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(54) **Title:** IMPROVED SYNTHESIS

(57) **Abstract:** The present invention provides an improved synthesis of a class of steroid saponins. Furthermore, the present invention provides a method of selectively discriminating between the C2 and C3 hydroxyl groups of a mono-glycosylated steroid saponin – a key step in the preparation of this class of compounds. Additionally, the present invention provides a range of steroid saponin derivatives, and methods of making them.

## Improved synthesis

### Field of the Invention

The present invention provides an improved synthesis of a class of steroid saponins. Furthermore, the present invention provides a method of selectively discriminating between the C2 and C3 hydroxyl groups of a mono-glycosylated steroid saponin – a key step in the preparation of this class of compounds. Additionally, the present invention provides a range of steroid saponin derivatives, and methods of making them.

### Cross Reference to Related Application(s)

The present invention is related to the following two international patent applications assigned to the present applicant, the disclosure of which is incorporated herein by cross reference: PCT/AU2007/001091 and PCT/AU2007/001092. The aforementioned applications describe advantageous therapeutic applications, compositions and uses of some steroid saponins that can be synthesised using the methods of the present invention.

### Background

There remains a great need for new compounds and therapies to treat all manner of pathogenic, deficiency, hereditary and physiological diseases. In particular, with rising life expectancies, there has been a significant rise in the incidence of non-infectious, age-related diseases, such as cancer.

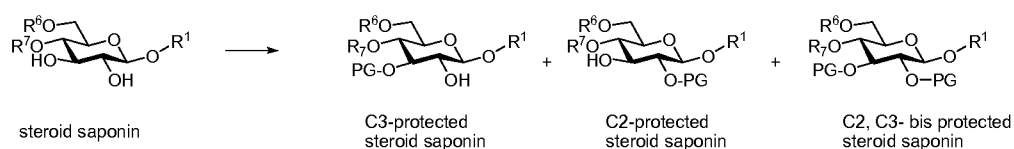
Furthermore, it is paramount that new, efficient methods for accessing these active agents are developed to provide access to them in sufficient quantities to provide broad availability of their novel therapies.

Steroid saponins, a class of secondary metabolites derived from various plant and marine species, are of particular interest as novel active agents due to their remarkable bioactivity. Some saponins have been shown to bind and cross cell membranes, others have been utilised as surfactants, still others have been used as adjuvants in vaccines. Saponins have also been utilised in Chinese medicine and as such, have been promoted as dietary supplements. Furthermore, some steroid saponins are known to enhance the activity of a number of chemotherapeutic and anti-cancer agents, ultimately inhibiting growth of cancerous cells. Other steroid saponins have demonstrated an ability to inhibit angiogenesis in a number of *in vivo* and *ex vivo* model systems. Accordingly, steroid saponins provide a class of interesting molecules with diverse biological activity.

Some naturally occurring steroid saponins have been extracted from plant sources. However, the relatively small and seasonally variable amounts of the compound available from plant sources via extraction limits the potential commercial development of this class of compounds and its physiologically active analogues and derivatives as therapeutic agents. Obtaining access to the steroid saponins via synthetic routes is thus paramount to their further development as pharmaceutical agents.

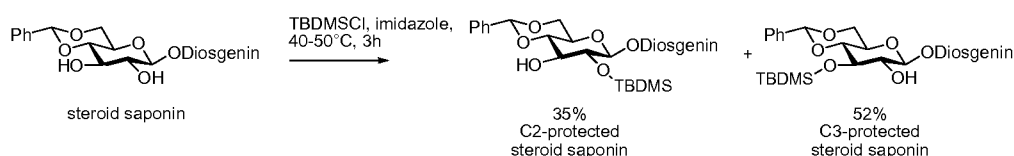
Nevertheless, the synthesis of these complex molecules remains challenging. Broadly speaking, some problems associated with achieving an efficient synthesis include the large number of stereocentres; the contrasting physico-chemical properties of the lipophilic sapogenin/aglycone moiety and the more hydrophilic glycoside moiety; and their comparatively high molecular weights.

More specifically, the construction of the di- and/or poly-glycoside moiety has proved particularly difficult to achieve. In particular, discrimination of the C2 / C3 hydroxyl groups of the mono-glycoside saponin has proved difficult, but is nevertheless a key step in order to selectively and efficiently functionalise the derived saponin with further glycosidic or other moieties at either of the C2 or C3 positions.

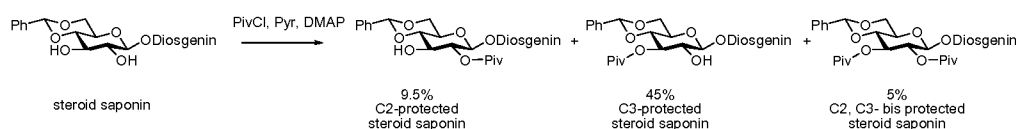


The C2 and C3 hydroxyl groups of the mono-glycosylated steroid saponin have similar physico-chemical properties, and thus substantially similar reactivities (*Journal of Organic Chemistry* (1997), **62**, pp. 8406-8418). Achieving regio-selectivity at one or other of these positions is thus challenging. In view of this, previous syntheses towards steroid saponin derivatives have utilised elaborate and inefficient protecting group strategies comprising many steps in order to discriminate between these two groups. Alternative syntheses have utilised non- or semi-selective conditions to obtain mixtures of regio-isomers which subsequently require complicated separation and purification techniques in order to access one or other preferred regioisomer. However, separation of C2-mono protected and C3-mono protected compounds, is often difficult due to their similar physico-chemical properties. All of these strategies are inefficient, require separation of complex mixtures and/or necessitate removal of contaminating by-products and thus ultimately lead to reduced yields of the desired steroid saponin. This is clearly undesirable when access to commercial scale quantities of the active agent is required.

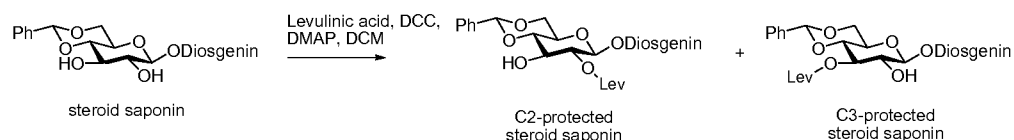
Previously described attempts provide low yields and/or poor selectivity for one or other regioisomer. For example, previous attempts to selectively protect the C3-hydroxyl of a steroid saponin with a silyl protecting group, namely tertbutyldimethylsilyl chloride, provided 35% of the undesired C2-protected steroid saponin, and 52% of the desired C3-steroid saponin. This mixture of products ultimately requires extensive separation and purification in order to access one or other preferred regioisomer (*Carbohydrate Research*, (1998) **306**, pp. 189–195).



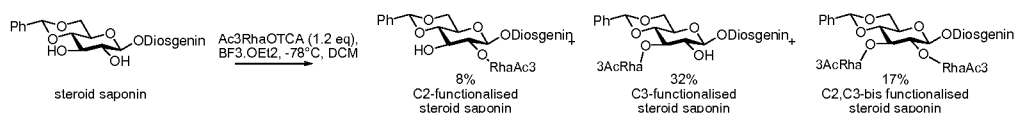
Similarly poor selectivity has been reported with other protecting groups, such as pivaloyl chloride, wherein 9.5% of the undesired C2-protected steroid saponin, 45% of the desired C3-steroid saponin and 5% of the C2,C3-bis protected steroid saponin were obtained (*Bioorganic & Medicinal Chemistry* (2008), **16**, pp. 2063–2076).



Furthermore, attempts to selectively prepare a C3-protected steroid saponin using known coupling reagents also reportedly result in mixtures of products. For example, use of levulinic acid with N,N'-dicyclohexylcarbodiimide and DMAP in DCM provided the C3- and C2-protected material in 75% yield and a ratio of 2:1 (*Bioorganic & Medicinal Chemistry Letters* (2006), **16**, pp. 2454–2458).



Still other attempts to subsequently functionalise similar steroid saponins, without protection at either of C2 or C3 have resulted in, for example, no selectivity and complex mixtures comprising the starting steroid saponin (43%), C2-mono functionalised (8%), C3-mono functionalised (32%) and C2,C3-bis functionalised compounds (17%) (*Journal of Carbohydrate Chemistry* (1999), **18**, pp. 1107-1120).



In view of the inefficient synthesis described in the art, development of a new method for efficient and selective discrimination of the C2 / C3 hydroxyl groups, especially of mono-glycosylated saponins is paramount.

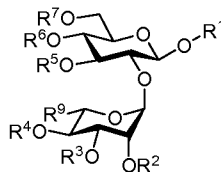
The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification (including the claims) they are to be interpreted as specifying the presence of the stated features, integers, steps or components, but not precluding the presence of one or more other features, integers, steps or components, or group thereof.

#### **Summary of the Invention**

In seeking new efficient synthesis of a range of active agents, specifically steroid saponins, the Applicant has surprisingly found a method of discriminating between the C2 / C3 hydroxyl groups of the mono-glycoside saponin. The new method advantageously provides efficient, regio- and stereo-selective access to this class of compounds. Furthermore, the method also provides access to a range of new saponin derivatives, not previously known or characterised. As such, the method described by the Applicant provides the desired C3-protected mono-glycosylated saponin in excellent selectivity. In some embodiments, none of the undesired C2-protected mono-glycoside is observed. In view of this surprising and unexpected result, the applicant's method advantageously provides a more efficient route to access this class of active agents, and thus provides access to a range of novel physiologically active analogues and derivatives thereof. The present invention thus extends to such novel steroid saponin analogues and derivatives.

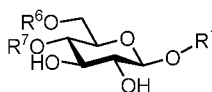
Accordingly, in one aspect, the present invention provides a method for the preparation of a compound of Formula X

**Formula X**

- wherein
- R<sup>1</sup> is a sapogenin;
  - R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup> are each independently an oxygen protecting group;
  - R<sup>5</sup> is an acyl group;
  - R<sup>6</sup> and R<sup>7</sup> are H;
  - R<sup>9</sup> is selected from the group consisting of H, CH<sub>3</sub>, and an oxygen protected by an oxygen protecting group;

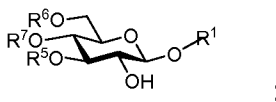
the method comprising

- (i) reacting a compound of Formula A with an acylating agent in an acylation reaction in the presence of a base;

**Formula A**

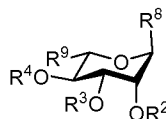
- wherein
- R<sup>1</sup> is a sapogenin;
  - R<sup>6</sup> and R<sup>7</sup> are each independently an oxygen protecting group, or when taken together form a cyclic di-oxygen protecting group;

to provide a compound of Formula B

**Formula B**

- R<sup>1</sup> is sapogenin;
- R<sup>5</sup> is an acyl group;
- R<sup>6</sup> and R<sup>7</sup> are each independently an oxygen protecting group, or when taken together form a cyclic di-oxygen protecting group;

- (ii) reacting a compound of Formula B with a compound of Formula C under coupling conditions

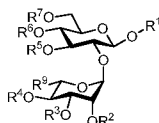


**Formula C**

- wherein  $R^2$ ,  $R^3$ , and  $R^4$  are each independently an oxygen protecting group;  
 $R^8$  is a leaving group; and  
 $R^9$  is selected from the group consisting of H,  $CH_3$ , and an oxygen protected by an oxygen protecting group;

- (iii) selectively removing the oxygen protecting groups at  $R^6$  and  $R^7$  to provide a compound of Formula X.

In another another aspect, the present invention provides a method for the preparation of a compound of Formula Y

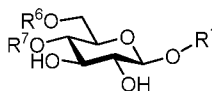


**Formula Y**

- wherein  $R^1$  is a sapogenin;  
 $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$  and  $R^7$  are each H,  
 $R^6$  is H or a saccharide,  
 $R^9$  is selected from the group consisting of H, OH and  $CH_3$ ;  
 pharmaceutically acceptable salts, isomers, hydrates and solvates thereof;

the method comprising

- (i) reacting a compound of Formula A with an acylating agent in an acylation reaction in the presence of a base;

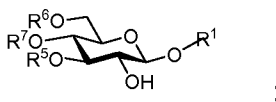
**Formula A**

wherein

$R^1$  is a sapogenin;

$R^6$  and  $R^7$  are each independently an oxygen protecting group, or when taken together form a cyclic di-oxygen protecting group;

to provide a compound of Formula B

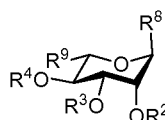
**Formula B**

$R^1$  is a sapogenin;

$R^5$  is an acyl group;

$R^6$  and  $R^7$  are each independently an oxygen protecting group, or when taken together form a cyclic di-oxygen protecting group;

- (ii) reacting a compound of Formula B with a compound of Formula C under coupling conditions

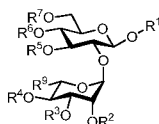
**Formula C**

wherein  $R^2$ ,  $R^3$ , and  $R^4$  are each independently an oxygen protecting group;

$R^8$  is a leaving group; and

$R^9$  is selected from the group consisting of H,  $CH_3$ , and an oxygen protected by an oxygen protecting group;

- (iii) selectively removing the oxygen protecting groups at  $R^6$  and  $R^7$  to provide a compound of Formula X

**Formula X**

- wherein  $R^1$  is a sapogenin;  
 $R^2$ ,  $R^3$ , and  $R^4$  are each independently an oxygen protecting group;  
 $R^5$  is an acyl group;  
 $R^6$  and  $R^7$  are H;  
 $R^9$  is selected from the group consisting of H,  $CH_3$ , and an oxygen protected by an oxygen protecting group;
- (iv) converting a compound of Formula X into a compound of Formula Y, pharmaceutically acceptable salts, isomers, hydrates or solvates thereof.

### Detailed Description of the Invention

Various terms that will be used throughout the specification have meanings that will be well understood by a skilled addressee. However, for ease of reference, some of these terms will now be defined.

The term "protected" as used throughout the specification in the context of any compound, residue or functional group will, unless the context otherwise requires, be taken to refer to the use of a removable protecting group to prevent an undesirable reaction of the respective compound, residue or functional group. The expression "protecting group" and like expressions will be understood correspondingly. Protecting groups, if present, may if desired be simultaneously or subsequently removed in conventional manner. The use and removal of appropriate protecting groups will be well within the capacity of those skilled in this art.

The term "oxygen protecting group" as used throughout the specification means a group that can prevent the oxygen moiety reacting during further derivatisation of the protected compound and which can be readily removed when desired. Examples of oxygen protecting groups include but are not limited to acyl groups (such as acetyl and benzoyl), optionally substituted alkoxy (such as methoxy methyl ether (MOM), p-methoxy ethoxy methyl ether (MEM), p-methoxy benzyl ether (PMB), methylthio methyl ether, Pivaloyl (Piv), Tetrahydropyran (THP)), and silyl ethers (such as Trimethylsilyl (TMS) tert-butyl dimethyl silyl (TBDMS) and triisopropylsilyl (TIPS)).

The terms "alcohol", "hydroxyl" or "hydroxy" are all understood to refer to a functional group of the formula  $-OH$ , and may be used interchangeably throughout the specification. Where a molecule or compound comprises more than one functional group of the formula  $-OH$ , these may be differentiated based on the order of attachment to, or systematic numbering of, the molecule or compound. Where possible, the order of attachment is

determined by standard International Union of Pure and Applied Chemistry (IUPAC) nomenclature. For example reference to C2-hydroxyl of a monoglycoside saponin, refers to the secondary alcohol at the 2-position of the glycoside ring. Similarly, reference to C3-hydroxyl of a monoglycoside saponin refers to the secondary alcohol at the 3-position of the glycoside ring.

“Acyl” and “acyl group” as used throughout the specification, will be understood to refer to a group of the following formula:  $R'-C(=O)-$ ; wherein R' is independently selected from the group consisting of H, halogen, optionally substituted  $C_1-C_{12}$  alkyl, optionally substituted  $C_2-C_{12}$  alkenyl, optionally substituted  $C_2-C_{12}$  alkynyl, optionally substituted  $C_2-C_{10}$  heteroalkyl, optionally substituted  $C_3-C_{12}$  cycloalkyl, optionally substituted bicycloalkyl, optionally substituted  $C_3-C_{12}$  cycloalkenyl, optionally substituted  $C_2-C_{12}$  heterocycloalkyl, optionally substituted  $C_2-C_{12}$  heterocycloalkenyl, optionally substituted  $C_6-C_{18}$  aryl, optionally substituted  $C_1-C_{18}$  heteroaryl, optionally substituted aroyl, optionally substituted phenyl, optionally substituted  $C_1-C_{10}$  alkoxyphenyl, optionally substituted  $C_1-C_{10}$  dialkoxyphenyl, optionally substituted  $C_1-C_{10}$  alkylphenyl, or optionally substituted  $C_1-C_{10}$  dialkylphenyl. Examples of suitable acyl groups include acetoxy, propionyl, trichloroacetyl, benzoyl, 2-chlorobenzoyl, 4-chlorobenzoyl, 4-nitrobenzoyl and 4-methoxybenzoyl.

The term “acylating agent” as used throughout the specification, will be understood to mean a reagent or compound that provides an acyl group upon reaction with a nucleophile. In some embodiments, the nucleophile is oxygen as part of an alcohol functional group.

The term “leaving group” is any atom, group of atoms or molecular fragment, that is displaced during heterolytic bond cleavage, and takes with it the pair of bonding electrons. The leaving group may be charged or uncharged, but is generally stable. Accordingly in any situation the choice of leaving group will depend upon the ability of the particular group to be displaced by the incoming chemical moiety. Suitable leaving groups are well known in the art, see for example “Advanced Organic Chemistry” Jerry March 4<sup>th</sup> Edn. pp 351-357, Oak Wick and Sons NY (1997). Examples of suitable leaving groups include, but are not limited to, halogen, alkoxy (such as ethoxy, methoxy), sulphonyloxy, optionally substituted arylsulfonyl, optionally substituted silyl, and optionally substituted acyl (such as acetyl and trichloroacetyl), optionally substituted alkylthio, and optionally substituted aryl thio.

The terms “coupling reaction”, “coupling conditions” and the like, as used throughout the specification are understood to mean reactions and/or conditions that may be used to couple a saccharide molecule to another saccharide molecule, which may be the same or a

different saccharide to assemble larger polysaccharides; or to couple a saccharide to a sapogenin to form a mono-glycoside steroid saponin, or similarly to couple additional saccharides to form di- or poly-saccharide saponins; or to couple a saccharide to a compound of any one of Formulas A, B, C X, Y, Y<sup>A</sup>, Y<sup>B</sup>, Y<sup>C</sup>, E, F and G.

"Alkenyl" as a group or part of a group denotes an aliphatic hydrocarbon group containing at least one carbon-carbon double bond and which may be straight or branched having 2-12 carbon atoms, typically 2-10 carbon atoms, typically 2-6 carbon atoms, in the normal chain. The group may contain a plurality of double bonds in the normal chain and the orientation about each is independently E or Z. The alkenyl group is typically a 1-alkenyl group. Exemplary alkenyl groups include, but are not limited to, ethenyl, propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl and nonenyl.

"Alkyl" as used throughout the specification as a group or part of a group refers to a straight or branched aliphatic hydrocarbon group, of for example one to twenty carbon atoms, typically a C<sub>1</sub>-C<sub>10</sub> alkyl, typically C<sub>1</sub>-C<sub>6</sub> alkyl unless otherwise noted. Examples of suitable straight and branched C<sub>1</sub>-C<sub>6</sub> alkyl substituents include methyl, ethyl, n-propyl, 2-propyl, n-butyl, sec-butyl, t-butyl, hexyl, and the like.

"Alkyloxy" and "alkoxy" as used throughout the specification refer to an alkyl-O- group in which alkyl is as defined herein. Examples include, but are not limited to, methoxy and ethoxy.

"Aryl" as a group or part of a group denotes (i) an optionally substituted monocyclic, or fused polycyclic, aromatic carbocycle (ring structure having ring atoms that are all carbon) preferably having from 5 to 12 atoms per ring. Examples of aryl groups include phenyl, naphthyl, and the like; (ii) an optionally substituted partially saturated bicyclic aromatic carbocyclic moiety in which a phenyl and a C<sub>5</sub>-C<sub>7</sub> cycloalkyl or C<sub>5</sub>-C<sub>7</sub> cycloalkenyl group are fused together to form a cyclic structure, such as tetrahydronaphthyl, indenyl or indanyl. Typically an aryl group is a C<sub>6</sub>-C<sub>8</sub> aryl group and most preferably is phenyl.

"Arylalkyl" means an aryl-alkyl- group in which the aryl and alkyl moieties are as defined herein. Preferred arylalkyl groups contain a C<sub>1</sub>-C<sub>5</sub> alkyl moiety. Exemplary arylalkyl groups include benzyl, phenethyl, 1-naphthalenemethyl and 2-naphthalenemethyl.

"Arylamino" includes both mono-arylamino and di-arylamino unless specified. Mono-arylamino means a group of formula arylNH-, in which aryl is as defined herein,

di-arylamino means a group of formula  $(\text{aryl})_2\text{N}$ - where each aryl may be the same or different and are each as defined herein for aryl.

"Aryloxy" refers to an aryl-O- group in which the aryl is as defined herein. Preferably the aryloxy is a  $\text{C}_6$ - $\text{C}_{18}$ aryloxy, more preferably a  $\text{C}_6$ - $\text{C}_{10}$ aryloxy.

A "bond" is a covalent linkage between atoms in a compound or molecule. The bond may be a single bond, a double bond, or a triple bond.

"Cycloalkenyl" means a non-aromatic monocyclic or multicyclic ring system containing at least one carbon-carbon double bond and preferably having from 5-10 carbon atoms per ring. Exemplary monocyclic cycloalkenyl rings include cyclopentenyl, cyclohexenyl or cycloheptenyl. The cycloalkenyl group may be substituted by one or more substituent groups. A cycloalkenyl group typically is a  $\text{C}_3$ - $\text{C}_{12}$  alkenyl group.

"Cycloalkyl" refers to a saturated monocyclic or fused or spiro polycyclic, carbocycle preferably containing from 3 to 9 carbons per ring, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like, unless otherwise specified. It includes monocyclic systems such as cyclopropyl and cyclohexyl, bicyclic systems such as decalin, and polycyclic systems such as adamantane. A cycloalkyl group typically is a  $\text{C}_3$ - $\text{C}_{12}$  alkyl group.

"Haloalkyl" refers to an alkyl group as defined herein in which one or more of the hydrogen atoms has been replaced with a halogen atom selected from the group consisting of fluorine, chlorine, bromine and iodine. A haloalkyl group typically has the formula  $\text{C}_n\text{H}_{(2n+1-m)}\text{X}_m$  wherein each X is independently selected from the group consisting of F, Cl, Br and I. In groups of this type n is typically from 1 to 10, more preferably from 1 to 6, most preferably 1 to 3. m is typically 1 to 6, more preferably 1 to 3. Examples of haloalkyl include fluoromethyl, difluoromethyl and trifluoromethyl.

"Haloalkenyl" refers to an alkenyl group as defined herein in which one or more of the hydrogen atoms has been replaced with a halogen atom independently selected from the group consisting of F, Cl, Br and I.

"Halogen" or "halo" represents chlorine, fluorine, bromine or iodine.

"Heteroalkyl" refers to a straight- or branched-chain alkyl group preferably having from 2 to 12 carbons, typically 2 to 6 carbons in the chain, in which one or more of the carbon

atoms (and any associated hydrogen atoms) are each independently replaced by a heteroatomic group selected from S, O, P and NR' where R' is selected from the group consisting of H, optionally substituted C<sub>1</sub>-C<sub>12</sub> alkyl, optionally substituted C<sub>3</sub>-C<sub>12</sub> cycloalkyl, optionally substituted C<sub>6</sub>-C<sub>18</sub> aryl, and optionally substituted C<sub>1</sub>-C<sub>18</sub> heteroaryl. Exemplary heteroalkyls include alkyl ethers, secondary and tertiary alkyl amines, amides, alkyl sulfides, and the like. Examples of heteroalkyl also include hydroxyl C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkyloxy C<sub>1</sub>-C<sub>6</sub> alkyl, amino C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkylamino C<sub>1</sub>-C<sub>6</sub> alkyl, and di(C<sub>1</sub>-C<sub>6</sub> alkyl)amino C<sub>1</sub>-C<sub>6</sub> alkyl.

"Heteroaryl" either alone or part of a group refers to groups containing an aromatic ring (preferably a 5 or 6 membered aromatic ring) having one or more heteroatoms as ring atoms in the aromatic ring with the remainder of the ring atoms being carbon atoms. Suitable heteroatoms include nitrogen, oxygen and sulphur. Examples of heteroaryl include thiophene, benzothiophene, benzofuran, benzimidazole, benzoxazole, benzothiazole, benzisothiazole, naphtho[2,3-bi]thiophene, furan, isoindolizine, xantholene, phenoxatine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, tetrazole, indole, isoindole, 1H-indazole, purine, quinoline, isoquinoline, phthalazine, naphthyridine, quinoxaline, cinnoline, carbazole, phenanthridine, acridine, phenazine, thiazole, isothiazole, phenothiazine, oxazole, isooxazole, furazane, phenoxazine, 2-, 3- or 4- pyridyl, 2-, 3-, 4-, 5-, or 8- quinolyl, 1-, 3-, 4-, or 5- isoquinolyl 1-, Z-, or 3- indolyl, and 2-, or 3- thienyl. A heteroaryl group is typically a C<sub>1</sub>-C<sub>18</sub> heteroaryl group.

"Heterocyclic" refers to saturated, partially unsaturated or fully unsaturated monocyclic, bicyclic or polycyclic ring system containing at least one heteroatom selected from the group consisting of nitrogen, sulfur and oxygen as a ring atom. Examples of heterocyclic moieties include heterocycloalkyl, heterocycloalkenyl and heteroaryl.

"Heterocycloalkyl" refers to a saturated monocyclic, bicyclic, or polycyclic ring containing at least one heteroatom selected from nitrogen, sulfur or oxygen, preferably from 1 to 3 heteroatoms in at least one ring. Each ring is preferably from 3 to 10 membered, more preferably 4 to 7 membered. Examples of suitable heterocycloalkyl substituents include pyrrolidyl, tetrahydrofuryl, tetrahydrothiofuranyl, piperidyl, piperazyl, tetrahydropyranyl, morpholino, 1,3-diazapane, 1,4-diazapane, 1,4-oxazepane, and 1,4-oxathiapane. A heterocycloalkyl group typically is a C<sub>2</sub>-C<sub>12</sub> heterocycloalkyl group.

The term "glycoside" as used throughout the specification is to be understood to mean a compound that contains a saccharide (sugar) moiety (monosaccharide, disaccharide or polysaccharide), linked to a triterpene or steroid or steroid alkaloid aglycone (non-saccharide)

component. In most circumstances, the saccharide (sugar) moiety is linked to the C-3 position of the aglycone, although other linkages are contemplated within the scope of the present invention. For example the furostanol glycosides, which contain a saccharide attached to the C-26 position, and spirostanol glycosides are both sub-classes of the steroid saponins.

"Lower alkyl" as a group means unless otherwise specified, an aliphatic hydrocarbon group which may be straight or branched having 1 to 6 carbon atoms in the chain, more preferably 1 to 4 carbons such as methyl, ethyl, propyl (n-propyl or isopropyl) or butyl (n-butyl, isobutyl or tertiary-butyl).

The term "saponin" as used throughout the specification is to be understood to mean a glycoside including a saccharide (sugar) attached to the aglycone, generally through the C-3 position of the aglycone.

The term "steroid saponin" as used throughout the specification is to be understood to mean a glycoside including one or more saccharide units (including one or more monosaccharide, disaccharide or polysaccharide units) attached to an aglycone which does not contain a nitrogen atom.

In this regard, it will be understood that the term "steroid saponin" includes within its scope any salts or any other derivatives of the compounds that are functionally equivalent, in particular with respect of therapeutic active agents. As such, they may be pharmaceutically acceptable salts. Furthermore, they may be naturally-occurring or synthetic steroid saponins.

