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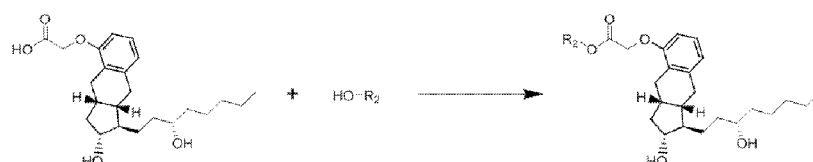
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(54) Title: METHODS OF MANUFACTURING TREPROSTINIL AND TREPROSTINIL DERIVATIVE PRODRUGS

FIGURE 1



(57) Abstract: Methods for making prodrugs of trepreostinil and treprostinil derivatives are provided. Specifically, methods are provided herein for producing prostacyclin compounds comprising treprostinil covalently linked to a linear C₅-C₁₈ alkyl, branched C₅-C₁₈ alkyl, linear C₂-C₁₈ alkenyl, branched C₃-C₁₈ alkenyl, aryl, aryl-C₁-C₁₈ alkyl or an amino acid or a peptide (e.g., dipeptide, tripeptide, tetrapeptide). The linkage, in one embodiment, is via an amide or ester bond. Prostacyclin compounds provided herein can also include at least one hydrogen atom substituted with at least one deuterium atom. The compounds provided herein can be used to treat pulmonary hypertension (e.g., pulmonary arterial hypertension) and portopulmonary hypertension.

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METHODS OF MANUFACTURING TREPROSTINIL AND TREPROSTINIL DERIVATIVE PRODRUGS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority from U.S. Provisional Application Serial No. 62/081,515, the disclosure of which is incorporated by reference herein in its entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] Pulmonary hypertension (PH) is characterized by an abnormally high blood pressure in the lung vasculature. It is a progressive, lethal disease that leads to heart failure and can occur in the pulmonary artery, pulmonary vein, or pulmonary capillaries. Symptomatically patients experience shortness of breath, dizziness, fainting, and other symptoms, all of which are made worse by exertion. There are multiple causes, and can be of unknown origin, idiopathic, and can lead to hypertension in other systems, for example, portopulmonary hypertension in which patients have both portal and pulmonary hypertension.

[0003] Pulmonary hypertension has been classified into five groups by the World Health Organization (WHO). Group I is called pulmonary arterial hypertension (PAH), and includes PAH that has no known cause (idiopathic), inherited PAH (*i.e.*, familial PAH or FPAH), PAH that is caused by drugs or toxins, and PAH caused by conditions such as connective tissue diseases, HIV infection, liver disease, and congenital heart disease. Group II pulmonary hypertension is characterized as pulmonary hypertension associated with left heart disease. Group III pulmonary hypertension is characterized as PH associated with lung diseases, such as chronic obstructive pulmonary disease and interstitial lung diseases, as well as PH associated with sleep-related breathing disorders (*e.g.*, sleep apnea). Group IV PH is PH due to chronic thrombotic and/or embolic disease, *e.g.*, PH caused by blood clots in the lungs or blood clotting disorders. Group V includes PH caused by other disorders or conditions, *e.g.*, blood disorders (*e.g.*, polycythemia vera, essential thrombocythemia), systemic disorders (*e.g.*, sarcoidosis, vasculitis), metabolic disorders (*e.g.*, thyroid disease, glycogen storage disease).

[0004] Pulmonary arterial hypertension (PAH) afflicts approximately 200,000 people globally with approximately 30,000-40,000 of those patients in the United States. PAH patients experience constriction of pulmonary arteries which leads to high pulmonary arterial

pressures, making it difficult for the heart to pump blood to the lungs. Patients suffer from shortness of breath and fatigue which often severely limits the ability to perform physical activity.

[0005] The New York Heart Association (NYHA) has categorized PAH patients into four functional classes, used to rate the severity of the disease. Class I PAH patients as categorized by the NYHA, do not have a limitation of physical activity, as ordinary physical activity does not cause undue dyspnoea or fatigue, chest pain, or near syncope. Treatment is not needed for class I PAH patients. Class II PAH patients as categorized by the NYHA have a slight limitation on physical activity. These patients are comfortable at rest, but ordinary physical activity causes undue dyspnoea or fatigue, chest pain or near syncope. Class III PAH patients as categorized by the NYHA have a marked limitation of physical activity. Although comfortable at rest, class III PAH patients experience undue dyspnoea or fatigue, chest pain or near syncope as a result of less than ordinary physical activity. Class IV PAH patients as categorized by the NYHA are unable to carry out any physical activity without symptoms. Class IV PAH patients might experience dyspnoea and/or fatigue at rest, and discomfort is increased by any physical activity. Signs of right heart failure are often manifested by class IV PAH patients.

[0006] Patients with PAH are treated with an endothelin receptor antagonist (ERA), phosphodiesterase type 5 (PDE-5) inhibitor, a guanylate cyclase stimulator, a prostanoid (*e.g.*, prostacyclin), or a combination thereof. ERAs include abrisentan (Letairis®), sitaxentan, bosentan (Tracleer®), and macitentan (Opsumit®). PDE-5 inhibitors indicated for the treatment of PAH include sildenafil (Revatio®), tadalafil (Adcirca®). Prostanoids indicated for the treatment of PAH include iloprost, epoprostenol and treprostil (Remodulin®, Tyvaso®). The one approved guanylate cyclase stimulator is riociguat (Adempas®). Additionally, patients are often treated with combinations of the aforementioned compounds.

[0007] Portopulmonary hypertension is defined by the coexistence of portal and pulmonary hypertension, and is a serious complication of liver disease. The diagnosis of portopulmonary hypertension is based on hemodynamic criteria: (1) portal hypertension and/or liver disease (clinical diagnosis-ascites/varices/splenomegaly), (2) mean pulmonary artery pressure > 25 mmHg at rest, (3) pulmonary vascular resistance > 240 dynes s/cm⁵, (4) pulmonary artery occlusion pressure < 15mmHg or transpulmonary gradient > 12 mmHg. PPH is a serious

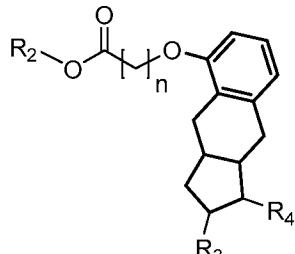
complication of liver disease, and is present in 0.25 to 4% of patients suffering from cirrhosis. Today, PPH is comorbid in 4-6% of those referred for a liver transplant.

[0008] Despite there being treatments for PAH and PPH, the current prostacyclin therapies are associated with severe toxicity and tolerability issues, as well as the requirement for inconvenient dosing schedules. The present invention overcomes addresses these factors by providing compounds and that provide for less toxicity, better tolerability and more convenient dosing schedules, and methods for manufacturing the same.

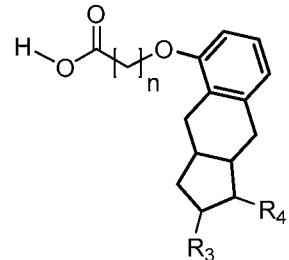
SUMMARY OF THE INVENTION

[0009] Methods for the manufacture of treprostinil prodrugs and treprostinil derivative prodrugs are provided herein, e.g., compounds of the Formulae (I), (II) or (III). The treprostinil or treprostinil derivative prodrug, in one embodiment, comprises an ester or amide linkage to the prodrug moiety.

[0010] One aspect of the invention relates to the synthesis of a carboxylic acid derivative of treprostinil. In one embodiment, a treprostinil ester derivative of the formula

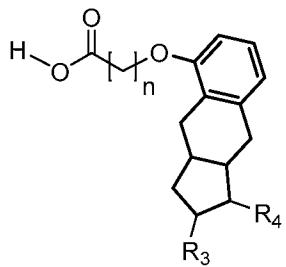


is esterified by mixing the appropriate alcohol (*i.e.*, R₂-OH where the R₂ is a linear or branched C₅-C₁₈ alkyl, a linear C₂-C₁₈ alkenyl or a branched C₃-C₁₈



alkenyl) with treprostinil or a compound of the formula in the presence of an acid catalyst. The acid catalyst in one embodiment is a resin or in some other solid form. The acid catalyst in one embodiment is sulfuric acid or sulfonic acid. Other acid catalysts (in solid, *e.g.*, a resin, or liquid form) include but are not limited to hydrofluoric acid, phosphoric acid, toluenesulfonic acid, polystyrene sulfonate, heteropoly acid, zeolites, metal oxides, and graphene oxygen.

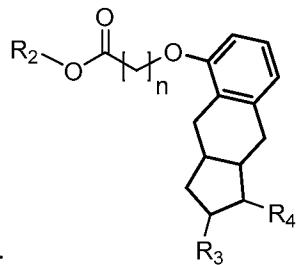
[0011] In some embodiments, the treprostinil or treprostinil compound of the formula



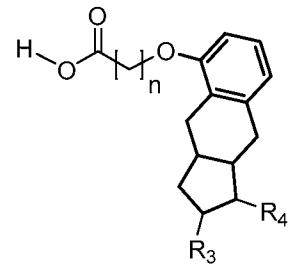
(where R₃, R₄ and n are defined above) and/or alcohol R₂-OH is dissolved in a solvent prior to the esterification reaction.

[0012] In another embodiment, the Mitsunobu reaction can be used, where a mixture of triphenylphosphine (PPh₃) and diisopropyl azodicarboxylate (DIAD or its diethyl analogue, DEAD) convert an alcohol and carboxylic acid to the ester to form one of the carboxylic acid ester prodrugs provided herein.

[0013] In yet another embodiment, N, N'-dicyclohexylcarbodiimide (DCC) or N, N'-diisopropylcarbodiimide (DIC) is used in combination with 4-dimethylaminopyridine (DMAP) in an esterification reaction (sometimes referred to as Steglich esterification).

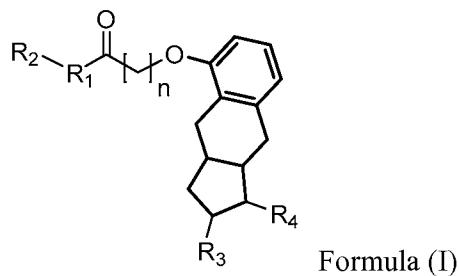


[0014] Treprostinil amide derivatives (e.g., of the formula:) can be manufactured according to well known protocols of amide functionalization of a carboxylic



acid group. For example, treprostinil (or a compound of the formula (for example, dissolved in dioxane) is combined with HATU or PyBOP and alkylamine R₂-NH₂. R₂, R₃, R₄ and n are defined herein.

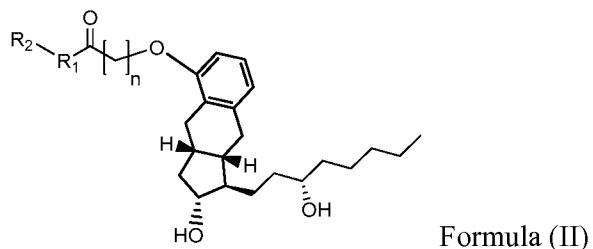
[0015] The methods provided herein in one embodiment, are used to manufacture a prostacyclin compound of Formula (I), or a pharmaceutically acceptable salt:



Formula (I)

wherein R₁ is NH, O or S; R₂ is H, a linear C₅-C₁₈ alkyl, branched C₅-C₁₈ alkyl, linear C₂-C₁₈ alkenyl, branched C₃-C₁₈ alkenyl, aryl; aryl-C₁-C₁₈ alkyl; an amino acid or a peptide; R₃ is H, OH, O-alkyl or O-alkenyl; R₄ is an optionally substituted linear or branched C₁-C₁₅ alkyl, or an optionally substituted linear or branched C₂-C₁₅ alkenyl; and n is an integer from 0 to 5, with the proviso that the prostacyclin compound is not treprostinil.

[0016] In another embodiment, a method provided herein is used to manufacture a prostacyclin compound of Formula (II), or a pharmaceutically acceptable salt:

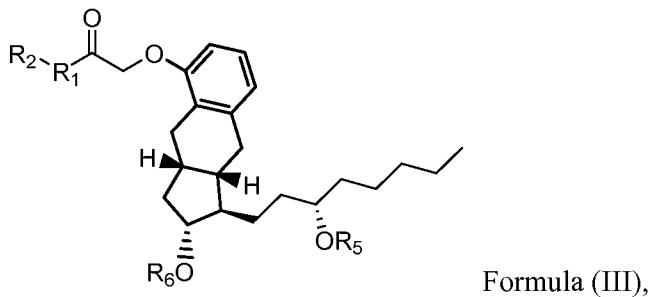


Formula (II)

wherein R₁ is NH, O or S; R₂ is a linear or branched C₅-C₁₈ alkyl, a linear C₂-C₁₈ alkenyl or a branched C₃-C₁₈ alkenyl, aryl, aryl-C₁-C₁₈ alkyl, an amino acid or a peptide; and n is an integer from 0 to 5.

[0017] In one embodiment, a compound of Formula (I) and/or (II) is manufactured by a method described herein, wherein one or more hydrogen atoms is substituted with a deuterium. Accordingly, in one embodiment, the present invention relates to an isotopologue of Formula (I) and/or (II), substituted with one or more deuterium atoms. The isotopologue of Formula (I) and/or (II) may be used to accurately determine the concentration of compounds of Formula (I) and/or (II) in biological fluids and to determine metabolic patterns of compounds of Formula (I) and/or (II) and its isotopologues.

[0018] Yet another embodiment of the invention relates to a method for manufacturing the prostacyclin compound of Formula (III), or a pharmaceutically acceptable salt,:



wherein R₁ and R₂ are defined above, and

R₅ and R₆ are independently selected from H, optionally substituted linear or branched C₁-C₁₅ alkyl, optionally substituted linear or branched C₂-C₁₅ alkenyl, (C=O)-optionally substituted linear or branched C₁-C₁₅ alkyl, or (C=O)-optionally substituted linear or branched C₂-C₁₅ alkenyl, with the proviso that the prostacyclin compound of Formula (III) is not treprostinil.

BRIEF DESCRIPTION OF THE FIGURES

[0019] Figure 1 is an esterification scheme for an alkyl ester-TR prodrug compound provided herein.

[0020] Figure 2 is a general scheme for synthesis of acylated treprostinil derivative prodrugs.

DETAILED DESCRIPTION OF THE INVENTION

[0021] The term “alkyl” as used herein refers to both a straight chain alkyl, wherein alkyl chain length is indicated by a range of numbers, and a branched alkyl, wherein a branching point in the chain exists, and the total number of carbons in the chain is indicated by a range of numbers. In exemplary embodiments, “alkyl” refers to an alkyl chain as defined above containing 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 carbons (*i.e.*, C₆-C₁₆ alkyl).

[0022] The term “alkenyl” as used herein refers to a carbon chain containing one or more carbon-carbon double bonds.

