



(43) International Publication Date

26 May 2016 (26.05.2016)

(51) International Patent Classification:

C07J 9/00 (2006.01)

(21) International Application Number:

PCT/GB2015/053518

(22) International Filing Date:

19 November 2015 (19.11.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1420593.4 19 November 2014 (19.11.2014)

GB

1420594.2 19 November 2014 (19.11.2014)

GB

1505676.5 1 April 2015 (01.04.2015)

GB

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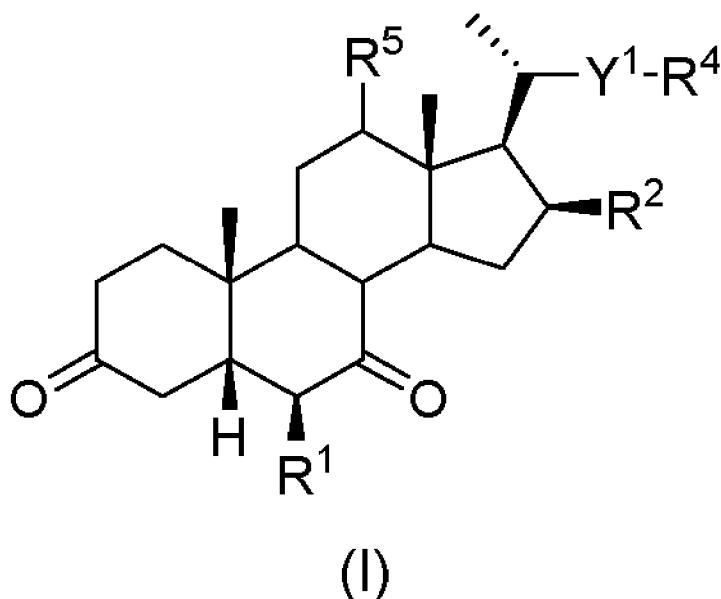
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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(54) Title: 6.ALPHA.-ALKYL-3,7-DIONE STEROIDS AS INTERMEDIATES FOR THE PRODUCTION OF STEROIDAL FXR MODULATORS

(57) Abstract: The invention relates to compounds of formula (I) wherein R¹, R², Y¹, R⁴ and R⁵ are as defined herein. The compounds are intermediates in the synthesis of synthetic bile acids.





(84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE,

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

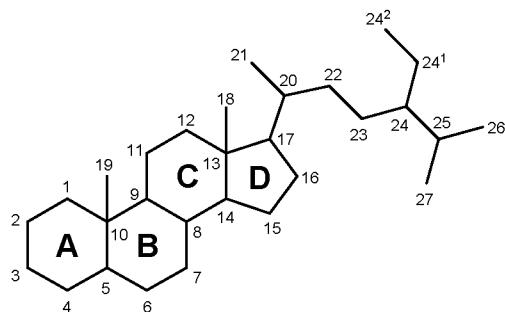
6.ALPHA.-ALKYL-3,7-DIONE STEROIDS AS INTERMEDIATES FOR THE
PRODUCTION OF STEROIDAL FXR MODULATORS

The present invention relates to compounds which are intermediates in the synthesis of bile acid derivatives with pharmacological activity. In particular, the invention 5 relates to intermediates in the synthesis of obeticholic acid and its analogues. In addition, the invention relates to a method of synthesizing these intermediates and a method of preparing obeticholic acid and obeticholic acid analogues from the compounds of the invention.

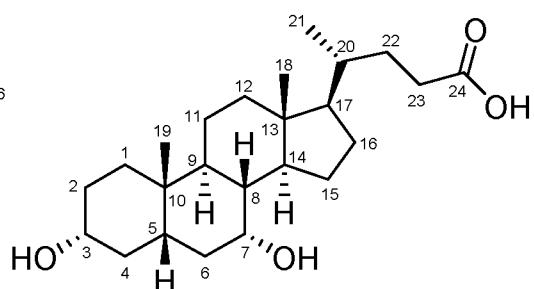
10 Bile acids are steroid acids which are found in the bile of mammals and include compounds such as cholic acid, chenodeoxycholic acid, lithocholic acid and deoxycholic acid, all of which are found in humans. Many bile acids are natural ligands of the farnesoid X receptor (FXR) which is expressed in the liver and intestine of mammals, including humans.

15

Bile acids are derivatives of steroid and are numbered in the same way. The following shows the general numbering system for steroids and the numbering of the carbon atoms in chenodeoxycholic acid.



General steroid numbering



CDCA numbering

20

Agonists of FXR have been found to be of use in the treatment of cholestatic liver disorders including primary biliary cirrhosis and non-alcoholic steatohepatitis (see review by Jonker *et al*, in *Journal of Steroid Biochemistry & Molecular Biology*, 2012, 130, 147-158).

25

Ursodeoxycholic acid (UDCA), a bile acid originally isolated from the gall bladder of bears, is currently used in the treatment of cholestatic liver disorders, although it appears to be inactive at the FXR.

30 As well as their action at the FXR, bile acids and their derivatives are also modulators

of the G protein-coupled receptor TGR5. This is a member of the rhodopsin-like superfamily of G-protein coupled receptors and has an important role in the bile acid signalling network, which complements the role of the FXR.

5 Because of the importance of FXR and TGR5 agonists in the treatment of cholestatic liver disorders, efforts have been made to develop new compounds which have agonist activity at these receptors. One particularly active compound is obeticholic acid, which is a potent agonist of both FXR and TGR5. Obeticholic acid is described in WO 02/072598 and EP1568706, both of which describe a process for the preparation of obeticholic acid from 7-keto lithocholic acid, which is derived from 10 cholic acid. Further processes for the production of obeticholic acid and its derivatives are described in WO 2006/122977, US 2009/0062256 and WO 2013/192097 and all of these processes also start from 7-keto lithocholic acid.

15 It is clear from the number of patent publications directed to processes for the production of obeticholic acid that it is by no means simple to synthesise this compound and indeed the process which is currently used starts from cholic acid and has 12 steps and an overall yield of only 5-10%.

20 In addition to the inefficiency and high cost of this process, there are also problems with the cost and availability of the starting materials. Cholic acid, the current starting material for the production of obeticholic acid, is a natural bile acid which is usually obtained from the slaughter of cows and other animals. This means that the availability of cholic acid and other bile acids is limited by the number of cattle 25 available for slaughter and, moreover, the price of bile acids is extremely high. Since the incidence of cholestatic liver disease is increasing worldwide, the demand for synthetic bile acids such as obeticholic acid is also likely to increase and it is doubtful whether the supply of naturally derived bile acids will continue to be sufficient to meet demand.

30 Furthermore, the use of a starting material derived from animals means that there is the possibility of the contamination of the material which infectious agents such as viruses, which can not only be hazardous to workers but could potentially contaminate the end products if steps are not taken to prevent this.

35 Although some patients with cholestatic liver disease can be treated with ursodeoxycholic acid, this is also a natural bile acid and faces the same problems of

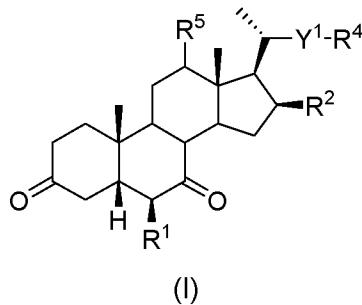
limited availability and high cost.

In an attempt to solve the problems associated with the use of bile acids as starting materials, the present inventors have devised a process for the synthesis of synthetic bile acid derivatives such as obeticholic acid which uses plant sterols as starting materials.

Plant sterols are widely available at significantly lower cost than bile acids and, indeed, are often waste products of other processes. The inventors have developed a process for the preparation of synthetic bile acids starting from bis-norcholenol (also known as 20-hydroxymethylpregn-4-en-3-one), which proceeds via novel intermediates.

Therefore, in the present invention there is provided a compound of general formula

15 (I):



wherein:

R¹ is C₁₋₄ alkyl optionally substituted with one or more substituents selected from

20 halo, OR⁶ or NR⁶R⁷;

where each of R⁶ and R⁷ is independently selected from H or C₁₋₄ alkyl;

R² is H, halo or OH or a protected OH;

Y¹ is a bond or an alkylene linker group having from 1 to 20 carbon atoms and optionally substituted with one or more groups R³;

25 each R³ is independently halo, OR⁸ or NR⁸R⁹;

where each of R⁸ and R⁹ is independently selected from H or C₁₋₄ alkyl;

and

R⁴ is C(O)OR¹⁰, OC(O)R¹⁰, C(O)NR¹⁰R¹¹, OR¹⁰, OSi(R¹³)₃, S(O)R¹⁰, SO₂R¹⁰, OSO₂R¹⁰, SO₃R¹⁰, or OSO₃R¹⁰;

30 where each R¹⁰ and R¹¹ is independently:

a. hydrogen or

b. C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, C₂₋₂₀ alkynyl, -O-C₁₋₂₀ alkyl, -O-C₂₋₂₀ alkenyl or

-O-C₂₋₂₀ alkynyl, any of which is optionally substituted with one or more substituents selected from halo, NO₂, CN, OR¹⁹, SR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂, or a 6- to 14- membered aryl or 5 to 14-membered heteroaryl group, either of which is optionally substituted with C₁₋₆ alkyl, C₁₋₆ haloalkyl, halo,

5 NO₂, CN, OR¹⁹, SR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂; or

c. a 6- to 14- membered aryl or 5 to 14-membered heteroaryl group either of which is optionally substituted with one or more substituents selected from C₁₋₆ alkyl, C₁₋₆ haloalkyl, halo, NO₂, CN, OR¹⁹, SR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂;

10 d. a polyethylene glycol residue;

each R¹⁹ is independently selected from H, C₁₋₆ alkyl, C₁₋₆ haloalkyl, or a 6- to 14- membered aryl or 5 to 14-membered heteroaryl group either of which is optionally substituted with halo, C₁₋₆ alkyl or C₁₋₆ haloalkyl;

15

each R¹³ is independently

a. C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl or C₂₋₂₀ alkynyl optionally substituted with one or more substituents selected from halo, NO₂, CN, OR¹⁹, SR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂, a 6- to 14- membered aryl or 5 to 14-membered heteroaryl group, either of which is optionally substituted with C₁₋₆ alkyl, C₁₋₆ haloalkyl, halo, NO₂, CN, OR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂; or

b. a 6- to 14- membered aryl or 5 to 14-membered heteroaryl group optionally substituted with one or more substituents selected from C₁₋₆ alkyl, C₁₋₆ haloalkyl, halo, NO₂, CN, OR¹⁹, SR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂;

25 each R¹⁹ is independently selected from H, C₁₋₆ alkyl or C₁₋₆ haloalkyl;

R⁵ is H or OH or a protected OH;

or a salt or an isotopic variant thereof.

Compounds of general formula (I) are intermediates in the synthesis of

30 pharmaceutically active compounds such as obeticholic acid and its derivatives.

In the present specification, except where the context requires otherwise due to express language or necessary implication, the word "comprises", or variations such as "comprises" or "comprising" is used in an inclusive sense i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

5

In the present application the term “C₁₋₂₀” alkyl refers to a straight or branched fully saturated hydrocarbon group having from 1 to 20 carbon atoms. The term encompasses methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl and t-butyl. Other alkyl groups, for example C₁₋₂₀ alkyl, C₁₋₆ alkyl or C₁₋₃ alkyl are as defined above but contain different numbers of carbon atoms.

The term “C₁₋₆ haloalkyl” refers to a straight or branched alkyl group as defined above having from 1 to 6 carbon atoms and substituted with one or more halo atoms, up to perhalo substitution. Examples include trifluoromethyl, chloroethyl and 1,1-difluoroethyl.

The term “C₂₋₂₀ alkenyl” refers to a straight or branched hydrocarbon group having from 2 to 20 carbon atoms and at least one carbon-carbon double bond. Examples include ethenyl, prop-1-enyl, hex-2-enyl etc.

20

The term “C₂₋₂₀ alkynyl” refers to a straight or branched hydrocarbon group having from 2 to 20 carbon atoms and at least one carbon-carbon triple bond. Examples include ethynyl, prop-1-ylyn, hex-2-yynyl etc.

25

The term “alkylene” refers to a straight or branched fully saturated hydrocarbon chain. Examples of alkylene groups include -CH₂-, -CH₂CH₂-, CH(CH₃)-CH₂-, CH₂CH(CH₃)-, -CH₂CH₂CH₂-, -CH₂CH(CH₂CH₃)- and -CH₂CH(CH₂CH₃)CH₂-.

30

The term “alkenylene” refers to a straight or branched hydrocarbon chain containing at least one carbon-carbon double bond. Examples of alkenylene groups include -CH=CH-, -CH=C(CH₃)-, -CH₂CH=CH-, -CH=CHCH₂-, CH₂CH₂CH=CH-, CH₂CH=CH(CH₃)- and -CH₂CH=C(CH₂CH₃)-.

35

The term “alkynylene” refers to a straight or branched hydrocarbon chain containing at least one carbon-carbon triple bond. Examples of alkenylene groups include -C≡C-, -CH₂C≡C-, -C≡C-CH₂-, CH₂CH₂C≡C-, CH₂C≡CCH₂- and -CH₂CH≡C-CH₂CH₂-.

The terms “aryl” and “aromatic” refer to a cyclic group with aromatic character having from 6 to 14 ring carbon atoms (unless otherwise specified) and containing up to three rings. Where an aryl group contains more than one ring, not all rings must be aromatic in character. Examples include phenyl, naphthyl and anthracenyl as well as 5 partially saturated systems such as tetrahydronaphthyl, indanyl and indenyl.

