THERAPEUTIC PEPTIDES

FIELD OF THE INVENTION

This invention relates to therapeutic peptides useful in the treatment of obesity and metabolic disorders. More specifically, the invention relates to novel analogs of Peptide YY (PYY) and their use.

BACKGROUND OF THE INVENTION

The prevalence of obesity in the United States is increasing, with 35.7% of adults considered obese (BMI ≥ 30) and 68.8% considered overweight (BMI ≥ 25) in 2009-2010. See, for example, Flegal *et al.* (2012) *JAMA* 307(5):491-7. Worldwide, over 300 million people are considered obese. Obesity-related diseases, including Type 2 Diabetes Mellitus, hypertension, heart disease, joint disease, and some types of cancer have increased in prevalence as the population has grown heavier.

Prevention of obesity through diet and exercise is of critical importance to control these trends, but once patients become obese, the body's resistance to weight loss can be considerable. Diet and exercise alone may be insufficient to bring about significant weight change in severely obese patients, and both pharmacologic therapy and surgery have proven to be effective as additional aids to weight loss. Prevention and treatment of obesity are areas of high unmet medical need, with few medications currently available for chronic weight loss therapy.

Peptide YY (PYY) belongs to the PP-fold family of peptides together with pancreatic polypeptide and neuropeptide Y, which have a role in controlling appetite. See, for example, Schwartz *et al.* (2002) *Nature* :418(6898):595-7. PYY is secreted as a 36 amino acid, straight chain polypeptide and then cleaved by dipeptidyl peptidase IV to produce PYY(3-36). Fasting and post-prandial concentrations of PYY in morbidly obese individuals after gastric bypass surgery are suggested as playing a role in their dramatic weight loss. See, for example, le Roux (2006) *Ann Surg.*243(1):108-14. Peripheral infusion of PYY(3-36) has been shown to increase energy expenditure and fat oxidation rates in obese and lean subjects. See, for example, Batterham *et al.* (2003) *N Engl J Med.* 349(10):941-8, and Sloth *et al.* (2007) *Am J Physiol Endocrinol Metab.*: 293(2):E604-9. Administration of a PYY(3-36) nasal spray reduced daily caloric intake of obese individuals by 2713 kJ, resulting in a weight loss of 0.6 kg over a six-day study period. See, for example, Gantz *et al.* (2007) *J Clin Endocrinol Metab.* 92(5):1754-7. These results demonstrate that obese subjects retain sensitivity to PYY(3-36), in contrast to leptin, where resistance limits its therapeutic usefulness in obesity.

Accordingly, there remains a need in the art for improved PYY compositions for use in the treatment of obesity and obesity-related disorders.

BRIEF SUMMARY OF INVENTION

The present invention relates to novel analogs of PYY that have an improved therapeutic profile when compared to native human PYY. These novel PYY analogs are useful in the treatment of obesity, diabetes, and other disorders.

Briefly, in one aspect, the invention provides a polypeptide comprising the amino acid sequence:

ProLysProGluXaa$_1$ProGlyXaa$_2$AspAlaSerXaa$_3$GluGluXaa$_4$Xaa$_5$Xaa$_6$TyrTyrAla Xaa$_7$LeuArgXaa$_8$TyrXaa$_9$ AsnTrpXaa$_{10}$ThrArgGlnArgTyr (SEQ ID NO:1)

or a salt thereof, wherein:

Xaa$_1$ is Ala, His, or Ser;

Xaa$_2$ is Glu or Lys;

Xaa$_3$ is Pro or Ala;

Xaa$_4$ is Leu or Trp;

Xaa$_5$ is Asn, Ala, or Thr;

Xaa$_6$ is Arg or Lys;

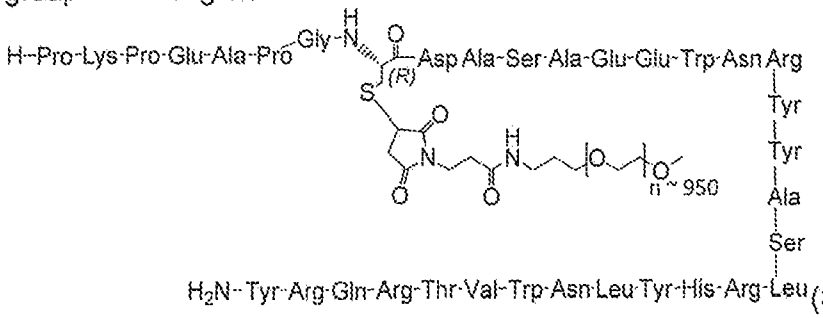
Xaa$_7$ is Ser, Asp, or Ala;

Xaa$_8$ is His or Lys;

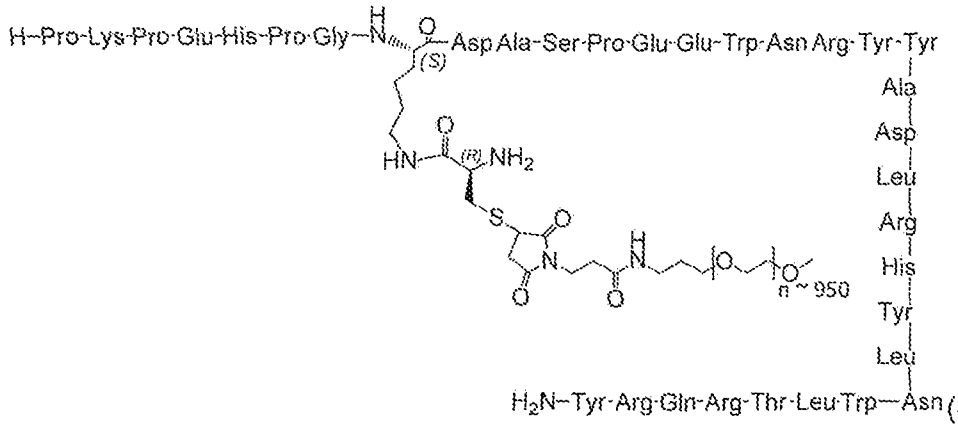
Xaa$_9$ is Leu or Ile; and

Xaa$_{10}$ is Val or Leu.

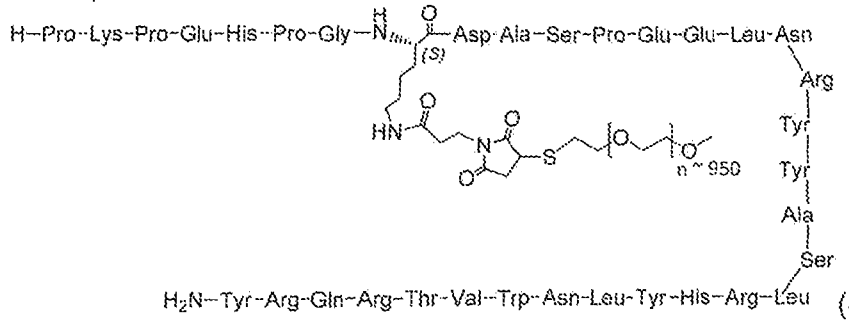
In another aspect, the invention provides a polypeptide selected from the group consisting of:



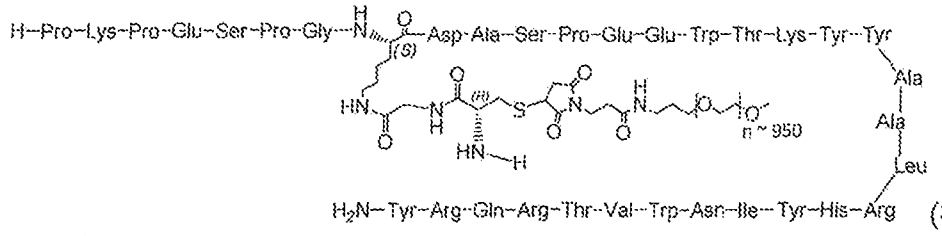
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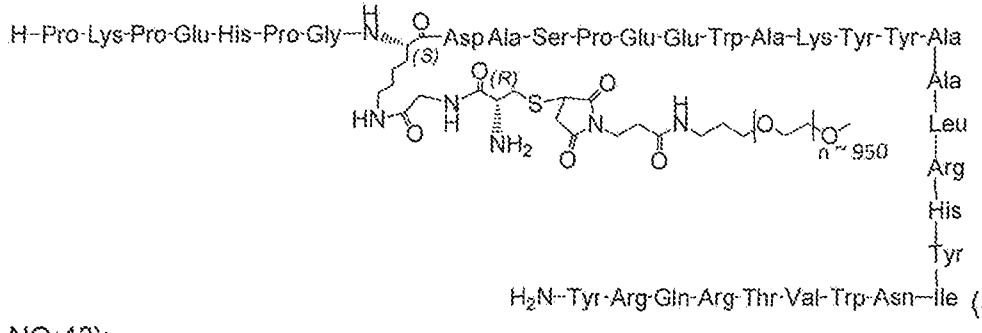
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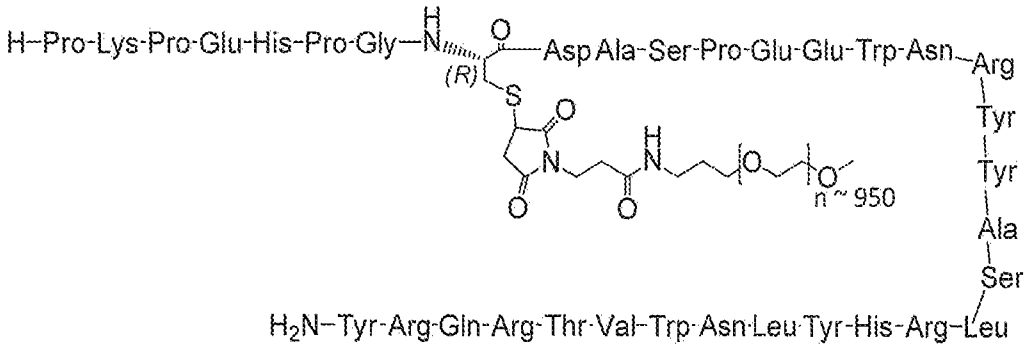
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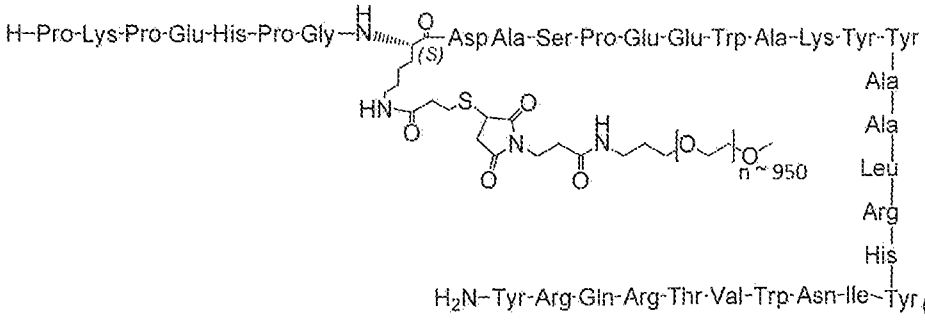
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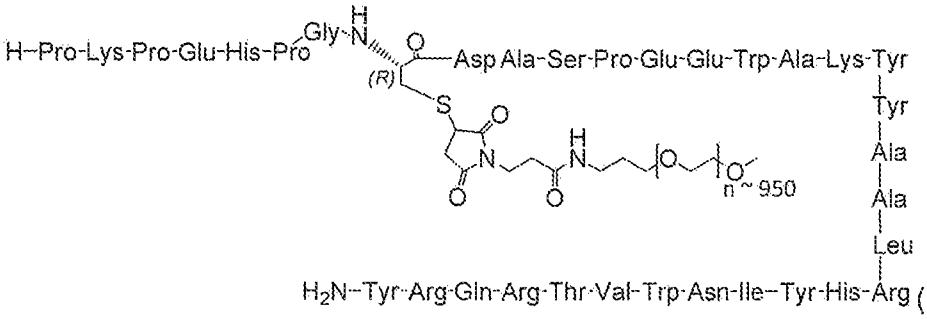
(SEQ ID NO:43);



(SEQ ID NO:44);



(SEQ ID NO:43);



(SEQ ID NO:45);

and salts thereof.

In another aspect, the invention provides a nucleic acid molecule encoding a polypeptide of the invention

In yet another aspect, the invention includes an expression vector comprising a nucleic acid molecule encoding a polypeptide of the invention.

In a further aspect, the invention encompasses a host cell containing an expression vector comprising a nucleic acid molecule encoding a polypeptide of the invention.

In another aspect, the invention provides a pharmaceutical combination comprising a novel PYY polypeptide of the invention and exendin-4.

In yet another aspect, the invention provides a pharmaceutical combination comprising a novel PYY polypeptide of the invention and GLP-1.

In an additional aspect the invention provides a pharmaceutical composition comprising a novel PYY polypeptide of the invention and one or more pharmaceutically acceptable excipients.

In a further aspect, the invention encompasses a method of treating a metabolic disorder or obesity, the method comprising administering a novel PYY polypeptide or pharmaceutical combination of the invention to a subject in need thereof.

The invention also provides the use of a polypeptide or pharmaceutical combination of the invention in the preparation of a medicament for use in the treatment of obesity.

In addition, the invention provides a polypeptide or pharmaceutical combination of the invention for use in the treatment of a metabolic disorder or obesity.

BREIF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the effects of the peptide shown in Example 5 (Analog # 5), PYY(3-36)NH_2 (PYY3-36), and exendin-4 singly and in combination on changes in body weight in diet-induced obese (DIO) Long Evans (LE) rats.

Figure 2 shows the effects of the peptide shown in Example 5 (Analog # 5), PYY(3-36)NH_2 (PYY3-36), and exendin-4 singly and in combination on body composition changes in DIO LE rats.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides novel analogs of PYY that have an improved therapeutic profile when compared to native human PYY. The novel PYY analogs of the invention show improved effects on food intake when compared with the native PYY sequence.

