

Claims:

1. A conjugate comprising a PTH peptide, and a self-cleaving dipeptide covalently bound to said PTH peptide via an amide bond,
said PTH peptide comprising an amino acid sequence selected from the group consisting of

SVSEIQLMHX₁₀LGX₁₃HLX₁₆SX₁₈ERVEWLRX₂₆X₂₇LQDX₃₁H-Z, (SEQ ID NO: 133);

SVSEIQLMHX₁₀LX₁₂KHLX₅₆X₁₇X₁₈ERVEWLRKKLQDVH-Z; (SEQ ID NO: 134);

SVSEIQLMHX₁₀LGKHLX₁₆SX₁₈ERVEWLRKKLQDVH-Z (SEQ ID NO: 135) and

SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVH-Z (SEQ ID NO: 7);

wherein Z is X₃₃, X₃₃F, X₃₃FX₃₅, X₃₃FVX₃₅, X₃₃FVAX₃₅, X₃₃FVALX₃₅, X₃₃FVALGX₃₅, X₅₃X₃₅, X₅₃FX₃₅, X₅₃FVX₃₅, X₅₃FVAX₃₅, X₅₃FVALX₃₅, or X₅₃FVALGX₃₅, optionally wherein Z is X₃₃, X₅₃X₃₅, X₅₃FX₃₅, X₅₃FVX₃₅, X₅₃FVAX₃₅, X₅₃FVALX₃₅, or X₅₃FVALGX₃₅;

X₁₀ and X₁₆ are independently Asp, Gln or Asn;

X₁₂ is Gly or Aib;

X₅₆ is amino isobutyric acid (Aib) or Asn

X₁₇ is amino isobutyric acid (Aib) or Ser;

X₁₈ is Met, Met(O), Leu, or Nleu;

X₁₃, X₂₆, and X₂₇ are independently selected from the group consisting of Arg, Glu, Asp and Lys;

X₃₁ is Gly or Val;

X₃₃ and X₃₅ each comprise an acylated amino acid comprising a C16-C30 fatty acid or C16-C30 diacid covalently linked to the side chain of the amino acid, optionally via a spacer, optionally wherein the acylated amino acid is selected from the group consisting of Lys, dLys, ornithine, Cys and homocysteine;

X₅₃ is Gln or Asn, optionally with the proviso that no more than one of X₁₂, X₁₆ and X₁₇ is Aib, and optionally wherein the C-terminal amino acid is modified to replace the carboxy terminus with an amide;

wherein said self-cleaving dipeptide comprises the structure A-B
 where A is an amino acid; and
 B is an N-alkylated amino acid.

2. The conjugate of claim 1 wherein Z is X_{33} , $X_{53}X_{35}$, $X_{53}FX_{35}$, $X_{53}FVX_{35}$, $X_{53}FVAX_{35}$, $X_{53}FVALX_{35}$, or $X_{53}FVALGX_{35}$.

3. A conjugate comprising a PTH peptide and a self-cleaving dipeptide covalently bound to said PTH peptide via an amide bond,
 said PTH peptide comprising an amino acid sequence selected from the group consisting of
 $SVSEIQLMHNLGX_{13}HLNSMERVEWLRX_{26}X_{27}LQDX_{31}H-Z$, (SEQ ID NO: 5);
 $SVSEIQLMHX_{10}LGKHLX_{16}SX_{18}ERVEWLRKKLQDVH-Z$ (SEQ ID NO: 135);
 $SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVH-Z$ (SEQ ID NO: 7), and
 a peptide that differs from the peptide of SEQ ID NO: 7 by 1, 2 or 3 amino acid substitutions;

wherein
 Z is $X_{33}F$, $X_{53}X_{35}$, $X_{53}FX_{35}$, or X_{33} ;
 X_{10} and X_{16} are independently Asp, Gln or Asn;
 X_{18} is Met, Met(O), Leu, or Nleu;
 X_{13} , X_{26} , and X_{27} are independently selected from the group consisting of Arg, Glu, Asp and Lys;
 X_{31} is Gly or Val;
 X_{33} and X_{35} each comprise an acylated amino acid;
 X_{53} is Gln or Asn; and
 said self-cleaving dipeptide comprising the general structure
 A-B-;

wherein
 A is an amino acid or an acylated amino acid;
 B is an N-alkylated amino acid;
 wherein said acylated amino acid of each of X_{33} , X_{35} and A is independently selected from an amino acid comprising a C16-C30 fatty acid or C16-C30 diacid

covalently linked to the amino acid side chain, optionally via a spacer, and said self-cleaving dipeptide is linked to said PTH peptide through formation of an amide bond between B and the N-terminal alpha amine of said PTH peptide, further wherein said optional spacer of each of X_{33} , X_{35} and A comprises one or more linker moieties independently selected from the group consisting of a gamma glutamic acid, and $\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{NH}$, wherein k is an integer selected from the range of 1-8;

with the proviso that when A is a non-acylated amino acid, then A is an amino acid in the D-stereochemical configuration.