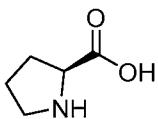
[0023] The term “aryl” as used herein refers to a cyclic hydrocarbon, where the ring is characterized by delocalized π electrons (aromaticity) shared among the ring members, and wherein the number of ring atoms is indicated by a range of numbers. In exemplary embodiments, “aryl” refers to a cyclic hydrocarbon as described above containing 6, 7, 8, 9, or 10 ring atoms (*i.e.*, C₆-C₁₀ aryl). Examples of an aryl group include, but are not limited to, benzene, naphthalene, tetralin, indene, and indane.

[0024] The term “alkoxy” as used herein refers to $-\text{O}-(\text{alkyl})$, wherein “alkyl” is as defined above.

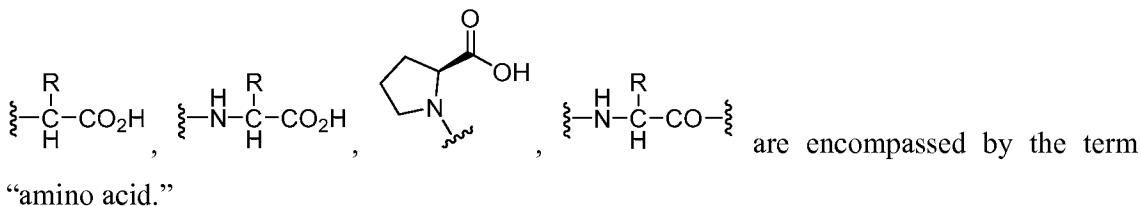
[0025] The term “substituted” in connection with a moiety as used herein refers to a further substituent which is attached to the moiety at any acceptable location on the moiety. Unless otherwise indicated, moieties can bond through a carbon, nitrogen, oxygen, sulfur, or any other acceptable atom.

[0026] The term “amino acid” refers to both natural (genetically encoded) and non-natural (non-genetically encoded) amino acids, and moieties thereof. Of the twenty natural amino

acids, 19 have the general structure: $\text{H}_2\text{N}-\underset{\text{H}}{\text{C}}-\text{R}-\text{CO}_2\text{H}$, where R is the amino acid sidechain. The 20th amino acid, proline, is also within the scope of the present invention, and has the



following structure: Of the twenty natural amino acids, all but glycine is chiral, and both the D- and L- amino acid isomers, as well as mixtures thereof, are amenable for use with the prostacyclin compounds described herein. It is also noted that an amino acid moiety is encompassed by the term "amino acid." For example, the amino acid moieties



[0027] Examples of non-natural amino acids amenable for use with the present invention include β -alanine (β -Ala); 2,3-diaminopropionic acid (Dpr); nipecotic acid (Nip); pipecolic acid (Pip); ornithine (Om); citrulline (Cit); t-butylalanine (t-BuA); 2-tbutylglycine (t-BuG); N-methylisoleucine (Melle); phenylglycine (PhG); cyclohexylalanine (ChA); norleucine (Nle); naphthylalanine (Nal); 4-chlorophenylalanine (Phe(4-Cl)); 2-fluorophenylalanine (Phe(2-F)); 3-fluorophenylalanine (Phe(3-F)); 4-fluorophenylalanine (Phe(4-F)); penicillamine (Pen); 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic); β -2-thienylalanine (Thi); methionine sulfoxide (MSO); homoarginine (hArg); N-acetyllysine (AcLys); 2,4-diaminobutyric acid (Dbu); 2,3-diaminobutyric acid (Dab); p-aminophenylalanine (Phe (pNH₂)); N-methyl valine (MeVal); homocysteine (hCys), homophenylalanine (hPhe); homoserine (hSer); hydroxyproline (Hyp); homoproline (hPro);

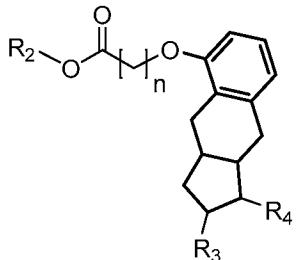
and the corresponding D-enantiomer of each of the foregoing. Other non-genetically encoded amino acid residues include 3-aminopropionic acid; 4-aminobutyric acid; isonipeptic acid (Inp); aza-pipeolic acid (azPip); aza-proline (azPro); α -aminoisobutyric acid (Aib); ϵ -aminohexanoic acid (Aha); δ -aminovaleric acid (Ava); N-methylglycine (MeGly).

[0028] A “peptide” is a polymer of amino acids (or moieties thereof) linked by a peptide bond. Peptides for use with the present invention, comprise from about two to about fifteen amino acids, for example, two, three, four, five, six, seven, eight, nine or ten amino acids (or moieties thereof).

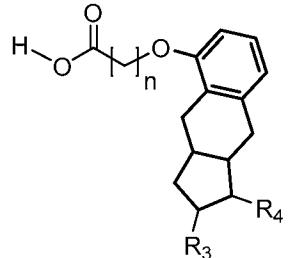
[0029] The term “salt” or “salts” as used herein encompasses pharmaceutically acceptable salts commonly used to form alkali metal salts of free acids and to form addition salts of free bases. The nature of the salt is not critical, provided that it is pharmaceutically acceptable. Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Exemplary pharmaceutical salts are disclosed in Stahl, P.H., Wermuth, C.G., Eds. *Handbook of Pharmaceutical Salts: Properties, Selection and Use*; Verlag Helvetica Chimica Acta/Wiley-VCH: Zurich, 2002, the contents of which are hereby incorporated by reference in their entirety. Specific non-limiting examples of inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Appropriate organic acids include, without limitation, aliphatic, cycloaliphatic, aromatic, arylaliphatic, and heterocyclyl containing carboxylic acids and sulfonic acids, for example formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, stearic, salicylic, *p*-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, toluenesulfonic, 2-hydroxyethanesulfonic, sulfanilic, cyclohexylaminosulfonic, algenic, 3-hydroxybutyric, galactaric or galacturonic acid. Suitable pharmaceutically acceptable salts of free acid-containing compounds disclosed herein include, without limitation, metallic salts and organic salts. Exemplary metallic salts include, but are not limited to, appropriate alkali metal (group Ia) salts, alkaline earth metal (group IIA) salts, and other physiological acceptable metals. Such salts can be made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc. Exemplary organic salts can be made from primary amines, secondary amines, tertiary amines and quaternary ammonium salts, for example, tromethamine, diethylamine, tetra-*N*-methylammonium, *N,N'*-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (*N*-methylglucamine) and procaine.

[0030] Methods are provided herein for the synthesis of treprostinil prodrugs, as well as treprostinil derivative prodrugs, for example prodrugs of Formulae (I), (II) and (III). The prodrugs find utility in the treatment of pulmonary hypertension, for example, pulmonary arterial hypertension and portopulmonary hypertension, as well as other indicaitons, as described in U.S. Patent Application Publication No. 2015/0148414, published May 28, 2015, the disclosure of which is incorporated by reference in its entirety for all purposes. For example, the treprostinil derivative prodrug or treprostinil prodrug, or a composition comprising the same, is effective when employed in a once-daily, twice-daily or three-times daily dosing regimen, for example, for the treatment of pulmonary arterial hypertension or portopulmonary hypertension in a patient in need thereof. The prostacyclin compound provided herein, in one embodiment, can be administered less frequently than treprostinil, with equal or greater efficacy. Moreover, in one embodiment, the side effect profile of the compounds provided herein is less deleterious than the side effect profile resulting from treprostinil administration.

[0031] One aspect of the invention relates to the synthesis of a carboxylic acid derivative of treprostinil. In one embodiment, a treprostinil ester derivative of the formula



is esterified by mixing the appropriate alcohol (*i.e.*, R₂-OH where the R₂ is a linear or branched C₅-C₁₈ alkyl, a linear C₂-C₁₈ alkenyl or a branched C₃-C₁₈



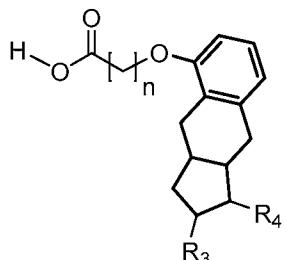
alkenyl) with treprostinil or a compound of the formula in the presence of an acid catalyst. As provided herein, R₃ is H, OH, optionally substituted linear or branched C₁-C₁₅ alkoxy, O-optionally substituted linear or branched C₂-C₁₅ alkenyl, O-(C=O)-optionally substituted linear or branched C₁-C₁₅ alkyl, or O-(C=O)-optionally substituted linear or branched C₂-C₁₅ alkenyl; R₄ is an optionally substituted linear or branched C₁-C₁₅ alkyl, or an optionally substituted linear or branched C₂-C₁₅ alkenyl; and n is

an integer from 0 to 5. It will be appreciated by one of ordinary skill in the art that the purity of the final product will depend in part on the purity of the reagents employed in the esterification reaction, and/or the cleanup procedure after the reaction has completed. For example, a high purity alcohol will give a higher purity treprostinil ester derivative than a lower purity alcohol. Similarly, a higher purity product is obtained through clean-up procedures such as HPLC, diafiltration, etc.

[0032] The acid catalyst in one embodiment is a resin or in some other solid form. However, in other embodiment, the acid catalyst is in liquid form. The acid catalyst in one embodiment is sulfuric acid or sulfonic acid. Other acid catalysts (in solid, e.g., a resin, or liquid form) include but are not limited to hydrofluoric acid, phosphoric acid, toluenesulfonic acid, polystyrene sulfonate, heteropoly acid, zeolites, metal oxides, and graphene oxygene.

[0033] Acid catalyst resins, e.g., sulfonic acid resin catalysts are available commercially, e.g., from Sigma-Aldrich, under the trade name AMBERLYST. Other resins are available commercially, e.g., from Purolite®, and are amenable for use with the methods described herein.

[0034] In some embodiments, the treprostinil or the treprostinil compound of the formula



(where R₃, R₄ and n are defined above) and/or alcohol R₂-OH is dissolved in a solvent prior to the esterification reaction. For example, in one embodiment, where treprostinil is esterified with an alkyl group having 12 carbon atoms or more, treprostinil is first dissolved in a solvent such as dioxane prior to the esterification reaction. Other solvents besides dioxane, or in combination with dioxane can also be used. For example, acetonitrile (MeCN), N,N'-dimethylformamide (DMF), dichloromethane (DCM), or a combination thereof can be used. Various examples of solvents are provided in Table 1 below.

Table 1
Solvents amenable for use in esterification reactions.
Dioxane

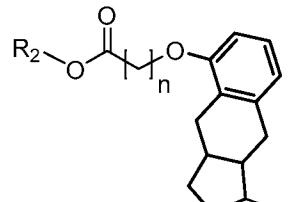
Dioxane (2 mL/100 μ mol TRP)
Dioxane (1 mL/100 μ mol TRP)
DMF
DCM
MeCN
1:1 Dioxane:MeCN
DMF/DCM
10% DMF/DCM
20% DMF/DCM

[0035] Carboxylic acid esterification reactions other than the ones described above are known to those of ordinary skill in the art and are amenable for use in manufacturing the treprostinil alkyl esters described herein. For example, the Mitsunobu reaction can be used, where a mixture of triphenylphosphine (PPh_3) and diisopropyl azodicarboxylate (DIAD or its diethyl analogue, DEAD) convert an alcohol and carboxylic acid to the ester. In this reaction, the DIAD is reduced as it serves as the hydrogen acceptor, and the PPh_3 is oxidized to OPPh_3 .

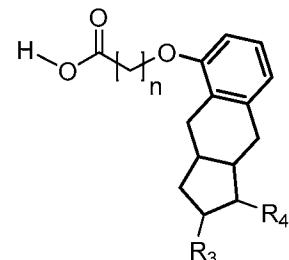
[0036] In yet another embodiment, N, N'-dicyclohexylcarbodiimide (DCC) or N, N'-diisopropylcarbodiimide (DIC) is used in combination with 4-dimethylaminopyridine (DMAP) (additive) in an esterification reaction (sometimes referred to as Steglich esterification). In this reaction, DCC or DIC and the carboxylic acid (treprostinil or its non-esterified derivative) are able to form an O-acylisourea activated carboxylic acid intermediate. The alcohol is added to the activated compound to form the stable dicyclohexylurea and the ester. In one embodiment, the treprostinil or its non-esterified derivative is first dissolved in solvent, *e.g.*, one of the solvents described above, prior to performing the Steglich esterification.

[0037] Other esterification reactions can be employed. For example, 1-[Bis (dimethylamino) methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxid hexafluorophosphate (HATU) or benzotriazol-1-yl-oxytritypyrrolidinophosphonium hexafluorophosphate (PyBOP) can be used as a coupling reagent. These reagents can be used with or without an additive to facilitate the coupling. For example, triethylamine (TEA) can be used in some embodiments in conjunction with either HATU or PyBOP coupling reagents to form a treprostinil alkyl ester. As with the other esterification reactions described herein, the treprostinil or non-esterified treprostinil derivative can first be dissolved in solvent prior to performing the esterification reaction.

[0038] In yet another embodiment, an esterification of treprostinil or a treprostinil derivative proceeds through steps 1 through 5 of the reaction scheme set forth in Example 3 of PCT Publication No. WO 2011/153363, incorporated by reference herein in its entirety for all purposes.



[0039] Treprostinil amide derivatives (e.g., of the formula:) can be manufactured according to protocols of amide functionalization of a carboxylic acid group.



For example, treprostinil (or a compound of the formula (for example, dissolved in dioxane) is combined with HATU or PyBOP and alkylamine R2-NH2. R2, R3, R4 and n are defined above.

[0040] Other reaction conditions for forming treprostinil amide derivatives with alkylamine R2-NH2, are provided below in Table 2.