The terms “heteroaryl” and “heteroaromatic” refer to a cyclic group with aromatic character having from 5 to 14 ring atoms (unless otherwise specified), at least one of which is a heteroatom selected from N, O and S, and containing up to three rings., 10 Where a heteroaryl group contains more than one ring, not all rings must be aromatic in character. Examples of heteroaryl groups include pyridine, pyrimidine, indole, benzofuran, benzimidazole and indolene.

The term “halogen” refers to fluorine, chlorine, bromine or iodine and the term “halo” 15 to fluoro, chloro, bromo or iodo groups.

The term “isotopic variant” refers to isotopically-labelled compounds which are identical to those recited in formula (I) but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the 20 atomic mass or mass number most commonly found in nature, or in which the proportion of an atom having an atomic mass or mass number found less commonly in nature has been increased (the latter concept being referred to as “isotopic enrichment”). Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, fluorine, iodine and 25 chlorine such as 2H (deuterium), 3H, 11C, 13C, 14C, 18F, 123I or 125I (e.g. 3H, 11C, 14C, 18F, 123I or 125I), which may be naturally occurring or non-naturally occurring isotopes.

Polyethylene glycol (PEG) is a polyether compound, which in linear form has general 30 formula H-[O CH₂-CH₂]_n-OH. A polyethylene glycol residue is a PEG in which the terminal H is replaced by a bond linking it to the remainder of the molecule.

Branched versions, including hyperbranched and dendritic versions are also contemplated and are generally known in the art. Typically, a branched polymer has 35 a central branch core moiety and a plurality of linear polymer chains linked to the central branch core. PEG is commonly used in branched forms that can be prepared by addition of ethylene oxide to various polyols, such as glycerol, glycerol oligomers,

5 pentaerythritol and sorbitol. The central branch moiety can also be derived from several amino acids, such as lysine. The branched poly (ethylene glycol) can be represented in general form as R(-PEG-OH)_m in which R is derived from a core moiety, such as glycerol, glycerol oligomers, or pentaerythritol, and m represents the number of arms. Multi-armed PEG molecules, such as those described in US 5,932,462; US 5,643,575; US 5,229,490; US 4,289,872; US 2003/0143596; WO 96/21469; and WO 93/21259 may also be used.

10 The PEG polymers may have an average molecular weight of, for example, 600-2,000,000 Da, 60,000-2,000,000 Da, 40,000-2,000,000 Da, 400,000-1,600,000 Da, 800-1,200,000 Da, 600-40,000 Da, 600-20,000 Da, 4,000-16,000 Da, or 8,000-12,000 Da.

15 The term "protected OH" relates to an OH group protected with any suitable protecting group. For example, the protected OH may be a group R⁴ as defined above.

20 Suitable protecting groups include esters such that, for example when R² and/or R⁵ is a protected OH, R² and/or R⁵ may independently be a group OC(O)R¹⁴, where R¹⁴ is a group R¹⁰ as defined above. Silyl ethers are also suitable, and in this case, R² and/or R⁵ may independently be a group OSi(R¹⁶)₃, where each R¹⁶ is independently a group R¹³ as defined above.

25 Other suitable protecting groups for OH are well known to those of skill in the art (see Wuts, PGM and Greene, TW (2006) "Greene's Protective Groups in Organic Synthesis", 4th Edition, John Wiley & Sons, Inc., Hoboken, NJ, USA).

30 References to a protecting group which is stable in basic conditions mean that the protecting group cannot be removed by treatment with a base.

35 Appropriate salts of the compounds of general formula (I) include basic addition salts such as sodium, potassium, calcium, aluminium, zinc, magnesium and other metal salts as well as choline, diethanolamine, ethanolamine, ethyl diamine, meglumine and other well-known basic addition salts as summarised in Paulekuhn *et al.*, *J. Med. Chem.* 2007, 50, 6665-6672 and/or known to those skilled in the art.

In some suitable compounds of general formula (I):

R^1 is C_{1-4} alkyl optionally substituted with one or more substituents selected from halo, OR^6 or NR^6R^7 ;

where each of R^6 and R^7 is independently selected from H or C_{1-4} alkyl;

R^2 is H, halo or OH;

5 Y^1 is a bond or an alkylene linker group having from 1 to 6 carbon atoms and optionally substituted with one or more group R^3 ;

each R^3 is independently halo, OR^8 or NR^8R^9 ;

where each of R^8 and R^9 is independently selected from H or C_{1-4} alkyl;

and

10 R^4 is $C(O)OR^{10}$, $C(O)NR^{10}R^{11}$, $S(O)R^{10}$, SO_2R^{10} , OSO_2R^{10} , SO_3R^{10} , or OSO_3R^{10} ;

where each R^{10} is hydrogen or C_{1-6} alkyl or benzyl, either of which may optionally be substituted with one or more halo substituents and R^{11} is hydrogen or C_{1-6} alkyl, benzyl, $-C_{1-4}$ alkylene- SO_3H or $-C_{1-4}$ alkylene- $SO_3(C_{1-4}$ alkyl), any of which may optionally be substituted with one or more halo

15 substituents;

R^5 is H or OH;

or a salt thereof.

In suitable compounds of general formula (I), R^1 may be C_{1-4} alkyl optionally

20 substituted with one or more substituents selected from halo, OR^6 or NR^6R^7 , where R^6 and R^7 are each independently H, methyl or ethyl, especially H or methyl.

More suitably, R^1 is unsubstituted C_{1-4} alkyl.

25 In particularly suitable compounds, R^1 is ethyl.

In some compounds of general formula (I), Y^1 is a bond.

30 Suitably in compounds of general formula (I), Y^1 is an alkylene linker group having from 1 to 15 carbon atoms, more suitably 1 to 12, 1 to 10 or 1 to 8 carbon atoms and optionally substituted with one or more groups R^3 as defined above. Typically each R^3 is independently halo, OR^8 or NR^8R^9 ; where each of R^8 and R^9 is independently selected from H, methyl or ethyl, especially H or methyl.

35 In some suitable compounds, Y^1 is an unsubstituted alkylene linker having from 1 to 15 carbon atoms, more suitably 1 to 12, 1 to 10 or 1 to 8 carbon atoms.

In some suitable compounds of general formula (I), R² is H.

In other suitable compounds of general formula (I), R² is OH.

5 In still other suitable compounds of general formula (I), R² is a protected OH group. When R² is a protected OH group, it may be a group which is not stable in a basic environment such that treatment with a base converts the protected OH group to OH. Examples of such groups are well known in the art and include a group OC(O)R¹⁴ as defined above in which R¹⁴ is a group R¹⁰ as defined above for general formula (I).

10

Particularly suitable R¹⁴ groups are as defined for R¹⁰ below.

Alternatively, R² may be a protected OH group which is stable in a basic environment. Examples of such groups include OSi(R¹⁶)₃, where each R¹⁶ is 15 independently a group R¹³ as defined above.

Particularly suitable R¹⁶ groups are as defined for R¹³ below.

In the compounds of general formula (I), R⁴ is C(O)OR¹⁰, OC(O)R¹⁰, C(O)NR¹⁰R¹¹, 20 OR¹⁰, OSi(R¹³)₃, S(O)R¹⁰, SO₂R¹⁰, OSO₂R¹⁰, SO₃R¹⁰, or OSO₃R¹⁰.

Suitably, is C(O)OR¹⁰, OR¹⁰, SO₃R¹⁰, or OSO₃R¹⁰

More suitably, R⁴ is C(O)OR¹⁰, SO₃R¹⁰, or OSO₃R¹⁰

25

Suitably, each R¹⁰ and R¹¹ is independently:

- a. hydrogen or
- b. C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, -O-C₁₋₁₀ alkyl, -O-C₂₋₁₀ alkenyl or -O-C₂₋₁₀ alkynyl, any of which is optionally substituted with one or more substituents as described above; or
- c. a 6- to 10- membered aryl or 5 to 10-membered heteroaryl group optionally substituted with one or more substituents as described above.
- d. a polyethylene glycol residue.

35 More suitably, each R¹⁰ and R¹¹ is independently

- a. hydrogen or

- b. C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl or $-O-C_{1-10}$ alkyl optionally substituted with one or more substituents as described above or
- c. a 6- to 10-membered aryl group optionally substituted with one or more substituents as described above.

5

Suitably each R^{13} is independently selected from:

- a. C_{1-10} alkyl, C_{2-10} alkenyl or C_{2-10} alkynyl optionally substituted with one or more substituents as described above; or

- b. a 6- to 10- membered aryl or 5 to 10-membered heteroaryl group optionally

10 substituted with one or more substituents as described above.

More suitably, each R^{13} is independently selected from:

- a. C_{1-10} alkyl, C_{2-10} alkenyl or C_{2-10} alkynyl optionally substituted with one or more substituents as described above; or

15 b. a 6- to 10- membered aryl group optionally substituted with one or more substituents as described above.

Still more suitably, each R^{13} is independently selected from C_{1-10} alkyl or phenyl, either of which is optionally substituted as described above.

20

Suitable substituents for alkyl, alkenyl, alkynyl, alkoxy, alkenyloxy and alkynyloxy R^{10} and R^{11} groups and alkyl, alkenyl and alkynyl R^{13} groups include halo, NO_2 , CN , OR^{19} , SR^{19} , SO_2R^{19} , SO_3R^{19} or $N(R^{19})_2$, or a 6- to 10- membered aryl or 5 to 14-membered heteroaryl group, either of which is optionally substituted with C_{1-6} alkyl, C_{1-6} haloalkyl, halo, NO_2 , CN , OR^{19} , SO_2R^{19} , SO_3R^{19} or $N(R^{19})_2$; where R^{19} is as defined above.

More suitable substituents for these R^{10} , R^{11} and R^{13} groups include halo, OR^{19} , $N(R^{19})_2$ or a 6- to 10-membered aryl group optionally substituted as described above, 30 more suitably optionally substituted with halo, C_{1-4} alkyl, C_{1-4} haloalkyl, $-O-C_{1-4}$ alkyl, $-O-C_{1-4}$ haloalkyl, $-NH(C_{1-4}$ alkyl) or $-N(C_{1-4}$ alkyl) $_2$; for example fluoro, chloro, methyl, ethyl, trifluoromethyl, methoxy, ethoxy, trifluoromethoxy, amino, methyl amino and dimethylamino.

35 Suitable substituents for aryl and heteroaryl R^{10} , R^{11} and R^{13} groups include C_{1-6} alkyl, C_{1-6} haloalkyl, halo, NO_2 , CN , OR^{19} , SR^{19} or $N(R^{19})_2$.

More suitable substituents for these R¹⁰, R¹¹ and R¹³ groups include C₁₋₄ alkyl, C₁₋₄ haloalkyl, halo, OR¹⁹ or N(R¹⁹)₂; in particular, halo, C₁₋₄ alkyl, C₁₋₄ haloalkyl, -O-C₁₋₄ alkyl, -O-C₁₋₄ haloalkyl, -NH(C₁₋₄ alkyl) or -N(C₁₋₄ alkyl)₂.

5 Specific examples of substituents for aryl and heteroaryl R¹⁰, R¹¹ and R¹³ groups include fluoro, chloro, methyl, ethyl, trifluoromethyl, methoxy, ethoxy, trifluoromethoxy, amino, methyl amino and dimethylamino.

As set out above, each R¹⁹ is independently selected from H, C₁₋₆ alkyl, C₁₋₆ haloalkyl, or a 6- to 14- membered aryl or 5 to 14-membered heteroaryl group either of which is optionally substituted with one or more halo, C₁₋₆ alkyl or C₁₋₆ haloalkyl substituents.

10 Suitably, R¹⁹ is H, C₁₋₆ alkyl, C₁₋₆ haloalkyl, or a 6- to 10- membered aryl or 5 to 10- membered heteroaryl group optionally substituted with one or more halo, C₁₋₄ alkyl or C₁₋₄ haloalkyl substituents.

15 More suitably, R¹⁹ is H, C₁₋₆ alkyl, C₁₋₆ haloalkyl or phenyl optionally substituted with one or more halo, C₁₋₄ alkyl or C₁₋₄ haloalkyl substituents.

20 20 Specific examples of R¹⁹ include H, methyl, ethyl, trifluoromethyl or phenyl optionally substituted with one or more fluoro, chloro, methyl, ethyl or trifluoromethyl groups.

In some suitable compounds of general formula (I), R⁵ is H.

25 25 In other suitable compounds of general formula (I), R⁵ is OH.

In still other suitable compounds of general formula (I), R⁵ is a protected OH group.

30 30 In still other suitable compounds of general formula (I), R⁵ is a protected OH group. When R⁵ is a protected OH group, it may be a group which is not stable in a basic environment such that treatment with a base converts the protected OH group to OH. Examples of such groups are well known in the art and include a group OC(O)R¹⁴ as defined above in which R¹⁴ is a group R¹⁰ as defined above for general formula (I).

35 35 Particularly suitable R¹⁴ groups are as defined for R¹⁰ above.

Alternatively, R^5 may be a protected OH group which is stable in a basic environment. Examples of such groups include $OSi(R^{16})_3$, where each R^{16} is independently a group R^{13} as defined above.

In some suitable compounds of general formula (I), independently or in any

5 combination:

Y^1 is a bond or an alkylene group having 1 to 3 carbon atoms and is optionally substituted with one or two R^3 groups;

R^4 is $C(O)OR^{10}$, SO_3R^{10} , or OSO_3R^{10} , where R^{10} is as defined above but is more suitably H, C_{1-6} alkyl or benzyl;

10 R^5 is H or OH.

In some more suitable compounds, independently or in any combination:

R^1 is ethyl; and/or

R^2 is H; and/or

15 Y^1 is a bond, $-CH_2-$ or $-CH_2CH_2-$; and/or

R^4 is $C(O)OR^{10}$, where R^{10} is H, C_{1-6} alkyl or benzyl; and/or

R^5 is H.

