Claims

Claim 1. A method of enzymatic peptide ligation, said method comprising providing

i) a peptidyl asparaginyl ligase (PAL) and a glutaminyl cyclase (QC);

ii) a first peptide or protein having a P1-P1'-P2' tripeptide PAL motif as an acyl donor, wherein P1 is Asn or Asp, P1' is Gln or Glu and P2′ is a hydrophobic amino acid or a β-branched amino acid;

iii) a second peptide or protein which may be the same or different to the first peptide or protein, having a P1″-P2″ motif as an acyl acceptor at the N-terminus, wherein P1″ is any amino acid and P2″ is a hydrophobic amino acid or a β-branched amino acid;

iv) contacting the PAL and the QC with said first and second peptides or proteins;

wherein the PAL cleaves the first peptide or protein after P1 in the tripeptide PAL motif and ligates said first peptide or protein to the P1″-P2″ motif of said second peptide or protein to form a ligated product, and the QC cyclizes P1' in the released P1'-P2′ dipeptide motif to pyroglutamyl (pGlu).

Claim 2. The method of claim 1, wherein P2′ is selected from the group comprising Leu, Met, Phe, Tyr, Trp, Val, Ile and Thr.

Claim 3. The method of either claim 1 or claim 2, wherein the PAL is selected from the group comprising butelase1 comprising the amino acid sequence set forth in SEQ ID NO: 1, butelase-2 comprising the amino acid sequence set forth in SEQ ID NO: 2 or 3, VyPAL2 comprising the amino acid sequence set forth in SEQ ID NO: 4, VyPAL3 comprising the amino acid sequence set forth in SEQ ID NO: 5, OaAEP1b-C247A comprising the amino acid sequence set forth in SEQ ID NO: 6, HeAEP3 comprising the amino acid sequence set forth in SEQ ID NO: 7, AtLEGγ comprising the amino acid sequence set forth in SEQ ID NO: 8, VuPAL1 comprising the amino acid sequence set forth in SEQ ID NO: 9, HaPAL1 comprising the amino acid sequence set forth in SEQ ID NO: 10, OaAEP1b comprising the amino acid sequence set forth in SEQ ID NO: 11 and a functional fragment or a variant thereof.

Claim 4. The method of any one of claims 1 to 3, wherein the QC is selected from the group comprising Human glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 12, Mouse glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 13, Drosophila glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 14, Arabidopsis glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 15, Conus glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 16, Sistrurus glutaminyl cyclase comprising the amino

acid sequence set forth in SEQ ID NO: 17, Bacterial glutaminyl cyclase comprising the amino acid sequence set forth in SEQ ID NO: 18 and a functional fragment or a variant thereof.

Claim 5. The method of any one of claims 1 to 4, wherein the ratio of QC: PAL: first peptide or protein is in the range of 0.1:1:1000 to 1:1:50, respectively, preferably in the range of 0.1:1:1000 to 0.1:1:50, respectively.

Claim 6. The method of any one of claims 1 to 5, wherein one of said first and second peptides or proteins is an epitope-binding peptide or protein and the other peptide or protein comprises a payload.

Claim 7. A ligated product comprising a first peptide or protein and a second peptide or protein which may be the same or different to the first peptide or protein, the second peptide or protein having a N-terminus, wherein one of said first and second peptides or proteins is an epitope-binding peptide or protein, and the other peptide or protein comprises a payload, and wherein the first peptide or protein is ligated to the N-terminus of the second peptide or protein via -P1-P1"-P2"-, wherein:

P1 is Asn or Asp;

P1" is any amino acid;

P2" is a hydrophobic amino acid or β-branched amino acid.

Claim 8. The method of claim 6 or the ligated product of claim 7, wherein the epitope-binding peptide or protein is an antibody or functional fragment thereof., an affibody such as Z_{EGFR} or Z_{EGFR}-Fc, and DARPin.

Claim 9. The method of claim 8, wherein the antibody or functional fragment thereof is selected from minibody, diabody, scFv, nanobody and $F(ab')_2$.

Claim 10. The method according to anyone of claims 6, 8, or 9, or the ligated product according to anyone of claims 7 to 9, wherein P2" is selected from: Leu, Phe, Tyr, Trp, Val, Ile and Thr, preferably P2" is Val or Ile.

Claim 11. The method according to anyone of claims 6 or 8 to 10, or the ligated product according to anyone of claims 7 to 10, wherein the payload further comprises a payload-releasing linkage, and/or wherein the payload is an imaging agent or a therapeutic agent.

Claim 12. The method or ligated product of claim 11, wherein the imaging agent is a radiolabel chelator or an optical label.

Claim 13. The method or ligated product of claim 12, wherein the radiolabel chelator is selected from 1,4,7-triazacyclononanetriacetic acid (NOTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and 1,4,7-triazacyclononane-1-glutaric acid-4,7-diacetic acid (NODAGA), and/or wherein the therapeutic agent is selected from

a) Monomethyl auristatin E (MMAE);

b) radiolabelled DOTA;

c) Exatecan;

d) Glycolyl-exatecan;

d) Maytansine;

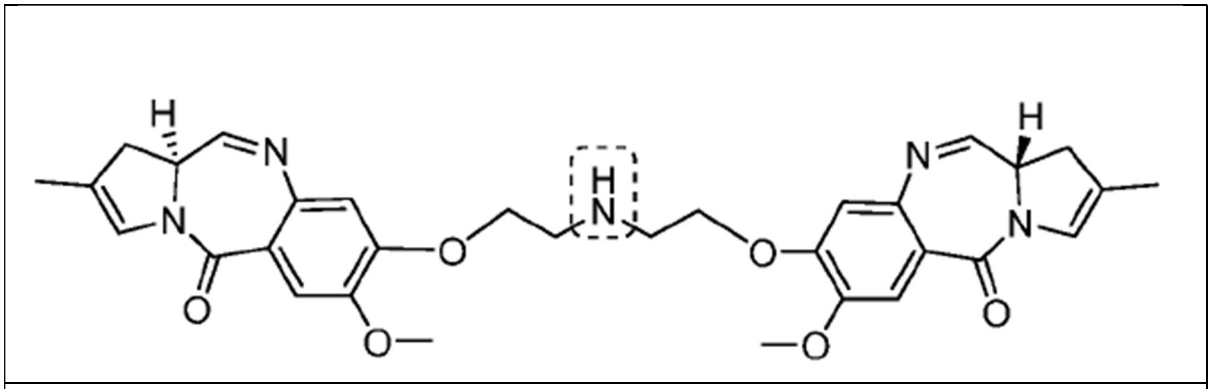
e) PBD dimer;

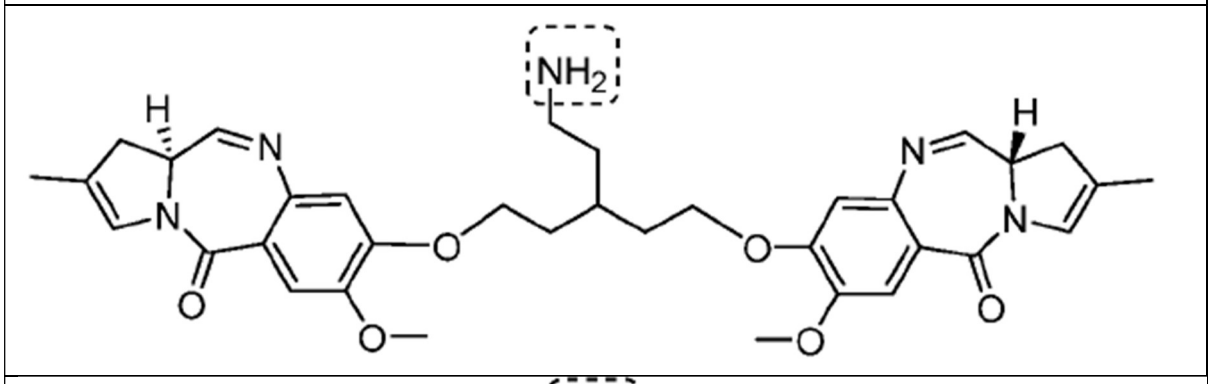
f) Auristatin E;

