PEPTIDOMIMETIC COMPOUNDS AND ANTIBODY-DRUG CONJUGATES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This non-provisional application filed under 37 CFR §1.53(b), claims the benefit under 35 USC §119(e) of U.S. Provisional Application Serial No. 61/916,661 filed on 16 December 2013, and U.S. Provisional Application Serial No. 61/916,691 filed on 16 December 2013, all of which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 12, 2014, is named P5760PCT_SL.txt and is 35,047 bytes in size.

FIELD OF INVENTION

This invention relates to novel peptidomimetic compounds which are useful as linkers of antibody-drug conjugates(ADC). This invention also relates to ADCs containing peptidomimetic linkers. This invention also relates to methods of treating diseases in humans.

BACKGROUND OF THE INVENTION

The use of monoclonal antibodies (mABs) to deliver anticancer drugs directly to tumor cells has attracted a great deal of focus in recent years. Two new antibody-drug conjugates have been approved by the FDA for the treatment of cancer. Adcetris® (brentuximab vedotin) is a CD30-directed antibody-drug conjugate (ADC) indicated for the treatment of relapsed or refractory Hodgkin lymphoma and systemic anaplastic large cell lymphoma (ALCL). Kadcyla® (ado-trastuzumab emtansine), is a new therapy approved for patients with HER2-positive, late-stage (metastatic) breast cancer. To obtain a therapeutic both potent anti-tumor activity and acceptable therapeutic index in an ADC, several aspects of design may be optimized. Particularly, it is well known that the chemical structure of the linker can have significant impact on both the efficacy and the safety of ADC (Ducry & Stump, Bioconjugate Chem, 2010, 21, 5-13). Choosing the right linker influences proper drug delivery to the intended cellular compartment of cancer cells. Linkers can be generally divided into two categories: cleavable (such as peptide, hydrzone, or disulfide) or non-cleavable (such as thioether). Peptide linkers, such as Valine-Citrulline (Val-

Cit), that can be hydrolyzed by lysosomal enzymes (such as Cathepsin B) have been used to connect the drug with the antibody (US6214345). They have been particularly useful, due in part to their relative stability in systemic circulation and the ability to efficiently release the drug in tumor. ADCs containing the Val-Cit linker have been shown to be relatively stable in vivo (t1/2 for drug release ~7 days (Doronina et al (2008), Bioconjugate Chem., 19, 1960-1963). However, the chemical space represented by natural peptides is limited; therefore, it is desirable to have a variety of non-peptide linkers which act like peptides and can be effectively cleaved by lysosomal proteases. The greater diversity of non-peptide structures may yield novel, beneficial properties that are not afforded by the peptide linkers. Provided herein are different types of non-peptide linkers for ADC that can be cleaved by lysosomal enzymes.

SUMMARY OF THE INVENTION

This invention relates to antibody-drug conjugates represented by Formula (I)

Ab— (L—D)$_p$,

Ab is an antibody;

L is a peptidomimetic linker represented by the following formula

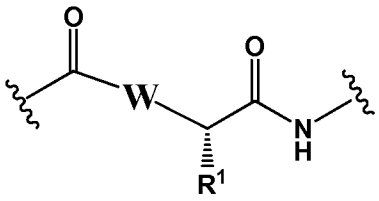
—Str—(PM)—Sp—

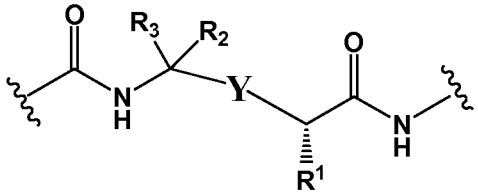
wherein

Str is a stretcher unit covalently attached to Ab;

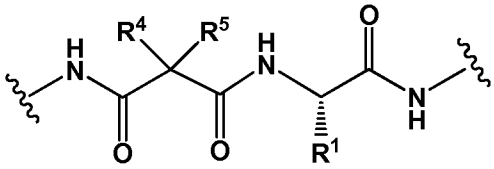
Sp is a bond or spacer unit covalently attached to a drug moiety;

PM is a non-peptide chemical moiety selected from the group consisting of:





and



W is –NH-heterocycloalkyl- or heterocycloalkyl;

Y is heteroaryl, aryl, -C(O)C_1-C_6alkylene, C_1-C_6alkylene-NH_2, C_1-C_6alkylene-NH-CH_3, C_1-C_6alkylene-N-$(CH_3)_2$, C_1-C_6alkenyl or C_1-C_6alkylenyl;

each R^1 is independently C_1-C_{10}alkyl, C_1-C_{10}alkenyl, (C_1-C_{10}alkyl)NHC(NH)NH_2 or (C_1-C_{10}alkyl)NHC(O)NH_2;

R^3 and R^2 are each independently H, C_1-C_{10}alkyl, C_1-C_{10}alkenyl, arylalkyl or heteroarylalkyl, or R^3 and R^2 together may form a C_3-C_7cycloalkyl;

R^4 and R^5 are each independently C_1-C_{10}alkyl, C_1-C_{10}alkenyl, arylalkyl, heteroarylalkyl, (C_1-C_{10}alkyl)OCH_2-, or R^4 andR^5 may form a C_3-C_7cycloalkyl ring;

p is an integer from 1 to 8;

D is a drug moiety.

This invention also relates to pharmaceutical compositions of antibody-drug conjugates of Formula (I).

This invention also relates to a method of treating cancer, use of antibody-drug conjugates of Formula (I) in therapy, and use of compounds of Formula (I) in manufacturing a medicament for treating cancer.

This invention also relates to method of preparing antibody-drug conjugates of Formula (I).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows efficacy comparison of CD33 ADCs (CD33 CBI-PBD ADC3-2 and ADC2-2) in SCID mice with HL-60 human acute myeloid leukemia tumors.

Figure 2 shows efficacy comparison of NaPi2b ADCs (NaPi2b CBI-PBD ADC2-1 and ADC3-1) in SCID-beige mice with OVCAR3X2.1 human ovarian tumors.

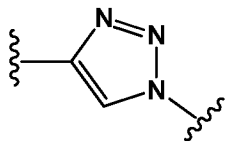
DETAILED DESCRIPTION OF THE INVENTION

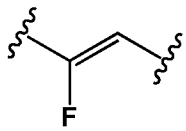
Provided herein are different types of non-peptide linkers for ADC that are cleavable by lysosomal enzymes. For example, the amide bond in the middle of a dipeptide (e.g. Val-Cit) was repaced with an amide mimic; and/or entire amino acid (e.g., valine amino acid in Val-Cit dipeptide) was replaced with a non-amino acid moiety (e.g., cycloalkyl dicarbonyl structures (for example, ring size = 4 or 5)).

This invention relates to antibody-drug conjugates of Formula (I).

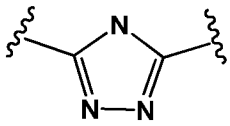
This invention also relates to antibody-drug conjugates of Formula (I), wherein Y is heteroaryl; R^4 and R^5 together form a cyclobutyl ring.

This invention also relates to antibody-drug conjugates of Formula (I), wherein Y is a moiety selected from the group consisting of



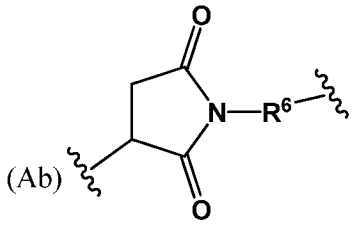


AND



This invention also relates to antibody-drug conjugates of Formula (I), wherein

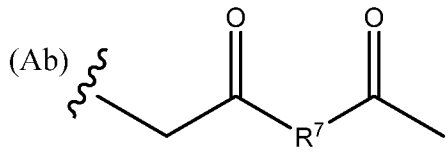
Str is a chemical moiety represented by the following formula:



wherein R^6 is selected from the group consisting of C_1-C_{10}alkylene, C_1-C_{10}alkenyl, C_3-C_8cycloalkyl, (C_1-C_8alkylene)O-, and C_1-C_{10}alkylene–C(O)N(R^a)–C_2-C_6alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, C_3-C_8cycloalkyl, C_4-C_7heterocycloalkyl,

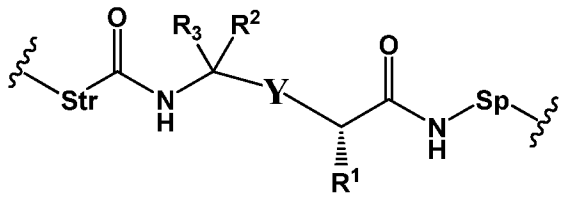
aryl, arylalkyl, heteroarylalkyl and heteroaryl each R^a is independently H or C_1-C_6alkyl; Sp is —Ar—R^b—, wherein Ar is aryl or heteroaryl, R^b is (C_1-C_{10}alkylene)O-.

This invention also relates to antibody-drug conjugates of Formula (I), wherein Str has the formula:



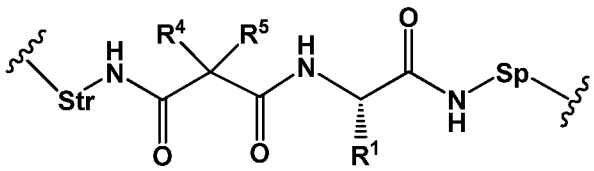
wherein R^7 is selected from C_1-C_{10}alkylene, C_1-C_{10}alkenyl, (C_1-C_{10}alkylene)O-, N(R^c)–(C_2-C_6 alkylene)–N(R^c) and N(R^c)–(C_2-C_6alkylene); where each R^c is independently H or C_1-C_6 alkyl; Sp is —Ar—R^b—, wherein Ar is aryl or heteroaryl, R^b is (C_1-C_{10}alkylene)O- or Sp -C_1-C_6alkylene-C(O)NH-.

This invention also relates to antibody-drug conjugates of Formula (I),
wherein Ab is an antibody; L is non-peptide chemical moiety represented by the following formula



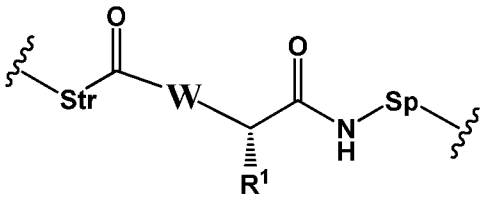
R^1 is C_1-C_6alkyl, C_1-C_6alkenyl, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;
R^3 and R^2 are each independently H or C_1-C_{10}alkyl.

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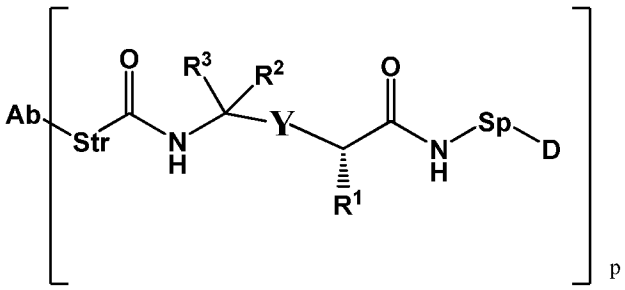
R^1 is C_1-C_6alkyl, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;
R^4 and R^5 together form a C_3-C_7cycloalkyl ring.

This invention also relates to antibody-drug conjugates of Formula (I),
wherein Ab is an antibody;
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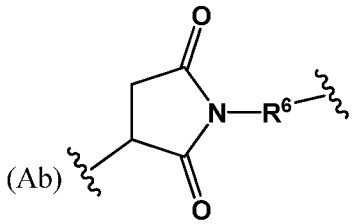
This invention also relates to antibody-drug conjugates of Formula (I), which is represented by the following formula



(I)(A1)

wherein

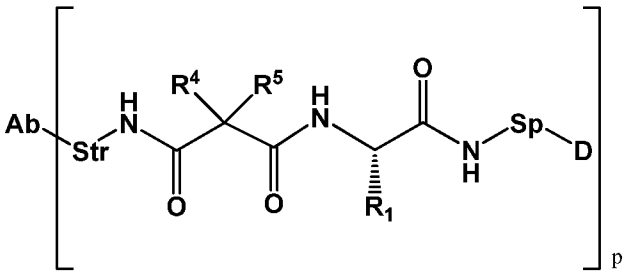
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wherein R^6 is selected from the group consisting of C_1-C_{10}alkylene, and C_1-C_{10}alkylene–C(O)N(R^a)–C_2-C_6alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, C_3-C_8cycloalkyl, C_4-C_7heterocycloalkyl, aryl, arylalkyl, heteroarylalkyl and heteroaryl, each R^a is independently H or C_1-C_6alkyl;

p is 1, 2, 3 or 4.

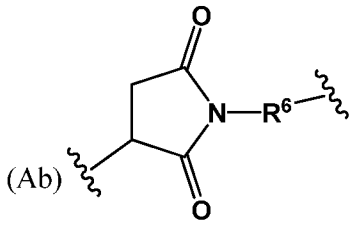
This invention also relates to antibody-drug conjugates of Formula (I) represented by the following formula:



(I)(B1)

wherein

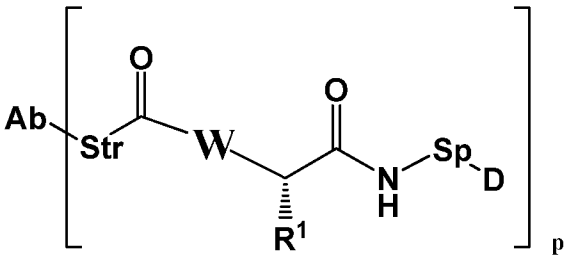
Str is a chemical moiety represented by the following formula:



wherein R^6 is selected from the group consisting of C_1-C_{10}alkylene, and C_1-C_{10}alkylene–C(O)N(R^a)–C_2-C_6alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, C_3-C_8cycloalkyl, C_4-C_7heterocycloalkyl, aryl, arylalkyl, heteroarylalkyl and heteroaryl, each R^a is independently H or C_1-C_6alkyl;

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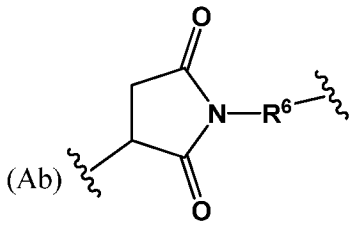
This invention also relates to antibody-drug conjugates of Formula (I) represented by the following formula:



(I)(C1)

wherein

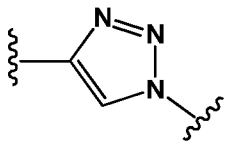
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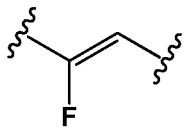
wherein R^6 is selected from the group consisting of C_1-C_{10}alkylene, and C_1-C_{10}alkylene–C(O)N(R^a)–C_2-C_6alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, C_3-C_8cycloalkyl, C_4-C_7heterocycloalkyl, aryl, arylalkyl, heteroarylalkyl and heteroaryl, each R^a is independently H or C_1-C_6alkyl;
p is 1, 2, 3 or 4.

This invention also relates to any one of the above antibody-conjuagates, wherein Y is heteroaryl, aryl or alkenyl; R^6 is C_1-C_{10}alkylene.

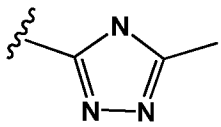
This invention also relates to any one of the above antibody-conjuagates (I) and (I)(A1), wherein Y is



This invention also relates to any one of the above antibody-conjuagates (I) and (I)(A1), wherein Y is

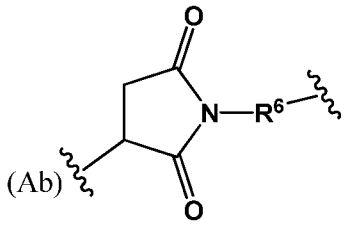


This invention also relates to any one of the above antibody-conjuagates (I) and (I)(A1), wherein Y is



This invention also relates to any one of the above antibody-conjuagates (I)(A1), (I)(B1), and (I)(C1), wherein

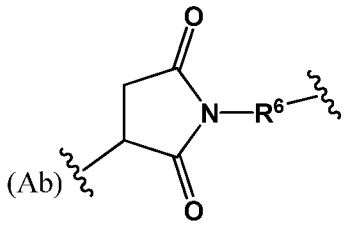
Str is a chemical moiety represented by the following formula:



,

R^6 is C_1-C_6alkylene which may be substituted with 1-3 groups selected from aryl and heteroaryl;
Sp is -C_1-C_6alkylene-C(O)NH- or —Ar—R^b—, where Ar is aryl, R^b is (C_1-C_3alkylene)O-.

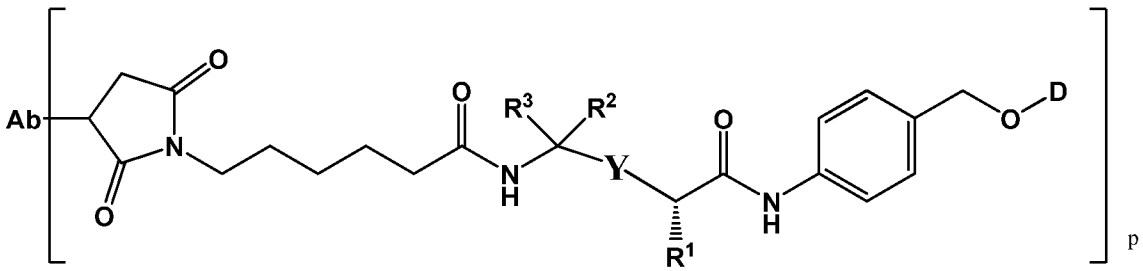
This invention also relates to any one of the above antibody-conjuagates (I)(A1), (I)(B1), and (I)(C1), wherein
Str is a chemical moiety represented by the following formula:



,

R^6 is C_1-C_6alkylene which may be substituted with 1-3 groups selected from aryl and heteroaryl;
Sp is -C_1-C_6alkylene-C(O)NH-.

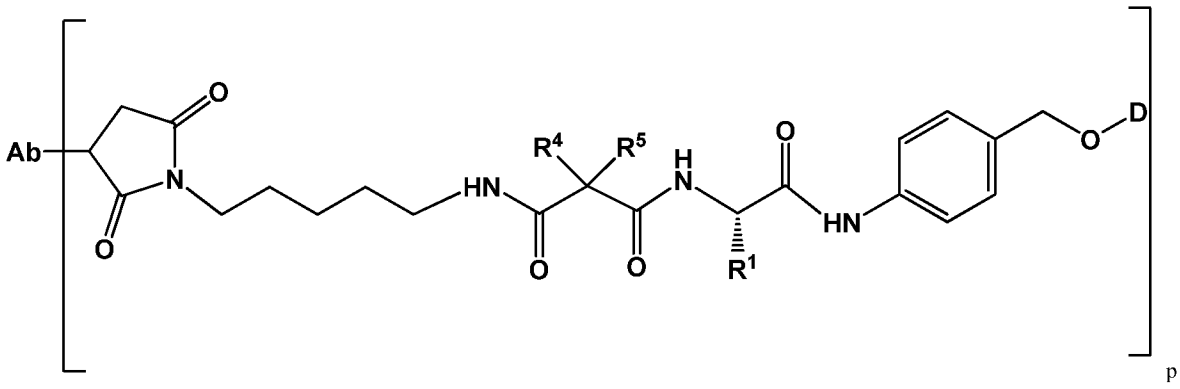
This invention also relates to any one of the above antibody-conjuagates (I) and (I)(A1), represented by the following formula:



(I)(A2)

wherein
R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2
p is 1, 2 ,3 or 4.

This invention also relates to any one of the above antibody-conjuagates (I) and (I)(B1), represented by the following formula:



(I)(B2)

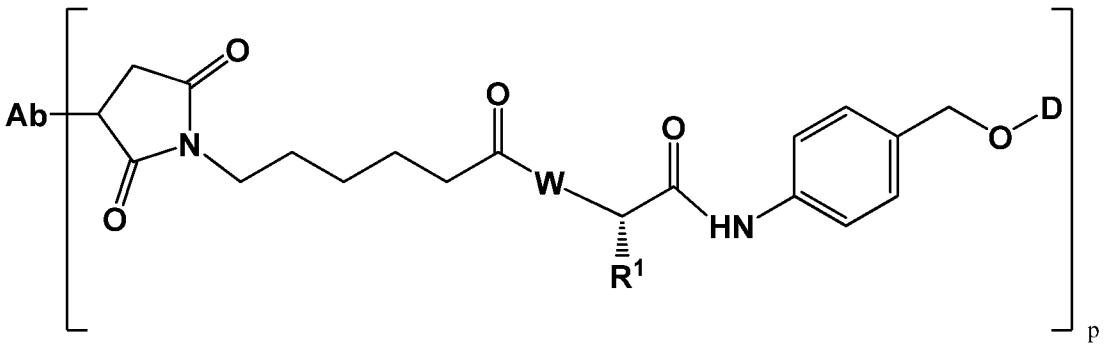
wherein

p is 1, 2, 3 or 4;

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

R^4 and R^5 are each independently C_1-C_6alkyl, wherein said alkyl are unsubstituted, or R^4 andR^5 may form a C_3-C_7cycloalkyl ring.

This invention also relates to any one of the above antibody-conjuagates (I) and (I)(C1), represented by the following formula:



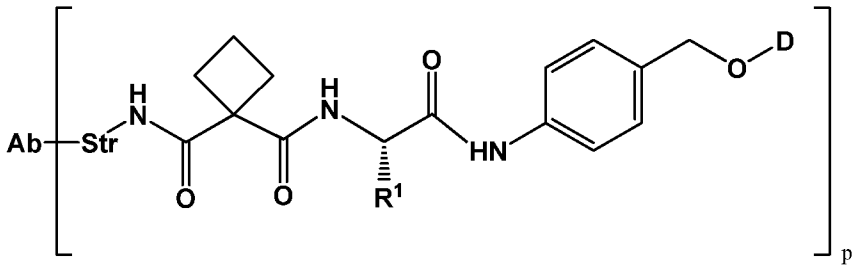
(I)(C2)

wherein

p is 1, 2, 3 or 4;

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2.

This invention also relates to antibody-conjuagates of (I), which is represented by the following formula:



(I)(B3)

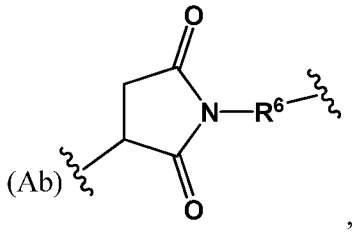
wherein

p is 1, 2, 3 or 4;

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

This invention also relates to antibody-drug conjugates of (I)(B3), wherein

Str is a chemical moiety represented by the following formula:



,

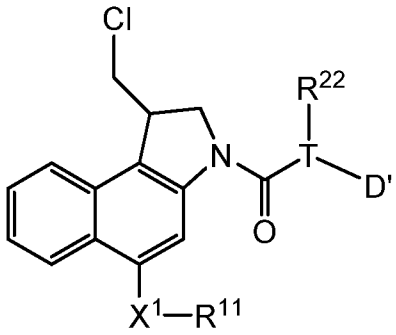
R^6 is C_1-C_6alkylene which may be substituted with 1-3 groups selected from aryl and heteroaryl;

This invention also relates to antibody-drug conjugates of (I)(B3) wherein R^1 is $(CH_2)_3NHC(O)NH_2$.

This invention also relates to antibody-drug conjugates of (I)(B3) wherein R^1 is $(CH_2)_4NH_2$.

This invention also relates to antibody-drug conjugates of (I), (I)(B1), (I)(B2) and (I)(B3), wherein R^1 is (C_1-C_6alkyl)NHC(NH)NH_2.

This invention also relates to any one of the above antibody-drug conjugates, wherein D is the dimer drug moiety having the formula:



wherein

R^{11} is selected from H, $P(O)_3H_2$, $C(O)NR^{aa}R^{bb}$, or a bond to L;

R^{22} is selected from H, $P(O)_3H_2$, $C(O)NR^{aa}R^{bb}$, or a bond to L;

R^{aa} and R^{bb} are independently selected from H and C_1–C_6alkyl optionally substituted with one or more F,

or R^{aa} and R^{bb} form a five or six membered heterocycloalkyl group;

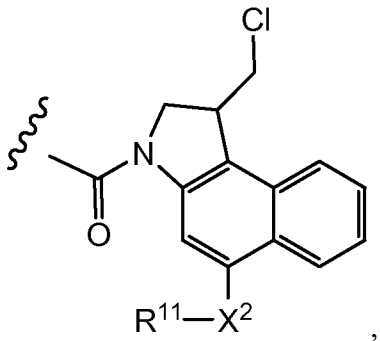
T is a tether group selected from C_3–C_{12}alkylene, Y^1, (C_1–C_6alkylene)– Y^1–(C_1–C_6 alkylene), (C_1–C_6alkylene)– Y^1–(C_1–C_6alkylene)– Y^1–(C_1–C_6alkylene), (C_2–C_6alkenylene)– Y^1–(C_2–C_6alkenylene), and (C_2–C_6alkynylene)– Y^1–(C_2–C_6alkynylene);

where Y^1 is independently selected from O, S, NR^{11}, aryl, and heteroaryl;

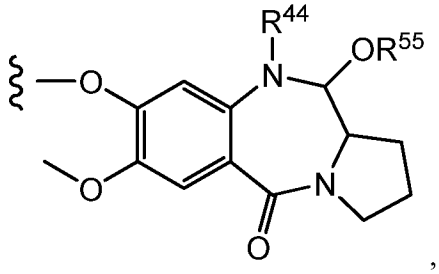
where alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with F, OH, O(C_1–C_6alkyl), NH_2, $NHCH_3$, $N(CH_3)_2$, $NHC(O)(C_1$-C_6alkylene)$_m$, $OP(O)_3H_2$, and C_1–C_6alkyl, where alkyl is optionally substituted with one or more F, m is 0 or 1;

or alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with a bond to L, wherein the bond to L may connect through one of the optional substituents;

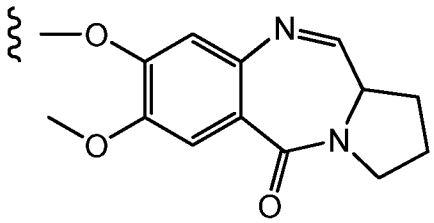
D' is a drug moiety selected from:



,



, and



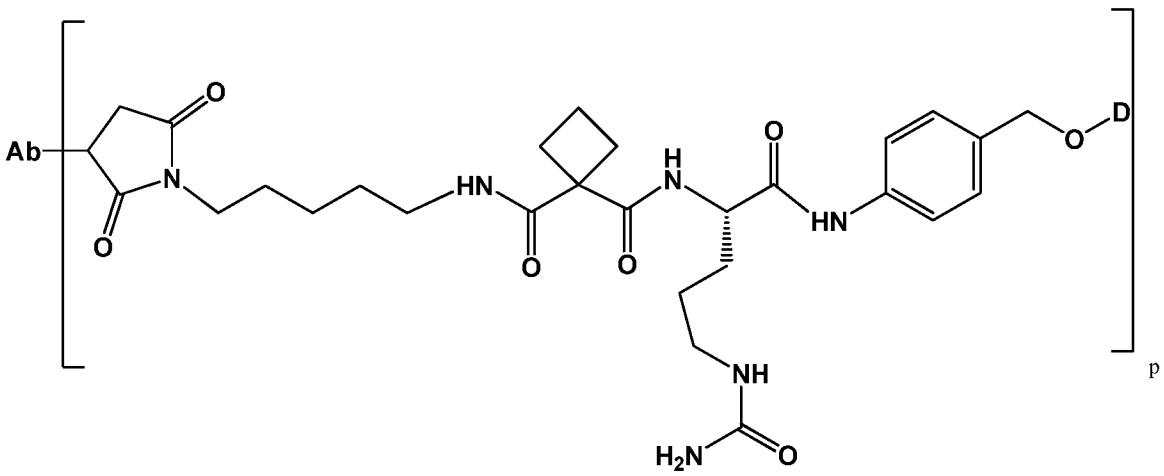
where the wavy line indicates the site of attachment to T;

X^1 and X^2 are independently selected from O and NR^{33}, where R^{33} is selected from H, C(O), and C_1–C_6alkyl optionally substituted with one or more F, or X^1 and X^2 are each independently absent;

R^{44} is H, CO_2R, C(O), or a bond to L, where R is C_1–C_6alkyl or benzyl; and

R^{55} is H or C_1–C_6alkyl.

This invention also relates to antibody-drug conjugates of formula (I), which is represented by the following formula:



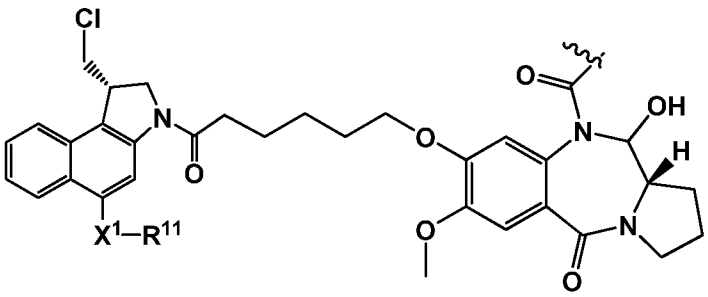
(I)(B4)

wherein,

Ab is an antibody that binds to a target seleted from Her2, CLL1, CD33, CD22 and NaPi2b;

P is 1-4;

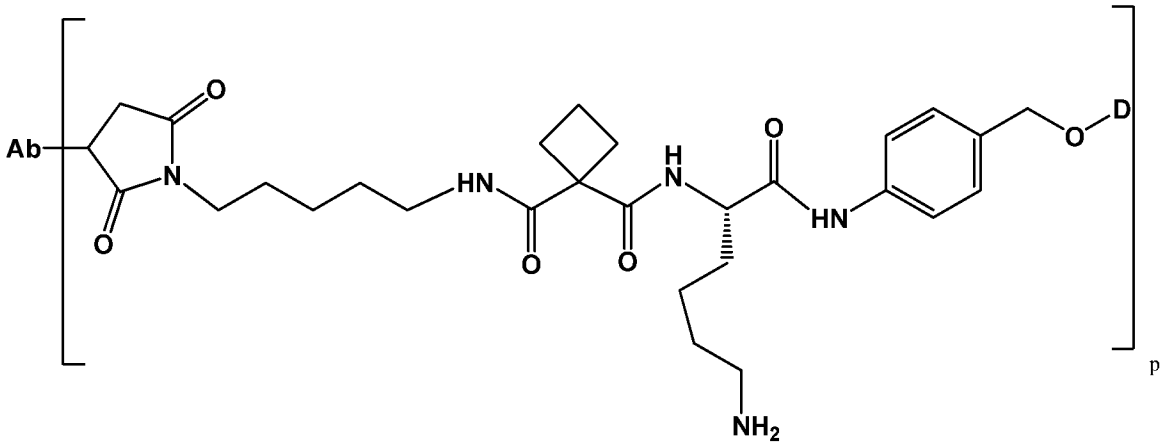
D is



X^1 is absent or O;

R^{11} is C(O)N-piperazine(CH_3) or $P(O)_3H_2$.

This invention also relates to antibody-drug conjugates of formula (I), which is represented by the following formula:



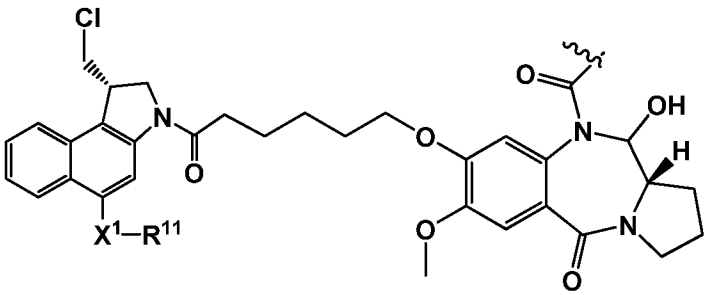
(I)(B5)

wherein,

Ab is an antibody that binds to a target seleted from Her2, CLL1, CD33, CD22 and NaPi2b;

P is 1-4;

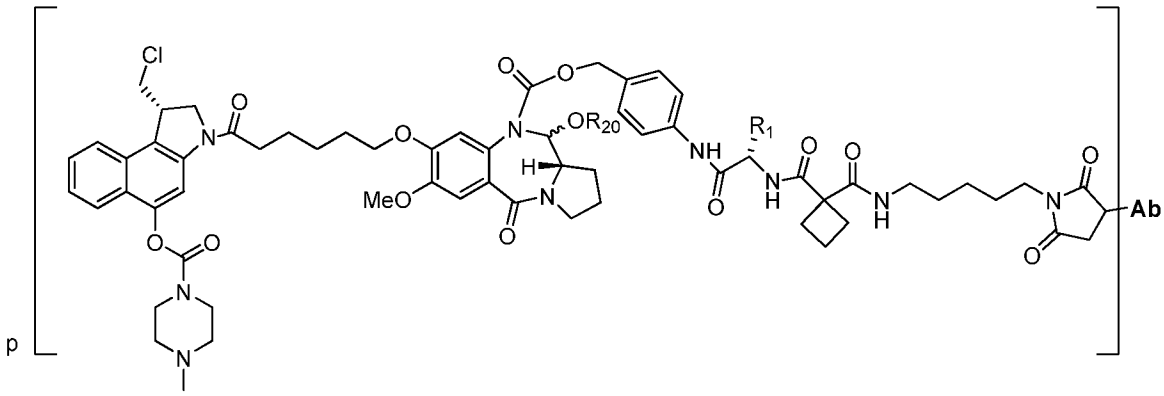
D is



X^1 is absent or O;

R^{11} is C(O)N-piperazine(CH_3) or $P(O)_3H_2$.

This invention also relates to antibody-drug conjugates of formula (I), which is represented by the following formula:



(I)(B6)

wherein,

Ab is an antibody that binds to a target seleted from Her2, CLL1, CD33, CD22 and NaPi2b;

P is 1-4; and

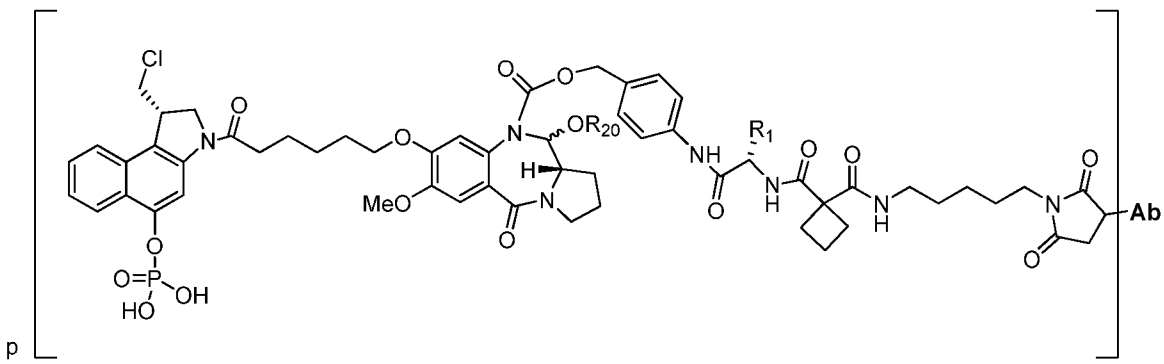
R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

R^{20} is H or Me;

This invention also relates to antibody-drug conjugates of formula (I)(B6), wherein R^{20} is H, R^1 is $(CH_2)_4NH_2$.

This invention also relates to antibody-drug conjugates of formula (I)(B6), wherein R^{20} is Me, R^1 is $(CH_2)_4NH_2$.

This invention also relates to antibody-drug conjugates of formula (I), which is represented by the following formula:



(I)(B7)

wherein,

Ab is an antibody that binds to a target seleted from Her2, CLL1, CD33, CD22 and NaPi2b;

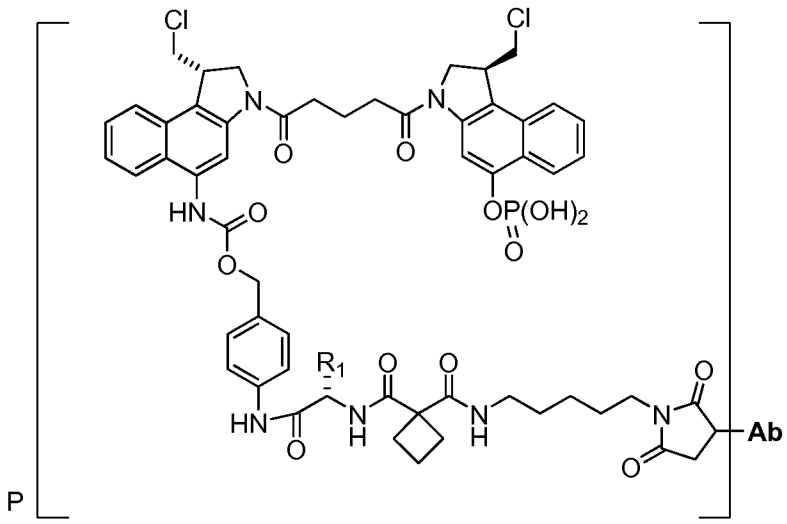
P is 1-4;

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;
R^{20} is H or Me;

This invention also relates to antibody-drug conjugates of formula (I)(B7), wherein
R^{20} is H, R^1 is $(CH_2)_4NH_2$.

This invention also relates to antibody-drug conjugates of formula (I)(B7), wherein
R^{20} is Me, R^1 is $(CH_2)_4NH_2$.

This invention also relates to antibody-drug conjugates of formula (I), which is represented by the following formula:

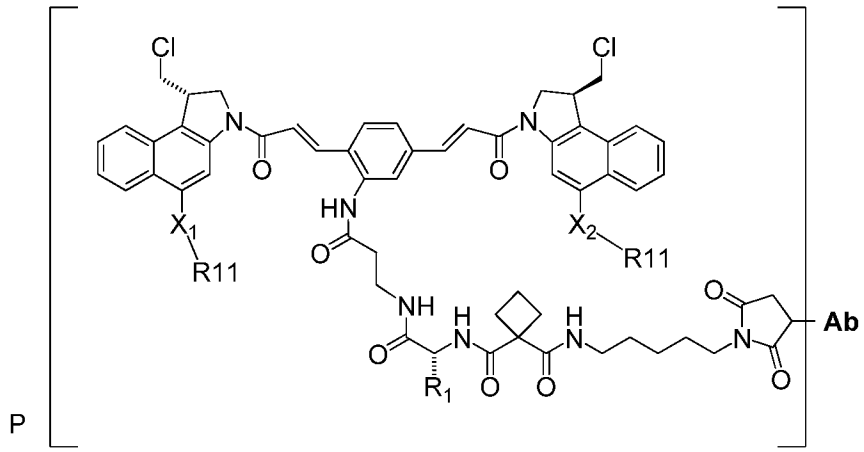


(I)(B8)

wherein,
Ab is an antibody that binds to a target seleted from Her2, CLL1, CD33, CD22 and NaPi2b;
P is 1-4; and
R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

This invention also relates to antibody-drug conjugates of formula (I)(B8), wherein
R^1 is $(CH_2)_4NH_2$.

This invention also relates to antibody-drug conjugates of formula (I), which is represented by the following formula:



(I)(B9)

wherein,

Ab is an antibody that binds to a target seleted from Her2, CLL1, CD33, CD22 and NaPi2b;

P is 1-4;

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

X^1 and X^2 are each independently absent or O;

Each R^{11} is independently C(O)N-piperazine(CH_3) or $P(O)_3H_2$.

This invention also relates to antibody-drug conjugates of formula (I)(B9), wherein X^1 is absent, R^{11} is $P(O)_3H_2$, R^1 is $(CH_2)_4NH_2$.

This invention also relates to any of the above antibody-drug conjugates of formula (I)(B6), (I)(B7), (I)(B8) and (I)(B9), wherein the Ab is an antibody that binds to Her2.

This invention also relates to any of the above antibody-drug conjugates of formula (I)(B6), (I)(B7), (I)(B8) and (I)(B9), wherein the Ab is an antibody that binds to CLL1.

This invention also relates to any of the above antibody-drug conjugates of formula (I)(B6), (I)(B7), (I)(B8) and (I)(B9), wherein the Ab is an antibody that binds to CD33.

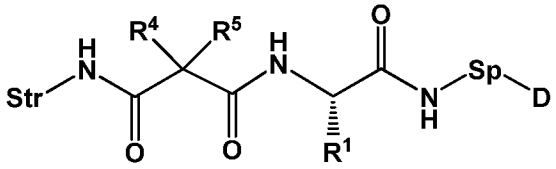
This invention also relates to any of the above antibody-drug conjugates of formula (I)(B6), (I)(B7), (I)(B8) and (I)(B9), wherein the Ab is an antibody that binds to CD22.

This invention also relates to any of the above antibody-drug conjugates of formula (I)(B6), (I)(B7), (I)(B8) and (I)(B9), wherein the Ab is an antibody that binds to NaPi2b.

This invention also relates to any of the above antibody-drug conjugates, wherein

where Y^1 is phenyl which is optionally substituted with F, OH, O(C_1–C_6alkyl), NH_2, $NHCH_3$, $N(CH_3)_2$, NHC(O)(C_1-C_6alkylene)$_m$, $OP(O)_3H_2$, and C_1–C_6alkyl, where alkyl is optionally substituted with one or more F, m is 0 or 1;

This invention also relates to non-peptide compounds of Formula (I)(B)(LD1):



(I)(B)(LD1)

wherein

Str is a stretcher unit which can be covalently attached to an antibody;

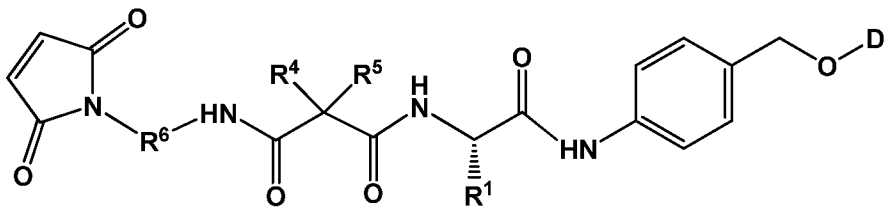
Sp is a bond or a spacer unit covalently attached to a drug moiety;

R^1 is C_1-C_{10}alkyl, (C_1-C_{10}alkyl)NHC(NH)NH_2 or (C_1-C_{10}alkyl)NHC(O)NH_2;

R^4 and R^5 are each independently C_1-C_{10}alkyl, arylalkyl, heteroarylalkyl, (C_1-C_{10}alkyl)OCH_2-, or R^4 and R^5 may form a C_3-C_7cycloalkyl ring;

D is a drug moiety.

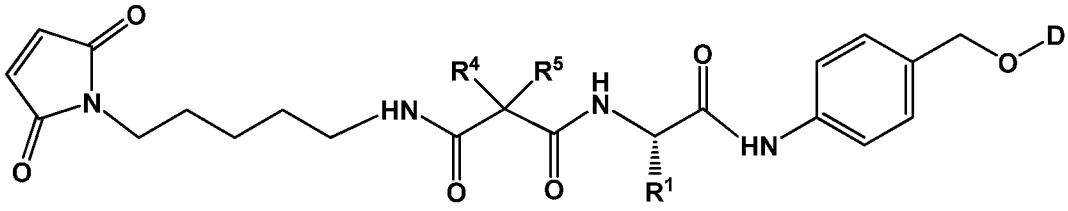
This invention also relates to non-peptide compounds represented by the following formula



(I)(B)(LD2)

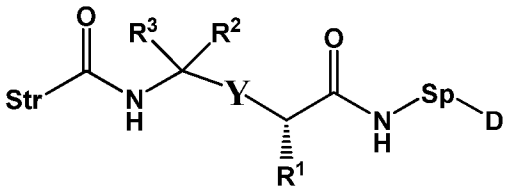
wherein R_6 is C_1-C_{10}alkylene; R^4 and R^5 together form a C_3-C_7cycloalkyl ring.

This invention also relates to non-peptide compounds represented by the following formula



(I)(B)(LD3)

This invention also relates to non-peptide compounds of Formula:



(I)(A)(LD1)

wherein

Str is a stretcher unit which can be covalently attached to an antibody;

Sp is an optional spacer unit covalently attached to a drug moiety;

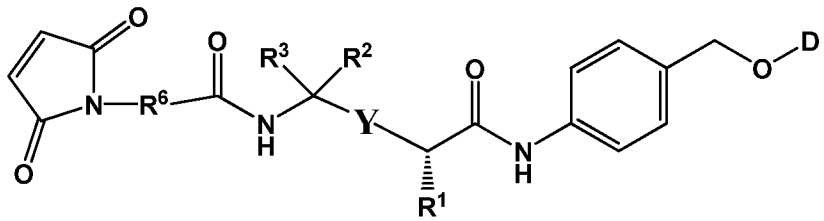
Y is heteroaryl, aryl, -C(O)C_1-C_6alkylene, C_1-C_6alkylene-NH_2, C_1-C_6alkylene-NH-CH_3, C_1-C_6alkylene-N-$(CH_3)_2$, C_1-C_6alkenyl or C_1-C_6alkylenyl;

R^1 is C_1-C_{10}alkyl, (C_1-C_{10}alkyl)NHC(NH)NH_2 or (C_1-C_{10}alkyl)NHC(O)NH_2;

R^3 and R^2 are each independently H, C_1-C_{10}alkyl, arylalkyl or heteroarylalkyl, or R^3 and R^2 together may form a C_3-C_7cycloalkyl;

D is a drug moiety.

This invention also relates to non-peptide compounds represented by the following formula:

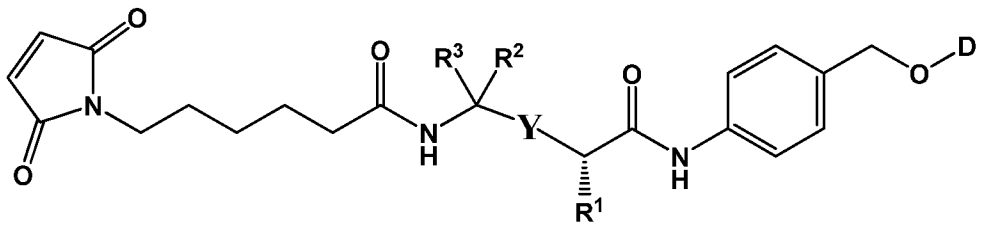


(I)(A)(LD2)

wherein

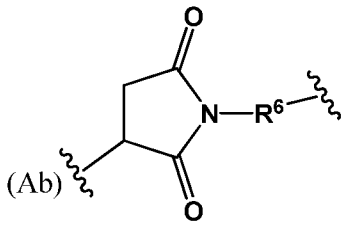
R_6 is C_1-C_{10}alkylene.

This invention also relates to non-peptide compounds represented by the following formula:



(I)(A)(LD3)

This invention also relates to any of the above non-peptide compounds, wherein Str has the following formula:

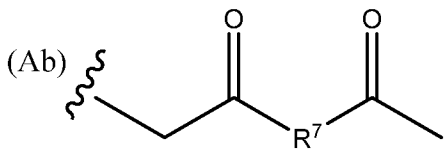


wherein R^6 is selected from the group consisting of C_1-C_{10}alkylene, C_3-C_8cycloalkyl, O-(C_1-C_8alkylene), and C_1-C_{10}alkylene–C(O)N(R^a)–C_2-C_6alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, C_3-C_8cycloalkyl, C_4-C_7heterocycloalkyl aryl, arylalkyl, heteroarylalkyl and heteroaryl; each R^a is independently H or C_1-C_6alkyl; Sp is —Ar—R^b—, wherein Ar is aryl or heteroaryl, R^b is (C_1-C_{10}alkylene)O-.

This invention also relates to non-peptide compounds, wherein R^6 is C_1-C_{10}alkylene, Sp is —Ar—R^b—, wherein Ar is aryl R^b is (C_1-C_6alkylene)O-.

This invention also relates to non-peptide compounds, where R_6 is $-(CH_2)_q$ is 1-10;

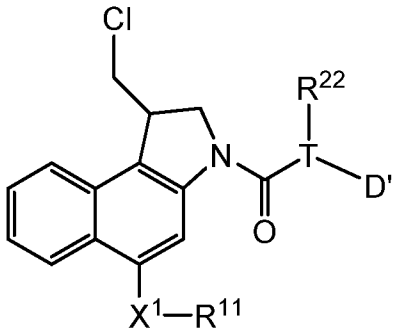
This invention also relates to non-peptide compounds, wherein Str has the formula:



wherein R^7 is selected from C_1-C_{10}alkylene, C_1-C_{10}alkylene–O, N(R^c)–(C_2-C_6 alkylene)–N(R^c) and N(R^c)–(C_2-C_6alkylene); where each R^c is independently H or C_1-C_6 alkyl; Sp is —Ar—R^b—, wherein Ar is aryl or heteroaryl, R^b is (C_1-C_{10} alkylene)O-.

This invention also relates to non-peptide compounds, wherein R^6 is C_1-C_{10} alkylene, Sp is —Ar—R^b—, wherein Ar is aryl R^b is (C_1-C_6 alkylene)O-.

This invention also relates to any one of the above non-peptide compounds of Formula (IV), (IV)(A), (IV)(B), (V), (V)(A) and (V)(B), wherein D is the dimer drug moiety having the formula:



wherein

R^{11} is selected from H, $P(O)_3H_2$, $C(O)NR^{aa}R^{bb}$, or a bond to L;

R^{22} is selected from H, $P(O)_3H_2$, $C(O)NR^{aa}R^{bb}$, or a bond to L;

R^{aa} and R^{bb} are independently selected from H and C_1–C_6alkyl optionally substituted with one or more F,

or R^{aa} and R^{bb} form a five or six membered heterocycloalkyl group;

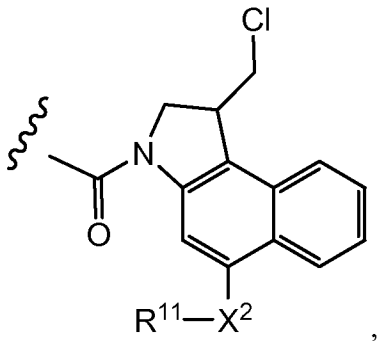
T is a tether group selected from C_3–C_{12}alkylene, Y^1, (C_1–C_6alkylene)– Y^1–(C_1–C_6 alkylene), (C_1–C_6alkylene)– Y^1–(C_1–C_6alkylene)– Y^1–(C_1–C_6alkylene), (C_2–C_6alkenylene)– Y^1–(C_2–C_6alkenylene), and (C_2–C_6alkynylene)– Y^1–(C_2–C_6alkynylene);

where Y^1 is independently selected from O, S, NR^{11}, aryl, and heteroaryl;

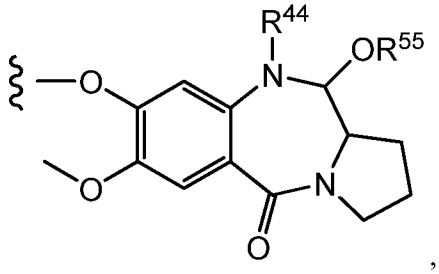
where alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with F, OH, O(C_1–C_6alkyl), NH_2, $NHCH_3$, $N(CH_3)_2$, $NHC(O)(C_1$-C_6alkylene)$_m$, $OP(O)_3H_2$, and C_1–C_6alkyl, where alkyl is optionally substituted with one or more F, m is 0 or 1;

or alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with a bond to L, wherein the bond to L may connect through one of the optional substituents;

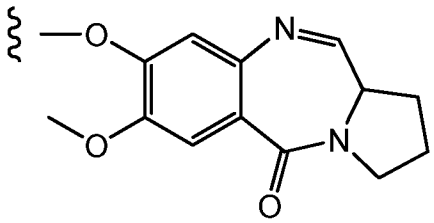
D' is a drug moiety selected from:



,



, and



where the wavy line indicates the site of attachment to T;

X^1 and X^2 are independently selected from O and NR^{33}, where R^{33} is selected from H, C(O), and C_1–C_6alkyl optionally substituted with one or more F, or X^1 and X^2 are each independently absent;

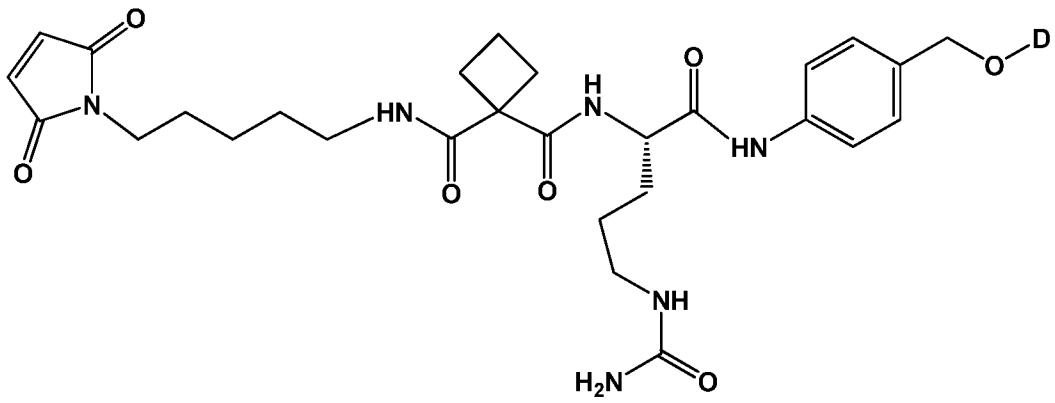
R^{44} is H, CO_2R, C(O), or a bond to L, where R is C_1–C_6alkyl or benzyl; and

R^{55} is H or C_1–C_6alkyl.

This invention also relates to any of the above linker drug comounds, wherein

where Y^1 is phenyl which is optionally substituted with F, OH, O(C_1–C_6alkyl), NH_2, $NHCH_3$, $N(CH_3)_2$, $NHC(O)(C_1$-C_6alkylene$)_m$, $OP(O)_3H_2$, and C_1–C_6alkyl, where alkyl is optionally substituted with one or more F, m is 0 or 1;

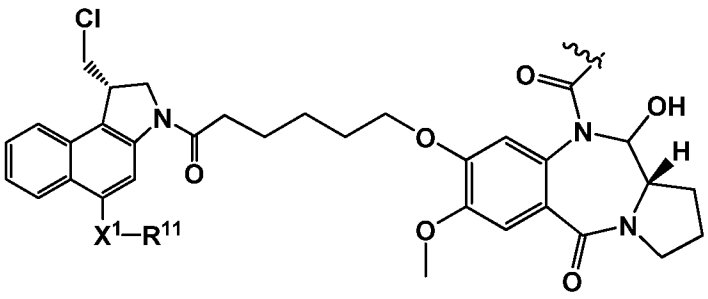
This invention also relates to compounds of the following formula:



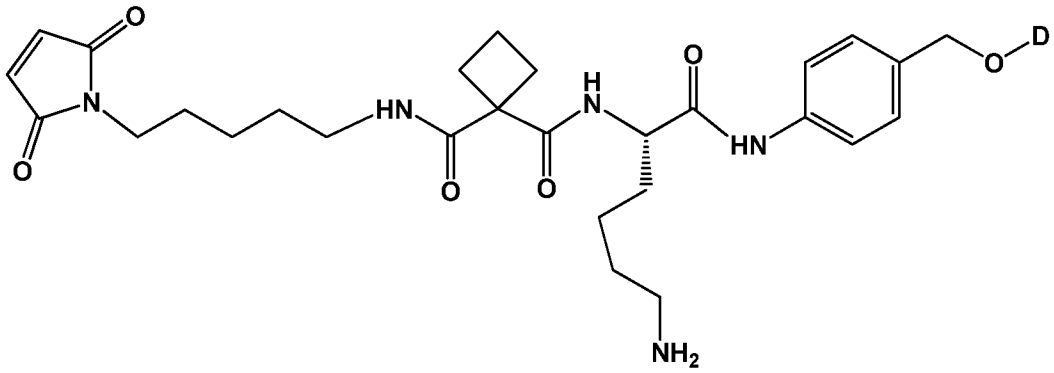
(I)(B)(LD4)

wherein,

D is a drug moiety of the following formula



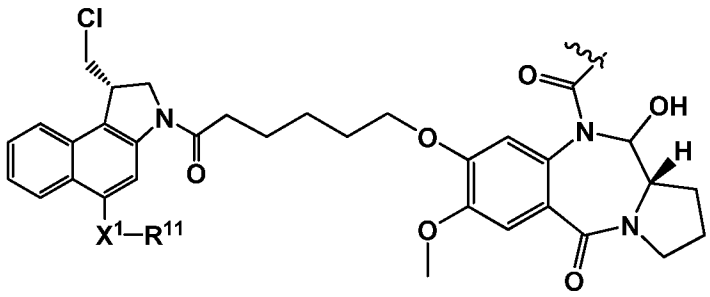
This invention also relates to compounds of the following formula:



(I)(B)(LD5)

wherein,

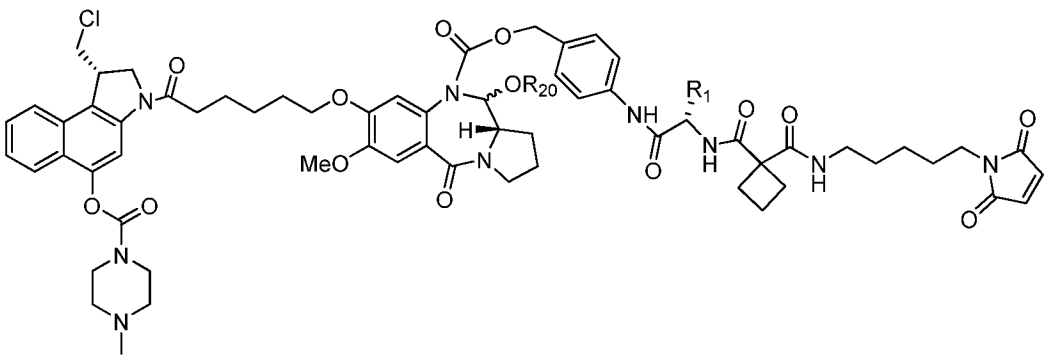
D is a drug moiety of the following formula



X^1 is absent or O;

R^{11} is C(O)N-piperazine(CH_3) or $P(O)_3H_2$.

This invention also relates to compounds of the following formula:



(I)(B)(LD6)

wherein,

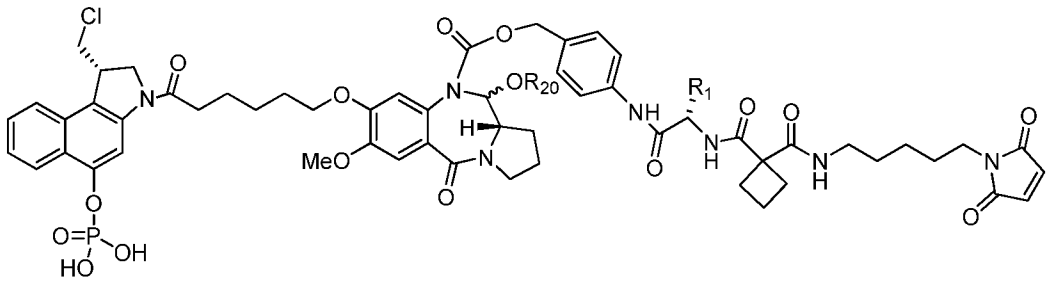
R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

R^{20} is H or Me;

This invention also relates to compounds of formula (I)(B((LD6), wherein R^{20} is H, R^1 is $(CH_2)_4NH_2$.

This invention also relates to compounds of formula (I)(B((LD6), wherein R^{20} is Me, R^1 is $(CH_2)_4NH_2$.

This invention also relates to compounds of the following formula:



(I)(B)(LD7)

wherein,

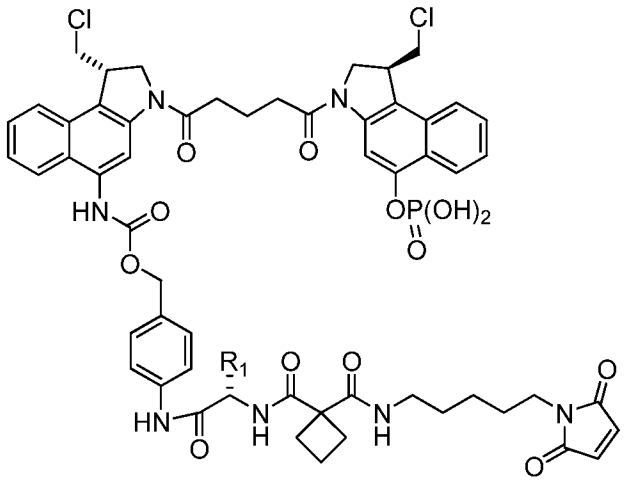
R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

R^{20} is H or Me;

This invention also relates to compounds of formula (I)(B((LD7), wherein R^{20} is H, R^1 is $(CH_2)_4NH_2$.

This invention also relates to compounds of formula (I)(B((LD7), wherein R^{20} is Me, R^1 is $(CH_2)_4NH_2$.

This invention also relates to compounds of the following formula:



(I)(B)(LD8)

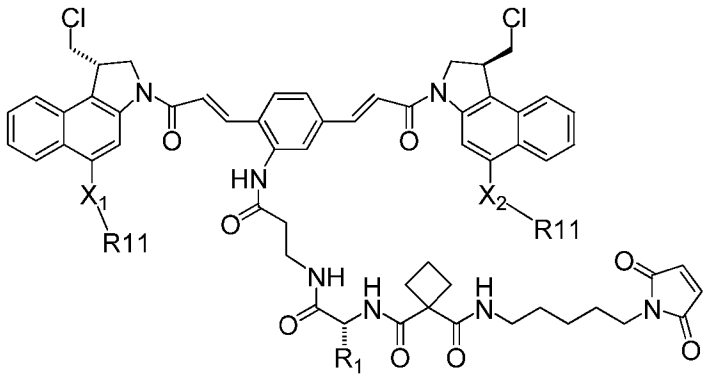
wherein,

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

This invention also relates to compounds of formula (I)(B)(LD8), wherein

R^1 is $(CH_2)_4NH_2$.

This invention also relates to compounds of the following formula:



(I)(B)(LD9)

wherein,

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

X^1 and X^2 are each independently absent or O;

each R^{11} is independently C(O)N-piperazine(CH_3) or $P(O)_3H_2$.

This invention also relates to any one of the above conjugates, wherein D is an antibiotic moiety selected from the group consisting of clindamycin, novobiocin, retapamulin, daptomycin, GSK-2140944, CG-400549, sitafloxacin, teicoplanin, triclosan, napthyridone, radezolid, doxorubicin,

ampicillin, vancomycin, imipenem, doripenem, gemcitabine, dalbavancin, and azithromycin. The mechanisms of bactericidal and bacteriostatic action of such antibiotics include, but are not limited to: (i) inhibition of cell wall, peptidoglycan elongation (vancomycin, teicoplanin, dalbavancin); (ii) inhibition of cell wall, penicillin-binding protein crosslinks (imipenem, doripenem, ampicillin); (iii) cell membrane depolarization (daptomycin); (iv) disruption of DNA replication (gemcitabine); (v) DNA binding (doxorubicin); (vi) enoyl ACP-reductase FABI (CG-400549, triclosan, napthyridone); (vii) inhibition of ribosomal protein synthesis, ribosome 30S (clindamycin, retapamulin, radezolid); and (viii) topoisomerase (topoIIA) inhibitors (novobiocin, sitafloxacin, GSK-2140944). Structurally, most antibiotics can be grouped into: (i) aminoglycosides; (ii) beta-lactams; (iii) macrolides/cyclic peptides; (iv) tetracyclines; (v) fluoroquinolines/fluoroquinolones; (vi) and oxazolidinones. See: Shaw, K. and Barbachyn, M. (2011) Ann. N.Y. Acad. Sci. 1241:48-70; Sutcliffe, J. (2011) Ann. N.Y. Acad. Sci. 1241:122-152.

This invention also relates to any one of the above antibody-drug conjugates or antibody-antibiotic conjugates described herein, wherein p is 1.

This invention also relates to any one of the above antibody-drug conjugates or antibody-antibiotic conjugates described herein, wherein p is 2.

This invention also relates to any one of the above antibody-drug conjugates or antibody-antibiotic conjugates described herein, wherein p is 3.

This invention also relates to any one of the above antibody-drug conjugates or antibody-antibiotic conjugates described herein, wherein p is 4.

The invention provides novel antibacterial therapy that aims to prevent antibiotic escape by targeting populations of bacteria that evade conventional antibiotic therapy. The novel antibacterial therapy is achieved with an Antibody Antibiotic Conjugate (AAC) in which an antibody specific for cell wall components found on *S. aureus* (including MRSA) is chemically linked to a potent antibiotic. The antibiotic is joined to the antibody via a protease cleavable, peptide linker that is designed to be cleaved by cathepsin B, a lysosomal protease found in most mammalian cell types (Dubowchik et al (2002) Bioconj. Chem. 13:855-869). The AAC acts as a pro-drug in that the antibiotic is inactive (due to the large size of the antibody) until the linker is cleaved. Since a significant proportion of *S. aureus* found in a natural infection is taken up by host cells, primarily neutrophils and macrophages, at some point during the course of infection in the host, and that the time spent inside host cells provides a significant opportunity for the bacterium to evade antibiotic activity. The AACs of the invention are designed to bind to S. aureus and release the antibiotic inside the phagolysosome after bacteria are taken up by host cells. By this mechanism, AAC are able to concentrate the active antibiotic specifically in a location where S. aureus is poorly treated by conventional antibiotics. While the invention is not limited or defined

by an particular mechanism of action, the AAC improve antibiotic activity via three potential mechanisms: (1) The AAC delivers antibiotic inside mammalian cells that take up the bacteria, thereby increasing the potency of antibiotics that diffuse poorly into the phagolysosomes where bacteria are sequestered. (2) AAC opsonize bacteria - thereby increasing uptake of free bacteria by phagocytic cells - and release the antibiotic locally to kill the bacteria while they are sequestered in the phagolysosome. (3) AAC improve the half-life of antibiotics *in vivo* (improved pharmacokinetics) by linking the antibiotic to an antibody. Improved pharmacokinetics of AAC enable delivery of sufficient antibiotic in regions where *S. aureus* is concentrated while limiting the overall dose of antibiotic that needs to be administered systemically. This property should permit long-term therapy with AAC to target persistent infection with minimal antibiotic side effects.

This inventin also relates to antibody-drug conjugates which are antibody-antibiotic conjugates, wherein the antibody binds to bacteria and D is an antibiotic moiety.

This invention also relates to any one of the above antibody-antibiotic conjugates, wherein the D is a drug selected from the group comprising clindamycin, novobiocin, retapamulin, daptomycin, GSK-2140944, CG-400549, sitafloxacin, teicoplanin, triclosan, napthyridone, radezolid, doxorubicin, ampicillin, vancomycin, imipenem, doripenem, gemcitabine, dalbavancin, and azithromycin.

Another aspect of the invention is a pharmaceutical composition comprising an antibody-antibiotic conjugate compound of the invention.

Another aspect of the invention is a method of treating a bacterial infection by administering to a patient a therapeutically-effective amount of an antibody-antibiotic conjugate compound of the invention. In one embodiment, the patient is a human. In one embodiment the bacterial infection is a Staphylococcus aureus infection. In some embodiments, the patient has been diagnosed with a Staph aureus infection. In some embodiments, treating the bacterial infection comprises reducing bacterial load.

In another embodiment, the method of treatment further comprises administering a second therapeutic agent. In a further embodiment, the second therapeutic agent is an antibiotic.

In one embodiment, the second antibiotic administered in combination with the antibody-antibiotic conjugate compound of the invention is selected from the structural classes: : (i) aminoglycosides;

(ii) beta-lactams; (iii) macrolides/cyclic peptides; (iv) tetracyclines; (v) fluoroquinolines/fluoroquinolones; (vi) and oxazolidinones.

In one embodiment, the second antibiotic administered in combination with the antibody-antibiotic conjugate compound of the invention is selected from rifamycin, clindamycin, novobiocin, retapamulin, daptomycin, GSK-2140944, CG-400549, sitafloxacin, teicoplanin, triclosan, napthyridone, radezolid, doxorubicin, ampicillin, vancomycin, imipenem, doripenem, gemcitabine, dalbavancin, and azithromycin.

Another aspect of the invention is a process for making an antibody or an antibody-antibiotic conjugate compound of the invention.

Another aspect of the invention is a kit for treating a bacterial infection comprising a pharmaceutical composition of the invention and instructions for use.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to one or more of polypeptides selected from the group consisting of:

CLL1
BMPR1B;
E16;
STEAP1;
0772P;
MPF;
NaPi2b;
Sema 5b;
PSCA hlg;
ETBR;
MSG783;
STEAP2;
TrpM4;
CRIPTO;
CD21;
CD79b;
FcRH2;
HER2;
NCA;
MDP;

IL20Rα;
Brevican;
EphB2R;
ASLG659;
PSCA;
GEDA;
BAFF-R;
CD22;
CD79a;
CXCR5;
HLA-DOB;
P2X5 ;
CD72;
LY64;
FcRH1;
IRTA2;
TENB2;
PMEL17;
TMEFF1;
GDNF-Ra1;
Ly6E;
TMEM46;
Ly6G6D;
LGR5;
RET;
LY6K;
GPR19;
GPR54;
ASPHD1;
Tyrosinase;
TMEM118;
GPR172A;
MUC16 and
CD33.

This invention also relates to methods of treating a disease in a human in need thereof, comprising administering to said human an effective amount of an Antibody-drug conjugate of claim 1.

This invention also relates to pharmaceutical compositions comprising a compound of claim 1 and a pharmaceutically acceptable carrier thereof.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to one or more of polypeptides selected from the group consisting of:

CLL1;
STEAP1;
NaPi2b;
STEAP2;
TrpM4;
CRIPTO;
CD21;
CD79b;
FcRH2;
HER2;
CD22;
CD79a;
CD72;
LY64;
Ly6E;
MUC16; and
CD33.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD33.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD22.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to NaPi2b.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CLL1.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to Her2.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD33 and the anti-CD33 antibody comprise an HVR-L1 comprising the amino acid sequence of SEQ ID NO:11, an HVR-L2 comprising the amino acid sequence of SEQ ID

NO:12, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:13, an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 14, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:15, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 16.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD33 and the anti-CD33 antibody comprises a VL domain comprising the amino acid sequence of SEQ ID NO:17 and a VH domain comprising the amino acid sequence of SEQ ID NO:18.

In some embodiments, the antibody of the antibody-drug conjugate binds CD33. In some embodiments, the antibody of the antibody-drug conjugate comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:22; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:23; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:24; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:19; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:20; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:21.

In some embodiments, the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VL and VH sequences in SEQ ID NO:25 and SEQ ID NO:26, respectively, including post-translational modifications of those sequences.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to NaPi2b.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to NaPi2b and the NaPi2b antibody comprise an HVR-L1 comprising the amino acid sequence of SEQ ID NO:1, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:2, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:3, an HVR-H1 comprising the amino acid sequence of SEQ ID NO:4, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:5, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to NaPi2b and the NaPi2b antibody comprise s a VL domain comprising the amino acid sequence of SEQ ID NO:7 and a VH domain comprising the amino acid sequence of SEQ ID NO:8.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to NaPi2b and the NaPi2b antibody comprises an amino acid sequence of SEQ ID NO:9 and an amino acid sequence of SEQ ID NO: 10.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD22.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD22 and the CD22 antibody comprise an HVR-L1 comprising the amino acid sequence of SEQ ID NO:41, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:42, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:43, an HVR-H1 comprising the amino acid sequence of SEQ ID NO:44, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:45, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 46.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD22 and the CD22 antibody comprise s a VL domain comprising the amino acid sequence of SEQ ID NO:47 and a VH domain comprising the amino acid sequence of SEQ ID NO:48.

This invention also relates to any one of the above antibody-drug conjugates, wherein the antibody binds to CD22 and the CD22 antibody comprises an amino acid sequence of SEQ ID NO:49 and an amino acid sequence of SEQ ID NO: 50.

DEFINITIONS

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings: when trade names are used herein, applicants intend to independently include the trade name product formulation, the generic drug, and the active pharmaceutical ingredient(s) of the trade name product.

The term “peptidomimetic” or PM as used herein means a non-peptide chemical moiety. Peptides are short chains of amino acid monomers linked by peptide (amide) bonds, the covalent chemical bonds formed when the carboxyl group of one amino acid reacts with the amino group of another. The shortest peptides are dipeptides, consisting of 2 amino acids joined by a single peptide bond, followed by tripeptides, tetrapeptides, etc. A peptidomimetic chemical moiety includes non-amino acid chemical moieties. A peptidomimetic chemical moiety may also include one or more amino acid that are separated by one or more non-amino acid chemical units. A peptidomimetic chemical

moiety does not contain in any portion of its chemical structure two or more adjacent amino acids that are linked by peptide bonds.

The term "amino acid" as used herein means glycine, alanine, valine, leucine, isoleucine, phenylalanine, proline, serine, threonine, tyrosine, cysteine, methionine, lysine, arginine, histidine, tryptophan, aspartic acid, glutamic acid, asparagine, glutamine or citrulline.

The term "antibiotic" (abx or Abx) includes any molecule that specifically inhibits the growth of or kill micro-organisms, such as bacteria, but is non-lethal to the host at the concentration and dosing interval administered. In a specific aspect, an antibiotic is non-toxic to the host at the administered concentration and dosing intervals. Antibiotics effective against bacteria can be broadly classified as either bactericidal (*i.e.,* directly kills) or bacteriostatic (*i.e.,* prevents division). Anti-bactericidal antibiotics can be further subclassified as narrow-spectrum or broad-spectrum. A broad-spectrum antibiotic is one effective against a broad range of bacteria including both Gram-positive and Gram-negative bacteria, in contrast to a narrow-spectrum antibiotic, which is effective against a smaller range or specific families of bacteria. Examples of antibiotics include: (i) aminoglycosides, *e.g.*, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, paromycin, (ii) ansamycins, e.g., geldanamycin, herbimycin, (iii) carbacephems, e.g., loracarbef, (iv), carbapenems, *e.g.,* ertapenum, doripenem, imipenem/cilastatin, meropenem, (v) cephalosporins (first generation), *e.g.,* cefadroxil, cefazolin, cefalotin, cefalexin, (vi) cephalosporins (second generation), e.g., ceflaclor, cefamandole, cefoxitin, cefprozil, cefuroxime, (vi) cephalosporins (third generation), e.g., cefixime, cefdinir, cefditoren, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, (vii) cephalosporins (fourth generation), e.g., cefepime, (viii), cephalosporins (fifth generation), *e.g.*, ceftobiprole, (ix) glycopeptides, e.g., teicoplanin, vancomycin, (x) macrolides, *e.g.*, axithromycin, clarithromycin, dirithromycine, erythromycin, roxithromycin, troleandomycin, telithromycin, spectinomycin, (xi) monobactams, *e.g.*, axtreonam, (xii) penicilins, *e.g.*, amoxicillin, ampicillin, axlocillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, meticillin, nafcilin, oxacillin, penicillin, peperacillin, ticarcillin, (xiii) antibiotic polypeptides, *e.g.*, bacitracin, colistin, polymyxin B, (xiv) quinolones, e.g., ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, lemefloxacin, moxifloxacin, norfloxacin, orfloxacin, trovafloxacin, (xv) sulfonamides, *e.g.*, mafenide, prontosil, sulfacetamide, sulfamethizole, sulfanilamide, sulfasalazine, sulfisoxazole, trimethoprim, trimethoprim-sulfamethoxazole (TMP-SMX), (xvi) tetracyclines, *e.g.*, demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline and (xvii) others such as arspenamine, chloramphenicol, clindamycin, lincomycin, ethambutol, fosfomycin, fusidic acid, furazolidone, isoniazid, linezolid, metronidazole, mupirocin, nitrofurantoin, platensimycin, pyrazinamide, quinupristin/dalfopristin, rifampin/rifampicin or tinidazole.

The term "methicillin-resistant *Staphylococcus aureus"* (MRSA), alternatively known as multidrug resistant *Staphyloccus aureus* or *oxacillin-resistant Staphylococcus aureus* (ORSA), refers to any strain of *Staphyloccus aureus* that is resistant to beta-lactam antibiotics, which in include the penicillins (*e.g.*, methicillin, dicloxacillin, nafcillin, oxacillin, *etc.*) and the cephalosporins. "Methicillin-sensitive *Staphylococcus aureus"(MSSA)* refers to any strain of *Staphyloccus aureus* that is sensitive to beta-lactam antibiotics.

The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity (Miller et al (2003) Jour. of Immunology 170:4854-4861). Antibodies may be murine, human, humanized, chimeric, or derived from other species. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) Immuno Biology, 5th Ed., Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by CDRs on multiple antibodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody. An antibody includes a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, i.e., a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin disclosed herein can be of any type (e.g., IgG, IgE, IgM, IgD, and IgA), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The immunoglobulins can be derived from any species. In one aspect, however, the immunoglobulin is of human, murine, or rabbit origin.

The term "antibody fragment(s)" as used herein comprises a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; minibodies (Olafsen et al (2004) Protein Eng. Design & Sel. 17(4):315-323), fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR (complementary determining region), and epitope-binding fragments of any of the above which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens, single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.,* the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor

amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al (1975) Nature, 256:495, or may be made by recombinant DNA methods (see for example: US 4816567; US 5807715). The monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in Clackson et al (1991) Nature, 352:624-628; Marks et al (1991) J. Mol. Biol., 222:581-597; for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (US 4816567; and Morrison et al (1984) Proc. Natl. Acad. Sci. USA, 81:6851-6855). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (*e.g.,* Old World Monkey, Ape, etc.) and human constant region sequences.

The term "intact antibody" as used herein is one comprising a VL and VH domains, as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (*e.g.,* human native sequence constant domains) or amino acid sequence variant thereof. The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc constant region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; and down regulation of cell surface receptors such as B cell receptor and BCR.

The term "Fc region" as used hererin means a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc

regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

The term "framework" or "FR" as used herein refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4. Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes." There are five major classes of intact immunoglobulin antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Ig forms include hinge-modifications or hingeless forms (Roux et al (1998) J. Immunol. 161:4083-4090; Lund et al (2000) Eur. J. Biochem. 267:7246-7256; US 2005/0048572; US 2004/0229310).

The term "human antibody" as used herein refers to an anitbody which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

The term "human consensus framework" as used herein refers to a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra.* In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

The term "humanized antibody" as used herein refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term "hypervariable region" or "HVR," as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).) With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

The term "variable region" or "variable domain" as used herein refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated

using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The term "vector" as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

The term "free cysteine amino acid" as used herein refers to a cysteine amino acid residue which has been engineered into a parent antibody, has a thiol functional group (-SH), and is not paired as an intramolecular or intermolecular disulfide bridge.

Ther term "Linker", "Linker Unit", or "link" as used herein means a chemical moiety comprising a chain of atoms that covalently attaches a drug moiety to an antibody. In various embodiments, a linker is a divalent radical, specified as L.

The term "drug moiety" as used herein means a substance that that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; and the various antitumor or anticancer agents disclosed below.

As used herein, unless defined otherwise in a claim, the term "acyl" refers to the group -C(O)R', where R' is alkyl, C_3-C_6cycloalkyl, or heterocyclyl, as each is defined herein.
As used herein, unless defined otherwise in a claim, the term "alkoxy" refers to the group –OR', where R' is C_1-C_4alkyl or C_3-C_6cycloalkyl as defined above. Examples of "alkoxy" include methoxy, ethoxy, isopropoxy, propoxy, butoxy, t-butoxy, isobutoxy, cyclopropoxy, and cyclobutoxy, and halogenated forms thereof, e.g. fluoromethoxy and difluoromethoxy.

As used herein, unless defined otherwise in a claim, the term "alkyl" refers to a straight or branched, monovalent or divalent hydrocarbon chain radical having from one to twelve(C_1-C_{12}) carbon atoms, which may be unsubstituted or substituted with multiple degrees of substitution, for

example one, two, three, four, five or six included within the present invention. Examples of substituents are selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid and alkylthio. Examples of "alkyl" as used herein include, but are not limited to, methyl (Me, $-CH_3$), ethyl (Et, $-CH_2CH_3$), 1-propyl (n-Pr, n-propyl, $-CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl, $-CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, $-CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl, $-CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, $-CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, $-C(CH_3)_3$), 1-pentyl (n-pentyl, $-CH_2CH_2CH_2CH_2CH_3$), 2-pentyl ($-CH(CH_3)CH_2CH_2CH_3$), 3-pentyl ($-CH(CH_2CH_3)_2$), 2-methyl-2-butyl ($-C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl ($-CH(CH_3)CH(CH_3)_2$), 3-methyl-1-butyl ($-CH_2CH_2CH(CH_3)_2$), 2-methyl-1-butyl ($-CH_2CH(CH_3)CH_2CH_3$), 1-hexyl ($-CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl ($-CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl ($-CH(CH_2CH_3)(CH_2CH_2CH_3)$), 2-methyl-2-pentyl ($-C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl ($-CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl ($-CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl ($-C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl ($-CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl ($-C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl ($-CH(CH_3)C(CH_3)_3$, as well as the divalent ("alkylene") and substituted versions thereof. Examples of substituted alkyl include but are not limited to, hydroxymethyl, difluoromethyl and trifluoromethyl.

As used herein unless otherwise defined in a claim, the term "alkenyl" means a linear or branched, monovalent or divalent hydrocarbon chain radical of any length from two to eight carbon atoms (C_2–C_{10}) with at least one site of unsaturation, i.e., a carbon-carbon, sp^2 double bond, wherein the alkenyl radical may be optionally substituted independently with one or more substituents described above in the definition of "alkyl", and includes radicals having "cis" and "trans" orientations, or alternatively, "E" and "Z" orientations. Examples of alkenyl include, but are not limited to, ethenyl or vinyl ($-CH=CH_2$), prop-1-enyl ($-CH=CHCH_3$), prop-2-enyl ($-CH_2CH=CH_2$), 2-methylprop-1-enyl, but-1-enyl, but-2-enyl, but-3-enyl, buta-1,3-dienyl, 2-methylbuta-1,3-diene, hex-1-enyl, hex-2-enyl, hex-3-enyl, hex-4-enyl, hexa-1,3-dienyl as well as the divalent ("alkenylene") and substituted versions thereof.

As used herein unless otherwise defined in a claim, the term "alkynyl" refers to a linear or branched, monovalent or divalent hydrocarbon radical of any length from two to eight carbon atoms (C_2–C_{10}) with at least one site of unsaturation, i.e., a carbon-carbon, sp triple bond, wherein the alkynyl radical may be optionally substituted independently with one or more substituents described above in the definition of alkyl, examples of alkynyl includes, but not limited to, ethynyl

(-C≡CH), prop-1-ynyl (-C≡CCH$_3$), prop-2-ynyl (propargyl, -CH$_2$C≡CH), but-1-ynyl, but-2-ynyl and but-3-ynyl, as well as the divalent ("alkynylene") and substituted versions thereof.

As used herein, unless defined otherwise in a claim, the term "alkylamino" refers to the group –NR'R", wherein R' is H, C$_1$-C$_6$alkyl or C$_3$-C$_6$cycloalkyl, and R" is C$_1$-C$_6$alkyl or C$_3$-C$_6$cycloalkyl, examples of alkylamino include, but are not limited to, methylamino, dimethylamino, ethylamino, diethylamino, propylamino and cyclopropylamino.

As used herein, unless defined otherwise in a claim, the term "amide" refers to the group –C(O)NR'R", wherein R' and R" are each independently H, C$_1$-C$_6$alkyl, or C$_3$-C$_6$cycloalkyl; examples of amide include, but are not limited to, -C(O)NH$_2$, -C(O)NHCH$_3$, and -C(O)N(CH$_3$)$_2$.

As used herein, unless defined otherwise in a claim, the term "aryl" refers to an aromatic, hydrocarbon, ring system. The ring system may be monocyclic or fused polycyclic (e.g., bicyclic, tricyclic, etc.), substituted or unsubstituted. In various embodiments, the monocyclic aryl ring is C$_5$-C$_{10}$, or C$_5$-C$_7$, or C$_5$-C$_6$, where these carbon numbers refer to the number of carbon atoms that form the ring system. A C$_6$ ring system, i.e. a phenyl ring, is an aryl group. In various embodiments, the polycyclic ring is a bicyclic aryl group, where examples of bicyclic aryl groups include are C$_8$-C$_{12}$, or C$_9$-C$_{10}$. A naphthyl ring, which has 10 carbon atoms, is a polycyclic aryl group. Examples of substituents for aryl are described below in the definition of "optionally substituted".

As used herein, unless defined otherwise in a claim, the term "cyano" refers to the group -CN.

As used herein, unless defined otherwise in a claim, "cycloalkyl" refers to a non-aromatic, substituted or unsubstituted, saturated or partially unsaturated hydrocarbon ring group. Examples of substituents are described in the definition of "optionally substituted". In one example, the cycloalkyl group is 3 to 12 carbon atoms (C$_3$-C$_{12}$). In other examples, cycloalkyl is C$_3$-C$_8$, C$_3$-C$_{10}$ or C$_5$-C$_{10}$. In other examples, the cycloalkyl group, as a monocycle, is C$_3$-C$_8$, C$_3$-C$_6$ or C$_5$-C$_6$. In another example, the cycloalkyl group, as a bicycle, is C$_7$-C$_{12}$. In another example, the cycloalkyl group, as a spiro system, is C$_5$-C$_{12}$. Examples of monocyclic cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, perdeuteriocyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cyclohexadienyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl and cyclododecyl. Exemplary arrangements of bicyclic cycloalkyls having 7 to 12 ring atoms include, but are not limited to, [4,4], [4,5], [5,5], [5,6] or [6,6] ring systems. Exemplary bridged bicyclic cycloalkyls include, but are not limited to, bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane and

bicyclo[3.2.2]nonane. Examples of spiro cycloalkyl include, spiro[2.2]pentane, spiro[2.3]hexane, spiro[2.4]heptane, spiro[2.5]octane and spiro[4.5]decane.

As used herein, unless defined otherwise in a claim, the term "ester" refers to the group -C(O)OR', where R' is C_1-C_6alkyl, or C_3-C_6cycloalkyl.

As used herein, unless defined otherwise in a claim, the term "heterocycle" "heterocycloalkyl" or "heterocyclyl" refers to unsubstituted and substituted mono- or polycyclic non-aromatic ring system containing 2 to 12 ring carbon atoms and 1 to 3 ring hetero atoms. Polycyclic ring systems can be fused bi- or tri-cyclic, spiro or bridged. Examples of heteroatoms include N, O, and S, including N-oxides, sulfur oxides, and dioxides. In one embodiment, the ring is three to eight-membered and is either fully saturated or has one or more degrees of unsaturation. Multiple degrees of substitution are included within the present definition. Examples of substituents are defined hereunder. Examples of "heterocyclic" groups include, but are not limited to tetrahydrofuranyl, pyranyl, 1,4-dioxanyl, 1,3-dioxanyl, oxolanyl, oxetanyl, 2-oxa-6-azaspiro[3.3]heptan-6-yl, piperidinyl, pyrrolidinyl, morpholinyl, azetidinyl, piperazinyl, pyrrolidinonyl, piperazinonyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, and their various tautomers.

As used herein, unless defined otherwise in a claim, the term "heteroaryl", unless defined otherwise in a claim, refers to an aromatic ring system containing 1 to 9 carbon(s) and at least one heteroatom. Examples of heteroatoms include N, O, and S. Heteroaryl may be monocyclic or polycyclic, substituted or unsubstituted. A monocyclic heteroaryl group may have 2 to 6 ring carbon atoms and 1 to 3 ring hetero atoms in the ring, while a polycyclic heteroaryl may contain 3 to 9 ring carbon atoms and 1 to 5 ring hetero atoms. A polycyclic heteroaryl ring may contain fused, spiro or bridged ring junctions, for example, bicyclic heteroaryl is a polycyclic heteroaryl. Bicyclic heteroaryl rings may contain from 8 to 12 member atoms. Monocyclic heteroaryl rings may contain from 5 to 8 member atoms (carbons and heteroatoms). Exemplary heteroaryl groups include but are not limited to: benzofuranyl, benzothiophenyl, furanyl, imidazolyl, indolyl, azaindolyl, azabenzimidazolyl, benzoxazolyl, benzthiazolyl, benzothiadiazolyl, benzotriazolyl, benzoimidazolyl, tetrazinyl, tetrazolyl, isothiazolyl, oxazolyl, isoxazolyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridinyl, pyrimidinyl, pyrrolyl, quinolinyl, quinazolinyl, quinoxalinyl, triazinyl, triazolyl, thiazolyl and thiophenyl. Examples of substituents for heteroaryl are described below in the definition of "optionally substituted".

As used herein, unless defined otherwise in a claim, the term "heteroarylalkyl" means the group (heteroaryl)C_1-C_3alkyl.

As used herein, unless defined otherwise in a claim, the term "arylalkyl" means the group (aryl)C_1-C_3alkyl.

As used herein, unless defined otherwise in a claim, the term "urea" refers to the group –NR'C(O)NR", wherein R' and R" are each independently H, C_1-C_6alkyl, or C_3-C_6cycloalkyl.

As used herein, unless defined otherwise in a claim, the term "optionally" means that the subsequently described event(s) may or may not occur, and includes both event(s) that occur and event(s) that do not occur.

As used herein, unless defined otherwise, the phrase "optionally substituted", "substituted" or variations thereof denote an optional substitution, including multiple degrees of substitution, with one or more substituent group, for example, one, two or three. The phrase should not be interpreted as duplicative of the substitutions herein described and depicted. Exemplary optional substituent groups include acyl, C_1-C_6alkyl, sulfonyl, amino, sulfonamide, sulfoxide, alkoxy, cyano, halo, urea, ester, carboxylic acid, amide, hydroxy, oxo, and nitro.

As used herein, unless defined otherwise in a claim, the term "treatment" refers to alleviating the specified condition, eliminating or reducing one or more symptoms of the condition, slowing or eliminating the progression of the condition

As used herein, unless defined otherwise in a claim, the term "effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal, or human that is being sought, for instance, by a researcher or clinician.

As used herein, unless defined otherwise in a claim, the term "therapeutically effective amount" means any amount which, as compared to a corresponding subject who has not received such amount, results in treatment of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function. For use in therapy, therapeutically effective amounts of a compound of Formula I, as well as salts thereof, may be administered as the raw chemical. Additionally, the active ingredient may be presented as a pharmaceutical composition.

This invention also relates to any one of the examples in the Experimental section.
The phrase "pharmaceutically acceptable salt," as used herein, refers to pharmaceutically acceptable organic or inorganic salts of an antibody-drug conjugate (ADC) or a linker-drug

moiety. Exemplary salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, and pamoate (*i.e.*, 1,1'-methylene-bis -(2-hydroxy-3- naphthoate)) salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure. Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterion.

Other salts, which are not pharmaceutically acceptable, may be useful in the preparation of compounds of this invention and these should be considered to form a further aspect of the invention. These salts, such as oxalic or trifluoroacetate, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable salts.

Compounds of the present invention may exist in solid or liquid form. In the solid state, it may exist in crystalline or noncrystalline form, or as a mixture thereof. The skilled artisan will appreciate that pharmaceutically acceptable solvates may be formed for crystalline or non-crystalline compounds. In crystalline solvates, solvent molecules are incorporated into the crystalline lattice during crystallization. Solvates may involve non-aqueous solvents such as, but not limited to, ethanol, isopropanol, DMSO, acetic acid, ethanolamine, or ethyl acetate, or they may involve water as the solvent that is incorporated into the crystalline lattice. Solvates wherein water is the solvent incorporated into the crystalline lattice are typically referred to as "hydrates." Hydrates include stoichiometric hydrates as well as compositions containing variable amounts of water. The invention includes all such solvates.

The skilled artisan will further appreciate that certain compounds of the invention that exist in crystalline form, including the various solvates thereof, may exhibit polymorphism (i.e. the capacity to occur in different crystalline structures). These different crystalline forms are typically known as "polymorphs." The invention includes all such polymorphs. Polymorphs have the same chemical composition but differ in packing, geometrical arrangement, and other descriptive properties of the crystalline solid state. Polymorphs, therefore, may have different physical properties such as shape, density, hardness, deformability, stability, and dissolution properties.

Polymorphs typically exhibit different melting points, IR spectra, and X-ray powder diffraction patterns, which may be used for identification. The skilled artisan will appreciate that different polymorphs may be produced, for example, by changing or adjusting the reaction conditions or reagents, used in making the compound. For example, changes in temperature, pressure, or solvent may result in polymorphs. In addition, one polymorph may spontaneously convert to another polymorph under certain conditions.

Compounds of the present invention or a salt thereof may exist in stereoisomeric forms (e.g., it contains one or more asymmetric carbon atoms). The individual stereoisomers (enantiomers and diastereomers) and mixtures of these are included within the scope of the present invention. Likewise, it is understood that a compound or salt of Formula (I) may exist in tautomeric forms other than that shown in the formula and these are also included within the scope of the present invention. It is to be understood that the present invention includes all combinations and subsets of the particular groups defined hereinabove. The scope of the present invention includes mixtures of stereoisomers as well as purified enantiomers or enantiomerically/diastereomerically enriched mixtures. It is to be understood that the present invention includes all combinations and subsets of the particular groups defined hereinabove.

The subject invention also includes isotopically-labelled forms of the compounds of the present invention, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention and pharmaceutically acceptable salts thereof include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, sulphur, fluorine, iodine, and chlorine, such as 2H, 3H, 11C, 13C, 14C, 15N, 17O, 18O, 31P, 32P, 35S, 18F, 36Cl, 123I and 125I.

Compounds of the present invention and pharmaceutically acceptable salts of said compounds that contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of the present invention. Isotopically-labelled compounds of the present invention, for example those into which radioactive isotopes such as 3H, 14C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i.e., 3H, and carbon-14, i.e., 14C, isotopes are commonly used for their ease of preparation and detectability. 11C and 18F isotopes are useful in PET (positron emission tomography), and 125I isotopes are useful in SPECT (single photon emission computerized tomography), all useful in brain imaging. Further, substitution with heavier isotopes such as deuterium, i.e., 2H, can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labelled compounds of formula I

and following of this invention can generally be prepared by carrying out the procedures disclosed in the Schemes and/or in the Examples below, by substituting a readily available isotopically labelled reagent for a non-isotopically labelled reagent.

PHARMACEUTICAL COMPOSITION OF ADCS

Pharmaceutical formulations of therapeutic antibody-drug conjugates (ADC) of the invention are typically prepared for parenteral administration, i.e. bolus, intravenous, intratumor injection with a pharmaceutically acceptable parenteral vehicle and in a unit dosage injectable form. An antibody-drug conjugate (ADC) having the desired degree of purity is optionally mixed with pharmaceutically acceptable diluents, carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences (1980) 16th edition, Osol, A. Ed.), in the form of a lyophilized formulation or an aqueous solution.

CYSTEINE ENGINEERED ANTIBODIES

The compounds of the invention include antibody-drug conjugates comprising cysteine engineered antibodies where one or more amino acids of a wild-type or parent antibody are replaced with a cysteine amino acid. Any form of antibody may be so engineered, i.e. mutated. For example, a parent Fab antibody fragment may be engineered to form a cysteine engineered Fab, referred to herein as "ThioFab." Similarly, a parent monoclonal antibody may be engineered to form a "ThioMab." It should be noted that a single site mutation yields a single engineered cysteine residue in a ThioFab, while a single site mutation yields two engineered cysteine residues in a ThioMab, due to the dimeric nature of the IgG antibody. Mutants with replaced ("engineered") cysteine (Cys) residues are evaluated for the reactivity of the newly introduced, engineered cysteine thiol groups. The thiol reactivity value is a relative, numerical term in the range of 0 to 1.0 and can be measured for any cysteine engineered antibody. Thiol reactivity values of cysteine engineered antibodies of the invention are in the ranges of 0.6 to 1.0; 0.7 to 1.0; or 0.8 to 1.0.
To prepare a cysteine engineered antibody by mutagenesis, DNA encoding an amino acid sequence variant of the starting polypeptide is prepared by a variety of methods known in the art. These methods include, but are not limited to, preparation by site-directed (or oligonucleotide-mediated) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared DNA encoding the polypeptide. Variants of recombinant antibodies may be constructed also by restriction fragment manipulation or by overlap extension PCR with synthetic oligonucleotides. Mutagenic primers encode the cysteine codon replacement(s). Standard mutagenesis techniques can be employed to generate DNA encoding such mutant cysteine engineered antibodies. General guidance can be found in Sambrook et al Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; and Ausubel et al Current Protocols in Molecular Biology, Greene Publishing and Wiley-Interscience, New York, N.Y., 1993.

Cysteine amino acids may be engineered at reactive sites in an antibody and which do not form intrachain or intermolecular disulfide linkages (Junutula, et al., 2008b Nature Biotech., 26(8):925-932; Dornan et al (2009) Blood 114(13):2721-2729; US 7521541; US 7723485; WO2009/052249, Shen et al (2012) Nature Biotech., 30(2):184-191; Junutula et al (2008) Jour of Immun. Methods 332:41-52). The engineered cysteine thiols may react with linker reagents or the linker-drug intermediates of the present invention which have thiol-reactive, electrophilic groups such as maleimide or alpha-halo amides to form ADC with cysteine engineered antibodies (ThioMabs) and the drug (D) moiety. The location of the drug moiety can thus be designed, controlled, and known. The drug loading can be controlled since the engineered cysteine thiol groups typically react with thiol-reactive linker reagents or linker-drug intermediates in high yield. Engineering an antibody to introduce a cysteine amino acid by substitution at a single site on the heavy or light chain gives two new cysteines on the symmetrical antibody. A drug loading near 2 can be achieved and near homogeneity of the conjugation product ADC.

Cysteine engineered antibodies of the invention preferably retain the antigen binding capability of their wild type, parent antibody counterparts. Thus, cysteine engineered antibodies are capable of binding, preferably specifically, to antigens. Such antigens include, for example, tumor-associated antigens (TAA), cell surface receptor proteins and other cell surface molecules, transmembrane proteins, signaling proteins, cell survival regulatory factors, cell proliferation regulatory factors, molecules associated with (for e.g., known or suspected to contribute functionally to) tissue development or differentiation, lymphokines, cytokines, molecules involved in cell cycle regulation, molecules involved in vasculogenesis and molecules associated with (for e.g., known or suspected to contribute functionally to) angiogenesis. The tumor-associated antigen may be a cluster differentiation factor (i.e., a CD protein). An antigen to which a cysteine engineered antibody is capable of binding may be a member of a subset of one of the above-mentioned categories, wherein the other subset(s) of said category comprise other molecules/antigens that have a distinct characteristic (with respect to the antigen of interest).
Cysteine engineered antibodies are prepared for conjugation with linker-drug intermediates by reduction and reoxidation of intrachain disulfide groups.

TUMOR-ASSOCIATED ANTIGENS:

Antibodies, including but not limited to cysteine engineered antibodies, which may be useful in the antibody-drug conjugates of the invention in the treatment of cancer include, but are not limited to, antibodies against cell surface receptors and tumor-associated antigens (TAA). Certain tumor-associated antigens are known in the art, and can be prepared for use in generating antibodies using methods and information which are well known in the art. In attempts to discover effective cellular targets for cancer diagnosis and therapy, researchers have sought to identify

transmembrane or otherwise tumor-associated polypeptides that are specifically expressed on the surface of one or more particular type(s) of cancer cell as compared to on one or more normal non-cancerous cell(s). Often, such tumor-associated polypeptides are more abundantly expressed on the surface of the cancer cells as compared to on the surface of the non-cancerous cells. The identification of such tumor-associated cell surface antigen polypeptides has given rise to the ability to more specifically target cancer cells for destruction via antibody-based therapies. Examples of tumor-associated antigens TAA include, but are not limited to, those listed below. For convenience, information relating to these antigens, all of which are known in the art, is listed below and includes names, alternative names, Genbank accession numbers and primary reference(s), following nucleic acid and protein sequence identification conventions of the National Center for Biotechnology Information (NCBI). Nucleic acid and protein sequences corresponding to TAA listed below are available in public databases such as GenBank. Tumor-associated antigens targeted by antibodies include all amino acid sequence variants and isoforms possessing at least about 70%, 80%, 85%, 90%, or 95% sequence identity relative to the sequences identified in the cited references, and/or which exhibit substantially the same biological properties or characteristics as a TAA having a sequence found in the cited references. For example, a TAA having a variant sequence generally is able to bind specifically to an antibody that binds specifically to the TAA with the corresponding sequence listed. The sequences and disclosure in the reference specifically recited herein are expressly incorporated by reference.

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203)
ten Dijke,P., et al Science 264 (5155):101-104 (1994), Oncogene 14 (11):1377-1382 (1997)); WO2004063362 (Claim 2); WO2003042661 (Claim 12); US2003134790-A1 (Page 38-39); WO2002102235 (Claim 13; Page 296); WO2003055443 (Page 91-92); WO200299122 (Example 2; Page 528-530); WO2003029421 (Claim 6); WO2003024392 (Claim 2; Fig 112); WO200298358 (Claim 1; Page 183); WO200254940 (Page 100-101); WO200259377(Page 349-350); WO200230268 (Claim 27; Page 376); WO200148204 (Example; Fig 4)
NP_001194 bone morphogenetic protein receptor, type IB /pid=NP_001194.1 - Cross-references: MIM:603248; NP_001194.1; AY065994

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486)
Biochem. Biophys. Res. Commun. 255 (2), 283-288 (1999), Nature 395 (6699):288-291 (1998), Gaugitsch, H.W., et al (1992) J. Biol. Chem. 267 (16):11267-11273); WO2004048938 (Example 2); WO2004032842 (Example IV); WO2003042661 (Claim 12); WO2003016475 (Claim 1); WO200278524 (Example 2); WO200299074 (Claim 19; Page 127-129); WO200286443 (Claim 27; Pages 222, 393); WO2003003906 (Claim 10; Page 293); WO200264798 (Claim 33; Page 93-

95); WO200014228 (Claim 5; Page 133-136); US2003224454 (Fig 3); WO2003025138 (Claim 12; Page 150);

NP_003477 solute carrier family 7 (cationic amino acid transporter, y+ system), member 5 /pid=NP_003477.3 - Homo sapiens

Cross-references: MIM:600182; NP_003477.3; NM_015923; NM_003486_1

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449)

Cancer Res. 61 (15), 5857-5860 (2001), Hubert, R.S., et al (1999) Proc. Natl. Acad. Sci. U.S.A. 96 (25):14523-14528); WO2004065577 (Claim 6); WO2004027049 (Fig 1L); EP1394274 (Example 11); WO2004016225 (Claim 2); WO2003042661 (Claim 12); US2003157089 (Example 5); US2003185830 (Example 5); US2003064397 (Fig 2); WO200289747 (Example 5; Page 618-619); WO2003022995 (Example 9; Fig 13A, Example 53; Page 173, Example 2; Fig 2A);

NP_036581 six transmembrane epithelial antigen of the prostate

Cross-references: MIM:604415; NP_036581.1; NM_012449_1

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486)

J. Biol. Chem. 276 (29):27371-27375 (2001)); WO2004045553 (Claim 14); WO200292836 (Claim 6; Fig 12); WO200283866 (Claim 15; Page 116-121); US2003124140 (Example 16); Cross-references: GI:34501467; AAK74120.3; AF361486_1

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823) Yamaguchi, N., et al Biol. Chem. 269 (2), 805-808 (1994), Proc. Natl. Acad. Sci. U.S.A. 96 (20):11531-11536 (1999), Proc. Natl. Acad. Sci. U.S.A. 93 (1):136-140 (1996), J. Biol. Chem. 270 (37):21984-21990 (1995)); WO2003101283 (Claim 14); (WO2002102235 (Claim 13; Page 287-288); WO2002101075 (Claim 4; Page 308-309); WO200271928 (Page 320-321); WO9410312 (Page 52-57); Cross-references: MIM:601051; NP_005814.2; NM_005823_1

(6) Napi3b/NaPi2b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b,Genbank accession no. NM_006424)

J. Biol. Chem. 277 (22):19665-19672 (2002), Genomics 62 (2):281-284 (1999), Feild, J.A., et al (1999) Biochem. Biophys. Res. Commun. 258 (3):578-582); WO2004022778 (Claim 2); EP1394274 (Example 11); WO2002102235 (Claim 13; Page 326); EP875569 (Claim 1; Page 17-

19); WO200157188 (Claim 20; Page 329); WO2004032842 (Example IV); WO200175177 (Claim 24; Page 139-140);
Cross-references: MIM:604217; NP_006415.1; NM_006424_1

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878)
Nagase T., et al (2000) DNA Res. 7 (2):143-150); WO2004000997 (Claim 1); WO2003003984 (Claim 1); WO200206339 (Claim 1; Page 50); WO200188133 (Claim 1; Page 41-43, 48-58); WO2003054152 (Claim 20); WO2003101400 (Claim 11);
Accession: Q9P283; EMBL; AB040878; BAA95969.1. Genew; HGNC:10737;

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628); Ross et al (2002) Cancer Res. 62:2546-2553; US2003129192 (Claim 2); US2004044180 (Claim 12); US2004044179 (Claim 11); US2003096961 (Claim 11); US2003232056 (Example 5); WO2003105758 (Claim 12); US2003206918 (Example 5); EP1347046 (Claim 1); WO2003025148 (Claim 20);
Cross-references: GI:37182378; AAQ88991.1; AY358628_1

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);
Nakamuta M., et al Biochem. Biophys. Res. Commun. 177, 34-39, 1991; Ogawa Y., et al Biochem. Biophys. Res. Commun. 178, 248-255, 1991; Arai H., et al Jpn. Circ. J. 56, 1303-1307, 1992; Arai H., et al J. Biol. Chem. 268, 3463-3470, 1993; Sakamoto A., Yanagisawa M., et al Biochem. Biophys. Res. Commun. 178, 656-663, 1991; Elshourbagy N.A., et al J. Biol. Chem. 268, 3873-3879, 1993; Haendler B., et al J. Cardiovasc. Pharmacol. 20, s1-S4, 1992; Tsutsumi M., et al Gene 228, 43-49, 1999; Strausberg R.L., et al Proc. Natl. Acad. Sci. U.S.A. 99, 16899-16903, 2002; Bourgeois C., et al J. Clin. Endocrinol. Metab. 82, 3116-3123, 1997; Okamoto Y., et al Biol. Chem. 272, 21589-21596, 1997; Verheij J.B., et al Am. J. Med. Genet. 108, 223-225, 2002; Hofstra R.M.W., et al Eur. J. Hum. Genet. 5, 180-185, 1997; Puffenberger E.G., et al Cell 79, 1257-1266, 1994; Attie T., et al, Hum. Mol. Genet. 4, 2407-2409, 1995; Auricchio A., et al Hum. Mol. Genet. 5:351-354, 1996; Amiel J., et al Hum. Mol. Genet. 5, 355-357, 1996; Hofstra R.M.W., et al Nat. Genet. 12, 445-447, 1996; Svensson P.J., et al Hum. Genet. 103, 145-148, 1998; Fuchs S., et al Mol. Med. 7, 115-124, 2001; Pingault V., et al (2002) Hum. Genet. 111, 198-206; WO2004045516 (Claim 1); WO2004048938 (Example 2); WO2004040000 (Claim 151); WO2003087768 (Claim 1); WO2003016475 (Claim 1); WO2003016475 (Claim 1); WO200261087 (Fig 1); WO2003016494 (Fig 6); WO2003025138 (Claim 12; Page 144); WO200198351 (Claim 1; Page 124-125); EP522868 (Claim 8; Fig 2); WO200177172 (Claim 1;

Page 297-299); US2003109676; US6518404 (Fig 3); US5773223 (Claim 1a; Col 31-34); WO2004001004;

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763); WO2003104275 (Claim 1); WO2004046342 (Example 2); WO2003042661 (Claim 12); WO2003083074 (Claim 14; Page 61); WO2003018621 (Claim 1); WO2003024392 (Claim 2; Fig 93); WO200166689 (Example 6);
Cross-references: LocusID:54894; NP_060233.2; NM_017763_1

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138)
Lab. Invest. 82 (11):1573-1582 (2002)); WO2003087306; US2003064397 (Claim 1; Fig 1); WO200272596 (Claim 13; Page 54-55); WO200172962 (Claim 1; Fig 4B); WO2003104270 (Claim 11); WO2003104270 (Claim 16); US2004005598 (Claim 22); WO2003042661 (Claim 12); US2003060612 (Claim 12; Fig 10); WO200226822 (Claim 23; Fig 2); WO200216429 (Claim 12; Fig 10);
Cross-references: GI:22655488; AAN04080.1; AF455138_1

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636)
Xu, X.Z., et al Proc. Natl. Acad. Sci. U.S.A. 98 (19):10692-10697 (2001), Cell 109 (3):397-407 (2002), J. Biol. Chem. 278 (33):30813-30820 (2003)); US2003143557 (Claim 4); WO200040614 (Claim 14; Page 100-103); WO200210382 (Claim 1; Fig 9A); WO2003042661 (Claim 12); WO200230268 (Claim 27; Page 391); US2003219806 (Claim 4); WO200162794 (Claim 14; Fig 1A-D);
Cross-references: MIM:606936; NP_060106.2; NM_017636_1

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212)
Ciccodicola, A., et al EMBO J. 8 (7):1987-1991 (1989), Am. J. Hum. Genet. 49 (3):555-565 (1991)); US2003224411 (Claim 1); WO2003083041 (Example 1); WO2003034984 (Claim 12); WO200288170 (Claim 2; Page 52-53); WO2003024392 (Claim 2; Fig 58); WO200216413 (Claim 1; Page 94-95, 105); WO200222808 (Claim 2; Fig 1); US5854399 (Example 2; Col 17-18); US5792616 (Fig 2);
Cross-references: MIM:187395; NP_003203.1; NM_003212_1

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792 Genbank accession no. M26004)
Fujisaku et al (1989) J. Biol. Chem. 264 (4):2118-2125); Weis J.J., et al J. Exp. Med. 167, 1047-1066, 1988; Moore M., et al Proc. Natl. Acad. Sci. U.S.A. 84, 9194-9198, 1987; Barel M., et al Mol. Immunol. 35, 1025-1031, 1998; Weis J.J., et al Proc. Natl. Acad. Sci. U.S.A. 83, 5639-5643, 1986; Sinha S.K., et al (1993) J. Immunol. 150, 5311-5320; WO2004045520 (Example 4); US2004005538 (Example 1); WO2003062401 (Claim 9); WO2004045520 (Example 4); WO9102536 (Fig 9.1-9.9); WO2004020595 (Claim 1);
Accession: P20023; Q13866; Q14212; EMBL; M26004; AAA35786.1.

(15) CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626 or 11038674)
Proc. Natl. Acad. Sci. U.S.A. (2003) 100 (7):4126-4131, Blood (2002) 100 (9):3068-3076, Muller et al (1992) Eur. J. Immunol. 22 (6):1621-1625); WO2004016225 (claim 2, Fig 140); WO2003087768, US2004101874 (claim 1, page 102); WO2003062401 (claim 9); WO200278524 (Example 2); US2002150573 (claim 5, page 15); US5644033; WO2003048202 (claim 1, pages 306 and 309); WO 99/558658, US6534482 (claim 13, Fig 17A/B); WO200055351 (claim 11, pages 1145-1146);
Cross-references: MIM:147245; NP_000617.1; NM_000626_1

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764, AY358130)
Genome Res. 13 (10):2265-2270 (2003), Immunogenetics 54 (2):87-95 (2002), Blood 99 (8):2662-2669 (2002), Proc. Natl. Acad. Sci. U.S.A. 98 (17):9772-9777 (2001), Xu, M.J., et al (2001) Biochem. Biophys. Res. Commun. 280 (3):768-775; WO2004016225 (Claim 2); WO2003077836; WO200138490 (Claim 5; Fig 18D-1-18D-2); WO2003097803 (Claim 12); WO2003089624 (Claim 25);
Cross-references: MIM:606509; NP_110391.2; NM_030764_1

(17) HER2 (ErbB2, Genbank accession no. M11730)
Coussens L., et al Science (1985) 230(4730):1132-1139); Yamamoto T., et al Nature 319, 230-234, 1986; Semba K., et al Proc. Natl. Acad. Sci. U.S.A. 82, 6497-6501, 1985; Swiercz J.M., et al J. Cell Biol. 165, 869-880, 2004; Kuhns J.J., et al J. Biol. Chem. 274, 36422-36427, 1999; Cho H.-S., et al Nature 421, 756-760, 2003; Ehsani A., et al (1993) Genomics 15, 426-429; WO2004048938 (Example 2); WO2004027049 (Fig 1I); WO2004009622; WO2003081210; WO2003089904 (Claim 9); WO2003016475

(Claim 1); US2003118592; WO2003008537 (Claim 1); WO2003055439 (Claim 29; Fig 1A-B); WO2003025228 (Claim 37; Fig 5C); WO200222636 (Example 13; Page 95-107); WO200212341 (Claim 68; Fig 7); WO200213847 (Page 71-74); WO200214503 (Page 114-117); WO200153463 (Claim 2; Page 41-46); WO200141787 (Page 15); WO200044899 (Claim 52; Fig 7); WO200020579 (Claim 3; Fig 2); US5869445 (Claim 3; Col 31-38); WO9630514 (Claim 2; Page 56-61); EP1439393 (Claim 7); WO2004043361 (Claim 7); WO2004022709; WO200100244 (Example 3; Fig 4); Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761; AAA35808.1.

(18) NCA (CEACAM6, Genbank accession no. M18728);
Barnett T., et al Genomics 3, 59-66, 1988; Tawaragi Y., et al Biochem. Biophys. Res. Commun. 150, 89-96, 1988; Strausberg R.L., et al Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903, 2002; WO2004063709; EP1439393 (Claim 7); WO2004044178 (Example 4); WO2004031238; WO2003042661 (Claim 12); WO200278524 (Example 2); WO200286443 (Claim 27; Page 427); WO200260317 (Claim 2);
Accession: P40199; Q14920; EMBL; M29541; AAA59915.1. EMBL; M18728;

(19) MDP (DPEP1, Genbank accession no. BC017023)
Proc. Natl. Acad. Sci. U.S.A. 99 (26):16899-16903 (2002)); WO2003016475 (Claim 1); WO200264798 (Claim 33; Page 85-87); JP05003790 (Fig 6-8); WO9946284 (Fig 9);
Cross-references: MIM:179780; AAH17023.1; BC017023_1

(20) IL20Rα (IL20Ra, ZCYTOR7, Genbank accession no. AF184971);
Clark H.F., et al Genome Res. 13, 2265-2270, 2003; Mungall A.J., et al Nature 425, 805-811, 2003; Blumberg H., et al Cell 104, 9-19, 2001; Dumoutier L., et al J. Immunol. 167, 3545-3549, 2001; Parrish-Novak J., et al J. Biol. Chem. 277, 47517-47523, 2002; Pletnev S., et al (2003) Biochemistry 42:12617-12624; Sheikh F., et al (2004) J. Immunol. 172, 2006-2010; EP1394274 (Example 11); US2004005320 (Example 5); WO2003029262 (Page 74-75); WO2003002717 (Claim 2; Page 63); WO200222153 (Page 45-47); US2002042366 (Page 20-21); WO200146261 (Page 57-59); WO200146232 (Page 63-65); WO9837193 (Claim 1; Page 55-59);
Accession: Q9UHF4; Q6UWA9; Q96SH8; EMBL; AF184971; AAF01320.1.

(21) Brevican (BCAN, BEHAB, Genbank accession no. AF229053)
Gary S.C., et al Gene 256, 139-147, 2000; Clark H.F., et al Genome Res. 13, 2265-2270, 2003; Strausberg R.L., et al Proc. Natl. Acad. Sci. U.S.A. 99, 16899-16903, 2002; US2003186372 (Claim 11); US2003186373 (Claim 11); US2003119131 (Claim 1; Fig

52); US2003119122 (Claim 1; Fig 52); US2003119126 (Claim 1); US2003119121 (Claim 1; Fig 52); US2003119129 (Claim 1); US2003119130 (Claim 1); US2003119128 (Claim 1; Fig 52); US2003119125 (Claim 1); WO2003016475 (Claim 1); WO200202634 (Claim 1);

(22) EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5, Genbank accession no. NM_004442) Chan,J. and Watt, V.M., Oncogene 6 (6), 1057-1061 (1991) Oncogene 10 (5):897-905 (1995), Annu. Rev. Neurosci. 21:309-345 (1998), Int. Rev. Cytol. 196:177-244 (2000)); WO2003042661 (Claim 12); WO200053216 (Claim 1; Page 41); WO2004065576 (Claim 1); WO2004020583 (Claim 9); WO2003004529 (Page 128-132); WO200053216 (Claim 1; Page 42); Cross-references: MIM:600997; NP_004433.2; NM_004442_1

(23) ASLG659 (B7h, Genbank accession no. AX092328) US20040101899 (Claim 2); WO2003104399 (Claim 11); WO2004000221 (Fig 3); US2003165504 (Claim 1); US2003124140 (Example 2); US2003065143 (Fig 60); WO2002102235 (Claim 13; Page 299); US2003091580 (Example 2); WO200210187 (Claim 6; Fig 10); WO200194641 (Claim 12; Fig 7b); WO200202624 (Claim 13; Fig 1A-1B); US2002034749 (Claim 54; Page 45-46); WO200206317 (Example 2; Page 320-321, Claim 34; Page 321-322); WO200271928 (Page 468-469); WO200202587 (Example 1; Fig 1); WO200140269 (Example 3; Pages 190-192); WO200036107 (Example 2; Page 205-207); WO2004053079 (Claim 12); WO2003004989 (Claim 1); WO200271928 (Page 233-234, 452-453); WO 0116318;

(24) PSCA (Prostate stem cell antigen precursor, Genbank accession no. AJ297436) Reiter R.E., et al Proc. Natl. Acad. Sci. U.S.A. 95, 1735-1740, 1998; Gu Z., et al Oncogene 19, 1288-1296, 2000; Biochem. Biophys. Res. Commun. (2000) 275(3):783-788; WO2004022709; EP1394274 (Example 11); US2004018553 (Claim 17); WO2003008537 (Claim 1); WO200281646 (Claim 1; Page 164); WO2003003906 (Claim 10; Page 288); WO200140309 (Example 1; Fig 17); US2001055751 (Example 1; Fig 1b); WO200032752 (Claim 18; Fig 1); WO9851805 (Claim 17; Page 97); WO9851824 (Claim 10; Page 94); WO9840403 (Claim 2; Fig 1B); Accession: O43653; EMBL; AF043498; AAC39607.1.

(25) GEDA (Genbank accession No. AY260763); AAP14954 lipoma HMGIC fusion-partner-like protein /pid=AAP14954.1 - Homo sapiens Species: Homo sapiens (human)

WO2003054152 (Claim 20); WO2003000842 (Claim 1); WO2003023013 (Example 3, Claim 20); US2003194704 (Claim 45);
Cross-references: GI:30102449; AAP14954.1; AY260763_1

(26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3, Genbank accession No. AF116456); BAFF receptor /pid=NP_443177.1 - Homo sapiens
Thompson, J.S., et al Science 293 (5537), 2108-2111 (2001); WO2004058309; WO2004011611; WO2003045422 (Example; Page 32-33); WO2003014294 (Claim 35; Fig 6B); WO2003035846 (Claim 70; Page 615-616); WO200294852 (Col 136-137); WO200238766 (Claim 3; Page 133); WO200224909 (Example 3; Fig 3);
Cross-references: MIM:606269; NP_443177.1; NM_052945_1; AF132600

(27) CD22 (B-cell receptor CD22-B isoform, BL-CAM, Lyb-8, Lyb8, SIGLEC-2, FLJ22814, Genbank accession No. AK026467);
Wilson et al (1991) J. Exp. Med. 173:137-146; WO2003072036 (Claim 1; Fig 1);
Cross-references: MIM:107266; NP_001762.1; NM_001771_1

(28) CD79a (CD79A, CD79α, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation), pI: 4.84, MW: 25028 TM: 2 [P] Gene Chromosome: 19q13.2, Genbank accession No. NP_001774.10)
WO2003088808, US20030228319; WO2003062401 (claim 9); US2002150573 (claim 4, pages 13-14); WO9958658 (claim 13, Fig 16); WO9207574 (Fig 1); US5644033; Ha et al (1992) J. Immunol. 148(5):1526-1531; Mueller et al (1992) Eur. J. Biochem. 22:1621-1625; Hashimoto et al (1994) Immunogenetics 40(4):287-295; Preud'homme et al (1992) Clin. Exp. Immunol. 90(1):141-146; Yu et al (1992) J. Immunol. 148(2) 633-637; Sakaguchi et al (1988) EMBO J. 7(11):3457-3464;

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia); 372 aa, pI: 8.54 MW: 41959 TM: 7 [P] Gene Chromosome: 11q23.3, Genbank accession No. NP_001707.1)
WO2004040000; WO2004015426; US2003105292 (Example 2); US6555339 (Example 2); WO200261087 (Fig 1); WO200157188 (Claim 20, page 269); WO200172830 (pages 12-13); WO200022129 (Example 1, pages 152-153, Example 2, pages 254-256); WO9928468 (claim 1, page 38); US5440021 (Example 2, col 49-52); WO9428931 (pages 56-58); WO9217497 (claim 7,

Fig 5); Dobner et al (1992) Eur. J. Immunol. 22:2795-2799; Barella et al (1995) Biochem. J. 309:773-779;

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+ T lymphocytes); 273 aa, pI: 6.56 MW: 30820 TM: 1 [P] Gene Chromosome: 6p21.3, Genbank accession No. NP_002111.1)
Tonnelle et al (1985) EMBO J. 4(11):2839-2847; Jonsson et al (1989) Immunogenetics 29(6):411-413; Beck et al (1992) J. Mol. Biol. 228:433-441; Strausberg et al (2002) Proc. Natl. Acad. Sci USA 99:16899-16903; Servenius et al (1987) J. Biol. Chem. 262:8759-8766; Beck et al (1996) J. Mol. Biol. 255:1-13; Naruse et al (2002) Tissue Antigens 59:512-519; WO9958658 (claim 13, Fig 15); US6153408 (Col 35-38); US5976551 (col 168-170); US6011146 (col 145-146); Kasahara et al (1989) Immunogenetics 30(1):66-68; Larhammar et al (1985) J. Biol. Chem. 260(26):14111-14119;

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability); 422 aa), pI: 7.63, MW: 47206 TM: 1 [P] Gene Chromosome: 17p13.3, Genbank accession No. NP_002552.2)
Le et al (1997) FEBS Lett. 418(1-2):195-199; WO2004047749; WO2003072035 (claim 10); Touchman et al (2000) Genome Res. 10:165-173; WO200222660 (claim 20); WO2003093444 (claim 1); WO2003087768 (claim 1); WO2003029277 (page 82);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2) PROTEIN SEQUENCE Full maeaity...tafrfpd (1..359; 359 aa), pI: 8.66, MW: 40225 TM: 1 [P] Gene Chromosome: 9p13.3, Genbank accession No. NP_001773.1)
WO2004042346 (claim 65); WO2003026493 (pages 51-52, 57-58); WO200075655 (pages 105-106); Von Hoegen et al (1990) J. Immunol. 144(12):4870-4877; Strausberg et al (2002) Proc. Natl. Acad. Sci USA 99:16899-16903;

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosis); 661 aa, pI: 6.20, MW: 74147 TM: 1 [P] Gene Chromosome: 5q12, Genbank accession No. NP_005573.1)
US2002193567; WO9707198 (claim 11, pages 39-42); Miura et al (1996) Genomics 38(3):299-304; Miura et al (1998) Blood 92:2815-2822; WO2003083047; WO9744452 (claim 8, pages 57-61); WO200012130 (pages 24-26);

(34) FcRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C2 type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation); 429 aa, pI: 5.28, MW: 46925 TM: 1 [P] Gene Chromosome: 1q21-1q22, Genbank accession No. NP_443170.1)
WO2003077836; WO200138490 (claim 6, Fig 18E-1-18-E-2); Davis et al (2001) Proc. Natl. Acad. Sci USA 98(17):9772-9777; WO2003089624 (claim 8); EP1347046 (claim 1); WO2003089624 (claim 7);

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies); 977 aa, pI: 6.88 MW: 106468 TM: 1 [P] Gene Chromosome: 1q21, Genbank accession No. Human:AF343662, AF343663, AF343664, AF343665, AF369794, AF397453, AK090423, AK090475, AL834187, AY358085; Mouse:AK089756, AY158090, AY506558; NP_112571.1
WO2003024392 (claim 2, Fig 97); Nakayama et al (2000) Biochem. Biophys. Res. Commun. 277(1):124-127; WO2003077836; WO200138490 (claim 3, Fig 18B-1-18B-2);

(36) TENB2 (TMEFF2, tomoregulin, TPEF, HPP1, TR, putative transmembrane proteoglycan, related to the EGF/heregulin family of growth factors and follistatin); 374 aa, NCBI Accession: AAD55776, AAF91397, AAG49451, NCBI RefSeq: NP_057276; NCBI Gene: 23671; OMIM: 605734; SwissProt Q9UIK5; Genbank accession No. AF179274; AY358907, CAF85723, CQ782436
WO2004074320 (SEQ ID NO 810); JP2004113151 (SEQ ID NOS 2, 4, 8); WO2003042661 (SEQ ID NO 580); WO2003009814 (SEQ ID NO 411); EP1295944 (pages 69-70); WO200230268 (page 329); WO200190304 (SEQ ID NO 2706); US2004249130; US2004022727; WO2004063355; US2004197325; US2003232350; US2004005563; US2003124579; Horie et al (2000) Genomics 67:146-152; Uchida et al (1999) Biochem. Biophys. Res. Commun. 266:593-602; Liang et al (2000) Cancer Res. 60:4907-12; Glynne-Jones et al (2001) Int J Cancer. Oct 15;94(2):178-84;

(37) PMEL17 (silver homolog; SILV; D12S53E; PMEL17; (SI); (SIL); ME20; gp100) BC001414; BT007202; M32295; M77348; NM_006928; McGlinchey, R.P. et al (2009) Proc. Natl. Acad. Sci. U.S.A. 106 (33), 13731-13736; Kummer, M.P. et al (2009) J. Biol. Chem. 284 (4), 2296-2306;

(38) TMEFF1 (transmembrane protein with EGF-like and two follistatin-like domains 1; Tomoregulin-1; H7365; C9orf2; C9ORF2; U19878; X83961) NM_080655; NM_003692; Harms, P.W. (2003) Genes Dev. 17 (21), 2624-2629; Gery, S. et al (2003) Oncogene 22 (18):2723-2727;

(39) GDNF-Ra1 (GDNF family receptor alpha 1; GFRA1; GDNFR; GDNFRA; RETL1; TRNR1; RET1L; GDNFR-alpha1; GFR-ALPHA-1; U95847; BC014962; NM_145793) NM_005264; Kim, M.H. et al (2009) Mol. Cell. Biol. 29 (8), 2264-2277; Treanor, J.J. et al (1996) Nature 382 (6586):80-83;

(40) Ly6E (lymphocyte antigen 6 complex, locus E; Ly67,RIG-E,SCA-2,TSA-1) NP_002337.1; NM_002346.2; de Nooij-van Dalen, A.G. et al (2003) Int. J. Cancer 103 (6), 768-774; Zammit, D.J. et al (2002) Mol. Cell. Biol. 22 (3):946-952;

(41) TMEM46 (shisa homolog 2 (Xenopus laevis); SHISA2) NP_001007539.1; NM_001007538.1; Furushima, K. et al (2007) Dev. Biol. 306 (2), 480-492; Clark, H.F. et al (2003) Genome Res. 13 (10):2265-2270;

(42) Ly6G6D (lymphocyte antigen 6 complex, locus G6D; Ly6-D, MEGT1) NP_067079.2; NM_021246.2; Mallya, M. et al (2002) Genomics 80 (1):113-123; Ribas, G. et al (1999) J. Immunol. 163 (1):278-287;

(43) LGR5 (leucine-rich repeat-containing G protein-coupled receptor 5; GPR49, GPR67) NP_003658.1; NM_003667.2; Salanti, G. et al (2009) Am. J. Epidemiol. 170 (5):537-545; Yamamoto, Y. et al (2003) Hepatology 37 (3):528-533;

(44) RET (ret proto-oncogene; MEN2A; HSCR1; MEN2B; MTC1; (PTC); CDHF12; Hs.168114; RET51; RET-ELE1) NP_066124.1; NM_020975.4; Tsukamoto, H. et al (2009) Cancer Sci. 100 (10):1895-1901; Narita, N. et al (2009) Oncogene 28 (34):3058-3068;

(45) LY6K (lymphocyte antigen 6 complex, locus K; LY6K; HSJ001348; FLJ35226) NP_059997.3; NM_017527.3; Ishikawa, N. et al (2007) Cancer Res. 67 (24):11601-11611; de Nooij-van Dalen, A.G. et al (2003) Int. J. Cancer 103 (6):768-774;

(46) GPR19 (G protein-coupled receptor 19; Mm.4787) NP_006134.1; NM_006143.2; Montpetit, A. and Sinnett, D. (1999) Hum. Genet. 105 (1-2):162-164; O'Dowd, B.F. et al (1996) FEBS Lett. 394 (3):325-329;

(47) GPR54 (KISS1 receptor; KISS1R; GPR54; HOT7T175; AXOR12) NP_115940.2; NM_032551.4; Navenot, J.M. et al (2009) Mol. Pharmacol. 75 (6):1300-1306; Hata, K. et al (2009) Anticancer Res. 29 (2):617-623;

(48) ASPHD1 (aspartate beta-hydroxylase domain containing 1; LOC253982) NP_859069.2; NM_181718.3; Gerhard, D.S. et al (2004) Genome Res. 14 (10B):2121-2127;

(49) Tyrosinase (TYR; OCAIA; OCA1A; tyrosinase; SHEP3) NP_000363.1; NM_000372.4; Bishop, D.T. et al (2009) Nat. Genet. 41 (8):920-925; Nan, H. et al (2009) Int. J. Cancer 125 (4):909-917;

(50) TMEM118 (ring finger protein, transmembrane 2; RNFT2; FLJ14627) NP_001103373.1; NM_001109903.1; Clark, H.F. et al (2003) Genome Res. 13 (10):2265-2270; Scherer, S.E. et al (2006) Nature 440 (7082):346-351

(51) GPR172A (G protein-coupled receptor 172A; GPCR41; FLJ11856; D15Ertd747e) NP_078807.1; NM_024531.3; Ericsson, T.A. et al (2003) Proc. Natl. Acad. Sci. U.S.A. 100 (11):6759-6764; Takeda, S. et al (2002) FEBS Lett. 520 (1-3):97-101.

In one embodiment, the antibody binds to one or more of the following polypeptides: BMPR1B; E16; STEAP1; 0772P; MPF; NaPi2b; Sema 5b; PSCA hlg; ETBR; MSG783; STEAP2; TrpM4; CRIPTO; CD21; CD79b; FcRH2; HER2; NCA; MDP; IL20Rα; Brevican; EphB2R; ASLG659; PSCA; GEDA; BAFF-R; CD22; CD79a; CXCR5; HLA-DOB; P2X5; CD72; LY64; FcRH1; IRTA2; TENB2; PMEL17; TMEFF1; GDNF-Ra1; Ly6E; TMEM46; Ly6G6D; LGR5; RET; LY6K; GPR19; GPR54; ASPHD1; Tyrosinase; TMEM118; GPR172A; and CD33.
In one embodiment, the antibody binds to BMPR1B;
In one embodiment, the antibody binds to E16;
In one embodiment, the antibody binds to STEAP1;
In one embodiment, the antibody binds to 0772P;
In one embodiment, the antibody binds to MPF;
In one embodiment, the antibody binds to NaPi2b;
In one embodiment, the antibody binds to Sema 5b;
In one embodiment, the antibody binds to PSCA hlg;
In one embodiment, the antibody binds to ETBR;
In one embodiment, the antibody binds to MSG783;
In one embodiment, the antibody binds to STEAP2;
In one embodiment, the antibody binds to TrpM4;
In one embodiment, the antibody binds to CRIPTO;
In one embodiment, the antibody binds to CD21;
In one embodiment, the antibody binds to CD79b;

In one embodiment, the antibody binds to FcRH2;

In one embodiment, the antibody binds to HER2;

In one embodiment, the antibody binds to NCA;

In one embodiment, the antibody binds to MDP;

In one embodiment, the antibody binds to IL20Rα;

In one embodiment, the antibody binds to Brevican;

In one embodiment, the antibody binds to EphB2R;

In one embodiment, the antibody binds to ASLG659;

In one embodiment, the antibody binds to PSCA;

In one embodiment, the antibody binds to GEDA;

In one embodiment, the antibody binds to BAFF-R;

In one embodiment, the antibody binds to CD22;

In one embodiment, the antibody binds to CD79a;

In one embodiment, the antibody binds to CXCR5;

In one embodiment, the antibody binds to HLA-DOB;

In one embodiment, the antibody binds to P2X5 ;

In one embodiment, the antibody binds to CD72;

In one embodiment, the antibody binds to LY64;

In one embodiment, the antibody binds to FcRH1;

In one embodiment, the antibody binds to IRTA2;

In one embodiment, the antibody binds to TENB2;

In one embodiment, the antibody binds to PMEL17;

In one embodiment, the antibody binds to TMEFF1;

In one embodiment, the antibody binds to GDNF-Ra1;

In one embodiment, the antibody binds to Ly6E;

In one embodiment, the antibody binds to TMEM46;

In one embodiment, the antibody binds to Ly6G6D;

In one embodiment, the antibody binds to LGR5;

In one embodiment, the antibody binds to RET;

In one embodiment, the antibody binds to LY6K;

In one embodiment, the antibody binds to GPR19;

In one embodiment, the antibody binds to GPR54;

In one embodiment, the antibody binds to ASPHD1;

In one embodiment, the antibody binds to Tyrosinase;

In one embodiment, the antibody binds to TMEM118;

In one embodiment, the antibody binds to GPR172A;

In one embodiment, the antibody binds to CD33.

The parent antibody may also be a fusion protein comprising an albumin-binding peptide (ABP) sequence (Dennis et al. (2002) "Albumin Binding As A General Strategy For Improving The Pharmacokinetics Of Proteins" J Biol Chem. 277:35035-35043; WO 01/45746). Antibodies of the invention include fusion proteins with ABP sequences taught by: (i) Dennis et al (2002) J Biol Chem. 277:35035-35043 at Tables III and IV, page 35038; (ii) US 20040001827 at [0076]; and (iii) WO 01/45746 at pages 12-13, and all of which are incorporated herein by reference. Antibodies may be produced using recombinant methods and compositions, e.g., as described in US 4816567 and known in the art. In some embodiments, the antibody is produced in a eukaryotic host cell (e.g., mammalian host cell). In some embodiments, the antibody is produced in a prokaryotic host cell (e.g., E. coli).

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability.

DRUG LOADING OF ADC

The drug loading is the average number of drug moieties per antibody. Drug loading may range from 1 to 8 drugs (D) per antibody (Ab), i.e. where 1, 2, 3, 4, 5, 6, 7, and 8 drug moieties are covalently attached to the antibody. Compositions of ADC include collections of antibodies conjugated with a range of drugs, from 1 to 8. The average number of drugs per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, electrophoresis, and HPLC. The quantitative distribution of ADC in terms of p may also be determined. By ELISA, the averaged value of p in a particular preparation of ADC may be determined (Hamblett et al (2004) Clin. Cancer Res. 10:7063-7070; Sanderson et al (2005) Clin. Cancer Res. 11:843-852). However, the distribution of p (drug) values is not discernible by the antibody-antigen binding and detection limitation of ELISA. Also,

ELISA assay for detection of antibody-drug conjugates does not determine where the drug moieties are attached to the antibody, such as the heavy chain or light chain fragments, or the particular amino acid residues. In some instances, separation, purification, and characterization of homogeneous ADC where p is a certain value from ADC with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. Higher drug loading, e.g. p >5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates.