In one aspect, the novel PYY analogs comprise the amino acid sequence:

ProLysProGluXaa$_1$ProGlyXaa$_2$AspAlaSerXaa$_3$GluGluXaa$_4$Xaa$_5$Xaa$_6$TyrTyrAla Xaa$_7$LeuArgXaa$_8$TyrXaa$_9$ AsnTrpXaa$_{10}$ThrArgGlnArgTyr (SEQ ID NO:1)

or a salt thereof, wherein:

Xaa$_1$ is Ala, His, or Ser;

Xaa_2 is Glu or Lys;

Xaa_3 is Pro or Ala;

Xaa_4 is Leu or Trp;

Xaa_5 is Asn, Ala, or Thr;

Xaa_6 is Arg or Lys;

Xaa_7 is Ser, Asp, or Ala;

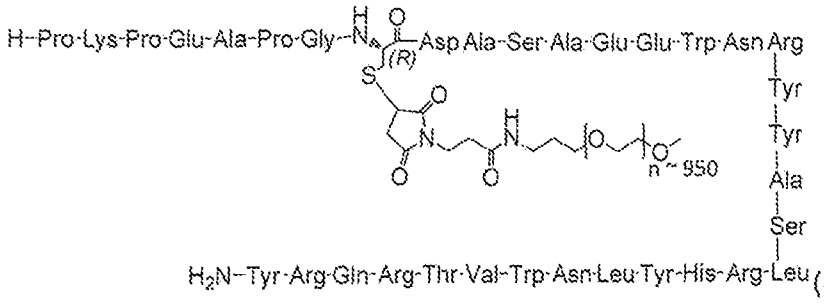
Xaa_8 is His or Lys;

Xaa_9 is Leu or Ile; and

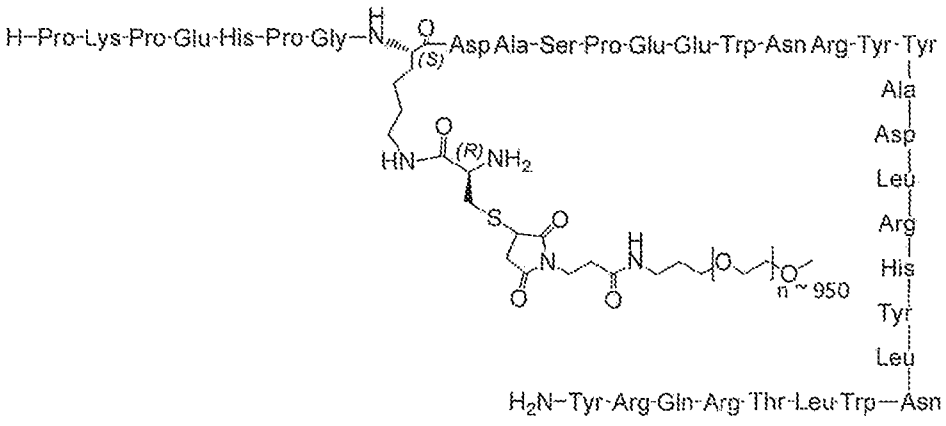
Xaa_{10} is Val or Leu.

The novel polypeptides of the invention show a statistically significant increase in the reduction of food intake in either a lean and/or diet-induced obesity animal model when compared with human PYY(3-36). Preferably the polypeptides of the invention reduce the intake of food in a lean and/or diet-induced obesity animal model by at least 20%, at least 30%, or at least 40%. More preferably, the polypeptides reduce the intake of food in a lean and/or diet-induced obesity animal model by at least 50%.

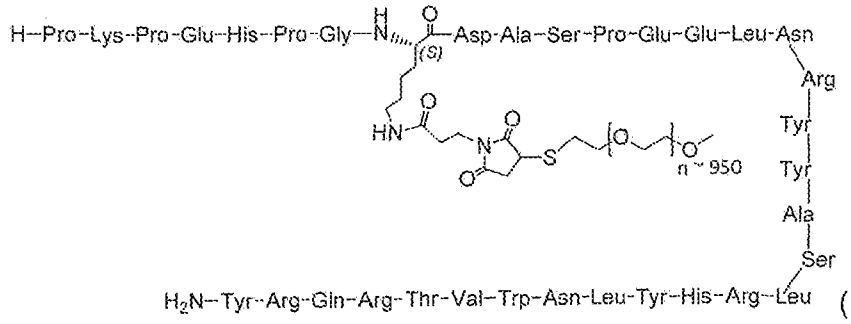
In another aspect, the invention provides a polypeptide selected from the group consisting of:



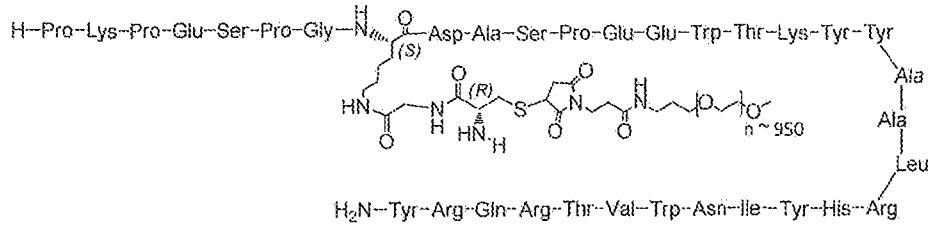
(SEQ ID NO:39);



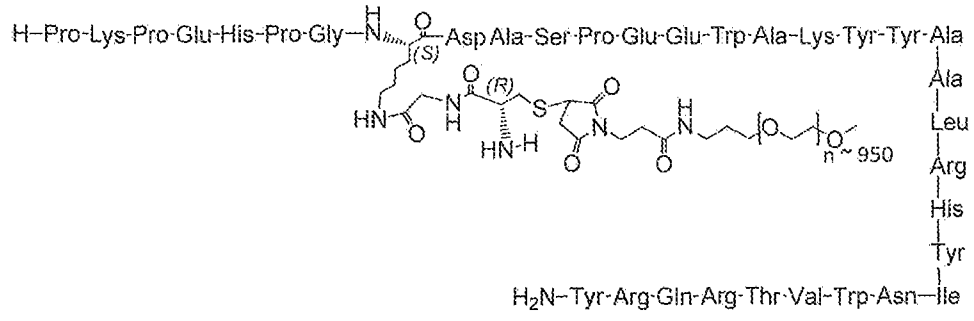
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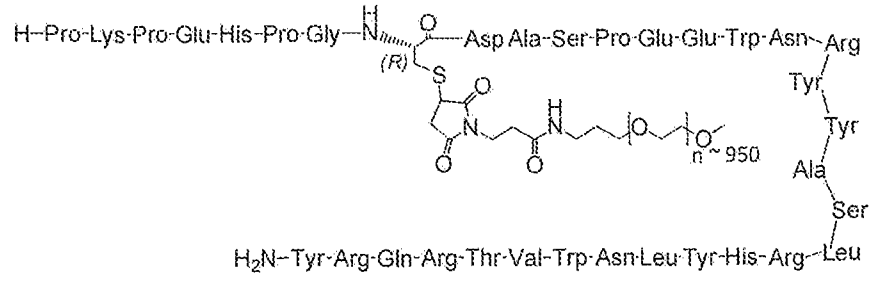
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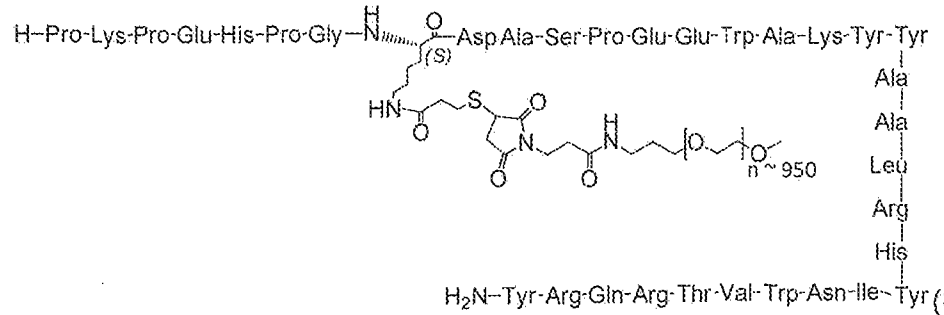
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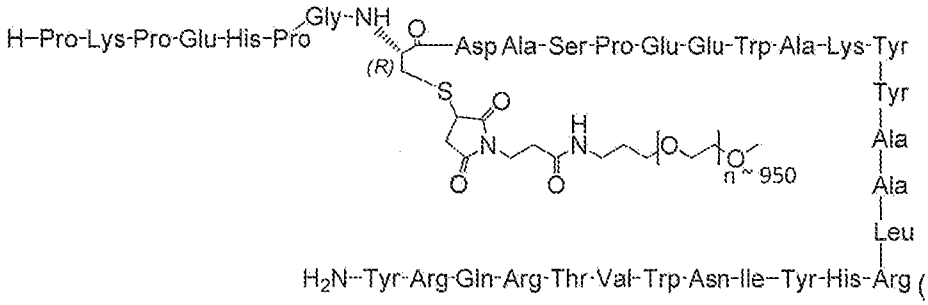
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(SEQ ID NO:43);



(SEQ ID NO:45);

and salts thereof.

Unless otherwise indicated, the polypeptides of the invention may have either a carboxamide or carboxylic acid at the end of the amino acid chain.

The invention encompasses salts of the recited polypeptides, including pharmaceutically acceptable salts. Examples of such salts include, but are not limited to, including inorganic and organic acids and bases, including but not limited to, sulfuric, citric, maleic, acetic, oxalic, hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfite, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Also included are salts formed with free amino groups such as, for example, hydrochloric, phosphoric, acetic, trifluoroacetic, oxalic, and tartaric acids. Also included are salts that may form with free carboxy groups such as, for example sodium, potassium, ammonium, sodium, lithium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, and procaine salts.

The polypeptides of the invention may be prepared using standard recombinant expression or chemical peptide synthesis techniques known in the art. See, for example, Chan, Weng C., and Peter D. White, eds. *Fmoc Solid Phase Peptide Synthesis: A Practical Approach.* New York: Oxford UP, 2000, and Howl, John, ed. *Peptide Synthesis and Applications (Methods in Molecular Biology).* Totowa, NJ: Humana, 2005.

The compositions and pharmaceutical combinations of the invention are useful for the treatment of metabolic disorders including, for example, hyperglycemia , impaired glucose tolerance, beta cell deficiency, diabetes (including type 1 diabetes,

type 2 diabetes, and gestational diabetes), non-alcoholic steatotic liver disease, steatosis of the liver, polycystic ovarian syndrome, hyperlipidemia, and Metabolic Syndrome. The compositions and pharmaceutical combinations may be used for treating obesity or diseases characterized by overeating and for the suppression of appetite. The methods comprise administering to a subject a therapeutically effective amount of a composition of the invention to a subject in need thereof, preferably a human subject.

Other disorders that may be treated with the compositions and combinations of the invention include, but are not limited to, insulin resistance, insulin deficiency, hyperinsulinemia, hyperglycemia, dyslipidemia, hyperlipidemia, hyperketonemia, hyperglucagonemia, pancreatitis, pancreatic neoplasms, cardiovascular disease, hypertension, coronary artery disease, atherosclerosis, renal failure, neuropathy (e.g., autonomic neuropathy, parasympathetic neuropathy, and polyneuropathy), diabetic retinopathy, cataracts, endocrine disorders, and sleep apnea, polycystic ovarian syndrome, neoplasms of the breast, colon, prostate, rectum and ovarian, osteoarthritis steatosis of the liver.

The invention further encompasses methods of regulating insulin responsiveness in a patient, as well as methods of increasing glucose uptake by a cell, and methods of regulating insulin sensitivity of a cell, using the conjugates or fusions of the invention. Also provided are methods of stimulating insulin synthesis and release, enhancing adipose, muscle or liver tissue sensitivity towards insulin uptake, stimulating glucose uptake, slowing digestive process, slowing of gastric emptying, inhibition of gastric acid secretion, inhibition of pancreatic enzyme secretion, reducing appetite, inhibition of food intake, modifying energy expenditure, or blocking the secretion of glucagon in a patient, comprising administering to said patient a composition of the invention e.g. comprising administering at least one dose of a composition *e.g.* a pharmaceutical composition or pharmaceutical combination of the present invention.

The invention also provides for use of a composition of the invention in the manufacture of a medicament for treatment of a metabolic disease such as those described herein. The invention also relates to use of any of the compositions described herein for use in therapy.

The polypeptides of the present invention and their salts may be employed alone or in combination with other therapeutic agents (a "pharmaceutical combination") for the treatment of the above-mentioned conditions. In some embodiments, the polypeptide of the present invention and the additional therapeutic agent or agents are administered together, while in other embodiments, the

polypeptide of the invention and the additional therapeutic agent or agents are administered separately. When administered separately, administration may occur simultaneously or sequentially, in any order. The amounts of the polypeptides(s) of the present invention and the other therapeutic agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect. The administration in combination of a compound of the present invention with other treatment agents may be in combination by administration concomitantly in: (1) a unitary pharmaceutical composition including both therapeutic agents; or (2) separate pharmaceutical compositions each including one of the therapeutic agents. Alternatively, the combination may be administered separately in a sequential manner wherein one treatment agent is administered first and the other second or vice versa. Such sequential administration may be close in time or remote in time.

In one embodiment, the pharmaceutical combinations of the invention include a polypeptide according to the invention and an exendin-4 peptide (see, for example, US patent no. 5,424,286) or a fragment or analog thereof. Exendin-4 (Ex -4) and analogs thereof that are useful for the present invention include Byetta® and Bydureon® (exenatide), Victoza® (liraglutide), lixisenatide, LY2189265 (dulaglutide), PF-4856883, ZYD-1, and HM11260C (LAPS exendin) as well as those described in PCT patent publications WO 99/25728 (Beeley *et al.*), WO 99/25727 (Beeley *et al.*), WO 98/05351 (Young *et al.*), WO 99/40788 (Young *et al.*), WO 99/07404 (Beeley *et al.*), and WO 99/43708 (Knudsen *et al.*).

In another embodiment, the pharmaceutical combinations of the invention include a polypeptide according to the invention and GLP-1 (see, for example, Gutniak, M., *et al.* (1992) *N. Engl. J. Bled.* 326:1316-22), or a fragment or analog thereof, for example, GLP-1(7-37), GLP-1(7-36) , GLP-1(7-35), GLP-1(7-38), GLP-1(7-39), GLP-1(7-40), GLP-1(7-41).

Further GLP-1 analogues are described in International Patent Application No. 90/11299, which relates to peptide fragments which comprise GLP-1(7-36) and functional derivatives thereof and have an insulinotropic activity which exceeds the insulinotropic activity of GLP-1(1-36) or GLP-1(1-37) and to their use as insulinotropic agents (incorporated herein by reference, particularly by way of examples of drugs for use in the present invention).

International Patent Application No. WO 91/11457 (Buckley *et al.*) discloses analogues of the active GLP-1 peptides GLP-1(7-34), GLP-1(7-35), GLP-1(7-36), and GLP-1(7-37) which can also be useful as GLP-1 drugs according to the present invention (incorporated herein by reference, particularly by way of examples of drugs or agents for use in the present invention).

The pharmaceutical combinations of the invention also include a polypeptide according to the invention and albiglutide.

In another embodiment, the pharmaceutical combinations include a polypeptide according to the invention and an enhancer of GLP-1 action such as a DPP-IV inhibitor (*e.g.* sitagliptin and/or saxagliptin).

In other embodiments, the pharmaceutical combination comprises a PYY analog of the present invention and one or more therapeutic agents that are direct or indirect stimulators of GLP-1 secretion such as metformin, bile acid sequestrants (*e.g.* colestipol, cholestryramine, and/or colesevelam), ileal bile acid transport (iBAT) Inhibitors (*e.g.* ALBI-3309, AZD-7806, S-8921, SAR-58304, or those described in US20130029938), and SGLT-1 Inhibitors (*e.g.* DSP-3235 and/or LX-4211).

The invention provides for methods of treatment where a "therapeutically effective amount" of a polypeptide of the invention is administered to a subject in need of such treatment. The term "therapeutically effective amount" means any amount which, as compared to a corresponding subject who has not received such amount, results in improved treatment, healing or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. As will be recognized by those in the field, an effective amount of therapeutic agent will vary with many factors including the age and weight of the patient, the patient's physical condition, the blood sugar level, the weight level to be obtained, and other factors

In one embodiment, a therapeutically effective amount of a polypeptide of the present invention is the amount required to suppress appetite in the subject to a desired degree. The effective daily appetite-suppressing dose of the compounds will typically be in the range of about 0.01 µg to about 500 µg /day, preferably about 0.05 µg to about 100 µg/day and more preferably about 1 µg to about 50 µg /day, most preferably about 5 µg to about 25 µg/day, for a 70 kg patient, administered in a single or divided doses.

