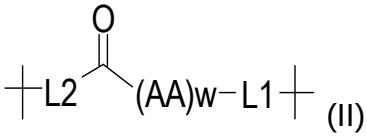
CLAIMS

1. Compounds of formula (I):

$$RCG1\text{-}L\text{-}P \quad (I)$$

wherein

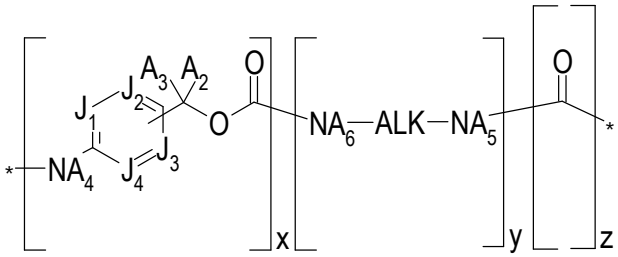
- **RCG1** represents a reactive chemical group that is reactive towards a chemical group present on a polypeptide such as an antibody;
- **P** represents a hydrogen atom, -OH or an activated O;
- **L** represents a linker of formula (II):



(II)

wherein:

- **L1** is of formula (III):



(III)

wherein:

- when P represents a hydrogen atom, then x = 0 or 1 and y = 1 and z = 0;
- when P represents -OH, then x = y = z = 0;
- when P represents an activated O, then x = 1 and y = z = 0, or x = y = z = 1;
- **J_1, J_2, J_3** and **J_4** are chosen, independently of each other, from CA_1 and N;
- **ALK** represents a (C_1-C_{12})alkylene group, for instance (C_1-C_6)alkylene, such as of the form -$(CH_2)_n$-, n being an integer ranging from 1 to 12 and for example ranging from 1 to 6;
- **A_1, A_2, A_3, A_4, A_5**, and **A_6** represent, independently of each other, a hydrogen atom or a (C_1-C_6)alkyl group, such as a hydrogen atom or a methyl group.

- **(AA)w** represents a sequence of w substituted **AA_s** or non-substituted amino acids **AA_{ns}** connected together *via* peptide bonds;
- **w** represents an integer ranging from 2 to 12, for instance from 2 to 6, such as 2 or 3;

• if **(AA)w** contains at least one substituted amino acid **AA_s**, then **L2** represents a (C_1-C_6)alkyl group, a C(=O)-(C_1-C_6)alkyl group, a (C_1-C_6)alkyl-NA_7-(C_1-C_6)alkyl group, or a C(=O)-(C_1-C_6)alkyl-NA_7-(C_1-C_6)alkyl group;

• if (**AA)w** represents a sequence of w non-substituted amino acids **AA_{ns}**, then **L2** represents a (C_1-C_6)alkyl-NA_7-(C_1-C_6)alkyl group;

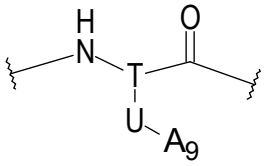
▪ **A_7** representing a straight or branched, saturated or unsaturated, C_1-C_{160} hydrocarbon chain wherein the C_1-C_{160} hydrocarbon is optionally substituted by 1 or more substituents independently selected from the group consisting of halogen, alkyl, NH_2, NH(alkyl), N(alkyl)$_2$, OH, and O(alkyl), and further wherein 1 or more methylene units of the C_1-C_{160} hydrocarbon are optionally and independently replaced by 1 or more atoms or groups independently selected from the group consisting of -NHC(=O)-, -N(alkyl)C(=O)-, -C(=O)NH-, -C(=O)N(alkyl)-, -OC(=O)-, -C(=O)O-, -OC(=O)O-, -CH(OH)-, -CH(SO_3H)- , -O-, -C(=O)-, -S(=O)-, -S(=O)$_2$-, -NHS(=O)$_2$-, -N(alkyl)S(=O)$_2$-, -S(=O)$_2$NH-, -S(=O)$_2$N(alkyl)-, -P(=O)(OH)-, -P(=O)(OH)O-, -O-P(=O)(OH)-, and -O-P(=O)(OH)-O-, or a heterocycloalkyl group, wherein the heterocycloalkyl is optionally substituted by 1 or more substituents independently selected from the group consisting of -OH, -Oalkyl, -alkyl, a halogen atom, -NH_2, -NHalkyl, and -N(alkyl)$_2$, being understood that each **A_7** comprising a SO_3H function can be under salt forms such as alkali metal salts, for instance sodium salts (SO_3^- $^+$Na);

▪ **A_8** representing a hydrogen atom or a (C_1-C_6)alkyl group, for instance a hydrogen atom or a methyl group;

▪ **i** representing an integer ranging from 1 to 50, for instance ranging from 1 to 35;

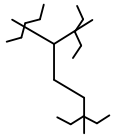
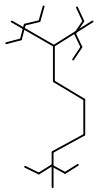
- each (AA_{ns}) is a natural or non-natural amino acid independently selected from the group consisting of alanine, γ-aminobutyric acid, 2-amino-2-cyclohexylacetic acid, 2-amino-2-phenylacetic acid, arginine, asparagine, aspartic acid, citrulline, cysteine, α,α-dimethyl-γ-aminobutyric acid, 3,3-dimethyl-γ-aminobutyric acid, glutamine, glutamic acid, glycine, homocysteine, selenocysteine, homoselenocysteine, histidine, isoleucine, leucine, lysine, ε-(acetyl)-lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine

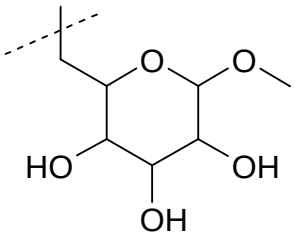
- each (**AA_s**) is independently of formula (VI):



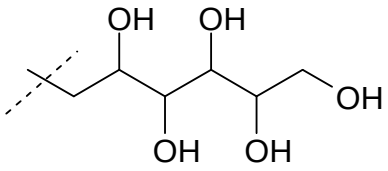
(VI)

wherein:

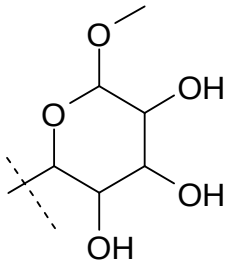
- **T** represents a saturated or unsaturated, linear or branched, (C_1-C_8) trivalent alkyl group, preferably T is  , or  ;
- **U** group represents a single bond, -NHC(=O)-, -N(alkyl)C(=O)-, -C(=O)NH-, -C(=O)N(alkyl)-, -NHC(=O)NH-, -NHC(=NH)NH-, -OC(=O)-, -C(=O)O-, -OC(=O)O-, -S-, -Se-, -O-, -NH-, -N(alkyl)-, -C(=O)-, -OP(=O)-, -S(=O)-, -$S(=O)_2$-, -$NHS(=O)_2$-, -$N(alkyl)S(=O)_2$-, -$S(=O)_2NH$-, -$S(=O)_2N(alkyl)$-, -P(=O)(OH)-, -P(=O)(OH)O-, -O-P(=O)(OH)-, or -O-P(=O)(OH)-O, such as **U** group represents -NH-C(=O)-, or -C(=O)NH-;
- **A_9** represents a straight or branched, saturated or unsaturated, C_1-C_{160} hydrocarbon chain, wherein the C_1-C_{160} hydrocarbon is optionally substituted by 1 or more substituents independently selected from the group consisting of halogen, alkyl, NH_2, NH(alkyl), $N(alkyl)_2$, OH, and O(alkyl), and further wherein one or more methylene unit of the C_1-C_{160} hydrocarbon are optionally and independently replaced by 1 or more atoms or groups independently selected from -NH-C(=O)-, -N(alkyl)C(=O)-, -C(=O)NH-, -C(=O)N(alkyl)-, -NHC(=O)NH-, -NHC(=NH)NH-, -OC(=O)-, -C(=O)O-, -OC(=O)O-, -CH(OH)-, -$CH(SO_3H)$-, -CH(Oalkyl)-, -CHF-, -CF_2-, -S-, -Se-, -O-, -NH-, -N(alkyl)-, -$N^+H(alkyl)$-, -$N^+(alkyl)_2$-, -C(=O)-, -OP(=O)-, -S(=O)-, -$S(=O)_2$-, -$NHS(=O)_2$-, -$N(alkyl)S(=O)_2$-, -$S(=O)_2NH$-, -$S(=O)_2N(alkyl)$-, -P(=O)(OH)-, -P(=O)(OH)O-, -O-P(=O)(OH)-, and -O-P(=O)(OH)-O-, or a heterocycloalkyl group, wherein the heterocycloalkyl is optionally substituted by one or more substituents independently selected from the group consisting of -OH, -Oalkyl, -alkyl, a halogen atom, -NH_2, -NHalkyl, and -$N(alkyl)_2$, for instance **A_9** is –



$[(CH_2)_2\text{-}O]_4\text{-}CH_3$, $\text{-}[(CH_2)_2\text{-}O]_{24}\text{-}CH_3$, ,



,



,

or $\text{-}[(CH_2)_2\text{-}O)]_4\text{-}(CH_2)_2\text{-}C(=O)\text{-}NH\text{-}(CH_2)_2\text{-}SO_3H$;

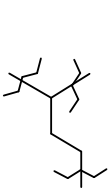
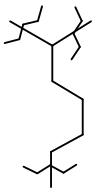
being understood that each **A_9** comprising a SO_3H function can be under salt forms such as alkali metal salts, for instance sodium salts ($SO_3^- {}^+Na$),

with the proviso that:

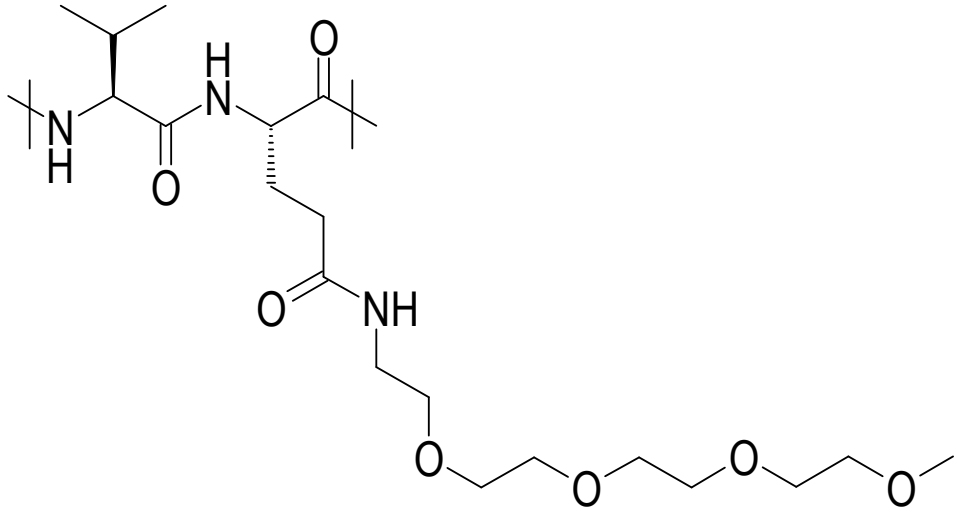
the substituted amino acid, $(AA)_s$, of formula (VI) is not selected from the group consisting of alanine, γ-aminobutyric acid, 2-amino-2-cyclohexylacetic acid, 2-amino-2-phenylacetic acid, arginine, asparagine, aspartic acid, citrulline, cysteine, α,α-dimethyl-γ-aminobutyric acid, 3,3-dimethyl-γ-aminobutyric acid, glutamine, glutamic acid, glycine, homocysteine, selenocysteine, homoselenocysteine, histidine, isoleucine, leucine, lysine, ε-(acetyl)-lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

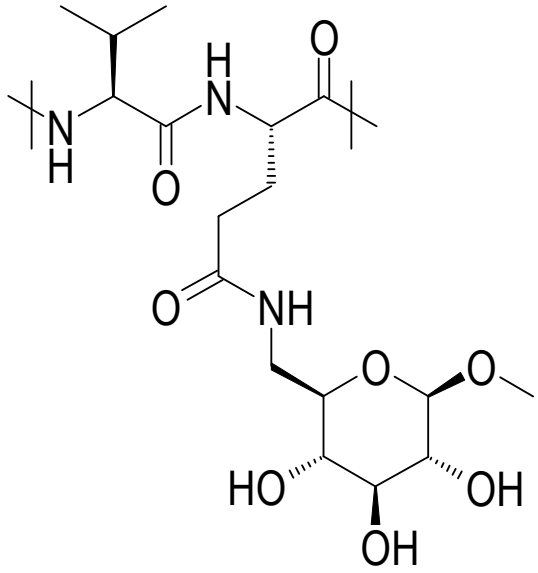
2. Compounds of formula (I) according to claim 1, wherein said sequence **(AA)w** of non-substituted amino acids **AA_{ns}** is selected from the following list: Gly-Gly, Phe-Lys, Val-Lys, Val-AcLys, Val-Cit, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Ala-Lys, Val-Ala, Phe-Cit, Phe-Gly, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Ala-Phe, Gly-Gly-Gly, Gly-Ala-Phe, Gly-Phe-Gly, Gly-Val-Cit, Gly-Phe-Leu-Cit, Gly-Phe-Leu-Gly, and Ala-Leu-Ala-Leu, such as Val-Ala and Val-Cit, for instance Val-Ala.

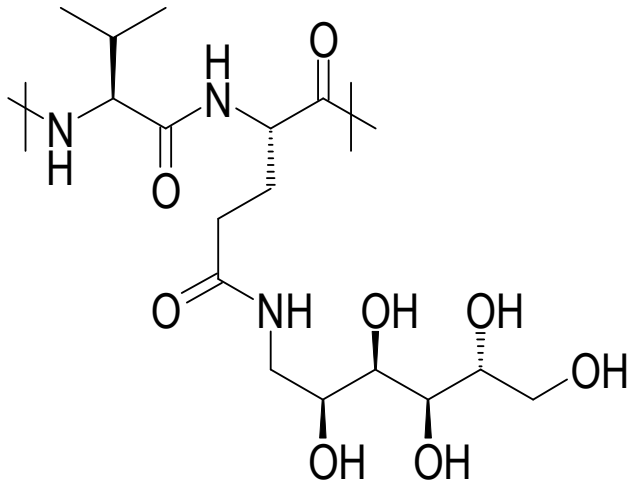
3. Compounds of formula (I) according to claim 1, wherein the substituted amino acids **AA_s** have the formula (VI) as defined in claim 1 in which;

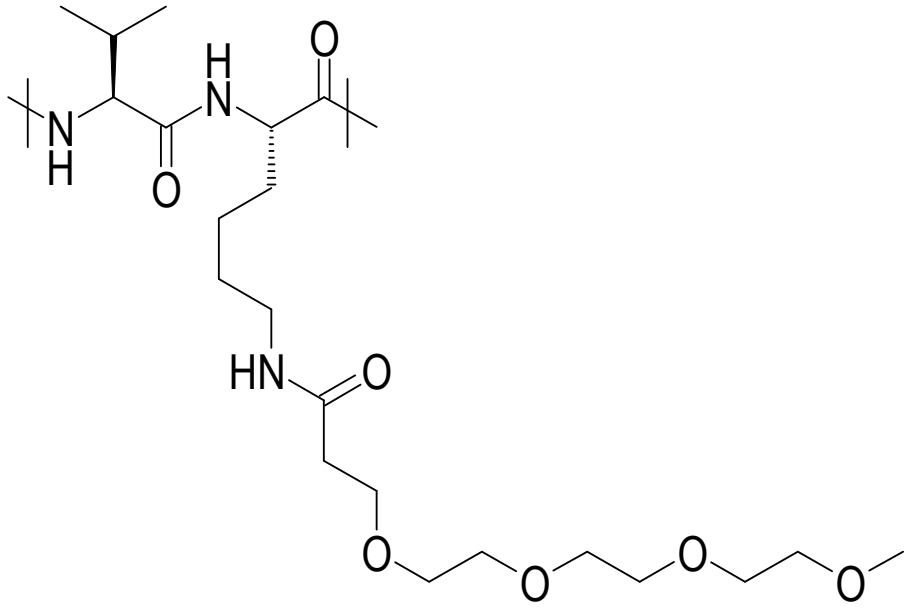
- **T** represents  , or  ;
- **U** group represents -NH-C(=O)-, or -C(=O)NH-;
- **A_9** represents $–[(CH_2)_2\text{-}O]_b\text{-}CH_3$ wherein b represents an integer ranging from 1 to 50, for instance ranging from 1 to 24, such as 4, 7 and 24, for instance 4 and 24.

