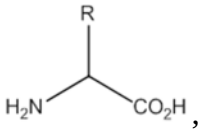
CLAIMS

What is claimed is:

1. A modified relaxin polypeptide comprising a non-naturally encoded amino acid, wherein:

(a) the modified relaxin polypeptide comprises the relaxin A chain polypeptide of SEQ ID NO: 4 and the relaxin B chain polypeptide of SEQ ID NO: 5 or SEQ ID NO: 6, substituted with a non-naturally encoded amino acid at A chain residue 1, and having zero, one, or two additional amino acid changes in said relaxin A chain and having zero, one, or two additional amino acid changes in said relaxin B chain, wherein each of said amino acid changes is independently a substitution, insertion or deletion;

(b) said non-naturally encoded amino acid has the structure:



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wherein the R group is any substituent other than the side chain found in alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine; and

(c) said non-naturally encoded amino acid is linked to a pharmacokinetic enhancer comprising a peptide component having between 4 and 8 amino acids and a half-life extending moiety comprising a fatty acid of Formula I:

—Cn—COOH (Formula I)

wherein n is between 12 and 16, wherein said peptide component comprises Glu^{γ}.

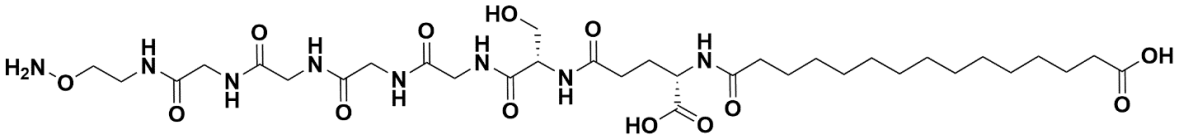
2. The modified relaxin polypeptide of claim 1, wherein said non-naturally encoded amino acid comprises a carbonyl group, an aminooxy group, a hydrazide group, a hydrazine group, a semicarbazide group, an azide group, or an alkyne group.
3. The modified relaxin polypeptide of claim 1, wherein said non-naturally encoded amino acid comprises a phenylalanine derivative.
4. The modified relaxin polypeptide of claim 1, wherein said non-naturally encoded amino acid is directly linked to said peptide component.

5. The modified relaxin polypeptide of claim 1, wherein said non-naturally encoded amino acid is linked to said pharmacokinetic enhancer through an oxime linkage or triazole linkage.
6. The modified relaxin polypeptide of claim 1, wherein said relaxin A chain polypeptide comprises SEQ ID NO: 35, and said relaxin B chain polypeptide comprises SEQ ID NO: 5 or SEQ ID NO: 6.
7. The modified relaxin polypeptide of claim 1, wherein said peptide component comprises Glu, GGGGS-Glu$^{\gamma}$ (SEQ ID NO: 139), DRDDRD (SEQ ID NO: 102), KKKKKK-Glu$^{\gamma}$ (SEQ ID NO: 103), GGGEEE-Glu$^{\gamma}$ (SEQ ID NO: 105), EEEGGG-Glu$^{\gamma}$ (SEQ ID NO: 106), KKKGGG-Glu$^{\gamma}$ (SEQ ID NO: 107), GGGKKK-Glu$^{\gamma}$ (SEQ ID NO: 109), Sar-Sar-Sar-Sar-Ser-Glu$^{\gamma}$ (SEQ ID NO: 112), Sar-Sar-Sar-Glu-Glu-Glu$^{\gamma}$ (SEQ ID NO: 113), KSGGSGG-Glu$^{\gamma}$ (SEQ ID NO: 117), dKdKdKdKdKdK-Glu$^{\gamma}$ (SEQ ID NO: 120), EEEGGG-dGlu$^{\gamma}$ (SEQ ID NO: 128), EGGGGSK-Glu$^{\gamma}$ (SEQ ID NO: 130), EEEEEE-Glu$^{\gamma}$ (SEQ ID NO: 131), KK-Glu$^{\gamma}$ (SEQ ID NO: 140), KGPKGP-Glu$^{\gamma}$ (SEQ ID NO: 146), SGGGS-Glu$^{\gamma}$ (SEQ ID NO: 147), KGGGS-Glu$^{\gamma}$ (SEQ ID NO: 148), KGGGSE-Glu$^{\gamma}$ (SEQ ID NO: 149), GSPGSP-Glu$^{\gamma}$ (SEQ ID NO: 150), GGGGP-Glu$^{\gamma}$ (SEQ ID NO: 151), EGGS-Glu$^{\gamma}$ (SEQ ID NO: 152), EGGGP-Glu$^{\gamma}$ (SEQ ID NO: 153), KGPGSE-Glu$^{\gamma}$ (SEQ ID NO: 154), Spermine-Glu$^{\gamma}$, or KKGGS-Glu$^{\gamma}$ (SEQ ID NO: 156).
8. The modified relaxin polypeptide of claim 1, wherein the relaxin polypeptide comprises the relaxin A chain polypeptide of SEQ ID NO: 4 and the relaxin B chain polypeptide of SEQ ID NO: 5 or SEQ ID NO: 6, substituted with a non-naturally encoded amino acid at A chain residue 1, wherein said non-naturally encoded amino acid is linked to said pharmacokinetic enhancer and said pharmacokinetic enhancer comprises the peptide component GGGGS-Glu$^{\gamma}$ (SEQ ID NO: 139).
9. The modified relaxin polypeptide of claim 1, wherein said half-life extending moiety comprises a fatty acid or derivative thereof, wherein the fatty acid or derivative thereof is covalently linked to the peptide component.
10. The modified relaxin polypeptide of claim 1, wherein said half-life extending moiety comprises —C14—COOH.
11. The modified relaxin polypeptide of claim 1, wherein said half-life extending moiety comprises a fatty acid of Formula I:

    —Cn—COOH (Formula I)

wherein n is between 12 and 14.

12. The modified relaxin polypeptide of claim 1, wherein said pharmacokinetic enhancer comprises:



GGGGS-Glu$^{\gamma}$-C14-COOH

(Formula II)

wherein the aminooxy group is linked to the non-natural amino acid in said modified relaxin polypeptide.

13. The modified relaxin polypeptide of claim 1, wherein said half-life extending moiety is conjugated to said peptide component through an amide bond.

14. The modified relaxin polypeptide of claim 1, wherein the relaxin polypeptide comprises the relaxin A chain polypeptide of SEQ ID NO: 4 and the relaxin B chain polypeptide of SEQ ID NO: 5 or SEQ ID NO: 6, substituted with a non-naturally encoded amino acid at A chain residue 1;

wherein said non-naturally encoded amino acid comprises para-acetyl-L-phenylalanine linked to said pharmacokinetic enhancer which comprises

a peptide component comprising GGGGS-Glu$^{\gamma}$ (SEQ ID NO: 139); and

a half-life extending moiety comprising -C14-COOH.

15. The modified relaxin polypeptide of claim 1, wherein said modified relaxin polypeptide exhibits an increased in vivo half-life by at least 2-fold compared to the wild-type relaxin polypeptide consisting of the relaxin A chain of SEQ ID NO: 4 linked to the relaxin B chain of SEQ ID NO: 5.

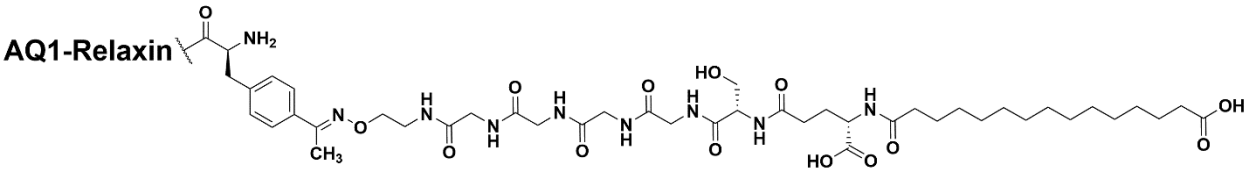
16. A pharmaceutical composition comprising the modified relaxin polypeptide of claim 1 and a pharmaceutically acceptable carrier.