In one aspect, the invention provides a pharmaceutical composition comprising a polypeptide of the invention, and a pharmaceutically acceptable carrier, excipient or diluent.

The pharmaceutical compositions and pharmaceutical combinations of the invention can be administered by any route, including intravenously, intraperitoneal, subcutaneous, and intramuscular, orally, topically, transmucosally, or by pulmonary inhalation. For example, polypeptides of the invention can be provided in the form of formulations suitable for parenteral (including intravenous, intramuscular and subcutaneous), nasal or oral administration.

Methods for formulating and delivering polypeptides for various routes of administration are known in the art. See, for example, Swain *et al.* (2013) *Recent Pat. Biotechnol.* 1 Feb 2013 Epub ahead of print, Hovgaard, Lars, Sven Frokiaer, and Marco Van De Weert, eds. *Pharmaceutical Formulation Development of Peptides and Proteins*. 2nd ed. Boca Raton: CRC Press, 2012, and Van Der Walle, Chris, ed. *Peptide and Protein Delivery*. London: Academic, 2011.

In one embodiment, the invention encompasses a slow release formulation. Such formulations allow for therapeutically effective amounts of the therapeutic polypeptide or polypeptides to be delivered into the bloodstream over many hours or days following injection or delivery to the subcutaneous space.

Slow release formulations of the invention may include one or more polymers useful in delaying the release of the therapeutic polypeptide. Non-limiting examples of such polymers include poly(lactic-*co*-glycolic acid) PLGA, polycaprolactone, polydioxanone, polytrimethylene carbonate, polyanhydrides, PEG-PLGA, polyglutamic acid, polyethylene glycol terphthalate/polybutylene terphthalate/polybutylene terphthalate, poly(aminoacid)-Leu/Glu copolymer, polytyrosine carbonates, polyesteramides, poly (alpha aminoacid) based polymeric micelles, polyhydroxypropylmethacrylamide, polyalkylcyanoacrylate, collagen, hyaluronic acide, albumin, carboxymethylcellulose, fleximer, chitosan, maltodextrin, dextran, or dextran sulfate,

In one aspect, the polypeptides of the invention may be delivered via a miniature device such as an implantable infusion pump which is designed to provide long-term continuous or intermittent drug infusion. Such devices can be used to administer a therapeutic polypeptide of the invention via intravenous, intra-arterial, subcutaneous, intraperitoneal, intrathecal, epidural, or intraventricular routes. Such devices may be erodible, non-erodible and/or durable. Non-limiting examples of such devices include the Durasert™ device (pSivida), the DUROS® osmotic delivery system (Intarcia Therapeutics), MedLaunch™ Polymer Technology (Endo Health).

Other devices that could be used according to the present invention include the SnychroMed® pump (Medtronic), and the Codman® 3000 infusion pump(Johnson & Johnson), the V-Go® delivery system (Valeritas), the OmniPod® pump (Insulet), and the JewelPump™ (Debiotech).

The polypeptides of the invention may be administered in an *in situ* gel formulation. Such formulations typically are administered as liquids which form a gel either by dissipation of the water miscible organic solvent or by aggregation of hydrophobic domains present in the matrix. Non-limiting examples include the FLUID CRYSTAL technology (Camurus) and the SABER technology (Durect), and

the formulations described in US Patent serial nos. 5612051, 5714159, 6413539, 6004573, and 6117949.

The therapeutic polypeptides of the invention may also be encapsulated into a microsphere-based pharmaceutical formulation suitable for subcutaneous injection. Non-limiting examples of microsphere-based formulations for the delivery of peptides include Chroniject™ (Oakwood Labs), Medusa® (Flamel's), Q-Sphera (Q-CHIP), as well as those described in US patent serial nos. 4675189, 6669961, and Amin *et al.* (2001) *J of Controlled Release* 73: 49-57.

The formulation may contain antibacterial or antifungal agents such as meta-cresol, benzyl alcohol, parabens (methyl, propyl, butyl), chlorobutanol, phenol, phenylmercuric salts such as acetate, borate, or nitrate, or sorbic acid.

The compositions of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. Any suitable lyophilization method (*e.g.*, spray drying, cake drying) and/or reconstitution techniques can be employed. In a particular embodiment, the invention provides a composition comprising a lyophilized (freeze dried) polypeptide as described herein.

In certain aspects, the invention provides a nucleic acid encoding a polypeptide of the invention and recombinant expression vectors containing such nucleic acids. Recombinant expression vectors of the invention include a nucleic acid encoding a polypeptide of the invention operably linked to one more expression control elements such as, e.g., a promoter.

Host cells containing a nucleic acid or recombinant expression vector encoding a polypeptide of the invention are also included. Suitable host cells according to the invention include both prokaryotic host cells and eukaryotic hosts cells. Possible host cells include, but are not limited to, mammalian host cells, bacterial host cells (*e.g. E. coli*), yeast host cells, and plant host cells.

Nucleic acids and recombinant expression vectors encoding a polypeptide of the invention can be introduced into a suitable host cell to create a recombinant host cell using any method appropriate to the host cell selected, *e.g.*, transformation, transfection, electroporation, or infection. In some embodiments, the nucleic acid or recombinant expression vector is integrated into the host cell genome. The resulting recombinant host cell can be maintained under conditions suitable for expression (*e.g.*, in the presence of an inducer, in a suitable animal, in suitable culture media supplemented with appropriate salts, growth factors, antibiotics, nutritional supplements, etc.), whereby the encoded polypeptide is produced. If desired, the encoded peptide or polypeptide can be isolated or recovered.

The invention further provides a method for producing a polypeptide of the present invention where the method comprises maintaining a host cell such as those described above that comprises a nucleic acid or recombinant expression vector that encodes a polypeptide of the invention under conditions suitable for expression of said nucleic acid or recombinant expression vector. Methods for recombinant expression of polypeptides in host cells are well known in the art. See, for example, Rosalyn M. Bill, ed. *Recombinant Protein Production in Yeast: Methods and Protocols (Methods in Molecular Biology, Vol. 866)*, Humana Press 2012; James L. Hartley, ed. *Protein Expression in Mammalian Cells: Methods and Protocols (Methods in Molecular Biology)*, Humana Press 2012, Löic Faye and Veronique Gomord, eds. *Recombinant Proteins From Plants: Methods and Protocols (Methods in Molecular Biology)*, Humana Press 2008; and Argelia Lorence, ed. *Recombinant Gene Expression (Methods in Molecular Biology)*, Humana press 2011.

In certain embodiments, the nucleic acids of the invention are "isolated." Nucleic acids referred to herein as "isolated" are nucleic acids which have been separated away from other material (*e.g.*, other nucleic acids such as genomic DNA, cDNA and/or RNA) in its original environment (*e.g.*, in cells or in a mixture of nucleic acids such as a library). An isolated nucleic acid can be isolated as part of a recombinant expression vector.

The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way.

EXAMPLES

The examples make use of the following abbreviations:

amu	atomic mass unit
Fmoc	9-fluorenylmethoxycarbonyl
HBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate
HCTU	2-(6-chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate
DMF	N,N-dimethylformamide
NMM	N-methylmorpholine
DIPEA	N,N-diisopropylethylamine
TFA	trifluoroacetic acid
Trt	trityl
t-Bu	tert-butyl
Boc	tert-butylcarbonyl

Pbf	2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl
MAL	maleimide
PBS	phosphate buffered saline
ivDde	1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl
MALDI	matrix assisted laser desorption/ionization
BMPS	N-β-maleimidopropyloxysuccinimide ester
DODT	2,2'-(ethylenedioxy)diethanethiol
TIPS	triisopropylsilane
MPA	mercaptopropionic acid
rt	retention time
RPM	revolutions per minute

Peptide Synthesis

The peptides shown in the following examples were synthesized by solid-phase methods using Fmoc strategy with 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HBTU) or 2-(6-chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) activation (5 fold molar excess) in N,N-dimethylformamide (DMF), and N-methylmorpholine (NMM) as base, 20% piperidine/DMF for Fmoc deprotection, on an automated peptide synthesizer (model Prelude or Overture; Protein Technologies, Tucson, AZ). The resin was Rink Amide MBHA LL (Novabiochem) or Rink Amide AM LL (Novabiochem) with a loading of 0.29 - 0.38 mmol/g on a 20 - 400 µmol scale. The side chain protection groups used were Trt for Asn, Gln, Cys and His; *t*-Bu for Ser, Thr, and Tyr; Boc for Lys and Trp; O*t*-Bu for Asp and Glu; and Pbf for Arg. Cleavage of peptide-resin was completed with a mixture of trifluoroacetic acid (TFA):anisole:water:triisopropylsilane (88:5:5:2). The crude peptide was precipitated in cold diethyl ether, the diethyl ether was decanted and the solids triturated again with cold diethyl ether. The crude solids were then purified by reverse phase HPLC on a Waters XBridge™ BEH 130, C18, 10 µm, 130Å, 30 X 250 mm ID column, using a gradient within the ranges of 5 - 75% acetonitrile/water with 0.1% TFA over 30 - 45 minutes at a flow rate of 30 mL/min, λ – 215 nm.

LC/MS Conditions

Method A:Performed using a Phenomenex UPLC Aeris™ Peptide XB C18 column, 1.7 µm, 2.1 X 100 mm or ACQUITY BEH300 or BEH130 C18 column, 1.77 µm, 2.1 X 100 mm using 5 - 65% acetonitrile/water with 0.05% TFA over 30 minutes with a flow rate 0.5 mL/min, λ – 215 nm, 280 nm.

C18 HPLC Conditions:

Method A: Performed using a Waters XBridge™ BEH130 C18 column, 5 µm, 4.6 X 250 mm, with 5 - 70% acetonitrile/water with 0.1% TFA over 15 minutes with a flow rate 1.5 mL/min, 40° C, λ – 215 nm, 280 nm.

Method B: Performed using a Waters XBridge™ BEH130 C18 column, 5 µm, 4.6 X 250 mm, 5 - 75% acetonitrile/water with 0.1% TFA over 20 minutes with a flow rate 1.5 mL/min, λ – 215 nm, 280 nm.

Method C: Performed using a Waters XBridge™ BEH130 C18 column, 5 µm, 4.6 X 250 mm, 20 - 37.5% acetonitrile/water with 0.1% TFA over 15 minutes with a flow rate 1.0 mL/min, 60° C, λ – 215 nm, 280 nm.

Method D: Performed using a Waters XBridge™ BEH300 C18 column, 5 µm, 4.6 X 250 mm, 5 - 70% acetonitrile/water with 0.1% TFA over 15 minutes with a flow rate 1.5 mL/min, λ – 215 nm, 280 nm.

Example 1. PKPEAPGKDASPEELNRYYASLRHYLNWVTRQRY-NH_2 (SEQ ID NO:3)

Example 1 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1369 amu and (M+4)/4 – 1027 amu, which corresponds to a peptide with the parent molecular weight of 4105 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.90 min) for the isolated peptide (25 mg, as the 8 trifluoroacetic acid salt).

Example 2. PKPEAPGKDASPEELNRYYASLRKYLNWLTRQRY-NH_2 (SEQ ID NO:4)

Example 2 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1371 amu and (M+4)/4 – 1028 amu, which corresponds to a peptide with the parent molecular weight of 4111 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.46 min) for the isolated peptide (20 mg, as the 8 trifluoroacetic acid salt).

Example 3. PKPEAPGKDASPEELNRYYASLRHYLNWLTRQRY-NH_2 (SEQ ID NO:5)

Example 3 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by

fragment ions (M+3)/3 – 1374 amu and (M+4)/4 – 1031 amu, which corresponds to a peptide with the parent molecular weight of 4120 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.42 min) for the isolated peptide (16 mg, as the 8 trifluoroacetic acid salt).

Example 4. PKPEAPGKDASPEEWNRYYADLRKYLNWLTRQRY-NH_2 (SEQ ID NO:6)

Example 4 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1405 amu and (M+4)/4 – 1054 amu, which corresponds to a peptide with the parent molecular weight of 4212 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.54 min) for the isolated peptide (18 mg, as the 8 trifluoroacetic acid salt).

Example 5. PKPEAPGKDASPEEWNRYYADLRHYLNWLTRQRY-NH_2 (SEQ ID NO:7)

Example 5 was prepared on a 6X50 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1407 amu and (M+4)/4 – 1056 amu, which corresponds to a peptide with the parent molecular weight of 4221 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.27 min) for the isolated peptide (180 mg, as the 8 trifluoroacetic acid salt).

Alternatively, Example 5 was prepared via manual synthesis using a 250 mL jacketed reactor that was cooled to 15°C. Rink Amide AM Resin LL (100-200 mesh, 13.8 g, 0.29 mmol/g loading) was swelled with DMF (50 mL) 3 times for 10 min each with nitrogen sparge. The Fmoc group was removed with 20% piperidine in DMF (200 mL) over 5 min with nitrogen sparge, followed by 20% piperidine in DMF (200 mL) over 12 min with nitrogen sparge. The resin was then washed with DMF (100 mL) and then twice with DMF (100 mL) for 1 min with nitrogen sparge. Following Fmoc deprotecion and DMF washing, the first amino acid (100 mL, 200 mM solution in DMF) was added, followed by DIPEA solution (50 mL, 800 mM solution in DMF). A solution of HCTU in DMF (50 mL, 400 mM) was added over a 12 min period via peristaltic pump. After a minimum of 15 min, a Kaiser test on an aliquot of resin was performed to ensure complete reaction. The resin was then washed with DMF (100 mL) and then twice with DMF (100 mL) for 1 min with nitrogen sparge. This sequence (Fmoc deprotection with 20% piperidine/DMF; washes; amino acid coupling; Kaiser test; washes) was performed for the remaining sequence of the

peptide, except for the histidine at position 24. The amino acid (His) and DIPEA solutions were cooled to ~10°C and added to the reactor. The reactor's chiller was set to 5°C and the reaction mixture cooled to 6.3°C in ~15 min. A cooled (~10°C) solution of HCTU in DMF (50 mL) was added dropwise over a 25 min period via peristaltic pump at 2 mL/min, during which time the solution warmed to 7.8°C. After 15 min, a Kaiser test showed complete reaction. The remaining amino acids were coupled using the standard protocol. At the completion of the synthesis (proline 1 was coupled), the resin was then washed twice with DMF (100 mL) for 1 min with nitrogen sparge, then washed three time with DCM (200 mL) for 5 min with nitrogen sparge, and then finally three times with methanol (200 mL) for 5 min with nitrogen sparge. The resin was dried with nitrogen sparge for 30 min to give 37.5 g of dry resin. The resin was cleaved in portions. Ten grams of resin was swelled with 120 mL DMF for 45 min with nitrogen sparge. The DMF was drained off and the final N-terminal Fmoc was removed with 20% piperidine in DMF (150 mL) over 5 min with nitrogen sparge, followed by 20% piperidine in DMF (150 mL) over 12 min with nitrogen sparge. The resin was then washed with DMF (100 mL) and then twice with DMF (100 mL) for 1 min with nitrogen sparge, then washed three time with DCM (120 mL) for 5 min with nitrogen sparge, and then finally three times with methanol (120 mL) for 5 min with nitrogen sparge. The resin was dried with nitrogen sparge for 30 min. Cleavage of peptide from the resin was performed using 100 – 120 mL of cleavage cocktail: TFA:phenol:DODT:water:TIPS (90:2.5:2.5:2.5:2.5) for 2.5 - 3 h. . The filtrates were split into vessels and treated with cold diethyl ether. The vessels were centrifuged for 10 min at 3000 RPM and the supernatant was poured off. The material was treated with cold diethyl ether again, shaken and then centrifuged for another 10 min at 3000 RPM. The supernatant was poured off again. The solids from the vessels were combined using 0.1% aqueous TFA and lyophilized to give batch 1. The resin was subjected to second cleavage using the same procedure to give batch 2. This process (Fmoc deprotection; washes; cleavage from resin, trituration/resuspension, and lyophilization) was repeated three times with ~9 g of resin to afford a total of ~11 g of crude peptide after lyophilization. This material was dissolved in 0.1% aqueous TFA to give an approximate concentration of 75 mg/mL and the material was purified by reverse phase HPLC using multiple injections (between 2 and 3 mL each) using the following step gradient: 5 – 41.25% acetonitrile/water with 0.1% TFA over 75 min,; XBridge™ Prep C18, 50 x 250 mm, 10 µm, flow rate 50 mL/min. Fractions containing product with >93% purity (HPLC Method C) were combined. Impure fractions (purity of ~88 – 93%) were also collected and resubjected to the purification conditions. All pure fractions (>93%)

were then combined and freeze-dried to give desired peptide as a white solid. A purity of > 93% was determined by C18 HPLC (C18 HPLC Method C, rt = 14.12 min) for the isolated peptide (2.8g, as the 8 trifluoroacetic acid salt).