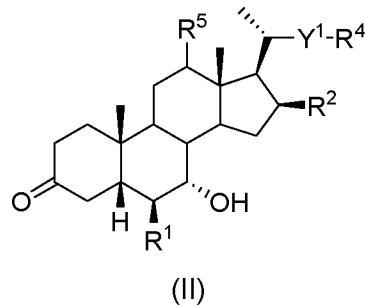
A particularly suitable compounds of the present invention is

20 (6 β , 5 β , 7 α)-6-ethyl-7-hydroxy-3,7-dioxo-cholan-24-oic acid

and C_{1-6} alkyl and benzyl esters thereof and salts thereof, especially the methyl and ethyl esters.

Compounds of general formula (I) may be prepared by oxidising a compound of

25 general formula (II):



Wherein Y^1 , R^1 , R^2 , R^4 and R^5 are as defined for general formula (I).

30 The oxidation reaction may be carried out using any suitable method. One suitable method is a Dess-Martin periodinane (1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol) oxidation, which may be carried out in a chlorinated solvent such as chloroform or

Alternatively, R^5 may be a protected OH group which is stable in a basic environment. Examples of such groups include $OSi(R^{16})_3$, where each R^{16} is independently a group R^{13} as defined above.

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10 R^5 is H or OH.

In some more suitable compounds, independently or in any combination:

R^1 is ethyl; and/or

R^2 is H; and/or

15 Y^1 is a bond, - CH_2 - or - CH_2CH_2 -; and/or

R^4 is $C(O)OR^{10}$, where R^{10} is H, C₁₋₆ alkyl or benzyl; and/or

R^5 is H.

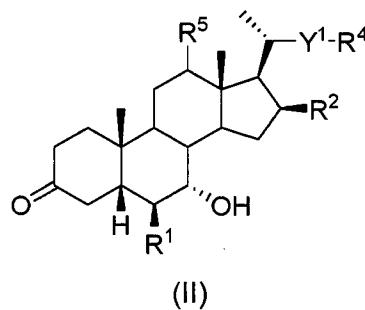
A particularly suitable compounds of the present invention is

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Compounds of general formula (I) may be prepared by oxidising a compound of

25 general formula (II):



Wherein Y^1 , R^1 , R^2 , R^4 and R^5 are as defined for general formula (I).

30 The oxidation reaction may be carried out using any suitable method. One suitable method is a Dess-Martin periodinane (1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol) oxidation, which may be carried out in a chlorinated solvent such as chloroform or

dichloromethane at a temperature of about 15 to 25 °C, suitably at room temperature.

An alternative oxidation method is oxidation using a hypochlorite, for example sodium hypochlorite, under acidic conditions, for example provided by acetic acid. The 5 reaction may be carried out in an aqueous solvent and at a temperature 0 to 15 °C, more usually at about 0 to 10 °C.

Other oxidation methods include a Jones reaction using sodium dichromate or, more 10 usually, chromic trioxide in dilute sulfuric acid. This process is known to be reliable for the clean conversion of bile acid hydroxyl groups to the corresponding keto derivatives (Bortolini *et al*, *J. Org. Chem.*, 2002, 67, 5802). Alternatively oxidation may be carried out using TEMPO ((2,2,6,6-Tetramethyl-piperidin-1-yl)oxy) or a derivative thereof.

15 The method is particularly suitable for the preparation of compounds of general formula (I) in which R⁴ is C(O)OR¹⁰ from compounds of general formula (II) where R⁴ is also C(O)OR¹⁰, where R¹⁰ is as defined above but is especially H, C₁₋₆ alkyl or benzyl.

20 The process for preparing a compound of formula (I) from a compound of formula (II) is new and itself forms a part of the invention.

Alternatively, compounds of formula (I) can be prepared from other compounds of 25 general formula (I). For example, a compound of general formula (I) in which R⁴ is C(O)OR¹⁰ may be converted to a compound of general formula (I) in which R⁴ is C(O)NR¹⁰R¹¹, S(O)R¹⁰, SO₂R¹⁰, OSO₂R¹⁰, SO₃R¹⁰, or OSO₃R¹⁰.

Compounds of general formula (I) in which R⁴ is SO₃R¹⁰ may be synthesised from 30 compounds of general formula (I) in which R⁴ is C(O)OH by the methods taught in WO2008/002573, WO2010/014836 and WO2014/066819.

Thus a compound of formula (I) in which R⁴ is C(O)OH may be reacted with a C₁₋₆ alkanoyl or benzoyl chloride or with a C₁₋₆ alkanoic anhydride to protect the OH groups. The protected compound may then be reacted with a reducing agent such 35 as a hydride, suitably lithium aluminium hydride or sodium borohydride in order to reduce the carboxylic acid group to OH. The alcohol group may be replaced by a halogen, for example bromine or iodine, using the triphenyl

phosphine/imidazole/halogen method described by Classon *et al*, *J. Org. Chem.*, **1988**, 53, 6126-6130. The halogenated compound may then be reacted with sodium sulphite in an alcoholic solvent to give a compound with a $\text{SO}_3^- \text{Na}^+$ substituent.

A compound of general formula (I) in which R^4 is $\text{OSO}_3\text{R}^{10}$ can be obtained by

5 reacting the alcohol obtained from reducing the protected carboxylic acid as described above with chlorosulfuric acid in the presence of a base such as triethylamine to yield the protected triethylammonium salt. Protecting groups can be removed using base hydrolysis as described above. Reduction of the carboxylic acid followed by reaction of the resultant alcohol with chlorosulfuric acid yields a 10 compound of general formula (I) in which R^4 is $\text{OSO}_2\text{R}^{10}$.

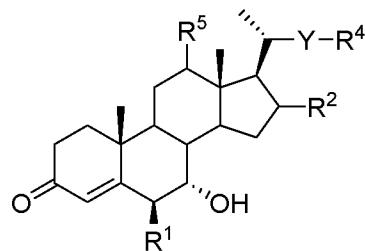
Compounds of general formula (I) in which R^4 is $\text{C}(\text{O})\text{NR}^{10}\text{R}^{11}$ may be prepared from the carboxylic acid by reaction with an amine of formula $\text{H}-\text{NR}^{10}\text{R}^{11}$ in a suitable solvent with heating. Compounds of general formula (I) in which R^4 is $\text{C}(\text{O})\text{NR}^{10}\text{R}^{11}$

15 or $\text{OSO}_3\text{R}^{10}$ may also be prepared by methods similar to those described by Festa *et al*, *J. Med. Chem.*, **2014**, 57, 8477-8495.

Compounds of general formula (I) with other R^4 groups may be prepared from the above compounds of general formula (I) by methods which are familiar to those of

20 skill in the art. These methods also form an aspect of the invention.

Compounds of general formula (II) may be prepared from compounds of general formula (III):



(III)

wherein R^1 , R^2 , R^4 and R^5 are as defined for general formula (I); and

Y is a bond or an alkylene, alkenylene or alkynylene linker group having from 1 to 20 carbon atoms and optionally substituted with one or more groups R^3 , wherein R^3 is as defined for general formula (I);

30 by reduction.

In some compounds of general formula (II), Y is a bond.

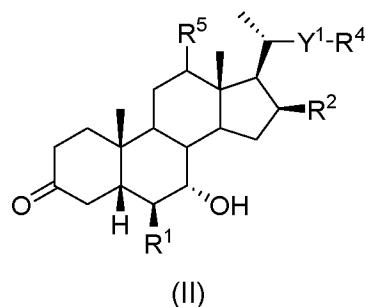
Suitably in compounds of general formula (II), Y is an alkylene or alkenylene linker group having from 1 to 15 carbon atoms, more suitably 1 to 12, 1 to 10 or 1 to 8 carbon atoms and optionally substituted with one or more groups R³ as defined above. Typically each R³ is independently halo, OR⁸ or NR⁸R⁹; where each of R⁸ and R⁹ is independently selected from H, methyl or ethyl, especially H or methyl.

In some suitable compounds of general formula (II), Y is an unsubstituted alkylene or alkenylene linker having from 1 to 15 carbon atoms, more suitably 1 to 12, 1 to 10 or 1 to 8 carbon atoms.

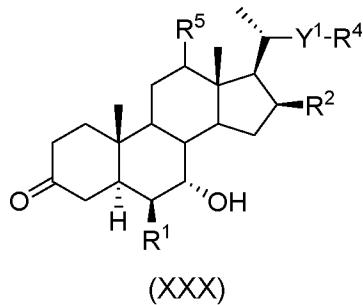
Specific examples of Y groups include a bond, -CH₂-, -CH₂CH₂-, -CH=CH- or -CH=C(CH₃)-.

The reduction may be hydrogenation, usually catalytic hydrogenation. Suitable catalysts for the catalytic hydrogenation include a palladium/carbon, palladium/calcium carbonate, palladium/aluminium oxide, platinum/palladium or Raney nickel catalyst. The reaction may be carried out in an organic solvent, which may be an alcoholic solvent such as methanol, ethanol or isopropanol; ethyl acetate; pyridine; acetic acid; cyclopentyl methyl ether (CPME) or N,N-dimethylformamide (DMF). The organic solvent may optionally be mixed with a co-solvent such as acetone or water and/or a base such as triethylamine may also be added.

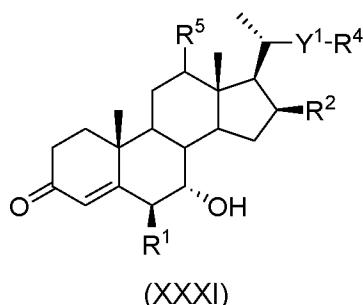
The choice of catalyst and solvent affects the ratio of the required product of general formula (II):



to its isomer of general formula (XXX):



It also affects the rate of conversion of the intermediate of formula (XXXI):



to the product.

More suitably, a palladium/carbon or palladium/calcium carbonate catalyst is used.

10 Typically, in the catalyst the palladium is present in an amount of 5-10% by weight with respect to the weight of the matrix (where the matrix is the carbon, calcium carbonate etc).

15 Solvents which give superior ratios of (II): (XXX) include methanol, ethanol and DMF, particularly methanol and DMF.

When methanol is used as the solvent, it may be used alone or in the presence of a base such as triethylamine. Suitably, the amount of triethylamine used is a substoichiometric amount, typically 0.1 to 0.5 equivalents with respect to the amount 20 of starting material of general formula (III).

Methanol in the presence of triethylamine gave a particularly high ratio of the required product of general formula (II) to isomer of general formula (XXX).

25 Reactions conducted with methanol as the solvent may be carried out at a temperature of about -30 to 25 °C and the temperature has little effect on the ratio of (II): (XXX).

When DMF is used as a solvent, it may be mixed with a co-solvent such as acetone, TBME, THF, acetonitrile or acetone/water. Optionally, the solvent contains a base such as triethylamine in a stoichiometric amount, typically 0.1 to 0.5 equivalents with respect to the amount of starting material of general formula (III).

5

Reactions conducted using DMF as solvent appear to be more sensitive to temperature than reactions carried out in methanol and the ratio of (XIX) : (XXX) decreases with increasing temperature. Suitably, therefore the reaction is conducted at a temperature of -30 to 0 °C, more suitably -20 to -10 °C.

10

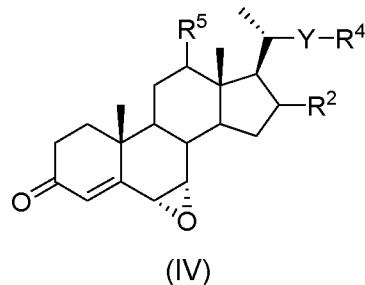
It has been found that the pressure of hydrogen has little effect on the selectivity and therefore the hydrogen pressure is suitably about 1 atmosphere.

Similarly dilution does not appear to have a major impact on the selectivity and

15 therefore the solvent may be used in any convenient amount.

Hydrogenation of a compound of formula (III) will also reduce any alkene bonds, if present, in the linker Y.

20 Compounds of general formula (III) may be prepared from compounds of general formula (IV):



wherein R², R⁴ and R⁵ are as defined in general formula (I) and Y is as defined for

25 general formula (III);

by selective alkylation with an organometallic reagent.

Suitable organometallic reagents include Gilman reagents formed by reaction of an alkyl lithium compound of formula (XXIV):

30



wherein R¹ is as defined for general formula (I);

and a copper (I) salt, particularly a copper (I) halide such as copper (I) iodide.

The reaction may be conducted in an organic solvent such as tetrahydrofuran, other ethers such as diethylether or a mixture thereof.

5 Alternatively, the addition can be carried out using Grignard reagents R^1MgX , where R^1 is as defined for general formula (I) and X is a halide, for example ethylmagnesium bromide and the reaction is suitably conducted in the presence of a zinc (II) salt such as zinc chloride and a catalytic amount of a copper (I) or copper(II) salt or complex, for example copper (I) chloride, copper (II) chloride or a copper(I) or 10 copper (II) acetylacetone (acac) complex.

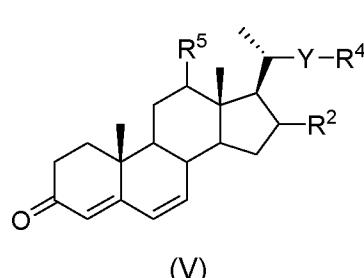
The reaction may be carried out in an organic solvent, for example an ether such as THF, 2-methyl THF, methyl *tert*-butyl ether (tBME), diethyl ether. Surprisingly, the reaction temperature is not particularly significant and while in some cases the

15 reaction may be carried out at reduced temperature, for example at about -25 to 0 °C, it has also been successfully conducted at higher temperatures of up to about 55°C.

The process for preparing a compound of formula (I) from a compound of formula (II) is new and itself forms a part of the invention.

