ	<LLOQ
Liver	R10k	209.4	134.0
	Example 3k	26.0	6.3
Kidney	R10k	107.6	10.2
	Example 3k	49.3	10.2

The ADCs from Example 3k and Reference Example R10k (with and without legumain-cleavable group) show a comparable *in vivo* effect in the Ku-19-19 model (cf. Chapter C-6b). The ADC from Example 3h1 likewise shows an *in vivo* effect in the A498 model (cf. Chapter C-6c) which is at least comparable to Reference Example R10h1 (with and without legumain-cleavable group).

After administration of the ADC from Example 3k, however, much lower concentrations of the active metabolite are measured, especially in the liver, than after application of the comparative ADC R10k. The low concentrations of the active metabolite M26 in the liver after administration of the ADC from Example 3k (26 µg/l) compared to the reference ADC without an enzymatically cleavable group from Example R10k (209.4 µg/l) are in accordance with the high stability of model compound B described in chapter C1c (Reference Example R3r-LDL) in rat liver lysosomes.

C-6 Activity test in vivo

The activity of the conjugates according to the invention was tested, for example, using xenograft models. The person skilled in the art is familiar with methods in the prior art which allow the activity of the compounds according to the invention to be tested (see, for

example, WO 2005/081711; Polson et al., Cancer Res. 2009 Mar 15;69(6):2358-64). To this end, a tumour cell line expressing the target molecule of the binder was implanted into rodents (for example mice). A conjugate according to the invention, an isotype antibody control conjugate, a control antibody or isotonic saline was then administered to the implant animals. The administration took place once or more than once. Following an incubation time of several days, the size of the tumour was determined by comparing conjugate-treated animals and the control group. The conjugate-treated animals displayed a smaller tumour size.

C-6a. Growth inhibition / regression of experimental tumours in the mouse

Human tumour cells which express the antigen for the antibody-drug conjugate are inoculated subcutaneously into the flank of immunosuppressed mice, for example NMRi nude or SCID mice. 1-10 million cells are detached from the cell culture, centrifuged and resuspended in medium or medium / matrigel. The cell suspension is injected under the skin of the mouse.

Within a few days, a tumour grows. Treatment is commenced after the tumour is established, at a tumour size of approximately 40 mm^2. To examine the effect on larger tumours, treatment may be initiated only at a tumour size of 50-100 mm^2.

Treatment with APDCs and ADCs is carried out via the intravenous (i.v.) route into the tail vein of the mouse. The ADC is administered in a volume of 5 ml/kg.

The treatment protocol depends on the pharmacokinetics of the antibody. As standard, treatment takes place three times in succession every fourth day. In the case of slow-growing tumours, weekly treatment is an option. For a quick assessment, a protocol with a single treatment may be employed. However, the treatment may also be continued, or a second cycle of three treatment days may follow at a later time.

As standard, 8 animals are used per treatment group. In addition to the groups to which the active substances are administered, one group is treated as control group only with the buffer, according to the same protocol.

During the experiment, the tumour area is measured regularly in two dimensions (length / width) using a caliper. The tumour area is determined as length x width. The ratio of the

mean tumour area of the treatment group to that of the control group is stated as T/C area.

When, after the end of the treatment, all groups of the experiment are terminated at the same time, the tumours can be removed and weighed. The ratio of the mean tumour weights of the treatment group to that of the control group is stated as T/C weight.

C-6b. Efficacy of the anti-TWEAKR APDCs in various xenograft models

The tumour cells (e.g. KU-19-19, NCI-H292, SCC4) are inoculated subcutaneously into the flank of female NMRI-nude or NOD.SCID mice (Janvier). At a tumour size of ~ 40 mm², intravenous treatment is effected with the antibody-drug conjugate. After the treatment, monitoring of the tumour growth continues if appropriate.

Treatment with anti-TWEAKR antibody-prodrug conjugates (APDCs) leads to distinct and long-lasting inhibition of tumour growth compared to the control group and isotype-drug conjugate, and is comparable to the inhibition of growth of the corresponding ADC without a legumain-cleavable group (R10k). Table 5 states the T/C values determined over the tumour area on the day of the last measurement of the control group, calculated after the start of treatment. Legumain-cleavable anti-TWEAKR ADCs (e.g. Example 24l1) also showed a potent and specific anti-tumour effect.

Table 5:

Example	Tumour model	Dose	Dose scheme	T/C area
3k	KU-19-19 (human bladder carcinoma)	5 mg/kg	Q7dx3	0.39 (Day 15)
R10k		5mg/kg	Q7dx3	0.32 (Day 15)
Isotype-drug conjugate		5 mg/kg	Q7dx3	0.81 (Day 15)
3k	NCI-H292 (human lung carcinoma)	5 mg/kg	SD (single dose)	0.63 (Day 20)
3L1	NCI-H292	5 mg/kg	Q7dx3	0.09 (Day 25)
3L1	KU-19-19 KU-19-19 KU-19-19	5 mg/kg 2.5 mg/kg 1 mg/kg	Q7dx3 Q7dx3 Q7dx3	0.30 (Day 25) 0.67 (Day 25) 1.01 (Day 25)
3L2	NCI-H292	10 mg/kg	SD	0.39 (Day 29)
16I2	NCI-H292	10 mg/kg	SD	0.18 (Day 29)
24I1	NCI-H292	5 mg/kg	SD	0.38 (Day 21)
28I1	KU-19-19	5 mg/kg	Q7dx3	0.25 (Day 25)
28I1	NCI-H292	5 mg/kg	SD	0.31 (Day 21)
28I1	SCC4 (human tongue base carcinoma)	5 mg/kg	Q7dx3	0.30 (Day 39)
31I1	KU-19-19 KU-19-19 KU-19-19	5 mg/kg 2.5 mg/kg 1 mg/kg	Q7dx3 Q7dx3 Q7dx3	0.44 (Day 25) 0.68 (Day 25) 0.97 (Day 25)
31I1	NCI-H292 NCI-H292	5 mg/kg 2.5 mg/kg	SD SD	0.29 (Day 21) 0.64 (Day 21)
35I1	NCI-H292	5 mg/kg	SD	0.27 (Day 21)
37I1	NCI-H292	5 mg/kg	SD	0.30 (Day 21)

C-6c. Efficacy of the anti-B7H3 APDCs in various xenograft models

The tumour cells (e.g. U251-MG, NCI-H292, SCC4, NCI-H1703) are inoculated subcutaneously into the flank of female NMRI-nude or NOD.SCID mice (Janvier). At a

tumour size of ~ 40 mm², intravenous treatment is effected with the antibody-drug conjugate. After the treatment, monitoring of the tumour growth continues if appropriate.

Treatment with anti-B7H3 antibody-prodrug conjugates (APDCs) results in marked and long-lasting inhibition of tumour growth compared to the control group and the isotype-drug conjugate. Table 6 states the T/C values of various anti-B7H3 APDCs and the T/C value of the reference compound R10h1 determined over the tumour area on the day of the last measurement of the control group, calculated after the start of treatment, in various xenograft models.

Table 6:

Example	Tumour model	Dose	Dose scheme	T/C area
3h1	A498 (human renal cell carcinoma)	10 mg/kg	Q7dx3	0.33 (Day 52)
R10h1	A498	10 mg/kg	Q7dx3	0.43 (Day 52)
3h1	U251 MG (human glioblastoma)	5 mg/kg	Q7dx3	0.28 (Day 34)
3h1	SCC4	10 mg/kg	Q7dx3	0.28 (Day 31)
3h2	NCI-H1703 (human lung carcinoma)	10 mg/kg	Q7dx3	0.10 (Day 33)
3h2	SCC4	10 mg/kg	Q7dx3	0.001 (Day 51)
16h1	U251-MG	5 mg/kg	Q7dx3	0.17 (Day 47)
16h1	NCI-H292	10 mg/kg	SD	0.29 (Day 22)
22h1	U251-MG	5 mg/kg	Q7dx3	0.38 (Day 47)
22h1	NCI-H292	10 mg/kg	SD	0.83 (Day 22)

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Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asn Tyr
            20                  25                  30

Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45

Gly Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr Pro Phe Thr
    50                  55                  60

Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65                  70                  75                  80

Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys Ala
                85                  90                  95

Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ala
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Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Ser Ile Gly Thr Asn
20 25 30

Ile His Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
35 40 45

Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser
65 70 75 80

Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Asn Asn Asn Trp Pro Thr
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Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

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Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Asn Tyr
20 25 30

Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Ser Gly Gly Asn Thr Asp Tyr Asn Thr Pro Phe Thr
50 55 60

Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80

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Lys Met Asn Ser Leu Gln Ser Asn Asp Thr Ala Ile Tyr Tyr Cys Ala
                85                  90                  95

Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser Val Phe
        115                 120                 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
    130                 135                 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145                 150                 155                 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
                165                 170                 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
            180                 185                 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
        195                 200                 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
    210                 215                 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225                 230                 235                 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
                245                 250                 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            260                 265                 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        275                 280                 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    290                 295                 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305                 310                 315                 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
                325                 330                 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            340                 345                 350
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Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
        355                 360                 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    370                 375                 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385                 390                 395                 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
                405                 410                 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            420                 425                 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445

Lys
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Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Ser Ile Gly Thr Asn
            20                  25                  30

Ile His Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
        35                  40                  45

Lys Tyr Ala Ser Glu Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Ser
65                  70                  75                  80

Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Asn Asn Asn Trp Pro Thr
                85                  90                  95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
            100                 105                 110
```

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
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20 25 30

Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
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1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
            20                  25                  30

Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270
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Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

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            20                  25                  30
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Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
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35 40 45

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Ser Tyr Ile Ser Pro Ser Gly Gly Ser Thr His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

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Gly Thr Leu Val Thr Val Ser Ser
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Gly
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1               5                   10
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Gly Tyr
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35 40 45

Tyr Gln Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Ser Pro Phe
85 90 95

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100 105

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20 25 30

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35 40 45

Ser Tyr Ile Ser Pro Ser Gly Gly Ser Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Asp Thr Tyr Phe Asp Tyr Phe Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
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Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly Lys
450

<210> 30
<211> 215
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 30

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Gly Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Gln Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Ser Pro Phe
                85                  90                  95

Ile Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
            100                 105                 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 31
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 31

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Pro Tyr
            20                  25                  30

Pro Met Ile Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Tyr Ile Ser Pro Ser Gly Gly Ser Thr His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Gly Asp Thr Tyr Phe Asp Tyr Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 32
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 32

```
Pro Tyr Pro Met Ile
1               5
```

<210> 33
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 33

```
Tyr Ile Ser Pro Ser Gly Gly Ser Thr His Tyr Ala Asp Ser Val Lys
1               5                  10                  15
```

Gly

<210> 34
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 34

Gly Gly Asp Thr Tyr Phe Asp Tyr Phe Asp Tyr
1 5 10

<210> 35
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 35

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Gly Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Gln Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Ser Pro Phe
85 90 95

Ile Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 36
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 36

```
Arg Ala Ser Gln Ser Ile Ser Gly Tyr Leu Asn
1               5                   10
```

<210> 37
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 37

```
Gln Ala Ser Ser Leu Gln Ser
1               5
```

<210> 38
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 38

```
Gln Gln Ser Tyr Thr Ser Pro Phe Ile Thr
1               5                   10
```

<210> 39
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 39

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Pro Tyr
            20                  25                  30

Pro Met Ile Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Tyr Ile Ser Pro Ser Gly Gly Ser Thr His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Gly Asp Thr Tyr Phe Asp Tyr Phe Asp Tyr Trp Gly Gln
            100                 105                 110
```

```
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380
```

```
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly
```

<210> 40
<211> 215
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 40

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Gly Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Gln Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Ser Pro Phe
                85                  90                  95

Ile Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
            100                 105                 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140
```

```
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 41
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 41

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Pro Tyr
            20                  25                  30

Pro Met Ile Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Tyr Ile Ser Pro Ser Gly Gly Ser Thr His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Gly Asp Thr Tyr Phe Asp Tyr Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 42
<211> 5
<212> PRT
<213> Artificial Sequence

<220>

<223> Antibody sequence

<400> 42

Pro Tyr Pro Met Ile
1 5

<210> 43
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 43

Tyr Ile Ser Pro Ser Gly Gly Ser Thr His Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 44
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 44

Gly Gly Asp Thr Tyr Phe Asp Tyr Phe Asp Tyr
1 5 10

<210> 45
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 45

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Gly Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Gln Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Ser Pro Phe
85 90 95

Ile Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 46
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 46

Arg Ala Ser Gln Ser Ile Ser Gly Tyr Leu Asn
1 5 10

<210> 47
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 47

Gln Ala Ser Ser Leu Gln Ser
1 5

<210> 48
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 48

Gln Gln Ser Tyr Thr Ser Pro Phe Ile Thr
1 5 10

<210> 49
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 49

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Pro Tyr
20 25 30

```
Pro Met Ile Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Tyr Ile Ser Pro Ser Gly Gly Ser Thr His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Gly Asp Thr Tyr Phe Asp Tyr Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Gln Ser Thr Tyr Arg
    290                 295                 300
```

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly

<210> 50
<211> 215
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 50

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Gly Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Gln Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

```
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Ser Pro Phe
                85                  90                  95

Ile Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
            100                 105                 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys
    210                 215


<210>  51
<211>  120
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Antibody sequence

<400>  51

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
            20                  25                  30

Ile Ile Ala Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
    50                  55                  60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Asn Thr Ala Tyr
65                  70                  75                  80
```

Met Gln Leu Ser Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Leu Thr Val Ser Ser
115 120

<210> 52
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 52

Asp Phe Ile Ile Ala
1 5

<210> 53
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 53

Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe Arg
1 5 10 15

Gly

<210> 54
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 54

Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr
1 5 10

<210> 55
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 55

Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro Val Thr Leu Gly
1 5 10 15

Thr Ser Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
85 90 95

Leu Glu Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys
100 105 110

<210> 56
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 56

Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr
1 5 10 15

<210> 57
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 57

Gln Met Ser Asn Leu Ala Ser
1 5

<210> 58
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 58

Ala His Asn Leu Glu Leu Pro Trp Thr
1 5

<210> 59
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 59

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
20 25 30

Ile Ile Ala Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
50 55 60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Asn Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215 220

```
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly
```

<210> 60
<211> 219
<212> PRT
<213> Artificial Sequence

<220>

<223> Antibody sequence

<400> 60

```
Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro Val Thr Leu Gly
1               5                  10                  15

Thr Ser Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
            20                  25                  30

Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
                85                  90                  95

Leu Glu Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130                 135                 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 61
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 61

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
20 25 30

Ile Ile Ala Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
50 55 60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 62
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 62

Asp Phe Ile Ile Ala
1 5

<210> 63
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 63

Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe Arg
1 5 10 15

Gly

<210> 64
<211> 11

<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 64

Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr
1 5 10

<210> 65
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 65

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
85 90 95

Leu Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 66
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 66

Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr
1 5 10 15

<210> 67
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 67

Gln Met Ser Asn Leu Ala Ser
1 5

<210> 68
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 68

Ala His Asn Leu Glu Leu Pro Trp Thr
1 5

<210> 69
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 69

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
20 25 30

Ile Ile Ala Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
50 55 60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140

```
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415
```

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly

<210> 70
<211> 219
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 70

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
85 90 95

Leu Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

```
Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 71
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 71

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
            20                  25                  30

Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 72
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 72

```
Asp Thr Tyr Ile His
1               5
```

<210> 73
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 73

Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 74
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 74

Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr
1 5 10

<210> 75
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 75

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 76
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 76

Arg Ala Ser Gln Asp Val Asn Thr Ala Val Ala
1 5 10

<210> 77
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 77

Ser Ala Ser Phe Leu Tyr Ser
1 5

<210> 78
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 78

Gln Gln His Tyr Thr Thr Pro Pro Thr
1 5

<210> 79
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 79

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30

Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
50 55 60

```
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335
```

```
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly
```

<210> 80
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 80

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95
```

```
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys
    210
```

<210> 81
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 81

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
            20                  25                  30

Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110
```

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 82
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 82

Asp Thr Tyr Ile His
1 5

<210> 83
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 83

Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 84
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 84

Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr
1 5 10

<210> 85
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 85

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 86
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 86

Arg Ala Ser Gln Asp Val Asn Thr Ala Val Ala
1 5 10

<210> 87
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 87

Ser Ala Ser Phe Leu Tyr Ser
1 5

<210> 88
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 88

Gln Gln His Tyr Thr Thr Pro Pro Thr
1 5

<210> 89
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 89

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
            20                  25                  30

Tyr Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Arg Ile Tyr Pro Thr Asn Gly Tyr Thr Arg Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255
```

```
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Gln Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly
```

<210> 90
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 90

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15
```

```
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys
    210
```

<210> 91
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 91

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30
```

```
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Ser Ile Ser Gly Ser Gly Gly Ser Thr Leu Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Leu Thr Gly Thr Ser Phe Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser
        115
```

<210> 92
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 92

```
Ser Tyr Ala Met Ser
1               5
```

<210> 93
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 93

```
Ser Ile Ser Gly Ser Gly Gly Ser Thr Leu Tyr Ala Asp Ser Val Lys
1               5                   10                  15

Gly
```

<210> 94
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 94

Leu Thr Gly Thr Ser Phe Asp Tyr
1 5

<210> 95
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 95

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Phe Asp Ser Ser Leu
85 90 95

Lys Lys Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> 96
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 96

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn
1 5 10

<210> 97
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 97

Ser Asn Asn Gln Arg Pro Ser
1 5

<210> 98
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 98

Gln Ser Phe Asp Ser Ser Leu Lys Lys Val
1 5 10

<210> 99
<211> 446
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 99

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Gly Ser Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Leu Thr Gly Thr Ser Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

```
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445
```

<210> 100
<211> 215
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 100

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1               5                  10                  15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20                  25                  30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45

Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65                  70                  75                  80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Phe Asp Ser Ser Leu
                85                  90                  95

Lys Lys Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro
            100                 105                 110

Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu
        115                 120                 125

Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro
    130                 135                 140

Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala
145                 150                 155                 160

Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala
                165                 170                 175

Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg
            180                 185                 190

Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr
        195                 200                 205

Val Ala Pro Thr Glu Cys Ser
    210                 215
```

<210> 101
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 101

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Gly Ser Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Leu Thr Gly Thr Ser Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 102
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 102

Ser Tyr Ala Met Ser
1 5

<210> 103
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 103

Ser Ile Ser Gly Ser Gly Gly Ser Thr Leu Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 104
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 104

Leu Thr Gly Thr Ser Phe Asp Tyr
1 5

<210> 105
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 105

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Phe Asp Ser Ser Leu
85 90 95

Lys Lys Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> 106
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 106

Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn Pro Val Asn
1 5 10

<210> 107
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 107

Ser Asn Asn Gln Arg Pro Ser
1 5

<210> 108
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 108

Gln Ser Phe Asp Ser Ser Leu Lys Lys Val
1 5 10

<210> 109
<211> 446
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 109

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Gly Gly Ser Thr Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Leu Thr Gly Thr Ser Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

```
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        115                 120                 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    130                 135                 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145                 150                 155                 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                165                 170                 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            180                 185                 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        195                 200                 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
    210                 215                 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225                 230                 235                 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
                245                 250                 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
            260                 265                 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
        275                 280                 285

Thr Lys Pro Arg Glu Glu Gln Tyr Gln Ser Thr Tyr Arg Val Val Ser
    290                 295                 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305                 310                 315                 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
                325                 330                 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
            340                 345                 350

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
        355                 360                 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
    370                 375                 380
```

```
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385                 390                 395                 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
                405                 410                 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
            420                 425                 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        435                 440                 445
```

<210> 110
<211> 215
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 110

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1               5                   10                  15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
            20                  25                  30

Pro Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45

Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65                  70                  75                  80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Phe Asp Ser Ser Leu
                85                  90                  95

Lys Lys Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro
            100                 105                 110

Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu Leu
        115                 120                 125

Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr Pro
    130                 135                 140

Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys Ala
145                 150                 155                 160
```

```
Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr Ala
                165                 170                 175

Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His Arg
            180                 185                 190

Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys Thr
        195                 200                 205

Val Ala Pro Thr Glu Cys Ser
    210                 215
```

<210> 111
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 111

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Pro Tyr
            20                  25                  30

Pro Met Ile Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Tyr Ile Ser Pro Ser Gly Gly Ser Thr His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Gly Asp Thr Tyr Phe Asp Tyr Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 112
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 112

Pro Tyr Pro Met Ile
1 5

<210> 113
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 113

Tyr Ile Ser Pro Ser Gly Gly Ser Thr His Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 114
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 114

Gly Gly Asp Thr Tyr Phe Asp Tyr Phe Asp Tyr
1 5 10

<210> 115
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 115

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Gly Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Gln Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Ser Pro Phe
85 90 95

Ile Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 116
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 116

Arg Ala Ser Gln Ser Ile Ser Gly Tyr Leu Asn
1 5 10

<210> 117
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 117

Gln Ala Ser Ser Leu Gln Ser
1 5

<210> 118
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 118

Gln Gln Ser Tyr Thr Ser Pro Phe Ile Thr
1 5 10

<210> 119
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 119

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Pro Tyr
20 25 30

Pro Met Ile Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

```
Ser Tyr Ile Ser Pro Ser Gly Gly Ser Thr His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Gly Asp Thr Tyr Phe Asp Tyr Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Asn Tyr Gln Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320
```

```
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly
```

<210> 120
<211> 215
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 120

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Gly Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Gln Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
```

```
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Thr Ser Pro Phe
                85                  90                  95

Ile Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
            100                 105                 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 121
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 121

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
            20                  25                  30

Ile Ile Ala Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
    50                  55                  60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95
```

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 122
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 122

Asp Phe Ile Ile Ala
1 5

<210> 123
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 123

Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe Arg
1 5 10 15

Gly

<210> 124
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 124

Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr
1 5 10

<210> 125
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 125

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
85 90 95

Leu Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 126
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 126

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr
1 5 10 15

<210> 127
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 127

Gln Met Ser Asn Arg Ala Ser
1 5

<210> 128
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 128

Ala His Asn Leu Glu Leu Pro Trp Thr
1 5

<210> 129
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 129

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
            20                  25                  30

Ile Ile Ala Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
    50                  55                  60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240
```

```
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly
```

<210> 130
<211> 219
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 130

```
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                  10                  15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
            20                  25                  30

Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
                85                  90                  95

Leu Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130                 135                 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 131
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 131

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                  10                  15
```

```
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
            20                  25                  30

Ile Ile Ala Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
    50                  55                  60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 132
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 132

```
Asp Phe Ile Ile Ala
1               5
```

<210> 133
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 133

```
Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe Arg
1               5                   10                  15

Gly
```

<210> 134
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<223> Antibody sequence

<400> 134

Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr
1 5 10

<210> 135
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 135

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Ile Asn Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
85 90 95

Leu Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 136
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 136

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Ile Asn Tyr Leu Tyr
1 5 10 15

<210> 137
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 137

Gln Met Ser Asn Arg Ala Ser
1 5

<210> 138
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 138

Ala His Asn Leu Glu Leu Pro Trp Thr
1 5

<210> 139
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 139

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
20 25 30

Ile Ile Ala Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
50 55 60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

```
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430
```

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly

<210> 140
<211> 219
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 140

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Ile Asn Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
85 90 95

Leu Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195 200 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 141
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 141

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
20 25 30

Ile Ile Ala Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
50 55 60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 142
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 142

Asp Phe Ile Ile Ala
1 5

<210> 143
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 143

Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe Arg
1 5 10 15

Gly

<210> 144
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 144

Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr
1 5 10

<210> 145
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 145

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Trp Ile Asn Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
85 90 95

Leu Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 146
<211> 16

<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 146

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Trp Ile Asn Tyr Leu Tyr
1 5 10 15

<210> 147
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 147

Gln Met Ser Asn Arg Ala Ser
1 5

<210> 148
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 148

Ala His Asn Leu Glu Leu Pro Trp Thr
1 5

<210> 149
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 149

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
20 25 30

Ile Ile Ala Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
50 55 60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

```
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly
```

<210> 150
<211> 219
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 150

```
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
            20                  25                  30

Asn Trp Ile Asn Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
                85                  90                  95

Leu Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105                 110
```

```
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130                 135                 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 151
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 151

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
            20                  25                  30

Ile Ile Ala Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
    50                  55                  60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 152
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 152

Asp Phe Ile Ile Ala
1 5

<210> 153
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 153

Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe Arg
1 5 10 15

Gly

<210> 154
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 154

Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr
1 5 10

<210> 155
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 155

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Trp Ile Asn Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Gln Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
85 90 95

Leu Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 156
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 156

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Trp Ile Asn Tyr Leu Tyr
1 5 10 15

<210> 157
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 157

Gln Gly Ser Asn Arg Ala Ser
1 5

<210> 158
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 158

Ala His Asn Leu Glu Leu Pro Trp Thr
1 5

<210> 159
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 159

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Phe
            20                  25                  30

Ile Ile Ala Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Tyr Pro Gly Thr Gly Arg Thr Tyr Tyr Ser Glu Lys Phe
    50                  55                  60

Arg Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

Ala Arg Arg Thr Ile Tyr Tyr Asp Tyr Asp Gly Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270
```

```
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly
```

<210> 160
<211> 219
<212> PRT
<213> Artificial Sequence

<220>
<223> Antibody sequence

<400> 160

```
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
            20                  25                  30
```

```
Asn Trp Ile Asn Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Gln Gly Ser Asn Arg Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala His Asn
                85                  90                  95

Leu Glu Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
        115                 120                 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
    130                 135                 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
                165                 170                 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
            180                 185                 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
        195                 200                 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

<210> 161
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> c(RGDfK)

<400> 161

```
His Ser Asp Ala Val Phe Thr Asp Asn Tyr Thr Arg Leu Arg Lys Gln
1               5                   10                  15

