ANTICANCER DERIVATIVES, PREPARATION THEREOF AND THERAPEUTIC USE THEREOF

The present invention relates to conjugates of pyrrolo[1,4]benzodiazepine (PBD) dimers, to compositions containing them and to their therapeutic use, especially as anticancer agents. The invention also relates to the process for preparing the conjugates and to their use as anticancer agents, and also to the dimers themselves.

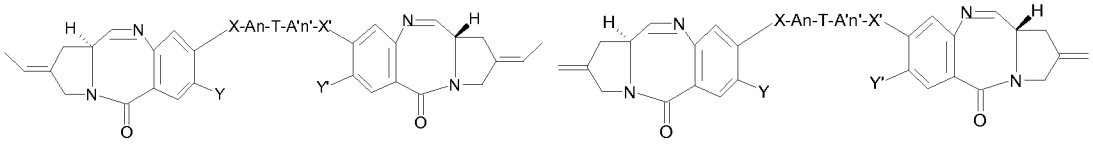
[Technical field]

Pyrrolo[1,4]benzodiazepine dimers are anticancer agents that act by covalently binding to cellular DNA. These derivatives have been described in patent applications **WO 00/12508** and **WO 2005/085260** and also in the following publications: *Eur. J. Med. Chem.* **2005**, *40*, 641–654; *Tetrahedron Letters* **1988**, *29*(40), 5105-5108.

Conjugates chemistry has been known for many years and has been applied to several families of cytotoxic agents, for instance maytansinoids (**WO 04103272**), taxanes (**WO 06061258**), leptomycins (**WO 07144709**), **CC-1065** and analogues thereof (**WO 2007102069**); with regard to conjugates, see also Monneret C. et al., *Bulletin du Cancer* **2000**, *87*(11), 829-38; Ricart A.D. et al., *Nature Clinical Practice Oncology* **2007**, *4*, 245-255; Singh R. and Rickson H.K., *Therapeutic Antibodies: Methods and Protocols*, **2009**, 525, 445-467.

[Prior art]

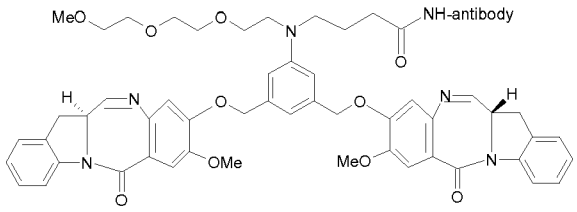
Conjugates of pyrrolo[1,4]benzodiazepine dimers have been described in patent applications **WO 07085930** and **WO 2009/016516**. The dimers used have the formulae:



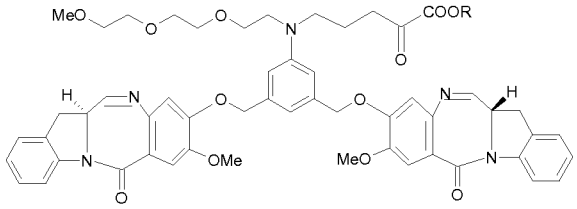
in which T may represent an aryl or heteroaryl group substituted with –G-D-$(Z)_p$-SZ_a or –G-D-$(Z)_p$-$C(=O)Z_bR_b$. G represents a single or double bond or alternatively –O-, -S- or –NR-. D represents a single bond or one of the following groups: –E-, -E-NR-, -E-NR-F-, -E-O-, -E-O-F-, -E-NR-CO-, -E-NR-CO-F-, -E-CO-, -CO-E-, -E-CO-F, -E-S-, -E-S-F-, -E-NR-C-S-, -E-NR-CS-F- for which E and F are chosen from -$(OCH_2CH_2)_i$alkyl$(OCH_2CH_2)_j$-, -alkyl$(OCH_2CH_2)_i$-alkyl, -$(OCH_2CH_2)_i$, -$(OCH_2CH_2)_i$cycloalkyl$(OCH_2CH_2)_j$-, -$(OCH_2CH_2)_i$heterocyclyl-$(OCH_2CH_2)_j$-, -$(OCH_2CH_2)_i$aryl$(OCH_2CH_2)_j$-, -$(OCH_2CH_2)_i$heteroaryl$(OCH_2CH_2)_j$-, –alkyl-$(OCH_2CH_2)_i$alkyl$(OCH_2CH_2)_j$-, -alkyl-$(OCH_2CH_2)_i$-, -alkyl-$(OCH_2CH_2)_i$cycloalkyl-$(OCH_2CH_2)_j$-, -alkyl$(OCH_2CH_2)_i$heterocyclyl$(OCH_2CH_2)_j$-, -alkyl-$(OCH_2CH_2)_i$aryl-

$(OCH_2CH_2)_j$-, -alkyl$(OCH_2CH_2)_i$heteroaryl$(OCH_2CH_2)_j$-, -cycloalkyl-alkyl-, -alkyl-cycloalkyl-, -heterocyclyl-alkyl-, -alkyl-heterocyclyl-, -alkyl-aryl-, -aryl-alkyl-, -alkyl-heteroaryl-, -heteroaryl-alkyl-. i and j represent integers ranging from 0 to 2000. Z represents an alkyl group and p is an integer equal to 0 or 1. In these compounds, the R of the group NR does not comprise a PEG chain.

Immunogen described in November 2009 at the EORTC Congress (Abstract B-126) the following conjugate:

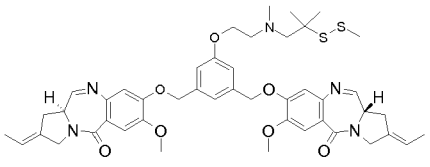


which is distinguished by the nature of the linker and of the cytotoxic compound. This compound was redescribed at the Sixth Annual PEGS Congress in Boston which took place on 17 to 21 May 2010, and also the following precursors:

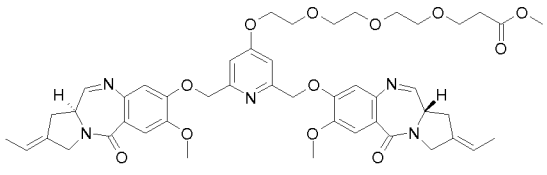


with R = N-succinimidyl or methyl.

The following dimers are especially described, respectively, in **WO 07085930** and **WO 2009/016516**:



(Ex. 28)



(Ex. 5)

These three applications do not describe or suggest the novel linkers of the invention, which comprise one or two pegylated chains.

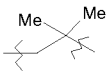
[Technical problem]

The technical problem that the present invention intends to solve is that of proposing novel conjugates of pyrrolo[1,4]benzodiazepine dimers.

[Definitions]

The following definitions apply:

- conjugate: a cell binding agent to which is covalently attached at least one molecule of a cytotoxic compound;
- cell binding agent: a molecule that has affinity for a biological target: it may be, for example, a ligand, a protein, an antibody, more particularly a monoclonal antibody, a protein or antibody fragment, a peptide, an oligonucleotide or an oligosaccharide. The binding agent has the function of directing the biologically active compound as a cytotoxic agent to the biological target;
- biological target: an antigen (or group of antigens) preferentially located at the surface of cancer cells or stromal cells associated with this tumour; these antigens possibly being, for example, a growth factor receptor, a mutated "tumour suppressant" gene or oncogene product, an angiogenesis-related molecule, an adhesion molecule;
- linker: a set of atoms for covalently attaching a cytotoxic compound to the binding agent;
- alkyl group: a saturated aliphatic hydrocarbon-based group obtained by removing a hydrogen atom from an alkane. The alkyl group may be linear or branched. Examples that may be mentioned include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, 2,2-dimethylpropyl, hexyl;
- cycloalkyl group: a cyclic alkyl group comprising between 3 and 8 carbon atoms engaged in the ring structure. Examples that may be mentioned include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl groups;
- aryl group: a monocyclic or bicyclic aromatic group not containing any heteroatoms. It is more particularly a phenyl or naphthyl group;
- heteroaryl group: a monocyclic or bicyclic aromatic group comprising at least one heteroatom (O, S, N) engaged in the ring and connected to the carbon atoms forming the ring. It is more particularly a pyridyl, pyrrolyl, thienyl, furyl, pyrimidinyl or triazolyl group;
- heterocycloalkyl group: a cycloalkyl group comprising at least one heteroatom (O, S, N) engaged in the ring and connected to the carbon atoms forming the ring. It is more particularly a piperazino, N-methylpiperazino, morpholino, piperidino or pyrrolidino group;
- alkoxy group: a group -O-alkyl, in which the alkyl group is as defined above;
- alkanoyloxy group: a group –O-CO-alkyl, in which the alkyl group is as defined above;
- alkylene group: a saturated divalent group of empirical formula $–C_mH_{2m}$-, obtained by removing two hydrogen atoms from an alkane. Examples that may be mentioned include methylene ($-CH_2-$), ethylene ($-CH_2CH_2-$), propylene ($-CH_2CH_2CH_2-$), butylene

(-$CH_2CH_2CH_2CH_2$-), isobutylene () and hexylene (-$CH_2CH_2CH_2CH_2CH_2CH_2$-) groups. The alkylene group may more particularly be of formula –$(CH_2)_m$-, m representing an integer;

- in the ranges of values, the limits are included (e.g. a range of the type "i ranging from 1 to 6" includes the limits 1 and 6. Furthermore, the range also describes all points in the range; thus, "i ranging from 1 to 6" describes the values 1, 2, 3, 4, 5 and 6.

Abbreviations used

EtOAc: ethyl acetate; ALK: alkylene group; (C_x-C_y)ALK: group (C_x-C_y)alkylene; TLC: thin-layer chromatography; MSC: methanesulfonyl chloride; DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; DCC: N,N'-dicyclohexylcarbodiimide; DCM: dichloromethane; DEAD: diethyl azodicarboxylate; DIC: N,N'-diisopropylcarbodiimide; DIPEA: N,N-diisopropylethylamine; DMA: dimethylacetamide; DMAP: 4-dimethylaminopyridine; DME: dimethoxyethane; DMF: dimethylformamide; DMSO: dimethyl sulfoxide; $e_{WL\ nm}$: molar extinction coefficient at the wavelength WL; EEDQ: 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline; EDCI: N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide; EDTA: ethylenediaminetetraacetic acid; Fmoc: fluorenylmethoxycarbonyl; Hal: halogen atom; HOBt: 1-hydroxybenzotriazole; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; WL: wavelength; Me: methyl; NHS: N-hydroxysuccinimide; NMP: N-methylpyrrolidinone; RP: reduced pressure; Rf: retention factor; SEC: steric exclusion chromatography; RT: room temperature; TBDMS: *tert*-butyldimethylsilyl; TEA: triethylamine; TFA: trifluoroacetic acid; TIPS: triisopropylsilyl; THF: tetrahydrofuran; t_R: retention time.

[Figures]

Figure 1: modification of the binding agent by SPDP;

Figure 2: modification of the binding agent by an iminothiolane;

Figure 3: deconvoluted high resolution mass spectrum of the conjugate of Ex. 8 after deglycosylation;

Figure 4: deconvoluted high resolution mass spectrum (HRMS) of the non-deglycosylated conjugate of Ex. 9;

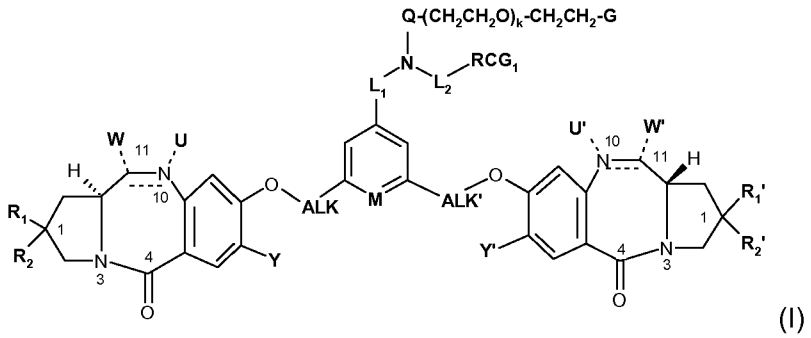
Figure 5: deconvoluted high resolution mass spectrum (HRMS) of the non-deglycosylated conjugate of Ex. 10;

Figure 6: deconvoluted high resolution mass spectrum (HRMS) of the non-deglycosylated conjugate of Ex. 11.

These figures show for each conjugate the distribution of the species bearing from 0 to 8 tomaymycin dimers (D_0: no dimers; D_x: x dimers).

[Brief description of the invention]

The invention relates to compounds of formula (I):



(I)

in which:

- ---- represents a single bond or a double bond, with the condition that

  if ---- represents a single bond, then:

  - **U** and/or **U'**, which may be identical or different, represent, independently of each other, H;
  - **W** and/or **W'**, which may be identical or different, represent, independently of each other: OH, -OR, –OCOR, -COOR, -OCOOR, –OCONRR', a cyclic carbamate such that N10 and C11 are included in a ring, –NRCONRR', -OCSNHR, a cyclic thiocarbamate such that N10 and C11 are included in a ring, -SH, -SR, -SOR, -SOOR, $–SO_3^-$, -NRSOOR', –NRR', a cyclic amine such that N10 and C11 are included in a ring, -NROR', –NRCOR', $–N_3$, -CN, Hal, a trialkylphosphonium or triarylphosphonium group;

  if ---- represents a double bond, then:

  - **U** and **U'** are absent;
  - **W** and/or **W'**, which may be identical or different, represent, independently of each other, H;

- **R_1**, **R_2**, **R_1'**, **R_2'**, which may be identical or different, represent, independently of each other: H, Hal or a group (C_1-C_6)alkyl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, an aryl or heteroaryl group, $S(O)_qR$ with q = 0, 1 or 2;

  or alternatively

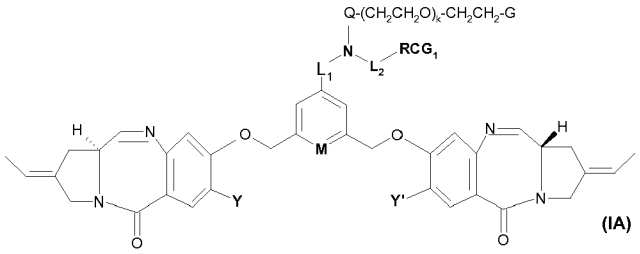
  R_1 and **R_2** and/or **R_1'** and **R_2'** together form, respectively, a double bond $=CH_2$ or $=CH-CH_3$;

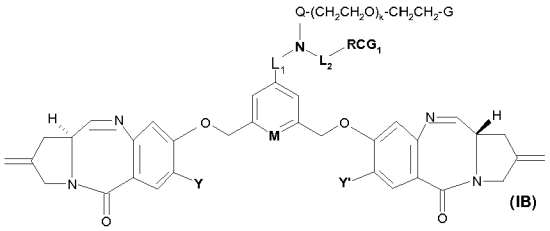
- **Y** and **Y'**, which may be identical or different, represent, independently of each other, H or -OR;
- **M** represents CH or N;
- **ALK** and **ALK'** denote a group (C_1-C_6)alkylene;

- **R** and **R'** represent, independently of each other, H or a group (C_1-C_6)alkyl or aryl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, an aryl or heteroaryl group;
- L_1 represents:
  - a single bond;
    or
  - the group -$(OCH_2CH_2)_i$-, attached to the phenyl or pyridyl ring via the oxygen atom, i representing an integer ranging from 2 to 40, preferably from 2 to 10, preferably equal to 3;
    or
  - the group -D-(C_1-C_6)ALK- attached to the phenyl or pyridyl ring via D, in which D represents –O-, –NH- or –N(C_1-C_4)alkyl-;
- L_2 represents:
  - a group -(C_1-C_6)ALK-;
    or
  - the group –$(CH_2CH_2O)_j$-CH_2CH_2-, **j** representing an integer ranging from 1 to 40, preferably from 1 to 10, preferably equal to 2 or 3;
    or
  - a group -$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-, attached to the nitrogen atom via the unit -(CH_2CH_2O)-, **j** representing an integer ranging from 1 to 40, preferably from 1 to 10, preferably equal to 2 or 3, and **R"** representing H or a group (C_1-C_4)alkyl;
- **Q** represents a single bond or the group C(=O);
- **k** represents an integer ranging from 0 to 40, preferably from 1 to 40, rather from 1 to 10;
- **G** represents a group –OR or -NRR', R and R' being as defined previously or being such that they form, with the nitrogen atom to which they are attached, a group (C_4-C_{10})heterocycloalkyl which may comprise in the ring another heteroatom chosen from N, O and S and which may be optionally substituted with at least one substituent chosen from a group (C_1-C_4)alkyl, a halogen atom and a hydroxyl group;
- **RCG1** represents the group -SZ_a or –C(=O)-Z_bR_b;
- Z_a represents Ac, R_a or SR_a;
- R_a represents H, or a group (C_1-C_6)alkyl, (C_3-C_8)cycloalkyl, aryl, heteroaryl or (C_4-C_{10})heterocycloalkyl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, NO_2, an aryl or heteroaryl group;
- Z_b represents a single bond, -O- or -NH- and R_b representing H or a group (C_1-C_6)alkyl, (C_3-C_8)cycloalkyl, aryl, heteroaryl or (C_4-C_{10})heterocycloalkyl or alternatively Z_b represents a single bond and R_b represents Hal;

with the condition that if L_1 represents a single bond, then RCG1 represents $-SZ_a$.

More particularly, they are compounds of formula (IA) or (IB):

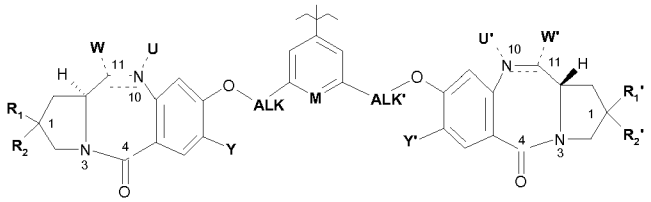




The invention also relates to the process for preparing a conjugate, which consists in: (i) placing in contact and leaving to react an aqueous solution, optionally buffered, of the binding agent, optionally modified with a modifying agent, and a solution of a compound of formula (I), (ii) and then in optionally separating the conjugate formed in step (i) from the compound of formula (I) and/or the unreacted binding agent and/or the aggregates that may possibly have formed. The chemical group RCG1 of the compound of formula (I) must be reactive towards the chemical groups RCG2 present on the binding agent, especially towards the amino groups present on the antibodies, the said chemical groups RCG2 having been introduced, where appropriate, by the modifying agent so as to attach the compound of formula (I) to the binding agent by formation of a covalent bond.

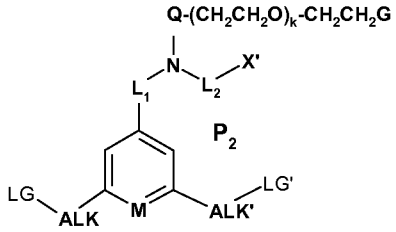
The invention also relates to a conjugate and to a conjugate solution that may be obtained via the process described above.

The invention also relates to the use of a derivative of formula (I) for the preparation of a binding agent to which is covalently attached in the para position of M the dimer of formula:



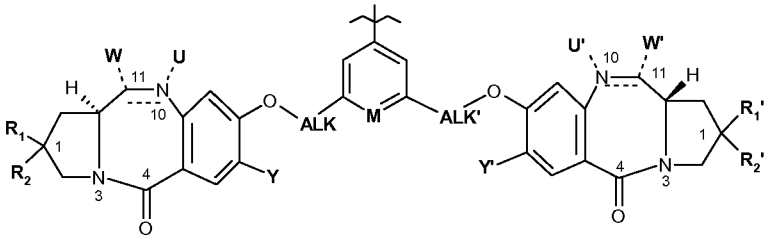
The compound, the conjugate and the conjugate solution may be used as anticancer agents.

The invention also relates to the compound of formula P_2:



in which L_1, L_2, **Q**, **k**, **ALK**, **ALK'** and **G** are as defined previously; **LG** and **LG'** represent a leaving group and **X'** represents RCG1.

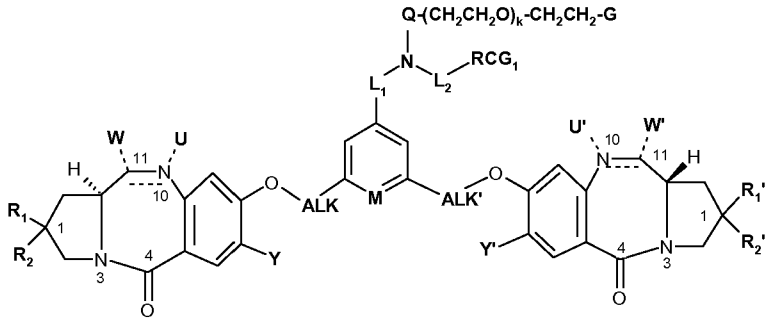
The invention also relates to a binding agent to which has been covalently attached in the para position of M the dimer of formula:



after reaction of a compound of the invention with the binding agent.

[Description of the invention]

The invention relates to compounds of formula (I):



(I)

in which:

- ---- represents a single bond or a double bond, with the condition that

  if ---- represents a single bond, then:

  - **U** and/or **U'**, which may be identical or different, represent, independently of each other, H;
  - **W** and/or **W'**, which may be identical or different, represent, independently of each other: OH, -OR, –OCOR, -COOR, -OCOOR, –OCONRR', a cyclic carbamate such that N10 and C11 are included in a ring, –NRCONRR', -OCSNHR, a cyclic thiocarbamate such that N10 and C11 are included in a ring,

-SH, -SR, -SOR, -SOOR, $-SO_3^-$, -NRSOOR', –NRR', a cyclic amine such that N10 and C11 are included in a ring, -NROR', –NRCOR', $-N_3$, -CN, Hal, a trialkylphosphonium or triarylphosphonium group;

if ---- represents a double bond, then:

- ❖ **U** and **U'** are absent;
- ❖ **W** and/or **W'**, which may be identical or different, represent, independently of each other, H;

- **R_1**, **R_2**, **R_1'**, **R_2'**, which may be identical or different, represent, independently of each other: H, Hal or a group $(C_1\text{-}C_6)$alkyl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, an aryl or heteroaryl group, $S(O)_qR$ with q = 0, 1 or 2;

or alternatively

R_1 and **R_2** and/or **R_1'** and **R_2'** together form, respectively, a double bond $=CH_2$ or $=CH\text{-}CH_3$;

- **Y** and **Y'**, which may be identical or different, represent, independently of each other, H or -OR;
- **M** represents CH or N;
- **ALK** and **ALK'** denote a group $(C_1\text{-}C_6)$alkylene;
- **R** and **R'** represent, independently of each other, H or a group $(C_1\text{-}C_6)$alkyl or aryl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, an aryl or heteroaryl group;
- **L_1** represents:
  - a single bond;

    or
  - the group $-(OCH_2CH_2)_i-$, attached to the phenyl or pyridyl ring via the oxygen atom, i representing an integer ranging from 2 to 40, preferably from 2 to 10, preferably equal to 3;

    or
  - the group -D-$(C_1\text{-}C_6)$ALK- attached to the phenyl or pyridyl ring via D, in which D represents –O-, –NH- or $-N(C_1\text{-}C_4)$alkyl-;
- **L_2** represents:
  - a group $-(C_1\text{-}C_6)$ALK-;

    or
  - the group $-(CH_2CH_2O)_j\text{-}CH_2CH_2-$, **j** representing an integer ranging from 1 to 40, preferably from 1 to 10, preferably equal to 2 or 3;

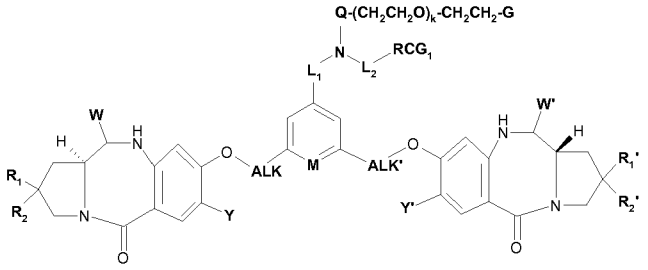
    or
  - a group $-(CH_2CH_2O)_j\text{-}CH_2CH_2NR''\text{-}(C_1\text{-}C_6)$ALK-, attached to the nitrogen atom via the unit $-(CH_2CH_2O)-$, **j** representing an integer ranging from 1 to

40, preferably from 1 to 10, preferably equal to 2 or 3, and **R"** representing H or a group (C_1-C_4)alkyl;

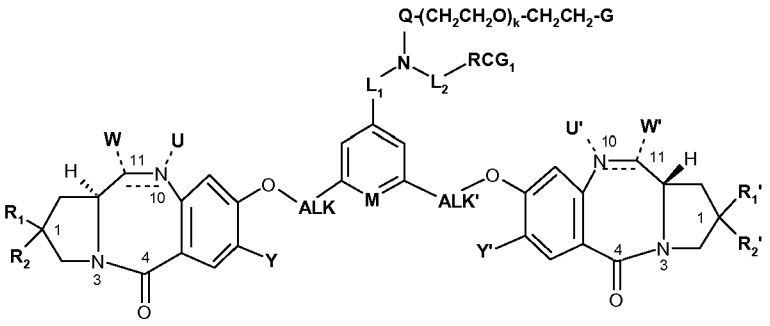
- **Q** represents a single bond or the group C(=O);
- **k** represents an integer ranging from 0 to 40, preferably from 1 to 40, rather from 1 to 10;
- **G** represents a group –OR or -NRR', R and R' being as defined previously or being such that they form, with the nitrogen atom to which they are attached, a group (C_4-C_{10})heterocycloalkyl which may comprise in the ring another heteroatom chosen from N, O and S and which may be optionally substituted with at least one substituent chosen from a group (C_1-C_4)alkyl, a halogen atom and a hydroxyl group;
- **RCG1** represents the group -SZ_a or –C(=O)-Z_bR_b;
- **Z_a** represents Ac, R_a or SR_a;
- **R_a** represents H, or a group (C_1-C_6)alkyl, (C_3-C_8)cycloalkyl, aryl, heteroaryl or (C_4-C_{10})heterocycloalkyl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, NO_2, an aryl or heteroaryl group;
- **Z_b** represents a single bond, -O- or -NH- and **R_b** representing H or a group (C_1-C_6)alkyl, (C_3-C_8)cycloalkyl, aryl, heteroaryl or (C_4-C_{10})heterocycloalkyl or alternatively **Z_b** represents a single bond and **R_b** represents Hal;

with the condition that if **L_1** represents a single bond, then RCG1 represents –SZ_a.

The compounds of formula (I) may thus exist in the form:



The invention also relates to compounds of formula (I'):



(I')

in which:

- ---- represents a single bond or a double bond, with the condition that if ---- represents a single bond, then:
  - ❖ ---- represents a single bond;
  - ❖ **U** and/or **U'**, which may be identical or different, represent, independently of each other, H;
  - ❖ **W** and/or **W'**, which may be identical or different, represent, independently of each other: OH, -OR, –OCOR, -COOR, -OCOOR, –OCONRR', a cyclic carbamate such that N10 and C11 are included in a ring, –NRCONRR', -OCSNHR, a cyclic thiocarbamate such that N10 and C11 are included in a ring, -SH, -SR, -SOR, -SOOR, $–SO_3^-$, -NRSOOR', –NRR', a cyclic amine such that N10 and C11 are included in a ring, -NROR', –NRCOR', $–N_3$, -CN, Hal, a trialkylphosphonium or triarylphosphonium group;
- **R_1**, **R_2**, **R_1'**, **R_2'**, which may be identical or different, represent, independently of each other: H, Hal or a group $(C_1$-$C_6)$alkyl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, an aryl or heteroaryl group, $S(O)_qR$ with q = 0, 1 or 2;

or alternatively

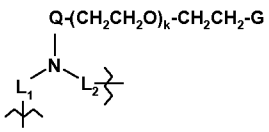
R_1 and **R_2** and/or **R_1'** and **R_2'** together form, respectively, a double bond $=CH_2$ or $=CH\text{-}CH_3$;

- **Y** and **Y'**, which may be identical or different, represent, independently of each other, H or -OR;
- **M** represents CH or N;
- **ALK** and **ALK'** denote a group $(C_1$-$C_6)$alkylene;
- **R** and **R'** represent, independently of each other, H or a group $(C_1$-$C_6)$alkyl or aryl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, an aryl or heteroaryl group;
- **L_1** represents:
  - a single bond;
    or
  - the group $-(OCH_2CH_2)_i-$, attached to the phenyl or pyridyl ring via the oxygen atom, i representing an integer ranging from 2 to 40, preferably from 2 to 10;
    or
  - the group -D-$(C_1$-$C_6)$ALK- attached to the phenyl or pyridyl ring via D, in which D represents –O-, –NH- or –N$(C_1$-$C_4)$alkyl-;
- **L_2** represents:
  - a group -$(C_1$-$C_6)$ALK-;
    or

- the group $-(CH_2CH_2O)_j-CH_2CH_2-$, **j** representing an integer ranging from 1 to 40, preferably from 1 to 10;
  or
- a group $-(CH_2CH_2O)_j-CH_2CH_2NR''-(C_1-C_6)ALK-$, attached to the nitrogen atom via the unit $-(CH_2CH_2O)-$, **j** representing an integer ranging from 1 to 40, preferably from 1 to 10, and **R"** representing H or a group (C_1-C_4)alkyl;

- **Q** represents a single bond or the group C(=O);
- **k** represents an integer ranging from 0 to 40, preferably from 1 to 40, rather from 1 to 10;
- **G** represents a group –OR or -NRR', R and R' being as defined previously or being such that they form, with the nitrogen atom to which they are attached, a group (C_4-C_{10})heterocycloalkyl which may comprise in the ring another heteroatom chosen from N, O and S and which may be optionally substituted with at least one substituent chosen from a group (C_1-C_4)alkyl, a halogen atom and a hydroxyl group;
- **RCG1** represents the group $-SZ_a$ or $-C(=O)-Z_bR_b$;
- $\mathbf{Z_a}$ represents Ac, R_a or SR_a;
- $\mathbf{R_a}$ represents H, or a group (C_1-C_6)alkyl, (C_3-C_8)cycloalkyl, aryl, heteroaryl or (C_4-C_{10})heterocycloalkyl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, NO_2, an aryl or heteroaryl group;
- $\mathbf{Z_b}$ represents a single bond, -O- or -NH- and $\mathbf{R_b}$ representing H or a group (C_1-C_6)alkyl, (C_3-C_8)cycloalkyl, aryl, heteroaryl or (C_4-C_{10})heterocycloalkyl or alternatively $\mathbf{Z_b}$ represents a single bond and $\mathbf{R_b}$ represents Hal;

with the condition that if $\mathbf{L_1}$ represents a single bond, then RCG1 represents $-SZ_a$.

