Sustained release composition of prostacyclin

Pulmonary arterial hypertension (PAH) is an increase in blood pressure in the pulmonary artery, pulmonary vein, or pulmonary capillaries, leading to shortness of breath, dizziness, fainting, and other symptoms, all of which are exacerbated by exertion. PAH is a severe disease with a markedly decreased exercise tolerance and heart failure. It is an orphan disease with an incidence of about 2-3 per million per year and a prevalence of about 15 per million. Median survival of patients with untreated PAH is in the range of 2–3 years from time of diagnosis, with the cause of death usually being right ventricular failure.

Pulmonary arterial hypertension involves the vasoconstriction or tightening of blood vessels connected to and within the lungs. Over time, fibrosis causes the affected blood vessels to become both stiffer and thicker which further increases the blood pressure within the lungs and impairs their blood flow. In addition, the increased workload of the heart causes hypertrophy of the right ventricle which ultimately causes right heart failure. As the blood flowing through the lungs decreases, the left side of the heart receives less blood and thus oxygen supply is below the required level, especially during physical activity.

A number of agents have been introduced for the treatment of PAH of which prostacyclins are commonly considered to be the most effective. One prostacyclin is Epoprostenol which is a synthetic prostacyclin and marketed as Flolan® (GlaxoSmithKline). It is given to patients via continuous infusion and requires a semi-permanent central venous catheter which can cause sepsis and thrombosis. Flolan® is unstable, and therefore has to be kept on ice during administration. Since it has a half-life of only 3 to 5 minutes, the infusion has to be continuous night and day and any interruption can be fatal. Thus, treatment of PAH with Flolan® is a huge burden for the patient.

Therefore, there was a need to develop other prostanoids, as has been described for example in US4306075A and EP159784B1. One such prostaglandin is treprostinil with the trade name Remodulin® (United Therapeutics). The half-life of treprostinil is 4 hours but treprostinil is still required to be administered as a continuous subcutaneous infusion or continuous intravenous infusion via an infusion pump that the patient must wear at all times.

As continuous infusion is also required for treprostinil, any interruption of drug delivery can be fatal, and there is therefore a need to develop porstacyclin compounds with an even longer duration of action than that of treprostinil.

Subcutaneous infusion of treprostinil is frequently painful to the extent that the patient cannot tolerate the pain and consequently the mode of administration is switched to intravenous infusion. However, an increased risk of sepsis with intravenous Remodulin® has been reported.

As subcutaneous infusion is associated with pain, there is a need for developing a prostacyclin that can be administered by subcutaneous administration but with reduced rates of pain.

Another prostacyclin, Iloprost (Ilomedin) which is marketed as Ventavis® (Baier), was the only inhaled form of prostacyclin approved for use in the US and Europe, until the inhaled form of treprostinil was approved by the FDA in July 2009 which is marketed under the trade name TYVASO® (United Therapeutics).

Inhaled prostacyclin suffers from the drawback of not providing fully efficacious plasma levels of drug throughout the dosing period, making inhaled therapy less desired in severe patients.

Prostacyclins are the standard treatment of PAH, particularly in more severe patients. Although inhaled treprostinil is more convenient and without the strong pain that is frequently associated with subcutaneously infused treprostinil, inhalation is considered to be less effective and therefore less often prescribed.

Therefore, there exists a need to provide a more efficacious and comfortable prostacyclin treatment for patients.

This object is achieved with a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients, which is characterized by having a concentration of the prostacyclin compound that is sufficient to maintain a therapeutically effective level of prostacyclin in blood plasma for at least 12 hours after a single subcutaneous or intramuscular injection.

In a further embodiment, the present invention relates to a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients, for use in the treatment and/or prevention of diseases wherein the concentration of the prostacyclin compound is sufficient to maintain a therapeutically effective level of prostacyclin in blood plasma for at least 12 hours after a single subcutaneous or intramuscular injection.

This object is further achieved with a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients which is characterized by having a pharmacokinetic profile in vivo in a mammal, in particular in a human, with substantially no burst of the prostacyclin compound.

In a further embodiment, the present invention relates to a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients, for use in the treatment and/or prevention of diseases wherein a prostacyclin compound which is characterized by having a pharmacokinetic profile in vivo in a mammal, in particular in a human, with substantially no burst of the prostacyclin compound.

This object is further achieved with a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients which is characterized by exhibiting a peak to trough ratio of less than 5, such as less than 3 or less than 2.

In a further embodiment, the present invention relates to a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients, for use in the treatment and/or prevention of diseases wherein the peak to trough ratio of the prostacyclin compound less than 5, such as less than 3 or less than 2.

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This object is further achieved with a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients, wherein the prostacyclin compound has an activity of < 20%, preferably < 10%, more preferably < 5% of the activity of free prostacyclin.

In a further embodiment, the present invention relates to a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients, for use in the treatment and/or prevention of diseases, wherein the prostacyclin compound has an activity of < 20%, preferably < 10%, more preferably < 5% of the activity of free prostacyclin.

This object is further achieved with a pharmaceutical composition comprising a prostacyclin prodrug and optionally one or more pharmaceutically acceptable excipients which is characterized in that after subcutaneous or intramuscular administration of said prostacyclin prodrug more than 50% of the administered prostacyclin dose is releasable within the blood compartment.

In a preferred embodiment, the promoiety of the prodrug comprises a linear or branched PEG moiety.

In a further embodiment, the present invention relates to a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients, for use in the treatment and/or prevention of diseases, wherein after subcutaneous or intramuscular administration of said prostacyclin prodrug more than 50% of the administered prostacyclin dose is releasable within the blood compartment.

The prostacyclin compounds and pharmaceutical compositions and pharmaceutical compositions for use of the present invention reduce the pain associated with subcutaneous injections due to a protective carrier moiety which inhibits binding of the prostacyclin to its receptors in the subcutaneous tissue, while at the same time providing relatively constant prostacyclin levels in the blood plasma of the patient.

The object is also achieved with a prostacyclin compound and a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients, which when administered to a patient in need thereof is characterized by having an absorption of the compound that is sufficiently fast and the release of prostacyclin from the compound is sufficiently slow, so that the subcutaneous exposure to free prostacyclin is minimized.

In a further embodiment, the present invention relates to a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients, for use in the treatment and/or prevention of diseases, wherein the absorption of the compound in a patient, preferably a human patient, is sufficiently fast and the release of prostacyclin from the compound is sufficiently slow, so that the subcutaneous exposure to free prostacyclin is minimized.

The prostacyclin compounds and pharmaceutical compositions and pharmaceutical compositions for use of the present invention are to be administered to mammals, in particular humans. In particular, they are administered to patients, in particular human patient, in need of treatment with prostacyclin.

Such prostacyclin compounds elicit less of the known adverse prostacyclin effects on tissue after administration (such as erythema, edema, fibrosis and hemorrhage) upon subcutaneous or intramuscular administration than compared to subcutaneous or intramuscular infusion of the same amount of the corresponding free prostacyclin.

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

The terms "drug", "biologically active molecule", "biologically active moiety", "biologically active agent", "active agent", “active substance” and the like mean any substance which can affect any physical or biochemical properties of a biological organism, including but not limited to viruses, bacteria, fungi, plants, animals, and humans. In particular, as used herein, the terms include any substance intended for diagnosis, cure, mitigation, treatment, or prevention of disease in organisms, in particular humans or other animals, or to otherwise enhance physical or mental well-being of organisms, in particular humans or animals. According to the present invention, prostacyclins are drugs.

“Total polymer content of a pharmaceutical composition” means the total polymer concentration after complete removal of prostacyclin from said pharmaceutical composition.

As used herein the term “prostacyclin” is intended to mean a biologically active moiety, including non-prostanoid biologically active moieties, capable of eliciting a therapeutic effect similar to prostacyclin, by stimulating prostanoid receptors, such as, but not limited to, DP_1,

DP_2, EP_1, EP_2, EP_3, EP_4, FP, IP and TP, such as epoprostenol, iloprost, treprostinil, beraprost, ONO-1301 and NS-304/ACT-293987/selexipag, prostacyclin conjugated to low-molecular weight PEG, wherein low-molecular-weight PEG has a molecular weight smaller than 10 kDa, beraprost sodium, epoprostenol sodium, iloprost in combination with bosentan, iloprost in combination with sildenafil citrate, treprostinil, pegylated treprostinil, treprostinil diethanolamine and treprostinil sodium, 2-{4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}-N-(methylsulfonyl)acetamide, {4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}acetic acid, 8-[1,4,5-triphenyl-1 H-imidazol-2-yl-oxy]octanoic acid, isocarbacyclin, cicaprost, [4-[2-(1,1-Diphenylethylsulfanyl)-ethyl]-3,4-dihydro-2H-benzo[1,4]oxazin-8-yloxy]-acetic acid N-methyl-d-glucamine, 7,8-dihydro-5-(2-(1-phenyl-1-pyrid-3-yl-methiminoxy)-ethyl)-a-naphthyloxyacetic acid, (5-(2-diphenylmethyl aminocarboxy)-ethyl)-a-naphthyloxyacetic acid, 2-[3-[2-(4,5-diphenyl-2-oxazolyl)ethyl]phenoxy]acetic acid, [3-[4-(4,5-diphenyl-2-oxazolyl)-5-oxazolyl]phenoxy]acetic acid, bosentan, 17[alpha], 20-dimethyl-[DELTA]6,6a6a-carba PGI1, 15-deoxy-16[alpha]-hydroxy-16[beta],20-dimethyl-[DELTA]6,6a-6a-carba PGI1 and pentoxifylline (1-{5-oxohexyl}-3,7-dimethylxanthine). The prostacyclin compound may be in the form of a prodrug, in which case the compound to be released in plasma is the active prostacyclin which forms after administration of the prodrug.

The term "prostacyclin compound" refers to free prostacyclin, prostacyclin conjugates, prostacyclin depots with non-covalently or covalently bound prostacyclin, and prostacyclin prodrugs, including polymer carrier-linked prostacyclin prodrug.

Targeting moieties are moieties that when present in a molecule, such as for example a prodrug, allow preferential localization of such larger molecule in specific target areas of the organism to which it has been administered. Such specific target areas might be organs, certain cell types or subcellular compartments. "Preferential localization" means that at least 10%, preferably at least 20% and more preferably at least 30% of the biologically active moieties administered to a patient reach said specific target areas.

Targeting moieties may be divided into 3 classes according to size:

- small molecular targeting moieties, for example C-glucuronide, cobalamin, vitamins such as folic acid (folate) and analogs and derivatives, carbohydrates, bisphosphonates, N-acetylgalactosamine,

- peptides, for example bombesin, somatostatin, LHRH, EGF, VEGF, hCG, fragments of luteinizing hormone (LH), octreotide, vapreotide, lanreotide, RC-3940 series, decapeptyl, lupron, zoladex, cetrorelix, peptides or peptidomimetics containing the NGR or RGD motifs or derived from these motifs such as CNGRC (linear), GNGRG (cyclic), ACDC RGD CFCG (cyclic), CDCRGDCFC, CNGRC (cyclic), CRGDCGG, CNGRC, or other peptides such as ATWLPPR, thrombospondin (TSP)-1 mimetics, (RGD peptidomimetic), CTTHWGFTLC, CGNKRTRGC, neuropeptide substance P, SSP, the Sar9, Met(O2)11 analog of substance P, cholecystokinin (CCK), corticotropin-releasing hormone/factor (CRH/CRF), dermorphin, FGF-2 or basic fibroblast growth factor, galanin, melanopsin, neurotensin,

- and protein or macro- molecular targeting moieties, for example IL-2, GM-CSF, TNF-a, transferrin, immunoglobulins, acetylated-LDL, lactoferrin (Lf) (also called lactotransferrin) and lactoferricin (Lcin), gambogic acid (GA), antibody fragments and affinity scaffold proteins.

In principle, any ligand of a cell surface receptor may be advantageously used as a targeting moiety. For instance, ATWLPPR peptide is a potent antagonist of VEGF; thrombospondin-1 (TSP-1) induces apoptosis in endothelial cells, RGD-motif mimics block integrin receptors, NGR-containing peptides inhibit aminopeptidase N, and cyclic peptides containing the sequence of HWGF selectively inhibit MMP-2 and MMP-9. LyP-1 peptide specifically binds to tumor lymphatic vessels. Illustrative other ligands include peptide ligands identified from library screens, tumor cell-specific peptides, tumor cell-specific aptamers, tumor cell-specific carbohydrates, tumor cell-specific monoclonal or polyclonal antibodies, Fab or scFv (i.e., a single chain variable region) fragments of antibodies such as, for example, a Fab fragment of an antibody directed to EphA2 or other proteins specifically expressed or uniquely accessible on metastatic cancer cells, small organic molecules derived from combinatorial libraries, growth factors, such as EGF, FGF, insulin, and insulin-like growth factors, and homologous polypeptides, somatostatin and its analogs, transferrin, lipoprotein complexes, bile salts, selecting, steroid hormones, Arg-Gly-Asp containing peptides, retinoids, various Galectins, δ-opioid receptor ligands, cholecystokinin A receptor ligands, ligands specific for angiotensin AT1 or AT2 receptors, peroxisome proliferator-activated receptor λ ligands, β-lactam antibiotics such as penicillin, small organic molecules including antimicrobial drugs, and other molecules that bind specifically to a receptor preferentially expressed on the surface of

tumor cells or on an infectious organism, antimicrobial and other drugs designed to fit into the binding pocket of a particular receptor based on the crystal structure of the receptor or other cell surface protein, ligands of tumor antigens or other molecules preferentially expressed on the surface of tumor cells, or fragments of any of these molecules. Examples of tumor-specific antigens that can function as targeting moieties include extracellular epitopes of a member of the ephrin family of proteins, such as EphA2. EphA2 expression is restricted to cell-cell junctions in normal cells, but EpbA2 is distributed over the entire cell surface in metastatic tumor cells. Thus, EphA2 on metastatic cells would be accessible for binding to, for example, a Fab fragment of an antibody conjugated to an immunogen, whereas the protein would not be accessible for binding to the Fab fragment on normal cells, resulting in a targeting moiety specific for metastatic cancer cells.

Further examples for such targeting moieties are: FSH-33, allatostatin 1, hepatocarcinoma targeting peptide, peptide GFE, anti-EGFR antibodies and/or antibody fragments, in particular cetuximab, CendR, iRGD peptide (RGD-CendR hybrid peptide), small molecules, antibodies and/or antibody fragments binding to cancer-specific epitopes like e.g. CEA, gastrin-releasing peptide receptors, somatostatin receptors, galanin receptors, follicle-stimulating hormone receptors, p32 protein, fibroblast growth factor receptors, HepG2, epidermal growth factor receptors, integrin $\alpha v\beta 6$, neuropilin-1 receptor and VEGF receptors.

"Free form" of a drug refers to the drug in its unmodified, pharmacologically active form, such as after being released from a carrier-linked prodrug. "Free form" of a prostacyclin refers to a prostacyclin in its unmodified, pharmacologically active form, such as after being released from a conjugate, carrier-linked prodrug, or depot.

As used herein, the term "prodrug" is intended to mean a prostacyclin compound that undergoes biotransformation before exhibiting its pharmacological effects. Prodrugs can thus be viewed as biologically active moieties containing specialized non-toxic protective groups used in a transient manner to alter or to eliminate undesirable properties in the parent molecule. For instance, the prodrug may be a biohydrolyzable amide and biohydrolyzable ester and also encompasses a) compounds in which the biohydrolyzable functionality in such a prodrug is encompassed in the compound, and b) compounds which may be oxidized or reduced biologically at a given functional group. Typical prodrugs may be a carrier-linked prodrug that contains a temporary linkage of a given active substance with a transient carrier

group that produces improved physicochemical or pharmacokinetic properties and that can be easily removed in vivo, usually by a hydrolytic cleavage; a cascade prodrug for which the cleavage of the carrier group becomes effective only after unmasking an activating group.

To enhance physicochemical or pharmacokinetic properties of a drug, such as prostacyclin, *in vivo* said drug can be conjugated with a carrier. If the drug is reversibly bound to a carrier and/or a linker, such system is commonly assigned as "carrier-linked prodrug". According to the definitions provided by IUPAC (as given under http://www.chem.qmul.ac.uk/iupac/medchem/ah.html, accessed on March 7, 2011), a carrier-linked prodrug is a prodrug that contains a temporary linkage of a given active substance with a reversible carrier group that produces improved physicochemical or pharmacokinetic properties and that can be easily removed *in vivo*, usually by a hydrolytic cleavage.

The term "promoiety" refers to the part of the prodrug which is not the prostacyclin drug, thus meaning for example the carrier as well as the reversible prodrug linker moiety and/or one or more spacer moiety/moieties, if present.

The term "reversible prodrug linker" or "transient prodrug linkers" refers to a moiety which on its one end is attached to a prostacyclin through a reversible linkage and at another end is attached through a permanent bond to either a spacer moiety permanently attached to a carrier moiety or is directly attached through a permanent bond to a carrier moiety. Such reversible prodrug linkers are non-enzymatically hydrolytically degradable, i.e. cleavable, under physiological conditions (aqueous buffer at pH 7.4, 37°C) with half-lives ranging from, for example, one hour to three months. Reversible linkages are, for example, aconityls, acetals, amides, carboxylic anhydrides, esters, imines, hydrazones, maleamic acid amides, ortho esters, phosphamides, phosphoesters, phosphosilyl esters, silyl esters, sulfonic esters, aromatic carbamates, and combinations thereof.

Permanent linkages are non-enzymatically hydrolytically degradable under physiological conditions (aqueous buffer at pH 7.4, 37°C) with half-lives of six months or longer, such as, for example, amides.

If, for example, a functional group is coupled to another functional group, the resulting chemical structure is referred to as “linkage”. For example, the reaction of an amine group with a carboxyl group results in an amide linkage.

As used herein the term “substantially no burst” or “substantially burstless” (both terms are used interchangeably in the present description) is intended to mean that upon administration of a prostacyclin compound to a mammal, in particular a human, the ratio of the peak concentration of a detectable prostacyclin compound in blood plasma during the first 48 hours after administration, such as subcutaneous or intramuscular, to the lowest concentration of a detectable prostacyclin compound in blood plasma after the peak concentration during the first 48 hours after administration is less than 2 (substantially no burst detectable), preferred less than 1.5 (no burst detectable).

As used herein the term “burst” is intended to mean that upon administration of a prostacyclin compound, which may be a prodrug or an active prostacyclin compound, to a mammal, in particular a human, the ratio of the peak concentration of a detectable prostacyclin compound in blood plasma during the first 48 hours after administration, such as subcutaneous or intramuscular, to the lowest concentration of a detectable prostacyclin compound in blood plasma after the peak concentration during the first 48 hours after administration is 2 or higher.

A "therapeutically effective amount" of a prostacyclin compound as used herein means an amount sufficient to cure, alleviate or partially arrest the clinical manifestations of a given disease and its complications in a mammal, in particular a human. An amount adequate to accomplish this is defined as "therapeutically effective amount". Effective amounts for each purpose will depend on the severity of the disease or injury as well as the weight and general state of the subject. It will be understood that determining an appropriate dosage may be achieved using routine experimentation, by constructing a matrix of values and testing different points in the matrix, which is all within the ordinary skills of a trained physician.

As used herein the term “a hydrogel” is intended to mean a three-dimensional, hydrophilic or amphiphilic polymeric network capable of taking up large quantities of water. The networks are composed of homopolymers or copolymers, are insoluble due to the presence of covalent chemical or physical (ionic, hydrophobic interactions, entanglements) crosslinks. The

crosslinks provide the network structure and physical integrity. Hydrogels exhibit a thermodynamic compatibility with water which allows them to swell in aqueous media. The chains of the network are connected in such a fashion that pores exist and that a substantial fraction of these pores are of dimensions between 1 nm and 1000 nm.

The term "gel" refers to a non-crosslinked, jelly-like polymer solution.

As used herein the term "a depot" is intended to mean a drug delivery system, typically injected as a subcutaneous or intramuscular injection, of a prostacyclin compound, capable of consistently releasing the active compound over an extended period of time.

As used herein the term "a peak concentration" is intended to mean the highest concentration obtained after administration of a prostacyclin compound.

As used herein the term "peak to trough ratio" is intended to mean the ratio between the highest steady state plasma concentration of a compound administered to a mammal, in particular a human, and the lowest steady state plasma concentration of a prostacyclin compound within a given period between administrations.

"Alkyl" means a straight-chain (linear, unbranched) or branched carbon chain (unsubstituted alkyl). Optionally, one or more hydrogen atom(s) of an alkyl carbon may be replaced by a substituent as indicated herein. In general, a preferred alkyl is C_{1-6} alkyl.

"C_{1-4} alkyl" means an alkyl chain having 1 to 4 carbon atoms (unsubstituted C_{1-4} alkyl), e.g. if present at the end of a molecule: methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl tert-butyl, or e.g. $-CH_2-$, $-CH_2-CH_2-$, $-CH(CH_3)-$, $-CH_2-CH_2-CH_2-$, $-CH(C_2H_5)-$, $-C(CH_3)_2-$, when two moieties of a molecule are linked by the alkyl group (also referred to as C_{1-4} alkylene). Optionally, one or more hydrogen atom(s) of a C_{1-4} alkyl carbon may be replaced by a substituent as indicated herein. Accordingly, "C_{1-50} alkyl" means an alkyl chain having 1 to 50 carbon atoms.

"C_{1-6} alkyl" means an alkyl chain having 1 - 6 carbon atoms, e.g. if present at the end of a molecule: C_{1-4} alkyl, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, n-hexyl, or e.g. $-CH_2-$, $-CH_2-CH_2-$, $-CH(CH_3)-$, $-C(CH_2)-$, $-CH_2-CH_2-CH_2-$, -

$CH(C_2H_5)$-, $-C(CH_3)_2$-, when two moieties of a molecule are linked by the alkyl group (also referred to as C_{1-6} alkylene). One or more hydrogen atom(s) of a C_{1-6} alkyl carbon may be replaced by a substituent as indicated herein. The terms C_{1-15} alkyl or C_{1-15} alkylene are defined accordingly.

"C_{2-6} alkenyl" means an alkenyl chain having 2 to 6 carbon atoms, e.g. if present at the end of a molecule: $-CH=CH_2$, $-CH=CH-CH_3$, $-CH_2-CH=CH_2$, $-CH=CH-CH_2-CH_3$, $-CH=CH-CH=CH_2$, or e.g. -CH=CH-, when two moieties of a molecule are linked by the alkenyl group. One or more hydrogen atom(s) of a C_{2-6} alkenyl carbon may be replaced by a substituent as indicated herein.

The term C_{2-4} alkenyl is defined accordingly.

"C_{2-6} alkynyl" means an alkynyl chain having 2 to 6 carbon atoms, e.g. if present at the end of a molecule: $-C\equiv CH$, $-CH_2-C\equiv CH$, $CH_2-CH_2-C\equiv CH$, $CH_2-C\equiv C-CH_3$, or e.g. $-C\equiv C-$ when two moieties of a molecule are linked by the alkynyl group. One or more hydrogen atom(s) of a C_{2-6} alkynyl carbon may be replaced by a substituent as indicated herein. The term C_{2-4} alkynyl is defined accordingly.

"C_{2-50} alkenyl" means a branched or unbranched alkenyl chain having 2 to 50 carbon atoms (unsubstituted C_{2-50} alkenyl), e.g. if present at the end of a molecule: $-CH=CH_2$, $-CH=CH-CH_3$, $-CH_2-CH=CH_2$, $-CH=CH-CH_2-CH_3$, $-CH=CH-CH=CH_2$, or e.g. -CH=CH-, when two moieties of a molecule are linked by the alkenyl group. Optionally, one or more hydrogen atom(s) of a C_{2-50} alkenyl carbon may be replaced by a substituent as further specified. Accordingly, the term "alkenyl" relates to a carbon chain with at least one carbon carbon double bond. Optionally, one or more triple bonds may occur. The term "C_{2-15} alkenyl" is defined accordingly.

"C_{2-50} alkynyl" means a branched or unbranched alkynyl chain having 2 to 50 carbon atoms (unsubstituted C_{2-50} alkynyl), e.g. if present at the end of a molecule: $-C\equiv CH$, $-CH_2-C\equiv CH$, $CH_2-CH_2-C\equiv CH$, $CH_2-C\equiv C-CH_3$, or e.g. $-C\equiv C-$ when two moieties of a molecule are linked by the alkynyl group. Optionally, one or more hydrogen atom(s) of a C_{2-50} alkynyl carbon may be replaced by a substituent as further specified. Accordingly, the term "alkynyl" relates

to a carbon chain with at least one carbon triple bond. Optionally, one or more double bonds may occur.

"C_{3-7} cycloalkyl" or "C_{3-7} cycloalkyl ring" means a cyclic alkyl chain having 3 to 7 carbon atoms, which may have carbon-carbon double bonds being at least partially saturated (unsubstituted C_{3-7} cycloalkyl), e.g. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl, cycloheptyl. Optionally, one or more hydrogen atom(s) of a cycloalkyl carbon may be replaced by a substituent as indicated herein. The term "C_{3-7} cycloalkyl" or "C_{3-7} cycloalkyl ring" also includes bridged bicycles like norbonane (norbonanyl) or norbonene (norbonenyl). Accordingly, "C_{3-5} cycloalkyl" means a cycloalkyl having 3 to 5 carbon atoms. Accordingly, "C_{3-10} cycloalkyl" means a cycloalkyl having 3 to 10 carbon atoms.

"Halogen" means fluoro, chloro, bromo or iodo. It is generally preferred that halogen is fluoro or chloro.

"4 to 7 membered heterocyclyl" or "4 to 7 membered heterocycle" means a ring with 4, 5, 6 or 7 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 (unsubstituted 4 to 7 membered heterocyclyl). For the sake of completeness it is indicated that in some embodiments of the present invention, 4 to 7 membered heterocyclyl has to fulfill additional requirements. Examples for a 4 to 7 membered heterocycles are 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 or homopiperazine. Optionally, one or more hydrogen atom(s) of a 4 to 7 membered heterocyclyl may be replaced by a substituent.

"8 to 11 membered heterobicyclyl" or "8 to 11 membered heterobicycle" means a heterocyclic system 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 (unsubstituted 8 to 11 membered heterobicyclyl). Examples for a 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 or 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. The term “9 to 11 membered heterobicyclyl" or "9 to 11 membered heterobicycle" is defined accordingly.

The term “aliphatic” means fully saturated.

The term ”interrupted“ means that between two carbon atoms of, for example, a linker or a spacer or at the respective end of the carbon chain between the respective carbon atom and the hydrogen atom a group (such a -O- or -NH-) is inserted.

In general the term “substituted” preferably refers to substituents, which are the same or different and which are independently selected from the group consisting of halogen, CN, $COOR^{b9}$, OR^{b9}, $C(O)R^{b9}$, $C(O)N(R^{b9}R^{b9a})$, $S(O)_2N(R^{b9}R^{b9a})$, $S(O)N(R^{b9}R^{b9a})$, $S(O)_2R^{b9}$, $S(O)R^{b9}$, $N(R^{b9})S(O)_2N(R^{b9a}R^{b9b})$, SR^{b9}, $N(R^{b9}R^{b9a})$, NO_2, $OC(O)R^{b9}$, $N(R^{b9})C(O)R^{b9a}$, $N(R^{b9})S(O)_2R^{b9a}$, $N(R^{b9})S(O)R^{b9a}$, $N(R^{b9})C(O)OR^{b9a}$, $N(R^{b9})C(O)N(R^{b9a}R^{b9b})$, $OC(O)N(R^{b9}R^{b9a})$, T^b, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl,

wherein T^b, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more R^{b10}, 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^b, -C(O)O-; -O-; -C(O)-; $-C(O)N(R^{b11})$-; $-S(O)_2N(R^{b11})$-;

-S(O)N(R^{b11})-; -S(O)$_2$-; -S(O)-; -N(R^{b11})S(O)$_2$N(R^{b11a})-; -S-; -N(R^{b11})-; -OC(O)R^{b11}; -N(R^{b11})C(O)-; -N(R^{b11})S(O)$_2$-; -N(R^{b11})S(O)-; -N(R^{b11})C(O)O-; -N(R^{b11})C(O)N(R^{b11a})-; and -OC(O)N($R^{b11}R^{b11a}$);

R^{b9}, R^{b9a}, R^{b9b} are independently selected from the group consisting of H; T^b; and C_{1-50} alkyl; C_{2-50} alkenyl; and C_{2-50} alkynyl,

wherein T^b, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more R^{b10}, 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^b, -C(O)O-, -O-, -C(O)-, -C(O)N(R^{b11})-, -S(O)$_2$N(R^{b11})-, -S(O)N(R^{b11})-, -S(O)$_2$-, -S(O)-, -N(R^{b11})S(O)$_2$N(R^{b11a})-, -S-, -N(R^{b11})-, -OC(O)R^{b11}, -N(R^{b11})C(O)-, -N(R^{b11})S(O)$_2$-, -N(R^{b11})S(O)-, -N(R^{b11})C(O)O-, -N(R^{b11})C(O)N(R^{b11a})-, and -OC(O)N($R^{b11}R^{b11a}$),

T^b is selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 4- to 7-membered heterocyclyl, and 9- to 11-membered heterobicyclyl, wherein T^b is optionally substituted with one or more R^{b10}, which are the same or different,

R^{b10} is halogen, CN, oxo (=O), COOR^{b12}, OR^{b12}, C(O)R^{b12}, C(O)N($R^{b12}R^{b12a}$), S(O)$_2$N($R^{b12}R^{b12a}$), S(O)N($R^{b12}R^{b12a}$), S(O)$_2R^{b12}$, S(O)R^{b12}, N(R^{b12})S(O)$_2$N($R^{b12a}R^{b12b}$), SR^{b12}, N($R^{b12}R^{b12a}$), NO_2, OC(O)R^{b12}, N(R^{b12})C(O)R^{b12a}, N(R^{b12})S(O)$_2R^{b12a}$, N(R^{b12})S(O)R^{b12a}, N(R^{b12})C(O)OR^{b12a}, N(R^{b12})C(O)N($R^{b12a}R^{b12b}$), OC(O)N($R^{b12}R^{b12a}$), or C_{1-6} alkyl, wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different,

R^{b11}, R^{b11a}, R^{b12}, R^{b12a}, R^{b12b} are independently selected from the group consisting of H; or C_{1-6} alkyl, wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different.

The term “interrupted” means that between two carbons a group is inserted or that at the end of the carbon chain between the carbon and hydrogen.

In general the term “comprise” or “comprising” also encompasses “consist of” or “consisting of”.

The term “pharmaceutically acceptable” 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 in humans.

"Pharmaceutical composition" or “composition” means a composition containing one or more drugs or prodrugs, in particular a prostacyclin compound, and one or more inert ingredients, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, 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 obtainable by admixing a water-soluble carrier-linked prodrug of the present invention and a pharmaceutically acceptable excipient.

“Dry composition” means that a pharmaceutical composition comprising a prostacyclin compound is provided in a dry form in a container. Suitable methods for drying are spray-drying and lyophilization (freeze-drying). Such dry composition comprising a prostacyclin compound 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). The preferred method of drying is lyophilization. “Lyophilized composition” means that the pharmaceutical composition comprising a prostacyclin compound was first frozen and subsequently subjected to water reduction by means of reduced pressure. This terminology does not exclude additional drying steps which occur in the manufacturing process prior to filling the composition into the final container.

“Lyophilization” (freeze-drying) is a dehydration process, characterized by freezing a composition and then reducing the surrounding pressure and, optionally, adding heat to allow the frozen water in the composition to sublime directly from the solid phase to gas. Typically, the sublimed water is collected by desublimation.

“Reconstitution” means the addition of a liquid to bring back the original form of a composition.

"Reconstitution solution" refers to the liquid used to reconstitute the dry composition of a pharmaceutical composition comprising a prostacyclin compound prior to administration to a patient in need thereof.

"Container" means any container in which the pharmaceutical composition comprising a prostacyclin compound is comprised and can be stored until reconstitution.

A "therapeutically effective amount" of a prostacyclin compound as used herein means an amount sufficient to cure, alleviate or partially arrest the clinical manifestations of a given disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective amount". Effective amounts for each purpose will depend on the severity of the disease or injury as well as the weight and general state of the subject. It will be understood that determining an appropriate dosage may be achieved using routine experimentation, by constructing a matrix of values and testing different points in the matrix, which is all within the ordinary skills of a trained physician or veterinary.

"Buffer" or "buffering agent" refers to chemical compounds that maintain the pH in a desired range. Physiologically tolerated buffers are, for example, sodium phosphate, succinate, histidine, bicarbonate, citrate and acetate, sulphate, nitrate, chloride, pyruvate. Antacids such as $Mg(OH)_2$ or $ZnCO_3$ may be also used. Buffering capacity may be adjusted to match the conditions most sensitive to pH stability.

"Excipients" refers to compounds administered together with the therapeutic agent, for example, buffering agents, isotonicity modifiers, preservatives, stabilizers, anti-adsorption agents, oxidation protection agents, or other auxiliary agents. However, in some cases, one excipient may have dual or triple functions.

A "lyoprotectant" is a molecule which, when combined with a protein of interest, significantly prevents or reduces chemical and/or physical instability of the protein upon drying in general and especially during lyophilization and subsequent storage. Exemplary lyoprotectants include sugars, such as sucrose or trehalose; amino acids such as monosodium glutamate or histidine; methylamines such as betaine; lyotropic salts such as magnesium sulfate; polyols such as trihydric or higher sugar alcohols, e.g. glycerin, erythritol, glycerol, arabitol, xylitol,

sorbitol, and mannitol; ethylene glycol; propylene glycol; polyethylene glycol; pluronics; hydroxyalkyl starches, e.g. hydroxyethyl starch (HES), and combinations thereof.

"Surfactant" refers to wetting agents that lower the surface tension of a liquid.

"Isotonicity modifiers" refer to compounds which minimize pain that can result from cell damage due to osmotic pressure differences at the injection depot.

The term "stabilizers" refers to compouds used to stabilize the polymeric prodrug. 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.

"Anti-adsorption agents" refers to mainly ionic or non-ionic surfactants or other proteins or soluble polymers used to coat or adsorb competitively to the inner surface of the composition´s container. 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.

"Oxidation protection agents" refers to antioxidants such as ascorbic acid, ectoine, methionine, glutathione, monothioglycerol, morin, polyethylenimine (PEI), propyl gallate, vitamin E, chelating agents such aus citric acid, EDTA, hexaphosphate, thioglycolic acid.

"Antimicrobial" refers to a chemical substance that kills or inhibits the growth of microorganisms, such as bacteria, fungi, yeasts, protozoans and/or destroys viruses.

"Sealing a container" means that the container is closed in such way that it is airtight, allowing no gas exchange between the outside and the inside and keeping the content sterile.

"Pharmaceutically acceptable" is meant to encompass any excipient and/or additive, which does not interfere with the effectiveness of the biological activity of the active ingredient and that, is not toxic to the host to which it is administered.

The term "reagent" refers to an intermediate or starting material used in the assembly process of a molecule.

The term "derivatives" refers to chemical functional groups suitably substituted with protecting and/or activation groups or to activated forms of a corresponding chemical functional group which are known to the person skilled in the art. For example, activated forms of carboxyl groups include but are not limited to active esters, such as succinimidyl ester, benzotriazyl ester, nitrophenyl ester, pentafluorophenyl ester, azabenzotriazyl ester, acyl halogenides, mixed or symmetrical anhydrides, acyl imidazole.

The term "non-enzymatically cleavable linker" refers to linkers that are hydrolytically degradable under physiological conditions without enzymatic activity.

"Non-biologically active linker" means a linker which does not show the pharmacological effects of the drug (D-H) derived from the biologically active moiety.

The terms "spacer", "spacer group", "spacer molecule", and "spacer moiety" are used interchangeably and refers to any moiety suitable for connecting two moieties, such as C_{1-50} alkyl, C_{2-50} alkenyl or C_{2-50} alkinyl, which fragment is optionally interrupted by one or more groups selected from -NH-, -N(C_{1-4} alkyl)-, -O-, -S-, -C(O)-, -C(O)NH-, -C(O)N(C_{1-4} alkyl)-, -O-C(O)-, -S(O)-, -$S(O)_2$-, 4 to 7 membered heterocyclyl, phenyl and naphthyl.

The phrases "in bound form" or "moiety" refer to sub-structures which are part of a larger molecule. The phrase "in bound form" is used to simplify reference to moieties by naming or listing reagents, starting materials or hypothetical starting materials well known in the art, and whereby "in bound form" means that for example one or more hydrogen radicals (–H), or one or more activating or protecting groups present in the reagents or starting materials are not present in the moiety.

"Continuous release" refers to an uninterrupted release of prostacyclin compound.

As used herein a single prostacyclin compound dose is given in mg and concentration of a prostacyclin compound in a pharmaceutical composition is given in mg/mL. If the prostacyclin compound is a prodrug, the concentration is based on quantitative release of free prostacyclin from said prodrug. By methods well-known in the art, aliquots of a composition are subjected to prostacyclin-releasing conditions (aqueous buffer pH 7.4, 37°C, or accelerated conditions at elevated pH), until no significant increase in prostacyclin

concentration is observed and the total amount of released prostacyclin is determined. It is understood that in the case of soluble carriers, quantitative release is synonymous to quantitative hydrolysis.

"Activity" of a prostacyclin compound is measured by ADP and collagen-Induced Aggregation of human and/or rat platelets. The effects of prostacyclin compounds on human and rat platelet aggregation can be evaluated by methods known in the art. Generally, platelets are obtained from healthy human volunteers or male Wistar rats. Prior to induction of aggregation with either ADP or collagen, platelets are pre-incubated with the appropriate test article for 1 minute. Platelets are incubated with prodrugs of prostacyclin compounds and the free form of the prostacyclin in parallel at concentrations sufficient to determine IC_{50}.
Prodrugs of prostacyclin compounds are considered inactive when the IC_{50} value is at least 20 fold higher compared to the IC_{50} value of the corresponding free form of prostacyclin.

The present invention relates to a pharmaceutical composition comprising a prostacyclin compound, and optionally one or more pharmaceutically acceptable excipients, which is characterized by having a concentration of the prostacyclin compound that is sufficient to maintain a therapeutically effective level of prostacyclin in blood plasma for at least 12 hours after a single subcutaneous or intramuscular injection.

In a further embodiment, the present invention relates to a pharmaceutical composition comprising a prostacyclin compound and optionally one or more pharmaceutically acceptable excipients, for use in the treatment and/or prevention of diseases wherein the concentration of the prostacyclin compound is sufficient to maintain a therapeutically effective level of prostacyclin in blood plasma for at least 12 hours after a single subcutaneous or intramuscular injection.

Such a concentration will vary from subject to subject and depends on the therapeutic window in an individual subject, but needs to be sufficient in order for the therapeutic effect to be present during at least 12 hours.

Preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound is further characterized in that it comprises a prostacyclin compound in a concentration of at least 0.05 mg/ml.

Even more preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound is characterized in that it further comprises a prostacyclin compound in a concentration of at least 0.05 mg/ml, and wherein a single dose of said pharmaceutical composition comprises at least 0.05 mg of the prostacyclin compound.

Even more preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound further exhibits a time period between administration of a pharmaceutical composition of at least about 12 hours, such as at least 16 hours, typically at least one day.

Even more preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound further exhibits a pharmacokinetic profile in vivo in a mammal, in particular a human with substantially no burst of the prostacyclin compound.

Even more preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound is further characterized by exhibiting a peak to trough ratio in a mammal, in particular a human of less than 5, such as less than 3, or less than 2.

Even more preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound is further characterized in that the prostacyclin compound has an activity of < 20%, preferably < 10%, more preferably < 5% of the activity of free prostacyclin.

It is understood that the activity of free prostacyclin is understood as the activity of the corresponding free prostacyclin which free prostacyclin is obtained after release from e.g. the prodrug or depot.

