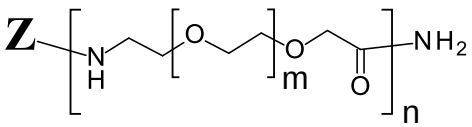
environmentally friendly organic solvent. Polyethylene glycol (PEG) has been reported as a hydrophilic support for liquid-phase peptide synthesis (see *e.g.*, Fischer, P.M.; Zheleva, D.I. (2002) *Journal of Peptide Science* 8:529-542). However, PEG derivatives used in these applications are often polydisperse and lack a fixed molecular weight. Variable PEG chain lengths can create complications in analysis and purification of high molecular weight PEG conjugation products. Accordingly, it would be an improvement to find new hydrophilic linker systems with a fixed molecular weight for LPPS which would address these deficiencies, especially in the commercial manufacturing of peptides. It would be a further advancement if such a system could enable a liquid phase peptide synthetic method for long peptides (15-mer and above). In fact, the present embodiments will specifically provide hydrophilic linker systems for tag-assisted LPPS and methods of use thereof for the synthesis of peptides on a commercial scale. Such methods and systems are disclosed herein.

SUMMARY

In a first aspect of the present disclosure, there is provided a compound of the formula



wherein "m" is 0 to 20, "n" is 1 to 50, and "Z" is a linker compound.

In another aspect of the present disclosure, there is provided a peptide of SEQ ID NO: 1, 3, 5, 6, 8, 9, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23.

In yet another aspect of the present disclosure, there is provided a method of preparation of the peptide of SEQ ID NO: 2, comprising preparing the peptide of SEQ ID NO: 1 using liquid-phase peptide synthesis, and then treating the prepared peptide of SEQ ID NO: 1 with an acid to give the peptide of SEQ ID NO: 2.

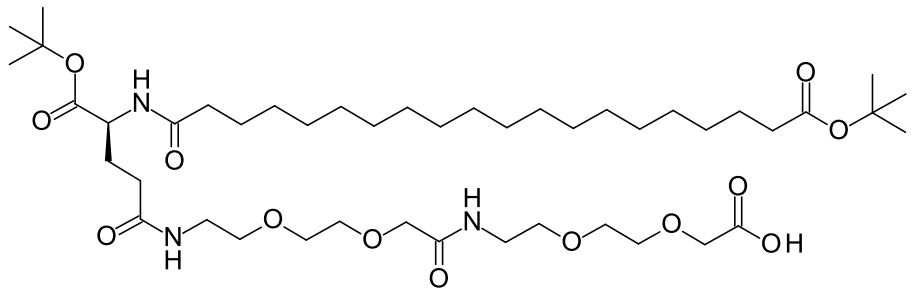
In a further aspect of the present disclosure, there is provided a method of preparation of the peptide of SEQ ID NO: 4, comprising preparing the peptide of SEQ ID NO: 3 using liquid-phase peptide synthesis, and then treating the prepared peptide of SEQ ID NO: 3 with an acid to give the peptide of SEQ ID NO: 4.

In another aspect of the present disclosure, there is provided a method of preparation of the peptide of SEQ ID NO: 7, comprising preparing the peptide of SEQ ID NO: 6 using liquid-phase peptide synthesis, and then treating the prepared peptide of SEQ ID NO: 6 with an acid to give the peptide of SEQ ID NO: 7.

In yet another aspect of the present disclosure, there is provided a method of preparation of the peptide of SEQ ID NO: 10, comprising preparing the peptide of SEQ ID NO: 9 using liquid-phase peptide synthesis, and then treating the prepared peptide of SEQ ID NO: 9 with an acid to give the peptide of SEQ ID NO: 10.

In a further aspect of the present disclosure, there is provided a method of preparation of the peptide of SEQ ID NO: 13, comprising preparing the peptide of SEQ ID NO: 11, 12, 14, 15, 16, 17, or 18 using liquid-phase peptide synthesis, and then treating the prepared peptide of SEQ ID NO: 11, 12, 14, 15, 16, 17, or 18 with an acid to give the peptide of SEQ ID NO: 13.