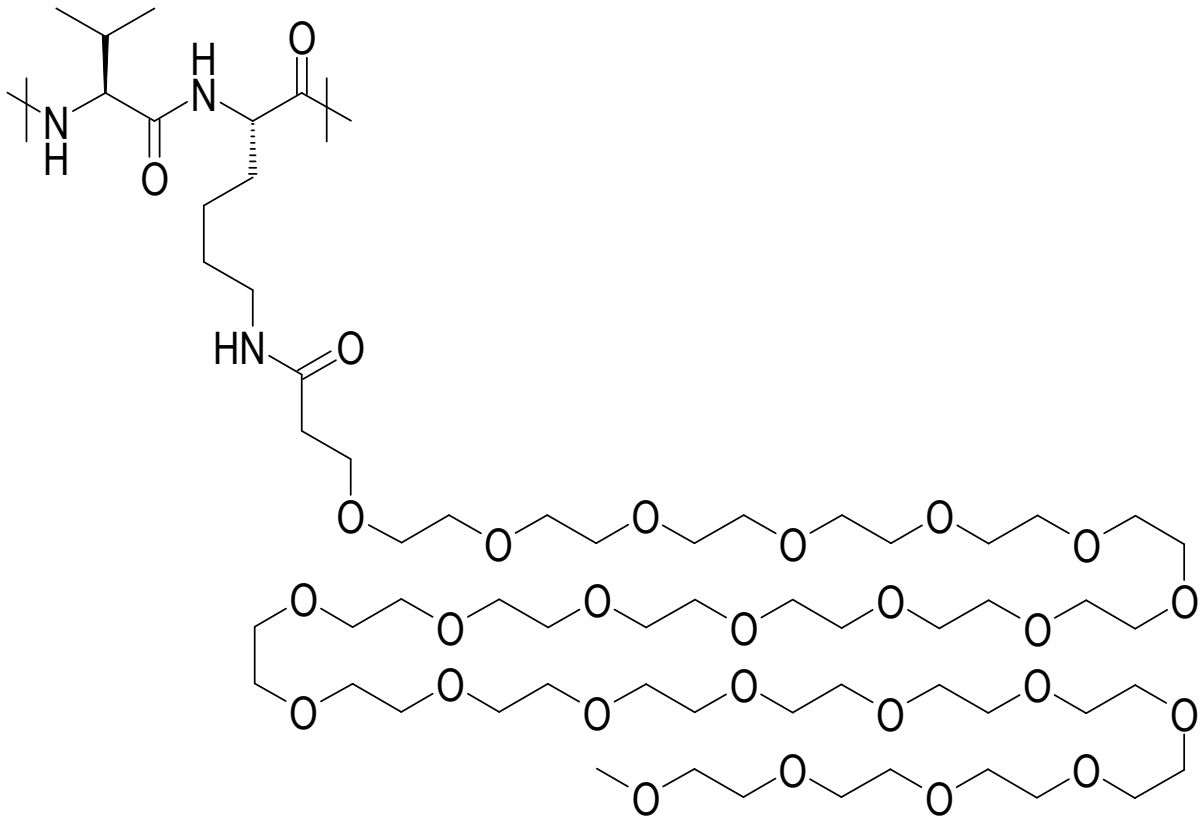
4. Compounds of formula (I) according to claim 1, wherein the sequence **$(AA_S)w$** containing at least one substituted amino acid **AA_S**, is selected from the list:

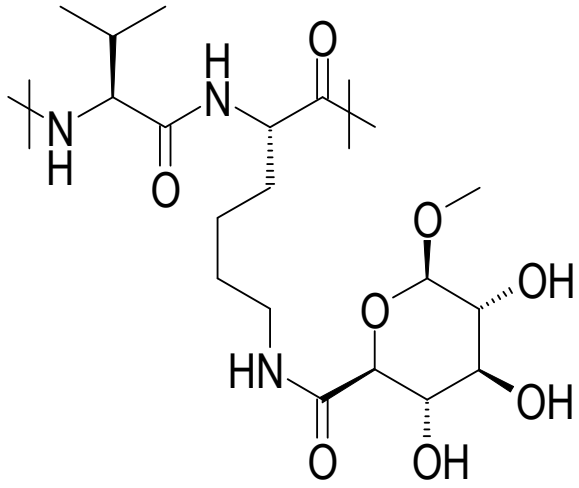




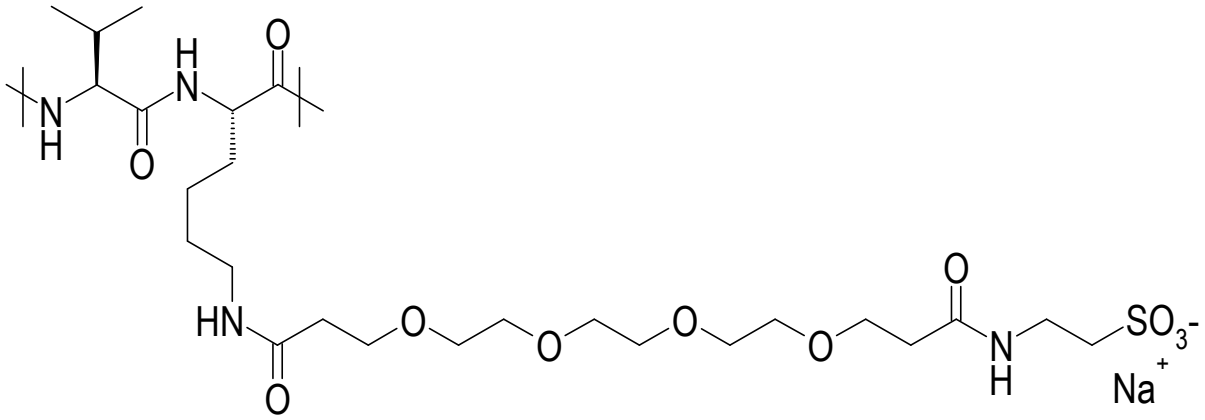








or



5. Compounds of formula (I) according to claim 1, wherein **(AA)w** contains at least one substituted amino acid $\mathbf{AA_s}$ and **L2** represents:

- a -(C_1-C_6)alkyl- group, such as a –$(CH_2)_3$- group;
- a -C(=O)-(C_1-C_6)alkyl- group, such as a -C(=O)-$(CH_2)_3$- group; or
- a -(C_1-C_6)alkyl-NA_7-(C_1-C_6)alkyl- group, such as a -$(CH_2)_2$-NA_7-$(CH_2)_2$- group

in which $\mathbf{A_7}$ is a -C(=O)-$(CH_2)_2$-C(=O)-NH-$(CH_2)_2$-SO_3H group;

being understood that each $\mathbf{A_7}$ comprising a SO_3H function can be under salt forms such as alkali metal salts, for instance sodium salts (SO_3^- $^+$Na).

6. Compounds of formula (I) according to claim 1, wherein **(AA)w** contains w non-substituted amino acid $\mathbf{AA_{ns}}$ and **L2** represents :

a -(C_1-C_6)alkyl-NA_7-(C_1-C_6)alkyl- group, such as a -$(CH_2)_2$-NA_7-$(CH_2)_2$- group in which $\mathbf{A_7}$ represents:

- a –C(=O)-$[(CH_2)_2$-$O]_a$-CH_3 group wherein **“a”** represents an integer ranging

from 1 to 50, such as ranging from 1 to 24, for instance 4, 7 and 24, such as **A_7** is a -C(=O)-[$(CH_2)_2$-O]$_4$-CH_3 group, a -C(=O)-[$(CH_2)_2$-O]$_7$-CH_3 group, or a -C(=O)-[$(CH_2)_2$-O]$_{24}$-CH_3 group;

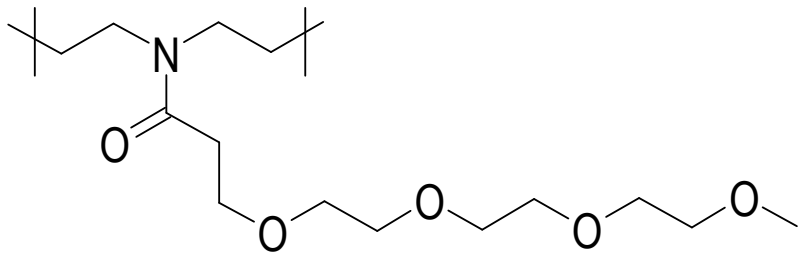
-a -C(=O)-$(CH_2)_2$-C(=O)-NH-$(CH_2)_2$-SO_3H group; or

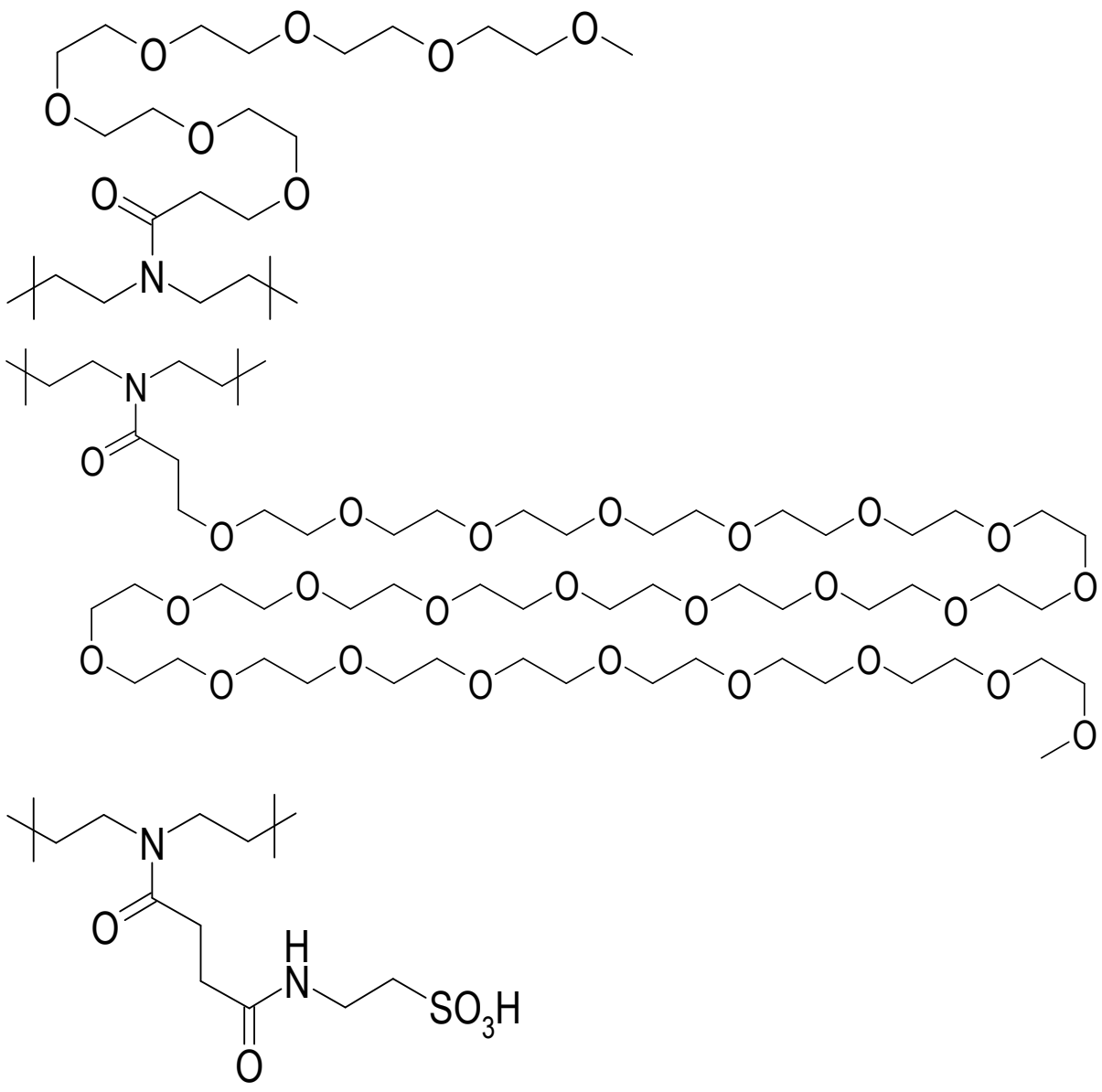
-a -C(=O)-$(CH_2)_2$-C(=O)-NH-[$(CH_2)_2$-O]$_a$-$(CH_2)_2$-C(=O)-NH-$(CH_2)_2$-SO_3H group wherein **"a"** represents an integer ranging from 1 to 50, such as ranging from 1 to 24, for instance 4, such as **A_7** is -C(=O)-$(CH_2)_2$-C(=O)-NH-[$(CH_2)_2$-O]$_4$-$(CH_2)_2$-C(=O)-NH-$(CH_2)_2$-SO_3H group;
being understood that each **A_7** comprising a SO_3H function can be under salt forms such as alkali metal salts, for instance sodium salts (SO_3^- $^+$Na).

7. Compounds of formula (I) according to claim 1, wherein **A_7** represents
a -C(=O)-[$(CH_2)_2$-O]$_4$-CH_3 group; a -C(=O)-[$(CH_2)_2$-O]$_7$-CH_3 group,
a -C(=O)-[$(CH_2)_2$-O]$_{24}$-CH_3 group; a -C(=O)-$(CH_2)_2$-C(=O)-NH-$(CH_2)_2$-SO_3H group;
a -C(=O)-$(CH_2)_2$-C(=O)-NH-[$(CH_2)_2$-O-]$_4$-$(CH_2)_2$-C(=O)-NH-$(CH_2)_2$-SO_3H group;
being understood that each **A_7** comprising a SO_3H function can be under salt forms such as alkali metal salts, for instance sodium salts (SO_3^- $^+$Na).

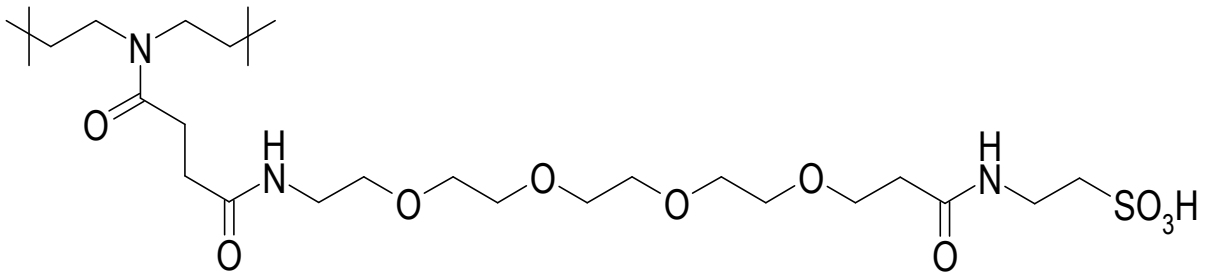
8. Compounds of formula (I) according to claim 1, wherein **(AA)w** contains at least one substituted amino acid **AA_s** and/or w non-substituted amino acid **AA_{ns}** and **L2** represents:
a (C_1-C_6)alkyl-NA_7-(C_1-C_6)alkyl group, or a C(=O)-(C_1-C_6)alkyl-NA_7-(C_1-C_6)alkyl group.

9. Compounds of formula (I) according to claim 1, wherein **(AA)w** contains at least one substituted amino acid **AA_s** and/or w non-substituted amino acid **AA_{ns}** and **L2** represents :





or

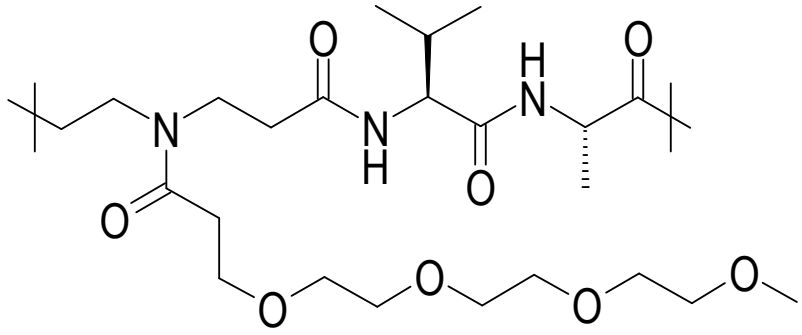


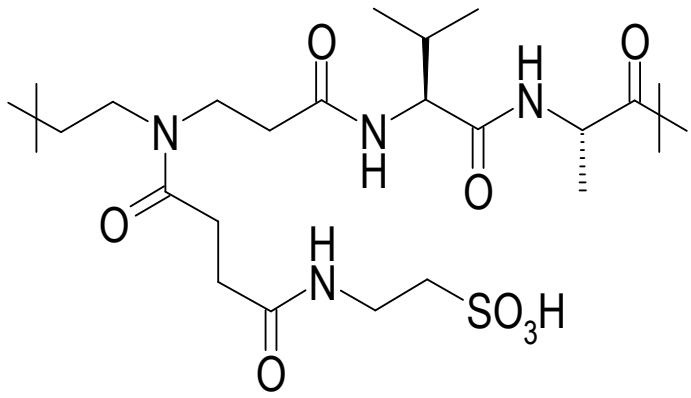
10. Compounds of formula (I) according to claim 1, wherein **(AA)w** contains at least one substituted amino acid $\mathbf{AA_s}$ and/or w non-substituted amino acid $\mathbf{AA_{ns}}$ and **L2** comprises a $\mathbf{A_7}$ representing :

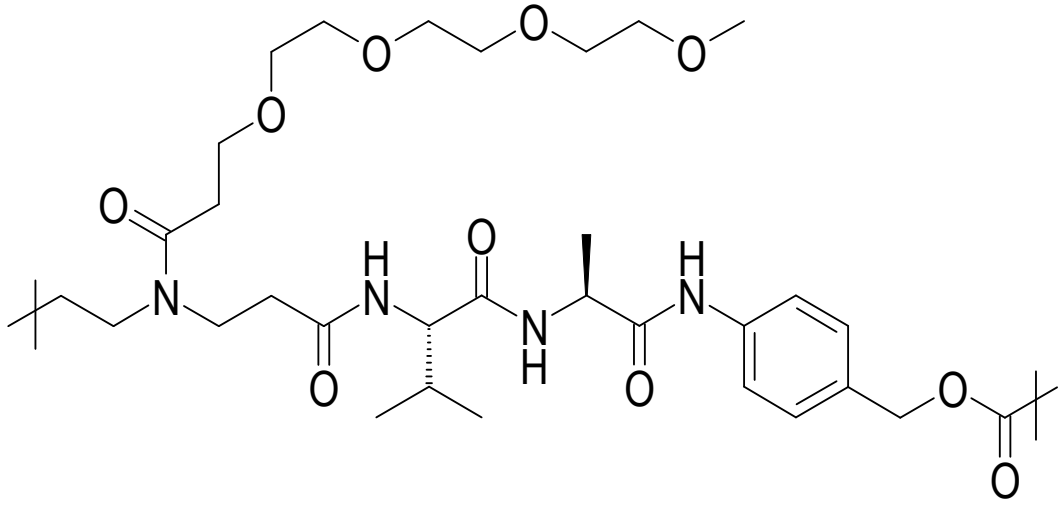
a -C(=O)-$[(CH_2)_2$-O$]_4$-CH_3 group; a -C(=O)-$[(CH_2)_2$-O$]_7$-CH_3 group; a -C(=O)-$[(CH_2)_2$-O$]_{24}$-CH_3 group; a -C(=O)-$(CH_2)_2$-C(=O)-NH-$(CH_2)_2$-SO_3H group; a -

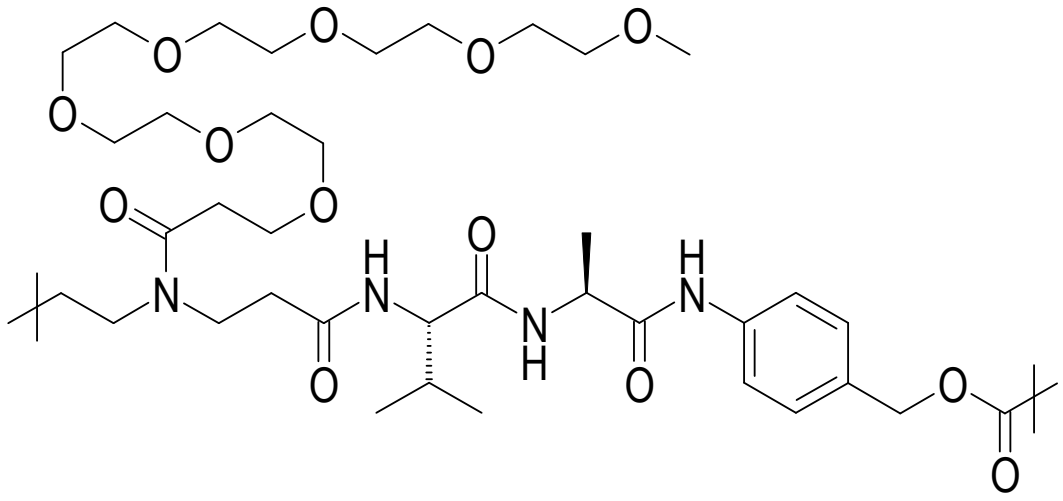
C(=O)-$(CH_2)_2$-C(=O)-NH-[$(CH_2)_2$-O]$_4$-$(CH_2)_2$-C(=O)-NH-$(CH_2)_2$-SO_3H group; being understood that each **A_7** comprising a SO_3H function can be under salt forms such as alkali metal salts, for instance sodium salts (SO_3^- $^+$Na).