4. The conjugate of any one of claims 1-3 wherein said PTH peptide comprises the sequence SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVH-Z (SEQ ID NO: 7),

wherein

Z is $X_{33}\text{F}$, $X_{53}X_{35}$, $X_{53}\text{FX}_{35}$, or X_{33} ;

X_{33} and X_{35} are each independently an amino acid comprising a C16-C30 fatty acid or C16-C30 diacid covalently linked to the acid side chain of the amino acid, optionally via a spacer; and

X_{53} is Asn.

5. The conjugate of any one of claims 1-3 wherein said PTH peptide comprises the sequence of SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHX₃₃ (SEQ ID NO: 16) or SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF₃₅ (SEQ ID NO: 12)

wherein X_{33} and X_{35} are each independently an amino acid comprising a C16-C22 fatty acid or C16-C22 diacid covalently linked to the side chain of the amino acid, optionally via a spacer.

6. The conjugate of any one of claims 1-5 wherein A is selected from the group consisting of Lys, dLys, acylated-Lys and acylated-dLys wherein said self-cleaving dipeptide is covalently linked to the N-terminal alpha amine of the PTH peptide.

7. The conjugate of any one of claims 1-6 wherein said acylated amino acid of each of X_{33} , X_{35} and A comprises a C16-C30 fatty acid or C16-C30 diacid covalently linked to the amino acid side chain via a spacer, wherein the spacer of each of X_{33} , X_{35} and A is independently selected from a gamma glutamic acid-gamma glutamic acid dipeptide, $(X_{aa})-[COCH_2(OCH_2CH_2)_kNH]_q$ -gamma glutamic acid and a gamma glutamic acid- $[COCH_2(OCH_2CH_2)_kNH]_q$ -gamma glutamic acid, wherein

Xaa is selected from Arg, Tyr(OPO_3H_2), and hCys(SO_3H);

k is an integer selected from the range of 1-8; and

q is an integer selected from the range of 1-8, optionally wherein k is 2 and q is selected from the range of 1-4.

8. The conjugate of any one of claims 1-7 wherein the acylated amino acid of A, X_{33} and X_{35} is independently selected from cysteine, homocysteine, ornithine lysine and d-lysine wherein the side chain of said cysteine, homocysteine, ornithine lysine or d-lysine is covalently linked to a C16-C22 fatty acid or C16-C22 diacid, optionally through a spacer, wherein the optional spacer of each of A, X_{33} and X_{35} comprises a gamma glutamic acid linkage.

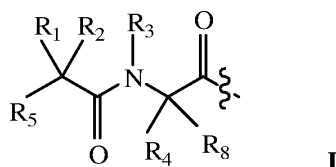
9. The conjugate of any one of claims 1-8 wherein the acylated amino acid of A, X_{33} and X_{35} are each independently selected from lysine or d-lysine wherein the side chain of said lysine or d-lysine is covalently linked to a C16-C22 fatty acid or C16-C22 diacid, optionally through a spacer comprising a gamma glutamic acid linkage.

10. The conjugate of claim 9 wherein the acylated amino acid of A is d-lysine, X_{33} and X_{35} are each lysine.

11. The conjugate of claim 9 or 10 wherein said spacer of A, X_{33} and X_{35} are each independently selected from compounds comprising the structure: gamma glutamic acid- $[COCH_2(OCH_2CH_2)_kNH]_q$ -gamma glutamic acid, wherein k is an integer selected from 2, 4 or 8 and q is an integer selected from 1, 2, 4 or 8.

12. The conjugate of claim 11 wherein k is 2 and q is 2 or 4.

13. The conjugate of any one of claims 1-5 wherein A-B comprises the structure:



wherein

R₁, comprises a side chain selected from the group consisting of C₁-C₈ alkyl, (C₁-C₄ alkyl)OH, (C₁-C₄ alkyl)SH, (C₁-C₄ alkyl)COOH, and (C₁-C₄ alkyl)NH₂, optionally wherein a C16-C30 fatty acid or C16-C30 diacid is covalently linked to said side chain, optionally via a spacer selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a gamma glutamic acid-[COCH₂(OCH₂CH₂)_kNH]_q-gamma glutamic acid, wherein

k is an integer selected from the range of 1-8; and

q is an integer selected from the range of 1-8, optionally wherein k is 2 and q is selected from the range of 1-8;

R₂, R₄ and R₈ are independently H, or C₁-C₄ alkyl;

R₃ is C₁-C₆ alkyl; and

R₅ is NH₂.

