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DESCRIPTION

Description

[0001] The present invention relates to pharmaceutical compositions comprising a controlled-release PTH compound or a pharmaceutically acceptable salt, hydrate, or solvate thereof, for use in a method of treating hypoparathyroidism, wherein the pharmaceutical composition comprising the controlled-release PTH compound is administered in accordance with a dosage regimen in which dose adjustment in response to hypocalcemia or hypercalcemia is performed in increments of no more than 25%.

[0002] Hypoparathyroidism is a rare endocrine disorder of calcium and phosphate metabolism that most often arises as a result of parathyroid gland damage or removal during surgery of the thyroid gland. Hypoparathyroidism is unusual among endocrine disorders in that it has not been treated, until recently, by replacement with the missing hormone, parathyroid hormone, or PTH. Conventional therapy for hypoparathyroidism involves large doses of vitamin D and oral calcium supplementation, which, although often effective, is associated with marked swings in blood Ca^{2+} resulting in hypercalcemia and hypocalcemia, excess urinary calcium excretion, nephrocalcinosis, and ectopic calcifications, including vascular, basal ganglia, and lens of eye.

[0003] Calcium is the most abundant mineral in the human body, and its tight regulation is required for many critical biological functions, such as bone mineralization, muscle contraction, nerve conduction, hormone release, and blood coagulation. It is particularly important to maintain calcium concentration as stable as possible, because of the high sensitivity of a variety of cell systems or organs, including the central nervous system, muscle, and exo/endocrine glands, to small variations in Ca^{2+}. PTH is a major regulator of calcium homeostasis.

[0004] The inappropriately low or frankly low PTH levels in relation to serum Ca^{2+} concentration, characteristic of hypoparathyroidism, leads to decreased renal tubular reabsorption of Ca^{2+} and simultaneously, to increased renal tubular reabsorption of phosphate. Thus, the main biochemical abnormalities of hypoparathyroidism are hypocalcemia and hyperphosphatemia. Clinical features of the disease include symptoms of hypocalcemia, such as perioral numbness, paresthesias, and carpal/pedal muscle spasms. Laryngeal spasm, tetany, and seizures are serious and potentially life-threatening complications. Hyperphosphatemia and an elevated calcium x phosphate product contributes to ectopic deposition of insoluble calcium phosphate complexes in soft tissues, including vasculature, brain, kidneys, and other organs.

[0005] Standard therapy of hypoparathyroidism is oral calcium and vitamin D supplementation. The goals of therapy are to a) ameliorate symptoms of hypocalcemia; b) maintain fasting serum calcium within or slightly below to the low-normal range; c) maintain fasting serum phosphorus within the high normal range or only slightly elevated; d) avoid or minimize hypercalciuria; e) maintain a calcium-phosphate product at levels well below the upper limit of normal and f) avoid ectopic calcification of the kidney (stones and nephrocalcinosis) and other soft tissues.

[0006] Several concerns arise with prolonged use of calcium and active vitamin D in large doses, particularly with regard to hypercalciuria, kidney stones, nephrocalcinosis and ectopic soft tissue calcification. In addition, conventional therapy with calcium and active vitamin D does not alleviate quality of life complaints nor does it reverse abnormalities in bone remodeling characteristic of the disease. In short, there is a high need for improved therapies for hypoparathyroidism.

[0007] In 2015, Natpara, PTH(1-84), was approved for once-daily subcutaneous injection as an adjunct to vitamin D and calcium in patients with hypoparathyroidism. Natpara, PTH(1-84), was approved to control hypocalcemia based on a pivotal trial demonstrating that 42 percent of PTH(1-84) treated participants achieved normal blood calcium levels on reduced doses of calcium supplements and active forms of vitamin D, compared to 3 percent of placebo-treated participants. Following a time course in which serum calcium was monitored after injection, 71 percent of patients treated with PTH(1-84) developed hypercalcemia at one or more measurements during a 24-hour period. PTH(1-84) reduced urinary calcium excretion 2-8 hours after injection but over the 24-hour period, urinary calcium excretion did not change. Similarly, urinary phosphate excretion increased only during the first 8 hours after PTH(1-84) injection.

[0008] While this represents an important advance in the treatment of the disease, Natpara has not demonstrated an ability to reduce incidences of hypercalcemia (elevated serum calcium levels), hypocalcemia (low serum calcium), or hypercalciuria (elevated urinary calcium) relative to conventional therapy in treated patients.

[0009] As such, there is a high need for improved PTH based therapies for hypoparathyroidism.

[0010] PTH(1-34), or teriparatide, was approved by the FDA in 2002 for the treatment of osteoporosis. Despite not being approved for this indication, PTH(1-34) has historically been used for treatment of hypoparathyroidism with patients receiving twice- or thrice-daily injections. To facilitate more physiological PTH levels, clinical studies have been conducted with PTH(1-34) administered by pump delivery in comparison with twice-daily injections. Over 6-months, pump delivery produced normal, steady state calcium levels with minimal fluctuation and avoided the rise in serum and urine calcium levels that are evident soon after PTH injection. The marked reduction in urinary calcium excretion when PTH(1-34) is administered by pump may indicate that PTH must be continuously exposed to the renal tubule for the renal

calcium-conserving effects to be realized. Pump delivery of PTH(1-34) achieved simultaneous normalization of markers of bone turnover, serum calcium, and urine calcium excretion. These results were achieved with a 65 percent lower daily PTH(1-34) dose and a reduced need for magnesium supplementation compared with the twice daily PTH(1-34) injection regimen.

[0011] However, continuous pump therapy is inconvenient and challenging for patients, and it is an object of the current invention to provide for a more convenient therapeutic option of providing continuous exposure to PTH.

[0012] Long-term daily administration of PTH is associated with a progressive cortical bone loss due to increased bone metabolism. In a 6-year follow-up of patients treated with PTH(1-84) (Rubin, JCEM 2016) bone turnover markers remained greater than pretreatment values, peaking at the early years after PTH(1-84) initiation and declining thereafter but remaining significantly higher than baseline values by year 6. BMD by DXA was consistent with known site-specific effects of PTH, namely increases in lumbar spine and declines in distal 1/3 radius. The decrease observed at the distal one third radius is consistent with the known effects of intermittent PTH to increase cortical porosity and endosteal resorption.

[0013] It is an object of this invention to provide for a method of intermittently administering PTH, with improved control of serum and urine calcium, serum phosphorus, and lower elevation of bone turnover markers than currently applied PTH therapies. Preferably intermittent means with daily intervals, or more preferred with weekly intervals.

[0014] In the preclinical development program of both Forteo, PTH(1-34), and Natpara, PTH(1-84), a dose dependent increase in osteosarcoma rate was observed in rats treated with daily injections of the PTH compound. In the Natpara study, dosing of the high dose rats were discontinued due to excessive deaths in this group, primarily from metastatic osteosarcoma. This is felt to be due to the sensitivity of rats to the anabolic effects of intermittent PTH. In contrast, continuous exposure to PTH is known to lack significant bone anabolic activity. As such is an object of this invention to provide for an intermittent PTH replacement therapy that provides for an infusion-like profile of PTH, resulting in improved symptom control with a lower administered dose. Preferably intermittent means with daily intervals, or more preferred with weekly intervals.

[0015] WO 2004/024758 discloses PTH-containing compounds for use in the treatment of hypoparathyroidism, which may be formulated for sustained release.

[0016] In summary, there is a need for a more convenient and safer treatment of hypoparathyroidism with reduced side-effects.

[0017] It is therefore an object of the present invention to at least partially overcome the shortcomings described above.

[0018] This object is achieved with a pharmaceutical composition comprising a controlled-

release PTH compound or a pharmaceutically acceptable salt, hydrate, or solvate thereof, for use in a method of treating hypoparathyroidism as described in the claims.

[0019] It was surprisingly found that such controlled-release PTH compound has a higher potency than PTH 1-84, so dose adjustment for a pharmaceutical composition comprising such controlled-release PTH compounds can be performed in smaller increments than for pharmaceutical compositions comprising PTH 1-84, in which doses in response to hypocalcemia or hypercalcemia are doubled or halved, respectively, in order to achieve the right dose. The typical starting dose is 50 µg/day, which is titrated down to 25 µg/day (50%) in the event of hypercalcemia, or titrated up to 75 µg/day at first (50%) and then 100 µg/day (33.3%) in the event of hypocalcemia, with most subjects requiring either 75 or 100 µg/day.

[0020] Within the present invention the terms are used having the meaning as follows.

[0021] As used herein the term "increment" refers to the increase or decrease in the amount of controlled-release PTH compound based on a previously administered dose of the same controlled-release PTH compound which increase or decrease is given as a certain percentage based on the weight amount of the controlled-release PTH compound.

[0022] As used herein the term "controlled-release PTH compound" refers to any compound, conjugate, crystal or admixture that comprises at least one PTH molecule or moiety and from which the at least one PTH molecule or moiety is released with a release half-life of at least 12 hours.

[0023] As used herein the terms "release half-life" and "half-life" refer to the time required under physiological conditions (i.e. aqueous buffer, pH 7.4, 37°C) until half of all PTH or PTH moieties, respectively, comprised in a controlled-release PTH compound are released from said controlled-release PTH compound.

[0024] As used herein the term "PTH" refers to all PTH polypeptides, preferably from mammalian species, more preferably from human and mammalian species, more preferably from human and murine species, as well as their variants, analogs, orthologs, homologs, and derivatives and fragments thereof, that are characterized by raising serum calcium and renal phosphorus excretion, and lowering serum phosphorus and renal calcium excretion. The term "PTH" also refers to all PTHrP polypeptides, such as the polypeptide of SEQ ID NO:121, that bind to and activate the common PTH/PTHrP1 receptor. Preferably, the term "PTH" refers to the PTH polypeptide of SEQ ID NO:51 as well as its variants, homologs and derivatives exhibiting essentially the same biological activity, i.e. raising serum calcium and renal phosphorus excretion, and lowering serum phosphorus and renal calcium excretion.

[0025] Preferably, the term "PTH" refers to the following polypeptide sequences:

SEQ ID NO:1 (PTH 1-84)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLTKAKSQ

SEQ ID NO:2 (PTH 1-83)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLTKAKS

SEQ ID NO:3 (PTH 1-82)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLTKAK

SEQ ID NO:4 (PTH 1-81)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLTKA

SEQ ID NO:5 (PTH 1-80)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLTK

SEQ ID NO:6 (PTH 1-79)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLT

SEQ ID NO:7 (PTH 1-78)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVL

SEQ ID NO:8 (PTH 1-77)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNV

SEQ ID NO:9 (PTH 1-76)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVN

SEQ ID NO:10 (PTH 1-75)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADV

SEQ ID NO:11 (PTH 1-74)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKAD

SEQ ID NO:12 (PTH 1-73)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKA

SEQ ID NO:13 (PTH 1-72)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADK

SEQ ID NO:14 (PTH 1-71)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLVESHEKSLGEAD

SEQ ID NO:15 (PTH 1-70)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLVESHEKSLGEA

SEQ ID NO:16 (PTH 1-69)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLVESHEKSLGE

SEQ ID NO:17 (PTH 1-68)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLVESHEKSLG

SEQ ID NO:18 (PTH 1-67)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLVESHEKSL

SEQ ID NO:19 (PTH 1-66)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLVESHEKS

SEQ ID NO:20 (PTH 1-65)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLVESHEK

SEQ ID NO:21 (PTH 1-64)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLVESHE

SEQ ID NO:22 (PTH 1-63)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLVESH

SEQ ID NO:23 (PTH 1-62)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLVES

SEQ ID NO:24 (PTH 1-61)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLVE

SEQ ID NO:25 (PTH 1-60)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVLV

SEQ ID NO:26 (PTH 1-59)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

NVL

SEQ ID NO:27 (PTH 1-58)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NV

SEQ ID NO:28 (PTH 1-57)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
N

SEQ ID NO:29 (PTH 1-56)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED

SEQ ID NO:30 (PTH 1-55)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKE

SEQ ID NO:31 (PTH 1-54)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKK

SEQ ID NO:32 (PTH 1-53)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRK

SEQ ID NO:33 (PTH 1-52)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPR

SEQ ID NO:34 (PTH 1-51)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRP

SEQ ID NO:35 (PTH 1-50)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQR

SEQ ID NO:36 (PTH 1-49)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQ

SEQ ID NO:37 (PTH 1-48)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGS

SEQ ID NO:38 (PTH 1-47)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAG

SEQ ID NO:39 (PTH 1-46)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDA

SEQ ID NO:40 (PTH 1-45)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRD

SEQ ID NO:41 (PTH 1-44)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPR

SEQ ID NO:42 (PTH 1-43)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAP

SEQ ID NO:43 (PTH 1-42)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLA

SEQ ID NO:44 (PTH 1-41)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPL

SEQ ID NO:45 (PTH 1-40)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAP

SEQ ID NO:46 (PTH 1-39)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGA

SEQ ID NO:47 (PTH 1-38)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALG

SEQ ID NO:48 (PTH 1-37)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVAL

SEQ ID NO:49 (PTH 1-36)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVA

SEQ ID NO:50 (PTH 1-35)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFV

SEQ ID NO:51 (PTH 1-34)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF

SEQ ID NO:52 (PTH 1-33)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHN

SEQ ID NO:53 (PTH 1-32)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVH

SEQ ID NO:54 (PTH 1-31)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDV

SEQ ID NO:55 (PTH 1-30)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQD

SEQ ID NO:56 (PTH 1-29)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQ

SEQ ID NO:57 (PTH 1-28)
SVSEIQLMHNLGKHLNSMERVEWLRKKL

SEQ ID NO:58 (PTH 1-27)
SVSEIQLMHNLGKHLNSMERVEWLRKK

SEQ ID NO:59 (PTH 1-26)

SVSEIQLMHNLGKHLNSMERVEWLRK

SEQ ID NO:60 (PTH 1-25)
SVSEIQLMHNLGKHLNSMERVEWLR

SEQ ID NO:61 (amidated PTH 1-84)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLTKAKSQ; wherein the C-terminus is amidated

SEQ ID NO:62 (amidated PTH 1-83)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLTKAKS; wherein the C-terminus is amidated

SEQ ID NO:63 (amidated PTH 1-82)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLTKAK; wherein the C-terminus is amidated

SEQ ID NO:64 (amidated PTH 1-81)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLTKA; wherein the C-terminus is amidated

SEQ ID NO:65 (amidated PTH 1-80)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLTK; wherein the C-terminus is amidated

SEQ ID NO:66 (amidated PTH 1-79)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVLT; wherein the C-terminus is amidated

SEQ ID NO:67 (amidated PTH 1-78)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNVL; wherein the C-terminus is amidated

SEQ ID NO:68 (amidated PTH 1-77)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVNV; wherein the C-terminus is amidated

SEQ ID NO:69 (amidated PTH 1-76)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADVN; wherein the C-terminus is amidated

SEQ ID NO:70 (amidated PTH 1-75)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKADV; wherein the C-terminus is amidated

SEQ ID NO:71 (amidated PTH 1-74)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKAD; wherein the C-terminus is amidated

SEQ ID NO:72 (amidated PTH 1-73)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADKA; wherein the C-terminus is amidated

SEQ ID NO:73 (amidated PTH 1-72)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEADK; wherein the C-terminus is amidated

SEQ ID NO:74 (amidated PTH 1-71)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEAD; wherein the C-terminus is amidated

SEQ ID NO:75 (amidated PTH 1-70)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGEA; wherein the C-terminus is amidated

SEQ ID NO:76 (amidated PTH 1-69)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLGE; wherein the C-terminus is amidated

SEQ ID NO:77 (amidated PTH 1-68)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSLG; wherein the C-terminus is amidated

SEQ ID NO:78 (amidated PTH 1-67)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKSL; wherein the C-terminus is amidated

SEQ ID NO:79 (amidated PTH 1-66)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEKS; wherein the C-terminus is amidated

SEQ ID NO:80 (amidated PTH 1-65)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHEK; wherein the C-terminus is amidated

SEQ ID NO:81 (amidated PTH 1-64)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED
NVLVESHE; wherein the C-terminus is amidated

SEQ ID NO:82 (amidated PTH 1-63)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED NVLVESH;
wherein the C-terminus is amidated

SEQ ID NO:83 (amidated PTH 1-62)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED NVLVES;
wherein the C-terminus is amidated

SEQ ID NO:84 (amidated PTH 1-61)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED NVLVE; wherein the C-terminus is amidated

SEQ ID NO:85 (amidated PTH 1-60)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED NVLV; wherein the C-terminus is amidated

SEQ ID NO:86 (amidated PTH 1-59)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED NVL; wherein the C-terminus is amidated

SEQ ID NO:87 (amidated PTH 1-58)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED NV; wherein the C-terminus is amidated

SEQ ID NO:88 (amidated PTH 1-57)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED N; wherein the C-terminus is amidated

SEQ ID NO:89 (amidated PTH 1-56)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKED ; wherein the C-terminus is amidated

SEQ ID NO:90 (amidated PTH 1-55)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKKE; wherein the C-terminus is amidated

SEQ ID NO:91 (amidated PTH 1-54)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRKK; wherein the C-terminus is amidated

SEQ ID NO:92 (amidated PTH 1-53)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRK; wherein the C-terminus is amidated

SEQ ID NO:93 amidated PTH 1-52)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPR; wherein the C-terminus is amidated

SEQ ID NO:94 (amidated PTH 1-51)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRP; wherein the C-terminus is amidated

SEQ ID NO:95 (amidated PTH 1-50)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQR; wherein the C-terminus is amidated

SEQ ID NO:96 (amidated PTH 1-49)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQ; wherein the C-terminus is amidated

SEQ ID NO:97 (amidated PTH 1-48)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGS; wherein the C-terminus is amidated

SEQ ID NO:98 (amidated PTH 1-47)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAG; wherein the C-terminus is amidated

SEQ ID NO:99 (amidated PTH 1-46)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDA; wherein the C-terminus is amidated

SEQ ID NO:100 (amidated PTH 1-45)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRD; wherein the C-terminus is amidated

SEQ ID NO:101 (amidated PTH 1-44)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPR; wherein the C-terminus is amidated

SEQ ID NO:102 (amidated PTH 1-43)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLAP; wherein the C-terminus is amidated

SEQ ID NO:103 (amidated PTH 1-42)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPLA; wherein the C-terminus is amidated

SEQ ID NO:104 (amidated PTH 1-41)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAPL; wherein the C-terminus is amidated

SEQ ID NO:105 (amidated PTH 1-40)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGAP; wherein the C-terminus is amidated

SEQ ID NO:106 (amidated PTH 1-39)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALGA; wherein the C-terminus is amidated

SEQ ID NO:107 (amidated PTH 1-38)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVALG; wherein the C-terminus is amidated

SEQ ID NO:108 (amidated PTH 1-37)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVAL; wherein the C-terminus is amidated

SEQ ID NO:109 (amidated PTH 1-36)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVA; wherein the C-terminus is amidated

SEQ ID NO:110 (amidated PTH 1-35)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFV; wherein the C-terminus is amidated

SEQ ID NO:111 (amidated PTH 1-34)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNF; wherein the C-terminus is amidated

SEQ ID NO:112 (amidated PTH 1-33)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHN; wherein the C-terminus is amidated

SEQ ID NO:113 (amidated PTH 1-32)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVH; wherein the C-terminus is amidated

SEQ ID NO:114 (amidated PTH 1-31)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQDV; wherein the C-terminus is amidated

SEQ ID NO:115 (amidated PTH 1-30)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQD; wherein the C-terminus is amidated

SEQ ID NO:116 (amidated PTH 1-29)
SVSEIQLMHNLGKHLNSMERVEWLRKKLQ; wherein the C-terminus is amidated

SEQ ID NO:117 (amidated PTH 1-28)
SVSEIQLMHNLGKHLNSMERVEWLRKKL; wherein the C-terminus is amidated

SEQ ID NO:118 (amidated PTH 1-27)
SVSEIQLMHNLGKHLNSMERVEWLRKK; wherein the C-terminus is amidated

SEQ ID NO:119 (amidated PTH 1-26)
SVSEIQLMHNLGKHLNSMERVEWLRK; wherein the C-terminus is amidated

SEQ ID NO:120 (amidated PTH 1-25)
SVSEIQLMHNLGKHLNSMERVEWLR; wherein the C-terminus is amidated

SEQ ID NO:121 (PTHrP)
AVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHTAEIRATSEVSPNSKPSPNTKNHPVRF
GSDDEGRYLTQETNKVETYKEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLDS
GVTGSGLEGDHLSDTSTTSLELDSRRH

[0026] More preferably, the term "PTH" refers to the sequence of SEQ ID:NOs 47, 48, 49, 50, 51, 52, 53, 54, 55, 107, 108, 109, 110, 111, 112, 113, 114 and 115. Even more preferably, the term "PTH" refers to the sequence of SEQ ID:NOs 50, 51, 52, 110, 111 and 112. In a particularly preferred embodiment the term "PTH" refers to the sequence of SEQ ID NO:51.