Typically, fewer than the theoretical maximum of drug moieties is conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, many lysine residues that do not react with the linker-drug intermediate (X-L-D) or linker reagent. Only the most reactive lysine groups may react with an amine-reactive linker reagent. Also, only the most reactive cysteine thiol groups may react with a thiol-reactive linker reagent or linker-drug intermediate. Generally, antibodies do not contain many, if any, free and reactive cysteine thiol groups which may be linked to a drug moiety. Most cysteine thiol residues in the antibodies of the compounds exist as disulfide bridges and must be reduced with a reducing agent such as dithiothreitol (DTT) or TCEP, under partial or total reducing conditions. The loading (drug/antibody ratio, "DAR") of an ADC may be controlled in several different manners, including: (i) limiting the molar excess of linker-drug intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

Where more than one nucleophilic or electrophilic group of the antibody reacts with a linker-drug intermediate, or linker reagent followed by dimer drug moiety reagent, then the resulting product is a mixture of Antibody-drug conjugate s with a distribution of drug moieties attached to an antibody, e.g. 1, 2, 3, etc. Liquid chromatography methods such as polymeric reverse phase (PLRP) and hydrophobic interaction (HIC) may separate compounds in the mixture by drug loading value. Preparations of ADC with a single drug loading value (p) may be isolated, however, these single loading value ADCs may still be heterogeneous mixtures because the drug moieties may be attached, via the linker, at different sites on the antibody. Thus the antibody-drug conjugate compositions of the invention include mixtures of antibody-drug conjugate compounds where the antibody has one or more drug moieties and where the drug moieties may be attached to the antibody at various amino acid residues.

EXEMPLARY DRUG MOIETIES

Maytansine and maytansinoids

In some embodiments, an immunoconjugate comprises an antibody conjugated to one or more maytansinoid molecules. Maytansinoids are derivatives of maytansine, and are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Patent No. 3896111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinoids are disclosed, for example, in U.S. Patent Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

Maytansinoid drug moieties are attractive drug moieties in antibody-drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification or derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

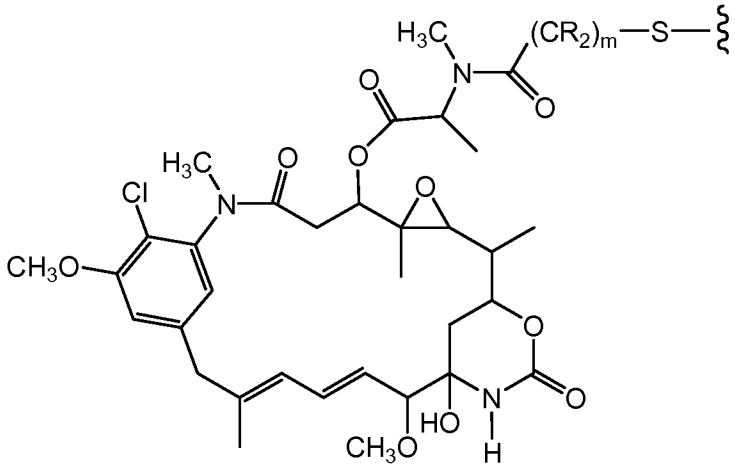
Certain maytansinoids suitable for use as maytansinoid drug moieties are known in the art and can be isolated from natural sources according to known methods or produced using genetic engineering techniques (see, e.g., Yu et al (2002) PNAS 99:7968-7973). Maytansinoids may also be prepared synthetically according to known methods.

Exemplary maytansinoid drug moieties include, but are not limited to, those having a modified aromatic ring, such as: C-19-dechloro (US Pat. No. 4256746) (prepared, for example, by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (US Pat. Nos. 4361650 and 4307016) (prepared, for example, by demethylation using *Streptomyces* or *Actinomyces* or dechlorination using LAH); and C-20-demethoxy, C-20-acyloxy (-OCOR), +/-dechloro (U.S. Pat. No. 4,294,757) (prepared, for example, by acylation using acyl chlorides), and those having modifications at other positions of the aromatic ring.

Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (US Pat. No. 4424219) (prepared, for example, by the reaction of maytansinol with H_2S or P_2S_5); C-14-alkoxymethyl(demethoxy/CH_2 OR)(US 4331598); C-14-hydroxymethyl or acyloxymethyl (CH_2OH or CH_2OAc) (US Pat. No. 4450254) (prepared, for example, from Nocardia); C-15-hydroxy/acyloxy (US 4364866) (prepared, for example, by the conversion of maytansinol by Streptomyces); C-15-methoxy (US Pat. Nos. 4313946 and 4315929) (for example, isolated from Trewia nudlflora); C-18-N-demethyl (US Pat. Nos. 4362663 and 4322348) (prepared, for example, by the demethylation of maytansinol by Streptomyces); and 4,5-deoxy (US 4371533) (prepared, for example, by the titanium trichloride/LAH reduction of maytansinol).

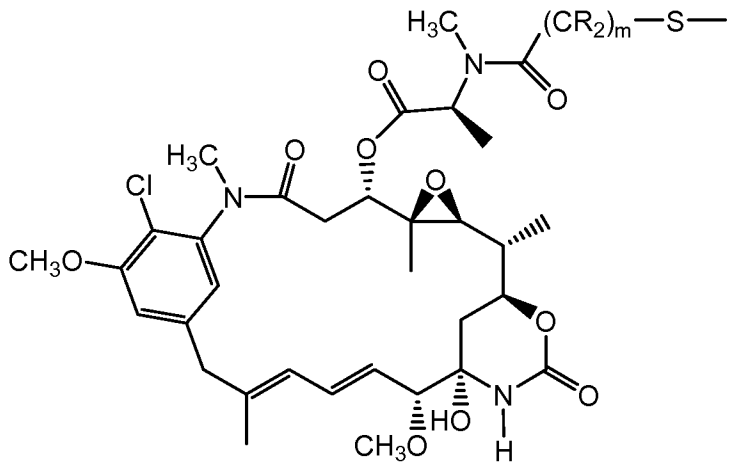
Many positions on maytansinoid compounds are useful as the linkage position. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. In some embodiments, the reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In some embodiments, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

Maytansinoid drug moieties include those having the structure:

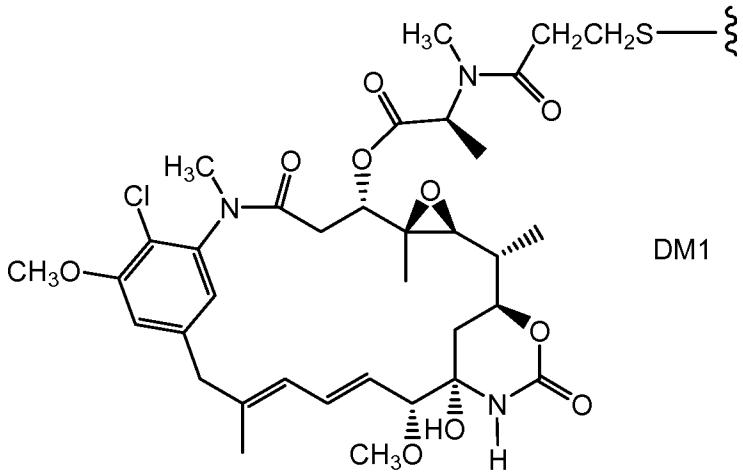


where the wavy line indicates the covalent attachment of the sulfur atom of the maytansinoid drug moiety to a linker of an ADC. Each R may independently be H or a C_1–C_6 alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or propyl, i.e., m is 1, 2, or 3 (US 633410; US 5208020; Chari et al (1992) *Cancer Res.* 52:127-131; Liu et al (1996) *Proc. Natl. Acad. Sci USA* 93:8618-8623).

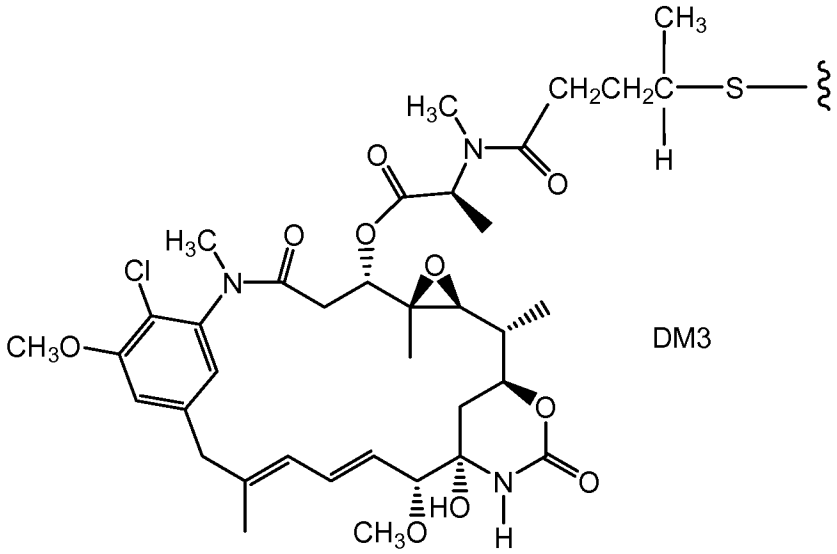
All stereoisomers of the maytansinoid drug moiety are contemplated for the ADC of the invention, i.e. any combination of *R* and *S* configurations at the chiral carbons (US 7276497; US 6913748; US 6441163; US 633410 (RE39151); US 5208020; Widdison et al (2006) J. Med. Chem. 49:4392-4408, which are incorporated by reference in their entirety). In some embodiments, the maytansinoid drug moiety has the following stereochemistry:



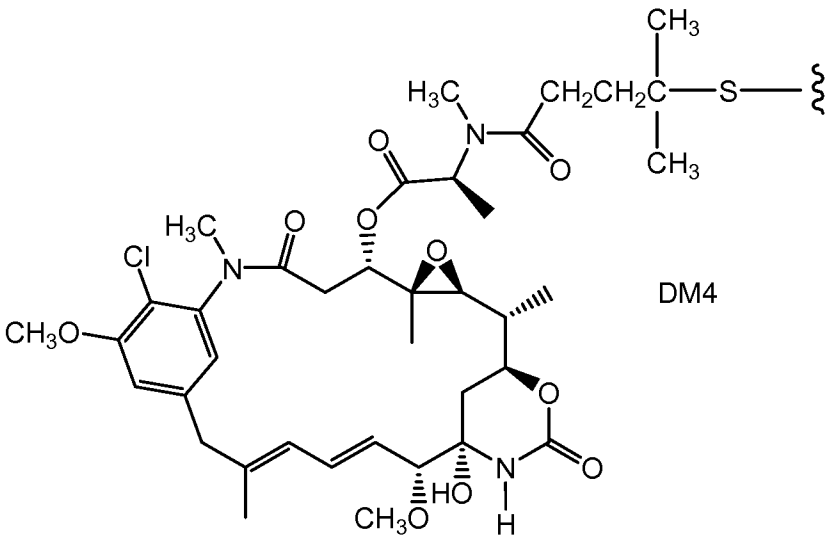
Exemplary embodiments of maytansinoid drug moieties include, but are not limited to, DM1; DM3; and DM4, having the structures:



DM1



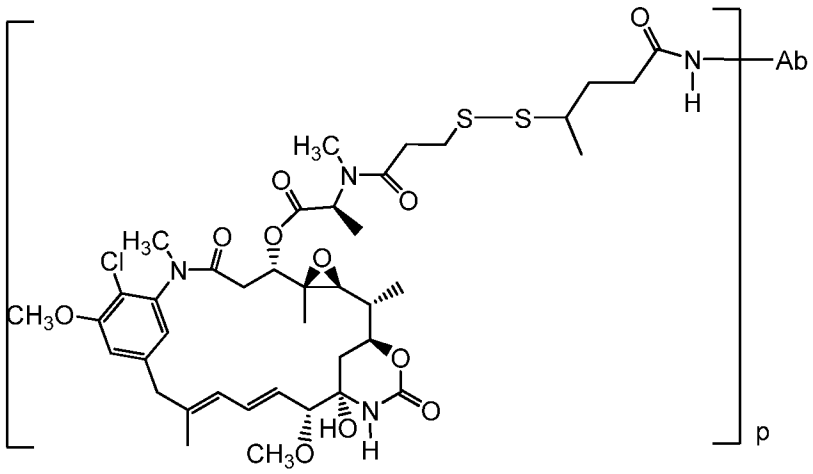
DM3



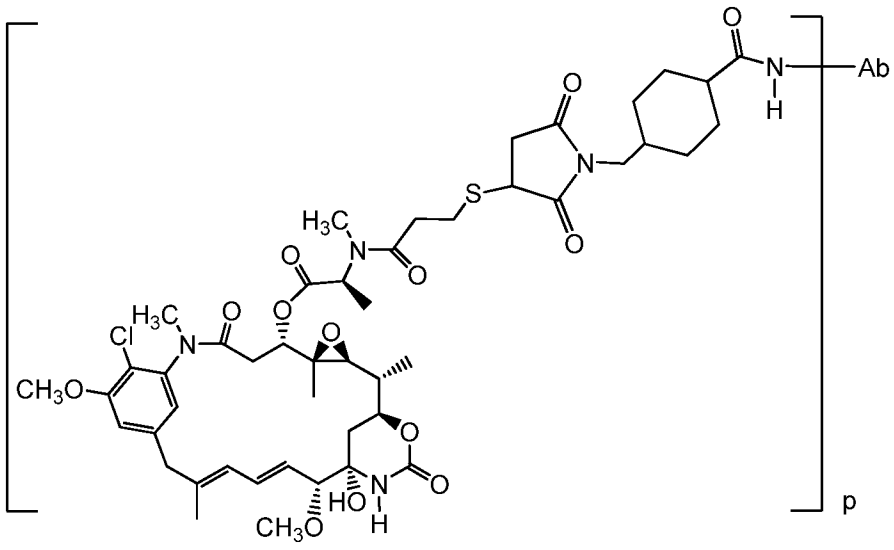
DM4

wherein the wavy line indicates the covalent attachment of the sulfur atom of the drug to a linker (L) of an antibody-drug conjugate.

Other exemplary maytansinoid antibody-drug conjugates have the following structures and abbreviations (wherein Ab is antibody and p is 1 to about 20. In some embodiments, p is 1 to 10, p is 1 to 7, p is 1 to 5, or p is 1 to 4):

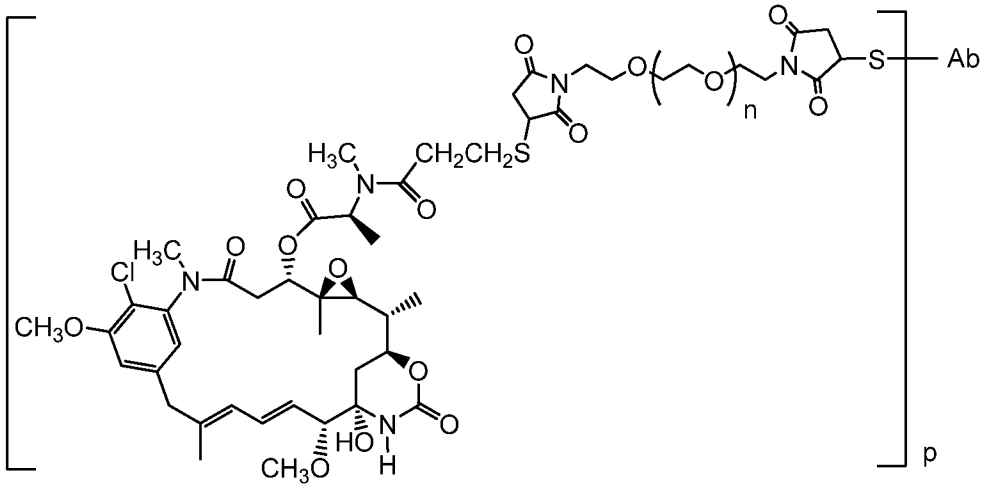


Ab -SPP-DM1



Ab-SMCC-DM1

Exemplary antibody-drug conjugates where DM1 is linked through a BMPEO linker to a thiol group of the antibody have the structure and abbreviation:



where Ab is antibody; n is 0, 1, or 2; and p is 1 to about 20. In some embodiments, p is 1 to 10, p is 1 to 7, p is 1 to 5, or p is 1 to 4.

Immunoconjugates containing maytansinoids, methods of making the same, and their therapeutic use are disclosed, for example, in U.S. Patent Nos. 5,208,020 and 5,416,064; US 2005/0276812 A1; and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. *See also* Liu et al. *Proc. Natl. Acad. Sci. USA* 93:8618-8623 (1996); and Chari et al. *Cancer Research* 52:127-131 (1992).

In some embodiments, antibody-maytansinoid conjugates may be prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. *See, e.g.*, U.S. Patent No. 5,208,020 (the disclosure of which is hereby expressly incorporated by reference). In some embodiments, ADC with an average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody. In some instances, even one molecule of toxin/antibody is expected to enhance cytotoxicity over the use of naked antibody.

Exemplary linking groups for making antibody-maytansinoid conjugates include, for example, those described herein and those disclosed in U.S. Patent No. 5208020; EP Patent 0 425 235 B1; Chari et al. *Cancer Research* 52:127-131 (1992); US 2005/0276812 A1; and US 2005/016993 A1, the disclosures of which are hereby expressly incorporated by reference.

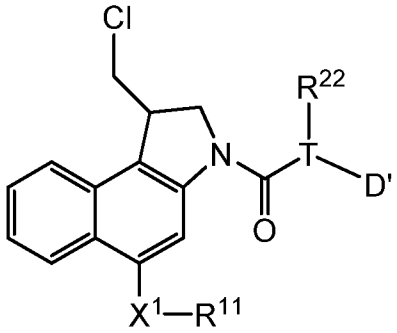
Calicheamicin

In some embodiments, the immunoconjugate comprises an antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics, and analogues thereof, are capable of producing double-stranded DNA breaks at sub-picomolar concentrations (Hinman et al., (1993) *Cancer Research* 53:3336-3342; Lode et al., (1998) *Cancer Research* 58:2925-2928). Calicheamicin has intracellular sites of action but, in certain instances, does not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody-mediated internalization may, in some embodiments, greatly enhances their cytotoxic effects. Nonlimiting

exemplary methods of preparing antibody-drug conjugates with a calicheamicin drug moiety are described, for example, in US 5712374; US 5714586; US 5739116; and US 5767285.

CBI dimers

In some embodiments, the drug moiety is a CBI dimer having the following the formula:



wherein

R^{11} is selected from H, $P(O)_3H_2$, $C(O)NR^{aa}R^{bb}$, or a bond to L;

R^{22} is selected from H, $P(O)_3H_2$, $C(O)NR^{aa}R^{bb}$, or a bond to L;

R^{aa} and R^{bb} are independently selected from H and C_1–C_6alkyl optionally substituted with one or more F,

or R^{aa} and R^{bb} form a five or six membered heterocycloalkyl group;

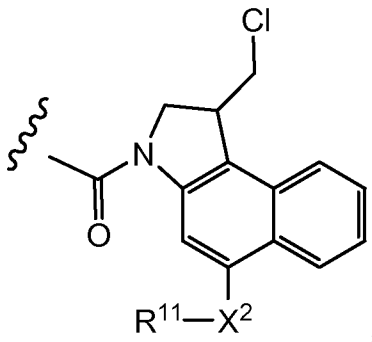
T is a tether group selected from C_3–C_{12}alkylene, Y^1, (C_1–C_6alkylene)– Y^1–(C_1–C_6 alkylene), (C_1–C_6alkylene)– Y^1–(C_1–C_6alkylene)– Y^1–(C_1–C_6alkylene), (C_2–C_6alkenylene)– Y^1–(C_2–C_6alkenylene), and (C_2–C_6alkynylene)– Y^1–(C_2–C_6alkynylene);

where Y^1 is independently selected from O, S, NR^{11}, aryl, and heteroaryl;

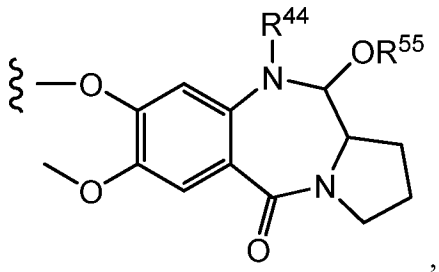
where alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with F, OH, O(C_1–C_6alkyl), NH_2, $NHCH_3$, $N(CH_3)_2$, $OP(O)_3H_2$, and C_1–C_6alkyl, where alkyl is optionally substituted with one or more F;

or alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with a bond to L;

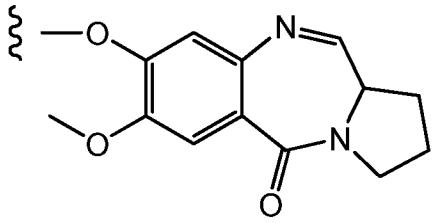
D' is a drug moiety selected from:



,



, and



where the wavy line indicates the site of attachment to T;

X^1 and X^2 are independently selected from O and NR^{33}, where R^{33} is selected from H and C_1–C_6alkyl optionally substituted with one or more F, or X^1 and X^2 are each independently absent;

R^{44} is H, CO_2R, C(O) or a bond to L, where R is C_1–C_6alkyl or benzyl; and

R^{55} is H or C_1–C_6alkyl.

Other Drug Moieties

Drug moieties also include geldanamycin (Mandler et al (2000) *J. Nat. Cancer Inst.* 92(19):1573-1581; Mandler et al (2000) *Bioorganic & Med. Chem. Letters* 10:1025-1028; Mandler et al (2002) *Bioconjugate Chem.* 13:786-791); and enzymatically active toxins and fragments thereof, including, but not limited to, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, e.g., WO 93/21232. Drug moieties also include compounds with nucleolytic activity (e.g., a ribonuclease or a DNA endonuclease).

Antibiotics

Antibiotics that can be conjugated to an antibody includes clindamycin, novobiocin, retapamulin, daptomycin, GSK-2140944, CG-400549, sitafloxacin, teicoplanin, triclosan, napthyridone, radezolid, doxorubicin, ampicillin, vancomycin, imipenem, doripenem, gemcitabine, dalbavancin, and azithromycin. The mechanisms of bactericidal and bacteriostatic action of such antibiotics include, but are not limited to: (i) inhibition of cell wall, peptidoglycan elongation (vancomycin, teicoplanin, dalbavancin); (ii) inhibition of cell wall, penicillin-binding protein crosslinks (imipenem, doripenem, ampicillin); (iii) cell membrane depolarization (daptomycin); (iv) disruption of DNA replication (gemcitabine); (v) DNA binding (doxorubicin); (vi) enoyl ACP-

reductase FABI (CG-400549, triclosan, napthyridone); (vii) inhibition of ribosomal protein synthesis, ribosome 30S (clindamycin, retapamulin, radezolid); and (viii) topoisomerase (topoIIA) inhibitors (novobiocin, sitafloxacin, GSK-2140944). Structurally, most antibiotics can be grouped into: (i) aminoglycosides; (ii) beta-lactams; (iii) macrolides/cyclic peptides; (iv) tetracyclines; (v) fluoroquinolines/fluoroquinolones; (vi) and oxazolidinones. See: Shaw, K. and Barbachyn, M. (2011) Ann. N.Y. Acad. Sci. 1241:48-70; Sutcliffe, J. (2011) Ann. N.Y. Acad. Sci. 1241:122-152.

In certain embodiments, an immunoconjugate may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. In some embodiments, when an immunoconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc^{99} or I^{123}, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, e.g., for PET imaging (WO 2011/056983). The radio- or other labels may be incorporated in the immunoconjugate in known ways. For example, a peptide may be biosynthesized or chemically synthesized using suitable amino acid precursors comprising, for example, one or more fluorine-19 atoms in place of one or more hydrogens. In some embodiments, labels such as Tc^{99}, I^{123}, Re^{186}, Re^{188} and In^{111} can be attached via a cysteine residue in the antibody. In some embodiments, yttrium-90 can be attached via a lysine residue of the antibody. In some embodiments, the IODOGEN method (Fraker et al (1978) *Biochem. Biophys. Res. Commun.* 80: 49-57 can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes certain other methods.

In certain embodiments, an immunoconjugate may comprise an antibody conjugated to a prodrug-activating enzyme. In some such embodiments, a prodrug-activating enzyme converts a prodrug (e.g., a peptidyl chemotherapeutic agent, see WO 81/01145) to an active drug, such as an anti-cancer drug. Such immunoconjugates are useful, in some embodiments, in antibody-dependent enzyme-mediated prodrug therapy ("ADEPT"). Enzymes that may be conjugated to an antibody include, but are not limited to, alkaline phosphatases, which are useful for converting phosphate-containing prodrugs into free drugs; arylsulfatases, which are useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase, which is useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), which are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, which are useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-

cleaving enzymes such as β-galactosidase and neuraminidase, which are useful for converting glycosylated prodrugs into free drugs; β-lactamase, which is useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase and penicillin G amidase, which are useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. In some embodiments, enzymes may be covalently bound to antibodies by recombinant DNA techniques well known in the art. See, e.g., Neuberger et al., *Nature* 312:604-608 (1984).

INDICATIONS AND METHODS OF TREATMENT

It is contemplated that the antibody-drug conjugates (ADC) of the present invention may be used to treat various diseases or disorders, e.g. characterized by the overexpression of a tumor antigen. Exemplary conditions or hyperproliferative disorders include benign or malignant solid tumors and hematological disorders such as leukemia and lymphoid malignancies. Others include neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, inflammatory, angiogenic and immunologic, including autoimmune, disorders.

In certain embodiments, an ADC of the invention comprising an anti-NaPi2b antibody, such as those described above, is used in a method of treating solid tumor, e.g., ovarian,
In another embodiment, an ADC of the invention comprising an anti-CD33 antibody, such as those described herein, is used in a method of treating hematological malignancies such as non-Hodgkin's lymphoma (NHL), diffuse large hematopoietic lymphoma, follicular lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia (AML), and myeloid cell leukemia (MCL), and including B-cell related cancers and proliferative disorders. See: US 8226945; Li et al (2013) Mol. Cancer. Ther. 12(7):1255-1265; Polson et al (2010) Leukemia 24:1566-1573; Polson et al (2011) Expert Opin. Investig. Drugs 20(1):75-85, the contents of which are incorporated by reference.

In another embodiment, an ADC of the invention comprising an anti-MUC16 antibody, such as those described herein, is used in a method of treating ovarian, breast and pancreatic cancers. The cancer may be associated with the expression or activity of a MUC16/CA125/O772P polypeptide. See: WO 2007/001851; US 7989595; US 8449883; US 7723485; Chen et al (2007) Cancer Res. 67(10): 4924-4932; Junutula, et al., (2008) Nature Biotech., 26(8):925-932, the contents of which are incorporated by reference.

In certain embodiments, an ADC of the invention comprising an anti-HER2 antibody, such as those described above, is used in a method of treating cancer, e.g., breast or gastric cancer, more specifically HER2+ breast or gastric cancer, wherein the method comprises administering such

ADC to a patient in need of such treatment. In one such embodiment, the ADC comprises the anti-HER2 antibody trastuzumab or pertuzumab.

Generally, the disease or disorder to be treated is a hyperproliferative disease such as cancer. Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

Autoimmune diseases for which the Antibody-drug conjugate s may be used in treatment include rheumatologic disorders (such as, for example, rheumatoid arthritis, Sjögren's syndrome, scleroderma, lupus such as systemic lupus erythematosus (SLE) and lupus nephritis, polymyositis/dermatomyositis, cryoglobulinemia, anti-phospholipid antibody syndrome, and psoriatic arthritis), osteoarthritis, autoimmune gastrointestinal and liver disorders (such as, for example, inflammatory bowel diseases (e.g., ulcerative colitis and Crohn's disease), autoimmune gastritis and pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and celiac disease), vasculitis (such as, for example, ANCA-associated vasculitis, including Churg-Strauss vasculitis, Wegener's granulomatosis, and polyarteriitis), autoimmune neurological disorders (such as, for example, multiple sclerosis, opsoclonus myoclonus syndrome, myasthenia gravis, neuromyelitis optica, Parkinson's disease, Alzheimer's disease, and autoimmune polyneuropathies), renal disorders (such as, for example, glomerulonephritis, Goodpasture's syndrome, and Berger's disease), autoimmune dermatologic disorders (such as, for example, psoriasis, urticaria, hives, pemphigus vulgaris, bullous pemphigoid, and cutaneous lupus erythematosus), hematologic disorders (such as, for example, thrombocytopenic purpura, thrombotic thrombocytopenic purpura, post-transfusion purpura, and autoimmune hemolytic anemia), atherosclerosis, uveitis, autoimmune hearing diseases (such as, for example, inner ear disease and hearing loss), Behcet's disease, Raynaud's syndrome, organ transplant, and autoimmune endocrine disorders (such as, for example, diabetic-related autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM), Addison's disease, and autoimmune thyroid disease (*e.g.*, Graves' disease and thyroiditis)). More preferred such diseases include, for example, rheumatoid arthritis, ulcerative colitis, ANCA-associated vasculitis, lupus,

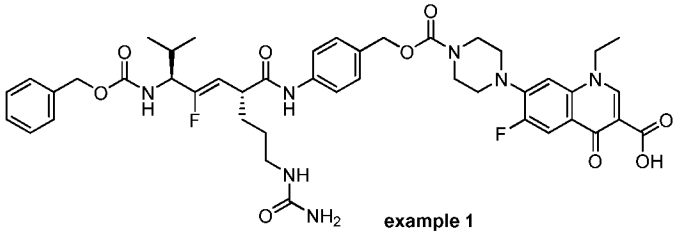
multiple sclerosis, Sjögren's syndrome, Graves' disease, IDDM, pernicious anemia, thyroiditis, and glomerulonephritis.

For the prevention or treatment of disease, the appropriate dosage of an ADC will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the molecule is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The molecule is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1-20 mg/kg) of molecule is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. An exemplary dosage of ADC to be administered to a patient is in the range of about 0.1 to about 10 mg/kg of patient weight.

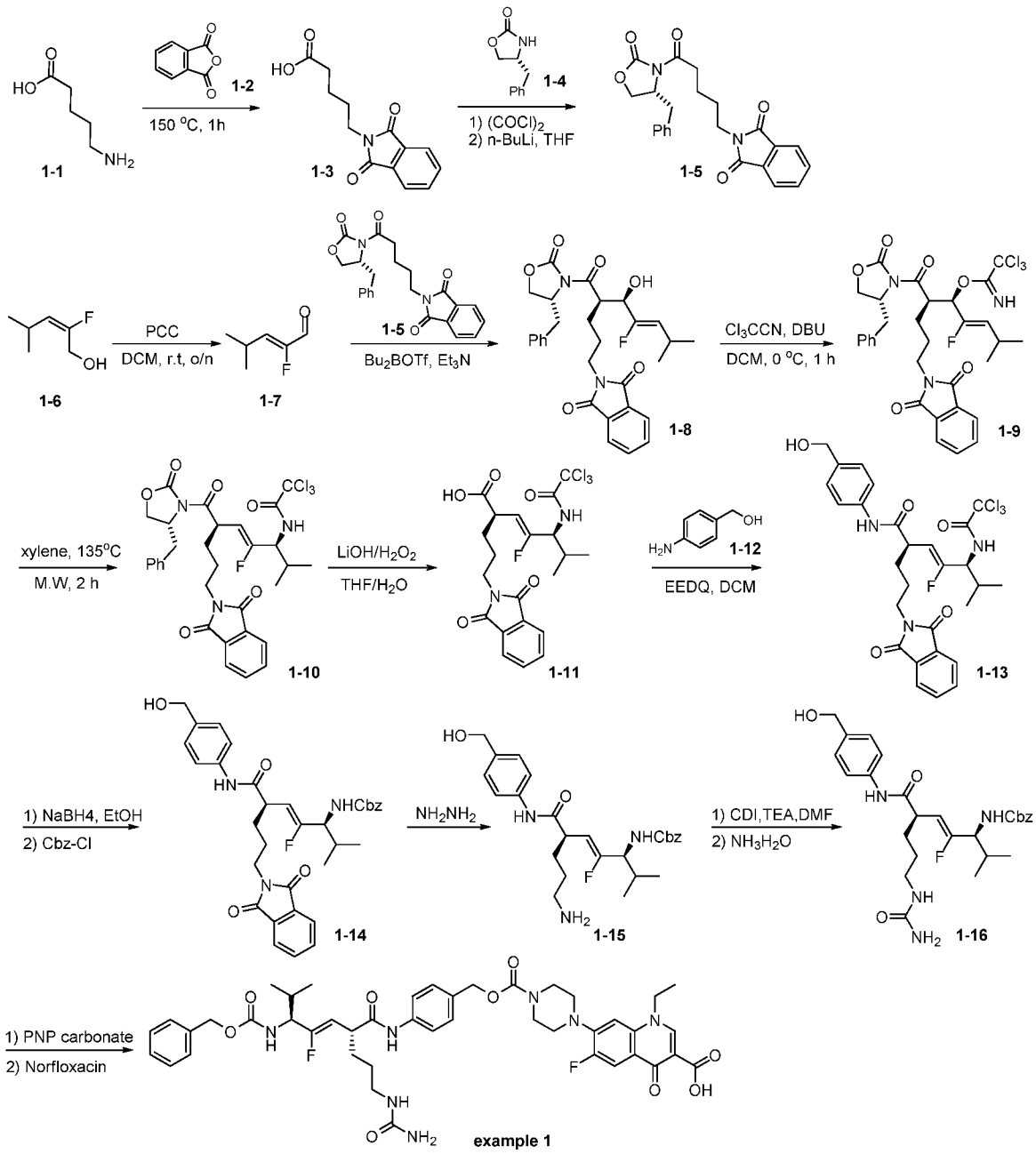
EXPERIMENTALS

Method of preparing Peptidomimetic Linker Drug moieties; PCT/US2014/042560 is incorporated by reference herein.

Example 1. 7-(4-((4-((2R,5S,Z)-5-(benzyloxycarbonylamino)-4-fluoro-6-methyl-2-(3-ureidopropyl)hept-3-enamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



example 1



Step 1. A mixture of **1-1** (10.0 g, 85.36 mmol), **1-2** (13.3 g, 89.79 mmol) was stirred at 150 °C for 1 h. The mixture was cooled to 25 °C, the solid was dissolved in hot water. The mixture was cooled in an ice bath and the precipitate was collected by filtration and washed with water. The filter cake was dried to give **1-3** as white solid (19.0 g, 90.0 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (br, 1H), 7.78 - 7.77 (m, 4H), 3.52 (t, J = 6.8 Hz, 2H), 2.18 (t, J = 7.2 Hz, 2H), 1.59 - 1.51 (m, 2H), 1.47 - 1.41 (m, 2H).

Step 2. To a mixture of **1-3** (9.0 g, 36.40 mmol) in anhydrous DCM (100 mL) were added $(COCl)_2$ (15.0 mL, 157.76 mmol), DMF (1 mL) dropwise at r.t. After the reaction mixture was stirred at r.t for 0.5 h, it was concentrated under reduced pressure. The residue co-evaporated with anhydrous THF (60 mL) to give the acyl chlorine as yellow solid.

To a mixture of **1-4** (6.6 g, 37.25 mmol) in anhydrous THF (60 mL) was added n-BuLi (15.0 mL, 2.5 M, 37.5 mmol) dropwise at -78 °C under N_2. The acyl chloride in THF (40 mL) was added slowly into the mixture at -78 °C. The reaction mixture was stirred at -78 °C for 15 min, and quenched with aq. NH_4Cl solution (30 mL). The mixture waspartitioned between EtOAc and water. The combined organic layers was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (PE/EtOAc 3:1) to give crude compound **1-5** as white solid (13.0 g, 87.9%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 - 7.83 (m, 4H), 7.32 - 7.28 (m, 2H), 7.25 - 7.22 (m, 1H), 7.19 - 7.17 (m, 2H), 4.66 - 4.60 (m, 1H), 4.30 (t, J = 8.4 Hz, 1H), 4.17 (dd, J = 9.2, 2.8 Hz, 1H), 3.61 (t, J = 6.4 Hz, 2H), 3.00 – 2.78 (m, 4H), 1.70 - 1.60 (m, 4H).

Step 3. To a solution of **1-6** (3.0 g, 25.39 mmol) in DCM (100 mL) was added PCC (10.9 g, 50.78 mmol). After the mixture was stirred at 25 °C for 16 h under N_2, it was filtered through a silica plug. The filtrate was concentrated under reduced pressure at a bath temperature of 25 °C to give compound **1-7** as an oil (1.8 g, 61.0 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18 (d, J = 18.4Hz, 1 H), 5.79 (dd, J =32.8, 9.2 Hz, 1 H), 3.02 - 2.93 (m, 1 H), 1.13 (d, J = 6.8 Hz, 6 H).

Step 4. A solution of **1-5** (6.0 g, 14.7 mmol) in DCM (20 mL) was cooled to 0°C with an ice bath. Bu_2BOTf in DCM (1M, 15 mL, 15 mmol) was added dropwise, followed by Et_3N (3.03 g, 30 mmol) at a rate to keep the internal temperature below 3°C. The ice bath was replaced by a dry ice-acetone bath. When the internal temperature dropped below -65°C, compound **1-7** (1.5 g, 12.9 mmol) in DCM (10 mL) was added dropwise. The solution was stirred for 20 min in the dry ice-acetone bath, then for 1 h at ice bath temperature. The reaction mixture was quenched with aqueous phosphate buffer (pH = 7.0, 20 mL) and MeOH (10 mL). To this cloudy solution was added a mixture of MeOH/30% H_2O_2 (2:1, 20 mL) at a rate to keep the internal temperature below 10°C. After the solution was stirred for an additional 1 h, the volatile was removed on a rotary evaporator at a bath temperature of 25-30 °C. The slurry was extracted with EtOAc (50 mL × 3). The combined organic layer was washed with saturated Na_2SO_3 solution (15 mL), 5% $NaHCO_3$ solution (30 mL) and brine (25 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EtOAc 3:1) to give crude **1-8** as oil (4.0 g, 59.7%).

LCMS (ESI): m/z 505.0 [M-17].

Step 5. To a solution of **1-8** (4.0 g, 7.65 mmol) and Cl_3CCN (1.67 g, 11.48 mmol) in DCM (20 mL) was added DBU (234 mg, 1.53 mmol) at 0 °C under N_2. The mixture was stirred at 0 °C for 1 h. After removal of the solvent, the residue was purified by column chromatography on silica gel (5%-20% petroleum in EtOAc) to give **1-9** (3.0 g, 58.8%).

LCMS (ESI): m/z 505.1 [M-160].

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.83 - 7.80 (m, 2H), 7.72 - 7.69 (m, 2H), 7.36 - 7.28 (m, 2H), 7.28 - 7.22 (m, 3H), 5.69 - 5.63 (q, 1H), 4.89 (dd, J = 37.6, 9.6 Hz, 1H), 4.63 - 4.58 (m, 2H), 4.20 - 4.11 (m, 2H), 3.74 - 3.69 (m, 2H), 3.35 (dd, J = 13.2, 3.2 Hz, 1H), 2.78 - 2.69 (m, 2H), 1.99 - 1.85 (m, 2H), 1.80 - 1.76 (m, 2H), 0.96 - 0.92 (q, 6H).

Step 6. A solution of **1-9** (3.0 g, 4.50 mmol) in xylene (5 mL) was heated in microwave for 2 h at 135°C. The mixture was cooled to 25 °C and purified by column chromatography on silica gel (5%-10%-50% PE in EtOAc) to give **1-10** (1.4 g, 46.7%).

LCMS (ESI): m/z 685.0 [M+H_2O].

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 - 7.81 (m, 2H), 7.71 - 7.69 (m, 2H), 7.36 - 7.32 (m, 2H), 7.29 - 7.25 (m, 1H), 7.21 - 7.19 (m, 2H), 6.90 (d, J = 8.8 Hz, 1H), 5.11 (dd, J = 36.4, 9.6 Hz, 1H), 4.81 - 4.76 (m, 1H), 4.68 - 4.64 (m, 1H), 4.30 - 4.16 (m, 3H), 3.75 - 3.68 (m, 2H), 3.27 (dd, J = 13.2, 3.2 Hz, 1H), 2.80 - 2.74 (q, 1H), 2.08-2.05 (m, 1H), 1.93 - 1.90 (m, 1H), 1.76 - 1.70 (m, 2H), 1.65 - 1.62 (m, 1H), 1.00 (dd, J = 6.8, 3.2 Hz, 6H).

Step 7. To a solution of compound **1-10** (1.4 g, 2.1 mmol) in THF/H_2O (v/v 4:1, 10 mL) was added H_2O_2 (1.43 g, 30 % in water, 12.6 mmol), followed by $LiOH{\cdot}H_2O$ (264.6 mg, 6.3 mmol). After the solution was stirred for 1.5 h at 25 °C, saturated Na_2SO_3 solution (8 mL) was added. Solvent was removed and the residue was extracted with DCM (20 mL × 2). The aqueous solution was acidified to pH = 1 with 1M HCl, extracted with EtOAc/MeOH (10/1, 25 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated to give compound **1-11** (1.0 g, 93.4%).

LCMS (ESI): m/z 527.0 [M+Na^+].

Step 8. To a solution of compound **1-11** (1.0 g, 1.97 mmol) and (4-aminophenyl) methanol (364 mg, 2.96 mmol) in DCM/MeOH (v/v 2:1, 7.5 mL) was added EEDQ (732 mg, 2.96 mmol) at 0°C under N_2. The mixture was stirred at 25°C for 16 h. The solvent was removed, the residue was purified by column chromatography on silica gel (30% petroleum in EtOAc) to give crude compound **1-13** (1.0 g, 82.8 %).

LCMS (ESI): m/z 614.0 [M+H^+].

Step 9. To a solution of compound **1-13** (1.0 g, 1.63 mmol) in EtOH (15 mL) was added $NaBH_4$ (364.8 mg, 9.60 mmol) at 0°C. The mixture was stirred at 25°C for 2 h and water (10 mL) was added to quench the reaction. Then Cbz-Cl (410.4 mg, 2.40 mmol) was added dropwise at 0°C. The mixture was stirred at 0°C for 1 h. The mixture was diluted with water, and extracted with DCM (25 mL × 3). The organic layer was dried over Na_2SO_4, concentrated under reduced

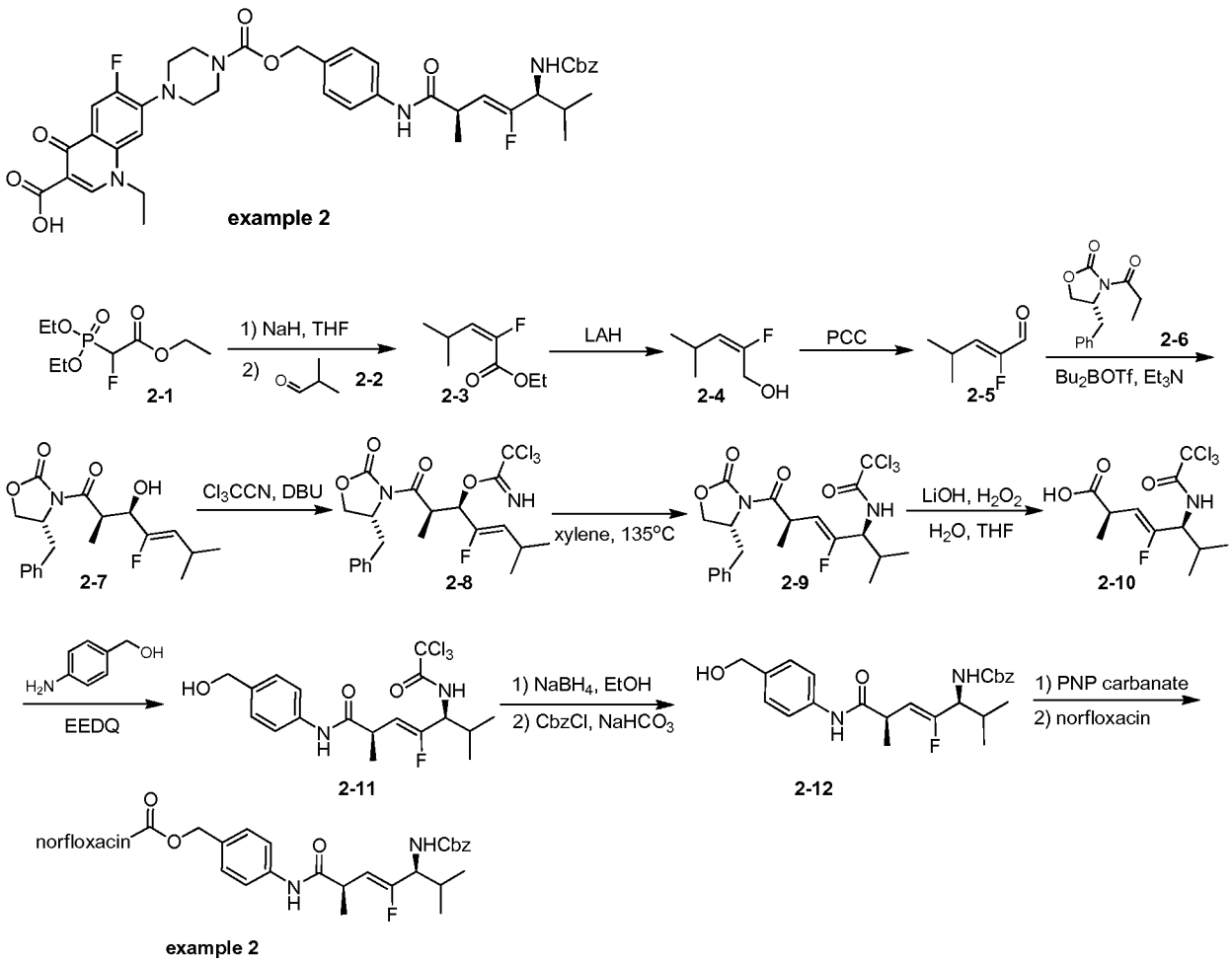
pressure. The residue was purified by column chromatography on silica gel (DCM/MeOH = 20:1~5:1) to give crude compound **1-14** (300 mg, 31.2%).
LCMS (ESI): m/z 602.0 [M+H].

Step 10. To a solution of compound **1-14** (300 mg, 0.498 mmol) in EtOH (10 mL) was added $NH_2NH_2.xH_2O$ (48 mg, 50 %, 0.748 mmol) dropwise. The mixture was heated at reflux for 1 h. The reaction mixture was concentrated, and the residue (**1-15)** was used directly to the next reaction without further purification.

Step 11. To a stirred solution of compound **1-15** (281.0 mg, 0.496 mmol) in DMF (5 mL) was added TEA (100 mg, 0.992 mmol) and CDI (162.7 mg, 0.992 mmol). After the mixture was stirred at 29°C for 1 h, NH_3H_2O (5 mL) was added, and the mixture was stirred at 29 °C for 16 h. The reaction mixture was filtered and the filtrate was purified by prep-HPLC to give compound **1-16** (50 mg, 19.6 %) as white solid.
LCMS (ESI): m/z 515.1 [M+1].
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, 1H), 7.66 (d, J = 9.2 Hz, 1H), 7.53 (d, J = 8.4 Hz, 2H), 7.35 - 7.29 (m, 5H), 7.22 (d, J = 8.4 Hz, 2H), 5.94 (t, J = 6.0 Hz, 1H), 5.36 (s, 2H), 5.10 (t, J = 6.0 Hz, 1H), 5.05 - 4.94 (m, 3H), 4.42 (d, J = 4.2 Hz, 2H), 3.92 - 3.83 (m, 1H), 3.46 - 3.44 (m, 1H), 3.01 - 2.90 (m, 2H), 1.92 - 1.85 (m, 1H), 1.65 - 1.63 (m, 1H), 1.44 - 1.28 (m, 3H), 0.88 (t, J = 6.4 Hz, 6H).

Step 12. To the mixture of compound **1-16** (50 mg, 0.097 mmol) in anhydrous DMF (3 mL) was added DIPEA (63 mg, 0.485 mmol). PNP carbonate (87 mg, 0.291 mmol) was added at 25 °C. The reaction mixture was stirred at 25 °C for 8 h, and norfloxacin (93 mg, 0.291 mmol) was added. After the mixture was stirred at 25 °C for another 3 h, it was filtered, and the filtrate was purified by prep-HPLC to give **example 1** (35 mg, 42.0 %).
LCMS (ESI): RT = 0.885 min, M/2+H$^+$ = 430.7. method = 5-95 /2 min.
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.32 (s, 1H), 10.04 (s, 1H), 8.95 (s, 1H), 7.94 (d, J = 12.8 Hz, 1H), 7.66 (d, J = 8.8 Hz, 1H), 7.60 (d, J = 8.8 Hz, 2H), 7.34 - 7.28 (m, 7H), 7.21 (d, J = 7.2 Hz, 1H), 5.94 (t, J = 5.2 Hz, 1H), 5.36 (s, 2H), 5.06 - 4.94 (m, 5H), 4.59 - 4.57 (q, 2H), 3.92 - 3.84 (m, 1H), 3.61 (s, 4H), 3.49 - 3.43 (q, 1H), 3.33 - 3.31 (m, 4H), 3.01 - 2.90 (m, 2H), 1.90 - 1.83 (m, 1H), 1.68 - 1.65 (m, 1H), 1.41 (d, J = 6.8 Hz, 5H), 1.33 - 1.23 (m, 1H), 0.86 (t, J = 6.8 Hz, 6H).

Example 2. 7-(4-((4-((2R,5S,Z)-5-(benzyloxycarbonylamino)-4-fluoro-2,6-dimethylhept-3-enamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



Step 1. To a mixture of NaH (1.82 g, 45.5 mmol) in THF (200 mL) was added **2-1** (10.0 g, 41.3 mmol) in THF (20 mL) dropwise at 0°C. The mixture was stirred at 0°C for 1 h and then cooled to -78°C. Compound **2-2** (2.98 g, 41.3 mmol) in THF (5 mL) was added dropwise and the mixture was warmed to 25°C slowly, and stirred at 25°C for 16 h. Saturated NH_4Cl solution was slowly added at 0°C followed by water (50 mL) and the mixture was extracted with EtOAc (80 mL×2). The organic layer was dried over Na_2SO_4, filtered, concentrated and purified by column chromatography on silica gel (PE/EtOAc = 50:1) to give **2-3** (2.2 g, 33%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (dd, J = 10.4, 22.0 Hz, 1 H), 4.30 (q, J = 7.2 Hz, 2 H), 3.37-3.31 (m, 1 H), 1.35 (t, J = 7.2 Hz, 3 H), 1.08-1.04 (m, 6 H).

Step 2. To a solution of **2-3** (12.0 g, 74.91 mmol) in THF (80 mL) was added $LiAlH_4$ (5.69 g, 149.82 mmol) at 0°C. After the mixture was stirred for 2 h at 0°C, it was quenched with saturated NH_4Cl solution (1 mL). The organic solvent was removed under reduced pressure and the mixture was diluted with water (50 mL) and extracted with EtOAc (100 mL × 2). The organic layer was dried over Na_2SO_4, filtered, concentrated to give crude product **2-4** (8.0 g).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.06 (dd, J = 10.4, 21.2 Hz, 1 H), 4.24 (d, J = 21.2 Hz, 2 H), 2.42-2.37 (m, 1 H), 1.03-0.99 (m, 6 H).

Step 3. To a solution of **2-4** (8.0 g, 33.86 mmol) in DCM (100 mL) was added PCC (29.2 g, 67.72 mmol) at 25 °C. After the mixture was heated at reflux for 3 h under N_2, it was cooled to 25 °C and filtered through a plug of silica gel. The filtrate concentrated to give crude **2-5** (5.1 g).
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (d, J = 18.0Hz, 1 H), 5.79 (dd, J = 9.6, 32.8 Hz, 1 H), 3.01-2.95 (m, 1 H), 1.15-1.13 (m, 6 H).

Step 4. Bu_2BOTf in DCM (1M, 66 mL, 66 mmol) was added dropwise to solution of **2-6** (15.37 g, 65.88 mmol) in DCM (200 mL), followed by Et_3N (8.89 g, 87.84 mmol) at a rate as to keep the internal temperature below 3°C. It was cooled to -65°C, and **2-5** (5.1 g, 43.92 mmol) in DCM (10 mL) was added dropwise. The solution was stirred for 20 min in the dry ice-acetone bath, then for 1 h at ice bath. The reaction mixture was quenched with a pH 7 aqueous phosphate buffer (50 mL) and MeOH (150 mL). To this cloudy solution was added a mixtureof MeOH/30% H_2O_2 (2:1, 90 mL) at such a rate as keep the internal temperature below 10°C. After the solution was stirred for an additional 1h, the volatile was removed on a rotary evaporator at a bath temperature of 25-30°C. The resulting slurry was extracted with EtOAc (150 mL×3). The combined organic layer was washed with saturated Na_2SO_3 solution (150 mL), 5% $NaHCO_3$ solution (150 mL) and brine (150 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EtOAc 3:1) to give **2-7** (8.8 g, 57%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.27 (m, 3 H), 7.21-7.19 (m, 2 H), 4.85 (dd, J = 9.2, 38.4 Hz, 1 H), 4.73-4.67 (m, 1 H), 4.53-4.50 (m, 1 H), 4.27-4.19 (m, 2 H), 3.99-3.95 (m, 1 H), 3.24 (dd, J = 3.2, 13.6 Hz, 1 H), 3.17-3.16 (m, 1 H), 2.83-2.74 (m, 2 H), 1.29 (d, J = 6.8Hz, 3 H), 1.02-0.99 (m, 6 H).

Step 5. To a solution of **2-7** (2.0 g, 5.72 mmol) and Cl_3CCN (1.24 g, 8.58 mmol) in DCM (15 mL) was added DBU (174 mg, 1.14 mmol) at 0 °C under N_2. After the mixture was stirred at 0°C for 1 h, solvent was removed and the residue was purified by column chromatography on silica gel (5%-20% petroleum in EtOAc) to give **2-8** (1.55 g, 55%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1 H), 7.35-7.27 (m, 3 H), 7.25-7.19 (m, 2 H), 5.81-5.75 (m, 1 H), 4.98-4.85 (m, 1 H), 4.64-4.59 (m, 1 H), 4.40-4.36 (m, 1 H), 4.22-4.17 (m, 2 H), 3.27-3.24 (m, 1 H), 2.83-2.71 (m, 2 H), 1.40-1.38 (m, 3 H), 0.99-0.95 (m, 6 H).

Step 6. A solution of **2-8** (1.55 g, 3.14 mmol) in xylene (10 mL) was heated at 135°C for 30 h. It was cooled to r.t. and the solvent was removed under reduced pressure. The residue was purified

by column chromatography on silica gel (5%-10%-50% of petroleum in EtOAc) to give **2-9** (450 mg, 29%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.28 (m, 3 H), 7.21-7.20 (m, 2 H), 6.75 (d, J = 8.4 Hz, 1 H), 5.20 (dd, J = 9.2, 37.6 Hz, 1 H), 4.76-4.65 (m, 2 H), 4.28-4.17 (m, 3 H), 3.27-3.23 (m, 1 H), 2.79 (dd, J = 9.6, 13.2 Hz, 1 H), 2.07-2.00 (m, 1 H), 1.33 (d, J = 7.2Hz, 3 H), 1.00-0.94 (m, 6 H).

Step 7. To a solution of **2-9** (950 mg, 1.92 mmol) in THF and H_2O (v/v 4:1, 10 mL) was added H_2O_2 (1.38 mg, 11.52 mmol), followed by $LiOH{\cdot}H_2O$ (242 mg, 5.76 mmol) in water (2 mL). After the solution was stirred at 10 °C for 1 h, saturated Na_2SO_3 solution (8 mL) was added. Solvent was removed, and the residue was washed with DCM (20 mL × 2). The aqueous solution was acidified to pH 1 with 1M HCl, and extracted with EtOAc (25 mL × 3). The organic layer was dried over Na_2SO_4, filtered, concentrated to give **2-10** (420 mg, 65%).

LCMS (ESI): m/z 334.0 [M+H$^+$].

Step 8. To a solution of **2-10** (420 mg, 1.26 mmol) and (4-aminophenyl)methanol (233 mg, 1.89 mmol) in DCM (10 mL) was added EEDQ (623 mg, 2.52 mmol) at 0°C under N_2. After the mixture was stirred at 10°C for 3 h, solvent was removed, and the residue was purified by column chromatography on silica gel (30% of petroleum in ethyl acetate) to give **2-11** (300 mg, 54%).

LCMS (ESI): m/z 439.0 [M+H$^+$].

Step 9. To a solution of **2-11** (300 mg, 0.682 mmol) in EtOH (6 mL) was added $NaBH_4$ (300 mg, 7.94 mmol) at 0°C. The mixture was stirred at 10°C for 3 h. Water (0.5 mL) was added, and EtOH was removed under reduced pressure. The crude was dissolved in a mixture of THF (4 mL) and saturated $NaHCO_3$ solution (4 mL), and CbzCl (140 mg, 8.18 mmol) was added at 10°C. After the mixture was stirred at 10°C for 2 h, solvent was removed and the residue was extracted with DCM (8 mL×2). The organic layer was dried over Na_2SO_4, concentrated, and purified by column chromatography on silica gel (PE/EtOAc = 2:1) to give **2-12** (125 mg, 43%).

^{1}H NMR (400 MHz, MeOD) δ 7.53-7.46 (m, 2 H), 7.36-7.25 (m, 7 H), 5.11-5.00 (m, 3 H), 4.56 (s, 2 H), 3.94-3.87 (m, 1 H), 3.63-3.59 (m, 1 H), 1.98-1.92 (m, 1 H), 1.27 (d, J = 6.8 Hz, 3 H), 0.96-0.93 (m, 6 H).

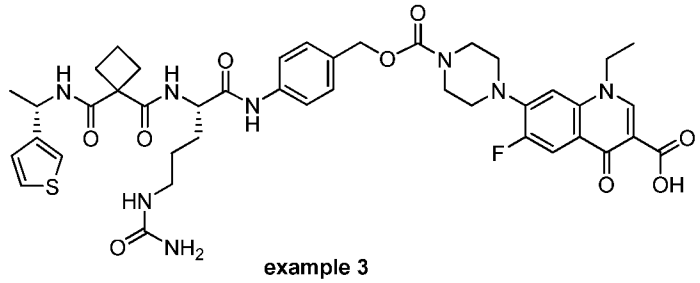
LCMS (ESI): m/z 429.2 [M+H$^+$].

Step 10. To a solution of **2-12** (20 mg, 0.0467 mmol) in DCM (2 mL) was added PNP carbonate (43 mg, 0.140 mmol) and DIEA (24 mg, 0.187 mmol) at 10 °C. After the mixture was heated at reflux for 16 h, solvent was removed, and the residue was dissolved in DMF (2 mL). To this solution were added DIEA (24 mg, 0.187 mmol) and norfloxacin (22 mg, 0.070 mmol) at 10°C, and the resulting solution was stirred at 10°C for 1 h. After the solvent was removed, the residue was purified by prep-HPLC to give **example 2** (14.1 mg, 39%).

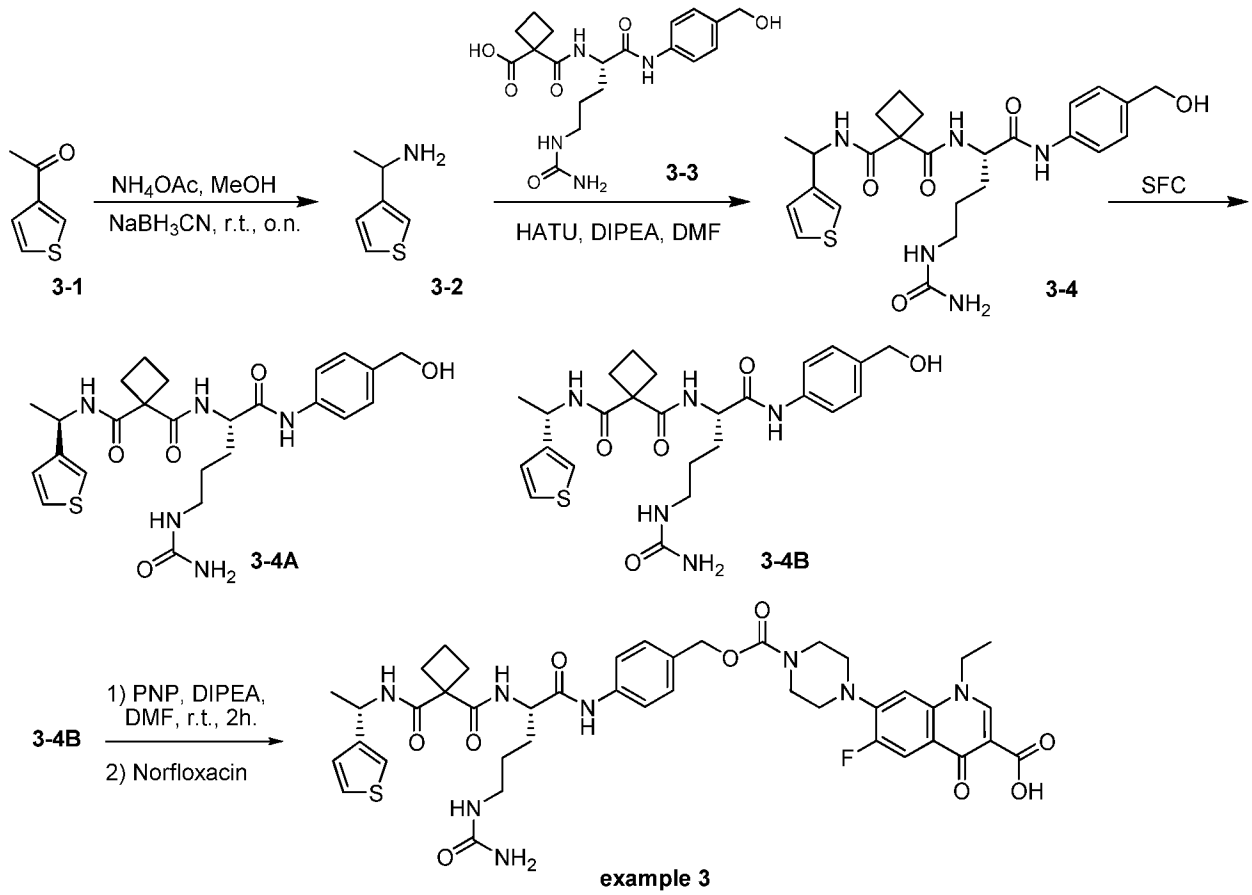
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.35 (br, 1 H), 10.02 (s, 1 H), 8.95 (s, 1 H), 7.95-7.91 (m, 1 H), 7.69-7.58 (m, 3 H), 7.34-7.19 (m, 8 H), 5.12-5.00 (m, 5 H), 4.59-4.57 (m, 2 H), 3.87-3.81 (m, 1 H), 3.60-3.51 (m, 5 H), 3.30 (s, 4 H), 1.89-1.83 (m, 1 H), 1.40 (t, J = 7.2 Hz, 3 H), 1.20-1.81 (m, 3 H), 0.87 (m, 6 H).

LCMS (ESI): m/z 774.8 [M+H$^+$]

Example 3. 1-ethyl-6-fluoro-4-oxo-7-(4-((4-((S)-2-(1-((S)-1-(thiophen-3-yl)ethylcarbamoyl) cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.



example 3



example 3

Step 1. To a solution of compound **3-1** (2 g, 15.9 mmol) in MeOH (20 mL) was added NH_4OAc (12.2 g, 0.159 mol) and $NaBH_3CN$ (3.5 g, 55.5 mmol) at 20°C. After the mixture was stirred at 20°C for 16 h, it was concentrated. The residual was partitioned between EtOAc (50 mL) and

NaOH solution (5 M, 5 mL) (adjust pH>13). The organic layer was washed with brine (20 mL×2), concentrated and purified by column (20%-30% MeOH in DCM) to give the crude compound **3-2** (800 mg, 40% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 - 7.61 (d, J = 8.0 Hz, 1 H), 7.58 - 5.57 (m, 1 H), 7.25 - 7.23 (d, J = 8.0 Hz , 2 H), 4.53 - 4.48 (m, 1 H), 1.51 - 1.49 (d, J = 8.0 Hz , 3 H).

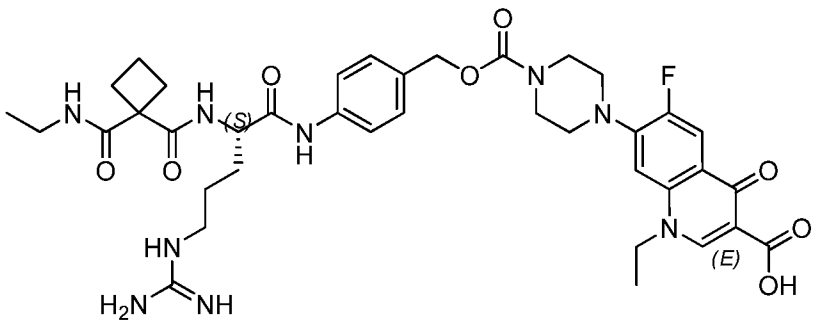
Step 2. To a solution of compound **3-3** (500 mg, 1.23 mmol) in DMF (3 mL) was added compound **3-2** (600 mg, 4.7 mmol), DIPEA (0.5 mL, 3 mmol) and HATU (740 mg, 2 mmol) at 20°C. After the mixture was stirred at 20°C for 2 h, it was purified by prep-HPLC to give **3-4** (360 mg, 57% yield). Compound **3-4** (300 mg) was separated by SFC separation to give 2 isomers **3-4A** and **3-4B**.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1 H), 8.12 - 8.10 (m, 1H), 7.9 -7.75 (m, 1 H), 7.55 - 7.52 (m, 2 H), 7.45 - 7.35 (m, 2 H), 7.3 - 7.2 (m, 3 H), 7.04 (m, 1 H), 6.0 (m, 1 H), 5.5 - 5.2 (m, 2 H), 5.1 - 5.0 (m, 1 H), 4.45 - 4.35 (m, 3 H), 3.1 - 2.9 (m, 4 H), 2.43 - 2.39 (m, 4 H), 1.8 - 1.7 (m, 3 H), 1.7 - 1.5 (m, 1 H), 1.5-1.33 (m, 5 H).

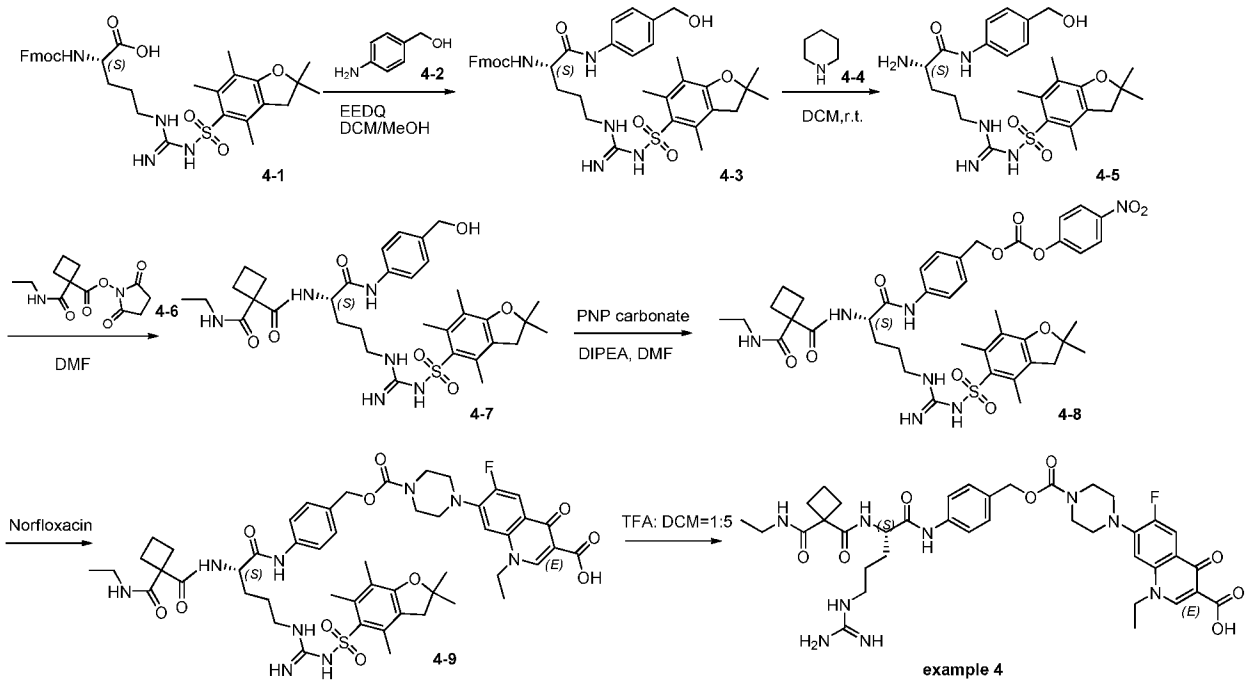
Step 3. To a solution of compound **3-4B** (110 mg, 0.21 mmol) in dry DMF (3 mL) was added PNP (130 mg, 0.43 mmol) and DIPEA (0.5 ml, 3 mmol) at 20°C. After the mixture was stirred at 20°C for 1.5 h under N_2, norfloxacin (100 mg, 0.31 mmol) was added. The mixture was stirred at 20°C for another 1 h and purified by prep-HPLC (FA), to give **example 3** (102.1 mg, 57%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H), 8.93 (s, 1 H), 8.10 - 8.08 (d, 1H), 8.04 (s, 1 H), 7.93 - 7.90 (m, 2 H), 7.60 - 7.58 (d, J = 8.0 Hz , 2 H), 7.41 - 7.39 (d, , 2 H), 7.32 - 7.30 (d, J = 8.0 Hz , 2 H), 7.25 (d, 1H), 7.20 (d, 1H), 7.04 - 7.02(d, 1H), 6.0 (m, 1 H), 5.40 (s, 2 H), 5.1-5.0 (m, 3 H), 4.6 - 4.5 (m, 2 H), 4.5 - 4.35 (m, 1 H), 3.59 (s, 1 H), 3.2 (s, 4 H), 3.1 - 2.9 (m, 2 H), 2.42 - 2.40 (m, 4 H), 1.8 - 1.7 (m, 3 H), 1.7 - 1.6 (m, 1 H), 1.5 - 1.3 (m, 8 H).

Example 4. (S)-1-ethyl-7-(4-((4-(2-(1-(ethylcarbamoyl)cyclobutanecarboxamido)-5-guanidinopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 4



Step 1. To a solution of compound **4-1** (1.3 g, 2 mmol) in DCM / MeOH (20 mL / 20 mL) were added 4-amino-phenyl)-methanol (370 mg, 3 mmol) and EEDQ (989 mg, 4 mmol). The mixture was stirred at r.t. for 16 h. The residue was purified by column (PE/EtOAc=1/3) to give **4-3** (1.94 g, Yield: 90 %)

To a solution of **4-3** (1.44 g, 2 mmol) in DCM (50 mL) were added **4-4** (1.2 ml, 9.56 mmol) via syringe at r.t. The mixture was stirred at r.t. for 16 h. The residue was concentrated and washed by methyl tert-butyl ether, then filtered and the filtrate cake was combined to give **4-5** (700 mg, Yield: 70 %).

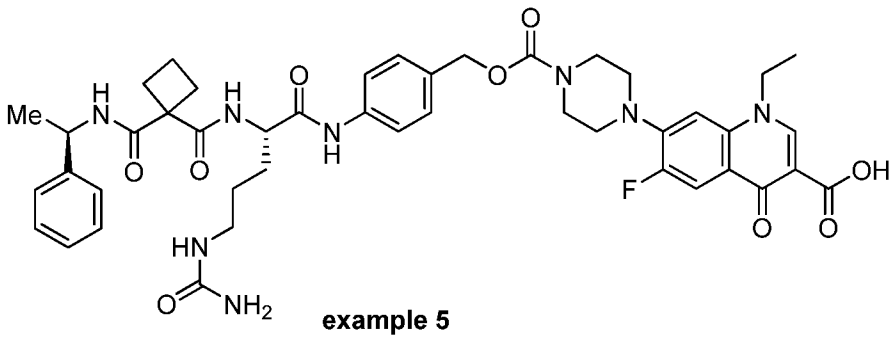
Step 2. To a solution of **4-5** (700 mg, 1.32 mmol) in DME (10 ml) was added a solution of **4-6** (424 mg, 1.58 mmol) and $NaHCO_3$ (222 mg, 2.64 mmol) in water (10 ml). The mixture was stirred at r.t. for 16 h. The mixture was washed with EtOAc and adjusted to pH = 3 with 10% HCl. The resulting suspension was extracted with EtOAc. The combined organic layers was concentrated and purified by column (PE/EtOAc=1/3) to give **4-7** (400 mg, Yield: 37 %)

Step 3. To a solution of **4-7** (250 mg, 0.365 mmol), PNP carbonate (223 mg, 0.731 mmol) in DMF (4 mL) was added DIPEA (142 mg, 1.095 mmol) at 0°C. The mixture was stirred at r.t. for 16 h. The mixture (**4-8**) was used for next step without further purification.

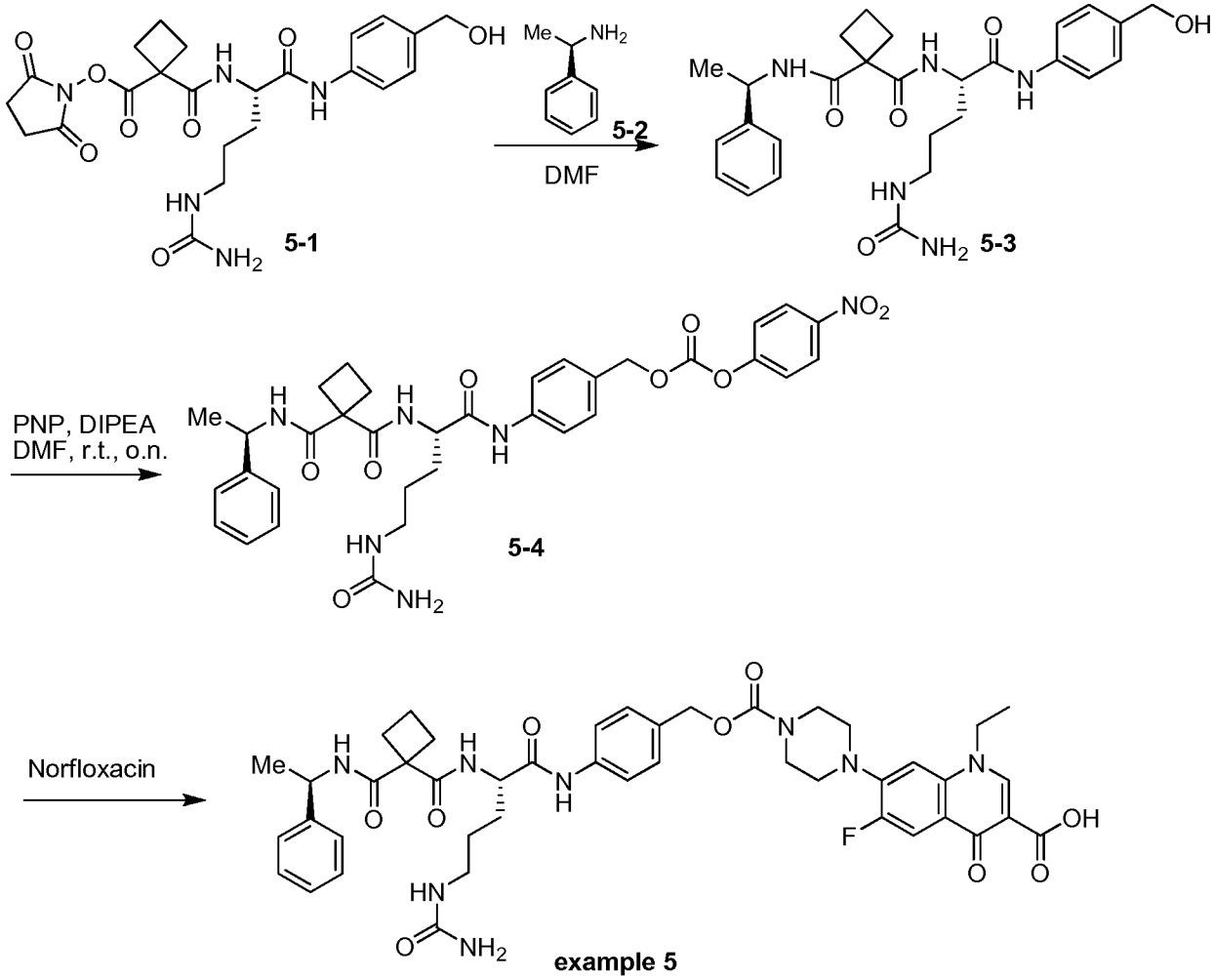
Step 4. To the mixture from last step was added norfloxacin (234 mg, 0.73 mmol) at r.t. After the mixture was stirred at r.t. for 1 h, it was purified by prep-HPLC and SFC to give **4-9** (Yield: 30 %).

Step 5. To compound **4-9** (100 mg, 0.1 mmol) was added a solution of TFA in DCM (1:5) at 0 °C. The mixture was stirred at r.t. for 3 h. The mixture was basified to pH = 9 by $NH_3.H_2O$. The residue was purified by prep-HPLC then SFC to give **example 4** (16.0 mg, Yield: 15 %). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.83 (s, 1 H), 8.55 (s, 1 H), 7.97 (d, J = 13.2 Hz, 1 H), 7.62 (d, J = 7.6 Hz, 2 H), 7.35 (d, J = 7.6 Hz, 2 H), 7.17 (s, 1 H), 5.13 (s, 2 H), 4.55 - 4.52 (m, 3 H), 3.72 (s, 4 H), 3.34 (s, 4 H), 3.31 – 3.24 (m, 4 H), 2.58 - 2.54 (m, 4 H), 2.00 – 1.69 (m, 6 H), 1.52 (s, 3 H), 1.15 – 0.11 (m, 3 H).

Example 5. 1-ethyl-6-fluoro-4-oxo-7-(4-((4-((S)-2-(1-((S)-1-phenylethylcarbamoyl) cyclobutanecarboxamido)-5 ureidopentanamido)benzyloxy)carbonyl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid



example 5



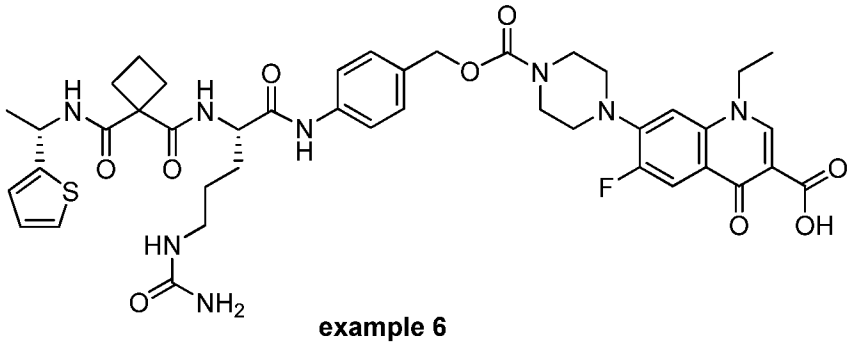
Step 1. A mixture of compound **5-1** (50 mg, 0.1 mmol) and **5-2** (15 mg, 0.12 mmol) in DMF (10 mL) was stirred at r.t. for 3 h. The mixture was concentrated and purified by prep-TLC (MeOH/ DCM=1/10) to give the **5-3** (50 mg, 99 %).

Step 2. To a solution of compound **5-3** (40 mg, 0.078 mmol), PNP carbonate (48 mg, 0.157 mmol) in DMF (4 mL) was added DIPEA (30 mg, 0.236 mmol) at 0°C. The mixture was stirred at r.t. for 16 h. The mixture (**5-4**) was used for next step without further purification.

Step 3. To the mixture from last step was added norfloxacin (51 mg, 0.157 mmol) at r.t. and stirred for 1 h. The residue was purified by prep-HPLC to give **example 5**. (7 mg, Yield: 8 %)

^{1}H NMR (400 MHz, MeOH-d_4) δ 8.86 (s, 1 H), 8.46 (s, 1 H), 8.00 (d, J = 12.8 Hz, 1 H), 7.61 (d, J = 8 Hz, 2 H), 7.35 – 7.33 (m, 2 H), 7.30 (d, J = 7.2 Hz, 2 H), 7.24 (d, J = 7.2 Hz, 2 H), 7.20 (d, J = 14 Hz, 2 H), 7.16 (s, 2 H), 5.12 (s, 2 H), 5.04 (d, J = 7.2 Hz, 1 H), 4.50 – 4.47 (m, 4 H), 3.71 (s, 4 H), 3.34 (s, 4 H), 3.22 – 3.03 (m, 2 H), 2.60 – 2.51 (m, 4 H), 1.94 – 1.86 (m, 3 H), 1.74 – 1.71 (m, 1 H), 1.60 – 1.45 (m, 7 H).

Example 6. 1-ethyl-6-fluoro-4-oxo-7-(4-((4-((S)-2-(1-((S)-1-(thiophen-2-yl)ethylcarbamoyl) cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

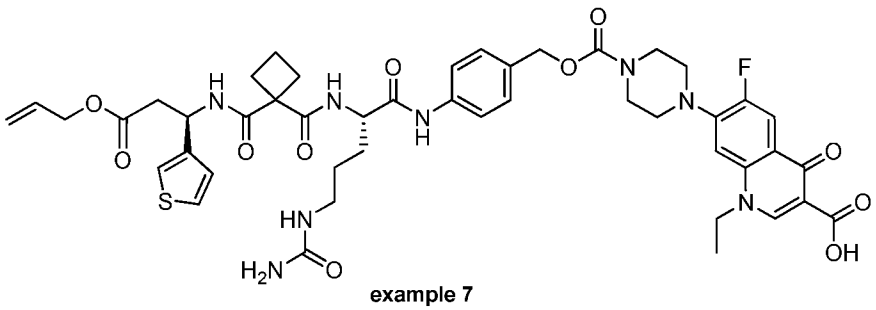


example 6

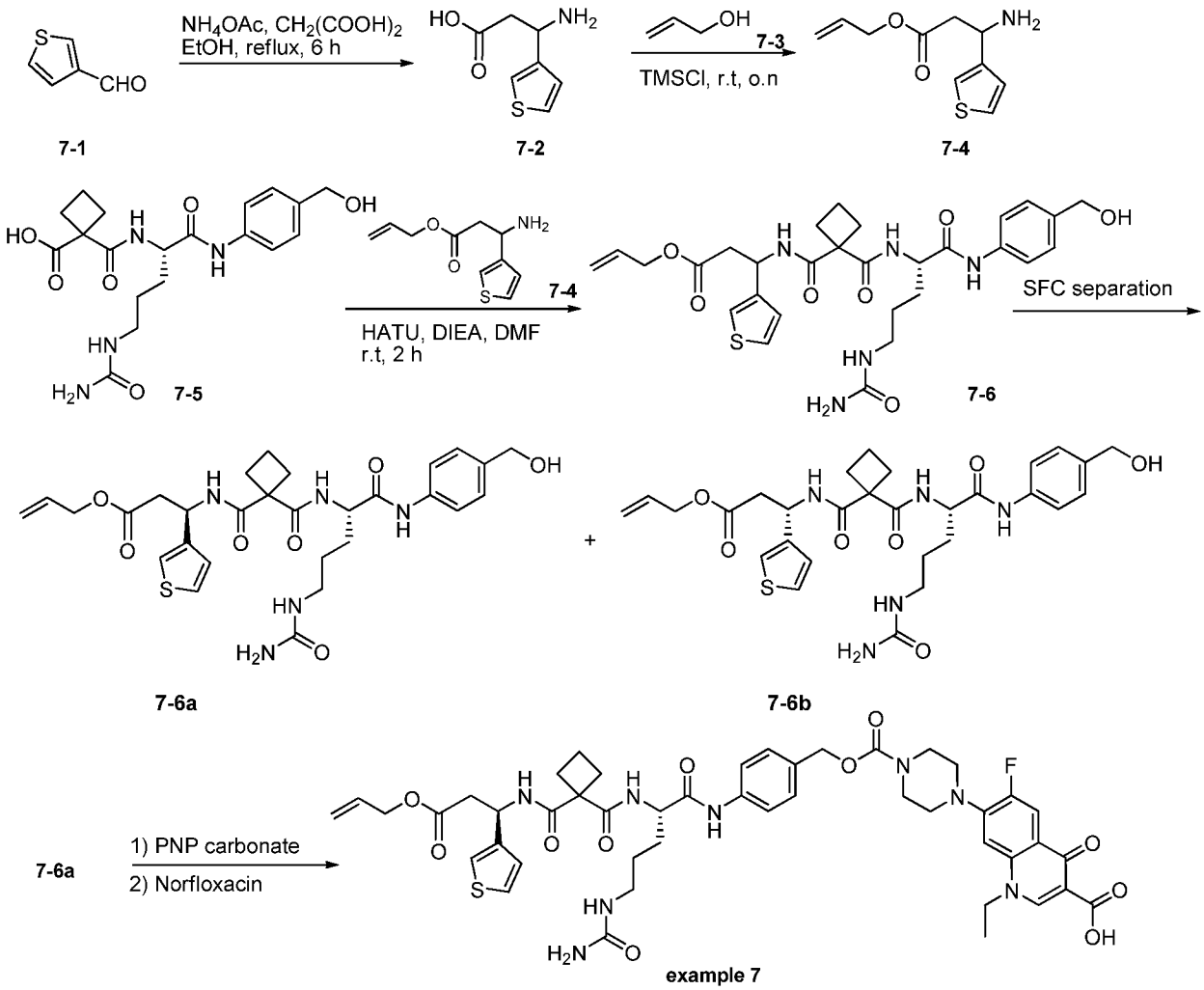
Example 6 was made using the procedure as **Example 3**. It shared the intermediate with **Example 10**.

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.3 (s, 1H), 10.13 (s, 1H), 8.93 (s, 1 H), 8.25 (d, 1H), 7.94 - 7.91 (d, J = 12.0 Hz, 1H), 7.8 (d, 1 H), 7.60 - 7.58 (d, J = 8.0 Hz, 2 H), 7.33 - 7.30 (d, J = 8.0 Hz , 3H), 7.20 (d, 1 H), 6.92 - 6.91 (m, 2 H), 5.95 (m, 1 H), 5.40 (s, 1H), 5.25 - 5.15 (m, 1H), 5.04 (s, 2 H), 4.65 - 4.4 (m, 2 H), 4.5 - 4.4 (m, 1 H), 3.7 - 3.5 (s, 4 H), 3.3 (s, 4 H), 3.1 - 2.85 (m, 2 H), 2.44 - 2.42 (m, 4 H), 1.8 - 1.7 (m, 3 H), 1.7 - 1.55 (m, 1 H), 1.46 - 1.45 (d, J = 4.0 Hz , 1 H), 1.45 - 1.3 (m, 5 H).

Example 7. 7-(4-((4-((S)-2-(1-((R)-3-(allyloxy)-3-oxo-1-(thiophen-3-yl)propylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 7



Step 1. To a solution of compound **7-1** (10.0 g, 89.17 mmol) and $CH_2(COOH)_2$ (9.3 g, 89.17 mmol) in EtOH (100 mL) was added NH_4OAc (13.7 g, 178.33 mmol). The mixture was stirred at 80 °C for 6 h. It was cooled to r.t, filtered, and washed with EtOH (100 mL). The filter cake was collected, and concentrated under reduced pressure to afford compound **7-2** (8.0 g, 52.6 %) as white solid.

^{1}H NMR D_2O 400 MHz, δ 7.44 (s, 2H), 7.13 (d, J = 3.2 Hz, 1H), 4.69 (t, J = 7.2 Hz, 1H), 2.84 - 2.73 (m, 2H).

Step 2. To a mixture of compound **7-2** (6.0 g, 35.04 mmol) in compound **7-3** (60.0 g) was added TMSCl (13.4 mL, ρ = 0.85, 105.13 mmol) dropwise. The reaction mixture was stirred at r.t. under N_2 overnight. The mixture was concentrated under reduced pressure, and the residue was diluted with H_2O (50 mL), adjusted to pH 1 by HCl solution, and washed with EtOAc (50 mL × 3). The water phase was adjusted to pH 12 by NH_3H_2O, and extracted with EtOAc (50 mL × 3). The combined EtOAc layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford compound **7-4** as an oil (4.0 g, 54.1 %).

^{1}H NMR DMSO-d_6 400 MHz, δ 7.45 - 7.43 (q, 1H), 7.31 (s, 1H), 7.15 (d, J =4.8 Hz, 1H), 5.91 - 5.85 (m, 1H), 5.27 (dd, J = 17.2, 1.6 Hz, 1H), 5.20 - 5.17 (m, 1H), 4.53 (d, J = 5.2 Hz, 2H), 4.31 - 4.27 (m, 1H), 2.73 - 2.61 (m, 2H).

Step 3. To a mixture of compound **7-5** (1.0 g, 2.46 mmol) in DMF (20 mL) was added DIPEA (636 mg, 4.92 mmol), followed by HATU (1.4 g, 3.69 mmol). The mixture was stirred at r.t. for 15 min, and compound **7-4** (624 mg, 2.95 mmol) was added. The reaction mixture was stirred at r.t. for 2 h and purified by prep-HPLC to afford compound **7-6** as white solid (800 mg, 54.1 %). Compound **7-6** was scaled up to 1.5 g, and was separated by SFC to afford **7-6a** (580 mg) and **7-6b** (560 mg).

7-6a

LCMS: (5-95, AB, 1.5 min, ESI), 0.773 min, MS = 600.1 [M+1]

^{1}H NMR DMSO-d_6 400 MHz, δ 10.05 (s, 1H), 8.25 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 7.9 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 7.45 - 7.43 (q, 1H), 7.31 (d, J = 2.6 Hz, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.08 (d, J = 4.0 Hz, 1H), 5.97 (t, J = 5.6 Hz, 1H), 5.90 - 5.80 (m, 1H), 5.43 - 5.37 (m, 3H), 5.27 (dd, J = 17.4, 1.5 Hz, 1H), 5.17 (dd, J = 10.4 Hz, 1.3 Hz, 1H), 5.17 (t, J = 5.7 Hz, 1H), 4.51 (d, J = 5.3 Hz, 2H), 4.44 - 4.39 (m, 3H), 3.06 - 2.98 (m, 1H), 2.97 - 2.88 (m, 3H), 2.48 - 2.39 (m, 4H), 1.78 - 1.70 (m, 3H), 1.64 - 1.55 (m, 1H), 1.42 - 1.28 (m, 2H).

7-6b

LCMS: (5-95, AB, 1.5 min, ESI), 0.762 min, MS = 600.1 [M+1]

^{1}H NMR DMSO-d_6 400 MHz, δ 10.04 (s, 1H), 8.26 (d, J = 8.4 Hz, 1H), 7.79 (d, J = 7.7 Hz, 1H), 7.57 (d, J = 8.4 Hz, 2H), 7.39 - 7.37 (q, 1H), 7.31 (s, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 4.9 Hz, 1H), 5.97 (t, J = 5.5 Hz, 1H), 5.91 - 5.81 (m, 1H), 5.43 - 5.36 (m, 3H), 5.26 (dd, J = 17.3, 1.4 Hz, 1H), 5.18 (d, J = 10.4 Hz, 1H), 5.11 (t, J = 5.7 Hz, 1H), 4.53 - 4.52 (m, 2H), 4.44 - 4.40 (m, 3H), 3.05 - 2.98 (m, 1H), 2.97 - 2.85 (m, 3H), 2.46 - 2.33 (m, 4H), 1.77 - 1.72 (m, 3H), 1.64 - 1.56 (m, 1H), 1.42 - 1.30 (m, 2H).

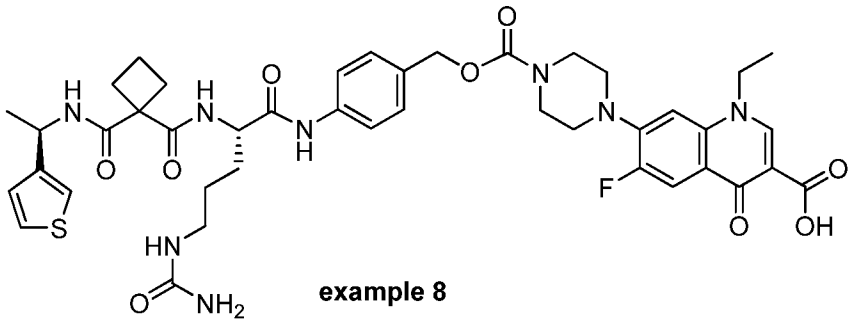
Step 4. To a solution of **7-6a** (50 mg, 0.083 mmol) in anhydrous DMF (5 mL) was added DIPEA (107 mg, 0.83 mmol), followed by PNP carbonate (60 mg, 0.20 mmol). The reaction mixture was stirred at r.t. overnight. Then norfloxacin (64 mg, 0.20 mmol) was added. The reaction mixture was stirred at r.t. for another 2 h. The mixture was filtered, and the filtrate was purified by prep-HPLC to afford **example 7** as a white solid (41.7 g, 53.5 %).

LCMS: (5-95, AB, 1.5 min, ESI), 0.858 min, MS = 473.3 [1/2M+1]

^{1}H NMR DMSO-d_6 400 MHz, δ 15.32 (br, 1H), 10.15 (s, 1H), 8.95 (s, 1H), 8.23 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 13.0 Hz, 1H), 7.77 (d, J = 7.5 Hz, 1H), 7.62 (d, J = 8.0 Hz, 2H), 7.44 - 7.42 (q, 1H), 7.35 - 7.31 (m, 3H), 7.19 (br, 1H), 7.08 (d, J = 4.5 Hz, 1H), 5.98 (br, 1H), 5.89 - 5.80 (m, 1H), 5.44 - 5.37 (m, 3H), 5.23 (d, J = 17.6 Hz, 1H), 5.16 (d, J = 10.5 Hz, 1H), 5.07 (s, 2H), 4.57

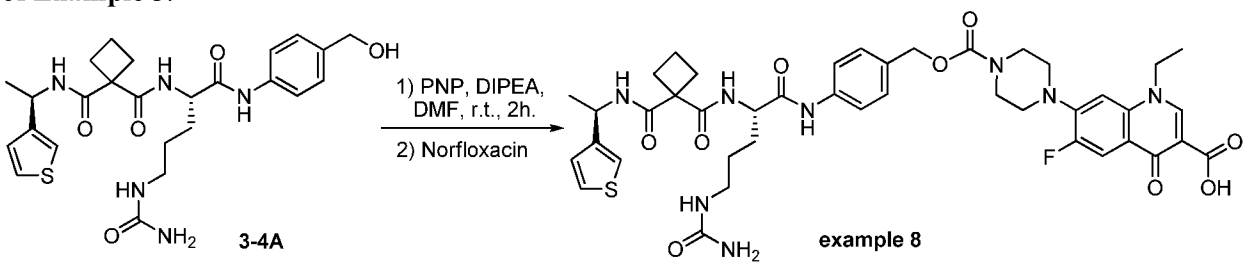
(br, 2H), 4.51 (d, J = 5.5 Hz, 2H), 4.45 - 4.40 (m, 1H), 3.61 (br, 4H), 3.31 (br, 4H), 3.06 - 3.01 (m, 1H), 2.95 - 2.90 (m, 3H), 2.47 - 2.40 (m, 4H), 1.76 - 1.72 (m, 3H), 1.62 - 1.59 (m, 1H), 1.40 - 1.35 (m, 5H).

Example 8. 1-ethyl-6-fluoro-4-oxo-7-(4-((4-((S)-2-(1-((R)-1-(thiophen-3-yl)ethylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid



example 8

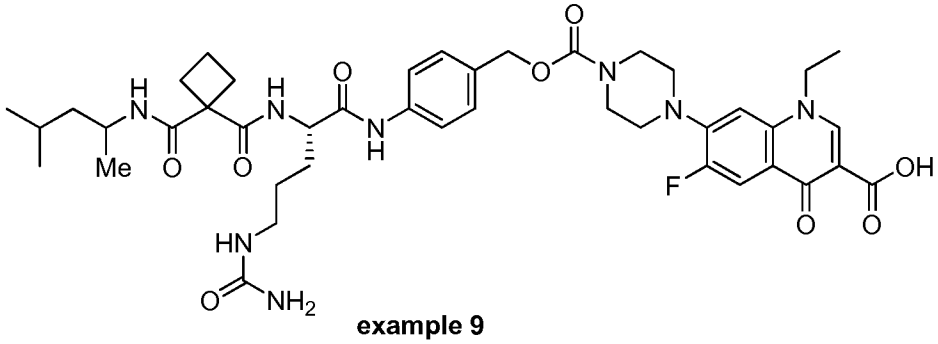
Example 8 was made using the procedure as **Example 3**, with the intermediate from the synthesis of **Example 3**.



3-4A example 8

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H), 8.92 (s, 1 H), 8.05 - 8.03 (d, 1H), 7.93 - 7.90 (d, J = 12.0 Hz, 1 H), 7.85 - 7.83 (d, 1 H), 7.61 - 7.59 (d, J = 8.0 Hz , 1H), 7.36 - 7.35 (m , 1 H), 7.33 - 7.31 (d, J = 8.0 Hz , 2 H), 7.24 (s, 1 H), 7.20 (m, 1 H), 5.5 (m, 1 H), 7.04 - 7.03 (m, 1H), 6.0 (m, 1H), 5.40 (s, 2 H), 5.1 - 5.0 (m, 3 H), 4.6 - 4.5 (m, 2 H), 4.5 - 4.35 (m, 1 H), 3.59 (s, 1 H), 3.2 (s, 4 H), 3.05 - 2.9 (m, 2 H), 2.44 - 2.41 (m, 4 H), 1.8 - 1.7 (m, 3 H), 1.7 - 1.6 (m, 1 H), 1.5 - 1.35 (m, 8 H).

Example 9. 1-ethyl-6-fluoro-7-(4-((4-((2S)-2-(1-(4-methylpentan-2-ylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



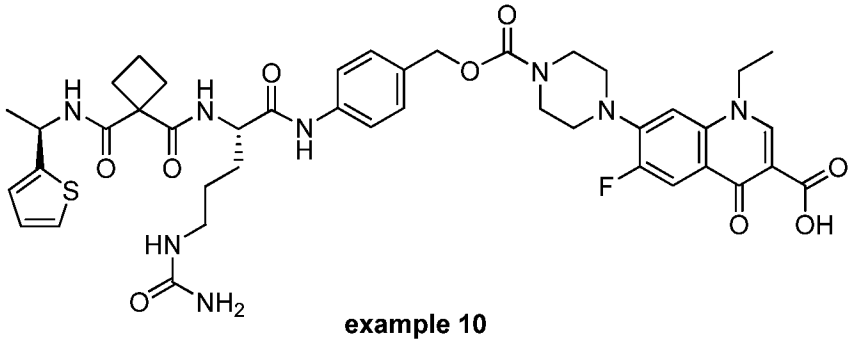
example 9

Example 9 was made using the procedure as **Example 5**

LCMS : (5-95 AB, 1.5 min), T = 0.858 min, M = 8.352 (M+1);
^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.22 (s, 1H), 8.85 (s, 1H), 7.89 - 7.71 (m, 2H), 7.65 - 7.64 (d, *J* = 8.8 Hz, 2H), 7.53 - 7.50 (m, 1H), 7.34 - 7.31 (d, *J* = 8.8 Hz, 2H), 7.18 - 7.13 (m, 1H), 6.06 (s, 1H), 5.45 (d, *J* = 3.2 Hz, 2H), 5.05 (s, 2H), 4.60 - 4.42 (m, 2H), 3.91 - 3.88 (t, *J* = 6.4 Hz, 1H), 3.59 (s, 4H), 3.26 (s, 4H), 3.03 - 2.99 (m, 1H), 2.97 - 2.91 (m, 1H), 2.41 - 2.39 (m, 5H), 1.74 - 1.71 (m, 3H), 1.7 - 1.5 (m, 2H), 1.5 - 1.3 (m, 6H), 1.2 - 1.1 (m, 1H), 1.03 -1.00 (m, 3H), 0.81 (m, 6H).

.

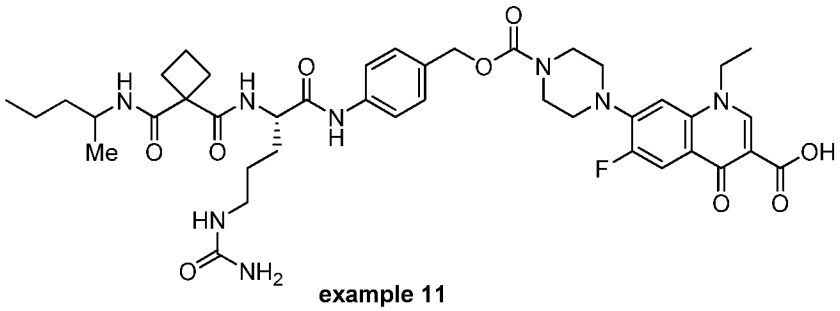
Example 10. 1-ethyl-6-fluoro-4-oxo-7-(4-((4-((S)-2-(1-((R)-1-(thiophen-2-yl)ethylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid



example 10

Example 10 was made using the procedure as **Example 3**. It shared the intermediate with **Example 6**.
^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ 15.3 (s, 1H), 10.13 (s, 1H), 8.94 (s, 1 H), 8.25 (d, 1H), 7.94 - 7.91 (d, *J* = 12.0 Hz, 1H), 7.8 (d, 1 H), 7.62 - 7.60 (d, *J* = 8.0 Hz, 2 H), 7.34 - 7.31 (d, *J* = 8.0 Hz , 2H), 7.28 - 7.27 (d , *J* = 4.0 Hz , 1 H), 7.20 (d, 1 H), 6.93 (s, 1 H), 6.90 - 6.88 (m, 1 H), 5.95 (m, 1 H), 5.40 (s, 1H), 5.25 - 5.15 (m, 1H), 5.05 (s, 2 H), 4.65 - 4.4 (m, 2 H), 4.5 - 4.4 (m, 1 H), 3.7 - 3.5 (s, 4 H), 3.3 (s, 4 H), 3.1 - 2.85 (m, 2 H), 2.44 - 2.42 (m, 4 H), 1.85 - 1.7 (m, 3 H), 1.7 - 1.55 (m, 1 H), 1.47 - 1.46 (d, *J* = 4.0 Hz , 1 H), 1.46 - 1.3 (m, 5 H).

Example 11. 1-ethyl-6-fluoro-4-oxo-7-(4-((4-((2S)-2-(1-(pentan-2-ylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

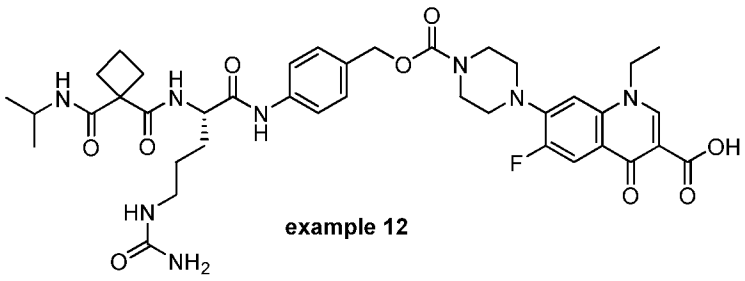


example 11

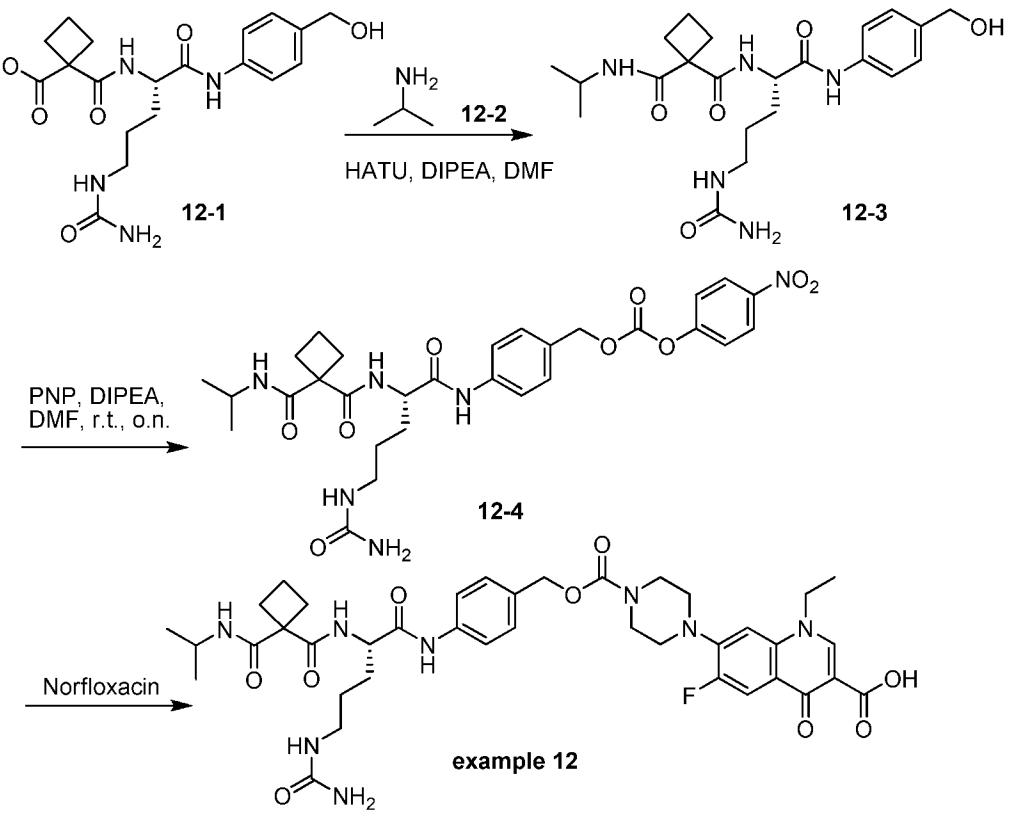
Example11 was made using the procedure as Example 5.
LCMS: (5-95 AB, 1.5 min), T = 0.848 min, M = 821.2 (M+1);

^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.15 - 10.13 (m, 1H), 8.96 (s, 1H), 7.97 - 7.93 (d, J = 13.2 Hz, 1H), 7.81 - 7.74 (m, 1H), 7.63 (d, J = 8.8 Hz, 2H), 7.50 - 7.46 (m, 1H), 7.34 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 7.2 Hz, 1H), 5.98 - 5.95 (t, J = 5.6 Hz, 1H), 5.42 (s, 2H), 5.06 (s, 2H), 4.60 - 4.57 (q, J = 6.8 Hz, 2H), 4.44 - 4.42 (m, 1H), 3.81(m, 1H), 3.60 (s, 4H), 3.49 - 3.36 (s, 4H), 3.33 - 3.29 (m, 2H), 3.1 - 2.9(m, 2H), 2.42 - 2.39 (m, 4H), 1.73 (m, 4H), 1.63 - 1.58 (m, 1H), 1.41 (m, 5H), 1.35 - 1.30 (m, 2H), 1.04 - 1.02 (m, 3H), 0.84 - 0.78 (m, 3H).

Example 12. (S)-1-ethyl-6-fluoro-7-(4-((4-(2-(1-(isopropylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 12



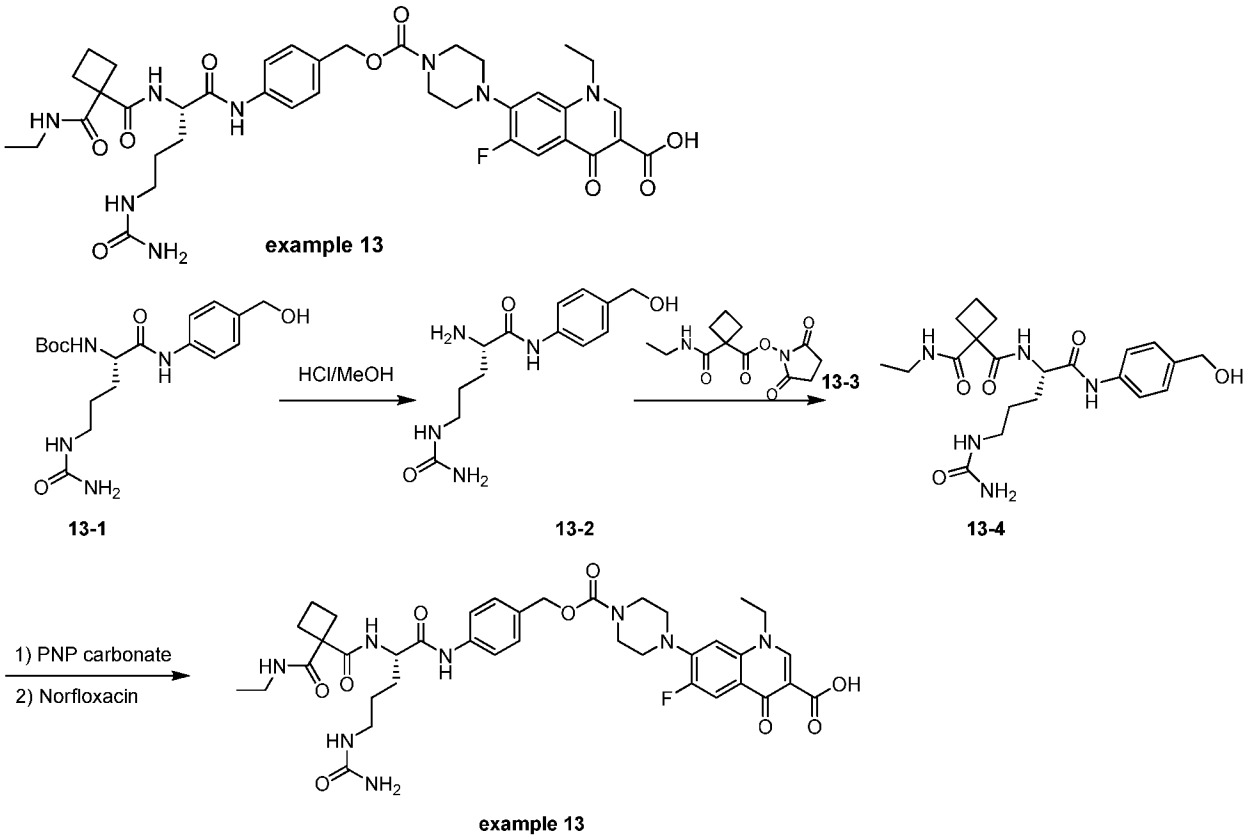
Step 1. A mixture pf compound **12-1** (120 mg, 0.3 mmol), HATU (171 mg, 0.45 mmol), DIPEA (195 mg, 1.5 mmol) in DMF (10 mL) was stirred at r.t. for 30 min. Then compound **12-2** (9 mg, 0.3 mmol) was added. The reaction mixture was stirred at r.t. for 3 h.The mixture was concentrated and purified by Prep-TLC (MeOH/ DCM=1/10) to give **12-3** (180 mg, 136 %).

Step 2. To a solution of compound **12-3** (180 mg, 0.4 mmol), PNP carbonate (245 mg, 0.81 mmol) in DMF (4 mL) was added DIPEA (156 mg, 1.21 mmol) at 0°C . The mixture was stirred at r.t. for 16 h. The mixture (**12-4**) was used for next step without further purification.

Step 3. To the mixture of last step was added norfloxacin (259 mg, 0.81 mmol) at r.t. The mixture was stirred at r.t. for 1 h. The residue was purified by prep-HPLC to give **exampel 12** (18.5 mg, Yield: 6 % over two steps).

^{1}H NMR (400 MHz, MeOH-d_4) δ 8.88 (s, 1 H), 8.02 - 7.99 (d, J = 13.2 Hz, 1 H), 7.65 - 7.62 (d, J = 8.8 Hz, 3 H), 7.37 - 7.35 (d, J = 8.4 Hz, 2 H), 7.19 (d, J = 6.4 Hz, 1 H), 5.13 (s, 2 H), 4.54 - 4.50 (m, 4 H), 4.06 - 4.01 (m, 1 H), 3.73(s, 4 H), 3.35 (s, 4 H), 3.26 - 3.19 (m, 2 H), 2.57 - 2.52 (m, 4 H), 1.94 - 1.90 (m, 2 H), 1.81 - 1.74 (m, 2 H), 1.59 - 1.53 (m, 3 H), 1.21 - 1.13 (m, 6 H).

Example 13. (S)-1-ethyl-7-(4-((4-(2-(1-(ethylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



Step 1. A mixture of compound **13-1** (1 g, 2.63 mmol) and HCl in dioxane (4 M, 10 mL) was stirred at r.t. for 2 h. The solution was concentrated to give the **13-2**.

Step 2. To a solution of compound **13-2** (651 mg, 2.326 mmol) in DME-H_2O (10 mL / 2 mL) were added a mixture of compound **13-3** (623 mg, 2.326 mmol) and sat. $NaHCO_3$ (10 mL). The mixture was stirred at r.t. for 16 h. The solution was concentrated and the residue was purified by prep-HPLC to give **13-4.**

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.62 (d, J = 8.4 Hz, 2 H), 7.33 (d, J = 8.8 Hz, 2 H), 4.57-4.52 (m, 3 H), 3.29-3.10 (m, 4 H), 2.60-2.53 (m, 4 H), 2.05-1.50 (m, 6 H), 1.16 (t, J = 7.6 Hz, 3 H).

LCMS (ESI): m/z 434.1 [M+H$^+$].

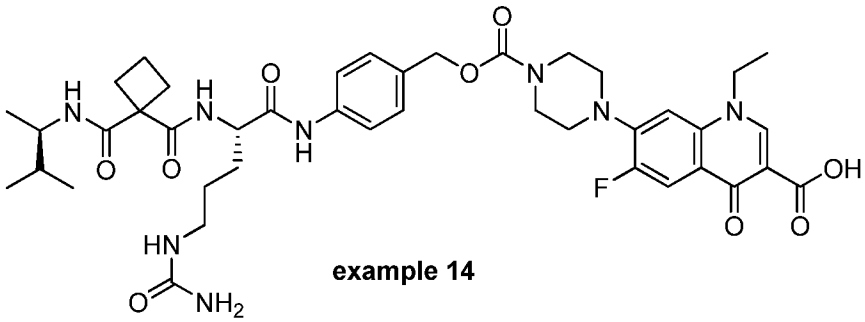
Step 3. To a solution of **13-4** (60 mg, 0.139 mmol) in DCM (10 mL) were added DIPEA (90 mg, 0.695 mmol) and PNP carbonate (84 mg, 0.277 mmol). The mixture was stirred at 25°C for 2 days. The mixture was concentrated and dissolved in DMF (5 mL) and norfloxacin (88 mg, 0.278 mmol) was added. The mixture was stirred at 25°C for 1.5 h. After removal of the solvent, the residue was purified by prep-HPLC to give **example 13**.

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.86 (s, 1 H), 8.00 (d, J = 13.2 Hz, 1 H), 7.63 (d, J = 8.4 Hz, 2 H), 7.35 (d, J = 8.8 Hz, 2 H), 7.18 (d, J = 6.8 Hz, 1 H), 5.11 (s, 2 H), 4.57-4.48 (m, 3 H), 3.71 (s, 4

H), 3.33-3.32 (m, 4 H), 3.26-3.08 (m, 4 H), 2.60-2.47 (m, 4 H), 1.93-1.50 (m, 10 H), 1.14 (t, J = 7.2 Hz, 3 H).

LCMS (ESI): m/z 779.4.[M+H$^+$].

Example 14. 1-ethyl-6-fluoro-7-(4-((4-((S)-2-(1-((R)-3-methylbutan-2-ylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

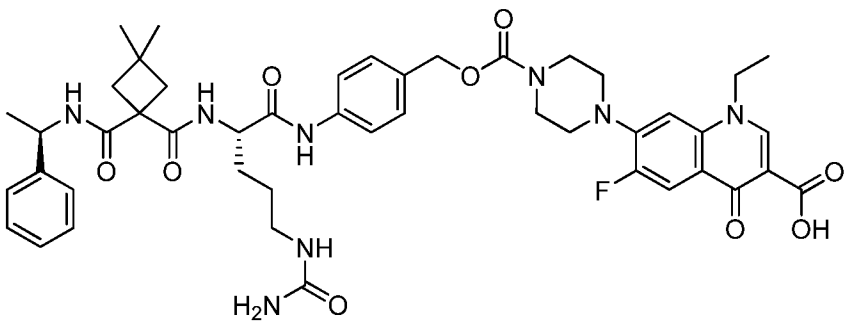


example 14

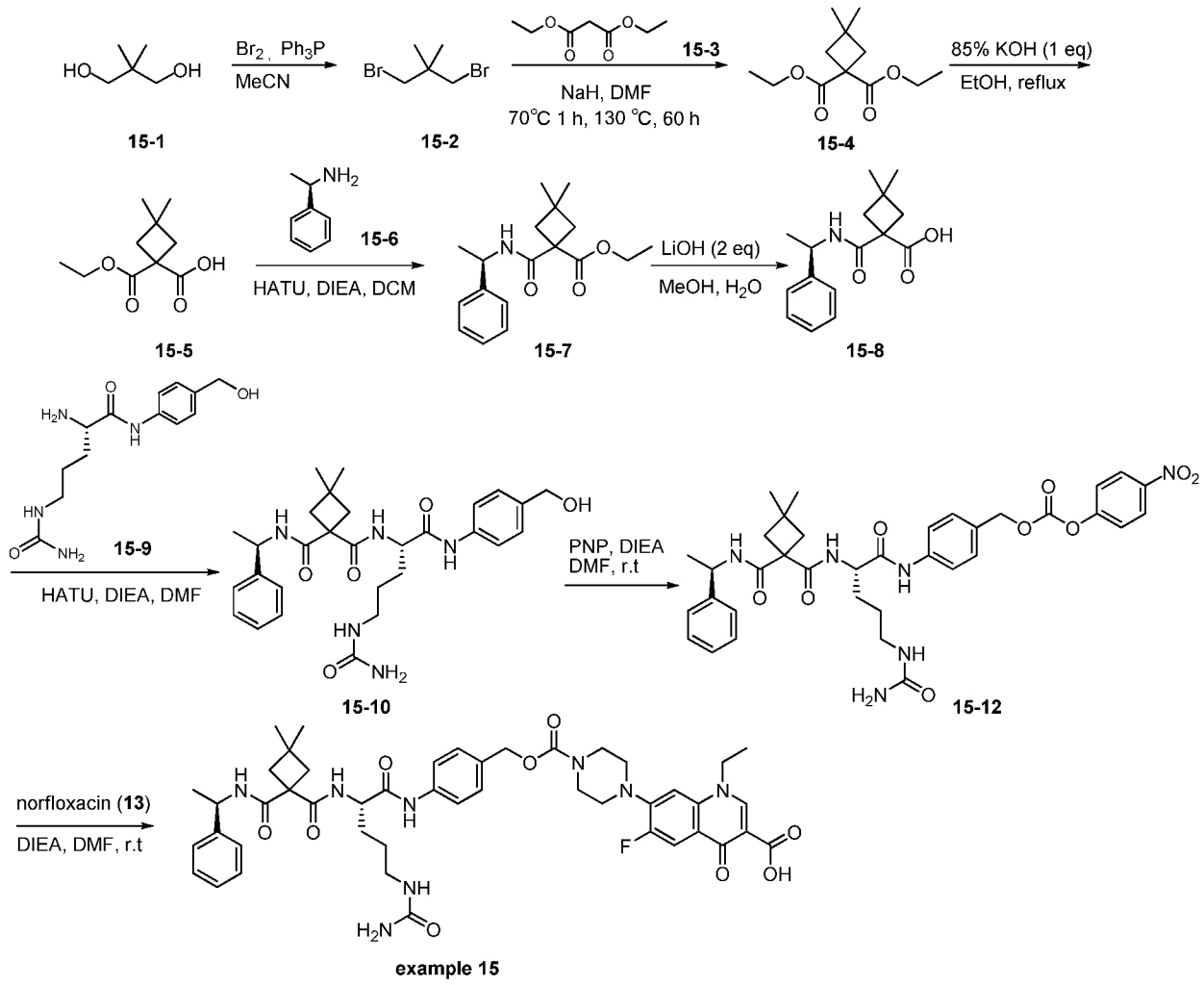
Example 14 was made using the procedure as **Example 5**.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1 H), 8.96 (s, 1 H), 7.97 - 7.93 (d, J = 13.2 Hz, 1 H), 7.85 - 7.83 (d, J = 7.6 Hz, 1 H), 7.63 - 7.61 (d, J = 8.8 Hz, 2 H), 7.43 - 7.41 (d, J = 8.8 Hz, 1 H), 7.35 - 7.33 (d, J = 8.8 Hz, 2 H), 7.22 - 7.21 (d, J = 6.8 Hz, 1 H), 6.00 - 5.97 (m, 1 H), 5.43 (s, 2 H), 5.07 (s, 2 H), 4.61 - 4.56 (m, 2 H), 4.46 - 4.41 (m, 1 H), 3.62 (m, 6 H), 3.41 (s, 2 H), 3.30 (s, 4 H), 3.12 - 2.89 (m, 2 H), 2.45 - 2.39 (m, 4 H), 1.76 - 1.62 (m, 4 H), 1.44 - 1.39 (m, 3 H) , 1.00 (d, J = 6.8 Hz, 3 H) , 0.82 (d, J = 5.6 Hz, 6 H).

Example 15. 7-(4-((4-((S)-2-(3,3-dimethyl-1-((R)-1-phenylethylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 15



Step 1. To a stirred solution of Ph_3P (91.8 g, 350 mmol) in MeCN (250 mL) was added dropwise a solution of bromine (56 g, 350 mmol) in MeCN (40 mL) at 0 °C. Then compound **15-1** (18.2 g, 175 mmol) was added to the mixture. The reaction was heated at reflux for 16 h. The solvent was removed and the residue wad distilled to give **15-2** as a light yellow oil (14 g, 35%).

^{1}H NMR $CDCl_3$ 400 MHz, δ 3.40 (s, 4 H), 1.17 (s, 6 H).

Step 2. To a suspension of NaH (3.4 g, 60%, 84.9 mmol) in DMF (40 mL), compound **15-3** (11.14 g, 69.6 mmol) was added dropwise at 0 °C. The mixture was heated at 70 °C for 1 h under N_2. Then compound **15-2** (8.0 g, 34.8 mmol) was added and stirred for 60 h under N_2. The mixture was poured into an aqueous solution of NH_4Cl (20 g in 300 mL) and extracted with PE (50 mL x 5). The organic phase was concentrated and purified by chromatography (PE) to afford compound **15-4** (3.0 g, Yield: 37.9%).

^{1}H NMR $CDCl_3$ 400 MHz δ 4.21- 4.16 (m, 4 H), 2.36 (s, 4 H), 1.26 - 1.22 (m, 6H), 1.11 (s, 6 H).

Step 3. To a stirred solution of compound **15-4** (3.0 g, 13.9 mmol) in EtOH (10 mL) was added aqueous KOH (85%, 779 mg, 13.9 mmol) at r.t. The reaction mixture was stirred at 76 °C for 3 h. The mixture was concentrated and partitioned between EtOAc (75 mL) and H_2O (125 mL). The

aqueous phase was acidified with 1N HCl till pH = 3 and extracted with EtOAc (75 mL x 2). The organic layer was concentrated to give compound **15-5** (1.9 g, 68.3%) as oil.

^{1}H NMR $CDCl_3$ 400 MHz, δ 11.62 (s, 1 H), 4.26 - 4.21 (m, 2 H), 2.42 (s, 4 H), 1.29 - 1.26 (m, 3H), 1.15 - 1.13 (d, J = 7.2 Hz, 6 H).

Step 4. To a stirred solution of compound **15-5** (500 mg, 0.5 mmol) in DCM (8 mL) was added HATU (285.0 mg, 0.75 mmol) and DIPEA (193.5 mg, 1.5 mmol) at r.t. After the reaction mixture was stirred at r.t. for 30 min, **15-6** (90.9 mg, 0.75 mmol) was added and stirred at r.t. for 16 h. The mixture was concentrated and purified by chromatography (DCM: MeOH = 90: 10) give compound **15-7** as white solid (70 mg, 46.2%)

LCMS: (5-95, AB, 1.5 min, ESI), 0.807 min, MS = 303.9[M+1]

^{1}H NMR $CDCl_3$ 400 MHz, δ 7.34 - 7.22 (m, 5 H), 6.19 (d, J = 7.2 Hz, 1 H), 5.14 - 5.06 (m, 1 H), 4.22 - 4.16 (m, 2 H), 2.46 - 2.31 (m, 4 H), 1.46 (d, J = 6.8 Hz, 3 H), 1.25 - 1.21 (m, 3 H), 1.09 (s, 6 H).

Step 5. To a stirred solution of compound **15-7** (70 mg, 0.23 mmol) in MeOH / THF /H_2O (1 mL / 2 mL/2 mL) was added LiOH·H_2O (19.4 mg, 0.462 mmol) at r.t. The reaction mixture was stirred at r.t. for 1 h. Organic solvent was removed and partitioned between EtOAc (30 mL) and H_2O (25 mL). The aqueous phase was acidified with 1N HCl to pH = 3 and extracted with EtOAc (30 mL). The organic phase was concentrated to afford compound **15-8** as white solid (50 mg, 78.7%).

^{1}H NMR $CDCl_3$ 400 MHz, δ 7.34 - 7.23 (m, 5 H), 6.30 (d, J = 7.2 Hz, 1 H), 5.14 - 5.07 (m, 1 H), 2.50 - 2.31 (m, 4 H), 1.48 (d, J = 6.8 Hz, 3 H), 1.11 (d, J = 16 Hz, 6 H).

Step 6. To a stirred solution of compound **15-8** (50 mg, 0.18 mmol) in DCM (10 ml) was added HATU (102.6 mg, 0.27 mmol) and DIPEA (46.44 mg, 0.36 mmol) at r.t. and stirred for 30 min. Compound **15-9** (53 mg, 0.19 mmol) was added into the reaction mixture and stirred at r.t. for 16 h. The mixture was concentrated and purified by chromatography (DCM: MeOH = 85: 15) to afford compound **15-10** (60 mg, 61.8%) as white solid.

LCMS: (5-95, AB, 1.5 min, ESI), 0.803 min, MS = 538.2[M+1]

^{1}H NMR Methanol-d_4 400 MHz, δ 8.19 (d, J = 8 Hz, 1 H), 7.58 (d, J = 8.4 Hz, 2 H), 7.31 - 7.17 (m, 7 H), 5.07 - 5.00 (m, 1 H), 4.56 (s, 2 H), 4.51 - 4.47 (m, 1 H), 3.21 - 3.17 (m, 1 H), 2.45 - 2.35 (m, 4 H), 1.89 - 1.85 (m, 1 H), 1.75 - 1.72 (m, 1 H),1.55 - 1.47 (m, 5H), 1.08(d, J = 2 Hz, 6 H).

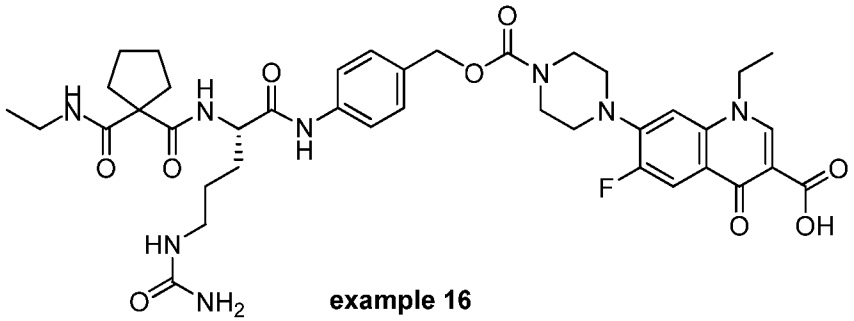
Step 7. To a stirred solution of compound **15-10** (60 mg, 0.11 mmol) in dry DMF (3 mL) was added PNP carbonate (**15-11**) (66.9 mg, 0.22 mmol) and DIPEA (70.9 mg, 0.55 mmol) at r.t. and stirred at r.t. for 3 h. The mixture (**15-12**) was used for next step without further purification.

Step 8. To the reaction mixture of last step was added norfloxacin (70.2 mg, 0.22 mmol) at r.t. The mixture was stirred at r.t. for 16 h and purified by prep-HPLC to give **example 15** (14.4 mg, Yield: 14.8 %).

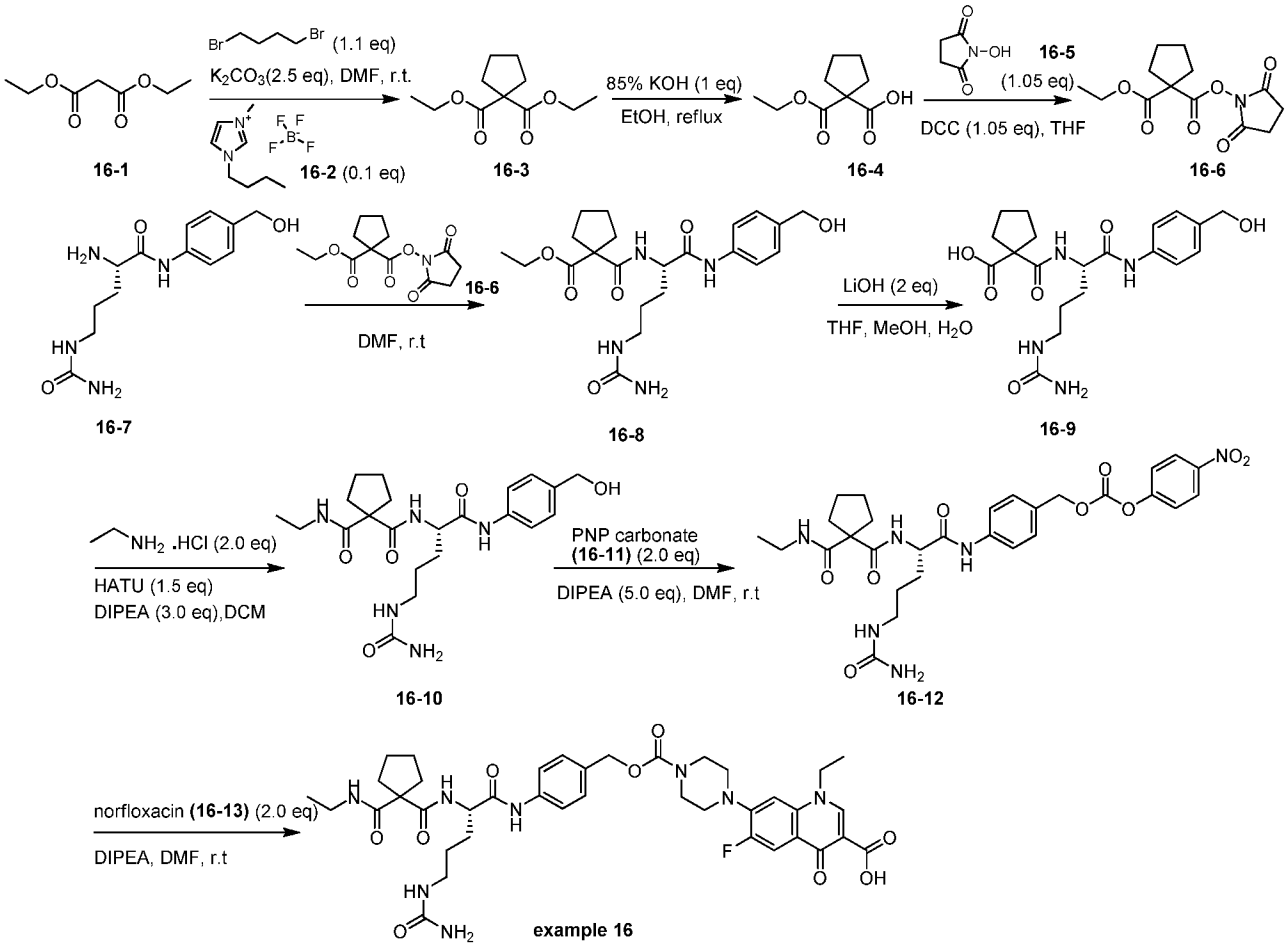
LCMS: (5-95, AB, 1.5 min, ESI), 0.887 min, MS = 883.4.4[M+1], 442.4 [1/2M+1]

^{1}H NMR DMSO - d_6 400 MHz, δ 10.24 (s, 1 H), 8.94 (s, 1 H), 8.39 (s, 1 H), 8.13 (d, J = 2 Hz, 1 H), 7.965 - 7.963 (m, 2 H), 7.63 (d, J = 8.4 Hz, 2 H), 7.47 (d, J = 8.8 Hz, 2 H), 7.30 - 7.26 (m, 4 H), 7.19 - 7.18 (m, 2 H), 6.05 (s, 1 H), 5.46 (s, 2 H), 5.07 (s, 2 H), 4.97 - 4.91 (m, 1 H), 4.57 (s, 2 H), 4.45 (s, 1 H), 3.62 (s, 4 H), 3.35 (s, 4 H), 3.05 - 3.02 (m, 1 H), 2.96 - 2.92 (m, 1 H), 2.32 - 2.26 (m, 4 H), 1.73 - 1.71 (m, 1 H), 1.63 - 1.61 (m, 1 H), 1.42 - 1.37 (m, 8 H), 0.99 (s, 6 H).

Example 16. (S)-1-ethyl-7-(4-((4-(2-(1-(ethylcarbamoyl)cyclopentanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 16



Step 1. To a solution of compound **16-1** (10 g, 62.4 mmol) in DMF (50 mL) was added 1,4-dibromobutane (14.8 g, 68.6 mmol), K_2CO_3 (21.5 g, 155.8 mmol) and compound **16-2** (1.4 g, 6.2 mmol) at r.t. and stirred at r.t. for 16 h. The solvent was removed under reduced pressure, and the residue was partitioned between EtOAc (200 mL) and H_2O (80 mL), the combined organic phase was dried and concentrated to give compound **16-3** as oil (10.0 g, Yield: 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 - 4.10 (m, 4 H), 2.14 - 2.10 (m, 4 H), 1.64 - 1.61 (m, 4 H), 1.20 - 1.17 (m, 6 H).

Step 2. To a solution of compound **16-3** (6.0 g, 28.0 mmol) in EtOH (20 mL) was added 85% aqueous KOH solution (1.85 g, 28.0 mmol) at r.t. and stirred at 76 °C for 3 h. Solvent was removed and the residue was partitioned between EtOAc (20 mL) and H_2O (30 mL). The aqueous phase was acidified with 1N HCl to pH = 3 and extracted with EtOAc (20 mL×2). The organic layer was dried and concentrated to give compound **16-4** as oil (2.5 g, yield: 48.0%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.61 - 10.60 (m, 1 H), 4.21 - 4.15 (m, 2 H), 2.21 - 2.18 (m, 4 H), 1.72 - 1.65 (m, 4 H), 1.26 - 1.22 (m, 3 H).

Step 3. To a solution of compound **16-4** (2.5 g, 13.4 mmol) and compound **16-5** (1.62 g, 14.1 mmol) in dry THF (20 mL) was added DCC (3.04 g, 14.74 mmol) at 0°C. The mixture was stirred at r.t. for 16 h under N_2. The mixture was filtered and the filtrate was concentrated to give crude compound **16-6** which was used for next step without further purification.

^{1}H NMR (400 MHz, MeOH-d_4) δ 4.23 - 4.18 (m, 2 H), 2.79 (s, 4 H), 2.36 - 2.23 (m, 4 H), 1.76 - 1.72 (m, 4 H), 1.30 - 1.28 (t, J = 7.2 Hz, 3 H).