Met Ala Val Lys Lys Tyr Leu Asn Ser Ile Leu Asn
            20                  25
```

<210> 162
<211> 20

<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-targeting peptide

<400> 162

Ala Thr Glu Pro Arg Lys Gln Tyr Ala Thr Pro Arg Val Phe Trp Thr
1 5 10 15

Asp Ala Pro Gly
20

<210> 163
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-targeting peptide

<400> 163

Leu Gln Trp Arg Arg Asp Asp Asn Val His Asn Phe Gly Val Trp Ala
1 5 10 15

Arg Tyr Arg Leu
20

<210> 164
<211> 129
<212> PRT
<213> Homo sapiens

<400> 164

Met Ala Arg Gly Ser Leu Arg Arg Leu Leu Arg Leu Leu Val Leu Gly
1 5 10 15

Leu Trp Leu Ala Leu Leu Arg Ser Val Ala Gly Glu Gln Ala Pro Gly
20 25 30

Thr Ala Pro Cys Ser Arg Gly Ser Ser Trp Ser Ala Asp Leu Asp Lys
35 40 45

Cys Met Asp Cys Ala Ser Cys Arg Ala Arg Pro His Ser Asp Phe Cys
50 55 60

Leu Gly Cys Ala Ala Ala Pro Pro Ala Pro Phe Arg Leu Leu Trp Pro
65 70 75 80

Ile Leu Gly Gly Ala Leu Ser Leu Thr Phe Val Leu Gly Leu Leu Ser
85 90 95

Gly Phe Leu Val Trp Arg Arg Cys Arg Arg Arg Glu Lys Phe Thr Thr
100 105 110

Pro Ile Glu Glu Thr Gly Gly Glu Gly Cys Pro Ala Val Ala Leu Ile
115 120 125

Gln

<210> 165
<211> 534
<212> PRT
<213> Homo sapiens

<400> 165

Met Leu Arg Arg Arg Gly Ser Pro Gly Met Gly Val His Val Gly Ala
1 5 10 15

Ala Leu Gly Ala Leu Trp Phe Cys Leu Thr Gly Ala Leu Glu Val Gln
20 25 30

Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu
35 40 45

Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
50 55 60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala
65 70 75 80

Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
85 90 95

Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
100 105 110

Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
115 120 125

Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
130 135 140

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr
145 150 155 160

Val Thr Ile Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val
165 170 175

Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
180 185 190

Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Ile Leu
195 200 205

```
Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
    210                 215                 220

Pro Val Leu Gln Gln Asp Ala His Ser Ser Val Thr Ile Thr Pro Gln
225                 230                 235                 240

Arg Ser Pro Thr Gly Ala Val Glu Val Gln Val Pro Glu Asp Pro Val
                245                 250                 255

Val Ala Leu Val Gly Thr Asp Ala Thr Leu Arg Cys Ser Phe Ser Pro
            260                 265                 270

Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn Leu Ile Trp Gln Leu Thr
        275                 280                 285

Asp Thr Lys Gln Leu Val His Ser Phe Thr Glu Gly Arg Asp Gln Gly
    290                 295                 300

Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe Pro Asp Leu Leu Ala Gln
305                 310                 315                 320

Gly Asn Ala Ser Leu Arg Leu Gln Arg Val Arg Val Ala Asp Glu Gly
                325                 330                 335

Ser Phe Thr Cys Phe Val Ser Ile Arg Asp Phe Gly Ser Ala Ala Val
            340                 345                 350

Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys Pro Ser Met Thr Leu Glu
        355                 360                 365

Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr Val Thr Ile Thr Cys Ser
    370                 375                 380

Ser Tyr Arg Gly Tyr Pro Glu Ala Glu Val Phe Trp Gln Asp Gly Gln
385                 390                 395                 400

Gly Val Pro Leu Thr Gly Asn Val Thr Thr Ser Gln Met Ala Asn Glu
                405                 410                 415

Gln Gly Leu Phe Asp Val His Ser Val Leu Arg Val Val Leu Gly Ala
            420                 425                 430

Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn Pro Val Leu Gln Gln Asp
        435                 440                 445

Ala His Gly Ser Val Thr Ile Thr Gly Gln Pro Met Thr Phe Pro Pro
    450                 455                 460