11. Compounds of formula (I) according to anyone of claims 1 to 10, wherein said linker **L** is selected from the following list:

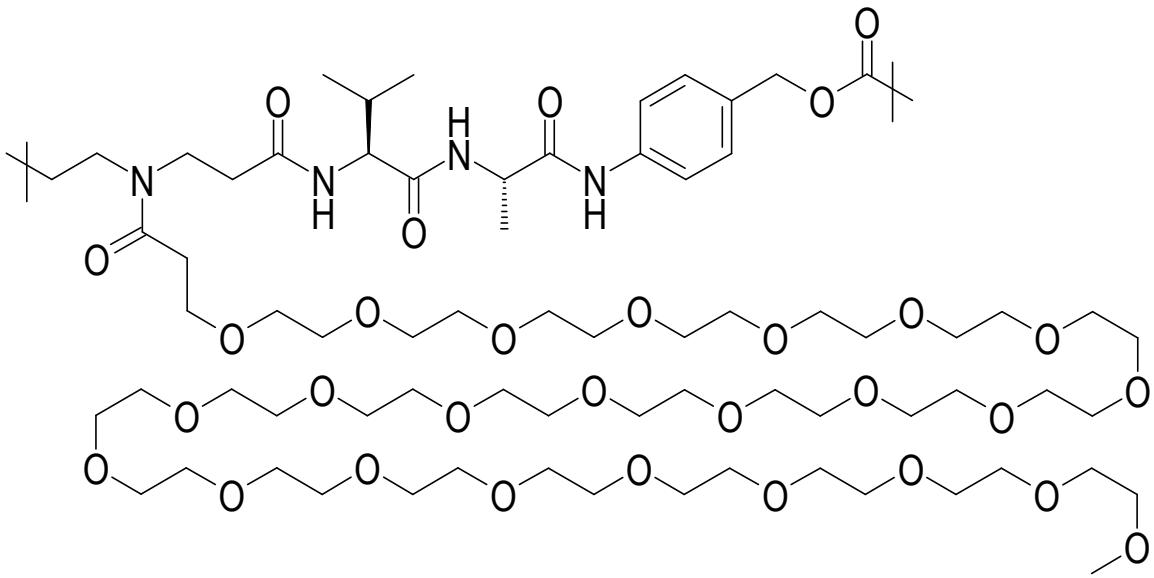

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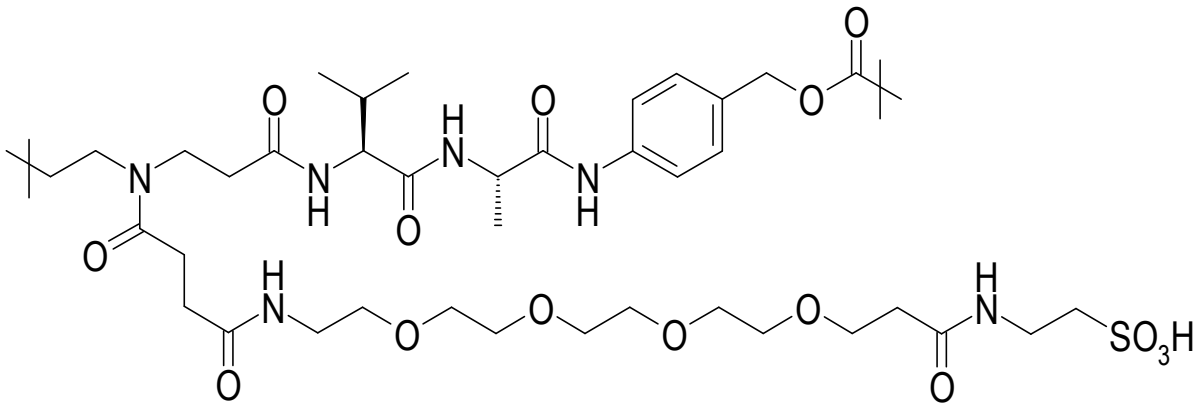

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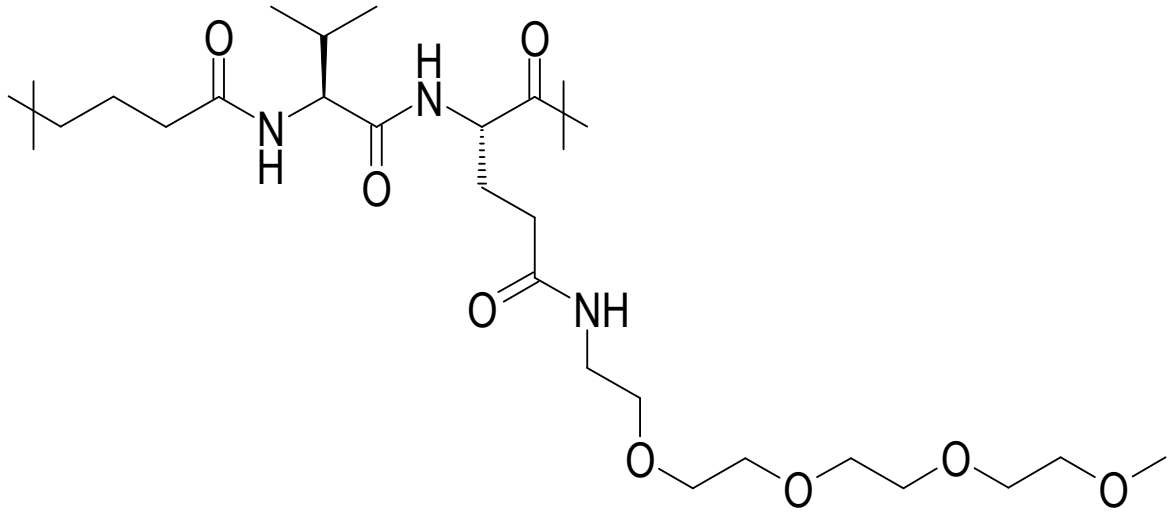
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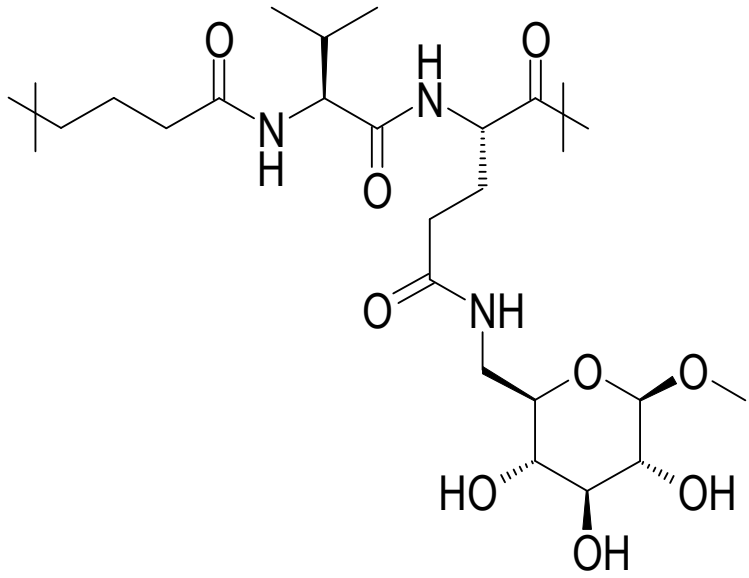
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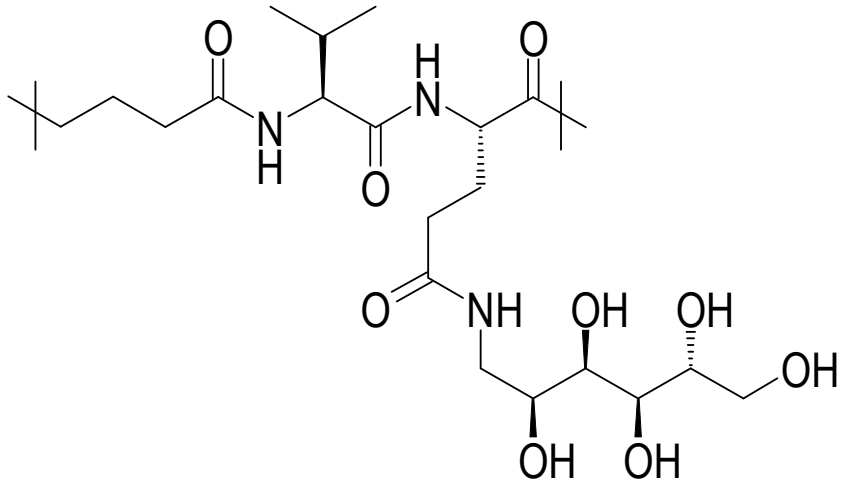
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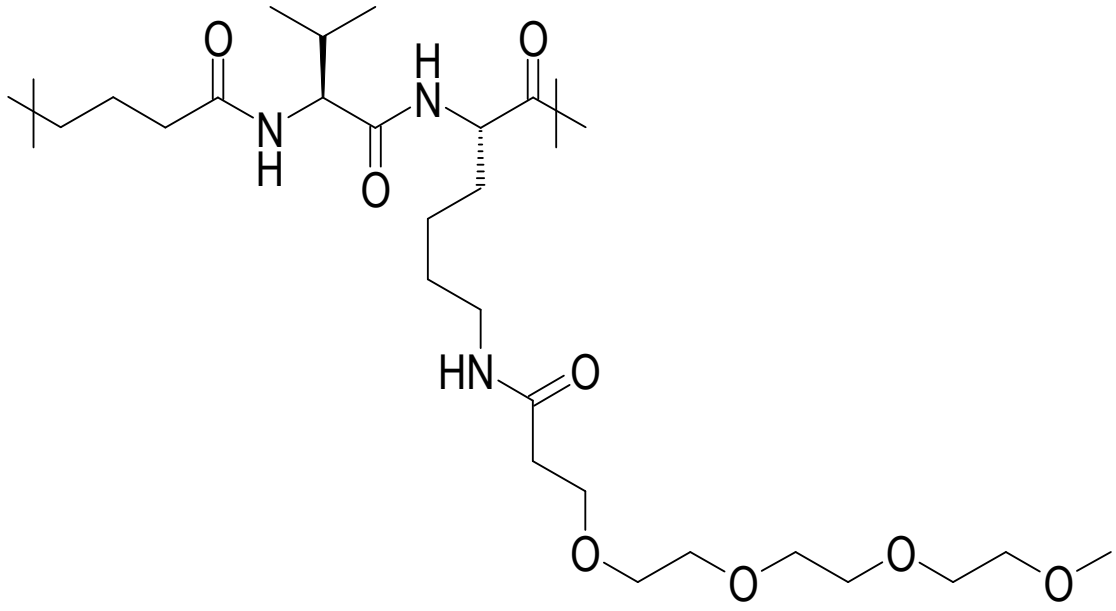
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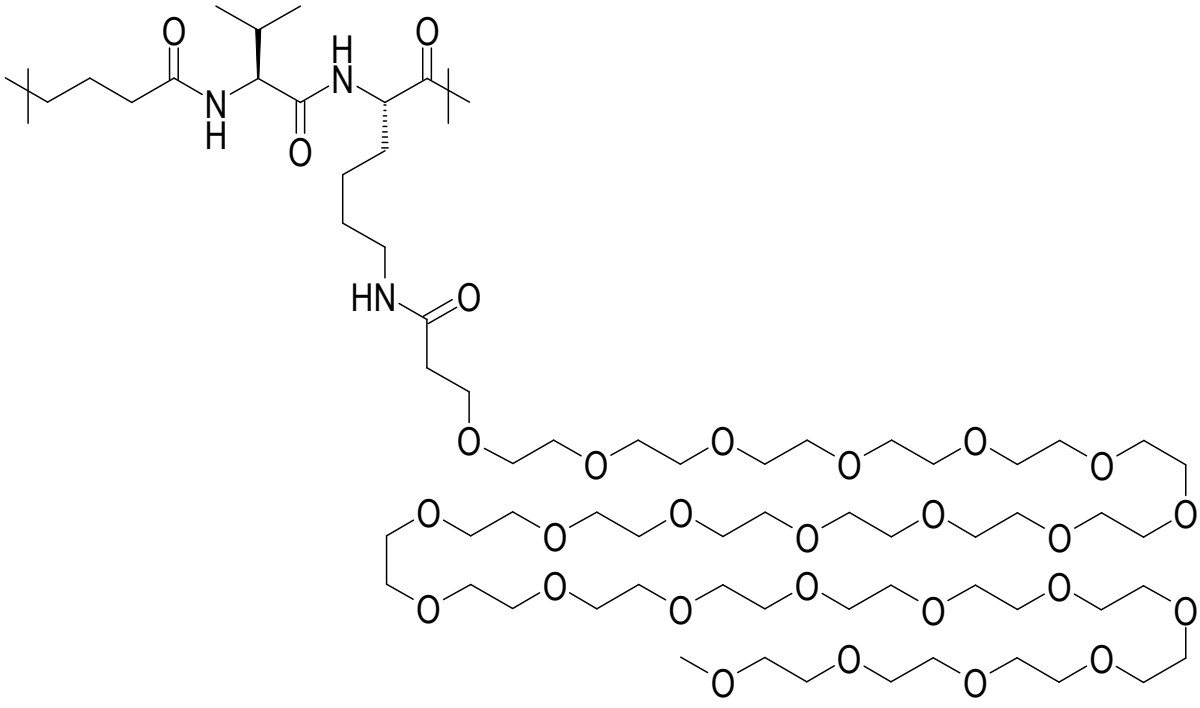
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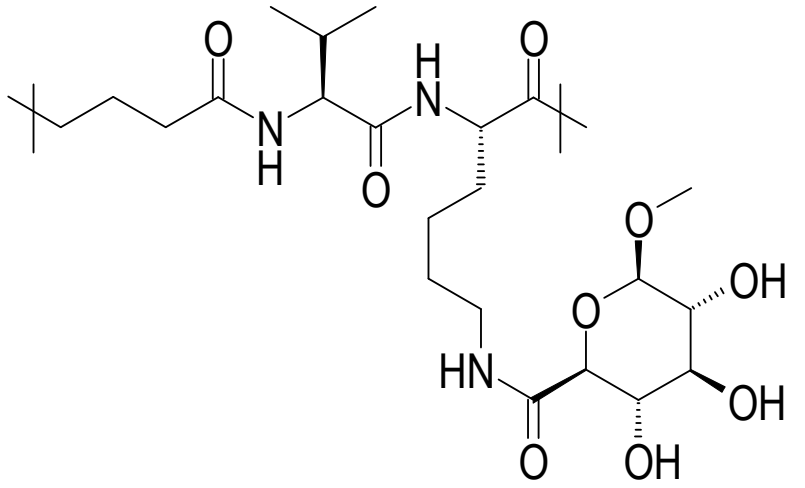
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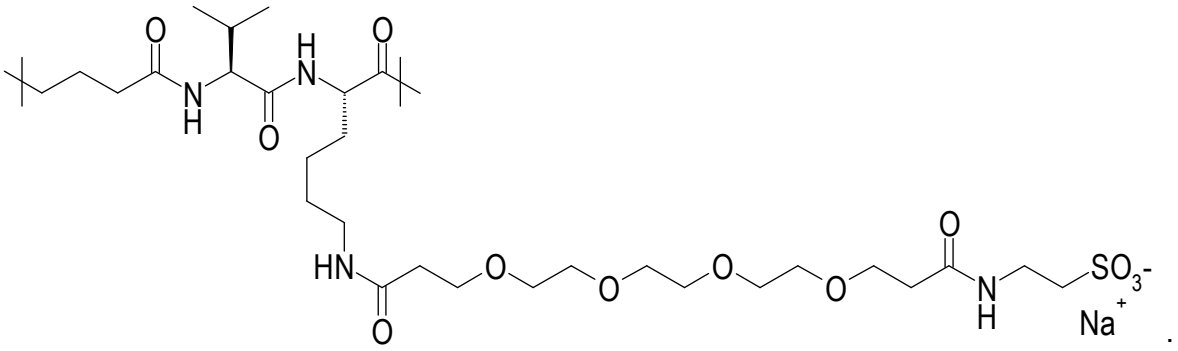
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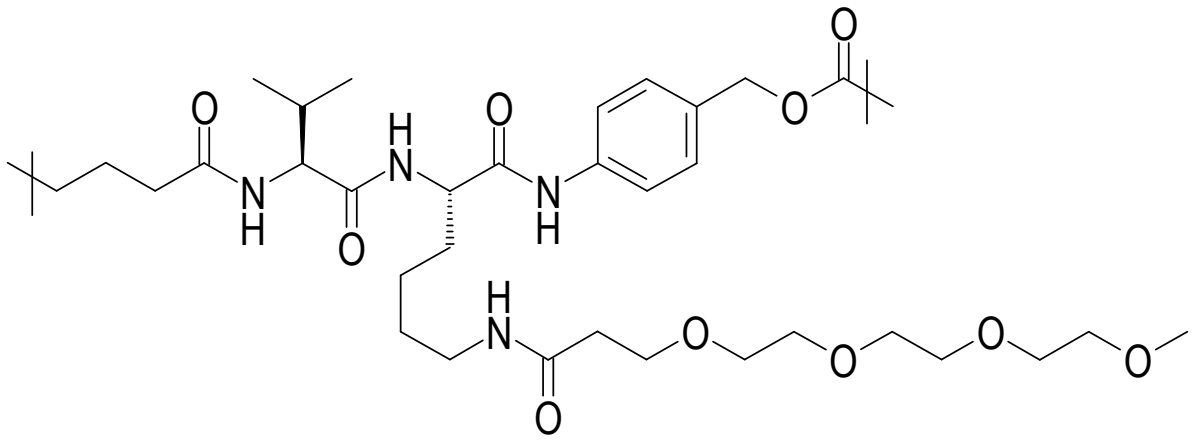
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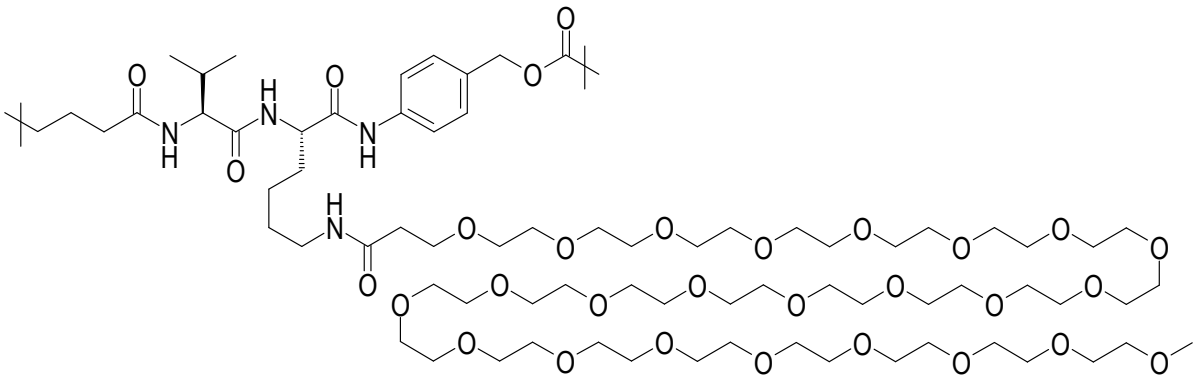
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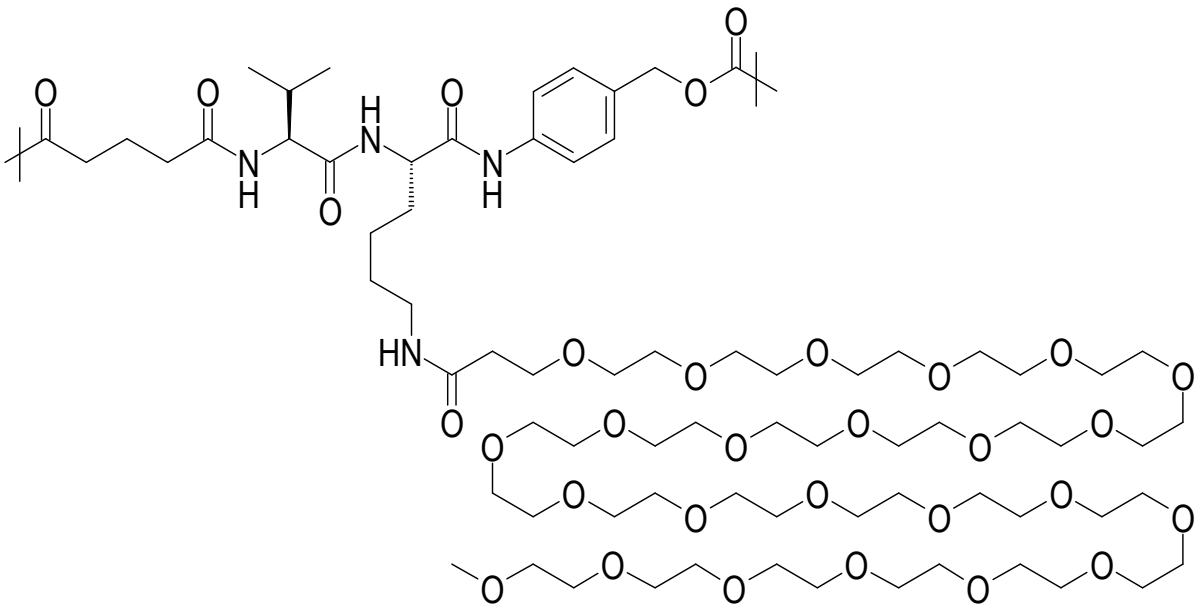
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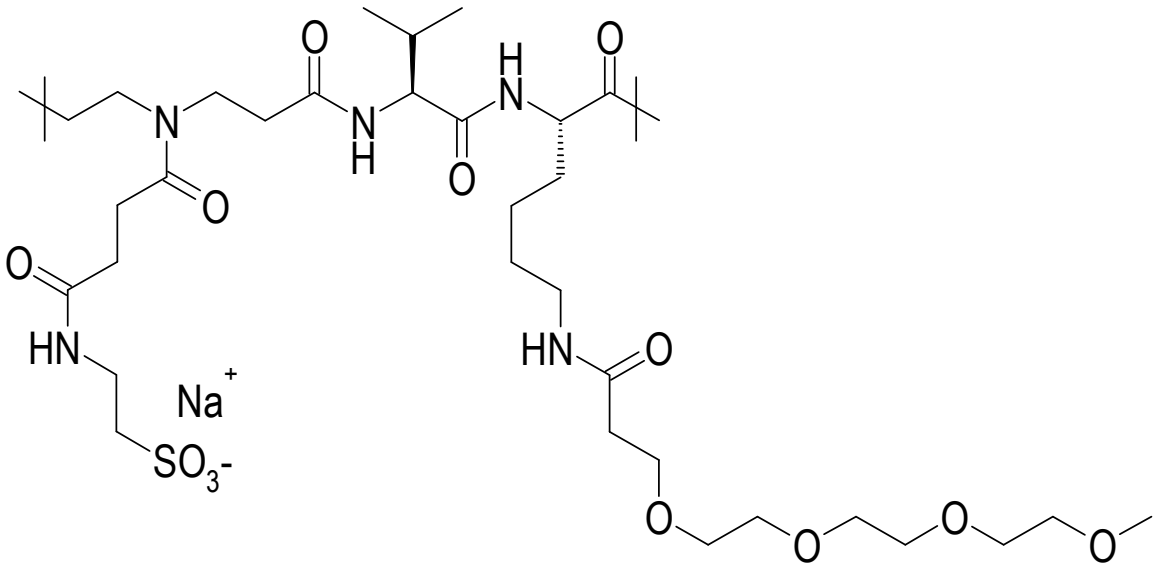
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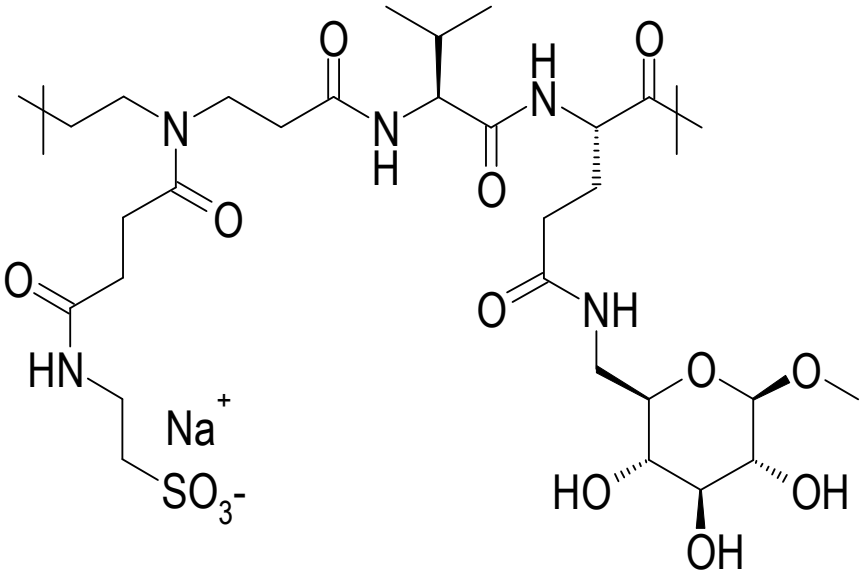
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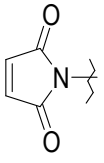
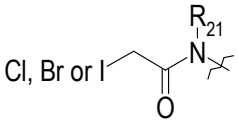
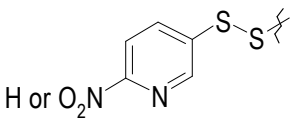