The term **L** denotes the linker defined by 

.

More particularly, if $\mathbf{L_1}$ represents a single bond, $\mathbf{L_2}$ represents $-(CH_2CH_2O)_j-CH_2CH_2-$ or $-(CH_2CH_2O)_j-CH_2CH_2NR''-(C_1-C_6)ALK-$ and/or $\mathbf{k}\neq 2$.

More particularly, when **M** represents N, $-D-(C_1-C_6)ALK-$ rather represents $-O-(C_1-C_6)ALK-$.

More particularly, the two groups ALK and ALK' attached to the phenyl or pyridyl nucleus both denote a methylene group.

More particularly, D represents –O-, -NH- or –NMe-.

In formulae (I) and (I') above, each alkylene group (thus, for example, the two groups ALK and ALK') attached to the phenyl or pyridyl nucleus may be identical or different; according

to another example, the group ALK of –D-(C_1-C_6)ALK- may be identical to or different from the group ALK attached to the phenyl or pyridyl nucleus. ALK may be chosen, for example, from one of the following: -CH_2-, –CH_2CH_2-, CH_2CMe_2-, -$CH_2CH_2CH_2$-.

Y and **Y'** more particularly represent a group (C_1-C_4)alkoxy, especially the methoxy group.
R and **R'** may more particularly represent, independently of each other, H or a group (C_1-C_6)alkyl.

According to one particular embodiment, **U=U'** and/or **W=W'** and/or **R_1=R_1'** and/or **R_2=R_2'** and/or **Y=Y'** and/or both the groups **ALK** and **ALK'** attached to the phenyl or pyridyl nucleus are identical (symmetrical dimer, which is easier to prepare).

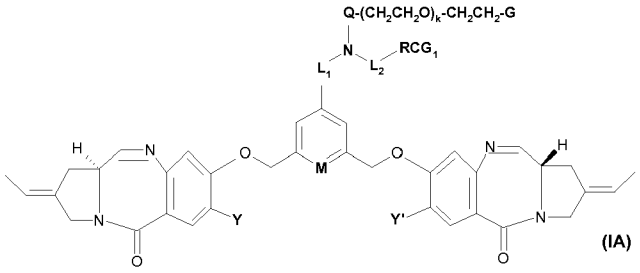
More particularly, **W** and **W'** are identical or different and represent OH, OMe, OEt, $NHCONH_2$, SMe.
R'' may represent H or a group (C_1-C_4)alkyl, especially Me.
M more particularly represents a nitrogen atom (N).
More particularly, **R_1** and **R_2** and/or **R_1'** and **R_2'** together form, respectively, a double bond $=CH_2$ or $=CH\text{-}CH_3$, more specifically $=CH\text{-}CH_3$.

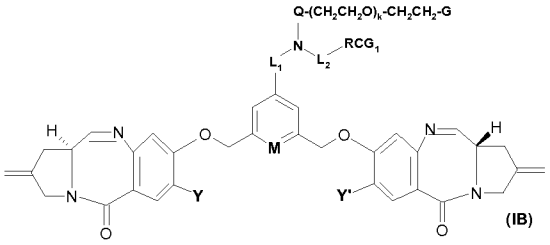
G may represent a group –OR, more particularly a group –OH or –O(C_1-C_6)alkyl, especially –OMe. **G** may also represent a group -NRR' in which R and R' represent, independently of each other, H or a group (C_1-C_6)alkyl. More particularly, **G** may thus represent a group $–NH_2$, -NH(C_1-C_6)alkyl or –N(C_1-C_6)$alkyl_2$, especially $–N(CH_3)_2$. **G** may also represent a group -NRR' in which R and R' form, together with the nitrogen atom to which they are attached, a group (C_4-C_{10})heterocycloalkyl which may comprise in the ring another heteroatom chosen from N, O and S and which may be optionally substituted with at least one substituent chosen from a group (C_1-C_4)alkyl, a halogen atom and a hydroxyl group. The heterocycloalkyl group may be chosen especially from piperazino, N-methylpiperazino, morpholino, piperidino and pyrrolidino.

k represents an integer ranging from 0 to 40. **k** may take the value 0 (no PEG chain ending with G attached to the nitrogen atom). Among the compounds of the invention, a distinction may be made for those comprising a PEG chain ending with G attached to the nitrogen atom and **k** ranges from 1 to 40, rather from 1 to 10, rather from 1 to 5. A distinction may also be made for those comprising at least one PEG chain, i.e. those for which **k** ranges from 1 to 40, preferably from 1 to 10, rather from 1 to 5, and/or **L_1**= -$(OCH_2CH_2)_i$- and/or **L_2**= -$(CH_2CH_2O)_j$-CH_2CH_2- or alternatively –$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-.

The compounds of formulae (I) and (I'), including those given as examples, may exist in the form of bases or of addition salts with pharmaceutically acceptable acids, and also in the form of hydrates or solvates of these bases or of these salts.

More particularly, a distinction is made for those of formula **(IA)** or **(IB)**:





Furthermore, the following are distinguished:

- a first subgroup of compounds for which L_1= -D-(C_1-C_6)ALK- and L_2= -(C_1-C_6)ALK-;
- a second subgroup of compounds for which L_1= -(OCH_2CH_2)$_i$- and L_2= -(C_1-C_6)ALK-;
- a third subgroup of compounds for which L_1= single bond and L_2= –(CH_2CH_2O)$_j$-CH_2CH_2NR''-(C_1-C_6)ALK-;
- a fourth subgroup of compounds for which L_1= -D-(C_1-C_6)ALK-, and L_2= –(CH_2CH_2O)$_j$-CH_2CH_2NR''-(C_1-C_6)ALK-;
- a fifth subgroup of compounds for which L_1= -D-(C_1-C_6)ALK- and L_2= -(CH_2CH_2O)$_j$-CH_2CH_2-.

The following groups of compounds may also be distinguished:

- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q = single bond, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q=CO, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= -(OCH_2CH_2)$_i$-, L_2= -(C_1-C_6)ALK-, Q = single bond, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= -(OCH_2CH_2)$_i$-, L_2= -(C_1-C_6)ALK-, Q=CO, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q = single bond, k=1-10, G=NRR', RCG1=-SZ_a;
- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q=CO, k=1-10, G=NRR', RCG1=-SZ_a;
- L_1= single bond, L_2= –(CH_2CH_2O)$_j$-CH_2CH_2NR''-(C_1-C_6)ALK-, Q = single bond, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= single bond, L_2= –(CH_2CH_2O)$_j$-CH_2CH_2NR''-(C_1-C_6)ALK-, Q=CO, k=1-10, G=OR, RCG1=-SZ_a;

- L_1= single bond, L_2= $-(CH_2CH_2O)_j-CH_2CH_2NR''-(C_1-C_6)ALK-$, Q = single bond, k=0-10, G=NRR', RCG1=$-SZ_a$;
- L_1= single bond, L_2= $-(CH_2CH_2O)_j-CH_2CH_2NR''-(C_1-C_6)ALK-$, Q=CO, k=0-10, G=NRR', RCG1=$-SZ_a$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2= $-(CH_2CH_2O)_j-CH_2CH_2NR''-(C_1-C_6)ALK-$, Q = single bond, k=0-10, G=NRR', RCG1=$-SZ_a$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2= $-(CH_2CH_2O)_j-CH_2CH_2NR''-(C_1-C_6)ALK-$, Q=CO, k=0-10, G=NRR', RCG1=$-SZ_a$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2=$-(CH_2CH_2O)_j-CH_2CH_2NR''-(C_1-C_6)ALK-$, Q = single bond, k=1-10, G=OR, RCG1=$-SZ_a$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2=$-(CH_2CH_2O)_j-CH_2CH_2NR''-(C_1-C_6)ALK-$, Q=CO, k=1-10, G=OR, RCG1=$-SZ_a$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2= $-(C_1-C_6)ALK-$, Q = single bond, k=1-10, G=OR, RCG1 = $-C(=O)Z_bR_b$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2= $-(C_1-C_6)ALK-$, Q=CO, k=1-10, G=OR, RCG1 = $-C(=O)Z_bR_b$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2= $-(C_1-C_6)ALK-$, Q = single bond, k=1-10, G=NRR', RCG1 = $-C(=O)Z_bR_b$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2= $-(C_1-C_6)ALK-$, Q=CO, k=1-10, G=NRR', RCG1 = $-C(=O)Z_bR_b$;
- L_1= $-(OCH_2CH_2)_i-$, L_2= $-(C_1-C_6)ALK-$, Q = single bond, k=1-10, G=OR, RCG1 = $-C(=O)Z_bR_b$;
- L_1= $-(OCH_2CH_2)_i-$, L_2= $-(C_1-C_6)ALK-$, Q=CO, k=1-10, G=OR, RCG1 = $-C(=O)Z_bR_b$;
- L_1= $-(OCH_2CH_2)_i-$, L_2= $-(C_1-C_6)ALK-$, Q = single bond, k=1-10, G=NRR', RCG1 = $-C(=O)Z_bR_b$;
- L_1= $-(OCH_2CH_2)_i-$, L_2= $-(C_1-C_6)ALK-$, Q=CO, k=1-10, G=NRR', RCG1 = $-C(=O)Z_bR_b$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2= $-(CH_2CH_2O)_j-CH_2CH_2-$, Q = single bond, k=1-10, G=OR, RCG1 = $-C(=O)Z_bR_b$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2= $-(CH_2CH_2O)_j-CH_2CH_2-$, Q=CO, k=1-10, G=OR, RCG1 = $-C(=O)Z_bR_b$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2= $-(CH_2CH_2O)_j-CH_2CH_2-$, Q = single bond, k=0-10, G=NRR', RCG1 = $-C(=O)Z_bR_b$;
- L_1= -D-$(C_1-C_6)ALK-$, L_2= $-(CH_2CH_2O)_j-CH_2CH_2-$, Q=CO, k=0-10, G=NRR', RCG1 = $-C(=O)Z_bR_b$.

In the groups of compounds defined above:

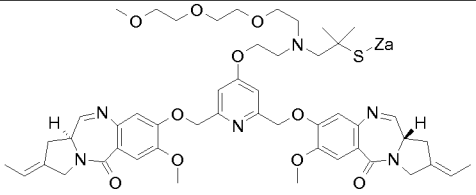
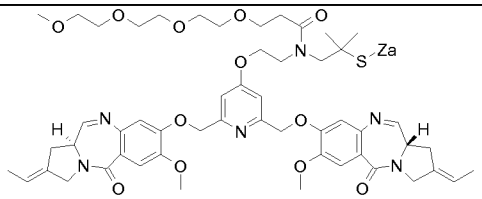
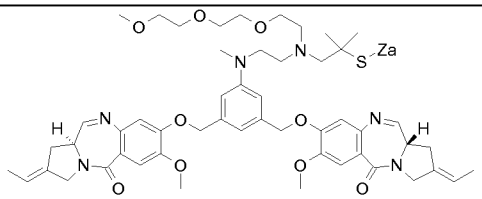
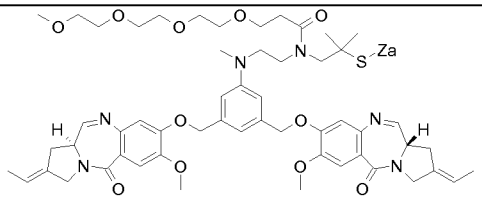
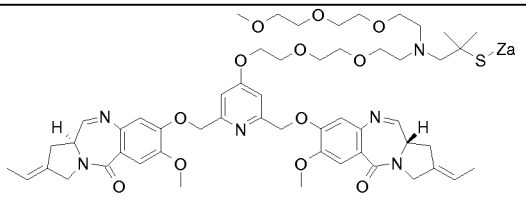
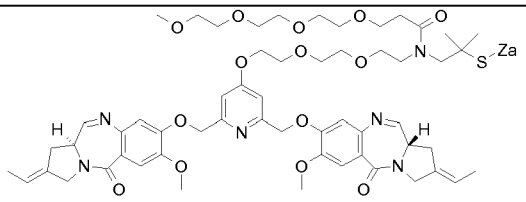
i more particularly represents an integer ranging from 2 to 10, especially an integer equal to 3;

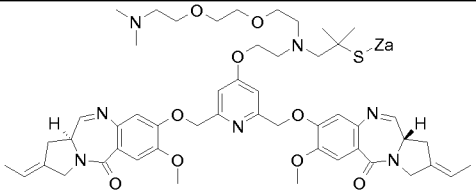
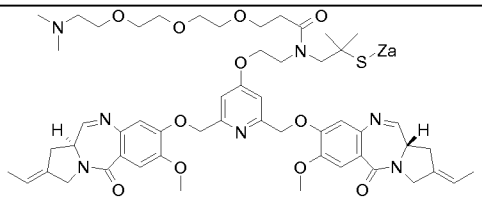
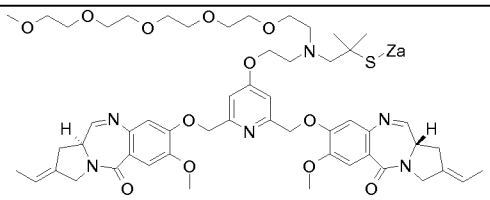
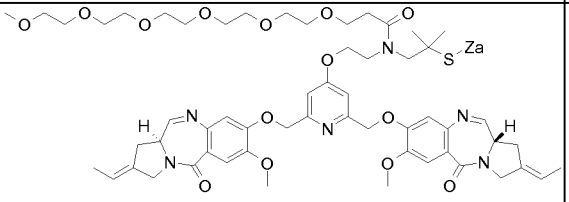
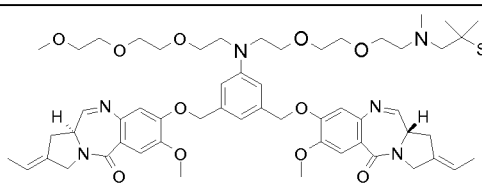
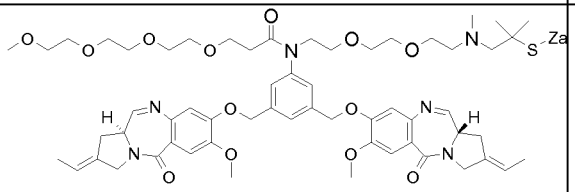
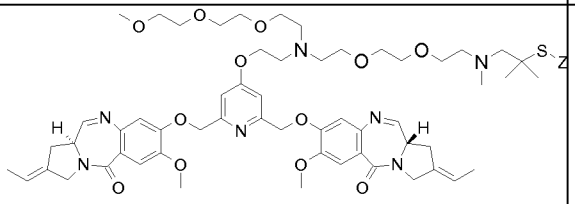
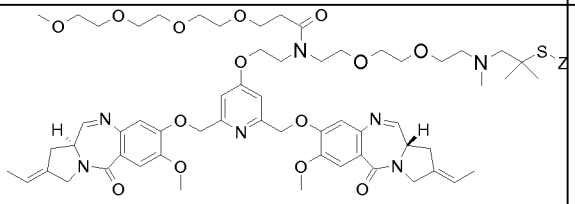
j more particularly represents an integer ranging from 1 to 10, especially an integer equal to 2 or 3;

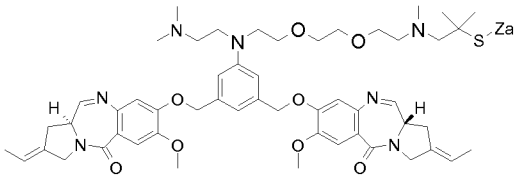
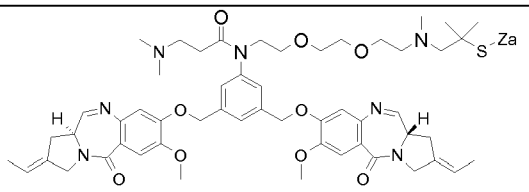
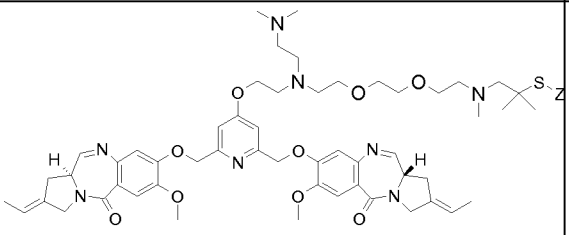
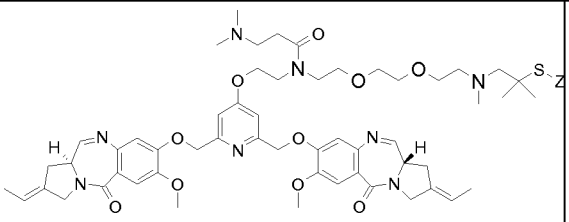
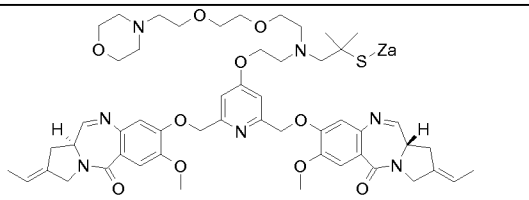
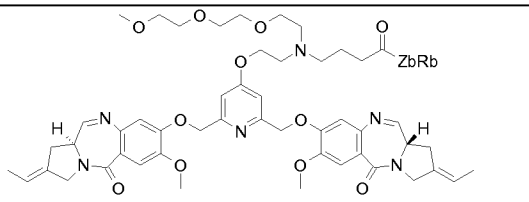
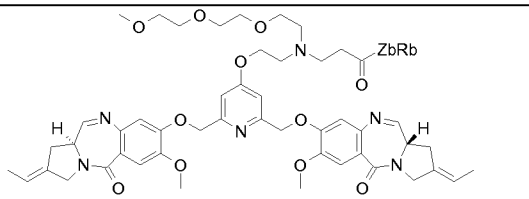
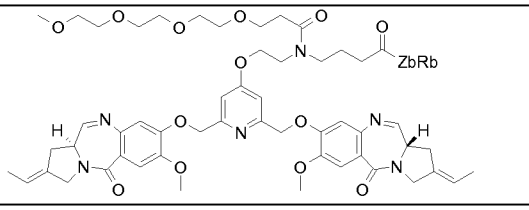
k more particularly represents an integer equal to 0, or ranging from 1 to 5.

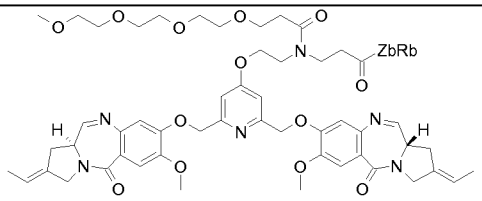
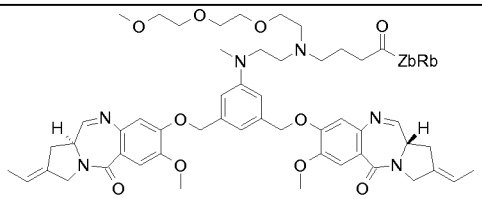
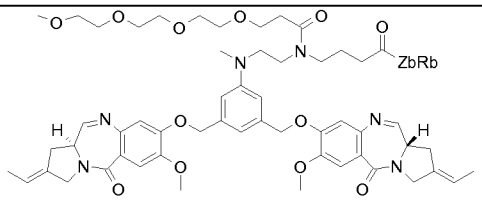
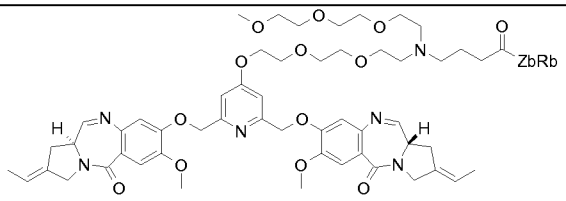
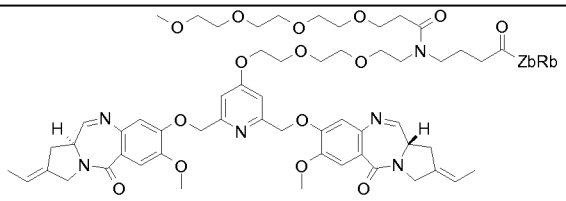
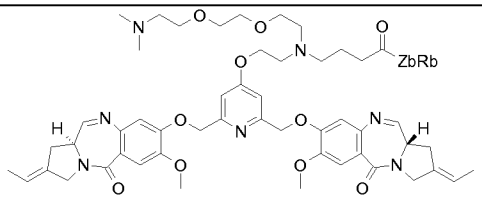
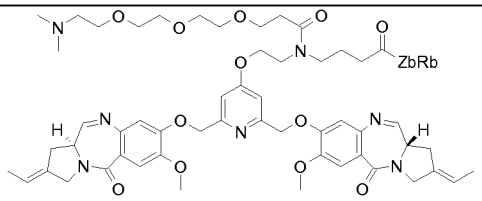
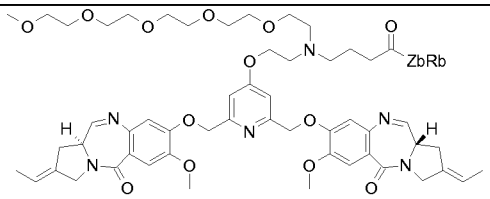
Table I describes particular linkers **L** and also examples of compounds corresponding thereto. Each compound of this table may exist in the form with **M**=CH (benzene) or alternatively **M**=N (pyridine). The compounds with **M**=N are more water-soluble. In this table, the examples are given in a form (IA) but may also exist in a form (IB). **L**, L_1, L_2, **k** and **G** may be chosen from those described in **Table I** or among the examples.

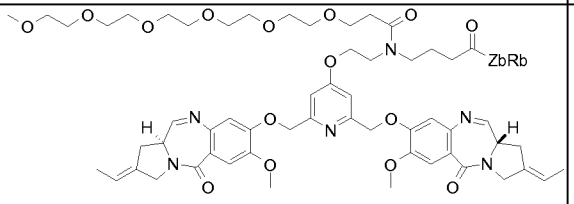
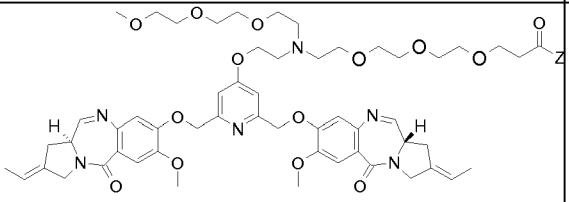
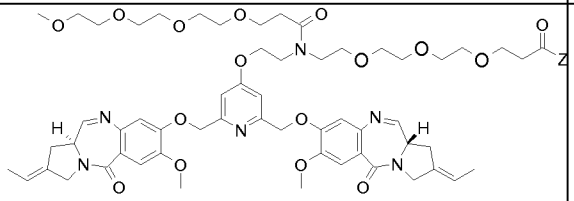
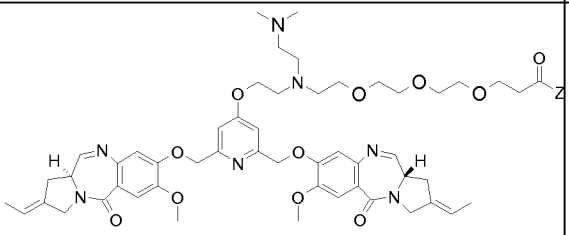
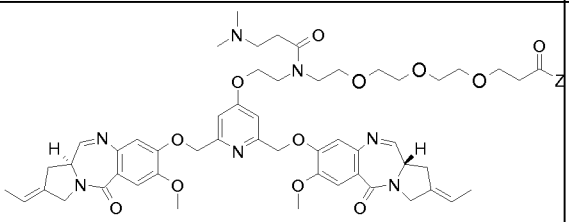
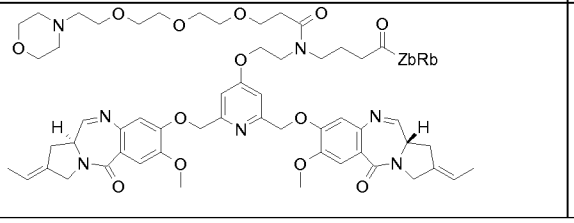
Table I

L_1	L_2	Q	G	Compound of formula (IA)	n°
-D-ALK-	ALK	-	OR		1
-D-ALK-	ALK	CO	OR		2
-D-ALK-	ALK	-	OR		3
-D-ALK-	ALK	CO	OR		4
$-(OCH_2CH_2)_i-$	ALK	-	OR		5
$-(OCH_2CH_2)_i-$	ALK	CO	OR		6

L_1	L_2	Q	G	Compound of formula (IA)	n°
-D-ALK-	ALK	-	NRR'		7
-D-ALK-	ALK	CO	NRR'		8
-D-ALK-	ALK	-	OR		9
-D-ALK-	ALK	CO	OR		10
single bond	$-(CH_2CH_2O)_j-CH_2CH_2NR''-ALK-$	-	OR		11
single bond	$-(CH_2CH_2O)_j-CH_2CH_2NR''-ALK-$	CO	OR		12
-D-ALK-	$-(CH_2CH_2O)_j-CH_2CH_2NR''-ALK-$	-	OR		13
-D-ALK-	$-(CH_2CH_2O)_j-CH_2CH_2NR''-ALK-$	CO	OR		14

L_1	L_2	Q	G	Compound of formula (IA)	n°
single bond	$-(CH_2CH_2O)_j-CH_2CH_2NR''-ALK-$	-	NRR'		15
single bond	$-(CH_2CH_2O)_j-CH_2CH_2NR''-ALK-$	CO	NRR'		16
-D-ALK-	$-(CH_2CH_2O)_j-CH_2CH_2NR''-ALK-$	-	NRR'		17
-D-ALK-	$-(CH_2CH_2O)_j-CH_2CH_2NR''-ALK-$	CO	NRR'		18
-D-ALK-	ALK	-	NRR'		19
-D-ALK-	ALK	-	OR		20
-D-ALK-	ALK	-	OR		21
-D-ALK-	ALK	CO	OR		22

L_1	L_2	Q	G	Compound of formula (IA)	n°
-D-ALK-	ALK	CO	OR		23
-D-ALK-	ALK	-	OR		24
-D-ALK-	ALK	CO	OR		25
-$(OCH_2CH_2)_i$-	ALK	-	OR		26
-$(OCH_2CH_2)_i$-	ALK	CO	OR		27
-D-ALK-	ALK	-	NRR'		28
-D-ALK-	ALK	CO	NRR'		29
-D-ALK-	ALK	-	OR		30

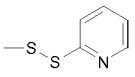
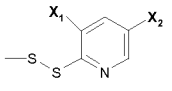
L_1	L_2	Q	G	Compound of formula (IA)	n°
-D-ALK-	ALK	CO	OR		31
-D-ALK-	$-(CH_2CH_2O)_j-CH_2CH_2-$	-	OR		32
-D-ALK-	$-(CH_2CH_2O)_j-CH_2CH_2-$	CO	OR		33
-D-ALK-	$-(CH_2CH_2O)_j-CH_2CH_2-$	-	NRR'		34
-D-ALK-	$-(CH_2CH_2O)_j-CH_2CH_2-$	CO	NRR'		35
-D-ALK-	ALK	CO	NRR'		36

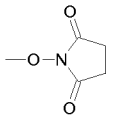
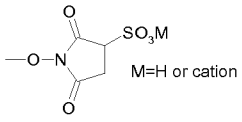
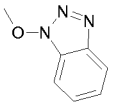
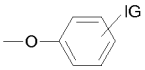
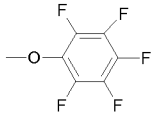
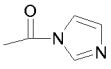
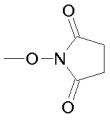
The compounds described comprise a reactive chemical group (RCG1) that is reactive towards a reactive chemical group (RCG2) present on the binding agent. The reaction between RCG1 and RCG2 brings about the attachment of the compound of formula (I) to the binding agent by formation of a covalent bond. Thus, the compounds of formula (I) can be conjugated to a binding agent.