Glu Ala Leu Trp Val Thr Val Gly Leu Ser Val Cys Leu Ile Ala Leu
465                 470                 475                 480
```

Leu Val Ala Leu Ala Phe Val Cys Trp Arg Lys Ile Lys Gln Ser Cys
485 490 495

Glu Glu Glu Asn Ala Gly Ala Glu Asp Gln Asp Gly Glu Gly Glu Gly
500 505 510

Ser Lys Thr Ala Leu Gln Pro Leu Lys His Ser Asp Ser Lys Glu Asp
515 520 525

Asp Gly Gln Glu Ile Ala
530

<210> 166
<211> 1255
<212> PRT
<213> Homo sapiens

<400> 166

Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu
1 5 10 15

Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
20 25 30

Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
35 40 45

Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
50 55 60

Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65 70 75 80

Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
85 90 95

Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
100 105 110

Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
115 120 125

Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
130 135 140

Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
145 150 155 160

Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
165 170 175

Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys

180 185 190

His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
195 200 205

Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
210 215 220

Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
225 230 235 240

Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
245 250 255

His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
260 265 270

Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
275 280 285

Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
290 295 300

Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
305 310 315 320

Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
325 330 335

Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
340 345 350

Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
355 360 365

Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
370 375 380

Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385 390 395 400

Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
405 410 415

Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
420 425 430

Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
435 440 445

Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly

450 455 460

Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
465 470 475 480

Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
485 490 495

Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
500 505 510

Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
515 520 525

Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
530 535 540

Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
545 550 555 560

Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
565 570 575

Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
580 585 590

Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
595 600 605

Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
610 615 620

Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
625 630 635 640

Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Ile Ser
645 650 655

Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
660 665 670

Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
675 680 685

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Leu Ser Leu Gln Arg Met Phe Asn Asn Cys Glu Val Val Leu Gly Asn
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Leu Glu Ile Thr Tyr Val Gln Arg Asn Tyr Asp Leu Ser Phe Leu Lys
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Thr Ile Gln Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Thr Val
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Glu Arg Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg Gly Asn Met Tyr
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Tyr Glu Asn Ser Tyr Ala Leu Ala Val Leu Ser Asn Tyr Asp Ala Asn
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Lys Thr Gly Leu Lys Glu Leu Pro Met Arg Asn Leu Gln Glu Ile Leu
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180 185 190

Ser Cys Pro Asn Gly Ser Cys Trp Gly Ala Gly Glu Glu Asn Cys Gln
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Cys Asn Gly Ile Gly Ile Gly Glu Phe Lys Asp Ser Leu Ser Ile Asn
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Pro Lys Ile Pro Ser Ile Ala Thr Gly Met Val Gly Ala Leu Leu Leu
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Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn Gln Ala Leu Leu
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Arg Ile Leu Lys Glu Thr Glu Phe Lys Lys Ile Lys Val Leu Gly Ser
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Gly Ala Phe Gly Thr Val Tyr Lys Gly Leu Trp Ile Pro Glu Gly Glu
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Lys Val Lys Ile Pro Val Ala Ile Lys Glu Leu Arg Glu Ala Thr Ser
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Tyr Val Arg Glu His Lys Asp Asn Ile Gly Ser Gln Tyr Leu Leu Asn
                805                 810                 815

Trp Cys Val Gln Ile Ala Lys Gly Met Asn Tyr Leu Glu Asp Arg Arg
            820                 825                 830

Leu Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Thr Pro
        835                 840                 845

Gln His Val Lys Ile Thr Asp Phe Gly Leu Ala Lys Leu Leu Gly Ala
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Glu Glu Lys Glu Tyr His Ala Glu Gly Gly Lys Val Pro Ile Lys Trp
865                 870                 875                 880

Met Ala Leu Glu Ser Ile Leu His Arg Ile Tyr Thr His Gln Ser Asp
                885                 890                 895

Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ser
            900                 905                 910

Lys Pro Tyr Asp Gly Ile Pro Ala Ser Glu Ile Ser Ser Ile Leu Glu
        915                 920                 925

Lys Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr
    930                 935                 940

Met Ile Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys
945                 950                 955                 960

Phe Arg Glu Leu Ile Ile Glu Phe Ser Lys Met Ala Arg Asp Pro Gln
                965                 970                 975

Arg Tyr Leu Val Ile Gln Gly Asp Glu Arg Met His Leu Pro Ser Pro
            980                 985                 990

Thr Asp Ser Asn Phe Tyr Arg Ala  Leu Met Asp Glu Glu  Asp Met Asp
        995                 1000                1005

Asp Val  Val Asp Ala Asp Glu  Tyr Leu Ile Pro Gln  Gln Gly Phe
    1010                1015                1020

Phe Ser  Ser Pro Ser Thr Ser  Arg Thr Pro Leu Leu  Ser Ser Leu
    1025                1030                1035

Ser Ala  Thr Ser Asn Asn Ser  Thr Val Ala Cys Ile  Asp Arg Asn
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Tyr Ser Ser Asp Pro Thr Gly Ala Leu Thr Glu Asp Ser Ile Asp
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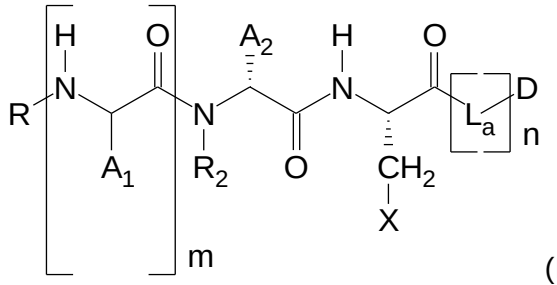
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Gly Ser Thr Ala Glu Asn Ala Glu Tyr Leu Arg Val Ala Pro Gln
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Ser Ser Glu Phe Ile Gly Ala
    1205                1210
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Claims

1. Compound of the following formula Ia:



(Ia)

in which

m		is 0 to 2;
n		is 0 or 1;
X		is –C(=O)-NH_2 or –COOH,
L_a		is a self-immolative linker,
A_1		is a radical which derives from one of the amino acids Gly, Pro, Ala, Val, Nva, Leu, Ile, Met, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Asp, Glu, Lys, Arg, citrulline and His, or one of the respective N-alkyl amino acids,
A_2		is a radical which derives from one of the amino acids D-Ala, D-Pro, D- Val, D-Nva, D-Leu, D-Ile, D-Met, D-Phe, D-Tyr, D-Trp, D-Ser, D-Thr, D-Cys, D-Asn, D-Gln, D-Asp, D-Glu, D-Lys, D-Arg, D-citrulline or D-His, or one of the respective N-alkyl amino acids,
R_2		is –H- or C_1-C_3-alkyl,
	or	
R_2		is the bond to the methylene group of the proline ring if A_2 is a radical which derives from D-Pro,

D is $-D_1-(L_b)_o-(LIG)_p$,

D_1 is a cytotoxic drug,

LIG is a binder which, after binding to a target molecule of a tumour cell, is internalized by the tumour cell and processed intracellularly, preferably lysosomally,

L_b is a linker,

o and p are each independently 0 or 1,

R is $Z_1-(C=O)q-$,

q is 0 or 1,

Z_1 is a C_{1-10}-alkyl, C_{5-10}-aryl or C_{6-10}-aralkyl, C_{5-10}-heteroalkyl, C_{1-10}-alkyl-O-C_{6-10}-aryl, C_{5-10}-heterocycloalkyl, heteroaryl, heteroarylalkyl, C_{5-10}-heteroarylalkoxy, C_{1-10}-alkoxy, C_{6-10}-aryl-C_{1-10}-alkyloxy, C_{6-10}-aryloxy or C_{6-10}-aralkoxy-, C_{5-10}-heteroalkoxy, C_{1-10}-alkyl-O-C_{6-10}-aryloxy- or C_{5-10}-heterocycloalkoxy group which may be mono- or polysubstituted by $-NH_2$, -C(=O)-, -NH-alkyl, $-N(alkyl)_2$, -NH-C(=O)-alkyl, -N(alkyl)-C(=O)-alkyl, $-S(=O)_3-H$, $-S(=O)_2-NH_2$, $-S(=O)_2-N(alkyl)_2$, -COOH, $-C(=O)NH_2$, -C(=O)-$N(alkyl)_2$ or –OH,