The term "pharmaceutically acceptable salts" used throughout the specification is understood to mean salts that retain the desired biological activity of the above-identified compounds, and include pharmaceutically acceptable acid addition salts and base addition salts. Suitable pharmaceutically acceptable acid addition salts of compounds of the present invention may be prepared from an inorganic acid or from an organic acid. Examples of such inorganic acids are hydrochloric, sulfuric, and phosphoric acid. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, heterocyclic carboxylic and sulfonic classes of organic acids, examples of which are formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, fumaric, maleic, alkyl sulfonic arylsulfonic. Suitable pharmaceutically acceptable base addition salts include metallic salts made from lithium, sodium, potassium, magnesium, calcium, aluminium, and zinc, and organic salts made from organic bases such as benzathine, chlorprocaine, choline, diethanolamine, ethanolamine, ethyldiamine, meglumine and procaine. Other examples of organic salts are: ammonium



cycloalkyloxy, cycloalkenyloxy, heterocycloalkyloxy, heterocycloalkenyloxy, aryloxy, phenoxy, benzyloxy, heteroaryloxy, arylalkyloxy, amino, alkylamino, acylamino, aminoalkyl, arylamino, sulfonylamino, sulfinylamino, sulfonyl, alkylsulfonyl, arylsulfonyl, aminosulfonyl, sulfinyl, alkylsulfinyl, arylsulfinyl, aminosulfinylaminoalkyl,  $-C(=O)OH$ ,  $-C(=O)R^a$ ,  $-C(=O)OR^a$ ,  $C(=O)NR^aR^b$ ,  $C(=NOH)R^a$ ,  $C(=NR^a)NR^bR^c$ ,  $NR^aR^b$ ,  $NR^aC(=O)R^b$ ,  $NR^aC(=O)OR^b$ ,  $NR^aC(=O)NR^bR^c$ ,  $NR^aC(=NR^b)NR^cR^d$ ,  $NR^aSO_2R^b$ ,  $-SR^a$ ,  $SO_2NR^aR^b$ ,  $-OR^a$ ,  $OC(=O)NR^aR^b$ ,  $OC(=O)R^a$  and acyl,

wherein  $R^a$ ,  $R^b$ ,  $R^c$  and  $R^d$  are each independently selected from the group consisting of H, optionally substituted  $C_1$ - $C_{12}$  alkyl, optionally substituted  $C_1$ - $C_{12}$  haloalkyl, optionally substituted  $C_2$ - $C_{12}$  alkenyl, optionally substituted  $C_2$ - $C_{12}$  alkynyl, optionally substituted  $C_2$ - $C_{10}$  heteroalkyl, optionally substituted  $C_3$ - $C_{12}$  cycloalkyl, optionally substituted  $C_3$ - $C_{12}$  cycloalkenyl, optionally substituted  $C_2$ - $C_{12}$  heterocycloalkyl,  $C_2$ - $C_{12}$  heterocycloalkenyl, optionally substituted  $C_6$ - $C_{18}$  aryl, optionally substituted  $C_1$ - $C_{18}$  heteroaryl, and acyl, or any two or more of  $R^a$ ,  $R^b$ ,  $R^c$  and  $R^d$ , when taken together with the atoms to which they are attached form a heterocyclic ring system with 3 to 12 ring atoms.

It is recognized that the steroid saponins of the present invention are advantageous therapeutic agents for the treatment of disease. In using the compounds of the invention they can be administered in any form or mode which makes the compound available to provide a biological effect. One skilled in the art of preparing formulations can readily select the proper form and mode of administration depending upon the particular characteristics of the compound selected, the condition to be treated, the stage of the condition to be treated and other relevant circumstances. As previously described, and in view of the advantageous therapeutic effect(s), compounds referred to herein are intended to cover, where applicable, any form, isomer, pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof that may advantageously provide or improve the bioavailability and/or administration of the compound of the present invention to obtain a desired therapeutic effect.

Saponins are conventionally divided into three major classes: (i) triterpene glycosides; (ii) steroidal glycosides; and (iii) steroidal alkaloid glycosides. They all have in common the attachment of one or more saccharide units to the aglycone, generally at the C-3 position. Steroid saponins are generally as described in Hostettmann K and Marston A (2005). *Chemistry & pharmacology of natural products: Saponins*. Cambridge University Press.

As discussed previously herein, steroid saponins do not contain a nitrogen atom in the aglycone moiety.

It will be appreciated that the steroid saponin in the various embodiments of the present invention include naturally occurring steroid saponins and non-naturally occurring steroid saponins (ie chemically synthesized steroid saponins). In addition, it will also be appreciated that the steroid saponin in the various embodiments of the present invention also includes pro-drugs of the steroid saponin, derivatives of steroid saponins, including for example, any esters, ketones, carboxylic acids, salts, substituted forms, halogenated forms or other heteroatom containing forms, unsaturated forms, or any other functional derivative.

The saccharide portion of the steroid saponins in the various embodiments of the present invention may include one or more saccharide units, such as a monosaccharide, a disaccharide unit or a polysaccharide unit.

It will also be appreciated that the steroid saponin of the various embodiments of the present invention may also include an aglycone with a saccharide attached at one or more positions of the aglycone moiety.

In one embodiment, the steroid saponin includes a saccharide attached to a single position of the sapogenin component of the steroid saponin.

As discussed above, the saccharide unit may be a monosaccharide, a disaccharide or a polysaccharide. The saccharide may be composed of a suitable monosaccharide, such as D-glucose (Glc), L-rhamnose (Rha), D-galactose (Gal), D-glucuronic acid (GlcA), D-xylose (Xyl), L-arabinose (Ara), D-fucose (Fuc), D-galacturonic acid (GalA). The saccharide unit may also be a substituted saccharide, such as an amino saccharide, a sulfated saccharide, an acylated saccharide, an *N*-acylated amino saccharide, and functional derivatives of any of the aforementioned monosaccharides.

Similarly, a disaccharide may be any combination of two monosaccharides, as described above.

The polysaccharides in the various embodiments of the present invention may be linear or branched, and include any combination of two or more monosaccharide, including the monosaccharide described previously herein.

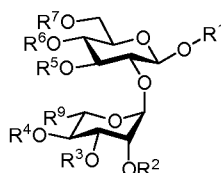
In one embodiment, the polysaccharide is composed of 1 to 6 monosaccharide units.

In this regard, and as described previously herein, polysaccharides are generally described in the context of the arrangement of the component monosaccharides.

The method of the invention can be performed on any scale from laboratory through pilot plant to commercial (kilogram) scale.

As previously described, in some aspects, the present invention provides a new method for accessing a range of steroid saponins.

With regard to the new method described herein, the present invention provides a method for the preparation of a compound of Formula X

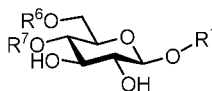


**Formula X**

- wherein
- $R^1$  is a sapogenin;
  - $R^2$ ,  $R^3$ , and  $R^4$  are each independently an oxygen protecting group;
  - $R^5$  is an acyl group;
  - $R^6$  and  $R^7$  are H;
  - $R^9$  is selected from the group consisting of H,  $\text{CH}_3$ , and an oxygen protected by an oxygen protecting group;

the method comprising

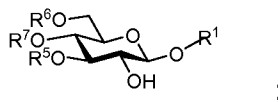
- (i) reacting a compound of Formula A with an acylating agent in an acylation reaction in the presence of a base;



**Formula A**

- wherein
- $R^1$  is a sapogenin;
  - $R^6$  and  $R^7$  are each independently an oxygen protecting group, or when taken together form a cyclic di-oxygen protecting group;

to provide a compound of Formula B



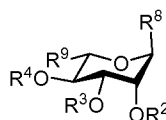
**Formula B**

R<sup>1</sup> is sapogenin;

R<sup>5</sup> is an acyl group;

R<sup>6</sup> and R<sup>7</sup> are each independently an oxygen protecting group or when taken together form a cyclic di-oxygen protecting group;

- (ii) reacting a compound of Formula B with a compound of Formula C under coupling conditions

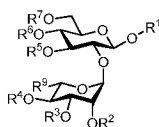


**Formula C**

wherein R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup> are each independently an oxygen protecting group;  
R<sup>8</sup> is a leaving group; and  
R<sup>9</sup> is selected from the group consisting of H, CH<sub>3</sub>, and an oxygen protected by an oxygen protecting group;

- (iii) selectively removing the oxygen protecting groups at R<sup>6</sup> and R<sup>7</sup> to provide a compound of Formula X.

In another aspect, the present invention provides a method for the preparation of a compound of Formula Y



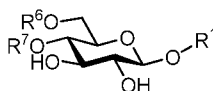
**Formula Y**

wherein R<sup>1</sup> is a sapogenin;  
R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>7</sup> are each H;  
R<sup>6</sup> is H or a saccharide;  
R<sup>9</sup> is selected from the group consisting of H, OH and CH<sub>3</sub>;

pharmaceutically acceptable salts, isomers, hydrates and solvate thereof;

the method comprising

- (i) reacting a compound of Formula A with an acylating agent in an acylation reaction in the presence of a base;



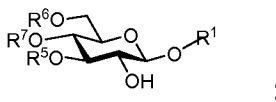
**Formula A**

wherein

$R^1$  is a sapogenin;

$R^6$  and  $R^7$  are each independently an oxygen protecting group, or when taken together form a cyclic di-oxygen protecting group;

to provide a compound of Formula B



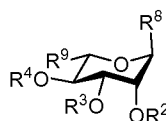
**Formula B**

$R^1$  is a sapogenin;

$R^5$  is an acyl group;

$R^6$  and  $R^7$  are each independently an oxygen protecting group, or when taken together form a cyclic di-oxygen protecting group;

- (ii) reacting a compound of Formula B with a compound of Formula C under coupling conditions



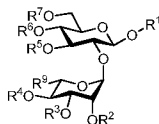
**Formula C**

wherein  $R^2$ ,  $R^3$ , and  $R^4$  are each independently an oxygen protecting group;

$R^8$  is a leaving group; and

$R^9$  is selected from the group consisting of H,  $CH_3$ , and an oxygen protected by an oxygen protecting group;

- (iii) selectively removing the oxygen protecting groups at R<sup>6</sup> and R<sup>7</sup> to provide a compound of Formula X



**Formula X**

- wherein
- R<sup>1</sup> is a sapogenin;
  - R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup> are each independently an oxygen protecting group;
  - R<sup>5</sup> is an acyl group;
  - R<sup>6</sup> and R<sup>7</sup> are H;
  - R<sup>9</sup> is selected from the group consisting of H, CH<sub>3</sub>, and an oxygen protected by an oxygen protecting group;

- (iv) converting a compound of Formula X into a compound of Formula Y, pharmaceutically acceptable salts, isomers, hydrates or solvates thereof.

In some embodiments due to the nature of the protecting groups at R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>6</sup> and R<sup>7</sup>, the oxygen protecting group that can be a component of R<sup>9</sup> and the acyl group at R<sup>5</sup> steps (III) and (IV) can be carried out concurrently as all protecting groups can be removed in a single operation. In these embodiments a compound of formula X is not isolated as a single operation removes all protecting groups.

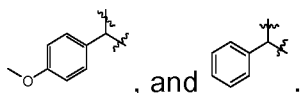
Where R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup> are each oxygen protecting groups, they may be any suitable oxygen protecting group. In some embodiments of Formula C, R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup> are each independently an acyl group. In other embodiments, R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup> of Formula C are each independently selected from an optionally substituted benzoyl and acetyl; in other embodiments R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup> are each independently an optionally substituted benzoyl.

R<sup>5</sup> of Formula B may be any acyl group. In some embodiments R<sup>5</sup> of Formula B is an acyl group selected from the group consisting of an optionally substituted C<sub>1</sub>-C<sub>12</sub> alkyl acyl, an optionally substituted C<sub>3</sub>-C<sub>12</sub> cycloalkyl acyl, an optionally substituted C<sub>6</sub>-C<sub>18</sub> aryl acyl, or an optionally substituted C<sub>5</sub>-C<sub>12</sub> heteroaryl acyl. In some embodiments the acyl group of R<sup>5</sup> may be selected from the group consisting of acetoxy, propionyl, benzoyl, 2-chlorobenzoyl, 4-chlorobenzoyl, 4-nitrobenzoyl and 4-methoxybenzoyl. In further embodiments the acyl group

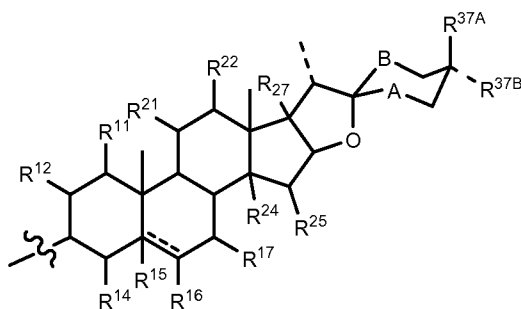
of R<sup>5</sup> is an optionally substituted benzoyl. In other embodiments the acyl group of R<sup>5</sup> is benzoyl.

In some embodiments, the choice of leaving group at R<sup>8</sup> will depend upon the ability of the particular group to be displaced by the incoming chemical moiety. In some embodiments the leaving group is chosen such as to make Formula C an activated donor. Examples of suitable leaving groups are halogen, optionally substituted acyl (such as acetate), optionally substituted alkoxy (such as ethoxy, methoxy), optionally substituted acetimidate (such as trichloroacetimidate or *N*-(phenyl)trifluoroacetimidate), sulphonyloxy, optionally substituted arylsulfonyl, optionally substituted silyl, optionally substituted alkylthio, optionally substituted arylthio. In some embodiments R<sup>8</sup> of Formula C is selected from the group consisting chloro, iodo, bromo, fluoro, ethoxy, methoxy, mesylate, tosylate, triflate, trimethyl silyl, *t*-butyldimethyl silyl, methanethio, ethanethio, *t*-butylthio, trichloroacetyl, trichloroacetimidate and *N*-(phenyl)trifluoroacetimidate. In other embodiments R<sup>8</sup> is trichloroacetimidate or *N*-(phenyl)trifluoroacetimidate.

In some embodiments, R<sup>6</sup> and R<sup>7</sup> of Formula A may each independently be any oxygen protecting group. Suitable oxygen protecting groups have been described as discussed for R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> above and are equally applicable to R<sup>6</sup> and R<sup>7</sup>. In some embodiments, the R<sup>6</sup> and R<sup>7</sup> of Formula A are each independently an acyl or alkoxy protecting group. In further embodiments, the R<sup>6</sup> and R<sup>7</sup> are an acetal, or when taken together, form a cyclic acetal. In still further embodiments, R<sup>6</sup> and R<sup>7</sup> when taken together, form a cyclic group selected from the group consisting of:



In some aspects, the sapogenin at R<sup>1</sup> of Formula A, Formula X, and Formula Y may be a compound of Formula E, F or G as follows:



**Formula E**

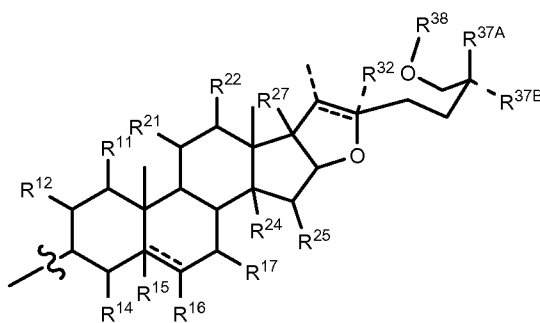
wherein

$R^{11}$ ,  $R^{12}$ ,  $R^{14}$ ,  $R^{16}$ ,  $R^{17}$ ,  $R^{21}$ ,  $R^{22}$ ,  $R^{24}$ ,  $R^{25}$  and  $R^{27}$  are independently H, OH, =O, pharmacologically acceptable ester groups or pharmacologically acceptable ether groups;

$R^{15}$  is H when C-5,C-6 is a single bond, and nothing when C-5,C-6 is a double bond; A is either O concurrently with B being  $CH_2$ , or B is O concurrently with A being  $CH_2$ ;

$R^{37A}$  is H concurrently with  $R^{37B}$  being  $CH_3$ , or  $R^{37A}$  is  $CH_3$  concurrently with  $R^{37B}$  being H;

or a pharmaceutically acceptable salt, or derivative thereof.



**Formula F**

wherein

$R^{11}$ ,  $R^{12}$ ,  $R^{14}$ ,  $R^{16}$ ,  $R^{17}$ ,  $R^{21}$ ,  $R^{22}$ ,  $R^{24}$ ,  $R^{25}$  and  $R^{27}$  are independently H, OH, =O, pharmacologically acceptable ester groups or pharmacologically acceptable ether groups;

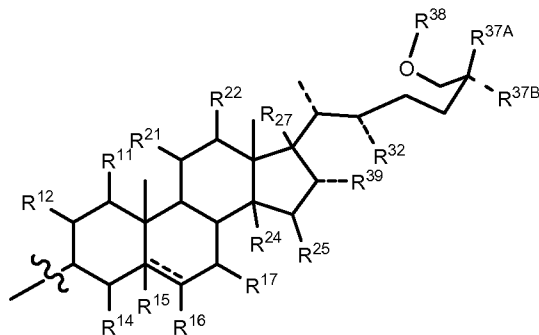
$R^{15}$  is H when C-5,C-6 is a single bond, and nothing when C-5,C-6 is a double bond;

$R^{32}$  is either a hydroxyl or an alkoxy group when C-20, C-22 is a single bond, or nothing when C-20, C-22 is a double bond;

$R^{37A}$  is H concurrently with  $R^{37B}$  being  $CH_3$ , or  $R^{37A}$  is  $CH_3$  concurrently with  $R^{37B}$  being H;

$R^{38}$  is H or a saccharide; or a pharmaceutically acceptable salt, or derivative thereof;

or a pharmaceutically acceptable salt, or derivative thereof.



**Formula G**

wherein

$R^{11}$ ,  $R^{12}$ ,  $R^{14}$ ,  $R^{16}$ ,  $R^{17}$ ,  $R^{21}$ ,  $R^{22}$ ,  $R^{24}$ ,  $R^{25}$  and  $R^{27}$  are each independently H, OH, =O, pharmacologically acceptable ester groups or pharmacologically acceptable ether groups;

$R^{15}$  is H when C-5,C-6 is a single bond, and nothing when C-5,C-6 is a double bond;

$R^{32}$  and  $R^{39}$  are each independently H, OH, =O, pharmacologically acceptable ester groups or pharmacologically acceptable ether groups;

$R^{37A}$  is H concurrently with  $R^{37B}$  being  $CH_3$ , or  $R^{37A}$  is  $CH_3$  concurrently with  $R^{37B}$  being H;

$R^{38}$  is H or a saccharide; or a pharmaceutically acceptable salt, or derivative thereof;

or a pharmaceutically acceptable salt, or derivative thereof.

In some aspects, the sapogenin at  $R^1$  of Formula A is selected from the group consisting of spirostanol aglycones and furostanol aglycones. In other aspects,  $R^1$  of Formula A is a spirostanol aglycone or a furostanol aglycone selected from the group consisting of diosgenin, yamogenin (neodiosgenin), yuccagenin, sarsasapogenin, tigogemmn, smilagenin, hecogenin, gitogemmn, convallamarogenin, neoruscogenin, solagenin, protodiosgenin, pseudoprotodiosgenin, methyl protodiosgenin, protoyamogenin, methyl protoyamogenin, and pharmaceutically acceptable salts, isomers and hydrates thereof.

It is understood that compounds referred to herein are intended to include pharmaceutically acceptable salts, hydrates and solvates thereof. As such, in some aspects the compound of the present invention may be any pharmaceutically acceptable salt that

retains the desired biological activity of the above-identified compounds. In some aspects pharmaceutically acceptable salts include acid addition salts and base addition salts. Where the salt is an acid addition salt, the salt may be prepared from either organic or inorganic acids. Similarly, where the salt is a base addition salt, it may be prepared by addition of organic or inorganic bases. In some aspects, compounds of the present invention may be prepared from inorganic acids such as hydrochloric, sulfuric, and phosphoric acid. In other aspects, the pharmaceutically acceptable salts of the present invention may be prepared from organic acids such as aliphatic, cycloaliphatic, aromatic, heterocyclic carboxylic and sulfonic classes of organic acids, examples of which are formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, fumaric, maleic, alkyl sulfonic and arylsulfonic. Furthermore, in other aspects the compounds of the present invention may be prepared by addition of metallic salts made including lithium, sodium, potassium, magnesium, calcium, aluminium, and zinc, or by addition of organic bases such as benzathine, chlorprocaine, choline, diethanolamine, ethanolamine, ethyldiamine, meglumine, and procaine. In further aspects compounds of the present invention may be organic salts including ammonium salts, quaternary salts such as tetramethylammonium salt; amino acid addition salts such as salts with histidine, glycine, lysine and arginine. In some other aspects compounds of the present invention may be a salt selected from the group consisting of sodium, potassium, ammonium, tetraalkyl ammonium, ethanolamine, diethanolamine, phosphate, and choline.

The base in step (i) may be selected from any nucleophilic or non-nucleophilic base, including organic and inorganic bases. Examples of suitable inorganic bases include alkali earth metal carbonates, alkali earth metal acetates, alkali earth metal hydroxides and alkali earth metal alkyloxides. Specific examples of suitable inorganic bases include sodium carbonate, sodium bicarbonate, potassium carbonate, cesium carbonate, potassium bicarbonate, sodium acetate, potassium acetate, sodium hydroxide, potassium hydroxide, sodium methoxide, and sodium ethoxide. Suitable organic bases include alkyl and aromatic bases, especially nitrogen-containing alkyl and aromatic bases. In some embodiments, the base is a tertiary amine or an aromatic amine, especially a hindered tertiary amine. Examples of suitable organic bases include trialkyl amines, such as trimethylamine, triethylamine, and diisopropylethylamine; heteroaromatic bases, typically nitrogen-containing heteroaromatic bases, such as optionally substituted imidazoles, optionally substituted pyridines such as pyridine, 4-dimethylaminopyridine and 2,6-lutidine; and cyclic- and poly-cyclic nitrogen-containing bases such as optionally substituted piperidine, including *N*-formyl piperidine, 2,2,6,6-tetramethylpiperidine, and 1,8-diazabicycloundec-7-ene. In some embodiments the base is pyridine or an optionally substituted pyridine derivative such as 4-dimethylaminopyridine.

The amount of base chosen will depend upon the steroid saponin, the acylating agent, the solvent (if any), the temperature, and the desired speed of reaction; but is chosen to ensure the method provides the desired regioisomer. Typically an excess of base on a molar equivalent is used. In some embodiments the amount of base 1 to 3 molar equivalents. In other embodiments the amount is from 1 to 2 molar equivalents. In still other embodiments the amount of base is 1 to 1.5 molar equivalents. In other embodiments the amount of base is 1.2 molar equivalents.

Furthermore, method step (i) may be carried out in the presence of a suitable solvent or may be conducted solvent-less. In some cases the base in step (i) may also be the solvent, for example, where the base is an optionally substituted pyridine or optionally substituted piperidine. In some embodiments a suitable solvent is a solvent capable of solvating a glycosylated steroid saponin. In other embodiments, the suitable solvent is a solvent capable of solvating a compound of Formula A. In other embodiments, a suitable solvent is selected from hydrocarbon solvents, halogenated solvents or mixtures thereof. Examples of suitable solvents include, but are not limited to, acetonitrile, acetone, benzene, benzonitrile, 1-butanol, 2-butanol, tert-butyl alcohol, butyl acetate, carbon tetrachloride, chlorobenzene, chloroform, cyclohexane, cyclopentane, 1,2-dichlorobenzene, dichloromethane, 1,2-dichloroethane, diethyl amine, diethyl ether, diethyl ketone, dimethoxyethane diethylene glycol, diethylene glycol dimethyl ether, N,N-dimethylacetamide, N,N-dimethylformamide, dimethyl sulfoxide, 1,4-dioxane, ethylene glycol, ethyl acetate, hexane, heptanes, 2-methoxyethanol, 2-methoxyethyl acetate, methyl acetate, 1-octanol, isopropanol, 1-propanol, ethanol, methanol, tetrachloroethylene, 1,1,2-trichlorotrifluoroethane, 2,2,2-trifluoroethanol, tetrahydrofuran, toluene, triethyl amine, pentane, petroleum ether, pyridine, water, o-xylene, p-xylene, m-xylene, and mixtures thereof. In other embodiments a suitable solvent is selected from the group consisting of dichloromethane, tetrahydrofuran, 1,4-dioxane, dichloroethane, chloroform, carbon tetrachloride and pyridine and mixture thereof. In some embodiments, the solvent is a halogenated solvent. In some embodiments, the solvent is dichloromethane. In other embodiments, the solvent is a dry solvent, or is substantially free from water.

Method step (i) may be carried out at any suitable temperature although it is typically conducted at from -100 to 80°C. In other embodiments, a suitable temperature to conduct method step (i) may be at from -85 to 40°C. In other embodiments, a suitable temperature to carry out method step (i) at from -80 to 25°C. In some embodiments, the method is conducted over a range of temperatures, especially where the temperature is increased over the course of the reaction. In some preferred embodiments the temperature of the reaction in

method step (i) is initially in the range of  $-85$  to  $-70^{\circ}\text{C}$ , and the temperature is subsequently increased over the course of the reaction to a temperature in the range of  $10$  to  $25^{\circ}\text{C}$ . In some embodiments the temperature of the reaction in method step (i) is initially in the range of  $-10$  to  $20^{\circ}\text{C}$  and then subsequently increased over the course of the reaction to a temperature in the range of  $10$  to  $25^{\circ}\text{C}$ . In some embodiments the temperature of the reaction in method step (i) is in the range of from  $0$  to  $20^{\circ}\text{C}$ . In some embodiments the temperature of the reaction in method step (i) is in the range of from  $5$  to  $15^{\circ}\text{C}$ . In some embodiments the temperature of the reaction in method step (i) is in the range of from  $8$  to  $13^{\circ}\text{C}$ .

The method of step (i) may be conducted at atmospheric pressures or may be conducted at pressures greater than or less than atmospheric pressures.

In some embodiments, method of step (i) may be conducted under an inert atmosphere. Where the method step (i) is conducted under an inert atmosphere, the atmosphere may be Nitrogen ( $\text{N}_2$ ) or Argon (Ar).

The method of step (i) of the present invention typically takes from less than 1 minute to 6 hours, more typically from 10 minutes to 3 hours, most typically from 30 minutes to 2.5 hours. In some aspects, the method of the present invention is conducted over 1 to 2 hours. As will be appreciated, however, it is quite easy for a skilled addressee to monitor the reaction.

Furthermore, the acylating agent of step (i) may be any acylating agent. In some embodiments the acylating agent is an acid anhydride or an acyl halide. In other embodiments, the acylating agent is an acyl chloride. In some embodiments the acylating agent is an optionally substituted benzoylating agent. In some embodiments the acylating agent is selected from the group consisting of acetyl chloride, propionyl chloride, benzoyl chloride, 2-chlorobenzoyl chloride, 4-chlorobenzoyl chloride, 4-nitrobenzoyl chloride and 4-methoxybenzoyl chloride. In other embodiments the acylating agent is benzoyl chloride.

The acylating agent and the compound of Formula A may be reacted in any of a number of ratios although the ratio is typically from 5:1 to 0.8:1. In other aspects, the ratio of acylating agent to a compound of Formula A is from 3:1 to 1:1. In other aspects, the ratio of acylating agent to a compound of Formula A is from 2:1 to 1:1. In other aspects, the ratio of acylating agent to a compound of Formula A is from 1.3:1 to 1:1. In other aspects, the ratio of

acylating agent to a compound of Formula A is from 1.2:1 to 1:1. In still other aspects, the ratio of acylating agent to a compound of Formula A is from 1.1:1 to 1:1.

As previously described, in some aspects the present invention provides, in part, methods of coupling a saccharide molecule to another saccharide molecule, which may be the same or a different saccharide to assemble larger saccharide molecules; or methods to couple a saccharide to a sapogenin to form a mono-glycoside steroid saponin, or similarly to couple additional saccharides to form di- or poly-steroid saponins; or to couple a saccharide to a compound of any one of Formulas A, B, C X, Y, Y<sup>A</sup>, Y<sup>B</sup>, Y<sup>C</sup>, E, F and G.

