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(54) Title: PEPTIDE CONTROLLED-RELEASE FORMULATIONS

(57) Abstract: The present invention relates to compositions forming a low viscosity mixture of lipid-based components with a peptide agonist active agent wherein the pre- formulation forms, or is capable of forming, at least one liquid crystalline phase structure upon contact with excess aqueous fluid. The invention further relates to methods of treatment comprising administration of such compositions, and to pre-filled administration devices and kits containing the formulations.

Peptide Controlled-Release Formulations

FIELD OF THE INVENTION

The present invention relates to formulation precursors (pre-formulations) for the *in situ* generation of compositions for the controlled release of certain hormone active agents and methods of treatment with such formulations. In particular, the invention relates to high-loading pre-formulations of amphiphilic components and at least one hybrid dual agonist polypeptide hormone active agent for parenteral application, which undergo phase transition upon exposure to aqueous fluids, such as body fluids, thereby forming a controlled release composition.

BACKGROUND TO THE INVENTION

Many bioactive agents including pharmaceuticals, nutrients, vitamins and so forth have a "functional window". That is to say that there is a range of concentrations over which these agents can be observed to provide a beneficial or desirable biological effect. Where the concentration in the appropriate part of the body (e.g. locally or as demonstrated by serum concentration) falls below a certain level, no beneficial effect can be attributed to the agent. Similarly, there is generally an upper concentration level above which no further benefit is derived by increasing the concentration. In some cases increasing the concentration above a particular level results in undesirable or even dangerous effects.

Some bioactive agents have a long biological half-life and/or a wide functional window and thus may be administered occasionally, maintaining a functional biological concentration over a substantial period of time (e.g. 6 hours to several days). In other cases the rate of clearance is high and/or the functional window is narrow and thus to maintain a biological concentration within this window regular (or even continuous) doses of a small amount are required. This can be particularly difficult where non-oral routes of administration (e.g. parenteral administration) are desirable or necessary, since self-administration may be difficult and thus cause inconvenience and/or poor compliance. Agents for treatment or maintenance in

chronic conditions also benefit considerably from slow-release formulations because the dose is potentially more stable and the disruption to the lifestyle of a subject living with a chronic or long-term condition is minimised. In such cases it would be advantageous for a single administration to provide active agent at a therapeutic level over the whole period during which activity is needed or over a period of days or weeks in chronic conditions.

There is an enormous potential in the use of peptides for treating various disease states, as well as in prophylaxis and in improving general health and well-being of subjects. However, the performance of administered peptide agents is generally limited due to poor bioavailability, which in turn is caused by their rapid degradation in biological fluids. This increases the dose which must be administered and in many cases restricts the effective routes of administration. These effects are further exaggerated by the often limited permeability of peptides and proteins across biological membranes.

Peptides and proteins that are administered to the mammalian body (e.g. orally, intramuscularly etc.) are subject to degradation by various proteolytic enzymes and systems present throughout the body. Well known sites of peptidase activity include the stomach (e.g. pepsin), and the intestinal tract (e.g. trypsin, chymotrypsin, and others) but other peptidases (e.g. aminopeptidases, carboxypeptidases, etc.) are found throughout the body. Upon oral administration, gastric and intestinal degradation reduces the amount of peptide or protein which potentially could be absorbed through the intestinal surface lining and thereby decreases their bioavailability. Similarly, free peptides and proteins in the mammalian blood stream are also subject to enzymatic degradation (e.g. by plasma proteases etc.).

Some patients undergoing treatment, particularly for long-term or chronic conditions will typically require ongoing treatment for many months or years. Thus a depot system allowing loading and controlled release of a larger dose over a longer period would offer a considerable advantage over conventional delivery systems. Diabetes

is one such condition that may benefit by long-term or chronic treatment, as well as by use of a depot formulation.

For treatment of diabetes, glucagon-like peptide (GLP)-1, which is a potent glucoregulatory hormone that is released from intestinal L cells into the circulation in response to nutrient ingestion and neural and endocrine stimuli, has been well explored. Structurally, GLP-1 is a 37-amino acid peptide with a MW of 4.2 KDa, having a sequence highly conserved between different species. GLP-1 is involved in modification of glucose homeostasis through actions that include potentiation of glucose-stimulated insulin secretion and biosynthesis and suppression of glucagon secretion, gastric emptying, and food intake. The abilities of GLP-1 to stimulate insulin secretion and inhibit glucagon release are glucose-dependent; thus, the risk of hypoglycemia with GLP-1 administration is low. GLP-1 also increases beta-cell mass in preclinical models of diabetes through mechanisms that include stimulation of beta-cell proliferation and neogenesis and inhibition of beta-cell apoptosis. Studies in both animals and humans indicate that GLP-1 may also play a protective role in the cardiovascular system.

The combined actions of GLP-1 have generated substantial interest in using this peptide as a therapeutic agent for the treatment of type 2 diabetes. However, the therapeutic potential of native GLP-1 is limited by its very short plasma half-life (below 2 minutes). This is due to both rapid inactivation by the proteolytic enzyme dipeptidyl peptidase (DPP)-IV and renal clearance. Consequently, long-acting, DPP-IV-resistant GLP-1 receptor analogs and agonists have been developed for clinical use, including analogs liraglutide (Novo Nordisk), CJC-1131 (ConjuChem), LY548806 (Lilly), and TH-0318 (TheraTechnologies) and agonists exenatide (*Byetta*, Amylin-Lilly) and AVE010 (Zealand Pharma – Sanofi-Aventis). All these are once- or twice-daily administration products; a controlled-release (one week) exenatide product (*Exenatide LAR* Alkermes-Amylin-Lilly) is currently under clinical investigation. These GLP-1 mimetics bind to GLP-1 receptors with similar affinity and produce biological actions overlapping to those of native GLP-1 but are resistant to DPP-IV-mediated inactivation and, in some cases more resistant to renal

clearance. These compounds are able to exert more sustained GLP-1-like activity for longer periods of time in vivo. An alternative therapeutic approach for prolonging the action of native GLP-1 is to inhibit DPP-IV activity, thereby preventing GLP-1 degradation. Several orally active agents that inhibit DPP-IV activity are also being evaluated for the treatment of type 2 diabetes.

With regard to administration, conditions such as type-2 diabetes are ongoing, and any treatment regime will typically involve long-term, ongoing therapy, for periods of months or years. Currently available GLP-1 therapies are typically injectables which require administration around twice a day for the period of treatment. This will generally be by patient self-administration. Since frequent injection over a long period is not an optimal administration strategy, there is clearly scope for GLP-1 users to benefit from long-acting, sustained formulations, which might be administered much less frequently.

DPP-IV inhibitors are often associated with undesirable side-effects. Furthermore, controlling or reducing obesity, a co-morbidity often associated with diabetes, e.g. type II diabetes, is extremely desirable, but is not generally obtained by DPP-IV inhibitors. On the other hand while GLP-1 receptor agonists can provide some weight control benefit, their primary benefit is glucose control, and further weight control is desirable.

Attempts to treat the multiple abnormalities and co-morbidities often associated with diabetes, such as obesity and overweight which aggravate the diabetes and lead to further cardiovascular diseases, have prompted the administration of several different anti-diabetic medicaments in order to address these abnormalities in the same patient, necessitating multiple injections and dosing regimens and multiple opportunities for adverse advents. Examples of such anti-diabetic medicaments are insulin and insulin analogues, and small molecules such as insulin sensitizers, insulin secretagogues and appetite regulators.

Nevertheless, there remains a need to develop therapeutic formulations that are more useful and patient friendly in the above described metabolic diseases, conditions, and disorders.

The present invention addresses the above needs by providing long-acting depot formulations of actives that address multiple aspects of metabolic diseases.

SUMMARY OF THE INVENTION

The present invention provides a pharmaceutical formulation comprising an appropriate combination of lipid excipients, organic alcoholic solvent, aqueous solvent, hybrid polypeptide hormone active agent and certain optional components, that can be used as a depot-precursor formulation (referred to herein for brevity as a pre-formulation) to address one or more of the needs described above.

In a first aspect, the invention provides a pre-formulation comprising a low viscosity mixture of:

a) 20-80 wt.% of at least one diacyl glycerol and/or a tocopherol;
b) 20-80 wt.% of at least one phosphatidyl choline (PC);
c) 5-35 wt.% of at least one biocompatible, organic alcoholic solvent;
d) up to 20 wt.% aqueous solvent
e) at least one dual amylin receptor/GLP-1 receptor agonist active agent
f) optionally at least one antioxidant;

wherein the pre-formulation forms, or is capable of forming, at least one liquid crystalline phase structure upon contact with excess aqueous fluid.

In a further embodiment of each of the embodiments herein, a preservative, e.g. antimicrobial or microbial-static agent, is present. The preservative may be an additional agent to the formulations herein or, preferably a preservative activity is provided by one of or any combination of c), d) or f) above.

Preferably, component a) comprises glycerol dioleate (GDO). Preferably, component a) is present at a level of 30-40% by weight.

Preferably component b) comprises PC. Component b) preferably is present at a level of 30-40% by weight. Preferably the PC is derived from soy. Preferably the PC comprises 18:2 fatty acids as the primary fatty acid component with 16:0 and/or 18:1 as the secondary fatty acid components. These are preferably present in the PC at a ratio of between 1.5:1 and 6:1. PC having approximately 60-65% 18:2, 10 to 20% 16:0, 5-15% 18:1, with the balance predominantly other 16 carbon and 18 carbon fatty acids is preferred and is typical of soy PC.

Preferably the ratio of components a:b is in the range 40:60 to 70:30

In the pre-formulation of the invention it is preferred that component c) comprises at least one mono-ol, diol or triol. Preferably component c) comprises ethanol or propylene glycol or mixtures thereof. Component c) can be in the range of 5 to 35%, even 10-30%. Preferably component c) is present at a level of 10-25% by weight.

Preferably component d) comprises water. Preferably component d) is present at a level of 1.2 to 15% by weight.

Preferably the antioxidant (component f)) is ascorbic acid, EDTA or citric acid, more preferably EDTA.

Preferably the ratio of components d:f is in the range 100:1 to 10000:1, preferably 1000:1 to 5000:1.

It is preferred if the dual amylin receptor/GLP-1 receptor agonist active agent is as defined herein below.

Preferably the pre-formulation according to the invention has an L_2 phase structure.

Preferred embodiments of the pre-formulation according to the invention are listed in Table 1. Preferably the pre-formulation of the invention comprises or consists of at least one formulation selected from those listed in Table 1. Each composition listed in Table 1 of Example 1 below forms a separate and independent preferred embodiment of the present invention.

In another aspect the invention provides a process for the formation of a pre-formulation suitable for the administration of a hybrid polypeptide hormone bioactive agent to a (preferably mammalian) subject, said process comprising forming a low viscosity mixture of:

a) 20-80 wt.% of at least one diacyl glycerol and/or a tocopherol;
b) 20-80 wt.% of at least one phosphatidyl choline (PC);
c) 5-35 wt.% of at least one biocompatible, organic alcoholic solvent;
d) up to 20 wt.% aqueous solvent
e) at least one dual amylin receptor/GLP-1 receptor agonist active agent;
f) optionally at least one antioxidant;

and dissolving or dispersing at least one dual amylin receptor/GLP-1 receptor agonist active agent in the low viscosity mixture, or in at least one of components a), b), c), d) and optionally f) prior to forming the low viscosity mixture.

A further aspect of the invention is the use of a low viscosity mixture of:

a) 20-80 wt.% of at least one diacyl glycerol and/or a tocopherol;
b) 20-80 wt.% of at least one phosphatidyl choline (PC);
c) 5-35 wt.% of at least one biocompatible, organic alcoholic solvent;
d) up to 20 wt.% aqueous solvent
e) at least one dual amylin receptor/GLP-1 receptor agonist active agent;
f) optionally at least one antioxidant;

in the manufacture of a pre-formulation for use in the sustained administration of said hybrid polypeptide hormone active agent.

Preferably this will be the use of a pre-formulation of the invention as defined herein.

In another aspect, the invention provides a method for the treatment of a human or non-human mammalian subject comprising administering to said subject a pre-formulation according to the invention.

Preferably, said method is for the treatment of a human or non-human mammalian subject in need thereof to combat at least one condition selected from diabetes, type I diabetes, type II diabetes, abnormal glucose control, excess bodyweight, obesity, hypertension, arthrosclerosis, dyslipidemia, congestive heart failure, stroke, hypercholesterolemia, cardiovascular disease, myocardial ischemia, myocardial reperfusion, eating disorders, gestational diabetes, diabetic neuropathy, pulmonary hypertension, or a metabolic disease or disorder associated with insufficient pancreatic beta cell mass, wherein the pre-formulation preferably comprises a dual-functional GLP-1 receptor/ amylin receptor agonist, more preferably Cmpd 1, described herein. Preferably, the subject is in need of treatment for at least one of the above conditions that benefits by glucose lowering, delay of gastric emptying, insulin secretion, glucagons suppression and/or HbA1c lowering (as may be provided by a GLP-1 receptor agonist) and for at least one of the above conditions that benefits by further reducing body weight, inhibiting food intake or further delaying gastric emptying (as may be provided by an amylin receptor agonist). Preferably the treatments provide improved patient compliance due to reduced adverse events and side effects, such as flushing, nausea, and/or to a more convenient, patient friendly administration regimen such as by once weekly dosing.

In another aspect, the invention provides a method of cosmetic treatment of a human or non-human mammalian subject comprising administering to said subject a pre-formulation according to the invention

In yet a further aspect, the invention provides the use of:

a) 20-80 wt.% of at least one diacyl glycerol and/or a tocopherol;
b) 20-80 wt.% of at least one phosphatidyl choline (PC);
c) 5-35 wt.% of at least one biocompatible, organic alcoholic solvent;
d) up to 20 wt.% aqueous solvent
e) at least one dual amylin receptor/GLP-1 receptor agonist active agent;
f) optionally at least one antioxidant;

in the manufacture of a low viscosity pre-formulation medicament for use in the *in vivo* formation of a depot for treatment of at least one of type I diabetes, type II diabetes, abnormal glucose control, excess bodyweight, obesity, hypertension, arthrosclerosis, dyslipidemia, congestive heart failure, stroke, hypercholesterolemia,

cardiovascular disease, myocardial ischemia, myocardial reperfusion, eating disorders, gestational diabetes, diabetic neuropathy, pulmonary hypertension, or a metabolic disease or disorder associated with insufficient pancreatic beta cell mass. Preferably, the subject is in need of treatment for at least one of the above conditions that benefits by glucose lowering, delay of gastric emptying, insulin secretion, glucagons suppression and/or HbA1c lowering (as may be provided by a GLP-1 receptor agonist) and for at least one of the above conditions that benefits by further reducing body weight, inhibiting food intake or further delaying gastric emptying (as may be provided by an amylin receptor agonist). Preferably the treatments provide improved patient compliance due to reduced adverse events and side effects, such as flushing, nausea, and/or to a more convenient, patient friendly administration regimen such as by once weekly dosing.

In another aspect, the invention provides a pre-filled administration device containing a pre-formulation according to the invention. The device may be single- or multi-use.

Preferably in said device the pre-formulation according to the invention delivers a dosage of active agent in the range of 1 μg to 10 mg/day, or about 0.5 mg to 5 mg/day, or alternatively as described herein, in the range for example of 3 mg to 60 mg/week or 5 to 40 mg/week, and even 10 to 30 mg/week, whether from a single use or multi-use device.

Preferably, with any of the concentrations active described herein, each administered dose is a total volume for administration of no more than or equal to 3 ml, preferably no more than or equal to 1 ml, more preferably no more than or equal to 0.5 ml, and even more preferably no more than or equal to 0.1 ml. Further, the volume is preferably no less or equal to than 0.03 ml, more preferably no less than or equal to 0.06 ml, and even no less than or equal to 0.1 ml. A preferred volume for administration is approximately 0.5 ml.

In a further aspect the invention provides a kit comprising said administration device according to the invention.

The kit can optionally contain instructions for subcutaneous or intramuscular administration of said composition. All compositions described herein are suitable for use in such a kit and may thus be contained therein.

The kits of the invention can optionally include additional administration components such as needles, swabs, and the like and will optionally contain instructions for administration. The kits may additionally contain other devices and/or medicaments relating to blood glucose control in a diabetic subject, such as blood- or urine-glucose testing devices (e.g. strips or sticks), insulin formulations, carbohydrate preparations etc. Where the kit is for use in the event of and emergency hypoglycemic situation, it may additionally contain glucose and/or other carbohydrate preparation(s) and/or blood glucose testing devices. Where the kit is for routine administration, it can optionally be combined with the subject's routine insulin medication, such that the kit contains compositions of the invention plus one or more insulin preparations and/or one or more blood or urine glucose testing devices.

The formulations can be provided as single use or multi-use formats, which can include for example, vials with syringes, prefilled syringes, prefilled cartridges, prefilled injection pens and/or needle-free injection pens and devices. “Multi-use” is meant as providing for more than one injection, for example 2, 3, 4, 5, 6 or more injections, from the same device. These may be from the same reservoir of pre-formulation or by means of multiple smaller reservoirs.. The injections are preferably made over a period of at least at or about two weeks, three weeks, four weeks and even 8 weeks, more preferably over at least 4 weeks, most preferably as once weekly injections over any of those periods. The injections are made at appropriate dosing intervals as discussed herein.

Single-dose formats must remain stable and potent in storage prior to use, but are disposable after the single use. Multi-dose formats must not only remain stable and potent in storage prior to use, but must also remain stable, potent and relatively free of bacteria over the multiple-dose use regimen administration period after the first use in which a seal has been compromised. For this reason multi-dose formats often require a anti-microbial or microbial-static agent, e.g. bacteriostatic agent, preservative.

However, the production of preserved pharmaceutical preparations containing protein or peptide actives has often proven difficult, as when preservatives are used, these give rise to stability problems. Often the proteins are inactivated and aggregates are formed, which may sometimes lead to reported injection site intolerance or immunogenicity to the active. This can be further aggravated by additional excipients or formulation components.

In one aspect each of the embodiments herein can optionally contain an antimicrobial or microbial-static agent, which includes bacteriostatic agents and preservative. Such agents include benzalkonium chloride, m-cresol, benzyl alcohol or other phenolic preservatives. Typical concentrations as known in the art can be used.

However, surprisingly it has been found that the present formulations with dual receptor agonist do not require an additional preservative, anti-microbial or microbial-static agent, e.g. bacteriastatic or bacteriacide or additional amount of such agent to provide a multi-use format. The formulations as described herein provide a preservative effect with an acceptable peptide stability and formulation stability. They can be used for single-dose as well as for multiple-dose use. In this regard, preferred formulations herein for multi-use format can contain ethanol, propylene glycol, citric acid and/or EDTA as described, preferably in sufficient concentrations to not only provide their primary benefit as taught herein but also at sufficient concentration, either alone or in any combination, to provide the preservative effect while maintaining stability of the active and the formulation.

BRIEF SUMMARY OF THE ATTACHED FIGURES

Figure 1 shows the change in peptide concentration (vs. start value measured directly after manufacturing) after storage of the formulations with compositions presented in Table 1 for 3 months (formulation 16: 2 months) under different conditions.

Figures 2A and 2B: Figure 2A is a graph depicting change in body weight in DIO (diet induced obese) rats administered Cmpd 2 or the other compounds in the amounts shown in Figure 1A as described in the Examples. Figure 2B is a graph depicting change in body weight in DIO rats administered Cmpd 1 or the compounds in the amounts shown in Figure 1B as described in the Examples. Groups not sharing a superscript are significantly different from each other; $p<0.05$, e.g. the vehicle controls of Figures 1A and 1B are not significantly different from each other.

Figures 3A and 3B: Figure 3A is a graph depicting total cumulative food intake for DIO rats administered Cmpd 2 or the other compounds in the amounts shown in Figure 2A as described in the Examples. Figure 3B is a graph depicting total cumulative food intake for DIO rats administered Cmpd 1 or the other compounds in the amounts shown in Figure 2B as described in the Examples. Groups not sharing a superscript are significantly different from each other; $p<0.05$, e.g. the vehicle controls of Figures 2A and 2B are not significantly different from each other.

Figure 4 shows mean body weights of male rats following administration of a single subcutaneous dose of Cmpd1 formulated as set out in the Examples below.

Figure 5 shows the variation in plasma concentration over time from a Single Dose Subcutaneous Pharmacokinetic Study in Rats using Cmpd1. Figure 5A shows results over a timescale up to 312 hours. Figure 5B shows results over the first 24 hours.

Figures 6A to 6C depict Pharmacokinetic (Fig. 6A and 6B) and Pharmacodynamics (Fig. 6C) profiles in dogs following a single subcutaneous administration of two exemplary and preferred sustained release formulations containing Cmpd 1 over a 312 hour (two week) time course. Fig. 6A depicts the Cmpd 1 plasma concentration in pg/ml of plasma over the 312 hour time course for formulation B10 and formulation B12. Fig. 6B is a plot expanding the 0 to 36 hour period of the pharmacokinetic profile. Fig. 6C depicts percent change in body weight for each

individual animal over the 312 hour time course. For Figs. 6A-6B, B10 (**closed circle**); B12 (**closed square**). For Fig. 6C, open symbols and star represent B10 and closed symbols represent B12.

DETAILED DESCRIPTION OF THE INVENTION

The formulations of the present invention generate a non-lamellar liquid crystalline phase following administration. The use of non-lamellar phase structures (such as liquid crystalline phases) in the delivery of bioactive agents is now relatively well established. However, the present inventors have now established that the combination of particular lipid controlled-release compositions with certain peptide active agents serves to provide a still further and unexpected improvement in performance in one or more key criteria.

The present inventors have now established that by providing a pre-formulation comprising at least one diacyl glycerol, at least one phosphatidyl choline, at least one biocompatible, organic alcohol solvent, at least one active agent as described herein and optionally at least one antioxidant in a low viscosity phase, such as molecular solution, a pre-formulation may be generated addressing many of the shortfalls of known depot formulations. Such a formulation may be applied to provide a depot of the active agent in situ. In particular, the pre-formulation is easy to manufacture, may be sterile-filtered, has low viscosity (allowing easy and less painful administration typically through a narrow needle), allows a higher level of bioactive agent to be incorporated than has previously been demonstrated (thus potentially allowing a smaller amount of composition to be used), contains a highly effective bioactive agent (again reducing the necessary volume), requires shallow injection and/or forms a desired non-lamellar depot composition *in vivo* having a controllable "burst" or "non-burst" release profile. The compositions are also formed from materials that are non-toxic, biotolerable and biodegradable, which can be administered by i.m., or s.c. and are suitable for self-administration. Preferably once weekly administration.

Certain of the formulations of the present invention generate a non-lamellar liquid crystalline phase following administration. The use of non-lamellar phase structures (such as liquid crystalline phases) in the delivery of bioactive agents is now relatively well established. A most effective lipid depot system is described in

WO2005/117830, and a highly preferred lipid depot for use in the present invention is a development from that described in that document, the full disclosure of which is hereby incorporated herein by reference. For a description of the most favourable phase structures of such formulations, attention is drawn to the discussion in WO2005/117830 and particularly to page 29 thereof.

The present compositions have numerous advantages over polymer based formulations, such as those using PLGA microspheres.

Hybrid, dual agonist polypeptides have many advantages in terms of activity and may be delivered by systems consisting of microspheres of biodegradable polymers. However, such polymer microsphere formulations must generally be administered by means of a sizable needle, typically of 20-gauge or wider. This is necessary as a result of the nature of the polymeric dosing systems used, which are typically polymer suspensions. Similarly, such systems typically require suspension or multi-step preparation at the point of care prior to administration, making such administration relatively time-consuming and requiring a healthcare professional.

In comparison, the present formulations advantageously provide a system of low viscosity, such as a homogeneous solution, dispersion of fine particles, or L_2 phase, which could be administered easily through a narrow needle, thus decreasing the discomfort of the patient during the procedure. In the case of chronic conditions such as type II diabetes, particularly when obesity, overweight or a need for body weight reduction is a co-morbidity and complicating factor, this ease and comfort of administration is particularly significant because most patients will typically be on a self-administration regime. Providing a sustained formulation with a duration of a few days, but which is sufficiently complex to administer that it requires treatment by a healthcare professional will not be an advantage to all patients over twice-daily or daily self-administration, and is likely to be more costly. Providing a formulation which gives sufficiently long duration to justify a visit to a health professional for administration and/or a preparation which can be self-administered, and reducing preparation time of health-care professionals or patients prior to the actual administration are all important issues. The present formulations surprisingly provide these advantages by means of a sterile, ready-to-administer composition requiring little or no preparation and which can be provided in pre-filled administration devices suitable for use by heathcare professionals or patients.