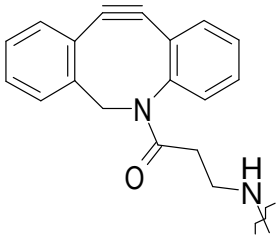
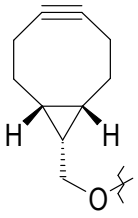
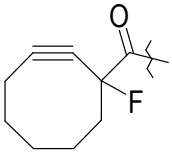
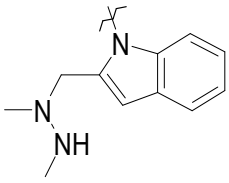
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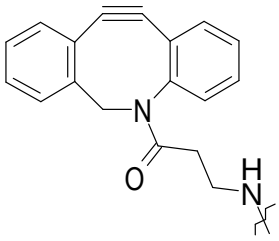
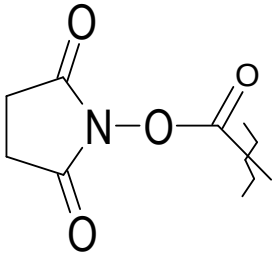
12. Compounds of formula (I) according to anyone of claims 1 to 11, wherein **RCG1** is selected from the following list:

(i) a R_aZ_a-C(=O) reactive group for which $\mathbf{Z_a}$ represents a single bond, -O- or -NH-, for instance -O-, and $\mathbf{R}_a$ represents a hydrogen atom, a (C_1-C_6)alkyl group, a (C_3-C_7)cycloalkyl group, an alkenyl group, an aryl group, a heteroaryl group or a (C_3-C_7)heterocycloalkyl group, the aryl group, the heteroaryl group and/or the (C_3-C_7)heterocycloalkyl group being optionally substituted by 1 to 5 atoms/groups chosen from a halogen atom, for instance a fluorine atom, an alkyl group, an alkoxy group, a hydroxyl group, an oxo group, a nitro group and a cyano group;

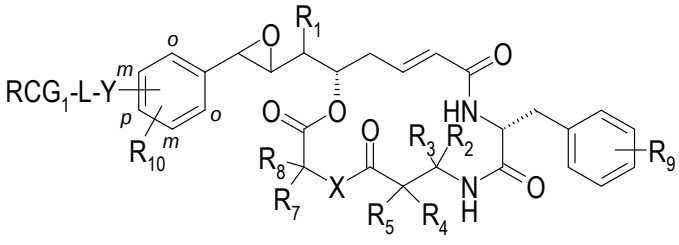
(ii) one of the following reactive groups: a maleimido  group; a haloacetamido  group with $\mathbf{R_{21}}$ representing a hydrogen atom or a (C_1-C_6)alkyl group, such as Me; Cl-; N_3-; HO-; HS-; an activated disulfide such as  ; H_2N-; HC≡C- group or an activated C≡C group such as a

cyclooctyne moiety for instance a DBCO-amine  or BCN  or MFCO  ; an O-alkyl hydroxylamine or a Pictet-Spengler reaction substrate such as  ;

for instance **RCG1** is selected from the following list:

 -COOH, and  .

13. Cryptophycin payloads of formula (IV):



(IV)

wherein:

- **R_1** represents a (C_1-C_6)alkyl group;
- **R_2** and **R_3** represent, independently of each other, a hydrogen atom or a (C_1-C_6)alkyl group;

  or alternatively **R_2** and **R_3** form together with the carbon atom to which they are attached a (C_3-C_6)cycloalkyl or a (C_3-C_6)heterocycloalkyl group;

▪ **R_4** and **R_5** represent, independently of each other, a hydrogen atom or a (C_1-C_6)alkyl group or a (C_1-C_6)alkyl-NH(R_{12}) group or a (C_1-C_6)alkyl-OH group or a (C_1-C_6)alkyl-SH group or a (C_1-C_6)alkyl-CO_2H group;
or alternatively **R_4** and **R_5** form together with the carbon atom to which they are attached a (C_3-C_6)cycloalkyl or a (C_3-C_6)heterocycloalkyl group;

▪ **X** represents O or N(R_6);

▪ **R_6** represents a hydrogen atom or a (C_1-C_6)alkyl group;

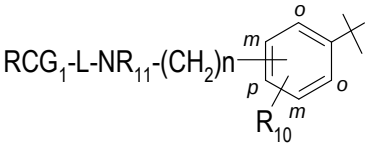
▪ **R_7** and **R_8** represent, independently of each other, a hydrogen atom or a (C_1-C_6)alkyl group or a (C_1-C_6)alkyl-CO_2H group or a (C_1-C_6)alkyl-N(C_1-C_6)alkyl$_2$ group;
or alternatively **R_7** and **R_8** form together with the carbon atom to which they are attached a (C_3-C_6)cycloalkyl group or a (C_3-C_6)heterocycloalkyl group;

▪ **R_9** represents at least one substituent of the phenyl nucleus chosen, independently of each other, from: a hydrogen atom, -OH, (C_1-C_4)alkoxy, a halogen atom, -NH_2, -NH(C_1-C_6)alkyl or -N(C_1-C_6)alkyl$_2$ or -NH(C_1-C_6)cycloalkyl or (C_3-C_6)heterocycloalkyl group;

▪ **R_{10}** represents at least one substituent of the phenyl nucleus chosen from a hydrogen atom and a (C_1-C_4)alkyl group;

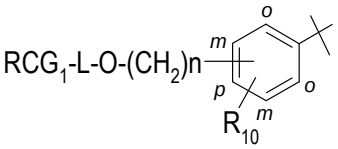
▪ **Y** represents

- -NR_{11}-(C_1-C_6)alkyl-, such as -NR_{11}-(CH_2)$_n$- like



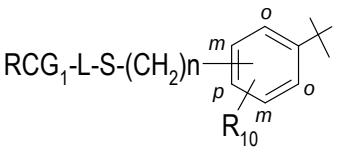
;

- -O-(C_1-C_6)alkyl-, such as -O-(CH_2)$_n$- like



;

- -S-(C_1-C_6)alkyl-, such as -S-(CH_2)$_n$- like



;

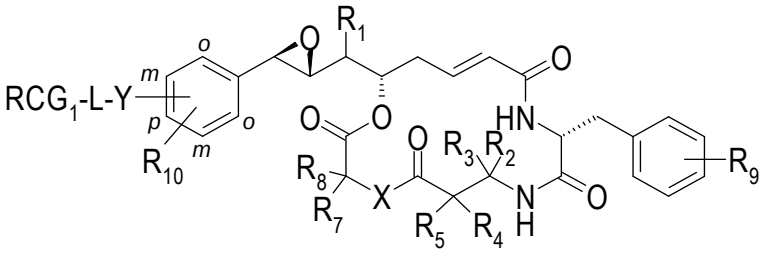
Y being positioned in an *ortho* (*o*), *meta* (*m*) or *para* (*p*) position of the phenyl nucleus;

▪ **R_{11}** and **R_{12}** represent, independently of each other, a hydrogen atom or (C_1-

C_6)alkyl, such as a hydrogen atom or a methyl group;

- **n** represents an integer ranging from 1 to 5;
- **L** is defined as in formula (I) according to claim 1 and represents a linker of formula (II) as defined in any one of claims 1 to 11;
- **RCG1** represents a reactive chemical group present at the end of the linker **L**, **RCG1** being reactive towards a chemical group present on a polypeptide such as an antibody.

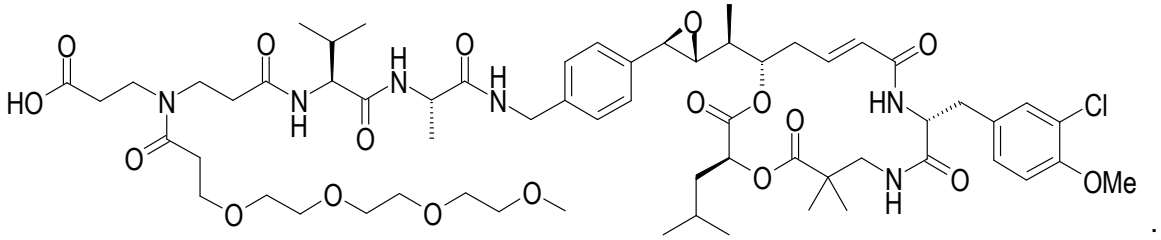
14. Cryptophycin payloads of formula (IV) according to claim 13, having the following structure:



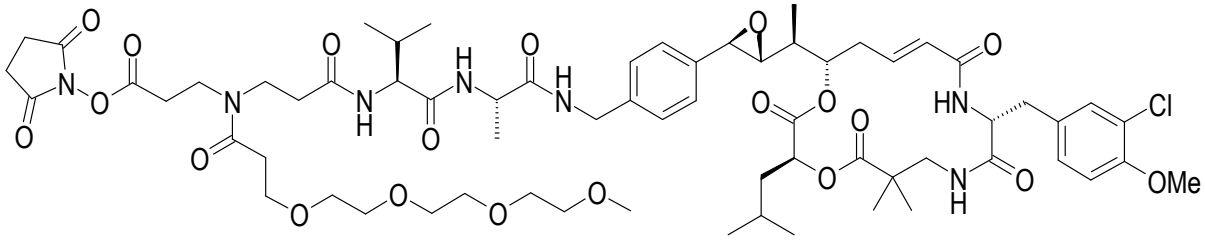
wherein:

- **R_1**, **R_2**, **R_3**, **R_4**, **R_5**, **R_6**, **R_7**, **R_8**, **R_9**, **R_{10}**, **R_{11}**, **R_{12}**, **n**, **RCG_1**, **X, Y** and **L** are as defined in formula (IV) according to claim 14.