Table 2

<u>Entry</u>	<u>Solvent</u>	<u>Time</u>	<u>Coupling Reagent</u>	<u>Additive</u>	<u>Base</u>	<u>Amine (Equiv)</u>	<u>Amine Delay</u>
1	10% DMF/DCM	68 h	DCC	DMAP	--	5.0	--
2	Dioxane	68 h	DSC	--	--	5.0	30 min
3	Dioxane	92 h	DSC	--	--	5.0	68 h
4	Dioxane	68 h	--	--	--	5.0	--
5	Dioxane	92 h	DSC	--	DIPEA	5.0	68 h
6	10% DMF/DCM	68 h	MIBA	Mol. Sieve	--	1.0	--
7	10% DMF/DCM	68 h	DSC	--	--	5.0	30 min
8	Dioxane	18 h	DCC	--	--	5.0	--
9	Dioxane	48 h	DSC	--	DIPEA	5.0	24 h
10	10% DMF/DCM	18 h	DCC	--	--	5.0	--
11	DMF	18 h	DCC	--	--	5.0	--

Table 2

<u>Entry</u>	<u>Solvent</u>	<u>Time</u>	<u>Coupling Reagent</u>	<u>Additive</u>	<u>Base</u>	<u>Amine (Equiv)</u>	<u>Amine Delay</u>
12	DMF	48 h	DSC	--	DIPEA	5.0	24 h
13	Dioxane	48 h	DCC	DMAP	--	10.0	--
14	Dioxane	48 h	DCC	--	--	10.0	--
15	Dioxane	115 h	DSC	--	DIPEA	5.0	91 h
16	Dioxane	18 h	DSC	DMAP	DIPEA	5.0	150 m
17	Dioxane	115 h	DSC	DMAP	DIPEA	5.0	91 h
18	Dioxane	86 h	DSC	--	DIPEA	7.5	68 h
19	DMF	1 h	HATU	--	DIPEA	1.2	--
20	DMF	1 h	PyBOP	--	DIPEA	1.2	--
21	1:2 Dioxane:MeCN	1h	PyBOP	--	DIPEA	1.2	--
22	Dioxane	18 h	DCC	HOEt	DIPEA	1.2	--
23	Dioxane	18 h	DIC	HOEt	DIPEA	1.2	--
24	Dioxane	18 h	EDC	HOEt	DIPEA	1.2	--
25	Dioxane	48 h	DCC	NHS	DIPEA	1.2	24 h
26	Dioxane	48 h	DIC	NHS	DIPEA	1.2	24 h
27	Dioxane	48 h	EDC	NHS	DIPEA	1.2	24 h
28	Dioxane	48 h	DCC	PfpOH	DIPEA	1.2	24 h
29	Dioxane	48 h	DIC	PfpOH	DIPEA	1.2	24 h
30	Dioxane	48 h	EDC	PfpOH	DIPEA	1.2	24 h

DCC = N,N'-Dicyclohexylcarbodiimide

DIC = N,N'-Diisopropylcarbodiimide

DSC = N,N'-Disuccinimidyl carbonate

DIPEA = N,N-Diisopropylethylamine

DMF = N,N'-dimethylformamide

EDC = N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride

HATU = 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate

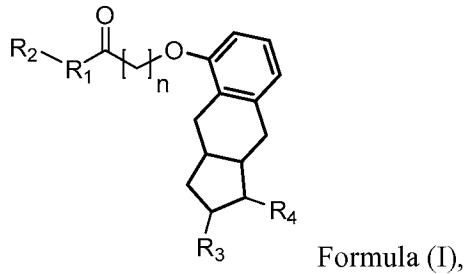
HOEt = 1-Hydroxybenzotriazole hydrate

MIBA = 5-methoxy-2-iodophenylboronic acid

PyBOP = benzotriazol-1-yl-oxytritypyrrolidinophosphonium hexafluorophosphate

PfpOH = 2,2,3,3,3-Pentafluoro-1-propanol

[0041] In one aspect of the invention described herein, a prostacyclin compound of Formula (I), or a pharmaceutically acceptable salt thereof, is manufactured by a method described herein:



wherein R₁ is NH, O or S;

R₂ is H, a linear C₅-C₁₈ alkyl, branched C₅-C₁₈ alkyl, linear C₂-C₁₈ alkenyl, branched C₃-C₁₈ alkenyl, aryl, aryl-C₁-C₁₈ alkyl; an amino acid or a peptide;

R₃ is H, OH, optionally substituted linear or branched C₁-C₁₅ alkoxy, O-optionally substituted linear or branched C₂-C₁₅ alkenyl, O-(C=O)-optionally substituted linear or branched C₁-C₁₅ alkyl, or O-(C=O)-optionally substituted linear or branched C₂-C₁₅ alkenyl;

R₄ is an optionally substituted linear or branched C₁-C₁₅ alkyl, or an optionally substituted linear or branched C₂-C₁₅ alkenyl; and

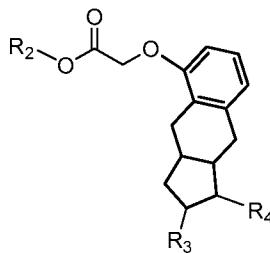
n is an integer from 0 to 5, with the proviso that the prostacyclin compound of Formula (I) is not treprostinil.

[0042] In a further embodiment, a prostacyclin compound of Formula (I) is manufactured, wherein R₃ is OH and n is 0 or 1. In even a further embodiment, R₄ is an optionally substituted linear or branched C₁-C₁₅ alkyl. In even a further embodiment, R₁ is NH or O.

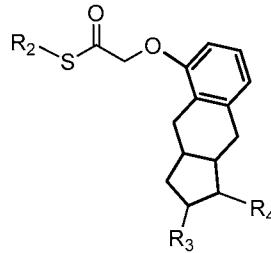
[0043] In one embodiment, a prostacyclin compound of Formula (I) is manufactured, wherein R₁ is NH, O or S; R₂ is a linear C₅-C₁₈ alkyl, branched C₅-C₁₈ alkyl, linear C₂-C₁₈ alkenyl, branched C₃-C₁₈ alkenyl; R₃ is H, OH or O-alkyl; R₄ is an optionally substituted linear or branched C₁-C₁₅ alkyl, or an optionally substituted linear or branched C₂-C₁₅ alkenyl; and n is an integer from 0 to 5. In even a further embodiment, R₁ is NH or O and R₂ is a linear C₅-C₁₈ alkyl or a branched C₅-C₁₈ alkyl.

[0044] In one embodiment, R₂ is aryl or aryl-C₁-C₁₈ alkyl; R₃ is OH and n is 0 or 1. In even a further embodiment, R₄ is an optionally substituted linear or branched C₁-C₁₅ alkyl.

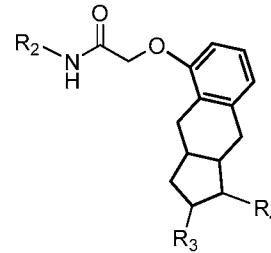
[0045] In one embodiment, the present invention provides a method for manufacturing a prostacyclin compound of Formula (I), wherein the compound is a compound of one of Formulae (Ia), (Ib), (Ic) or (Id), or a pharmaceutically acceptable salt thereof:



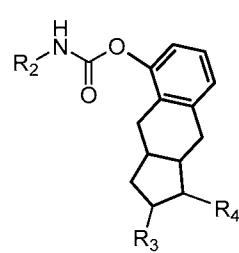
Formula (Ia)



Formula (Ib)



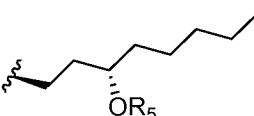
Formula (Ic)

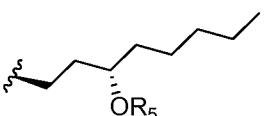


Formula (Id)

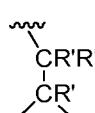
wherein, R₂ is H, a linear or branched C₅-C₁₈ alkyl, linear C₂-C₁₈ alkenyl, or a branched C₃-C₁₈ alkenyl;

R₃ is H, OH, optionally substituted linear or branched C₁-C₁₅ alkoxy, O-optionally substituted linear or branched C₂-C₁₅ alkenyl, -O(C=O)-optionally substituted linear or branched C₁-C₁₅ alkyl, or -O(C=O)-optionally substituted linear or branched C₂-C₁₅ alkenyl; and

R₄ is  , an optionally substituted linear or branched C₁-C₁₅ alkyl, or an optionally substituted linear or branched C₂-C₁₅ alkenyl, where R₅ is H, optionally substituted linear or branched C₁-C₁₅ alkyl, optionally substituted linear or branched C₂-C₁₅ alkenyl, (C=O)-optionally substituted linear or branched C₁-C₁₅ alkyl, or (C=O)-optionally substituted linear or branched C₂-C₁₅ alkenyl. In a further embodiment, R₄ is

 , with the proviso that the compound is not treprostinil, *i.e.*, R₂ and R₅ cannot both be H.

[0046] In one embodiment of Formula (Ia), Formula (Ib), Formula (Ic) and Formula (Id), R₂

is a linear or branched C₅-C₁₈ alkyl. In even a further embodiment, R₂ is $(\text{---} \text{C}(\text{R}')\text{---})^{m1} \text{---} \text{C}(\text{R}')\text{---})^{m2}$ or  , where m₁ and m₂ are each independently an integer selected from 1 to 9 and each

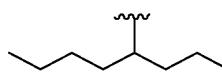
occurrence of R' is independently H, a linear or branched C₁-C₈ alkyl, or a linear or branched

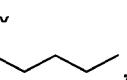
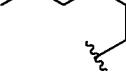
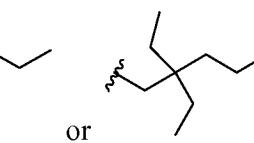
C₁-C₈ alkenyl. In even a further embodiment, R₂ is $m_1^{m_1} (\text{ })^{m_2} \text{ }^{m_2}$ and m₁ and m₂ are both 4.

In another embodiment, R₂ is $m_1^{m_1} (\text{ })^{m_2} \text{ }^{m_2}$ and m₁ is 3 and m₂ is 4, or m₁ is 2 and m₂ is 3.

[0047] When m₁ and/or m₂ is an integer from 2-9, the m₁/m₂ at the end of the carbon chain is CH₃, while the remaining m₁/m₂ groups are CH₂.

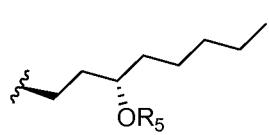
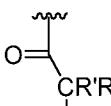
[0048] In one embodiment of Formula (Ia), Formula (Ib), Formula (Ic) and Formula (Id), R₂

is  ,  ,  ,  ,

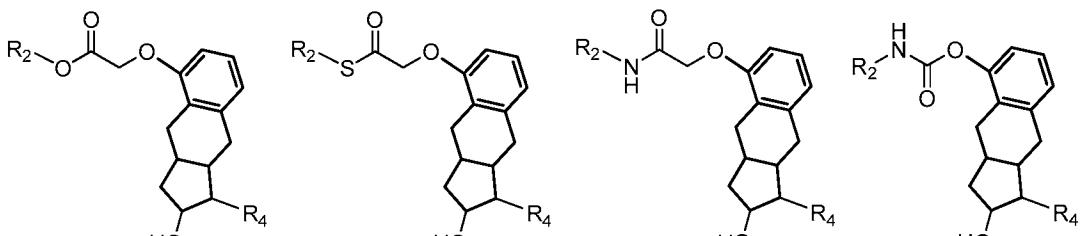
 ,  ,  or  . In a further

embodiment, R₃ is OH and R₄ is  , where R₅ is H, optionally substituted linear or branched C₁-C₁₅ alkyl, optionally substituted linear or branched C₂-C₁₅ alkenyl, (C=O)-optionally substituted linear or branched C₁-C₁₅ alkyl, or (C=O)-optionally substituted linear or branched C₂-C₁₅ alkenyl.

[0049] In one embodiment of Formulae (Ia), (Ib), (Ic) or (Id), R₂ is H, R₃ is OH and R₄ is

 , and R₅ is $m_1^{m_1} (\text{ })^{m_2} \text{ }^{m_2}$ or  , where m₁ and m₂ are each independently an integer selected from 1 to 9 and each occurrence of R' is independently H, a linear or branched C₁-C₈ alkyl, or a linear or branched C₁-C₈ alkenyl. When m₁ and/or m₂ is an integer from 2-9, the m₁/m₂ at the end of the carbon chain is CH₃, while the remaining m₁/m₂ groups are CH₂.

[0050] In another embodiment, a method for manufacturing a prostacyclin compound of one of Formula (Ia), (Ib), (Ic) or (Id) is provided wherein R₃ is OH, as provided in one of Formulae (Ia'), (Ib'), (Ic') or (Id'):



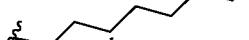
Formula (Ia')

Formula (Ib')

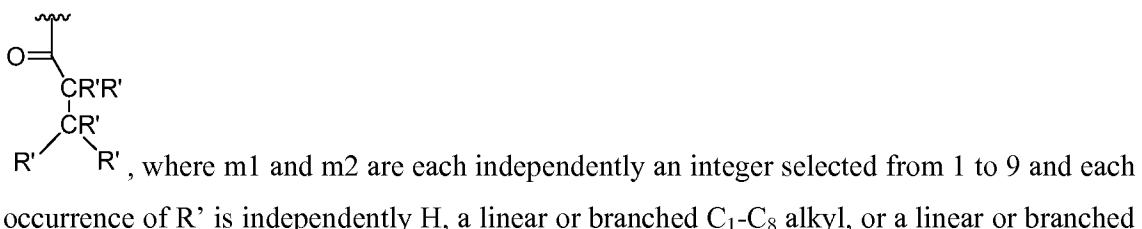
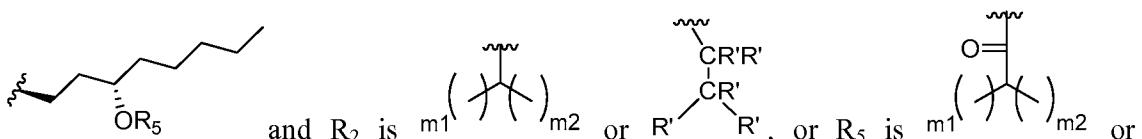
Formula (Ic')

Formula (Id')

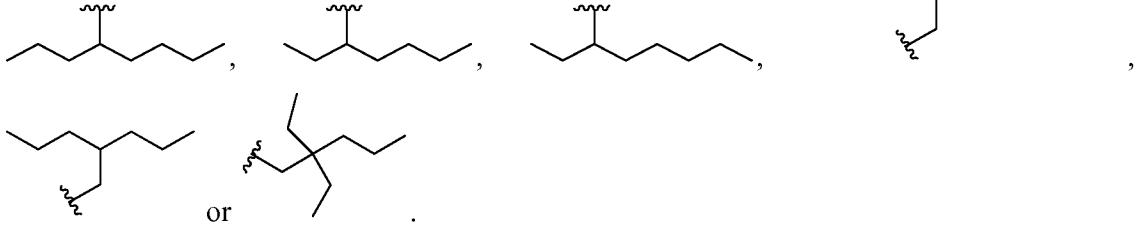
wherein, R₂ is H, a linear or branched C₅-C₁₈ alkyl, or a linear or branched C₅-C₁₈ alkenyl;

and R_4 is  , an optionally substituted linear or branched C_1 - C_{15} alkyl, or an optionally substituted linear or branched C_2 - C_{15} alkenyl, wherein R_5 is H, optionally substituted linear or branched C_1 - C_{15} alkyl, optionally substituted linear or branched C_2 - C_{15} alkenyl, (C=O)-optionally substituted linear or branched C_1 - C_{15} alkyl, or (C=O)-optionally substituted linear or branched C_2 - C_{15} alkenyl, with the proviso that R_2 and R_5 are not both H.

