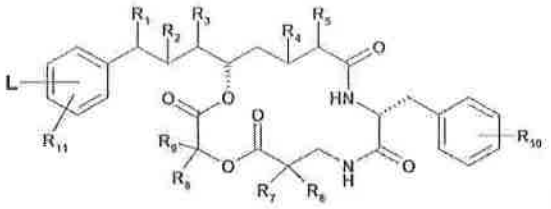
CLAIMS

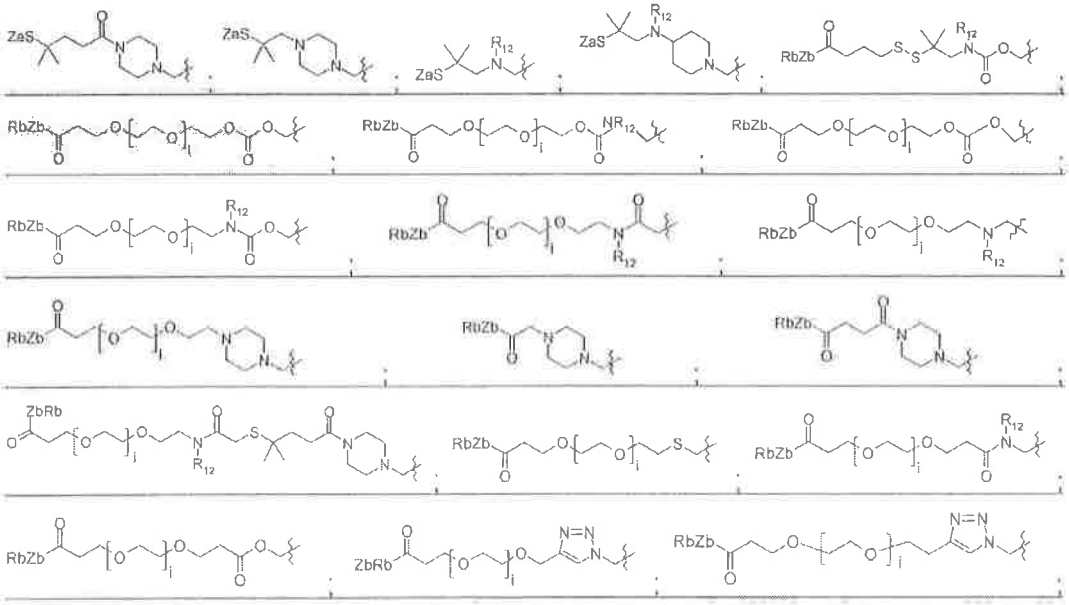
1. Cryptophycin derivative of formula (II):

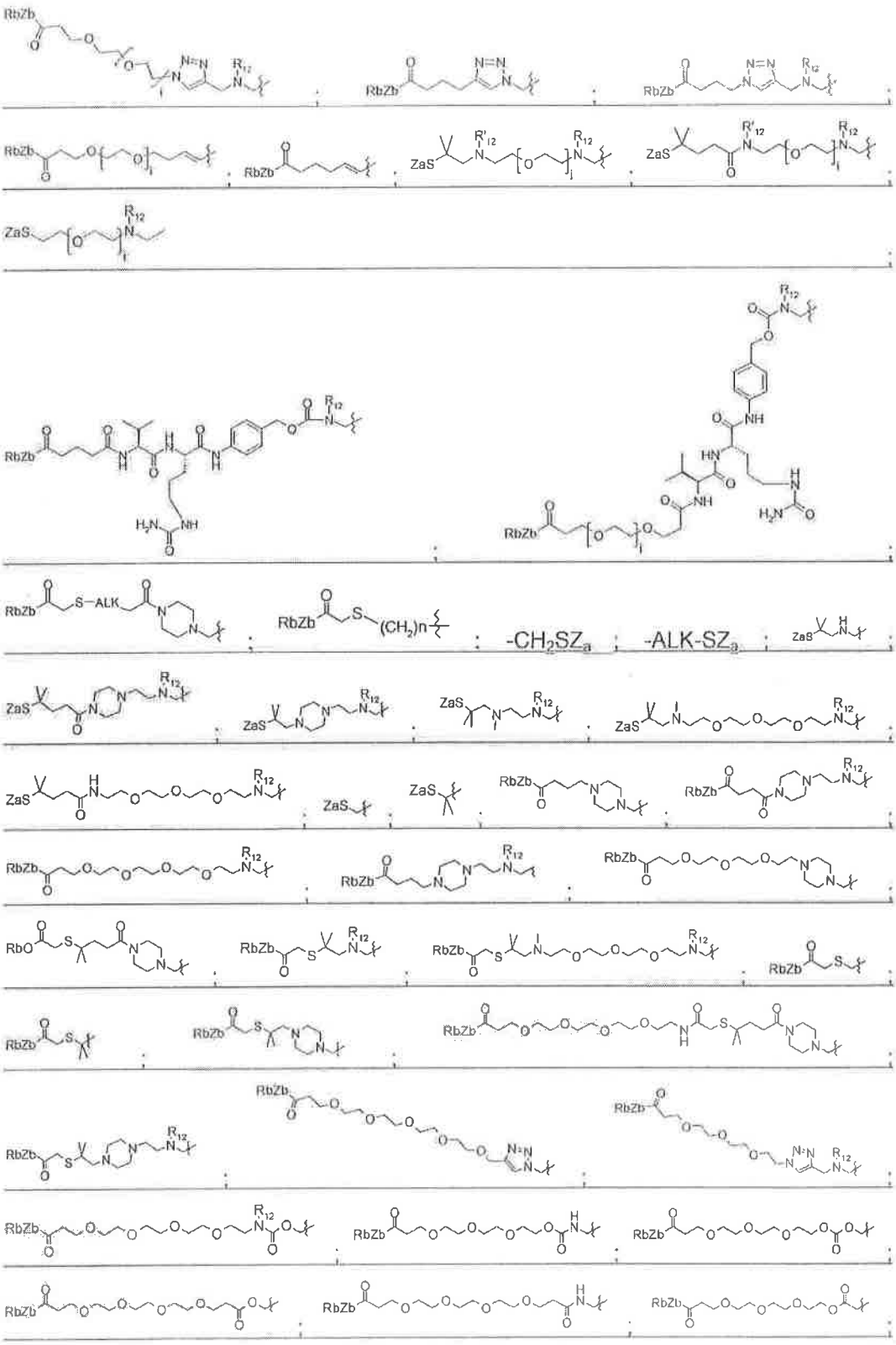


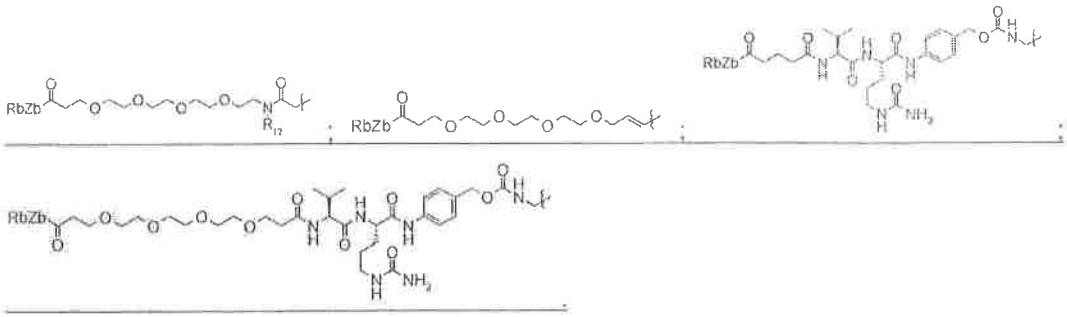
(II)

in which:

- **R_1** represents a halogen atom and **R_2** represents a group –OH, an acyl group derived from an amino acid **AA** or a group (C_1-C_4)alkanoyloxy;
  or alternatively **R_1** and **R_2** form an epoxide unit;
- **AA** denotes a natural or unnatural amino acid;
- **R_3** represents a group (C_1-C_6)alkyl;
- **R_4** and **R_5** both represent H or together form a double bond CH=CH between C13 and C14;
- **R_6** and **R_7** represent, independently of each other, H or a group (C_1-C_6)alkyl;
- **R_8** and **R_9** represent, independently of each other, H or a group (C_1-C_6)alkyl;
- **R_{10}** represents at least one substituent of the phenyl nucleus chosen from: H, a group OH, (C_1-C_4)alkoxy, a halogen atom or a group -NH_2, -NH(C_1-C_6)alkyl or -N(C_1-C_6)$alkyl_2$;
- **R_{11}** represents at least one substituent of the phenyl nucleus chosen from H and a group (C_1-C_4)alkyl;
- **L** represents a linker in the ortho (o), meta (m) or para (p) position, preferably in the para position, of the phenyl nucleus wherein L is ~~bearing the unit RCG_1~~ chosen from:




RbZb
ZaS
-CH2SZa
-ALK-SZa



- R_{12} and R'_{12} represent, independently of each other, H or a group $(C_1\text{-}C_6)$alkyl, more particularly a methyl group;
- i represents an integer ranging from 1 to 20, more particularly from 1 to 10, even more particularly from 1 to 8, or from 1 to 6, and even more particularly from 2 to 5;
- Z_a represents H or a group $-SR_a$, R_a representing a group $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_7)$cycloalkyl, aryl, heteroaryl or $(C_3\text{-}C_7)$heterocycloalkyl;
- Z_b represents a single bond, -O- or -NH-, R_b representing H or a group $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_7)$cycloalkyl, aryl, heteroaryl or $(C_3\text{-}C_7)$heterocycloalkyl.