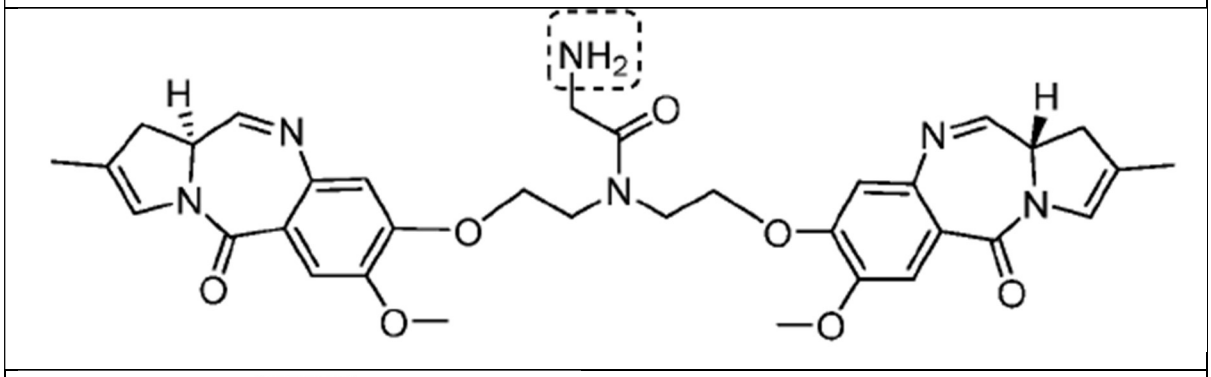
g) SN-38; and

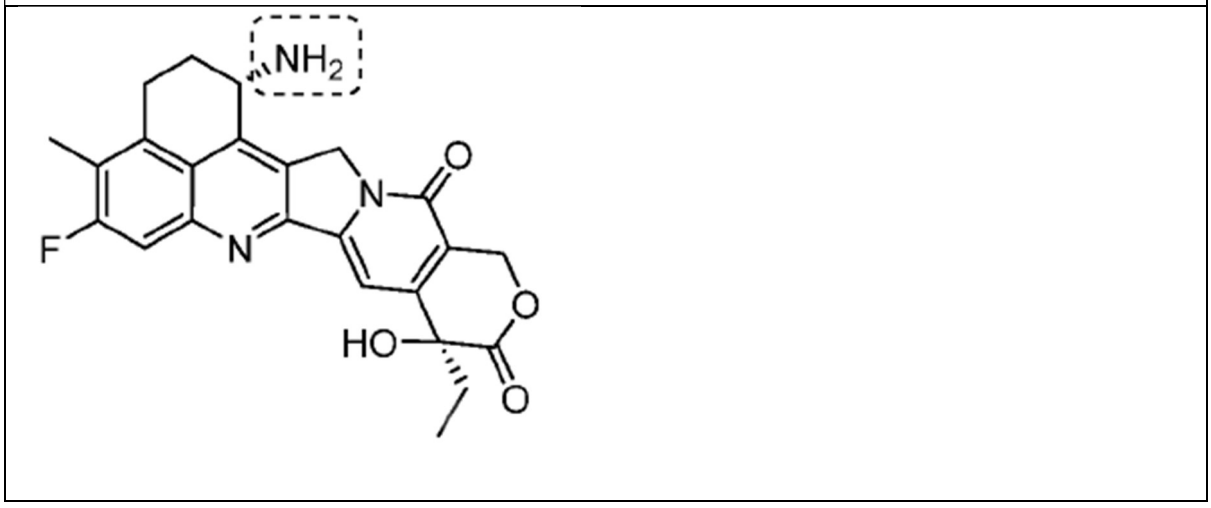
h) α-amanitin.

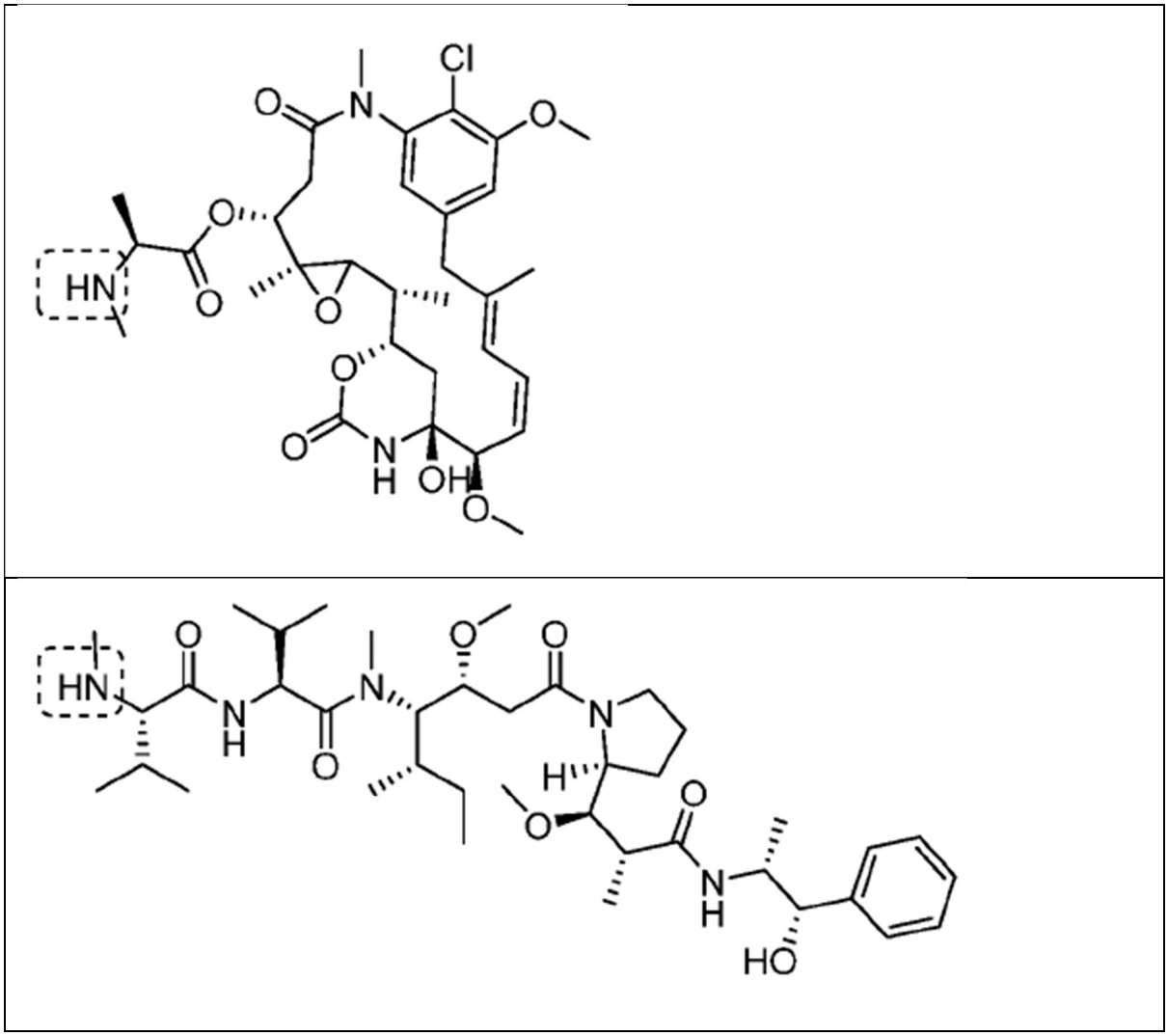
Claim 14. The method according to any of claims 6 and 8 to 13 or the ligated product according to any one of claims 7 to 13, wherein the payload comprises a drug linked to the ligated product via an amine group in the drug, and the drug is selected from:





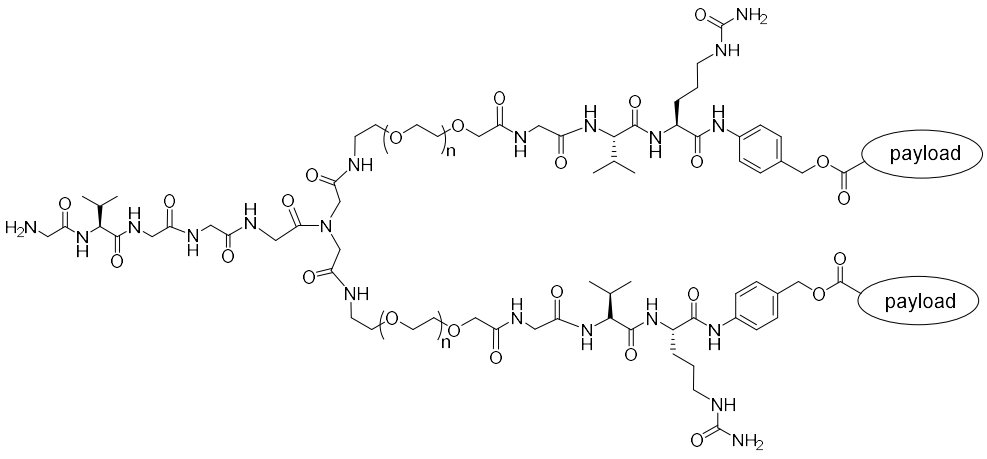


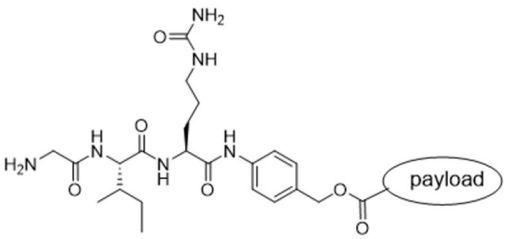


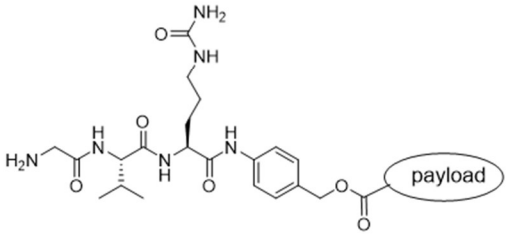


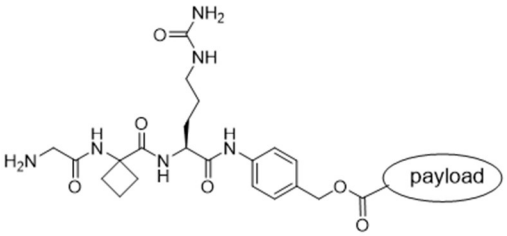
wherein [ ] indicates the amine group, and the amine group can be modified with a linker.