In addition, another aspect of the present invention relates to a pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound which is

characterized by having a pharmacokinetic profile in vivo in a mammal, in particular a human with substantially no burst of the prostacyclin compound.

Preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound exhibits a peak to trough ratio in a mammal, in particular a human of less than 5, such as less than 3, or less than 2

Even more preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound is further characterized in that the prostacyclin compound has an activity of < 20%, preferably < 10%, more preferably < 5% of the activity of free prostacyclin.

Even more preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound is further characterized by being administered to a mammal, preferably a human, more preferably a human patient by injection, such as subcutaneous or intramuscular injection.

The volume to be administered in order to administer to a mammal, preferably a human, more preferably a human patient an effective dose, for example by a syringe, to a subject, such as a human, is preferably less than 3 ml, typically 2 ml or less.

In addition, another aspect of the present invention further relates to a pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound in which the prostacyclin compound exhibits a peak to trough ratio in a mammal, in particular a human of less than 5, such as less than 3, or less than 2.

Even more preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound is further characterized in that the prostacyclin compound has an activity of < 20%, preferably < 10%, more preferably < 5% of the activity of free prostacyclin.

Even more preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound is further characterized by being administered to a

mammal, preferably a human, more preferably a human patient by injection, such as subcutaneous or intramuscular.

In addition, another aspect of the present invention further relates to a pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound wherein the prostacyclin compound has an activity of < 20%, preferably < 10%, more preferably < 5% of the activity of free prostacyclin.

Even more preferably, such pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin compound is further characterized by being administered by injection, such as subcutaneous or intramuscular injection.

In another preferred embodiment, the present invention relates to a pharmaceutical composition comprising a prostacyclin compound for use in subcutaneous or intramuscular injection.

In another preferred embodiment, the present invention relates to a pharmaceutical composition comprising a prostacyclin compound, wherein said pharmaceutical composition is characterized in that the prostacyclin compound releases prostacyclin in a plasma-independet manner. Preferably, the prostacyclin compound releases prostacyclin in an enzyme-independent manner.

The term “plasma-indpendent” means that the release kinetics of prostacyclin from the prostacyclin compound measured at 37°C independently in buffer at pH 7.4 and in buffered 80% plasma at pH 7.4 varies by no more than 50%, preferably by no more than 40%, more preferably by no more than 30%, even more preferably by no more than 20% and most preferably by no more than 10%.

The term “enzyme-independent” means that the release of prostacyclin from the prostacyclin compound does not require the presence of enzymes.

Preferably, the prostacyclin compound of all above described aspects and embodiments of the present invention comprises a prostacyclin compound or pharmaceutical composition for use

which is in a depot, preferably a polymer gel, more preferably a hydrogel, e.g. a well hydrated polymer matrix.

In an alternatively preferred embodiment, the prostacyclin compound or pharmaceutical composition for use of all above described aspects and embodiment of the present invention is covalently linked to a compound in the depot, preferably to the polymer gel, even more preferably the hydrogel, most preferably to the well hydrated polymer matrix.

Even more preferably, the pharmaceutical compositions or pharmaceutical composition for use comprising a prostacyclin compound of the present invention are further characterized by being administered to a mammal, preferably a human, more preferably a human patient by injection, such as subcutaneous or intramuscular injection.

Even more preferably, the prostacyclin compound comprised in a pharmaceutical composition or pharmaceutical composition for use of all above described aspects and embodiments of the present invention is a prodrug and which is further characterized in that after subcutaneous or intramuscular administration of said prostacyclin prodrug more than 50% of the administered prostacyclin dose is releasable within the blood compartment.

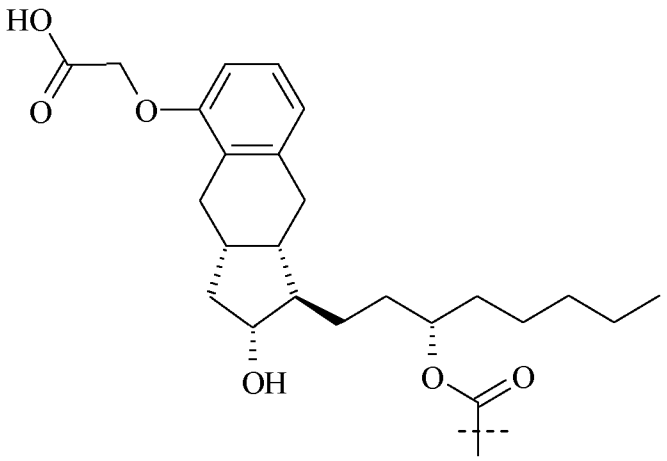
In addition, another aspect of the present invention is a pharmaceutical composition or pharmaceutical composition for use comprising a prostacyclin prodrug which is characterized in that after subcutaneous or intramuscular administration of said prostacyclin prodrug more than 50% of the administered prostacyclin dose is releasable within the blood compartment.

More preferably, the prostacyclin compound is a prodrug of prostacyclin, even more preferably a carrier-linked prostacyclin prodrug, even more preferably a polymer carrier-linked prostacyclin prodrug, which means that the carrier moiety of the carrier-linked prostacyclin prodrug comprises at least one polymer.

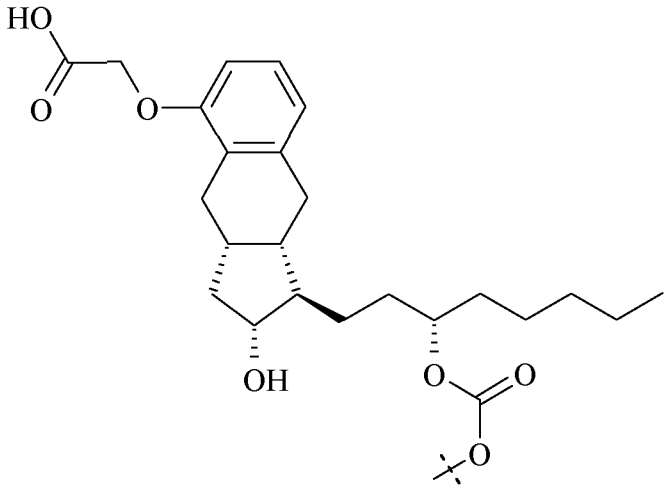
Preferably, the prostacyclin compound is a carrier-linked prostacyclin prodrug of formula (I):

$$Z^1 \text{-} (X^0 - T)_y \qquad (I),$$

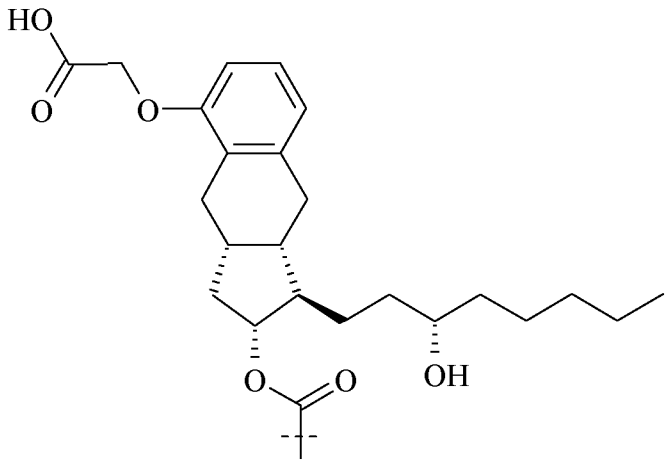
wherein each T is independently a prostacyclin-comprising moiety, preferably the carrier-linked prostacyclin prodrug is a carrier-linked treprostinil prodrug of formula (I), wherein T is a moiety selected from structures (i) to (v):



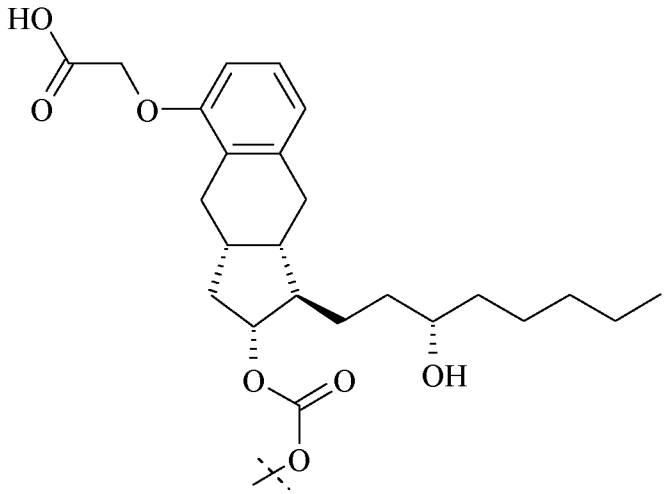
(i),



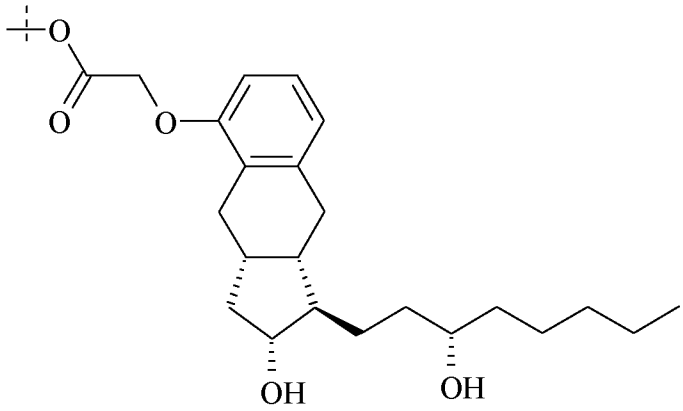
(ii),



(iii),



(iv),



(v),

wherein dashed lines indicating attachment to X^0;

y is an integer ranging of from 1 to 64, preferably ranging from 1 to 16, more preferably y selected from 1, 2, 3, 4, 5, 6, 7 8, 9, 10, 11, 12, 13, 14, 15 and 16, even more preferably y is 8,

each X^0 is independently $(X^{0A})_{m1}$-$(X^{0B})_{m2}$;

m1; m2 are independently 0; or 1;

X^{0A} is T^0;

X^{0B} is a branched or unbranched C_{1-15} alkylene group which is unsubstituted or substituted with one or more R^3, which are the same or different;

R^3 is halogen; C_{1-6} alkyl; CN; $C(O)R^4$; $C(O)OR^4$; OR^4; $C(O)R^4$; $C(O)N(R^4R^{4a})$; $S(O)_2N(R^4R^{4a})$; $S(O)N(R^4R^{4a})$; $S(O)_2R^4$; $S(O)R^4$; $N(R^4)S(O)_2N(R^{4a}R^{4b})$; SR^4; $N(R^4R^{4a})$; NO_2; $OC(O)R^4$; $N(R^4)C(O)R^{4a}$; $N(R^4)SO_2R^{4a}$; $N(R^4)S(O)R^{4a}$; $N(R^4)C(O)N(R^{4a}R^{4b})$; $N(R^4)C(O)OR^{4a}$; $OC(O)N(R^4R^{4a})$; or T^0;

R^4, R^{4a}, R^{4b} are independently selected from the group consisting of H; T^0; C_{1-6} alkyl; C_{2-6} alkenyl; and C_{2-6} alkynyl, wherein C_{1-6} alkyl; C_{2-6} alkenyl; and C_{2-6} alkynyl are optionally substituted with one or more R^5, which are the same of different;

R^5 is halogen; CN; $C(O)R^6$; $C(O)OR^6$; OR^6; $C(O)R^6$; $C(O)N(R^6R^{6a})$; $S(O)_2N(R^6R^{6a})$; $S(O)N(R^6R^{6a})$; $S(O)_2R^6$; $S(O)R^6$; $N(R^6)S(O)_2N(R^{6a}R^{6b})$; SR^6; $N(R^6R^{6a})$; NO_2; $OC(O)R^6$; $N(R^6)C(O)R^{6a}$; $N(R^6)SO_2R^{6a}$; $N(R^6)S(O)R^{6a}$; $N(R^6)C(O)N(R^{6a}R^{6b})$; $N(R^6)C(O)OR^{6a}$; $OC(O)N(R^6R^{6a})$;

R^6, R^{6a}, R^{6b} are independently selected from the group consisting of H; C_{1-6} alkyl; C_{2-6} alkenyl; and C_{2-6} alkynyl, wherein C_{1-6} alkyl; C_{2-6} alkenyl; and C_{2-6} alkynyl are optionally substituted with one or more halogen, which are the same of different;

T^0 is phenyl; naphthyl; azulenyl; indenyl; indanyl; C_{3-7} cycloalkyl; 3 to 7 membered heterocyclyl; or 8 to 11 membered heterobicyclyl, wherein T^0, is optionally substituted with one or more R^7, which are the same or different;

R^7 is halogen; CN; $COOR^8$; OR^8; $C(O)R^8$; $C(O)N(R^8R^{8a})$; $S(O)_2N(R^8R^{8a})$; $S(O)N(R^8R^{8a})$; $S(O)_2R^8$; $S(O)R^8$; $N(R^8)S(O)_2N(R^{8a}R^{8b})$; SR^8; $N(R^8R^{8a})$; NO_2; $OC(O)R^8$; $N(R^8)C(O)R^{8a}$; $N(R^8)S(O)_2R^{8a}$; $N(R^8)S(O)R^{8a}$; $N(R^8)C(O)OR^{8a}$; $N(R^8)C(O)N(R^{8a}R^{8b})$; $OC(O)N(R^8R^{8a})$; oxo (=O), where the ring is at least partially saturated; C_{1-6} alkyl; C_{2-6} alkenyl; or C_{2-6} alkynyl, wherein C_{1-6} alkyl; C_{2-6} alkenyl; and C_{2-6} alkynyl are optionally substituted with one or more R^9, which are the same or different;

R^8, R^{8a}, R^{8b} are independently selected from the group consisting of H; C_{1-6} alkyl; C_{2-6} alkenyl; and C_{2-6} alkynyl, wherein C_{1-6} alkyl; C_{2-6} alkenyl; and C_{2-6} alkynyl are optionally substituted with one or more R^{10}, which are the same of different;

R^9, R^{10} are independently selected from the group consisting of halogen; CN; $C(O)R^{11}$; $C(O)OR^{11}$; OR^{11}; $C(O)R^{11}$; $C(O)N(R^{11}R^{11a})$; $S(O)_2N(R^{11}R^{11a})$; $S(O)N(R^{11}R^{11a})$; $S(O)_2R^{11}$; $S(O)R^{11}$; $N(R^{11})S(O)_2N(R^{11a}R^{11b})$; SR^{11}; $N(R^{11}R^{11a})$; NO_2; $OC(O)R^{11}$; $N(R^{11})C(O)R^{11a}$; $N(R^{11})SO_2R^{11a}$; $N(R^{11})S(O)R^{11a}$; $N(R^{11})C(O)N(R^{11a}R^{11b})$; $N(R^{11})C(O)OR^{11a}$; and $OC(O)N(R^{11}R^{11a})$;

R^{11}, R^{11a}, R^{11b} are independently selected from the group consisting of H; C_{1-6} alkyl; C_{2-6} alkenyl; and C_{2-6} alkynyl, wherein C_{1-6} alkyl; C_{2-6} alkenyl; and C_{2-6} alkynyl are optionally substituted with one or more halogen, which are the same of different;

Z^1 is a carrier comprising a covalently bound polymer, preferably a pharmaceutically acceptable polymer,
wherein the carrier is covalently attached to a moiety X^0, provided that one of m1, m2 is 1 and wherein the carrier is covalently attached to T in case m1, m2 = 0,

or a pharmaceutically acceptable salt thereof.

It was surprisingly found that such carrier-linked treprostinil prodrugs can be used to obtain dosage forms of treprostinil which at least partially overcome the above mentioned shortcomings.

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

In the present invention, the carrier-linked treprostinil prodrug or a pharmaceutically acceptable salt thereof does not contain treprostinil in its free form or as a pharmaceutically acceptable salt thereof, but in bound form. Treprostinil is bound via one of its functional groups, e.g. via a hydroxyl or carboxyl, to the rest of the molecule and is as part of a moiety T which is connected to a moiety X^0 or - if m1 and m2 are both 0 - to a moiety Z^1 of formula (I). This means that the carrier-linked treprostinil prodrug according to the present invention contains treprostinil as a biologically active moiety. Due to the cleavage of the biologically

active moiety from the carrier-linked treprostinil prodrug when administered to a patient in need thereof, treprostinil is released either in its free form or as a pharmaceutically acceptable salt thereof. In other words, the carrier-linked treprostinil prodrug contains one or more moieties T, which moiety T is each substituted with a moiety X^0 (provided that at least one of m1 and m2 is 1), which in turn is covalently bound to a carrier Z^1. Said carrier comprises a covalently bound polymer, preferably a pharmaceutically acceptable polymer with a molecular weight of at least 500 Dalton.

In another preferred embodiment, the molecular weight of the polymer, preferably a pharmaceutically acceptable polymer is up to 160 kDa, preferably up to about 100 kDa, even more preferably up to about 50 kDa.

Preferably, a moiety X^0 (provided that at least one of m1 and m2 is 1) and a moiety T are connected through a carbonate or ester linkage, most preferably a moiety X^0 and a moiety T are connected through an ester linkage.

Preferably, a moiety X^0 is unsubstituted. More preferably, each moiety X^0 is unsubstituted.

In one preferred embodiment, m1 is 0 and m2 is 1.

In another preferred embodiment, both m1 and m2 are 0.

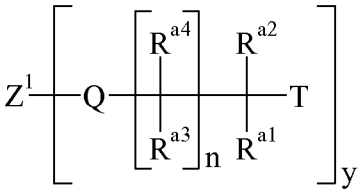
Preferably, a sub-structure X^0-Z^1 is $C(R^1R^2)$-CH_2-Z^1, wherein R^1, R^2 are independently selected from the group consisting of H and C_{1-4} alkyl, provided that at least one of R^1, R^2 is other than H; or $(CH_2)_n$-Z^1, wherein n is 2, 3, 4, 5, 6, 7 or 8.

Preferably, the carrier Z^1 is covalently attached to a moiety X^0 via an amide group.

Preferably, R^3 is halogen; CN; $C(O)R^4$; $C(O)OR^4$; OR^4; $C(O)R^4$; $C(O)N(R^4R^{4a})$; $S(O)_2N(R^4R^{4a})$; $S(O)N(R^4R^{4a})$; $S(O)_2R^4$; $S(O)R^4$; $N(R^4)S(O)_2N(R^{4a}R^{4b})$; SR^4; $N(R^4R^{4a})$; NO_2; $OC(O)R^4$; $N(R^4)C(O)R^{4a}$; $N(R^4)SO_2R^{4a}$; $N(R^4)S(O)R^{4a}$; $N(R^4)C(O)N(R^{4a}R^{4b})$; $N(R^4)C(O)OR^{4a}$; $OC(O)N(R^4R^{4a})$; or T^0.

Preferably, R^4, R^{4a}, R^{4b} are independently selected from the group consisting of H; T^0; C_{1-4} alkyl; C_{2-4} alkenyl; and C_{2-4} alkynyl, wherein C_{1-4} alkyl; C_{2-4} alkenyl; and C_{2-4} alkynyl are optionally substituted with one or more R^5, which are the same of different.

More preferably, the carrier-linked treprostinil prodrug is of formula (II):



(II),

wherein T, y and Z^1 are used as defined in formula (I), and

R^{a1} is selected from the group of unsubstituted alkyl; substituted alkyl; unsubstituted phenyl; substituted phenyl; unsubstituted naphthyl; substituted naphthyl; unsubstituted indenyl; substituted indenyl; unsubstituted indanyl; substituted indanyl; unsubstituted tetralinyl; substituted tetralinyl; unsubstituted C_{3-10} cycloalkyl; substituted C_{3-10} cycloalkyl; unsubstituted 4- to 7-membered heterocyclyl; substituted 4- to 7-membered heterocyclyl; unsubstituted 9- to 11-membered heterobicyclyl; and substituted 9- to 11-membered heterobicyclyl;

R^{a2} is selected from H, unsubstituted alkyl, and substituted alkyl;

R^{a3} and R^{a4} are independently selected from the group consisting of H, unsubstituted alkyl, and substituted alkyl;

n is 0 or 1;

optionally, R^{a1} and R^{a3} are joined together with the atoms to which they are attached to form a ring A;

A is selected from the group consisting of C_{3-10} cycloalkyl; 4- to 7-membered aliphatic heterocyclyl; and 9- to 11-membered aliphatic heterobicyclyl, wherein A is unsubstituted or substituted;

Q is a spacer moiety;

Preferably, R^{a1} is C_{1-6} alkyl or substituted C_{1-6} alkyl, more preferably C_{1-4} alkyl or substituted C_{1-4} alkyl.

More preferably, R^{a1} is selected from methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, and benzyl.

Preferably, R^{a2} is H.

Preferably, R^{a3} is H, C_{1-6} alkyl or substituted C_{1-6} alkyl, more preferably C_{1-4} alkyl or substituted C_{1-4} alkyl. More preferably, R^{a3} is selected from methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, and benzyl.

More preferably, R^{a3} is H.

Preferably, R^{a4} is selected from H, C_{1-6} alkyl or substituted C_{1-6} alkyl, more preferably C_{1-4} alkyl or substituted C_{1-4} alkyl. More preferably, R^{a4} is selected from methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, and benzyl.

More preferably, R^{a4} is H.

In another preferred embodiment, R^{a1} and R^{a3} are joined together with the atoms to which they are attached to form a ring A; wherein A is selected from the group consisting of cyclopropane, cyclobutane, cyclopentane, cyclohexane, cycloheptane.

Preferably, Q in formula (II) is selected from $COOR^{a9}$; OR^{a9}; $C(O)R^{a9}$; $C(O)N(R^{a9}R^{a9a})$; $S(O)_2N(R^{a9}R^{a9a})$; $S(O)N(R^{a9}R^{a9a})$; $S(O)_2R^{a9}$; $S(O)R^{a9}$; $N(R^{a9})S(O)_2N(R^{a9a}R^{a9b})$; SR^{a9}; $N(R^{a9}R^{a9a})$; $OC(O)R^{a9}$; $N(R^{a9})C(O)R^{a9a}$; $N(R^{a9})S(O)_2R^{a9a}$; $N(R^{a9})S(O)R^{a9a}$; $N(R^{a9})C(O)OR^{a9a}$; $N(R^{a9})C(O)N(R^{a9a}R^{a9b})$; $OC(O)N(R^{a9}R^{a9a})$; W; C_{1-50} alkyl; C_{2-50} alkenyl; and C_{2-50} alkynyl,

wherein W, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more R^{a10}, 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 -W-, -C(O)O-; -O-; -C(O)-; -C(O)N(R^{a11})-; -S(O)$_2$N(R^{a11})-; -S(O)N(R^{a11})-; -S(O)$_2$-; -S(O)-; -N(R^{a11})S(O)$_2$N(R^{a11a})-; -S-; -N(R^{a11})-; -OC(O)R^{a11}; -N(R^{a11})C(O)-; -N(R^{a11})S(O)$_2$-; -N(R^{a11})S(O)-; -N(R^{a11})C(O)O-; -N(R^{a11})C(O)N(R^{a11a})-; and -OC(O)N($R^{a11}R^{a11a}$);

R^{a9}, R^{a9a}, R^{a9b} are independently selected from the group consisting of H; W; and C_{1-50} alkyl; C_{2-50} alkenyl; and C_{2-50} alkynyl,

wherein W, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more R^{a10}, which are the same or different,

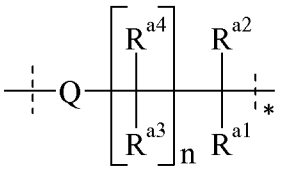
and wherein C_{1-50} alkyl; C_{2-50} alkenyl; and C_{2-50} alkynyl are optionally interrupted by one or more groups selected from the group consisting of W, -C(O)O-; -O-; -C(O)-; -C(O)N(R^{a11})-; -S(O)$_2$N(R^{a11})-; -S(O)N(R^{a11})-; -S(O)$_2$-; -S(O)-; -N(R^{a11})S(O)$_2$N(R^{a11a})-; -S-; -N(R^{a11})-; -OC(O)R^{a11}; -N(R^{a11})C(O)-; -N(R^{a11})S(O)$_2$-; -N(R^{a11})S(O)-; -N(R^{a11})C(O)O-; -N(R^{a11})C(O)N(R^{a11a})-; and -OC(O)N($R^{a11}R^{a11a}$);

W is selected from the group consisting of phenyl; naphthyl; indenyl; indanyl; tetralinyl; C_{3-10} cycloalkyl; 4- to 7-membered heterocyclyl; or 9- to 11-membered heterobicyclyl, wherein W is optionally substituted with one or more R^{a10}, which are the same or different;

R^{a10} is halogen; CN; oxo (=O); COOR^{a12}; OR^{a12}; C(O)R^{a12}; C(O)N($R^{a12}R^{a12a}$); S(O)$_2$N($R^{a12}R^{a12a}$); S(O)N($R^{a12}R^{a12a}$); S(O)$_2R^{a12}$; S(O)R^{a12}; N(R^{a12})S(O)$_2$N($R^{a12a}R^{a12b}$); SR^{a12}; N($R^{a12}R^{a12a}$); NO_2; OC(O)R^{a12}; N(R^{a12})C(O)R^{a12a}; N(R^{a12})S(O)$_2R^{a12a}$; N(R^{a12})S(O)R^{a12a}; N(R^{a12})C(O)OR^{a12a}; N(R^{a12})C(O)N($R^{a12a}R^{a12b}$); OC(O)N($R^{a12}R^{a12a}$); or C_{1-6} alkyl, wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different;

R^{a11}, R^{a11a}, R^{a12}, R^{a12a}, R^{a12b} are 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.

In formula (II) the moiety X^0 is of formula (IIa):



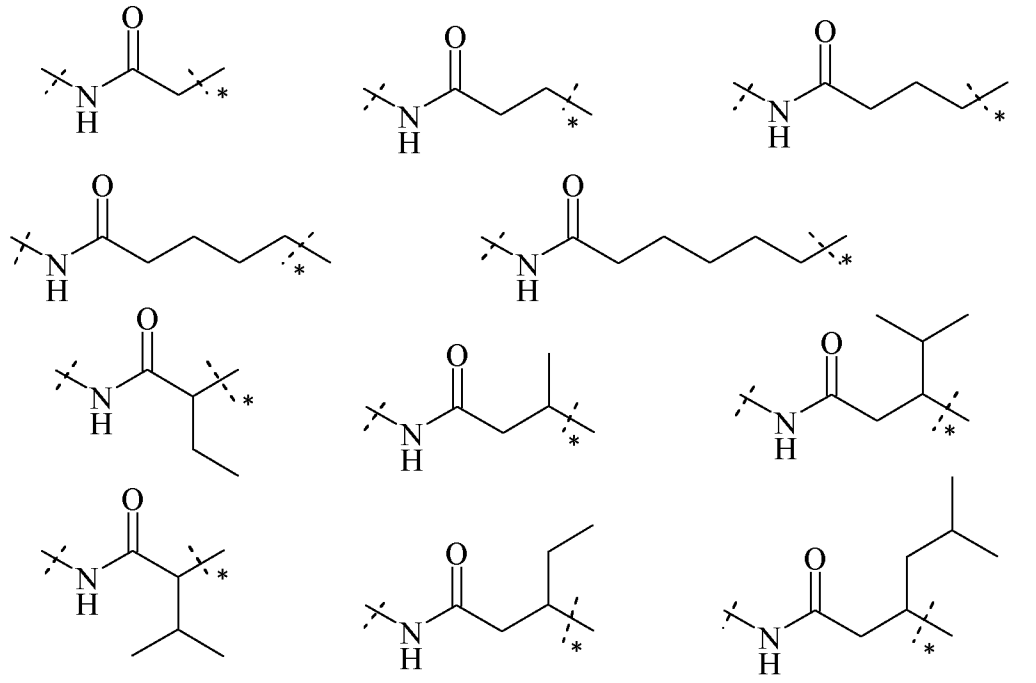
(IIa),

wherein

the dashed line marked with the asterisk indicates attachment to T and the unmarked dashed line indicates attachment to the rest of the carrier-linked treprostinil prodrug; and

wherein Q, R^{a1}, R^{a2}, R^{a3}, and R^{a4} are used as defined in formula (II).

Preferably, X^0 is selected from the following structures:



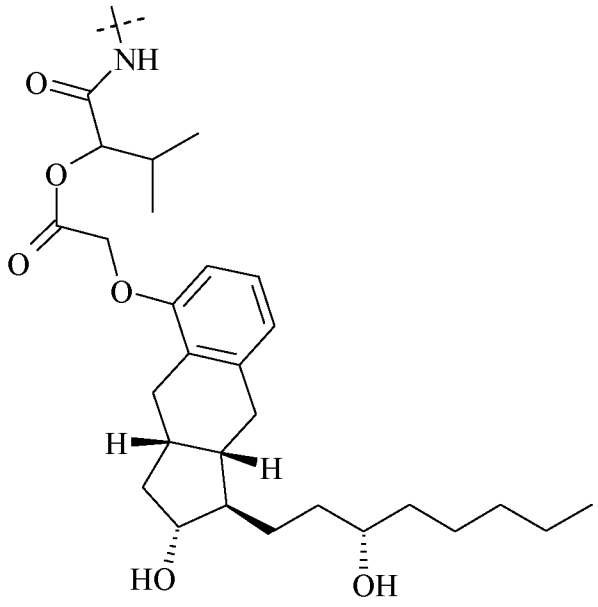
wherein dashed lines marked with an asterisk indicate attachment to T and

unmarked dashed lines indicate attachment to the rest of the carrier-linked treprostinil prodrug.

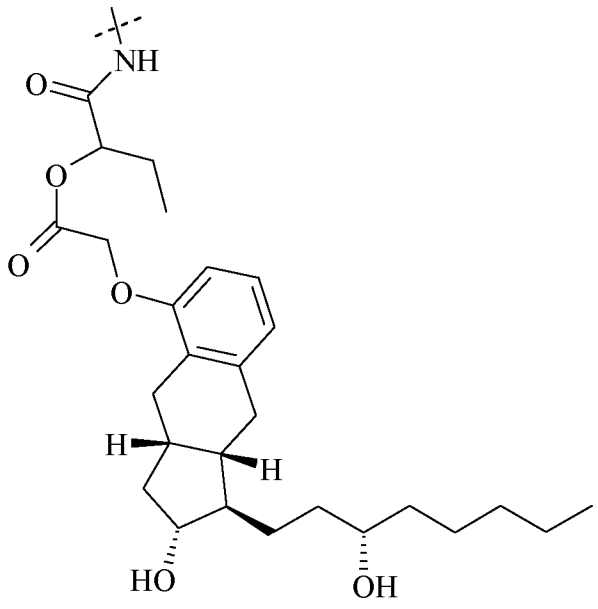
Preferably, all moieties T of the carrier-linked treprostinil prodrug of formula (I) have the same structure.

Preferably, all moieties T of formula (I) have the structure of formula (v).

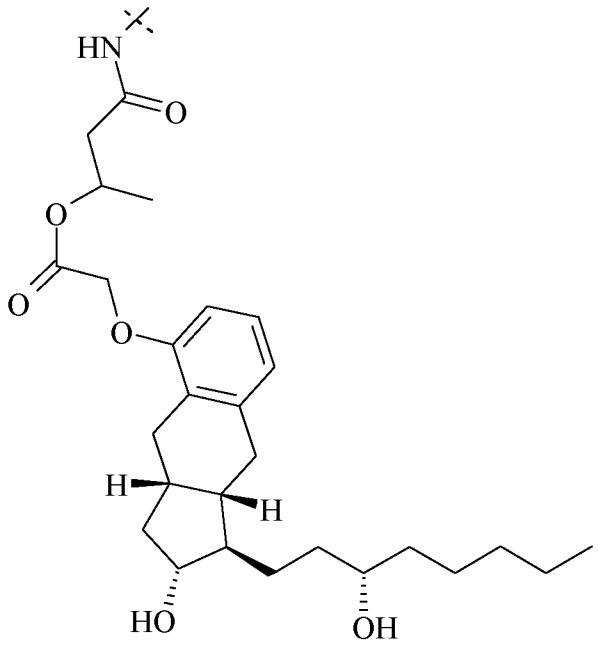
Preferred sub-structures $-X^0-T$ of formula (I) are selected from the following structures:



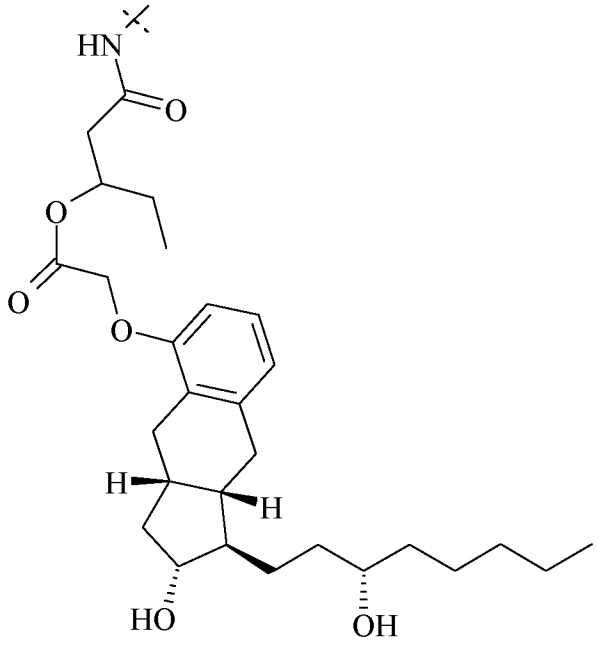
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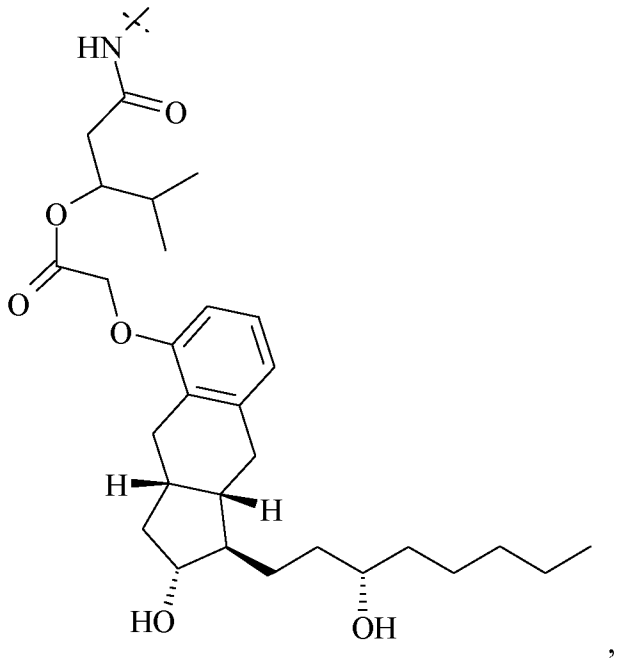
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HN
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O
O
O
H
H
HO
OH

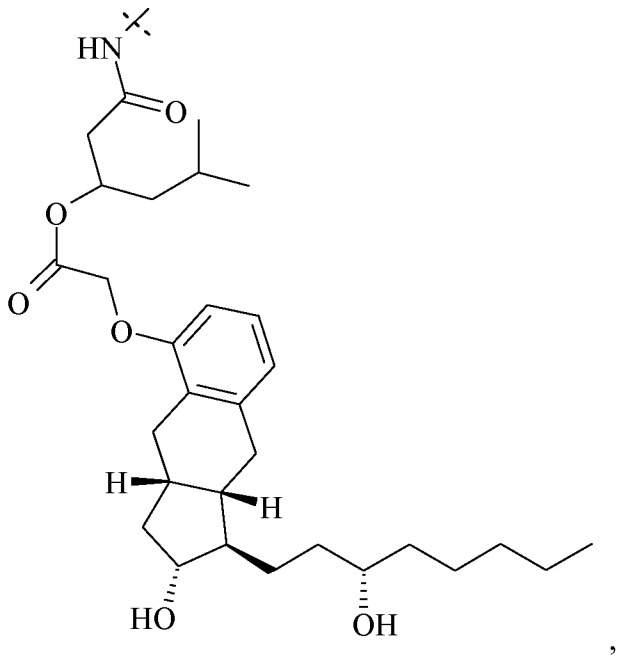
,


HN
O
O
O
O
H
H
HO
OH

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wherein the dashed lines indicate attachment to Z^1.

The carrier Z^1 comprises a covalently bound polymer, preferably a pharmaceutically acceptable polymer.

Preferred polymers are selected from 2-methacryloyl-oxyethyl phosphoyl cholins, hydrogels, PEG-based hydrogels, poly(acrylic acids), poly(acrylates), poly(acrylamides), poly(alkyloxy)

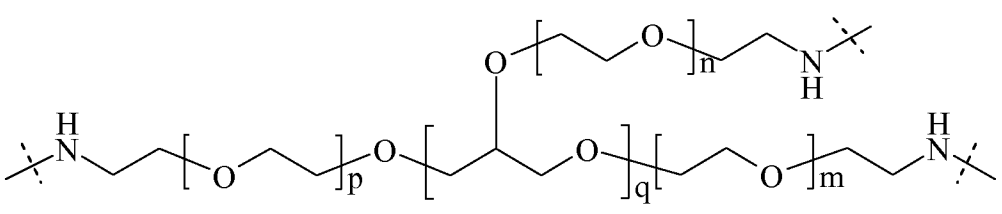
polymers, poly(amides), poly(amidoamines), poly(amino acids), poly(anhydrides), poly(aspartamides), poly(butyric acids), poly(glycolic acids), polybutylene terephthalates, poly(caprolactones), poly(carbonates), poly(cyanoacrylates), poly(dimethylacrylamides), poly(esters), poly(ethylenes), poly(ethyleneglycols), poly(ethylene oxides), poly(ethyl phosphates), poly(ethyloxazolines), poly(glycolic acids), poly(hydroxyethyl acrylates), poly(hydroxyethyloxazolines), poly(hydroxymethacrylates), poly(hydroxypropylmethacrylamides), poly(hydroxypropyl methacrylates), poly(hydroxypropyloxazolines), poly(iminocarbonates), poly(lactic acids), poly(lactic-co-glycolic acids), poly(methacrylamides), poly(methacrylates), poly(methyloxazolines), poly(organophosphazenes), poly(ortho esters), poly(oxazolines), poly(propylene glycols), poly(siloxanes), poly(urethanes), poly(vinyl alcohols), poly(vinyl amines), poly(vinylmethylethers), poly(vinylpyrrolidones), silicones, celluloses, carbomethyl celluloses, hydroxypropyl methylcelluloses, chitins, chitosans, dextrans, dextrins,gelatins, hyaluronic acids and derivatives, mannans, pectins, rhamnogalacturonans, starches, hydroxyalkyl starches, hydroxyethyl starches and other carbohydrate-based polymers, xylans, and copolymers thereof.

Preferably, the carrier Z^1 comprises a poly(oxazoline) or a PEG-based polymer. Most preferably, the carrier Z^1 comprises a PEG-based polymer.

In one embodiment the carrier Z^1 may be a hydrogel (as one option for a polymer) which are known in the art. Suitable hydrogels are described in WO-A 2006/003014 or EP-A 1 625 856. If the carrier Z^1 is a hydrogel it is preferred that it is a PEG-based hydrogel as disclosed in WO-A 2011/012715 which is incorporated by reference herewith.

Preferably, the carrier Z^1 is a water-soluble carrier.