~~-G'-X-$(CR_{13}R_{14})_t(OCH_2CH_2)_y(CR_{15}R_{16})_u$-Q-RCG$_1$;~~
~~-G'-X-$(CR_{13}R_{14})_t(OCH_2CH_2)_y$-Y'-$(CR_{15}R_{16})_u$-Q-RCG$_1$;~~
~~-G'-X-$(CR_{13}R_{14})_t(CR_{17}=CR_{18})(CR_{15}R_{16})_u(OCH_2CH_2)_y$-Q-RCG$_1$;~~
~~-G'-X-$(CR_{13}R_{14})_t(OCH_2CH_2)_y(CR_{17}=CR_{18})(CR_{15}R_{16})_u$-Q-RCG$_1$;~~
~~-G'-X-$(CR_{13}R_{14})_t$-phenyl-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$; -G'-X-$(CR_{13}R_{14})_t$-furyl-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$;~~
~~-G'-X-$(CR_{13}R_{14})_t$-oxazolyl-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$; -G'-X-$(CR_{13}R_{14})_t$-thiazolyl-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$; -G'-X-$(CR_{13}R_{14})_t$-thienyl-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$; -G'-X-$(CR_{13}R_{14})_t$-imidazolyl-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$; -G'-X-$(CR_{13}R_{14})_t$-piperazinyl-CO-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$; -G'-X-$(CR_{13}R_{14})_t$-piperidyl-methyl-NR_{12}-CO-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$; -G'-X-$(CR_{13}R_{14})_t$-piperidyl-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$; -G'-X-$(CR_{13}R_{14})_t$-piperidyl-NR_{12}-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$; -G'-X-$(CR_{13}R_{14})_t$-triazolyl-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$; -G'-X-$(CR_{13}R_{14})_t$-triazolyl-$(CR_{15}R_{16})_u$-Y'-Q-RCG$_1$;~~

-G' X-($CR_{13}R_{14}$)$_t$-phenyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G' X-($CR_{13}R_{14}$)$_t$-furyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G' X-($CR_{13}R_{14}$)$_t$-oxazolyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G' X-($CR_{13}R_{14}$)$_t$-thiazolyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G' X-($CR_{13}R_{14}$)$_t$-thienyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G' X-($CR_{13}R_{14}$)$_t$-imidazolyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G' X-($CR_{13}R_{14}$)$_t$-piperazinyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G' X-($CR_{13}R_{14}$)$_t$-piperidyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G' X-($CR_{13}R_{14}$)$_t$-piperidyl-methyl-NR_{12}-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G' X-($CR_{13}R_{14}$)$_t$-piperidyl-NR_{12}-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G' X-($CR_{13}R_{14}$)$_t$-triazolyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$;

or

-G" Y-($CR_{13}R_{14}$)$_t$(OCH_2CH_2)$_y$($CR_{15}R_{16}$)$_u$-Q-RCG$_1$;

-G" Y-($CR_{13}R_{14}$)$_t$(OCH_2CH_2)$_y$-Y'-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$;

-G" Y-($CR_{13}R_{14}$)$_t$(CR_{17}=CR_{18})($CR_{15}R_{16}$)$_u$(OCH_2CH_2)$_y$-Q-RCG$_1$;

-G" Y-($CR_{13}R_{14}$)$_t$(OCH_2CH_2)$_y$(CR_{17}=CR_{18})($CR_{15}R_{16}$)$_u$-Q-RCG$_1$;

-G" Y-($CR_{13}R_{14}$)$_t$-phenyl-($CR_{15}R_{16}$)$_u$-Y'-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-furyl-($CR_{15}R_{16}$)$_u$-Y'-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-oxazolyl-($CR_{15}R_{16}$)$_u$-Y'-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-thiazolyl-($CR_{15}R_{16}$)$_u$-Y'-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-thienyl-($CR_{15}R_{16}$)$_u$-Y'-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-imidazolyl-($CR_{15}R_{16}$)$_u$-Y'-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-piperazinyl-CO($CR_{15}R_{16}$)$_u$-Y'-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-piperidyl-methyl-NR_{12}-CO($CR_{15}R_{16}$)$_u$-Y'-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-piperidyl-($CR_{15}R_{16}$)$_u$-Y'-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-piperidyl-NR_{12}-($CR_{15}R_{16}$)$_u$-Y'-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-triazolyl-($CR_{15}R_{16}$)$_u$-Y'-Q-RCG$_1$;

-G" Y-($CR_{13}R_{14}$)$_t$-phenyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-furyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-oxazolyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-thiazolyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-thienyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-imidazolyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-piperazinyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-piperazinyl-($CR_{15}R_{16}$)$_u$-Q-CCR$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-piperidyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-piperidyl-methyl-NR_{12}-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-piperidyl-NR_{12}-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$; -G" Y-($CR_{13}R_{14}$)$_t$-triazolyl-($CR_{15}R_{16}$)$_u$-Q-RCG$_1$;

in which formulae:

G' represents a group -CH=CH- or -(CH_2)$_n$-;

G" represents a group -(CH_2)$_n$-;

n represents an integer ranging from 1 to 6;

X represents a single bond or a group -CO-, -COO- or -$CONR_{12}$-, the group CO being attached to G';

Y represents a group -O-, -OCO-, -OCOO-, -$OCONR_{12}$-, -NR_{12}-, -$NR_{12}CO$-, -$NR_{12}CONR'_{12}$-, -$NR_{12}COO$- or -S(O)$_q$-, the atom O and the group NR_{12} being attached to G";

q represents an integer that may be 0, 1 or 2;

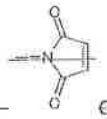
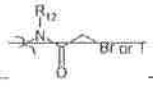
Y' represents a group -O-, -OCO-, -OCOO-, -$OCONR_{12}$-, -NR_{12}-, -$NR_{12}CO$-, -$NR_{12}CONR'_{12}$-, -$NR_{12}COO$-, -S(O)$_q$-, -CO-, -COO-, or -$CONR_{12}$-;

R_{12}, R'_{12}, R_{13}, R_{14}, R_{15}, R_{16}, R_{17} and R_{18} represent, independently of each other, H or a group $(C_1$-$C_6)$alkyl;