or is -H or a $-(CH_2)_{0-1}-Ox-(CH_2CH_2O)v-R^1$ group,

x is 0 or 1,

v is a number from 1 to 20,

R^1 is –H, –alkyl, $-CH_2-COOH$, $-CH_2-CH_2-COOH$ or

$-CH_2-CH_2-NH_2$,

or

R is $LIG-(L_c)_r-$,

	LIG	is a binder which, after binding to a target molecule on a tumour cell, is internalized by the tumour cell and processed intracellularly, preferably lysosomally,
	L_c	is a linker and
	r	is 0 or 1.

2. Compound according to Claim 1, where A_2 is a radical derived from one of the amino acids D-Ala, D-Pro, D-Asp, D-Asn, D-His and D-Ser.

3. Compound according to one of more of the preceding claims, where R_2 is -H or a methyl group, preferably -H.

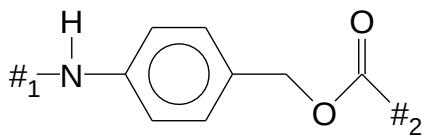
4. Compound according to one or more of the preceding claims, where m is 1.

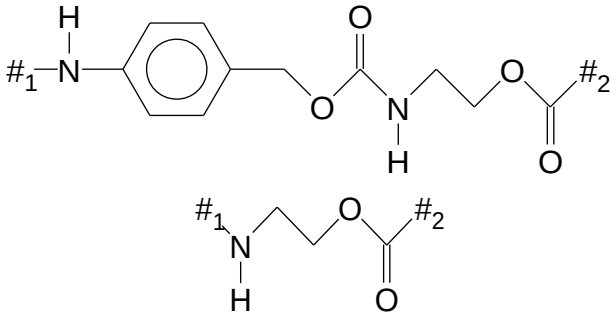
5. Compound according to one or more of the preceding claims, where A_1 is in the L configuration or D configuration.

6. Compound according to one or more of the preceding claims, where A_1 derives from Ala or Val.

7. Compound according to one or more of the preceding claims, where q is 1, and Z_1 represents a C_{1-10}-alkyl which may be interrupted once or more than once by an oxygen atom (for example a methyl group or an optionally alkylated oligoalkylene oxide chain), C_{6-10}-aralkyl, C_{5-10}-heteroarylalkyl, C_{6-10}-aralkoxy or C_{5-10}-heteroarylalkoxy group.

8. Compound according to one or more of the preceding claims, where L_a is selected from the following groups:

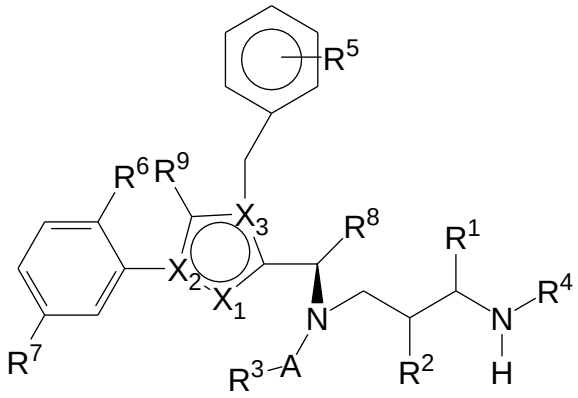




where $\#_1$ represents the bond to the carbonyl group and $\#_2$ the bond to the hydroxyl or amino group of D_1.

9. Compound according to one or more of the preceding claims, where D_1 is a drug selected from mitomycin, doxorubicin, aminopterin, actinomycin, bleomycin, 9-aminocamptothecin, n8-acetylspermidine, 1-(2-chloroethyl)-1,2-dimethanesulphonyl hydrazide, tallysomycin, cytarabine, etoposide, camptothecin, taxol, esperamicin, podophyllotoxin, anguidine, vincristine, vinblastine, morpholine-doxorubicin, n-(5,5-diacetoxypentyl)doxorubicin, duocarmycin, auristatin, pyrrolobenzodiazepine derivatives, calicheamicin, daunorubicin, camptophecin DX8951 (exatecan) or a kinesin spindle protein inhibitor (KSP inhibitor), the drug being bonded via its hydroxyl or amino group to L_a (when n = 1) or the carbonyl group (when n = 0) of formula I.

10. Compound according to Claim 9, where the KSP inhibitor is a compound of the following formula (IIa):



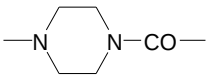
(IIa)

in which

X_1 is N,

	X_2	is N and
	X_3	is C,
	or	
	X_1	is N,
	X_2	is C and
	X_3	is N,
	or	
	X_1	is CH or CF,
	X_2	is C and
	X_3	is N,
	or	
	X_1	is NH,
	X_2	is C and
	X_3	is C,
	or	
	X_1	is CH,
	X_2	is N and
	X_3	is C.
	A	is –C(=O)-, -S(=O)-, -S(=O)$_2$-, or -S(=O)$_2$-NH-,
	R^1	is -H, –L-#1, –MOD or -$(CH_2)_{0-3}$Z,
	Z	is -H, -NHY^3, -OY^3, -SY^3, halogen, -C(=O)-NY^1Y^2, or –C(=O)-OY^3,
	Y^1 and Y^2	are independently -H, -NH_2,-$(CH_2CH_2O)_{0-3}$-$(CH_2)_{0-3}$Z' or -$CH(CH_2W)$Z',
	Y^3	is -H or -$(CH_2)_{0-3}$Z',
	Z'	is -H, -NH_2, -S(=O)$_3$H, -COOH, -NH-C(=O)-CH_2-CH_2-$CH(NH_2)$C(=O)- or -(C(=O)-NH-CHY^4)$_{1-3}$COOH,
	W	is -H or -OH,
	Y^4	is linear or branched C_{1-6} alkyl, optionally substituted by -NH-C(=O)-NH_2, or is aryl or benzyl optionally substituted by –NH_2,
	R^2	is -H, –L-#1 , -MOD, -C(=O)-CHY^4-NHY^5 or -$(CH_2)_{0-3}$Z,
	Z	is -H, halogen, -OY^3, -SY^3, -NHY^3, -C(=O)-NY^1Y^2, or

	–C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}Z'$,
Y^3	is -H or -$(CH_2)_{0-3}Z'$,
Z'	is -H, -$S(=O)_3H$, -NH_2 or –COOH,
Y^4	is linear or branched C_{1-6} alkyl- optionally substituted by –NHC(=O)-NH_2, or is aryl or benzyl optionally substituted by –NH_2,
Y^5	is -H or –C(=O)-CHY^6-NH_2,
Y^6	is linear or branched C_{1-6}-alkyl,
R^3	is -MOD, –L-#1, or an optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, heteroalkyl, heterocycloalkyl group which may be substituted by one to three OH groups, one to three halogen atoms, one to three mono-, di- or trihalogenated alkyl groups, one to three -O-alkyl groups, one to three -SH groups, one to three -S-alkyl groups, one to three -O-C(=O)-alkyl groups, one to three -O-C(=O)-NH-alkyl groups, one to three -NH-C(=O)-alkyl groups, one to three -NH-C(=O)-NH-alkyl groups, one to three -$S(=O)_n$-alkyl groups, one to three –$S(=O)_2$-NH-alkyl groups, 1-3 -NH-alkyl groups, one to three -$N(alkyl)_2$ groups, one to three NH_2 groups or one to three -$(CH_2)_{0-3}Z$ groups,
n	is 0, 1 or 2,
Z	is -H, halogen, -OY^3, -SY^3, -NHY^3, -C(=O)-NY^1Y^2 or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}Z'$,
Y^3	is -H, -$(CH_2)_{0-3}$-$CH(NHC(=O)CH_3)Z'$,-$(CH_2)_{0-3}$-$CH(NH_2)Z'$ or -$(CH_2)_{0-3}Z'$,
Z'	is - H, -$S(=O)_3H$, -NH_2 or –C(=O)-OH,
R^4	is the legumain-cleavable group of the formulae Ia', Ia'' and Ia''',
R^5	is -H, -NH_2, -NO_2, halogen, -CN, CF_3, -OCF_3, -CH_2F, -CH_2F, -SH or -$(CH_2)_{0-3}Z$,
Z	is -H, -OY^3, -SY^3, halogen, -NHY^3, -C(=O)-NY^1Y^2, or –C(=O)-OY^3,
Y^1 and Y^2	are independently -H, -NH_2, or -$(CH_2)_{0-3}Z'$,

Y^3	is -H or $-(CH_2)_{0-3}Z'$,
Z'	is -H, $-S(=O)_3H$, $-NH_2$ or –COOH,
R^6 and R^7	are independently -H, -CN, C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, hydroxyl, $-NO_2$, $-NH_2$, -COOH or halogen,
R^8	is C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, C_{4-10}-cycloalkyl, fluoro-C_{4-10}-cycloalkyl or $-(CH_2)_{0-2}-(HZ^2)$,
HZ^2	is a 4- to 7-membered heterocycle having up to two heteroatoms selected from N, O and S, which may be substituted by –OH, –COOH, $-NH_2$ or –L-#1,
R^9	is -H, -F, $-CH_3$, $-CF_3$, $-CH_2F$ or $-CHF_2$,
–L-#1	is $-(L_b)_o-(LIG)_p$,
LIG	is a binder which, after binding to a target molecule of a tumour cell, is internalized by the tumour cell and processed intracellularly and preferably lysosomally,
L_b	is a linker,
o and p	are independently 0 or 1,
–MOD	is $–(NR^{10})_n-(G1)_o-G2-G3$,
R^{10}	is -H or C_1-C_3-alkyl,
G1	is –NH-C(=O)- , -C(=O)-NH- or  ,
n	is 0 or 1;
o	is 0 or 1 and
G2	is a straight-chain or branched hydrocarbon chain which has 1 to 20 carbon atoms and may be interrupted once or more than once by -O-, -S-, -S(=O)-, $-S(=O)_2-$, $-NR^y-$, $-NR^yC(=O)-$, $-C(=O)NR^y-$, $-NR^yNR^y-$, $-S(=O)_2-NR^yNR^y-$, $-C(=O)-NR^yNR^y-$, -C(=O)-,

	$-CR^x=N-O-$, and the straight-chain or branched hydrocarbon chain may be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid,
R^y	is -H, phenyl, C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl or C_2-C_{10}-alkynyl, each of which may be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, sulphonamide, sulphone, sulphoxide, or sulphonic acid,
Rx	is -H, C_1-C_3-alkyl or phenyl,
G3	is –H or –COOH and
–MOD	has at least one –COOH group,

and the salts, solvates and salts of the solvates thereof.

11. Compound according to Claim 10, where R^1 or R^3 is –L-#1.

12. Compound according to one or more of Claims 10 and 11, where X_1 is CH, X_2 is C and X_3 is N.

13. Compound according to one or more of Claims 10 to 12, where R^6 and R^7 are independently -H, C_{1-3}-alkyl or halogen.

14. Compound according to Claim 13, where R^6 and R^7 are F.

15. Compound according to one of more or Claims 10 to 14, where R^8 is C_{1-4}-alkyl (preferably tert-butyl) or cyclohexyl.

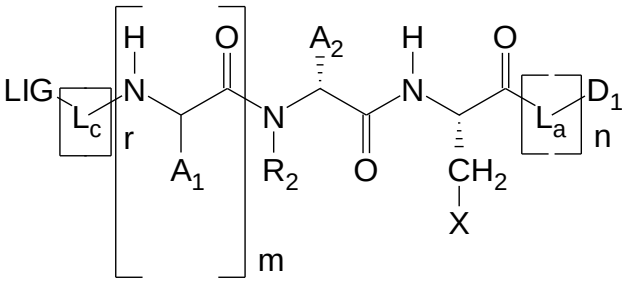
16. Compound according to one or more of Claims 10 to 15, where R^9 is -H.

17. Compound according to one or more of the preceding claims, where either D or R contains the binder LIG.

18. Compound according to Claim 17, where LIG is a peptide, protein or derivative thereof, selected from octreotide, GnRH-III, [D-Tyr^6, β-Ala^{11}, Phe^{13}, Nle^{14}]BN(6-14), NT(8-13), c(RGDfK), HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2 (SEQ ID NO: 161), NAPamide, [Phe^7, Pro^{34}]NPY, HER2-targeting peptide, ATEPRKQYATPRVFWTDAPG (SEQ ID NO: 162), LQWRRDDNVHNFGVWARYRL (SEQ ID NO: 163), or an antibody or a fragment

or derivative thereof which binds to an extracellular target molecule of a tumour cell and which is conjugated to further cytotoxic drugs.

19. Compounds according to one or more of the preceding claims, where the compounds have the following formula III'

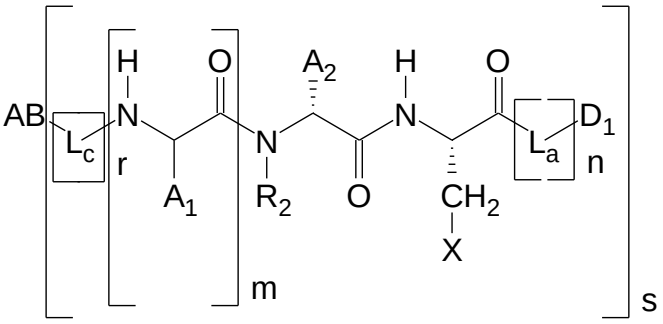


III'

in which

m, n, r, LIG, L_a, L_c, D_1,
X, A_2, R_2 and A_1 have the definitions given in Claim 1.

20. Compounds of the formula IIIa'



IIIa'

in which

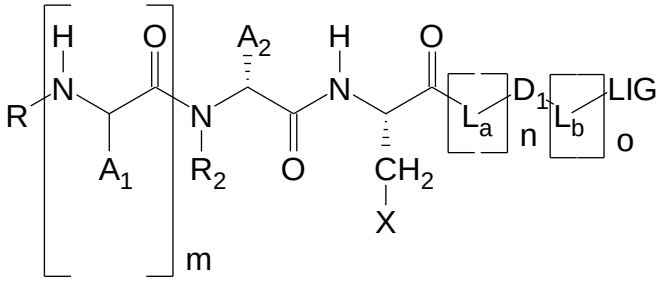
m, n, r, L_a, L_c,D_1, X,

R_2, A_2 and A_1 have the same definition as in Claim 1,

AB is an antibody or an antigen-binding antibody fragment and

s is 1 to 20,
preferably 2 to 8,
more preferably 2 to 6.