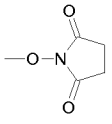
RCG1 represents:

(i) the reactive group $-SZ_a$ for which Z_a represents H or the group $-SR_a$ and R_a representing a group $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_7)$cycloalkyl, aryl, heteroaryl or $(C_4\text{-}C_{10})$heterocycloalkyl;

(ii) the reactive group $-C(=O)\text{-}Z_bR_b$ for which Z_b represents a single bond, -O- or -NH-, more particularly -O-, and R_b representing H or a group $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_7)$cycloalkyl, aryl, heteroaryl or $(C_4\text{-}C_{10})$heterocycloalkyl.

More particularly, $-SZ_a$ may represent –SH or $-SS(C_1\text{-}C_6)$alkyl, especially –SSMe, or –SS-heteroaryl, especially  or  (**X_1** and **X_2** being defined hereinbelow). More particularly, $-SZ_a$ may represent –SH or $-SS(C_1\text{-}C_6)$alkyl, especially –SSMe.

More particularly, $-Z_bR_b$ may represent –OH (acid function), $-O(C_1\text{-}C_6)$alkyl, especially $-OCH_3$, $-OCH_2CH_3$, $-OCH_2CH{=}CH_2$ (ester function) or alternatively $-Z_bR_b$ may represent , ,  or the group  in which IG represents at least one electroinductive group such as $-NO_2$ or -Hal, especially -F. It may be, for example, one of the following groups:  or . Another type of group $-C(=O)Z_bR_b$ is the following: . The reactive groups –SH and  show good reactivity.

More particularly, $-Z_bR_b$ may represent –OH (acid function), $-O(C_1\text{-}C_6)$alkyl, especially $-OCH_3$, $-OCH_2CH_3$, - or alternatively $-Z_bR_b$ may represent .

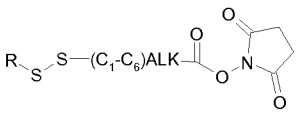
More particularly, RCG1 may be chosen from one of those described in the examples.

As examples of RCG2, mention may be made of the ε-amino groups of lysines borne by the side chains of lysine residues that are present at the surface of an antibody, the saccharide groups of the hinge region or the thiols of cysteines by reduction of intra-chain disulfide bonds (Garnett M.C. et al., *Advanced Drug Delivery Reviews* **2001**, *53*, 171-216). More recently, other approaches have been considered, such as the introduction of cysteines by mutation (Junutula J.R. et al., *Nature Biotechnology* **2008**, *26*, 925-932; **WO 09026274**) or the introduction of unnatural amino acids allowing other types of chemistry (de Graaf A.J. et

al., *Bioconjugate Chem.* **2009**, Publication Date (Web): February 3, 2009 (Review); **DOI:** 10.1021/bc800294a; **WO 2006/069246** and according to Chin J.W. et al., *JACS* **2002**, *124*, 9026-9027 (ReCode® technology)). These modes of attachment used with antibodies are applicable to all the known targeting agents as a function of their structure.

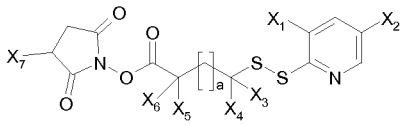
It is also possible to chemically modify the targeting agent so as to introduce novel reactive chemical groups RCG2. Thus, it is well known to those skilled in the art how to modify an antibody using a modifying agent (see especially **WO 2005/077090** page 14). The modification makes it possible to improve the conjugation reaction and to use a wider variety of groups RCG1.

Modifying agents for introducing disulfide groups

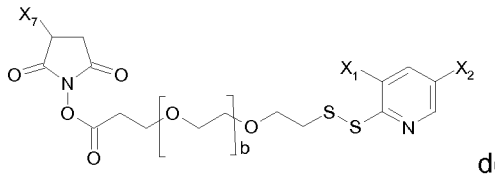


The modifying agent may be an activated ester NHS of formula

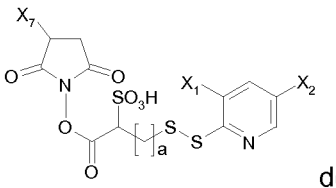
in which R represents a group $(C_1\text{-}C_6)$alkyl, aryl, heteroaryl, $(C_3\text{-}C_7)$cycloalkyl, $(C_4\text{-}C_{10})$heteroycloalkyl; for example, it is possible to use N-pyridyldithiopropionate (SPDP) or N-succinimidyl pyridyldithiobutyrate (SPDB or the N-hydroxysuccinimidyl ester of 4-(2-pyridyldithio)butanoic acid) so as to introduce dithiopyridyl reactive groups RCG2 (see Bourdon M.A. et al., *Biochem. J.* **1978**, *173*, 723-737; **US 5208020**) which can then react with a reactive chemical group RCG1 of the type –SH present on the linker of the pyrrolo[1,4]benzodiazepine dimer so as to form a new –S-S- bond (see Ex. 1) for a conjugate bearing a disulfide bond. The N-hydroxysuccinimide group preferentially reacts on the amino groups present on the antibody so as to form amide bonds. Another example of a modifying agent is described in **WO 2004/016801** of formula



; for example, it is possible to use N-succinimidyl 4-(5-nitro-2-pyridyldithio)pentanoate (SNPP), or a pegylated analogue of formula



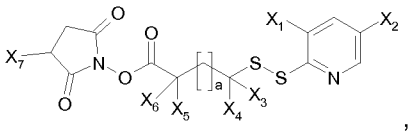
described in **WO 2009/134976** or a sulfonic analogue of formula



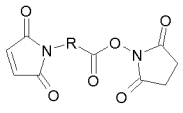
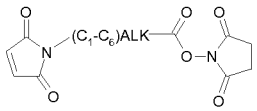
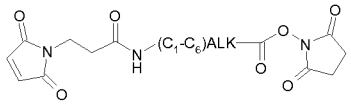
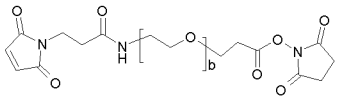
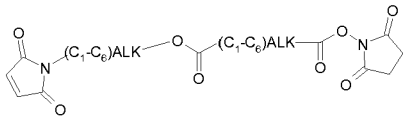
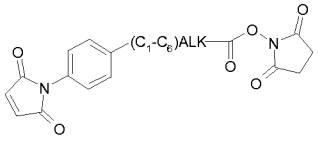
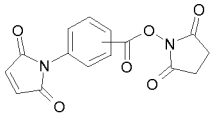
described in **WO 2009/134977**, in which formulae:

- X_3, X_4, X_5, X_6 represent H or a group $(C_1\text{-}C_6)$alkyl,
- X_1 and X_2 represent -H, $-CONX_8X_9$, $-NO_2$, X_8 and X_9 representing H or a group $(C_1\text{-}C_6)$alkyl,

- X_7 represents $-SO_3^-M^+$ or H or alternatively a quaternary ammonium group;
- a denotes an integer ranging from 0 to 4 and b denotes an integer ranging from 0 to 2000, preferably between 1 and 200; a and b may take all the values between, respectively, 0 and 4 or between 0 and 2000.

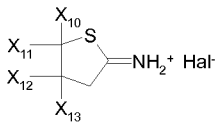
Preferably, among the compounds of formula , a=1, X_3=Me and X_2=NO_2 and X_1=X_4=X_5=X_6=X_7=H).

Modifying agents for introducing maleimido groups

Another modifying agent may be an activated ester NHS of formula  in which R represents a group $-(CH_2)_n-$, $-(CH_2)_n$-cyclohexyl-, -cyclohexyl-$(CH_2)_n-$ and n represents an integer ranging from 1 to 10; for example, it is possible to use succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) (according to **EP 0306943),** or a sulfo-SMCC (sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate). Other examples that may be mentioned include:  such as N-succinimidyl 3-maleimidopropanoate;  for instance N-succinimidyl 6-(3-maleimidopropionamido)hexanoate;  b being an integer between 0 and 2000, preferably between 1 and 200 (b may take all the values between 0 and 2000), for instance N-succinimidyl 3-(2-{2-[3-maleimidopropionylamino]-ethoxy}ethoxy)propanoate or $SM(PEG)_2$;  for instance maleimidoethyl N-succinimidyl succinate;  for instance N-succinimidyl 4-(4-maleimidophenyl)butanoate or  for instance N-succinimidyl 3-maleimidobenzoate.

Modifying agents for introducing thiol groups

Another example of a modifying agent described in **WO 90/06774** has the formula



in which:

- Hal represents a halogen atom;
- X_{10} represents a halogen atom or the group $COOX_{14}$, nitro, unsubstituted or halogenated $(C_1\text{-}C_8)$alkyl, unsubstituted or halogenated $(C_1\text{-}C_8)$alkoxy, unsubstituted or halogenated $(C_2\text{-}C_8)$alkenyl, unsubstituted or halogenated $(C_2\text{-}C_8)$ alkynyl, unsubstituted $(C_3\text{-}C_8)$cycloalkyl, aryl which is unsubstituted or substituted with one to three substituents selected from amino, a halogen atom, an unsubstituted or halogenated group $(C_1\text{-}C_8)$alkyl, or an unsubstituted or halogenated $(C_1\text{-}C_8)$alkoxy;
- each of the groups X_{11}, X_{12}, X_{13} independently represents a hydrogen atom or alternatively may represent X_{10};

or X_{10} and X_{11} together form a $(C_2\text{-}C_5)$alkylene ring, which is unsubstituted or substituted with one to five groups $(C_1\text{-}C_4)$alkyl;

or X_{10} or X_{11} form, together with X_{12}, a $(C_1\text{-}C_5)$alkylene ring, which is unsubstituted or substituted with one to five groups $(C_1\text{-}C_4)$ alkyl;

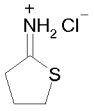
and X_{14} is -H or a group $(C_1\text{-}C_8)$alkyl;

or $X_{10}=X_{11}=X_{12}=X_{13}=H$.

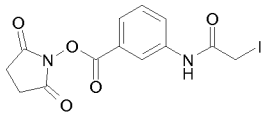
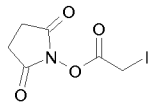
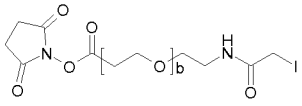
Preferably, Hal represents a chlorine or bromine atom. Possibilities for X_{10}-X_{13} will be found in the table below:

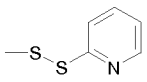
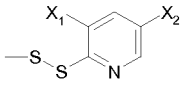
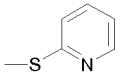
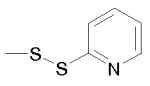
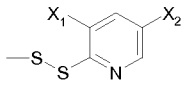
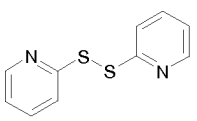
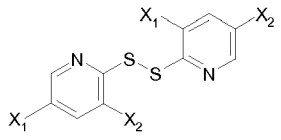
X_{10}	X_{11}	X_{12}	X_{13}	Hal
Me	H	H	H	Cl
Ph	H	H	H	Cl
t-Bu	H	H	H	Cl
Me	Me	H	H	Cl
$(-CH_2(CH_2)_3CH_2-)$		H	H	Cl
H	$(-CH_2(CH_2)_3CH_2-)$		H	Cl
Et	H	H	H	Br
Et	Me	H	H	Cl
-CH-CH_2-CH	H	H	H	Cl
Me	H	Me	H	Cl
H	H	Me	Me	Cl
Ph	Me	H	H	Cl
4-ClPh	H	H	H	Cl
3-furanyl	H	H	H	Cl
i-Pr	H	H	H	Cl
Me	Me	Me	Me	Cl
C_6H_{11}	H	H	H	Cl

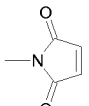
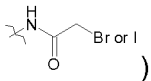
X_{10}	X_{11}	X_{12}	X_{13}	Hal
CH_2Br	H	H	H	Cl
CF_3	H	H	H	Cl
$CH=CH_2$	H	H	H	Cl
2-NH_2Ph	H	H	H	Cl

An example of a preferred iminothiolane is the following: .

Modifying agents for introducing haloacetamido groups

Another example of a modifying agent is succinimidyl-4-(N-iodoacetyl)aminobenzoate (SIAB) , or similar compounds, including succinimidyl-N-iodoacetate (SIA) , succinimidyl-N-bromoacetate (SBA), or succinimidyl-3-(N-bromoacetamido)propionate (SBAP) or a similar pegylated compound described in **WO 2009/134976** , **b** being as described previously. Figures 1 and 2 illustrate the modification of an amino group of a binding agent with SPDP or alternatively the preferred iminothiolane above.

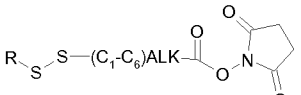
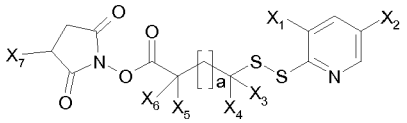
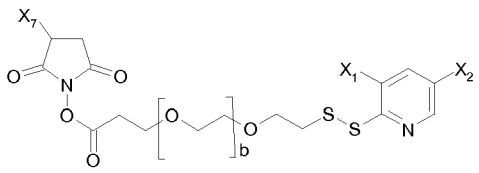
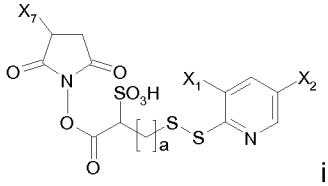
Thus, it is possible to introduce onto the binding agent disulfide RCG2 groups (–SSR), especially of pyridyldisulfide type  or , in the case where RCG1 represents –SH. Similarly, it is possible to introduce onto the binding agent thiol (-SH) RCG2 groups, for example with an iminothiolane, in the case where RCG1 represents disulfide (i.e. RCG1= $–SZ_a$ with $Z_a \neq H$, for example Z_a= ). It is also possible to modify these thiol (-SH) RCG2 groups into disulfide (-SSR) RCG2 groups, especially of pyridyldisulfide type  or  by reaction with the corresponding aromatic disulfides  or . In both cases, the covalent bond that forms by reaction between RCG1 and RCG2 is a cleavable disulfide bond.

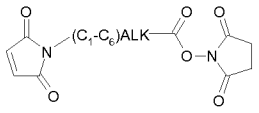
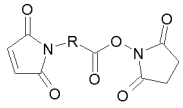
It is also possible, in the case where RCG1 represents -SH, to introduce at the surface of the binding agent RCG2 groups of maleimido () or haloacetamido type (e.g. bromo- or iodoacetamido ). In this case, the covalent bond that forms by reaction between RCG1 and RCG2 is an uncleavable sulfide bond.

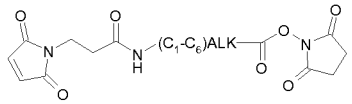
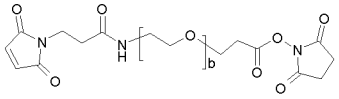
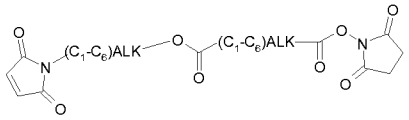
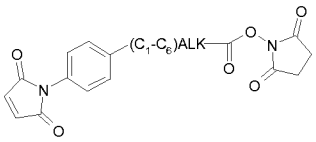
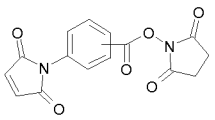
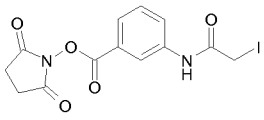
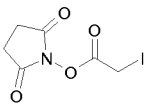
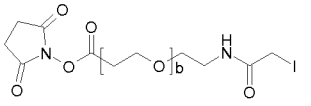
Thus,

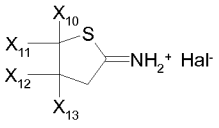
- in the presence of a derivative of formula (I) comprising a reactive chemical group RCG1 of the type $-SZ_a$, the binding agent comprises:
  - disulfide chemical groups in the case where RCG1 represents –SH;
  - thiol chemical groups in the case where RCG1 represents $-SZ_a$ with $Z_a \neq H$;
  - maleimido or haloacetamido chemical groups in the case where RCG1 represents -SH;
- in the presence of a derivative of formula (I) comprising a reactive chemical group RCG1 of the type $-C(=O)-Z_bR_b$, the derivative of formula (I) is reacted with the amino functions of the binding agent, especially the ε-amino groups borne by the side chains of the lysine (Lys) residues of an antibody.

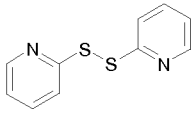
More particularly,

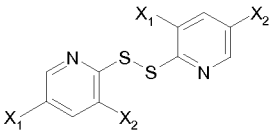
- when the reactive chemical group RCG1 is of the type -SH, and when the binding agent bears amino functions, especially ε-amino groups borne by the side chains of the lysine residues of an antibody, the latter is modified by means of a modifying agent chosen from a compound of formula:  in which R represents a group $(C_1$-$C_6)$alkyl, aryl, heteroaryl, $(C_3$-$C_7)$cycloalkyl, $(C_4$-$C_{10})$heterocycloalkyl; , a pegylated analogue of formula:  or a sulfonic analogue of formula  in which X_3 X_4, X_5, X_6 represent H or a group $(C_1$-$C_6)$alkyl, X_1 and

X_2 represent -H, -$CONX_8X_9$, -NO_2, X_8 and X_9 representing H or a group (C_1-C_6)alkyl, X_7 represents -$SO_3^-M^+$ or H or alternatively a quaternary ammonium group and a denotes an integer ranging from 0 to 4 and b denotes an integer ranging from 0 to 2000; or chosen from succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate; sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate;  ;  in which R represents a group -$(CH_2)_n$-, -$(CH_2)_n$-cyclohexyl-, -cyclohexyl-$(CH_2)_n$- and n represents an integer ranging from 1 to 10;

 ;  ;  ;  ;  **b** being an integer between 0 and 2000;  ;  ; succinimidyl-N-bromoacetate; succinimidyl-3-(N-bromoacetamido)propionate;  , **b** being an integer between 0 and 2000.

➢ when the reactive chemical group RCG1 is of the type -SZ_a with Z_a≠H, and when the binding agent bears amino functions, especially ε-amino groups borne by the side chains of the lysine residues of an antibody, the latter is modified by means of a modifying agent of formula  described previously.

➢when the reactive chemical group RCG1 is of the type -SH, and when the binding agent contains thiol functions, especially following the introduction of cysteines by mutation or by chemical modification of a binding agent containing amino functions, the binding agent is modified such that its thiol functions are converted into disulfide functions. It is possible, for example, to use a modifying agent chosen from a compound of formula  or



in which X_1 and X_2 represent -H, $-CONX_8X_9$ or $-NO_2$, X_8 and X_9 representing H or a group $(C_1\text{-}C_6)$alkyl.

Table II below illustrates the modification of an amino group of a binding agent according to the preceding methods. For the sake of simplicity, the following abbreviations are used:

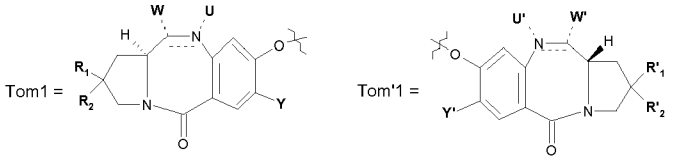
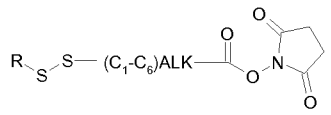
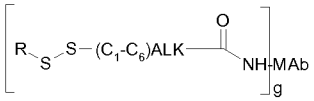
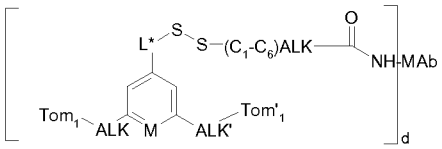
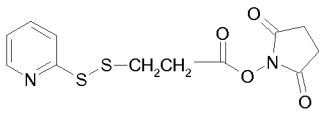
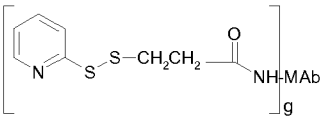
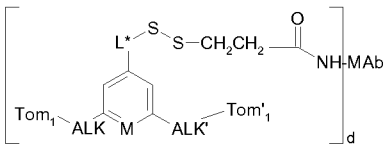
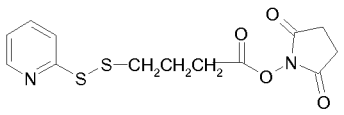
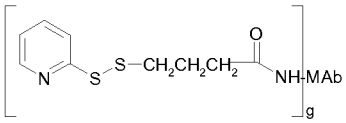
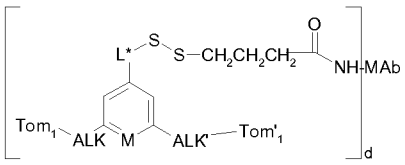
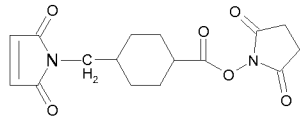
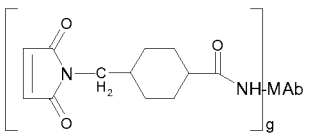
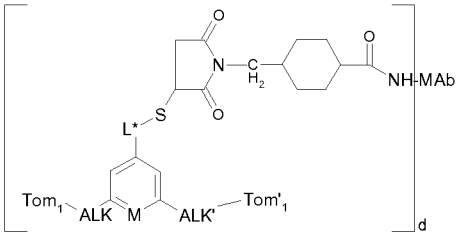
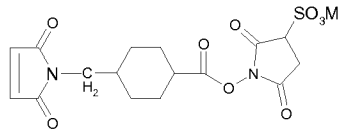
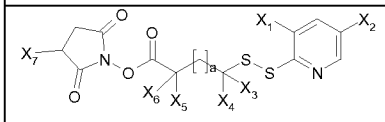
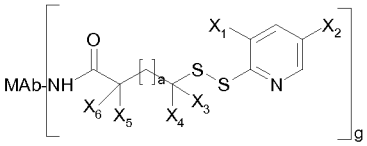
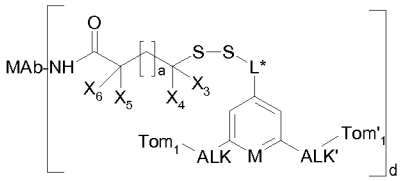
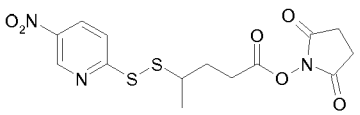
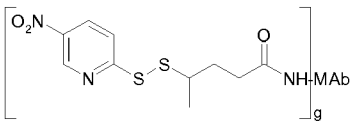
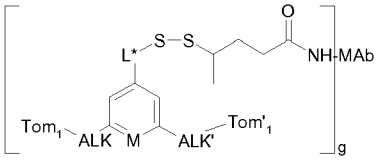
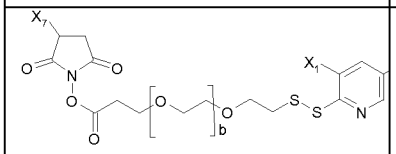
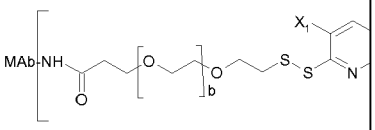
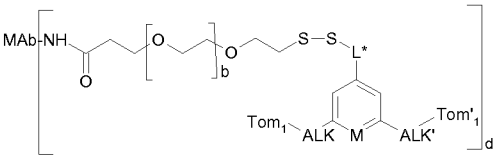
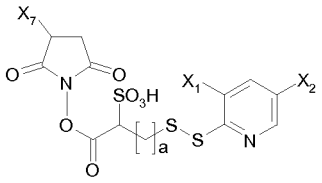
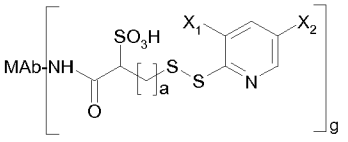
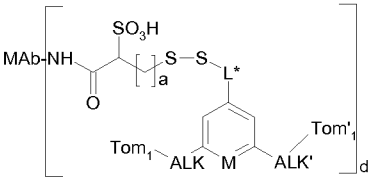
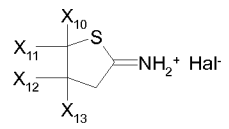
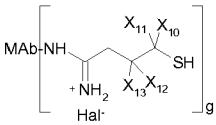
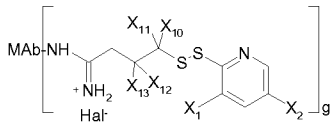
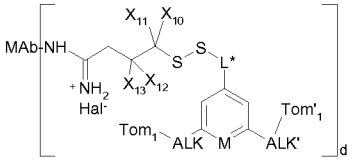
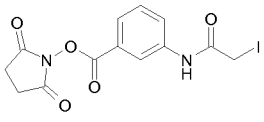
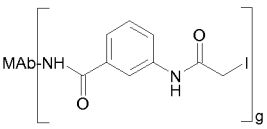
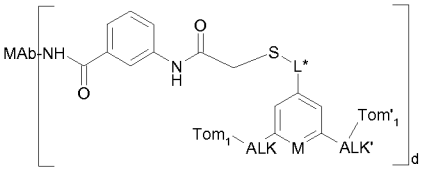
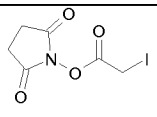
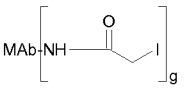
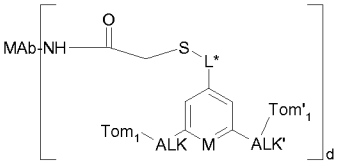
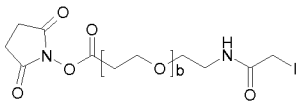
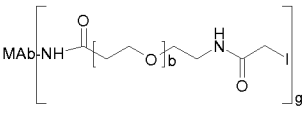
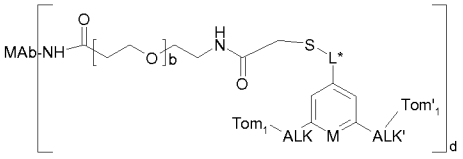


Table II: examples of modifications of binding agents when RCG1=-SZ_a

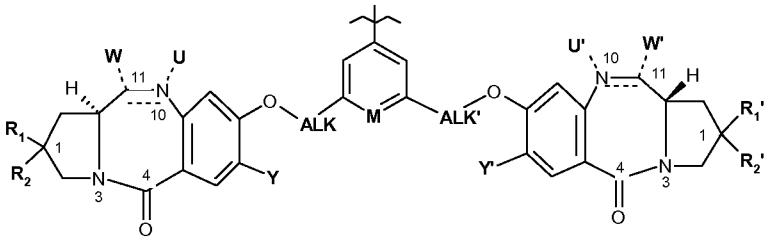
Modifying agent	Example after reaction on an amino group, especially lysine, of an antibody, noted MAb	Conjugate
		
SPDP= 		
SPDB= 		
SMCC= 		
sulfo-SMCC= 		

Modifying agent	Example after reaction on an amino group, especially lysine, of an antibody, noted MAb	Conjugate
		
SNPP= 		
		
		
	 	
SIAB= 		
SIA= 		
		

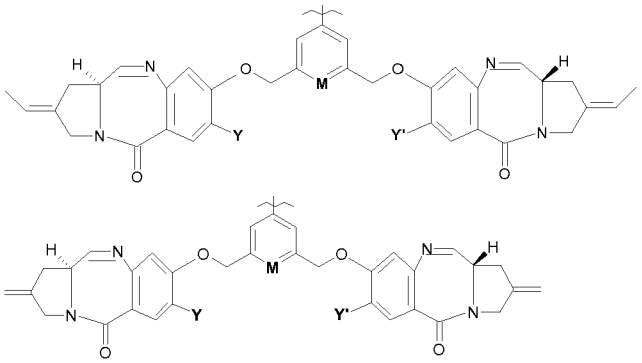
g: number of functions RCG2 on a modified binding agent; d: number of pyrrolo[1,4]benzodiazepine dimers on the binding agent Mab

L* represents $–L_1-N(Q-CH_2CH_2O)_k-CH_2CH_2-G)-L_2–$

The compounds according to the invention may thus be used for the preparation of a binding agent to which is covalently attached in the para position of **M** the dimer of formula:

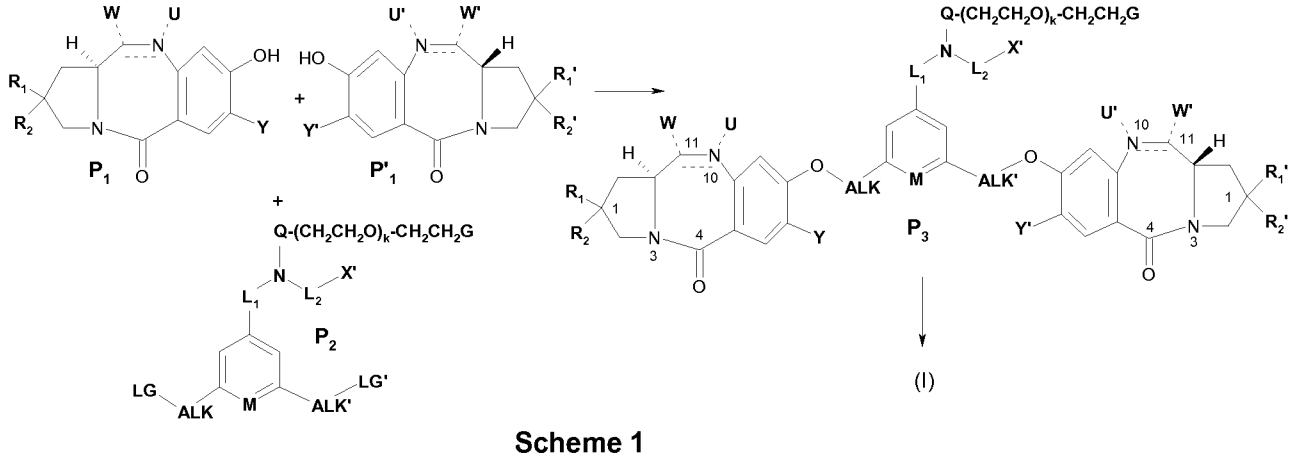


More particularly, the binding agent is an antibody. More particularly, the dimer has the formula:



Process for preparing the compounds of formula (I)

The compounds of formula (I) may be prepared according to **Scheme 1**:



Scheme 1

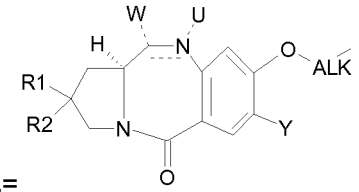
Compounds P_1, P'_1 and P_2 are reacted together to give P_3. **LG** and **LG'** denote a leaving group. The term "leaving group" denotes an atom or a group of atoms which, in the heterolytic reaction between P_2 and P_1 or P'_1, leaves taking the lone pair of electrons of the covalent bond connecting ALK and LG or LG'. The leaving group is more particularly chosen

from a halogen atom, especially chlorine or bromine, a mesylate, tosylate or nosylate group or -$OPPh_3^+$. The intermediate compounds **P_2** also form part of the invention.