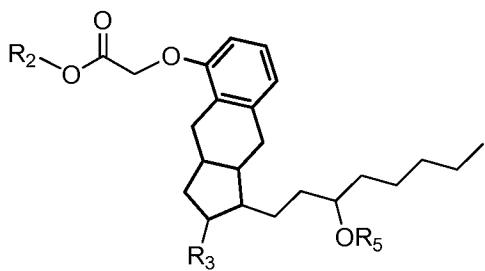
In one embodiment of Formula (Ia'), Formula (Ib'), Formula (Ic') and Formula (Id'), R_4 is



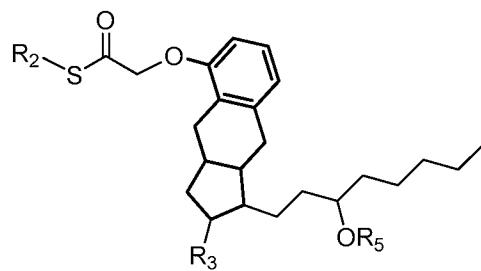
C₁-C₈ alkenyl. In even a further embodiment, R₂ is



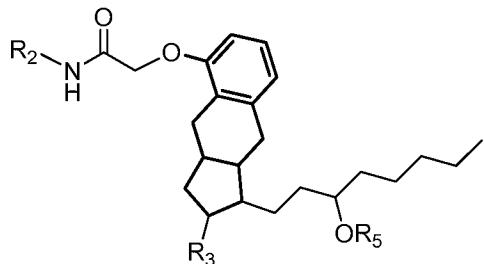
[0051] Yet another embodiment of the invention relates to a method for manufacturing a prostacyclin compound of one of Formula (Ia''), (Ib''), (Ic'') or (Id''), or a pharmaceutically acceptable salt thereof:



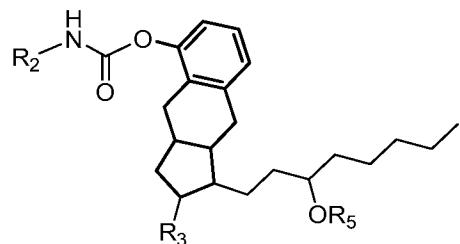
Formula (Ia'')



Formula (Ib'')



Formula (Ic'')



Formula (Id'')

wherein,

R_2 is H, a linear or branched C_5 - C_{18} alkyl, linear C_2 - C_{18} alkenyl, branched C_3 - C_{18} alkenyl, aryl, aryl- C_1 - C_{18} alkyl; an amino acid or a peptide; and

R_3 is H, OH, optionally substituted linear or branched C_1 - C_{15} alkoxy, O-optionally substituted linear or branched C_2 - C_{15} alkenyl, O-(C=O)-optionally substituted linear or branched C_1 - C_{15} alkyl, or O-(C=O)-optionally substituted linear or branched C_2 - C_{15} alkenyl; and

R_5 is H, optionally substituted linear or branched C_1 - C_{15} alkyl, optionally substituted linear or branched C_2 - C_{15} alkenyl, (C=O)-optionally substituted linear or branched C_1 - C_{15} alkyl, or (C=O)-optionally substituted linear or branched C_2 - C_{15} alkenyl, with the proviso that R_2 and R_5 are not both H. In a further embodiment, R_3 is OH and R_2 is 5-nonanyl, 4-heptyl, 4-octyl, 3-octyl, 2-dimethyl-1-propyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, 3-pentyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl or octadecyl. In even a further embodiment, R_2 is decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl or octadecyl. In even a further embodiment, R_2 is a linear alkyl.

[0052] One embodiment of the present invention is directed to a method for manufacturing a compound of Formula (Ic), (Ic') and (Ic''). In a further embodiment, R_2 is a linear C_5 - C_{18}

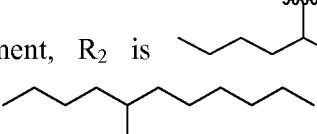
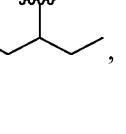
alkyl or a branched C₅-C₁₈ alkyl. In even a further embodiment, R₂ is a linear C₆-C₁₈ alkyl or a branched C₆-C₁₈ alkyl. In yet a further embodiment, R₂ is a linear C₆-C₁₄ alkyl, *e.g.*, a linear C₆ alkyl, C₈ alkyl, C₁₀ alkyl, C₁₂ alkyl or C₁₄ alkyl.

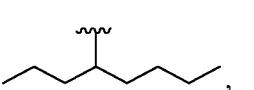
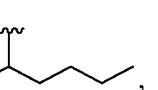
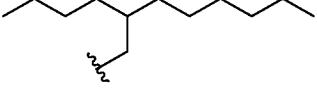
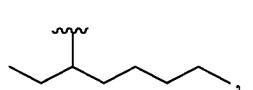
[0053] In one embodiment, a compound of Formula (Ic'') is provided wherein R₂ is a linear C₅-C₁₈ alkyl; R₃ is OH and R₅ is H. In another embodiment, a compound of Formula (Ic'') is provided wherein R₂ is a linear C₆-C₁₈ alkyl; R₃ is OH and R₅ is H. In yet embodiment, a compound of Formula (Ic'') is provided wherein R₂ is a linear C₆-C₁₆ alkyl; R₃ is OH and R₅ is H. In even another embodiment, a compound of Formula (Ic'') is provided wherein R₂ is a linear C₈-C₁₄ alkyl; R₃ is OH and R₅ is OH.

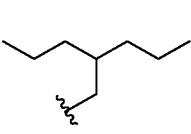
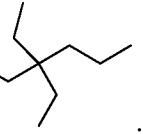
[0054] In one embodiment, a method for manufacturing a prostacyclin compound of Formula (Ic'') is provided wherein R₂ is a linear C₅-C₁₈ alkyl; R₃ is OH and R₅ is H. In another embodiment, a compound of Formula (Ic'') is provided wherein R₂ is a branched C₆-C₁₈ alkyl; R₃ is OH and R₅ is H. In yet embodiment, a compound of Formula (Ic'') is provided wherein R₂ is a branched C₆-C₁₆ alkyl; R₃ is OH and R₅ is H. In even another embodiment, a compound of Formula (Ic'') is provided wherein R₂ is a branched C₈-C₁₄ alkyl; R₃ is OH and R₅ is H.

[0055] In yet another embodiment a method for manufacturing a prostacyclin compound of Formula (Ia''), (Ib''), (Ic'') or (Id'') is provided, wherein R₃ is OH, R₅ is H and R₂ is

$m_1(\text{---})^{m_2}$ or $\text{R}'\text{---}\begin{array}{c} \text{---} \\ \text{CR}'\text{R}' \\ \text{---} \\ \text{CR}' \\ \text{---} \\ \text{R}' \end{array}$, and m₁ and m₂ are each independently an integer selected from

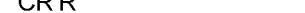
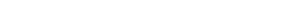
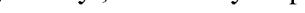
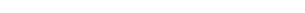
1 to 9. In even a further embodiment, R₂ is , ,

, , , ,

 or .

[0056] In yet another embodiment of Formula (Ia''), (Ib''), (Ic'') or (Id''), R₂ is H, R₃ is OH,

$\text{O}=\text{---}(\text{---})^{m_2}$ or $\text{R}'\text{---}\begin{array}{c} \text{---} \\ \text{O}=\text{---} \\ \text{---} \\ \text{CR}'\text{R}' \\ \text{---} \\ \text{CR}' \\ \text{---} \\ \text{R}' \end{array}$, where m₁ and m₂ are each independently an integer

selected from 1 to 9. In even a further embodiment, R_2 is  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , ,

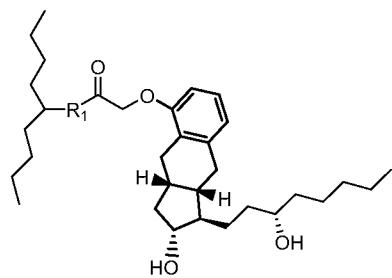
[0057] In one embodiment, a method for manufacturing a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id) is provided where R_2 is a linear or branched C_5 - C_{18} alkyl. In a further embodiment, R_2 is 5-nonanyl, 4-heptanyl, 4-octanyl, 3-octanyl, 2-dimethyl-1-propanyl, 3,3-dimethyl-1-butanyl, 2-ethyl-1-butanyl, 3-pentanyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl or octadecyl.

[0058] In one embodiment, a method for manufacturing a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic), (Id), (Ia'), (Ib'), (Ic'), (Id'), (Ia''), (Ib''), (Ic'') or (Id'') is provided where R₂ is a linear or branched C₅-C₁₈ alkyl. In even a further embodiment, R₂ is a linear C₅-C₁₈

alkyl. In another embodiment, R_2 is $(\text{---} \text{C}(\text{---}) \text{---})^{m1} \text{---} \text{C}(\text{---}) \text{---})^{m2}$ or $\text{---} \text{C}(\text{---} \text{R}' \text{---} \text{R}' \text{---}) \text{---} \text{C}(\text{---} \text{R}' \text{---} \text{R}' \text{---}) \text{---}$, where $m1$ and $m2$ are each independently an integer selected from 1 to 9 and each occurrence of R' is independently H, a linear or branched C_1 - C_8 alkyl, or a linear or branched C_1 - C_8 alkenyl. In even a further embodiment, R_2 is

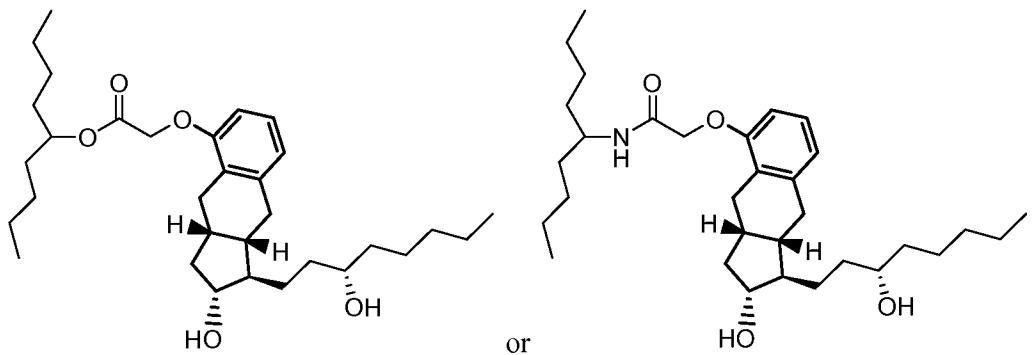
[0059] In another embodiment, a method for manufacturing a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id) is provided wherein R₂ is a branched C₅-C₁₈ alkyl. In a further embodiment, R₂ is 5-nonanyl, 4-heptyl, 4-octyl, 3-octyl, 2-dimethyl-1-propyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, 3-pentyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl or octadecyl.

[0060] In one embodiment of the invention, the prostacyclin compound manufactured by the methods provided herein has the following structure:



wherein R_1 is NH, O or S.

[0061] For example, R₁ is O or N, and one of the following compounds (5-nonanyl treprostinil (alkyl ester, 5C₉-TR) or 5-nonanyl treprostinil (amide linked; 5C9-TR-A), is provided:



[0062] In one embodiment, a method for manufacturing a prostacyclin compound of Formula

(I), (Ia), (Ib), (Ic) or (Id) is provided wherein R_2 is $\left(\begin{array}{c} \text{---} \\ | \\ \text{---} \end{array}\right)^{m1} \left(\begin{array}{c} \text{---} \\ | \\ \text{---} \end{array}\right)^{m2}$ or $\text{R}' \begin{array}{c} \text{---} \\ | \\ \text{---} \end{array} \text{CR}' \begin{array}{c} \text{---} \\ | \\ \text{---} \end{array} \text{R}'$, where $m1$ and $m2$ are each independently each an integer selected from 1 to 9 and each occurrence of R' is independently H, a linear or branched $\text{C}_1\text{-C}_8$ alkyl, or a linear or branched $\text{C}_1\text{-C}_8$ alkenyl.

[0063] When m1 and/or m2 is an integer from 2-9, the m1/m2 at the end of the carbon chain is CH₃, while the remaining m1/m2 groups are CH₂.

[0064] In even another embodiment, a method for manufacturing a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic), (Id), (Ia'), (Ib'), (Ic'), (Id'), (Ia''), (Ib''), (Ic'') or (Id'') is

provided and R_2 is  ,  ,  ,  ,  ,  ,  , or  .

[0065] The compounds provided herein can include a symmetrical branched alkyl or an

asymmetrical branched alkyl as the R₂ moiety. For example, where R₂ is $\text{---}(\text{---})^{\text{m1}}\text{---}(\text{---})^{\text{m2}}\text{---}$, m1 and m2 can be the same integer and R₂ is therefore a symmetrical branched alkyl. R₂ is an assymetrical branched alkyl when m1 and m2 are different.

[0066] In another embodiment, a compound of Formula (I), (Ia), (Ib), (Ic), (Id), (Ia'), (Ib'),

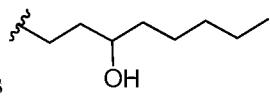
(Ic'), (Id'), (Ia''), (Ib''), (Ic'') or (Id'') is provided, R₂ is $\text{---}(\text{---})^{\text{m1}}\text{---}(\text{---})^{\text{m2}}\text{---}$, m1 is 2 and m2 is 3, m1 and m2 are each independently 4, or m1 and m2 are each independently 3 is provided via one of the methods described herein.

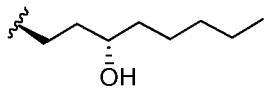
[0067] In another embodiment, the prostacyclin compound manufactured by the disclosed methods comprises an assymetrical branched alkyl at the R₂ position, such as, for example, 3-hexanyl (3C₆), 2-heptanyl (2C₇), 3-heptanyl (3C₇), 2-octanyl (2C₈), 3-octanyl (3C₈), or 4-octanyl (4C₈).

[0068] In another embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id) is manufactured by the disclosed methods, wherein R₂ is a branched alkyl selected from 2,2-diethyl-1-pentyl, 3-pentyl, 4-octyl, 5-nonanyl, 2-ethyl-1-butyl, 2-propyl-1-pentyl, 12-butyl-1-octyl, 2-dimethyl-1-propyl, and 3,3-dimethyl-1-butyl.

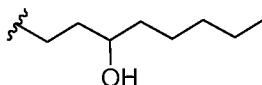
[0069] In yet another embodiment, a method for manufacturing a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic), (Id), (Ia'), (Ib'), (Ic') or (Id') is provided, wherein, R₂ is a linear or branched C₅-C₁₈ alkenyl. For example, in one embodiment, R₂ is a linear C₅-C₁₈ alkenyl selected from pentenyl, hexenyl, heptenyl, octenyl, nonenyl, decenyl, undecenyl, tridecenyl, tetradecenyl, pentadecenyl, hexadecenyl, heptadecenyl or octadecenyl. In a further embodiment, R₃ is OH. In another embodiment, R₂ is a branched C₅-C₁₈ alkenyl selected from pentenyl, hexenyl, heptenyl, octenyl, nonenyl, decenyl, undecenyl, tridecenyl, tetradecenyl, pentadecenyl, hexadecenyl, heptadecenyl or octadecenyl. In a further embodiment, R₃ is OH.