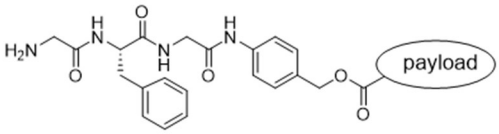
Claim, 15. The method according to any of claims 6 and 8 to 13 or the ligated product according to any one of claims 7 to 13, wherein the peptide or protein comprising a payload is selected from:

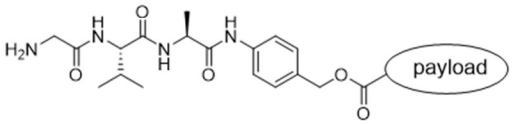


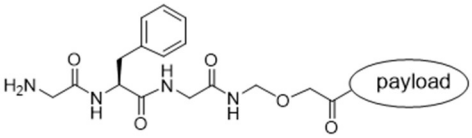

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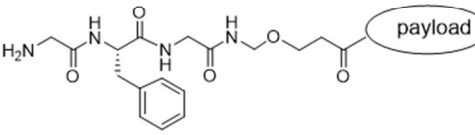

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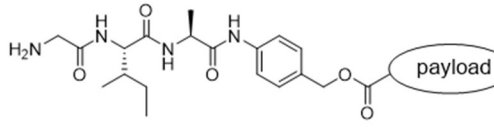

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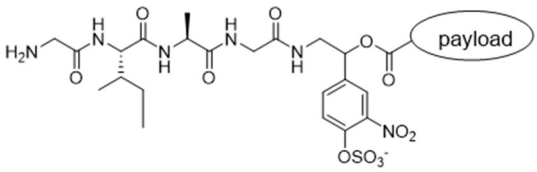

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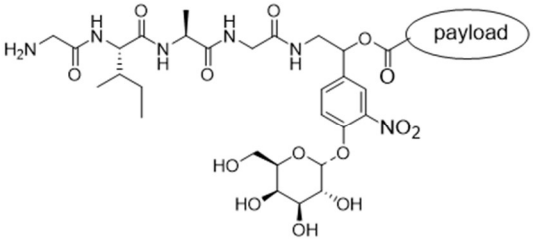

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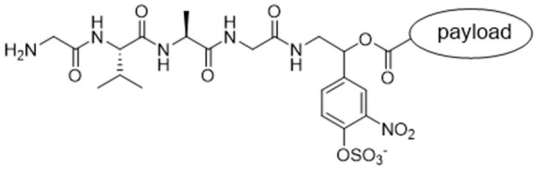

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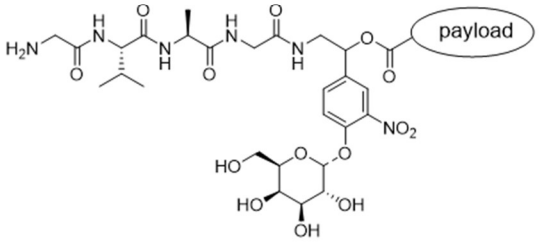

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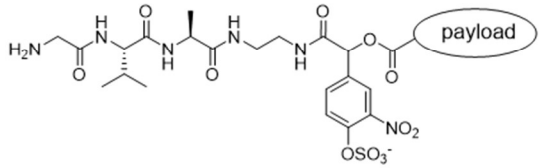
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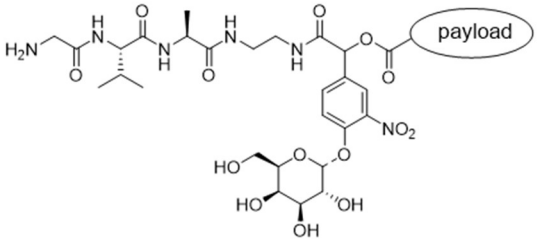

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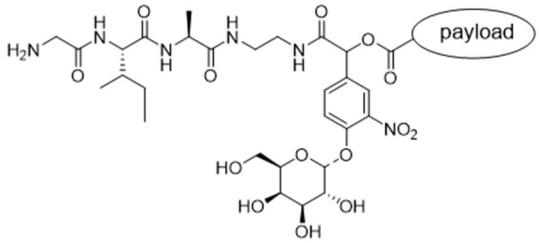

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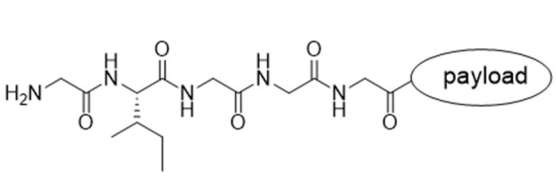

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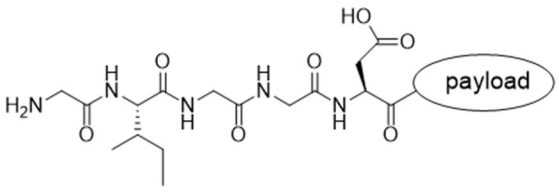

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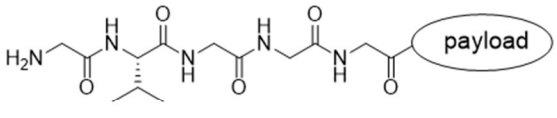

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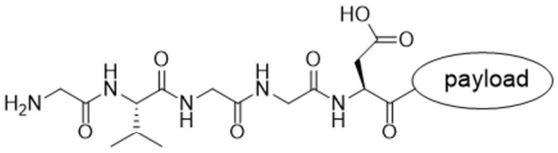

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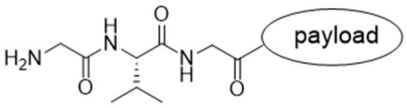

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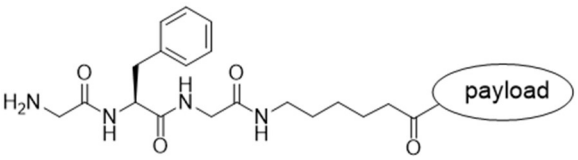


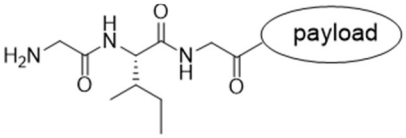


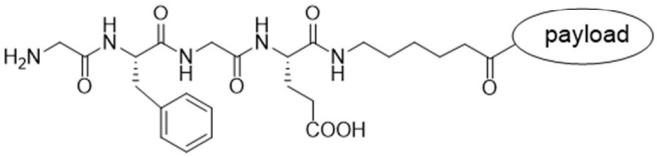


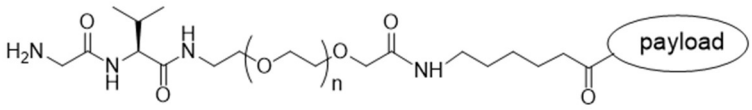




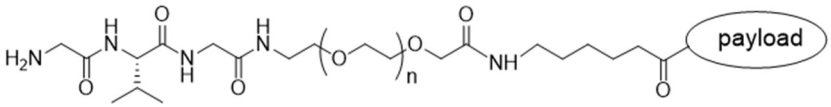






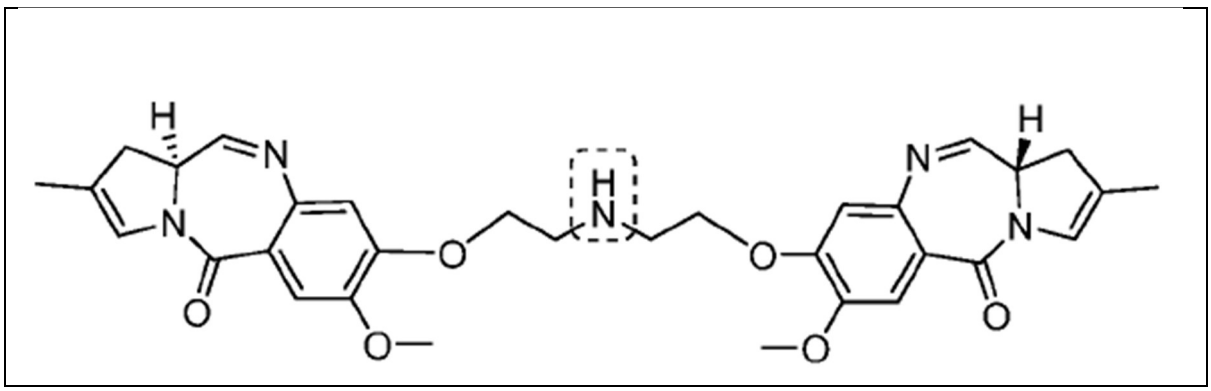


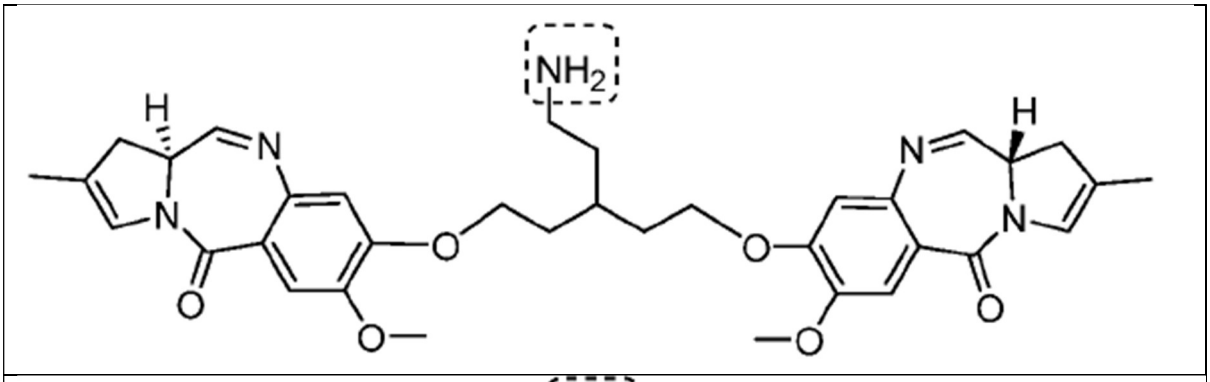
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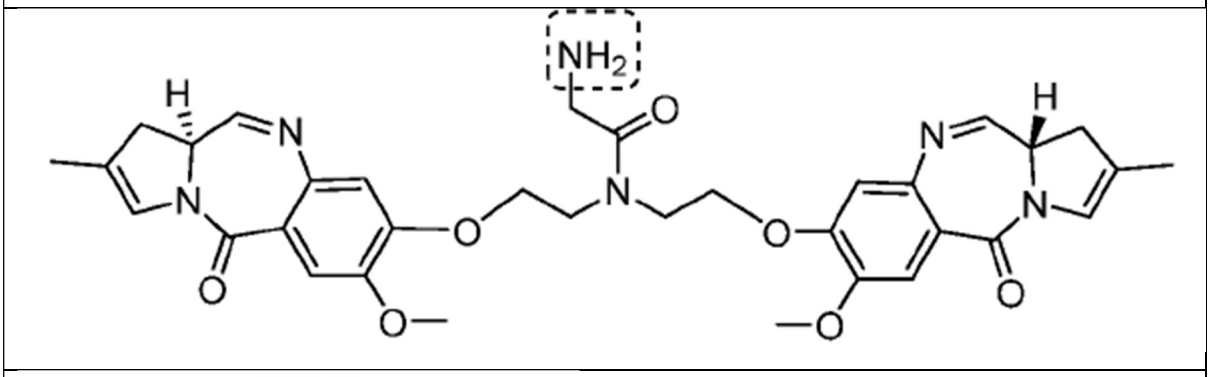