In one embodiment the carrier Z^1 has the structure of formula (III):



(III),

wherein dashed lines indicate attachment to X^0 and

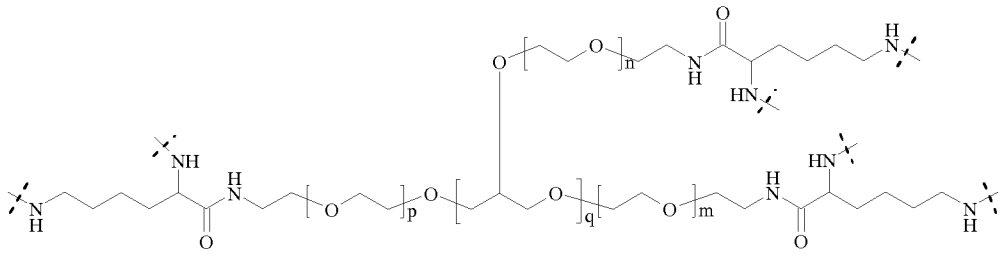
wherein each of m, n, and p of formula (III) are independently an integer ranging of from 5 to 500,

and wherein q of formula (III) ranges of from 2 to 32.

Preferably, q in formula (II) is an integer ranging of from 2 to 14 and more preferably q of formula (II) is 6.

Preferably, each of m, n, and p in formula (III) independently range of from 10 to 250, more preferably from 50 to 150. Preferably, m, n, and p in formula (III) are the same.

In an alternative embodiment the carrier Z^1 has the structure of formula (IV):



(IV),

wherein dashed lines indicate attachment to X^0, provided that one of m1, m2 is 1 and wherein the carrier is covalently attached to T in case m1, m2 = 0, and

wherein each of m, n, and p of formula (IV) are independently an integer ranging of from 5 to 500 and wherein q of formula (IV) ranges of from 0 to 14.

Preferably, q in formula (IV) is an integer ranging of from 2 to 6 and more preferably q of formula (IV) is 2.

Preferably, each of m, n, and p in formula (IV) independently range of from 10 to 250, more preferably from 50 to 150. Preferably, m, n, and p in formula (IV) are the same.

In another preferred embodiment Z^1 has the structure of formula (V):

$Hyp^1{}_{mx} - POL^x - Hyp^2$ (V),

wherein

POL^x is a polymeric moiety having a molecular weight ranging from 0.5 kDa to 160 kDa,

Hyp^1 and Hyp^2 are independently a hyperbranched moiety, and

mx is 0 or 1.

The polymeric moiety POL^x has a molecular weight of from 0.5 kDa to 160 kDa, preferably of from 2 kDa to 80 kDa and more preferably of from 5 kDa to 40kDa.

POL^x may be selected from the group of polymers consisting of, for example, polypeptides, 2-methacryloyl-oxyethyl phosphoyl cholins, water-soluble hydrogels, water-soluble PEG-based hydrogels, water-soluble hyaluronic acid-based hydrogels, poly(acrylic acids), poly(acrylates), poly(acrylamides), poly(alkyloxy) polymers, poly(amides), poly(amidoamines), poly(amino acids), poly(anhydrides), poly(aspartamides), poly(butyric acids), poly(glycolic acids), polybutylene terephthalates, poly(caprolactones), poly(carbonates), poly(cyanoacrylates), poly(dimethylacrylamides), poly(esters), poly(ethylenes), poly(ethyleneglycols), poly(ethylene oxides), poly(ethyl phosphates), poly(ethyloxazolines), poly(glycolic acids), poly(hydroxyethyl acrylates), poly(hydroxyethyloxazolines), poly(hydroxymethacrylates), poly(hydroxypropylmethacrylamides), poly(hydroxypropyl methacrylates), poly(hydroxypropyloxazolines), poly(iminocarbonates), poly(lactic acids), poly(lactic-co-glycolic acids), poly(methacrylamides), poly(methacrylates), poly(methyloxazolines), poly(organophosphazenes), poly(ortho esters), poly(oxazolines), poly(propylene glycols), poly(siloxanes), poly(urethanes), poly(vinyl alcohols), poly(vinyl amines), poly(vinylmethylethers), poly(vinylpyrrolidones), silicones, celluloses, carbomethyl celluloses, hydroxypropyl methylcelluloses, chitins, chitosans, dextrans, dextrins, gelatins, hyaluronic acids and derivatives, functionalized hyaluronic acids, mannans, pectins, rhamnogalacturonans, starches, hydroxyalkyl starches, hydroxyethyl starches and other carbohydrate-based polymers, xylans, and copolymers thereof.

The polymeric moiety POL^x of formula (V) may comprise a linear or branched polymer. Preferably, POL^x of formula (V) comprises, in particular consists of a linear polymer.

In one preferred embodiment, POL^x of formula (V) comprises, in particular consists of a PEG-based polymer or a poly(oxazoline)-based polymer, more preferably a linear PEG-based polymer. Even more preferably, POL^x of formula (V) consists of a PEG-based linear polymer.

If m in formula (V) is 0, it is preferred that POL^x of formula (V) comprises, preferably consists of a structure of the formula $X1\text{-}(OCH_2CH_2)_p\text{-}O\text{-}(CH_2)_n\text{-}X2\text{-}$, wherein n is selected from 2, 3, or 4; p is an integer in the range of from 5 to 2000, preferably p is an integer in the range of from 10 to 1000, more preferably p is an integer in the range of from 100 to 1000; and X2 is a functional group covalently linking POL^x and Hyp^2 of formula (V); and X1 is selected from H, CH_3 and C_2H_5.

If m in formula (V) is 1, it is preferred that POL^x of formula (V) comprises, preferably consists of a structure of the formula $X3\text{-}(CH_2)_{n1}\text{-}(OCH_2CH_2)_p\text{-}O\text{-}(CH_2)_{n2}\text{-}X2\text{-}$, wherein n1 and n2 are independently selected from 2, 3, and 4; p is an integer in the range of from 5 to 2000, preferably p is an integer in the range of from 10 to 1000, more preferably p is an integer in the range of from 100 to 1000; and X2 and X3 are functional groups covalently linking POL^x to Hyp^1 and Hyp^2 of formula (V), respectively.

In a preferred embodiment mx in formula (V) is 0.

In another preferred embodiment, POL^x of formula (V) is a polypeptide (or protein), in particular a non-immunogenic polypeptide as described below.

Preferably, the polymeric moiety POL^x of formula (V) is a polypeptide which comprises at least about 100 amino acid residues, in particular which consists of at least about 100 amino acid residues. In a preferred embodiment, amino acids selected from alanine, serine and/or proline residues are present, in particular are mainly present, and which polypeptide moiety preferably has a random coil conformation at physiological conditions. It is understood that such a polypeptide moiety POL^x of formula (V) may transiently or temporarily not form a random coil, for example when present in a lyophilisate or dried composition.

A polypeptide moiety POL^x of formula (V) may have a random coil conformation with an amino acid sequence consisting of maximally about 1000 amino acid residues, preferably of maximally about 900 amino acid residues, more preferably of maximally about 800 amino acid residues, even more preferably of maximally about 700 amino acid residues, particularly preferably of maximally about 600 amino acid residues. Thus, the amino acid sequence forming random coil conformation may consist of maximally about 500 amino acid residues or of maximally about 450 amino acid residues.

It is also envisaged herein that the amino acid sequence forming random coil conformation may consist of maximally about 1200 and up to about 1500 amino acid residues. Accordingly, the amino acid sequence forming random coil conformation may consist of about 100 to about 1500 amino acid residues.

In particular embodiments said amino acid sequence forming random coil conformation consists of about 100 to 1000 amino acid residues as characterized herein, i.e. comprising alanine, serine and/or proline as main or unique residues as defined below.

In a preferred embodiment, a polypeptide moiety POL^x of formula (V) consists mainly of one, two or three of the amino acid residues alanine, serine and proline, whereby proline residues represent preferably about 4 % to about 40 % of the polypeptide moiety POL^x of formula (V). The alanine and serine residues comprise the remaining at least 60 % to 96 % of the polypeptide moiety POL^x of formula (V). However, as will be detailed herein below said polypeptide moiety POL^x of formula (V) may also comprise further amino acids differing from alanine, serine, and proline, i.e. as minor constituents.

The term "minor constituent" as used herein means that maximally 10 % (i.e. maximally 10 of 100 amino acids) may be different from alanine, serine and proline, preferably maximally 8 % (i.e. maximally 8 of 100 amino acids) may be different than alanine, serine and proline, more preferably maximally 6 % (i.e. maximally 6 of 100 amino acids) may be different from alanine, serine and proline, even more preferably maximally 5 % (i.e. maximally 5 of 100 amino acids) may be different from alanine, serine and proline, particularly preferably maximally 4 % (i.e. maximally 4 of 100 amino acids) may be different from alanine, serine and proline, more particularly preferably maximally 3 % (i.e. maximally 3 of 100 amino acids) may be different from alanine, serine and proline, even more particularly preferably

maximally 2 % (i.e. maximally 2 of 100 amino acids) may be different from alanine, serine and proline and most preferably maximally 1 % (i.e. maximally 1 of 100 of the amino acids) may be different from alanine, serine and proline. Said amino acids different from alanine, serine and proline may be selected from the group consisting of different from alanine, serine and proline may be selected from the group of natural or proteinogenic amino-acids comprising Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Tyr, Val, selenocystein, selenomethionin, and hydroxyproline. Minor constituents may also be selected from non-naturally occurring amino acids.

The term "at least about 100/150/200/250/300/300/350 (etc) amino acid residues" is not limited to the concise number of amino acid residues but also comprises amino acid stretches that comprise an additional 10 % to 20 % or comprise 10 % to 20 % less residues. For example "at least about 100 amino acid residues" may also encompass 80 to 100 and about 100 to 120 amino acid residues without deferring from the gist of the present invention.

In one embodiment, the polypeptide moiety POL^x of formula (V) comprises a plurality of polymer cassettes wherein said polymer cassettes consist of one, two or three of the amino acids selected from Ala, Ser, and Pro and wherein no more than 6 consecutive amino acid residues are identical and wherein said proline residues constitute more than 4 % and less than 40 % of the amino acids of said polypeptide moiety POL^x of formula (V).

A polypeptide moiety POL^x of formula (V) may comprise a plurality, in particular 2, 3, 4, 5 or more of identical polymer cassettes or a plurality of non-identical polymer cassettes. Non-limiting examples of polymer cassettes consisting of Ala, Ser and Pro residues are provided herein below; see SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13 and SEQ ID NO:14 or peptide fragments or multimers of these sequences. A polymer cassette may consist of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more amino acid residues, wherein each polymer cassette comprises (an) Ala, Ser, and Pro residue(s).

In one embodiment, the polymer cassette according to the present invention does not comprise more than 100 amino acid residues. Preferably, a polymer cassette as defined herein comprises more than about 4 %, preferably more than about 5 %, even more preferably more than about 6%, particularly preferably more than about 8 %, more particularly preferably

more than about 10 %, even more particularly preferably more than about 15 % and most preferably more than about 20 % proline residues. Such polymer cassette as defined herein preferably comprises less than about 40 % or less than about 35 % proline residues.

In one preferred embodiment the polypeptide moiety POL^x of formula (V) comprises, in particular consists of formula (a):

$Ser_x[Ala_y\ Ser_z]_n$ (a),

which formula further comprises proline residues as defined herein and wherein

x is independently selected from integer 0 to 6,

each y is independently selected from integer ranging of from 1 to 6,

each z is independently selected from integer ranging of from 1 to 6.

n is any integer so that a polypeptide moiety POL^x of formula (V) consists of at least about 100 amino acid residues, and in particular of at least about 100 to about 3000 amino acid residues, preferably to about 2000 and more preferably to about 1000 amino acid residues.

In another preferred embodiment, a polypeptide moiety POL^x of formula (V) comprises no more than 5 identical consecutive amino acid residues, more preferably no more than 4 identical consecutive amino acid residues and most preferably no more than 3 identical consecutive amino acid residues.

As already indicated herein above, a polypeptide moiety POL^x of formula (V) comprises in one embodiment proline residues, wherein said proline residues constitute more than about 4 %, preferably more than about 5 %, even more preferably more than about 6 %, particularly preferably more than about 8 %, more particularly preferably more than about 10 %, even more particularly preferably more than about 15 % and most preferably more than about 20 % of the amino acids of POL^x of formula (V).

In another preferred embodiment, a polypeptide moiety POL^x of formula (V) comprises more than about 4 % but less than about 50 %, preferably more than about 10 % but less than about 50 % and most preferably more than about 20 % but less than about 50 % alanine residues of the amino acids constituting the polypeptide moiety POL^x of formula (V).

In a further preferred embodiment, a polypeptide moiety POL^x of formula (V) comprises more than about 4 % and less than about 50 %, preferably more than about 10 % but less than about 50 % and most preferably more than about 20 % but less than about 50 % serine residues of the amino acids constituting the polypeptide moiety POL^x of formula (V).

Preferably, a polypeptide moiety POL^x of formula (V) comprises about 35 % proline residues, about 50 % alanine residues and about 15 % serine residues of the amino acids constituting the polypeptide moiety POL^x of formula (V). Alternatively, a polypeptide moiety POL^x of formula (V) may comprise about 35 % proline residues, about 15 % alanine residues and about 50 % serine residues of the amino acids constituting the polypeptide moiety POL^x of formula (V).

Preferably, a polypeptide moiety POL^x of formula (V) comprises one or more of the following alanine-serine polymer cassettes:

SEQ ID NO:1
AAAASSASSASSSSSAAASA

SEQ ID NO:2
AASAAASSAAASAAAASASS

SEQ ID NO:3
ASASASASASASSAASAASA

SEQ ID NO:4
SAASSSASSSSAASSASAAA

SEQ ID NO:5
SSSSAASAASAAAAASSSAS

SEQ ID NO:6
SSASSSAASSSASSSSASAA

SEQ ID NO:7
SASASASASASAASSASSAS

SEQ ID NO:8
ASSAAASAAAASSAASASSS

The multimers of these alanine-serine polymer cassettes may form random coil conformation in case the resulting amino acid sequence further comprises proline residues as defined herein above.

In a preferred embodiment, a polypeptide moiety POL^x of formula (V) comprises one or more of the following polymer cassettes:

SEQ ID NO:9
ASPAAPAPASPAAPAPSAPA

SEQ ID NO:10
AAPASPAPAAPSAPAPAAPS

SEQ ID No:11
APSSPSPSAPSSPSPASPSS

SEQ ID NO:15
SAPSSPSPSAPSSPSPASPS

SEQ ID NO:15 corresponds to the herein provided SEQ ID No:11 in a circularly permuted form, wherein the last serine was removed and another serine was appended as starting amino acid. As a consequence, multimers of this modified sequence possess essentially the same internal repeating unit as multimers of the non-modified sequence, except for the very first and the very last residue. Accordingly, SEQ ID NO:15 may be considered as an example of a further polymer cassette for a polypeptide moiety POL^x of formula (V). It is clear for the

person skilled in the art that also other polymer cassettes and (shorter) peptide fragments or circularly permuted versions of the herein provided amino acid polymers may be used as polymer cassettes for a polypeptide moiety POL^x of formula (V).

Yet, even further and illustrative amino acid polymers forming random coil conformation may comprise amino acid sequences that may be selected from the group consisting of the following sequences:

SEQ ID NO:12
SSPSAPSPSSPASPSPSSPA

SEQ ID NO:13
AASPAAPSAPPAAASPAAPSAPPA

SEQ ID NO:14
ASAAAPAAASAAASAPSAAA

Therefore, preferred polymer cassettes for a polypeptide moiety POL^x of formula (V) are selected from the following sequences:

ASPAAPAPASPAAPAPSAPA (SEQ ID NO:9),
AAPASPAPAAPSAPAPAAPS (SEQ ID NO:10),
APSSPSPSAPSSPSPASPSS (SEQ ID NO:11),
SSPSAPSPSSPASPSPSSPA (SEQ ID NO:12),
AASPAAPSAPPAAASPAAPSAPPA (SEQ ID NO:13), and
ASAAAPAAASAAASAPSAAA (SEQ ID NO:14);

or circular permuted versions or (a) multimer(s) of these sequences as a whole or parts of these sequences.

Again, also (a) peptide fragment(s) or (a) multimer(s) or circularly permuted versions of these sequences and the sequences provided herein above may be employed in context of the present invention as polymer cassettes for a polypeptide moiety POL^x of formula (V). The person skilled in the art is readily in a position to generate further amino acid polymer

cassettes that form random coil conformation under physiological conditions and are constituted of mainly alanine, serine, and proline as defined herein. Such other and further examples of random coil conformation forming amino acid polymer cassettes to be used for a polypeptide moiety POL^x of formula (V) may, inter alia, comprise combinations and/or peptide fragments or circularly permuted versions of the specific polymer cassettes shown above.

Accordingly, the exemplified polymer cassettes may also provide for individual peptide fragments which may be newly combined to form further polymer cassettes.

In accordance with the above, a polypeptide moiety POL^x of formula (V) may comprise a multimer of sequences consisting of either one of the amino acid sequences with SEQ ID NO:9, 10, 11, 12, 13 or 14 as disclosed herein above or may comprise a multimer of sequences consisting of more than one of amino acid sequences SEQ ID NOs:9, 10, 11, 12, 13 and 14. Furthermore, it is envisaged that also peptide fragments or circularly permuted versions of these exemplified sequences may be used to build up further polymer cassettes of a polypeptide moiety POL^x of formula (V).

In another embodiment, a polypeptide moiety POL^x of formula (V) may comprise a multimer of sequences consisting of a (circular) permutation of the amino acid sequence selected from the group consisting of SEQ ID NO:9, 10, 11, 12, 13, 14, 15 or (a) multimers(s) of these (circular) permutated sequences.

In yet another embodiment, a polypeptide moiety POL^x of formula (V) may comprise a multimer consisting of a peptide fragment/part of the amino acid sequence selected from the group consisting of SEQ ID NOs: 9, 10, 12, 13, 14, 15 or (a) multimers(s) of these exemplified polymer cassettes.

Peptide fragments of these sequences to be employed for the generation of a polypeptide moiety POL^x of formula (V) may consist of at least 3, preferably of at least 4, more preferably of at least 5, even more preferably of at least 6, still more preferably of at least 8, particularly preferably of at least 10, more particularly preferably of at least 12, even more particularly preferably of at least 14, preferably of at least 6, still more preferably of at least 8, particularly preferably of at least 10, more particularly preferably of at least 12, even more particularly

preferably of at least 14, even more particularly preferably of at least 16, and most preferably of at least 18 consecutive amino acids of the amino acid sequence selected from the group consisting of said SEQ ID NOs: 9, 10, 11, 12, 13 and 14.

For example, individual peptide fragments of the inventive polymer cassettes may be combined to further individual polymer cassettes as long as the above-identified rules for the overall distribution and amount of alanine, serine and proline are respected. Again, these polymer cassettes may also comprise further amino acid residues, however only as minimal or minor constituents, i. e. maximally 10 %, preferably maximally 2 % of the individual polymer cassette. POL^x of formula (V) moieties comprising polymer cassettes consist, in one embodiment of the present invention, of at least about 100 amino acid residues. Individual polymer cassettes may be combined in order to form longer random coil forming amino acid polymers, whereby a maximal length of a polypeptide moiety POL^x of formula (V) is about 3000 amino acids.

Preferably, POL^x of formula (V) is covalently linked to Hyp^1 and Hyp^2 of formula (V), in particular by a permanent linkage, more preferably by a permanent amide linkage.

In the carrier-linked treprostinil prodrugs of the present invention functional groups of Hyp^1 and Hyp^2 of formula (V) are connected to a moiety X^0 of formula (I).

The hyperbranched moieties Hyp^1 and Hyp^2 of formula (V) are each independently selected from the group comprising, in particular consisting of, in bound form glycerol, pentaerythritol, dipentaerythritol, tripentaerythritol, hexaglycerine, sucrose, sorbitol, fructose, mannitol, glucose, cellulose, amyloses, starches, hydroxyalkyl starches, polyvinylalcohols, dextranes, hyualuronans, dilysine, trilysine, tetralysine, pentalysine, hexalysine, heptalysine, octalysine, nonalysine, decalysine, undecalysine, dodecalysine, tridecalysine, tetradecalysine, pentadecalysine, hexadecalysine, heptadecalysine, octadecalysine, nonadecalysine, triornithine, tetraornithine, pentaornithine, hexaornithine, heptaornithine, octaornithine, nonaornithine, decaornithine, undecaornithine, dodecaornithine, tridecaornithine, tetradecaornithine, pentadecaornithine, hexadecaornithine, heptadecaornithine, octadecaornithine, nonadecaornithine, tridiaminobutyric acid, tetradiaminobutyric acid, pentadiaminobutyric acid, hexadiaminobutyric acid, heptadiaminobutyric acid, octadiaminobutyric acid, nonadiaminobutyric acid, decadiaminobutyric acid,

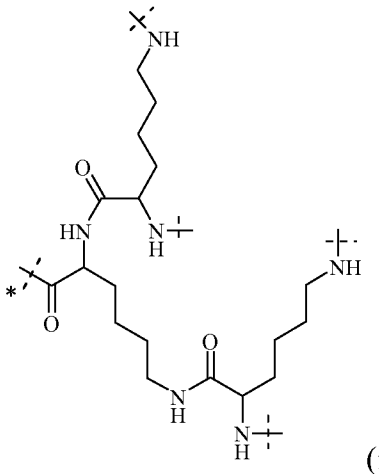
undecadiaminobutyric acid, dodecadiaminobutyric acid, tridecadiaminobutyric acid, tetradecadiaminobutyric acid, pentadecadiaminobutyric acid, hexadecadiaminobutyric acid, heptadecadiaminobutyric acid, octadecadiaminobutyric acid, nonadecadiaminobutyric acid, di(glutamic acid), tri(glutamic acid), tetra(glutamic acid), penta(glutamic acid), hexa(glutamic acid), hepta(glutamic acid), octa(glutamic acid), nona(glutamic acid), deca(glutamic acid), undeca(glutamic acid), dodeca(glutamic acid), trideca(glutamic acid), tetradeca(glutamic acid), pentadeca(glutamic acid), hexadeca(glutamic acid), heptadeca(glutamic acid), octadeca(glutamic acid), nonadeca(glutamic acid), di(aspartic acid), tri(aspartic acid), tetra(aspartic acid), penta(aspartic acid), hexa(aspartic acid), hepta(aspartic acid), octa(aspartic acid), nona(aspartic acid), deca(aspartic acid), undeca(aspartic acid), dodeca(aspartic acid), trideca(aspartic acid), tetradeca(aspartic acid), pentadeca(aspartic acid), hexadeca(aspartic acid), heptadeca(aspartic acid), octadeca(aspartic acid), nonadeca(aspartic acid), polyethyleneimines, and low-molecular weight PEI.

In a preferred embodiment, the hyperbranched moieties Hyp^1 and Hyp^2 of formula (V) are each independently selected from the group comprising, in particular consisting of, in bound form dilysine, trilysine, tetralysine, pentalysine, hexalysine, heptalysine, octalysine, nonalysine, decalysine, undecalysine, dodecalysine, tridecalysine, tetradecalysine, pentadecalysine, hexadecalysine, heptadecalysine, octadecalysine, nonadecalysine, triornithine, tetraornithine, pentaornithine, hexaornithine, heptaornithine, octaornithine, nonaornithine, decaornithine, undecaornithine, dodecaornithine, tridecaornithine, tetradecaornithine, pentadecaornithine, hexadecaornithine, heptadecaornithine, octadecaornithine, nonadecaornithine, tridiaminobutyric acid, tetradiaminobutyric acid, pentadiaminobutyric acid, hexadiaminobutyric acid, heptadiaminobutyric acid, octadiaminobutyric acid, nonadiaminobutyric acid, decadiaminobutyric acid, undecadiaminobutyric acid, dodecadiaminobutyric acid, tridecadiaminobutyric acid, tetradecadiaminobutyric acid, pentadecadiaminobutyric acid, hexadecadiaminobutyric acid, heptadecadiaminobutyric acid, octadecadiaminobutyric acid, nonadecadiaminobutyric acid, di(glutamic acid), tri(glutamic acid), tetra(glutamic acid), penta(glutamic acid), hexa(glutamic acid), hepta(glutamic acid), octa(glutamic acid), nona(glutamic acid), deca(glutamic acid), undeca(glutamic acid), dodeca(glutamic acid), trideca(glutamic acid), tetradeca(glutamic acid), pentadeca(glutamic acid), hexadeca(glutamic acid), heptadeca(glutamic acid), octadeca(glutamic acid), nonadeca(glutamic acid), di(aspartic acid), tri(aspartic acid), tetra(aspartic acid), penta(aspartic acid), hexa(aspartic acid), hepta(aspartic acid),

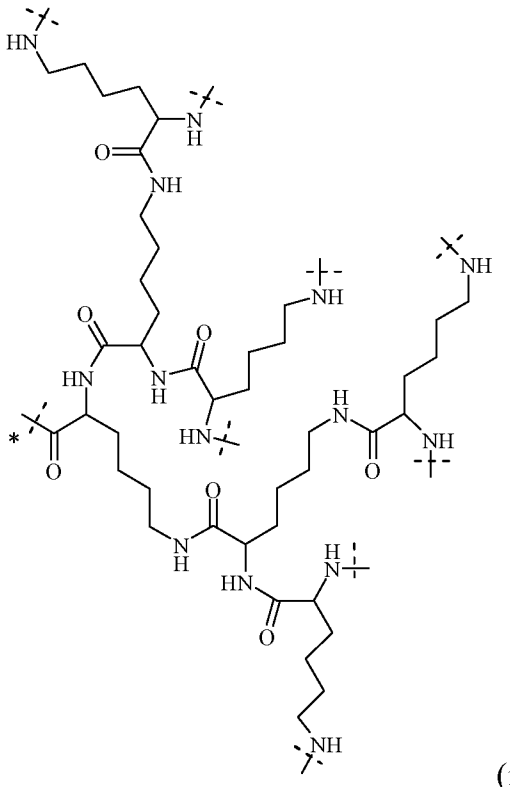
octa(aspartic acid), nona(aspartic acid), deca(aspartic acid), undeca(aspartic acid), dodeca(aspartic acid), trideca(aspartic acid), tetradeca(aspartic acid), pentadeca(aspartic acid), hexadeca(aspartic acid), heptadeca(aspartic acid), octadeca(aspartic acid), nonadeca(aspartic acid), polyethyleneimines, and low-molecular weight PEI.

More preferably, the hyperbranched moieties Hyp^1 and Hyp^2 of formula (V) are independently selected from the group comprising, more preferably consisting of, in bound form, trilysine, tetralysine, pentalysine, hexalysine, heptalysine, octalysine, nonalysine, decalysine, undecalysine, dodecalysine, tridecalysine, tetradecalysine, pentadecalysine, hexadecalysine, and heptadecalysine, even more preferably Hyp^1 and Hyp^2 are independently comprising, preferably consisting of, in bound form, trilysine, heptalysine or pentadecalysine.

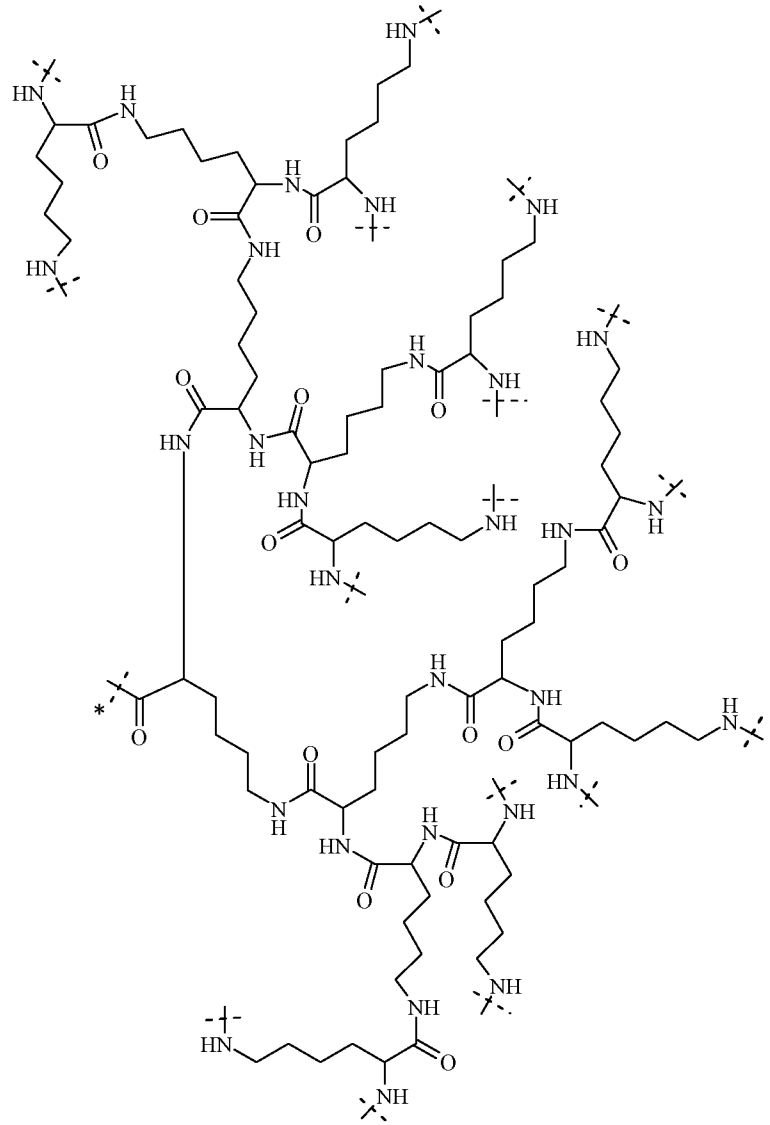
More preferably, Hyp^1 and Hyp^2 of formula (V) are independently selected from any one of the following structures:



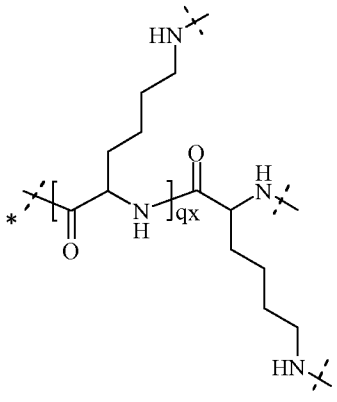
(i-x),



(ii-x),



(iii-x),



(iv-x),

wherein

dashed lines marked with an asterisk indicate attachment to POL^x of formula (V),

unmarked dashed lines indicate attachment to X^0, and

qx is an integer of from 0 to 15, preferably 3 to 7, and even more preferably 6.

Preferably, Hyp^1 and Hyp^2 of formula (V) are each a heptalysinyl group, in particular Hyp^1 and Hyp^2 of formula (V) each have the structure of formula (ii-x) above.

Preferably, Hyp^1 and Hyp^2 of formula (V) have the same structure.

Functional groups of Hyp^1 and Hyp^2 of formula (V) serve as attachment points for direct linkage of Hyp^1 and Hyp^2 of formula (V) to X^0. Remaining functional groups which are not connected to X^0 may, independently of each other, be capped with suitable capping reagents or may optionally be connected to at least one targeting moiety, in particular through permanent linkages

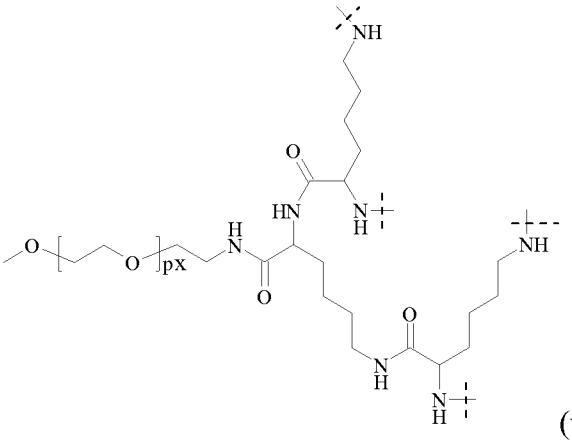
Therefore, in the water-soluble carrier-linked prodrugs of the present invention the hyperlinked moieties Hyp^1 and Hyp^2 of formula (V) are connected to POL^x of formula (V) and functional groups of Hyp^1 and Hyp^2 of formula (V) are connected to X^0 of formula (I), permanent linkages, targeting moieties and/or capping groups.

In a preferred embodiment, all functional groups of the hyperbranched moieties Hyp^1 and Hyp^2 of formula (V) are connected to X^0 of formula (I).

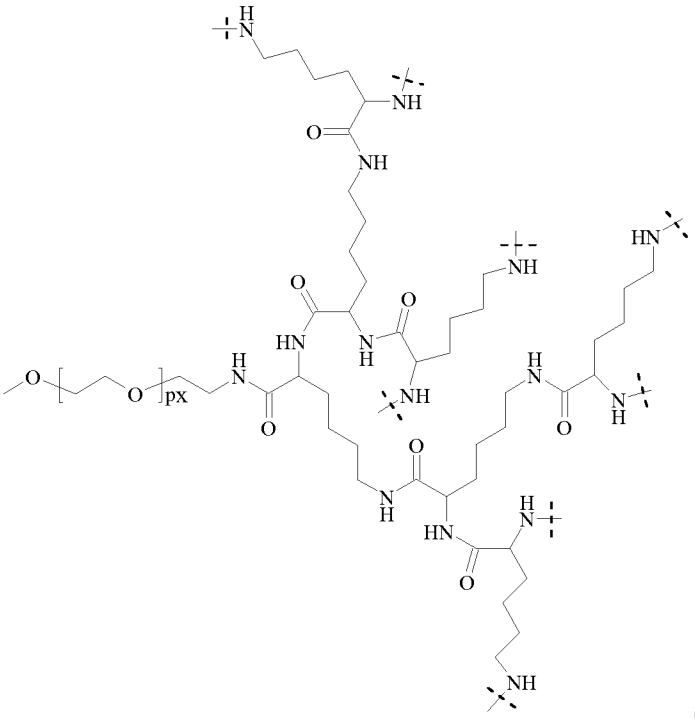
Preferably, the hyperbranched moieties Hyp^1 and Hyp^2 of formula (V) have independently a molecular weight in the range of from 0.1 kDa to 4 kDa, more preferably 0.4 kDa to 2 kDa. Preferably, the hyperbranched moieties Hyp^1 and Hyp^2 of formula (V) have each independently at least 3 branchings and are each independently conjugated to at least 4 X^0, permanent linkages, and/or capping groups and each independently have at most 63 branchings and are each independently at most conjugated to 64 X^0, permanent linkages, and/or capping groups. It is preferred that the hyperbranched moieties Hyp^1 and Hyp^2 of formula (V) have each independently at least 7 branchings and are each independently conjugated to at least 8 X^0, permanent linkages, and/or capping groups and have each independently at most 31 branchings and are each independently at most conjugated to 32 X^0, permanent linkages, and/or capping groups.

Preferably, the hyperbranched moieties Hyp^1 and Hyp^2 of formula (V) are each independently a hyperbranched polypeptide. Preferably, such hyperbranched polypeptide comprises lysine in bound form. Preferably, each hyperbranched moiety Hyp^1 and Hyp^2 of formula (V) independently have a molecular weight in the range of from 0.1 kDa to 4 kDa, in particular 0.4 kDa to 2 kDa.

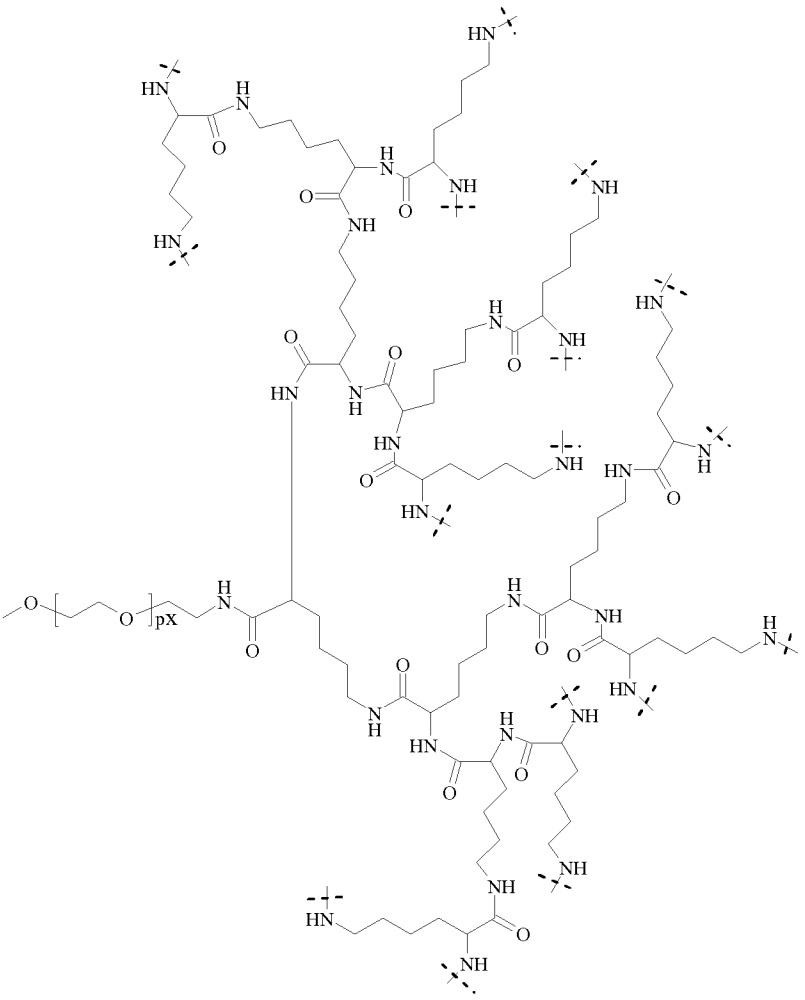
Preferably, mx is 0 and POL-Hyp^2- of formula (V) is selected from the following structures:



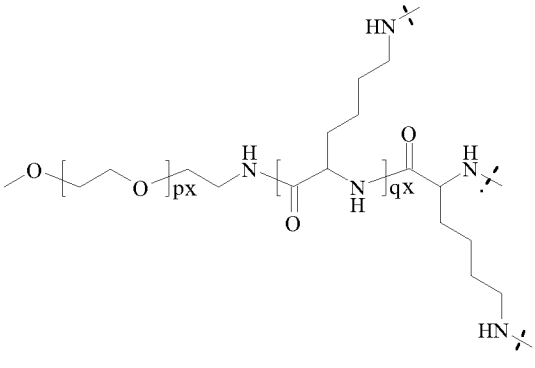
(v-x),



(vi-x),



(vii-x),



(viii-x),

wherein

dashed lines indicate attachment to X^0, provided that one of m1, m2 is 1 and wherein the carrier is covalently attached to T in case m1, m2 = 0,

px is an integer of from 5 to 2000, preferably 10 to 1000, in particular 100 to 1000, and

qx is an integer of from 0 to 15, preferably 3 to 7, more preferably, qx is 6.

In another preferred embodiment Z^1 of formula (I) has the structure of formula (VI):

$B-(A\text{-}Hyp^y)_n$ (VI),

wherein

B is a branching core,

each A is independently a poly(ethylene glycol)-based polymeric chain,

each Hyp^y is independently a branched moiety, and

n is an integer of from 3 to 32;

In a preferred embodiment, the branching core B of formula (VI) comprises, preferably consists of a moiety selected from:

- a polyalcohol comprising at least 2 hydroxyl groups (preferably further comprising a functional group, which is preferably an additional amino group or a carboxylic acid group, more preferably an additional carboxylic acid group),

  preferably B is selected from glycerol, pentaerythritol, dipentaerythritol, tripentaerythritol, hexaglycerine, sucrose, sorbitol, fructose, mannitol, glucose, cellulose, amyloses, starches, hydroxyalkyl starches, polyvinylalcohols, dextranes, and hyualuronans,

- or a polyamine comprising at least 2 amine groups (preferably further comprising a functional group, which is preferably an additional hydroxyl group or a carboxylic acid group, more preferably a carboxylic acid group),

  preferably selected from ornithine, diornithine, triornithine, tetraornithine, pentaornithine, hexaornithine, heptaornithine, octaornithine, nonaornithine,

decaornithine, undecaornithine, dodecaornithine, tridecaornithine, tetradecaornithine, pentadecaornithine, hexadecaornithine, heptadecaornithine, octadecaornithine, nonadecaornithine, diaminobutyric acid, di(diaminobutyric acid), tri(diaminobutyric acid), tetra(diaminobutyric acid), penta(diaminobutyric acid), hexa(diaminobutyric acid), hepta(diaminobutyric acid), octa(diaminobutyric acid), nona(diaminobutyric acid), deca(diaminobutyric acid), undeca(diaminobutyric acid), dodeca(diaminobutyric acid), trideca(diaminobutyric acid), tetradeca(diaminobutyric acid), pentadeca(diaminobutyric acid), hexadeca(diaminobutyric acid), heptadeca(diaminobutyric acid), octadeca(diaminobutyric acid), nonadeca(diaminobutyric acid), lysine, dilysine, trilysine, tetralysine, pentalysine, hexalysine, heptalysine, octalysine, nonalysine, decalysine, undecalysine, dodecalysine, tridecalysine, tetradecalysine, pentadecalysine, hexadecalysine, heptadecalysine, octadecalysine, nonadecalysine, oligolysines, polyethyleneimines, and polyvinylamines;

wherein the polyalcohol or polyamine is in bound form.