In another aspect of the present disclosure, there is provided a method for preparing a compound of the formula:



comprising preparing the compound using liquid phase synthesis, wherein the liquid phase synthesis includes coupling an AEEA containing molecule to a compound as disclosed herein.

In yet another aspect of the present disclosure, there is provided a method for preparing the peptide of SEQ ID NO: 32, comprising preparing part or all of the peptide sequence by liquid-phase peptide synthesis using a compound as disclosed herein.

In a further aspect of the present disclosure, there is provided a method for preparing the peptide of SEQ ID NO: 20, comprising coupling the peptide of SEQ ID NO: 19 with the peptide of SEQ ID NO: 7.

In another aspect of the present disclosure, there is provided a method for preparing the peptide of SEQ ID NO: 22, comprising coupling the peptide of SEQ ID NO: 21 with the peptide of SEQ ID NO: 10.

In yet another aspect of the present disclosure, there is provided a method for preparing the peptide of SEQ ID NO: 32, comprising the following steps:

a. preparing the peptide of SEQ ID NO: 19 by liquid-phase peptide synthesis using a compound as disclosed herein;

b. coupling the peptide of SEQ ID NO: 19 to the peptide of SEQ ID NO: 7 to generate the peptide of SEQ ID NO: 20;

c. deprotecting the peptide of SEQ ID NO: 20 to generate the peptide of SEQ ID NO: 21;

d. coupling the peptide of SEQ ID NO: 10 to the peptide of SEQ ID NO: 21; and

e. deprotecting the peptide from step d. to generate the peptide of SEQ ID NO: 22.

In a further aspect of the present disclosure, there is provided a method for preparing the peptide of SEQ ID NO: 32 comprising the following steps:

a. coupling the peptide of SEQ ID NO: 24 to a compound as disclosed herein and deprotecting the resulting peptide;

b. coupling the peptide from step a. to the peptide of SEQ ID NO: 25 and deprotecting the resulting peptide;

c. coupling the peptide from step b. with the peptide of SEQ ID NO: 26 and deprotecting the resulting peptide;

d. coupling the peptide from step c. with the peptide of SEQ ID NO: 27 and deprotecting the resulting peptide;

e. coupling the peptide from step d. with the peptide of SEQ ID NO: 28 and deprotecting the resulting peptide;

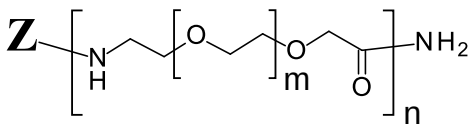
f. coupling the peptide from step e. with the peptide of SEQ ID NO: 29 and deprotecting the resulting peptide;

g. coupling the peptide from step f. with the peptide of SEQ ID NO: 30 and deprotecting the resulting peptide; and

h. coupling the peptide of step g. with the peptide of SEQ ID NO: 31 and deprotecting the resulting peptide.

The present examples provide compounds of a fixed molecular weight which are useful as hydrophilic linker constructs for liquid phase organic synthesis such as LPPS. Compounds of the present disclosure feature repeating heterobifunctional PEG-like units attached to a linker, upon which a polypeptide or other molecule can be built through coupling (e.g., amino acid coupling) and deprotection steps. In particular, compounds of the present disclosure can be used to build polypeptides or other molecules through repeated synthetic steps, e.g., amino acid coupling and deprotection steps.

An example of the present disclosure comprises hydrophilic linker compounds of Formula 1:



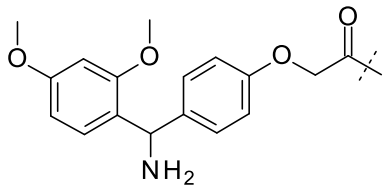
(1)

wherein "m" is 0 to 20, “n” is 1 to 50, and “Z” is a linker group. “Z” is a functional group which can form a covalent bond to an optionally protected compound such as an amino acid, which can in turn undergo iterative deprotection and coupling steps onto one or more optionally protected compounds such as amino acids or peptides, and then the

resulting product such as a polypeptide product is able to be liberated from the “Z” group through chemical transformation.