20 The method is particularly suitable for the preparation of compounds of general formula (II) in which R^4 is $C(O)OR^{10}$ from compounds of general formula (III) where R^4 is also $C(O)OR^{10}$, where R^{10} is as defined above but is especially H, C₁₋₆ alkyl or benzyl.

25 Compounds of general formula (IV) may be prepared from compounds of formula (V):

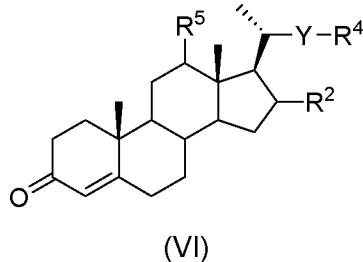


30 wherein R², R⁴ and R⁵ are as defined in general formula (I) and Y is as defined for general formula (III);
by oxidation, for example using monoperoxyphthalate (MMPP) or 3-Chloroperoxybenzoic acid, (mCPBA).

The reaction using MMPP may be carried out in an organic solvent such as ethyl acetate and if *m*CPBA is used, the reaction may be carried out in a solvent such as dichloromethane or toluene. Suitably, the reaction is conducted at or just below the reflux temperature of the solvent.

5

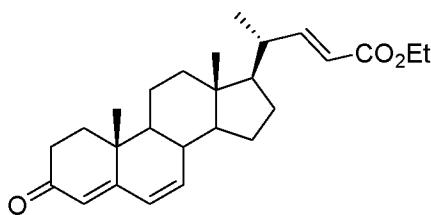
Compounds of general formula (V) may be prepared from compounds of general formula (VI):



10 wherein R², R⁴ and R⁵ are as defined in general formula (I) and Y is as defined for general formula (III);
by reaction with an oxidizing agent such as chloranil.

15 The reaction may be carried out under acidic conditions, for example in the presence of acetic acid, and in an organic solvent such as toluene.

Some compounds of general formulae (IV), (V) and (VI) are known and, for example Uekawa *et al* in *Biosci. Biotechnol. Biochem.*, 2004, 68, 1332-1337 describe the synthesis of (22*E*)-3-oxo-4,22-choladien-24-oic acid ethyl ester from stigmasterol followed by its conversion to (22*E*)-3-oxo-4,6,22-cholatrien-24-oic acid ethyl ester, 20 which has the formula:



25 Uekawa *et al* then go on to describe the conversion of this compound to (6*α*, 7*α*, 22*E*)-6,7-epoxy-3-oxo-4,22-choladien-24-oic acid ethyl ester, a compound of general formula (III) in which R² and R⁵ are H, Y is -CH=CH-, and R⁴ is C(O)OCH₂CH₃.

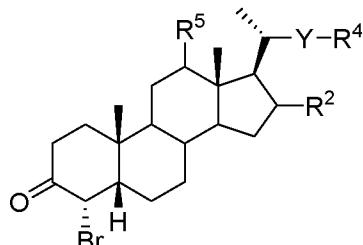
Other compounds of general formulae (IV), (V) and (VI) may be prepared by

analogous methods from phytosterols similar to stigmasterol.

Stigmasterol and other phytosterols are plant sterols and are readily available or may be prepared by known routes.

5

Compounds of general formula (VI) may also be prepared from compounds of general formula (VIIa):

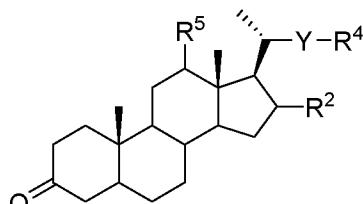


(VIIa)

10 wherein R², R⁴ and R⁵ are as defined in general formula (I) and Y is as defined for general formula (III);
by reaction with lithium bromide and a base such as lithium carbonate. The reaction may be carried out in a solvent such as *N,N*-dimethylformamide (DMF) and at a temperature of about 120 to 180 °C.

15

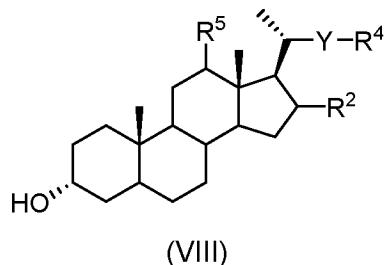
Compounds of general formula (VIIa) may be obtained by bromination of a compound of general formula (VII):



(VII)

20 wherein R², R⁴ and R⁵ are as defined in general formula (I) and Y is as defined for general formula (III);
using, for example bromine in acetic acid.

25 Compounds of general formula (VII) may be prepared from compounds of general formula (VIII):



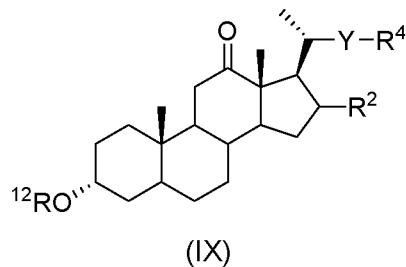
wherein R², R⁴ and R⁵ are as defined in general formula (I) and Y is as defined for general formula (III);

5 by oxidation, typically with a chromium-based oxidizing agent or with sodium hypochlorite.

Compounds of general formula (VIII) in which R⁴ is C(O)OR¹⁰, where R¹⁰ is C₁₋₆ alkyl or benzyl may be prepared from compounds of general formula (VII) in which R⁴ is

10 C(O)OH by esterification, typically by reaction with an appropriate alcohol under acidic conditions.

Compounds of general formula (VIII) in which R⁴ is C(O)OH and R⁵ is H may be prepared from compounds of general formula (IX):



wherein R² and Y are as defined in general formula (I);

R⁴ is C(O)OR¹⁰, where R¹⁰ is C₁₋₆ alkyl or benzyl; and

20 R¹² is a protected OH;
by reaction with a reducing agent, typically hydrazine under basic conditions and in an alcoholic or glycolic solvent, for example diethylene glycol.

Where R¹² is a protected OH group which is stable under basic conditions, the reaction may be followed by a reaction to remove the protecting group R¹² to leave an OH group.

Protecting groups for OH are discussed above and, for example, R¹² may be a group C(O)R¹⁴, where R¹⁴ is as defined above, in particular, C₁₋₆ alkyl or benzyl. Silyl ethers

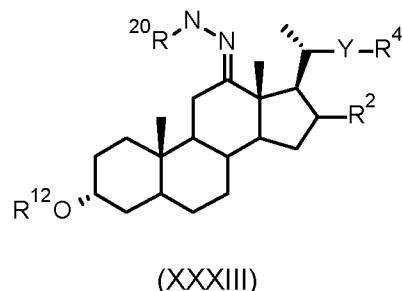
are also suitable, and in this case, R² and/or R⁵ may independently be a group Si(R¹⁶)₃, where R¹⁶ is as defined above but is especially C₁₋₆ alkyl or phenyl. Other suitable protecting groups for OH are well known to those of skill in the art (see Wuts, PGM and Greene, TW (2006) "Greene's Protective Groups in Organic Synthesis", 4th Edition, John Wiley & Sons, Inc., Hoboken, NJ, USA).

Particularly suitable R¹² groups include groups which are not stable in the presence of a base since this removes the need for the additional step of removing the protecting group. An example of a group R¹² which is not stable in basic conditions is 10 a group C(O)R¹⁴, where R¹⁴ is as defined above, and is particularly C₁₋₆ alkyl or benzyl.

Alternatively, the reaction may be carried out in 2 steps such that the compound of general formula (VIII) is reacted with a compound of general formula (XXXII):



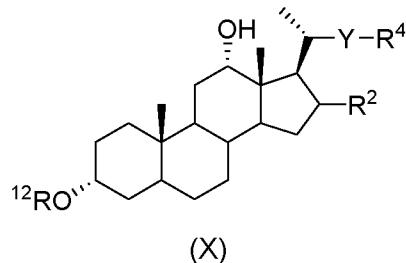
wherein R²⁰ is a leaving group such as toluene sulfonyl or methane sulfonyl; to give a compound of general formula (XXXIII):



followed by reduction with a suitable reducing agent. Examples of reducing agents which can be used in this reaction include hydrides such as sodium borohydride, sodium cyanoborohydride, lithium aluminum hydride etc.

25

Compounds of general formula (IX) may be prepared from compounds of general formula (X):



wherein R² is as defined in general formula (I) and Y is as defined for general formula (III);

R⁴ is C(O)OR¹⁰, where R¹⁰ is C₁₋₆ alkyl or benzyl; and

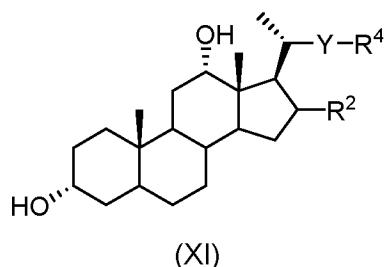
R¹² is as defined above, especially -C(O)C₁₋₆ alkyl;

5 by reaction with an oxidizing agent, for example sodium hypochlorite.

The reaction may be carried out under acidic conditions, for example in the presence of acetic acid, and in an organic solvent such as ethyl acetate.

Compounds of general formula (X) may be prepared from compounds of general

10 formula (XI):



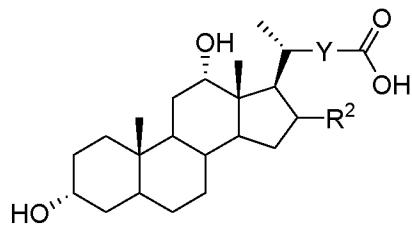
wherein R² is as defined in general formula (I) and Y is as defined for general formula (III);

15 R⁴ is C(O)OR¹⁰, where R¹⁰ is C₁₋₆ alkyl or benzyl;

by reaction with an agent suitable to introduce the protecting group R¹². For example, when R¹² is C(O)R¹⁴, the compound of general formula (XI) may be reacted with a carboxylic acid anhydride or an acid chloride in the presence of a weak base such as pyridine, suitably catalysed by 4-dimethylaminopyridine (DMAP). The reaction may

20 be conducted in a solvent such as ethyl acetate.

Compounds of general formula (XI) may be prepared by the esterification of compounds of general formula (XII):



25

wherein R² is as defined in general formula (I) and Y is as defined for general formula (III);

The reaction may be carried out by reacting the acid of general formula (XII) with a

suitable alcohol under acidic conditions.

Compounds of general formula (XII) are known. For example, the compound of general formula (XII) in which Y is $-\text{CH}_2\text{CH}_2-$ and R^2 is H is deoxycholic acid, which

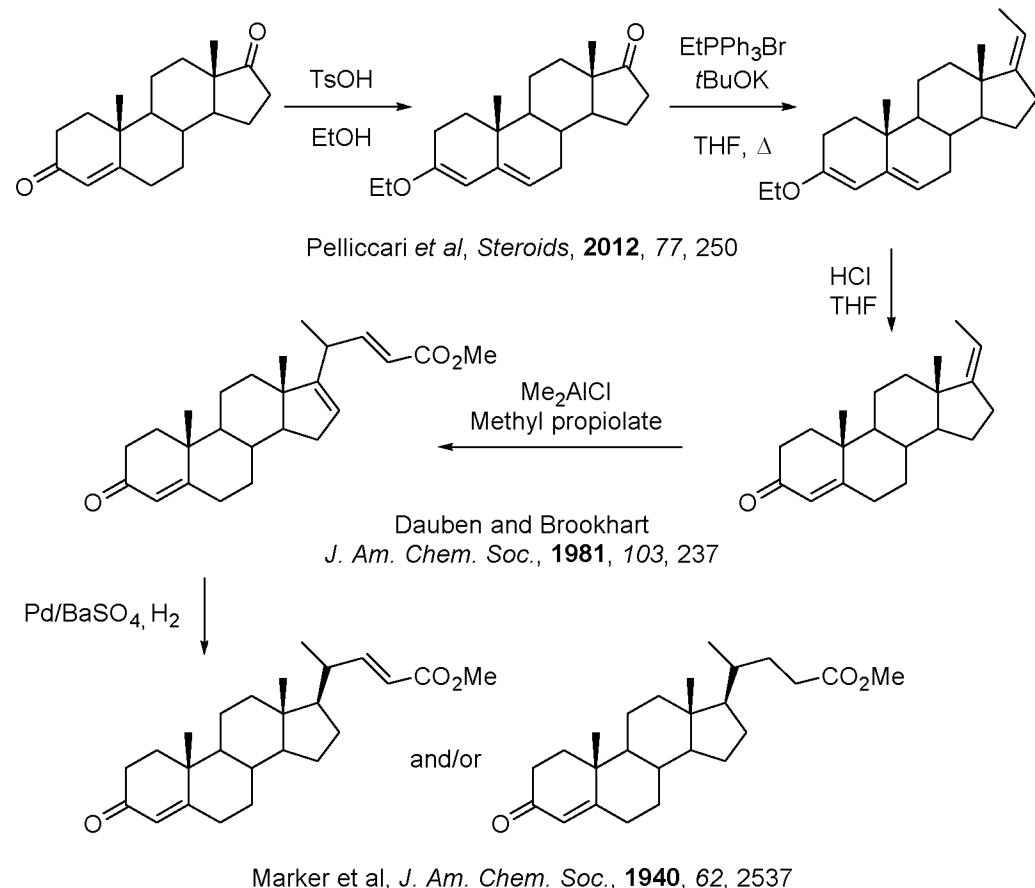
5 is readily available from a number of sources.

Other bile acids with different values for Y and R^2 can be used as alternative starting materials.

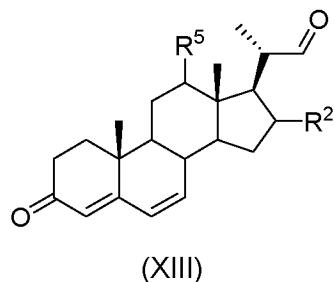
10 An alternative route to compounds of general formula (VI) is as shown in Scheme 1, in which androstenedione is converted to a compound of general formula (V) in which R^2 and R^5 are H; R^4 is $-\text{C}(\text{O})\text{OCH}_3$ and Y is either $-\text{CH}_2\text{CH}_2-$ or $-\text{CH}=\text{CH}-$.