[0027] As used herein, the term "PTH polypeptide variant" refers to a polypeptide from the same species that differs from a reference PTH or PTHrP polypeptide. Preferably, such reference is a PTH polypeptide sequence and has the sequence of SEQ ID NO:51. Generally, differences are limited so that the amino acid sequence of the reference and the variant are closely similar overall and, in many regions, identical. Preferably, PTH polypeptide variants are at least 70%, 80%, 90%, or 95% identical to a reference PTH or PTHrP polypeptide, preferably to the PTH polypeptide of SEQ ID NO:51. By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. These alterations of the reference sequence may occur at the amino (N-terminal) or carboxy terminal (C-terminal) positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. The query sequence may be an entire amino acid sequence of the reference sequence or any fragment specified as described herein. Preferably, the query sequence is the sequence of SEQ ID NO:51.

[0028] Such PTH polypeptide variants may be naturally occurring variants, such as naturally occurring allelic variants encoded by one of several alternate forms of a PTH or PTHrP occupying a given locus on a chromosome or an organism, or isoforms encoded by naturally occurring splice variants originating from a single primary transcript. Alternatively, a PTH polypeptide variant may be a variant that is not known to occur naturally and that can be made by mutagenesis techniques known in the art.

[0029] It is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus of a bioactive polypeptide without substantial loss of biological function. Such N- and/or C-terminal deletions are also encompassed by the term PTH polypeptide variant.

[0030] It is also recognized by one of ordinary skill in the art that some amino acid sequences of PTH or PTHrP polypeptides can be varied without significant effect of the structure or function of the polypeptide. Such mutants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al. (1990), Science 247:1306-1310, wherein the authors indicate that there are two main approaches for studying the tolerance of the amino acid sequence to change.

[0031] The term "PTH polypeptide" also encompasses all PTH and PTHrP polypeptides encoded by PTH and PTHrP analogs, orthologs, and/or species homologs. It is also recognized by one of ordinary skill in the art that PTHrP and PTHrP analogs bind to an activate the common PTH/PTHrP1 receptor, so the term PTH polypeptide also encompasses all PTHrP analogs. As used herein, the term "PTH analog" refers to PTH and PTHrP of different and unrelated organisms which perform the same functions in each organism but which did not

originate from an ancestral structure that the organisms' ancestors had in common. Instead, analogous PTH and PTHrP arose separately and then later evolved to perform the same or similar functions. In other words, analogous PTH and PTHrP polypeptides are polypeptides with quite different amino acid sequences but that perform the same biological activity, namely raising serum calcium and renal phosphorus excretion, and lowering serum phosphorus and renal calcium excretion.

[0032] As used herein the term "PTH ortholog" refers to PTH and PTHrP within two different species which sequences are related to each other via a common homologous PTH or PTHrP in an ancestral species, but which have evolved to become different from each other.

[0033] As used herein, the term "PTH homolog" refers to PTH and PTHrP of different organisms which perform the same functions in each organism and which originate from an ancestral structure that the organisms' ancestors had in common. In other words, homologous PTH polypeptides are polypeptides with quite similar amino acid sequences that perform the same biological activity, namely raising serum calcium and renal phosphorus excretion, and lowering serum phosphorus and renal calcium excretion. Preferably, PTH polypeptide homologs may be defined as polypeptides exhibiting at least 40%, 50%, 60%, 70%, 80%, 90% or 95% identity to a reference PTH or PTHrP polypeptide, preferably the PTH polypeptide of SEQ ID NO:51.

[0034] Thus, a PTH polypeptide according to the invention may be, for example: (i) one in which at least one of the amino acids residues is substituted with a conserved or non-conserved amino acid residue, preferably a conserved amino acid residue, and such substituted amino acid residue may or may not be one encoded by the genetic code; and/or (ii) one in which at least one of the amino acid residues includes a substituent group; and/or (iii) one in which the PTH polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); and/or (iv) one in which additional amino acids are fused to the PTH polypeptide, such as an IgG Fc fusion region polypeptide or leader or secretory sequence or a sequence which is employed for purification of the above form of the polypeptide or a pre-protein sequence.

[0035] As used herein, the term "PTH polypeptide fragment" refers to any polypeptide comprising a contiguous span of a part of the amino acid sequence of a PTH or PTHrP polypeptide, preferably the polypeptide of SEQ ID NO:51.

[0036] More specifically, a PTH polypeptide fragment comprises at least 6, such as at least 8, at least 10 or at least 17 consecutive amino acids of a PTH or PTHrP polypeptide, more preferably of the polypeptide of SEQ ID NO:51. A PTH polypeptide fragment may additionally be described as sub-genuses of PTH or PTHrP polypeptides comprising at least 6 amino acids, wherein "at least 6" is defined as any integer between 6 and the integer representing the C-terminal amino acid of a PTH or PTHrP polypeptide, preferably of the polypeptide of SEQ ID No:51. Further included are species of PTH or PTHrP polypeptide fragments at least 6 amino acids in length, as described above, that are further specified in terms of their N-terminal and

C-terminal positions. Also encompassed by the term "PTH polypeptide fragment" as individual species are all PTH or PTHrP polypeptide fragments, at least 6 amino acids in length, as described above, that may be particularly specified by a N-terminal and C-terminal position. That is, every combination of a N-terminal and C-terminal position that a fragment at least 6 contiguous amino acid residues in length could occupy, on any given amino acid sequence of a PTH or PTHrP polypeptide, preferably the PTH polypeptide of SEQ ID:NO51, is included in the present invention.

[0037] The term "PTH" also includes poly(amino acid) conjugates which have a sequence as described above, but having a backbone that comprises both amide and non-amide linkages, such as ester linkages, like for example depsipeptides. Depsipeptides are chains of amino acid residues in which the backbone comprises both amide (peptide) and ester bonds. Accordingly, the term "side chain" as used herein refers either to the moiety attached to the alpha-carbon of an amino acid moiety, if the amino acid moiety is connected through amine bonds such as in polypeptides, or to any carbon atom-comprising moiety attached to the backbone of a poly(amino acid) conjugate, such as for example in the case of depsipeptides. Preferably, the term "PTH" refers to polypeptides having a backbone formed through amide (peptide) bonds.

[0038] As the term "PTH" includes the above-described variants, analogs, orthologs, homologs, derivatives and fragments of PTH and PTHrP, all references to specific positions within a reference sequence also include the equivalent positions in variants, analogs, orthologs, homologs, derivatives and fragments of a PTH or PTHrP moiety, even if not specifically mentioned.

[0039] As used herein the term "micelle" means an aggregate of amphiphilic molecules dispersed in a liquid colloid. In aqueous solution a typical micelle forms an aggregate with the hydrophilic moiety of the surfactant molecules facing the surrounding solvent and the hydrophobic moiety of the surfactant molecule facing inwards, also called "normal-phase micelle". "Invers micelles" have the hydrophilic moiety facing inwards and the hydrophobic moiety facing the surrounding solvent.

[0040] As used herein the term "liposome" refers to a vesicle, preferably a spherical vesicle, having at least one lipid bilayer. Preferably, liposomes comprise phospholipids, even more preferably phosphatidylcholine. The term "liposome" refers to various structures and sizes, such as, for example, to multilamellar liposome vesicles (MLV) having more than one concentric lipid bilayer with an average diameter of 100 to 1000 nm, small unilamellar liposome vesicles (SUV) having one lipid bilayer and an average diameter of 25 to 100 nm, large unilamellar liposome vesicles (LUV) having one lipid bilayer and an average diameter of about 1000 µm and giant unilamellar vesicles (GUV) having one lipid bilayer and an average diameter of 1 to 100 µm. The term "liposome" also includes elastic vesicles such as transferosomes and ethosomes, for example.

[0041] As used herein the term "aquasome" refers to spherical nanoparticles having a diameter of 60 to 300 nm that comprise at least three layers of self-assembled structure,

namely a solid phase nanocrystalline core coated with an oligomeric film to which drug molecules are adsorbed with or without modification of the drug.

[0042] As used herein the term "ethosome" refers to lipid vesicles comprising phospholipids and ethanol and/or isopropanol in relatively high concentration and water, having a size ranging from tens of nanometers to micrometers.

[0043] As used herein the term "LeciPlex" refers to positively charged phospholipid-based vesicular system which comprises soy PC, a cationic agent, and a bio-compatible solvent like PEG 300, PEG 400, diethylene glycol monoethyl ether, tetrahydrofurfuryl alcohol polyethylene glycol ether or 2-pyrrolidoneor N-methyl-2-pyrrolidone.

[0044] As used herein the term "niosome" refers to unilamellar or multilamellar vesicles comprising non-ionic surfactants.

[0045] As used herein the term "pharmacosome" refers to ultrafine vesicular, micellar or hexagonal aggregates from lipids covalently bound to biologically active moieties.

[0046] As used herein the term "proniosome" refers to dry formulations of surfactant-coated carrier which on rehydration and mild agitation gives niosomes.

[0047] As used herein the term "polymersome" refers to an artificial spherical vesicle comprising a membrane formed from amphiphilic synthetic block copolymers and may optionally comprise an aqueous solution in its core. A polymersome has a diameter ranging from 50 nm to 5 μm and larger. The term also includes syntosomes, which are polymersomes engineered to comprise channels that allow certain chemicals to pass through the membrane into or out of the vesicle.

[0048] As used herein the term "sphingosome" refers to a concentric, bilayered vesicle in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic sphingolipid.

[0049] As used herein the term "transferosome" refers to ultraflexible lipid vesicles comprising an aqueous core that are formed from a mixture of common polar and suitable edge-activated lipids which facilitate the formation of highly curved bilayers which render the transferosome highly deformable.

[0050] As used herein the term "ufasome" refers to a vesicle comprising unsaturated fatty acids.

[0051] As used herein the term "polypeptide" refers to a peptide comprising up to and including 50 amino acid monomers.

[0052] As used herein the term "protein" refers to a peptide of more than 50 amino acid

residues. Preferably a protein comprises at most 20000 amino acid residues, such as at most 15000 amino acid residues, such as at most 10000 amino acid residues, such as at most 5000 amino acid residues, such as at most 4000 amino acid residues, such as at most 3000 amino acid residues, such as at most 2000 amino acid residues, such as at most 1000 amino acid residues.

[0053] As used herein the term "physiological conditions" refers to an aqueous buffer at pH 7.4, 37°C.

[0054] As used herein the term "pharmaceutical composition" refers to a composition containing one or more active ingredients, such as for example at least one controlled-release PTH compounds, and one or more excipients, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients of the composition, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing one or more controlled-release PTH compound and a pharmaceutically acceptable excipient.

[0055] As used herein the term "liquid composition" refers to a mixture comprising water-soluble controlled-release PTH compound and one or more solvents, such as water.

[0056] The term "suspension composition" relates to a mixture comprising water-insoluble controlled-release PTH compound and one or more solvents, such as water.

[0057] As used herein, the term "dry composition" means that a pharmaceutical composition is provided in a dry form. Suitable methods for drying are spray-drying and lyophilization, i.e. freeze-drying. Such dry composition of prodrug has a residual water content of a maximum of 10 %, preferably less than 5% and more preferably less than 2%, determined according to Karl Fischer. Preferably, the pharmaceutical composition of the present invention is dried by lyophilization.

[0058] The term "drug" as used herein refers to a substance used in the treatment, cure, prevention, or diagnosis of a disease or used to otherwise enhance physical or mental well-being. If a drug is conjugated to another moiety, the moiety of the resulting product that originated from the drug is referred to as "biologically active moiety".

[0059] As used herein the term "prodrug" refers to a conjugate in which a biologically active moiety is reversibly and covalently connected to a specialized protective group through a reversible linker moiety, also referred to as "reversible prodrug linker moiety", which comprises a reversible linkage with the biologically active moiety and wherein the specialized protective group alters or eliminates undesirable properties in the parent molecule. This also includes the enhancement of desirable properties in the drug and the suppression of undesirable properties. The specialized non-toxic protective group is referred to as "carrier". A prodrug releases the reversibly and covalently bound biologically active moiety in the form of its

corresponding drug. In other words, a prodrug is a conjugate comprising a biologically active moiety which is covalently and reversibly conjugated to a carrier moiety via a reversible prodrug linker moiety, which covalent and reversible conjugation of the carrier to the reversible prodrug linker moiety is either directly or through a spacer. Such conjugate releases the formerly conjugated biologically active moiety in the form of a free unmodified drug.

[0060] A "biodegradable linkage" or a "reversible linkage" is a linkage that is hydrolytically degradable, i.e. cleavable, in the absence of enzymes under physiological conditions (aqueous buffer at pH 7.4, 37°C) with a half-life ranging from one hour to three months, preferably from one hour to two months, even more preferably from one hour to one month, even more preferably from one hour to three weeks, most preferably from one hour to two weeks. Accordingly, a stable linkage is a linkage having a half-life under physiological conditions (aqueous buffer at pH 7.4, 37°C) of more than three months.

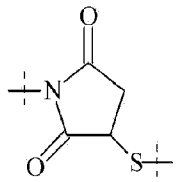
[0061] As used herein, the term "traceless prodrug linker" means a reversible prodrug linker, i.e. a linker moiety reversibly and covalently connecting the biologically active moiety with the carrier, which upon cleavage releases the drug in its free form. As used herein, the term "free form" of a drug means the drug in its unmodified, pharmacologically active form.

[0062] As used herein, the term "excipient" refers to a diluent, adjuvant, or vehicle with which the therapeutic, such as a drug or prodrug, is administered. Such pharmaceutical excipient can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, including peanut oil, soybean oil, mineral oil and sesame oil. Water is a preferred excipient when the pharmaceutical composition is administered orally. Saline and aqueous dextrose are preferred excipients when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions are preferably employed as liquid excipients for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, mannitol, trehalose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water and ethanol. The pharmaceutical composition, if desired, can also contain minor amounts of wetting or emulsifying agents, pH buffering agents, like, for example, acetate, succinate, tris, carbonate, phosphate, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), MES (2-(A-morpholino)ethanesulfonic acid), or can contain detergents, like Tween, poloxamers, poloxamines, CHAPS, Igepal, or amino acids like, for example, glycine, lysine, or histidine. These pharmaceutical compositions can take the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders and sustained-release formulations. The pharmaceutical composition can be formulated as a suppository, with traditional binders and excipients such as triglycerides. Oral formulation can include standard excipients such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such compositions will contain a therapeutically effective amount of the drug or biologically active moiety, together with a suitable amount of excipient so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

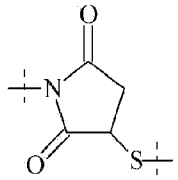
[0063] As used herein, the term "reagent" means a chemical compound which comprises at least one functional group for reaction with the functional group of another chemical compound or drug. It is understood that a drug comprising a functional group (such as a primary or secondary amine or hydroxyl functional group) is also a reagent.

[0064] As used herein, the term "moiety" means a part of a molecule, which lacks one or more atom(s) compared to the corresponding reagent. If, for example, a reagent of the formula "H-X-H" reacts with another reagent and becomes part of the reaction product, the corresponding moiety of the reaction product has the structure "H-X-" or "-X-", whereas each "-" indicates attachment to another moiety. Accordingly, a biologically active moiety is released from a prodrug as a drug.

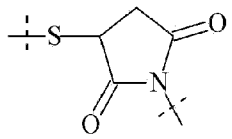
[0065] It is understood that if the sequence or chemical structure of a group of atoms is provided which group of atoms is attached to two moieties or is interrupting a moiety, said sequence or chemical structure can be attached to the two moieties in either orientation, unless explicitly stated otherwise. For example, a moiety "-C(O)N(R^1)-" can be attached to two moieties or interrupting a moiety either as "-C(O)N(R^1)-" or as "-N(R^1)C(O)-". Similarly, a moiety



can be attached to two moieties or can interrupt a moiety either as



or as



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[0066] As used herein, the term "functional group" means a group of atoms which can react with other groups of atoms. Functional groups include but are not limited to the following groups: carboxylic acid (-(C=O)OH), primary or secondary amine (-NH_2, -NH-), maleimide, thiol (-SH), sulfonic acid (-(O=S=O)OH), carbonate, carbamate (-O(C=O)N<), hydroxyl (-OH), aldehyde (-(C=O)H), ketone (-(C=O)-), hydrazine (>N-N<), isocyanate, isothiocyanate, phosphoric acid (-O(P=O)OHOH), phosphonic acid (-O(P=O)OHH), haloacetyl, alkyl halide, acryloyl, aryl fluoride, hydroxylamine, disulfide, sulfonamides, sulfuric acid, vinyl sulfone, vinyl ketone, diazoalkane, oxirane, and aziridine.