Another example of the present disclosure comprises a compound of Formula 1 wherein “m” is 0, 1, 2, or 3 and “n” is 1 to 50. Another example of the present disclosure comprises a compound of Formula 1 wherein “m” is 0, 1, 2, or 3 and “n” is 1 to 10. Another example of the present disclosure comprises a compound of Formula 1 wherein “m” is 1 and “n” is 2 to 10.

A further example of the present disclosure comprises a compound of Formula 1 wherein “Z” is selected from: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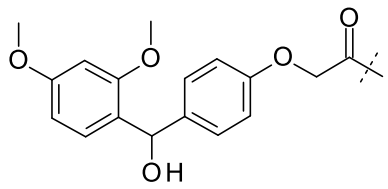
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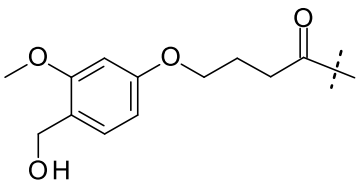
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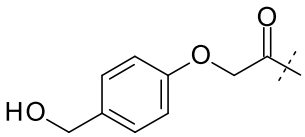
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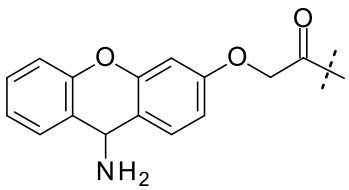
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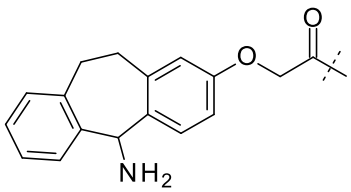
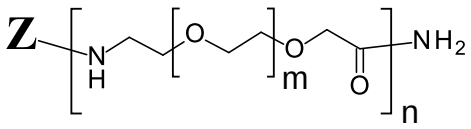

; and “m” and “n” are as defined above.

The hydrophilic linkers of the present disclosure are useful for the synthesis of peptides and other compounds, wherein the activated ester of compounds such as an amino acid or peptide fragment is first coupled onto the free alcohol -OH or amine -NH_2 of a linker of Formula 1. Thereafter the compound, e.g., peptide, is grown by coupling activated esters of individual molecule components such as amino acids or peptide fragments consecutively after intermediary deprotections. The finished compounds, e.g., peptides are cleaved from the linker with acidic conditions.

DETAILED DESCRIPTION

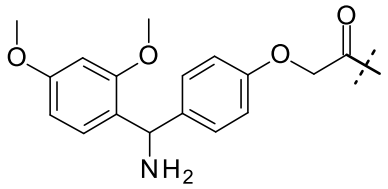
What is claimed is:

1. A compound of the formula



wherein “m” is 0 to 20, “n” is 1 to 50, and “Z” is a linker compound.

2. The compound of claim 1, wherein **Z** is selected from the group consisting of: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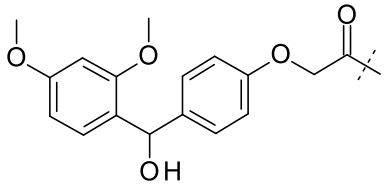
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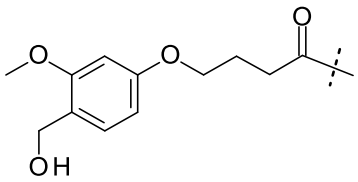
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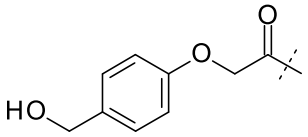
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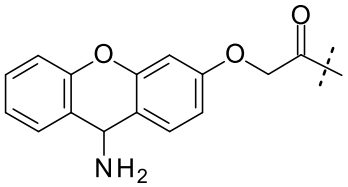
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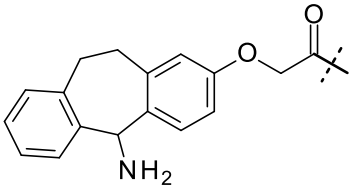
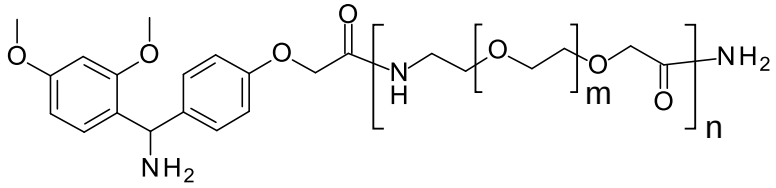

