

risk. As a result, hypoglycemia remains a key medical risk with huge burden on patients and causes significant morbidity and mortality.

BRIEF SUMMARY

5 The present disclosure provides compounds comprising to two insulin molecules covalently linked to form an insulin molecule dimer that may activate the insulin receptor with regular insulin-like potency but with reduced maximum activity. These compounds are insulin receptor partial agonists (IPRAs): they behave like other insulin analogs to lower glucose effectively but with lower risk of hypoglycemia.

10 Provided are insulin receptor partial agonist covalent insulin dimers formulated as novel and transformative basal insulins (once daily administration) that manifest an improved therapeutic index (TI) over current standard of care (SOC) basal insulins. In one example, the IPRAs of the present disclosure may lower glucose effectively with reduced risk of hypoglycemia in diabetic minipig and has the property of a once daily (QD) basal insulin. The improved TI may empower practitioners to more aggressively dose IPRAs of the present disclosure to achieve target goals for control of fasting glucose. Tight control of fasting glucose and HbA1c by an IRPA may allow it to serve as 1) a stand-alone long-acting insulin with an enhanced efficacy and safety profile in T2DM and 2) an improved foundational basal insulin in T1DM (and some T2DM) for use with additional prandial rapid-acting insulin analogs (RAA) doses. Thus, the present disclosure provides the following examples.

20 The present disclosure provides an insulin receptor partial agonist or insulin dimer comprising a first insulin or insulin analog heterodimer and a second insulin or insulin analog heterodimer each heterodimer including an A-chain polypeptide and a B-chain polypeptide, wherein the A-chain polypeptide and the B-chain polypeptide are linked together through
25 interchain disulfide bonds; wherein the first and second insulin or insulin analog heterodimers are covalently linked together through a linking moiety joining the side chain of an amino acid at or near the carboxy terminus of the two respective B-chain polypeptides; and wherein at least one amino terminus of the A-chain polypeptides and the B-chain polypeptides is covalently linked to a substituent, with the proviso that the linking moiety does not include a disulfide bond.
30 In particular examples, at least the amino terminus of the A-chain polypeptide and the B-chain polypeptide of the first insulin or insulin analog are covalently linked to a substituent.

In particular examples of the insulin receptor partial agonist or insulin dimer, the amino terminus of each A-chain polypeptide and each B-chain polypeptide is covalently linked to a substituent. In particular examples, the amino terminus of the A-chain polypeptide and the B-chain polypeptide of the first insulin or insulin analog and the amino terminus of the A-chain polypeptide and B-chain polypeptide of the second insulin or insulin analog are each covalently linked to a substituent. In examples in which the amino termini of the first and second insulin or insulin analogs are covalently linked to a substituent, the substituent on the amino termini of the A-chain and B-chain polypeptides of the first insulin or insulin analog may be the same as the substituent on the amino termini of the A-chain and B-chain polypeptides of the second insulin or insulin analog. In examples in which the amino termini of the first and second insulin or insulin analogs are covalently linked to a substituent, the substituent on the amino termini of the A-chain and B-chain polypeptides of the first insulin or insulin analog may be different from the substituent on the amino termini of the A-chain and B-chain polypeptides of the second insulin or insulin analog.

In a further example of the insulin receptor partial agonist or insulin dimer, the first and second insulin or insulin analog heterodimers are the same or wherein the first and second insulin or insulin analog heterodimers are different.

In a further still example of the insulin receptor partial agonist or insulin dimer, the linking moiety covalently links the first insulin or insulin analog heterodimer and the second insulin or insulin analog heterodimer via the epsilon amino group of a lysine residue at or near the carboxy terminus of their respective B-chain polypeptides.

In a further still example of the insulin receptor partial agonist or insulin dimer, the substituent has a general formula $RC(O)-$, where R can be $R'CH_2$, $R'NH$, $R'O$, and R' can be H, linear alkyl chain, amino acid, peptide, PEG, saccharides, which in particular examples $RC(O)-$ may be acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, or alkoxycarbonyl. In particular examples, the substituent is selected from the group consisting of acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, isobutyl, methoxy acetyl, glycine, aminoethylglucose (AEG), AEG-C6, PEG1, PEG2, *N*-dimethyl, and alkoxycarbonyl.

In particular examples of the insulin receptor partial agonist or insulin dimer, each A-chain polypeptide independently comprises the amino acid sequence $GX_2X_3EQCCX_8SICSLYQLX_{17}NX_{19}CX_{23}$ (SEQ ID NO:3) and each B-chain polypeptide

independently comprises the amino acid sequence

X₂₅LCGX₂₉X₃₀LVEALYLVCGERGFX₂₇YTX₃₁X₃₂ (SEQ ID NO:4) or

X₂₂VNQX₂₅X₂₆CGX₂₉X₃₀LVEALYLVCGERGFX₂₇YTX₃₁X₃₂X₃₃X₃₄X₃₅ (SEQ ID

NO:5) wherein X₂ is isoleucine or threonine; X₃ is valine, glycine, or leucine; X₈ is threonine or

5 histidine; X₁₇ is glutamic acid or glutamine; X₁₉ is tyrosine, 4-methoxy-phenylalanine, alanine, or 4-amino phenylalanine; X₂₃ is asparagine or glycine; X₂₂ is or phenylalanine and desamino-phenylalanine; X₂₅ is histidine or threonine; X₂₆ is leucine or glycine; X₂₇ is phenylalanine or aspartic acid; X₂₉ is alanine, glycine, or serine; X₃₀ is histidine, aspartic acid, glutamic acid, homocysteic acid, or cysteic acid; X₃₁ is aspartic acid, proline, or lysine; X₃₂ is lysine or
10 proline; X₃₃ is threonine, alanine, or absent; X₃₄ is arginine or absent; and X₃₅ is arginine or absent; with the proviso at least one of X₃₁ or X₃₂ is lysine.

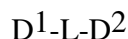
In particular examples of the insulin receptor partial agonist or insulin dimer, the first and second insulins or insulin analogs are independently native human insulin, insulin lispro, insulin aspart, desB30 insulin, or insulin glargine.

15 In particular examples of the insulin receptor partial agonists or insulin dimers, the linking moiety may be an optionally substituted group selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, heteroaryl, and heterocyclic. The linking moiety may be a bivalent, straight or branched, saturated or unsaturated, optionally substituted C1-C20 hydrocarbon chain wherein one or more methylene units are optionally and independently
20 replaced by -O-, -S-, -N(R)-, -C(O)-, C(O)O-, OC(O)-, -N(R)C(O)-, -C(O)N(R)-, -S(O)-, -S(O)₂-, -N(R)SO₂-, SO₂N(R)-, a heterocyclic group, an aryl group, or a heteroaryl group, wherein each occurrence of R is independently hydrogen, a suitable protecting group, an acyl moiety, arylalkyl moiety, aliphatic moiety, aryl moiety, heteroaryl moiety, or heteroaliphatic moiety.

25 In a further still example of the insulin receptor partial agonist or insulin dimer, the linking moiety is an acyl moiety, -C(O)RC(O)-, where R is alkyl chain, poly(ethylene glycol) (PEG) chain, amide-containing chain, triazole(s)-containing chain, cyclooctyne-containing moiety, a substituted acyl chain, or a polyethylene glycol (PEG) chain.

In a further example, the linking moiety is an alkylidioyl, $-C(O)(CH_2)_n C(O)-$, wherein $n = 0-45$, including but not limited to an oxalyl (C2) moiety, a succinyl (C4) moiety, an adipoyl (C6) moiety, a suberyol (C8) moiety, a decanedioyl (C10) moiety, a dodecanedioyl (C12) moiety, a tetradecanedioyl (C14) moiety, or a hexadecanedioyl (C16) moiety.

5 The present disclosure further provides an insulin receptor partial agonist or insulin dimer comprising the formula



wherein D^1 and D^2 are each independently an insulin or insulin analog polypeptide, wherein each insulin polypeptide is a heterodimer comprising an A-chain polypeptide and a B-chain polypeptide linked together through interchain disulfide bonds; L is a
10 linking moiety wherein one end of the linker moiety is attached to an amino acid residue at or near the carboxyl group of D^1 and the other end of the linker moiety is attached to an amino acid residue at or near the carboxyl end of D^2 with the proviso that L does not include a disulfide linkage; and wherein the first and second insulin or insulin analog polypeptides include a
15 substituent attached to the amino terminus of the A-chain polypeptide and the B-chain polypeptide.

In a further example of the insulin receptor partial agonist or insulin dimer, D^1 and D^2 are the same or wherein D^1 and D^2 are different.

In a further example of the insulin receptor partial agonist or insulin dimer, the
20 linking moiety covalently links D^1 and D^2 via the epsilon amino group of a lysine residue at or near the carboxy terminus of D^1 and D^2 .