The present formulations further allow for the formation of a depot in-situ by the administration of a suitable active agent, as described herein, in an administration system formed from low-toxicity, non-immunogenic, non-inflammatory lipids and biotolerable solvents. Not only does this formulation (in combination with low viscosity) provide minimal pain at the injection site but no irritant degradation products are generated as the active agent is released and thus very little build-up of inflammation or scar tissue is observed even following repeated administration (for example in treating a chronic condition such as diabetes).

From a drug delivery point of view, polymer depot compositions generally have the disadvantage of accepting only relatively low drug loads and having a "burst/lag" release profile. The nature of the polymeric matrix, especially when applied as a solution or pre-polymer, causes an initial burst of drug release when the composition is first administered. This is followed by a period of low release, while the degradation of the matrix begins, followed finally by an increase in the release rate to the desired sustained profile. This burst/lag release profile can cause the *in vivo* concentration of active agent to burst above the functional window immediately following administration, and then drop back through the bottom of the functional window during the lag period before reaching a sustained functional concentration for a period of time. Evidently, from a functional and toxicological point of view this burst/lag release profile is undesirable and could be dangerous. It may also limit the equilibrium concentration which can be provided due to the danger of adverse effects at the "peak" point. The presence of a lag phase may furthermore require supplementary dosing with repeat injections during the start-up period of depot treatment in order to maintain a therapeutic dose while the concentrations of active provided from the depot are sub-functional. For certain polypeptides in particular, it would be advantageous to minimise the immediate "burst" effect upon administration of a composition in order to avoid side effects such as hypoglycaemia.

The manufacture of PLGA microbeads and suspensions is additionally a considerable difficulty with certain existing depot systems. In particular, since the beads are particulate, and polymers clog membranes, they cannot generally be sterile-filtered and furthermore, since the PLGA copolymer melts at around 40°C,

they cannot be heat-treated for sterility. As a result, a complex manufacturing process must all be conducted under conditions of high sterility.

Further issues with biodegradable polymer microspheres include complex reconstitution prior to injection and limited storage stability, due both to aggregation and degradation of the delivery system and/or active. The present formulations surprisingly addresses these disadvantages by providing a formulation that is a low-viscosity fluid and can thus be readily sterile-filtered, thereby providing a straightforward method of sterile manufacture.

Another advantage of the compositions of the present invention over polymer formulations, such as PLGA spheres, is the low initial release ("non-burst profile") of active agent, as demonstrated herein. This may be defined such that the area under a plasma concentration against time the curve during an initial period (e.g. the first 6 hours) is less than a certain proportion of the area under the curve for the entire curve (measured or extrapolated from time 0 to infinity or from time 0 to the last sampling time point, such as day 7 (about 168 hours) for a once weekly injection or day 1 (about 24 hours) for a once daily injection, and so forth). This amount might be, for example, less than 15%, particularly less than 10% in the first 6 hours of a 7-day composition. For very low burst and highly preferred compositions, this may be less than 7% or less than 6% in the first 6 hours. The low burst behaviour of the current invention is demonstrated in figures 5A-C. In particular formulations B12 and B16 have exceptional low-burst performance and as such form particularly advantageous embodiments of the present invention. Furthermore, as can be seen from Table 8 below, the compositions of the invention have an AUC of less than 15% in the first 6 hours of a 7 day composition and five of the six compositions (those with chloride counter-ion) have a AUC of less than 11%. The two lowest-burst compositions which form highly preferred embodiments have an AUC of less than 6% in the first 6 hours of a 7-day formulation.

The data observed *in vivo* in rats and shown in Figure 5 is furthermore supported by corresponding result in dog studies as indicated in Example 13 and Figures 6A-6C below.

Furthermore, it is important to control the peak concentration (Cmax) of drug in the plasma to a level equal to or less than that tolerable to the subject, for example to

avoid side-effects such as flushing or severe nausea, while providing or achieving a therapeutically effective level over the desired period of release. Generally, the average concentration during the period of release before the next dose is administered, Cave, falls within the therapeutic range, maximal and minimum concentrations to achieve the desired treatment over time. Preferably the initial burst is not the Cmax of the release profile, as demonstrated for some of the formulations described herein.

Whether or not the initial burst is also the Cmax, preferably the Cmax/Cave ratio is less than 15, preferably less than or equal to 12, more preferably less than or equal to 10, even more preferably less than or equal to 5. Cmax is defined as is known in the art, as the peak or maximal plasma concentration observed during the period of release before the next dose is adminsitered and Cave is defined as the average plasma concentration during that period of release. Cave can be calculated by calculating the drug present in the plasma as area under the curve (AUC) over the selected period of time, generally the entire period of release before the administration of the next dose, and dividing by that period of time.

What has been found is that the initial burst and/or Cmax can be minimized by use of the present formulations, which minimizes initial dilution and/or diffusion of the active agent prior to transition to solid phase, as well as by their relatively rapid time to phase transition to solid, as discussed herein.

The formulations of the present invention are advantageous even to previously reported lipid formulations. Compared to previously reported lipid-based, slow-release composition described in WO2006/131730 for GLP-1, the present formulations provide a higher concentration of active agent that allows for the possibility of longer duration depot products, products maintaining a higher systemic concentration, and products having a smaller injection volume, all of which factors are of considerable advantage under appropriate circumstances.

Furthermore, the present formulations can have the advantage to provide a slow-release composition comprising a highly effective active agent which may show longer residence times *in vivo* and may furthermore provide more functional effect per unit mass of injected active, as well as providing dual receptor pharmacology to provide multiple biological effects with improved benefits to the patient as described

herein. This is demonstrated by the examples herein, in particular table 8, in which the bioavailability of pre-formulations according to the current invention is consistently shown to be higher than that provided by control subcutaneous injections. It is an aspect of the invention that the pre-formulations provided have increased bioavailability in comparison with direct subcutaneous administration of the active agent.

All % are specified by weight herein throughout, unless otherwise indicated. Furthermore, the % by weight indicated is the % of the total pre-formulation including all of the components indicated herein. The pre-formulations can optionally consist of essentially only the components indicated herein (including where appropriate additional optional components indicated herein below and in the attached claims) and in one aspect consist entirely of such components.

The lipid-based systems described herein comprise lipid components a) and b), plus organic solvent (c), aqueous solvent (d), dual amylin receptor/GLP-1 receptor agonist active agent e) and optional antioxidant (f) components.

Component a) - Diacyl Glycerol

Preferable ranges for component a) are 20-80 wt.%, preferably 25-70 wt.%, more preferably 28-60% (e.g. 30-55%), particularly 30-45% (e.g. 30-40%). Preferable ranges of component b) are 20-80 wt.%, preferably 25-70 wt.%, more preferably 28-60% (e.g. 30-55%), particularly 30-45% (e.g. 30 to 40%).

Ratios of a:b are typically 40:60 to 70:30, preferably 45:55 to 60:40 and more preferably 48:52 to 55:45. Ratios of around 50:50 are highly effective.

Component "a" as indicated herein is preferably at least one diacyl glycerol (DAG) and thus has two non-polar "tail" groups. The two non-polar groups may have the same or a differing number of carbon atoms and may each independently be saturated or unsaturated. Examples of non-polar groups include C_6-C_{32} alkyl and alkenyl groups, which are typically present as the esters of long chain carboxylic acids. These are often described by reference to the number of carbon atoms and the number of unsaturations in the carbon chain. Thus, CX:Z indicates a hydrocarbon chain having X carbon atoms and Z unsaturations. Examples particularly include

caproyl (C6:0), capryloyl (C8:0), capryl (C10:0), lauroyl (C12:0), myristoyl (C14:0), palmitoyl (C16:0), phytanoyl (C16:0), palmitoleoyl (C16:1), stearoyl (C18:0), oleoyl (C18:1), elaidoyl (C18:1), linoleoyl (C18:2), linolenoyl (C18:3), arachidonoyl (C20:4), behenoyl (C22:0) and lignoceroyl (C24:9) groups. Thus, typical non-polar chains are based on the fatty acids of natural ester lipids, including caproic, caprylic, capric, lauric, myristic, palmitic, phytanic, palmitolic, stearic, oleic, elaidic, linoleic, linolenic, arachidonic, behenic or lignoceric acids, or the corresponding alcohols. Preferable non-polar chains are palmitic, stearic, oleic and linoleic acids, particularly oleic acid.

Mixtures of any number of diacyl lipids may be used as component a). Preferably this component will include at least a portion of glycerol dioleate (GDO). A highly preferred example is DAG comprising at least 50%, preferably at least 80% and even comprising substantially 100% GDO.

Since GDO and other diacyl glycerols are products derived from natural sources, there is generally a certain proportion of "contaminant" lipid having other chain lengths etc. In one aspect, GDO as used herein is thus used to indicate any commercial grade of GDO with concomitant impurities (i.e. GDO of commercial purity). These impurities may be separated and removed by purification but providing the grade is consistent this is rarely necessary. If necessary, however, "GDO" may be essentially chemically pure GDO, such as at least 80% pure, preferably at least 85% pure and more preferably at least 90% pure GDO.

Component b) - Phosphatidyl Choline

Component "b" in the preferred lipid matrices of the present invention is at least one phosphatidyl choline (PC). As with component a), this component comprises a polar head group and at least one non-polar tail group. The difference between components a) and b) lies principally in the polar group. The non-polar portions may thus suitably be derived from the fatty acids or corresponding alcohols considered above for component a. As with component a), the PC will contain two non-polar groups.

The phosphatidyl choline portion, even more suitably than any diacyl glycerol portion, may be derived from a natural source. Suitable sources of phospholipids

include egg and plant sources including soybean. Such sources may provide one or more constituents of component b, which may comprise any mixture of phospholipids. Any single PC or mixture of PCs from these or other sources may be used, but mixtures comprising soy PC or egg PC are highly suitable. The PC component preferably contains at least 50% soy PC or egg PC, more preferably at least 75% soy PC or egg PC and most preferably essentially pure soy PC or egg PC.

In an alternative but equally preferred embodiment, the PC component may comprise synthetic PC, such as synthetic dioleoyl PC (DOPC). This may provide increased stability and so will be particularly preferable for compositions needing to be stable to long term storage, and/or having a long release period *in vivo*. In this embodiment the PC component preferably contains at least 50% synthetic dioleoyl PC, more preferably at least 75% synthetic dioleoyl PC and most preferably essentially pure synthetic dioleoyl PC. Any remaining PC is preferably soy PC as above. The synthetic dioleoyl PC is most preferably 1,2-dioleoyl-sn-glycero-3-phosphocholine, and other synthetic PC components include DDPC (1,2-Didecanoyl-sn-glycero-3-phosphocholine); DEPC(1,2-Dierucoyl-sn-glycero-3-phosphocholine); DLOPC(1,2-Dilinoleoyl-sn-glycero-3-phosphocholine); DLPC(1,2-Dilauroyl-sn-glycero-3-phosphocholine); DMPC(1,2-Dimyristoyl-sn-glycero-3-phosphocholine); DOPC(1,2-Dioleoyl-sn-glycero-3-phosphocholine); DPPC(1,2-Dipalmitoyl-sn-glycero-3-phosphocholine); DSPC(1,2-Distearoyl-sn-glycero-3-phosphocholine); MPPC(1-Myristoyl-2-palmitoyl-sn-glycero 3-phosphocholine); MSPC(1-Myristoyl-2-stearoyl-sn-glycero-3–phosphocholine); PMPC(1-Palmitoyl-2-myristoyl-sn-glycero-3–phosphocholine); POPC(1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine); PSPC(1-Palmitoyl-2-stearoyl-sn-glycero-3–phosphocholine); SMPC(1-Stearoyl-2-myristoyl-sn-glycero-3–phosphocholine); SOPC(1-Stearoyl-2-oleoyl-sn-glycero-3-phosphocholine); and SPPC(1-Stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine), or any combination thereof.

Since the pre-formulations of the invention are to be administered to a subject for the controlled release of a hybrid polypeptide active agent, it is important that the components are biocompatible. In this regard, the preferred lipid matrices for use in the pre-formulations of the present invention are highly advantageous since both PC and DAGs are well tolerated and are broken down *in vivo* into components that are naturally present in the mammalian body.

A particularly favoured combination of components a) and b) are GDO with PC, especially GDO with soy PC. Appropriate amounts of each component suitable for the combination are those amounts indicated herein for the individual components in any combination. This applies also to any combinations of components indicated herein, where context allows.

Component c) - organic alcoholic solvent

Component c) of the pre-formulations of the invention is an organic alcoholic solvent. Since the pre-formulation is to generate a depot composition following administration (e.g. *in vivo),* typically upon contact with excess aqueous fluid, it is desirable that this solvent be tolerable to the subject and be capable of mixing with the aqueous fluid, and/or diffusing or dissolving out of the pre-formulation into the aqueous fluid. Solvents having at least moderate water solubility are thus preferred.

Most preferably component c) comprises or consists of ethanol or propylene glycol or mixtures thereof.

In a preferred version, the solvent is such that a relatively small addition to a mixture comprising a) and b) (i.e. preferably below 25%) gives large viscosity reductions, of one order of magnitude or more. As described herein, the addition of 25%, 20%, 15% or even 10% solvent can give a reduction of two or more orders of magnitude in viscosity over the solvent-free composition, or over a depot containing a viscosity-increasing solvent such as glycerol.

The amount of solvent component c) in the pre-formulation will have a considerable effect upon several features. In particular, the solubility of the active substance, viscosity and the rate (and duration) of release may alter significantly with the solvent level. The amount of solvent will thus be at least sufficient to provide the required solubility of the drug substance, a low viscosity mixture but will additionally be determined so as to provide the desired release rate. This may be determined by routine methods in view of the Examples below. Typically a level of 0.1 to 35%, particularly 5 to 35% solvent will provide suitable release and viscosity properties, as will 5 to 25%. This will preferably be 8 to 28% (e.g. 10 to 25%) and a total amount of around 15%, or 20% or even 25% is highly effective, whether one organic solvent alone or a combination of two or more than two organic solvents.

As indicated above, the amount of component c) in the pre-formulations of the invention will be at least sufficient to provide the required drug load in solution, a low viscosity mixture (e.g. a molecular solution, see above) of components a), b), c) and d) and optionally f) and will be easily determined for any particular combination of components by standard methods.

It has surprisingly been established that the solubility of the active agents described herein is significantly affected by the presence of water and/or propylene glycol in the pre-formulations that relate to all aspects of the invention. Thus, in one embodiment, at least a portion of the solvent component c) comprises propylene glycol (PG). PG may be present, for example, in an amount of 1 to 15% by weight, preferably 2 to 15% (e.g. 10 to 15%). The amount of PG may be selected to achieve an appropriate level of solubility in combination with the water content, which also increases active agent solubilisation, as discussed below. In any event, the amount of PG will be consistent the total amount of c) as described above and herein. When PG is absent, another organic alcoholic solvent is present to provide viscosity reduction benefits, and optionally water may be increased as needed

The phase behaviour may be analysed by techniques such as visual observation in combination with polarized light microscopy, nuclear magnetic resonance, and cryo-transmission electron microscopy (cryo-TEM) to look for solutions, L_2 or L_3 phases, or liquid crystalline phases or as in the case of cryoTEM, dispersed fragments of such phases. Viscosity may be measured directly by standard means. As described above, an appropriate practical viscosity is that which can effectively be syringed and particularly sterile filtered. This will be assessed easily as indicated herein.

Typical solvents suitable for use in the invention include at least one solvent selected from mono-ols, diols and triols. Examples of suitable alcohols include ethanol, propylene glycol, isopropanol, benzyl alcohol and glycerol formal, particularly ethanol. Monools are preferred to diols and polyols. Most preferred is a mixture of at least one diol and at least one mono-ol. Ethanol and/or propylene glycol are most preferred.

A highly preferred combination for the lipid matrix aspect is PC, GDO and ethanol/propylene glycol or mixtures thereof. As indicated above, appropriate

amounts of each component suitable for the combination are those amounts indicated herein for the individual components, in any combination.

It is preferable that little or none of component c) contains halogen substituted hydrocarbons since these tend to have lower biocompatibility. Where a portion of halogenated solvent such as dichloromethane or chloroform is necessary, this proportion will generally be minimised.

Component c) as used herein may be a single solvent or a mixture of suitable solvents but will generally be of low viscosity. This is important because one of the key aspects of the present invention is that it provides pre-formulations that are of low viscosity and one of the primary roles of a suitable solvent is to reduce this viscosity. This reduction will be a combination of the effect of the lower viscosity of the solvent and the effect of the molecular interactions between solvent and lipid composition. One observation of the present inventors is that the oxygen-containing solvents of low viscosity described herein have highly advantageous and unexpected molecular interactions with the lipid parts of the composition, thereby providing a non-linear reduction in viscosity with the addition of a small volume of solvent.

Surprisingly, some embodiments preferably contain propylene glycol and further surprisingly can contain PG and substantial water. In contrast WO2006/131730, which discloses lipid-based, slow-release compositions containing GLP-1, reported that the formulations should contain glycerol, ethylene glycol or propylene glycol and contain no more than a trace of water. There have not previously been proposed depot compositions based upon lipid precursors having substantial amounts of water (e.g. greater than 2% water) or showing the benefits attributable to this water content as described herein.

Component d) - Aqueous solvent

The pre-formulations of the present invention must also contain an aqueous solvent, component d). A suitable amount will typically be greater than 1% by weight of the pre-formulation, for example 1-15 wt.%, particularly 1.2-15 wt.%, especially 2 to

15%, and even more as 2-13 wt.%. Component d) is preferably water, such as purified water for injection. In one preferred aspect, the pre-formulations of the invention contain ethanol and/or propylene glycol as component c) with water as component d). Correspondingly, in one embodiment, aqueous component d) does not include any organic solvent (that solvent being present in component c).

It has been surprisingly established that the presence of a certain amount of water in the pre-formulations increases the solubility of the dual agonist peptide active agent (as described herein). This function is also served to some extent by the propylene glycol component and thus the amount of water necessary may be reduced when greater amounts of PG are employed. Thus, in one embodiment, the total concentration of water and PG in the formulations may be in the range of 8 to 20% by weight, with 10 to 18%, and 10 to 17% being preferred. This is believed to provide a loading of active agent that may be 2% or more.

A highly preferred combination for the lipid matrix aspect is soy PC and/or DOPC, GDO, ethanol/propylene glycol or mixtures thereof, and water. As indicated above, appropriate amounts of each component suitable for the combination are those amounts indicated herein for the individual components, in any combination.

Component e) - Dual Amylin Receptor/GLP-1 Receptor Agonist Active Agent

The formulations of the present invention comprise as an active agent one or more dual amylin receptor/GLP-1 receptor agonist compounds--compounds having a component that is an amylin receptor agonist linked to a component that is a GLP-1 receptor agonist. The GLP-1 receptor agonist component refers to a compound that has GLP-1 receptor binding and activation activity and elicits a biological activity of an exendin reference peptide (e.g. exendin-4) or a GLP-1(7-37) reference peptide when evaluated by art-known measures including receptor binding studies, in vitro cAMP generation or insulin secretion, or in vivo blood glucose control, insulin secretion, delay of gastric emptying and/or glucagon suppression assays as known in the art. See for example, Hargrove et al, Regulatory Peptides, 141:113-119 (2007).

GLP-1 receptor agonist compounds include, for example, native exendins and exendin analogues and native GLP-1, for example GLP-1(7-37) and GLP-1 analogues. The amylin receptor agonist component refers to a compound that has amylin receptor binding and activation and elicits a biological activity of an amylin reference peptide (preferably rat amylin or davalintide), when evaluated by art-known measures including receptor binding, amylin agonist activity in soleus muscle assays, in vivo blood glucose control, body weight loss and gastric emptying delay as known in the art. See for example Mack et al., Int. J. Obesity 34:385–395 (2009). Amylin receptor agonist compounds include, for example, native amylins, e.g. human and rat amylins, and amylin analogues, e.g. pramlintide and davalintide.

The dual amylin receptor/GLP-1 receptor agonist polypeptide comprises an amylin or amylin analogue, including davalintide and pramlintide, conjugated or fused either to an exendin or exendin analogue or to a GLP-1 or GLP-1 analogue. The two peptides can be conjugated or fused directly or through a linker. Either or both peptides can be conjugated to the other through a side-chain of an internal or terminal amino acid or through the peptide's backbone, i.e. via the terminal alpha amino or carboxy groups. In a preferred embodiment of the dual amylin receptor/GLP-1 receptor agonist polypeptide, the N-terminal amino acid of the amylin or amylin analogue, e.g. davalintide, is conjugated or fused at the C-terminal amino acid of the exendin or exendin analogue or to the GLP-1 or GLP-1 analogue. More preferably the linkage is via the N-terminal alpha amino group of the amylin or amylin analogue and the C-terminal alpha carboxy group of the exendin or exendin analogue or the GLP-1 or GLP-1 analogue. In one such embodiment a peptide linker fuses the two peptides creating a fusion polypeptide that can be recombinantly produced. Exendin-4 and exendin-4 analogues are preferred as the GLP-1 receptor agonist. Exendin analogues having a leucine substitution for methionine at position 14 are even more preferred. The most preferred dual amylin receptor/GLP-1 receptor agonist polypeptide is a fusion polypeptide comprising the C-terminally amidated polypeptide having the sequence HGEGTFTSDLSKQLEEEAVRLFIEWLKNGGPSSGAPPPSGGGKCNTATCVLG RLSQELHRLQTYPRTNTGSNTY-NH2 (herein designated **Cmpd 1**). The

polypeptide is a fusion of davalintide covalently attached inframe through its peptide backbone to the peptide backbone of Leu(14) exendin-4 via a glycine-glycine-glycine peptide linker. Less preferred is its C-terminal free acid form. Another dual receptor agonist is a fusion polypeptide comprising C-terminally amidated polypeptide having the sequence HGEGTFTSDLSKQLEEEAVRLFIEWLKQGGPSKEIISGGGKCNTATCVLGRLS QELHRLQTYPRTNTGSNTY-NH2 (**Cmpd 2**). The polypeptide is a fusion of davalintide covalently attached in-frame to HGEGTFTSDLSKQLEEEAVRLFIEWLKQGGPSKEIIS through a glycine-glycine-glycine peptide linker. Also suitable but less preferred is its C-terminal free acid form. **Cmpd 5** is the amide form of the preceding exendin analog: HGEGTFTSDLSKQLEEEAVRLFIEWLKQGGPSKEIIS-NH2

GLP-1 Receptor Agonist Component—GLP-1 and GLP-1 Analogues.

By "GLP-1" is meant a GLP-1 receptor agonist that is a naturally-occurring form of glucagon like peptide-1, either human or from any other species. A straightforward system is used to describe fragments and analogues of GLP-1, which is based on full-length GLP-1: GLP-1(1-37). For example, Arg34-GLP-1(7-37) designates an analogue of GLP-1 formally derived from full-length GLP-1 by deleting the amino acid residues Nos. 1 to 6 and substituting the naturally occurring amino acid residue in position 34 (Lys) by Arg. The following are sequences two equipotent naturally-occurring forms. Native human GLP-1(7-37) has the sequence His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly. Native human GLP-1(7-36)amide has the sequence His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-NH2. Also included are their C-terminal amide and acid forms, and physiologically acceptable salts, esters and derivatives.