In the preparation of a compound of formula (I) comprising the group RCG1, **X'** may represent the said group RCG1, in which case **P_3** represents a compound of formula (I). **X'** may also be a precursor of the said group RCG1 of the type -SZ_a or alternatively -$C(=O)Z_bR_b$ and in this case, it is necessary to convert X' into RCG1 by means of one or more chemical reactions. According to one variant, the conversion **X'** → RCG1 may also be performed on **P_2**.

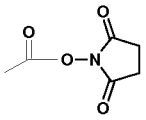
Thus, for the preparation of a compound **P_3** (or according to variant **P_2**) for which Z_a=H, it is preferred to introduce a group **X'**=-SZ_a for which Z_a=-$S(C_1$-$C_6)$alkyl using the precursor of the corresponding linker, and then to reduce the disulfide function –$SS(C_1$-$C_6)$alkyl to a thiol function –SH. To do this, use may be made, for example, of tris(2-carboxyethyl)phosphine: see in this respect Burns J.A. et al., *J. Org. Chem.* **1991**, *56*(8), 2648-2650. This conversion -$SS(C_1$-$C_6)$alkyl → -SH may apply especially to compounds **1** to **19** of **Table I**.

In the case of preparation of a compound of formula (I) from **P_3** according to the conversion **X'** → RCG1 such that RCG1 represents the group –SH, a compound **P_3** may also be formed,

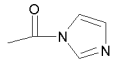


such that **X'** may also represent the group –SZ_a with Z_a= corresponding to the adduct of a thiol function to the imine function.

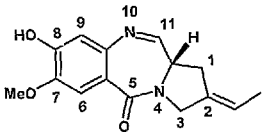
Similarly, for the production of a compound of formula (I) comprising a group RCG1= -$C(=O)Z_bR_b$, it is possible to convert a group X'= -$C(=O)Z_bR_b$ into a group RCG1= -$C(=O)Z_bR_b$ by means of one or more chemical reactions. In particular, in the case



where –$C(=O)Z_bR_b$= , it is possible to introduce onto a compound **P_3** (or according to variant **P_2**) the group **X'** = -$C(=O)O$-$(C_1$-$C_4)$alkyl or –C(=O)O-allyl, which is then converted into a group –C(=O)OH, which finally reacts with N-N'-disuccinimidyl carbonate or NHS. The conversion –COOalkyl/allyl to –COOH may be performed by treatment with a base such as LiOH or a palladium catalyst, for example tetrakis(triphenylphosphine)palladium in the presence of an amine "scavenger", for example morpholine. The reaction with N,N'-disuccinimidyl is performed in the presence of a base, for example DIPEA; the reaction with NHS is performed in the presence of a coupling agent, for

example DCC. Similarly, in the case where $-C(=O)Z_bR_b=$ , it is possible to introduce a group $-C(=O)Z_bR_b=$ -COOH, which then reacts with N,N'-carbonyldiimidazole (*JACS* **1958**, *80*, 4423; *JACS* **1960**, *82*, 4596). This conversion **X' =** $-C(=O)Z_bR_b$ → RCG1 = $-C(=O)Z_bR_b$ may especially apply to the compounds of Examples **20** to **36** of **Table I.**

Compounds $\mathbf{P_1}$ and $\mathbf{P'_1}$ are described in patent applications **WO 00/12508**, **WO 00/12507**, **WO 2005/040170**, **WO 2005/085260**, **WO 07085930** or **WO 2009/016516** or are accessible via total synthesis (Mori M. et al., *Tetrahedron*, **1986**, *42*, 3793-3806). In the case where $\mathbf{P_1}$ and/or $\mathbf{P'_1}$ represent(s) tomaymycin of formula:

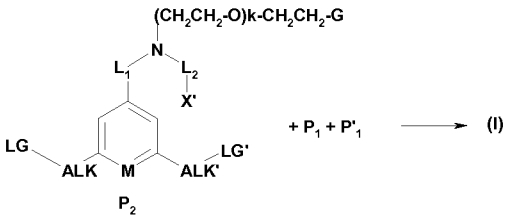


Tomaymycin

, the latter may be prepared with the aid of the strain *Streptomyces croceus* by following the teaching of **FR 1516743** or alternatively by total synthesis (see *J. Antibiotics* **1983**, *XXXVI*(3), 276-282 Z. Tozuka "Studies on tomaymycin. Total syntheses of the antitumor antibiotics E- and Z- tomaymycins"). Commercial compounds $\mathbf{P_1/P'_1}$ also exist.

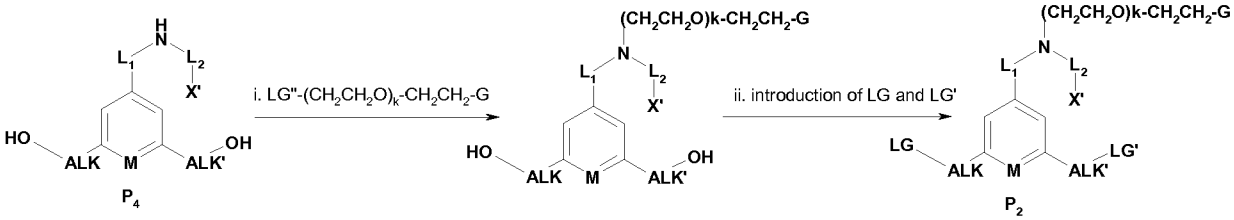
For the introduction of the groups W/W', the imine function (---- = double bond) is capable of adding various compounds HW/HW' (for example H_2O, alcohol ROH).

Case where Q = single bond



Scheme 2

Preparation of P_2

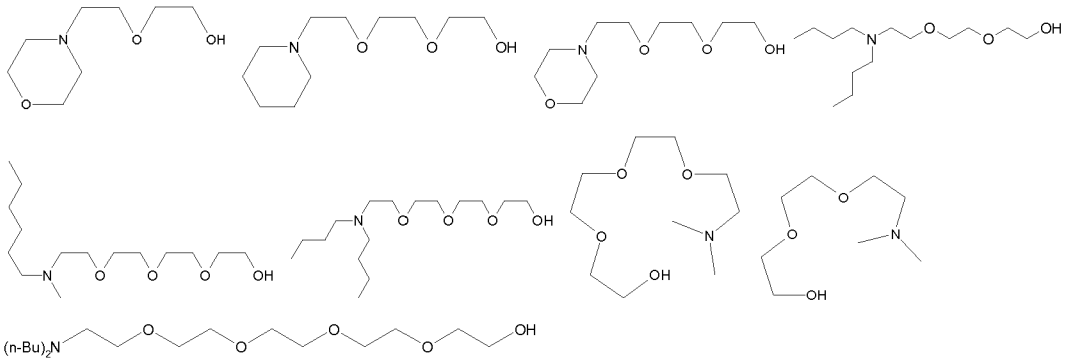


Scheme 3

i. nucleophilic reaction between the secondary amine function –NH- of $\mathbf{P_4}$ and a reagent of formula LG''-$(CH_2CH_2O)_k$-CH_2CH_2-G (LG'' = leaving group) in the presence of a base, for instance K_2CO_3 in a polar solvent such as DMF or THF.

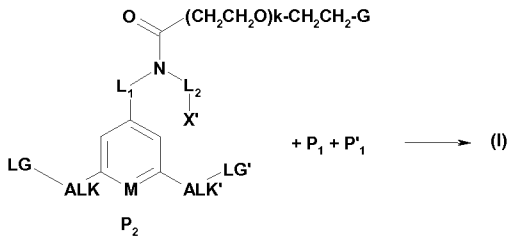
The reagent LG''-$(CH_2CH_2O)_k$-CH_2CH_2-G is obtained from a compound of formula HO-$(CH_2CH_2O)_k$-CH_2CH_2-G by replacing the group -OH with the leaving group LG'' by means of chemical reactions known to those skilled in the art. For example, in the case where LG'' represents a mesylate group, use is made of methanesulfonyl chloride in the presence of a base such as a tertiary amine (for example TEA). In the case where LG'' represents I, the mesylate is substituted with I, for example using sodium iodide, according to D. Marquis et al. *J. Org. Chem.* **1995**, *24*, 7984-96.

The PEG-alcohols of formula $HO(CH_2CH_2O)_k$-$CH_2CH_2OCH_3$ are commercially available (see for example the catalogue of the American company QuantaBioDesign, Ltd.). Other PEG-alcohols $HO(CH_2CH_2O)_k$-CH_2CH_2OR with R≠Me are commercially available or alternatively are available from $HO(CH_2CH_2O)_k$-CH_2CH_2OH by means of chemical reactions known to those skilled in the art. Similarly, certain compounds for which G=NRR' and k>=1 are commercially available, for example:



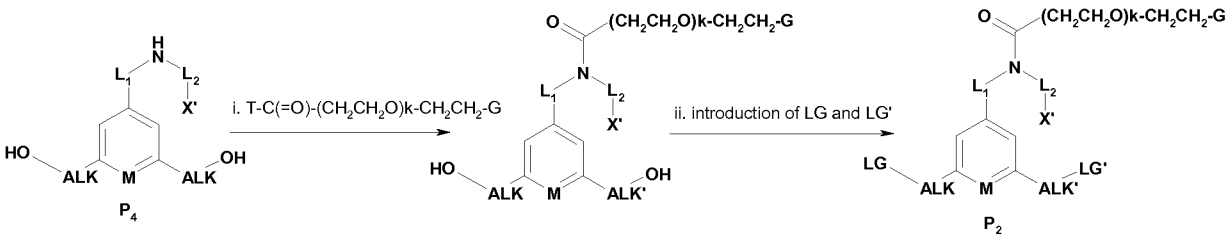
ii. introduction of **LG** and **LG'**. In the case of a mesylate group, use is made of MSC in the presence of a base such as a tertiary amine (for example TEA).

Case where Q=-C(=O)



Scheme 4

Preparation of P_2



Scheme 5

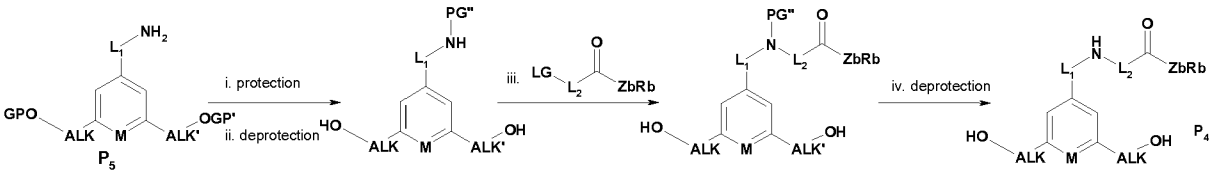
i. Amidation reaction between **P_4** and a carboxylic acid derivative of formula T-C(=O)-$(CH_2CH_2O)_k$-CH_2CH_2-G.

The carboxylic acid derivative may be an acyl halide (T = -Hal). According to one variant, use is made of an activated ester (for example T = -ONHS) or alternatively the carboxylic acid (T = -OH) in the presence of a coupling agent. The PEG-acids of formula HOC(=O)-$(CH_2CH_2O)_k$-CH_2CH_2OR may be prepared from the corresponding PEG-alcohols of formula HO-$(CH_2CH_2O)_{k-1}$-CH_2CH_2OR, which are commercially available for k= 1 to 11, by addition to sodium acrylate, according to J. Huskens, J.A. Peters, H. van Bekkum, *Tetrahedron* **1993**, *15*, 3149-64. This is likewise the case when G is the group NRR' for k>=1 starting with the corresponding compounds of formula H-$(OCH_2CH_2)_{k-1}$-CH_2CH_2NRR'. Similarly, the compounds HOC(=O)-(CH_2CH_2)-NRR' (k=0) are commercially available.

ii. introduction of **LG** and **LG'**. In the case of a mesylate group, use is made of MSC in the presence of a base such as a tertiary amine (for example TEA).

Preparation of P_4

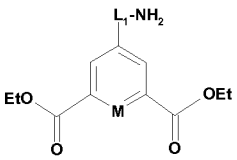
Case where X'= -C(=O)-Z_bR_b



Scheme 6

i. introduction of the protecting group PG". In the case of a nosylate group, use is made of 2-nitrobenzenesulfonyl chloride in the presence of a base such as a tertiary amine (for example TEA) or pyridine;

ii. deprotection of the groups PG and PG'. For example in the presence of hydrochloric acid or TFA when the groups PG and PG' are TBDMS.

According to one variant, in the case where ALK=ALK'=-CH_2-, **P_5** may represent . The deprotection step is then replaced with a step of reduction of the

ester function to a $-CH_2OH$, for example with sodium borohydride; to do this, the reduction conditions given on pages 62-63 of **WO 2007/085930** may be applied. As described later, this variant consisting in using a diester and in then applying a reduction may be generalized to the other $\mathbf{P_5}$. Furthermore, the reduction of the ester functions may be performed on a $\mathbf{P_4}$ but optionally also, according to one variant, on a $\mathbf{P_5}$.

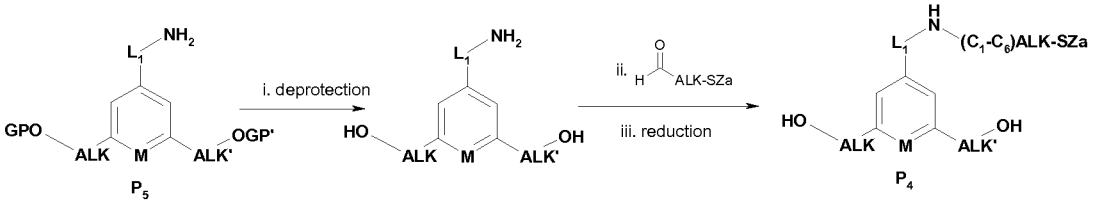
iii. Nucleophilic reaction between the protected amine function –NH(PG'') and a reagent of formula $LG\text{-}L_2\text{-}C(=O)\text{-}Z_bR_b$ in the presence of a base, for instance K_2CO_3 in a polar solvent such as DMF or THF.

For the case where $L_2=(C_1\text{-}C_6)ALK$, the bromo-alkyl esters of formula $Br\text{-}(C_1\text{-}C_6)ALK\text{-}C(=O)\text{-}OMe$ are commercially available. For the case where $L_2=\text{-}(CH_2CH_2O)_j\text{-}CH_2CH_2\text{-}$, LG may be introduced starting with the corresponding PEG-alcohols of formula $HO\text{-}(CH_2CH_2O)_j\text{-}CH_2CH_2\text{-}C(=O)Z_bR_b$. Such compounds are commercially available or alternatively may be obtained from the corresponding PEG-diols of formula $HO\text{-}(CH_2CH_2O)_j\text{-}H$, which are commercially available for j= 1 to 11, by addition to sodium acrylate, according to J. Huskens, J.A. Peters, H. van Bekkum, *Tetrahedron* **1993**, *15*, 3149-64.

iv. deprotection of the group PG''. For example in the presence of thiophenol and a base such as caesium carbonate when PG'' is the nosylate group.

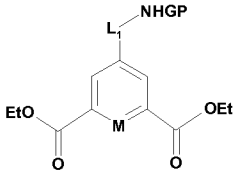
Case where X'= $-SZ_a$

Case where $L_2=(C_1\text{-}C_6)ALK$



Scheme 7

i. deprotection of the protecting groups PG and PG', preferably in acidic medium, for example in the presence of hydrochloric acid or TFA when the groups PG and PG' are TBDMS.

According to one variant, in the case where ALK=ALK'=$-CH_2-$, $\mathbf{P_5}$ may represent 

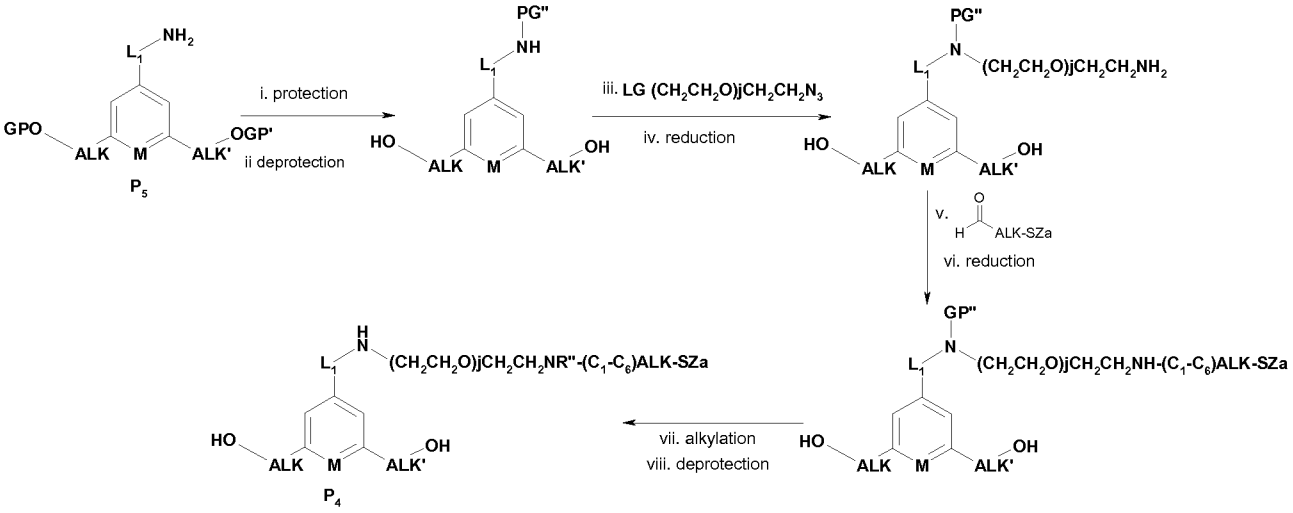
. The step of deprotection of the alcohol functions is then replaced with a reaction for reduction of the ester function to a function $-CH_2OH$, for example with sodium borohydride, followed by a step of deprotection of the amine function;

to do this, the reduction conditions given on pages 62-63 of **WO 2007/085930** may be applied.

ii. reductive amination with the aldehyde of formula HC(=O)-ALK-SZa;

iii. the intermediate amine is reduced *in situ* with a reducing agent, for instance sodium triacetoxyborohydride according to A.F. Abdel-Magid et al., J. Org. Chem. **1996**, *61*, 3849-62, preferably in acetic acid.

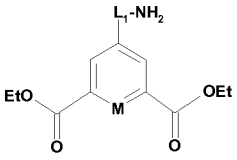
Case where L_2=-$(CH_2CH_2O)_j$-CH_2CH_2NR''-ALK-



Scheme 8

i. introduction of the protecting group PG". In the case of a nosylate group, use is made of 2-nitrobenzenesulfonyl chloride in the presence of a base such as a tertiary amine (for example TEA) or pyridine;

ii. deprotection of the groups PG and PG'. For example in the presence of hydrochloric acid or TFA when the groups PG and PG' are TBDMS.

According to one variant, in the case where ALK=ALK'=-CH_2-, **P_5** may represent . The deprotection step is then replaced with a step of reduction of the ester function to a function –CH_2OH, for example with sodium borohydride; to do this, the reduction conditions given on pages 62-63 of **WO 2007/085930** may be applied.

iii. nucleophilic reaction between the protected amine function –NH(PG'') and a reagent of formula LG-$(CH_2CH_2O)_jCH_2CH_2N_3$ in the presence of a base, for instance K_2CO_3 in a polar solvent such as DMF or THF. Such compounds may be obtained according to **WO 07/085930**, starting with the corresponding PEG-diols of formula HO-$(CH_2CH_2O)_{j+1}$-H, which are commercially available for j= 0 to 10.

iv. reduction of the azido group, for example via the Staudinger reaction in the presence of triphenylphosphine and water.

v. reductive amination with the aldehyde of formula HC(=O)-ALK-SZa;

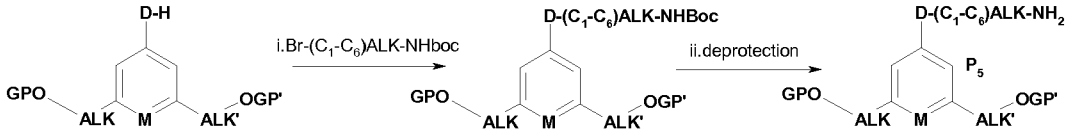
vi. the intermediate amine is reduced *in situ* with a reducing agent, for instance sodium triacetoxyborohydride, according to A.F. Abdel-Magid et al., J. Org. Chem. **1996**, *61*, 3849-62, preferably in acetic acid.

vii. alkylation of the secondary amine function.

viii. deprotection of the group PG''. For example in the presence of thiophenol and a base such as caesium carbonate when PG'' is the nosylate group.

Preparation of P_5

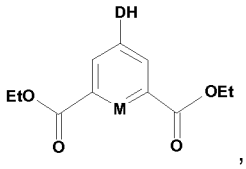
Case where L_1=-D-(C_1-C_6)ALK-, D = O or NH

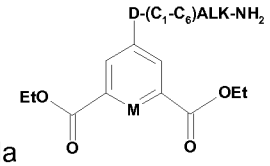


Scheme 9

i. nucleophilic reaction between the function –DH and a boc-protected bromo-amine of formula Br(C_1-C_6)ALK-NHboc in the presence of a base, for instance K_2CO_3 in a polar solvent such as DMF or THF (see for example the conditions on page 63 of **WO 07085930**).

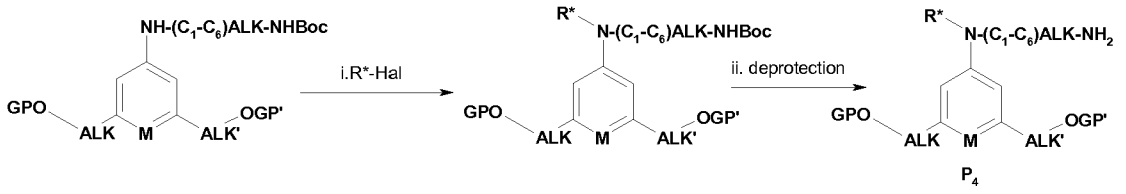
ii. selective deprotection of the amine, preferably in acidic medium, for example in the presence of hydrochloric acid or TFA. For the cases where a selective deprotection cannot be performed, for example when the groups PG and PG' are TBDMS, a step of selective reprotection of the alcohols is necessary.

According to one variant, in the case where ALK=ALK'=-CH_2-, it is possible to perform the nucleophilic substitution of the bromo-amine with a diester of formula: 

, so as to obtain a compound **P_5** of formula 

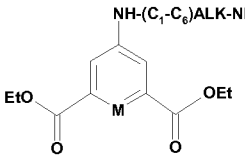
which is used in the form as obtained, for the preparation of **P_4**.

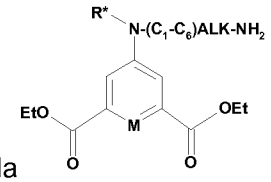
Case where L_1=-N((C_1-C_4)alkyl)- (C_1-C_6)ALK-



Scheme 9'

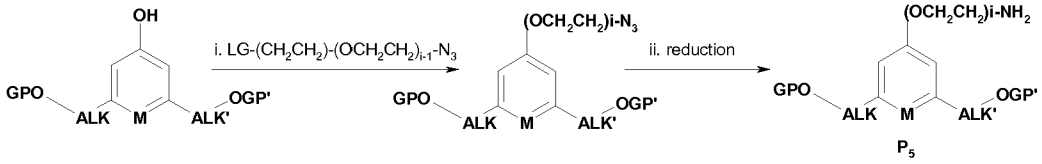
i. nucleophilic reaction between the function –NH- and an alkyl halide of formula R*-Hal with R* = (C_1-C_4)alkyl in the presence of a base, for instance K_2CO_3 in a polar solvent such as DMF or THF.

According to one variant, in the case where ALK=ALK'=-CH_2-, it is possible to perform the nucleophilic substitution of the alkyl halide with a diester of formula: 

, so as to obtain a compound **P_5** of formula 

, which is used in the form as obtained, for the preparation of **P_4**.

Case where L_1= -$(OCH_2CH_2)_i$

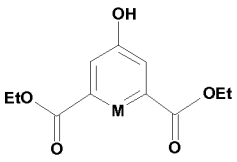


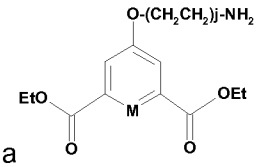
Scheme 10

i. nucleophilic reaction between one of the –OH functions (the two others being protected with PG which denotes a protecting group) and an azido-PEG reagent of formula LG-(CH_2CH_2)-$(OCH_2CH_2)_{i-1}$-N_3 bearing a nucleofugal group (LG) such as Hal or mesylate, in the presence of a base, for instance K_2CO_3 in a polar solvent such as DMF or THF (see for example the conditions on page 63 of **WO 07085930**).

ii. reduction of the azido group, for example with triphenylphosphine in the presence of water in a polar solvent such as THF.

According to one variant, in the case where ALK=ALK'=-CH_2-, it is possible to perform the nucleophilic substitution of the azido-PEG reagent with the hydroxy-diester of formula:



, so as to obtain a compound P_5 of formula 

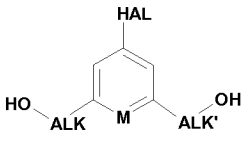
, which is used in the form as obtained, for the preparation of P_4.

Case where L_1 = single bond



Scheme 11

i. protection of the alcohol groups

P_5 may be obtained from the halo-diol of formula 

. An example of a halo-diol and of the corresponding protected diol is described in Scheme 1 on page 48 of **WO 2009/016516** (compounds 2 and 3 of Scheme 1). Two examples of protected diols are those of CAS Nos. 181225-40-1 and 181225-41-2. The halo-diol may be obtained by reduction of the corresponding diacid or diester compound, for example that of CAS No. 193010-40-1. See also in the case of a pyridine (M=N): *Liebigs Annalen der Chemie* **1991**, *10*, 987-988 or *Tetrahedron* **2005**, *61*(7), 1755-1763 (compound 3 of Scheme 1).