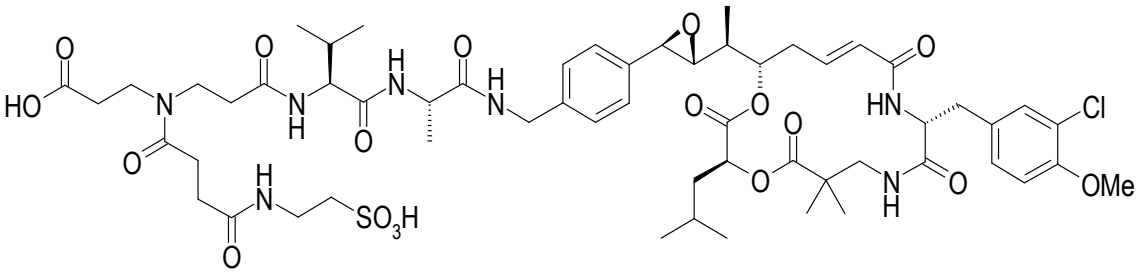
15. Cryptophycin payloads of formula (IV) according to claim 13 which are selected from the following list:



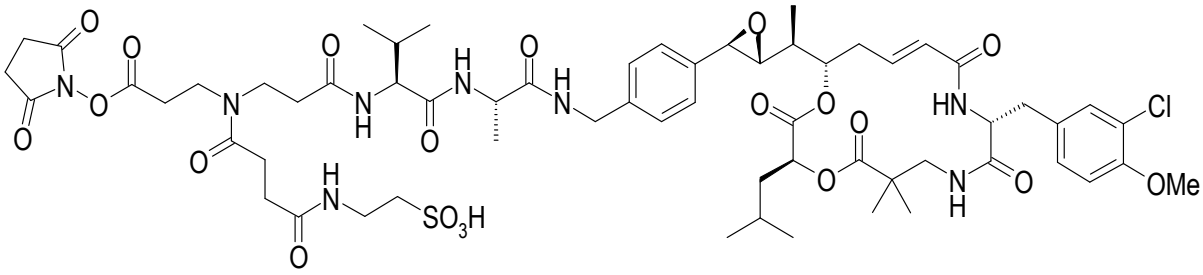
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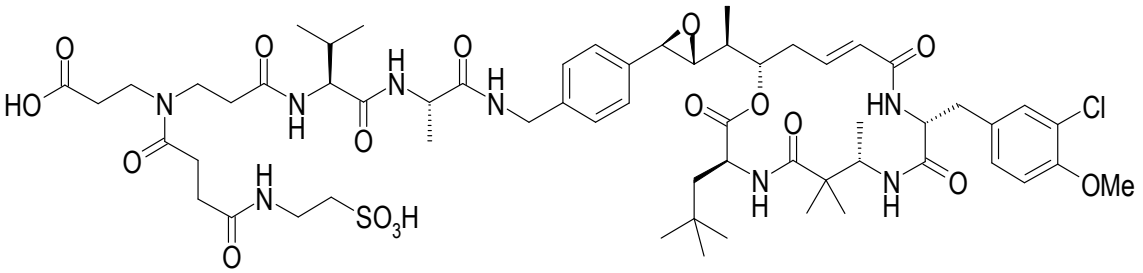
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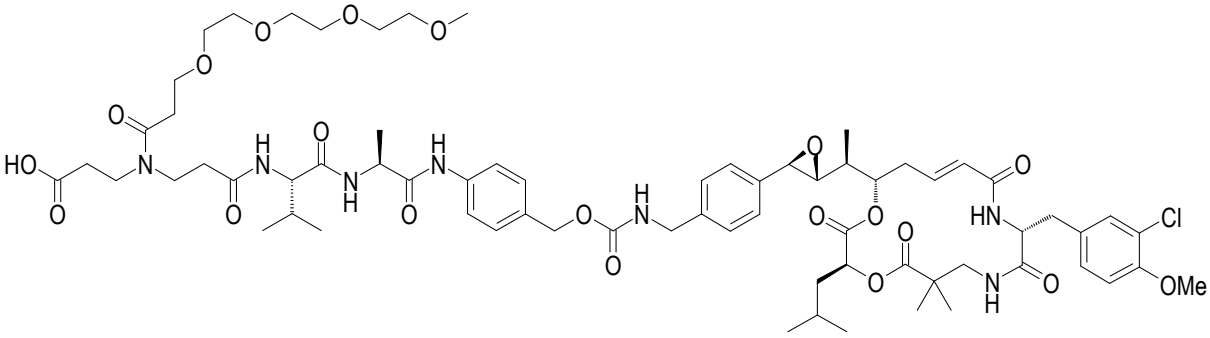
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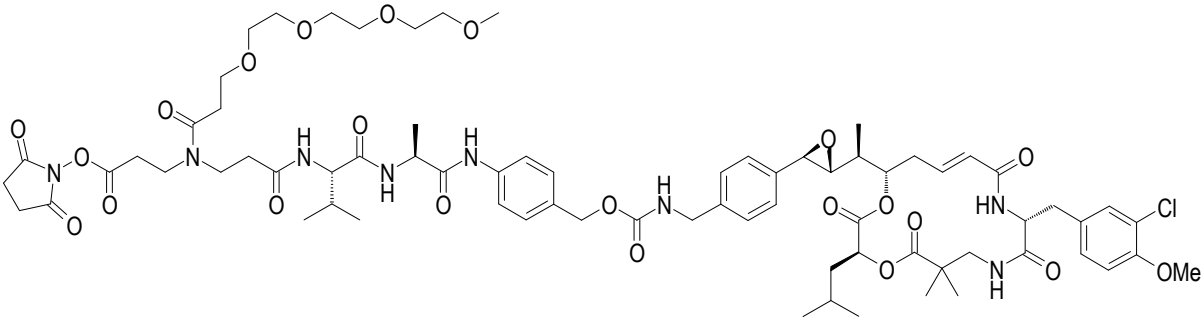
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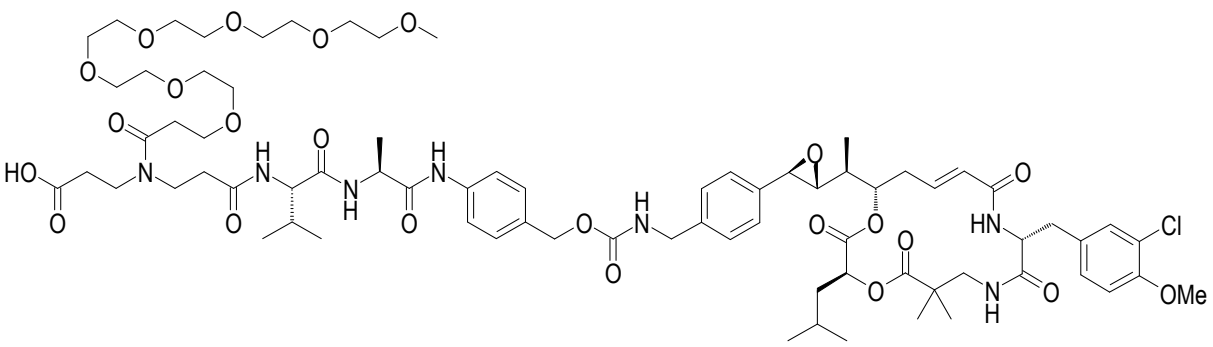
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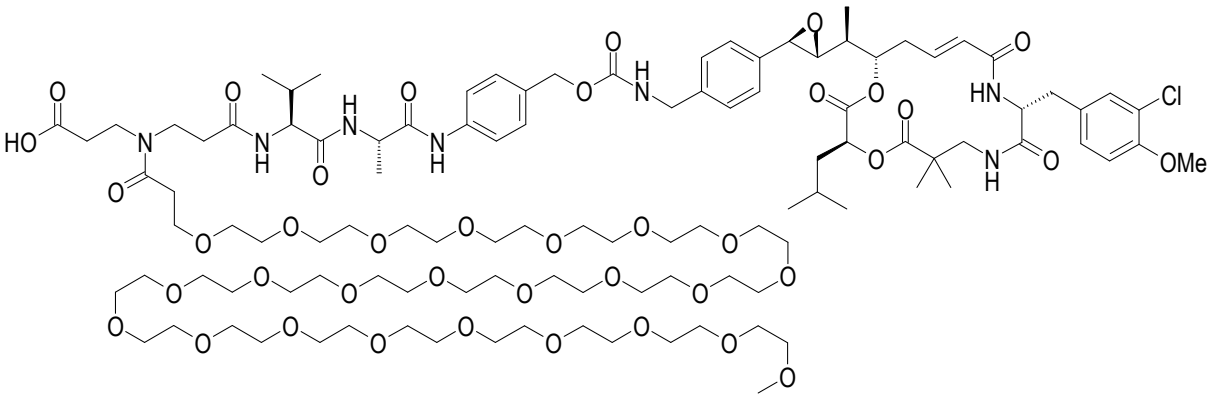
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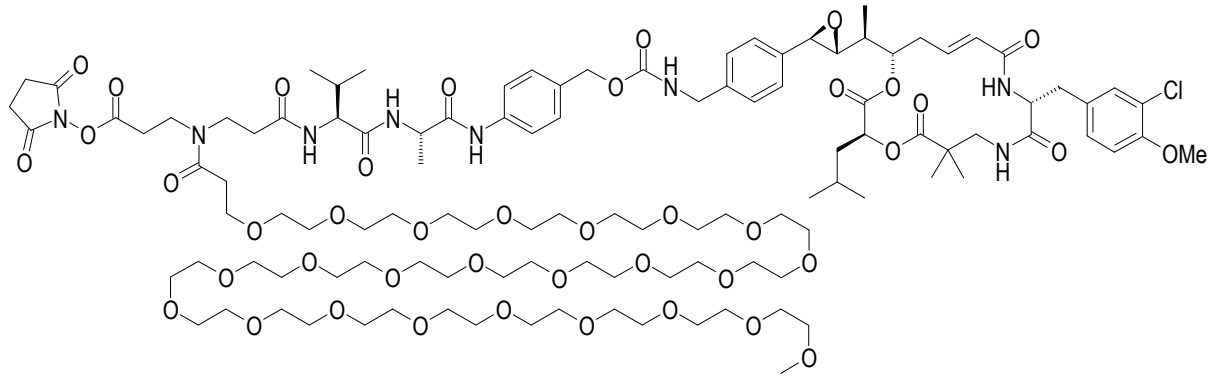
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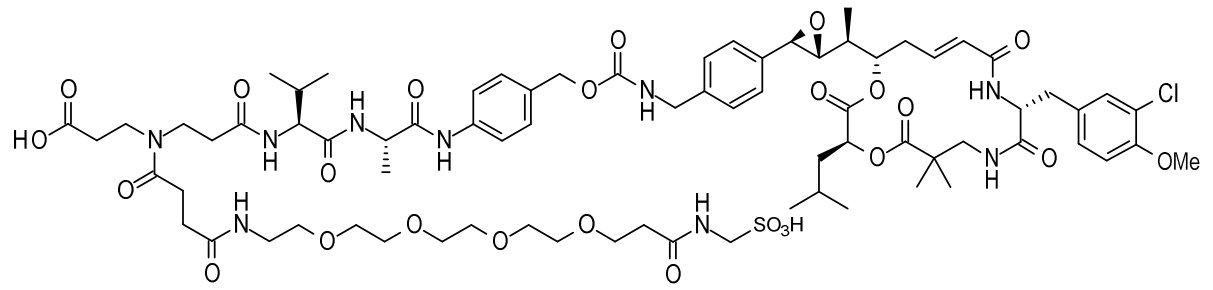
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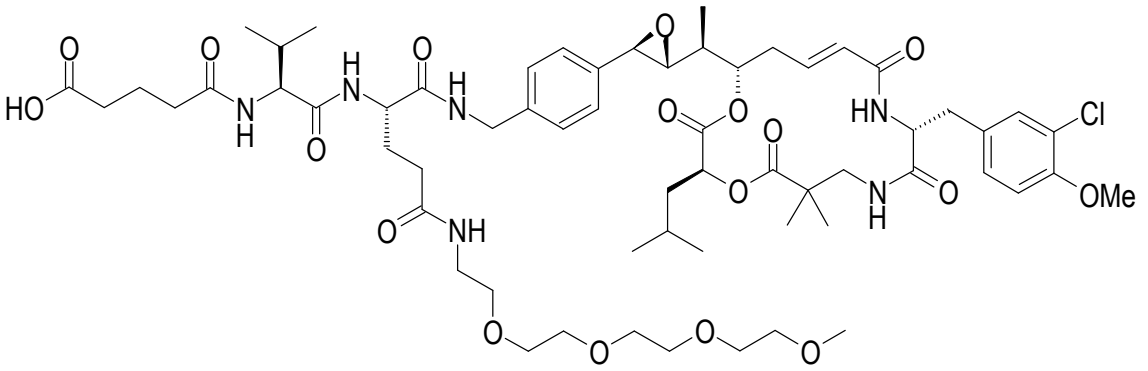
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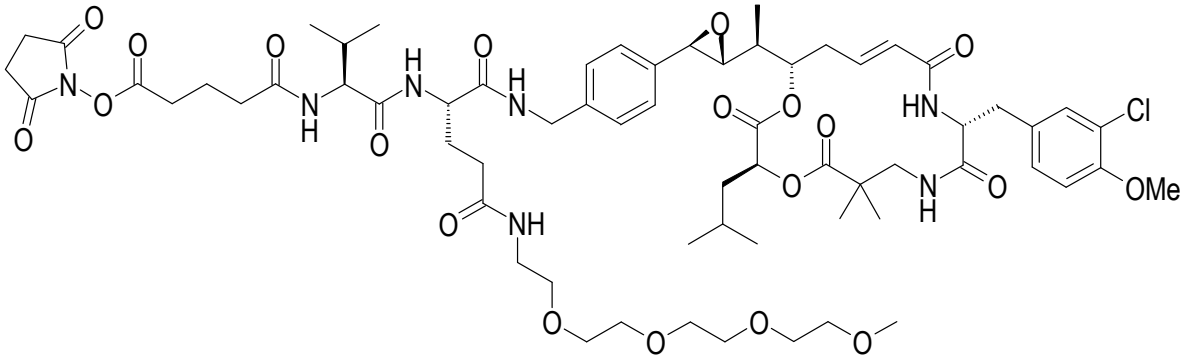
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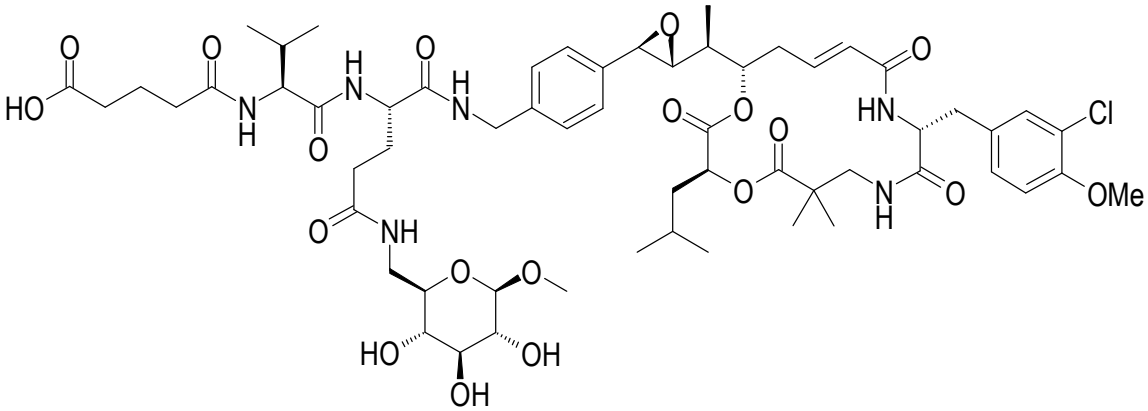
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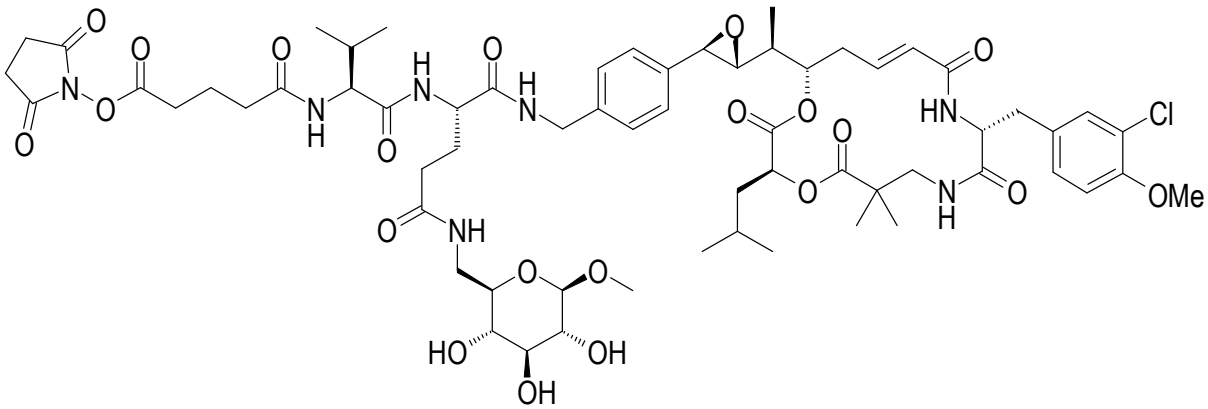