17. A method of manufacturing a modified relaxin polypeptide according to claim 1, comprising:

(a) providing a polypeptide comprising said relaxin A chain and said relaxin B chain, wherein said polypeptide comprises said non-naturally encoded amino acid; and

(b) linking said non-naturally encoded amino acid to said pharmacokinetic enhancer.

18. The modified relaxin polypeptide of claim 1, wherein said modified relaxin polypeptide exhibits an increased in vivo half-life by at least 5-fold compared to the wild-type relaxin polypeptide consisting of the relaxin A chain of SEQ ID NO: 4 linked to the relaxin B chain of SEQ ID NO: 5.
19. The modified relaxin polypeptide of claim 1, wherein said modified relaxin polypeptide exhibits an increased in vivo half-life by at least 10-fold compared to the wild-type relaxin polypeptide consisting of the relaxin A chain of SEQ ID NO: 4 linked to the relaxin B chain of SEQ ID NO: 5.
20. A modified relaxin polypeptide comprising:

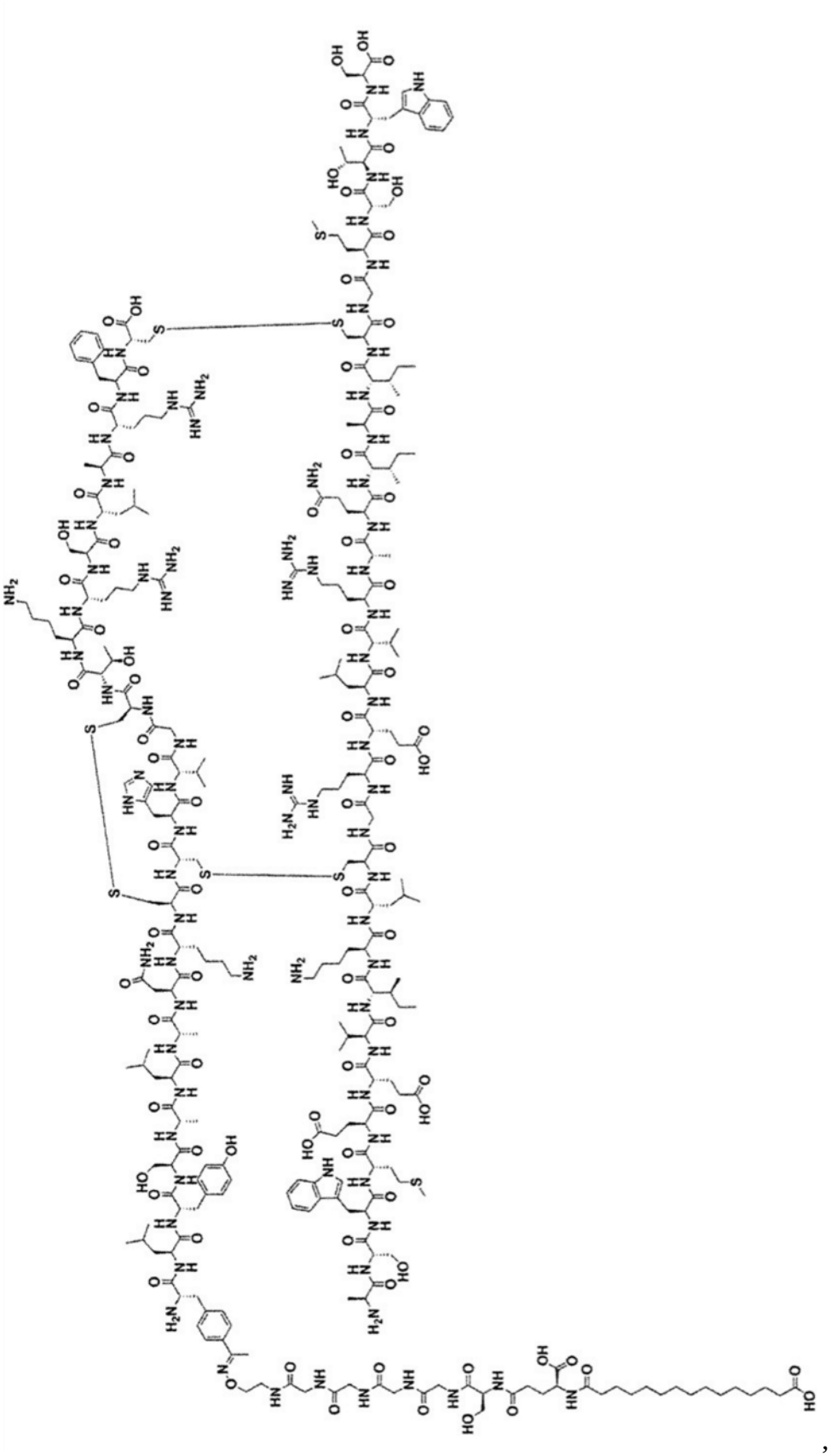


(Formula III),