A person skilled in the art may be inspired by the operating conditions of the examples described below which are given for particular linkers L_1 and L_2 and may adapt them to other linkers L_1 and L_2.

Process for preparing the conjugate

The conjugate is obtained via the process that consists in:

(i) placing in contact and leaving to react an aqueous solution, optionally buffered, of the binding agent, optionally modified with a modifying agent, and a solution of a compound of formula (I);

(ii) and then in optionally separating the conjugates formed in step (i) from the compound of formula (I) and/or the unreacted binding agent and/or any aggregates that may have formed.

The chemical group RCG1 of the compound of formula (I) must be reactive towards the chemical groups RCG2 present on the binding agent, especially towards the amino groups present on antibodies, the said chemical groups RCG2 having been introduced, where appropriate, by the modifying agent, so as to attach the compound of formula (I) to the

binding agent via formation of a covalent bond.

According to one variant, in step (ii) the conjugate formed in step (i) is separated from the unreacted binding agent and from any aggregates that may be present in the solution. According to another variant, in step (ii) the conjugate from step (i) is separated only from the unreacted compound of formula (I) and from the aggregates that may have formed, and any unreacted binding agent is left in solution.

The aqueous solution of the binding agent may be buffered with at least one buffer, for instance potassium phosphate or N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES buffer). The buffer depends on the nature of the binding agent. The compound of formula (I) is dissolved in a polar organic solvent, for example DMSO or DMA.

The reaction takes place at a temperature generally of between 20 and 40°C. The reaction time may range between 1 and 24 hours. The reaction between the binding agent and the compound of formula (I) may be monitored by SEC with a refractometric and/or ultraviolet detector, so as to determine its progress. If the degree of grafting is insufficient, the reaction may be left for longer and/or compound of formula (I) may be added. Reference may be made to the general method given in the examples section for further details regarding the particular conditions that may be used for the conjugation.

A person skilled in the art has at his disposal various chromatographic techniques for the separation of step (ii): the conjugate may be purified, for example, by steric exclusion chromatography (SEC), by adsorption chromatography (such as ion exchange, IEC), by hydrophobic interaction chromatography (HIC), by affinity chromatography, by chromatography on mixed supports such as ceramic hydroxyapatite, or by HPLC. Purification by dialysis or diafiltration may also be used.

The term "aggregates" means associations that may form between two or more binding agents, the binding agents having possibly been modified by conjugation. Aggregates are capable of being formed under the influence of a large number of parameters such as a high concentration of binding agent in the solution, the pH of the solution, high shear forces, the number of grafted dimers and their hydrophobic nature, the temperature (see the references cited in the introduction of *J. Membrane Sci.* **2008**, *318*, 311–316), the influence of some of them occasionally not being explained with precision. In the case of proteins or antibodies, reference may be made to *AAPS Journal*, "Protein Aggregation and Bioprocessing" **2006**, 8(3), E572-E579. The content of aggregates may be determined by means of known techniques such as SEC (see in this respect *Analytical Biochemistry* **1993**, *212*(2), 469-480).

After step (i) or (ii), the solution of the conjugate may undergo a step (iii) of ultrafiltration and/or diafiltration. The conjugate in aqueous solution is thus obtained after these steps.

Antibody

The antibody (see in this respect Janeway et al. "Immunobiology", 5th edition, 2001, Garland Publishing, New York) may be chosen from those described especially in patent applications **WO 04043344**, **WO 08010101**, **WO 08047242**, **WO 05009369** (anti-CA6). The antibody may especially be monoclonal, polyclonal or multispecific. It may also be an antibody fragment. It may also be a murine, human, humanized or chimeric antibody.

Conjugate

A conjugate generally comprises from about 1 to 10 pyrrolo[1,4]benzodiazepine dimers attached to the binding agent (this is the degree of grafting or the "drug-to-antibody ratio" (or "DAR")). This number varies as a function of the nature of the binding agent and of the dimer, and also of the operating conditions used for the conjugation (for example the number of equivalents of dimer relative to the binding agent, the reaction time, the nature of the solvent and of any cosolvent). Placing the binding agent and the dimer in contact leads to a mixture comprising: several conjugates that are individually distinguished from each other by different DARs; possibly the unreacted binding agent (in the case of an incomplete reaction); possibly aggregates. The DAR, which is determined on the final solution, for example by UV spectroscopy, thus corresponds to an average DAR.

In the case where the binding agent is an antibody, UV spectroscopy may be a method used for determining the DAR. This method is inspired by that presented in Antony S. Dimitrov (ed), LLC, 2009, "Therapeutic Antibodies and Protocols", vol. 525, 445, Springer Science. It consists in measuring the absorbance of a solution of conjugate after the separation step (ii) at two wavelengths noted WL1 and WL2. The following molar extinction coefficients of the naked antibody and of the pyrrolo[1,4]benzodiazepine dimer prior to conjugation are used.

The absorbances of the solution of conjugate at WL1 and WL2 (A_{WL1}) and (A_{WL2}) are measured either on the corresponding peak of the SEC spectrum (which makes it possible to calculate a "DAR(SEC)") or by using a standard UV spectrophotometer (which makes it possible to calculate a "DAR(UV)"). The absorbances may be expressed in the form:

$$A_{WL1} = (c_D \times e_{D\,WL1}) + (c_A \times e_{A\,WL1})$$

$$A_{WL2} = (c_D \times e_{D\,WL2}) + (c_A \times e_{A\,WL2})$$

for which equations:

- c_D and c_A denote, respectively, the concentrations in the solution of the part of the conjugate relating to the pyrrolo[1,4]benzodiazepine dimer and the part of the conjugate relating to the antibody;
- $e_{D\,WL1}$ and $e_{D\,WL2}$ denote, respectively, the molar extinction coefficients of the pyrrolo[1,4]benzodiazepine dimer before conjugation at the wavelengths WL1 and WL2;
- $e_{A\,WL1}$ and $e_{A\,WL2}$ denote, respectively, the molar extinction coefficients of the naked antibody at the two wavelengths WL1 and WL2.

The term "naked antibody" means the antibody to which no pyrrolo[1,4]benzodiazepine dimer is attached, i.e. the antibody before the conjugation step.

Resolution of these two equations leads to:

$$c_D = [(e_{A\,WL1} \times A_{WL2}) - (e_{A\,WL2} \times A_{WL1})] / [(e_{D\,WL2} \times e_{A\,WL1}) - (e_{A\,WL2} \times e_{D\,WL1})]$$

$$c_A = [A_{WL1} - (c_D \times e_{D\,WL1})] / e_{A\,WL1}$$

The average DAR then corresponds to c_D/c_A. In the case of the pyrrolo[1,4]benzodiazepine dimers, the two wavelengths considered are: WL1= 280 nm and WL2= 320 nm. The average DAR is preferably between 1 and 10, and preferably between 1.5 and 7.

The conjugate may be used as an anticancer agent. By virtue of the presence of the binding agent, the conjugate is made very selective towards tumour cells rather than healthy cells. This makes it possible to direct the compound of formula (I) which has anticancer activity into an environment close to these tumour cells or directly therein (see in this respect the following publications that describe the use of monoclonal antibody conjugates in cancer treatment: “Antibody-drug conjugates for cancer therapy” Carter P.J. et al., *Cancer J.* **2008**, *14*, 154–169; “Targeted cancer therapy: conferring specificity to cytotoxic drugs” Chari R., *Acc. Chem. Res.* **2008**, *41*, 98–107). It is possible to treat solid or liquid cancers.

The conjugate is formulated in the form of a buffered aqueous solution at a concentration generally of between 1 and 10 mg/ml. This solution may be injected in perfusion form as such, or may be rediluted to form a perfusion solution.

[Examples]

Method A: High-pressure liquid chromatography - Mass spectrometry (LMSC)

The spectra were acquired on a Waters UPLC-SQD machine in positive and/or negative electrospray ionization mode (ES+/-). Chromatographic conditions: column: ACQUITY BEH C18- 1.7 µm – 2.1x50 mm; solvents: A: H_2O (0.1% formic acid) B: CH_3CN (0.1% formic

acid); column temperature: 50°C; flow rate: 1 ml/min; gradient (2 min): from 5 to 50% B in 0.8 min; 1.2 min: 100% B; 1.85 min: 100% B; 1.95: 5% B.

Method B: High-pressure liquid chromatography - Mass spectrometry (LMSC)

The spectra were acquired on a Waters ZQ machine in positive and/or negative electrospray mode (ES+/-) with a U.V. DAD 200<WL<400 nm detector. Chromatographic conditions: column: Phenomenex Kinetex C18 100A 3x50 mm, particle diameter 2.6 µm; solvents: A: H_2O (0.1% formic acid) B: CH_3CN; column temperature: 50°C; flow rate: 1 ml/min; gradient (6 min): 6% B for 0.80 min; from 6 to 100% B in 3.9 min; 4.80 min: 100% B; 5 min: 6% B; 6 min: 6% B.

Method C: High-pressure liquid chromatography - Mass spectrometry (LMSC)

The spectra were acquired on a Waters ZQ machine in positive and/or negative electrospray mode (ES+/-) with a U.V. DAD 200<WL<400 nm detector. Chromatographic conditions: column: Phenomenex Kinetex C18 3x100 mm column, particle diameter 2.6 µm; solvents: A: H_2O (0.1% formic acid) B: CH_3CN; column temperature: 50°C; flow rate: 0.8 ml/min; gradient (8.2 min): 4% B for 0.15 min; from 6 to 100% B in 6.85 min; 7.1 min: 100% B; 7.4 min: 4% B; 8.2 min: 4% B.

Method D: deglycosylation and mass spectrometry (HRMS) of a conjugate

Deglycosylation is an enzymatic digestion technique using glycosidase. It is performed starting with 500 µl of conjugate + 100 µl of Tris HCl 50 mM buffer + 10 µl of glycanase-F enzyme (100 units of lyophilized enzyme/100 µl of water). The mixture is vortexed and maintained overnight at 37°C. The deglycosylated sample is then ready to be analysed by HRMS. Depending on the case, the HRMS analysis of the sample may also be performed without prior deglycosylation. In both cases, the mass spectra were obtained on a Waters Xévo Q-Tof machine in positive electrospray mode (ES+). Chromatographic conditions: Acquity UPLC Waters BEH 300 C4 2.1x150 mm column, particle diameter 1.7 µm; solvents: A: H_2O + 0.1% formic acid: B: CH_3CN + 0.1% formic acid; column temperature 70°C: flow rate 0.5 ml/min; gradient (10 min): 20% B for 2 min 50 sec; from 20 to 80% B in 2 min 5 sec; 8 min 50 sec: 80% B; 8 min 55 sec: 20% B; 10 min: 20% B.

Method E: High-pressure liquid chromatography - Mass spectrometry (LMSC)

The spectra were acquired on a Waters UPLC-SQD line in positive and/or negative electrospray ionization mode (ES+/-) with a U.V. DAD 210<WL<400 nm detector. Chromatographic conditions: column: ACQUITY UPLC BEH C18- 1.7 µm – 2.1x30 mm; solvents: A: H_2O (0.1% formic acid) B: CH_3CN (0.1% formic acid); column temperature:

45°C; flow rate: 0.6 ml/min; gradient (2 min): from 5 to 50% B in 1 min; from 50 to 100% B in 0.3 min; 1.45 min: 100% B; from 100 to 5% B in 0.3 min; 2 min: 100% B.

Method F: High-pressure liquid chromatography - Mass spectrometry (LMSC)

The spectra were acquired on a Waters ZQ line in positive and/or negative electrospray mode (ES+/-) with a U.V. DAD 200<WL<400 nm detector. Chromatographic conditions: column: XSelect CSH Waters C18 3x75 mm, particle diameter 3.5 µm; solvents: A: H_2O (0.1% formic acid) B: CH_3CN (0.1% formic acid); column temperature: 50°C; flow rate: 0.8 ml/min; gradient (6 min): 6% B for 0.80 min; from 6 to 100% B in 3.9 min; 4.80 min: 100% B; 5 min: 6% B; 6 min: 6% B.

Method G: High-pressure liquid chromatography - Mass spectrometry (LMSC)

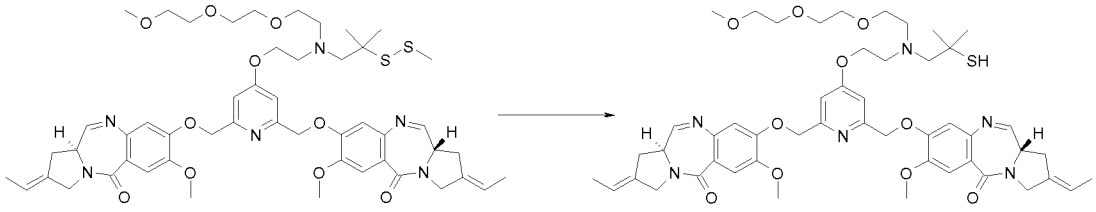
The spectra were acquired on a Waters UPLC-SQD machine in positive and/or negative electrospray ionization mode (ES+/-). Chromatographic conditions: column: ACQUITY BEH C18- 1.7 µm – 2.1x50 mm; solvents: A: H_2O (0.1% formic acid) B: CH_3CN (0.1% formic acid); column temperature: 50°C; flow rate: 0.8 ml/min; gradient (2.5 min): from 5 to 100% B in 1.8 min; 2.40 min: 100% B; 2.45 min: 100% B; from 100 to 5% B 0.05 min.

The antibody hu2H11 (also known as hu53 2H11 on page 15 of **WO 2008010101;** it is an antibody comprising a Vh having the amino acid sequence SEQ ID No. 24) or the antibody hu2H11R35R74 obtained by directed mutagenesis of hu53 2H11 (referenced on page 20 of **WO 2011039721**; it is an antibody comprising a Vh having the amino acid sequence SEQ ID No. 18 and a Vl having the sequence SEQ No. 16) is used.

Chapter 1: Novel tomaymycin derivatives

Example 1:

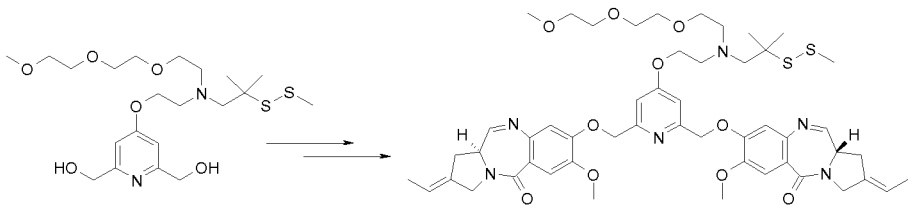
1.1. 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-(2-methyl-2-mercapto-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1,4] benzodiazepin-5-one-8-yloxymethyl]-pyridine



To 20 mg of 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-2-methyldisulfanyl-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1,4] benzodiazepin-5-one-8-yloxymethyl]-pyridine dissolved in 900 µL of MeOH and 400 µL of DMF is added a solution of 17.5 mg of tris(2-carboxyethyl)phosphine

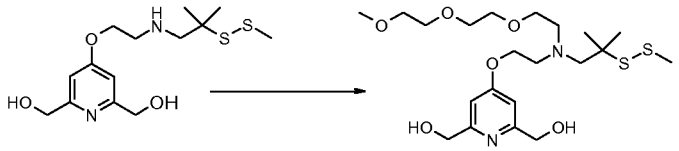
hydrochloride and 15.8 mg of $NaHCO_3$ in 370 µL of water. The mixture obtained is stirred for 1 hour at room temperature and then concentrated under reduced pressure and purified by flash chromatography on silica (Interchrom Puriflash Silica 15/35U 2G), using a gradient of 0 to 10% MeOH in a 9:1 DCM/acetonitrile mixture. The fractions containing the desired product are combined and concentrated under reduced pressure. 7 mg of 4-{2-[methyl-(2-methyl-2-mercapto-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c][1.4] benzodiazepin-5-one-8-yloxymethyl]-pyridine are thus obtained: LC/MS (A): tr= 1.06 min; [M+H]+: m/z 941; [M+H_2O+H]+: m/z 959.

1.2. 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-2-methyldisulfanyl-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c] [1.4] benzodiazepin-5-one-8-yloxymethyl]-pyridine



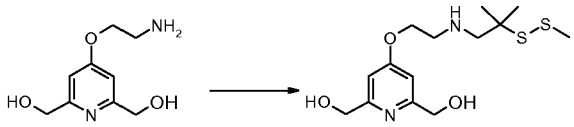
To a solution of 30 mg of 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-2-methyldisulfanyl-propyl)-amino]-ethoxy}-2,6-bis-(hydroxy methyl)-pyridine and 65.5 µL of diisopropylethylamine in 200 µL of DCM cooled to -20°C are added 19.4 µL of MSC. After stirring for 20 minutes, the mixture is hydrolysed and the organic phase is washed with water and then dried over $MgSO_4$ and concentrated under reduced pressure. The residue obtained (36 mg) dissolved in 400 µL of DMF is added to a solution of 26 mg of tomaymycin in 425 µL of DMF, along with 39.6 mg of K_2CO_3 and 15.8 mg of KI. The mixture is stirred for 12 hours at 30°C and then hydrolysed until precipitation takes place. The insoluble matter is removed by filtration on a sinter funnel, washed with DCM and the combined organic phases are then concentrated under reduced pressure and purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF25-8g) using a gradient of 0 to 10% MeOH in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure, taken up in a 1/1 dioxane/water mixture and concentrated again under reduced pressure. 23 mg of 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-2-methyldisulfanyl-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridine are thus obtained. ^1H NMR (500 MHz, chloroform-d): broad signals: 1.20 to 1.78 (m, 12 H); 2.40 (s, 3 H); 2.70 to 3.10 (m, 10 H); 3.34 (m, 4 H); 3.49 to 3.70 (m, 8 H); 3.71 (s, 3 H); 3.91 (m, 2 H); 4.00 (s, 6 H); 4.27 (m, 4 H); 5.27 (m, 4 H); 5.60 (m, 2 H); 6.86 (s, 2 H); 7.00 (m, 2H); 7.56 (s, 2 H); 7.65 (d, J=4.4 Hz, 2 H). LC/MS (A): Tr= 0.81 min; [M+H]+: m/z 987.

1.3. 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-2-methyldisulfanyl-propyl)-amino]-ethoxy}-2,6-bis-(hydroxy methyl)-pyridine



To a solution of 100 mg of 4-[2-(2-methyl-2-methyldisulfanyl-propylamino)-ethoxy]-2,6-bis-(hydroxymethyl)-pyridine in 2 mL of DMF are added 99 mg of 1-iodo-2-[2-(2-methoxy-ethoxy)-ethoxy]-ethane (B. Ben Aroya Bar Nir, J.F. Kadla *Carbohydrate Polymers*, **2009**, *76*, 60-67) and 54 mg of K_2CO_3. After 12 hours at 60°C, the mixture is supplemented with 40 mg of 1-iodo-2-[2-(2-methoxy-ethoxy)-ethoxy]-ethane and a further 55 mg of K_2CO_3. The mixture obtained is stirred for a further 24 hours at 80°C. After concentration under reduced pressure, the crude product thus obtained is dissolved in a minimum amount of MeOH and applied on Mega BE-SCX, 1GM 6ML (Varian). After washing the phase with MeOH, the product of interest is eluted with a 2N solution of ammonia in MeOH. The MeOH phase is concentrated under reduced pressure and then reapplied on Mega BE-SCX, 2GM 12ML (Varian) according to the same protocol. The methanol/NH_3 phases are concentrated under reduced pressure and the residue obtained is purified by flash chromatography on silica (Merck SuperVarioFlash 10g column, Si60 15-40 μm), using a gradient of 0 to 10% MeOH in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 30 mg of 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-2-methyldisulfanyl-propyl)-amino]-ethoxy}-2,6-bis-(hydroxymethyl)-pyridine are thus obtained. ^{1}H NMR (400 MHz, DMSO-d_6): 1.27 (s, 6 H); 2.40 (s, 3 H); 2.75 (s, 2 H); 2.80 (t, J=5.9 Hz, 2 H); 3.00 (t, J=5.9 Hz, 2 H); 3.23 (s, 3 H); 3.40 (m, 2 H); 3.47 to 3.55 (m, 8 H); 4.12 (t, J=5.9Hz, 2 H); 4.45 (d, J=5.9 Hz, 4 H); 5.30 (t, J=5.9 Hz, 2 H); 6.85 (s, 2 H). LC/MS (A): tr= 0.44 min; [M+H]+: m/z 479; [M-H+HCO2H]-: m/z 523.

1.4. 4-[2-(2-methyl-2-methyldisulfanyl-propylamino)-ethoxy]-2,6-bis-(hydroxymethyl)-pyridine

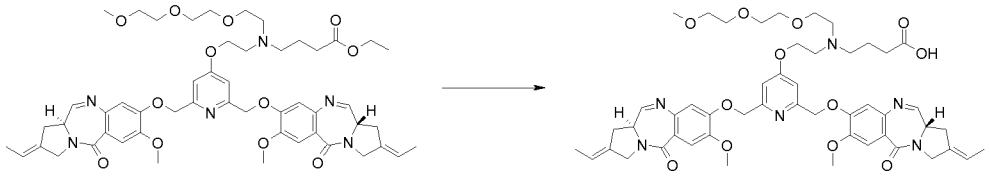


To a suspension of 390 mg of 4-[2-amino-ethoxy]-2,6-bis-(hydroxymethyl)-pyridine (prepared after deprotection of the boc group of 4-(2-tert-butoxycarbonylamino-ethoxy)-2,6-bis-(hydroxymethyl)-pyridine described on page 101 of **WO 07085930**) in 2 mL of THF are added 270 µl of 2-(methyldithio)-isobutyraldehyde and 730 µL of titanium isopropoxide. After 20 min, a further 270 µl of 2-(methyldithio)-isobutyraldehyde and 730 µL of titanium isopropoxide are added and the mixture is stirred for 2 hours at room temperature. The mixture is then supplemented with 6 mL of ethanol, stirred for 20 min at room temperature

and then supplemented with 124 mg of sodium cyanoborohydride. After stirring for 45 minutes, a further 124 mg of sodium cyanoborohydride are added and after stirring for 1 hour, the mixture is concentrated under reduced pressure, and diluted with EtOAc and water. The resulting precipitate is filtered off, and dissolved in aqueous 1M HCl solution. The aqueous phase obtained is brought to basic pH with aqueous 5M sodium hydroxide solution, extracted 3x with DCM and the combined organic phases are concentrated under reduced pressure. 322 mg of 4-[2-(2-methyl-2-methyldisulfanyl-propylamino)-ethoxy]-2,6-bis-(hydroxymethyl)-pyridine are obtained . ^{1}H NMR (400 MHz, DMSO-d_6): 1.26 (s, 6 H); 1.81 (broad m, 1 H); 2.39 (s, 3 H); 2.67 (broad s, 2 H); 2.94 (broad t, J=5.7 Hz, 2 H); 4.11 (t, J=5.7 Hz, 2 H); 4.45 (d, J=5.5 Hz, 4 H); 5.32 (t, J=5.5 Hz, 2 H); 6.85 (s, 2 H). LC/MS (A): tr= 0.24 min; [M+H]+: m/z 347.

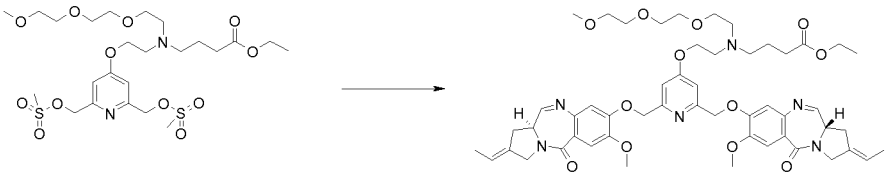
Example 2

2.1. 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-butanoic acid



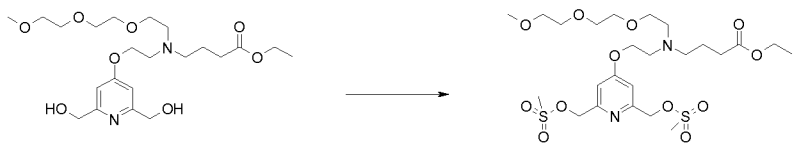
To a solution of 28 mg of ethyl 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-butanoate in 527 µl of THF and 61 µl of water are added 32 µl of an aqueous 1 M lithium hydroxide solution. The mixture is stirred for 1 hour 30 minutes at room temperature and a further 5 µl of aqueous 1 M lithium hydroxide solution are added thereto. After stirring for 1 hour 30 minutes at room temperature, the mixture is acidified to a pH close to 3 by adding 800 µl of potassium phosphate buffer (pH=3) and then extracted 5x with DCM. The organic phases are combined, dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF10-4g), using a gradient of 3 to 20% MeOH in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 10.2 mg of 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c][1.4]benzo-diazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-butanoic acid are obtained. LC/MS (B): tr= 3.08min; $[M+H]^+$: m/z 939.

2.2. Ethyl 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-butanoate



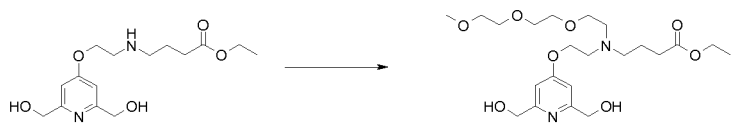
To a solution of 50.4 mg of (S)-2-eth-(E)-ylidene-8-hydroxy-7-methoxy-1,2,3,11a-tetrahydro-pyrrolo[2.1-c][1.4]benzodiazepin-5-one in 4 ml of DMF are added 77 mg of K_2CO_3, 30.7 mg of potassium iodide and 67 mg of ethyl 4-([2-(2,6-bis-methanesulfonyloxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-butyrate. The mixture is heated for 18 hours at 30°C and then cooled to room temperature, filtered through a 0.45 µm membrane, concentrated under reduced pressure and purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF15-12g), using a gradient of 0 to 10% MeOH in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 51 mg of ethyl 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c][1.4] benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy] ethyl}-amino)-butanoate are obtained. LC/MS (B): tr= 3.20 min; $[M+H]^+$: m/z 967.

2.3. Ethyl ([2-(2,6-bis-methanesulfonyloxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-butyrate



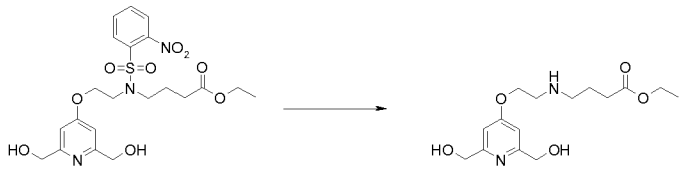
To a solution of 51 mg of ethyl 4-([2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-butanoate in 5 ml of DCM, precooled to -25°C, are added 110 µl of diisopropylethylamine and 34 µl of MSC. The mixture is stirred for 1 hour at -15°C and then washed with 5 ml of water. The aqueous phase is extracted with 5 ml of DCM. The organic phases are combined, dried over $MgSO_4$ and concentrated under reduced pressure. 69 mg of ethyl 4-([2-(2,6-bis-methanesulfonyloxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-butyrate are obtained. LC/MS (B): tr= 2.87 min; $[M+H]^+$: m/z 615.

2.4. Ethyl 4-([2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-butanoate



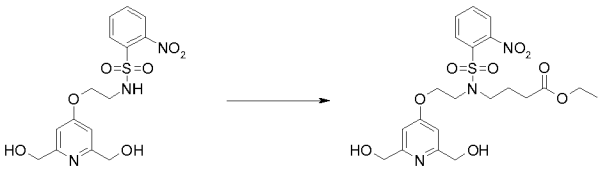
To a solution of 180 mg of ethyl 4-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethylamino]-butanoate in 18 ml of acetonitrile are added 207 mg of 1-iodo-2-[2-(2-methoxy-ethoxy)-ethoxy]-ethane and 192 µl of diisopropylethylamine. The mixture is heated for 3 days at 80°C and then concentrated under reduced pressure and purified by flash chromatography on silica (Merck SuperVarioFlash 15 g column, Si60 15-40 µm), using a DCM/MeOH/water mixture (40/5/0.5). The fractions containing the desired product are combined and concentrated under reduced pressure. 51 mg of ethyl 4-([2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-butanoate are obtained. LC/MS (B): tr= 0.70 min; $[M+H]^+$: m/z 459.