Scheme 1

15

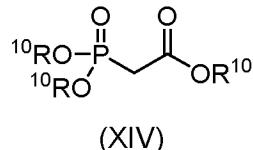


An alternative route to compounds of general formula (V) in which Y is an alkenylene group is by use of an olefination reaction, for example a Horner-Wadsworth-Emmons (HWE) olefination of a compound of general formula (XIII):



wherein R² and R⁵ are as defined for general formula (I);

using a compound of general formula (XIV):



wherein R¹⁰ is as defined for general formula (I).

The reaction may be carried out under standard HWE conditions, for example using a base such as sodium hydride.

10

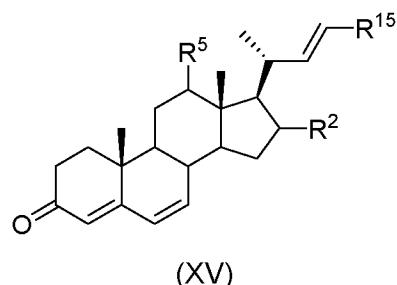
Compounds of general formula (XV) are readily available or may be prepared by methods known to those of skill in the art.

15

Other olefination reactions such as a Tebbe olefination, a Wittig reaction or a Julia-Kocienski olefination would also give rise to compounds of general formula (III) in which Y is an alkenylene group. These olefination reactions are familiar to a chemist of skill in the art.

20

Compounds of general formula (XIII) may be prepared by reaction of a compound of general formula (XV) with ozone



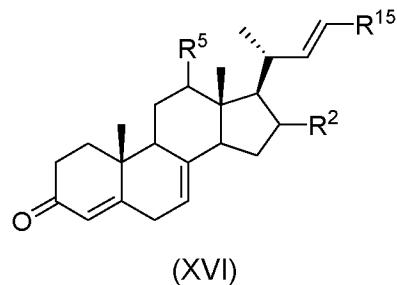
wherein R² and R⁵ are as defined for general formula (I) and R¹⁵ is C₁₋₆ alkyl.

25

An example of a reaction of this type is given in US 2,624,748.

Compounds of general formula (XV) may be prepared by reaction of a compound of

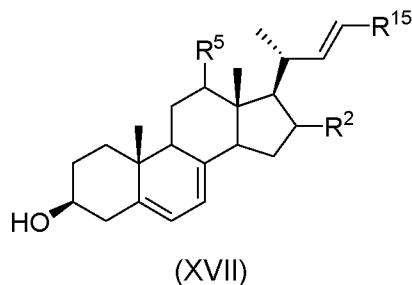
general formula (XVI):



wherein R² and R⁵ are as defined for general formula (I) and R¹⁵ is C₁₋₆ alkyl

5 with an acid in a solvent such as methanol.

Compounds of general formula (XVI) may be prepared by oxidation of a compound of general formula (XVII):



10 wherein R² and R⁵ are as defined for general formula (I) and R¹⁵ is C₁₋₆ alkyl using an Oppenauer oxidation.

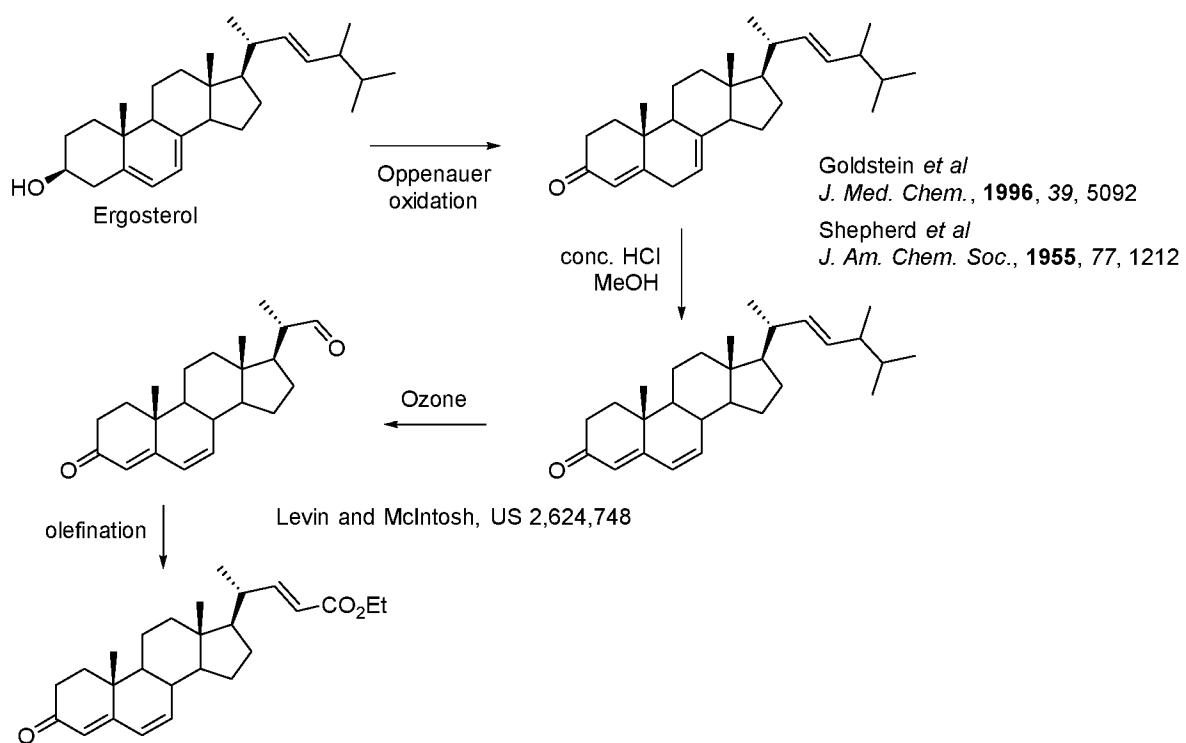
Examples of the conversion of compounds of general formula (XVII) to compounds of

15 general formula (XV) are taught by Shepherd *et al*, *J. Am. Chem. Soc.* **1955**, *77*, 1212-1215 and Goldstein, *J. Med. Chem.* **1996**, *39*, 5092-5099.

One example of a compound of general formula (XVI) is ergosterol, which is a fungal sterol and Scheme 2 below shows the conversion of ergosterol to a compound of

20 general formula (IV) in which both R² and R⁵ are H, Y is CH=CH₂ and R⁴ is C(O)OR¹⁰, where R¹⁰ is ethyl.

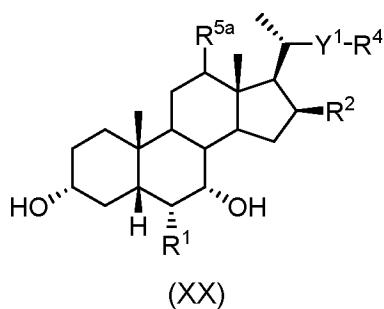
Scheme 2



5 As with the compounds of general formula (I), compounds of general formulae (II) to (XII), (VIIa) and (XXXIII) in which R^4 is $C(O)R^{10}$, $C(O)NR^{10}R^{11}$, $S(O)R^{10}$, SO_3R^{10} , or OSO_3R^{10} may be prepared from the corresponding compounds in which R^4 is $C(O)OR^{10}$ by reaction with an appropriate reagents using methods well known to those of skill in the art. For example, the methods described in WO2008/002573 and

10 WO2010/014836 or methods similar to those described by Classon *et al*, *J. Org. Chem.*, **1988**, *53*, 6126-6130 and Festa *et al*, *J. Med. Chem.*, **2014**, *57*, 8477-8495.

Compounds of general formula (I) are synthetic precursors of compounds of general formula (XX):

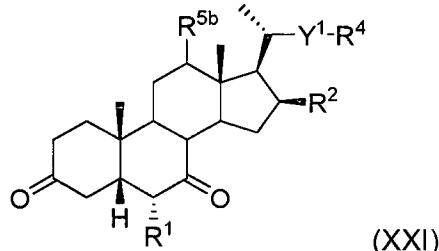


wherein R^1 , R^4 and Y^1 are as defined in general formula (I);
 R^2 is H, halo or OH; and
 R^{5a} is H or OH.

The compounds of general formula (I) may be converted to compounds of general formula (XX) in two steps via an intermediate of general formula (XXI) as described below.

5 Therefore, in a further aspect of the invention there is provided a process for the preparation of a compound of general formula (XX), the process comprising:

i. epimerisation of a compound of general formula (I) to give a compound of general formula (XXI):



10 wherein R¹, R⁴ and Y¹ are as defined in general formula (I); and
 R² is H, halo or OH or a protected OH group which is stable under basic conditions; and
 R^{5b} is H or OH or a protected OH group which is stable under basic conditions; and

15 ii. reduction of the compound of general formula (XXI) using a suitable reducing agent and, where R² and/or R^{5b} is a protected OH, removal of the protecting group(s), to give a compound of general formula (XX) as defined above, wherein removal of the protecting group can take place before or after the reduction; and optionally

20 iii. conversion of a compound of general formula (XX) to another compound of general formula (XX).

Compounds of general formula (XX) are potent agonists of FXR and TGR5 and
 25 include obeticholic acid, which is a compound of formula (XX) in which R¹ is ethyl, R² and R^{5a} are both H, Y¹ is -CH₂CH₂- and R⁴ is C(O)OH.

In the compounds of general formulae (XIX) to (XXI), more suitable values for Y¹, R¹ and R⁴ are as defined for general formula (I).

30 The epimerisation reaction of step (i) suitably comprises treating the compound of general formula (I) with a base. The compound of general formula (I) may be

dissolved in an alcoholic solvent, optionally mixed with water and contacted with a base, for example sodium or potassium hydroxide or a sodium or potassium alkoxide, typically an ethoxide.

5 In the case of compounds of general formula (I) in which R⁴ is C(O)OR¹⁰, where R¹⁰ is C₁₋₆ alkyl or benzyl and where a strong base such as sodium or potassium hydroxide is used, the epimerization reaction of step (i) may be accompanied by hydrolysis to give a compound of general formula (XXI) in which R⁴ is C(O)OH.

10 If, in the compound of general formula (I) R² and/or R⁵ is a protected OH, for example a group OC(O)OR¹⁴, where R¹⁴ is as defined above but is especially C₁₋₆ alkyl or benzyl, this will be removed during the epimerisation step to give a compound of general formula (XXI) in which R² and/or R^{5b} is OH. Other protected OH groups which are stable in basic conditions (for example a group OSi(R¹⁶)₃ where each R¹⁶ is independently as defined above but is especially C₁₋₆ alkyl or phenyl) may be removed before or after step (ii).

In step (ii), the reducing agent is typically a hydride, such as sodium borohydride which may be used in a solvent such as a mixture of tetrahydrofuran and water.

20 Typically, this reaction is carried out under basic conditions, for example in the presence of a strong base such as sodium or potassium hydroxide and at a temperature of about 0 to 110 °C, more usually 60 to 100 °C. A compound of general formula (XX) in which R⁴ is C(O)OH may be produced by the reduction of a compound of general formula (XXI) in which R⁴ is C(O)OH.

25 Compounds of general formulae (XX) and (XXI) in which R⁴ is C(O)R¹⁰, C(O)NR¹⁰R¹¹, S(O)R¹⁰, SO₂R¹⁰, or OSO₂R¹⁰ may be prepared from the corresponding compounds in which R⁴ is C(O)OR¹⁰ by reaction with an appropriate reagents using methods well known to those of skill in the art.

30 Compounds of general formulae (XX) and (XXI) in which R⁴ is SO₃R¹⁰ may be synthesised from compounds of general formulae (XX) and (XXI) in which R⁴ is C(O)OH by the methods taught in WO2008/002573, WO2010/014836 and WO2014/066819.

35 Thus a compound of formula (XX) or (XXI) in which R⁴ is C(O)OH may be reacted with a C₁₋₆ alkanoyl or benzoyl chloride or with a C₁₋₆ alkanoic anhydride to protect

the OH groups. The protected compound may then be reacted with a reducing agent such as a hydride, suitably sodium borohydride in order to reduce the carboxylic acid group to OH. The alcohol group may be replaced by a halogen, for example bromine or iodine, using the triphenyl phosphine/imidazole/halogen method described by 5 Classon *et al*, *J. Org. Chem.*, **1988**, 53, 6126-6130. The halogenated compound may then be reacted with sodium sulphite in an alcoholic solvent to give a compound with a $\text{SO}_3^- \text{Na}^+$ substituent.

Compounds of general formulae (XX) or (XXI) in which R^4 is $\text{OSO}_3\text{R}^{10}$ can be 10 obtained by reacting the alcohol obtained from reducing the protected carboxylic acid with chlorosulfuric acid in the presence of a base such as triethylamine to yield the protected triethylammonium salt. Protecting groups can be removed using base hydrolysis as described above. Reduction of the carboxylic acid followed by reaction of the resultant alcohol with chlorosulfuric acid yields a compound of general 15 formulae (XX) or (XXI) in which R^4 is $\text{OSO}_2\text{R}^{10}$.

Compounds of general formulae (XX) or (XXI) in which R^4 is $\text{C}(\text{O})\text{NR}^{10}\text{R}^{11}$ may be prepared from the carboxylic acid by reaction with an amine of formula $\text{H}-\text{NR}^{10}\text{R}^{11}$ in a suitable solvent with heating. Compounds of general formulae (XIX) to (XXI) in 20 which R^4 is $\text{C}(\text{O})\text{NR}^{10}\text{R}^{11}$ or $\text{OSO}_3\text{R}^{10}$ may also be prepared by methods similar to those described by Festa *et al*, *J. Med. Chem.*, **2014**, 57 (20), 8477-8495. These methods also form an aspect of the invention.