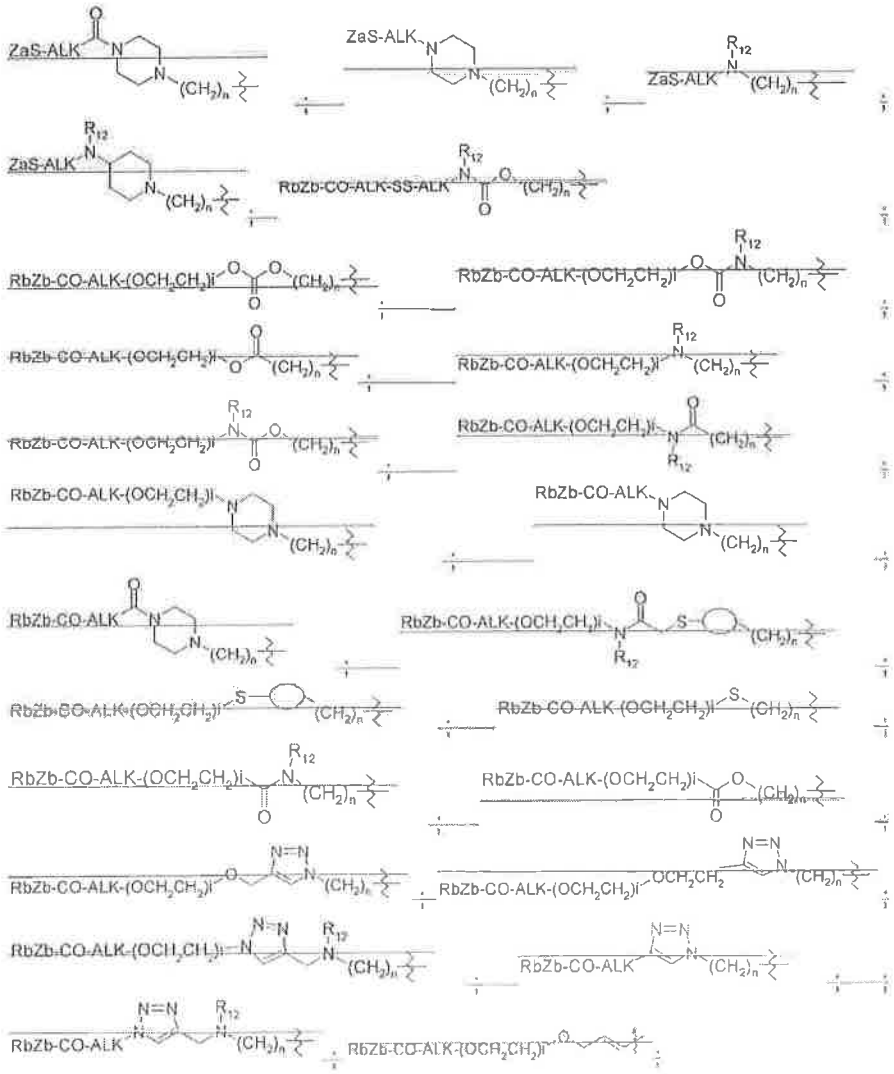
t, u and y represent integers that may range from 0 to 20 and such that t+u+y is greater than or equal to 1;

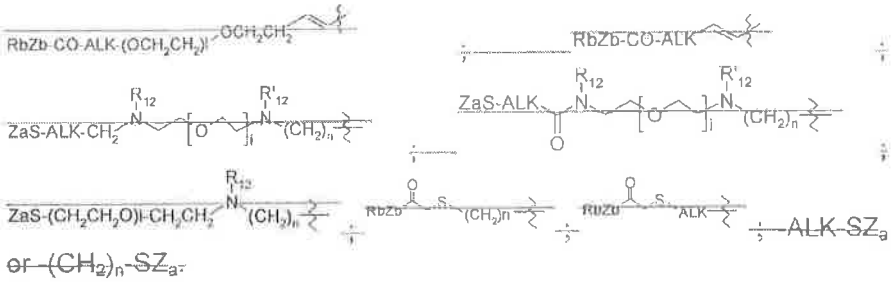
in the case of the linker of formula $-G''Y(CR_{13}R_{14})_t(OCH_2CH_2)_y Y'(CR_{15}R_{16})_u Q\text{-}RCG_1$, if y is 0 and Q represents a single bond, then u cannot be 0;

Q represents a single bond, a group $(C_1$-$C_{10})$alkylene or a group $(OCH_2CH_2)_i$, i being an integer ranging from 1 to 20, more particularly from 1 to 10, even more particularly from 1 to 8 or from 1 to 6, and even more particularly from 2 to 5;

- RCG1 represents $-SZ_a$, $-C(=O)Z_bR_b$,  or  with R_{12} representing H or $(C_1$-$C_6)$alkyl, more particularly a methyl group;

or L is chosen from:

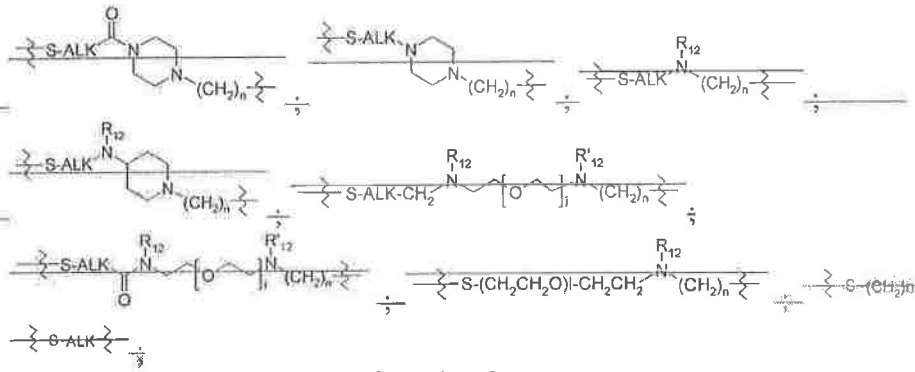




or $-(CH_2)_n-SZ_a$.

in which formulae:

-  represents one of the following 9 groups:



- n represents an integer ranging from 1 to 6;
- ALK represents a group (C_1-C_{12})alkylene;
- R_{12} and R'_{12} represent, independently of each other, H or a group (C_1-C_6)alkyl, more particularly a methyl group;
- i represents an integer ranging from 1 to 20, more particularly from 1 to 10, even more particularly from 1 to 8, or from 1 to 6, and even more particularly from 2 to 5;

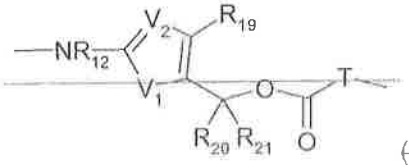
or alternatively L is a linker of formula (IV):



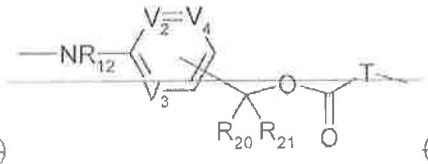
RbZb-CO-ALK (AA)w (IV)

in which:

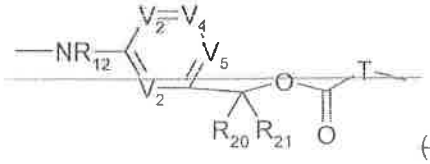
- $(AA)_w$ represents a sequence of w amino acids AA connected together via peptide bonds;
- w represents an integer ranging from 1 to 12 and preferably from 1 to 6;
- n represents an integer ranging from 1 to 6;
- D represents one of the following units:



(D1)



(D2)



(D3)

~~for which:~~

~~R_{12} represents H or a group $(C_1$-$C_6)$alkyl;~~

~~R_{19}, R_{20}, R_{21} and R_{22} represent, independently of each other, H, a halogen atom, -OH, -CN or a group $(C_1$-$C_4)$alkyl;~~

~~T attached to $(CH_2)_n$ represents NR_{12} or O;~~

~~V_1 represents O, S or NR_{12};~~

~~V_2 represents CR_{22} or N;~~

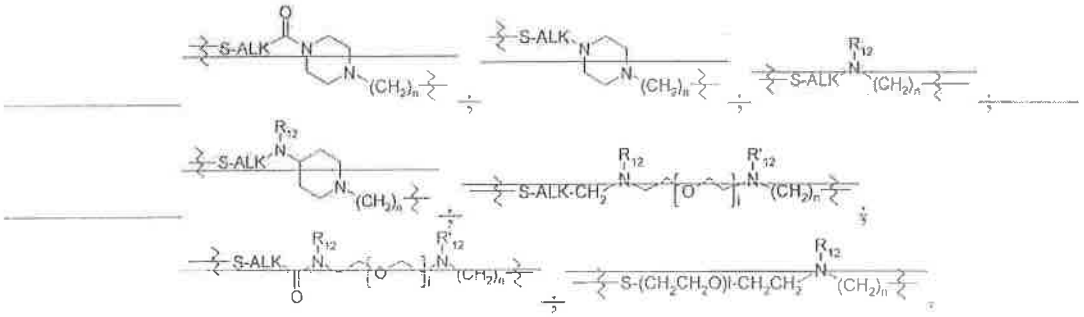
~~V_3, V_4 and V_5 are chosen, independently of each other, from CR_{22} and N;~~

- ~~Z_a represents H or the group $-SR_a$, R_a represents a group $(C_1$-$C_6)$alkyl, $(C_3$-$C_7)$cycloalkyl, aryl, heteroaryl or $(C_3$-$C_7)$heterocycloalkyl;~~
- ~~Z_b represents a single bond, -O- or -NH-, R_b representing H or a group $(C_1$-$C_6)$alkyl, $(C_3$-$C_7)$cycloalkyl, aryl, heteroaryl or $(C_3$-$C_7)$heterocycloalkyl.~~

2. Cryptophycin derivative according to Claim 1, in which R_{10} represents at least one substituent on the phenyl nucleus chosen from: H, a group OH, $(C_1$-$C_4)$alkoxy, a halogen atom.