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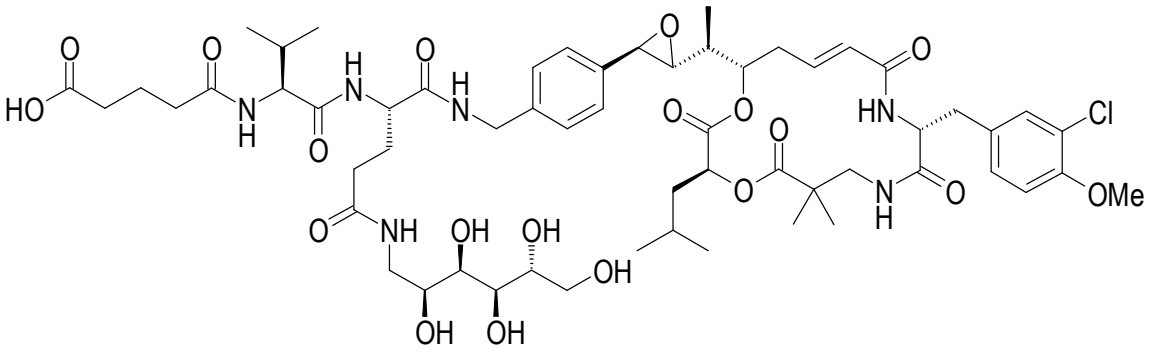
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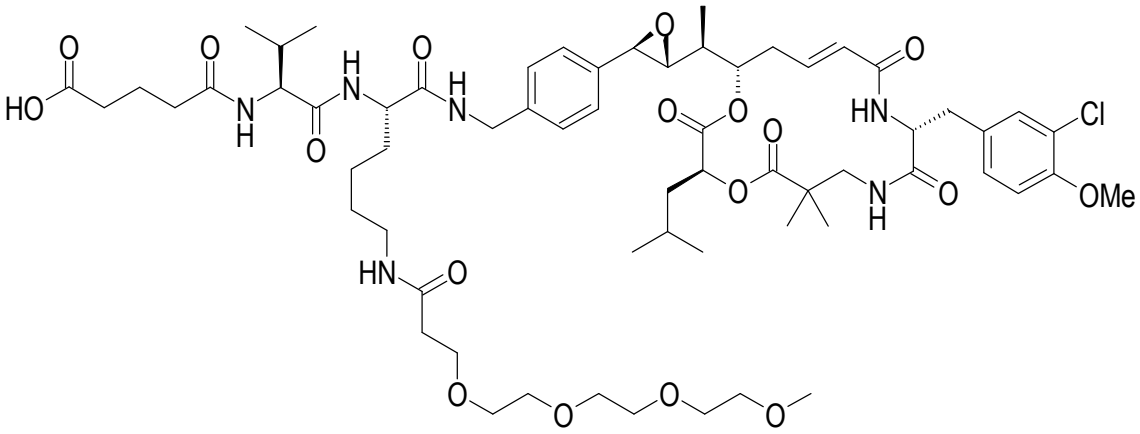
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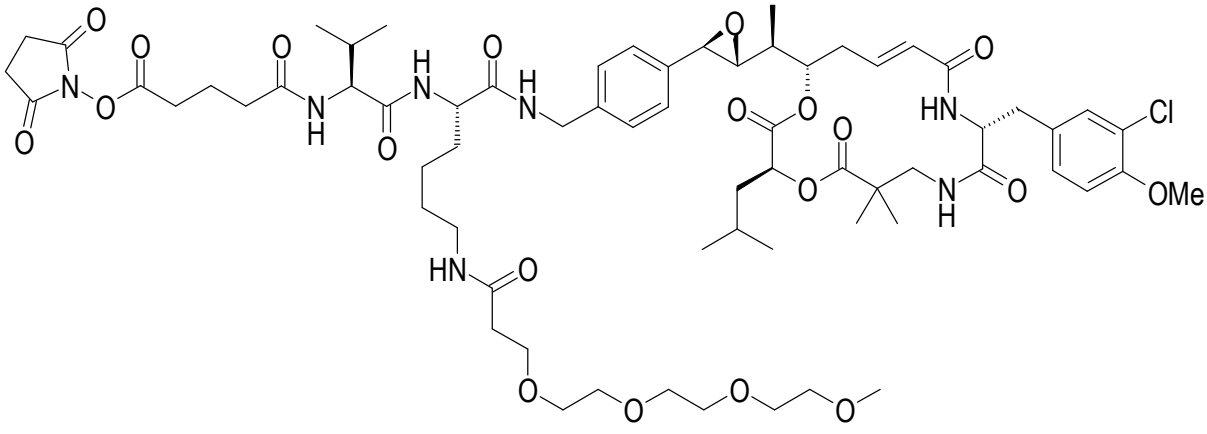
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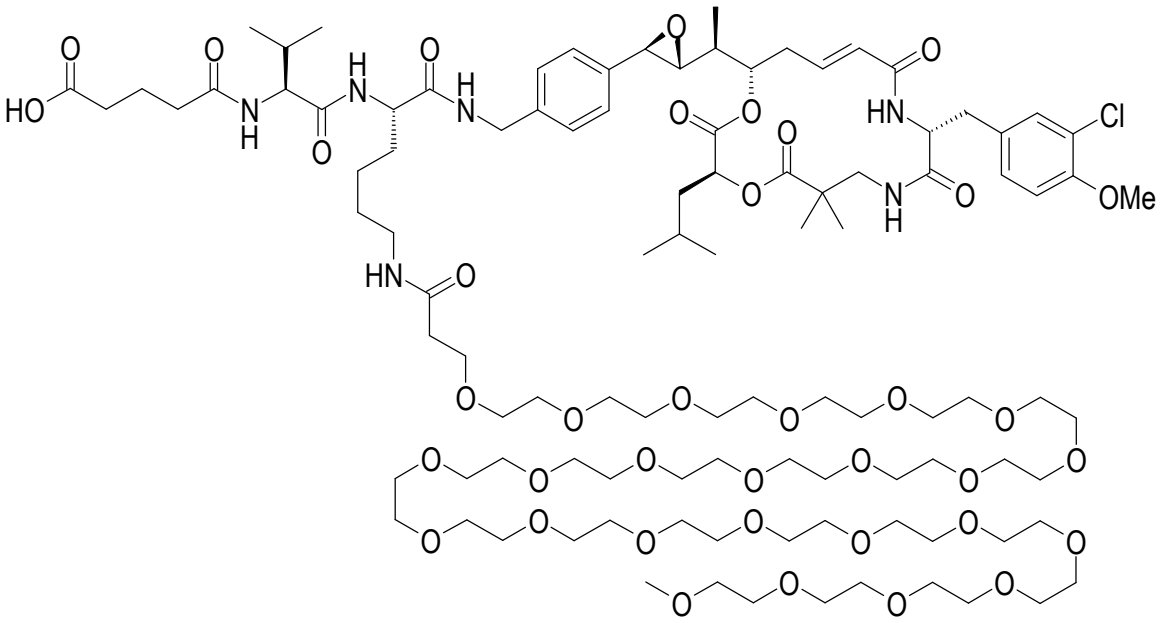
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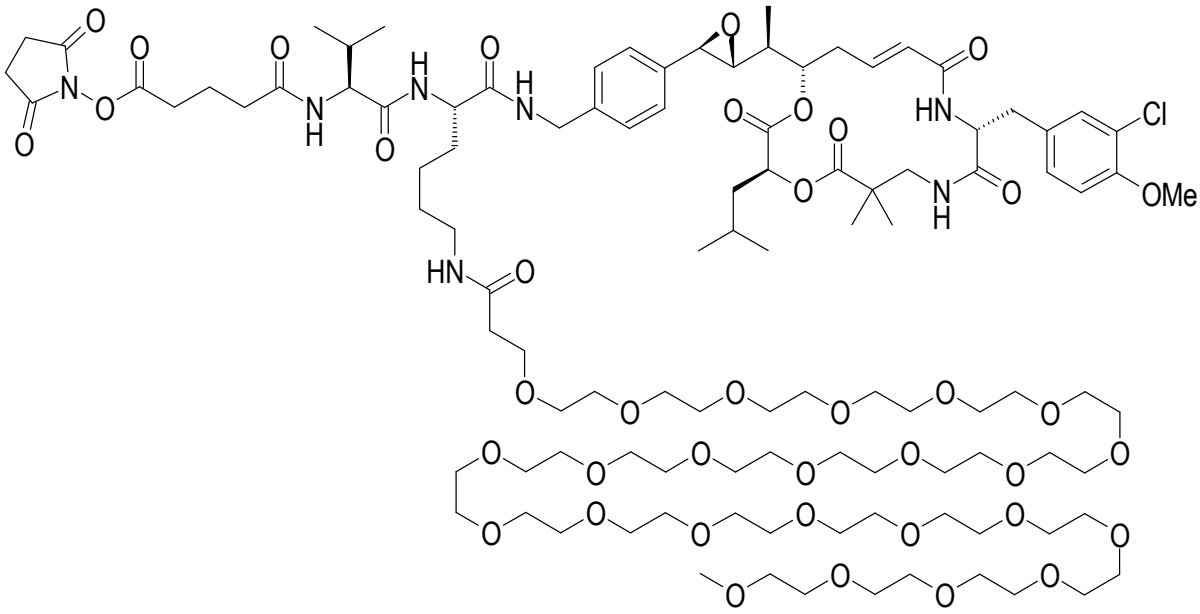
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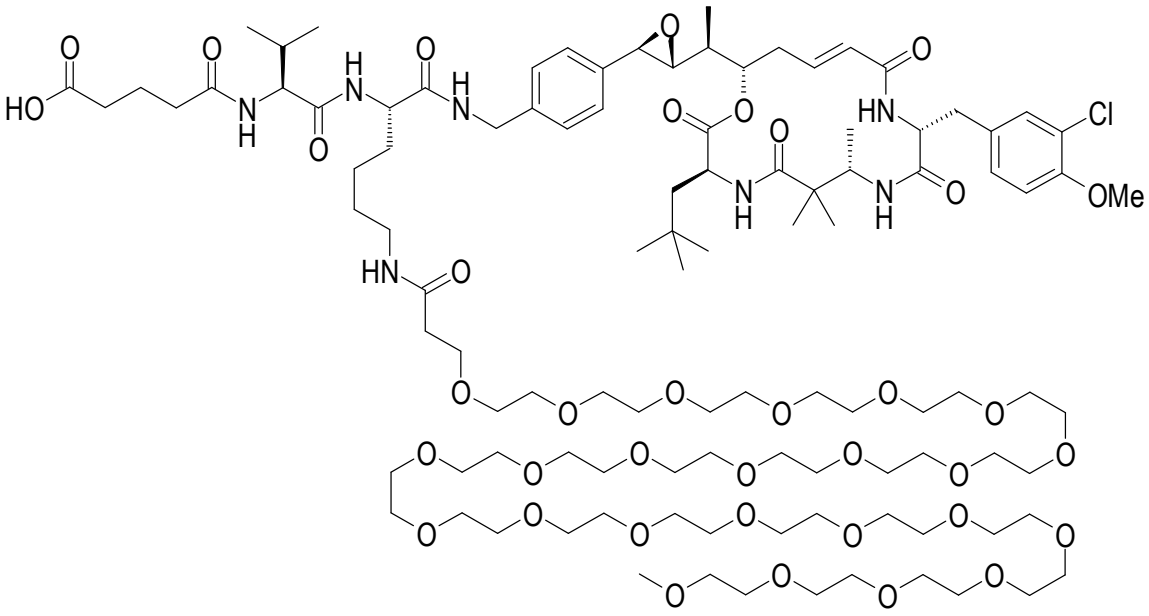
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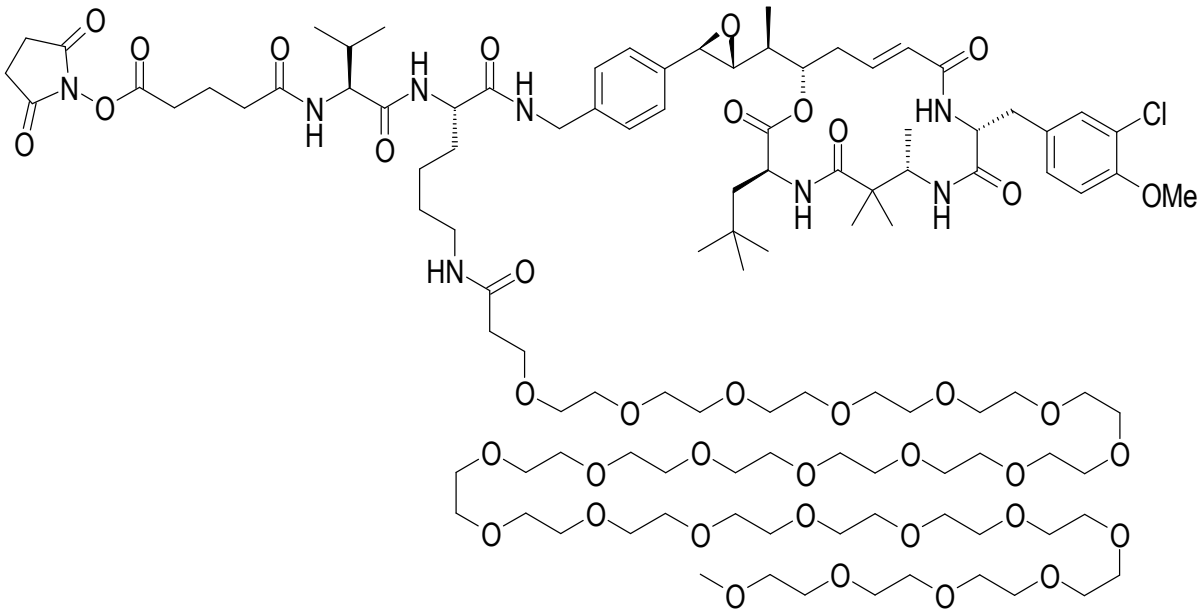
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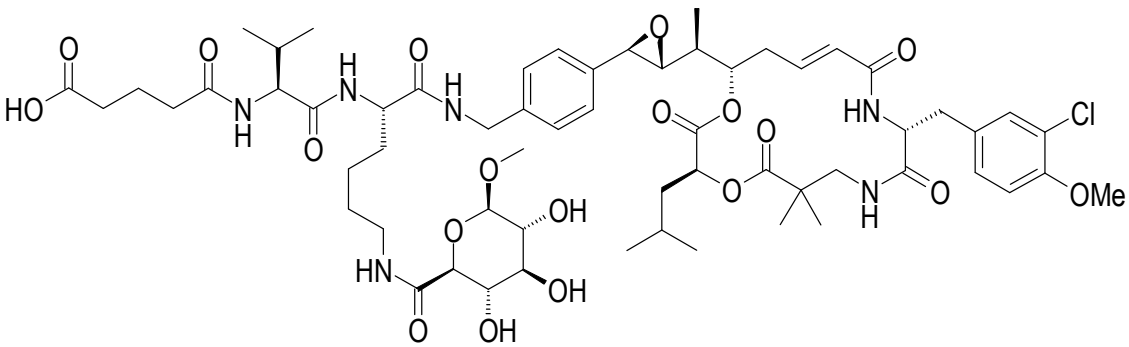
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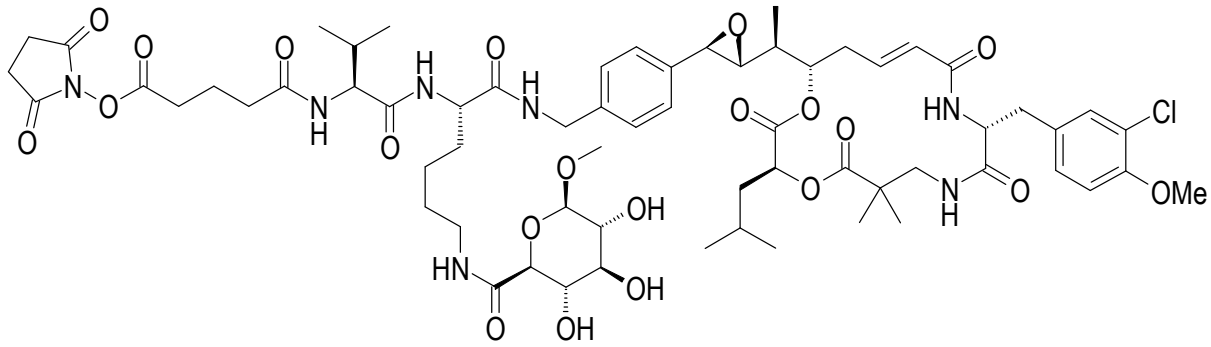
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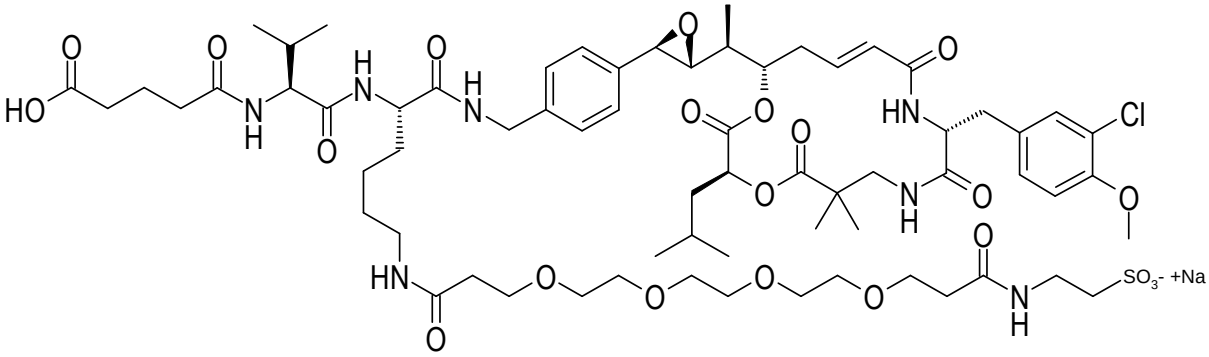
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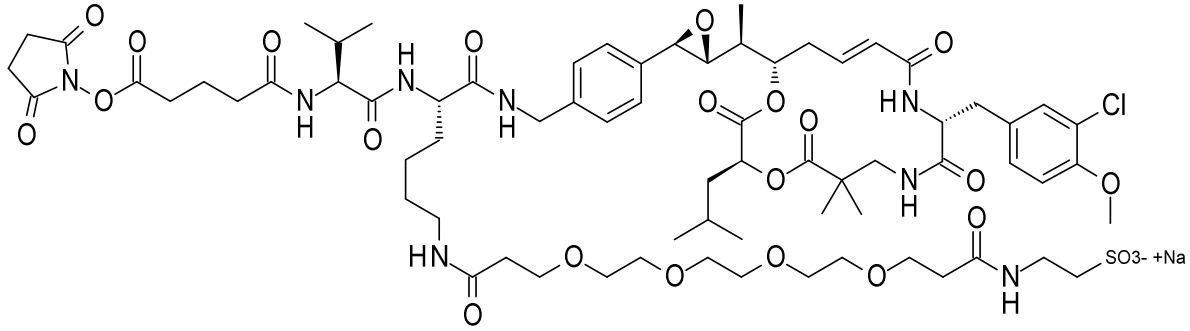
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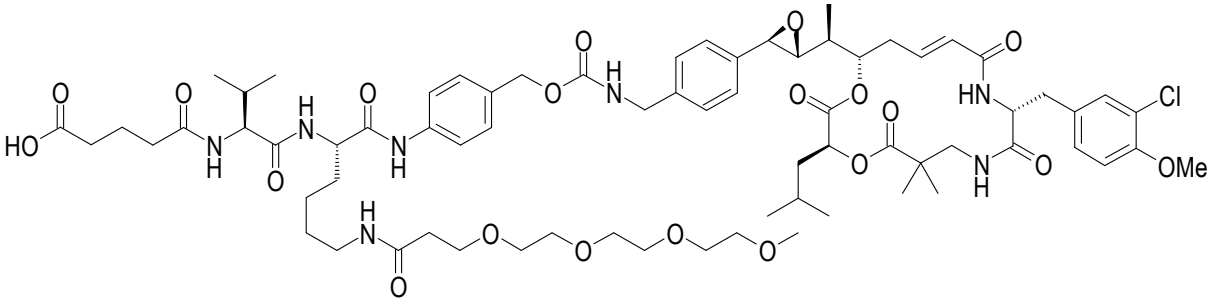
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SO3- +Na