In a further still example of the insulin receptor partial agonist or insulin dimer, the substituent has a general formula $RC(O)-$, where R can be $R'CH_2$, $R'NH$, $R'O$, and R' can be H, linear alkyl chain, amino acid, peptide, PEG, saccharides, which in particular examples
25 $RC(O)-$ may be acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, or alkoxycarbonyl. In particular examples, the substituent is selected from the group consisting of acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, isobutyl, methoxy acetyl, glycine, aminoethylglucose (AEG), AEG-C6, PEG1, PEG2, *N*-dimethyl, and alkoxycarbonyl.

In particular examples of the insulin receptor partial agonist or insulin dimer,
30 each A-chain polypeptide independently comprises the amino acid sequence

GX₂X₃EQCCX₈SICSLYQLX₁₇NX₁₉CX₂₃ (SEQ ID NO:3) and each B-chain polypeptide independently comprises the amino acid sequence

X₂₅LCGX₂₉X₃₀LVEALYLVCGERGFX₂₇YTX₃₁X₃₂ (SEQ ID NO:4) or

X₂₂VNQX₂₅X₂₆CGX₂₉X₃₀LVEALYLVCGERGFX₂₇YTX₃₁X₃₂X₃₃X₃₄X₃₅ (SEQ ID

5 NO:5) wherein X₂ is isoleucine or threonine; X₃ is valine, glycine, or leucine; X₈ is threonine or histidine; X₁₇ is glutamic acid or glutamine; X₁₉ is tyrosine, 4-methoxy-phenylalanine, alanine, or 4-amino phenylalanine; X₂₃ is asparagine or glycine; X₂₂ is or phenylalanine and desamino-phenylalanine; X₂₅ is histidine or threonine; X₂₆ is leucine or glycine; X₂₇ is phenylalanine or aspartic acid; X₂₉ is alanine, glycine, or serine; X₃₀ is histidine, aspartic acid, glutamic acid,
10 homocysteic acid, or cysteic acid; X₃₁ is aspartic acid, proline, or lysine; X₃₂ is lysine or proline; X₃₃ is threonine, alanine, or absent; X₃₄ is arginine or absent; and X₃₅ is arginine or absent; with the proviso at least one of X₃₁ or X₃₂ is lysine.

In a further example of the insulin receptor partial agonist or insulin dimer, wherein D¹ and D² are independently native human insulin, insulin lispro, insulin aspart, desB30
15 insulin, or insulin glargine.

In particular examples of the insulin receptor partial agonists or insulin dimers, the linking moiety may be an optionally substituted group selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, heteroaryl, and heterocyclic. The linking moiety may be a bivalent, straight or branched, saturated or unsaturated, optionally substituted C1-C20
20 hydrocarbon chain wherein one or more methylene units are optionally and independently replaced by -O-, -S-, -N(R)-, -C(O)-, C(O)O-, OC(O)-, -N(R)C(O)-, -C(O)N(R)-, -S(O)-, -S(O)₂-, -N(R)SO₂-, SO₂N(R)-, a heterocyclic group, an aryl group, or a heteroaryl group, wherein each occurrence of R is independently hydrogen, a suitable protecting group, an acyl moiety, arylalkyl moiety, aliphatic moiety, aryl moiety, heteroaryl moiety, or heteroaliphatic
25 moiety.

In a further still example of the insulin receptor partial agonist or insulin dimer, the linking moiety is an acyl moiety, -C(O)RC(O)-, where R is alkyl chain, poly(ethylene glycol) (PEG) chain, amide-containing chain, triazole(s)-containing chain, cyclooctyne-containing moiety, a substituted acyl chain, or a polyethylene glycol (PEG) chain. .

In a further example of the insulin receptor partial agonist or insulin dimer, the linking moiety is an alkylidioyl, $-C(O)(CH_2)_n C(O)-$, wherein $n = 0-45$, including but not limited to an oxalyl (C2) moiety, a succinyl (C4) moiety, an adipoyl (C6) moiety, a suberyol (C8) moiety, a decanedioyl (C10) moiety, a dodecanedioyl (C12) moiety, a tetradecanedioyl (C14) moiety, or a hexadecanedioyl (C16) moiety.

Further provided are compositions comprising any one of the aforementioned insulin receptor partial agonists or insulin dimer and a pharmaceutically acceptable carrier.

The present disclosure further provides an insulin receptor partial agonist or insulin dimer comprising a first insulin or insulin analog heterodimer and a second insulin or insulin analog heterodimer each heterodimer including an A-chain polypeptide and a B-chain polypeptide, wherein the A-chain polypeptide and the B-chain polypeptide are linked together through interchain disulfide bonds; wherein the first and second insulin or insulin analog heterodimers are covalently linked together through a linking moiety joining the side chain of an amino acid at or near the carboxy terminus of the two respective B-chain polypeptides; and optionally wherein the amino terminus of at least one of the A-chain polypeptides and the B-chain polypeptides of the first insulin polypeptide or second insulin polypeptide is covalently linked to a substituent, with the proviso that (1) the linking moiety does not include a disulfide bond and (2) when the insulin or insulin analog is not a human insulin or insulin analog and the amino terminus of the A-chain polypeptide and the B-chain polypeptide do not include a substituent then the linking moiety is not an oxalyl (C2) moiety, a suberyol (C8) moiety, or a dodecanedioyl (C12) moiety.

In a further example of the insulin receptor partial agonist or insulin dimer, the first and second insulin or insulin analog heterodimers are the same or wherein the first and second insulin or insulin analog heterodimers are different.

In a further example of the insulin receptor partial agonist or insulin dimer, the linking moiety covalently links the first insulin or insulin analog heterodimer and the second insulin or insulin analog heterodimer via the epsilon amino group of a lysine residue at or near the carboxy terminus of their respective B-chain polypeptides.

In a further still example of the insulin receptor partial agonist or insulin dimer, the substituent has a general formula $RC(O)-$, where R can be $R'CH_2$, $R'NH$, $R'O$, and R' can be H, linear alkyl chain, amino acid, peptide, PEG, saccharides, which in particular examples $RC(O)-$

may be acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, or alkoxycarbonyl. In particular examples, the substituent is selected from the group consisting of acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, isobutyl, methoxy acetyl, glycine, aminoethylglucose (AEG), AEG-C6, PEG1, PEG2, *N*-dimethyl, and alkoxycarbonyl.

5 In a further example of the insulin receptor partial agonist or insulin dimer, the first and second insulins or insulin analogs are independently native human insulin, insulin lispro, insulin aspart, desB30 insulin, or insulin glargine.

In particular examples of the insulin receptor partial agonist or insulin dimer, each A-chain polypeptide independently comprises the amino acid sequence

10 GX₂X₃EQCCX₈SICSLYQLX₁₇NX₁₉CX₂₃ (SEQ ID NO:3) and each B-chain polypeptide independently comprises the amino acid sequence

X₂₅LCGX₂₉X₃₀LVEALYLVCGERGFX₂₇YTX₃₁X₃₂ (SEQ ID NO:4) or

X₂₂VNQX₂₅X₂₆CGX₂₉X₃₀LVEALYLVCGERGFX₂₇YTX₃₁X₃₂X₃₃X₃₄X₃₅ (SEQ ID NO:5) wherein X₂ is isoleucine or threonine; X₃ is valine, glycine, or leucine; X₈ is threonine or histidine; X₁₇ is glutamic acid or glutamine; X₁₉ is tyrosine, 4-methoxy-phenylalanine, alanine, or 4-amino phenylalanine; X₂₃ is asparagine or glycine; X₂₂ is or phenylalanine and desamino-phenylalanine; X₂₅ is histidine or threonine; X₂₆ is leucine or glycine; X₂₇ is phenylalanine or aspartic acid; X₂₉ is alanine, glycine, or serine; X₃₀ is histidine, aspartic acid, glutamic acid, homocysteic acid, or cysteic acid; X₃₁ is aspartic acid, proline, or lysine; X₃₂ is lysine or proline; X₃₃ is threonine, alanine, or absent; X₃₄ is arginine or absent; and X₃₅ is arginine or absent; with the proviso at least one of X₃₁ or X₃₂ is lysine.

In particular examples of the insulin receptor partial agonists or insulin dimers, the linking moiety may be an optionally substituted group selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, heteroaryl, and heterocyclic. The linking moiety may be a bivalent, straight or branched, saturated or unsaturated, optionally substituted C1-C20 hydrocarbon chain wherein one or more methylene units are optionally and independently replaced by -O-, -S-, -N(R)-, -C(O)-, C(O)O-, OC(O)-, -N(R)C(O)-, -C(O)N(R)-, -S(O)-, -S(O)₂-, -N(R)SO₂-, SO₂N(R)-, a heterocyclic group, an aryl group, or a heteroaryl group, wherein each occurrence of R is independently hydrogen, a suitable protecting group, an acyl

moiety, arylalkyl moiety, aliphatic moiety, aryl moiety, heteroaryl moiety, or heteroaliphatic moiety.