In a preferred embodiment, the branching core B of formula (VI) comprises, preferably consists of pentaerithritol.

Preferably, a poly(ethylene glycol)-based polymeric chain A connected to the branching core B of formula (VI) consists of a linear PEG chain, of which one terminus is connected to B of formula (VI) and the other terminus is connected to Hyp^y of formula (VI). It is understood that a PEG-based chain A of formula (VI) may optionally be terminated in case of a branched PEG chain and/or may optionally be interrupted in case of a branched or linear PEG chain by alkyl or aryl groups and may optionally be substituted with heteroatoms and/or functional groups.

Each sub-structure A-Hyp^y of formula (VI) extending from the branching core B of formula (VI) may be independently of each other the same or different sub-structures A-Hyp^y. In a preferred embodiment, the all sub-structures A-Hyp^y of formula (VI) are the same.

Each A and each Hyp^y of formula (VI) may be independently selected from the other moieties A and Hyp^y. Preferably, all sub-structures A-Hyp^y connected to B of formula (VI) have an identical structure.

Preferably, the PEG-based polymeric chains A of formula (VI) are connected to B through permanent linkages.

n of formula (VI) is an integer from 3 to 32. Preferably, n is an integer from 3 to 16, more preferably n is an integer from 4 to 8 and most preferably n is 4.

In a preferred embodiment n of formula (VI) is 4 and m is 2.

In one embodiment, a PEG-based polymeric chain A of formula (VI) is selected from a linear or branched PEG-based polymeric chain. Preferably, A is a linear PEG-based polymeric chain.

Preferably, each A of formula (VI) is independently selected from the formula

$$-X3-(CH_2)_{n1}-(OCH_2CH_2)_p-O-(CH_2)_{n2}-X2-,$$

wherein

n1 and n2 are independently selected from 1, 2, 3, and 4, preferably from 1, 2, and 3;

p is an integer in the range of from 5 to 2000, preferably p is an integer in the range of from 10 to 1000, more preferably p is an integer in the range of from 100 to 1000; and

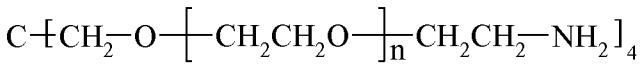
X3 and X2 are independently functional groups covalently linked to B or Hyp^y, respectively.

Preferably, a linkage between a moiety A and a moiety Hyp^y of formula (VI) is a permanent linkage, more preferably a permanent linkage comprising a linkage group comprising, in particular consisting of a group selected from amine groups, amide groups, carbamate groups,

thioether groups, ether groups, and most preferably a permanent linkage between a moiety A and a moiety Hyp^y of formula (VI) is an amide linkage.

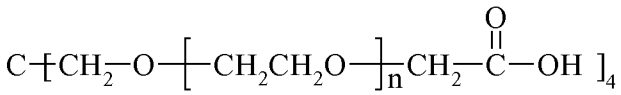
In a preferred embodiment, a sub-structure $B-(A)_n$ of formula (VI) is a multi-arm PEG derivative as, for instance, detailed in the products list of JenKem Technology, USA (accessed by download from http://jenkemusa.net/pegproducts2.aspx on March 8, 2011), such as a 4-arm-PEG derivative, in particular comprising a pentaerythritol core, an 8-arm-PEG derivative comprising a hexaglycerin core, and an 8-arm-PEG derivative comprising a tripentaerythritol core. Most preferred are sub-structures $B-(A)_n$ of formula (VI) comprising, in particular consisting of, moieties selected from:

a 4-arm PEG Amine comprising a pentaerythritol core:



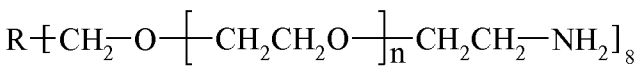
with n ranging from 400 to 2000;

a 4-arm PEG Carboxyl comprising a pentaerythritol core:



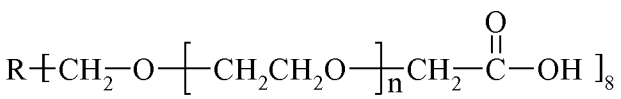
with n ranging from 400 to 2000;

an 8-arm PEG Amine comprising a hexaglycerin core:



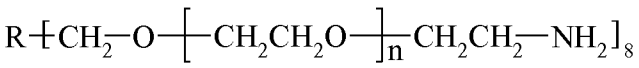
with n ranging from 400 to 2000 and
R = hexaglycerin core structure;

an 8-arm PEG Carboxyl comprising a hexaglycerin core:



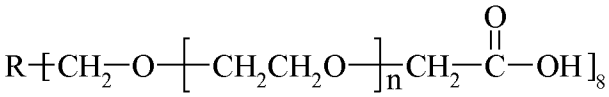
with n ranging from 400 to 2000 and
R = hexaglycerin core structure;

an 8-arm PEG Amine comprising a tripentaerythritol core:



with n ranging from 400 to 2000
and R = tripentaerythritol core structure;

and an 8-arm PEG Carboxyl comprising a tripentaerythritol core:



with n ranging from 400 to 2000 and
R = tripentaerythritol core structure;

each in bound form.

In a preferred embodiment, the molecular weight of a sub-structure B-$(A)_n$ of formula (VI) ranges from 1 kDa to 80 kDa, more preferably 1 kDa to 40 kDa and even more preferably 10 kDa to 40 kDa. It is understood that the terminal amine groups or carboxyl groups, respectively, are used for conjugation to a moiety Hyp^y of formula (VI).

Functional groups of a moiety Hyp^y of formula (VI) are connected to moieties X^0 of formula (I).

In a preferred embodiment, a moiety Hyp^y of formula (VI) is connected to a moiety X^0 of formula (I) through a functional group selected from amide groups, carbamate groups, ester groups, ether groups, amine groups, thioether groups. Preferably, a moiety Hyp^y of formula (VI) is connected to a moiety X^0 of formula (I) through amide groups, thioether groups and/or ether groups, even more preferably through amide groups.

Optionally, functional groups of a moiety Hyp^y of formula (VI) which are not connected to a moiety X^0 of formula (I) may be capped with suitable capping reagents and/or may optionally be connected to at least one targeting moiety, in particular through permanent linkages. Therefore, a moiety Hyp^y of formula (VI) may be connected to a moiety X^0 of formula (I), capping moieties and/or targeting moieties. Preferably, functional groups of a moiety Hyp^y of

formula (VI) are connected to a moiety X^0 of formula (I) and are not connected to capping moieties and/or targeting moieties. Targeting moieties, if present, may be conjugated to a moiety Hyp^y of formula (VI) either directly or indirectly through spacer moieties.

Examples of suitable capping moieties are linear, branched or cyclic C_{1-8} alkyl groups.

In one embodiment, each branched moiety Hyp^y of formula (VI) is directly or indirectly connected to at least two moieties X^0 of formula (I). More preferably, each branched moiety Hyp^y of formula (VI) is directly or indirectly connected to at least three moieties X^0 of formula (I). Most preferably, each branched moiety Hyp^y of formula (VI) is directly or indirectly connected to at least four moieties X^0 of formula (I).

The branched moiety Hyp^y of formula (VI) comprises, preferably consists of a moiety in bound form selected from:

- a polyalcohol in bound form comprising at least 2 hydroxyl groups (preferably further comprising a functional group, which is preferably an additional hydroxyl group or a carboxylic acid group, more preferably an additional hydroxyl group),

  preferably selected from glycerol, pentaerythritol, dipentaerythritol, tripentaerythritol, hexaglycerine, sucrose, sorbitol, fructose, mannitol, glucose, cellulose, amyloses, starches, hydroxyalkyl starches, polyvinylalcohols, dextranes, and hyualuronans,

- or a polyamine in bound form comprising at least 2 amine groups (preferably further comprising a functional group, which is preferably an additional amine group or a carboxylic acid group, more preferably a carboxylic acid group),

  preferably selected from ornithine, diornithine, triornithine, tetraornithine, pentaornithine, hexaornithine, heptaornithine, octaornithine, nonaornithine, decaornithine, undecaornithine, dodecaornithine, tridecaornithine, tetradecaornithine, pentadecaornithine, hexadecaornithine, heptadecaornithine, octadecaornithine, nonadecaornithine, diaminobutyric acid, di(diaminobutyric acid), tri(diaminobutyric acid), tetra(diaminobutyric acid), penta(diaminobutyric

acid), hexa(diaminobutyric acid), hepta(diaminobutyric acid), octa(diaminobutyric acid), nona(diaminobutyric acid), deca(diaminobutyric acid), undeca(diaminobutyric acid), dodeca(diaminobutyric acid), trideca(diaminobutyric acid), tetradeca(diaminobutyric acid), pentadeca(diaminobutyric acid), hexadeca(diaminobutyric acid), heptadeca(diaminobutyric acid), octadeca(diaminobutyric acid), nonadeca(diaminobutyric acid), lysine, dilysine, trilysine, tetralysine, pentalysine, hexalysine, heptalysine, octalysine, nonalysine, decalysine, undecalysine, dodecalysine, tridecalysine, tetradecalysine, pentadecalysine, hexadecalysine, heptadecalysine, octadecalysine, nonadecalysine, oligolysines, triornithine, tetraornithine, pentaornithine, hexaornithine, heptaornithine, octaornithine, nonaornithine, decaornithine, undecaornithine, dodecaornithine, tridecaornithine, tetradecaornithine, pentadecaornithine, hexadecaornithine, heptadecaornithine, octadecaornithine, nonadecaornithine, tridiaminobutyric acid, tetradiaminobutyric acid, pentadiaminobutyric acid, hexadiaminobutyric acid, heptadiaminobutyric acid, octadiaminobutyric acid, nonadiaminobutyric acid, decadiaminobutyric acid, undecadiaminobutyric acid, dodecadiaminobutyric acid, tridecadiaminobutyric acid, tetradecadiaminobutyric acid, pentadecadiaminobutyric acid, hexadecadiaminobutyric acid, heptadecadiaminobutyric acid, octadecadiaminobutyric acid, nonadecadiaminobutyric acid,

- or a polycarboxylate in bound form comprising at least 2 carboxylate groups (preferably further comprising a functional group, which is preferably an additional amino group or a carboxylic acid group, more preferably an additional carboxylic acid group),

  preferably selected from di(glutamic acid), tri(glutamic acid), tetra(glutamic acid), penta(glutamic acid), hexa(glutamic acid), hepta(glutamic acid), octa(glutamic acid), nona(glutamic acid), deca(glutamic acid), undeca(glutamic acid), dodeca(glutamic acid), trideca(glutamic acid), tetradeca(glutamic acid), pentadeca(glutamic acid), hexadeca(glutamic acid), heptadeca(glutamic acid), octadeca(glutamic acid), nonadeca(glutamic acid), di(aspartic acid), tri(aspartic acid), tetra(aspartic acid), penta(aspartic acid), hexa(aspartic acid), hepta(aspartic acid), octa(aspartic acid), nona(aspartic acid), deca(aspartic acid), undeca(aspartic

acid), dodeca(aspartic acid), trideca(aspartic acid), tetradeca(aspartic acid), pentadeca(aspartic acid), hexadeca(aspartic acid), heptadeca(aspartic acid), octadeca(aspartic acid), nonadeca(aspartic acid), polyethyleneimines, and polyvinylamines.

In a preferred embodiment, a moiety Hyp^y of formula (VI) is selected from the group comprising, in particular consisting of, in bound form, dilysine, trilysine, tetralysine, pentalysine, hexalysine, heptalysine, octalysine, nonalysine, decalysine, undecalysine, dodecalysine, tridecalysine, tetradecalysine, pentadecalysine, hexadecalysine, heptadecalysine, octadecalysine, nonadecalysine, triornithine, tetraornithine, pentaornithine, hexaornithine, heptaornithine, octaornithine, nonaornithine, decaornithine, undecaornithine, dodecaornithine, tridecaornithine, tetradecaornithine, pentadecaornithine, hexadecaornithine, heptadecaornithine, octadecaornithine, nonadecaornithine, tridiaminobutyric acid, tetradiaminobutyric acid, pentadiaminobutyric acid, hexadiaminobutyric acid, heptadiaminobutyric acid, octadiaminobutyric acid, nonadiaminobutyric acid, decadiaminobutyric acid, undecadiaminobutyric acid, dodecadiaminobutyric acid, tridecadiaminobutyric acid, tetradecadiaminobutyric acid, pentadecadiaminobutyric acid, hexadecadiaminobutyric acid, heptadecadiaminobutyric acid, octadecadiaminobutyric acid, nonadecadiaminobutyric acid, di(glutamic acid), tri(glutamic acid), tetra(glutamic acid), penta(glutamic acid), hexa(glutamic acid), hepta(glutamic acid), octa(glutamic acid), nona(glutamic acid), deca(glutamic acid), undeca(glutamic acid), dodeca(glutamic acid), trideca(glutamic acid), tetradeca(glutamic acid), pentadeca(glutamic acid), hexadeca(glutamic acid), heptadeca(glutamic acid), octadeca(glutamic acid), nonadeca(glutamic acid), di(aspartic acid), tri(aspartic acid), tetra(aspartic acid), penta(aspartic acid), hexa(aspartic acid), hepta(aspartic acid), octa(aspartic acid), nona(aspartic acid), deca(aspartic acid), undeca(aspartic acid), dodeca(aspartic acid), trideca(aspartic acid), tetradeca(aspartic acid), pentadeca(aspartic acid), hexadeca(aspartic acid), heptadeca(aspartic acid), octadeca(aspartic acid), nonadeca(aspartic acid), polyethyleneimines, and low-molecular weight PEI.

More preferably, a moiety Hyp^y of formula (VI) is selected from the group comprising, more preferably consisting of, in bound form, trilysine, tetralysine, pentalysine, hexalysine, heptalysine, octalysine, nonalysine, decalysine, undecalysine, dodecalysine, tridecalysine, tetradecalysine, pentadecalysine, hexadecalysine, and heptadecalysine, even more preferably

a moiety Hyp^y of formula (VI) comprises, preferably consists of, in bound form, trilysine, heptalysine or pentadecalysine.

In a preferred embodiment, a moiety Hyp^y of formula (VI) has a molecular weight in the range of from 0.1 kDa to 4 kDa, more preferably 0.2 kDa to 2 kDa.

In a further preferred embodiment, each branched moiety Hyp^y of formula (VI) has at least 1 branching and is conjugated to at least 2 moieties X^0 of formula (I) and has at most 63 branchings and is at most conjugated to 64 moieties X^0 of formula (I), more preferably each branched moiety Hyp^y of formula (VI) has at least 1 branching and is conjugated to at least 2 moieties X^0 of formula (I) and has at most 31 branchings and is at most conjugated to 32 moieties X^0 of formula (I).

In a preferred embodiment, Z^1 of formula (VI) comprises a quaternary carbon, in particular a quaternary carbon of a branching core moiety B, wherein B of formula (VI) is pentarythritol in bound form. Preferably, each A of formula (VI) is independently a PEG-based polymeric chain terminally attached to the quaternary carbon of pentaerythritol via the $-CH_2-O-$ moieties of the branching core moiety pentaerythritol by a permanent covalent linkage, and the distal end of the PEG-based polymeric chain is covalently bound to a branched moiety Hyp^y of formula (VI), each branched moiety Hyp^y of formula (VI) is conjugated to the moieties X^0 of formula (I).

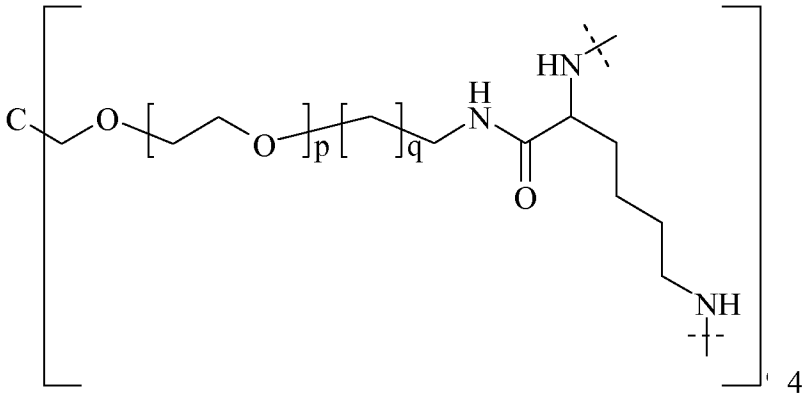
In one preferred embodiment, a branched moiety Hyp^y of formula (VI) comprises, preferably consists of branched polyamines comprising at least 2 amine groups. Preferably, the branched polyamine comprising at least 2 amine groups, comprises one or more lysine residues in bound form. Preferably, each branched moiety Hyp^y of formula (VI) has a molecular weight in the range of from 0.1 kDa to 4 kDa, particular 0.2 to 2 kDa. In a preferred embodiment, a moiety $B(A\text{-}Hyp^y)_n$ of formula (VI), wherein n = 4, consist of the same or different branched moieties Hyp^y and that each moiety Hyp^y can be chosen independently. In a preferred embodiment, all moieties Hyp_y of formula (VI) are the same.

In a preferred embodiment, a moiety Hyp^y of formula (VI) comprises, in particular consists of, between 1 and 32 lysines in bound form, preferably of 1, 3, 7 or 15 lysines in bound form,

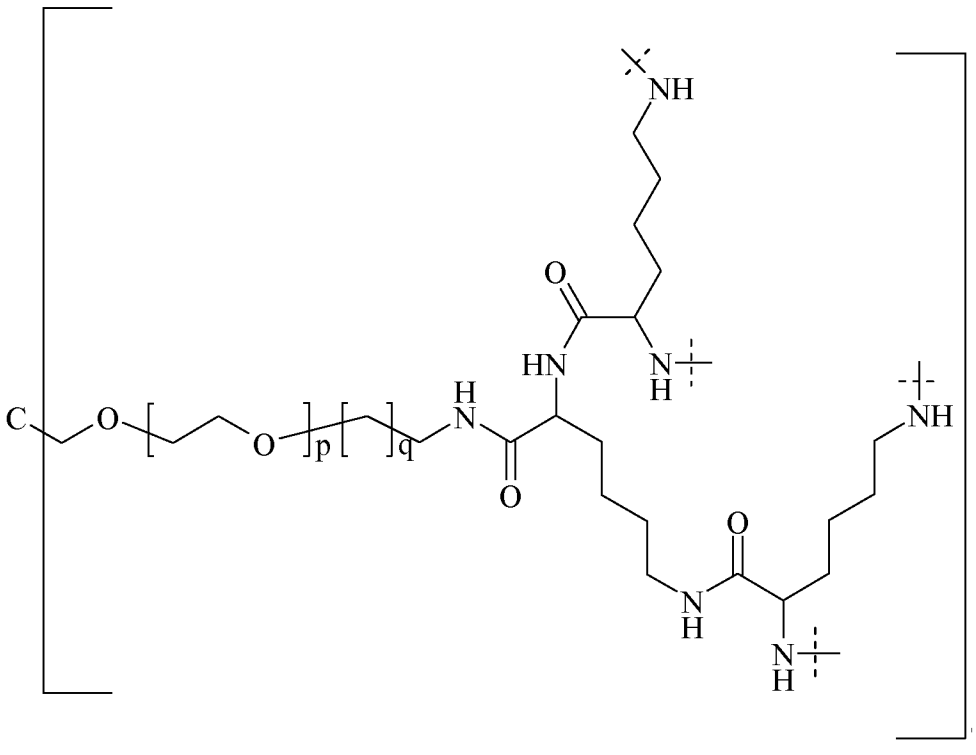
more preferably of 1, 3 or 7 lysines in bound form. Most preferably, Hyp^y of formula (VI) comprises, in particular consists of heptalysinyl.

Preferably, the moiety $B\text{-}(A\text{-}Hyp^y)_n$ of formula (VI), wherein n is preferably 4, has a molecular weight in the range of from 1 kDa to 160 kDa, more preferably 1 kDa to 80 kDa and even more preferably 10 kDa to 40 kDa.

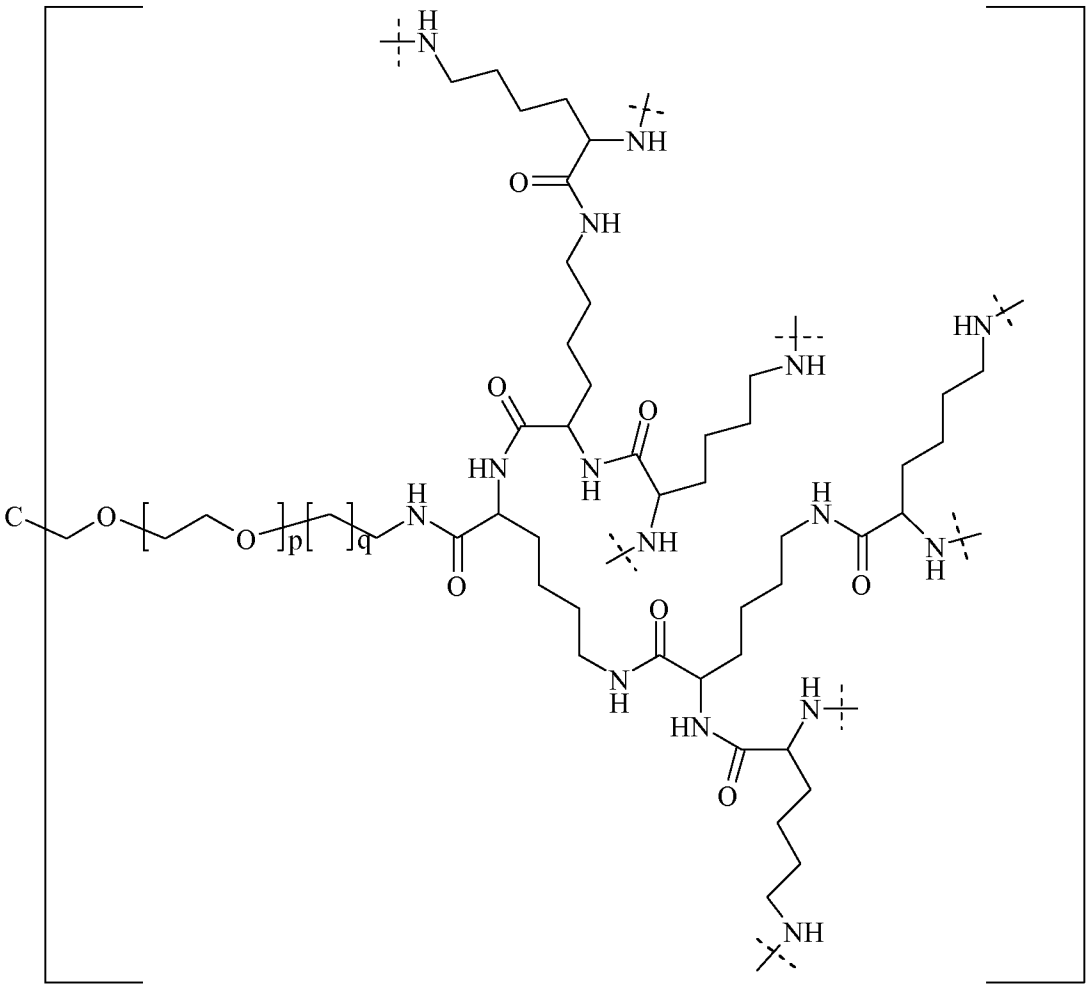
Preferred moieties $B\text{-}(A\text{-}Hyp^y)_4$ of formula (VI) are selected from structures (i-y) to (iii-y):



(i-y),



(ii-y),



4

(iii-y);

wherein

dashed lines indicate attachment to a moiety X^0 of formula (I), provided that one of m1, m2 is 1 and wherein the carrier is covalently attached to T in case m1, m2 = 0,,

p is an integer of from 5 to 2000, preferably from 10 to 1000, more preferably from 10 to 500, most preferably from 100 to 1000,

q is 1 or 2.

In a preferred embodiment, B of formula (VI) is pentaerythritol.

In another preferred embodiment, Z^1 of formula (I) is a protein carrier which comprises, in particular consists of an amino acid sequence of at least 100 amino acid residues.

In another preferred embodiment, the protein carrier Z^1 of formula (I) is in random coil conformation.

In another preferred embodiment, the protein carrier Z^1 of formula (I) comprises, in particular consists of alanine, serine and proline residues.

In the preferred embodiment, the protein carrier Z^1 of formula (I) comprises, in particular consists of an amino acid sequence of at least 100 amino acid residues, and

wherein the amino acid sequence of at least 100 amino acid residues is in random coil conformation, and,

wherein the amino acid sequence of at least 100 amino acid residues comprises alanine, serine and proline residues.

Preferably, the protein carrier a protein carrier Z^1 of formula (I) is composed of an amino acid sequence comprising at least about 100 amino acid residues, at least 100 amino acid residues, consisting of alanine, serine and proline residues which have a random coil conformation at physiological conditions. It is understood that the protein carrier Z^1 of formula (I) may transiently or temporarily not form a random coil, for example when present in a lyophilisate or dried composition.

In one embodiment the protein carrier Z^1 of formula (I) has a random coil conformation with an amino acid sequence of maximally about 3000 amino acid residues, preferably of maximally about 1500 amino acid residues, more preferably of maximally about 900 amino acid residues, even more preferably of maximally about 700 amino acid residues, particularly preferably of maximally about 600 amino acid residues. Thus, the amino acid sequence forming random coil conformation is maximally about 500 amino acid residues or of maximally about 450 amino acid residues in length.

Accordingly, the protein carrier Z^1 of formula (I), in particular the amino acid sequence forming random coil conformation of the protein carrier Z^1 of formula (I) is about 100 to about 3000 amino acid residues in length.

In particular embodiments said amino acid sequence forming random coil conformation of about 100 to 1000 amino acid residues is as characterized herein, i.e. comprising alanine, serine and proline as main or unique residues as defined below.

The protein carrier moiety Z^1 of formula (I) consists mainly of the three amino acid residues alanine, serine and proline, and wherein all three amino acids are present in a protein carrier moiety Z^1 of formula (I), whereby proline residues represent preferably about 4 % to about 40 % of the protein carrier Z^1 of formula (I). The alanine and serine residues preferably comprise the remaining at least 60 % to 96 % of the protein carrier Z^1 of formula (I). However, as will be detailed herein below said protein carrier Z^1 of formula (I) may also comprise further amino acids differing from alanine, serine, and proline, i.e. as minor constituents.

The term "minor constituent" as used herein means that maximally 10 % (i.e. maximally 10 of 100 amino acids) may be different from alanine, serine and proline, preferably maximally 8 % (i.e. maximally 8 of 100 amino acids) may be different than alanine, serine and proline, more preferably maximally 6 % (i.e. maximally 6 of 100 amino acids) may be different from alanine, serine and proline, even more preferably maximally 5 % (i.e. maximally 5 of 100 amino acids) may be different from alanine, serine and proline, particularly preferably maximally 4 % (i.e. maximally 4 of 100 amino acids) may be different from alanine, serine and proline, more particularly preferably maximally 3 % (i.e. maximally 3 of 100 amino acids) may be different from alanine, serine and proline, even more particularly preferably maximally 2 % (i.e. maximally 2 of 100 amino acids) may be different from alanine, serine and proline and most preferably maximally 1 % (i.e. maximally 1 of 100 of the amino acids) that encode the protein carrier Z^1 of formula (I) may be different from alanine, serine and proline. Said amino acids different from alanine, serine and proline may be selected from the group of natural or proteinogenic amino-acids consisting of Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Tyr, and Val. Minor constituents may also be selected from non-naturally occurring amino acids, such as, for example, hydroxyproline or selenomethionine or other modified natural amino acids.

The term "at least about 100/150/200/250/300/300/350 (etc) amino acid residues" is not limited to the concise number of amino acid residues but also comprises amino acid stretches that comprise an additional 10 % to 20 % or comprise 10 % to 20 % less residues. For

example "at least about 100 amino acid residues" may also comprise 80 to 100 and about 100 to 120 amino acid residues.

In one embodiment, the protein carrier Z^1 of formula (I) comprises a plurality of polymer cassettes wherein said polymer cassettes consist of Ala, Ser, and/or Pro, and wherein no more than 6 consecutive amino acid residues of the polymer cassettes, preferably of the protein carrier Z^1 of formula (I) are identical and wherein said proline residues constitute more than 4 % and less than 40 % of the amino acids of said protein carrier Z^1 of formula (I).

In one embodiment, the protein carrier moiety Z^1 of formula (I) comprises, preferably consists of a plurality of amino acid repeats,

wherein said repeats consist of Ala, Ser, and Pro residues,

and wherein no more than 6 consecutive amino acid residues of the carrier moiety Z^1 of formula (I) are identical.

In a preferred embodiment, said proline residues constitute more than 4 % and less than 40 % of the amino acids of the protein carrier moiety Z^1 of formula (I).

In a further preferred embodiment, the protein carrier moiety Z^1 of formula (I) comprises, in particular consists of an amino acid sequence of about 100 to 3000 amino acid residues forming random coil conformation.

The protein carrier Z^1 of formula (I) may comprise a plurality of identical polymer cassettes or a plurality of non-identical polymer cassettes. Non-limiting examples of polymer cassettes consisting of Ala, Ser and/or Pro residues are provided herein below; see SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13 and SEQ ID NO:14 or peptide fragments or multimers of these sequences. A polymer cassette may consist of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more amino acid residues, wherein each polymer cassette comprises (an) Ala, Ser, and/or Pro residue(s), preferably (an) Ala, Ser, and Pro residue(s).

In one embodiment, the polymer cassette does not comprise more than 100 amino acid residues. Preferably, a polymer cassette as defined herein comprises more than about 4 %, preferably more than about 5 %, even more preferably more than about 6%, particularly preferably more than about 8 %, more particularly preferably more than about 10 %, even more particularly preferably more than about 15 % and most preferably more than about 20 % proline residues. Such polymer cassette as defined herein preferably comprises less than about 40 % or less than about 35 % proline residues.

In one embodiment the protein carrier Z^1 of formula (I) is of formula (b):

$Ser_x[Ala_y\ Ser_z]_v$ (b),

which formula further comprises proline residues as defined herein and wherein

x is independently selected from integer 0 to 6,

each y is independently selected from integer ranging of from 1 to 6,

each z is independently selected from integer ranging of from 1 to 6.

v is any integer so that the protein carrier Z^1 consists of at least about 100 amino acid residues, and in particular of at least about 100 to about 3000 amino acid residues, preferably to about 2000 and more preferably to about 1000 amino acid residues.

In one embodiment, all y of formula (b) and z of formula (b) of the v $Ala_y\ Ser_z$ monomer moieties of formula (b) are identical. In another embodiment, the y of formula (b) and z of formula (b) of the v $Ala_y\ Ser_z$ monomer moieties of formula (b) are different.

In preferred embodiments, the protein carrier Z^1 of formula (I) comprises no more than 5 identical consecutive amino acid residues, more preferably no more than 4 identical consecutive amino acid residues and most preferably no more than 3 identical consecutive amino acid residues.

As already indicated herein above, the protein carrier Z^1 of formula (I) comprises proline residues, wherein said proline residues constitute more than about 4 %, preferably more than about 5 %, even more preferably more than about 6 %, particularly preferably more than about 8 %, more particularly preferably more than about 10 %, even more particularly preferably more than about 15 % and most preferably more than about 20 % of the amino acids constituting the protein carrier Z^1 of formula (I). Such proline residues may be introduced at any position in formula (b). Preferably, the proline residues may be present in one or more of the v Ala_y Ser_z monomers of formula (b), and they may be present at the same or at different positions.

In another preferred embodiment, the protein carrier Z^1 of formula (I) comprises more than about 4 % but less than about 50 %, preferably more than about 10 % but less than about 50 % and most preferably more than about 20 % but less than about 50 % alanine residues of the amino acids constituting the protein carrier Z^1 of formula (I).

In a further preferred embodiment, the protein carrier Z^1 of formula (I) comprises more than about 4 % and less than about 50 %, preferably more than about 10 % but less than about 50 % and most preferably more than about 20 % but less than about 50 % serine residues of the amino acids constituting the protein carrier Z^1 of formula (I).

Accordingly, the protein carrier Z^1 of formula (I) comprises about 35 % proline residues, about 50 % alanine residues and about 15 % serine residues of the amino acids constituting the protein carrier Z^1 of formula (I). Alternatively, the protein carrier Z^1 of formula (I) may comprise about 35 % proline residues, about 15 % alanine residues and about 50 % serine residues of the amino acids constituting the protein carrier Z^1 of formula (I).

Preferably, the protein carrier Z^1 of formula (I) is comprises one or more of the following alanine-serine polymer cassettes:

SEQ ID NO:1
AAAASSASSASSSSSAAASA

SEQ ID NO:2
AASAAASSAAASAAAASASS

SEQ ID NO:3
ASASASASASASSAASAASA

SEQ ID NO:4
SAASSSASSSSAASSASAAA

SEQ ID NO:5
SSSSAASAASAAAAASSSAS

SEQ ID NO:6
SSASSSAASSSASSSSASAA

SEQ ID NO:7
SASASASASASAASSASSAS

SEQ ID NO:8
ASSAAASAAAASSAASASSS

provided that the protein carrier Z^1 of formula (I) further comprises proline residues as described herein.

The multimers of these alanine-serine polymer cassettes may form random coil conformation in case the resulting amino acid sequence further comprises proline residues as defined herein above.

In a preferred embodiment, the protein carrier Z^1 of formula (I) comprises, preferably consists of one or more of the following polymer cassettes:

SEQ ID NO:9
ASPAAPAPASPAAPAPSAPA

SEQ ID NO:10
AAPASPAPAAPSAPAPAAPS

SEQ ID No:11
APSSPSPSAPSSPSPASPSS

SEQ ID NO:15
SAPSSPSPSAPSSPSPASPS

SEQ ID NO:15 corresponds to the herein provided SEQ ID No:11 in a circularly permuted form, wherein the last serine was removed and another serine was appended as starting amino acid. As a consequence, multimers of this modified sequence possess essentially the same internal repeating unit as multimers of the non-modified sequence, except for the very first and the very last residue. Accordingly, SEQ ID NO:15 may be considered as an example of a further polymer cassette for the protein carrier Z^1 of formula (I). It is clear for the person skilled in the art that also other polymer cassettes and (shorter) peptide fragments or circularly permuted versions of the herein provided amino acid polymers may be used as polymer cassettes for the protein carrier Z^1 of formula (I).

Yet, even further and illustrative amino acid polymers forming random coil conformation may comprise amino acid sequences that may be selected from the group consisting of:

SEQ ID NO:12
SSPSAPSPSSPASPSPSSPA,

SEQ ID NO:13
AASPAAPSAPPAAASPAAPSAPPA, and

SEQ ID NO:14
ASAAAPAAASAAASAPSAAA.

Therefore, preferred polymer cassettes for Z^1 of formula (I) are selected from the following sequences:

ASPAAPAPASPAAPAPSAPA (SEQ ID NO:9),
AAPASPAPAAPSAPAPAAPS (SEQ ID NO:10),
APSSPSPSAPSSPSPASPSS (SEQ ID NO:11),

SSPSAPSPSSPASPSPSSPA (SEQ ID NO:12),
AASPAAPSAPPAAASPAAPSAPPA (SEQ ID NO:13), and
ASAAAPAAASAAASAPSAAA (SEQ ID NO:14);

or circular permuted versions or (a) multimer(s) of these sequences as a whole or parts of these sequences.

In one embodiment, the protein carrier moiety Z^1 of formula (I) comprises at least one amino acid sequence selected from the group consisting of:

ASPAAPAPASPAAPAPSAPA (SEQ ID NO:9),
AAPASPAPAAPSAPAPAAPS (SEQ ID NO:10),
APSSPSPSAPSSPSPASPSS (SEQ ID NO:11),
SSPSAPSPSSPASPSPSSPA (SEQ ID NO:12),
AASPAAPSAPPAAASPAAPSAPPA (SEQ ID NO:13), and
ASAAAPAAASAAASAPSAAA (SEQ ID NO:14);

and circular permuted versions or (a) multimer(s) of these sequences as a whole or parts of these sequences.

Again, also (a) peptide fragment(s) or (a) multimer(s) or circularly permuted versions of these sequences and the sequences provided herein above may be employed as polymer cassettes for the protein carrier Z^1 of formula (I).

Accordingly, the exemplified polymer cassettes may also provide for individual peptide fragments which may be newly combined to form further polymer cassettes.

In accordance with the above, the protein carrier Z^1 of formula (I) may comprise a multimer consisting of either one of the amino acid sequences with SEQ ID NO:9, 10, 11, 12, 13 or 14 as disclosed herein above or may comprise a multimer consisting of more than one of amino acid sequences SEQ ID NO:9, 10, 11, 12, 13 and 14. Furthermore, it is envisaged that also peptide fragments or circularly permuted versions of these exemplified sequences may be used to build up further polymer cassettes of the protein carrier Z^1 of formula (I).

In another embodiment, the protein carrier Z^1 of formula (I) may comprise a multimer comprising, preferably consisting of a (circular) permutation of the amino acid sequence selected from the group consisting of SEQ ID NOs:9, 10, 11, 12, 13, 14, 15 and (a) multimers(s) of these (circular) permutated sequences.

In yet another embodiment, the protein carrier Z^1 of formula (I) may comprise, preferably consist of a multimer consisting of a peptide fragment/part of the amino acid sequence selected from the group consisting of SEQ ID NO: 9, 10, 12, 13, 14, 15 and (a) multimers(s) of these exemplified polymer cassettes.

Peptide fragments of these sequences to be employed for the generation of the protein carrier Z^1 of formula (I) may consist of at least 3, preferably of at least 4, more preferably of at least 5, even more preferably of at least 6, still more preferably of at least 8, particularly preferably of at least 10, more particularly preferably of at least 12, even more particularly preferably of at least 14, preferably of at least 6, still more preferably of at least 8, particularly preferably of at least 10, more particularly preferably of at least 12, even more particularly preferably of at least 14, even more particularly preferably of at least 16, and most preferably of at least 18 consecutive amino acids of the amino acid sequence selected from the group consisting of said SEQ ID NOs: 9, 10, 11, 12, 13 and 14.

For example, individual peptide fragments of the polymer cassettes may be combined to further individual polymer cassettes as long as the above-identified rules for the overall distribution and amount of alanine, serine and proline are respected. Again, these polymer cassettes may also comprise further amino acid residues, however only as minimal or minor constituents, i. e. maximally 10 %, preferably maximally 2 % of the individual polymer cassette. Said individual polymer cassettes consist of at least about 100 amino acid residues. Individual polymer cassettes may be combined in order to form longer random coil forming amino acid polymers, whereby a maximal length of the protein carrier Z^1 of formula (I) is about 3000 amino acids. A preferred minor constituent of the protein carrier Z^1 is lysine.