[0067] In case the controlled-release PTH compound of the present invention comprise one or more acidic or basic groups, the invention also comprises their corresponding pharmaceutically or toxicologically acceptable salts, in particular their pharmaceutically utilizable salts. Thus, the controlled-release PTH compound of the present invention comprising acidic groups can be used according to the invention, for example, as alkali metal salts, alkaline earth metal salts or as ammonium salts. More precise examples of such salts include sodium salts, potassium salts, calcium salts, magnesium salts or salts with ammonia or organic amines such as, for example, ethylamine, ethanolamine, triethanolamine or amino acids. Controlled-release PTH compound of the present invention comprising one or more basic groups, i.e. groups which can be protonated, can be present and can be used according to the invention in the form of their addition salts with inorganic or organic acids. Examples for suitable acids include hydrogen chloride, hydrogen bromide, phosphoric acid, sulfuric acid, nitric acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acids, oxalic acid, acetic acid, tartaric acid, lactic acid, salicylic acid, benzoic acid, formic acid, propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, malic acid, sulfaminic acid, phenylpropionic acid, gluconic acid, ascorbic acid, isonicotinic acid, citric acid, adipic acid, and other acids known to the person skilled in the art. For the person skilled in the art further methods are known for converting the basic group into a cation like the alkylation of an amine group resulting in a positively-charge ammonium group and an appropriate counterion of the salt. If the controlled-release PTH compound of the present invention simultaneously comprise acidic and basic groups, the invention also includes, in addition to the salt forms mentioned, inner salts or betaines (zwitterions). The respective salts can be obtained by customary methods which are known to the person skilled in the art like, for example by contacting these compounds with an organic or inorganic acid or base in a solvent or dispersant, or by anion exchange or cation exchange with other salts. The present invention also includes all salts of the compounds of the present invention which, owing to low physiological compatibility, are not directly suitable for use in pharmaceuticals but which can be used, for example, as intermediates for chemical reactions or for the preparation of pharmaceutically acceptable salts.

[0068] The term "pharmaceutically acceptable" means a substance that does cause harm when administered to a patient and preferably means approved by a regulatory agency, such as the EMA (Europe) and/or the FDA (US) and/or any other national regulatory agency for use in animals, preferably for use in humans.

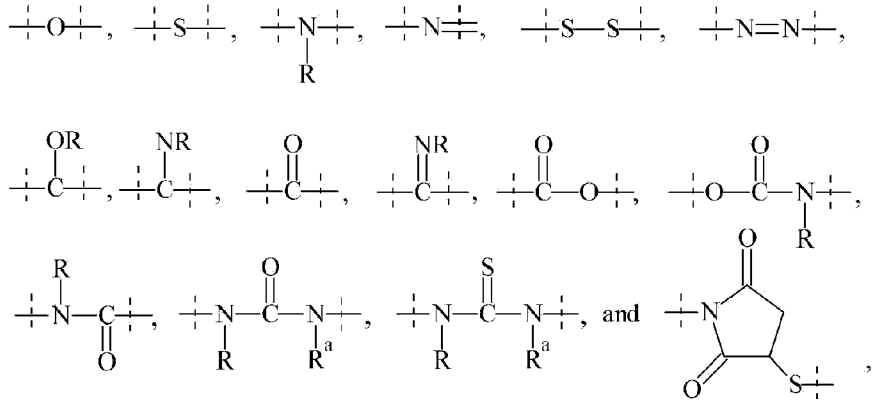
[0069] As used herein the term "about" in combination with a numerical value is used to indicate a range ranging from and including the numerical value plus and minus no more than 10% of said numerical value, more preferably no more than 8% of said numerical value, even more preferably no more than 5% of said numerical value and most preferably no more than 2% of said numerical value. For example, the phrase "about 200" is used to mean a range ranging from and including 200 +/- 10%, i.e. ranging from and including 180 to 220; preferably 200 +/-8%, i.e. ranging from and including 184 to 216; even more preferably ranging from and including 200 +/-5%, i.e. ranging from and including 190 to 210; and most preferably 200 +/-2%, i.e. ranging from and including 196 to 204. It is understood that a percentage given as

"about 20%" does not mean "20% +/- 10%", i.e. ranging from and including 10 to 30%, but "about 20%" means ranging from and including 18 to 22%, i.e. plus and minus 10% of the numerical value which is 20.

[0070] As used herein, the term "polymer" means a molecule comprising repeating structural units, i.e. the monomers, connected by chemical bonds in a linear, circular, branched, crosslinked or dendrimeric way or a combination thereof, which may be of synthetic or biological origin or a combination of both. It is understood that a polymer may also comprise one or more other chemical groups and/or moieties, such as, for example, one or more functional groups. Preferably, a soluble polymer has a molecular weight of at least 0.5 kDa, e.g. a molecular weight of at least 1 kDa, a molecular weight of at least 2 kDa, a molecular weight of at least 3 kDa or a molecular weight of at least 5 kDa. If the polymer is soluble, it preferable has a molecular weight of at most 1000 kDa, such as at most 750 kDa, such as at most 500 kDa, such as at most 300 kDa, such as at most 200 kDa, such as at most 100 kDa. It is understood that for insoluble polymers, such as hydrogels, no meaningful molecular weight ranges can be provided. It is understood that also a protein is a polymer in which the amino acids are the repeating structural units, even though the side chains of each amino acid may be different.

[0071] As used herein, the term "polymeric" means a reagent or a moiety comprising one or more polymers or polymer moieties. A polymeric reagent or moiety may optionally also comprise one or more other moiety/moieties, which are preferably selected from the group consisting of:

- C_{1-50} alkyl, C_{2-50} alkenyl, C_{2-50} alkynyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicyclyl, phenyl, naphthyl, indenyl, indanyl, and tetralinyl; and
- linkages selected from the group comprising



wherein

dashed lines indicate attachment to the remainder of the moiety or reagent, and

-R and -R^a are independently of each other selected from the group consisting of -H, methyl, ethyl, propyl, butyl, pentyl and hexyl.

[0072] The person skilled in the art understands that the polymerization products obtained from a polymerization reaction do not all have the same molecular weight, but rather exhibit a molecular weight distribution. Consequently, the molecular weight ranges, molecular weights, ranges of numbers of monomers in a polymer and numbers of monomers in a polymer as used herein, refer to the number average molecular weight and number average of monomers, i.e. to the arithmetic mean of the molecular weight of the polymer or polymeric moiety and the arithmetic mean of the number of monomers of the polymer or polymeric moiety.

[0073] Accordingly, in a polymeric moiety comprising "x" monomer units any integer given for "x" therefore corresponds to the arithmetic mean number of monomers. Any range of integers given for "x" provides the range of integers in which the arithmetic mean numbers of monomers lies. An integer for "x" given as "about x" means that the arithmetic mean numbers of monomers lies in a range of integers of x +/- 10%, preferably x +/- 8%, more preferably x +/- 5% and most preferably x +/- 2%.

[0074] As used herein, the term "number average molecular weight" means the ordinary arithmetic mean of the molecular weights of the individual polymers.

[0075] As used herein the term "water-soluble" with reference to a carrier means that when such carrier is part of the controlled-release PTH compound of the present invention at least 1 g of the controlled-release PTH compound comprising such water-soluble carrier can be dissolved in one liter of water at 20°C to form a homogeneous solution. Accordingly, the term "water-insoluble" with reference to a carrier means that when such carrier is part of a controlled-release PTH compound of the present invention less than 1 g of the controlled-release PTH compound comprising such water-insoluble carrier can be dissolved in one liter of water at 20°C to form a homogeneous solution.

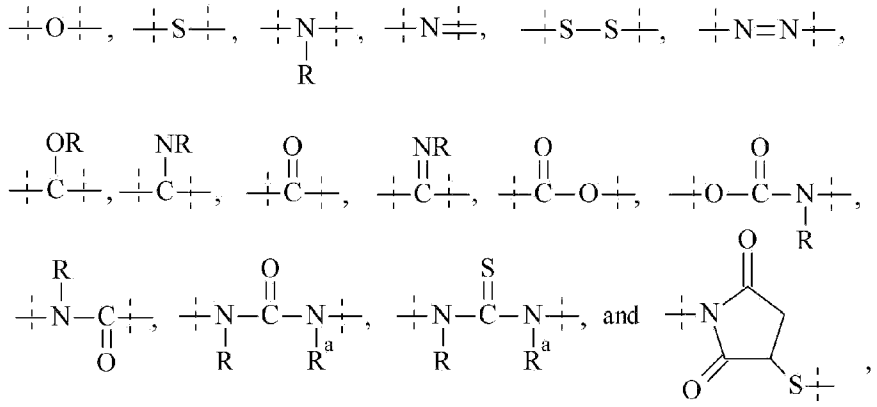
[0076] As used herein, the term "hydrogel" means a hydrophilic or amphiphilic polymeric network composed of homopolymers or copolymers, which is insoluble due to the presence of covalent chemical crosslinks. The crosslinks provide the network structure and physical integrity.

[0077] As used herein the term "thermogelling" means a compound that is a liquid or a low viscosity solution having a viscosity of less than 500 cps at 25°C at a shear rate of about 0.1 /second at a low temperature, which low temperature ranges between about 0°C to about 10°C, but which is a higher viscosity compound of less than 10000 cps at 25°C at a shear rate of about 0.1/second at a higher temperature, which higher temperature ranges between about 30°C to about 40°C, such as at about 37°C.

[0078] As used herein, the term "PEG-based" in relation to a moiety or reagent means that said moiety or reagent comprises PEG. Preferably, a PEG-based moiety or reagent comprises at least 10% (w/w) PEG, such as at least 20% (w/w) PEG, such as at least 30% (w/w) PEG, such as at least 40% (w/w) PEG, such as at least 50% (w/w), such as at least 60 (w/w) PEG,

such as at least 70% (w/w) PEG, such as at least 80% (w/w) PEG, such as at least 90% (w/w) PEG, such as at least 95%. The remaining weight percentage of the PEG-based moiety or reagent are other moieties preferably selected from the following moieties and linkages:

- C_{1-50} alkyl, C_{2-50} alkenyl, C_{2-50} alkynyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicyclyl, phenyl, naphthyl, indenyl, indanyl, and tetralinyl; and
- linkages selected from the group comprising



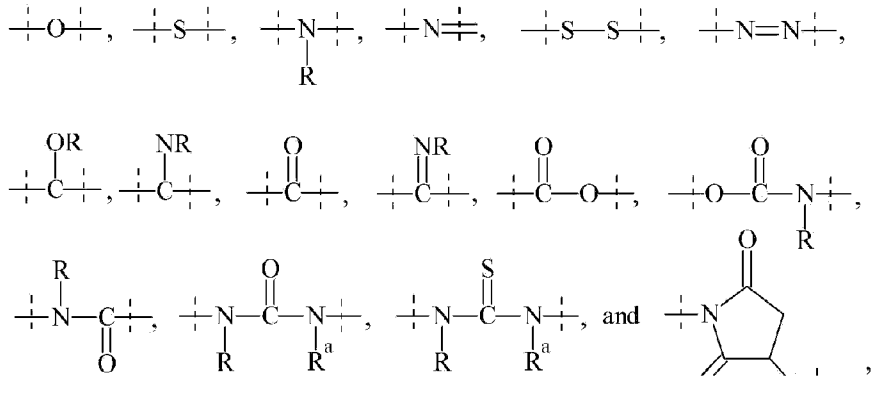
wherein

dashed lines indicate attachment to the remainder of the moiety or reagent, and

-R and -R^a are independently of each other selected from the group consisting of -H, methyl, ethyl, propyl, butyl, pentyl and hexyl.

[0079] As used herein, the term "PEG-based comprising at least X% PEG" in relation to a moiety or reagent means that said moiety or reagent comprises at least X% (w/w) ethylene glycol units (-CH_2CH_2O-), wherein the ethylene glycol units may be arranged blockwise, alternating or may be randomly distributed within the moiety or reagent and preferably all ethylene glycol units of said moiety or reagent are present in one block; the remaining weight percentage of the PEG-based moiety or reagent are other moieties preferably selected from the following moieties and linkages:

- C_{1-50} alkyl, C_{2-50} alkenyl, C_{2-50} alkynyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicyclyl, phenyl, naphthyl, indenyl, indanyl, and tetralinyl; and
- linkages selected from the group comprising



wherein

dashed lines indicate attachment to the remainder of the moiety or reagent, and

-R and -R^a are independently of each other selected from the group consisting of -H, methyl, ethyl, propyl, butyl, pentyl and hexyl.

[0080] The term "hyaluronic acid-based comprising at least X% hyaluronic acid" is used accordingly.

[0081] The term "substituted" as used herein means that one or more -H atom(s) of a molecule or moiety are replaced by a different atom or a group of atoms, which are referred to as "substituent".

[0082] Preferably, the one or more further optional substituents are independently of each other selected from the group consisting of halogen, -CN, -$COOR^{x1}$, -OR^{x1}, -$C(O)R^{x1}$, -$C(O)N(R^{x1}R^{x1a})$, -$S(O)_2N(R^{x1}R^{x1a})$, -$S(O)N(R^{x1}R^{x1a})$, -$S(O)_2R^{x1}$, -$S(O)R^{x1}$, -$N(R^{x1})S(O)_2N(R^{x1a}R^{x1b})$, -$SR^{x1}$, -$N(R^{x1}R^{x1a})$, -$NO_2$, -$OC(O)R^{x1}$, -$N(R^{x1})C(O)R^{x1a}$, -$N(R^{x1})S(O)_2R^{x1a}$, -$N(R^{x1})S(O)R^{x1a}$, -$N(R^{x1})C(O)OR^{x1a}$, -$N(R^{x1})C(O)N(R^{x1a}R^{x1b})$, -$OC(O)N(R^{x1}R^{x1a})$, -$T^0$, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl; wherein -T^0, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more -R^{x2}, which are the same or different and wherein C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally interrupted by one or more groups selected from the group consisting of -T^0-, -C(O)O-, -O-, -C(O)-, -$C(O)N(R^{x3})$-, -$S(O)_2N(R^{x3})$-, -$S(O)N(R^{x3})$-, -$S(O)_2$-, -S(O)-, -$N(R^{x3})S(O)_2N(R^{x3a})$-, -S-, -$N(R^{x3})$-, -$OC(OR^{x3})(R^{x3a})$-, -$N(R^{x3})C(O)N(R^{x3a})$-, and -$OC(O)N(R^{x3})$-;

-R^{x1}, -R^{x1a}, -R^{x1b} are independently of each other selected from the group consisting of -H, -T^0, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl; wherein -T^0, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more -R^{x2}, which are the same or different and wherein C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally interrupted by one or more groups selected from the group consisting of -T^0-, -C(O)O-, -O-, -C(O)-, -$C(O)N(R^{x3})$-, -$S(O)_2N(R^{x3})$-, -$S(O)N(R^{x3})$-; -$S(O)_2$-, -S(O)-, -$N(R^{x3})S(O)_2N(R^{x3a})$-, -S-, -$N(R^{x3})$-, -$OC(OR^{x3})(R^{x3a})$-, -$N(R^{x3})C(O)N(R^{x3a})$-, and -$OC(O)N(R^{x3})$-;

each T^0 is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, and 8- to 11-membered heterobicyclyl; wherein each T^0 is independently optionally substituted with one or more -R^{x2},

which are the same or different;

each -R^{x2} is independently selected from the group consisting of halogen, -CN, oxo (=O), -$COOR^{x4}$, -OR^{x4}, -$C(O)R^{x4}$, -$C(O)N(R^{x4}R^{x4a})$, -$S(O)_2N(R^{x4}R^{x4a})$, -$S(O)N(R^{x4}R^{x4a})$, -$S(O)_2R^{x4}$, -$S(O)R^{x4}$, -$N(R^{x4})S(O)_2N(R^{x4a}R^{x4b})$, -$SR^{x4}$, -$N(R^{x4}R^{x4a})$, -$NO_2$, -$OC(O)R^{x4}$, -$N(R^{x4})C(O)R^{x4a}$, -$N(R^{x4})S(O)_2R^{x4a}$, -$N(R^{x4})S(O)R^{x4a}$, -$N(R^{x4})C(O)OR^{x4a}$, -$N(R^{x4})C(O)N(R^{x4a}R^{x4b})$, -$OC(O)N(R^{x4}R^{x4a})$, and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different;

each -R^{x3}, -R^{x3a}, -R^{x4}, -R^{x4a}, -R^{x4b} is independently selected from the group consisting of -H and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different.