Saccharide coupling reactions generally fall into two categories, according to how the saccharide moiety to be coupled is activated.

The first general category of reaction uses a saccharide trihaloacetimidate (e.g. trichloroacetimidate) as the activated saccharide moiety for coupling. The activated saccharide is coupled, via the activated carbon centre, to a glycoside acceptor, namely another saccharide molecule (via an unprotected OH group thereof) or a diketone (via an unprotected OH group thereof, including an unprotected OH group of a saccharide moiety of the diketone). In some embodiments, a suitable catalyst may also be used.

The second general category of reaction uses a thioglycoside (e.g. thioethyl saccharide or thio-2-propyl saccharide) as the activated saccharide moiety for coupling, in the presence of a suitable catalyst such as a catalytic amount of N-iodosuccinimide (NIS) and an iodine, silyl or silver cocatalyst.

The methods of the present invention encompass each general category of coupling reaction.

It is possible to first assemble a di- or higher polysaccharide and then subsequently couple the di- or higher polysaccharide to a steroid or sapogenin molecule. Alternately, it is possible to first assemble a mono-glycoside saponin and then couple additional saccharides to form di- or poly-glycoside saponins of the present invention. Either of these methods is applicable to the present invention. Typically, the present invention provides initial assembly of a mono-glycoside saponin and then subsequent coupling of additional saccharides to form the desired di- or poly-glycoside saponins.

Examples of suitable saccharides include a mono aldose or ketose having 5 or 6 carbon atoms, preferably in the cyclised furanose or pyranose form, either as  $\alpha$ - or  $\beta$ -anomer and having D or L optical isomerism. Examples of suitable saccharides include glucose, mannose, fructose, galactose, maltose, cellobiose, sucrose, rhamnose, xylose, arabinose, fucose, quinovose, apiose, lactose, galactose-glucose, glucose-arabinose, fucose-glucose, rhamnose-glucose, rhamnose-galactose, glucose-glucose-glucose, glucose-glucose-galactose, gluctose-rhamnose, mannose-glucose, rhamnose-(glucose)-glucose, rhamnose-(rhamnose)-glucose, glucose-(rhamnose)-glucose, glucose-(rhamnose)-galactose, giucose-(rhamnose)-rhamnose, galactose-(rhamnose)-galactose, and protected, typically acylated, derivatives thereof.

As such, the coupling conditions of method of step (ii) may be any suitable coupling conditions. As previously described, in some embodiments, the compound of Formula C in method step (ii) is an activated saccharide. The choice of activated saccharide will depend upon the desired steroid saponin. Suitable leaving groups for activation of the saccharide of Formula C have been discussed with respect to R<sup>8</sup>.

In some embodiments, method of step (ii) uses a saccharide trihaloacetimidate (e.g. trichloroacetimidate or *N*-(phenyl)trifluoroacetimidate) as the activated saccharide of Formula C. In other embodiments, method of step (ii) uses a thioglycoside as the activated saccharide of Formula C. In still other embodiments, method step C uses a haloglycoside as the the activated saccharide of Formula C. In still other embodiments, the activated saccharide of Formula C may be any of suitable saccharide previously described, or derivatives thereof.

Furthermore, method step (ii) may be carried out in the presence of a suitable solvent or may be conducted solvent-less. In some embodiments a suitable solvent is a solvent capable of solvating a glycosylated steroid saponin. In other embodiments, the suitable solvent is a solvent capable of solvating a compound of Formula A or a compound of Formula C. In other embodiments, a suitable solvent is selected from hydrocarbon solvents, halogenated solvents or mixtures thereof. Examples of suitable solvents include, but are not limited to, acetonitrile, acetone, benzene, benzonitrile, 1-butanol, 2-butanol, tert-butyl alcohol, butyl acetate, carbon tetrachloride, chlorobenzene, chloroform, cyclohexane, cyclopentane, 1,2-dichlorobenzene, dichloromethane, 1,2-dichloroethane, diethyl amine, diethyl ether, diethyl ketone, dimethoxyethane diethylene glycol, diethylene glycol dimethyl ether, N,N-dimethylacetamide, N,N-dimethylformamide, dimethyl sulfoxide, 1,4-dioxane, ethylene glycol, ethyl acetate, hexane, heptanes, 2-methoxyethanol, 2-methoxyethyl acetate, methyl acetate, 1-octanol, isopropanol, 1-propanol, ethanol, methanol, tetrachloroethylene, 1,1,2-

trichlorotrifluoroethane, 2,2,2-trifluoroethanol, tetrahydrofuran, toluene, triethyl amine, pentane, petroleum ether, pyridine, water, o-xylene, p-xylene, m-xylene, and mixtures thereof. In other embodiments a suitable solvent is selected from the group consisting of dichloromethane, tetrahydrofuran, 1,4-dioxane, dichloroethane, chloroform, carbon tetrachloride and pyridine and mixture thereof. In some embodiments, the solvent is a halogenated solvent. In some embodiments, the solvent is dichloromethane. In other embodiments, the solvent is a dry solvent, or is substantially free from water.

Method step (ii) may be carried out at any suitable temperature although it is typically conducted at from  $-100$  to  $80^{\circ}\text{C}$ . In other embodiments, a suitable temperature to conduct method step (ii) may be at from  $-85$  to  $40^{\circ}\text{C}$ . In other embodiments, a suitable temperature to carry out method step (i) at from  $-80$  to  $25^{\circ}\text{C}$ . In some embodiments, the method is conducted over a range of temperatures, especially where the temperature is decreased and/or increased over the course of the reaction. In some preferred embodiments the temperature of the reaction in method step (ii) is initially in the range of  $0$  to  $40^{\circ}\text{C}$ , the temperature is then decreased to a temperature in the range of  $-80$  to  $-25^{\circ}\text{C}$ , and subsequently increased to a temperature in the range of  $-25$  to  $10^{\circ}\text{C}$ . In other embodiments the temperature of the reaction in method step (ii) is initially in the range of  $-50$  to  $0^{\circ}\text{C}$ , and the temperature is subsequently increased to a temperature in the range of  $0$  to  $40^{\circ}\text{C}$ .

The method of step (i) may be conducted at atmospheric pressures or may be conducted at pressures greater than or less than atmospheric pressures.

The method of step (i) of the present invention typically takes from less than 1 minute to 48 hours, more typically from 5 minutes to 3 hours, most typically from 30 minutes to 2.5 hours. In some aspects, the method of the present invention is conducted over 1 to 2 hours. As will be appreciated, however, it is quite easy for a skilled addressee to monitor the reaction.

In some embodiments, method of step (ii) may be conducted under an inert atmosphere. Where the method step (ii) is conducted under an inert atmosphere, the atmosphere may be Nitrogen ( $\text{N}_2$ ) or Argon (Ar).

As previously described, the method of step (ii) may be conducted in the presence of a catalyst. The catalyst may be any suitable catalyst. In some embodiments, the catalyst may be a Lewis acid, a Lewis base, a Brønsted acid or a Brønsted base. In still other embodiments, the catalyst may be a reagent that can activate a saccharide, for example, the catalyst may be a reagent that forms a leaving group *in situ*. In some embodiments, the catalyst may be a silylating agent. In still other embodiments, the catalyst may be both a Lewis acid and a silylating agent. In other embodiments, the catalyst is trimethylsilyl trifluoromethanesulfonate.

As previously described, removal of protecting groups can be achieved in conventional manner according to the nature of the protecting group. In some embodiments, one protecting group may be selectively removed, whilst other protecting groups remain intact. The use and removal of appropriate protecting groups will be well within the capacity of those skilled in this art.

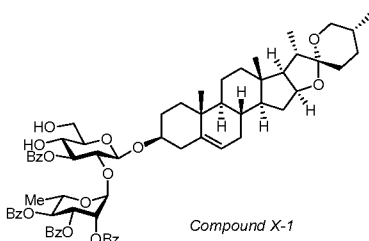
For example, treatment of a compound which comprises a saccharide moiety with residues protected by acetyl or benzoyl protecting groups, with NaOMe in methanol at around pH 10 with stirring, for a period of time, typically 1-8 hours, at room temperature results in complete de-O-acetylation or de-O-benzoylation, that is, removal of the acetyl and/or benzoyl protecting groups. Such a deprotection provides, in this instance, free hydroxyl groups.

As discussed previously therefore the de-protection regime chosen in each instance will depend upon the exact nature of the oxygen protecting groups located at R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>6</sup> and R<sup>7</sup>, and as a possible component of R<sup>9</sup> and the acyl group at R<sup>5</sup>. In some instances all oxygen protecting groups can be removed in a single operation thus leading to manufacturing efficiencies. In other instances due to the nature and identity of these groups it is necessary to manipulate the protecting groups in a multi-step operation to arrive at the desired product. It is noted that a skilled addressee equipped with a competent working knowledge of protecting group chemistry would be readily able to analyse the compounds to be de-protected and from the identity of the protecting groups devise a de-protection protocol to arrive at the desired end product.

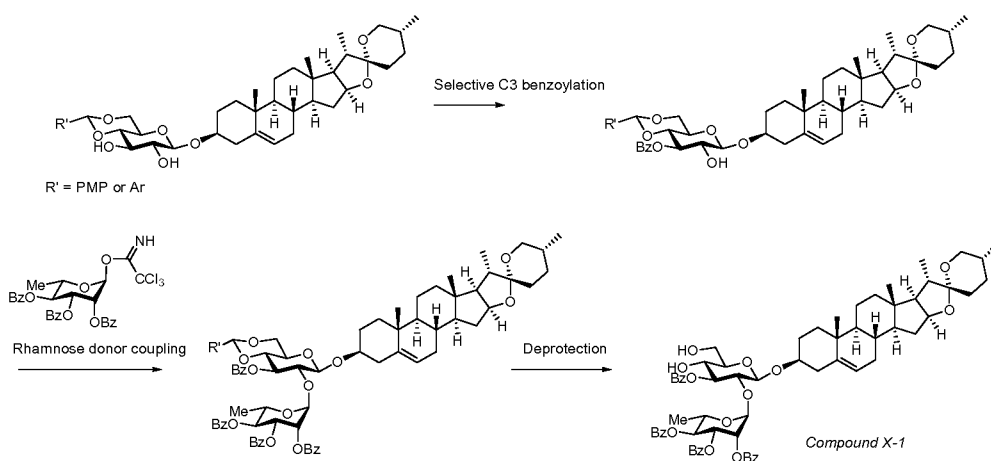
In other embodiments, for example, treatment of a compound which comprises a saccharide moiety protected, in part, by an acetal, such as para-methoxy phenyl (PMP), with anionic ion exchange resin in methanol and tetrahydrofuran results in selective removal of the para-methoxy phenyl protecting group only. As such, where the compound comprising a saccharide moiety also comprises other protected residues, such as benzoyl and acetyl

protected hydroxyl groups, these other protecting groups remain intact. Suitable anionic ion exchange resins include those sold under the names Amberjet™, Amberlite™ and Ambersep™.

In some aspects, the compound of formula X is Compound X-1:

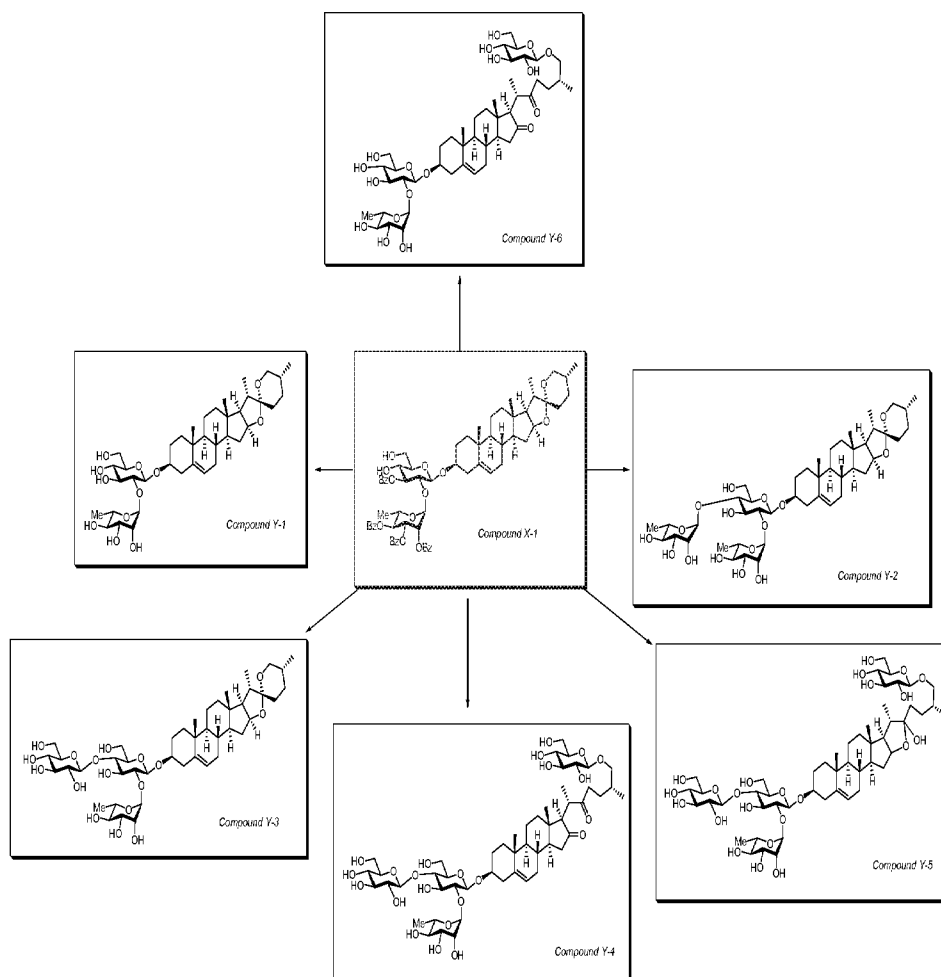


Where the compound of Formula X is Compound X-1, it may be accessed by the method of the present invention, as follows:



That is the acetal protected compound is subjected to selective C3 benzoylation to achieve the desired mono-benzoylated derivative. This is then subjected to Rhamnose coupling with a suitably activated saccharide to produce the desired coupled product which is then reacted to remove the acetal protecting group. This product can be further elaborated by a number of different reactions to provide entry to a large number of structurally related compounds.

Advantageously, the present method provides access to a range of steroid saponins. For example, wherein the compound of Formula X is Compound X-1, the present method provides access to the following desired steroid saponins:



In some aspects, the present invention provides a method for preparing a compound of Formula Y. Typically, a compound of Formula Y of the present invention may be prepared converting a compound of Formula X into a compound of Formula Y. A compound of Formula X may be converted to a compound of Formula Y by methods recognised in the art, including but not limited to functional group interconversions, oxidations, reductions, alkylation, protections, deprotections and the coupling of additional saccharide units.

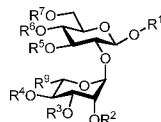
For example, in some aspects, the compound of Formula Y may be prepared, according to the present methods, wherein steps (i) to (iv) and  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$  and  $R^9$  are as previously described for Formula A, B, C, X and Y.

In some aspects, a compound of Formula X may be converted to a compound of Formula Y by removing any protecting groups from a compound of Formula X to provide a compound of Formula Y. In some aspect, removal of protecting groups can be achieved in conventional manner according to the nature of the protecting group. In some aspects, treating a compound of Formula X with a base will provide a compound of Formula Y. The base may be selected from any suitable base. In some embodiments the base may be selected from any nucleophilic or non-nucleophilic base, including organic and inorganic bases. In still other embodiments the base may be selected from those previously described in respect of in step (i) of the present invention. In still other embodiments, the base may be used in combination with a solvent, or may be used in the absence of a solvent. Where a solvent is used, the solvent may be selected from any suitable solvent. For example, in some embodiments, treating a compound of Formula X with  $K_2CO_3$  in methanol will provide a compound of Formula Y. In other embodiments treating a compound of Formula X with  $NH_3$  in methanol will provide a compound of Formula Y. In still other embodiments, treating a compound of Formula X with NaOH in dichloromethane and/or methanol will provide a compound of Formula Y.

In further aspects, a compound of Formula X may be converted to a Formula Y by

- (a) selectively introducing an oxygen protecting group at  $R^7$ ;
- (b) coupling a suitable saccharide under coupling conditions such that  $R^6$  is a saccharide;

to provide a compound of Formula X'

**Formula X'**

- wherein
- R<sup>1</sup> is a sapogenin;
  - R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup> and R<sup>7</sup> are each independently an oxygen protecting group;
  - R<sup>5</sup> is an acyl group;
  - R<sup>6</sup> is a saccharide;
  - R<sup>9</sup> is selected from the group consisting of H, CH<sub>3</sub>, and an oxygen protected by an oxygen protecting group; and
- (c) converting a compound of Formula X' into a compound of Formula Y, a pharmaceutically acceptable salt, isomer, hydrate or solvate thereof .

In other aspects, a compound of Formula X may be converted to a compound of Formula Y by:

- (a) selectively introducing an oxygen protecting group at R<sup>7</sup>;
- (b) coupling a suitable saccharide under coupling conditions such that R<sup>6</sup> is a saccharide to provide a compound of Formula X' as previously defined; and
- (c) removing any protecting groups to provide a compound of Formula Y.

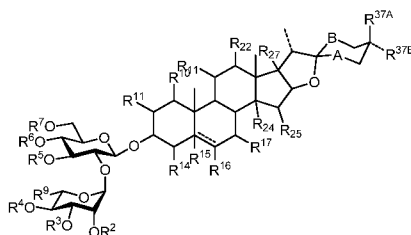
In other aspects, a compound of Formula X may be converted to a compound of Formula Y by:

- (a) selectively introducing an oxygen protecting group at R<sup>7</sup>;
- (b) coupling a suitable saccharide under coupling conditions such that R<sup>6</sup> is a saccharide to provide a compound of Formula X' as previously defined;
- (c) converting the sapogenin at R<sup>1</sup> into a sapogenin of Formula G as previously defined; and
- (d) removing any protecting groups to provide a compound of Formula Y.

In still other embodiments, a compound of Formula X may be converted to a compound of Formula Y by:

- (a) selectively introducing an oxygen protecting group at R<sup>7</sup>;
- (b) coupling a suitable saccharide under coupling conditions such that R<sup>6</sup> is a saccharide to provide a compound of Formula X' as previously defined;
- (c) converting the sapogenin at R<sup>1</sup> into a sapogenin of Formula F as previously defined; and
- (d) removing any protecting groups to provide a compound of Formula Y.

In some embodiments, a compound of Formula Y wherein R<sup>1</sup> is a sapogenin of Formula G, may be prepared from a compound of Formula X by oxidising a compound of the Formula Y<sup>A</sup>



**Formula Y<sup>A</sup>**

R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, and R<sup>7</sup> are each independently an oxygen protecting group;

R<sup>6</sup> is an oxygen protecting group or a saccharide;

R<sup>9</sup> is selected from the group consisting of H, Me, and an oxygen protected by an oxygen protecting group;

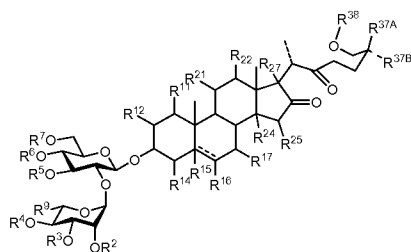
R<sup>11</sup>, R<sup>12</sup>, R<sup>14</sup>, R<sup>16</sup>, R<sup>17</sup>, R<sup>21</sup>, R<sup>22</sup>, R<sup>24</sup>, R<sup>25</sup> and R<sup>27</sup> are independently H, OH, =O, pharmacologically acceptable ester groups or pharmacologically acceptable ether groups;

R<sup>15</sup> is H when C-5,C-6 is a single bond, and nothing when C-5,C-6 is a double bond;

A is either O concurrently with B being CH<sub>2</sub>, or B is O concurrently with A being CH<sub>2</sub>;

R<sup>37A</sup> is H concurrently with R<sup>37B</sup> being CH<sub>3</sub>, or R<sup>37A</sup> is CH<sub>3</sub> concurrently with R<sup>37B</sup> being H;

to provide a compound of Formula Y<sup>B</sup>



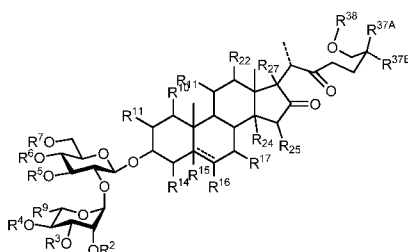
**Formula Y<sup>B</sup>**

wherein  $R^2, R^3, R^4, R^5, R^6, R^7, R^9, R^{11}, R^{12}, R^{14}, R^{15}, R^{16}, R^{17}, R^{21}, R^{22}, R^{24}, R^{25}, R^{27}, R^{37A}$  and  $R^{37B}$  are as defined for Formula  $Y^A$ ; and

$R^{38}$  is H or a saccharide; or a pharmaceutically acceptable salt, or derivative thereof;

followed by removable of any protecting groups to provide a compound of Formula Y.

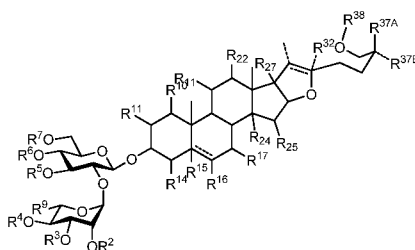
In other embodiments, a compound of Formula Y wherein  $R^1$  is a saponin of Formula F, may be prepared by selectively reducing a compound of Formula  $Y^B$



**Formula  $Y^B$**

$R^2, R^3, R^4, R^5, R^6, R^7, R^9, R^{11}, R^{12}, R^{14}, R^{15}, R^{16}, R^{17}, R^{21}, R^{22}, R^{24}, R^{25}, R^{27}, R^{37A}$  and  $R^{37B}$  are as defined for Formula  $Y^A$ ;

$R^{38}$  is H or a saccharide; or a pharmaceutically acceptable salt, or derivative thereof; to provide a compound of Formula  $Y^C$

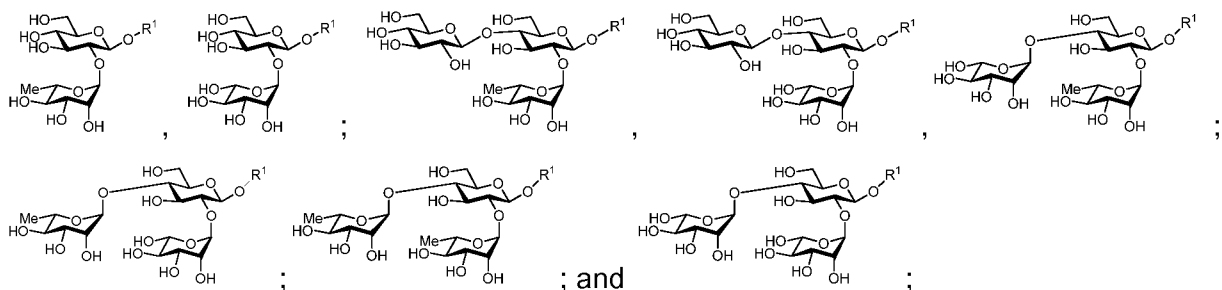


**Formula  $Y^C$**

$R^2, R^3, R^4, R^5, R^6, R^7, R^9, R^{11}, R^{12}, R^{14}, R^{15}, R^{16}, R^{17}, R^{21}, R^{22}, R^{24}, R^{25}, R^{27}, R^{37A}$  and  $R^{37B}$  and  $R^{38}$  are as defined for Formula  $Y^B$ ;

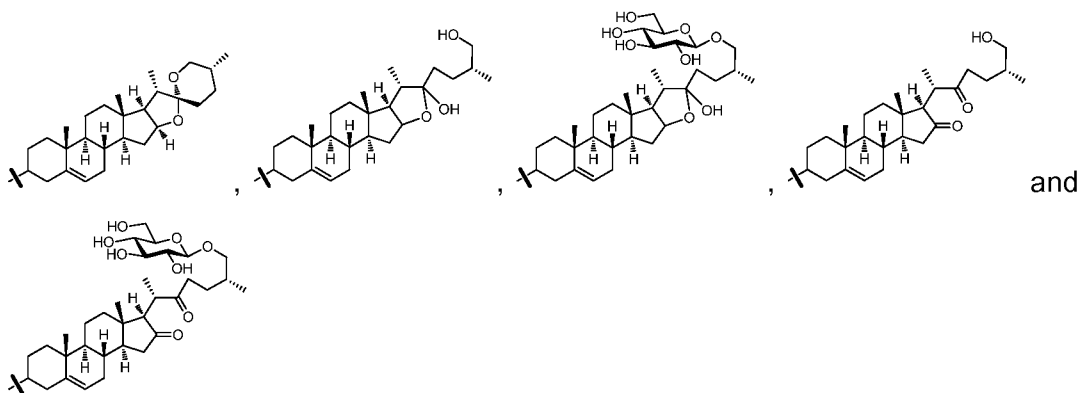
followed by removable of any protecting groups to provide a compound of Formula Y.

In some embodiments, the compound of Formula Y is selected from the group consisting of

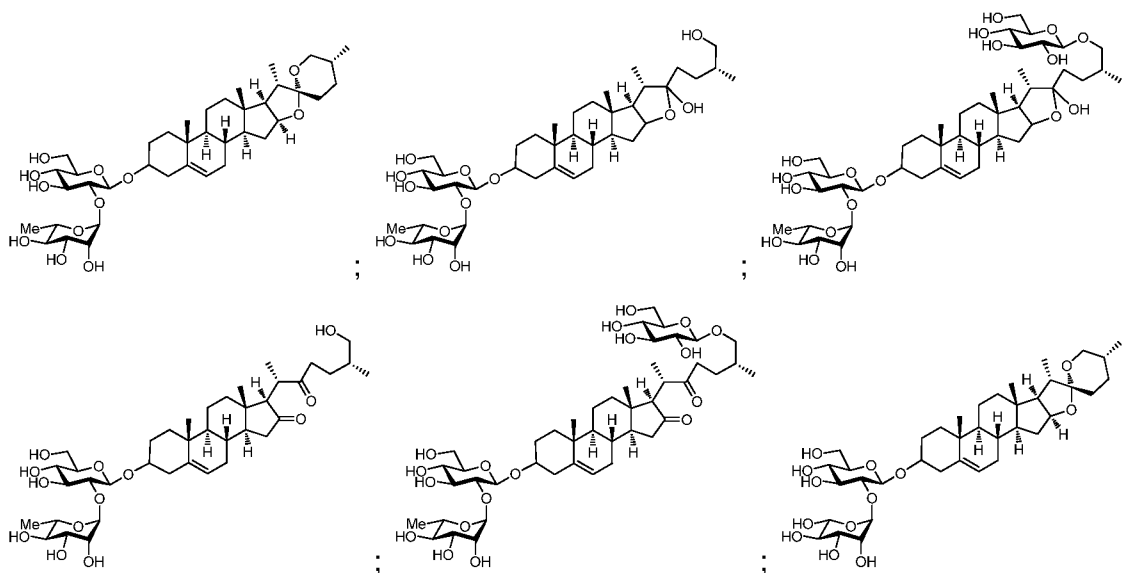


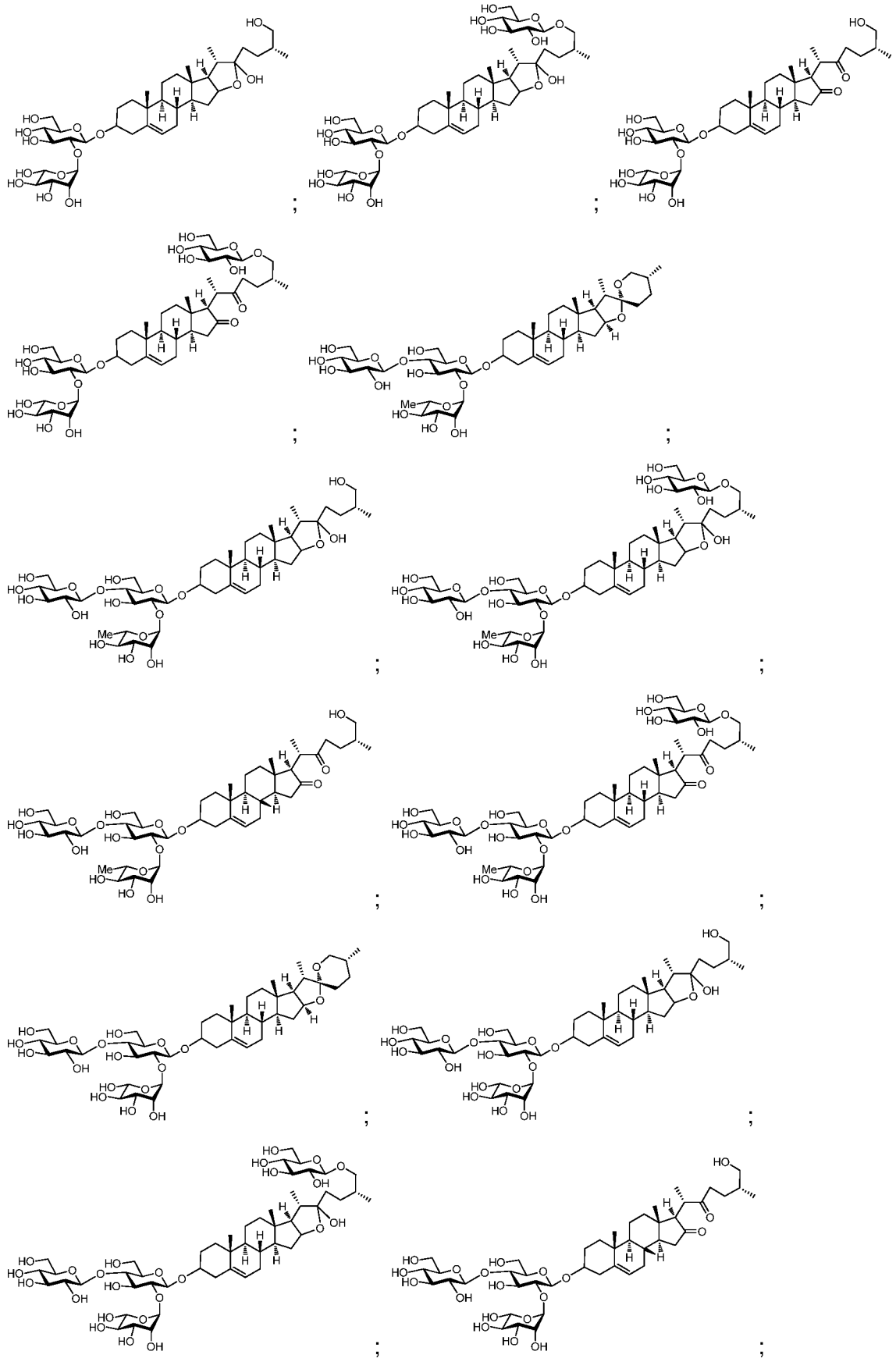
where R<sup>1</sup> is a sapogenin.