By “GLP-1 analogue" as used herein is meant a GLP-1 receptor agonist that has at least 70% sequence identity to naturally occurring forms of GLP-1, either human or from any other species, and is derived from GLP-1 by modifications including

insertions, substitutions, extensions, and/or deletions of the reference GLP-1 amino acid sequence. More preferably the analogue sequence has at least 75%, 80%, 90%, or 95% amino acid sequence identity with the reference GLP-1 peptide. In one aspect the analogue has no more than 10, 6, 5, 4, 3, 2, and/or 1 insertions, substitutions, extensions, and/or deletions relative to the reference compound. An extension at the C-terminal end can be all or part of the exendin-4 "tail" (i.e. PSSGAPPPS) or frog GLP-1 "tail" (PSKEIIS or PKKIRYS) or can be positively charged amino acids, e.g. Lys, Arg, of 1 to 10 amino acids long; such exemplary extensions include KNGGPSSGAPPPS, PSSGAPPPS, FIEWLKNGGPSSGAPPPS, PKKIRYS, KKKKKK, and their analogs. In one embodiment, the GLP-1 analogue may comprise conservative or non-conservative amino acid substitutions (including non-natural amino acids and L and D forms). These analogues are preferably peptides, peptide derivatives or less preferably peptide mimics. Since GLP-1 is a peptide hormone, typical GLP-1 analogues will be peptides, especially of around 30 amino acids, e.g. 27 to 45, especially 28 to 38. Peptide derived GLP-1 agonists are most preferred, such as those indicated herein, especially Val8-GLP-1(7-37), Val8-GLP-1(7-36)amide, Aib8-GLP1(7-37), Aib8-GLP-1(7-37) and liraglutide. Other GLP-1 analogues include 9Gln-GLP-1(7-37) , D-9Gln GLP-1(7-37), 16Thr-18 Lys GLP-1(7-37), 18Lys-GLP-1(7-37), 8Gly-GLP-1 (7-36), 9Gln-GLP-1 (7-37), D-9Gln-GLP-1 (7-37), acetyl-9Lys-GLP-1(7-37), 9Thr-GLP-1(7-37), D-9Thr-GLP-1 (7-37), 9Asn-GLP-1 (7-37), D-^{9}Asn-GLP-1 (7-37), 22Ser23Arg24Arg26Gln-GLP-1(7-37), 16Thr18Lys-GLP-1(7-37), 18Lys-GLP-1(7-37), 23Arg-GLP-1(7-37), and 24Arg-GLP-1(7-37). Also included are their C-terminal amide and acid forms, and physiologically acceptable salts, esters and derivatives. An analogue may have superior chemical stability, physical stability, protease resistance (e.g. DPPIV resistance), solubility, efficacy, half-life, and the like.

GLP-1 Receptor Agonist Component—Exendin and Exendin Analogues. By "exendin" is meant the sequences of naturally-occurring exendin peptides, exendin-3 and exendin-4, that are found in the salivary secretions of the Gila monster. Exendins are not the species homolog of mammalian GLP-1 (Chen & Drucker *J. Biol. Chem.* **272**:4108-15 (1997)); the Gila monster has separate genes

for proglucagons (from which GLP-1 is processed). Exenatide (exendin-4) is a 39 amino acid GLP-1 receptor agonist currently indicated for the treatment of type 2 diabetes, and also exerts glucose lowering, glucagon suppression, delay of gastric emptying, body weight loss and other metabolic actions. The sequence of exendin-4 is HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2, where –NH2 indicates the presence of an amidated C-terminal alpha-carboxy amino acid. Exendin-4 is also active in its free acid form. Exendin-3 is identical to exendin-4 but for Ser and Asp at positions 2 and 3 respectively. Also included are C-terminal amide and acid forms, and physiologically acceptable salts, esters and derivatives.

By "exendin analogue" as used herein is meant a GLP-1 receptor agonist that has at least 70% sequence identity to naturally occurring forms of exendin, either exendin-3 or exendin-4, and is derived from the exendin by modifications including insertions, substitutions, extensions, and/or deletions of the reference exendin amino acid sequence. More preferably the analogue sequence has at least 75%, 80%, 90%, or 95% amino acid sequence identity with the reference exendin peptide. In one aspect the analogue has no more than 10, 6, 5, 4, 3, 2, and/or 1 insertions, substitutions, extensions, and/or deletions relative to the reference compound. An extension at the C-terminal end can be all or part of the exendin-4 "tail" (i.e. PSSGAPPPS) or frog GLP-1 "tail" (PSKEIIS or PKKIRYS) or can be positively charged amino acids, e.g. Lys, Arg, of 1 to 10 amino acids long; such exemplary extensions include KNGGPSSGAPPPS, PSSGAPPPS, FIEWLKNGGPSSGAPPPS, PKKIRYS, KKKKKK, and their analogs. In one embodiment, such exendin analogues may comprise conservative or non-conservative amino acid substitutions (including non-natural amino acids and L and D forms). These analogues are preferably peptides, peptide derivatives or less preferably peptide mimics. Since exendin-4 is a peptide, typical exendin analogues will be peptides, especially of around 39 amino acids, e.g. 27 to 45, especially 28 to 39. Peptide derived GLP-1 receptor agonists are most preferred, such as those indicated herein, especially ^{14}Leu-exendin-4, despite Hargrove et al. having reported that this exendin-4 analogue is remarkably inferior to exendin-4 with respect to its delaying of gastric emptying, anti-obesity properties and half-life. As part of a dual amylin

receptor/GLP-1 receptor agonist, this analogue is surprisingly effective in its exendin activities. Additional analogues include a chimera of the first 32 amino acids of exendin-4 having amino acid substitutions at positions 14 and 28 followed by a 5 amino acid sequence from the C-terminus of a non-mammalian (frog) GLP1, with the HGEGTFTSDLSKQLEEEAVRLFIEWLKQGGPSKEIIS, truncated biologically active forms of exendin-4 including exendin-4(1-27), exendin-4(1-28), exendin-4(1-29), exendin-4(1-30), exendin-4(1-28), ^{14}Leu,^{25}Phe-exendin-4(1-28), ^{5}Ala,^{14}Leu,^{25}Phe-exendin-4(1-28), exendin(7-15) and its Ser2 analog, HSEGTFTSD, ^{14}Leu,^{25}Phe-exendin-4, ^{5}Ala,^{14}Leu,^{25}Phe-exendin-4, desPro38 exendin-4, and AVE-010 (also known as ZP10) having the sequence His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Ser-Lys-Lys-Lys-Lys-Lys-Lys. Further analogues described below. Also included are their C-terminal amide and acid forms, and physiologically acceptable salts, esters and derivatives. An analogue may have superior chemical stability, physical stability, protease resistance (e.g. DPPIV resistance), solubility, efficacy, half-life, and the like.

Exendin analogues include compounds comprising the structure of Formula (I) following:

Xaa_1 Xaa_2 Xaa_3 Gly Thr Xaa_6 Xaa_7 Xaa_8 Xaa_9 Xaa_{10}
Ser Lys Gln Xaa_{14} Glu Glu Glu Ala Val Arg Leu
Xaa_{22} Xaa_{23} Xaa_{24} Xaa_{25} Leu Lys Asn Gly Gly Xaa_{31}
Ser Ser Gly Ala Xaa_{36} Xaa_{37} Xaa_{38} Xaa_{39}

wherein Xaa_1 is His, Arg or Tyr; Xaa_2 is Ser, Gly, Ala or Thr; Xaa_3 is Asp or Glu; Xaa_6 is Phe, Tyr or naphthylalanine; Xaa_7 is Thr or Ser; Xaa_8 is Ser or Thr; Xaa_9 is Asp or Glu; Xaa_{10} is Leu, Ile, Val, pentylglycine or Met; Xaa_{14} is Leu, Ile, pentylglycine, Val or Met; Xaa_{22} is Phe, Tyr or naphthylalanine; Xaa_{23} is Ile, Val, Leu, pentylglycine, tert-butylglycine or Met; Xaa_{24} is Glu or Asp; Xaa_{25} is Trp, Phe, Tyr, or naphthylalanine; Xaa_{31}, Xaa_{36}, Xaa_{37} and Xaa_{38} are independently Pro, homoproline, 3Hyp, 4Hyp, thioproline, N-alkylglycine, N-alkylpentylglycine or N-alkylalanine or absent; and Xaa_{39} is Ser, Thr or Tyr or absent. Optionally, the C-

terminus of the peptide is amidated, or is attached to the linker or directly to the amylin receptor agonist. Exendin analogues also include those described in U.S. Patent No. 7,223,725 (incorporated herein by reference and for all purposes), such as compounds of Formula (II) following:

Xaa_1 Xaa_2 Xaa_3 Gly Xaa_5 Xaa_6 Xaa_7 Xaa_8 Xaa_9 Xaa_{10} Xaa_{11} Xaa_{12} Xaa_{13} Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17} Ala Xaa_{19} Xaa_{20} Xaa_{21} Xaa_{22} Xaa_{23} Xaa_{24} Xaa_{25} Xaa_{26} Xaa_{27} Xaa_{28}

In Formula (II), Xaa_1 is His, Arg or Tyr; Xaa_2 is Ser, Gly, Ala or Thr; Xaa_3 is Ala, Asp or Glu; Xaa_5 is Ala or Thr; Xaa_6 is Ala, Phe, Tyr; Xaa_7 is Thr or Ser; Xaa_8 is Ala, Ser or Thr; Xaa_9 is Asp or Glu; Xaa_{10} is Ala, Leu, Ile, Val, or Met; Xaa_{11} is Ala or Ser; Xaa_{12} is Ala or Lys; Xaa_{13} is Ala or Gln; Xaa_{14} is Ala, Leu, Ile, , Val or Met; Xaa_{15} is Ala or Glu; Xaa_{16} is Ala or Glu; Xaa_{17} is Ala or Glu; Xaa_{19} is Ala or Val; Xaa_{20} is Ala or Arg; Xaa_{21} is Ala or Leu; Xaa_{22} is Ala, Phe, Tyr; Xaa_{23} is Ile, Val, Leu, or Met; Xaa_{24} is Ala, Glu or Asp; Xaa_{25} is Ala, Trp, Phe, Tyr; Xaa_{26} is Ala or Leu; Xaa_{27} is Ala or Lys; Xaa_{28} is Ala or Asn. In some embodiments of Formula (II), the C-terminus of the peptide is modified by $-Z_1$ is -OH or $-NH_2$, or is attached to the linker or directly to the amylin receptor agonist, or the C-terminus of the peptide further includes Gly-Z_2, Gly Gly-Z_2, Gly Gly Xaa_{31}-Z_2, Gly Gly Xaa_{31} Ser-Z_2, Gly Gly Xaa_{31} Ser Ser-Z_2, Gly Gly Xaa_{31} Ser Ser Gly-Z_2, Gly Gly Xaa_{31} Ser Ser Gly Ala-Z_2, Gly Gly Xaa_{31} Ser Ser Gly Ala Xaa_{36}-Z_2, Gly Gly Xaa_{31} Ser Ser Gly Ala Xaa_{36} Xaa_{37}-Z_2 or Gly Gly Xaa_{31} Ser Ser Gly Ala Xaa_{36} Xaa_{37} Xaa_{38}-Z_2; Xaa_{31}, Xaa_{36}, Xaa_{37} and Xaa_{38} are independently Pro or are absent; and the C-terminus of the peptide is modified by $-Z_2$, wherein $-Z_2$ is -OH or $-NH_2$ or is attached to the linker or directly to the amylin receptor agonist. In any and each of the exendin analogues described herein and above, specifically contemplated are those wherein a replacement for the histidine corresponding to Xaa1 is made with any of D-histidine, desamino-histidine, 2-amino-histidine, beta-hydroxy-histidine, homohistidine. N-alpha-acetyl-histidine, alpha-fluoromethyl-histidine, alpha-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine. Further specifically contemplated herein are exendin analogues described herein and above wherein a replacement for the glycine at Xaa2 is made with any of D-Ala, Val, Leu, Lys, Aib (aminoisobutyric acid), (1-amino cyclopropyl)carboxylic acid, (1-

aminocyclobutyl)carboxylic acid, l-aminocyclopentyl)carboxylic acid, (1-aminocyclohexyl)carboxylic acid, (1-aminocycloheptyl)carboxylic acid, or (1-amino cyclooctyl)carboxylic acid.

Additional examples of exendin analogues include those described in U.S. Patent 6528486 (incorporated herein by reference and for all purposes). Specifically, exendin analogues include those consisting of an exendin or exendin analogue having at least 90% homology to exendin-4 having optionally between one and five deletions at positions 34-39, and a C-terminal extension of a peptide sequence of 4-20 amino acid units covalently bound to said exendin wherein each amino acid unit in said peptide extension sequence is selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Asn, Gln, Asp, Glu, Lys, Arg, His, and Met. More preferably the extension is a peptide sequence of 4-20 amino acid residues, e.g., in the range of 4-15, more preferably in the range of 4-10 in particular in the range of 4-7 amino acid residues, e.g., of 4, 5, 6, 7, 8 or 10 amino acid residues, where 6 amino acid residues are preferred. Most preferably, according to U.S. Patent 6528486 the extension peptide contains at least one Lys residue, and is even more preferably from 3 to 7 lysines and even most preferably 6 lysines. For example, one analogue is HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPP SKKKKKK (also designated ([des-^{36}Pro]exendin-4(1-39)-Lys_6 or lixisenatide). Additional exemplary analogues include Lys_6-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Ser-$(Lys)_6$ (H-Lys_6-des Pro ^{36}exendin-4(1-39)-Lys_6); His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Ser (H-[des ^{36}Pro, ^{37,38}Pro]exendin-4(1-39)-NH_2); Lys-Lys-Lys-Lys-Lys-Lys-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Ser (H-$(Lys)_6$-[des ^{36}Pro, ^{37,38}Pro]exendin-4(1-39); Asn-Glu-Glu-Glu-Glu-Glu-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Ser (H-Asn-$(Glu)_5$-[des ^{36}Pro, ^{37,38}Pro]exendin-4(1-39); His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-

Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Ser-$(Lys)_6$ ([des ^{36}Pro, ^{37,38}Pro]exendin-4(1-39)-$(Lys)_6$); $(Lys)_6$-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Ser-$(Lys)_6$ (H-$(Lys)_6$-[des ^{36}Pro, ^{37,38}Pro]exendin-4(1-39)-$(Lys)_6$); and Asp-Glu-Glu-Glu-Glu-Glu-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Ser-$(Lys)_6$ (Asn-$(Glu)_5$-[des ^{36}Pro, ^{37,38}Pro]exendin-4(1-39)-$(Lys)_6$). As customary in the art, repetition of an amino acid can be indicated by a subscripted number setting forth the number of repetitions; i.e., Lys_6, $(Lys)_6$ and the like refer to hexalysyl. In any and each of the exendin analogues described above, specifically contemplated are those wherein a replacement for the histidine corresponding to position 1 is made with any of D-histidine, desamino-histidine, 2-amino-histidine, beta-hydroxy-histidine, homohistidine. N-alpha-acetyl-histidine, alpha-fluoromethyl-histidine, alpha-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine. Further specifically contemplated herein are exendin analogues described herein wherein a replacement for the glycine at position 2 is made with any of D-Ala, Val, Leu, Lys, Aib, (1-aminocyclopropyl)carboxylic acid, (1-aminocyclobutyl)carboxylic acid, l-aminocyclopentyl)carboxylic acid, (1-amino cyclohexyl)carboxylic acid, (1-aminocycloheptyl)carboxylic acid, or (1-aminocyclooctyl) carboxylic acid.

Further examples of exendin analogues suitable for use in the methods described herein are described in published PCT application WO2004035623 (incorporated herein by reference and for all purposes), particularly those comprised of naturally-occurring amino acids, which describes exendin analogues having at least one modified amino acid residue particularly at positions ^{13}Gln, ^{14}Met, ^{25}Trp or ^{28}Asn with reference to the corresponding positions of exendin-4(1-39). According to that publication are additional such analogues further comprising a 1 to 7 amino acid C-terminal extension that comprises at least one Lys amino acid and more preferably at least five Lys amino acid units such as six or seven Lys amino acid units.

In any and each of the exendin analogues and formulas described herein and above, specifically contemplated are those wherein a replacement for the histidine corresponding to position 1 is made with any of L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, beta-hydroxy-histidine, homohistidine. N-alpha-acetyl-histidine, alpha-fluoromethyl-histidine, alpha-methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine. Further specifically contemplated herein are exendin analogues described herein wherein a replacement for the glycine at position 2 is made with any of D-Ala, Val, Leu, Lys, Aib, (1-aminocyclopropyl)carboxylic acid, (1-aminocyclobutyl)carboxylic acid, l-aminocyclopentyl)carboxylic acid, (1-amino cyclohexyl)carboxylic acid, (1-aminocycloheptyl)carboxylic acid, or (1-aminocyclooctyl) carboxylic acid.

Amylin Receptor Agonist Component.

Amylin is a peptide hormone co-secreted with insulin by pancreatic beta-cells after nutrient ingestion whose primary physiological roles involve the inhibition of feeding behavior and gastric emptying, and subsequently reduced body weight, as well as lowering meal-related blood glucose levels. Amylin (also referred to islet (or insulinoma) amyloid polypeptide) is a 37-residue, amidated peptide hormone. Secreted from pancreatic beta cells in response to meals, its overall effect is to slow the rate of appearance (Ra) from the meal, which is mediated via a coordinate reduction of food intake, slowing of gastric emptying, inhibition of digestive secretion [gastric acid, pancreatic enzymes, and bile ejection]. Appearance of new glucose is slowed by inhibiting secretion of the gluconeogenic hormone glucagon. These actions, which are mostly mediated via a glucose-sensitive part of the brain stem, the area postrema, may be over-ridden during hypoglycemia. They collectively reduce the total insulin demand. Amylin also acts in bone metabolism, along with the related peptides calcitonin and calcitonin gene related peptide.

The human form of IAPP has the amino acid sequence KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH2, with a disulfide bridge between cysteine residues 2 and 7. Both the amidated C-terminus and the disulfide bridge are necessary for the full biological activity of amylin.

A synthetic analog of human amylin with proline substitutions in positions 25, 26 and 29, or pramlintide, was recently approved for adult use in patients with both diabetes mellitus type 1 and diabetes mellitus type 2. Insulin and pramlintide, injected separately but both before a meal, work together to control the post-prandial glucose excursion. Amylin compounds, including rat amylin and pramlintide and davalintide are reported to reduce body weight in animals and/or humans, and thus have been proposed for treating obesity and obesity-related disorders.

By "amylin" is meant an amylin receptor agonist that is a naturally-occurring form of amylin, either rat, human or from any other species. The sequence of amylin is highly preserved across mammalian species, with structural similarities to calcitonin. The sequence of rat amylin (amidated) is KCNTATCATQRLANFLVRSSNNLGPVLPPTNVGSNTY-NH2 and that of human amylin (amidated) is KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH2. Also included are their C-terminal amide and acid forms, and physiologically acceptable salts, esters and derivatives.

By “amylin analogue" as used herein is meant an amylin receptor agonist that has at least 50% sequence identity, preferably at least 70% sequence identity, to a naturally-occurring form of amylin, either rat or human or from any other species, or to davalintide, and is derived from them by modifications including insertions, substitutions, extensions, and/or deletions of the reference amino acid sequence. Davalintide is an amylin agonist peptide chimera of amylin and sCT, having the sequence KCNTATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2 (**Cmpd 6**). The amylin analogue sequence can have at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, or 95% amino acid sequence identity with the reference amylin or davalintide. In one aspect the analogue has 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or even 16 amino acid substitutions, insertions, extensions, and/or deletions relative to the reference compound. In one embodiment, the amylin analogue may comprise conservative or non-conservative amino acid substitutions (including non-

natural amino acids and L and D forms). These analogues are preferably peptides, peptide derivatives or less preferably peptide mimics. Since amylin and davalintide are peptides, typical amylin analogues will be peptides, especially of 32-37 amino acids, e.g. 27 to 45, especially 28 to 38, and even 31-36.

Amylin analogues with identity to rat and human amylin include ^{25,28,29}Pro-h-amylin (pramlintide); des-^{1}Lys-h-amylin; ^{25}Pro,^{26}Val,^{28,29}Pro-h-amylin; ^{18}Arg,^{25,28}Pro-h-amylin; des-^{1}Lys,^{18}Arg,^{25,28}Pro-h amylin; ^{18}Arg,^{25,28,29}Pro-h-amylin; des-^{1}Lys,^{18}Arg,^{25,28,29}Pro-h-amylin; des-^{1},Lys^{25,28,29}Pro-h-amylin; ^{25}Pro,^{26}Val,^{28,29}Pro-h-amylin; ^{28}Pro-h-amylin, 2,7-Cyclo-[^{2}Asp,^{7}Lys]-h-amylin; $^{2\text{-}37}$h-amylin; ^{1}Ala-h-amylin; ^{2}Ala-h-amylin; ^{2,7}Ala-h-amylin; ^{1}Ser-h-amylin; ^{29}Pro-h-amylin; ^{25,28}Pro-h-amylin; des-^{1}Lys,^{25,28}Pro-h-amylin; ^{23}Leu,^{25}Pro,^{26}Val,^{28,29}Pro-h-amylin; ^{23}Leu^{25}Pro^{26}Val^{28}Pro-h-amylin; des-^{1}Lys,^{23}Leu,^{25}Pro,^{26}Val,^{28}Pro-h-amylin; ^{18}Arg,^{23}Leu,^{25}Pro,^{26}Val,^{28}Pro-h-amylin; ^{18}Arg,^{23}Leu,^{25,28,29}Pro-h-amylin; ^{18}Arg^{23}Leu,^{25,28}Pro-h-amylin; ^{17}Ile,^{23}Leu,^{25,28,29}Pro-h-amylin; ^{17}Ile,^{25,28,29}Pro-h-amylin; des-^{1}Lys,^{17}Ile,^{23}Leu,^{25,28,29}Pro-h-amylin ; ^{17}Ile,^{18}Arg,^{23}Leu-h-amylin ; ^{17}Ile,^{18}Arg,^{23}Leu,^{26}Val,^{29}Pro-h-amylin; ^{17}Ile,^{18}Arg,^{23}Leu,^{25}Pro,^{26}Val,^{28,29}Pro-h-amylin; ^{13}Thr,^{21}His,^{23}Leu,^{26}Ala,^{28}Leu,^{29}Pro,^{31}Asp-h-amylin; ^{13}Thr,^{21}His,^{23}Leu,^{26}Ala,^{29}Pro,^{31}Asp-h-amylin; des-^{1}Lys,^{13}Thr,^{21}His,^{23}Leu,^{26}Ala,^{28}Pro,^{31}Asp-h-amylin; ^{13}Thr,^{18}Arg,^{21}His,^{23}Leu,^{26}Ala,^{29}Pro,^{31}Asp-h-amylin; ^{13}Thr,^{18}Arg,^{21}His,^{23}Leu,^{28,29}Pro,^{31}Asp-h-amylin; and ^{13}Thr,^{18}Arg,^{21}His,^{23}Leu,^{25}Pro,^{26}Ala,^{28,29}Pro,^{31}Asp-h-amylin.

In some embodiments, the amylin analogue component includes an amino acid sequence of residues 1-37 of Formula (III) following, wherein up to 25% of the amino acids set forth in Formula (III) may be deleted or substituted with a different amino acid:

X'-Xaa^{1}-Cys^{2}-Asn^{3}-Thr^{4}-Ala^{5}-Thr^{6}-Cys^{7}-Ala^{8}-Thr^{9}-
Gln^{10}-Arg^{11}-Leu^{12}-Ala^{13}-Asn^{14}-Phe^{15}-Leu^{16}-Val^{17}-
His^{18}-Ser^{19}-Ser^{20}- Xaa^{21}-Asn^{22}-Phe^{23}- Xaa^{24}- Xaa^{25}-

Xaa^{26}- Xaa^{27}- Xaa^{28}- Xaa^{29}-Thr^{30}- Xaa^{31}-Val^{32}-Gly^{33}-
Ser^{34}-Asn^{35}-Thr^{36}-Tyr^{37}-X (III).

In Formula (III), X' is hydrogen, an N-terminal capping group, a bond to an exendin or exendin analogue optionally via a linker. Xaa^{1} is Lys or a bond, Xaa^{21} is Lys, Cys, or Asn, Xaa^{24} is Lys, Cys, or Gly, Xaa^{25} is Lys, Cys, or Pro, Xaa^{26} is Lys, Cys, or Ile, Xaa^{27} is Lys, Cys, or Leu, Xaa^{28} is Lys, Cys, or Pro, Xaa^{29} is Lys, Cys, or Pro and Xaa^{31} is Lys, Cys, or Asn. Further regarding Formula (III), the variable X represents a C-terminal functionality (e.g., a C-terminal cap). X is substituted or unsubstituted amino, substituted or unsubstituted alkylamino, substituted or unsubstituted dialkylamino, substituted or unsubstituted cycloalkylamino, substituted or unsubstituted arylamino, substituted or unsubstituted aralkylamino, substituted or unsubstituted alkyloxy, substituted or unsubstituted aryloxy, substituted or unsubstituted aralkyloxy, or hydroxyl. In some embodiments, the exendin or exendin analogue is covalently linked, optionally through a linker, to a side chain of an internal amino acid residue of the amylin analogue. If the C-terminal of the polypeptide component with the sequence of residues 1-37 of Formula (III) is capped with a functionality X, then X is preferably amine thereby forming a C-terminal amide. In some embodiments, up to 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% or even 50% of the amino acids of residues 1-37 of Formula (III) are deleted or substituted in a polypeptide component according to Formula (III). In some embodiments, the amylin analogue component has 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or even 16 amino acid substitutions relative to the amino acid sequence set forth in Formula (III). In some embodiments, the amylin analogue has a sequence which has a defined sequence identity with respect to the residues 1-37 of the amino acid sequence according to Formula (III). In some embodiments, the sequence identity between a polypeptide component described herein and residues 1-37 of Formula (I) is 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or even higher. In some embodiments, up to 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5% or even less of the amino acids set forth in residues 1-37 of Formula (III) may be deleted or substituted with a different amino acid. In some embodiments, the sequence identity is within the range 75%-100%. In some

embodiments, the sequence identity is within the range 75%-90%. In some embodiments, the sequence identity is within the range 80%-90%. In some embodiments, the sequence identity is at least 75%. In some embodiments, the polypeptide component of the conjugate has the sequence of residues 1-37 of Formula (III). In some embodiments, the amylin analogue of Formula (III) has the sequence of pramlintide, in others the sequence of davalintide. It has reported that davalintide, a second-generation analogue of amylin for the treatment of obesity, did not provide an improved weight loss efficacy and tolerability profile over pramlintide in a Phase 2 human clinical study. However, the dual receptor polypeptides of the present formulations preferably comprise davalintide or closely related analogue thereof, surprisingly providing superior potency and efficacy, with potentially fewer side effects, than those containing amylin or pramlintide, particularly when compared as a fusion polypeptide with exendin-4 or analogue including Leu14 exendin-4 (**Cmpd 4**).