A salt exchange from TFA to HOAc using Example 5 prepared by peptide synthesizer and manual synthesis was performed using a 2 x 60 mL Agilent StratoSpheres™ PL-HCO_3 MP SPE column. The column was equilibrated by first treating with 50 mL of MeOH, followed by 50 mL of DI water. The column was then treated with 2 x 50 mL 1 N HOAc and then with 2 x 50 mL 0.1 N HOAc, and the filtrate was monitored to ensure pH ~ 3 (pH paper). A solution of Example 5 (~3.5 g including 2.8 g prepared as described above and 0.7 g derived from previously-prepared batches) in 0.1 N HOAc was split equally between the SPE columns and then eluted with 5 x 50 mL of 0.1 N HOAc. The column was then washed with 5 x 50 mL of MeOH. The methanol fractions containing product (as determined by HPLC, Method C) were concentrated via rotary evaporator to ~40 mL, which was added to the 0.1 N HOAc washes. The solution was freeze-dried over 3 d to afford the desired isolated peptide as a white solid. A purity of > 95% was determined by C18 HPLC (C18 HPLC Method C, rt = 14.14 min) for the isolated peptide (2.95g, as the 8 acetic acid salt).

Example 6. PKPEAPGKDASPEEWNRYYASLRKYLNWLTRQRY-NH_2 (SEQ ID NO:8)

Example 6 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1395 amu and (M+4)/4 – 1047 amu, which corresponds to a peptide with the parent molecular weight of 4184 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.45 min) for the isolated peptide (18 mg, as the 8 trifluoroacetic acid salt).

Example 7. PKPEAPGKDASPEEWNRYYASLRHYLNWLTRQRY-NH_2 (SEQ ID NO:9)

Example 7 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1398 amu and (M+4)/4 – 1049 amu, which corresponds to a peptide with the parent molecular weight of 4193 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.47 min) for the isolated peptide (27 mg, as the 8 trifluoroacetic acid salt).

Example 8. PKPEAPGKDASPEEWNRYYADLRKYLNWVTRQRY-NH_2 (SEQ ID NO:10)

Example 8 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1400 amu and (M+4)/4 – 1050 amu, which corresponds to a peptide with the parent molecular weight of 4198 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.35 min) for the isolated peptide (21 mg, as the 8 trifluoroacetic acid salt).

Example 9. PKPEAPGKDASPEEWNRYYADLRHYLNWVTRQRY-NH_2 (SEQ ID NO:11)

Example 9 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1403 amu and (M+4)/4 – 1052 amu, which corresponds to a peptide with the parent molecular weight of 4207 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.36 min) for the isolated peptide (22 mg, as the 8 trifluoroacetic acid salt).

Example 10. PKPEAPGKDASPEEWNRYYASLRKYLNWVTRQRY-NH_2 (SEQ ID NO:12)

Example 10 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1391 amu and (M+4)/4 – 1043 amu, which corresponds to a peptide with the parent molecular weight of 4170 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.23 min) for the isolated peptide (23 mg, as the 8 trifluoroacetic acid salt).

Example 11. PKPEAPGKDASPEEWNRYYASLRHYLNWVTRQRY-NH_2 (SEQ ID NO:13)

Example 11 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1393 amu and (M+4)/4 – 1045 amu, which corresponds to a peptide with the parent molecular weight of 4179 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.24 min) for the isolated peptide (22 mg, as the 8 trifluoroacetic acid salt).

Example 12. PKPEAPGEDASPEELNRYYASLRHYLNWVTRQRY-NH_2 (SEQ ID NO:14)

Example 12 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1369 amu and (M+4)/4 – 1027 amu, which corresponds to a peptide with the parent molecular weight of 4106 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.60 min) for the isolated peptide (22 mg, as the 7 trifluoroacetic acid salt).

Example 13. PKPEHPGKDASPEEWNRYYAALRKYLNWVTRQRY-NH_2 (SEQ ID NO:15)

Example 13 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1407 amu and (M+4)/4 – 1056 amu, which corresponds to a peptide with the parent molecular weight of 4220 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.46 min) for the isolated peptide (22 mg, as the 9 trifluoroacetic acid salt).

Example 14. PKPEHPGKDASPEELNKYYAALRHYLNWVTRQRY-NH_2 (SEQ ID NO:16)

Example 14 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1377 amu and (M+4)/4 – 1033 amu, which corresponds to a peptide with the parent molecular weight of 4128 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.88 min) for the isolated peptide (27 mg, as the 9 trifluoroacetic acid salt).

Example 15. PKPEHPGKDASPEELNRYYASLRHYINWVTRQRY-NH_2 (SEQ ID NO:17)

Example 15 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1391 amu and (M+4)/4 – 1044 amu, which corresponds to a peptide with the parent molecular weight of 4172 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.77 min) for the isolated peptide (29 mg, as the 9 trifluoroacetic acid salt).

Example 16. PKPEHPGKDASPEELARYYASLRHYLNWVTRQRY-NH_2 (SEQ ID NO:18)

Example 16 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1377 amu and (M+4)/4 – 1033 amu, which corresponds to a peptide with the parent molecular weight of 4129 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.89 min) for the isolated peptide (30 mg, as the 9 trifluoroacetic acid salt).

Example 17. PKPEHPGKDASPEEWNRYYASLRHYINWVTRQRY-NH_2 (SEQ ID NO:19)

Example 17 was prepared on a 35 µmol scale as a white solid using the general method. The isolated crude solid was stirred for several hours in 8 mL of 25% acetic acid to minimize the tryptophan CO_2 adduct formed during cleavage from the resin. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1416 amu and (M+4)/4 – 1062 amu, which corresponds to a peptide with the parent molecular weight of 4245 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.88 min) for the isolated peptide (35 mg, as the 9 trifluoroacetic acid salt).

Example 18. PKPEHPGKDASPEEWNRYYADLRHYINWVTRQRY-NH_2 (SEQ ID NO:20)

Example 18 was prepared on a 35 µmol scale as a white solid using the general method. The isolated crude solid was stirred for several hours in 8 mL of 25% acetic acid to minimize the tryptophan CO_2 adduct formed during cleavage from the resin. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1425 amu and (M+4)/4 – 1069 amu, which corresponds to a peptide with the parent molecular weight of 4273 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.98 min) for the isolated peptide (17 mg, as the 9 trifluoroacetic acid salt).

Example 19. PKPEHPGKDASPEEWNRYYADLRHYLNWVTRQRY-NH_2 (SEQ ID NO:21)

Example 19 was prepared on a 35 µmol scale as a white solid using the general method. The isolated crude solid was stirred for several hours in 8 mL of 25% acetic acid to minimize the tryptophan CO_2 adduct formed during cleavage from the resin. The molecular mass of the isolated peptide was confirmed by fragment

ions (M+3)/3 – 1425 amu and (M+4)/4 – 1069 amu, which corresponds to a peptide with the parent molecular weight of 4273 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.98 min) for the isolated peptide (34 mg, as the 9 trifluoroacetic acid salt).

Example 20. PKPESPGKDASPEEWNRYYADLRHYINWVTRQRY-NH_2 (SEQ ID NO:22)

Example 20 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1408 amu and (M+4)/4 – 1057 amu, which corresponds to a peptide with the parent molecular weight of 4223 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.30 min) for the isolated peptide (28 mg, as the 8 trifluoroacetic acid salt).

Example 21. PKPESPGKDASPEEWNRYYADLRHYLNWVTRQRY-NH_2 (SEQ ID NO:23)

Example 21 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1408 amu and (M+4)/4 – 1057 amu, which corresponds to a peptide with the parent molecular weight of 4223 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.68 min) for the isolated peptide (28 mg, as the 8 trifluoroacetic acid salt).

Example 22. PKPEHPGKDASPEEWNRYYADLRHYLNWLTRQRY-NH_2 (SEQ ID NO:24)

Example 22 was prepared on a 40 µmol scale as a white solid using the general method. The isolated crude solid was stirred for several hours in 8 mL of 25% acetic acid to minimize the tryptophan CO_2 adduct formed during cleavage from the resin. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1430 amu and (M+4)/4 – 1073 amu, which corresponds to a peptide with the parent molecular weight of 4287 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.65 min) for the isolated peptide (24 mg, as the 9 trifluoroacetic acid salt).

Example 23. PKPEHPGKDASPEEWAKYYAALRHYINWVTRQRY-NH_2 (SEQ ID NO:25)

Example 23 was prepared on a 40 μmol scale as a white solid using the general method. The isolated crude solid was stirred for several hours in 8 mL of 25% acetic acid to minimize the tryptophan CO_2 adduct formed during cleavage from the resin. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1386 amu and (M+4)/4 – 1040 amu, which corresponds to a peptide with the parent molecular weight of 4158 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.28 min) for the isolated peptide (20 mg, as the 9 trifluoroacetic acid salt).

Example 24. PKPEAPGKDASPEEWNRYYADLRHYINWVTRQRY-NH_2 (SEQ ID NO:26)

Example 24 was prepared on a 40 μmol scale as a white solid using the general method. The isolated crude solid was stirred for several hours in 8 mL of 25% acetic acid to minimize the tryptophan CO_2 adduct formed during cleavage from the resin. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1403 amu and (M+4)/4 – 1053 amu, which corresponds to a peptide with the parent molecular weight of 4207 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.22 min) for the isolated peptide (28 mg, as the 8 trifluoroacetic acid salt).

Example 25. PKPEHPGKDASPEEWNRYYASLRKYLNWVTRQRY-NH_2 (SEQ ID NO:27)

Example 25 was prepared on a 40 μmol scale as a white solid using the general method. The isolated crude solid was stirred for several hours in 8 mL of 25% acetic acid to minimize the tryptophan CO_2 adduct formed during cleavage from the resin. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1412 amu and (M+4)/4 – 1060 amu, which corresponds to a peptide with the parent molecular weight of 4236 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.09 min) for the isolated peptide (21 mg, as the 9 trifluoroacetic acid salt).

Example 26. PKPEHPGKDASAEEWAKYYAALRHYINWVTRQRY-NH_2 (SEQ ID NO:28)

Example 26 was prepared on a 20 μmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1378 amu and (M+4)/4 – 1034 amu, which corresponds to a peptide with the parent molecular weight of 4132 amu (ESI-MS, LC/MS Method A).

A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.44 min) for the isolated peptide (19 mg, as the 9 trifluoroacetic acid salt).

Example 27. PKPEAPGKDASAEEWNRYYASLRHYLNWVTRQRY-NH_2 (SEQ ID NO:29)

Example 27 was prepared on a 20 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1385 amu and (M+4)/4 – 1039 amu, which corresponds to a peptide with the parent molecular weight of 4153 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.31 min) for the isolated peptide (16 mg, as the 8 trifluoroacetic acid salt).

Example 28. PKPEHPGKDASAEELARYYASLRHYLNWVTRQRY-NH_2 (SEQ ID NO:30)

Example 28 was prepared on a 20 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1368 amu and (M+4)/4 – 1027 amu, which corresponds to a peptide with the parent molecular weight of 4103 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.27 min) for the isolated peptide (14 mg, as the 9 trifluoroacetic acid salt).

Example 29. PKPEAPGKDASAEEWNRYYASLRKYLNWVTRQRY-NH_2 (SEQ ID NO:31)

Example 29 was prepared on a 20 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1382 amu and (M+4)/4 – 1037 amu, which corresponds to a peptide with the parent molecular weight of 4144 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.31 min) for the isolated peptide (27 mg, as the 8 trifluoroacetic acid salt).

Example 30. PKPESPGKDASAEEWTKYYAALRHYINWVTRQRY-NH_2 (SEQ ID NO:32)

Example 30 was prepared on a 20 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1371 amu and (M+4)/4 – 1029 amu, which corresponds to a peptide with the parent molecular weight of 4112 amu (ESI-MS, LC/MS Method A).

A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 9.59 min) for the isolated peptide (33 mg, as the 8 trifluoroacetic acid salt).

Example 31. PKPEAPGKDASPEELNRYYASLRKYLNWVTRQRY-NH_2 (SEQ ID NO:33)

Example 31 was prepared on a 35 µmol scale as a white solid using the general method. The isolated crude solid was stirred for several hours in 8 mL of 25% acetic acid to minimize the tryptophan CO_2 adduct formed during cleavage from the resin. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1366 amu and (M+4)/4 – 1025 amu, which corresponds to a peptide with the parent molecular weight of 4097 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.93 min) for the isolated peptide (21 mg, as the 8 trifluoroacetic acid salt).

Example 32. PKPEHPGEDASPEEWAKYYAALRHYINWVTRQRY-NH_2 (SEQ ID NO:34)

Example 32 was prepared on a 20 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1387 amu and (M+4)/4 – 1041 amu, which corresponds to a peptide with the parent molecular weight of 4159 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by LC/MS (LC/MS Method A, rt = 13.58 min) for the isolated peptide (9.4 mg, as the 8 trifluoroacetic acid salt).

Example 33. PKPEAPGEDASAEEWNRYYASLRHYLNWVTRQRY-NH_2 (SEQ ID NO:35)

Example 33 was prepared on a 20 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1385 amu and (M+4)/4 – 1039 amu, which corresponds to a peptide with the parent molecular weight of 4154 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by LC/MS (LC/MS Method A, rt = 13.35 min) for the isolated peptide (7.7 mg, as the 7 trifluoroacetic acid salt).