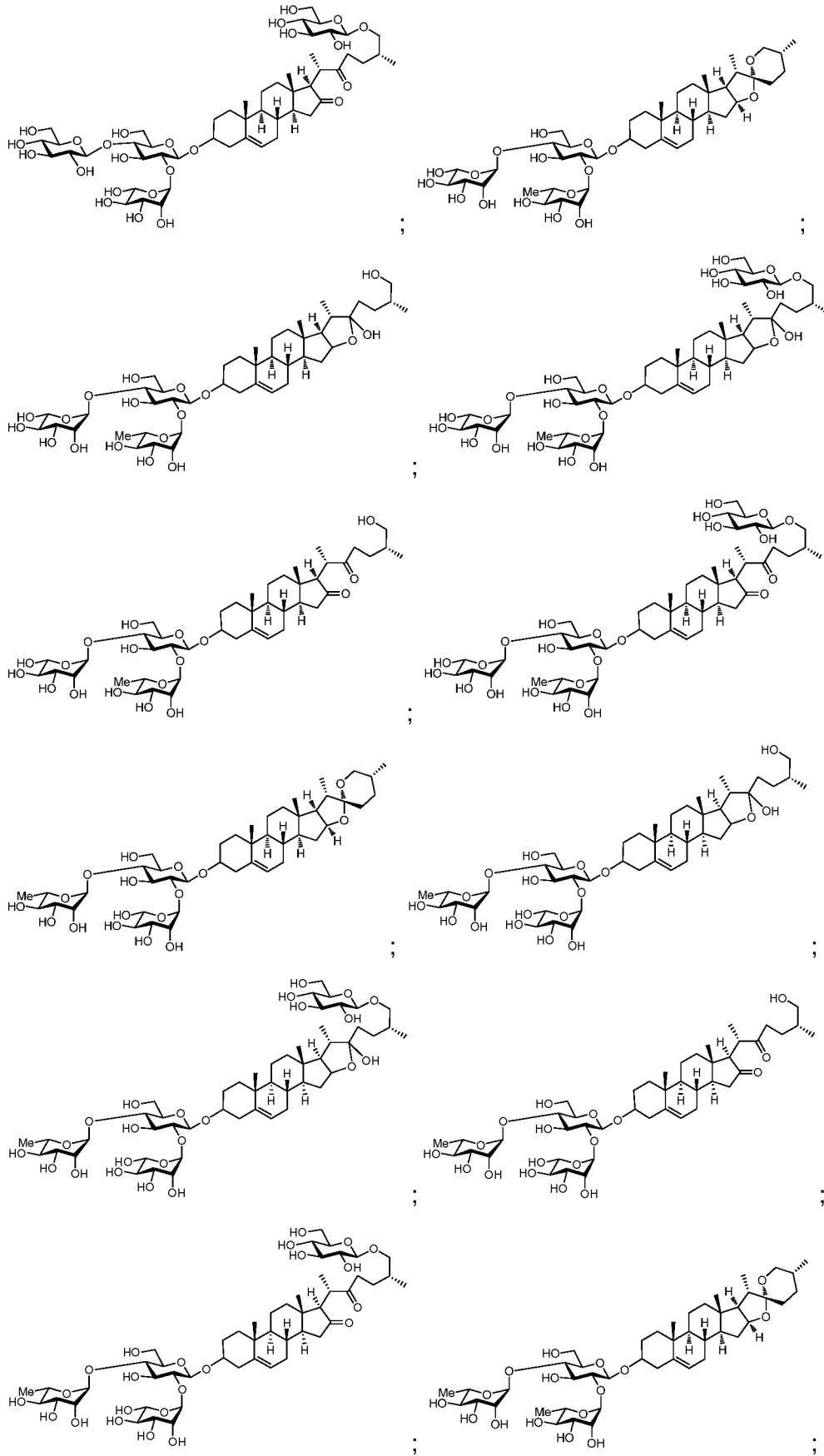
In other embodiments, the sapogenin at R<sup>1</sup> is selected from the group consisting of:



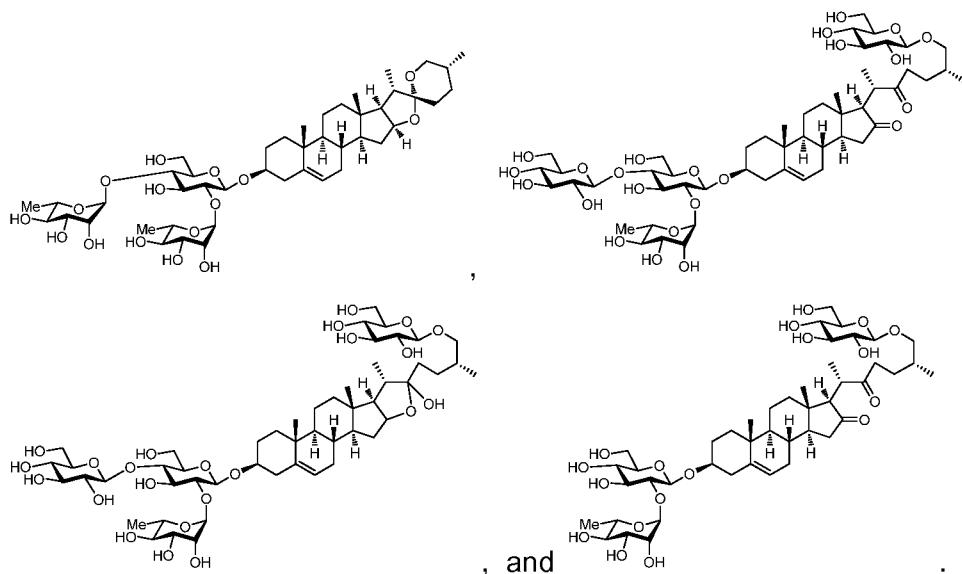
Accordingly, in some embodiments the compound of Formula Y may be selected from the group consisting of:











The invention will now be illustrated by way of examples; however, the examples are not to be construed as being limitations thereto. Additional compounds, other than those described below, may be prepared using methods and synthetic protocols or appropriate variations or modifications thereof, as described herein.

### Examples

In the examples described below, unless otherwise indicated, all temperatures in the following description are in degrees Celsius and all parts and percentages are by weight, unless indicated otherwise.

Various starting materials and other reagents were purchased from commercial suppliers, such as Aldrich Chemical Company or Lancaster Synthesis Ltd., and used without further purification, unless otherwise indicated. All solvents were purified by using standard methods in the art, unless otherwise indicated.

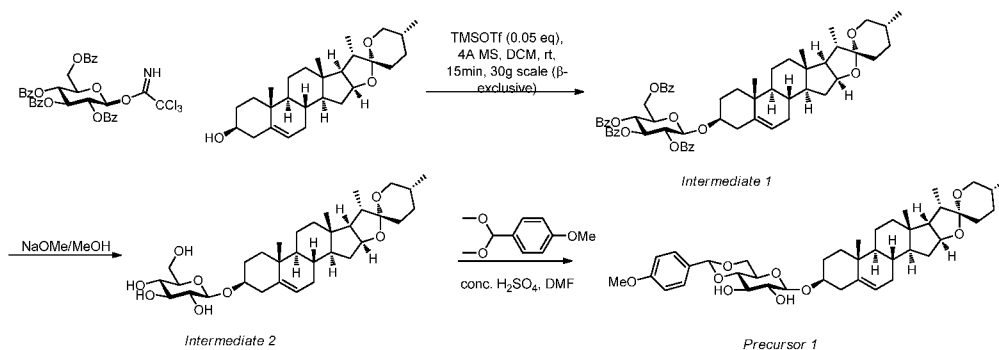
$^1\text{H}$  NMR spectra were recorded on a Bruker Avance III-500 at 500 MHz, and  $^{13}\text{C}$ -NMR spectra were recorded on a Bruker Avance III-500 at 126 MHz. When peak multiplicities are reported, the following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br = broadened, dd = doublet of doublets, dt = doublet of triplets. Coupling constants, when given, are reported in Hertz.

Mass spectra were obtained using Waters Q-TOF Premier™ Tandem Mass Spectrometer with electro-spray ionisation.

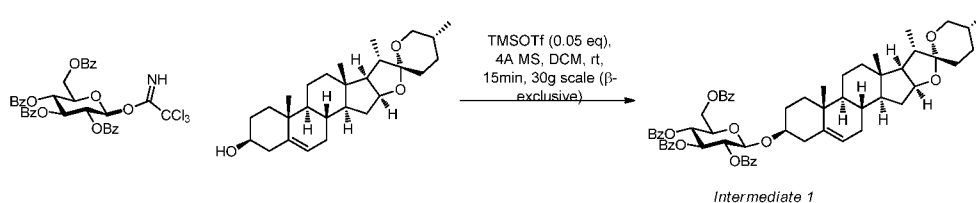
### Example (1):

#### Preparation of Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)-β-D-glucopyranoside (Precursor 1)

#### Scheme 1:



#### Intermediate 1: Preparation of 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranose (Intermediate 1)

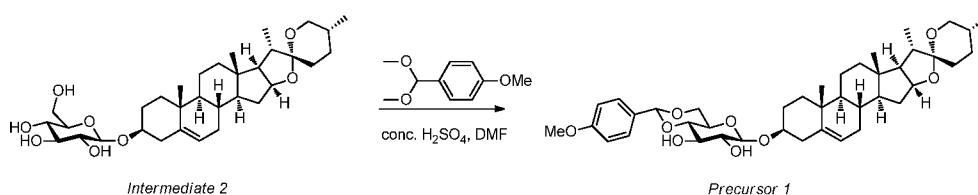


2,3,4,6-Tetra-O-benzoyl-D-glucopyranoside trichloroacetimidate (50.3 g, 67.9 mmol) and Diosgenin (26.0 g, 63 mmol) were dissolved in a mixture of dichloromethane (anhydrous, 11 mL) and toluene (anhydrous, 314 mL) and the solution dried by rotary evaporation at 40°C. The product was dissolved in dichloromethane (anhydrous, 222 mL) and cooled to 0°C under dry nitrogen. TMSOTf (0.250 mL, 1.38 mmol) was added and the solution warmed to ambient temperature and stirred for 1 h. The reaction was then quenched with *N*-methylmorpholine (0.343 mL, 3.1 mmol). Additional DCM was added (20 mL) and the product precipitated by the slow addition of methanol (450 mL) and the subsequent slow addition of a mixture of methanol and water (200 mL of 3:1 methanol:water). The product was collected by filtration, washed with a mixture of methanol and water (450 mL of 4:1 methanol:water) and dried under vacuum to give diosgenyl 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranose (Intermediate 1).



31.54, 30.89, 30.00, 22.18, 20.56, 18.25, 17.50, 15.60. ES-MS  $m/z$   $C_{33}H_{52}O_8Na$  calcd 599.3560, found 599.3554.

### Precursor 1: Preparation of Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Precursor 1)



To a solution of Diosgenyl- $\beta$ -D-glucopyranoside (35.95 g, 62.3 mmol) in DMF (270 mL) was added anisaldehyde dimethyl acetal (42.5 mL, 249 mmol) and 5 drops of concentrated H<sub>2</sub>SO<sub>4</sub>, pH ~ 2.5. The solution was heated at 60°C under house vacuum for 8 h to remove the methanol. The reaction was cooled and transferred to a separating funnel with ethyl acetate (400 mL), where it was washed iteratively with H<sub>2</sub>O (3 x 300 mL), 0.5 M aqueous HCl (2 x 200 mL) and then sat. aq. NaHCO<sub>3</sub> (200 mL) which caused the precipitation of a grey material at interface of organic and aqueous layers.

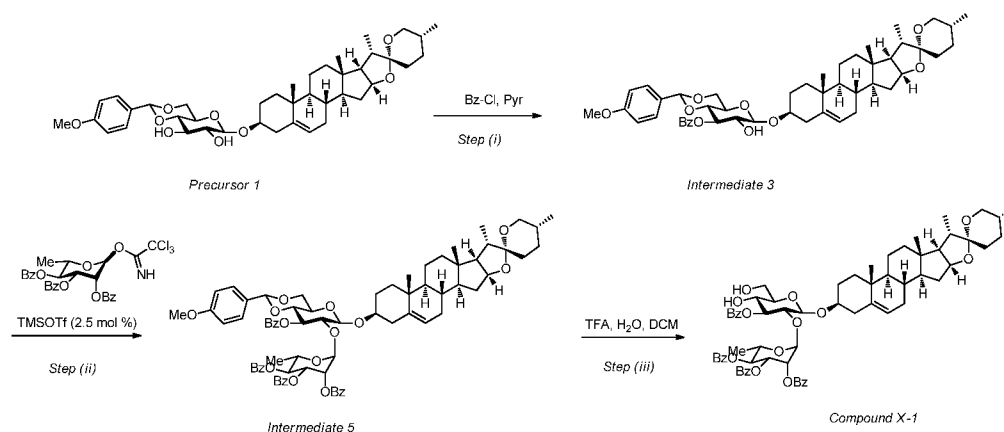
This grey material was identified as the desired product contaminated with a small amount of DMF, and was taken up in ethyl acetate and precipitated out with hexanes to give a grey powder (13.89 g, 32%).

The ethyl acetate layer was evaporated under reduced pressure to yield an orange oil which solidified on standing. This orange solid where dissolved in ethyl acetate and precipitated with hexanes to yield a grey powder (13.47 g, 31%).

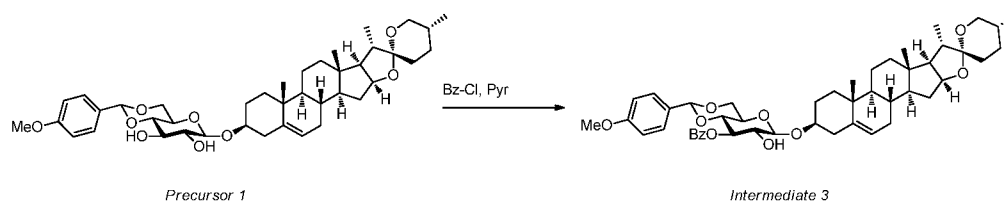
The orange filtrate could not be induced to precipitate more product instead resulting in an oiling-out due to high 4-methoxybenzaldehyde content which acts as a solvent, so consequently was absorbed onto Celite and columned through a short silica plug eluting with a gradient of 3:1 - 2:1 PE/EA, then 3:1 - 1:1 toluene/EA to give an additional portion of yellow solid (12.62 g 29%; cumulative 39.98 g, 92%).

**Example 2:**

**Preparation of a compound of Formula X; Diosgenyl-(2,3,4-tribenzoyl)-a-L-rhamnopyranosyl-(1→2)-3-benzoyl)-β-D-glucopyranoside (Compound X-1)**

**Scheme 2:****Intermediate 3: Step (i)**

**Selective benzoylation of Precursor 1 to provide Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)-β-D-glucopyranoside (Intermediate 3)**



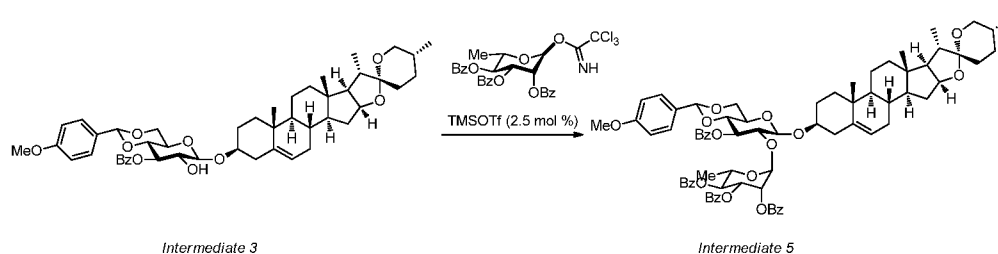
To a solution of Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)-β-D-glucopyranoside (18.37 g, 26.4 mmol) and DMAP (0.161 g, 1.322 mmol) dissolved in pyridine (42.8 mL) and Dichloromethane (68.0 mL) cooled to -78°C was added benzoyl chloride (3.38 mL, 29.1 mmol, 1.1 equiv) dropwise (transiently forming a chunky solid, which could be Pyr.HCl, before stirring into the reaction volume). The solution was allowed to warm to room temperature with stirring overnight.

The reaction was quenched with the addition of MeOH (10 mL), diluted with 200 mL of DCM and washed with 0.5N HCl (4 x 250 mL), NaHCO<sub>3</sub> (200mL), brine (200 mL), and dried with MgSO<sub>4</sub>. The crude material was absorbed onto Celite evaporating with 50 mL of toluene to drive off residual DCM. This was loaded as a slurry in toluene (200 mL) to the top of a silica column and eluted with stepwise gradient of 2% EA/toluene (diBz elution), 5% EA/toluene (intermediate) and then 10% EA/toluene (monoBz).

Collected fractions were combined to yield Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 3) (13.11, 62%). Diosgenyl-(4,6-O-(4-methoxybenzylidene)-2,3-dibenzoyl)- $\beta$ -D-glucopyranoside was also isolated, 3.17 g, 13.3%.

### Intermediate 5: Step (ii)

**Coupling to rhamnose moiety at provide Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 5)**



To a solution of Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 3) (18.4g, 23.03 mmol), 2,3,4-tri-O-benzoyl- $\alpha/\beta$ -L-rhamnopyranoside trichloroacetimidate (17.87 g, 28.8 mmol, 1.25 equiv) and 4 Å MS sieves (2 g/g acceptor; 37 g) in DCM (450 mL) stirred at  $-78^{\circ}\text{C}$  was added trimethylsilyl trifluoromethanesulfonate (0.104 mL, 0.576 mmol) dropwise immediately forming a bright yellow solution. The reaction was allowed to warm room temperature overnight in the cold bath (lagged with foil) with stirring.

A small aliquot, quenched with 1 drop  $\text{NEt}_3$  (- yellow colour disappeared) and evaporated showed the complete consumption of starting materials by  $^1\text{H}$  NMR.

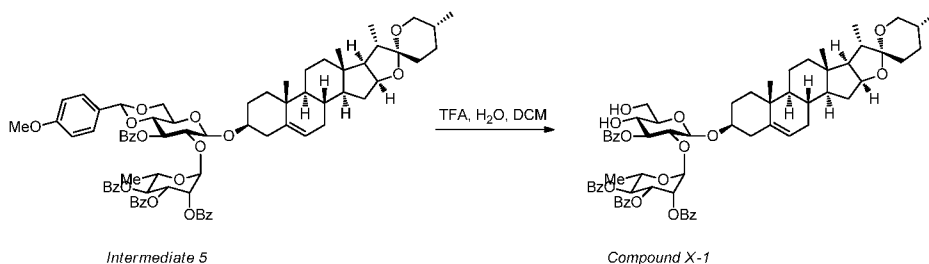
The reaction was quenched with addition of  $\text{NEt}_3$  (2 mL) and filtered through a bed of Celite to separate the sieves. The solid support was washed with DCM (2 x 50 mL). Evaporation of the organic solution gave a white foam. The foam was slurried with  $\text{Et}_2\text{O}$  (~200 mL), filtered, and washed with cold  $\text{Et}_2\text{O}$  (2 x 50 mL) to yield Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside as white powder in excellent purity (22.75 g, 79%).

The yellow filtrate was determined to be comprised of decomposed rhamnose donor and a small amount of the desired product. Absorption onto Celite and elution through silica

eluting with a gradient of EA/toluene (2%, 4% then 6%) gave an additional 3.07 g (10.6%) of product (cumulative yield 25.82 g, 90%).

### Compound X-1: Step (iii)

**Deprotection of Intermediate 5 to provide a compound of Formula X; Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-3-benzoyl)- $\beta$ -D-glucopyranoside (Compound X-1)**



Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (20 g, 15.91 mmol) was dissolved in dichloromethane (125 mL, 1943 mmol) and water (45 mL). The biphasic mixture was stirred as trifluoroacetic acid (15.91 mL) was added at 0°C forming a bright yellow / green fluorescent coloured solution.

The reaction was allowed to stir for 3.5 hours. The reaction was quenched by washing with water (2 x 150 mL), NaHCO<sub>3</sub> (2 x 200 mL), brine (200 mL), dried with MgSO<sub>4</sub> and evaporated to give a white foam.

The crude material was dissolved in a minimum of hot EA (~30 mL) and added dropwise to a PE (500 mL) causing the precipitation of a white solid as a 'stringy' material. The solution was allowed to stir overnight resulting in the formation of a gel. Filtration gave a white solid which was washed with cold 10% EA/PE. (18.202 g, 100%).

Alternatively, Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (1.76 g, 1.43 mmol) and Amberjet® 1200 H (8.8 g) were slurried in methanol (24 mL) and tetrahydrofuran (12 mL) in a 100 mL round bottomed flask. The reaction was heated to reflux for 15 h. The reaction was then quenched with triethylamine (0.2 mL). The resin was removed by filtration and the solvent evaporated under reduced pressure. The crude product was dissolved in methanol (25 mL) and water added dropwise (15 mL) resulting in the crystallisation of a white solid. The solid product was isolated by filtration and the cake washed with 1:1 methanol/water (2 x 15 mL)

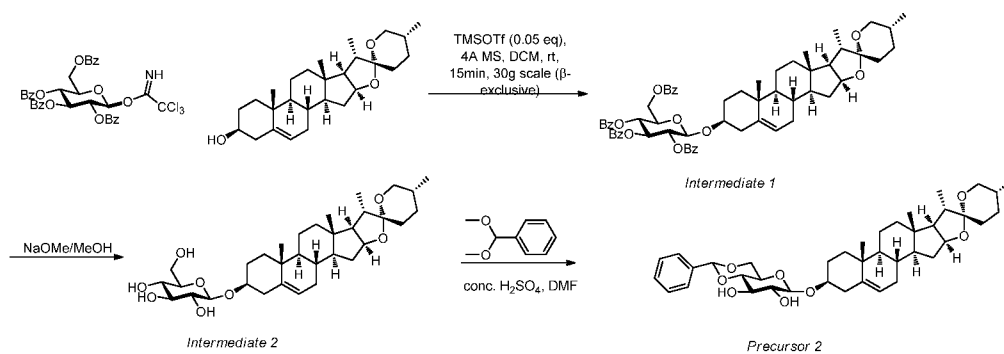
followed by petroleum ether 60-80 (2 x 15 mL). The product was dried overnight under vacuum at 45°C, to give Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-3-benzoyl)- $\beta$ -D-glucopyranoside (Compound X-1), 1.33 g, 81%.

$^1\text{H}$  NMR 500 MHz ( $\text{CDCl}_3$ )  $\delta$  8.03 (d, 2H), 7.90 (d, 2H), 7.77 (d, 2H), 7.74 (d, 2H), 7.56 (t,  $J = 7.5$  Hz, 1H), 7.53 (t,  $J = 7.5$  Hz, 1H), 7.41 (m, 3H), 7.33 (m, 3H), 7.28 (t,  $J = 7.5$  Hz, 2H), 7.23 (t,  $J = 7.5$  Hz, 2H), 5.74 (dd,  $J = 3.6, 10.0$  Hz, 1H), 5.48-5.57 (m, 3H), 5.44 (dd,  $J = 1.6, 3.6$  Hz, 1H), 5.16 (d,  $J = 1.3$  Hz, 1H), 4.82 (d,  $J = 7.9$  Hz, 1H), 4.77 (m, 1H), 4.45 (q, 1H), 3.73-3.95 (m, 6H), 3.47-3.54 (m, 2H), 3.38-3.41 (m, 2H), 2.64 (app ddd, 1H), 2.45 (t, 1H), 2.03 (m, 3H), 1.09-1.93 (m, 20H), 1.34 (d,  $J = 6.4$  Hz, 3H), 0.99 (d,  $J = 6.4$  Hz, 3H), 0.95 (s, 3H), 0.81 (d,  $J = 6.4$  Hz, 3H), 0.80 (s, 3H).  $^{13}\text{C}$  NMR 126 MHz ( $\text{CDCl}_3, \text{MeOD}$  3:1)  $\delta$  166.3, 165.8, 165.3, 164.7, 139.9, 133.24, 133.17, 133.0, 132.9, 129.61, 129.55, 129.5, 129.3, 129.0, 128.9, 128.23, 128.17, 128.1, 122.0, 109.4, 99.4, 97.6, 80.8, 79.0, 78.4, 75.9, 75.5, 71.8, 70.2, 69.6, 68.7, 66.7, 66.6, 61.9, 61.4, 56.3, 49.9, 41.4, 40.1, 39.5, 38.6, 37.0, 36.7, 32.0, 31.6, 31.3, 31.1, 30.0, 29.7, 28.5, 20.6, 19.0, 17.1, 16.8, 16.0. ES-MS  $m/z$   $\text{C}_{67}\text{H}_{78}\text{O}_{16}\text{Na}$  calcd: 1161.5188, found 1161.5186

### Example 3:

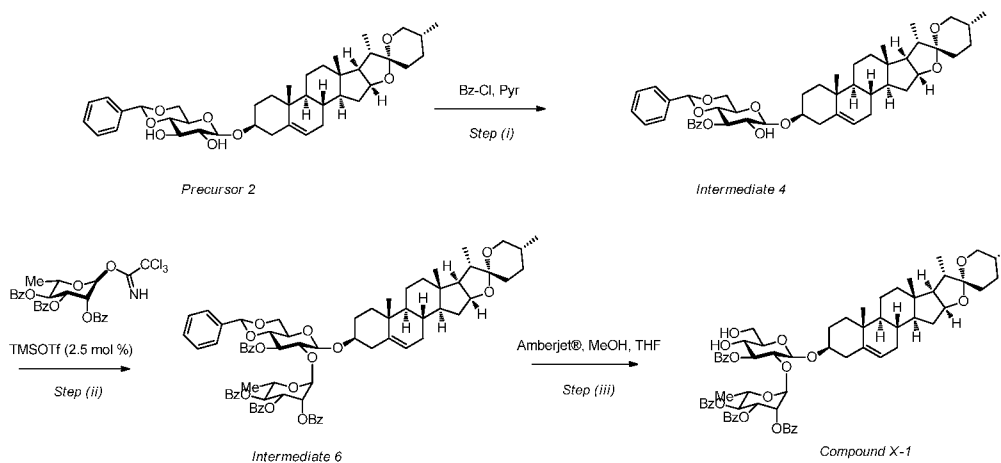
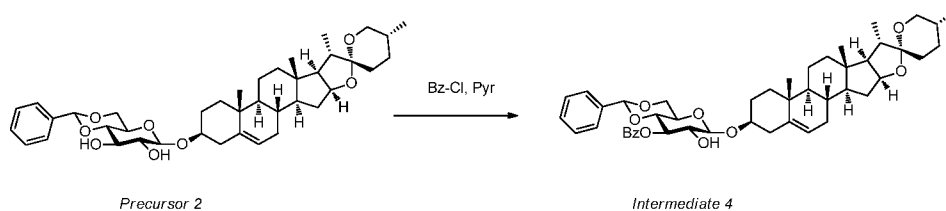
#### Preparation of Diosgenyl-(4,6-O-(benzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Precursor 2)

#### Scheme 3:



Preparation of 2,3,4,6-tetra-O-benzoyl- $\beta$ -D-glucopyranoside (Intermediate 1) and Diosgenyl- $\beta$ -D-glucopyranoside (Intermediate 2) are as described above.