and .

3. The compound of claim 2, wherein the compound is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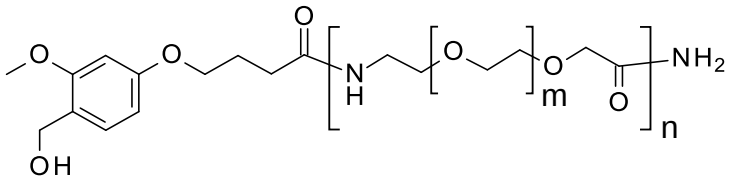

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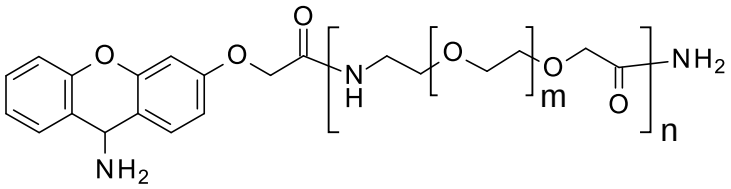

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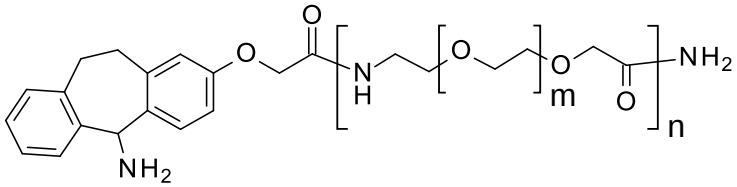

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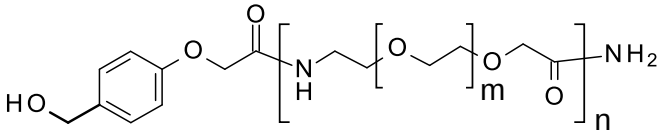

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4. The compound of any of claims 1 to 3, wherein

1) "m" is 0, 1, 2, or 3, and "n" is 1 to 50;

2) "m" is 0, 1, 2, or 3, and "n" is 1 to 10; or

3) "m" is 1 and "n" is 2, 3, 4, 5, 6, 7, 8, 9, or 10.

5. The compound of claim 1, wherein the compound is used in liquid phase synthesis.

6. The compound of claim 1, wherein the liquid phase synthesis is Liquid Phase Peptide Synthesis (LPPS).

7. The compound of claim 1, wherein the compound is hydrophilic.

8. The compound of claim 1, wherein "Z" is a functional group which forms a covalent bond to an optionally protected amino acid, which can in turn undergo iterative

deprotection and coupling steps onto one or more optionally protected amino acids or peptides, and then the resulting polypeptide product is able to be liberated from the "Z" group through chemical transformation.

9. A peptide of SEQ ID NO: 1, 3, 5, 6, 8, 9, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23.

10. A method of preparation of the peptide of SEQ ID NO: 2, comprising preparing the peptide of SEQ ID NO: 1 using liquid-phase peptide synthesis, and then treating the prepared peptide of SEQ ID NO: 1 with an acid to give the peptide of SEQ ID NO: 2.