21. Compounds according to one or more of Claims 1 to 18, where the compounds have the following formula IV'

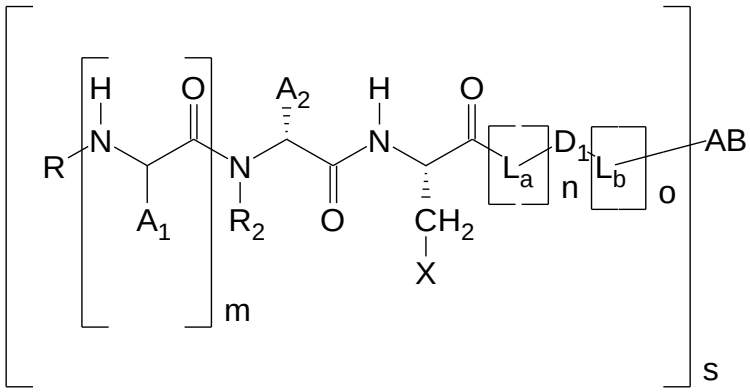


IV'

in which

m, n, o, R, LIG, L_a, L_b,
D_1, X, R_2, A_2 and A_1 have the same definition as in Claim 1.

22. Compounds of the formula IVa'



IVa'

in which
m, n, o, R, L_a, L_b,
D_1, X and A_1 have the same definition as in Claim 1,
AB is an antibody or an antigen-binding antibody fragment and
s is 1 to 20,
preferably 2 to 8,
more preferably 2 to 6.

23. Compounds according to one or more of the preceding claims, where the linker L_b or L_c is bound to a cysteine side chain or a cysteine residue of a binder, for example of an antibody or an antigen-binding antibody fragment, and has the following formula:

§-(C=O)m-L1-L2-§§

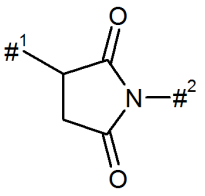
where

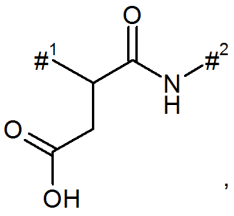
m is 0 or 1;

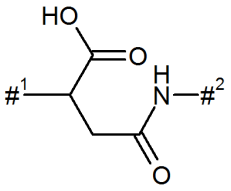
§ is the bond to the drug molecule or the legumain-cleavable group and

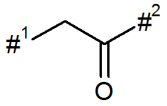
§§ is the bond to the binder,

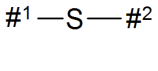
-L2- is the group



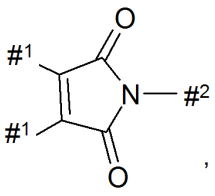
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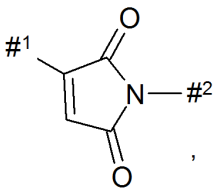
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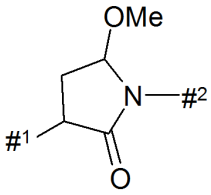
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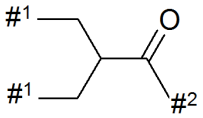
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or


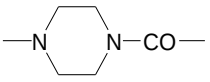
where

$\#^1$ denotes the linkage site to the sulphur atom of the binder,

$\#^2$ denotes the linkage site to the L^1 group,

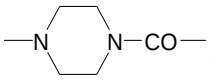
L1 is $–(NR10)_n-(G1)_o-G2-$,

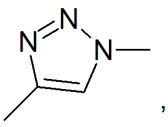
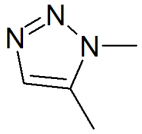
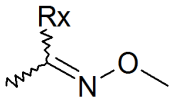
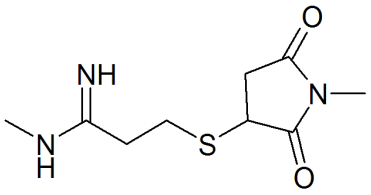
R^{10} is -H, $-NH_2$ or C_1-C_3-alkyl,

G1 is –NHC(=O)- or ,

n is 0 or 1,

o is 0 or 1,

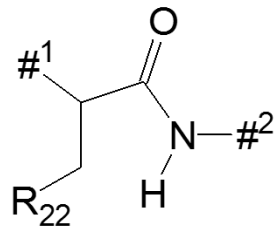
G2 is a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms, composed of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups, which may be interrupted once or more than once, by one or more of the groups -O-, -S-, -S(=O)-, $-S(=O)_2$, -NH-, -C(=O)-, -Nme-, -NHNH-, $-S(=O)_2$-NHNH-, -NH-C(=O)-, -C(=O)-NH-, -C(=O)-NHNH- and a 5- to 10-membered aromatic or nonaromatic heterocycle having up to 4 heteroatoms selected from N, O and S, -S(=O)- or $–S(=O)_2$- (preferably ), and where the side chains, if present, may be substituted by -NH-C(=O)-NH_2, -COOH, -OH, -NH_2, NH-CNNH_2, sulphonamide, sulphone, sulphoxide or sulphonic acid, or one of the following groups:

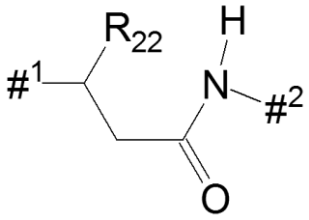
, ,  or 

where

Rx is -H, C_1-C_3-alkyl or phenyl.

24. Compounds according to Claim 23, where L2 is one or both of the following formulae:





where

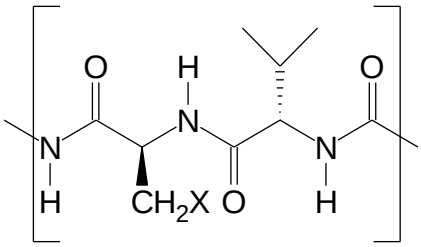
#[1] is the linkage site to the sulphur atom of the binder,

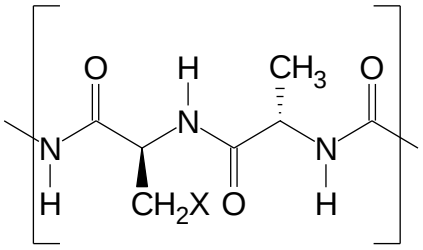
#[2] is the linkage site to the L[1] group,

R[22] is -COOH and

the bonds to the sulphur atom of the binder are present in one of these two formulae to an extent of more than 80% (based on the total number of bonds in the linker to the binder).

25. Compounds according to Claim 23 or 24, wherein the hydrocarbon chain is interrupted by one of the following groups:

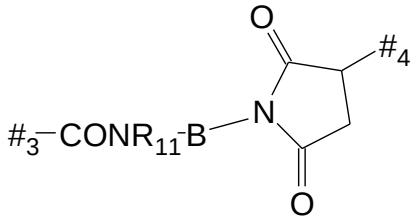




where

X is -H or a C_{1-10}-alkyl group which may optionally be substituted by –NH-C(=O)-NH_2, -COOH, -OH, -NH_2, -NH-CNNH_2, sulphone, sulphoxide or sulphonic acid.

26. Compounds according to any of Claims 23 to 25, where the linker has the following formula:



in which

#3 is the bond to the drug molecule,

#4 is the bond to the binder,

R^{11} is -H or -NH_2,

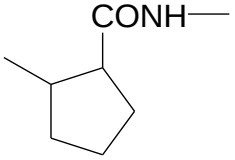
B is the –[$(CH_2)_x$-$(X^4)_y$]w-$(CH_2)_z$- group,

w is 0 to 20;

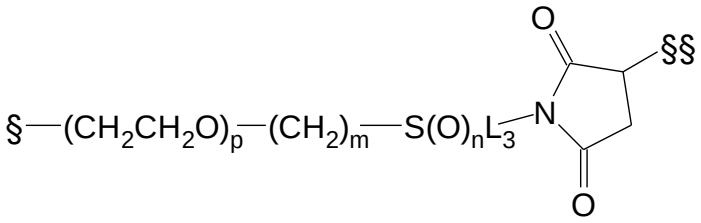
x is 0 to 5;

y is 0 or 1;

z is 0 to 5; and

X^4 is –O-, -C(=O)-NH-,–NH-C(=O)- or  .

27. Compounds according to one or more of the preceding claims, where the linker -L_b- and/or –L_c- is bonded to a cysteine side chain or a cysteine residue and has the following formula:



in which

§ is the bond to the drug molecule or the legumain-cleavable group,

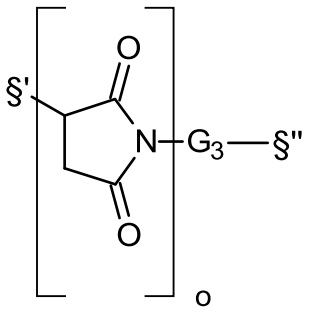
§§ is the bond to the binder,

m is 0, 1, 2, or 3,

n is 0, 1 or 2,

p is 0 to 20,

L3 is the group

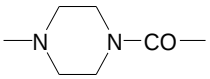


in which

o is 0 or 1,

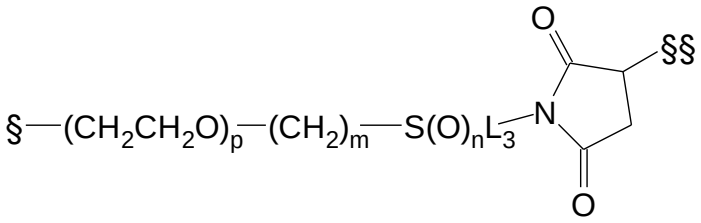
§' is the bond to the –S(O)n group and

§'' is the bond to the nitrogen atom in the ring.

G3 is a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms, composed of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups which may be interrupted once or more than once by one or more of the groups -O-, -S-, -S(=O)-, $S(=O)_2$-, -NH-, -C(=O)-, -NH-C(=O)-, -C(=O)-NH- and a 5- to 10-membered aromatic or nonaromatic heterocycle having up to 4 heteroatoms selected from N, O and S, -Nme-, -NHNH-, $-S(=O)_2$-NHNH-, -C(=O)-NHNH-, -S(=O)- or $-S(=O)_2$- (preferably ), where the side chains, if present,

may be substituted by –NHC(=O)-NH_2, -COOH, -OH, sulphone, sulphoxide or sulphonic acid.

28. Conjugate according to Claim 27, where the linker –L_b- or -L_c- is bonded to a cysteine side chain or a cysteine residue and has the following formula:



where

§ is the bond to the drug molecule or the legumain-cleavable group,

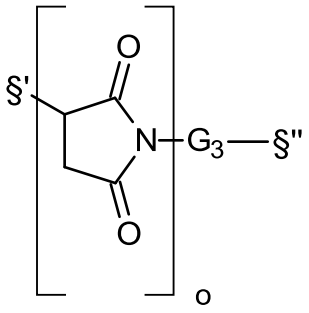
§§ is the bond to the binder,

m is 1;

p is 0;

n is 0,

L3 is the group



in which

o is 0 or 1,

G3 is $–(CH_2CH_2O)_s - (CH_2)_t - (C(=O)-NH)_u - CH_2CH_2O)_v - (CH_2)_w$-,

s, t, v and w are independently 0 to 20,

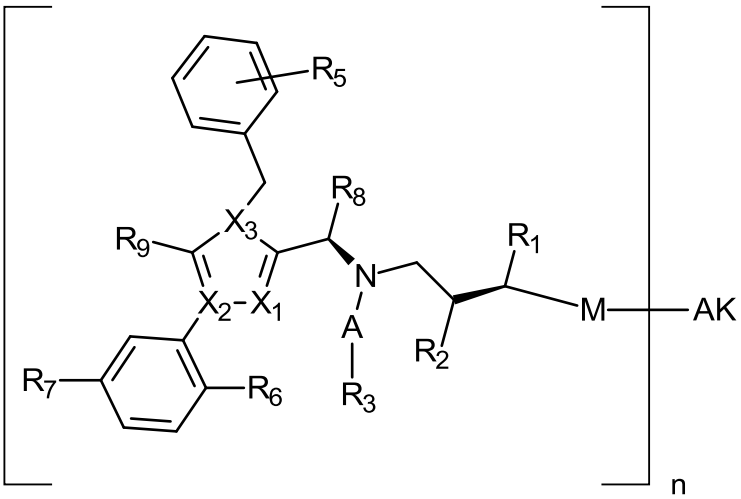
u is 0 or 1,

§' is the bond to the –S(O)n group and

§'' is the bond to the nitrogen atom in the ring.

29. Compound according to Claim 26 or 27, where R^2 or R^3 is –L-#1.

30. Conjugate composed of a binder or a derivative thereof (preferably an antibody or antigen-binding antibody fragment) with a compound according to one or more of the preceding claims, of the formula (X)



(X)

in which

AK	is the binder, preferably an antibody or antigen-binding antibody fragment,
n	is a number from 1 to 50, preferably 1 to 20, more preferably 2 to 8 and especially 2 to 6,
X_1	is N,
X_2	is N and
X_3	is C;
or	
X_1	is N,
X_2	is C and
X_3	is N;

or

X_1 is CH or CF,

X_2 is C and

X_3 is N;

or

X_1 is NH,

X_2 is C and

X_3 is C;

or

X_1 is CH,

X_2 is N and

X_3 is C.

A is –C(=O)-, -S(=O)-, -S(=O)$_2$- or -S(=O)$_2$-NH-,

M is the group

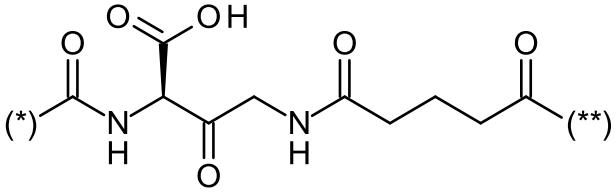
(*)-C(=O)-NH-(CH$_2$)$_3$-C(=O)-(**),

(*)-C(=O)-NH-(CH$_2$)$_2$-NH-C(=O)-(CH$_2$)$_3$-C(=O)-(**),

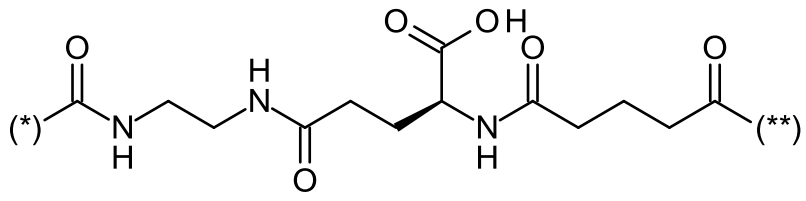
(*)-C(=O)-NH-(CH$_2$)$_2$-NH-C(=O)-CH$_2$-NH-C(=O)-(CH$_2$)$_3$-C(=O)-(**),

(*)-NH-C(=O)-CH(CH$_2$-CH$_2$-COOH)-NH-C(=O)-CH(CH$_3$)-NH-C(=O)-CH(CH$_3$)-NH-C(=O)-(CH$_2$)$_3$-C(=O)-(**),

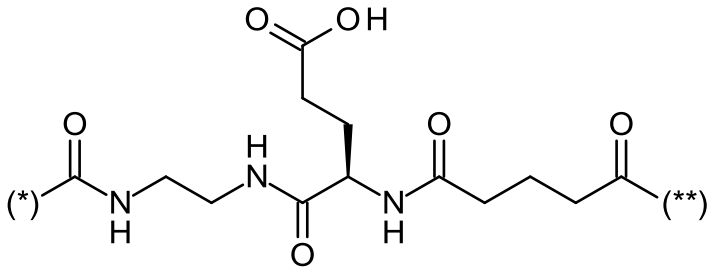
(*)-NH-C(=O)-CH(CH$_2$-C(=O)-NH$_2$)-NH-C(=O)-CH(CH$_3$)-NH-C(=O)-CH(CH$_3$)-NH-C(=O)-(**),



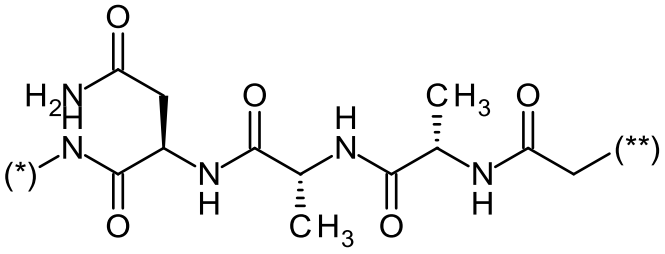
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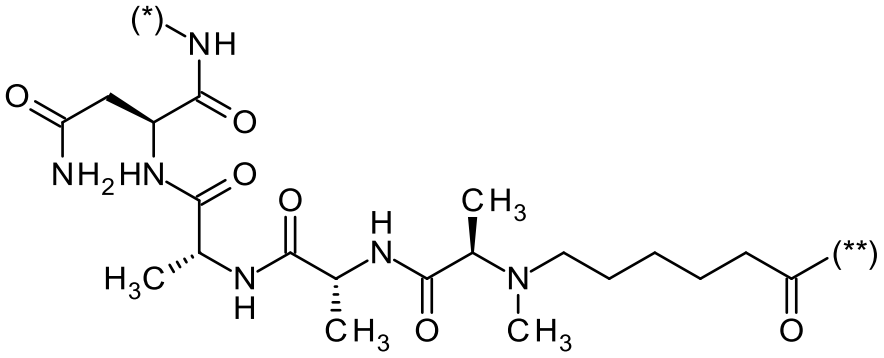
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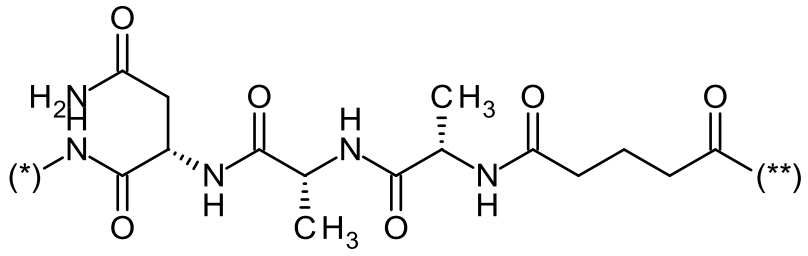
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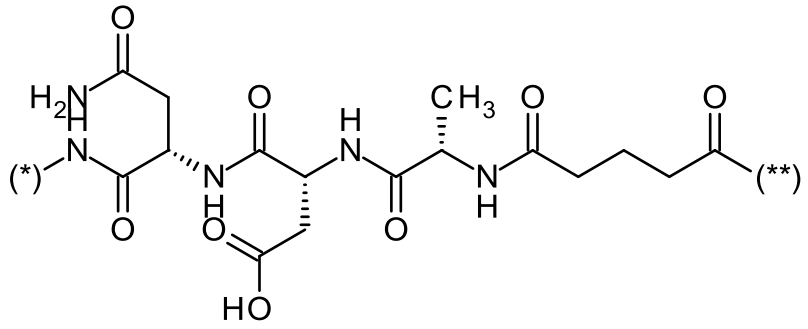
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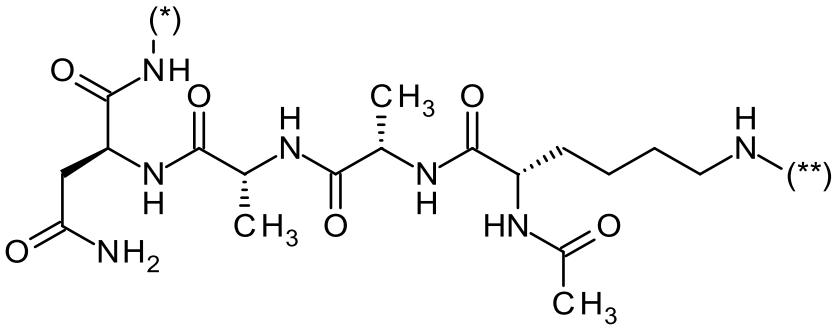
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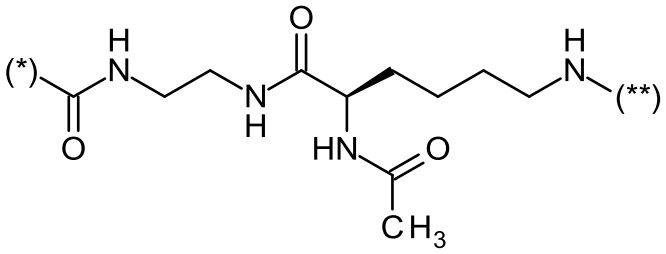
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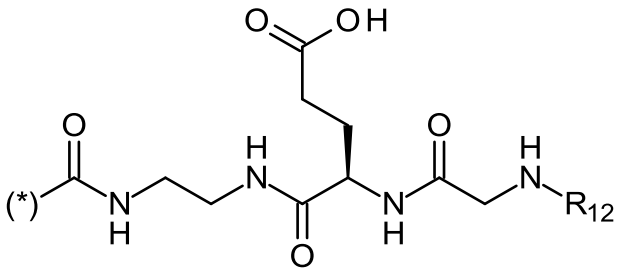


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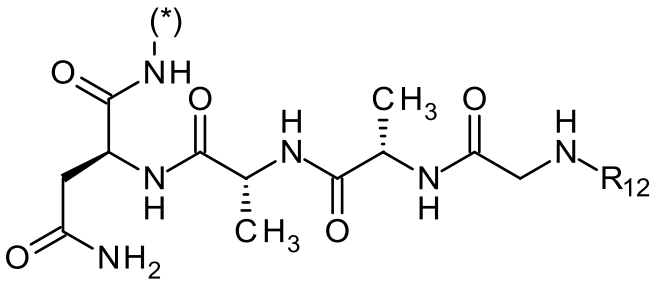
(*)-C(=O)-NH-$(CH_2)_2$-NH-C(=O)-CH_2-NH-R^{12},

(*)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH_2-NH-R^{12},

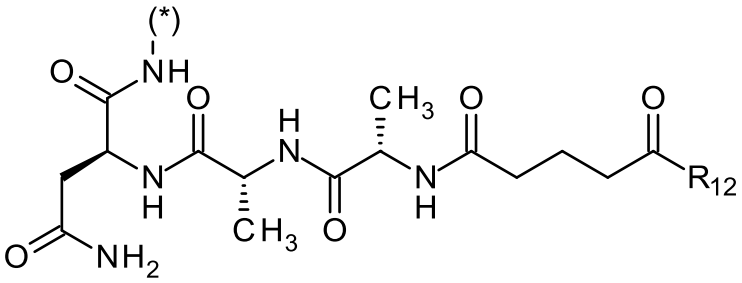
(*)-NH-C(=O)-CH(CH_2-C(=O)-NH_2)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH_2-C(=O)-NH-R^{12},



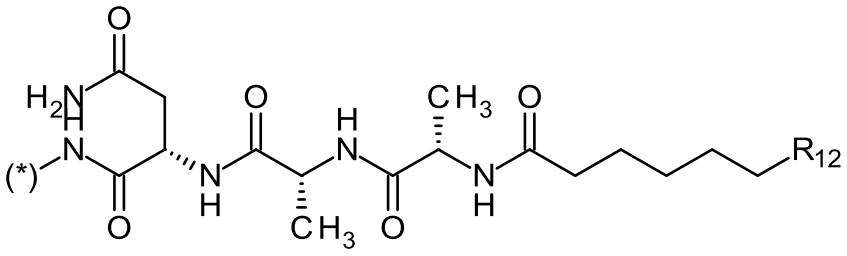
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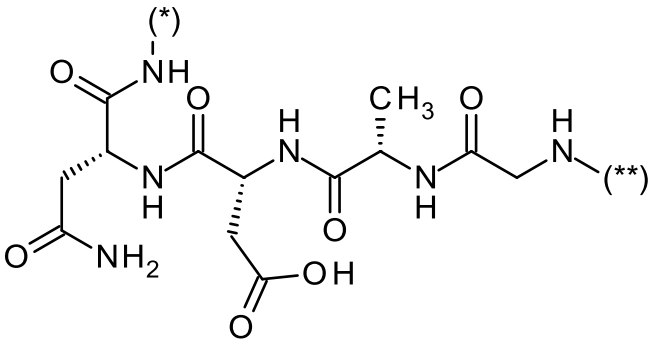
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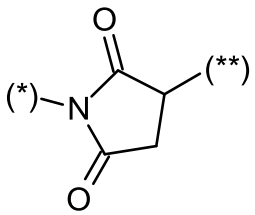
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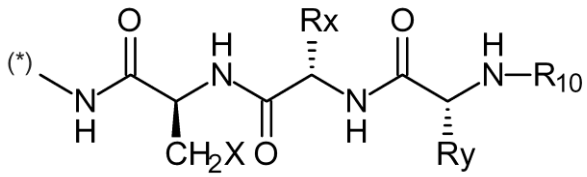
,

R^{12} is the group

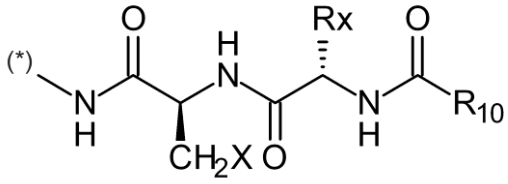
(*)-C(=O)-CH(**)-CH_2-COOH, (*)-C(=O)-CH_2-CH(**)-COOH or



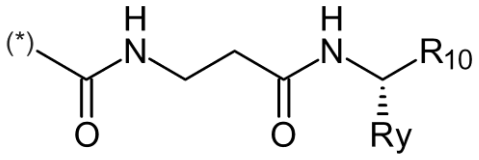
R^1 is hydrogen or the group



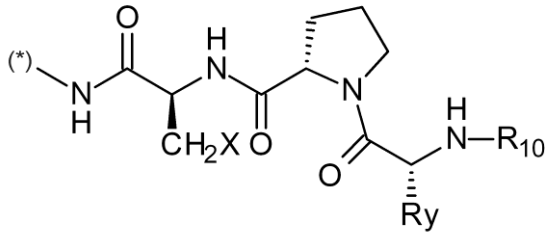
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X is $–C(=O)-NH_2$,

Rx and Ry are independently $–CH_3$, $-CH_2-COOH$, $-(CH_2)_2-COOH$, $-CH_2-C(=O)-NH_2$, $-CH_2OH$ or $–CH_2R^{11}$,