The pharmaceutical compositions comprising a prostacyclin compound may comprise one or more excipients. Excipients may be categorized as buffering agents, isotonicity modifiers, preservatives, stabilizers, anti-adsorption agents, oxidation protection agents, viscosifiers/viscosity enhancing agents, or other auxiliary agents. In some cases, these

ingredients may have dual or triple functions. The pharmaceutical compositions comprising a prostacyclin compound according to the present invention contain one or more excipients, selected from the groups consisting of:

(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. Buffering capacity may be adjusted to match the conditions most sensitive to pH stability

(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

(iii) Preservatives and/or antimicrobials: multidose parenteral preparations 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

(iv) Stabilizers: Stabilization is achieved by strengthening of the protein-stabilizing forces, by destabilization 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

(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 composition´s or composition´s container. Suitable surfactants are e.g., alkyl sulfates, such as ammonium lauryl sulfate and sodium lauryl sulfate; alkyl ether sulfates, such as sodium laureth sulfate and sodium myreth sulfate; sulfonates such as dioctyl sodium sulfosuccinates, perfluorooctanesulfonates, perfluorobutanesulfonates, alkyl benzene sulfonates; phosphates, such as alkyl aryl ether phosphates and alkyl ether phosphates; carboxylates, such as fatty acid salts (soaps) or sodium stearate, sodium lauroyl sarcosinate, perfluorononanoate, perfluorooctanoate; octenidine dihydrochloride; quaternary ammonium cations such as cetyl trimethylammonium bromide, cetyl trimethylammonium chloride, cetylpyridinium chloride, polyethoxylated tallow amine, benzalkonium chloride, benzethonium chloride, 5-bromo-5-nitor-1,3-dioxane, dimethyldioctadecylammonium chloride, dioctadecyldimethylammonium bromide; zwitterionics, such as 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, cocamidopropyl hydroxysultaine, amino acids, imino acids, cocamidopropyl betaine, lecithin; fatty alcohols, such as cetyl alcohol, stearyl alcohol, cetostearyl alcohol, oleyl alcohol; polyoxyethylene glycol alkyl ethers, such as octaethylene glycol monododecyl ether, pentaethylene glycol monododecyl ether; polyoxypropylene glycol alkyl ethers; glucoside alkyl ethers, such as decyl glucoside, lauryl glucoside, octyl glucoside; polyoxyethylene glycol octylphenol ethers such as Triton X-100; polyoxyethylene glycol alkylphenol ethers such as nonoxynol-9; glycerol alkyl esters such as glyceryl laurate; polyoxyethylene glycol sorbitan alkyl esters such as polysorbates; sorbitan alkyl esters; cocamide MEA and cocamide DEA; dodecyl dimethylamine oxide; block copolymers of polyethylene glycol and polypropylene glycol, such as poloxamers (Pluronic F-68), PEG dodecyl ether (Brij 35), polysorbate 20 and 80; other anti-absorption agents are dextran, polyethylene glycol, PEG-polyhistidine, BSA and HSA and gelatines. 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

(vi) Lyo- and/or cryoprotectants: During freeze- or spray drying, excipients may counteract the destabilizing effects caused by hydrogen bond breaking and water removal. For this purpose sugars and polyols may be used but corresponding positive effects have also been observed for surfactants, amino acids, non-aqueous solvents,

and other peptides. Trehalose is particulary efficient at reducing moisture-induced aggregation and also improves thermal stability potentially caused by exposure of protein hydrophobic groups to water. Mannitol and sucrose may also be used, either as sole lyo/cryoprotectant or in combination with each other where higher ratios of mannitol:sucrose are known to enhance physical stability of a lyophilized cake. Mannitol may also be combined with trehalose. Trehalose may also be combined with sorbitol or sorbitol used as the sole protectant. Starch or starch derivatives may also be used

(vii) Oxidation protection agents: antioxidants such as ascorbic acid, ectoine, methionine, glutathione, monothioglycerol, morin, polyethylenimine (PEI), propyl gallate, vitamin E, chelating agents such aus citric acid, EDTA, hexaphosphate, thioglycolic acid

(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 as but not limited to hyaluronic acid, a polysaccharide found in the intercellular space of connective tissue. A spreading agent such as but not limited to hyaluronidase temporarily decreases the viscosity of the extracellular matrix and promotes diffusion of injected drugs.

(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.

In a general embodiment the pharmaceutical composition comprising a prostacyclin compound of the present invention in either dry or liquid form may be provided as a single or multiple dose composition.

In one embodiment of the present invention, the liquid or dry pharmaceutical composition comprising a prostacyclin compound is provided as a single dose, meaning that the container in which it is supplied contains one pharmaceutical dose.

Alternatively, the liquid or dry pharmaceutical composition a prostacyclin compound is a multiple dose composition, meaning that the container in which it is supplied contains more

than one therapeutic dose, i.e., a multiple dose composition contains at least 2 doses. Such multiple dose composition comprising a prostacyclin compound can either be used for different patients in need thereof or can be used for one patient, wherein the remaining doses are stored after the application of the first dose until needed.

In another aspect of the present invention the pharmaceutical composition comprising a prostacyclin compound is comprised in a container. Suitable containers for liquid or dry compositions are, for example, syringes, vials, vials with stopper and seal, ampoules, and cartridges. In particular, the liquid or dry composition comprising a prostacyclin compound according to the present invention is provided in a syringe. If the pharmaceutical composition comprising a prostacyclin compound is a dry pharmaceutical composition the container preferably is a dual-chamber syringe. In such embodiment, said dry pharmaceutical composition is provided in a first chamber of the dual-chamber syringe and reconstitution solution is provided in the second chamber of the dual-chamber syringe.

Prior to applying the dry composition comprising a prostacyclin compound to a patient in need thereof, the dry composition is reconstituted. Reconstitution can take place in the container in which the dry composition comprising a prostacyclin compound is provided, such as in a vial, syringe, dual-chamber syringe, ampoule, and cartridge. Reconstitution is done by adding a predefined amount of reconstitution solution to the dry composition. Reconstitution solutions are sterile liquids, such as water or buffer, which may contain further additives, such as preservatives and/or antimicrobials, such as, for example, benzyl alcohol and cresol. Preferably, the reconstitution solution is sterile water. When a dry composition is reconstituted, it is referred to as a "reconstituted pharmaceutical composition" or "reconstituted composition".

An additional aspect of the present invention relates to the method of administration of a reconstituted or liquid pharmaceutical composition comprising a prostacyclin compound of the present invention. The pharmaceutical composition comprising a prostacyclin compound may be administered by methods of inhalation, injection or infusion, including intradermal, subcutaneous, intramuscular, intravenous, intraosseous, and intraperitoneal. Preferably, the pharmaceutical composition comprising a prostacyclin compound is administered subcutaneously.

The preferred method of administration for dry pharmaceutical compositions comprising a prostacyclin compound of the present invention is via inhalation.

In another embodiment, a reconstituted or liquid pharmaceutical composition comprising a prostacyclin compound of the present invention is administered via a first method of administration and a second reconstituted or liquid pharmaceutical composition comprising a prostacyclin compound of the present invention is administered via a second method of administration, either simultaneously or consecutively. Said first and second method of administration can be any combination of topical, enteral administration, parenteral administration, inhalation, injection, or infusion, intraarticular, intradermal, subcutaneous, intramuscular, intravenous, intraosseous, and intraperitoneal, intrathecal, intracapsular, intraorbital, intracardiac, transtracheal, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intraventricular or intrasternal administration.

A further aspect is a method of preparing a reconstituted composition comprising a therapeutically effective amount of a prostacyclin compound of the present invention, and optionally one or more pharmaceutically acceptable excipients, the method comprising the step of

- contacting the pharmaceutical composition comprising a prostacyclin compound of the present invention with a reconstitution solution.

Another aspect is a reconstituted pharmaceutical composition comprising a therapeutically effective amount of a prostacyclin compound of the present invention, and optionally one or more pharmaceutically acceptable excipients.

Another aspect of the present invention is the method of manufacturing a dry composition of a prostacyclin compound. In one embodiment, such dry composition is made by

(i) admixing the prostacyclin compound with one or more excipients,
(ii) transfering amounts equivalent to single or multiple doses into a suitable container,
(iii) drying the composition in said container, and
(iv) sealing the container.

Suitable containers are vials, syringes, dual-chamber syringes, ampoules, and cartridges.

Another aspect of the present invention is a kit of parts.

If the administration device is simply a hypodermic syringe then the kit may comprise the syringe, a needle and a container comprising the dry pharmaceutical composition comprising a prostacyclin compound for use with the syringe and a second container comprising the reconstitution solution.

If the pharmaceutical composition is a liquid composition then the kit may comprise the syringe, a needle and a container comprising the liquid composition comprising a prostacyclin compound for use with the syringe.

In more preferred embodiments, the injection device is other than a simple hypodermic syringe and so the separate container with reconstituted or liquid prostacyclin compound is adapted to engage with the injection device such that in use the liquid composition in the container is in fluid connection with the outlet of the injection device. Examples of administration devices include but are not limited to hypodermic syringes and pen injector devices. Particularly preferred injection devices are the pen injectors in which case the container is a cartridge, preferably a disposable cartridge. Optionally, the kit of parts comprises a safety device for the needle which can be used to cap or cover the needle after use to prevent injury.

A preferred kit of parts comprises a needle and a container containing the composition according to the present invention and optionally further containing a reconstitution solution, the container being adapted for use with the needle. Preferably, the container is a dual-chamber syringe.

In another aspect, the invention provides a cartridge comprising a pharmaceutical composition comprising a prostacyclin compound as hereinbefore described for use with a pen injector device. The cartridge may contain a single dose or multiplicity of doses of the prostacyclin compound.

Yet another aspect of the present invention is a pharmaceutical composition comprising a prostacyclin compound of the present invention or a pharmaceutical composition of the present invention for use as a medicament.

In a further embodiment, the present invention relates to a prostacyclin compound or pharmaceutical composition of the present invention for the preparation of a medicament, in particular for the preparation of a medicament with one or more of the features described herein.

In case a prostacyclin compound comprised in the pharmaceutical compositions according to the invention contain 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 pharmaceutical composition comprising a prostacyclin compound according to the invention which contains 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. Pharmaceutical compositions comprising a prostacyclin compound according to the invention which contain 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. If the pharmaceutical compositions comprising a prostacyclin compound according to the invention simultaneously contain acidic and basic groups in the molecule, 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 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 prodrugs 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.

Yet another aspect of the present invention is a method of treating, controlling, delaying or preventing in a mammalian patient, preferably in a human, in need of the treatment of one or more conditions comprising administering to said patient a therapeutically effective amount of a prostacyclin compound or a pharmaceutical composition comprising a prostacyclin compound of the present invention or a pharmaceutically acceptable salt thereof.
The invention further relates to a method for treating pulmonary hypertension, wherein the method comprises the step of subcutaneous or intramuscular administration, to a patient with pulmonary hypertension, of a pharmaceutical composition comprising

(a) a polymer carrier-linked prostacyclin prodrug, and optionally one or more pharmaceutically acceptable excipients, or
(b) a prostacyclin compound and at least one polymer, and optionally one or more pharmaceutically acceptable excipients,

wherein the pharmaceutical composition releases therapeutically effective amounts of free prostacyclin compound for a period of time of at least 12 hours.

In a preferred embodiment, the pharmaceutical composition formulation of (b) comprises from about 0.05 to about 10 weight percent of the prostacyclin compound, more preferably 0.01 to 5 weight percent of the prostacyclin compound and most preferably from 0.1 to about 2 weight percent of the prostacyclin compound and from about 0.5 to about 20 weight percent total polymer content, preferably from about 1 to about 10 weight percent total polymer content and most preferably from about 1 to about 7 weight percent total polymer.

In a preferred embodiment, the pharmaceutical compositions of the present invention are sustained release formulations of a prostacyclin.

The prostacyclin compounds, and pharmaceutical compositions comprising a prostacyclin compound, and pharmaceutical compositions comprising a prostacyclin compound for use of the present invention can be used for the treatment and/or prevention of, for example, pulmonary hypertension, ischemic diseases (e.g. peripheral vascular disease including peripheral arterial disease, Raynaud's phenomenon including Raynaud's disease and

Raynaud's syndrome, scleroderma including systemic sclerosis, myocardial ischemia, ischemic stroke, renal insufficiency), ischemic ulcers including digital ulcers, heart failure (including congestive heart failure), portopulmonary hypertension, interstitial lung disease, idiopathic pulmonary fibrosis, conditions requiring anticoagulation (e.g., post MI, post cardiac surgery), thrombotic microangiopathy, extracorporeal circulation, central retinal vein occlusion, atherosclerosis, inflammatory diseases (e.g., COPD, psoriasis), hypertension (e.g., preeclampsia), reproduction and parturition, cancer or other conditions of unregulated cell growth, cell/tissue preservation and other emerging therapeutic areas where prostacyclin treatment appears to have a beneficial role.

Therefore, the present invention relates to a pharmaceutical composition of the present invention for use in the treatment or prevention of pulmonary hypertension, ischemic diseases, preferably peripheral vascular disease including peripheral arterial disease, Raynaud's phenomenon including Raynaud's disease and Raynaud's syndrome, scleroderma including systemic sclerosis, myocardial ischemia, ischemic stroke, renal insufficiency, ischemic ulcers including digital ulcers, heart failure, in particular congestive heart failure, portopulmonary hypertension, interstitial lung disease, idiopathic pulmonary fibrosis, conditions requiring anticoagulation, in particular post MI, post cardiac surgery, thrombotic microangiopathy, extracorporeal circulation, central retinal vein occlusion, atherosclerosis, inflammatory diseases, in particular COPD, psoriasis, hypertension, in particular preeclampsia, reproduction and parturition, cancer or other conditions of unregulated cell growth, cell/tissue preservation and other emerging therapeutic areas where prostacyclin treatment appears to have a beneficial role.

Fig. 1 shows the treprostinil release in buffer and buffered rat plasma at different time points expressed as % treprostinil release compared to total treprostinil content (see Example 29).

Fig. 2 shows a prolonged duration of circulation of treprostinil conjugate **25** for more than two weeks in monkeys after subcutaneous injection (see Example 30).

Fig. 3 shows a single dose iv injection of compound **25** and subsequent plasma analysis for total treprostinil and carrier content (see Example 30).

Fig. 4 shows a single dose iv injection of compound **25** and subsequent plasma analysis for free treprostinil (see Example 32).

Fig. 5 shows a single dose subcutaneous injection of compound **25** and subsequent plasma analysis for free treprostinil (see Example 32).

Materials, Methods and Analytics:

Product purification

Normal phase purification was performed on a Biotage "Isolera one" purification system Biotage AB, Sweden. Biotage KP-Sil silica cartridges. Gradients of Heptane/Ethylacetate or Dichloromethane/Methanol were used. Products were detected and collected at 254 and 280nm.

For preparative RP-HPLC, a Waters 600 controller and a 2487 Dual Absorbance Detector was used equipped with a 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. Gradients of eluents A (water containing 0.05 % TFA v/v or 0.01 % HCl v/v) and B (acetonitrile containing 0.05 % TFA v/v or 0.01 % HCl v/v) were used.

HPLC fractions containing product were pooled and lyophilized if not stated otherwise.

Automated Flash Chromatography

Automated Flash Chromatography was performed on a Biotage "Isolera one" purification system Biotage AB, Sweden, using Biotage KP-Sil silica cartridges. Products were detected and collected at 254 and 280nm.

LC/MS Analytics

Analytical RP-HPLC/ESI-MS was performed on waters equipment consisting of a 2695 sample manager, a 2487 Dual Absorbance Detector, and a ZQ 4000 ESI instrument equipped with a 5 µm Reprosil Pur 300 Å ODS-3 column (75 x 1.5 mm) (Dr. Maisch, Ammerbuch, Germany; flow rate: 350 µl/min, typical gradient: 10-90% MeCN in water, 0.05 % TFA over 5 min) or on a Waters Acquity UPLC with an Acquity PDA detector coupled to a Thermo LTQ Orbitrap Discovery high resolution/high accuracy mass spectrometer equipped with a Waters ACQUITY UPLC BEH300 C18 RP column (2.1 x 50 mm, 300 Å, 1.7 µm, flow: 0.25 mL/min; solvent A: UP-H_20 + 0.04% TFA, solvent B: UP-Acetonitrile + 0.05 % TFA.

RP-UPLC/ESI-MS was performed on Waters/Thermo equipment consisting of a Waters Acquity UPLC with an Acquity PDA detector coupled to a Thermo LTQ Orbitrap Discovery high resolution/high accuracy mass spectrometer equipped with a ACQUITY UPLC®

BEH300 C18 RP column (Waters Corporation, 2.1 x 50 mm, 300 Å, 1.7 µm, Flow: 0.25 mL/min; solvent A: UP-H_20 + 0.04% TFA, solvent B: UP-MeCN + 0.05 % TFA.
Typical gradients for determination of released treprostinil from TransCon 5 kDa PEG linker treprostinil are: 0.25 mL flow rate, gradient: 30-50 % B over 10 min

RP-HPLC purification:

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 0.05 % TFA v/v or 0.01 % HCl v/v) and solvent system B (acetonitrile containing 0.05 % TFA v/v or 0.01 % HCl v/v)

Typical gradients for purification procedures are:

- 6 mL/min flow rate, solvent A: H_20 + 0.05% TFA, solvent B: MeCN + 0.05 % TFA, typical gradient: 1-95 % B over 14 min
- 6 mL/min flow rate, solvent A: H_20 + 0.05% TFA, solvent B: MeCN + 0.05 % TFA, typical gradient: 10-80 % B over 14 min
- 40 mL/min flow rate, solvent A: H_20 + 0.05% TFA, solvent B: MeCN + 0.05 % TFA, typical gradient: 40-95 % B over 14 min

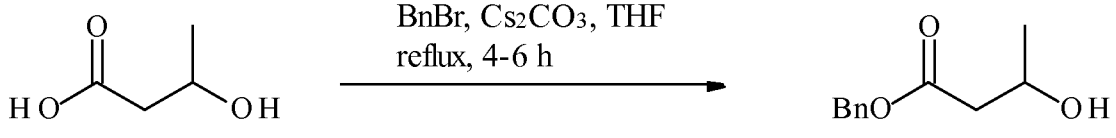
HPLC fractions containing product were pooled and lyophilized if not stated otherwise.

Chemicals and drug substances:

Treprostinil acid was purchased from Shanghai Techwell Biopharmaceutical Co., Ltd., Shanghai, Peoples Republic of China or Chirogate International Inc. Yangmei, Taiwan. 6-(*S*-Tritylmercapto)hexanoic acid was purchased from Polypeptide, Strasbourg, France. *Cis*-cyclohexanedicarboxylic anhydride was purchased from Alfa Aesar GmbH & Co KG, Karlsruhe, Germany. 2-Chlorotrityl chloride resin (1%, Novabiochem® DVB) was obtained from Merck Biosciences GmbH, Germany. 6-(*S*-Tritylsulfanyl)-hexaneamine was synthesized according to WO-A 2009/133137. PEGs used in this work were acquired from NOF Europe N.V., Grobbendonk, Belgium. All other chemicals were purchased from Sigma Aldrich GmbH, Taufkirchen, Germany. Water and acetonitrile for analytical RP-HPLC were purchased from Biosolve B.V. and TFA from Thermo scientific.

Example 1:

Benzyl protection of 3-hydroxybutanoic acid **1**:



1 **2**

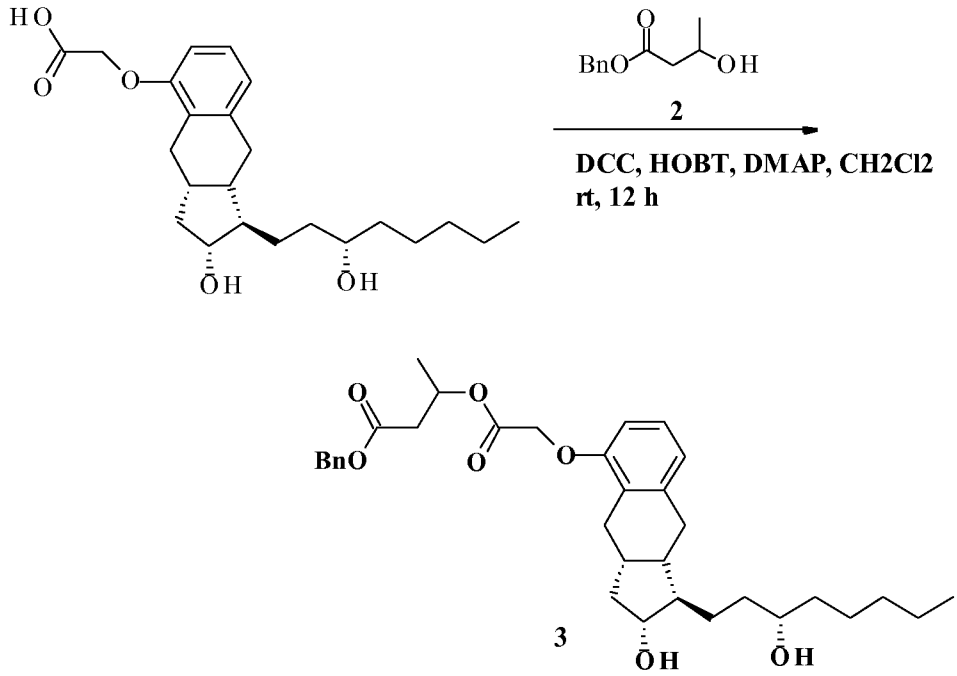
3-Hydroxybutanoic acid **1** (434 mg, 4.17 mmol) was dissolved in THF (10 mL) and BnBr (700 µL, 5.89 mmol) and Cs_2CO_3 (2.5 g, 7.67 mmol) were added. The reaction mixture was refluxed in a sealed tube for 4-6 hours. After cooling down to room temperature the reaction mixture was filtrated and the residue was washed several times with EtOAc. The organic solvents were removed and the product was purified by automated flash chromatography on silica in one portion (SNAP 25 g cartridge, flow 30 ml/min, solvent A: DCM, solvent B: MeOH; gradient: 0 – 5 % B over 19 CV) to remove starting material and obtain desired benzyl protected 3-hydroxybutanoic acid **2** as yellow oil.

Yield: 361 mg (45 %)

MS: m/z 217.1 = $[M+Na]^+$ (MW+Na calculated = 217.2).

Example 2:

Coupling reaction of benzylated 3-hydroxybutanoic acid **2** with treprostinil:



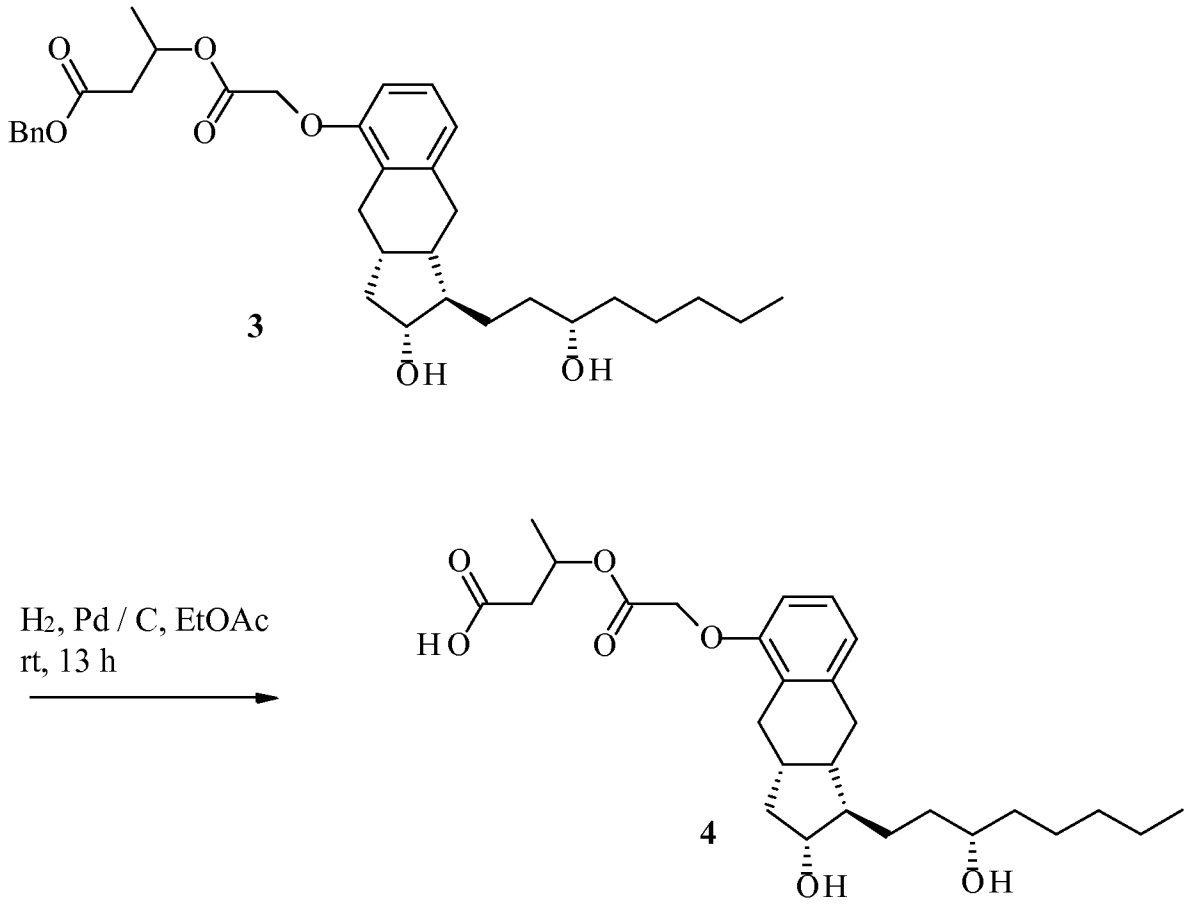
Treprostinil acid (10.5 mg, 0.0268 mmol) was dissolved in DCM (4.5 mL) and DCC (9.4 mg, 0.0455 mmol), HOBT (7.5 mg, 0.0489 mmol) and DMAP (7.5 mg, 0.0613 mmol) were added to the solution. Then benzylated 3-hydroxybutanoic acid **2** (15 mg, 0.0772 mmol) was dissolved in DCM (0.5 mL) and added to the reaction mixture. The mixture was stirred at RT until the consumption was complete (analytical RP-HPLC). Volatile solvents were removed *in vacuo* and the residue was purified over a small silica column (3 ml silica, DCM / MeOH (100 : 0) – DCM / MeOH (95 : 5) to obtain the desired linker treprostinil **3** as yellow oil.

Yield: 8 mg (50 %)

MS: m/z 589.3 = $[M+Na]^+$ (MW+Na calculated = 589.7)

Example 3:

Hydrogenation reaction of benzylester **3**:



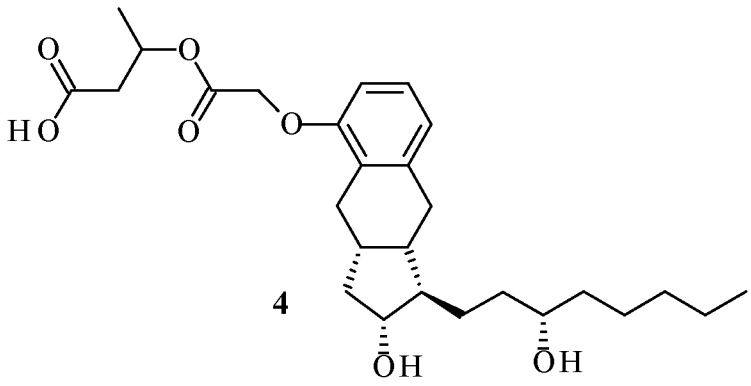
Benzylester **3** (13 mg, 0.0229 mmol) was dissolved in EtOAc (4 Å MS, 2 mL) and 5 % palladium on charcoal (5 % Pd, 15 mg) was added. Hydrogen was bubbled through the solution for 30 min. The reaction mixture was stirred further 12.5 h under hydrogen atmosphere until the consumption was complete (analytical RP-HPLC). The mixture was filtered over celite and washed several times with EtOAc. Organic solvents were removed *in vacuo* and the residue was purified using RP-HPLC (solvent A: H_2O with 0.05 % TFA, solvent B: MeCN with 0.05 % TFA, gradient: 1-95 % B over 20 min, flow: 6 mL/min). The product containing fractions were pooled and lyophilized to obtain **4** as white solid.

Yield: 1.9 mg (29 %).

MS: m/z 499.3 = $[M+Na]^+$ (MW+Na calculated = 499.6).

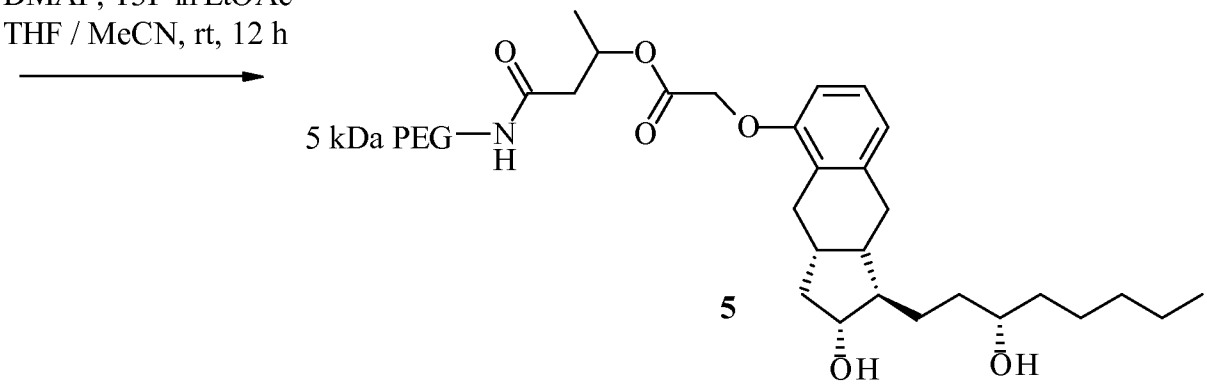
Example 4:

Coupling reaction of linear PEG 5 kDa amine with linker treprostinil **4**:



4

5 kDa PEG-amine, Et_3N
DMAP, T3P in EtOAc
THF / MeCN, rt, 12 h



5

Linker treprostinil **4** (1.9 mg, 3.98 µmol) and linear PEG 5 kDa amine (86 mg, 17.2 µmol) were dissolved in THF/MeCN (4 Å MS; 1.5 mL : 0.5 mL) and Et_3N (40 µL), a catalytic amount of DMAP and T3P (50% in EtOAc, 50 µL, 73.2 µmol) were successively added. The reaction mixture was allowed to stir at rt for 12 h. The reaction mixture was diluted with 20 µL H_2O and volatile solvents were removed *in vacuo*. The residue was purified using RP-HPLC (solvent A: H_2O with 0.05 % TFA, solvent B: MeCN with 0.05 % TFA, gradient: 10-80 % B over 20 min, flow: 6 mL/min). The product containing fractions were pooled and lyophilized to obtain TransCon PEG linker treprostinil **5** as white solid.

Yield: 12.5 mg (58 %).

MS: m/z 1378.6 = $[M+4H]^{4+}$ (calculated = 1378.9) for one representative peak in the polymer distribution.

Example 5:

Treprostinil release kinetics of TransCon PEG linker treprostinil **5**:

TransCon PEG linker treprostinil **5** (0.5 – 1.5 mg) was incubated in pH 7.4 hydrolysis buffer (60 mM sodium phosphate, 3 mM EDTA, 0.05 % Tween-20, 1 mL) at 37 °C and aliquots were analyzed by UPLC at various time points for released treprostinil.

Half life determination of hydrolysis kinetics of TransCon PEG linker treprostinil **5**:

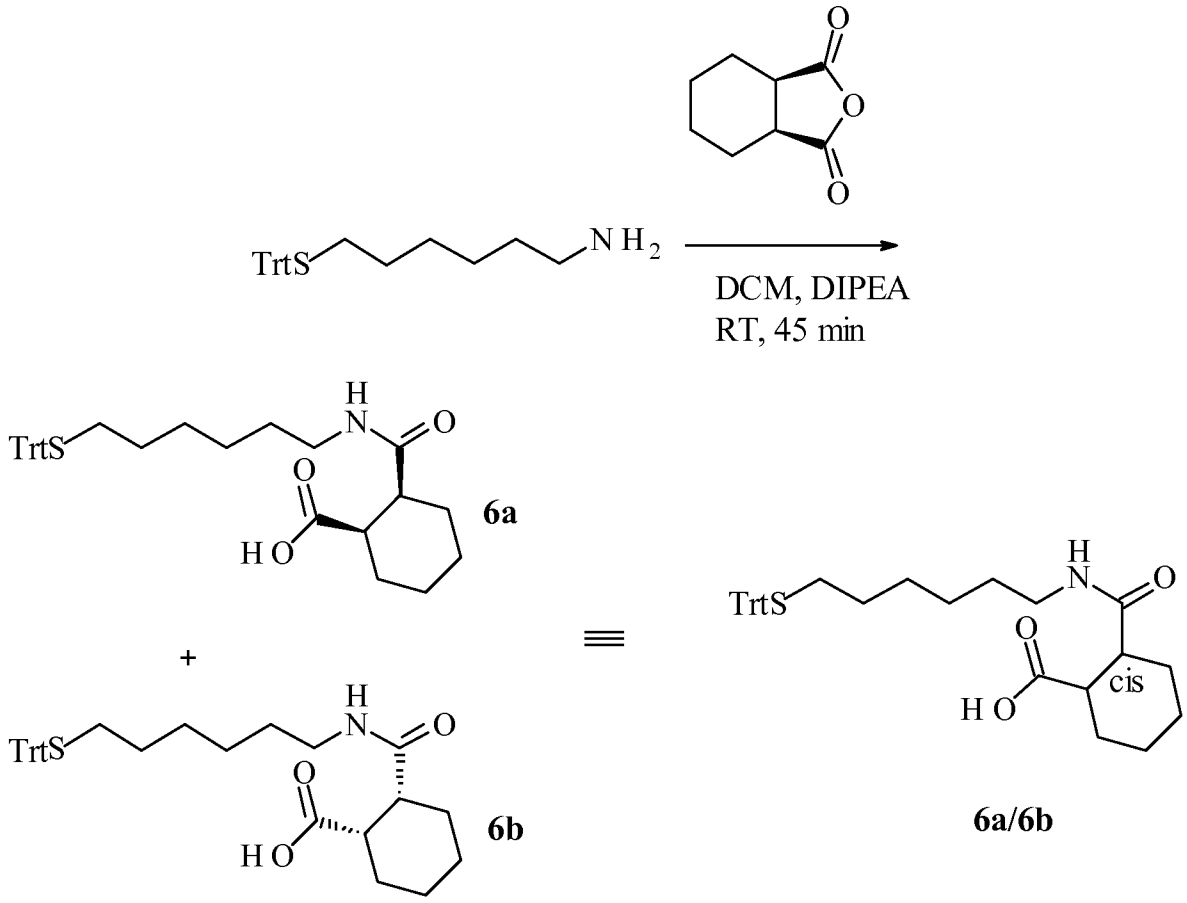
The percentage of released treprostinil after incubation at pH 7.4 and 37°C for a given time period was determined by integrating the corresponding peaks (released material versus conjugate) in the RP-UPLC chromatogram. The data as shown in table 1 were subsequently plotted against time. By using a first order kinetics fit a half life of 4.20 d for the treprostinil release from **5** was obtained.

Table 1:

entry	Incubation time [d]	released treprostinil [%]
1	0.000	2
2	0.83	5
3	1.11	18
4	1.81	27
5	2.06	29
6	5.13	59
7	6.10	64
8	8.80	77
9	11.90	86

Example 6:

Synthesis of intermediates **6a**/**6b**:



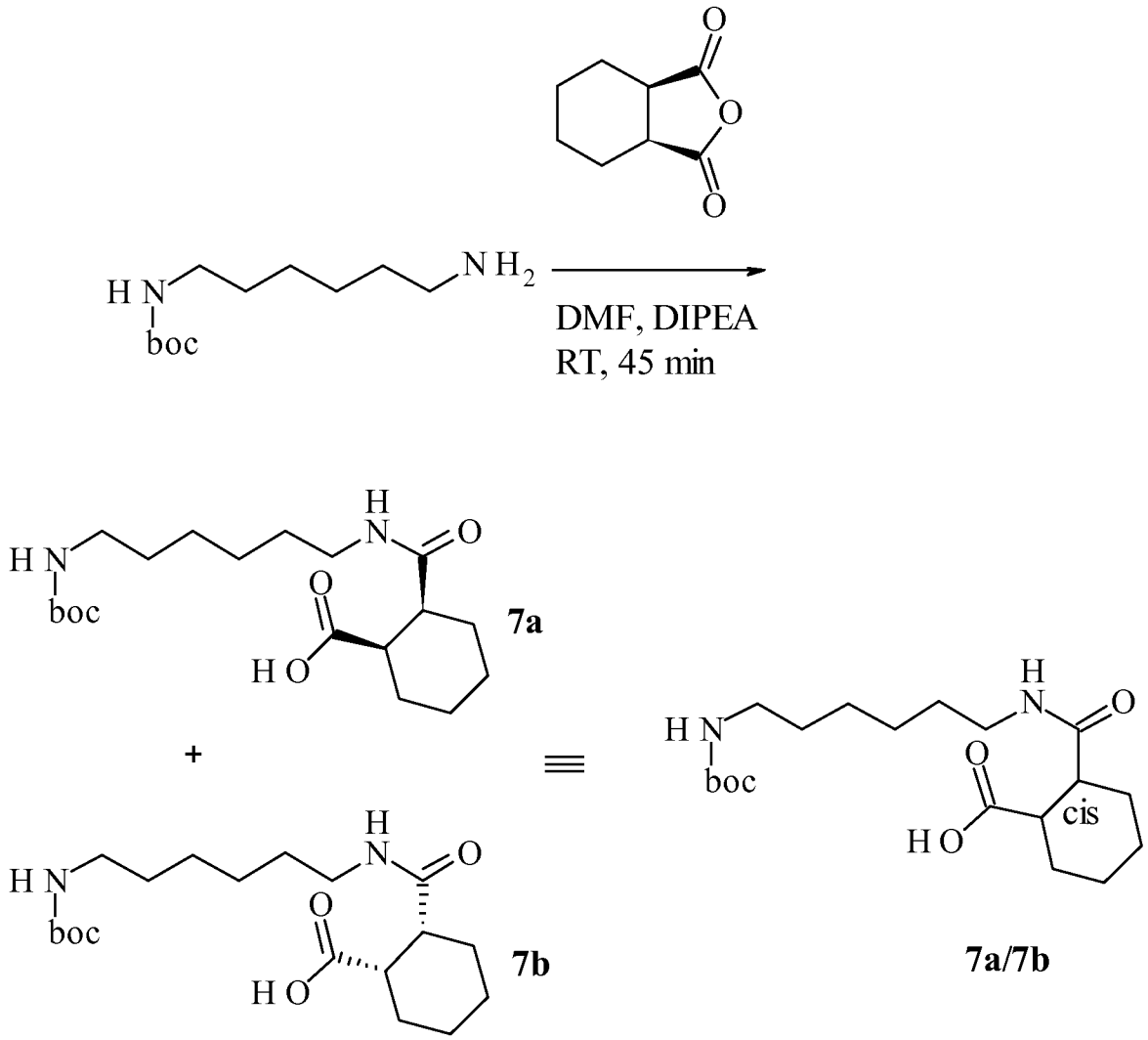
6-(*S*-Tritylsulfanyl)-hexaneamine (for synthesis see WO-A 2009/133137) (507 mg, 1.35 mmol) was dissolved in DCM (4 ml) and cis-1,2-cyclohexanedicarboxylic anhydride (251 mg, 1.63 mmol) was added to the reaction mixture at RT. DIPEA (0.70 mL, 4.06 mmol) was added and the mixture was stirred at RT until complete consumption of 6-(*S*-Tritylsulfanyl)-hexaneamine (LC/MS). Volatile solvents were removed *in vacuo*, the residue was dissolved in H_2O/MeCN (6:1, 18 mL) and the product was purified by RP-HPLC (solvent A: H_2O with 0.05 % TFA, solvent B: MeCN with 0.05 % TFA, gradient: 40-95 % B over 16 min, flow: 40 ml/min). The pooled fractions were neutralized with sat. $NaHCO_3$ soln. (pH approx. 6) and the organic solvents were removed *in vacuo*. The remaining aqueous phase was extracted twice with DCM. Combined organic layers were dried with $MgSO_4$ and the solvent was removed *in vacuo* obtaining **6a**/**6b** as a racemic mixture.

