CLAIMS

What is claimed is:

1. Method of forming a peptide of Formula (I)

$$P^1\text{-Asx-Xaa}^1\text{-Xaa}^2\text{-}P^2 \quad \text{(I)}$$

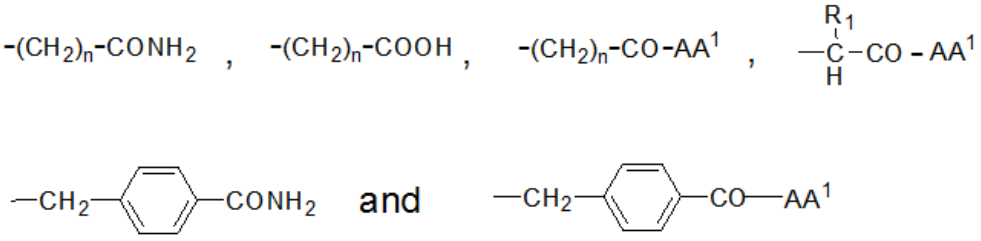
by ligating a first peptide of Formula (II)

$$P^1\text{-Asx-X-R} \quad \text{(II)}$$

to a second peptide of Formula (III)

$$\text{Xaa}^1\text{-Xaa}^2\text{-}P^2 \quad \text{(III)},$$

wherein P^1 and P^2 are each independently any peptide, modified or unmodified; Asx is Asp or Asn, preferably Asn; X is O or S, preferably S; R is a substituted or unsubstituted alkyl, preferably selected from the group consisting of



wherein n is an integer of 1 to 10, R_1 is H, or any other side chain of a naturally occurring amino acid, and AA^1 is any amino acid or is absent; Xaa^1 is any naturally occurring amino acid with the exception of Pro; Xaa^2 is any naturally occurring amino acid, but preferably is a hydrophobic amino acid or Cys, more preferably Val, Ile, Leu, or Cys,

by enzymatically cleaving the bond between “Asx” and “X” in the first peptide of Formula (II) and ligating the fragment P^1-Asx of the first peptide to the second peptide of Formula (III) to form a ligated peptide of Formula (I), wherein the enzymatic cleavage and ligation reaction is catalyzed by a polypeptide having the activity of butelase 1 (SEQ ID NO:1) under conditions allowing said ligation.

2. The method according to claim 1, wherein Asx is Asn, X is S, R is -CH(R_1)-CO-AA^1, R_1 is H, and AA^1 is any amino acid or absent.

3. The method according to claim 1 or 2, wherein Asx is Asn, X is S, R is -CH(R_1)-CO-AA^1, R_1 is H, and AA^1 is Val.

4. The method according to any one of claims 1-3, wherein the polypeptide having the ligase activity of butelase 1 (SEQ ID NO:1) comprises or consists of:
   (a) the amino acid sequence as set forth in SEQ ID NO:1 (butelase 1);
   (b) an amino acid sequence that shares at least 60, preferably at least 70, even more preferably at least 80, most preferably at least 90 % sequence identity with the amino acid sequence as set forth in SEQ ID NO:1; or
   (c) an amino acid sequence that shares at least 80, preferably at least 90, more preferably at least 95 % sequence homology with the amino acid sequence as set forth in SEQ ID NO:1; or
   (d) a fragment of any one of (a)-(c).

5. The method according to any one of claims 1-4, wherein the polypeptide having the ligase activity of butelase 1 (SEQ ID NO:1) comprises or consists of the amino acid sequence as set forth in SEQ ID NO:2.

6. The method according to any one of claims 1-5, wherein the polypeptide having the ligase activity of butelase 1 (SEQ ID NO:1) comprises
   (a) the amino acid residue Asn at the position corresponding to position 19 of SEQ ID NO:1; and/or
   (b) the amino acid residue His at the position corresponding to position 124 of SEQ ID NO:1; and/or
   (c) the amino acid residue Cys at the position corresponding to position 166 of SEQ ID NO:1.

7. The method according to any one of claims 1-6, wherein the polypeptide having the ligase activity of butelase 1 (SEQ ID NO:1) is the polypeptide of any one of claims 4-6 or comprises or consists of

(a) any one of the amino acid sequences as set forth in SEQ ID Nos:3-109;

(b) an amino acid sequence that shares at least 60, preferably at least 70, more preferably at least 80, most preferably at least 90 % sequence identity with any one of the amino acid sequences of (a) over its entire length;

(c) an amino acid sequence that shares at least 80, preferably at least 90, more preferably at least 95 % sequence homology with any one of the amino acid sequences of (a) over its entire length; or

(d) a fragment of any one of (a)-(c).

8. The method according to any one of claims 1-7, wherein P^1 or P^2 is modified by an affinity tag, a detectable label, a solid support material, or a scaffold molecule.

9. The method according to any one of claims 1-8, wherein P^1 or P^2 is modified by a biotin, a fluorescent marker, a polymer resin, or a dendrimer.

10. The method according to any one of claims 1-9, wherein P^1 or P^2 is modified by a dendrimer.

11. The method according to claim 10, wherein each dendrimer is conjugated to 2 or more copies of the second peptide via P^2, such that the ligation of the first peptide and the second peptide results in a dendrimeric peptide assembly comprising 2 or more copies of the ligated peptide P^1-Asx-Xaa^1-Xaa^2-P^2.

12. The method according to any one of claims 9-11, wherein the dendrimer is a lysyl dendrimer.

13. The method according to any one of claims 1-12, wherein the first peptide is P^1-Asn-thioglc-Val, and P^1 is an antimicrobial peptide.

14. The method according to claim 13, wherein P^1 is an antimicrobial peptide comprising a BHHB tetrapeptide motif.

15. The method according to claim 14, wherein P^1 is an antimicrobial peptide comprising an Arg-Leu-Tyr-Arg (SEQ ID NO:121) tetrapeptide.

16. The method according to claim 15, wherein the first peptide is Ac-Arg-Leu-Tyr-Arg-Asn-thioglc-Val (SEQ ID NO:120).

17. The method according to any one of claims 1-16, wherein the second peptide is Arg-Ile-βAla conjugated to a lysyl dendrimer via βAla.

18. The method according to any one of claims 12-17, wherein the first peptide is Ac-Arg-Leu-Tyr-Arg-Asn-thioglc-Val (SEQ ID NO:120), and each lysyl dendrimer is conjugated to 2 or more copies of the second peptide Arg-Ile-βAla via βAla, such that the ligation of the first peptide and the second peptide results in a dendrimeric peptide assembly comprising 2 or more copies of Ac-Arg-Leu-Tyr-Arg-Asn-Arg-Ile-βAla (SEQ ID NO:131).