[0070] In one embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id) is

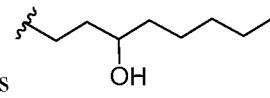
provided and R₄ is  is synthesized by one of the methods provided

herein. In a further embodiment, R₄ is .

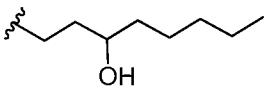
[0071] In one embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id) is synthesized by one of the methods provided herein and R₂ a linear C₅-C₁₈ alkyl, R₃ is OH

and R₄ is . In a further embodiment, R₂ is 5-nonanyl, 4-heptyl, 4-octanyl, 3-octanyl, 2-dimethyl-1-propyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, 3-pentyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl or octadecyl.

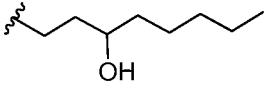
[0072] In one embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id) is synthesized by one of the methods provided herein and R₂ hexyl, dodecyl, tetradecyl, hexadecyl, 5-nonanyl, 4-heptanyl, 4-octanyl, 3-octanyl, 2-dimethyl-1-propyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, 3-pentyl, R₃ is OH and R₄ is

.

[0073] In one embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id) is synthesized by one of the methods provided herein and R₂ hexyl, R₃ is OH and R₄ is

.

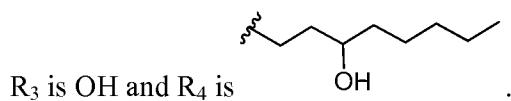
[0074] In one embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id) is synthesized by one of the methods described herein and R₂ hexyl, R₃ is OH and R₄ is

.

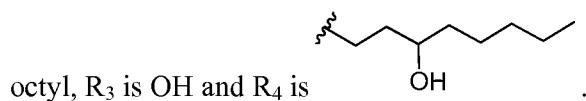
[0075] In another embodiment, a method for manufacturing a prostacyclin compound of Formula (Ia''), (Ib''), (Ic'') or (Id'') is provided and R₂ hexyl, R₃ is OH R₄ is H. In a further embodiment, the prostacyclin compound is a compound of Formula (Ic''). In yet another embodiment, a prostacyclin compound of Formula (Ia''), (Ib''), (Ic'') or (Id'') is provided and R₂ dodecyl, tetradecyl, pentadecyl or hexadecyl, R₃ is OH R₄ is H. In a further embodiment, the compound is a compound of Formula (Ia''). In even a further embodiment,

the compound is present in a lipid nanoparticle formulation as described in more detail below.

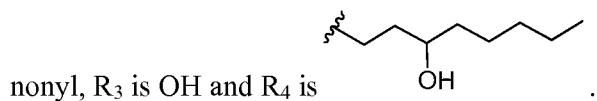
[0076] In one embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id), or pharmaceutically acceptable salt, is synthesized by a method described herein, and R₂ heptyl,



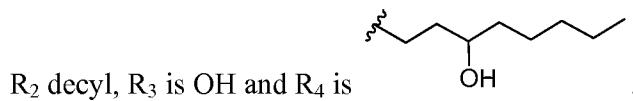
[0077] In one embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id), or pharmaceutically acceptable salt, is synthesized by a method described herein, and R₂



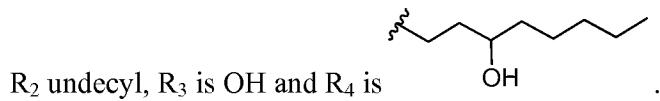
[0078] In one embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id), or pharmaceutically acceptable salt, is synthesized by a method described herein, and R₂



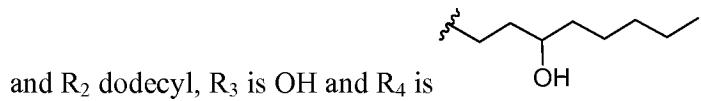
[0079] In another embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id), or pharmaceutically acceptable salt, is synthesized by a method described herein, and



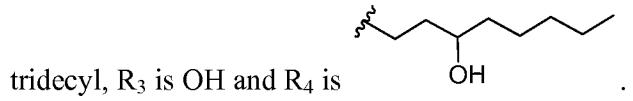
[0080] In yet another embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id), or pharmaceutically acceptable salt, is synthesized by a method described herein, and



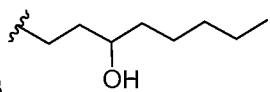
[0081] In even another embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id), or pharmaceutically acceptable salt, is synthesized by a method described herein,



[0082] In one embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id), or pharmaceutically acceptable salt, is synthesized by a method described herein, and R₂

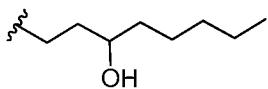


[0083] In another embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic), or (Id), or pharmaceutically acceptable salt, is synthesized by a method described herein, and



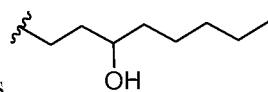
R₂ tetradecyl, R₃ is OH and R₄ is

[0084] In even another embodiment, a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id), or pharmaceutically acceptable salt, is synthesized by a method described herein,



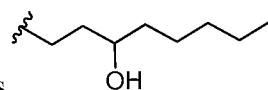
and R₂ pentadecyl, R₃ is OH and R₄ is

[0085] Another embodiment of the invention concerns a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id), or pharmaceutically acceptable salt is synthesized by a method



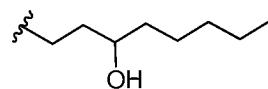
described herein, wherein R₂ hexadecyl, R₃ is OH and R₄ is

[0086] Yet another embodiment of the invention concerns a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id), a or pharmaceutically acceptable salt is synthesized by a



method described herein, wherein R₂ heptadecyl, R₃ is OH and R₄ is

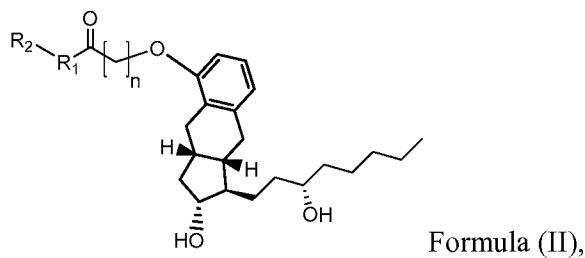
[0087] Yet another embodiment of the invention concerns a prostacyclin compound of Formula (I), (Ia), (Ib), (Ic) or (Id), or a pharmaceutically acceptable salt is synthesized by a



method described herein, wherein R₂ octadecyl, R₃ is OH and R₄ is

[0088] In one embodiment, a method is provided for manufacturing a compound of Formula (I), (Ia), (Ib), (Ic) or (Id), or a pharmaceutically acceptable salt, wherein one or more hydrogen atoms is substituted with a deuterium. Accordingly, in one embodiment, the present invention relates to an isotopologue of Formula (I), (Ia), (Ib), (Ic) or (Id), substituted with one or more deuterium atoms. The isotopologue of Formula (I), (Ia), (Ib), (Ic) or (Id) may be used to accurately determine the concentration of compounds of Formula (I), (Ia), (Ib), (Ic) or (Id) in biological fluids and to determine metabolic patterns of compounds of Formula (I), (Ia), (Ib), (Ic) or (Id) and its isotopologues.

[0089] In another embodiment of the invention, a method for manufacturing a prostacyclin compound of Formula (II), or a pharmaceutically acceptable salt thereof, is provided:



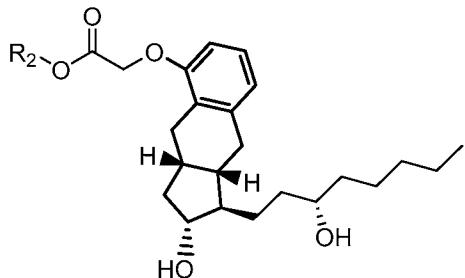
Formula (II),

wherein R_1 is NH , O or S ;

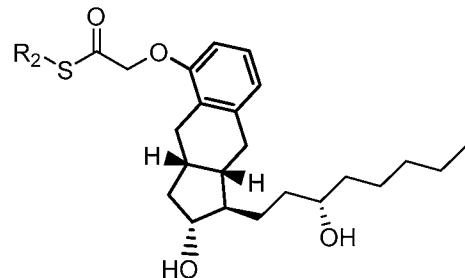
R_2 is a linear or branched C_5 - C_{18} alkyl, a linear C_2 - C_{18} alkenyl or a branched C_3 - C_{18} alkenyl, aryl, aryl- C_1 - C_{18} alkyl, an amino acid or a peptide; and
 n is an integer from 0 to 5.

[0090] In one embodiment, the method comprises manufacturing a prostacyclin compound of Formula (II), or a pharmaceutically acceptable salt thereof, wherein R_1 is NH , O or S ; R_2 is a linear or branched C_5 - C_{18} alkyl, a linear C_2 - C_{18} alkenyl or a branched C_3 - C_{18} alkenyl; and n is an integer from 0 to 5. In a further embodiment, n is 1 and R_1 is NH or O .

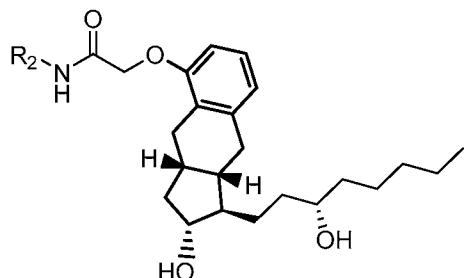
[0091] In one embodiment, the method comprises manufacturing a prostacyclin compound of Formula (II), wherein the prostacyclin compound is a compound of formula (IIa), (IIb), (IIc) or (IId), or a pharmaceutically acceptable salt thereof:



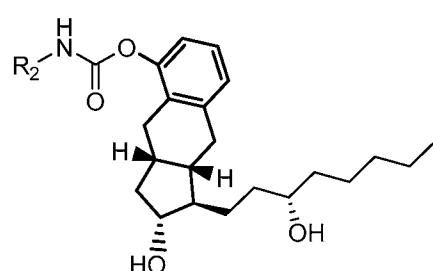
Formula (IIa)



Formula (IIb)



Formula (IIc)



Formula (IId)

wherein R₂ is a linear or branched C₅-C₁₈ alkyl, a linear C₂-C₁₈ alkenyl or a branched C₃-C₁₈ alkenyl, aryl, aryl-C₁-C₁₈ alkyl, an amino acid or a peptide. In a further embodiment, a compound of formula (IIa), (IIb), (IIc) or (IId) is provided wherein R₂ is a linear or branched C₅-C₁₈ alkyl, a linear C₂-C₁₈ alkenyl or a branched C₃-C₁₈ alkenyl. In one embodiment, a compound of Formula (II), (IIa), (IIb), (IIc) or (IId) is provided, wherein one or more hydrogen atoms is substituted with a deuterium. Accordingly, in one embodiment, the present invention relates to an isotopologue of Formula (II), (IIa), (IIb), (IIc) or (IId), substituted with one or more deuterium atoms. The isotopologue of Formula (II), (IIa), (IIb), (IIc) or (IId) may be used to accurately determine the concentration of compounds of Formula (II), (IIa), (IIb), (IIc) or (IId) in biological fluids and to determine metabolic patterns of compounds of Formula (II), (IIa), (IIb), (IIc) or (IId) and its isotopologues. The invention further provides compositions comprising these deuterated isotopologues and methods of treating diseases and conditions, as set forth herein.

[0092] In another embodiment, the method comprises manufacturing a prostacyclin compound of Formula (IIc). In a further embodiment, R₂ is a linear C₅-C₁₈ alkyl or a branched C₅-C₁₈ alkyl. For example, in one embodiment, R₂ is a linear C₆-C₁₈ alkyl. In another embodiment of Formula (IIc), R₂ is a linear C₆-C₁₀ alkyl. In even a further embodiment of Formula (IIc), R₂ is a hexyl, heptyl or octyl.

[0093] Compounds of Formula (IIa) and Formula (IId) that can be manufactured by the methods described herein are provided in tables 3 and 4 below.

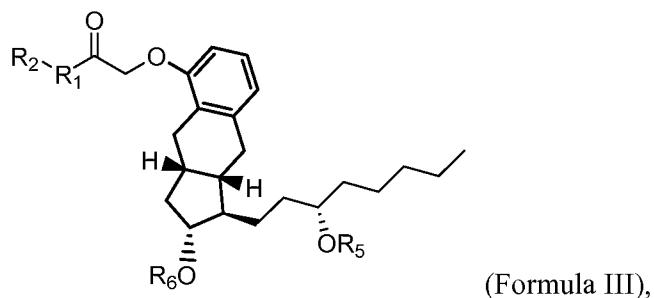
Table 3. Compounds of Formula (IIa)

R ₂ = linear C ₅ -C ₁₈ alkyl	R ₂ = branched C ₅ -C ₁₈ alkyl	R ₂ = linear C ₈ alkyl	R ₂ = branched C ₆ alkyl
R ₂ = linear C ₆ -C ₁₈ alkyl	R ₂ = branched C ₆ -C ₁₈ alkyl	R ₂ = linear C ₉ alkyl	R ₂ = branched C ₇ alkyl
R ₂ = linear C ₇ -C ₁₈ alkyl	R ₂ = branched C ₇ -C ₁₈ alkyl	R ₂ = linear C ₁₀ alkyl	R ₂ = branched C ₈ alkyl
R ₂ = linear C ₈ -C ₁₈ alkyl	R ₂ = branched C ₈ -C ₁₈ alkyl	R ₂ = linear C ₁₁ alkyl	R ₂ = branched C ₉ alkyl
R ₂ = linear C ₉ -C ₁₈ alkyl	R ₂ = branched C ₉ -C ₁₈ alkyl	R ₂ = linear C ₁₂ alkyl	R ₂ = branched C ₁₀ alkyl
R ₂ = linear C ₁₀ -C ₁₈ alkyl	R ₂ = branched C ₁₀ -C ₁₈ alkyl	R ₂ = linear C ₁₃ alkyl	R ₂ = branched C ₁₁ alkyl
R ₂ = linear C ₁₁ -C ₁₈ alkyl	R ₂ = branched C ₁₁ -C ₁₈ alkyl	R ₂ = linear C ₁₄ alkyl	R ₂ = branched C ₁₂ alkyl
R ₂ = linear C ₁₂ -C ₁₈ alkyl	R ₂ = branched C ₁₂ -C ₁₈ alkyl	R ₂ = linear C ₁₅ alkyl	R ₂ = branched C ₁₃ alkyl

Table 4. Compounds of Formula (IIc)