Example 34. PKPESPGEDASPEEWTKYYAALRHYINWVTRQRY-NH_2 (SEQ ID NO:36)

Example 34 was prepared on a 20 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1381 amu and (M+4)/4 – 1036 amu, which corresponds to a

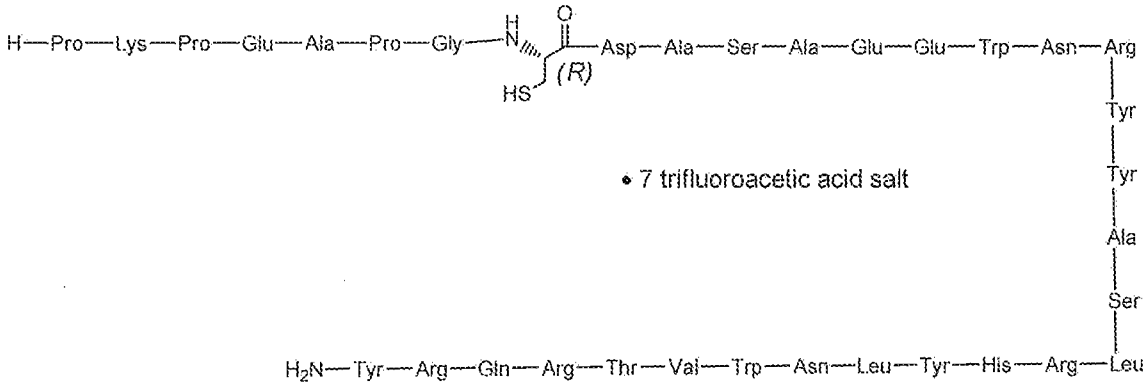
peptide with the parent molecular weight of 4139 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by LC/MS (LC/MS Method A, rt = 13.98 min) for the isolated peptide (8.2 mg, as the 7 trifluoroacetic acid salt).

Example 35. PKPEAPGEDASPEEWNRYYADLRHYLNWLTRQRY-NH_2 (SEQ ID NO:37)

Example 35 was prepared on a 20 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1408 amu and (M+4)/4 – 1056 amu, which corresponds to a peptide with the parent molecular weight of 4222 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by LC/MS (LC/MS Method A, rt = 13.84 min) for the isolated peptide (7.5 mg, as the 7 trifluoroacetic acid salt).

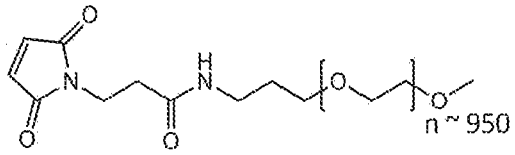
Examples 36-43 make reference to the following intermediates:

Intermediate 1



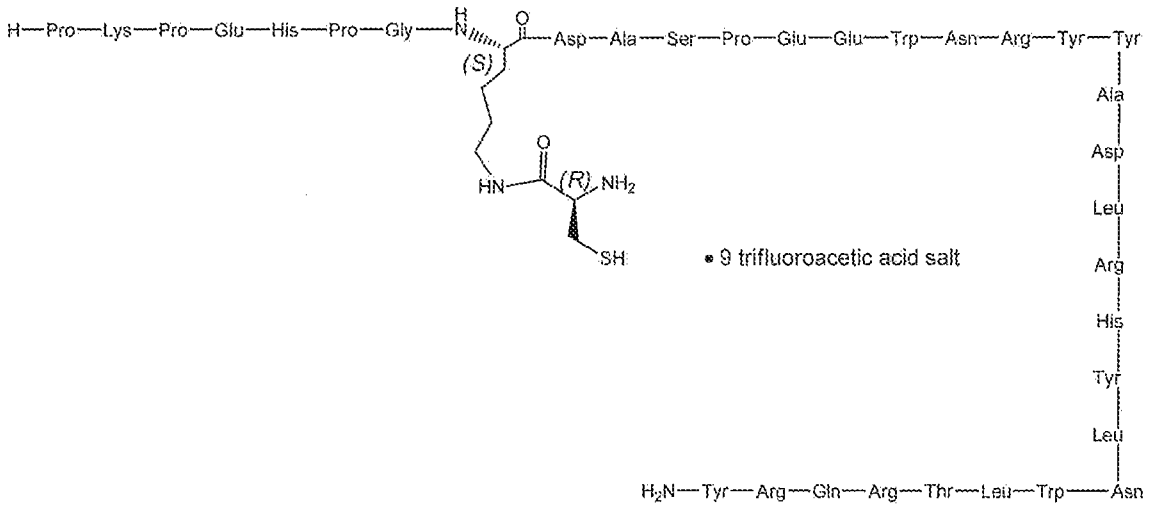
(SEQ ID NO:39)

Intermediate 2



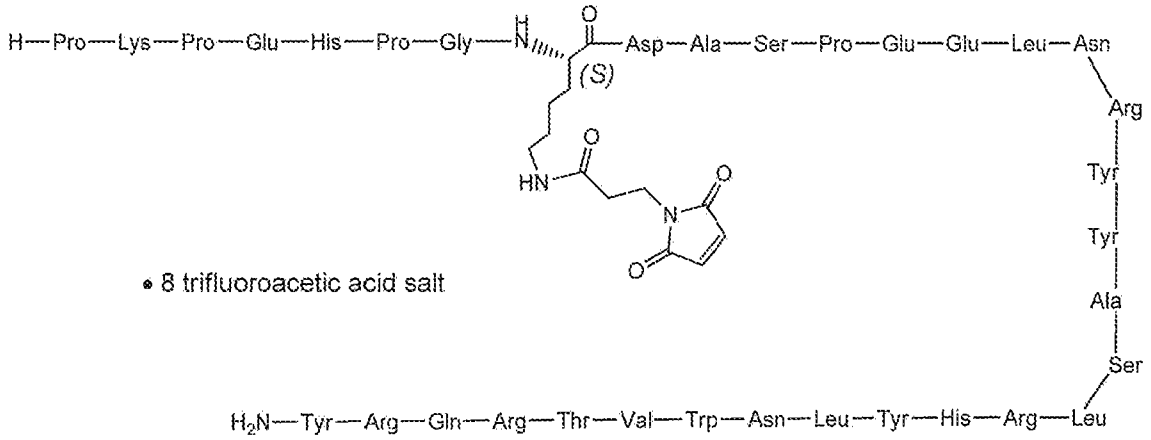
α-[3-(3-maleimido-1-oxopropyl)amino]propyl-ω-methoxy, polyoxyethylene (available from NOF Corporation or JenKEM Technology USA Inc.)

Intermediate 3



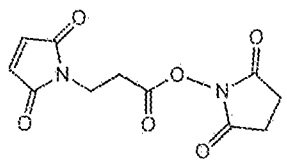
(SEQ ID NO:40)

Intermediate 4



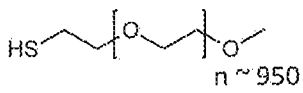
(SEQ ID NO:41)

Intermediate 5



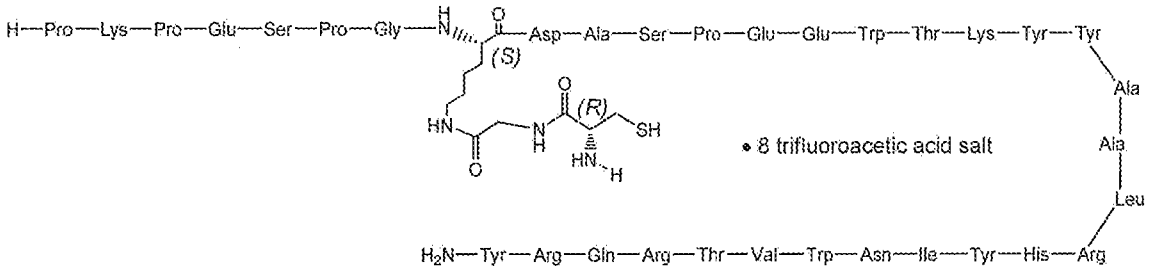
N-succinimidyl-3-maleinimidopropionate

Intermediate 6



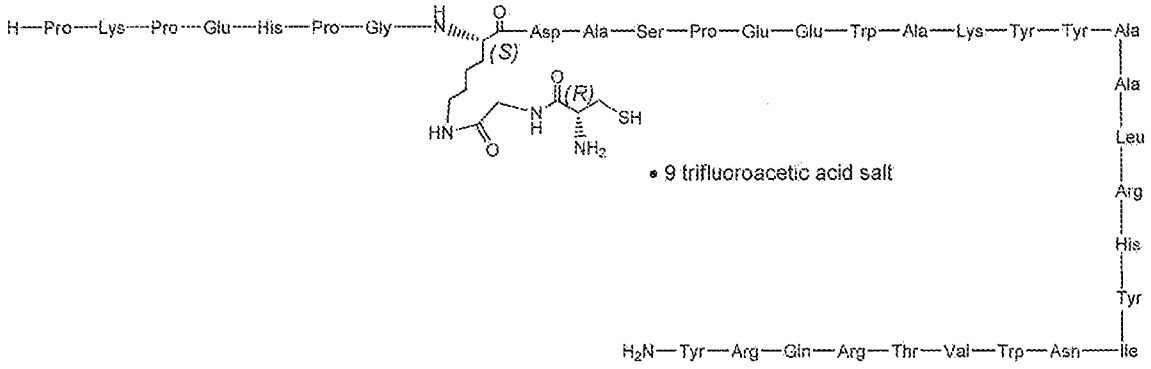
M-SH-40K, available from JenKem Technology USA Inc.

Intermediate 7



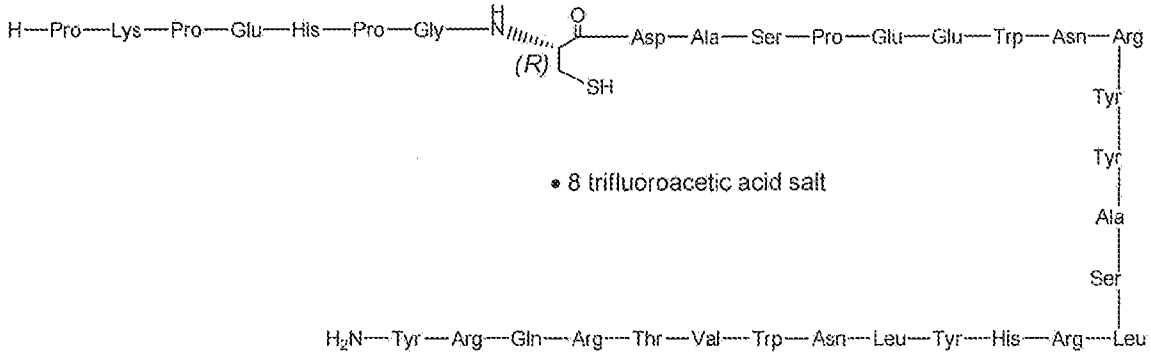
(SEQ ID NO:42)

Intermediate 8



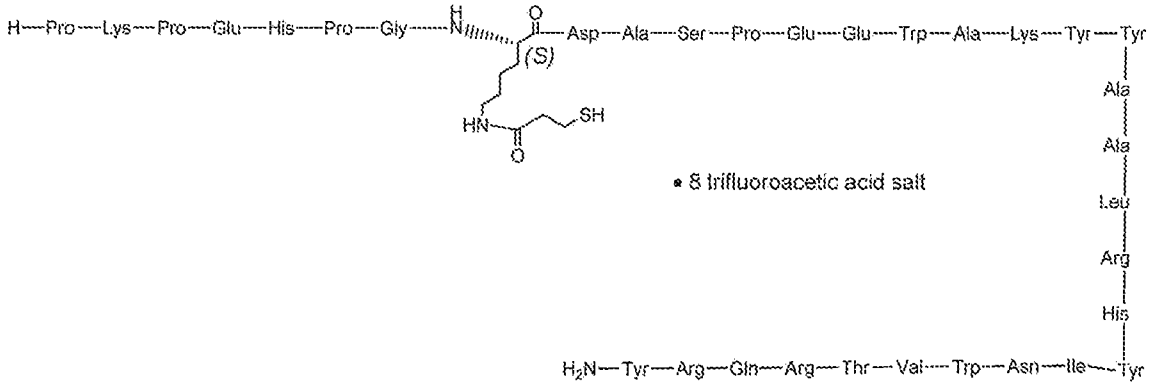
(SEQ ID NO:43)

Intermediate 9



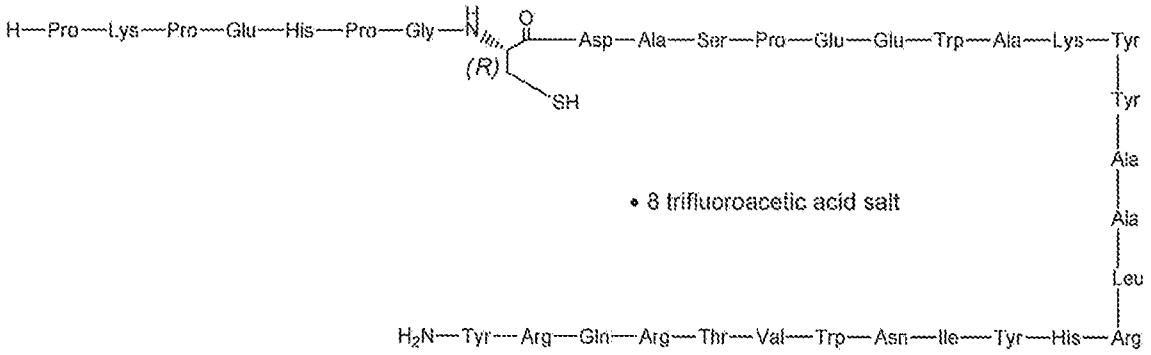
(SEQ ID NO:44)

Intermediate 10



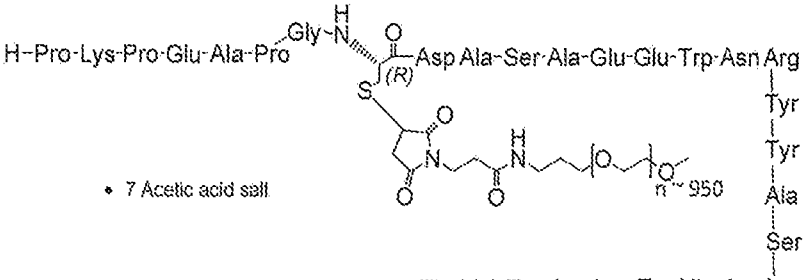
(SEQ ID NO:43)

Intermediate 11



(SEQ ID NO:45)

Example 36

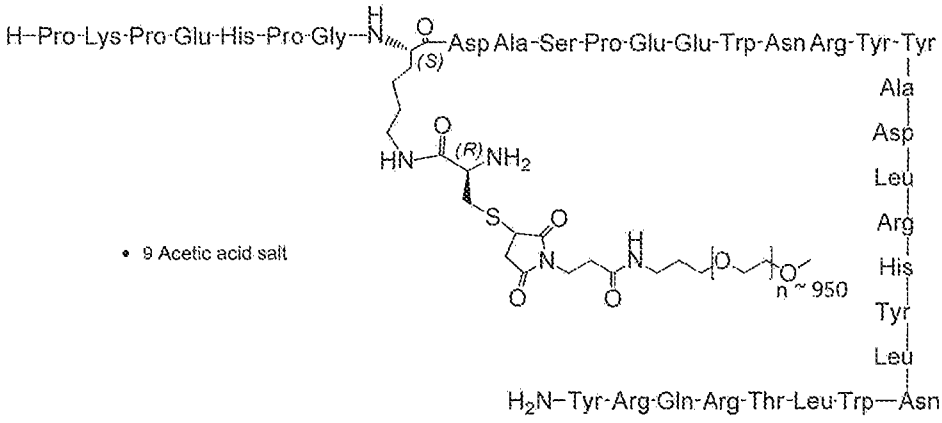


(SEQ ID NO:39)

Intermediate 1 was prepared on a 400 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1377 amu and (M+4)/4 – 1033 amu, which corresponds to a peptide with the parent molecular weight of 4128 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method B, rt = 10.98 min) for the isolated peptide (76 mg, as the 7 trifluoroacetic acid salt).