Further amylin analogues, which are derivable, from davalintide include those disclosed in publications WO 2006/083254 and WO 2007/114838, each of which is incorporated by reference herein in its entirety and for all purposes. For purposes of the present invention, amylin analogues also include 32 amino acid human calcitonin (hCT) having the sequence CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAP and species variants thereof, including salmon calcitonin (sCT) having the sequence CSNLSTCVLGKLSQELHKLQTYPRTNTGSGTP. Also included are their C-terminal amide and acid forms, and physiologically acceptable salts, esters and derivatives. An analogue may have superior chemical stability, physical stability, protease resistance, solubility, efficacy, half-life, and the like

The amylin or amylin analogue can be conjugated or fused to the exendin or exendin analogue or to the GLP-1 or GLP-1 analogue by a covalent linker in any manner known in the art. When present the linker structure, which since serves primarily as a spacer, is preferably flexible, chemically and proteolytically stable, capable of little or no interaction with amino acids in either the exendin or amylin component,

and more preferably the linker is also a peptide comprising naturally-occurring amino acids compatible with recombinant production. Thus the linker can affect and be useful in optimizing pharmacological activity in some embodiments as described herein. A linker can be one or more amino acid residues, typically from about 1 to about 50 amino acid residues, more typically 1-30 amino acid residues, preferably about 1-20 amino acid residues, more preferably about 1-10, more preferred about 3-10, and even more preferably 3 amino acids, with Gly-Gly-Gly being most preferred, as used in the most preferred dual receptor agonist described above. Preferably, but not necessarily, the amino acid residues in the linker are from among the twenty canonical (i.e., physiologic, naturally-occurring) amino acids, more preferably, cysteine, glycine, alanine, proline, asparagine, glutamine, and /or serine. Even more preferably, a peptidyl linker is made up of a majority of amino acids that are sterically unhindered, such as glycine, serine, and alanine linked by a peptide bond. It is also desirable that, if present, a peptidyl linker be selected that avoids rapid proteolytic turnover in circulation in vivo. Thus, preferred linkers include polyglycines, polyserines, and polyalanines, or combinations of any of these. In certain embodiments, beta-amino acids (e.g., β-ala) as known in the art are included in the linker; where such exemplary linkers include $(\beta\text{-ala})_n$, where n is 1 to 20, preferably 1 to 10, more preferably 1 to 4. Additional linkers or moieties for use in a linker include a short or medium chain alkyl; 1-10 PEG repeating units; bifunctional linker (see, *e.g.*, Pierce catalog, Rockford, Il); aminocaproyl ("Aca") and 8-amino-3,6-dioxaoctanoyl.

Some additional exemplary peptidyl linkers are poly$(Gly)_{1-8}$, particularly $(Gly)_3$, $(Gly)_4$, $(Gly)_5$ $(Gly)_6$ and $(Gly)_7$, as well as, poly$(Gly)_4Ser$, poly$(Gly\text{-}Ala)_{2-4}$ and poly$(Ala)_{2-8}$. Other specific examples of peptidyl linkers include $(Gly)_5Lys$ and $(Gly)_5LysArg$. Other specific examples of linkers include: $(Gly)_3Lys(Gly)_4$, $(Gly)_3AsnGlySer(Gly)_2$, $(Gly)_3Cys(Gly)_4$; and GlyProAsnGlyGly. Additional preferred linkers include GGGGS, GGGGSGGGGS, GGGGSGGGGSGGGGSGGGGSGGGGS. In some embodiments, the linkers include the following peptide linker sequences: GGEGGG, GGEEEGGG, GEEEG, GEEE, GGDGGG, GGDDDGG, GDDDG, GDDD,

GGGGSDDSDEGSDGEDGGGGS, WEWEW, FEFEF, EEEWWW, EEEFFF, WWEEEWW or FFEEEFF. In another embodiment, the linker contains a cysteine or homocysteine residue, or other 2-amino- ethanethiol or 3-amino-propanethiol moiety for conjugation to maleimide, iodoacetaamide or thioester, functionalized half-life extending moiety. Another useful peptidyl linker is a large, flexible linker comprising a random Gly/Ser/Thr sequence, for example: GSGSATGGSGSTASSGSGSATH or HGSGSATGGSGSTASSGSGSAT, that is estimated to be about the size of a 1 kDa polyethylene glycol (PEG) molecule. Alternatively, a useful peptidyl linker may be comprised of amino acid sequences known in the art to form rigid helical structures (e.g., Rigid linker: -AEAAAKEAAAKEAAAKAGG. Additionally, a peptidyl linker can also comprise a non-peptidyl segment such as a 6 carbon aliphatic molecule of the formula – $(CH_2)_6$-. Optionally, non-peptidyl linkers are also useful for conjugating the two components. For example, alkyl linkers such as -NH-$(CH_2)_s$-C(O)-, wherein s = 2-20 can be used. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C_1-C_6) lower acyl, halogen (e.g., Cl, Br), CN, NH2, phenyl, etc. Exemplary non-peptidyl linkers are PEG linkers as known in the art and/or described herein.

Either or both peptides in the dual receptor agonist can be conjugated to the other through a side-chain of an internal or terminal amino acid or through the peptide's backbone, i.e. via the terminal alpha amino or carboxy groups. In a preferred embodiment the N-terminal amino acid of the amylin or amylin analogue, e.g. davalintide, is conjugated or fused at the C-terminal amino acid of the exendin or exendin analogue or to the GLP-1 or GLP-1 analogue. More preferably the linkage is via the N-terminal alpha amino group of the amylin or amylin analogue and the C-terminal alpha carboxy group of the exendin or exendin analogue or the GLP-1 or GLP-1 analogue, even more preferably through a peptide linker creating a fusion polypeptide that can be recombinantly produced. Generally, the dual receptor agonist compound will have a peptide backbone from 50 to 120 amino acids, preferably from 55 to 105, more preferably 60 to 100, even more preferably 65 to 80, and most preferably 74 to 76 amino acids. Generally, the dual receptor agonist

compound will have a GLP1 receptor agonism potency from 0.1 to 10 times that of the most preferred dual receptor agonist Cmpd 1, preferably from 0.2 to 5 times, more preferably from 0.4 to 3 times, even more preferably 0.5 to 2 times. Generally, the dual receptor agonist compound will have an amylin receptor agonism potency from 0.1 to 10 times that of the most preferred dual receptor agonist Cmpd 1, preferably from 0.2 to 5 times, more preferably from 0.4 to 3 times, even more preferably 0.5 to 2 times. Preferably the dual receptor agonist compound will have potency from 0.1 to 10 times, preferably from 0.2 to 5 times, more preferably from 0.4 to 3 times, even more preferably 0.5 to 2 times that of the most preferred dual receptor agonist Cmpd 1 for at least one or more in vivo biological activities of body weight loss, acute plasma glucose lowering (as in an oral glucose tolerance test), insulin secretion, delaying of gastric emptying, HbA1c lowering and/or inhibition of food intake. Such lengths and potencies are believed useful to minimize variation of protein concentration compared to that of Cmpd 1 in the pre-formulations and thus the desired properties, e.g. injectability, of the pre-formulations as disclosed herein. Additonal dual receptor agonists include previously described conjugates of an exendin analog and an amylin analogue include **Cmpd 3** having the sequence HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGGKCNTATCVLGRLSQELHRLQ TYPRTNTGSNTY-NH2 (**Cmpd 3**) where the polypeptide is C-terminally amidated, and **Cmpd 7** having the sequence HGEGTFTSDLSKQMEEEAVRLFIEWLKN(beta-A)(beta-A)KCNTATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2 (**Cmpd 7**) where the polypeptide is C-terminally amidated and contains a beta-alanine, beta-alanine unnatural amino acid dipeptide linker. The above compounds comprise an active C-terminally truncated form of exendin-4: exendin-4(1-28) amide (**Cmpd 10**).

As noted Cmpd 1 is the most preferred dual amylin receptor/GLP-1 receptor agonist. Surprisingly, despite nausea associated with exendin-4 and with davalintide, Cmpd 1 displayed no significant kaolin intake in a rat study, which is accepted as a predictor of nausea in humans. In addition Cmpd 1 displays a high potency for both the GLP-1 receptor and the amylin and calcitonin receptors, demonstrating that their exendin-like and davalintide moieties retain their biological activities. The activities for

these target receptors are only moderately attenuated compared with the parent compounds, exendin-4 and davalintide. Surprisingly, Cmpd 1 binds the CGRP receptor poorly if at all, and thus displays better selectivity than prior dual receptor agonist compounds for the amylin receptor (> 600 fold versus > 100 fold, respectively) and calcitonin receptors (> 1600 fold versus > 280 fold) against the CGRP receptor, and even better selectivity than davalintide for binding to calcitonin and amylin receptors against the CGRP receptor. Despite davalintide being a potent adrenomedullin receptor antagonist (IC50 = 18 nM), Cmpd 1 did not display functional activation or antagonism of the adrenomedullin receptor at concentrations up to 10 uM. Accordingly, Cmpd 1 presents a surprisingly different pharmacological profile compared to davalintide with respect to cellular receptors that recognize amylin and amylinomimetics. Cmpd 1 has fewer off-target activities than the parent peptide. This improved pharmacological profile for Cmpd 1 is expected to result in decreased side-effects, such as reduced severe flushing, nausea and/or vomiting, particularly with human subjects, as compared to davalintide. For example, CGRP and CGRP agonists have been reported to induce severe flushing, and even nausea and vomiting, in human subjects, which is believed in part due to activation of CGRP receptors and which is relieved by CGRP antagonists. It is expected that Cmpd 1 will have increased patient compliance and/or allow increased dosing as needed compared to previous compounds, for example compared to davalintide, resulting in improved commercial success.

The active agents used for and in connection with all aspects of the present invention are dual amylin receptor/GLP-1 receptor agonist compounds. These are referred to herein as "dual receptor agonists" and "peptide active agents". Dual receptor agonists as indicated herein are peptide compounds having at least two distinct domains wherein one domain serves as an agonist for amylin receptor and another serves as an agonist for GLP-1 receptor. Such dual agonists are distinct from a single non-specific agonist in that, although the domains may and preferably will be covalently bound together, the domain serving as amylin receptor agonist resides on a distinct portion of the peptide sequence from the domain serving at the GLP-1 receptor agonist. That is to say, the dual agonist is a compound in which a peptide sequence having amylin receptor function and substantially no GLP-1 agonist

function is chemically linked (directly or indirectly) to a sequence having GLP-1 agonist function and substantially no amylin receptor agonist function.

In one typical embodiment, the dual amylin receptor/GLP-1 receptor agonist active agent (e.g. Cmpd 1) will generally be formulated as 0.02 to 12% by weight of the total formulation. Typical values will be 0.1 to 10%, preferably 0.2 to 8% and more preferably 0.5 to 6%, and most preferably 1 to 3%, by weight of the formulation. These levels may be applied to all aspects of the invention, where context allows.

In a related embodiment, the dual amylin receptor/GLP-1 receptor agonist active agent (e.g. Cmpd 1) will be formulated at a level which cannot easily be achieved in the absence of water and/or propylene glycol. In such an embodiment, the dual amylin receptor/GLP-1 receptor agonist active agent (e.g. Cmpd 1) content is typically at least 0.7%, preferably at least 1%, more preferably at least 2% by weight of formulation, and even more preferably at 2.5%, such that also preferable load of active is 1 to 3 % by weight. Levels of at least 3% and at least 4% are achievable with the present invention, as are loading levels up to 8, 10 or 12%. Such compositions of the present invention typically not only contain a very high level of dual amylin receptor/GLP-1 receptor agonist active agent (especially Cmpd 1), as indicated, but are additionally stable to storage with minimal degradation of the active agent for considerable periods, as indicated herein. Such periods will generally be at least a month at 25°C or at 5°C, preferably at least 3 months, and more preferably at least 6 months at 5°C or alternatively at 25°C. These degrees of stability are applicable to all aspects of the invention, where context allows.

In one embodiment, the compositions of the present invention rely upon the effect of the water and/or propylene glycol to allow for a loading of dual amylin receptor/GLP-1 receptor agonist active agent (e.g. Cmpd 1) at a level above that which could be achieved in the absence of that solvent or solvent mixture. Obviously, a high loading level is highly advantageous and has been surprisingly established by the present inventors that by including the water and/or PG as specified herein, in the amounts indicated, a much higher loading of hybrid polypeptide active agents (particularly Cmpd 1) can be obtained (see examples). The level of dual amylin receptor/GLP-1 receptor agonist active agent (e.g. Cmpd 1) which could be loaded in a composition is easily established by equilibration of the composition with excess active agent (e.g. by slow end-over-end rotation for 5 days

at 25°C). The present compositions can and preferably do contain a greater amount of dual amylin receptor/GLP-1 receptor agonist active agent(particularly Cmpd 1) than can be achieved by equilibration in the absence of the water and/or PG component. This can apply in all aspects of the present invention, where context allows.

Suitable doses of dual amylin receptor/GLP-1 receptor agonist active agent (such as Cmpd 1) for inclusion in the formulation, and thus the volume of formulation used, will depend upon the release rate (as controlled, for example by the solvent type and amount used, and so forth) and release duration, as well as the desired therapeutic level, the activity of the specific agent, and the rate of clearance of the particular active chosen. Typically a sustained delivery of an amount of active around 0.5 to 5 mg active over one day, or a corresponding amount adjusted for longer sustained duration of delivery such as over one week, is desirable. Typically an amount of active around 0.05 to 60 mg per week of depot duration, even 0.5 mg to 60 mg per week, 0.05 to 40 mg per week, or 5 to 35 mg per week duration, and even 5 to 25 mg per week, for a period of administration of at least 1 to 24 weeks, preferably at least 2 to 16 (e.g. 8, 10 or 12) weeks. Evidently, the stability of the active and linearity of the release rate will mean that the loading to duration may not be a linear relationship. A depot administered every 7 days might have, for example 0.05 to 60 mg of active agent (e.g. Cmpd 1), while a 14 day depot may have more than twice the amount in the event that not 100% is released or may have less than twice that amount in the event that higher release in the earlier part of the cycle consumes additional active agent. Evidently also, the biological half-life of the specific active will be particularly important.

In a further embodiment applicable to all aspects of the invention, the dose of dual amylin receptor/GLP-1 receptor agonist active agent may be insufficient to provide a therapeutic concentration of active agent during the dosing period when administered in isolation but sufficient to provide a therapeutic concentration when a regime of regular dosing is undertaken. Such a regime will typically be administration once every dosing period (as discussed herein), such as once each day or even once each week. It may thus be that the Cmax < Cther, and optionally Caver < Cther where Cmax is the maximum plasma concentration generated by a single administration and Cther is the desired therapeutic concentration. However, in such an embodiment, CmaxM > Cther and CaverM > Cther, where CmaxM is the

maximum plasma concentration achieved after multiple periodic dosings (once every dosing period) and CaverM is the average plasma concentration achieved after multiple periodic dosings (once every dosing period).

Like essentially all organic molecules, lipids and biologically active agents are thermodynamically unstable to oxidation. As a result, many lipid formulations, including those comprising bioactive agents such as APIs are susceptible to degradation upon storage, especially by oxidation.

In a highly preferred embodiment, the lipid matrix aspect is soy PC, GDO, ethanol/propylene glycol or mixtures thereof, and water, and the hybrid polypeptide hormone active agent. As indicated above, appropriate amounts of each component suitable for the combination are those amounts indicated herein for the individual components, in any combination.

Optional Component f) - Antioxidant

Component f) is an antioxidant. In a most preferred embodiment, component f) is present (i.e. it is mandatory not optional) and is selected from ascorbic acid, ethylenediaminetetraacetic acid (EDTA) and citric acid.

In all aspects of the invention, component f) is typically present at a molar ratio of antioxidant to dual amylin receptor/GLP-1 receptor agonist active agent of 1:10 to 1:10000 (e.g. 1:50 to 1:5000), preferably 1:100 to 1:1300, and most preferably 1:150 to 1:1250. Since typical antioxidants are of lower molecular weight that the hybrid polypeptide active agents, the proportion by weight of antioxidant may be relatively small. For example, with EDTA and similar agents (e.g. less than 500 amu), 0.001 to 5% of the composition may be antioxidant, preferably 0.002 to 2%, more preferably 0.002 to 0.15%, e.g. 0.002 to 0.15%.

Unfortunately, many common antioxidants are not highly compatible with lipid systems. Indeed, the present inventors have previously established that some antioxidants commonly used in previous systems can cause increased degradation of active agents in a lipid system. This applies particularly to peptide active agents. The present inventors have therefore analysed a variety of potential antioxidant compounds and classes for use with lipid based matrix systems and have

surprisingly found that one particular class of antioxidants is unusually well suited for use in these systems.

The antioxidant component is generally included in the range 0.001 to 5% by weight of the total pre-formulation. Around 0.002 to 0.15% of antioxidant (particularly EDTA) is particularly preferred, especially in combination with the other preferred components and ranges indicated herein above and below.

Stability data using a number of different antioxidants demonstrate that EDTA antioxidants are surprisingly more efficient than other antioxidants in suppressing the oxidative degradation of the dual receptor agonist, especially of Cmpd 1. EDTA as antioxidant can also show a synergistic effect in combination with the antioxidants of the present invention, in maintaining the chemical and physical stability of the dual amylin receptor/GLP-1 receptor agonist active agent and complete pre-formulation. EDTA has a stabilising effect on the active agent. This is demonstrated by the examples, particularly figure 1, in which the stability of formulations B10, B12 and B16 are demonstrably better than those of the corresponding formulations not containing EDTA. It is notable that composition B09 and B10 differ only by the presence or absence of EDTA and compositions B11 and B12 differ from each other in the same way.

By "stabilising" is indicated an increase in the stability of the composition, especially with regard to the physical and chemical stability of the dissolved or dispersed active agent. Such an increase in stability may be demonstrated by the chemical and/or physical stability of a dual amylin receptor/GLP-1 receptor agonist active agentin a lipid formulation for a greater period than would be observed in the absence of an antioxidant. This would preferably be tested under conditions of typical storage, such as 0-5°C, 25°C and/or ambient temperature. This is further described herein below.

Administration

The pre-formulations of the present invention are generally formulated to be administered parenterally. This administration will generally not be an intra-vascular method but will preferably be subcutaneous (s.c.), intracavitary or intramuscular (i.m.). Typically the administration will be by injection, which term is

used herein to indicate any method in which the formulation is passed through the skin, such as by needle, catheter, or needle-less (needle-free) injector.

Preferred parenteral administration is by i.m or s.c. injection, most preferably by deep s.c. injection. An important feature of the composition of the invention is that it can be administered both by i.m. and s.c. and other routes without toxicity or significant local effects. It is also suitable for intracavital administration. The deep s.c. injection has the advantage of being less deep and less painful to the subject than the (deep) i.m. injection used for some current depots and is technically most suitable in the present case as it combines ease of injection with low risk of local side effects. It is a surprising observation of the present inventors that the formulations provide sustained release of active agent over a predictable time period by subcutaneous injection. This therefore allows the site of injection to be varied widely and allows the dose to be administered without detailed consideration of the tissue depth at the site of injection.

The preferred lipid pre-formulations of the present invention provide non-lamellar liquid crystalline depot compositions upon exposure to aqueous fluids, especially *in vivo*. As used herein, the term "non-lamellar" is used to indicate a normal or reversed liquid crystalline phase (such as a cubic or hexagonal phase) or the L3 phase or any combination thereof. The term liquid crystalline indicates all hexagonal, all cubic liquid crystalline phases and/or all mixtures thereof. Hexagonal as used herein indicates "normal" or "reversed" hexagonal (preferably reversed) and "cubic" indicates any cubic liquid crystalline phase unless specified otherwise. The skilled reader will have no difficulty in identifying those compositions having appropriate phase behaviour by reference to the description and Examples provided herein, and to WO2005/117830, but the most favoured compositional area for phase behaviour is where ratio of components a:b are in the region of equality (e.g. ranges around 30:40 to 40:30, more preferably 33:38 to 38:33). Accordingly, in the present formulations the ratio of components a:b are in the region of equality (1:1), including 1:2 to 2:1 ratios, but each of a) and b) are also preferably expressed as the range of absolute weight percentages of 30 to 40%, more preferably 33 to 38%, and even specifically 33.7, 34.8, 35.0 and 37.7%. Accordingly, for example, preferred formulation ratios of a:b that are in the region of equality when expressed as the desired absolute weight percentages are ratios of a:b where component a) is 30-40% and b) is 30-40%, where a) is 33 to 38% and b) is 33 to 38%, and even more

specifically where a) and b) are each any one of 33.7, 34.8, 35.0 and 37.7%, for example more preferably where ratio of a:b is 37.7:37.7, 35.0:35.0, 34.8:34.8 and 33.7:33.7.

It is important to appreciate that the pre-formulations of the present invention are of low viscosity. As a result, these pre-formulations must not be in any bulk liquid crystalline phase since all liquid crystalline phases have a viscosity significantly higher than could be administered by syringe or spray dispenser. The pre-formulations of the present invention will thus be in a non-liquid crystalline state, such as a solution, L_2 or L_3 phase, particularly solution or L_2. The L_2 phase as used herein throughout is preferably a "swollen" L_2 phase containing greater than 10 wt% of solvent (component c) having a viscosity reducing effect. This is in contrast to a "concentrated" or "unswollen" L_2 phase containing no solvent, or a lesser amount of solvent, or containing a solvent (or mixture) which does not provide the decrease in viscosity associated with the oxygen-containing, low viscosity solvents specified herein.

As used herein, the term "low viscosity mixture" is used to indicate a mixture which may be readily administered to a subject and in particular readily administered by means of a standard syringe and needle arrangement. This may be indicated, for example by the ability to be dispensed from a 1 ml disposable syringe through a small gauge needle. Preferably, the low viscosity mixtures can be dispensed through a needle of 19 awg, preferably smaller than 19 awg, more preferably 23 awg (or most preferably even 27 awg) needle by manual pressure. In a particularly preferred embodiment, the low viscosity mixture should be a mixture capable of passing through a standard sterile filtration membrane such as a 0.22 μm syringe filter. A typical range of suitable viscosities would be, for example (all at 20°C), 0.1 to 5000 mPas, (e.g. 5 to 2000 mPas) preferably 1 to 1000 mPas, more preferably 1 to 500 mPas and most preferably 1 to 350 mPas at 20°C, for example 250-350 mPas at 20 °C.

Upon administration, the preferred lipid-based pre-formulations of the present invention undergo a phase structure transition from a low viscosity mixture to a high viscosity (generally tissue adherent) depot composition. Generally this will be a transition from a molecular mixture, swollen L_2 and/or L_3 phase to one or more

(high viscosity) liquid crystalline phases such as normal or reversed hexagonal or cubic liquid crystalline phases or mixtures thereof. Further phase transitions may also take place following administration. Obviously, complete phase transition is not necessary for the functioning of the invention but at least a surface layer of the administered mixture will form a liquid crystalline structure. Generally this transition will be rapid for at least the surface region of the administered formulation (that part in direct contact with air, body surfaces and/or body fluids). This will most preferably be over a few seconds or minutes (e.g. from 1 second up to 30 minutes, preferably up to 10 minutes, more preferably 5 minutes of less). The remainder of the composition may change phase to a liquid crystalline phase more slowly by diffusion and/or as the surface region disperses.