A compound of general formula (XX) or (XXI) in which R^4 is $\text{C}(\text{O})\text{R}^{10}$ can be obtained 25 by reduction of a compound in which R^4 is $\text{C}(\text{O})\text{OR}^{10}$ using one equivalent of diisobutyl aluminium hydride (DIBAL) to obtain an aldehyde in which R^4 is $\text{C}(\text{O})\text{H}$ (see, for example, WO2011/014661).

Alternatively, the aldehyde may be prepared by oxidation of a protected compound in 30 which R^4 is OH prepared as described above. The oxidation may be Swern oxidation carried out using oxalyl chloride and dimethyl sulfoxide followed by trimethylamine (see, for example Xiang-Dong Zhou *et al*, *Tetrahedron*, **2002**, 58, 10293-10299). Alternatively, the oxidation may be carried out using an oxidising agent such as 35 pyridinium chlorochromate (PCC) as described by Carnell *et al* (*J. Med. Chem.*, **2007**, 50, 2700-2707).

A compound of general formula (I) in which R^4 is $\text{C}(\text{O})\text{R}^{10}$ where R^{10} is other than

hydrogen can be obtained by known methods, for example by the reaction of the aldehyde in which R⁴ is C(O)H with a suitable Grignard reagent, followed by oxidation. Such methods are well known to those of skill in the art.

5 The invention will now be described in greater detail with reference to the examples.

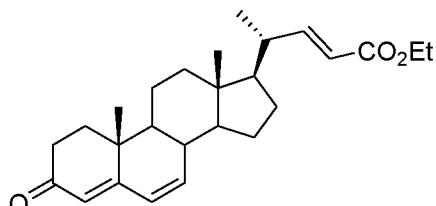
In the examples, the following abbreviations were used:

AcOH	Acetic acid
CPME	Cyclopentyl methyl ether
DMF	N,N-dimethylformamide
EtOAc	Ethyl acetate
EtOH	Ethanol
IPA	Isopropyl alcohol
MeOH	Methanol
NEt ₃	Triethylamine
nBuOAc	n-butyl acetate
TBME	t-butyl methyl ether
THF	Tetrahydrofuran
TLC	Thin layer chromatography

10

Examples 1 to 4 – Synthesis of (6 β , 5 β , 7 α)-6-ethyl-7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester from Stigmasterol.

Example 1 – Synthesis of (22E)-3-oxo-4,6,22-cholatrien-24-oic acid ethyl ester



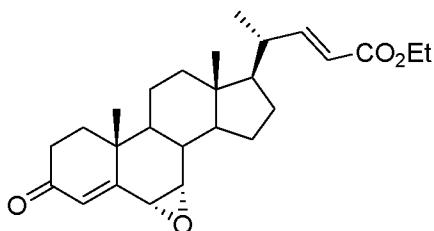
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The starting material, (22E)-3-oxo-4,22-choladien-24-oic acid ethyl ester, was prepared from stigmasterol according to the method described by Uekawa *et al* in *Biosci, Biotechnol, Biochem.*, **2004**, 68, 1332-1337.

20 (22E)-3-oxo-4,22-choladien-24-oic acid ethyl ester (1.00 kg, 2.509 mol; 1 eq) was charged to a reaction vessel, followed by AcOH (3 vol, 3.0 L) and toluene (1 vol, 1.0

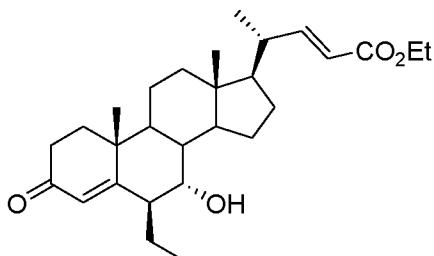
L) with stirring. Chloranil (0.68 kg, 2.766 mol; 1.1 eq) was then charged and the reaction mixture heated to 100 °C and maintained at this temperature for 1-2 h (IPC by TLC on silica, eluent 3:7 EtOAc : Heptane; Starting Material: R_f 0.50, Product: R_f 0.46; visualise with anisaldehyde stain). The mixture was then cooled in an ice/water bath to 10 °C and the resulting solid was filtered off. The filter-cake was washed with premixed 3:1 AcOH : Toluene (4 x 0.5 vol) at 5 °C ± 4 °C and the filtrate concentrated *in vacuo* at up to 70 °C. The residue was dissolved in acetone (3 vol), then 3% w/w aq. NaOH (10 vol) was charged dropwise with stirring, maintaining the temperature below 30 °C (exothermic). The resulting suspension was cooled to 10-15 °C and stirred for 30 mins. The solids were collected by filtration and the filter cake was washed with premixed 1:1 acetone : water (1 x 2 vol then 3 x 1 vol). The filter cake (tan solid) was dried under vacuum at 70-75 °C, 672 g (68% yield). Characterisation of the compound agrees with the data published in the literature.

15 **Example 2 – (6 α , 7 α , 22 E)-6,7-epoxy-3-oxo-4,22-choladien-24-oic acid ethyl ester**



To a solution of (22 E)-3-oxo-4,6,22-cholatrien-24-oic acid ethyl ester (58.0 g, 146.3 mmol) in EtOAc (1.0 L) at reflux was added 80% MMPP (magnesium bis(monoperoxyphthalate) hexahydrate, 197.0 g, ca. 318.6 mmol) in four equal portions at 30 min intervals. The suspension was vigorously stirred at reflux for 5 h and at ambient temperature for a further 16 h. The reaction was then heated to reflux and stirred for an additional 6 h. The mixture was cooled to ca. 50 °C and the solids were filtered and rinsed with hot EtOAc (200 mL). The filtrate was subsequently washed with 20% aq. NaHSO₃ (100 mL), 1M aq. NaOH (100 mL then 200 mL) and 10% aq. NaCl (250 mL), dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue (yellow solid) was crystallised from minimum volume of EtOAc at 60 °C to give the epoxide product as off white/pale yellow crystals (25.7 g, 43% yield, prisms). Characterisation of the compound agrees with the data published in the literature.

Example 3 – Synthesis of (6 β , 7 α , 22E)-6-ethyl-7-hydroxy-3-oxo-4,22-choladien-24-oic acid ethyl ester



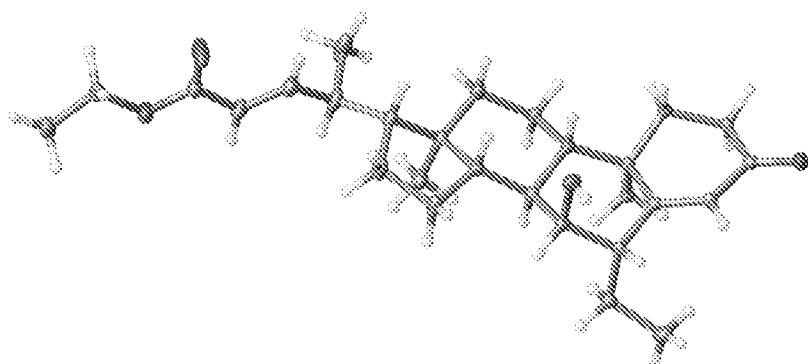
Method 1:

5 To a suspension of CuI (1.40 g, 7.35 mmol) in diethyl ether (10 mL), cooled to -78 °C under an argon blanket was charged EtLi (28.8 mL, 14.4 mmol, 0.5 M solution in benzene / cyclohexane). The thick white suspension formed was allowed to warm to 0 °C, stirred for 5 mins (forming a dark solution) and cooled to -78 °C. A solution of (6 α , 7 α , 22E)-6,7-epoxy-3-oxo-4,22-choladien-24-oic acid ethyl ester (1.00 g, 2.42 mmol) in diethyl ether / THF (24 mL, 3:1) was prepared and charged to the vessel containing the organocuprate. THF (1 mL) was used to rinse the vessel that contained the solution of the epoxide and this was also charged to the organocuprate. The reaction mixture was allowed to warm to -4 °C over 30 mins after which time the reaction was complete by TLC (silica, 1:1 EtOAc : heptane).

10 After a further 30 mins of stirring at c.a. -4 °C a solution of aq. sat. NH₄Cl was charged and the mixture was stirred over 30 mins. The mixture was transferred to a separating funnel and the aqueous phase was removed, along with solid material present at the interface. The organic phase was washed with 5 wt % aq NaHCO₃ (2 x 50 mL,.) and water (1 x 50 mL). TBME (50 mL) was used to extract the original aqueous phase from the reaction and the combined washes. The combined organic phases were concentrated and the residue was purified by chromatography using silica (25 g) as the stationary phase (gradient elution with 0-30 % EtOAc in heptane) to give (6 β , 7 α , 22E)-6-ethyl-7-hydroxy-3-oxo-4,22-choladien-24-oic acid ethyl ester (0.63 g, 59 %).

15

20



¹H NMR (400 MHz, CDCl₃): δ = 6.82 (1H, dd, *J* = 15.6, 8.9, C22H), 5.75 (1H, s, C4H), 5.74 (1H, d, *J* = 15.6, C23H), 4.17 (2H, q, *J* = 7.1, OCH₂CH₃), 3.72 (1H, br s, C7H), 2.52-2.25 (5H, m), 2.05-1.98 (2H, m), 1.82-1.10 (23H, m), 0.91 (3H, t, *J* = 7.4, CH₃), 0.77 (3H, s, CH₃). ¹³C NMR (100 MHz, CDCl₃): δ = 199.2, 171.2, 167.1, 154.5, 5 128.4, 119.0, 71.9, 60.1, 55.3, 54.9, 49.9, 44.3, 42.7, 39.6, 39.1, 38.3, 37.4, 35.6, 34.0, 28.0, 26.3, 23.6, 20.8, 19.7, 19.2, 14.2, 12.8, 12.0; (IR) ν_{max}(cm⁻¹): 3467, 2939, 2870, 1716, 1651, 1457, 1268, 1229, 1034; HRMS (ESI-TOF) *m/z*: (M+H)⁺ calcd for C₂₈H₄₃O₄ 443.3161; found: 443.3156. mp = 59.4 - 62.9 °C

10 Method 2

ZnCl₂ (32.84 g, 240.9 mmol) was dried under vacuum with slow stirring at 180 °C for 2 h. The flask was cooled to room temperature under an argon atmosphere and the residue was dissolved in THF (520 mL) and transferred via cannula into a three neck reaction flask equipped with mechanical stirrer and temperature probe. The solution 15 was cooled in an ice bath to 0-3 °C and a 3M solution of EtMgBr in Et₂O (80 mL, 240.0 mmol) was added dropwise over 20 mins, maintaining the internal temperature below 10 °C. Formation of a white precipitate (active zincate species) was observed after addition of ca. 1/3 of the Grignard solution. The mixture was stirred for 1.2 h at 0 °C before a solution of the epoxide (6 α , 7 α , 22 E)-6,7-epoxy-3-oxo-4,22-choladien-20 24-oic acid ethyl ester (43.0 g, 104.2 mmol) in THF (300 mL) was added dropwise, maintaining the internal temperature below 10 °C. Solid CuCl (1.03 g, 0.104 mmol) was then added in two equal portions with vigorous stirring. After 10 mins the cooling bath was removed and stirring continued at ambient temperature for an additional 1.2 h. The reaction was quenched by dropwise addition of sat. aq. NH₄Cl (800 mL) at < 25 15 °C and stirred for 0.5 h. The mixture was filtered and the solid rinsed with TBME (150 mL). The phases were separated and the aqueous phase extracted with TBME 2 \times 250 mL. The combined organic extracts were washed with 10% aq. NaCl (2 \times 200 mL), dried over Na₂SO₄, filtered and concentrated *in vacuo* to give 43.7 g of the crude (6 β , 7 α , 22 E)-6-ethyl-7-hydroxy-3-oxo-4,22-choladien-24-oic acid ethyl ester 30 as a yellow foam.

Method 3

To a solution of ZnCl₂ in THF (0.5 M, 8.7 mL, 4.85 mmol, 0.9 eq) was charged anhydrous THF (8.0 mL) and the contents then cooled to -25 °C. A solution of 35 EtMgBr in TBME (1.0 M, 8.7 mL, 8.70 mmol, 1.8 eq) was added over 30 mins and the mixture stirred for 45 mins at -25 °C. Solid CuCl (24 mg, 0.49 mmol, 0.05 eq)

was added in one portion and a solution of (6 α , 7 α , 22 E)-6,7-epoxy-3-oxo-4,22-choladien-24-oic acid ethyl ester (2.0 g, 4.85 mmol) in THF (8.0 mL) was added dropwise over 30 mins. The remaining solid CuCl (24 mg, 0.49 mmol, 0.05 eq) was added half way through the addition of (6 α , 7 α , 22 E)-6,7-epoxy-3-oxo-4,22-choladien-24-oic acid ethyl ester. The reaction was stirred for 1 h at -25 °C, (TLC 1:1 Heptane:EtOAc, visualised by UV and developed using Ceric Ammonium Molybdate stain) and then additional of EtMgBr in TBME (1.0 M, 2.9 mL, 2.91 mmol, 0.6 eq) was added over 10 mins. The reaction was stirred for 0.5 h at -25 °C and then quenched by the addition of sat. aq. NH₄Cl (5 mL), maintaining the temperature below -5 °C. The inorganic salts were filtered off, rinsed with TBME and the filtrate phases were separated. The aqueous layer extracted with TBME and then the combined organic extracts were washed with sat. aq. NH₄Cl (3 × 5 mL) and 10% brine (3 × 6 mL). The organic phase was concentrated *in vacuo* at 40 °C to give crude (6 β , 7 α , 22 E)-6-ethyl-7-hydroxy-3-oxo-4,22-choladien-24-oic acid ethyl ester as a yellow foam (1.91 g).