[0083] More preferably, the one or more further optional substituents are independently of each other selected from the group consisting of halogen, -CN, -$COOR^{x1}$, -OR^{x1}, -$C(O)R^{x1}$, -$C(O)N(R^{x1}R^{x1a})$, -$S(O)_2N(R^{x1}R^{x1a})$, -$S(O)N(R^{x1}R^{x1a})$, -$S(O)_2R^{x1}$, -$S(O)R^{x1}$, -$N(R^{x1})S(O)_2N(R^{x1a}R^{x1b})$, -$SR^{x1}$, -$N(R^{x1}R^{x1a})$, -$NO_2$, -$OC(O)R^{x1}$, -$N(R^{x1})C(O)R^{x1a}$, -$N(R^{x1})S(O)_2R^{x1a}$, -$N(R^{x1})S(O)R^{x1a}$, -$N(R^{x1})C(O)OR^{x1a}$, -$N(R^{x1})C(O)N(R^{x1}aR^{x1b})$, -$OC(O)N(R^{x1}R^{x1a})$, -$T^0$, C_{1-10} alkyl, C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein -T^0, C_{1-10} alkyl, C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more -R^{x2}, which are the same or different and wherein C_{1-10} alkyl, C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally interrupted by one or more groups selected from the group consisting of -T^0-, -C(O)O-, -O-, -C(O)-, -$C(O)N(R^{x3})$-, -$S(O)_2N(R^{x3})$-, -$S(O)N(R^{x3})$-, -$S(O)_2$-, -S(O)-, -$N(R^{x3})S(O)_2N(R^{x3a})$-, -S-, -$N(R^{x3})$-, -$OC(OR^{x3})(R^{x3a})$-, -$N(R^{x3})C(O)N(R^{x3a})$-, and -$OC(O)N(R^{x3})$-;

each -R^{x1}, -R^{x1a}, -R^{x1b}, -R^{x3}, -R^{x3a} is independently selected from the group consisting of -H, halogen, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl;

each T^0 is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, and 8- to 11-membered heterobicyclyl; wherein each T^0 is independently optionally substituted with one or more -R^{x2}, which are the same or different;

each -R^{x2} is independently selected from the group consisting of halogen, -CN, oxo (=O), -$COOR^{x4}$, -OR^{x4}, -$C(O)R^{x4}$, -$C(O)N(R^{x4}R^{x4a})$, -$S(O)_2N(R^{x4}R^{x4a})$, -$S(O)N(R^{x4}R^{x4a})$, -$S(O)_2R^{x4}$, -$S(O)R^{x4}$, -$N(R^{x4})S(O)_2N(R^{x4a}R^{x4b})$, -$SR^{x4}$, -$N(R^{x4}R^{x4a})$, -$NO_2$, -$OC(O)R^{x4}$, -$N(R^{x4})C(O)R^{x4a}$, -

$N(R^{x4})S(O)_2R^{x4a}$, $-N(R^{x4})S(O)R^{x4a}$, $-N(R^{x4})C(O)OR^{x4a}$, $-N(R^{x4})C(O)N(R^{x4a}R^{x4b})$, $-OC(O)N(R^{x4}R^{x4a})$, and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different;

each $-R^{x4}$, $-R^{x4a}$, $-R^{x4b}$ is independently selected from the group consisting of -H, halogen, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl;

Even more preferably, the one or more further optional substituents are independently of each other selected from the group consisting of halogen, -CN, $-COOR^{x1}$, $-OR^{x1}$, $-C(O)R^{x1}$, $-C(O)N(R^{x1}R^{x1a})$, $-S(O)_2N(R^{x1}R^{x1a})$, $-S(O)N(R^{x1}R^{x1a})$, $-S(O)_2R^{x1}$, $-S(O)R^{x1}$, $-N(R^{x1})S(O)_2N(R^{x1a}R^{x1b})$, $-SR^{x1}$, $-N(R^{x1}R^{x1a})$, $-NO_2$, $-OC(O)R^{x1}$, $-N(R^{x1})C(O)R^{x1a}$, $-N(R^{x1})S(O)_2R^{x1a}$, $-N(R^{x1})S(O)R^{x1a}$, $-N(R^{x1})C(O)OR^{x1a}$, $-N(R^{x1})C(O)N(R^{x1a}R^{x1b})$, $-OC(O)N(R^{x1}R^{x1a})$, $-T^0$, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl; wherein $-T^0$, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl are optionally substituted with one or more $-R^{x2}$, which are the same or different and wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl are optionally interrupted by one or more groups selected from the group consisting of $-T^0-$, -C(O)O-, -O-, -C(O)-, $-C(O)N(R^{x3})-$, $-S(O)_2N(R^{x3})-$, $-S(O)N(R^{x3})-$, $-S(O)_2-$, -S(O)-, $-N(R^{x3})S(O)_2N(R^{x3a})-$, -S-, $-N(R^{x3})-$, $-OC(OR^{x3})(R^{x3a})-$, $-N(R^{x3})C(O)N(R^{x3a})-$, and $-OC(O)N(R^{x3})-$;

each $-R^{x1}$, $-R^{x1a}$, $-R^{x1b}$, $-R^{x2}$, $-R^{x3}$, $-R^{x3a}$ is independently selected from the group consisting of -H, halogen, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl;

each T^0 is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, and 8- to 11-membered heterobicyclyl; wherein each T^0 is independently optionally substituted with one or more $-R^{x2}$, which are the same or different.

[0084] Preferably, a maximum of 6 -H atoms of an optionally substituted molecule are independently replaced by a substituent, e.g. 5 -H atoms are independently replaced by a substituent, 4 -H atoms are independently replaced by a substituent, 3 -H atoms are independently replaced by a substituent, 2 -H atoms are independently replaced by a substituent, or 1 -H atom is replaced by a substituent.

[0085] The term "interrupted" means that a moiety is inserted between two carbon atoms or - if the insertion is at one of the moiety's ends - between a carbon or heteroatom and a hydrogen atom, preferably between a carbon and a hydrogen atom.

[0086] As used herein, the term "C_{1-4} alkyl" alone or in combination means a straight-chain or

branched alkyl moiety having 1 to 4 carbon atoms. If present at the end of a molecule, examples of straight-chain or branched C_{1-4} alkyl are methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl. When two moieties of a molecule are linked by the C_{1-4} alkyl, then examples for such C_{1-4} alkyl groups are $-CH_2-$, $-CH_2-CH_2-$, $-CH(CH_3)-$, $-CH_2-CH_2-CH_2-$, $-CH(C_2H_5)-$, $-C(CH_3)_2-$. Each hydrogen of a C_{1-4} alkyl carbon may optionally be replaced by a substituent as defined above. Optionally, a C_{1-4} alkyl may be interrupted by one or more moieties as defined below.

[0087] As used herein, the term "C_{1-6} alkyl" alone or in combination means a straight-chain or branched alkyl moiety having 1 to 6 carbon atoms. If present at the end of a molecule, examples of straight-chain and branched C_{1-6} alkyl groups are methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl, 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl and 3,3-dimethylpropyl. When two moieties of a molecule are linked by the C_{1-6} alkyl group, then examples for such C_{1-6} alkyl groups are $-CH_2-$, $-CH_2-CH_2-$, $-CH(CH_3)-$, $-CH_2-CH_2-CH_2-$, $-CH(C_2H_5)-$ and $-C(CH_3)_2-$. Each hydrogen atom of a C_{1-6} carbon may optionally be replaced by a substituent as defined above. Optionally, a C_{1-6} alkyl may be interrupted by one or more moieties as defined below.

[0088] Accordingly, "C_{1-10} alkyl", "C_{1-20} alkyl" or "C_{1-50} alkyl" means an alkyl chain having 1 to 10, 1 to 20 or 1 to 50 carbon atoms, respectively, wherein each hydrogen atom of the C_{1-10}, C_{1-20} or C_{1-50} carbon may optionally be replaced by a substituent as defined above. Optionally, a C_{1-10} or C_{1-50} alkyl may be interrupted by one or more moieties as defined below.

[0089] As used herein, the term "C_{2-6} alkenyl" alone or in combination means a straight-chain or branched hydrocarbon moiety comprising at least one carbon-carbon double bond having 2 to 6 carbon atoms. If present at the end of a molecule, examples are $-CH=CH_2$, $-CH=CH-CH_3$, $-CH_2-CH=CH_2$, $-CH=CHCH_2-CH_3$ and $-CH=CH-CH=CH_2$. When two moieties of a molecule are linked by the C_{2-6} alkenyl group, then an example for such C_{2-6} alkenyl is -CH=CH-. Each hydrogen atom of a C_{2-6} alkenyl moiety may optionally be replaced by a substituent as defined above. Optionally, a C_{2-6} alkenyl may be interrupted by one or more moieties as defined below.

[0090] Accordingly, the term "C_{2-10} alkenyl", "C_{2-20} alkenyl" or "C_{2-50} alkenyl" alone or in combination means a straight-chain or branched hydrocarbon moiety comprising at least one carbon-carbon double bond having 2 to 10, 2 to 20 or 2 to 50 carbon atoms. Each hydrogen atom of a C_{2-10} alkenyl, C_{2-20} alkenyl or C_{2-50} alkenyl group may optionally be replaced by a substituent as defined above. Optionally, a C_{2-10} alkenyl, C_{2-20} alkenyl or C_{2-50} alkenyl may be interrupted by one or more moieties as defined below.

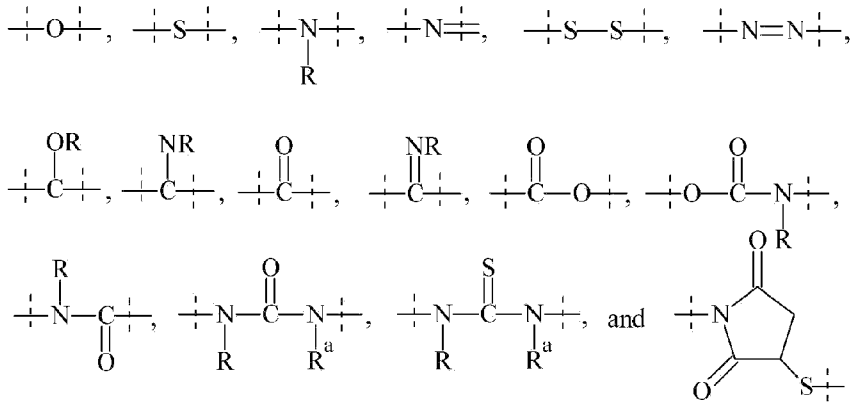
[0091] As used herein, the term "C_{2-6} alkynyl" alone or in combination means straight-chain or

branched hydrocarbon moiety comprising at least one carbon-carbon triple bond having 2 to 6 carbon atoms. If present at the end of a molecule, examples are -C=CH, -CH_2-C≡CH, CH_2-CH_2-C=CH and CH_2-C≡C-CH_3. When two moieties of a molecule are linked by the alkynyl group, then an example is -C=C-. Each hydrogen atom of a C_{2-6} alkynyl group may optionally be replaced by a substituent as defined above. Optionally, one or more double bond(s) may occur. Optionally, a C_{2-6} alkynyl may be interrupted by one or more moieties as defined below.

[0092] Accordingly, as used herein, the term "C_{2-10} alkynyl", "C_{2-20} alkynyl" and "C_{2-50} alkynyl" alone or in combination means a straight-chain or branched hydrocarbon moiety comprising at least one carbon-carbon triple bond having 2 to 10, 2 to 20 or 2 to 50 carbon atoms, respectively. Each hydrogen atom of a C_{2-10} alkynyl, C_{2-20} alkynyl or C_{2-50} alkynyl group may optionally be replaced by a substituent as defined above. Optionally, one or more double bond(s) may occur.

[0093] Optionally, a C_{2-10} alkynyl, C_{2-20} alkynyl or C_{2-50} alkynyl may be interrupted by one or more moieties as defined below.

[0094] As mentioned above, a C_{1-4} alkyl, C_{1-6} alkyl, C_{1-10} alkyl, C_{1-20} alkyl, C_{1-50} alkyl, C_{2-6} alkenyl, C_{2-10} alkenyl, C_{2-20} alkenyl, C_{2-50} alkenyl, C_{2-6} alkynyl, C_{2-10} alkynyl, C_{2-20} alkenyl or C_{2-50} alkynyl may optionally be interrupted by one or more moieties which are preferably selected from the group consisting of



wherein dashed lines indicate attachment to the remainder of the moiety or reagent; and -R and -R^a are independently of each other selected from the group consisting of -H, methyl, ethyl, propyl, butyl, pentyl and hexyl.

[0095] As used herein, the term "C_{3-10} cycloalkyl" means a cyclic alkyl chain having 3 to 10 carbon atoms, which may be saturated or unsaturated, e.g. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl, cycloheptyl, cyclooctyl, cyclononyl or cyclodecyl. Each hydrogen atom of a C_{3-10} cycloalkyl carbon may be replaced by a substituent as defined above. The term "C_{3-10} cycloalkyl" also includes bridged bicycles like norbornane or norbornene.

[0096] The term "8- to 30-membered carbopolycyclyl" or "8- to 30-membered carbopolycycle" means a cyclic moiety of two or more rings with 8 to 30 ring atoms, where two neighboring rings share at least one ring atom and that may contain up to the maximum number of double bonds (aromatic or non-aromatic ring which is fully, partially or un-saturated). Preferably a 8- to 30-membered carbopolycyclyl means a cyclic moiety of two, three, four or five rings, more preferably of two, three or four rings.

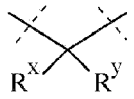
[0097] As used herein, the term "3- to 10-membered heterocyclyl" or "3- to 10-membered heterocycle" means a ring with 3, 4, 5, 6, 7, 8, 9 or 10 ring atoms that may contain up to the maximum number of double bonds (aromatic or non-aromatic ring which is fully, partially or un-saturated) wherein at least one ring atom up to 4 ring atoms are replaced by a heteroatom selected from the group consisting of sulfur (including -S(O)-, $-S(O)_2-$), oxygen and nitrogen (including =N(O)-) and wherein the ring is linked to the rest of the molecule via a carbon or nitrogen atom. Examples for 3- to 10-membered heterocycles include but are not limited to aziridine, oxirane, thiirane, azirine, oxirene, thiirene, azetidine, oxetane, thietane, furan, thiophene, pyrrole, pyrroline, imidazole, imidazoline, pyrazole, pyrazoline, oxazole, oxazoline, isoxazole, isoxazoline, thiazole, thiazoline, isothiazole, isothiazoline, thiadiazole, thiadiazoline, tetrahydrofuran, tetrahydrothiophene, pyrrolidine, imidazolidine, pyrazolidine, oxazolidine, isoxazolidine, thiazolidine, isothiazolidine, thiadiazolidine, sulfolane, pyran, dihydropyran, tetrahydropyran, imidazolidine, pyridine, pyridazine, pyrazine, pyrimidine, piperazine, piperidine, morpholine, tetrazole, triazole, triazolidine, tetrazolidine, diazepane, azepine and homopiperazine. Each hydrogen atom of a 3- to 10-membered heterocyclyl or 3- to 10-membered heterocyclic group may be replaced by a substituent as defined below.

[0098] As used herein, the term "8- to 11-membered heterobicyclyl" or "8- to 11-membered heterobicycle" means a heterocyclic moiety of two rings with 8 to 11 ring atoms, where at least one ring atom is shared by both rings and that may contain up to the maximum number of double bonds (aromatic or non-aromatic ring which is fully, partially or un-saturated) wherein at least one ring atom up to 6 ring atoms are replaced by a heteroatom selected from the group consisting of sulfur (including -S(O)-, $-S(O)_2-$), oxygen and nitrogen (including =N(O)-) and wherein the ring is linked to the rest of the molecule via a carbon or nitrogen atom. Examples for an 8- to 11-membered heterobicycle are indole, indoline, benzofuran, benzothiophene, benzoxazole, benzisoxazole, benzothiazole, benzisothiazole, benzimidazole, benzimidazoline, quinoline, quinazoline, dihydroquinazoline, quinoline, dihydroquinoline, tetrahydroquinoline, decahydroquinoline, isoquinoline, decahydroisoquinoline, tetrahydroisoquinoline, dihydroisoquinoline, benzazepine, purine and pteridine. The term 8- to 11-membered heterobicycle also includes spiro structures of two rings like 1,4-dioxa-8-azaspiro[4.5]decane or bridged heterocycles like 8-aza-bicyclo[3.2.1]octane. Each hydrogen atom of an 8- to 11-membered heterobicyclyl or 8- to 11-membered heterobicycle carbon may be replaced by a substituent as defined below.

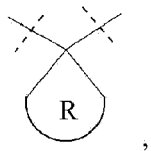
[0099] Similary, the term "8- to 30-membered heteropolycyclyl" or "8- to 30-membered heteropolycycle" means a heterocyclic moiety of more than two rings with 8 to 30 ring atoms, preferably of three, four or five rings, where two neighboring rings share at least one ring atom

and that may contain up to the maximum number of double bonds (aromatic or non-aromatic ring which is fully, partially or unsaturated), wherein at least one ring atom up to 10 ring atoms are replaced by a heteroatom selected from the group of sulfur (including -S(O)-, -$S(O)_2$-), oxygen and nitrogen (including =N(O)-) and wherein the ring is linked to the rest of a molecule via a carbon or nitrogen atom.

[0100] It is understood that the phrase "the pair R^x/R^y is joined together with the atom to which they are attached to form a C_{3-10} cycloalkyl or a 3- to 10-membered heterocyclyl" in relation with a moiety of the structure



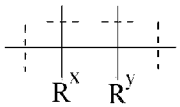
means that R^x and R^y form the following structure:



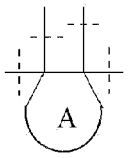
,

wherein R is C_{3-10} cycloalkyl or 3- to 10-membered heterocyclyl.

[0101] It is also understood that the phrase "the pair R^x/R^y is joint together with the atoms to which they are attached to form a ring A" in relation with a moiety of the structure



means that R^x and R^y form the following structure:



.

[0102] As used herein, "halogen" means fluoro, chloro, bromo or iodo. It is generally preferred that halogen is fluoro or chloro.

[0103] In general, the term "comprise" or "comprising" also encompasses "consist of" or "consisting of '.

[0104] Preferably, the pharmaceutical composition comprising a controlled-release PTH compound is administered in accordance with a dosage regimen in which dose adjustment in response to hypocalcemia or hypercalcemia is performed in increments of no more than 20%, more preferably in increments of no more than 15% and most preferably in increments of no more than 10%.

[0105] In on embodiment the pharmaceutical composition comprising a controlled-release PTH compound is administered in accordance with a dosage regimen in which dose adjustment in

response to hypocalcemia or hypercalcemia is performed in increments of 25%.

[0106] More preferably, the pharmaceutical composition comprising a controlled-release PTH compound is administered in accordance with a dosage regimen in which dose adjustment in response to hypocalcemia or hypercalcemia is performed in increments of 20%.

[0107] Even more preferably, the pharmaceutical composition comprising a controlled-release PTH compound is administered in accordance with a dosage regimen in which dose adjustment in response to hypocalcemia or hypercalcemia is performed in increments of 15%.

[0108] Most preferably, the pharmaceutical composition comprising a controlled-release PTH compound is administered in accordance with a dosage regimen in which dose adjustment in response to hypocalcemia or hypercalcemia is performed in increments of 10%.

[0109] Preferably, the pharmaceutical comprising the controlled-release PTH compound it administered to the patient no more often than once every 24 hours, such as every 24 hours, every 36 hours, every 48 hours, every 60 hours, every 72 hours, every 84 hours, every 96 hours, every 108 hours, every 120 hours, every 132 hours, every 144 hours, every 156 hours, once a week, once every two weeks.

[0110] In one embodiment the pharmaceutical composition comprising the controlled-release PTH compound is administered every 24 hours.

[0111] In another embodiment the pharmaceutical composition comprising the controlled-release PTH compound is administered every 48 hours.

[0112] In another embodiment the pharmaceutical composition comprising the controlled-release PTH compound is administered every 72 hours.

[0113] In another embodiment the pharmaceutical composition comprising the controlled-release PTH compound is administered every 96 hours.

[0114] In another embodiment the pharmaceutical composition comprising the controlled-release PTH compound is administered every 120 hours.

[0115] In another embodiment the pharmaceutical composition comprising the controlled-release PTH compound is administered every 144 hours.

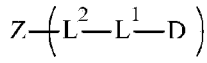
[0116] In another embodiment the pharmaceutical composition comprising the controlled-release PTH compound is administered once every week.