Step 4. To a solution of compound **16-6** (1.0 g, 3.53 mmol) in DMF (15 mL) was added compound **16-7** (658.7 mg, 2.35 mmol) at r.t. The reaction mixture was stirred at r.t. for 16 h. The mixture was concentrated and purified by silica gel column chromatography (DCM : MeOH = 10:1) to give compound **16-8** as white solid (150 mg, Yield: 14.2%).

LCMS (ESI): m/z 449.0 [M+1].

Step 5. To a solution of compound **16-8** (150 mg, 0.33 mmol) in THF/ MeOH /H_2O(3 mL / 3 mL/1.5 mL) was added $LiOH·H_2O$ (28.14 mg, 0.67 mmol) at r.t. The reaction mixture was stirred at r.t. for 16 h. The mixture was concentrated and partitioned between EtOAc (15 mL) and H_2O (20 mL). The aqueous phase was acidified with 1N HCl to pH = 3, extracted with EtOAc (15 mL × 3) and concentrated to give crude compound **16-9** as white solid which was used to the next step without further purification.

LCMS (ESI): m/z 420.9 [M+1].

Step 6. To a solution of compound **16-9** (250 mg, 0.595 mmol) in DMF (15 mL) was added HATU (339.2 mg, 0.89 mmol) and DIPEA (268.6 mg, 2.08 mmol) at r.t. and stirred for 30 min. Ethylamine hydrochloride (96.98 mg, 1.19 mmol) was added into the reaction mixture and stirred at r.t. for 16 h. The mixture was filtered and purified by prep-HPLC and SFC to give compound **16-10** as white solid (30 mg, Yield: 11.3%).

LCMS (ESI): m/z 447.9 [M+1].

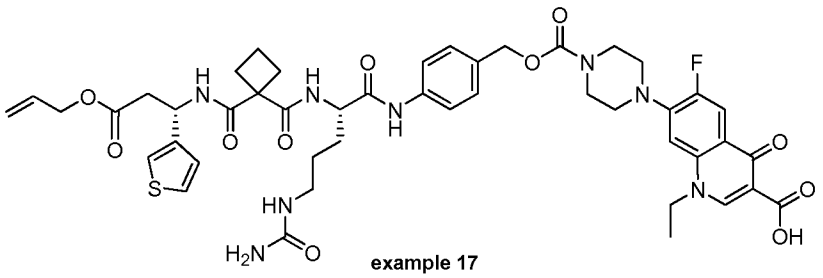
^{1}H NMR (400 MHz, DMSO - d_6) δ 9.99 (s, 1 H), 7.75 - 7.73 (m, 2 H), 7.57 - 7.55 (m, 2 H), 7.23 - 7.21 (m, 2 H), 5.97 - 5.94 (m, 1 H), 5.39 (s, 2 H), 5.11 - 5.08 (m, 1 H), 4.41 - 4.36 (m, 2 H), 4.35 - 4.32 (s, 1 H), 3.13 - 3.06 (m, 2 H), 2.97 - 2.90 (m, 2 H), 2.16 - 2.14 (m,2 H), 2.11 - 2.05 (m, 1 H), 1.95 - 1.93 (m, 1 H), 1.92 - 1.91 (m, 1 H), 1.51 - 1.49 (m, 4 H), 1.39 - 1.37 (m, 1 H), 1.20 - 1.19 (m, 2 H), 1.02 - 0.98 (t, J = 7.2 Hz, 3 H).

Step 7. To a solution of compound **16-10** (30 mg, 0.067 mmol) in dry DMF (3 mL) was added compound **16-11** (40.7 mg, 0.134 mmol) and DIPEA (43.22 mg, 0.335 mmol) at 0°C. After the mixture was stirred at r.t. for 5 h, it was used for next step directly without further purification.

Step 8. To the mixture from last step (**16-12**) was added compound **16-13** (42.8 mg, 0.134 mmol) at r.t. The mixture was stirred at r.t. for 1 h and then purified by prep-HPLC to give the desired product **example 16**. (35.0 mg Yield: 66.0 %)

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.32 (s, 1 H), 10.09 (s, 1 H), 8.95 (s, 1 H), 7.93 (d, J = 13.2 Hz, 1 H), 7.80 - 7.71 (m, 2 H), 7.62 (d, J = 8.4 Hz, 2 H), 7.32 (d, J = 8.4 Hz, 2 H), 7.18 (d, J = 8.0 Hz, 1 H), 5.98 - 5.91 (m, 1 H), 5.39 (s, 2 H), 5.04 (s, 2 H), 4.60 - 4.52 (m, 2 H), 4.38 - 4.32 (m, 1 H), 3.59 (s, 4 H), 3.30 (s, 4 H), 3.12 - 3.08 (m, 2 H), 2.96 - 2.85 (m, 2 H), 2.14 - 2.11 (m, 2 H), 2.10 - 2.00 (m, 1 H), 1.94 - 1.89 (m, 1 H), 1.63 - 1.40 (m, 5 H), 1.38 - 1.30 (m, 5 H), 1.02 - 0.98 (t, J = 7.2 Hz, 3 H).

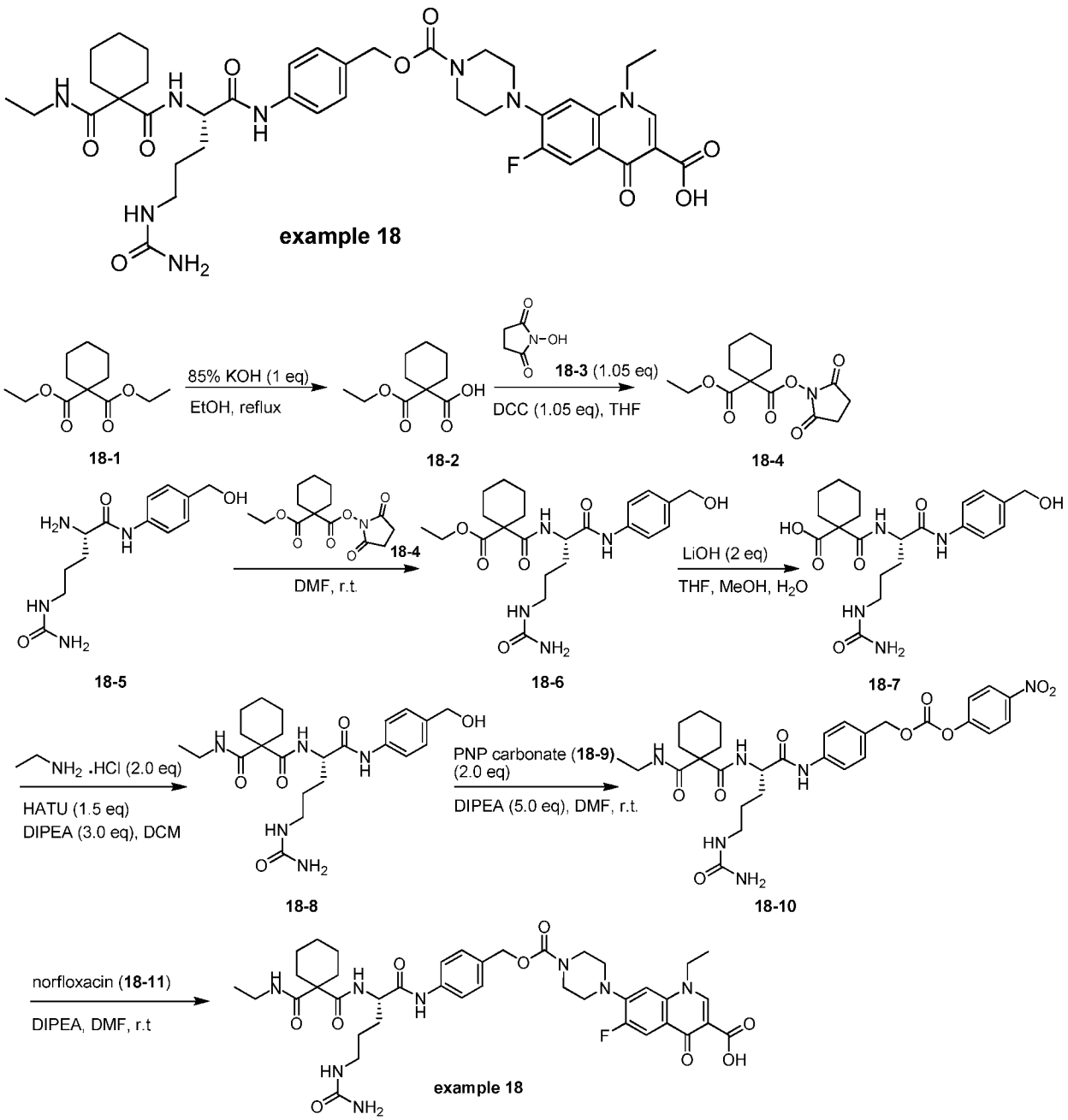
Example 17. 7-(4-((4-((S)-2-(1-((S)-3-(allyloxy)-3-oxo-1-(thiophen-3-yl)propylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 17

Example 17 was made using the procedure as **Example 7**, with the intermediates from the synthesis of **Example 7**.

Example 18. (S)-1-ethyl-7-(4-((4-(2-(1-(ethylcarbamoyl)cyclohexanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



Step 1. To a solution of compound **18-1** (4.0 g, 17.5 mmol) in EtOH (20 mL) was added 85% aqueous KOH solution (1.15 g, 17.5 mmol) at r.t. After the reaction mixture was stirred at 76 °C for 3 h, it was concentrated and partitioned between EtOAc (15 mL) and H_2O (25 mL). The aqueous phase was acidified with 1N HCl to pH = 3 and extracted with EtOAc (15 mL × 2). The organic layer was concentrated to give compound **18-2** as an oil (2.5 g, yield: 71.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 - 4.15 (m, 2 H), 2.03 - 1.90 (m, 4 H), 1.56 - 1.47 (m, 6 H), 1.45 - 1.43 (t, 4.0 Hz, 3 H).

Step 2. To a solution of compound **18-2** (2.5 g, 12.5 mmol) and **18-3** (1.51 g, 13.13 mmol) in dry THF (20 mL) was added DCC (2.83 g, 13.35 mmol) at 0°C. The mixture was stirred at r.t. for 16 h

under N_2. The mixture was filtered and the filtrate was concentrated to give the crude compound **18-4** which was used for next step without further purification (2.0 g, Yield: 53.8%).

Step 3. To a stirred solution of compound **18-4** (2.0 g, 6.7 mmol) in DMF (15 mL) was added compound **18-5** (1.26 g, 4.5 mmol) at r.t. The reaction mixture was stirred at r.t. for 16 h. The mixture was concentrated and purified by column chromatography (DCM : MeOH = 10:1) to give compound **18-6** as white solid (700 mg, Yield: 22.4%).

LCMS (ESI): m/z 463.0 [M+1].

Step 4. To a solution of compound **18-6** (700 mg, 1.50 mmol) in THF/ MeOH /H_2O(4 mL / 4 mL/2 mL) was added LiOH·H_2O (126.0 mg, 3.0 mmol) at r.t. After the reaction mixture was stirred at r.t. for 16 h, it was concentrated and partitioned between EtOAc (25 mL) and H_2O (30 mL), the aqueous phase was acidified with 1N HCl to pH = 3, extracted with EtOAc (25 mL × 2) and concentrated to give crude compound **18-7** as white solid which was used in the next step without further purification.

LCMS (ESI): m/z 435.0 [M+1].

Step 5. To a solution of compound **18-7** (300 mg, 0.69 mmol) in DMF (15 mL) was added HATU (395.2 mg, 1.04 mmol) and DIPEA (267.03 mg, 2.07 mmol) at r.t. and stirred at r.t. for 30 min. Ethylamine (112.47 mg, 1.38 mmol) was added into the reaction mixture and stirred at r.t. for 16 h. The mixture was filtered and purified by prep-HPLC and SFC to give compound **18-8** (60 mg, Yield: 18.8%) as white solid.

LCMS (ESI): m/z 462.0 [M+1].

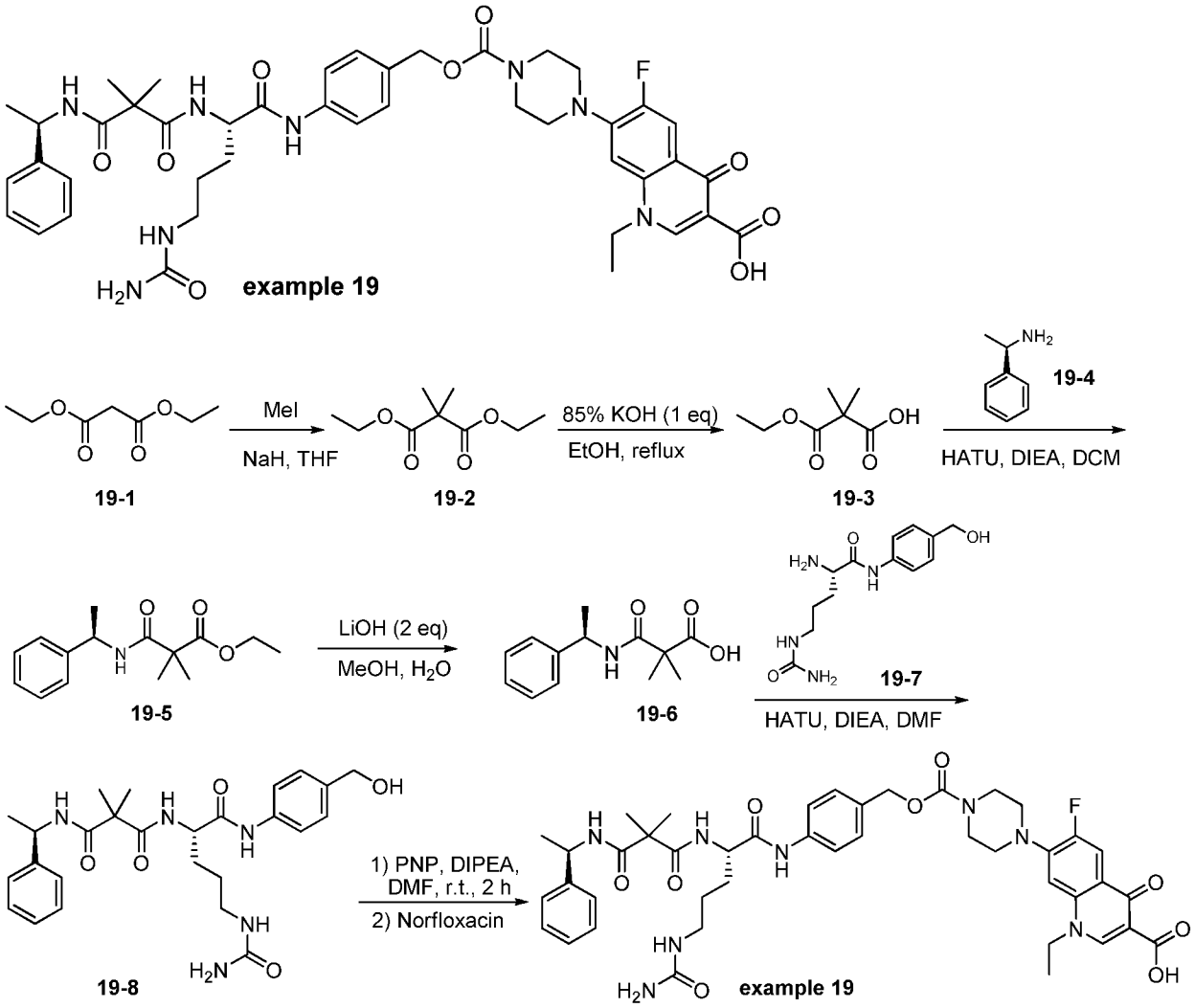
^{1}H NMR (400 MHz, MeOH – d_4) δ 7.62 (d, J =8.4 Hz, 2 H), 7.28 (d, J =8.8 Hz, 2 H), 4.58 (s, 1 H), 4.54 - 4.50 (m, 3 H), 3.62 - 3.57 (m, 1 H), 3.26 - 3.18 (s, 2 H), 3.10 - 3.07 (m, 2 H), 2.16 - 2.02 (m, 2 H), 1.85 -1.81 (m, 3 H), 1.73 - 1.71 (m, 1 H), 1.59 - 1.50 (m, 5 H), 1.18 - 1.14 (m, 1 H), 1.12 - 1.09 (m, 3 H).

Step 6. To a stirred solution of compound **18-8** (100 mg, 0.216 mmol) in dry DMF (3 ml) was added compound **18-9** (131.3 mg, 0.432 mmol) and DIPEA (139.3 mg, 1.08 mmol) at 0°C. The mixture (**18-10**) was stirred at r.t. for 3 h and used for next step without further purification.

Step 7. To the mixture (**18-10**) from last step was added norfloxacin (**18-11**) (137.9 mg, 0.432 mmol) at r.t. and stirred at r.t. for 1 h. The mixture was purified by prep-HPLC to give **example 18** (98.0 mg Yield: 56.2 %).

^{1}H NMR (400 MHz, DMSO - d_6) δ 15.30 (s, 1 H), 10.09 (s, 1 H), 8.93 (s, 1 H), 7.92 (d, J = 13.2 Hz, 1 H), 7.78 - 7.72 (m, 1 H), 7.67 (d, J = 7.6 Hz, 1 H), 7.63 (d, J = 8.8 Hz, 2 H), 7.32 (d, J = 8.8 Hz, 2 H), 7.18 (d, J = 8.0 Hz, 1 H), 5.98 - 5.92 (m, 1 H), 5.38 (s, 2 H), 5.04 (s, 2 H), 4.57 - 4.55 (m, 2 H), 4.39 - 4.31 (m, 1 H), 3.59 (s, 4 H), 3.31 (s, 4 H), 3.31 - 3.08 (m, 2 H), 3.00 - 2.86 (m, 2 H), 2.12 - 2.03 (m, 1 H), 2.01 - 1.91 (m, 1 H), 1.90 - 1.83 (m, 3 H), 1.58 - 1.67 (m, 1 H), 1.40 - 1.25 (m, 11 H), 1.01 - 0.97 (t, J = 7.2 Hz, 3 H).

Example 19. 7-(4-((4-((S)-2-(2,2-dimethyl-3-oxo-3-((R)-1-phenylethylamino)propanamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



Step 1. To a solution of compound **19-1** (5.0 g, 31.22 mmol) in anhydrous THF (70 mL) was added NaH (3.75 g, 93.65 mmol, c = 60 %) slowly at 0 °C. After the mixture was stirred at 0 °C for 10 m. MeI (6.15 mL, 124.88 mmol) was added dropwise at 0 °C, and the reaction mixture was stirred at 0 °C for 2 h. The mixture was diluted with EtOAc (50 mL), filtered, the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (50 mL), filtered, and the filtrate was concentrated to afford compound **19-2** (3.5 g, 59.6 %) as an oil.

^{1}H NMR (400 MHz, DMSO-d_6) δ 4.13 - 4.08 (q, 4H), 1.33 (s, 6H), 1.16 (t, J = 6.8 Hz, 6H).

Step 2. To a solution of compound **19-2** (3.0 g, 15.94 mmol) in EtOH (20 mL) was added aq. KOH solution (85%, 894 mg, 15.94 mmol). The reaction mixture was heated at reflux for 1 h. After organic solvent was removed under reduced pressure, it was diluted with H_2O (20 mL), and washed with PE (10 mL × 2). The aqueous phase was adjusted to pH 2 with conc. HCl solution

and extracted with EtOAc (20 mL × 3). The combined EtOAc layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford compound **19-3** (1.5 g, 58.8 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.98 - 3.93 (q, 4H), 1.17 (s, 6H), 1.12 (t, J = 7.2 Hz, 3H).

Step 3. To a mixture of compound **19-3** (1.0 g, 6.24 mmol) in anhydrous DCM (20 mL) was added DIPEA (1.61 g, 12.48 mmol), followed by HATU (2.85 g, 7.49 mmol). After the mixture was stirred at r.t. for 15 min, and compound **19-4** (908 mg, 7.49 mmol) was added. The reaction mixture was stirred at r.t. for 3 h, and washed with citric acid solution (10 mL × 3), brine (10 mL × 1), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (PE/EtOAc = 5:1) to afford compound **19-5** as a white solid (1.30 g, 79.3 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (d, J = 8.0 Hz, 1H), 7.31 - 7.28 (m, 4H), 7.22 - 7.18 (m, 1H), 4.97 - 4.90 (m, 1H), 4.12 - 4.01 (m, 2H), 1.36 (d, J = 6.8 Hz, 3H), 1.33 (d, J = 2.4 Hz, 6H), 1.14 (d, J = 6.8 Hz, 3H).

Step 4. To a solution of compound **19-5** (1.5 g, 5.70 mmol) in a mixture of MeOH/H_2O (15 mL/ 5 mL) was added LiOH·H_2O (478 mg, 11.40 mmol). The reaction mixture was heated at reflux for 2 h. Organic solvent was removed under reduced pressure, and the water slurry was washed with DCM (5 mL × 3). It was adjusted to pH 1 with con. HCl solution and extracted with EtOAc (10 mL × 3). The combined EtOAc layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford compound **19-6** as white solid (800 mg, 59.7 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.45 (br, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.32 - 7.27 (m, 4H), 7.23 - 7.17 (m, 1H), 4.97 - 4.90 (m, 1H), 1.36 (d, J = 6.8 Hz, 3H), 1.31 (d, J = 3.6 Hz, 6H).

Step 5. To a solution of compound **19-6** (167 mg, 0.71 mmol) in DMF (5 mL) was added DIPEA (183 mg, 1.42 mmol), followed by HATU (323 mg, 0.85 mmol). The mixture was stirred at r.t. for 10 min, and compound **19-7** (200 mg, 0.71mmol) was added. After the reaction mixture was stirred at r.t. for 2 h, it was purified by prep-HPLC to afford compound **19-8** as white solid (80 mg, 22.7 %).

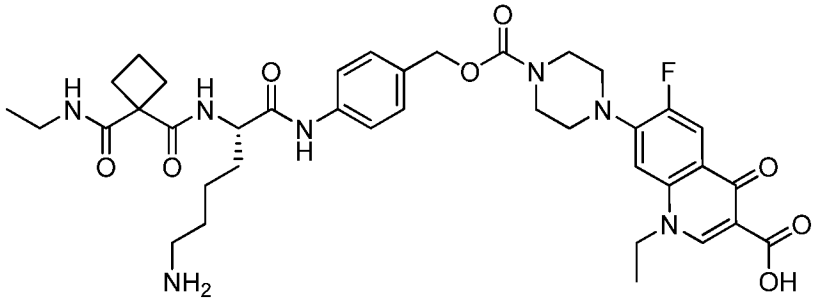
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 7.6 Hz, 1H), 7.57 - 7.54 (m, 2H), 7.32 - 7.25 (m, 4H), 7.22 - 7.15 (m, 3H), 5.95 (t, J = 5.6 Hz, 1H), 5.39 (s, 2H), 5.08 (br, 1H), 4.98 - 4.94 (m, 1H), 4.41 (s, 2H), 4.36 - 4.31 (m, 1H), 3.00 - 2.89 (m, 2H), 1.78 - 1.75 (m, 1H), 1.63 - 1.59 (m, 1H), 1.40 - 1.33 (m, 8H), 1.30 (s, 3H).

Step 6. To a solution of compound **19-8** (80 mg, 0.16 mmol) in anhydrous DMF (4 mL) was added DIPEA (103 mg, 0.80 mmol), followed by PNP carbonate (97 mg, 0.32 mmol). After it was stirred at r.t. under N_2 for 2 h, norfloxacin (102 mg, 0.32 mmol) was added. The mixture was stirred at r.t. for another 1 h, filtered, and the filtrate was purified by prep-HPLC to afford **example 19** as white solid (81 mg, 60.0 %).

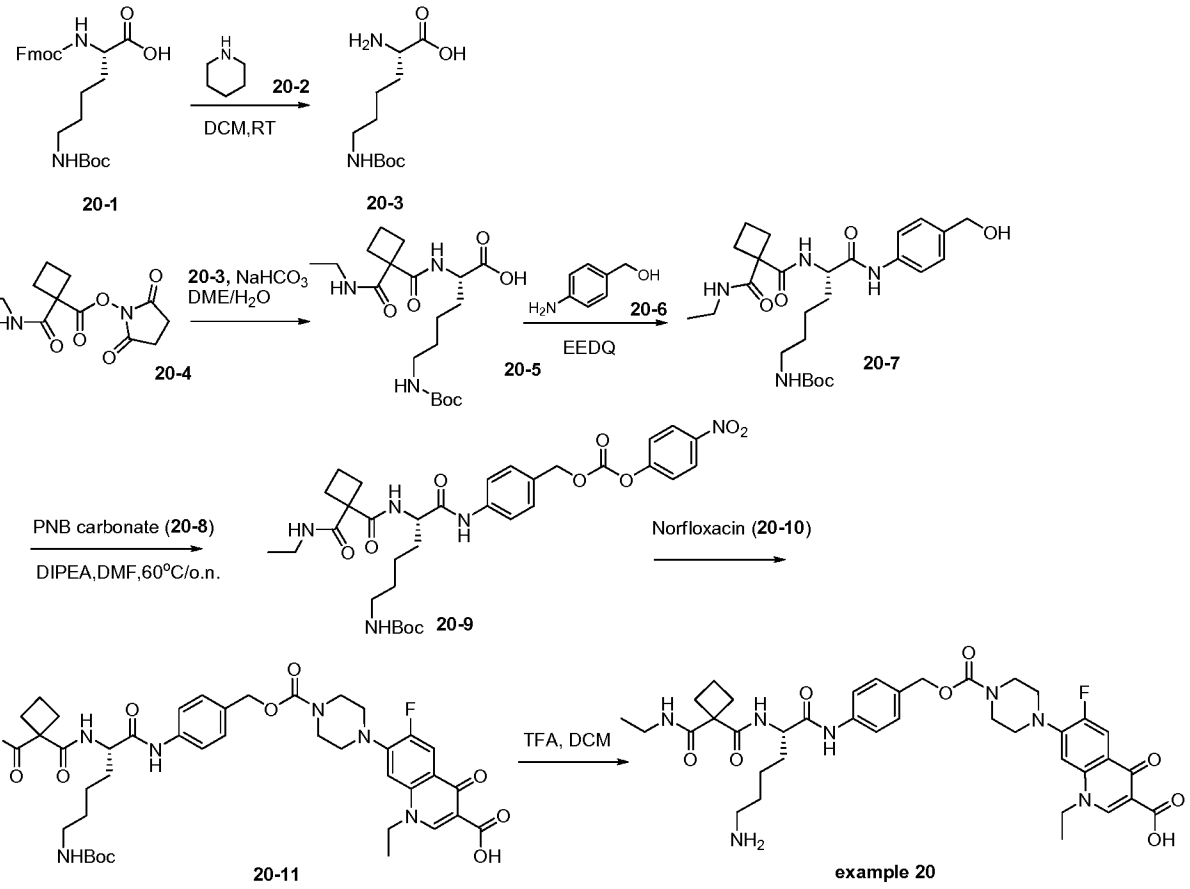
LCMS (ESI): RT = 0.854 min, M/2+H^+ = 422.2. method = 5-95 /1.5 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.30 (br, 1H), 10.16 (s, 1H), 8.95 (s, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.95 - 7.91 (m, 2H), 7.64 (d, J = 8.8 Hz, 2H), 7.35 - 7.27 (m, 6H), 7.21 - 7.17 (m, 2H), 5.97 (t, J = 5.6 Hz, 1H), 5.42 (s, 2H), 5.07 (s, 2H), 5.00 - 4.97 (m, 1H), 4.58 - 4.55 (m, 2H), 4.39 - 4.33 (m, 1H), 3.61 (s, 4H), 3.31 (s, 4H), 3.02 - 2.92 (m, 2H), 1.80 - 1.78 (m, 1H), 1.64 - 1.62 (m, 1H), 1.42 - 1.33 (m, 14H).

Example 20. (S)-7-(4-((4-(6-amino-2-(1-(ethylcarbamoyl)cyclobutanecarboxamido)hexanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 20



Step 1. To a stirred solution of compound **20-1** (5 g, 10.7 mmol) in DCM was added compound **20-2** (5.5 ml, 53 mmol) at r.t. The mixture was stirred at r.t. for 16 h. The reaction mixture was

extracted with H_2O and the combined aqueous layers were concentrated to give **20-3**. (Yield: 95 %)

Step 2. To a solution of compound **20-4** (1.5 g, 5.6 mmol) in DME (50 ml) was added a solution of compound **20-3** (2.75 g, 11.2 mmol) and $NaHCO_3$ (940 mg, 11.2 mmol) in water (50 mL). The mixture was stirred at r.t. for 16 h. The mixture was washed with EtOAc and acidified to pH 3 with 10% HCl. The resulting suspension was extracted with EtOAc. The combined organic layer was concentrated to give compound **20-5**. (Yield: 80 %)

LCMS (ESI): m/z 400.0 [M+H$^+$].

Step 3. To a solution of compound **20-5** (1 g, 2.5 mmol) in a mixture of DCM and MeOH (20 mL, 10 mL) were added 4-amino-phenyl)methanol (**20-6**) (462 mg, 3.75 mmol) and EEDQ (1.236 g, 5 mmol). The mixture was stirred at r.t. for 16 h. The residue was purified by column (PE/EtOAc= 1/3) to give **20-7** (Yield: 60 %).

LCMS (ESI): m/z 505.1 [M+H$^+$].

Step 4. To a solution of compound **20-7** (180 mg, 0.36 mmol) in DMF (6 mL) was added compound **20-8** (219 mg, 0.72 mmol) and DIPEA (140 mg, 1.08 mmol) at 0°C. The mixture was stirred at r.t. for 16 h. The mixture (**20-9**) was used in next step without further purification. (Yield: 100 %)

LCMS (ESI): m/z 670.6 [M+H$^+$].

Step 5. To the mixture of crude **20-9** was added norfloxacin (230 mg, 0.72 mmol) at r.t. and stirred at r.t. for 1 h. The residue was purified by prep-HPLC to give compound **20-11**. (Yield: 60 %)

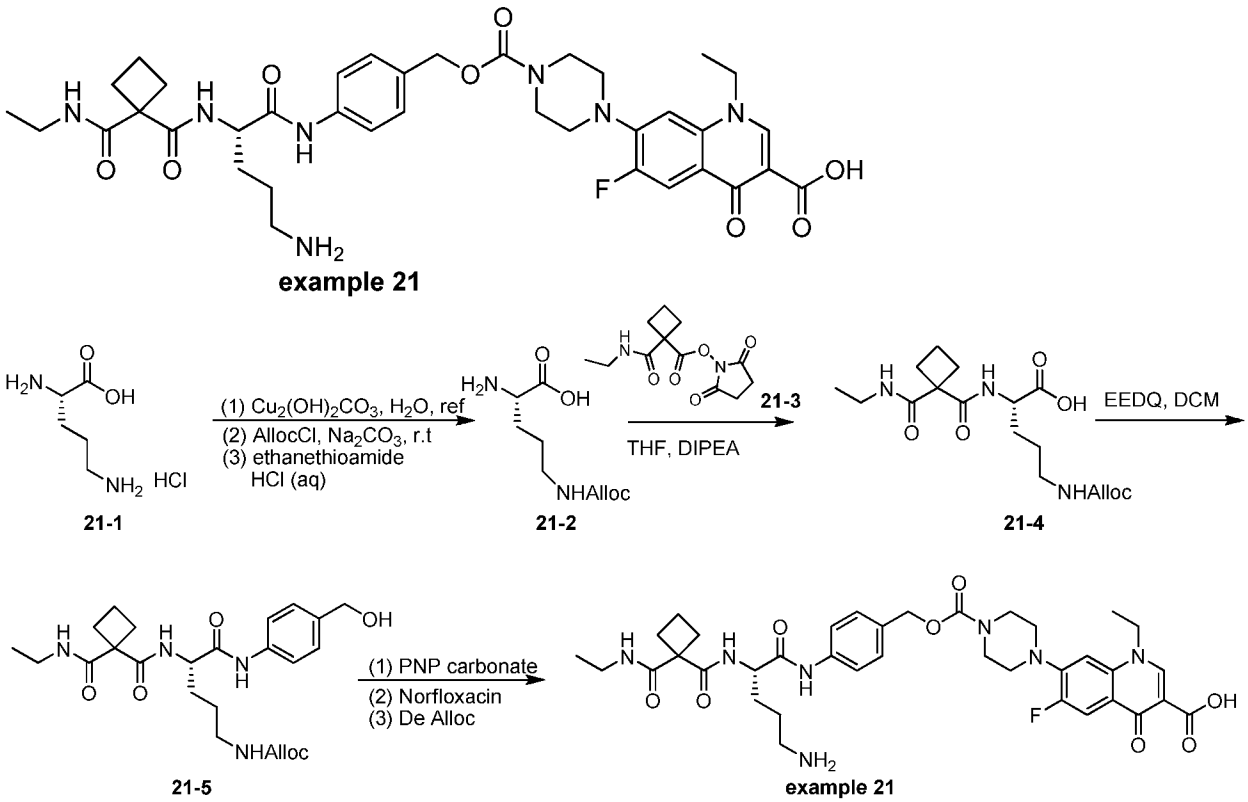
LCMS (ESI): m/z 850.5 [M+H$^+$].

Step 6. To compound **20-11** was added a solution of TFA and DCM (1:1) at 0 °C. The mixture was stirred at r.t. for 1 h. The mixture was basified to pH = 9 with $NH_3.H_2O$. The residue was purified by prep-HPLC and SFC to give **example 20** (Yield: 30 %).

^{1}H NMR (400 MHz, DMSO-d_6)

δ 10.26 (s, 1 H), 9.43 (HCOOH), 8.97 (d, J = 7.6 Hz, 1 H), 7.99 - 7.89 (m, 6 H), 7.67 - 7.65 (m, 2 H), 7.39 - 7.33 (m, 2 H), 7.23 - 7.21 (m, 1 H), 5.06 (s, 2 H), 4.72 - 4.60 (m, 2 H), 4.40 (s, 1 H), 3.80 - 3.55 (m, 6 H), 3.31 (s, 4 H), 3.15 - 3.12 (m, 2 H), 2.76 - 2.73 (m, 2 H), 2.48 - 2.41 (m, 2 H), 1.81 - 1.69 (m, 4 H), 1.59 - 1.55 (m, 2 H), 1.43 - 1.34 (m, 5 H), 1.07 - 0.98 (m, 3 H).

Example 21. (S)-7-(4-((4-(5-amino-2-(1-(ethylcarbamoyl)cyclobutanecarboxamido) pentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



Step 1. To a solution of **21-1** (10.0 g, 0.06 mol) in H_2O (300 mL) was added $Cu_2(OH)_2CO_3$ (24.0 g, 0.12 mol). After the mixture was stirred at 100°C for 1 h, it was filtrated quickly. Na_2CO_3 (20.0 g, 0.18 mol) was added to the filtrate. After it was stirred at r.t. for 20 min, AllocCl (12.0 g, 0.10 mol) was added to dropwise at r.t. and stirred at r.t. for 3 h. The mixture was filtered and washed with H_2O, then ethanethioamide (7.5 g, 0.10 mol) was added. After it was stirred at 50°C for 3 h, HCl (aq) was added to adjust pH = 3-4. The mixture was heated at reflux for 1 h. After the hot mixture was filtered, the filtrate was concentrated until white solid precipitated out. It was cooled to r.t. and filtered to give **21-2** as white solid (4.5 g, 35%)

Step 2. To a solution of **21-2** (1.0 g, 4.6 mmol) in THF (30 mL) was added DIPEA (1.9 g, 15.0 mmol) and compound **21-3** (1.2 g, 4.6 mmol). After it was stirred at 100°C for 4 h, the mixture was cooled to r.t. Water (100 mL) was added, followed by HCl (aq) to adjust pH = 2-3. The mixture was extracted with EtOAc (50 mL×2). The organic layer was washed with brine (50 mL), dried over Na_2SO_4 and concentrated to give the crude **21-4** as brown solid (1.3 g, crude).

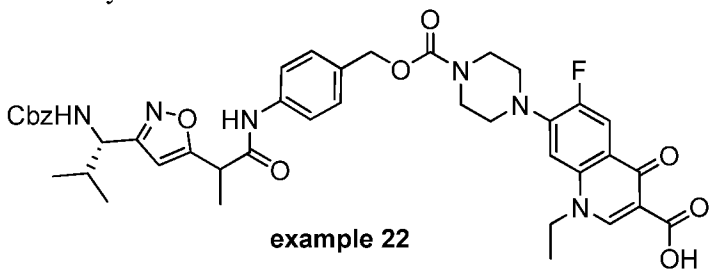
Step 3. To a solution of **21-4** (1.2 g, 3.2 mmol) in DCM (50 mL) was added EEDQ (1.1 g, 4.5 mmol) and (4-aminophenyl)methanol (750 mg, 6.0 mmol). After the mixture was stirred at r.t. for 4 h, water (50 mL) was added. The mixture was extracted with DCM (60 mL×2), dried over Na_2SO_4, concentrated, and purified by column (PE: EtOAc = 1:20) to give **21-5** as yellow oil (500 mg, 30%).

Step 4. To a solution of **21-5** (500 mg, 1.05 mmol) in DCM (30 mL) was added DIPEA (650 mg, 5.0 mmol) and PNP carbonate (320 mg, 1.5 mmol). The mixture was heated at reflux for 16 h. Solvent was removed, and the residue was dissolved in DMF (20 mL). DIPEA (400 mg, 3.0 mol) and norfloxacin (380 mg, 1.2 mmol) were added. The mixture was stirred at 100 °C for 5 h, then cooled to r.t. Saturated NaCl (100 mL) was added to and filtered to give the crude product as yellow solid (300 mg, MS = 820.2, M +1).

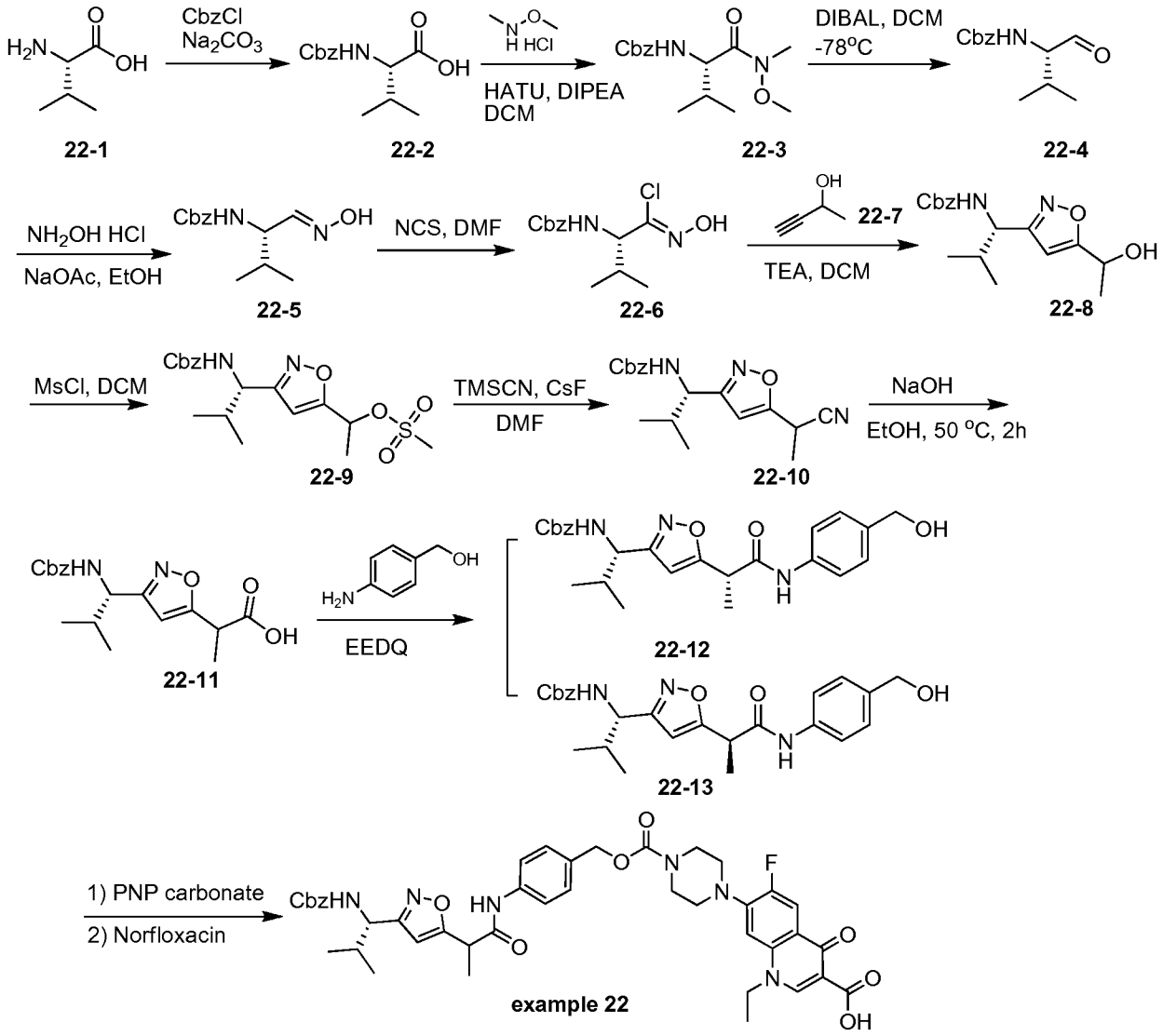
To a solution of the above crude product (300 mg) in THF (30 mL) was added $Pd(PPh_3)_4$ (80 mg, 0.07 mmol) and 1, 3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione (1.0 g, 6.4 mmol). The mixture was stirred at 50°C for 16 h under N_2 and cooled to r.t. The mixture was filtered, concentrated and purified by prep-HPLC to give **example 21** (24 mg, 8%).

^{1}H NMR (400MHz, DMSO-d_6) δ 10,37 (s, 1 H), 8.94 (s, 1 H), 7.94 - 7.89 (m, 6 H), 7.65 - 7.63 (d, J = 8.4 Hz, 2 H), 7.37 - 7.31 (m, 2 H), 7.19 (d, J = 7.6 Hz, 1 H), 5.04 (s, 2 H), 4.63 - 4.54 (m, 2 H), 4.47 - 4.41 (m, 1 H), 3.68 - 3.53 (m, 6 H), 3.29 (s, 4 H), 3.14 - 3.08 (m, 2 H), 2.80 - 2.77 (m, 2 H), 2.48 - 2.42 (m, 2 H), 1.88 - 1.63 (m, 6 H), 1.48 (t, J = 6.8 Hz, 3 H), 1.05 - 0.95 (t, J = 7.2 Hz, 3 H).

Example 22. 7-(4-((4-(2-(3-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)isoxazol-5-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 22



Step 1. To a suspension of compound **22-1** (50.0 g, 0.43 mol) and Na_2CO_3 (90.0 g, 0.85 mol) in THF and H_2O (300 mL/300 mL) at 0°C was added CbzCl (84.0 g, 0.49 mol) slowly. The reaction mixture was allowed to warm to r.t. and stirred at r.t. for 16 h. The organic solvent was removed and the aqueous solution was extracted with EtOAc (200 mL). The aqueous solution was acidified to pH = 2 with 1M HCl, and then extracted with EtOAc (200 mL × 2). The combined organic layer was washed with brine (150 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to afford the crude **22-2** as a white solid, which was used in next step without further purification.

Step 2. To the solution of compound **22-2** (20 g, 79.6 mmol) in dry DCM (200 mL) was added HATU (35 g, 92.1 mmol) and DIPEA (28 g, 217 mmol). After it was stirred at r.t. for 15 min, *N*-methoxymethanamine hydrochloride (11 g, 112.8 mmol) was added. After the solution was stirred for 3 h, it was washed with 1M HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and purified by the column chromatography on silica gel to give compound **22-3** (12 g, 51.2 %).

Step 3. DIBAL-H (30 mL, 1M in toluene) was added dropwise to a solution of compound **22-3** (4 g, 13.6 mmol) in dry DCM (150 mL) at -78°C under N_2. After the solution was stirred at -78°C for 6 h, it was quenched with MeOH (100 mL) and water (10 mL). The suspension was filtered off and the filtrate was dried over Na_2SO_4. Solvent was removed and the residue was purified on silica gel column to afford compound **22-4** as colorless oil (1.1 g, 34.4 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1 H), 7.37-7.32 (m, 5 H), 5.35-5.33 (m, 1 H), 5.12 (s, 2 H), 4.38-4.32 (m, 1 H), 2.36-2.28 (m, 1 H), 1.05 - 1.03 (d, J = 7.2 Hz, 3 H), 0.95 (d, J = 6.8 Hz, 3 H).

Step 4. To the solution of compound **22-4** (110 mg, 0.47 mmol) in EtOH (10 mL) was added sodium acetate (57.5 mg, 0.7 mmol) and hydroxylamine hydrochloride (49 mg, 0.7 mmol). After the reaction was stirred at 80°C for 16 h, the solvent was removed and the residue was dissolved in water and extracted with EtOAc (30 mL×2). The combined organic layers were washed with brine (30 mL), dried over anhydrous sodium sulfate, filtered and concentrated to give compound **22-5** as a white solid which was used in next step without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.97 (s, 0.6 H), 10.73 (s, 0.3 H), 7.49-7.45 (m, 1 H), 7.39-7.29 (m, 5 H), 7.18 (d, J = 7.2 Hz, 0.3 H), 6.54 (d, J = 6.8 Hz, 0.6 H), 5.02 (s, 2 H), 4.63-4.58 (m, 0.6 H), 3.88-3.82 (m, 0.3 H), 1.89-1.78 (m, 1 H), 0.84 (q, J = 6.8 Hz, 6 H).

LCMS (ESI): m/z 251.0 [M+H$^+$].

Step 5. To a solution of compound **22-5** (800 mg, 3.2 mmol) in DMF (5 mL) was added NCS (470 mg, 3.5 mmol). After the mixture was stirred for 1 h at 40°C, it was diluted with EtOAc (50 mL) and water (20 mL). The organic layer separated and washed with brine (30 mL × 5), dried over Na_2SO_4, filtered and concentrated to afford compound **22-6**, which was used directly in the next step.

Step 6. To a stirred solution of compound **22-7** (168 mg, 2.4 mmol) in DCM (10 mL) was added TEA (240 mg, 2.4 mmol) at 0°C. After it was stirred for 30 min, a solution of compound **22-6** (1.2 mmol) in DCM (10 mL) was added slowly. The reaction mixture was warmed to r.t. and stirred at r.t. for 16 h. Water was added to the reaction mixture and the layers were separated and the aqueous layer was extracted with DCM (20 mL × 3). The combined organic layers were washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified on silica gel column to give compound **22-8** as a pale yellow solid (150 mg, 39.3 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.31 (m, 5 H), 6.09 (s, 1 H), 5.41 (d, J = 8.0 Hz, 1 H), 5.14-5.06 (m, 2 H), 4.96 (d, J = 6.4 Hz, 1 H), 4.78 - 4.74 (q, J = 9.2 Hz, 1 H), 2.17-2.08 (m, 1 H), 1.55 (d, J = 6.8 Hz, 3 H), 1.03 (d, J = 6.8 Hz, 3 H), 0.96-0.91 (t, J = 9.6 Hz, 6 H).

Step 7. To the solution of compound **22-8** (1 g, 3.14 mmol) in dry DCM (50 mL) was added MsCl (5 g, 43.9 mmol) at 0 °C. The reaction mixture was allowed to warm to r.t. and stirred at r.t. for 3 h. Water was added to the reaction mixture and the layers were separated and the aqueous layer was extracted with DCM (50 mL×3). The combined organic layers were washed with brine (50

mL), dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified on silica gel column to afford compound **22-9** as colorless oil (1.2 g, 96.4 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 - 7.33 (m, 5 H), 6.26 (s, 1 H), 5.82 - 5.77 (q, J = 13.6 Hz, 1 H), 5.32 - 5.31 (d, J = 8.8 Hz, 1 H), 5.14 - 5.08 (m, 2 H), 4.80 - 4.77 (m, 1 H), 3.00 (s, 3 H), 2.20 - 2.12 (m, 1 H), 1.79 - 1.77 (d, J = 6.8 Hz, 3 H), 0.97 - 0.93 (q, J = 10 Hz, 6 H).

Step 8. A flask containing CsF (690 mg, 4.54 mmol) was purged with N_2, TMSCN (750 mg, 7.57 mmol) and dry DMF (2.5 mL) was added. The mixture was stirred at r.t. for 15 min and a pale yellow suspension formed. The solution of compound **22-9** (600 mg, 1.51 mmol) in dry DMF (2 mL) was added. After the mixture was stirred at 50°C for 16 h under N_2, water (50 mL) and EtOAc (50 mL) was added and the layers were separated. The aqueous layer was extracted with EtAc (50 mL × 2). The combined organic layers were washed with aq. $NaHCO_3$, brine and dried over Na_2SO_4. Solvent was removed, and the residue was purified by prep-HPLC to give compound **22-10** (60 mg, 12.1 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 - 7.33 (m, 5 H), 6.22 (s, 1 H), 5.28 - 5.26 (m, 1 H), 5.15 - 5.08 (m, 2 H), 4.80 - 4.76 (m, 1 H), 4.12 - 4.06 (m, J = 14.8 Hz ,1 H), 2.19 - 2.14 (m, 1 H), 1.74 - 1.70 (d, J = 7.2 Hz, 3 H), 0.98 - 0.93 (m, 6 H).

LCMS (ESI): m/z 327.9 [M+H$^+$].

Step 9. To a stirred solution of compound **22-10** (500 mg, 1.5 mmol) in EtOH (10 mL) was added NaOH aqueous solution (4 M, 5 mL). After the reaction mixture was stirred at 50 °C for 2 h, organic solvent was removed and the aqueous layer was diluted with H_2O (20 mL) and EtOAc (30 mL). The layers were separated and the aqueous layer was acidified to pH = 2 with 1 M HCl. It was extracted with EtOAc (30 mL × 2). The combined organic layers were washed with brine, dried and concentrated to afford the crude compound **22-11** which was used in next step without further purification.

Step 10. EEDQ (300 mg, 1.22 mmol) was added to the solution of compound **22-11** (220 mg, 0.61 mmol) and (4-aminophenyl)methanol (155 mg, 1.22 mmol) in dry DCM (10 mL) at 0 °C under N_2. The reaction mixture was warmed to r.t. and stirred at r.t. for 1 h under N_2. The solvent was removed and the residue was purified by prep-HPLC and SFC separation to afford **22-12 and 22-13.**

^{1}H NMR (400 MHz, MeOD-*d*$_4$) δ 7.49 - 7.47 (d, J = 8.4 Hz, 2 H), 7.27 - 7.23 (m, 7 H), 6.23 (s, 1H), 5.05 - 4.97 (m, 2 H), 4.48 (s, 3 H), 4.00 - 3.94 (q, J = 14.0 Hz, 1 H), 2.04 - 1.95 (m, 1 H), 1.52 1.51 (d, J = 7.2 Hz, 3 H), 0.91 - 0.90 (d, J = 6.8 Hz, 3 H), 0.82 - 0.80 (d, J = 6.8 Hz, 3 H).

LCMS (ESI): m/z 473.9 [M+Na$^+$], 497.0 [M+2Na$^+$].

^{1}H NMR (400 MHz, MeOD) δ 7.54 - 7.52 (d, J = 8.4 Hz, 2 H), 7.31 - 7.29 (m, 7 H), 6.29 (s, 1 H), 5.05 (s, 2 H), 4.55 (s, 3 H), 4.05 - 4.00 (m, 1 H), 2.10 - 2.00 (m, 1 H), 1.58 - 1.56 (d, J = 7.2 Hz, 3 H), 0.97 - 0.96 (d, J = 6.8 Hz, 3 H), 0.87 - 0.85 (d, J = 6.8 Hz, 3 H).

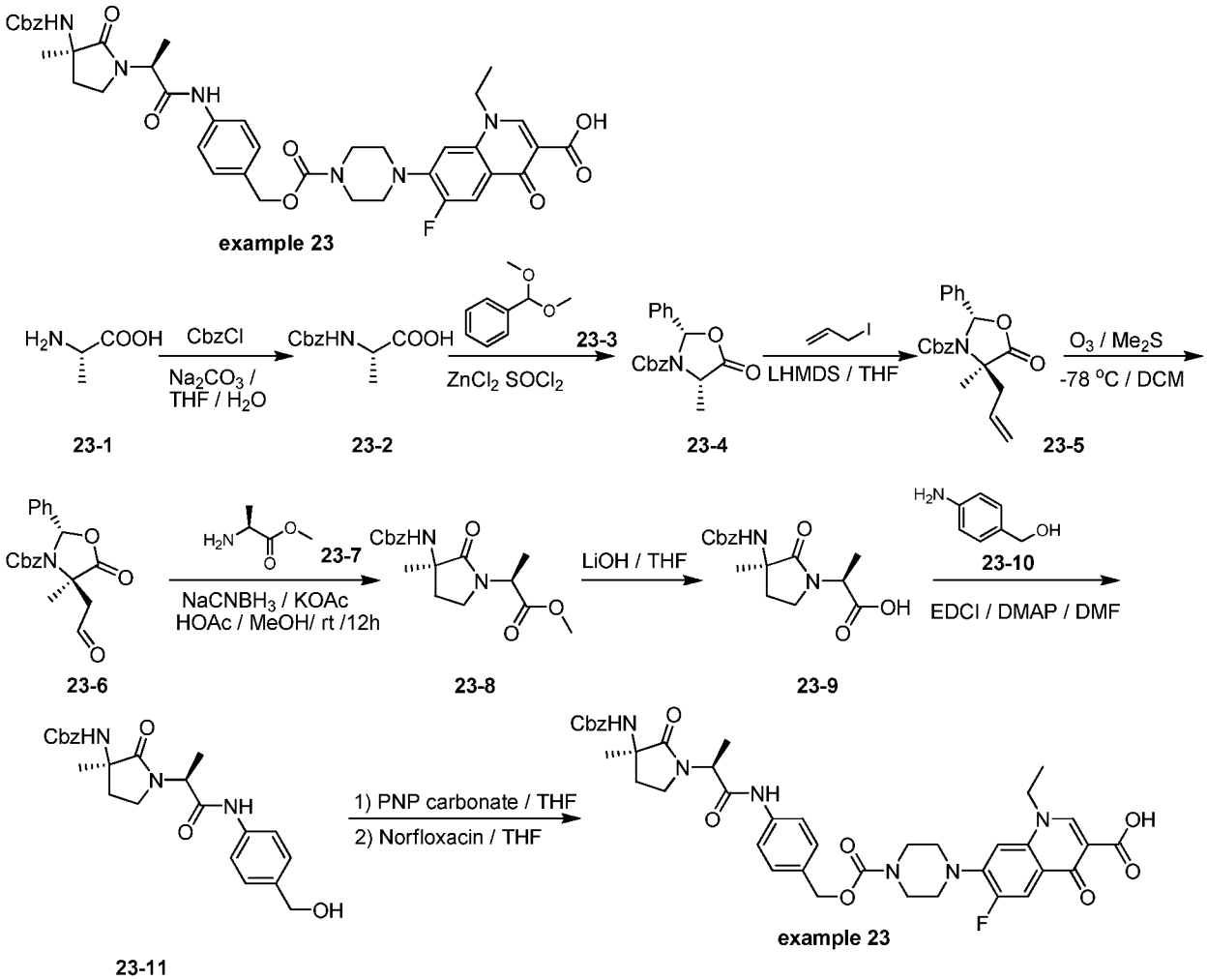
LCMS (ESI): m/z 473.8 [M+Na$^+$], 496.9 [M+2Na$^+$].

Step 11. To the solution of **22-12 or 22-13** (30 mg, 0.066 mmol) in dry DCM (5 mL) was added PNP carbonate (40.4 mg, 0.13 mmol) and DIPEA (0.5 mL). The mixture was heated at reflux for 20 h. After the solvent was removed, the residue was dissolved in DMF (3 mL). DIPEA (0.5 mL) and norfloxacin (63.5 mg, 0.2 mmol) were added. After the mixture was stirred at r.t. for 2 h, solvent was removed, and the residue was purified by prep-HPLC to give **example 22**.

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.32 (s, 1 H), 10.37 (s, 1 H), 8.97 (s, 1 H), 7.97 - 7.94 (d, J = 13.2 Hz, 1 H), 7.83 - 7.81 (d, J = 10.8 Hz, 1 H), 7.62 - 7.60 (d, J = 8.4 Hz, 2 H), 7.37 - 7.21 (m, 8 H), 6.34 (s, 1 H), 5.07 - 5.01 (m, 4 H), 4.60 - 4.56 (m, 2 H), 4.47 - 4.43 (m, 1 H), 4.08 - 4.03 (m, 1 H), 3.61 (s, 4 H), 3.33 (s, 4 H), 1.97 - 1.92 (m, 1 H), 1.49 - 1.47 (d, J = 7.2 Hz, 3 H), 1.43 - 1.39 (t, J = 7.2 Hz, 3 H), 0.91 - 0.90 (d, J = 4.8 Hz, 3 H), 0.77 - 0.76 (d, J = 6.8 Hz, 3 H).

LCMS (ESI): m/z 797.0 [M+H$^+$].

Example 23. 7-(4-((4-((S)-2-((R)-3-(benzyloxycarbonylamino)-3-methyl-2-oxopyrrolidin-1-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



Step 1. To the solution of L-alanine **23-1** (18 g, 0.2 mol) in water (100 mL) was added sodium carbonate (32 g, 0.30 mol). The solution became clear and was cooled to 0°C. CbzCl (40 g, 235 mmol) in THF (200 mL) was added in 1h, maintaining the temperature below 5 °C. After it was stirred at r.t. for another 3 h, the solution was washed with EtOAc. Aq. solution was then acidified and extracted with EtOAc (300 mL x 3). The organic layer was dried over Na_2SO_4 and concentrated to give compound **23-2**.

Step 2. Thionyl chloride (3.27 mL, 44.8 mmol) was added to the stirring mixture of CBz-L-alanine **23-2** (10.0 g, 44.8 mmol) and benzaldehyde dimelthyl acetal (6.73 mml, 44.8 mmol) in dry THF at 0 °C. After it was stirred for 30 min, anhydrous $ZnCl_2$ (6.11 g, 44.8 mmol) was added. The mixture was stirred at 0 °C for 3 h and antoher 0.2 equiv. of $ZnCl_2/SOCl_2$ was then added. The mixture was then quenched with water (below 10°C) and extracted with MTBE (150 mL x 3). The organic layer was dried over Na_2SO_4 and purified by column on silica gel to give compound **23-4**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.39 (m, 10 H), 6.65 (s, 1 H), 5.18-5.16 (m, 2 H), 4.47-4.52 (m, 1 H), 1.63-1.57 (m, 3 H).

Step 3. To a solution of compound **23-4** (15.0 g, 48.2 mmol) in dry THF (80 mL) at -78°C was added LiHMDS (1M, 63 mL) dropwise in 1 h and the solution was stirred at -78°C for 20 min. Allyl iodide (6.3 mL, 68.9 mmol) was then added slowly and the reaction was stirred at -78°C for 3 h. The mixture was warmed to r.t. and stirred for another 12 h. The mixture was diluted with ether and quenched with aq. NH_4Cl (100 mL). The mixture was extracted with ether (150 mL x 3). The organic layer was dried over Na_2SO_4 and purified by column on silica gel to give compound **23-5** (15 g, 88%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.14 (m, 9 H), 6.82-6.81 (m, 1 H), 6.28-6.21 (m, 2 H), 5.62-5.56 (m, 1 H), 5.23-5.08 (m, 2 H), 5.01-4.84 (m, 2 H), 3.36-3.19 (m, 1 H), 2.50-2.45 (m, 1 H), 1.65 (s, 2 H), 1.62-1.60 (m, 1 H).

Step 4. Ozone was bubbled to a solution of compound **23-5** (7.8 g, 22.2 mmol) in DCM at -78°C, until the solution turned to blue. N_2 was bubbled to the solution util it turned to colorless and Me_2S (33 mL) was then added and stirred at -78°C for 1 h. After it was warmed to r.t., solvent was evaporated to give compound **23-6**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1 H), 7.42-7.16 (m, 9 H), 6.80-6.78 (m, 2 H), 6.57 (s, 1 H), 4.99-4.96 (m, 1 H), 4.86-4.83 (m, 1 H), 4.13-4.02 (m, 1 H), 3.10-3.05 (m, 1 H), 1.76 (s, 3 H).

Step 5. To a solution of compound **23-6** (1.0 g, 2.83 mmol) in MeOH (20 mL) was added compound **23-7** (438 mg, 4.24 mmol), $NaCNBH_3$ (263 mg, 4.24 mmol) and NaOAc (100 mg). Acetic acid was added to adjust pH to 6.0. The reaction was stirred at r.t. under N_2 for 24 h. After solvent was removed, the residue was extracted with DCM (50 mL x 2), washed with 10 % HCl and water (30 mL). The organic layer was dried over Na_2SO_4, concentrated and purified with column to give compound **23-8**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.29 (m, 5 H), 5.35 (s, 1 H), 5.11-5.03 (m, 2 H), 4.89-4.88 (m, 1 H), 3.71 (s, 3 H), 3.41-3.35 (m, 2 H), 2.45-2.43 (m, 1 H), 2.32-2.31 (m, 1 H), 1.47-1.39 (m, 6 H).

Step 6. To the solution of compound **23-8** (334 mg, 1 mmol) in THF (1 mL) was added aq. LiOH (4 equiv.). The mixture was stirred at r.t. for 3 h. After solvent was removed, the residue was dissolved in water and acidified to pH 3.0 with aq. HCl. It was extracted with ether (20 mL x 3). The organic layer was combined, dried and concentrated to afford compound **23-9**, which was used directly in the next step.

Step 7. To the solution of compound **23-9** (320 mg, 1.0 mmol) in DCM (20 mL) was added EEDQ (247 mg, 1.0 mmol) and compound **23-10** (123 mg, 1.0 mmol). After the reaction was stirred at r.t. for 1 h, solvent was removed and the residue was purified with prep-HPLC to give **23-11**.

^{1}H NMR (400 MHz, MeOD) δ 7.55-7.52 (m, 2 H), 7.34-7.27 (m, 7 H), 5.05 (s, 2 H), 4.65-4.58 (m, 1 H), 4.54 (s, 2 H), 3.61-3.59 (m, 2 H), 2.60-2.45 (m, 1 H), 2.10-2.00 (m, 1 H), 1.56-1.54 (m, 3 H), 1.33 (s, 3 H).

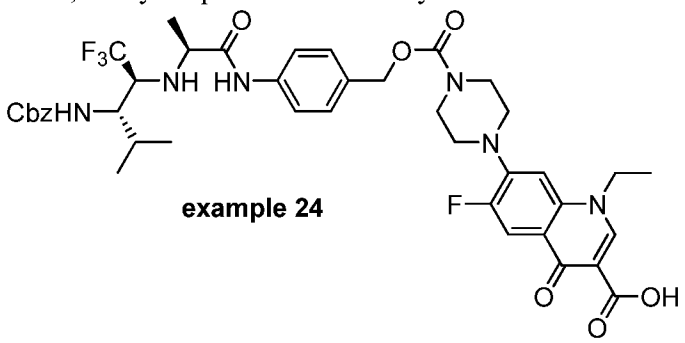
LCMS (ESI): m/z 448.1 [M+Na^+].

Step 8. To the solution of **23-11** (42 mg, 0.1 mmol) in DCM (20 mL) was added PNP carbonate (2 equiv.) and DIPEA (0.2 mL). The solution was heated at reflux for 16 h. Solvents were evaporated and the residue was dissolved in dry DMF (5 mL). DIPEA (0.2 mL) and norfloxacin (4 eq.) was added. The mixture was stirred at r.t. for 30 min, after the solvent was removed, the residue was purified by the prep-HPLC to give **example 23**.

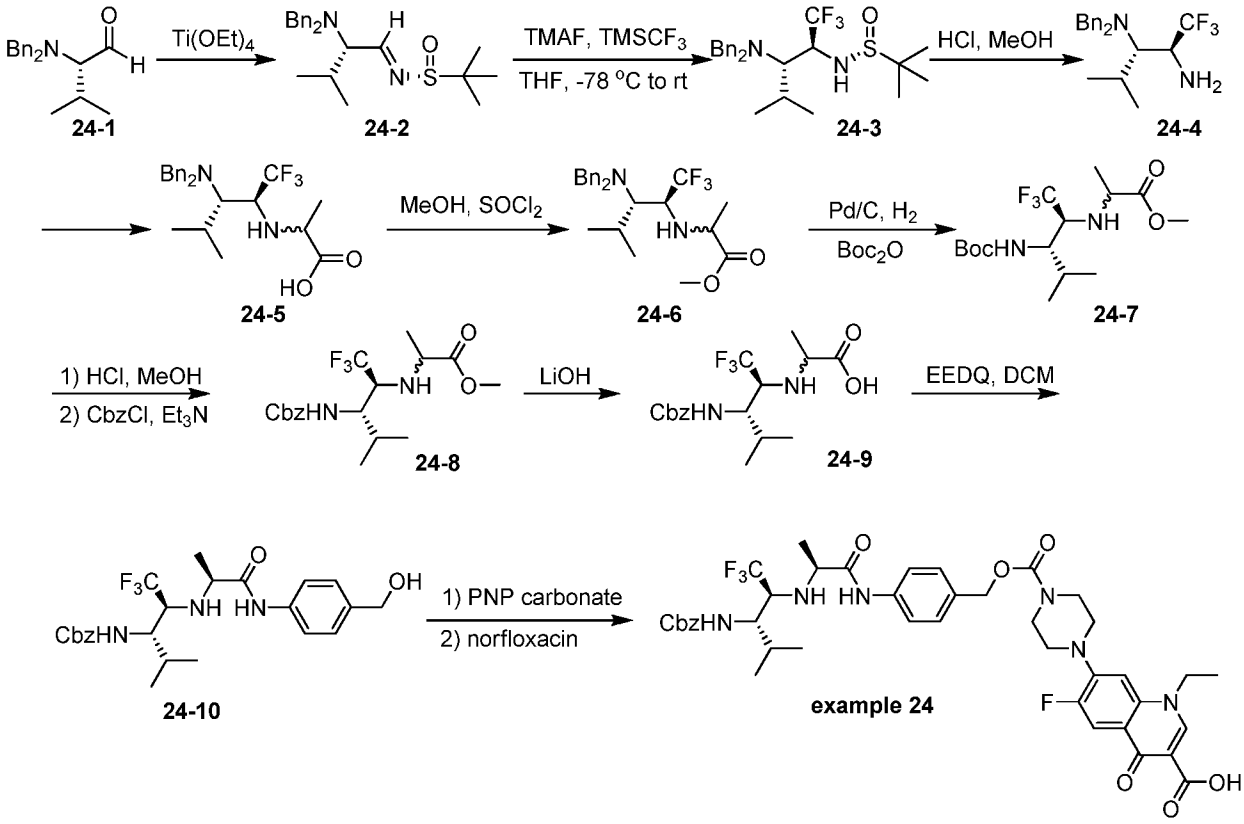
^{1}H NMR (400 MHz, MeOD) δ 8.77 (s, 1 H), 7.91-7.88 (m, 1 H), 7.50-7.48 (m, 2 H), 7.25-7.23 (m, 7 H), 7.10-7.00 (m, 1 H), 5.02 (s, 2 H), 4.96 (s, 2 H), 4.61-4.42 (m, 6 H), 4.15-4.08 (m, 1 H), 3.62 (s, 4 H), 3.52-3.50 (m, 2 H), 2.50-2.40 (m, 1 H), 2.00-1.92 (m, 1 H), 1.47-1.41 (m, 6 H), 1.27 (s, 3 H).

LCMS (ESI): m/z 771.1 [M+H^+].

Example 24. 7-(4-((4-((S)-2-((2S,3S)-3-(benzyloxycarbonylamino)-1,1,1-trifluoro-4-methylpentan-2-ylamino)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 24



Step 1. To a solution of compound **24-1** (9.93 g, 35.28 mmol) and 2-methylpropane-2-sulfinamide (4.28 g, 35.28 mmol) in THF (100 mL) was added $Ti(OEt)_4$ (32.19 g, 141.12 mmol) dropwise at 0°C. After the mixture was stirred at 25°C for 16 h, water (25 mL) was added dropwise at 0°C. The mixture was filtered and the filtrate was extracted with EtOAc (50 mL × 3). Solvent was removed to give crude product **24-2** (11 g, 81.1%).

LCMS (ESI): m/z 385.3 [$M+H^+$].

Step 2. $TMSCF_3$ (3.55 g, 24.96 mmol) was added dropwise at -78 °C to a solution of **24-2** (6.4 g, 16.64 mmol) and TMAF (1.86 g, 19.97 mmol) in THF (30 mL). After the solution was stirred at -78 °C for 2 h, it was quenched slowly with water (2 mL), then diluted with water (50 mL). The mixture was extracted with EtOAc (60 mL × 2). The organic layer was concentrated and the residue was purified by column chromatography on silica gel to give **24-3** (3.3 g, 44%).

^1H NMR (400 MHz, $CDCl_3$) δ 7.37-7.20 (m, 10 H), 4.21-4.11 (m, 1 H), 3.98-3.93 (m, 3 H), 3.65-3.61 (m, 2 H), 2.88-2.85 (m, 1 H), 2.29-2.20 (m, 1 H), 1.22 (s, 9 H), 1.13-1.11 (m, 3 H), 0.95-0.91 (m, 3 H).

Step 3. A mixture of **24-3** (3.3 g, 7.26 mmol) and 4M HCl in MeOH (25 mL) was stirred at 25 °C for 6 h. Solvent was removed to give crude product of **24-4** (2.8 g).

LCMS (ESI): m/z 351.2 [$M+H^+$].

Step 4. To a solution of **24-4** (900 mg, 2.57 mmol), 2-oxopropanoic acid (905 mg, 10.28 mmol) and HOAc (617 mg, 10.28 mmol) in DCE (12 mL) was added $NaBH(AcO)_3$ (2.18 g, 10.28 mmol)

at 25 °C. After the mixture was stirred at 25 °C for 16 h, solvent was removed and the residue was purified by column chromatography to give **24-5** (700 mg, 64.5%).

LCMS (ESI): m/z 423.3 [M+H$^+$].

Step 5. To a solution of **24-5** (700 mg, 1.66 mmol) in MeOH (5 mL) was added $SOCl_2$ (395 mg, 3.32 mmol) dropwise at 0 °C. After the solution was stirred at 50 °C for 16 h, it was cooled to 25 °C and solvent was removed under reduced pressure to give crude product of **24-6** (620 mg, crude).

LCMS (ESI): m/z 437.3 [M+H$^+$].

Step 6. To a solution of **24-6** (520 mg, 1.19 mmol) and Boc_2O (260 mg, 1.19 mmol) in MeOH (8 mL) was added Pd/C (100 mg). After the mixture was stirred at 25 °C for 16 h under H_2, it was filtered, solvent was removed and the residue was purified by column chromatography on silica gel (PE/EtOAc 5:1) to give **24-7** (390 mg, 92%).

LCMS (ESI): m/z 357.2 [M+H$^+$].

Step 7. To a solution of **24-7** (395 mg, 1.08 mmol) in MeOH (5 mL) was added 4M HCl in MeOH (5 mL, 20 mmol). After the solution was stirred at 25°C for 2 h, solvent was removed and the residue was dissolved in DCM (8 mL). CbzCl (276 mg, 1.62 mmol) and Et_3N (219 mg, 2.16 mmol) were added at 0°C and the mixture was stirred at 25 °C for 6 h. Solvent was removed and the residue was purified by prep-TLC to give compound **8** (110 mg, 26%).

LCMS (ESI): 391.1 [M+H$^+$].

Step 8. To a solution of compound **8** (110 mg, 0.282 mmol) in THF/MeOH/H_2O (0.5 mL: 0.5 mL : 0.5 mL) was added $LiOH{\cdot}H_2O$ (42 mg, 1 mmol) at 25°C. The solution was stirred at 25 °C for 2 h. Solvent Removed, and the residue was taken up with water (3 mL). The aqueous solution was acidified to pH 2 with 1M HCl and extracted with EtOAc (15 mL×3). The organic layer was dried over Na_2SO_4, filtered, concentrated to give compound **9** (100 mg, 94%).

LCMS (ESI): 377.1 m/z [M+H$^+$].

Step 9. To a solution of **24-9** (120 mg, 0.319 mmol) and (4-aminophenyl) methanol (79 mg, 0.638 mmol) in DCM (3 mL) was added EEDQ (158 mg, 0.638 mmol) at 0°C under N_2. The mixture was stirred at r.t. for 6 h. After removal of the solvent, the residue was purified by prep-TLC, then SFC separation to give the major isomer (72 mg) **24-10** and minor isomer (26 mg). The absolute configuration of each isomer is not determined.

^{1}H NMR (400 MHz, MeOD) δ 7.65-7.63 (m, 2 H), 7.37-7.27 (m, 7 H), 5.14-5.07 (m, 2 H), 4.56 (s, 2 H), 3.81-3.79 (m, 1 H), 3.59-3.57 (m, 1 H), 3.37-3.35 (m, 1 H), 2.11-2.10 (m, 1 H), 1.33 (d, J = 6.8 Hz, 3 H), 0.93 (d, J = 6.8 Hz, 3 H), 0.87 (d, J = 6.4 Hz, 3 H).

LCMS (ESI): 482.2 m/z [M+H$^+$].

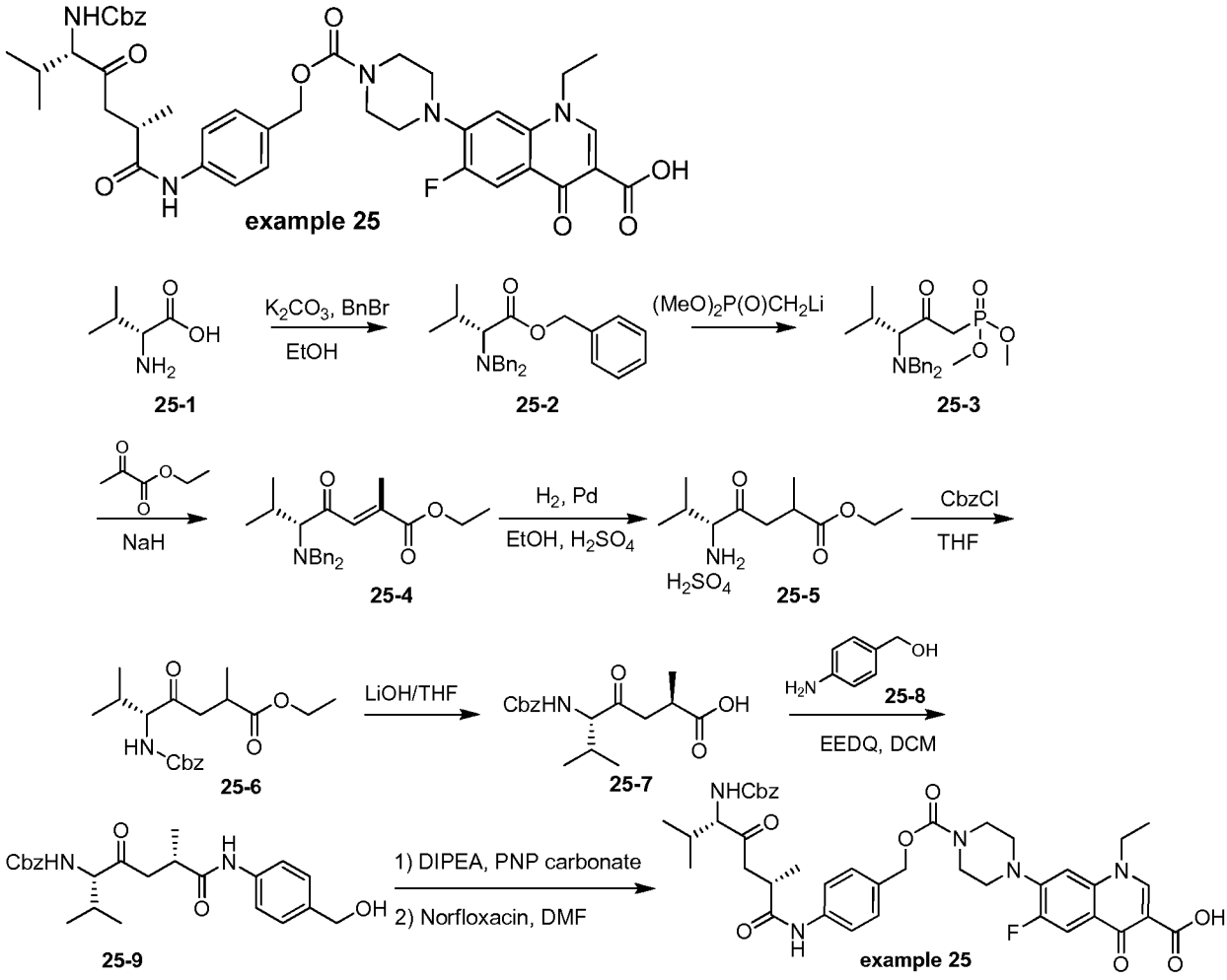
Step 10. To a solution of **24-10** (20 mg, 0.042 mmol) in THF (1.5 mL) was added PNP carbonate (38 mg, 0.125 mmol) and DIPEA (21 mg, 0.166 mmol) at 25°C. The mixture was heated at 50°C for 18 h. Solvent was removed, and the residue was dissolved in DMF (1.5 mL). Norfloxacin (20

mg, 0.062 mmol) was added and the mixture was stirred at 25°C for 2 h. The mixture was purified by prep-HPLC to give **example 24** (11.2 mg, 33%).

^{1}H NMR (400 MHz, MeOD) δ 8.86 (s, 1 H), 8.41 (s, 1 H), 8.1-7.94 (m, 1 H), 7.70-7.68 (m, 2 H), 7.37-7.09 (m, 9 H), 5.13-5.09 (m, 4 H), 4.89-4.86 (m, 2 H), 3.80-3.57 (m, 6H), 3.38-3.28 (m, 3 H), 2.20-2.07 (m, 1 H), 1.52 (s, 3 H), 1.33 (t, J = 6.8 Hz, 3 H), 0.937 (d, J = 6.8 Hz, 3 H), 0.87(d, J = 6.8 Hz, 3 H).

LCMS (ESI): 827.3 m/z [M+H$^+$].

Example 25: 7-(4-((4-((2S,5R)-5-(benzyloxycarbonylamino)-2,6-dimethyl-4-oxoheptanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



Step 1. To the solution of compound **25-1** (2 g, 17.07 mmol) and K_2CO_3 (7.066 g, 51.2mmol) in EtOH (50 mL) was added benzyl bromide (8.7 g, 51.2 mmol). After the mixture was heated at reflux for 5 h, solid was filtered off and the filtrate was concentrated under reduced pressure and purified by flash chromatography on silica gel (EtOAc: hexane = 1:10) to give **25-2** (3.52 g, 58.1%).

Step 2. To the solution of dimethyl methylphosphonate (6.78 g, 54.7 mmol) in dry THF (60 mL) was added dropwise LDA (2 mol/L, 27 mL) at -78°C over 1 h. After it was stirred at -78°C for 1h, a solution of **25-2** (3.52 g, 9.12 mmol) in dry THF (10 mL) was added dropwise at -78°C. The mixture was stirred for 1 h and it was extracted with EtOAc (100 mL × 3). The organic layer was washed with brine (60 mL) and concentrated to give the crude product of **25-3**, which was used for next step without further purification (3.12 g, 84.9%).

LCMS (ESI): m/z 403.9 [M+H$^+$], 425.9 [M+Na$^+$]

Step 3. To a solution of **25-3** (3.12 g, 7.75 mmol) in dry THF (60 mL) was added sodium hydride (372 mg, 9.3 mmol, 60%) at 0°C. After it was stirred at 0°C for 30 min, a solution of 2-oxo-propionic acid ethyl ester (1.349 g, 11.6 mmol) in dry THF (5 mL) was added dropwise. The mixture was stirred at r.t. for 16 h under N_2. Solvent was removed, the residue was purified by prep-HPLC to give **25-4** (2.85 g, 93.6%).

LCMS (ESI): m/z 394.2 [M+H$^+$].

Step 4. To the solution of **25-4** (2.85 g, 7.3 mmol) and sulfuric acid (750 mg, 7.3 mmol) in EtOH (60 mL) was added 10% palladium on activated carbon (1 g). After it was stirred under hydrogen for 6 h, the reaction mixture was filtered through a pad of celite and concentrated to give the desired product **25-5** (3.65 g, 100%).

Step 5. To the solution of **25-5** (3.65 g, 7.3 mmol) and triethylamine (2.21 g, 21.9 mmol) in CH_2Cl_2 (150 mL) was added dropwise a solution of CbzCl (1.36 g, 8.03 mmol) in ice bath. After the mixture was stirred at r.t. for 2 h, it was extracted with EtOAc (50 mL × 3). The organic layer was washed with brine (50 mL), dried over Na_2SO_4, concentrated in vacuo to give the crude product **25-6**.

LCMS (ESI): m/z 350.2 [M+H$^+$], 372.2 [M+Na$^+$].

Step 6. To the solution of **25-6** (1.6 g, 4.58 mmol) in a mixture of H_2O and THF (40 mL, 1:3) was added lithium hydroxide hydrate (1.93 g, 45.8 mmol). After the reaction mixture was stirred at r.t. for 16 h, it was extracted with EtOAc (50 mL × 3). The organic layer was washed with brine (50 mL), dried over Na_2SO_4, concentrated in vacuo to give the crude product **25-7** (1.43 g, 97.3%).

LCMS (ESI): m/z 344.1 [M+Na$^+$].

Step 7. To a solution of compound **25-7** (1.0 g, 3.11 mmol) in dry DCM (20 mL) was added EEDQ (1.52 g, 6.22 mmol) and **25-8** (765 mg, 6.22 mmol). The mixture was stirred at 0°C for 16 h under N_2. After the solvent was removed, the residue was purified by prep-HPLC and SFC to give **25-9**, **25-10**, **25-11**, and **25-12.**

^{1}H NMR (400 MHz, MeOD) δ 7.49 (d, J = 8.8 Hz, 2 H), 7.35-7.25 (m, 7 H), 5.06 (s, 2 H), 4.53 (s, 2 H), 4.12 (d, J = 5.6 Hz, 1 H), 3.07-3.92 (m, 2 H), 2.62-2.57 (m, 1 H), 2.26-2.21 (m, 1 H), 1.18 (d, J = 6.8 Hz, 3 H), 0.95 (d, J = 6.8 Hz, 3 H), 0.81 (d, J = 6.8 Hz, 3 H). LCMS (ESI): m/z 427.2 [M+H$^+$], 449.1 [M+Na$^+$], 409.2 [M-OH].

^{1}H NMR (400 MHz, MeOD) δ 7.49 (d, J = 8.8 Hz, 2 H), 7.33-7.20 (m, 7 H), 5.07 (s, 2 H), 4.53 (s, 2 H), 4.12 (d, J = 5.6 Hz, 1 H), 3.28-2.92 (m, 2 H), 2.63-2.57 (m, 1 H), 2.27-2.19 (m, 1 H), 1.18 (d, J = 6.8 Hz, 3 H), 0.95 (d, J = 6.8 Hz, 3 H), 0.81 (d, J = 6.8 Hz, 3 H). LCMS (ESI): m/z 427.2 [M+H$^+$], 449.1 [M+Na$^+$], 409.2 [M-OH].

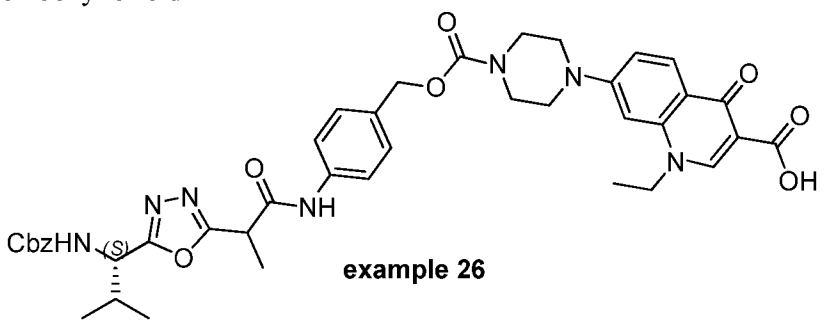
^{1}H NMR (400 MHz, MeOD) δ 7.41 (d, J = 6.8 Hz, 2 H), 7.39-7.16 (m, 7 H), 5.03-4.96 (m, 2 H), 4.47 (s, 2 H), 3.89 (d, J = 6.4 Hz, 1 H), 2.93-2.82 (m, 2 H), 2.49-2.43 (m, 1 H), 2.10-2.05 (m, 1 H), 1.07 (d, J = 6.8 Hz, 3 H), 0.82 (d, J = 6.8 Hz, 3 H), 0.77 (d, J = 6.8 Hz, 3 H). LCMS (ESI): m/z 427.2 [M+H$^+$], 449.1 [M+Na$^+$], 409.2 [M-OH].

^{1}H NMR (400 MHz, MeOD) δ 8.02 (d, J = 18.8 Hz, 2 H), 7.79-7.29 (m, 7 H), 5.16-5.08 (m, 2 H), 4.56 (s, 2 H), 4.01 (d, J = 6.0 Hz, 1 H), 3.32-2.94 (m, 2 H), 2.60-2.55 (m, 1 H), 2.24-2.12 (m, 1 H), 1.19 (d, J = 6.8 Hz, 3 H), 0.95-0.88 (m, 6 H). LCMS (ESI): m/z 409.0 [M-OH].

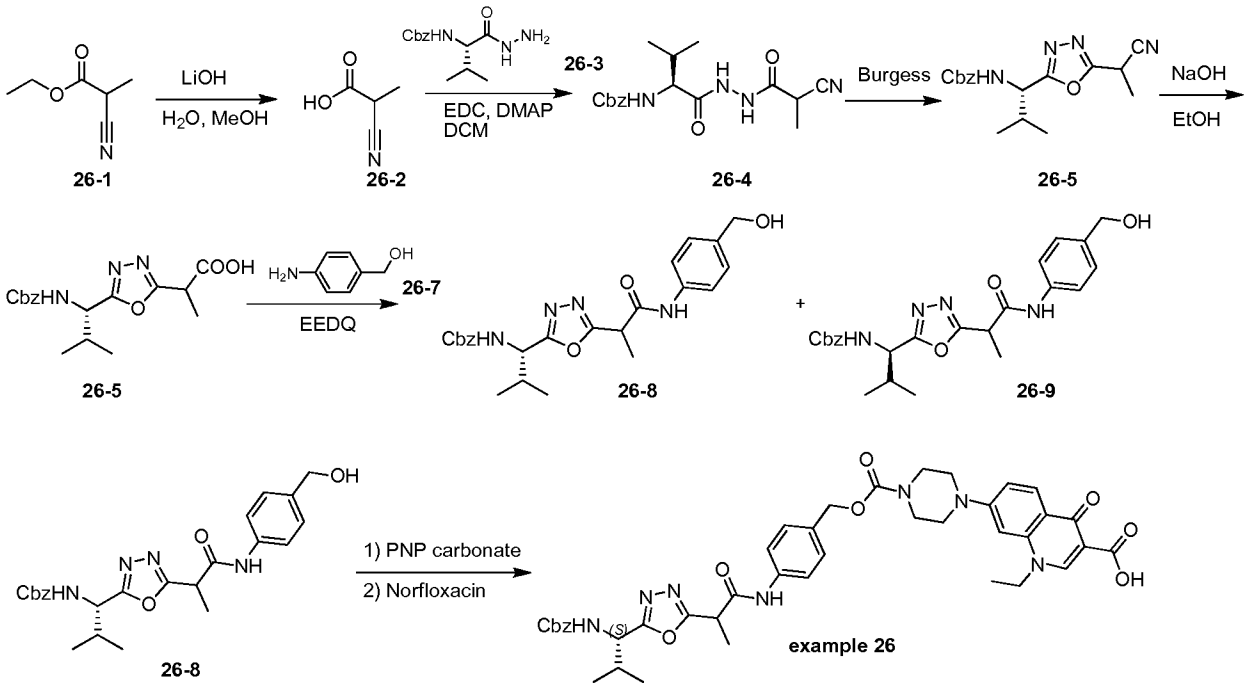
Step 8. To a solution of **25-9** (100 mg, 0.235 mmol) in dry DCM (2 mL) was added PNP carbonate (147 mg, 0.47 mmol) and DIPEA (61 mg). The mixture was heated at reflux for 16 h. After the solvent was removed, the residue was dissolved in DMF (3 mL). DIPEA (61 mg, 0.47 mmol) and norfloxacin (150 mg, 0.47 mmol) was added. The mixture was stirred at r.t. for 1 h. After solvent was removed, the residue was purified by prep-HPLC to give **example 25** as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.33 (s, 1 H), 10.01 (s, 1 H), 8.96 (s, 1 H), 7.94 (d, J = 12.8 Hz, 1 H), 7.72 (d, J = 8.0 Hz, 1 H), 7.58 (d, J = 8.4 Hz, 2 H), 7.37-7.30 (m, 7 H), 7.21 (d, J = 7.2 Hz, 1 H), 5.05-5.04 (m, 4 H), 4.59-4.58 (m, 2 H), 3.84-3.80 (m, 1 H), 3.61 (s, 4 H), 2.94-2.89 (m, 2 H), 2.12-2.05 (m, 1 H), 1.42-1.39 (m, 3 H), 1.07 (d, J = 6.0 Hz, 3 H), 0.82 (d, J = 6.4 Hz, 6 H). LCMS (ESI): m/z 772.6 [M+H$^+$], 386.7 [M/2+H$^+$].

Example 26. 7-(4-((4-(2-(5-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)-1,3,4-oxadiazol-2-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 26



Step 1. To a solution of **26-1** (10.0 g, 78.7 mmol) in MeOH (180 mL) and H_2O (180 mL) was added $LiOH \cdot H_2O$ (16.9 g, 393.3 mmol). After the mixture was stirred at r.t. for 24 h, it was acidified with conc. HCl to pH = 6. The crude was then extracted with (200 mL×3) and concentrated to give **26-2**.

Step 2. To a stirred solution of **26-2**(2 g, 20.2 mmol) and DMAP (247 mg, 2.02 mmol) in DCM (400 mL) was added **26-3** (5.89 g, 22.2 mmol) and EDCI (4.26 g, 22.2 mmol). After the solution was stirred at r.t. for 2 h, it was washed successively with water (200 mL×3), brine (100 mL), dried over Na_2SO_4 and concentrated to give **26-4**.

Step 3. A mixture of **26-4** (3 g, 8.7 mmol) and Burgess regent (3.1 g, 13.1 mmol) in THF (50 mL) was stirred at r.t. for 24 h. After removal of the solvent, the residue was extracted with EtOAc (100 mL×3). The organic layers were washed with brine (100 mL), concentrated and purified by column chromatography on silica gel (PE: EtOAc = 2:1) to give **26-5**.

Step 4. A mixture of **26-5**(1.67 g, 5.09 mmol) and a solution of NaOH (66 mmol) in EtOH (66 mL) and H_2O (66 mL) was heated at 80°C for 12 h. After it was cooled to r.t., the mixture was washed with EtOAc (50 mL) and the aqueous layer was adjusted to pH 5-6 and extracted with EtOAc (60 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated to give **26-6**.

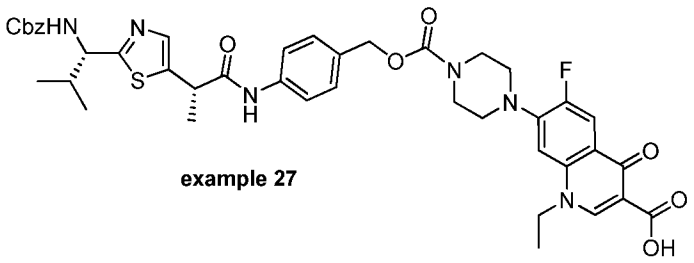
Step 5. A mixture of **26-6** (1.47 g, 4.23 mmol), **26-7** (1.56 g, 12.7 mmol) and EEDQ (3.14 g, 12.7 mmol) in DCM (50 mL) was stirred at r.t. for 2 h. Solvent was removed, and the residue was purified by prep-HPLC and SFC to give **26-8** and **26-9**.

Step 6. A mixture of **26-8** (40 mg, 0.088 mmol), PNP (57 mg, 0.18 mmol) and DIPEA (34 mg, 0.26 mmol) in DCM (3 mL) was stirred at 50°C for 12 h. It was concentrated and was mixed with Norfloxacin (82 mg, 0.26 mmol) and DIPEA (34 mg, 0.26 mmol) in DMF (5 mL). After it was stirred at r.t. for 2 h, solvent was removed, and the residue was purified by prep-HPLC to give **example 26** (30.5 mg, 43.6%).

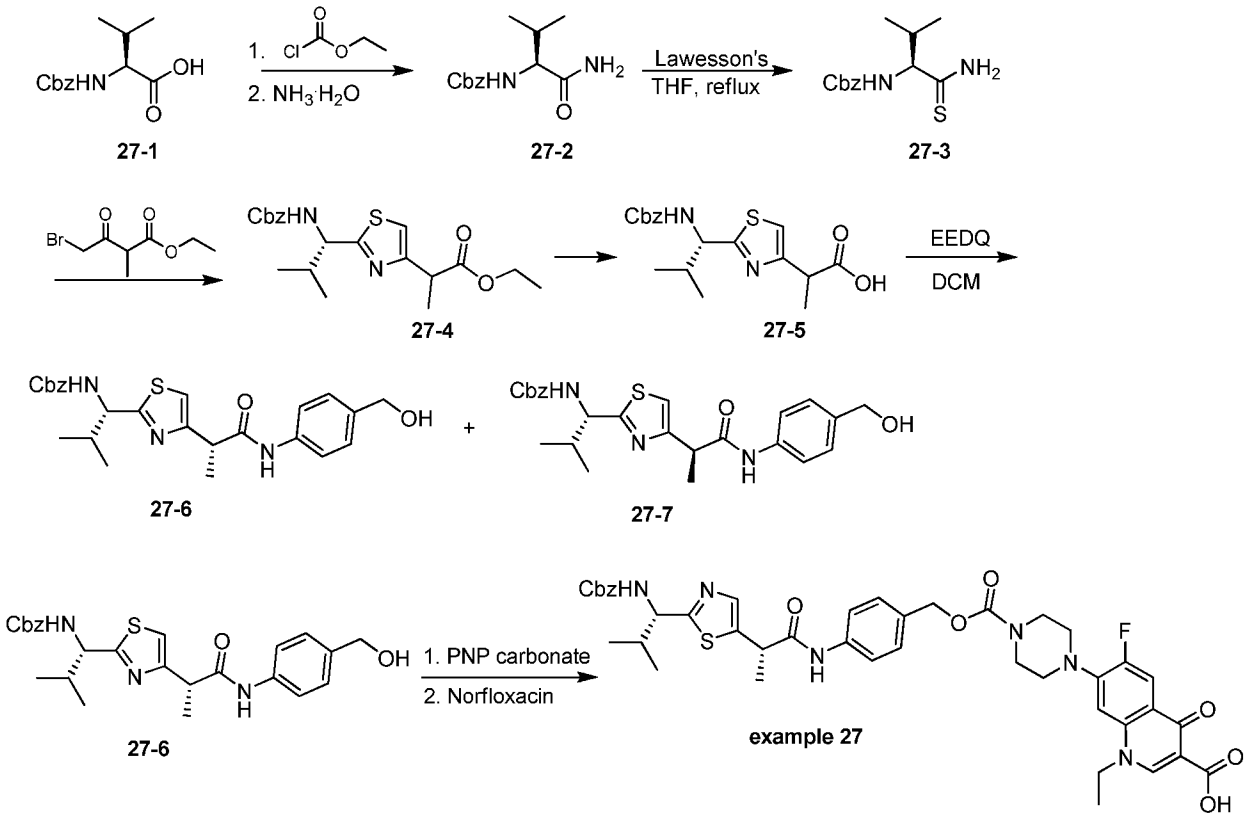
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.33 (s, 1 H), 10.44 (s, 1 H), 8.97 (s, 1 H), 8.12-8.08 (m, 1 H), 7.95 (d, J = 13.2 Hz, 1 H), 7.58 (d, J = 8.4 Hz, 2 H), 7.37-7.34 (m, 7 H), 7.21 (d, J =7.2 Hz, 1 H), 5.05 (d, J = 14.8 Hz, 4 H), 4.59-4.58 (m, 3 H), 4.19-4.17 (m, 1 H), 3.61 (s, 4 H), 3.38 (s, 4 H), 2.10-2.07 (m, 1 H), 1.58 (dd, J =2.0, 7.2 Hz, 3 H), 1.40 (t, J = 6.8 Hz, 3 H), 0.93 (d, J = 6.8 Hz, 3 H), 0.82 (d, J = 6.8 Hz, 3 H).

LCMS (ESI): m/z 798.3 [M+H$^+$], 820.2 [M+Na$^+$].

Example 27. 7-(4-((4-((S)-2-(2-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)thiazol-5-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 27



Step 1. To a solution of **27-1** (8 g, 31.8 mmol) and Et_3N (6.43 g, 63.6 mmol) in dry THF (100 mL) at -78°C was added dropwise a solution of ethyl chloroformate (3.45 g, 31.8 mmol) in THF (10 mL). After the reaction was stirred at -78°C for 1 h, $NH_3.H_2O$ (5 mL) was added. The reaction was warmed to r.t. over 2 h. White solid was collected by filtration and dried to give **27-2** (5.1 g, 63.8%).

Step 2. A solution of **27-2** (3 g, 12 mmol) and Lawesson's Reagent (9.7 g, 24 mmol) in dry THF (60 mL) was stirred 70°C for 16 h. Solvent was removed and residue was purified by column (EtOAc: henaxe= 1:3) to give **27-3** (2.9 g, 90.6%).

LCMS (ESI): m/z 267.1 [$M+H^+$], 289.1 [$M+Na^+$].

Step 3. To the solution of **27-3** (3 g, 11.26 mmol), $KHCO_3$ (3.38 g, 33.8 mmol) in dry DME (40 mL) was added dropwise a solution of **27-3a** (11.31 g, 22.52 mmol) in DME (10 mL). After the reaction was stirred at -40°C for 16 h, water (50 mL) and EtOAc (100 mL) was added, and the organic layer was washed with brine, dried over Na_2SO_4, concentrated in vacuo. To the mixture of crude intermediate and 2, 6-dimethylpyridine (3.3 g, 30.4 mmol) in THF (20 mL) was added dropwise a solution of 2,2,2-trifluoroacetic anhydride (3.2 g, 15.2 mmol) at -40°C slowly. After the reaction was stirred at -10°C for 2 h, water (100 mL) and EtOAc (100 mL) was added. The organic layer was washed with brine, dried over Na_2SO_4, concentrated in vacuo. The crude product was purified by prep-HPLC to afford **27-4** (2 g, 67.7%).

LCMS (ESI): m/z 391.2 [$M+H^+$].

Step 4. To the solution of **27-4** (2 g, 5.13 mmol) in a mixture of CH_3OH and H_2O (60 mL/20 mL) was added lithium hydroxide (2.15g, 51.3 mmol). After the mixture was stirred at r.t. for 16 h, pH was adjusted to 5 with diluted HCl (5%) and the mixture was extracted with EtOAc (60 mL × 2). The organic layer was washed with brine (60 mL), dried over Na_2SO_4, concentrated in vacuo to give **27-5**.

LCMS (ESI): m/z 363.2 [M+H$^+$].

Step 5. To the solution of **27-5** (1 g, 2.76 mmol) and EEDQ (750 mg, 5.52 mmol) in CH_2Cl_2 (20 mL) was added (4-aminophenyl)methanol (680 mg, 5.52 mmol) at 0°C. The reaction mixture was stirred at 0°C for 2 h and then at r.t. for 16 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with brine (50 mL), dried over Na_2SO_4, filtered, concentrated, purified by prep-HPLC and SFC to give **27-6 and 27-7** (370 mg each, 28.7%).

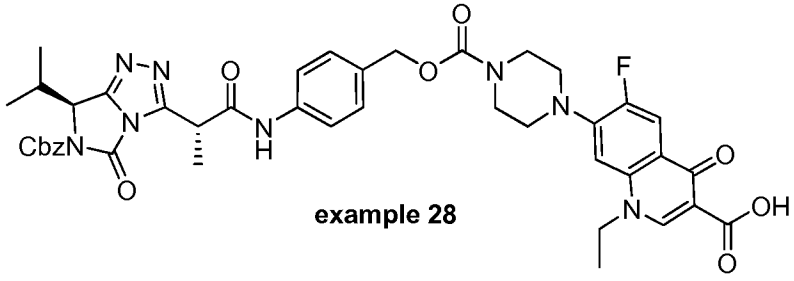
^{1}H NMR (400 MHz, MeOD) δ 7.55 (d, J = 8.4 Hz, 2 H), 7.36-7.29 (m, 8 H), 5.11 (s, 2 H), 4.80 (d, J = 6.8 Hz, 1 H), 4.56 (s, 2 H), 4.07-4.01 (m, 1 H), 2.33-2.25 (m, 1 H), 1.60 (d, J = 6.8 Hz, 3 H), 0.96-0.91 (m, 6 H). LCMS (ESI): m/z 468.0 [M+H$^+$], 490.2 [M+Na$^+$].

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1 H), 7.49-7.23 (m, 9 H), 7.05 (s, 1 H), 5.48-5.45 (m, 1 H), 5.14-5.10 (m, 2 H), 5.03-4.99 (m, 1 H), 4.61 (s, 2 H), 3.94-3.88 (m, 1 H), 2.42-2.34 (m, 1 H), 1.63 (d, J = 7.2 Hz, 3 H), 1.02-1.94 (m, 6H). LCMS (ESI): m/z 489.9 [M+Na$^+$].

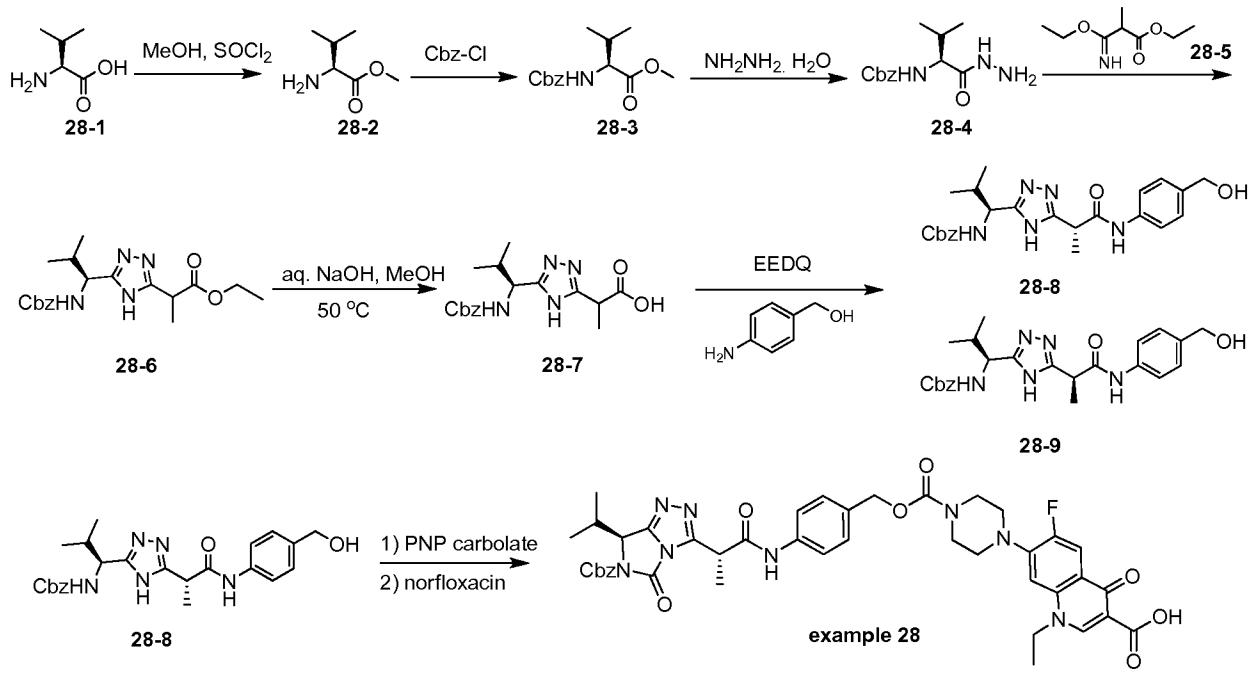
Step 6. To the solution of **27-6** (50 mg, 0.11 mmol) in dry DCM (2 mL) was added PNP carbonate (67 mg, 0.22 mmol) and DIPEA (28 mg). The mixture was heated at reflux for 16 h. After the solvent was removed, the residue was dissolved in dry DMF (5 mL). DIPEA (60 mg, 047 mmol) and norfloxacin (60 mg, 0.188 mmol) was added. After the mixture was stirred at r.t. for 1 h, solvent was removed and the residue was purified by prep-HPLC to afford **example 27**.

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.34 (s, 1 H), 10.17 (s, 1 H), 8.97 (s, 1 H), 8.10 (d, J = 8.4 Hz, 1 H), 7.95 (d, J = 8.4 Hz, 1 H), 7.60 (d, J = 8.4 Hz, 2 H), 7.36-7.31 (m, 7 H), 7.21 (d, J = 7.2 Hz, 1 H), 5.05 (s, 4 H), 4.66-4.56 (m, 3 H), 4.03-3.97 (m, 1 H), 3.61 (s, 4 H), 3.35 (s, 4 H), 2.20-2.14 (m, 1 H), 1.47-1.39 (m, 6 H), 0.87-0.80 (m, 6 H). LCMS (ESI): m/z 813.1, [M+H$^+$], 407.1 [M/2+H$^+$].

Example 28. 7-(4-((4-((R)-2-((S)-6-(benzyloxycarbonyl)-7-isopropyl-5-oxo-6,7-dihydro-5H-imidazo[5,1-c][1,2,4]triazol-3-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 28



Step 1. To the solution of **28-1** (12 g, 0.1 mol) in MeOH (200 mL) was added $SOCl_2$ (10 mL) in 10 min at 0°C. After the mixture was stirred at r.t. for 12 h, solvent was removed, to give the desired product **28-2**.

Step 2. To the solution of **28-2** (2.6 g, 5 mmol) in DCM (50 mL) was added TEA (2 mL) and stirred at 0 °C until the solution became clear. CbzCl (10.2 g, 6 mmol) was then added dropwise in 1 h and maintain the temperature below 5 °C. The mixture was then stirred at r.t. for another 10 h. Solution was taken up with DCM (100 mL) and washed with aq. HCl, aq. $NaHCO_3$ and water, organic layer was dried over anhydrous Na_2SO_4, and solvents were then evaporated to give **28-3**. LCMS (ESI): m/z 265.9 [$M+H^+$].

Step 3. To the solution of **28-3** (2.65 g, 10 mmol) in MeOH (25 mL) was added $NH_2NH_2.H_2O$ (5 mL, 80%). After the mixture was stirred at r.t. for 6 h, the solid was collected by filtration to give **28-4**.

^{1}H NMR (400 MHz, MeOD) δ 7.36-7.30 (m, 5 H), 5.07 (s, 2 H), 4.59 (s, 1 H), 3.84-3.82 (m, 1 H), 0.94-0.91 (m, 6 H).

Step 4. Compound **28-4** (2.65g, 10 mmol), **28-5** (4.2 g, 20.2 mmol) and dry MeOH (50 mL) was heated at 150°C in a sealed container for 24 h. Solvent was removed to the desired product **28-6**.

Step 5. To the solution of **28-6** (740 mg, 2 mmol) in EtOH (20 mL) and water (10 mL) was added NaOH (400 mg, 10mmol). After the mixture was stirred at 50°C for 1 h, solvent was removed, and the residue was purified by column to give **28-7**.

^{1}H NMR (400 MHz, MeOD) δ 7.32-7.26 (m, 5 H), 5.10-5.05 (m, 2 H), 4.61-4.59 (m, 1 H), 3.94-3.92 (m, 1 H), 2.16-2.14 (m, 1 H), 1.55-1.53 (m, 3 H), 0.99-0.82 (m, 6 H).

Step 6. To the solution of **28-7** (100 mg, 0.28 mmol) in dry DCM (5 mL) was added (4-aminophenyl)methanol (250 mg, 2 mmol) and EEDQ (247 mg, 1 mmol). After the mixture was stirred at 0°C for 2 h, solvent was removed, and the residue was purified by prep-HPLC and SFC to give **28-8** and **28-9**.

^{1}H NMR (400 MHz, MeOD) δ 7.57 (d, J = 8.4 Hz, 2 H), 7.35-7.30 (m, 7 H), 5.13-5.05 (m, 2 H), 4.63 (d, J = 7.6 Hz, 1 H), 4.57 (s, 2 H), 4.07-4.02 (m, 1 H), 2.23-2.17 (m, 1 H), 1.66 (d, J = 7.2 Hz, 3 H), 0.98 (d, J = 6.8 Hz, 3 H), 0.87 (d, J = 6.8 Hz, 3 H).

LCMS (ESI): m/z 452.0 [M+H$^+$].

^{1}H NMR (400 MHz, MeOD) δ 7.57 (d, J = 8.4 Hz, 2 H), 7.35-7.30 (m, 7 H), 5.13-5.05 (m, 2 H), 4.63 (d, J = 7.6 Hz, 1 H), 4.57 (s, 2 H), 4.00-3.99 (m, 1 H), 2.19-2.17 (m, 1 H), 1.66 (s, 3 H), 0.98 (d, J = 5.6 Hz, 3 H), 0.87 (d, J = 6.8 Hz, 3 H).

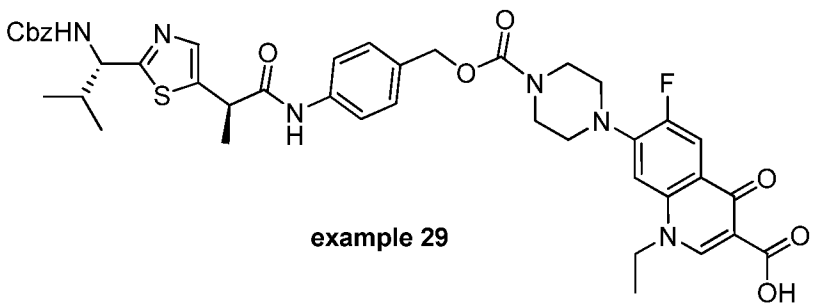
LCMS (ESI): m/z 473.9 [M+Na$^+$].

Step 7. To the solution of **28-8** (45 mg, 0.1 mmol) in dry DCM (30 mL) was added PNP carbonate (62 mg, 0.2 mmol) and DIPEA (1 mL). After the mixture was heated at reflux for 16 h, solvent was removed, and the residue was dissolved in DMF (5 mL). DIPEA (1.0 mL) and norfloxacin (65 mg, 0.2 mmol) were added. The mixture was stirred at r.t. for 1 h. After removal of the solvent, the residue was purified by prep-HPLC to give **example 28.**

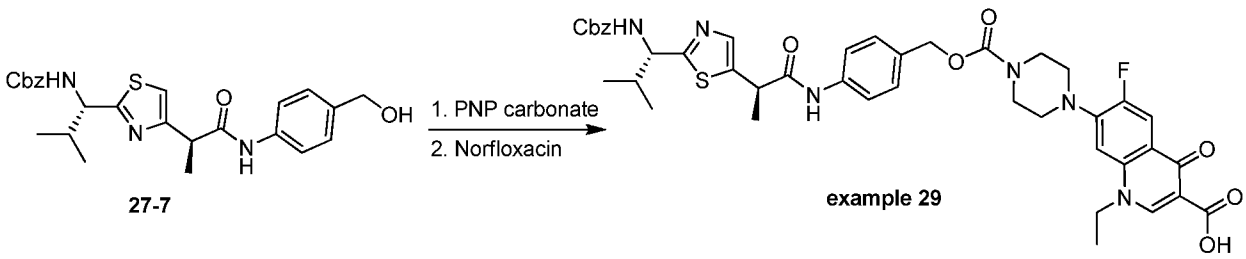
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (s, 1 H), 7.97-7.94 (m, 2 H), 7.48-7.23 (m, 10 H), 5.17 (s, 2 H), 5.04 (s, 2 H), 4.61-4.58 (m, 2 H), 4.50-4.40 (m, 1 H), 3.69-3.63 (m, 4 H), 3.40-3.35 (m, 1 H), 3.29-3.26 (m, 4 H), 2.09-2.08 (m, 1 H), 1.91-1.88 (m, 2 H), 1.41 (t, J = 7.2 Hz, 3 H), 0.96-0.89 (m, 6 H).

LCMS (ESI): 823.3 m/z [M+H$^+$].

Example 29. 7-(4-((4-((R)-2-(2-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)thiazol-5-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

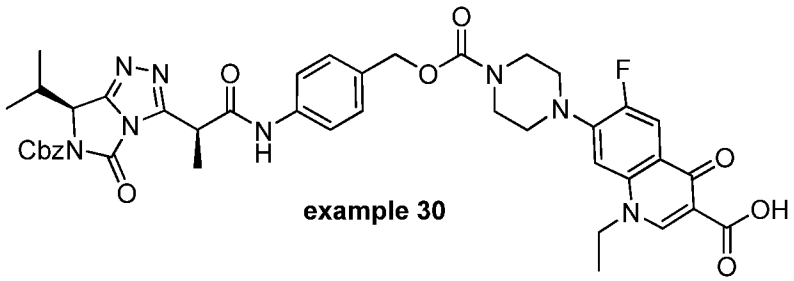


example 29

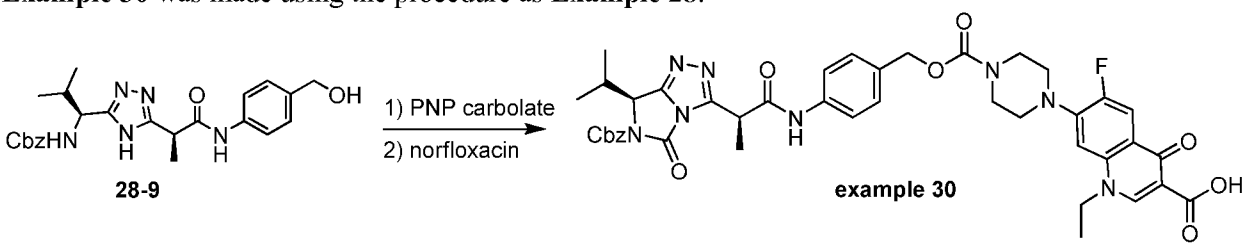


Example 29 was made using the procedure as **Example 27**.
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.33 (brs, 1 H), 10.17 (s, 1 H), 8.96 (s, 1 H), 8.10 (d, J = 8.4 Hz, 1 H), 7.95 (d, J = 8.4 Hz, 1 H), 7.60 (d, J = 8.4 Hz, 2 H), 7.36-7.31 (m, 7 H), 7.21 (d, J = 7.2 Hz, 1 H), 5.05 (s, 4 H), 4.66-4.56 (m, 3 H), 4.03-3.97 (m, 1 H), 3.61 (s, 4 H), 3.35 (s, 4 H), 2.20-2.14 (m, 1 H), 1.47-1.39 (m, 6 H), 0.87-0.80 (m, 6 H). LCMS (ESI): m/z 813.1, [M+H$^+$], 407.3 [M/2+H$^+$].

Example 30. 7-(4-((4-((S)-2-((S)-6-(benzyloxycarbonyl)-7-isopropyl-5-oxo-6,7-dihydro-5H-imidazo[5,1-c][1,2,4]triazol-3-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

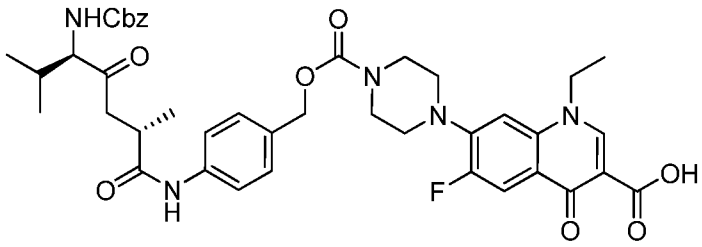


Example 30 was made using the procedure as **Example 28**.

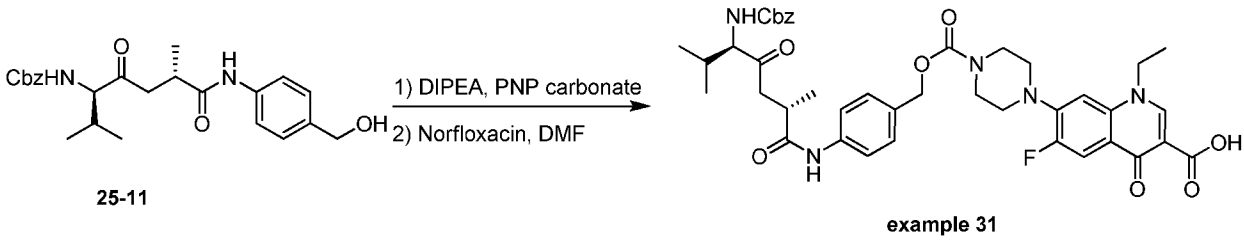


^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1 H), 8.14-7.93 (m, 2 H), 7.57-7.19 (m, 10 H), 5.19 (s, 2 H), 5.11-5.03 (m, 2 H), 4.62-4.56 (m, 2 H), 4.51-4.47 (m, 1 H), 3.67-3.57 (m, 4 H), 3.52-3.50 (m, 1 H), 3.40-3.35 (m, 4 H), 2.25-2.15 (m, 1 H), 1.90 (s, 2 H), 1.41 (t, J = 7.2 Hz, 3 H), 0.98-0.84 (m, 6 H).

Example 31: 7-(4-((4-((2S,5S)-5-(benzyloxycarbonylamino)-2,6-dimethyl-4-oxoheptanamido)benzyloxy) carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



example 31



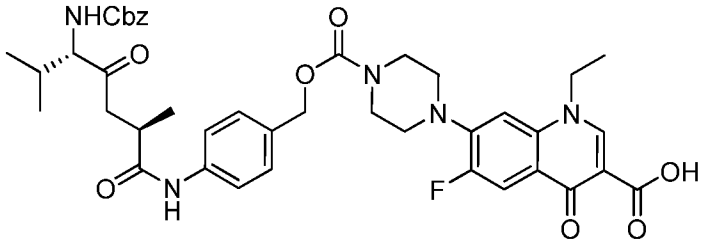
25-11

example 31

Step 1. Example 31 was made using the procedure as **Example 25**.

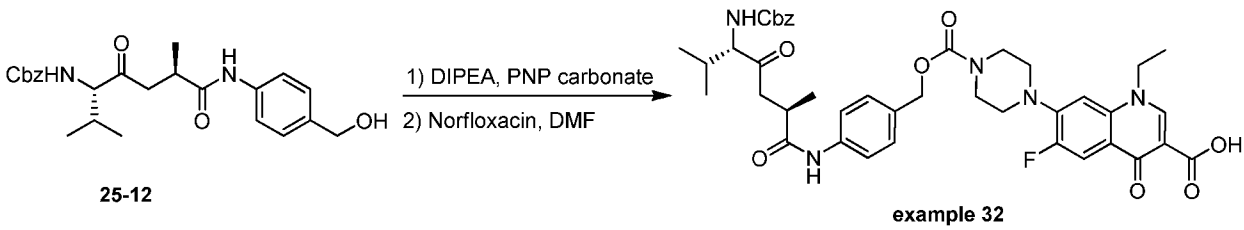
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.29 (s, 1 H), 9.98 (s, 1 H), 8.92 (s, 1 H), 7.90 (d, J = 13.2 Hz, 1 H), 7.57-7.54 (m, 3 H), 7.32-7.24 (m, 7 H), 7.17 (d, J = 7.6 Hz, 1 H), 5.05-4.97 (m, 4 H), 4.56-4.54 (m, 2 H), 3.95-3.92 (m, 1 H), 3.58 (s, 4 H), 3.26 (s, 4 H), 2.93-2.83 (m, 2 H), 2.57-2.52 (m, 1 H), 2.16-2.08 (m, 1 H), 1.45-1.35 (m, 3 H), 1.07 (d, J = 6.4 Hz, 3 H), 0.85 (d, J = 8.2 Hz, 3 H), 0.75 (d, J = 8.0 Hz, 3 H). LCMS (ESI): m/z 772.1 [M+H$^+$].

Example 32: 7-(4-((4-((2R,5R)-5-(benzyloxycarbonylamino)-2,6-dimethyl-4-oxoheptanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



example 32

Step 1. Example 32 was made using the procedure as **Example 25**, with the intermediate from the synthesis of **Example 25**.



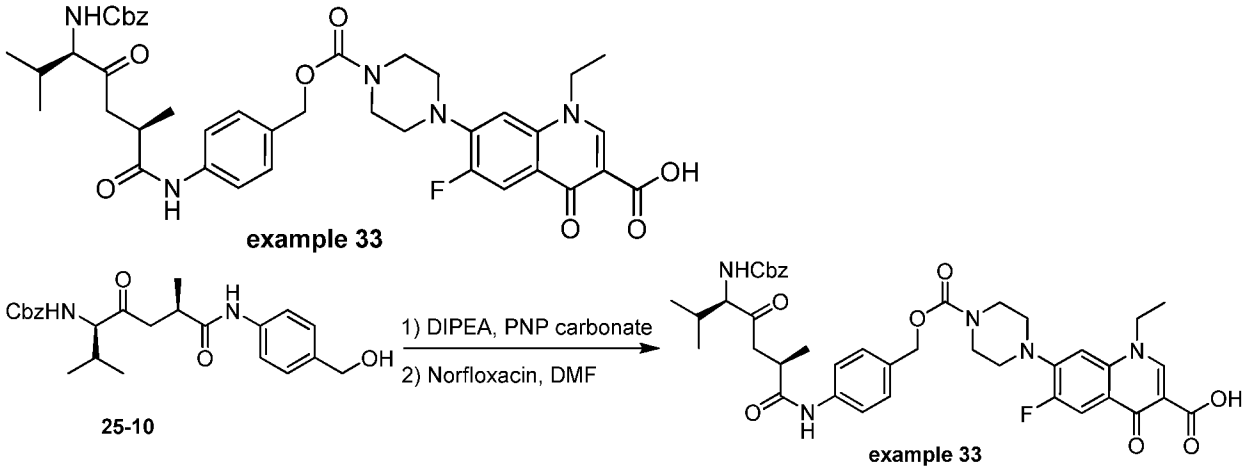
25-12

example 32

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.30 (s, 1 H), 10.03 (s, 1 H), 8.97 (s, 1 H), 7.95 (d, J = 12.8 Hz, 1 H), 7.74 (d, J = 8.4 Hz, 1 H), 7.58 (d, J = 8.4 Hz, 2 H) 7.37-7.31 (m, 7 H), 7.21 (d, J = 7.2 Hz, 1 H) 5.09-5.02 (m, 4 H), 4.60-4.56 (m, 2 H), 3.84-3.80 (m, 1 H), 3.61 (s, 4 H), 3.34 (s, 4 H), 2.92-

2.85 (m, 2 H), 2.12-2.08 (m, 1 H), 2.05 (s, 1 H), 1.42-1.39 (m, 3 H), 1.07 (d, J = 6.8 Hz, 3 H), 0.82 (d, J = 6.8 Hz, 6 H). LCMS (ESI): m/z 772.4 [M+H$^+$].

Example 33: 7-(4-((4-((2R,5S)-5-(benzyloxycarbonylamino)-2,6-dimethyl-4-oxoheptanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

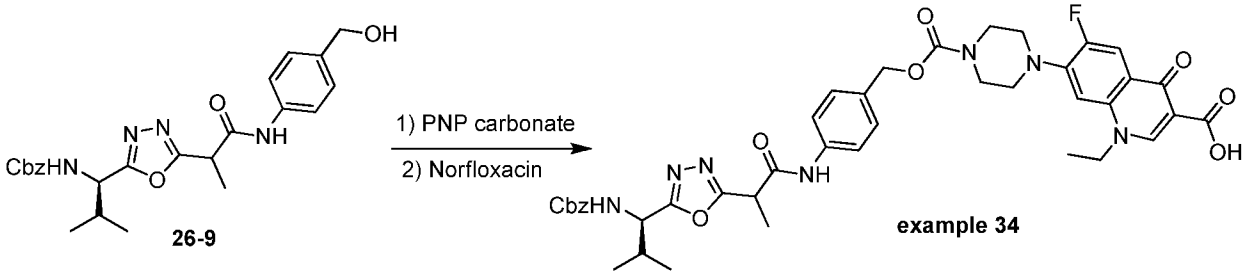


Step 1. Example 33 was made using the procedure as **Example 25**, with the intermediate from the synthesis of **Example 25**.

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.34 (s, 1 H), 10.02 (s, 1 H), 8.97 (s, 1 H), 7.95 (d, J = 12.8 Hz, 1 H), 7.58 (d, J = 8.4 Hz, 3 H), 7.36-7.29 (m, 7 H), 7.21 (d, J = 7.2 Hz, 1 H), 5.08-5.00 (m, 4 H), 4.61-4.56 (m, 2 H), 3.99-3.95 (m, 1 H), 3.61 (s, 4 H), 3.31 (s, 4 H), 2.96-2.85 (m, 2 H), 2.50-2.47 (m, 1 H), 2.19-2.13 (m, 1 H), 1.42-1.39 (m, 3 H), 1.08 (d, J = 6.0 Hz, 3 H), 0.90 (d, J = 6.0 Hz, 3 H), 0.82 (d, J = 6.4 Hz, 3 H). LCMS (ESI): m/z 772.0 [M+H$^+$].

Example 34. 7-(4-((4-(2-(5-((R)-1-(benzyloxycarbonylamino)-2-methylpropyl)-1,3,4-oxadiazol-2-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

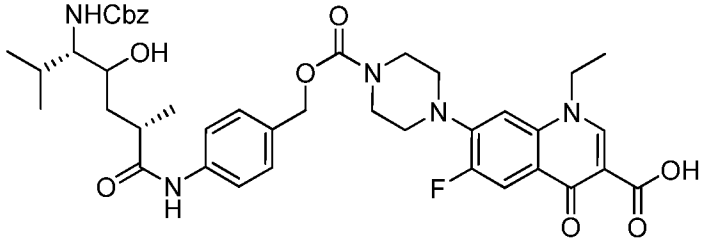
Example 34 was made using the procedure as **Example 26**, with the intermediate from the synthesis of **Example 26**.



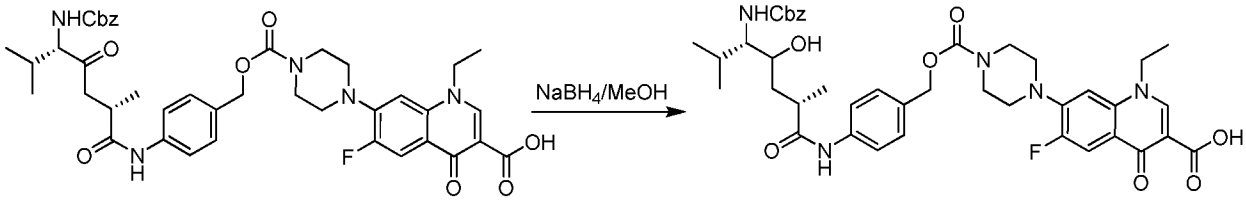
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.32 (s, 1 H), 10.46 (s, 1 H), 8.95 (s, 1 H), 8.09 (s, 1 H), 7.94 (d, J = 13.2 Hz, 1 H), 7.58 (d, J = 8.0 Hz, 2 H), 7.36-7.33 (m, 7 H), 7.20 (d, J = 6.4 Hz, 1 H), 5.04 (d, J =15.2 Hz, 4 H), 4.62-4.57 (m, 3 H), 4.22-4.21 (m, 1 H), 3.61 (s, 4 H), 3.35 (s, 4 H), 2.13-2.11 (m, 1 H), 1.58 (d, J = 5.6 Hz, 3 H), 1.40 (t, J = 6.8 Hz, 3 H), 0.93 (d, J = 6.0 Hz, 3 H), 0.81 (d, J =6.4 Hz, 3H).

LCMS (ESI): m/z 798.1 [M+H$^+$].

Example 35: 7-(4-((4-((2S,5S)-5-(benzyloxycarbonylamino)-4-hydroxy-2,6-dimethylheptanamido) benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



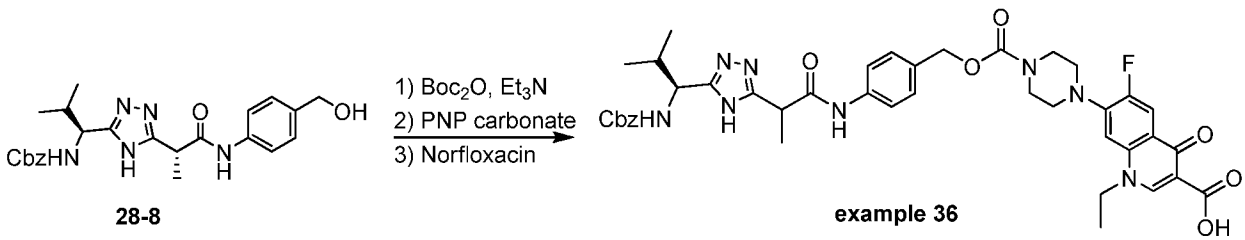
example 35



example 25 **example 35**

Step 1. To the solution of **example 25** (120 mg, 0.156 mmol) in dry CH_3OH (20 mL) was added $NaBH_4$ (6 mg, 0.158 mmol) in ice bath. After the mixture was stirred at r.t. for 2 h, the solvent was removed, and the residue was purified by prep-HPLC to give **example 35** as a solid (40 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.33 (s, 1 H), 9.88 (s, 1 H), 8.96 (s, 1 H), 7.94 (d, J = 12.8 Hz, 1 H), 7.62 (d, J = 8.4 Hz, 2 H), 7.37-7.20 (m, 8 H), 6.95 (d, J = 10.0 Hz, 1 H), 5.05-4.99 (m, 4 H), 4.59-4.57 (m, 3 H), 3.61 (s, 4 H), 3.47-3.42 (m, 1 H), 3.30 (s, 4 H), 2.72-2.66 (m, 1 H) 2.07-2.04 (m, 1 H), 1.59-1.51 (m, 2 H), 1.45-1.35 (m, 3 H), 1.05 (d, J = 8.0 Hz, 3 H), 0.85-0.75 (m, 6 H). LCMS (ESI): m/z 387.7 [M/2+H$^+$].

Example 36. 7-(4-((4-(2-(5-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)-4H-1,2,4-triazol-3-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



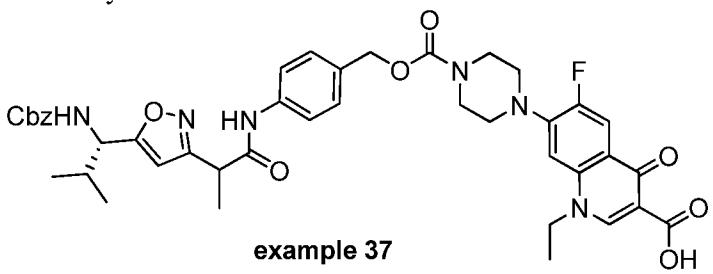
28-8 **example 36**

Step 1. To a solution of **28-8** (80 mg, 0.177 mmol) in DCM (3 mL) was added Boc_2O (232 mg, 1.062 mmol) and Et_3N (107 mg, 1.062mmol) at 15°C. After the solution was stirred at 15°C for 16 h, DCM was removed under reduced pressure and the residue was washed with petroleum ether (5 mL × 3). The resulting solid was dissolved in DCM (3 mL), and PNP carbonate (108 mg, 0.354

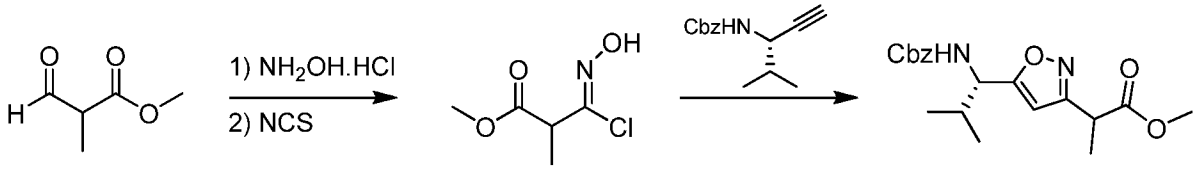
mmol) DIPEA (92 mg, 0.708 mmol) were added. After the solution was stirred at 15°C for 2 h, solvent was removed under reduced pressure and the residue was dissolved DMF (3 mL) and norfloxacin (170 mg, 0.531 mmol) and DIPEA (92 mg, 0.708 mmol) were added. It was stirred at 15°C for 1 h. After the solvent was removed, the residue was purified by prep-HPLC to give a mixture of **example 36** (37 mg, 26%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (s, 1 H), 7.95 (d, J = 13.2 Hz, 1 H), 7.60 (d, J = 8.4 Hz, 2 H), 7.34-7.20 (m, 8 H), 5.06-5.00 (m, 4 H), 4.59-4.41 (m, 3 H), 4.08-3.82 (m, 1 H), 3.61 (s, 4 H), 3.29 (s, 4 H), 2.15-2.07 (m, 1 H), 1.50-1.49 (m, 3 H), 1.40 (t, J = 7.2 Hz, 3 H), 0.88-0.87 (m, 3 H), 0.75-0.73 (m, 3 H).

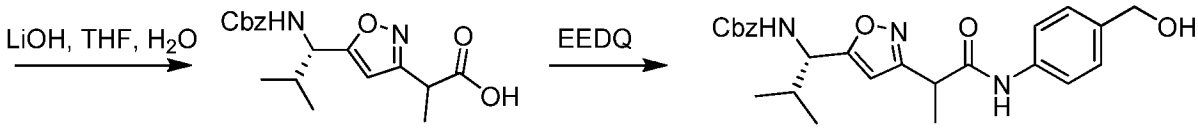
Example 37. 7-(4-((4-(2-(5-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)isoxazol-3-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



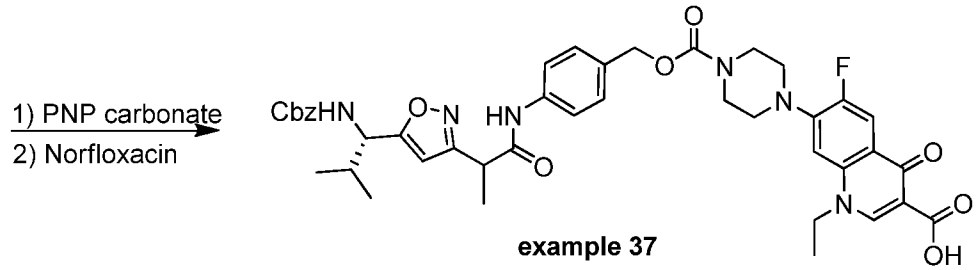
example 37



37-1 37-2 37-3



37-4 37-5



example 37

Step 1. A mixture of **37-1** (5 g, 43.1 mmol) and hydroxylamine hydrochloride (3.02 g, 43.1 mmol) in pyridine (50 mL) was stirred at 70°C for 16 h. The solvent was removed and residue was dissolved in DMF (30 mL) and NCS (5.73 g, 43.1 mmol) was added at 0°C. The mixture was stirred at r.t. for 16 h. After the solvent was removed, the residue (**37-2**) was used directly in next step without purification.