A mixture of Intermediate 1 (24.1 mg, 4.89 µmol) and Intermediate 2 (NOF Corporation, ME-400MA, 226 mg, 5.14 µmol) in 3.5 mL of 1X PBS buffer at pH 7.4 was shaken for 45 minutes, during which time the reaction became homogenous. The reaction was then diluted with a solution of 20% MeOH in 0.1 M aqueous HCl and purified by ion exchange chromatography (Sepharose FF Media, 5-50% 1 M NaCl in 20% methanol/10 mM aqueous HCl and over 5 column volumes, flow rate 5mL/min, λ – 254 nm). The purified conjugate was desalted using size exclusion chromatography (GE HiPrep 26/10 Desalting column, 0.1 M acetic acid-, λ – 254 nm) to afford a white solid after lyophilization. The molecular mass of the isolated peptide was confirmed by positive fragment ion distribution with the apex at 47379 amu (MALDI). Example 36 (107 mg, as the 7 acetic acid salt) gave a retention time equal to 9.95 min using size exclusion HPLC (Phenomenex BioSep-SEC-S3000 column, 7.8 x 300 mm, 5 µm, 50% acetonitrile/water with 0.5% TFA over 20 min, flow rate 0.75 mL/min, λ – 220 nm).

Example 37

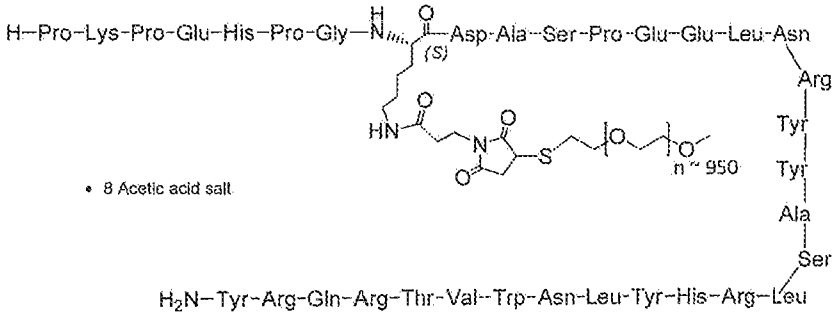


(SEQ ID NO:40)

Intermediate 3 was prepared on a 40 µmol scale as a white solid using the general method, except the lysine at position 8 of the peptide was protected with an ivDde group, and proline at position 1 was protected with a Boc. After the coupling of the last amino acid (proline 1), the ivDde was removed with repeated treatments of 4% hydrazine in DMF, and Fmoc-Cys(Trt)-OH was coupled. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1464 amu and (M+4)/4 – 1098 amu, which corresponds to a peptide with the parent molecular weight of 4390 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method D, rt = 9.61 min) for the isolated peptide (32 mg, as the 9 trifluoroacetic acid salt).

A mixture of Intermediate 3 (10 mg, 1.85 µmol) and Intermediate 2 (JenKem Technology USA Inc., 74 mg, 1.85 µmol) in 5 mL of 1X PBS buffer at pH 7.4 was shaken overnight, during which time the reaction became homogenous. The reaction was then diluted with 5 mL of 20% MeOH in 10 mM aqueous HCl and purified by ion exchange chromatography (Sepharose FF Media, 0 – 60% 1 M NaCl in 20% methanol/10 mM aqueous HCl over 7 column volumes, flow rate 5 mL/min, λ - 254 nm). The purified conjugate was desalted using size exclusion chromatography (Sephadex G 25 Fine Desalting column, 0.1 M acetic acid, λ - 254 nm) to afford a white solid after lyophilization. The molecular mass of the isolated peptide was confirmed by positive fragment ion distribution with the apex at 44568 amu (MALDI). Example 37 (35 mg, as the 9 acetic acid salt) gave a retention time equal to 11.58 min using size exclusion HPLC (Phenomenex BioSep-SEC-S3000 column, 7.8 x 300 mm, 5 µm, 0.15 mM NaCl in 30 mM PBS over 20 min, pH 6.8, flow rate 0.75 mL/min, λ - 215 nm).

Example 38

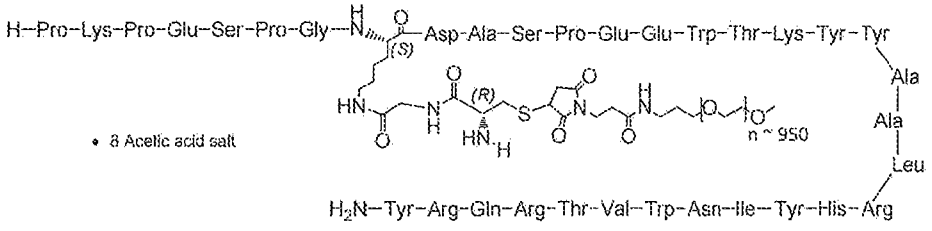


(SEQ ID NO:41)

Intermediate 4 was prepared on a 40 µmol scale as a white solid using the general method, except the lysine at position 8 of the peptide was protected with an ivDde group, and proline at position 1 was protected with a Boc. After the coupling of the last amino acid (proline 1), the ivDde was removed with repeated treatments of 4% hydrazine in DMF, and the linker was coupled using the activated succinimide ester reagent Intermediate 5, N-β-maleimidopropyloxysuccinimide ester, without the use of activator (HCTU) or base (NMM). The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1442 amu and (M+4)/4 – 1082 amu, which corresponds to a peptide with the parent molecular weight of 4323 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.79 min) for the isolated peptide (29 mg, as the 8 trifluoroacetic acid salt).

A mixture of Intermediate 4 (10 mg, 1.91 µmol) and Intermediate 6 (JenKem Technology USA Inc., 76 mg, 1.91 µmol) in 5 mL of 1X PBS buffer at pH 7.4 was stirred overnight, during which time the reaction became homogenous. The reaction was then diluted with 5 mL of 20% MeOH in 10 mM aqueous HCl and purified by ion exchange chromatography (Sepharose FF Media , 0 – 60% 1 M NaCl in 20% methanol/10 mM aqueous HCl over 7 column volumes, flow rate 5 mL/min, λ - 254 nm). The purified conjugate was desalted using size exclusion chromatography (Sephadex G 25 Fine Desalting column, 0.1 M acetic acid, λ - 254 nm) to afford a white solid after lyophilization. The molecular mass of the isolated peptide was confirmed by positive fragment ion distribution with the apex at 44346 amu (MALDI). Example 38 (27 mg, as the 8 acetic acid salt) gave a retention time equal to 12.19 min using size exclusion HPLC (Phenomenex BioSep-SEC-S3000 column, 7.8 x 300 mm, 5 µm, 0.15 mM NaCl in 30 mM PBS over 20 min, pH 6.8, flow rate 0.75 mL/min, λ - 215 nm).

Example 39



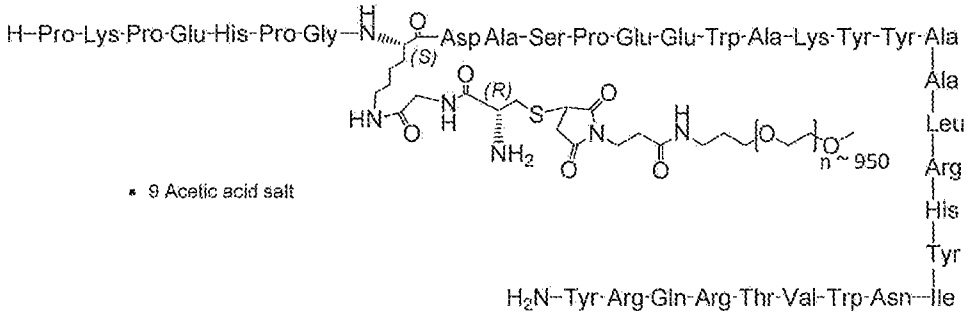
(SEQ ID NO:42)

Intermediate 7 was prepared on a 40 µmol scale as a white solid using the general method, except the lysine at position 8 of the peptide was protected with an ivDde group, while proline at position 1 was protected with a Boc. After the coupling of the last amino acid (proline 1), the ivDde was removed with repeated treatments of 4% aqueous hydrazine in DMF and Fmoc-Gly-OH and Fmoc-Cys(Trt)-OH were coupled. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1433 amu and (M+4)/4 – 1075 amu, which corresponds to a peptide with the parent molecular weight of 4298 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by LC/MS (LC/MS Method A, rt = 13.68 min) for the isolated peptide (28.4 mg, as the 8 trifluoroacetic acid salt).

A mixture of Intermediate 7 (10.1 mg, 1.94 µmol) and Intermediate 2 (JenKem Technology USA Inc., 78 mg, 1.94 µmol) in 10 mL of 1X PBS buffer at pH 7.4 was stirred overnight. The reaction was then diluted with 10 mL of a solution of 20% MeOH in 10 mM aqueous HCl and purified by ion exchange chromatography

(Sepharose FF Media, 0 - 60% 1 M NaCl in 20% methanol/10 mM aqueous HCl over 7 column volumes, flow rate 5 mL/min, λ - 215 nm). The purified conjugate was desalted using size exclusion chromatography (Sephadex G 25 Fine, 50 x 130 mm column, 0.1 Macetic acid, λ - 254 nm) to afford a white solid after lyophilization. The molecular mass of the isolated peptide was confirmed by positive fragment ion distribution with the apex at 44384 amu (MALDI). Example 39 (26.7 mg, as the 8 acetic acid salt) gave a retention time equal to 12.30 min using size exclusion HPLC (Phenomenex BioSep-SEC-S3000 column, 7.8 x 300 mm, 5 µm, 0.15 mM NaCl in 30 mM PBS over 20 min, pH 6.8, flow rate 0.75 mL/min, λ - 215 nm), and a retention time equal to 12.31 min by C18 HPLC (C18 HPLC Method A).

Example 40



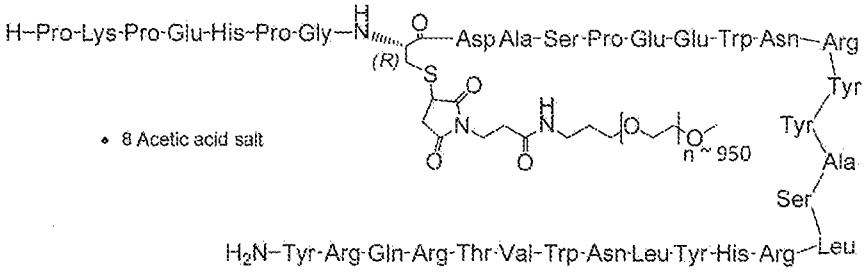
(SEQ ID NO:43);

Intermediate 8 was prepared on a 40 µmol scale as a white solid using the general method, except the lysine at position 8 of the peptide was protected with an ivDde group, while proline at position 1 was protected with a Boc. After the coupling of the last amino acid (proline 1), the ivDde was removed with repeated treatments of 4% aqueous hydrazine in DMF and Fmoc-Gly-OH and Fmoc-Cys(Trt)-OH were coupled. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1440 amu and (M+4)/4 – 1080 amu, which corresponds to a peptide with the parent molecular weight of 4318 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by LC/MS (LC/MS Method A, rt = 13.16 min) for the isolated peptide (39 mg, as the 9 trifluoroacetic acid salt).

A mixture of Intermediate 8 (10.43 mg, 1.95 µmol) and Intermediate 2 (JenKem Technology USA Inc., 86 mg, 2.15 µmol) in 10 mL of 1X PBS buffer at pH 7.4 was stirred for 2 h. The reaction was then diluted with 10 mL of a solution of 20% MeOH in 10 mM aqueous HCl and purified by ion exchange chromatography (Sepharose FF Media, 0 - 60% 1 M NaCl in 20% methanol/10 mM aqueous HCl over 7 column volumes, flow rate 5 mL/min, λ - 215 nm). The purified conjugate was

desalted using size exclusion chromatography (Sephadex G 25 Fine, 50 x 130 mm column, 0.1 M acetic acid, λ - 254 nm) to afford a white solid after lyophilization. The molecular mass of the isolated peptide was confirmed by positive fragment ion distribution with the apex at 44514 amu (MALDI). Example 40 (35 mg, as the 9 acetic acid salt) gave a retention time equal to 14.90 min using size exclusion HPLC (Phenomenex BioSep-SEC-S3000 column, 7.8 x 300 mm, 5 µm, 0.15 mM NaCl in 30 mM PBS over 20 min, pH 6.8, flow rate 0.75 mL/min, λ - 215 nm), and a retention time equal to 12.08 min by C18 HPLC (C18 HPLC Method A).

Example 41.

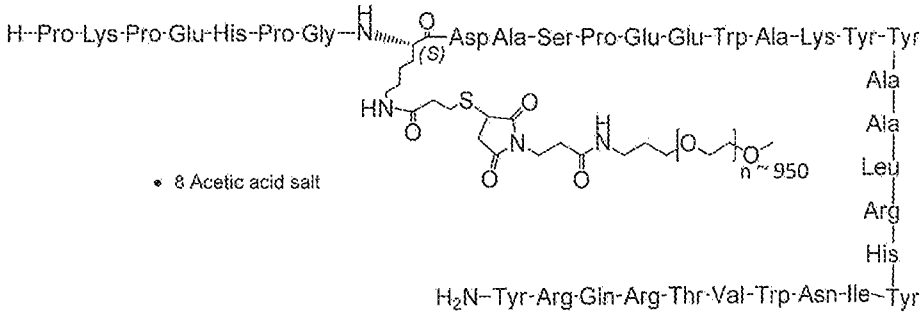


(SEQ ID NO:44);

Intermediate 9 was prepared on a 40 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1407 amu and (M+4)/4 – 1056 amu, which corresponds to a peptide with the parent molecular weight of 4220 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by C18 HPLC (C18 HPLC Method A, rt = 8.98 min) for the isolated peptide (30 mg, as the 8 trifluoroacetic acid salt).