In a further still example of the insulin receptor partial agonist or insulin dimer, the linking moiety is an acyl moiety, $-C(O)RC(O)-$, where R is alkyl chain, poly(ethylene glycol) (PEG) chain, amide-containing chain, triazole(s)-containing chain, cyclooctyne-containing moiety, a substituted acyl chain, or a polyethylene glycol (PEG) chain.

In a further example of the insulin receptor partial agonist or insulin dimer, the linking moiety is a C2-C20 acyl moiety.

In particular examples, the linking moiety is an alkyldioyl, $-C(O)(CH_2)_nC(O)-$, wherein $n = 0-45$, including but not limited to an oxalyl (C2) moiety, a succinyl (C4) moiety, an adipoyl (C6) moiety, a suberyol (C8) moiety, a decanedioyl (C10) moiety, a dodecanedioyl (C12) moiety, a tetradecanedioyl (C14) moiety, or a hexadecanedioyl (C16) moiety.

Further provided are compositions comprising any one of the aforementioned insulin receptor partial agonists or insulin dimers and a pharmaceutically acceptable carrier.

The present disclosure further provides an insulin receptor partial agonist or insulin dimer comprising the formula



wherein D^1 and D^2 are each independently an insulin or insulin analog polypeptide, wherein each insulin polypeptide is a heterodimer comprising an A-chain polypeptide and a B-chain polypeptide linked together through interchain disulfide bonds; L is a linking moiety wherein one end of the linker moiety is attached to an amino acid residue at or near the carboxyl group of D^1 and the other end of the linker moiety is attached to an amino acid residue at or near the carboxyl end of D^2 with the proviso that L does not include a disulfide linkage; and optionally, wherein at least one of D^1 or D^2 includes a substituent attached to the amino terminus of the A-chain polypeptide or the B-chain polypeptide of D^1 or D^2 ; with the proviso that (1) the linking moiety does not include a disulfide bond and (2) when the amino terminus of the A-chain polypeptide and the B-chain polypeptide do not include a substituent then the linking moiety is not an oxalyl (C2) moiety, a suberyol (C8) moiety, or a dodecanedioyl (C12) moiety.

In a further example of the insulin receptor partial agonist or insulin dimer, D¹ and D² are the same or wherein D¹ and D² are different.

In a further example of the insulin receptor partial agonist or insulin dimer, the linking moiety covalently links D¹ and D² via the epsilon amino group of a lysine residue at or
5 near the carboxy terminus of D¹ and D².

In a further still example of the insulin receptor partial agonist or insulin dimer, the substituent has a general formula RC(O)-, where R can be R'CH₂, R'NH, R'O, and R' can be H, linear alkyl chain, amino acid, peptide, PEG, saccharides, which in particular examples RC(O)- may be acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, or alkoxycarbonyl. In
10 particular examples, the substituent is selected from the group consisting of acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, isobutyl, methoxy acetyl, glycine, aminoethylglucose (AEG), AEG-C6, PEG1, PEG2, *N*-dimethyl, and alkoxycarbonyl.

In a further example of the insulin receptor partial agonist or insulin dimer, D¹ and D² are independently native human insulin, insulin lispro, insulin aspart, desB30 insulin, or
15 insulin glargine.

In particular examples of the insulin receptor partial agonist or insulin dimer, each A-chain polypeptide independently comprises the amino acid sequence
GX₂X₃EQCCX₈SICSLYQLX₁₇NX₁₉CX₂₃ (SEQ ID NO:3) and each B-chain polypeptide independently comprises the amino acid sequence

X₂₅LCGX₂₉X₃₀LVEALYLVCGERGFX₂₇YTX₃₁X₃₂ (SEQ ID NO:4) or
20 X₂₂VNQX₂₅X₂₆CGX₂₉X₃₀LVEALYLVCGERGFX₂₇YTX₃₁X₃₂X₃₃X₃₄X₃₅ (SEQ ID NO:5) wherein X₂ is isoleucine or threonine; X₃ is valine, glycine, or leucine; X₈ is threonine or histidine; X₁₇ is glutamic acid or glutamine; X₁₉ is tyrosine, 4-methoxy-phenylalanine, alanine, or 4-amino phenylalanine; X₂₃ is asparagine or glycine; X₂₂ is or phenylalanine and desamino-phenylalanine; X₂₅ is histidine or threonine; X₂₆ is leucine or glycine; X₂₇ is phenylalanine or
25 aspartic acid; X₂₉ is alanine, glycine, or serine; X₃₀ is histidine, aspartic acid, glutamic acid, homocysteic acid, or cysteic acid; X₃₁ is aspartic acid, proline, or lysine; X₃₂ is lysine or proline; X₃₃ is threonine, alanine, or absent; X₃₄ is arginine or absent; and X₃₅ is arginine or absent; with the proviso at least one of X₃₁ or X₃₂ is lysine.

In particular examples of the insulin receptor partial agonists or insulin dimers, the linking moiety may be an optionally substituted group selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, heteroaryl, and heterocyclic. The linking moiety may be a bivalent, straight or branched, saturated or unsaturated, optionally substituted C1-C20

hydrocarbon chain wherein one or more methylene units are optionally and independently replaced by -O-, -S-, -N(R)-, -C(O)-, C(O)O-, OC(O)-, -N(R)C(O)-, -C(O)N(R)-, -S(O)-, -S(O)₂-, -N(R)SO₂-, SO₂N(R)-, a heterocyclic group, an aryl group, or a heteroaryl group, wherein each occurrence of R is independently hydrogen, a suitable protecting group, an acyl moiety, arylalkyl moiety, aliphatic moiety, aryl moiety, heteroaryl moiety, or heteroaliphatic moiety.

In a further still example of the insulin receptor partial agonist or insulin dimer, the linking moiety is an acyl moiety, -C(O)RC(O)-, where R is alkyl chain, poly(ethylene glycol) (PEG) chain, amide-containing chain, triazole(s)-containing chain, cyclooctyne-containing moiety, a substituted acyl chain, or a polyethylene glycol (PEG) chain.

In a further example of the insulin receptor partial agonist or insulin dimer, the linking moiety is a C2-C20 acyl moiety.

In particular examples, the linking moiety is an alkyldioyl, -C(O)(CH₂)_nC(O)-, wherein n = 0-45, including but not limited to an oxalyl (C2) moiety, a succinyl (C4) moiety, an adipoyl (C6) moiety, a suberyol (C8) moiety, a decanedioyl (C10) moiety, a dodecanedioyl (C12) moiety, a tetradecanedioyl (C14) moiety, or a hexadecanedioyl (C16) moiety.

The present disclosure further provides an insulin analog dimer comprising:

a first insulin analog heterodimer and a second insulin or insulin analog heterodimer each heterodimer including an A-chain polypeptide and a B-chain polypeptide, wherein the A-chain polypeptide and the B-chain polypeptide are linked together through interchain disulfide bonds; wherein the first and second insulin or insulin analog heterodimers are covalently linked together through a linking moiety joining the side chain of an amino acid at or near the carboxy terminus of the two respective B-chain polypeptides; wherein the insulin analog is selected from insulin lispro, insulin aspart, and insulin glargine; and optionally wherein the amino terminus of at least one of the A-chain polypeptides and the B-chain polypeptides of the first insulin polypeptide or second insulin polypeptide is covalently linked to a substituent, with the proviso that the linking moiety does not include a disulfide bond.

In a further example of the insulin receptor partial agonist, the first and second insulin or insulin analog heterodimers are the same or wherein the first and second insulin or insulin analog heterodimers are different.

In a further example of the insulin receptor partial agonist, the linking moiety covalently links the first insulin or insulin analog heterodimer and the second insulin or insulin analog heterodimer via the epsilon amino group of a lysine residue at or near the carboxy terminus of their respective B-chain polypeptides.

In a further still example of the insulin receptor partial agonist, the substituent has a general formula $RC(O)-$, where R can be $R'CH_2$, $R'NH$, $R'O$, and R' can be H, linear alkyl chain, amino acid, peptide, PEG, saccharides, which in particular examples $RC(O)-$ may be acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, or alkoxycarbonyl. In particular examples, the substituent is selected from the group consisting of acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, isobutyl, methoxy acetyl, glycine, aminoethylglucose (AEG), AEG-C6, PEG1, PEG2, *N*-dimethyl, and alkoxycarbonyl.

In a further example of the insulin receptor partial agonist, at least one of the first and second insulin or insulin analog is further conjugated to polyethylene glycol, a sugar moiety, or a heterocycle.