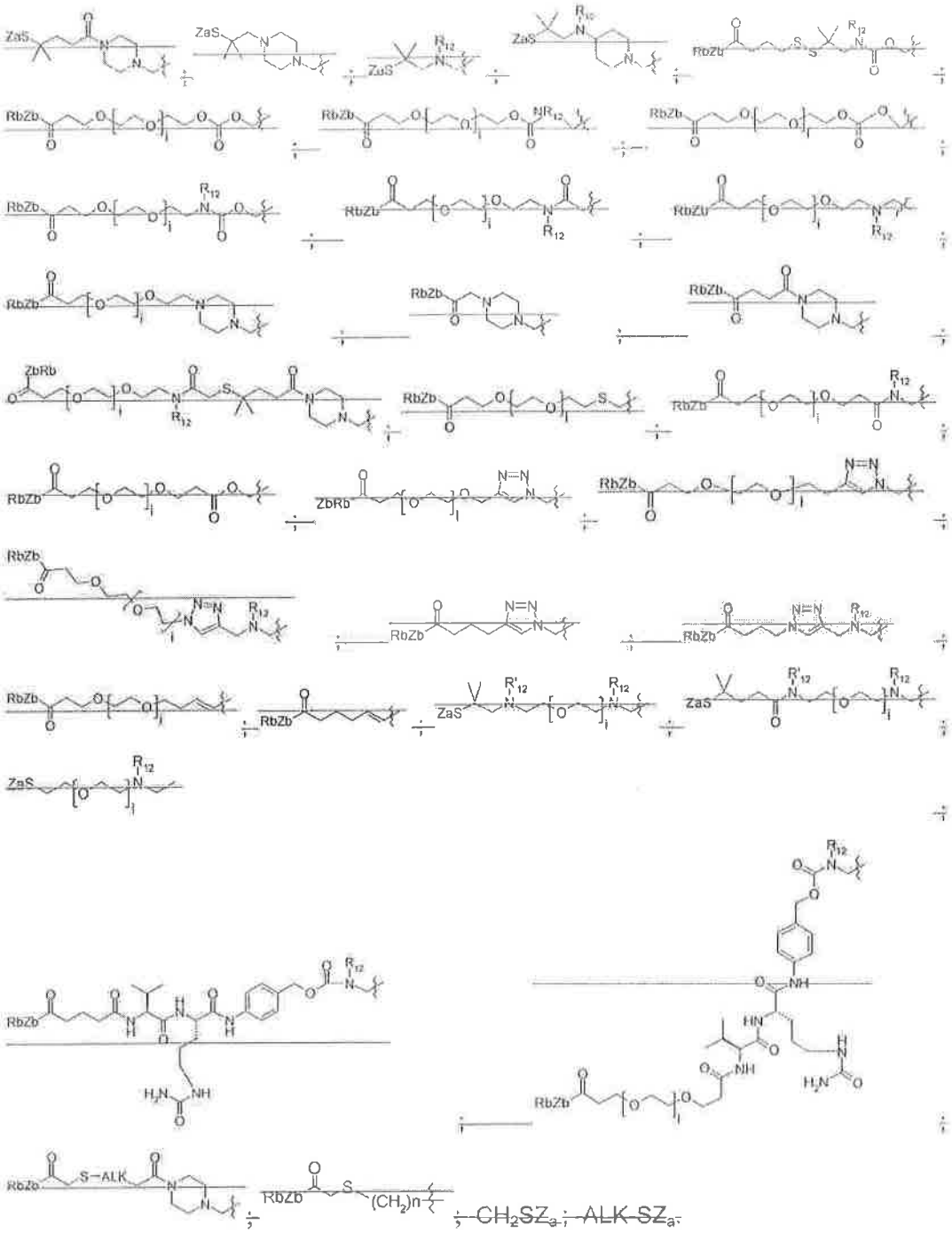
~~3. Cryptophycin derivative according to Claim 1 or 2, in which RCG1 represents $-SZ_a$ or $-C(=O)-Z_bR_b$.~~

~~4. Cryptophycin derivative according to Claims 1 to 3, in which~~  ~~represents one of the following 7 groups:~~



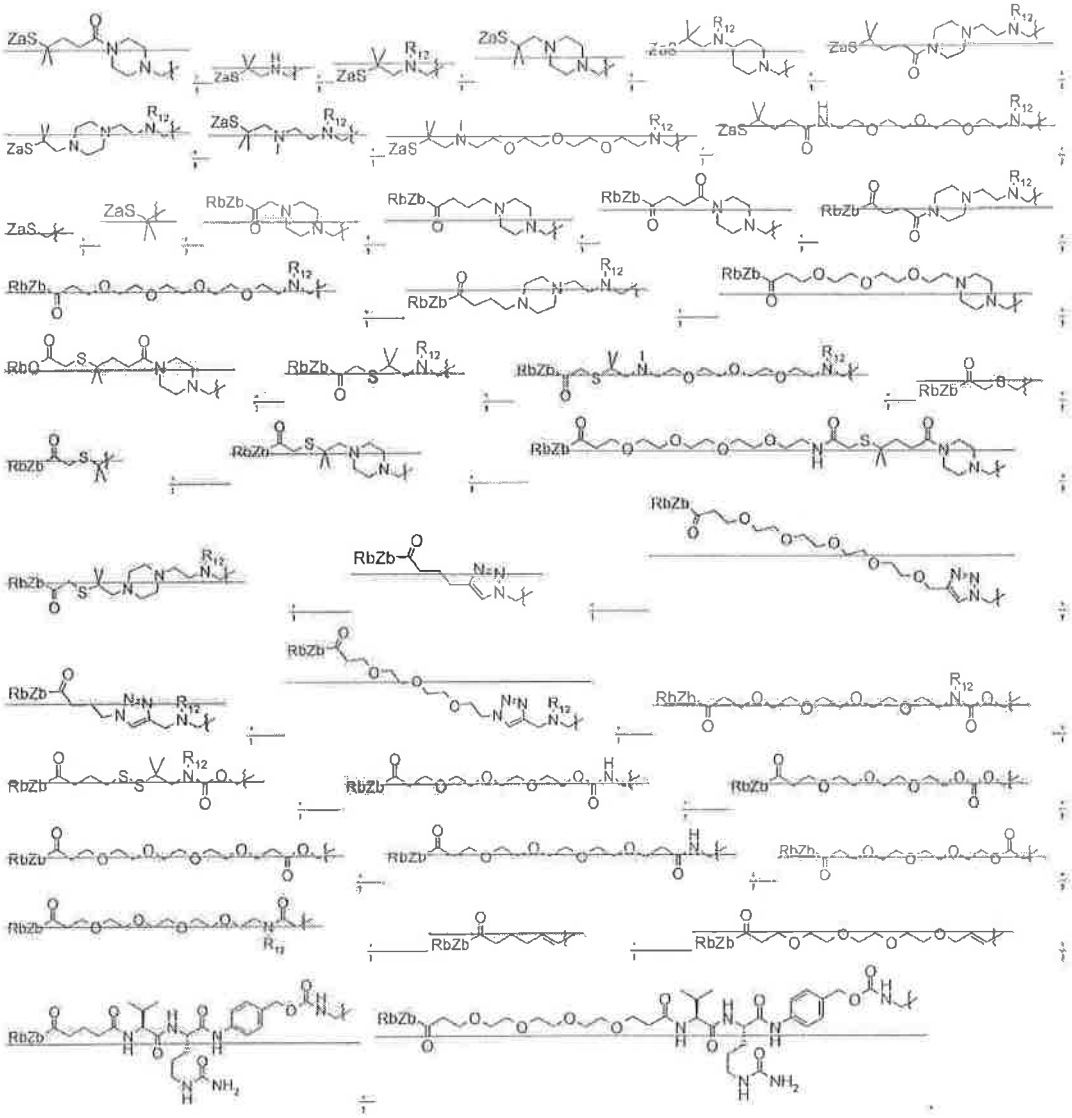
~~5. Cryptophycin derivative according to Claims 1 to 4, in which n is 1.~~

~~6. Cryptophycin derivative according to Claim 1, in which L is chosen from:~~



- R_{12} and R'_{12} represent, independently of each other, H or a group (C_1-C_6)alkyl, more particularly a methyl group;
- i represents an integer ranging from 1 to 20, more particularly from 1 to 10, even more particularly from 1 to 8, or from 1 to 6, and even more particularly from 2 to 5;
- Z_a represents H or a group $-SR_a$, R_a representing a group (C_1-C_6)alkyl, (C_3-C_7)cycloalkyl, aryl, heteroaryl or (C_3-C_7)heterocycloalkyl;
- Z_b represents a single bond, -O- or -NH-, R_b representing H or a group (C_1-C_6)alkyl, (C_3-C_7)cycloalkyl, aryl, heteroaryl or (C_3-C_7)heterocycloalkyl.