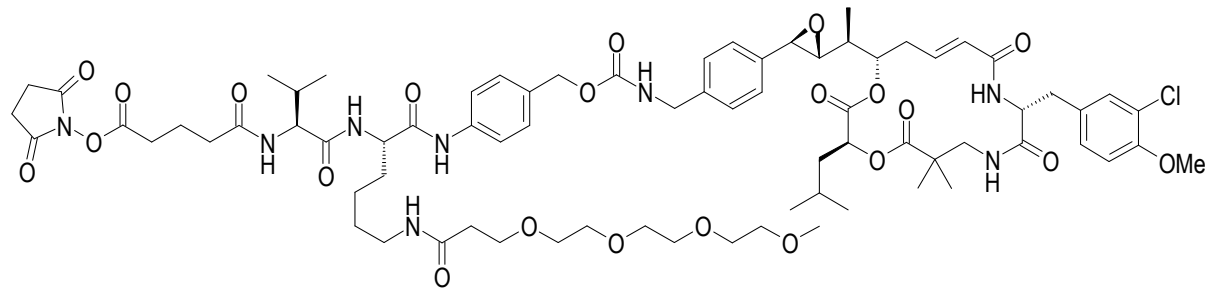
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SO3- +Na

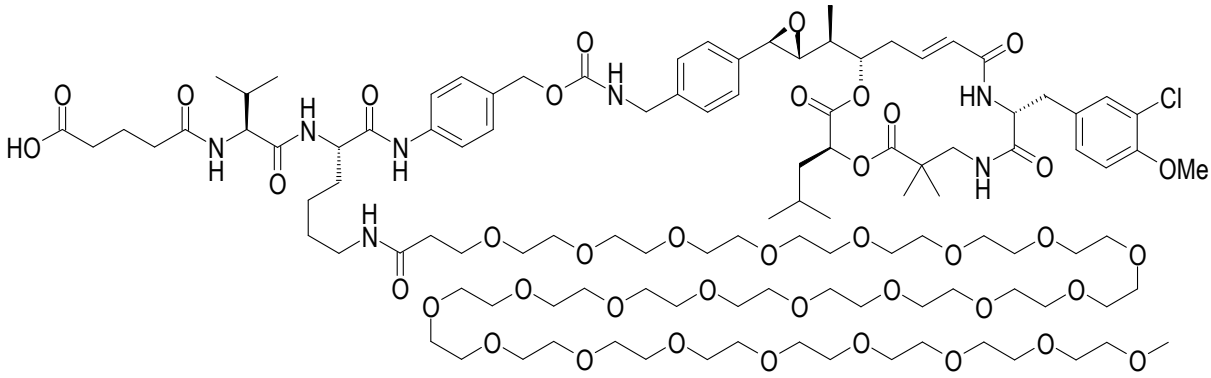
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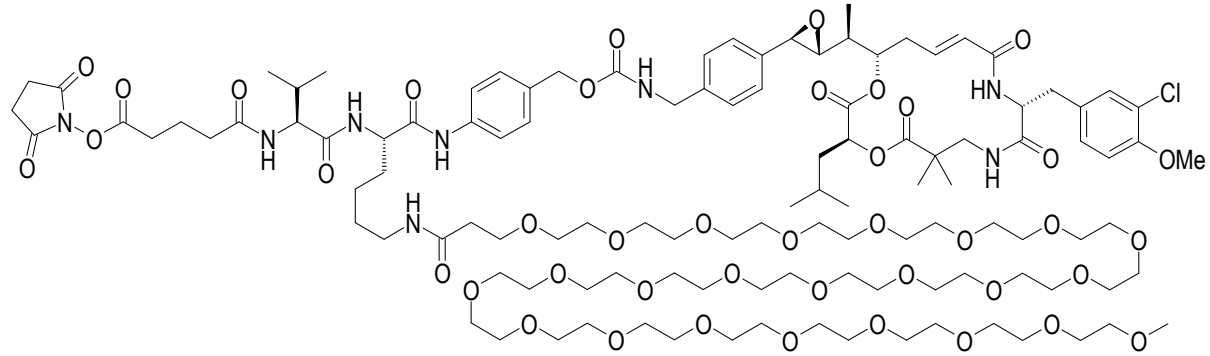
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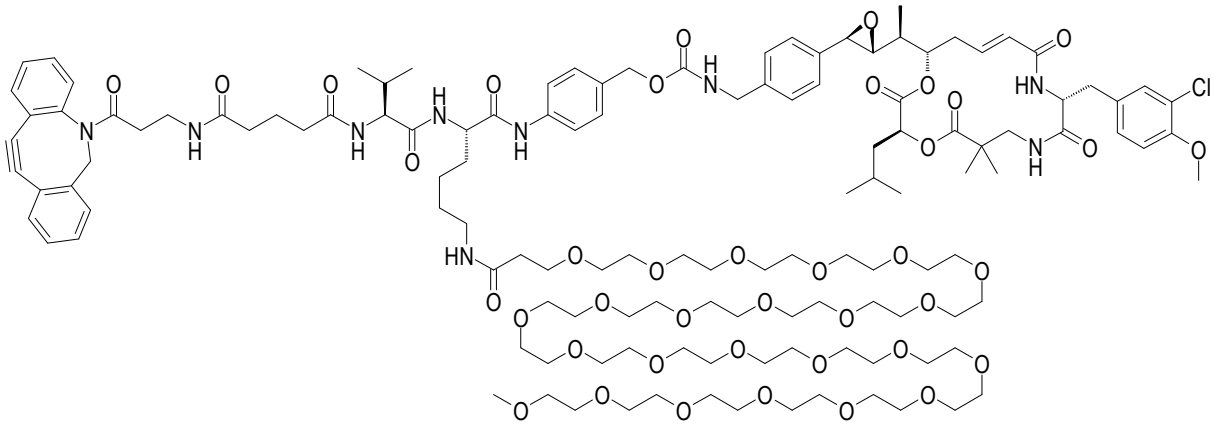
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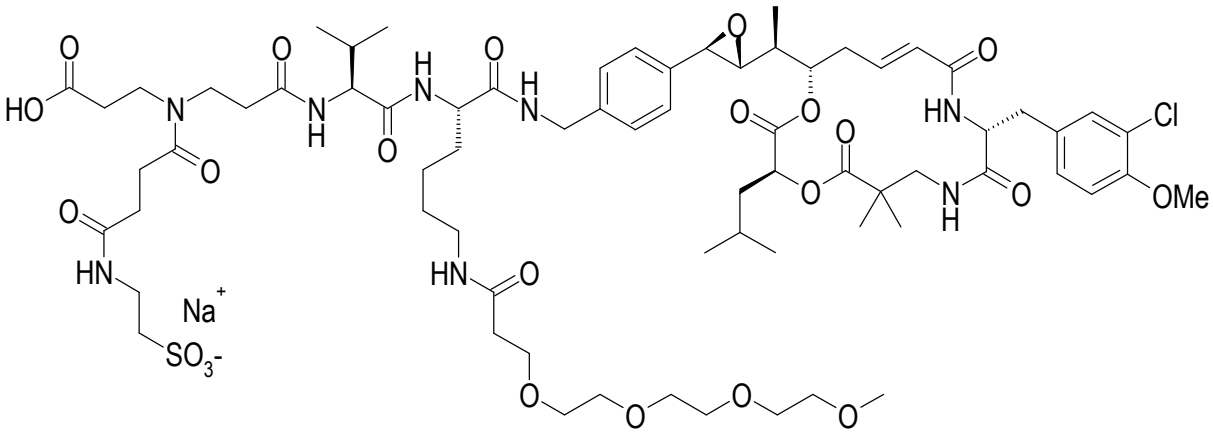
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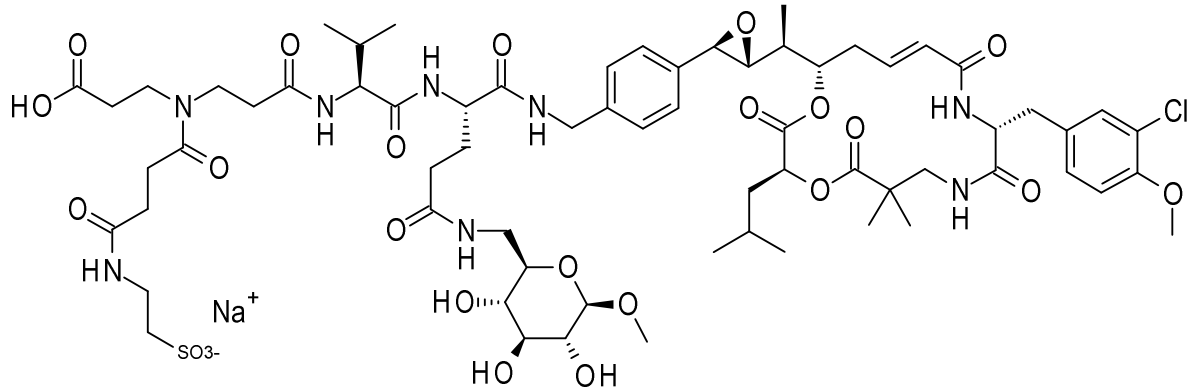
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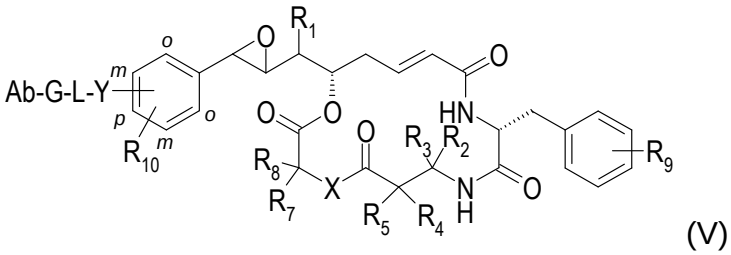
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and



16. Conjugate of formula (V):

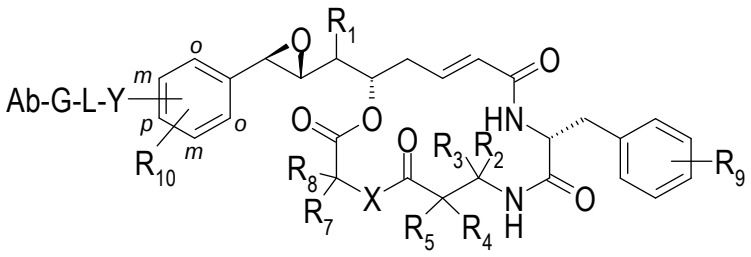


(V)

wherein:

- **R_1**, **R_2**, **R_3**, **R_4**, **R_5**, **R_6**, **R_7**, **R_8**, **R_9** ,**R_{10}**, **R_{11}**, **R_{12}**, **n, X, Y** and **L** are as defined in formula (IV) according to claims 13 or 14;
- **G** represents the product of reaction between **RCG1** as defined in claim 12, a reactive chemical group present at the end of the linker **L** of formula (II) as defined in claim any one of claims 1 to 11, and **RCG2**, an orthogonal reactive chemical group present on a polypeptide such as the antibody (Ab); and
- **Ab** represents an antibody.

17. Conjugates of formula (V) according to claim 16, having the following structure:



wherein:

- **R_1**, **R_2**, **R_3**, **R_4**, **R_5**, **R_6**, **R_7**, **R_8**, **R_9** ,**R_{10}**, **R_{11}**, **R_{12}**, **n, X, Y, L**, **G, RCG1, RCG2** and **Ab** are as in formula (V) according to claim 16.

18. Conjugates of formula (V) according to claim 16 or 17, wherein **RCG2** is selected from:

(i) ε-amino groups borne by the side chains of lysine residues that are present at the surface of the antibody;

(ii) α-amino groups of N-terminal amino acids of heavy and light chains of the antibody;

(iii) the saccharide groups of the hinge region of the antibody;

(iv) the thiols of cysteines generated by reducing intra-chain disulfide bonds of the antibody or the thiols of engineered cysteines of the antibody;

(v) amide groups borne by side chains of glutamine residues that are present at the surface of the antibody; and

(vi) aldehyde groups introduced into the antibody using a formylglycine generating enzyme.

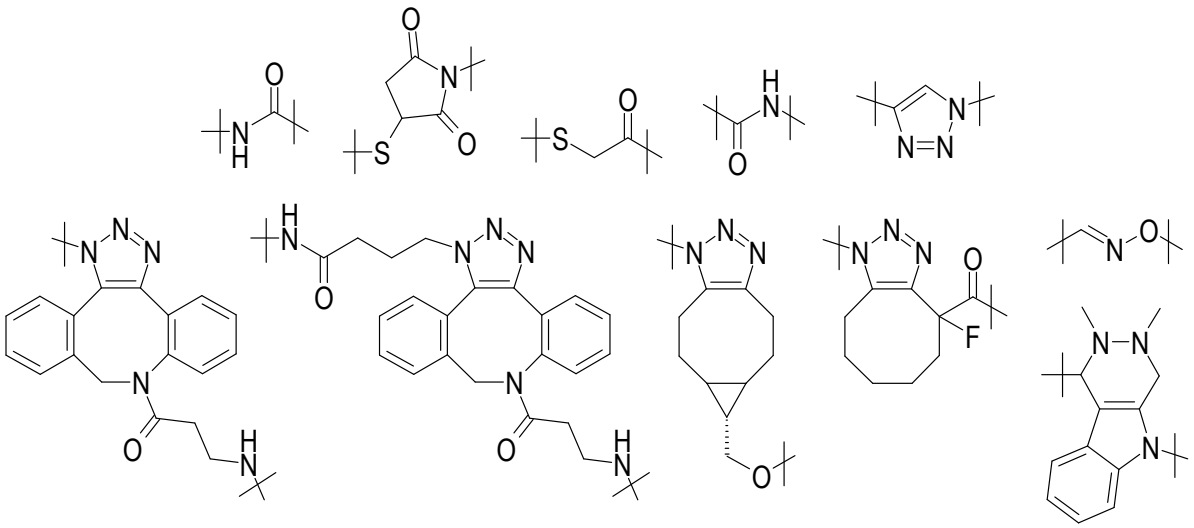
19. Conjugates of formula (V) according to claim 16 or 17, wherein:

- when **RCG1** represents a N-hydroxysuccinimidyl ester, **RCG2** represents a NH_2 group;
- when **RCG1** represents a maleimido function, a haloacetamido function, a chlorine atom or an activated disulfide, **RCG2** represents a -SH group;
- when **RCG1** represents a N_3 group, **RCG2** represents a C≡CH group or an activated C≡C group such as a cyclooctyne moiety;
- when **RCG1** represents a OH or NH_2 group, **RCG2** represents a carboxylic acid or amide function;
- when **RCG1** represents a SH group, **RCG2** represents a maleimido function, a

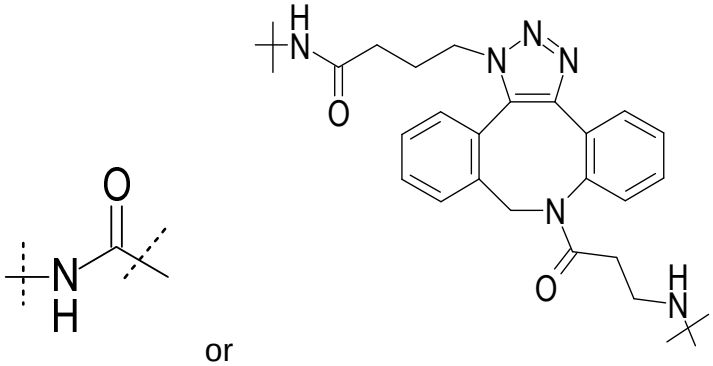
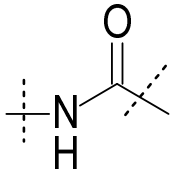
haloacetamido function or an activated disulfide function;

- when **RCG1** represents a C≡CH group or an activated C≡C group, **RCG2** represents a N_3 group;
- when **RCG1** represents a O-alkyl hydroxylamine function or a Pictet-Spengler reaction substrate, **RCG2** represents an aldehyde or ketone function.