Without being bound by theory, it is believed that upon exposure to excess aqueous fluid, the pre-formulations of the invention lose some or all of the organic solvent included therein (e.g. by diffusion) and take in aqueous fluid from the bodily environment (e.g. the *in vivo* environment). For lipid pre-formulations, at least a part of the formulation preferably generates a non-lamellar, particularly liquid crystalline phase structure. In most cases these non-lamellar structures are highly viscous and are not easily dissolved or dispersed into the *in vivo* environment. The result is a monolithic "depot" generated *in vivo* with only a limited area of exposure to body tissue. Furthermore, because the non-lamellar structure has large polar, apolar and boundary regions, the lipid depot is highly effective in solubilising and stabilising active agents such as peptides and protecting these from degradation mechanisms. As the depot composition formed from the pre-formulation gradually degrades over a period of days, weeks or months, the active agent is gradually released and/or diffuses out from the composition. Since the environment within the depot composition is relatively protected, the pre-formulations of the invention are highly suitable for active agents with a relatively low biological half-life (see above).

The depot systems formed by the formulations of the present invention are highly effective in protecting the active agent from degradation and thus allow an extended release period. The formulations of the present invention give very low degradation under simulated *in vivo* conditions. The formulations of the invention thus may provide *in vivo* depots of dual amylin receptor/GLP-1 receptor agonist active agents which require administration only once every 3 to 60 days (e.g. 5 to 30 days),

preferably 5 to 14 days (e.g. 7 to 14 days), preferably once every 7 days (once every "dosing period"). Evidently, a longer stable release period is desirable for patient comfort and compliance, as well as demanding less time from health professionals if the composition is not to be self-administered. Where the composition is to be self-administered, patient compliance may be aided by a weekly (e.g. every 7 days, optionally ±1 day) or monthly (e.g. every 28 or 30 days (optionally ± 7 days)) administration so that the need to administer is not forgotten.

Preferably the administration is once weekly.

A considerable advantage of the depot precursors of the present invention is that they are stable homogeneous phases. That is to say, they may be stored for considerable periods (preferably at least 6 months) at refrigerator temperature (e.g. 0-4°C, 0-5°C, and even 2-8°C), without phase separation. As well as providing advantageous storage and facile administration, without the need for mixing, this allows for the dose of hybrid polypeptide hormone active agent (e.g. Cmpd 1) to be selected by reference to the species, age, sex, weight, and/or physical condition of the individual subject, by means of injecting a selected volume.

The present invention thus provides for methods comprising the selection of a dosing amount specific to an individual, particularly by subject weight. The means for this dose selection is the choice of administration volume.

For particular applications it may be necessary for the composition to equilibrate repeatedly at differing temperatures. For example in a multi-dose device it may be expected that the device will be refrigerated, then equilibrated, then re-refrigerated several times over the lifetime of the device. Such devices therefore will have a longer time at high temperature and the high temperature stability of the formulation must be sufficient for the relevant number of equilibration cycles, for example, up to 12, preferably up to 6. Preferably the active agent counterion (if present/necessary) is a halide, particularly chloride, as this has been observed to give improved high temperature stability, as demonstrated in the current examples.

In one preferred aspect, the present invention provides a pre-formulation comprising components a), b), c), d), f) and at least one dual amylin receptor/GLP-1 receptor agonist active agent (e.g. Cmpd 1) as indicated herein. The amounts of these

components will typically be in the range 30-70% a), 30-60% b), 5-35% c) and 0.1-20% d), with the dual amylin receptor/GLP-1 receptor agonist active agent (e.g. Cmpd 1) present at 0.01% to 10%. A highly preferred combination is 30-40% a), 30-40% b), 15-25% c) and 2-15% d), with the hybrid polypeptide hormone active agent (e.g. Cmpd 1) present at 1% to 3%.

Typically, component f) is present at an antioxidant to dual amylin receptor/GLP-1 receptor agonist active agent molar ratio of 1:10 to 1:10000, preferably 1:100 to 1:1300, and most preferably 1:150 to 1:1250. Since typical antioxidants are of lower molecular weight than hybrid polypeptide hormone active agent (e.g. Cmpd 1), the proportion by weight of antioxidant may be relatively small. For example, with a small molecular weight antioxidant (e.g. less than 500 amu), 0.001 to 5% of the composition may be antioxidant, preferably 0.002 to 2%, more preferably 0.002 to 0.15%, e.g. 0.002 to 0.015%.

The pre-formulations of the present invention are highly advantageous in that they are stable to prolonged storage in their final "administration ready" form. As a result, they may readily be supplied for administration either by health professionals or by patients or their carers, who need not be fully trained health professionals and may not have the experience or skills to make up complex preparations. This is particularly important in long-duration, slow-effecting diseases such as diabetes.

Devices

In a yet further aspect, the present invention provides a disposable administration device (which is also to include a device component) pre-loaded with a measured dose of a pre-formulation of the present invention. Such a device will typically contain a single dose ready for administration, and will generally be sterile-packed such that the composition is stored within the device until administration. Suitable devices include cartridges, ampoules and particularly syringes and syringe barrels, either with integral needles or with standard (e.g. luer) fittings adapted to take a suitable disposable needle, or prefilled syringes with preattached needles.

The pre-filled devices of the invention may also suitably be included in an administration kit, which kit also forms a further aspect of the invention. In a still further aspect, the invention thus provides a kit for the administration of at least one

hybrid polypeptide active agent, said kit containing a measured dose of a formulation of the invention and optionally an administration device or component thereof. Preferably the dose will be held within the device or component, which will be suitable for i.m. or preferably s.c. administration. The kits may include additional administration components such as needles, swabs, etc. and will optionally and preferably contain instructions for administration. Such instructions will typically relate to administration by a route as described herein and/or for the treatment of a disease indicated herein above. The device may be single- or multi-use. The multi-use device may provide once-weekly doses for a period of at least 2, 3, 4 or even 8 weeks.

The device of the invention will preferably be loaded with a composition having any of the concentrations of active described herein. Preferably each administered dose is a total volume for administration of no more than 3 ml, preferably no more than 1 ml more preferably no more than 0.5 ml, and more preferably no more than 0.1 ml. Further, the volume is preferably no less than 0.04 ml, more preferably no less than 0.06 ml, and even not less than 0.1 ml.

Preferred Features and Combinations

In combination with the features and preferred features indicated herein, the pre-formulations of the invention may have one or more of the following preferred features independently or in combination:

All proportions indicated herein may optionally be varied by up to 10% of the amount specified, optionally and preferably by up to 5%;

Component a) comprises, consists essentially of or preferably consists of GDO;

Component b) comprises, consists essentially of or preferably consists of soy PC;

Component c) comprises, consists essentially of or preferably consists of a 1, 2, 3 or 4 carbon alcohol, preferably isopropanol or more preferably ethanol, propylene glycol or mixtures thereof;

Component f) comprises, consists essentially of or preferably consists of ethylenediaminetetraacetic acid (EDTA). Preferably component f) is present.

The pre-formulation contains CMPD 1 as defined herein.

The pre-formulation has a low viscosity as indicated herein.

The pre-formulation forms a liquid crystalline phase as indicated herein upon *in vivo* administration.

The pre-formulation generates a depot following *in vivo* administration, which depot releases at least one active agent at a therapeutic level over a period of at least 3 days, preferably at least 5 days, more preferably at least 7 days

The pre-formulation has a higher loading of dual amylin receptor/GLP-1 receptor agonist active agent (e.g. Cmpd 1) than is stable in the same formulation in the absence of component e).

The pre-formulation has a higher loading of dual amylin receptor/GLP-1 receptor agonist active agent (e.g. Cmpd 1) than is obtainable by equilibration at 25°C of the same formulation in the absence of water and propylene glycol.

In combination with the features and preferred features indicated herein, the method(s) of treatment of the present invention may have one or more of the following preferred features independently or in combination:

The method comprises the administration of at least one formulation with one or more preferred features as indicated above;

The method comprises the administration of at least one formulation as indicated herein by i.m., s.c. or preferably deep s.c. injection;

The method comprises administration by means of a pre-filled administration device as indicated herein;

The method comprises administration through a needle no larger than 20 gauge, preferably smaller than 20 gauge, and most preferably 23 gauge or smaller, including 25 gauge;

The method comprises a single administration each dosing period, such as every 3 to 30 days, preferably 5 to 14 days, more preferably 7 ±1 days.

In combination with the features and preferred features indicated herein, the use(s) of the pre-formulations indicated herein in the manufacture of medicaments may have one or more of the following preferred features independently or in combination:

The use comprises the use of at least one formulation with one or more preferred features as indicated above;

The use comprises the manufacture of a medicament for administration of at least one formulation as indicated herein by i.m., s.c. or preferably deep s.c. injection;

The use comprises the manufacture of a medicament for administration by means of a pre-filled administration device as indicated herein;

The use comprises the manufacture of a medicament for administration through a needle no larger than 20 gauge, preferably smaller than 20 gauge, and most preferably 23 gauge or smaller, including 25 gauge;

The use comprises the manufacture of a medicament for administration once every dosing period of 3 to 30 days, preferably 5 to 14 days, more preferably 7 ±1 days.

In combination with the features and preferred features indicated herein, the pre-filled devices of the invention may have one or more of the following preferred features independently or in combination:

They contain a preferred formulation as indicated herein;

They comprise a needle smaller than 20 awg, preferably no larger than 23 awg;

They contain a single dose of 0.05 to 250 mg of hybrid polypeptide hormone active agent (e.g. Cmpd 1), preferably 0.1 to 100 mg and more preferably 1-50 mg

They contain a homogeneous mixture of a composition of the invention in ready-to-inject form.

They contain a total volume for administration of no more than 3 ml, preferably no more than 1 ml more preferably no more than 0.5 ml, and more preferably no more than 0.1 ml. Further, the volume is no less than 0.04 ml, and preferably no less than 0.06 ml.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention the at least one diacyl glycerol and/or a tocopherol is present at 33-38% by weight or a value from Table 1.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention the at least one diacyl glycerol is GDO.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention the at least one PC is present at 33-38% by weight or a value from Table 1.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention the at least one PC is soy PC or DOPC.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention the at least one biocompatible, organic alcoholic solvent is present at 33-38% by weight or a value from table 1.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention the at least one biocompatible, organic alcoholic solvent is ethanol and/or PG.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention the at least one biocompatible, organic alcoholic solvent is ethanol and is present at 15% and PG is absent; or the at least one biocompatible, organic alcoholic solvent is a mixture of ethanol and PG wherein ethanol is present at 5% and PG is present at 15%.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention the total aqueous solvent is present in a 15% to 20% range and/or a value from table 1.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention the antioxidant is present in a 0.002 % to 0.015 % range and/or a value from Table 1.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention the active agent is Cmpd 1.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention comprises a pre-formulation selected from the group B9, B10, B11, B12, B15 and B16.

In a most preferred combination of the pre-formulation, use, method, device or kit of the invention comprises a pre-formulation selected from B12 or B16.

The Invention will now be further illustrated by reference to the following non-limiting Examples and the attached Figures. The following examples are for purposes of illustration only and are not intended to limit the scope of the claims.

EXAMPLES

Example 1

Depot precursors with the compositions presented in Table 1 were prepared by first preparing an excess of lipid stock solution, by mixing all the components except Cmpd 1 until a homogeneous solution was obtained; when EDTA was included in the samples, it was first dissolved in the required amount of water (final concentration 0.5 – 1 mg EDTA/mL) prior to mixing with the other excipients. The final formulations were obtained by adding 14.625 g of the corresponding lipid stock solution to 0.375 g Cmpd 1 powder, mixing by brief vortex followed by end-over-end rotation for up to 24h giving clear, homogeneous and low viscosity lipid/Cmpd 1 formulations.

Table 1: Sample compositions according to the invention.

									Compd1*	
Formulation	SPC/ wt.%	GDO/ wt.%	EtOH/ wt.%	PG/ wt.%	Water/ wt.%	Citric acid/ wt.%	HCl/ wt.%	EDTA/ wt.%	Conc./ wt.%	Salt
B08	37.7	37.7	5.0	15.0	2.0			0.002	2.50	Cl
B09	35.0	35.0	10.0	10.0	7.5		0.045		2.50	Cl
B10	35.0	35.0	10.0	10.0	7.5		0.045	0.015	2.50	Cl
B11	33.7	33.7	15.0		15.0		0.045		2.50	Cl
B12	33.7	33.7	15.0		15.0		0.045	0.015	2.50	Cl
B15	34.8	34.8	10.0	15.0	2.0	1.0			2.50	Ac
B16	37.7	37.7	5.0	15.0	2.0		0.045	0.002	2.50	Cl

SPC = soy PC; GDO = glycerol dioleate; EtOH = ethanol; PG = propylene glycol; EDTA = ethylenediaminetetraacetic acid; Cmpd 1 = (as defined herein above) * = recombinant source.

Example 2

Formulations with compositions presented in Table 1 were filled into 0.5 mL glass syringes, which were then placed in cabinets at controlled storage conditions (5°C; 25°C/60% RH, or 40°C/75% RH). The content and purity of Cmpd 1 in the samples were evaluated (after equilibration of the samples to ambient temperature, when necessary) directly after manufacturing, and after 1; 2; and 3 months of storage in the mentioned condition, using an HPLC-UV/DAD method based on a

Polysulfoethyl A (PolyLC Inc.) analytical column (cation-exchange) and a gradient of $NaClO_4$ in a mixture of acetonitrile, methanol, water and HCl.

The results obtained for up to 3 months of storage in the above mentioned conditions are presented in Figure 1.

Example 3: Methods for In Vivo Studies in DIO Rats

The present examples characterized the metabolic actions of Cmpd 1, parent compounds and other dula receptor agonists, demonstrating activity of the dual receptor agonists and surprising superiority of Cmpd 1. As disclosed in the Examples, the effect of 4 weeks of constant subcutaneous infusion of Cmpd 1 and Cmpd 2 (at 3, 10, 30 and 100 nmol/kg/d) were compared to single and co-administration of the parent peptides, Cmpd 6, Cmpd 5 and Cmpd 4 (davalintide) (at 2.8, 15 and 7.2 nmol/kg/; maximum efficacious dose for weight loss) in diet-induced obese (DIO) male Sprague Dawley rats. Various metabolic and PK parameters were evaluated.

Animals. Male Sprague Dawley rats (CRL:CD rats, Charles River Laboratories, Wilmington, MA) were individually housed and maintained on high fat diet (32% kcal from fat; D12266B Research Diets, Brunswick, NJ) for approximately 8 weeks prior to the study. At the start of testing (day 0) average body weight of the rats was 545 ± 3.8 g.

Compounds. Cmpds used in the examples include the following:
Cmpd 4, Leu14 exendin-4 amide. Cmpd 5, a chimera of the first 32 amino acids of exendin-4 having amino acid substitutions at positions 14 and 28 followed by a 5 amino acid sequence from the C-terminal of a non-mammalian (frog) GLP1;
Cmpd 6, davalintide; Cmpd 2, the dual receptor agonist of Cmpd 5 covalently attached in-frame to Cmpd 6 through an glycine-glycine-glycine peptide linker; and the most preferred dual receptor agonist Cmpd 1, a fusion conjugate of Cmpd 4 covalently attached inframe to Cmpd 6 through a glycine-glycine-glycine peptide

linker.

Study Design. The compounds were tested relative to vehicle (50% DMSO in sterile water) and parent compounds alone and in combination (see Table 1). The doses of Cmpd 5, Cmpd 4 and Cmpd 6 are maximally efficacious for weight loss in this model (data not shown). On day 1 rats were surgically implanted with two osmotic mini-pumps (Alzet, Durect Corporation, Cupertino, CA) that delivered either vehicle, Cmpd 2, Cmpd 1, Cmpd 5, Cmpd 4 or Cmpd 6 at a constant rate (nanomoles per kilogram of rat per day) for 4 weeks. See Table 2.

Table 2: Group Allocation and Treatments

Group #	Treatment (nmol/kg/day)	
1	Vehicle	Vehicle
2	Vehicle	Cmpd 2 (3)
3	Vehicle	Cmpd 2 (10)
4	Vehicle	Cmpd 2 (30)
5	Vehicle	Cmpd 2 (100)
6	Vehicle	Cmpd 1 (3)
7	Vehicle	Cmpd 1 (10)
8	Vehicle	Cmpd 1 (30)
9	Vehicle	Cmpd 1 (100)
10	Vehicle	Cmpd 5 (15)
11	Vehicle	Cmpd 4 (7.2)
12	Vehicle	Cmpd 6 (2.8)
13	Cmpd 5 (15)	Cmpd 6 (2.8)
14	Cmpd 4 (7.2)	Cmpd 6 (2.8)

Food intake and body weight were measured weekly. Body composition was assessed on day -1 and day 28 using an NMR instrument (Echo Medical Systems, Houston, TX). Adiposity (percent fat mass) was defined as the amount of fat mass relative to body weight (fat mass/body weight x 100). Blood was collected via tail vein on day 14. On day 28 a sample of blood was drawn via the jugular vein and animals were euthanized by isoflurane overdose. Mini-pumps were immediately removed, animals were subjected to a brief NMR scan, and tissues were collected for future histological examination and preliminary toxicological assessment.

Statistical Analysis. Data were analyzed using one-way analysis of variance (ANOVA) with Newman-Keuls post-hoc comparisons. Significance was assumed for $p<0.05$. Graphs were generated using Prism 4 for Windows (Graphpad Software, San Diego, CA). All data points are expressed as mean ± SEM. For the highest doses of Cmpd 1 and Cmpd 2, several of the animals had minimal food intake and this data was included in the analysis.

Hormone and metabolite analyses. Plasma levels of study drug were measured at day 14 and termination by an ELISA. Whole blood percent hemoglobin A1c (%HbA1c), plasma triglyceride, total cholesterol, HDL cholesterol and plasma glucose at day 14 and at termination were measured using an Olympus bioanalyzer (Olympus America Diagnostics). Plasma insulin levels at day 14 and at termination were analyzed by an ELISA kit (Rat/Mouse Insulin ELISA, Linco Diagnostics).

Example 4: Compound 1 Provides Superior Body Weight Loss

Following the method of Example 3, Cmpd 1 and Cmpd 2 dose-dependently reduced body weight significantly ($p<0.05$ relative to vehicle) at 3, 10, 30 and 100 nmol/kg/d over the 28 day treatment (see Figures 2A and 2B). Co-administration of the parent compounds (Cmpd 5+Cmpd 6 and Cmpd 4+Cmpd 6) significantly reduced body weight compared to vehicle controls and compared to Cmpd 6 alone ($p<0.05$). See Figures 2A and 2B.

As indicated in Figure 2A, four week vehicle-corrected weight loss was: -31.5 ± 2.7% for Cmpd 2 at 100 nmol/kg/d, -16.6 ± 3.6% for Cmpd 5, -12.3 ± 1.3% for Cmpd 6, -24.3 ± 1.3% for Cmpd 5+Cmpd 6, $p<0.05$ for Cmpd 2 vs. Cmpd 6, but not different from Cmpd 5 or co-administration of Cmpd 5+Cmpd 6. For Cmpd 2 there was no dose-effect beyond 10 nmol/kg/d; further weight loss was not observed with doses of 30 or 100 nmol/kg/d. Maximal weight loss was achieved in the 100 nmol/kg/d group, at -27.8 ± 5.3%. See Figure 2A. Cmpd 2 was also several fold more potent at reducing body weight via sustained infusion than Cmpd 3.

As indicated in Figure 2B, four week vehicle-corrected weight loss was: -37.3 ± 4.8% for Cmpd 1 at 100 nmol/kg/d, -13.5 ± 1.5% for Cmpd 4 and -25.8 ± 1.5% for Cmpd 4+Cmpd 6; $p<0.05$ for Cmpd 1 versus both parent peptides alone and in combination. For Cmpd 1 dose-dependent weight loss was observed up to the highest dose tested [100 nmol/kg/d group at -37.3 ± 4.8%]. Weight loss with Cmpd 1 at 100 nmol/kg/d was also significantly greater than weight loss observed with co-administration of maximally efficacious doses of parent compounds. See Figure 3A. Although both dual receptor agonists elicited profound weight loss over the 28 day treatment period, for Cmpd 1 the weight loss at even the lowest dose tested was significantly greater than via single administration of either parent peptide. Cmpd 1 was also remarkably more potent and more efficacious than Cmpd 2. Cmpd 1 was also approximately 10-fold more potent at reducing body weight via sustained infusion than Cmpd 3.

Example 5: Compound 1 Provides Superior Metabolic Parameters

Plasma parameters. The effects of the parent peptides and the dual receptor agonist compounds on plasma parameters including lipids (triglycerides, total and HDL cholesterol) and glucose and concentration of the dual receptor agonist in plasma, at 14 and 28 days.

After 28 days, no change in glucose, total or HDL cholesterol was observed with Cmpd 2 treatment. Likewise there was no significant effect of Cmpd 1 on total or HDL cholesterol after 28 days. Plasma glucose levels were significantly lowered by Cmpd 1 at some doses compared to vehicle controls. Plasma triglycerides were significantly reduced by all peptide treatments compared to vehicle after 28 days of treatment. Plasma levels of Cmpd 1 and Cmpd 2, measured by a specific immunoassay, were detected at increasing levels corresponding to treatment dose after both 2 and 4 weeks of treatment. All doses of Cmpd 2 and parent peptides administered separately or together did not alter glucose, or total or HDL cholesterol after 28 days. Triglycerides were significantly reduced by all groups compared to vehicle (see Table 3).

Table 3: Plasma glucose and lipids: Cmpd 2

Treatment (nmol/kg/day)	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)
Vehicle	136 ± 7	34 ± 1	533 ± 24[a]	136 ± 4
Cmpd 2 (3)	116 ± 8	33 ± 2	295 ± 75[b]	132 ± 9
Cmpd 2 (10)	115 ± 14	33 ± 5	146 ± 41[b]	115 ± 6
Cmpd 2 (30)	112 ± 4	32 ± 1	128 ± 25[b]	111 ± 2
Cmpd 2 (100)	107 ± 3	30 ± 1	139 ± 17[b]	110 ± 4
Cmpd 5 (15)	110 ± 8	32 ± 2	195 ± 41[b]	125 ± 2
Cmpd 6 (2.8)	142 ± 11	39 ± 2	223 ± 35[b]	126 ± 5
Cmpd 5+Cmpd 6	127 ± 5	35 ± 2	159 ± 16[b]	173 ± 62

In contrast to all other compounds including Cmpd 2, Cmpd 1 decreased HDL cholesterol at 10 and 30 nmol/kg/d, e.g. as compared to Cmpd 6. Cmpd 1 did not alter total cholesterol. See Table 4. All doses of Cmpd 1, and parent peptides, decreased triglycerides relative to vehicle, and lowered glucose at 10 and 30 nmol/kg/d, as did Cmpd 4A+Cmpd 6A (see Table 4).

Table 4: Plasma glucose and lipids: Cmpd 1

Treatment (nmol/kg/day)	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)
Vehicle	136 ± 7	34 ± 1	533 ± 24[a]	136 ± 4[a]
Cmpd 1 (3)	119 ± 4	33 ± 1	192 ± 39[b]	109 ± 3[b]
Cmpd 1 (10)	108 ± 6	29 ± 1[a]	121 ± 24[b]	111 ± 2[b]
Cmpd 1 (30)	110 ± 8	29 ± 1[a]	98 ± 15[b]	123 ± 15
Cmpd 1 (100)	143 ± 15	35 ± 1	100 ± 9[b]	122 ± 8
Cmpd 4 (7.2)	121 ± 5	33 ± 2	157 ± 13[b]	116 ± 1
Cmpd 6 (2.8)	142 ± 11	39 ± 2[b]	223 ± 35[b]	126 ± 5
Cmpd 4+Cmpd 6	120 ± 9	33 ± 2	142 ± 49[b]	107 ± 4[b]

Example 6: Dual Receptor Agonists are Active in Basal Glucose Lowering

Dual receptor agonists and parent compounds were analyzed in an in vivo basal glucose lowering assay. This assay reflects the conjugate polypeptide's ability to enhance insulin-mediated glucose clearance of orally administered glucose challenge (Oral Glucose Tolerance Test; OGTT). The OGTT is used to diagnose diabetes, although the simpler fasting plasma glucose test, which measures a subject's plasma glucose level after fasting for at least eight hours, is preferred. The following procedure was used: Test compound at various concentrations was injected intraperitoneally (IP) at t = -5 min to 4-hour fasted NIH/Swiss female mice. Glucose gavage (1.5 g/kg) was given at t = 0. Sample was taken at t = 30 minutes as tail blood glucose using a OneTouch® Ultra® (LifeScan, Inc., Milpitas, CA). Significant effects were identified by ANOVA ($p<0.05$), followed by Dunnett's post test using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego CA). ED50 values were calculated. As expected exendin-4 and exendin-4 peptide analogs Cmpd 4, Cmpd 5 and Cmpd 10, are active in glucose lowering. Cmpd 2 is surprisingly more potent than either previously known dual receptor agonist Cmpd 3 and Cmpd 7. Cmpd 1 is surprisingly more potent than Cmpd 2 as well as Cmpd 3 and Cmpd 7.