14. The conjugate of claim 13 wherein the chemical cleavage half-life ($t_{1/2}$) of A-B from said PTH peptide is at least about 48 to 168 hours in standard PBS solution under physiological conditions.

15. The conjugate of claim 13 wherein

R₁, is (C₁-C₄ alkyl)NH;

R₂ and R₈ are each H;

R₄ is H, or CH₃;

R₃ is CH₃ and

R₅ is NH₂.

16. The conjugate of claim 13 wherein

R₁ is (C₁-C₄ alkyl)NH-[spacer]-CO(CH₂)₁₄₋₂₀COOH, (C₁-C₄ alkyl)NH-[spacer]-CO(CH₂)₁₄₋₂₀CH₃, (C₁-C₄ alkyl)NH-CO(CH₂)₁₄₋₂₀COOH, or (C₁-C₄ alkyl)NH-CO(CH₂)₁₄₋₂₀CH₃;

R₂ and R₈ are each H;

R₄ is H, or CH₃;

R₃ is CH₃ and

R₅ is NH₂, wherein said [spacer] is a linking moiety selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a gamma glutamic acid-[COCH₂(OCH₂CH₂)_kNH]_q-gamma glutamic acid, wherein k is an integer selected from the range of 2-4 and q is an integer selected from the range of 1-8.

17. The conjugate of claim 16 wherein R₁ is (C₁-C₄ alkyl)NH-[spacer]-CO(CH₂)₁₄₋₂₀COOH, wherein said [spacer] is a linking moiety comprising the structure gamma glutamic acid-[COCH₂(OCH₂CH₂)_kNH]_q-gamma glutamic acid, wherein k is 2 or 4 and q is 1, 2 or 4.

18. The conjugate of claim 16 wherein

R₁ is (C₄ alkyl)NH-{ gamma glutamic acid-[COCH₂(OCH₂CH₂)₂NH-COCH₂(OCH₂CH₂)₂NH-gamma glutamic acid]-CO(CH₂)₁₄₋₂₀COOH.

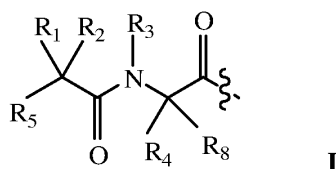
19. The conjugate of any one of claims 1-18 wherein the first amino acid of the cleavable dipeptide is an amino acid in the D-stereochemical configuration.

20. A conjugate comprising a PTH peptide, and a self-cleaving dipeptide covalently linked to the N-terminal alpha amine of said PTH peptide via an amide bond, wherein

said PTH peptide comprises the amino acid sequence of

SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHX₃₃ (SEQ ID NO: 16), SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF₃₅ (SEQ ID NO: 12), or a peptide that differs from the peptide of SEQ ID NO: 16 or SEQ ID NO: 12 by 1 or 2 amino acid substitutions wherein

X₃₃ and X₃₅ are each an amino acid comprising a side chain of (C₁-C₄ alkyl)NH-CO(CH₂)₁₄₋₂₀COOH, (C₁-C₄ alkyl)NH-[spacer]-CO(CH₂)₁₄₋₂₀COOH, (C₁-C₄ alkyl)NH-CO(CH₂)₁₄₋₂₀CH₃ or (C₁-C₄ alkyl)NH-[spacer]-CO(CH₂)₁₄₋₂₀CH₃; and said self-cleaving dipeptide comprises the general structure:



wherein

R₁ is (C₁-C₄ alkyl)NH, (C₁-C₄ alkyl)NH-CO(CH₂)₁₄₋₂₀COOH, (C₁-C₄ alkyl)NH-[spacer]-CO(CH₂)₁₄₋₂₀COOH, (C₁-C₄ alkyl)NH-CO(CH₂)₁₄₋₂₀CH₃ or (C₁-C₄ alkyl)NH-[spacer]-CO(CH₂)₁₄₋₂₀CH₃;

R₂ and R₈ are each H;

R₄ is H, or CH₃;

R₃ is C₁-C₃ alkyl and

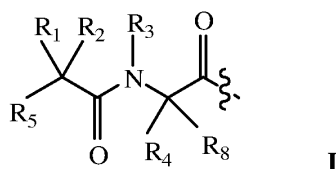
R₅ is NH₂, wherein said spacer of R₁, X₃₃ and X₃₅ is each independently selected from the group consisting of gamma glutamic acid, gamma glutamic acid-gamma glutamic acid dipeptide, and gamma glutamic acid-[COCH₂(OCH₂CH₂)_kNH]_q-gamma glutamic acid wherein k is an integer selected from the range of 2-4 and q is an integer selected from the range of 1-4.