Yield: 580 mg (81 %).

MS: m/z 552.23 = $[M+Na]^+$ (MW+Na calculated = 552.62 g/mol).

Example 7:

Synthesis of intermediates **7a**/**7b**:



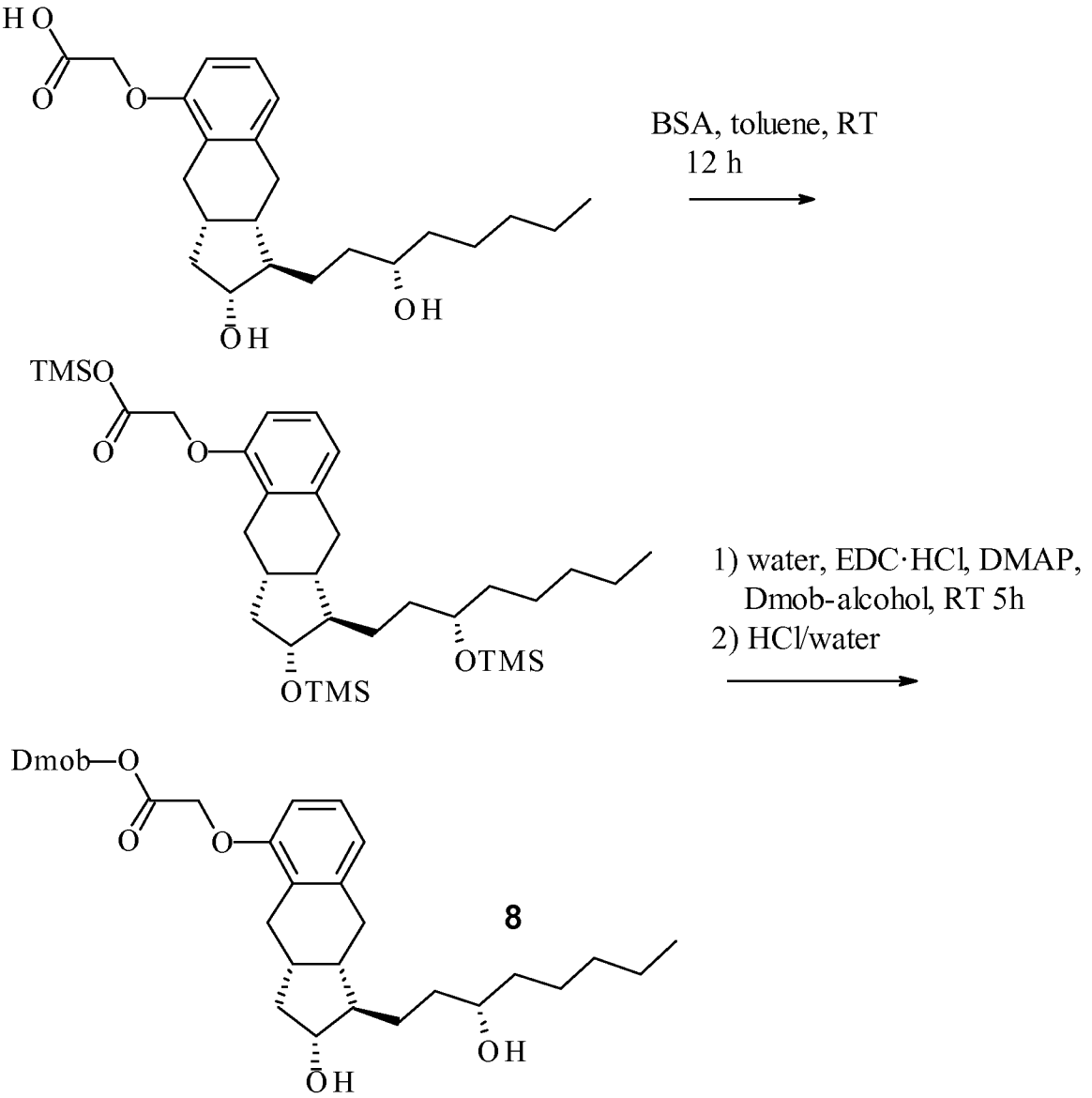
N-Boc-1,6-hexanediamine (270 mg, 1.25 mmol) was dissolved in DMF (2 ml) and cis-1,2-cyclohexanedicarboxylic anhydride (231 mg, 1.50 mmol) was added to the reaction mixture at RT. DIPEA (0.65 mL, 3.76 mmol) was added and the mixture was stirred at RT until consumption of *N*-Boc-1,6-hexanediamine (LC/MS). The reaction mixture was diluted with H_2O/MeCN (9:1) and the product was purified by RP-HPLC (solvent A: H_2O with 0.05 % TFA, solvent B: MeCN with 0.05 % TFA, gradient: 10-80 % B over 16 min, flow: 40 ml/min). The pooled fractions were neutralized with sat. $NaHCO_3$ soln. (pH approx. 6) and the organic solvents were removed *in vacuo*. The remaining aqueous phase was extracted several times with DCM. The organic layers were dried with $MgSO_4$ and the solvent was removed *in vacuo* obtaining 7**a**/7**b** as a racemic mixture.

Yield: 410 mg (88 %).

MS: m/z 371.39 = $[M+H]^+$ (MW+H calculated = 371.27 g/mol).

Example 8:

Synthesis of Dmob protected treprostinil **8**:



Treprostinil (61 mg, 0.156 mmol) was dissolved in toluene (dry, molecular sieve, 2.5 ml) and silylation reagent BSA (0.6 mL, 0.245 mmol) was added. The reaction mixture was stirred for 12 h at RT. Volatile solvents were removed *in vacuo* and the TMS protected treprostinil was used without further purification.

TMS protected treprostinil was dissolved in DCM (2.5 mL) and H_2O (60 µL). DMAP (76 mg, 0.624 mmol), EDC·HCl (119 mg, 0.624 mmol) and Dmob-alcohol (105 mg, 0.624 mmol) dissolved in DCM (1 ml) were added. The reaction mixture was stirred at RT until reaction was complete (LC/MS). The solution was diluted with DCM and quenched by addition of 0.1

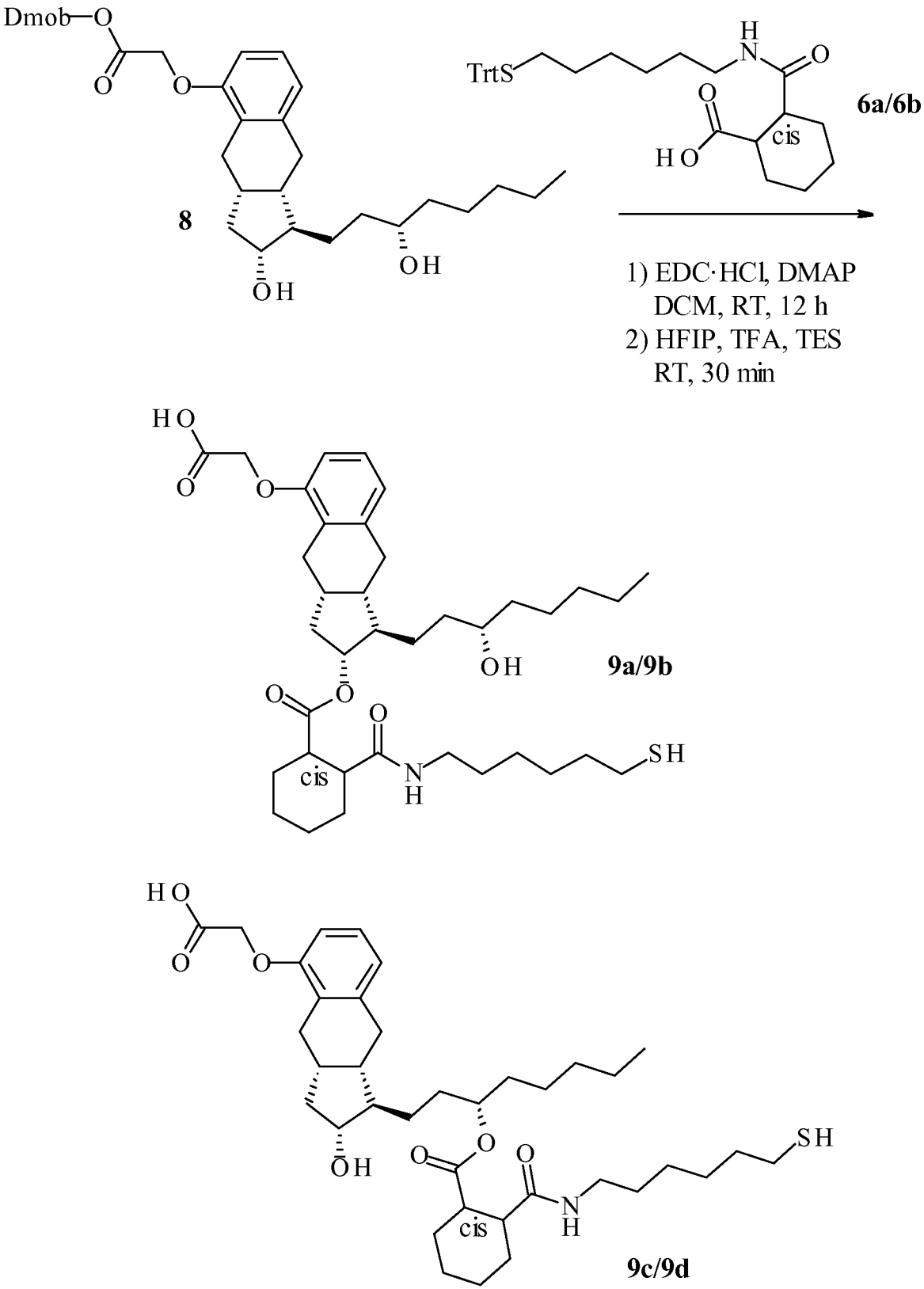
N HCl solution saturated with NaCl. The aqueous phase was extracted several times with DCM. Combined organic layers were dried with $MgSO_4$ and the solvent was removed *in vacuo* obtaining crude product **8**. Crude product was purified using RP-HPLC (solvent A: H_2O with 0.05 % TFA, solvent B: MeCN with 0.05 % TFA, gradient: 35-85 % B over 16 min, flow: 40 ml/min). Combined HPLC fractions were adjusted to a pH of approx. 7 by adding sat. $NaHCO_3$ soln. MeCN was removed *in vacuo*. The remaining H_2O layer was extracted several times with DCM and the combined organic phases were dried with $MgSO_4$, filtered and the solvent was removed *in vacuo* obtaining product **8** as colorless solid.

Yield: 69 mg (82 %).

MS: m/z 563.20 g/mol= $[M+Na]^+$ (MW+Na calculated = 563.67 g/mol).

Example 9:

Synthesis of treprostinil linker thiol



Carboxylic acid **6a/6b** (147 mg, 0.277 mmol), EDC·HCl (53 mg, 0.277 mmol) and DMAP (34 mg, 0.277 mmol) were dissolved in 0.5 mL DCM. Dmob protected treprostinil **8** (43 mg, 0.08 mmol) was dissolved in 0.5 mL DCM and added to the reaction mixture. The mixture

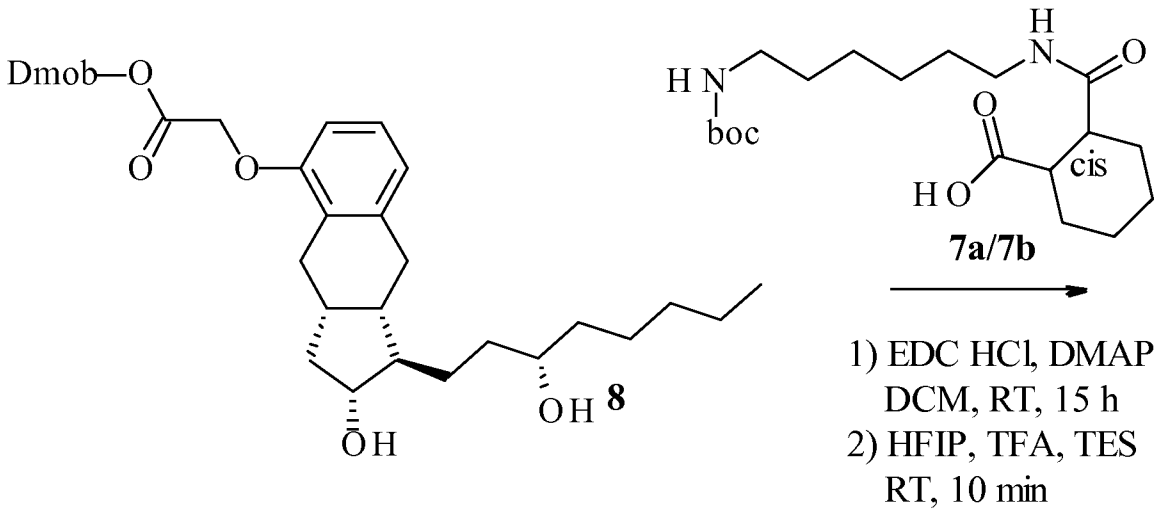
was stirred at RT until consumption of **8** was complete (over night, LC/MS). Volatile solvents were removed *in vacuo*. The residue was dissolved in HFIP (2 mL), TFA (100 µL) and TES (50 µL) and stirred for 30 min at RT (LC/MS). Volatiles were removed *in vacuo*. The residue was dissolved in H_2O/MeCN (9/1, 0.05 % TFA, 2 mL) and the mixture of four possible isomers was purified by RP-HPLC (solvent A: H_2O with 0.05 % TFA, solvent B: MeCN with 0.05 % TFA, gradient: 60-85 % B over 16 min, flow: 6 mL/min). Product isomers eluted as three separable peaks. Fractions containing the peak with the shortest elution time (compound "**9x**") were pooled and used in the PEGylation step without further processing. Structural assignment of **9x** to the possible isomers **9a**, **9b**, **9c** or **9d** was not performed in this experiment. Yield of **9x** was determined by using Ellman test.

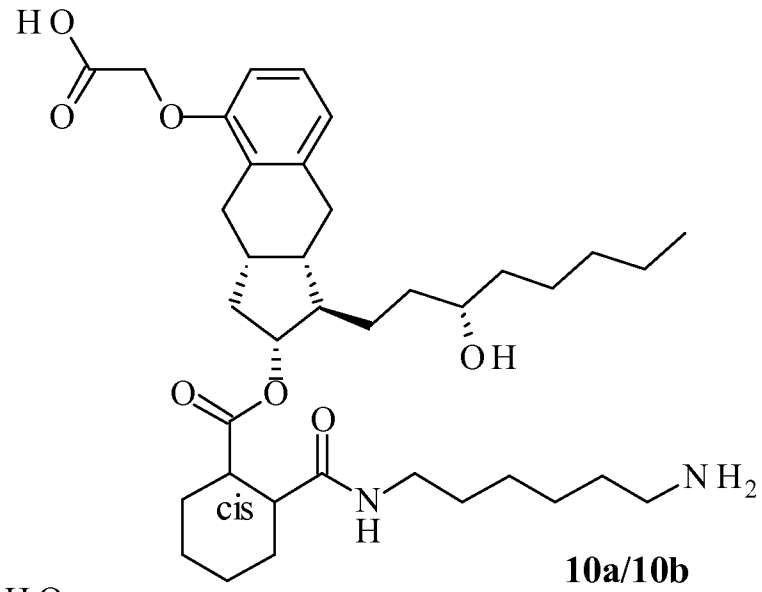
Yield: 8.1 mg (26 %)

MS: m/z 682.21 g/mol = $[M+Na]^+$ (MW+Na calculated = 682.40 g/mol).

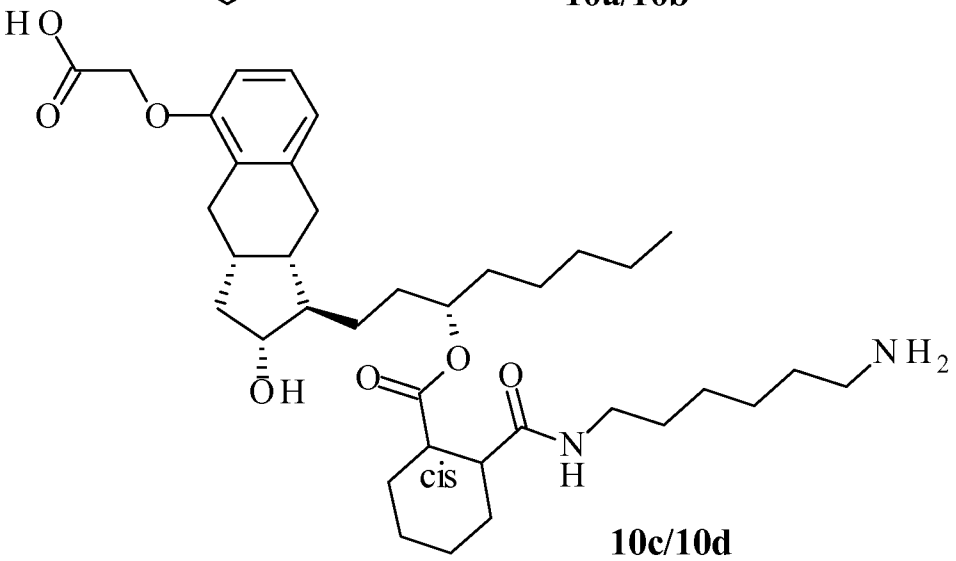
Example 10:

Synthesis of treprostinil linker amine





10a/10b



10c/10d

Carboxylic acid **7a**/**7b** (50 mg, 0.134 mmol), EDC HCl (26 mg, 0.134 mmol) and DMAP (16 mg, 0.134 mmol) were dissolved in DCM (0.3 mL). Dmob protected treprostinil **8** (36 mg, 0.066 mmol) was dissolved in DCM (0.5 mL) and added to the reaction mixture. The mixture was stirred at RT until the consumption was complete (LC/MS). Volatile solvents were removed *in vacuo*. The residue was dissolved in H_2O/MeCN (9/1, 0.05 % TFA, 2 mL) and the mono coupling products (treprostinil coupled to one **7a**/**7b** molecule) were separated from the double coupling products (treprostinil coupled to two **7a**/**7b** molecules) by RP-HPLC: Thermo Fisher Hypersil Gold PFP column, 150 x 10 mm, solvent A: H_2O with 0.05 % TFA, solvent B: MeCN with 0.05 % TFA, gradient: 35-55 % B over 16 min, flow: 6 mL/min. HPLC fractions containing mono coupling products were pooled and lyophilized. Lyophilizate was dissolved in HFIP (0.9 mL), DCM (0.1 mL), TFA (100 µL) and TES (20 µL) and stirred for 10 min at RT. Volatiles were removed *in vacuo*, the residue was dissolved

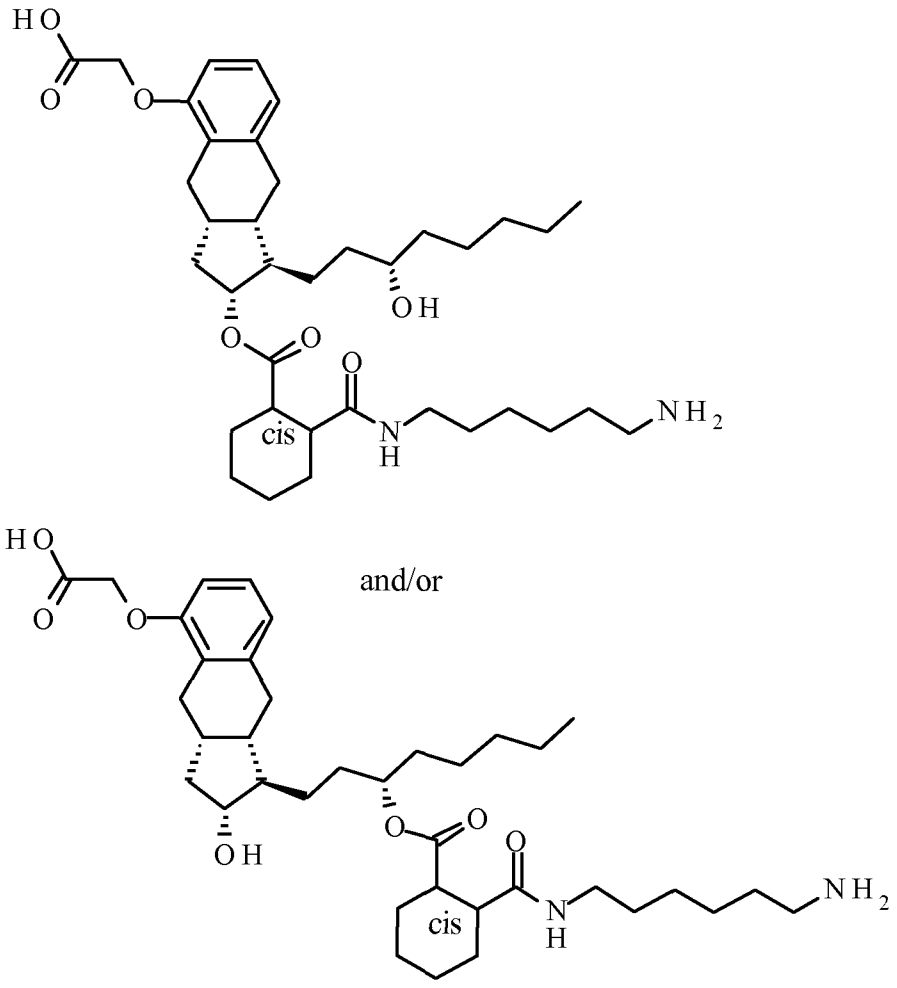
in H_2O/MeCN (9/1, 0.05 % TFA, 2 mL) and the and the mixture of four possible isomers was purified by RP-HPLC (solvent A: H_2O with 0.05 % TFA, solvent B: MeCN with 0.05 % TFA, gradient: 35-55 % B over 16 min, flow: 6 mL/min). Product isomers eluted as three separable peaks. Fractions containing the peak with the shortest elution time (compound "**10x**") were pooled and used in the PEGylation step without further processing. Structural assignment of the **10x** to the possible isomers **10a**, **10b**, **10c** or **10d** was not performed in this experiment. Yield of **10x** was estimated by HPLC by using a treprostinil calibration curve (280 nm).

Yield: 3.0 mg

MS: m/z 643.28 g/mol = $[M+Na]^+$ (MW+Na calculated = 643.45 g/mol).

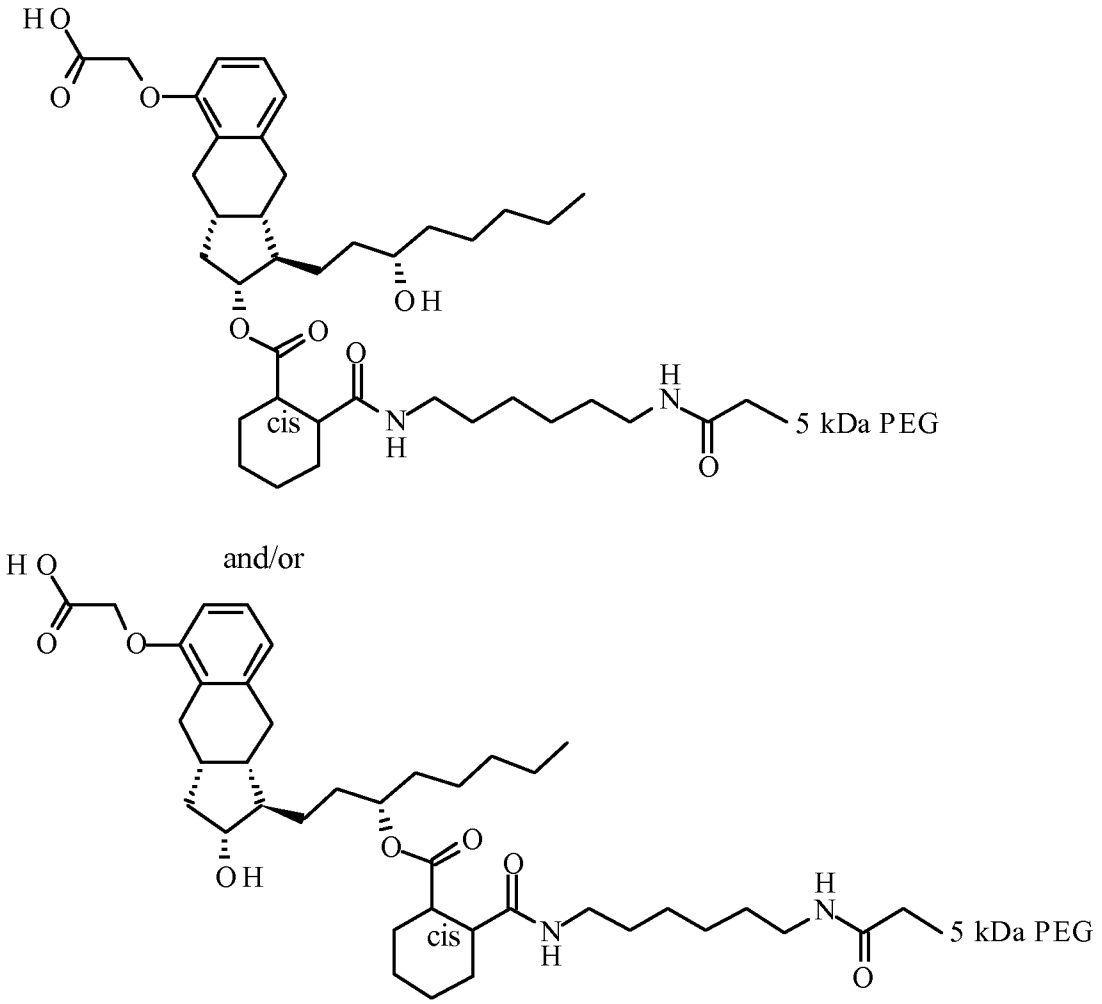
Example 11:

PEGylation reaction of treprostinil linker amine with linear PEG 5 kDa NHS



10x

linear 5 kDa NHS-PEG
MeCN, H_2O
pH 7.4 phosphate buffer
RT, 1 h



11

To treprostinil linker amine **10x** (0.6 mg, 1 µmol in solution, MeCN/H_2O, 0.05 % TFA, 5 mL) linear PEG 5 kDa NHS (23 mg, 4.6 µmol) was added. The solution was neutralized by addition of 0.5 M pH 7.4 buffer (0.5 M phosphate, 0.6 mL). H_2O (1 mL) was added for obtaining a clear solution, and reaction mixture was incubated at RT for 1 h. Then the reaction mixture was purified by RP-HPLC (solvent A: H_2O with 0.01 % HCl, solvent B: MeCN with 0.01 % HCl, gradient: 10-70 % B over 16 min, flow: 6 mL/min) to obtain after lyophilization TransCon linear 5 kDa PEG treprostinil **11**.

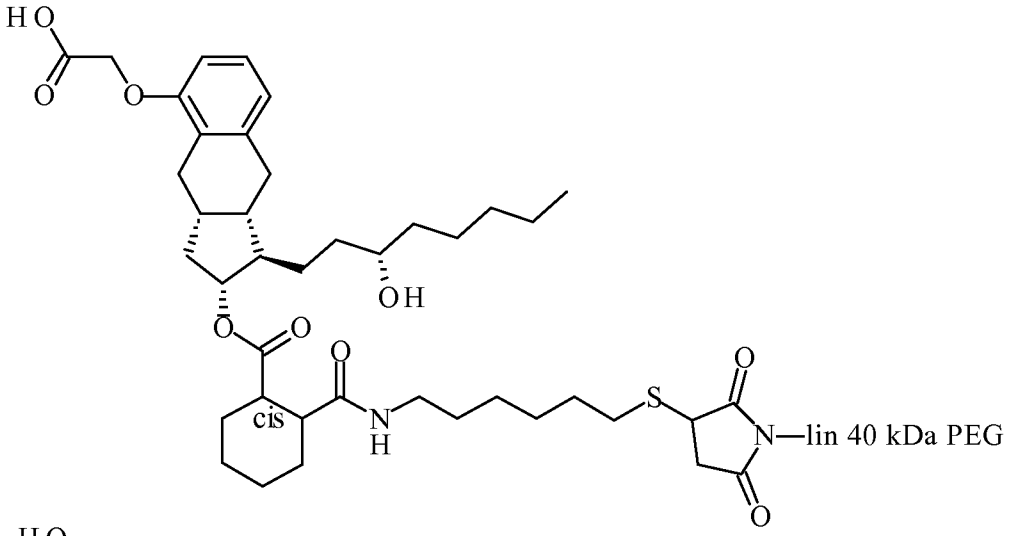
Yield: 3 mg

Example 12:

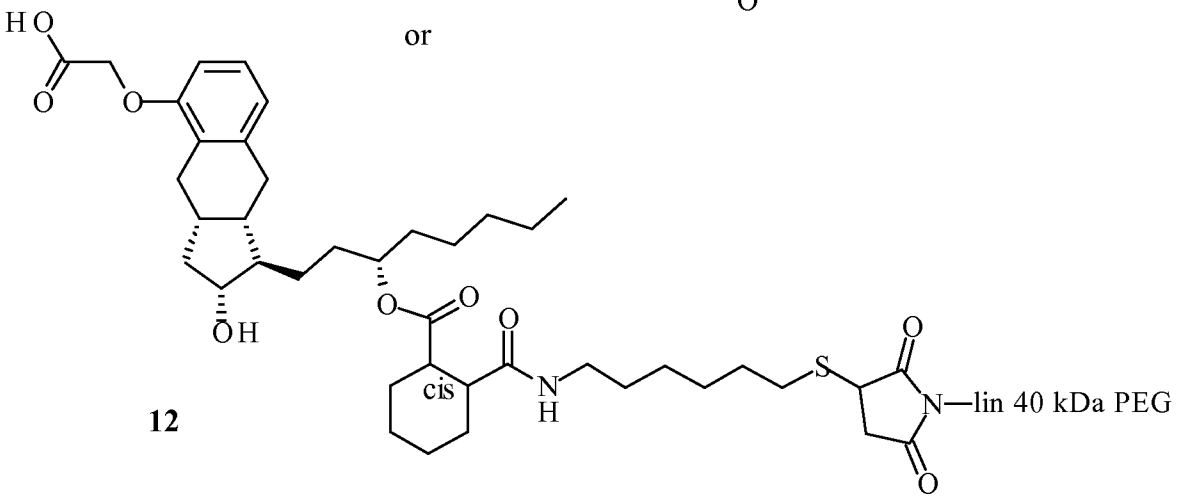
PEGylation reaction of treprostinil linker thiol with linear PEG 40 kDa maleimide

linear 40 kDa Mal-PEG
MeCN, H_2O
pH 7.4 phosphate buffer
RT, 3 h

9x →



or



12

To the treprostinil linker thiol **9x** (6.2 mg, 9.42 µmol) solution in MeCN/H_2O (0.05 % TFA, 87 mL) linear PEG 40 kDa maleimide (463 mg, 11.3 µmol) was added. The solution was neutralized by addition of pH 7.4 buffer (0.5 M phosphate, 4.4 mL). After 1 h incubation time another portion of linear 40 kDa Mal-PEG (73 mg, 178 µmol) and H_2O (5 mL) was added and the reaction solution was incubated for another 1.5 h. The reaction mixture was purified by RP-HPLC (solvent A: H_2O with 0.01 % HCl, solvent B: MeCN with 0.01 % HCl, gradient: 30-50 % B over 16 min, flow: 40 mL/min) to obtain after lyophilization TransCon linear 40 kDa PEG treprostinil **12**.

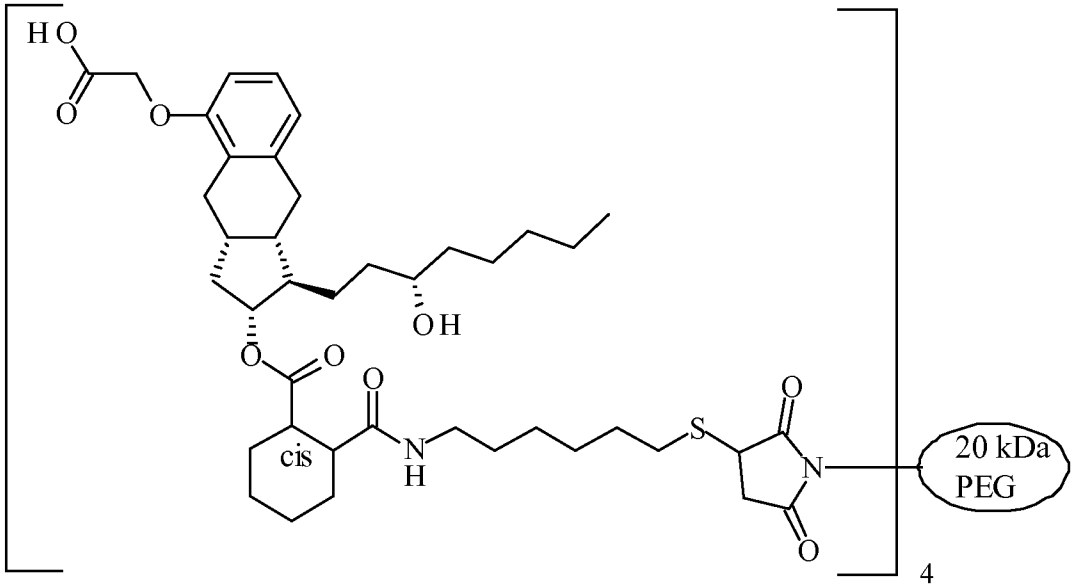
Yield: 321 mg (82 %)

Example 13:

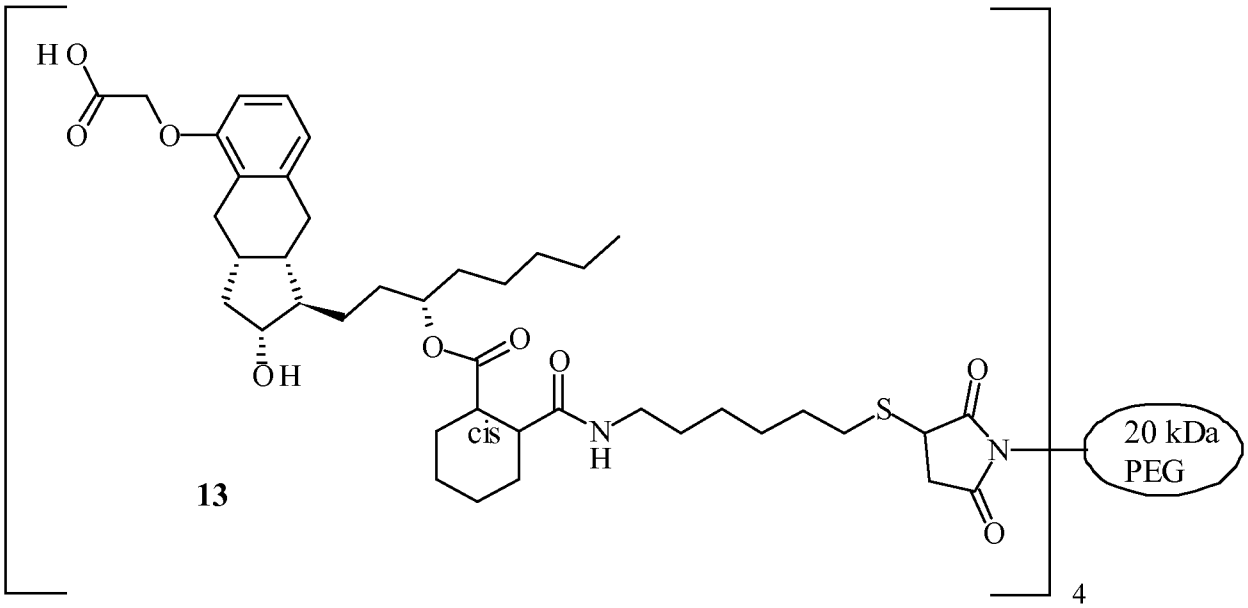
PEGylation reaction of treprostinil linker thiol with 4-arm PEG 20 kDa maleimide

9x

4 arm 20 kDa Mal-PEG
MeCN, H_2O
pH 7.4 phosphate buffer
RT, 2 h



or



13

To the treprostinil linker thiol **9x** (2.54 mg, 3.84 μmol) solution in MeCN/H_2O (0.05 % TFA, 5.7 mL) 4-arm PEG 20 kDa maleimide (21 mg, 0.98 μmol) was added. The solution was neutralized by addition of pH 7.4 buffer (0.5 M phosphate, 3.0 mL). H_2O (3 mL) was added until the reaction mixture became a clear solution again. The reaction mixture was incubated at RT for 2 h and then purified by RP-HPLC (solvent A: H_2O with 0.01 % HCl, solvent B: MeCN with 0.01 % HCl, gradient: 45-85 % B over 16 min, flow: 40 mL/min) to obtain after lyophilization TransCon 4-arm PEG 20 kDa treprostinil **13**.
Yield: 14 mg (66 %).

Example 14:

Treprostinil release kinetics of TransCon PEG linker treprostinil compounds **11** and **12**:

Release kinetics were determined according to Example 5. A treprostinil release half life time of 4.3 days (± 0.7 days) was obtained for compounds **11** and **12.**

Example 15:

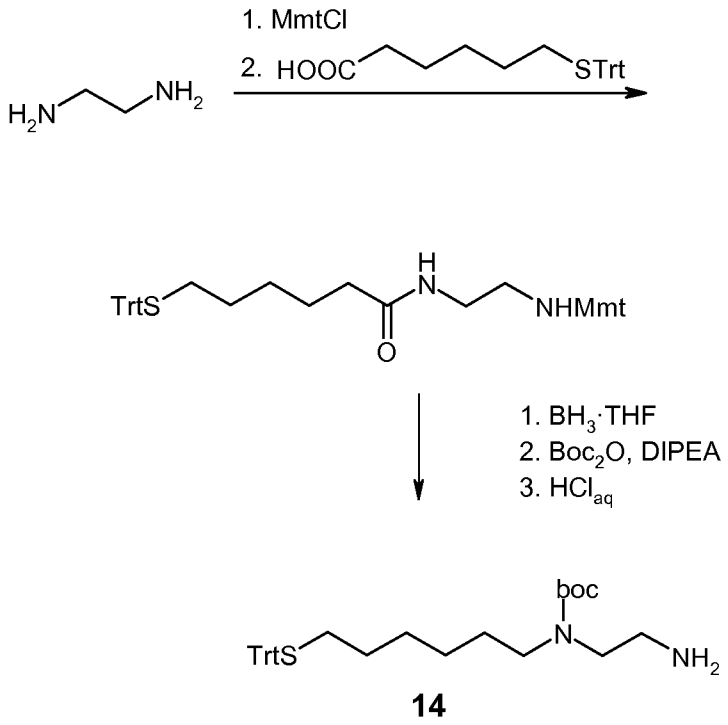
Treprostinil release kinetics of TransCon PEG linker treprostinil compound **13**

TransCon PEG linker treprostinil **13** (2.5 mg) was incubated in pH 7.4 hydrolysis buffer (60 mM sodium phosphate, 3 mM EDTA, 0.05 % Tween-20, 1 mL) at 37 °C and aliquots were analyzed by UPLC at various time points for released treprostinil. The percentage of released treprostinil was determined in relation to the area of treprostinil after total hydrolysis of an aliquot (50 μl hydrolysis solution and 25 μl 5 N NaOH were mixed for 20 min. 25 μl AcOH was added and the resulting solution was analyzed by LCMS).
By using a first order kinetics fit, a half life of 5 d for treprostinil release from **13** was obtained.