R^{10} is methyl, $–(C(=O))_q-O-R^{11}$, $–C(=O)-(CH_2)_m-R^{11}$,

q	is 0 or 1,
m	is 0, 1 or 2,
R^{11}	is hydrogen, methyl, benzyl, pyridyl, imidazolyl or the group $–(O\text{-}CH_2\text{-}CH_2)_p\text{-}O\text{-}CH_3$,
p	is 1 to 11,
R^2	is -H,
R^3	is an optionally substituted alkyl, cycloalkyl, aryl, heteroaryl, heteroalkyl, heterocycloalkyl group which may be substituted by one to three OH groups, one to three halogen atoms, one to three mono-, di- or trihalogenated alkyl groups, one to three –O-alkyl groups, one to three -SH groups, one to three -S-alkyl groups, one to three -O-C(=O)-alkyl groups, one to three -O-C(=O)-NH-alkyl groups, one to three -NH-C(=O)-alkyl groups, one to three -NH-C(=O)-NH-alkyl groups, one to three $-S(=O)_n$-alkyl groups, one to three $–S(=O)_2$-NH-alkyl groups, 1-3 -NH-alkyl groups, one to three -$N(alkyl)_2$ groups, one to three NH_2 groups or one to three $-(CH_2)_{0\text{-}3}Z$ groups,
n	is 0, 1 or 2,
Z	is -H, halogen, $-OY^3$, $-SY^3$, $-NHY^3$, $-C(=O)-NY^1Y^2$ or $–C(=O)-OY^3$,
Y^1 and Y^2	are independently -H, $-NH_2$, or $-(CH_2)_{0\text{-}3}Z'$,
Y^3	is -H, $-(CH_2)_{0\text{-}3}$-CH-$(NHC(=OCH_3)Z'$, $-(CH_2)_{0\text{-}3}$-$CH(NH_2)Z'$ or $-(CH_2)_{0\text{-}3}Z'$,
Z'	is -H, $-S(=O)_3H$, $-NH_2$ or –COOH,
R^5	is -H, $-NH_2$, $-NO_2$, halogen, -CN, $-CF_3$, $-OCF_3$, $-CH_2F$, $-CH_2F$, -SH or $-(CH_2)_{0\text{-}3}Z$,
Z	is -H, $-OY^3$, $-SY^3$, halogen, $-NHY^3$, $-C(=O)-NY^1Y^2$, or $–C(=O)-OY^3$,
Y^1 and Y^2	are independently -H, $-NH_2$, or $-(CH_2)_{0\text{-}3}Z'$,
Y^3	is -H or $-(CH_2)_{0\text{-}3}Z'$,
Z'	is -H, $-S(=O)_3H$, $-NH_2$ or –COOH,
R^6 and R^7	are independently -H, -CN, $C_{1\text{-}10}$-alkyl, fluoro-$C_{1\text{-}10}$-alkyl, $C_{2\text{-}10}$-alkenyl, fluoro-$C_{2\text{-}10}$-alkenyl, $C_{2\text{-}10}$-alkynyl, fluoro-$C_{2\text{-}10}$-alkynyl, hydroxyl, $-NO_2$, $-NH_2$, -COOH or halogen,

R^8	is C_{1-10}-alkyl, fluoro-C_{1-10}-alkyl, C_{2-10}-alkenyl, fluoro-C_{2-10}-alkenyl, C_{2-10}-alkynyl, fluoro-C_{2-10}-alkynyl, C_{4-10}-cycloalkyl, fluoro-C_{4-10}-cycloalkyl or -$(CH_2)_{0-2}$-(HZ^2),
HZ^2	is a 4- to 7-membered heterocycle having up to two heteroatoms selected from N, O and S, which may be substituted by –OH, –COOH or -NH_2,
R^9	is -H, -F, -CH_3, -CF_3, -CH_2F or -CHF_2,
(*)	is the bond to the drug molecule or the legumain-cleavable group,
(**)	is the bond to the binder,

and the salts, solvates and salts of the solvates thereof.

31. Conjugates of the formula (X) according to Claim 30, in which

AK	is the binder, preferably an antibody or antigen-binding antibody fragment,
n	is a number from 1 to 50, preferably 1 to 20, more preferably 2 to 8 and especially 2 to 6,
X_1	is CH,
X_2	is C and
X_3	is N;
A	is –C(=O)-,
M	is the group

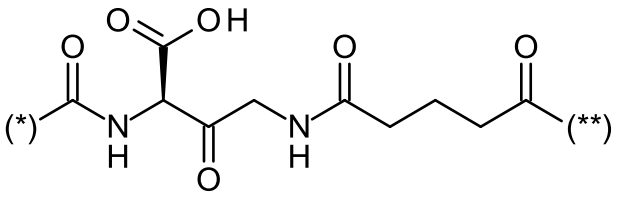
(*)-C(=O)-NH-$(CH_2)_3$-C(=O)-(**),

(*)-C(=O)-NH-$(CH_2)_2$-NH-C(=O)-$(CH_2)_3$-C(=O)-(**),

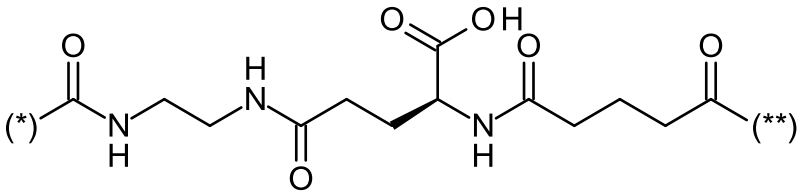
(*)-C(=O)-NH-$(CH_2)_2$-NH-C(=O)-CH_2-NH-C(=O)-$(CH_2)_3$-C(=O)-(**),

(*)-NH-C(=O)-CH(CH_2-CH_2-COOH)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-$(CH_2)_3$-C(=O)-(**),

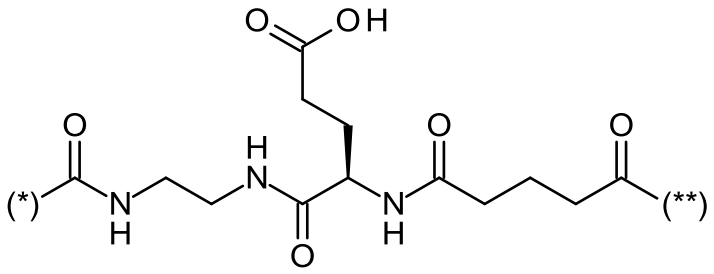
(*)-NH-C(=O)-CH(CH_2-C(=O)-NH_2)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-(**),



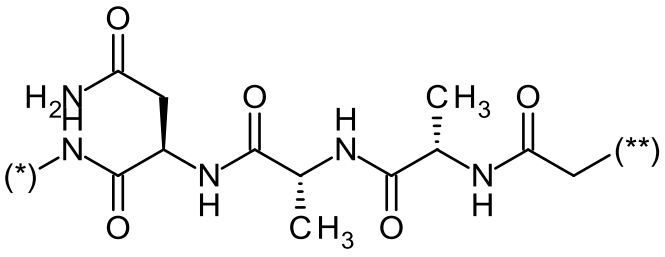
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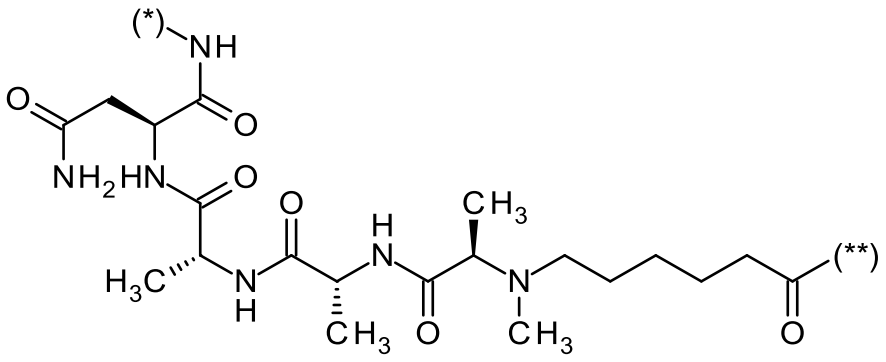
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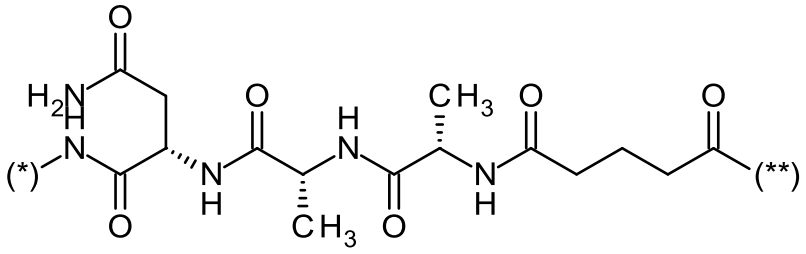
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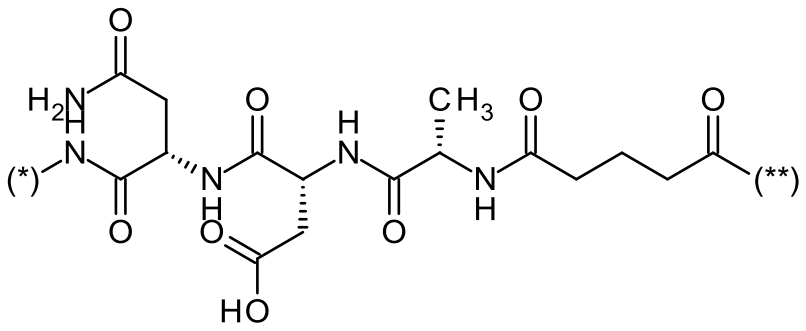
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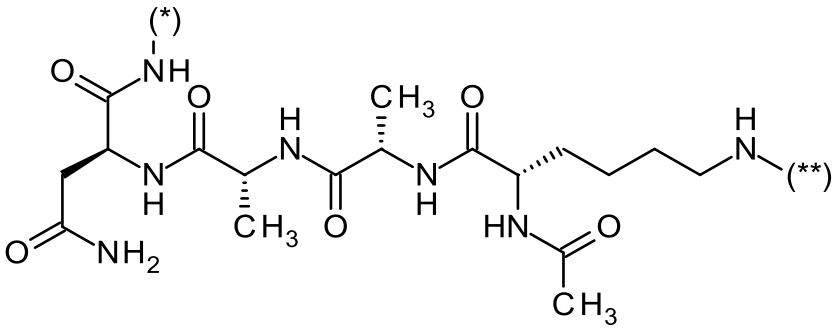
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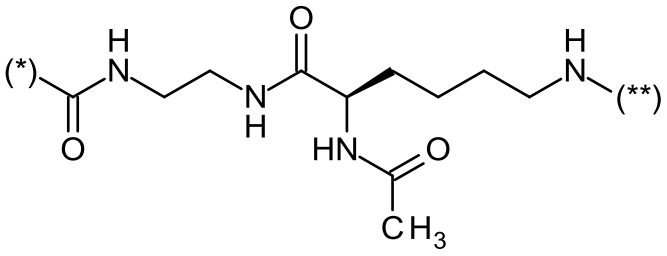
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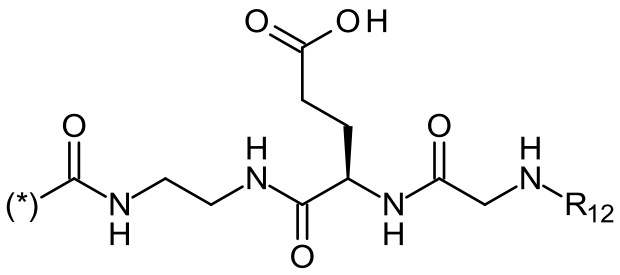


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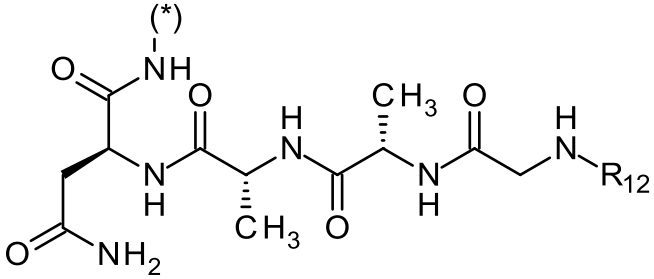
(*)-C(=O)-NH-$(CH_2)_2$-NH-C(=O)-CH_2-NH-R^{12},

(*)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH_2-NH-R^{12},

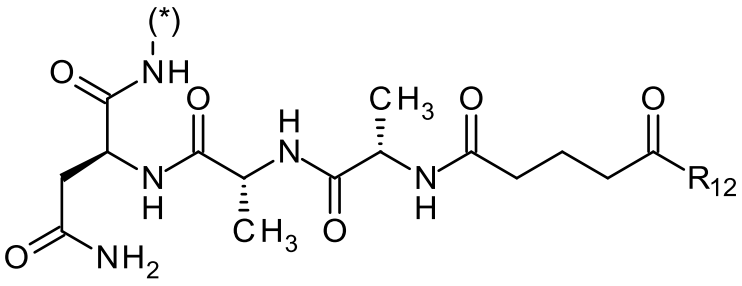
(*)-NH-C(=O)-CH(CH_2-C(=O)-NH_2)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH_2-C(=O)-NH-R^{12},



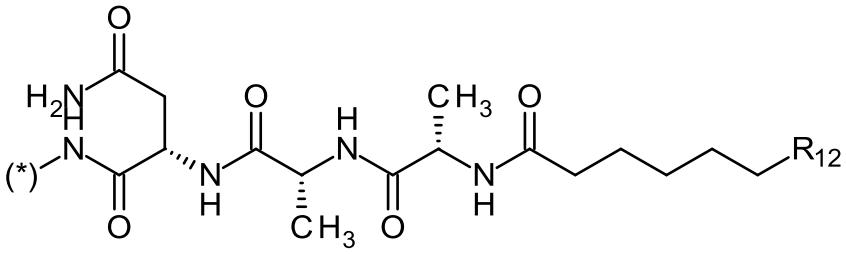
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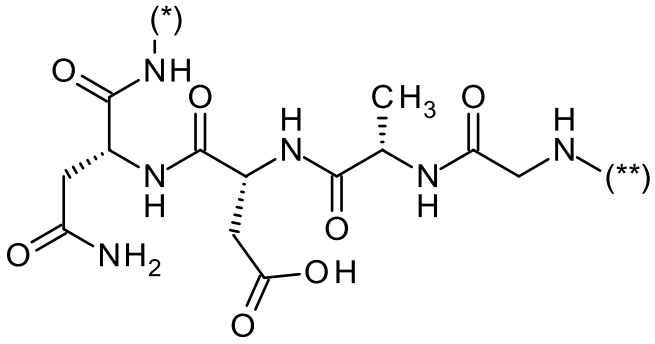
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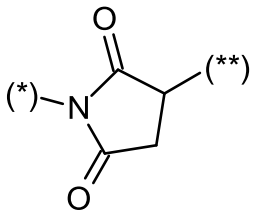


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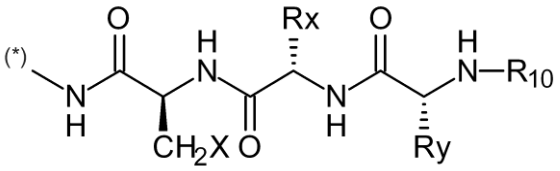


R^{12} is the group

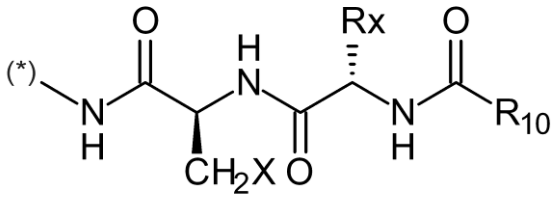
(*)-C(=O)-CH(**)-CH_2-COOH, (*)-C(=O)-CH_2-CH(**)-COOH or

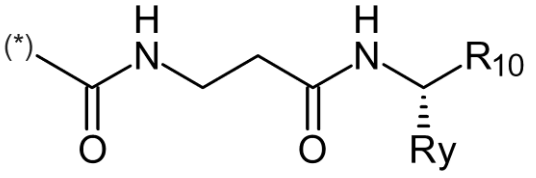


R^1 is hydrogen or the group

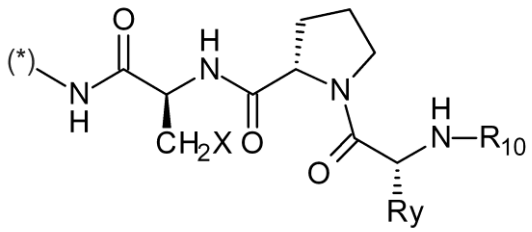


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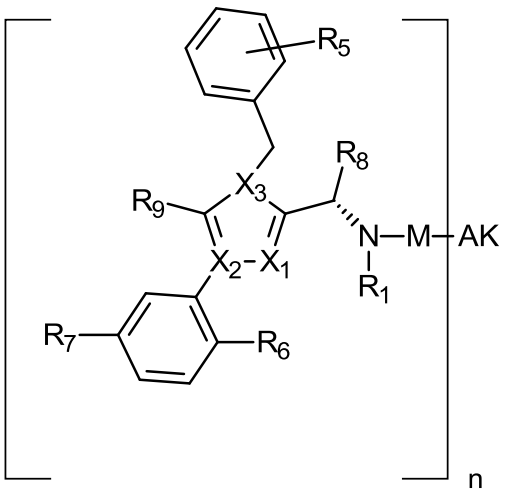
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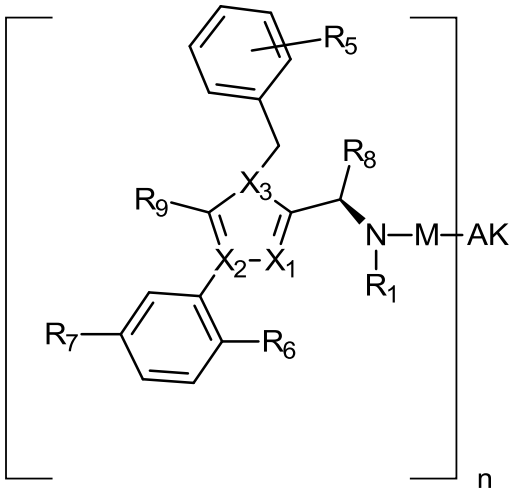
	X	is –C(=O)-NH_2,
	Rx and Ry	are independently –CH_3, -CH_2-COOH, -$(CH_2)_2$-COOH, -CH_2-C(=O)-NH_2, -CH_2OH or –CH_2R^{11},
	R^{10}	is methyl, –$(C(=O))_q$-O-R^{11}, –C(=O)-$(CH_2)_m$-R^{11},
	q	is 0 or 1,
	m	is 0, 1 or 2,
	R^{11}	is hydrogen, methyl, benzyl, pyridyl, imidazolyl or the group –$(O\text{-}CH_2\text{-}CH_2)_p$-O-$CH_3$,
	p	is 1 to 11,
	R^2	is -H,
	R^3	is a –CH_2-OH group,
	R^5	is –H,
	R^6 and R^7	are independently fluorine,
	R^8	is t-butyl,
	R^9	is -H,
	(*)	is the bond to the drug molecule or the legumain-cleavable group,
	(**)	is the bond to the binder,

and the salts, solvates and salts of the solvates thereof.

32. Conjugate composed of a binder, preferably an antibody or antigen-binding antibody fragment, with a compound according to one or more of the preceding claims, of the formulae (XI) and (XI')



(XI),



(XI')

in which

AK	is the binder, preferably an antibody or antigen-binding antibody fragment,
n	is a number from 1 to 50, preferably 1 to 20, more preferably 2 to 8 and especially 2 to 6,
X_1	is N,
X_2	is N and
X_3	is C;

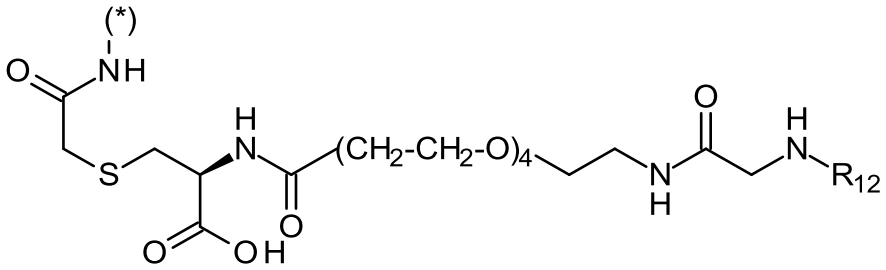
or

X_1	is N,
X_2	is C and
X_3	is N;

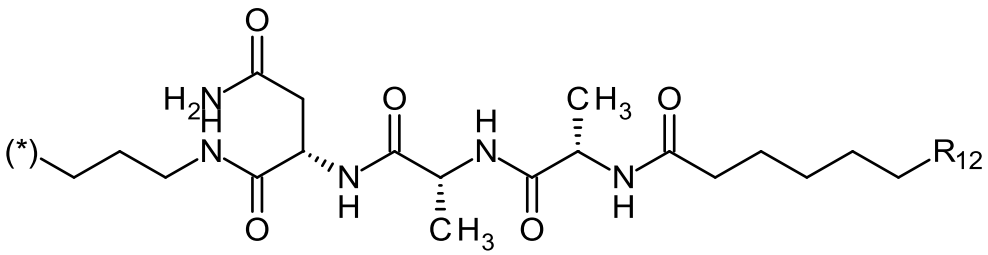
or

X_1 is CH or CF,
X_2 is C and
X_3 is N;
or
X_1 is NH,
X_2 is C and
X_3 is C;
or
X_1 is CH,
X_2 is N and
X_3 is C.

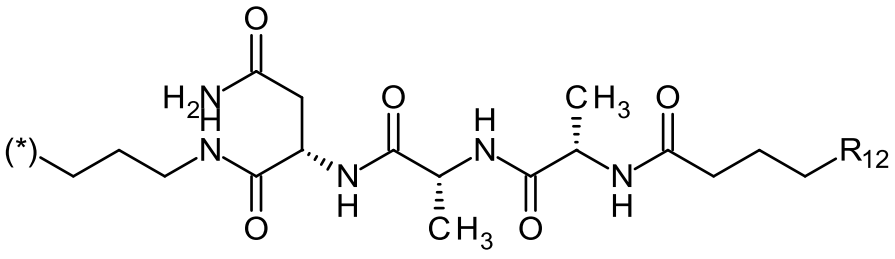
M is the group



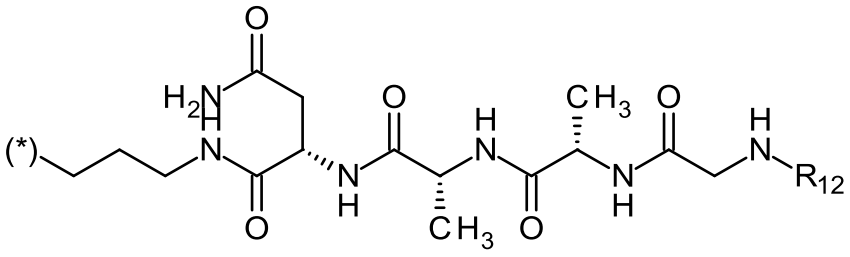
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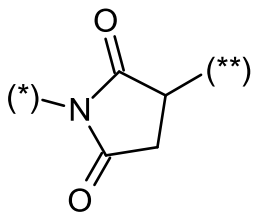


and

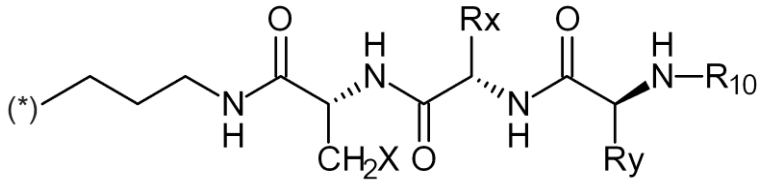
$-(CH_2)_3-NH-C(=O)-CH(CH_2-C(=O)-NH_2)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-(CH_2)_3-R^{12}$

R^{12} is the group

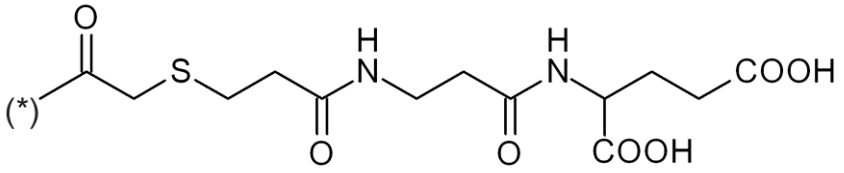
(*)-C(=O)-(**), (*)-C(=O)-CH(**)-CH_2-COOH, (*)-C(=O)-CH_2-CH(**)-COOH



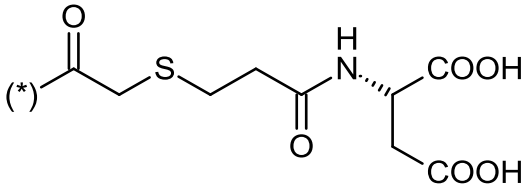
R^1 is the group



,



,



X is $–CH_2-C(=O)-NH_2$, $–C(=O)-NH_2$,

Rx and Ry are $–CH_3$, propyl,

R^{10} is $–C(=O)-(CH_2)-R^{11}$, $–C(=O)-(CH_2-CH_2-O)_p-CH_3$,

R^{11} is hydrogen, pyridyl,

p is 8 to 12,

R^5 is –H,

R^6 and R^7 are independently fluorine,

R^8 is t-butyl,

R^9 is -H,

(*) is the bond to the drug molecule or the legumain-cleavable group,

(**) is the bond to the binder,

and the salts, solvates and salts of the solvates thereof.

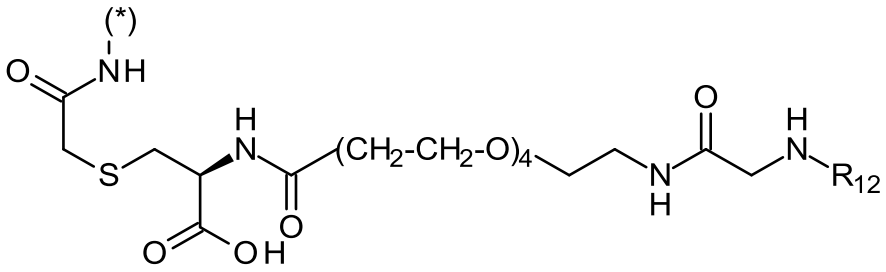
33. Conjugates of the formulae (XI) and (XI') according to Claim 32, in which

AK is the binder, preferably an antibody or antigen-binding antibody fragment,

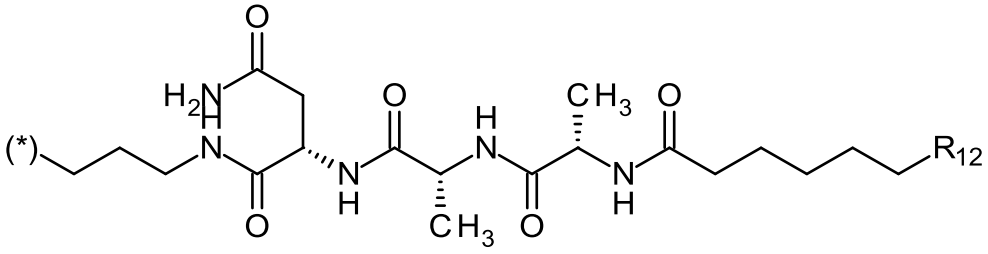
n is a number from 1 to 50, preferably 1 to 20, more preferably 2 to 8 and especially 2 to 6,

X_1 is CH,
X_2 is C and
X_3 is N;

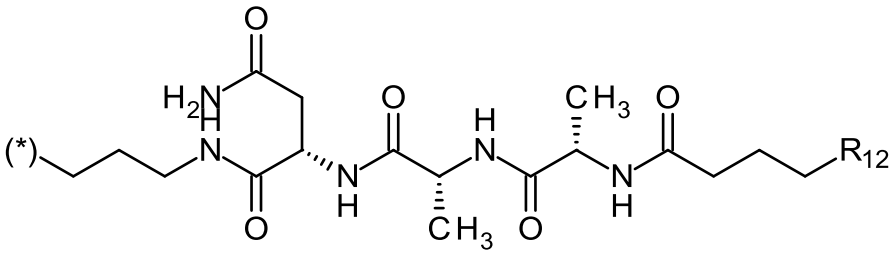
M is the group



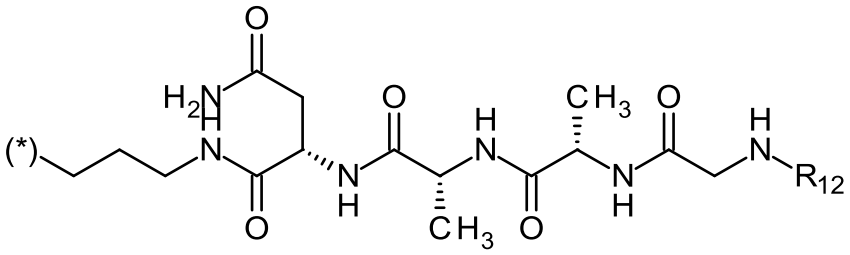
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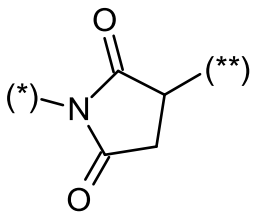


and

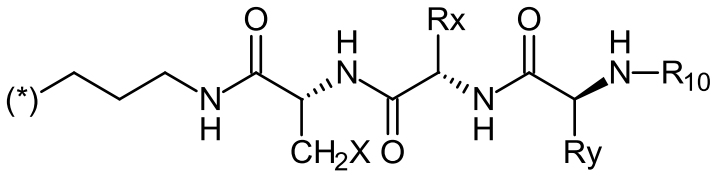
$-(CH_2)_3-NH-C(=O)-CH(CH_2-C(=O)-NH_2)-NH-C(=O)-CH(CH_3)-NH-C(=O)-CH(CH_3)-NH-C(=O)-(CH_2)_3-R^{12}$

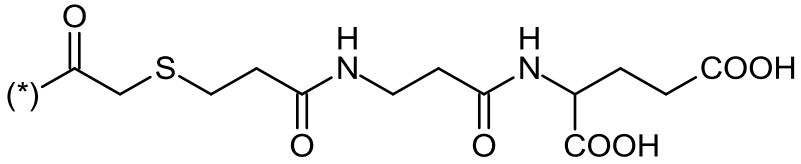
R^{12} is the group

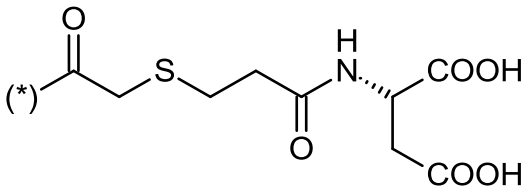
(*)-C(=O)-(**), (*)-C(=O)-CH(**)-CH_2-COOH,
(*)-C(=O)-CH_2-CH(**)-COOH



R^1 is the group







X	is $–CH_2$-C(=O)-NH_2, –C(=O)-NH_2,
Rx and Ry	are $–CH_3$, propyl,
R^{10}	is $–C(=O)-(CH_2)-R^{11}$, $–C(=O)-(CH_2-CH_2-O)_p-CH_3$,
R^{11}	is hydrogen, pyridyl,
p	is 8 to 12,
R^5	is –H,
R^6 and R^7	are independently fluorine,
R^8	is t-butyl,
R^9	is -H,
(*)	is the bond to the drug molecule or the legumain-cleavable group,
(**)	is the bond to the binder,

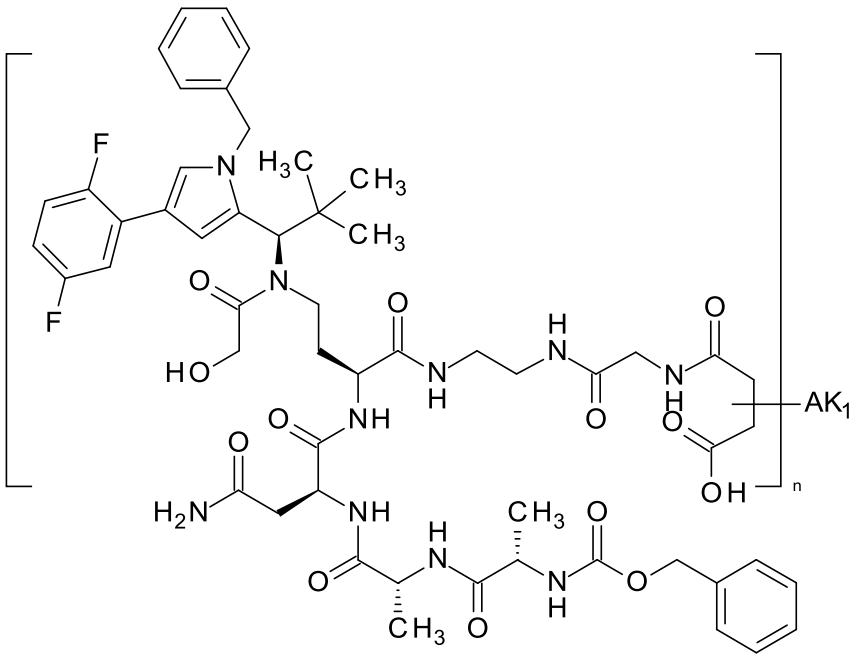
and the salts, solvates and salts of the solvates thereof.

34. Conjugates according to one or more of the preceding claims, of the following structures in which:

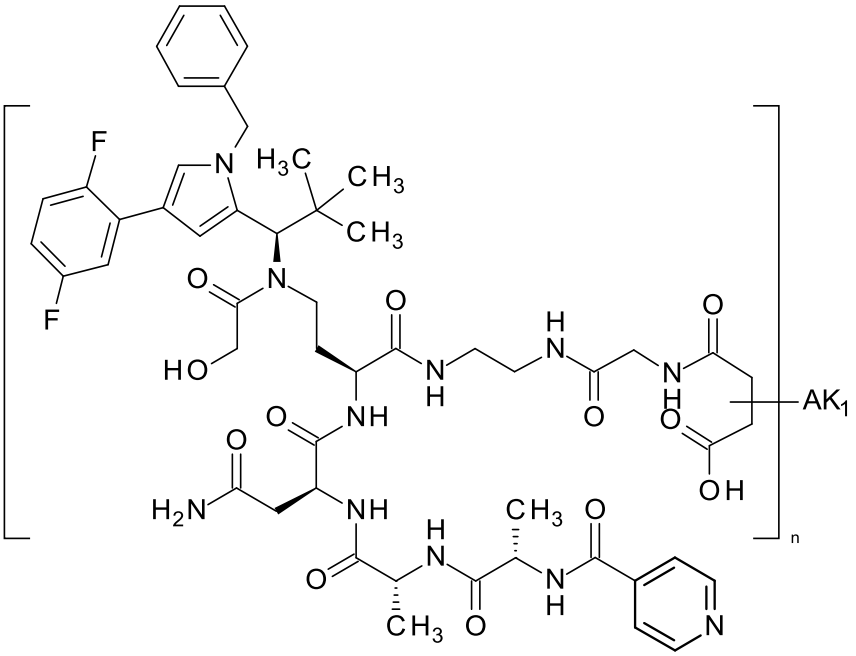