R ₂ = linear C ₅ -C ₁₈ alkyl	R ₂ = branched C ₅ -C ₁₈ alkyl	R ₂ = linear C ₆ alkyl	R ₂ = branched C ₆ alkyl
R ₂ = linear C ₆ -C ₁₈ alkyl	R ₂ = branched C ₆ -C ₁₈ alkyl	R ₂ = linear C ₇ alkyl	R ₂ = branched C ₇ alkyl
R ₂ = linear C ₇ -C ₁₈ alkyl	R ₂ = branched C ₇ -C ₁₈ alkyl	R ₂ = linear C ₈ alkyl	R ₂ = branched C ₈ alkyl
R ₂ = linear C ₈ -C ₁₈ alkyl	R ₂ = branched C ₈ -C ₁₈ alkyl	R ₂ = linear C ₉ alkyl	R ₂ = branched C ₉ alkyl
R ₂ = linear C ₉ -C ₁₈ alkyl	R ₂ = branched C ₉ -C ₁₈ alkyl	R ₂ = linear C ₁₀ alkyl	R ₂ = branched C ₁₀ alkyl
R ₂ = linear C ₁₀ -C ₁₈ alkyl	R ₂ = branched C ₁₀ -C ₁₈ alkyl	R ₂ = linear C ₁₁ alkyl	R ₂ = branched C ₁₁ alkyl
R ₂ = linear C ₅ -C ₁₂ alkyl	R ₂ = branched C ₅ -C ₁₂ alkyl	R ₂ = linear C ₁₂ alkyl	R ₂ = branched C ₁₂ alkyl
R ₂ = linear C ₆ -C ₁₀ alkyl	R ₂ = branched C ₆ -C ₁₀ alkyl	R ₂ = linear C ₁₃ alkyl	R ₂ = branched C ₁₃ alkyl

[0094] Yet another embodiment of the invention relates to a method for manufacturing a prostacyclin compound of Formula (III), or a pharmaceutically acceptable salt thereof:



wherein R₁ and R₂ are defined as provided for Formula (I) and (II), and R₅ and R₆ are independently selected from H, optionally substituted linear or branched C₁-C₁₅ alkyl, optionally substituted linear or branched C₂-C₁₅ alkenyl, (C=O)-optionally substituted linear or branched C₁-C₁₅ alkyl, or (C=O)-optionally substituted linear or branched C₂-C₁₅ alkenyl, with the proviso that the prostacyclin compound of Formula (III) is not treprostинil.

[0095] In one embodiment, the manufacturing methods provide prostacyclin compounds that contain a chiral moiety at one or more of the R₂, R₅ and/or R₆ positions. For example, the moiety at position R₂, in one embodiment, is a chiral moiety and comprises either the R isomer, the S isomer, or a mixture thereof. An optical isomer at position R₂, R₅ and/or R₆ can also be classified with the D/L nomenclature. For example, where R₂ is an amino acid or an

amino acid moiety, the amino acid or amino acid moiety can be the D-isomer, L-isomer, or a mixture thereof.

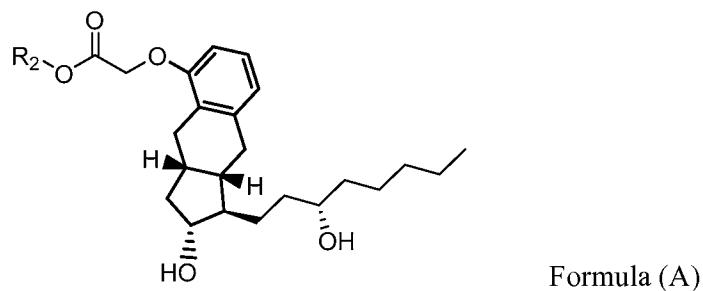
[0096] In one embodiment, one or more of the R₂, R₅ and/or R₆ moieties is the R isomer or S isomer. In another embodiment, one or more of the R₂, R₅ and/or R₆ moieties provided herein comprise a mixture of R and S moieties. The “R isomer” or “S isomer” as used herein refers to an enantiomerically pure isomer. An “enantiomerically pure isomer” has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% pure R- or S- isomer or when using the D/L nomenclature, D- or L-isomer. A racemic compound is a compound having a mixture in equal amounts of both enantiomers.

EXAMPLES

[0097] The present invention is further illustrated by reference to the following Examples. However, it should be noted that these Examples, like the embodiments described above, are illustrative and are not to be construed as restricting the scope of the invention in any way.

Example 1 – Synthesis of treprostinil alkyl esters

[0098] Treprostinil compounds derivatized with alkyl groups at the carboxylic acid moiety were prepared. Specifically, treprostinil was derivatized at the carboxylic acid moiety with C₂, C₃, C₄, C₅, C₆, C₈, C₁₀, C₁₂, C₁₆, and C₁₈ alkyl chains (*i.e.*, R₂ in Formula (A), below, is C₂, C₃, C₄, C₅, C₆, C₈, C₁₀, C₁₂, C₁₆ or C₁₈ alkyl) to make treprostinil alkyl esters of various ester chain lengths. Treprostinil can be synthesized, for example, by the methods disclosed in U.S. Patent Nos. 6,765,117 and 8,497,393. Synthesis of prostaglandin derivatives is described in U.S. Patent No. 4,668,814. The disclosures of U.S. Patent Nos. 6,765,117; 8,497,393 and 4,668,814 are each incorporated by reference in their entireties for all purposes.



[0099] Scheme 1:

[00100] Treprostinil esterification was catalyzed by strongly acidic resin Amberlyst® 15 (Rohm and Haas). Treprostinil acid was dissolved in anhydrous dioxane/alcohol at a concentration 10 mg/mL (typically 4 mL). Alcohol (R₂-OH) added was appropriate to make corresponding chain length at the R₂ group. By way of example, for the C₂ (ethyl ester) compound, the alcohol was ethanol. The molar amount of alcohol in the solvent was ten times the molar amount of treprostinil.

[00101] Treprostinil in dioxane/alcohol solution was added to washed and dry Amberlyst resin. Per each 40 mg treprostinil, 1 g resin in a glass vial was added. The mixture was placed on a shaker and incubated overnight at 40 °C. Next, the liquid portion was taken out of the vial, washed twice with 3 mL dioxane. All recovered solvent was then collected. The solvent was dried by nitrogen stream until the evaporation stopped. The remaining treprostinil alkyl ester and nonvolatile alcohol (if long chain alcohol used) was dissolved in 2 mL hexane/ethyl acetate 1:1, and cleaned by liquid-liquid extraction vs. equal volume of phosphate buffer, and then water. Next, the organic layer was separated and dried by nitrogen stream and further in vacuum. If a long chain alcohol used, an additional purification step was required to separate alcohol by liquid chromatography. ACE CN, 5 µm, Ultra-Inert HPLC Column, 100x21.2 mm was used, with mobile phase of hexane/propanol 98:2%.

[00102] Scheme 2:

[00103] To a solution of (1R,2R,3aS,9aS)-[[2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3S)-3-hydroxyoctyl]-1H-benz[f]inden-5-yl]oxy]acetic acid (treprostinil) (78.1 mg, 200 µmoles) dissolved in 1,4-dioxane (2.0 mL) was added Amberlyst® 15 resin (2.0 g) and alcohol R₂-OH (2.0 mmoles, 10 equivalents). The reaction mixture was heated to 40 °C and allowed to shake at approximately 100 rpm for 18-196 hours. Solvent was removed and the resin was washed with acetonitrile (MeCN) (3 x 3 mL). The 1,4-dioxane and MeCN extracts were combined and dried using a gentle stream of warmed N₂ gas and gentle heat to yield a thick waxy solid. The crude material was dissolved in 20% ⁷PrOH/Hexanes and submitted to preparatory HPLC purification. Solvent was removed from the purified material using a gentle stream of warmed N₂ gas and gentle heat to yield an off-white waxy solid. The pure material was suspended in ethyl lactate for storage and was submitted to analytical HPLC for concentration determination.

[00104] By way of example, the following compounds of Formula (A), set forth in Table 5 were synthesized by the method of scheme 2.

Table 5.

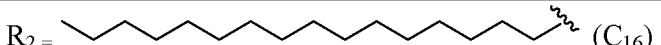
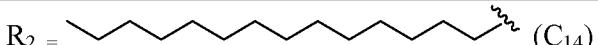
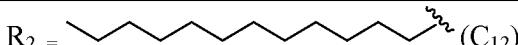
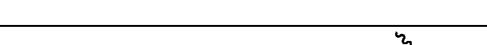
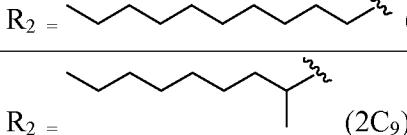
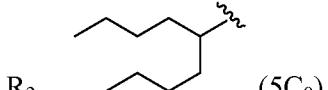
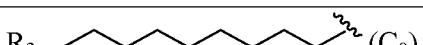
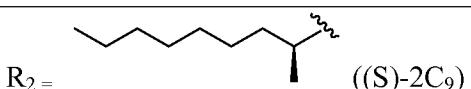
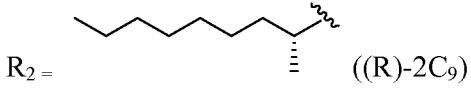
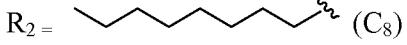
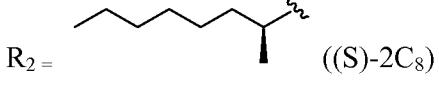
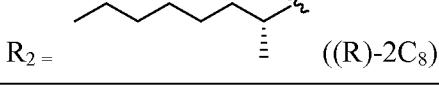
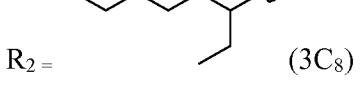
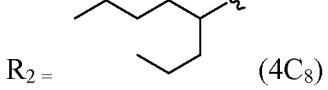
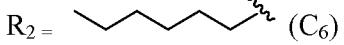
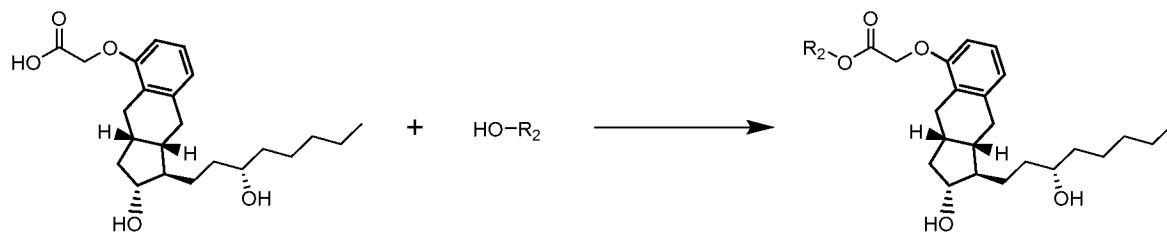
R₂ group	Compound abbreviation
R ₂ =  (C ₁₆)	C ₁₆ -TR
R ₂ =  (C ₁₄)	C ₁₄ -TR
R ₂ =  (C ₁₂)	C ₁₂ -TR
R ₂ =  (C ₁₁)	C ₁₁ -TR
R ₂ =  (C ₁₀)	C ₁₀ -TR
R ₂ =  (2C ₉)	C ₉ -TR
R ₂ =  (5C ₉)	5C ₉ -TR
R ₂ =  (C ₉)	2C ₉ -TR
R ₂ =  ((S)-2C ₉)	(S)-2C ₉ -TR
R ₂ =  ((R)-2C ₉)	(R)-2C ₉ -TR
R ₂ =  (C ₈)	C ₈ -TR
R ₂ =  ((S)-2C ₈)	(S)-2C ₈ -TR
R ₂ =  ((R)-2C ₈)	(R)-2C ₈ -TR
R ₂ =  (3C ₈)	3C ₈ -TR
R ₂ =  (4C ₈)	4C ₈ -TR
R ₂ =  (C ₆)	C ₆ -TR

Table 5.

R₂ group	Compound abbreviation
R ₂ =	C ₅ -TR
R ₂ =	C ₄ -TR
R ₂ =	C ₃ -TR
R ₂ =	C ₂ -TR

[00105] A general diagram for synthesis of the alkyl ester of treprostinil is shown in Scheme 1, below as well as Figure 1. The alcohol can be modified based on the desired alkyl ester chain length (e.g., C₅-C₁₈ alkyl esters of even or odd chain length, straight chain or branched). Other reaction conditions used to synthesize treprostinil ester prodrugs are provided in Table 6, below.



Scheme 1: Esterification Mechanism for alkyl ester-TR Compounds

Table 6.

Entry	Ester	Solvent	Coupling Reagent	Additive	Time
1	C12TR	Dioxane (2 mL/100 µmol TRP)	DCC	DMAP	84 h
2	C12TR	10% DMF/DCM	DCC	DMAP	84 h
3	C12TR	Dioxane (2 mL/100 µmol TRP)	Amberlyst-15	-	72 h
4	C12TR	Dioxane (1 mL/100 µmol TRP)	Amberlyst-15	-	18h
5	C14TR	Dioxane (1 mL/100 µmol TRP)	Amberlyst-15	-	18 h
6	C14TR	Dioxane (1 mL/100 µmol TRP)	Amberlyst-15	-	18 h
7	C14TR	DMF	DCC	DMAP	18 h
8	C14TR	DMF	PyBOP	TEA	18 h
9	C14TR	DMF	HATU	TEA	18 h
					168
10	C14TR	Dioxane (2 mL/100 µmol TRP)	Amberlyst-15	-	h
11	5C9TR	Dioxane (1 mL/100 µmol TRP)	Amberlyst-15	-	72 h
12	5C9TR	Dioxane (1 mL/100 µmol TRP)	Amberlyst-15	-	72 h
13	5C9TR	Dioxane (1 mL/100 µmol TRP)	Amberlyst-15	-	168

Table 6.