wherein said AQ1-Relaxin comprises a relaxin A chain polypeptide having the amino acid sequence of SEQ ID NO: 35 and a relaxin B chain polypeptide having the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6, and having zero, one, or two amino acid changes in said relaxin A chain and having zero, one, or two amino acid changes in said relaxin B chain, wherein the modified para-acetyl-phenylalanine depicted in Formula III is located at the N-terminus of said relaxin A chain polypeptide.

21. The modified relaxin polypeptide of claim 20, wherein AQ1-Relaxin comprises a relaxin A chain polypeptide of SEQ ID NO: 35 and a relaxin B chain polypeptide of SEQ ID NO: 5 or SEQ ID NO: 6.
22. The modified relaxin polypeptide of claim 20, wherein said AQ1-Relaxin comprises a relaxin A chain polypeptide of SEQ ID NO: 35 and a relaxin B chain polypeptide of SEQ ID NO: 6, wherein the modified para-acetyl-L-phenylalanine depicted in Formula III is located at the N-terminus of said relaxin A chain polypeptide.
23. A pharmaceutical composition comprising the modified relaxin polypeptide of claim 20 or 22 and a pharmaceutically acceptable carrier.
24. A modified relaxin polypeptide comprising AQ1-GGGGS-Glu$^{\gamma}$ (SEQ ID NO: 139)-C14-COOH