Method 4

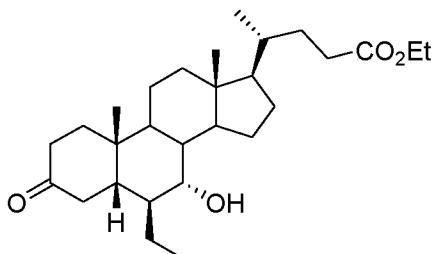
To a solution of ZnCl₂ in THF (0.5 M, 8.7 mL, 4.85 mmol, 0.9 eq) was charged anhydrous THF (8.0mL) and the contents then heated to 40 °C. A solution of EtMgBr in TBME (1.0 M, 8.7 mL, 8.70 mmol, 1.8 eq) was added over 30 mins and the mixture stirred for 45 mins at 40 °C. Solid CuCl (24 mg, 0.49 mmol, 0.05 eq) was added in one portion and a solution of (6 α , 7 α , 22 E)-6,7-epoxy-3-oxo-4,22-choladien-24-oic acid ethyl ester (2.0 g, 4.85 mmol) in THF (8.0 mL) was added dropwise over 30 mins. The remaining solid CuCl (24 mg, 0.49 mmol, 0.05 eq) was added half way through the addition of (6 α , 7 α , 22 E)-6,7-epoxy-3-oxo-4,22-choladien-24-oic acid ethyl ester. The reaction was stirred for 1 h at 40 °C, (TLC 1:1 Heptane:EtOAc, visualised by UV and developed using Ceric Ammonium Molybdate stain) and then quenched by the dropwise addition of sat. aq. NH₄Cl (5 mL). The inorganic salts were filtered off, rinsed with TBME and the filtrate phases were separated. The aqueous layer was extracted with TBME and then the combined organic extracts were washed with sat. aq. NH₄Cl (3 × 5 mL) and 10% brine (3 × 6 mL). The organic phase was concentrated *in vacuo* at 40 °C to give crude (6 β , 7 α , 22 E)-6-ethyl-7-hydroxy-3-oxo-4,22-choladien-24-oic acid ethyl ester as a yellow foam (2.08 g).

Method 5

To a solution of ZnCl₂ in THF (0.5 M, 8.7 mL, 4.85 mmol, 0.9 eq) was charged

anhydrous THF (8.0 mL) and the contents then cooled to -15 °C. A solution of EtMgBr in THF (1.0 M, 8.7 mL, 8.70 mmol, 1.8 eq) was added over 30 mins and the mixture stirred for 45 mins at -15 °C. Solid CuCl (24 mg, 0.49 mmol, 0.05 eq) was added in one portion and a solution of (6 α , 7 α , 22E)-6,7-epoxy-3-oxo-4,22-choladien-24-oic acid ethyl ester (2.0 g, 4.85 mmol) in THF (8.0 mL) was added dropwise over 30 mins. The remaining solid CuCl (24 mg, 0.49 mmol, 0.05 eq) was added half way through the addition of (6 α , 7 α , 22E)-6,7-epoxy-3-oxo-4,22-choladien-24-oic acid ethyl ester. The reaction stirred for 1 h at -15 °C, (TLC 1:1 Heptane:EtOAc, visualised by UV and developed using Ceric Ammonium Molybdate stain) and then additional EtMgBr in THF (1.0 M, 4.35 mL, 4.36 mmol, 0.9 eq) was added over 15 mins and then quenched by the dropwise addition of sat. aq. NH₄Cl (5 mL). The inorganic salts were filtered off, rinsed with TBME and the filtrate phases were separated. The aqueous phase was extracted with TBME and then the combined organic extracts were washed with sat. aq. NH₄Cl (3 \times 5 mL) and 10% brine (3 \times 6 mL). The organic phase was concentrated *in vacuo* at 40 °C to give crude (6 β , 7 α , 22E)-6-ethyl-7-hydroxy-3-oxo-4,22-choladien-24-oic acid ethyl ester as a yellow foam (1.94 g).

Example 4 – Synthesis of (6 β , 5 β , 7 α)-6-ethyl-7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester



20

Method 1

To a suspension of 10 wt. % Pd/C (50% wet, 20 mg, 8.6 mol%) in DMF (2 mL) was added a solution of (6 β , 7 α , 22E)-6-ethyl-7-hydroxy-3-oxo-4,22-choladien-24-oic acid ethyl ester (50 mg, 0.11 mmol) in DMF (3 mL) and the reaction mixture was cooled to 0 °C. The flask was evacuated then filled with hydrogen three times with vigorous stirring. After 3 h the flask was evacuated then filled with argon and the mixture filtered *via* syringe filter. The mixture was partitioned between TBME (30 mL) and H₂O (20 mL). The organic phase was dried (Na₂SO₄) and concentrated *in vacuo*. The crude product (50 mg) was a 14:1 mixture of 5 β to 5 α isomers (analysed by ¹H NMR) of (6 β , 5 β , 7 α)-6-ethyl-7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester, yield 92%.

¹H NMR (700 MHz, CDCl₃): δ = 4.12 (2H, q, *J* = 7.1, OCH₂CH₃), 3.71 (1H, br s, C7H), 3.34 (1H, dd, *J* = 15.5, 13.6, C4H), 2.39-2.32 (2H, m), 2.24-2.20 (1H, m), 2.14-2.09

(2H, m), 2.03-1.91 (4H, m), 1.83-1.79 (2H, m), 1.68-1.63 (2H, m), 1.58 (1H, s), 1.55-1.12 (19H, m), 1.04 (3H, s), 0.95-0.93 (6H, m), 0.88 (1H, J = 7.0), 0.71 (3H, s). ^{13}C NMR (100 MHz, CDCl_3): δ = 213.5, 174.2, 72.1, 60.2, 55.9, 50.2, 49.8, 47.0, 46.7, 42.7, 39.5, 37.7, 36.3, 36.0, 35.7, 35.3, 34.2, 31.3, 31.0, 28.1, 27.7, 24.4, 23.8, 20.8, 18.3, 14.2, 13.9, 11.8. (IR) $\nu_{\text{max}}(\text{cm}^{-1})$: 3514, 2939, 2870, 1710, 1462, 1377, 1159, 1099, 1032; HRMS (ESI-TOF) m/z : (M-H₂O+H)⁺ calcd for $\text{C}_{28}\text{H}_{45}\text{O}_3$ 429.3369; found: 429.3363.

Method 2

10 (6 β , 7 α , 22 E)-6-ethyl-7-hydroxy-3-oxo-4,22-choladien-24-oic acid ethyl ester (20.0 g) was dissolved in DMF (400 mL) and added under argon to solid 10 wt. % Pd/C (50% wet, 10.0 g). The mixture was cooled in an ice-salt bath to approximately -15 °C and the flask was evacuated then filled with hydrogen three times with vigorous stirring. The mixture was stirred under an atmosphere of hydrogen for 6 h then the flask was 15 evacuated, filled with argon and filtered through a pad of celite. The catalyst was rinsed with 400 mL of TBME. The filtrate was washed with 10% aq. NaCl (400 mL) and the aqueous phase extracted with TBME (400 mL). The combined organic phases were washed with 10% aq. NaCl (3 \times 200 mL), dried over Na_2SO_4 , filtered and concentrated *in vacuo* to give crude (6 β , 5 β , 7 α)-6-ethyl-7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester (20.0 g, ca. 28:1 5H β :5H α ratio) as pale yellow oil.

Method 3

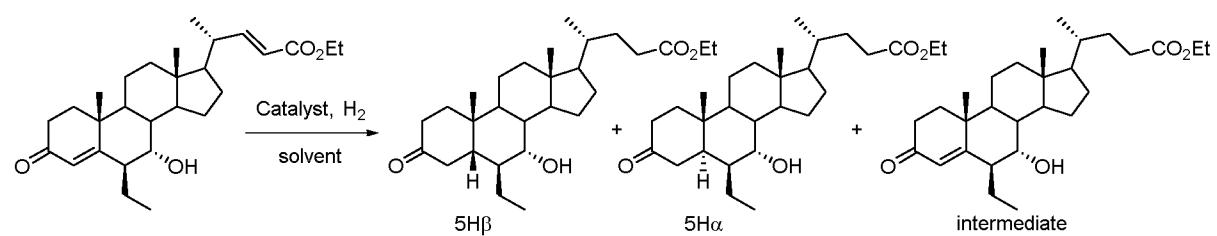
10% Pd/C was charged to a stainless steel jacketed reaction vessel under an argon atmosphere; DMF was added (20 mL), followed by a solution of crude (6 β , 7 α , 22 E)-6-ethyl-7-hydroxy-3-oxo-4,22-choladien-24-oic acid ethyl ester from Example 3 (approximately 72.6 mmol) in DMF (130 mL). The reaction mixture was cooled to -25 °C (over approximately 40 mins) with vigorous stirring (1200 rpm). The reaction vessel was evacuated and charged with hydrogen (10-12 bar) three times. The mixture was stirred for 16 h under an atmosphere of hydrogen (10-12 bar). The 25 vessel was evacuated, purged with argon and warmed to 20 °C with stirring. TLC of the reaction mixture (1:1 Heptane:EtOAc, developed using Ceric Ammonium Molybdate or vanillin dip, R_f values: starting material = 0.42, product = 0.67) indicated complete consumption of the starting material. The suspension was diluted with CH₃CN (120 mL) and H₂O (30 mL) and the suspension filtered *via* a double GFA 30 filter paper and the filter cake rinsed with CH₃CN (60 mL). The mixture was telescoped to the next step without further purification. The mixture contained 35

approximately 5% of the 5H α isomer.

Optimisation

The hydrogenation reaction of this example proceeds *via* the intermediate shown

5 below and produces both the required 5H β compound and its 5H α isomer. A solvent and catalyst screen was carried out to determine reaction conditions which led to the highest yield and the highest ratios of 5H β isomer to 5H α isomer.



The solvent screen was performed using 10 wt. % Pd/C catalyst and the reactions were run at room temperature under atmospheric pressure of hydrogen. The reaction run in MeOH in the presence of NEt₃ was more selective than the one run in neat MeOH, whilst the addition of 10% of H₂O decreased the 5 β H selectivity. The reaction in DMF provided the best β : α ratio. The reaction in pyridine gave poor conversion to the required product with mainly starting material and intermediate present in the mixture.

	Solvent	5H β : α ratio
A	MeOH	4 : 1
B	MeOH:H ₂ O	2 : 1
C	MeOH:NEt ₃	7 : 1
D	EtOH	3 : 1
E	IPA	2 : 1
F	EtOAc	2 : 1
G	Pyridine	2 : 1
H	AcOH	1 : 1
I	CPME	1 : 1
J	DMF	9 : 1

Reactions in DMF and MeOH were tested at a range of temperatures. For reactions run in DMF temperature has substantial impact on selectivity (the selectivity decreases with increasing temperature), while little difference was observed for reactions in MeOH.

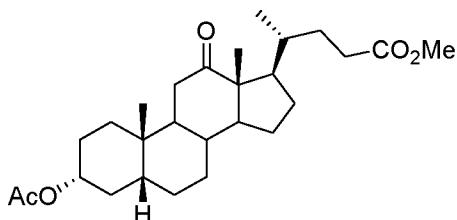
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Reactions in DMF and MeOH were tested at a range of commercially available 5 and 10 wt. % Pd catalysts, on carbon, calcium carbonate, barium sulfate and aluminium oxide support.

10 The reactions were run in 10 volumes of solvent at -15 °C under atmospheric pressure of hydrogen gas. For reactions run in DMF pressure has lower impact on the selectivity than the temperature. The effect of dilution on the selectivity is negligible.

15 **Examples 5 to 14 – Synthesis of (6 β , 5 β , 7 α)-6-ethyl-7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester from Deoxycholic Acid**

Example 5 – Synthesis of (3 α , 5 β)-3-acetoxy-12-oxo-cholan-24-oic acid methyl ester



20

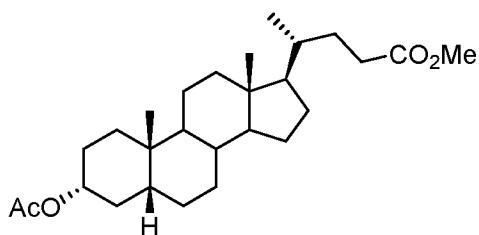
To a solution of deoxycholic acid (500 g, 1.27 mol) in MeOH (1.5 L) was charged H₂SO₄ (0.68 mL, 12.7 mmol) and the reaction heated to 64 °C until complete. The reaction was cooled to 55 °C and pyridine (2.06mL, 25.4 mmol) was charged. MeOH (800 mL) was removed by distillation and the reaction cooled to 50 °C. EtOAc (500 mL) was charged and the distillation continued. This co-evaporation was repeated until the MeOH content was <0.5%. The reaction was cooled to 40 °C and EtOAc (1.0 L) was charged followed by Pyridine (134 mL, 1.65 mol) and DMAP (1.1 g, 8.89 mmol). Acetic anhydride (150 mL, 1.58 mmol) was added dropwise and the reaction vessel stirred at 40 °C until complete. The reaction was cooled to 22 °C and 2M aq.

25 H₂SO₄ (1500 mL) added maintaining the temperature below 25 °C. The aqueous phase was removed and the organic phase washed with water (1.2 L), sat. aq.