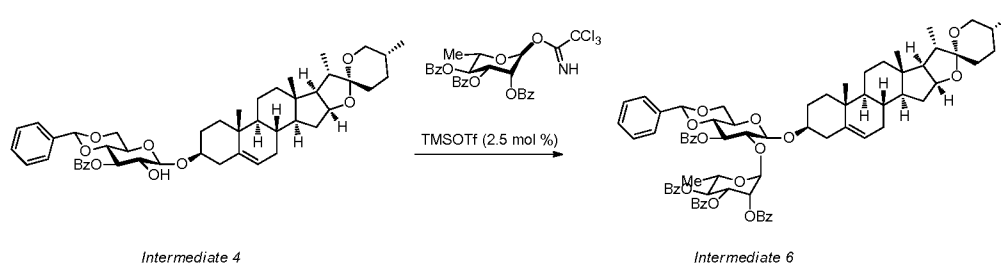
**Example 4:****Preparation of a compound of Formula X; Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-3-benzoyl)- $\beta$ -D-glucopyranoside (Compound X-1)****Scheme 4:****Intermediate 4: Step (i)****Selective benzoylation of Precursor 2 to provide Diosgenyl-(4,6-O-(benzylidene))-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 4)**

Diosgenyl-(4,6-O-(benzylidene))- $\beta$ -D-glucopyranoside (Precursor 2) (16.0 g, 24.1 mmol) was dissolved in pyridine (60 mL) and DCM (60 mL) and the solvent removed under reduced pressure to azeotropically dry starting material. The resulting solid was dissolved in pyridine (39 mL) and DCM (62 mL) at ambient temperature under argon. Benzoyl chloride (3.35 mL, 28.8 mmol) was added dropwise maintaining a batch temperature below 22°C. The reaction mixture was stirred at ambient temperature for 35 minutes then quenched with methanol (5.0 mL, 120 mmol). The solvent was removed under reduced pressure and the resulting crude product dissolved in DCM (200 mL) and washed with 5% citric acid solution (3 x 70 mL), water (70 mL) and saturated sodium bicarbonate (70 mL). The organic layer was dried ( $\text{MgSO}_4$ ), filtered and concentrated *in vacuo*. The resulting crude product was purified by flash chromatography (2 to 8% ethyl acetate in toluene) to afford diosgenyl-(4,6-O-(4-methoxybenzylidene))-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 4) (10.12 g, 55% yield) as a white solid.

$^1\text{H}$  NMR (500 MHz, 3:1  $\text{CDCl}_3/\text{CD}_3\text{OD}$ ):  $\delta$ 8.07 (dd,  $J = 1.3, 8.5$  Hz, 2 H), 7.57 (tt,  $J = 1.3, 7.5$  Hz, 1 H), 7.45 (t,  $J = 7.5$  Hz, 2 H), 7.40 (m, 2 H), 7.29 (m, 3 H), 5.52 (s, 1 H), 5.46 (t,  $J = 9.4$  Hz, 1 H), 5.39 (dd,  $J = 1.9, 3.4$  Hz, 1 H), 4.66 (d,  $J = 7.7$  Hz, 1 H), 4.42 (q,  $J = 7.7$  Hz, 1 H), 4.37 (dd,  $J = 4.9, 10.5$  Hz, 1 H), 3.83 (t,  $J = 10.3$  Hz, 1 H), 3.78 (t,  $J = 9.4$  Hz, 1 H), 3.63 (m, 3 H), 3.48 (dd,  $J = 2.0, 4.3, 10.7$  Hz, 1 H), 3.37 (t,  $J = 11.0$  Hz, 1 H), 2.46 (ddd,  $J = 2.0, 4.5, 13.2$  Hz, 1 H), 2.30 (m, 1 H), 2.06-0.95 (m, 22 H), 1.04 (s, 3 H), 0.98 (d,  $J = 6.9$  Hz, 3 H), 0.80 (s, 3 H), 0.79 (d,  $J = 7.9$  Hz, 3 H);  $^{13}\text{C}$  NMR (126 MHz, 3:1  $\text{CDCl}_3/\text{CD}_3\text{OD}$ ):  $\delta$ 167.91, 141.65, 138.29, 138.29, 134.52, 131.25, 131.11, 130.32, 129.69, 129.47, 127.45, 123.25, 110.95, 103.51, 102.80, 82.35, 80.95, 80.21, 75.82, 74.33, 70.10, 68.23, 67.70, 63.39, 57.85, 51.48, 43.00, 41.64, 41.09, 39.97, 38.57, 38.20, 33.41, 33.03, 32.79, 32.65, 31.55, 30.94, 30.01, 22.20, 20.59, 18.28, 17.52; HRMS (TOF ES+)  $m/z$  calcd for  $\text{C}_{47}\text{H}_{60}\text{O}_9\text{Na}$  791.4135, found 791.4127.

### Intermediate 6: Step (ii)

**Coupling to rhamnose moiety to provide Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-(4,6-O-(benzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 6)**



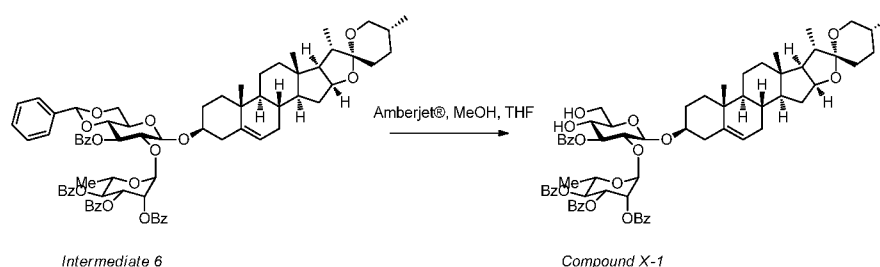
### Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside

(Intermediate 4) (10.0 g, 13.0 mmol), 2,3,4-tri-O-benzoyl- $\alpha/\beta$ -L-rhamnopyranoside trichloroacetimidate (11.0 g, 17.7 mmol) and 4 $\text{\AA}$  molecular sieves were slurried in DCM (250 mL) and cooled to  $-40^\circ\text{C}$  under argon. Trifluoromethane sulfonate (72  $\mu\text{L}$ , 0.4 mmol) was added dropwise and the reaction mixture stirred at  $-40^\circ\text{C}$  (batch temperature) for a further 90 minutes. Triethylamine (1.54 mL, 11.0 mmol) was added and the reaction mixture allowed to warm to ambient temperature. The molecular sieves were removed by filtration and the solvent evaporated under reduced pressure. The crude product, a pale yellow oil, was triturated with acetonitrile (200 mL) and slurried for one hour at ambient temperature. The solid product was isolated by filtration, washed with acetonitrile (2 x 30 mL) dried under vacuum at  $30^\circ\text{C}$  for 16 h to afford diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-(4,6-O-(benzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 6) (13.7 g, 86% yield) as a white solid;

$^1\text{H}$  NMR (500 MHz, 3:1  $\text{CDCl}_3/\text{CD}_3\text{OD}$ ):  $\delta$ 8.02 (dd,  $J = 1.3, 8.5$  Hz, 2 H), 7.90 (dd,  $J = 1.2, 8.5$  Hz, 2 H), 7.77 (dd,  $J = 1.3, 8.5$  Hz, 2 H), 7.74 (dd,  $J = 1.2, 8.5$  Hz, 2 H), 7.57 (tt,  $J = 1.3, 7.5$  Hz, 1 H), 7.53 (tt,  $J = 1.3, 7.5$  Hz, 1 H), 7.39-7.4- (m, 4 H), 7.22-7.37 (m, 11 H), 5.78 (t,  $J = 9.2$  Hz, 1 H), 5.75 (dd,  $J = 3.5, 10.1$  Hz, 1 H), 5.57 (t,  $J = 10.1$  Hz, 1 H), 5.52 (s, 1 H), 5.51 (m, 1 H), 5.48 (dd,  $J = 1.7, 3.5$  Hz, 1 H), 5.22 (d,  $J = 1.5$  Hz, 1 H), 4.92 (d,  $J = 7.6$  Hz, 1 H), 4.76 (m, 1 H), 4.42 (m, 2 H), 4.04 (dd,  $J = 7.7, 9.3$  Hz, 1 H), 3.66-3.88 (m, 4 H), 3.49 (m, 1 H), 3.38 (t,  $J = 11.2$  Hz, 1 H), 2.65 (ddd,  $J = 2.2, 4.5, 13.1$  Hz, 1 H), 2.46 (m, 1 H), 2.04 (m, 3 H), 2.09-1.00 (m, 20 H), 1.36 (d,  $J = 6.6$  Hz, 3 H), 0.99 (d,  $J = 7.2$  Hz, 3 H), 0.95 (s, 3 H), 0.81 (d,  $J = 6.5$  Hz, 3 H), 0.79 (s, 3 H);  $^{13}\text{C}$  NMR (126 MHz, 3:1  $\text{CDCl}_3/\text{CD}_3\text{OD}$ ):  $\delta$ 167.36; 167.04, 166.85, 166.29, 141.33, 138.16, 134.77, 134.70, 134.50, 134.47, 131.17, 131.09, 131.02, 130.91, 130.64, 130.50, 130.42, 130.33, 129.76, 129.70, 129.58, 129.47, 127.45, 123.67, 110.95, 102.83, 101.68, 99.27, 82.34, 80.88, 80.14, 77.56, 76.08, 73.29, 71.78, 71.02, 70.02, 68.27, 68.24, 67.66, 63.40, 57.79, 51.42, 43.01, 41.65, 41.03, 40.04, 38.50, 38.20, 33.48, 33.09, 32.87, 32.67, 31.55, 31.22, 30.02, 22.15, 20.56, 18.62, 18.28, 17.51, 15.63; HRMS (TOF ES+)  $m/z$  calcd for  $\text{C}_{74}\text{H}_{82}\text{O}_{16}\text{Na}$  1249.5501, found 1249.5505.

### Compound X-1: Step (iii)

**Deprotection of Intermediate 6 to provide a compound of Formula X; Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-3-benzoyl)- $\beta$ -D-glucopyranoside (Compound X-1)**



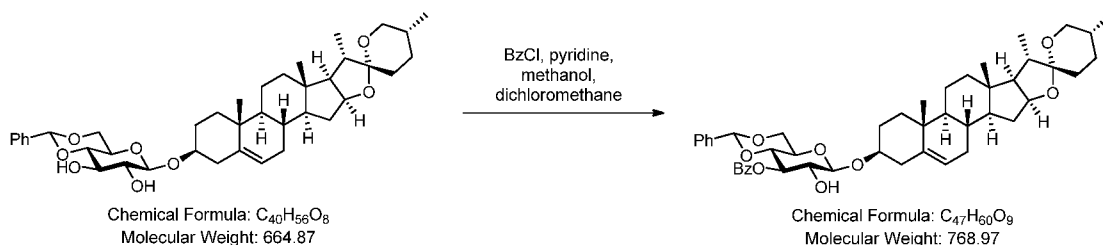
Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-(4,6-O-(benzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 6) (1.76 g, 1.43 mmol) and Amberjet® 1200 H (8.8 g) were slurried in methanol (24 mL) and tetrahydrofuran (12 mL) in a 100 mL round bottomed flask. The reaction was heated to reflux for 15 h with monitoring of the reaction by  $^1\text{H}$  NMR. Upon completion the reaction was quenched with triethylamine (0.2 mL). The resin was removed by filtration and the solvent evaporated under reduced pressure. The crude product was dissolved in methanol (25 mL) and water added drop wise (15 mL) resulting in the crystallisation of a white solid. The solid product was isolated by filtration and the cake washed with 1:1 methanol/water (2 x 15 mL) followed by petroleum ether 60-80 (2 x 15 mL).

The product was dried overnight under vacuum at 45°C, to give Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-3-benzoyl)- $\beta$ -D-glucopyranoside (Compound X-1), 1.33 g, 81%.

$^1\text{H}$  NMR 500 MHz ( $\text{CDCl}_3$ ):  $\delta$  8.03 (d, 2H), 7.90 (d, 2H), 7.77 (d, 2H), 7.74 (d, 2H), 7.56 (t, J = 7.5 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 7.41 (m, 3H), 7.33 (m, 3H), 7.28 (t, J = 7.5 Hz, 2H), 7.23 (t, J = 7.5 Hz, 2H), 5.74 (dd, J = 3.6, 10.0 Hz, 1H), 5.48-5.57 (m, 3H), 5.44 (dd, J = 1.6, 3.6 Hz, 1H), 5.16 (d, J = 1.3 Hz, 1H), 4.82 (d, J = 7.9 Hz, 1H), 4.77 (m, 1H), 4.45 (q, 1H), 3.73-3.95 (m, 6H), 3.47-3.54 (m, 2H), 3.38-3.41 (m, 2H), 2.64 (app ddd, 1H), 2.45 (t, 1H), 2.03 (m, 3H), 1.09-1.93 (m, 20H), 1.34 (d, J = 6.4 Hz, 3H), 0.99 (d, J = 6.4 Hz, 3H), 0.95 (s, 3H), 0.81 (d, J = 6.4 Hz, 3H), 0.80 (s, 3H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3/\text{MeOD}$  3:1):  $\delta$  166.3, 165.8, 165.3, 164.7, 139.9, 133.24, 133.17, 133.0, 132.9, 129.61, 129.55, 129.5, 129.3, 129.0, 128.9, 128.23, 128.17, 128.1, 122.0, 109.4, 99.4, 97.6, 80.8, 79.0, 78.4, 75.9, 75.5, 71.8, 70.2, 69.6, 68.7, 66.7, 66.6, 61.9, 61.4, 56.3, 49.9, 41.4, 40.1, 39.5, 38.6, 37.0, 36.7, 32.0, 31.6, 31.3, 31.1, 30.0, 29.7, 28.5, 20.6, 19.0, 17.1, 16.8, 16.0. ES-MS  $m/z$   $\text{C}_{67}\text{H}_{78}\text{O}_{16}\text{Na}$  calcd: 1161.5188, found 1161.5186

### Example 5

#### Selective benzoylation of Precursor 2 to provide Diosgenyl-(4,6-O-(benzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 4) with crystallisation



In a 50 L Buchi, precursor 2 (2.642 kg) was dissolved in dichloromethane (DCM) (16.5 kg) and pyridine (12.59 kg) by heating to 40-45°C. After dissolution, the solvent was evaporated (azeotropic drying). The precursor 2 was then dissolved in dry DCM (8.21 kg,  $\leq$  0.01% water) and dry pyridine (3.90 kg,  $\leq$  0.01% water) by stirring overnight at ambient temperature under nitrogen. The solution was then transferred into a 60 L reactor and stirred under nitrogen. The bowl was rinsed into the reactor with dry dichloromethane (5.26 kg) and dry pyridine (2.53 kg).

The jacket temperature of the reactor was then set to -5°C and Benzoyl chloride (BzCl) (694 g, 1.2 equivs) was added slowly to the solution at such a rate that the

temperature of the reaction mixture was kept below 12°C. After the addition was complete the reaction mixture was stirred at 20°C for 35 min. The reaction mixture was sampled for completion and no additional BzCl was required (Additional benzoyl chloride can be added if required).

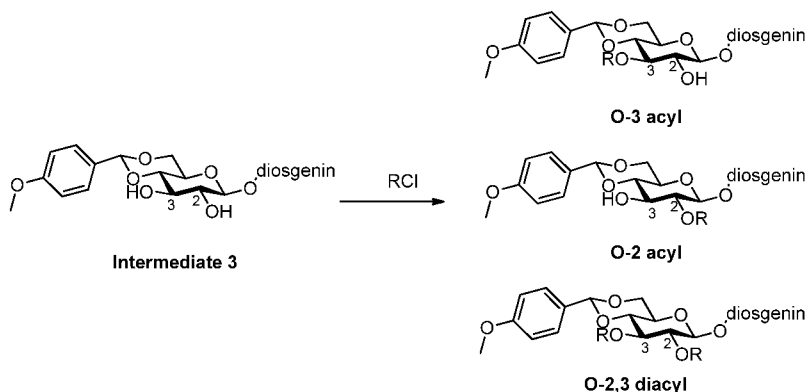
The reaction mixture was quenched by adding methanol (592 g) (over ~ 5 min) and the mixture stirred for an additional 30 min at 20°C. The solution was then concentrated to dryness. The resulting crude material was dissolved in DCM (33.7 kg) and the resulting organic layer washed with cold 9% citric acid (5 x 16.5 kg) and then water (10.9 L). Additional DCM (10.6 kg) was added and the organic layer washed with 5% sodium bicarbonate (10.4 L). The bicarbonate wash was back extracted with DCM (10.64 kg). The organic phases were combined and the solvent removed on the rotary evaporator.

The crude intermediate 4 was slurried in DCM (20.8 kg). Toluene (13.0 kg) was added and the solvent removed by evaporation. The residue is slurried a second time with DCM (26.3 kg) while warming to 45°C for 30 min. Toluene (14.1 kg) was then added and the solution evaporated to give a mixture that had a net weight of 14.5 kg. Additional toluene was then added (39.4 kg) and the suspension stirred overnight at 20°C. Stirring was stopped and the suspension allowed to settle for 2 days at 20°. The product was recovered by filtration and dried under vacuum (2.032 kg, 67% yield, 96% pure by NMR & HPLC).

### **Example (6):**

#### **Scope study of selective acylation (Step i) with Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)-β-D-glucopyranoside (Intermediate 3)**

The scope of selective acylation at C3 was examined with Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)-β-D-glucopyranoside (Intermediate 3). Specifically, the influence of each of the acylating agent, solvent, base, temperature and time on the selectivity was examined. Results are summarised below in Table 1.



Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 3) (50 mg) was dissolved in solvent (anhydrous, 2 mL) and base (10 eq) was added. The solution was brought to the temperature, unless otherwise stated acyl chloride (3 eq) was added and the reaction stirred for the required time. Water (0.5 mL) was added and volatiles were removed under reduced pressure. The residue was partitioned between ethyl acetate and saturated aqueous bicarbonate and then the organic phase was washed with water, dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure.

Table 1:

Experiment No. and conditions				Ratio of Products		
Expt	Acyl group (R)	Base/solvent	Temperature/time	O-2 acyl	O-3 acyl	O-2,3 diacyl
1	1.0 eq benzoyl	pyridine, DCM	-78-18 °C then 18 °C, 1 h	0.0	4.7	1.0
2	1.2 eq benzoyl	pyridine, DCM	up to 25 °C	0.0	4.0	1.0
3	1.2 eq benzoyl	pyridine, DCM	-78-18 °C then 18 °C, 1 h	0.0	9.0	1.0
4	benzoyl	pyridine, DCM	-78-18 °C then 18 °C, 1 h	1.0	27.8	1.0
5	4-chlorobenzoyl	pyridine, DCM	-78-18 °C then 18 °C, 2 h	5.0	192.0	1.0
6	4-nitrobenzoyl	pyridine, DCM	-78-18 °C then 18 °C, 2 h	1.0	72.5	7.5
7	4-methoxybenzoyl	pyridine, DCM	-78-18 °C then rt overnight	21.0	276.0	1.0
8	2-chlorobenzoyl	pyridine, DCM	-78-18 °C then 18 °C, 2 h	1.0	22.5	4.0
9	acetyl	pyridine, DCM	-78-18 °C then 18 °C, 2 h	1.0	22.5	3.6
10	acetyl	pyridine, DCM	-78-18 °C then 18 °C, 1 h	1.0	3.5	0.0
11	propionoyl	pyridine, DCM	-78-18 °C then 18 °C, 2 h	1.0	15.1	2.5
12	benzoyl	triethylamine, DCM	-78-18 °C then 18 °C, 2 h	1.0	6.5	0.0
13	benzoyl	triethylamine, DCM	18 °C, 5 h	24.7	178.3	1.0
14	benzoyl	DIPEA, DCM	-78-18 °C then 18 °C, 2 h	1.0	2.1	0.0
15	benzoyl	DIPEA, DCM	rt, 24 h	1.0	1.8	0.0
16	benzoyl	K <sub>2</sub> CO <sub>3</sub>	-78-18 °C then 18 °C, 2 h	0.0	0.0	0.0
17	benzoyl	pyridine, DCM	18 °C, 2 h	1.0	16.1	1.6

18	benzoyl	pyridine, DCM	40 °C, 80 min	1.2	12.7	1.0
19	benzoyl	pyridine, DCM	0 °C, 3 h	3.1	51.0	1.0
20	benzoyl	pyridine, neat	18 °C, 110 min	0.0	2.0	1.0
21	benzoyl	pyridine, THF	rt, 24 h	10.3	33.4	1.0
22	benzoyl	pyridine, dioxane	rt, 24 h	3.5	11.1	1.0
23	benzoyl	pyridine, DCM	18 °C, 2 h	1.0	17.5	1.6
24	benzoyl	benzoic acid, DCC, DMAP, DCM	18 °C, 2 h	1.0	3.0	1.1

Advantageously, where the acylation was conducted with 1.1 to 1.2 equivalents of benzoyl chloride, in the presence of pyridine as a base and DCM as a solvent, none of the undesired C2-benzoylated product was observed (Expt No. 1 to 3).

Similarly, where the acylation was conducted with benzoyl chloride and in the presence of pyridine as both a solvent and base (Expt No. 20), none of the undesired C2-benzoylated product was observed.

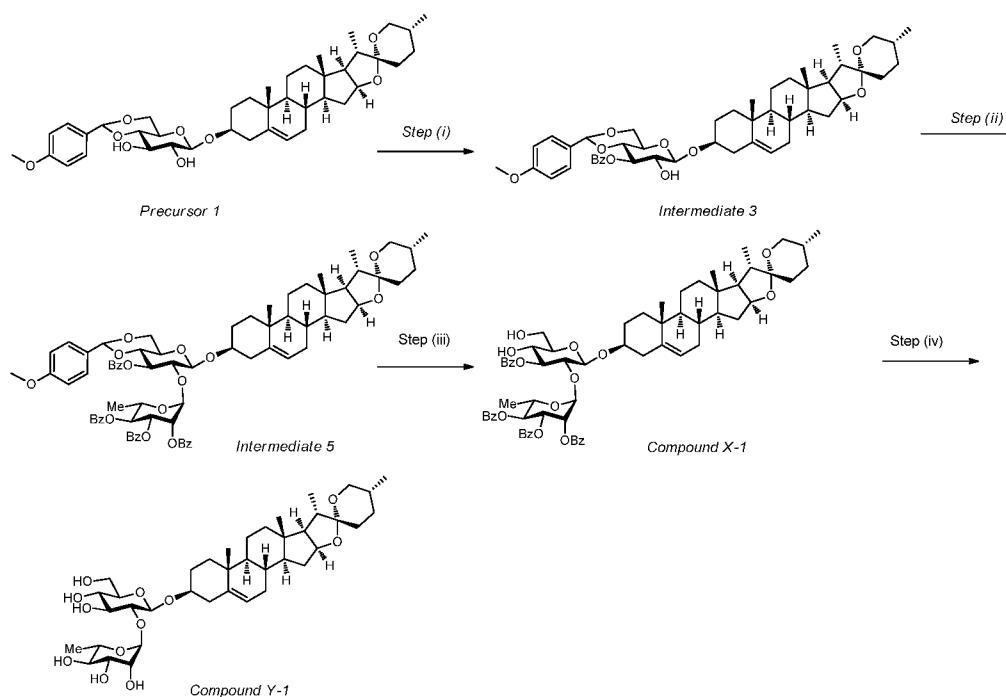
The method step (i) of the present invention was able to be conducted over a range of temperatures, typically in the range of -78 and 40°C.

The method step (i) of the present invention also provides a range of acylating agents (Expt No. 4 to 11), typically optionally substituted benzoylating agents provide advantageous ratios of C2- to C3- protected products.

Advantageously, where any undesired O-2,3-bis-diacyl product is isolated, it may be recycled to recover Intermediate 3 by removal of the two acyl groups at C2- and C3-. The recovered Intermediate 3 may then be subjected to the selective acylation conditions to provide the desired O-3-acyl product.

**Example (7):**

**Preparation of a compound of Formula Y; Diosgenyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranoside (Compound Y-1)**

**Scheme 5:**

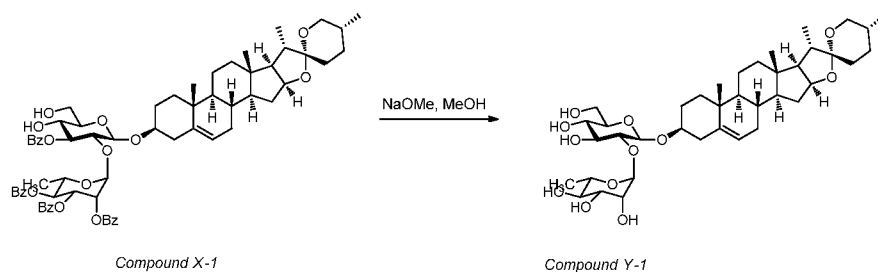
**Step (i):** Preparation of Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 3) as described above.

**Step (ii):** Preparation of Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 5) as described above.

**Step (iii):** Preparation of Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-3-benzoyl)- $\beta$ -D-glucopyranoside (Compound X-1) as described above.

**Compound Y-1: Step (iv)**

**Deprotection of a compound of Formula X to provide a compound of Formula Y; Diosgenyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranoside (Compound Y-1)**



To a solution of Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-3-benzoyl)- $\beta$ -D-glucopyranoside (Compound X-1) (16.542 g, 14.52 mmol) in MeOH (125 mL) was added 30 drops of NaOMe (5.4M in MeOH) and the pH was checked to be  $\sim$  10. Monitoring of the mixture by TLC indicated that the reaction was complete within 90 mins. The reaction was quenched with the addition of DOWEX 50W-X 400 until the pH  $\sim$  7 causing the DOWEX to change from a cream to light yellow colour. The resin was washed with MeOH and then 1:1 MeOH/CHCl<sub>3</sub>.

The filtrate was evaporated to yield a dry white solid which was washed with EA (9.58g, 91%). The crude material was columned on silica eluting with 10% MeOH/DCM then 20% MeOH/DCM to give Compound Y-1 (8.67 g, 94% pure via HPLC).