Example 16:

Synthesis of building block **14**

Building block **14** was synthesized according to the following scheme:



14

Mmt-chloride (3 g, 9.71 mmol) was dissolved in DCM (20 mL) and added dropwise to a solution of ethylenediamine (6.5 mL, 97.1 mmol) in DCM (20 mL). After two hours the solution was poured into diethyl ether (300 mL) and washed three times with 30/1 (v/v) brine/0.1 M NaOH solution (50 ml each) and once with brine (50 mL). The organic phase was dried over Na_2SO_4 and volatiles were removed under reduced pressure. Mmt-protected amine (3.18 g, 9.56 mmol) was used in the next step without further purification.

The Mmt-protected amine (3.18 g, 9.56 mmol) was dissolved in anhydrous DCM (30 mL). 6-(*S*-Tritylmercapto)hexanoic acid (4.48 g, 11.47 mmol), PyBOP (5.96 g, 11.47 mmol) and DIPEA (5.0 mL, 28.68 mmol) were added and the mixture was agitated for 30 min at RT. The solution was diluted with diethyl ether (250 mL) and washed three times with 30/1 (v/v) brine/0.1 M NaOH solution (50 mL each) and once with brine (50 mL). The organic phase was dried over Na_2SO_4 and volatiles were removed under reduced pressure. Amide was purified by flash chromatography eluting with heptane/ethyl acetate containing 0.02 % (v/v) diethylmethylamine.

Yield: 5.69 g (8.07 mmol).

MS: m/z 705.4 = $[M+H]^+$ (MW = 705.0).

Amide (3.19 g, 4.53 mmol) was dissolved in anhydrous THF (50 mL) and BH_3•THF (1 M solution, 8.5 mL, 8.5 mmol) was added. Solution was stirred for 16 h at RT. Further BH_3•THF

(1 M solution, 14 mL, 14 mmol) was added and stirred for further 16 h at RT. The reaction was quenched by addition of methanol (8.5 mL). *N,N*-dimethyl-ethylenediamine (3 mL, 27.2 mmol) was added, the solution was heated to reflux and stirred for 3 h. Reaction mixture was allowed to cool down to RT and was then diluted with ethyl acetate (300 mL), washed with saturated, aqueous Na_2CO_3 solution (2 x 100 mL) and saturated, aqueous $NaHCO_3$ solution (2 x 100 mL). The organic phase was dried over Na_2SO_4 and volatiles were removed under reduced pressure to obtain crude amine intermediate (3.22 g).

The amine intermediate (3.22 g) was dissolved in DCM (5 mL). Boc_2O (2.97 g, 13.69 mmol) dissolved in DCM (5 mL) and DIPEA (3.95 mL, 22.65 mmol) were added and the mixture was agitated at RT for 30 min. Boc- and Mmt-protected intermediate was purified by flash chromatography.

Yield: 3.00 g (3.79 mmol).

MS: m/z 791.4 = $[M+H]^+$, 519.3 = $[M\text{-}Mmt+H]^+$ (MW calculated = 791.1).

0.4 M aqueous HCl (48 mL) was added to a solution of the Boc- and Mmt-protected intermediate in acetonitrile (45 mL). The mixture was diluted with acetonitrile (10 mL) and stirred for 1 h at RT. Subsequently, the pH value of the reaction mixture was adjusted to 5.5 by addition of an aqueous 5 M NaOH solution. Acetonitrile was removed under reduced pressure and the aqueous solution was extracted with DCM (4 x 100 mL). The combined organic phases were dried over Na_2SO_4 and volatiles were removed under reduced pressure. Crude amine **14** was used without further purification.

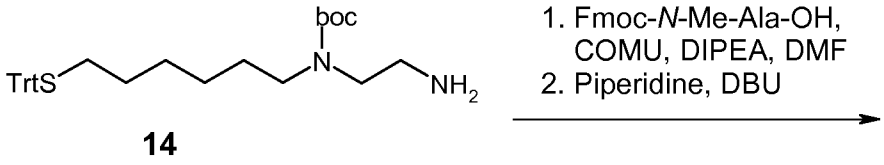
Yield: 2.52 g (3.19 mmol). A MW of 791.1 g/mol of crude amine **14** was assumed

MS: m/z 519.3 = $[M+H]^+$ (MW calculated = 519.8 g/mol).

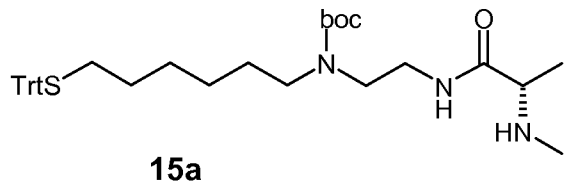
Example 17:

Synthesis of linker building blocks **15a**, **15b**, and **15c**

Linker building block **15a** was synthesized according to the following scheme:



14



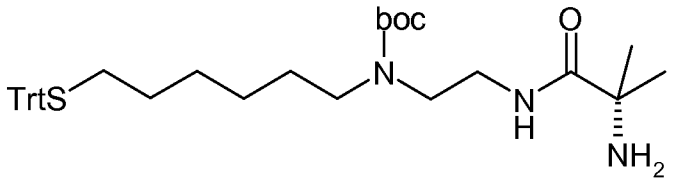
15a

Amine **14** (503 mg, 0.635 mmol, assuming a MW of 791.1 g/mol of crude **1**) was dissolved in 4 mL DMF (anhydrous, mol. sieve). Fmoc-*N*-Me-Ala-OH (310 mg, 0.953 mmol), COMU (408 mg, 0.953 mmol) and DIPEA (332 µl, 1.906 mmol) were added and the reaction was allowed to stir for 3 h at RT. 150 µl piperidine and 150 µl DBU were added to the mixture and stirring was continued for further 60 min. 400 µl acetic acid were added and product was purified by HPLC. HPLC fractions containing product **15a** were neutralized with a saturated $NaHCO_3$ solution and extracted twice with DCM. Combined organic phases were dried over Na_2SO_4 and volatiles were removed under reduced pressure.

Yield: 203 mg (0.336 mmol).

MS: m/z 604.1 = $[M+H]^+$ (MW calculated = 603.9 g/mol).

Linker building block **15b**



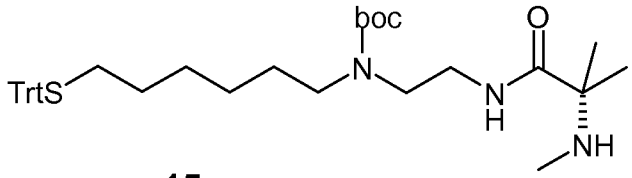
15b

Linker building block **15b** was synthesized as described for **15a** except that Fmoc-Aib-OH was used instead of Fmoc-*N*-Me-Ala-OH.

Yield: 95 mg (0.161 mmol).

MS: m/z 604.2 = $[M+H]^+$ (MW calculated = 603.9 g/mol).

Linker building block **15c**



15c

Linker building block **15c** was synthesized as described for **15a** except that Fmoc-*N*-Me-Aib-OH was used instead of Fmoc-*N*-Me-Ala-OH.

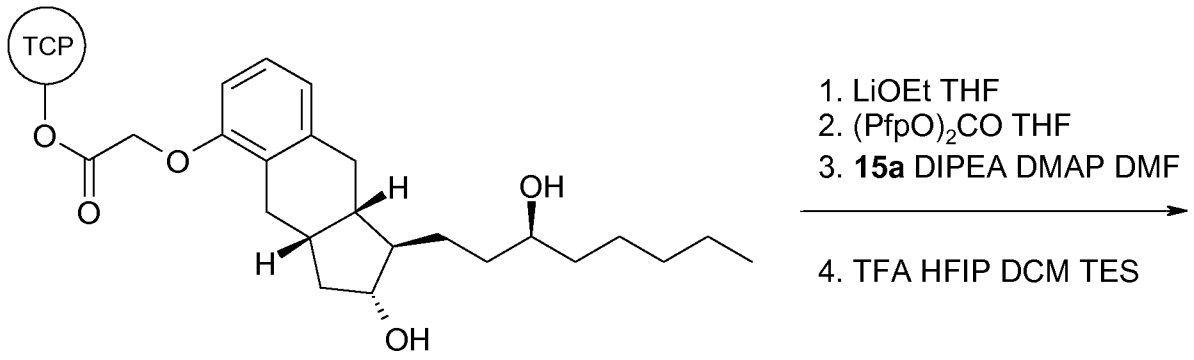
Yield: 149 mg (0.241 mmol).

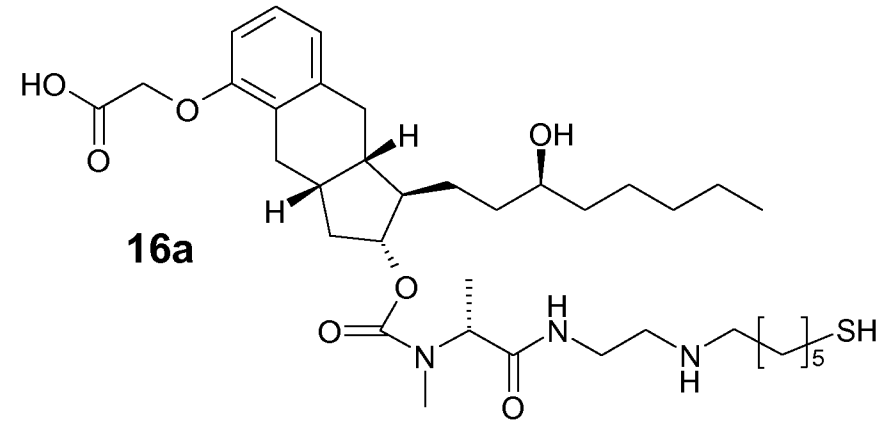
MS: m/z 619.0 = $[M+H]^+$ (MW calculated = 617.9 g/mol).

Example 18:

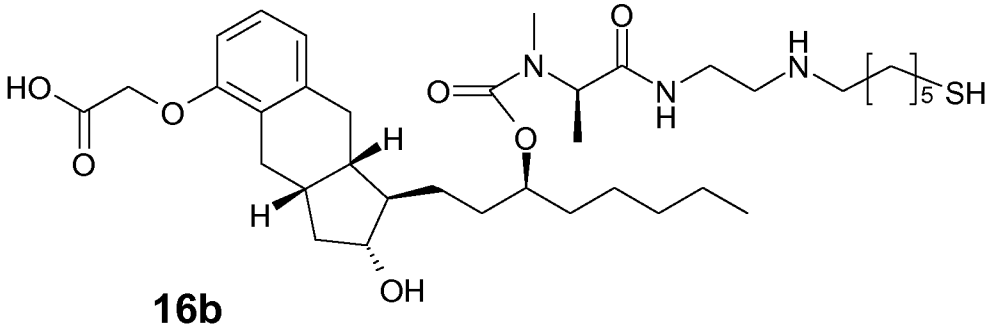
Synthesis of treprostinil-linker thiols **16a**, **16b**, **16c**, **16d**, **16e** and **16f**

Treprostinil-linker thiols **16a/16b** were synthesized according to the following scheme:





16a



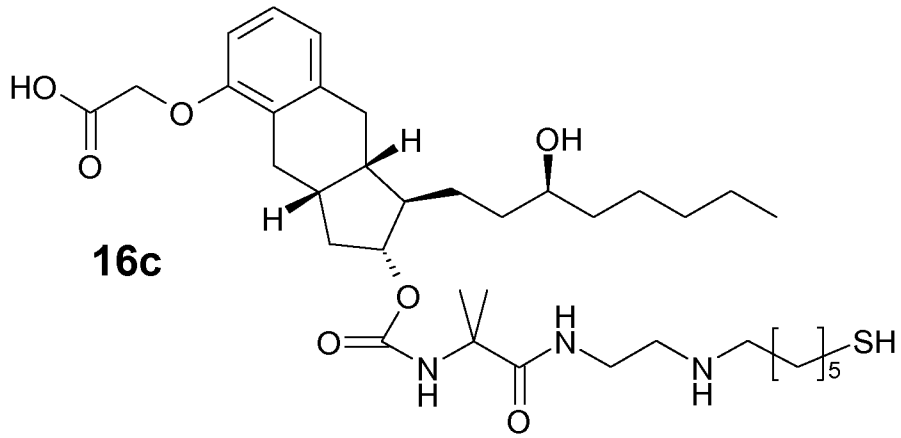
16b

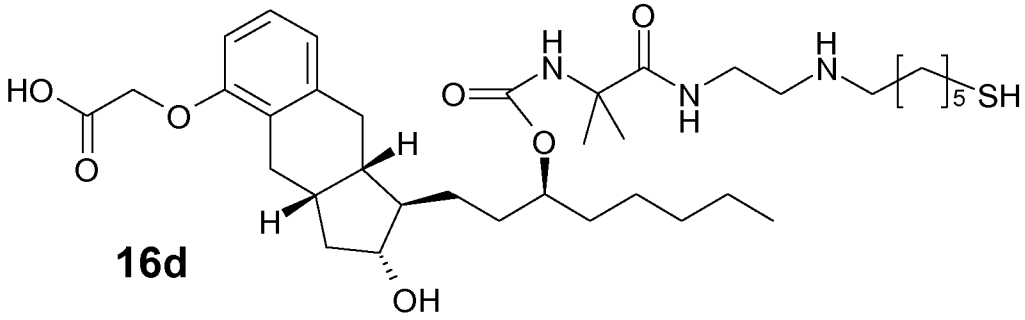
A 10 mL single use syringe reactor equipped with a PE frit was loaded with 2-chlorotrityl chloride (TCP) resin (153 mg, loading 1.22 mmol/g, 0.186 mmol). A solution of treprostinil (54 mg, 0.138 mmol) and DIPEA (60 µl, 0.346 mmol) in DCM (anhydrous, mol. sieve) was drawn into the reactor. Reactor was agitated for 2 h at RT. 200 µl methanol were added and reactor was agitated for further 10 min. Solution was dispelled and resin was washed with DCM (5x), DMF (5x) and DCM (10x). Resin was dried under vacuum (1 mbar). Based on weight, a treprostinil loading of 0.72 mmol/g TCP resin was obtained.

900 µl THF (anhydrous, mol. sieve) and 300 µl of a 1 M LiOEt solution in THF (300 µmol) were drawn to 30 mg treprostinil loaded TCP resin (21,6 µmol) in a single use 2 mL syringe reactor equipped with a PE frit. Reactor was agitated for 40 min at RT. Solution was dispelled and resin was washed with THF (2x). A solution of bis(pentafluorophenyl)carbonate (100mg, 254 µmol) in 1 mL THF was drawn into the syringe which was agitated for 90 min at RT. Solution was dispelled and resin was washed with THF (5x) and DMF (5x). A solution of linker building block **15a** (50 mg, 83 µmol), DIPEA (50 µl, 287 µmol) and DMAP (1 mg, 8 µmol) in 300 µl DMF (anhydrous, mol. sieve) was drawn into the syringe. Syringe was agitated for 3 h at RT. Solution was dispelled and resin was washed with DMF (10x) and DCM (10x). Product was cleaved from resin by incubation with 500 µl of cleavage cocktail HFIP/DCM/TES 90/10/2 v/v/v for 10 min (3x). Resin was washed with 500 µl DCM (2x).

TFA (250 µL) was added to the combined cleavage and washing solutions and the mixture was incubated at RT for 10 min. Volatiles were removed under reduced pressure. Residue was subjected to HPLC purification which gave thiols **16a/16b** as a mixture of the two regioisomers. HPLC eluate was used in the next step without further processing.
MS: m/z 678.1 = $[M+H]^+$ (MW calculated = 678.0 g/mol).

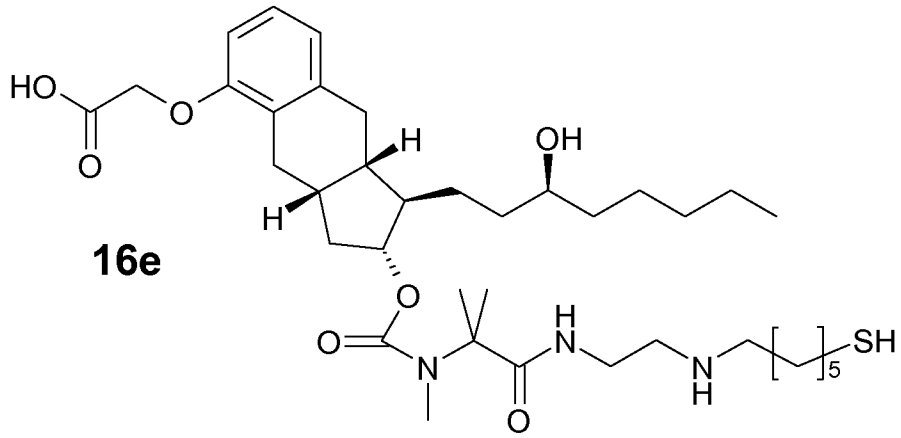
Treprostinil linker thiols **16c/16d**



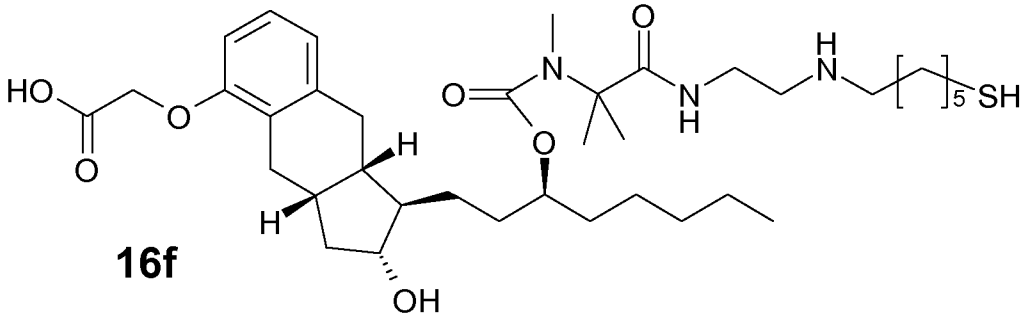


Treprostinil linker thiols **16c/16d** were synthesized as described for **16a/16b** except that linker building block **15b** was used instead of **15a**. Thiols **16c/16d** were obtained as a mixture of isomers. HPLC eluate was used in the next step without further processing.
MS: m/z 678.1 = $[M+H]^+$ (MW calculated = 678.0 g/mol).

Treprostinil linker thiols **16e** and **16f**



16e



16f

Treprostinil linker thiols **16e** and **16f** were synthesized as described for **16a/16b** except that linker building block **15c** was used instead of **15a**. Two isomers assigned to structures **16e** and **16f** were separated by HPLC. HPLC eluates were used in the next step without further processing.

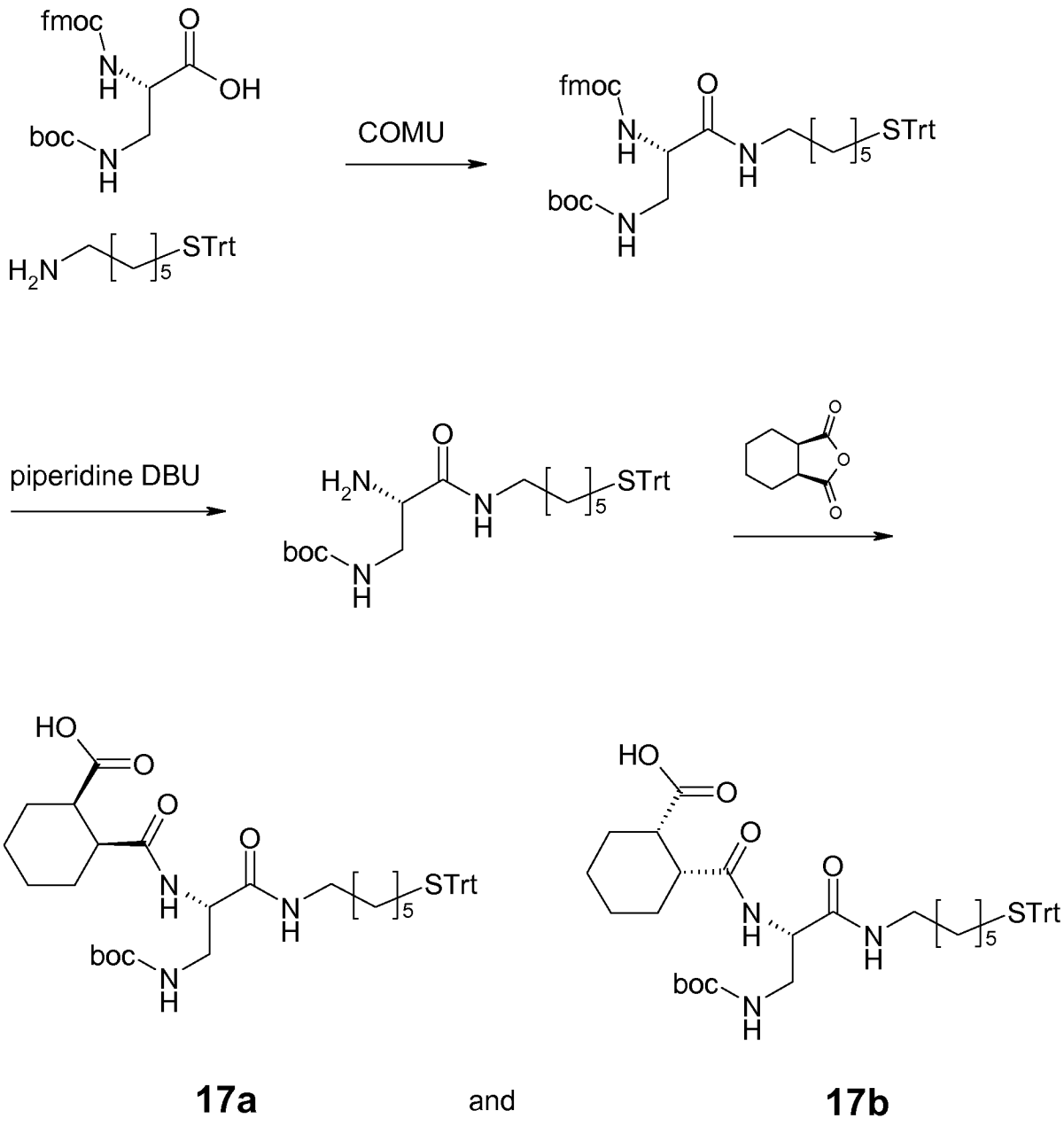
15e MS: m/z 693.0 = $[M+H]^+$ (MW calculated = 692.0 g/mol).

15f MS: m/z 693.0 = $[M+H]^+$ (MW calculated = 692.0 g/mol).

Example 19:

Synthesis of linker building blocks **17a** and **17b**

Linker building blocks **17a** and **17b** were synthesized according to the following scheme:



17a and **17b**

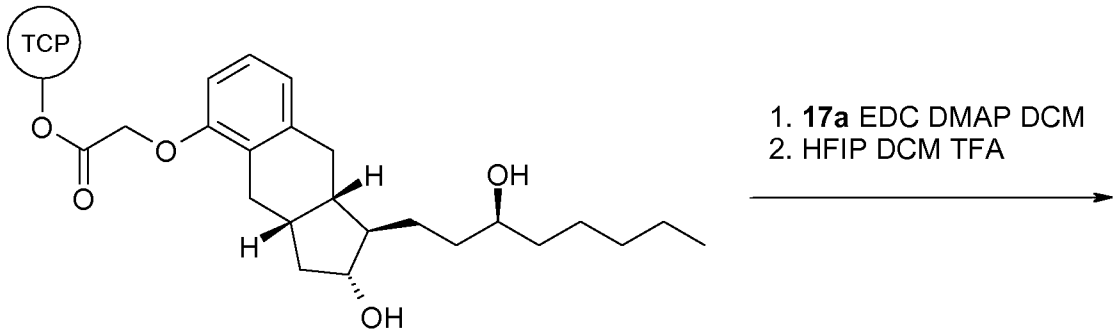
L-Fmoc-Dpr(Boc)-OH (100 mg, 0.234 mmol) was dissolved in 0.5 mL DMF (anhydrous, mol. sieve). 6-(*S*-Tritylsulfanyl)-hexaneamine (71 mg, 0.189 mmol), COMU (97 mg, 0.227 mmol) and DIPEA (66 µl, 0.378 mmol) were added and mixture was stirred for 1 h at RT. Piperidine (50 µl, 0.505 mmol) and DBU (40 µl, 0.336 mmol) were added and stirring was continued for 10 h. *cis*-Cyclohexanedicarboxylic anhydride (600 mg, 3.89 mmol) was added and stirring was continued for 1 h. Solution was quenched with water/acetonitrile and acidified with acetic acid Building blocks were purified by RP-HPLC. Structures assignment of the earlier eluting diastereomer **17a** and the later eluting diastereomer **17b** was done arbitrarily and could also be reverse.

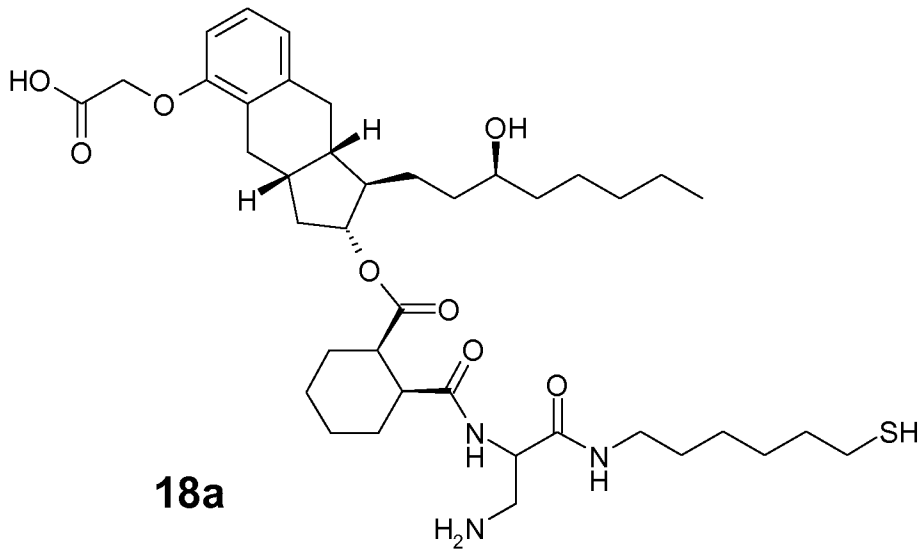
Yield: **17a** 30 mg (0.042 mmol), **17b** 42 mg (0.059 mmol)

MS: m/z 716.2 = $[M+H]^+$ (MW calculated = 716.0 g/mol).

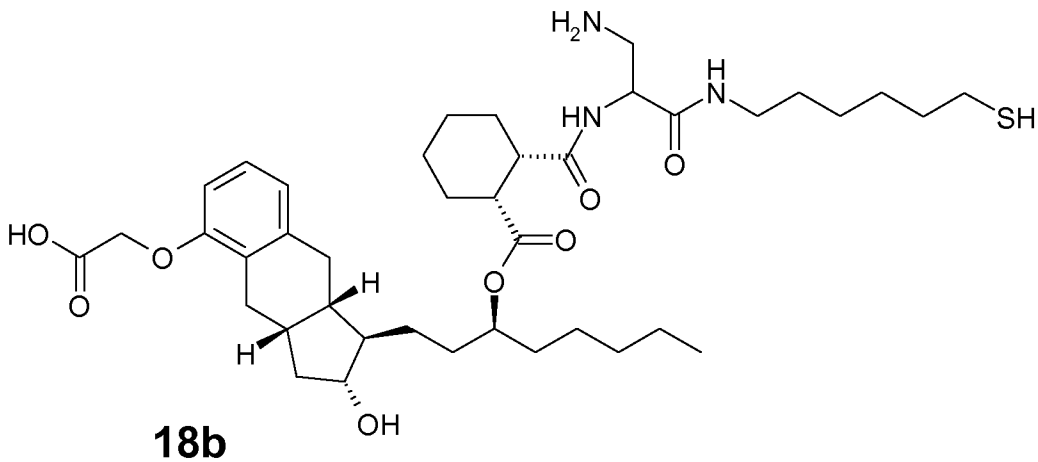
Example 20:

Synthesis of treprostinil linker thiols **18a/18b**





18a



18b

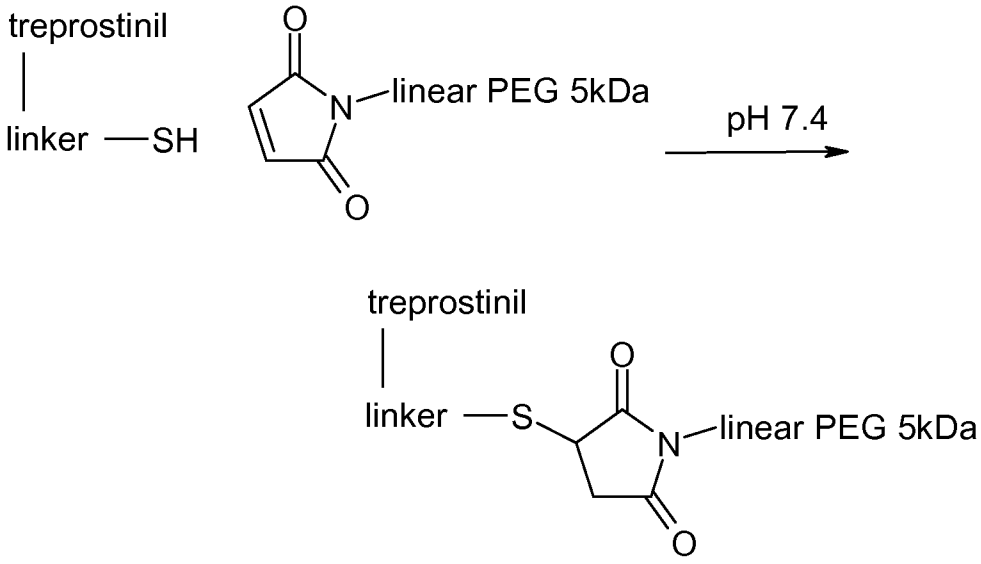
Linker building block **17a** (11 mg, 12 µmol), EDC HCl (7.4 mg, 38.5 µmol) and DMAP (4.7 mg, 38.5 µmol) were dissolved in 300 µl DCM (anhydrous, mol. sieve). Solution was drawn

to 15 mg treprostinil loaded TCP resin (10.8 µmol, 0.72 mmol/g see Example 3) in a single use 2 mL syringe reactor equipped with a frit. Reactor was agitated for 15 h at RT. Solution was dispelled and resin was washed with DCM (10x). Product was cleaved by incubating resin with 500 µl HFIP/DCM 30/70 v/v for 10 min (3x). Resin was washed with 500 µl DCM (2x). To the combined cleavage and washing solutions were added 250 µl TFA and the mixture was incubated at RT for 10 min. Volatiles were removed under reduced pressure. Residue was subjected to RP-HPLC purification which gave thiols **18a/18b** as a mixture of the two regioisomers. HPLC eluate was used in the next step without further processing.
Yield: **18a/18b** 1.5 mg (2 µmol) as determined by thiol quantification by Ellman Test.
MS: m/z 746.2 = $[M+H]^+$ (MW calculated = 746.0 g/mol).

Example 21:

Synthesis of PEG-linker-drug conjugates **19a/b**, **19c/6d**, **19e**, **19f** and **19g/19h**

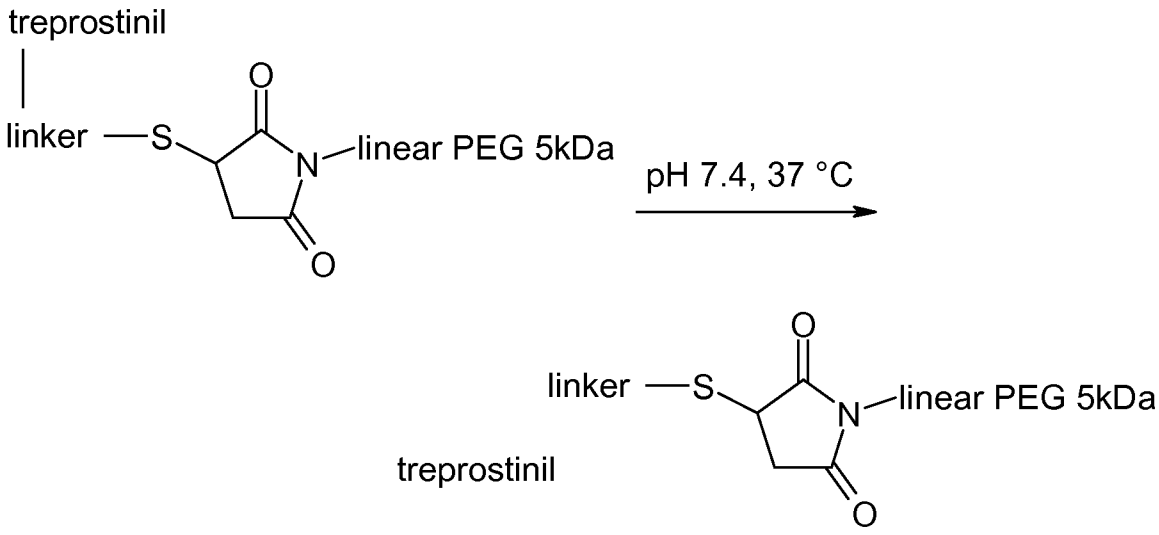
PEG-linker-drug conjugates were prepared according to the following scheme:



To HPLC eluates of treprostinil linker thiols **16a/16b**, **16c/16d**, **16e**, **16f** and **18a/18b** was given an excess of linear PEG 5 kDa maleimide. Mixtures were neutralized by addition of pH 7.4 buffer (0.5 M phosphate) and incubated at RT. After complete consumption of thiol (approx. 1 h) mixtures were acidified with acetic acid and separated from excess PEG-maleimide by RP-HPLC. HPLC eluates were lyophilized to yield PEG-linker-drug conjugates **19a/b**, **19c/19d**, **19e**, **19f** and **19g/19h** respectively.

Example 22:

Determination of drug release half life time from PEG conjugates **19a/b**, **19c/19d**, **19e**, **19f** and **19g/19h**:



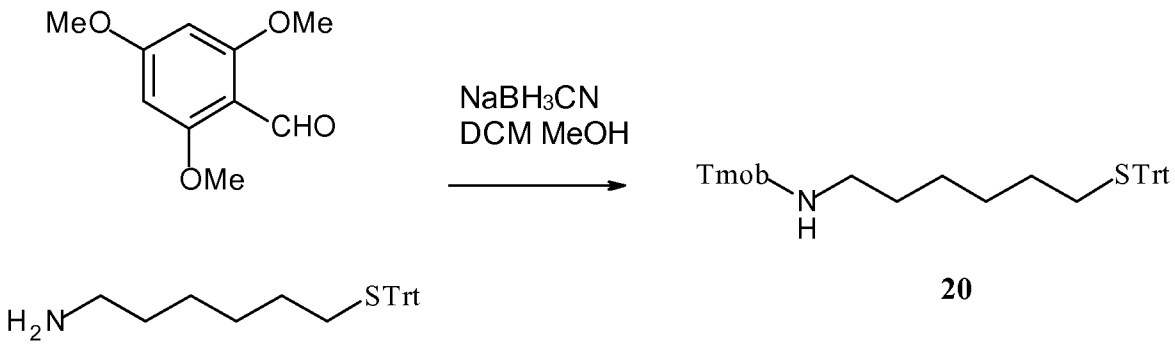
PEG-linker-drug conjugates **19a/b**, **19c/19d**, **19e**, **19f** and **19g/19h** were dissolved in pH 7.4 buffer (60 mM sodium phosphate, 3 mM EDTA, 0.05 % Tween-20, 1 mL) and incubated at 37 °C. At various time points aliquots were analyzed by UPLC to determine the amount of released treprostinil which was plotted against time. Drug release was found to follow first order kinetics. Curve fitting software was used to determine half life time of drug release from the respective conjugates (Table 1)

Table 2:

entry	PEG-linker-drug conjugate	drug release half life time
1	**19a/b**	31 d
2	**19c/19d**	17 d
3	**19e**	24 d
4	**19f**	37 d
5	**19g/19h**	35 min

Example 23:

Synthesis of intermediate **20**:



20

The amino group of 6-(*S*-Tritylsulfanyl)-hexaneamine was Tmob (2,4,6-Trimethoxybenzyl) protected by dropwise addition of a solution of 2,4,6-trimethoxybenzaldehyde (4.22 g, 21.51 mmol) in 88 mL methanol/DCM 1/1 (v/v) to 6-(*S*-tritylsulfanyl)-hexaneamine (6.74 g, 17.95 mmol) and sodium cyanoborohydride (1.58 g, 25.14 mmol) in 44 mL methanol. The mixture was stirred for 1.5 h at RT and quenched with 95 mL of 0.4 N aqueous HCl solution. After further stirring at RT for 30 min mixture was extracted with ethyl acetate (4x). Combined organic layers were washed with sat. aqueous $NaHCO_3$ solution (2x) and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure.

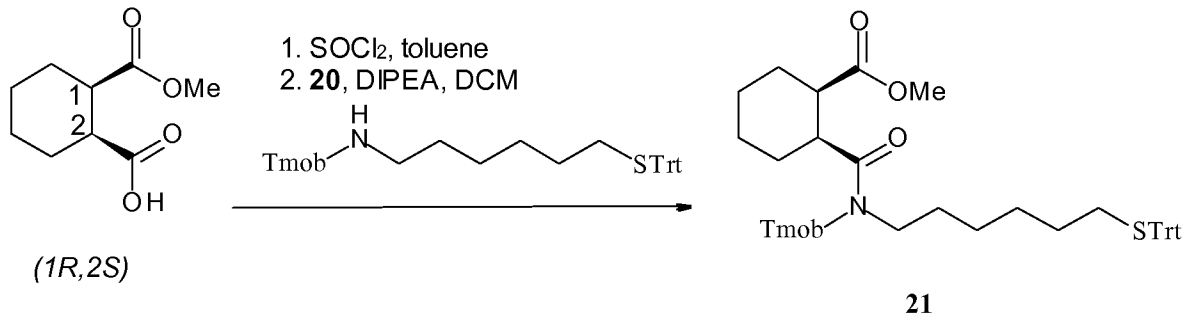
Tmob protected amine **20** was purified by flash chromatography eluting with DCM/methanol containing 0.1 % (v/v) triethylamine.

Yield: 5.88 g (55 %).

MS: m/z 556.3 = $[M+H]^+$ (MW calculated = 555.79 g/mol).

Example 24:

Synthesis of intermediate **21**:



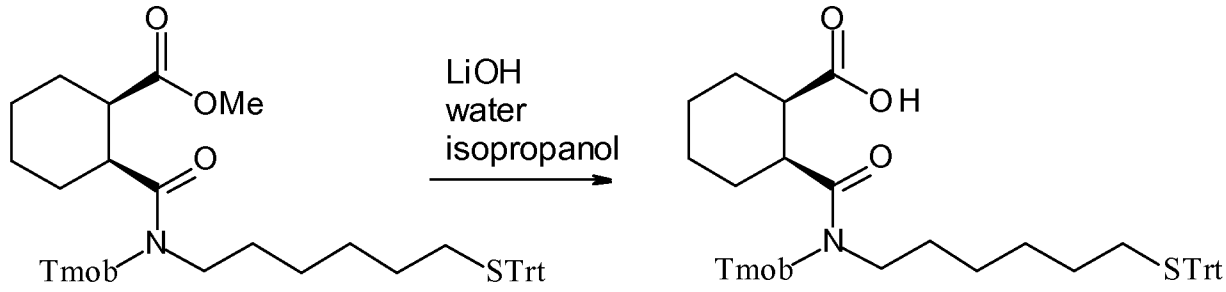
(1R,2S)

21

(1R,2S)-Cyclohexanedicarboxylic acid 1-methyl ester, CAS no. 88335-92-6 (for synthesis see R. Manzano et al. *J. Org. Chem.* **2010**, *75(15)*, 5417-5420) (506 mg, 2.72 mmol) was dissolved in toluene (11 ml, anhydrous). Thionyl chloride (1.09 mL, 15.0 mmol) was added and mixture was heated for 1 h at 60 °C in a pressure tube. Volatiles were removed *in vacuo*. A solution of Tmob protected amine **20** (1.66 g, 2.99 mmol) and DIPEA (1.12 mL, 6.43 mmol) in DCM (30 mL, anhydrous) was added and mixture was stirred for 2 h at RT. Ethyl acetate was added and the organic layer was washed with 0.1 N aqueous HCl (2x). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Methyl ester **21** was purified by flash chromatography eluting with ethyl acetate/heptane.
Yield: 1.55 g (79 %).
MS: m/z 746.1 = $[M+Na]^+$ (MW calculated = 723.98 g/mol).