[0117] Preferably, the pharmaceutical composition comprising the PTH compound is administered to a patient via subcutaneous administration, preferably via subcutaneous injection.

[0118] In one embodiment the pharmaceutical composition for use of the present invention is performed with a syringe. In another embodiment the pharmaceutical composition for use of the present invention is administered with a pen injector. In another embodiment the pharmaceutical composition for use of the present invention is administered with an auto injector.

[0119] The controlled-release PTH compound is water-soluble.

[0120] Such water-soluble controlled-release PTH compound is a compound of formula (Ia)

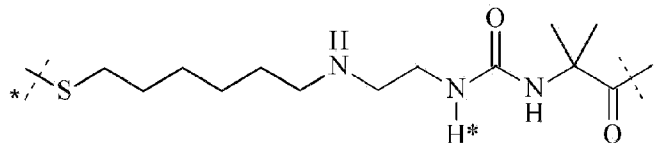


(Ia)

wherein

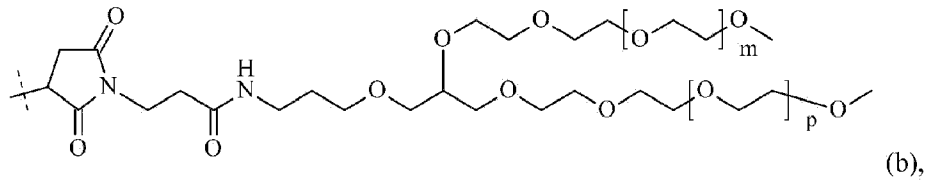
-D has the sequence of SEQ ID NO:51;

-L^1-L^2- has the formula:



(IIcb-iii);

wherein the unmarked dashed line indicates attached by an amide bond to a nitrogen of -D, and the dashed line marked with an asterisk indicates attachment to -Z ;-Z comprises a moiety of formula (b)



(b),

wherein

the dashed line indicates attachment to -L^2- or to the remainder of -Z; and

m and p are independently of each other an integer ranging from and including 400 to 500; and

x is 1.

[0121] It is understood that the compounds of formula (Ia) are PTH prodrugs, more specifically water-soluble PTH prodrugs.

[0122] -D has the sequence of SEQ ID NO:51.

[0123] The moiety -L^1- is either conjugated to a functional group of the side chain of an amino

acid residue of -D or to the N-terminal amine functional group of -D.

[0124] Preferably, the amino acid residue of -D to which -L^1- is conjugated comprises a primary or secondary amine functional group. Most preferably the amino acid residue of -D to which -L^1- is conjugated comprises a primary amine functional group.

[0125] In certain embodiments -L^1- is conjugated to a functional group of the side chain of an amino acid residue of -D. Preferably said amino acid is selected from the group consisting of histidine, lysine, tryptophan and arginine. Even more preferably said amino acid is selected from the group consisting of lysine and arginine.

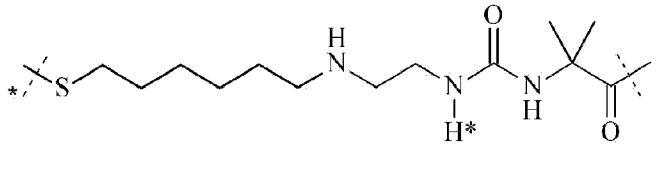
[0126] In one embodiment -L^1- is conjugated to a functional group of the side chain of a histidine of -D.

[0127] In another embodiment -L^1- is conjugated to a functional group of the side chain of a lysine of -D.

[0128] In another embodiment -L^1- is conjugated to a functional group of the side chain of a tryptophan of -D.

[0129] In another embodiment -L^1- is conjugated to a functional group of the side chain of an arginine of -D.

[0130] In a preferred embodiment -L^1- is conjugated to the N-terminal amine functional group of -D. The moiety -L^1-L^2- is



(IIcb-iii);

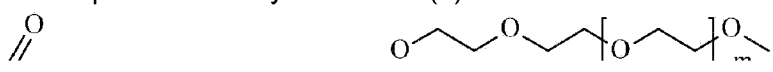
wherein

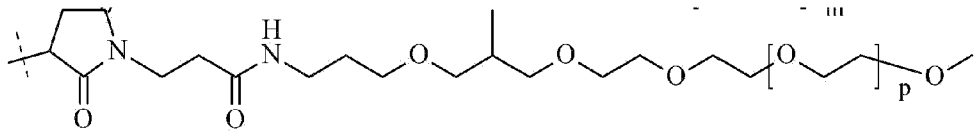
the unmarked dashed line indicates the attachment to a nitrogen of -D by forming an amide bond; and

the dashed line marked with the asterisk indicates attachment to -Z.

[0131] The controlled-release PTH compound of the present invention is of formula (Ia) with x = 1.

[0132] -Z comprises a moiety of formula (b)





(b),

wherein

the dashed line indicates attachment to -L^2- or to the remainder of -Z; and

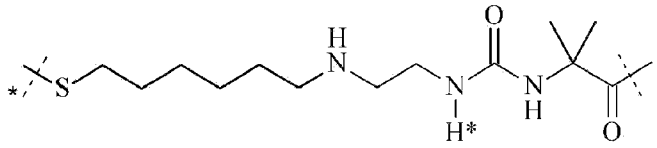
m and p are independently of each other an integer ranging from and including 400 to 500.

[0133] Preferably, m and p of formula (b) are the same integer.

[0134] Most preferably m and p of formula (b) are about 450.

[0135] Preferably, -Z is a moiety of formula (b).

[0136] In a preferred embodiment the PTH compound in the pharmaceutical composition of the present invention is of formula (IIf-i):

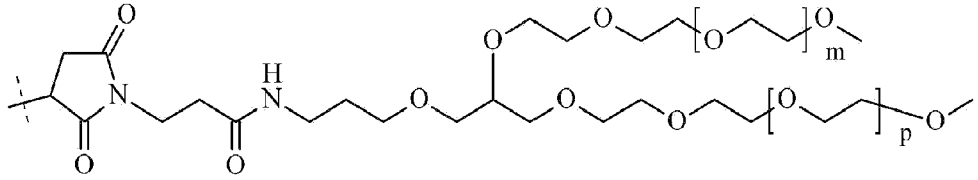


(IIf-i),

wherein

the unmarked dashed line indicates the attachment to a nitrogen of -D which is a PTH moiety by forming an amide bond; and

the dashed line marked with the asterisk indicates attachment to a moiety



wherein

m and p are independently an integer ranging from and including 400 to 500.

[0137] Preferably, -D is attached to the moiety of formula (IIf-i) through the N-terminal amine functional group of the PTH moiety.

[0138] In a preferred embodiment the residual activity of the controlled-release PTH compound in the form of a PTH prodrug is less than 10%, more preferably less than 1%, even more preferably less than 0.1%, even more preferably less than 0.01%, even more preferably less than 0.001% and most preferably less than 0.0001%.

[0139] As used herein the term "residual activity" refers to the activity exhibited by the PTH prodrug with the PTH moiety bound to a carrier in relation to the activity exhibited by the corresponding free PTH. In this context the term "activity" refers to binding to an activation of the PTH/PTHrP 1 receptor resulting in activation of adenylate cyclase to generate cAMP, phospholipase C to generate intracellular calcium, or osteoblastic expression of RANKL (which binds to RANK (Receptor Activator of Nuclear Factor kB) on osteoclasts. It is understood that measuring the residual activity of the PTH prodrug of the present invention takes time during which a certain amount of PTH will be released from the PTH prodrug of the present invention and that such released PTH will distort the results measured for the PTH prodrug. It is thus accepted practice to test the residual activity of a prodrug with a conjugate in which the drug moiety, in this case PTH, is non-reversibly, i.e. stably, bound to a carrier, which as closely as possible resembles the structure of the PTH prodrug for which residual activity is to be measured.

[0140] Preferably, the pharmaceutical composition comprising at least one controlled-release PTH compound of the present invention has a pH ranging from and including pH 3 to pH 8. More preferably, the pharmaceutical composition has a pH ranging from and including pH 4 to pH 6. Most preferably, the pharmaceutical composition has a pH ranging from and including pH 4 to pH 5.

[0141] In one embodiment the pharmaceutical composition comprising at least one controlled-release PTH compound of the present invention is a liquid or suspension formulation. It is understood that the pharmaceutical composition is a suspension formulation if the controlled-release PTH compound of the present invention is water-insoluble.

[0142] In another embodiment the pharmaceutical composition comprising at least one controlled-release PTH compound of the present invention is a dry formulation which is reconstituted before administration to a patient.

[0143] Such liquid, suspension, dry or reconstituted pharmaceutical composition comprises at least one excipient. Excipients used in parenteral formulations may be categorized as, for example, buffering agents, isotonicity modifiers, preservatives, stabilizers, anti-adsorption agents, oxidation protection agents, viscosifiers/viscosity enhancing agents, or other auxiliary agents. However, in some cases, one excipient may have dual or triple functions. Preferably, the at least one excipient comprised in the pharmaceutical composition of the present invention is selected from the group consisting of

1. (i) Buffering agents: physiologically tolerated buffers to maintain pH in a desired range, such as sodium phosphate, bicarbonate, succinate, histidine, citrate and acetate, sulphate, nitrate, chloride, pyruvate; antacids such as $Mg(OH)_2$ or $ZnCO_3$ may be also used;
2. (ii) Isotonicity modifiers: to minimize pain that can result from cell damage due to osmotic pressure differences at the injection depot; glycerin and sodium chloride are examples;

effective concentrations can be determined by osmometry using an assumed osmolality of 285-315 mOsmol/kg for serum;

3. (iii) Preservatives and/or antimicrobials: multidose parenteral formulations require the addition of preservatives at a sufficient concentration to minimize risk of patients becoming infected upon injection and corresponding regulatory requirements have been established; typical preservatives include m-cresol, phenol, methylparaben, ethylparaben, propylparaben, butylparaben, chlorobutanol, benzyl alcohol, phenylmercuric nitrate, thimerosol, sorbic acid, potassium sorbate, benzoic acid, chlorocresol, and benzalkonium chloride;
4. (iv) Stabilizers: Stabilisation is achieved by strengthening of the protein-stabilising forces, by destabilisation of the denatured state, or by direct binding of excipients to the protein; stabilizers may be amino acids such as alanine, arginine, aspartic acid, glycine, histidine, lysine, proline, sugars such as glucose, sucrose, trehalose, polyols such as glycerol, mannitol, sorbitol, salts such as potassium phosphate, sodium sulphate, chelating agents such as EDTA, hexaphosphate, ligands such as divalent metal ions (zinc, calcium, etc.), other salts or organic molecules such as phenolic derivatives; in addition, oligomers or polymers such as cyclodextrins, dextran, dendrimers, PEG or PVP or protamine or HSA may be used;
5. (v) Anti-adsorption agents: Mainly ionic or non-ionic surfactants or other proteins or soluble polymers are used to coat or adsorb competitively to the inner surface of the formulation's container; e.g., poloxamer (Pluronic F-68), PEG dodecyl ether (Brij 35), polysorbate 20 and 80, dextran, polyethylene glycol, PEG-polyhistidine, BSA and HSA and gelatins; chosen concentration and type of excipient depends on the effect to be avoided but typically a monolayer of surfactant is formed at the interface just above the CMC value;
6. (vi) Oxidation protection agents: antioxidants such as ascorbic acid, ectoine, methionine, glutathione, monothioglycerol, morin, polyethylenimine (PEI), propyl gallate, and vitamin E; chelating agents such as citric acid, EDTA, hexaphosphate, and thioglycolic acid may also be used;
7. (vii) Viscosifiers or viscosity enhancers: in case of a suspension retard settling of the particles in the vial and syringe and are used in order to facilitate mixing and resuspension of the particles and to make the suspension easier to inject (i.e., low force on the syringe plunger); suitable viscosifiers or viscosity enhancers are, for example, carbomer viscosifiers like Carbopol 940, Carbopol Ultrez 10, cellulose derivatives like hydroxypropylmethylcellulose (hypromellose, HPMC) or diethylaminoethyl cellulose (DEAE or DEAE-C), colloidal magnesium silicate (Veegum) or sodium silicate, hydroxyapatite gel, tricalcium phosphate gel, xanthans, carrageenans like Satia gum UTC 30, aliphatic poly(hydroxy acids), such as poly(D,L- or L-lactic acid) (PLA) and poly(glycolic acid) (PGA) and their copolymers (PLGA), terpolymers of D,L-lactide, glycolide and caprolactone, poloxamers, hydrophilic poly(oxyethylene) blocks and hydrophobic poly(oxypropylene) blocks to make up a triblock of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) (e.g. Pluronic®), polyetherester copolymer, such as a polyethylene glycol terephthalate/polybutylene terephthalate copolymer, sucrose acetate isobutyrate (SAIB), dextran or derivatives thereof, combinations of dextrans and

PEG, polydimethylsiloxane, collagen, chitosan, polyvinyl alcohol (PVA) and derivatives, polyalkylimides, poly (acrylamide-co-diallyldimethyl ammonium (DADMA)), polyvinylpyrrolidone (PVP), glycosaminoglycans (GAGs) such as dermatan sulfate, chondroitin sulfate, keratan sulfate, heparin, heparan sulfate, hyaluronan, ABA triblock or AB block copolymers composed of hydrophobic A-blocks, such as polylactide (PLA) or poly(lactide-co-glycolide) (PLGA), and hydrophilic B-blocks, such as polyethylene glycol (PEG) or polyvinyl pyrrolidone; such block copolymers as well as the abovementioned poloxamers may exhibit reverse thermal gelation behavior (fluid state at room temperature to facilitate administration and gel state above sol-gel transition temperature at body temperature after injection);

8. (viii) Spreading or diffusing agent: modifies the permeability of connective tissue through the hydrolysis of components of the extracellular matrix in the intrastitial space such hyaluronic acid, a polysaccharide found in the intercellular space of connective tissue; a spreading agent such as hyaluronidase temporarily decreases the viscosity of the extracellular matrix and promotes diffusion of injected drugs; and
9. (ix) Other auxiliary agents: such as wetting agents, viscosity modifiers, antibiotics, hyaluronidase; acids and bases such as hydrochloric acid and sodium hydroxide are auxiliary agents necessary for pH adjustment during manufacture.

[0144] Preferably, the pharmaceutical composition comprising the PTH compound is administered to a patient via subcutaneous administration, preferably via subcutaneous injection.

[0145] Preferably, the patient treated for hypoparathyroidism of the present invention is a mammalian patient, preferably a human patient.

Examples

Materials and Methods

[0146] Side chain protected PTH(1-34) (SEQ ID NO:51) on TCP resin having Boc protected N-terminus and ivDde protected side chain of Lys26 (synthesized by Fmoc-strategy) was obtained from a custom peptide synthesis supplier.

[0147] Side chain protected PTH(1-34) on TCP resin having Fmoc protected N-terminus (synthesized by Fmoc-strategy) was obtained from a custom peptide synthesis supplier.

[0148] PEG 2x20 kDa maleimide, Sunbright GL2-400MA was purchased from NOF Europe N.V., Grobbendonk, Belgium. S-Trityl-6-mercaptohexanoic acid was purchased from Polypeptide, Strasbourg, France. HATU was obtained from Merck Biosciences GmbH,

Schwalbach/Ts, Germany. Fmoc-N-Me-Asp(OBn)-OH was obtained from Peptide International Inc., Louisville, KY, USA. Fmoc-Aib-OH was purchased from Iris Biotech GmbH, Marktredwitz, Germany. All other chemicals and reagents were purchased from Sigma Aldrich GmbH, Taufkirchen, Germany, unless a different supplier is mentioned.

[0149] Compound 11a (examples 11-15) was synthesized following the procedure described in patent WO29095479A2, example 1.

[0150] Syringes equipped with polyethylenene frits (MultiSynTech GmbH, Witten, Germany) were used as reaction vessels or for washing steps of peptide resins.

[0151] General procedure for the removal of ivDde protecting group from side chain protected PTH on resin: The resin was pre-swollen in DMF for 30 min and the solvent was discarded. The ivDde group was removed by incubating the resin with DMF/hydrazine hydrate 4/1 (v/v, 2.5 mL/g resin) for 8 x 15 min. For each step fresh DMF/hydrazine hydrate solution was used. Finally, the resin was washed with DMF (10 x), DCM (10 x) and dried *in vacuo.*

[0152] General procedure for the removal of Fmoc protecting group from protected PTH on resin: The resin was pre-swollen in DMF for 30 min and the solvent was discarded. The Fmoc group was removed by incubating the resin with DMF/piperidine/DBU 96/2/2 (v/v/v, 2.5 mL/g resin) for 3 x 10 min. For each step fresh DMF/piperidine/DBU hsolution was used. Finally, the resin was washed with DMF (10 x), DCM (10 x) and dried *in vacuo.*

RP-HPLC purification:

[0153] For preparative RP-HPLC a Waters 600 controller and a 2487 Dual Absorbance Detector was used, equipped with the following columns: Waters XBridge™ BEH300 Prep C18 5 µm, 150 x 10 mm, flow rate 6 mL/min, or Waters XBridge™ BEH300 Prep C18 10 µm, 150 x 30 mm, flow rate 40 mL/min. Linear gradients of solvent system A (water containing 0.1 % TFA v/v) and solvent system B (acetonitrile containing 0.1 % TFA v/v) were used. HPLC fractions containing product were pooled and lyophilized if not stated otherwise.

Flash Chromatography:

[0154] Flash chromatography purifications were performed on an Isolera One system from Biotage AB, Sweden, using Biotage KP-Sil silica cartridges and n-heptane and ethyl acetate as eluents. Products were detected at 254 nm.

[0155] Ion exchange chromatography:

[0156] Ion exchange chromatography (IEX) was performed using an Amersham Bioscience

AEKTAbasic system equipped with a MacroCap SP cation exchanger column (Amersham Bioscience/GE Healthcare). 17 mM acetic acid pH 4.5 (solvent A) and 17 mM acetic acid, 1 M NaCl, pH 4.5 (solvent B) were used as mobile phases.

Size exclusion chromatography:

[0157] Size exclusion chromatography (SEC) was performed using an Amersham Bioscience AEKTAbasic system equipped with HiPrep 26/10 desalting columns (Amersham Bioscience/GE Healthcare). 0.1 % (v/v) acetic acid was used as mobile phase.