~~7.~~ ~~Cryptophycin derivative according to Claim 1, in which L is chosen from:~~

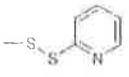
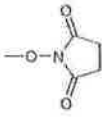
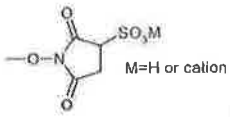
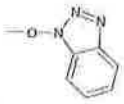
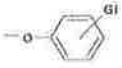


~~R_{12} representing H or a group (C_1-C_6)alkyl, more particularly a methyl group.~~

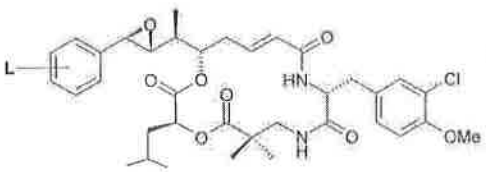
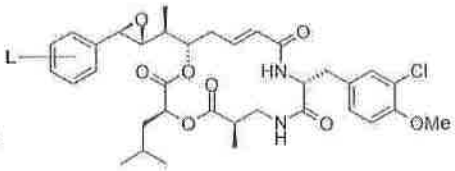
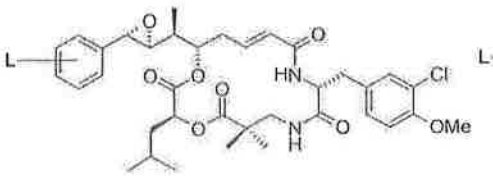
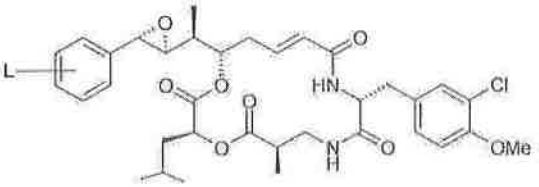
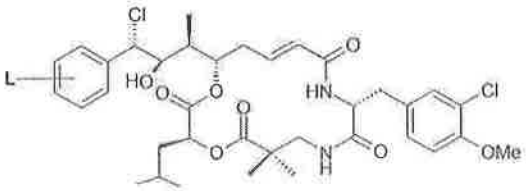
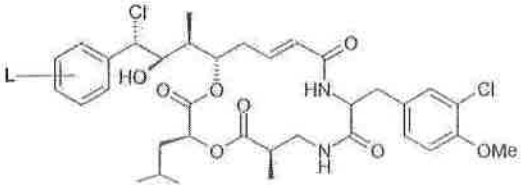
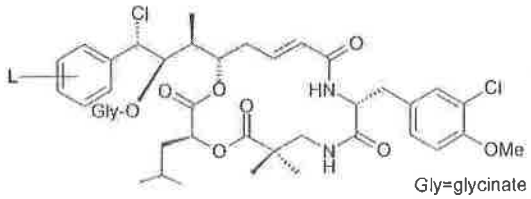
~~8~~3. Cryptophycin derivative according to Claim ~~7~~1, in which R_{12} represents H.

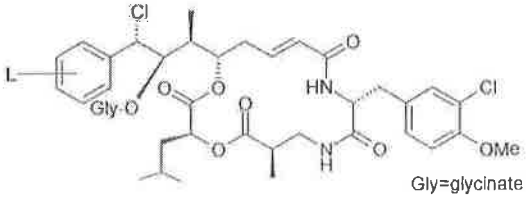
~~9~~4. Cryptophycin derivative according to Claim ~~7~~1, in which R_{12} represents (C_1-C_6)alkyl, more particularly a methyl group.

~~10~~5. Cryptophycin derivative according to Claims 1 to ~~9~~4, in which Z_a represents H or

–S(C_1-C_6)alkyl, especially –SMe, ou –S-heteroaryl, especially  or Z_bR_b represents –O(C_1-C_6)alkyl, -OH, -OCH_3, -$OCH_2CH{=}CH_2$,  or   or the group  in which **Gl** represents at least one electroinductive group, or alternatively in which –C(=O)Z_bR_b represents .

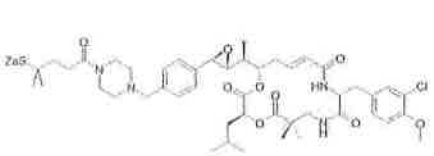
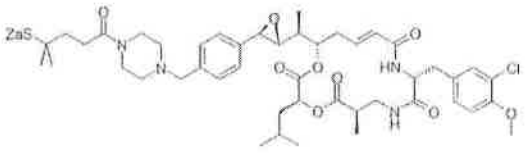
~~116~~. Cryptophycin derivative according to one of Claims 1 to ~~10~~ 5 defined according to one of the following formulae, **L** preferably being in the para position:

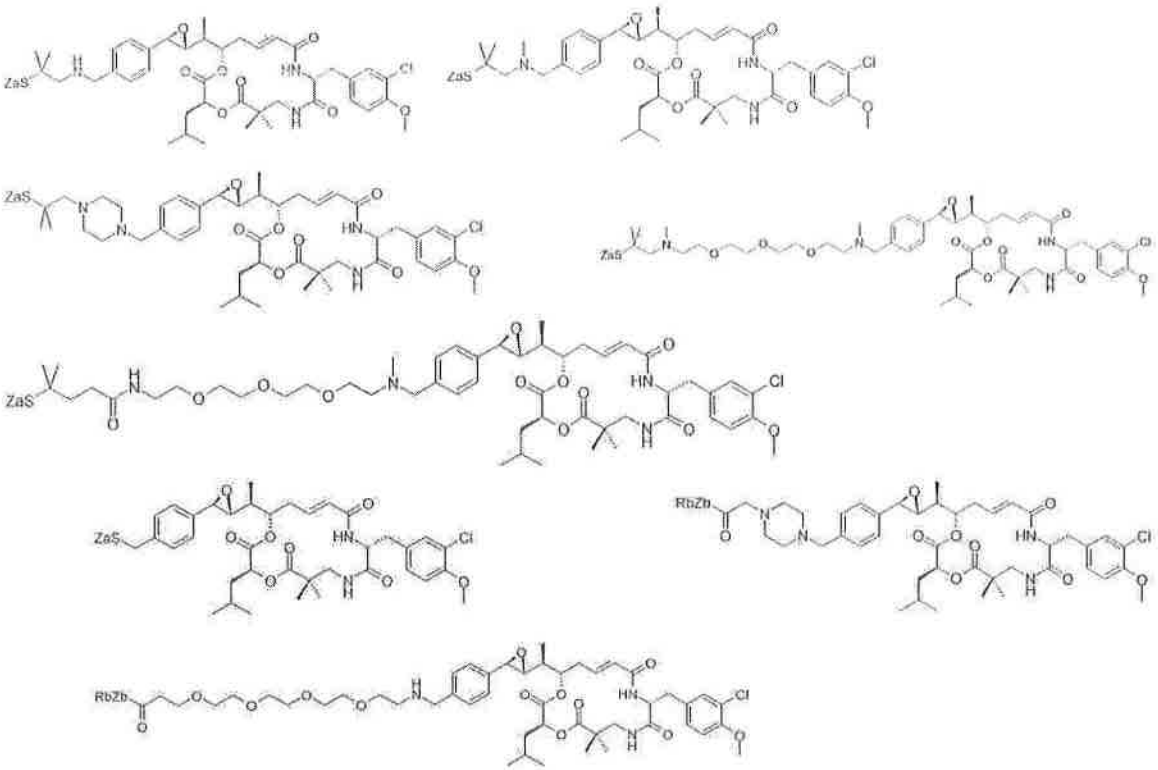








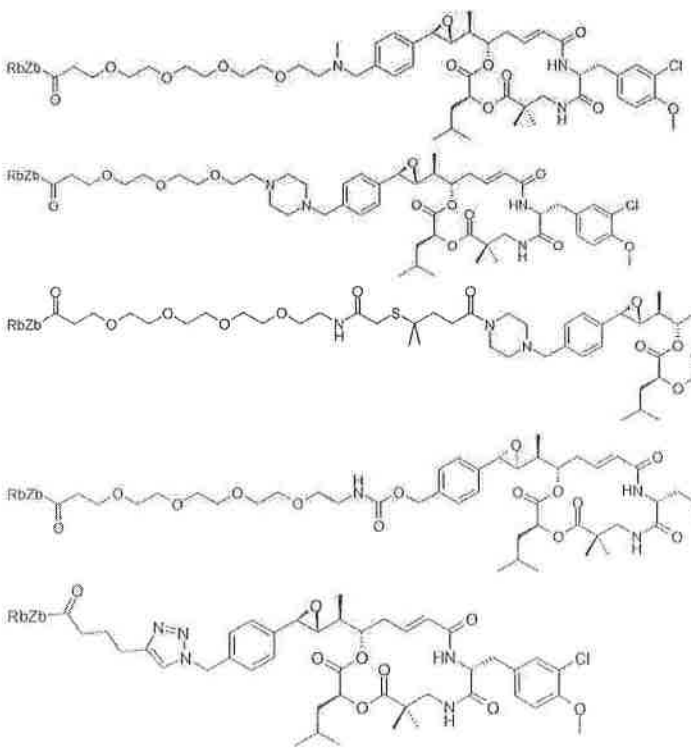
Gly=glycinate


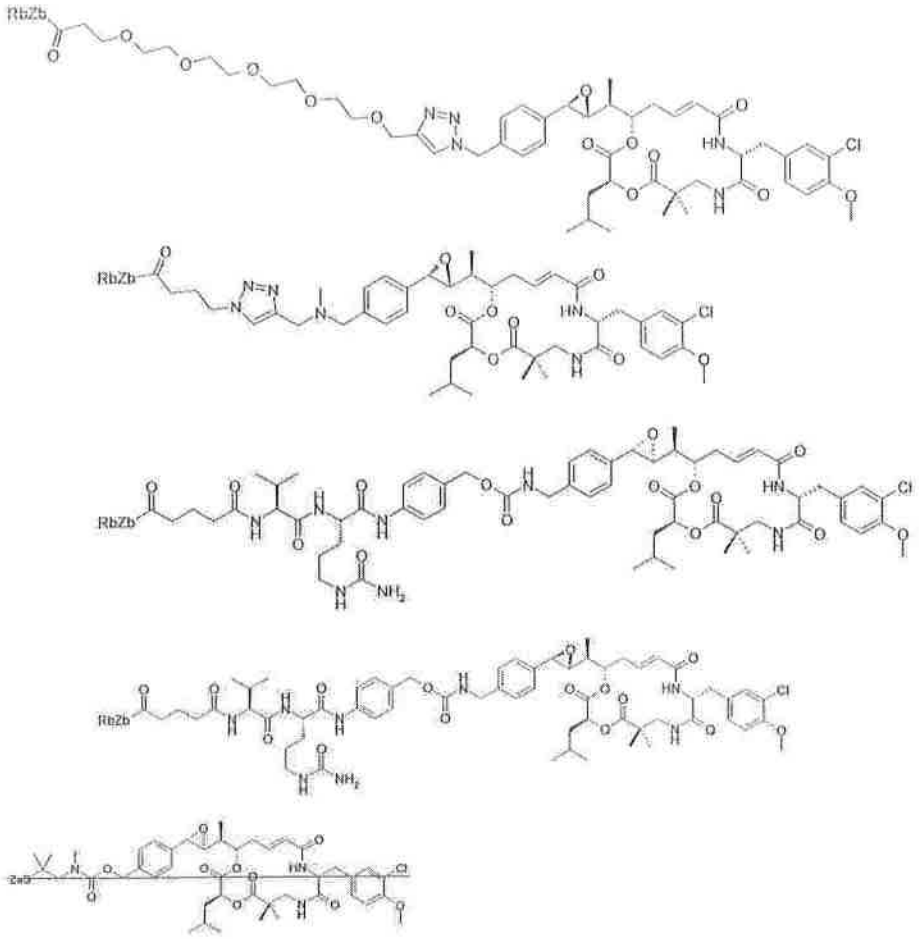
Gly=glycinate

~~117~~. Cryptophycin derivative according to Claim 1, chosen from:

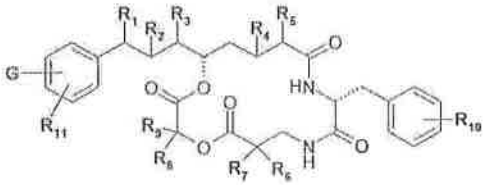




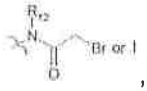


Z_a, Z_b and R_b being as defined in Claim 1 or ~~10~~5.

~~13~~8. Cryptophycin derivative of formula (III):



in which the groups R_1 to R_{11} have the same meanings as in Claim 1, ~~11~~ 6 or ~~12~~ 7 and **G** represents a group $-(CH_2)_nY$, which is in an ortho (o), meta (m) or para (p) position on the phenyl nucleus bearing the unit CR_1, preferably in the para position,

n being an integer ranging from 1 to 6 and Y denoting $-N_3$; $-NR_{12}-CH_2-C\equiv CH$ in which R_{12} represents H or a group (C_1-C_6)alkyl; -OMs; -OC(=O)-O-(4-nitrophenyl),  or , R_{12} representing H or (C_1-C_6)alkyl, more particularly a methyl group.

~~14~~9. Cryptophycin derivative according to Claim ~~13~~8, in which Y represents $-N_3$; $-NR_{12}-CH_2-C\equiv CH$ in which R_{12} represents H or a group (C_1-C_6)alkyl; -OMs or -OC(=O)-O-(4-nitrophenyl).

~~15~~10. Cryptophycin derivative according to one of Claims 1 to ~~12~~7 or according to formula (III) of Claim ~~13~~8 with G= $-(CH_2)_nY$ with Y= -Cl, $-N_3$, -OH, $-NH_2$, maleimido, haloacetamido intended to be conjugated to a binding agent.

~~16~~11. Cryptophycin derivative according to Claim ~~15~~10, in which the binding agent is a ligand, a protein, an antibody, more particularly a monoclonal antibody, a protein or antibody fragment, a peptide, an oligonucleotide or an oligosaccharide.

~~17~~12. Process for preparing a binding agent to which is attached at least one cryptophycin derivative, referred to as a conjugate, which consists in:

(i) placing in contact and leaving to react an aqueous solution of an optionally buffered binding agent and a solution of the cryptophycin derivative as defined in one of Claims 1 to ~~16~~11;

(ii) and then in optionally separating the conjugate formed in step (i) from the cryptophycin derivative and/or the unreacted binding agent and/or any aggregates formed,

wherein

- in the presence of a cryptophycin derivative comprising a reactive chemical group of the type $-SZ_a$ in the linker L, the binding agent comprises:
  - disulfide chemical groups in the case where $-SZ_a$ represents –SH;
  - thiol chemical groups in the case where $Z_a \neq H$;
  - maleimido or iodoacetamido chemical groups in the case where $-SZ_a$ represents –SH;
- in the presence of a cryptophycin derivative comprising a reactive chemical group of the type $-C(=O)-Z_bR_b$ in the linker L, the cryptophycin derivative is reacted with the amino functions of the binding agent, especially the ε-amino groups borne by the side chains of the lysine residues (Lys) of an antibody;
- in the presence of a cryptophycin derivative of formula (III) with G= $-(CH_2)_nY$, the binding agent comprises groups –SH when Y= -Cl or -maleimido, groups -C≡CH when Y= $-N_3$ or carboxylic acid groups when Y= -OH or $-NH_2$.