which comprises the structure



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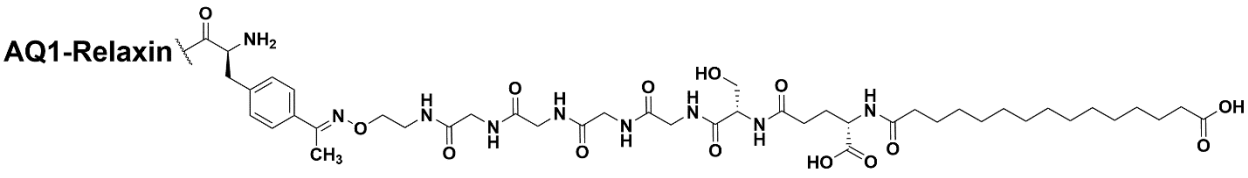
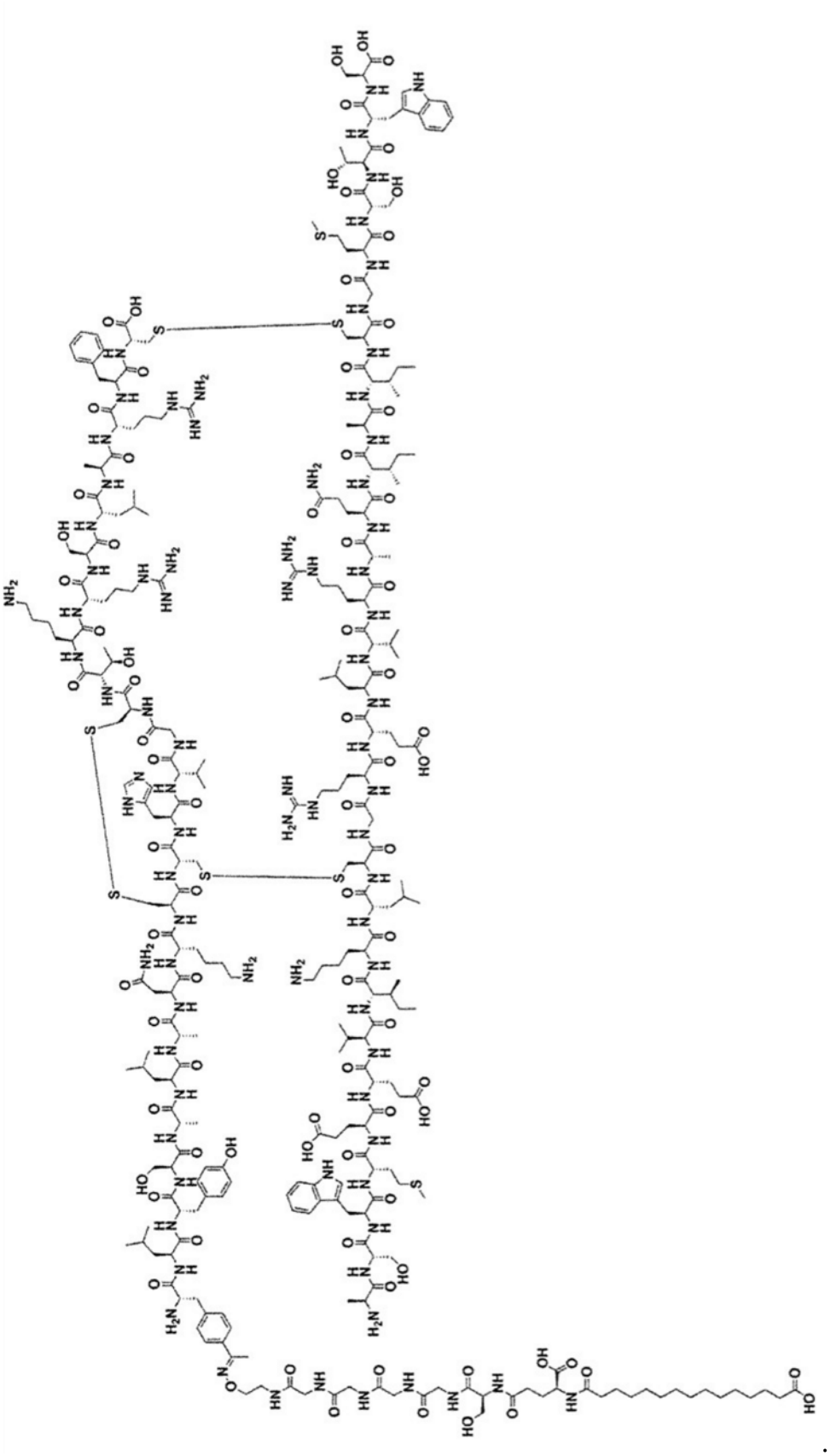
or

which comprises a relaxin A chain polypeptide of SEQ ID NO: 35 and a relaxin B chain polypeptide of SEQ ID NO: 6, wherein the modified para-acetyl-phenylalanine located at the N-terminus of said relaxin A chain polypeptide is linked to pharmacokinetic enhancer depicted in Formula III:

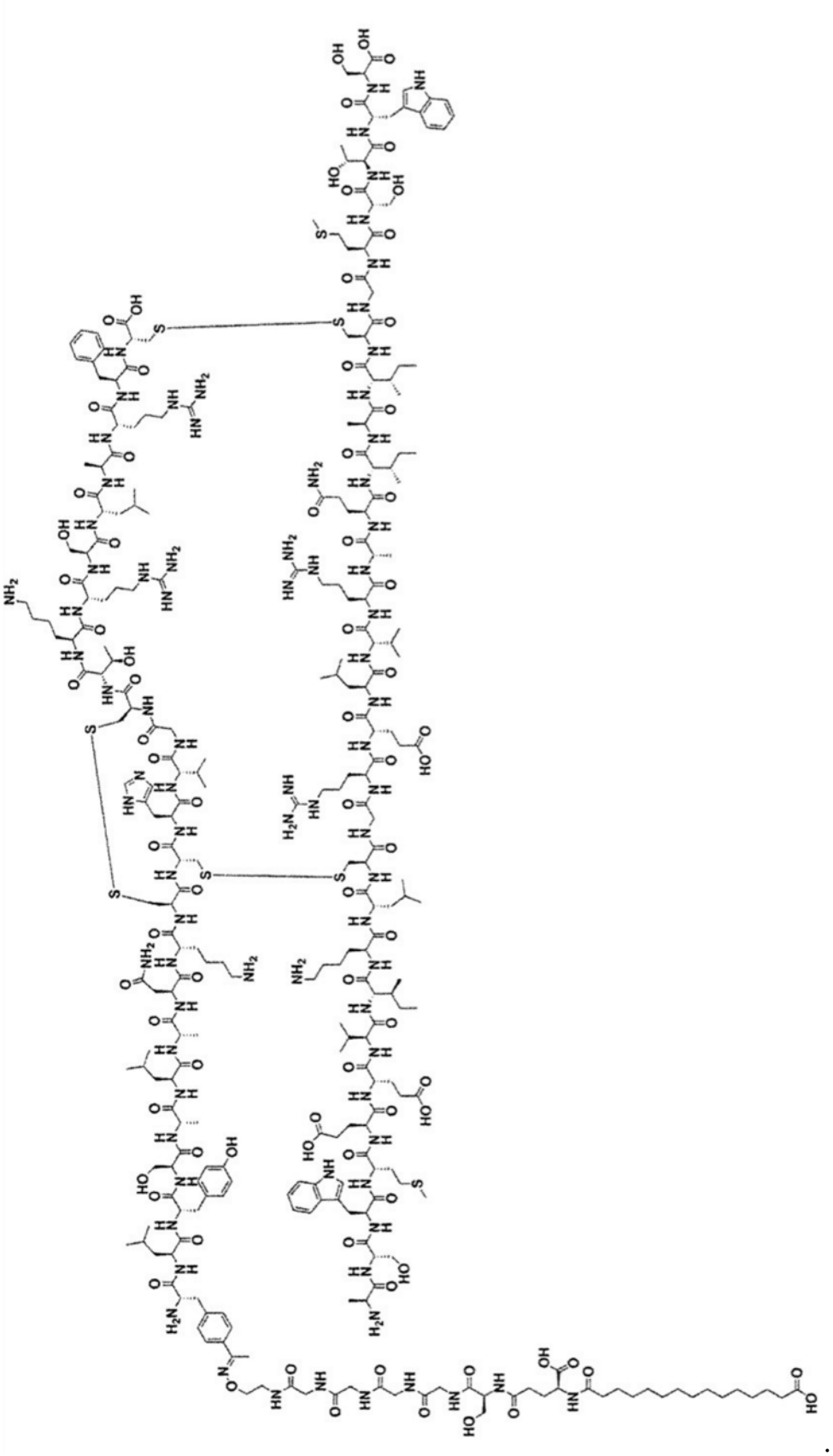


(Formula III).

25. The modified relaxin polypeptide of claim 24, which comprises the structure



26. The modified relaxin polypeptide of claim 25, which consists of the structure



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27. The modified relaxin polypeptide of claim 25 or 26, wherein said modified relaxin polypeptide exhibits an increased in vivo half-life by at least 2-fold compared to the wild-type relaxin polypeptide consisting of the relaxin A chain of SEQ ID NO: 4 linked to the relaxin B chain of SEQ ID NO: 5.
28. A pharmaceutical composition comprising the modified relaxin polypeptide of any one of claims 24 to 26 and a pharmaceutically acceptable carrier.