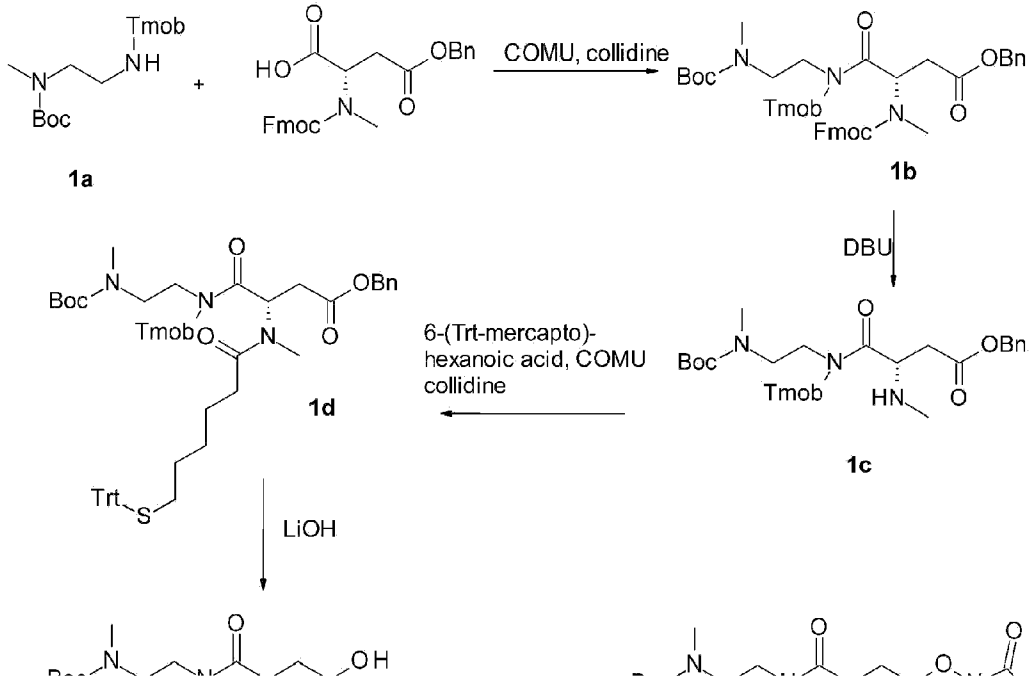
Analytical methods

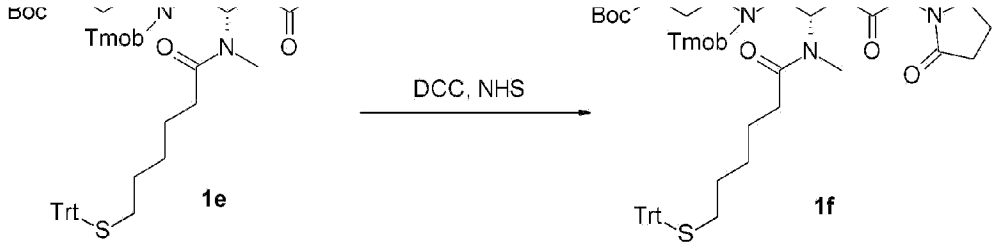
[0158] Analytical ultra-performance LC (UPLC)-MS was performed on a Waters Acquity system equipped with a Waters BEH300 C18 column (2.1 × 50 mm, 1.7 µm particle size, flow: 0.25 mL/min; solvent A: water containing 0.04% TFA (v/v), solvent B: acetonitrile containing 0.05% TFA (v/v)) coupled to a LTQ Orbitrap Discovery mass spectrometer from Thermo Scientific or coupled to a Waters Micromass ZQ.

Example 1 - not according to the invention

Synthesis of linker reagent If

[0159] Linker reagent **If** was synthesized according to the following scheme:





[0160] To a solution of N-methyl-N-Boc-ethylenediamine (2 g, 11.48 mmol) and $NaCNBH_3$ (819 mg, 12.63 mmol) in MeOH (20 mL) was added 2,4,6-trimethoxybenzaldehyde (2.08 g, 10.61 mmol) portion wise. The mixture was stirred at rt for 90 min, acidified with 3 M HCl (4 mL) and stirred further 15 min. The reaction mixture was added to saturated $NaHCO_3$ solution (200 mL) and extracted 5 x with DCM. The combined organic phases were dried over Na_2SO_4 and the solvents were evaporated *in vacuo.* The resulting N-methyl-N-Boc-N'-Tmob-ethylenediamine 1a was dried in high vacuum and used in the next reaction step without further purification.

Yield:	3.76 g (11.48 mmol, 89 % purity, **1a:** double Tmob protected product = 8 :1)
MS:	m/z 355.22 = $[M+H]^+$, (calculated monoisotopic mass = 354.21).

[0161] To a solution of 1a (2 g, 5.65 mmol) in DCM (24 mL) COMU (4.84 g, 11.3 mmol), N-Fmoc-N-Me-Asp(OBn)-OH (2.08 g, 4.52 mmol) and 2,4,6-collidine (2.65 mL, 20.34 mmol) were added. The reaction mixture was stirred for 3 h at rt, diluted with DCM (250 mL) and washed 3 x with 0.1 M H_2SO_4 (100 mL) and 3 x with brine (100 mL). The aqueous phases were re-extracted with DCM (100 mL). The combined organic phases were dried over Na_2SO_4, filtrated and the residue concentrated to a volume of 24 mL. **1b** was purified using flash chromatography.

Yield:	5.31 g (148 %, 6.66 mmol)
MS:	m/z 796.38 = $[M+H]^+$, (calculated monoisotopic mass = 795.37).

[0162] To a solution of **1b** (5.31 g, max. 4.52 mmol ref. to N-Fmoc-N-Me-Asp(OBn)-OH) in THF (60 mL) DBU (1.8 mL, 3 % v/v) was added. The solution was stirred for 12 min at rt, diluted with DCM (400 mL) and washed 3 x with 0.1 M H_2SO_4 (150 mL) and 3 x with brine (150 mL). The aqueous phases were re-extracted with DCM (100 mL). The combined organic phases were dried over Na_2SO_4 and filtrated. **1c** was isolated upon evaporation of the solvent and used in the next reaction without further purification.

MS:	m/z 574.31 = $[M+H]^+$, (calculated monoisotopic mass = 573.30).

[0163] **1c** (5.31 g, 4.52 mmol, crude) was dissolved in acetonitrile (26 mL) and COMU (3.87 g,

9.04 mmol), 6-tritylmercaptohexanoic acid (2.12 g, 5.42 mmol) and 2,4,6-collidine (2.35 mL, 18.08 mmol) were added. The reaction mixture was stirred for 4 h at rt, diluted with DCM (400 mL) and washed 3 x with 0.1 M H_2SO_4 (100 mL) and 3 x with brine (100 mL). The aqueous phases were re-extracted with DCM (100 mL). The combined organic phases were dried over Na_2SO_4, filtered and **1d** was isolated upon evaporation of the solvent. Product **1d** was purified using flash chromatography.

Yield:	2.63 g (62 %, 94 % purity)
MS:	m/z 856.41 = $[M+H]^+$, (calculated monoisotopic mass = 855.41).

[0164] To a solution **of 1d** (2.63 g, 2.78 mmol) in i-PrOH (33 mL) and H_2O (11 mL) was added LiOH (267 mg, 11.12 mmol) and the reaction mixture was stirred for 70 min at rt. The mixture was diluted with DCM (200 mL) and washed 3 x with 0.1 M H_2SO_4 (50 mL) and 3 x with brine (50 mL). The aqueous phases were re-extracted with DCM (100 mL). The combined organic phases were dried over Na_2SO_4, filtered and **1e** was isolated upon evaporation of the solvent. **1e** was purified using flash chromatography.

Yield:	2.1 g (88%)
MS:	m/z 878.4 = $[M+Na]^+$, (calculated monoisotopic mass = 837.40).

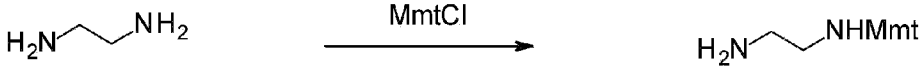
[0165] To a solution of **1e** (170 mg, 0.198 mmol) in anhydrous DCM (4 mL) were added DCC (123 mg, 0.59 mmol), and a catalytic amount of DMAP. After 5 min, N-hydroxy-succinimide (114 mg, 0.99 mmol) was added and the reaction mixture was stirred at rt for 1 h. The reaction mixture was filtered, the solvent was removed *in vacuo* and the residue was taken up in 90 % acetonitrile plus 0.1 % TFA (3.4 mL). The crude mixture was purified by RP-HPLC. Product fractions were neutralized with 0.5 M pH 7.4 phosphate buffer and concentrated. The remaining aqueous phase was extracted with DCM and **If** was isolated upon evaporation of the solvent.

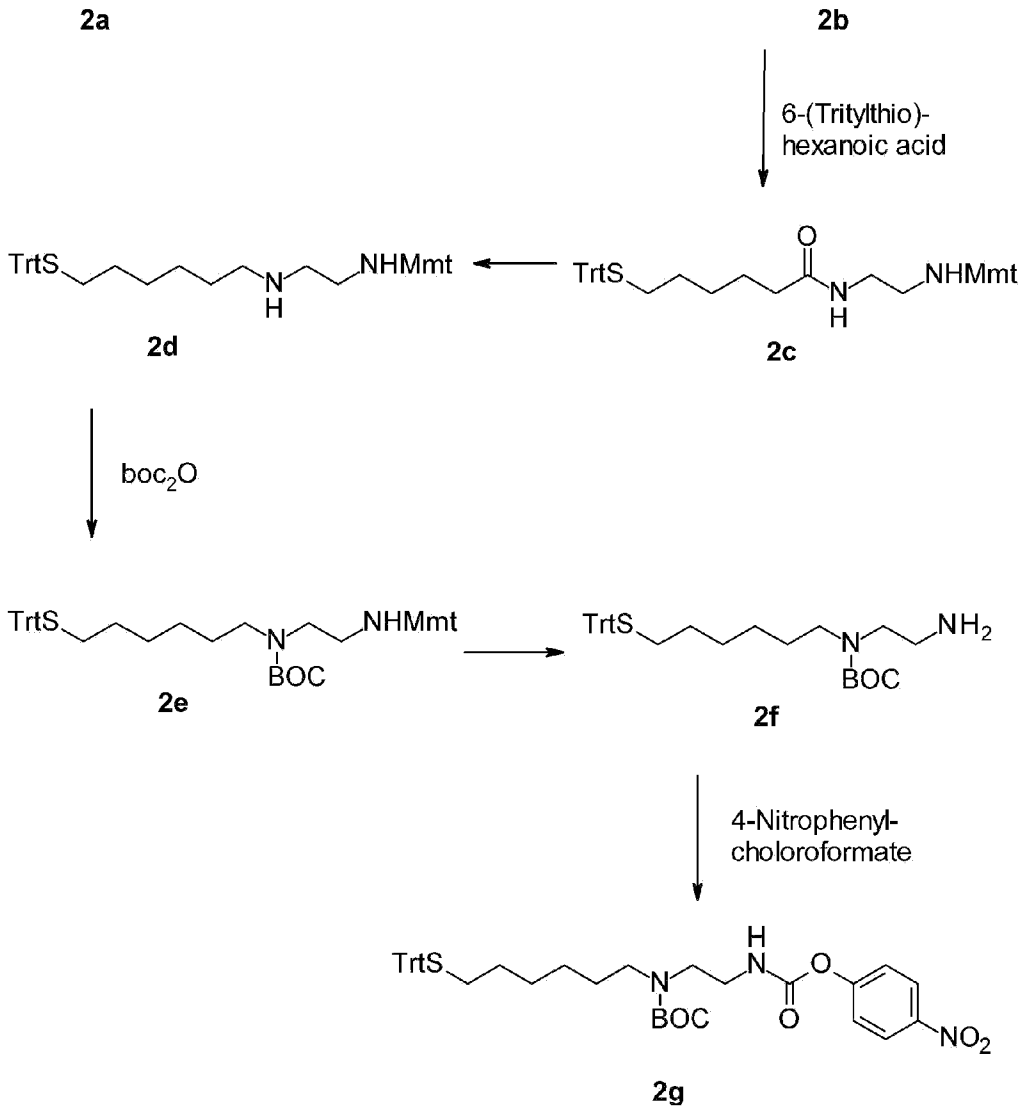
Yield:	154 mg (81%)
MS:	m/z 953.4 = $[M+H]^+$, (calculated monoisotopic mass = 952.43)

Example 2

Synthesis of linker reagent 2g

[0166]





[0167] 4-Methoxytriphenylmethyl chloride (3.00 g, 9.71 mmol) was dissolved in DCM (20 mL) and added dropwise under stirring to a solution of ethylenediamine **2a** (6.5 mL, 97.3 mmol) in DCM (20 mL). The reaction mixture was stirred for 2 h at rt after which it was diluted with diethyl ether (300 mL), washed 3 x with brine/0.1 M NaOH 30/1 (v/v) and once with brine. The organic phase was dried over Na_2SO_4 and **2b** was isolated upon evaporation of the solvent.

Yield:	3.18 g (98%)

[0168] Mmt protected intermediate **2b** (3.18 g, 9.56 mmol) was dissolved in DCM (30 mL). 6-(Tritylthio)-hexanoic acid (4.48 g, 11.5 mmol), PyBOP (5.67 g, 10.9 mmol) and DIPEA (5.0 mL, 28.6 mmol) were added and the mixture was stirred for 30 min at rt. The solution was diuted with diethyl ether (250 mL), washed 3 x with brine/0.1 M NaOH 30/1 (v/v) and once with brine. The organic phase was dried over Na_2SO_4 and the solvent was removed in vacuo. **2c** was purified using flash chromatography.

Yield:	5.69 g (85 %)

MS:	m/z 705.4 = $[M+H]^+$, (calculated monoisotopic mass = 704.34).

[0169] Compound **2c** (3.19 g, 4.53 mmol) was dissolved in abhydrous THF (50 mL), 1 M BH_3·THF solution in THF (8.5 mL, 8.5 mmol) was added and the mixture was stirred for 16 h at rt. More 1 M BH_3·THF solution in THF (14 mL, 14.0 mmol) was added and the mixture was stirred for further 16 h at rt. Methanol (8.5 mL) and N,N'-dimethyl-ethylendiamine (3.00 mL, 27.9 mmol) were added and the mixture was heated under reflux for 3 h. The mixture was allowed to cool down and ethyl acetate (300 mL) was added. The solution was washed 2 x with aqueous Na_2CO_3 and 2 x with aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4 and the solvent was removed in vacuo to obtain **2d.**

Yield:	3.22 g (103 %)
MS:	m/z 691.4 = $[M+H]^+$, (calculated monoisotopic mass = 690.36).

[0170] Di-tert-butyl dicarbonate (2.32 g, 10.6 mmol) and DIPEA (3.09 mL, 17.7 mmol) were dissolved in DCM (5 mL) and added to a solution **of 2d** (2.45 g, 3.55 mmol) in DCM (5 mL). The mixture was stirred for 30 min at rt. The solution was concentrated in vacuo and purified by flash chromatography to obtain product **2e.**

Yield:	2.09 g (74 %)
MS:	m/z 791.4 = $[M+H]^+$, (calculated monoisotopic mass = 790.42).

[0171] Compound **2e** (5.01 g, 6.34 mmol) was dissolved in acetonitrile (80 mL). 0.4 M aqueous HCl (80 mL) followed by acetonitrile (20 mL) was added and the mixture was stirred for 1 h at rt. The pH was adjusted to pH 5.5 by addition of aqueous 5 M NaOH. The organic solvent was removed in vacuo and the remaining aqueous solution was extracted 4 x with DCM. The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo to obtain product **2f.**

Yield:	4.77 g (95 %)
MS:	m/z 519.3 = $[M+H]^+$, (calculated monoisotopic mass = 518.30).

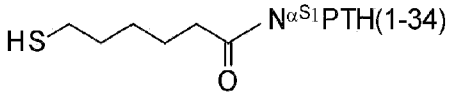
[0172] Compound **2f** (5.27 g, 6.65 mmol) was dissolved in DCM (30 mL) and added to a solution of p-nitrophenyl chloroformate (2.01 g, 9.98 mmol) in DCM (25 mL). 2,4,6-trimethylpyridine (4.38 mL, 33.3 mmol) was added and the solution was stirred for 45 min at rt. The solution was concentrated *in vacu* and purified by flash chromatography to obtain product **2g.**

Yield:	4.04 g (89 %)
MS:	m/z 706.32 = $[M+Na]^+$, (calculated monoisotopic mass = 683.30).

Example 3 - not according to the invention

Synthesis of permanent S1 PTH(1-34) conjugate 3

[0173]



3

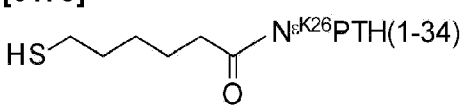
[0174] Side chain protected PTH(1-34) on TCP resin having Fmoc protected N-terminus was Fmoc deprotected according to the procedure given in Materials and Methods. A solution of 6-tritylmercaptohexanoic acid (62.5 mg, 160 µmol), PyBOP (80.1 mg, 154 µmol) and DIPEA (53 µL, 306 µmol) in DMF (2 mL) was added to 0.21 g (51 µmol) of the resin. The suspension was agitated for 80 min at rt. The resin was washed 10 x with DMF, 10 x with DCM and dried *in vacuo.* Cleavage of the peptide from the resin and removal of protecting groups was achieved by adding 10 mL cleavage cocktail 100/3/3/2/1 (v/w/v/v/v) TFA/DTT/TES/water/thioanisole and agitating the suspension for 1 h at rt. Crude 3 was precipitated in pre-cooled diethyl ether (-18 °C). The precipitate was dissolved in ACN/water and purified by RP-HPLC. The product fractions were freeze-dried.

Yield:	36 mg (14 %), **3***8 TFA
MS:	m/z 1062.31 = $[M+4H]^{4+}$, (calculated monoisotopic mass for $[M+4H]^{4+}$ = 1062.30).

Example 4

Synthesis of permanent K26 PTH(1-34) conjugate 4 - not according to the invention

[0175]



4

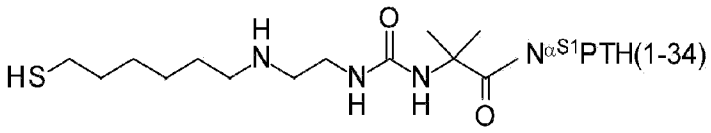
[0176] Side chain protected PTH(1-34) on TCP resin having Boc protected N-terminus and ivDde protected side chain of Lys26 was ivDde deprotected according to the procedure given in Materials and Methods. A solution of 6-tritylmercaptohexanoic acid (107 mg, 273 µmol), PyBOP (141 mg, 273 µmol) and DIPEA (95 µL, 545 µmol) in DMF (3 mL) was added to 0.80 g (90.9 µmol) of the resin. The suspension was agitated for 1 h at rt. The resin was washed 10 x with DMF, 10 x with DCM and dried *in vacuo.* Cleavage of the peptide from the resin and removal of protecting groups was achieved by adding 6 mL cleavage cocktail 100/3/3/2/1 (v/w/v/v/v) TFA/DTT/TES/water/thioanisole and agitating the suspension for 1 h at rt. Crude **4** was precipitated in pre-cooled diethyl ether (-18 °C). The precipitate was dissolved in ACN/water and purified by RP-HPLC. The product fractions were freeze-dried.