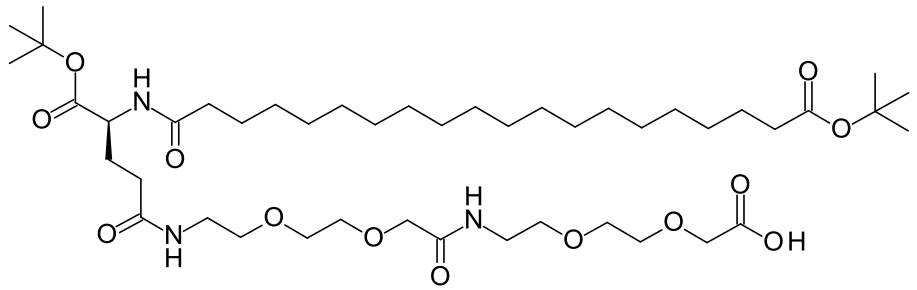
11. A method of preparation of the peptide of SEQ ID NO: 4, comprising preparing the peptide of SEQ ID NO: 3 using liquid-phase peptide synthesis, and then treating the prepared peptide of SEQ ID NO: 3 with an acid to give the peptide of SEQ ID NO: 4.

12. A method of preparation of the peptide of SEQ ID NO: 7, comprising preparing the peptide of SEQ ID NO: 6 using liquid-phase peptide synthesis, and then treating the prepared peptide of SEQ ID NO: 6 with an acid to give the peptide of SEQ ID NO: 7.

13. A method of preparation of the peptide of SEQ ID NO: 10, comprising preparing the peptide of SEQ ID NO: 9 using liquid-phase peptide synthesis, and then treating the prepared peptide of SEQ ID NO: 9 with an acid to give the peptide of SEQ ID NO: 10.

14. A method of preparation of the peptide of SEQ ID NO: 13, comprising preparing the peptide of SEQ ID NO: 11, 12, 14, 15, 16, 17, or 18 using liquid-phase peptide synthesis, and then treating the prepared peptide of SEQ ID NO: 11, 12, 14, 15, 16, 17, or 18 with an acid to give the peptide of SEQ ID NO: 13.

15. A method for preparing a compound of the formula:



comprising preparing the compound using liquid phase synthesis, wherein the liquid phase synthesis includes coupling an AEEA containing molecule to a compound of claim 1.

16. The method of claim 15, comprising:

1)

a. coupling Fmoc-AEEA-OH onto a linker compound of any of claims 1-8 and deprotecting the resulting product using a base;

b. coupling a second Fmoc-AEEA-OH onto the product from step a. and deprotecting the resulting product using a base;

c. coupling Fmoc-Glu-O*t*Bu onto the product of step b. and deprotecting the resulting product using a base;

d. coupling 20-(*tert*-butoxy)-20-oxoicosanoic acid onto the product of step c.; and

e. removing the linker compound from the product of step d. with an acid,

2)

a. coupling Fmoc-$(AEEA)_2$-OH onto a linker compound of any of claims 1-8 and deprotecting the resulting product using a base;

b. coupling Fmoc-Glu-O*t*Bu onto the product of step a. and deprotecting the resulting product using a base;

c. coupling 20-(*tert*-butoxy)-20-oxoicosanoic acid onto the resulting product of step b.; and