21. The conjugate of claim 20 wherein said PTH peptide comprises the amino acid sequence of

SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF₃₅ (SEQ ID NO: 12), wherein

X₃₅ is an amino acid comprising a side chain of (C₁-C₄ alkyl)NH-{gamma glutamic acid-[COCH₂(OCH₂CH₂)₂NH-COCH₂(OCH₂CH₂)₂]NH-gamma glutamic acid}-CO(CH₂)₁₄₋₂₀COOH; and

said self-cleaving dipeptide comprises the general structure:



wherein

R_1 is $(C_1-C_4 \text{ alkyl})NH-\{\text{gamma glutamic acid}-[COCH_2(OCH_2CH_2)_2NH-COCH_2(OCH_2CH_2)_2]NH-\text{gamma glutamic acid}\}-CO(CH_2)_{14-20}COOH$;

R_2 , R_4 and R_8 are each H;

R_3 is CH_3 ; and

R_5 is NH_2 .

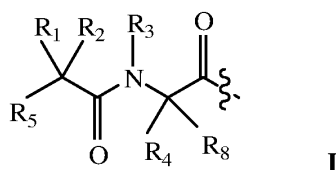
22. The conjugate of claim 19 wherein said PTH peptide comprises the amino acid sequence of

SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHX₃₃ (SEQ ID NO: 16),

wherein

X_{33} is an amino acid comprising a side chain of $(C_1-C_4 \text{ alkyl})NH-\{\text{gamma glutamic acid}-[COCH_2(OCH_2CH_2)_k-NH]_q-\text{gamma glutamic acid}\}-CO(CH_2)_{14-20}COOH$; and

said self-cleaving dipeptide comprises the general structure:



wherein

R_1 is $(C_1-C_4 \text{ alkyl})NH-\{\text{gamma glutamic acid}-[COCH_2(OCH_2CH_2)_kNH]_q-\text{gamma glutamic acid}\}-CO(CH_2)_{16-18}COOH$;

R_2 , R_4 and R_8 are each H;

R_3 is CH_3 ;

R_5 is NH_2 ;

q is 2 or 4 and

k is 2.

23. The conjugate of claim 22 wherein

X_{33} is an amino acid comprising a side chain of $(C_4 \text{ alkyl})NH-\{\text{gamma glutamic acid}-[COCH_2(OCH_2CH_2)_2NH-COCH_2(OCH_2CH_2)_2]NH-\text{gamma glutamic acid}\}-CO(CH_2)_{16}COOH$; and

R₁ is (C₄ alkyl)NH-{gamma glutamic acid-[COCH₂(OCH₂CH₂)₂NH-COCH₂(OCH₂CH₂)₂]NH-gamma glutamic acid}-CO(CH₂)₁₆COOH.

24. The conjugate of any one of claims 21, 22 or 23 wherein the first amino acid of the self-cleaving dipeptide is in the D-stereochemical configuration.

25. A pharmaceutical composition comprising the conjugate of any one of claims 1-24, or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

26. The pharmaceutical composition of claim 25 formulated for oral delivery wherein said composition further comprises sodium N[8-(2-hydroxybenoyl)aminocaprylate], optionally wherein the composition is formulated in a tablet form.

27. The pharmaceutical composition of claim 25 or 26 further comprising a peptide of SEQ ID NO: 7, SEQ ID NO: 31, or SEQ ID NO: 32 and optionally calcitonin.

28. A method of treating hypoparathyroidism, said method comprising administering an effective amount of a pharmaceutical composition of claim 23, 24 or 25 to a patient in need of treatment.

29. A method of treating osteoporosis or osteopenia, said method comprising administering an effective amount of a pharmaceutical composition of claim 22, 23 or 24 to a patient in need of treatment.

30. The method of claim 28 or 29 wherein the composition is administered once per week.

31. The method of claim 28 or 29 wherein the composition is administered daily.

32. The method of any one of claims 28-31 wherein the composition is administered orally.

33. Use of the composition of any one of claims 25-27, for the treatment of hypoparathyroidism.

34. Use of the composition of any one of claims 25-27 for the treatment of osteoporosis or osteopenia.