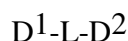
In particular examples of the insulin receptor partial agonists, the linking moiety may be an optionally substituted group selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, heteroaryl, and heterocyclic. The linking moiety may be a bivalent, straight or branched, saturated or unsaturated, optionally substituted C1-C20 hydrocarbon chain wherein one or more methylene units are optionally and independently replaced by $-O-$, $-S-$, $-N(R)-$, $-C(O)-$, $C(O)O-$, $OC(O)-$, $-N(R)C(O)-$, $-C(O)N(R)-$, $-S(O)-$, $-S(O)_2-$, $-N(R)SO_2-$, $SO_2N(R)-$, a heterocyclic group, an aryl group, or a heteroaryl group, wherein each occurrence of R is independently hydrogen, a suitable protecting group, an acyl moiety, arylalkyl moiety, aliphatic moiety, aryl moiety, heteroaryl moiety, or heteroaliphatic moiety.

In a further still example of the insulin receptor partial agonist, the linking moiety is an acyl moiety, $-C(O)RC(O)-$, where R is alkyl chain, poly(ethylene glycol) (PEG) chain, amide-containing chain, triazole(s)-containing chain, cyclooctyne-containing moiety, a substituted acyl chain, or a polyethylene glycol (PEG) chain.

In a further example of the insulin receptor partial agonist, the linking moiety is a C2-C20 acyl moiety.

In particular examples, the linking moiety is an alkylidioyl, $-C(O)(CH_2)_nC(O)-$, wherein $n = 0-45$, including but not limited to an oxalyl (C2) moiety, a succinyl (C4) moiety, an adipoyl (C6) moiety, a suberyol (C8) moiety, a decanedioyl (C10) moiety, a dodecanedioyl (C12) moiety, a tetradecanedioyl (C14) moiety, or a hexadecanedioyl (C16) moiety.

The present disclosure further provides an insulin analog dimer comprising the formula



wherein D^1 and D^2 are each independently an insulin or insulin analog polypeptide, wherein each insulin polypeptide is a heterodimer comprising an A-chain polypeptide and a B-chain polypeptide linked together through interchain disulfide bonds; L is a linking moiety wherein one end of the linker moiety is attached to an amino acid residue at or near the carboxyl group of D^1 and the other end of the linker moiety is attached to an amino acid residue at or near the carboxyl end of D^2 with the proviso that L does not include a disulfide linkage; wherein the insulin analog is selected from insulin lispro, insulin aspart, and insulin glargine; and optionally, wherein at least one of D^1 or D^2 includes a substituent attached to the amino terminus of the A-chain polypeptide or the B-chain polypeptide of D^1 or D^2 ; with the proviso that the linking moiety does not include a disulfide bond.

In a further example of the insulin receptor partial agonist, D^1 and D^2 are the same or wherein D^1 and D^2 are different.

In a further example of the insulin receptor partial agonist, the linking moiety covalently links D^1 and D^2 via the epsilon amino group of a lysine residue at or near the carboxy terminus of D^1 and D^2 .

In a further still example of the insulin receptor partial agonist, the substituent has a general formula $RC(O)-$, where R can be $R'CH_2$, $R'NH$, $R'O$, and R' can be H, linear alkyl chain, amino acid, peptide, PEG, saccharides, which in particular examples $RC(O)-$ may be acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, or alkoxycarbonyl. In particular examples, the substituent is selected from the group consisting of acetyl, phenylacetyl, carbamoyl, *N*-alkyl

carbamoyl, isobutyl, methoxy acetyl, glycine, aminoethylglucose (AEG), AEG-C6, PEG1, PEG2, *N*-dimethyl, and alkoxycarbonyl.

In particular examples of the insulin receptor partial agonists, the linking moiety may be an optionally substituted group selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, heteroaryl, and heterocyclic. The linking moiety may be a bivalent, straight or branched, saturated or unsaturated, optionally substituted C1-C20 hydrocarbon chain wherein one or more methylene units are optionally and independently replaced by -O-, -S-, -N(R)-, -C(O)-, C(O)O-, OC(O)-, -N(R)C(O)-, -C(O)N(R)-, -S(O)-, -S(O)₂-, -N(R)SO₂-, SO₂N(R)-, a heterocyclic group, an aryl group, or a heteroaryl group, wherein each occurrence of R is independently hydrogen, a suitable protecting group, an acyl moiety, arylalkyl moiety, aliphatic moiety, aryl moiety, heteroaryl moiety, or heteroaliphatic moiety.

In a further still example of the insulin receptor partial agonist, the linking moiety is an acyl moiety, -C(O)RC(O)-, where R is alkyl chain, poly(ethylene glycol) (PEG) chain, amide-containing chain, triazole(s)-containing chain, cyclooctyne-containing moiety, a substituted acyl chain, or a polyethylene glycol (PEG) chain.

In a further example of the insulin receptor partial agonist, the linking moiety is a C2-C20 acyl moiety.

In particular examples, the linking moiety is an alkyldioyl, -C(O)(CH₂)_nC(O)-, wherein n = 0-45, including but not limited to an oxalyl (C2) moiety, a succinyl (C4) moiety, an adipoyl (C6) moiety, a suberyol (C8) moiety, a decanedioyl (C10) moiety, a dodecanedioyl (C12) moiety, a tetradecanedioyl (C14) moiety, or a hexadecanedioyl (C16) moiety.

The present disclosure provides an insulin receptor partial agonist, comprising a first insulin or insulin analog heterodimer and a second insulin or insulin analog heterodimer each heterodimer including an A-chain polypeptide and a B-chain polypeptide, wherein the A-chain polypeptide and the B-chain polypeptide are linked together through interchain disulfide bonds; wherein the first and second insulin or insulin analog heterodimers are covalently linked together through a linking moiety joining the side chain of an amino acid at or near the carboxy terminus of the two respective B-chain polypeptides; optionally wherein the amino terminus of at least one of the A-chain polypeptides and the B-chain polypeptides of the first insulin polypeptide or second insulin polypeptide is covalently linked to a substituent; and wherein the insulin receptor partial agonist has a maximal response towards the human insulin

or near the carboxy terminus of the two respective B-chain polypeptides; optionally wherein the amino terminus of at least one of the A-chain polypeptides and the B-chain polypeptides of the first insulin polypeptide or second insulin polypeptide is covalently linked to a substituent; and wherein the insulin receptor partial agonist has a maximal response towards the human insulin receptor (IR) that is less than 70% of the maximal response of native human insulin towards the IR as determined by a functional phosphorylation assay; or

a first insulin or insulin analog heterodimer and a second insulin or insulin analog heterodimer each heterodimer including an A-chain polypeptide and a B-chain polypeptide, wherein the A-chain polypeptide and the B-chain polypeptide are linked together through interchain disulfide bonds; wherein the first and second insulin or insulin analog heterodimers are covalently linked together through a linking moiety joining the side chain of an amino acid at or near the carboxy terminus of the two respective B-chain polypeptides; optionally wherein the amino terminus of at least one of the A-chain polypeptides and the B-chain polypeptides of the first insulin polypeptide or second insulin polypeptide is covalently linked to a substituent; and wherein the insulin receptor partial agonist has a maximal response towards the human insulin receptor (IR) that is about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70% of the maximal response of native human insulin towards the IR as determined by a functional phosphorylation assay.

In the above examples, the functional phosphorylation assay may be an Insulin Receptor (IR) AKT-Phosphorylation assay.

In a further example of the insulin receptor partial agonist, the linking moiety does not include a disulfide bond and when the amino terminus of the A-chain polypeptide and the B-chain polypeptide do not include a substituent the linking moiety is not an oxalyl (C2) moiety, a suberyol (C8) moiety, or a dodecanedioyl (C12) moiety.

In a further example of the insulin receptor partial agonist, the first and second insulin or insulin analog heterodimers are the same or wherein the first and second insulin or insulin analog heterodimers are different.

In a further example of the insulin receptor partial agonist, wherein the linking moiety covalently links the first insulin or insulin analog heterodimer and the second insulin or

insulin analog heterodimer via the epsilon amino group of a lysine residue at or near the carboxy terminus of their respective B-chain polypeptides.

In a further still example of the insulin receptor partial agonist, the substituent has a general formula $RC(O)-$, where R can be $R'CH_2$, $R'NH$, $R'O$, and R' can be H, linear alkyl chain, amino acid, peptide, PEG, saccharides, which in particular examples $RC(O)-$ may be acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, or alkoxycarbonyl. In particular examples, the substituent is selected from the group consisting of acetyl, phenylacetyl, carbamoyl, *N*-alkyl carbamoyl, isobutyl, methoxy acetyl, glycine, aminoethylglucose (AEG), AEG-C6, PEG1, PEG2, *N*-dimethyl, and alkoxycarbonyl.

In a further example of the insulin receptor partial agonist, the first and second insulins or insulin analogs are independently native human insulin, insulin lispro, insulin aspart, desB30 insulin, or insulin glargine.