d. removing the linker compound from the product of step c. with an acid, or

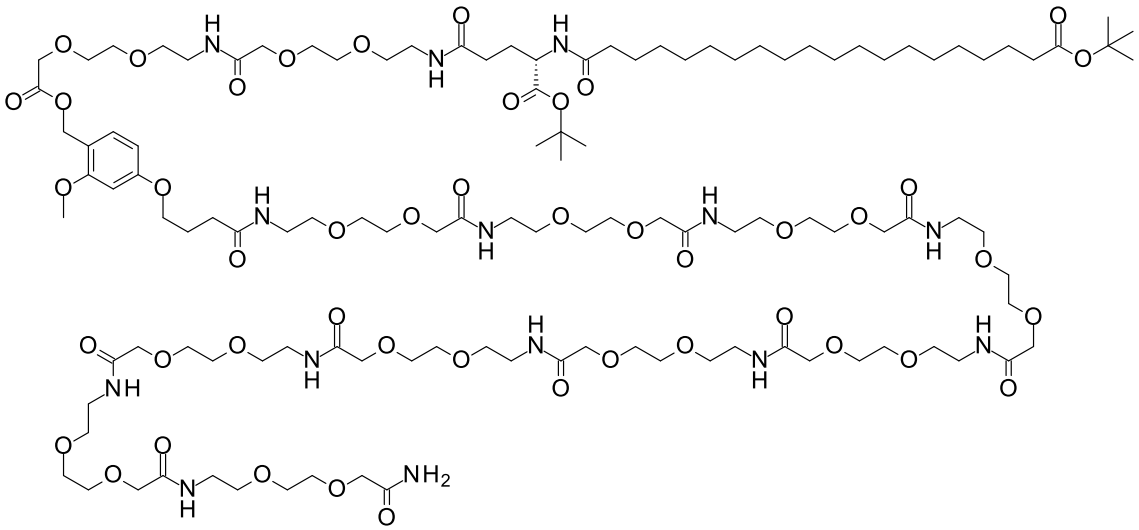
3)

a. coupling Fmoc-$(AEEA)_2$-OH onto the linker compound of any of claims 1-8 and deprotecting the resulting product using a base;

b. coupling O1-*tert*-butyl O5-(2,5-dioxopyrrolidin-1-yl) (2S)-2-[(20-*tert*-butoxy-20-oxo-icosanoyl)amino]pentanedioate onto the resulting product of step a.; and

c. removing the linker compound from the product of step b. with an acid.

17. The compound which is



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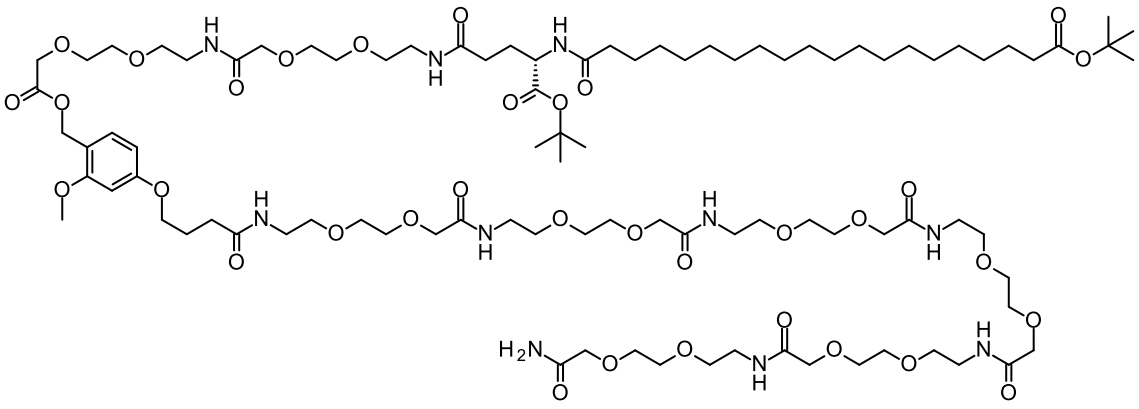