AK	is the binder, preferably an antibody or antigen-binding antibody fragment (AK_1, AK_2, AK_3), and
AK_1	is preferably an antibody bonded to the KSP inhibitor via a cysteine residue,
AK_2	is preferably an antibody bonded to the KSP inhibitor via a lysine residue and
AK_3	is preferably an antibody bonded to the KSP inhibitor via a glutamine residue,

and

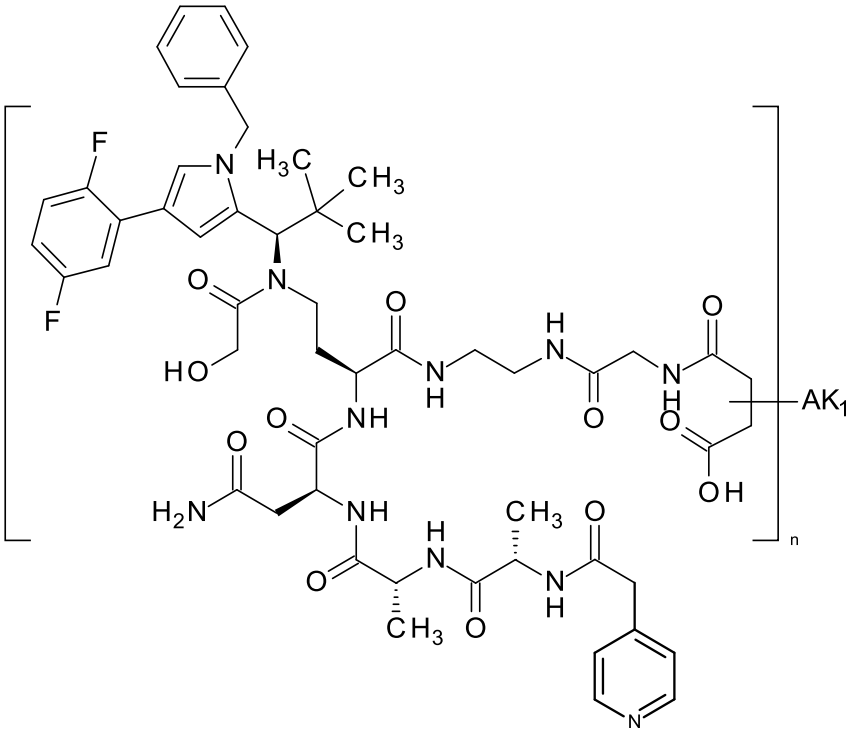
n	is a number from 1 to 50, preferably 1 to 20, more preferably 2 to 8 and especially 2 to 6:



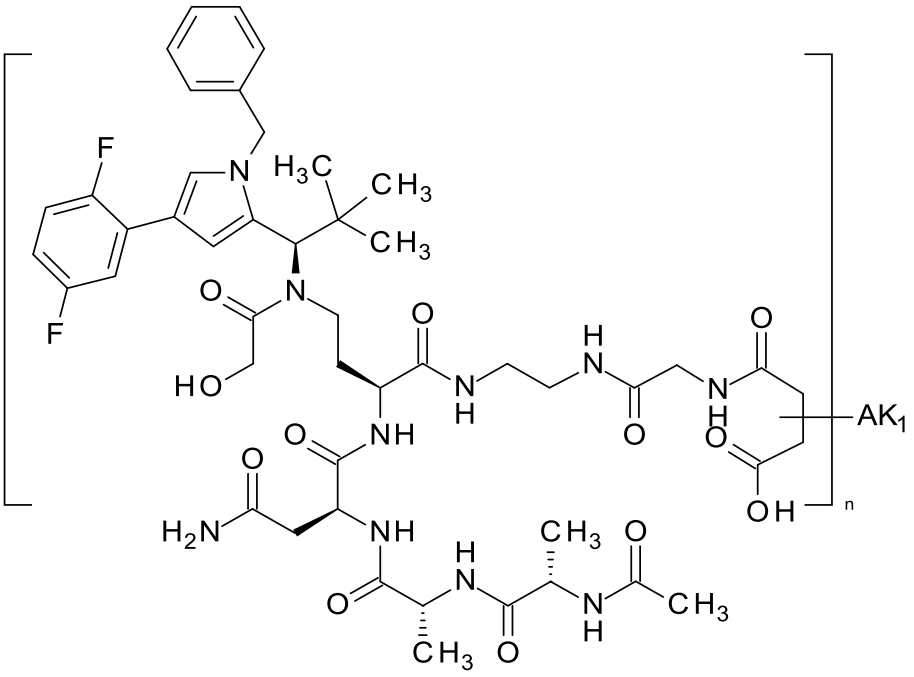
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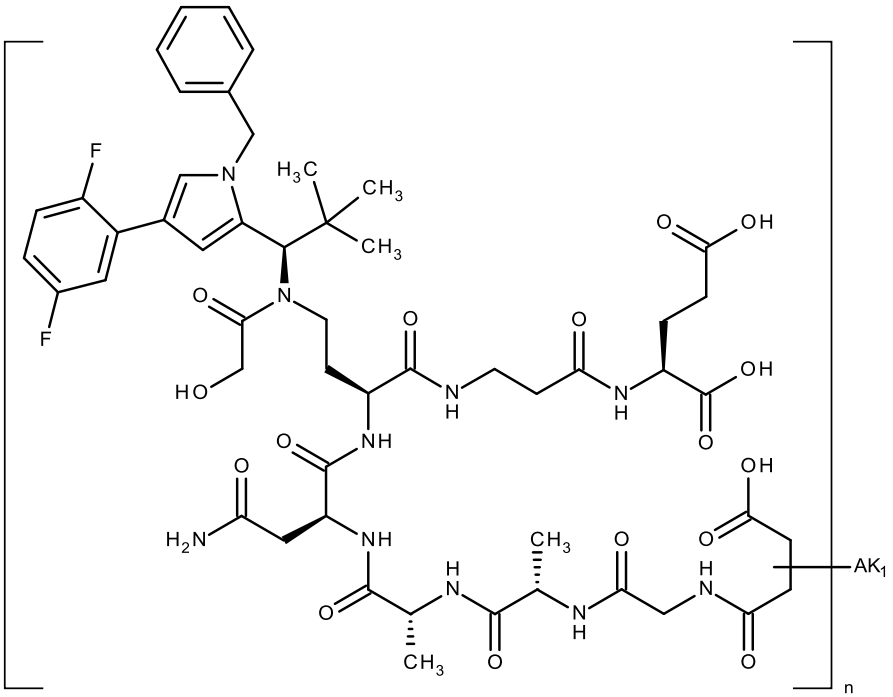
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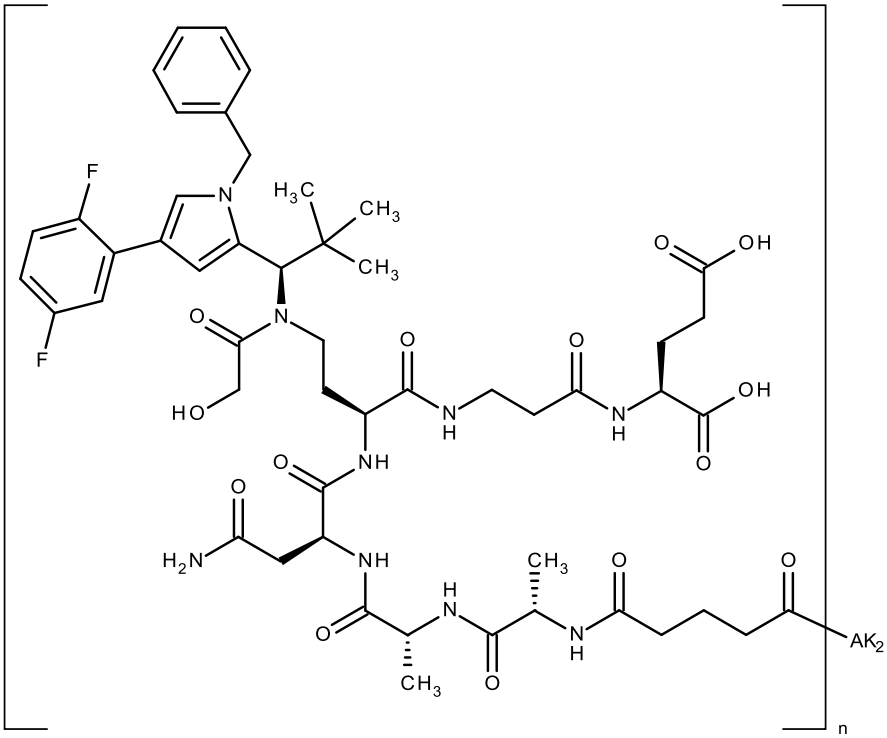
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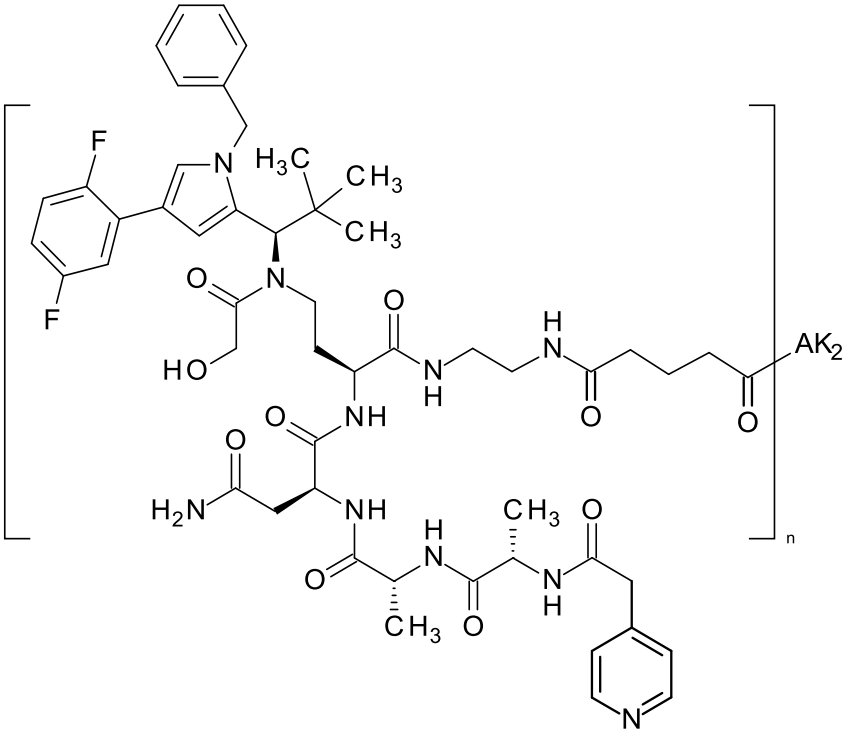
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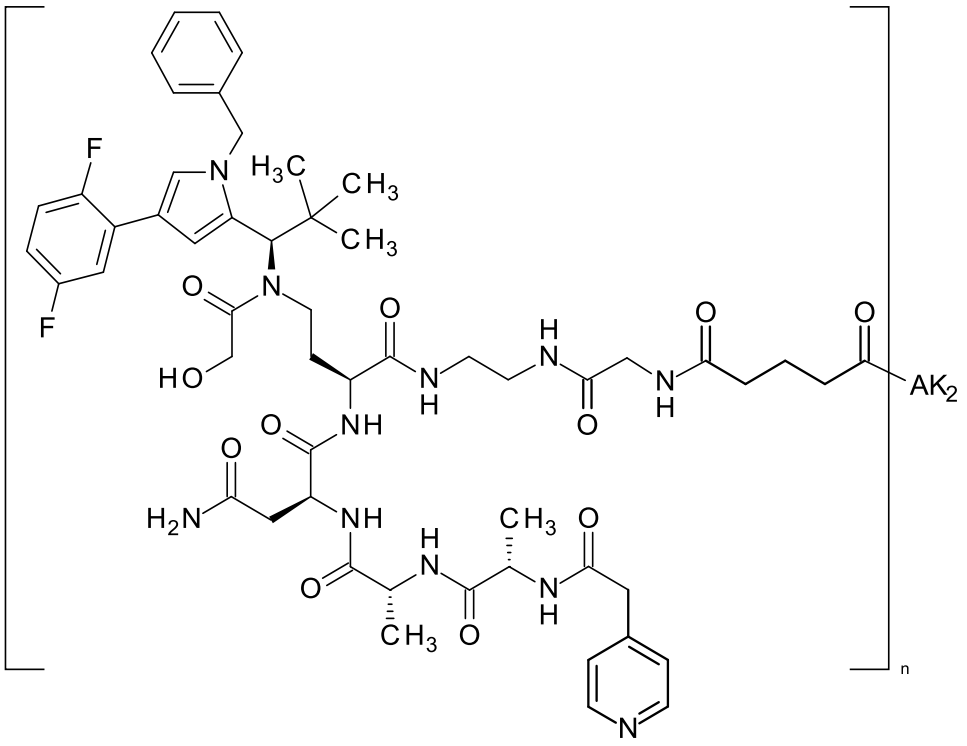
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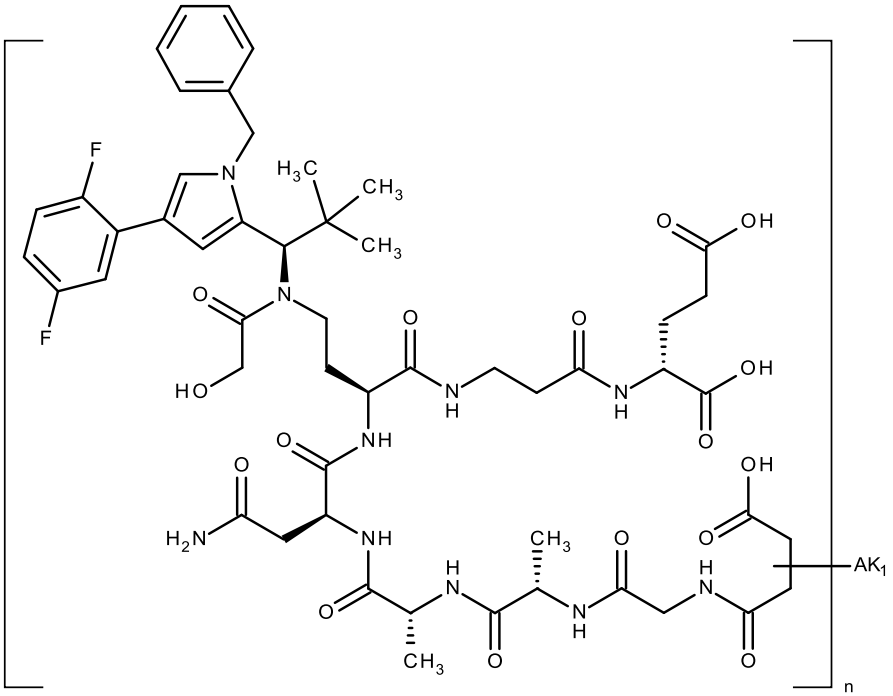
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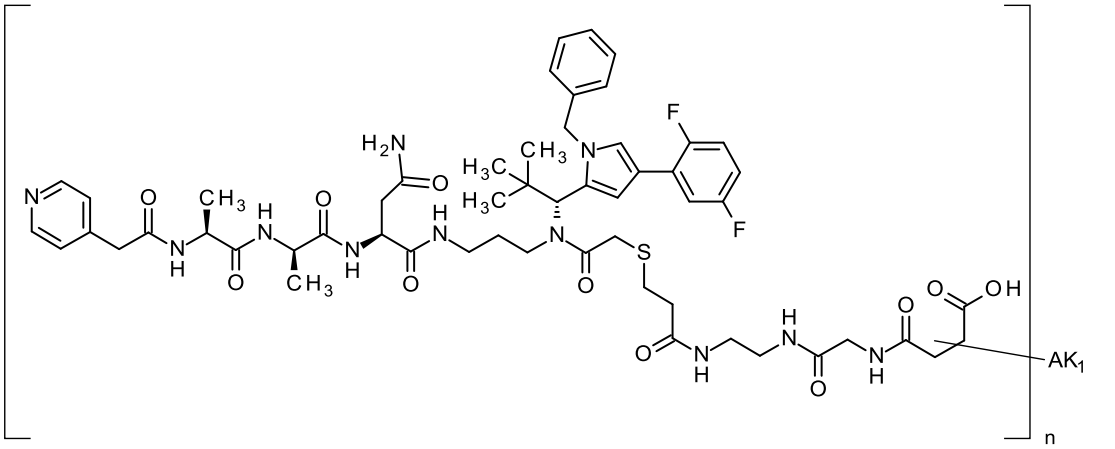
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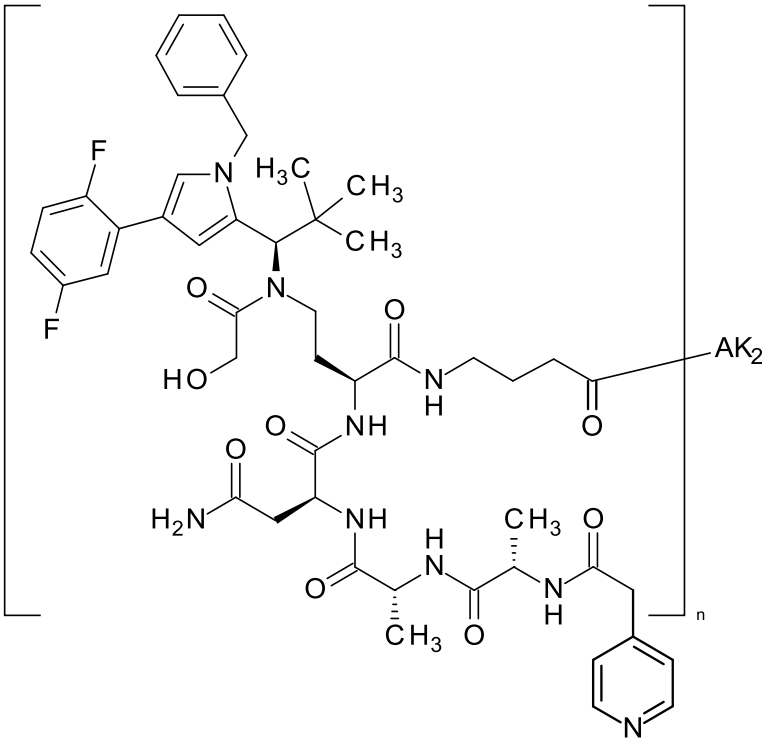
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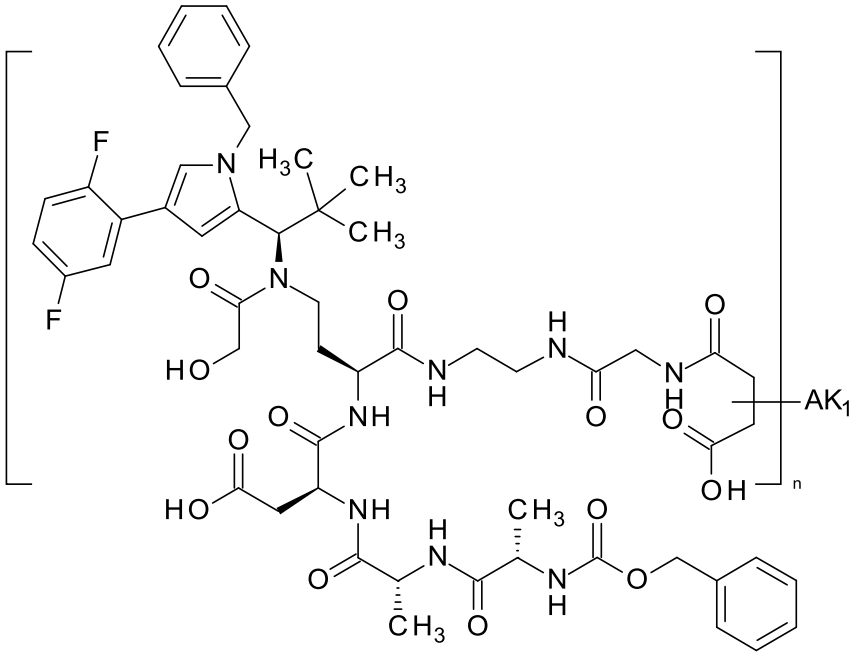
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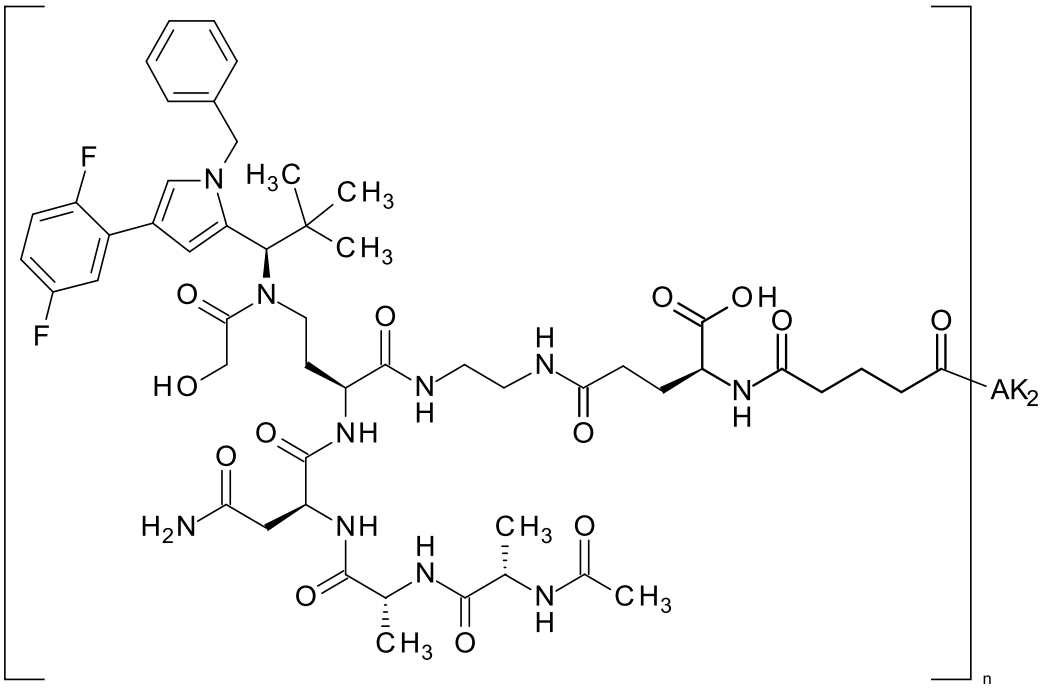
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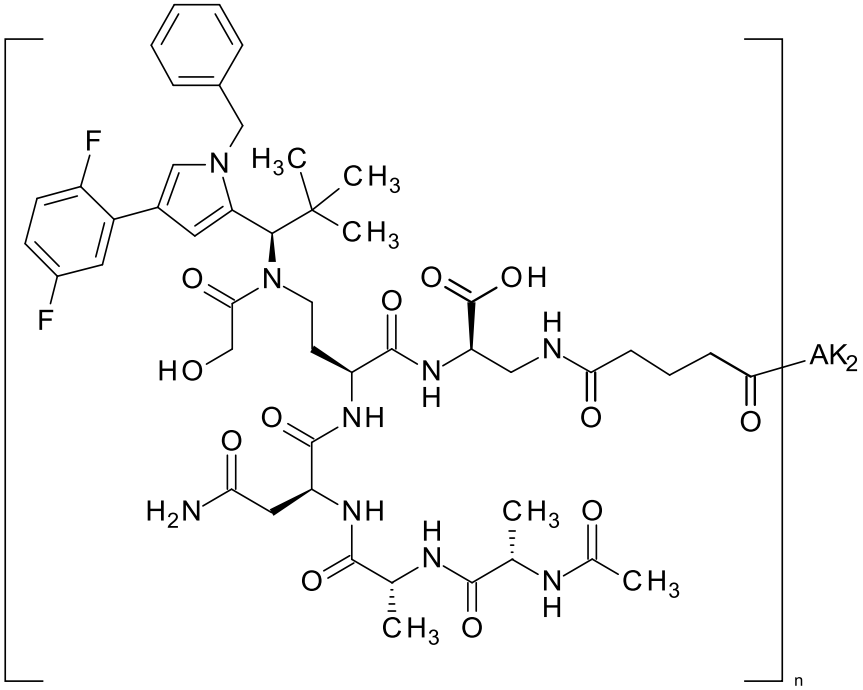
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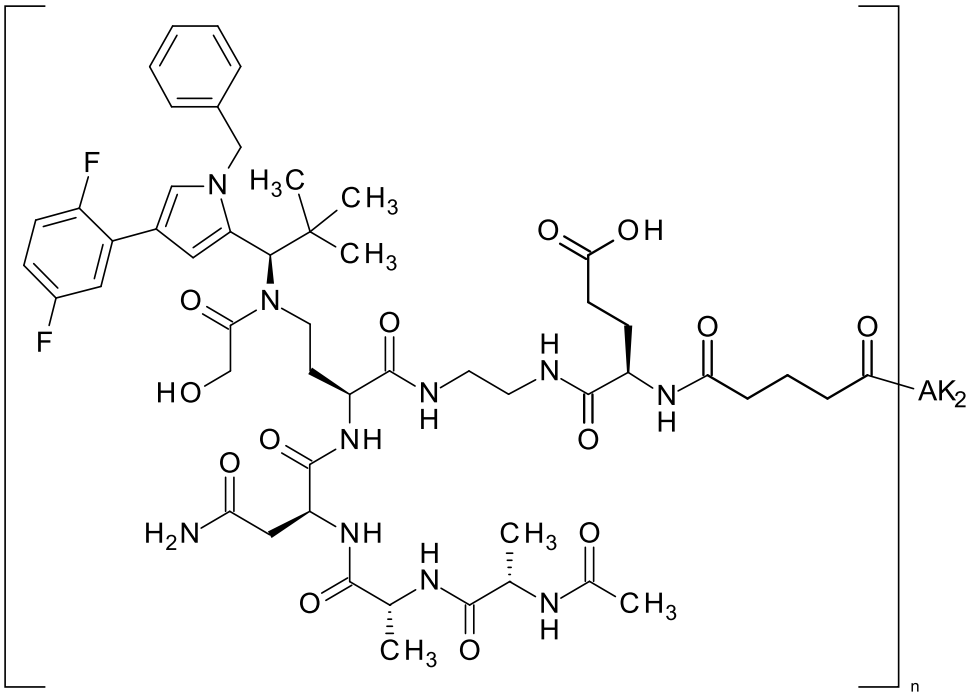
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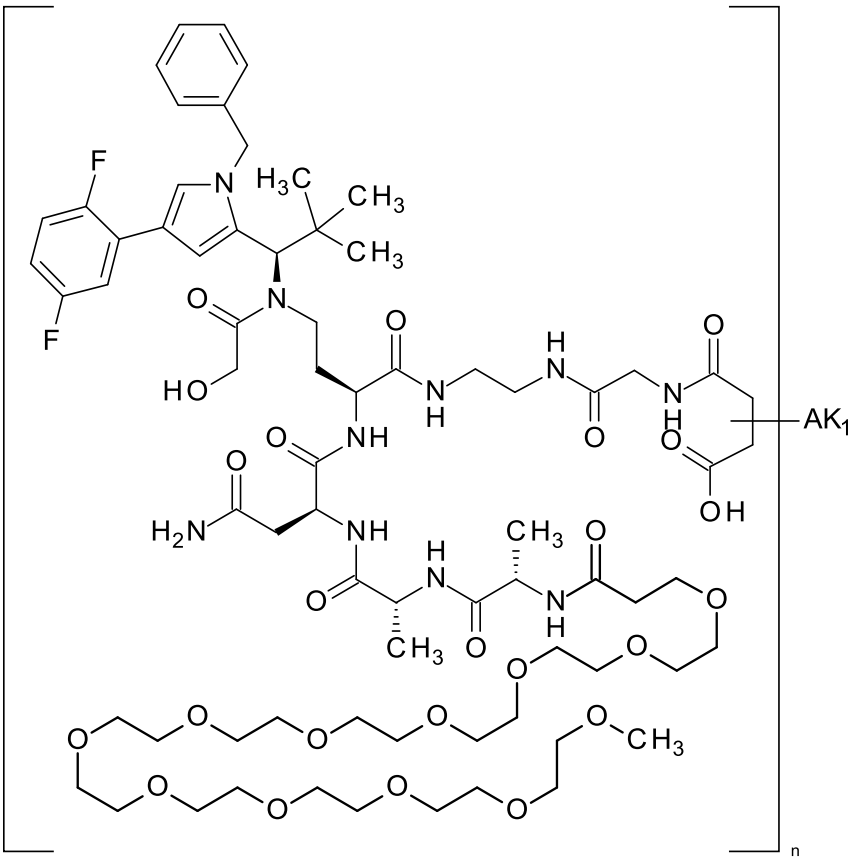
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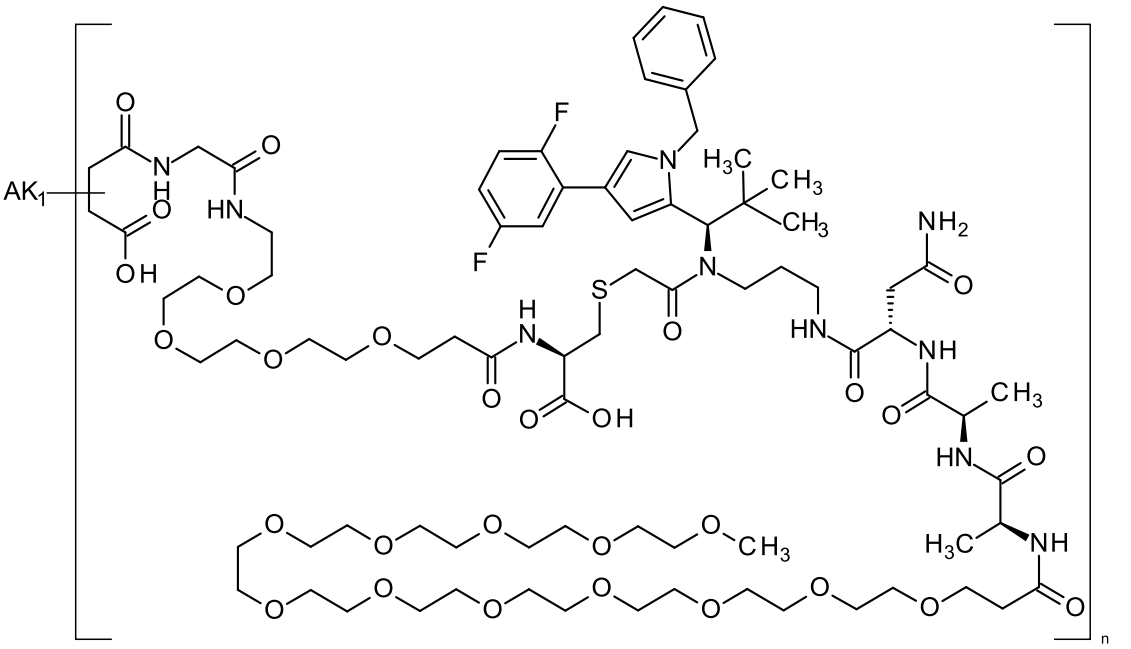
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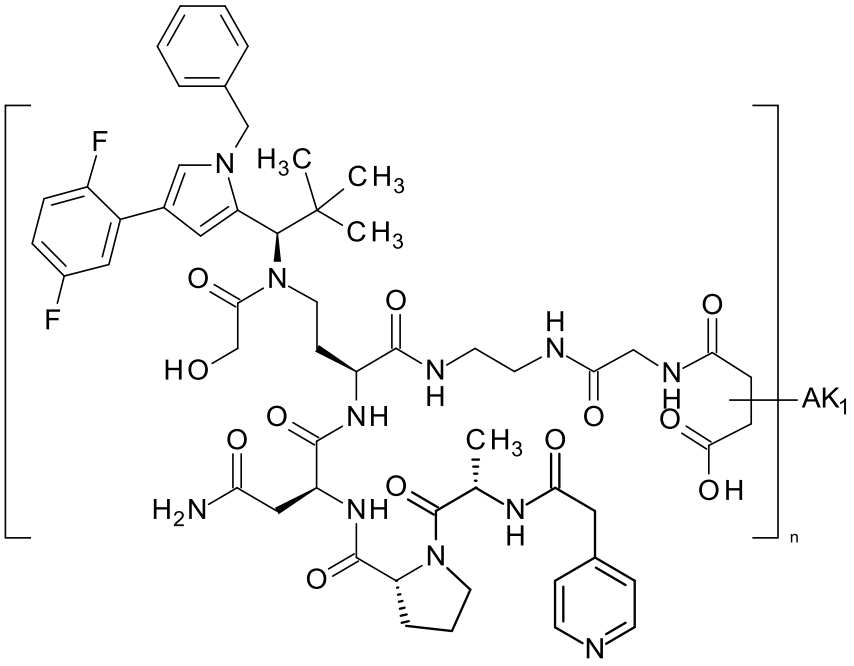
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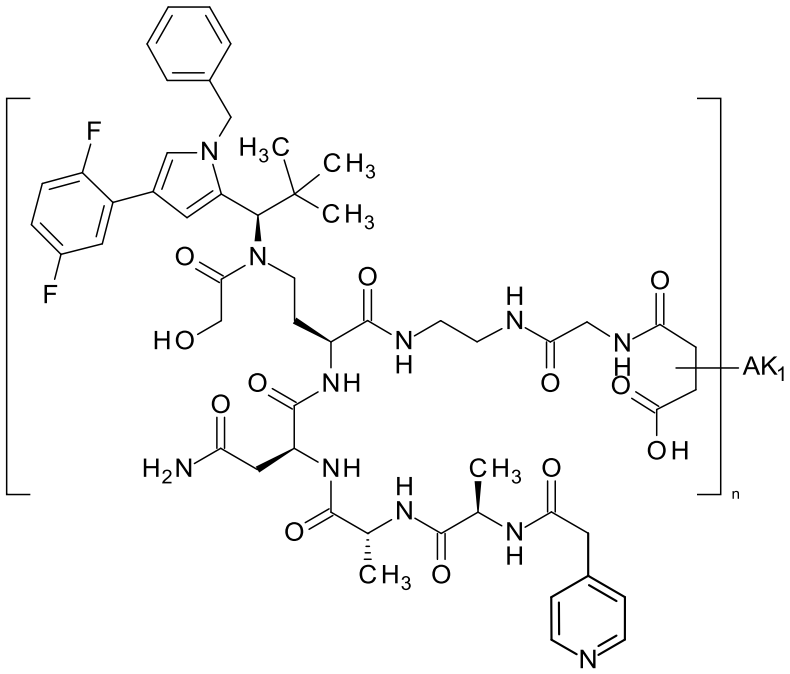
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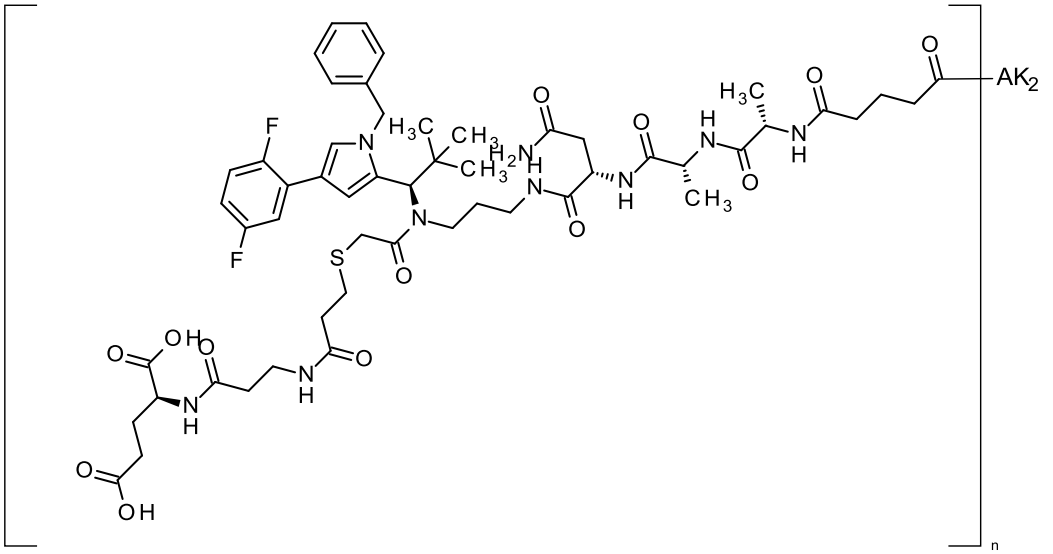
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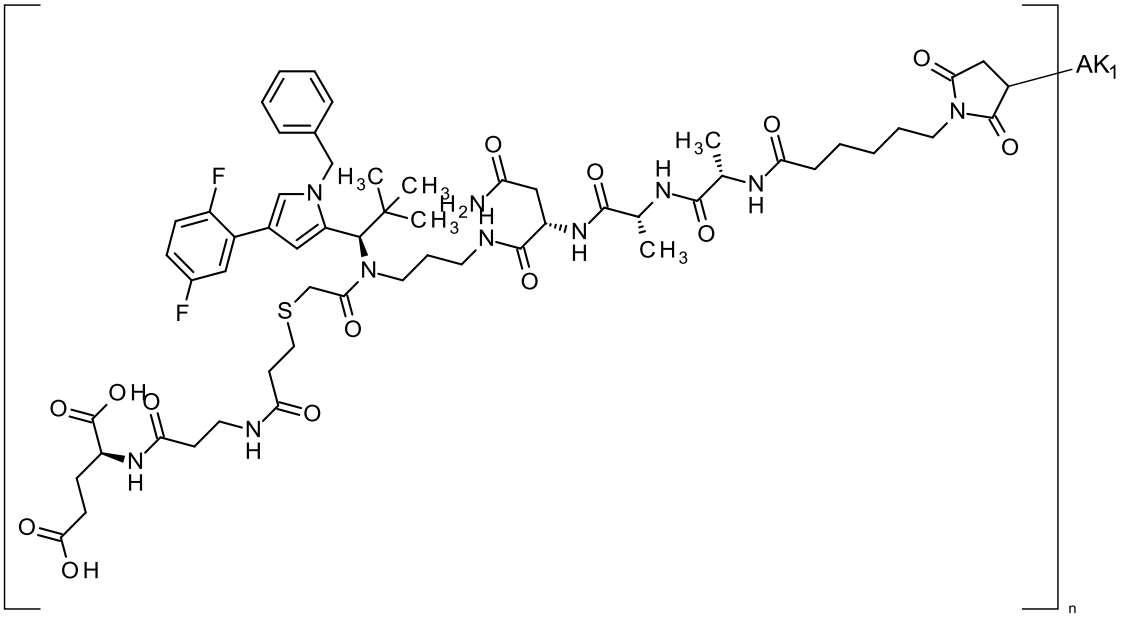
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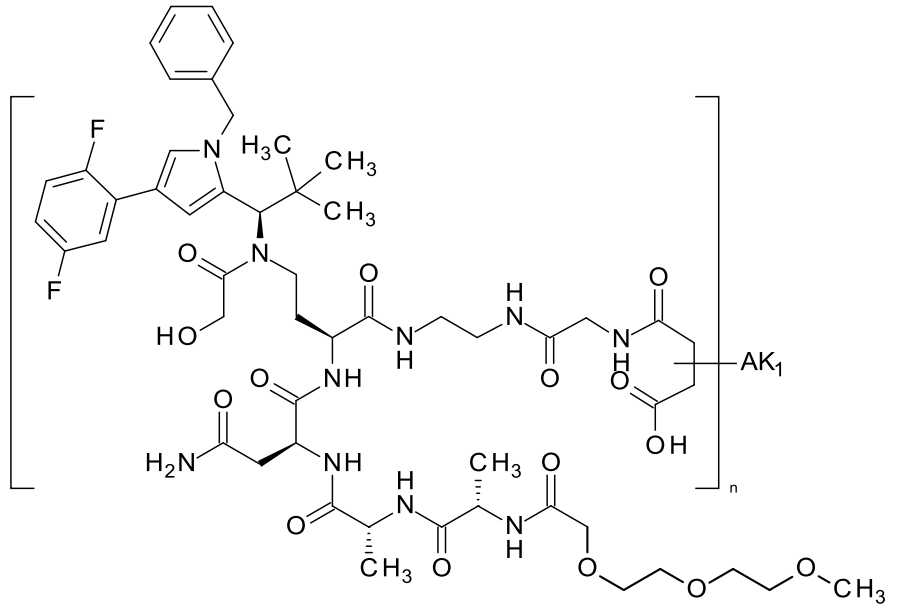
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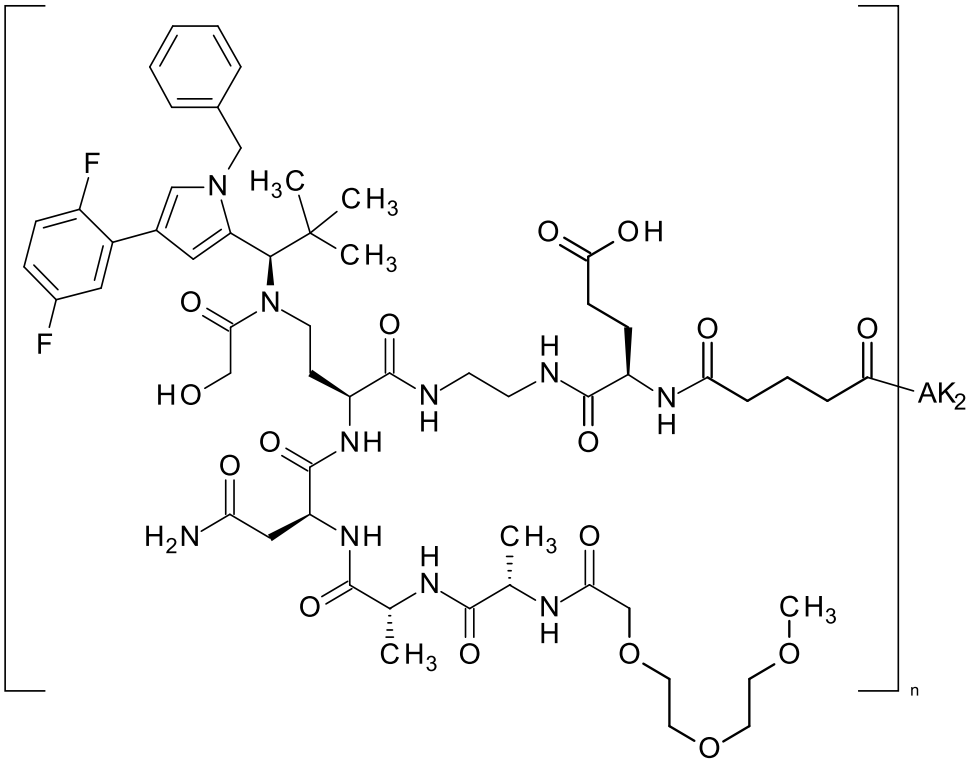
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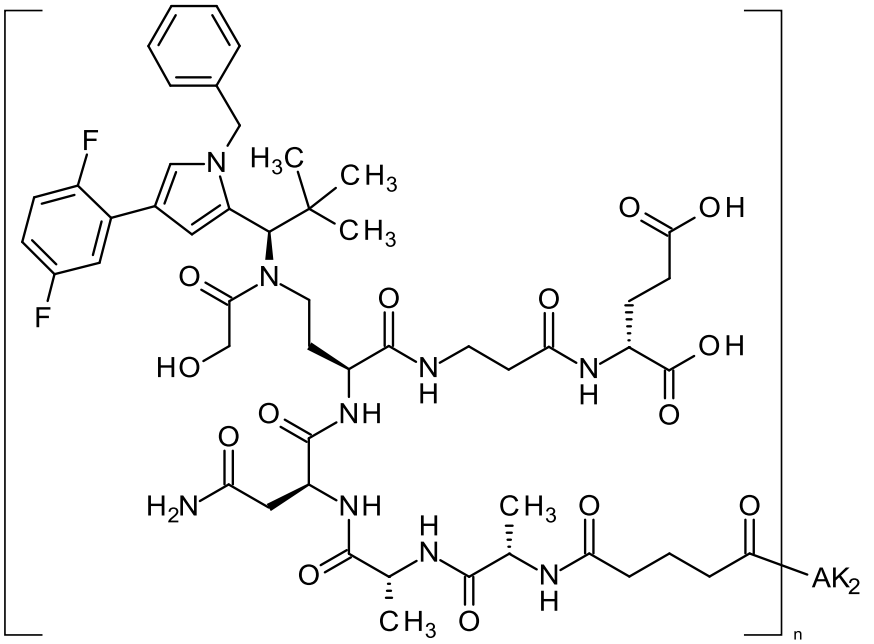
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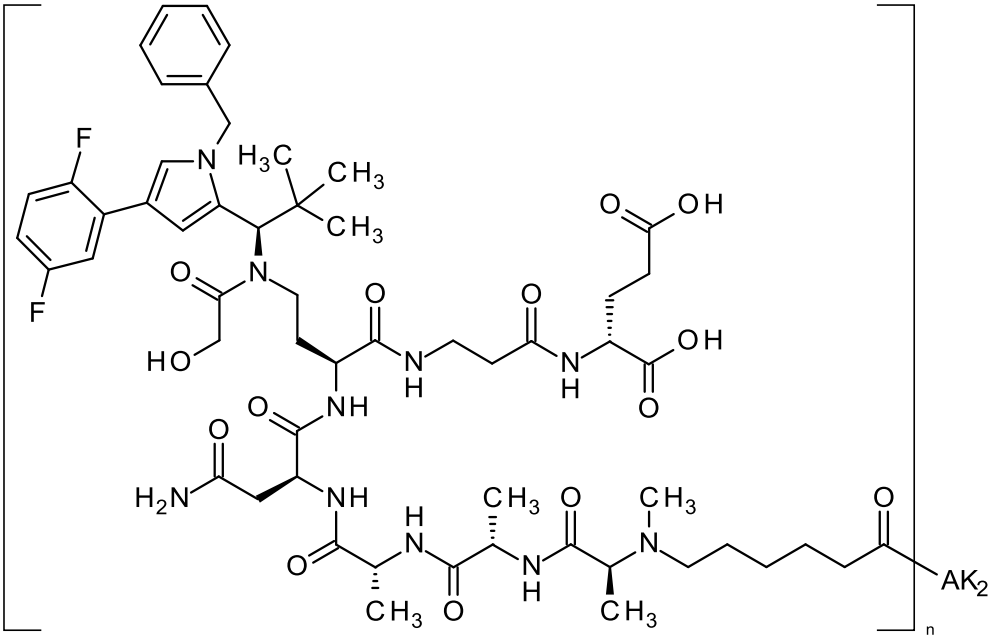
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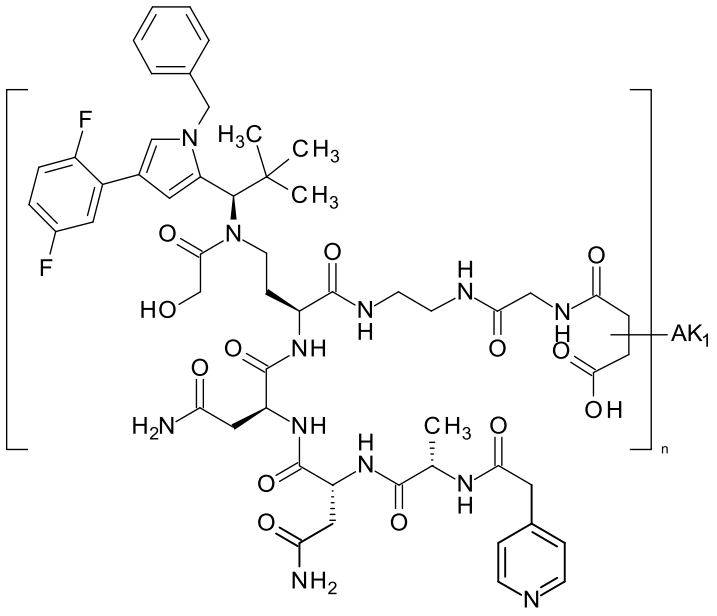
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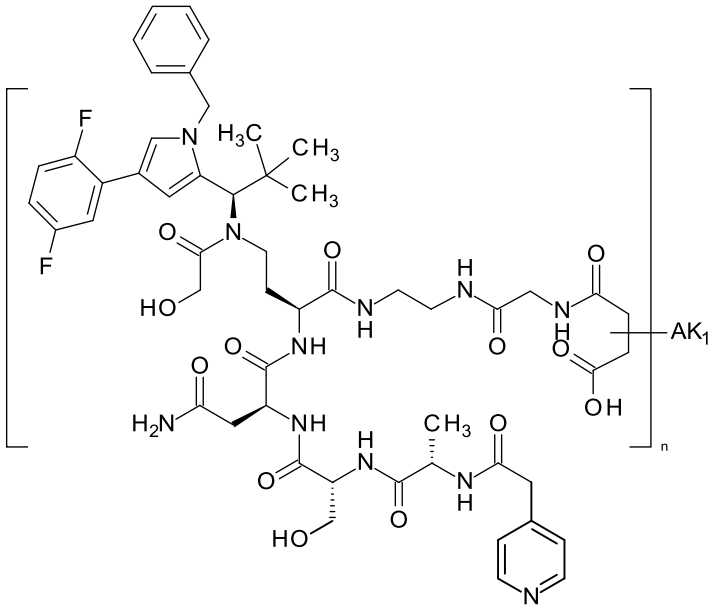
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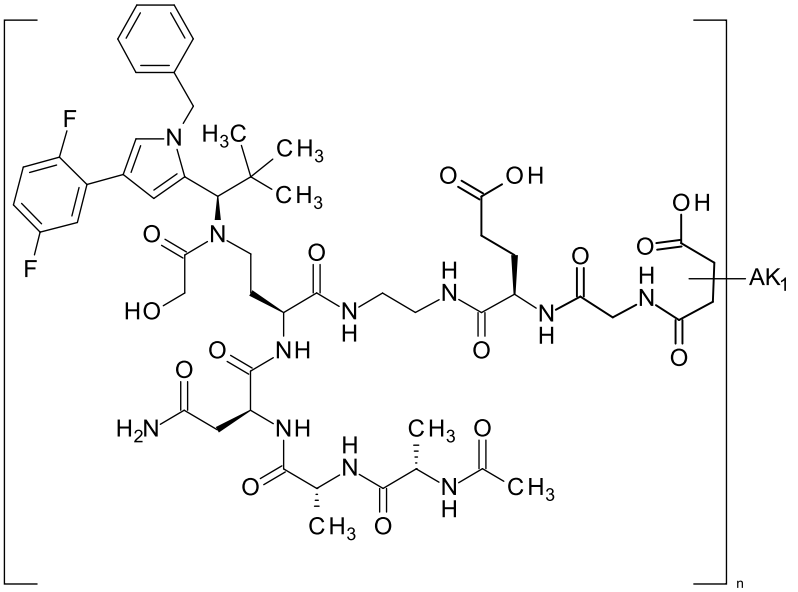
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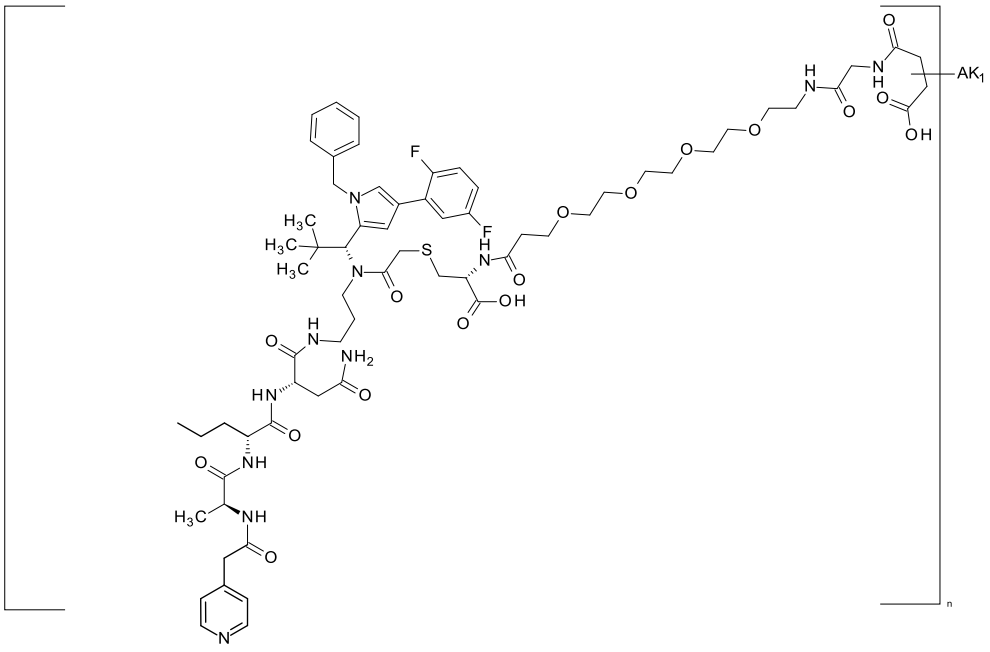
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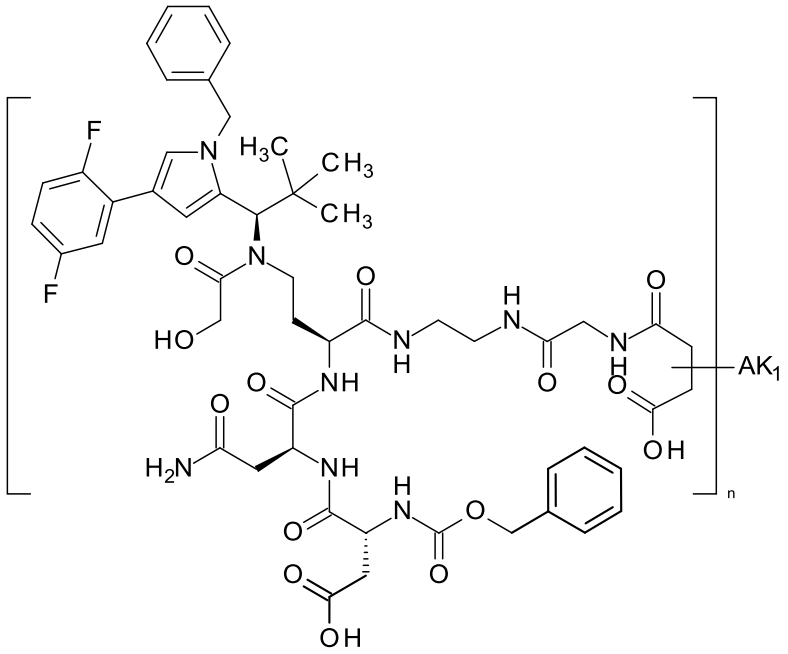
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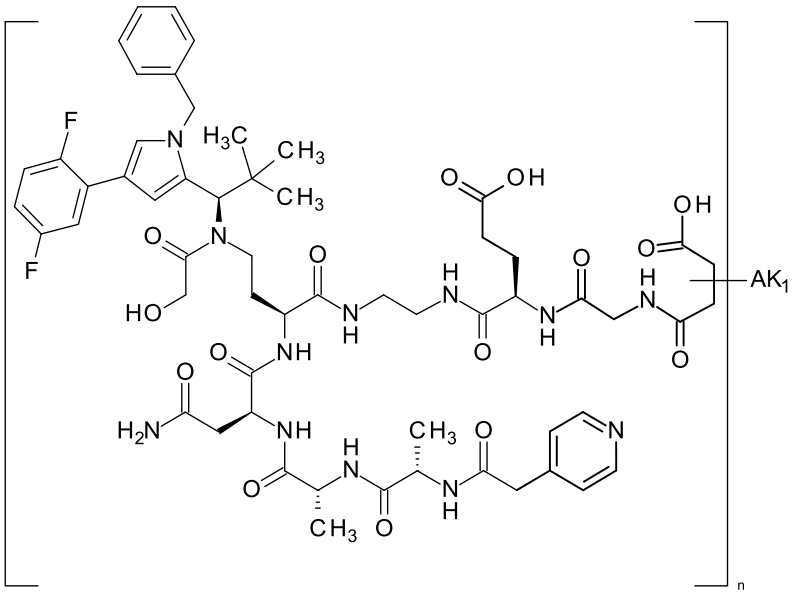
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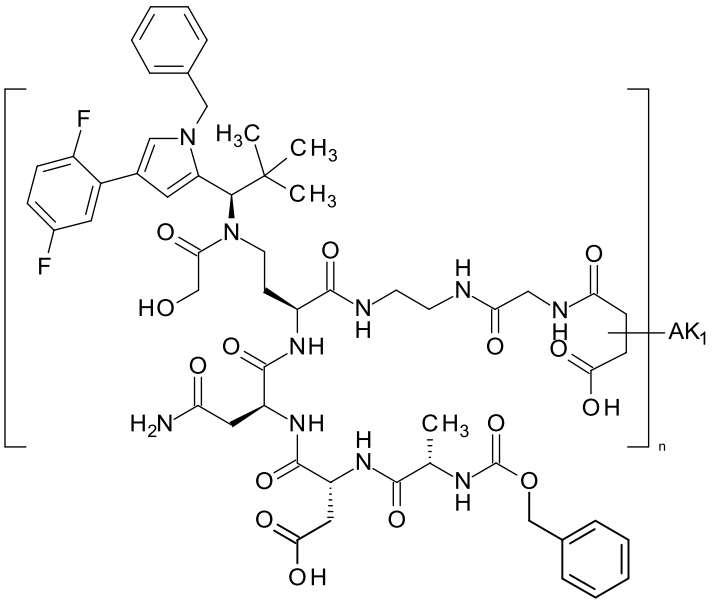
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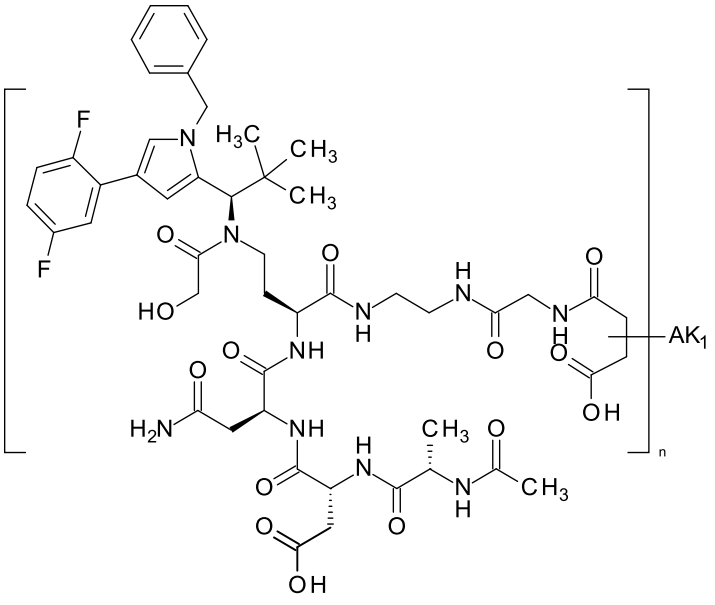
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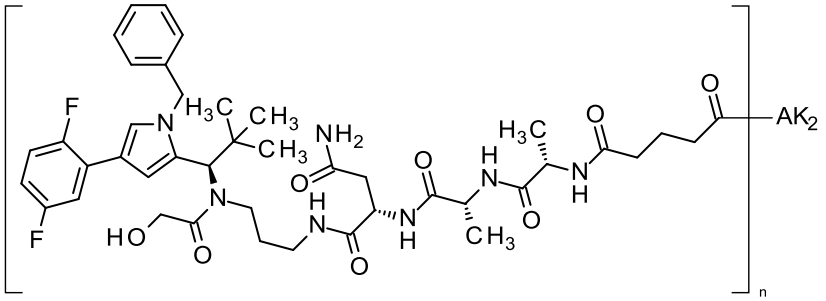
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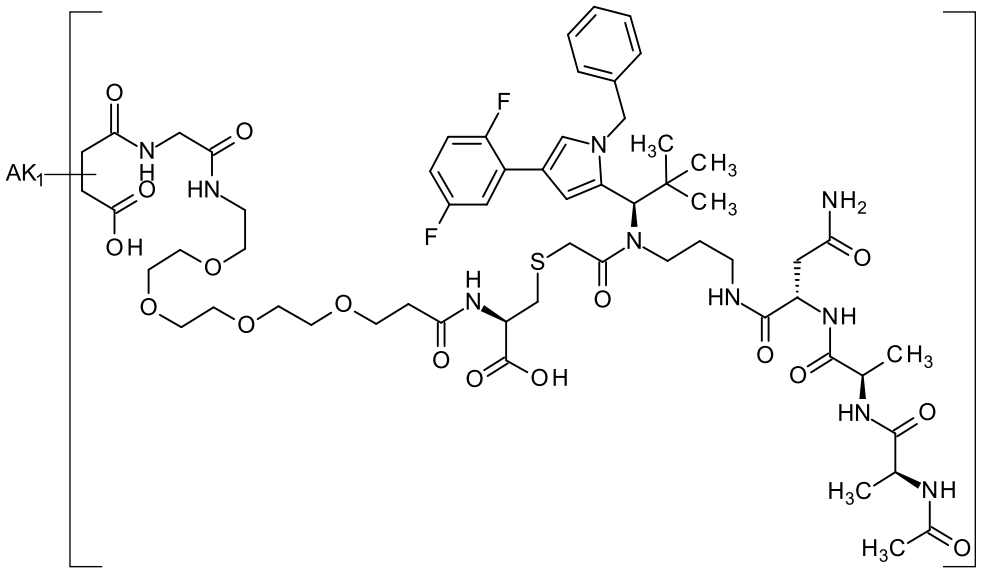
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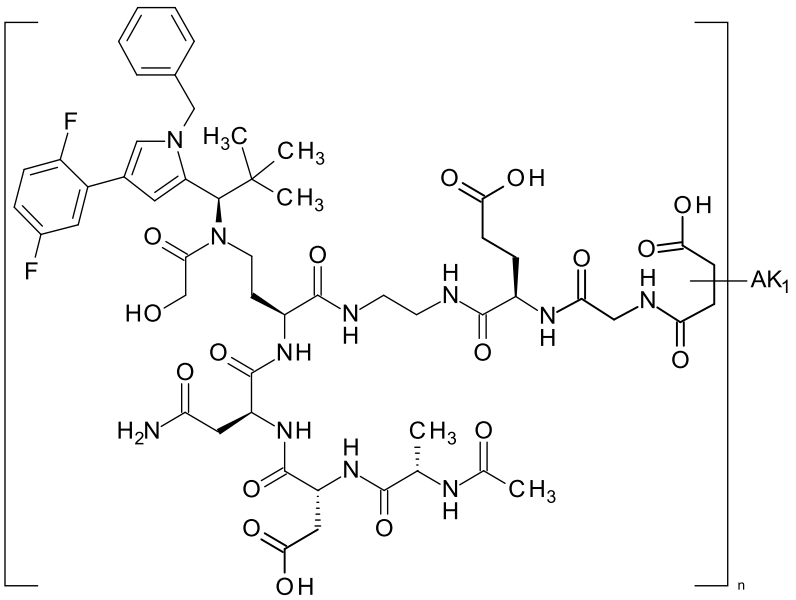
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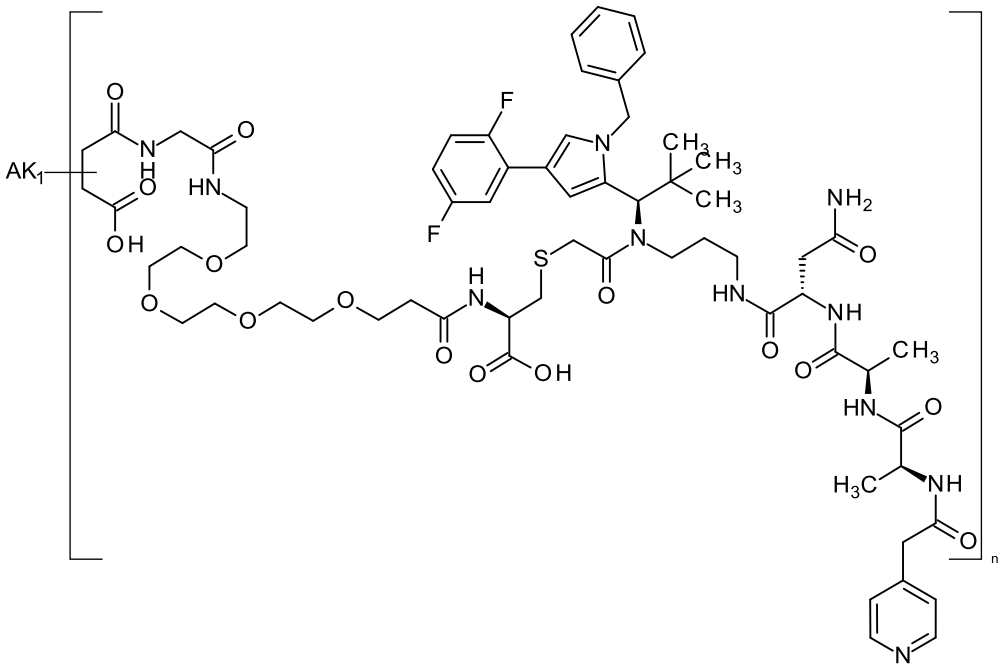
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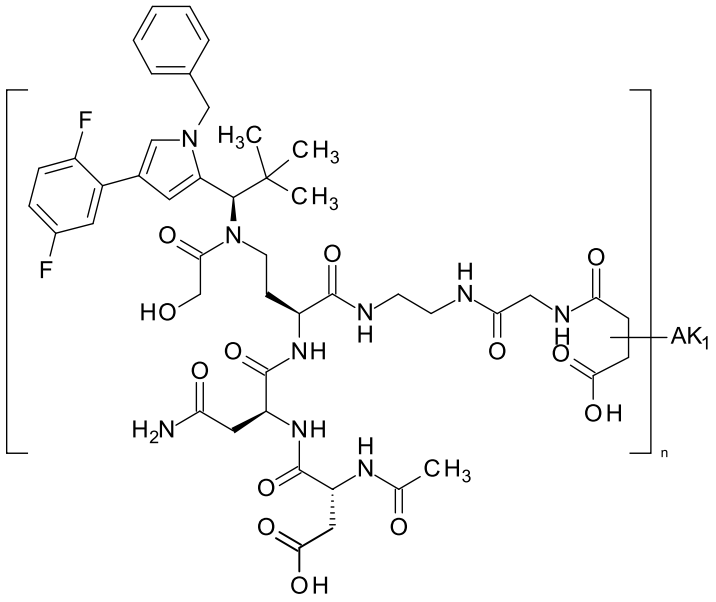
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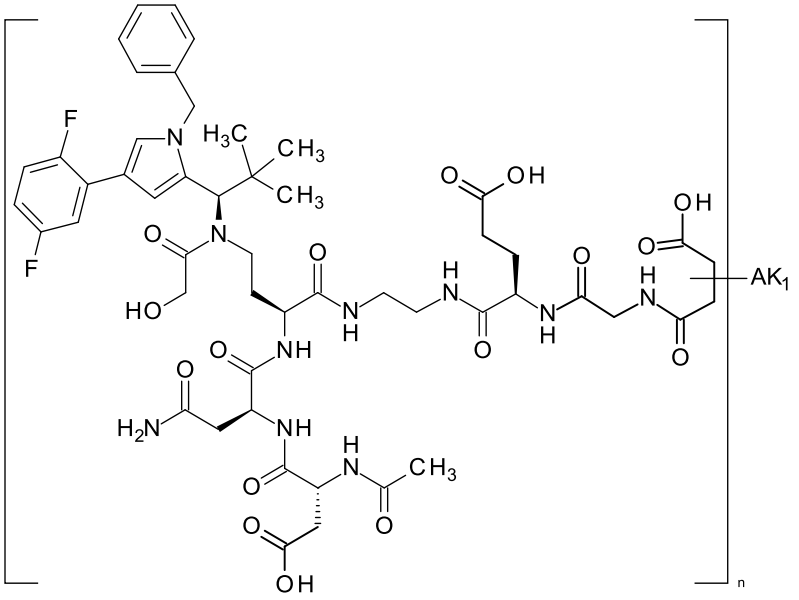
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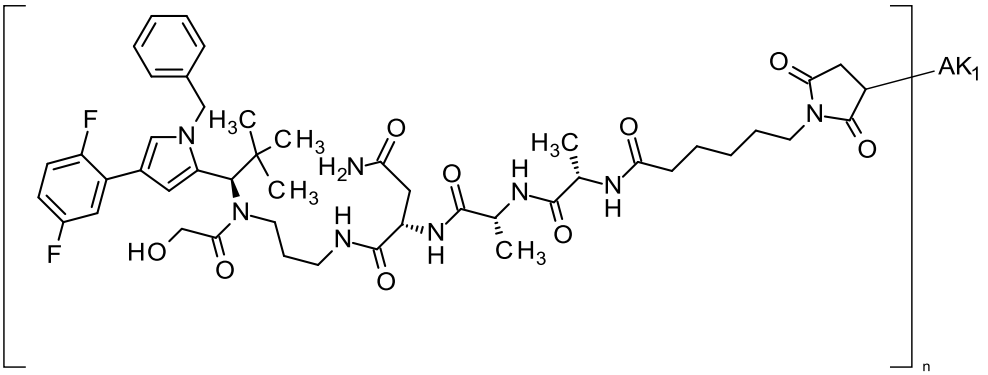
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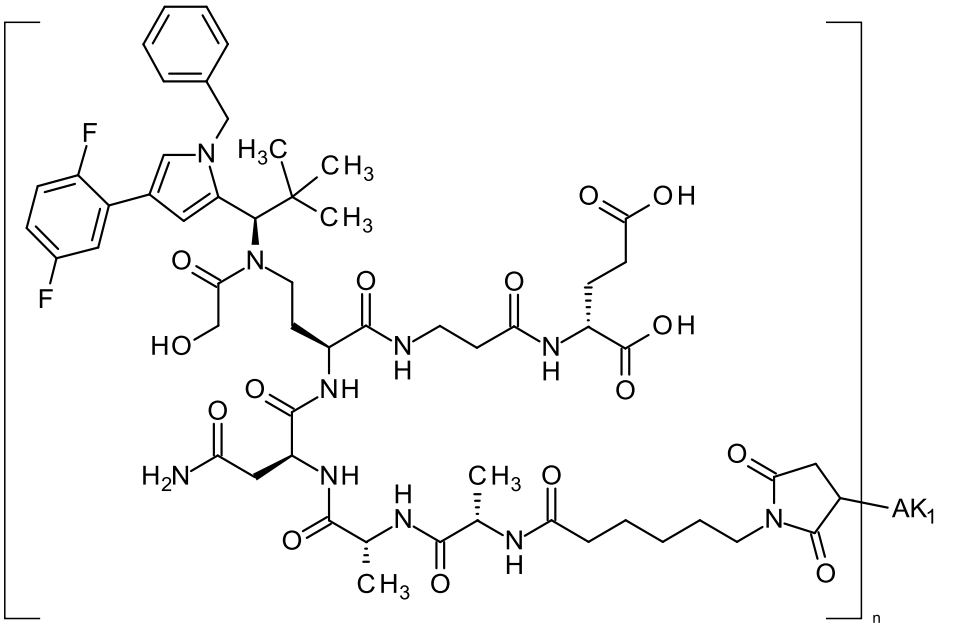
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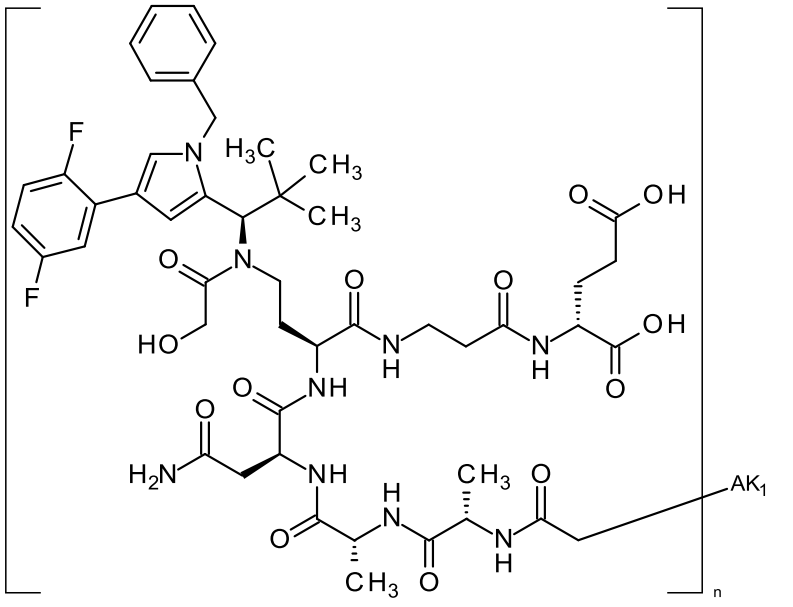
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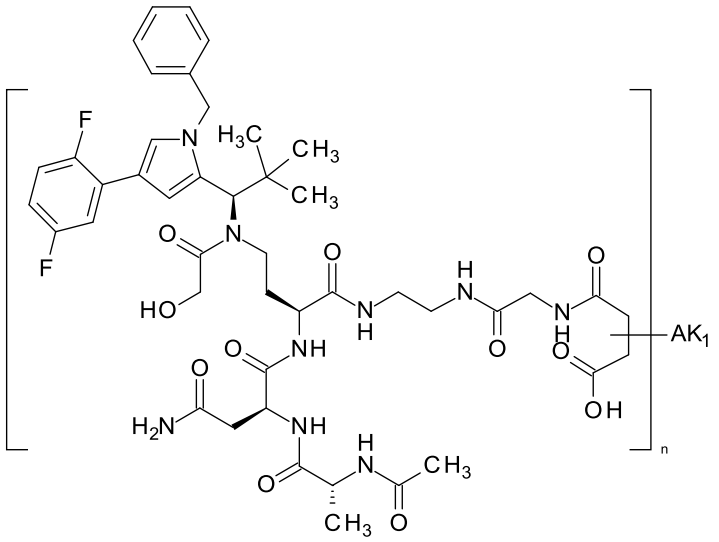
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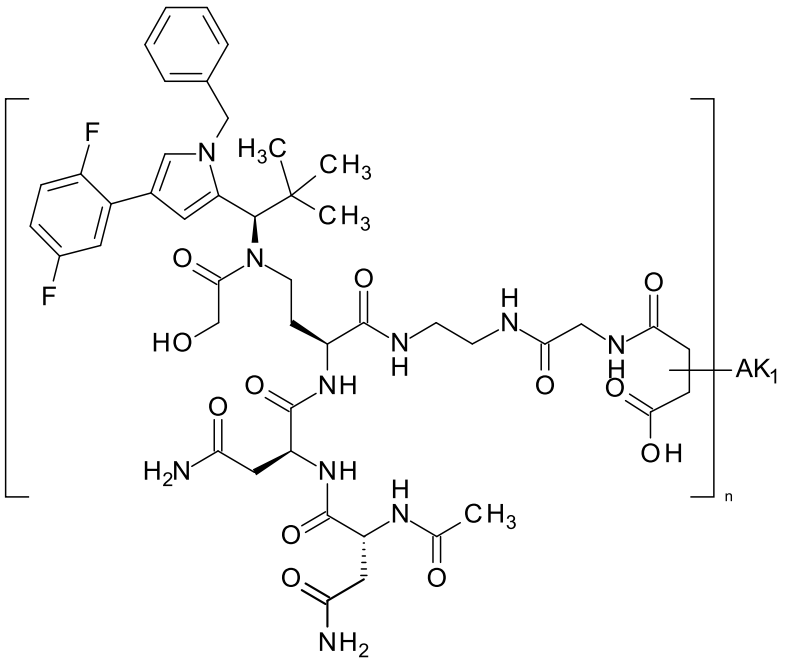
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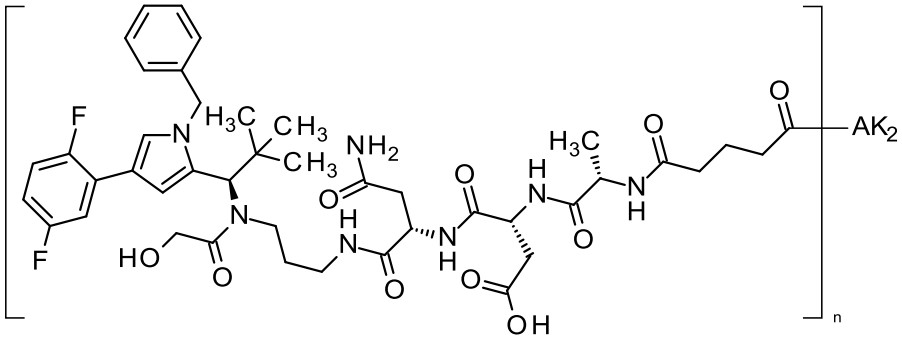


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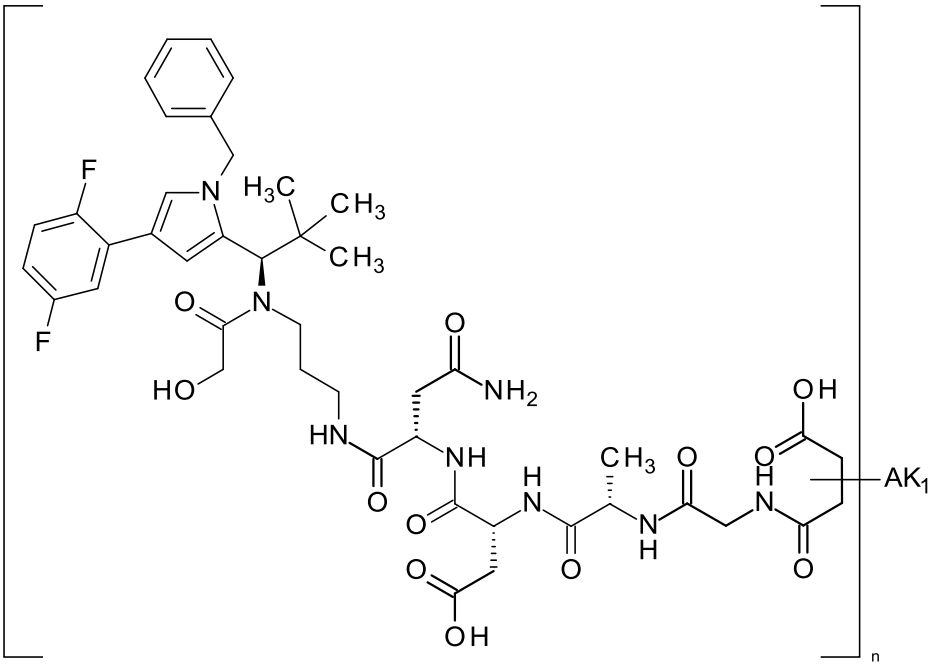


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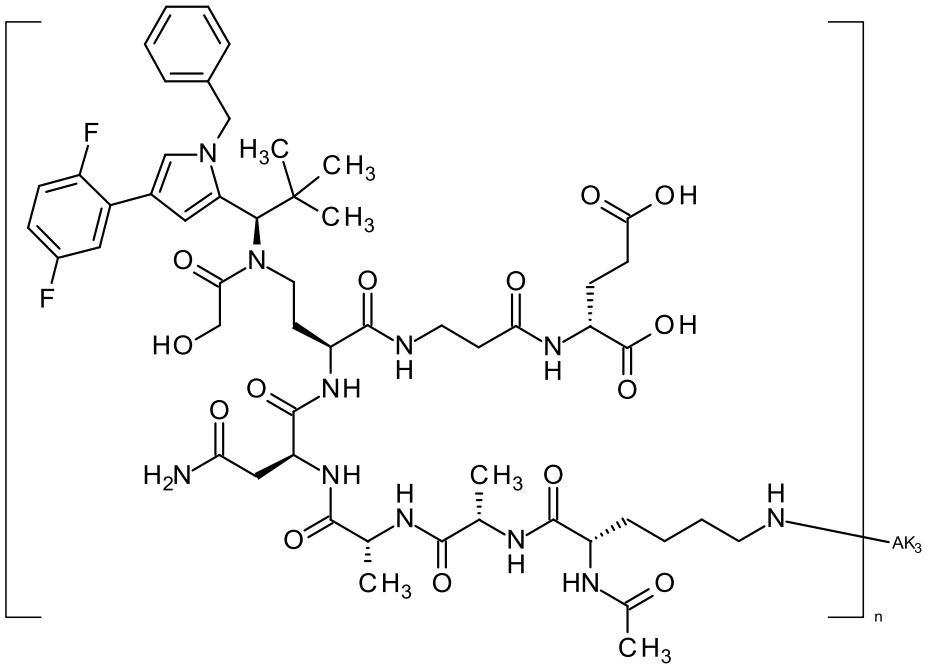
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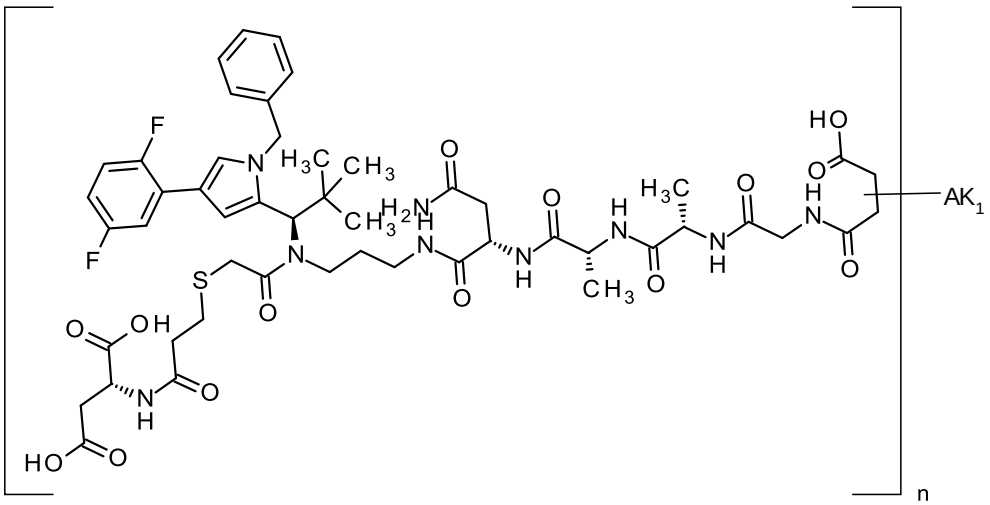
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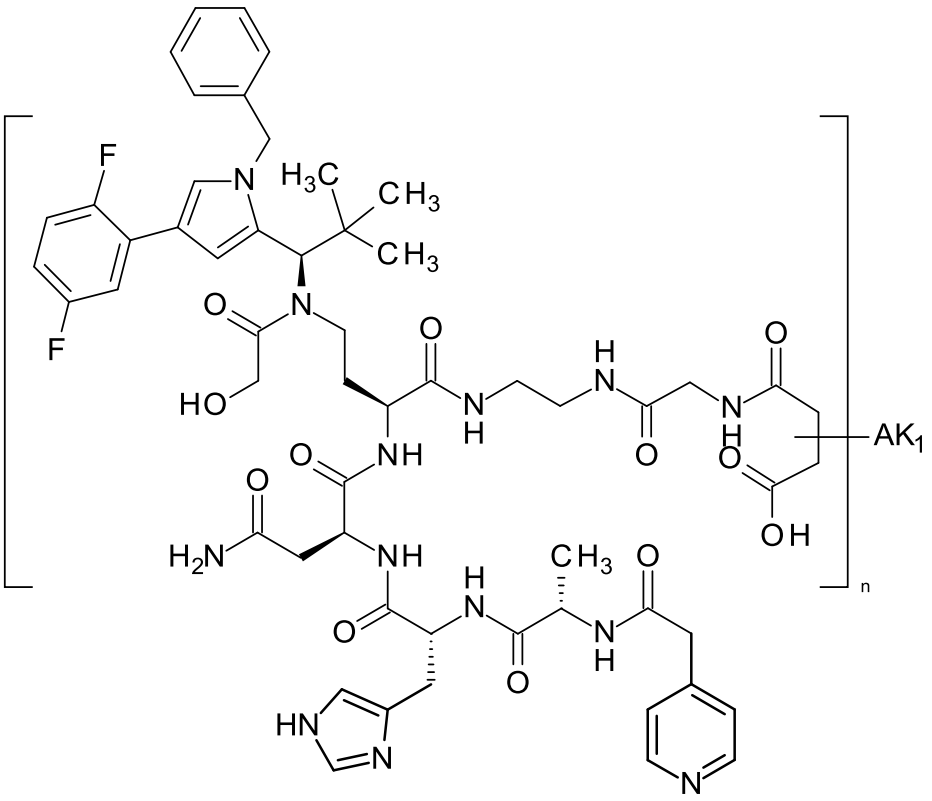
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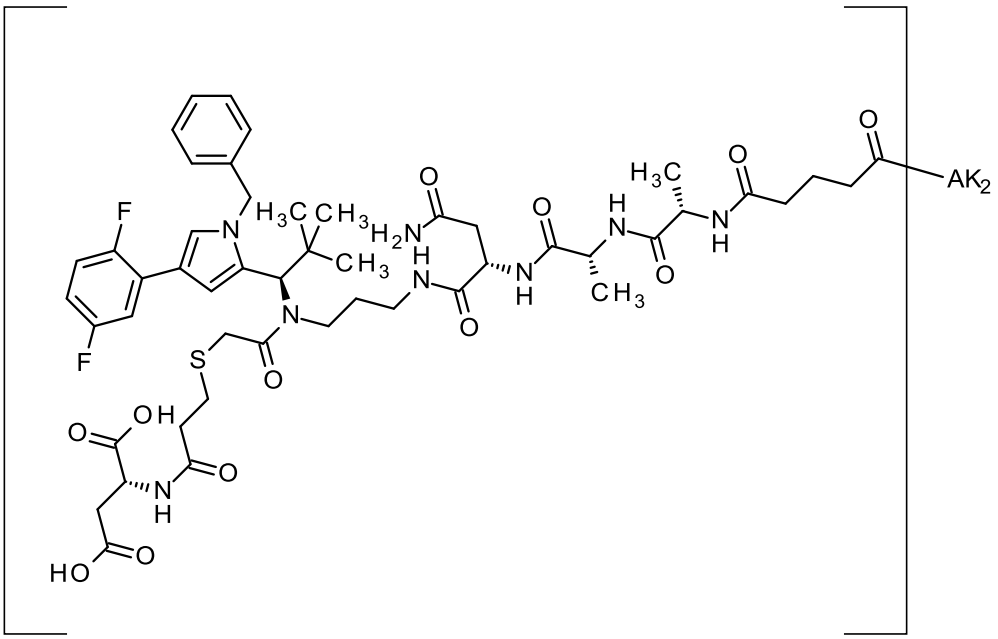
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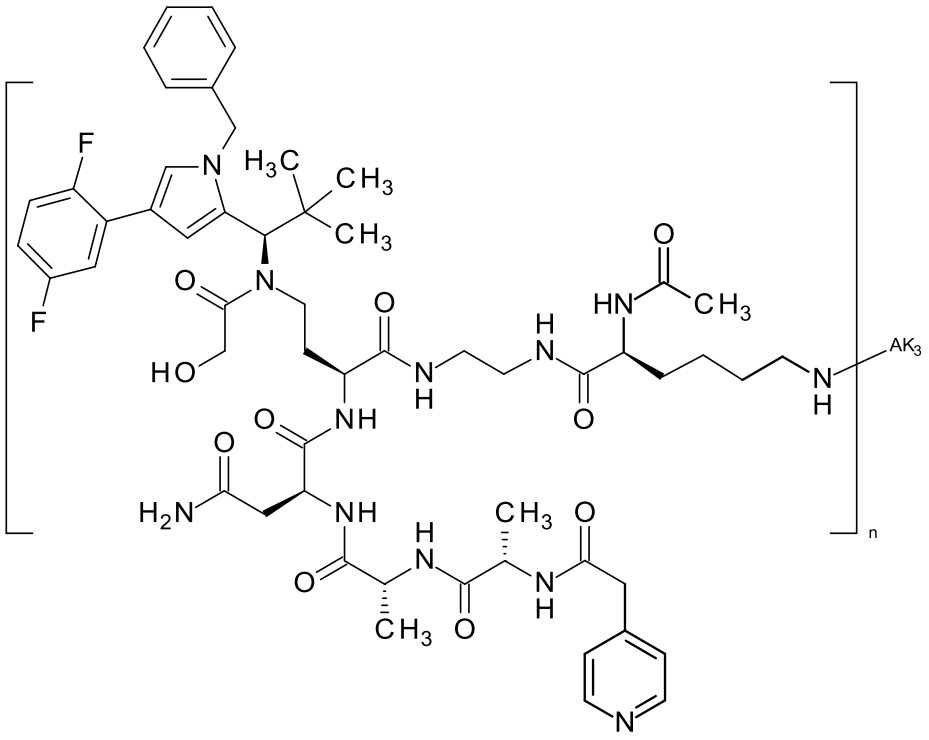
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,



and



35. Compound or conjugate according to one or more of the preceding claims, wherein the antibody or the antigen-binding antibody fragment binds to an extracellular cancer target molecule.

36. Compound or conjugate according to one or more of the preceding claims, wherein the antibody or the antigen-binding antibody fragment, after binding to its extracellular target molecule on the target cell, is internalized by the target cell through the binding.

37. Compound or conjugate according to one or more of the preceding claims, wherein the antibody is an anti-HER2 antibody, an anti-EGFR antibody, an anti-B7H3 antibody, an anti-TWEAKR antibody, or an antigen-binding antibody fragment of these.

38. Compound or conjugate according to Claim 37, wherein the anti-TWEAKR antibody is selected from the group consisting of TPP-7006, TPP-7007, TPP-

10336 and TPP-10337, the anti-B7H3 antibody is selected from the group consisting of TPP-8382 and TPP-8567, the anti-EGFR antibody is cetuximab (TPP-981) and the anti-HER2 antibody is selected from the group consisting of trastuzumab and TPP-1015.

39. Compound or conjugate according to one or more of the preceding claims, wherein the antibody (AK, AB, AK1, AK2, AK3)

(i) is an anti-EGFR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 2, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 3 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 4, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 6, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 7 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 8,

(ii) is an anti-HER2 antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 12, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 13 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 14, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 16, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 17 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 18,

(iii) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 22, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 23 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 24, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 26, the variable CDR2 sequence of the light

chain (L-CDR2), as shown by SEQ ID NO: 27 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 28,

(iv) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 32, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 33 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 34, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 36, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 37 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 38,

(v) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 42, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 43 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 44, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 46, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 47 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 48,

(vi) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 52, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 53 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 54, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 56, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 57 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 58,

(vii) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 62, the variable CDR2 sequence of the heavy chain (H-

CDR2), as shown by SEQ ID NO: 63 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 64, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 66, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 67 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 68,