In particular examples of the insulin receptor partial agonist, each A-chain polypeptide independently comprises the amino acid sequence

$GX_2X_3EQCCX_8SICSLYQLX_{17}NX_{19}CX_{23}$ (SEQ ID NO:3) and each B-chain polypeptide independently comprises the amino acid sequence

$X_{25}LCGX_{29}X_{30}LVEALYLVCGERGFX_{27}YTX_{31}X_{32}$ (SEQ ID NO:4) or

$X_{22}VNQX_{25}X_{26}CGX_{29}X_{30}LVEALYLVCGERGFX_{27}YTX_{31}X_{32}X_{33}X_{34}X_{35}$ (SEQ ID NO:5) wherein X_2 is isoleucine or threonine; X_3 is valine, glycine, or leucine; X_8 is threonine or histidine; X_{17} is glutamic acid or glutamine; X_{19} is tyrosine, 4-methoxy-phenylalanine, alanine, or 4-amino phenylalanine; X_{23} is asparagine or glycine; X_{22} is or phenylalanine and desamino-phenylalanine; X_{25} is histidine or threonine; X_{26} is leucine or glycine; X_{27} is phenylalanine or aspartic acid; X_{29} is alanine, glycine, or serine; X_{30} is histidine, aspartic acid, glutamic acid, homocysteic acid, or cysteic acid; X_{31} is aspartic acid, proline, or lysine; X_{32} is lysine or proline; X_{33} is threonine, alanine, or absent; X_{34} is arginine or absent; and X_{35} is arginine or absent; with the proviso at least one of X_{31} or X_{32} is lysine.

In particular examples of the insulin receptor partial agonists, the linking moiety may be an optionally substituted group selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, heteroaryl, and heterocyclic. The linking moiety may be a bivalent, straight or branched, saturated or unsaturated, optionally substituted C1-20 hydrocarbon chain wherein

one or more methylene units are optionally and independently replaced by -O-, -S-, -N(R)-, -C(O)-, C(O)O-, OC(O)-, -N(R)C(O)-, -C(O)N(R)-, -S(O)-, -S(O)₂-, -N(R)SO₂-, SO₂N(R)-, a heterocyclic group, an aryl group, or a heteroaryl group, wherein each occurrence of R is independently hydrogen, a suitable protecting group, an acyl moiety, arylalkyl moiety, aliphatic moiety, aryl moiety, heteroaryl moiety, or heteroaliphatic moiety.

In a further still example of the insulin receptor partial agonist, the linking moiety is an acyl moiety, -C(O)RC(O)-, where R is alkyl chain, poly(ethylene glycol) (PEG) chain, amide-containing chain, triazole(s)-containing chain, cyclooctyne-containing moiety, a substituted acyl chain, or a polyethylene glycol (PEG) chain.

In a further example of the insulin receptor partial agonist, the linking moiety is a C2-C20 acyl moiety.

In particular examples, the linking moiety is an alkyldioyl, -C(O)(CH₂)_nC(O)-, wherein n = 0-45, including but not limited to an oxalyl (C2) moiety, a succinyl (C4) moiety, an adipoyl (C6) moiety, a suberyol (C8) moiety, a decanedioyl (C10) moiety, a dodecanedioyl (C12) moiety, a tetradecanedioyl (C14) moiety, or a hexadecanedioyl (C16) moiety.

The present disclosure further provides an insulin dimer comprising a first B29 or B28 Lys of a first insulin heterodimer molecule having a first A-chain polypeptide and first B-chain polypeptide and a second B29 or B28 Lys of a second insulin heterodimer having a second A-chain polypeptide and second B-chain polypeptide conjugated together by a bifunctional linker selected from the group consisting **Linker 1, Linker 2, Linker 3, Linker 10, Linker 11, Linker 12, Linker 13, Linker 14, Linker 15, Linker 16, Linker 17, Linker 18, Linker 19, Linker 20, Linker 21, Linker 22, Linker 23, Linker 24, Linker 25, Linker 26, Linker 27, Linker 28, Linker 29, Linker 30, Linker 31, Linker 32, Linker 33, Linker 34, Linker 35, Linker 36, Linker 37, Linker 38, Linker 39, Linker 40, Linker 41, Linker 42, Linker 43, Linker 44, Linker 45, Linker 46, Linker 47, Linker 48, Linker 49, and Linker 50** with the proviso that when the bifunctional linker is **Linker 10, Linker 11, Linker 12, Linker 13, or Linker 14**, at least one of the first or second A-chain or B-chain polypeptides is conjugated at its *N*-terminal amino acid to a substituent or at least the *N*-terminal amino acids of the first insulin heterodimer molecule are conjugated to a substituent or the *N*-terminal amino acids of both the first insulin heterodimer and second insulin heterodimer are conjugated to a substituent.

In particular examples, the substituent comprises an *N*-hydroxysuccinimide ester linked to a group having the general formula $RC(O)-$, where R can be $R'CH_2$, $R'NH$, $R'O$, and R' can be H, linear alkyl chain, amino acid, peptide, polyethylene glycol (PEG), saccharides. In particular examples, the substituent is a carbamoyl group, acetyl group, glycine, methyl group, methoxy group, dimethyl group, isobutyl group, PEG1 group, AEG group, AEG-C6 alkyl group, or PEG2 group.

The present disclosure further provides an insulin dimer comprising a first B29 or B28 Lys of a first insulin heterodimer molecule having a first A-chain polypeptide and first B-chain polypeptide is conjugated to a first linker selected from the group consisting of **Linker 5** and **Linker 7** and a second B29 or B28 Lys of a second insulin heterodimer having a second A-chain polypeptide and second B-chain polypeptide conjugated to a second linker selected from the group consisting of **Linker 4**, **Linker 6**, **Linker 8**, and **Linker 9** conjugated together via the first linker and the second linker.

In a further example, the present disclosure provides an insulin analog dimer, comprising a first B29 or B28 Lys of a first insulin heterodimer molecule having a first A-chain polypeptide and first B-chain polypeptide conjugated to a first linker selected from the group consisting of **Linker 5** and **Linker 7** and a second B29 or B28 Lys of a second insulin heterodimer having a second A-chain polypeptide and second B-chain polypeptide conjugated to a second linker selected from the group consisting of **Linker 5** and **Linker 7**, wherein the first and second linkers are conjugated together via a bridging linker having a structure



wherein R is a covalent bond, a carbon atom, a phenyl, a heteroatom, or an optionally substituted group selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, heteroaryl, and heterocyclic. In particular examples R is a C2, C3, C4, C6, C7, C8, C9 or C10 acyl group or a PEG2, PEG3, PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, PEG11, PEG12, PEG13, or PEG25.

In particular examples, the substituent comprises an *N*-hydroxysuccinimide ester linked to a group having the general formula $RC(O)-$, where R can be $R'CH_2$, $R'NH$, $R'O$, and R' can be H, linear alkyl chain, amino acid, peptide, polyethylene glycol (PEG), saccharides. In particular examples, the substituent is a carbamoyl group, acetyl group, glycine, methyl

group, methoxy group, dimethyl group, isobutyl group, PEG1 group, AEG group, AEG-C6 alkyl group, or PEG2 group.

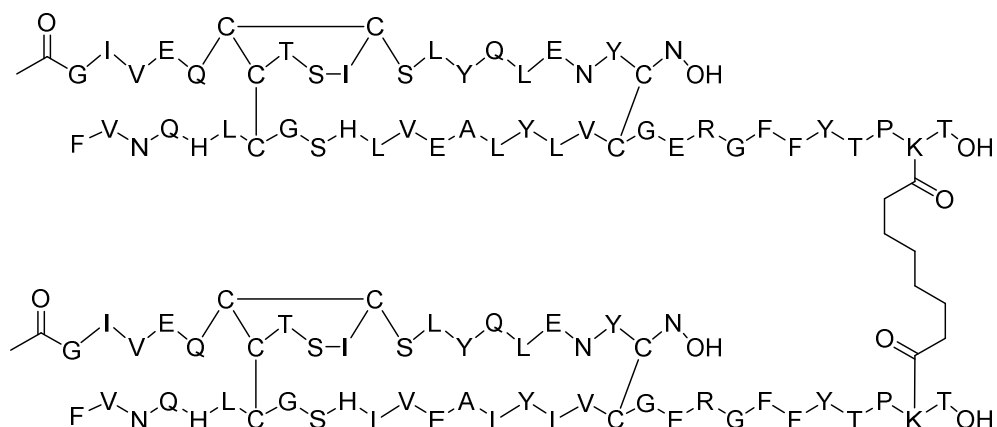
In a further example, the present disclosure provides an insulin analog dimer, comprising a first B29 or B28 Lys of a first insulin heterodimer molecule having a first A-chain polypeptide and first B-chain polypeptide conjugated to a first linker selected from the group consisting of **Linker 4**, **Linker 6**, **Linker 8**, and **Linker 9** and a second B29 or B28 Lys of a second insulin heterodimer having a second A-chain polypeptide and second B-chain polypeptide conjugated to a second linker selected from the group consisting of **Linker 4**, **Linker 6**, **Linker 8**, and **Linker 9**, wherein the first and second linkers are conjugated together via a bridging linker having a structure