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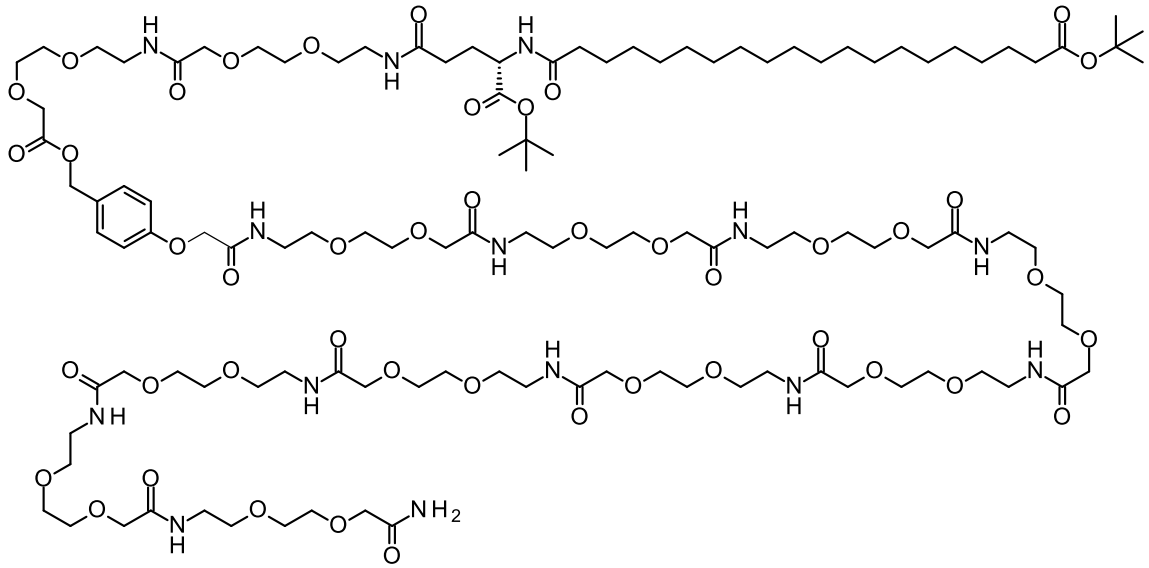

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18. A method for preparing the peptide of SEQ ID NO: 32, comprising preparing part or all of the peptide sequence by liquid-phase peptide synthesis using a compound according to claim 1.

19. A method for preparing the peptide of SEQ ID NO: 20, comprising coupling the peptide of SEQ ID NO: 19 with the peptide of SEQ ID NO: 7.

20. A method for preparing the peptide of SEQ ID NO: 22, comprising coupling the peptide of SEQ ID NO: 21 with the peptide of SEQ ID NO: 10.

21. A method for preparing the peptide of SEQ ID NO: 32, comprising the following steps:

a. preparing the peptide of SEQ ID NO: 19 by liquid-phase peptide synthesis using a compound according to claim 1;

b. coupling the peptide of SEQ ID NO: 19 to the peptide of SEQ ID NO: 7 to generate the peptide of SEQ ID NO: 20;

c. deprotecting the peptide of SEQ ID NO: 20 to generate the peptide of SEQ ID NO: 21;

d. coupling the peptide of SEQ ID NO: 10 to the peptide of SEQ ID NO: 21; and

e. deprotecting the peptide from step d. to generate the peptide of SEQ ID NO: 22.

22. The method of claim 21, further comprising:

f. elongating the peptide of SEQ ID NO: 22 from step e. by coupling individual amino acids, peptide fragments, or mixtures thereof;

g. treating the resulting peptide with an acid; and

h. deprotecting the resulting peptide.

23. A method for preparing the peptide of SEQ ID NO: 32 comprising the following steps:

a. coupling the peptide of SEQ ID NO: 24 to a compound according to claim 1 and deprotecting the resulting peptide;

b. coupling the peptide from step a. to the peptide of SEQ ID NO: 25 and deprotecting the resulting peptide;

c. coupling the peptide from step b. with the peptide of SEQ ID NO: 26 and deprotecting the resulting peptide;

d. coupling the peptide from step c. with the peptide of SEQ ID NO: 27 and deprotecting the resulting peptide;

e. coupling the peptide from step d. with the peptide of SEQ ID NO: 28 and deprotecting the resulting peptide;

f. coupling the peptide from step e. with the peptide of SEQ ID NO: 29 and deprotecting the resulting peptide;

g. coupling the peptide from step f. with the peptide of SEQ ID NO: 30 and deprotecting the resulting peptide; and

h. coupling the peptide of step g. with the peptide of SEQ ID NO: 31 and deprotecting the resulting peptide.