Step 2. To a mixture of **37-2** (5 g, 30.2 mmol), $CuSO_4 \cdot 5H_2O$ (250 mg, 1 mmol), sodium ascorbate (198 mg, 1 mmol), Na_2CO_3 (1.1 g, 10.4 mmol) in a mixture of *t*-BuOH and H_2O (20 mL/20 mL) was added (1-isopropyl-prop-2-ynyl)-carbamic acid benzyl ester (1.2 g, 5.2 mmol) at 0°C. After the mixture was stirred at 60°C for 16 h, it was extracted with EtOAc (80 mL × 3). The organic layer was washed with brine (60 mL), dried over Na_2SO_4, concentrated in vacuo and purified by prep-HPLC to give **37-3**.

LCMS (ESI): m/z 361.1 [$M+H^+$]

Step 3. To the solution of **37-3** (150 mg, 0.417 mmol) in a mixture of THF and H_2O (8 mL/4 mL) was added lithium hydroxide hydrate (145 mg, 4.17 mmol) and the mixture was stirred at r.t. for 16 h. The solvent was removed and the mixture was washed with EtOAc. After it was acidified, it was extracted with EtOAc (30 mL×3). The organic layer was washed with brine (30 mL), dried over Na_2SO_4, concentrated to give the crude product **37-4**, which was used in the next step without further purification (123 mg, 84.8%).

LCMS (ESI): m/z 347.2 [$M+H^+$].

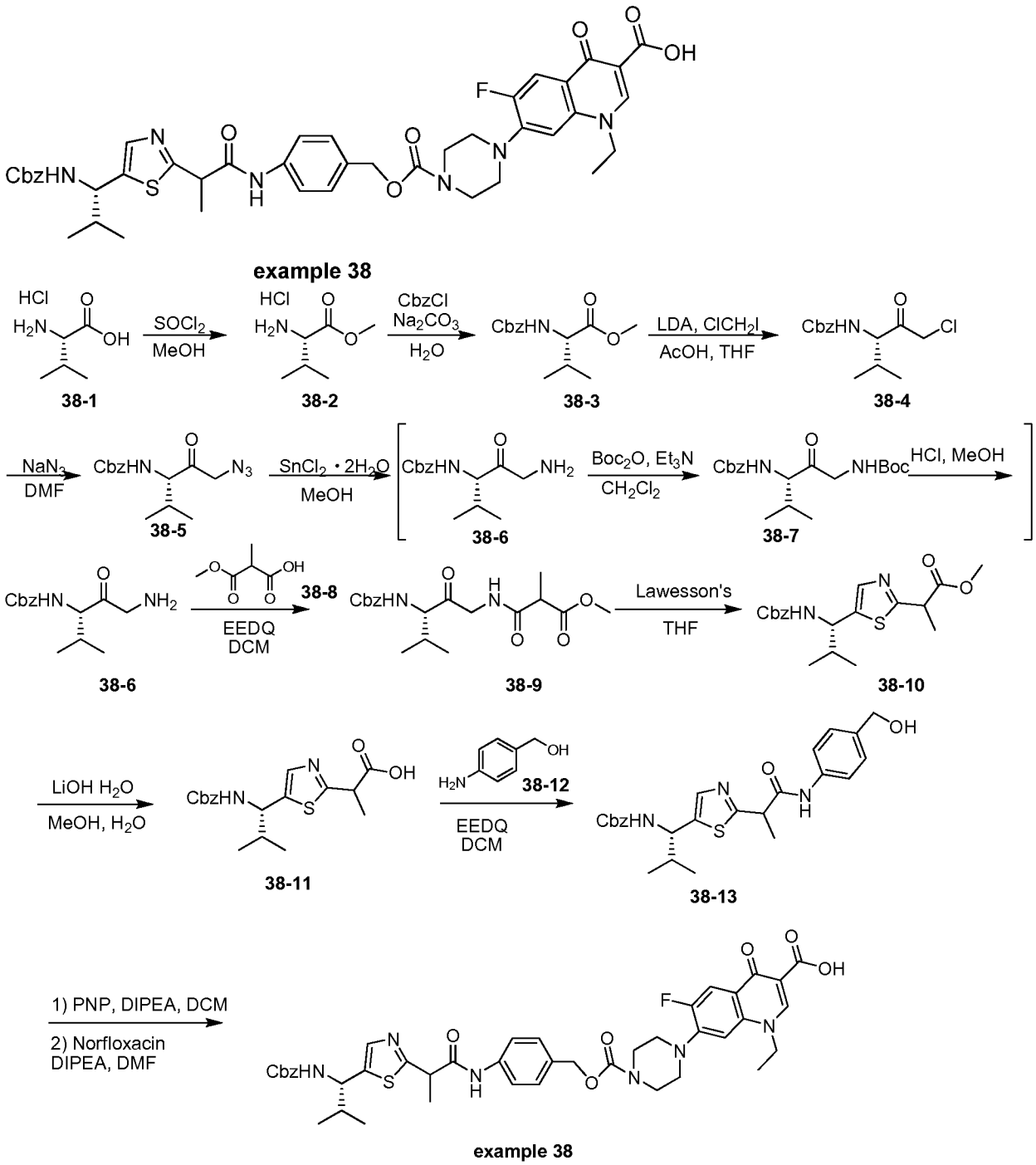
Step 4. To a mixture of **37-4** (123 mg, 0.35 mmol) and (4-aminophenyl)methanol (87 mg, 0.71 mmol) in CH_2Cl_2 (5 mL) was added EEDQ (174 mg, 0.71 mmol) at 0°C. The mixture was stirred at r.t. for 16 h and extracted with EtOAc (30 mL × 3). The organic layer was washed with brine (30 mL), dried over Na_2SO_4, concentrated and purified by prep-HPLC to give **37-5** (63 mg, 39.4%).

^{1}H NMR (400 MHz, MeOD) δ 7.44-7.42 (m, 2 H), 7.23-7.19 (m, 7 H), 6.23 (d, J = 2.8 Hz, 1 H), 4.99-4.98 (m, 2 H), 4.58-4.54 (m, 1 H), 4.46 (s, 2 H), 3.90-3.85 (m, 1 H), 2.10-2.01 (m, 1 H), 1.45-1.43 (m, 3 H), 089-0.79 (m, 6 H). LCMS (ESI): m/z 434.1 [M-OH].

Step 5. To a solution of **37-5** (40 mg, 0.089 mmol) in dry DCM (2 mL) was added PNP carbonate (57 mg, 0.177 mmol) and DIPEA (23 mg). The mixture was heated at reflux for 16 h. After the solvent was removed, the residue was dissolved in DMF (2 mL), DIPEA (23 mg) and norfloxacin (54 mg, 0.178 mmol) was added. The mixture was stirred at r.t. for 1 h. After the solvent was removed, the residue was purified by prep-HPLC to give **example 37**.

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.30 (s, 1 H), 10.28 (s, 1 H), 8.93 (s, 1 H), 7.95-7.89 (m, 2 H), 7.57 (d, J = 8.8 Hz, 2 H), 7.35-7.12 (m, 8 H), 6.32 (d, J = 4.4 Hz, 1 H), 5.02 (d, J = 11.6 Hz, 4 H), 4.56-4.52 (m, 3 H), 3.97-3.91 (m, 1 H), 3.57 (s, 4 H), 3.29 (s, 4 H), 2.03-1.96 (m, 1 H), 1.41-1.35 (m, 6 H), 0.85-0.75 (m, 6 H). LCMS (ESI): m/z 797.3 [$M+H^+$], 399.3 [$M/2+H^+$].

Example 38. 7-(4-((4-(2-(5-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)thiazol-2-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



Step 1. To a solution of **38-1** (40 g, 0.34 mol) in MeOH (200 mL) at 0°C was added the $SOCl_2$ (28 mL, 0.38 mol) under N_2. After the mixture was stirred at r.t. for 12 h, solvent was removed to give the crude product **38-2**.

Step 2. CbzCl (53 mL, 0.37 mol) was added dropwise over 20 min to a mixture of **38-2** (57 g, 0.34 mol) and Na_2CO_3 (72 g, 0.68 mol) in water (300 mL). After the mixture was stirred at r.t. for 12 h, it was extracted with EtOAc (500 mL × 3). The combined organic layers were dried over Na_2SO_4 and concentrated to give the crude product, which was purified by column chromatography on silica gel (PE: EtOAc = 5:1) to give compound **38-3**.

Step 3. To the solution of compound **38-3** (10 g, 37.7 mmol) in THF (20 mL) was added $ClCH_2I$ (26.6 g, 151 mmol). After it was cooled to -78°C, LDA was added slowly in 2 h. After it was stirred at -78°C for 30 min under N_2, HOAc (21 mL, 377 mmol) in THF was added under -70°C. It was warmed to r.t., and EtOAc (30 mL) was added and the mixture was poured to brine. It was extracted with EtOAc (80 mL × 3), dried over Na_2SO_4, concentrated, and purified by column chromatography on silica gel (PE: EtOAc= 5:1) to give compound **38-4**.

Step 4. After a mixture of compound **38-4** (5 g, 17.6 mmol) and NaN_3 (1.8 g, 26.4 mmol) was stirred at r.t. for 1 h, it was poured to water and extracted with EtOAc (150 mL × 3). The combined organic layers were dried over Na_2SO_4 and concentrated under pressure reduce to give the crude product of compound **38-5**.

Step 5. To the solution of compound **38-5** (4.84 g, 16.7 mmol) in MeOH (50 mL) was added $SnCl·2H_2O$ (7.5 g, 33.3 mmol) and the mixture was stirred at r.t. for 2 h. After the solvent was removed; the residue (**38-6**) was used directly in the next step.

Step 6. A mixture of compound **38-6** (3.75 g, 14.2 mmol), $NaHCO_3$ (11.93 g, 142 mmol), Boc_2O (3.41 g, 15.6 mmol) in H_2O (15 mL) and dioxane (15 mL) was stirred at r.t. for 1 h. The mixture was extracted with EtOAc (150 mL × 3), dried over Na_2SO_4, concentrated and purified by column chromatography on silica gel (PE: EtOAc = 5:1 to 2:1) to give compound **38-7**. After a mixture of compound **38-7** (1.6 g, 4.43 mmol) and 4 M HCl in MeOH (20 mL) was stirred at r.t. for 1 h, it was evaporated to give compound **38-6**, which was used for the next step without further purification.

Step 7. To the solution of compound **38-6** (1.17 g, 4.43 mmol) and compound **38-8** (643 mg, 4.87 mmol) in DCM (20 mL) was added EEDQ (1.31 g, 5.31 mmol). After the mixture was stirred at r.t. for 1 h, solvent was removed, and the residue was purified by prep-HPLC to give compound **38-9**.

Step 8. To a solution of compound **38-9** (1 g, 2.64 mmol) in THF (20 mL) under N_2 was added Lawesson's reagent (1.18 g, 2.91 mmol). After the mixture was stirred at 60°C for 1 h, solvent was removed, and the residue was purified by prep-HPLC to give compound **38-10**.

Step 9. To a solution of compound **38-10** (630 mg, 1.67 mmol) in MeOH (5 mL) and H_2O (1 mL) was added $LiOH·H_2O$ (105 mg, 2.51 mmol). After the suspension was stirred at r.t. for 1 h, it was acidified with conc. HCl to pH = 6. The mixture was extracted with (50 mL × 3) and concentrated under reduced pressure to give compound **38-11**.

Step 10. A mixture of compound **38-11** (438 mg, 1.21 mmol), compound **38-12** (446 mg, 3.62 mmol), HATU (690 mg, 1.82 mmol), DIPEA (468 mg, 3.62 mmol) and EEDQ (895 mg, 3.62 mmol) in DCM (10 mL) was stirred at r.t. for 2 h. After the solvent was removed, it was purified by prep-HPLC to give **38-13** (200 mg, 35.4%).

^{1}H NMR (400 MHz, MeOD) δ 7.54 (d, J = 8.4 Hz, 3 H), 7.31-7.25 (m, 7 H), 5.06-5.04 (m, 2 H), 4.64 (d, J = 8.0 Hz, 1 H), 4.55 (s, 2 H), 4.21 (m, 1 H), 2.14-2.04 (m, 1 H), 1.63 (d, J = 6.8 Hz, 3 H), 0.99 (d, J = 6.8 Hz, 3 H), 0.89-0.86 (m, 3 H).

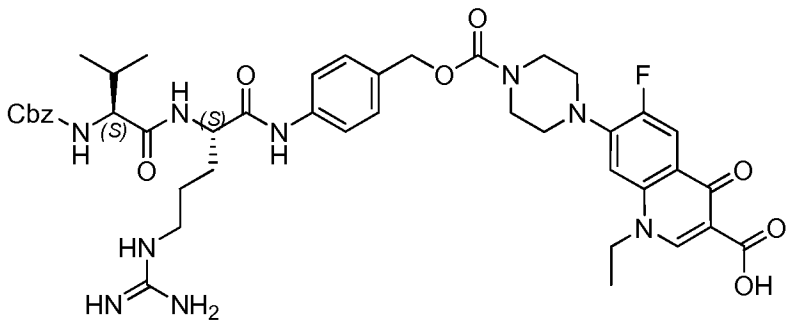
LCMS (ESI): m/z 467.9 [M+H$^+$].

Step 11. A mixture of **38-13** (50 mg, 0.107 mmol), PNP (65 mg, 0.214 mmol) and DIPEA (41 mg, 0.321 mmol) in DCM (5 mL) was stirred at 50°C for 12 h. It was concentrated and added to a mixture of norfloxacin (102 mg, 0.321 mmol) and DIPEA (41 mg, 0.321 mmol) in DMF (5 mL). After it was stirred at r.t. for 2 h, solvent was removed and the residue was purified by prep-HPLC to give **example 38** (24 mg, 27.6%).

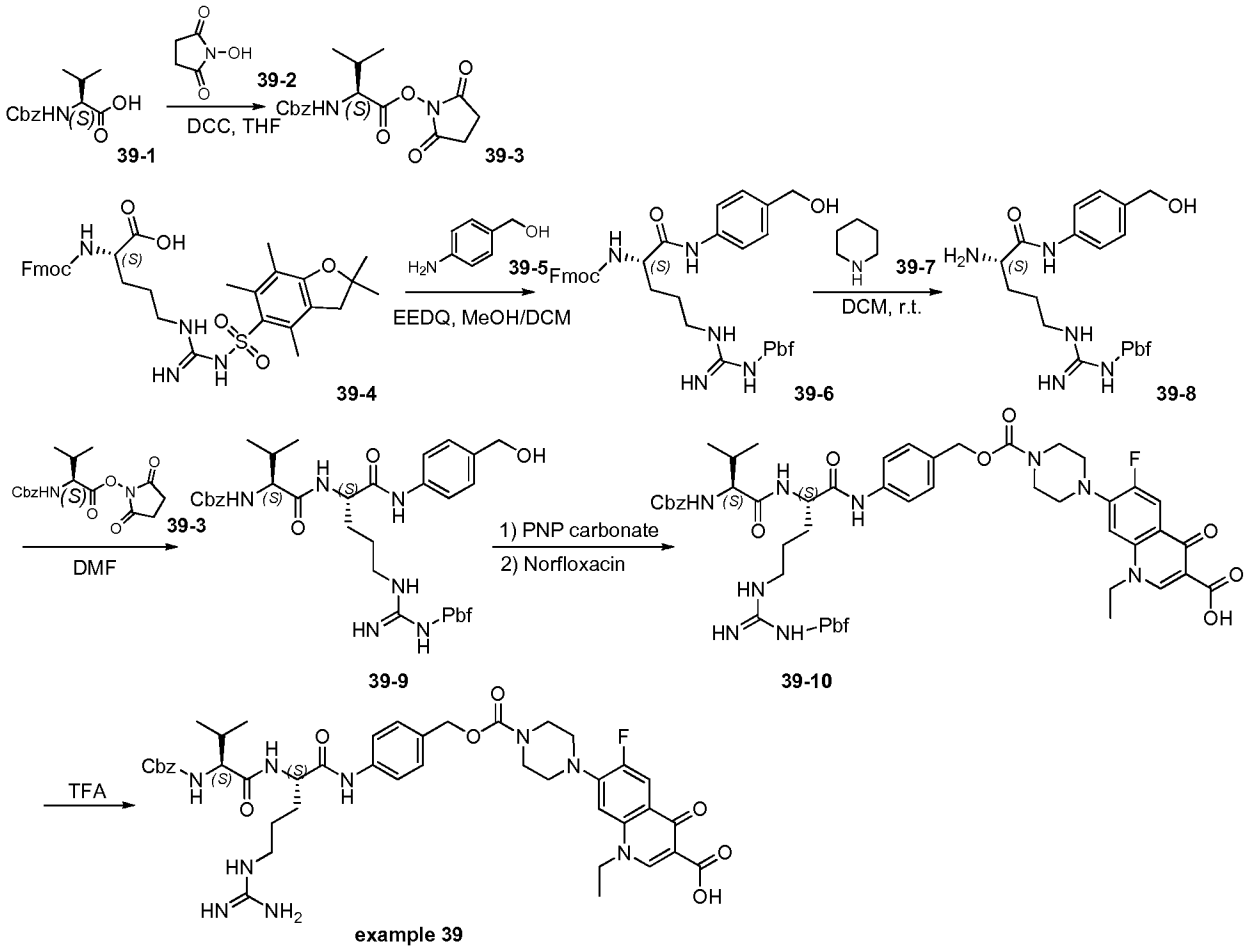
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.32 (s, 1 H), 10.40 (s, 1 H), 8.96 (s, 1 H), 7.96-7.88 (m, 2 H), 7.61 (d, J = 8.4 Hz, 2 H), 7.51 (s, 1 H), 7.36-7.20 (m, 8 H), 5.07 (s, 2 H), 5.02-5.00 (m, 2 H), 4.59-4.55 (m, 3 H), 4.24-4.22 (m, 1 H), 3.61 (s, 4 H), 3.32 (s, 4 H), 1.97-1.92 (m, 1 H), 1.52 (d, J = 6.8 Hz, 3 H), 1.40 (t, J = 6.8 Hz, 3 H), 0.92 (d, J = 6.0 Hz, 3 H), 0.79-0.77 (m, 3 H).

LCMS (ESI): m/z 813.2 [M+H$^+$].

Example 39. 7-(4-((4-((S)-2-((S)-2-(benzyloxycarbonylamino)-3-methylbutanamido)-5-guanidinopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 39



Step 1. To a stirred solution of compound **39-1** (1 g, 4 mmol), compound **39-2** (459 mg, 4 mmol) in THF (20 mL) was added DCC (908 mg, 4.4 mmol) at 0°C. The reaction mixture was stirred at r.t. for 16 h. The mixture was filtered and the filtrate was concentrated to give compound **39-3** (Yield: 90 %).

Step 2. To a solution of compound **39-4** (2 g, 3.1 mmol) in DCM / MeOH (20 mL / 20 mL) were added 4-amino-phenyl-methanol (**39-60**) (570 mg, 4.6 mmol) and EEDQ (1.532 mg, 6.2 mmol). The mixture was stirred at r.t. for 16 h and purified by column chromatography (PE/EtOAc = 1/3) to give compound **39-6** (2 g, Yield: 86 %).

Step 3. To a solution of compound **39-6** (2 g, 2.65 mmol) in DCM (50 mL) was added compound **39-7** (1.3 mL, 13.3 mmol) at r.t. After the mixture was stirred at r.t. for 16 h, it was concentrated and washed with MTBE, and filtrated to give compound **39-8** (Yield: 80 %).

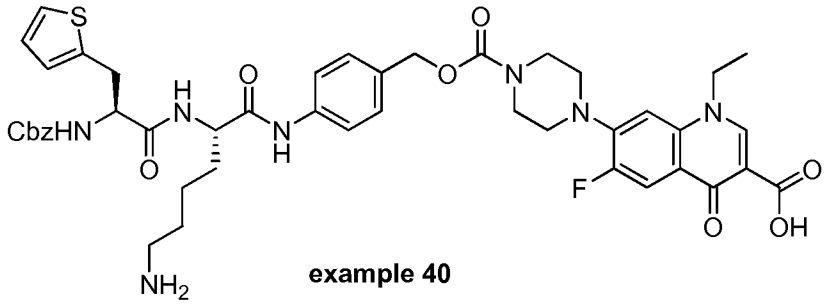
Step 4. To a solution of compound **39-8** (640 mg, 1.2 mmol) in DME (10 mL) was added a solution of compound **39-3** (640 mg, 1.8 mmol) and $NaHCO_3$ (304 mg, 3.6 mmol) in water (10 mL). After the mixture was stirred at r.t. for 16 h, it was washed with EtOAc and acidified to pH 3 with 10% HCl. The resulting suspension was extracted with EtOAc. The combined organic layer was concentrated and purified by column chromatography (PE/EtOAc=1/2) to give **39-9** (510 mg, Yield: 55 %).

Step 5. To a solution of compound **39-9** (200 mg, 0.26 mmol), PNP carbonate (158 mg, 0.52 mmol) in DMF (4 mL) was added DIPEA (101 mg, 0.78 mmol) at 0°C. The mixture was stirred at r.t. for 16 h. To the mixture was added norfloxacin (170 mg, 0.52 mmol) at r.t. The mixture was stirred at r.t. for 1 h and was purified by prep-HPLC and SFC to give Compound **39-10** (Yield: 30 % over two steps).

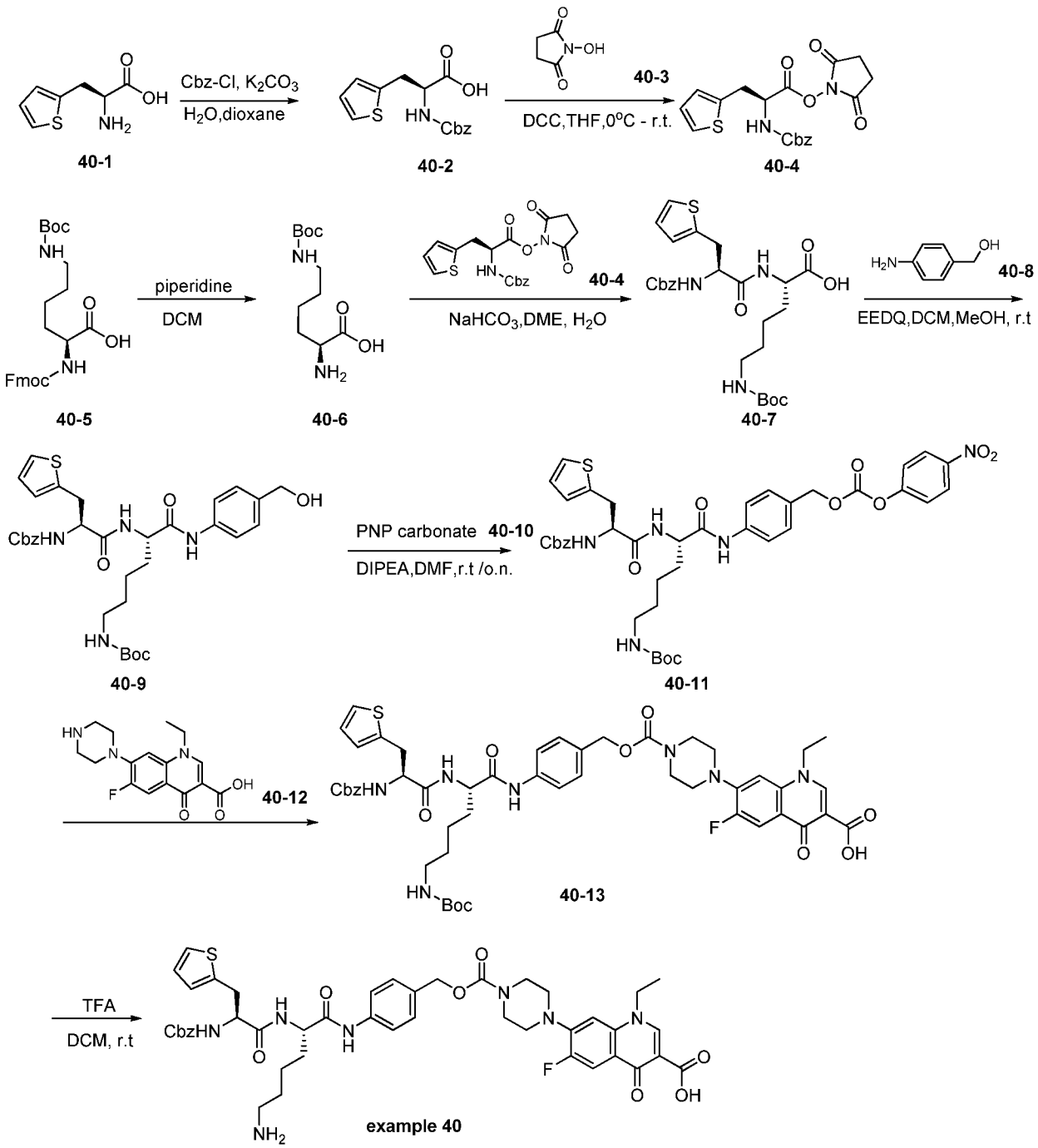
Step 6. To the compound **39-10** (100 mg, 0.1 mmol) was added a mixture of TFA and DCM (TFA/DCM=1/5) at 0 °C. After the mixture was stirred at r.t. for 3 h, it was basified to pH = 9 by $NH_3.H_2O$. The residue was purified by prep-HPLC and SFC to give **example 39** (13.8 mg, 15 %). LCMS: (5-95, AB, 1.5 min, ESI), 0.826 min, MS = 858.2[M+1]

^{1}H NMR Methanol-d_4 400 MHz, δ 8.80 (s, 1 H), 8.55 (s, 1 H), 7.98 (d, J = 13.2 Hz, 1 H), 7.58 (s, 2 H), 7.36 - 7.27 (m, 9 H),7.15 (m, 1H), 5.11 (d, J = 16.4 Hz, 4 H), 4.57 - 4.50 (m, 4 H), 3.94 (d, J = 7.2 Hz, 1 H), 3.71 (m, 4 H), 3.22 (s, 2 H), 2.07 (d, J = 6.8 Hz, 1 H), 1.95 (s, 1 H), 1.83 - 1.79 (m, 1 H), 1.70 (d, J = 1.6 Hz, 2 H), 1.51 (s, 3 H), 0.97 - 0.96 (m, 6 H).

Example 40. 7-(4-((4-((S)-6-amino-2-((S)-2-(benzyloxycarbonylamino)-3-(thiophen-2-yl)propanamido)hexanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 40



Step 1. To the mixture of compound **40-1** (1 g, 5.85 mmol) in H_2O (6 mL) and dioxane (9 mL) was added K_2CO_3 (2.02 g, 14.63 mmol), and Cbz-Cl (1.2 g, 7.01 mmol) at 0°C. The reaction mixture was stirred at r.t. for 2 h. The mixture was washed with EtOAc (20 mL). The aqueous phase was acidified with 1N HCl to pH = 3 and was extracted with EtOAc (25 ml x 2), the organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude compound **40-2** as an oil (1.5 g, yield: 84.3%).

LCMS: (10-80, AB, 2 min, ESI), 0.954 min, MS = 305.8[M+1], 327.8[M+Na^+]

^{1}H NMR DMSO-d_6 400 MHz δ 7.65 - 7.63 (d, J = 8.0 Hz, 1H), 7.37 - 7.29 (m, 6 H), 6.94 - 6.90 (m, 2H), 5.01 (s, 2 H), 4.2 - 4.1 (m, 1 H), 3.31 - 3.27 (m, 1H), 3.12 - 3.06 (m, 1 H).

Step 2. To a solution of compound **40-2** (2.0 g, 6.55 mmol), compound **40-3** (829.15 mg, 7.2 mmol) in dry THF (25 mL) was added DCC (1.49 g, 7.21 mmol) at 0°C. The mixture was stirred at r.t. for 16 h under N_2. The mixture was filtered and the filtrate was concentrated to give crude compound **40-4** which was used for next step directly without further purification (2.2 g, Yield: 84.6%).

LCMS: (10-80, AB, 2 min, ESI), 1.040 min, MS = 424.8[M+Na$^+$]

Step 3. To a stirred solution of compound **40-5** (5 g, 10.7 mmol) in DCM (80 mL) was added piperidine (4.5 g, 52.8 mmol) dropwise via syringe at r.t. After the mixture was stirred at r.t. for 30 min, it was concentrated under reduced pressure, and the residue was partitioned between EtOAc (30 mL) and H_2O (50 ml). The aqueous phase was concentrated to give crude compound **40-6** (2.0 g, 76%) as white solid which was used in the next step directly.

Step 4. To a solution of compound **40-4** (980 mg, 2.44 mmol) in DME (10 mL) was added a solution of compound **40-6** (500 mg, 2.03 mmol) and $NaHCO_3$ (374 mg, 4.46 mmol) in water (10 mL). After the mixture was stirred at r.t. for 16 h, it was washed with EtOAc (20 mL) and the aqueous phase was acidified to pH=3 with 15% citric acid solution. The resulting suspension was extracted with EtOAc (30 mL x 2). The organic layer was purified by prep-HPLC to give compound **40-7** as white solid (200 mg, 18.4%).

LCMS: (10-80, AB, 2 min, ESI), 1.123 min, MS = 434.1[M+1-Boc$^+$], 556.1[M+Na$^+$]

Step 5. To a solution of compound **40-7** (300 mg, 0.563 mmol) in DCM / MeOH (20 mL / 10 mL) were added 4-amino-phenyl-methanol (**40-8**) (103.9 mg, 0.845 mmol) and EEDQ (208.82 mg, 0.845 mmol). After the mixture was stirred at r.t. for 16 h, it was concentrated. The residue was purified by column chromatography (PE : EtOAc = 2:3) to give compound **40-9** as white solid (260 mg, Yield: 72.3 %).

LCMS: (5-95, AB, 1.5 min, ESI), 0.880 min, MS = 539.0[M+1-Boc$^+$], 661.0[M+Na$^+$]

^{1}H NMR $CDCl_3$ 400 MHz, δ 8.51 (s, 1 H), 7.44 (d, J = 8 Hz, 2 H), 7.24 - 7.19 (m, 6 H), 7.05 - 7.03 (m, 1 H), 6.78 - 6.73 (m, 3 H), 5.52 (d, J = 3.2 Hz, 1 H), 5.03 (s, 2 H), 4.56 (s, 2 H), 4.46 - 4.39 (m, 2 H), 3.25 (d, J = 5.6 Hz, 2 H), 2.98 (s, 2 H), 1.87 - 1.83 (m, 1 H), 1.39 (d, J = 7.6 Hz, 2 H), 1.35 (s, 9 H), 1.25 - 1.17 (m, 2 H).

Step 6. To a solution of compound **40-9** (250 mg, 0.39 mmol) in dry DMF (5 mL) was added PNP carbonate (237.14 mg, 0.78 mmol) and DIPEA (251.6 mg, 1.95 mmol) at 0°C . The mixture was stirred at r.t. for 16 h. The mixture (**40-11**) was used for next step directly without further purification.

Step 7. To the mixture of **40-11** was added norfloxacin (249.1 mg, 0.78 mmol) at r.t. After the mixture was stirred at r.t. for 1 h, it was purified by prep-HPLC to give crude compound **40-13** (220 mg Yield: 57.3 %).

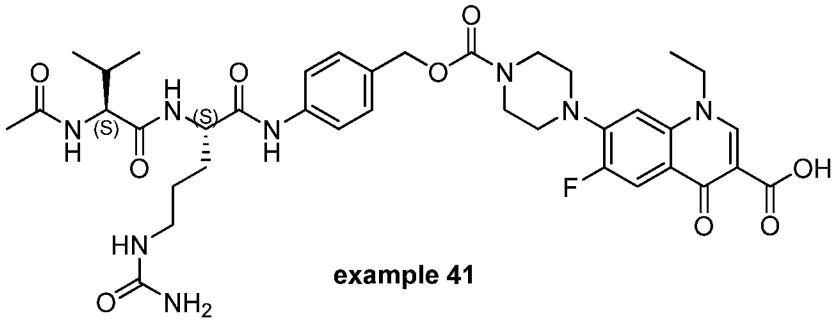
LCMS: (5-95, AB, 1.5 min, ESI), 0.964 min, MS = 442.6 [1/2M+1], 984.4[M+1]

Step 8. To compound **40-13** (120 mg, 0.12 mmol) was added a mixture of DCM:TFA (5:1, 3 mL) at r.t. After the mixture was stirred at r.t. for 1 h, it was adjusted to pH=7 with ammonia, then concentrated, the residual was purified by prep-HPLC to give **example 40** (29 mg, yield: 16.9%).

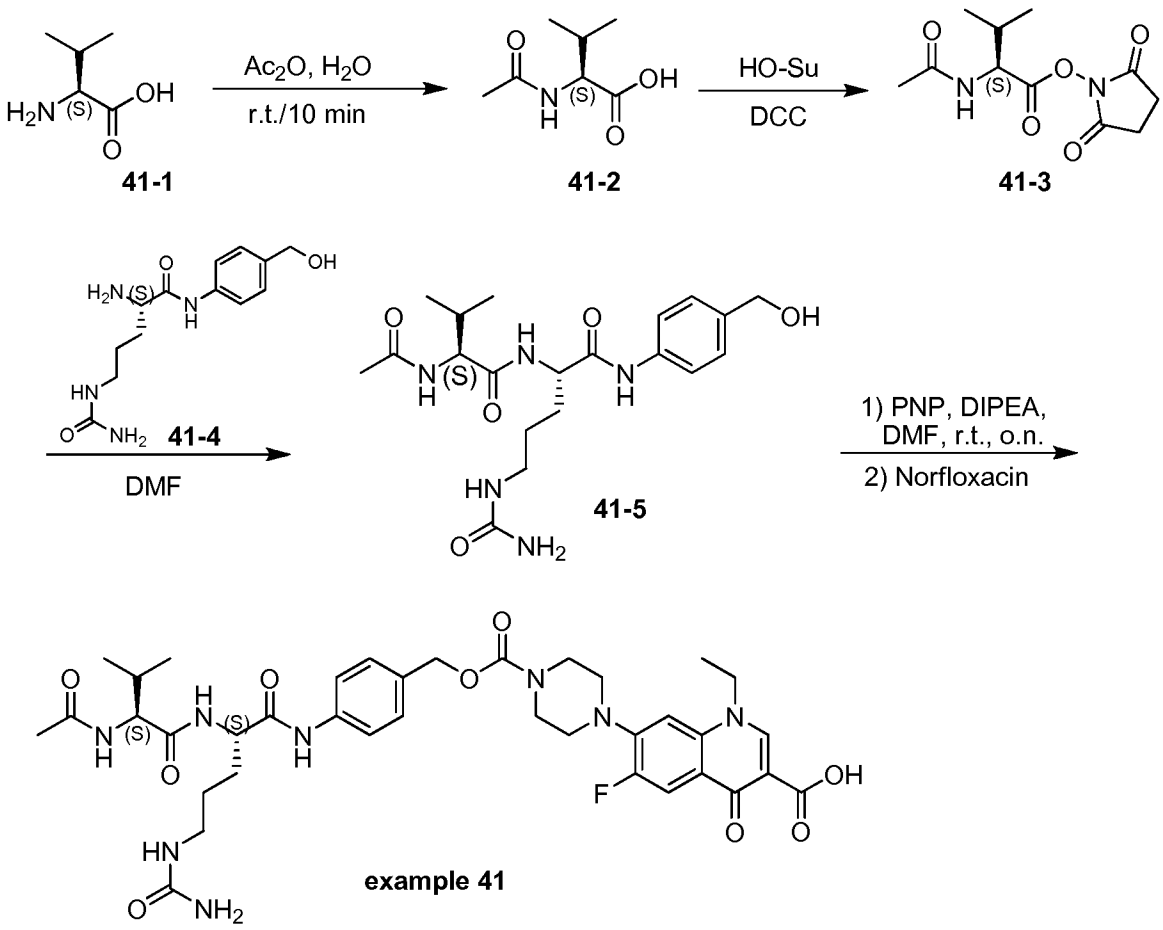
LCMS: (5-95, AB, 1.5 min, ESI), 0.826 min, MS = 884.3[M+1], 442.8[1/2M+1]

^{1}H NMR DMSO-d_6 400 MHz, δ 10.23 (s, 1 H), 8.93 (s, 1 H), 8.44 (s, 1 H), 8.42 (s, 0.5 H, HCOOH), 7.92 (d, J = 13.2 Hz, 1 H), 7.93 - 7.61 (m, 3 H), 7.33 - 7.25 (m, 8 H), 7.20 - 7.16 (m, 1 H), 6.88 - 6.86 (m, 2 H), 5.63 (s, 2 H), 4.97 (s, 2 H), 4.58 - 4.53 (m, 2 H), 4.40 - 4.31 (m, 1 H), 4.30 - 4.19 (m, 1 H), 3.58 (s, 4 H), 3.28 - 3.19 (m, 4 H), 3.19 - 3.03 (m, 2 H), 3.04 - 2.96 (m, 2 H), 2.69 - 2.64 (m, 2 H), 1.73 - 1.67 (m, 2 H), 1.57 - 1.49 (m, 2 H), 1.47 - 1.34 (m, 3 H), 1.32 - 1.28 (m, 2 H).

Example 41. 7-(4-((4-((S)-2-((S)-2-acetamido-3-methylbutanamido)-5-ureidopentanamido)benzyloxy) carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 41



Step 1. After compound **41-1** (470.6 mg, 4 mmol) in water (10 mL) was sonicated for 6 min, Ac_2O was added over 4 min. The mixture was concentrated and the residue was dissolved in MeOH, filtered, concentrated to give crude **41-2** as a white solid (350 mg, 55%).

^{1}H NMR DMSO-d_6 400 MHz, δ 7.94 - 7.92 (d, J = 8.0 1 H), 4.10 - 4.08 (m, 1H), 2.01 - 1.99 (m, 1 H), 1.84 (s, 1 H), 0.83 - 0.80 (m, 6 H)

Step 2. To a solution of compound **41-2** (160 mg, 1 mmol) and HO-Su (122 mg, 1.05 mmol) in THF (10 mL) was added DCC (218 mg, 1.05 mmol) at r.t. The mixture was stirred at r.t. for 16 h under N_2. The mixture was filtered and the filtrate was concentrated to give **41-3** (242 mg, Yield: 95 %).

Step 3. Compound **41-3** (242 mg, 0.94 mmol), **41-4** (265 mg, 0.94 mmol) were dissolved in DMF (15 mL). After the reaction mixture was stirred at r.t. for 3 h, it was filtered and purified by prep-HPLC to give **41-5** (70 mg, Yield: 17.7 %).

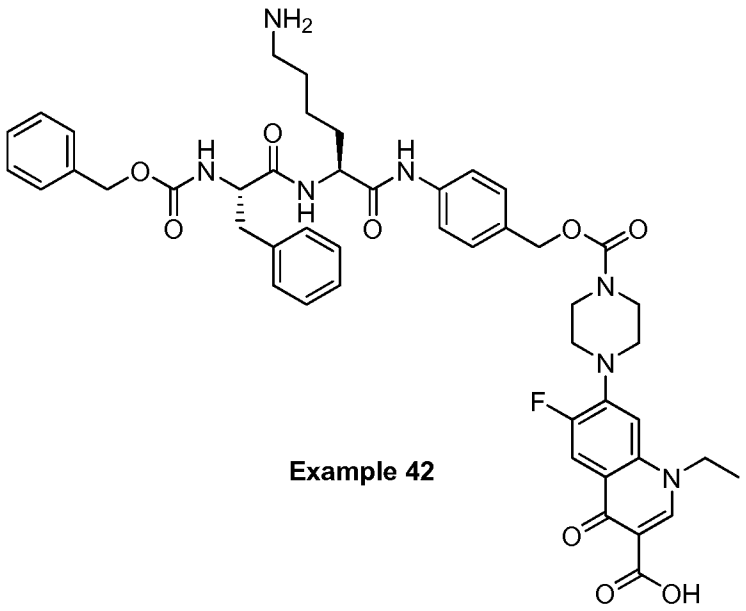
Step 4. To a solution of compound **41-5** (60 mg, 0.142 mmol) in dry DMF (3 mL) was added PNP carbonate (87 mg, 0.285 mmol) and DIPEA (56 mg, 0.427 mmol) at r.t. The mixture was stirred at r.t. for 16 h. Norfloxacin (91 mg, 0.285 mmol) was added. The mixture was stirred at r.t. for

another 1 h. The mixture was concentrated, filtered and purified by prep-HPLC (FA) to give **example 41** (40 mg, yield: 37%).

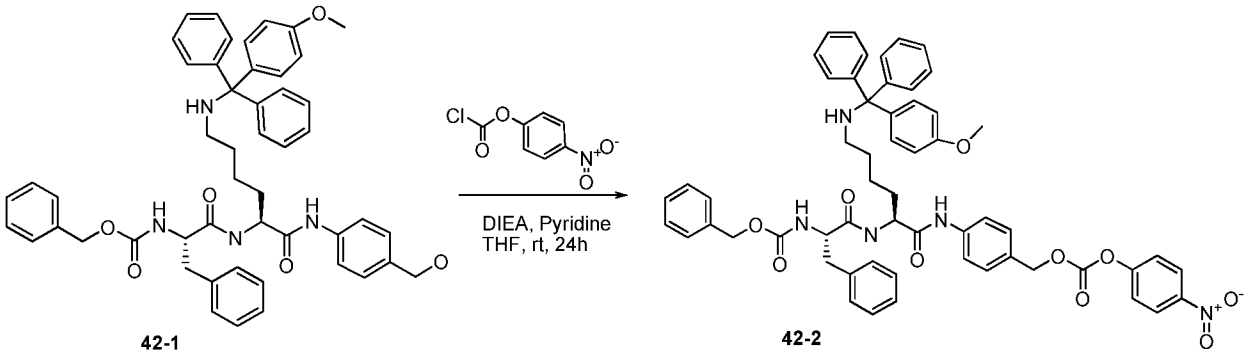
LCMS: (5-95, AB, 1.5 min, ESI), 0.784 min, MS = 767.1[M+1]

^{1}H NMR DMSO-d_6 400 MHz, δ 9.96 (s, 1 H), 8.93 (s, 1 H), 8.10 (d, J = 7.6 Hz, 1 H), 7.92 (d, J = 12.4 Hz, 1 H), 7.86 (d, J = 8.8 Hz, 1 H), 7.58 (d, J = 8.4 Hz, 2 H), 7.31 (d, J = 8.8 Hz, 2 H), 7.18 (d, J = 7.2 Hz, 1 H), 5.96 - 5.94 (m, 1 H), 5.38 (s, 2 H), 5.03 (s, 2 H), 4.58 - 4.53 (m, 2 H), 4.38 - 4.32 (m, 1 H), 4.17 - 4.14 (m, 1 H), 3.58 (s, 4 H), 3.26 (s, 4 H), 3.00 - 2.91 (m, 2 H), 1.96 - 1.92 (m, 1 H), 1.86 (s, 3 H) , 1.68 - 1.55 (m, 2 H) , 1.38 (m, 5 H), 0.85 - 0.80 (m, 6 H).

Example 42: 7-(4-{4-[(S)-6-Amino-2-((S)-2-benzyloxycarbonylamino-3-phenyl-propionylamino)-hexanoylamino]-benzyloxycarbonyl}-piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid



Example 42

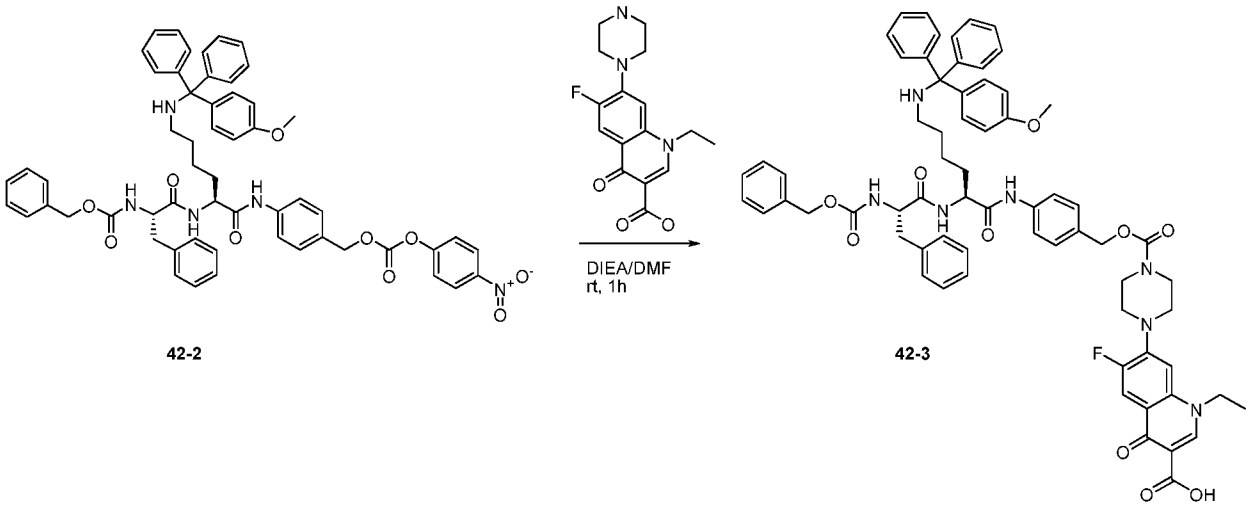


42-1 **42-2**

Step 1. To the 5ml THF solution of **42-1** (200 mg, 0.25 mmol) was added DIEA (0.18 ml, 0.99 mmol), then added (4-nitrophenyl) carbonochloridate (150 mg, 0.75 mmol), followed by pyridine (0.03 ml, 0.32 mmol). The reaction mixture was stirred at 28°C for overnight. The mixture was concentrated down to dry, dissolved into EtOAc, washed by Sat. NH4Cl, brine. The organic layer

was dried over MgSO4, was concentrated down. The crude was triturated with ether, filtered to afford a light yellow solid 42-2 (190 mg, 79%).

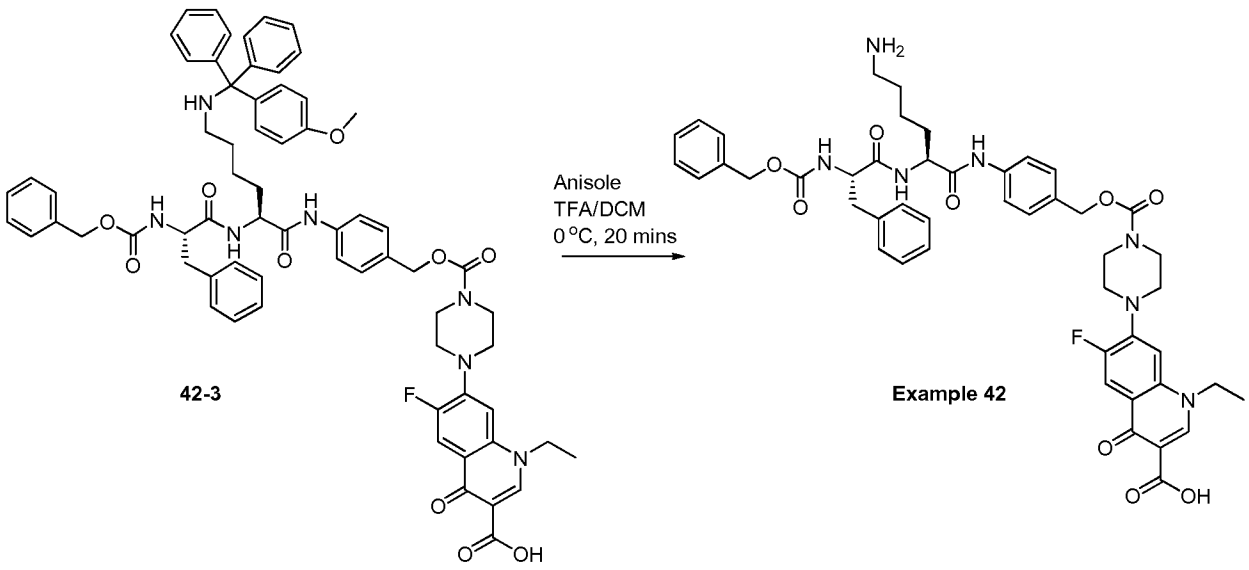
LCMS (ESI): m/z 970.7 [M+H$^+$].



42-2 42-3

Step 2. To the suspension of 1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (59 mg, 0.18 mmol) in 2ml DMF, was added DIEA (0.11 mL, 0.62 mmol), then added 42-2 (120 mg, 0.12 mmol) as one portion, slowly became yellow solution till reaction completed. The reaction mixture was poured into ice water, extracted with DCM. The organic layer washed by Sat. NH_4Cl, brine, dried over $MgSO_4$, was concentrated down to yield 42-3 (140 mg, 99%).

LCMS (ESI): m/z 1150.9 [M+H$^+$].



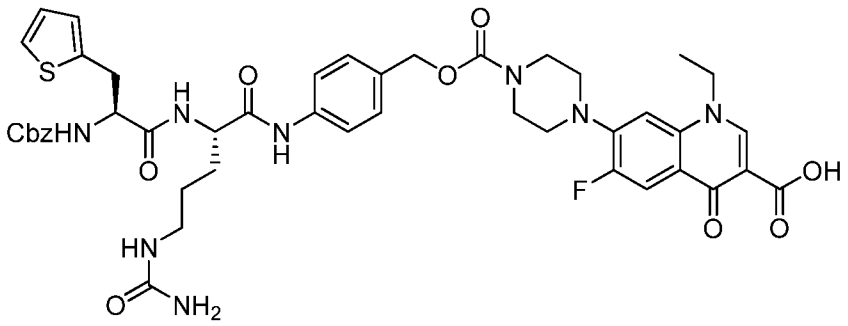
42-3 Example 42

Step 3. To the 10 ml DCM solution of **42-3** (145 mg, 0.13 mmol) was added anisole (0.05 ml, 0.50 mmol), cooled to ice bath, then dropwisely added TFA (0.1 ml, 1.00 mmol). After 10 mins the reaction was completed, removed the ice bath. The reaction mixture was concentrated down to dry, dissolved into DCM, washed by Sat. NaHCO3/water, brine. The organic layer was dried over MgSO4, was concentrated down. The crude was triturated with DCM, filtered to afford a light yellow solid 42 (38 mg, 34%).

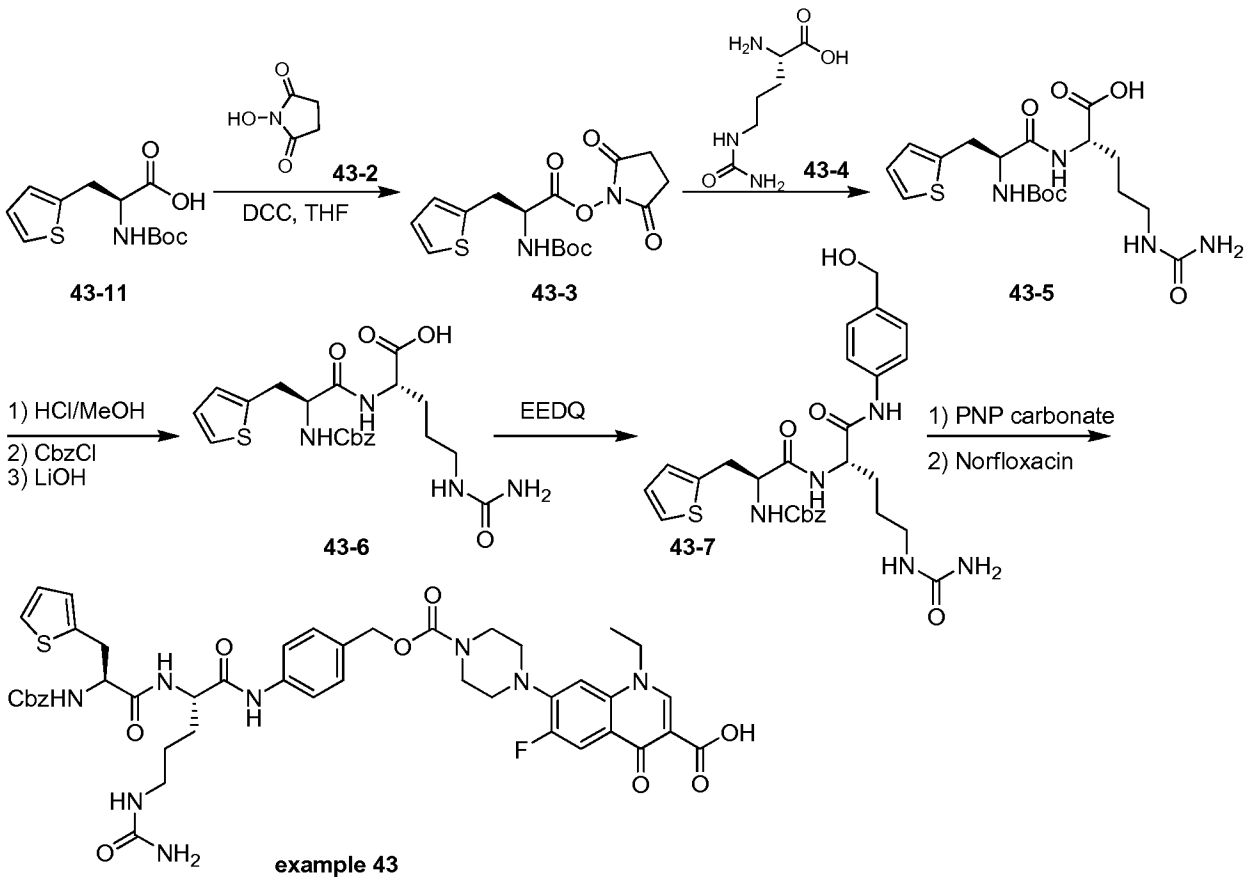
LCMS (ESI): m/z 878.7 [M+H$^+$].

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H), 8.86 (s, 1H), 8.26 (s, 1H), 7.91 (d, J = 12.7 Hz, 1H), 7.62 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 9.2 Hz, 1H), 7.38 – 7.10 (m, 13H), 5.07 (s, 2H), 4.95 (s, 2H), 4.51 (s, 2H), 4.40 (s, 1H), 4.33 (s, 1H), 3.61 (s, 4H), 3.03 (d, J = 12.2 Hz, 2H), 1.69 (d, J = 15.5 Hz, 3H), 1.39 (t, J = 6.8 Hz, 8H).

Example 43. 7-(4-((4-((S)-2-((S)-2-(benzyloxycarbonylamino)-3-(thiophen-2-yl)propanamido) -5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 43



Step 1. To a solution of compound **43-1** (178 mg, 0.665 mmol) and compound **43-2** (79 mg, 0.688 mmol) in THF (3 mL) was added DCC (149 mg, 0.721 mmol) in THF (0.5 mL) at 10°C. The solution was stirred at 10°C for 6 h. The solid was filtered, and solvent was removed. The residue was dissolved in DCM (5 mL). The mixture was allowed to stand for 1 h and was filtered to remove more DCU. The filtrate was evaporated to give crude compound **43-3** (245 mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 5.2Hz, 1 H), 7.02-6.96 (m, 2 H), 5.30-4.93 (m, 1 H), 3.54-3.40 (m, 2 H), 2.82 (s, 4 H), 1.44 (s, 9 H).

Step 2. To a solution of compound **43-3** (245 mg, 0.665 mmol) in DME (2 mL) was added a solution of compound **43-4** (175 mg, 0.998 mmol) in water (2 mL). The solution was stirred at 10°C for 16 h. Saturated $NaHCO_3$ solution (3 mL) was added, and the mixture was washed with DCM (15 mL x 2). The aqueous layer was acidified to pH 3 with 1M HCl solution, and extracted with EtOAc (15 mL x 3). The organic layer was dried over Na_2SO_4, filtered, concentrated to give crude product **43-5** (270 mg).

LCMS (ESI): m/z429.2 [M+H$^+$].

Step 3. A solution of compound **43-5** (270 mg, 0.63 mmol) in a MeOH of HCl (4 M, 5 mL) was stirred for 2 h at 10°C. Solvent was removed under reduced pressure and the residue was dissolved

in H_2O/THF (v/v 1:1, 3 mL), then Na_2CO_3 (134 mg, 1.26 mmol) and CbzCl (167 mg, 0.979 mmol) were added. The mixture was stirred for 2 h at 10°C, then $LiOH{\cdot}H_2O$ (79 mg, 1.89 mmol) was added and the resulting solution was stirred for 1 h at 10°C. Organic solvent was removed and the aqueous solution was washed with DCM (15 mL x 2). The aqueous layer was acidified to pH 2 with 1M HCl solution and extracted with EtOAc (15 mL x 3). The organic layer was dried over Na_2SO_4, filtered, concentrated to give crude product (182 mg).

LCMS (ESI): m/z 463.2 [$M+H^+$].

Step 4. To a solution of compound **43-6** (180 mg, 0.389 mmol) and (4-aminophenyl) methanol (144 mg, 1.17 mmol) in DMF (4 mL) was added EEDQ (289 mg, 1.17 mmol) under N_2. The mixture was stirred at 30°C for 16 h. The mixture was purified by prep-HPLC to give **43-6** (80 mg, 36%).

^{1}H NMR (400 MHz, MeOD) δ 7.55-7.53 (m, 2 H), 7.32-7.27 (m, 7 H), 7.17-7.15 (m, 1 H), 6.88-6.84 (m, 2 H), 5.11-5.03 (m, 2 H), 4.55 (s, 2 H), 4.50-4.39 (m, 2 H), 3.38-3.36 (m, 1 H), 3.19-3.05 (m, 3 H), 1.89-1.50 (m, 4 H).

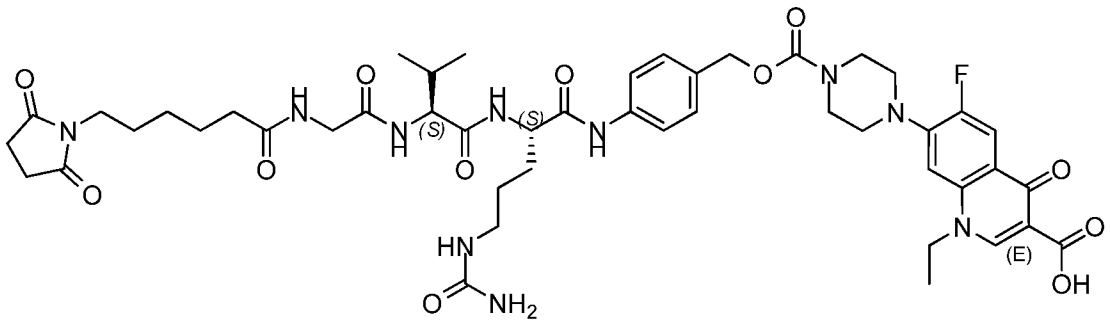
LCMS (ESI): m/z 568.1 [$M+H^+$].

Step 5. To a solution of **43-7** (20 mg, 0.035 mmol) in DMF (2 mL) were added PNP carbonate (21 mg, 0.070 mmol) and DIPEA (18 mg, 0.141 mmol) at 10°C. After the mixture was stirred for 24 h at 10°C, norfloxacin (17 mg, 0.053 mmol) and DIPEA (9 mg, 0.0695 mmol) were added. The resulting solution was stirred at 10°C for 1 h and purified by prep-HPLC to give **example 43** (7 mg, 22%).

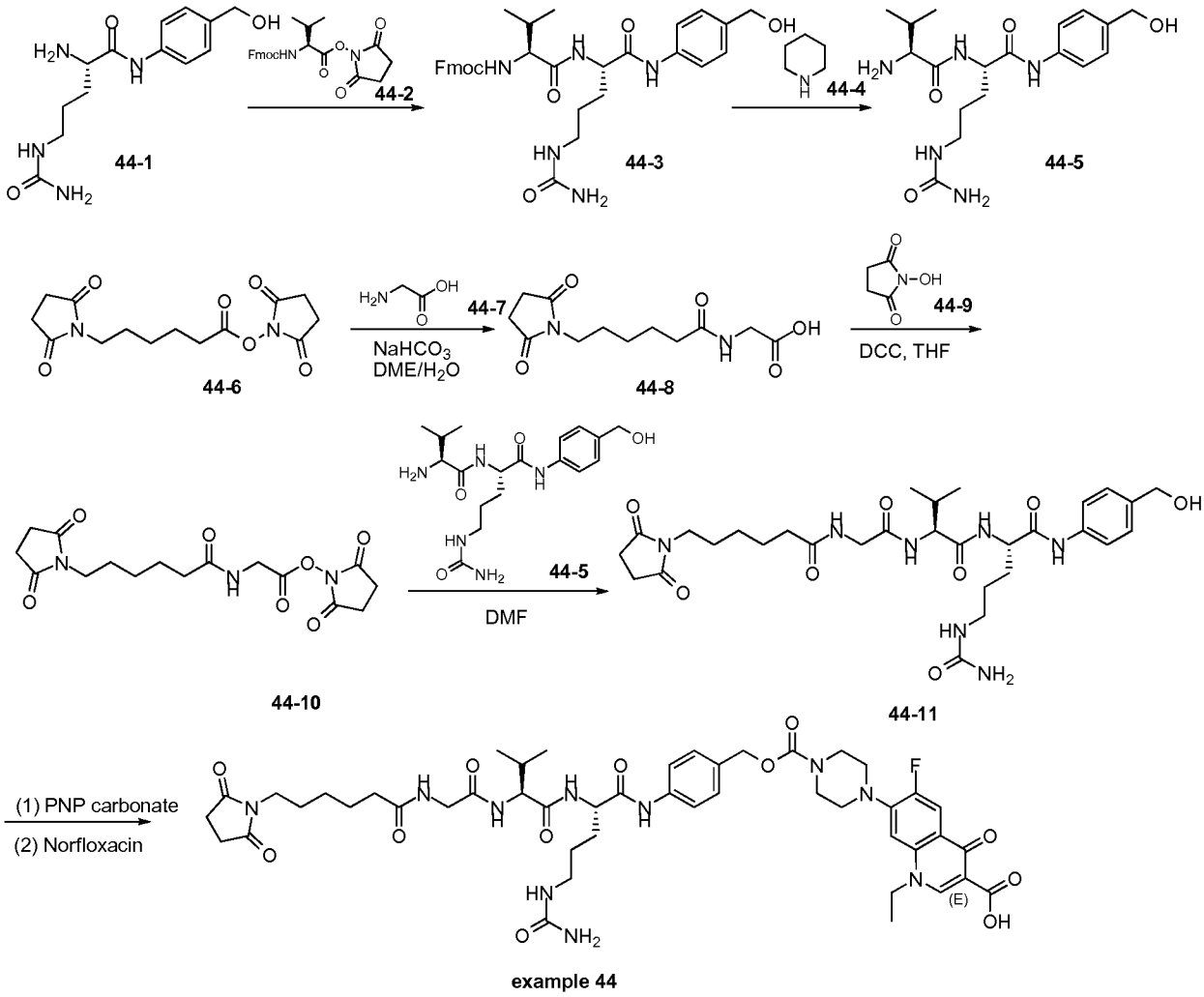
^{1}H NMR (400 MHz, DMSO–d_6) δ 10.16 (s, 1 H), 8.95 (s, 1 H), 8.33-8.32 (m, 1 H), 7.94 (d, J = 12.0Hz, 1 H), 7.63-7.57 (m, 3 H), 7.35-7.19 (m, 10 H), 6.91-6.90 (m, 2 H), 6.04 (m, 1 H), 5.44 (s, 2 H), 5.06 (s, 2 H), 5.00 (s, 2 H), 4.59-4.29 (m, 4 H), 3.61 (s, 4 H), 3.21 (s, 4 H), 3.04-2.94 (m, 4 H), 1.71-1.61 (m, 2 H), 1.45-1.38 (m, 5 H).

LCMS (ESI): m/z 913.3 [$M+H^+$].

Example 44. 7-(4-((4-((S)-2-((S)-2-(2-(6-(2,5-dioxopyrrolidin-1-yl)hexanamido)acetamido)-3-methylbutanamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 44



example 44

Step 1. Compound **44-1** (600 mg, 2.14 mmol) and compound **44-2** (936 mg, 2.14 mmol) were dissolved in DMF (5 mL) and stirred at r.t. for 3 h to give **44-3** as a mixture.

Step 2. To a solution of compound **44-3** (2.14 mmol) in DMF (10 mL) was added compound **44-4** (0.6 mL, 4.5 mmol) and stirred at r.t. for 1 h. The mixture was concentrated and washed with MTBE (30 mL x 3), filtered and the filtrate was concentrated to give **44-5** (597 mg, Yield: 70 %).

Step 3. To a solution of compound **44-7** (75 mg, 1 mmol) in DME (10 mL) was added a solution of compound **44-6** (340 mg, 1.1 mmol) and $NaHCO_3$ (252 mg, 3 mmol) in water (10 mL). The mixture was stirred at r.t. for 16 h. The mixture was washed with EtOAc and acidified to pH = 3 with 10% HCl. The resulting suspension was extracted with EtOAc. The combined organic layer was concentrated to give crude compound **44-8** (350 mg)

Step 4. To a solution of **44-8** (350 mg, 1.3 mmol) and **44-9** (157 mg, 1.36 mmol) in THF (15 mL) was added DCC (281 mg, 1.36 mmol). After it was stirred for 16 h under nitrogen, it was concentrated to give **44-10** (480 mg, Yield: 100 %).

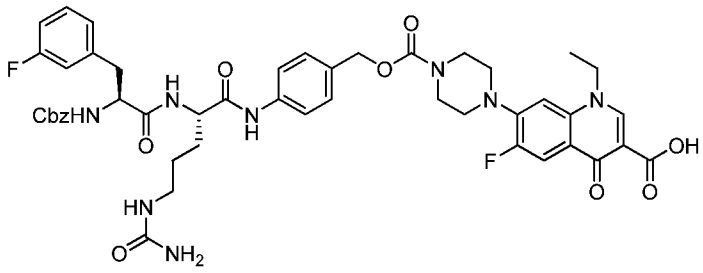
Step 5. Compound **44-10** (404 mg, 1.1 mmol), compound **44-5** (835 mg, 2.2 mmol) were dissolved in DMF (6 mL). After the reaction mixture was stirred at r.t. for 3 h, it was filtered and purified by prep-HPLC to give compound **44-11** (110 mg, Yield: 16 %).

Step 6. To a solution of compound **44-11** (85 mg, 0.135 mmol) in dry DMF (5 mL) was added PNP carbonate (82 mg, 0.27 mmol) and DIPEA (52 mg, 0.40 mmol) at r.t. It was stirred at r.t. for 16 h. Norfloxacin (85 mg, 0.27 mmol) was added and the mixture was stirred at r.t. for another 1 h. The mixture was concentrated, filtered and purified by prep-HPLC (FA) to give **example 44** (70 mg, yield: 51%).

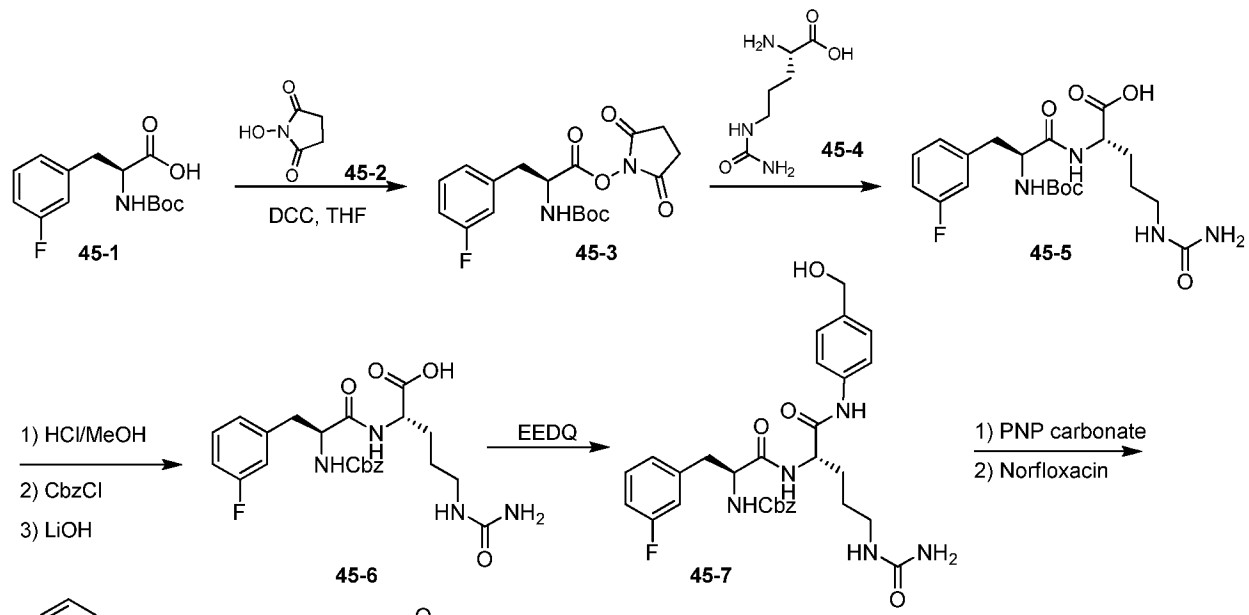
LCMS: (5-95, AB, 1.5 min, ESI), 0.794 min, MS = 977.3[M+1],489.3 [1/2M+1]

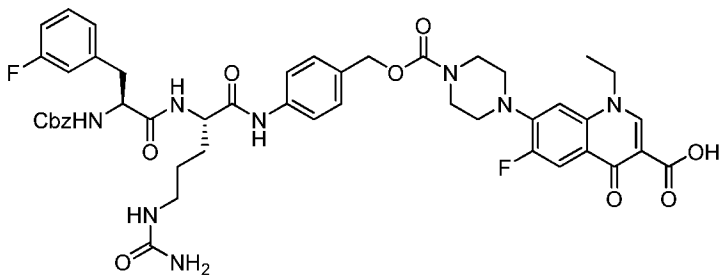
^{1}H NMR DMSO-d_6 400 MHz, δ 15.32 (s, 1 H), 9.90 (s, 1 H), 8.96 (s, 1 H), 8.17 (s, 2 H), 8.11 (s, 1 H), 7.95 (d, J = 13.6 Hz, 1 H), 7.82 (d, J = 8.4 Hz, 1 H), 7.62 (d, J = 8.8 Hz, 2 H), 7.33 (d, J = 8.4 Hz, 2 H), 7.21 (d, J = 9.6 Hz, 1 H), 5.98 (s, 1 H), 5.41 (s, 2 H), 5.06 (s, 2 H), 4.59 (d, J = 7.2 Hz, 2 H), 4.35 (s, 1 H), 4.22 (d, J = 14.8 Hz, 1 H), 3.76 - 3.73 (m, 2 H), 3.61 (s, 4 H), 3.28 (s, 4 H), 3.12 - 2.86 (m, 2 H), 2.60 (s, 4 H), 2.07 (d, J = 6.8 Hz, 2 H), 1.98 (s, 1 H) , 1.80 - 1.56 (m, 2 H), 1.47 - 1.39 (m, 9 H), 1.18 (s, 2 H), 0.87 - 0.82 (m, 6 H).

Example 45. 7-(4-((4-((S)-2-((S)-2-(benzyloxycarbonylamino)-3-(3-fluorophenyl)propanamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 45





example 45

Step 1. To a solution of compound **45-1** (178 mg, 0.63 mmol) and compound **45-2** (76 mg, 0.662 mmol) in THF (3 mL) was added DCC (143 mg, 0.693 mmol) in THF (0.5 mL) at 10°C. After the solution was stirred at 10°C for 3 h, solid was filtered, and solvent was removed. The residue was dissolved in DCM (5 mL). The mixture was allowed to stand for a 1 hour and then filtered to remove more DCU. The filtrate was evaporated to give crude compound **45-3** (250 mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69-7.27 (m, 1 H), 7.23-6.95 (m, 3 H), 4.98-4.85 (m, 1 H), 3.33-3.14 (m, 2 H), 2.82 (s, 4 H), 1.37 (s, 9 H).

Step 2. To a solution of compound **45-3** (250 mg, 0.63 mmol) in DME (2 mL) was added a solution of compound **45-4** (173 mg, 0.986 mmol) in water (2 mL). The solution was stirred at

10°C for 16 h. Saturated $NaHCO_3$ solution (3 mL) was added, and the mixture was washed with DCM (5 mL x 2). The aqueous layer was acidified to pH 3 with 1M HCl solution, extracted with EtOAc (10 mL x 3). The organic layer was dried over Na_2SO_4, filtered, concentrated to give the crude product **45-5** (280 mg).

LCMS (ESI): m/z 441.2 [M+H$^+$].

Step 3. After a solution of compound **45-5** (240 mg, 0.545 mmol) in 4M HCl in MeOH (5 mL) was stirred for 2 h at 10°C, solvent was removed under reduced pressure and the residue was dissolved in H_2O/THF (v/v 1:1, 3 mL). Na_2CO_3 (116 mg, 1.09 mmol) and CbzCl (139 mg, 0.817 mmol) was added. The mixture was stirred for 2 h at 10°C, then $LiOH{\cdot}H_2O$ (67 mg, 1.63 mmol) was added and the resulting solution was stirred for 1 h at 10°C. Solvent was removed, and the aqueous solution was washed with DCM (5 mL x 2) and acidified to pH 2 with 1M HCl solution. It was extracted with EtOAc (10 mL x 3). The combined organic layer was dried over Na_2SO_4, filtered, concentrated to give of crude product **45-6** (198 mg).

LCMS (ESI): m/z 475.2 [M+H$^+$].

Step 4. To a solution of compound **45-6** (192 mg, 0.405 mmol) and (4-aminophenyl) methanol (150 mg, 1.21 mmol) in DMF (4 mL) was added EEDQ (300 mg, 1.21 mmol) under N_2. After the mixture was stirred at 30°C for 16 h, it was purified by prep-HPLC to give **45-7** (70 mg, 30%).

^{1}H NMR (400 MHz, MeOD) δ 7.55-7.53 (m, 2 H), 7.31-7.17 (m, 8 H), 7.05-7.00 (m, 2 H), 6.91-6.86 (m, 1 H), 5.07-4.99 (m, 2 H), 4.56 (s, 2 H), 4.49-4.41 (m, 2 H), 3.17-3.06 (m, 3 H), 2.91-2.85 (m, 1 H), 1.87-1.50 (m, 4 H).

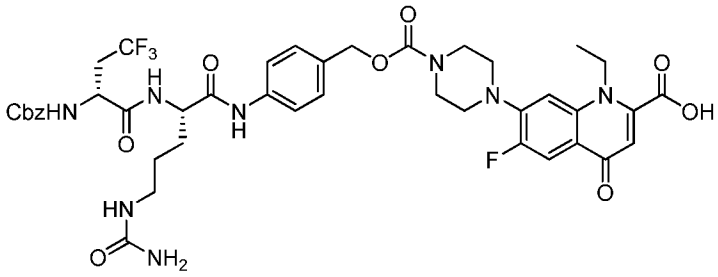
LCMS (ESI): m/z 580.2 [M+H$^+$].

Step 5. To a solution of **45-7** (20 mg, 0.0345 mmol) in DMF (2 mL) was added PNP carbonate (21 mg, 0.069 mmol) and DIPEA (18 mg, 0.139 mmol) at 10°C. After the mixture was stirred for 24 h at 10°C, norfloxacin (17 mg, 0.053 mmol) and DIPEA (9 mg, 0.0695 mmol) were added. The reaction mixture was stirred at 10°C for 1 h and purified by prep-HPLC to give **example 45** (17 mg, 53%).

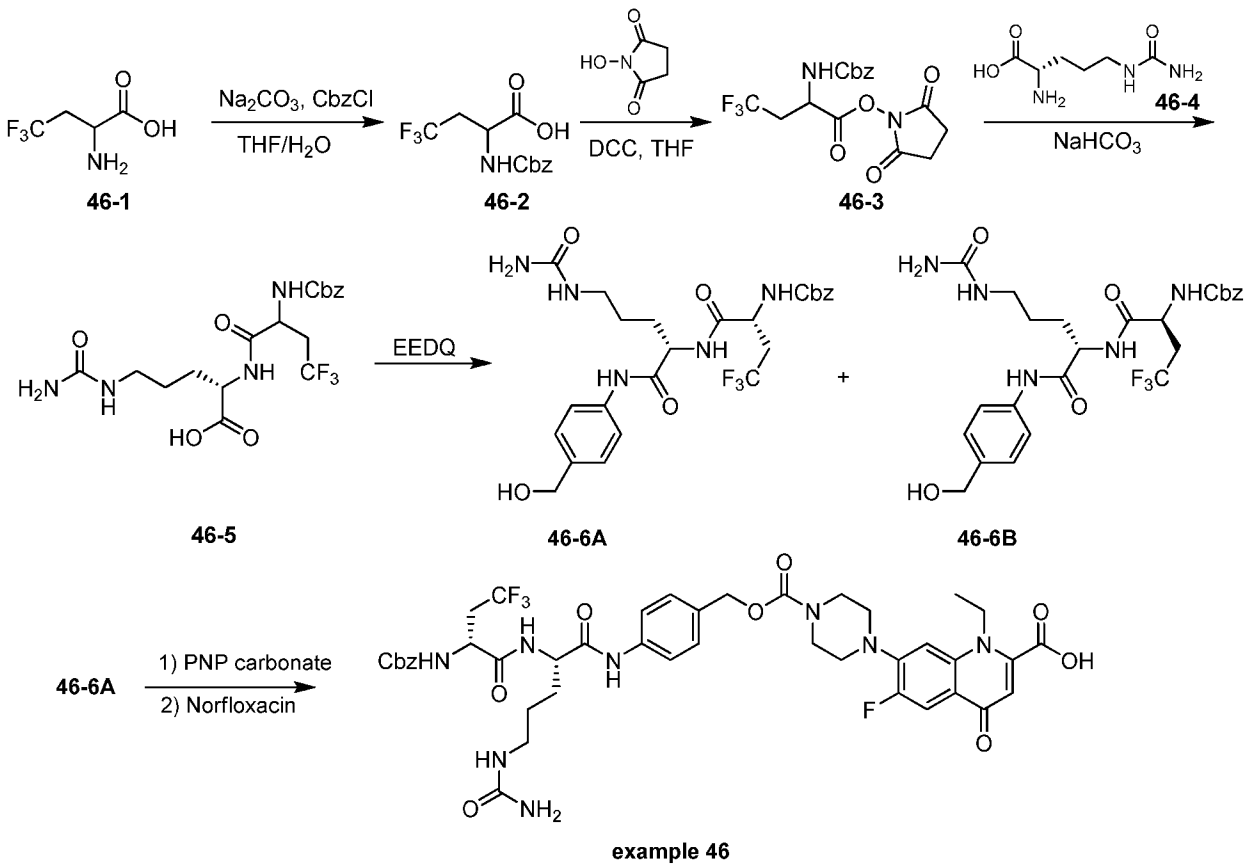
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.32 (br, 1 H), 10.12 (s, 1 H), 8.96 (s, 1 H), 8.30-8.28 (m, 1 H), 7.94 (d, J = 13.2 Hz, 1 H), 7.62 (d, J = 8.4 Hz, 2 H), 7.52 (d, J = 8.8 Hz, 1 H),7.35-7.13 (m, 11 H), 7.04-7.00 (m, 1 H), 5.99 (m, 1 H), 5.43 (s, 2 H), 5.06 (s, 2 H), 4.95 (s, 2 H), 4.61-4.33 (m, 4 H), 3.61 (s, 4 H), 3.32 (s, 4 H), 3.06-2.66 (m, 4 H), 1.75-1.60 (m, 2 H), 1.45-1.35 (m, 5 H).

LCMS (ESI): m/z 925.5 [M+H$^+$].

Example 46. 7-(4-((4-((S)-2-((R)-2-(benzyloxycarbonylamino)-4,4,4-trifluorobutanamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-2-carboxylic acid



example 46



example 46

Step 1. To the solution of compound **46-1** (200 mg, 1.27 mmol) in THF (10 mL) was added benzyl carbonochloridate (226 mg, 1.33 mmol) in an ice bath. A solution of Na_2CO_3 (270 mg, 2.54 mmol) in water (10 mL) was added dropwise with vigorous stirred at 7°C or lower. After mixture was stirred for 16 h, the organic layer was separated, washed with diluted HCl (0.1 M, 60 mL) and saturated $NaHCO_3$ solution (60 mL), dried over anhydrous Na_2SO_4, concentrated to afford compound **46-2** as a white solid (250 mg, 67.6%).

Step 2. To a mixture of compound **46-2** (250 mg, 0.887 mmol), 1-hydroxypyrrolidine-2, 5-dione (102 mg, 0.887 mmol) in dry THF (10 mL) was added DCC (183 mg, 0.887 mmol) at 0°C. The mixture was stirred at r.t. for 16 h, and filtered. The filtrate was concentrated in vacuo to give the crude product of compound **46-3**, which was used for next step without further purification.

Step 3. To the solution of compound **46-3** (710 mg, 1.87 mmol) in a mixture of DME and H_2O (10 mL/10 mL) was added compound **46-4** (327 mg, 1.87 mmol) and $NaHCO_3$ (236 mg, 2.8 mmol) at 0°C. After the mixture was stirred at r.t. for 16 h, it was extracted with EtOAc (50 mL x 3). The organic layer was washed with brine (50 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by prep-HPLC to afford the desired product of compound **46-5** as a white solid (421 mg, 50.2%).

LCMS (ESI): m/z 449.2 [M+H$^+$].

Step 4. To the solution of compound **46-5** (410 mg, 0.915 mmol) in DCM (10 mL) was added EEDQ (448 mg, 1.83 mmol) and 4-aminophenyl-methanol (225 g, 1.83 mmol). After the mixture was stirred at 0°C for 1 h, solvent was removed and the residue was purified with prep-HPLC and SFC separation to afford **46-6A** and **46-6B.**

46-6A

^{1}H NMR (400 MHz, MeOD-d_4) δ 7.54 (d, J = 8.4 Hz, 2 H), 7.35-7.29 (m, 7 H), 5.12 (s, 2 H), 4.55-4.49 (m, 4 H), 3.30-3.03 (m, 2 H), 2.83-2.53 (m, 2 H), 1.93-1.75 (m, 4 H). LCMS: m/z 553.9 [M+H$^+$].

46-6B

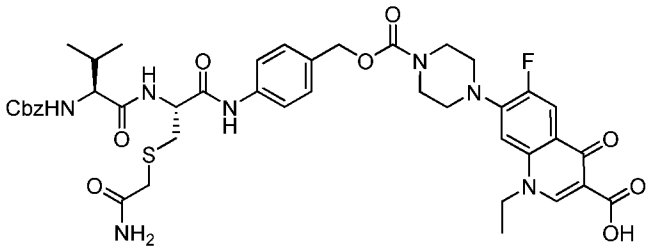
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1 H), 9.31(d, J = 9.2 Hz, 1 H), 8.53 (d, J = 9.6 Hz, 1 H), 8.26(d, J = 9.6 Hz, 2H), 8.00-7.96 (m, 5 H), 7.90 (d, J = 9.6 Hz, 2 H), 6.45-6.44 (m, 1 H), 5.81 (s, 1 H), 5.48-5.36 (m, 3 H), 4.77-4.69 (m, 4 H), 3.09-2.95 (m, 2 H), 2.79-2.64 (m, 2 H), 1.64-1.20 (m, 4 H). LCMS (ESI): m/z 553.9 [M+H$^+$].

Step 5. To the solution of **46-6A** (20 mg, 0.036 mmol) in dry DCM (2 mL) was added PNP carbonate (22 mg, 0.072 mmol) and DIPEA (14 mg). The mixture was heated at reflux for 16 h. After solvent was removed, the residue was dissolved in DMF (5 mL). DIPEA (9 mg, 0.072 mmol) and norfloxacin (23 mg, 0.072 mmol) was added. The mixture was stirred at r.t. for 1 h. Solvent was removed and the residue was purified by prep-HPLC to afford **example 46.**

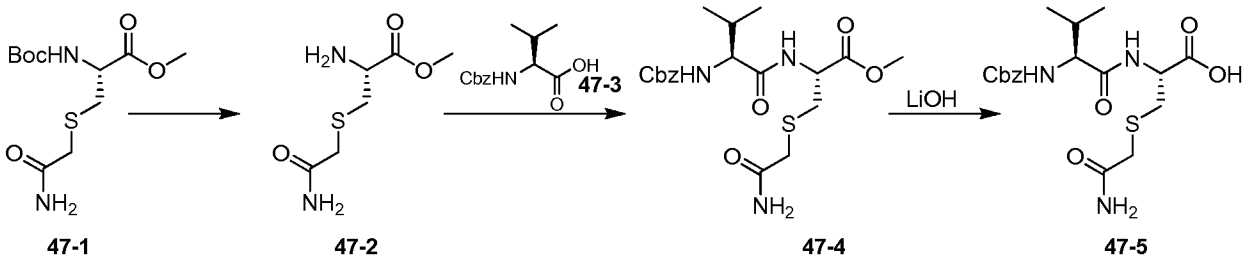
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.33 (s, 1 H), 10.11 (s, 1 H), 8.97 (s, 1 H), 8.34 (d, J = 7.6 Hz, 1 H), 7.95 (d, J = 12.8 Hz, 1 H), 7.78 (d, J = 8.4 Hz, 1 H), 7.60 (d, J = 8.4 Hz, 2 H), 7.37-7.21 (m,

8 H), 6.00-5.98 (m, 1 H), 5.97 (s, 2 H), 5.43-5.03 (m, 4 H), 4.61-4.37 (m, 4 H), 3.61 (s, 4 H), 3.31 (s, 4 H), 3.04-2.49 (m, 4 H), 1.70-1.20 (m, 7 H). LCMS (ESI): m/z 450.3 [M/2+H$^+$].

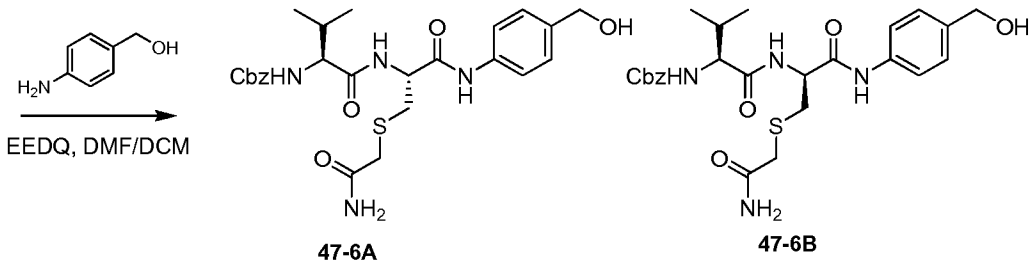
Example 47. 7-(4-((4-((R)-3-(2-amino-2-oxoethylthio)-2-((S)-2-(benzyloxycarbonylamino)-3-methylbutanamido)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



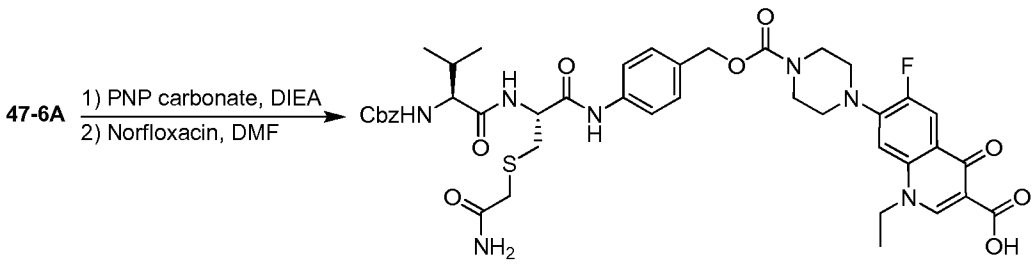
example 47



47-1 47-2 47-3 47-4 47-5



47-6A 47-6B



example 47

Step 1. The mixture of compound **47-1** (120 mg, 0.411 mmol) in 4 M HCl / 1, 4-dioxane (5 mL) was stirred at r.t. for 2 h. The mixture was concentrated to give **47-2**.

LCMS (ESI): m/z 193.0 [M+H$^+$].

Step 2. To a solution of compound **47-2** (1.3 g, 6.85 mmol) in DCM (20 mL) was added compound **47-3** (2.065 g, 8.22 mmol), DIPEA (4.418 g, 34.25 mmol), HOBt (1.018 g, 7.535 mmol) and EDCI (1.7 g, 8.905 mmol). The mixture was stirred at r.t. for 16 h. The solution was filtered and the solid was used in next step without further purification (**47-4**).

LCMS (ESI): m/z 425.9 [M+H$^+$].

Step 3. To a solution of compound **47-4** (500 mg, 1.176 mmol) in THF / MeOH / H_2O (6 mL /2 mL / 2 mL) was added LiOH (85 mg, 7.05 mmol). The mixture was stirred at r.t. for 1.5 h. The mixture was concentrated and the residue was purified by prep-HPLC to give compound **47-5**.

LCMS (ESI): m/z 412.0 [M+H$^+$].

Step 4. To a solution of compound **47-5** (600 mg, 1.46 mmol) in DCM / DMF (10 mL / 2 mL) were added 4-amino-phenyl-methanol (216 mg, 1.75 mmol) and EEDQ (541 mg, 2.19mmol). The mixture was stirred at r.t. for 16 h. The mixture was quenched with H_2O. The residue was purified by prep-HPLC and then purified by SFC to give **47-6A** and **47-6B**.

47-6A

^{1}H NMR (400 MHz, MeOD-d_4) δ 7.57 (d, J = 8.4 Hz, 2 H), 7.35-7.26 (m, 7 H), 5.06 (s, 2 H), 4.71-4.68 (m, 1 H), 4.54 (s, 2 H), 3.97 (d, J = 6.4 Hz, 2 H), 3.25 (m, 2 H), 3.13-3.08 (m, 1 H), 3.0-2.93 (m, 1 H), 2.13-2.01 (m, 1 H), 0.98-0.95 (m, 6 H).

LCMS (ESI): m/z 517.2 [M+H$^+$].

47-6B

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (m, 1 H), 8.597-8.56 (m, 1 H), 7.61-7.1 (m, 11 H), 5.13-5.00 (m, 3 H), 4.63-4.59 (m, 1 H), 4.44-4.41 (m, 2 H), 3.92-3.90 (m, 2 H), 3.17-2.84 (m, 4 H), 2.00-1.96 (m, 1 H), 0.90-0.89 (m, 6 H).

LCMS (ESI): m/z 517.1 [M+H$^+$].

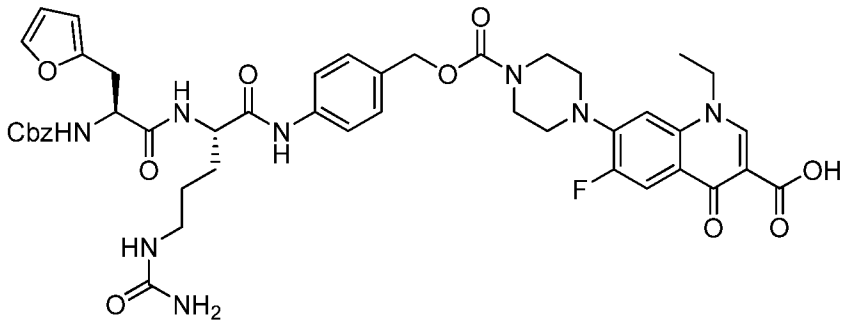
Step 5. To a solution of **47-6A** (40 mg, 0.0775 mmol) in DCM (3 mL) was added carbonic acid bis-(4-nitro-phenyl) ester (20 mg, 0.093 mmol) and DIPEA (15 mg, 0.116 mmol). The mixture was mixture was stirred at 45°C for 16 h. The solution was concentrated and dissolved in DMF (3 mL), and norfloxacin (50 mg, 0.155 mmol) was added. The mixture was stirred at r.t. for 1 h. After removal of the solvent, the residue was purified by pre-HPLC to give **example 47** (5.5 mg).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1 H), 8.93-8.89 (m, 1 H), 8.40-8.34 (m, 2 H), 7.63-7.61 (m, 4 H), 7.36-7.35 (m, 8 H), 7.13 (m, 2 H), 5.06-5.03 (4 H), 4.62-4.52 (m, 3 H), 3.94-3.91 (m, 1

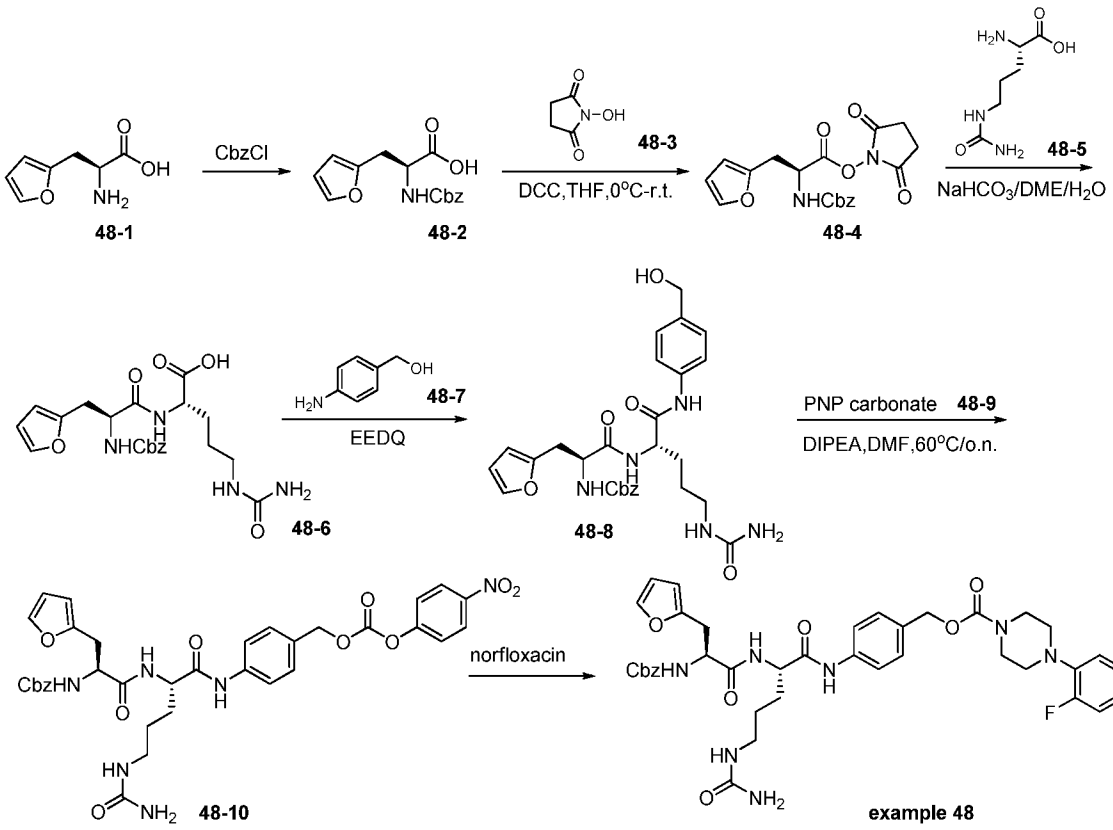
H), 3.36 (m, 4 H), 3.19 (m, 4 H), 3.00-2.86 (m, 3 H), 2.03-1.95 (m, 1 H), 1.38 (m, 3 H), 0.88-0.83 (m, 6 H).

LCMS (ESI): m/z 862.4 [M+H$^+$].

Example 48. 7-(4-((4-((S)-2-((S)-2-(benzyloxycarbonylamino)-3-(furan-2-yl)propanamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 48



Step 1. To the mixture of compound **48-1** (1.0 g, 6.45 mmol) in THF and $NaHCO_3$ solution was added CbzCl via syring at 0°C. The reaction mixture was stirred at r.t. for 2 h and purified by prep-HPLC to give **48-2** (Yield: 32 %).

Step 2. To a solution of compound **48-2** (380 mg, 1.31 mmol), compound **48-3** (151 mg, 1.31 mmol) in THF (25 mL) was added DCC (271 mg, 1.31 mmol) at 0°C. The mixture was stirred at r.t. for 16 h under N_2. The solution was filtered and the filtrate was concentrated to give **48-4** which used in the next step without further purification (Yield: 90 %).

Step 3. To a solution of compound **48-4** (540 mg, 1.38 mmol) in DME (15 ml) was added a solution of compound **48-5** (364 mg, 2.07 mmol) and $NaHCO_3$ (174 mg, 2.07 mmol) in water (15 mL). The mixture was stirred at r.t. for 16 h. The mixture was washed with EtOAc and acidified to pH 3.0 with 10% HCl. The resulting suspension was extracted with EtOAc. The combined organic layer was concentrated to give compound **48-6** (Yield: 90 %).

LCMS: (10-80, AB, 2 min, ESI), 0.826 min, MS = 447.1 [M+1]

Step 4. To a solution of compound **48-6** (420 mg, 0.94 mmol) in DCM / MeOH (20 mL / 10 mL) were added 4-amino-phenyl-methanol (174 mg, 1.4 mmol) and EEDQ (495 mg, 1.88 mmol). The mixture was stirred at r.t. for 16 h. The mixture was quenched with H_2O. The residue was purified by prep-HPLC and SFC to give **48-8** (Yield: 50 %).

^{1}H NMR DMSO-d_6 400 MHz, δ 9.98 (s, 1 H), 8.20 (d, J = 8 Hz, 1 H), 7.56 - 7.49 (m, 3 H), 7.37 (s, 1 H), 7.35 - 7.30 (m, 5 H), 7.24 (d, J = 8.8 Hz, 3 H), 6.33 - 6.32 (m, 1 H), 6.13 (d, J = 3.2 Hz, 1 H), 5.98 - 5.95 (t, J = 6.0 Hz, 1 H), 5.41 (s, 2 H), 5.11 - 5.08 (t, J = 5.6 Hz, 1 H), 4.99 (d, J = 13.2 Hz, 2 H), 4.44 - 4.35 (m, 3 H), 3.33 - 2.83 (m, 4 H), 1.71 - 1.58 (m, 2 H), 1.44 - 1.34 (m, 2 H).

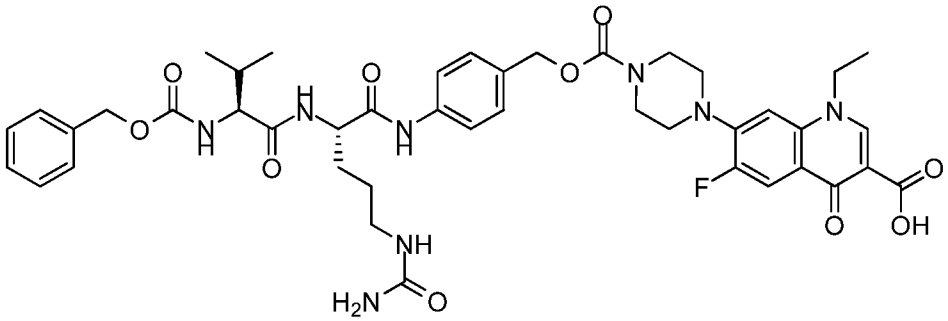
Step 5. To a solution of compound **48-9** (30 mg, 0.06 mmol) in DMF (5 mL) was added a solution of PNP carbonate (37 mg, 0.12 mmol) and DIPEA (24 mg, 0.18 mmol) at 0°C. The mixture was stirred at r.t. for 16 h. The mixture (**48-10**) was used for next step without further purification (Yield: 95 %)

LCMS: (10-80, AB, 2 min, ESI), 1.092 min, MS = 717.1[M+1]

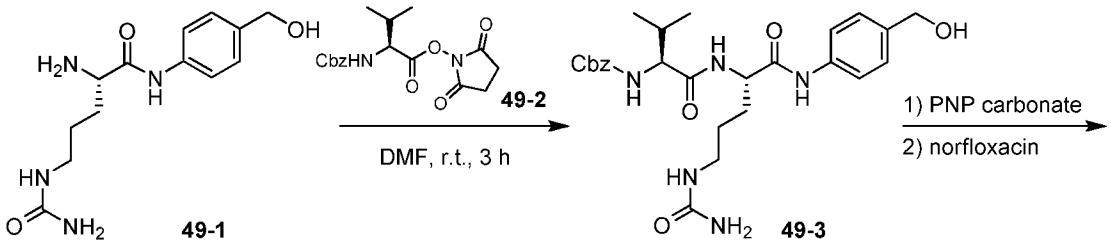
Step 6. A mixture of **48-10** from last step and norfloxacin was stirred at r.t. for 1 h. The residue was purified by prep-HPLC and then purified by SFC to give **example 48**. (Yield: 30 %)

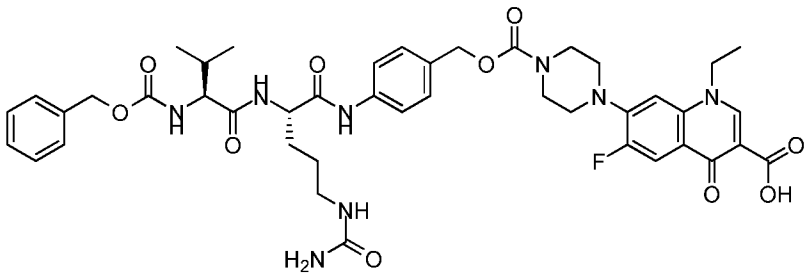
^{1}H NMR DMSO-d_6 400 MHz δ 10.08 (s, 1 H), 8.96 (s, 1 H), 8.22 (d, J = 7.2 Hz, 1 H), 7.95 (d, J = 13.2 Hz, 1 H), 7.62 (s, 1 H), 7.60 (s, 1 H), 7.53 (d, J = 8.4 Hz, 1 H), 7.50 (s, 1 H), 7.36 - 7.33 (m, 5 H), 7.30 (d, J = 7.6 Hz, 4 H), 7.21 (d, J = 7.2 Hz, 1 H), 6.33 - 6.32 (m, 1 H), 6.13 (d, J = 2.4 Hz, 1 H), 5.99 - 5.96 (m, 1 H), 5.42 (s, 2 H), 5.07 (s, 2 H), 5.01 (d, J = 1.6 Hz, 2 H), 4.61 - 4.56 (m, 2 H), 4.45 - 4.35 (m, 2 H), 3.62 (d, J = 10.4 Hz, 5 H), 3.06 - 3.01 (m, 2 H), 2.89 - 2.83 (m, 2 H), 2.55 - 2.45 (m, 2 H), 1.71 - 1.58 (m, 2 H), 1.42 (s, 5 H).

Example 49. 7-(4-((4-((S)-2-((S)-2-(benzyloxycarbonylamino)-3-methylbutanamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 49





example 49

Step 1. Compound **49-1** (100 mg, 0.26 mmol) and compound **49-2** (138 mg, 0.38 mmol) were dissolved in DMF (5 mL) at r.t. The reaction mixture was stirred at r.t. for 3 h, filtered and purified by prep-HPLC to give **49-3** (100 mg, Yield: 54 %).

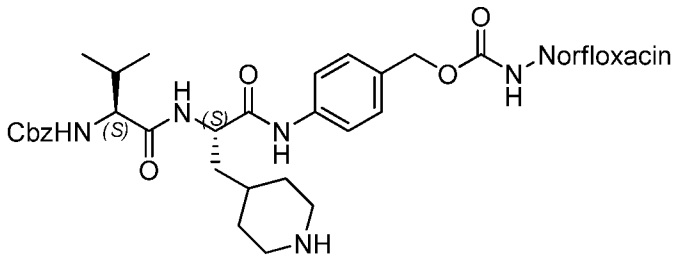
Step 2. To a solution of compound **49-3** (60 mg, 0.142 mmol) in dry DMF (3 mL) was added PNP carbonate (87 mg, 0.285 mmol) and DIPEA (56 mg, 0.427 mmol) at r.t. The mixture was stirred at r.t. for 16 h. Norfloxacin (91 mg, 0.285 mmol) was added. The mixture was stirred at r.t. for another 1 h. The mixture was concentrated, filtered and purified by prep-HPLC (FA) to give **example 49** (40 mg, yield: 37%).

LCMS: (5-95, AB, 1.5 min, ESI), 0.866 min, MS = 859.2[M+1]

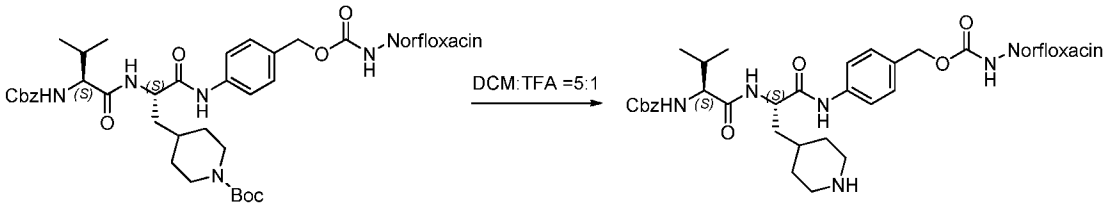
^{1}H NMR DMSO-d_6 400 MHz, δ 15.32 - 15.28 (m, 1 H), 10.08 (s, 1 H) , 8.96 (s, 1 H), 8.11 (d, J = 7.2 Hz, 1 H), 7.94 (d, J = 13.2 Hz, 1 H), 7.61 (d, J = 8.4 Hz, 2 H), 7.37 - 7.30 (m, 8 H), 7.21 (d, J =

7.2 Hz, 1 H), 5.78 (d, *J* = 4.8 Hz, 1 H), 5.41 (s, 2 H), 5.05 (d, *J* = 9.6 Hz, 4 H), 4.58 (d, *J* = 7.2 Hz, 2 H), 4.41 (d, *J* = 5.6 Hz, 1 H), 3.93 (s, 1 H), 3.33 (s, 4 H), 3.04 - 2.93 (m, 2 H), 2.00 - 1.95 (m, 1 H), 1.68 (s, 2 H), 1.60 - 1.58 (m, 5 H), 1.42 (d, *J* = 7.2 Hz, 6 H).

Example 50. 7-(4-((4-((S)-2-((S)-2-(benzyloxycarbonylamino)-3-methylbutanamido)-3-(piperidin-4-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



example 50



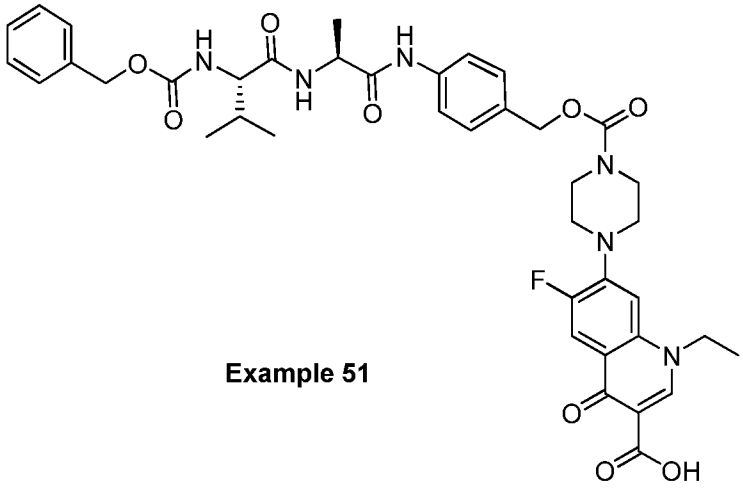
example 56 **example 50**

Step 1. A mixture of DCM/TFA (5:1,v:v) (1 mL) was added to **example 56** (50 mg, 52 mmol) at 0°C. The mixture was allowed to stir at 0°C for 1 h. LCMS showed 80% desired product and 20% STM. The mixture was purified by prep-HPLC (FA), to give **example 50** (31.2 mg, 70%).

LCMS: (5-95, AB, 1.5 min, ESI), 0.811 min, MS = 856.2[M+1], 428.8[1/2M+1]

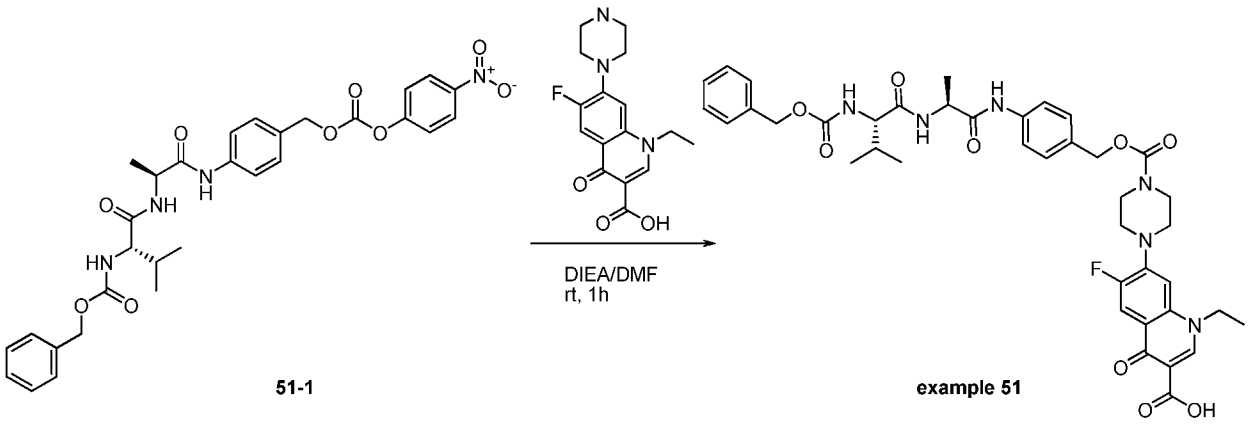
^{1}H NMR Methanol-d_4 400MHz, δ 8.81 (s, 1H), 8.54(s, 1H), 7.98 - 7.95(m, 1H), 7.59 - 7.57 (m, 2H), 7.36 - 7.32 (m, 7H), 7.2 - 7.1(m, 1H), 5.12 - 5.09 (m, 4H), 4.7 - 4.4 (m, 3H), 3.88 - 3.86 (m, 1H), 3.70 (m, 4H), 3.3(m, 4H), 3.0 - 2.8 (m, 2H), 2.1 - 1.7 (m, 6H), 1.6 - 1.3 (m, 5H), 0.98 - 0.96 (m, 6H).

Example 51: 7-(4-{4-[(S)-2-((S)-2-Benzyloxycarbonylamino-3-methyl-butyrylamino)-propionylamino]-benzyloxycarbonyl}-piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid



Example 51

Example 51 was made using the procedure as **Example 42**.



51-1

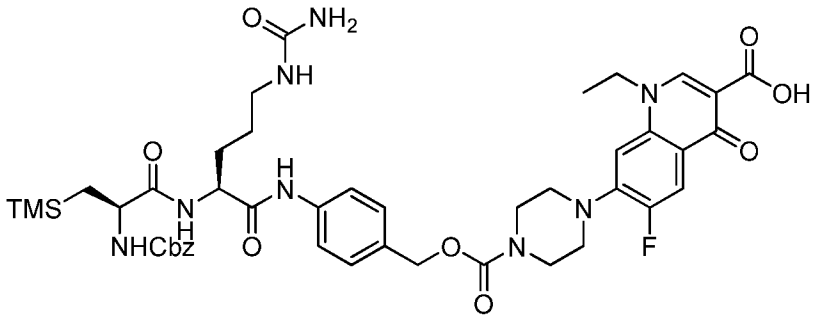
example 51

Step 1. To the suspension of 1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (129 mg, 0.40 mmol) in 2ml DMF, was added DIEA (0.30 ml, 1.69 mmol), then added 51-1 (120 mg, 0.34 mmol) as one portion, slowly became yellow solution till reaction completed. The reaction mixture was poured into ice water, extracted with DCM. The organic layer washed by Sat. NH4Cl, brine, dried over MgSO4, was concentrated down, purified by Pre-HPLC to yield 51 (125 mg, 48%).

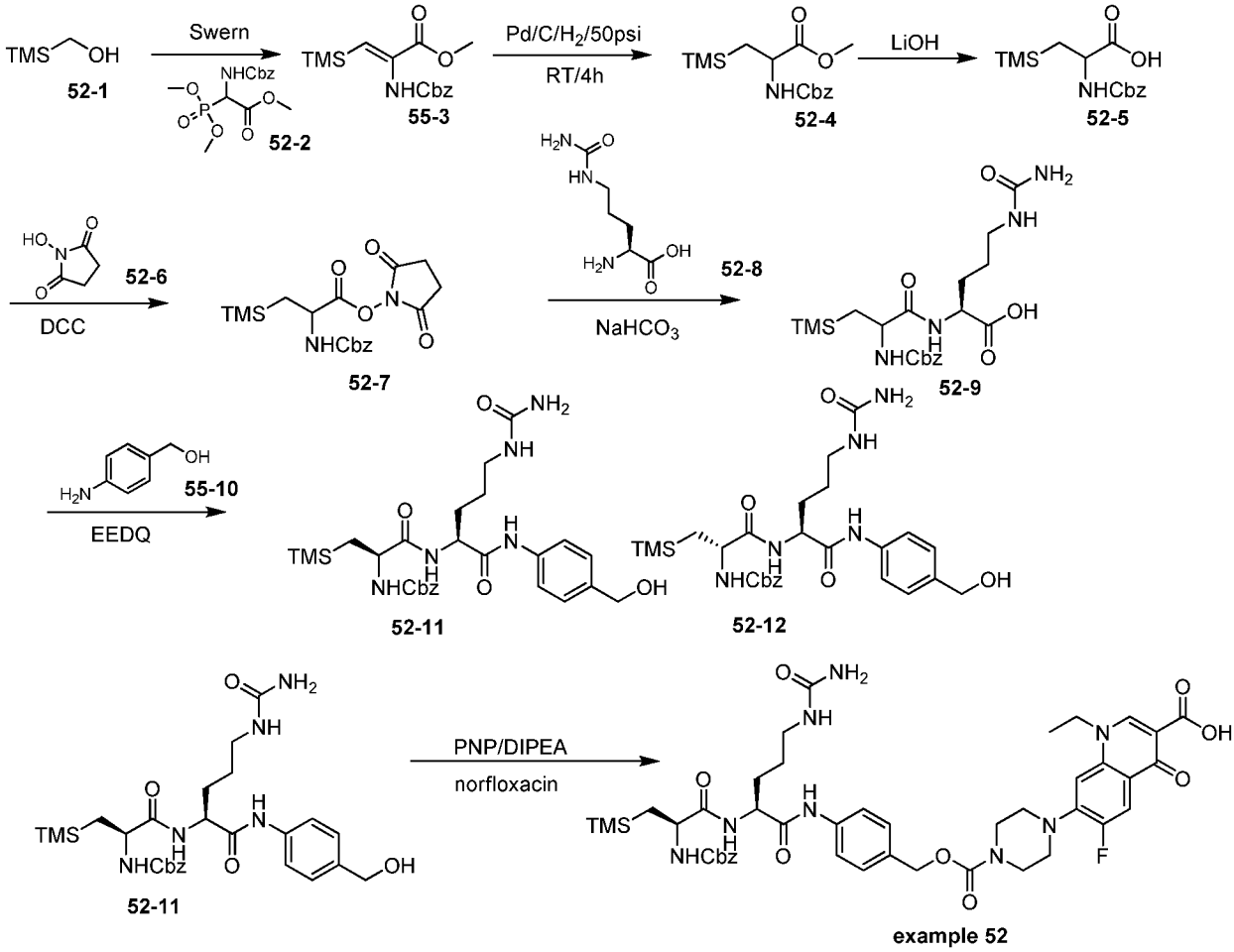
LCMS (ESI): m/z 773.3 [M+H$^+$].

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.29 (s, 1H), 9.98 (s, 1H), 8.95 (s, 1H), 8.14 (d, J = 6.8 Hz, 1H), 7.95 (d, J = 13.1 Hz, 1H), 7.59 (d, J = 8.3 Hz, 2H), 7.47 – 7.12 (m, 8H), 5.05 (d, J = 9.9 Hz, 4H), 4.70 – 4.48 (m, 2H), 4.42 (t, J = 7.0 Hz, 1H), 3.91 (t, J = 7.8 Hz, 1H), 3.61 (s, 4H), 1.98 (d, J = 6.8 Hz, 1H), 1.53 – 1.14 (m, 6H), 0.86 (dd, J = 17.7, 6.7 Hz, 6H).

Example 52. 7-(4-((4-((S)-2-((R)-2-(benzyloxycarbonylamino)-3-(trimethylsilyl)propanamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 52



Step 1. To a stirred solution of **52-1** (3.31 g, 10.0 mmol) in DCM (10 mL) at 10°C was added DBU (1.67 g, 11.0 mmol) and stirred at r.t. for 1 h. To a stirred solution of $(COCl)_2$ (1.83 g, 14.4 mmol) in DCM (30 mL) at -78°C was added DMSO (1.28 g, 16.32 mmol). After 15 min, a mixture of compound **52-1** (1.0 g, 9.60 mmol) with DBU was added over 5 min. After 30 min, Et_3N (3.59 g, 35.52 mmol) was added and after 30 min, the ylide **52-2** was added at -78°C. After it was for 30

min, the reaction mixture was warmed to r.t. and stirred at r.t. for 6 h. The mixture was quenched with 1M HCl solution. After removal of the solvent, the residue was extracted with EtOAc (60 mL x 3). The organic layer was washed with brine (60 mL), dried with Na_2SO_4 and concentrated. The residue was purified by prep-HPLC to give compound **52-3** (940 mg, 32%).

^{1}H NMR: 400 MHz, $CDCl_3$, δ 7.37 - 7.30 (m, 5 H), 6.58 (s, 1 H), 5.15 (s, 2 H), 3.76 (s, 3 H), 0.16 - 0.13 (s, 9 H). LCMS (ESI): m/z 308.1 [M+H$^+$].

Step 2. Wet Pd/C (0.1 g) was added to the solution of compound **52-3** (0.5 g, 1.6 mmol) in MeOH (50 mL) at r.t. The reaction mixture was stirred under H_2 (50 psi) at r.t. for 4 h. The reaction mixture was filtered and the filtrate was concentrated to afford compound **52-4**, which was used directly in the next step without further purification.

^{1}H NMR: $CDCl_3$, 400 MHz, δ 7.36 - 7.30 (m, 5 H), 5.10 (s, 2 H), 4.42 - 4.36 (m, 1 H), 3.72 (s, 3 H), 1.17 - 1.11 (dd, J = 6.4, 14.8 Hz, 1 H), 0.99 - 0.93 (dd, J = 9.2, 14.8 Hz, 1 H), 0.04 (s, 9 H). LCMS (ESI): m/z 310.1 [M+H$^+$].

Step 3. To a solution of compound **52-4** (220 mg, 0.7 mmol) in THF/H_2O (5 mL/5 mL) was added LiOH (44 mg, 1 mmol) at 0°C. The reaction mixture was warmed to r.t. and stirred for 20 h. After removal of the solvent, the residue was extracted with EtOAc (20 mL) and the aqueous layer was acidified with 1 M HCl to pH = 2 and extracted with EtOAc (30 mL x 2). The organic layer was washed with brine (20 mL x 2), dried over sodium sulfate and concentrated. The residue was purified by prep-HPLC to afford compound **52-5**.

LCMS (ESI): m/z 280.1 [M+H$^+$].

Step 4. To a solution of compound **52-5** (145 mg, 0.49 mmol) and **52-6** (57 mg, 0.495 mmol) in dry THF (20 mL) was added a solution of DCC (102.3 mg, 0.495 mmol) in dry THF (10 mL) dropwise at 0°C. The reaction mixture was stirred at 0-5 °C for 3 h. After removal of the solvent, the residue was dissolved in DCM (20 mL). The precipitate was filtered and the filtrate was concentrated to afford the crude product **52-7** as a white solid, which was used in next step without further purification.

Step 5. To the solution of compound **52-7** (obtained above, 0.49 mmol) in DME (15 mL) and H_2O (15 mL) was added compound **52-8** (129.5 mg, 0.74 mmol) and $NaHCO_3$ (61.8 mg, 0.74 mmol). The mixture was stirred at r.t. for 16 h under N_2. After removal of the solvent, the residue (**52-9**) was used in next step without further purification.

^{1}H NMR: DMSO-d_6, 400 MHz, δ 7.97 (dd, J = 7.6, 29.2 Hz, 1 H), 7.35 - 7.29 (m, 5 H), 5.93 - 5.92 (m, 1 H), 5.36 (s, 2 H), 5.07 - 4.98 (m, 2 H), 4.15 - 4.07 (m, 2 H), 2.95 - 2.90 (m, 2 H), 1.75 - 1.64 (m, 1 H), 1.58 - 1.49 (m, 1 H), 0.98 - 0.87 (m, 2 H), -0.01 (s, 9 H).

Step 6. To the solution of compound **52-9** (160 mg, 0.35 mmol) and (4-aminophyl)methanol (65.2 mg, 0.53 mmol) in dry DCM (10 mL) was added EEDQ (175 mg, 0.71 mmol) at 0 °C under N_2. The reaction mixture was warmed to r.t. and stirred for 1 h under N_2. After removal of the solvent, the residue was purified by prep-HPLC and SFC to give **52-10** and **52-11.**

LCMS: (10-80, AB, 2 min, ESI), 1.151 min, MS = 558.1[M+1]

^{1}H NMR Methanol-d_4, 400MHz, δ 9.99 (s, 1 H), 7.92 (d, J = 8.0 Hz, 1 H), 7.55 (d, J = 8.8 Hz, 1 H), 7.45 - 7.24 (m, 8 H), 6.01 - 5.98 (m, 1 H), 5.41 (s, 2 H), 5.11 (t, J = 5.2 Hz, 1 H), 5.05 (d, J = 4.8 Hz, 2 H), 4.44 (d, J = 5.6 Hz, 3 H), 4.15 - 4.08 (m, 1 H), 3.08 - 2.87 (m, 2 H), 1.72 - 1.41 (m, 4 H), 1.02 - 0.90 (m, 2 H), 0 (s, 9 H).

LCMS: (10-80, AB, 2 min, ESI), 1.130 min, MS = 558.1[M+1]

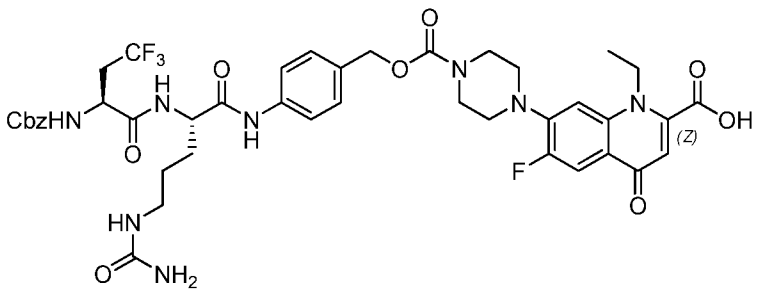
^{1}H NMR Methanol-d_4, 400MHz, δ 9.89 (s, 1 H), 8.16 (d, J = 8.0 Hz, 1 H), 7.59 - 7.57 (d, J = 8.8 Hz, 2 H), 7.45 - 7.24 (m, 8 H), 6.01 - 5.95 (m, 1 H), 5.41 (s, 2 H), 5.11 (t, J = 5.6 Hz, 1 H), 5.03 (d, J = 11.2 Hz, 2 H), 4.43 (d, J = 5.6 Hz, 2 H), 4.42 - 4.38 (m, 1 H), 4.20 - 4.12 (m, 1 H), 3.05 - 2.87 (m, 2 H), 1.72 - 1.41 (m, 4 H), 0.95 (d, J = 8.0 Hz, 2 H), 0 (s, 9 H).

Step 7. To the solution **52-11** (30 mg, 0.066 mmol) in dry DCM (5 mL), was added PNP carbonate (40.4 mg, 0.13 mmol) and DIPEA (0.5 mL). The mixture was heated at reflux for 20 h. After removal of the solvent, the residue was dissolved in DMF (3 mL). DIPEA (0.5 mL) and norfloxacin (63.5 mg, 0.2 mmol) were added. The mixture was stirred at r.t. for 2 h. The reaction mixture was purified by prep-HPLC to give **example 52**.

LCMS: (10-80, AB, 2 min, ESI), 1.269min, MS = 903.0[M+1]

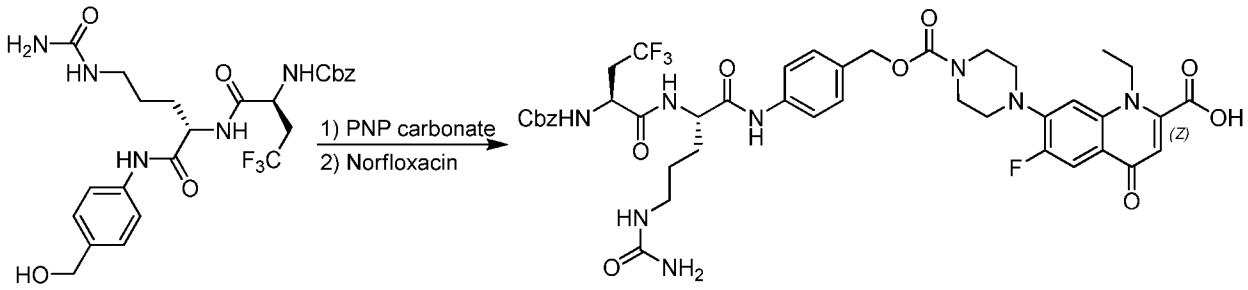
^{1}H NMR DMSO-d_6, 400 MHz, δ 10.00 (s, 1 H), 8.97 (s, 1 H), 8.18 (d, J = 10.4 Hz, 1 H), 7.94 (d, J = 13.2 Hz, 1 H), 7.63 (d, J = 8.4 Hz, 2 H), 7.40 (d, J = 8.4 Hz, 1 H), 7.35 - 7.30 (m, 7 H), 7.21 (d, J = 7.6 Hz, 1 H), 5.96 (t, J = 6.4 Hz, 1 H), 5.41 (s, 2 H), 5.06 (s, 2 H), 5.02 (d, J = 9.6 Hz, 2 H), 4.61 - 4.55 (m, 2 H), 4.41 - 4.36 (m, 1 H), 4.21 - 4.12 (m, 1 H), 3.61 (s, 4 H), 3.33 (s, 4 H), 3.03 - 2.90 (m, 2 H), 1.71 - 1.68 (m, 1 H), 1.58 - 1.53 (m, 1 H), 1.41 (t, J = 7.2 Hz, 4 H), 1.42 - 1.31 (m, 1 H), 0.93 (d, J = 6.8 Hz, 2 H), -0.01 (s, 9 H).

Example 53. 7-(4-((4-((S)-2-((S)-2-(benzyloxycarbonylamino)-4,4,4-trifluorobutanamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-2-carboxylic acid



example 53

Example 53 was made using the procedure as **Example 46**, with the intermediate from the synthesis of **Example 46**.

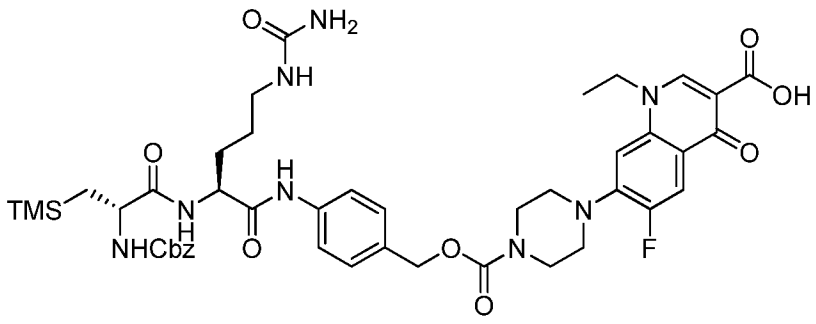


46-6B **example 53**

Step 1. To the solution of **46-6B** (23 mg, 0.041 mmol) in dry DCM (2 mL) was added PNP carbonate (25 mg, 0.082 mmol) and of DIPEA (16 mg). The mixture was heated at reflux for 16 h. After the solvent was removed, the residue was dissolved in of DMF (5 mL). DIPEA (16 mg, 0.123 mmol) and norfloxacin (26 mg, 0.082 mmol) was added, and the mixture was stirred at r.t. for 1 h. Solvent was removed and the residue was purified by prep-HPLC to afford **example 53.**

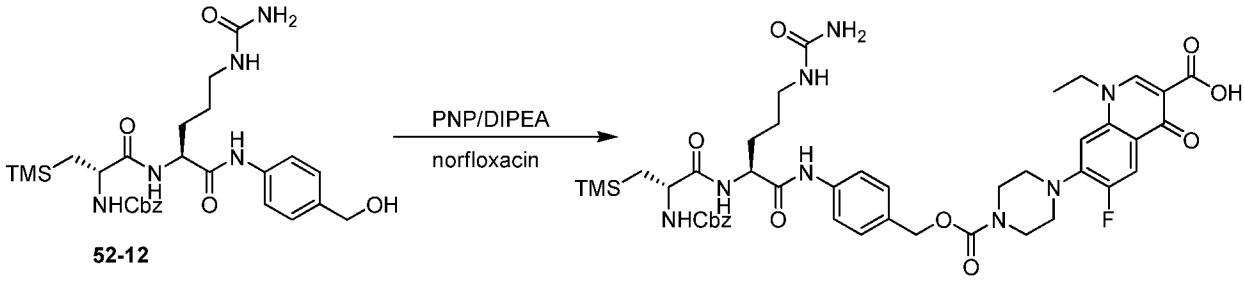
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.33 (s, 1 H), 10.03 (s, 1 H), 8.97 (s, 1 H), 8.51 (d, J = 8.0 Hz, 1 H), 7.45 (d, J = 13.2 Hz, 1 H), 7.79(d, J = 8.4 Hz, 1 H), 7.63 (d, J = 8.4 Hz, 2 H), 7.36-7.21(m, 8 H), 5.98-5.96 (m, 1 H), 5.41 (s, 2 H), 5.07-5.01 (m, 4 H), 4.59-4.42 (m, 4 H), 3.61(s, 4 H), 3.31(s, 4 H), 3.17-2.50 (m, 4 H), 1.71-1.60 (m, 7 H). LCMS (ESI): m/z 898.8 [M+H$^+$], 450.3 [M/2+H$^+$].

Example 54. 7-(4-((4-((S)-2-((S)-2-(benzyloxycarbonylamino)-3-(trimethylsilyl)propanamido)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 54

Example 54 was made using the procedure as **Example 52**, with the intermediate from the synthesis of **Example 52**.



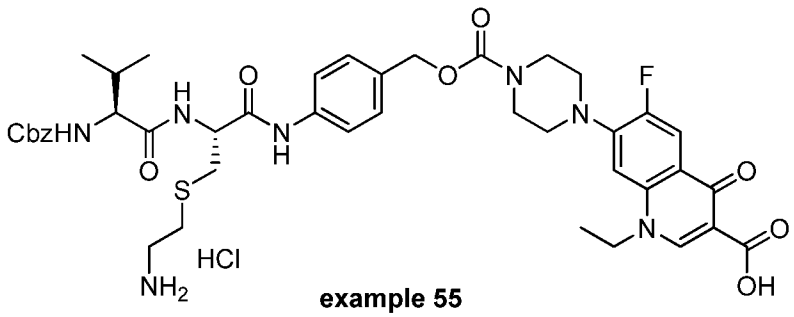
52-12

example 54

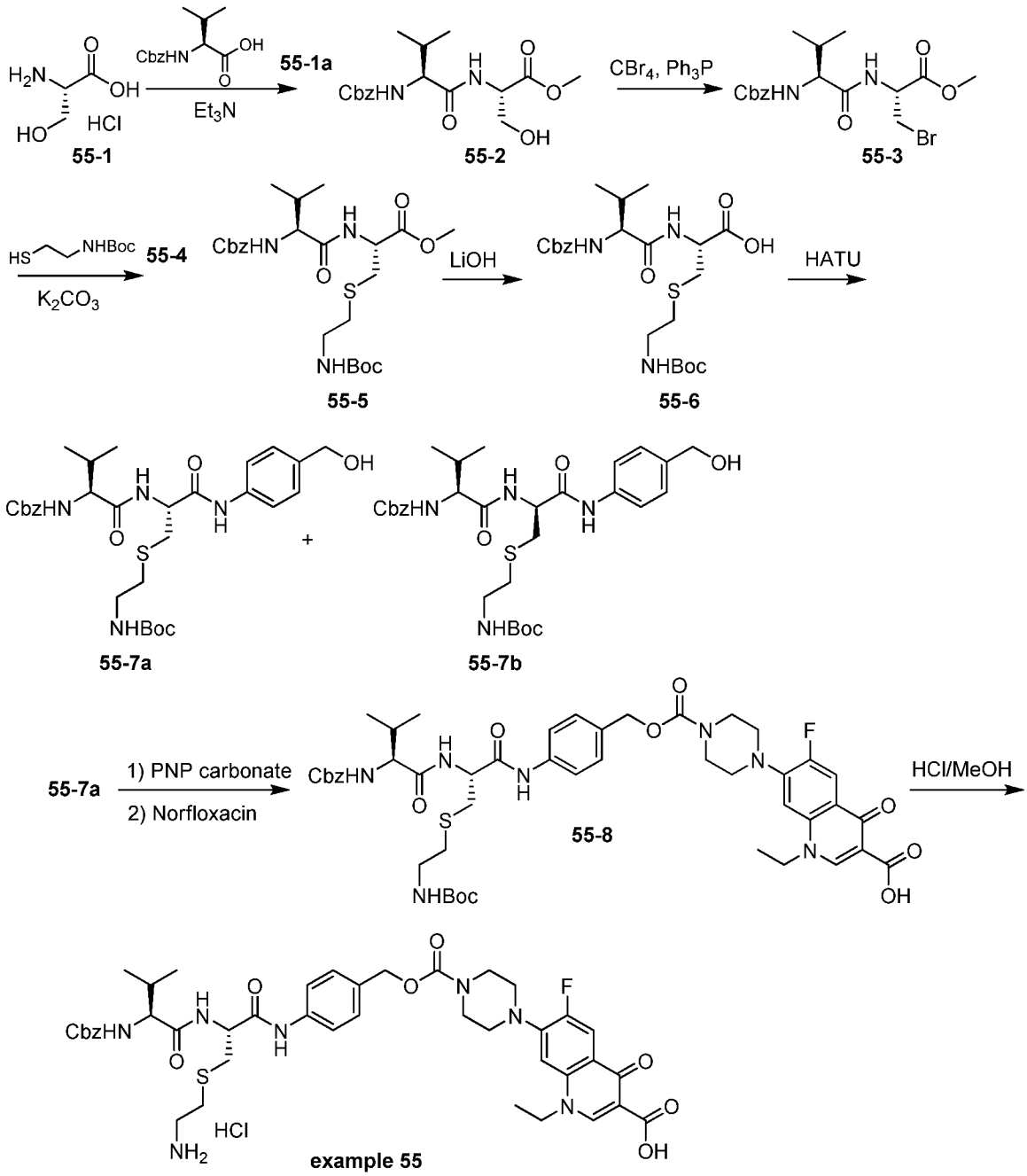
LCMS: (10-80, AB, 2 min, ESI), 1.274min, MS = 903.0[M+1]

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.10 (s, 1 H), 8.97 (s, 1 H), 7.95 (d, J = 13.2 Hz, 2 H), 7.61 (d, J = 8.4 Hz, 2 H), 7.40 (d, J = 8.4 Hz, 1 H), 7.37 - 7.29 (m, 7 H), 7.21 (d, J = 10.8 Hz, 1 H), 5.98 (t, J = 6.4 Hz, 1 H), 5.05 (d, J = 8.8 Hz, 2 H), 5.01 (d, J = 13.2 Hz, 2 H), 4.62 - 4.56 (m, 2 H), 4.43 - 4.40 (m, 1 H), 4.14 - 4.08 (m, 1 H), 3.61 (s, 4 H), 3.33 (s, 4 H), 3.05 - 2.91 (m, 2 H), 1.76 - 1.52 (m, 2 H), 1.48 - 1.39 (m, 5 H), 1.01 - 0.89 (m, 2 H), -0.01 (s, 9 H).