A mixture of Intermediate 9 (13.2 mg, 2.57 µmol) and Intermediate 2 (JenKem Technology USA Inc., 113 mg, 2.83 µmol) in 5 mL of 1X PBS buffer at pH 7.4 was shaken for 1 h, during which time the reaction became homogenous. The reaction was then diluted with 5 mL of 20% MeOH in 10 mM aqueous HCl and purified by ion exchange chromatography (Sepharose FF Media, 0 - 60% 1 M NaCl in 20% methanol/10 mM aqueous HCl over 7 column volumes, flow rate 5 mL/min, λ - 254 nm). The purified conjugate was desalted using size exclusion chromatography (Sephadex G 25 Fine Desalting column, 0.1 M acetic acid,λ - 254 nm) to afford a white solid after lyophilization. The molecular mass of the isolated peptide was confirmed by positive fragment ion distribution with the apex at 44239 amu (MALDI). Example 41 (41 mg, as the 8 acetic acid salt) gave a retention time equal to 9.20 min using size exclusion HPLC (Phenomenex BioSep-SEC-S3000 column, 7.8 x 300 mm, 5 µm, 0.15 mM NaCl in 30 mM PBS over 20 min, pH 6.8, flow rate 0.75 mL/min, λ - 215 nm).

Example 42.

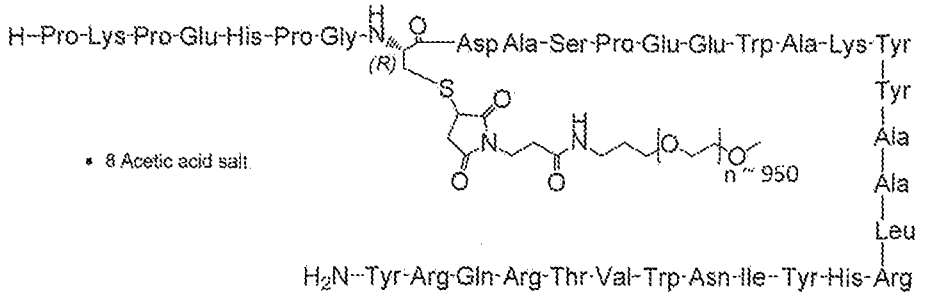


(SEQ ID NO:43);

Intermediate 10 was prepared on a 40 µmol scale as a white solid using the general method, except the lysine at position 8 of the peptide was protected with an ivDde group, while proline 1 was protected with a Boc. After the coupling of the last amino acid (proline 1), the ivDde was removed with repeated treatments of 4% aqueous hydrazine in DMF and Trt-mercaptopropionic acid (MPA) was coupled. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1416 amu and (M+4)/4 – 1062 amu, which corresponds to a peptide with the parent molecular weight of 4246 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by LC/MS (LC/MS Method A, rt = 13.88 min) for the isolated peptide (22.2 mg, as the 8 trifluoroacetic acid salt).

A mixture of Intermediate 10 (10.2 mg, 1.98 µmol) and Intermediate 2 (JenKem Technology USA Inc., 87 mg, 2.18 µmol) in 10 mL of 1X PBS buffer at pH 7.4 was stirred overnight. The reaction was then diluted with 10 mL of a solution of 20% MeOH in 10 mM aqueous HCl and purified by ion exchange chromatography (Sepharose FF Media, 0 - 60% 1 M NaCl in 20% methanol/10 mM aqueous HCl over 7 column volumes, flow rate 5 mL/min, λ - 215 nm). The purified conjugate was desalted using size exclusion chromatography (Sephadex G 25 Fine, 50 x 130 mm column, 0.1 M acetic acid,λ - 254 nm) to afford a white solid after lyophilization. The molecular mass of the isolated peptide was confirmed by positive fragment ion distribution with the apex at 44392 amu (MALDI). Example 42 (32.2 mg, as the 8 acetic acid salt) gave a retention time equal to 13.83 min using size exclusion HPLC (Phenomenex BioSep-SEC-S3000 column, 7.8 x 300 mm, 5 µm, 0.15 mM NaCl in 30 mM PBS over 20 min, pH 6.8, flow rate 0.75 mL/min, λ - 215 nm), and a retention time equal to 12.06 min by C18 HPLC (C18 HPLC Method A).

Example 43.



(SEQ ID NO:45)

Intermediate 11 was prepared on a 35 µmol scale as a white solid using the general method. The molecular mass of the isolated peptide was confirmed by fragment ions (M+3)/3 – 1378 amu and (M+4)/4 – 1034 amu, which corresponds to a peptide with the parent molecular weight of 4133 amu (ESI-MS, LC/MS Method A). A purity of >90% was determined by LC/MS (LC/MS Method A, rt = 13.77 min) for the isolated peptide (13 mg, as the 8 trifluoroacetic acid salt).

A mixture of Intermediate 11 (9.54 mg, 1.89 µmol) and Intermediate 2 (JenKem Technology USA Inc., 83 mg, 2.08 µmol) in 10 mL of a solution of 1X PBS buffer at pH 7.4 was stirred at ambient temperature overnight. The reaction was then diluted with 10 mL of a solution of 20% MeOH in 10 mM aqueous HCl and purified by ion exchange chromatography (Sepharose FF Media, 0 - 60% 1 M NaCl in 20% methanol/10 mM aqueous HCl over 7 column volumes, flow rate 5 mL/min, λ - 215 nm). The purified conjugate was desalted using size exclusion chromatography (Sephadex G 25 Fine, 50 x 130 mm column, 0.1 M acetic acid, λ - 254 nm) to afford a white solid after lyophilization. The molecular mass of the isolated peptide was confirmed by positive fragment ion distribution with the apex at 44117 amu (MALDI). Example 43 (32 mg, as the 8 acetic acid salt) gave a retention time equal to 10.65 min using size exclusion HPLC (Phenomenex BioSep-SEC-S3000 column, 7.8 x 300 mm, 5 µm, 0.15 mM NaCl in 30 mM PBS over 20 min, pH 6.8, flow rate 0.75 mL/min, λ - 215 nm), and a retention time equal to 12.10 min by C18 HPLC (C18 HPLC Method A).

BIOLOGICAL EXAMPLES

Potency of PYY analogs at the human neuropeptide Y receptor type 2

The relative potency of PYY analogs at the human Neuropeptide Y receptor type 2 was determined using a melanophore assay essentially as described in Jayawickreme *et al.* (2005) *Current Protocols in Pharmacology* 12.9.1-12.

Effects of PYY analogs on food intake

Cumulative food intake after 6 h was determined for the PYY analogs in either lean (Model A) or diet-induced obese (DIO) (Model B) C57BL/6 mice in a BioDaQ system for continuous monitoring of food intake (Research Diets Inc., New Brunswick, NJ). Model A utilized 10 week old male C57BL/6 mice (Taconic, Germantown, New York) fed a normal chow (Purina PMI 5001) whereas Model B utilized 25 week old male C57BL/6 mice fed a 45% high fat chow for 20 weeks (Research Diets D12451). Mice were placed singly into the BioDaQ cages and acclimatized for a minimum of 6 days and were allowed *ad libitum* access to food and water. Approximately 1 hour prior to lights-out, animals were dosed subcutaneously with either vehicle (20 mM Acetate buffer, pH 4.9 or 20% DMSO in water) or analogs dissolved in vehicle (1 mg/kg) (8 animals per group). Once all animals have been dosed, feeder gates were opened providing *ad-libitum* access to food. Continuous food intake was monitored and collected for 15 hours. Hourly food intake, as well as 6 and 15-hour cumulative food intake, was summarized as % inhibition relative to vehicle controls. The data were analyzed in JMP 6.0.0 (SAS Institute, Cary, NC) using a pooled variance t-test *vs.* groups treated with human PYY(3-36)NH_2. P-values < 0.05 were considered to indicate a significant difference between treatment groups.

Table 1 shows potency at the human Neuropeptide Y receptor and food intake reduction for the PYY analogs shown in Examples 1-35.

Table 1

Example	hNPY Y2 pEC_{50}	% reduction in food intake, model A	% reduction in food intake, model B	p value (pooled variance t-test vs hPYY[3-36])
Example 1	9.9	-81		<0.0001
Example 2	9.8	-50		0.0038
Example 3	9.5	-89		<0.0001
Example 4	10.7	-83		<0.0001
Example 5	10.5	-89		<0.0001
Example 6	10.1	-88		<0.0001

Example 7	10.1	-91		<0.0001
Example 8	10.7	-74		<0.0001
Example 9	10.7	-86		<0.0001
Example 10	10.4	-88		<0.0001
Example 11	10.3	-86		<0.0001
Example 12	10.2	-88		<0.0001
Example 13	9.9	-85		<0.0001
Example 14	10.5	-89		<0.0001
Example 15	10	-90		<0.0001
Example 16	10.4	-90		<0.0001
Example 17	9.9	-90		<0.0001
Example 18	10.5	-92		<0.0001
Example 19	10.2	-89		<0.0001
Example 20	10.5	-86		<0.0001
Example 21	10.5	-90		<0.0001
Example 22	10.2		-80	<0.0001
Example 23	9.8		-86	<0.0001
Example 24	10.6		-56	0.0035
Example 25	9.6		-68	0.0003
Example 26	9.7		-81	<0.0001
Example 27	9.8		-69	<0.0001
Example 28	10		-65	<0.0001
Example 29	9.7		-54	0.0006
Example 30	9.7		-73	<0.0001
Example 31	9.8	-59		0.0004
Example 32	10.1		-77	<0.0001
Example 33	10		-67	<0.0001
Example 34	10		-69	<0.0001
Example 35	10.3		-70	<0.0001

Table 2 shows examples of PYY analogs which have potency at the human Neuropeptide Y receptor but do not show food intake reduction greater than human PYY(3-36)NH_2 at the 6 h time point.

5

Table 2

Peptide	Peptide Sequence	hNPY Y2 pEC_{50}	% reduction in food intake, model A	SEQ ID NO
Peptide 1	PKPEAPGCDASPEEWNRYYASLRKYLNWVTRQNY-NH_2	7.9	5	46
Peptide 2	IKPEAPLSKQLEEEAVRYYASLRHYLNLVTRQRY-NH_2	8.6	-12	47
Peptide 3	PKPEAPGEDASPKEWNRYYASLRKYLNWVTRQRY-NH_2	9.2	-30	48
Peptide 4	PKPEHPGEDASPEELNRYHAALRAYLNLVTRQRY-NH_2	11.1	-26	49
Peptide 5	PKPEHPGEDASPEELNRYYAALRAYLNLVTRQKY-NH_2	8.5	-7	50
Peptide 6	PKPEHPGEDASPEELNRYYAALRAYLNLVTKQRY-NH_2	9.7	-14	51
Peptide 7	PQPESPGCNASPEELAKYHAALRHYVNLITRQRY-NH_2	10.2	-25	52
Peptide 8	IKPPYPGCDASPEEQNKYYASLRAYWNLVTRQRY-NH_2	9.3	-19	53
Peptide 9	PKPESPGSNASPEDWAKYQAAVRHYVNLITRQRY-NH_2	10.6	-24	54
Peptide 10	PEPEHPGCDASPEDQNKYHASLRKYLNWVTRQRY-NH_2	9.5	-21	55
Peptide 11	IKPPEPGCDASPEEQNKYYASLRHYWNLVTRQRY-NH_2	9.5	-5	56
Peptide 12	IEPEAPGEDASPEELNRYYASLRHYLNLVTRQRY-NH_2	9.8	-15	57
Peptide 13	PKPESPGSDASPEDLAKYHAAVRHYVNLITRQRY-NH_2	10.9	-23	58
Peptide 14	PKPEAPGCDASPEEWNRYYASLRKYLNWVTRQHY-NH_2	8.2	24	59
Peptide 15	PKPVAPGCDASPAELNRQYSDLRNYWNLVTRQRY-NH_2	8.9	-17	60
Peptide 16	IQPEAPGEDASPEELNRYYASLRHYLNLVTRQRY-NH_2	10	-32	61
Peptide 17	PKPESPGKDASPEDLAKYHAAVRHYVNLITRQRY-NH_2	11	-36	62
Peptide 18	PQPESPEGNASPEDWACYHAAVRHYVNLITRQRY-NH_2	9.7	-17	63
Peptide 19	IHPEAPGEDASPEELNRYYASLRHYLNLVTRQRY-NH_2	10	-26	64
Peptide 20	IKPEAPGEDASPEQLMAQYASLRHYLNLVTRQRY-NH_2	9.9	-16	65
Peptide 21	PKPEAPLSKQLEEEAVRYYASLRHYLNLVTRQRY-NH_2	8.7	-2	66
Peptide 22	PKPEAPGCDASPEELNRYQASLRHYLNLVTRQRY-NH_2	10.3	-16	67

Effects of Example 5 in combination with Exendin-4 on body weight, body composition and food intake reduction

A chronic (41 days) *in vivo* efficacy study was conducted in a rodent model for obesity (diet- induced obese (DIO) Long Evans rat) to investigate the efficacy and durability of Example 5 singly and in combination with exendin-4 as anti-obesity agents.

Male Diet-Induced Obese (DIO) Long Evans (LE) rats were used (Harlan Laboratories, Inc., Indianapolis, IN) and beginning at weaning (about 3 weeks of

age), the rats were fed a high fat chow (Teklad TD 95217, 40% kcal from fat, Harlan Laboratories, Madison, WI). Rats were 17 weeks old at the start of the study. The rats were housed 1 per cage and given *ad libitum* access to TD.95217 chow and water, maintained on a 12 h light/dark cycle from 5:00 AM to 5:00 PM at 21°C and 50% relative humidity and allowed to acclimate for at least 7 days prior to baseline measurements. Baseline fat mass and non-fat mass measurements were taken 3 days before the start of peptide infusion and on day 40 of treatment using a QMR instrument (Echo Medical Systems, Houston, TX). Rats were randomized according to their percent body fat mass into 6 groups: (1) vehicle (sterile water, n=8), (2) Exendin-4 (ED_{50}=0.15 mg/kg/day, n=8), (3) Example 5 (ED_{50}=0.03mg/kg/day, n=8), (4) PYY(3-36)NH_2 (1.5 mg/kg/day, n=8), (5) Exendin-4+ Example 5 (n=8) and (6) Exendin-4+ PYY(3-36)NH_2 (n=8). ALZET® mini-osmotic pumps (6 week; Model 2006, Durect Corporation, Cupertino, CA) were filled under sterile condition with either vehicle or peptide one day prior to the surgery. Each rat was implanted with two osmotic pumps subcutaneously in the scapula region containing vehicle or peptide according to their treatment group. Body weight and food intake were measured twice per week beginning three days before the 41-day treatment period. On day 41 of treatment, whole blood was collected by cardiac stick under isoflurane anesthesia. Plasma and serum were then prepared from the whole blood for serum chemistry analysis. All the data are presented as mean ± SEM. The data were analyzed in either Prism (GraphPad Software, Inc., La Jolla, CA) or Excel using a Student's T-test to compare each group to the appropriate control group. P-values < 0.05 were considered to indicate a significant difference between treatment groups.

All procedures were performed in compliance with the Animal Welfare Act, USDA regulations and approved by the GlaxoSmithKline Institutional Animal Care and Use Committee.