Example 25:

Synthesis of intermediate **22**:

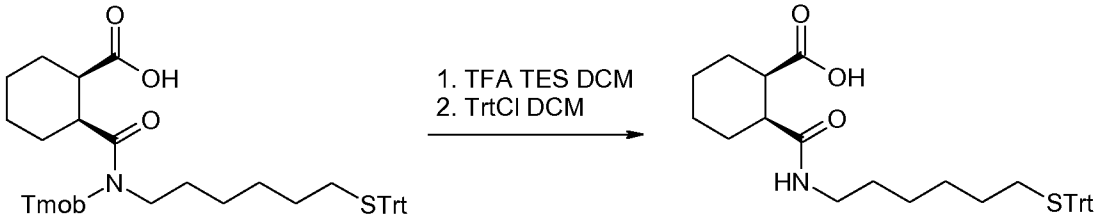


21 **22**

Methyl ester **21** (3.12 g, 4.31 mmol) was dissolved in isopropanol (10 ml). 35 mL of a 1 M aqueous LiOH solution were added and the mixture was stirred for 5 d at RT. Ethyl acetate was added and the organic layer was washed with 0.05 N aqueous HCl (2x) and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. **22** was purified by flash chromatography eluting with ethyl acetate/heptane containing 0.1 % formic acid (v/v).
Yield: 2.41 g (79 %).
MS: m/z 710.1 = $[M+H]^+$ (MW calculated = 709.95 g/mol).

Example 26:

Synthesis of intermediate **6a**:



22 **6a**

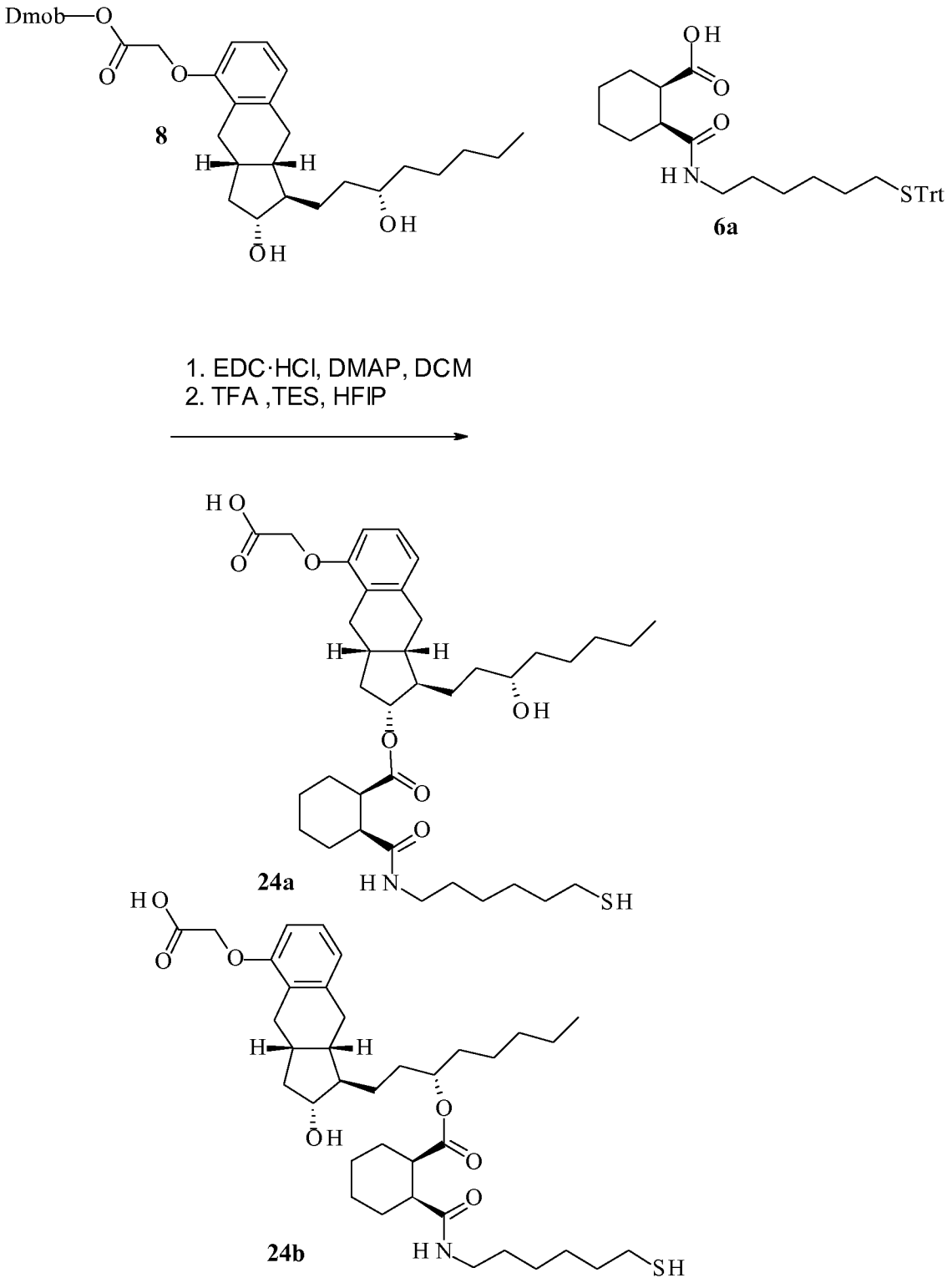
Compound **22** (1.23 g, 1.74 mmol) was dissolved in DCM (18 ml). TFA (2 mL) and TES (600 µl) were added and the mixture was stirred for 40 min at RT. Volatiles were removed *in vacuo*. The residue was dissolved in DCM (20 mL) and tritylchloride (728 mg, 2.61 mmol) was added. The mixture was stirred for 2 h at RT. DCM was removed under reduced pressure. Carboxylic acid **6a** was purified by flash chromatography using ethyl acetate/heptane containing 0.1 % formic acid (v/v) as eluent, followed by RP-HPLC purification.

Yield: 615 mg (67 %).

MS: m/z 552.2 = $[M+Na]^+$ (MW calculated = 529.75g/mol).

Example 27:

Synthesis of treprostinil linker thiol **24a**



Dmob protected treprostinil **8** (100 mg, 0.185 mmol), carboxylic acid **6a** (195 mg, 0.368 mmol), EDC·HCl (72 mg, 0.376 mmol) and DMAP (43 mg, 0.352 mmol) were dissolved in DCM (1.8 mL, anhydrous, mol. sieve). The mixture was stirred at RT for 1 d. Ethyl acetate was added and the organic layer was washed with 0.1 N aqueous HCl (3x) and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure.

The residue was dissolved in HFIP (5 mL), TFA (250 µL) and TES (250 µL) and stirred for 30 min at RT. The precipitate was filtered off and the filtrate was evaporated *in vacuo*.

UPLC analysis revealed a 4/1 ratio of regioisomers **24a** and **24b** (column: Kinetex 100 x 2.1 mm, 1.7 µm XB-C18 silica, pore size 100Å, Phenomonex Ltd, Aschaffenburg, Germany; flow rate 0.25 mL/min; solvent A: water + 0.05 % TFA (v/v), solvent B: acetonitrile + 0.04 % TFA; gradient: 30-58 % B (10 min), 58 % B isocratic (10 min), 58-80% B (5 min), 80-99% (5 min), wavelength 280 nm). **24a** turned out to be identical with compound **9x**.

The residue was taken up in acetonitrile/water and **24a** was purified by RP-HPLC (solvent A: H_2O + 0.01 % HCl, solvent B: MeCN + 0.01 % HCl, gradient: 60-85 % B over 16 min). Isomer **24a** eluted first, followed by isomer **24b**. Fractions containing pure **24a** were combined and lyophilized. Mixed fractions containing **24a** and **24b** were subjected to repurification.

Yield **24a**: 29.5 mg (24 %)

MS: m/z 660.3 = $[M+H]^+$ (MW calculated = 659.9 g/mol).

^{1}H-NMR ($CDCl_3$, δ[ppm]): 7.07 (t, 1H), 6.80 (d, 1H), 6.71 (d, 1H), 5.86 (bs, 1H), 4.78-4.63 (m, 3H), 3.53 (bs, 1H), 3.14-3.03 (m, 1H), 3.03-2.83 (m, 2H), 2.82-2.66 (m, 2H), 2.66-2.58 (m, 1H), 2.58-2.46 (m, 4H), 2.46-2.31 (m, 1H), 2.31-2.13 (m, 1H), 2.13-1.92 (m, 2H), 1.92-1.81 (m, 1H), 1.75-1.51 (m, 7H), 1.51-1.21 (m, 21H), 1.21-1.08 (m, 1H), 0.90 (t, 3H).

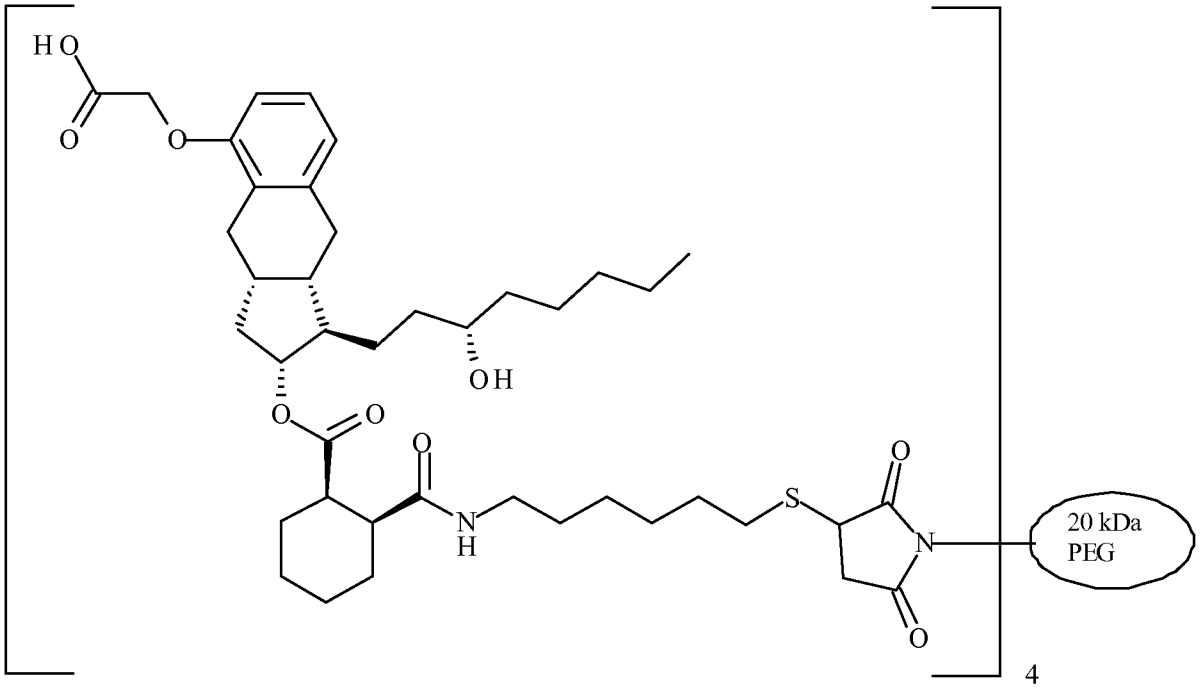
^{13}C-NMR (126 MHz, $CDCl_3$, δ[ppm]): 174.7, 174.2, 171.7, 155.2, 140.6, 127.5, 126.3, 121.8, 109.71, 78.8, 72.4, 65.8, 48.3, 43.9, 42.5, 40.4, 39.7, 37.4, 37.3, 35.4, 34.00, 33.0, 32.9, 32.1, 29.3, 28.4, 28.1, 27.0, 26.4, 25.5, 24.6, 24.2, 23.2, 22.8, 14.2.

Example 28:

PEGylation reaction of treprostinil linker thiol **24a** with 4-arm PEG 20 kDa maleimide

4 Arm 20 kDa Mal-PEG
MeCN, H_2O
pH 7.4 phosphate buffer
RT, 2 h

24a ⟶



25

A solution of treprostinil linker thiol **24a** (7.5 mg, 11.3 µmol) in 2 mL of acetonitrile/water 9/1 (v/v) was mixed with a solution of 4-arm PEG 20 kDa maleimide (53.5 mg, 2.54 µmol) in 2 mL of acetonitrile/water 1/1 (v/v). The pH was adjusted to 7.0 by addition of pH 7.4 buffer (50 mM phosphate, 0.8 mL). The reaction mixture was stirred at RT for 1.5 h and then purified by RP-HPLC (solvent A: H_2O with 0.01 % HCl, solvent B: MeCN with 0.01 % HCl, gradient: 45-85 % B over 16 min). Product containing fractions were pooled and acetonitrile was removed under reduced pressure. The solution was neutralized by addition of pH 7.4 buffer (phosphate, 0.5 M). The solution was concentrated and the buffer was exchanged with 10 mM pH 7.0 phosphate containing 46 g/l mannitol by ultrafiltration (Vivaspin centrifugal concentrator, PES membrane with 10 kDa cut off) to obtain 8.5 mL of the final solution of **25**. UPLC and SEC analysis revealed a uniform material. The concentration was determined by quantification of treprostinil content after basic hydrolysis: 30 µL aliquots were treated with 35 µL 0.5 M NaOH. After 30 min incubation at RT 35 µL acetic acid was added. The

trerostinil content was determined by UPLC by using a treprostinil calibration curve. A total treprostinil content of 2.0 mg was found, corresponding to 30 mg **25**. Yield: 50 % based on PEG starting material.

Example 29:

Treprostinil release kinetics of TransCon PEG linker treprostinil compound **25**

Treprostinil release kinetics from **25** was determined as described in example 15 and compared with the results obtained from compound **13**. No difference in half life time (5 d) was observed.

Example 30:

Treprostinil release kinetics of TransCon PEG linker treprostinil compound **13** in rat plasma

150 µl of a pH 7.5 HEPES buffer (1 M HEPES, 3 mM EDTA) were mixed with 1.2 mL rat plasma (WISTAR rat Li heparin plasma, Innovative Research, Novi, MI, USA). 150 µl of a of TransCon PEG linker treprostinil **13** solution (0.15 mg **13** in 1.5 mL 10 mM phosphate 46 g/l mannitol buffer pH 7.0) were added. A pH of 7.4 of the mixture was confirmed by means of a pH electrode. Mixture was incubated at 37 °C. At given time points 100 µl aliquots were withdrawn. 100 µl aliquots were analyzed for released and total treprostinil content.

For analysis of released treprostinil, 100 µl aliquots were spiked with 20 µl internal standard (2.8 µg/mL tolbutamide in methanol/water 1/1 (v/v)) and transferred to a Ostro 96 well plate (Waters GmbH, Eschborn, Germany). Plasma proteins were precipitated by addition of three volumes of pre-cooled (0-5°C) acetonitrile containing 1 % formic acid. Positive pressure was applied (4 bar, Waters Positive Pressure-96 Processor) and eluate was lyophilized. Lyophilizate was dissolved in 40 µl of 10 mM ammonium formiate pH 4.0/acetonitrile 7/3 (v/v). Solution was centrifuged and supernatant was assayed for released treprostinil by UPLC-MS/MS.

For analysis of total treprostinil content (sum of released and carrier bound treprostinil), 100 µl aliquots were spiked with 20 µl internal standard (2.8 µg/mL tolbutamide in methanol/water 1/1 (v/v)) and 50 µl of 0.5 M LiOH were added. Mixture was incubated in a shaker for 2 h at room temperature. After addition 25 µL 1 M HCl the mixture was transferred to a Ostro 96 well plate (Waters GmbH, Eschborn, Germany). Plasma proteins were

precipitated by addition of three volumes of pre-cooled (0-5°C) acetonitrile containing 1 % formic acid. Positive pressure was applied (4 bar, Waters Positive Pressure-96 Processor) and eluate was lyophilized. Lyophilizate was dissolved in 100 µl of 10 mM ammonium formiate pH 4.0/acetonitrile 7/3 (v/v). Solution was centrifuged and supernatant was assayed for total treprostinil content by UPLC-MS/MS.

UPLC-MS/MS method for determination of treprostinil content:

The quantification of plasma treprostinil concentrations were carried out using a Waters Acquity UPLC coupled to a Thermo LTQ Orbitrap Discovery mass spectrometer via an ESI probe and with Waters BEH C18 (50 x 2.1 mm I.D., 1.7 µm particle size) as analytical column (mobile phase A: 10 mM ammonium formate pH 4.6, mobile phase B: methanol, T = 22°C). The gradient system comprised a linear gradient from 0.1 % B to 95 % B in 4 min, an isocratic washing phase with 95% B (0.5 min), and a reconditioning phase (2.4 min) with a flow rate of 0.25 mL/min. Detection of the ions was performed in the selected reaction monitoring (SRM, negative ionization) mode, monitoring the transition pairs at the m/z 389.2 precursor ions to the m/z 331.2 product ions for treprostinil and m/z 269.1 precursor ions to the m/z 170.0 product ions for the internal standard (IS) tolbutamide.

The calibration curve was acquired by plotting the extracted peak area ratio area $_{\text{treprostinil}}$/area $_{\text{tolbutamide}}$ against the nominal trepostinil concentrations of calibration standards. The results were fitted to linear regression using standard software.

The extracted peak area ratio area $_{\text{treprostinil}}$/area $_{\text{tolbutamide}}$ of the quantification experiments at different time points were used to calculate the treprostinil content according to the calibration curve.

Treprostinil release at time points was expressed as % treprostinil release compared to total treprostinil content (see Fig. 1). By using a first order kinetics fit a half life of 4.5 d for the release kinetics of treprostinil from **25** in buffered rat plasma at 37 °C was obtained, which is in good agreement to release kinetics in pH 7.4 buffer at 37 °C (Example 15).

Example 31

PK of PEG treprostinil conjugate **25** in monkeys

25 (3 mg/mL in 10 mM pH 7.0 phosphate, 46 g/L mannitol) was given at a dose level of 0.5 mg/kg as a single dose by sc and iv injection in three male cynomolgus monkeys each. Blood samples were collected at given time points over two weeks. The plasma was assayed for PEG content and total treprostinil content (sum of released and carrier bound treprostinil). Due to the fast elimination of free treprostinil compared to carrier bound treprostinil, treprostinil plasma levels reflect the presence of treprostinil conjugate rather than free treprostinil levels.

For the analysis of total treprostinil content, 100 µL plasma samples and treprostinil standards in cynomolgus monkey plasma were spiked with 20 µL internal standard (2.8 µg/mL tolbutamide in methanol/water 1/1 (v/v)) and 50 µl of 0.5 M LiOH were added. The mixture was incubated in a shaker at RT for 2.5 h. After addition of 25 µL 1 M HCl the mixture was transferred to an Ostro 96 well plate (Waters GmbH, Eschborn, Germany). Plasma proteins were precipitated by addition of three volumes of pre-cooled (0-5°C) acetonitrile containing 1 % formic acid. Positive pressure was applied (4 bar, Waters Positive Pressure-96 Processor) and the eluate was lyophilized. The lyophilizate was dissolved in 100 µl of 10 mM ammonium formiate pH 4.0/acetonitrile 7/3 (v/v). The solution was centrifuged and the supernatant was assayed for total treprostinil content by UPLC-MS/MS.

UPLC-MS/MS method for determination of treprostinil content:
The quantification of plasma treprostinil concentrations were carried out using a Waters Acquity UPLC coupled to a Thermo LTQ Orbitrap Discovery mass spectrometer via an ESI probe and with a Waters BEH C18 (50 x 2.1 mm I.D., 1.7 µm particle size) as analytical column (mobile phase A: 10 mM ammonium formate pH 4.6, mobile phase B: methanol, T = 22°C). The gradient system comprised a linear gradient from 0.1 % B to 95 % B in 4 min, an isocratic washing phase with 95% B (0.5 min), and a reconditioning phase (2.4 min) with a flow rate of 0.25 mL/min. Detection of the ions was performed in the selected reaction monitoring (SRM, negative ionization) mode, monitoring the transition pairs at the m/z 389.2 precursor ions to the m/z 331.2 product ions for treprostinil and m/z 269.1 precursor ions to the m/z 170.0 product ions for the internal standard (IS) tolbutamide.
The calibration curve was acquired by plotting the extracted peak area ratio area $_{\text{treprostinil}}$/area $_{\text{tolbutamide}}$ against the nominal trepostinil concentrations of calibration standards which were

prepared in cynomolgus monkey plasma. The results were fitted to a linear regression using standard software.

The extracted peak area ratio area $_{treprostinil}$/area $_{tolbutamide}$ of the quantification experiments at different time points were used to calculate the treprostinil content according to the calibration curve.

For analysis of total PEG content, plasma samples underwent basic preincubation in order to generate a uniform PEG material from **25**. This was based on the fact that after injection of **25** different treprostinil carrier species are generated due to the sequencial release of 4 treprostinils from carrier molecule over time.

50 µl plasma samples and **25** PEG treprostinil conjugate standards in cynomolgus monkey plasma were diluted with 50 µL of 200 mM HEPES solution (pH 7.5) and 50 µl of 0.5 M LiOH were added. Mixture was incubated in a shaker for 2 h at room temperature. After addition of 50 µL 1 M HCl the mixture was assayed using the high sensitivity PEG ELISA kit P-0003 from Life Diagnostics Inc. West Chester, PA, USA, according to the manufacturer's instructions.

The calibration curve was acquired by plotting the absorption values at 450 nm against the nominal PEG concentrations of calibration standards. The results were fitted to a sigmoidal curve using standard software.

The absorption values at 450 nm of the quantification experiments at different time points were used to calculate the PEG content according to the calibration curve.

Result: Total treprostinil content analysis after a single dose sc injection of **25** reveals a prolonged duration of circulation of treprostinil conjugate for more than two weeks in monkeys. **(Fig 2)**

Single dose iv injection of **25** and plasma analysis for total treprostinil content revealed similar duration of circulation **(Fig 3)**. An apparent first order total treprostinil elimination half life time of 2.9 days (rate constant $k_{apparent}$: 0.239 d^{-1}) was obtained by using standard software.

In contrast plasma analysis for PEG carrier content revealed a much slower elimination **(Fig 3)**. By fitting as a first order kinetics using standard software an PEG carrier elimination half life time of 6.6 d (rate constant $k_{PEGelim}$: 0.105 d^{-1}) was obtained.
Equal elimination rate constant of different treprostinil carrier species e.g. generated by sequential linker hydrolysis/release of one to four treprostinils from PEG carrier are assumed.

The apparent faster elimination half life time of total treprostinil compared to PEG carrier is based on the combination of elimination of the PEG carrier and treprostinil release by linker hydrolysis. From the determined rate constant values of $k_{apparent}$ (0.239 d^{-1}) and $k_{PEGelim}$ (0.105 d^{-1}) a first order treprostinil release by linker cleavage rate constant k_{linker} can be calculated:
$\exp(-k_{apparent}\, t) = \exp(-k_{PEGelim}\, t) * \exp(-k_{linker}\, t) = \exp(-t[k_{PEGelim} + k_{linker}])$

After logmarithizing and rearrangement k_{linker} can be calculated according to:
$k_{linker} = k_{apparent} - k_{PEGelim}$; $k_{linker} = 0.239\ d^{-1} - 0.105\ d^{-1} = 0.134\ d^{-1}$

By help of the equation $t_{half\ life\ time} = \ln(2)/k$ the half life time of treprostinil release by linker hydrolysis was calculated as 5.2 d, which is in good agreement with the 5 d linker treprostinil release half life time determined in vitro.

Example 32

PK of PEG treprostinil conjugate **25** and free treprostinil in rats

25 (3 mg/mL buffer (10 mM pH 7.0 phosphate, 46 g/L mannitol)) was injected at a dose level of 5.5 mg/kg as a single dose in male Wistar rats each. Three animals received sc injection and three animals received iv injection. Blood samples were collected at given time points over two weeks. Blood samples (250 µL) were given directly into collection tubes containing 50 µL acidic citrate buffer (0.5 M sodium citrate, pH 4.0). The plasma was assayed for free treprostinil content and total treprostinil content (sum of free and carrier bound treprostinil).

For the analysis of free treprostinil, 50 µL plasma were thawed on ice and mixed with 5 µl acidic citrate buffer and 10 µL internal standard (0.28 µg/mL tolbutamide in methanol/water 1/1 (v/v)). Samples were transferred to Ostro 96 well plates (Waters GmbH, Eschborn, Germany), and plasma proteins were precipitated by rapid addition of 400 µL pre-cooled (0-5 °C) acetonitrile containing 1 % formic acid. Positive pressure was applied (4 bar, Waters

Positive Pressure-96 Processor) and the eluates were collected. Subsequently, the well plates were rinsed two times with 100 µL ice-cold acetonitrile containing 1 vol. % formic acid. The eluates were transferred into 2 mL vials, placed into an Eppendorf Thermomixer (at 10°C) and eluates were concentrated under a soft stream of nitrogen over 45 min to a final volume of 60-80 µL. 30 µl solvent mixture (10 mM aqueous ammonium formiate adjusted to pH 4.0 with formic acid/acetonitrile 7/3 (v/v)) were added to each vial and the solutions were analyzed by UHPLC-MS/MS.

For preparation of calibration standards, blank plasma samples were spiked with treprostinil and treated likewise.

UHPLC-MS/MS method for determination of free treprostinil content:

The quantification of plasma treprostinil concentrations were carried out using an Agilent 1290 UHPLC coupled to an Agilent Triplequad 6460 system (MassHunter Xcalibur software) in the ES- mode. As analytical column a Waters BEH C18 was used (50 x 2.1 mm I.D., 1.7 µm particle size. Mobile phase A: 10 mM ammonium formate pH 5.7, mobile phase B: methanol. The gradient system comprised a linear gradient from 35 % B to 99 % B in 8 min, an isocratic washing phase with 99% B (0.9 min), and a reconditioning phase (3 min) with a flow rate of 0.200 mL/min (T = 40°C).

Detection of the ions was performed in the SRM mode, monitoring the transition pairs at the m/z 389.1 precursor ions to the m/z 331.1 product ions for treprostinil and m/z 269.0 precursor ions to the m/z 169.9 product ions for the internal standard (IS) tolbutamide.

The calibration curve was acquired by plotting the extracted peak area ratio area treprostinil/area tolbutamide against the nominal trepostinil concentrations of calibration standards. The results were fitted to a linear regression using standard software.

The extracted peak area ratio area treprostinil/area tolbutamide of the quantification experiments at different time points were used to calculate the treprostinil content according to the calibration curve.

Total treprostinil plasma content was determined as given in Example 31.

Result: Free and total treprostinil content analysis after a single dose sc injection of **25** reveals a prolonged circulation of treprostinil conjugate and a burstless release of free treprostinil for more than four days in rats after iv **(Fig 4)** or sc injection **(Fig 5)**.

Example 33

Isolation of intermediate **6a** by enantioseparation of racemic mixture **6a/6b**

Racemic mixture **6a/6b** (107 g) was separated on a Chiralpak IA column (250 x 76 mm, 20 µm, flow rate 270 mL/min) using acetonitrile/acetic acid 1000/1 (v/v) as eluent. Combined eluates of second eluting enantiomer (**6a**) were mixed with 5 vol% water and evaporated under reduced pressure. The residue was taken up in DCM (500 mL) and extracted with 0.1 M HCl (500 mL, 2 x) and brine (500 mL). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure.

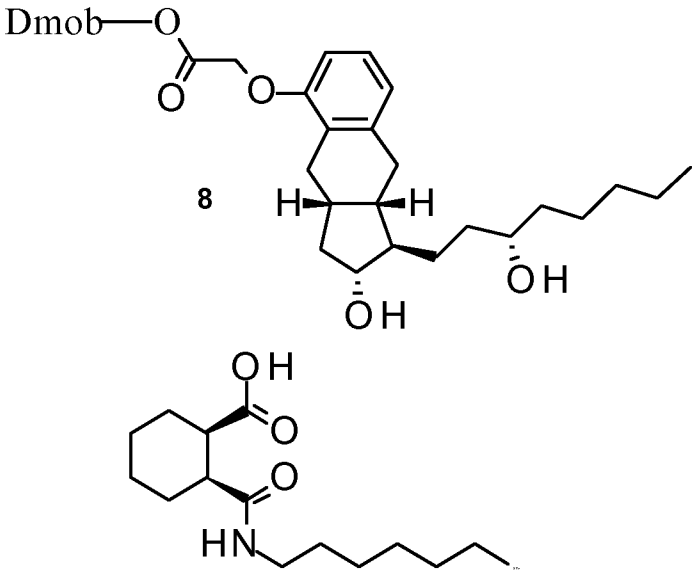
Yield: 28.9 g (27 %).

MS: m/z 552.2 = $[M+Na]^+$ (MW calculated = 529.75g/mol).

Enantiomeric ratio of **6a**/**6b** as determined by Chiralpak IC column (4.5 x 250 mm, 5 µm, eluent acetonitrile/acetic acid 1000/1 (v/v), flow rate 1 mL/min, 230 nm): 97.5/2.5

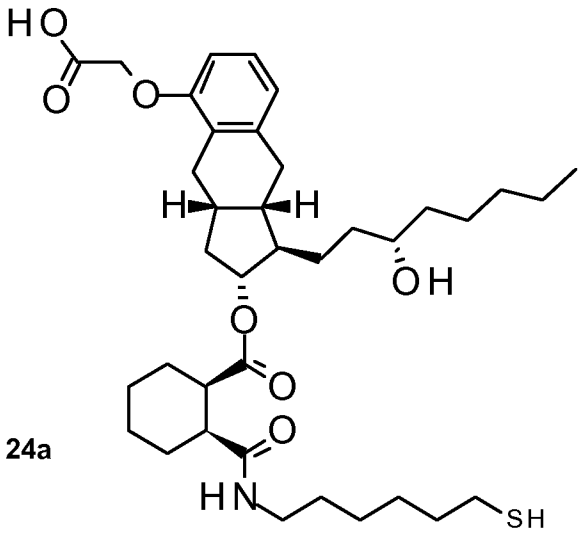
Example 34:

Improved synthesis of treprostinil linker thiol **24a**



23

1. EDC·HCl, DMAP, DCM
2. TFA, TES, HFIP



24a

Dmob protected treprostinil **8** (200 mg, 0.370 mmol), carboxylic acid **23** (294 mg, 0.555 mmol), EDC·HCl (248 mg, 1.295 mmol) and DMAP (158 mg, 1.295 mmol) were dissolved in DCM (2.9 mL, anhydrous, mol. sieve). The mixture was stirred at RT for 1 d. Ethyl acetate was added and the organic layer was washed with 0.1 N aqueous HCl (3x) and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure.

The residue was dissolved in HFIP (8 mL). After addition of TFA (200 µL) and TES (200 µL) the mixture was stirred for 30 min at RT. The solution was extracted with heptane (16 mL, 6x) and diluted with DCM (16 mL). Solution was extracted with water (16 mL, 3x). Combined water phases were back extracted with DCM (8 mL). The ombined DCM phases were evaporated under reduced pressure.

UPLC analysis revealed a 9/1 ratio of regioisomers **24a** and **24b** (column: Kinetex 100 x 2.1 mm, 1.7 µm XB-C18 silica, pore size 100 Å, Phenomonex Ltd, Aschaffenburg, Germany; flow rate 0.25 mL/min; solvent A: water + 0.05 % TFA (v/v), solvent B: acetonitrile + 0.04 % TFA; gradient: 30-58 % B (10 min), 58 % B isocratic (10 min), 58-80 % B (5 min), 80-99 % (5 min), wavelength 280 nm).

The residue was taken up in acetonitrile/water and **24a** was isolated by RP-HPLC (solvent A: H_2O + 0.01 % HCl, solvent B: MeCN + 0.01 % HCl, gradient: 57-62 % B over 15 min). Mixed fractions were subjected to repurification. Fractions containing pure **24a** were combined and lyophilized.
Yield **24a**: 98 mg (39 %)
MS: m/z 660.3 = $[M+H]^+$ (MW calculated = 659.9 g/mol).

Abbreviations

AcOH	acetic acid
AIB	2-Aminoisobutyric acid
BnBr	benzylbromide
Boc	tert-Butoxycarbonyl-
BSA	*N,O*-Bis-(trimethylsilyl)-acetamide
COMU	(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate
d	day
DIPEA	diisopropylethylamine
DCM	dichloromethane
DMAP	4-(Dimethylamino)pyridine
DMF	*N,N*-Dimethylformamide
Dmob	2,4-dimethoxybenzyl

Abbreviations

DMSO	dimethyl sulfoxide
Dpr	2,3-Diaminopropionic acid
EDC	*N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide
EDTA	ethylenediamine tetraacetic acid disodium salt dihydrate
EtOAc	ethyl acetate
eq	equivalent
h	Hour
HFIP	1,1,1,3,3,3-Hexafluoroisopropanol
HPLC	high performance liquid chromatography
LC/MS	mass spectrometry-coupled liquid chromatography
Mal	maleimido
MeOH	methanol
MeCN	acetonitrile
min	Minute
Mmt	4-Methoxytriphenylmethyl
mol.	Molecular
m/z	Mass/charge
NaOH	Sodium hydroxide
NHS	*N*-hydroxysuccinimide
PEG	Polyethylene glycol
Pfp	Pentafluorophenyl
PyBOB	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
PP	polypropylene
RT	room temperature
RP	reversed phase
sat.	saturated
soln.	solution
T	temperature
T3P	propyl phosphonic anhydride
TCP	2-Chlorotrityl chloride resin
TES	Triethylsilane
Trt	Trityl

Abbreviations

Tmob	2,4,6-trimethoxybenzyl
TMS	trimethylsilyl
TransCon	transiently conjugated
THF	tetrahydrofuran
TFA	trifluoroacetic acid
UPLC	ultra performance liquid chromatography
UV	ultra violet

Claims

1. A pharmaceutical composition comprising a prostacyclin compound, and optionally one or more pharmaceutically acceptable excipients, wherein the concentration of the prostacyclin compound is sufficient to maintain a therapeutically effective level of prostacyclin in blood plasma for at least 12 hours after a single subcutaneous or intramuscular injection.

2. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition comprises the prostacyclin compound in a concentration of at least 0.05 mg/ml.

3. The pharmaceutical composition comprising a prostacyclin compound of claim 1 or 2 in a concentration of at least 0.05 mg/ml, and wherein a single dose of the pharmaceutical composition comprises at least 0.05 mg of the prostacyclin compound.

4. The pharmaceutical composition of any one of claims 1 to 3, wherein the time period between administration of a pharmaceutical composition is at least about 12 hours, such as at least 16 hours, typically at least one day.

5. A pharmaceutical composition comprising a prostacyclin compound, and optionally one or more pharmaceutically acceptable excipients, wherein the pharmaceutical composition is characterized by having a pharmacokinetic profile in vivo in a mammal, in particular in a human, with substantially no burst of the prostacyclin compound.

6. The pharmaceutical composition of any one of claims 1 to 4, wherein the pharmaceutical composition is characterized by having a pharmacokinetic profile in vivo in a mammal, in particular in a human, with substantially no burst of the prostacyclin compound.

7. A pharmaceutical composition comprising a prostacyclin compound, and optionally one or more pharmaceutically acceptable excipients, wherein the pharmaceutical composition is characterized by exhibiting a peak to trough ratio of the prostacyclin

compound in a mammal, in particular in a human, of less than 5, such as less than 3, or less than 2.

8. The pharmaceutical composition of any one of claims 1 to 6, wherein the pharmaceutical composition is characterized by exhibiting a peak to trough ratio of the prostacyclin compound in a mammal, in particular in a human, of less than 5, such as less than 3, or less than 2.

9. A pharmaceutical composition comprising a prostacyclin compound, and optionally one or more pharmaceutically acceptable excipients, wherein the prostacyclin compound has an activity of < 20%, preferably < 10%, more preferably < 5% of the activity of free prostacyclin.

10. The pharmaceutical composition of any one of claims 1 to 8, wherein the prostacyclin compound has an activity of < 20%, preferably < 10%, more preferably < 5% of the activity of free prostacyclin.

11. A pharmaceutical composition comprising a prostacyclin prodrug, and optionally one or more pharmaceutically acceptable excipients, wherein the pharmaceutical composition is characterized in that after subcutaneous or intramuscular administration of said prostacyclin prodrug more than 50% of the administered prostacyclin dose is releasable within the blood compartment.

12. The pharmaceutical composition of any one of claims 1 to 10 wherein the prostacylin compound is a prostacyclin prodrug characterized in that after subcutaneous or intramuscular administration of said prostacyclin prodrug more than 50% of the administered prostacyclin dose is releasable within the blood compartment.

13. The pharmaceutical composition of any one of claims 1 to 10, wherein the prostacyclin compound is in a depot, preferably a polymer gel, even more preferably a hydrogel, most preferably a hydrated polymer matrix.

14. The pharmaceutical composition of claim 13, wherein the prostacyclin compound is covalently linked to a compound in the depot, preferably to the polymer gel, even more preferably to the hydrogel, most preferably tothe well hydrated polymer matrix.

15. The pharmaceutical composition of any of claims 1 to 15, wherein the prostacyclin compound is a prodrug of prostacyclin.

16. The pharmaceutical composition of claim 15, wherein the promoiety of the prodrug comprises a linear or branched PEG moiety.

17. A pharmaceutical composition comprising a prostacyclin prodrug, wherein said pharmaceutical composition is characterized in that the prostacyclin compound releases prostacyclin in a plasma-independet manner

18. The pharmaceutical composition of any one of claims 5 to 17, characterized by being administered by injection, preferably subcutaneous or intramuscular injection.

19. A pharmaceutical composition of any of claims 1 to 18, for use in the treatment or prevention of pulmonary hypertension, ischemic diseases, preferably peripheral vascular disease including peripheral arterial disease, Raynaud's phenomenon including Raynaud's disease and Raynaud's syndrome, scleroderma including systemic sclerosis, myocardial ischemia, ischemic stroke, renal insufficiency, ischemic ulcers including digital ulcers, heart failure, in particular congestive heart failure, portopulmonary hypertension, interstitial lung disease, idiopathic pulmonary fibrosis, conditions requiring anticoagulation, in particular post MI, post cardiac surgery, thrombotic microangiopathy, extracorporeal circulation, central retinal vein occlusion, atherosclerosis, inflammatory diseases, in particular COPD, psoriasis, hypertension, in particular preeclampsia, reproduction and parturition, cancer or other conditions of unregulated cell growth, cell/tissue preservation and other emerging therapeutic areas where prostacyclin treatment appears to have a beneficial role.

20. A method for treating pulmonary hypertension, wherein the method comprises the step of subcutaneous or intramuscular administration, to a patient with pulmonary hypertension, of a pharmaceutical composition comprising

(a) a polymer carrier-linked prostacyclin prodrug, and optionally one or more pharmaceutically acceptable excipients, or

(b) a prostacyclin compound and at least one polymer, and optionally one or more pharmaceutically acceptable excipients,

wherein the pharmaceutical composition releases therapeutically effective amounts of free prostacyclin compound for a period of time of at least 12 hours.

21. The method of claim 20, wherein the pharmaceutical composition of (b) comprises from about 0.05 to about 10weight percent the prostacyclin compound and from about 0.5 to about 20 weight percent total polymer content.