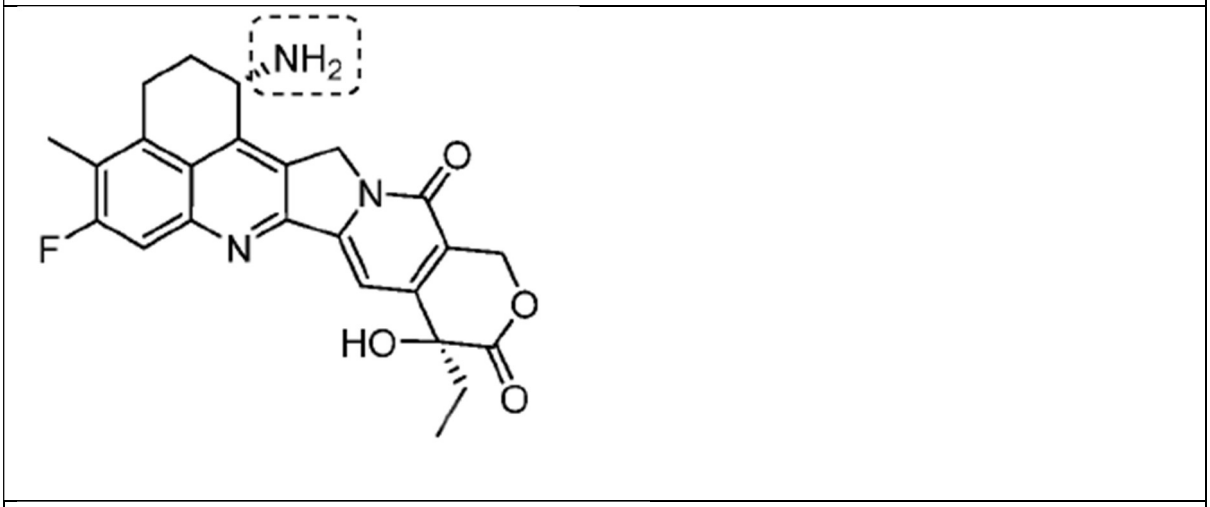
Claim 16. The method or ligated product according to claim 15, wherein 

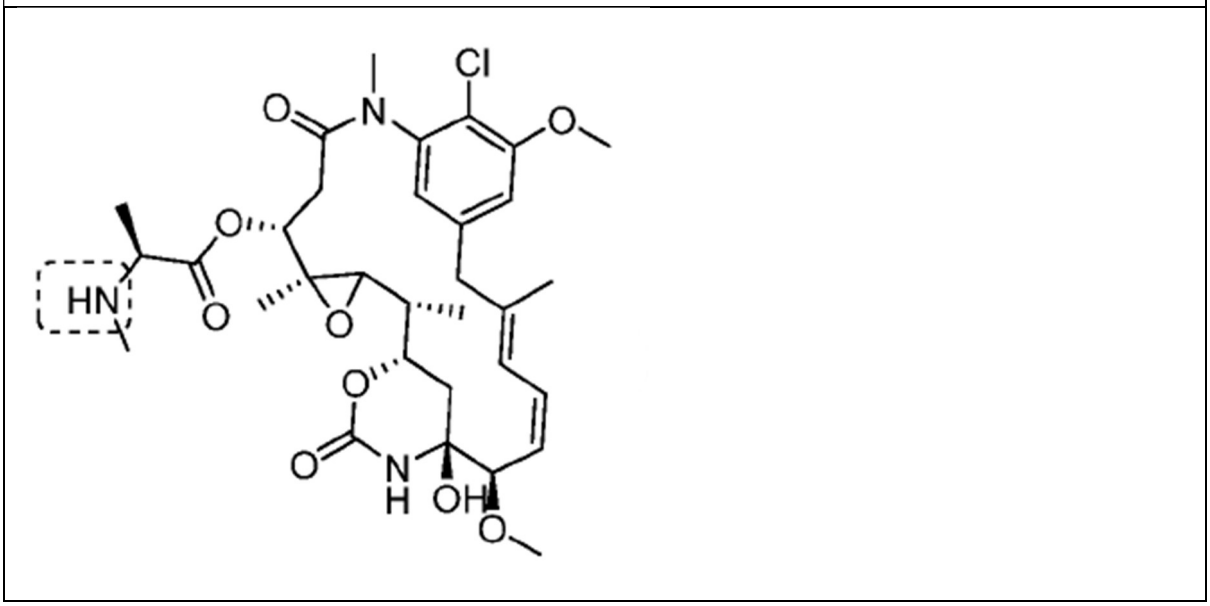
is a drug linked via an amine group in the drug, and the drug is selected from:

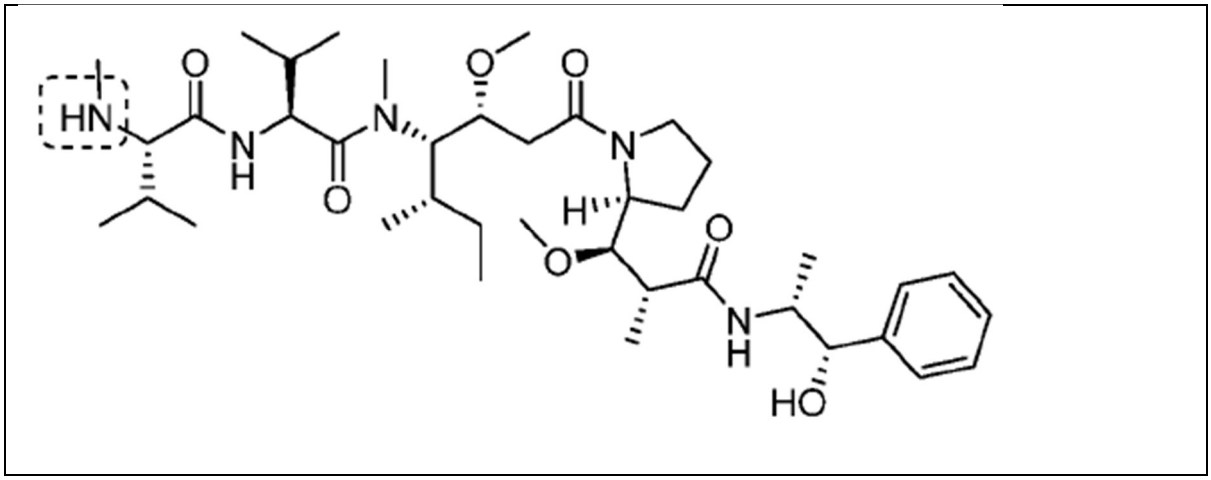



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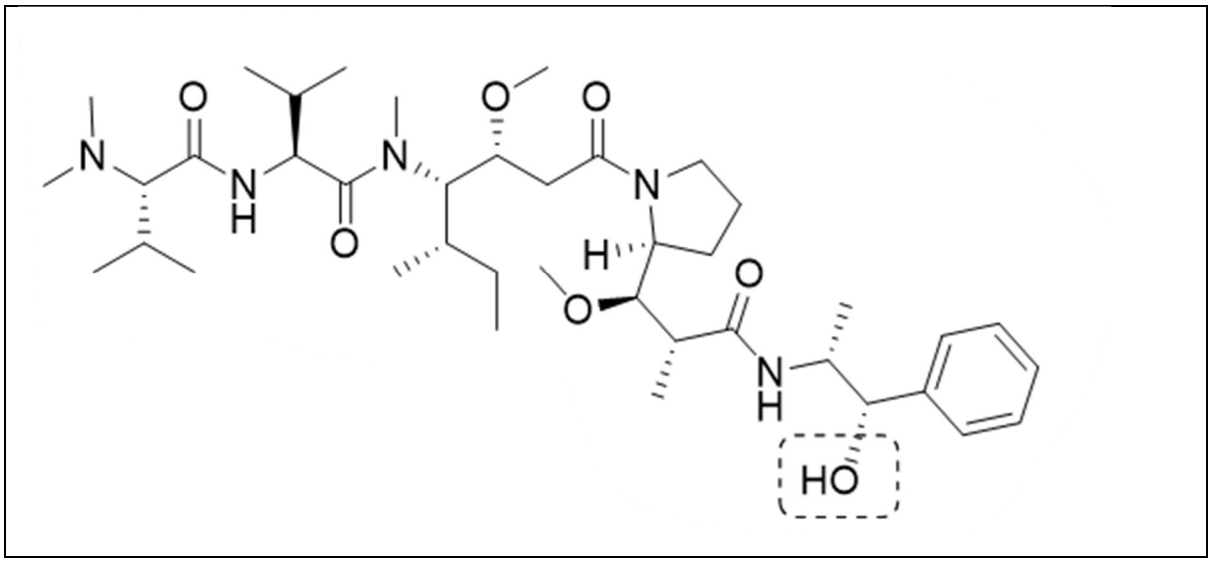