Example 55. 7-(4-((4-((R)-3-(2-aminoethylthio)-2-((S)-2-(benzyloxycarbonylamino)-3-methylbutanamido) propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 55



Step 1. To a mixture of compound **55-1** (1.55 g, 10 mmol), **55-1a** (2.51 g, 10 mmol) and Et_3N (3.03 g, 30 mmol) in DMF (20 mL) was added HATU (3.8 g, 10 mmol). After the mixture was stirred at r.t. for 3 h, it was extracted with EtOAc (60 mL x 3). The organic layer was washed with brine (60 mL x 3), dried over $NaSO_4$, concentrated in vacuo and purified by column (20% EtOAc in hexane) to give compound **55-2** (3.21 g, 91.2%).

LCMS (ESI): m/z 353.1 [$M+H^+$].

Step 2. Triphenylphosphine (2.87 g, 10.94 mmol) in dry DCM (60 mL) was added dropwise over 10-15 min to a well stirred solution of compound **55-2** (3.21 g, 9.12 mmol) and tetrabromomethane (4.54 g, 13.68 mmol) in dry DCM at 0°C. After the mixture was stirred at r.t. for 9-10 h, it was

treated with pentane (200 mL) and the resulting precipitate was removed by filtration and washed several times with pentane. The combined pentane solution was washed with 5% $NaHCO_3$, water, brine and dried over Na_2SO_4. The solvent was evaporated and the oil was purified by column chromatography on silica gel (hexane: EtOAc = 4:1) to give compound **55-3** (3.43 g, 90.7%).

LCMS (ESI): m/z 414.9 [M+H$^+$].

Step 3. To the solution of compound **55-3** (3.43 g, 8.3 mmol) in DMF (5 mL) was added compound **55-4** (1.12 g, 4.0 mmol) and K_2CO_3 (1.15 g, 8.3 mmol). After the mixture was stirred at r.t. for 16 h, it was extracted with EtOAc (100 mL x 2). The organic layer was washed with brine (60 mL), dried over Na_2SO_4, and concentrated to give the crude product of compound **55-5** (3.67 g, 86.4%).

LCMS (ESI): m/z 412.0 [M-BOC+H$^+$], 534.1 [M+Na$^+$].

Step 4. To a solution of compound **55-5** (3.67 g, 6.22 mmol) in THF/H_2O (30 mL/10 mL) was added LiOH.H_2O (2.6 g, 62.2 mmol). After the mixture was stirred at r.t. for 16 h, solvent was removed, and water (30 mL) was added. It was extracted with EtOAc (80 mL x 3). The organic layer was washed with brine (60 mL), dried over Na_2SO_4, concentrated in vacuo to give compound **55-6**, which was used for next step without further purification (3 g, 96.9%).

LCMS (ESI): m/z 398.2 [M-BOC+H$^+$].

Step 5. To a solution of compound **55-6** (500 mg, 1.0 mmol), (4-amino-phenyl)-methanol (246 mg, 2 mmol) and DIPEA (258 mg, 2 mmol) in DCM (10 mL) was added HATU (380 mg, 1.0 mmol) at 0°C. After the mixture was stirred at 0°C for 2 h, solvent was removed, and the residue was purified with prep-HPLC and SFC separation to afford **55-7a** and **55-7b**.

55-7a

^{1}H NMR (400 MHz, MeOD) δ 7.60 (d, J = 8.8 Hz, 2 H), 7.31-7.24 (m, 7 H), 5.06 (s, 2 H), 4.67-4.64 (m, 1 H), 4.53 (s, 2 H), 3.88 (d, J = 7.6 Hz, 1 H), 3.28-3.11 (m, 3 H), 2.89-2.84 (m, 1 H), 2.67-2.57 (m, 2 H), 2.08-1.99 (m, 1 H), 1.41 (s, 9 H), 1.01-1.99 (m, 6 H). LCMS (ESI): m/z 503.0 [M+H$^+$-BOC].

55-7b

^{1}H NMR (400 MHz, MeOD) δ 7.56 (d, J = 8.8 Hz, 2 H), 7.34-7.25 (m, 7 H), 5.08 (s, 2 H), 4.66-4.63 (m, 1 H), 4.54 (s, 2 H), 3.96 (d, J = 7.6 Hz, 1 H), 3.22-3.04 (m, 3 H), 2.90-2.80 (m, 1 H), 2.69-2.57 (m, 2 H), 2.13-2.03 (m, 1 H), 1.40 (s, 9 H), 0.98-0.94 (m, 6 H). LCMS (ESI): m/z 502.9 [M+H$^+$-BOC], 624.5 [M+Na$^+$].

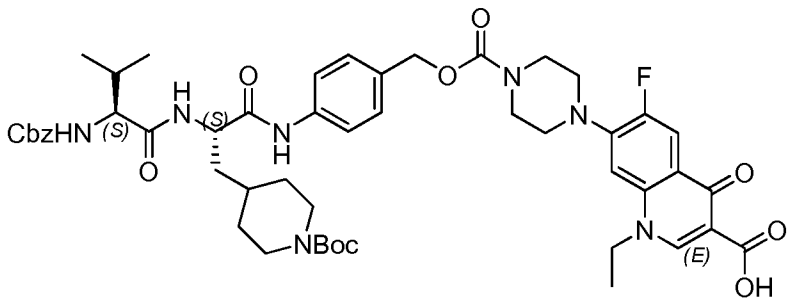
Step 6. To a solution of **55-7a** (28 mg, 0.047 mmol) in dry DCM (2 mL) was added PNP carbonate (30 mg, 0.094 mmol) and DIPEA (0.2 mL). The mixture was heated at reflux for 16 h. After solvent was removed, residue was dissolved in of DMF (2 mL). DIPEA (0.2 mL) and norfloxacin (29 mg, 0.094 mmol) was added. The mixture was stirred at r.t. for 1 h. Solvent was removed, and the residue was purified by prep-HPLC to give compound **55-8** (38 mg, 84.4%).

LCMS (ESI): m/z 948.4 [M+H$^+$].

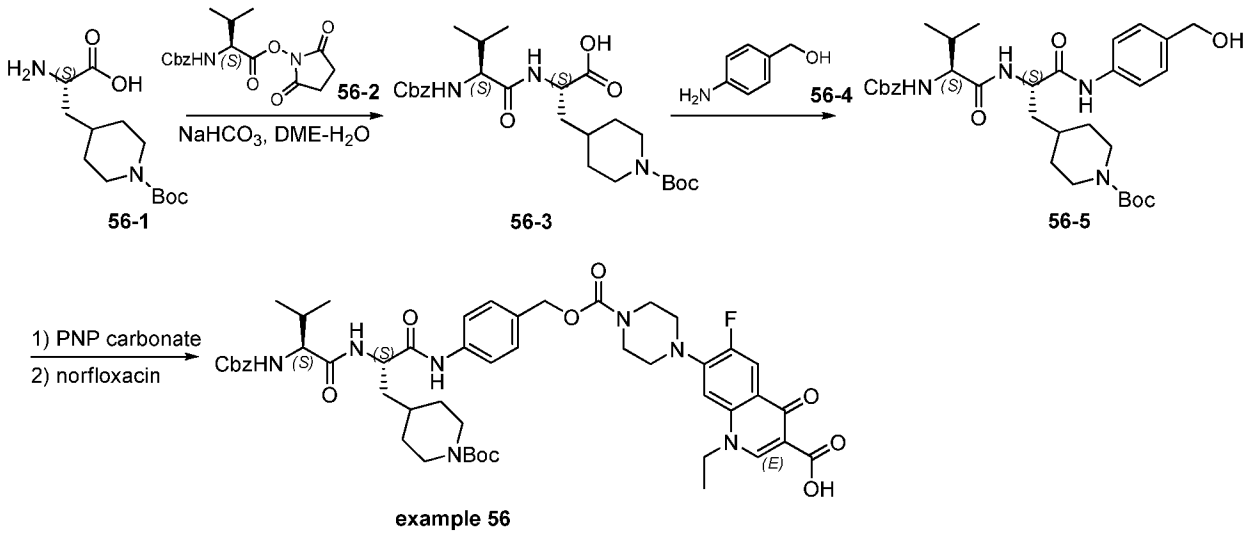
Step 7. To a solution of compound **55-8** (38 mg, 0.04 mmol) in dry DCM (10 mL) was added HCl/CH_3OH (4 mol/L) at 0°C over 10 min. After the mixture was stirred at r.t. for 1 h, solvent was removed and the residue was purified by prep-HPLC to give **example 55** as a white solid.

^{1}H NMR (400 MHz, MeOD) δ 8.86 (s, 1 H), 7.97 (d, J = 13.2 Hz, 1 H), 7.66 (d, J = 8.4 Hz, 2 H), 7.34 (d, J = 8.8 Hz, 2 H), 7.27-7.22 (m, 5 H), 7.14 (d, J = 7.2 Hz, 1 H), 5.08 (d, J = 21.6 Hz, 4 H), 4.73-4.51 (m, 1 H), 4.50-4.48 (m, 2 H), 3.81 (d, J = 8.0 Hz, 1 H), 3.69 (s, 4 H), 3.34 (s, 4 H), 3.25-2.91 (m, 3 H), 2.90-2.79 (m, 3 H), 2.09-2.00 (m, 1 H), 1.55-1.45 (m, 3 H), 1.09-1.00 (m, 6 H).
LCMS (ESI): m/z 848.1 [M+H$^+$].

Example 56. 7-(4-((4-((S)-2-((S)-2-(benzyloxycarbonylamino)-3-methylbutanamido)-3-(1-(tert-butoxycarbonyl)piperidin-4-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 56



Step 1. To a solution of compound **56-2** (150 mg, 0.551 mmol) in THF / H_2O (10 mL / 2 mL) were added $NaHCO_3$ (138 mg, 1.653 mmol) and compound **56-1** (287 mg, 0.827 mmol). After the mixture was stirred at 25°C overnight, it was acidified to pH = 6 with HCl (1 N), extracted with EtOAc (50 mL x 3). The organic layers were dried and concentrated to give **56-3**, which was purified by column chromatography on silica gel (PE / EtOAc =10 / 1 to 1 / 10).

LCMS: (0-60, AB, 2 min, ESI), 1.309 min, MS = 406.3 [M+1-Boc$^+$]

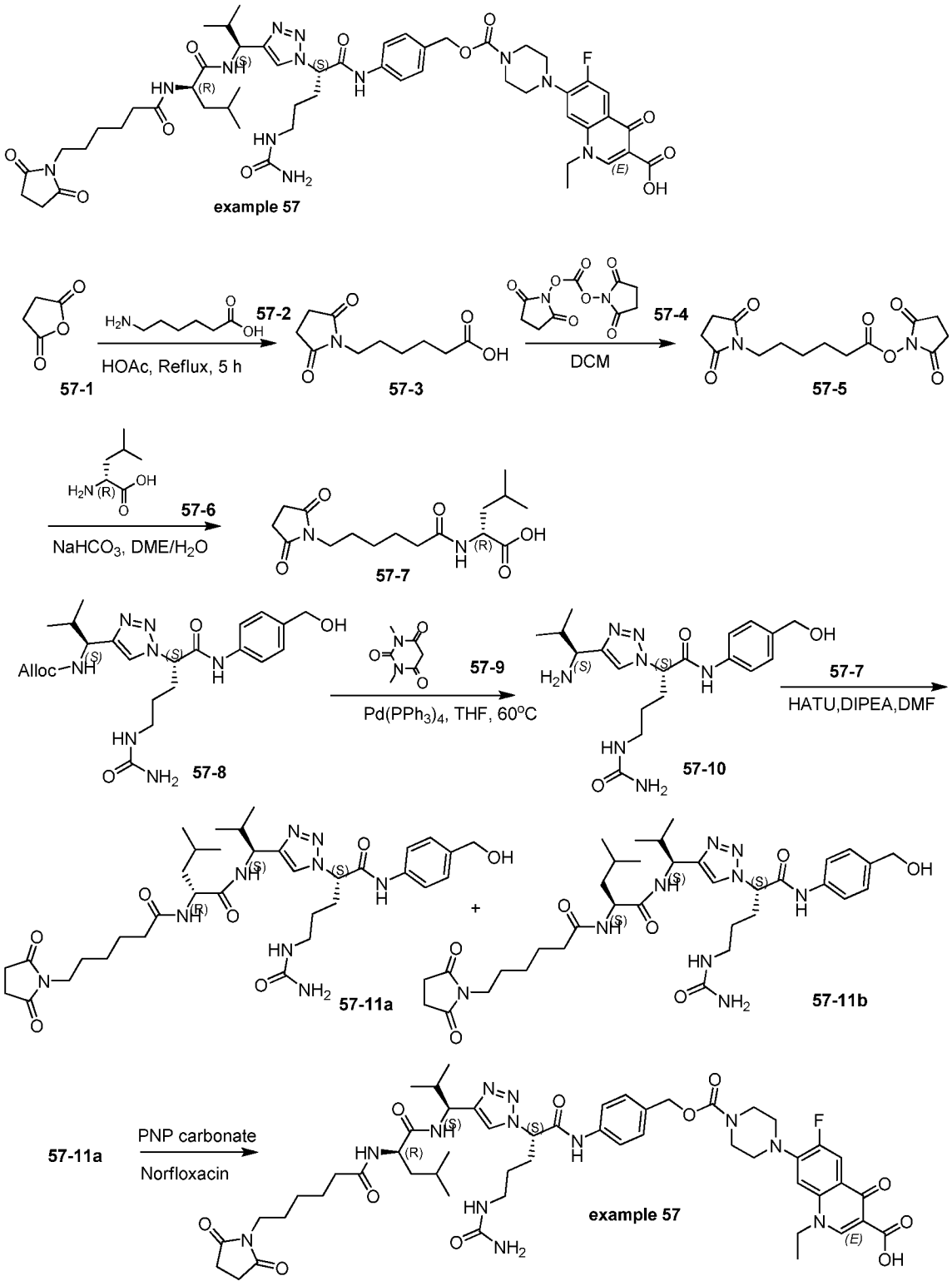
Step 2. To a solution of compound **56-3** (1.2 g, 2.376 mmol) in DCM (20 mL) were added compound **56-4** (439 mg, 3.56 mmol), and HATU (1.7 g, 5.064 mmol). The mixture was stirred at 25°C overnight. The solution was quenched with H_2O, and extracted with DCM (50 mL x 3). The organic layers were dried, and concentrated and purified by column chromatography on silica gel (PE / EtOAc = 10 / 1 to 1 / 10) to give **56-5**.

LCMS: (0-60, AB, 2 min, ESI), 1.413 min, MS = 511.2 [M+1-Boc$^+$]

Step 3. To compound **56-5** (100 mg, 0.16 mmol) in dry DMF (2 mL) at 0°C, was added DIPEA (0.5 mL, 3 mmol) and PNP carbonate (200 mg, 0.66 mmol, 4.1 eq) under N_2. The mixture was stirred at r.t. overnight. To the mixture was added norfloxacin (102 mg, 0.32 mmol, 2eq), and stirred for 1 h. The mixture was purified by prep-HPLC (FA), to give **example 56** (60 mg, 40%).

LCMS: (5-95, AB, 1.5 min, ESI), 1.064 min, MS = 956.3[M+1]

^{1}H NMR: MeOH-d_4 400MHz, δ 8.84 (s, 1H), 7.97 - 7.93 (dd, J = 16, 1H), 7.59 - 7.57 (m, 2H), 7.35 - 7.27 (m, 7H), 7.20 - 7.10 (m, 1H), 5.11 - 5.07 (m, 4H), 4.6 - 4.5 (m, 1H), 4.55 - 4.45 (m, 2H), 4.10 - 3.90 (m, 3H), 3.75 - 3.65 (m, 4H), 3.3 (m, 4H), 2.80 - 2.50 (m, 2H), 2.1 - 2.0 (m, 1H), 1.8 - 1.6 (m, 5H), 1.6 - 1.5 (m, 3H), 1.43 (s, 9H), 1.2 - 1.0 (m, 2H), 0.96 - 0.94 (d, J = 6.8 Hz, 6H).



Step 1. A solution of compound **57-2** (10 g, 100 mmol) in glacial acid (30 mL) was added to a solution of compound **57- 1** (13.1 g, 100 mmol) in glacial acid (150 mL) under N_2 over a period of 15 min. After it was stirred at r.t. for 1 h, the white heterogeneous reaction mixture was heated at reflux for 5 h. Most of the acetic acid was removed under vacuum. The residual was partitioned between water and DCM. DCM was removed and the brown oil was dissolved in small amount of

EtOAc and poured to PE. White precipitate was collected by filtration to give **57-3** (12 g, Yield: 56 %).

^{1}H NMR DMSO-d_6 400 MHz, δ 11.98 (s, 1 H), 3.34 - 3.31 (m, 2 H), 2.61 (m, 4 H), 2.19 - 2.16 (m, 2 H), 1.50 - 1.43 (m, 4 H), 1.23 (d, J = 7.2 Hz, 2 H).

Step 2. To a solution of compound **57-3** (12 g, 56.3 mmol) and excess of triethylamine (25 mL, 168.9 mmol) in DCM (100 mL) was added excess of compound **57-4** (36 g, 140.8 mmol) over 15 min. The mixture was stirred at r.t. for 6 h under N_2. The mixture became homogeneous and stirred at 40°C for 3 h. It was concentrated, washed with water and brine, dried over anhydrous $MgSO_4$. It was concentrated and purified by column chromatography (EtOAc), to give **57-5** (4 g, Yield: 23 %).

^{1}H NMR MeOD-d_4 400 MHz, δ 3.54 - 3.50 (m, 2 H), 2.86 (d, J = 3.2 Hz, 4 H), 2.71 (d, J = 2.8 Hz, 4 H), 2.66 - 2.63 (m, 2 H), 1.79 - 1.75 (m, 2 H), 1.65 - 1.59 (m, 2 H), 1.45 - 1.41 (m, 2 H).

Step 3. To a solution of compound **57-6** (375 mg, 2.86 mmol) in DME (10 mL) was added a solution of compound **57-5** (800 mg, 2.6 mmol) and $NaHCO_3$ (656 mg, 7.8 mmol) in water (10 mL). The mixture was stirred at r.t. for 16 h. The mixture was washed with EtOAc and acidified to pH = 3 with 10% HCl. The resulting suspension was extracted with EtOAc. The combined organic layers was concentrated to give crude compound **57-7** (1.2 g, contains impurity).

Step 4. To a mixture of **compound 57-8** (2.0 g, 4.10 mmol) in THF (50 mL) was added **compound 57-9** (3.8 g, 24.34 mol) and $Pd(PPh_3)_4$ (946 mg, 0.82 mmol). The reaction mixture was stirred at 60 °C for 3 h. The mixture was cooled to r.t. and filtered, the filtrate cake was purified by prep-HPLC to give **57-10** (1.0 g, 60.4 %).

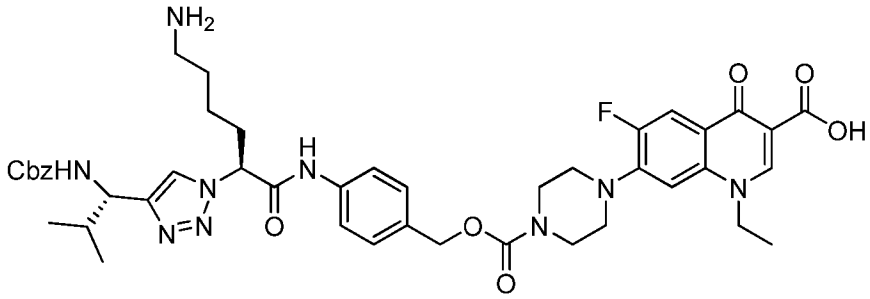
Step 5. Compound **57-7** (100 mg, 0.31mmol), HATU (175 mg, 0.46 mmol), DIPEA (119 mg, 0.92 mmol) were dissolved in DMF (10 ml). The reaction mixture was stirred at r.t. for 30 min. Then compound **57-10** (124 mg, 119 mmol) was added. The reaction mixture was stirred at r.t. for 3 h. Then the mixture was concentrated and purified by prep-HPLC to give **57-11a** and **57-11b** (30 mg each, 5 %).

Step 6. To a solution of compound **57-11a** (30 mg, 0.042 mmol) and PNP carbonate (26 mg, 0.084 mmol) in DMF (2 mL) was added DIPEA (17 mg, 0.127 mmol) at 0°C . The mixture was stirred at r.t. for 16 h and norfloxacin (27 mg, 0.084 mmol) was added at r.t. The mixture was stirred at r.t. for 1 h. The residue was purified by prep-HPLC to give **example 57** (14 mg, Yield: 15 %).

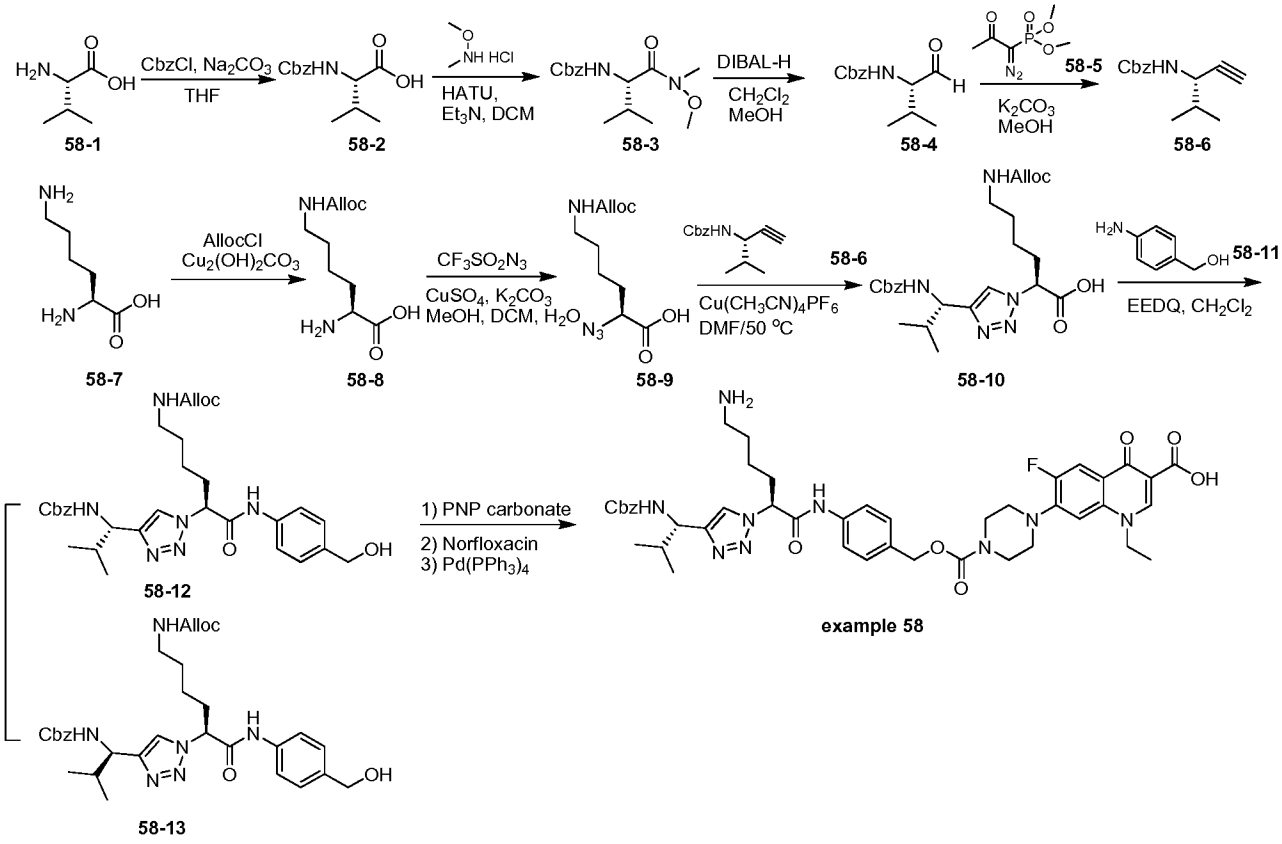
LCMS: (5-95, AB, 1.5 min, ESI), 0.832 min, MS = 529.4[1/2M+1]

^{1}H NMR Methanol-d_4 400 MHz, δ 8.89 (s, 1 H), 8.33 (d, J = 4.6 Hz, 1 H), 8.06 - 8.01 (m, 2 H), 7.63 (d, J = 8.4 Hz, 2 H), 7.50 - 7.40 (m, 1 H), 7.39 (d, J = 8.4 Hz, 2 H), 7.21 (s, 1 H), 5.55 - 5.51 (m, 1 H), 5.15 (s, 2 H), 4.92 (s, 1 H), 4.58 (s, 6 H), 3.74 (s, 4 H), 3.49 - 3.46 (m, 2 H), 3.35 - 3.33 (m, 4 H), 2.69 (s, 4 H), 2.26 - 2.22 (m, 4 H), 1.65 - 1.57 (m, 9 H), 1.56 - 1.29 (m, 5 H), 1.01 (d, J = 6.8 Hz, 3 H) , 0.93 (d, J = 6.4 Hz, 3 H) , 0.92 - 0.81 (m, 6 H)

Example 58. 7-(4-((4-((S)-6-amino-2-(4-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)hexanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 58



Step 1. CbzCl (26.7 mL, 0.19 mol) was added dropwise over 20 min to a mixture of **58-1** (20 g, 0.17 mol) and Na_2CO_3 (36 g, 0.34 mol) in water (100 mL). After it was stirred at r.t. for 12 h, it

was washed with EtOAc (200 mL × 2). The aqueous layer was adjusted to pH = 2 and extracted with EtOAc (200 mL × 4). The combined organic layers were dried over Na_2SO_4 and concentrated to give **58-2**.

Step 2. To a solution of **58-2** (20 g, 79.3 mmol), *N,O*-dimethylhydroxylamine (8.4 g, 87.2 mmol) and HATU (45.2 g, 118.9 mmol) in DCM (200 mL) was added Et_3N (45.8 mL, 317.1 mmol). The mixture was stirred at r.t. for 1 h. After removal of the solvent, the residue was extracted with EtOAc (200 mL × 3). The organic layer was washed with conc. HCl, aqueous $NaHCO_3$, saturated NaCl and concentrated. The crude was purified by column chromatography on silica gel (PE: EtOAc=2:1) to give **58-3**.

Step 3. Compound **58-3** (6.0 g, 20.4 mmol) in DCM (100 mL) was cooled to -78°C in a dry ice/acetone bath. DIBAL-H (30.6 mL, 30.6 mmol) was added dropwise and the mixture was stirred at -78°C for 4 h. Excess hydride was quenched with MeOH (5 mL) and the solution was warmed to r.t. After solvent was removed, **58-4** was used in next step without further purification.

Step 4. The mixture of **58-4** (4.80 g, 20.4 mmol), compound **5** (4.70 g, 24.5 mmol) and K_2CO_3 (5.64 g, 40.8 mmol) in MeOH (60 mL) was stirred at r.t. for 12 h. After removal of the solvent, the residue was extracted with EtOAc (100 mL × 3). The organic layer was washed with brine (60 mL) and dried over Na_2SO_4. The solvent was removed and the crude was purified by column chromatography on silica gel (PE: EtOAc = 10:1) to give **58-6**.

Step 5. A solution of **58-7** (10 g, 68.4 mmol) and $Cu(OH)_2CO_3$ (15.12 g, 68.4 mmol) in H_2O (100 mL) was heated at reflux for 30 min. Solids formed during reflux were removed by filtration while hot. The filtrate was cooled to 0°C and was adjusted to pH 9 by addition of solid Na_2CO_3 (1.0 g). AllocCl (10.8 mL, 102.6 mmol) was added dropwise, while the solution stirred at 0°C. During the addition, the reaction mixture was maintained at pH 9 by the addition of solid Na_2CO_3 (20 g). The reaction mixture was allowed to warm to room temperature and stirred for 12 h. The blue solid product formed during the reaction was collected by filtration in quantitative yield. The solid copper salt of Orn(Alloc) collected above was suspended in H_2O (200 mL) and two equivalents of thioacetamide (6.511 g, 86.66 mmol) were added to. The alkaline suspension was stirred at 50°C for 3 h, during which time, the solid slowly dissolved. The solution was then acidified to pH 2 with 2M HCl and was further boiled for 5 min. The precipitated CuS was removed by filtration. The filtrate was concentrated under vacuum to about 100 mL, at which point the product hydrochloride salt of Orn(Alloc) **58-8** precipitated as a white solid in quantitative yield.

Step 6. A solution of NaN_3 (8.5 g, 129.7 mmol) in distilled H_2O (45 mL) and CH_2Cl_2 (75 mL) was cooled on an ice bath. Tf_2O (4.4 mL, 25.94 mmol) was added slowly over 5 min with stirring continued for 2 h. The mixture was place in a separator funnel and the CH_2Cl_2 phase was removed.

The aqueous portion was extracted with CH_2Cl_2 (35 mL × 2). The organic fractions, containing the triflyl azide were pooled and washed once with saturated Na_2CO_3 and used without further purification. Compound **58-8** (2.99 g, 12.97 mmol) was combined with K_2CO_3 (2.69 g, 19.46 mmol) and $CuSO_4 \cdot 5H_2O$ (486 mg, 1.94 mmol) in distilled H_2O (90 mL) and MeOH (180 mL). The triflyl azide in CH_2Cl_2 (150 mL) was added and the mixture was stirred at r.t. for 12 h. Subsequently, the organic solvents were removed under pressure and the aqueous slurry was diluted with 0.2 M pH 6.2 phosphate buffers (100 mL) and extracted with EtOAc (200 mL × 2) to remove sulfonamide byproduct. The aqueous phase was then acidified to pH 2 with conc.HCl. The product was obtained from another round of EtOAc extractions (400 mL × 3). The EtOAc extracts were combined, dried over Na_2SO_4 and evaporated to give **58-9**, which was used for next step without further purification.

Step 7. The mixture of **58-9** (3.32 g, 12.97 mmol), **58-6** (1.50 g, 6.48 mmol) and $Cu(CH_3CN)_4PF_6$ (362 mg, 0.97 mmol) in DMF (10 mL) was stirred at 50°C for 2 h. After removal of the solvent, the residue was purified by prep-HPLC to give **58-10** (2.3 g, 36.4%).

Step 8. The mixture of **58-10** (2.33 g, 4.78 mmol), **58-11** (1.76 mg, 14.3 mmol) and EEDQ (3.55 g, 14.3 mmol) in DCM (50 mL) was stirred at r.t. for 1 h. After removal of the solvent, the residue was purified by prep-HPLC and SFC separation to afford **58-12** (326 mg) and **58-13** (30 mg).

^{1}H NMR (400 MHz, MeOD) δ 8.04 (s, 1 H), 7.55-7.53 (m, 2 H), 7.33-7.30 (m, 7 H), 5.91-5.87 (m, 1 H), 5.45-5.41 (m, 1 H), 5.27-5.23 (m, 1 H), 5.15-5.11 (m, 1 H), 5.07 (d, J = 2.8 Hz, 2 H), 4.70-4.68 (m, 1 H), 4.55 (s, 2 H), 4.48-4.46 (m, 2 H), 3.34-3.32 (m, 1 H), 3.09-3.07 (m, 2 H), 2.28-2.22 (m, 3 H), 1.54-1.50 (m, 2 H), 1.44-1.35 (m, 2 H), 0.93 (d, J = 6.8 Hz, 3 H), 0.87 (d, J = 6.8 Hz, 3 H).

LCMS (ESI): m/z 593.0 [M+H$^+$].

^{1}H NMR (400 MHz, MeOD) δ 8.04 (s, 1 H), 7.55-7.52 (m, 2 H), 7.32-7.27 (m, 7 H), 5.91-5.87 (m, 1 H), 5.43-5.42 (m, 1 H), 5.27-5.22 (m, 1 H), 5.15-5.12 (m, 1 H), 5.07 (d, J =3.6 Hz, 2 H), 4.70-4.68 (m, 1 H), 4.55 (s, 2 H), 4.47 (d, J = 5.2 Hz, 2 H), 3.07-3.05 (m, 2 H), 2.22-2.14 (m, 3 H), 1.54-1.50 (m, 2 H), 1.44-1.20 (m, 2 H), 0.92 (d, J = 6.8 Hz, 3 H), 0.87 (d, J = 6.8 Hz, 3 H).

LCMS (ESI): m/z 593.0 [M+H$^+$].

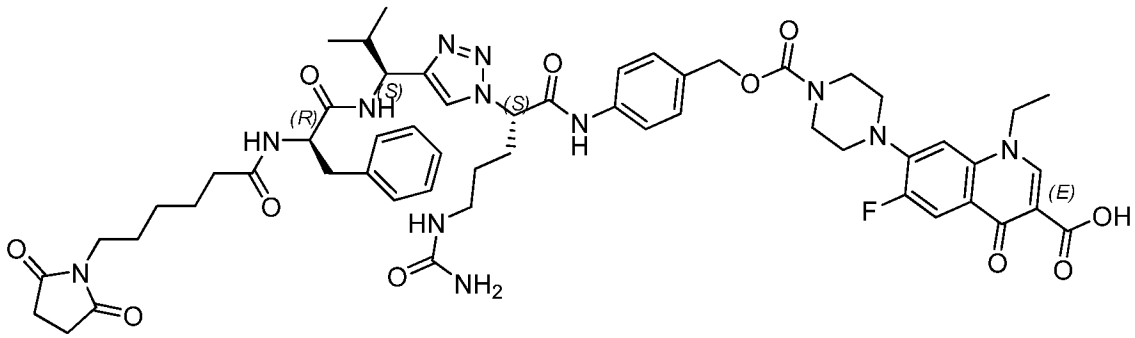
Step 9. The mixture of **58-12** (100 mg, 0.169 mmol), PNP (103 mg, 0.338 mmol) and DIPEA (66 mg, 0.507 mmol) in DCM (5 mL) was stirred at 50°C for 12 h and solvent was removed. A mixture of the above crude product (128 mg, 0.169 mmol), Norfloxacin (160 mg, 0.507 mmol) and DIPEA (66 mg, 0.507 mmol) in DMF (5 mL) was stirred at r.t. for 2 h. After removal of the solvent, the residue was purified by prep-HPLC to give 100 mg of intermediate. To the

intermediate (50 mg, 0.053 mmol) and 1,3-dimethylbarbituric acid (67 mg, 0.426 mmol) in THF (5 mL) was added $Pd(PPh_3)_4$ (12 mg, 0.0106 mmol). The mixture was stirred at 50°C for 12 h. After removal of the solvent, the residue was purified by prep-HPLC to give **example 58** (20.6 mg).

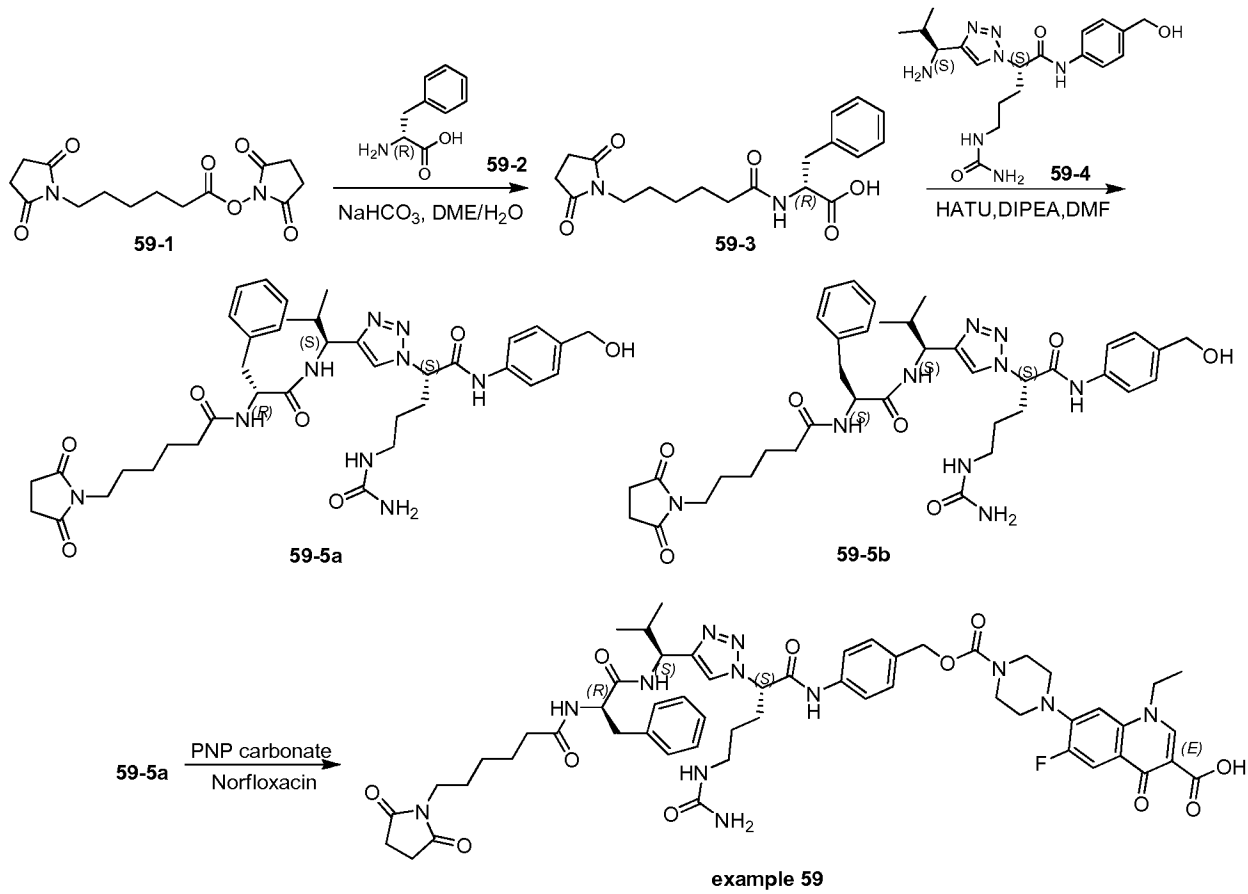
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (s, 1 H), 8.47-8.43 (m, 1 H), 8.09 (s, 1 H), 7.96-7.93 (m, 1 H), 7.74-7.72 (m, 1 H), 7.62-7.60 (m, 2 H), 7.38-7.29 (m, 7 H), 7.22-7.20 (m, 1 H), 5.52-5.49 (m, 1 H), 5.07 (s, 2 H), 5.02 (d, J = 4.8 Hz, 2 H), 4.62-4.58 (m, 3 H), 3.62 (s, 4 H), 3.15 (s, 4 H), 2,67-2.66 (m, 2 H), 2.15-2.09 (m, 3 H), 1.54-1.52 (m, 2 H), 1.40 (t, J = 7.2 Hz, 3 H), 1.23-1.18 (m, 2 H), 0.84 (d, J = 6.4 Hz, 3 H), 0.78 (d, J = 6.4 Hz, 3 H).

LCMS (ESI): m/z 854.1 [M+H$^+$].

Example 59. 7-(4-((4-((S)-2-(4-((S)-1-((R)-2-(6-(2,5-dioxopyrrolidin-1-yl)hexanamido)-3-phenylpropanamido)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy) carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 59



Step 1. To a solution of compound **59-2** (118 mg, 0.71 mmol) in DME (5 mL) was added a solution of compound **59-1** (200 mg, 0.71 mmol) and $NaHCO_3$ (122 mg, 1.42 mmol) in water (5 mL). The mixture was stirred at r.t. for 16 h. The mixture was washed with EtOAc and acidified to pH = 3 with 10% HCl. The resulting suspension was extracted with EtOAc. The combined organic layer was concentrated to give crude compound **59-3** (240 mg).

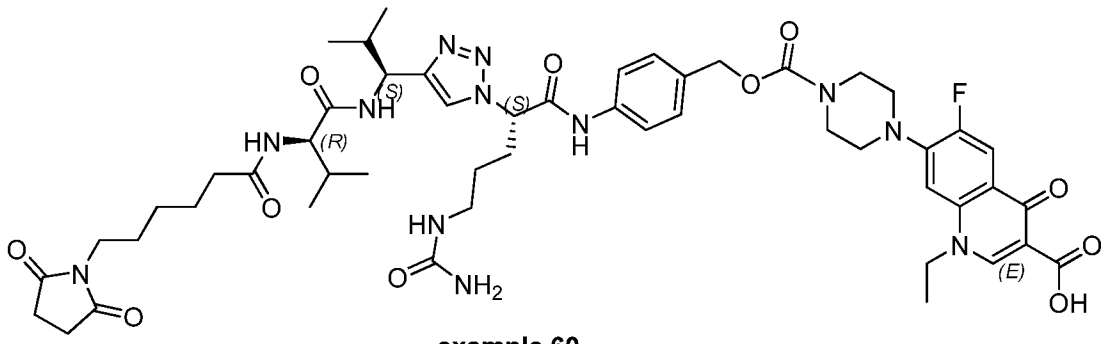
Step 2. Compound **59-3** (240 mg, 0.67mmol), HATU (506 mg, 1.34 mmol), DIPEA (258 mg, 2.01 mmol) were dissolved in DMF (5 mL) and stirred at r.t. for 30 min. Then compound **59-4** (269 mg, 0.67 mmol) was added. The reaction mixture was stirred at r.t. for 3 h. The mixture was concentrated and purified by prep-HPLC to give **59-5a** and **59-5b** (40 mg each, 8 %)

Step 3. To a solution of compound **59-5a** (40 mg, 0.054 mmol) in dry DMF (3 mL) was added PNP carbonate (34 mg, 0.11 mmol) and DIPEA (21 mg, 0.162 mmol) at r.t. and stir at r.t. for 1.5 h. Norfloxacin (35 mg, 0.11 mmol) was added. The mixture was stirred at r.t. for another 1 h and concentrated, filtered and purified by prep-HPLC (FA) (14 mg, Yield: 20 %).

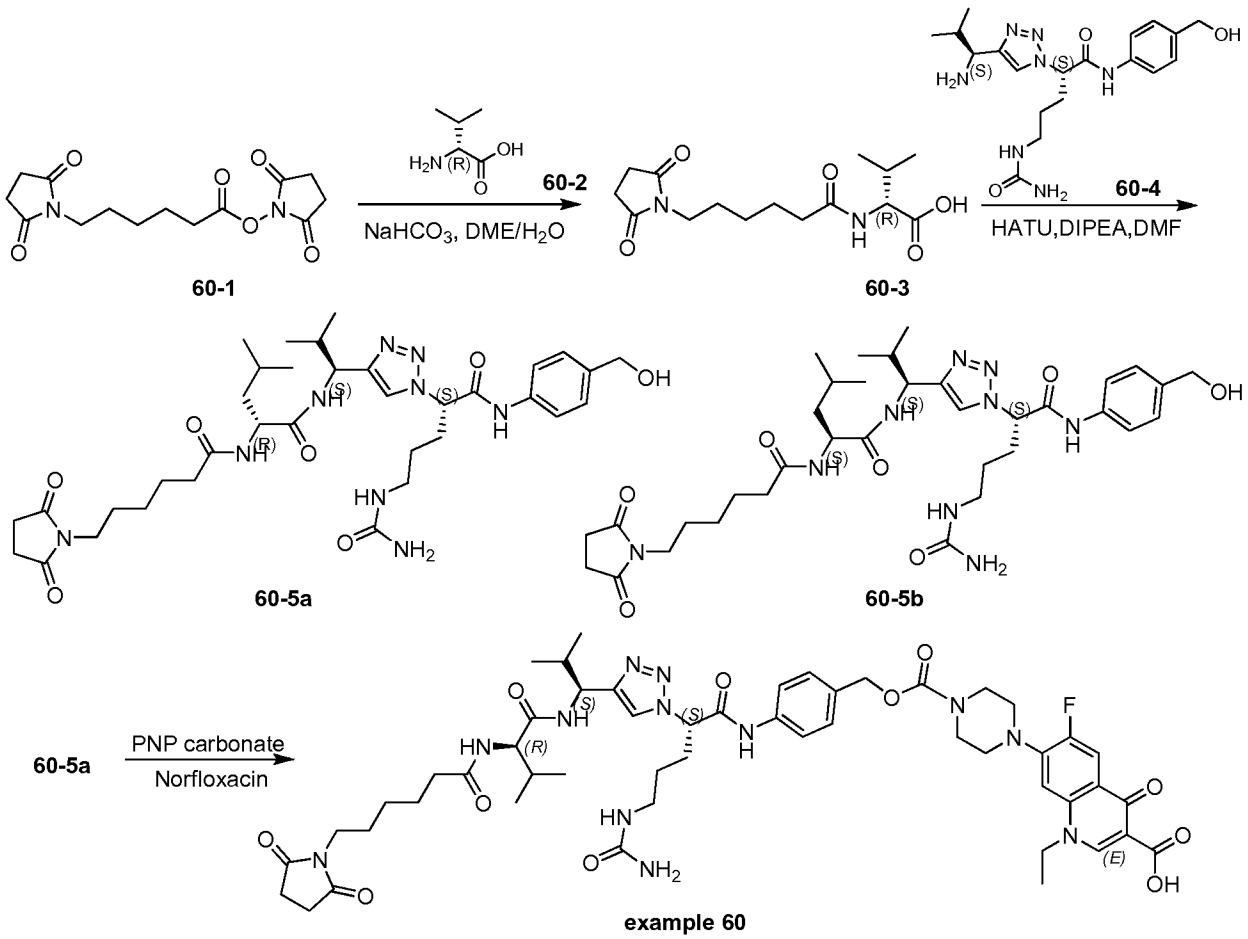
LCMS: (5-95, AB, 1.5 min, ESI), 0.838 min, MS = 546.5[1/2M+1]

^{1}H NMR DMSO-d_6 400 MHz, δ 10.64 (s, 1 H), 8.96 (s, 1H), 8.22 (d, J = 9.2 Hz, 1 H), 8.04 (d, J = 8.4 Hz, 1 H), 8.04 - 7.92 (m , 2 H), 7.61 (d, J = 8.4 Hz , 2 H), 7.36 (d, J = 8.4 Hz, 2 H), 7.22 - 7.20 (m, 5 H), 7.14 (s, 1 H), 6.06 (d, J = 6 Hz, 1 H), 5.50 - 5.47 (m, 1 H), 5.44 (s, 2 H), 5.07 (s, 2 H), 4.90 - 4.86 (m, 1 H), 4.61 - 4.58 (m, 3 H), 3.61 (s, 4 H), 3.34 - 3.25 (m, 4 H), 3.04 - 2.91 (m, 4 H), 2.75 - 2.71 (m, 2 H), 2.60 (s, 4 H), 2.14 - 2.11 (m , 2 H), 2.09 - 2.00 (m, 2H), 1.42 - 1.29 (m, 10H), 1.40 (d, J = 7.2 Hz, 2 H), 0.85 - 0.79 (m, 6H).

Example 60. 7-(4-((4-((S)-2-(4-((S)-1-((R)-2-(6-(2,5-dioxopyrrolidin-1-yl)hexanamido)-3-methylbutanamido)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy) carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 60



Step 1. To a solution of compound **60-2** (83 mg, 0.71 mmol) in DME (5 mL) was added a solution of compound **60-1** (200 mg, 0.71 mmol) and $NaHCO_3$ (122 mg, 1.42 mmol) in water (5 mL). After the mixture was stirred at r.t. for 16 h, it was washed with EtOAc and acidified to pH 3 with 10% HCl. The resulting suspension was extracted with EtOAc. The combined organic layer was concentrated to give compound **60-3** (160 mg, with impurity, Yield: 80 %.

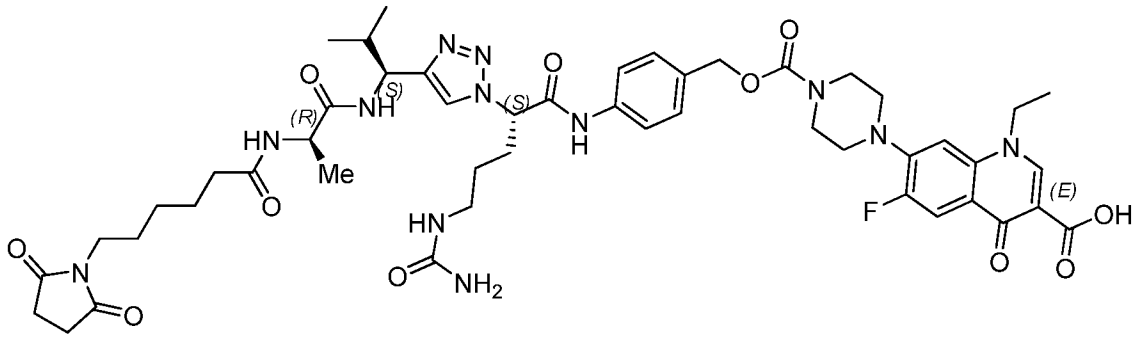
Step 2. Compound **60-3** (160 mg, 0.51mmol), HATU (390 mg, 1.02 mmol), DIPEA (200 mg, 0.68 mmol) were dissolved in DMF (5 mL) and stirred at r.t. for 30 min. Then compound **60-4** (207 mg, 0.51 mmol) was added. The reaction mixture was stirred at r.t. for 3 h. Then the mixture was concentrated and purified by prep-HPLC to give **60-5a** and **60-5b** (60 mg and 60 mg, 8 % and 8%, respectively)

Step 3. To a solution of compound **60-5a** (60 mg, 0.086 mmol) in dry DMF (3 mL) was added PNP carbonate (60 mg, 0.2 mmol) and DIPEA (0.5 mL, 3 mmol) at r.t., and the mixture was stirred at r.t. for 1.5 h. Norfloxacin (60 mg, 0.19 mmol) was added. The mixture was stirred at r.t. for additional 1 h. The mixture was concentrated, filtered and purified by prep-HPLC (FA) to give **example 60** (47.9 mg, yield: 54%).

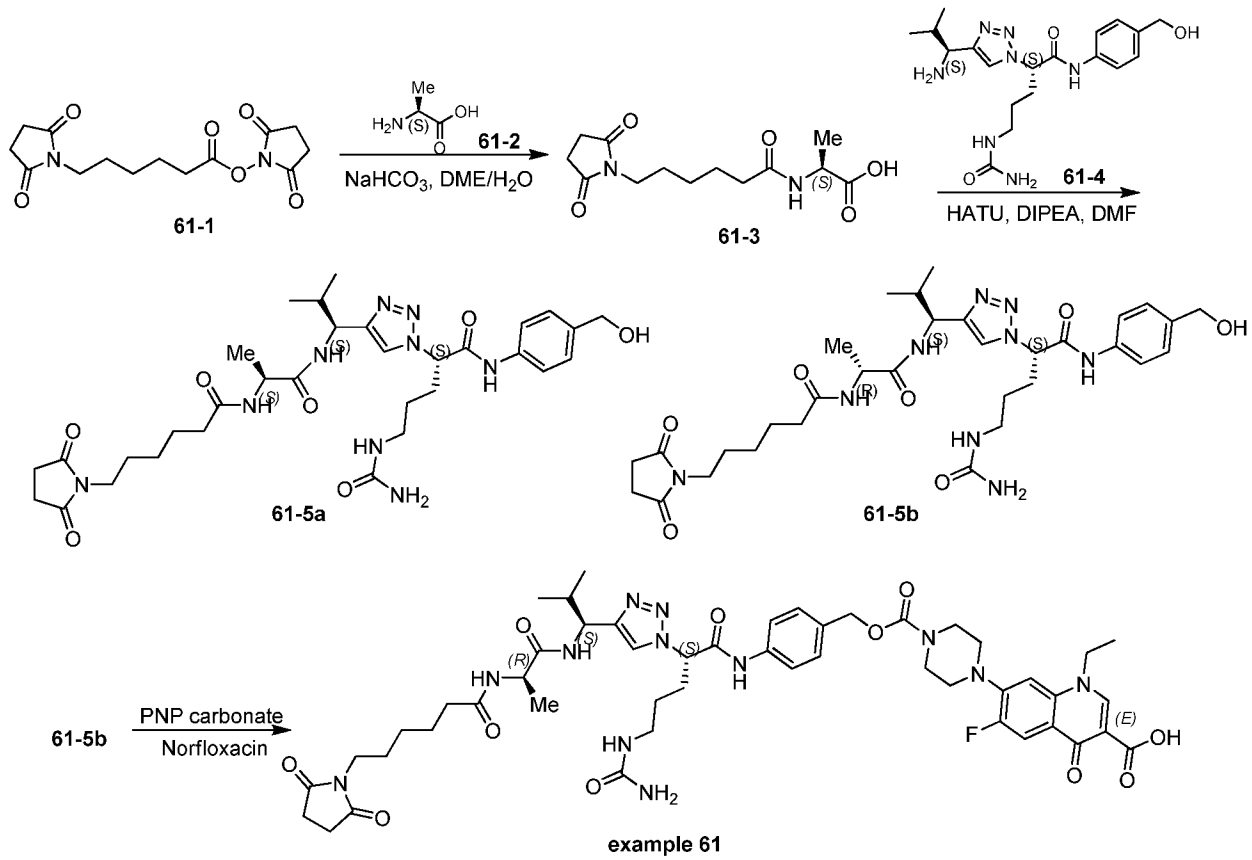
LCMS: (5-95, AB, 1.5 min, ESI), 0.824 min, MS = 522.4[1/2M+1]

^{1}H NMR DMSO-d_6 400 MHz, δ 10.1 (s, 1H), 8.93 (s, 1 H), 8.15 - 8.13 (d, 1H), 7.99 (s, 1 H), 7.93 - 7.90 (d, 1 H), 7.81 - 7.79 (d, 1H), 7.57 - 7.55 (d, J = 8.0 Hz , 2 H), 7.35 - 7.33 (d, J = 8.0 Hz , 2 H), 7.20 - 7.18 (m, 1 H), 6.0 (m, 1 H), 5.5 (m, 1 H), 5.40 (s, 2 H), 5.04 (s, 2 H), 4.8 (m, 1 H), 4.6 - 4.5 (m, 2 H), 4.2 - 4.1(m, 1 H), 3.6 (s, 4 H), 3.2 (m, 6 H), 3.05 - 2.9 (m, 2 H), 2.57 (s, 4 H), 2.15 - 2.0 (m, 5 H), 1.9 - 1.8 (m, 1 H), 1.5 - 1.35 (m, 7 H), 1.3 - 1.1 (m , 4 H), 0.82 - 0.80 (m, J = 8.0 Hz, 3H), 0.76 - 0.70 (m, 9H).

Example 61. 7-(4-((4-((S)-2-(4-((S)-1-((R)-2-(6-(2,5-dioxopyrrolidin-1-yl)hexanamido)propanamido)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 61



Step 1. To a solution of compound **61-2** (64 mg, 0.71 mmol) in DME (5 mL) was added a solution of compound **61-1** (200 mg, 0.71 mmol) and $NaHCO_3$ (122 mg, 1.42 mmol) in water (5 mL). After the mixture was stirred at r.t. for 16 h, it was washed with EtOAc and acidified to pH = 3 with 10% HCl. The resulting suspension was extracted with EA. The combined organic layer was concentrated to give compound **61-3**. (100 mg, contains impurity, Yield: 60 %)

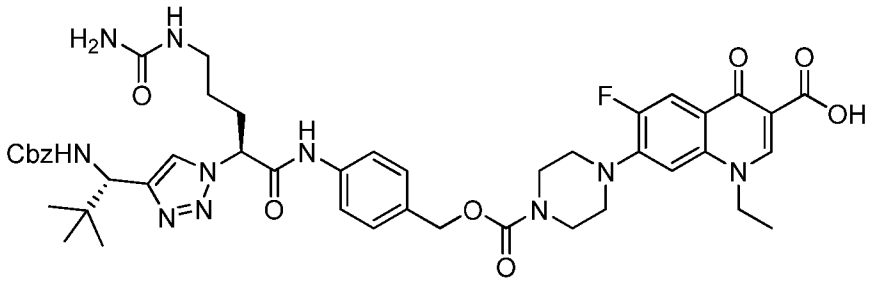
Step 2. Compound **61-3** (100 mg, 0.35mmol), HATU (267 mg, 0.7 mmol), DIPEA (136 mg, 1.05 mmol) were dissolved in DMF (5 mL) and stirred at r.t. for 30 min. Compound **61-4** (142 mg, 0.35 mmol) was added and the reaction mixture was stirred at r.t. for 3 h. The mixture was concentrated and purified by prep-HPLC to give **61-5a** and **61-5b** (43 mg, 22 mg, 6 %, 3 %, respectively)

Step 3. To a solution of compound **61-5b** (22 mg, 0.033 mmol) in dry DMF (2 mL) was added PNP carbonate (20 mg, 0.066 mmol) and DIPEA (0.2 mL, 1.2 mmol) at r.t., and the mixture was stirred at r.t. for 1.5 h. Norfloxacin (20 mg, 0.066 mmol) was added. The mixture was stirred at r.t. for another 1 h. The mixture was concentrated, filtered and purified by prep-HPLC (FA), to give **example 61** (14.6 mg yield: 43%).

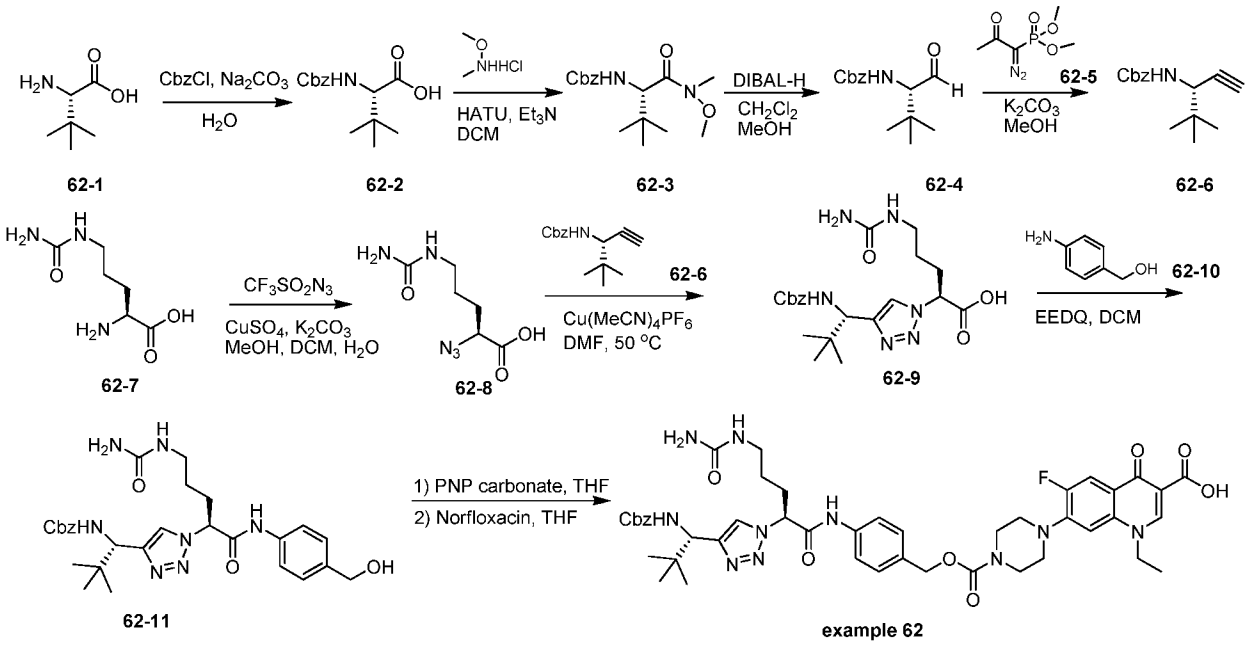
LCMS: (5-95, AB, 1.5 min, ESI), 0.805 min, MS = 508.3[1/2M+1]

^{1}H NMR methanol-d_4 + $CDCl_3$ 400 MHz, δ 8.83 (s, 1 H), 8.02 - 7.99 (m, 2 H), 7.77 (solvent:$CDCl_3$), 7.59 - 7.57 (d, J = 8.0 Hz , 2 H), 7.36 - 7.34 (d, J = 8.0 Hz , 2 H), 7.15 - 7.13 (m, 1 H), 5.5 (m, 1 H), 5.11 (s, 2 H), 4.95 - 4.9 (m, 1 H), 4.55 - 4.45 (m, 2 H), 4.4 - 4.35 (m, 1 H), 3.7 (s, 4 H), 3.45 (m, 2 H), 3.4 (s, 4 H), 3.25 - 3.05 (m, 2 H), 2.66 (s, 4 H), 2.3 - 2.1 (m, 5 H), 1.6 - 1.5 (m, 7 H), 1.5 - 1.3 (m, 2 H), 1.3 - 1.2 (m, 5 H), 0.96 - 0.94 (d, J = 8.0 Hz , 3 H), 0.87 - 0.85 (d, J = 8.0 Hz , 3 H).

Example 62. 7-(4-((4-((S)-2-(4-((S)-1-(benzyloxycarbonylamino)-2,2-dimethylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 62



Step 1. CbzCl (24 mL, 0.17 mol) was added to dropwise over 20 min to a mixture of **62-1** (20 g, 0.15 mol) and Na_2CO_3 (32 g, 0.30 mol) in water (100 mL). After the reaction mixture was stirred for 12 h, it was washed with EtOAc (200 mL × 2). The aqueous layer was adjusted to pH = 2 and

extracted with EtOAc (200 mL × 4). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give **62-2**, which was used for next step without further purification.

Step 2. To a mixture of **62-2** (10 g, 37.7 mmol), *N,O*-dimethylhydroxylamine (4.0 g, 41.5 mmol) and HATU (21.3 g, 56.0 mmol) in DCM (100 mL) was added Et_3N (21.8 mL, 150.8 mmol). After the mixture was stirred at r.t. for 1 h, the solvent was removed and the crude was taken up with water (200 mL). The aqueous layer was extracted with EtOAc (200 mL × 3). The extracts were washed with conc. HCl, aq. $NaHCO_3$, saturated NaCl. It was concentrated and purified by column chromatography on silica gel (PE: EtOAc = 2:1) to give the **62-3**.

Step 3. DIBAL-H (19.5 mL, 19.46 mmol) was added dropwise to a solution of compound **3** (4 g, 12.97 mmol) in DCM (60 mL) at -78°C. After the mixture was stirred at -78°C for 4 h, excess hydride was quenched with MeOH (5 mL) and the resulting solution was warmed to r.t. The solution was concentrated to give compound **4**, which was used for next step without further purification.

Step 4. After a mixture of **62-4** (3.23 g, 12.97 mmol), **62-5** (2.99 g, 15.56 mmol) and K_2CO_3 (3.58 g, 25.94 mmol) in MeOH (40 mL) was stirred at r.t. for 12 h, solvent was removed, and the residue was extracted with EtOAc (60 mL × 3). The organic layers were washed with brine, dried over Na_2SO_4, concentrated and purified by column (PE: EtOAc= 10:1) to give the **62-6**.

Step 5. Tf_2O (4.4 mL, 25.94 mmol) was added slowly over 5 min to a solution of NaN_3 (8.3 g, 129.7 mmol) in a mixture of H_2O (45 mL) and DCM (75 mL) at 0 °C. After it was stirred for 2 h, DCM layer was separated and the aqueous portion was extracted with DCM (35 mL×2). The organic fractions, containing the triflyl azide were pooled and washed once with saturated Na_2CO_3 and added to a mixture of **62-7** (2.27 g, 12.97 mmol), K_2CO_3 (2.69 g, 19.46 mmol) and $CuSO_4 \cdot 5H_2O$ (323 mg, 1.30 mmol) in H_2O (90 mL) and MeOH (180 mL). After the mixture was stirred for 12 h, organic solvents were removed under pressure and the aqueous slurry was diluted with phosphate buffers (0.2M, pH 6.2, 100 mL) and extracted with EtOAc (200 mL × 2). The aqueous phase was then acidified to pH = 2 with conc. HCl, and extracted with (400 mL × 3). The organic layers were combined, dried over Na_2SO_4 and concentrated to give **62-8**, which was used for next step without further purification.

Step 6. After a mixture of **62-8** (2.61 g, 12.97 mmol), **62-6** (1.59 g, 6.48 mmol) and $Cu(CH_3CN)_4PF_6$ (362 mg, 0.97 mmol) in DMF (5 mL) was stirred at 50°C for 2 h, solvent was removed, and the residue was purified by prep-HPLC to give **62-9**.

Step 7. After a mixture of **62-9** (968 mg, 2.17 mmol), **62-10** (801 mg, 6.50 mmol) and EEDQ (1.61 g, 6.50 mmol) in DCM (50 mL) was stirred at r.t. for 2 h, solvent was removed, and the residue was purified by prep-HPLC to give **62-11** (395.9 mg, 33.1%).

^{1}H NMR (400 MHz, MeOD) δ 8.11 (s, 1 H), 7.59-7.56 (m, 2 H), 7.37-7.27 (m, 7 H), 5.55-5.51 (m, 1 H), 5.13-5.05 (m, 2 H), 4.79 (s, 1 H), 4.58 (s, 2 H), 3.34-3.32 (m, 1 H), 3.25-3.11 (m, 1 H), 2.28-2.22 (m, 2 H), 1.48-1.41 (m, 2 H), 0.96 (s, 9 H).

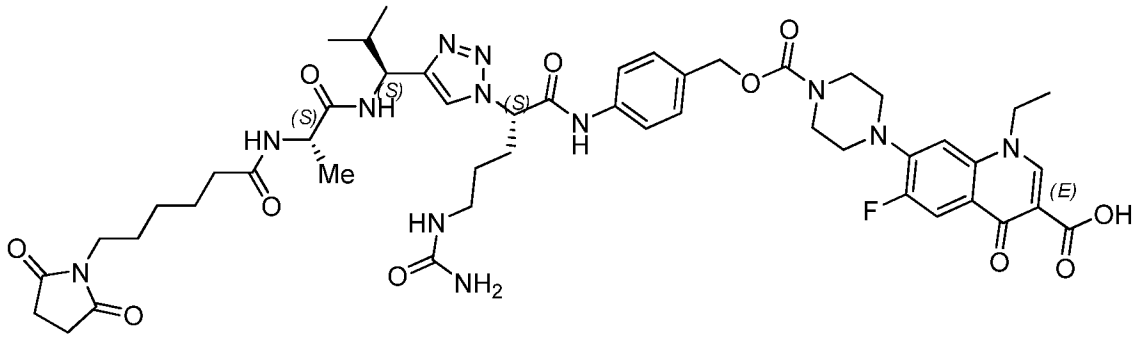
LCMS (ESI): m/z 551.9 [M+H$^+$].

Step 8. After a mixture of **62-11** (190 mg, 0.34 mmol), PNP carbonate (126 mg, 0.41 mmol) and DIPEA (132 mg, 1.02 mmol) in DCM (5 mL) was stirred at 50°C for 12 h, solvent was removed and added to a mixture of norfloxacin (323 mg, 1.02 mmol) and DIPEA (132 mg, 1.02 mmol) in DMF (5 mL). After it was stirred at r.t. for 2 h solvent was removed, and the residue was purified by prep-HPLC to give **example 62** (6.2 mg).

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.31 (s, 1 H), 10.66 (s, 1 H), 8.96 (s, 1 H), 8.15 (s, 1 H), 7.94 (d, J =13.2 Hz, 1 H), 7.72-7.70 (m, 1 H), 7.60 (d, J = 8.0 Hz, 2 H), 7.38-7.28 (m, 8 H), 6.04-6.03 (m, 1 H), 5.52-5.51 (m, 1 H), 5.42 (s, 2 H), 5.07 (s, 2 H), 5.03-5.01 (m, 2 H), 4.66 (d, J = 9.6 Hz, 1 H), 4.58-4.56 (m, 2 H), 3.61 (s, 4 H), 3.41 (s, 4 H), 3.02-2.98 (m, 2 H), 2.12-2.02 (m, 2 H), 1.42-1.39 (m, 3 H), 1.26-1.23 (m, 2 H), 0.84 (s, 9 H).

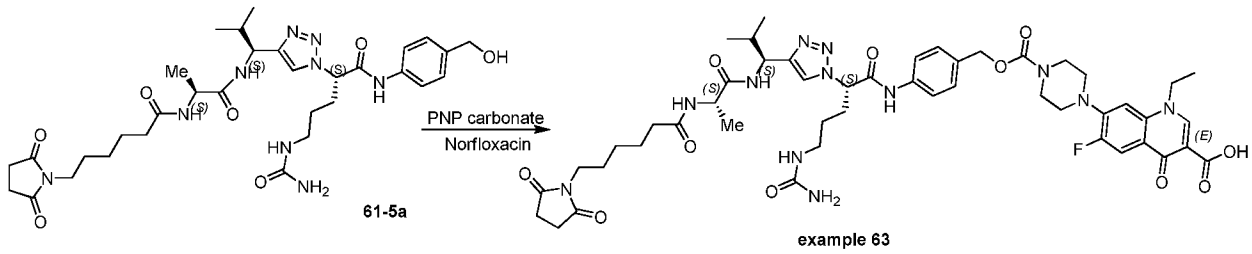
LCMS (ESI): m/z 897.1 [M+H$^+$].

Example 63. 7-(4-((4-((S)-2-(4-((S)-1-((S)-2-(6-(2,5-dioxopyrrolidin-1-yl)hexanamido)propanamido)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 63

Example 63 was made using the procedure as Example 61, with intermediate from the synthesis of **Example 61.**

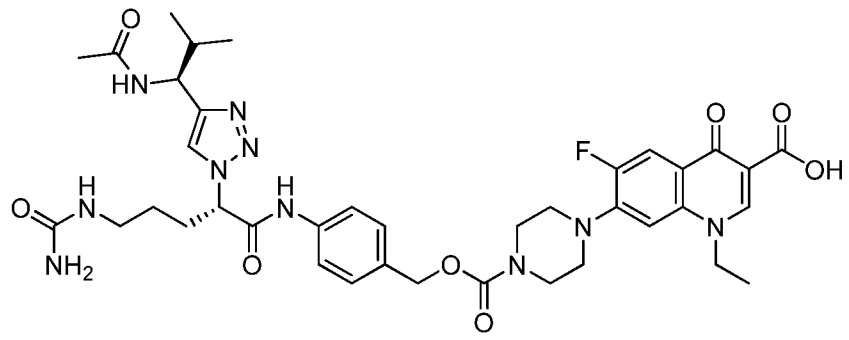


Step 1. To a solution of compound **61-5a** (43 mg, 0.064 mmol) in dry DMF (3 mL) was added PNP carbonate (40 mg, 0.128 mmol) and DIPEA (0.4 ml, 2.4 mmol) at r.t., and the mixture was allowed to stir at r.t. for 1.5 h. Norfloxacin (40 mg, 0.128 mmol) was added. The mixture was stirred at r.t. for another 1 h. The mixture was concentrated, filtered and purified by prep-HPLC (FA), to give **example 63** (33.8 mg, yield: 52%).

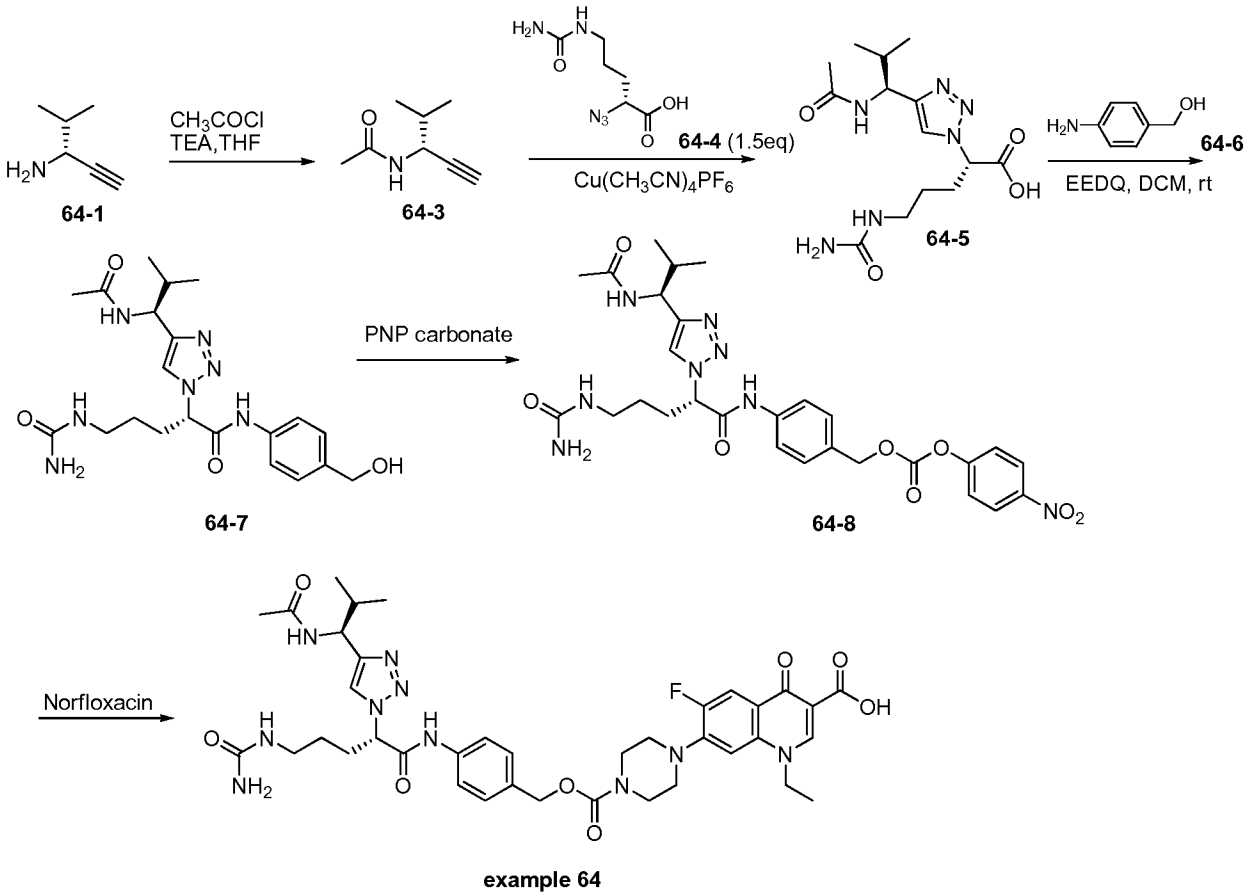
LCMS: (5-95, AB, 1.5 min, ESI), 0.803 min, MS = 508.4[1/2M+1]

^{1}H NMR Methanol-d_4 400 MHz, δ 8.86 (s, 1 H), 8.15 - 8.12 (d, 1H), 8.10 - 8.05 (m, 1 H), 8.05 - 7.98 (d, 1 H), 7.62 - 7.60 (d, J = 8.0 Hz , 2 H), 7.37 - 7.35 (d, J = 8.0 Hz , 2 H), 7.20 - 7.18 (m, 1 H), 5.5 (m, 1 H), 5.12 (s, 2 H), 4.95 - 4.9 (m, 1 H), 4.6 - 4.5 (m, 2 H), 4.42 - 4.38(m, 1 H), 3.7 (s, 4 H), 3.41 (m, 2 H), 3.3 (s, 4 H), 3.25 - 3.1 (m, 2 H), 2.63 (s, 4 H), 2.35 - 2.2 (m, 5 H), 1.6 - 1.5 (m, 7 H), 1.5 - 1.3 (m, 2 H), 1.35 - 1.33 (d, J = 8.0 Hz , 3 H), 1.3 - 1.2 (m, 2H), 0.95 - 0.93 (d, J = 8.0 Hz , 3 H), 0.91 - 0.88 (d, J = 8.0 Hz , 3 H).

Example 64. 7-(4-((4-((S)-2-(4-((S)-1-acetamido-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 64



64-1 64-3 64-5 64-7 64-8

example 64

Step 1. To a stirred solution of **64-1** (1.1 g, 11.4 mmol) in THF was added **64-2** (4.5 mL, 57 mmol), TEA (9 mL, 62.7 mmol) at 0°C. The mixture was stirred at r.t. for 3 h under N_2. The reaction mixture was poured into water (30 mL) and extracted with DCM, the combined organic layers were washed with aqueous NaCl and concentrated to give the **64-3** (Yield: 95 %).

Step 2. Compound **64-3** (139 mg, 1 mmol), **64-4** (302 mg, 1.5 mmol), $Cu(CH_3CN)_4PF_6$ (75 mg, 0.2 mmol) was dissolved in DMF (8 mL) at r.t. The mixture was stirred at 60 °C for 2 h. The mixture **64-5** was used for next step without further purification.

Step 3. To the crude mixture of **64-5** was added **64-6** (1.123 g, 9.12 mmol) and EEDQ (3 g, 12.2 mmol) at r.t. The mixture was stirred at r.t. for 16 h under N_2 atmosphere. The residue was purified by prep-HPLC and then purified by SFC to give **64-7**. (Yield: 80 %)

LCMS (ESI): m/z 446.0 [$M+H^+$].

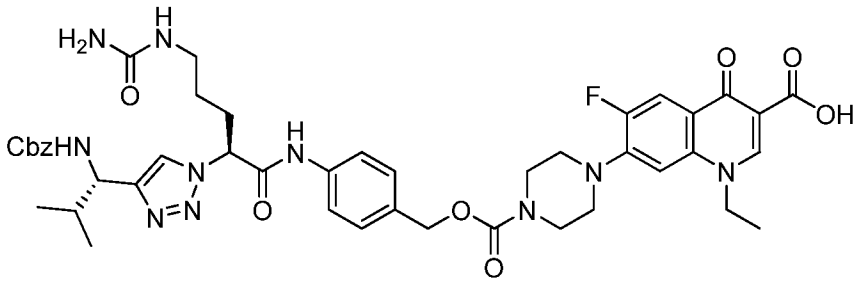
Step 4. To a solution of **64-7** (30 mg, 0.068 mmol) in DMF (2 mL) was added PNP carbonate (42 mg, 0.136 mmol) and DIPEA (27 mg, 0.21 mmol) at 0°C. The mixture was stirred at r.t. for 16 h. The mixture **64-8** was used for next step without further purification. (Yield: 95 %)

LCMS (ESI): m/z 611.2 [M+H$^+$].

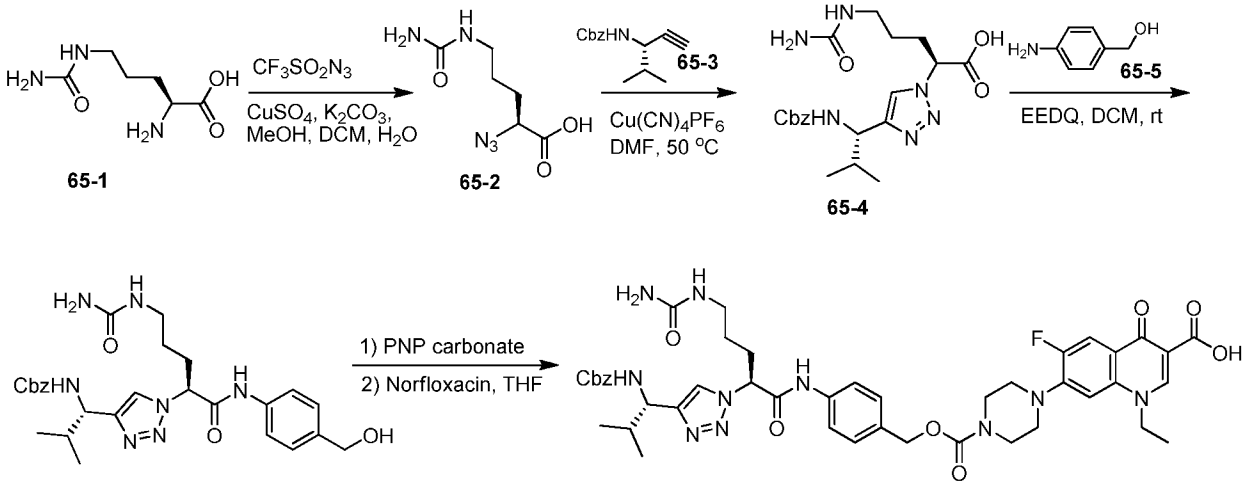
Step 5. To the mixture that **64-8** was added norfloxacin (44 mg, 0.136 mmol) at r.t. The mixture was stirred at r.t. for 1 h. The residue was purified by prep-HPLC and then purified by SFC to give **example 64** (Yield: 30 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, 1 H), 8.96 (s, 1 H), 8.17 (d, J = 9.2 Hz, 1 H), 8.06 (d, J = 6.8 Hz, 1 H), 7.94 (d, J = 13.2 Hz, 1 H), 7.60 (d, J = 8.4 Hz, 2 H), 7.36 (d, J = 8.0 Hz, 2 H), 7.21 (d, J = 6.0 Hz, 1 H), 6.02 (d, J = 4.4 Hz, 1 H), 5.51 - 5.42 (m, 3 H), 5.07 (s, 2 H), 4.90 - 4.86 (m, 1 H), 4.59 - 4.57 (m, 2 H), 3.65 - 3.61 (m, 4 H), 3.06 - 2.96 (m, 2 H), 2.12 - 1.87 (m, 3 H), 1.86 (s, 3 H), 1.48 - 1.35 (m, 3 H), 1.28 (s, 2 H), 0.89 - 0.72 (m, 6 H).

Example 65: 7-(4-((4-((S)-2-(4-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



example 65



Step 1. Tf_2O (8.8 mL, 51.9 mmol) was added slowly over 5 min to a solution of NaN_3 (16.9 g, 259.4 mmol) in a mixture of distilled H_2O (30 mL) and CH_2Cl_2 (45 mL) at 0 °C. After it was

stirred for 2 h, the organic phase was separate and aqueous portion was extracted with CH_2Cl_2 (40 mL×2). The organic fractions, containing the triflyl azide, were pooled and washed once with saturated Na_2CO_3 and added to a mixture of **65-1** (2.3 g, 12.97 mmol), K_2CO_3 (2.69 g, 19.46 mmol) and $CuSO_4.5H_2O$ (65 mg, 0.26 mmol) in H_2O (90 mL) and MeOH (180 mL). After the mixture was stirred at 26°C for 12 h, the organic solvents were removed under reduced pressure and the aqueous slurry was diluted with H_2O (50 mL). The mixture was acidified to pH = 6 with conc. HCl and then diluted with phosphate buffers (0.2 M, pH 6.2, 50 mL). The mixture was washed with EtOAc (100 mL × 2) and the aqueous phase was then acidified to pH = 2 with conc.HCl. The mixture was extracted with EtOAc (200 mL × 3). The organic layer was dried over Na_2SO_4 and concentrated to give **65-2**, which was used for next step without further purification.

Step 2. After a mixture of **65-2** (2.6 g, 12.97 mmol), **65-3** (1.5 g, 6.48 mmol) and $Cu(MeCN)_4PF_6$ (304 mg, 0.97mmol) in DMF (10 mL) was stirred at 50°C for 2 h, solvent was removed, and the residue was purified by prep-HPLC to give **65-4** (516 mg, 18%).

Step 3. After a mixture of **65-4** (285 mg, 0.66 mmol), **65-5** (244 mg, 1.98 mmol) and EEDQ (490 mg, 1.98 mmol) in DCM (30 mL) was stirred at 24°C for 2 h, solvent was removed, and the residue was purified by prep-HPLC to give **65-6** (250 mg, 70.4%).

^{1}H NMR (400 MHz, MeOD) δ 8.10 (s, 1 H), 7.58 (s, 2 H), 7.45-7.20 (m, 7 H), 5.55-5.52 (m, 1 H), 5.10-5.05 (m, 2 H), 4.77-4.58 (m, 3 H), 3.22-3.21 (m, 2 H), 2.33-2.15 (m, 3 H), 1.49-1.47 (m, 2 H), 0.97 (s, 3 H), 0.90 (s, 3 H).

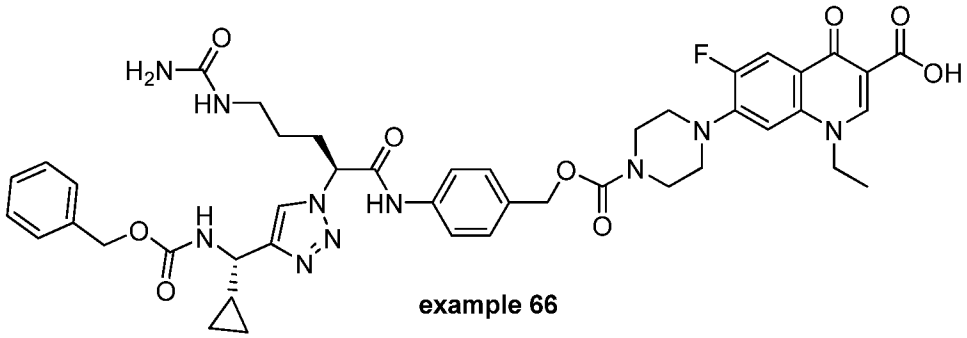
LCMS (ESI): m/z 538.3 [M+H$^+$].

Step 4. After a mixture of **65-6** (127 mg, 0.24 mmol), PNP (143 mg, 0.47 mmol) and DIPEA (92 mg, 0.71 mmol) in DCM (10 mL) was stirred at 50°C for 12 h, solvent was removed and the residue was mixed with DIPEA (93 mg, 0.72 mmol) and norfloxacin (230 mg, 0.72 mmol) in DMF (10 mL). After it was stirred at 24°C for 4 h, solvent was removed, and the residue was purified by prep-HPLC to give **example 65** (30 mg, 14.1%).

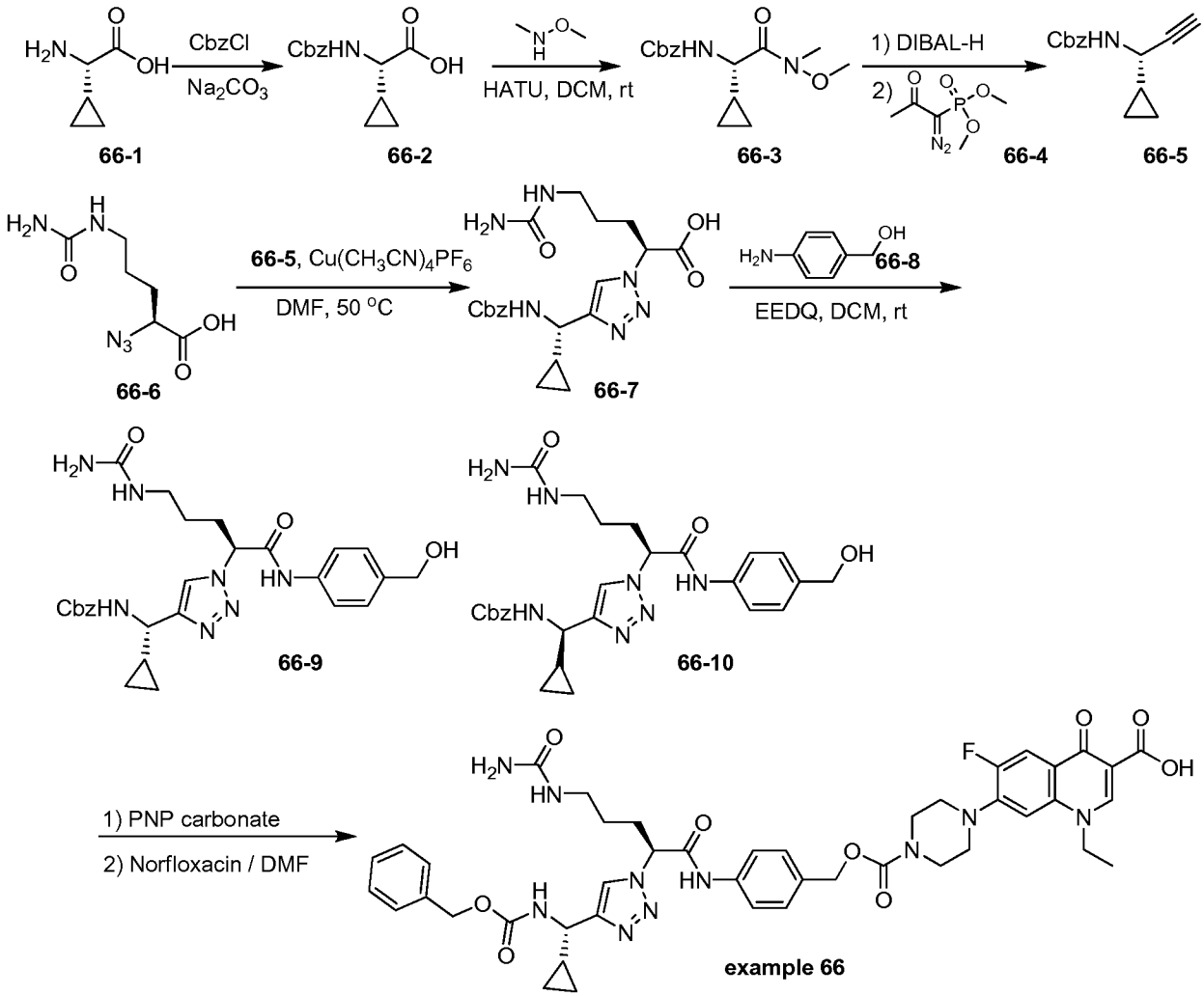
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.32 (s, 1 H), 10.63 (s, 1 H), 8.96 (s, 1 H), 8.08 (s, 1 H), 7.94 (d, J = 13.2 Hz, 1 H), 7.71 (d, J = 9.2 Hz, 1 H), 7.60 (d, J = 8.4 Hz, 2 H), 7.38-7.29 (m, 8 H), 7.21 (d, J = 7.6 Hz, 1 H), 6.02 (s, 1 H), 5.48-5.47 (m, 1 H), 5.42 (s, 2 H), 5.06 (s, 2 H), 5.02 (s, 2 H), 4.62-4.60 (m, 3 H), 3.61 (s, 4 H), 3.44 (s, 4 H), 3.02-2.99 (m, 2 H), 2.12-2.02 (m, 3 H), 1.40 (t, J = 6.8 Hz, 3 H), 1.27-1.24 (m, 2 H), 0.84 (d, J = 6.8 Hz, 3 H), 0.78 (d, J = 6.8 Hz, 3 H).

LCMS (ESI): m/z 883.7 [M+H$^+$].

Example 66. 7-(4-((4-((S)-2-(4-((S)-(benzyloxycarbonylamino)(cyclopropyl)methyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 66



Step 1. To a mixture of **66-1** (5.0 g, 43.43 mmol) and Na_2CO_3 (6.9 g, 65.15 mmol) in H_2O (50 mL) was added CbzCl (8.89 g, 52.12 mmol) dropwise at 0°C. After the reaction mixture was stirred at 25°C for 16 h, it was washed with EtOAc (30 mL × 2). The aqueous phase was acidified to pH 2

with conc. HCl, and extracted with EtOAc (50 mL × 2). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give **66-2** (11 g, crude).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.29 (m, 5 H), 5.13-5.08 (m, 2 H), 4.72-4.68 (m, 1 H), 1.12-1.09 (m, 1 H), 0.61-0.43 (m, 4 H).

Step 2. To a mixture of **66-2** (500 mg, 2.006 mmol), *N,O*-dimethylhydroxylamine hydrochloride (235 mg, 2.407 mmol) and Et_3N (609 mg, 6.018 mmol) in DCM (10 mL) was added HATU (1.14 g, 3.009 mmol) at 25°C. After it was stirred at 25°C for 2 h, solvent was removed and the residue was taken up with water (10 mL). The aqueous layer was extracted with EtOAc (8 mL × 2). The organic layer was washed with saturated Na_2CO_3 solution (10 mL), 1N HCl solution (10 mL), then water (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude was purified by column chromatography on silica gel (PE/EtOAc = 2:1) to give **66-3** (400 mg, 68%).

LCMS (ESI): m/z 293.1 [M+H$^+$].

Step 3. DIBAL-H in toluene (1M, 18 mL, 18 mmol) was added to a mixture of **66-3** (3.5 g, 11.97 mmol) in DCM (30 mL) at -78°C. After the mixture was stirred at -78°C for 3 h, MeOH (5 mL) was added dropwise. The mixture was warmed to 25°C. Solvent was removed and it was dissolved in MeOH (25 mL) and K_2CO_3 (3.31 g, 23.94 mmol) and **66-4** (2.76 g, 14.36 mmol) was added at 0°C. The mixture was stirred at 25°C for 16 h. After solvent was removed, the crude was taken up with 1N HCl solution (30 mL), extracted with EtOAc (20 mL × 3). The organic layer was dried over Na_2SO_4, concentrated and purified by column chromatography on silica gel (PE/EtOAc = 5:1-2:1) to give **66-5** (1.5 g, 55%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.30 (m, 5 H), 5.12-5.05 (m, 3 H), 4.47 (m, 1 H), 2.24 (s, 1 H), 1.18-1.11 (m, 1 H), 0.56-0.47 (m, 4 H).

Step 4. $Cu(CH_3CN)_4PF_6$ (366 mg, 0.981 mmol) was added to a solution of **66-6** (2.63 g, 13.08 mmol) and **66-5** (1.5 g, 6.54 mmol) in DMF (10 mL)at 25°C. The reaction mixture was stirred at 50°C for 2 h under N_2. Solvent was removed and the residue was purified by prep-HPLC to give **66-7** (3.0 g, 53%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (s, 1 H), 7.83-7.81 (m, 1 H), 7.37-7.14 (m, 5 H), 5.97 (br, 1 H), 5.40-5.38 (m, 1 H), 5.03-4.99 (m, 2 H), 4.32-4.28 (m, 1 H), 2.94 (s, 2 H), 2.17-2.07 (m, 2 H), 1.30-1.10 (m, 3 H), 0.50-0.26 (m, 4 H).

Step 5. A solution of **66-7** (1.0 g, 2.32 mmol), **66-8** (571 mg, 4.64 mmol) and EEDQ (1.15 g, 4.64 mmol) in DCM (15 mL) was stirred at 25°C for 2 h. After removal of the solvent, the residue was purified by prep-HPLC and SFC to give **66-9** (500 mg) and of **66-10** (112 mg).

^{1}H NMR (400 MHz, DMSO–d_6) δ 10.60 (s, 1 H), 8.10 (s, 1 H), 7.82 (d, J = 9.2 Hz, 1 H), 7.56-7.54 (m, 2 H), 7.36-7.25 (m, 7 H), 6.08-6.05 (m, 1 H), 5.51-5.43 (m, 3 H), 5.14 (t, J = 5.6 Hz, 1 H), 5.03 (s, 2 H), 4.44 (d, J = 5.6 Hz, 2 H), 4.31 (t, J = 8.4 Hz, 1 H), 3.04-2.97 (m, 2 H), 2.15-2.08 (m, 2 H), 1.30-1.23 (m, 3 H), 0.49-0.30 (m, 4 H).

LCMS (ESI): m/z 536.3 [M+H$^+$].

^{1}H NMR (400 MHz, DMSO–d_6) δ 10.54 (s, 1 H), 8.09 (s, 1 H), 7.82-7.80 (m, 1 H), 7.56-7.54 (m, 2 H), 7.36-7.26 (m, 7 H), 6.03-6.01 (m, 1 H), 5.49-5.42 (m, 3 H), 5.13 (t, J = 5.6 Hz, 1 H), 5.03 (s, 2 H), 4.44 (d, J = 5.6 Hz, 2 H), 4.29 (t, J = 8.4 Hz, 1 H), 3.04-2.97 (m, 2 H), 2.14-2.05 (m, 2 H), 1.30-1.25 (m, 3 H), 0.49-0.30 (m, 4 H).

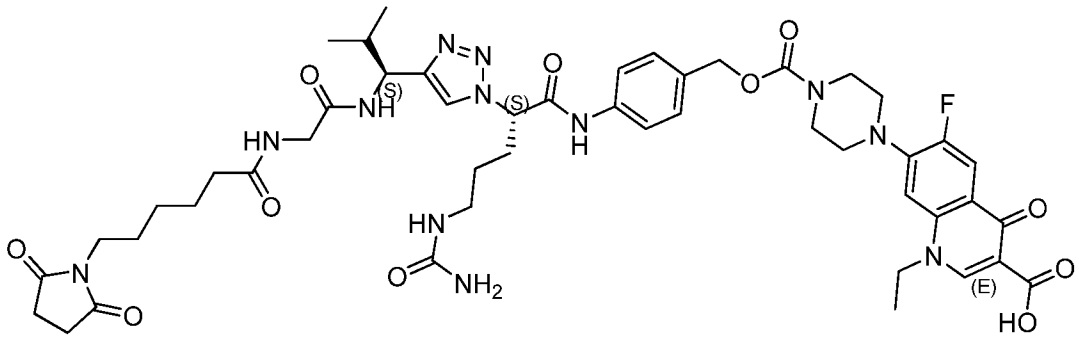
LCMS (ESI): m/z 536.4 [M+H$^+$].

Step 6. To a solution of **66-9** (80 mg, 0.149 mmol) in DCM (2 mL) were added PNP carbonate (136 mg, 0.447 mmol) and DIPEA (77 mg, 0.596 mmol) at 25°C. The mixture was heated at reflux for 20 h. Solvent was removed and residue was dissolved in DMF (2 mL) and norfloxacin (72 mg, 0.224 mmol) and DIEA (96 mg, 0.745 mmol) were added at 25°C, and mixture was stirred at 25°C for 2 h. After removal of the solvent, the residue was purified by prep-TLC (DCM/MeOH =10:1) to give **example 66** (23 mg, 18%).

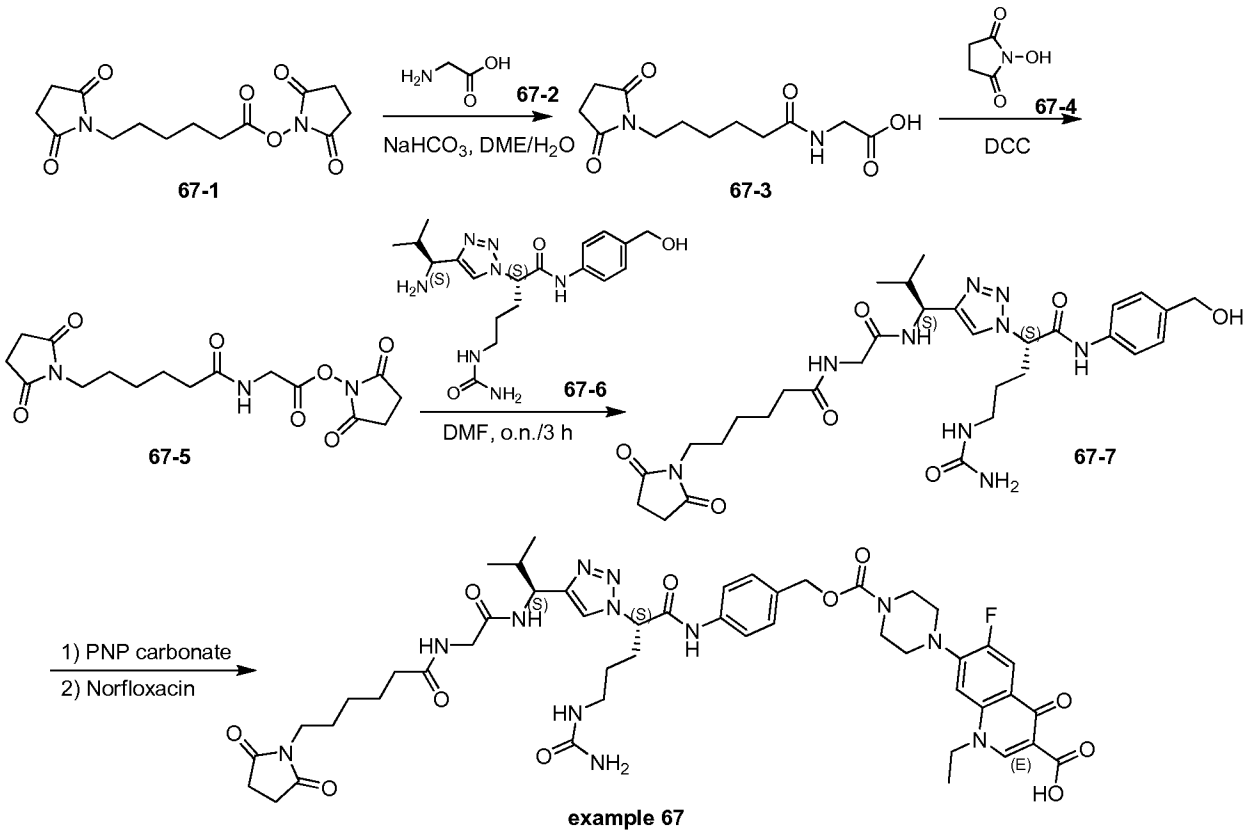
^{1}H NMR (400 MHz, MDSO-d_6) δ 15.33 (s, 1 H), 10.65 (s, 1 H), 8.96 (s, 1 H), 8.14 (s, 1 H), 8.09 (s, 1 H), 7.96-7.81 (m, 2 H), 7.62-7.60 (m, 2 H), 7.38-7.20 (m, 8 H), 6.04-6.00 (m, 1 H), 5.48-5.43 (m, 1 H), 5.07 (s, 2 H), 5.02 (s, 2 H), 4.59-4.57 (m, 2 H), 4.40-4.31 (m, 1 H), 3.61 (s, 4 H), 3.32 (s, 4 H), 3.03-2.99 (m, 2 H), 2.20-2.11 (m, 2 H), 1.40 (t, J = 7.2 Hz, 3 H), 1.30-1.23 (m, 3 H), 0.48-0.23 (m, 4 H).

LCMS (ESI): m/z 881.4 [M+H$^+$].

Example 67. 7-(4-((4-((S)-2-(4-((S)-1-(2-(6-(2,5-dioxopyrrolidin-1-yl)hexanamido)acetamido)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 67



example 67

Step 1. To a solution of compound **67-2** (75 mg, 1 mmol) in DME (10 mL) was added a solution of compound **67-1** (310 mg, 1 mmol) and $NaHCO_3$ (252 mg, 3 mmol) in water (10 mL). The mixture was stirred at r.t. for 16 h. The mixture was washed with EtOAc and acidified to pH = 3 with 10% HCl. The resulting suspension was extracted with EtOAc. The combined organic layer was concentrated to give compound **67-3** (270 mg, contains impurity).

Step 2. To a solution of compound **67-3** (200 mg, 0.74 mmol) and compound **67-4** (90 mg, 0.777 mmol) in THF (10 mL) was added DCC (161 mg, 0.777 mmol) at r.t. The mixture was stirred at r.t. for 16 h under N_2. It was concentrated and the filtrate was concentrated to give **67-5** (200 mg, Yield: 74 %).

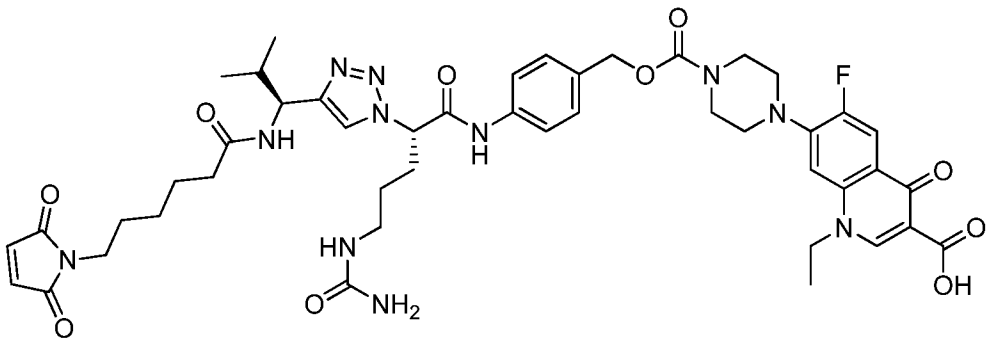
Step 3. Compound **67-5** (200 mg, 0.53 mmol), compound **67-6** (107 mg, 0.265 mmol) were dissolved in DMF (6 mL) and the reaction mixture was stirred at r.t. for 3 h. The mixture was filtered and purified by prep-HPLC to give **67-7** (25 mg, Yield: 6.3 %)

Step 4. To a solution of compound **67-7** (25 mg, 0.038 mmol) in dry DMF (3 mL) was added PNP carbonate (25 mg, 0.076 mmol) and DIPEA (15 mg, 0.114 mmol) at r.t. The mixture was stirred at r.t. for 1.5 h. Norfloxacin (30 mg, 0.076 mmol) was added. The mixture was stirred at r.t. for another 1 h. The mixture was concentrated, filtered and purified by prep-HPLC (FA), to give **example 67** (20 mg, yield: 48%).

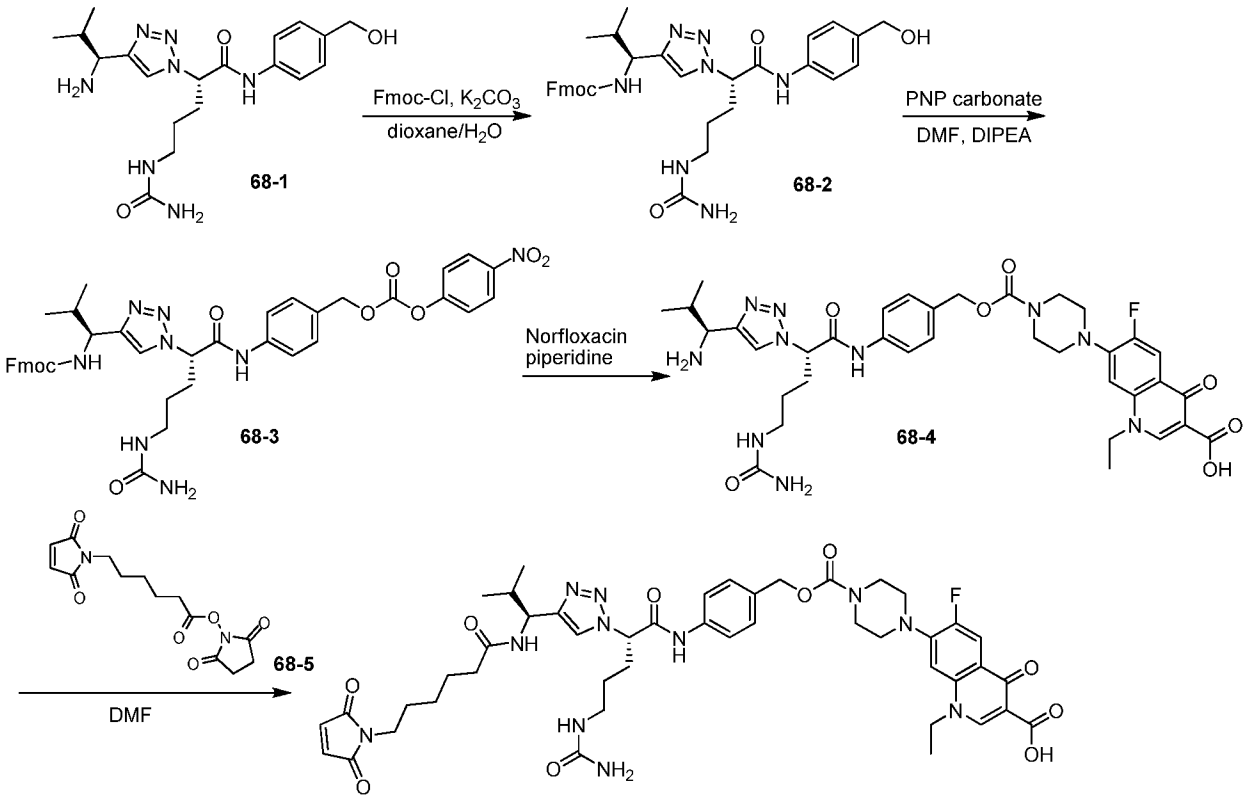
LCMS: (5-95, AB, 1.5 min, ESI), 0.802 min, MS = 501.3[1/2M+1]

^{1}H NMR Methanol-d_4 +$CDCl_3$ 400 MHz, δ 8.83 (s, 1 H), 7.99 (d, J = 13.2 Hz, 2 H), 7.59 (d, J = 6.8 Hz, 2 H), 7.35 (d, J = 8.0 Hz, 2 H), 7.11 (s, 1 H), 5.51 (s, 1 H), 5.12 (s, 2 H), 4.95 (s, 1 H), 4.47 (s, 2 H), 3.86 (s, 2 H), 3.71 (s, 4 H), 3.48 - 3.44 (m, 2 H), 3.31 (s, 4 H), 3.25 (s, 1 H), 3.15 (s, 1 H), 2.67 (s, 4 H), 2.26 - 2.20 (m, 5 H), 1.65 - 1.61 (m, 6 H), 1.58 - 1.52 (m, 1 H), 1.33 - 1.29 (m, 4 H), 0.95 (d, J = 6.8 Hz, 3 H) , 0.88 (d, J = 6.4 Hz, 3 H).

Example 68. 7-(4-((4-((S)-2-(4-((S)-1-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 68



example 68

Step 1. To a solution of crude compound **68-1** (500 mg, 1.24 mmol) in 1,4-dioxane/H_2O (10 mL/5 mL) was added K_2CO_3 (428 mg, 3 mmol) and Fmoc-Cl (416 mg, 1.6 mmol) in dioxane (10mL) dropwise. After the mixture was stirred at r.t. for 16 h, it was concentrated and purified by column chromatography (15%-20% MeOH in DCM) to give compound **68-2** (286 mg, 37%).

LCMS: (5-95, AB, 1.5 min, ESI), 0.709 min, MS = 626.1[M+1]

^{1}H NMR Methanol-d_4 400 MHz, δ 8.06 (s, 1H), 7.80 - 7.78 (d, J = 7.2 Hz, 2 H), 7.67 - 7.63 (t, J = 6.8 Hz, 2 H), 7.58 - 7.56 (d, J = 8.4 Hz, 2 H), 7.39 - 7.27 (m, 6 H), 5.6 - 5.5 (m, 1 H), 4.69 - 4.67 (d, J = 7.2 Hz, 1 H), 4.57 (s, 2 H), 4.40 - 4.36 (m, 2 H), 4.25 - 4.15 (m, 1 H), 3.3 - 3.2 (m, 1 H), 3.2 - 3.1 (m, 1 H), 2.35 - 2.15 (m, 3 H), 1.55 - 1.35 (m, 2 H), 0.98 - 0.97(d, J = 6.8 Hz, 3 H), 0.90 - 0.88(d, J = 6.8 Hz, 3 H).

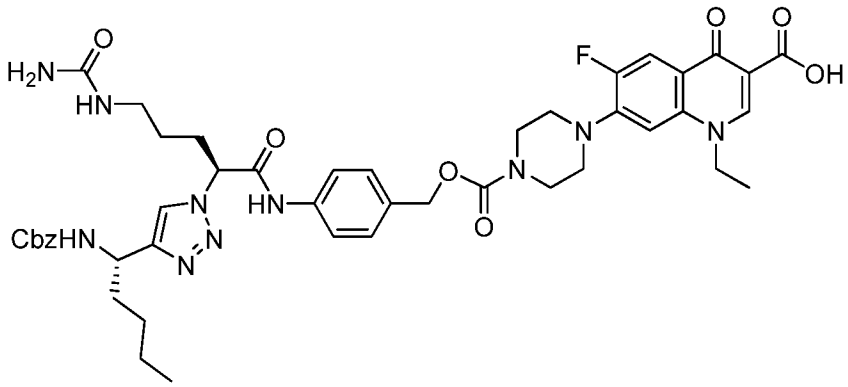
Step 2. To a solution of compound **68-2** (200 mg, 0.31 mmol) in dry DMF (5 mL) was added PNP carbonate (185 mg, 0.62 mmol) and DIPEA (0.5 mL, 3.0 mmol) at r.t., and the mixture was stirred at r.t. for 1.5 h under nitrogen. Norfloxacin (197 mg, 0.62 mmol) was added. The mixture was stirred at r.t. for another 1 h. Then piperidine (0.1 mL, 1 mmol) was added. After 30 min, the mixture was purified by prep-HPLC to give compound **68-4** (250 mg, contained 4-nitrophenol). It was used in next step without further purification.

Step 3. To a solution of compound **68-4** (100 mg, 0.134 mmol) in dry DMF (2 mL) was added compound **5** (50 mg, 0.162 mmol). The mixture stirred at 23°C for 2 h. The mixture was purified by prep-HPLC to give **example 68** (20 mg, yield 16 %).

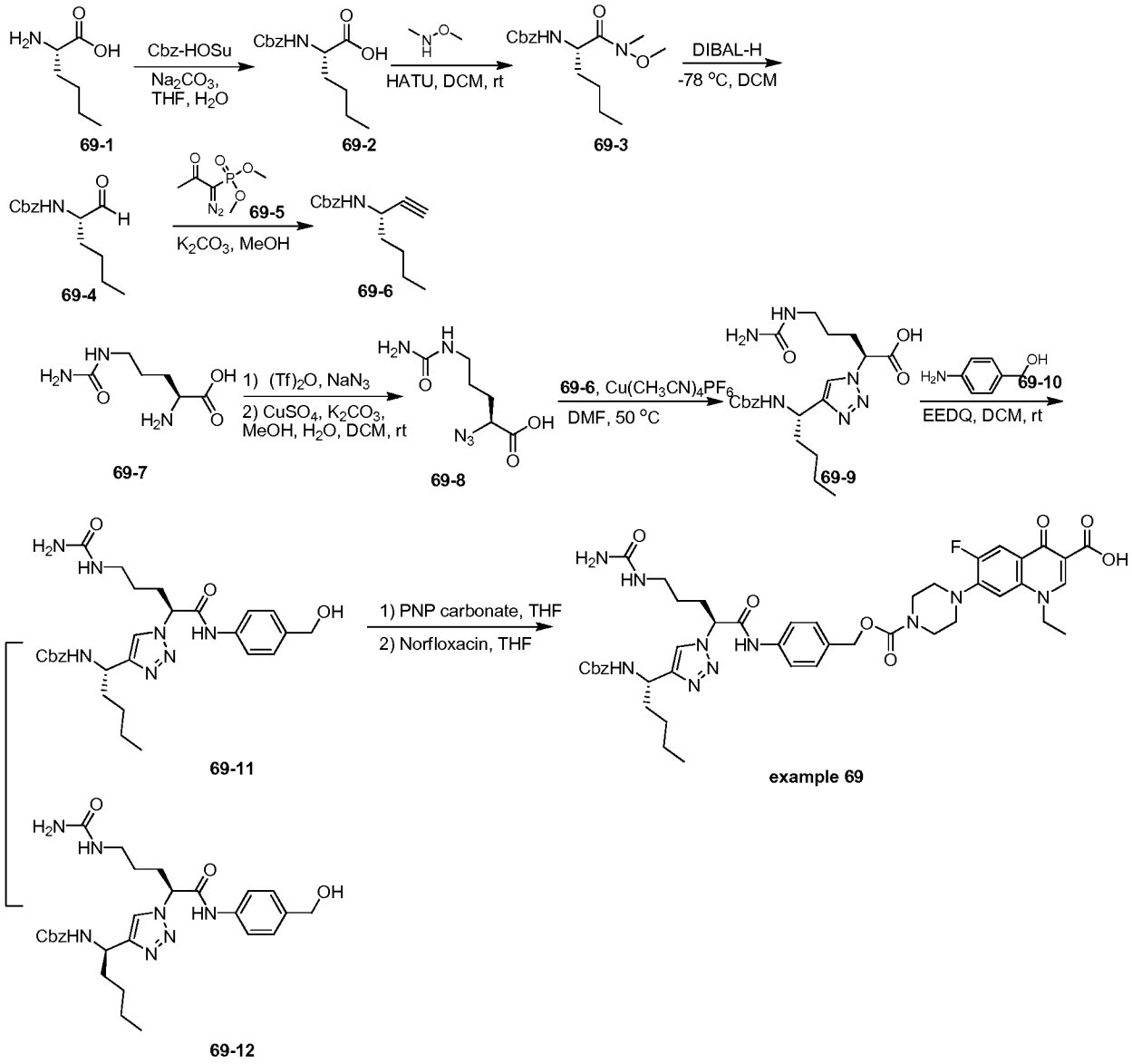
LCMS: (5-95, AB, 1.5 min, ESI), 0.803 min, MS = 942.4 [M+1],471.8[1/2M+1]

^{1}H NMR DMSO-d_6 400 MHz, δ 15.3 (s, 1H), 10.60 (s, 1H), 8.94 (s, 1H), 8.1(d, 1H) 8.01 (s, 1H),7.94 - 7.90 (dd, J = 12.8 Hz, 1 H), 7.58 - 7.56 (d, J = 8.8 Hz, 2 H), 7.34 - 7.32 (d, J = 8.8 Hz, 2 H), 7.2 (d, 1 H), 6.97 (s, 2 H), 6.0 (m, 1H), 5.5 - 5.4 (m, 1H), 5.40 (s, 2H), 5.04 (s, 2H), 4.9 - 4.85 (m, 1H), 4.6 - 4.5 (m, 2H), 3.58 (s, 4H), 3.4 - 3.3(m, 6H), 3.1 - 2.9 (m, 2H), 2.2 - 2.0 (m, 5H), 1.6 - 1.4 (m, 7H), 1.3 - 1.2 (m, 4H), 0.80 - 0.78 (d, J = 6.8 Hz, 3 H), 0.76 - 0.74 (d, J = 6.8 Hz, 3 H).

Example 69. 7-(4-((4-((S)-2-(4-((S)-1-(benzyloxycarbonylamino)pentyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 69



Step 1. To a mixture of aq Na_2CO_3 (16 g, 152.5 mmol) and **69-1** (5 g, 38.1 mmol) was added Cbz-OSu (11.4 g, 45.7 mmol) in THF (40 mL). After the mixture was stirred at r.t. for 16 h, it was adjusted to pH > 10 and washed with EtOAc (100 mL × 2). The aqueous layer was acidified to pH < 1 with conc. HCl. The solution was extracted with EtOAc (200 mL × 3). The organic layer was dried over Na_2SO_4 and concentrated to give **69-2** (9.7 g, 95.9%), which was used for next step without further purification.

Step 2. To a mixture of **69-2** (9.7 g, 36.6 mmol), *N,O*-dimethylhydroxylamine hydrochloride (3.9 g, 40.2 mmol) and HATU (20.8 g, 54.8 mmol) in DCM (100 mL) was added Et_3N (21 mL, 146.2 mmol). After the mixture was stirred at r.t. for 2 h, solvent was removed, and the crude was taken up with water (150 mL) and extracted with EtOAc (150 mL × 3). The organic layer was washed with saturated $NaHCO_3$, conc. HCl, saturated NaCl and concentrated. The residue was purified by column chromatography on silica gel (PE: EtOAc = 2: 1) to give **69-3** (10.1 g, 89.6%).

Step 3. DIBAL-H (19.5 mL, 1M in Tol) was added dropwise to a solution of **69-3** (4.0 g, 13.0 mmol) in anhydrous CH_2Cl_2 (50 mL) at -78°C. After the reaction mixture was stirred at -78°C for 2 h, excess DIBAL was quenched by anhydrous MeOH (15 mL) and the mixture was warmed to r.t. It was concentrated to give **69-4** (3.2 g, crude), which was used for next step without further purification.

Step 4. After a mixture of **69-4** (3.2 g, 13.0 mmol), **69-5** (3.0 g, 15.6 mmol) and K_2CO_3 (3.6 g, 26.0 mmol) in MeOH (60 mL) was stirred at r.t. for 16 h, solvent was removed, and the residue was extracted with EtOAc (80 mL × 3). The organic layers were washed with brine (60 mL) and dried over anhydrous Na_2SO_4, concentrated, and purified by column chromatography on silica gel (PE: EtOAc = 10: 1) to give **69-6** (1.9 g, 60.3%).

Step 5. Tf_2O (3.3 mL, 19.6 mmol) was added slowly to a solution of NaN_3 (6.3 g, 97.8 mmol) in H_2O (30 mL) and CH_2Cl_2 (48 mL) at 0°C. After it was stirred for 2 h, the organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (24 mL×2). The organic fractions, containing the triflyl azide, were pooled and washed once with saturated Na_2CO_3 and used without further purification. The triflyl azide solution in CH_2Cl_2 was added to a mixture of **69-7** (1.71 g, 13.9 mmol), K_2CO_3 (2.03 g, 14.67 mmol) and $CuSO_4.5H_2O$ (245 mg, 0.98 mmol) in H_2O (54 mL) and MeOH (108 mL). After the mixture was stirred at 26°C for 12 h, the organic solvents were removed under reduced pressure and the aqueous slurry was diluted with H_2O (200 mL). The mixture was acidified to pH 6 with conc. HCl and diluted with phosphate buffers (0.2M, pH 6.2, 200 mL) and washed with EtOAc (300 mL × 2). The aqueous phase was then acidified to pH = 2 with conc. HCl. It was extracted with (300 mL × 3), and the extracts were dried over Na_2SO_4 and concentrated to give **69-8**, which was used for next step without further purification.

Step 6. After a mixture of **69-8** (2.0 g, 9.78 mmol), **69-6** (1.2 g, 4.89 mmol) and $Cu(CH_3CN)_4PF_6$ (273 mg, 0.73 mmol) in DMF (5 mL) was stirred at 50°C for 2 h, solvent was removed, and the residue was purified by prep-HPLC to give **69-9** (600 mg, 27.5%).

Step 7. After a mixture of **69-9** (600 mg, 1.34 mmol), **69-10** (496 mg, 4.03 mmol) and EEDQ (996 mg, 4.03 mmol) in DCM (15 mL) was stirred at 23°C for 4 h, solvent was removed, and the residue was purified by prep-HPLC and SFC to give **69-11** (350 mg, 47.4%) and **69-12** (23.1 mg).

^{1}H NMR (400 MHz, MeOD) δ 8.02 (s, 1 H), 7.54 (d, J = 6.8 Hz, 2 H), 7.34-7.25 (m, 7 H), 5.49-5.46 (m, 1 H), 5.08 (s, 2 H), 4.55 (s, 2 H), 3.37-3.33 (m, 1 H), 3.24-3.16 (m, 1 H), 2.24-1.80 (m, 3 H), 1.45-1.43 (m, 7 H), 1.41 (s, 3 H).

LCMS (ESI): m/z 552.1 [M+H$^+$].

^{1}H NMR (400 MHz, MeOD) δ 10.53 (s, 1 H), 8.03 (s, 1 H), 7.55 (d, J = 9.2 Hz, 1 H), 7.35 (d, J = 4.0 Hz, 2 H), 7.31-7.26 (m, 7 H), 6.04 (s, 1 H), 5.50-5.43 (m, 3 H), 5.12 -5.03 (m, 3 H), 4.77-4.73 (m, 1 H), 4.44 (d, J = 3.2 Hz, 2 H), 3.08-2.91 (m, 2 H), 2.24-2.16 (m, 2 H), 1.90-1.71 (m, 2 H), 1.35-1.20 (m, 6 H), 0.86-0.83 (m, 3 H).

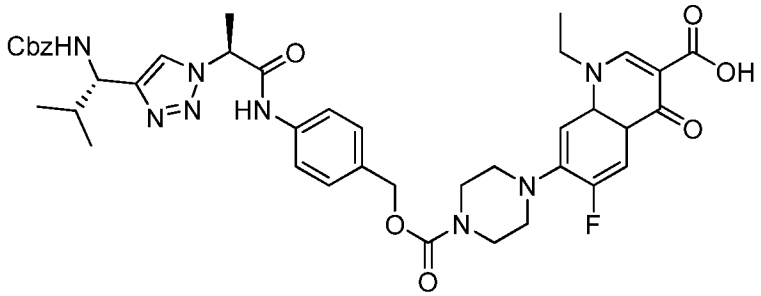
LCMS (ESI): m/z 552.2 [M+H$^+$].

Step 8. A mixture of **69-11** (100 mg, 0.18 mmol), PNP carbonate (109 mg, 0.36 mmol) and DIPEA (70 mg, 0.54 mmol) in DCM (5 mL) was stirred at 50°C for 12 h. It was concentrated and added to a mixture of DIPEA (70 mg, 0.54 mmol) and norfloxacin (172 mg, 0.54 mmol) in DMF (5 mL). After it was stirred at 23°C for 4 h, solvent was removed, and the residue was purified by prep-HPLC to give **example 69** (69.3 mg, 43%).

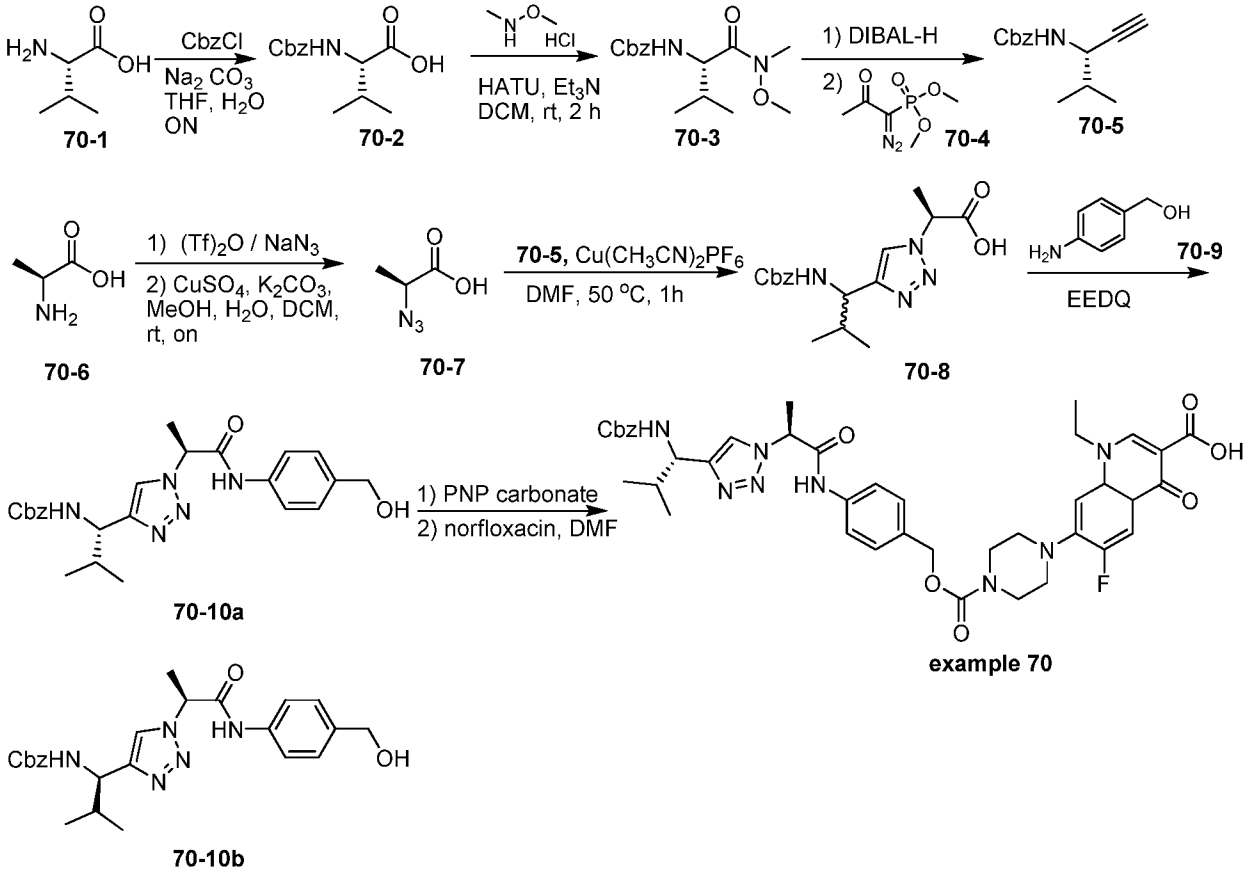
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.32 (s, 1 H), 10.63 (s, 1H), 8.96 (s, 1 H), 8.02 (s, 1 H), 7.94 (d, J =12.8 Hz, 1 H), 7.70 (d, J = 8.0 Hz, 1 H), 7.60 (d, J = 8.8 Hz, 2 H), 7.38-7.20 (m, 8 H), 6.03 (s, 1 H), 5.49-5.42 (m, 3 H), 5.07 (s, 2 H), 5.04 (d, J = 4.0 Hz, 2 H), 4.72-4.71 (m, 1 H), 4.58-4.57 (m, 2 H), 3.67 (s, 4 H), 3.61 (s, 4 H), 3.06-3.01 (m, 2 H), 2.12-2.07 (m, 2 H), 1.80-1.68 (m, 2 H), 1.42-1.38 (m, 3 H), 1.34 (s, 6 H), 0.84 (s, 3 H).

LCMS (ESI): m/z 897.4 [M+H$^+$].

Example 70: 7-(4-((4-((S)-2-(4-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)-1H-1,2,3 triazol-1-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4,4a,8a-tetrahydroquinoline-3-carboxylic acid



Example 70



Step 1. To a mixture of compound **70-1** (3.0 g, 25.61 mmol) in water (20 mL) was added Na_2CO_3 (2.71 g, 25.61 mmol). CbzCl (4.81 g, 28.17 mmol) was added and the reaction mixture was stirred at 28°C for 16 h. The mixture was filtered and extracted with EtOAc (15 mL × 2). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give compound **70-2** (5.9 g, 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21-7.30 (m, 5 H), 5.86 (br, 1 H), 5.15 (s, 2 H), 4.37-4.34 (m, 1 H), 2.28-2.21 (m, 1 H), 1.01-0.84 (m, 6 H).

Step 2. To a solution of compound **70-2** (5.9 g, 23.48 mmol), *N*-methoxymethanamine hydrochloride (2.52 g, 25.83 mmol) and Et_3N (7.13 g, 70.44 mmol) in DCM (60 mL) was added HATU (13.4 g, 35.22 mmol) at 28°C. After the mixture was stirred at 28°C for 3 h, solvent was removed and the residue was dissolved in water (30 mL). The aqueous layer was extracted with EtOAc (80 mL × 3). The organic layers were combined, dried over Na_2SO_4 and concentrated. The crude was purified by column chromatography on silica gel (PE/EtOAc = 2:1) to give compound **70-3** (4.5 g, 65%).

LCMS (ESI): m/z 295.1 [M+H$^+$].

Step 3. To a mixture of compound **70-3** (588 mg, 2.0 mmol) in DCM (8 mL) at -78°C was added dropwise DIBAL-H in toluene (1M, 2.4 mL, 2.4 mmol). After the mixture was stirred at -78°C for 2 h, MeOH (1 mL) was added dropwise. The mixture was allowed to warm up to r.t. Solvent was removed and the crude was used directly in the next step.

To a solution of the above crude in MeOH (5 mL) was added K_2CO_3 (553 mg, 4.0 mmol) and compound **70-4** (461 mg, 2.4 mmol). The reaction mixture was stirred at 26°C for 8 h. Solvent was removed and the residue was purified by column chromatography on silica gel (PE/EtOAc = 20:1) to give compound **70-5** (300 mg, 65.2%).

LCMS (ESI): m/z 232.1 [$M+H^+$].

Step 4. To mixture of NaN_3 (2.0 g, 30.76 mmol) in distilled H_2O (4.5 mL) and DCM (7.5 mL) was added Tf_2O (1.57 g, 5.55 mmol) dropwise at 0°C. The mixture was stirred at 0°C for 3 h. The aqueous layer was extracted with DCM (4 mL × 2), and washed with saturated Na_2CO_3 solution (8 mL). To a mixture of compound **70-6** (249 mg, 2.79 mmol), K_2CO_3 (578 mg, 4.19 mmol) and $CuSO_4$ $5H_2O$ (7 mg, 27.9 umol) in H_2O (9 mL) and MeOH (18 mL) was added the triflyl azide in DCM (15.5 mL) dropwise. After the mixture was stirred at 26°C overnight, the organic solvents were removed under reduced pressure and the aqueous slurry was diluted with H_2O (50 mL). It was acidified to pH 6 with conc. HCl, diluted with phosphate buffers (0.2 M, pH 6.2, 50 mL), and exacted with EtOAc (50 mL × 2). The aqueous phase was then acidified to pH 2 with conc. HCl and extracted with EtOAc (80 mL × 3). The organic layer was dried over Na_2SO_4 and evaporated to give **70-7**, which was used for next step without further purification.

Step 5. To a solution of compound **70-7** (321 mg, 2.79 mmol) and compound **70-5** (278 mg, 1.2 mmol) in DMF (3 mL), was added $Cu(CH_3CN)_4PF_6$ (67 mg, 0.18 mmol). The reaction mixture was heated at 50°C for 2 h under N_2. After removal of the solvent, the residue was purified by column chromatography on silica gel (2.5-5% of MeOH in DCM) to give compound **70-8** (90 mg, 22%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (s, 1 H), 7.37-7.32 (m, 5 H), 5.05 (s, 2 H), 4.64 (s, 1 H), 3.35 (s, 4 H), 2.02 (s, 1 H), 0.85-0.67 (m, 6 H).

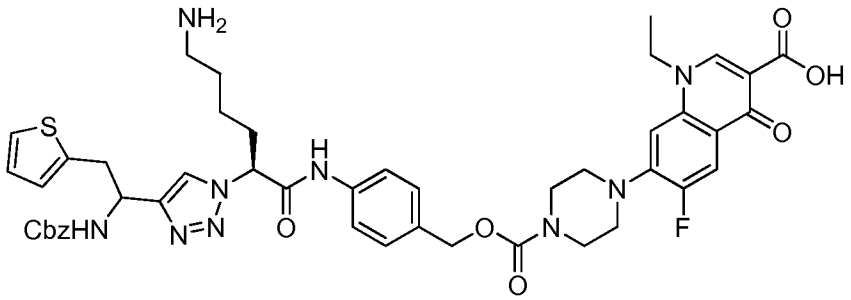
Step 6. To a solution of compound **70-8** (1.4 g, 4.04 mmol) and compound **70-9** (1.49 g, 12.12 mmol) in DCM (15 mL) was added EEDQ (3.0 g, 12.12 mmol) at 25°C. After the reaction mixture was stirred at 25°C for 2 h, the solvent was removed, the residue was purified by prep-HPLC to give a mixture (1.1 g). The two isomers were purified by SFC separation to give **70-10a** (250 mg) and other enantiomer **70-10b** (116 mg).

^{1}H NMR (400 MHz, MeOD) δ 8.03 (s, 1 H), 7.57-7.54 (m, 2 H), 7.35-7.28 (m, 7 H), 5.57 (q, J = 7.2 Hz, 1 H), 5.13-5.06 (m, 2 H), 4.72-4.65 (m, 1 H), 4.58 (s, 2 H), 2.21-2.17 (m, 1 H), 1.87 (d, J = 7.2 Hz, 3 H), 0.97 (d, J = 6.8 Hz, 3 H), 0.90 (d, J = 6.8 Hz, 3 H). LCMS (ESI): m/z 452.2 [M+H$^+$].