<u>Entry</u>	<u>Ester</u>	<u>Solvent</u>	<u>Coupling Reagent</u>	<u>Additive</u>	<u>Time</u>
14	5C9TR	10% DMF/DCM	DCC	DMAP	18 h
15	5C9TR	1:1 Dioxane:MeCN	HATU	-	18 h
16	5C9TR	1:1 Dioxane:MeCN	PyBOP	-	18 h
17	5C9TR	DMF	HATU	-	18 h
18	5C9TR	DMF	PyBOP	-	18 h
19	C16TR	Dioxane (1 mL/100 µmol TRP)	Amberlyst-15	-	18 h
20	C16TR	DCM	DCC	DMAP	18 h
21	C16TR	DCM		PPh ₃ , DEAD	-
					18h

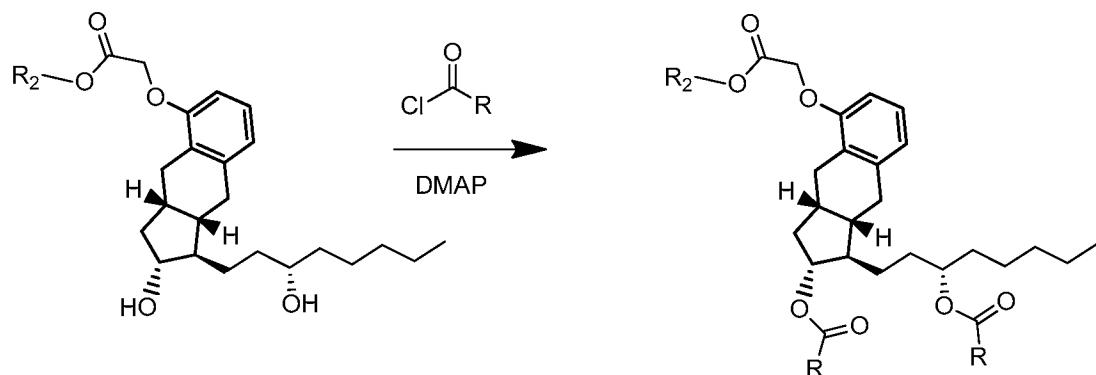
DCC = N,N'-Dicyclohexylcarbodiimide;
 DMAP = 4-dimethylaminopyridine
 DMF = N,N'-dimethylformamide;
 HATU = 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid Hexafluorophosphate;
 PyBOP = benzotriazol-1-yl-oxytritypyrrolidinophosphonium hexafluorophosphate

Example 2 – Acylation of Treprostinil Derivatives

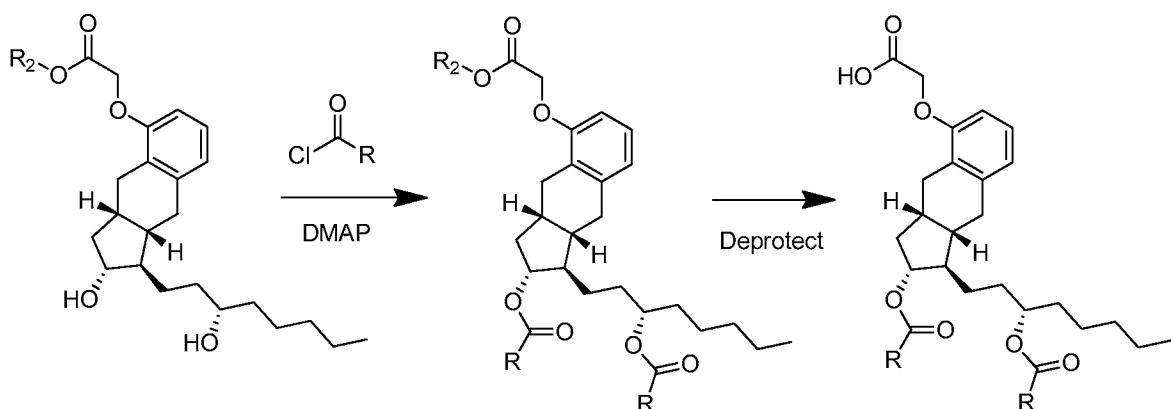
[00106] Treprostinil or treprostinil ester derivatives (e.g., derivatized with alkyl or alkenyl groups at the carboxylic acid moiety as prepared in Example 1) are acylated as follows.

[00107] The compound of Example 1 (0.05 mol) or treprostinil is dissolved in 10 mL of dichloromethane at 0 °C. Dimethylaminopyridine is added (20 mol%), and then a solution of an acyl chloride R(CO)Cl (2.1 equivalents) at 0 °C (wherein R is R₅ or R₆ as described herein) is added to the compound of Example 1 or treprostinil. The solution is allowed to stir and warm to 23 °C over 1 hour. The reaction is monitored by thin layer chromatography, and when no further change is observed, the reaction is quenched with NaHCO₃ (sat), and the quenched mixture is extracted with dichloromethane (3 x 10 mL). The combined organic extracts are dried over anhydrous sodium sulfate, and the solvent is removed under vacuum to afford the crude product. Purification is effected by column chromatography on silica gel with 2% methanol in dichloromethane.

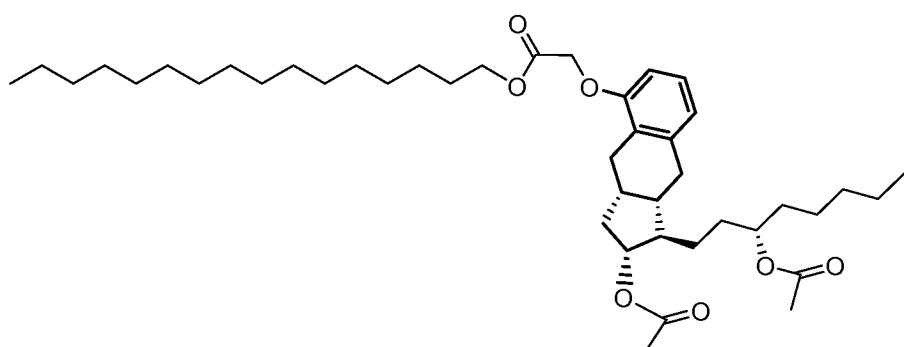
[00108] A general scheme for synthesis of the acylated treprostinil prodrugs and treprostinil derivative prodrugs is shown below and in Figure 2 (R₂ is described herein, for example as H or a linear or branched alkyl group):



[00109] Other acylation techniques known in the art, including selective acylation of each of the secondary alcohols, can be employed. In addition, R₂ can be selected such that the R₂ group can be selectively removed after acylation of the secondary hydroxyl functionalities. Such protecting group strategies are well known to those skilled in the art, and are described in, e.g., Peter G.M. Wuts and Theodora W. Greene, Greene's Protective Groups in Organic Synthesis, 4th Edition, Wiley (2006), which is incorporated herein by reference in its entirety for all purposes. An exemplary scheme of such a process is shown below:



[00110] Synthesis of C₁₆TR-OAc:



[00111] To a solution of Hexadecyl Treprostinil (C16TR) (78.1 mg, 200 μ moles) dissolved in 1,4-Dioxane (2.0 mL) was added triethylamine (TEA) (98 μ L, 700 μ moles, 3.5 equivalents), acetic anhydride (166 μ L, 1,760 μ moles, 8.8 equivalents), and a catalytic amount of dimethylaminopyridine (DMAP). The reaction mixture was allowed to shake at 40 °C for 72 hours. Solvent was removed under reduced pressure to yield a thick colorless oil. The crude material was dissolved in hexanes and washed with a solution of saturated NaHCO₃ (3 x 5 mL). The organic layers were combined and solvent was removed using a gentle stream of warmed N₂ gas and gentle heat to yield a thick colorless oil. The crude material was dissolved in 20% ²PrOH/Hexanes, passed through a 0.45 μ m syringe filter, and submitted to preparatory HPLC purification. Solvent was removed from the purified material using a gentle stream of warmed N₂ gas and gentle heat to yield a thick colorless oil. The pure material was suspended in ethyl lactate for storage and was submitted to analytical HPLC for concentration determination.

[00112] C₁₆-TR-OAc: 73% overall yield. The compound was also characterized by NMR spectroscopy:

[00113] ¹H NMR (500 MHz, CDCl₃) δ 0.89 (t, J = 7.0 Hz, 6H), 1.17-1.32 (m, 33H), 1.43-1.46 (m, 2H), 1.49-1.66 (m, 8H), 1.89-1.93 (m, 1H), 1.99 (s, 3H), 2.06 (s, 3H), 2.30-2.35 (m, 2H), 2.47 (d of d, J = 14.5 Hz, J = 6.0 Hz, 1H), 2.55 (d of d, J = 15.0 Hz, J = 6.0 Hz, 1H), 2.76 (d, of d, J = 14.5 Hz, J = 6.0 Hz, 1H), 2.90 (d of d, J = 15.0 Hz, J = 6.0 Hz, 1H), 4.19 (t, J = 7.0 Hz, 2H), 4.62 (s, 2H), 4.70-4.74 (m, 1H), 4.87 (p, J = 6.0 Hz, 1H), 6.63 (d, J = 8.0 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 7.08 (t, J = 8.0 Hz, 1H) ppm; ¹³C NMR (125 MHz, CDCl₃) δ 14.2, 14.3, 21.5 (2), 22.7, 22.9, 25.1, 26.0 (2), 28.3, 28.8, 29.4, 29.6, 29.7, 29.8, 29.9, 31.9, 32.1, 33.6, 33.7, 34.3, 37.8, 40.7, 49.0, 65.6, 66.2, 74.6, 79.0, 109.8, 121.8, 126.4, 127.6, 140.7, 155.1, 169.6, 171.0, 171.1 ppm.

Example 3 –Synthesis of treprostinil amide derivatives

[00114] Treprostinil is available commercially, and can be synthesized, for example, by the methods disclosed in U.S. Patent Nos. 6,765,117 and 8,497,393. Synthesis of prostaglandin derivatives is described in U.S. Patent No. 4,668,814. The disclosures of U.S. Patent Nos. 6,765,117; 8,497,393 and 4,668,814 are each incorporated by reference in their entireties for all purposes.

[00115] To a solution of (1R,2R,3aS,9aS)-[[2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3S)-3-hydroxyoctyl]-1H-benz[f]inden-5-yl]oxy]acetic acid (*i.e.*, treprostinil) (78.1 mg, 200

μmoles) dissolved in 1,4-Dioxane (2.0 mL) was added triethylamine (TEA) (98 μL, 700 μmoles, 3.5 equivalents), alkylamine R₁-NH₂ (240 μmoles, 1.2 equivalents), and a solution of PyBOP (364 mg, 700 μmoles, 3.5 equivalents) dissolved in 2.0 mL MeCN (acetonitrile).

[00116] The reaction mixture was heated to 40 °C and allowed to shake at approximately 100 rpm overnight. Solvent was removed under reduced pressure to yield the crude product as a thick yellow oil. The product was extracted (1:1 extraction) from the oil by repeated washings with 20% ²PrOH/Hexanes (3 x 3 mL). Solvent was removed from the organic extract using a gentle stream of warmed N₂ gas and gentle heat to yield a thick, slightly yellow oil. The crude material was dissolved in 20% ²PrOH/Hexanes, passed through a 0.45 μm syringe filter, and submitted to preparatory HPLC purification. Solvent was removed from the purified material using a gentle stream of warmed N₂ gas and gentle heat to yield a thick, colorless oil. The pure material was suspended in ethyl lactate for storage and was submitted to analytical HPLC for concentration determination.

[00117] The following treprostinil amide derivatives of Formula B were made by the synthesis scheme provided above. (Table 6) Percentage yield is also provided in parentheses.

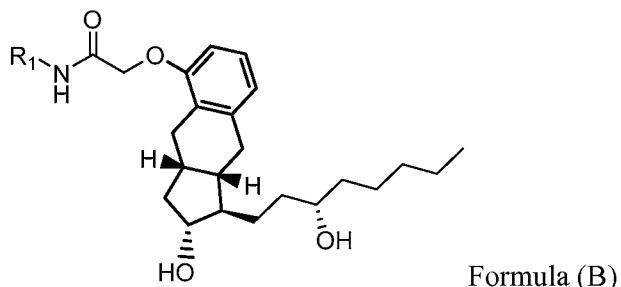
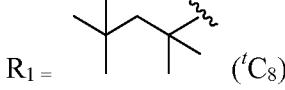
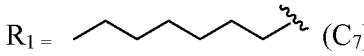
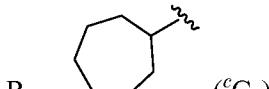
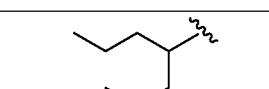
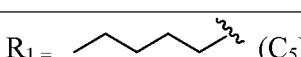
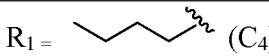
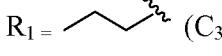
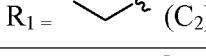
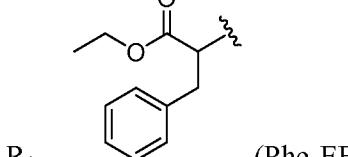
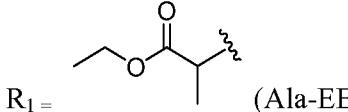
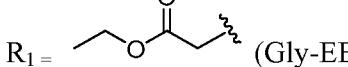
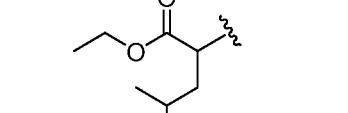


Table 6. Treprostinil amide derivatives

R₁ group	Yield	Compound abbreviation
R ₁ = (C ₁₆)	88 %	C ₁₆ -TR-A
R ₁ = (C ₁₄)	71 %	C ₁₄ -TR-A
R ₁ = (C ₁₂)	57 %	C ₁₂ -TR-A
R ₁ = (C ₁₀)	62 %	C ₁₀ -TR-A
R ₁ = (C ₈)	47 %	C ₈ -TR-A

Table 6. Treprostinil amide derivatives

R₁ group	Yield	Compound abbreviation
 R ₁ = (C ₈)	72 %	^t C ₈ -TR-A
 R ₁ = (C ₇)	50 %	C ₆ -TR-A
 R ₁ = (C ₇)	62 %	^c C ₇ -TR-A
 R ₁ = (4C ₇)	65 %	4C ₇ -TR-A
 R ₁ = (C ₆)	58 %	C ₆ -TR-A
 R ₁ = (C ₅)	77 %	C ₅ -TR-A
 R ₁ = (C ₄)	28 %	C ₄ -TR-A
 R ₁ = (C ₃)	12 %	C ₃ -TR-A
 R ₁ = (C ₂)	12 %	C ₂ -TR-A
 R ₁ = (Phe-EE)	60 %	Phe-EE-TR-A
 R ₁ = (Ala-EE)	Not determined	Ala-EE-TR-A
 R ₁ = (Gly-EE)	Not determined	Gly-EE-TR-A
 R ₁ = (Leu-EE)	Not determined	Leu-EE-TR-A

[00118] C₆-TR-A and C₁₂-TR-A were characterized by NMR spectroscopy.