2.5. Ethyl 4-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethylamino]-butanoate



To a solution of 390 mg of ethyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(2-nitro-benzenesulfonyl)-amino]-butanoate in 10 ml of acetonitrile, under argon, are added 766 mg of caesium carbonate and 160 µl of thiophenol. The mixture is stirred for 17 hours at room temperature and then filtered through a sinter of porosity 4. The cake is washed with EtOAc and the filtrate is concentrated under reduced pressure and purified on a Mega BE-SCX, 2GM 12ML cartridge (Varian), using washing with MeOH and detachment of the expected product with a 2N solution of ammonia in MeOH. The fractions containing the desired product are combined and concentrated under reduced pressure. 183 mg of ethyl 4-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethylamino]-butanoate are obtained. LC/MS (B): tr= 0.68 min; $[M+H]^+$: m/z 313.

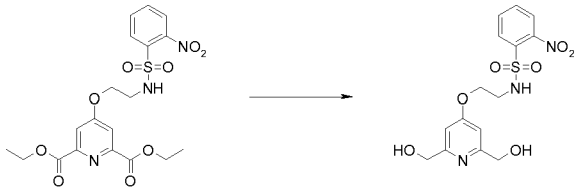
2.6. Ethyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(2-nitro-benzene-sulfonyl)-amino]-butanoate



To a solution of 767 mg of N-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-2-nitro-benzenesulfonamide in 15 ml of DMF, under argon, are added 344 µl of ethyl 3-bromo-butyrate and 1.38 g of K_2CO_3. The mixture is stirred for about 20 hours at 40°C and then filtered through a sinter of porosity 4. The cake is washed with EtOAc and the filtrate is concentrated under reduced pressure. The crude product is purified by flash chromatography on silica (Merck SuperVarioPrep 90 g column, Si60 15-40 µm), using a gradient of 0 to 10% MeOH in DCM. The fractions containing the desired product are

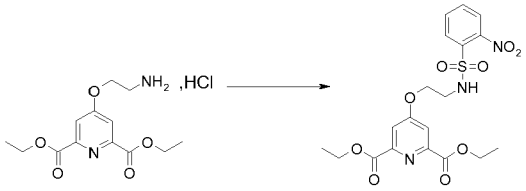
combined and concentrated under reduced pressure. 395 mg of ethyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(2-nitro-benzenesulfonyl)-amino]-butanoate are obtained. LC/MS (B): tr= 2.72 min; $[M+H]^+$: m/z 498; $[M+HCO_2H-H]^-$: m/z 542.

2.7. N-[2-(2,6-Bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-2-nitro-benzenesulfonamide



To a solution of 1.3 g of diethyl 4-[2-(2-nitro-benzenesulfonylamino)-ethoxy]-pyridine-2,6-dicarboxylate in 200 mL of ethanol are successively added 315 mg of sodium borohydride and 941 mg of $CaCl_2$. The mixture is stirred for 1 hour 30 minutes at room temperature and 50 ml of water are then added thereto. The ethanol is removed under reduced pressure and 50 ml of water are added to the residue obtained; the aqueous phase is extracted 3x with EtOAc. The organic phases are combined, washed with saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. 1.06 g of N-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-2-nitro-benzenesulfonamide are obtained. LC/MS (A): tr= 0.57 min; $[M+H]^+$: m/z 384.

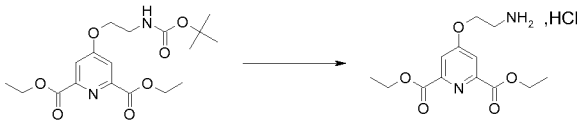
2.8. Diethyl 4-[2-(2-nitro-benzenesulfonylamino)-ethoxy]-pyridine-2,6-dicarboxylate



To a solution of 957 mg of diethyl 4-(2-amino-ethoxy)-pyridine-2,6-dicarboxylate monohydrochloride in 30 ml of DCM and 734 µl of pyridine, precooled to about 5°C, are added 798 mg of 2-nitrobenzenesulfonyl chloride. The mixture is warmed to room temperature and stirred for 2 hours. A further 244 µl of pyridine and 665 mg of 2-nitrobenzenesulfonyl chloride are then added thereto and stirring is continued for 15 hours. The mixture is washed with 25 ml of water and the aqueous phase is extracted 2x with 25 ml of DCM. The organic phases are combined, dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography on silica (Merck EasyVarioPrep 150 g column, Si60 15-40 µm), using a gradient of 0 to 10% EtOAc in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 960 mg of diethyl 4-[2-(2-nitro-benzenesulfonylamino)-ethoxy]-pyridine-2,6-dicarboxylate are obtained. ^1H NMR (400 MHz, DMSO-d_6): 1.35 (t, *J*=7.2 Hz, 6H); 3.41 (t, *J*=5.2 Hz, 2 H); 4.22

(t, *J*=5.2 Hz, 2 H); 4.39 (q, *J*=7.2 Hz, 4 H); 7.50 (s, 2 H); 7.80 (m, 2 H); 7.92 (m, 1 H); 8.04 (m, 2 H); 8.40 (m, 1 H). LC/MS (C): tr= 3.50 min; $[M+H]^+$: m/z 468.

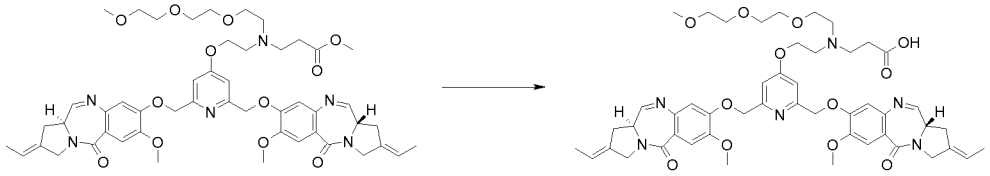
2.9. Diethyl 4-(2-amino-ethoxy)-pyridine-2,6-dicarboxylate monohydrochloride



To a solution of 2.64 g of diethyl 4-(2-tert-butoxycarbonylamino-ethoxy)-pyridine-2,6-dicarboxylate (described on page 101 of **WO 07085930**) in 27 ml of dioxane are added 20.7 ml of 4N hydrochloric acid in dioxane. The mixture is stirred for about 20 hours at room temperature and then concentrated under reduced pressure. The evaporation residue is taken up in about 70 ml of dioxane, and then concentrated again under reduced pressure. The operation is repeated 3x. The mixture is taken up in 50 ml of *tert*-butyl methyl ether and the suspension obtained is filtered through a sinter of porosity 4. The cake is washed with *tert*-butyl methyl ether, and dried in a desiccator under reduced pressure at room temperature. 2 g of diethyl 4-(2-amino-ethoxy)-pyridine-2,6-dicarboxylate monohydrochloride are obtained. ^{1}H NMR (400 MHz, DMSO-d_6): 1.35 (t, *J*=7.2 Hz, 6H); 3.26 (m, 2H); 4.39 (q, *J*=7.2 Hz, 4 H); 4.45 (m, 2 H); 7.77 (s, 2 H); 8.16 (broad m, 3 H). LC/MS (C): tr= 2.39 min; $[M+H]^+$: m/z 283.

Example 3

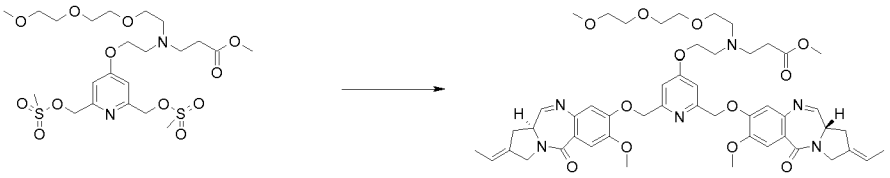
3.1. 3-([2-(2,6-Bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-propanoic acid



To a solution of 30 mg of methyl 3-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-propanoate in 576 µl of THF and 67 µl of water are added 35 µl of an aqueous 1 M lithium hydroxide solution. The mixture is stirred for 1 hour 30 minutes at room temperature and then taken up in 4 ml of DCM and acidified to a pH close to 3 by adding 1 ml of potassium phosphate buffer (pH=3). The mixture is extracted 4x with DCM and the organic phases are combined, dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF10-4g), using a gradient of 10 to 20% MeOH in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 13 mg of 3-([2-(2,6-bis-

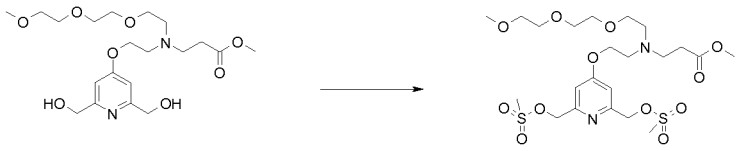
[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-propanoic acid are obtained. LC/MS (B): tr= 3.07 min; $[M+H]^+$: m/z 925

3.2. Methyl 3-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c] [1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-propanoate



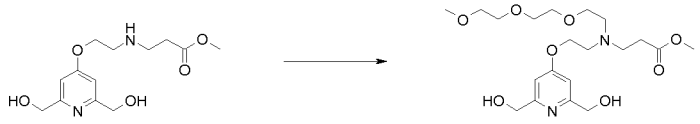
To a solution of 84 mg of (S)-2-eth-(E)-ylidene-8-hydroxy-7-methoxy-1,2,3,11a-tetrahydro-pyrrolo[2.1-c][1.4]benzodiazepin-5-one in 5 ml of DMF are added 128 mg of K_2CO_3, 51 mg of KI and 110 mg of methyl 3-([2-(2,6-bis-methanesulfonyloxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-propanoate. The mixture is heated for 18 hours at 30°C and then cooled to room temperature, filtered through a 0.45 µm membrane, concentrated under reduced pressure and purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF15-24g), using a gradient of 0 to 10% MeOH in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 33 mg of methyl 3-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-propanoate are obtained. LC/MS (B): tr= 3.21 min; $[M+H]^+$: m/z 939

3.3. Methyl 3-([2-(2,6-bis-methanesulfonyloxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-propanoate



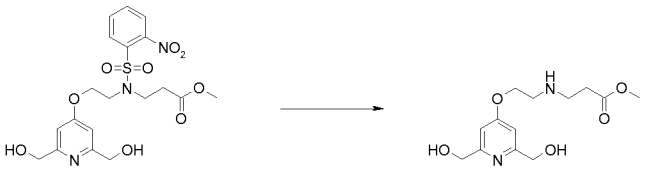
To a solution of 80 mg of methyl 3-([2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-propanoate in 8 ml of DCM, precooled to -25°C, are added 183 µl of diisopropylethylamine and 57 µl of methanesulfonyl chloride. The mixture is stirred for 1 hour at -15°C and then washed with 5 ml of water. The aqueous phase is extracted with 5 ml of DCM. The organic phases are combined, dried over $MgSO_4$ and concentrated under reduced pressure. 110 mg of methyl 3-([2-(2,6-bis-methanesulfonyloxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-propanoate are obtained. LC/MS (B): tr= 2.67 min; $[M+H]^+$: m/z 587

3.4. Methyl 3-([2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-propanoate



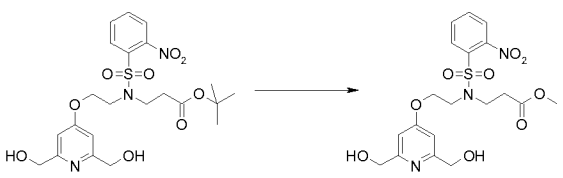
To a solution of 284 mg of methyl 3-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethylamino]-propanoate in 28 ml of acetonitrile are added 356 mg of 1-iodo-2-[2-(2-methoxy-ethoxy)-ethoxy]-ethane and 330 µl of diisopropylethylamine. The mixture is heated at 80°C for 3 days and then concentrated under reduced pressure and purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF25-40g), using a gradient of 5 to 10% MeOH in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 165 mg of methyl 3-([2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-amino)-propanoate are obtained. LC/MS (B): tr= 0.44 min; $[M+H]^+$: m/z 431.

3.5. Methyl 3-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethylamino]-propanoate



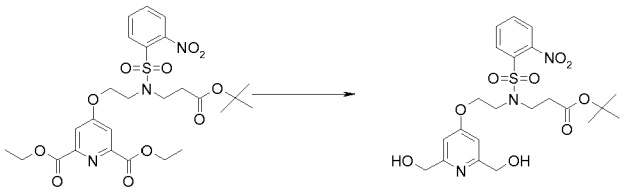
To a solution of 155 mg of methyl 3-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(2-nitro-benzenesulfonyl)-amino]-propanoate in 6 ml of acetonitrile, under argon, are added 325 mg of caesium carbonate and 67 µl of thiophenol. The mixture is stirred for 4 hours at room temperature and then filtered through a sinter of porosity 4. The cake is washed with EtOAc and the filtrate is concentrated under reduced pressure and purified on a Mega BE-SCX, 2GM 12ML cartridge (Varian), using washing with MeOH and detachment of the expected product with a 2N solution of ammonia in MeOH. The fractions containing the desired product are combined and concentrated under reduced pressure. 82 mg of methyl 3-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethylamino]-propanoate are obtained. LC/MS (B): tr= 0.33 min; $[M+H]^+$: m/z 285.

3.6. Methyl 3-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(2-nitro-benzenesulfonyl)-amino]-propanoate



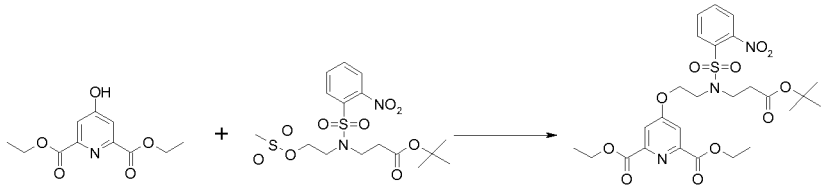
To a solution of 670 mg of *tert*-butyl 3-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethylamino]-propanoate in 20 ml of DCM are added 2 ml of TFA. The mixture is stirred for 6 hours at room temperature and then concentrated under reduced pressure, taken up in DCM and concentrated again under reduced pressure. To the residue obtained, dissolved in 10 ml of MeOH, are added, at 5°C, 7 ml of a 2M solution of (trimethylsilyl)diazomethane in hexane. The mixture is stirred for 1 hour 30 minutes at 5°C and then 200 µl of acetic acid are added. The mixture is taken up in 30 ml of water and 30 ml of EtOAc. The aqueous phase is extracted 2x with 30 ml of EtOAc. The organic phases are combined, washed with saturated NaCl solution, concentrated under reduced pressure and purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF15-24g), using a gradient of 0 to 10% MeOH in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 155 mg of methyl 3-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethylamino]-propanoate are obtained. LC/MS (C): tr= 2.56 min; $[M+H]^+$: m/z 470.

3.7. *tert*-Butyl 3-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(2-nitro-benzenesulfonyl)-amino]-propanoate



To a solution of 0.8 g of diethyl 4-{2-[(2-tert-butoxycarbonyl-ethyl)-(2-nitro-benzenesulfonyl)-amino]-ethoxy}-pyridine-2,6-dicarboxylate in 80 mL of ethanol are successively added 152 mg of sodium borohydride and 447 mg of $CaCl_2$. The mixture is stirred at room temperature and 20 ml of water are then added at the end of the reaction. The ethanol is removed under reduced pressure, 100 ml of water are added to the residue obtained and the aqueous phase is extracted 3x with EtOAc. The organic phases are combined, washed with saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. 670 mg of *tert*-butyl 3-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(2-nitro-benzenesulfonyl)-amino]-propanoate are obtained. LC/MS (C): tr= 3 min; $[M+H]^+$: m/z 512.

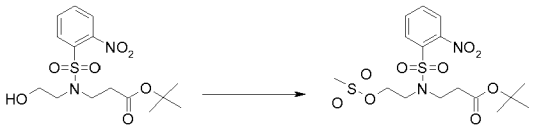
3.8. Diethyl 4-{2-[(2-tert-butoxycarbonyl-ethyl)-(2-nitro-benzenesulfonyl)-amino]-ethoxy}-pyridine-2,6-dicarboxylate



To a solution of 10.6 g of *tert*-butyl 3-[(2-methanesulfonyloxy-ethyl)-(2-nitro-benzenesulfonyl)-amino]-propionate in 220 ml of DMF are added 16.2 g of K_2CO_3 and 5.6 g

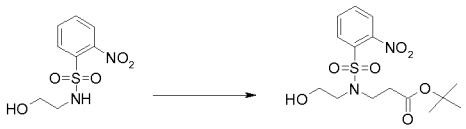
of diethyl 4-hydroxy-pyridine-2,6-dicarboxylate. The mixture is heated for 20 hours at 60°C and then concentrated under reduced pressure and taken up in 200 ml of water and 200 ml of EtOAc. The aqueous phase is extracted 2x with EtOAc. The organic phases are combined, washed with saturated NaCl solution, dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography on silica (Merck EasyVarioPrep 600 g column, Si60 15-40 µm), using a gradient of 0 to 20% EtOAc in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 6.56 g of diethyl 4-{2-[(2-tert-butoxycarbonyl-ethyl)-(2-nitro-benzenesulfonyl)-amino]-ethoxy}-pyridine-2,6-dicarboxylate are obtained. LC/MS (C): tr= 4.14 min; $[M+H]^+$: m/z 596.

3.9. *tert*-Butyl 3-[(2-methanesulfonyloxy-ethyl)-(2-nitro-benzenesulfonyl)-amino]-propionate



To a solution of 9.2 g of *tert*-butyl 3-[(2-hydroxy-ethyl)-(2-nitro-benzenesulfonyl)-amino]-propionate in 92 ml of DCM are added 8.1 ml of diisopropylethylamine. The mixture is cooled to -5°C and a solution of 2.34 ml of MSC in 10 ml of DCM is added dropwise. After warming to room temperature, the mixture is stirred for about 2 hours and is then supplemented with 100 ml of water. The aqueous phase is extracted twice with DCM and the organic phases are combined, dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF40-240g), using a gradient of 0 to 5% of ethyl acetate in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 10.62 g of *tert*-butyl 3-[(2-methanesulfonyloxy-ethyl)-(2-nitro-benzenesulfonyl)-amino]-propionate are obtained. LC/MS (C): tr= 3.77 min; $[M+Na]^+$: m/z 475.

3.10. *tert*-Butyl 3-[(2-Hydroxy-ethyl)-(2-nitro-benzenesulfonyl)-amino]-propionate



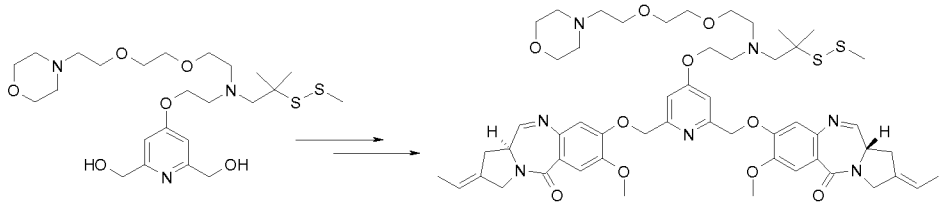
To a solution of 8.7 g of N-(2-hydroxy-ethyl)-2-nitro-benzenesulfonamide (Skerlj, R.T.; Nan, S.; Zhou, Y.; Bridger, G.J. *Tetrahedron Lett.* **2002** (*43*) 7569-7571) in 87 ml of DMF, under argon, are added 8.8 ml of *tert*-butyl 3-bromopropionate and 14.6 g of K_2CO_3. The mixture is stirred for 15 hours at 40°C and a further 5 ml of *tert*-butyl 3-bromopropionate are then added. The mixture is heated at 40°C overnight and then filtered through a sinter of porosity 4. The cake is washed with ethyl acetate and the filtrate is then concentrated under reduced pressure and purified by flash chromatography on silica (Merck EasyVarioPrep 400 g

column, Si60 15-40 µm), using a gradient of 0 to 10% EtOAc in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 9.54 g of *tert*-butyl 3-[(2-hydroxy-ethyl)-(2-nitro-benzenesulfonyl)-amino]-propionate are obtained. LC/MS (C): tr= 3.39 min; $[M+Na]^+$: m/z 397.

Example 4

4.1. 4-[2-((2-methyl-2-methyldisulfanyl-propyl)-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethyl}-amino)-ethoxy]-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro pyrrolo[2,1c][1.4] benzodiazepin-5-one-8-yloxymethyl]-pyridine

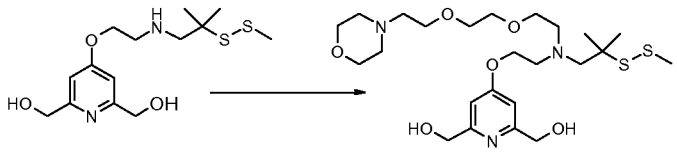
Prepared as for Ex. 1, starting with 4-[2-((2-methyl-2-methyldisulfanyl-propyl)-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethyl}-amino)-ethoxy]-2,6-bis-(hydroxy methyl)-pyridine:



^{1}H NMR (500 MHz, DMSO-d_6): 1.22 (s, 6 H); 1.69 (d, *J*=7.0 Hz, 6 H); 2.28 to 2.44 (m, 6 H); 2.36 (m, 3 H); 2.71 (s, 2 H); 2.77 (t, *J*=6.7 Hz, 2 H); 2.88 to 3.08 (m, 6 H); 3.42 to 3.55 (m, 12 H); 3.86 (s, 6 H); 3.88 (m, 2 H); 4.10 (s, 4 H); 4.15 (t, *J*=5.7 Hz, 2 H); 5.17 (d, *J*=13.2 Hz, 2 H); 5.23 (d, *J*=13.2 Hz, 2 H); 5.55 (m, 2 H); 6.93 (s, 2 H); 7.06 (s, 2 H); 7.38 (s, 2 H); 7.76 (d, *J*=4.4 Hz, 2 H). LC/MS (A): tr= 0.68 min; $[M+H]^{+:}$ m/z 1042; $[M+2H]^{2+}$: m/z 521.5 (base peak)

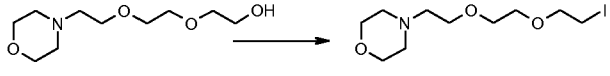
4.2. 4-[2-((2-methyl-2-methyldisulfanyl-propyl)-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethyl}-amino)-ethoxy]-2,6-bis-(hydroxy methyl)-pyridine

Prepared as for Ex. 1, starting with 4-{2-[2-(2-iodo-ethoxy)-ethoxy]-ethyl}-morpholine and 4-[2-(2-methyl-2-methyldisulfanyl-propylamino)-ethoxy]-2,6-bis-(hydroxymethyl)-pyridine:



^{1}H NMR (400 MHz, DMSO-d_6): 1.26 (s, 6 H); 2.36 (m, 4 H); 2.39 (s, 3 H); 2.43 (t, *J*=5.9 Hz, 2 H); 2.74 (s, 2 H); 2.79 (t, *J*=5.9 Hz, 2 H); 3.00 (t, *J*=5.9 Hz, 2 H); 3.46 to 3.55 (m, 12 H); 4.12 (t, *J*=5.9 Hz, 2 H); 4.45 (d, *J*=5.9 Hz, 4 H); 5.30 (t, *J*=5.9 Hz, 2 H); 6.84 (s, 2 H). LC/MS (E): tr= 0.33 min; $[M+H]^+$: m/z 534

4.3. 4-{2-[2-(2-Iodo-ethoxy)-ethoxy]-ethyl}-morpholine

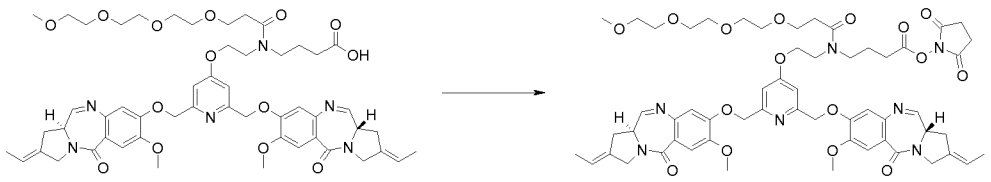


To a solution of 1.12 g of 2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethanol and 940 µl of diisopropylethylamine in 6 ml of DCM, cooled to 0°C, is added a solution of 440 µl of methanesulfonyl chloride in 3mL of DCM at the same temperature. The mixture is stirred for 1 hour at 0°C and then washed with 8 ml of water. The organic phase is dried over $MgSO_4$ and concentrated under reduced pressure.

The residue obtained is dissolved in 30 ml of acetone and then supplemented with 1.42 g of sodium iodide. After stirring at reflux for 5 hours, the insoluble matter is removed by filtration on a sinter funnel. The filtrate is concentrated under reduced pressure and then taken up in dichloromethane. The insoluble matter is again removed by filtration, and the filtrate is concentrated under reduced pressure and purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF25-40g), using a gradient of 0 to 5% MeOH in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 1.09 g of 4-{2-[2-(2-iodo-ethoxy)-ethoxy]-ethyl}-morpholine are obtained . ^{1}H NMR (400 MHz, DMSO-d_6): 2.39 (m, 4 H); 2.45 (t, *J*=5.9 Hz, 2 H); 3.32 (t, *J*=6.4 Hz, 2 H); 3.47 to 3.60 (m, 10 H); 3.67 (t, *J*=6.4 Hz, 2 H). LC/MS (A): tr= 0.29 min; $[M+H]^+$: m/z 330

Example 5

5.1. N-Hydroxysuccinimidyl 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate

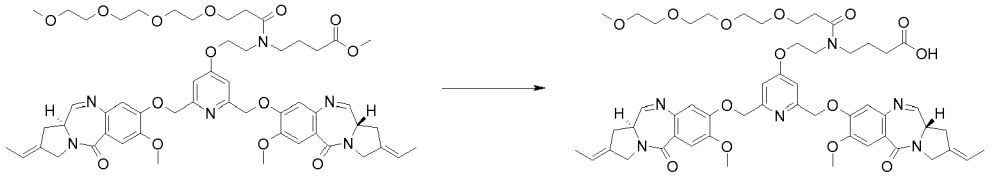


To 65 mg of 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoic acid dissolved in 3.8 mL of THF are added 67 µl of DIPEA and 33 mg of N,N'-disuccinimidyl carbonate. After 1 hour at room temperature, 8 mL of DCM are added and the resulting organic phase is washed twice with water, dried over $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF10-8g), using a gradient of 0 to 7.5% methanol in DCM. 35 mg of N-hydroxysuccinimidyl 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate are thus obtained.