(viii) is an anti-HER2 antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 72, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 73 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 74, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 76, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 77 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 78,

(ix) is an anti-HER2 antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 82, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 83 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 84, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 86, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 87 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 88,

(x) is an anti-B7H3 antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 92, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 93 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 94, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 96, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 97 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 98,

(xi) is an anti-B7H3 antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 102, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 103 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 104, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 106, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 107 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 108,

(xii) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 112, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 113 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 114, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 116, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 117 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 118,

(xiii) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 122, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 123 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 124, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 126, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 127 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 128,

(xiv) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 132, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 133 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 134, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-

CDR1), as shown by SEQ ID NO: 136, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 137 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 138,

(xv) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 142, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 143 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 144, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 146, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 147 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 148, or

(xvi) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) comprising the variable CDR1 sequence of the heavy chain (H-CDR1), as shown by SEQ ID NO: 152, the variable CDR2 sequence of the heavy chain (H-CDR2), as shown by SEQ ID NO: 153 and the variable CDR3 sequence of the heavy chain (H-CDR3), as shown by SEQ ID NO: 154, and a variable region of the light chain (VL) comprising the variable CDR1 sequence of the light chain (L-CDR1), as shown by SEQ ID NO: 156, the variable CDR2 sequence of the light chain (L-CDR2), as shown by SEQ ID NO: 157 and the variable CDR3 sequence of the light chain (L-CDR3), as shown by SEQ ID NO: 158,

or is an antigen-binding fragment of these antibodies.

40. Compound or conjugate according to one or more of the preceding claims, wherein the antibody (AK, AB, AK1, AK2, AK3)

(i) is an anti-EGFR antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 1 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 5,

(ii) is an anti-HER2 antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 11 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 15,

(iii) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 21 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 25,

(iv) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 31 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 35,

(v) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 41 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 45,

(vi) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 51 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 55,

(vii) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 61 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 65,

(viii) is an anti-HER2 antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 71 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 75,

(ix) is an anti-HER2 antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 81 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 85,

(x) is an anti-B7H3 antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 91 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 95,

(xi) is an anti-B7H3 antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 101 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 105,

(xii) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 111 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 115,

(xiii) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 121 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 125,

(xiv) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 131 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 135,

(xv) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 141 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 145, or

(xvi) is an anti-TWEAKR antibody comprising a variable region of the heavy chain (VH) corresponding to SEQ ID NO: 151 and a variable region of the light chain (VL) corresponding to SEQ ID NO: 155,

or is an antigen-binding fragment of these antibodies.

41. Compound or conjugate according to one or more of the preceding claims, wherein the antibody (AK, AB, AK1, AK2, AK3)

(i) is an anti-EGFR antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 9 and a region of the light chain corresponding to SEQ ID NO: 10,

(ii) is an anti-HER2 antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 19 and a region of the light chain corresponding to SEQ ID NO: 20,

(iii) is an anti-TWEAKR antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 29 and a region of the light chain corresponding to SEQ ID NO: 30,

(iv) is an anti-TWEAKR antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 39 and a region of the light chain corresponding to SEQ ID NO: 40,

(v) is an anti-TWEAKR antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 49 and a region of the light chain corresponding to SEQ ID NO: 50,

(vi) is an anti-TWEAKR antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 59 and a region of the light chain corresponding to SEQ ID NO: 60,

(vii) is an anti-TWEAKR antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 69 and a region of the light chain corresponding to SEQ ID NO: 70,

(viii) is an anti-HER2 antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 79 and a region of the light chain corresponding to SEQ ID NO: 80,

(ix) is an anti-HER2 antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 89 and a region of the light chain corresponding to SEQ ID NO: 90,

(x) is an anti-B7H3 antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 99 and a region of the light chain corresponding to SEQ ID NO: 100,

(xi) is an anti-B7H3 antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 109 and a region of the light chain corresponding to SEQ ID NO: 110,

(xii) is an anti-TWEAKR antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 119 and a region of the light chain corresponding to SEQ ID NO: 120,

(xiii) is an anti-TWEAKR antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 129 and a region of the light chain corresponding to SEQ ID NO: 130,

(xiv) is an anti-TWEAKR antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 139 and a region of the light chain corresponding to SEQ ID NO: 140,

(xv) is an anti-TWEAKR antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 149 and a region of the light chain corresponding to SEQ ID NO: 150, or

(xvi) is an anti-TWEAKR antibody comprising a region of the heavy chain corresponding to SEQ ID NO: 159 and a region of the light chain corresponding to SEQ ID NO: 160,

or is an antigen-binding fragment of these antibodies.

42. Pharmaceutical composition comprising a compound according to one or more of the preceding claims in combination with an inert non-toxic pharmaceutically suitable excipient.

43. Compound according to one or more of the preceding claims for use in a method for treatment and/or prophylaxis of diseases.

44. Compound according to one or more of the preceding claims for use in a method for treatment of hyperproliferative and/or angiogenic disorders.