Alternatively, Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-3-benzoyl)- $\beta$ -D-glucopyranoside (Compound X-1) (9.3 g, 8.2 mmol) was dissolved in anhydrous methanol (74 mL). Sodium methoxide (0.1 mL, 30% solution in methanol) was added and the reaction mixture stirred for 22 hours at ambient temperature under argon. Tetrahydrofuran (74 mL) was added and the reaction mixture adjusted to pH 7 using Amberjet  $\text{\textcircled{R}}$  1200H resin. The resin was removed by filtration and washed with tetrahydrofuran (2 x 30 mL). The resulting solution was concentrated in vacuo and redissolved in methanol (74 mL). The product crystallized upon stirring at ambient temperature and the slurry was diluted with water (15 mL). The solid product was isolated by filtration, washed with 20% water in methanol (2 x 30 mL), water (30 mL) and ethyl acetate (3 x 30 mL). The solid product dried under vacuum at 35 $^{\circ}$ C for 16 h to afford diosgenyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranoside (4.65 g, 79% yield) as a white solid.

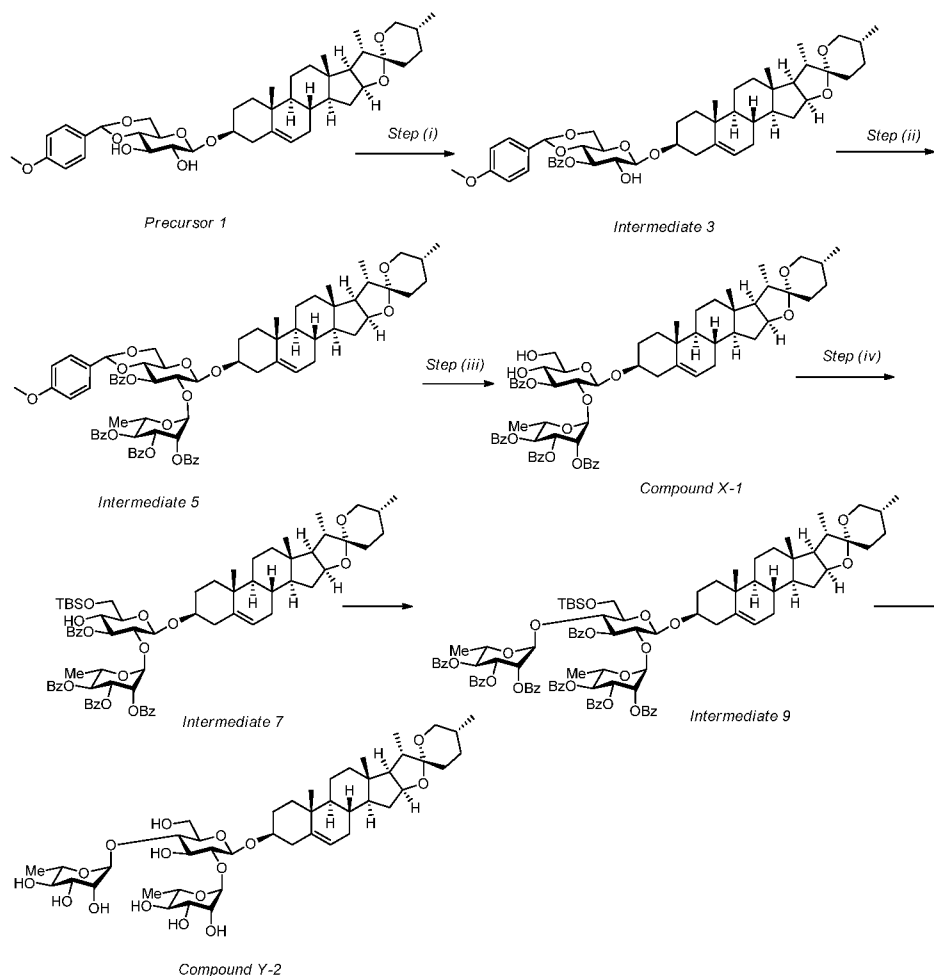
$^1\text{H}$  NMR (500 MHz, 3:1 CDCl<sub>3</sub>/CD<sub>3</sub>OD):  $\delta$ 5.35 (dd, J = 1.9, 3.2 Hz, 1 H), 5.19 (d, J = 1.5 Hz, 1 H), 4.46 (d, J = 7.6 Hz, 1 H), 4.41 (q, J = 7.6 Hz, 1 H), 4.08 (m, 1 H), 3.94 (dd, J = 1.5, 3.3 Hz, 1 H), 3.83 (dd, J = 3.0, 12.0 Hz, 1 H), 3.73 (dd, J = 4.7, 12.0 Hz, 1 H), 3.69 (dd, J = 3.5, 9.5 Hz, 1 H), 3.58 (m, 1 H), 3.49 (m, 2 H), 3.38 (m, 4 H), 3.25 (m, 1 H), 2.41 (ddd, J = 1.9, 4.7, 13.4 Hz, 1 H), 2.28 (m, 1 H), 2.00 (m, 2 H), 1.94-0.91 (m, 21 H), 1.27 (d, J = 6.2 Hz, 3 H), 1.02 (s, 3 H), 0.97 (d, J = 7.3 Hz, 3 H), 0.80 (d, J = 6.1 Hz, 3 H), 0.79 (s, 3 H);  $^{13}\text{C}$  NMR (126 MHz, 3:1 CDCl<sub>3</sub>/CD<sub>3</sub>OD):  $\delta$ 141.80, 123.05, 110.94, 101.93, 100.92, 82.35, 79.97, 79.09, 76.97, 74.19, 72.66, 71.93, 71.80, 69.69, 68.23, 63.38, 63.19, 57.86, 51.53, 42.99, 41.63, 41.09, 39.71, 38.60, 38.21, 33.40, 33.03, 32.78, 32.65, 31.54, 30.89, 30.01, 22.17,

20.47, 18.55, 18.28, 17.51, 15.62; HRMS (TOF ES+)  $m/z$  calcd for  $C_{39}H_{62}O_{12}Na$  745.4139, found 745.4141.

### Example (8):

#### Preparation of a compound of Formula Y

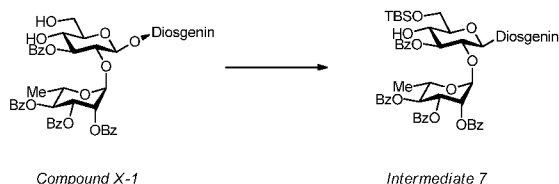
#### Scheme 6:



**Step (i):** Preparation of Diosgenyl-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 3) as previously described above.

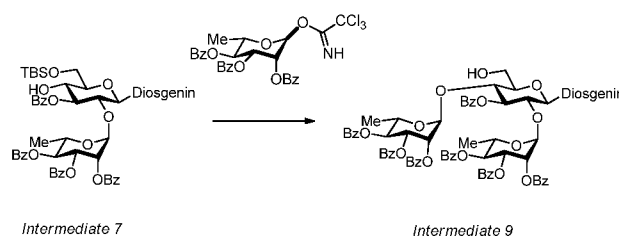
**Step (ii):** Preparation of Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-(4,6-O-(4-methoxybenzylidene)-3-benzoyl)- $\beta$ -D-glucopyranoside (Intermediate 5) as previously described above.

**Step (iii):** Preparation of Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-3-benzoyl)- $\beta$ -D-glucopyranoside (Compound X-1) as previously described above.

**Intermediate 7: Step (iv)****Selective protection at R<sup>7</sup> to provide Intermediate 7**

Diosgenyl-(2,3,4-tribenzoyl)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-3-benzoyl)- $\beta$ -D-glucopyranoside (Compound X-1) (1.26 g, 1.1 mmol) was combined with imidazole (0.49 g, 7.2 mmol), DMAP (0.10 g, 0.83 mmol) and *tert*-butyldimethylsilyl chloride (0.29 g, 1.9 mmol) at room temperature in a dry flask under an Ar atmosphere. Dimethylformamide (5 mL) was added to effect dissolution and the reaction heated to 40°C under an Ar atmosphere for 1 h. The reaction was quenched by addition to of ethyl acetate (100 mL) and washed successively with saturated aq. NH<sub>4</sub>Cl (2 x 100 mL), distilled water (2 x 100 mL), brine (100 mL), dried with MgSO<sub>4</sub> and evaporated. Columned chromatography upon silica with a gradient of ethyl acetate:toluene (5 to 10%) gave Intermediate 7 in near quantitative yield.

<sup>1</sup>H NMR 500 MHz (CDCl<sub>3</sub>)  $\delta$  8.04 (d, 2H), 7.91 (d, 2H), 7.78 (d, 2H), 7.74 (d, 2H), 7.53 (t, 1H), 7.49 (t, 1H), 7.39 (t, 2H), 7.35 (m, 1H), 7.31 (t, 2H), 7.28-7.15 (m, 7H), 5.75 (dd, J = 10.2, 3.5 Hz), 5.58 (t, J = 9.7 Hz), 5.47 (m, 2H), 5.44 (dd, J = 1.6, 3.4 Hz, 1H), 5.23 (d, J = 1.6 Hz, 1H), 4.75 (m, 2H), 4.43 (q, 1H), 3.92 (m, 3H), 3.76 (t, J = 9.2 Hz, 1H), 3.72 (m, 1H), 3.54 (dt, J = 5.4, 9.4 Hz, 1H), 3.48 (d of multiplets, J = 10.8 Hz, 1H), 3.39 (t, J = 10.8 Hz, 1H), 3.35 (s, 1H), 2.60 (d of multiplets, J = 13.1 Hz, 1H), 2.43 (t, 1H), 2.35 (s, 1H), 2.03 (m, 3H), 1.05-1.91 (m, 26H), 1.33 (d, J = 6.3 Hz, 3H), 0.98 (d, J = 6.8 Hz, 3H), 0.94 (s, 3H), 0.91 (s, 9H), 0.79 (d, J = 5.6 Hz, 3H), 0.79 (s, 3H), 0.11 (s, 3H), 0.10 (s, 3H); <sup>13</sup>C NMR 126 MHz (CDCl<sub>3</sub>)  $\delta$  167.1, 165.8, 165.4, 164.7, 140.3, 133.30, 133.25, 133.0, 130.1, 129.9, 129.9, 129.8, 129.5, 129.4, 129.1, 128.4, 128.4, 128.33, 128.26, 125.4, 122.2, 109.4, 99.9, 97.9, 80.9, 79.43, 79.41, 75.4, 75.2, 72.1, 72.0, 70.6, 69.7, 67.0, 66.9, 64.5, 62.3, 56.6, 50.2, 41.8, 40.4, 39.9, 38.9, 37.3, 37.0, 32.3, 32.0, 31.7, 31.6, 30.4, 30.3, 30.0, 29.8, 29.0, 26.0, 20.9, 19.4, 18.4, 17.5, 17.3, 16.4; ES-MS *m/z* C<sub>73</sub>H<sub>92</sub>O<sub>16</sub>NaSi calcd: 1275.6052; found: 1275.6040

**Intermediate 9: Step (iv)****Coupling of an additional saccharide at R<sup>6</sup> to provide Intermediate 9**

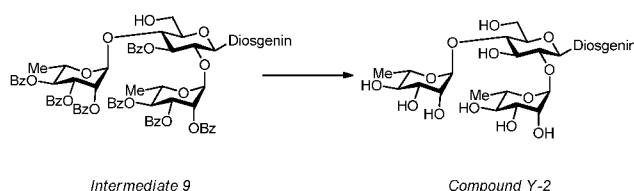
A solution of Intermediate 7 (116 mg, 0.09 mmol) and 4Å molecular sieve (0.49 g) in dry dichloromethane (5 mL) was stirred under Ar for 20 minutes at room temperature and then cooled to -78°C (internal) reaction temperature. Boron trifluoride diethyl etherate (60  $\mu$ L, 0.47 mmol) was added via syringe followed but a solution of 2,3,4-tri-O-benzoyl- $\alpha/\beta$ -L-rhamnopyranoside trichloroacetimidate (177 mg, 0.29 mmol) in dry dichloromethane (2 mL) via a cannula at -78°C. The mixture was allowed to warm to room temperature and stirred for 3 h. The reaction was neutralised with triethylamine (0.2 mL), filtered to remove the sieves and concentrated under reduced pressure. TLC (30% ethyl acetate/petroleum ether) indicated significant desilylation of the reaction product. Complete desilylation was achieved with addition of tetrabutylammonium fluoride (1 mL, 1 mmol) with stirring at room temperature for 10 h. The reaction was partitioned into saturated aq.  $\text{NH}_4\text{Cl}$  (100 mL) and ethyl acetate (50 mL). The organic layer was washed with water (2 x 100 mL), brine (100 mL), dried with  $\text{MgSO}_4$  and evaporated to dryness. Column chromatography was performed on silica eluting with a gradient of 0 to 15% ethyl acetate:toluene to give Intermediate 9; 76 mg, 69% yield.

$^1\text{H}$  NMR 500 MHz ( $\text{CDCl}_3$ )  $\delta$  8.11 (d, 2H), 8.07 (m, 2H), 8.04 (d, 2H), 7.91 (d, 2H), 7.86 (d, 2H), 7.83 (d, 2H), 7.74 (d, 4H), 7.61 (t, 1H), 7.56 (t, 1H), 7.34-7.54 (m, 12H), 7.32 (t, 2H), 7.15-7.27 (m, 5H), 5.81 (t,  $J = 9.3$  Hz, 1H), 5.76 (dd,  $J = 3.6, 10.2$  Hz, 1H), 5.64 (dd,  $J = 3.4, 10.4$  Hz, 1H), 5.58 (dd,  $J = 2.0, 3.7$  Hz, 1H), 5.54 (t,  $J = 10.4$  Hz, 1H), 5.51 (t,  $J = 10.4$  Hz, 1H), 5.48 (s, 1H), 5.42 (dd,  $J = 1.5, 3.4$  Hz, 1H), 5.23 (d,  $J = 1.5$  Hz, 1H), 5.12 (d,  $J = 1.3$  Hz, 1H), 4.84 (d,  $J = 7.4$  Hz, 1H), 4.74 (m, 1H), 4.44 (m, 1H), 4.26 (m, 1H), 4.20 (t,  $J = 9.8$  Hz, 1H), 4.08 (m, 2H), 3.97 (m, 2H), 3.75 (m, 1H), 3.72 (m, 1H), 3.49 (d of multiplets,  $J = 10.7$  Hz, 1H), 3.39 (t,  $J = 10.8$  Hz, 1H), 2.63 (d of multiplets,  $J = 13.2$  Hz, 1H), 2.44 (t, 1H), 2.17 (m, 1H), 2.04 (m, 4H), 1.05-1.97 (m, 21 H), 1.33 (d,  $J = 5.8$  Hz, 3H), 0.99 (d,  $J = 7.1$  Hz, 3H), 0.94 (s, 3H), 0.80 (d,  $J = 7.1$  Hz, 3H), 0.79 (s, 3H), 0.73 (d,  $J = 6.2$  Hz, 3H).  $^{13}\text{C}$  NMR 126 MHz ( $\text{CDCl}_3$ )  $\delta$  TBC; ES-MS  $m/z$   $\text{C}_{94}\text{H}_{100}\text{O}_{23}\text{Na}$ , calcd 1619.6548; found: ES-MS low res 1619.7.

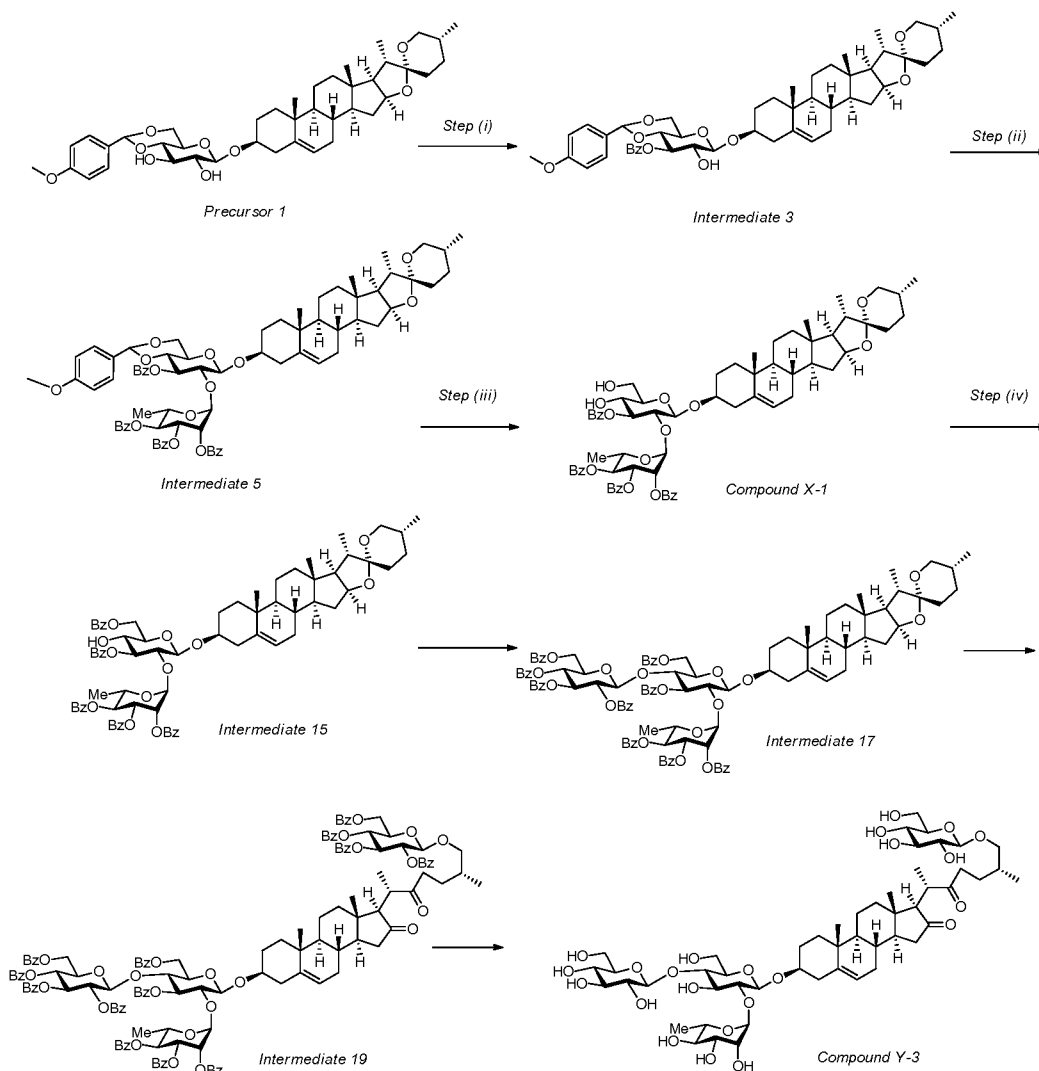
$^{13}\text{C}$  NMR 500 MHz ( $\text{CDCl}_3$ ) 165.9, 165.7, 165.6, 165.5, 165.4, 165.2, 164.5, 140.1, 133.5, 133.2, 133.2, 133.1, 133.0, 132.9, 130.0, 130.0, 129.8, 129.8, 129.7, 129.7, 129.4, 129.4, 129.3, 129.3, 129.0, 128.5, 128.3, 128.3, 128.2, 128.1, 122.3, 109.3, 99.8, 98.7, 98.0, 80.8, 79.4, 76.2, 76.1, 76.0, 75.4, 72.0, 71.3, 71.2, 70.4, 69.8, 69.6, 67.5, 66.9, 62.2, 61.3, 56.5, 50.1, 41.7, 40.3, 39.7, 38.7, 37.2, 36.9, 32.2, 31.9, 31.6, 31.5, 30.3, 29.9, 28.9, 20.8, 19.3, 17.4, 17.2, 17.1, 16.3, 14.5.

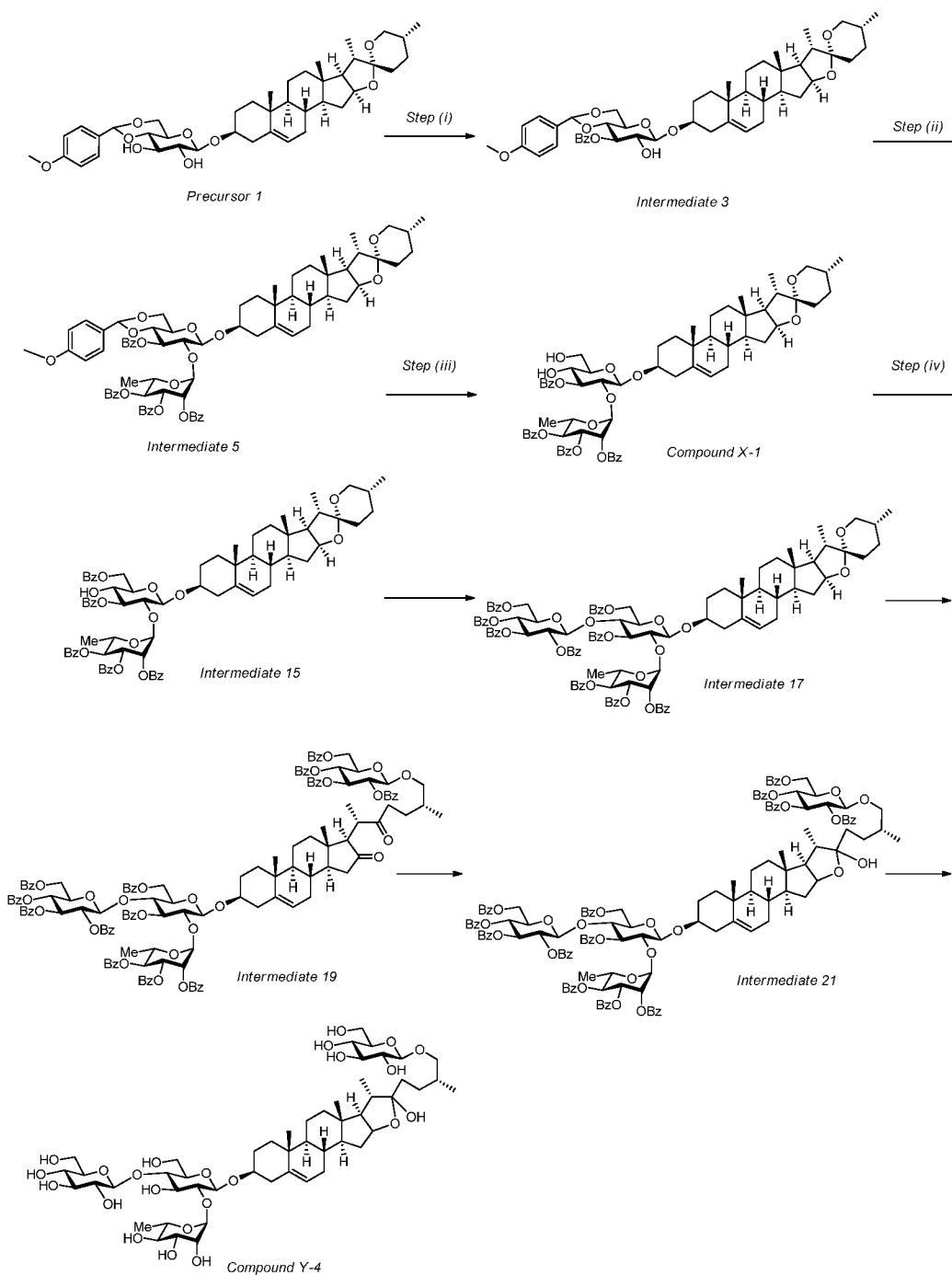
### Compound Y-2: Step (iv)

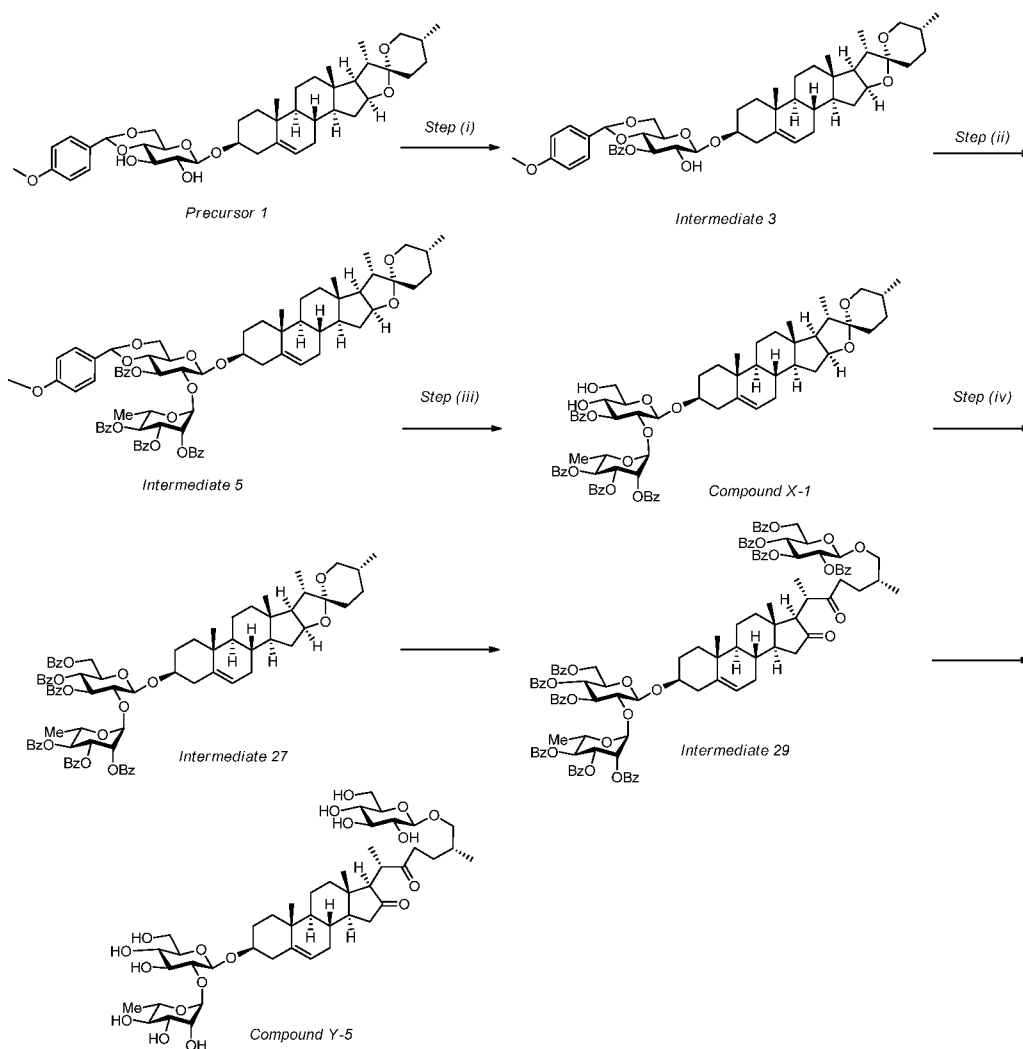
#### Deprotection to provide a compound of Formula Y



Intermediate 9 (82 mg, 0.051 mmol) was dissolved in 13 mL of methanol/tetrahydrofuran (8:5) at room temperature and 5.4 M sodium methoxide in methanol (0.011 mL, 0.059 mmol) was added to bring the reaction to ~ pH 10. The reaction was stirred overnight at room temperature resulting in 50% conversion of starting material to desired product as monitored via LCMS. An additional charge of 5.4 M sodium methoxide in methanol (0.011 mL, 0.059 mmol) was added and the reaction was stirred overnight at room temperature resulting in the complete conversion to product as monitored via LCMS. The material was neutralised with Amberjet 1200H resin, filtered and evaporated to give a white powder. Column chromatography on silica eluting with 5 to 25% methanol in dichloromethane led to the isolation of 38 mg (85%) of Y2. NMR 500 MHz (3:1  $\text{CDCl}_3/\text{CD}_3\text{OD}$ )  $\delta$  5.36 (dd,  $J = 1.9, 3.0$  Hz, 1H), 5.23 (d,  $J = 1.4$  Hz, 1H), 4.85 (d,  $J = 1.6$  Hz, 1H), 4.46 (d,  $J = 7.8$  Hz, 1H), 4.42 (q,  $J = 7.5$  Hz, 1H), 4.09 (m, 1H), 3.96 (dd,  $J = 1.6, 3.4$  Hz, 1H), 3.89-3.33 (m, 15H), 3.30 (dq,  $J = 2.2, 9.5$  Hz, 1H), 2.41 (ddd,  $J = 1.9, 4.6, 13.5$  Hz, 1H), 2.28 (t,  $J = 12.4$  Hz, 1H), 2.00 (m, 2 H), 1.94-0.91 (m, 21 H), 1.30 (d,  $J = 6.2$  Hz, 3 H), 1.27 (d,  $J = 6.2$  Hz, 3 H), 1.02 (s, 3 H), 0.98 (d,  $J = 7.1$  Hz, 3 H), 0.80 (d,  $J = 5.9$  Hz, 3 H), 0.79 (s, 3 H),  $^{13}\text{C}$  NMR 500 MHz ( $\text{CDCl}_3$ )  $\delta$  141.8, 123.1, 111.0, 103.2, 102.0, 100.7, 82.4, 80.8, 80.0, 78.0, 76.1, 74.2, 73.8, 72.7, 72.3, 72.2, 72.0, 70.9, 69.7, 68.2, 63.4, 62.3, 57.9, 51.5, 43.0, 41.6, 41.1, 39.7, 38.6, 38.2, 33.4, 33.0, 32.8, 32.6, 31.5, 30.9, 30.0, 22.2, 20.5, 18.5, 18.5, 18.3, 17.5, 15.6; ES-MS  $m/z$   $\text{C}_{45}\text{H}_{72}\text{O}_{16}\text{Na}$ , calcd 891.4718; found 891.4719.