Example 7: Dual Receptor Agonists Cmpd 1 and Cmpd 2 Reduce Food Intake with less Nausea

To investigate possible nausea effects of the fusion polypeptides, acute kaolin intake was measured in rats. Pica behavior (ingestion of dirt/clay) is a marker of nausea in rodents, typically associated with reduced food intake and weight loss. Pica can be assessed by measuring intake of the synthetic clay kaolin. Cisplatin, a chemotherapy drug that can act in the gut to produce emesis, was used to induce nausea-associated hypophagia as a positive control. Rats were acclimated to kaolin for 3 days as kaolin clay mixed in with regular chow. A 4 hr and a 24 hr baseline kaolin and chow intake were then measured. Subsequently, rats were fasted for approximately 16 hrs after which test compound was injected at the dose indicated below. At 24 hr post injection chow and kaolin consumption was measured. Table 5 presents the results of kaolin intake and correlation to (chow) food intake inhibition.

Table 5: Nausea

Compound Number	Dose	Inhibited food intake	Kaolin intake (g)
Cisplatin	5 mg/kg Pos. contr.	Yes ~20%	2.1
Cmpd 7	15 nmol/kg/d	Yes ~20%	2.1
Cmpd 3	15 nmol/kg/d	Yes ~20%	0.5
Cmpd 2	30 nmol/kg/d	Yes ~68%	0.3
Cmpd 1	30 nmol/kg/d	Yes ~64%	0.2

Cmpd 7, a previously known dual receptor agonist conjugate, at a dose that inhibited food intake, induced significant kaolin intake similar to the positive control, a sign of nausea in rats. Cmpd 3 at doses that suppressed food intake acutely similar to cisplatin injection, had only a modest, if any, effect on kaolin consumption. Cmpd 1 and Cmpd 2 at doses that elicited reductions in food intake even greater than the positive control, Cmpd 3 or 7, did not induce significant increases in kaolin consumption. Surprisingly, despite nausea associated with exendin-4 and davalintide, Cmpd 1 and Cmpd 2 displayed no significant kaolin intake in this study.

Cmpd 1 displays a high potency for both the GLP-1 receptor and the amylin and calcitonin receptors, demonstrating that their exendin-like and davalintide moieties retain their biological activities. The activities for these target receptors are only moderately attenuated compared with the parent compounds. Interestingly, Cmpd 1 binds the CGRP receptor with very low affinity, displaying better selectivity than Cmpd 3 for amylin receptor and calcitonin receptors against the CGRP receptor, and even better than davalintide selectivity for binding to calcitonin and amylin receptors against the CGRP receptor. Despite davalintide being a potent adrenomedullin receptor antagonist, Cmpd 1 did not display functional activation or antagonism of the adrenomedullin receptor at concentrations up to 10 uM. Accordingly, Cmpd 1 presents a surprisingly different pharmacological profile compared to davalintide with respect to cellular receptors that recognize amylin and amylinomimetics. Cmpd 1 has fewer off-target activities than the parent peptide. This improved pharmacological profile for Cmpd 1 is expected to result in decreased side-effects, such as reduced severe flushing, nausea and/or vomiting, particularly with human subjects, as compared to the parent peptide Cmpd 6. For example, CGRP and CGRP agonists have been reported to induce severe flushing, and even nausea and vomiting, in human subjects, which is believed in part due to activation of CGRP receptors and which is relieved by CGRP antagonists.

Example 8: Dual Receptor Agonists Delay Gastric Emptying

Dual receptor agonists and parent compounds were analyzed for their ability to delay gastric emptying in rats. Inhibition of gastric emptying is a physiological effect of GLP-1 receptor agonism as well as amylin receptor agonism, and a key pharmacological effect of exendin-4 and Cmpd 6 in glucose control. Fasted male Sprague Dawley rats (~250 grams, n=5 per group) received a single subcutaneous injection of saline, Cmpd 6A or test compound at t = 0 (1 nmol/kg). Rats then received an oral gavage of 33 mg acetaminophen/1ml Orablend (Paddock Laboratories, Inc., MN USA) at t=3.5 hr, 5.5 hr or 7.5 hr post-injection. Blood was collected for measurement of acetaminophen at 4 hr, 6 hr or 8 hr after SC injection.

Gastric emptying was assessed by the appearance of acetaminophen in plasma 30 min after oral gavage.

Table 6 presents percent inhibition of gastric emptying. Cmpd 1 and Cmpd 2 were as efficacious as Cmpd 6 at inhibiting gastric emptying up to six hours after a single injection. Cmpd 3 and Cmpd 7 did not significantly inhibit gastric emptying at the time point and doses tested. Surprisingly, Cmpd 1 provided a longer duration of action compared to Cmpd 2.

Table 6: Delayed Gastric Emptying

Test Compound	1 nmol/kg, Percent Inhibition of Gastric Emptying		
	4 hr	6 hr	8 hr
Cmpd 6	75	58	6
Cmpd 3	33	0	0
Cmpd 2	79	49	4
Cmpd 1	48	37	27

The effect of Cmpd 1, which comprises Cmpd4, in the assays provided herein is surprising. Hargrove et al. 2007 teaches that exendin analogue Cmpd 4 displays a markedly less potent delay of gastric emptying (4 fold less), a markedly less potent inhibition of food intake (8 fold less), a shorter half-life and a shorter duration of action compared to exendin-4. Despite the presence of the Leu14 exendin-4 peptide analog sequence in Cmpd 1, Cmpd 1 presents surprisingly superior pharmacological properties compared to Cmpd 2 and previously known conjugates, such as robust and longer acting inhibition of gastric emptying activity as well as a surprisingly robust and long acting reduction of food intake and reduction in body weight. Furthermore, Cmpd 1 and Cmpd 2 have a more stable (at least two fold) metabolic profile in vitro in human plasma and with human kidney brush border membrane matrices over a 5 hr incubation period compared to exendin-4, Cmpd 4 and Cmpd 6 (data not shown). No metabolites were detected for Cmpd 1 or Cmpd 2 during that period, which indicates that any unidentified metabolite would have been presents at levels below 10%. Surprisingly, Cmpd 1 and Cmpd 2 have a longer half-life with

similar bioavailability (subcutaneous injection in rats) compared to either exendin-4, Cmpd 4 or Cmpd 6 (data not shown). Compound 1 has a half-life of 72 minutes given subcutaneously (in male Sprague-Dawley rats) compared to exendin-4 of 20 minutes and davalintide of about 30 minutes, with similar absolute bioavailibility.

These superior pharmacological properties, coupled with an excellent PK profile and other favorable drug properties such as fewer off-target activities and reduced nausea, provide a surprisingly useful dual receptor agonist (Cmpd 2 and even more so Cmpd 1) for controlling glucose with further improvement in controlling body weight and composition in subjects in need of such treatment having diseases and conditions where such treatment is beneficial. Such conditions include subjects, e.g. human, having prediabetes, diabetes, diabetes with overweight or obesity, overweight or obesity, where such subjects are in need of and desirous of controlling blood glucose (e.g. anti-hyperglycemia) and/or having improved effect or control of body weight and body composition to reduce body weight, maintain body weight, prevent an increase in body weight and/or improve lean muscle to body fat ratio.

Example 9: Pharmacokinetic study

Materials and Methods

Animals

Thirty six male Sprague Dawley [Crl:CD(SD)IGS BR] rats were received from Charles River Laboratories. Each animal was identified with an individually numbered ear tag. The animals were acclimated for approximately 10 days. At the time of dose administration, animals were approximately 12 weeks of age and weighed 368 to 424 grams. During acclimation and the test period, animals were individually housed in suspended, stainless steel wire-mesh cages. Animals were assigned to groups on Day 1 based on overall health and body weights using computer-generated random numbers.

Environmental controls for the animal room were set to maintain a temperature of 18 to 26°C, a relative humidity of 50±20%, and a 12-hour light/12-hour dark cycle.

As necessary, the 12-hour dark cycle was temporarily interrupted to accommodate study procedures.

Certified Rodent Diet #8728C (Harlan Teklad, Inc.) was provided ad libitum. Water was provided fresh daily, ad libitum.

Test Articles

The test article formulations of Cmpd1- were prepared according to Example 1 above. Compositions B09, B10, B11, B12, B15 and B16 as set out in Table 1 were used in the following protocol. The dose formulations were stored at approximately -20°C and protected from light.

Dose Preparation

Prior to use, the formulations were allowed to thaw for approximately 1 hour. Formulations were not heated, sonicated, or vortexed. Care was taken to ensure that no air bubbles were present in the suspension contained in the dosing syringe. All dose formulations were used within 8 hours of thawing. Any dose formulations remaining following administration were stored at approximately 4°C and protected from light.

Dose Administration

The animals were not fasted prior to dosing. Individual doses were calculated based on body weights taken on the day of dose administration.

Prior to dosing, the back of each animal was clipped free of hair, and the injeciton site was prepared by carefully cleaning the area with an alcohol wipe. The injection site (on the dorsum, mid-scapular region) was marked with a permanent marker prior to injection to permit obervations and scoring. The marking of the injection site was done by drawing a circle around the injection site, taking care not to obscure the injection site itself or the immediate surrounding area of the injection site.

The subcutaneous doses were administered using a 0.5 mL (½ cc) syringe with an attached 27 G, 3/8-inch needle. A new syringe was used for each animal. Animals

were restrained in a manner that minimized irritation or pressure to the injection sites. To administer the dose, the total length of the needle was inserted and the plunger was pushed gently and slowly to administer the dose to the mid-scapular region.

In-Life Procedures

Clinical Observations (All Groups)

A detailed examination was performed once prior to dose administration and once daily through Day 14. Any unusual observations noted throughout the duration of the study were recorded in the raw data.

Animal Observations

Twice daily (a.m. and p.m.), animals were observed for mortality and signs of pain and distress, with the exception of Day 6, in which the p.m. check was not documented. Cageside observations for general health and appearance were done once daily. Any unusual observations noted throughout the duration of the study were recorded in the raw data.

Irritation Scoring

Injection site irritation was evaluated once daily beginning on Day 1 using a modified Draize technique.

Body Weights

Body weights were taken once daily beginning on Day 1.

Food Consumption

Quantitative individual food consumption was recorded twice per week.

Sample Collection and Protease Inhibitor Addition

Addition of Protease Inhibitor Solution

Prior to each sample collection, 4 μL of reconstituted Serine Protease Inhibitor Cocktail I (SPIC1 from A.G. Scientific; Fisher Catalog Number P1516 or

NC9600474, reconstituted by Covance) was added directly into the K2EDTA collection tube. Prepared tubes were stored at approximately 5°C no more than 24 hours prior to each blood sample collection.

Blood (approximately 0.4 mL) was collected from 3 animals/group predose and at 0.25, 1, 6, 24, 48, 72, 96 (Day 5), 144 (Day 7), 168 (Day 8), 216 (Day 10), and 312 (Day 14) hours postdose. Samples were collected according to the following table.

Groups	Subgroup	Number of Animals	Collection Timepoint (hr)
1-6	A	3	Predose, 1, 24, 72, 144, 216
	B	3	0.25, 6, 48, 96, 168, 312

Blood was collected from a jugular vein via syringe and needle followed by transfer into collection tubes containing K2EDTA + Serine Protease Inhibitor Cocktail I (SPIC1). After the addition of the whole blood, the collection tubes were gently mixed and placed immediately on wet ice.

Animal Disposition

After the final sample collection, animals were sacrificed by overdose of isoflurane. Carcasses were not retained.

Sample Handling and Storage

Following addition of the whole blood to the appropriate tubes, samples were gently mixed and immediately placed on wet ice prior to centrifugation at 2700 rpm for 10 minutes in a refrigerated centrifuge to obtain plasma. Centrifugation began within 30 minutes of collection and plasma was harvested. Plasma samples were transferred to clean storage tubes and placed on dry ice prior to storage at approximately -70°C.

RESULTS

Body Weights, Dose Administration, and Food Consumption

Individual animal body weights and dose administered are presented in Table 7.

Individual and group mean daily body weights (Days 1 through 14) are presented graphically in Figure 4.

Mean body weight for all groups decreased approximately 19 to 22% from Day 1 (predose) through Days 4-5, and then steadily increased to approximately the predose weights by Day 14. Mean daily food consumption was low on Days 1-5, and then increased through the end of the study.

Plasma Concentrations:

The following was observed with regard to the plasma concentration data (shown in Table 8 and Figures 5A-B):

- Cmax/Cave : 4 ~ 12
- Detectable plasma level through more than 7 days
- Bioavailability relative to immediate released formulation administrated subcutaneously: 114 ~ 140%

Injection-site scoring

Injection-site scoring (modified Draize technique) data are presented in Table 9. Edema scores generally ranged from none to slight. Erythema was not evident in any animals.

Sample Collections

According to Covance SOPs, all collections were made within the acceptable ranges. A summary of acceptable time ranges follows.

Scheduled Collection Time	Acceptable Deviation from Scheduled Time
0 – 15 minutes	± 1 minute
16 – 30 minutes	± 2 minutes

31 – 45 minutes	± 3 minutes
46 – 60 minutes	± 4 minutes
61 minutes – 2 hours	± 5 minutes
2 hours 1 minute – 8 hours	± 10 minutes
> 8 hours – 24 hours	± 20 minutes
> 24 hours	± 60 minutes

Table 7: Individual body weights of and doses administered to male rats given a single subcutan

Animal Number	Group	Active Compound	Formulation Number	Body Weight (g)	Dose Route	Dose Concentration (mg/mL)	Target Dose Level (mg/kg)	D Vo (mL/
B26947	1	Cmpd1	B09	396	SC	25	3.75	(
B26948	1	Cmpd1	B09	390	SC	25	3.75	(
B26949	1	Cmpd1	B09	389	SC	25	3.75	(
B26950	1	Cmpd1	B09	412	SC	25	3.75	(
B26951	1	Cmpd1	B09	404	SC	25	3.75	(
B26952	1	Cmpd1	B09	398	SC	25	3.75	(
B26953	2	Cmpd1	B10	408	SC	25	3.75	(
B26954	2	Cmpd1	B10	404	SC	25	3.75	(
B26955	2	Cmpd1	B10	376	SC	25	3.75	(
B26956	2	Cmpd1	B10	390	SC	25	3.75	(
B26957	2	Cmpd1	B10	382	SC	25	3.75	(
B26958	2	Cmpd1	B10	389	SC	25	3.75	(
B26959	3	Cmpd1	B11	384	SC	25	3.75	(
B26960	3	Cmpd1	B11	424	SC	25	3.75	(
B26961	3	Cmpd1	B11	396	SC	25	3.75	(
B26962	3	Cmpd1	B11	414	SC	25	3.75	(
B26963	3	Cmpd1	B11	419	SC	25	3.75	(
B26964	3	Cmpd1	B11	408	SC	25	3.75	(

SC Subcutaneous

Table 7 (continued): Individual body weights of and doses administered to male rats given a single sul

Animal Number	Group	Active Agent	Formulation Number	Body Weight (g)	Dose Route	Dose Concentration (mg/mL)	Target Dose Level (mg/kg)	D Vo (mL/
B26965	4	Cmpd1	B12	396	SC	25	3.75	(
B26966	4	Cmpd1	B12	414	SC	25	3.75	(
B26967	4	Cmpd1	B12	396	SC	25	3.75	(
B26968	4	Cmpd1	B12	387	SC	25	3.75	(
B26969	4	Cmpd1	B12	394	SC	25	3.75	(
B26970	4	Cmpd1	B12	379	SC	25	3.75	(
B26971	5	Cmpd1	B15	413	SC	25	3.75	(
B26972	5	Cmpd1	B15	401	SC	25	3.75	(
B26973	5	Cmpd1	B15	405	SC	25	3.75	(
B26974	5	Cmpd1	B15	421	SC	25	3.75	(
B26975	5	Cmpd1	B15	403	SC	25	3.75	(
B26976	5	Cmpd1	B15	401	SC	25	3.75	(
B26977	6	Cmpd1	B16	393	SC	25	3.75	(
B26978	6	Cmpd1	B16	399	SC	25	3.75	(
B26979	6	Cmpd1	B16	410	SC	25	3.75	(
B26980	6	Cmpd1	B16	385	SC	25	3.75	(
B26981	6	Cmpd1	B16	368	SC	25	3.75	(
B26982	6	Cmpd1	B16	390	SC	25	3.75	(

SC Subcutaneous

5

Table 8: Bioavailability data and plasma concentration data for male rats given a single subcuta

Test Article Lot#	Dose Level (mg/kg)*	Tmax (Hr)	Cmax (pg/mL)	AUC last (pg*hr/mL)	Cmax/Cave	Relative Percent Bioavailability	AUC 6hr (pg*hr/mL)
B09	3.75	1	191279	7019577	8.5	137	652,588
B10	3.75	1	208727	7044168	9.2	138	720,628
B11	3.75	1	167625	7131871	7.3	140	588,824
B12	3.75	1	90718	6636763	4.3	130	362,864
B15	3.75	1	220345	5800683	11.9	114	863,695
B16	3.75	72	86614	7019548	3.8	137	412,647

Table 9

Group/Sex:		1/M	2/M	3/M	4/M	5
Observation	Number in Group:	6	6	6	6	6
Test site A						
edema, none		1	2	3	2	1
edema, slight		4	6	4	4	4
edema, very slight		5	6	6	5	6
erythema, none		6	6	6	6	6

Test site A Area of observation (mid-scapular region surrounding injection site).

Example 10: Injectability

The following protocol was used to test the injectability of certain compositions of the invention:

- Draw 0.5 mL formulation into 1 mL syringe using 16G needle
- Switch to desired needle
- Place in Zwick force testing instrument
- Set instrument speed to deliver entire volume in 10 sec.
- Record maximum force, note any abnormalities
- Repeat three times, report average

Tested 25G RW 5/8" needles for all, 23G RW ¾" and 27G RW ½" for B09

Table 10: Results

	Maximum Force During Injection (N)					
Formulation	25G RW 5/8"				27G RW 1/2"	23G RW 3/4"
	1st Rep	2nd Rep	3rd Rep	average		
B09	26.7	27.7	27.7	27.4	>33**	11.3
B10	23.8	26.7*		25.2		
B11	24.2	23.7	23.2	23.7		
B12	26.1	26.7	23.1	25.3		
B15	25	17.1	15.7	19.2		
B16	29.9	28.1	28.3	28.8		

*Needle blew off on 2nd, not enough material left for 3rd repeat

**Needle blew off

Example 11: Solubility of Cmpd 1 in lipid formulations with different compositions

Formulations with the compositions presented in Table 11 were prepared by adding 975 mg of a lipid stock solution (previously manufactured by mixing all the

components except Cmpd 1 until a homogeneous solution was obtained) over 25 mg Cmpd 1 powder, mixing by brief vortex, followed by end-over-end rotation for up to 24h.

Table 11: Composition of samples used for solubility evaluation.

						Cmpd1*		Visual observation at 24h from preparation
Formulation	SPC/ wt.%	GDO/ wt.%	EtOH/ wt.%	PG/ wt.%	Water/ wt.%	Conc./ wt.%	Salt	
-424	38.8	38.8	4.9	15.0	--	2.50	Cl	Turbid, heterogeneous
-340	38.0	38.0	4.8	14.7	2.0	2.50	Cl	Clear, homogeneous
-425	33.8	33.8	14.9	--	15.0	2.50	Cl	Clear, homogeneous
-427	43.8	43.9	9.7	--	--	2.50	Cl	Turbid, heterogeneous

SPC = soy PC; GDO = glycerol dioleate; EtOH = ethanol; PG = propylene glycol; EDTA = ethylenediaminetetraacetic acid; * Cmpd1 from recombinant source.

The results in Table 11 clearly reflect the positive effect of water and/or PG for the solubility of Cmpd 1 in the lipid formulations.

Example 12: Pharmacokinetic and Pharmacodynamic Study in Dogs.

Pharmacokinetic and pharmacodynamic profiles were determined for a single dose subcutaneous administration of two exemplary, preferred sustained release formulations, B10 and B12, both containing Cmpd 1. The injectability of these compositions was studied in Example 10 with 2.5 wt% active agent. The results are shown in the relevant rows of Table 10 above. This study was conducted in accordance with generally recognized good laboratory practices, and all procedures were in compliance with the U.S. Animal Welfare Act Regulations (9 CFR 3).

Cmpd 1 was present in each formulation at a concentration of 25 mg/ml. A dose volume of 0.02 ml per kg of subject was administered subcutaneously, providing a Cmpd 1 dosage of 0.5 mg/kg, to each of four male dogs for each formulation. Eight male purebred beagles were acclimated and quarantined to the test site and study room for approximately two weeks prior to dose administration.

At the time of dose administration, animals were young adult/adult in age, approximately 9.2 kg at the start of the study. Animals were drug-naive prior to the first dose administration. During acclimation and the test period, animals were individually housed in stainless steel cages with plastic-coated flooring. Animals were not commingled for at least 24 hours after dose administration to allow monitoring of any test article-related effects. Environmental controls for the animal room were set to maintain a temperature of 20 to 26°C and a 12-hour light/12-hour dark cycle. As necessary, the 12-hour dark cycle was temporarily interrupted to accommodate study procedures. Approximately 5 cups of Harlan Teklad 2021 21% Protein Dog Diet (uncertified) were provided daily except as specified for dose administration (Protocol Deviation). Water was provided ad libitum.

Prior to use, the frozen formulations were allowed to thaw at ambient temperature for approximately 1 hour. Formulations were vortex mixed for approximately 10 seconds to ensure uniformity. Formulations for both groups were clear and yellow. Dose administration was completed within 8 hours after the formulations were removed from the freezer. For each group, the animals were fasted overnight through approximately 4 hours post dose. Individual doses were calculated based on body weights taken on the day of dosing. The subcutaneous doses were administered via sterile syringe and needle in the dorsal thoracic region. The dosing site was identified with a marker.

A detailed examination of the subjects was performed once prior to dose administration. Twice daily (a.m. and p.m.), animals were observed for mortality and signs of pain and distress. Cage side observations for general health and appearance were done once daily. Injection site irritation was evaluated approximately every 30 minutes through approximately 4 hours post dose and then once daily. Abnormalities or an indication of normal were recorded. Body weights were measured and recorded once daily beginning on the day of animal selection. Qualitative food consumption was recorded daily beginning on Day -1. Following the SC dose administration, blood (approximately 1 mL) was collected from each animal pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 (Day 2), 30, 36, 48 (Day 3), 60, 72 (Day 4), 84, 96 (Day 5), 120 (Day 6), 144 (Day 7), 168 (Day 8), 216 (Day 10), and 312 (Day 14) hours post-dose. Blood was collected from a jugular vein

into collection tubes containing potassium EDTA plus Serine Protease Inhibitor Cocktail I (SPIC1 from A.G.Scientific; Fisher Catalog Number P1516). After the addition of the whole blood, the collection tubes were gently mixed and placed immediately on wet ice, in chilled cryoracks, or at approximately 5°C prior to centrifugation to obtain plasma. Upon completion of the in-life portion of the study, animals were transferred to the stock colony. Blood was maintained on wet ice prior to centrifugation to obtain plasma. Centrifugation (3000 rpm for 10 minutes in a refrigerated centrifuge) began within 30 minutes of collection. Separated plasma was transferred into individual tubes for storage and shipment. Plasma samples were placed on dry ice prior to storage at approximately -70°C.