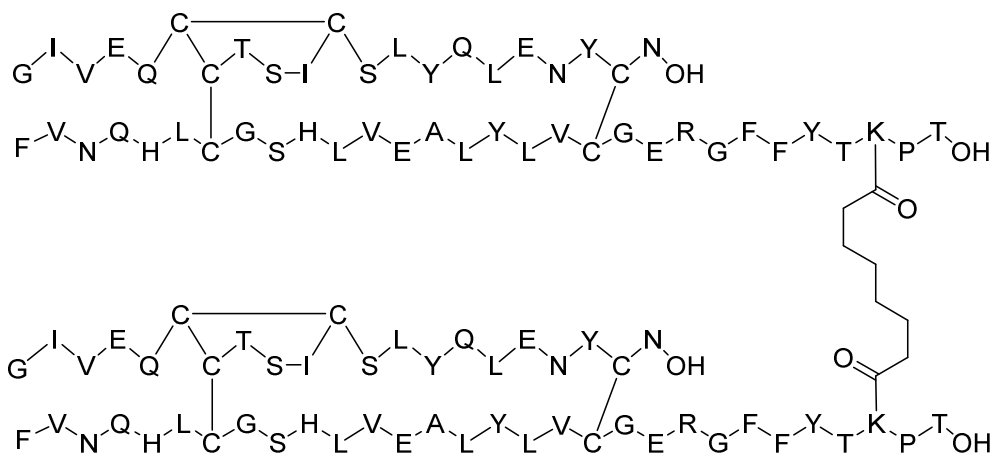
wherein R is a covalent bond, a carbon atom, a phenyl, a heteroatom, or an optionally substituted group selected from the group consisting of acyl, aliphatic, heteroaliphatic, aryl, heteroaryl, and heterocyclic. In particular examples R is a C2, C3, C4, C6, C7, C8, C9 or C10 acyl group or a PEG2, PEG3, PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, PEG11, PEG12, PEG13, or PEG 25.

In particular examples, the substituent comprises an *N*-hydroxysuccinimide ester linked to a group having the general formula RC(O)-, where R can be R'CH₂, R'NH, R'O, and R' can be H, linear alkyl chain, amino acid, peptide, polyethylene glycol (PEG), saccharides. In particular examples, the substituent is a carbamoyl group, acetyl group, glycine, methyl group, methoxy group, dimethyl group, isobutyl group, PEG1 group, AEG group, AEG-C6 alkyl group, or PEG2 group.

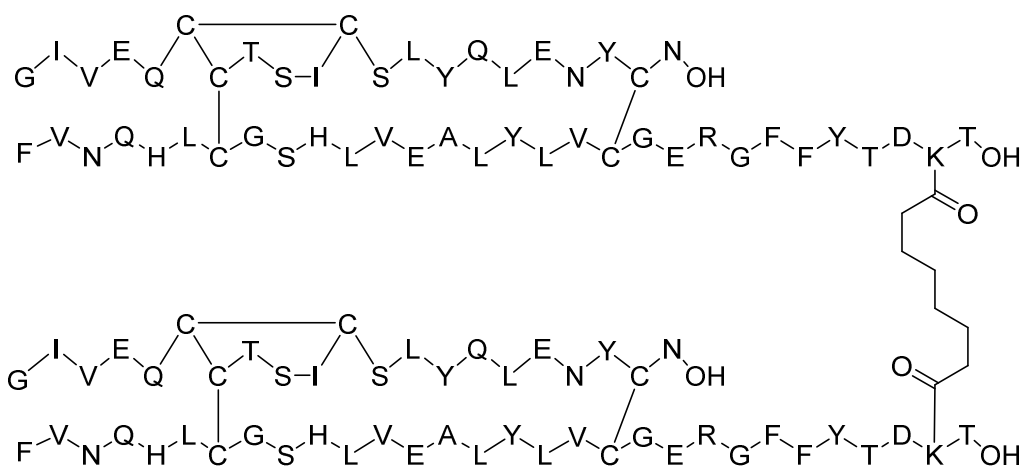
In one aspect, there is provided a compound selected from the group consisting of



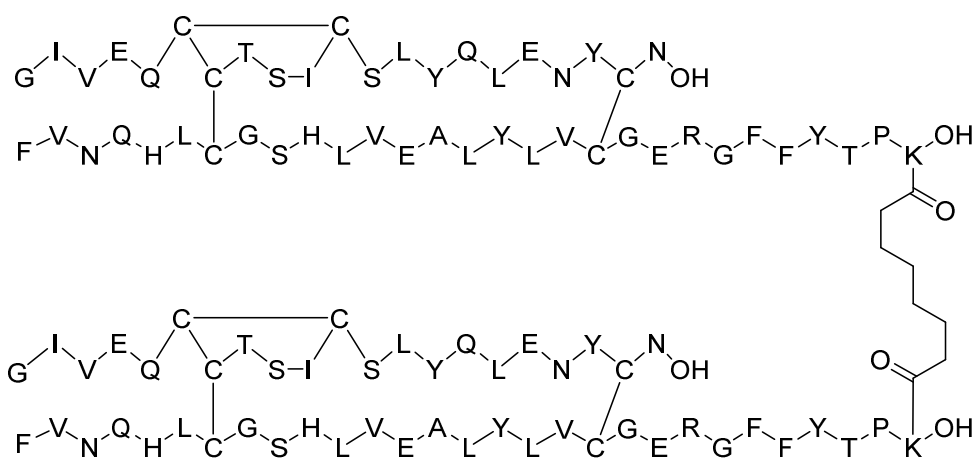
Dimer 13;



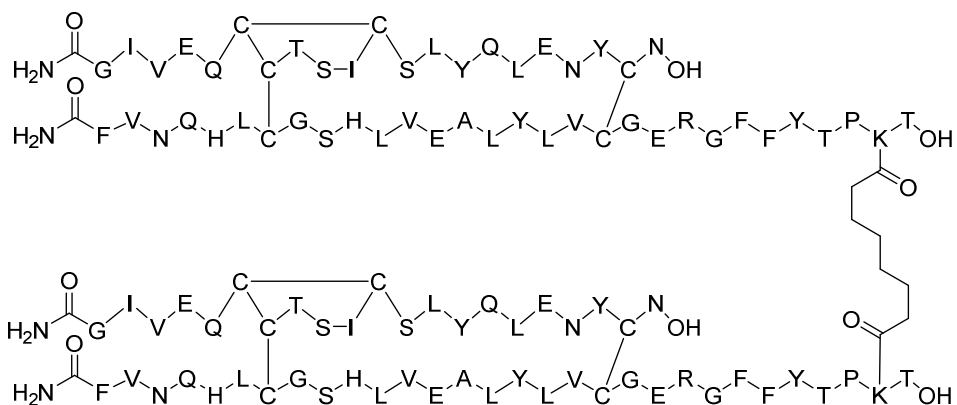
Dimer 18;



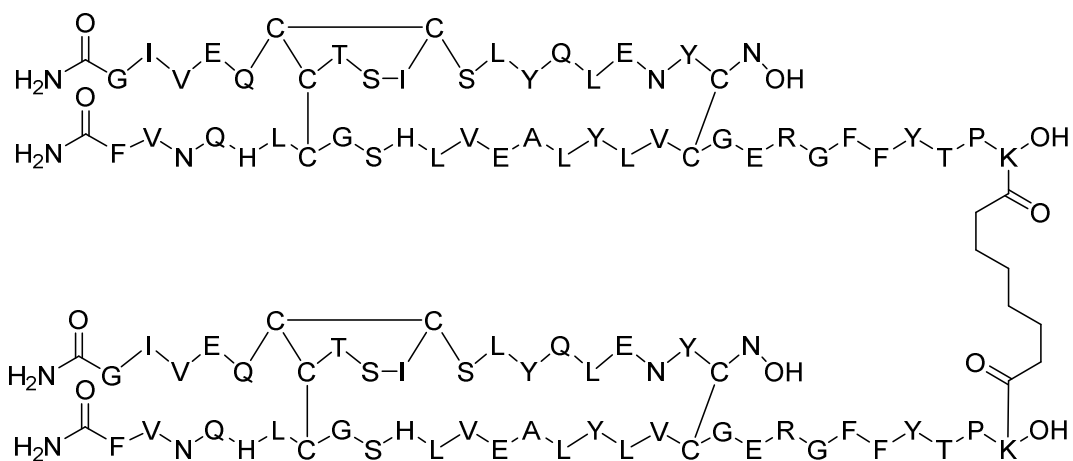
Dimer 19;