**Example (9):****Preparation of a compound of Formula Y****Scheme 7:**

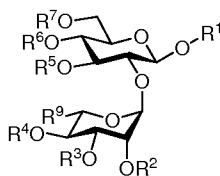
**Example (10):****Preparation of a compound of Formula Y****Scheme 8:**

**Example (11):****Preparation of a compound of Formula Y****Scheme 9:****Example (12):****Preparation of a compound of Formula Y utilising Precursor 2**

It will be appreciated that Compounds Y-1, Y-2, Y-3, Y-4, and Y-5 and other compounds of Formula Y can be prepared from Precursor 2 utilising the methods described for Example (3) to provide Compound X-1. Compound X-1 can then be elaborated by the methods described in Example 6 to Examples 11, to provide Compounds Y-1 to Y-5, respectively.

The claims defining the invention are as follows:

1. A method for the preparation of a compound of Formula X



**Formula X**

wherein

R<sup>1</sup> is a sapogenin;

R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup> are each independently an oxygen protecting group;

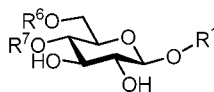
R<sup>5</sup> is an acyl group;

R<sup>6</sup> and R<sup>7</sup> are H;

R<sup>9</sup> is selected from the group consisting of H, CH<sub>3</sub>, and an oxygen protected by an oxygen protecting group;

the method comprising

- (i) reacting a compound of Formula A with an acylating agent in an acylation reaction in the presence of a base;



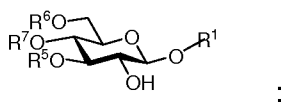
**Formula A**

wherein

R<sup>1</sup> is a sapogenin;

R<sup>6</sup> and R<sup>7</sup> are each independently an oxygen protecting group; or when taken together form a cyclic di-oxygen protecting group;

to provide a compound of Formula B



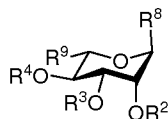
**Formula B**

R<sup>1</sup> is sapogenin;

R<sup>5</sup> is an acyl group;

R<sup>6</sup> and R<sup>7</sup> are each independently an oxygen protecting group or when taken together form a cyclic di-oxygen protecting group;

- (ii) reacting a compound of Formula B with a compound of Formula C under coupling conditions



**Formula C**

wherein

R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup> are each independently an oxygen protecting group;

R<sup>8</sup> is a leaving group; and

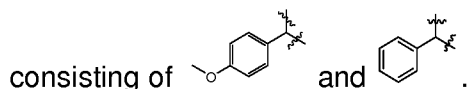
R<sup>9</sup> is selected from the group consisting of H, CH<sub>3</sub>, and an oxygen protected by an oxygen protecting group;

- (iii) selectively removing the oxygen protecting groups at R<sup>6</sup> and R<sup>7</sup> to provide a compound of Formula X.
2. A method according to claim 1 wherein the base in step (i) is selected from the group consisting of K<sub>2</sub>CO<sub>3</sub>, triethylamine, diisopropylethylamine, pyridine, 4-dimethylaminopyridine, and 1,8-diazabicycloundec-7-ene.
  3. A method according to claim 2 wherein the base is pyridine.
  4. A method according to any one of the preceding claims wherein step (i) is carried out in the presence of a solvent.
  5. A method according to claim 4, wherein the solvent is selected from the group consisting of dichloromethane, tetrahydrofuran, 1,2-dioxane, dichloroethane, chloroform, carbon tetrachloride and pyridine.
  6. A method according to claim 4 or claim 5, wherein the solvent is dichloromethane or pyridine.

7. A method according to any one of the preceding claims wherein step (i) is conducted at a temperature in the range of from  $-100$  to  $80^{\circ}\text{C}$ .
8. A method according to claim 7 wherein step (i) is conducted at a temperature in the range of from  $-85$  to  $40^{\circ}\text{C}$ .
9. A method according to claim 8 wherein step (i) is conducted at a temperature in the range of from  $-80$  to  $25^{\circ}\text{C}$ .
10. A method according to claim 7 the temperature of step (i) is initially in the range of  $-10$  to  $20^{\circ}\text{C}$ , and then subsequently increased over the course of the reaction to a temperature in the range of in the range of  $10$  to  $25^{\circ}\text{C}$ .
11. A method according to any one of the preceding claims wherein the acylating agent is an acid anhydride or an acyl halide.
12. A method according to claim 11 wherein the acylating agent is selected from the group consisting of acetyl chloride, propionyl chloride, benzoyl chloride, 2-chlorobenzoyl chloride, 4-chlorobenzoyl chloride, 4-nitrobenzoyl chloride and 4-methoxybenzoyl chloride.
13. A method according to claim 11 or claim 12 wherein the acylating agent is a benzoylating agent.
14. A method according to claim 12 or 13 wherein the acylating agent is benzoyl chloride.
15. A method according to claim any one of preceding claims wherein the ratio of acylating agent to a compound of Formula A is from 3:1 to 1:1.
16. A method according to claim 15 wherein the ratio of acylating agent to a compound of Formula A is from 2:1 to 1:1.
17. A method according to claim 16 wherein the ratio of acylating agent to a compound of Formula A is from 1.3:1 to 1:1.

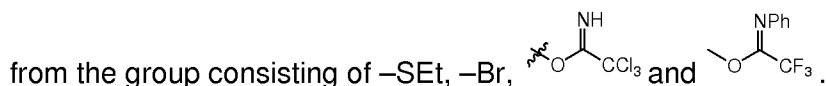
18. A method according to claim 17 wherein the ratio of acylating agent to a compound of Formula A is from 1.2:1 to 1:1.

19. A method according to any one of preceding claims wherein R<sup>6</sup> and R<sup>7</sup> of Formula A are taken together form a cyclic group selected from the group



20. A method according to any one of the preceding claims wherein R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup> are each independently acetyl or benzoyl.

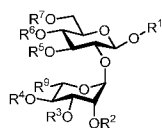
21. A method according to any one of the preceding claims wherein R<sup>8</sup> is selected



22. A method according to any one of the preceding claims wherein R<sup>1</sup> is selected from the group consisting of spirostanol aglycones and furostanol aglycones.

23. A method according to claim 21 wherein the spirostanol aglycones or furostanol aglycones are selected from the group consisting of diosgenin, yamogenin (neodiosgenin), yuccagenin, sarsasapogenin, tigogemmn, smilagenin, hecogenin, gitogemmn, convallamarogenin, neoruscogenin, solagenin, protodiosgenin, pseudoprotodiosgenin, methyl protodiosgenin, protoyamogenin, methyl protoyamogenin, and pharmaceutically acceptable salts, isomers and hydrates thereof.

24. A method for the preparation of a compound of Formula Y



**Formula Y**

wherein

R<sup>1</sup> is a sapogenin;

R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and R<sup>7</sup> are each H;

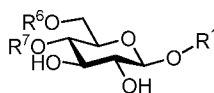
$R^6$  is H or a saccharide;

$R^9$  is selected from the group consisting of H, OH and  $CH_3$ ;

pharmaceutically acceptable salts, isomers, hydrates and solvate thereof;

the method comprising

- (i) reacting a compound of Formula A with an acylating agent in an acylation reaction in the presence of a base;



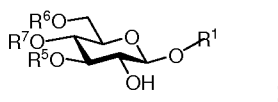
**Formula A**

wherein

$R^1$  is a sapogenin;

$R^6$  and  $R^7$  are each independently an oxygen protecting group; or when taken together form a cyclic di-oxygen protecting group;

to provide a compound of Formula B



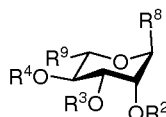
**Formula B**

$R^1$  is a sapogenin;

$R^5$  is an acyl group;

$R^6$  and  $R^7$  are each independently an oxygen protecting group, or when taken together form a cyclic di-oxygen protecting group;

- (ii) reacting a compound of Formula B with a compound of Formula C under coupling conditions



**Formula C**

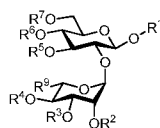
wherein

$R^2$ ,  $R^3$ , and  $R^4$  are each independently an oxygen protecting group;

$R^8$  is a leaving group; and

$R^9$  is selected from the group consisting of H,  $CH_3$ , and an oxygen protected by an oxygen protecting group;

- (iii) selectively removing the oxygen protecting groups at  $R^6$  and  $R^7$  to provide a compound of Formula X



**Formula X**

wherein

$R^1$  is a sapogenin;

$R^2$ ,  $R^3$ , and  $R^4$  are each independently an oxygen protecting group;

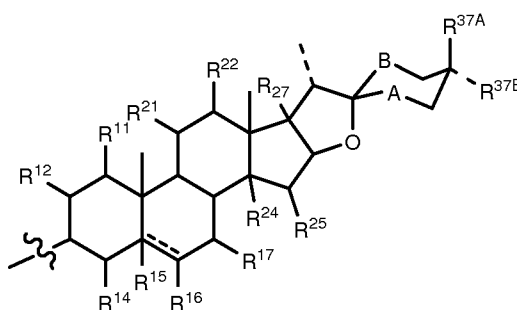
$R^5$  is an acyl group;

$R^6$  and  $R^7$  are H;

$R^9$  is selected from the group consisting of H,  $CH_3$ , and an oxygen protected by an oxygen protecting group;

- (iv) converting a compound of Formula X into a compound of Formula Y, pharmaceutically acceptable salts, isomers, hydrates or solvates thereof.

25. The method of claim 24 for preparing a compound of Formula Y wherein  $R^1$  is a sapogenin of Formula E, F or G:



**Formula E**

wherein

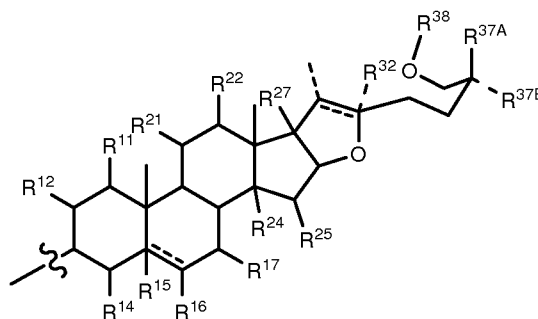
$R^{11}$ ,  $R^{12}$ ,  $R^{14}$ ,  $R^{16}$ ,  $R^{17}$ ,  $R^{21}$ ,  $R^{22}$ ,  $R^{24}$ ,  $R^{25}$  and  $R^{27}$  are independently H, OH, =O, pharmacologically acceptable ester groups or pharmacologically acceptable ether groups;

$R^{15}$  is H when C-5,C-6 is a single bond, and nothing when C-5,C-6 is a double bond;

A is either O concurrently with B being  $CH_2$ , or B is O concurrently with A being  $CH_2$ ;

$R^{37A}$  is H concurrently with  $R^{37B}$  being  $CH_3$ , or  $R^{37A}$  is  $CH_3$  concurrently with  $R^{37B}$  being H;

or a pharmaceutically acceptable salt, or derivative thereof;



**Formula F**

wherein

$R^{11}$ ,  $R^{12}$ ,  $R^{14}$ ,  $R^{16}$ ,  $R^{17}$ ,  $R^{21}$ ,  $R^{22}$ ,  $R^{24}$ ,  $R^{25}$  and  $R^{27}$  are independently H, OH, =O, pharmacologically acceptable ester groups or pharmacologically acceptable ether groups;

$R^{15}$  is H when C-5, C-6 is a single bond, and nothing when C-5, C-6 is a double bond;

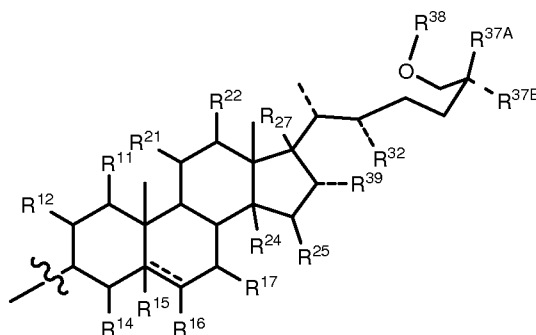
$R^{32}$  is either a hydroxyl or an alkoxy group when C-20, C-22 is a single bond, or nothing when C-20, C-22 is a double bond;

$R^{37A}$  is H concurrently with  $R^{37B}$  being  $CH_3$ , or  $R^{37A}$  is  $CH_3$  concurrently with  $R^{37B}$  being H;

$R^{38}$  is H or a saccharide; or a pharmaceutically acceptable salt, or derivative thereof;

$R^{13}$  is a bond to the C-1 oxygen of the mono-, di- or poly-glycoside;

or a pharmaceutically acceptable salt, or derivative thereof;



**Formula G**

wherein

$R^{11}$ ,  $R^{12}$ ,  $R^{14}$ ,  $R^{16}$ ,  $R^{17}$ ,  $R^{21}$ ,  $R^{22}$ ,  $R^{24}$ ,  $R^{25}$  and  $R^{27}$  are each independently H, OH, =O, pharmacologically acceptable ester groups or pharmacologically acceptable ether groups;

$R^{15}$  is H when C-5, C-6 is a single bond, and nothing when C-5, C-6 is a double bond;

$R^{32}$  and  $R^{39}$  are each independently H, OH, =O, pharmacologically acceptable ester groups or pharmacologically acceptable ether groups;

$R^{37A}$  is H concurrently with  $R^{37B}$  being  $CH_3$ , or  $R^{37A}$  is  $CH_3$  concurrently with  $R^{37B}$  being H;

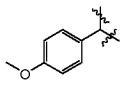

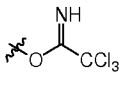
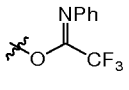
$R^{38}$  is H or a saccharide; or a pharmaceutically acceptable salt, or derivative thereof;

$R^{13}$  is a bond to the C-1 oxygen of the mono-, di- or poly-glycoside;

or a pharmaceutically acceptable salt, or derivative thereof.

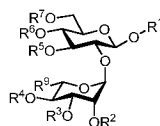
26. A method according to claim 24 wherein the base in step (i) is selected from the group consisting of  $K_2CO_3$ , triethyl amine, diisopropylethylamine, pyridine, 4-Dimethylaminopyridine, and 1,8-Diazabicycloundec-7-ene.
27. A method according to claim 26 wherein the base is pyridine.
28. A method according to any one of claims 24 to 26 wherein step (i) is carried out in the presence of a solvent.
29. A method according to claim 28, wherein the solvent is selected from the group consisting of dichloromethane, tetrahydrofuran, 1,2-dioxane, dichloroethane, chloroform, carbon tetrachloride and pyridine.
30. A method according to claim 28 or claim 29, wherein the solvent is dichloromethane or pyridine.
31. A method according to any one of claims 24 to 30 wherein step (i) is conducted at a temperature in the range of from  $-100$  to  $80^\circ C$ .
32. A method according to claim 31 wherein step (i) is conducted at a temperature in the range of from  $-85$  to  $40^\circ C$ .
33. A method according to claim 32 wherein step (i) is conducted at a temperature in the range of from  $-80$  to  $25^\circ C$ .
34. A method according to claim 31 the temperature of step (i) is initially in the range of  $-10$  to  $20^\circ C$ , and then subsequently increased over the course of the reaction to a temperature in the range of in the range of  $10$  to  $25^\circ C$ .
35. A method according to any one of claims 24 to 34 wherein the acylating agent is an anhydride or an acyl halide
36. A method according to claim 35 wherein the acylating agent is selected from the group consisting of acetyl chloride, propionyl chloride, benzoyl chloride, 2-

chlorobenzoyl chloride, 4-chlorobenzoyl chloride, 4-nitrobenzoyl chloride and 4-methoxybenzoyl chloride.

37. A method according to claim 35 or claim 36 wherein the acylating agent is a benzoylating agent.
38. A method according to claim 36 or 37 wherein the acylating agent is benzoyl chloride.
39. A method according to claim any one claims 24 to 38 wherein the ratio of acylating agent to a compound of Formula A is from 3:1 to 1:1.
40. A method according to claim 39 wherein the ratio of acylating agent to a compound of Formula A is from 2:1 to 1:1.
41. A method according to claim 40 wherein the ratio of acylating agent to a compound of Formula A is from 1.3:1 to 1:1.
42. A method according to claim 41 wherein the ratio of acylating agent to a compound of Formula A is from 1.2:1 to 1:1.
43. A method according to any one of claims 24 to 42 wherein  $R^6$  and  $R^7$  of Formula A are taken together form a cyclic group selected from the group consisting of  and .
44. A method according to any one of claims 24 to 43 wherein  $R^2$ ,  $R^3$ ,  $R^4$  are each independently acetyl or benzoyl.
45. A method according to any one of claims 24 to 44 wherein  $R^8$  is selected from the group consisting of  $-SEt$ ,  $-Br$ ,  and .
46. A method according to any one of claims 24 to 45 wherein  $R^1$  is selected from the group consisting of spirostanol aglycones and furostanol aglycones.

47. A method according to claim 46 wherein the spirostanol aglycones or furostanol aglycones are selected from the group consisting of diosgenin, yamogenin (neodiosgenin), yuccagenin, sarsasapogenin, tigogemn, smilagenin, hecogenin, gitogemn, convallamarogenin, neoruscogenin, solagenin, protodiosgenin, pseudoprotodiosgenin, methyl protodiosgenin, protoyamogenin, methyl protoyamogenin, and pharmaceutically acceptable salts, isomers and hydrates thereof.
48. The method of any one of claims 24 to 47 wherein step (iv) comprises removing any protecting groups to provide a compound of Formula Y.
49. The method of any one of claims 24 to 47 wherein step (iv) comprises
- selectively introducing an oxygen protecting group at R<sup>7</sup>;
  - coupling a suitable saccharide under coupling conditions such that R<sup>6</sup> is a saccharide;

to provide a compound of Formula X'



**Formula X'**

wherein

R<sup>1</sup> is a sapogenin;

R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup> and R<sup>7</sup> are each independently an oxygen protecting group;

R<sup>5</sup> is an acyl group;

R<sup>6</sup> is a saccharide;

R<sup>9</sup> is selected from the group consisting of H, CH<sub>3</sub>, and an oxygen protected by an oxygen protecting group; and

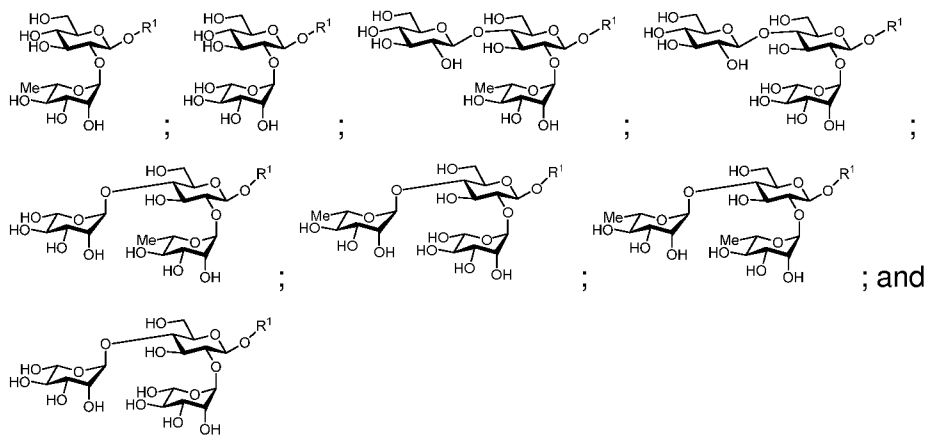
- converting a compound of Formula X' into a compound of Formula Y, a pharmaceutically acceptable salt, isomer, hydrate or solvate thereof .
50. The method of any one of claims 24 to 47 and 49 wherein step (iv) comprises
- selectively introducing an oxygen protecting group at R<sup>7</sup>;

- (b) coupling a suitable saccharide under coupling conditions such that R<sup>6</sup> is a saccharide to provide a compound of Formula X' as defined in claim 27; and
- (c) removing any protecting groups to provide a compound of Formula Y.

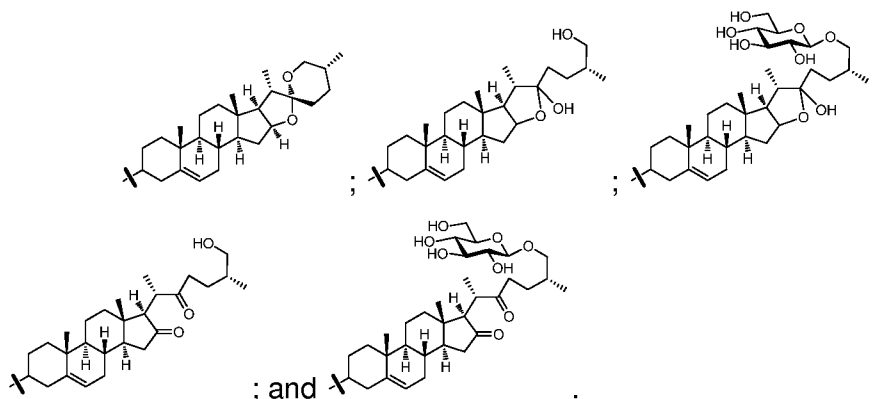
51. The method of any one of claims 24 to 47 and 49 wherein step (iv) comprises
- (a) selectively introducing an oxygen protecting group at R<sup>7</sup>;
  - (b) coupling a suitable saccharide under coupling conditions such that R<sup>6</sup> is a saccharide to provide a compound of Formula X' as defined in claim 27;
  - (c) converting the sapogenin at R<sup>1</sup> into a sapogenin of Formula G as defined in claim 25; and
  - (d) removing any protecting groups to provide a compound of Formula Y.

52. The method of any one of claims 24 to 47 and 49 wherein step (iv) comprises
- (a) selectively introducing an oxygen protecting group at R<sup>7</sup>;
  - (b) coupling a suitable saccharide under coupling conditions such that R<sup>6</sup> is a saccharide to provide a compound of Formula X' as defined in claim 27;
  - (c) converting the sapogenin at R<sup>1</sup> into a sapogenin of Formula F as defined in claim 25; and
  - (d) removing any protecting groups to provide a compound of Formula Y.

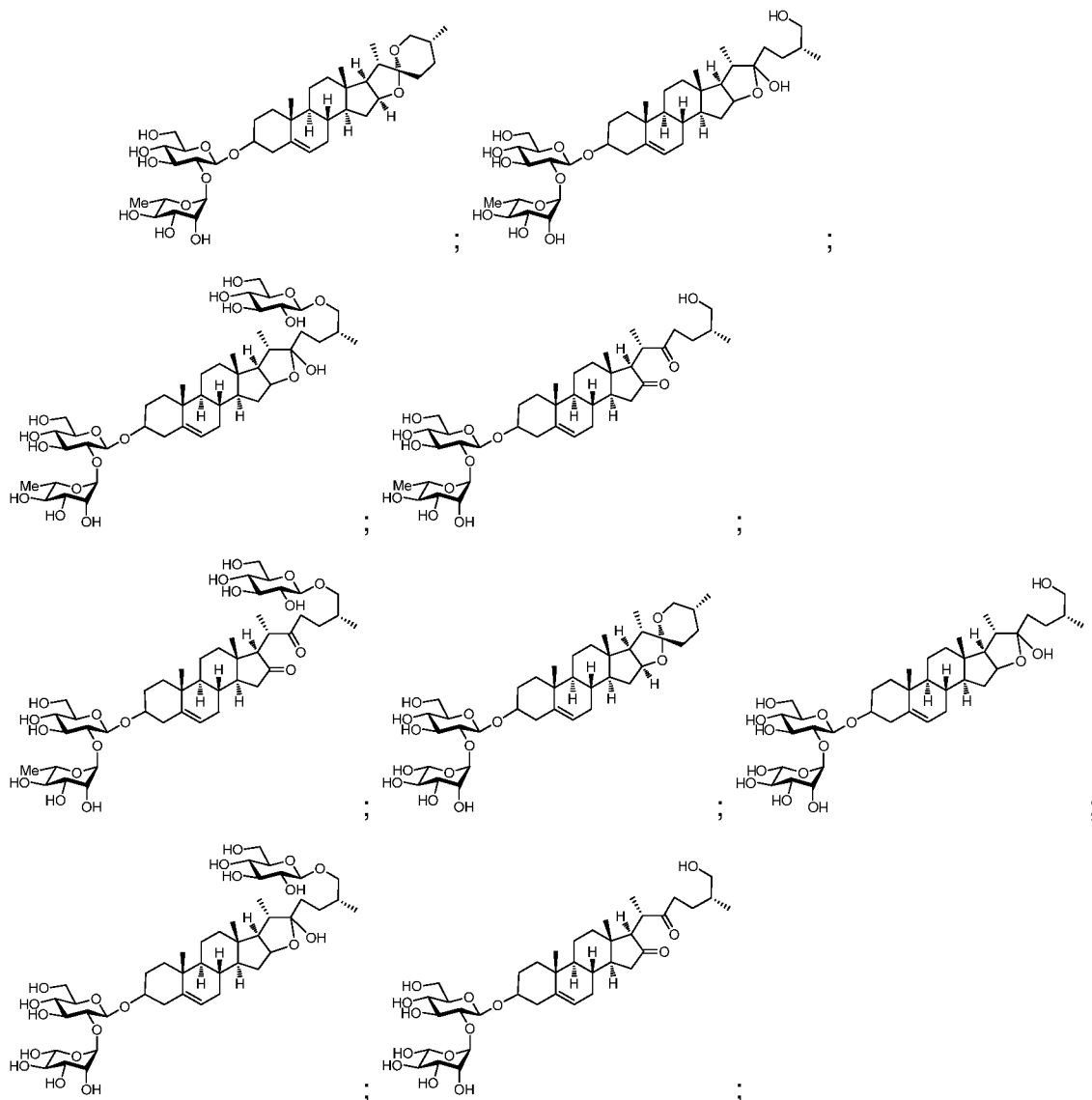
53. The method of any one of claims 24 to 52 wherein the compound of Formula Y is selected from the group consisting of selected from the group consisting of