Results. Percent change in body weight from baseline Day 1 is plotted in Fig. 6C. Body weights generally decreased from Day 1 to Days 3-5, leveled off by days 6-8, then increased to approximately Day 1 levels by the end of the 14-day post-dose study periods. Fig. 6C presents the percent body weight change induced in subjects by Cmpd 1 delivered in sustained release formulations B10 and B12. Both formulations significantly reduced body weight from baseline, and did so for at least 6, 7 or 8 days. In Fig. 6C open symbols and star represent formulation B10 results and closed symbols represent formulation B12 results. Thus a sustained weight loss effect was observed. Qualitative food consumption results (data not shown) indicate that food consumption generally decreased from Day -1 until Day 3-5, and then increased during the remainder of the in-life phase. Thus a sustained inhibition of food intake was observed. All animals appeared healthy prior to dosing and throughout the duration of the study, with the following exceptions of 1 dog treated with B10 vomiting on the first and second day, and 1 dog treated with B12 vomiting once mid-way thru the study. Injection site irritation was not observed.

Figures 6A and 6B provide the pharmacokinetic profile of Cmpd 1 plasma concentration over the 312 hour study. Dog subjects provide a PK profile and parameters, including Cmax, Cave and Cmax/Cave ratio, that more closely predicts and approximates human parameters than do rats. Rats provide a relatively rapid and less expensive means to compare and rank profiles and parameters.

Error! Reference source not found.6A presents the 0 to 312 hour (two week) drug (Cmpd 1) plasma concentration profile of formulation B10 and

formulation B12 following single subcutaneous administration in dogs. B10 is presented as closed circle and B12 as closed square in Figs. 6A and 6B. Both formulations provide detectable Cmpd 1 plasma levels through two weeks with no obvious terminal slope.

Figure presents the 0 to 36 hour time points from Fig. 6A, which expands the period of initial release ("initial burst") of drug. In Fig. 6B the T_{max} for B10 and B12 are 2 and 3 hours, respectively. The C_{max} for B10 and B12 are 62,309 and 30,639 pg/mL, respectively.

Table12 presents an analysis of the pharmacokinetic results for the single, subcutaneous administration of the two exemplary and preferred formulations in dog. Maximum drug plasma concentration is C_{max}, average drug plasma concentration is C_{ave}, and their ratio is C_{max}/C_{ave} ratio.

Table 12.

Form.	**Dose mg/kg**	**T_{max} (hr)**	**C_{max} (pg/mL)**	**AUC_{last} (pg*hr/mL)**	**AUC $_{30-312h}$ (pg*hr/mL)**	**C_{ave}* (pg/mL)**	**C_{max}/C_{ave}***	**%AUC SR**
B10	0.5	2.00	62,309	1,951,189	1,409,113	6,254	10.0	72%
B12	0.5	3.00	30,639	1,345,269	1,075,994	4,312	7.1	80%

* C_{ave} calculated as AUC $_{last\ (312hr)}$.

Both formulations provided a $C_{max}/C_{ave} \leq 10$, with formulation B10 yielding a Cmax/Cave ratio of 10.0 and B12 a more desirable ratio of 7.1. On the other hand, formulation B10 appears to provide a greater overall exposure (as AUC_{last}; 0 to 312 hr period) relative to B12. Assuming an immediate release to sustained release (IR to SR) division at t = 30 hour, then most of the drug exposure is in the sustained-release form. The SR portion AUC (from 30 to 312 hours) of the total AUC_{0-last} (from 0 to 312 hours) was 72% and 80% for B10 and B12, respectively.

CLAIMS:

1) A pre-formulation comprising a low viscosity mixture of:

a) 20-80 wt.% of at least one diacyl glycerol and/or a tocopherol;
b) 20-80 wt.% of at least one phosphatidyl choline (PC);
c) 5-35 wt.% of at least one biocompatible, organic alcoholic solvent;
d) up to 20 wt.% aqueous solvent
e) at least one dual amylin receptor/GLP-1 receptor agonist active agent
f) optionally at least one antioxidant;

wherein the pre-formulation forms, or is capable of forming, at least one liquid crystalline phase structure upon contact with excess aqueous fluid.

2) A pre-formulation as claimed in claim 1 wherein component a) comprises glycerol dioleate (GDO).

3) A pre-formulation as claimed in any of claims 1 to 2 wherein component b) comprises soy PC and/or synthetic DOPC.

4) A pre-formulation as claimed in any of claims 1 to 3 wherein component c) comprises at least one mono-ol, diol or triol; preferably component c) comprises ethanol or propylene glycol or mixtures thereof.

5) A pre-formulation as claimed in any of claims 1 to 4 wherein component d) comprises water.

6) A pre-formulation as claimed in any of claims 1 to 5 wherein the dual amylin receptor/GLP-1 receptor agonist active agent is CMPD1.

7) A pre-formulation as claimed in any of claims 1 to 6 wherein the antioxidant is ascorbic acid, EDTA or citric acid, preferably EDTA.

8) A pre-formulation as claimed in claims 1 to 7 wherein component a) is present at a level of 30-40% by weight.

9) A pre-formulation as claimed in claims 1 to 8 wherein component b) is present at a level of 30-40% by weight.

10) A pre-formulation as claimed in claims 1 to 9 wherein component c) is present at a level of 10-25% by weight.

11) A pre-formulation as claimed in claims 1 to 10 wherein component d) is present at a level of 1.2 to 15% by weight.

12) A pre-formulation as claimed in any of claims 1 to 11 wherein the ratio of components a:b is in the range 40:60 to 70:30.

13) A pre-formulation as claimed in any of claims 1 to 12 wherein the ratio of components d:f is in the range 100:1 to 10000:1, preferably 1000:1.

14) A pre-formulation as claimed in any of claims 1 to 13 wherein said pre-formulation has an L_2 phase structure.

15) A pre-formulation as claimed in any of claims 1 to 14 comprising at least one formulation selected from those listed in Table 1.

16) A pre-formulation as claimed in any preceding claim that releases a dose of at least one dual amylin receptor/GLP-1 receptor agonist active agent having a concentration greater than the therapeutic concentration for at least 7 days.

17) A pre-formulation as claimed in any of claims 1 to 16 that releases a dose of at least one dual amylin receptor/GLP-1 receptor agonist active agent such that Caver < Cther but CaveM > Cther, where Caver is the average plasma concentration following a single administration, CaveM is the average plasma concentration following multiple administrations and Cther is the minimum therapeutic concentration.

18) A process for the formation of a pre-formulation suitable for the administration of a dual amylin receptor/GLP-1 receptor agonist bioactive agent to a (preferably mammalian) subject, said process comprising forming a low viscosity mixture of:

a) 20-80 wt.% of at least one diacyl glycerol and/or a tocopherol;
b) 20-80 wt.% of at least one phosphatidyl choline (PC);
c) 5-35 wt.% of at least one biocompatible, organic alcoholic solvent;
d) up to 20 wt.% aqueous solvent
e) at least one dual amylin receptor/GLP-1 receptor agonist active agent
f) optionally at least one antioxidant;

and dissolving at least one dual amylin receptor/GLP-1 receptor agonist active agent (preferably Cmpd 1 in the low viscosity mixture, or in at least one of components a), b), c), d) and optionally f) prior to forming the low viscosity mixture.

19) Use of a low viscosity mixture of:

a) 20-80 wt.% of at least one diacyl glycerol and/or a tocopherol;
b) 20-80 wt.% of at least one phosphatidyl choline (PC);
c) 5-35 wt.% of at least one biocompatible, organic alcoholic solvent;
d) up to 20 wt.% aqueous solvent
e) at least one dual amylin receptor/GLP-1 receptor agonist active agent
f) optionally at least one antioxidant;

in the manufacture of a pre-formulation for use in the sustained administration of said dual amylin receptor/GLP-1 receptor agonist active agent.

20) Use as claimed in claim 19 wherein the low viscosity mixture is as defined in any of claims 1 to 17.

21) A method for the treatment of a human or non-human mammalian subject comprising administering to said subject a pre-formulation as claimed in any of claims 1 to 17.

22) The method of claim 21 for the treatment of a human or non-human mammalian subject in need thereof to combat at least one condition selected from diabetes, type I diabetes, type II diabetes, excess bodyweight, need for bodyweight reduction, obesity, hypertension, arthrosclerosis, dyslipidemia, congestive heart failure, stroke, hypercholesterolemia, cardiovascular disease, myocardial ischemia, myocardial reperfusion, eating disorders, gestational diabetes, diabetic neuropathy, pulmonary hypertension, or a metabolic disease or disorder associated with insufficient pancreatic beta cell mass..

23) The method of claim 22 to simultaneously combat of at least two of the conditions indicated in claim 22.

24) The method of claim 23 to combat obesity, overweight or a need for body weight reduction and at least one condition benefited from; reduction of hyperglycemia, plasma glucose lowering, delay of gastric emptying, increased insulin secretion, glucagon suppression and/or HbA1c lowering.

25) The method of claim 23 to combat both diabetes and at least one of obesity, overweight or need for body weight reduction.

26) The method of claim 24 to combat diabetes or type II diabetes and at least one of obesity, overweight or a need for body weight reduction.

27) A formulation as claimed in any of claims 1 to 17 for use in a method as claimed in any one of claims 21 to 26.

28) A method of cosmetic treatment of a human or non-human mammalian subject comprising administering to said subject a pre-formulation as claimed in any of claims 1 to 17.

29) Use of:

a) 20-80 wt.% of at least one diacyl glycerol and/or a tocopherol;
b) 20-80 wt.% of at least one phosphatidyl choline (PC);
c) 5-35 wt.% of at least one biocompatible, organic alcoholic solvent;
d) up to 20 wt.% aqueous solvent
e) at least dual amylin receptor/GLP-1 receptor agonist active agent
f) optionally at least one antioxidant;

in the manufacture of a low viscosity pre-formulation medicament for use in the *in vivo* formation of a depot for treatment of at least one of type I diabetes, type II diabetes, excess bodyweight, obesity, hypertension, arthrosclerosis, dyslipidemia, congestive heart failure, stroke, hypercholesterolemia, cardiovascular disease, myocardial ischemia, myocardial reperfusion, eating disorders, gestational diabetes, diabetic neuropathy, pulmonary hypertension, or a metabolic disease or disorder associated with insufficient pancreatic beta cell mass.

30) A pre-filled administration device containing a pre-formulation as claimed in any of claims 1 to 17.

31) A device according to claim 30 wherein said pre-formulation delivers a dosage in the range of 1 μg to 10 mg/day.

32) A device according to any of claims 30 to 31 wherein each administered dose is a total volume for administration of no more than 3 ml, preferably no more than 1 ml more preferably no more than 0.5 ml

33) A kit comprising an administration device as claimed in any of claims 30 to 32.

34) A pre-formulation, use, method, device or kit of any one of the preceding claims wherein the at least one diacyl glycerol and/or a tocopherol is present at 33-38% by weight or a value from Table 1.

35) A pre-formulation, use, method, device or kit of any one of the preceding claims wherein the at least one diacyl glycerol is GDO.

36) A pre-formulation, use, method, device or kit of any one of the preceeding claims wherein the at least one PC is present at 33-38% by weight or a value from Table 1.

37) A pre-formulation, use, method, device or kit of any one of the preceding claims wherein the at least one PC is soy PC or DOPC.

38) A pre-formulation, use, method, device or kit of any one of the preceding claims wherein the at least one biocompatible, organic alcoholic solvent is present at 33-38% by weight or a value from table 1.

39) A pre-formulation, use, method, device or kit of any one of the preceding claims wherein the at least one biocompatible, organic alcoholic solvent is ethanol and/or PG.

40) A pre-formulation, use, method, device or kit of any one of the preceding claims wherein the at least one biocompatible, organic alcoholic solvent is ethanol and is present at 15% and PG is absent; or the at least one biocompatible, organic alcoholic solvent is a mixture of ethanol and PG wherein ethanol is present at 5% and PG is present at 15%.

41) A pre-formulation, use, method, device or kit of any one of the preceding claims wherein the total aqueous solvent is present in a 15% to 20% range and/or a value from table 1.

42) A pre-formulation, use, method, device or kit of any one of the preceding claims wherein the antioxidant is present in a 0.002 % to 0.015 % range and/or a value from Table 1.

43) A pre-formulation, use, method, device or kit of any one of the preceding claims wherein the active agent is Cmpd 1.

44) A pre-formulation, use, method, device or kit of any one of the preceding claims that is selected from the group B9, B10, B11, B12, B15 and B16.

45) A pre-formulation, use, method, device or kit of any one of the preceding claims that is selected from B12 or B16.

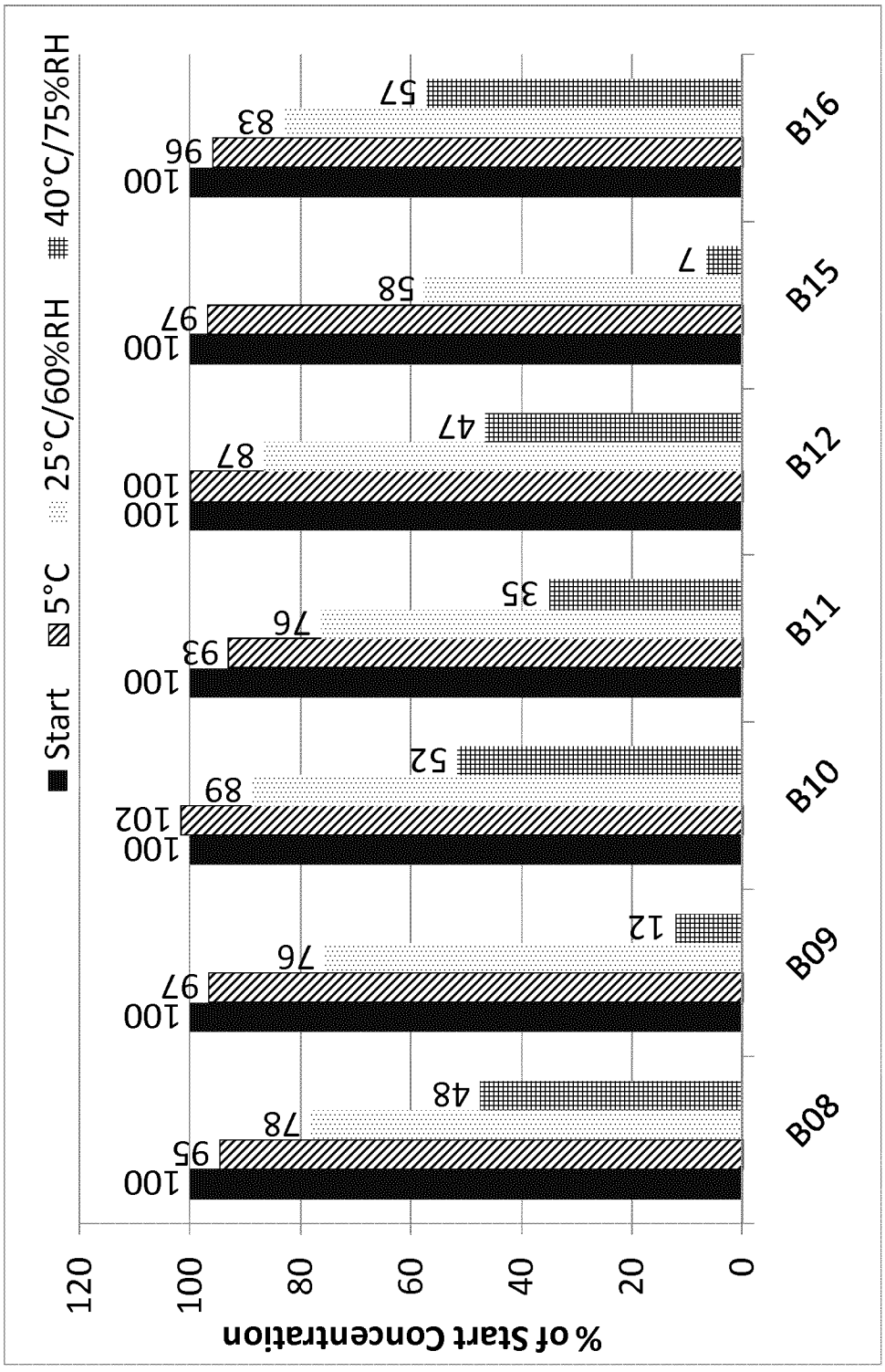


Figure 1 - Change in peptide concentration (vs. start value measured directly manufacturing) after storage of the formulations with compositions presented in Table 1 for 3 months (formulation 16: 2 months) in different conditions.

Figure 2A

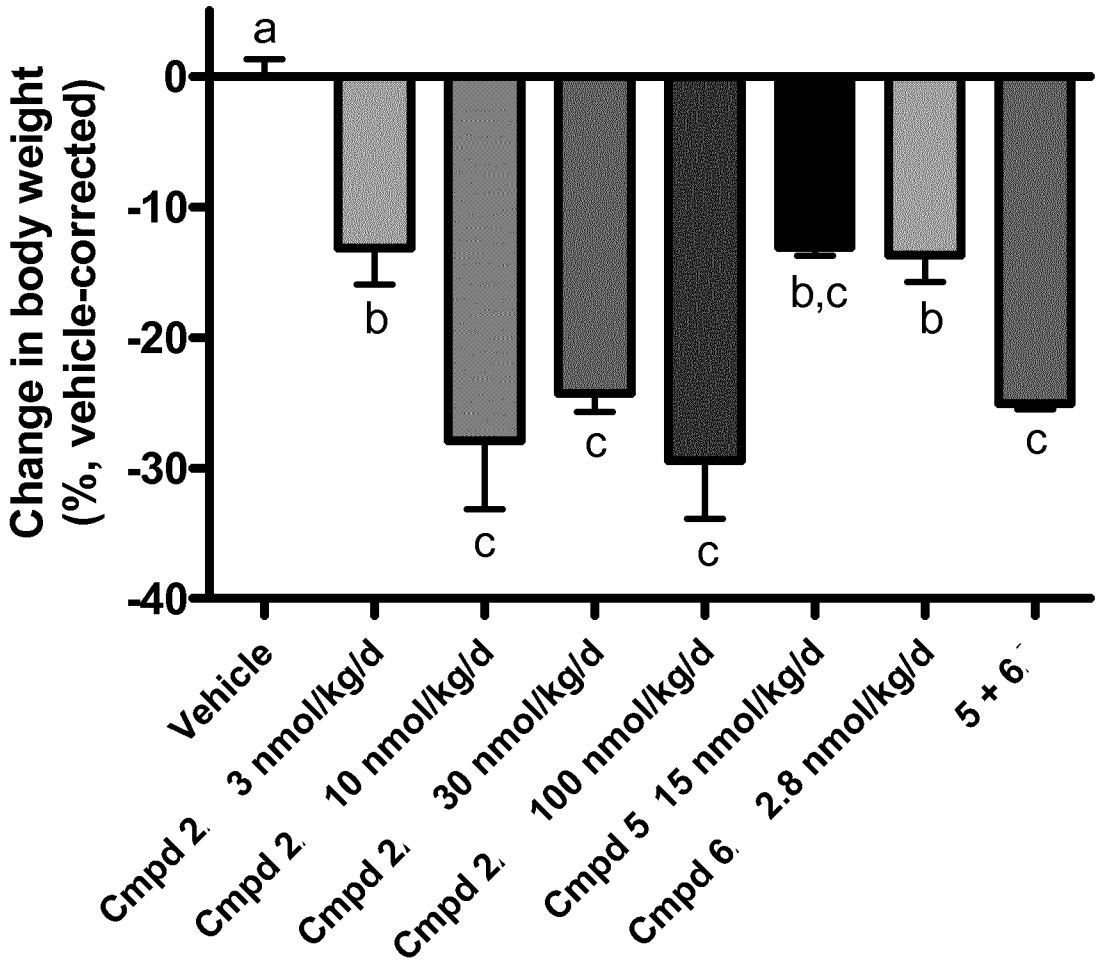

Change in body weight
(%, vehicle-corrected)
0
-10
-20
-30
-40
a
b
c
c
c
b,c
b
c
Vehicle
3 nmol/kg/d
10 nmol/kg/d
30 nmol/kg/d
100 nmol/kg/d
15 nmol/kg/d
2.8 nmol/kg/d
Cmpd 2
Cmpd 2
Cmpd 2
Cmpd 2
Cmpd 5
Cmpd 6
5 + 6

Figure 2B

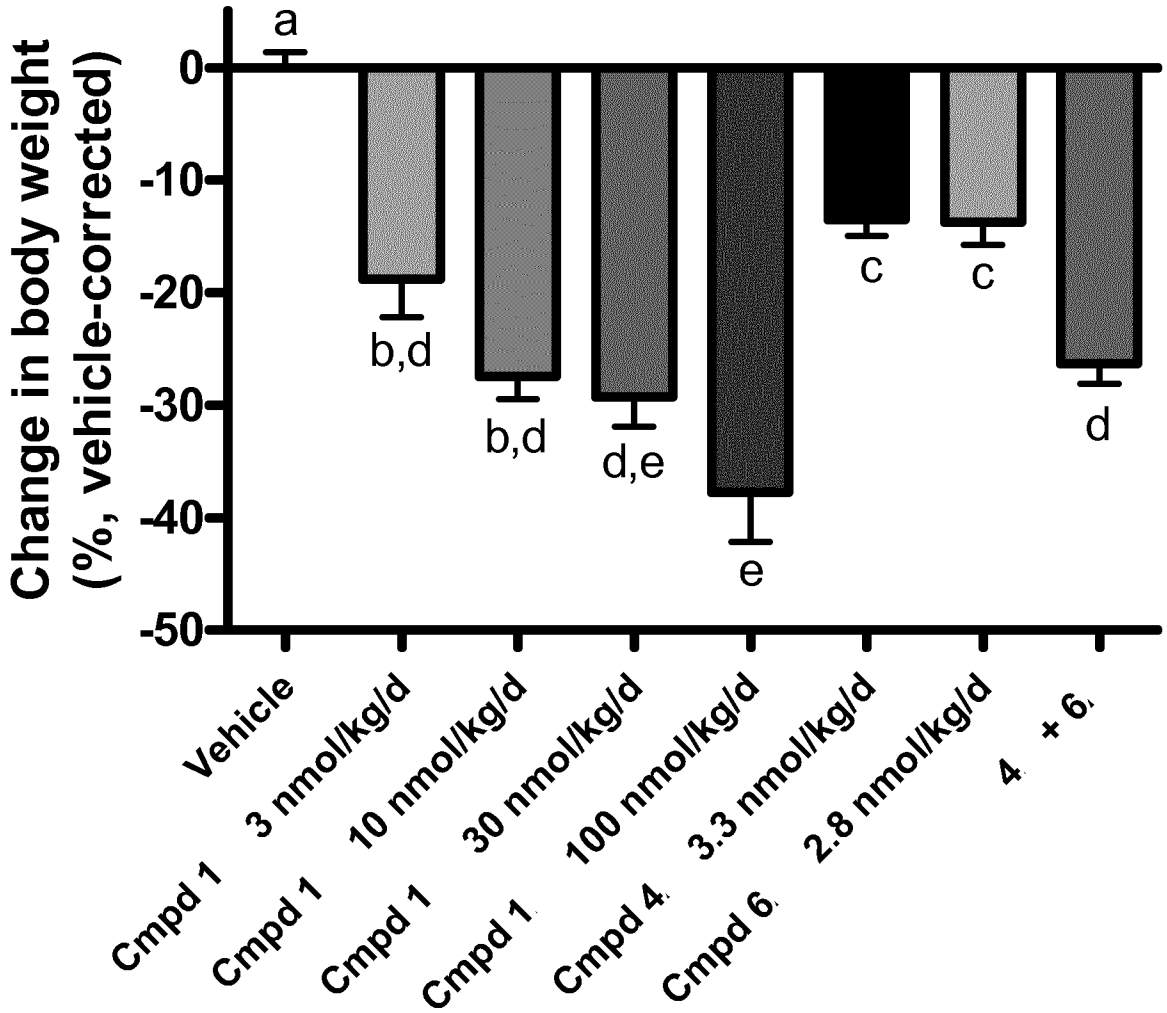

Change in body weight
(%, vehicle-corrected)
0
-10
-20
-30
-40
-50
a
b,d
b,d
d,e
e
c
c
d
Vehicle
3 nmol/kg/d
10 nmol/kg/d
30 nmol/kg/d
100 nmol/kg/d
3.3 nmol/kg/d
2.8 nmol/kg/d
Cmpd 1
Cmpd 1
Cmpd 1
Cmpd 1
Cmpd 4
Cmpd 6
4 + 6