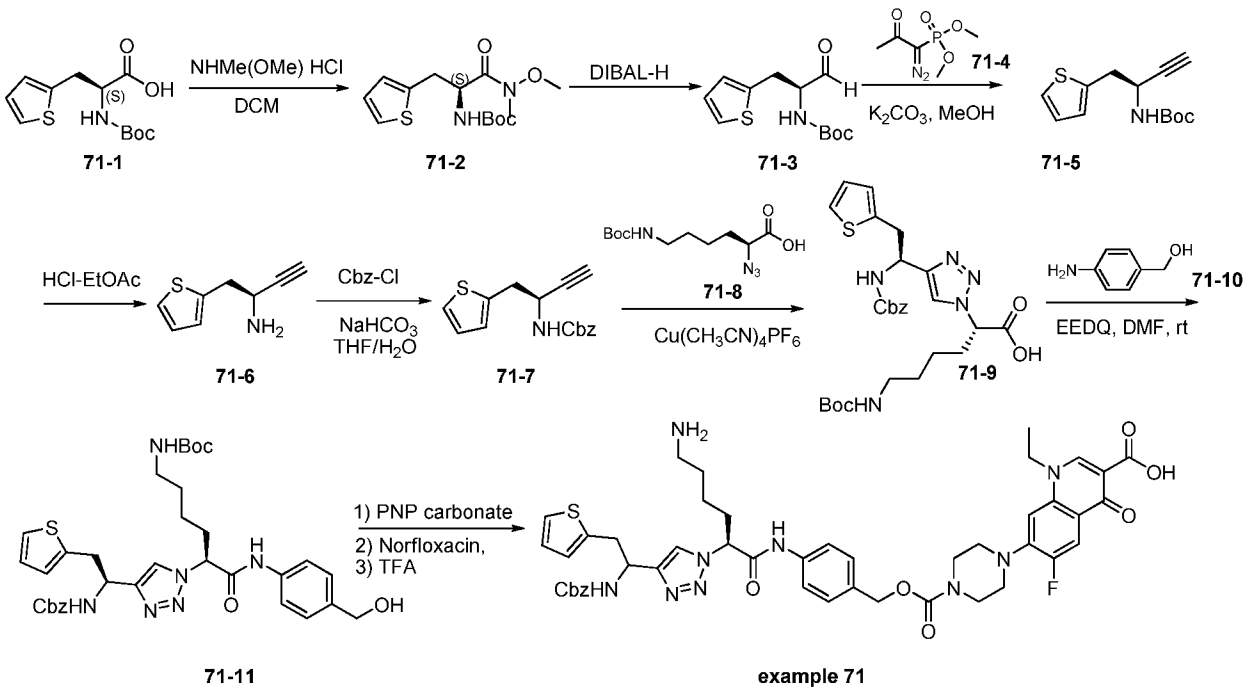
Step 7. To a solution of **70-10a** (100 mg, 0.221 mmol) in THF (3 mL) were added PNP carbonate (202 mg, 0.663 mmol) and DIPEA (114 mg, 0.884 mmol) at 25°C. After the mixture was heated at reflux for 24 h, solvent was removed, and the residue was purified by column chromatography on silica gel (DCM/MeOH = 20:1) to give the intermediate (90 mg). To a norfloxacin solution (70 mg, 0.219 mmol) in DMF (2 mL) was added DIPEA (94 mg, 0.73 mmol). After the solution was stirred at 25°C for 15 min, the above intermediate (90 mg, 0.219 mmol) was added. The mixture was stirred at 25°C for 2 h. Solvent was removed and the residue was purified by prep-TLC (DCM/MeOH = 10:1) to give **example 70** (26.5 mg, 23%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.32 (s, 1 H), 10.61 (s, 1 H), 8.98 (s, 1 H), 8.07 (s, 1 H), 7.95-7.92 (m, 1 H), 7.71-7.58 (m, 3 H), 7.38-7.16 (m, 8 H), 5.52 (m, 1 H), 5.10-5.00 (m, 4 H), 4.63-4.52 (m, 3 H), 3.60 (s, 4 H), 3.30 (s, 4 H), 2.10-2.00 (m, 1 H), 1.75 (d, J = 7.6 Hz, 3 H), 1.42 (t, J = 7.6 Hz, 3 H), 0.88-0.75 (m, 6H). LCMS (ESI): m/z 797.6 [M+H$^+$].

Example 71. 7-(4-((4-((2S)-6-amino-2-(4-(1-(benzyloxycarbonylamino)-2-(thiophen-2-yl)ethyl)-1H-1,2,3-triazol-1-yl)hexanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 71



Step 1. To a mixture of compound **71-1** (500 mg, 1.84 mmol) in anhydrous DCM (20 mL) was added Et_3N (559 mg, 5.52 mmol) and HATU (1.049 g, 2.76 mmol). The mixture was stirred at r.t. for 15 min, then NHMe(OMe)HCl (269 mg, 2.76 mmol) was added. The reaction mixture was stirred at r.t. overnight. The mixture was extracted with EtOAc (30 mL× 3) and water (30 mL), The combined organic layer was dried, concentrated, and purified by column chromatography on silica gel (PE: EtOAc = 2: 1) to give **71-2** (500 mg, 86.4 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.34 (d, J = 4.8 Hz, 1H), 7.22 (d, J = 9.2 Hz, 1H), 6.94 - 6.89 (m, 2H), 4.56 (s, 1H), 3.70 (s, 3H), 3.11 (s, 3H), 3.09 - 2.95 (m, 2H), 1.35 (s, 9H).

Step 2. Compound **71-2** (500 mg, 1.59 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) and was cooled to -78°C in a dryice/acetone bath. DIBAL-H (4.8 mL, 4.77 mmol, 1.0 M in toluene) was added dropwise and the resulting solution was stirred at -78°C for 3 h. Excess hydride was quenched with MeOH (5 mL) and the resulting solution was warmed to r.t. The solution was evaporated to give the compound **71-3** without further purification.

Step 3. To a solution of crude compound **71-3** (406 mg, 1.59 mmol), compound **71-4** (611 mg, 3.18 mmol) in MeOH (20 mL) was added K_2CO_3 (659 mg, 4.77 mmol). The reaction mixture was stirred at r.t overnight. The mixture was concentrated in vacuum, and purified by column chromatography on silica gel (PE: EtOAc = 5: 1) to give **71-5** (250 mg, 62.3 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (d, J = 8.4 Hz, 1H), 7.35 (dd, J = 4.8, 1.2 Hz, 1H), 6.96 - 6.92 (m, 2H), 4.35 - 4.31 (m, 1H), 3.19 (d, J = 1.2 Hz, 1H), 3.10 - 3.08 (m, 2H), 1.36 (s, 9H).

Step 4. To a solution of compound **71-5** (250 mg, 0.99 mmol) in DCM (5 mL) was added HCl-EtOAc (5 mL, 4.0 M, 20.00 mmol) dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. The mixture was concentrated in vacuum at 25 °C to give **71-6** as a HCl salt (180 mg, 96.9 %).

Step 5. Cbz-Cl (287 mg, 1.68 mmol) in THF (2 mL) was added dropwise into a solution of compound **71-6** (170 mg, 0.91 mmol) in saturated aq. $NaHCO_3$ solution (2 mL). The reaction mixture was stirred at 0 °C for 1 h. The mixture was extracted with EtOAc (10 mL × 3) and H_2O (10 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE: EtOAc = 5:1) to give compound **71-7** (250 mg, 96.3 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 (d, J = 8.4 Hz, 1H), 7.38 - 7.31 (m, 6H), 6.96 - 6.93 (m, 2H), 5.02 (s, 2H), 4.45 - 4.39 (m, 1H), 3.26 (d, J = 2.0 Hz, 1H), 3.13 (dd, J = 6.8, 2.0 Hz, 2H).

Step 6. To the solution of compound **71-7** (280 mg, 0.98 mmol) and Compound **71-8** (400 mg, 1.47 mmol) in DMF (5 mL) was added $Cu(CH_3CN)_4PF_6$ (37 mg, 0.1 mmol). The reaction mixture was stirred at 50°C for 2 h to give **71-9** which was used directly for next step.

Step 7. To the mixture of crude compound **71- 9** (547 mg, 0.98 mmol) in DMF (5 mL) was added EEDQ (484 mg, 1.96 mmol) and compound **71-10** (181 mg, 1.47 mmol). The reaction mixture was stirred at r.t. under N_2 overnight. The mixture was purified by prep-HPLC to give the **71-11** (100 mg, 15.4 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.11 – 8.06 (m, 1H), 7.90 (t, J = 8.4 Hz, 1H), 7.53 (d, J = 8.8 Hz, 2H), 7.36 - 7.26 (m, 8H), 6.92 - 6.89 (m, 1H), 6.85 (d, J = 3.2 Hz, 1H), 6.79 (t, J = 4.8 Hz, 1H), 5.44 - 5.40 (m, 1H), 5.14 (t, J = 6.0 Hz, 1H), 5.06 - 4.93 (m, 3H), 4.44 (d, J = 5.6 Hz, 2H), 3.43 - 3.38 (m, 1H), 3.01 - 3.24 (m, 1H), 2.90 - 2.85 (m, 2H), 2.12 - 2.07 (m, 2H), 1.43 - 1.39 (m, 2H), 1.37 (s, 9H), 1.18 - 1.10 (m, 2H).

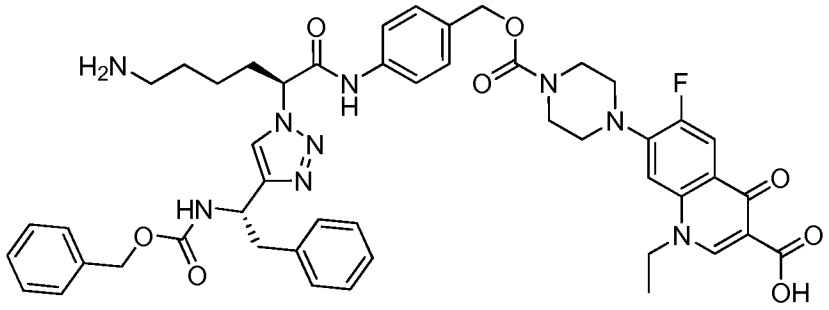
Step 8. To the solution of compound **71-11** (100 mg, 0.15 mmol) in anhydrous DMF (2 mL) was added DIPEA (116 mg, 0.90 mmol), PNP carbonate (91 mg, 0.30 mmol) 0 °C. After the reaction mixture was stirred at 0 °C for 2 h, norfloxacin was added. The mixture was stirred at °C for another 1 h, and it was purified by prep-HPLC to give compound **71-12** (120 mg, 79.4 %).

Step 9. To the mixture of compound **71-12** (100 mg, 0.10 mmol) in anhydrous DCM (2.5 mL) was added TFA (0.5 mL). The reaction mixture was stirred at 0°C for 1 h. Then the mixture was diluted with DMF, and was adjusted to pH 8 with NH_3H_2O dropwise. The resulting mixture was purified by prep-HPLC to give **example 71** (63 mg, 69.4 %) was obtained.

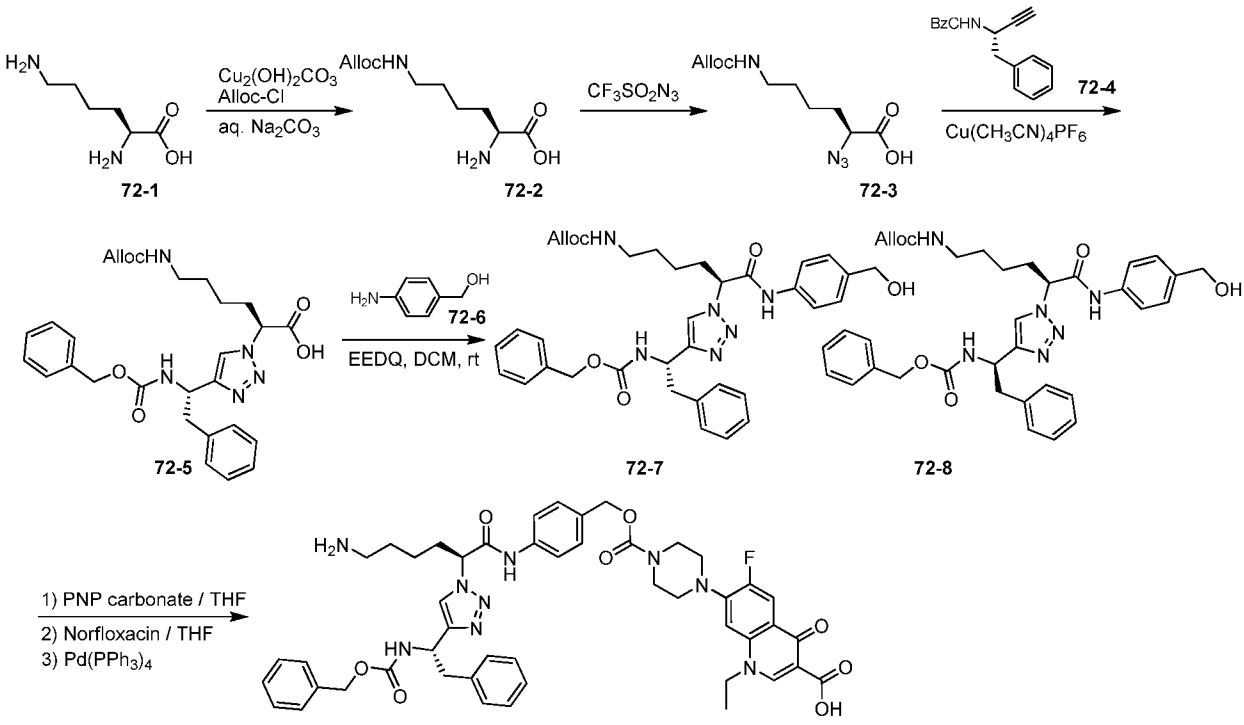
LCMS (ESI): RT = 0.830 min, M/2+H$^+$ = 454.8. method = 5-95 /2 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (bs, 1H), 8.96 (s, 1H), 8.46 (s, 1H), 8.13 - 8.09 (m, 1H), 7.96 - 7.89 (m, 2H), 7.61 (d, J = 8.8 Hz, 2H), 7.38 - 7.30 (m, 8H), 7.21 (d, J = 7.2 Hz, 1H), 6.92 - 6.90 (m, 1H), 6.86 (d, J = 2.0 Hz, 1H), 5.49 - 5.45 (m, 1H), 5.08 - 4.92 (m, 5H), 4.60 - 4.55 (m, 2H), 3.61 (s, 4H), 3.34 - 3.26 (m, 6H), 2.70 - 2.66 (m, 2H), 2.19 - 2.08 (m, 2H), 1.57 - 1.49 (m, 2H), 1.42 - 1.37 (m, 3H), 1.23 - 1.16 (m, 2H).

Example 72. 7-(4-((4-((S)-6-amino-2-(4-((S)-1-(benzyloxycarbonylamino)-2-phenylethyl)-1H-1,2,3-triazol-1-yl)hexanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



example 21



example 72

Step 1. To a solution of compound **72-1** (14.7 g, 0.1 mol) in water (300 mL) was added $Cu_2(OH)_2CO_3$ (38 g, 0.1 mol). It was heated at reflux for 1 h. Solid was filtered off, and the pH of filtrate was adjusted to 9.0 by addition of Na_2CO_3. Alloc-Cl (14.4 g, 0.12 mmol) was added dropwise at 0°C, while pH was maintained at 9.0 by addition of Na_2CO_3. The mixture was stirred for 12 h at r.t. The blue solid was collected by filtration and re-dissolved in water (300 mL). Thioacetamide (20 mmol) was added to and the solution was stirred at 50 °C for 3 h. The solution was acidified with HCl to pH 2.0 and boiled for 10 min. CuS was filtered off, and the solution was concentrated to 100 mL, and **72-2** was collected by filtration.

^{1}H NMR (400 MHz, MeOD) δ 5.94-5.88 (m, 1 H), 5.30-5.26 (m, 1 H), 5.18-5.15 (m, 1 H), 4.55-4.93 (m, 1 H), 3.50 (brs, 1 H), 3.13-3.09 (m, 2 H), 2.00-1.70 (m, 2 H), 1.54-1.41 (m, 4 H).

Step 2. To the solution of NaN_3 (1.78 g, 27.45 mmol) in a mixture of H_2O (5 mL) and DCM (7.5 mL) was added Tf_2O (0.93 mL, 5.55 mmol). After the mixture was stirred at r.t. for 2 h, it was extracted with DCM (50 mL×3). The organic layer was washed with aq. Na_2CO_3 and concentrated to 10 mL. Compound **72-2** (640 mg, 2.8 mmol) was added, followed by K_2CO_3 (577 mg, 4.19 mmol), $CuSO_4$ (7 mg, 0.028 mmol), H_2O (9 mL) and MeOH (18 mL). The mixture was stirred at r.t. for 12 h. The organic solvents were evaporated, solution was diluted with water, and pH was adjust to 6.0 with HCl and diluted with phosphate buffers (0.25 M, pH 6.2, 50 mL). The mixture was extracted with EtOAc (50 mL × 3). The organic layer was dried to give compound **72-3**, which was used for next step without further purification.

Step 3. To the solution of **72-3** (560 mg, 2 mmol) in DMF (5 mL) was added **72-4** (1.12 g, 4.0 mmol) and cat. $Cu(CH_3CN)_4PF_6$. The mixture was stirred at 50°C for 3 h under N_2. After the solvent was removed, the residue was purified by prep-HPLC to give **72-5**.

LCMS (ESI): m/z 536.2 [M+H$^+$]

Step 4. To the solution of **72-5** (480 mg, 1.0 mmol) in DCM (10 mL) was added EEDQ (247 mg, 1.0 mmol) and **72-6** (123 g, 1.0 mmol), and the mixture was stirred at 0°C for 1 h under N_2. After the solvent was removed, the residue was purified with prep-HPLC and SFC separation to give **72-7** and **72-8**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1 H), 7.55-7.52 (m, 2 H), 7.33-7.15 (m, 12 H), 5.89-5.87 (m, 1 H), 5.42-5.38 (m, 1 H), 5.28-5.23 (m, 1 H), 5.15-5.02 (m, 3 H), 4.58 (s, 2 H), 4.51-4.47 (m, 2 H), 3.31-3.05 (m, 4 H), 2.20-2.15 (m, 2 H), 1.53-1.50 (m, 2 H), 1.31-1.21 (m, 2 H). LCMS (ESI): m/z 641.1 [M+H$^+$].

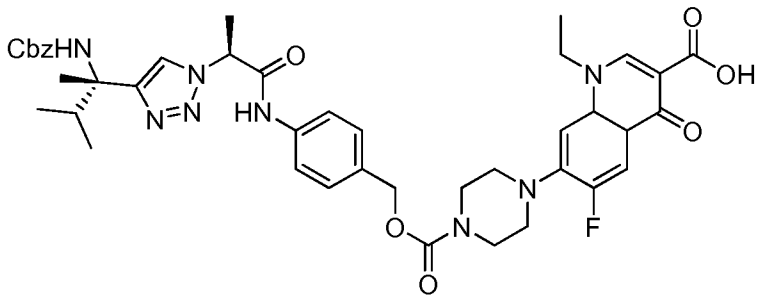
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1 H), 7.56 (d, J = 8.4 Hz, 2 H), 7.35-7.17 (m, 12 H), 5.95-5.88 (m, 1 H), 5.45-5.41 (m, 1 H), 5.30-5.26 (m, 1 H), 5.18-5.03 (m, 3 H), 4.58 (s, 2 H), 4.51-4.50

(m, 2 H), 3.31-3.23 (m, 1 H), 3.15-3.01 (m, 3 H), 2.23-2.15 (m, 2 H), 1.56-1.53 (m, 2 H), 1.32-1.22 (m, 2 H). LCMS: m/z 641.1 [M+H$^+$].

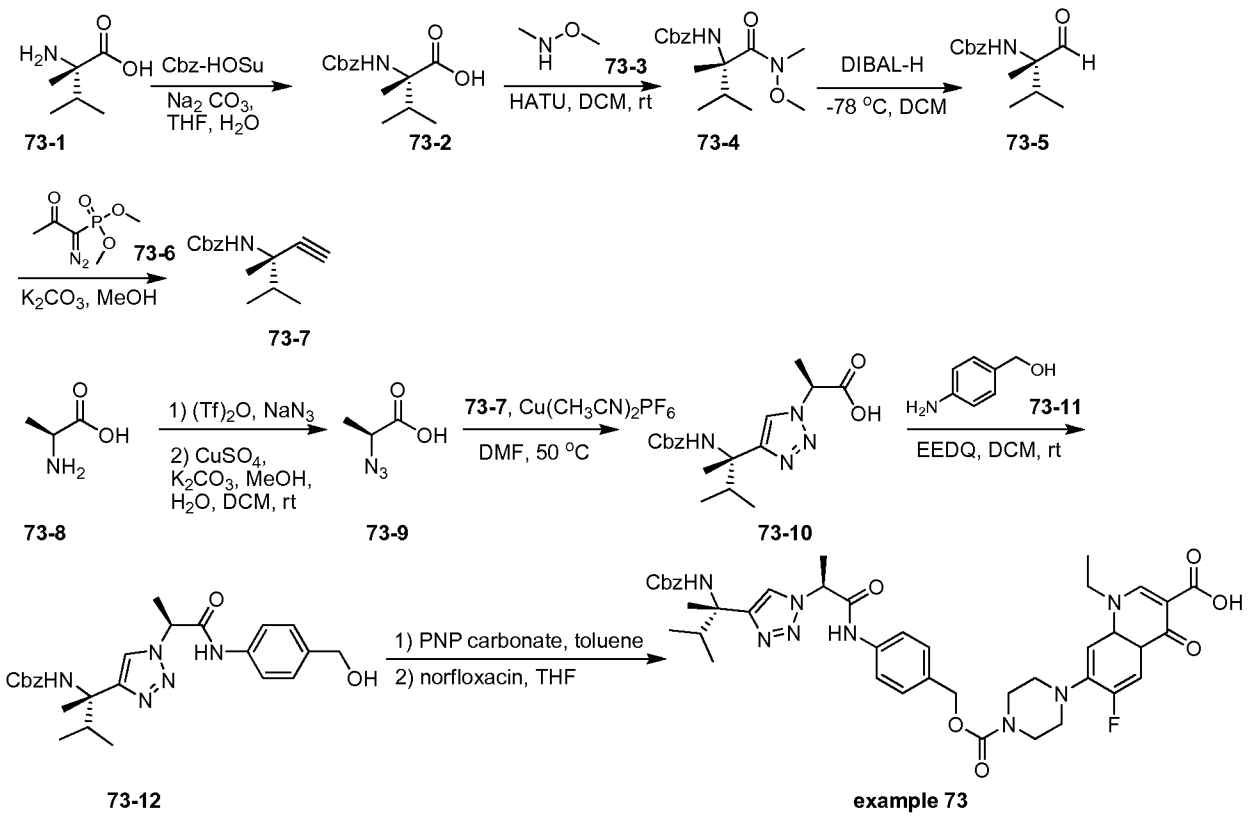
Step 5. To the solution of **72-7** (59 mg, 0.1 mmol) in dry DCM (30 mL) was added PNP carbonate (62 mg, 0.2 mmol) and DIPEA (1 mL). The mixture was heated at reflux for 16 h. After the solvent was removed, the residue was dissolved in DMF (5 mL). DIPEA (0.2 mL) and norfloxacin (65 mg, 0.2 mmol) were added. The mixture was stirred at r.t. for 1 h. After the solvent was removed, the residue was purified by column. To the intermediate in dry THF (10 mL) was added $Pd(PPh_3)_4$ (116 mg, 0.1 mmol). The mixture was stirred at r.t. for 12 h under N_2. After the solvent was removed, the residue was purified by column on silica gel to give **example 72**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (s, 1 H), 8.47 (s, 1 H), 8.01-7.93 (m, 2 H), 7.59-7.57 (m, 2 H), 7.39-7.37 (m, 2 H), 7.27-7.14 (m, 11 H), 5.41 (s, 1 H), 5.14 (s, 2 H), 5.09-4.92 (m, 3 H), 4.59-4.50 (m, 2 H), 3.72-3.71 (m, 4 H), 3.30 (s, 4 H), 3.21-3.19 (m, 2 H), 2.89-2.85 (m, 2 H), 2.25-2.23 (m, 2 H), 1.69-1.67 (m, 2 H), 1.52-1.49 (m, 3 H), 1.40-1.20 (m, 2 H). LCMS (ESI): m/z 902.5 [M+H$^+$].

Example 73: 7-(4-((4-((S)-2-(4-((S)-2-(benzyloxycarbonylamino)-3-methylbutan-2-yl)-1H-1,2,3-triazol-1-yl)propanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4,4a,8a-tetrahydroquinoline-3-carboxylic acid



example 73



Step 1. To a mixture of aq Na_2CO_3 (82 g, 0.78 mol) and compound **73-1** (20 g, 0.19 mol) was added Cbz-OSu (57 g, 0.23 mol) in THF (150 mL). After the mixture was stirred at r.t. for 16 h, it was adjusted to pH > 10 and the solution was extracted with EtOAc (400 mL × 2). The aqueous layer was acidified to pH < 1 with conc.HCl and the solution was extracted with EtOAc (500 mL × 2). The organic layers were dried over Na_2SO_4 and concentrated to give compound **73-2** (45 g, 0.19 mol), which was used for next step without further purification.

Step 2. To a mixture of compound **73-2** (20 g, 84.3 mmol), *N,O*-dimethylhydroxylamine Hydrochloride (8.9 g, 92.7 mmol) and HATU (48.1 g, 126.4 mmol) in DCM (200 mL) was added Et_3N (48.7 mL, 337.2 mmol). After the mixture was stirred at r.t. for 2 h, solvent was removed and the residue was taken up with water (300 mL). The aqueous layer was extracted with EtOAc (300 mL × 3). The organic layer was washed with saturated $NaHCO_3$ (100 mL), conc.HCl (100 mL), saturated NaCl (100 mL). Solvent was removed and the residue was purified by column chromatography on silica gel (PE: EtOAc = 2: 1) to give compound **73-4** (12.9 g, 46.0 mmol).

Step 3. DIBAL (17.8 mL, 1M in Tol) was added dropwise to a solution of compound **73-4** (4.15 g, 14.8 mmol) in anhydrous CH_2Cl_2 (100 mL) at-78°C. The resulting solution was stirred at -78°C for 2 h and excess DIBAL was quenched with anhydrous MeOH (5 mL) and the resulting solution was warmed to r.t. The solution was concentrated to give compound **73-5** (3.27 g, 14.8mmol), which was used for next step without further purification.

Step 4. After a mixture of compound **75-5** (3.27 g, 14.8 mmol), **73-6** (2.96 g, 17.8 mmol) and K_2CO_3 (4.1 g, 29.7 mmol) in MeOH (60 mL) was stirred at r.t. for 16 h, solvents were removed under reduced pressure and the crude residue was partitioned between EtOAc (200 mL) and water (100 mL). The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography on silica gel (PE: EtOAc = 10: 1) to give compound **73-7** (606 mg, 2.79 mmol).

Step 5. To a mixture of NaN_3 (3.2 g, 49.3mmol) in distilled H_2O (10 mL) and CH_2Cl_2 (16 mL) was added Tf_2O (1.7 mL, 9.9 mmol) slowly over 5 min at 0 °C. After it was stirred for 2 h the organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (8 mL × 2). The organic fractions, containing the triflyl azide, were washed once with saturated Na_2CO_3 and added to a mixture of Compound **73-8** (0.86 g, 4.93 mmol), K_2CO_3 (1.02 g, 7.40 mmol) and $CuSO_4.5H_2O$ (25 mg, 0.099 mmol) in distilled H_2O (18 mL) and MeOH (36 mL). After the mixture was stirred at 26°C for 12 h, the organic solvents were removed under reduced pressure and the aqueous slurry was diluted with H_2O (50 mL) and acidified to pH 6 with conc.HCl and diluted with 0.2M pH 6.2 phosphate buffers (50 mL). It was washed with EtOAc (100 mL × 2), and the aqueous phase was then acidified to pH 2 with conc. HCl. It was extracted with EtOAc (200 mL × 3) and the combined layers were dried over Na_2SO_4 concentrated and used for next step without further purification.

Step 6. A mixture of compound **73-9** (0.99 g, 4.93 mmol), compound **73-7 (**0.54 g, 2.46 mmol) and $Cu(CH_3CN)_2PF_6$ (115 mg, 0.37 mmol) in DMF (5 mL) was stirred at 50°C for 2 h. Solvent was removed and the residue was purified by prep-HPLC to give compound **73-10** (250 mg, 23%).

Step 7. A mixture of compound **73-10** (199 mg, 0.55 mmol), compound **73-11** (176 mg, 1.43 mmol) and EEDQ (353 mg, 1.43 mmol) in DCM (10 mL) was stirred at 24°C for 2 h. Solvent was removed and the residue was purified by prep-HPLC to give **73-12** (100 mg, 39.1%).

^{1}H NMR (400 MHz, MeOD) δ 7.98 (s, 1 H), 7.54 (d, J = 8.4 Hz, 2 H), 7.33-7.31 (m, 6 H), 7.10 (s, 1 H), 5.55-5.49 (m, 1 H), 4.98 (s, 2 H),4.55 (s, 3 H), 2.35 (s, 1 H), 1.84 (d, J = 6.4 Hz, 3 H), 1.69 (s, 3 H) ,0.94 (d, J = 6.8 Hz, 3 H), 0.79 (d, J = 6.8 Hz, 3 H).

LCMS (ESI): m/z 465.9 [M+H$^+$].

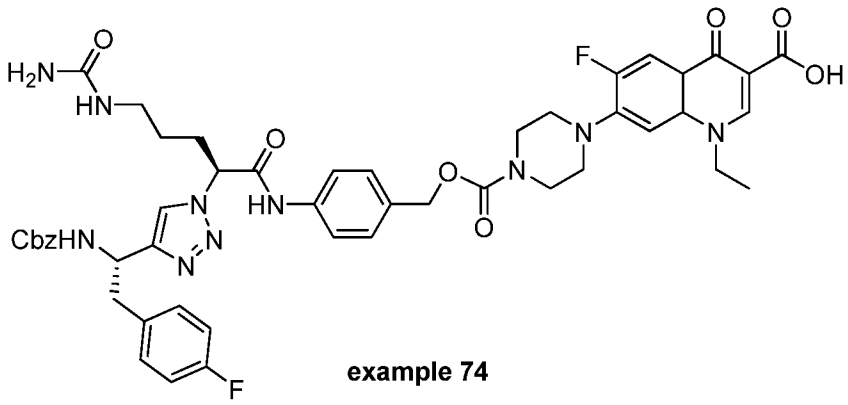
Step 8. A mixture of **73-12** (150 mg, 0.29 mmol), PNP (173 mg, 0.57 mmol) and DIPEA (111 mg, 0.86 mmol) in DCM (5 mL) was stirred at 50°C for 12 h. Solvent was removed and the residue was used in next step without further purification. The mixture (200 mg, 0.29 mmol), DIPEA (112 mg, 0.87 mmol) and norfloxacin (316 mg, 0.87 mmol) in DMF (5 mL) was stirred at 25°C for 4 h.

Solvent was removed and the residue was purified by prep-HPLC to give **example 73** (38.9 mg, 14.8%).

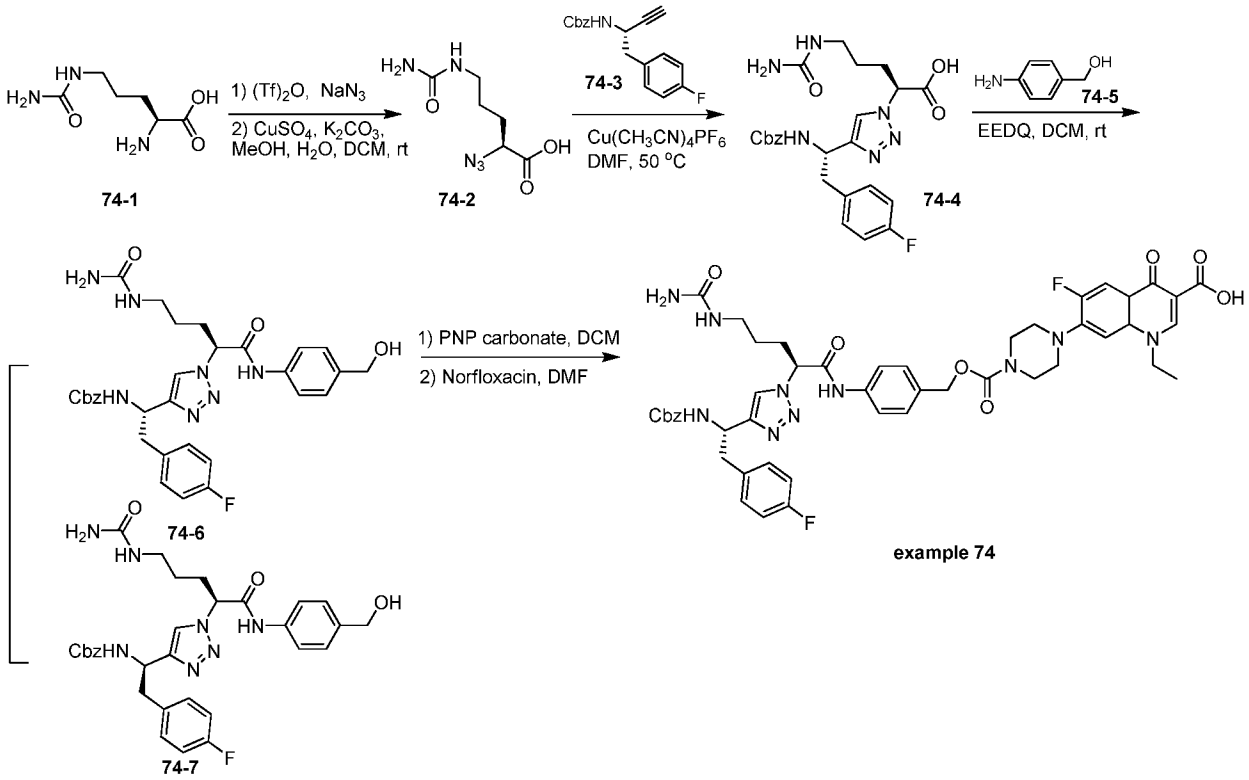
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1 H), 8.11 (d, J = 12.8 Hz, 1 H), 7.94 (s, 1 H), 7.59 (s, 1 H), 7.50 (d, J = 8.0 Hz, 2 H), 7.32-7.27 (m, 6 H), 6.82 (d, J = 6.4 Hz, 1 H), 5.45 (s, 1 H), 5.38-5.37 (m, 1 H). 5.10 (s, 2 H) ,4.97 (s, 2 H), 4.34-4.29 (m, 2 H), 3.71 (d, J = 2.4 Hz, 4 H), 3.25 (s, 4 H), 2.52-2.46 (m, 1 H), 1.94-1.91 (m, 3 H), 1.74 (s, 3 H) ,1.58-1.56 (m, 3 H), 0.93 (d, J = 7.2 Hz, 3 H), 0.82 (d, J = 6.8 Hz, 3 H).

LCMS (ESI): m/z 811.2 [M+H$^+$].

Example 74. 7-(4-((4-((S)-2-(4-((S)-1-(benzyloxycarbonylamino)-2-(4-fluorophenyl)ethyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4,4a,8a-tetrahydroquinoline-3-carboxylic acid.



example 74



Step 1. Tf_2O (3.15 mL, 18.4mmol) was added slowly over 5 min with stirring to a solution of NaN_3 (5.93 g, 91.3 mmol) in H_2O (18 mL) and DCM (30 mL) in icebath. After it was stirred at 0 °C for 2 h, the organic phase was separated. The aqueous portion was extracted with DCM (30 mL × 2). The organic fractions, containing the triflyl azide were pooled and washed once with saturated Na_2CO_3 and used without further purification. Compound **74-1** (1.6 g, 9.14 mmol), K_2CO_3 (1.90 g, 13.7 mmol) and $CuSO_4{\cdot}5H_2O$ (46 mg, 0.183 mmol) were mixed in H_2O (33 mL) and MeOH (66 mL). The triflyl azide in DCM was added and the mixture was stirred at 20°C for 12 h. Organic solvents were removed under reduced pressure and the aqueous slurry was diluted with H_2O (60 mL), acidified to pH 6 with conc.HCl and diluted with 0.2 M pH 6.2 phosphate buffers (60 mL). It was exacted with EtOAc (150 mL×2) to remove sulfonamide by-product. The aqueous phase was then acidified to pH 2 with conc. HCl. It was extracted with EtOAc (200 mL × 3) and the organic layers were combined, dried over Na_2SO_4 and evaporated to give **74-2**, which was used for next step without further purification.

Step 2. The mixture of **74-2** (1.84 g, 9.14 mmol), **74-3** (1.36 g, 4.57 mmol) and $Cu(CN)_4PF_6$ (213 mg, 0.687 mmol) in DMF (10 mL) was stirred at 50°C for 2 h. After removal of the solvent, the residue was purified by prep-HPLC to give **74-4** (470 mg, 20.6%).

LCMS (ESI): m/z 499.2 [$M+H^+$].

Step 3. The mixture of **74-4** (300 mg, 0.602 mmol), **74-5** (148 mg, 1.20 mmol) and EEDQ (294 mg, 1.20 mmol) in DCM (10 mL) was stirred at 20°C for 10 h. After removal of the solvent, the residue was purified by prep-HPLC and SFC to give **74-6** (160 mg, 44.1%) and **74-7** (95 mg, 26.2%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.5 (s, 1 H), 8.07 (s, 1 H), 7.80 (d, J = 9.2 Hz, 1 H), 7.53 (d, J = 6.8 Hz, 2 H), 7.51-7.18 (m, 8 H), 7.05-7.00 (m, 2 H), 6.03-6.00 (m, 1 H), 5.47-5.41 (m, 3 H), 5.12-5.09 (m, 1 H), 4.99-4.88 (m, 3 H) 4.42 (d, J = 5.6 Hz, 2 H), 3.17-2.92 (m, 4 H) ,2.14-2.05 (m, 2 H), 1.28-1.22 (m, 2 H).

LCMS (ESI): m/z 604.1 [M+H$^+$].

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1 H), 8.03 (s, 1 H), 7.81 (d, J = 9.2 Hz, 1 H), 7.51 (d, J = 6.8 Hz, 2 H), 7.28-7.18 (m, 8 H), 7.04-7.00 (m, 2 H), 6.06-6.03 (m, 1 H), 5.49-5.41 (m, 3 H), 5.13-5.10 (m, 1 H), 4.99-4.87 (m, 3 H) 4.42 (d, J = 5.6 Hz, 2 H), 3.18-2.92 (m, 4 H) ,2.13-2.02 (m, 2 H), 1.24-1.23 (m, 2 H).

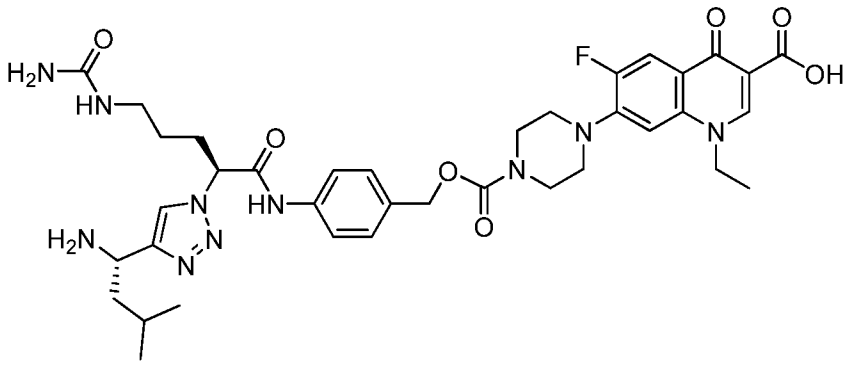
LCMS (ESI): m/z 604.1 [M+H$^+$].

Step 4. The mixture of **74-6** (50 mg, 0.082 mmol), PNP (53 mg, 0.166 mmol) and DIPEA (32 mg, 0.246 mmol) in DCM (5 mL) was stirred at 50°C for 12 h. After removal of the solvent, the residue was used without further purification. The mixture of above crude product (110 mg, crude, 0.082 mmol), DIPEA (32 mg, 0.246 mmol) and norfloxacin (51 mg, 0.166 mmol) in DMF (5 mL) was stirred at 20°C for 2 h. After removal of the solvent, the residue was purified by prep-HPLC to give **example 74** (25 mg, 32.1%).

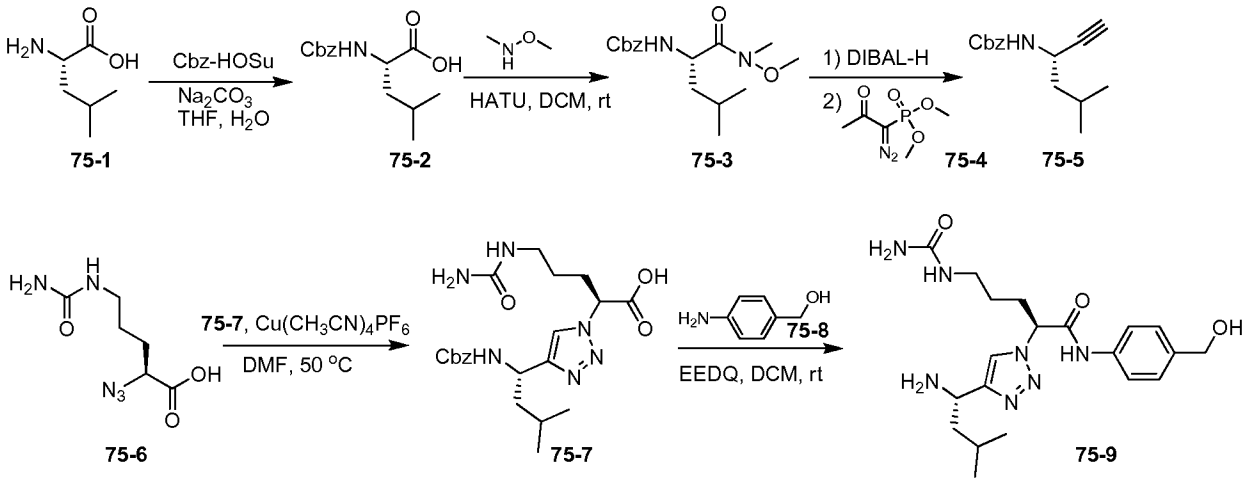
^{1}H NMR (400 MHz, DMSO-d_6) δ 15.64-15.33 (m, 1 H), 10.67 (s, 1 H), 8.96 (s, 1 H), 8.21-7.03 (m, 17 H), 6.08 (s, 1 H), 5.47 (s, 3 H), 5.08 (s, 2 H), 5.01-4.91 (m, 3 H), 4.58 (s, 2 H). 3.61 (s, 4 H) ,3.31 (s, 4 H), 3.20-2.96 (m, 4 H), 2.12 (s, 2 H), 1.41-1.24 (m, 5 H).

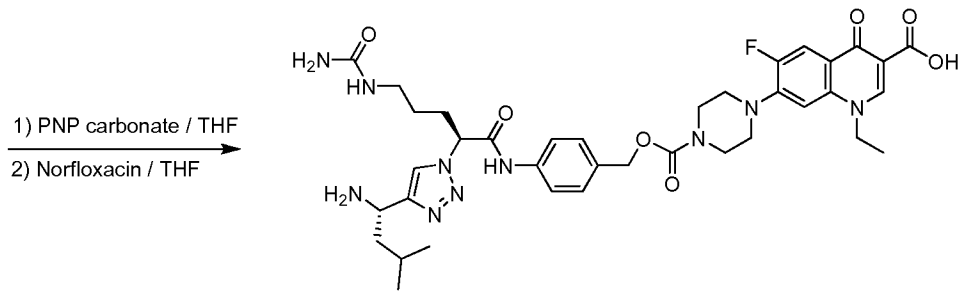
LCMS (ESI): m/z 949.1 [M+H$^+$], 475.3 [M/2+H$^+$].

Example 75. 7-(4-((4-((S)-2-(4-((S)-1-amino-3-methylbutyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



example 75





example 75

Step 1. Cbz-HOSu (22.8 g, 91.4 mmol) in THF (60 mL) was added dropwise over 15 min to a mixture of **75-1** (10.0 g, 76.2 mmol) in a mixture of water (60 mL) and THF (30 mL). The mixture was treated with Na_2CO_3 (24.2 g, 228.6 mmol). After the reaction mixture was stirred at 25°C for 16 h, solvent was removed and the residue was extracted with EtOAc (150 mL × 3). The aqueous phase was acidified to pH = 2 with conc.HCl, and extracted with EtOAc (60 mL×3). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give **75-2** (21.0 g crude).

LCMS (ESI): m/z 288.1 [M+Na^+].

Step 2. To a solution of **75-2** (5.0 g, 18.85 mmol), *N,O*-dimethylhydroxylamine hydrochloride (2.2 g, 22.62 mmol) and Et_3N (5.7 g, 56.55 mmol) in DCM (150 mL) was added HATU (10.8 g, 28.28 mmol) at 25°C. After the mixture was stirred at 25°C for 2 h, solvent was removed and the residue was taken up with water (80 mL). The aqueous layer was extracted with EtOAc (80 mL × 2). The organic layer was washed with 2N HCl solution (80 mL) and dried over Na_2SO_4, filtered and concentrated. The crude was purified by column chromatography on silica gel (PE/EtOAc = 2:1) to give **75-3** (4.1 g, 71%).

LCMS (ESI): m/z 309.2 [M+H$^+$].

Step 3. DIBAL-H in toluene (1M, 16 mL, 16 mmol) was added dropwise to a mixture of **75-3** (4.1 g, 13.3 mmol) in DCM (25 mL) at -78°C. After the mixture was stirred at -78°C for 2 h, MeOH (1 mL) was added dropwise. The mixture was warmed to 25°C, solvent was removed, and dissolved in MeOH (40 mL). To this solution K_2CO_3 (3.68 g, 26.6 mmol) and **75-4** (3.07 g, 16.0 mmol) were added at 0°C. After the solution was stirred at 25°C for 16 h, solvent was removed, and the crude was taken up with 1N HCl solution (30 mL) and extracted with EtOAc (360 mL × 3). The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by column chromatography on silica gel (PE/EtOAc = 20:1) to give **75-5** (1.9 g, 58%).

LCMS (ESI): m/z 246.1 [M+H$^+$].

Step 4. $Cu(CH_3CN)_4PF_6$ (432 mg, 1.16 mmol) was added to a solution of **75-6** (3.11 g, 15.48 mmol) and compound **5** (1.9 g, 7.74 mmol) in DMF (15 mL) at 25°C. After the reaction mixture was heated at 50°C for 2 h under N_2, solvent was removed and the residue was purified by prep-HPLC to give **75-7** (2.1 g, 61%).

^{1}H NMR (400 MHz, DMSO–d_6) δ 7.95 (s, 1 H), 7.69 (d, J = 8.8 Hz, 1 H), 7.36-7.29 (m, 5 H), 5.96 (br, 1 H), 5.38-5.35 (m, 1 H), 5.07-4.99 (m, 2 H), 4.82-4.77 (m, 1 H), 2.94 (s, 2 H), 2.16-2.06 (m, 2 H), 1.68-1.56 (m, 3 H), 1.28-1.05 (m, 2 H), 0.92-0.80 (m, 6 H).

Step 5. A mixture of **75-7** (500 mg, 1.12 mmol), **75-8** (414 mg, 3.36 mmol) and EEDQ (831 mg, 3.36 mmol) in DCM (10 mL) was stirred at 25°C for 2 h. Solvent was removed and the residue was purified by column chromatography on silica gel (DCM/MeOH = 5:1) to give **75-9** (350 mg, 57%).

^{1}H NMR (400 MHz, MeOD) δ 8.02 (s, 1 H), 7.56-7.54 (m, 2 H), 7.33-7.27 (m, 7 H), 5.50-5.46 (m, 1 H), 5.07 (s, 2 H), 4.95-4.91 (m, 1 H), 4.55 (s, 2 H), 3.25-3.07 (m, 2 H), 2.25-2.21 (m, 2 H), 1.77-1.64 (m, 3 H), 1.44-1.40 (m, 2 H), 0.96-0.95 (m, 6 H).

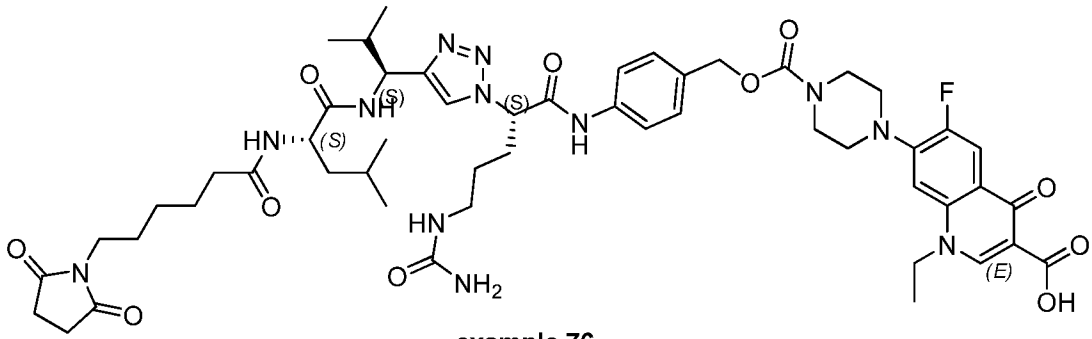
LCMS (ESI): m/z 552.2 [M+H$^+$].

Step 6. To a solution of **75-9** (120 mg, 0.218 mmol) in THF (3 mL) was added PNP carbonate (199 mg, 0.654 mmol) and DIPEA (113 mg, 0.872 mmol) at 25°C. After the mixture was heated at 50°C for 18 h, solvent was removed, and the residue was dissolved in DMF (3 mL). Norfloxacin (104 mg, 0.327 mmol) and DIPEA (141 mg, 1.09 mmol) was added and the mixture was stirred at 25°C for 6 h. Solvent was removed, and the residue was purified by prep-HPLC to give **example 75** (90 mg, 46%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.34 (s, 1 H), 10.64 (s, 1 H), 8.97 (s, 1 H), 8.13 (s, 1 H), 8.04 (s, 1 H), 7.97-7.93 (m, 1 H), 7.73-7.70 (m, 1 H), 7.61-7.59 (m, 2 H), 7.38-7.20 (m, 8 H), 6.03 (m, 1 H), 5.47-5.43 (m, 1 H), 5.07-5.03 (m, 4 H), 4.82-4.74 (m, 1 H), 4.59-4.57 (m, 2 H), 3.61 (s, 4 H), 3.34 (s, 4 H), 2.90-3.01 (m, 2 H), 2.10-2.07 (m, 2 H), 1.62-1.56 (m, 3 H), 1.42-1.38 (m, 3 H), 1.27 (m, 2 H), 0.90-0.89 (m, 6 H).

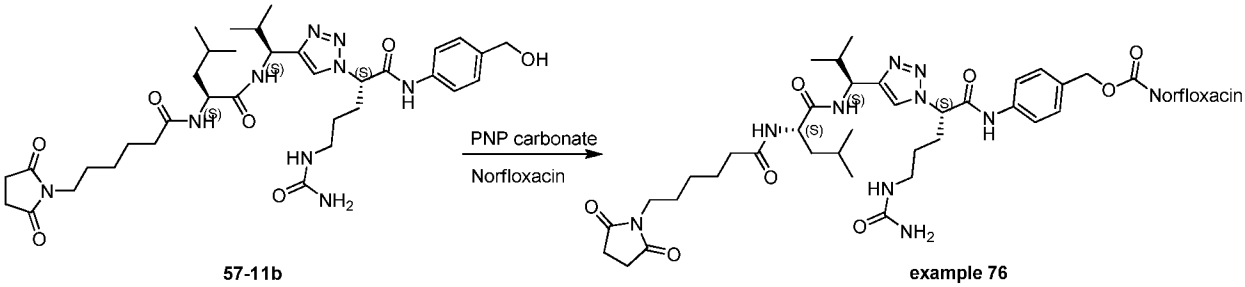
LCMS (ESI): m/z 897.4 [M+H$^+$].

Example 76. 7-(4-((4-((S)-2-(4-((S)-1-((S)-2-(6-(2,5-dioxopyrrolidin-1-yl)hexanamido)-4-methylpentanamido)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



example 76

Example 76 was made using the procedure as **Example 57**, with the intermediate from the synthesis of **Example 57**.

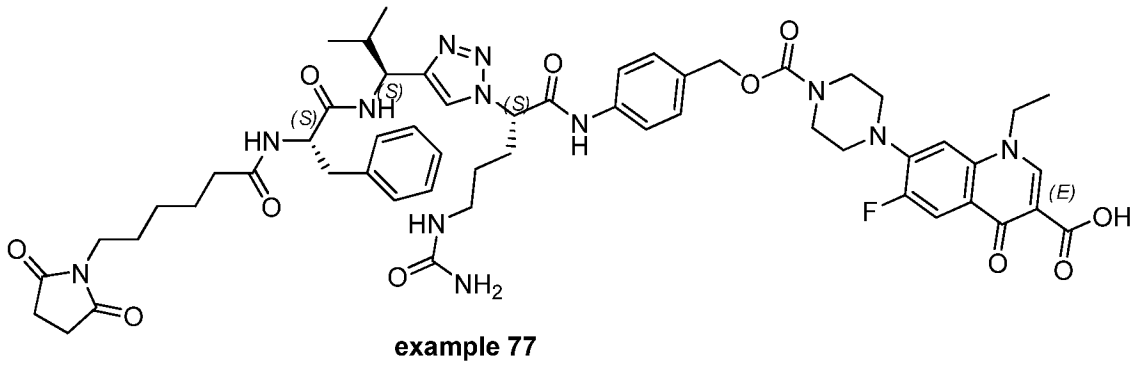


57-11b **example 76**

LCMS: (5-95, AB, 1.5 min, ESI), 0.828 min, MS = 529.4[1/2M+1]

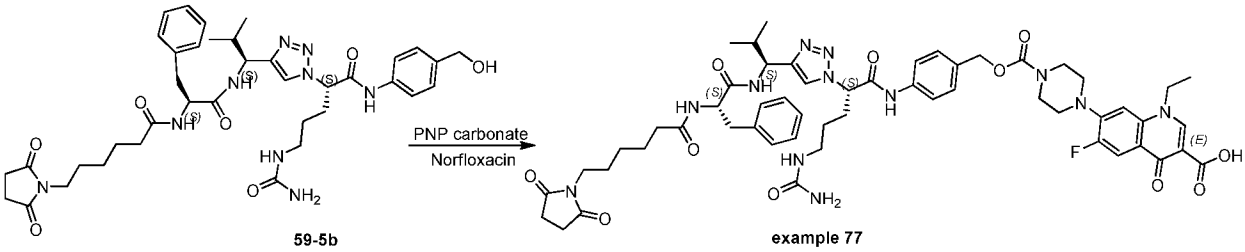
^{1}H NMR Methanol-d_4 400 MHz, δ 8.89 (s, 1 H), 8.09 (s, 1 H), 8.00 (d, J = 13.2 Hz, 1 H), 7.62 (d, J = 8 Hz, 2 H), 7.38 (d, J = 8 Hz, 2 H), 7.20 (s, 1 H), 5.51 (d, J = 5.2 Hz, 1 H), 5.15 (s, 2 H), 4.96 (d, J = 6.8 Hz, 1 H), 4.88 (s, 2 H), 4.60 (s, 4 H), 3.73 (s, 4 H), 3.46 - 3.42 (m, 2 H), 3.35 (s, 4 H), 2.65 (s, 4 H), 2.30 - 2.22 (m, 4 H), 1.65 - 1.56 (m, 9 H), 1.54 - 1.28 (m, 5 H), 1.00 - 0.97 (m, 12 H).

Example 77. 7-(4-((4-((S)-2-(4-((S)-1-((S)-2-(6-(2,5-dioxopyrrolidin-1-yl)hexanamido)-3-phenylpropanamido)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy) carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



example 77

Example 77 was made using the procedure as **Example 59**, with intermediates from the synthesis of **Example 59**.

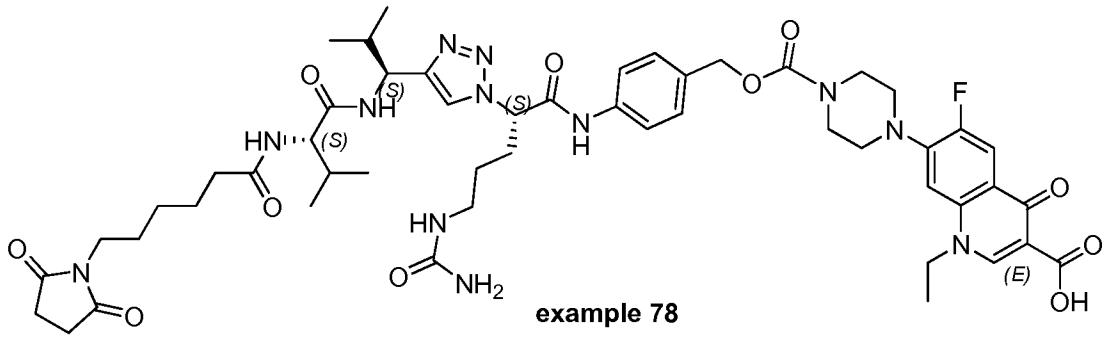


59-5b **example 77**

LCMS: (5-95, AB, 1.5 min, ESI), 0.841 min, MS = 546.5[1/2M+1]

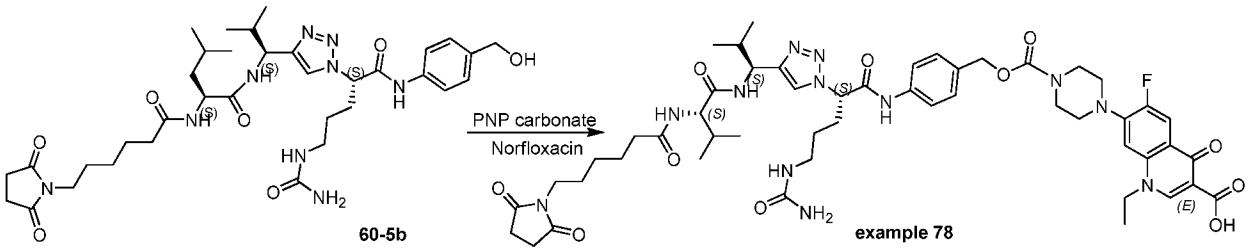
^{1}H NMR DMSO-d_6 400 MHz, δ 10.66 (s, 1 H), 8.96 (s, 1H), 8.34 (d, J = 9.2 Hz, 1 H), 8.09 (s, 1 H), 8.04 (d, J = 8.8 Hz , 1 H), 7.95 (d, J = 12.8 Hz , 1 H), 7.61 (d, J = 8.4 Hz, 2 H), 7.36 (d, J = 8.8 Hz, 2 H), 7.28 - 7.17 (m, 6 H), 6.05 (s, 1 H), 5.55 - 5.48 (m, 1 H), 5.43 (s, 2 H), 5.07 (s, 2 H), 4.90 - 4.86 (m, 1 H), 4.59 - 4.58 (m, 3 H), 3.61 (s, 4 H), 3.34 - 3.23 (m, 4 H), 3.04 - 2.95 (m, 4 H), 2.78 (s, 1 H), 2.59 (s, 4 H), 2.05 - 1.98 (m , 4 H), 1.43 - 1.22 (m, 10H), 1.04 (d, J = 7.2 Hz, 2 H) , 0.77 (d, J = 6.8 Hz, 3 H) , 1.42 (d, J = 6.8 Hz, 3 H),.

Example 78. 7-(4-((4-((S)-2-(4-((S)-1-((S)-2-(6-(2,5-dioxopyrrolidin-1-yl)hexanamido)-3-methylbutanamido)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido) benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 78

Example 78 was made using the procedure as **Example 60**, with intermediate from the synthesis of **Example 60**.



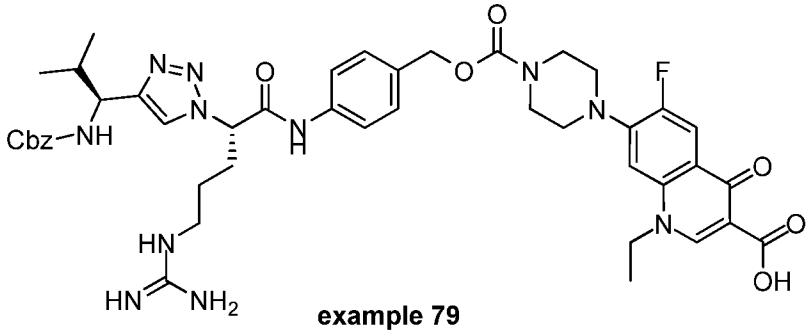
60-5b example 78

Step 1. To a solution of compound **60-5b** (50 mg, 0.072 mmol) in dry DMF (3 mL) was added PNP carbonate (50 mg, 0.16 mmol) and DIPEA (0.5 mL, 3 mmol) at r.t., and the mixture was stirred at r.t. for 1.5 h. Norfloxacin (50 mg, 0.16 mmol) was added. The mixture was stirred at r.t. for additional 1 h. The mixture was concentrated, filtered and purified by prep-HPLC (FA), to give **example 78** (38.8 mg, yield: 52%).

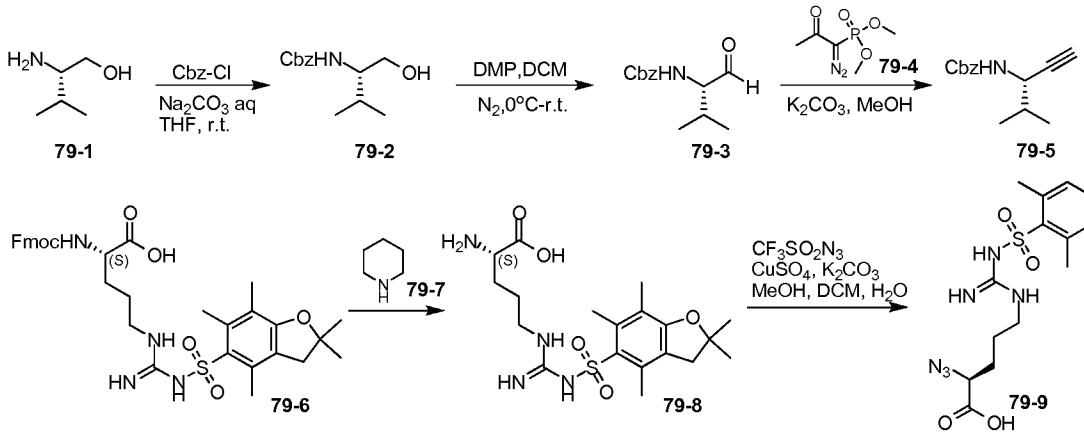
LCMS: (5-95, AB, 1.5 min, ESI), 0.833 min, MS = 522.4[1/2M+1]

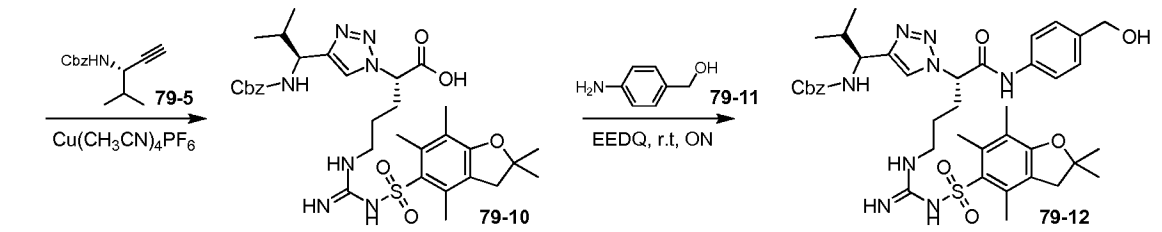
^{1}H NMR DMSO-d_6 400 MHz, δ 10.1 (s, 1H), 8.93 (s, 1 H), 8.25 - 8.22 (d, 1H), 8.04 (s, 1 H), 7.93 - 7.90 (d, 1 H), 7.81 - 7.79 (d, 1H), 7.58 - 7.56 (d, J = 8.0 Hz , 2 H), 7.35 - 7.33 (d, J = 8.0 Hz , 2 H), 7.20 - 7.18 (m, 1 H), 6.0 (m, 1 H), 5.5 (m, 1 H), 5.40 (s, 2 H), 5.04 (s, 2 H), 4.9 - 4.87 (m, 1 H), 4.6 - 4.5 (m, 2 H), 4.2 - 4.1(m, 1 H), 3.6 (s, 4 H), 3.2 (m, 6 H), 3.05 - 2.9 (m, 2 H), 2.55 (s, 4 H), 2.18 - 2.0 (m, 5 H), 1.9 - 1.8 (m, 1 H), 1.5 - 1.35 (m, 7 H), 1.3 - 1.05 (m , 4 H), 0.84 - 0.77 (m, 12H).

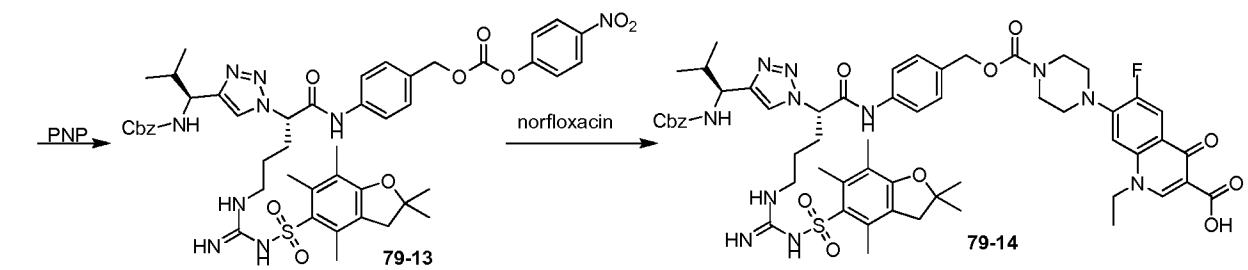
Example 79. 7-(4-((4-((S)-2-(4-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)-5-guanidinopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

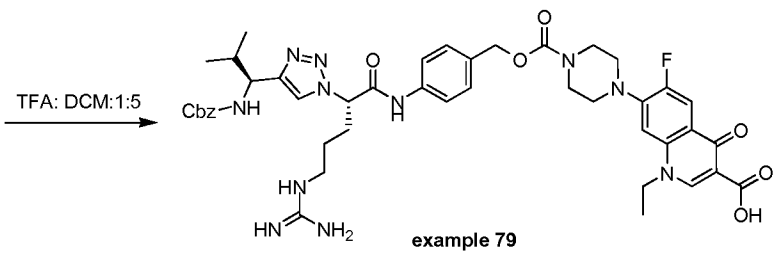


example 79









example 79

5 **Step 1.** To a solution of compound **79-1** (2 g, 19.4 mmol) in THF (4 mL) was added sat. aq. K_2CO_3 (30 mL), followed by Cbz-Cl (3.958 g, 23.2 mmol) at 0 °C. After the reaction mixture was

stirred at 28 °C for 1 h, it was extracted with EtOAc (20 mL × 3) and H_2O (20 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated to give compound **79-2** (5 g, 100%) which was used in the next step without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36 - 7.31 (m, 5H), 6.92 (d, J = 8.8 Hz, 1H), 5.01 (s, 2H), 3.39 - 3.32 (m, 3H), 1.80 - 1.76 (m, 1H), 0.85 - 0.80 (m, 6H).

Step 2. To a solution of compound **79-2** (6.8 g, 28.7 mmol) in DCM (100 mL) under N_2 was added DMP (14.59 mg, 343.4 mmol) at 0°C. After 5 min, the reaction mixture was warmed to r.t. and stirred at r.t. for 1 h. The reaction mixture was cooled to 0°C and washed with a saturated solution of $NaHCO_3$/Na_2SO_3 (1:1). After separation, the organic layer was washed once more. The combined aqueous layers were extracted with DCM (30 mL × 2). It was dried over Na_2SO_4, filtered and concentrated to give compound **79-3** (5.3 g, 79%), which was use in the next step without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (d, J = 0.8 Hz, 1H), 7.72 - 7.61 (m, 1H), 7.43 - 7.30 (m, 5H), 5.04 (s, 2H), 3.91 - 3.88 (m, 1H), 2.18 - 2.13 (m, 6H), 0.94 - 0.82 (m, 6H).

Step 3. To a solution of compound **79-3** (crude, 4.9 g, 12.76 mmol), compound **79-4** (4.9 g, 25.5 mmol) in MeOH (50 mL) was added K_2CO_3 (5.28 g, 38.28 mmol). The reaction mixture was stirred at r.t. for overnight. The mixture was concentrated and purified by column chromatography on silica gel (PE: EtOAc = 10: 1) to give the desired product **79-5** (1.30 g, 44 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74 (d, J = 8.8 Hz, 1H), 7.37 - 7.29 (m, 5H), 5.01 (s, 2H), 4.09 - 4.05 (m, 1H), 3.16 (d, J = 2.4 Hz, 1H), 1.76 (d, J = 6.8 Hz, 1H), 0.91 (d, J = 6.8 Hz, 3H) , 0.87 (d, J = 6.8 Hz, 3H).

Step 4. To a solution of compound **79-6** (2 g, 3.09 mmol) in DCM (30 mL) was added compound **79-7** (1.6 mL, 15.4 mmol). After the reaction mixture was stirred at r.t. for 16 h, it was concentrated in vacuum, and washed with MTBE, filtered to give the desired product **79-8** (1.315 g, 100 %).

Step 5. A mixture of NaN_3 (1.6 g, 15.45 mmol) in distilled H_2O (5 mL) and CH_2Cl_2 (10 mL) was cooled on an ice bath. Tf_2O (1.1 mL, 6.18 mmol) was added slowly over 5 min and stirred for 2 h. The CH_2Cl_2 phase was removed and the aqueous phase was extracted with CH_2Cl_2 (5 mL × 2). The organic fractions, containing the triflyl azide were pooled and washed once with saturated Na_2CO_3 (40 mL) and used without further purification. Compound **79-8** (1.315 g, 3.09 mmol), K_2CO_3 (641 mg, 4.635 mmol) and $CuSO_4$•$5H_2O$ (155 mg, 0.618 mmol) was added to distilled H_2O (10 mL) and MeOH (20 mL). The triflyl azide solution in CH_2Cl_2 (50 mL) was added and the mixture was stirred at r.t for overnight. Organic solvents were removed under reduced pressure and the aqueous

slurry was diluted with H_2O (50 mL) and acidified to pH 6 with conc. HCl then diluted with 0.2 M pH 6.2 phosphate buffer (50 mL) and exacted with EtOAc (3 × 100 mL) to remove sulfonamide by-product. The aqueous phase was then acidified to pH 2 with conc. HCl. The product was obtained from another round of EtOAc/MeOH (20: 1) extractions (4 × 100 mL). These EtOAc/MeOH extracts were combined, dried over Na_2SO_4 and evaporated to give compound **79-9** without further purification (600 mg, 43 %).

Step 6. To the solution of compound **79-9** (500 mg, 1.1 mmol) and **79-5** (307 g, 1.33 mmol) in DMF (5 mL) was added $Cu(CH_3CN)_4PF_6$ (165 mg, 0.44 mmol). The reaction mixture was stirred at 50°C for 2 h, the mixture (**79-10**) was used directly for next step.

Step 7. To the mixture of compound **79-10** (crude, 752 mg, 1.1 mmol) in DMF (5 mL) was added EEDQ (544 mg, 2.2 mmol) and compound **79-11** (203 g, 1.65 mmol). The reaction mixture was stirred at 0 °C and warmed to r.t. under N_2 overnight. The mixture was purified by prep-HPLC and SFC separation to give compound **79-12** (250 mg, 30 %).

Step 8. To the solution of compound **79-12** (90 mg, 0.114 mmol) and PNP carbonate (69 mg, 0.228 mmol) in DMF (3 mL) was added DIPEA (45 mg, 0.342 mmol). The reaction mixture was stirred at 21°C for 3 h under N_2. The mixture (**79-13**) was used directly for next step without purification.

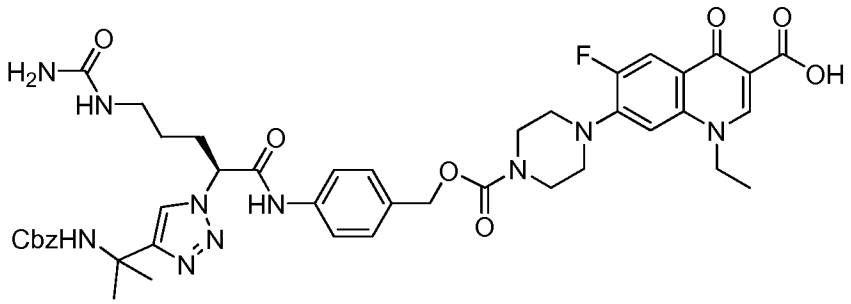
Step 9. To the solution from previous step was added norfloxacin (75 mg, 0.228 mmol). The reaction mixture was stirred at 21°C for 1 h. The mixture was purified by prep-HPLC to give compound **79-14** (80 mg, 62 %).

Step 10. To the solution of compound **79-14** (60 mg, 0.053 mmol) in DCM (5 mL) was added TFA (1 mL) at 0°C. The reaction mixture was stirred at 16°C for 5 h. The mixture was purified by prep-HPLC and SFC to give **example 79** (30 mg, 64 %).

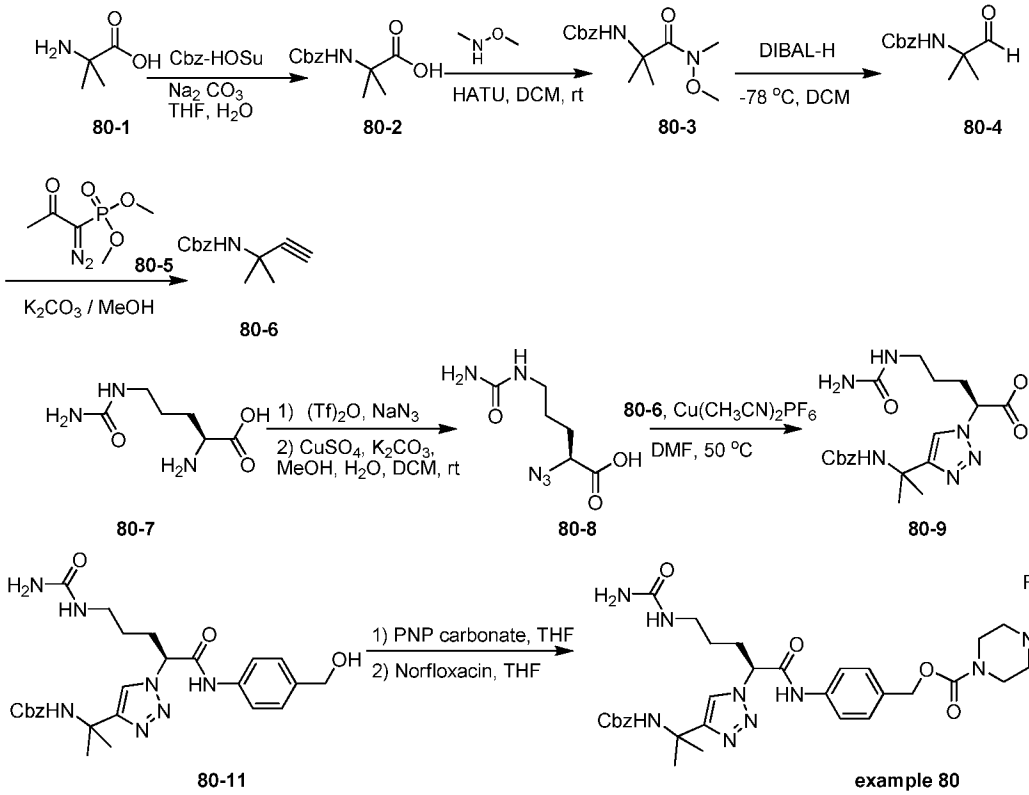
LCMS (ESI): RT = 0.837 min, $M+H^+$ = 882.2. method = 5-95 /1.5 min.

^1H NMR (400 MHz, DMSO-d_6) δ 8.94 (s, 1H), 8.48 (s, 1H), 8.12 (s, 1H), 7.92 (d, J = 12.4 Hz, 1H), 7.76 (d, J = 8.8 Hz, 1H), 7.61 (s, 1H), 7.34 (m, 10H), 5.07 - 4.99 (m, 5H), 4.59 (d, J = 7.2 Hz, 1H), 3.61 (s, 4H), 3.17 (m, 6H), 3.12 (s, 2H), 2.18 - 2.03 (m, 4H), 1.39 (s, 5H), 0.84 (d, J = 6.4 Hz, 3H), 0.78 (d, J = 6.0 Hz, 3H).

Example 80. (S)-7-(4-((4-(2-(4-(2-(benzyloxycarbonylamino)propan-2-yl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



example 80



Step 1. Cbz-OSu (57 g, 0.23 mol) in THF (150 mL) was added a mixture of aq Na_2CO_3 (82 g, 0.78 mol) and **80-1** (20 g, 0.19 mol). After it was stirred at r.t. for 16 h, it was adjusted to pH > 10 and washed with EtOAc (400 mL × 2). The aqueous layer was pooled and acidified to pH < 1 with conc. HCl. It was extracted with EtOAc (500 mL × 2). The organic layer was dried over Na_2SO_4 and evaporated to give **80-2** (45 g, 97.8%).

Step 2. To a mixture of **80-2** (20 g, 84.3 mmol), *N,O*-dimethylhydroxylamine hydrochloride (8.9 g, 92.7 mmol) and HATU (48.1 g, 126.4 mmol) in DCM (200 mL) was added Et_3N (48.7 mL, 337.2 mmol). The mixture was stirred at r.t. for 2 h. After the solvent was removed, the residue was taken up with water (300 mL). The aqueous layer was extracted with EtOAc (300 mL × 3) and the organic layers were washed with saturated $NaHCO_3$ (150 mL), conc.HCl (100 mL) and

saturated NaCl (100 mL). The organic layer was dried over Na_2SO_4, concentrated and purified by column chromatography on silica gel (PE: EtOAc = 2: 1) to give **80-3** (12.9 g, 54.6%).

Step 3. DIBAL-H (17.8 mL, 1M in toluene) was added dropwise to a solution of **80-3** (4.15 g, 14.8 mmol) in anhydrous CH_2Cl_2 (100 mL) at -78°C and the resulting solution was stirred at -78°C for 2 h. Excess DIBAL was quenched by anhydrous MeOH (5 mL) and the solution was warmed to r.t. The solution was concentrated to give **80-4** (3.27 g), which was used in next step without further purification.

Step 4. A mixture of **80-4** (3.27 g, 14.8 mmol), **80-5** (2.96 g, 17.8 mmol) and K_2CO_3 (4.1 g, 29.7 mmol) in MeOH (60 mL) was stirred at r.t. for 16 h. The solvents were removed under reduced pressure and the crude residue was partitioned in EtOAc (200 mL) and water (100 mL). The organic layer was washed with brine (50 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and purified by column chromatography on silica gel (PE: EtOAc= 10: 1) to give **80-6** (606 mg, 18.9%).

Step 5. Tf_2O (1.7 mL, 9.9 mmol) was added slowly over 5 min to a solution of NaN_3 (3.2 g, 49.3 mmol) in distilled H_2O (10 mL) and CH_2Cl_2 (16 mL) at 0 °C. After it was for 2 h, the organic phase was separated, and the aqueous was extracted with CH_2Cl_2 (20 mL × 2). The combined organic fractions, containing the triflyl azide were pooled and washed once with saturated Na_2CO_3 and used without further purification.

To a mixture of **80-7** (0.86 g, 4.93 mmol) and K_2CO_3 (1.02 g, 7.40 mmol), $CuSO_4.5H_2O$ (25 mg, 0.099 mmol) in H_2O (18 mL) and MeOH (36 mL) was added the triflyl azide in CH_2Cl_2 (32 mL) and the mixture was stirred at 26°C for 12 h. The organic solvents were removed under reduced pressure and the aqueous slurry was diluted with H_2O (50 mL). It was acidified to pH 6 with conc. HCl and diluted with phosphate buffers (0.2M, pH 6.2, 50 mL) and washed with EtOAc (100 mL × 2). The aqueous phase was then acidified to pH 2 with conc. HCl and extracted with EtOAc (100 mL × 2), dried over Na_2SO_4 and concentrated to give **80-8**, which was used in next step without further purification.

Step 6. A mixture of **80-8** (0.99 g, 4.93 mmol), **80-6** (0.54 g, 2.46 mmol) and $Cu(CH_3CN)_4PF_6$ (115 mg, 0.37 mmol) in DMF (5 mL) was stirred at 50°C for 2 h. After solvent was removed, the residue was purified by prep-HPLC to give **80-9** (250 mg, 23%).

Step 7. The mixture of **80-9** (199 mg, 0.48 mmol), **80-10** (176 mg, 1.43 mmol) and EEDQ (353 mg, 1.43 mmol) in DCM (10 mL) was stirred at 24°C for 2 h. After removal of the solvent, the residue was purified by prep-HPLC to give **80-11** (211 mg, 84%).

^{1}H NMR (400 MHz, MeOD) δ 8.00 (s, 1 H), 7.56 (dd, J =1.6, 8.4 Hz, 2 H), 7.33-7.31 (m, 7 H), 5.48-5.44 (m, 2 H), 4.99 (s, 2 H), 4.56 (s, 2 H), 3.23-3.16 (m, 1 H), 3.09-3.03 (m, 1 H), 2.26-2.20 (m, 2 H), 1.68 (s, 6 H), 1.51-1.45 (m, 1 H), 1.39-1.29 (m, 1 H).

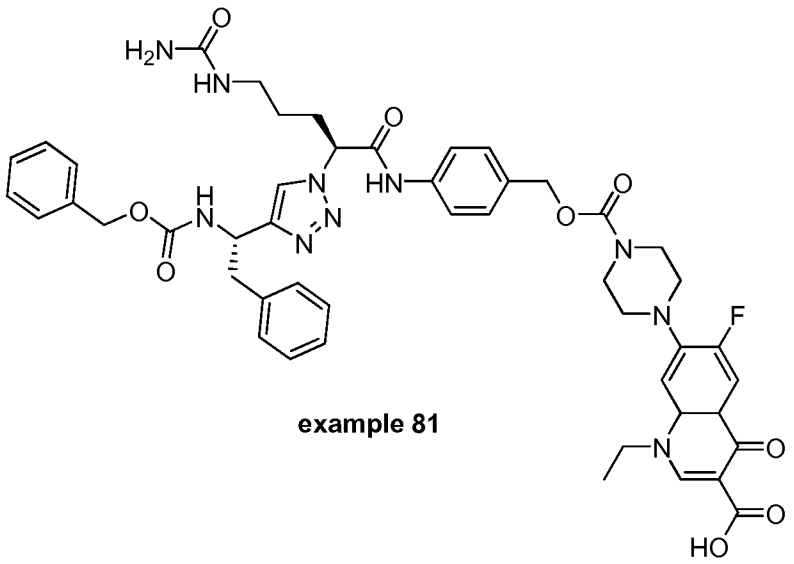
LCMS (ESI): m/z 524.3 [M+H$^+$].

Step 8. After a mixture of **80-11** (150 mg, 0.29 mmol), PNP carbonate (173 mg, 0.57 mmol) and DIPEA (111 mg, 0.86 mmol) in DCM (5 mL) was stirred at 50°C for 12 h solvent was removed and added to a solution of DIPEA (112 mg, 0.87 mmol) and norfloxacin (316 mg, 0.87 mmol) in DMF (5 mL), and stirred at 25°C for 4 h. Solvent was removed and the residue was purified by prep-HPLC to give **example 80** (113 mg, 45%).

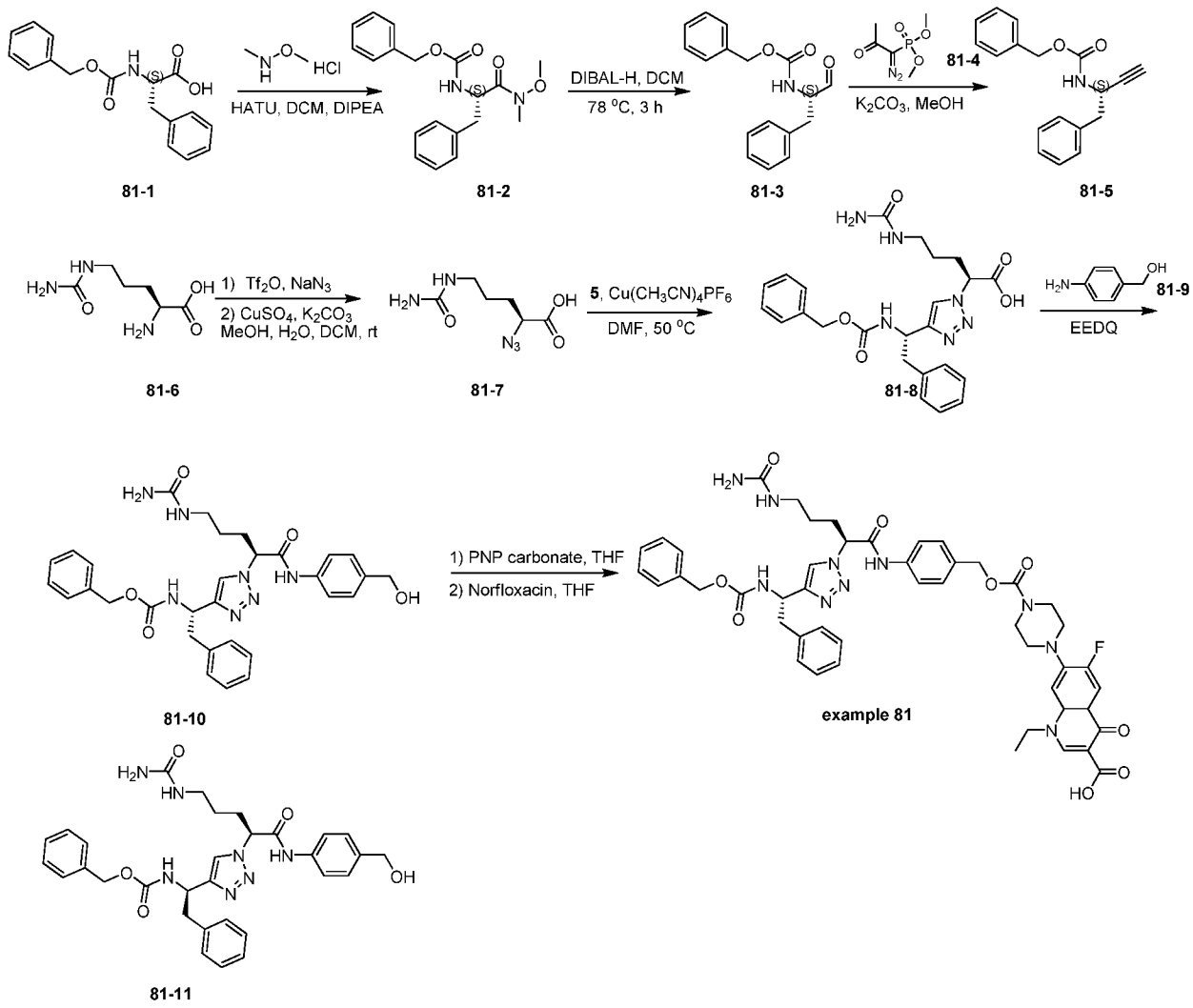
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1 H), 8.96 (s, 1 H), 7.98-7.93 (m, 2 H), 7.63-7.60 (m, 3 H), 7.38-7.20 (m, 9 H), 6.05 (s, 1 H), 5.44 (s, 3 H), 5.07 (s, 2 H), 4.93 (s, 2 H), 4.59-4.57 (m, 2 H), 3.61 (s, 4 H), 3.02-2.98 (m, 2 H), 2.12-2.02 (m, 2 H), 1.57 (d, J = 4.0 Hz, 6 H), 1.42-1.38 (m, 3 H), 1.28-1.23 (m, 2 H).

LCMS (ESI): m/z 869.3 [M+H$^+$].

Example 81. 7-(4-((4-((S)-2-(4-((S)-1-(benzyloxycarbonylamino)-2-phenylethyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4,4a,8a-tetrahydroquinoline-3-carboxylic acid.



example 81



Step 1. To the solution of Cbz-L-phenylalanine (3.0 g, 10 mmol) in DCM (100 mL) was added HATU (4.57 g, 12.0 mmol) and DIPEA (3.87 g, 30.0 mmol). After it was stirred at r.t. for 15 min, *N*-methoxymethanamine hydrochloride (1.2 g, 12 mmol) was added. After the solution was stirred for another 1 h, it was extracted with DCM (60 mL × 3), washed with 10% aq. HCl (60 mL) and water (60 mL). The organic layer was dried over Na_2SO_4, concentrated and purified by the column chromatography on silica gel to give **81-2** (3.2 g, 94%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.14 (m, 10 H), 5.42-5.40 (m, 1 H), 5.13-5.02 (m, 3 H), 3.69 (s, 3 H), 3.18 (s, 3 H), 3.11-3.06 (m, 1 H), 2.94-2.89 (m, 1 H).

Step 2. DIBAL-H (20 mL, 1M in toluene) was added to a solution of **81-2** (3.4 g, 10 mmol) in dry DCM (150 mL) at -78°C under N_2. After the solution was stirred at -78°C for 6 h, the mixture was quenched with MeOH (50 mL) and water (50 mL). It was filtered and the filtrate was dried over Na_2SO_4, concentrated and **81-3** used directly in the next step without further purification.

LCMS (ESI): m/z 284.1 [M+H$^+$].

Step 3. To the solution of **81-3** (10 mmol) in MeOH (100 mL) was added K_2CO_3 (2.76 g, 20 mmol) and **81-4** (2.31 g, 12 mmol) at 0°C. After the reaction was stirred at 0°C for 12 h, solvent was removed and the residue was purified by column chromatography on silica gel to give **81-5**.

LCMS (ESI): m/z 280.1 [M+H$^+$].

Step 4. To a solution of NaN_3 (1.78 g, 27.45 mmol) in a mixture of H_2O (5 mL) and DCM (7.5 mL) was added Tf_2O (0.93 mL, 5.55 mmol). After the mixture was stirred for 2 h at r.t., it was extracted with DCM (60 mL × 3). The organic layer was washed with aq. Na_2CO_3 and concentrated to 10 mL. **81-6** (500 mg, 2.8 mmol) was then added, followed by K_2CO_3 (577 mg, 4.19 mmol), $CuSO_4$ (7 mg, 0.028 mmol), H_2O (9 mL) and MeOH (18 mL). The mixture was stirred at r.t. for 12 h. After the organic solvents were evaporated, it was diluted with water (20 mL) and pH was adjusted to 6.0 with HCl and then diluted with phosphate buffers (0.25 M, pH 6.2, 50 mL). The mixture was extracted with EtOAc (50 mL × 3). The organic layer was dried over Na_2SO_4 to afford **81-7**, which was used directly in the next step.

Step 5. To the solution of **81-5** (560 mg, 2 mmol) in DMF (5 mL) was added **81-7** (804 mg, 4.0 mmol) and cat. $Cu(CH_3CN)_4PF_6$. The mixture was stirred at 50°C for 3 h under N_2. After the solvent was removed, the residue was purified by prep-HPLC to give **81-8**.

^{1}H NMR (400 MHz, MeOD) δ 8.00 (s, 1 H), 7.57 (d, J = 8.4 Hz, 2 H), 7.35-7.18 (m, 12 H), 5.50-5.45 (m, 1 H), 5.14-5.11 (m, 1 H), 5.05-5.03 (m, 2 H), 4.58 (s, 2 H), 3.26-3.24 (m, 2 H), 3.13-3.12 (m, 2 H), 2.35-2.15 (m, 2 H), 1.55-1.35 (m, 2 H).

Step 6. To a solution of **81-8** (480 mg, 1.0 mmol) in DCM (10 mL) was added EEDQ (247 mg, 1.0 mmol) and **81-9** (123 g, 1.0 mmol) under N_2 and the mixture was stirred at 0°C for 1 h. After the solvent was removed, the residue was purified by prep-HPLC and SFC separation to give **81-10** and **81-11**.

^{1}H NMR (400 MHz, MeOD) δ 8.00-7.95 (m, 1 H), 7.58-7.56 (m, 2 H), 7.35-7.18 (m, 12 H), 5.51-5.47 (m, 1 H), 5.15-5.09 (m, 1 H), 5.55-5.00 (m, 2 H), 4.58 (s, 2 H), 3.33-3.20 (m, 2 H), 3.16-3.09 (m, 2 H), 2.25-2.18 (m, 2 H), 1.43-1.38 (m, 2 H). LCMS (ESI): m/z 586.0 [M+H$^+$].

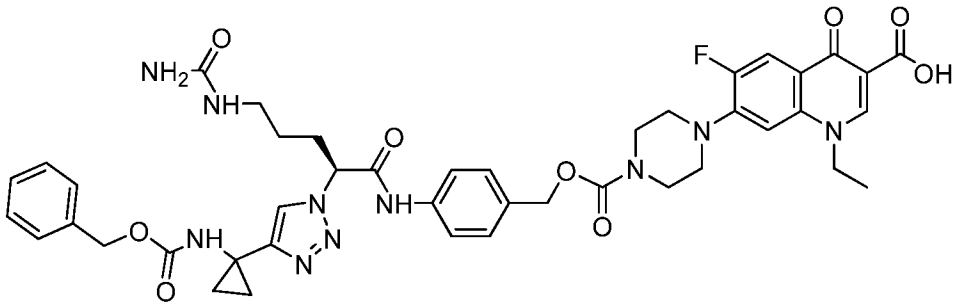
^{1}H NMR (400 MHz, MeOD) δ 7.96 (s, 1 H), 7.57 (d, J = 8.8 Hz, 2 H), 7.35-7.18 (m, 12 H), 5.52-5.48 (m, 1 H), 5.13-4.87 (m, 3 H), 4.58 (s, 2 H), 3.33-3.15 (m, 2 H), 3.14-3.07 (m, 2 H), 2.27-2.14 (m, 2 H), 1.46-1.31 (m, 2 H). LCMS (ESI): m/z 586.1 [M+H$^+$].

Step 7. To a solution of **81-10** (59 mg, 0.1 mmol) in dry DCM (30 mL) was added PNP carbonate (62 mg, 0.2 mmol) and DIPEA (1 mL). The mixture was heated at reflux for 16 h. After the

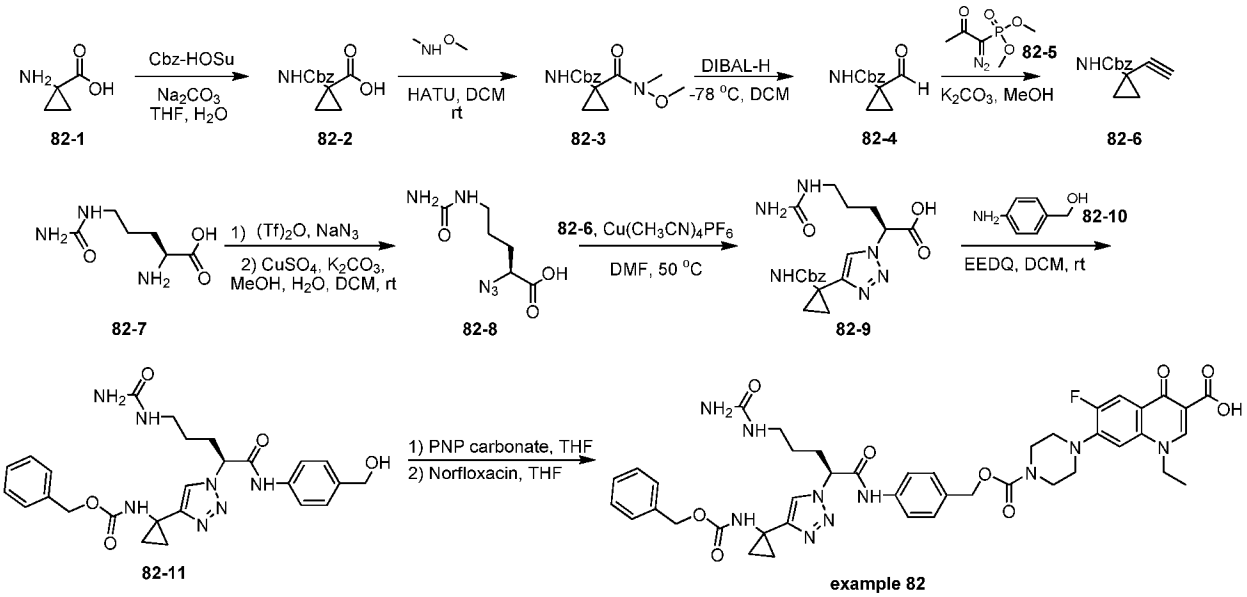
solvent was removed, the residue was dissolved in DMF (5 mL) and DIPEA (0.3 mL) and norfloxacin (65 mg, 0.2 mmol) were added. The mixture was stirred at r.t. for 1 h. After the solvent was removed, the residue was purified by prep-HPLC to give **example 81.**

^{1}H NMR (400 MHz, DMSO-d_6) δ 15.35 (br s, 1 H), 10.66 (s, 1 H), 8.97 (s, 1 H), 8.10 (s, 1 H), 7.95 (d, J = 12.8 Hz, 1 H), 7.85-7.83 (m, 1 H), 7.62 (d, J = 8.4 Hz, 2 H), 7.39-7.19 (m, 13 H), 6.07-6.02 (m, 1 H), 5.51-5.45 (m, 3 H), 5.07 (s, 2 H), 5.01-4.92 (m, 3 H), 4.59 (d, J = 7.6 Hz, 2 H), 3.61 (s, 4 H), 3.44-3.38 (m, 4 H), 3.21-3.16 (m, 1 H), 3.06-2.98 (m, 3 H), 2.12-2.00 (m, 2 H), 1.45-1.15 (m, 5 H). LCMS (ESI): m/z 931.2 [M+H$^+$].

Example 82. (S)-7-(4-((4-(2-(4-(1-(benzyloxycarbonylamino)cyclopropyl)-1H-1,2,3-triazol-1-yl)-5-ureidopentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid



example 82



Step 1. To a mixture of **82-1** (10 g, 98.9 mmol) in aq. solution Na_2CO_3 (41.9 g, 395.6 mmol) and was added Cbz-OSu (29.6 g, 118.7 mmol) in THF (150 mL). After the mixture was stirred at r.t. for 16 h, it was adjusted to pH > 10 and the solution was washed with EtOAc (200 mL × 2). The aqueous layer was acidified to pH < 1 with conc. HCl and the solution was extracted with EtOAc (250 mL × 2). The organic layer was dried over Na_2SO_4 and concentrated to give **82-2** (23 g, crude), which was used for next step without further purification.

Step 2. To a solution of **82-2** (10 g, 42.5 mmol), *N,O*-dimethylhydroxylamine Hydrochloride (4.5 g, 46.8 mmol) and HATU (24.2 g, 63.8 mmol) in DCM (100 mL) was added Et_3N (24.6 mL, 170.0 mmol). After the mixture was stirred at r.t. for 2 h, solvent was removed, and the crude was taken up with water (300 mL). The aqueous layer was extracted with EtOAc (300 mL × 3). The organic layer was washed with saturated $NaHCO_3$, diluted HCl, saturated NaCl, concentrated and purified by column chromatography on silica gel (PE: EtOAc = 2: 1) to give **82-3** (10.8 g, 91.3%).

Step 3. DIBAL-H (22.5 mL, 1M in toluene) was added to dropwise to a solution of **82-3** (4.2 g, 15.0 mmol) in anhydrous CH_2Cl_2 (50 mL) -78°C. After the mixture was stirred at -78°C for 2 h, excess DIBAL was quenched by anhydrous MeOH (15 mL) and the resulting solution was warmed to r.t. The solution was concentrated to give **82-4** (3.3 g, crude), which was used for next step without further purification.

Step 4. After a mixture of **82-4** (3.3 g, 15.0 mmol), **82-5** (3.0 g, 18.1 mmol) and K_2CO_3 (4.1 g, 30 mmol) in MeOH (60 mL) was stirred at r.t. for 16 h, solvents were removed under reduced pressure and the crude residue was partitioned between EtOAc (200 mL) and water (100 mL). The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography on silica gel (PE: EtOAc = 10: 1) to give **82-6** (1.8 g, 55.6%).

Step 5. Tf_2O (4.7 mL, 27.9 mmol) was added slowly to mixture of NaN_3 (9.2 g, 139.3 mmol) in distilled H_2O (30 mL) and DCM (48 mL) at 0 °C. After it was stirred for 2 h, DCM layer was separated and the aqueous portion was extracted with CH_2Cl_2 (24 mL × 2). The organic fractions, containing the triflyl azide were pooled and washed once with saturated Na_2CO_3 and used without further purification.

The triflyl azide in CH_2Cl_2 (96 mL) was added to a mixture of **82-7** (2.44 g, 13.9 mmol), K_2CO_3 (2.89 g, 20.9 mmol) and $CuSO_4.5H_2O$ (347 mg, 1.39 mmol) in H_2O (54 mL) and MeOH (108 mL). After the mixture was stirred at 26°C for 12 h, organic solvents were removed under reduced pressure and the aqueous slurry was diluted with H_2O (200 mL). The mixture was acidified to pH = 6 with conc. HCl and diluted with phosphate buffers (0.2 M, pH 6.2, 200 mL) and washed with EtOAc (300 mL×2). The aqueous phase was then acidified to pH = 2 with conc. HCl. It was

extracted with EtOAc (300 mL×3). The organic layer was dried over Na_2SO_4 and concentrated to give **82-8**, which was used for next step without further purification.

Step 6. After a mixture of **82-8** (2.8 g, 14.0 mmol), **82-6** (1.5 g, 7.0 mmol) and $Cu(CN)_4PF_6$ (779 mg, 2.1 mmol) in DMF (20 mL) was stirred at 50°C for 2 h, solvent was removed and the residue was purified by prep-HPLC to give **82-9** (200 mg, 6.9%).

Step 7. After a mixture of **82-9** (410 mg, 0.98 mmol), **82-10** (364 mg, 2.95 mmol) and EEDQ (730 mg, 2.95 mmol) in DCM (10 mL) was stirred at 24°C for 2 h, solvent was removed, and the residue was purified by prep-HPLC to give **82-11** (350 mg, 68%).

^{1}H NMR (400 MHz, MeOD) δ 7.96 (s, 1 H), 7.60 (d, J = 8.4 Hz, 2 H), 7.42-7.35 (m, 7 H), 5.50-5.46 (m, 1 H), 5.14 (s, 2 H), 4.61 (s, 2 H), 3.24-3.22 (m, 1 H), 3.15-3.13 (m, 1 H), 2.30-2.15 (m, 2 H), 1.46-1.40 (m, 4 H), 1.29 (s, 2 H).

LCMS (ESI): m/z 522.0 [M+H$^+$].

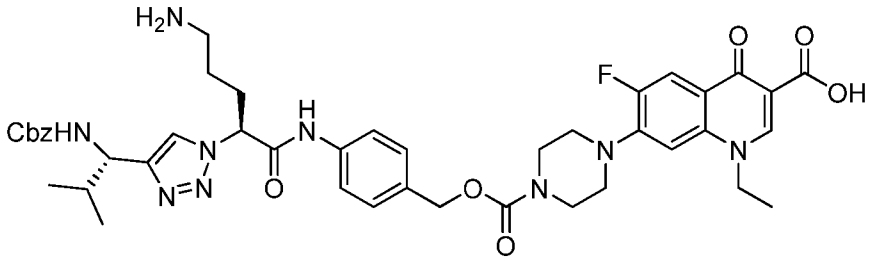
Step 8. After a mixture of **82-11** (200 mg, 0.38 mmol), PNP (234 mg, 0.77 mmol) and DIPEA (149 mg, 1.15 mmol) in DCM (10 mL) was stirred at 50 °C for 12 h, it was concentrated and added to a solution of DIPEA (149 mg, 1.15 mmol) and Norfloxacin (367 mg, 1.15 mmol) in DMF (8 mL). After it was stirred at 23 °C for 4 h, solvent was removed, and the residue was purified by prep-HPLC to give **example 82** (50 mg, 15.2%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, 1 H), 8.96 (s, 1 H), 8.18 (s, 1 H), 7.96-7.89 (m, 2 H), 7.62-7.60 (m, 2 H), 7.38-7.36 (m, 7 H), 7.22-7.20 (m, 1 H), 6.04 (s, 1 H), 5.43 (s, 3 H), 5.07 (s, 2 H), 5.03 (s, 2 H), 4.59-4.57 (m, 2 H), 3.67 (s, 6 H), 3.30--2.98 (m, 4 H), 2.20-2.09 (m, 2 H), 1.42-1.38 (m, 3 H), 1.25-1.22 (s, 4 H), 1.13 (s, 2 H).

MS (ESI): m/z 867.05 [M+H$^+$], 889.16 [M+Na$^+$].

Example 83. 7-(4-((4-((S)-5-amino-2-(4-((S)-1-(benzyloxycarbonylamino)-2-methylpropyl)-1H-1,2,3-triazol-1-yl)pentanamido)benzyloxy)carbonyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

Example 83 was made using the procedure as **Example 58**.



Example 83

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (s, 1 H), 8.47-8.39 (m, 1 H), 8.08 (s, 1 H), 7.94 (d, J = 13.2 Hz, 1 H), 7.74 (d, J = 9.2 Hz, 1 H), 7.61 (d, J = 8.4 Hz, 2 H), 7.38-7.29 (m, 8 H), 7.22-7.20 (m, 1 H), 5.49-5.47 (m, 1 H), 5.07 (s, 2 H), 5.02 (d, J = 6.0 Hz, 2 H), 4.62-4.55 (m, 3 H), 3.60 (s, 4 H), 3.16 (s, 4 H), 2.77-2.70 (m, 2 H), 2.20-2.15 (m, 2 H), 2.09-2.00 (m, 1 H), 1.40 (t, J = 6.8 Hz, 5 H), 1.23 (s, 1 H), 0.85 (d, J = 6.8 Hz, 3 H), 0.78 (d, J = 6.8 Hz, 3 H).

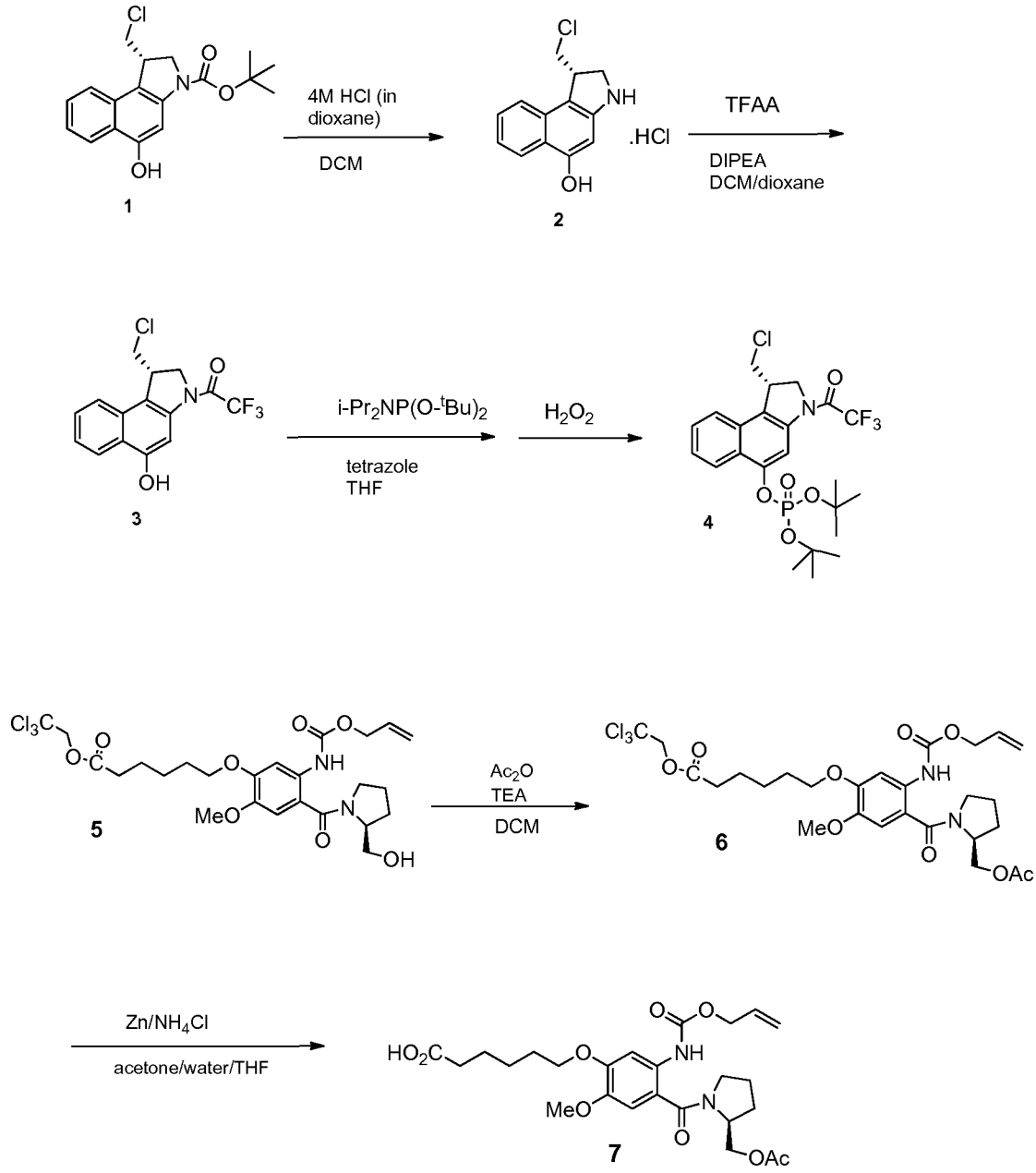
LCMS (ESI): m/z 840.2 [M+H$^+$].

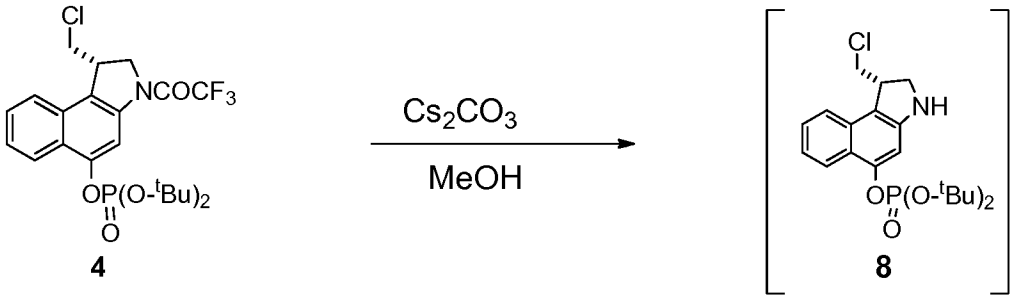
Method of Preparing Linker-drug compounds

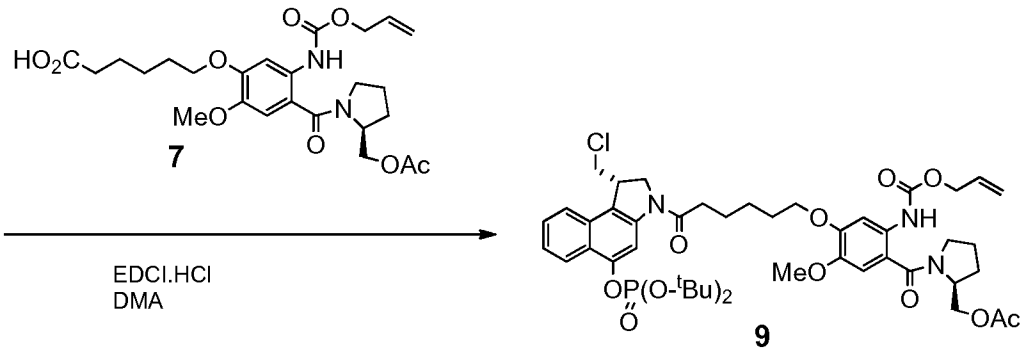
Preparation of CBI-PBD LD1

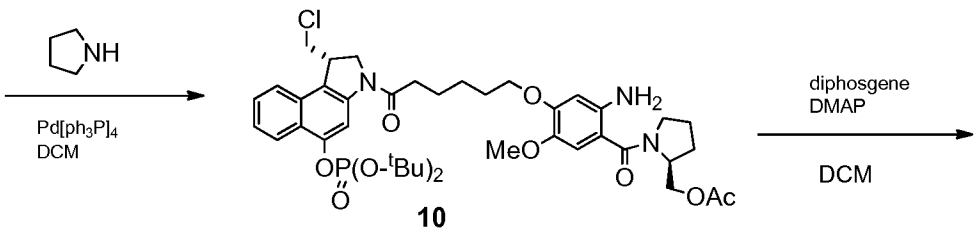
(11aS)-4-((S)-6-amino-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)hexanamido)benzyl 8-(6-((S)-1-(chloromethyl)-5-(phosphonooxy)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyloxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate

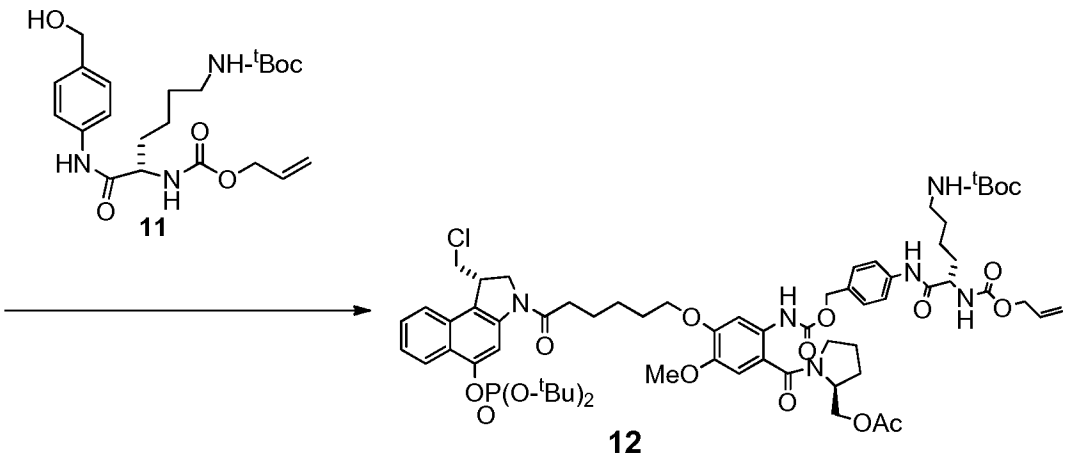
Synthetic Scheme



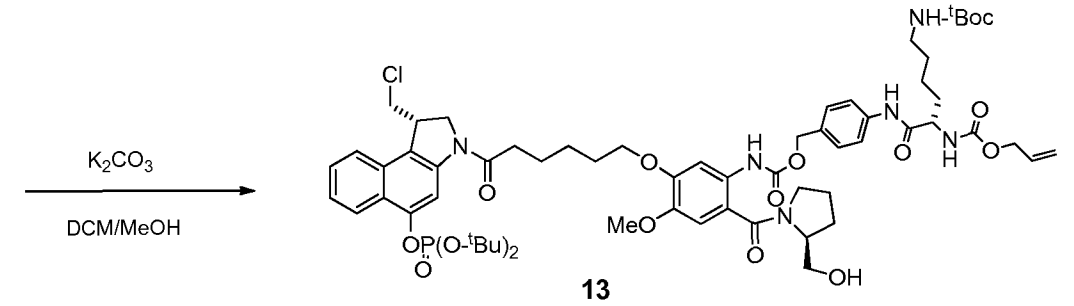

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Cl
NH
OP(O-tBu)2
O
8


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O
O
NH
MeO
N
O
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MeO
N
O
OAc
OP(O-tBu)2
O
9

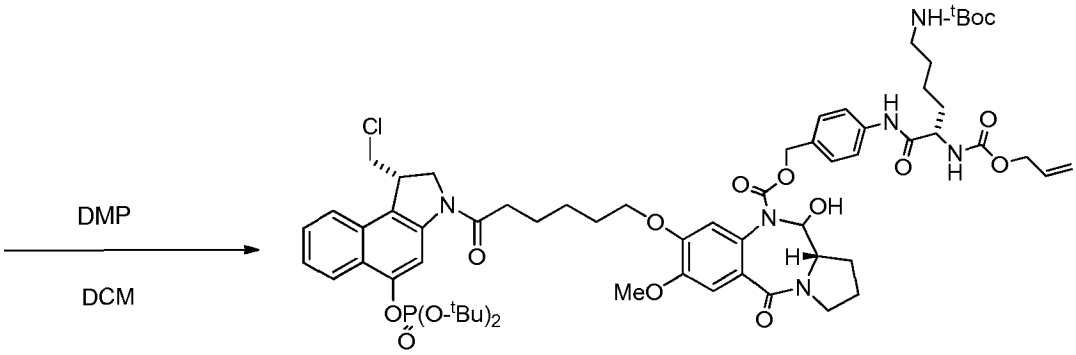

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OAc
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10
diphosgene
DMAP
DCM


HO
NH-tBoc
HN
O
N
H
O
O
11
Cl
N
O
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O
12

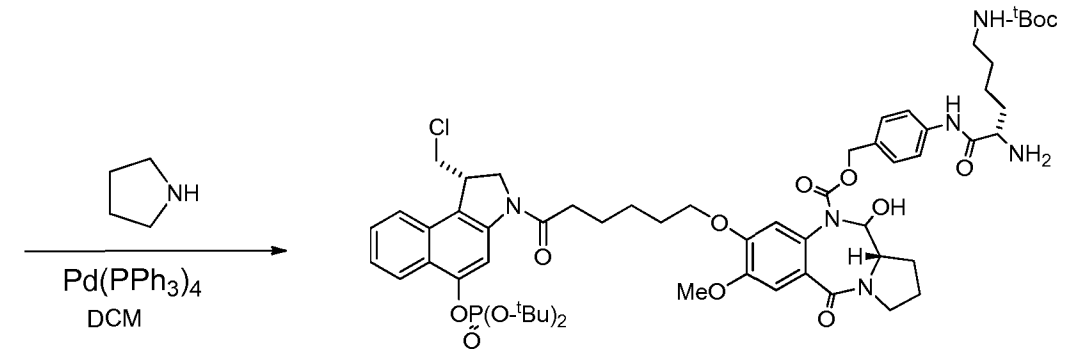
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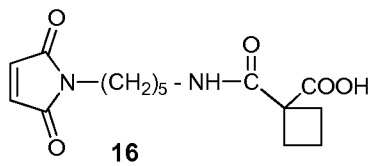
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14



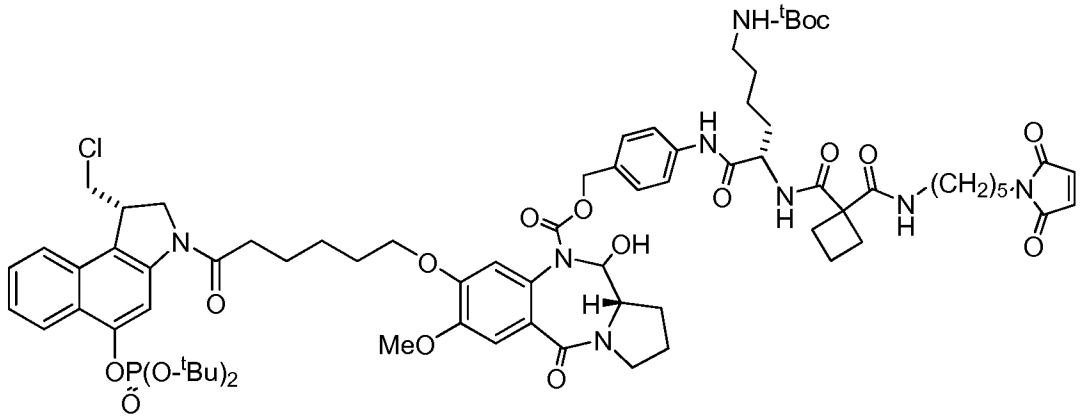
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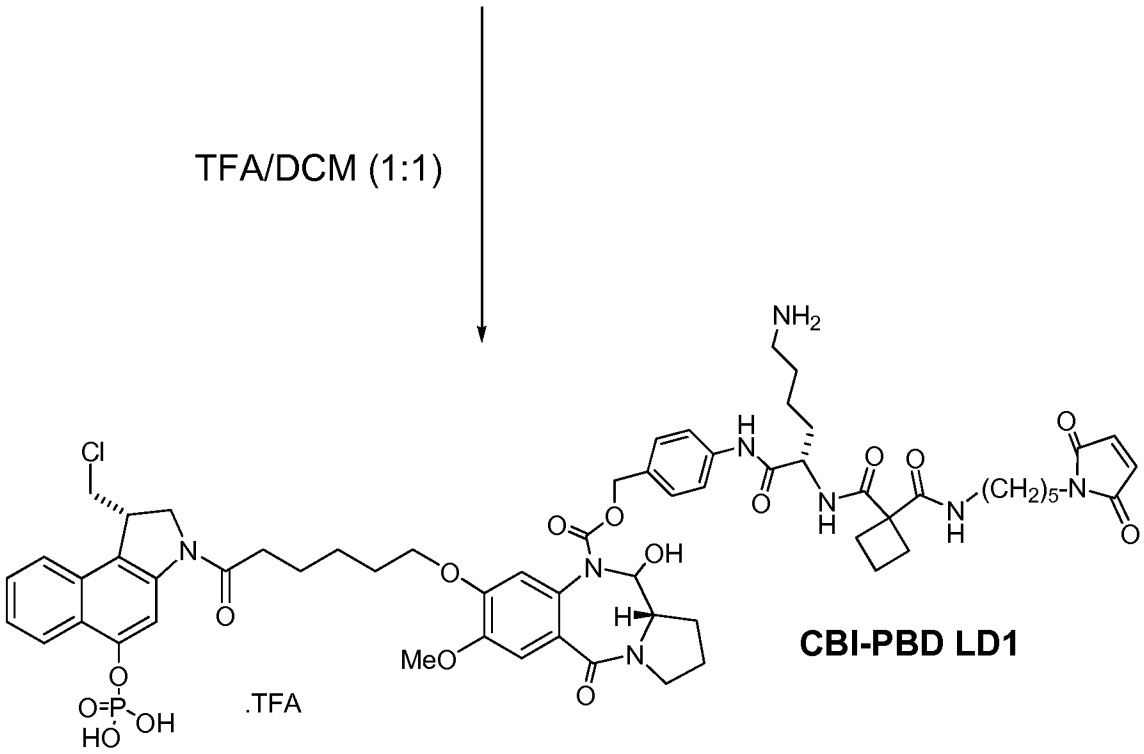
16

EDCI.HCl
DMA

5



17



Example 1. 2,2,2-Trifluoroacetic acid compound with (11aS)-4-((S)-6-amino-2-(1-((5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)carbamoyl) cyclobutanecarboxamido)hexanamido)benzyl 8-((6-((S)-1-(chloromethyl)-5-(phosphonooxy)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyl)oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (1:1) (**CBI-PBD LD1**).

To a stirred homogeneous solution of phenol **1** (3.34 g, 10.0 mmol) in dry DCM (25 mL) at 20 °C under a nitrogen atmosphere was added 4M HCl in dioxane (12.5 mL, 50.0 mmol). After addition the reaction mixture was stirred at 20 °C under nitrogen for a further 20 h. The mixture

was diluted with petroleum ether (250 mL) and stirred at 20 °C under nitrogen for 20 min. Solvents were decanted and the procedure was repeated once more with petroleum ether (250 mL). The resulting solid was dried under vacuum at 25 °C for 1h to give compound **2** (2.7 g, 100%); ^{1}H NMR [$(CD_3)_2SO$] δ 10.80 (s, 1 H), 8.15 (d, J = 8.3 Hz, 1 H), 7.87 (d, J = 8.2 Hz, 1 H), 7.58 (br t, J = 7.5 Hz, 1 H), 7.43 (br t, J = 7.4 Hz, 1 H), 6.81 (s, 1 H), 4.27-4.17 (m, 1 H), 4.01 (dd, J = 11.0, 3.2 Hz, 1 H), 3.93-3.74 (m, 3 H), 2 protons not observed. The crude product was used for the next step without further purification.

To a stirred heterogeneous mixture of amine **2** (2.7 g, 10.0 mmol) in dry DCM (10 mL) and dioxane (30 mL) at 0 °C under a nitrogen atmosphere was added trifluoroacetic anhydride (TFAA) (3.4 mL, 24.0 mmol), followed by diisopropylethylamine (DIPEA) (8.71 mL, 50.0 mmol). After addition the reaction mixture was stirred at 0 °C under nitrogen for a further 50 min. Ethyl acetate (400 mL) was added and 1N HCl (200 mL) were added at 0 °C and the mixture stirred for 20 min under nitrogen. The ethyl acetate layer was separated, washed successively with 1N HCl (200 mL) and water (2x200 mL), and then dried ($MgSO_4$) and evaporated under reduced pressure at a bath temperature of 25 °C to give **3** (3.3 g, 100%) as a green-grey solid. This material was used for the next step without further purification.

To a stirred homogeneous solution of phenol **3** (3.3 g, 10.0 mmol) in dry THF (40 mL) at 20 °C under a nitrogen atmosphere was added di-tert-butyl-N,N-diisopropylphosphoramidite (4.31 mL, 13.0 mmol). After addition the reaction mixture was stirred at 20 °C under nitrogen for 5-10 min and then tetrazole (3% solution in CH_3CN, 38.0 mL, 13.0 mmol) was added dropwise over 17 min. The final reaction mixture was stirred further at 20 °C under nitrogen for 19 h. The mixture was cooled in an ice-bath and 30% H_2O_2 (11.3 mL, 100.0 mmol) was added. After addition the reaction mixture was stirred at 20 °C for a further 1h 30 min. The mixture was diluted with ethyl acetate (300 mL) and 10% aqueous $Na_2S_2O_3$ (500 mL) at stirred at 0 °C for 20 min. The ethyl acetate layer was separated and washed successively with water (200 mL), saturated $NaHCO_3$ (200 mL), and water (200 mL) and then dried ($MgSO_4$) and evaporated under reduced pressure at a bath temperature of 25 °C to give an amber oil. Purification by chromatography on a silica gel (eluting with ethyl acetate:petroleum ether 1:3) gave compound **4** (4.7 g, 90%) as a colorless foamy solid, mp 39-42 °C; $[\alpha]_D$ −61.8° (c 1.02, $CHCl_3$). Anal. ($C_{23}H_{28}ClF_3NO_5P$) Calc: C, 52.93; H, 5.41; N, 2.68. Found: C, 53.05; H, 5.43; N, 2.80.

To a stirred solution of alcohol **5** (4.14 g, 6.95 mmol) (J. Med. Chem. 2003, *46*, 2132-2151) in dry DCM (25 mL) was added acetic anhydride (3.30 mL, 34.8 mmol) and triethylamine (5.81 mL, 41.7 mmol). The mixture was stirred at 20 °C for 3 h 30 min. Dry MeOH (4.0 mL) was added and the mixture was stirred for 30 min. The mixture was partitioned between EtOAc (400

mL) and water (400 mL). The EtOAc layer was separated, washed with water (2x200mL),and then dried ($MgSO_4$) and evaporated to give acetate **6** (4.28 g, 96%) as an oil; $[\alpha]_D$ −57.4° (*c* 0.21, $CHCl_3$); ^{1}H NMR [$(CD_3)_2SO$] δ 9.10 (s, 1 H), 7.17 (s, 1 H), 6.87 (s, 1 H), 6.01-5.87 (m, 1 H), 5.32 (dd, *J* = 17.2, 1.5 Hz, 1 H), 5.21 (dd, *J* = 10.4, 1.4 Hz, 1 H), 4.89 (s, 2 H), 4.54 (d, *J* = 5.4 Hz, 2 H), 4.39-4.20 (m, 3 H), 3.93 (t, *J* = 6.4 Hz, 2 H), 3.75 (s, 3 H), 3.46-3.27 (m, 2 H), 2.13-1.90 (m, 4 H), 1.89-1.60 (m, 7 H), 1.54-1.40 (m, 2 H), 2 protons obscured by DMSO peak. HRMS (ESI) m/z calc. for $C_{27}H_{36}Cl_3N_2O_9$: 637.1481, found: 637.1475 [MH^+]; calc. for $C_{27}H_{35}Cl_3N_2NaO_9$: 659.1300, found: 659.1303 [MNa^+]; calc. for $C_{27}H_{35}Cl_3KN_2O_9$: 675.1040, found: 675.1035 [MK^+].

To a stirred solution of acetate **6** (4.27 g, 6.69 mmol) in acetone (75 mL), water (50 mL), and THF (30 mL) was added zinc powder (17.5 g, 268 mmol) and NH_4Cl (28.6 g, 535 mmol). The mixture was stirred at 20 °C under a nitrogen atmosphere for 42 h. Acetone (100 mL) was added, the mixture was stirred for 10 min, and the supernatant was decanted. The procedure was repeated twice and the combined supernatants were evaporated under reduced pressure to remove acetone and THF. The residue was diluted with water (50 mL) and acidified with aqueous 1N HCl to pH ca. 1. The acidic mixture was washed with petroleum ether (2x200 mL) and extracted with EtOAc (400 mL). The EtOAc extract was washed with water (200 mL) and dried ($MgSO_4$) and the solvent was evaporated to give acid **7** (2.72 g, 80%) as an oil; $[\alpha]_D$ −73.5° (*c* 1.12, $CHCl_3$); ^{1}H NMR [$(CD_3)_2SO$] δ 11.99 (s, exchangeable with D_2O, 1 H), 9.10 (s, exchangeable with D_2O, 1 H), 7.17 (s, 1 H), 6.87 (s, 1 H), 6.00-5.86 (m, 1 H), 5.32 (dd, *J* = 17.2, 1.5 Hz, 1 H), 5.20 (dd, *J* = 10.4, 1.5 Hz, 1 H), 4.57-4.52 (m, 2 H), 4.37-4.03 (m, 3 H), 3.93 (t, *J* = 6.5 Hz, 2 H), 3.75 (s, 3 H), 3.40-3.10 (m, 2 H), 2.23 (t, *J* = 7.3 Hz, 2 H), 2.07-1.93 (m, 4 H), 1.89-1.66 (m, 5 H), 1.62-1.49 (m, 2 H), 1.47-1.34 (m, 2 H). HRMS (ESI) m/z calc. for $C_{25}H_{35}N_2O_9$: 507.2337, found: 507.2340 [MH^+]; calc. for $C_{25}H_{34}KN_2O_9$: 545.1896, found: 545.1906 [MK^+]; calc. for $C_{25}H_{34}N_2NaO_9$: 529.2157, found: 529.2169 [MNa^+].

To a stirred solution of trifluoroacetamide **4** (1.38 g, 2.64 mmol) in MeOH (10 mL) at 0 °C under a nitrogen atmosphere was added Cs_2CO_3 (1.03 g, 3.17 mmol). The mixture was stirred at 0 °C for 2 h 30 min and then partitioned between EtOAc (200 mL) and water (150 mL). The EtOAc layer was separated and washed again with water (100 mL), and then dried ($MgSO_4$) and evaporated under reduced pressure at a bathe temperature of 25 °C to give the unstable amine **8** (1.17 g) as a pale yellow foamy solid.

This crude material was treated with acid **7** (1.24 g, 2.45 mmol), EDCI.HCl (1.41 g, 7.35 mmol) and p-toluenesulfonic acid (84 mg, 0.49 mmol) in dry DMA (14 mL) at 0-20 °C for 22 h. The mixture was partitioned between EtOAc (400 mL) and water (300 mL). The EtOAc layer was

separated and washed again with water (100 mL), and then dried ($MgSO_4$) and evaporated. Purification by chromatography on silica gel (eluting with EtOAc:petroleum ether 2:1) gave amide **9** (1.49 g, 66%) as a pale yellow foamy solid, mp 55-59 °C; $[\alpha]_D$ −68.0° (*c* 1.00, $CHCl_3$); ^{1}H NMR [$(CD_3)_2SO$] δ 9.10 (s, exchangeable with D_2O, 1 H), 8.56 (s, 1 H), 8.03 (d, *J* = 8.1 Hz, 1 H), 7.92 (d, *J* = 8.4 Hz, 1 H), 7.57 (t, *J* = 8.1 Hz, 1 H), 7.47 (t, *J* = 7.6 Hz, 1 H), 7.19 (s, 1 H), 6.86 (s, 1 H), 5.99-5.86 (m, 1 H), 5.32 (dd, *J* = 17.2, 1.6 Hz, 1 H), 5.20 (dd, *J* = 10.4, 1.5 Hz, 1 H), 4.53 (d, *J* = 5.4 Hz, 2 H), 4.45-3.84 (m, 10 H), 3.74 (s, 3 H), 3.44-3.26 (m, 2 H), 2.68-2.47 (m, 2 H), 2.02 (br s, 3 H), 1.93-1.43 (m, 10 H), 1.474 and 1.469 (2 s, 18 H). HRMS (ESI) m/z calc. for $C_{46}H_{62}ClN_3O_{12}P$: 914.3754, found: 914.3749 [MH^+]; calc. for: $C_{46}H_{61}ClKN_3O_{12}P$: 952.3313, found: 952.3381 [MK^+]; calc. for $C_{46}H_{61}ClN_3NaO_{12}P$: 936.3574, found: 936.3589 [MNa^+].

To a stirred solution of carbamate **9** (548 mg, 0.60 mmol) in DCM (8 mL) at 20 °C under a nitrogen atmosphere was added $Pd(Ph_3P)_4$ (17.1 mg; 9.8% Pd) and pyrrolidine (0.49 mL, 6.00 mmol). The mixture was stirred at 20 °C for 30 min and then partitioned between EtOAc (200 mL) and water (150 mL). The EtOAc layer was separated and washed again with water (50 mL), and then dried ($MgSO_4$) and evaporated under reduced pressure at a bath temperature of 25 °C. The crude product was purified by chromatography on silica gel (eluting with EtOAc:MeOH 50:1) to give aniline **10** (323 mg, 65%) as a pale yellow foamy solid, mp 46-49 °C; $[\alpha]_D$ −85.2° (c 0.36, $CHCl_3$); ^{1}H NMR [$(CD_3)_2SO$] δ 8.56 (s, 1 H), 8.04 (d, *J* = 8.3 Hz, 1 H), 7.93 (d, *J* = 8.4 Hz, 1 H), 7.58 (t, *J* = 8.2 Hz, 1 H), 7.47 (t, *J* = 8.1 Hz, 1 H), 6.67 (s, 1 H), 6.37 (s, 1 H), 5.09 (s, exchangeable with D_2O, 2 H), 4.46-3.85 (m, 10 H), 3.63 (s, 3 H), 3.52-3.34 (m, 2 H), 2.69-2.50 (m, 2 H), 2.08-1.94 (m, 1 H), 2.01 (s, 3 H), 1.91-1.61 (m, 7 H), 1.58-1.44 (m, 2 H), 1.476 and 1.470 (2 s, 18 H). HRMS (ESI) m/z calc. for $C_{42}H_{58}ClN_3O_{10}P$: 830.3522, found: 830.3543 [MH^+].