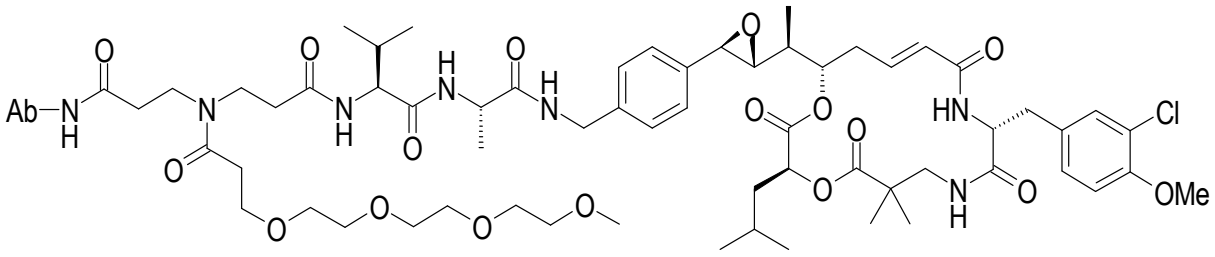
20. Conjugates of formula (V) according to any one of claims 16 to 19, wherein **G** is selected from:



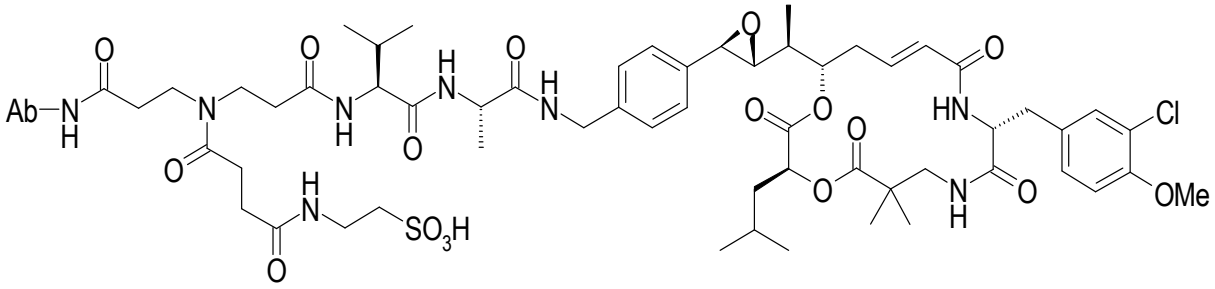
; for instance, G represents the following groups:

 or , such as  .

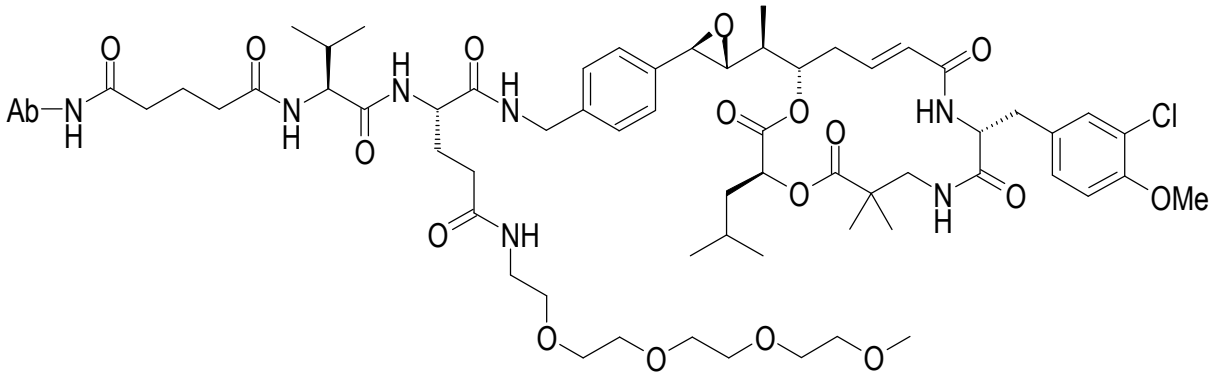
21. Conjugates of formula (V) according to claim 16, which is selected from the following list:



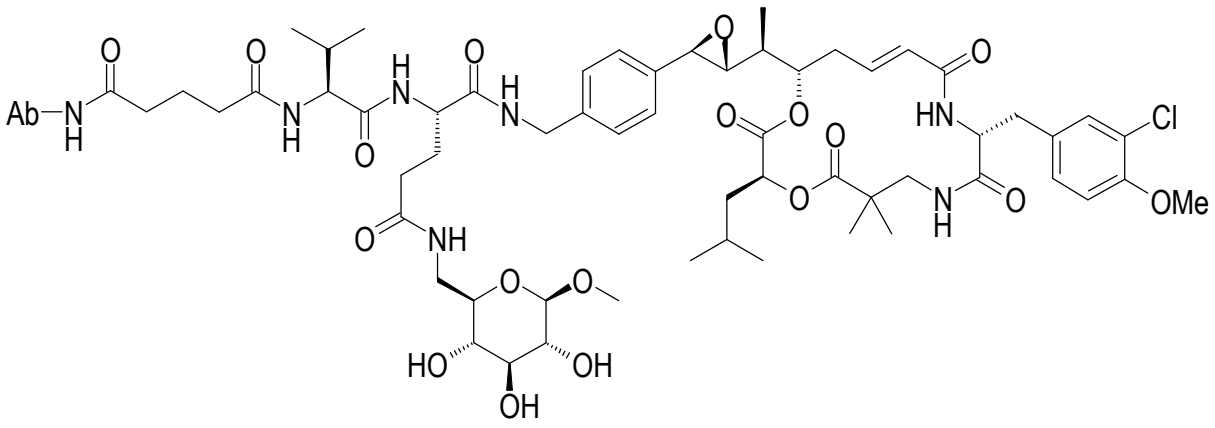
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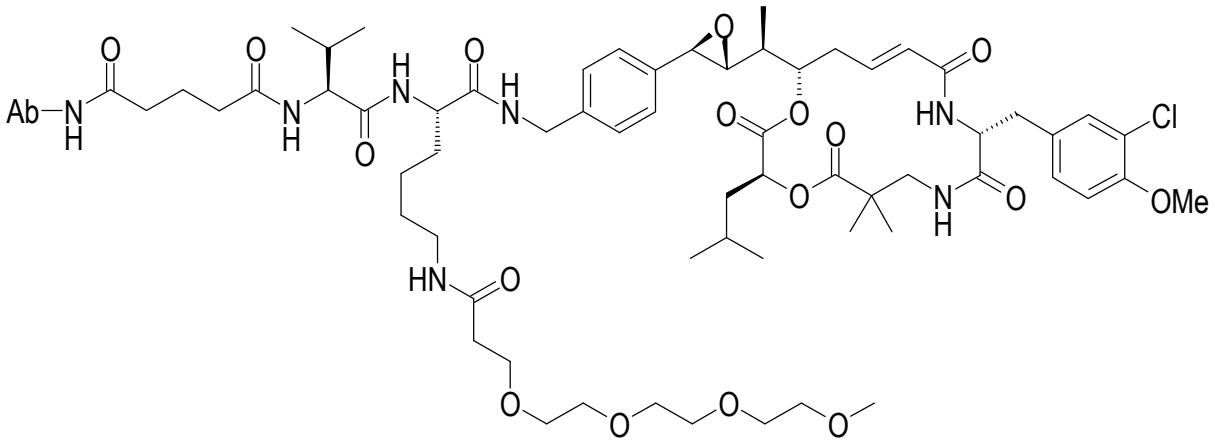
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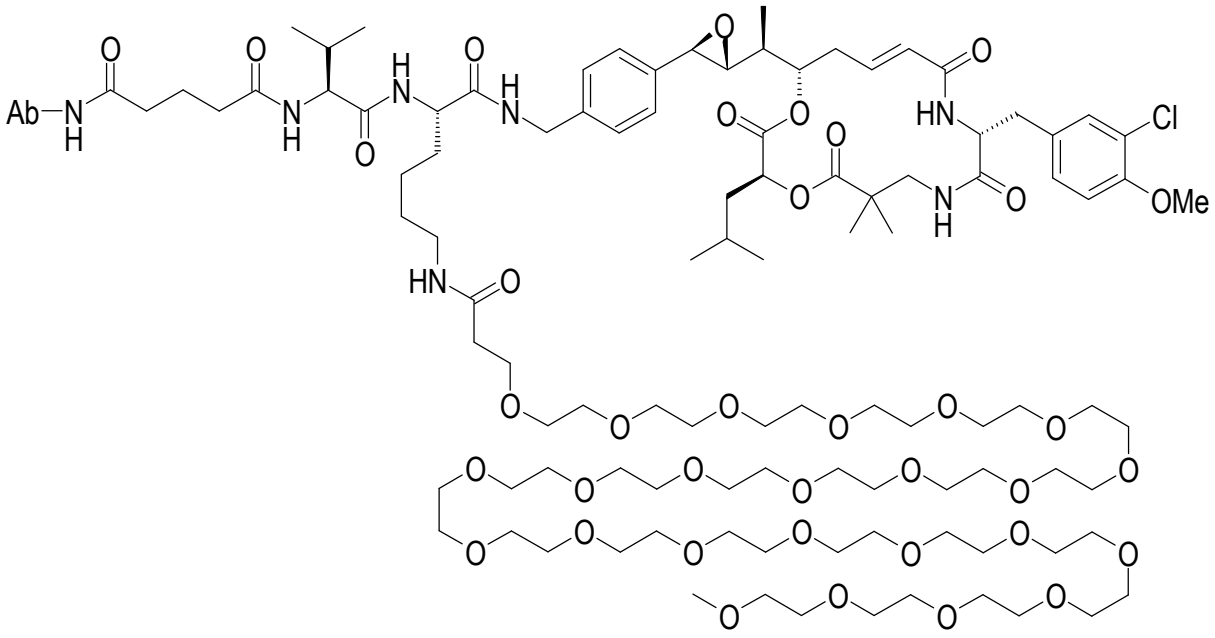
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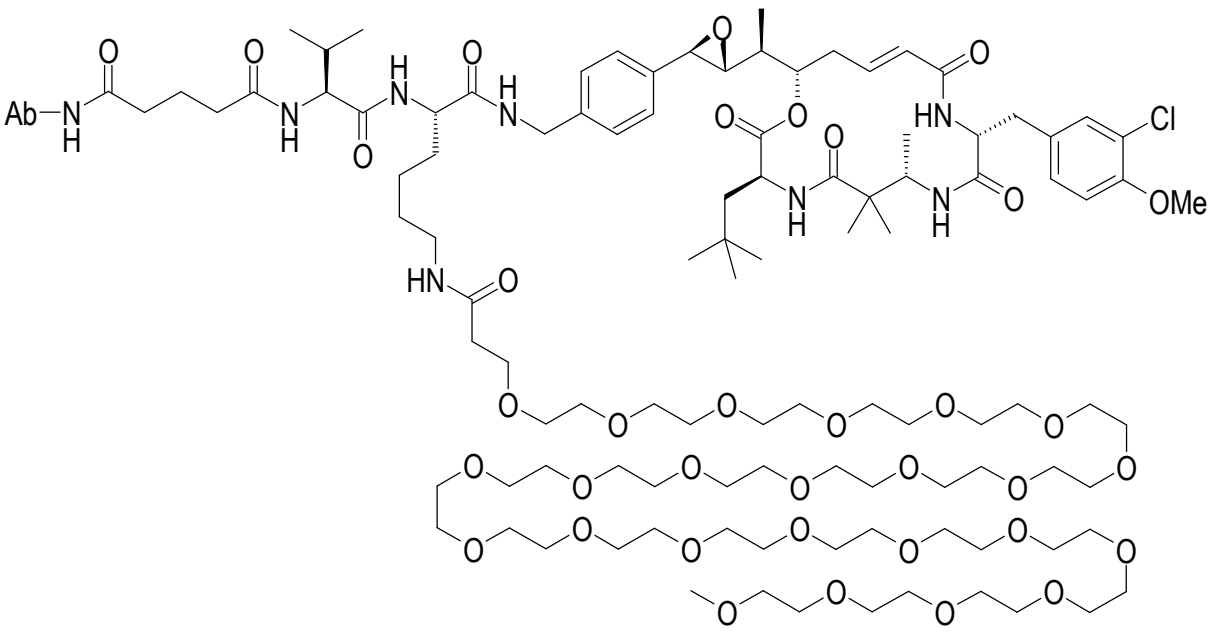
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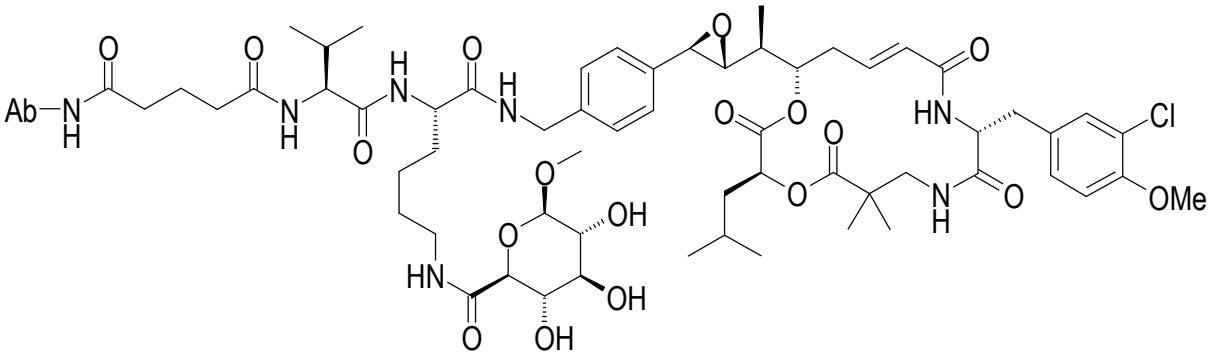
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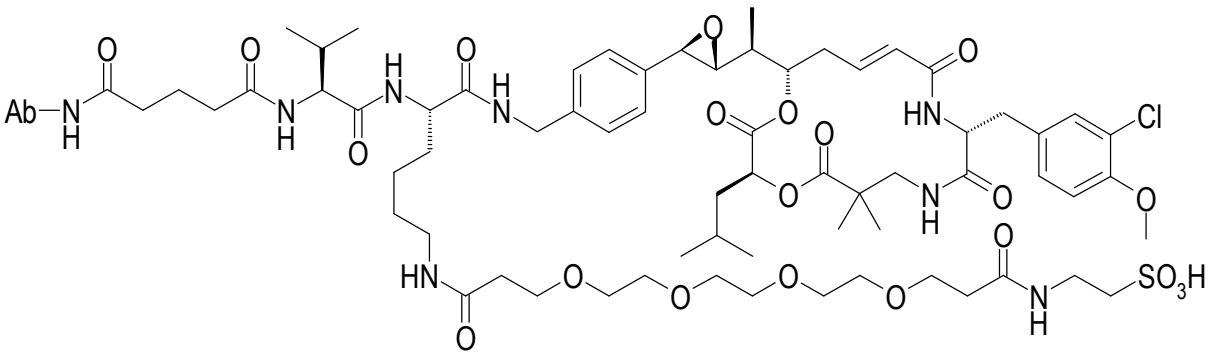
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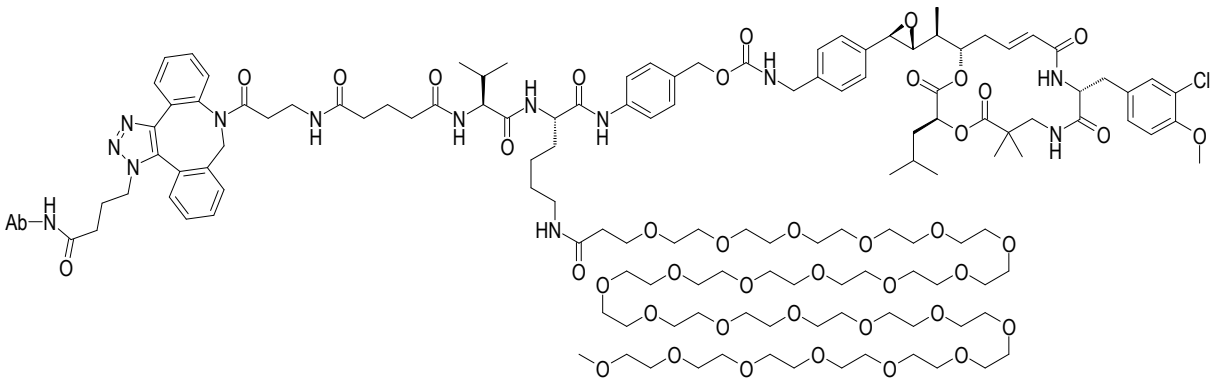
;



;



and



wherein Ab represents an antibody.

22. Process for preparing a conjugate of formula (V) as defined in any one of claims 16 to 21 comprising at least the steps of :

(i) placing in contact and leaving to react:

- an optionally buffered aqueous solution of an antibody, optionnally modified by means of a modifying agent,

and

- a solution of a cryptophycin payload of formula (IV) as defined in any one of claims 13 to 15,

the chemical group **RCG1** of the cryptophycin payload of formula (IV) being reactive towards the chemical groups **RCG2** present on the polypeptide such as the antibody 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 cryptophycin payload of formula (IV) to the antibody by formation of a covalent bond;

(ii) and then optionally to separate the conjugate formed in step (i) from the cryptophycin payload of formula (IV) and/or from the unreacted antibody and/or from any aggregates that may have formed.

23. Medicament, wherein it comprises at least one conjugate of formula (V) according to any one of claims 16 to 21.

24. Pharmaceutical composition, wherein it comprises at least one conjugate of formula (V) according to any one of claims 16 to 21, and also at least one pharmaceutically acceptable excipient.

25. Conjugates of formula (V) according to any one of claims 16 to 21 for use in the treatment of cancer.