NMR Characterization of C₆-TR-A

¹H NMR (500 MHz, CDCl₃) δ 0.90 (q, J = 7.0 Hz, 6 H), 1.17 (q, J = 12.0 Hz, 1H), 1.30-1.70 (m, 18 H), 1.81-1.83 (m, 1H), 1.80-1.93 (m, 1H), 2.20 (p, J = 6.0 Hz, 1H), 2.22-2.23 (m, 1H), 2.47-2.54 (m, 2H), 2.75-2.82 (m, 2H), 3.16 (sextet, J = 4.0 Hz, 1H), 3.35 (q, J = 7.0 Hz, 2H), 3.63 (s, 1H), 3.70-3.80 (m, 1H), 4.48 (s, 2H), 6.55 (s, 1H), 6.70 (d, J = 7.5 Hz, 1H), 6.85 (d, J = 7.5 Hz, 1H), 7.11 (t, J = 7.5 Hz, 1H) ppm; ¹³C NMR (125 MHz, CDCl₃) δ 14.2, 14.3, 22.8, 22.9, 25.6, 26.4, 26.7(2), 28.8, 29.7, 31.6, 32.1, 33.0, 33.8, 35.1, 37.7, 39.2, 41.4, 41.6, 46.5, 52.4, 68.4, 72.8, 110.4, 122.2, 126.8, 127.3, 141.2, 154.5, 168.7 ppm; HRMS (ESI, 2:2:1 MeCN, MeOH, H₂O): m/z = 474.35717 ([M+H]⁺).

NMR Characterization of C12-TR-A

HRMS (ESI, 2:2:1 MeCN, MeOH, H₂O): m/z = 558.45099 ([M+H]⁺).

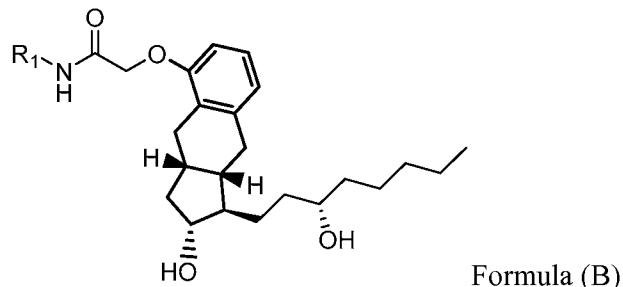
* * * * *

[00119] While the described invention has been described with reference to the specific embodiments thereof it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adopt a particular situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the described invention. All such modifications are intended to be within the scope of the claims appended hereto.

[00120] Patents, patent applications, patent application publications, journal articles and protocols referenced herein are incorporated by reference in their entireties, for all purposes.

CLAIMS

1. A method for making a treprostinil prodrug having the following formula:



comprising,

mixing, in the presence of a coupling reagent, a compound of Formula (B) with an compound of the formula R_1-NH_2 , wherein R_1 is a linear or branched C_5-C_{18} alkyl, a linear C_2-C_{18} alkenyl or a branched C_3-C_{18} alkenyl, aryl, aryl- C_1-C_{18} alkyl, an amino acid or a peptide,

incubating the mixture for a sufficient period of time to form the compound of Formula (B).

2. The method of claim 1, wherein the coupling reagent is 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*] pyridinium 3-oxid hexafluorophosphate (HATU).

3. The method of claim 1, wherein the coupling reagent is benzotriazol-1-yl-oxytritypyrrolidinophosphonium hexafluorophosphate (PyBOP).

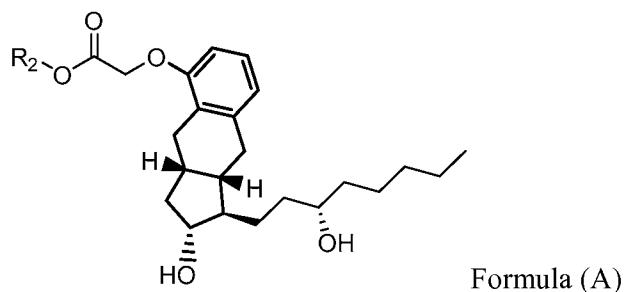
4. The method of claim 1, wherein the coupling reagent is N,N'-Disuccinimidyl carbonate (DSC).

5. The method of claim 1, wherein the coupling reagent is N,N'-Dicyclohexylcarbodiimide (DCC), DIC = N,N'-Diisopropylcarbodiimide (DIC) or N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC).

6. The method of any one of claims 1-5, wherein the compound of the formula R_2-NH_2 is added to the compound of Formula (B) and the coupling reagent after the of the compound of Formula (B) and the coupling reagent are mixed for a period of time.

7. The method of claim 6, wherein the period of time is from about 30 minutes to about 96 hours, from about 30 minutes to about 72 hours, from about 30 minutes to about 48 hours, from about 30 minutes to about 24 hours or from about 30 minutes to about 150 minutes.
8. The method of claim 7, wherein the period of time is about 30 minutes.
9. The method of claim 7, wherein the period of time is about 24 hr.
10. The method of claim 7, wherein the period of time is about 48 hr.
11. The method of claim 7, wherein the period of time is about 68 hr.
12. The method of claim 7, wherein the period of time is about 91 hr.
13. The method of any one of claims 1-12, wherein the compound of Formula (B) is dissolved in a solvent.
14. The method of claim 13, wherein the solvent comprises dioxane.
15. The method of claim 13, wherein the solvent comprises acetonitrile (MeCN), N,N'-dimethylformamide (DMF), dichloromethane (DCM), or a combination thereof
16. The method of claim 13, wherein the solvent comprises Dioxane (2 mL/100 μ mol TRP), Dioxane (1 mL/100 μ mol TRP), DMF, DCM, MeCN, 1:1 dioxane:MeCN, DMF/DCM, 10% DMF/DCM, or 20% DMF/DCM.
17. The method of any one of claims 1-8, wherein R₂ is a linear C₅-C₁₈ alkyl.
18. The method of any one of claims 1-8, wherein R₂ is a linear C₆-C₁₈ alkyl.
19. The method of any one of claims 1-8, wherein R₂ is a linear C₇-C₁₈ alkyl.
20. The method of any one of claims 1-8, wherein R₂ is a linear C₈-C₁₈ alkyl.
21. The method of any one of claims 1-8, wherein R₂ is a linear C₉-C₁₈ alkyl.
22. The method of any one of claims 1-8, wherein R₂ is a linear C₁₀-C₁₈ alkyl.
23. The method of any one of claims 1-8, wherein R₂ is a linear C₁₁-C₁₈ alkyl.
24. The method of any one of claims 1-8, wherein R₂ is a linear C₁₂-C₁₈ alkyl.

25. The method of any one of claims 1-8, wherein R₂ is a linear C₁₃-C₁₈ alkyl.
26. The method of any one of claims 1-8, wherein R₂ is a linear C₁₄-C₁₈ alkyl.
27. The method of any one of claims 1-8, wherein R₂ is a linear C₅-C₁₀ alkyl.
28. The method of any one of claims 1-8, wherein R₂ is a linear C₅-C₉ alkyl.
29. The method of any one of claims 1-8, wherein R₂ is a linear C₅-C₈ alkyl.
30. The method of any one of claims 1-8, wherein R₂ is a linear C₅-C₇ alkyl.
31. The method of any one of claims 1-8, wherein R₂ is an amino acid.
32. A method for making a treprostinil prodrug having the following formula:



comprising,
 mixing, in the presence of an acid catalyst, a compound of Formula (A) with an alcohol of the formula R₂-OH, wherein R₂ is a linear or branched C₅-C₁₈ alkyl, a linear C₂-C₁₈ alkenyl or a branched C₃-C₁₈ alkenyl, aryl, aryl-C₁-C₁₈ alkyl, an amino acid or a peptide, incubating the mixture for a sufficient period of time to form the compound of Formula (A).

33. The method of claim 32, wherein the compound of Formula (A) is dissolved in a solvent prior to mixing with the acid catalyst.

34. The method of claim 33, wherein the solvent comprises dioxane.

35. The method of claim 33, wherein the solvent comprises acetonitrile (MeCN), N,N'-dimethylformamide (DMF), dichloromethane (DCM), or a combination thereof

36. The method of claim 33, wherein the solvent comprises Dioxane (2 mL/100 μ mol TRP), Dioxane (1 mL/100 μ mol TRP), DMF, DCM, MeCN, 1:1 dioxane:MeCN, DMF/DCM, 10% DMF/DCM, or 20% DMF/DCM.

37. The method of any one of claims 32-36, wherein the acid catalyst is a solid.

38. The method of claim 37, wherein the solid is a solid resin.

39. The method of any one of claims 32-38, wherein the acid catalyst comprises sulfuric acid, sulfonic acid, hydrofluoric acid, phosphoric acid, toluenesulfonic acid, polystyrene sulfonate, heteropoly acid, zeolites, metal oxides, graphene oxygen or a combination thereof.

40. The method of any one of claims 32-39, wherein R_2 is a linear C_5-C_{18} alkyl.

41. The method of any one of claims 32-39, wherein R_2 is a linear C_6-C_{18} alkyl.

42. The method of any one of claims 32-39, wherein R_2 is a linear C_7-C_{18} alkyl.

43. The method of any one of claims 32-39, wherein R_2 is a linear C_8-C_{18} alkyl.

44. The method of any one of claims 32-39, wherein R_2 is a linear C_9-C_{18} alkyl.

45. The method of any one of claims 32-39, wherein R_2 is a linear $C_{10}-C_{18}$ alkyl.

46. The method of any one of claims 32-39, wherein R_2 is a linear $C_{11}-C_{18}$ alkyl.

47. The method of any one of claims 32-39, wherein R_2 is a linear $C_{12}-C_{18}$ alkyl.

48. The method of any one of claims 32-39, wherein R_2 is a linear $C_{13}-C_{18}$ alkyl.

49. The method of any one of claims 32-39, wherein R_2 is a linear $C_{14}-C_{18}$ alkyl.

50. The method of any one of claims 32-39, wherein R_2 is a linear C_5-C_{10} alkyl.

51. The method of any one of claims 32-39, wherein R_2 is a linear C_5-C_9 alkyl.

52. The method of any one of claims 32-39, wherein R_2 is a linear C_5-C_8 alkyl.

53. The method of any one of claims 32-39, wherein R_2 is a linear C_5-C_7 alkyl.

FIGURE 1

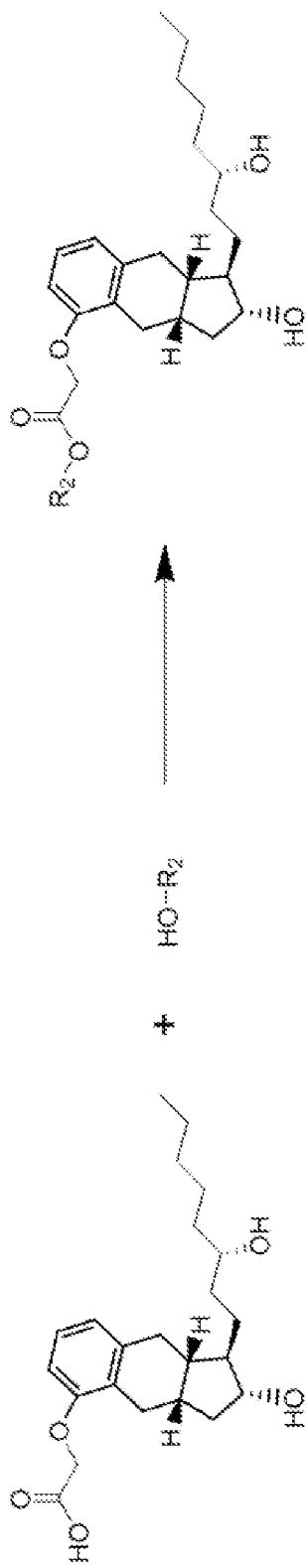
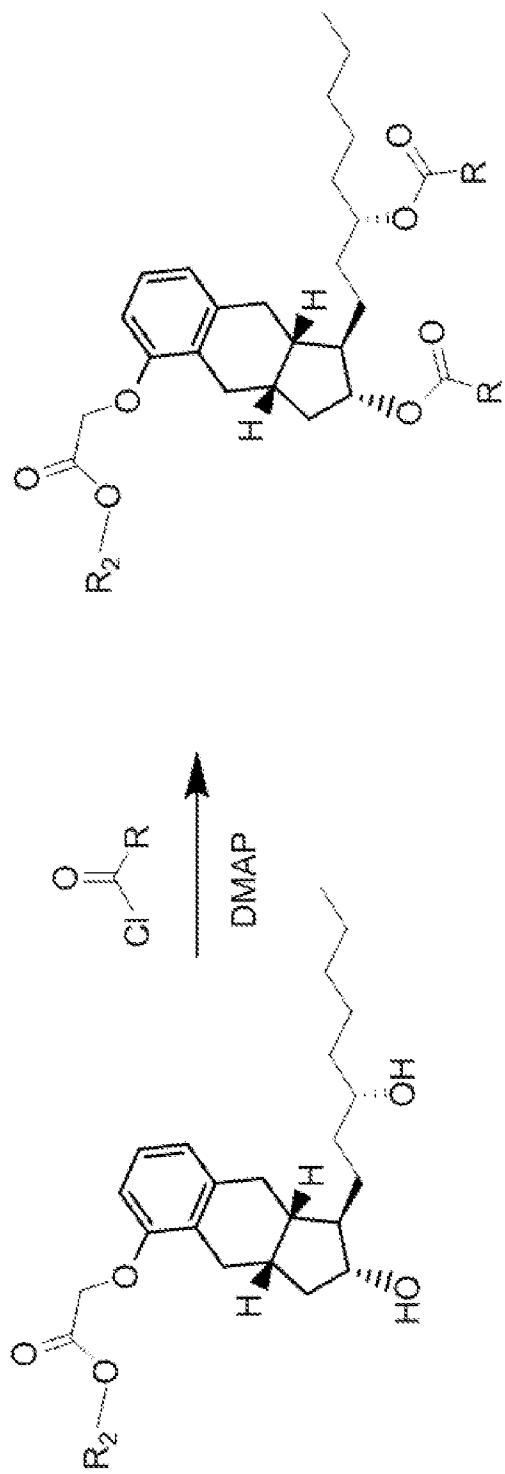


FIGURE 2



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/61427

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07C 65/00, C07C 62/00 (2015.01)

CPC - C07C 405/0075, C07C 59/72, C07C 51/412

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - C07C 65/00, C07C 62/00 (2015.01)

CPC - C07C 405/0075, C07C 59/72, C07C 51/412

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 562/466; 514/571

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Patbase, Google Patent, Google Web

Search terms used - trepostinil derivatives acid catalyst amberlyst dioxane sulfonic acid

Pubchem substructure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---	US 2014/0256730 A1 (Becker et al.) 11 September 2014 (11.09.2014); para [0075]: compound 2, [0091]:compound 17, [0159]	1-12 ----- 32-38
Y	US 7,417,070 B2 (Phares et al.) 26 August 2008 (26.08.2014); col 12, para 1	32-38
A,P	US 2015/0175529 A1 (Malinin et al.) 25 June 2015 (25.06.2015); entire document	1-12, 32-38
A	WO 2011/153363 A1 (United Therapeutics Corporation) 08 December 2011 (08.12.2011); entire document	1-12, 32-38

 Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 06 January 2016	Date of mailing of the international search report 02 FEB 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/61427

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 13-31; 39-53 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.