To a stirred solution of aniline **10** (293 mg, 0.35 mmol) and DMAP (202 mg, 1.65 mmol) in dry DCM (7 mL) at 20 °C under a nitrogen atmosphere was added a solution of diphosgene in dry DCM (0.05 M, 6.7 mL, 0.33 mmol). The mixture was stirred for 25 min and then a solution of alcohol **11** (1.54 g, 3.54 mmol) in dry DCM (20 mL) was added. The mixture was stirred at 20 °C under a nitrogen atmosphere for 68 h and then partitioned between EtOAc (300 mL) and water (200 mL). The EtOAc layer was separated, washed again with water (100 mL) and then dried ($MgSO_4$) and evaporated at a bath temperature of 30 °C. The resulting orange oil was purified by chromatography on silica gel (eluting with EtOAc:MeOH:petroleum ether 30:0.5:10) to afford carbamate **12** (385 mg, 84%) as a foamy solid, mp 72-75 °C; $[\alpha]_D$ −55.2° (c 0.53, $CHCl_3$); ^{1}H NMR [$(CD_3)_2SO$] δ 10.04 (s, exchangeable with D_2O, 1 H), 9.12 (br s, exchangeable with D_2O, 1 H), 8.56 (s, 1 H), 8.03 (d, *J* = 8.3 Hz, 1 H), 7.92 (d, *J* = 8.4 Hz, 1 H), 7.65-7.52 (m, 3 H, reduced to 2 H after D_2O), 7.46 (t, *J* = 7.8 Hz, 2 H), 7.31 (d, *J* = 8.5 Hz, 2 H), 7.20 (br s, 1 H), 6.86 (s, 1 H),

6.75 (poorly resolved t, exchangeable with D_2O, 1 H), 5.97-5.83 (m, 1 H), 5.30 (br d, J = 17.3 Hz, 1 H), 5.17 (br d, J = 10.6 Hz, 1 H), 5.18-4.97 (m, 2 H), 4.51-3.85 (m, 13 H), 3.74 (s, 3 H), 3.43-3.23 (m, 2 H, partially obscured by water peak), 2.94-2.83 (m, 2 H), 2.65-2.50 (m, 2 H, partially obscured by DMSO peak), 2.07-1.91 (m, 1 H), 2.01 (br s, 3 H), 1.88-1.43 (m, 11 H), 1.473-1.468 (2 s, 18 H), 1.43-1.20 (m, 4 H), 1.35 (s, 9 H). HRMS (ESI) m/z calc. for $C_{65}H_{89}ClN_6O_{17}P$: 1291.5665, found: 1291.5705 [MH^+]; calc. for $C_{65}H_{88}ClKN_6O_{17}P$: 1329.5262, found: 1329.5264 [MK^+]; calc. for $C_{65}H_{88}ClN_6NaO_{17}P$: 1313.5554, found: 1313.5524 [MNa^+].

A mixture of acetate **12** (366 mg, 0.28 mmol) and K_2CO_3 (1.14 g, 8.24 mmol) in DCM (9 mL) and MeOH (9 mL) was stirred at 0 °C for 3 h 30 min. The mixture was stirred with cold EtOAc (200 mL) and ice-water (150 mL) for 10 min. The EtOAc layer was separated, washed again with water (100 mL), and then dried ($MgSO_4$) and evaporated at a bath temperature of 25 °C to give alcohol **13** (343 mg, 97%) as a colorless foamy solid, mp 71-75 °C; $[\alpha]_D$ −58.2° (c 0.57, $CHCl_3$); ^{1}H NMR [$(CD_3)_2SO$] δ 10.04 (s, exchangeable with D_2O, 1 H), 9.11 (br s, exchangeable with D_2O, 1 H), 8.56 (s, 1 H), 8.03 (d, J = 8.3 Hz, 1 H), 7.92 (d, J = 8.4 Hz, 1 H), 7.65-7.53 (m, 3 H, reduced to 2 H after D_2O), 7.46 (t, J = 7.6 Hz, 2 H), 7.32 (d, J = 8.6 Hz, 2 H), 7.27 (br s, 1 H), 6.93 (s, 1 H), 6.75 (poorly resolved t, exchangeable with D_2O, 1 H), 5.97-5.82 (m, 1 H), 5.29 (br d, J = 17.2 Hz, 1 H), 5.17 (br d, J = 10.5 Hz, 1 H), 5.03 (br s, 2 H), 4.73 (t, J = 5.8 Hz, exchangeable with D_2O, 1 H), 4.50-3.82 (m, 11 H), 3.74 (s, 3 H), 3.62-3.44 (m, 2 H), 3.40-3.21 (m, 2 H, partially obscured by water peak), 2.95-2.80 (m, 2 H), 2.65-2.50 (m, 2 H, partially obscured by DMSO peak), 1.93-1.21 (m, 16 H), 1.473-1.468 (2 s, 18 H), 1.35 (s, 9 H). HRMS (ESI) m/z calc. for $C_{63}H_{86}ClKN_6O_{16}P$: 1287.5158, found: 1287.5113 [MK^+]; calc. for $C_{63}H_{86}ClN_6NaO_{16}P$: 1271.5419, found: 1271.5381 [MNa^+].

To a stirred solution of alcohol **13** (322 mg, 0.26 mmol) in dry DCM (14 mL) at 0 °C was added Dess-Martin periodinane (DMP) (131 mg, 0.31 mmol) portionwise over 3 min. The reaction mixture was stirred at 0 °C for a further 2 h, then at 20 °C for 50 h. The mixture was diluted with DCM (40 mL) and 10% $Na_2S_2O_3$ (40 mL), stirred at 20 °C for 10 min, and then partitioned between DCM (200 mL) and saturated $NaHCO_3$ solution (150 mL). The DCM layer was separated and the aqueous layer was further extracted with DCM (2x50 mL). The combined DCM extracts were washed with saturated $NaHCO_3$ solution (2x100 mL) and water (2x100 mL), and then dried ($MgSO_4$) and evaporated at a bath temperature of 25 °C. The resulting orange oil was purifed by chromatography on silica gel (eluting with $CHCl_3$:MeOH 40:1) to give **14** (228 mg, 71%) as a pale brown foamy solid, mp 98 °C (decomp); $[\alpha]_D$ +74.5° (c 0.26, $CHCl_3$); ^{1}H NMR [$(CD_3)_2SO$] δ 10.02 (s, exchangeable with D_2O, 1 H), 8.56 (s, 1 H), 8.04 (d, J = 8.3 Hz, 1 H), 7.92 (d, J = 8.4 Hz, 1 H), 7.65-7.47 (m, 5 H, reduced to 4 H after D_2O), 7.25-7.12 (m, 2 H, br s and 1 H on D_2O

exchange), 7.03 (s, 1 H), 6.83-6.64 (m, 2 H), 6.48 (br s, exchangeable with D_2O, 1 H), 5.96-5.80 (m, 1 H), 5.52-5.39 (m, d on D_2O exchange, J = 9.6 Hz, 1 H), 5.27 (br d, J = 16.8 Hz, 1 H), 5.21-5.10 (m, 2 H), 4.81 (br d, J = 12.3 Hz, 1 H), 4.54-3.85 (m, 8 H), 3.83-3.70 (m, 5 H), 3.53-3.21 (m, 3 H, partially obscured by water peak), 2.93-2.82 (m, 2 H), 2.64-2.47 (m, 2 H, partially obscured by DMSO peak), 2.10-1.20 (m, 16 H), 1.470 and 1.464 (2 s, 18 H), 1.34 (s, 9 H). HRMS (ESI) m/z calc. for $C_{63}H_{84}ClKN_6O_{16}P$: 1285.5002, found: 1285.4938 [MK^+]; calc. for $C_{63}H_{84}ClN_6NaO_{16}P$: 1269.5262, found: 1269.5220 [MNa^+].

To a stirred solution of **14** (125 mg, 0.10 mmol) in DCM (2 mL) at 20 °C under a nitrogen atmosphere was added $Pd(Ph_3P)_4$ (2.9 mg; 9.8% Pd) and pyrrolidine (0.08 mL, 1.00 mmol). The mixture was stirred at 20 °C and monitored by TLC (EtOAc:MeOH 20:1). After 40 min more $Pd(Ph_3P)_4$ (5.8 mg; 9.8% Pd) and pyrrolidine (0.16 mL, 2.00 mmol) were added and the mixture was stirred for another 3 h. The mixture was partitioned between EtOAc (100 mL) and water (100 mL). The EtOAc layer was separated and washed again with water (50 mL), and then dried ($MgSO_4$) and evaporated at a bath temperature of 25 °C. The crude product **15** (94 mg, 81%) was used for the next step without further purification. HRMS (ESI) m/z calc. for $C_{59}H_{81}ClN_6O_{14}P$: 1163.5231, found: 1163.5188 [MH^+].

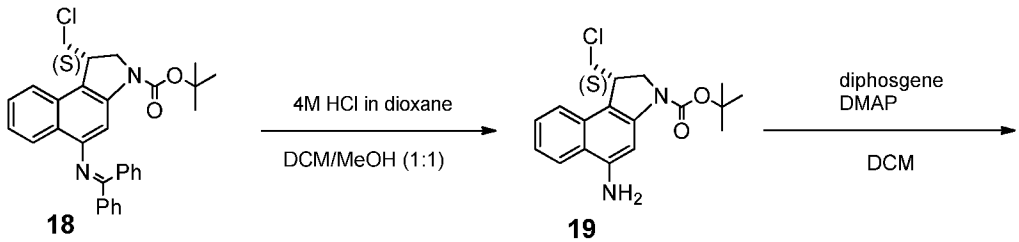
A solution of **15** (91 mg, 0.078 mmol) in dry DMA (1.0 mL) was treated with a pre-formed (at 20 °C for 10 min) mixture of acid **16** (36 mg, 0.12 mmol), EDCI.HCl (34 mg, 0.18 mmol), and TsOH (4.0 mg, 0.023 mmol) in dry DMA (0.5 mL) at 20 °C under a nitrogen atmosphere. After 10 min DIPEA (0.016 mL, 0.078 mmol) was added and the reaction mixture was stirred for 23 h. The mixture was partitioned between EtOAc (100 mL) and water (100 mL). The EtOAc layer was separated and washed further with saturated $NaHCO_3$ (50 mL), water (50 mL), and then dried ($MgSO_4$). Evaporation of solvent at a bath temperature of 25 °C gave a crude product which was purified by chromatography on silica gel (eluting with $CHCl_3$:EtOAc:MeOH 30:10:2) to give **17** (63 mg, 56%) as a pale brown foamy solid; mp 67-70 °C; $[\alpha]_D$ +23.9° (c 2.09, $CHCl_3$); ^1H NMR [$(CD_3)_2SO$] δ 10.05 (s, exchangeable with D_2O, 1 H), 8.56 (s, 1 H), 8.03 (d, J = 8.3 Hz, 1 H), 7.92 (d, J = 8.4 Hz, 1 H), 7.84-7.71 (m, 2 H, exchangeable with D_2O), 7.62-7.52 (m, 3 H), 7.46 (t, J = 7.7 Hz, 1 H), 7.22-7.13 (m, 2 H), 7.03 (br s, 1 H), 6.96 (s, 2 H), 6.71 (br s, 2 H, reduced to 1 H after D_2O), 6.49 (br s, exchangeable with D_2O, 1 H), 5.51-5.41 (m, but d on D_2O exchange with J = 9.5 Hz, 1 H), 5.15 (d, J = 12.2 Hz, 1 H), 4.82 (br d, J = 12.4 Hz, 1 H), 4.47-3.85 (m, 8 H), 3.77 (br s, 3 H), 3.52-3.20 (m, 3 H, partially obscured by water peak), 3.12-3.20 (m, but t on D_2O exchange with J = 6.7 Hz, 2 H), 2.92-2.80 (m, 2 H), 2.65-2.50 (m, 2 H, partially obscured by DMSO peak), 2.39 (t, J = 7.9 Hz, 2 H), 2.07-1.24 (m, 28 H), 1.469 and 1.463 (2 s, 18 H), 1.33 (s, 9 H). HRMS (ESI) m/z calc. for $C_{74}H_{98}ClN_8NaO_{18}P$: 1475.6317, found: 1475.6267 [MNa^+].

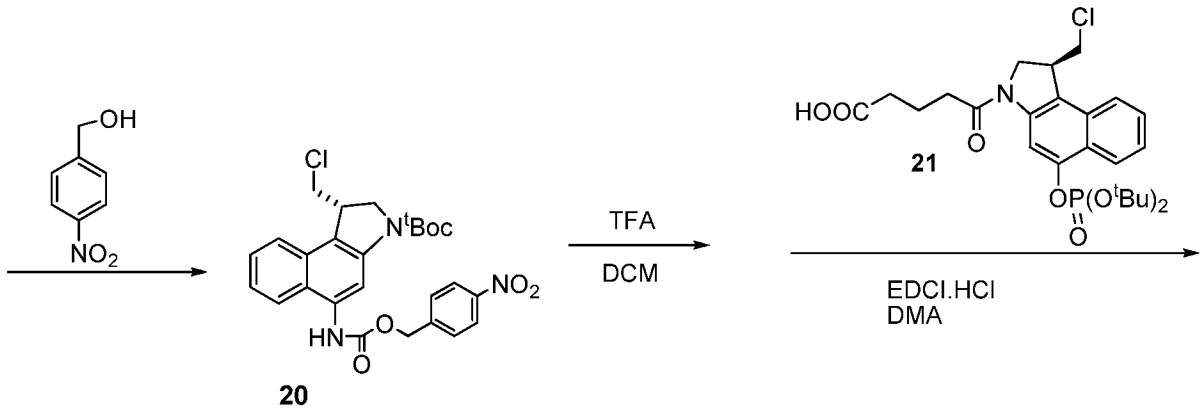
To a stirred solution of **17** (45 mg, 0.031 mmol) in DCM (1.0 mL) at 20 °C under nitrogen was added TFA (1.0 mL) and the mixture was stirred for 15 min. Petroleum ether (20 mL) was added and the mixture was stirred for 30 min. The supernatant was decanted and the procedure was repeated using EtOAc:petroleum ether (1:5) (2×20 mL). The resulting solid was collected and purified by preparative HPLC [Synergi PolarRP column; aqueous TFA (pH = 2.56; 90% to 2%)/10% water in CH_3CN (10% to 98%); gradient elution over 23 min with a flow rate of 12 mL/min] to give pure **CBI-PBD LD1** (17.5 mg, 38%) as a beige solid, purity (HPLC): 99.1%; $[\alpha]_D$ +54.9° (c 0.18, MeOH); ^{1}H NMR [$(CD_3)_2SO$] δ 10.20 (s, exchangeable with D_2O, 1 H), 8.50 (s, 1 H), 8.20-7.78 (m, 7 H, reduced to 1H after D_2O), 8.12 (d, J = 9.1 Hz, 1 H), 7.72-7.47 (m, 4 H, reduced to 3H after D_2O), 7.40 (t, J = 7.5 Hz, 1 H), 7.17 (br d, J = 7.3 Hz, 2 H), 7.03 (br s, 1 H), 6.97 (s, 2 H), 6.66 (br s, exchangeable with D_2O, 1 H), 5.51 (br s, 1 H), 5.48 (br d, J = 9.7 Hz, 1 H), 5.32-5.18 (m, but d after D_2O, J = 12.6 Hz, 1 H), 4.75 (br d, J = 12.4 Hz, 1 H), 4.44-3.81 (m, 8 H), 3.77 (s, 3 H), 3.52-3.21 (m, 5 H, partially obscured by water peak), 3.04 (q, but t after D_2O with J = 6.8 Hz, 2 H), 2.80-2.68 (m, 2 H), 2.39 (t, J = 7.7 Hz, 2 H), 2.12-1.08 (m, 28 H). HRMS (ESI) m/z calc. for $C_{61}H_{75}ClN_8O_{16}P$: 1241.4722, found: 1241.4700 [MH^+]; calc. for $C_{61}H_{74}ClN_8NaO_{16}P$: 1263.4541, found: 1263.4531 [MNa^+].

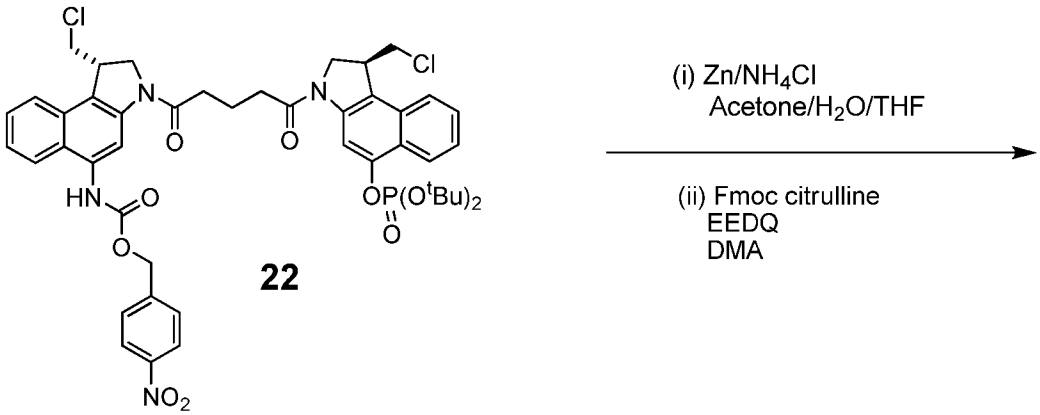
Synthesis of CBI-CBI LD4

4-((S)-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyl (S)-1-(chloromethyl)-3-(5-((S)-1-(chloromethyl)-5-(phosphonooxy)-1H-benzo[e]indol-3(2H)-yl)-5-oxopentanoyl)-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate

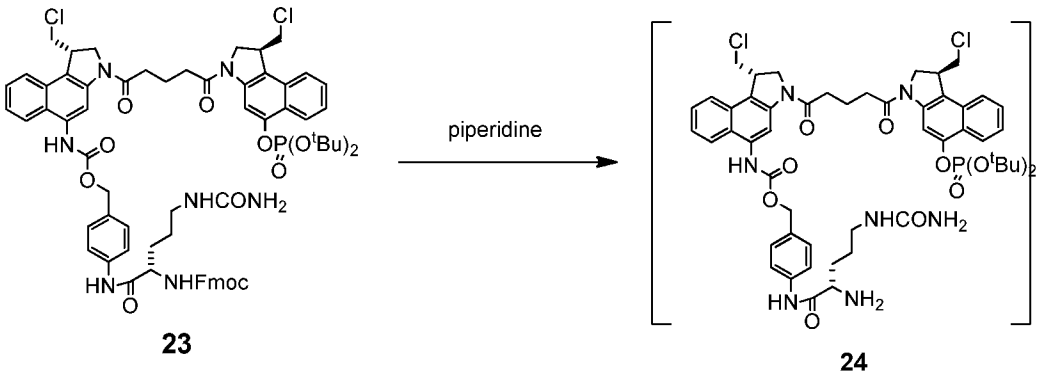
Synthetic Scheme



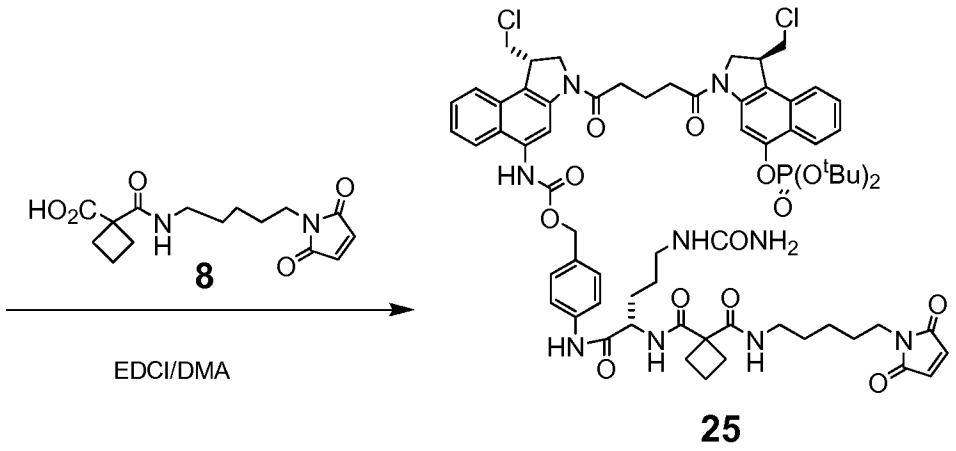

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NO2
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NO2
HN
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TFA
DCM
Cl
HOOC
N
O
21
OP(OtBu)2
O
EDCI.HCl
DMA

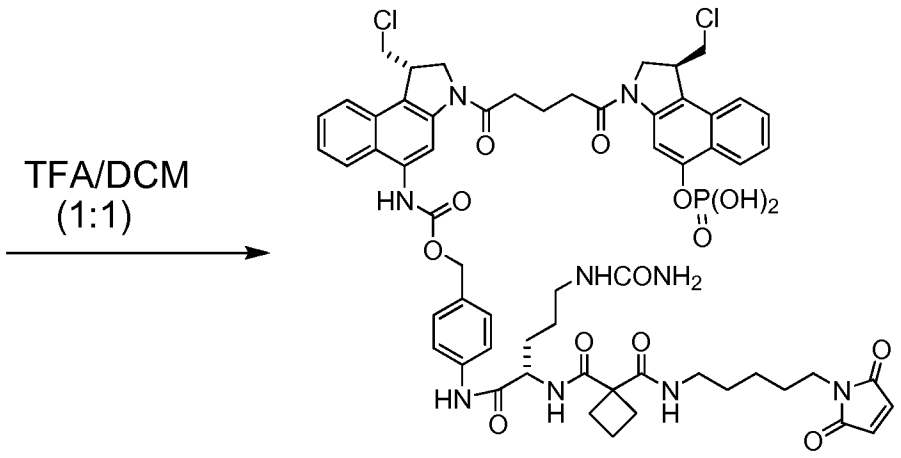

Cl
Cl
N
N
O
O
HN
O
O
OP(OtBu)2
O
22
NO2
(i) Zn/NH4Cl
Acetone/H2O/THF
(ii) Fmoc citrulline
EEDQ
DMA

5


Cl
Cl
N
N
O
O
HN
O
O
OP(OtBu)2
O
NHCONH2
HN
O
NHFmoc
23
piperidine
NH2
24

10





CBI-CBI LD4

Example 2. 4-((S)-2-(1-((5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)carbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyl ((S)-1-(chloromethyl)-3-(5-((S)-1-(chloromethyl)-5-(phosphonooxy)-1H-benzo[e]indol-3(2H)-yl)-5-oxopentanoyl)-2,3-dihydro-1H-benzo[e]indol-5-yl)carbamate (**CBI-CBI LD4).**

To a stirred solution of imine **18** (497 mg, 1.00 mmol) in dry DCM (10 mL) and MeOH (10 mL) at 20 °C under nitrogen was added 4M HCl in dioxane (0.63 mL, 2.5 mmol). After addition the reaction mixture was stirred at 20 °C for 1 h. The volatiles were evaporated under reduced pressure at 20 °C to give a yellow solid which was stirred with a mixture of EtOAc and

petroleum ether (1:10) (200 mL) at 20 °C for 30 min. The supernatant was decanted and the procedure was repeated. The resulting solid was stirred with a mixture of aqueous Na_2CO_3 (2N, 200 mL) and DCM (200 mL) at 0 °C for 15 min. The DCM layer was separated, washed with water (100 mL), and then dried ($MgSO_4$) and evaporated at 25 °C to give aniline **19** (322 mg, 97%) as an unstable solid; ^{1}H NMR [$(CD_3)_2SO$] δ 8.01 (d, J = 8.4 Hz, 1 H), 7.94-7.78 (m, 2 H), 7.52 (t, J = 7.3 Hz, 1 H), 7.39 (t, J = 7.5 Hz, 1 H), 4.20-3.72 (m, 5 H), 1.54 (s, 9 H), 2 protons not observed. HRMS (ESI) m/z calc. for $C_{18}H_{22}ClN_2O_2$: 333.1364, found: 333.1355 [MH^+]; calc. for $C_{18}H_{21}ClKN_2O_2$: 371.0923, found: 371.0920 [MK^+]; calc. for $C_{18}H_{21}ClN_2NaO_2$: 355.1184, found: 355.1179 [MNa^+]. This material was used for the next step without further purification.

To a stirred homogeneous mixture of aniline **19** (322 mg, 0.97 mmol) and DMAP (730 mg, 6.00 mmol) in dry DCM (30 mL) at 20 °C under nitrogen was added a solution of diphosgene in dry DCM (0.10 M, 11 mL, 1.10 mmol). The mixture was stirred for 20 min and then solid p-nitrobenzyl alcohol (1.53 g, 10.0 mmol) was added. The final reaction mixture was stirred at 20 °C under nitrogen for 18 h and then partitioned between EtOAc (300 mL) and water (300 mL). The EtOAc layer was separated, washed with brine (200 mL) and dried ($MgSO_4$). Evaporation of solvent at 30 °C gave a soft yellow solid which was purified by chromatography on silica gel (eluting with DCM:EtOAc:petroleum ether 20:1:10) to afford carbamate **20** (411 mg, 83%) as a pale yellow solid, mp 141-142 °C; $[\alpha]_D$ −15.0° (c 0.20, $CHCl_3$); ^{1}H NMR [$(CD_3)_2SO$] δ 9.85 (s, 1 H), 8.36-8.15 (m, 3 H), 8.05 (d, J = 8.4 Hz, 1 H), 7.88 (d, J = 8.3 Hz, 1 H), 7.72 (d, J = 8.6 Hz, 2 H), 7.57-7.49 (m, 1 H), 7.45-7.36 (m, 1 H), 5.35 (s, 2 H), 4.27-4.12 (m, 2 H), 4.10-3.96 (m, 2 H), 3.94-3.81 (m, 1 H), 1.52 (s, 9 H). Anal. ($C_{26}H_{26}ClN_3O_6$) Calc: C, 61.00; H, 5.12; N, 8.21. Found: C, 61.27; H, 5.05; N, 8.25.

To a stirred solution of **20** (282 mg, 0.55 mmol) in DCM (6 mL) at 0 °C under nitrogen was added trifluoroacetic acid (TFA) (3 mL). After addition the reaction mixture was stirred at 0 °C for 1h 15 min and then partitioned between DCM (300 mL) and cold aqueous Na_2CO_3 (2N, 300 ml). The DCM layer was separated, washed with cold aqueous Na_2CO_3 (2N, 100 mL) and water (100 mL) and then dried ($MgSO_4$) and evaporated at 25 °C to give an orange solid. This solid was dissolved in dry DMA (4 mL) and treated with acid **21** (297 mg, 0.55 mmol), EDCI.HCl (317 mg, 1.65 mmol) and TsOH (19 mg, 0.11 mmol). The mixture was stirred at 20 °C under nitrogen for 22 h and then partitioned between EtOAc (300 mL) and water (300 mL). The EtOAc layer was separated, washed with water (200 mL), and dried ($MgSO_4$). Evaporation of solvent at 25 °C gave an oil which was purified by chromatography on silica gel (eluting with DCM:EtOAc 2:1) to afford amide **22** (181 mg, 35%) as a sticky solid, $[\alpha]_D$ −26.8° (c 0.37, $CHCl_3$); ^{1}H NMR [$(CD_3)_2SO$] δ 9.84 (s, 1 H), 8.62 (s, 1 H), 8.59 (s, 1 H), 8.28 (d, J = 8.5 Hz, 2 H), 8.04 (d, J = 8.5

Hz, 2 H), 7.92 (d, *J* = 8.2 Hz, 2 H), 7.72 (d, *J* = 8.3 Hz, 2 H), 7.63-7.57 (m, 2 H), 7.51-7.39 (m, 2 H), 5.34 (s, 2 H), 4.48-4.17 (m, 6 H), 4.10-3.97 (m, 2 H), 3.97-3.84 (m, 2 H), 2.82-2.54 (m, 4 H), 2.04-1.91 (m, 2 H), 1.49 (s, 18 H). HRMS (ESI) m/z calc. for $C_{47}H_{51}Cl_2KN_4O_{10}P$: 971.2351, found: 971.2344 [MK^+]; calc. for $C_{47}H_{51}Cl_2N_4NaO_{10}P$: 955.2612, found: 955.2621 [MNa^+].

To a stirred solution of nitro compound **22** (47 mg, 0.05 mmol) in acetone:water:THF (10:5:1) (6 mL) at 0 °C under nitrogen was added Zn powder (65.4 mg, 1.00 mmol) and NH_4Cl (107 mg, 2.00 mmol). After addition the reaction mixture was stirred at 0 °C for 40 min. The reaction mixture was filtered through celite, washing several times with cold DCM. The combined filtrates were washed with cold water (50 mL), and then dried ($MgSO_4$) and evaporated at 25 °C to give a yellow foamy solid. This solid was dissolved in dry DMA (1 mL) and added to a preformed (10 min at 20 °C) mixture of Fmoc-L-citrulline (29.8 mg, 0.075 mmol) and EEDQ (18.5 mg, 0.075 mmol) in DMA (0.3 mL). The reaction mixture was stirred at 20 °C under nitrogen for 48 h and then partitioned between EtOAc (100 mL) and water (100 mL). The EtOAc layer was separated, washed with water (50 mL), and dried ($MgSO_4$). Evaporation of solvent at 25 °C gave an oil which was purified by chromatography on silica gel (EtOAc:MeOH 10:1) to give **23** (24 mg, 38%) as a sticky solid, $[\alpha]_D$ −31.9° (c 0.28, $CHCl_3$); ^{1}H NMR [$(CD_3)_2SO$] δ 10.09 (s, 1 H), 9.66 (s, 1 H), 8.62 (s, 1 H), 8.59 (s, 1 H), 8.10-7.25 (m, 21 H), 6.04-5.94 (m, 1 H), 5.53-5.29 (m, 2 H), 5.13 (s, 2 H), 4.51-4.08 (m, 10 H), 4.08-3.82 (m, 4 H), 3.11-2.90 (m, 2 H), 2.80-2.54 (m, 4 H), 2.04-1.92 (m, 2 H), 1.80-1.30 (m, 4 H), 1.49 (s, 18 H). HRMS (ESI) m/z calc. for $C_{68}H_{75}Cl_2N_7O_{12}P$: 1282.4583, found: 1282.4536 [MH^+]; calc. for $C_{68}H_{74}Cl_2KN_7O_{12}P$: 1320.4142, found: 1320.4119 [MK^+]; calc. for $C_{68}H_{74}Cl_2N_7NaO_{12}P$: 1304.4402, found: 1304.4388 [MNa^+].

To a stirred solution of **23** (86 mg, 0.067 mmol) in dry DMA (3 mL) at 0 °C under nitrogen was added a solution of piperidine in DMA (1.00 M, 0.58 mL, 0.58 mmol) and the mixture was stirred at 0 °C for 1 h 30 min. A mixture of EtOAc and petroleum ether (1:10, 60 mL) was added and the mixture was stirred at 0 °C for 40 min. The supernatant was decanted and the procedure was repeated with more EtOAc-petroleum ether (1:3, 50 mL then 30 mL). The residual oil was dried in vacuum at 20 °C for 1 h to give intermediate amine **24** (67 mg, 94%) as a sticky solid. This solid was used for the next step without further purification.

A mixture of acid **8** (30.4 mg, 0.098 mmol), EDCI.HCl (25.3 mg, 0.13 mmol) and TsOH (2.2 mg, 0.013 mmol) in dry DMA (0.7 mL) was stirred at 20 °C under nitrogen for 10 min. To this mixture was added a solution of the above amine **24** (67 mg, 0.060 mmol), followed by DIPEA (0.011 mL, 0.065 mmol. The reaction mixture was stirred at 20 °C under nitrogen for 20 h. Ice-water (30 mL) was added and the mixture was stirred at 0 °C for 30 min. The separated solid was filtered off, dried, and purified by chromatography on silica gel (eluting with

DCM:EtOAc;MeOH 10:10:1) to give **25** (44 mg, 54%) as a sticky solid, $[\alpha]_D$ −51.6° (c 0.16, $CHCl_3$); ^1H NMR [$(CD_3)_2SO$] δ 10.15 (s, exchangeable with D_2O, 1 H), 9.66 (s, exchangeable with D_2O, 1 H), 8.62 (s, 1 H), 8.59 (s, 1 H), 8.08-7.76 (m, 6 H, reduced to 4 H after D_2O exchange), 7.69-7.34 (m, 8 H), 6.97 (s, 2 H), 5.96 (t, J = 5.7 Hz, exchangeable with D_2O, 1 H), 5.40 (s, exchangeable with D_2O, 2 H), 5.11 (s, 2 H), 4.50-4.15 (m, 7 H), 4.02-3.82 (m, 4 H), 3.25 (t, partially obscured by water peak, J = 6.8 Hz, 2 H), 3.13-2.86 (m, 4 H), 2.81-2.56 (m, 4 H), 2.45-2.33 (m, 4 H), 2.05-1.91 (m, 2 H), 1.83-1.09 (m, 12 H), 1.49 (s, 18 H). HRMS (ESI) m/z calc. for $C_{68}H_{83}Cl_2N_9O_{14}P$: 1350.5169, found: 1350.5170 [MH^+]; calc. for $C_{68}H_{82}Cl_2KN_9O_{14}P$: 1388.4727, found: 1388.4771 [MK^+]; calc. for $C_{68}H_{82}Cl_2N_9NaO_{14}P$: 1372.4988, found: 1372.4992 [MNa^+].

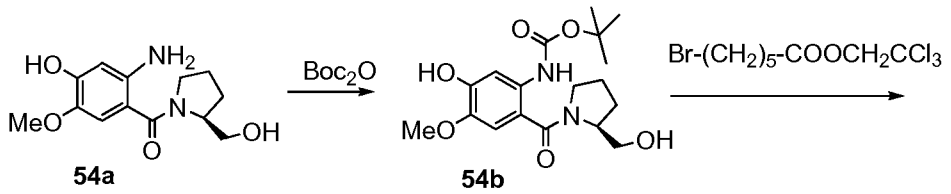
To a stirred solution of **25** (30.3 mg, 0.022 mmol) in DCM (1.0 mL) at 20 °C under nitrogen was added TFA (1.0 mL). After addition the mixture was stirred at this temperature for 1 min. Cold petroleum ether (20 mL) was added and the mixture was stirred at 0 °C for 15 min. The supernatant was decanted and the procedure was repeated with EtOAc-petroleum ether (1:3, 2×20 mL). The resulting solid was collected and dried in vacuum at 20 °C to give **CBI-CBI LD4** (25.3 mg, 93%) as a beige solid, $[\alpha]_D$ −186° (c 0.059, MeOH); ^1H NMR [$(CD_3)_2SO$] δ 10.13 (s, exchangeable with D_2O, 1 H), 9.66 (s, exchangeable with D_2O, 1 H), 8.62 (s, 1 H), 8.51 (s, 1 H), 8.10 (d, J = 8.4 Hz, 1 H), 8.00 (d, J = 8.5 Hz, 1 H), 7.94-7.73 (m, 4 H, reduced to 2 H after D_2O exchange), 7.65 (d, J = 8.4 Hz, 2 H), 7.60-7.32 (m, 6 H), 6.97 (s, 2 H), 6.00 (t, J = 5.5 Hz, exchangeable with D_2O, 1 H), 5.45 (br s, exchangeable with D_2O, 2 H), 5.13 (s, 2 H), 4.52-4.15 (m, 7 H), 4.10-3.82 (m, 4 H), 3.28 (t, partially obscured by water peak, J = 6.9 Hz, 2 H), 3.13-2.84 (m, 4 H), 2.81-2.56 (m, 4 H), 2.47-2.34 (m, 4 H), 2.08-1.89 (m, 2 H), 1.84-1.53 (m, 4 H), 1.53-1.09 (m, 8 H), 2 H not observed. HRMS (ESI) m/z calc. for $C_{60}H_{65}Cl_2N_9O_{14}P$: 1236.3771, found: 1236.3772 [$M-H]^-$.

Preparation of (11aS)-4-((S)-6-amino-2-(1-((5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)carbamoyl)cyclobutanecarboxamido)hexanamido)benzyl 8-((6-((S)-1-(chloromethyl)-5-((4-methylpiperazine-1-carbonyl)oxy)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyl)oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate bis(2,2,2-trifluoroacetate) (**CBI-PBD LD2**) and (11aS)-4-((S)-6-amino-2-(1-((5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)carbamoyl)cyclobutanecarboxamido)hexanamido)benzyl 8-((6-((S)-1-(chloromethyl)-5-((4-methylpiperazine-1-carbonyl)oxy)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyl)oxy)-7,11-dimethoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate bis(2,2,2-trifluoroacetate) (**CBI-PBD LD3**)

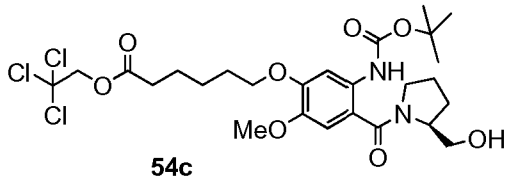
Step A:
Synthesis of Compound 15 (65j in PCT/US2014/042560) :

(S)-4-((S)-2-(allyloxycarbonylamino)-6-(tert-butoxycarbonylamino)hexanamido)benzyl 8-(6-((S)-1-(chloromethyl)-5-(4-methylpiperazine-1-carbonyloxy)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyloxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate

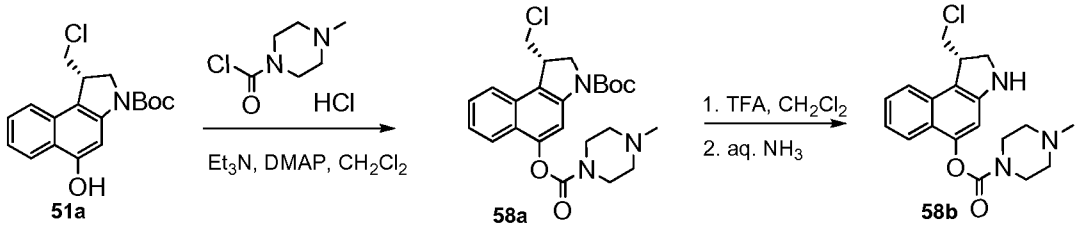
Scheme



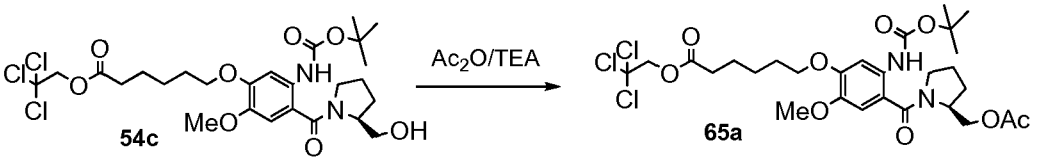
54a 54b



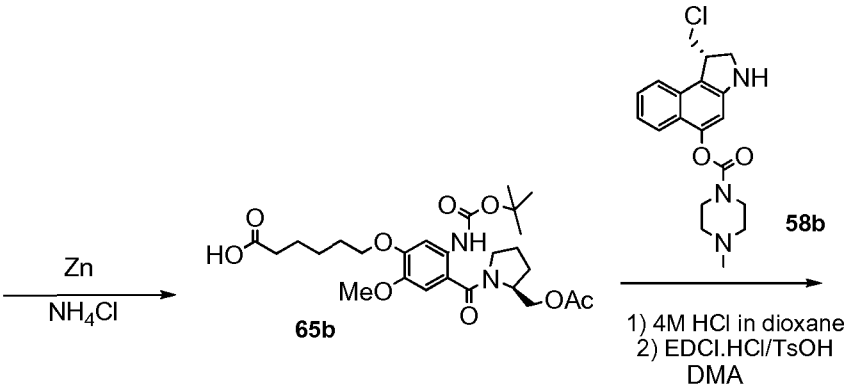
54c



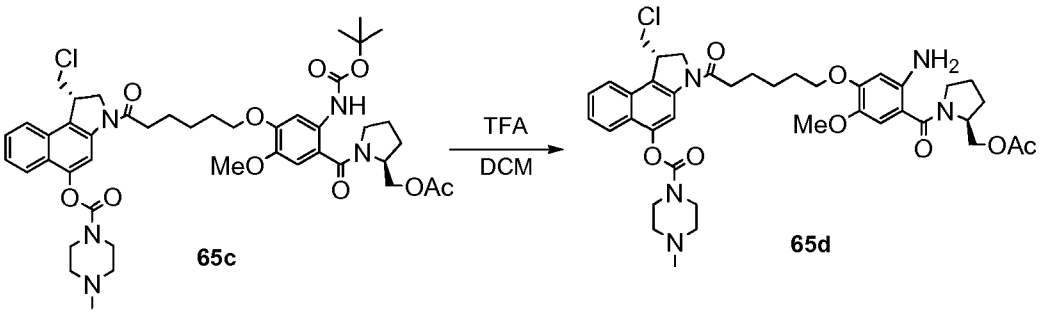
51a 58a 58b



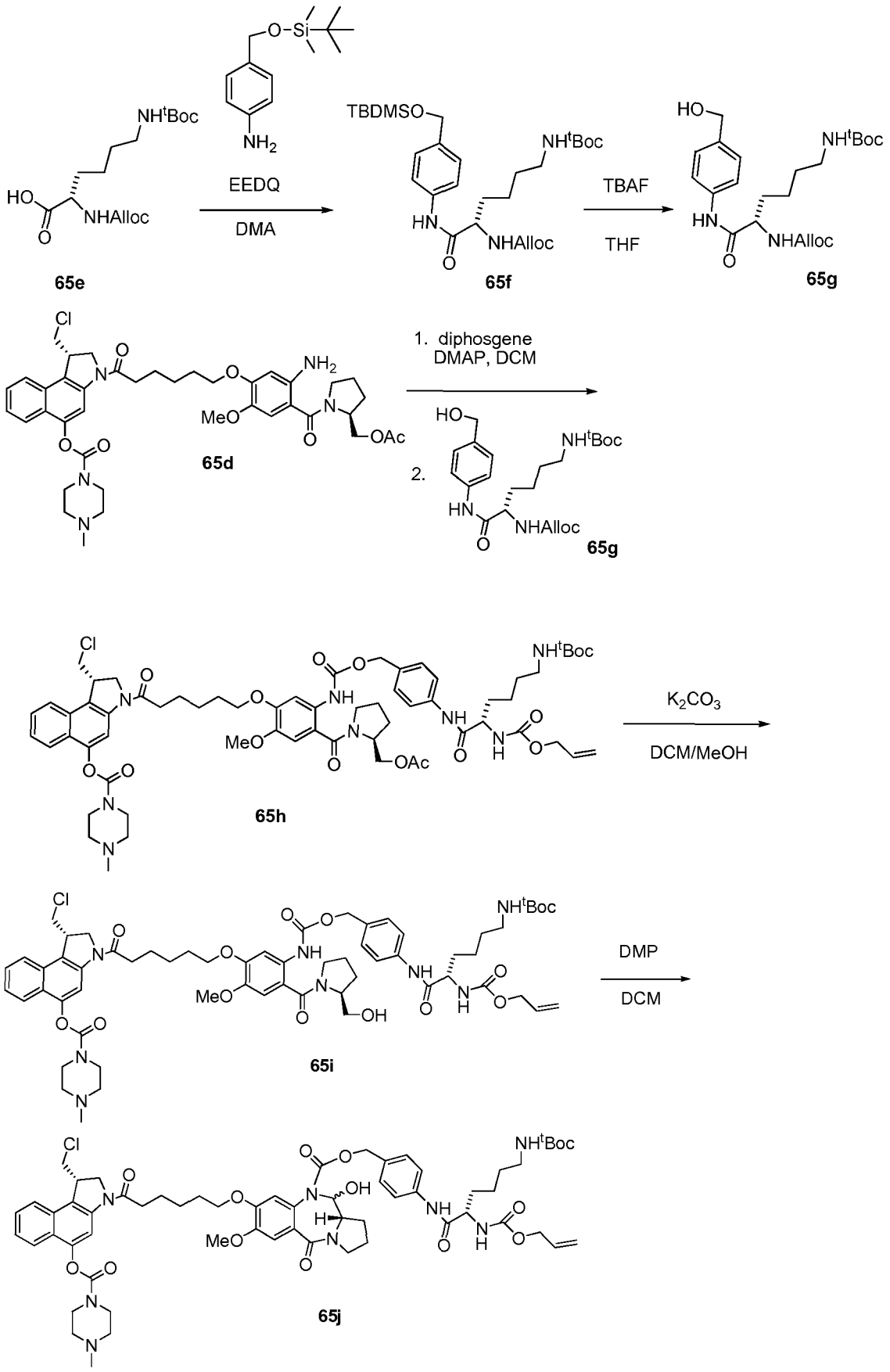
54c 65a



65b



65c 65d



Step 1. (S)-2,2,2-trichloroethyl 6-(5-(tert-butoxycarbonylamino)-4-(2-(hydroxymethyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)hexanoate **54c**

A mixture of (S)-(2-amino-4-hydroxy-5-methoxyphenyl)(2-(hydroxymethyl)pyrrolidin-1-yl)methanone **54a** (7.6 g, 28.6 mmol), prepared by the procedures of Tercel et al (2003) J. Med. Chem 46:2132-2151, and di-t-butyl dicarbonate (12.48 g, 57.2 mmol) in anhydrous THF (140 mL) was stirred under reflux in a nitrogen atmosphere for 18 h. The reaction mixture was cooled to r.t. and 2N NaOH (57.2 mL, 114 mmol) and MeOH (70 mL) were added. The mixture was stirred at r.t. for 6 h. Volatiles were evaporated under reduced pressure at 35-40 °C (bath temperature). Ice water (250 mL) was added and the pH was adjusted to 8-9 at 0 °C. The mixture was stirred with petroleum ether-ethyl acetate (20:1) (2x400 mL) at r.t. for 15 min. The organic layer was separated and discarded. The aqueous layer was extracted with DCM (4x300 mL) and the combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give (S)-tert-butyl 5-hydroxy-2-(2-(hydroxymethyl)pyrrolidine-1-carbonyl)-4-methoxyphenylcarbamate **54b** as a pink-white solid (9.36 g, 89%); mp 154-156 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.51 (s, 1 H), 8.90 (s, 1 H), 7.27 (s, 1 H), 6.91 (s, 1 H), 4.73 (t, J = 5.8 Hz, 1 H), 4.16-4.02 (m, 1 H), 3.73 (s, 3 H), 3.64-3.34 (m, 4 H), 1.99-1.60 (m, 4 H), 1.43 (s, 9 H). Anal. ($C_{18}H_{26}N_2O_6$) Calc: C, 59.00; H, 7.15; N, 7.65. Found: C, 58.94; H, 7.31; N, 7.39.

To a solution of **54b** (2.88 g, 7.87 mmol) and 2,2,2-trichloroethyl 6-bromohexanoate (3.86 g, 11.8 mmol), prepared by the procedures in Tercel et al (2003) J. Med. Chem 46:2132-2151, in dry DMA (7 mL) was added anhydrous K_2CO_3 (2.61 g, 18.9 mmol). The resulting mixture was stirred at r.t. for 68 h. It was poured into ice-water (600 mL) and the product was extracted into ethyl acetate (600 mL). The extracts were washed successively with cold (0 °C) aqueous 2N Na_2CO_3 solution (2x400 mL) and water (400 mL) and then dried ($MgSO_4$). Evaporation of the solvent gave a brown oil which was purifed by SiO_2 column chromatography (DCM-ethyl acetate = 2:1) to give pure (S)-2,2,2-trichloroethyl 6-(5-(tert-butoxycarbonylamino)-4-(2-(hydroxymethyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)hexanoate **54c** (3.62 g, 76%) as a pale yellow foam; mp 36-39 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.90 (s, 1 H), 7.33 (s, 1 H), 6.93 (s, 1 H), 4.89 (s, 2 H), 4.74 (t, J = 5.8 Hz, 1 H), 4.17-4.02 (m, 1 H), 3.94 (t, J = 6.4 Hz, 2 H), 3.73 (s, 3 H), 3.63-3.26 (m, 4 H), 2.55-2.46 (m, 2 H, partially obscured by DMSO peak), 2.00-1.55 (m, 8 H), 1.53-1.36 (m, 11 H). Anal. ($C_{26}H_{37}N_2O_8$) Calc: C, 51.03; H, 6.09; N, 4.58. Found: C, 51.33; H, 6.21; N, 4.35.

Step 2: (S)-1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indol-5-yl 4-methylpiperazine-1-carboxylate **58b**

A mixture of (*S*)-*tert*-butyl 1-(chloromethyl)-5-hydroxy-1*H*-benzo[*e*]indole-3(2*H*)-carboxylate **51a** (3.338 g, 10 mmol), 4-methylpiperazine-1-carbonyl chloride hydrochloride (5.98 g, 30 mmol), Et_3N (3.5 g, 35 mmol) and DMAP (1.34 g, 11 mmol) in CH_2Cl_2 (80 mL) was stirred at room temperature for 2 days. See Figure 12. The mixture was washed with water and the solvent was dried and removed under vacuum, to give (S)-tert-butyl 1-(chloromethyl)-5-(4-methylpiperazine-1-carbonyloxy)-1H-benzo[e]indole-3(2H)-carboxylate **58a** (Boger D.L. et al,

Synthesis, (1999), 1505-1509) in quantitative yield: mp 98 °C; ^{1}H NMR ($CDCl_3$) δ 8.11 (br, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.50 (ddd, J = 8.2, 6.9, 1.1 Hz, 1H), 7.37 (ddd, J = 8.1, 6.9, 1.0 Hz, 1H), 4.34-4.20 (m, 1H), 4.17-4.10 (m, 1H), 4.01-3.98 (m, 1H), 3.94 (dd, J = 9.6, 2.4 Hz, 1H), 3.87-3.80 (br, 2H), 3.68-3.60 (br, 2H), 3.47 (t, J = 10.8 Hz, 1H), 2.57-2.48 (m, 4H), 2.83 (s, 3H), 1.58 (s, 9H); MS (APCI+) m/z 461.2 MH^+. Anal. Calcd for $C_{24}H_{30}ClN_3O_4$: C, 62.7; H, 6.6; N, 9.1. Found: C, 62.5; H, 6.8; N, 9.2%.

A solution of **58a** (2.30 g, 5 mmol) in CH_2Cl_2 (50 mL) was treated with excess trifluoroacetic acid (TFA) at 0 °C for 4 h, and the mixture was neutralized with cold aq. NH_3. Dilution with hexanes resulted in the precipitation of a solid which was collected by filtration, washed with water and hexane, and dried to give (S)-1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indol-5-yl 4-methylpiperazine-1-carboxylate **58b** (1.60 g, 89%): mp 144-147 °C; ^{1}H NMR ($CDCl_3$) δ 7.69 (d, J = 8.4 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.45 (ddd, J = 8.3, 6.9, 1.2 Hz, 1H), 7.25 (ddd, J = 8.4, 6.8, 1.2 Hz, 1H), 6.82 (s, 1H), 5.30 (s, 1H), 4.17-4.05 (m, 2H), 4.03-3.96 (m, 2H), 3.89-3.77 (m, 4H), 3.54 (t, J = 10.9 Hz, 1H), 3.20-2.90 (m, 4H), 2.76 (s, 3H). Anal. Calcd for $C_{19}H_{22}ClN_3O_2$: C, 63.4; H, 6.2; N, 11.7. Found: C, 63.2; H, 6.2; N, 11.5%.

Step 3: (S)-3-(6-(4-((S)-2-(acetoxymethyl)pyrrolidine-1-carbonyl)-5-amino-2-methoxyphenoxy)hexanoyl)-1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indol-5-yl 4-methylpiperazine-1-carboxylate **65d**

To a stirred solution of (S)-2,2,2-trichloroethyl 6-(5-(tert-butoxycarbonylamino)-4-(2-(hydroxymethyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)hexanoate **54c** (1.66 g, 2.71 mmol) in dry DCM (10 mL) at r.t. was added acetic anhydride (1.29 mL, 13.6 mmol) and triethylamine (2.27 mL, 16.3 mmol). See Figure 17. The reaction mixture was stirred for a further 4 h. Dry MeOH (1.5 mL) was added and the mixture was stirred for 30 min. Ethyl acetate (200 mL) was added and the ethyl acetate layer was separated and then washed with water several times. The ethyl acetate solution was dried ($MgSO_4$) and evaporated to give (S)-2,2,2-trichloroethyl 6-(4-(2-(acetoxymethyl)pyrrolidine-1-carbonyl)-5-(tert-butoxycarbonylamino)-2-methoxyphenoxy)hexanoate **65a** (1.8 g, 100%) as a pale yellow glue; ^{1}H NMR [$(CD_3)_2SO$] δ 8.82 (br s, 1 H), 7.27 (s, 1 H), 6.86 (s, 1 H), 4.89 (s, 2 H), 4.39-4.20 (m, 3 H), 3.93 (t, J = 6.4 Hz, 2 H), 3.74 (s, 3 H), 3.50-3.33 (m, 2 H), 2.10-1.94 (m, 4 H), 1.92-1.61 (m, 7 H), 1.53-1.42 (m, 2 H), 1.43 (s, 9 H), 2 H obscured by DMSO peak. HRMS (ESI) *m/z* calc. for $C_{28}H_{39}Cl_3N_2NaO_9$: 675.1613, found: 675.1603 [MNa^+]. Calc. for $C_{28}H_{40}Cl_3N_2O_9$: 653.1794, found: 653.1778 [MH^+].

To a stirred solution of **65a** (1.76 g, 2.69 mmol) in a mixture of acetone (30 mL), water (20 mL), and THF (12 mL) under nitrogen was added Zn (7.06 g, 108 mmol) and NH_4Cl (11.6 g, 216 mmol). The mixture was stirred at r.t. for 23 h. Ethyl acetate (100 mL) was added and the mixture was stirred for 15 min. The organic layer was decanted. The extraction was repeated with

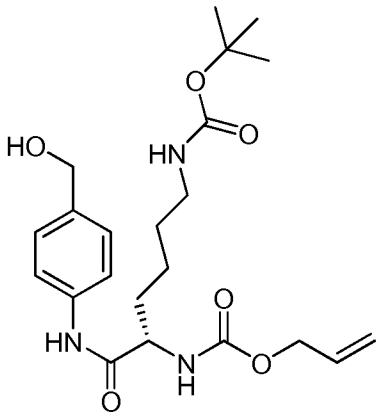
more ethyl acetate (2x100 mL). The combined organic solution was washed with water (2x100 mL), dried ($MgSO_4$), filtered through celite and evaporated to give (S)-6-(4-(2-(acetoxymethyl)pyrrolidine-1-carbonyl)-5-(tert-butoxycarbonylamino)-2-methoxyphenoxy)hexanoic acid **65b** (1.36 g, 96%) as a sticky colorless foam; ^{1}H NMR [$(CD_3)_2SO$] δ 11.49 (very br s, 1 H), 8.83 (s, 1 H), 7.27 (s, 1 H), 6.86 (br s, 1 H), 4.39-4.02 (m, 3 H), 3.93 (t, J = 6.4 Hz, 2 H), 3.74 (s, 3 H), 3.51-3.33 (m, 2 H, partially obscured by water peak), 2.21 (t, J = 7.1 Hz, 2 H), 2.11-1.93 (m, 4 H), 1.90-1.66 (m, 5 H), 1.62-1.50 (m, 2 H), 1.50-1.35 (m, 2 H), 1.43 (s, 9 H). Anal. ($C_{26}H_{38}N_2O_9$.) Calc: C, 59.76; H, 7.33; N, 5.36. Found: C, 59.66; H, 7.49; N, 5.29.

To a stirred solution of **65b** (0.87 g, 2.41 mmol) and (S)-1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indol-5-yl 4-methylpiperazine-1-carboxylate **58b** (1.26 g, 2.41 mmol) in dry DMA (5 mL) at 0 °C under a nitrogen atmosphere was added 4M HCl in p-dioxane (1.21 mL, 4.82 mmol), followed by EDCI.HCl (1.39 g, 7.23 mmol), and anhydrous TsOH (83 mg, 0.48 mmol). The reaction mixture was stirred at 0 °C under nitrogen for 21 hours then partitioned between ethyl acetate (500 mL) and water (500 mL). The ethyl acetate layer was separated and the aqueous layer was further extracted with more ethyl acetate (200 mL). The combined ethyl acetate extracts were washed successively with water (200 mL), saturated $NaHCO_3$ solution (2x200 mL) and water (200 mL). The ethyl acetate layer was dried and evaporated to give (S)-3-(6-(4-((S)-2-(acetoxymethyl)pyrrolidine-1-carbonyl)-5-(tert-butoxycarbonylamino)-2-methoxyphenoxy)hexanoyl)-1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indol-5-yl 4-methylpiperazine-1-carboxylate **65c** (1.66 g, 80%) as a beige solid-foam; mp 84-87 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.84 (br s, 1 H), 8.21 (s, 1 H), 7.95 (d, J = 8.3 Hz, 1 H), 7.81 (d, J = 8.3 Hz, 1 H), 7.58 (br t, J = 7.7, 1 H), 7.46 (br t, J = 8.1 Hz, 1 H), 7.29 (s, 1 H), 6.86 (s, 1 H), 4.40 (t, J = 10.0 Hz, 1 H), 4.36-3.86 (m, 10 H), 3.83-3.74 (m, 1 H), 3.73 (s, 3 H), 3.54-3.36 (m, 4 H), 2.67-2.34 (m, 6 H, partially obscured by DMSO peak), 2.26 (s, 3 H), 2.02 (br s, 3 H), 1.93-1.62 (m, 8 H), 1.60-1.47 (m, 2 H), 1.42 (s, 9 H). Anal. ($C_{45}H_{58}ClN_5O_{10}\cdot 1\frac{1}{2}H_2O$) Calc: C, 60.63; H, 6.90; N, 7.86. Found: C, 60.39; H, 6.66; N, 8.08.

To a stirred solution of **65c** (2.17 g, 2.51 mmol) in DCM (20 mL) at 0 °C under a nitrogen atmosphere was added TFA (20 mL). After addition, the mixture was stirred further at this temperature for 2.5 h. The mixture was poured into a cold (0 °C) mixture of $NaHCO_3$ (50 g), water (700 mL), and DCM (500 mL) and stirred for 15 min. (pH ca. 8). The DCM layer was separated and washed with more aqueous $NaHCO_3$ (200 mL) and water (200 mL) and then dried ($MgSO_4$). The solvent was evaporated to give (S)-3-(6-(4-((S)-2-(acetoxymethyl)pyrrolidine-1-carbonyl)-5-amino-2-methoxyphenoxy)hexanoyl)-1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indol-5-yl 4-methylpiperazine-1-carboxylate **65d** as a pale brown solid-foam (1.76 g, 92%); mp 62 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.21 (s, 1 H), 7.95 (d, J = 8.3 Hz, 1 H), 7.81 (d, J = 8.3 Hz, 1 H), 7.57 (br t, J = 7.6 Hz, 1 H), 7.46 (br t, J = 7.2 Hz, 1 H), 6.67 (s, 1 H), 6.37 (s, 1 H), 5.09 (s, 2 H), 4.41 (t, J =

9.7 Hz, 1 H), 4.36-4.20 (m, 3 H), 4.17-4.00 (m, 3 H), 3.97-3.86 (m, 3 H), 3.81-3.70 (m, 2 H), 3.63 (s, 3 H), 3.54-3.32 (m, 5 H), 2.66-2.34 (m, 6 H, partially obscured by DMSO peak), 2.26 (s, 3 H), 2.08-1.96 (m, 1 H), 2.10 (s, 3 H), 1.93-1.63 (m, 7 H), 1.57-1.45 (m, 2 H). Anal. ($C_{40}H_{50}ClN_5O_8 \cdot ½H_2O$) Calc: C, 62.13; H, 6.65; N, 9.06. Found: C, 62.12; H, 6.76; N, 8.77.

Step 4: 65g



A mixture of (S)-2-(allyloxycarbonylamino)-6-(tert-butoxycarbonylamino)hexanoic acid **65e** (3.30 g, 10.0 mmol) and EEDQ (3.71 g, 15.0 mmol) in dry DMA (10 mL) was stirred at r.t. under nitrogen for 15 min. See Figure 18. To this preformed mixture was added a solution of 4-((tert-butyldimethylsilyloxy)methyl)aniline (prepared from the corresponding p-nitrobenzyl alcohol and TBDMSCl in DMF; followed by reduction using Zn/NH_4Cl) (2.37 g, 10.0 mmol) in dry DMA (3 mL). The final reaction mixture was stirred further at r.t. under a nitrogen atmosphere for 23 h. The mixture was partitioned between ethyl acetate (500 mL) and water (500 mL). The ethyl acetate layer was separated and washed successively with saturated $NaHCO_3$ (2x300 mL) and water (300 mL) and then dried ($MgSO_4$). Evaporation of the solvent gave an orange oil which was purified by a silica column chromatography (petroleum ether-ethyl acetate gradient from 10-35%) to afford the TBDMS-protected lysine **65f** (4.87 g, 89%) as a sticky beige solid-foam; ^{1}H NMR [$(CD_3)_2SO$] δ 9.97 (s, 1 H), 7.55 (d, J = 8.50 Hz, 2 H), 7.44 (d, J = 7.8 Hz, 1 H), 7.21 (d, J = 8.5 Hz, 2 H), 6.75 (t, J = 5.3 Hz, 1 H), 5.99-5.82 (m, 1 H), 5.28 (br d, J = 17.2 Hz, 1 H), 5.17 (br d, J = 10.5 Hz, 1 H), 4.64 (s, 2 H), 4.46 (d, J = 5.2 Hz, 2 H), 4.12-4.02 (m, 1 H), 2.93-2.83 (m, 2 H), 1.70-1.52 (m, 2 H), 1.46-1.20 (m, 4 H), 1.35 (s, 9 H), 0.89 (s, 9 H), 0.06 (s, 6 H). HRMS (ESI) *m/z* calc. for $C_{28}H_{47}N_3NaO_6Si$: 572.3126, found: 572.3136 [MNa^+].

To a stirred solution of **65f** (4.81 g, 8.75 mmol) in THF (30 mL) at r.t. was added a 1M solution of tetrabutylammonium fluoride in THF (17.5 mL, 17.5 mmol). After addition, the mixture was stirred at this temperature for a further 2.5 h. Aqueous NH_4Cl (300 mL) was added and product was extracted into ethyl acetate (500 mL). The ethyl acetate was washed with water (2x100 mL) and dried ($MgSO_4$). The solvent was evaporated to give benzyl alcohol lysine **65g** (3.81 g, 100%) as a beige solid; mp 101-103 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.94 (s, 1 H), 7.52 (d, J =

8.4 Hz, 2 H), 7.44 (d, *J* = 7.8 Hz, 1 H), 7.23 (d, *J* = 8.4 Hz, 2 H), 6.76 (t, *J* = 5.4 Hz, 1 H), 5.97-5.84 (m, 1 H), 5.29 (br d, *J* = 17.2 Hz, 1 H), 5.17 (br d, *J* = 10.4 Hz, 1 H), 5.08 (t, *J* = 5.7 Hz, 1 H), 4.47 (d, *J* = 5.3 Hz, 2 H), 4.43 (d, *J* = 5.7 Hz, 2 H), 4.13-4.03 (m, 1 H), 2.96-2.82 (m, 2 H), 1.72-1.52 (m, 2 H), 1.46-1.20 (m, 4 H), 1.36 (s, 9 H). HRMS (ESI) *m/z* calc. for $C_{22}H_{33}N_3NaO_6$: 458.2262, found: 458.2272 [MNa^+]; calc. for $C_{22}H_{33}N_3KO_6$: 474.2001, found: 474.1998 [MK^+].

Step 5: (S)-3-(6-(4-((S)-2-(acetoxymethyl)pyrrolidine-1-carbonyl)-5-((4-((S)-2-(allyloxycarbonylamino)-6-(tert-butoxycarbonylamino)hexanamido)benzyloxy)carbonylamino)-2-methoxyphenoxy)hexanoyl)-1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indol-5-yl 4-methylpiperazine-1-carboxylate **65h**

To a stirred solution of **65d** (764 mg, 1.00 mmol) and DMAP (367 mg, 3.00 mmol) in dry DCM (15 mL) at r.t. under nitrogen was added a solution of diphosgene in dry DCM (0.05 mmol per mL, 12 mL, 0.60 mmol) and the mixture was stirred for a further 20 min. See Figure 19. To this mixture was added a solution of **65g** (3.97 g, 9.13 mmol) in dry DCM (80 mL). The final reaction mixture was stirred further at r.t. under a nitrogen atmosphere for 48 h. The mixture was partitioned between ethyl acetate (500 mL) and water (300 mL). The ethyl acetate layer was separated and the aqueous layer was further extracted with ethyl acetate (2x200 mL). The combined ethyl acetate solution was washed with more water (2x200 mL) and dried ($MgSO_4$). Evaporation of the solvent at 30 °C (bath temperature) gave an orange oil which was purified by silica column chromatography (ethyl acetate-MeOH = 10:1) to afford (S)-3-(6-(4-((S)-2-(acetoxymethyl)pyrrolidine-1-carbonyl)-5-((4-((S)-2-(allyloxycarbonylamino)-6-(tert-butoxycarbonylamino)hexanamido)benzyloxy)carbonylamino)-2-methoxyphenoxy)hexanoyl)-1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indol-5-yl 4-methylpiperazine-1-carboxylate **65h** (1.04 g, 85%) as a pale orange solid; mp 90-93 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.04 (s, 1 H), 9.10 (br s, 1 H), 8.21 (s, 1 H), 7.95 (d, *J* = 8.3 Hz, 1 H), 7.81 (d, *J* = 8.3 Hz, 1 H), 7.63-7.53 (m, 3 H), 7.51-7.42 (m, 2 H), 7.32 (d, *J* = 8.5 Hz, 2 H), 7.21 (br s, 1 H), 6.85 (br s, 1 H), 6.79-6.72 (m, 1 H), 5.97-5.83 (m, 1 H), 5.29 (br d, *J* = 17.2 Hz, 1 H), 5.17 (br d, *J* = 10.4 Hz, 1 H), 5.08-4.96 (m, 2 H), 4.52-4.37 (m, 3 H), 4.37-3.85 (m, 10 H), 3.83-3.66 (m, 2 H), 3.74 (s, 3 H), 3.54-3.41 (m, 2 H), 3.41-3.23 (m, 2 H, partially obscured by water peak), 2.95-2.83 (m, 2 H), 2.66-2.34 (m, 6 H, partially obscured by DMSO peak), 2.25 (s, 3 H), 2.07-1.92 (m, 4 H), 1.87-1.45 (m, 11 H), 1.45-1.20 (m, 4 H), 1.35 (s, 9 H). HRMS (ESI) *m/z* calc. for $C_{63}H_{82}ClN_8O_{15}$: 1225.5583, found: 1225.5557 [MH^+]; calc. for $C_{63}H_{81}ClN_8NaO_{15}$: 1247.5402, found: 1247.5401 [MNa^+]; calc. for $C_{63}H_{81}ClKN_8O_{15}$: 1263.5142, found: 1263.5141 [MK^+].

A mixture of **65h** (1.01 g, 0.824 mmol) and K_2CO_3 (1.14 g, 8.24 mmol) in DCM (20 mL) and MeOH (10 mL) was stirred at r.t. for 1 hour and 40 min. The mixture was diluted with DCM (200 mL) and stirred with ice-water (200 mL) for 10 min. The DCM layer was separated and the aqueous layer was further extracted with DCM (2x100 mL). The combined DCM solution was washed with more water (200 mL) and dried ($MgSO_4$). Evaporation of solvent at 25 °C (bath

temperature) gave (S)-3-(6-(5-((4-((S)-2-(allyloxycarbonylamino)-6-(tert-butoxycarbonylamino)hexanamido)benzyloxy)carbonylamino)-4-((S)-2-(hydroxymethyl)pyrrolidine-1-carbonyl)-2-methoxyphenoxy)hexanoyl)-1-(chloromethyl)-2,3-dihydro-1H-benzo[e]indol-5-yl 4-methylpiperazine-1-carboxylate **65i** (0.94 g, 96%) as a beige solid; mp 104-107 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.04 (s, 1 H), 9.17 (br s, 1 H), 8.21 (s, 1 H), 7.95 (d, *J* = 8.4 Hz, 1 H), 7.80 (d, *J* = 8.3 Hz, 1 H), 7.63-7.53 (m, 3 H), 7.51-7.42 (m, 2 H), 7.38-7.21 (m, 3 H), 6.93 (s, 1 H), 5.32 (t, *J* = 5.4 Hz, 1 H), 5.98-5.83 (m, 1 H), 5.30 (br d, *J* = 17.2 Hz, 1 H), 5.17 (br d, *J* = 11.7 Hz, 1 H), 5.03 (s, 2 H), 4.73 (t, *J* = 5.7 Hz, 1 H), 4.52-4.36 (m, 3 H), 4.36-4.17 (m, 2 H), 4.17-3.85 (m, 6 H), 3.83-3.66 (m, 2 H), 3.73 (s, 3 H), 3.61-3.40 (m, 4 H), 3.40-3.20 (m, 2 H, partially obscured by water peak), 2.94-2.83 (m, 2 H), 2.67-2.34 (m, 6 H, partially obscured by DMSO peak), 2.25 (s, 3 H), 1.96-1.45 (m, 12 H), 1.45-1.20 (m, 4 H), 1.35 (s, 9 H). HRMS (ESI) *m/z* calc. for $C_{61}H_{80}ClN_8O_{14}$: 1183.5477, found: 1183.5445 [MH^+]; calc. for $C_{61}H_{79}ClN_8NaO_{14}$: 1205.5296, found: 1205.5256 [MNa^+]; calc. for $C_{61}H_{79}ClKN_8O_{14}$: 1221.5036, found: 1221.5026 [MK^+].

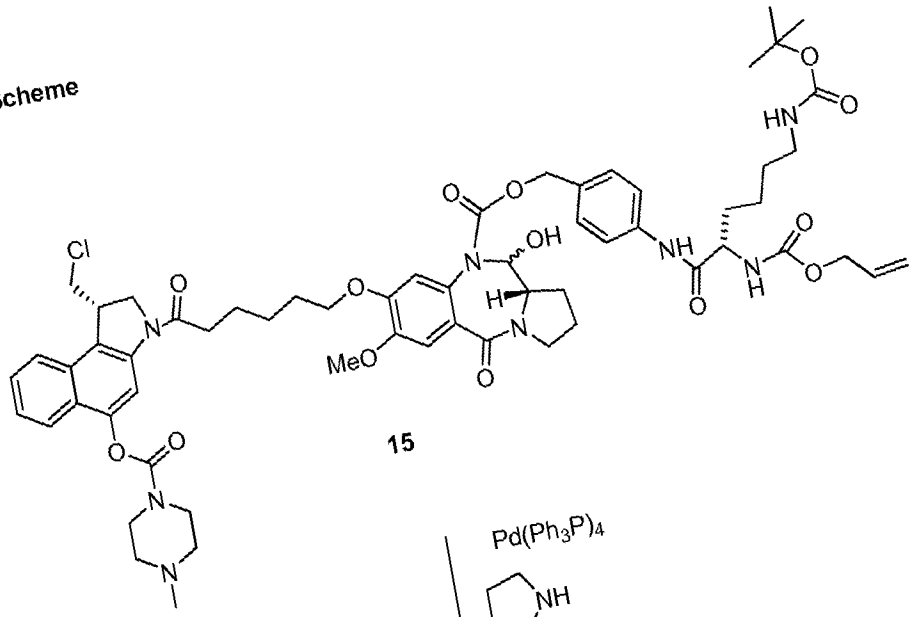
To a stirred solution of **65i** (0.92 g, 0.78 mmol) in dry DCM (20 mL) at 0 °C was added Dess-Martin periodinane (DMP, 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one, CAS Reg. No. 87413-09-0, 492 mg, 1.16 mmol) portionwise (over 8 min). After addition was complete the reaction mixture was stirred further at 0 °C for 2 h, then at r.t. for 45 h. The mixture was diluted with DCM (100 mL) and stirred with 10% $Na_2S_2O_3$ (100 mL) at r.t. for 10 min. The resulting mixture was partitioned between DCM (400 mL) and saturated $NaHCO_3$ solution (400 mL). The DCM layer was separated and the aqueous layer was further extracted with DCM (2x100 mL). The combined DCM solution was further washed with saturated $NaHCO_3$ solution (200 mL) and water (200 mL) and then dried ($MgSO_4$). Evaporation of solvent at 25 °C (bath temperature) gave a pale brown solid which was purifed by SiO_2 column chromatography (DCM-ethyl acetate-MeOH = 15:15:1, gradient to 15:15:3) to give (S)-4-((S)-2-(allyloxycarbonylamino)-6-(tert-butoxycarbonylamino)hexanamido)benzyl 8-(6-((S)-1-(chloromethyl)-5-(4-methylpiperazine-1-carbonyloxy)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyloxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate **65j** (0.64g, 70%) as a pale yellow solid; mp 137 °C (dec.); ^{1}H NMR [$(CD_3)_2SO$] δ 10.02 (s, 1 H), 8.21 (s, 1 H), 7.95 (d, *J* = 8.4 Hz, 1 H), 7.80 (d, *J* = 8.3 Hz, 1 H), 7.65-7.38 (m, 5 H), 7.18 (d, *J* = 7.0 Hz, 2 H), 7.03 (s, 1 H), 6.82-6.63 (m, 2 H), 6.49 (poorly resolved d, *J* = 4.7 Hz, exchangeable with D_2O, 1 H), 5.96-5.82 (m, 1 H), 5.46 (poorly resolved dd, *J* = 9.8, 4.7 Hz, became a d after D_2O, *J* = 10.1 Hz, 1 H), 5.27 (br d, *J* = 17.1 Hz, 1 H), 5.21-5.10 (m, 2 H), 4.81 (br d, *J* = 12.3 Hz, 1 H), 4.51-4.17 (m, 5 H), 4.13-3.84 (m, 4 H), 3.84-3.67 (m, 2 H), 3.77 (s, 3 H), 3.55-3.20 (m, 6 H, partially obscured by water peak), 2.66-2.30 (m, 6 H, partially obscured by DMSO peak), 2.26 (s, 3 H), 2.10-1.20 (m, 16 H), 1.35 (s, 9 H). HRMS (ESI) *m/z* calc. for $C_{61}H_{78}ClN_8O_{14}$:1181.5321, found:

1181.5286 [MH^+]; calc. for $C_{61}H_{77}ClN_8NaO_{14}$: 1203.5140, found: 1203.5130 [MNa^+]; calc. for $C_{61}H_{77}ClKN_8O_{14}$: 1219.4879, found: 1219.4861 [MK^+].

Step B:
(11aS)-4-((S)-6-amino-2-(1-((5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)carbamoyl)cyclobutanecarboxamido)hexanamido)benzyl 8-((6-((S)-1-(chloromethyl)-5-((4-methylpiperazine-1-carbonyl)oxy)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyl)oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate bis(2,2,2-trifluoroacetate) (**CBI-PBD LD2**) and (11aS)-4-((S)-6-amino-2-(1-((5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)carbamoyl)cyclobutanecarboxamido)hexanamido)benzyl 8-((6-((S)-1-(chloromethyl)-5-((4-methylpiperazine-1-carbonyl)oxy)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyl)oxy)-7,11-dimethoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate bis(2,2,2-trifluoroacetate) (**CBI-PBD LD3**)

Synthesis of CBI-PBD LD2 and CBI-PBD LD3

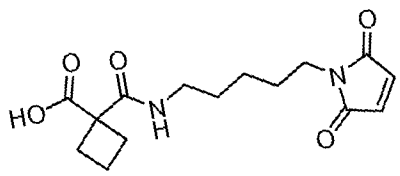
Synthetic Scheme



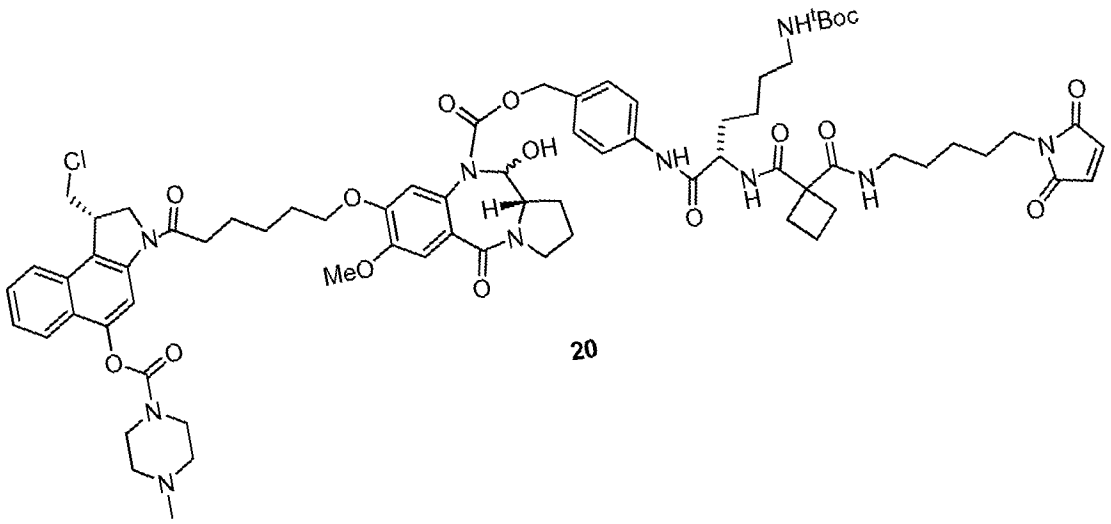
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$Pd(Ph_3P)_4$

DCM



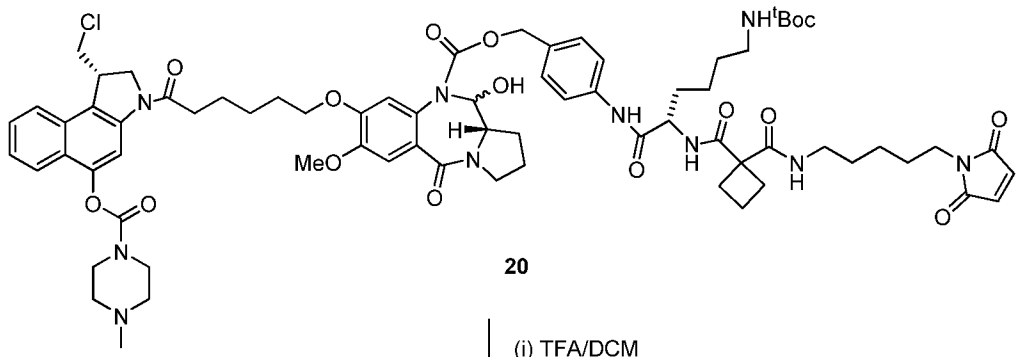
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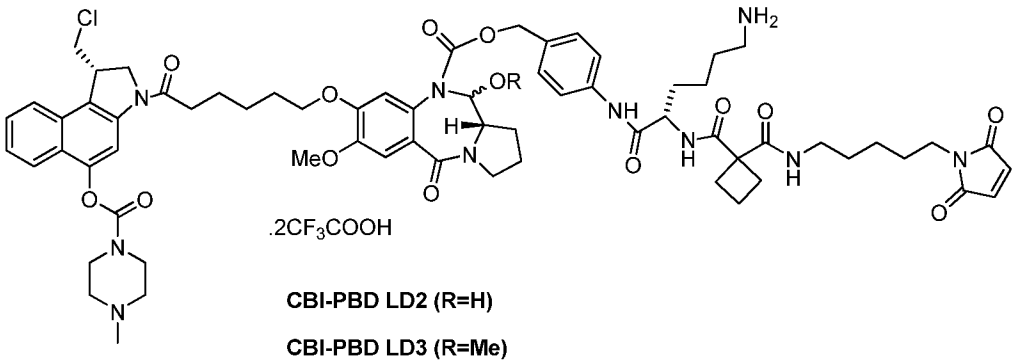
Synthesis of CBI-PBD LD2 and CBI-PBD LD3

Synthetic Scheme (cont'd)



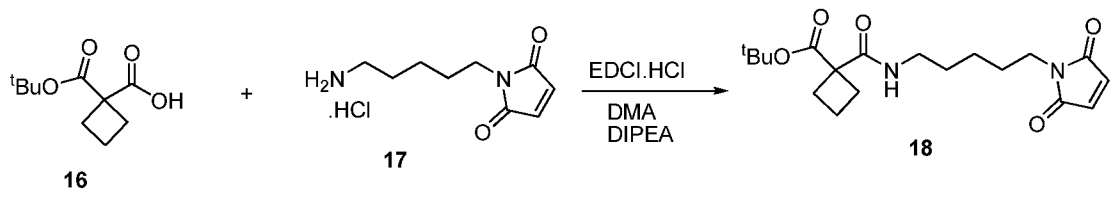
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(i) TFA/DCM
(ii) MeOH

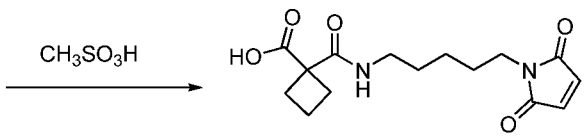


CBI-PBD LD2 (R=H)

CBI-PBD LD3 (R=Me)



16 **17** **18**



19

To a mixture of 1-(tert-butoxycarbonyl)cyclobutanecarboxylic acid (**16**) (200 mg, 1.00 mmol) (PCT Int. Appl. 2002, WO 2002076968 A1), 1-(5-aminopentyl)-1H-pyrrole-2,5-dione hydrochloride (**17**) (218 mg, 1.00 mmol) (J. Med. Chem. 2013, 56, 7890-7901), EDCI.HCl (576

mg, 3.00 mmol) and TsOH (35 mg, 0.20 mmol) was added DMA (2 mL). The mixture was stirred at 20 °C for 15 min and DIPEA (0.17 mL, 1.00 mmol) was added. The reaction mixture was stirred further for 20 h and partitioned between EtOAc (200 mL) and water (100 mL). The EtOAc layer was separated and washed successively with cold 1N HCl (100 mL), saturated $NaHCO_3$ (100 mL), and water (100 mL), and then dried ($MgSO_4$). Evaporation of solvent gave tert-butyl 1-((5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)carbamoyl)cyclobutanecarboxylate (**18**) (290 mg, 80%) as a pale yellow solid, mp 63-65 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.58 (t, J = 5.6 Hz, 1 H), 7.00 (s, 2 H), 3.37 (t, J = 7.0 Hz, 2 H), 3.03 (q, J = 6.0 Hz, 2 H), 2.40-2.23 (m, 4 H), 1.85-1.64 (m, 2 H), 1.55-1.32 (m, 4 H), 1.38 (s, 9 H), 1.26-1.01 (m, 2 H). HRMS (ESI) m/z calc. for $C_{19}H_{29}N_2O_5$: 365.2071, found: 365.2071 [MH^+]; calc. for $C_{19}H_{28}N_2NaO_5$: 387.1890, found: 387.1898 [MNa^+]; calc. for $C_{19}H_{28}KN_2O_5$: 403.1630, found: 403.1629 [MK^+].

To a stirred solution of **18** (794 mg, 2.18 mmol) in DCM (50 mL) was added methanesulfonic acid (2.83 mL, 43.6 mmol). The cloudy mixture was stirred at 20 °C for 2h 30 min. The mixture was diluted with DCM (200 mL) and washed with water (2×50 mL). The DCM solution was dried ($MgSO_4$) and evaporated at 25 °C (bath temperature) to give 1-((5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)carbamoyl)cyclobutanecarboxylic acid (**19**) (636 mg, 95%) as a pale yellow solid, mp 100-102 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 12.46 (br s, 1 H), 7.63 (t, J = 5.4 Hz, 1 H), 7.00 (s, 2 H), 3.37 (t, J = 7.0 Hz, 2 H), 3.02 (q, J = 5.9 Hz, 2 H), 2.42-2.28 (m, 4 H), 1.89-1.63 (m, 2 H), 1.55-1.32 (m, 4 H), 1.29-1.11 (m, 2 H). Anal. ($C_{15}H_{20}N_2O_5$) Calc: C, 58.43; H, 6.54; N, 9.09. Found: C, 58.54; H, 6.39; 8.84.

To a stirred homogeneous solution of (11aS)-4-((S)-2-(((allyloxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanamido)benzyl 8-((6-((S)-1-(chloromethyl)-5-((4-methylpiperazine-1-carbonyl)oxy)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyl)oxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate (**15**) (177 mg, 0.15 mmol) (refer filed patent: GENENLAW-#446508) in dry DCM (2 mL) at 20 °C and under a nitrogen atmosphere was added pyrrolidine (0.122 mL, 1.50 mmol), followed by $Pd(Ph_3P)_4$ (4.28 mg, 9.8% Pd). After addition the reaction mixture was stirred further at 20 °C (N_2) for 25 min. The mixture was diluted with petroleum ether (50 mL) and stirred at 20 °C (N_2) for 10 min. Solvents were decanted and the procedure was repeated with DCM-petroleum ether (1:10) (2×30 mL). The solid left behind was dissolved in DCM (100 mL) and washed with water (50 mL), brine (50 mL), and then dried ($MgSO_4$). Evaporation of solvent at 25 °C (bath temperature) gave the free amine as a beige solid (140 mg, 85%). Some of this material (110 mg, 0.10 mmol) was treated with a pre-formed (at 20 °C for 10 min) mixture of **19** (34 mg, 0.11 mmol), EDCI.HCl (58 mg, 0.30 mmol), and TsOH (3.4 mg, 0.02 mmol) in dry DMA (1 mL) at 20

°C (under a nitrogen atmosphere). After 10 min DIPEA (0.02 mL, 0.10 mmol) was added and the reaction mixture stirred further for 22 h. The mixture was partitioned between EtOAc (200 mL) and water (100 mL). The EtOAc layer was separated and washed further with saturated $NaHCO_3$ (100 mL), water (100 mL), and then dried ($MgSO_4$). Evaporation of solvent at 25 °C (bath temperature) gave a crude product which was purified by SiO_2 column chromatography (DCM-EtOAc-MeOH = 20:10:3) to give **20** (92 mg, 66%) as a pale yellow solid; mp 106-109 °C; $[\alpha]_D$ +43.1° (*c* 0.418, $CHCl_3$); ^1H NMR [$(CD_3)_2SO$] δ 10.06 (s, exchangeable with D_2O, 1 H), 8.21 (s, 1 H), 7.95 (d, *J* = 8.4 Hz, 1 H), 7.85-7.77 (m, 2 H, reduced to 1 H after D_2O), 7.75 (d, *J* = 8.0 Hz, exchangeable with D_2O, 1 H), 7.65-7.51 (m, 3 H), 7.46 (t, *J* = 7.6 Hz, 1 H), 7.24-7.11 (m, 2 H), 7.03 (s, 1 H), 6.96 (s, 2 H), 6.79-6.66 (m, 2 H, reduced to 1 H after D_2O), 6.54-6.43 (m, exchangeable with D_2O, 1 H), 5.52-5.41 (m, but d after D_2O with *J* = 9.5 Hz, 1 H), 5.15 (d, *J* = 12.0 Hz, 1 H), 4.81 (d, *J* = 12.0 Hz, 1 H), 4.47-4.18 (m, 5 H), 4.08-3.98 (m, 1 H), 3.97-3.86 (m, 2 H), 3.84-3.67 (m, 5 H), 3.56-3.21 (m, 8 H, partially obscured by water peak), 3.12-3.02 (m, but t after D_2O with *J* = 6.2 Hz, 2 H), 2.92-2.80 (m, 2 H), 2.65-2.33 (m, 10 H, partially obscured by DMSO peak), 2.25 (s, 3 H), 2.15-1.12 (m, 23 H), 1.33 (s, 9 H). HRMS (ESI) m/z calc. for $C_{72}H_{92}ClN_{10}O_{16}$: 1387.6376, found: 1387.6319 [MH^+]; calc. for $C_{72}H_{91}ClN_{10}NaO_{16}$: 1409.6195, found: 1409.6146 [MNa^+]; calc. for $C_{72}H_{91}ClKN_{10}O_{16}$: 1425.5935, found: 1425.5875 [MK^+].

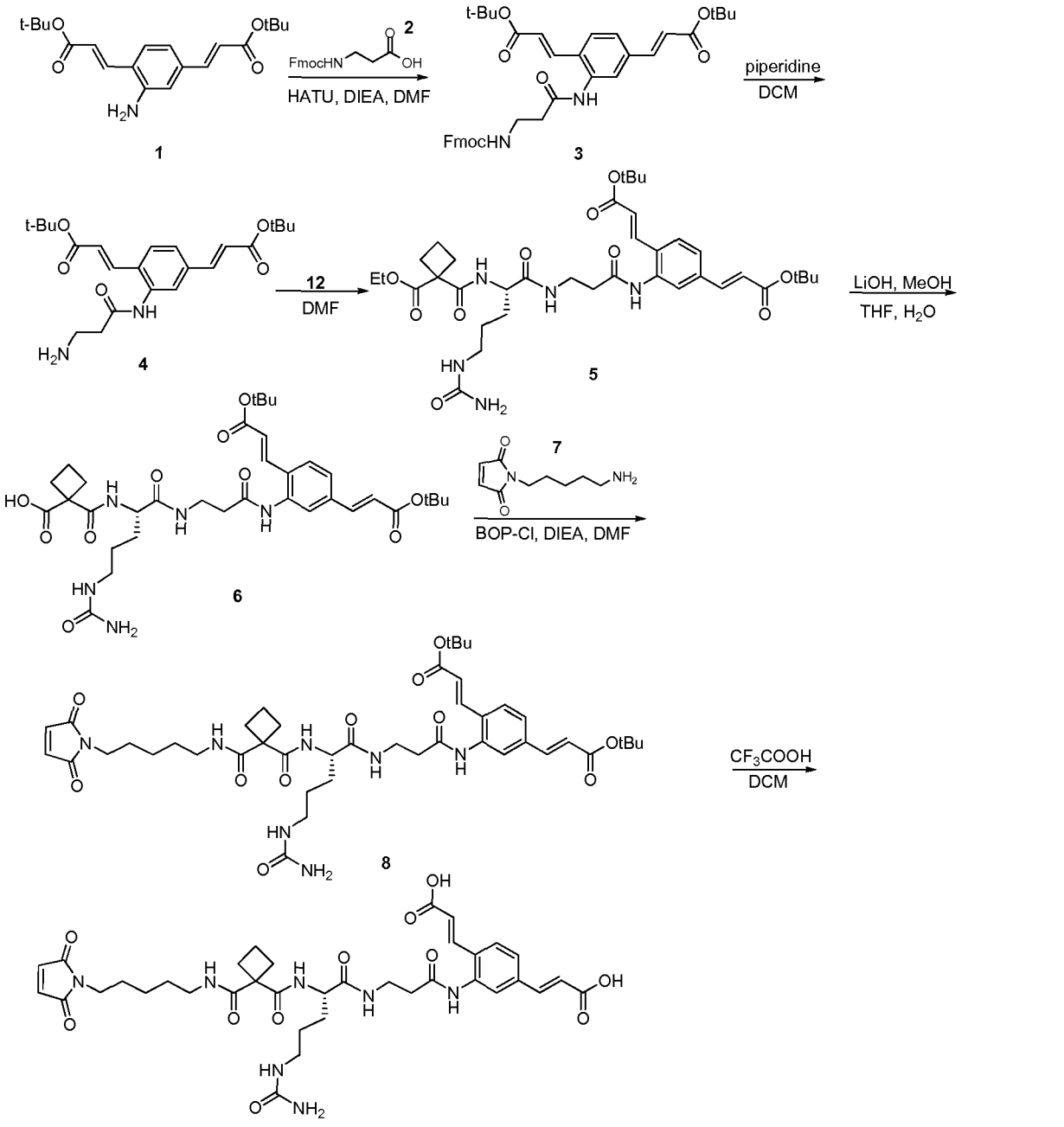
To a stirred solution of **20** (79 mg, 0.057 mmol) in DCM (5 mL) at 20 °C and under nitrogen was added TFA (5 mL). After addition the mixture was stirred further at this temperature for 20 min. Petroleum ether (100 mL) was added and the mixture was stirred at 20 °C for 30 min. Solvent was removed and the oil left behind was stirred with more EtOAc-petroleum ether (1:10) (3×50 mL). The residual oil was dissolved in MeOH and the solution was evaporated to give a glassy solid (76 mg) which was purified by preparative HPLC [SynergiMaxRP column, 4μ, 21×250 mm; water-TFA (pH = 2.56; 95% to 55%)/10% H_2O in CH_3CN (5% to 45%); gradient time 30 min; flow rate: 12 mL/min] to give (i) **CBI-PBD LD2** (26.2 mg, 30%) as a pale amber solid; HPLC purity: 98.5%; $[\alpha]_D$ +30.0° (*c* 0.233, MeOH); ^1H NMR [$(CD_3)_2SO$] δ 10.08 (s, exchangeable with D_2O, 1 H), 9.82 (br s, exchangeable with D_2O, 1 H), 8.27 (s, 1 H), 7.97 (d, *J* = 8.4 Hz, 1 H), 7.92-7.79 (m, 3 H, reduced to 1 H after D_2O), 7.67-7.52 (m, 5 H, reduced to 3 H after D_2O), 7.46 (t, *J* = 8.0 Hz, 1 H), 7.21-7.11 (m, 2 H), 7.04 (s, 1 H), 6.97 (s, 2 H), 6.75 (s, 1 H), 6.50 (br s, 1 H, exchangeable with D_2O), 5.45 (br d, but d after D_2O with *J* = 9.0 Hz, 1 H), 5.10 (br d, *J* = 13 Hz, 1 H), 4.87 (br d, *J* = 12 Hz, 1 H), 4.55-4.10 (m, 5 H), 4.50 (dd, *J* = 11.4, 3.4 Hz, 1 H), 4.01-3.88 (m, 2 H), 3.76 (s, 3 H), 3.39-3.22 (m, 4 H), 3.14-3.03 (m, 2 H), 2.89 (s, 3 H), 2.81-2.69 (m, 2 H), 2.65-2.55 (m, 1 H), 2.47-2.34 (m, 4 H), 2.07-1.13 (m, 23 H), remaining 12H obscured by water and DMSO peaks. HRMS (ESI) m/z calc. for $C_{67}H_{84}ClN_{10}O_{14}$:1287.5852, found: 1287.5845 [MH^+]; calc. for $C_{67}H_{83}ClN_{10}NaO_{14}$: 1309.5671, found: 1309.5654 [MNa^+].

Also purified was (ii) **CBI-PBD LD3** (11.2 mg, 15%) as a beige solid; HPLC purity: 92.6%; ^{1}H NMR [$(CD_3)_2SO$] δ 10.07 (s, exchangeable with D_2O, 1 H), 9.90 (br s, exchangeable with D_2O, 1 H), 8.27 (s, 1 H), 7.96 (d, J = 8.5 Hz, 1 H), 7.93-7.76 (m, 3 H, reduced to 1 H after D_2O), 7.73-7.51 (m, 5 H, reduced to 3 H after D_2O), 7.46 (t, J = 8.2 Hz, 1 H), 7.16-7.08 (m, 2 H), 7.04 (s, 1 H), 6.97 (s, 2 H), 6.85 (s, 1 H), 5.33 (br d, J = 7.8 Hz, 1 H), 5.07 (br d, J = 12 Hz, 1 H), 4.93 (br d, J = 13 Hz, 1 H), 4.56-4.10 (m, 5 H), 3.77 (s, 3 H), 3.62-3.23 (m, 6 H), 3.44 (s, 3 H), 3.15-3.01 (m, 2 H), 2.89 (s, 3 H), 2.82-2.69 (m, 2 H), 2.66-2.55 (m, 1 H), 2.46-2.34 (m, 4 H), 2.11-1.12 (m, 23 H), remaining 13H obscured by water and DMSO peaks. HRMS (ESI) m/z calc. for $C_{68}H_{86}ClN_{10}O_{14}$: 1301.6008, found: 1301.5952 [MH^+]; calc. for $C_{68}H_{85}ClN_{10}NaO_{14}$: 1323.5827, found: 1323.5778 [MNa^+].

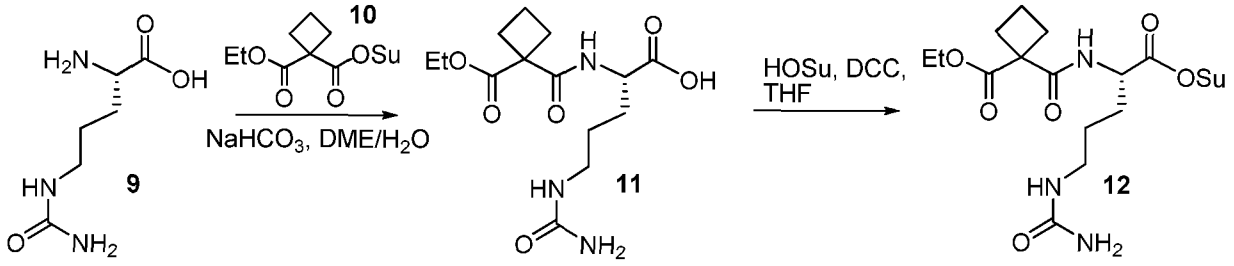
Preparation of

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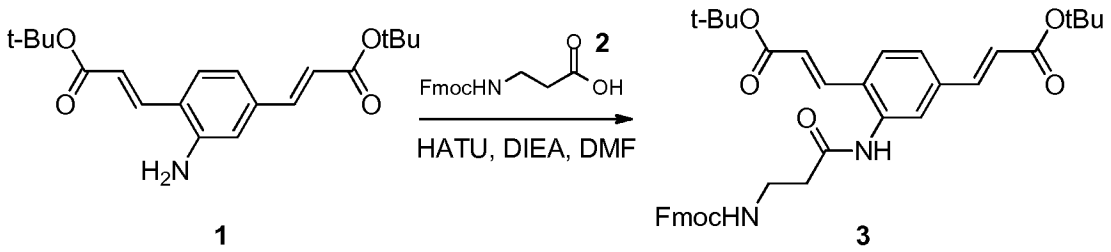
Scheme



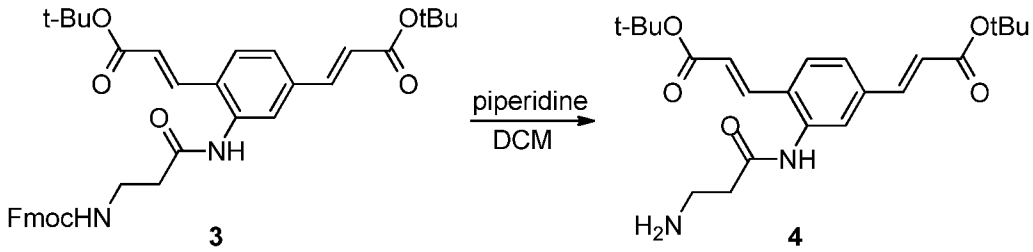
Synthesis of INT 12:



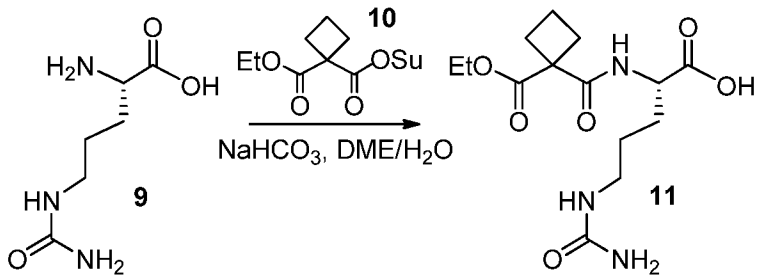
5



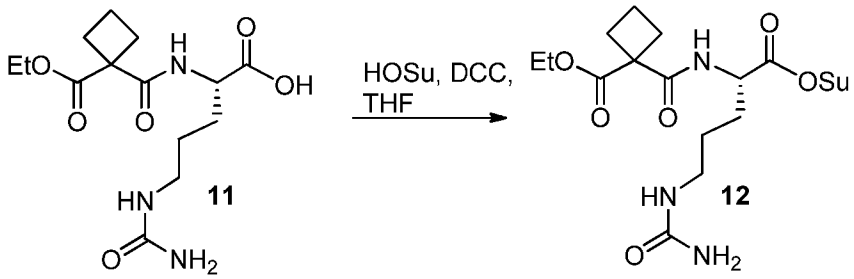
To a stirred solution of **2** (1.35 g, 4.34 mmol) in dry DMF (20 mL) was added HATU (2.20 g, 5.79 mmol), DIEA (1.12 g, 8.68 mmol). After the mixture was stirred at 25°C for 10 min, compound **1** (1.0 g, 2.89 mmol) was added. The reaction mixture was stirred at 25°C for 15 h under N_2. Water (20 mL) was added and it was extracted with EtOAc (30 mL×3). The combined the organic phase was washed with brine, dried over Na_2SO_4, and concentrated. It was purified by flash column (PE: EtOAc = 1:1) to give crude product **3** (2.25 g) as yellow solid. LCMS: (5-95 AB, 1.5 min), 1.075 min, $[M-114]^+$= 527.0.



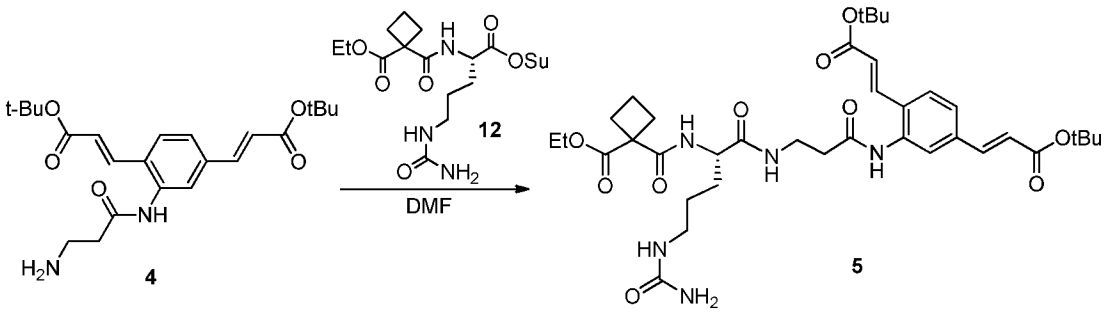
To a stirred solution of compound **3** (1.95 g, 3.05 mmol) in dry DCM (30 mL) was added piperidine (2.60 g, 30.5 mmol). The mixture was stirred at 25°C for 2.5 h under N_2. It was washed with H_2O (20 mL×3), brine (15 mL), and dried over Na_2SO_4. It was concentrated, washed with PE (20 mL×3), and dried to give crude product **4** (2.4 g) as a yellow solid.



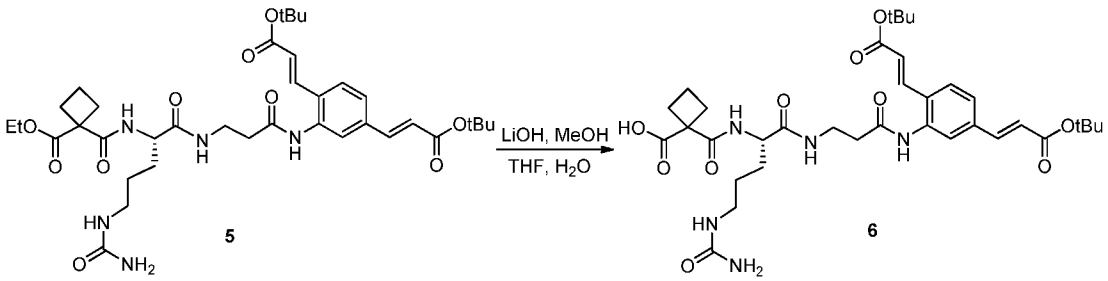
To a solution of compound **9** (3.0 g, 17.1 mmol) in DME/H_2O (40 mL / 20 mL) was added $NaHCO_3$ (2.88 g, 34.3 mmol). After the mixture was stirred at 25°C for 15 min, compound **10** (5.54 g, 20.6 mmol) was added. The mixture was stirred at 25°C for 16 h under N_2. Solvents was removed and H_2O (5 mL) was added. It was extracted with EtOAc (30 mL×3). The pH of water phase was adjusted to 3 with HCl solution, and it was extracted with EtOAc (120 mL×3). The combined organic phase was dried over Na_2SO_4, and concentrated to give crude product **11** as colorless oil.



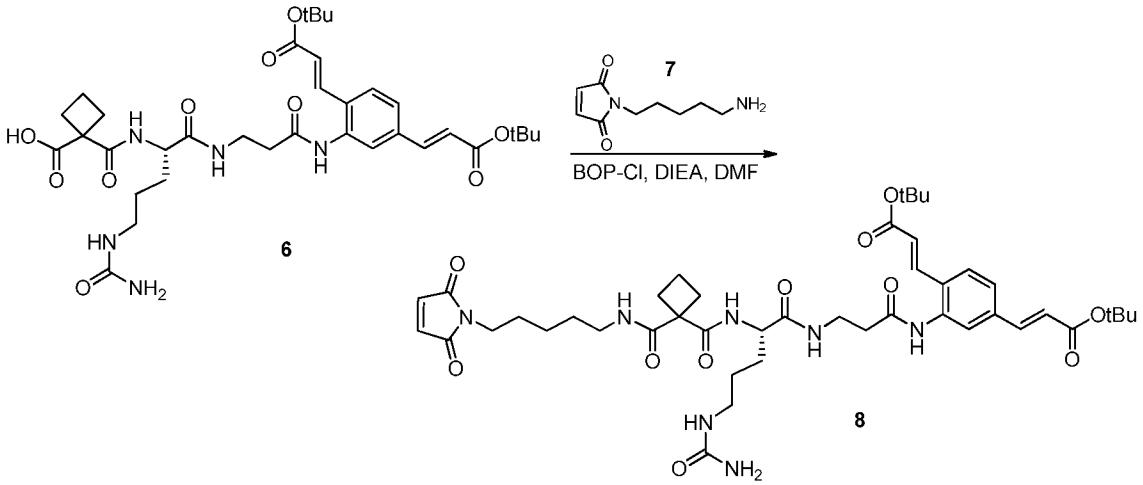
To a solution of compound **11** (5.64 g, 17.1 mmol) in dry THF (120 mL) was added HOSu (2.07 g, 17.98 mmol) and DCC (3.70 g, 17.98 mmol). The mixture was stirred at 25°C for 15 h under N_2. It was filtered and concentrated. The residue was washed the with PE (30 mL×3), dried and concenrated to give crude product **12** (8.30 g) as white solid.



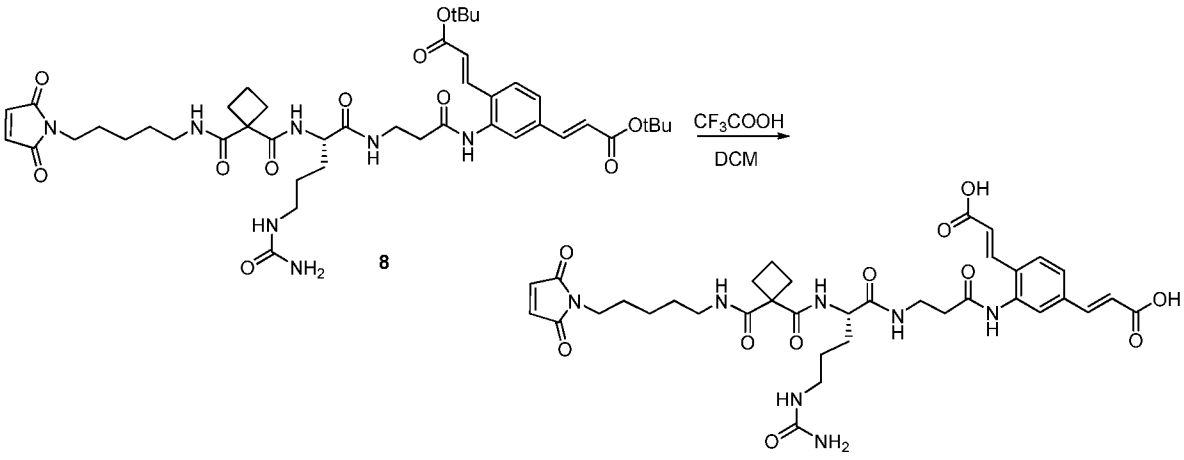
To a solution of compound **4** (1.65 g, 3.96 mmol) in dry DMF (20 mL) was added compoud **12** (2.03 g, 4.75 mmol). The mixture was stirred at 25°C for 15 h under N_2. Water (30 mL) was added and it was extracted with EtOAc (30 mL×3). The combined organic phase was washed with brine (30 mL), and dried over Na_2SO_4. It was concentrated to give crude product, which was washed the with PE (30 mL×4) and MTBE/PE (15 mL/45 mL ×2), and dried to give product **5** (0.96 g, yield: 33%) as light yellow solid.



To a solution of compound **5** (0.96 g, 1.32 mmol) in MeOH (4 mL), THF (8 mL) and H_2O (8 mL) was added LiOH-H_2O (111 mg, 2.64 mmol). The mixture was stirred at 25°C for 30 min under N_2. Organic solvents was removed under reduced pressure and H_2O (10 mL) was added. HCl solution was added to adjust pH to 3-4. It was extracted with EtOAc (50 mL×4), dried over Na_2SO_4, and concentrated to give crude product. The crude product was washed with PE (30 mL) and MTBE (10 mL × 3), and dried to give product **6** (620 mg, yield: 67%) as white solid.



To a solution of compound **6** (620 mg, 0.89 mmol) in dry DMF (10 mL) was added DIEA (573 mg, 4.43 mmol) and Bop-Cl (248 mg, 0.97 mmol) at 0°C. Compound 7 (177.59 mg, 0.97 mmol) was added. After the mixture was stirred at 0°C for 30 min under N_2, H_2O (20 mL) was added and it was extracted with EtOAc (30 mL×3). The combined organic phase was washed with brine (30 mL), dried over Na_2SO_4, and concentrated to give crude product. It was washed with MTBE (10 mL ×2) and PE (50 mL×3), and dried to give product **8** (690 mg, yield: 90%) as white solid. LCMS: (5-95 AB, 1.5 min), 0.875 min, MS=864.2 [M+1];



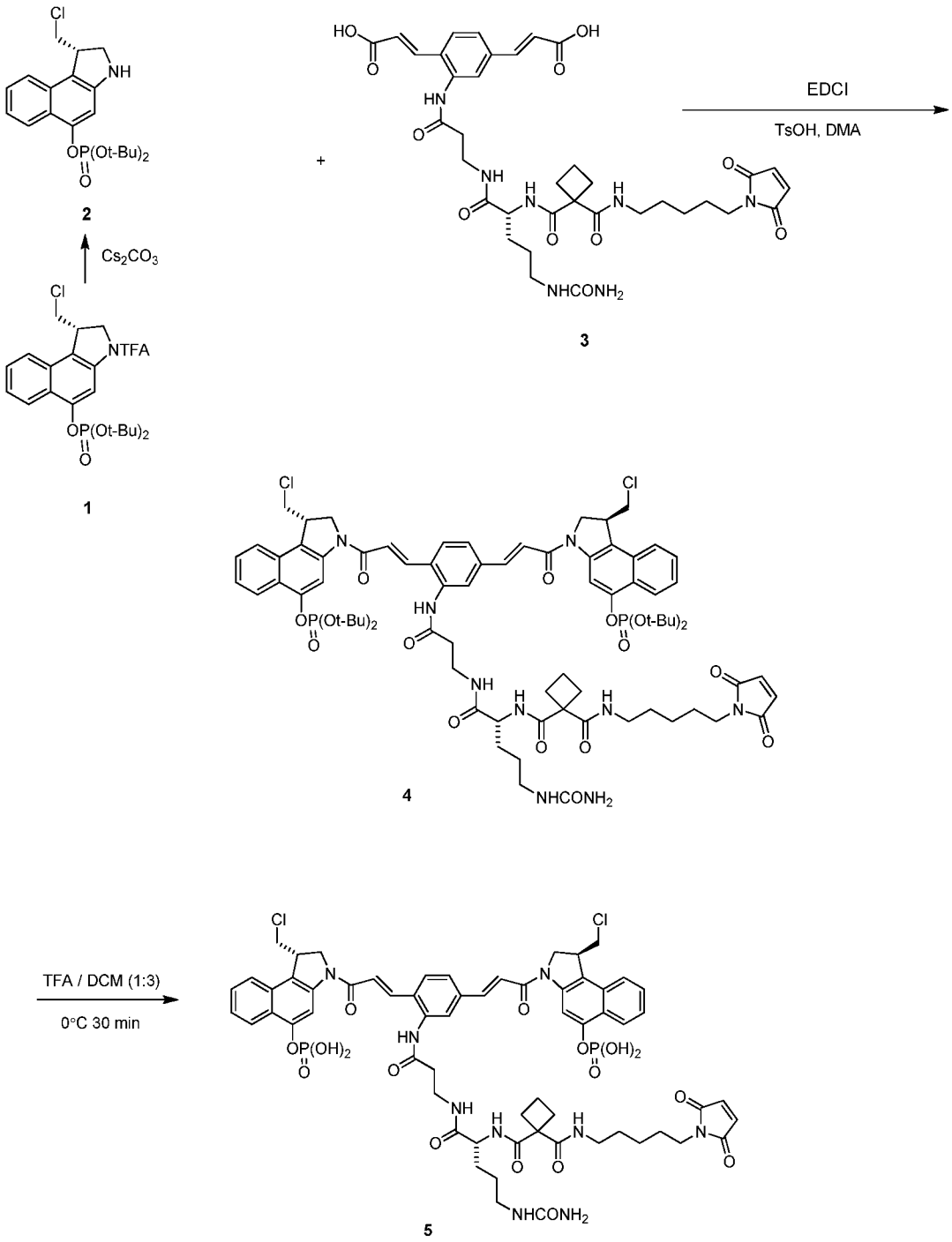
To a stirred solution of compound **8** (300 mg, 0.347 mmol) in dry DCM (4.0 mL) was added dropwise TFA (2.0 mL). After the mixture was stirred at 25°C for 30 min under N_2, solvent was removed. The residue was dissolved in DMF and purified by prep-HPLC (HCOOH) to give product (2E,2'E)-3,3'-(2-(3-((S)-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)propanamido)-1,4-phenylene)diacrylic acid

(81.4 mg, yield: 31%) as light yellow powder. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 9.93 (s, 2H), 6.13 (s, 1H), 7.82 – 7.46 (m, 8H), 6.98 (s, 2H), 6.55 – 6.51 (d, J = 16.0 Hz, 2H), 5.98 (s, 1H), 5.41

(s, 2H), 4.22 (s, 1H), 3.03 – 2.90 (m, 6H), 2.67 – 2.50 (m, 4H), 2.36 (s, 4H), 1.69 (s, 3H), 1.46 – 1.33 (m, 7H), 1.23 – 1.16 (d, *J* = 28 Hz, 2H).

Preparation of CBI-CBI LD5

4-((S)-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyl 2,5-bis((E)-3-((S)-1-(chloromethyl)-5-(phosphonooxy)-1H-benzo[e]indol-3(2H)-yl)-3-oxoprop-1-enyl)phenylcarbamate



CBI-CBI LD5

2

To a solution of **1** (230 mg, 0.44 mmol) in MeOH (2 mL) cooled in an ice bath was added Cs_2CO_3 (287 mg, 0.88 mmol) and several drops of water. The mixture was stirred in the ice bath for 1 h and then redistributed between ethyl acetate and water. The aqueous phase was extracted with ethyl acetate three times. The combined organic extracts were washed with water and brine, dried

over anhydrous Na_2SO_4, filtered through celite, and the solvent was removed. The resultant residue was dissolved in ethyl acetate and filtered through a pad of Florisil to give crude **2** as an off-white gum (188 mg, 100%) which was used directly without further purification.

4

To 180 mg (0.42 mmol) of **2** (freshly made by the procedure mentioned above) was added (2E,2'E)-3,3'-(2-(3-((S)-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)propanamido)-1,4-phenylene)diacrylic acid **3** (100 mg, 0.12 mmol), EDCI.HCl (185 mg, 0.96 mmol), toluenesulfonic acid (2.1 mg, 0.012 mmol) and DMA (0.5 mL). After the mixture was stirred overnight, most of the DMA was removed under vacuum and the residue was redistributed between ethyl acetate and aq. $NaHCO_3$. The aqueous phase was extracted with ethyl acetate three times. The combined organic extracts were washed with water followed by brine, dried over anhydrous Na_2SO_4, and filtered through a pad of Celite. The solvent was removed and the resultant residue was dissolved in the minimum DCM and precipitated by adding heptane to give crude product (207 mg), which was further purified by preparative HPLC [Column: Synergi-Max RP 4μ, 250 × 21.20 mm; Mobile phase: A/B = from 20% to 1% (A: ammonium formate pH 3.45, B: 90% acetonitrile in water); flow rate 12 mL/min] to give **4** (65 mg, 33%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 9.67 (br s, 1H), 8.67 (br s, 2H), 8.18-7.98 (m, 4H), 7.90-7.72 (m, 3H), 7.66-7.58 (m, 6H), 7.50-7.28 (m, 8H), 6.84-6.62 (m, 4H), 6.66 (s, 2H, maleimide), 6.00 (br s, 1H), 5.23-5.13 (m, 2H), 4.80-4.70 (m, 1H), 4.40-3.85 (m, 6H), 3.50-3.40 (m, 6H), 3.20-3.14 (m, 2H), 2.90-2.75 (m, 2H), 2.60-2.45 (m, 4H), 1.92-1.80 (m, 2H), 1.62, 1.60, 1.57, 1.56 (4s, 36H), 1.55-1.40 (m, 6H), 1.30-1.20 (m, 3H). ^{31}P NMR ($CDCl_3$) δ −15.44 (s), 15.82 (s). HRMS (ESI) found *m/z* 1666.6051 (M + Na). $C_{83}H_{101}Cl_2N_9NaO_{18}P_2$ requires 1666.6009.

5

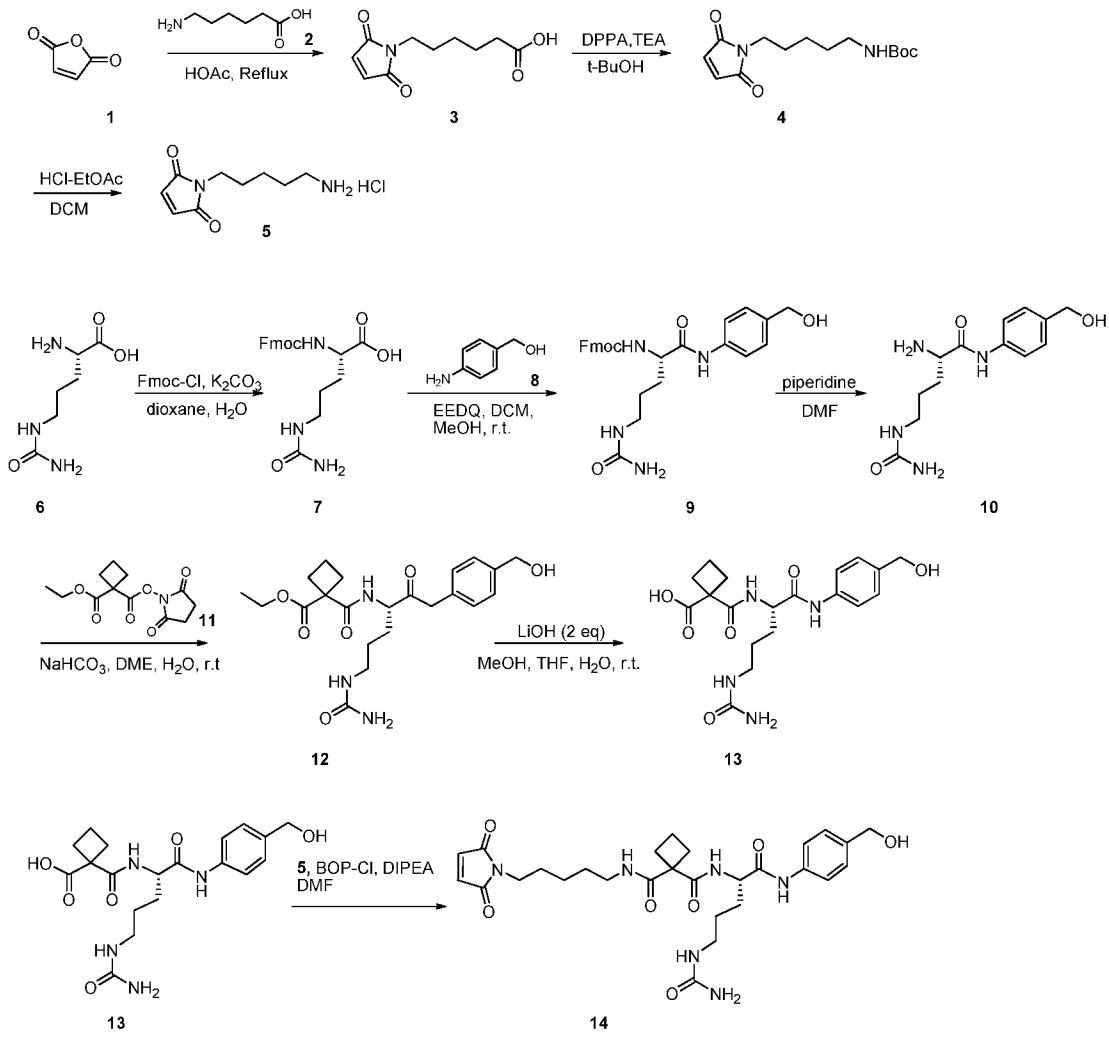
To a solution of **4** (25 mg, 0.015 mmol) in DCM (0.6 mL) cooled in an ice bath was added TFA (0.2 mL, 2.61mmol). The mixture was stirred in an ice bath for 0.5 h. All the volatile components were pumped off at 0 °C and the resultant residue was triturated with ethyl acetate, then washed with THF and petroleum ether to give **5** (**CBI-CBI LD5**) as a yellow solid (19 mg, 88%). ^{1}H NMR (DMSO) δ 10.33 (br s, 1H), 9.63 (s, 1H), 8.70 (s, 1H), 8.95 (s, 1H), 8.14-8.11 (m, 4H), 7.96-7.90 (m, 4H), 7.81-7.69 (m, 6H), 7.64-7.54 (m, 2H), 7.50-7.39 (m, 4H), 7.33-7.26 (m, 2H), 6.97 (s, 2H, maleimide), 6.13 (br s, 2H), 5.14 (s, 2H), 4.58 (s, 4H), 4.40-4.30 (m, 4H), 4.08-3.95 (m, 4H), 3.29 (t, J = 6.9 Hz, 2H), 2.99-2.94 (m, 4H), 2.39-2.33 (m, 2H), 1.71-1.67 (m, 4H), 1.40-1.35 (m, 6H), 1.12-1.10 (m, 3H). ^{31}P NMR (DMSO) δ −5.91 (s). HRMS (ESI) found *m/z* 1442.3438 (M + Na). $C_{67}H_{69}Cl_2N_9NaO_{18}P_2$ requires 1442.3505.

CBI-CBI LD6

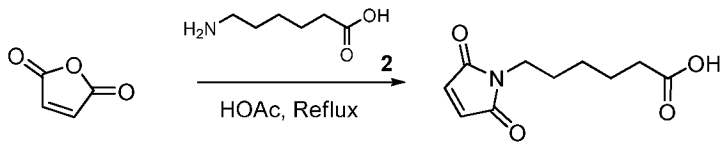
4-((S)-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyl 2,5-bis((E)-3-((S)-1-(chloromethyl)-5-(phosphonooxy)-1H-benzo[e]indol-3(2H)-yl)-3-oxoprop-1-enyl)phenylcarbamate

Step A: (S)-N-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentyl)-N-(1-(4-(hydroxymethyl)phenylamino)-1-oxo-5-ureidopentan-2-yl)cyclobutane-1,1-dicarboxamide

Scheme

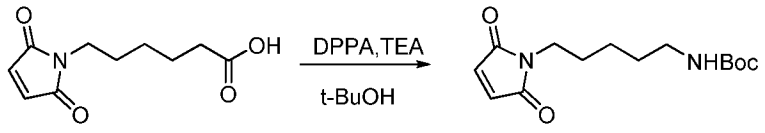


Procedure



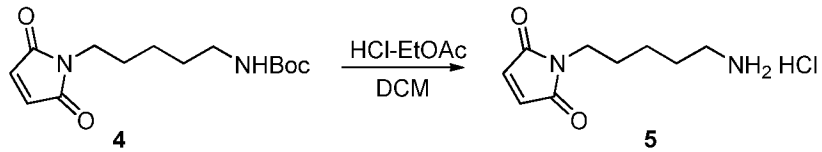
1 3

Compound **1** (150 g, 1.53 mol) was added to a stirred solution of Compound **2** (201 g, 1.53 mol) in HOAc (1000 mL). After the mixture was stirred at r.t. for 2 h, it was heated at reflux for 8 h. The organic solvents were removed under reduced pressure and the residue was extracted with EtOAc (500 mL × 3), washed with H_2O. The combined organic layers was dried over Na_2SO_4 and concentrated to give the crude product. It was washed with petroleum ether to give compound **3** as white solid (250 g, 77.4 %).



3 4

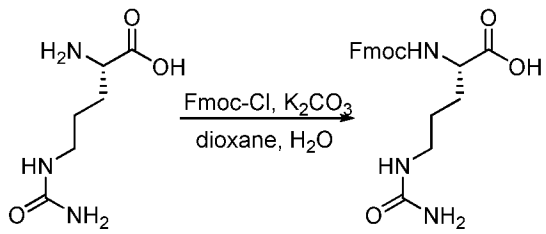
DPPA (130 g, 473 mmol) and TEA (47.9 g, 473 mmol) was added to a solution of compound **3** (100 g, 473 mmol) in t-BuOH (200 mL). The mixture was heated at reflux for 8 h under N_2. The mixture was concentrated, and the residue was purified by column chromatography on silica gel (PE:EtOAc= 3:1) to give compound **4** (13 g, 10 %).



4 5

To a solution of compound **4** (28 g, 992 mmol) in anhydrous EtOAc (30 mL) was added HCl/EtOAc (50 mL) dropwise. After the mixture was stirred at r.t. for 5 h, it was filtered and the solid was dried to give compound **5** (16 g, 73.7%).

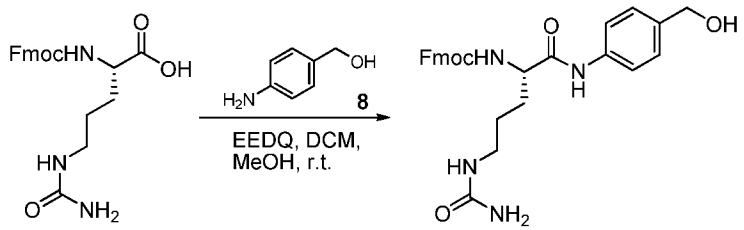
^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (s, 2H), 6.99 (s, 2H), 3.37-3.34 (m, 2H), 2.71-2.64 (m, 2H), 1.56-1.43 (m, 4H), 1.23-1.20 (m, 2H).



6 7

To a mixture of compound **6** (17.50 g, 0.10 mol) in a mixture of dioxane and H_2O (50 mL / 75 mL) was added K_2CO_3 (34.55 g, 0.25 mol). Fmoc-Cl (30.96 g, 0.12 mol) was added slowly at 0 °C. The reaction mixture was warmed to r.t. over 2 h. Organic solvent was removed under reduced

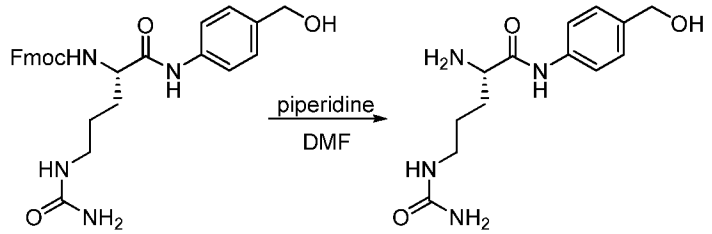
pressure, and the water slurry was adjusted to pH = 3 with 6 M HCl solution, and extracted with EtOAc (100 mL × 3). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the desired product **7** (38.0 g, 95.6 %). (Compound **7** later became commercially available.)



7 **9**

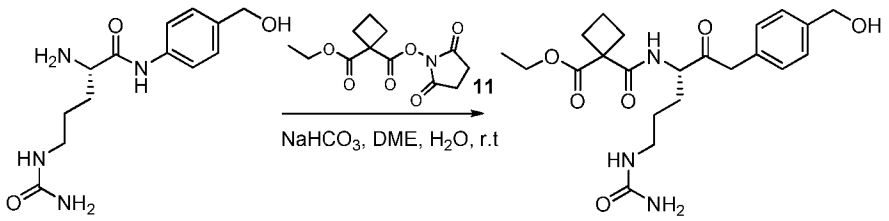
To a solution of compound **7** (4 g, 10 mmol) in a mixture of DCM and MeOH (100 mL / 50 mL) were added 4-amino-phenyl-methanol (**8**) (1.6 g, 13 mmol, 1.3 eq) and EEDQ (3.2 g, 13 mmol, 1.3 eq). After the mixture was stirred at r.t. for 16 h under N_2, it was concentrated to give a brown solid. MTBE (200 mL) was added and it was stirred at 15°C for 2 h. The solid was collected by filtration, washed with MTBE (50 mL × 2) to give the crude product **9** as an orange solid (4.2 g, 84%).

LCMS (ESI): m/z 503.0 [M+1].



9 **10**

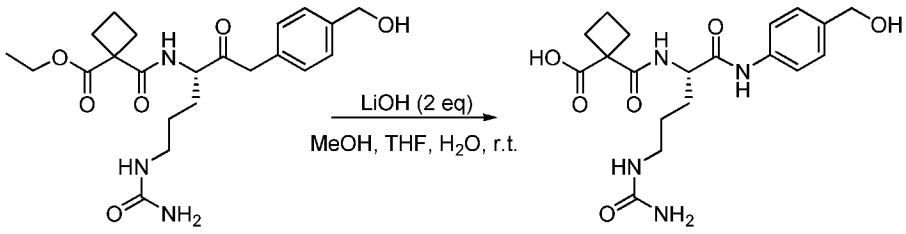
To a stirred solution of compound **9** （4.2 g, 8.3 mmol） in dry DMF (20 ml) was added piperidine (1.65 mL, 17 mmol, 2 eq) dropwise at r.t. The mixture was stirred at r.t. for 30 min, and solid precipitate formed. Dry DCM (50 mL) was added, and the mixture became transparent immediately. The mixture was stirred at r.t. for another 30 min, and LCMS showed compound **9** was consumed. It was concentrated to dryness under reduced pressure (make sure no piperidine remained), and the residue was partitioned between EtOAc and H_2O (50 mL / 20 mL). Aqueous phase was washed with EtOAc (50 mL × 2) and concentrated to give **10** as an oily residual (2.2 g, 94%) (contained small amount of DMF).



10 12

To a solution of compound **11** (8 g, 29.7 mmol) in DME (50 mL) was added a solution of compound **10** (6.0 g, 21.4 mmol) and $NaHCO_3$ (7.48 g, 89.0 mmol) in water (30 mL). After the mixture was stirred at r.t. for 16 h, it was concentrated to dryness under reduced pressure and the residue was purified by column chromatography (DCM:MeOH = 10:1) to give crude compound **12** as white solid (6.4 g, 68.7%).

LCMS (ESI): m/z 435.0 [M+1].

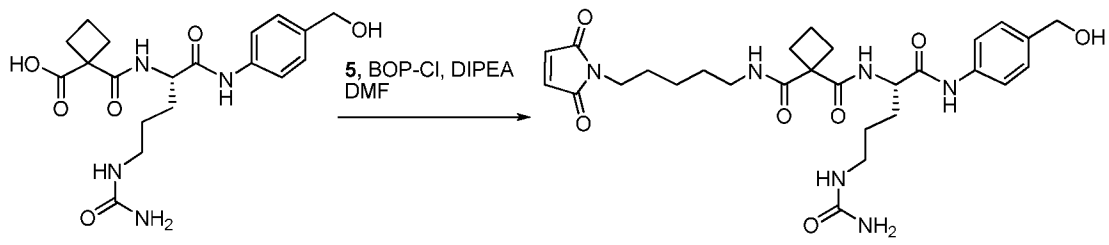


12 13

To a stirred solution of compound **12** (6.4 g, 14.7 mmol) in a mixture of THF and MeOH (20 mL / 10 mL) was added a solution of LiOH • H_2O (1.2 g, 28.6 mmol) in H_2O (20 mL) at r.t. After the reaction mixture was stirred at r.t. for 16 h, solvent was removed under reduced pressure, the residue obtained was purified by prep-HPLC to give compound **13** (3.5 g, yield: 58.5%).

LCMS (ESI): m/z 406.9 [M+1].

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.86 (d, J = 8.4 Hz, 2 H), 8.51 (d, J = 8.4 Hz, 2 H), 5.88 - 5.85 (m, 1 H), 5.78 (s, 2 H), 4.54 - 4.49 (m, 3 H), 4.38 - 4.32 (m, 1 H), 3.86 - 3.75 (m, 1 H), 3.84 - 3.80 (m, 2 H), 3.28 - 3.21 (m, 1 H), 3.30 - 3.24 (m, 1 H), 3.00 - 2.80 (m, 1 H), 2.37 - 2.28 (m, 2 H).



13 14

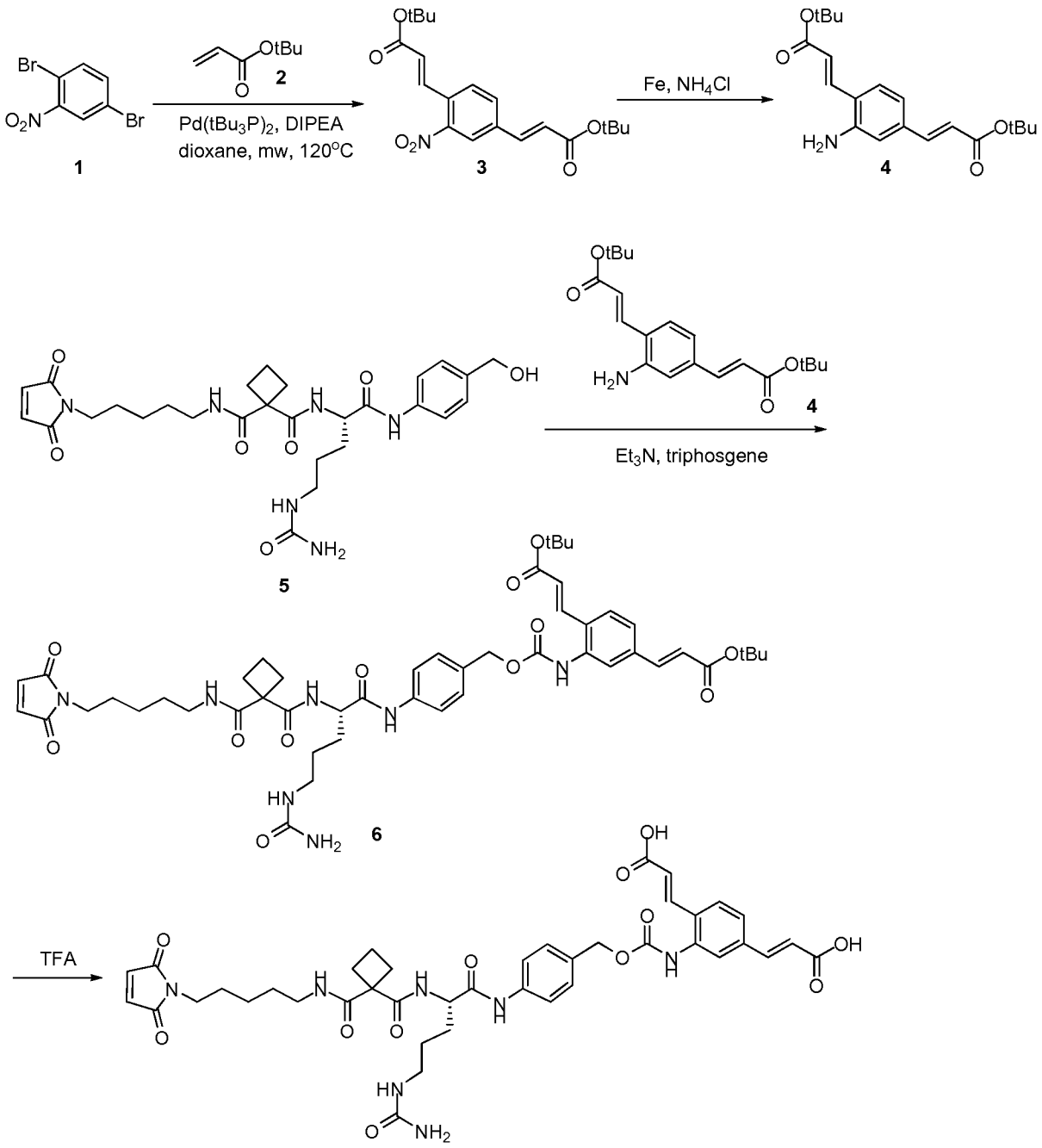
DIPEA (1.59 g, 12.3 mmol) and BOP-Cl (692 mg, 2.71 mmol) was added to a solution of compound **13** (1.0 g, 2.46 mmol) in DMF (10 mL) at 0°C, followed by compound **5** (592 mg, 2.71

mmol). The mixture was stirred at 0°C for 0.5 h. The reaction mixture was quenched with a citric acid solution (10 mL), extracted with DCM/MeOH (10:1). The organic layer was dried and concentrated, and the residue was purified by column chromatography on silica gel (DCM:MeOH = 10:1) to give compound **14** (1.0 g, 71 %).