Yield:	40 mg (8 %), **4***8 TFA
MS:	m/z 1062.30 = $[M+4H]^{4+}$, (calculated monoisotopic mass for $[M+4H]^{4+}$ = 1062.30).

Example 5

Synthesis of transient S1 PTH(1-34) conjugate

[0177]



5

[0178] Side chain protected PTH(1-34) on TCP resin having Fmoc protected N-terminus was Fmoc deprotected according to the procedure given in Materials and Methods. A solution of Fmoc-Aib-OH (79 mg, 244 µmol), PyBOP (127 mg, 244 µmol) and DIPEA (64 µL, 365 µmol) in DMF (1.5 mL) was added to 0.60 g (61 µmol) of the resin. The suspension was agitated for 16 h at rt. The resin was washed 10 x with DMF and Fmoc-deprotected as described above. A solution of **2g** (167 mg, 244 µmol) and DIPEA (64 µL, 365 µmol) in DMF (1.5 mL) was added to the resin. The suspension was agitated for 24 h at rt. The resin was washed 10 x with DMF, 10 x with DCM and dried *in vacuo.* Cleavage of the peptide from the resin and removal of protecting groups was achieved by adding 7 mL cleavage cocktail 100/3/3/2/1 (v/w/v/v/v) TFA/DTT/TES/water/thioanisole and agitating the suspension for 1 h at rt. Crude **5** was precipitated in pre-cooled diethyl ether (-18 °C). The precipitate was dissolved in ACN/water and purified by RP-HPLC. The product fractions were freeze-dried.

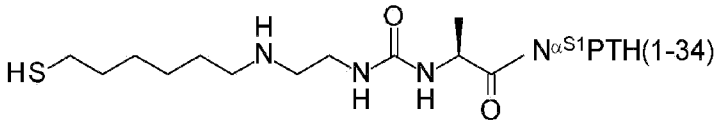
Yield:	78 mg (24 %), **5***9 TFA
MS:	m/z 1101.59 = $[M+4H]^{4+}$, (calculated monoisotopic mass for $[M+4H]^{4+}$ =

	1101.57).

Example 6

Synthesis of transient S1 PTH(1-34) conjugate 6 - not according to the invention

[0179]



6

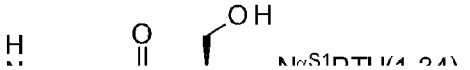
[0180] Side chain protected PTH(1-34) on TCP resin having Fmoc protected N-terminus was Fmoc deprotected according to the procedure given in Materials and Methods. A solution of Fmoc-Ala-OH (32 mg, 102 µmol), PyBOP (53 mg, 102 µmol) and DIPEA (27 µL, 152 µmol) in DMF (3 mL) was added to 0.25 g (25 µmol) of the resin. The suspension was shaken for 1 h at rt. The resin was washed 10 x with DMF, 10 x with DCM and dried under vacuum. Fmoc-deprotection was performed as described above. A solution **of 2g** (69 mg, 102 µmol) and DIPEA (27 µL, 152 µmol) in DMF (3 mL) was added to the resin. The suspension was agitated for 1.5 h at rt. The resin was washed 10 x with DMF, 10 x with DCM and dried *in vacuo.* Cleavage of the peptide from the resin and removal of protecting groups was achieved by adding 3 mL cleavage cocktail 100/3/3/2/1 (v/w/v/v/v) TFA/DTT/TES/water/thioanisole and agitating the suspension for 1 h at rt. Crude **6** was precipitated in pre-cooled diethyl ether (-18 °C). The precipitate was dissolved in ACN/water and purified by RP-HPLC. The product fractions were freeze-dried.

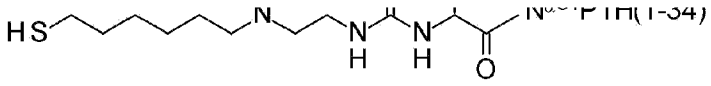
Yield:	25 mg (18 %), **6***9 TFA
MS:	m/z 1098.75 = $[M+4H]^{4+}$, (calculated monoisotopic mass for $[M+4H]^{4+}$ = 1098.07).

Example 7

Synthesis of transient S1 PTH(1-34) conjugate 7 - not according to the invention

[0181]





7

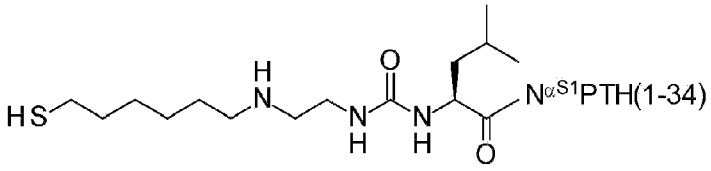
[0182] Side chain protected PTH(1-34) on TCP resin having Fmoc protected N-terminus was Fmoc deprotected according to the procedure given in Materials and Methods. A solution of Fmoc-Ser(Trt)-OH (117 mg, 205 µmol), PyBOP (108 mg, 207 µmol) and DIPEA (53 µL, 305 µmol) in DMF (2 mL) was added to 0.50 g (51 µmol) of the resin. The suspension was agitated for 1 h at rt. The resin was washed 10 x with DMF, 10 x with DCM and dried under vacuum. Fmoc-deprotection was performed as described above. A solution **of 2g** (144 mg, 211 µmol) and DIPEA (53 µL, 305 µmol) in DMF (1.8 mL) was added to the resin. The suspension was shaken for 7 h at rt. The resin was washed 10 x with DMF, 10 x with DCM and dried *in vacuo.* Cleavage of the peptide from the resin and removal of protecting groups was achieved by adding 6 mL cleavage cocktail 100/3/3/2/1 (v/w/v/v/v) TFA/DTT/TES/water/thioanisole and agitating the suspension for 1 h at rt. Crude **7** was precipitated in pre-cooled diethyl ether (-18 °C). The precipitate was dissolved in ACN/water and purified by RP-HPLC. The product fractions were freeze-dried.

Yield:	54 mg (20 %), **7***9 TFA
MS:	m/z 1102.08 = $[M+4H]^{4+}$, (calculated monoisotopic mass for $[M+4H]^{4+}$ = 1102.07).

Example 8

Synthesis of transient S1 PTH(1-34) conjugate 8 - not according to the invention

[0183]



8

[0184] Side chain protected PTH(1-34) on TCP resin having Fmoc protected N-terminus was Fmoc deprotected according to the procedure given in Materials and Methods. A solution of Fmoc-Leu-OH (36 mg, 102 µmol), PyBOP (53 mg, 102 µmol) and DIPEA (27 µL, 152 µmol) in DMF (3 mL) was added to 0.25 g (25 µmol) of the resin. The suspension was agitated for 1 h at rt. The resin was washed 10 x with DMF, 10 x with DCM and dried under vacuum. Fmoc-deprotection was performed as described above. A solution of **2g** (69 mg, 102 µmol) and

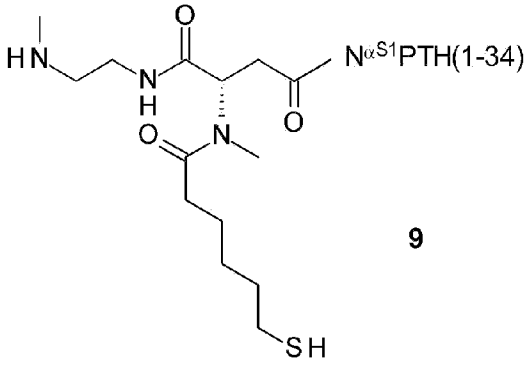
DIPEA (27 µL, 152 µmol) in DMF (3 mL) was added to the resin. The suspension was agitated for 1.5 h at rt. The resin was washed 10 x with DMF, 10 x with DCM and dried *in vacuo.* Cleavage of the peptide from the resin and removal of protecting groups was achieved by adding 3 mL cleavage cocktail 100/3/3/2/1 (v/w/v/v/v) TFA/DTT/TES/water/thioanisole and agitating the suspension for 1 h at rt. Crude **8** was precipitated in pre-cooled diethyl ether (-18 °C). The precipitate was dissolved in ACN/water and purified by RP-HPLC. The product fractions were freeze-dried.

Yield:	31 mg (22 %), **8***9 TFA
MS:	m/z 1109.32 = $[M+4H]^{4+}$, (calculated monoisotopic mass for $[M+4H]^{4+}$ = 1108.58).

Example 9

Synthesis of transient S1 PTH(1-34) conjugate 9 - not according to the invention

[0185]



9

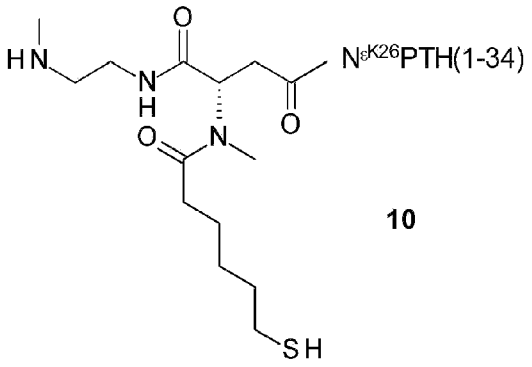
[0186] Side chain protected PTH(1-34) on TCP resin having Fmoc protected N-terminus was Fmoc deprotected according to the procedure given in Materials and Methods. A solution of **1e** (182 mg, 213 µmol), PyBOP (111 mg, 213 µmol) and DIPEA (93 µL, 532 µmol) in DMF (5 mL) was added to 2.00 g (107 µmol) of the resin. The suspension was agitated for 16 h at rt. The resin was washed 10 x with DMF, 10 x with DCM and dried under vacuum. Cleavage of the peptide from the resin and removal of protecting groups was achieved by adding 20 mL cleavage cocktail 100/3/3/2/1 (v/w/v/v/v) TFA/DTT/TES/water/thioanisole and agitating the suspension for 1 h at rt. Crude **9** was precipitated in pre-cooled diethyl ether (-18 °C). The precipitate was dissolved in ACN/water and purified by RP-HPLC. The product fractions were freeze-dried.

Yield:	47 mg (8 %), **9***9 TFA
MS:	m/z 1108.58 = $[M+4H]^{4+}$, (calculated monoisotopic mass for $[M+4H]^{4+}$ = 1108.57).

Example 10

Synthesis of transient K26 PTH(1-34) conjugate 10 - not according to the invention

[0187]



10

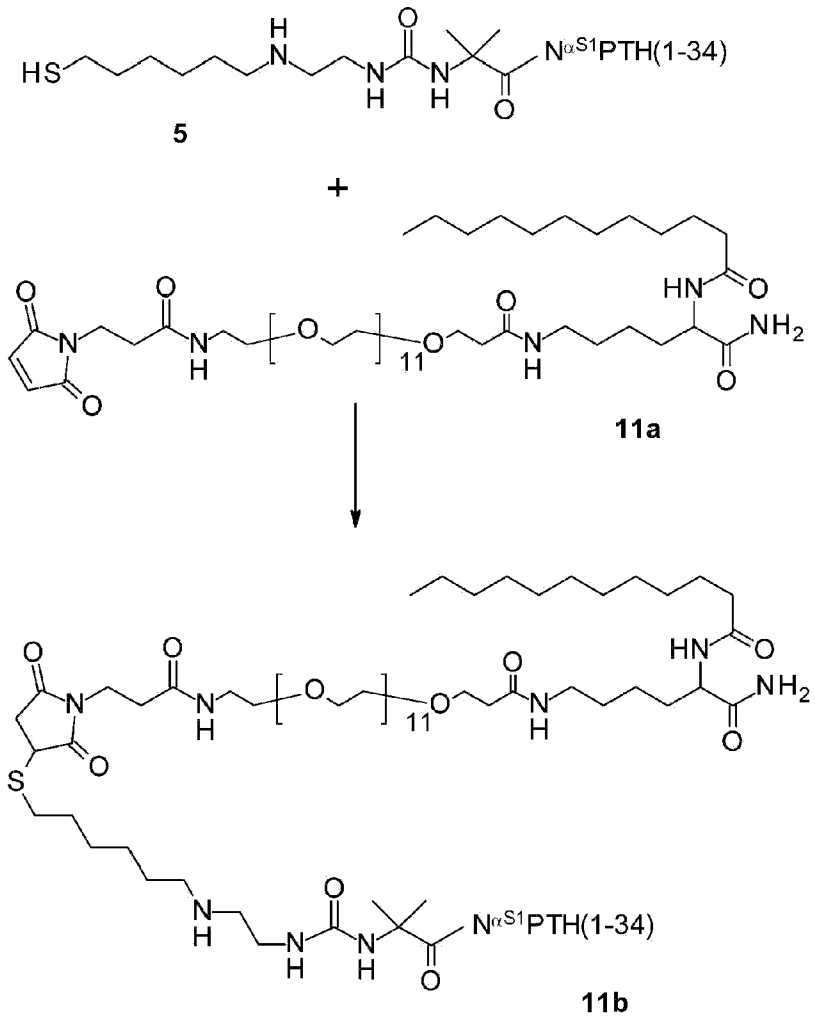
[0188] Side chain protected PTH(1-34) on TCP resin having Boc protected N-terminus and ivDde protected side chain of Lys26 was ivDde deprotected according to the procedure given in Materials and Methods. A solution of **1f** (867 mg, 910 µmol) and DIPEA (0.24 mL, 1.36 mmol) in DMF (5 mL) was added to 1.91 g (227 µmol) of the resin. The suspension was agitated for 1 h at rt. The resin was washed 10 x with DMF, 10 x with DCM and dried under vacuum. Cleavage of the peptide from the resin and removal of protecting groups was achieved by adding 20 mL cleavage cocktail 100/3/3/2/1 (v/w/v/v/v) TFA/DTT/TES/water/thioanisole and shaking the suspension for 1 h at rt. Crude **10** was precipitated in pre-cooled diethyl ether (-18 °C). The precipitate was dissolved in ACN/water and purified by RP-HPLC. The product fractions were freeze-dried.

Yield:	92 mg (7 %), **10***9 TFA
MS:	m/z 1108.58 = $[M+4H]^{4+}$, (calculated monoisotopic mass for $[M+4H]^{4+}$ = 1108.57).

Example 11

Synthesis of low molecular weight transient S1 PEG conjugate 11b - not according to the invention

[0189]



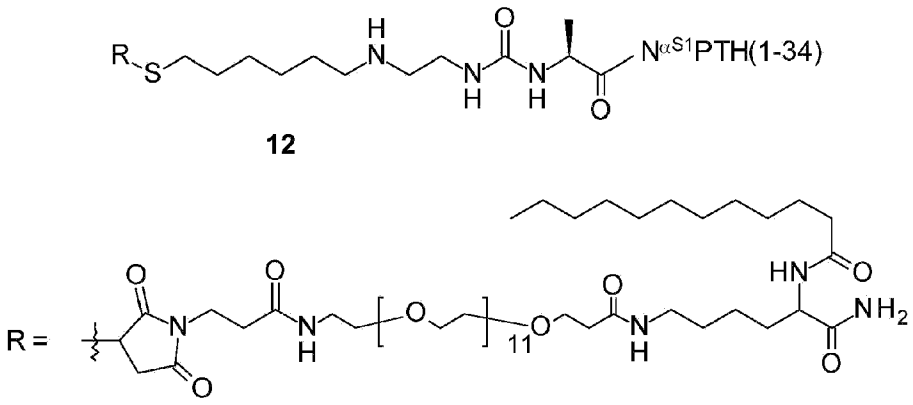
[0190] 0.15 mL of a 0.5 M NaH_2PO_4 buffer (pH 7.4) was added to 0.5 mL of a 20 mg/mL solution of thiol **5** (10 mg, 1.84 µmol) in 1/1 (v/v) acetonitrile/water containing 0.1 % TFA (v/v). The solution was incubated at rt for 10 min after which 238 µL of a 10 mg/mL solution of maleimide **11a** (2.4 mg, 2.21 µmol) in 1/1 (v/v) acetonitrile/water containing 0.1 % TFA (v/v) were added. The solution was incubated for 20 min at rt. 10 µL TFA was added and the mixture was purified by RP-HPLC. The product fractions were freeze-dried to obtain **11b.**

Yield:	3.1 mg (26 %), **11b*9** TFA
MS:	m/z 1097.00 = $[M+4H]^{4+}$, (calculated monoisotopic mass for $[M+5H]^{5+}$ = 1096.99).

Example 12

Synthesis of low molecular weight transient S1 PEG conjugate 12 - not according to the invention

[0191]



12

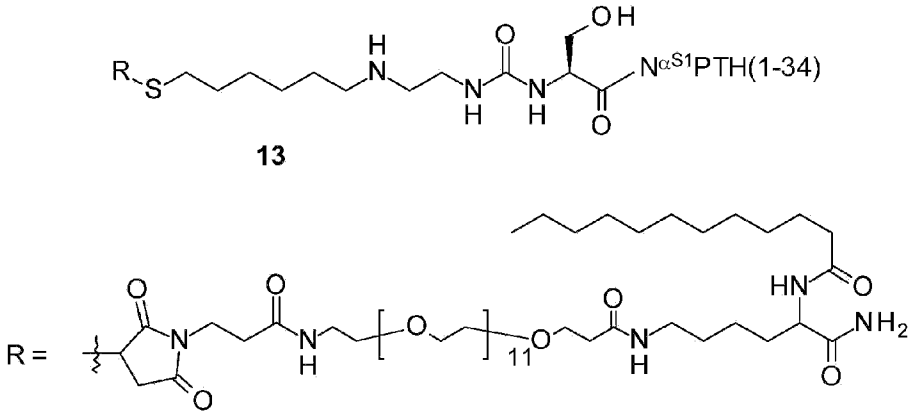
[0192] Conjugate **12** was synthesized as described for **11b** by using thiol **6** (10 mg, 1.85 µmol) and maleimide **11a** (2.4 mg, 2.21 µmol).

Yield:	10 mg (83 %), **12***9 TFA
MS:	m/z 1094.20 = $[M+4H]^{4+}$, (calculated monoisotopic mass for $[M+4H]^{4+}$ = 1094.19).