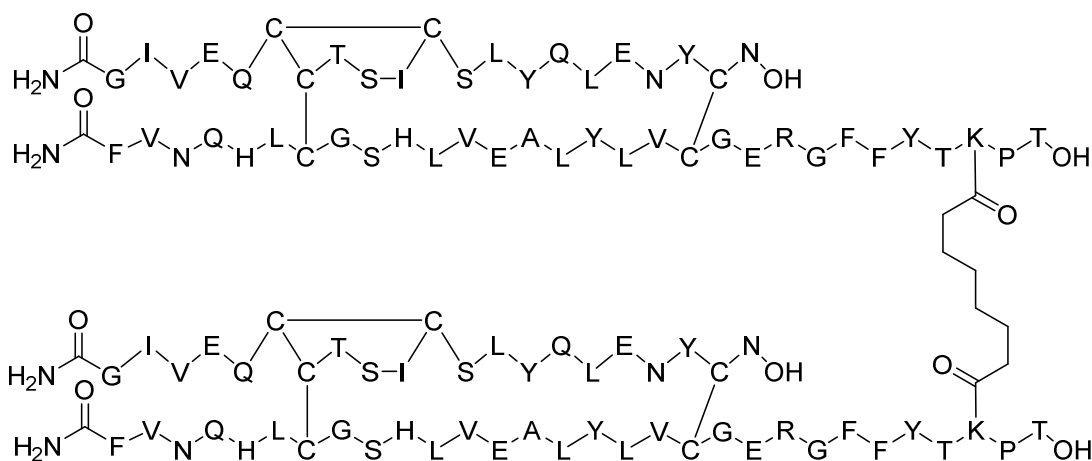
Dimer 20;



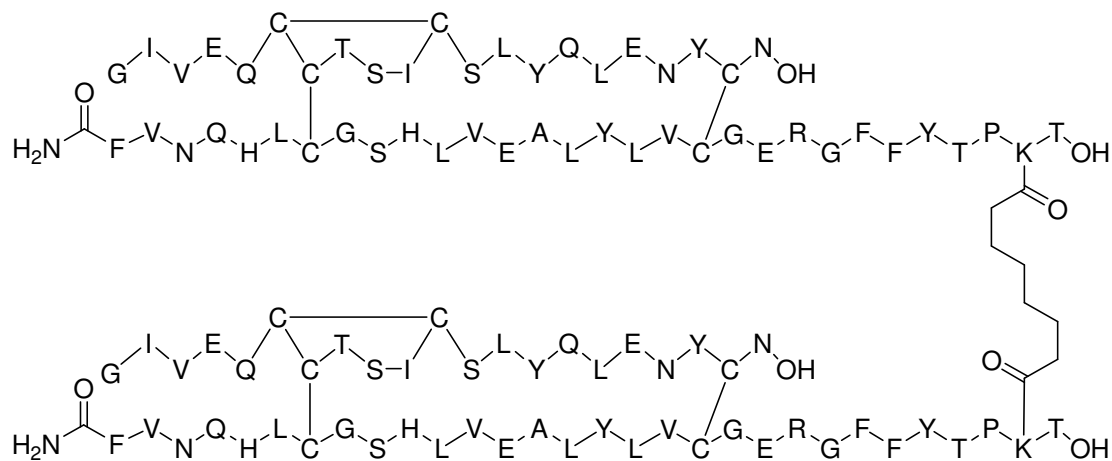
Dimer 24;



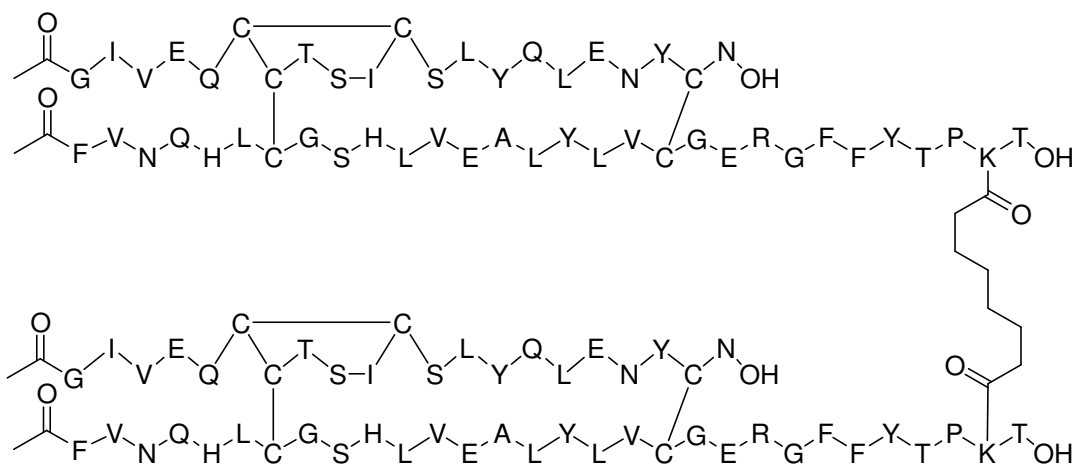
Dimer 28;



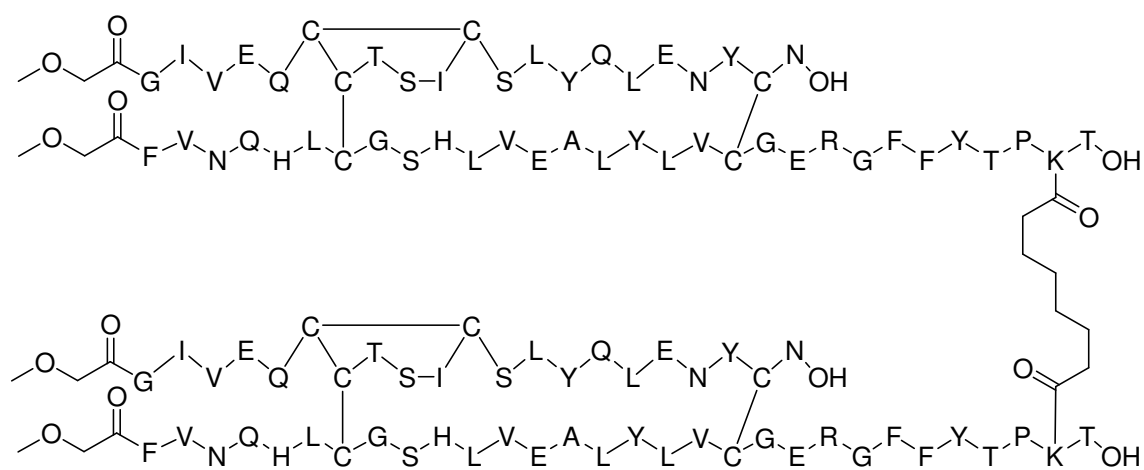
Dimer 32;



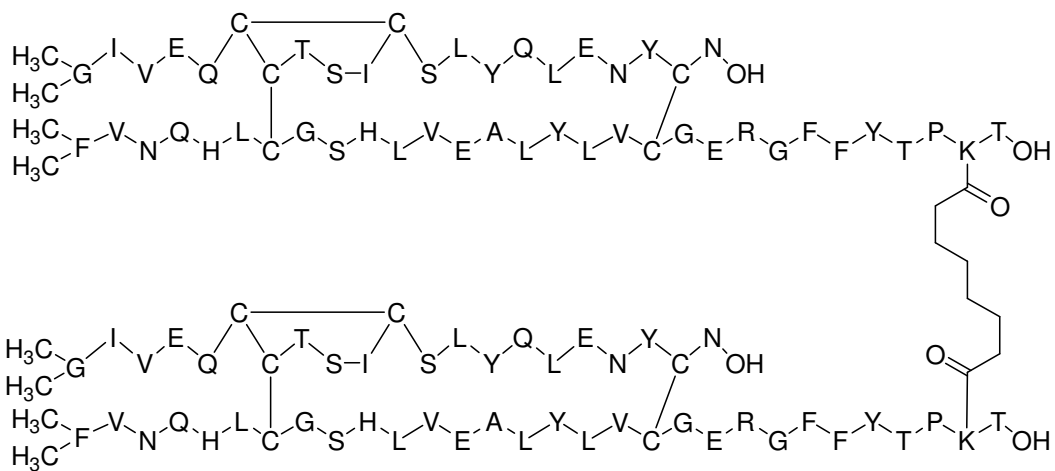
Dimer 54;