In DIO rats, administration of Example 5 at the ED_{50} for weight loss for 40 days resulted in -6.1% (p<0.05) weight loss whereas native PYY(3-36)NH_2 at the ED_{50} resulted in -1.3% (p=0.46) weight loss *vs.* vehicle (Figure 1). The combination of Example 5 and exendin-4 at combo ED_{50} doses for 40 days resulted in sustained and significant weight loss of -30.9% *vs.* vehicle (p<0.05), which far exceeded the expected additive effect based on weight loss of exendin-4 and Example 5 when administered alone (-11.3% and -6.1%, respectively, with a projected additive weight loss of -17.4%). Whereas, native PYY(3-36)NH_2 in combination with exendin-4 resulted in -10.2% weight loss *vs.* vehicle which was sub-additive based on weight loss of exendin-4 and PYY(3-36)NH_2 when administered alone (-11.3% and -1.3%, with a projected additive weight loss of -12.6%).

Changes in body composition were primarily driven by loss of body fat mass, with some changes in non-fat mass and mirrored the body weight changes in all treatment groups (Figure 2). Specifically, the animals treated with Example 5 lost -34.2 grams of fat mass from vehicle control ($p<0.05$), the PYY(3-36)NH_2 animals lost -10.9 grams fat mass (p=0.12 *vs.* vehicle control) and animals treated with exendin-4 lost -55.8 grams fat mass ($p<0.05$ *vs.* vehicle control) during the treatment period. The Example 5 + exendin-4 combination had a more than additive effect on fat mass where the combination lost -110.1 grams ($p<0.05$ *vs.* vehicle control), which was significantly greater than the predicted additivity value of -90 grams ($p<0.05$) (Figure 3). In contrast, PYY(3-36)NH_2 in combination with exendin-4 resulted in -54.0 grams fat mass loss *vs.* vehicle ($p<0.05$) which was less than the predicted additivity value of -66.7 grams.

In addition, a -57.1% inhibition of cumulative food intake ($p<0.05$ *vs.* vehicle control) was observed when Example 5 was co-administered with exendin-4 compared with -18.8% inhibition ($p=0.87$ *vs.* vehicle control) with the PYY(3-36)NH_2 + exendin-4 combination. There appears to be a more than additive efficacy with the Example 5 + exendin-4 combination based upon the food intake inhibition of each peptide administered alone (-11.5% and -20.1%, respectively, with a projected additive food intake inhibition of -31.6%). In contrast, the native PYY(3-36)NH_2 + exendin-4 combination resulted in sub-additive food intake inhibition based upon the food intake inhibition of each peptide administered alone (-0.7% for PYY(3-36)NH_2 and -20.1% for exendin-4, with a projected additive food intake inhibition of -20.8%).

Example 23 in Combination with exendin-4 causes more than additive effects on glucose parameters in Diabetic ZDF rats

A chronic (26 days) *in vivo* efficacy study was conducted in a rodent model for diabetes (Zucker Diabetic Fatty (ZDF) rat) to investigate the efficacy and durability of Example 23 singly and in combination with exendin-4 as anti-diabetes agents.

Male ZDF rats were 12 weeks old at the start of the study (Charles River, Inc., Boston, MA). The ZDF rats were housed 1 per cage and given *ad libitum* access to diet (Purina PMI 5008) and water, maintained on a 12 hr light/dark cycle from 5:00 AM to 5:00 PM at 21° C and 50% relative humidity and allowed to acclimate for at least 6 days prior to baseline measurements and 10 days prior to the surgeries. Baseline fat mass and non-fat mass measurements were taken 3 days before the start of peptide infusion and on day 26 of treatment using a QMR instrument (Echo Medical Systems, Houston, TX). Blood samples were taken via tail snip to measure fed glucose values and % HbA1c values two days before the start of drug dosing;

this data was used to randomize the animals into 7 groups: (1) Lean vehicle control (sterile phosphate buffered saline (PBS), pH 4.9, n=8), (2) ZDF vehicle control (sterile PBS, pH 4.9, n=8), (3) Exendin-4 (ED_{20}=0.0055 mg/kg/day, n=8), (4) Example 23 (ED_{20}=0.02mg/kg/day, n=8), (5) PYY(3-36)NH_2 (0.02 mg/kg/day, n=8), (6) Exendin-4+Example 23 (n=4) and (7) Exendin-4+ PYY(3-36)NH_2 (n=8). ALZET® mini-osmotic pumps (4-week; Model 2006, Durect Corporation, Cupertino, CA) were filled under sterile condition with either vehicle or peptide one day prior to the surgery. Similar surgical implantation of the mini-pumps was performed as described for the DIO rats above (except animals were injected ID with lidocaine (0.1 mL of 0.125% lidocaine). Body weight and food intake were measured twice per week beginning 3 days before the 26-day treatment period. On day 26 of treatment, whole blood was collected by cardiac stick under isoflurane anesthesia. The whole blood was used to determine the % HbA1c and the serum was used to measure glucose. The data were analyzed in either Prism (GraphPad Software, Inc., La Jolla, CA) or Excel using a Student's T-test to compare each group to the appropriate control group. P-values < 0.05 were considered to indicate a significant difference between treatment groups.

All procedures were performed in compliance with the Animal Welfare Act, USDA regulations and approved by the GlaxoSmithKline Institutional Animal Care and Use Committee.

Table 3 shows the glucose and glycosylated HbA1c changes from baseline and from vehicle control ZDF animals (ΔΔ) following chronic treatment (26 days) with Example 23, PYY(3-36)NH_2,or exendin-4 singly or in combination. Singly, only the exendin-4 and Example 23 achieved statistically significant glucose lowering from vehicle control (ΔΔ -53.9 and -54.5 mg/dL, respectively; p<0.05) compared to PYY(3-36)NH_2 (ΔΔ-33.1; p=0.11). Treatment with the combination of Example 23 and exendin-4 combo at ED_{20} doses for HbA1c lowering for 26 days resulted in significant glucose lowering ΔΔ -152.3 mg/dL (p<0.05 *vs.* vehicle control), which exceeded the expected additive effect based on glucose lowering of exendin-4 and Example 23 when administered alone (ΔΔ -53.9 and -54.5 mg/dL, with a projected additive glucose lowering of -108.4 mg/dL *vs.* vehicle control). The ΔΔ%HbA1c levels closely mirrored the glucose changes in all treatment groups, however, none of the groups were deemed statistically significant.

Table 3. Changes in glucose and HbA1c after 26 days of treatment with Example 23 and/or exendin-4 singly and in combination in ZDF rats

Treatment Group	ΔΔGlucose	ΔΔ %HbA1c
Exendin-4	**-53.9 ± 18.3**	-0.4 ± 0.2
Example 23	**-54.5 ± 13.7**	-0.4 ± 0.2
PYY(3-36)NH_2	-33.1 ± 17	0.01 ± 0.4
Ex-4 + Example 23	**-152.3 ± 91.5**	-1.3 ± 0.8
Ex-4 + PYY(3-36)NH_2	-17.6 ± 24.4	-0.4 ± 0.3

ΔΔ=Change in parameter from baseline and vehicle control

Bold= $p<0.05$ from vehicle control

CLAIMS

That which is claimed:

1. A polypeptide comprising the amino acid sequence:
ProLysProGluXaa$_1$ProGlyXaa$_2$AspAlaSerXaa$_3$GluGluXaa$_4$Xaa$_5$Xaa$_6$TyrTyrAla Xaa$_7$LeuArgXaa$_8$TyrXaa$_9$ AsnTrpXaa$_{10}$ThrArgGlnArgTyr (SEQ ID NO:1)
or a salt thereof, wherein:
Xaa$_1$ is Ala, His, or Ser;
Xaa$_2$ is Glu or Lys;
Xaa$_3$ is Pro or Ala;
Xaa$_4$ is Leu or Trp;
Xaa$_5$ is Asn, Ala, or Thr;
Xaa$_6$ is Arg or Lys;
Xaa$_7$ is Ser, Asp, or Ala;
Xaa$_8$ is His or Lys;
Xaa$_9$ is Leu or Ile; and
Xaa$_{10}$ is Val or Leu.

2. A polypeptide consisting of the amino acid sequence:
ProLysProGluXaa$_1$ProGlyXaa$_2$AspAlaSerXaa$_3$GluGlu Xaa$_4$Xaa$_5$ Xaa$_6$TyrTyrAlaXaa$_7$LeuArg Xaa$_8$Tyr Xaa$_9$ AsnTrp Xaa$_{10}$ThrArgGlnArgTyr –NH$_2$ (SEQ ID NO:2) or a salt thereof, wherein:
Xaa$_1$ is Ala, His, or Ser;
Xaa$_2$ is Glu or Lys;
Xaa$_3$ is Pro or Ala;
Xaa$_4$ is Leu or Trp;
Xaa$_5$ is Asn, Ala, or Thr;
Xaa$_6$ is Arg or Lys;
Xaa$_7$ is Ser, Asp, or Ala;
Xaa$_8$ is His or Lys;
Xaa$_9$ is Leu or Ile; and
Xaa$_{10}$ is Val or Leu.

3. The polypeptide of claim 2 which is selected from the group consisting of:
ProLysProGluAlaProGlyLysAspAlaSerProGluGluLeuAsnArgTyrTyrAla
SerLeuArgHisTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:3),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluLeuAsnArgTyrTyrAla
SerLeuArgLysTyrLeuAsnTrpLeuThrArgGlnArgTyr–NH_2 (SEQ ID NO:4),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluLeuAsnArgTyrTyrAla
SerLeuArgHisTyrLeuAsnTrpLeuThrArgGlnArgTyr–NH_2 (SEQ ID NO:5),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
AspLeuArgLysTyrLeuAsnTrpLeuThrArgGlnArgTyr–NH_2 (SEQ ID NO:6),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
AspLeuArgHisTyrLeuAsnTrpLeuThrArgGlnArgTyr–NH_2 (SEQ ID NO:7),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
SerLeuArgLysTyrLeuAsnTrpLeuThrArgGlnArgTyr–NH_2 (SEQ IDNO:8),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
SerLeuArgHisTyrLeuAsnTrpLeuThrArgGlnArgTyr–NH_2 (SEQ ID NO:9),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
AspLeuArgLysTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:10),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
AspLeuArgHisTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:11),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
SerLeuArgLysTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:12),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
SerLeuArgHisTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:13),
ProLysProGluAlaProGlyGluAspAlaSerProGluGluLeuAsnArgTyrTyrAla
SerLeuArgHisTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:14),
ProLysProGluHisProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
AlaLeuArgLysTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:15),
ProLysProGluHisProGlyLysAspAlaSerProGluGluLeuAsnLysTyrTyrAla
AlaLeuArgHisTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:16),
ProLysProGluHisProGlyLysAspAlaSerProGluGluLeuAsnArgTyrTyrAla
SerLeuArgHisTyrIleAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:17),
ProLysProGluHisProGlyLysAspAlaSerProGluGluLeuAlaArgTyrTyrAla
SerLeuArgHisTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:18),
ProLysProGluHisProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla

SerLeuArgHisTyrIleAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:19),
ProLysProGluHisProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
AspLeuArgHisTyrIleAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:20),
ProLysProGluHisProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
AspLeuArgHisTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:21),
ProLysProGluSerProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
AspLeuArgHisTyrIleAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:22),
ProLysProGluSerProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
AspLeuArgHisTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:23),
ProLysProGluHisProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
AspLeuArgHisTyrLeuAsnTrpLeuThrArgGlnArgTyr–NH_2 (SEQ ID NO:24),
ProLysProGluHisProGlyLysAspAlaSerProGluGluTrpAlaLysTyrTyrAla
AlaLeuArgHisTyrIleAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:25),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
AspLeuArgHisTyrIleAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:26),
ProLysProGluHisProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla
SerLeuArgLysTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:27),
ProLysProGluHisProGlyLysAspAlaSerAlaGluGluTrpAlaLysTyrTyrAla
AlaLeuArgHisTyrIleAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:28),
ProLysProGluAlaProGlyLysAspAlaSerAlaGluGluTrpAsnArgTyrTyrAla
SerLeuArgHisTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:29),
ProLysProGluHisProGlyLysAspAlaSerAlaGluGluLeuAlaArgTyrTyrAla
SerLeuArgHisTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:30),
ProLysProGluAlaProGlyLysAspAlaSerAlaGluGluTrpAsnArgTyrTyrAla
SerLeuArgLysTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:31),
ProLysProGluSerProGlyLysAspAlaSerAlaGluGluTrpThrLysTyrTyrAla
AlaLeuArgHisTyrIleAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:32),
ProLysProGluAlaProGlyLysAspAlaSerProGluGluLeuAsnArgTyrTyrAla
SerLeuArgLysTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:33),
ProLysProGluHisProGlyGluAspAlaSerProGluGluTrpAlaLysTyrTyrAla
AlaLeuArgHisTyrIleAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:34),
ProLysProGluAlaProGlyGluAspAlaSerAlaGluGluTrpAsnArgTyrTyrAla
SerLeuArgHisTyrLeuAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:35),
ProLysProGluSerProGlyGluAspAlaSerProGluGluTrpThrLysTyrTyrAla

AlaLeuArgHisTyrIleAsnTrpValThrArgGlnArgTyr–NH_2 (SEQ ID NO:36), and ProLysProGluAlaProGlyGluAspAlaSerProGluGluTrpAsnArgTyrTyrAla AspLeuArgHisTyrLeuAsnTrpLeuThrArgGlnArgTyr–NH_2 (SEQ ID NO:37).

4. The polypeptide of claim 1 which comprises the amino acid sequence: ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla AspLeuArgHisTyrLeuAsnTrpLeuThrArgGlnArgTyr (SEQ IDNO:38).

5. The polypeptide of claim 1 which consists of the amino acid sequence: ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla AspLeuArgHisTyrLeuAsnTrpLeuThrArgGlnArgTyr (SEQ IDNO:38).

6. The polypeptide of claim 2 which consists of the amino acid sequence: ProLysProGluAlaProGlyLysAspAlaSerProGluGluTrpAsnArgTyrTyrAla AspLeuArgHisTyrLeuAsnTrpLeuThrArgGlnArgTyr–NH_2 (SEQ ID NO:7).

7. A pharmaceutical combination comprising a polypeptide according to any one of claims 1-6 and exendin-4.

8. A pharmaceutical combination comprising a polypeptide according to any one of claims 1-6 and GLP-1.

9. A pharmaceutical composition comprising a polypeptide according to any one of claims 1-6 and a pharmaceutically acceptable carrier.

10. A method of treating a metabolic disorder in a human subject, said method comprising administering a polypeptide or pharmaceutical combination of any one of claims 1-8 to a subject in need thereof.

11. A method of treating obesity in a human subject, said method comprising administering a polypeptide or pharmaceutical combination of any one of claims 1-8 to a subject in need thereof.

12. Use of a polypeptide or pharmaceutical combination of any one of claims 1-8 in the preparation of a medicament for use in the treatment of a metabolic disorder.

13. Use of a polypeptide or pharmaceutical combination of any one of claims 1-8 in the preparation of a medicament for use in the treatment of obesity

14. A polypeptide or pharmaceutical combination according to any one of claims 1-8 for use in the treatment of a metabolic disorder.

15. A polypeptide or pharmaceutical combination according to any one of claims 1-8 for use in the treatment of obesity.

16. The method or use of any one of claims 10, 12, or 14 where the metabolic disorder is Type 2 Diabetes Mellitus.

17. A nucleic acid molecule encoding a polypeptide sequence according to any one of claims 1-6.

18. An expression vector comprising a nucleic acid molecule according to claim 17.

19. A host cell containing an expression vector according to claim 18.