Example 13

Synthesis of low molecular weight transient S1 PEG conjugate 13 - not according to the invention

[0193]



13

[0194] Conjugate **13** was synthesized as described for **11b** by using thiol **7** (10 mg, 1.84 µmol)

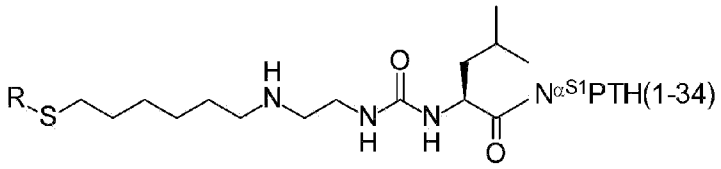
and maleimide **11a** (2.4 mg, 2.21 µmol).

Yield:	8 mg (67 %), **13***9 TFA
MS:	m/z 1097.40 = $[M+5H]^{5+}$, (calculated monoisotopic mass for $[M+5H]^{5+}$ = 1097.39).

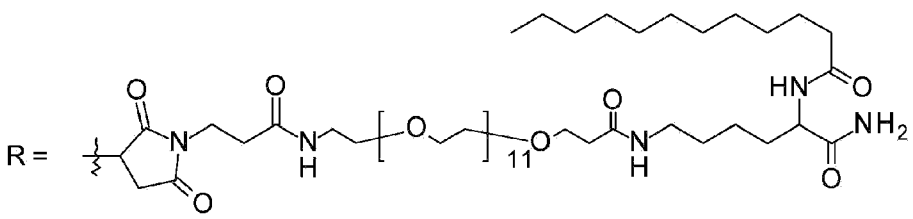
Example 14

Synthesis of low molecular weight transient S1 PEG conjugate 14 - not according to the invention

[0195]



14



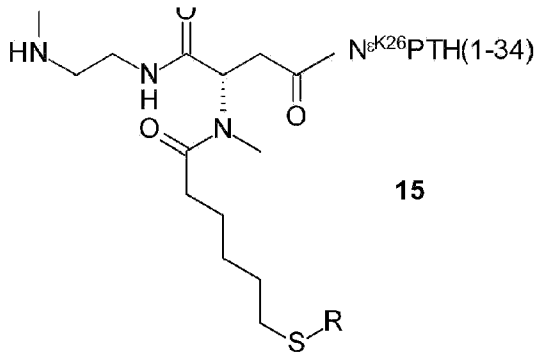
[0196] Conjugate **14** was synthesized as described for **11b** by using thiol **8** (10 mg, 1.83 µmol) and maleimide **11a** (2.4 mg, 2.21 µmol).

Yield:	4 mg (33 %), **14***9 TFA
MS:	m/z 1378.01 = $[M+4H]^{4+}$, (calculated monoisotopic mass for $[M+4H]^{4+}$ = 1378.00).

Example 15

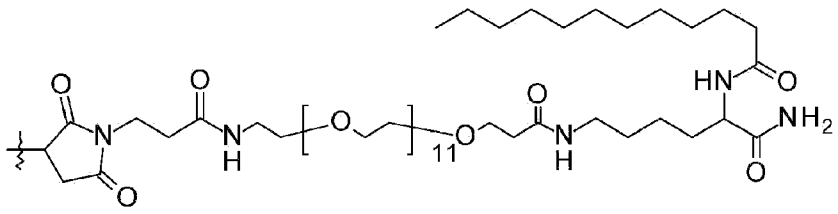
Synthesis of low molecular weight transient K26 PEG conjugate 15 - not according to the invention

[0197]



15

R =



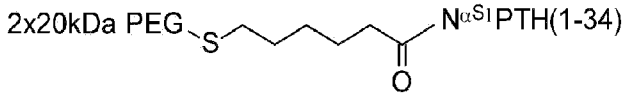
[0198] Conjugate **15** was synthesized as described for **11b** by using thiol **10** (5.2 mg, 0.95 µmol) and maleimide **11a** (1.23 mg, 1.14 µmol).

Yield:	2.1 mg (33 %), **15***9 TFA
MS:	m/z 1102.60 = $[M+5H]^{5+}$, (calculated monoisotopic mass for $[M+5H]^{5+}$ = 1102.59).

Example 16

Synthesis of permanent 2x20 kDa S1 PEG conjugate 16 - not according to the invention

[0199]



16

[0200] 772 µL of a solution containing thiol **3** (19.4 mg/mL, 15 mg, 3.54 µmol) and 2.5 mg/mL Boc-L-Met in 1/1 (v/v) acetonitrile/water containing 0.1 % TFA (v/v) were added to 1.87 mL of a solution containing PEG 2x20 kDa maleimide (Sunbright GL2-400MA, 187 mg, 4.32 µmol) and 2.5 mg/mL Boc-L-Met in water containing 0.1 % TFA (v/v). 0.5 M NaH_2PO_4 buffer (0.66 mL, pH 7.0) was added and the mixture was stirred for 30 min at rt. 10 µL of a 270 mg/mL solution of 2-mercaptoethanol in water was added. The mixture was stirred for 5 min at rt and 0.33 mL 1 M HCl were added. Conjugate **16** was purified by IEX followed by RP-HPLC using a linear

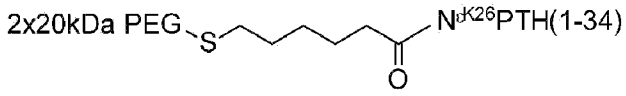
gradient of solvent system A (water containing 0.1 % AcOH v/v) and solvent system B (acetonitrile containing 0.1 % AcOH v/v). The product containing fractions were freeze-dried.

Yield:	97 mg (2.01 µmol, 57 %) conjugate **16***8 AcOH

Example 17

Synthesis of permanent 2x20 kDa K26 PEG conjugate 17 - not according to the invention

[0201]



17

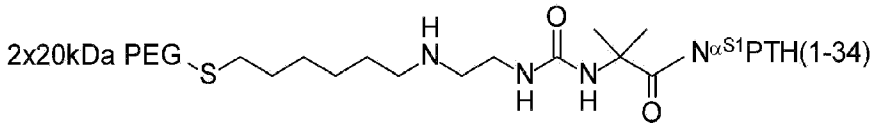
[0202] Conjugate **17** was prepared as described for **16** by reaction of thiol **4** (15 mg, 3.53 µmol) and PEG 2x20 kDa maleimide (Sunbright GL2-400MA, 187 mg, 4.32 µmol).

Yield:	80 mg (1.79 µmol, 51 %) conjugate **17***8 AcOH

Example 18

Synthesis of transient 2x20 kDa S1 PEG conjugate 18

[0203]



18

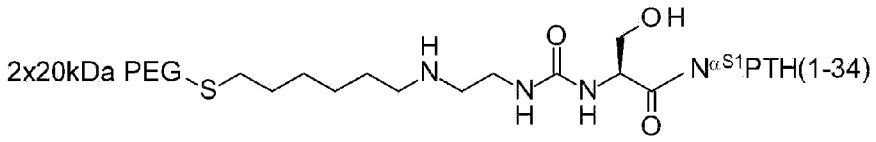
[0204] Conjugate **18** was prepared as described for **16** by reaction of thiol **5** (37 mg, 8.40 µmol) and PEG 2x20 kDa maleimide (Sunbright GL2-400MA, 445 mg, 9.24 µmol). The reaction was quenched by addition of 50 µL TFA without prior addition of 2-mercaptoethanol. Conjugate **18** was purified by IEX followed by SEC for desalting. The product containing fractions were freeze-dried.

Yield:	161 mg (3.33 µmol, 40 %) conjugate **18***9 AcOH

Example 19

Synthesis of transient 2x20 kDa S1 PEG conjugate 19 - not according to the invention

[0205]



19

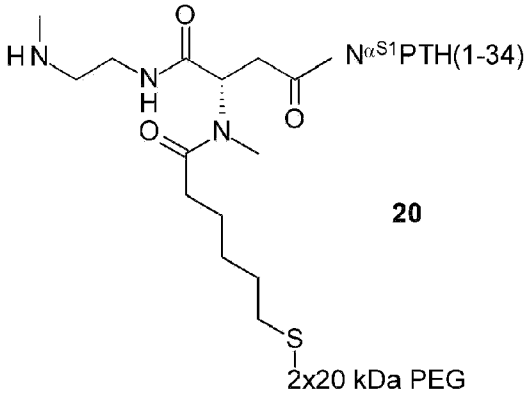
[0206] Conjugate **19** was prepared as described for **16** by reaction of thiol **7** (27 mg, 6.14 µmol) and PEG 2x20 kDa maleimide (Sunbright GL2-400MA, 325 mg, 7.50 µmol).

Yield:	249 mg (5.16 µmol, 84 %) conjugate **19***9 AcOH

Example 20

Synthesis of transient 2x20 kDa S1 PEG conjugate 20 - not according to the invention

[0207]



20

[0208] Conjugate **20** was prepared as described for **16** by reaction of thiol **9** (38 mg, 8.59 µmol) and PEG 2x20 kDa maleimide (Sunbright GL2-400MA, 455 mg, 9.45 µmol). The reaction was quenched by addition of 50 µL TFA without prior addition of 2-mercaptoethanol. Conjugate

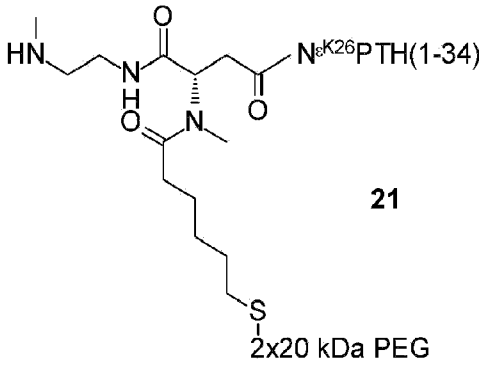
20 was purified by IEX followed by SEC for desalting. The product containing fractions were freeze-dried.

Yield:	194 mg (4.01 µmol, 47 %) conjugate **20***9 AcOH

Example 21

Synthesis of transient 2x20 kDa K26 PEG conjugate 21 - not according to the invention

[0209]



21

[0210] Conjugate **21** was prepared as described for **16** by reaction of thiol **10** (34 mg, 7.58 µmol) and PEG 2x20 kDa maleimide (Sunbright GL2-400MA, 401 mg, 9.26 µmol).

Yield:	256 mg (5.30 µmol, 70 %) conjugate **21***9 AcOH

Example 22

Dose range finding study - Dose adjustment study in Cynomolgus monkeys with daily subcutaneous injections with TransCon PTH

[0211] Method: This study was conducted in order to test the hypothesis of performing dose adjustments with TransCon PTH in 25% increments in response to hypocalcemia or hypercalcemia. The study was performed in male and female Cynomolgus monkeys. Four groups with one male and one female animal in each were dosed for four weeks starting at doses 0.2, 0.5, 1.0 and 2.0 µg/kg/day, respectively. Serum calcium (sCa) levels in the animals were measured pre-dose and post-dose on days -6, 1, 3, 7, 8, 12, 14, 16, 19, 23, 26, 28 and 29.

[0212] Results: Daily subcutaneous administration of TransCon PTH at doses of 0.2, 0.5 and 1.0 µg/kg/day for 28 days was well tolerated and was associated with expected sporadic slight increases in sCa levels (from 1.05x up to 1.12x compared to pre-study levels). On Day 3 for the male and female dosed at 2.0 µg/kg/day day sCa rose to severe hypercalcaemic levels (up to 1.42x and 1.34x, respectively, compared to pre-study levels). Dosing was discontinued in these two animals followed by a dosing holiday. On Day 23 dosing was resumed at 1.5 µg/kg/day (25% dose reduction) for one week. On Day 23 (first day of dosing after the dosing holiday), increases in calcium were noted at all timepoints for the male (from 1.05 x to 1.12 x to pre-study levels) with peak concentration achieved 4 hrs postdose. On Day 26, the increases were noted for male and female (up to 1.17 x to pre-study levels). On Day 29, calcium increases were still present in both sexes.

[0213] Conclusion: A dose of a controlled-release PTH compound administered subcutaneously to male and female cynomolgus monkeys was successfully adjusted (reduced) at a 25% increment as a response to hypercalcemia. The level of sCa was lower in the animals after dosing with the 25% reduced dose compared to dosing with the original higher dose.

Abbreviations:

[0214]

ACN
: acetonitrile

AcOH
: acetic acid

Aib
: 2-aminoisobutyric acid

BMD
: bone mineral density

Bn
: benzyl

Boc
: tert-butyloxycarbonyl

COMU
: (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate

cAMP
: cyclic adenosine monophosphate

d
: day

DBU
: 1,3-diazabicyclo[5.4.0]undecene

DCC

N,N'-dicyclohexylcarbodiimide

DCM
: dichloromethane

DIPEA
: N,N-diisopropylethylamine

DMAP
: dimethylamino-pyridine

DMF
: N,N-dimethylformamide

DMSO
: dimethylsulfoxide

DTT
: dithiothreitol

EDTA
: ethylenediaminetetraacetic acid

eq
: stoichiometric equivalent

ESI-MS
: electrospray ionization mass spectrometry

Et
: ethyl

Fmoc
: 9-fluorenylmethyloxycarbonyl

Glu-C
: endoproteinase Glu-C

h
: hour

HATU
: O-(7-azabenzotri azole-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate

HP
: hypoparathyroidism

HPLC
: high performance liquid chromatography

ivDde
: 4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl

LC
: liquid chromatography

LTQ
: linear trap quadrupole

Lys-C
: endoproteinase Lys-C

LLOQ
: lower limit of quantification

Mal

3-maleimido propyl

Me
: methyl

MeOH
: methanol

min
: minutes

Mmt
: monomethoxytrityl

MS
: mass spectrum / mass spectrometry

m/z
: mass-to-charge ratio

OtBu
: tert-butyloxy

PEG
: poly(ethylene glycol)

pH
: *potentia Hydrogenii*

PK
: pharmacokinetics

Pr
: propyl

PTH
: parathyroid hormone

PyBOP
: benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate

Q-TOF
: quadrupole time-of-flight

RP-HPLC
: reversed-phase high performance liquid chromatography

rt
: room temperature

sCa
: serum calcium

SIM
: single ion monitoring

SEC
: size exclusion chromatography

sc
: subcutaneous

sP
: serum phosphate

$t_{1/2}$

half life

TCP
: tritylchloride polystyrol

TES
: triethylsilane

TFA
: trifluoroacetic acid

THF
: tetrahydrofuran

Tmob
: 2,4,6-trimethoxybenzyl

TPTx
: thyroparathyroidectomy

Trt
: triphenylmethyl, trityl

ULOQ
: upper limit of quantification

UPLC
: ultra performance liquid chromatography

UV
: ultraviolet

ZQ
: single quadrupole

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patent documents cited in the description

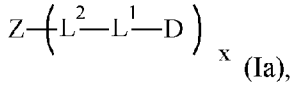
- WO2004024758A [0015]
- WO29095479A2 [0149]

Non-patent literature cited in the description

- **BOWIE et al.**Science, 1990, vol. 247, 1306-1310 [0030]

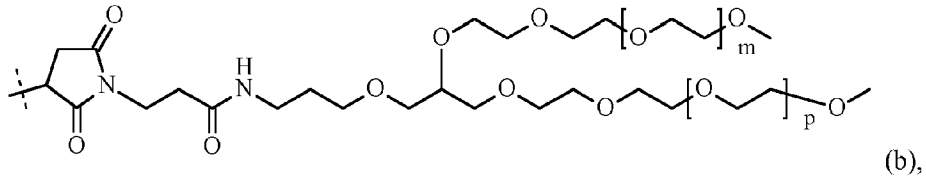
Patentkrav

1. Farmaceutisk sammensætning omfattende en PTH-forbindelse med kontrolleret frigivelse eller et farmaceutisk acceptabelt salt, hydrat eller solvat deraf til anvendelse i en fremgangsmåde til behandling af hypoparathyroidisme, hvor fremgangsmåden følger et doseringsregime, hvor dosisjustering som reaktion på hypocalcæmi eller hypercalcæmi udføres i stigninger på ikke mere end 25%, og hvor PTH-forbindelsen med kontrolleret frigivelse frigiver mindst ét PTH-molekyle med en frigivelseshalveringstid på mindst 12 timer i vandig buffer, pH 7,4 og 37°C, hvor PTH-forbindelsen med kontrolleret frigivelse er en forbindelse med formlen (Ia)



(Ia),

hvor -Z omfatter en enhed med formlen (b)



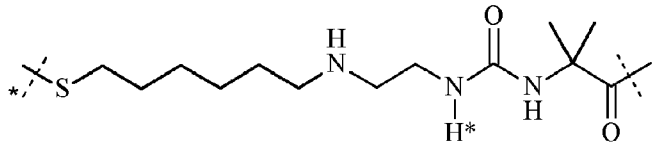
(b),

hvor

den stiplede linje angiver binding til -L^2- eller til resten af -Z; og

m og p er uafhængigt af hinanden et heltal fra og med 400 til 500, og

-L^1-L^2- har formlen:



(IIcb-iii);

hvor den umarkerede stiplede linje indikerer binding med en amidbinding til et nitrogen af -D, og den stiplede linje markeret med en stjerne indikerer binding til -Z, hvor -D har sekvensen SEQ ID NO:51 og x er 1.

2. Farmaceutisk sammensætning til anvendelse ifølge krav 1, hvor fremgangsmåden følger et dosisregimen, hvor dosisjustering som reaktion på hypocalcæmi eller hypercalcæmi udføres i stigninger på ikke mere end 20%.

3. Farmaceutisk sammensætning til anvendelse ifølge krav 1 eller 2, hvor fremgangsmåden følger et dosisregimen, hvor dosisjustering som reaktion på hypocalcæmi eller hypercalcæmi udføres i stigninger på ikke mere end 15%.

4. Farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 3, hvor fremgangsmåden følger et dosisregimen, hvor dosisjustering som reaktion på hypocalcæmi eller hypercalcæmi udføres i stigninger på ikke mere end 10%.

5. Farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 4, hvor fremgangsmåden følger et dosisregimen, hvor dosisjustering som reaktion på hypocalcæmi eller hypercalcæmi udføres i stigninger på 10%.

6. Farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 5, hvor den farmaceutiske sammensætning indgives til patienten ikke oftere end én gang hver 24. time.

7. Farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 6, hvor den farmaceutiske sammensætning indgives til patienten én gang hver 24. time.

8. Farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 6, hvor den farmaceutiske sammensætning indgives til patienten én gang hver 48. time.

9. Farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 6, hvor den farmaceutiske sammensætning indgives til patienten én gang om ugen.

10. Farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 9, hvor den farmaceutiske sammensætning indgives til en patient subkutant.

11. Farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 10, hvor -Z er en enhed med formlen (b).

12. Farmaceutisk sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 11, hvor $-L^1-L^2-$ er bundet med en amidbinding til den N-terminale aminfunktionelle gruppe af -D.

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.