Figure 3A

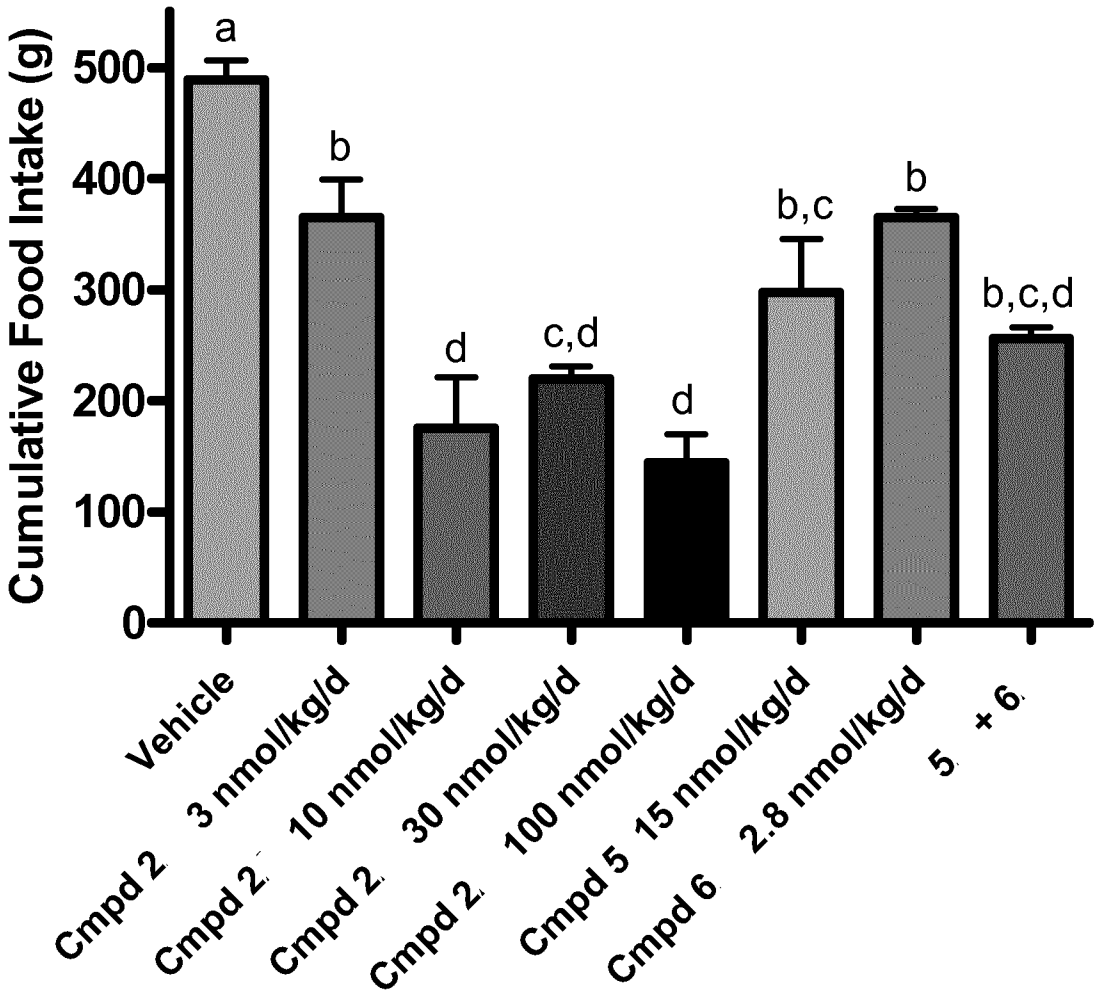

Cumulative Food Intake (g)
0
100
200
300
400
500
a
b
d
c,d
d
b,c
b
b,c,d
Vehicle
3 nmol/kg/d
10 nmol/kg/d
30 nmol/kg/d
100 nmol/kg/d
15 nmol/kg/d
2.8 nmol/kg/d
+ 6

Figure 3B

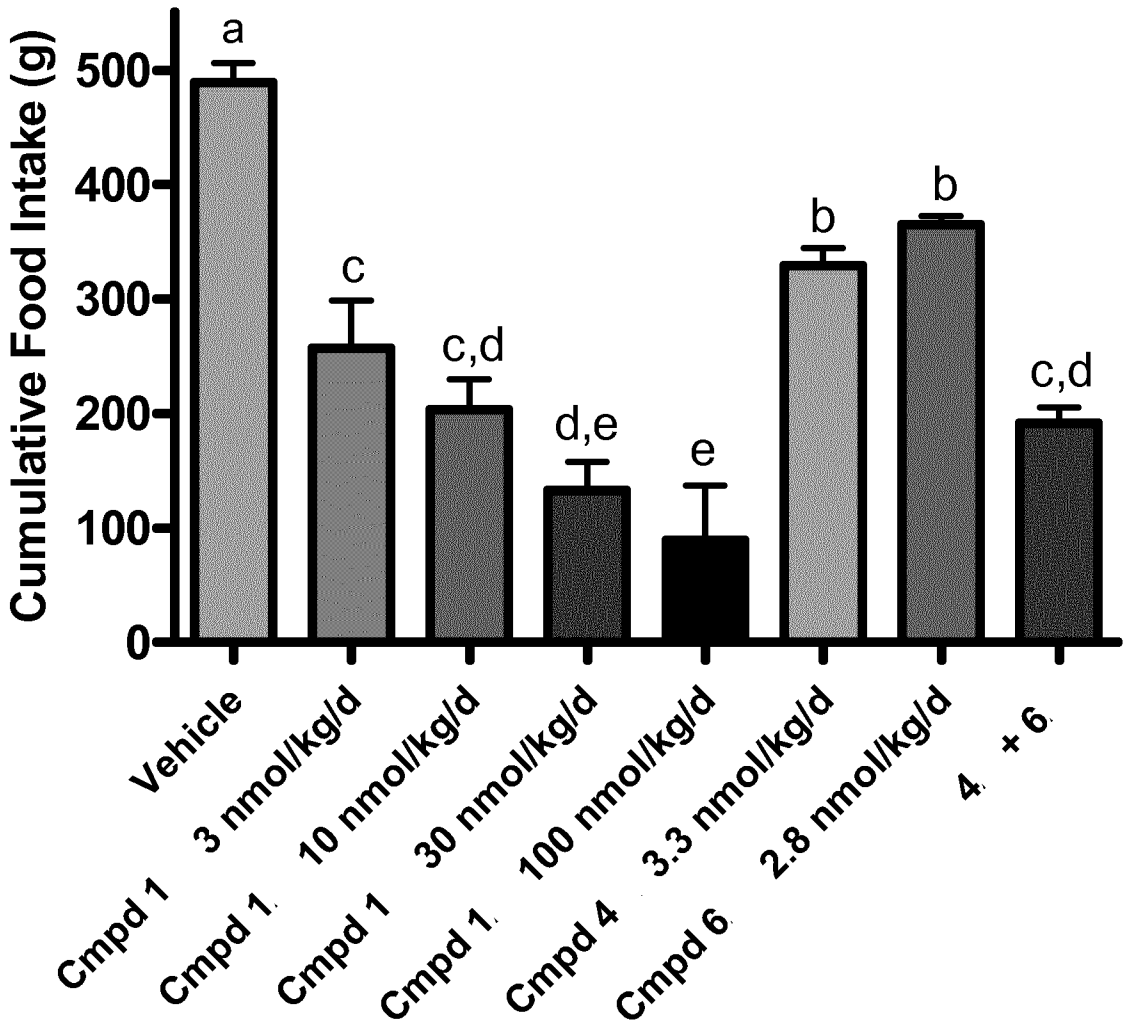

Cumulative Food Intake (g)
0
100
200
300
400
500
a
c
c,d
d,e
e
b
b
c,d
Vehicle
3 nmol/kg/d
10 nmol/kg/d
30 nmol/kg/d
100 nmol/kg/d
3.3 nmol/kg/d
2.8 nmol/kg/d
Cmpd 1
Cmpd 1
Cmpd 1
Cmpd 1
Cmpd 4
Cmpd 6
4 + 6

Figure 4 Mean body weights of male rats following administration of a single subcutaneous dose of Cmpd1

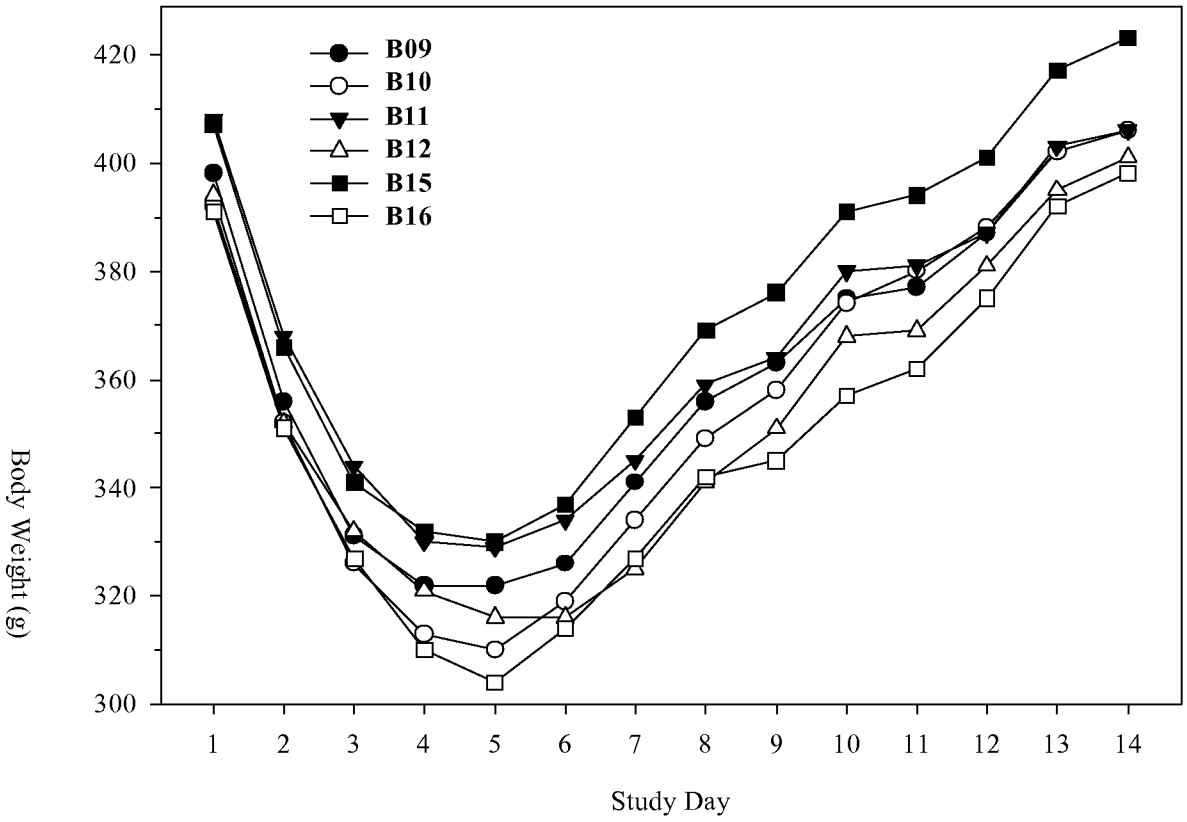


Figure 5
Cmpd 1: Single Dose Subcutaneous Pharmacokinetic Study in Rats, Plasma Concentration variation over time

Figure 5A - timescale up to 312 hours

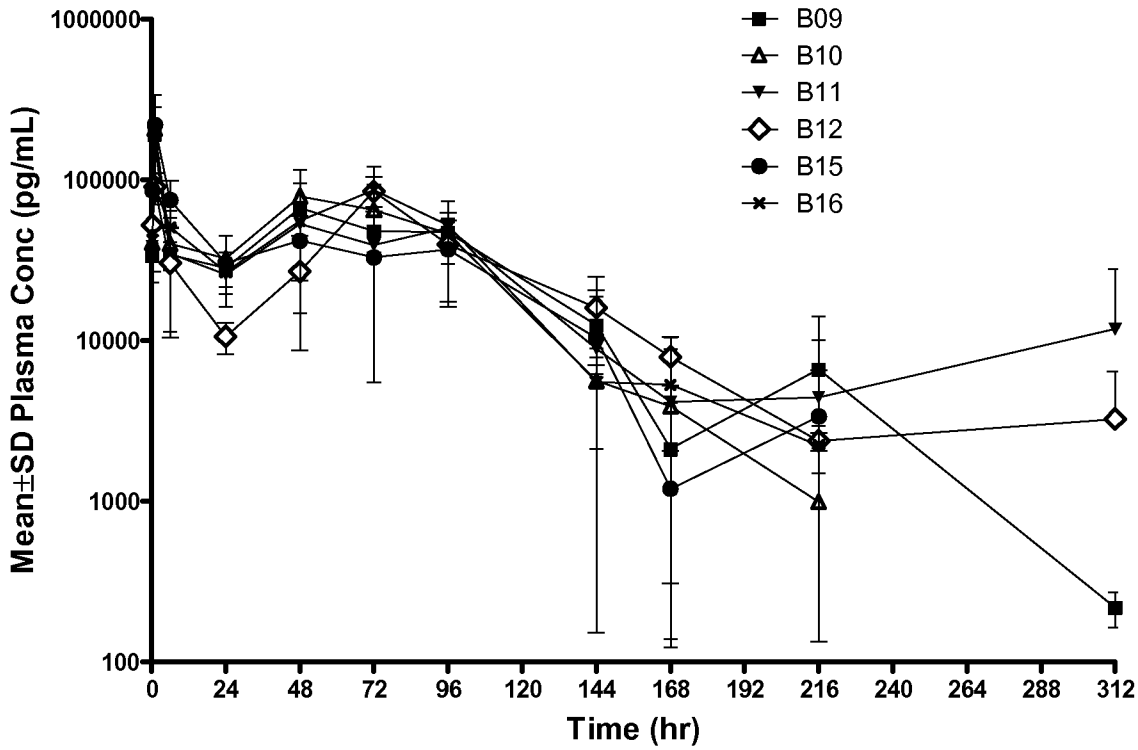


Figure 5B - timescale up to 24 hours

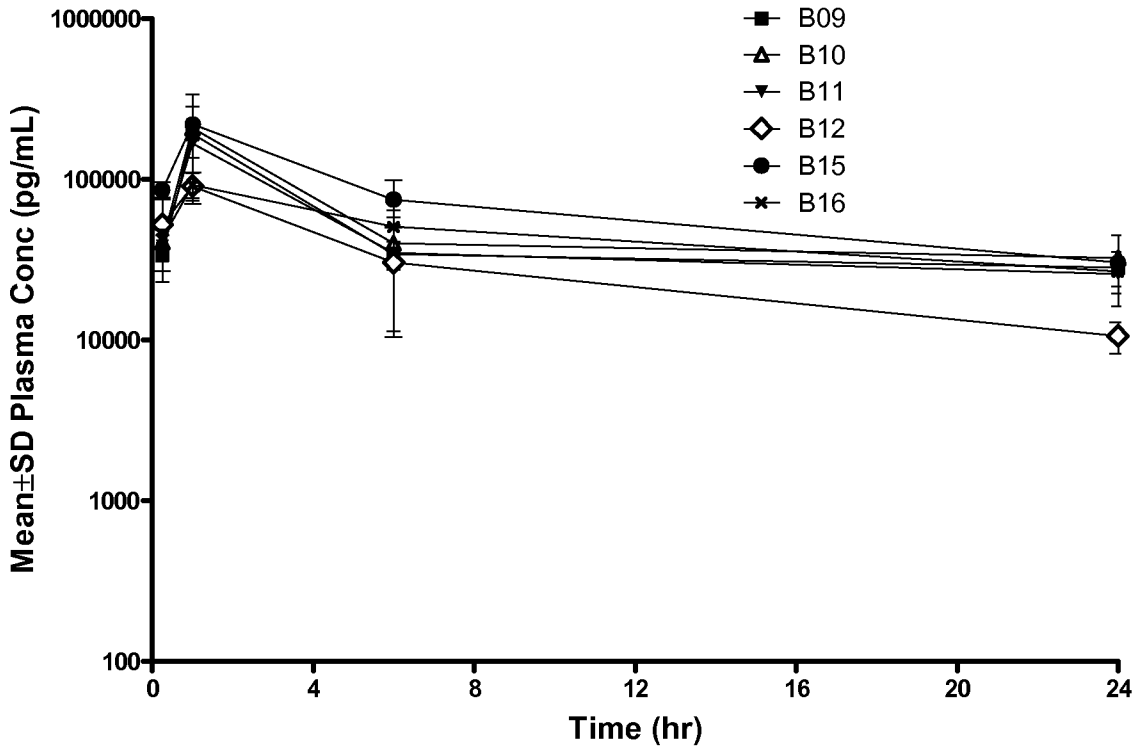


Figure 6A

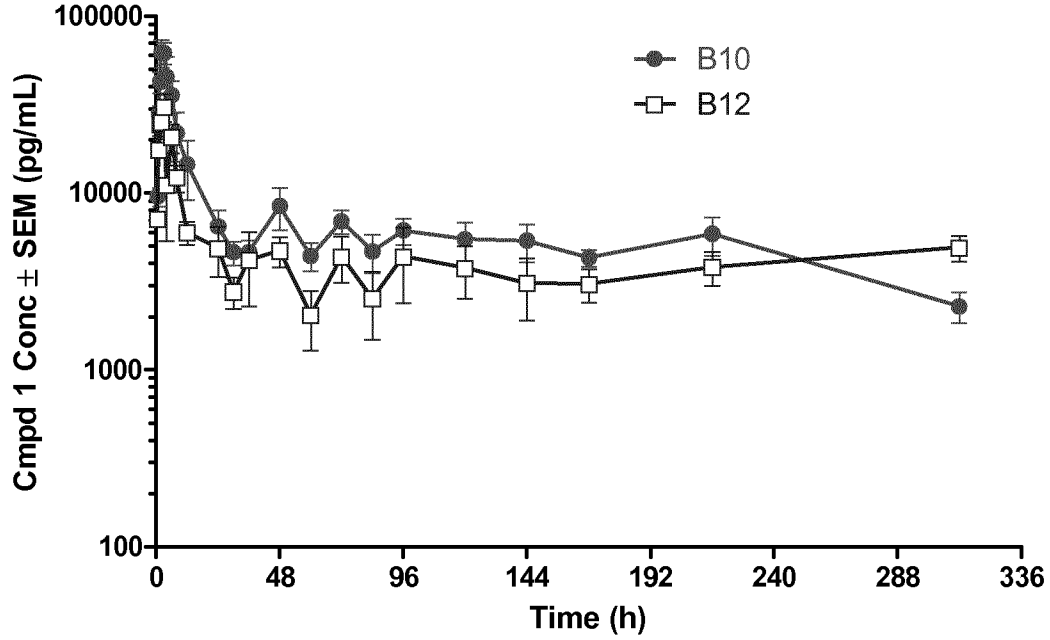


Figure 6B

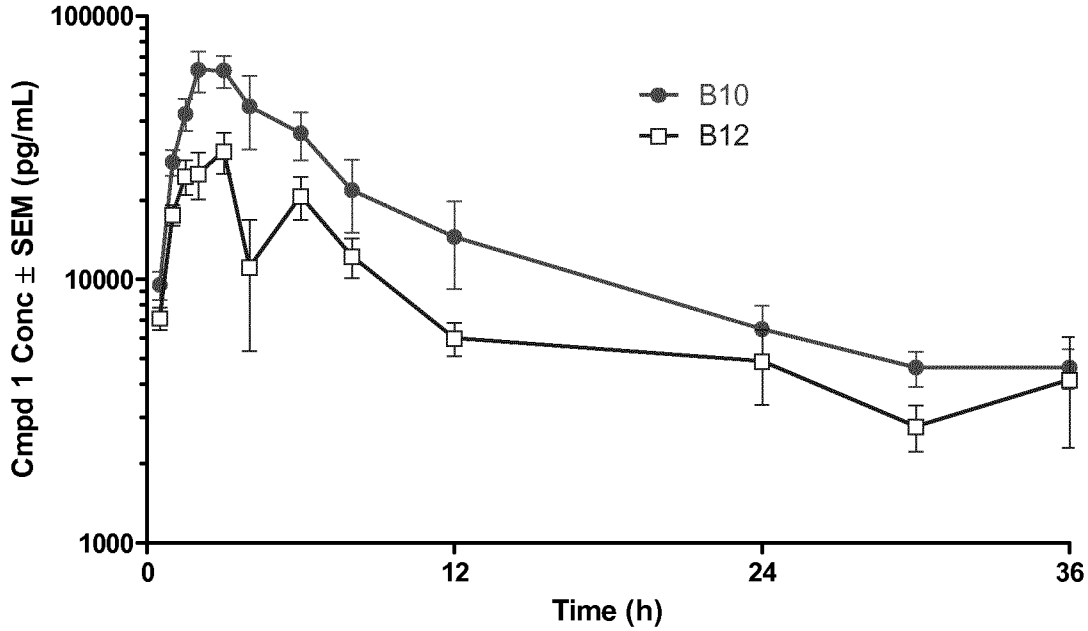
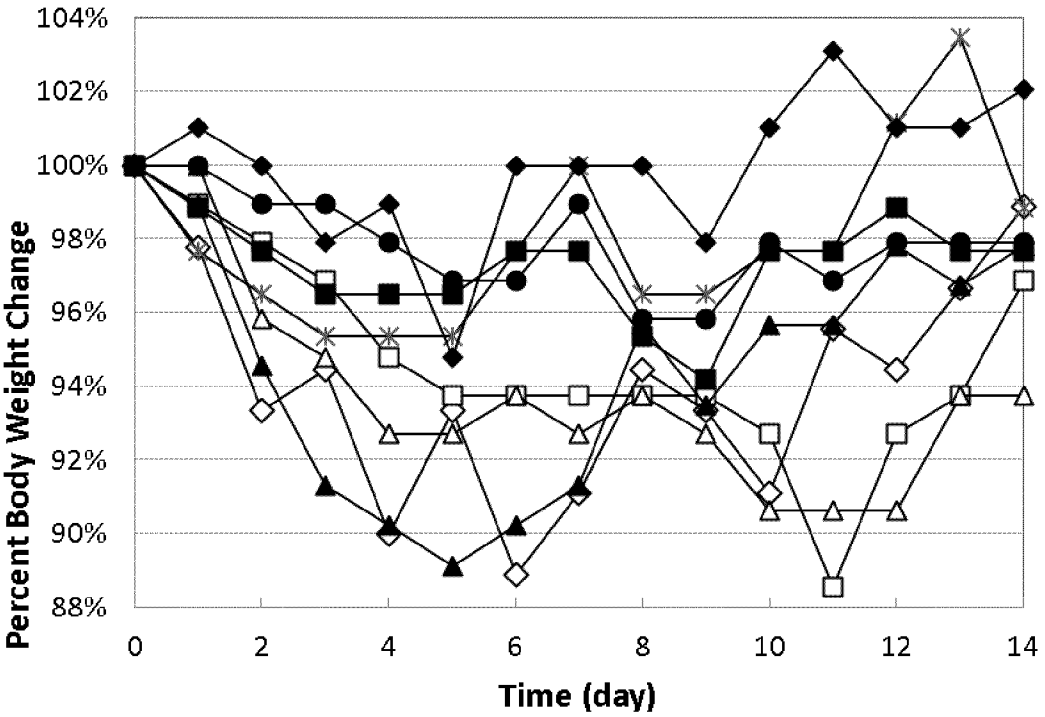


Figure 6C



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/059916

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/127 A61K38/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/131730 A1 (CAMURUS AB [SE]; GODDARD CHRISTOPHER [GB]; JOABSSON FREDRIK [SE]; JOHN) 14 December 2006 (2006-12-14) the whole document ----- -/--	1-14, 16-33, 35,37, 39,40, 43-45

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 October 2012	19/10/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Raposo, Antonio

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/059916

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	EP 2 052 716 A1 (CAMURUS AB [SE]) 29 April 2009 (2009-04-29) page 2, paragraph 1 page 3, paragraph 13-17 page 4, paragraph 21-25 pages 5-6 page 7, paragraph 44-46 page 8, paragraph 58-59 pages 9-10 page 11, paragraph 82-83	1-14, 16-33, 35,37, 39,40, 43-45
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A	SHAH JAYMIN C ET AL: "Cubic phase gels as drug delivery systems", ADVANCED DRUG DELIVERY REVIEWS, ELSEVIER BV, AMSTERDAM, NL, vol. 47, no. 2-3, 25 April 2001 (2001-04-25), pages 229-250, XP002320651, ISSN: 0169-409X, DOI: 10.1016/S0169-409X(01)00108-9 the whole document	1-14, 16-33, 35,37, 39,40, 43-45

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/059916

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