LC/MS (F): tr= 3.54 min; $[M+H]^+$: m/z 1108; $[M+H_2O+H]^+$: m/z 1126; $[M+2H_2O+H]^+$: m/z 1144

5.2. 4-([2-(2,6-Bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro-pyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoic acid

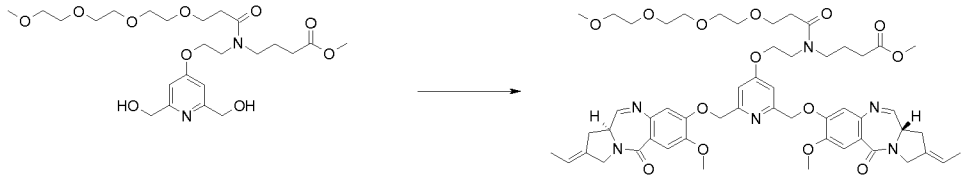
Prepared as for Ex. 2, starting with methyl 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate:



LC/MS (E): tr= 1.05 min; $[M+H]^+$: m/z 1011; $[M+H_2O+H]^+$: m/z 1029; $[M+2H_2O+H]^+$: m/z 1047

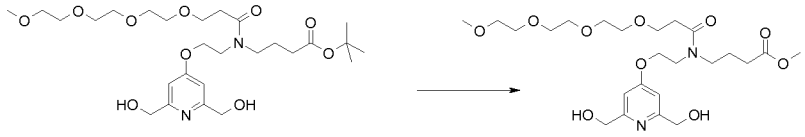
5.3. Methyl 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate

Prepared as for Ex. 2, starting with ethyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate:



LC/MS (E): tr= 1.14 min; $[M+H]^+$: m/z 1025; $[M+H_2O+H]^+$: m/z 1043; $[M+2H_2O+H]^+$: m/z 1061

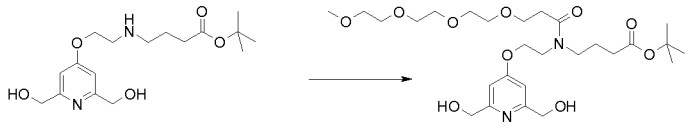
5.4. Methyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate



To a solution of 460 mg of *tert*-butyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate in 7 ml of DCM are added 1.14 ml of TFA. The mixture is stirred for 15 hours at room temperature and then concentrated under reduced pressure. To the residue obtained, dissolved in 3.8 ml of MeOH, are added, at 5°C, 3.1 ml of a 2M solution of (trimethylsilyl)diazomethane in hexane. The mixture is stirred for 1 hour at 5°C and 100 µl of acetic acid are then added. After concentration under reduced pressure, the residue obtained is purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF25-40g), using a gradient of 2 to 10% MeOH in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 265 mg of methyl 4-[[2-(2,6-bis-hydroxymethyl-

pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate are obtained. ^{1}H NMR (400 MHz, DMSO-d_6): 50%-50% mixture of conformers with: 1.67 to 1.86 (m, 2 H); 2.27 (t, J=7.3 Hz, 1 H); 2.36 (t, *J*=7.3 Hz, 1 H); 2.57 (t, *J*=6.7 Hz, 1 H); 2.66 (t, *J*=6.7 Hz, 1 H); 3.23 (s, 3 H); 3.31 to 3.75 (m, 21 H); 4.14 (t, *J*=5.6 Hz, 1 H); 4.19 (t, *J*=5.6 Hz, 1 H); 4.45 (m, 4 H); 5.30 (m, 2 H); 6.84 (s, 1 H); 6.86 (s, 1 H). LC/MS (G): tr= 0.62 min; $[M+H]^+$: m/z 517; $[M-H+HCO_2H]^-$: m/z 561

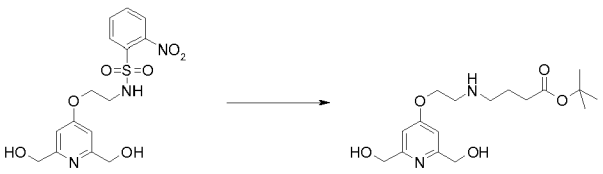
5.5. *tert*-Butyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate



To a solution of 560 mg of *tert*-butyl 3-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethylamino]-butanoate in 4 ml of DMF is added a solution of 658 mg of N-hydroxysuccinimidyl 3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propanoate (cf. M.A. Miller, N.B. Malkar, D. Severynse-Stevens, K.G. Yarbrough, M.J. Bednarcik, R.E. Dugdell, M.E. Puskas, R. Krishnan, K.D. James *Bioconjugate Chem.,* **2006**, *17*, 267-274) in 4 mL of DMF. The mixture is stirred at room temperature for 15 hours and then concentrated under reduced pressure and purified by flash chromatography on silica (Analogix Super Flash SiO_2 SF40-80g), using a gradient of 0 to 10% of a 10% solution of ammonia in MeOH, in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 460 mg of *tert*-butyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate are obtained. ^{1}H NMR (400 MHz, DMSO-d_6): 50%-50% mixture of conformers with: 1.39 (s, 4.5 H); 1.40 (s, 4.5 H); 1.63 to 1.81 (m, 2 H); 2.16 (t, *J*=7.3 Hz, 1 H); 2.25 (t, *J*=7.3 Hz, 1 H); 2.57 (t, *J*=6.7 Hz, 1 H); 2.66 (t, *J*=6.7 Hz, 1 H); 3.23 (s, 3 H); 3.31 to 3.74 (m, 18 H); 4.14 (t, *J*=5.7 Hz, 1 H); 4.19 (t, *J*=5.7 Hz, 1 H); 4.45 (m, 4 H); 5.30 (m, 2 H); 6.84 (s, 1 H); 6.86 (s, 1 H). LC/MS (G): tr= 0.80 min; $[M+H]^+$: m/z 559; $[M-H+HCO_2H]^-$: m/z 603.

5.6. *tert*-Butyl 3-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethylamino]-butanoate

Prepared as for Ex. 2, starting with *tert*-butyl 3-bromo-butyrate and N-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-2-nitro-benzenesulfonamide:



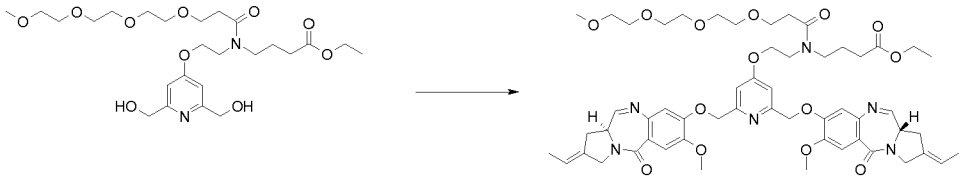
^{1}H NMR (400 MHz, DMSO-d_6): 1.39 (s, 9 H); 1.62 (t, *J*=7.2 Hz, 2 H); 1.84 (broad m, 1 H); 2.22 (t, *J*=7.2 Hz, 2 H); 2.55 (t, *J*=7.2 Hz, 2 H); 2.86 (t, *J*=5.7 Hz, 2 H); 4.07 (t, *J*=5.7 Hz, 2

H); 4.45 (d, *J*=5.7 Hz, 4 H); 5.29 (t, *J*=5.7 Hz, 2 H); 6.85 (s, 2 H). LC/MS (A): tr= 0.40 min; $[M+H]^+$: m/z 341; base peak: m/z 156.

Example 6

6.1. Ethyl 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate

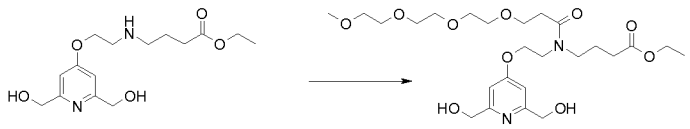
Prepared as for Ex. 5, starting with ethyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate:



^{1}H NMR (500 MHz, DMSO-d_6): 50%-50% mixture of conformers with: 1.11 to 1.18 (m, 3 H); 1.54 to 1.82 (m, 8 H); 2.24 (d, J=7.3 Hz, 1 H); 2.32 (d, J=7.3 Hz, 1 H); 2.53 to 2.68 (m, 4 H); 2.87 to 3.07 (m, 4 H); 3.20 (s, 1.5 H); 3.21 (s, 1.5 H); 3.31 to 4.27 (m, 32 H); 5.16 (d, *J*=13.2 Hz, 2 H); 5.22 (d, *J*=13.2 Hz, 2 H); 5.55 (q, *J*=7.0 Hz, 2 H); 6.94 (s, 2 H); 7.08 (s, 2 H); 7.37 (s, 2 H); 7.76 (d, *J*=4.4 Hz, 2 H). LC/MS (A): tr= 0.86 min; $[M+H]^+$: m/z 1039; base peak: m/z 376.

6.2. Ethyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate

Prepared as for Ex. 5, starting with ethyl 3-[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethylamino]-butanoate:

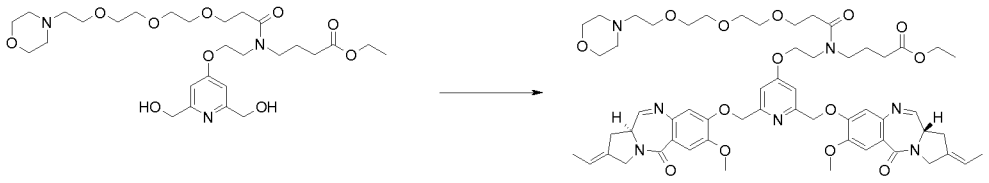


^{1}H NMR (400 MHz, DMSO-d_6) 50%-50% mixture of conformers with: 1.16 (t, J=7.1 Hz, 1.5 H); 1.17 (t, J=7.1 Hz, 1.5 H); 1.67 to 1.97 (m, 2 H); 2.25 (t, J=7.3 Hz, 1 H); 2.34 (t, J=7.3 Hz, 1 H); 2.57 (t, J=6.8 Hz, 1 H); 2.66 (t, J=6.8 Hz, 1 H); 3.23 (s, 3 H); 3.32 to 3.74 (m, 18 H); 4.04 (q, J=7.1 Hz, 1 H); 4.05 (q, J=7.1 Hz, 1 H); 4.14 (t, J=5.7 Hz, 1 H); 4.19 (t, J=5.7 Hz, 1 H); 4.45 (m, 4 H); 5.30 (m, 2 H); 6.84 (s, 1 H); 6.86 (s, 1 H). LC/MS (A): tr= 0.47 min; $[M+H]^+$: m/z 531; base peak: m/z 376; $[M-H+HCO_2H]^-$: m/z 575

Example 7

7.1. Methyl 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2- morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate

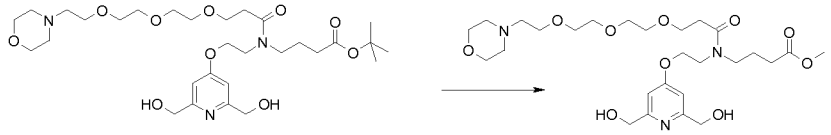
Prepared as for Ex. 5, starting with methyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2- morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate:



LC/MS (E): tr= 0.88 min; $[M+H]^+$: m/z 1080; $[M+H_2O+H]^+$: m/z 1098; $[M+2H_2O+H]^+$: m/z 1116

7.2. Methyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate

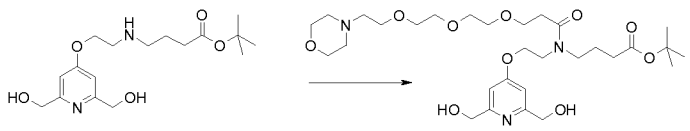
Prepared as for Ex. 5, starting with *tert*-butyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2- morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate:



^{1}H NMR (400 MHz, DMSO-d_6): 1.83 (m, 2 H); 2.33 (m, 2 H); 2.42 (m, 4 H); 2.48 (t, J=5.8 Hz, 2 H); 2.61 (m, 2 H); 3.40 (m, 2 H); 3.49 to 3.55 (m, 10 H); 3.56 (m, 4 H); 3.62 (s, 3 H); 3.69 (broad t, J=6.6 Hz, 4 H); 4.21 (t, J=5.6 Hz, 2 H); 4.49 (d, J=5.3 Hz, 4 H); 4.90 (t, J=5.3 Hz, 2 H); 6.87 (s, 2 H). LC/MS (G): tr= 0.50 min; $[M+H]^+$: m/z 572; base peak: m/z 198; $[M-H+HCO_2H]^-$: m/z 616

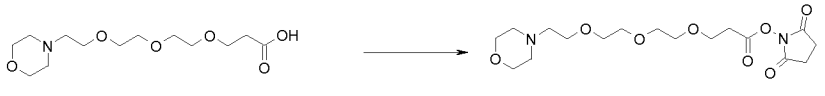
7.3. *tert*-Butyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate

Prepared as for Ex. 5, starting with N-hydroxysuccinimidyl 3-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propanoate:



^{1}H NMR (400 MHz, DMSO-d_6): 1.42 (s, 9 H); 1.78 (m, 2 H); 2.22 (m, 2 H); 2.42 (m, 4 H); 2.48 (t, J=5.8 Hz, 2 H); 2.61 (m, 2 H); 3.39 (m, 2 H); 3.48 to 3.55 (m, 10 H); 3.56 (m, 4 H); 3.69 (broad t, J=6.6 Hz, 4 H); 4.21 (t, J=5.6 Hz, 2 H); 4.49 (d, J=5.3 Hz, 4 H); 4.90 (t, J=5.3 Hz, 2 H); 6.87 (s, 2 H). LC/MS (G): tr= 0.65 min; [M+H]+: m/z 614; base peak: m/z 240

7.4. N-Hydroxysuccinimidyl 3-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propanoate



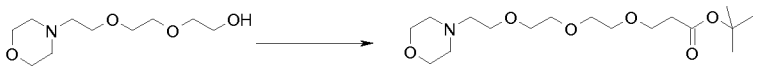
To 830mg of 3-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propanoic acid dissolved in 50 mL of THF are added 1.1 g of N,N'-disuccinimidyl carbonate and 1.5 mL of DIPEA. After 24 hours at room temperature, 200 mL of DCM are added and the resulting organic phase is concentrated to half its volume under reduced pressure and then washed twice with water, dried over $MgSO_4$ and concentrated under reduced pressure. 1.05 g of N-hydroxysuccinimidyl 3-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propanoate are thus obtained. ^1H NMR (400 MHz, DMSO-d_6): 2.39 (m, 4 H); 2.45 (t, J=5.9 Hz, 2 H); 2.81 (s, 4 H); 2.92 (t, J=6.0 Hz, 2 H); 3.45 to 3.57 (m, 14 H); 3.72 (t, J=6.0 Hz, 2 H). LC/MS (G): tr (ELSD) = 0.49 min; [M+H]+: m/z 389.

7.5. 3-{2-[2-(2-Morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propanoic acid



To a solution of 1g of *tert*-butyl 3-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propanoate in 50 ml of DCM are added 4 ml of TFA. The mixture is stirred for 10 hours at room temperature and then concentrated under reduced pressure and purified on a Mega BE-SCX, 25GM 150ML cartridge (Varian), using washing with MeOH and detachment of the expected product with a 2N solution of ammonia in MeOH. The fractions containing the desired product are combined and concentrated under reduced pressure. 830 mg of 3-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propanoic acid are obtained. LC/MS (E): tr (ELSD) = 0.16 min; $[M+H]^+$: m/z 292

7.6. *tert*-Butyl 3-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propanoate



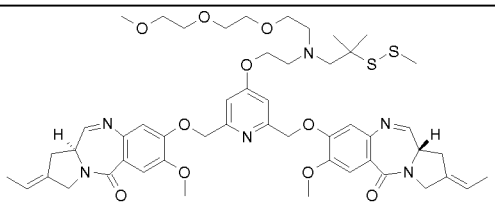
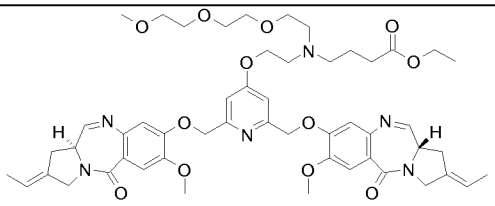
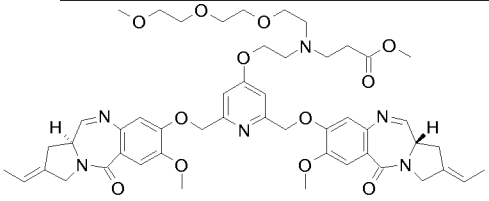
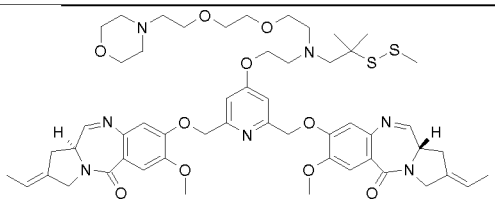
To a solution of 989 mg of 2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethanol in 2.8 mL of anhydrous THF are added 1.04 mg of sodium. After heating at 40°C for 2 hours 15 minutes, 793 µL of *tert*-butyl acrylate are added. After heating for a further two hours at 40°C, the reaction mixture is left for 20 hours at room temperature and then concentrated under reduced pressure and purified by flash chromatography on silica (Merck SuperVarioPrep 70 g column, Si60 15-40 µm), using a gradient of 0 to 6% MeOH in DCM. The fractions containing the desired product are combined and concentrated under reduced pressure. 1 g of *tert*-butyl 3-{2-[2-(2-morpholin-4-yl-ethoxy)-ethoxy]-ethoxy}-propanoate are obtained. LC/MS (E): tr (ELSD) = 0.59 min; $[M+H]^+$: m/z 348

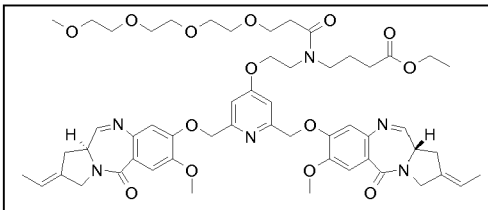
Evaluation of the inhibition of proliferation of the cell lines MDA-MB-231, MDA-A1 and HCT116 with the compounds of formula (IA) with RCG1=-SZ_a (Z_a=SMe) or RCG1 = -$C(=O)Z_bR_b$ (Z_bR_b=OMe)

MDA-MB-231, MDA-A1 or HCT116 cells in their exponential growth phase are trypsinized

and resuspended in their respective culture medium (DMEM/F12 Gibco #21331, 10% FCS Gibco #10500-056, 2 nM Glutamine Gibco #25030 for the MDA cells; DMEM Gibco #11960, 10% FCS Gibco #10500-056, 2 mM Glutamine Gibco #25030 for the HCT116 cells). The cell suspension is seeded in Cytostar 96-well culture plates (GE Healthcare Europe, #RPNQ0163) in the complete culture medium containing serum to a density of 5000 cells/well (MDA-MB-231, MDA-A1, HCT116). After incubation for 4 hours, successive dilutions of the tomaymycin dimers are added to the wells in triplicate for each concentration. The cells are cultured for 3 days at 37°C under an atmosphere of 5% CO_2 in the presence of the cytotoxic agents. On the fourth day, 10 µl of a solution of ^{14}C-thymidine (0.1 µCi/well, Perkin Elmer #NEC56825000) are added to each well. The incorporation of ^{14}C-thymidine is measured 96 hours after the start of the experiment with a Microbeta radioactivity counter (Perkin Elmer). The data are expressed in the form of a percentage of survival by determining the ratio between the Count obtained with the cells treated with the cytotoxic agents and that obtained with the cells of the control wells (treated with the culture medium alone).

Table II

Inhibition of proliferation (^{14}C-thymidine pulse at 96 h)	IC50 [pM]		
Structure of the compound of formula (IA)	HCT116	MDA-MB231	MDA-A1
	61	128	16270
	18	41	3465
	235	384	17849
	48	81	26474

	4984	5665	>50000

It is found that the test compounds, for which RCG1=-SZ_a, with Z_a=SMe) or RCG1 = -C(=O)Z_bR_b, with Z_bR_b=OMe, have powerful anticancer activity; this suggests that similar compounds characterized with another group Z_bR_b are liable to have at least identical activity.

Chapter 2: Novel tomaymycin conjugates

Example 8: preparation of a conjugate hu2H11 modified with SPDB with 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-(2-methyl-2-mercapto-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4] benzodiazepin-5-one-8-yloxymethyl]-pyridine

To 24 mg of hu2H11 (antibody described also by hu53 2H11 on page 15 of **WO 2008010101**; this is an antibody comprising a Vh having the amino acid sequence SED ID No. 24) in 2.37 ml of an aqueous buffer with a potassium phosphate concentration of 0.05 M, 0.05 M NaCl and 2 mM ethylenediaminetetraacetic acid (EDTA) of pH = 6.5, are added 320 µg of SPDB (described on page 7 of **WO 2010/076474**) dissolved in 62 µL of DMA with magnetic stirring. After 4 hours at room temperature, the modified antibody is purified by gel filtration on Sephadex G25 (PD-10 GE column) pre-equilibrated in an aqueous buffer with a concentration of 0.05 M N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid (HEPES), 0.05 M NaCl and 2 mM ethylenediaminetetraacetic acid (EDTA) of pH = 8. An aliquot of the modified antibody is treated with dithiothreitol (DTT) to cleave the dithiopyridine groups. The modified antibody along with the pyridinethiol released are assayed by spectrophotometry using the extinction coefficients of pyridinethiol ($e_{343\ nm}$= 8080 $M^{-1}cm^{-1}$), of the dithiopyridine group ($e_{280\ nm}$= 5100 M^{-1} cm^{-1}) and hu2H11 ($e_{280\ nm}$= 206941 $M^{-1}cm^{-1}$): an average of 3.2 dithiopyridine groups per antibody molecule was determined at a concentration of 7.08 mg/mL.

To 12 mg of the above modified antibody in 3.2 ml of an aqueous buffer with a concentration of 0.05 M N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid (HEPES), 0.05 M NaCl and 2 mM ethylenediaminetetraacetic acid (EDTA) of pH = 8 are added 713 µL of DMA and 1.22 mg of 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-(2-methyl-2-mercapto-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-

tetrahydropyrrolo[2,1c][1.4] benzodiazepin-5-one-8-yloxymethyl]-pyridine dissolved in 87 µL of dimethylacetamide (DMA) with magnetic stirring. After 12 hours at 30°C, the mixture is filtered on Millex^{R}-SV 0.45 µM (PVDF Durapore Millipore) and purified on a Superdex^{TM} 200 prep grade column (Hiload^{TM} 26/60 GE column) pre-equilibrated in a saline phosphate buffer containing 20% N-methylpyrrolidone (NMP). The fractions of interest are combined and concentrated on Amicon Ultra-15 (Ultracel 50k Millipore) and then filtered on Sephadex G-25 (PD10, GE columns) pre-equilibrated in an aqueous buffer at pH=6.5, with a concentration of 10 mM histidine containing 10% sucrose and 5% NMP.

The conjugate obtained (3.5 mL) is assayed by spectrophotometry using the extinction coefficients $e_{320\ nm}$= 7843M^{-1} cm^{-1} and $e_{280\ nm}$= 4436 M^{-1} cm^{-1} for 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-2-methyldisulfanyl-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridine and $e_{280\ nm}$ = 206941 $M^{-1}cm^{-1}$ for hu2H11: an average of 2.9 tomaymycin (4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-(2-methyl-2-mercapto-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridine) dimers per antibody molecule was determined at a concentration of 1.74 mg/mL.

Example 9: Preparation of a conjugate hu2H11R35R74 modified with SNPP with 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-(2-methyl-2-mercapto-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4] benzodiazepin-5-one-8-yloxymethyl]-pyridine

To 100 mg of hu2H11R35R74 (Ref on page 20 of **WO 2011039721**) in 8.2 ml of an aqueous buffer with a concentration of 0.05 M a potassium phosphate, 0.05 M NaCl and 2 mM ethylenediaminetetraacetic acid (EDTA) of pH = 6.5 are added 1.3 mg of SNPP (described on page 36 of **WO 2004/016801**) dissolved in 186 µL of DMA with magnetic stirring. After 4 hours at room temperature, the solution of modified antibody is fractionated into four and purified by gel filtration on four Sephadex G25 columns (PD-10 GE column) pre-equilibrated in an aqueous buffer with a concentration of 0.05 M N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid (HEPES), 0.05 M NaCl and 2 mM ethylenediaminetetraacetic acid (EDTA) of pH = 8. After mixing and homogenizing the four filtrates thus obtained, the modified antibody is assayed by spectrophotometry using the extinction coefficients of nitropyridinethiol ($e_{280\ nm}$= 3344 M^{-1} cm^{-1} and $e_{325\ nm}$= 10964 $M^{-1}cm^{-1}$), and hu2H11R35R74 ($e_{280\ nm}$= 219528 $M^{-1}cm^{-1}$): an average of 4.47 dithio-nitropyridine groups per antibody molecule was determined at a concentration of 6.27 mg/mL.

To 87 mg of modified antibody above in 23.24 ml of an aqueous buffer with a concentration of 0.05 M N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid (HEPES), 0.05 M NaCl and 2 mM ethylenediaminetetraacetic acid (EDTA) of pH = 8 are added 4.38 mL of DMA and 17.73 mg of 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-(2-methyl-2-mercapto-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydro pyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridine dissolved in 1.43 mL of dimethylacetamide (DMA) with magnetic stirring. After 17 hours at 30°C, the mixture is filtered on Steriflip^{R} 0.45 µM (PVDF Durapore Millipore) and purified by 3 injections on a Superdex^{TM} 200 prep grade column (Hiload^{TM} 26/60 GE column) pre-equilibrated in a saline phosphate buffer containing 20% N-methylpyrrolidone (NMP). The fractions of interest are combined and concentrated on Amicon Ultra-15 (Ultracel 50k Millipore) and then filtered on Sephadex G-25 (HiPrep 26/10 desalting, GE column) pre-equilibrated in an aqueous buffer of pH = 6.5 with a concentration of 10 mM histidine, containing 10% sucrose and 5% NMP.

The conjugate obtained (28 mL) is assayed by spectrophotometry using the extinction coefficient $e_{280\,nm}$ = 219528 $M^{-1}cm^{-1}$ for hu2H11R35R74: an average of 3.13 tomaymycin (4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-(2-methyl-2-mercapto-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridine) dimers per antibody molecule was determined at a concentration of 1.99 mg/mL.

Example 10: preparation of a conjugate hu2H11R35R74 modified with SPDB with 4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-(2-methyl-2-mercapto-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4] benzodiazepin-5-one-8-yloxymethyl]-pyridine

Prepared as for Ex. 8, starting with hu2H11R35R74: an average of 2.92 tomaymycin (4-{2-[{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethyl}-(2-methyl-(2-methyl-2-mercapto-propyl)-amino]-ethoxy}-2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo [2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridine) dimers per antibody molecule was determined.

Example 11: preparation of a conjugate hu2H11R35R74 with N-hydroxysuccinimidyl 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate

To a solution of 1.59 mg of hu2H11R35R74 in 150 µL an aqueous buffer with a concentration of 0.043 M of potassium phosphate of pH=6.6 are successively added 6 µL of

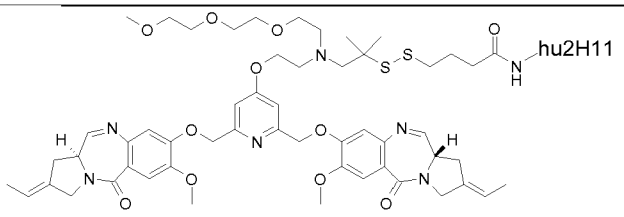
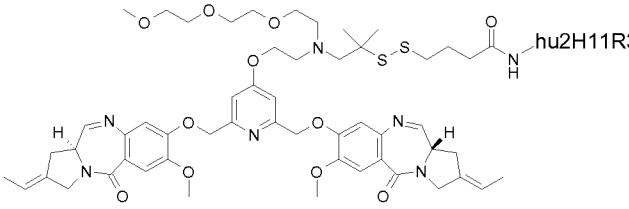
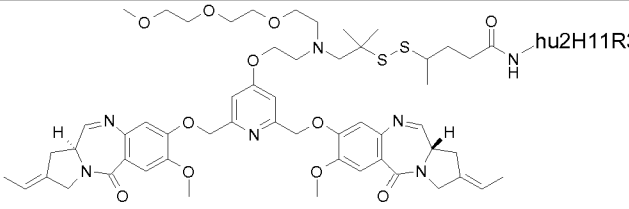
an aqueous 1 M solution of HEPES, followed by 267 µL of an aqueous buffer with a concentration of 0.05 M N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid (HEPES), 0.05 M NaCl and 2 mM ethylenediaminetetraacetic acid (EDTA) of pH = 8, 100 µL of DMA and then 83 µg of N-hydroxysuccinimidyl 4-([2-(2,6-bis-[(S)-2-eth-(E)-ylidene-7-dimethoxy-1,2,3,11a-tetrahydropyrrolo[2,1c][1.4]benzodiazepin-5-one-8-yloxymethyl]-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy-propionyl)-amino]-butanoate dissolved in 5 µl of DMA. After 4 hours at 30°C, the mixture is filtered on Sephadex G-25 (NAP-5 GE columns) pre-equilibrated in an aqueous buffer of pH = 6.5 with a concentration of 10 mM histidine, containing 10% sucrose and 5% NMP.

The conjugate obtained (1 mL) is assayed by spectrophotometry using the extinction coefficients of ethyl 4-[[2-(2,6-bis-hydroxymethyl-pyridin-4-yloxy)-ethyl]-(3-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-propionyl)-amino]-butanoate ($e_{322\ nm}$= 7971 M^{-1} cm^{-1} and $e_{280\ nm}$= 5219 M^{-1} cm^{-1}): an average of 4.18 tomaymycin dimers per antibody molecule was determined at a concentration of 1.14 mg/mL.

Evaluation of the inhibition of proliferation of the cell lines MDA-MB-231 with the cytotoxic conjugate hu2H11 (or hu2H11R35R74, respectively)

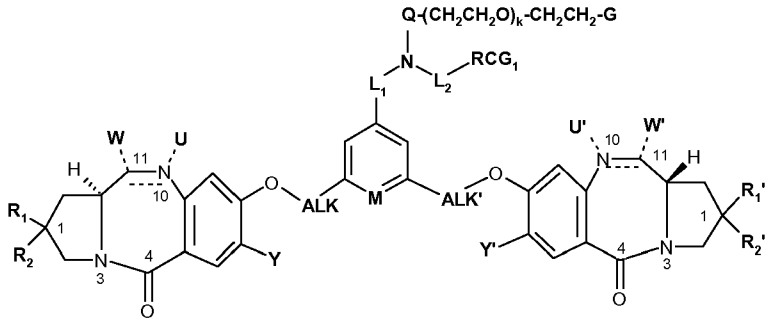
MDA-MB-231 cells in their exponential growth phase are trypsinized and resuspended in their culture medium (DMEM/F12 Gibco #21331, 10% FCS Gibco #10500-056, 2 nM Glutamine Gibco #25030). The cell suspension is seeded in Cytostar 96-well plates (GE Healthcare Europe, #RPNQ0163) in whole culture medium containing serum to a density of 5000 cells/well. After incubation for 4 hours, successive dilutions of the antibody-cytotoxic agent immunoconjugates are added to the wells at decreasing concentrations from 10^{-7} to 10^{-12} M (in triplicate for each concentration). The cells are cultured at 37°C under an atmosphere containing 5% CO_2 in the presence of the antibody-cytotoxic agent immunoconjugates for 3 days. On the fourth day, 10 µl of a solution ^{14}C-thymidine (0.1 µCi/well, Perkin Elmer #NEC56825000) are added to each well. The incorporation of ^{14}C-thymidine is measured 96 hours after the start of the experiment with a Microbeta radioactivity counter (Perkin Elmer). The data are expressed in the form of a percentage of survival by determining the ratio between accounts obtained with the cells treated with the immunoconjugate and that obtained with the cells of the control wells (treated with the culture medium alone). In certain experiments indicated with an asterisk (*), the naked antibody hu2H11 (or hu2H11R35R74, respectively) was added to the wells to a concentration of 1 µM at the start of the experiment and the inhibition of proliferation was measured as described previously.