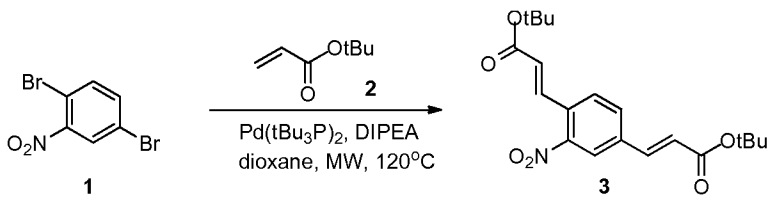
^{1}H NMR (400 MHz, DMSO-d_6): δ 10.00 (s, 1H), 7.82-7.77 (m, 2H), 7.53 (d, J = 8.4 Hz, 2 H), 7.19 (d, J = 8.4 Hz, 2 H), 6.96 (s, 2H), 5.95 (t, J = 6.4 Hz, 1H), 5.39 (s, 2H), 5.08 (t, J = 5.6 Hz, 1H), 4.40-4.35 (m, 3H), 4.09 (d, J = 4.8 Hz, 1 H), 3.01 (d, J = 3.2 Hz, 2 H), 3.05-2.72 (m, 4H), 2.68-2.58 (m, 3H), 2.40-2.36 (m, 4H), 1.72-1.70 (m, 3H), 1.44-1.42 (m, 1H), 1.40-1.23 (m, 6H), 1.21-1.16 (m, 4H).

Step B: Synthesis of (2E,2'E)-3,3'-(2-((4-((S)-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonylamino)-1,4-phenylene)diacrylic acid

Scheme

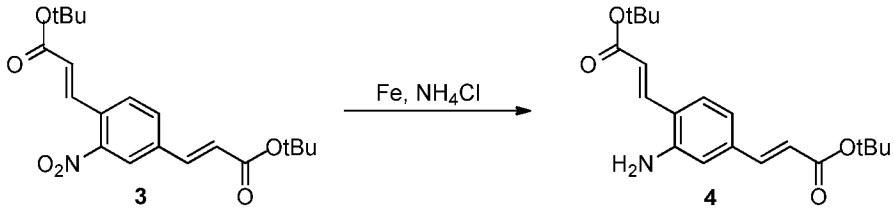


Procedure

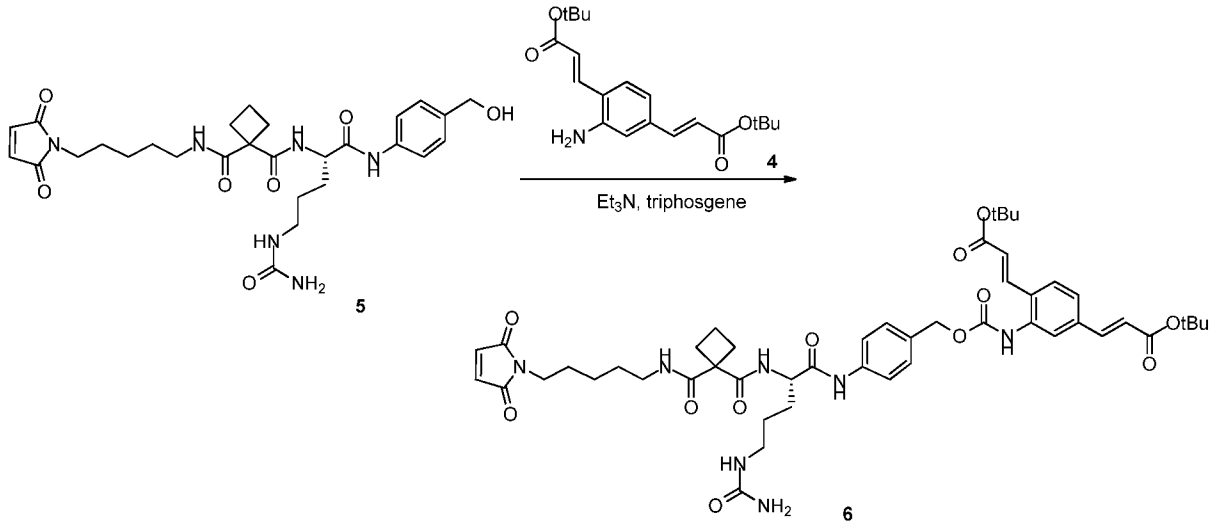


To a solution of compound **1** (1.5 g, 21.4 mmol) in dioxane (4.0 mL) was added compound **2** (2.74 g, 85.6 mmol), DIPEA (3.45 g, 107 mmol) and $Pd(t\text{-}Bu_3P)_2$(0.55 g, 4.30 mmol). The reaction was stirred at 120°C for 2.0 h under microwave irradiation. The reaction was repeated 4 times (total 6.0 g of **1** was used). The combined reaction mixture was concentrated, diluted with water (20 mL)

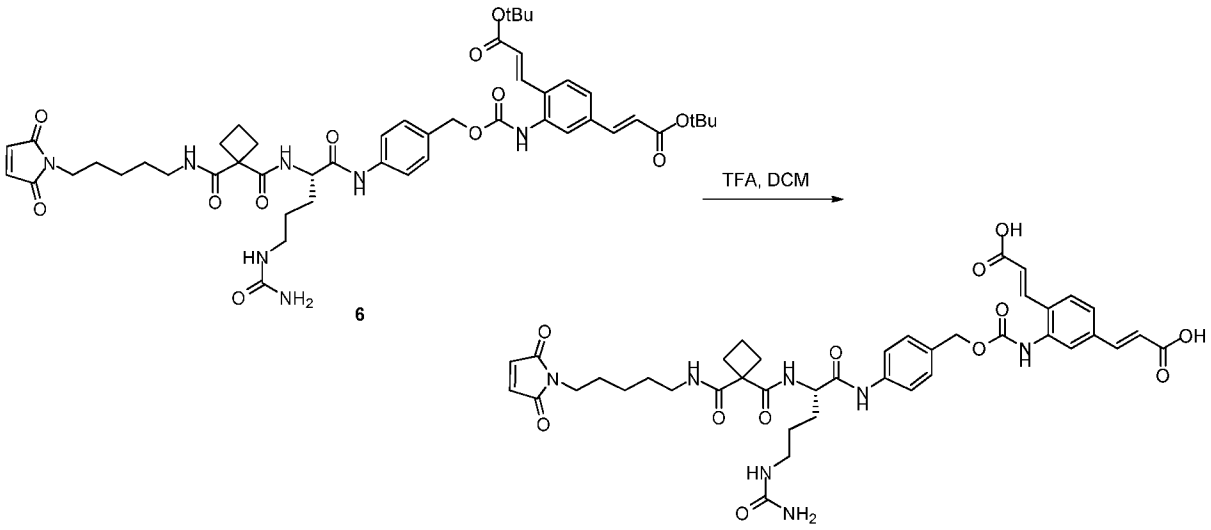
and extracted with EtOAc (100.0 mL x 3). The organic layer was combined, dried over Na_2SO_4. It was concentrated and purified by column (PE: EtOAc=10:1) to give the desired product (3.8 g, 47%).



To a solution of compound **3** (3.8 g, 10.1 mmol) in EtOH/H_2O (120.0 mL) was added Fe (2.83 g, 50.7 mmol), and NH_4Cl (5.4 g, 101 mmol), and the reaction mixture was stirred at 100°C for 2.0 h. The reaction mixture was filtered and the filtrate was concentrated and extracted with EtOAc (60.0 mL x 3). The organic layer was combined, dried over Na_2SO_4 and concentrated to give product (2.5 g, 72%).



To a solution of triphosgene (224 mg, 0.76 mmol) was added a solution of compound **4** (725 mg, 2.1 mmol) and Et_3N (530.3 mg, 5.25 mmol) in DCM (5.0 mL) dropwise in ice-bath. The reaction mixture was stirred at 21°C for 1.0 h until there was no starting material left. The reaction mixture was washed with water (5.0 mL x 2), and dried over Na_2SO_4. It was concentrated and dissolved in DCM (5.0 mL). A solution of the compound **5** (1.0 g, 1.75 mmol) was added and the reaction mixture was stirred at 21°C for 3.0 h. The reaction was quenched with MeOH (2.0 mL), and purified by column (DCM: MeOH=10:1) to give the desired product (380 mg, 23%).

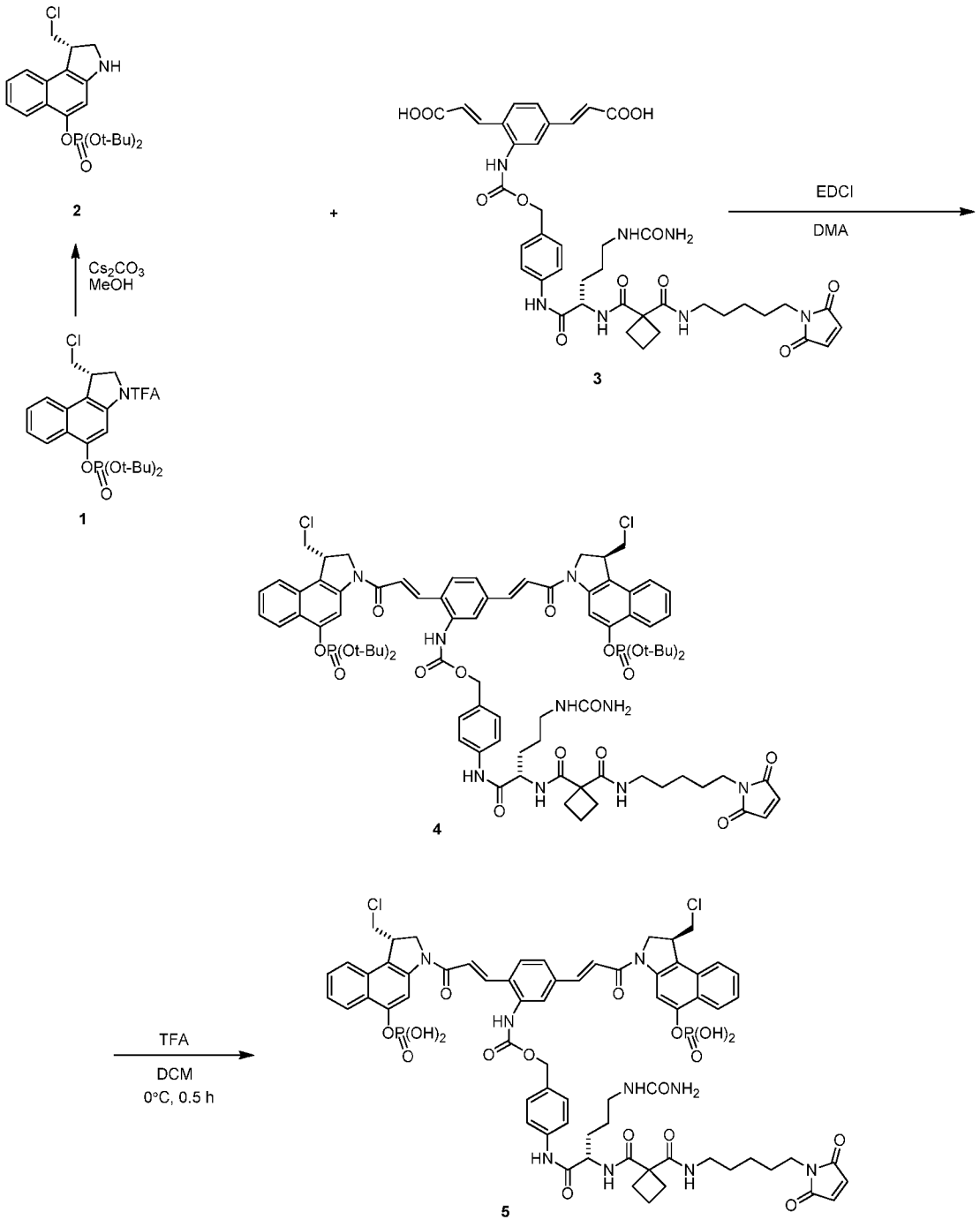


To a solution of compound **6** (300.0 mg, 0.32 mmol) in DCM (10.0 mL) was added TFA (2.0 mL), and the mixture was stirred at 21°C for 30.0 min. The mixture was adjusted to pH 6 with $NH_3 \cdot H_2O$. The precipitation was collected by filtration to give the product (2E,2'E)-3,3'-(2-((4-((S)-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyloxy)carbonylamino)-1,4-phenylene)diacrylic acid (112.0 mg, yield 42 %).

LCMS (10-80, AB, 2.0 min) RT = 0.962 min, $[M+1]^+$ = 830.0;

^1H NMR (400MHz, DMSO-d_6) δ 12.45 (br, 2H), 10.10 (s, 1H), 9.55 (s, 1H), 7.50-7.81 (m, 8H), 7.34 (m, 2H), 6.95 (s, 2H), 6.47-6.57 (m, 2H), 5.96 (s, 1H), 5.40 (s, 2H), 5.05 (s, 2H), 4.36-4.39 (m, 1H), 2.98-3.06 (m, 6H), 2.35-2.39 (m, 4H), 1.15-1.73 (m, 13H).

Step C:



CBI-CBI LD6

8

To 184 mg (0.43 mmol) of **2** (freshly made by the procedure mentioned above) was added **3** (80 mg, 0.11 mmol), EDCI.HCl (165 mg, 0.86 mmol), toluenesulfonic acid (2.0 mg, 0.011 mmol) and DMA (0.5 mL). After the mixture was stirred overnight, most of the DMA was removed under vacuum and the residue was redistributed between ethyl acetate and aq. $NaHCO_3$. The aqueous phase was extracted with ethyl acetate three times. The combined organic extracts were washed with water followed by brine, dried over anhydrous Na_2SO_4, and filtered through a pad of Celite.

The solvent was removed and the resultant residue was dissolved in the minimum DCM and precipitated by adding heptane to give crude product (195 mg), which was further purified by preparative HPLC [Column: Synergi-Max RP 4μ, 250 × 21.20 mm; Mobile phase: A/B = from 90% to 2% (A: ammonium formate pH 3.45, B: 90% acetonitrile in water); flow rate 12 mL/min] to give **4** (56 mg, 34%) as a yellow solid. ^{1}H NMR (DMSO) δ 10.02 (s, 1H), 8.67 (s, 2H), 8.14-8.06 (m, 4H), 7.97 (d, *J* = 8.4 Hz, 2H), 7.86-7.76 (m, 4H), 7.70 (d, *J* = 15.2 Hz, 1H), 7.63-7.59 (m, 2H), 7.53-7.49 (m, 2H), 7.29-7.23 (m, 2H), 6.96 (s, 2H, maleimide), 5.91 (br s, 1H), 5.36 (br s, 2H), 4.65-4.50 (m, 4H), 4.44-4.37 (m, 2H), 4.28-4.22 (m, 2H), 4.05-3.95 (m, 4H), 3.60 (t, *J* = 6.6 Hz, 1H), 3.07-3.00 (m, 2H), 2.95-2.88 (m, 2H), 2.68-2.58 (m, 2H), 2.42-2.32 (m, 3H), 1.78-1.62 (m, 4H), 1.51, 1.50, 1.49, 1.48 (4s, 36H), 1.49-1.28 (m, 11H). ^{31}P NMR ($CDCl_3$) δ −15.44 (s), 15.46 (s). HRMS (ESI) found *m/z* 1588.5827 (M + Na). $C_{78}H_{99}Cl_2N_9NaO_{17}P_2$ requires 1588.5903.

5

To a solution of **4** (25 mg, 0.015 mmol) in DCM (0.6 mL) cooled in an ice bath was added TFA (0.2 mL, 2.61 mmol). The mixture was stirred in an ice bath for 0.5 h. Ether was added and the resultant precipitate was collected by filtration and washed with ethyl acetate, THF and petroleum ether to give **5** (**CBI-CBI LD6**) as a brown solid (18 mg, 86%). ^{1}H NMR (DMSO) δ 10.01 (br s, 1H), 8.60 (br s, 2H), 8.16-8.09 (m, 4H), 7.96-7.93 (m, 2H), 7.88-7.58 (m, 8H), 7.46 (t, *J* = 7.7 Hz, 2H), 7.30-7.25 (m, 2H), 6.97 (s, 2H, maleimide), 6.10 (br s, 1H), 5.35 (br s, 2H), 4.60-4.18 (m, 6H), 4.05-3.95 (m, 4H), 3.45-3.29 (m, 5H), 3.04-2.87 (m, 4H), 2.68-2.60 (m, 2H), 2.40-2.30 (m, 4H), 1.72-1.57 (m, 4H), 1.43-1.28 (m, 5H), 1.20-1.07 (m, 3H). ^{31}P NMR (DMSO) δ −5.82 (s). HRMS (ESI) found *m/z* 1342.3562 (M + H). $C_{62}H_{68}Cl_2N_9O_{17}P_2$ requires 1342.3580.

Method of Preparing ADCs

Preparation of cysteine engineered antibodies for conjugation by reduction and reoxidation

Under certain conditions, the cysteine engineered antibodies may be made reactive for conjugation with linker-drug intermediates of the invention by treatment with a reducing agent such as DTT (Cleland's reagent, dithiothreitol) or TCEP (tris(2-carboxyethyl)phosphine hydrochloride; Getz et al (1999) Anal. Biochem. Vol 273:73-80; Soltec Ventures, Beverly, MA). Full length, cysteine engineered monoclonal antibodies (ThioMabs) expressed in CHO cells (Gomez et al (2010) Biotechnology and Bioeng. 105(4):748-760; Gomez et al (2010) Biotechnol. Prog. 26:1438-1445) were reduced, for example with about a 50 fold excess of DTT overnight at room temperature to reduce disulfide bonds which may form between the newly introduced cysteine residues and the cysteine present in the culture media.

Light chain amino acids are numbered according to Kabat (Kabat et al., *Sequences of proteins of immunological interest*, (1991) 5th Ed., US Dept of Health and Human Service, National Institutes of Health, Bethesda, MD). Heavy chain amino acids are numbered according to the EU numbering system (Edelman et al (1969) Proc. Natl. Acad. of Sci. 63(1):78-85), except where noted as the Kabat system. Single letter amino acid abbreviations are used.

Full length, cysteine engineered monoclonal antibodies (ThioMabs) expressed in CHO cells bear cysteine adducts (cystines) or glutathionylated on the engineered cysteines due to cell culture conditions. To liberate the reactive thiol groups of the engineered cysteines, the ThioMabs are dissolved in 500 mM sodium borate and 500 mM sodium chloride at about pH 8.0 and reduced with about a 50-100 fold excess of 1 mM TCEP (tris(2-carboxyethyl)phosphine hydrochloride (Getz et al (1999) Anal. Biochem. Vol 273:73-80; Soltec Ventures, Beverly, MA) for about 1-2 hrs at 37 °C. Alternatively, DTT can be used as reducing agent. The formation of inter-chain disulfide bonds was monitored either by non-reducing SDS-PAGE or by denaturing reverse phase HPLC PLRP column chromatography. The reduced ThioMab is diluted and loaded onto a HiTrap SP FF column in 10 mM sodium acetate, pH 5, and eluted with PBS containing 0.3M sodium chloride, or 50 mM Tris-Cl, pH 7.5 containing 150 mM sodium chloride.

Disulfide bonds were reestablished between cysteine residues present in the parent Mab by carrying out reoxidation. The eluted reduced ThioMab is treated with 15X or 2 mM dehydroascorbic acid (dhAA) at pH 7 for 3 hours or for 3 hrs in 50 mM Tris-Cl, pH 7.5, or with 2 mM aqueous copper sulfate ($CuSO_4$) at room temperature overnight. Other oxidants, i.e. oxidizing agents, and oxidizing conditions, which are known in the art may be used. Ambient air oxidation may also be effective. This mild, partial reoxidation step forms intrachain disulfides efficiently with high fidelity. The buffer is exchanged by elution over Sephadex G25 resin and eluted with PBS with 1mM DTPA. The thiol/Ab value is checked by determining the reduced antibody concentration from the absorbance at 280 nm of the solution and the thiol concentration by reaction with DTNB (Aldrich, Milwaukee, WI) and determination of the absorbance at 412 nm.

Liquid chromatography/Mass Spectrometric Analysis was performed on a TSQ Quantum Triple quadrupole™ mass spectrometer with extended mass range (Thermo Electron, San Jose California). Samples were chromatographed on a PRLP-S®, 1000 A, microbore column (50mm × 2.1mm, Polymer Laboratories, Shropshire, UK) heated to 75 °C. A linear gradient from 30-40% B (solvent A: 0.05% TFA in water, solvent B: 0.04% TFA in acetonitrile) was used and the eluent was directly ionized using the electrospray source. Data were collected by the Xcalibur® data system and deconvolution was performed using ProMass® (Novatia, LLC, New Jersey). Prior to LC/MS analysis, antibodies or drug conjugates (50 micrograms) were treated with PNGase F (2 units/ml; PROzyme, San Leandro, CA) for 2 hours at 37 °C to remove N-linked carbohydrates.

Hydrophobic Interaction Chromatography (HIC) samples were injected onto a Butyl HIC NPR column (2.5 micron particle size, 4.6 mm × 3.5 cm) (Tosoh Bioscience) and eluted with a linear gradient from 0 to 70% B at 0.8 ml/min (A: 1.5 M ammonium sulfate in 50 mM potassium phosphate, pH 7, B: 50 mM potassium phosphate pH 7, 20% isopropanol). An Agilent 1100 series HPLC system equipped with a multi wavelength detector and Chemstation software was used to resolve and quantitate antibody species with different ratios of drugs per antibody. Cysteine engineered antibodies of the present invention can be prepared according the general method described above.

Conjugation of linker-drug intermediates to antibodies (procedure 1)

Engineered antibody cysteines were blocked as mixed disulfides with glutathione and/or cysteine as expressed in CHO cells. These cysteines had to be "deblocked" prior to conjugation.

Deblocked antibody (5-12 mg/mL) in 20 mM succinate, 150 mM NaCl, 2 mM EDTA was brought to 75-100 mM Tris, pH 7.5-8 (using 1M Tris). Co-solvent (DMSO, DMF, or DMA) was added to the antibody solution, followed by linker-drug (in DMSO or DMF) to give a final %-organic solvent of 10-13% and final concentration of linker-drug 2.5-10X relative to antibody concentration. Reactions were allowed to proceed at room temperature for 1-12 hours (until maximum conjugation was achieved). Conjugation reactions were purified via cation exchange chromatography and/or gel filtration using disposable columns (S maxi or Zeba, respectively). Additional purification by preparative gel filtration (S200 columns) was performed if the crude conjugate was significantly aggregated according to analytical SEC (e.g., >10%). Conjugates were subsequently exchanged into formulation buffer (20 mM His-acetate, pH 5.5, 240 mM sucrose) using either gel filtration or dialysis. Tween-20 was subsequently added to the purified conjugate to reach a final concentration of 0.02%. Final conjugate concentrations ranged from 2.4 to 7.5 mg/mL (%Yield: 34-81% from deblocked antibody). Conjugates were analyzed by LCMS to obtain a measurement of the drug-antibody ratio (DAR), which ranged from 1.3 to 2.1 (average: 1.8). Conjugates were also analyzed for presence of high-molecular weight aggregates using analytical SEC (Zenix or Shodex columns); final, purified conjugates displayed aggregation ranging from 0-10%. Conjugates were also assessed for endotoxin contamination, which, in all cases, did not exceed 1.3 EU/mg. Free, unconjugated drug did not exceed 1% of the final conjugate.

Conjugation of linker-drug intermediates to antibodies (procedure 2, alternative procedure)

After the reduction and reoxidation procedures of the above example, the antibody is dissolved in PBS (phosphate buffered saline) buffer and chilled on ice. An excess, from about 1.5 molar to 20 equivalents of a linker-drug intermediate with a thiol-reactive functional group such as maleimido

or bromo-acetamide, is dissolved in DMSO, diluted in acetonitrile and water, and added to the chilled reduced, reoxidized antibody in PBS. After about one hour, an excess of maleimide is added to quench the reaction and cap any unreacted antibody thiol groups. The conjugation mixture may be loaded and eluted through a HiTrap SP FF column to remove excess drug-linker intermediate and other impurities. The reaction mixture is concentrated by centrifugal ultrafiltration and the cysteine engineered antibody drug conjugate is purified and desalted by elution through G25 resin in PBS, filtered through 0.2 μm filters under sterile conditions, and frozen for storage.

The ADCs of the present invention can be prepared according to the procedure described in the above section.

ASSAYS

Select linkers were then tested and found active in *in vitro* and *in vivo* assays. The cleavage data is shown in the table below

Cathepsin B cleavage Assay

Like peptide linkers, non-peptide linkers for ADC is expect to be cleavable in lysosome in order for proper drug release. As a digestive organelle of the cell, lysosome is enriched with some proteases which show optimal hydrolytic activity at an acidic pH. Cathepsin B is a representative lysosomal protease and has been shown to contribute to the activation of ADC peptide linkers (ref). As an initial screen, an assay was developed using purified cathepsin B to identify cleavable linker-drug constructs that are suitable for conjugation with antibody. Norfloxacin was used to represent the drug component of the linker-drug. The percentage of cleavage relative to the control peptides (such as Val-Cit) was measured at a given time point as well as the kinetic parameters of the cleavage reaction (Km and Vmax). Detailed description of the assay is shown below. From this assay, a variety of proteolytically active and structurally diverse linkers were identified and later used in making ADCs.

Cathepsin B cleavage activity using experimental linker-drugs as substrate was measured by monitoring the release of Norfloxacin using LC/MS. Varying concentrations of linker-drug (3-fold serial dilutions) were incubated in 20 uL reactions containing 20 nM Cathepsin B (EMD Millipore cat. #219364, human liver), 10 mM MES pH 6.0, 1 mM DTT, 0.03% CHAPS, and 25 nM Norfloxacin-d5 internal standard (Santa Cruz Biotechnology, cat. #sc-301482). Reactions were incubated for 1 hour at 37°C, followed by addition of 60 uL of 2% formic acid to quench the reactions. Samples were analyzed by injecting 2 uL of stopped reactions on a Waters Acquity

UPLC BEH Phenyl column (2.1 mm x 50 mm, Waters cat. #186002884). Samples were purified using a linear 2 minute gradient (0% to 80%) of acetonitrile, 0.1% formic acid on a Water Acquity UPLC. Norfloxacin and Norfloxacin-d5 internal standard were detected using an AB Sciex QTrap 5500 triple quadrupole mass spectrometer operating in positive MRM mode (Norfloxacin 320→233 m/z, Norfloxacin-d5 325 →233 m/z). The quantified norfloxacin (normalized with internal standard) was plotted against linker-drug concentration, and the resulting plot was curve fitted with a Michaelis-Menten fit using GraphPad Prism software for the kinetic constants Km and Vmax.

In vitro cell proliferation assay

Efficacy of ADC was measured by a cell proliferation assay employing the following protocol (CELLTITER GLO™ Luminescent Cell Viability Assay, Promega Corp. Technical Bulletin TB288; Mendoza et al (2002) Cancer Res. 62:5485-5488):

1. An aliquot of 100 µl of cell culture containing about 10^4 cells (SKBR-3, BT474, MCF7 or MDA-MB-468) in medium was deposited in each well of a 96-well, opaque-walled plate.

2. Control wells were prepared containing medium and without cells.

3. ADC was added to the experimental wells and incubated for 3-5 days.

4. The plates were equilibrated to room temperature for approximately 30 minutes.

5. A volume of CELLTITER GLO™ Reagent equal to the volume of cell culture medium present in each well was added.

6. The contents were mixed for 2 minutes on an orbital shaker to induce cell lysis.

7. The plate was incubated at room temperature for 10 minutes to stabilize the luminescence signal.

8. Luminescence was recorded and reported in graphs as RLU = relative luminescence units.

Data are plotted as the mean of luminescence for each set of replicates, with standard deviation error bars. The protocol is a modification of the CELLTITER GLO™ Luminescent Cell

Media: SK-BR-3 grow in 50/50/10%FBS/glutamine/250 µg/mL G-418 OVCAR-3 grow in RPMI/20%FBS/glutamine

In vivo assay

1. The efficacy of the anti-CD33 antibody-drug conjugates (ADCs) was investigated in a mouse xenograft model of HL-60 or EOL-1 (human acute myeloid leukemia). The HL-60 cell line was obtained from ATCC (American Type Culture Collection; Manassas, VA) and EOL-1 cell line was originated from DSMZ (German Collection of Microorganisms and Cell Cultures; Braunschweig, Germany).

Female C.B-17 SCID mice (Charles River Laboratories; Hollister, CA) were each inoculated subcutaneously in the flank area with five million cells of HL-60 or EOL-1. When the xenograft tumors reached an average tumor volume of 100-300 mm3 (referred to as Day 0), animals were randomized into groups of 7-10 mice each and received a single intravenous injection of the ADCs. Approximately 4 hours prior to administration of ADCs, animals were dosed intraperitoneally with excess amount (30mg/kg) of anti-gD control antibody to block possible nonspecific antibody binding sites on the tumor cells. Tumors and body weights of mice were measured 1-2 times a week throughout the study. Mice were promptly euthanized when body weight loss was >20% of their starting weight. All animals were euthanized before tumors reached 3000 mm3 or showed signs of impending ulceration.

2. The efficacy of the anti-Napi2B antibody-drug conjugates (ADCs) was investigated in a mouse xenograft model of OVCAR3-X2.1 (human ovarian cancer). The OVCAR3 cell line was obtained from ATCC (American Type Culture Collection; Manassas, VA) and a sub-line OVCAR3-X2.1 was generated at Genentech for optimal growth in mice.

Female C.B-17 SCID-beige mice (Charles River Laboratories; San Diego, CA) were each inoculated in the thoracic mammary fat pad area with ten million OVCAR3-X2.1 cells. When the xenograft tumors reached an average tumor volume of 100-300 mm3 (referred to as Day 0), animals were randomized into groups of 7-10 mice each and received a single intravenous injection of the ADCs. Tumors and body weights of mice were measured 1-2 times a week throughout the study. Mice were promptly euthanized when body weight loss was >20% of their starting weight. All animals were euthanized before tumors reached 3000 mm3 or showed signs of impending ulceration.

3. The efficacy of the anti-CD22 antibody-drug conjugates (ADCs) is investigated in a mouse xenograft model of BJAB-luc (human Burkitt's lymphoma) or WSU-DLCL2 (human diffuse large B-cell lymphoma). The BJAB cell line is obtained from DSMZ (German Collection of Microorganisms and Cell Cultures; Braunschweig, Germany), and a sub-line BJAB-luc is

generated at Genentech to stably express the luciferase gene. The WSU-DLCL2 cell line is also originated from DSMZ.

Female C.B-17 SCID mice (Charles River Laboratories; Hollister, CA) are each inoculated subcutaneously in the flank area with 20 million cells of BJAB-luc or WSU-DLCL2. When the xenograft tumors reached an average tumor volume of 100-300 mm3 (referred to as Day 0), animals are randomized into groups of 7-10 mice each and received a single intravenous injection of the ADCs. Tumors and body weights of mice are measured 1-2 times a week throughout the study. Mice are promptly euthanized when body weight loss is >20% of their starting weight. All animals are euthanized before tumors reached 3000 mm3 or showed signs of impending ulceration.

4. The efficacy of the anti-Her2 antibody-drug conjugates (ADCs) is investigated in a mouse allograft model of MMTV-HER2 Founder #5 (murine mammary tumor). The MMTV-HER2 Founder #5 (Fo5) model (developed at Genentech) is a transgenic mouse model in which the human HER2 gene, under transcriptional regulation of the murine mammary tumor virus promoter (MMTV-HER2), is overexpressed in mammary epithelium. The overexpression causes spontaneous development of mammary tumors that overexpress the human HER2 receptor. The mammary tumor from one of the founder animals (founder #5, Fo5) has been propagated in FVB mice (Charles River Laboratories) by serial transplantation of tumor fragments.

For efficacy studies, the Fo5 transgenic mammary tumor is surgically transplanted into the thoracic mammary fat pad of female nu/nu mice (Charles River Laboratories; Hollister, CA) as tumor fragments of approximately 2mm x 2mm in size. When the allograft tumors reached an average tumor volume of 100-300 mm3 (referred to as Day 0), animals are randomized into groups of 7-10 mice each and received a single intravenous injection of the ADCs. Tumors and body weights of mice are measured 1-2 times a week throughout the study. Mice are promptly euthanized when body weight loss is >20% of their starting weight. All animals are euthanized before tumors reached 3000 mm3 or showed signs of impending ulceration.

Biological Data

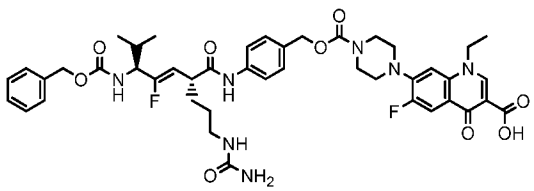
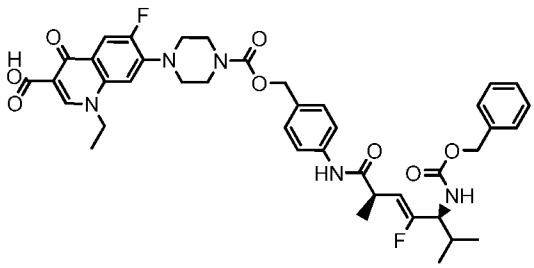
Linker-drug compound structures and Cathepsin B cleavage data

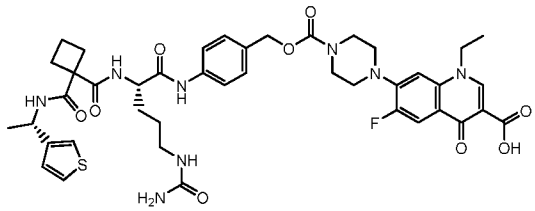
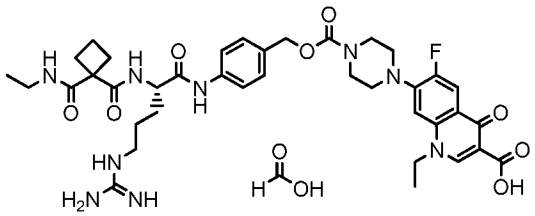
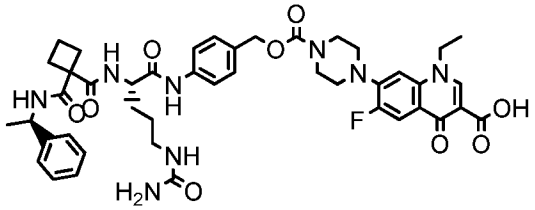
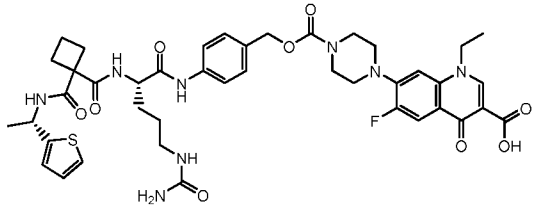
The Linker-drug compounds below can be generialized as the following formula

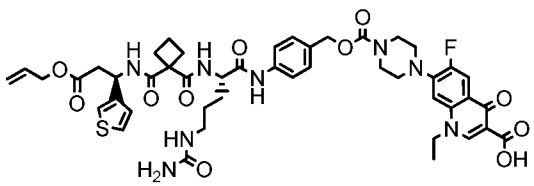
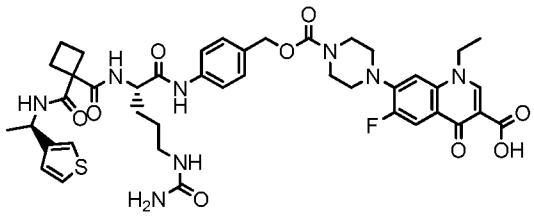
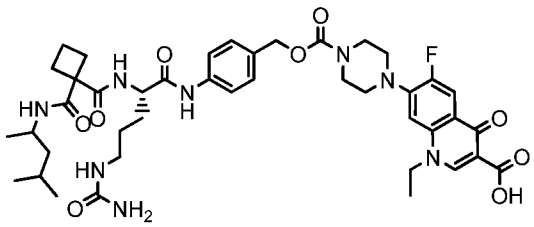
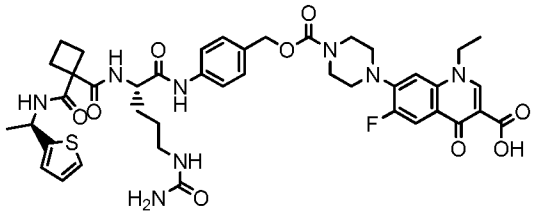
Cap—PM—Sp—T

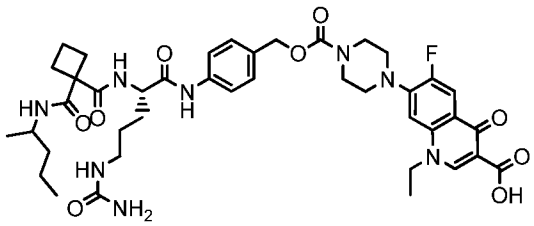
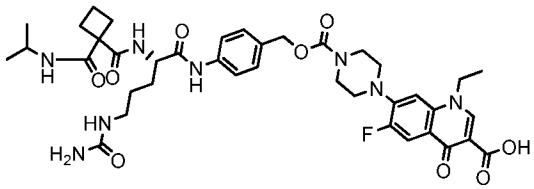
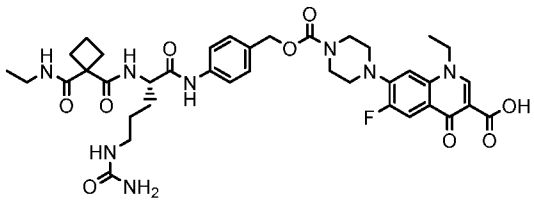
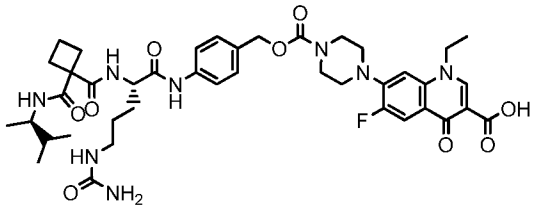
Where Cap is a capping group which protects the amino group in the Cathepsin B assay (for example, CBZ and ethyl); PM is a peptidomimetic moiety; sp is a spacer, T is substitute for drug moiety.

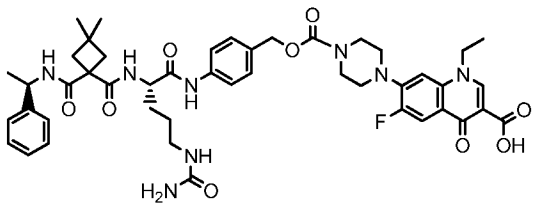
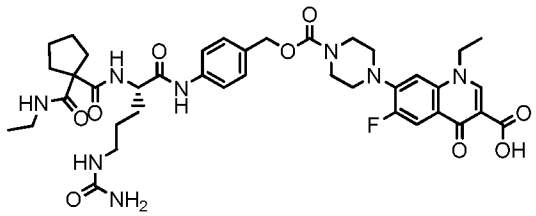
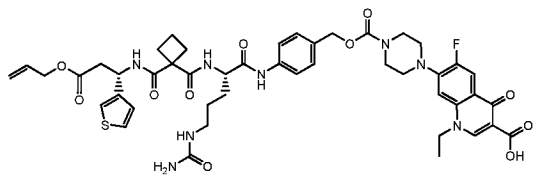
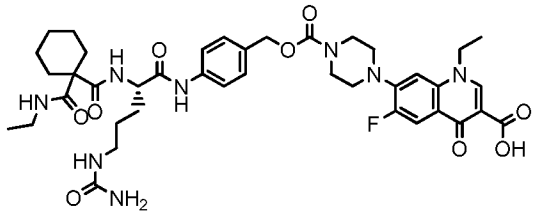
The CAT B cleavage data in the table below shows the cleavage rates of non-peptide linkers of the present inventions are comparable to peptide linkers (40-56). Peptide linkers have been widely used in antibody drug conjugates to release the active drug moiety; therefore, it is expected that conjugates comprising the present non-peptide linkers could achieve similar results *in vitro* and *in vivo*.

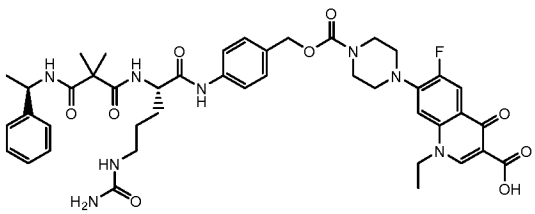
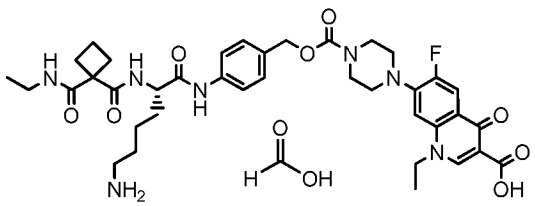
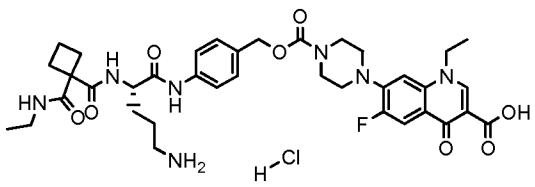
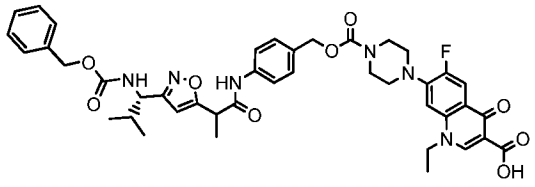
Example	Structure	Series	CATB Cleavage MS (Vmax/Km)	P3	CATB Cleavage MS (Vmax/Km) – background corrected	Stereo
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2		F-olefin	0.00132	Cbz	0.000189	SKS

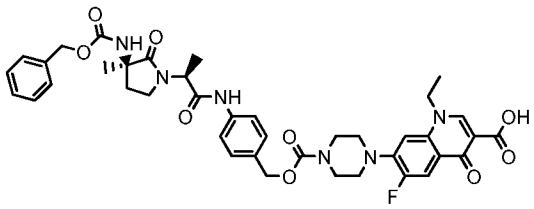
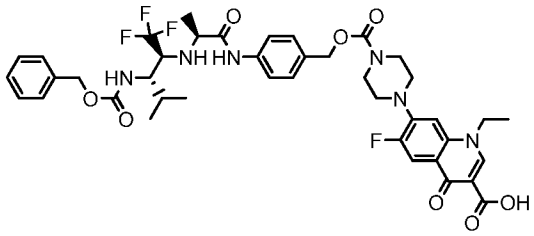
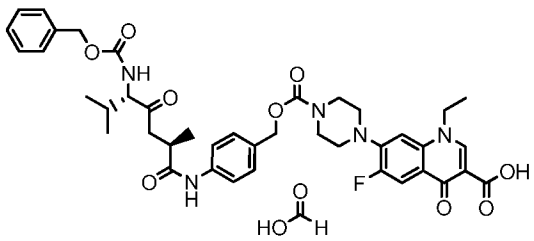
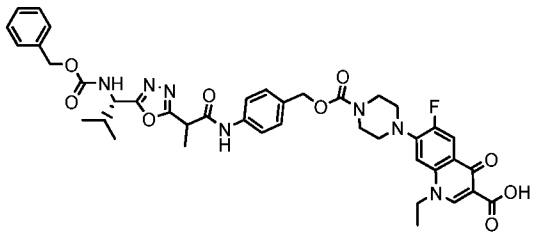
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5		cyclobutane-1,1-dicarbonyl	0.072	non-Cbz	0.014058	SKS
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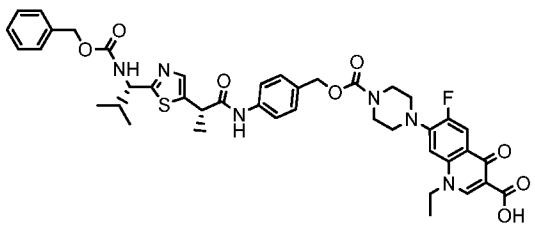
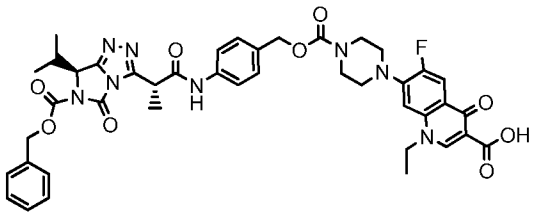
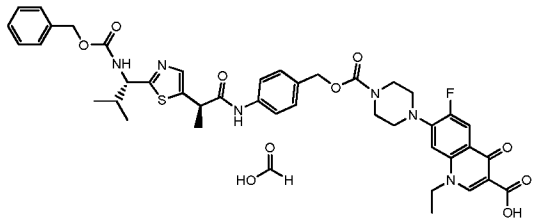
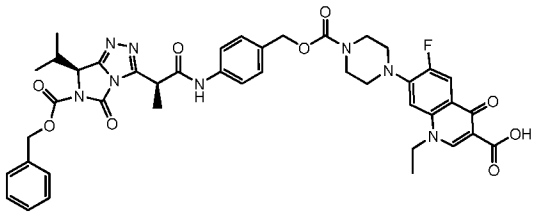
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8		cyclobutane-1,1-dicarbonyl	0.023	non-Cbz	0.004154	SUS
9		cyclobutane-1,1-dicarbonyl	0.023	non-Cbz	0.004148	MD
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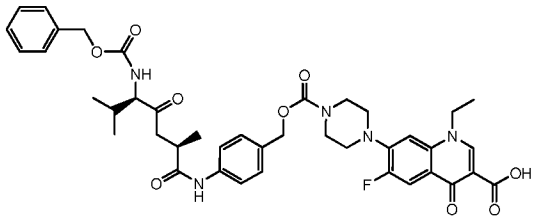
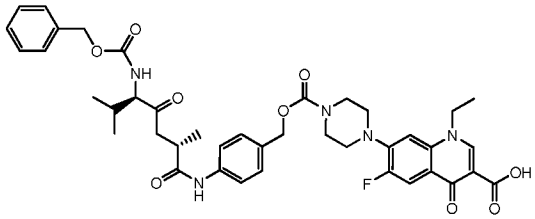
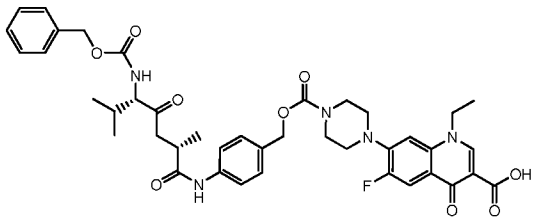
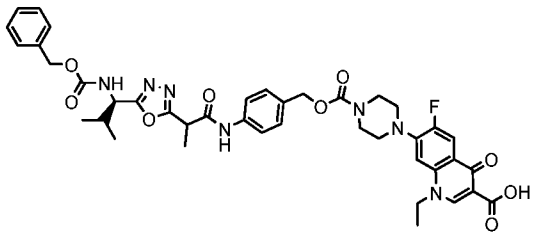
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13		cyclobutane-1,1-dicarbonyl	0.0101	non-Cbz	0.003088	SKS
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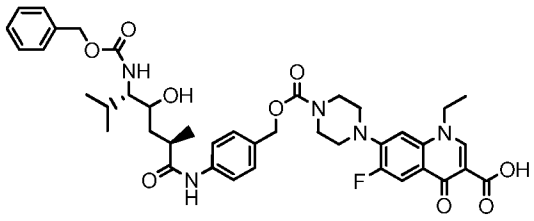
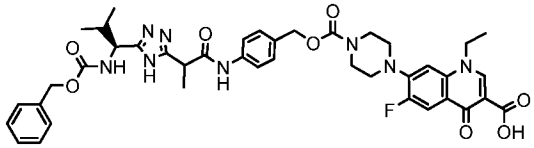
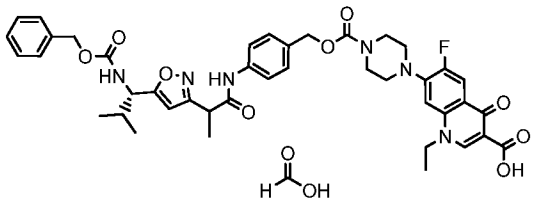
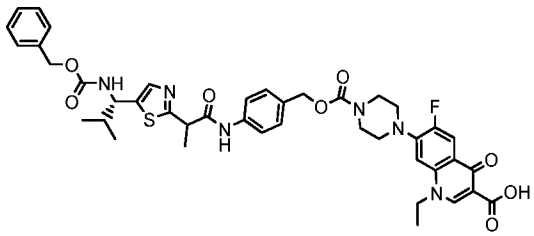
15		cyclobutane-1,1-dicarbonyl	0.00628	non-Cbz	0.002777	SKS
16		cyclobutane-1,1-dicarbonyl	0.0060	non-Cbz	0.001749	SKS
17		cyclobutane-1,1-dicarbonyl	0.00295	non-Cbz	0.002502	SUS
18		cyclobutane-1,1-dicarbonyl	0.000993	non-Cbz	0.000904	SKS

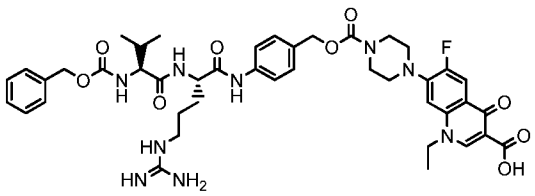
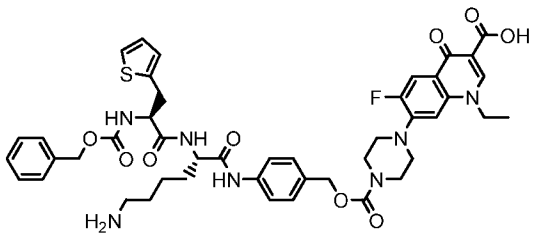
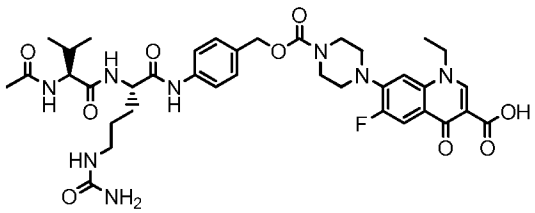
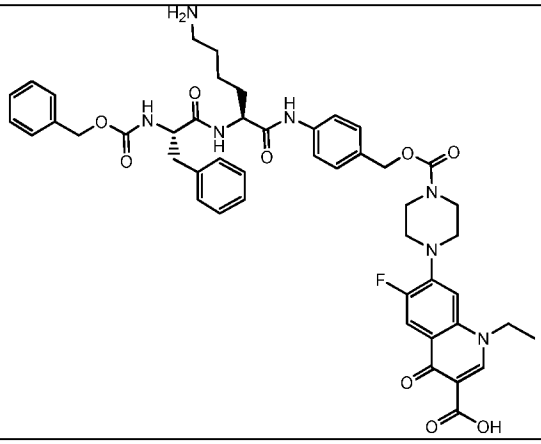
19		cyclobutane-1,1-dicarbonyl	0.000409	non-Cbz	0.000823	SKS
20		cyclobutane-1,1-dicarbonyl		non-Cbz	0.004020	SKS
21		cyclobutane-1,1-dicarbonyl		non-Cbz		SKS
22		isoxazole	0.000185	Cbz	0.000426	MD

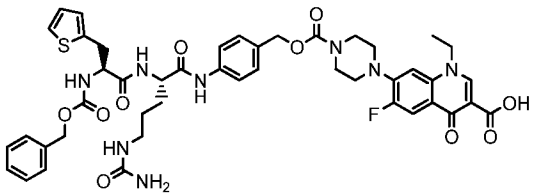
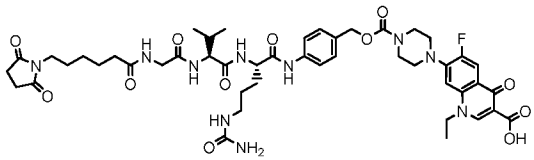
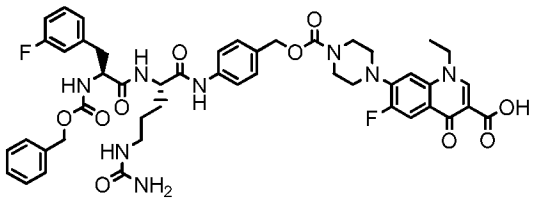
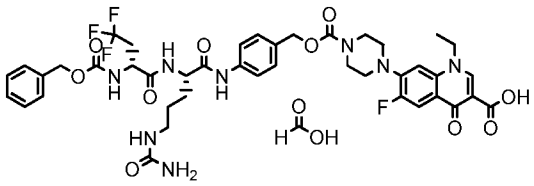
23		others		Cbz	0.000094	SKS
24		others		Cbz		SUS
25		others		Cbz		SUS
26		others		Cbz		MD

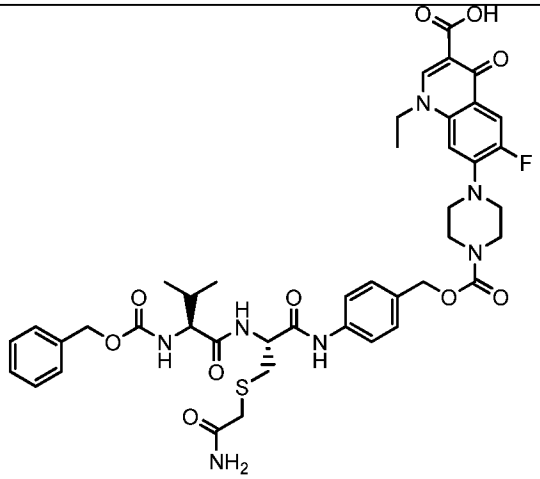
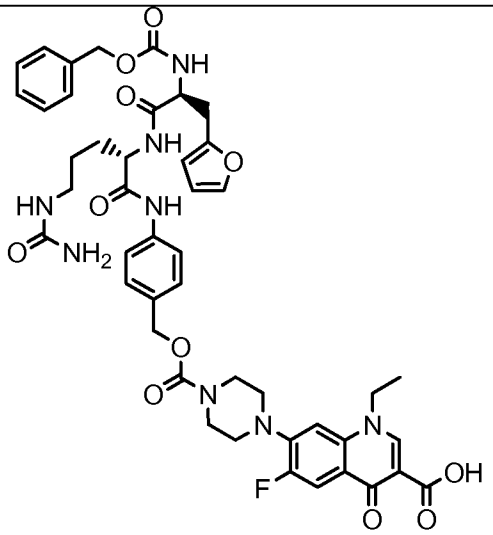
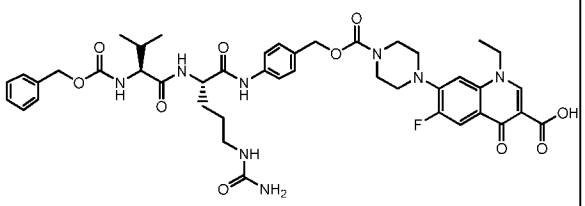
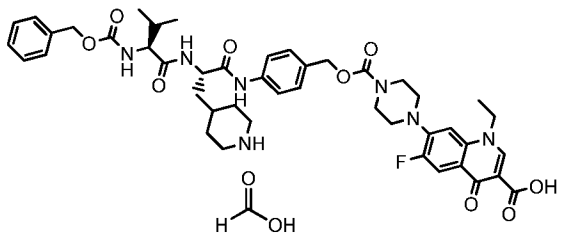
27		others		Cbz		SUS
28		others		Cbz		SUS
29		others		Cbz	0.000047	SUS
30		others		Cbz		SUS

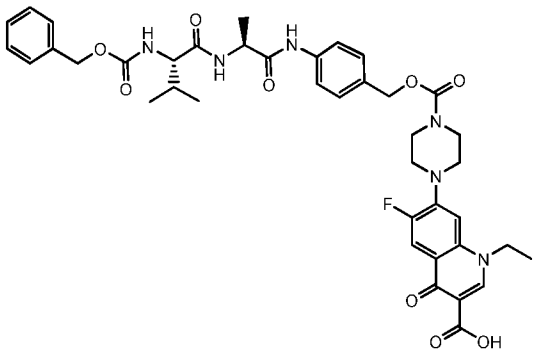
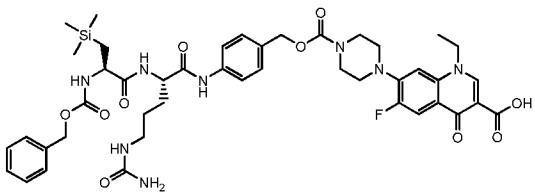
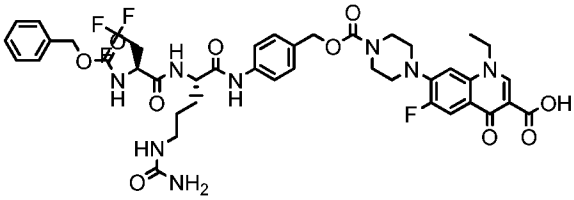
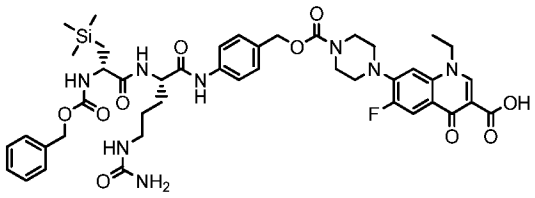
31		others		Cbz		SUS
32		others		Cbz		SUS
33		others		Cbz	0.000013	SUS
34		others		Cbz		ME

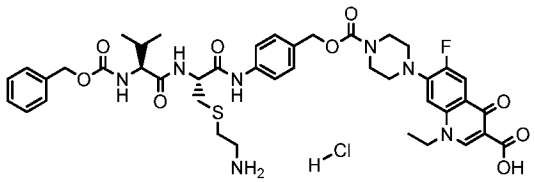
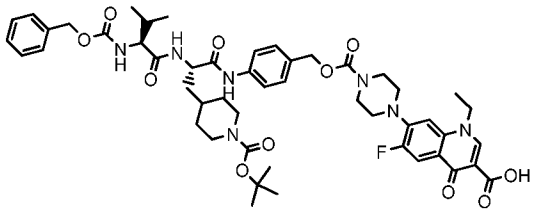
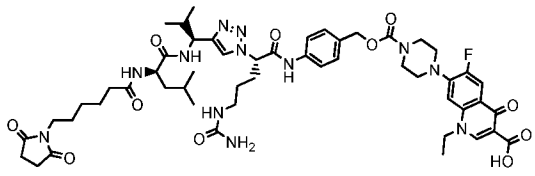
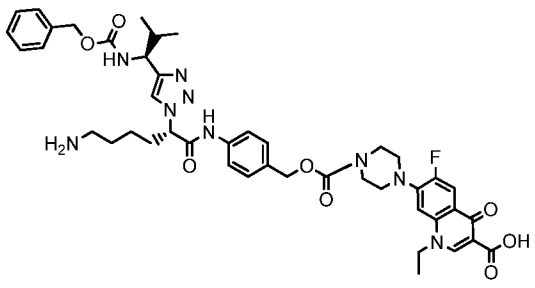
35		others		Cbz	0.009123	MD
36		others		Cbz	0.000014	MD
37		others		Cbz	0.000054	MD
38		others		Cbz		MD

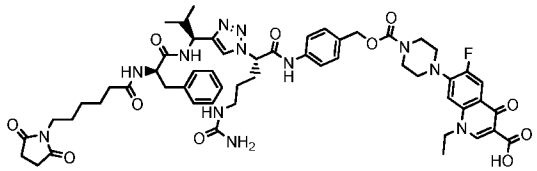
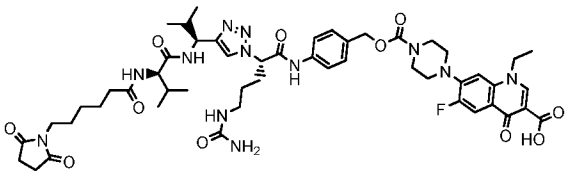
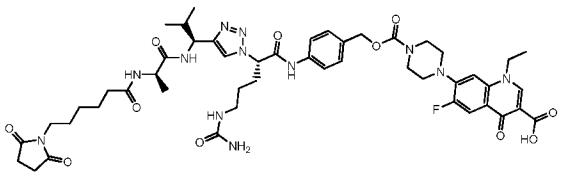
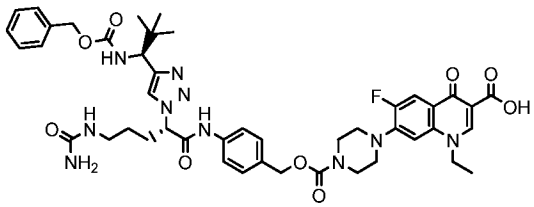
39		peptide	0.356	Cbz	0.375066	SKS
40		peptide	0.319	Cbz	0.257792	SKS
41		peptide	0.26	non-Cbz	0.101536	SKS
42		peptide	0.245	Cbz		SKS

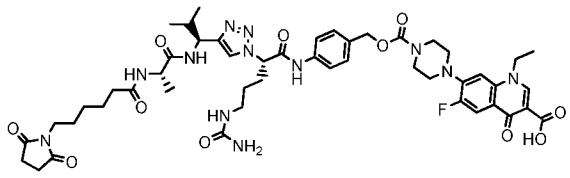
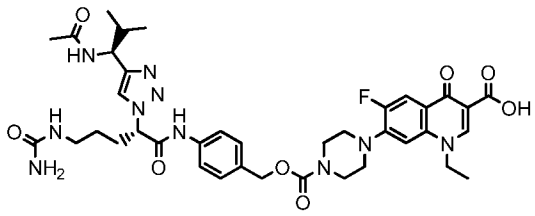
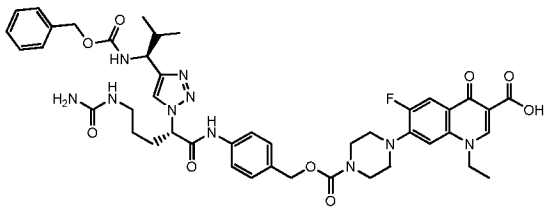
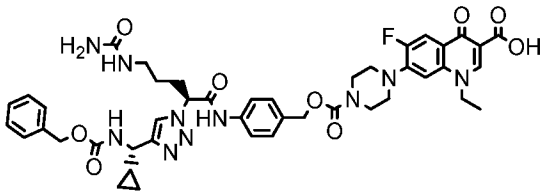
43		peptide	0.224	Cbz	0.032208	SKS
44		peptide	0.205	non-Cbz	0.118705	SKS
45		peptide	0.204	Cbz	0.021417	SKS
46		peptide	0.204	Cbz	0.041089	SKS

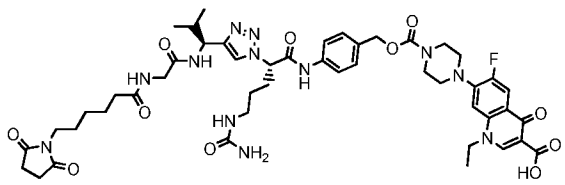
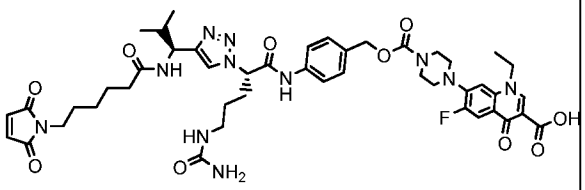
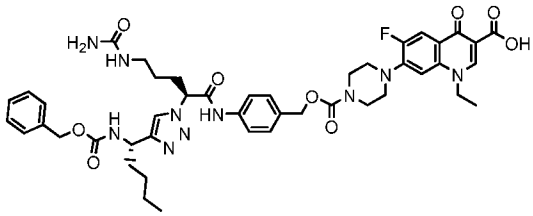
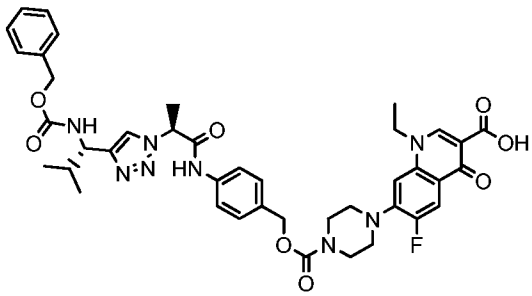
47		peptide	0.168	Cbz	0.037286	SKS
48		peptide	0.165	Cbz	0.068356	SKS
49		peptide	0.135	Cbz	0.041791	SKS
50		peptide	0.117	Cbz	0.025314	SKS

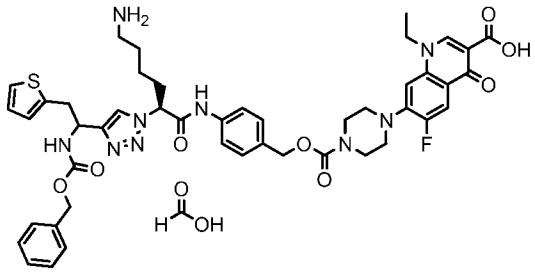
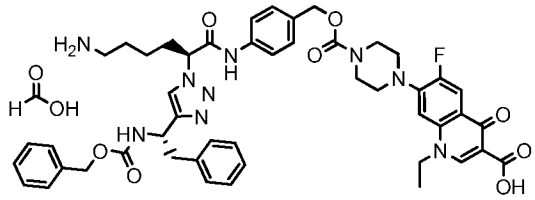
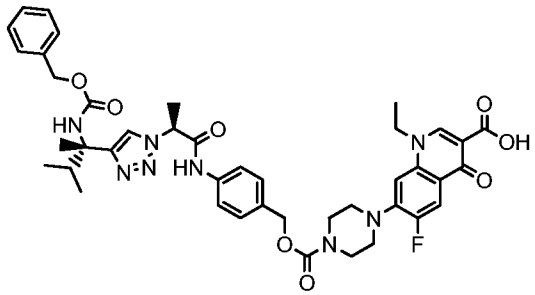
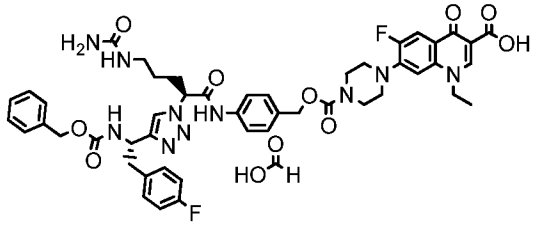
51		peptide	0.0786	Cbz	0.014192	SKS
52		peptide	0.0261	Cbz	0.005928	SUS
53		peptide	0.00262	Cbz	0.000848	SKS
54		peptide	0.00218	Cbz	0.000186	SUS

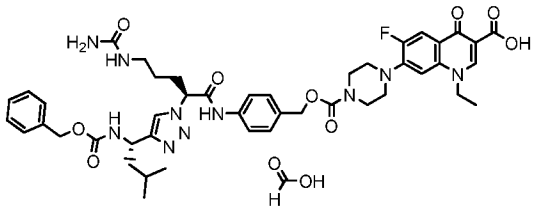
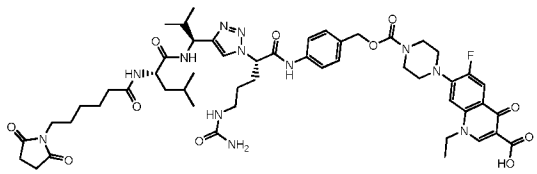
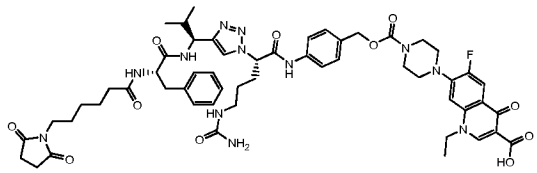
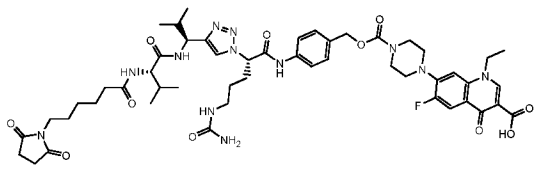
55		peptide		Cbz	0.002961	SKS
56		peptide		Cbz		SKS
57		triazole	0.036	non-Cbz	0.007780	SUS
58		triazole	0.0176	Cbz	0.003519	SKS

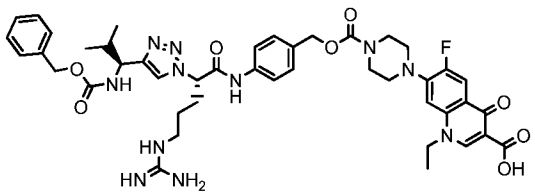
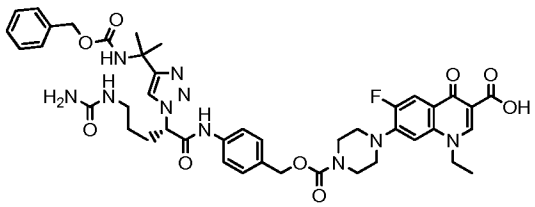
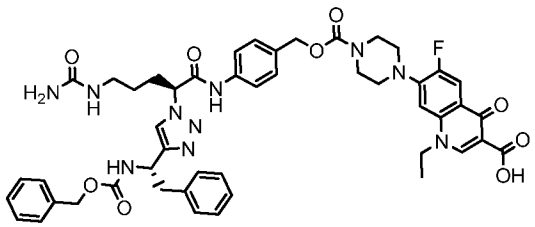
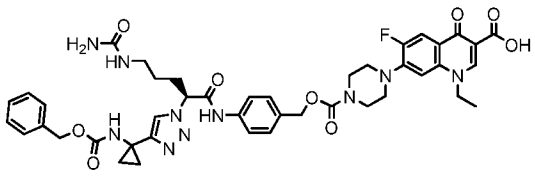
59		triazole	0.013	non-Cbz	0.003849	SUS
60		triazole	0.010	non-Cbz	0.004285	SUS
61		triazole	0.0050	non-Cbz	0.003133	SUS
62		triazole	0.00467	Cbz	0.000281	SKS

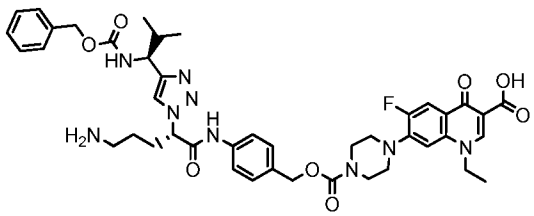
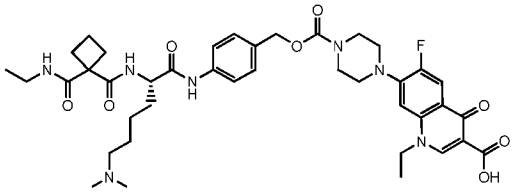
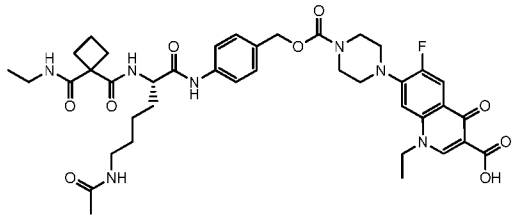
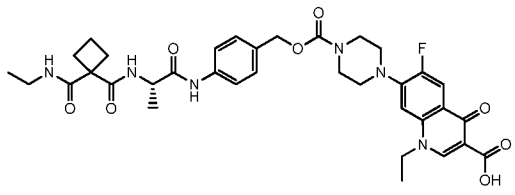
63		triazole	0.0030	non-Cbz	0.000638	SUS
64		triazole	0.00273	non-Cbz		SKS
65		triazole	0.00272	Cbz	0.000199	SKS
66		triazole	0.00218	Cbz	0.000169	SKS

67		triazole	0.0020	non-Cbz	0.000245	SKS
68		triazole	0.0012		0.001601	SKS
69		triazole	0.000852	Cbz		SKS
70		triazole	0.000603	Cbz		SKS

71		triazole	0.000571	Cbz	0.000156	ME
72		triazole	0.000437	Cbz	0.000108	SKS
73		triazole	0.000241	Cbz	0.000059	SKS
74		triazole	0.000195	Cbz		SKS

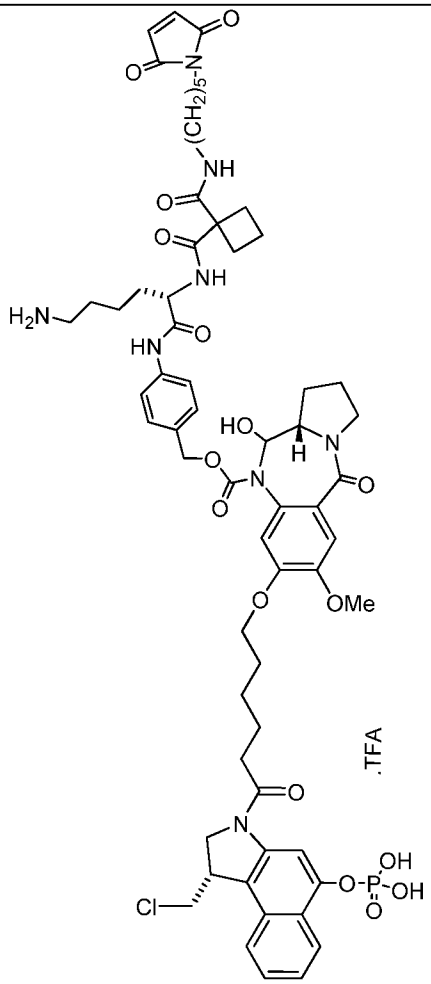
75		triazole	0.000152	Cbz		SKS
76		triazole	0.0	non-Cbz	0.000138	SUS
77		triazole	0.0	non-Cbz	0.000022	SUS
78		triazole	0.0	non-Cbz		SUS

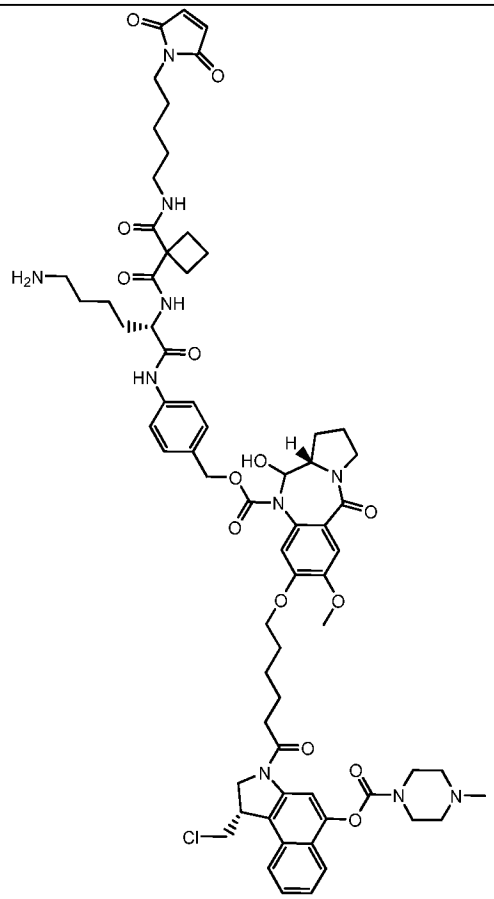
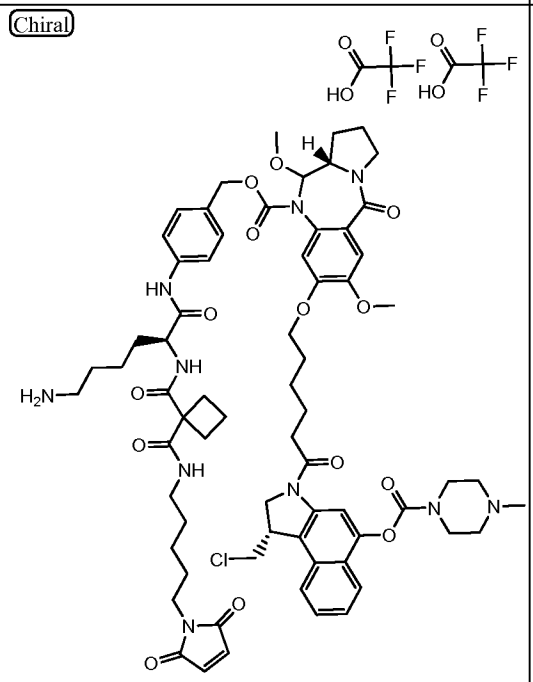
79		triazole	0.0	Cbz	0.005698	SKS
80		triazole		Cbz		SKS
81		triazole		Cbz	0.000019	SKS
82		triazole		Cbz		SKS

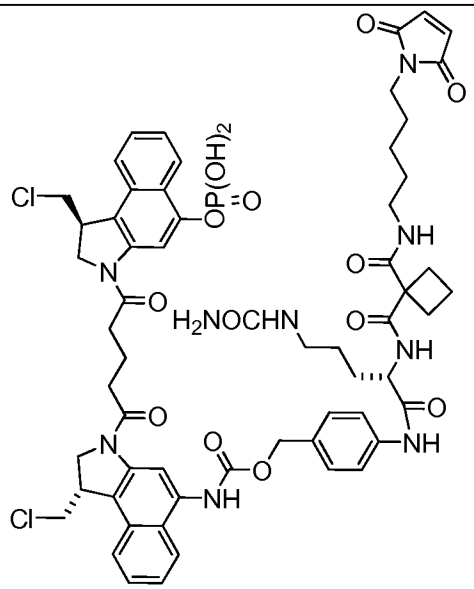
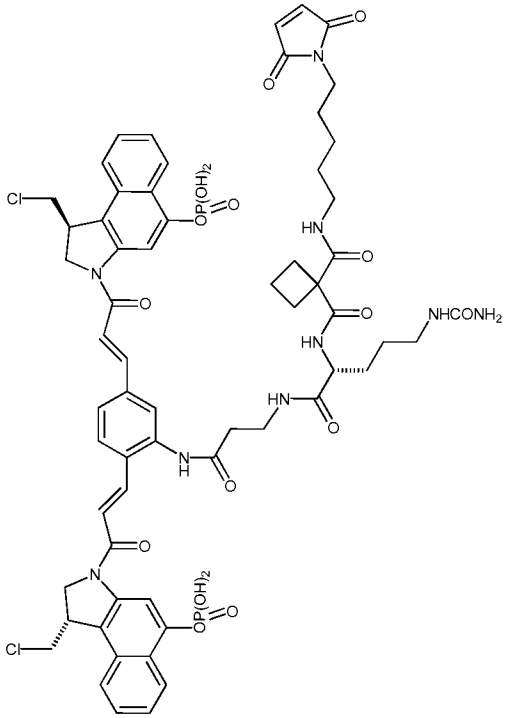
83		triazole		Cbz		SKS
84		cyclobutane-1,1-dicarbonyl		Non-Cbz	0.009691	SKS
85		cyclobutane-1,1-dicarbonyl		Non-Cbz	0.001715	SKS
86		cyclobutane-1,1-dicarbonyl		Non-Cbz	0.007420	SKS

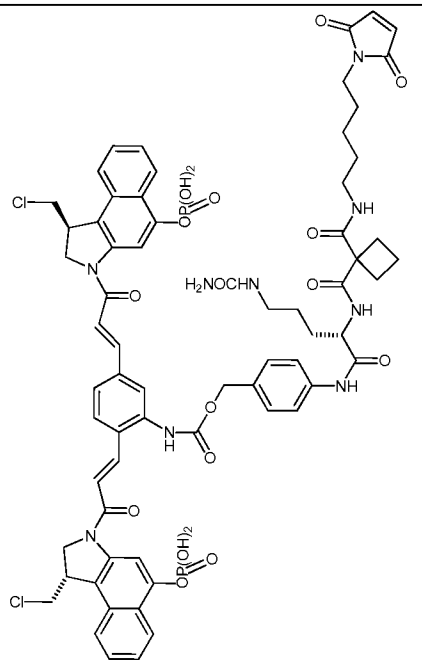
ADC Linker-Drug structures

Linker-	Correspondi	Structure	Name

drug compound	ng ADC		
CBI-PBD LD1			(11aS)-4-((S)-6-amino-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)hexanamido)benzyl 8-(6-((S)-1-(chloromethyl)-5-(phosphonooxy)-1H-benzo[e]indol-3(2H)-yl)-6-oxohexyloxy)-11-hydroxy-7-methoxy-5-oxo-2,3,11,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepine-10(5H)-carboxylate

CBI-PBD LD2	NaPi2b CBI-PBD ADC2-1 and CD33 CBI-PBD ADC2-2		[4-[[(2S)-6-amino-2-[[1-[5-(2,5-dioxopyrrol-1-yl)pentylcarbamoyl]cyclobutanecarbonyl]amino]hexanoyl]amino]phenyl]methyl (6aS)-3-[6-[(1S)-1-(chloromethyl)-5-(4-methylpiperazine-1-carbonyl)oxy-1,2-dihydrobenzo[e]indol-3-yl]-6-oxo-hexoxy]-6-hydroxy-2-methoxy-11-oxo-6a,7,8,9-tetrahydro-6H-pyrrolo[2,1-c][1,4]benzodiazepine-5-carboxylate
CBI-PBD LD3	NaPi2b CBI-PBD ADC3-1 and CD33 CBI-PBD ADC3-2		[4-[[(2S)-6-amino-2-[[1-[5-(2,5-dioxopyrrol-1-yl)pentylcarbamoyl]cyclobutanecarbonyl]amino]hexanoyl]amino]phenyl]methyl (6aS)-3-[6-[(1S)-1-(chloromethyl)-5-(4-methylpiperazine-1-carbonyl)oxy-1,2-dihydrobenzo[e]indol-3-yl]-6-oxo-hexoxy]-2,6-dimethoxy-11-oxo-6a,7,8,9-tetrahydro-6H-pyrrolo[2,1-c][1,4]benzodiazepine-5-carboxylate;2,2,2-trifluoroacetic acid

CBI-CBI LD4			4-((S)-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyl (S)-1-(chloromethyl)-3-(5-((S)-1-(chloromethyl)-5-(phosphonooxy)-1H-benzo[e]indol-3(2H)-yl)-5-oxopentanoyl)-2,3-dihydro-1H-benzo[e]indol-5-ylcarbamate
CBI-CBI LD5	anti-Napi2b 10H1.11.4B LC K149C (CBI-CBI LD5) and anti-CD22 10F4v3 LC K149C (CBI-CBI LD5)		4-((S)-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyl 2,5-bis((E)-3-((S)-1-(chloromethyl)-5-(phosphonooxy)-1H-benzo[e]indol-3(2H)-yl)-3-oxoprop-1-enyl)phenylcarbamate

CBI-CBI LD6	anti-Napi2b 10H1.11.4B LC K149C (CBI-CBI LD6) / anti-CD22 10F4v3 LC K149C (CBI-CBI LD6)		4-((S)-2-(1-(5-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)pentylcarbamoyl)cyclobutanecarboxamido)-5-ureidopentanamido)benzyl 2,5-bis((E)-3-((S)-1-(chloromethyl)-5-(phosphonooxy)-1H-benzo[e]indol-3(2H)-yl)-3-oxoprop-1-enyl)phenylcarbamate

SEQUENCES

NaPi2b humanized antibody:

In one embodiment, the NaPi2b antibody of ADCs of the present invention comprises three light chain hypervariable regions and three heavy chain hypervariable regions (SEQ ID NO:1-6), the sequences of which are shown below.

In one embodiment, the NaPi2b antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 7 and the variable heavy chain sequce of SEQ ID NO: 8

In one embodiment, the NaPi2b antibody of ADCs of the present invention comprises the light chain sequence of SEQ ID NO: 9 and the heavy chain sequence of SEQ ID NO: 10

10H1.11.4B HVR-L1	RSSETLVHSSGNTYLE	Seq ID No: 1
10H1.11.4B HVR-L2	RVSNRFS	Seq ID No: 2
10H1.11.4B HVR-L3	FQGSFNPLT	Seq ID No: 3

10H1.11.4B HVR-H1	GFSFSDFAMS	Seq ID No: 4
10H1.11.4B HVR-H2	ATIGRVAFHTYYPDSMKG	Seq ID No: 5
10H1.11.4B HVR-H3	ARHRGFDVGHFDF	Seq ID No: 6
10H1.11.4B V_L	DIQMTQSPSSLSASVGDRVTITCRSSETLVHSSGNTYLEWYQQK PGKAPKLLIYRVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCFQGSFNPLTFGQGTKVEIKR	SEQ ID NO: 7
10H1.11.4B V_H	EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGK GLEWVATIGRVAFHTYYPDSMKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCARHRGFDVGHFDFWGQGTLVTVSS	SEQ ID NO: 8
10H1.11.4B Light Chain	DIQMTQSPSSLSASVGDRVTITCRSSETLVHSSGNTYLEWYQQK PGKAPKLLIYRVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCFQGSFNPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 9
10H1.11.4B Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGFSFSDFAMSWVRQAPGK GLEWVATIGRVAFHTYYPDSMKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCARHRGFDVGHFDFWGQGTLVTVSSCSTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK	SEQ ID NO: 10

Anti-CD33 humanized antibody:

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises three light chain hypervariable regions and three heavy chain hypervariable regions, the sequences (SEQ ID NO:11-16) of which are shown below

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 17 and the variable heavy chain sequce of SEQ ID NO: 18

15G15.33-HVR L1	RSSQSLLHSNGYNYLD	SEQ ID NO:11
15G15.33-HVR L2	LGVNSVS	SEQ ID NO:12
15G15.33-HVR L3	MQALQTPWT	SEQ ID NO:13
15G15.33-HVR H1	NHAIS	SEQ ID NO:14
15G15.33-HVR H2	GIIPIFGTANYAQKFQG	SEQ ID NO:15
15G15.33-HVR H3	EWADVFDI	SEQ ID NO:16
15G15.33 V_L	EIVLTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQK PGQSPQLLIYLGVNSVSGVPDRFSGSGSGTDFTLKISRVEAEDV GVYYCMQALQTPWTFGQGTKVEIK	SEQ ID NO:17
15G15.33 V_H	QVQLVQSGAEVKKPGSSVKVSCKASGGIFSNHAISWVRQAPG QGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAFMELSS LRSEDTAVYYCAREWADVFDIWGQGTMVTVSS	SEQ ID NO:18

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the light chain sequence of SEQ ID NO: 19 and the heavy chain sequence of SEQ ID NO: 20

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises three light chain hypervariable regions and three heavy chain hypervariable regions, the sequences (Seq ID NO: 19-24) of which are shown below.

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 25 and the variable heavy chain sequce of SEQ ID NO: 26

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 27 and the variable heavy chain sequce of SEQ ID NO: 28

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 29 and the variable heavy chain sequce of SEQ ID NO: 30

In one embodiment, the anti-CD33 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 31 and the variable heavy chain sequce of SEQ ID NO: 32

9C3-HVR L1	RASQGIRNDLG	Seq ID NO:19
9C3-HVR L2	AASSLQS	Seq ID NO:20
9C3-HVR L3	LQHNSYPWT	Seq ID NO:21
9C3-HVR H1	GNYMS	Seq ID NO:22
9C3-HVR H2	LIYSGDSTYYADSVKG	Seq ID NO:23
9C3-HVR H3	DGYYVSDMVV	Seq ID NO:24
9C3 V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQK PGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP EDFATYYCLQHNSYPWTFGQGTKLEIK	Seq ID NO:25
9C3 V_H	EVQLVESGGALIQPGGSLRLSCVASGFTISGNYMSWVRQ APGKGLEWVSLIYSGDSTYYADSVKGRFNISRDISKNTVY LQMNSLRVEDTAVYYCVRDGYYVSDMVVWGKGTTVT VSS	Seq ID NO:26
9C3.2 V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQK PGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP EDFATYYCLQHNSYPWTFGQGTKLEIK	Seq ID NO:27
9C3.2 V_H	EVQLVESGGALIQPGGSLRLSCVASGFTISGNYMSWVRQ APGKGLEWVSLIYSGDSTYYADSVKGRFTISRDISKNTVY LQMNSLRVEDTAVYYCVRDGYYVSDMVVWGKGTTVT VSS	Seq ID NO:28
9C3.3 V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQK PGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP EDFATYYCLQHNSYPWTFGQGTKLEIK	Seq ID NO:29
9C3.3 V_H	EVQLVESGGALIQPGGSLRLSCVASGFTISGNYMSWVRQ APGKGLEWVSLIYSGDSTYYADSVKGRFSISRDISKNTVY LQMNSLRVEDTAVYYCVRDGYYVSDMVVWGKGTTVT VSS	Seq ID NO:30
9C3.4 V_L	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQK PGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP EDFATYYCLQHNSYPWTFGQGTKLEIK	Seq ID NO:31
9C3.4 V_H	EVQLVESGGALIQPGGSLRLSCVASGFTISGNYMSWVRQ APGKGLEWVSLIYSGDSTYYADSVKGRFAISRDISKNTVY LQMNSLRVEDTAVYYCVRDGYYVSDMVVWGKGTTVT VSS	Seq ID NO:32

Anti-CD22 humanized antibody:

In one embodiment, the anti-CD22 antibody of ADCs of the present invention comprises three light chain hypervariable regions and three heavy chain hypervariable regions (SEQ ID NO: 41-46), the sequences of which are shown below.

In one embodiment, the anti-CD22 antibody of ADCs of the present invention comprises the variable light chain sequence of SEQ ID NO: 47 and the variable heavy chain sequence of SEQ ID NO: 48

In one embodiment, the anti-CD22 antibody of ADCs of the present invention comprises the light chain sequence of SEQ ID NO: 49 and the heavy chain sequence of SEQ ID NO: 50

h10F4.V3.K149C HVR-L1	RSSQSIVHSVGNTFLE	Seq ID No: 41
h10F4.V3.K149C HVR-L2	KVSNRFS	Seq ID No: 42
h10F4.V3.K149C HVR-L3	FQGSQFPYT	Seq ID No: 43
h10F4.V3.K149C HVR-H1	GYEFSRSWMN	Seq ID No: 44
h10F4.V3.K149C HVR-H2	RIYPGDGDTNYSGKFKG	Seq ID No: 45
h10F4.V3.K149C HVR-H3	DGSSWDWYFDV	Seq ID No: 46
h10F4.V3.K149C V_L	DIQMTQSPSSLSASVGDRVTITCRSSQSIVHSVGNTFLEWYQQKP GKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCFQGSQFPYTFGQGTKVEIKR	SEQ ID NO: 47
h10F4.V3.K149C V_H	EVQLVESGGGLVQPGGSLRLSCAASGYEFSRSWMNWVRQAPG KGLEWVGRIYPGDGDTNYSGKFKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCARDGSSWDWYFDVWGQGTLVTVSS	SEQ ID NO: 48

h10F4.V3.K149C Light Chain	DIQMTQSPSSLSASVGDRVTITCRSSQSIVHSVGNTFLEWYQQKP GKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCFQGSQFPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWCVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 49
h10F4.V3.K149C Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGYEFSRSWMNWVRQAPG KGLEWVGRIYPGDGDTNYSGKFKGRFTISADTSKNTAYLQMNS LRAEDTAVYYCARDGSSWDWYFDVWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	SEQ ID NO: 50

ADC in vitro Data

The following ADCs were tested in *in vitro* assays described above and were found to be active. The activities of said ADCs are illustrated in the table below. The NaPi2b ADCs were used as a non-targeting control.

Code	Antibody ID	EOL-1 IC_{50} (ng/mL)
NaPi2b CBI-PBD ADC2-1	10H1.11.4B	2187
CD33 CBI-PBD ADC2-2	15G15.33	370
NaPi2b CBI-PBD ADC3-1	10H1.11.4B	590
CD33 CBI-PBD ADC3-2	15G15.33	18.1

Molecule Name	DAR	IGROV-1 IC50		OVCAR-3x2.1 IC50		NCI-H441 IC50	
		nM	ng/mL	nM	ng/mL	nM	ng/mL

anti-Napi2b 10H1.11.4B LC K149C (CBI-CBI LD5)	2.0	0.398	59.7	0.147	22.1	2.9	435
anti-CD22 10F4v3 LC K149C (CBI-CBI LD5)	2.0	7.6	1140	17.4	2610	4.0	600

Molecule Name	DAR	BJAB IC50		WSU-DLCL2 IC50		Jurkat IC50	
		nM	ng/mL	nM	ng/mL	nM	ng/mL
anti-CD22 10F4v3 LC K149C (CBI-CBI LD5)	2.0	0.0243	3.6	0.0130	1.95	50.2	7530
anti-Napi2b 10H1.11.4B LC K149C (CBI-CBI LD5)	2.0	62.2	9330	48.1	7215	47.8	7170

Molecule Name	DAR	IGROV-1 IC50		OVCAR-3x2.1 IC50		NCI-H441 IC50	
		nM	ng/mL	nM	ng/mL	nM	ng/mL
anti-Napi2b 10H1.11.4B LC K149C (CBI-CBI LD6)	2.0	0.189	28.4	3.7	555	11.0	1650
anti-CD22 10F4v3 LC K149C (CBI-CBI LD6)	2.0	2.9	435	>133.3	>20000	81.2	12180

Molecule Name	DAR	BJAB IC50		WSU-DLCL2 IC50		Jurkat IC50	
		nM	ng/mL	nM	ng/mL	nM	ng/mL
anti-CD22 10F4v3 LC K149C (CBI-CBI LD6)	2.0	0.0441	6.6	0.0306	4.6	>133.3	>20000
anti-Napi2b 10H1.11.4B LC K149C (CBI-CBI LD6)	2.0	>133.3	>20000	>133.3	>20000	>133.3	>20000

ADC in vivo data

Figure 1 shows efficacy comparison of CD33 ADCs in SCID mice with HL-60 human acute myeloid leukemia tumors. CD33 CBI-PBD ADC3-2 showed dose-dependent inhibition of tumor growth compared with vehicle group. The non-targeting control NaPi2b CBI-PBD ADC3-1 had minimal effect on the tumor growth.

Figure 2 shows efficacy comparison of NaPi2b ADCs in SCID-beige mice with OVCAR3X2.1 human ovarian tumors. NaPi2b CBI-PBD ADC2-1 and ADC3-1 demonstrated modest inhibition of tumor growth compared with vehicle group. The anti-tumor activity of NaPi2b CBI-PBD ADC2-1 and ADC3-1 was comparable, resulting in tumor growth delay at antibody dose of 3 mg/kg (= drug dose of 73 ug/m2).

Claims

1. An antibody-drug conjugate represented by Formula (I)

$$Ab—(L—D)_p,$$

Ab is an antibody;

L is a peptidomimetic linker represented by the following formula

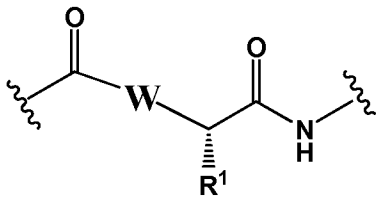
—Str—(PM)—Sp—

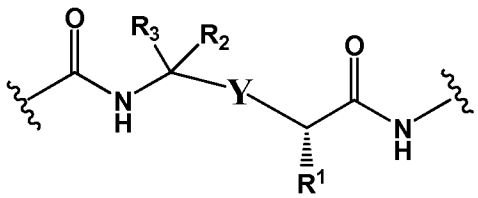
wherein

Str is a stretcher unit covalently attached to Ab;

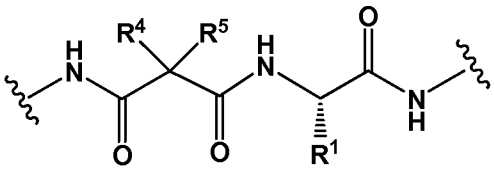
Sp is a bond or spacer unit covalently attached to a drug moiety;

PM is a non-peptide chemical moiety selected from the group consisting of:





and



W is –NH-heterocycloalkyl- or heterocycloalkyl;

Y is heteroaryl, aryl, -C(O)C_1-C_6alkylene, C_1-C_6alkylene-NH_2, C_1-C_6alkylene-NH-CH_3, C_1-C_6alkylene-N-$(CH_3)_2$, C_1-C_6alkenyl or C_1-C_6alkylenyl;

each R^1 is independently C_1-C_{10}alkyl, C_1-C_{10}alkenyl, (C_1-C_{10}alkyl)NHC(NH)NH_2 or (C_1-C_{10}alkyl)NHC(O)NH_2;

R^3 and R^2 are each independently H, C_1-C_{10}alkyl, C_1-C_{10}alkenyl, arylalkyl or heteroarylalkyl, or R^3 and R^2 together may form a C_3-C_7cycloalkyl;

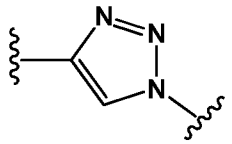
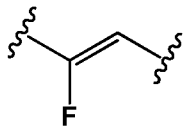
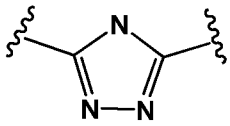
R^4 and R^5 are each independently C_1-C_{10}alkyl, C_1-C_{10}alkenyl, arylalkyl, heteroarylalkyl, (C_1-C_{10}alkyl)OCH_2-, or R^4 and R^5 together may form a C_3-C_7cycloalkyl ring;

p is an integer from 1 to 8;

D is a drug moiety.

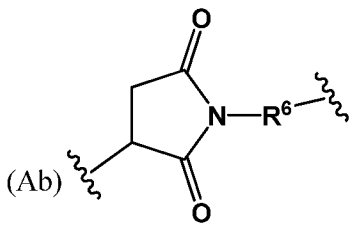
2. The antibody-drug conjugate of claim 1 wherein Y is heteroaryl; R^4 and R^5 together form a cyclobutyl ring.

3. The antibody-drug conjugate of claim 1 or 2, wherein Y is a moiety selected from the group consisting of



AND


4. An antibody-drug conjugate of claim 1 or 2, wherein

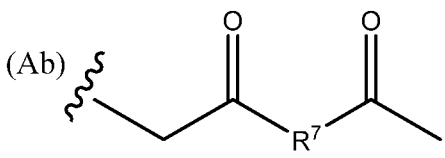
Str is a chemical moiety represented by the following formula:



wherein R^6 is selected from the group consisting of C_1-C_{10}alkylene, C_1-C_{10}alkenyl, C_3-C_8cycloalkyl, (C_1-C_8alkylene)O-, and C_1-C_{10}alkylene–C(O)N(R^a)–C_2-C_6alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, C_3-C_8cycloalkyl, C_4-C_7heterocycloalkyl, heteroarylalkyl, aryl arylalkyl, heteroarylalkyl and heteroaryl each R^a is independently H or C_1-C_6alkyl;

Sp is -C_1-C_6alkylene-C(O)NH- or —Ar—R^b—, wherein Ar is aryl or heteroaryl, R^b is (C_1-C_{10}alkylene)O-.

5. The antibody-drug conjugate of claim 1or 2, wherein Str has the formula:



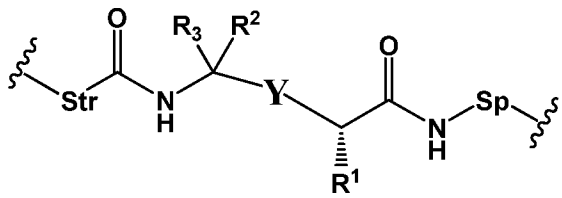
wherein R^7 is selected from C_1-C_{10}alkylene, C_1-C_{10}alkenyl, (C_1-C_{10}alkylene)O-, N(R^c)–(C_2-C_6 alkylene)–N(R^c) and N(R^c)–(C_2-C_6alkylene); where each R^c is independently H or C_1-C_6 alkyl;

Sp is -C_1-C_6alkylene-C(O)NH- or —Ar—R^b—, wherein Ar is aryl or heteroaryl, R^b is (C_1-C_{10}alkylene)O-.

6. The antibody-drug conjugate of claim 1 or 4,

wherein Ab is an antibody;

L is non-peptide chemical moiety represented by the following formula



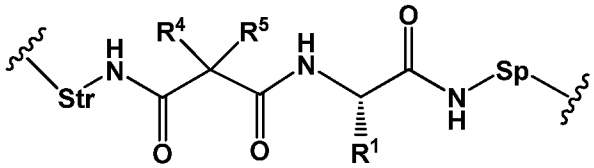
R^1 is C_1-C_6alkyl, C_1-C_6alkenyl, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

R^3 and R^2 are each independently H, C_1-C_{10}alkyl.

7. The antibody-drug conjugate of claim 1, 4 or 5,

wherein Ab is an antibody;

L is non-peptide chemical moiety represented by the following formula



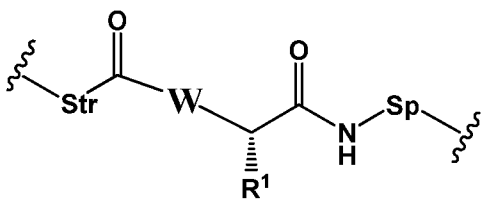
R^1 is C_1-C_6alkyl, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

R^4 and R^5 together form a C_3-C_7cycloalkyl ring.

8. The antibody-drug conjugate of claim 1 or 4,

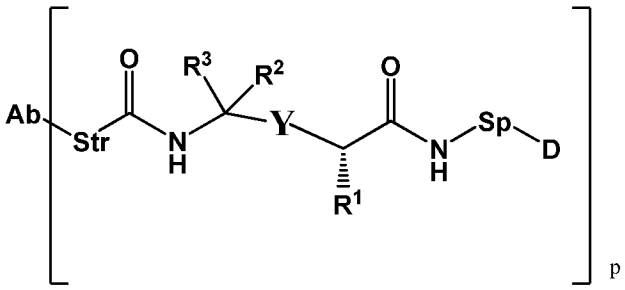
wherein Ab is an antibody;

L is non-peptide chemical moiety represented by the following formula



R^1 is C_1-C_6alkyl, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2.

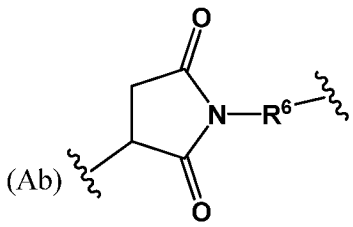
9. The antibody-drug conjugate compound of claim 1 represented by the following formula:



(I)(A1)

wherein

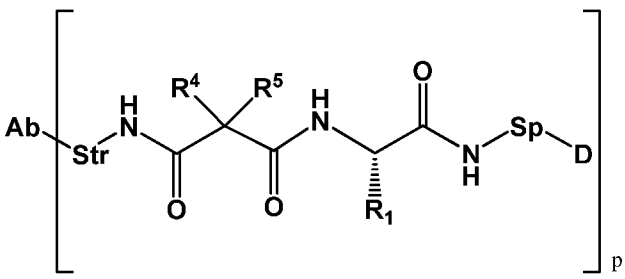
Str is a chemical moiety represented by the following formula:



R^6 is selected from the group consisting of C_1-C_{10}alkylene, and C_1-C_{10}alkylene–C(O)N(R^a)–C_2-C_6alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, C_3-C_8cycloalkyl, C_4-C_7heterocycloalkyl, heteroarylalkyl, aryl, arylalkyl, heteroarylalkyl and heteroaryl each R^a is independently H or C_1-C_6alkyl;

p is 1, 2, 3 or 4.

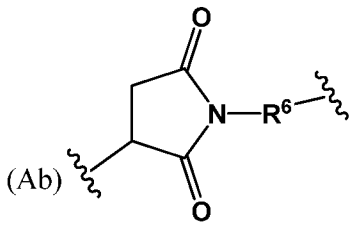
10. The antibody-drug conjugate of claim 1 represented by the following formula:



(I)(B1)

wherein

Str is a chemical moiety represented by the following formula:

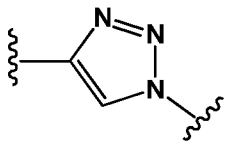


R^6 is selected from the group consisting of C_1-C_{10}alkylene, and C_1-C_{10}alkylene–C(O)N(R^a)–C_2-C_6alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, C_3-C_8cycloalkyl, C_4-C_7heterocycloalkyl, aryl, arylalkyl, heteroarylalkyl and heteroaryl each R^a is independently H or C_1-C_6alkyl;

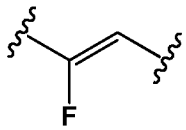
p is 1, 2, 3 or 4.

11. The antibody-drug conjugate of any one of claims 4-6, 9, wherein Y is heteroaryl, aryl or alkenyl; R^6 is C_1-C_{10}alkylene.

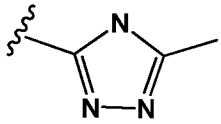
12. The antibody-drug conjugate of claim 1 or 9, wherein Y is



13. The antibody-drug conjugate of claim 1 or 9, wherein Y is



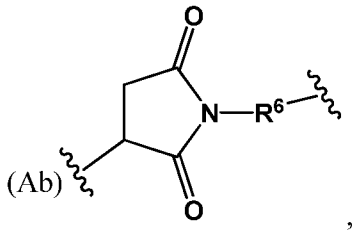
14. The antibody-drug conjugate of claim 1 or 9, wherein Y is



15. A compound of any one of claims 9-14,

wherein

Str is a chemical moiety represented by the following formula:

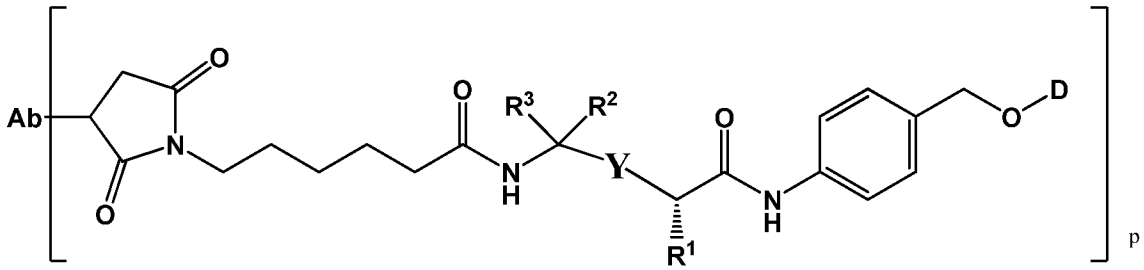


,

R^6 is C_1-C_6alkylene;

Sp is -C_1-C_6alkylene-C(O)NH- or —Ar—R^b—, where Ar is aryl, R^b is (C_1-C_3alkylene)O-.

16. The antibody-drug conjugate compound of claim 1, 4, 6 or 9, which is represented by the following formula:



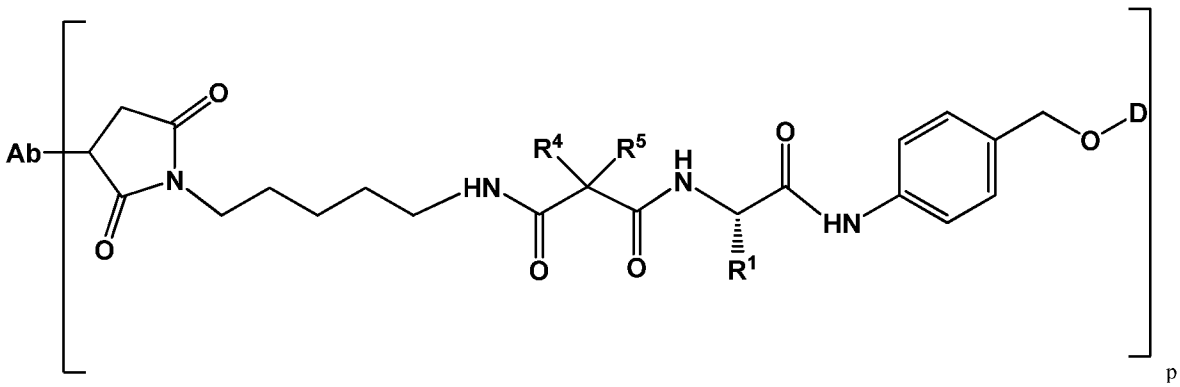
(I)(A2)

wherein

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2

p is 1, 2 ,3 or 4.

17. The antibody-drug conjugate compound of claim 1, 4, 7 or 10, which is represented by the following formula:



(I)(B2)

wherein

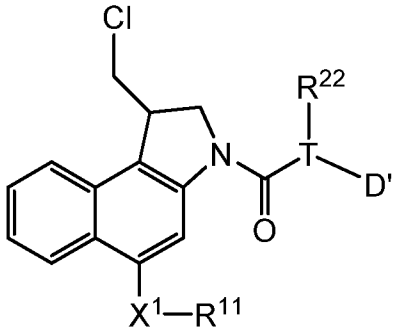
p is 1, 2, 3 or 4;

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

R^4 and R^5 are each independently C_1-C_6alkyl, wherein said alkyl are unsubstituted, or R^4 andR^5 may form a C_3-C_7cycloalkyl ring.

18. The antibody-drug conjugate of claim 1-17, wherein

D is the dimer drug moiety having the formula:



wherein

R^{11} is selected from H, $P(O)_3H_2$, $C(O)NR^{aa}R^{bb}$, or a bond to L;

R^{22} is selected from H, $P(O)_3H_2$, $C(O)NR^{aa}R^{bb}$, or a bond to L;

R^{aa} and R^{bb} are independently selected from H and C_1–C_6alkyl optionally substituted with one or more F,

or R^{aa} and R^{bb} form a five or six membered heterocycloalkyl group;

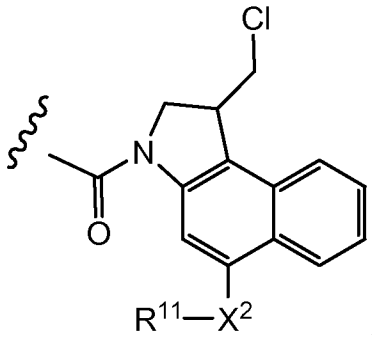
T is a tether group selected from C_3–C_{12}alkylene, Y^1, (C_1–C_6alkylene)– Y^1–(C_1–C_6 alkylene), (C_1–C_6alkylene)– Y^1–(C_1–C_6alkylene)– Y^1–(C_1–C_6alkylene), (C_2–C_6alkenylene)– Y^1–(C_2–C_6alkenylene), and (C_2–C_6alkynylene)– Y^1–(C_2–C_6alkynylene);

where Y^1 is independently selected from O, S, NR^{11}, aryl, and heteroaryl;

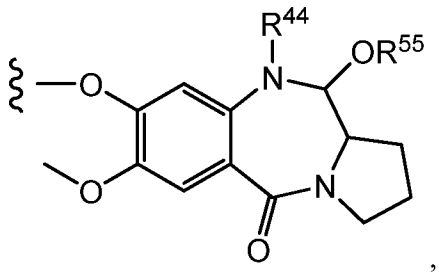
wh where alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with F, OH, O(C_1–C_6alkyl), NH_2, $NHCH_3$, $N(CH_3)_2$, $NHC(O)(C_1$-C_6alkylene$)_m$, $OP(O)_3H_2$, and C_1–C_6alkyl, where alkyl is optionally substituted with one or more F, m is 0 or 1;

or alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with a bond to L, wherein the bond to L may connect through one of the optional substituents;

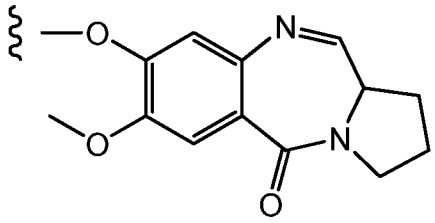
D' is a drug moiety selected from:



,



, and



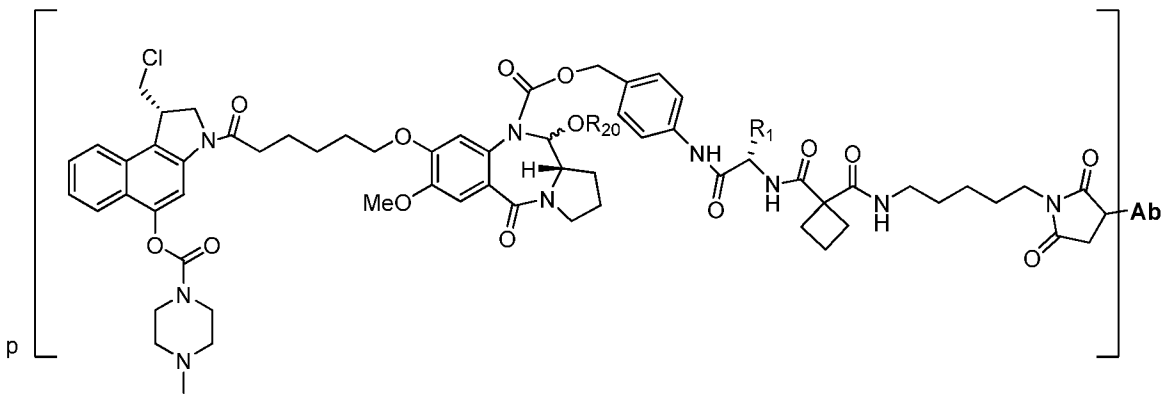
where the wavy line indicates the site of attachment to T;

X^1 and X^2 are independently selected from O and NR^{33}, where R^{33} is selected from H, C(O), and C_1–C_6alkyl optionally substituted with one or more F, or X^1 and X^2 are each independently absent;

R^{44} is H, CO_2R, C(O), or a bond to L, where R is C_1–C_6alkyl or benzyl; and

R^{55} is H or C_1–C_6alkyl.

19. The antibody-drug conjugate of claim 1, which is represented by the following formula:



(I)(B6)

wherein,

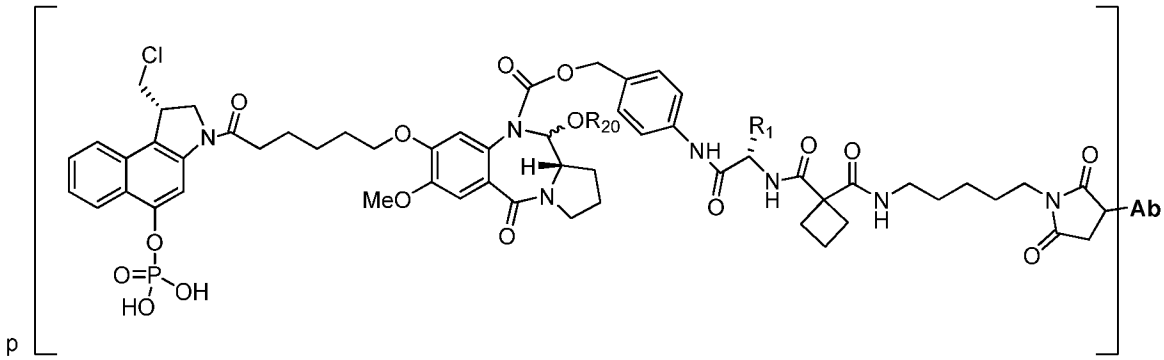
Ab is an antibody that binds to a target seleted from Her2, CLL1, CD33, CD22 and NaPi2b;

P is 1-4; and

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

R^{20} is H or Me;

20. The antibody-drug conjugate of claim 1, which is represented by the following formula:



(I)(B7)

wherein,

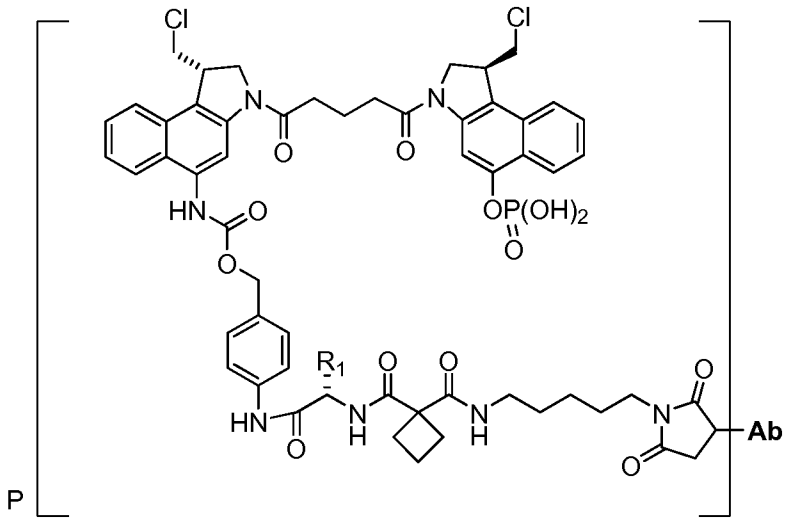
Ab is an antibody that binds to a target seleted from Her2, CLL1, CD33, CD22 and NaPi2b;

P is 1-4;

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

R^{20} is H or Me;

21. The antibody-drug conjugate of claim 1, which is represented by the following formula:



(I)(B8)

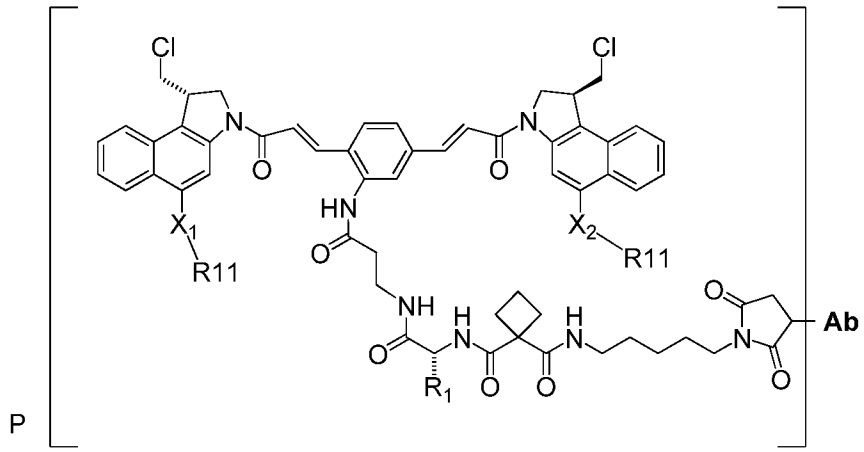
wherein,

Ab is an antibody that binds to a target seleted from Her2, CLL1, CD33, CD22 and NaPi2b;

P is 1-4; and

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

22. The antibody-drug conjugate of claim 1, which is represented by the following formula:



(I)(B9)

wherein,

Ab is an antibody that binds to a target seleted from Her2, CLL1, CD33, CD22 and NaPi2b;

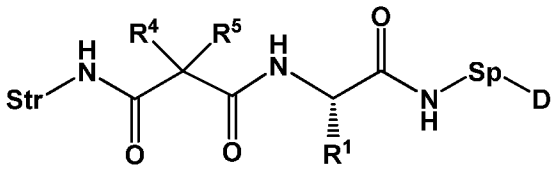
P is 1-4;

R^1 is C_1-C_6alkyl-NH_2, (C_1-C_6alkyl)NHC(NH)NH_2 or (C_1-C_6alkyl)NHC(O)NH_2;

X^1 and X^2 are each independently absent or O;

Each R^{11} is independently C(O)N-piperazine(CH_3) or $P(O)_3H_2$.

23. A non-peptide compound of Formula (I)(B)(LD1):



(I)(D)(LD1)

wherein

Str is a stretcher unit which can be covalently attached to an antibody;

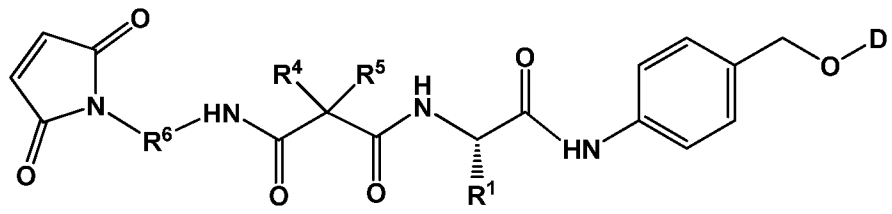
Sp is a bond or a spacer unit covalently attached to a drug moiety;

R^1 is C_1-C_{10}alkyl, (C_1-C_{10}alkyl)NHC(NH)NH_2 or (C_1-C_{10}alkyl)NHC(O)NH_2;

R^4 and R^5 are each independently C_1-C_{10}alkyl, arylalkyl, heteroarylalkyl, (C_1-C_{10}alkyl)OCH_2-, or R^4 and R^5 may form a C_3-C_7cycloalkyl ring;

D is a drug moiety.

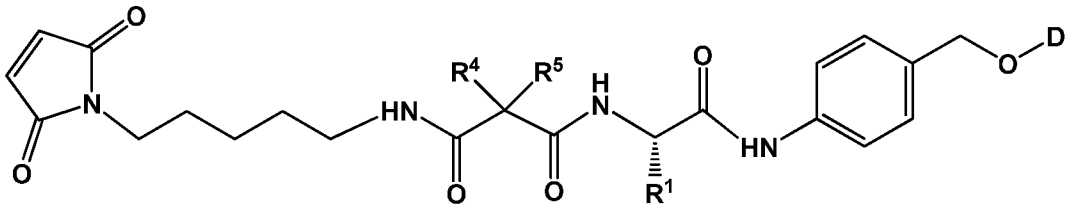
24. The compound of claim 23 represented by the following formula



(I)(B)(LD2)

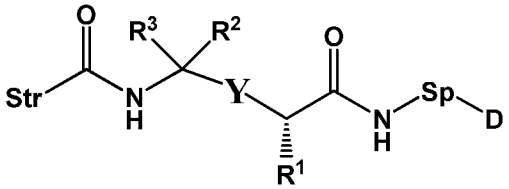
wherein R_6 is C_1-C_{10}alkylene; R^4 andR^5 together form a C_3-C_7cycloalkyl ring.

25. The compound of claim 23 represented by the following formula



(I)(B)(LD3)

26. A compound of Formula:



(I)(A)(LD1)

wherein

Str is a stretcher unit which can be covalently attached to an antibody;

Sp is an optional spacer unit covalently attached to a drug moiety;

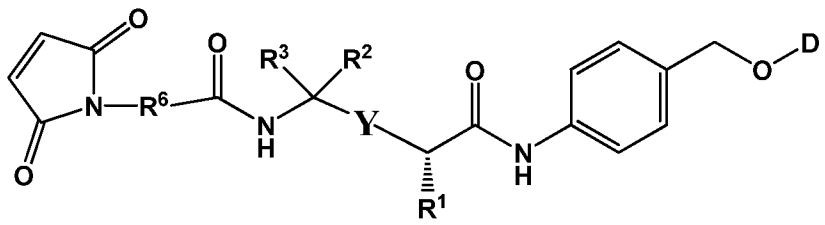
Y is heteroaryl, aryl, -C(O)C_1-C_6alkenyl, C_1-C_6alkenyl or - C_1-C_6alkenyl-NH-;

R^1 is C_1-C_{10}alkyl, (C_1-C_{10}alkyl)NHC(NH)NH_2 or (C_1-C_{10}alkyl)NHC(O)NH_2;

R^3 and R^2 are each independently H, C_1-C_{10}alkyl, arylalkyl or heteroarylalkyl, or R^3 and R^2 together may form a C_3-C_7cycloalkyl;

D is a drug moiety.

27. The compound of claim 26 represented by the following formula:

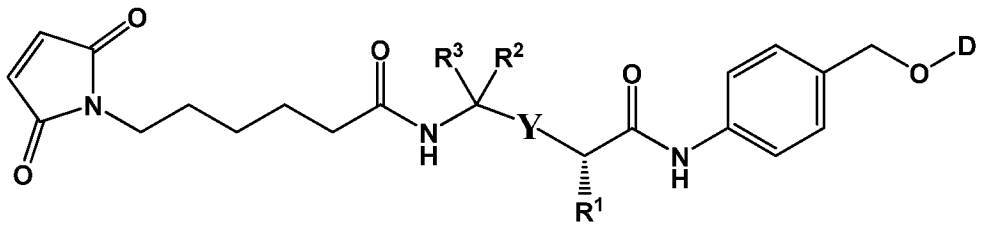


(I)(A)(LD2)

wherein

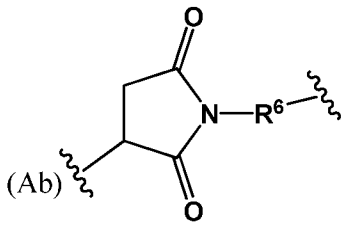
R_6 is C_1-C_{10}alkylene.

28. The compound of claim 20, which is represented by the following formula:



(I)(A)(LD3)

29. The compound of claim 23 or 26, wherein Str has the following formula:



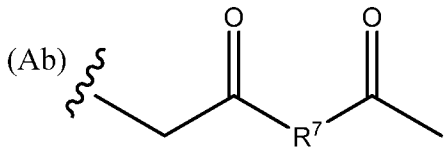
wherein R^6 is selected from the group consisting of C_1-C_{10}alkylene, C_3-C_8cycloalkyl, O-(C_1-C_8alkylene), and C_1-C_{10}alkylene–C(O)N(R^a)–C_2-C_6alkylene, where each alkylene may be substituted by one to five substituents selected from the group consisting of halo, trifluoromethyl, difluoromethyl, amino, alkylamino, cyano, sulfonyl, sulfonamide, sulfoxide, hydroxy, alkoxy, ester, carboxylic acid, alkylthio, C_3-C_8cycloalkyl, C_4-C_7heterocycloalkyl aryl, arylalkyl, heteroarylalkyl and heteroaryl each R^a is independently H or C_1-C_6alkyl;

Sp is -C_1-C_6alkylene-C(O)NH- or —Ar—R^b—, wherein Ar is aryl or heteroaryl, R^b is (C_1-C_{10}alkylene)O-.

30. The compound of claim 26, wherein R^6 is C_1-C_{10}alkylene, Sp is —Ar—R^b—, wherein Ar is aryl R^b is (C_1-C_6alkylene)O-.

31. The compound of claim 23 and 26, where R_6 is –$(CH_2)_q$ is 1-10;

32. The compound of claim 18 or 21, wherein Str has the formula:



wherein R^7 is selected from C_1-C_{10}alkylene, C_1-C_{10}alkylene–O, N(R^c)–(C_2-C_6 alkylene)–N(R^c) and N(R^c)–(C_2-C_6alkylene); where each R^c is independently H or C_1-C_6 alkyl; Sp is -C_1-C_6alkylene-C(O)NH- or —Ar—R^b—, wherein Ar is aryl or heteroaryl, R^b is (C_1-C_{10} alkylene)O-.

33. The compound of claim 26, wherein R^6 is C_1-C_{10} alkylene, Sp is —Ar—R^b—, wherein Ar is aryl R^b is (C_1-C_6 alkylene)O-.

34. The antibody-drug conjugate according to any one of claims 1-22, wherein p is 2.

35. The antibody conjugate of any one of claims 1-22, wherein the antibody binds to one or more of polypeptides selected from the group consisting of:

CLL1;
BMPR1B;
E16;
STEAP1;
0772P;
MPF;
NaPi2b;
Sema 5b;
PSCA hlg;
ETBR;
MSG783;
STEAP2;
TrpM4;
CRIPTO;
CD21;
CD79b;
FcRH2;
HER2;
NCA;
MDP;

IL20Rα;
Brevican;
EphB2R;
ASLG659;
PSCA;
GEDA;
BAFF-R;
CD22;
CD79a;
CXCR5;
HLA-DOB;
P2X5 ;
CD72;
LY64;
FcRH1;
IRTA2;
TENB2;
PMEL17;
TMEFF1;
GDNF-Ra1;
Ly6E;
TMEM46;
Ly6G6D;
LGR5;
RET;
LY6K;
GPR19;
GPR54;
ASPHD1;
Tyrosinase;
TMEM118;
GPR172A;
MUC16 and
CD33.

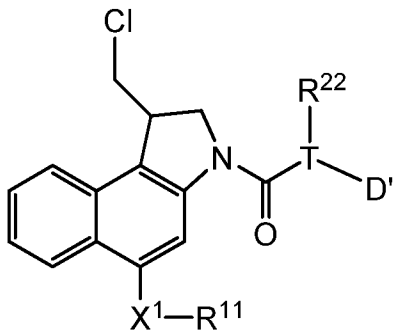
36. A compound according to any of the above claims, wherein D is an anti-tumor agent or an antibiotic moiety.

37. The compound of any one of the above claims, wherein D is selected from the group consisting of:

methotrexate, adriamicin, vincristine, vinblastine, etoposide, doxorubicin, melphalan, mitomycin C, chlorambucil and daunorubicin.

38. The compound of any one of the above claims 23-33, wherein

wherein D is the dimer drug moiety having the formula:



wherein

R^{11} is selected from H, $P(O)_3H_2$, $C(O)NR^{aa}R^{bb}$, or a bond to L;

R^{22} is selected from H, $P(O)_3H_2$, $C(O)NR^{aa}R^{bb}$, or a bond to L;

R^{aa} and R^{bb} are independently selected from H and C_1–C_6alkyl optionally substituted with one or more F,

or R^{aa} and R^{bb} form a five or six membered heterocycloalkyl group;

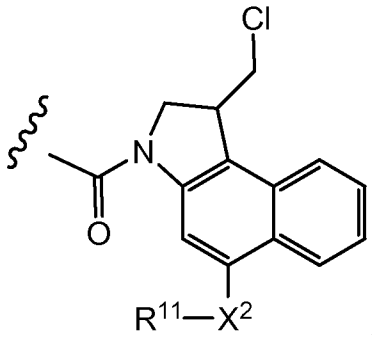
T is a tether group selected from C_3–C_{12}alkylene, Y^1, (C_1–C_6alkylene)– Y^1–(C_1–C_6 alkylene), (C_1–C_6alkylene)– Y^1–(C_1–C_6alkylene)– Y^1–(C_1–C_6alkylene), (C_2–C_6alkenylene)–Y^1–(C_2–C_6alkenylene), and (C_2–C_6alkynylene)– Y^1–(C_2–C_6alkynylene);

where Y^1 is independently selected from O, S, NR^{11}, aryl, and heteroaryl;

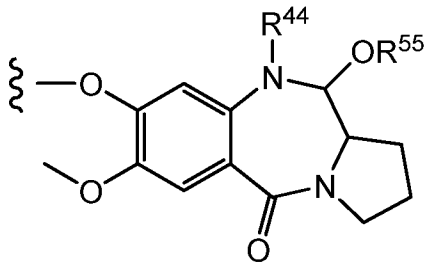
where alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with F, OH, O(C_1–C_6alkyl), NH_2, $NHCH_3$, $N(CH_3)_2$, $NHC(O)(C_1$-C_6alkylene$)_m$, $OP(O)_3H_2$, and C_1–C_6alkyl, where alkyl is optionally substituted with one or more F, m is 0 or 1;

or alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with a bond to L, wherein the bond to L may connect through one of the optional substituents;

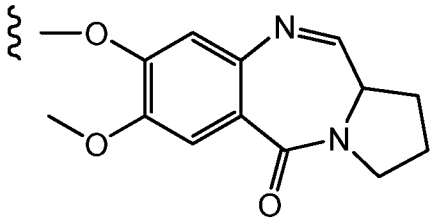
D' is a drug moiety selected from:



,



, and



where the wavy line indicates the site of attachment to T;

X^1 and X^2 are independently selected from O and NR^{33}, where R^{33} is selected from H, C(O), and C_1–C_6alkyl optionally substituted with one or more F, or X^1 and X^2 are each independently absent;

R^{44} is H, CO_2R, C(O), or a bond to L, where R is C_1–C_6alkyl or benzyl; and

R^{55} is H or C_1–C_6alkyl.

39. An antibody conjugate compound of which is prepared by covalently attaching an antibody with one to eight compounds of claims 23-33.

40. A method of treating a disease in a human in need thereof, comprising administering to said human an effective amount of an Antibody-drug conjugate of claim 1.

41. A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier thereof.

42. A compound of claim 30, wherein the antibody binds to one or more of polypeptides selected from the group consisting of:

CLL1;

STEAP1;

NaPi2b;
STEAP2;
TrpM4;
CRIPTO;
CD21;
CD79b;
FcRH2;
HER2;
CD22;
CD79a;
CD72;
LY64;
Ly6E;
MUC16; and
CD33.

43. The antibody-drug conjugate of any one of claims 1-22, wherein the antibody binds to CD33.

44. The antibody-drug conjugate of claim 34, wherein the anti-CD33 antibody comprise an HVR-L1 comprising the amino acid sequence of SEQ ID NO:11, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:13, an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 14, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:15, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 16.

45. The antibody-drug conjugate of claim 34, wherein said anti-CD33 antibody comprises a VL domain comprising the amino acid sequence of SEQ ID NO:17 and a VH domain comprising the amino acid sequence of SEQ ID NO:18.

46. The antibody-drug conjugate of any one of claims 1-22, wherein the antibody binds to NaPi2b.

47. The antibody-drug conjugate of claim 46, wherein the NaPi2b antibody comprise an HVR-L1 comprising the amino acid sequence of SEQ ID NO:1, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:2, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:3, an

HVR-H1 comprising the amino acid sequence of SEQ ID NO: 4, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:5, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 6.

48. The antibody-drug conjugate of claim 46, wherein said NaPi2b antibody comprises a VL domain comprising the amino acid sequence of SEQ ID NO:7 and a VH domain comprising the amino acid sequence of SEQ ID NO:8.

49. The antibody-drug conjugate of claim 46, wherein said NaPi2b antibody comprises an amino acid sequence of SEQ ID NO:9 and an amino acid sequence of SEQ ID NO: 10.

50. The antibody-drug conjugate of any one of claims 1-22, wherein the antibody binds to CD-22.

51. The antibody-drug conjugate of claim 50, wherein the CD-22 antibody comprise an HVR-L1 comprising the amino acid sequence of SEQ ID NO:41, an HVR-L2 comprising the amino acid sequence of SEQ ID NO:42, an HVR-L3 comprising the amino acid sequence of SEQ ID NO:43, an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 44, an HVR-H2 comprising the amino acid sequence of SEQ ID NO:45, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 46.

52. The antibody-drug conjugate of claim 50, wherein said CD-22 antibody comprises a VL domain comprising the amino acid sequence of SEQ ID NO:47 and a VH domain comprising the amino acid sequence of SEQ ID NO:48.

53. The antibody-drug conjugate of claim 50, wherein said CD-22 antibody comprises an amino acid sequence of SEQ ID NO:49 and an amino acid sequence of SEQ ID NO: 50.

54. The antibody-drug conjugate of claim 50, wherein said CD-22 antibody comprises an amino acid sequence that has at least 95% sequence identity with amino acid sequence of SEQ ID NO:49 and that comprises an amino acid sequence that has at least 95%sequence identity with an amino acid sequence of SEQ ID NO: 50.