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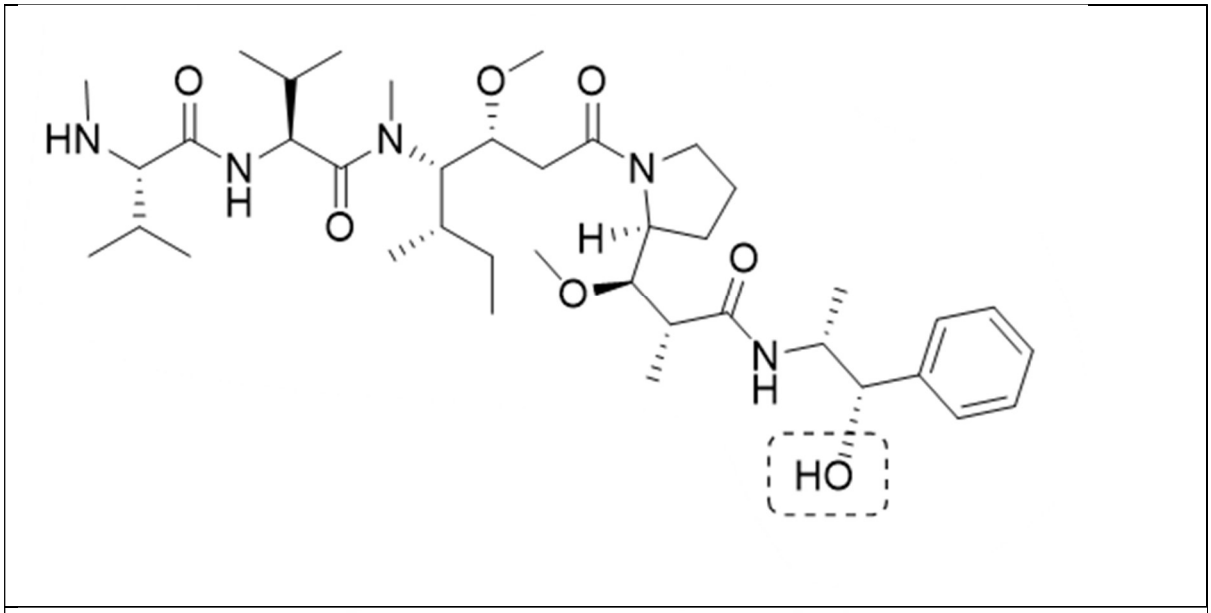

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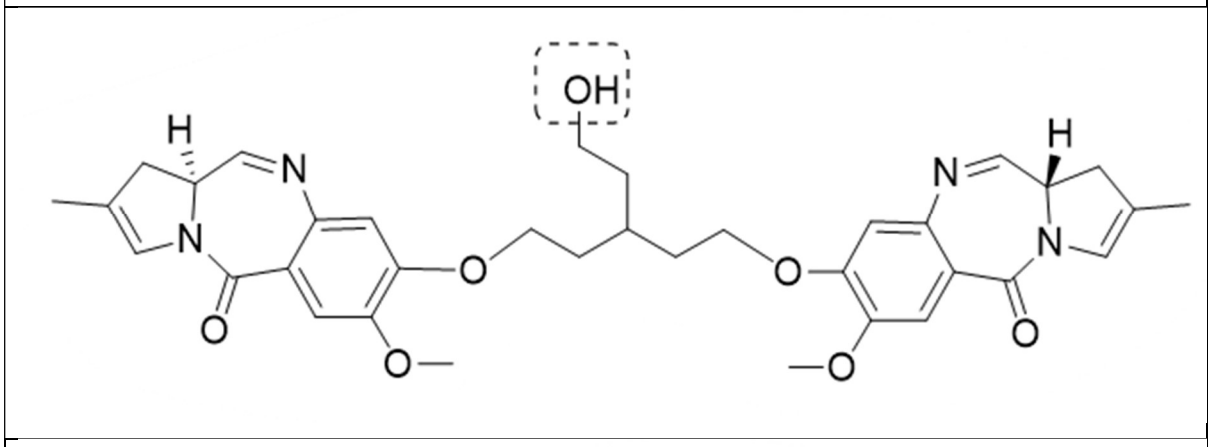


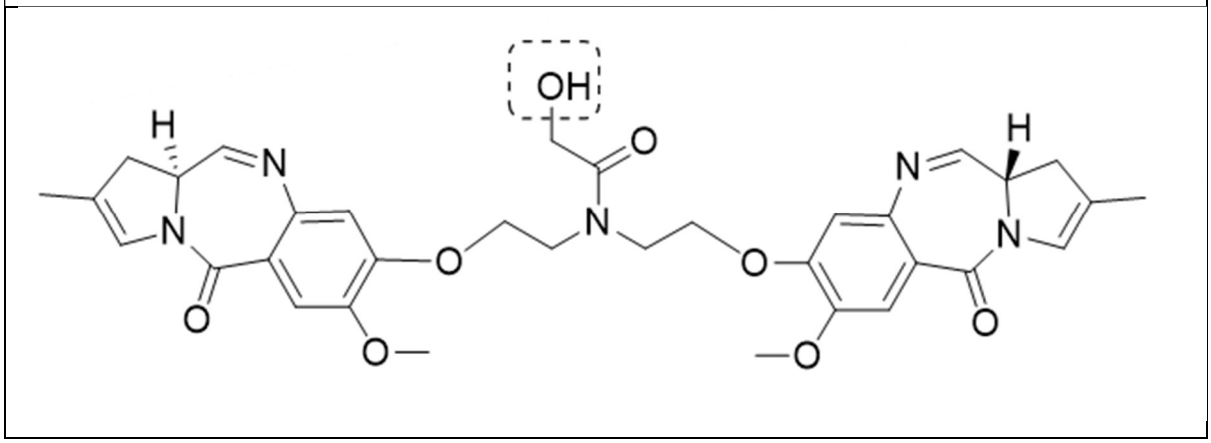
wherein ⬚ indicates the amine group.

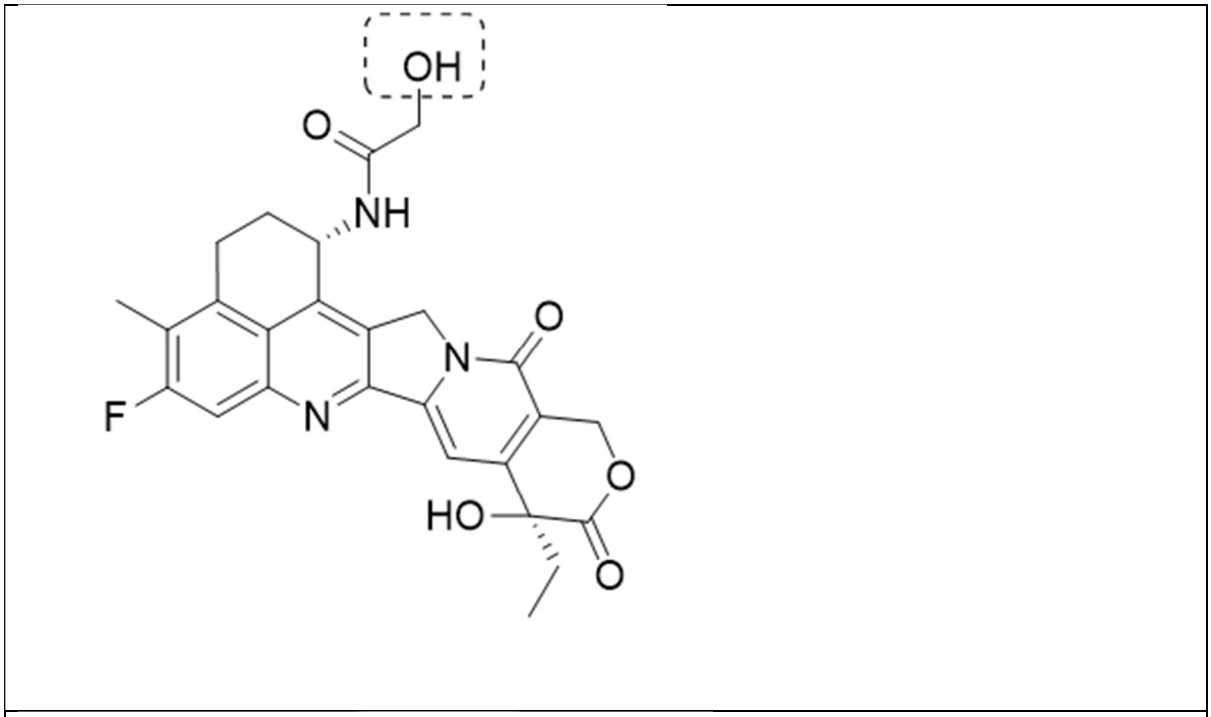
Claim 17. The method according to any of claims 6 and 8 to 13 or the ligated product according to any one of claims 7 to 13, wherein the payload comprises a drug linked to the ligated product via a hydroxy group in the drug, and the drug is selected from:

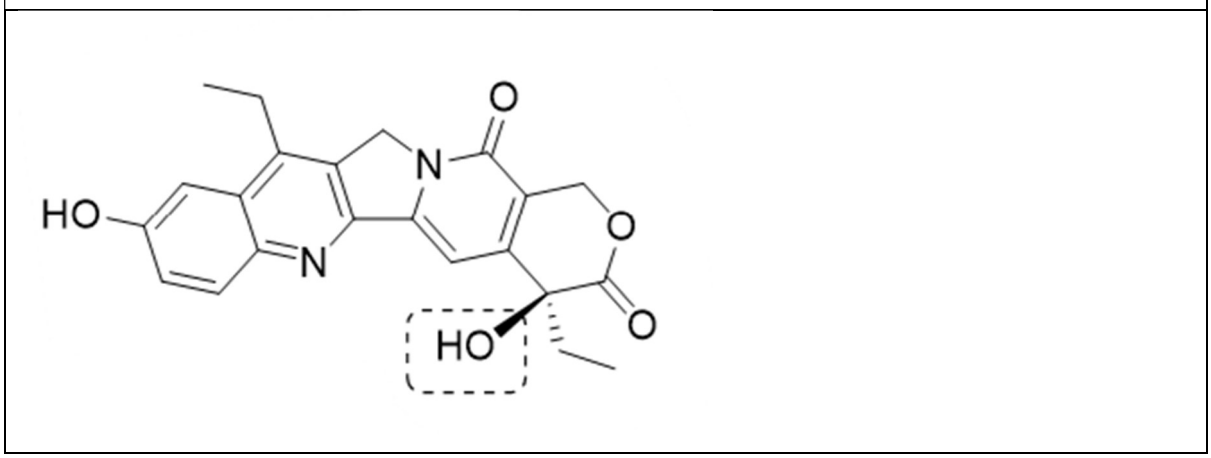


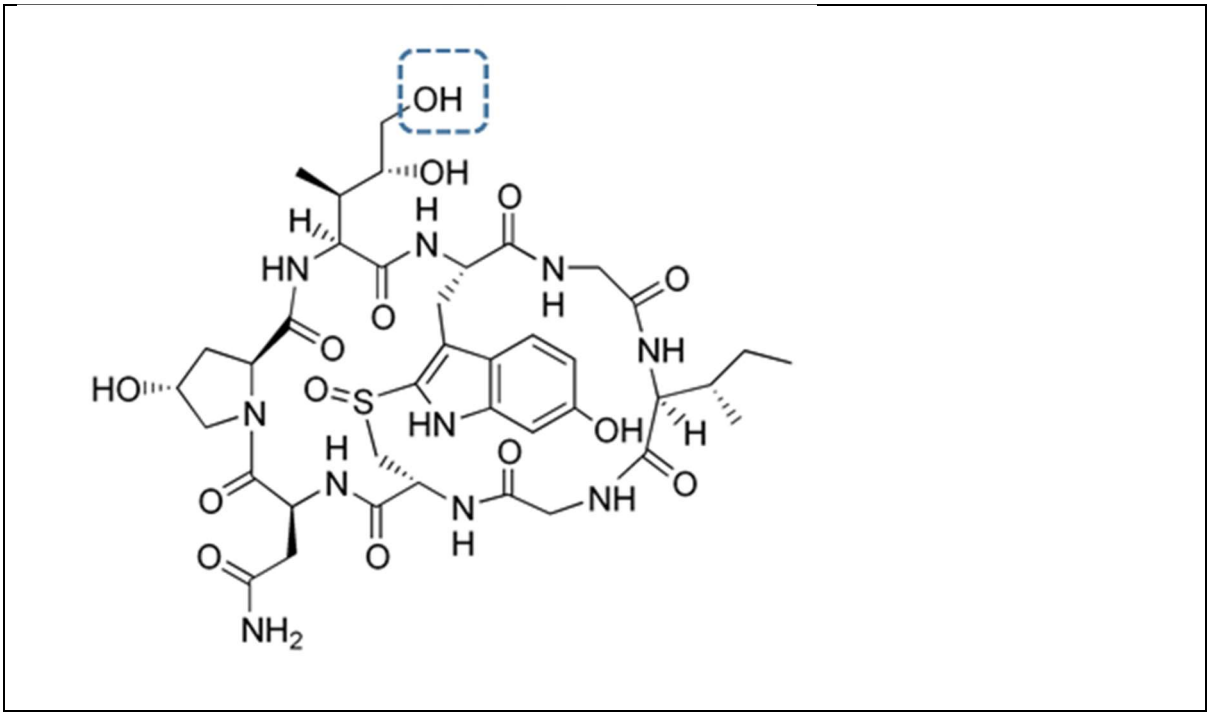

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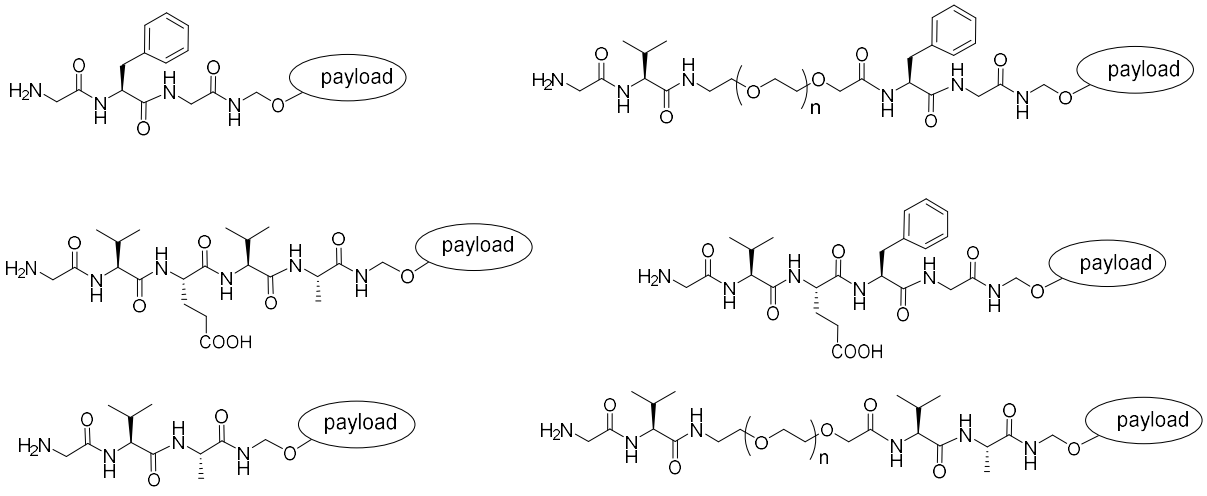

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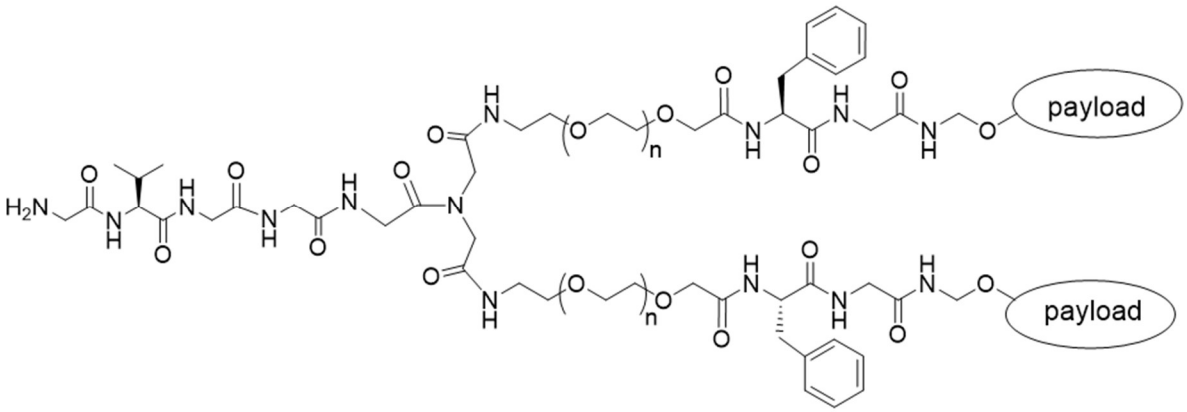


wherein ⬚ indicates the hydroxy group, and the hydroxy group can be modified with a linker.