Table III

Inhibition of proliferation (^{14}C-thymidine pulse at 96 h)	IC50 [pM]		
Structure	Mean IC50	Mean IC50 (+naked Ab*)	IC50 ratio
 hu2H11	58	760	13
 hu2H11R35R74	32	502	16
 hu2H11R35R74	72	3550	49

CLAIMS

1. Compound of formula (I):



(I)

in which:

- ---- represents a single bond or a double bond, with the condition that

  if ---- represents a single bond, then:

  - **U** and/or **U'**, which may be identical or different, represent, independently of each other, H;
  - **W** and/or **W'**, which may be identical or different, represent, independently of each other: OH, -OR, –OCOR, -COOR, -OCOOR, –OCONRR', a cyclic carbamate such that N10 and C11 are included in a ring, –NRCONRR', -OCSNHR, a cyclic thiocarbamate such that N10 and C11 are included in a ring, -SH, -SR, -SOR, -SOOR, $–SO_3^-$, -NRSOOR', –NRR', a cyclic amine such that N10 and C11 are included in a ring, -NROR', –NRCOR', $–N_3$, -CN, Hal, a trialkylphosphonium or triarylphosphonium group;

  if ---- represents a double bond, then:

  - **U** and **U'** are absent;
  - **W** and/or **W'**, which may be identical or different, represent, independently of each other, H;

- $\mathbf{R_1}$, $\mathbf{R_2}$, $\mathbf{R_1'}$, $\mathbf{R_2'}$, which may be identical or different, represent, independently of each other: H, Hal or a group $(C_1\text{-}C_6)$alkyl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, an aryl or heteroaryl group, $S(O)_qR$ with q = 0, 1 or 2;

or alternatively

$\mathbf{R_1}$ and $\mathbf{R_2}$ and/or $\mathbf{R_1'}$ and $\mathbf{R_2'}$ together form, respectively, a double bond $=CH_2$ or $=CH\text{-}CH_3$;

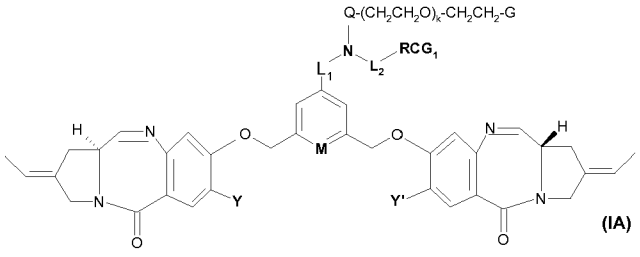
- **Y** and **Y'**, which may be identical or different, represent, independently of each other, H or -OR;
- **M** represents CH or N;
- **ALK** and **ALK'** denote a group $(C_1\text{-}C_6)$alkylene;

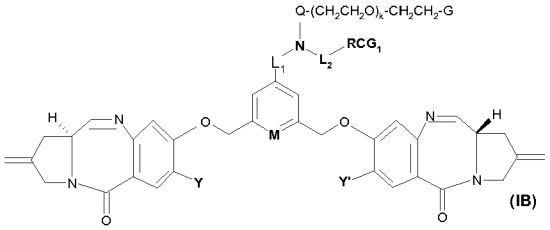
- **R** and **R'** represent, independently of each other, H or a group $(C_1\text{-}C_6)$alkyl or aryl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, an aryl or heteroaryl group;
- $\mathbf{L_1}$ represents:
  - a single bond;
    or
  - the group $-(OCH_2CH_2)_i-$, attached to the phenyl or pyridyl ring via the oxygen atom, i representing an integer ranging from 2 to 40;
    or
  - the group -D-$(C_1\text{-}C_6)$ALK- attached to the phenyl or pyridyl ring via D, in which D represents –O-, –NH- or $-N(C_1\text{-}C_4)$alkyl-;
- $\mathbf{L_2}$ represents:
  - a group $-(C_1\text{-}C_6)$ALK-;
    or
  - the group $-(CH_2CH_2O)_j-CH_2CH_2-$, **j** representing an integer ranging from 1 to 40;
    or
  - a group $-(CH_2CH_2O)_j-CH_2CH_2NR''-(C_1\text{-}C_6)$ALK-, attached to the nitrogen atom via the unit $-(CH_2CH_2O)-$, **j** representing an integer ranging from 1 to 40 and **R"** representing H or a group $(C_1\text{-}C_4)$alkyl;
- **Q** represents a single bond or the group C(=O);
- **k** represents an integer ranging from 0 to 40;
- **G** represents a group –OR or -NRR', R and R' being as defined previously or being such that they form, with the nitrogen atom to which they are attached, a group $(C_4\text{-}C_{10})$heterocycloalkyl which may comprise in the ring another heteroatom chosen from N, O and S and which may be optionally substituted with at least one substituent chosen from a group $(C_1\text{-}C_4)$alkyl, a halogen atom and a hydroxyl group;
- **RCG1** represents the group $-SZ_a$ or $-C(=O)-Z_bR_b$;
- $\mathbf{Z_a}$ represents Ac, R_a or SR_a;
- $\mathbf{R_a}$ represents H, or a group $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_8)$cycloalkyl, aryl, heteroaryl or $(C_4\text{-}C_{10})$heterocycloalkyl optionally substituted with one or more substituents chosen from: Hal, CN, NRR', CF_3, OR, NO_2, an aryl or heteroaryl group;
- $\mathbf{Z_b}$ represents a single bond, -O- or -NH- and $\mathbf{R_b}$ representing H or a group $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_8)$cycloalkyl, aryl, heteroaryl or $(C_4\text{-}C_{10})$heterocycloalkyl or alternatively $\mathbf{Z_b}$ represents a single bond and $\mathbf{R_b}$ represents Hal;

with the condition that if $\mathbf{L_1}$ represents a single bond, then RCG1 represents $-SZ_a$.

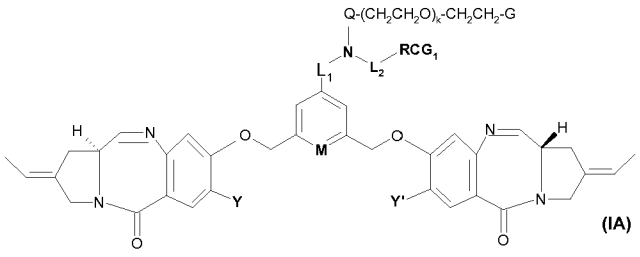
2. Compound according to Claim 1 in which U=U' and/or W=W' and/or R_1=R_1' and/or R_2=R_2' and/or Y=Y' and/or the two groups ALK and ALK' attached to the phenyl or pyridyl nucleus are identical.

3. Compound according to Claim 1 of formula **(IA)** or **(IB)**:





4. Compound according to Claim 3 of formula **(IA)**:



5. Compound according to Claim 1 to 4 in which **Y** and **Y'** represent a group (C_1-C_4)alkoxy.

6. Compound according to Claim 1 to 5 in which **M** represents N.

7. Compound according to one of Claims 1 to 6 in which if $\mathbf{L_1}$ represents a single bond, $\mathbf{L_2}$ represents $-(CH_2CH_2O)_j-CH_2CH_2-$ or $-(CH_2CH_2O)_j-CH_2CH_2NR''-(C_1-C_6)ALK-$.

8. Compound according to one of Claims 1 to 7 in which **G** represents a group -OH, -O(C_1-C_6)alkyl, $-NH_2$, -NH(C_1-C_6)alkyl or $-N(C_1-C_6)alkyl_2$ or **G** represents a group -NRR' in which R and R' form, together with the nitrogen atom to which they are attached, a group (C_4-C_{10})heterocycloalkyl which may comprise in the ring another heteroatom chosen from N, O and S and which may be optionally substituted with at least one substituent chosen from a group (C_1-C_4)alkyl, a halogen atom and a hydroxyl group.

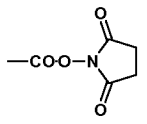
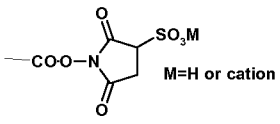
9. Compound according to one of Claims 1 to 8 in which:

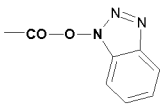
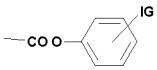
- L_1= -D-(C_1-C_6)ALK- and L_2= -(C_1-C_6)ALK-; or
- L_1= -$(OCH_2CH_2)_i$- and L_2= -(C_1-C_6)ALK-; or
- L_1= single bond and L_2= –$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-; or
- L_1= -D-(C_1-C_6)ALK-, and L_2= –$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-; or
- L_1= -D-(C_1-C_6)ALK- and L_2= -$(CH_2CH_2O)_j$-CH_2CH_2-.

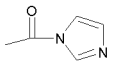
10. Compound according to Claim 1 to 8 in which:

- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q = single bond, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q=CO, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= -$(OCH_2CH_2)_i$-, L_2= -(C_1-C_6)ALK-, Q = single bond, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= -$(OCH_2CH_2)_i$-, L_2= -(C_1-C_6)ALK-, Q=CO, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q = single bond, k=1-10, G=NRR', RCG1=-SZ_a;
- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q=CO, k=1-10, G=NRR', RCG1=-SZ_a;
- L_1= single bond, L_2= –$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-, Q = single bond, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= single bond, L_2= –$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-, Q=CO, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= single bond, L_2= –$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-, Q = single bond, k=0-10, G=NRR', RCG1=-SZ_a;
- L_1= single bond, L_2= –$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-, Q=CO, k=0-10, G=NRR', RCG1=-SZ_a;
- L_1= -D-(C_1-C_6)ALK-, L_2= –$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-, Q = single bond, k=0-10, G=NRR', RCG1=-SZ_a;
- L_1= -D-(C_1-C_6)ALK-, L_2= –$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-, Q=CO, k=0-10, G=NRR', RCG1=-SZ_a;
- L_1= -D-(C_1-C_6)ALK-, L_2=–$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-, Q = single bond, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= -D-(C_1-C_6)ALK-, L_2=–$(CH_2CH_2O)_j$-CH_2CH_2NR''-(C_1-C_6)ALK-, Q=CO, k=1-10, G=OR, RCG1=-SZ_a;
- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q = single bond, k=1-10, G=OR, RCG1 = -C(=O)Z_bR_b;
- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q=CO, k=1-10, G=OR, RCG1 = -C(=O)Z_bR_b;
- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q = single bond, k=1-10, G=NRR', RCG1 = -C(=O)Z_bR_b;
- L_1= -D-(C_1-C_6)ALK-, L_2= -(C_1-C_6)ALK-, Q=CO, k=1-10, G=NRR', RCG1 = -C(=O)Z_bR_b;

- L_1= -$(OCH_2CH_2)_i$-, L_2= -$(C_1$-$C_6)$ALK-, Q = single bond, k=1-10, G=OR, RCG1 = -$C(=O)Z_bR_b$;
- L_1= -$(OCH_2CH_2)_i$-, L_2= -$(C_1$-$C_6)$ALK-, Q=CO, k=1-10, G=OR, RCG1 = -$C(=O)Z_bR_b$;
- L_1= -$(OCH_2CH_2)_i$-, L_2= -$(C_1$-$C_6)$ALK-, Q = single bond, k=1-10, G=NRR', RCG1 = -$C(=O)Z_bR_b$;
- L_1= -$(OCH_2CH_2)_i$-, L_2= -$(C_1$-$C_6)$ALK-, Q=CO, k=1-10, G=NRR', RCG1 = -$C(=O)Z_bR_b$;
- L_1= -D-$(C_1$-$C_6)$ALK-, L_2= -$(CH_2CH_2O)_j$-CH_2CH_2-, Q = single bond, k=1-10, G=OR, RCG1 = -$C(=O)Z_bR_b$;
- L_1= -D-$(C_1$-$C_6)$ALK-, L_2= -$(CH_2CH_2O)_j$-CH_2CH_2-, Q=CO, k=1-10, G=OR, RCG1 = -$C(=O)Z_bR_b$;
- L_1= -D-$(C_1$-$C_6)$ALK-, L_2= -$(CH_2CH_2O)_j$-CH_2CH_2-, Q = single bond, k=0-10, G=NRR', RCG1 = -$C(=O)Z_bR_b$;
- L_1= -D-$(C_1$-$C_6)$ALK-, L_2= -$(CH_2CH_2O)_j$-CH_2CH_2-, Q=CO, k=0-10, G=NRR', RCG1 = -$C(=O)Z_bR_b$.

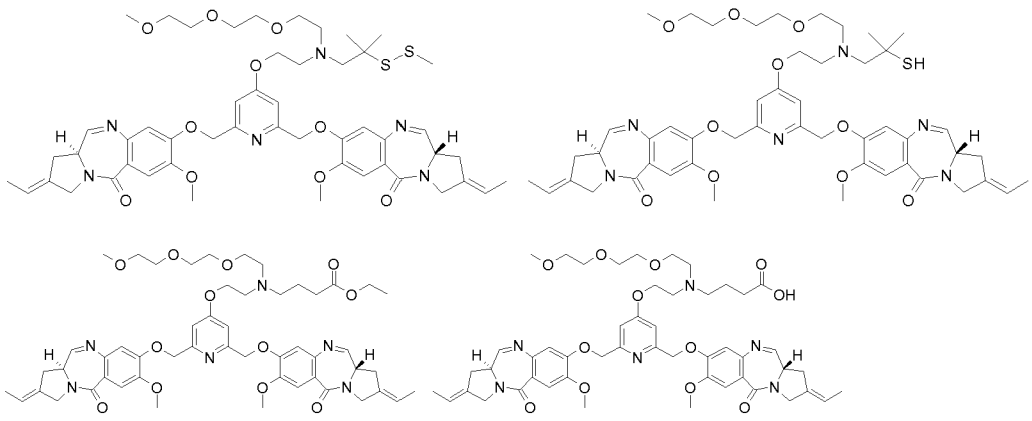
11. Compound according to Claim 1 to 10 in which $-COZ_bR_b$ represents -COOH, -$COO(C_1$-$C_6)$alkyl, , 

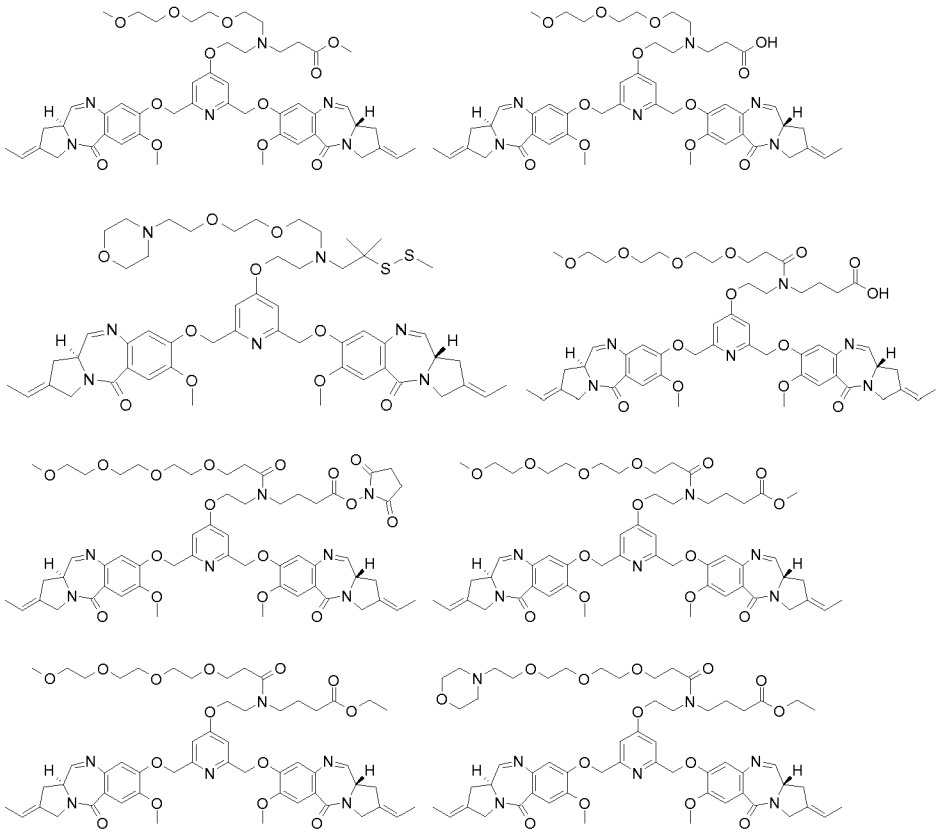
, , or the group 

in which **IG** represents at least one inductive group, or .

12. Compound according to Claim 1 to 10 in which $-SZ_a$ represents -SH or $-SS(C_1$-$C_6)$alkyl or –SS-heteroaryl.

13. Compound according to Claim 1 chosen from one of the following:





14. Process for preparing a conjugate, which consists in:

(i) placing in contact and leaving to react:

- an optionally buffered aqueous solution of the binding agent, optionally modified by means of a modifying agent,

and

- a solution of a compound of formula (I) as defined in one of Claims 1 to 13,

the chemical group RCG1 of the compound of formula (I) being reactive towards the chemical groups RCG2 present on the binding agent especially towards the amino groups present on antibodies, the said chemical groups RCG2 having been introduced, where appropriate, by the modifying agent,

So as to attach the compound of formula (I) to the binding agent with formation of a covalent bond;

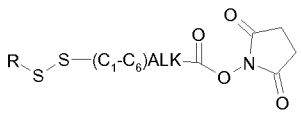
(ii) and then optionally to separate the conjugate formed in step (i) from the compound of formula (I) and/or from the unreacted binding agent and/or from any aggregates that may have formed.

15. Process according to Claim 14 in which:

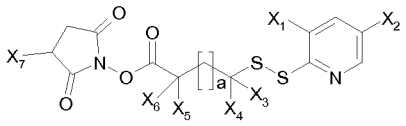
- in the presence of a derivative of formula (I) comprising a reactive chemical group RCG1 of the type $-SZ_a$, the binding agent comprises:
  - disulfide chemical groups in the case where RCG1 represents –SH;
  - thiol chemical groups in the case where RCG1 represents $-SZ_a$ with $Z_a \neq H$;
  - maleimido or haloacetamido chemical groups in the case where RCG1 represents –SH;
- in the presence of a derivative of formula (I) comprising a reactive chemical group RCG1 of the type $-C(=O)-Z_bR_b$, the derivative of formula (I) is reacted with the amino functions of the binding agent, especially the ε-amino groups borne by the side chains of the lysine (Lys) residues of an antibody.

16. Process according to Claim 14 or 15 in which:

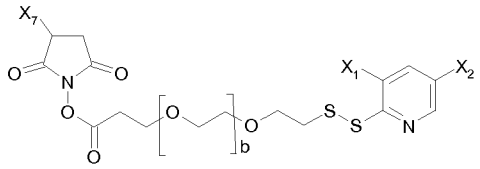
- when the reactive chemical group RCG1 is of the type -SH, and when the binding agent bears amino functions, especially ε-amino groups borne by the side chains of the lysine residues of an antibody, the latter is modified by means of a modifying agent chosen from a compound of formula:



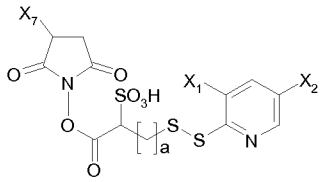
in which R represents a group $(C_1\text{-}C_6)$alkyl, aryl, heteroaryl, $(C_3\text{-}C_7)$cycloalkyl, $(C_4\text{-}C_{10})$heterocycloalkyl;



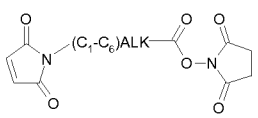
, a pegylated analogue of formula:



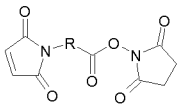
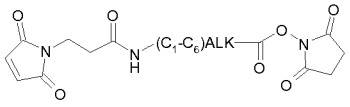
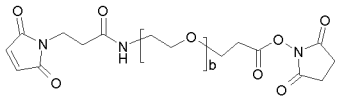
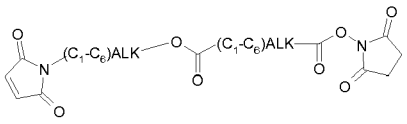
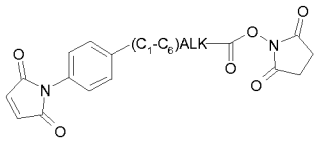
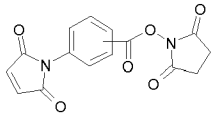
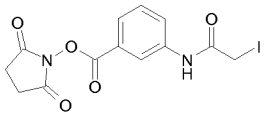
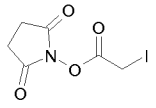
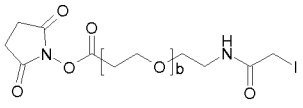
or a sulfonic analogue of formula



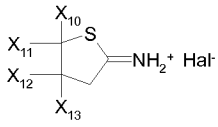
in which X_3 X_4, X_5, X_6 represent H or a group $(C_1\text{-}C_6)$alkyl, X_1 and X_2 represent -H, $-CONX_8X_9$, $-NO_2$, X_8 and X_9 representing H or a group $(C_1\text{-}C_6)$alkyl, X_7 represents $-SO_3^-M^+$ or H or alternatively a quaternary ammonium group and a denotes an integer ranging from 0 to 4 and b denotes an integer ranging from 0 to 2000; or chosen from succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate; sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate;



;


in which R represents a group $-(CH_2)_n-$, $-(CH_2)_n-$cyclohexyl-, -cyclohexyl-$(CH_2)_n-$ and n represents an integer ranging from 1 to 10;  ;  ;  ;  ;  **b** being an integer between 0 and 2000;  ;  ; succinimidyl-N-bromoacetate; succinimidyl-3-(N-bromoacetamido)propionate;  , **b** being an integer between 0 and 2000.

- when the reactive chemical group RCG1 is of the type $-SZ_a$ with $Z_a \neq H$, and when the binding agent bears amino functions, especially ε-amino groups borne by the side chains of the lysine residues of an antibody, the latter is modified by means of a modifying agent chosen from a compound of formula

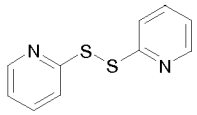
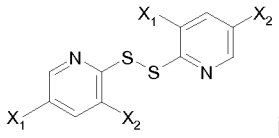

in which:

- Hal represents a halogen atom;
- X_{10} represents a halogen atom or the group $-COOX_{14}$, nitro, unsubstituted or halogenated (C_1-C_8)alkyl, unsubstituted or halogenated (C_1-C_8)alkoxy, unsubstituted or halogenated (C_2-C_8)alkenyl, unsubstituted or halogenated (C_2-C_8) alkynyl, unsubstituted (C_3-C_8)cycloalkyl, aryl which is unsubstituted or substituted with one to three substituents selected from amino, a halogen atom, unsubstituted or halogenated group (C_1-C_8)alkyl, unsubstituted or halogenated (C_1-C_8)alkoxy;
- each of the X_{11}, X_{12}, X_{13} independently represents a hydrogen atom or alternatively may represent X_{10};

or X_{10} and X_{11} together form a (C_2-C_5)alkylene ring, which is unsubstituted or substituted with one to five groups (C_1-C_4)alkyl;

or X_{10} or X_{11} form, together with X_{12}, a (C_1-C_5)alkylene ring, which is unsubstituted or substituted with one to five groups (C_1-C_4) alkyl

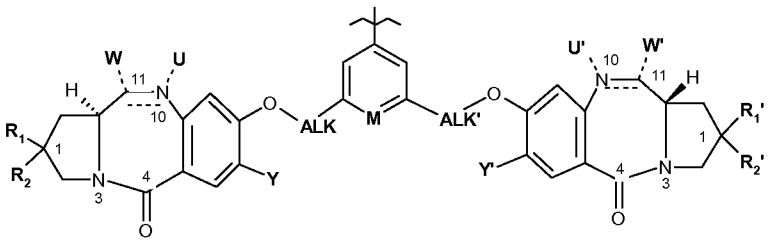
and X_{14} is -H or a group $(C_1\text{-}C_8)$alkyl
or $X_{10}=X_{11}=X_{12}=X_{13}=H$.

- when the reactive chemical group RCG1 is of the type -SH, and when the binding agent bears thiol functions, especially following the introduction of cysteines by mutation or by chemical modification of a binding agent bearing amino functions, the binding agent is modified such that its thiol functions are converted into disulfide functions, more particularly by means of a modifying agent chosen from a compound of formula  or  in which X_1 and X_2 represent -H, $-CONX_8X_9$ or $-NO_2$, X_8 and X_9 representing H or a group $(C_1\text{-}C_6)$alkyl.

17. Conjugate that may be obtained via the process according to one of Claims 14 to 16.

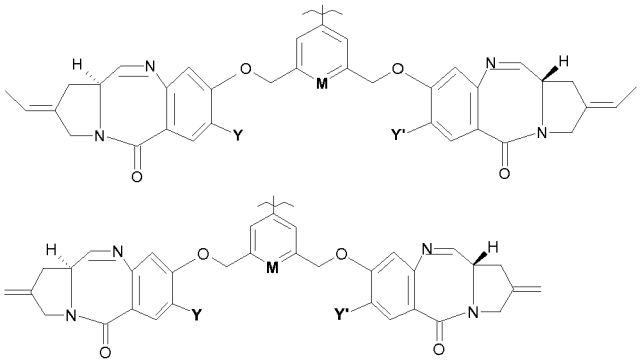
18. Conjugate solution that may be obtained via the process according to one of Claims 14 to 16 or comprising the conjugate as defined in Claim 17.

19. Use of a derivative of formula (I) according to one of Claims 1 to 13 for the preparation of a binding agent to which is covalently attached in the para position of M the dimer of formula



20. Use according to Claim 19 in which the binding agent is an antibody.

21. Use according to Claim 19 or 20 in which the dimer has the formula:

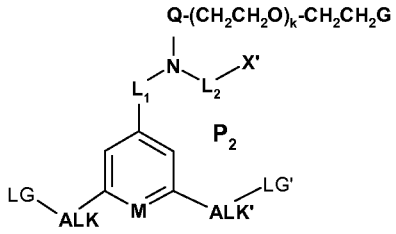


22. Compound according to one of Claims 1 to 13 for use as an anticancer agent.

23. Conjugate according to Claim 17 for use as an anticancer agent.

24. Conjugate solution according to Claim 18 for use as an anticancer agent.

25. Compound of formula $\mathbf{P_2}$:

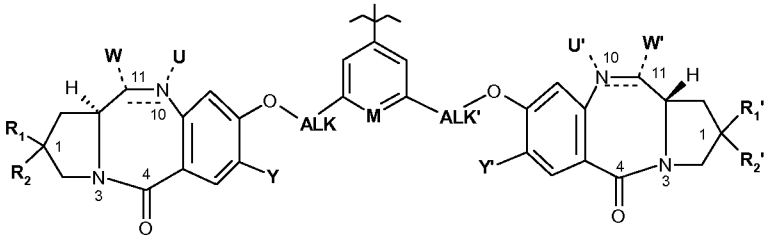


in which:

- $\mathbf{L_1}$, $\mathbf{L_2}$, **Q**, **k**, **ALK**, **ALK'** and **G** are as defined according to one of Claims 1 to 9;
- **LG** and **LG'** represent a leaving group;
- **X'** represents RCG1.

26. Use of a compound according to Claim 25 as an intermediate compound in the preparation of a compound according to one of Claims 1 to 13.

27. Binding agent to which has been covalently attached in the para position of M the dimer of formula:



after reaction of a compound as defined in one of Claims 1 to 13 with the binding agent.

28. Binding agent according to Claim 27, which has affinity for an antigen or a group of antigens located on cancer cells or stromal cells associated with a tumour.

29. Binding agent according to Claim 27 or 28, which is an antibody, preferably a monoclonal antibody.