~~18. Process according to Claim 17, in which:~~

~~- in the presence of a cryptophycin derivative 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;~~

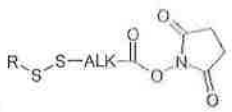
- thiol chemical groups in the case where RCG1 represents $-SZ_a$ with $Z_a \neq H$;
- maleimido or iodoacetamido chemical groups in the case where RCG1 represents –SH;

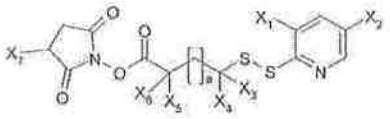
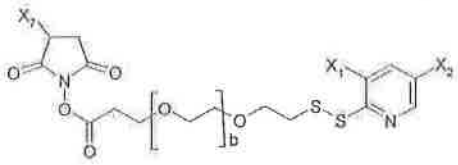
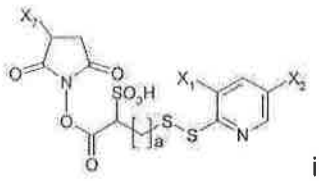
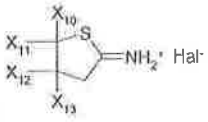
➢ in the presence of a cryptophycin derivative comprising a reactive chemical group RCG1 of the type $-C(=O)-Z_bR_b$, the cryptophycin derivative is reacted with the amino functions of the binding agent, especially the ε-amino groups borne by the side chains of the lysine residues (Lys) of an antibody;

➢ in the presence of a cryptophycin derivative of formula (III) with $G= -(CH_2)_nY$, the binding agent comprises groups –SH when Y= –Cl or –maleimido, groups –C≡CH when Y= $-N_3$ or carboxylic acid groups when Y= –OH or $-NH_2$;

➢ in the presence of a cryptophycin derivative comprising a reactive chemical group RCG1 of maleimido or haloacetamido type, the binding agent comprises thiol chemical groups.

~~19~~13. Process according to Claim ~~17~~ 12 ~~or 18~~, in which:

- in the presence of a cryptophycin derivative comprising a reactive chemical group ~~RCG1~~ of the type $-SZ_a$ in the linker L, the binding agent is modified with 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_3-C_7)heterocycloalkyl and ALK represents a group (C_1-C_6)alkylene;

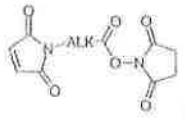
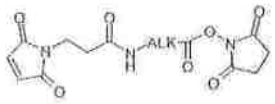
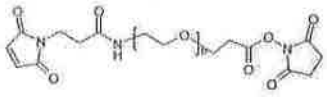
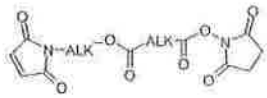
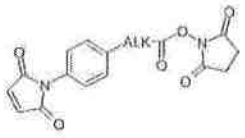
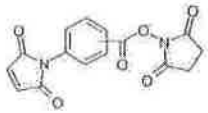
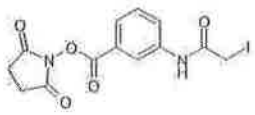
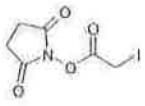
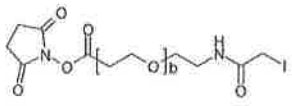
, a pegylated analogue of formula:  or a sulfonic analogue of formula  in which X_3, X_4, X_5 and X_6 represent H or a group (C_1-C_6)alkyl, X_1 and X_2 represent -H, $-CONX_8X_9$, $-NO_2$, X_8 and X_9 representing H or a group (C_1-C_6)alkyl, X_7 represents $-SO_3^-M^+$ or H or alternatively a quaternary ammonium group and a denotes an integer ranging from 0 to 4 and b denotes an integer ranging from 0 to 2000;  in which:

- Hal represents a halogen atom;
- X_{10} represents a halogen atom or a 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 that is unsubstituted or substituted with one to three substituents selected from amino, halogen atom, unsubstituted or halogenated (C_1-C_8)alkyl group, unsubstituted or halogenated (C_1-C_8)alkoxy;
- each of the X_{11}, X_{12} and X_{13} independently represents a hydrogen atom or may represent X_3;

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

or X_{10} and X_{11} form, together with X_{12}, a ring (C_1-C_5)alkylene, 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-C_8)alkyl;

- in the presence of a cryptophycin derivative comprising a reactive chemical group ~~RCG1~~ of the type —SH in the linker L, the binding agent is modified with a modifying agent chosen from succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate; sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate;  ;  ;  ;  ;  ;  ALK denoting a group (C_1-C_{12})alkylene and b being an integer between 0 and 2000;  ;  ; succinimidyl-N-bromoacetate; succinimidyl-3-(N-bromoacetamido)propionate;  , b being an integer between 0 and 2000.

~~20~~14. Conjugate that may be obtained via the process according to one of Claims ~~17~~ 12 to ~~19~~13.

~~21~~15. Conjugate according to Claim ~~20~~14, characterized by a DAR determined from the deconvolution of the HRMS spectrum of greater than 1, preferably between 2 and 10 and more particularly between 2 and 7.

~~22~~16. Conjugate according to Claim ~~20~~14, characterized by a DAR determined using a UV spectrophotometer of greater than 0.5, preferably greater than 1, more particularly between 1 and 10 and even more particularly between 2 and 7, the DAR being calculated by the equation:

$DAR = c_D / c_A$

in which:

$c_D = [(\varepsilon_{A\,\lambda1} \times A_{\lambda2}) - (\varepsilon_{A\,\lambda2} \times A_{\lambda1})] / [(\varepsilon_{D\,\lambda2} \times \varepsilon_{A\,\lambda1}) - (\varepsilon_{A\,\lambda2} \times \varepsilon_{D\,\lambda1})]$

$c_A = [A_{\lambda1} - (c_D \times \varepsilon_{D\,\lambda1})] / \varepsilon_{A\,\lambda1}$

and

$A_{\lambda 1}$ and $A_{\lambda 2}$ denoting, respectively, the absorbances of the conjugate solution, respectively, at the wavelengths λ1 and λ2;

$\varepsilon_{D\,\lambda 1}$ and $\varepsilon_{D\,\lambda 2}$ denoting, respectively, the molar absorption coefficients of the cryptophycin derivative before conjugation at the wavelengths λ1 and λ2, these coefficients being measured on the compounds of formula (II) of the type $-SZ_a$ with Z_a = -SMe or of the type $-C(=O)-Z_bR_b$ with Z_bR_b = -OMe or $-OCH_2-CH=CH_2$;

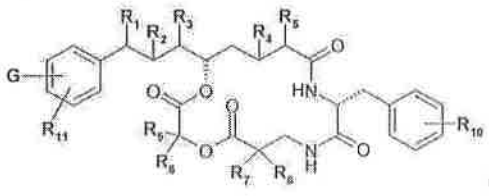
$\varepsilon_{A\,\lambda 1}$ and $\varepsilon_{A\,\lambda 2}$ denoting, respectively, the molar absorption coefficients of the naked antibody before conjugation at the two wavelengths λ1 and λ2.

~~23~~17. Conjugate according to Claim ~~22~~16, in which λ1= 280 nm and λ2 is chosen within the specific wavelength range 246 nm - 252 nm.

~~24~~18. Conjugate solution that may be obtained via the process according to one of Claims ~~17~~ 12 to ~~19~~ 13 or comprising the conjugate as defined in one of Claims ~~20~~ 14 to ~~23~~17.

~~25~~19. Use of a cryptophycin derivative according to one of Claims 1 to ~~13~~8, for the preparation of a binding agent to which is attached at least one of the said cryptophycin derivatives.

~~26~~20. Use of a cryptophycin derivative of formula (III):



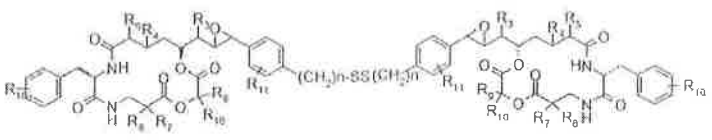
(III)

in which:

G represents a group $-CH=CH_2$ or $-(CH_2)_nY$, which is in an ortho (o), meta (m) or para (p) position on the phenyl nucleus bearing the unit CR_1, preferably in the para position;

Y represents –OH; -Cl; $-N_3$; $-NH_2$; -SH; -COOH; $-NR_{12}-CH_2-C\equiv CH$ in which $\mathbf{R_{12}}$ represents H or a group (C_1-C_6)alkyl, more particularly a methyl group; -OLG in which LG represents a leaving group; -OC(=O)-O-(4-nitrophenyl); maleimido,

or of formula:

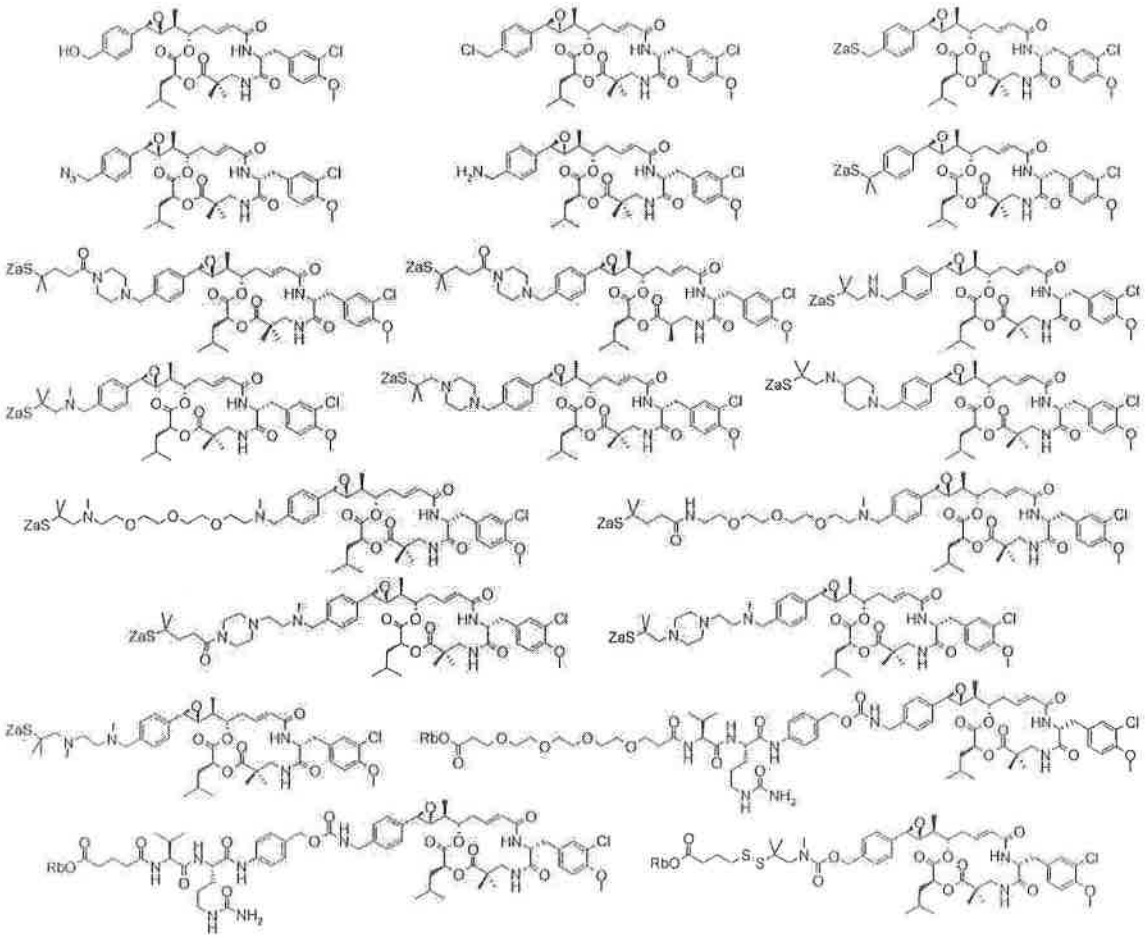


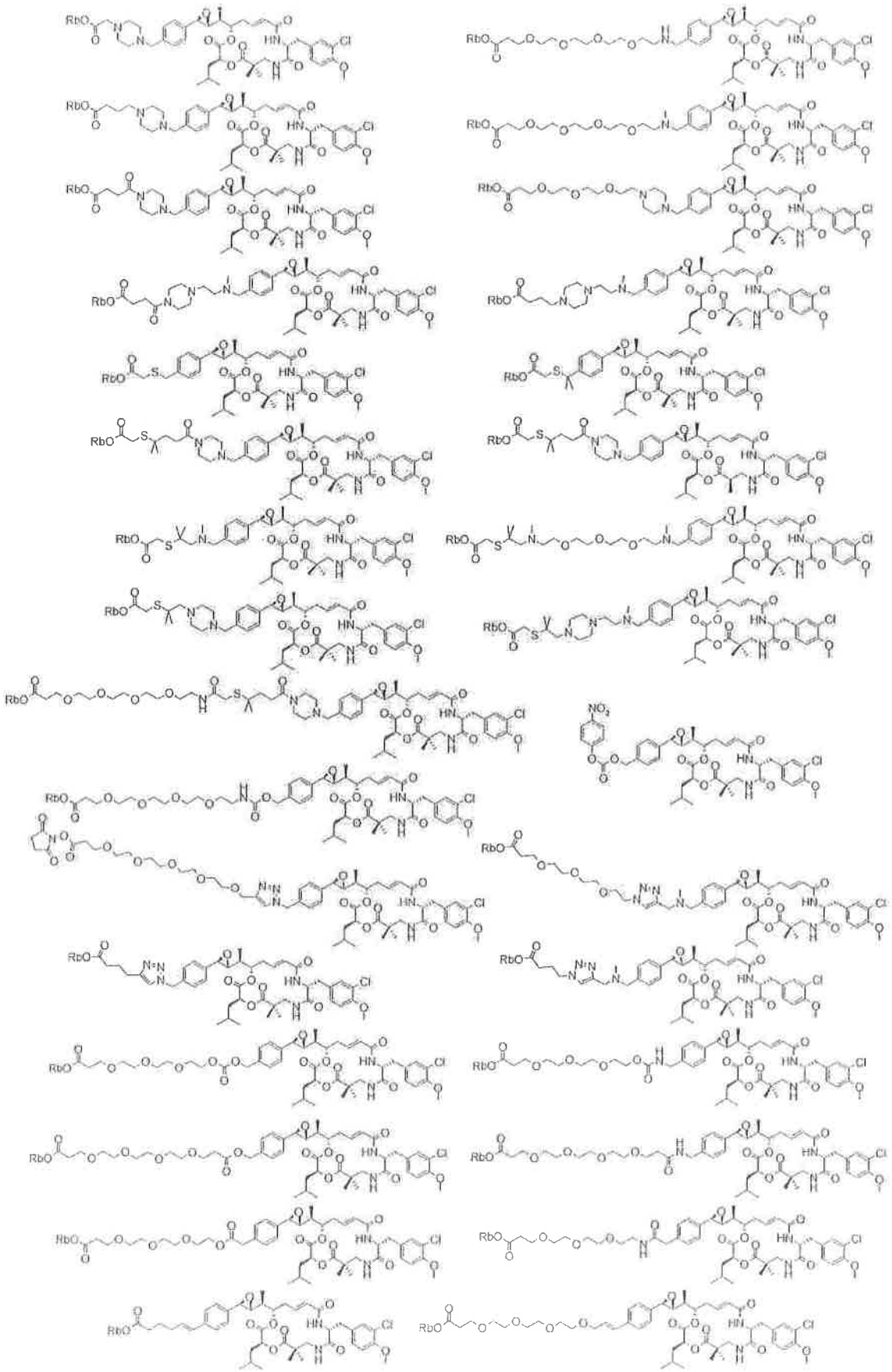
the groups $\mathbf{R_1}$ to $\mathbf{R_{11}}$ having the same meaning as in Claim 1, ~~11~~ 6 or ~~12~~ 7 and n being an

integer ranging from 1 to 6,

for the preparation of a binding agent to which is attached at least one of the said cryptophycin derivatives.

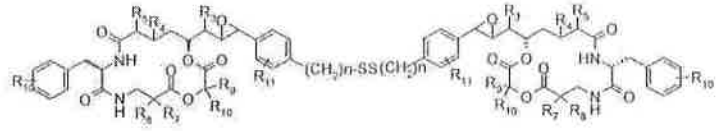
~~27~~21. Use of a cryptophycin derivative chosen from one of the following:





Z_a, Z_b and R_b being as defined in Claim 1 or ~~10~~5, for the preparation of a binding agent to which is attached at least one cryptophycin derivative.

~~28~~22. Cryptophycin derivative of formula:



the groups R_1 to R_{11} having the same meanings as in Claim 1, ~~11~~ 6 or ~~12~~ 7 and n being an integer ranging from 1 to 6.

~~29~~23. Cryptophycin derivative according to one of Claims 1 to ~~15~~10, for use as an anticancer agent.

~~30~~24. Conjugate according to Claims ~~20~~ 14 to ~~23~~17, for use as an anticancer agent.

~~31~~25. Conjugate solution according to Claim ~~24~~18, for use as an anticancer agent.

~~32~~26. Use of a cryptophycin derivative according to one of Claims 1 to ~~15~~10, for the preparation of an anticancer medicament.

~~33~~27. Use of a conjugate according to Claims ~~20~~ 14 to ~~23~~17, for the preparation of an anticancer medicament.

~~34~~28. Use of a conjugate solution according to Claim ~~24~~18, for the preparation of an anticancer medicament.