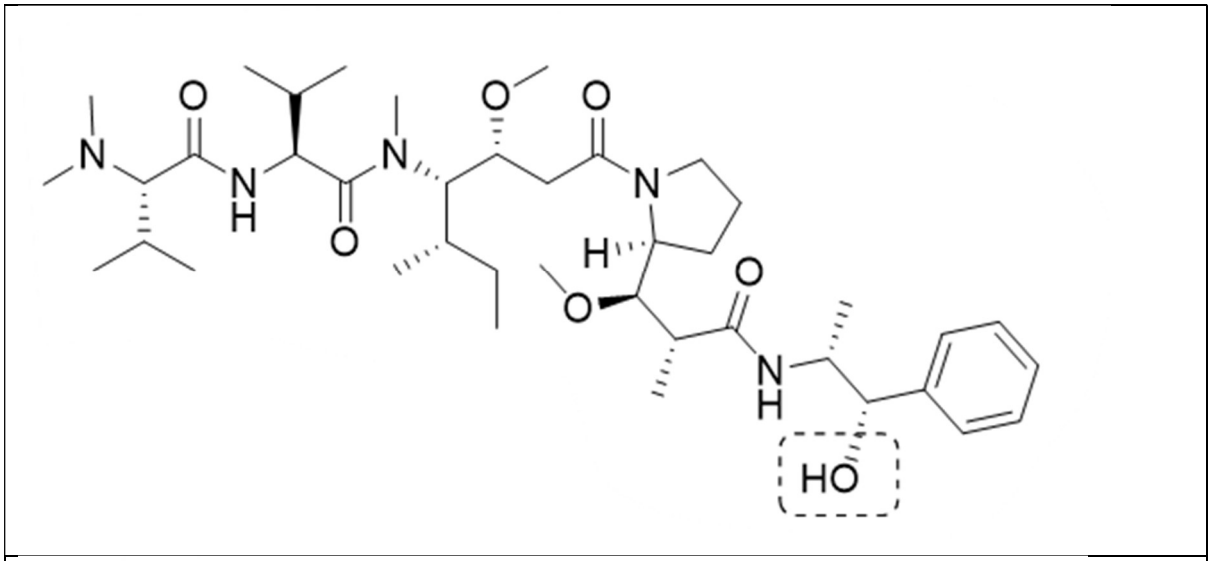
Claim 18. The method according to any of claims 6 and 8 to 13 or the ligated product according to any one of claims 7 to 13, wherein the peptide or protein comprising a payload is selected from:

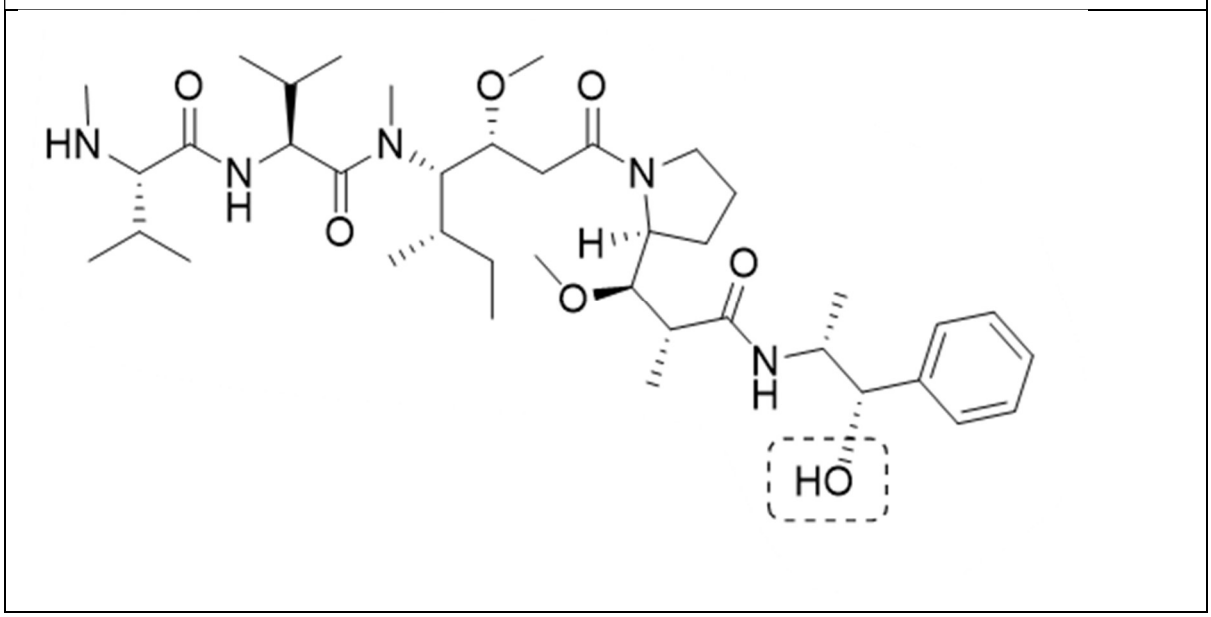


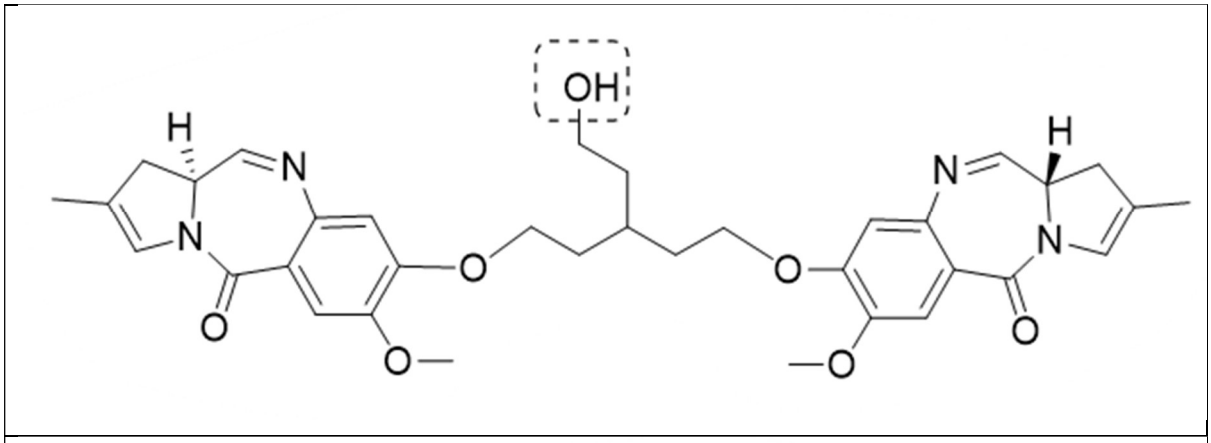
And

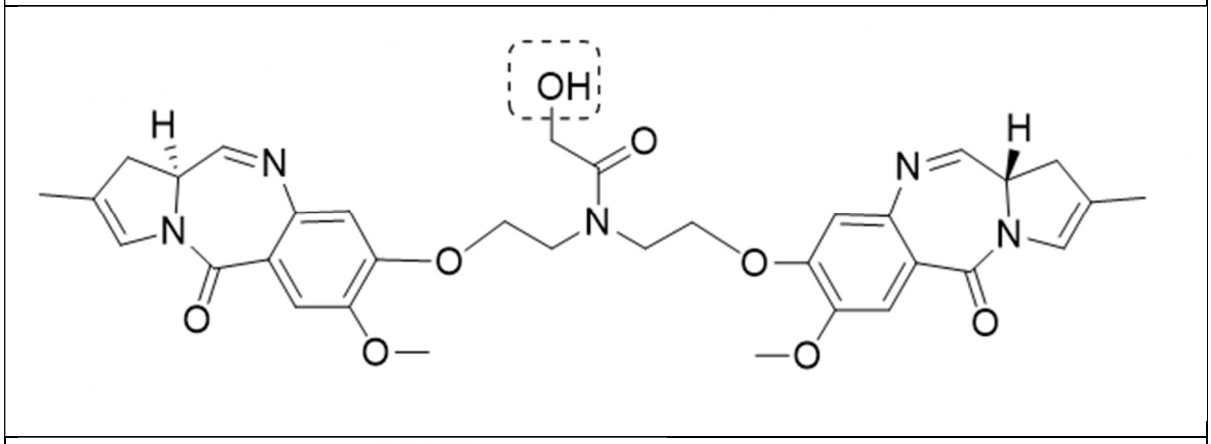


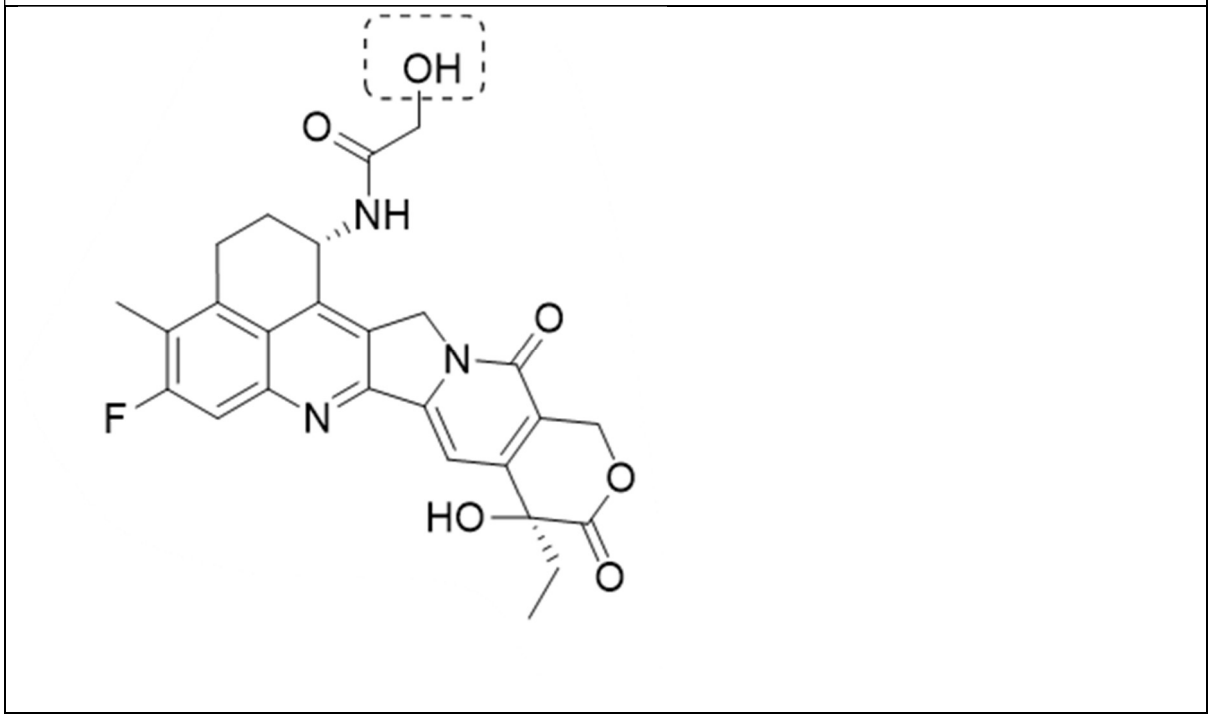
Claim 19. The method or ligated product according to claim 18, wherein payload is a drug linked via a hydroxy group in the drug, and the drug is selected from:

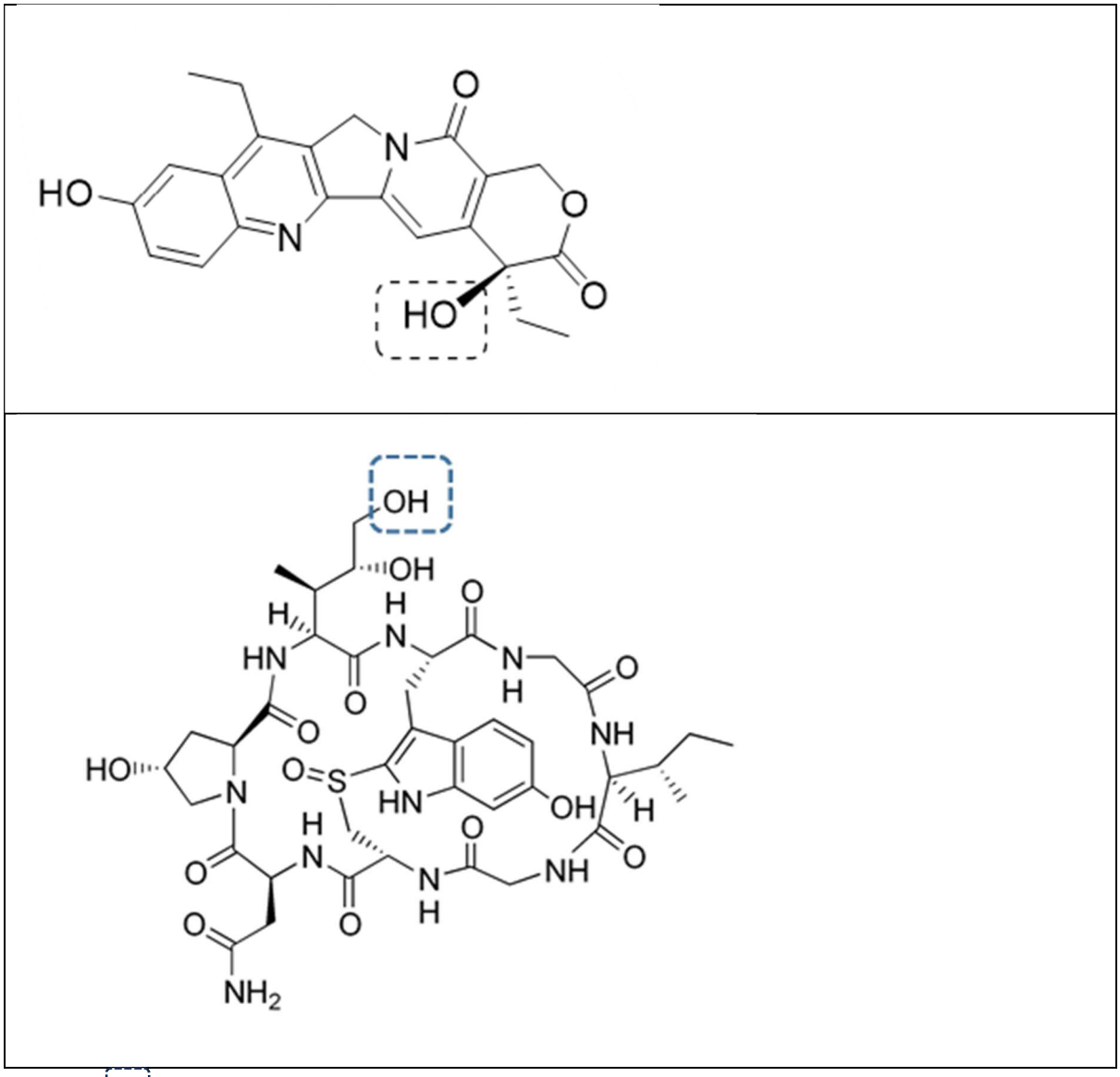





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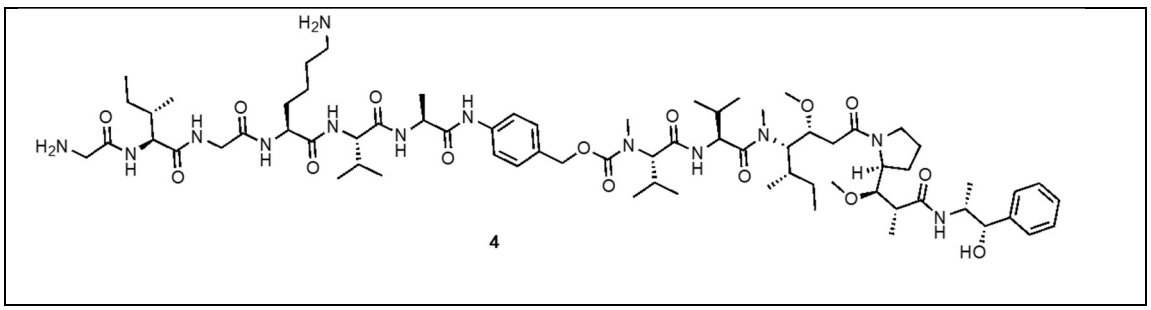

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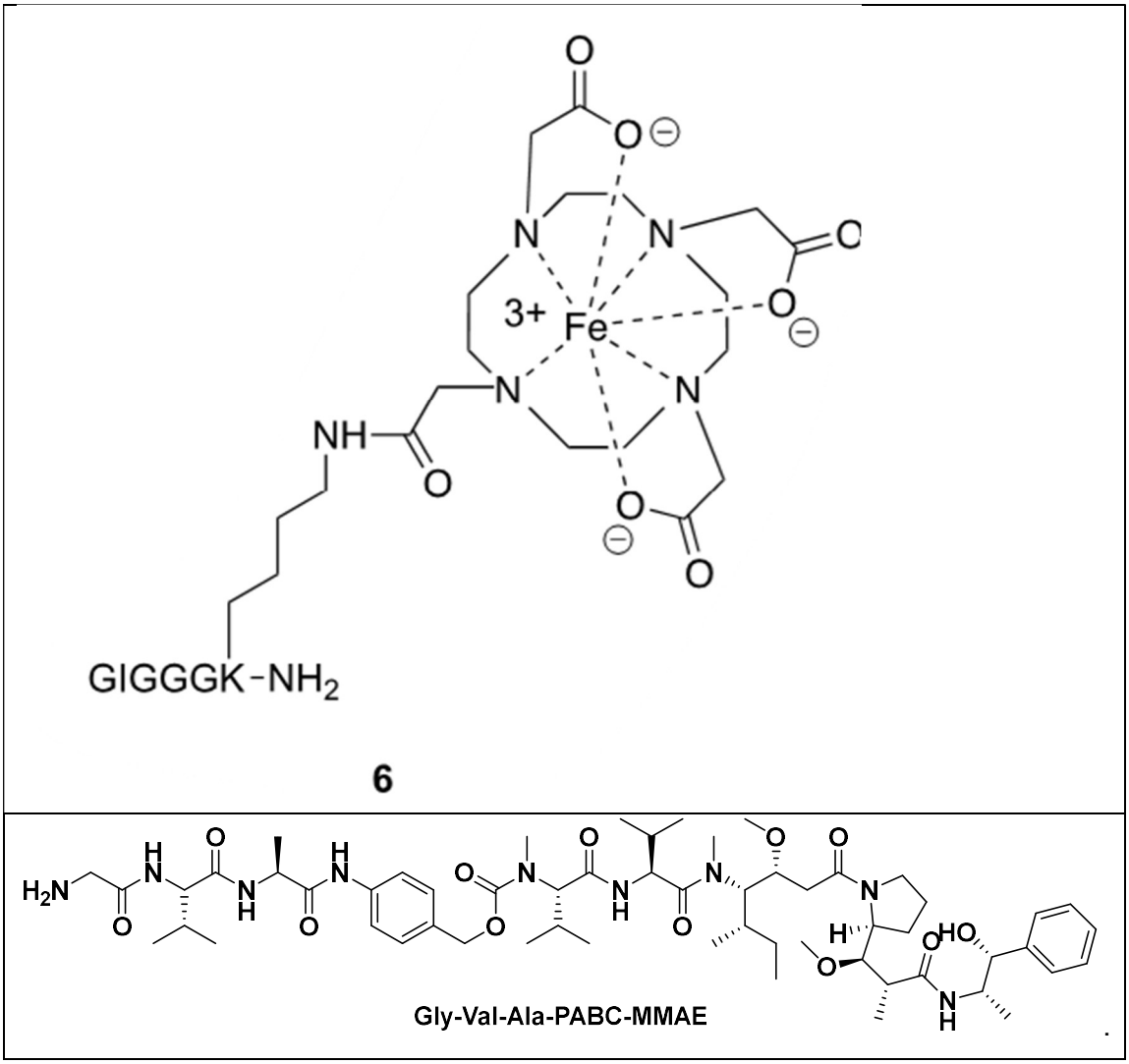


wherein [ ] indicates the hydroxy group.

Claim 20. The method according to any of claims 6 and 8 to 13 or the ligated product according to any one of claims 7 to 13, wherein the peptide comprising a payload is selected from:



4



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Gly-Val-Ala-PABC-MMAE