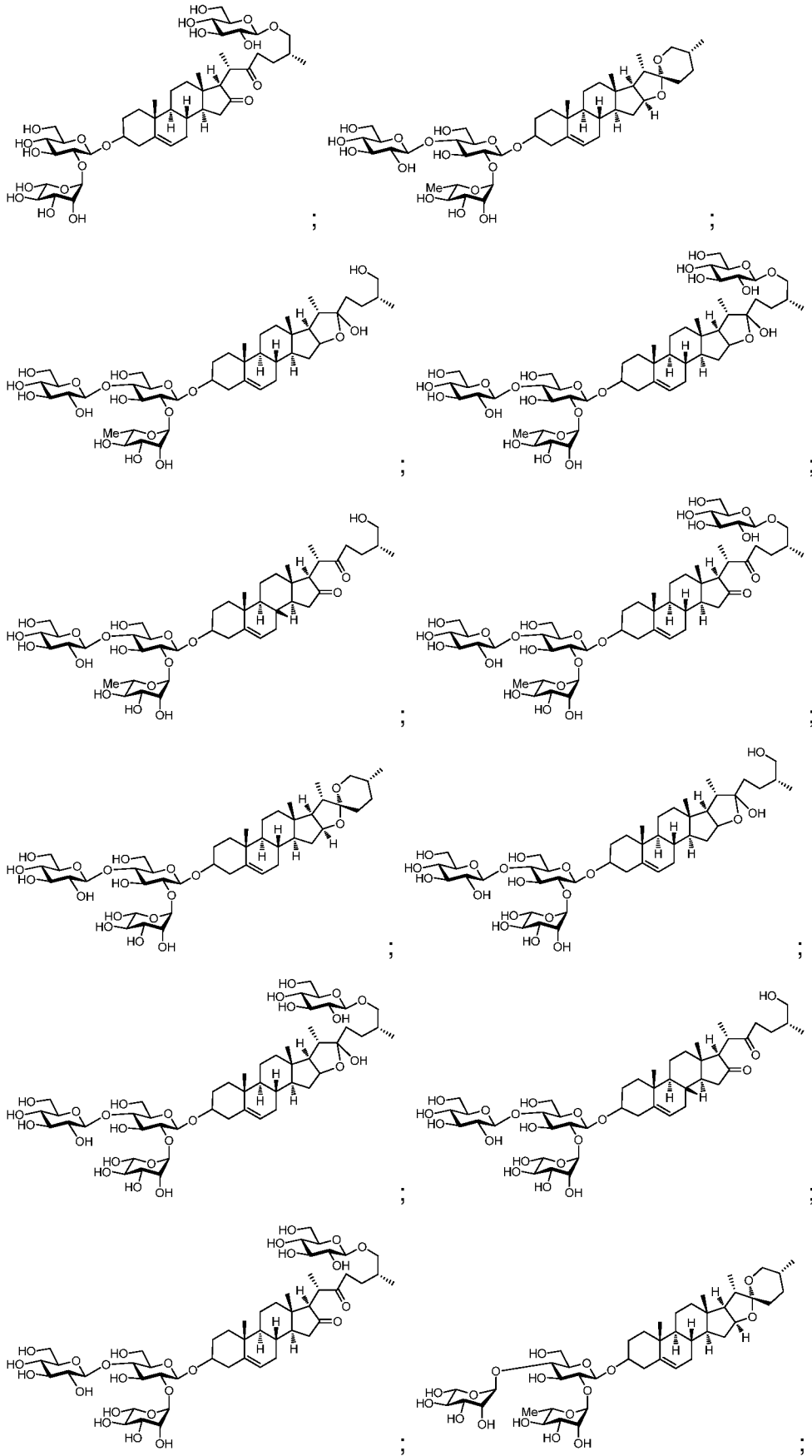


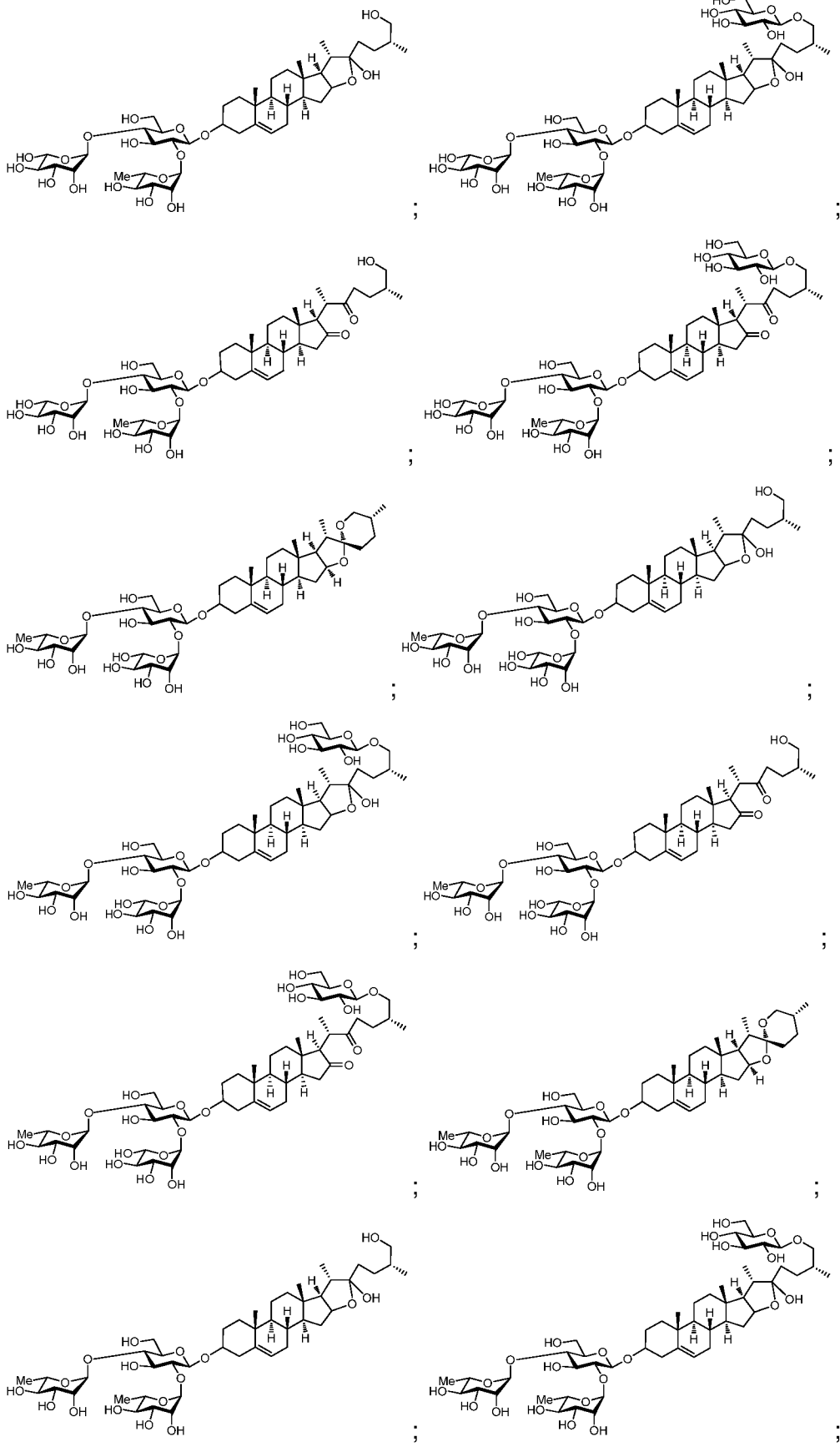
54. The method of any one of claims 24 to 53 wherein R<sup>1</sup> is selected from the group consisting of :



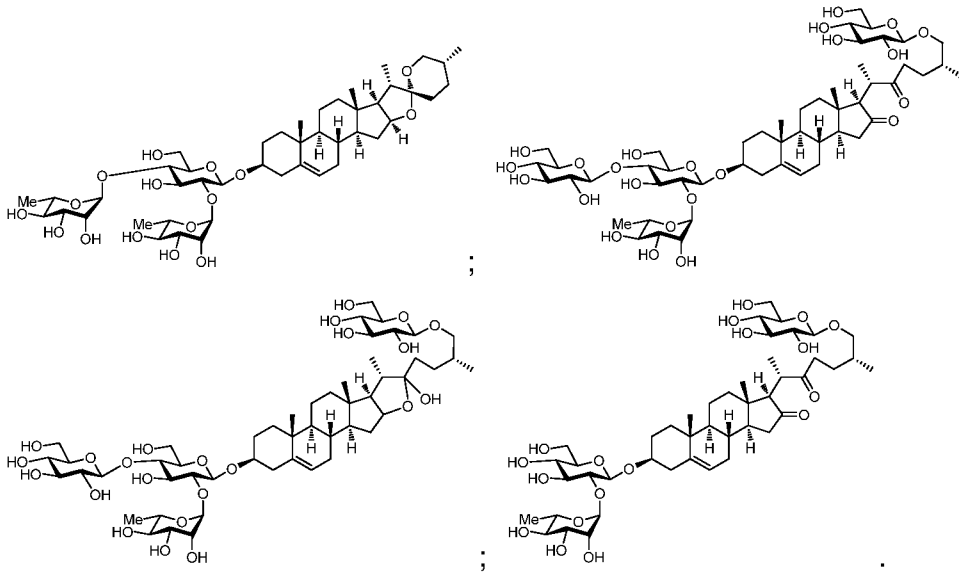
55. The method of any one claims 24 to 53 wherein the compound of Formula Y is selected from the group consisting of:





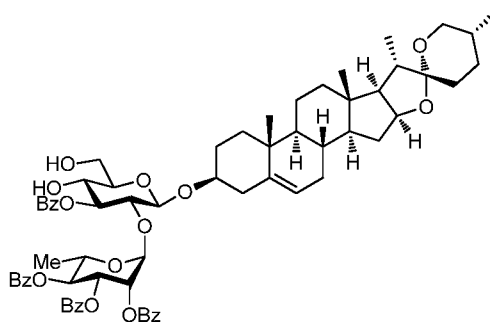




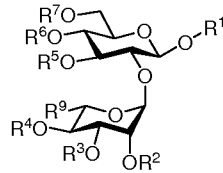


57. The method of any one of claims 24 to 56 wherein the compound of Formula Y is a pharmaceutically acceptable salt selected from the group consisting of salts of hydrochloric, sulfuric, phosphoric, formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, fumaric, maleic, alkyl sulfonic, and arylsulfonic acids; salts of lithium, sodium, potassium, magnesium, calcium, aluminium, zinc, benzathine, chlorprocaine, choline, diethanolamine, ethanolamine, ethyldiamine, meglumine, procaine,, alanine, glycine, lysine, histidine, arginine; and ammonium salts including tetra alkyl ammonium salts.

58. A method of any one of the preceding claims wherein the compound of formula X is



59. A compound of formula X



Formula X

wherein

R<sup>1</sup> is sapogenin;

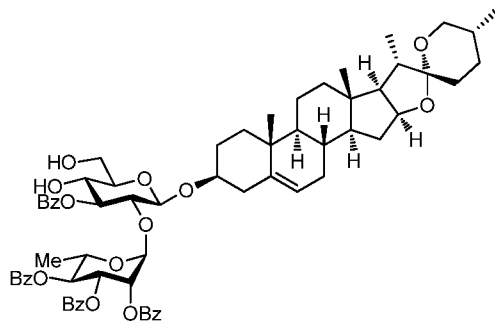
R<sup>2</sup>, R<sup>3</sup>, and R<sup>4</sup> are each independently an oxygen protecting group;

R<sup>5</sup> is an acyl group;

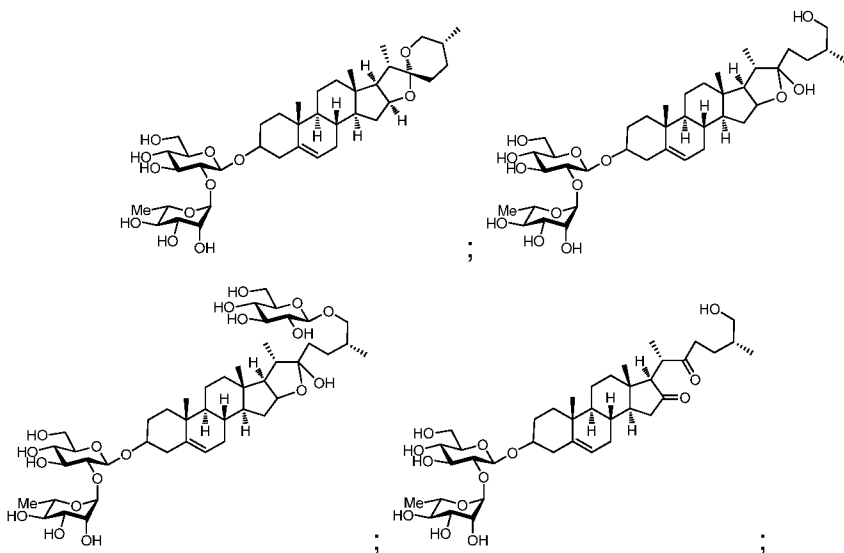
R<sup>6</sup> and R<sup>7</sup> are each H;

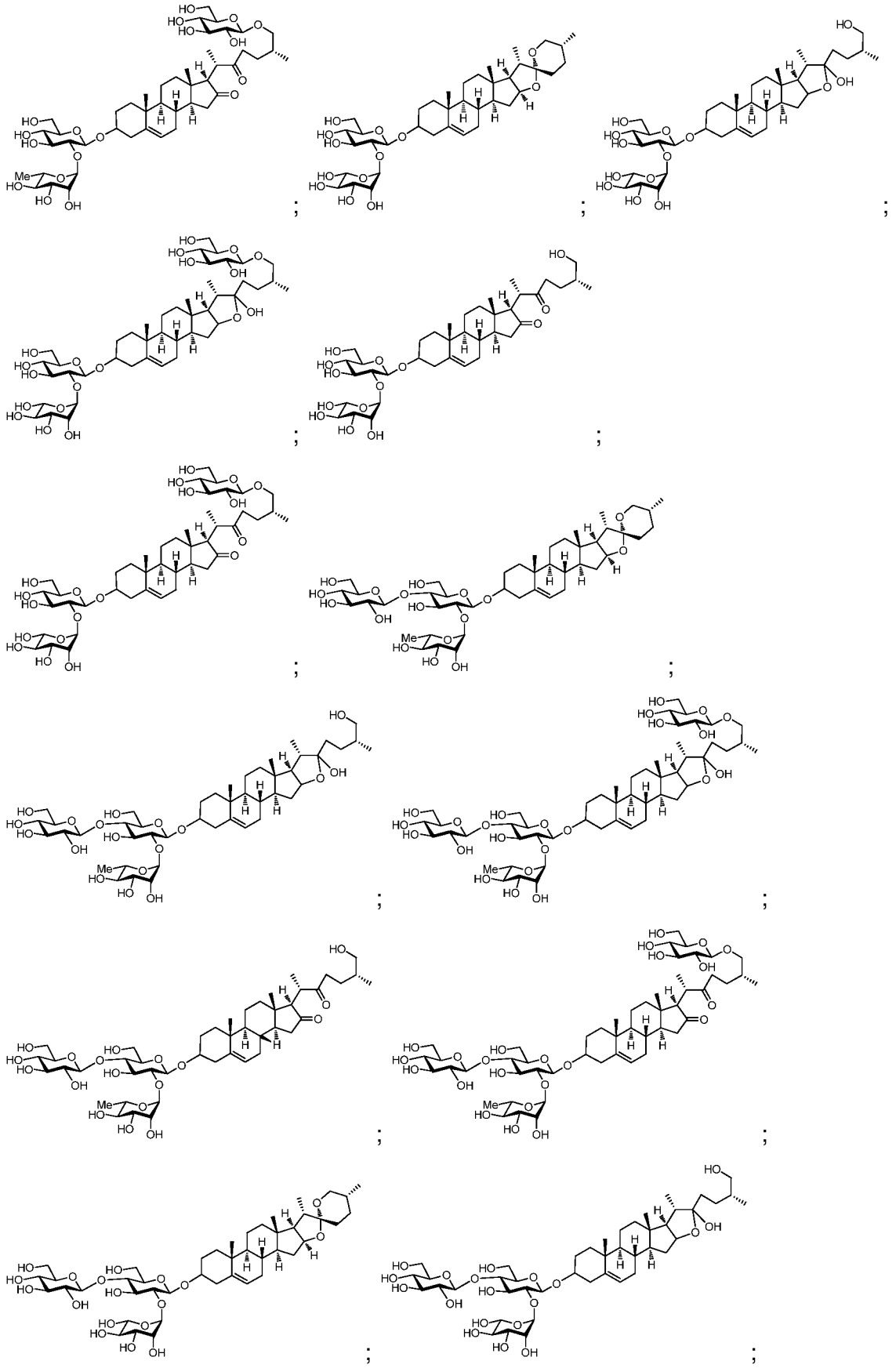
R<sup>9</sup> is CH<sub>3</sub>.

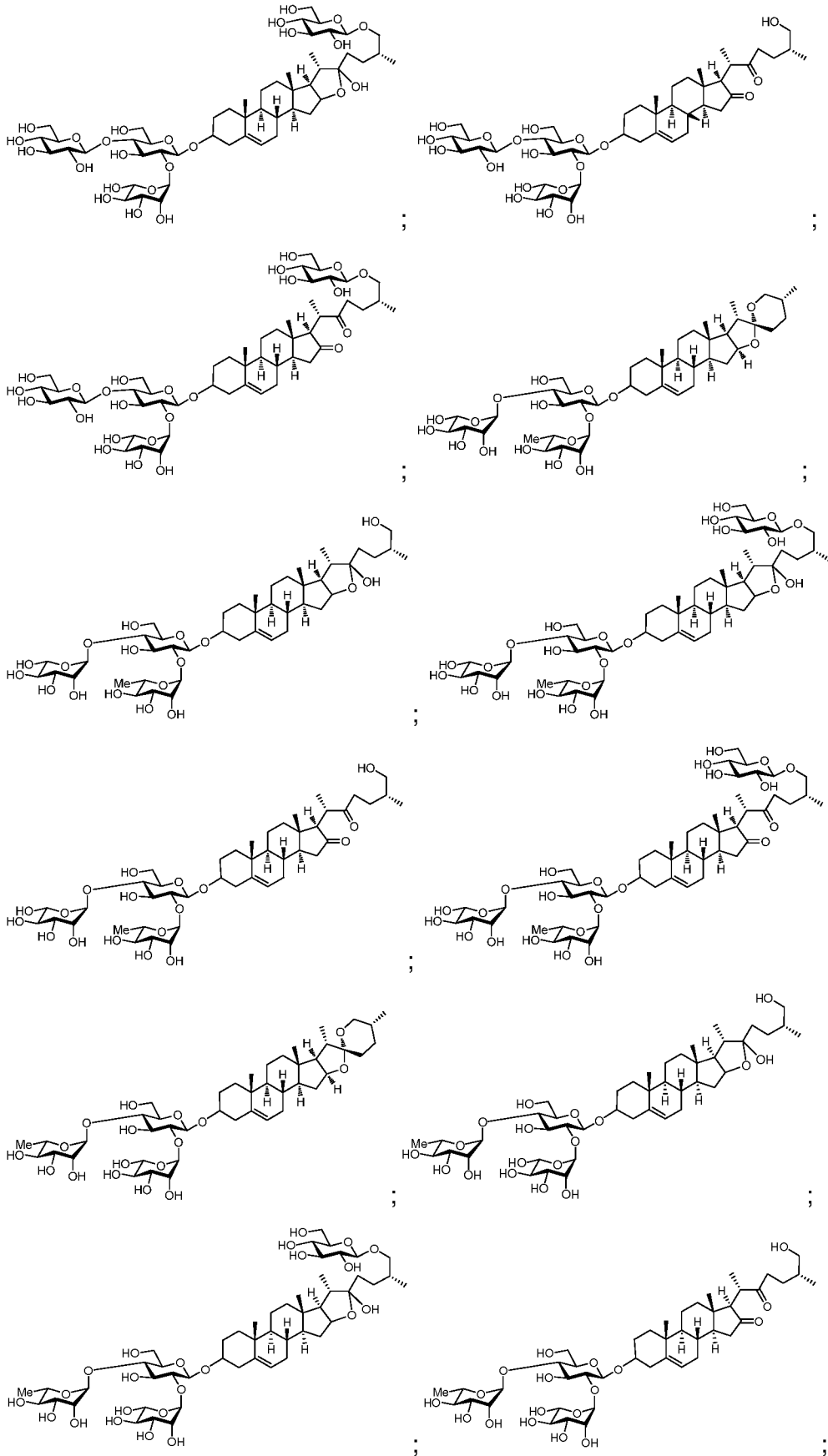
60. A compound of Formula X according to claim 59 which is:

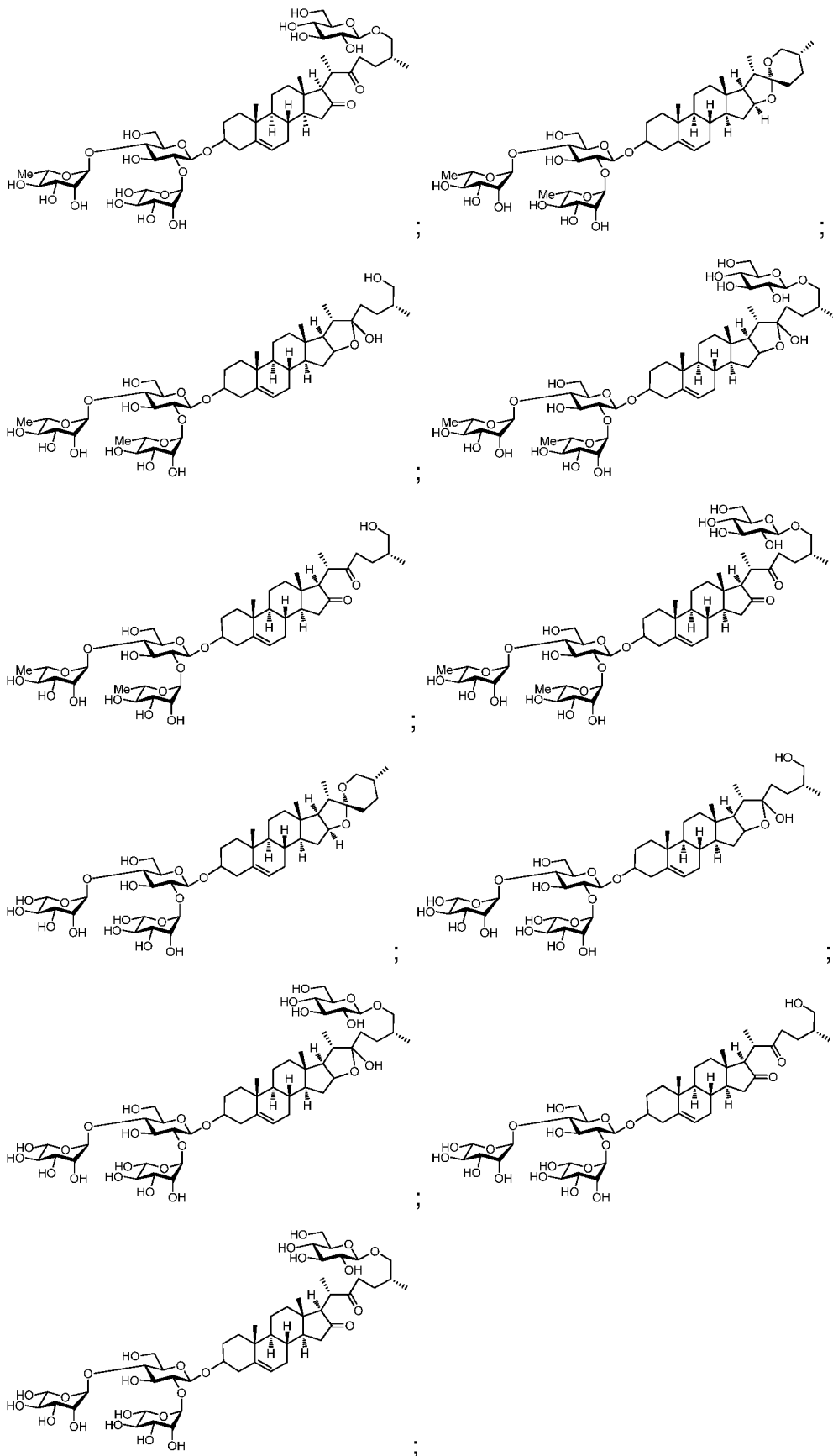


61. A compound of Formula X according to claim 59 or claim 60, when used in the preparation of a compound selected from the group consisting of:



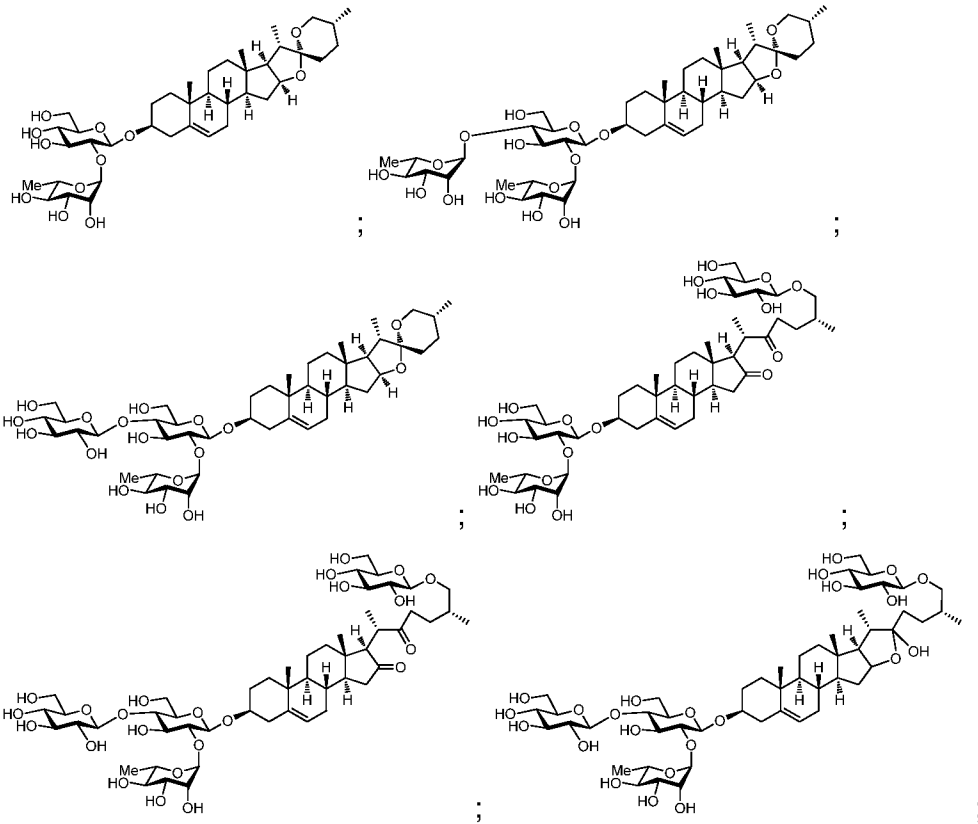






and pharmaceutically acceptable salts, isomers, hydrates and solvates thereof.

62. A compound of Formula X according to any one of claims 59 to 61 when used in the preparation of a compound selected from the group consisting of:



and pharmaceutically acceptable salts, isomers, hydrates and solvates thereof.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/AU2013/000416

## A. CLASSIFICATION OF SUBJECT MATTER

C07J 75/00 (2006.01) C07J 71/00 (2006.01) A61K 31/704 (2006.01) A61K 31/7048 (2006.01)

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)

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

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

STN Registry and CAPlus Sub structure search based on Formula X and prep/rl and SPN/RL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

 Further documents are listed in the continuation of Box C
  See patent family annex

* Special categories of cited documents:		
"A" document defining the general state of the art which is not considered to be of particular relevance	"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 30 May 2013	Date of mailing of the international search report 30 May 2013
<b>Name and mailing address of the ISA/AU</b>  AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au Facsimile No.: +61 2 6283 7999	<b>Authorised officer</b>  Ricky Fung AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262223648

**INTERNATIONAL SEARCH REPORT**

International application No.

C (Continuation).

DOCUMENTS CONSIDERED TO BE RELEVANT

**PCT/AU2013/000416**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Hernandez J. C., et al "Synthesis of novel spirostane saponins and their cytotoxic activity" Bioorganic & Medicinal Chemistry Vol 16 (2008) 2063-2076 See whole document esp. table 1 (such as compounds 24 and 25)	1-62

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

**PCT/AU2013/000416**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

**Patent Document/s Cited in Search Report****Patent Family Member/s****Publication Number****Publication Date****Publication Number****Publication Date****End of Annex**

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

Form PCT/ISA/210 (Family Annex)(July 2009)