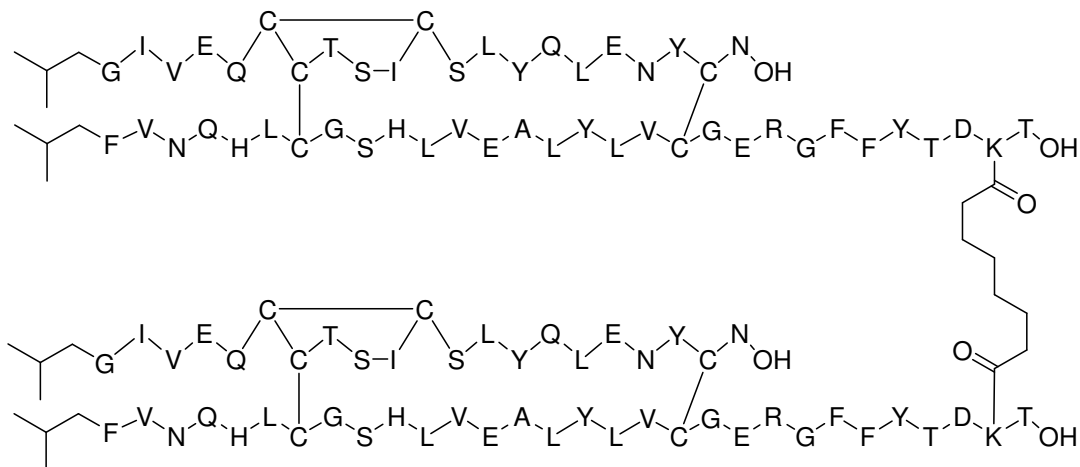
Dimer 55;



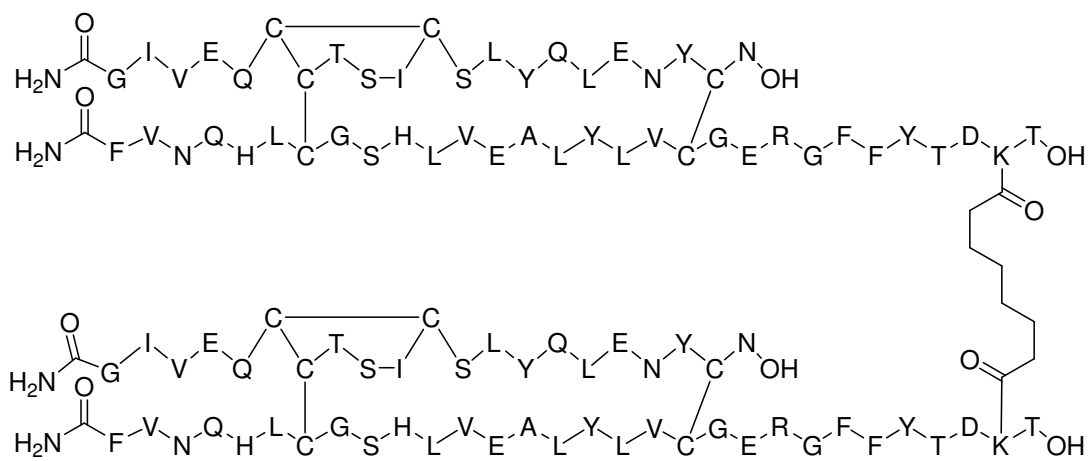
Dimer 56;



Dimer 58;



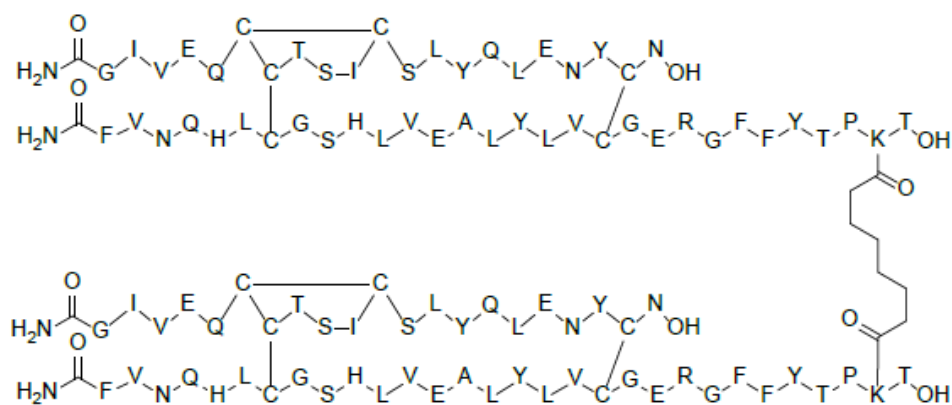
Dimer 65; or



Dimer 83;

wherein the disulfide linkages between the Cys₆ and Cys₁₁ residues of the A-chain polypeptide and the disulfide linkages between the Cys₇ and Cys₂₀ of the A-chain to the Cys₇ and Cys₁₉ of the B-chain polypeptide, respectively, are represented by the solid line therebetween; wherein the linking moieties are covalently linked to the epsilon amino acid of the shown lysine residue, wherein the A-chain polypeptide for **Dimers 13, 18, 19, 20, 24, 28, 32, 54, 55, 56, 58, 65 and 83** has the amino acid sequence shown in SEQ ID NO:1; the B-chain polypeptide for **Dimers 13, 24, 54, 55, 56 and 58** has the amino acid sequence shown in SEQ ID NO:2; the B-chain polypeptide for **Dimers 18 and 32** has the amino acid sequence shown in SEQ ID NO:6; the B-chain polypeptide for **Dimers 19, 65 and 83** has the amino acid sequence shown in SEQ ID NO:9; the B-chain polypeptide for **Dimers 20 and 28** has the amino acid sequence shown in SEQ ID NO:10.

In another aspect, there is provided a compound having the formula



Dimer 24;

wherein the disulfide linkages between the Cys₆ and Cys₁₁ residues of the A-chain polypeptide and the disulfide linkages between the Cys₇ and Cys₂₀ of the A-chain to the Cys₇ and Cys₁₉ of the B-chain polypeptide, respectively, are represented by the solid line therebetween; wherein the linking moieties are covalently linked to the epsilon amino acid of the shown lysine residue, wherein the A-chain polypeptide has the amino acid sequence shown in SEQ ID NO:1 and the B-chain polypeptide has the amino acid sequence shown in SEQ ID NO: 2.

The present disclosure further provides compositions comprising any one of the insulin receptor partial agonists disclosed herein and a pharmaceutically acceptable salt.

In another aspect, there is provided a composition comprising a compound as disclosed herein and a pharmaceutically acceptable carrier.

The present disclosure provides a method for treating diabetes comprising administering to an individual with diabetes a therapeutically effective amount of a composition
5 comprising any one of the aforementioned insulin receptor partial agonists. In particular examples the diabetes is Type 1 diabetes, Type 2 diabetes, or gestational diabetes.

In another aspect, there is provided a composition for use in the treatment of diabetes comprising a compound as described herein.

The present disclosure provides for the use of a composition for the treatment of
10 diabetes comprising any one of the aforementioned insulin receptor partial agonists. In particular examples the diabetes is Type 1 diabetes, Type 2 diabetes, or gestational diabetes.

The present disclosure provides for the use of any one of the insulin receptor partial agonists disclosed herein for the manufacture of a medicament for the treatment of diabetes. In another aspect, there is provided use of a compound as described herein in the manufacture of a medicament for the treatment of diabetes. In particular examples the diabetes is

5 Type 1 diabetes, Type 2 diabetes, or gestational diabetes.

Definitions

Insulin - as used herein, the term means the active principle of the pancreas that affects the metabolism of carbohydrates in the animal body and which is of value in the treatment of diabetes mellitus. The term includes synthetic and biotechnologically derived

10 products that are the same as, or similar to, naturally occurring insulins in structure, use, and intended effect and are of value in the treatment of diabetes mellitus. The term is a generic term that designates the 51 amino acid heterodimer comprising the A-chain peptide having the amino acid sequence shown in SEQ ID NO: 1 and the B-chain peptide having the amino acid sequence shown in SEQ ID NO: 2, wherein the cysteine residues at positions 6 and 11 of the A chain are

15 linked in a disulfide bond, the cysteine residues at position 7 of the A chain and position 7 of the B chain are linked in a disulfide bond, and the cysteine residues at position 20 of the A chain and 19 of the B chain are linked in a disulfide bond.

Insulin analog or analogue - the term as used herein includes any heterodimer analogue or single-chain analogue that comprises one or more modification(s) of the native A-chain peptide and/or B-chain peptide. Modifications include but are not limited to substituting

20 an amino acid for the native amino acid at a position selected from A4, A5, A8, A9, A10, A12, A13, A14, A15, A16, A17, A18, A19, A21, B1, B2, B3, B4, B5, B9, B10, B13, B14, B15, B16, B17, B18, B20, B21, B22, B23, B26, B27, B28, B29, and B30; deleting any or all of positions B1-4 and B26-30; or conjugating directly or by a polymeric or non-polymeric linker one or more

25 acyl, polyethylglycine (PEG), or saccharide moiety (moieties); or any combination thereof. As exemplified by the *N*-linked glycosylated insulin analogues disclosed herein, the term further includes any insulin heterodimer and single-chain analogue that has been modified to have at least one *N*-linked glycosylation site and in particular, embodiments in which the *N*-linked glycosylation site is linked to or occupied by an *N*-glycan. Examples of insulin analogues

30 include but are not limited to the heterodimer and single-chain analogues disclosed in published international application WO20100080606, WO2009/099763, and WO2010080609, the