30 NaHCO₃ solution (1.2 L x 2) and water (1.2 L). AcOH (1.0 L) was charged to the organic layer, followed by NaBr (6.6 g, 63.5 mmol). Aq. 16.4% NaOCl solution (958

mL, 2.54 mol) was charged dropwise maintaining the reaction temperature below 25 °C. The reaction was stirred until complete, then cooled to 10 °C and stirred for 90 mins. The resulting solids were collected by filtration, washed with water (3 x 500 mL) and the filter cake dried under vacuum at 40 °C. The solids were crystallised from 5 MeOH (10 vol) to give (3 α , 5 β)-3-acetoxy-12-oxo-cholan-24-oic acid methyl ester as an off white solid (268 g).

Example 6 – Synthesis of (3 α , 5 β)-3-acetoxy-cholan-24-oic acid methyl ester

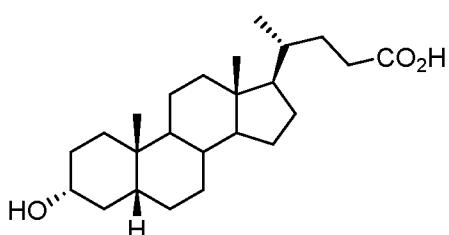


10

(3 α , 5 β)-3-acetoxy-12-oxo-cholan-24-oic acid methyl ester (268 g, 0.6 mol) was charged to the reaction vessel under argon, followed by AcOH (1.8 L). Tosyl hydrazide (190 g, 1.02 mol) was then added maintaining the reaction temperature at 25 °C. The reaction was stirred until complete and then NaBH₄ (113.5 g, 3.00 mol) 15 was charged portion-wise maintaining the temperature below 25 °C. The reaction mixture was stirred until complete and then quenched by the dropwise addition of water (1.34 L) maintaining the temperature below 25 °C. The reaction mixture was stirred for 30 mins, the resulting solids collected by filtration, washed with water (3 x 270 mL) and the solid dried under vacuum at 40 °C. The solids were crystallised from 20 MeOH (3 vol) to give (3 α , 5 β)-3-acetoxy-cholan-24-oic acid methyl ester as an off white solid (214.5g).

Example 7 – Synthesis of (3 α , 5 β)-3-hydroxy-cholan-24-oic acid (Lithocholic Acid)

25

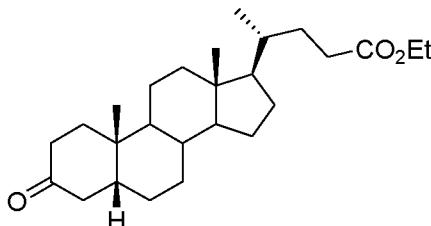


To a solution of (3 α , 5 β)-3-acetoxy-cholan-24-oic acid methyl ester (214.5 g, 0.50 mol) in IPA (536 mL) was charged water (536 mL) and 50% w/w NaOH (99 g, 1.24 mol). The reaction was heated to 50 °C and stirred until complete. 2M H₂SO₄ was

charged slowly with vigorous stirring until pH 2-3 was obtained and then the reaction cooled to 20 °C. The resulting solids were collected by filtration, washed with water (3 x 215 mL) and the resultant solid dried under vacuum at 40 °C to give (3 α , 5 β)-3-hydroxy-cholan-24-oic acid (176.53 g)

5

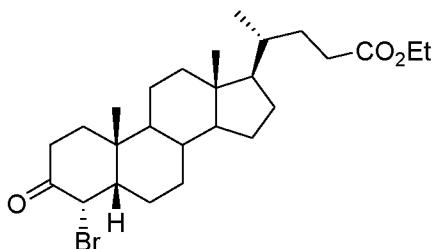
Example 8 – Synthesis of (5 β)-3-oxocholan-24-oic acid ethyl ester



To a solution of (3 α , 5 β)-3-hydroxy-cholan-24-oic acid (10 g, 26.5 mmol) in EtOH (50 mL) was charged H₂SO₄ 96% (14 μ L, 0.27 mmol) and the reaction mixture then

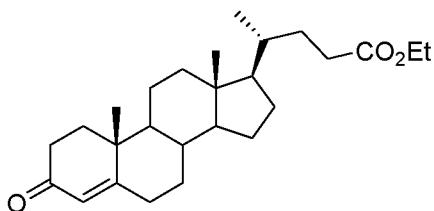
10 heated to reflux for 16 h. Pyridine was then charged, the mixture stirred for 30 mins and concentrated *in vacuo* at 40 °C. The residue was dissolved in EtOAc (30 mL) and AcOH (10 mL) and NaBr (136 mg, 1.33 mmol) was then charged. The solution was cooled to 5 °C and NaOCl 9% (27 mL, 39.8 mmol) was charged dropwise maintaining the temperature below 10 °C. The resulting suspension was warmed to 15 ambient temperature and stirred for 1 h. The reaction mixture was cooled to 0 °C for 10 mins, the solids collected by filtration and washed with water (3 x 3 vol). The resultant solid was dried under vacuum at 40 °C to give (5 β)-3-oxocholan-24-oic acid ethyl ester (7.83 g).

20 **Example 9 – Synthesis of (4 α , 5 β)-3-oxo-4-bromo-cholan-24-oic acid ethyl ester**



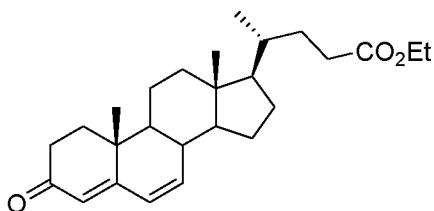
To a solution of (5 β)-3-oxocholan-24-oic acid ethyl ester (8.0 g, 19.9 mmol) in AcOH (84 mL) was added Br₂ in AcOH (16 mL, 21.9 mmol) dropwise over 15 mins. The reaction mixture was stirred for 10 mins, then diluted with EtOAc (250 mL), washed

25 with water (2 x 200 mL) and concentrated *in vacuo* at 40 °C. The crude material was purified by column chromatography (30% Heptane: EtOAc) and concentrated *in vacuo* at 40 °C to give (4 α , 5 β)-3-oxo-4-bromo-cholan-24-oic acid ethyl ester as a pale crystalline solid (7.49g).

Example 10 – Synthesis of (5 β)-3-oxo-4-cholene-24-oic acid ethyl ester

To a solution of (4 α , 5 β)-3-oxo-4-bromo-cholan-24-oic acid ethyl ester (4.0 g, 8.33 mmol) in DMF (40 mL) was charged Li₂CO₃ (4.0 g, 1 mass eq) and LiBr (2.0 g, 0.5 mass eq). The mixture was heated to 150 °C for 2 h then allowed to cool to ambient temperature and poured onto a mixture of water and ice (200 g, 50 volumes) and AcOH (8 mL). The resulting suspension was stirred for 15 mins, the solids collected by filtration and then purified by column chromatography (30% Heptane: EtOAc) to give 3-oxo-4-cholene-24-oic acid ethyl ester as a pale crystalline solid (1.68 g).

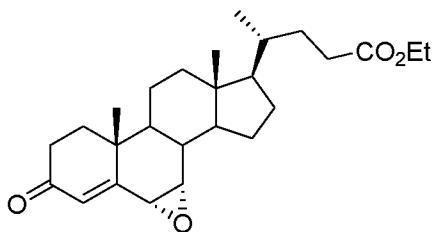
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Example 11 - Synthesis of 3-oxo-4,6-choladien-24-oic acid ethyl ester.

3-oxo-4-cholene-24-oic acid ethyl ester (2.23 g, 5.57 mmol) was charged to a reaction vessel, followed by AcOH (6.7 mL) and toluene (2.23 mL). Chloranil (1.5 g, 6.13 mmol) was charged and the reaction mixture heated to 100 °C for 2 h (IPC by TLC, 3:7 EtOAc: Heptane; visualized with Anisaldehyde stain). The reaction mixture was cooled to 10 °C for 10 mins and the resulting solid removed by filtration. The filter cake was washed with DCM (9 vol) and the resulting filtrate then concentrated *in vacuo* at 40 °C. The residue was dissolved in acetone (9 vol) then 3% w/w aq. NaOH (27 vol) was added dropwise maintaining the temperature below 30 °C. The resulting mixture was cooled in an ice bath for 10 mins and the solids collected by filtration. The filter cake was washed with water (2 x 9 vol) and acetone: water 2:1 (4 vol). Purification by column chromatography (0-30% Heptane: EtOAc) gave 3-oxo-4,6-choladien-24-oic acid ethyl ester as a pale crystalline solid (1.45 g)

25

Example 12 – Synthesis of (6 α , 7 α)-6,7-epoxy-3-oxo-4-chola-ene-24-oic acid ethyl ester

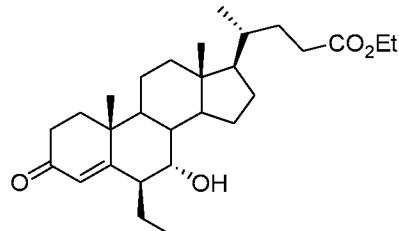


3-oxo-4,6-choladien-24-oic acid ethyl ester (1.37 g, 4.27 mmol) was charged to a

5 reaction vessel, followed by BHT (23 mg, 0.13 mmol), EtOAc (11mL) and water (3.4 mL) with stirring. The solution was heated to 80 °C and then a solution of *m*CPBA 70% (1.5 g, 7.51 mmol) in EtOAc (7.5 mL) was added dropwise over 15 mins. The reaction mixture was stirred at 70 °C for 2 h (IPC by TLC, 3:7 EtOAc: Heptane; visualized with Anisaldehyde stain), cooled to ambient temperature and then washed 10 with 1M aq.NaOH (2 x 20 mL) followed by 10% aq. NaS₂O₃: 2% NaHCO₃ (3 x 20 mL). The organic phases were dried over Na₂SO₄ and concentrated *in vacuo* at 40 °C. The crude solids were crystallized from EtOAc (3 vol) at 60 °C to give an off white solid which was dried under vacuum at 40 °C to give (6 α , 7 α)-6,7-epoxy-3-oxo-4-chola-ene-24-oic acid ethyl ester (0.90 g).

15

Example 13 – Synthesis of (6 β ,7 α)-6-ethyl-7-hydroxy-3-oxo-4-cholen-24-oic acid ethyl ester

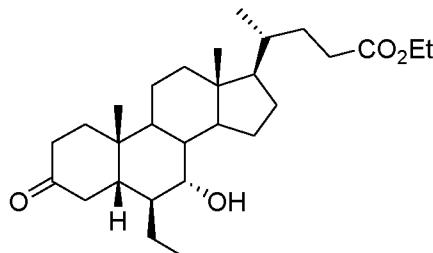


ZnCl₂ (600 mg, 4.25 mmol) was charged to a reaction vessel and dried under

20 vacuum at 180 °C for 1 h. The reaction vessel was cooled to ambient temperature, THF (15 mL) charged and the contents of the reaction vessel cooled to 3 °C. A solution of 3M EtMgBr in Et₂O (1.5 mL, 4.25 mmol) was charged to the reaction vessel over 40 mins maintaining the temperature below 5 °C. The reaction mixture was then stirred for 1 h. (6 α , 7 α)-6,7-epoxy-3-oxo-4-chola-ene-24-oic acid ethyl ester (0.80 g, 1.93 mmol) in THF (6 mL) was charged to the reaction vessel over 40 mins, maintaining the temperature below 5 °C. CuCl (20 mg, 0.19 mmol) was charged in one portion and the reaction stirred at ambient temperature for 16 h (IPC by TLC, 3:7 EtOAc: Heptane; visualized with Anisaldehyde stain). The reaction mixture was cooled in an ice bath and sat. aq.NH₄Cl was added dropwise, maintaining the

temperature below 10 °C. The reaction mixture was filtered and the filter cake washed with TBME (12.5 vol). The organic phase of the filtrate was separated and the aqueous phase extracted with TBME (2 x 12.5 vol). The combined organic phases were washed with 5% NaCl (3 x 12.5 vol) and concentrated *in vacuo* at 40 5 °C.

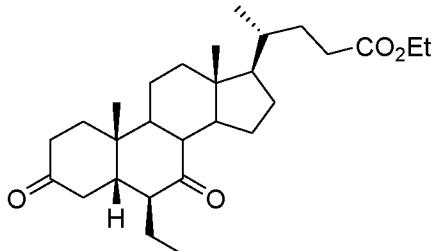
Example 14 – Synthesis of (6β, 5β, 7α)-6-ethyl-7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester



10 10% Pd/C (70 mg) was charged to a reaction vessel under an argon atmosphere followed by the crude material from Example 13 in DMF (14.6 mL). The mixture was cooled to -10 °C and the reaction vessel was evacuated then filled with hydrogen three times with vigorous stirring. The mixture was stirred under an atmosphere of hydrogen for 24 h while maintaining the temperature at -10 °C (IPC by TLC, eluent 1:1 EtOAc: Heptane; visualized with Anisaldehyde stain) then the flask was evacuated, filled with argon and filtered through a pad of celite and rinsed with DMF (7 mL). 10% Pd/C (70 mg) was recharged to the reaction vessel under an argon atmosphere followed by the DMF reaction mixture. The mixture was cooled to approximately -10 °C and the reaction vessel was evacuated then filled with 15 hydrogen three times with vigorous stirring. The mixture was stirred under an atmosphere of hydrogen for 24 h at -10 °C (IPC by TLC, 1:1 EtOAc: Heptane; visualized with Anisaldehyde stain) then the flask was evacuated, filled with argon and filtered through a pad of celite and washed with TBME (62.5 vol, 50 mL). The 20 mixture was washed with 10% aq. NaCl (4 x 25 vol), dried over Na₂SO₄, filtered and concentrated *in vacuo* at 40 °C. Purification by column chromatography (SiO₂, 0-30% 25 Heptane: EtOAc) gave (6β, 5β, 7α)-6-ethyl-7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester (0.17 g). The product was identical to the material obtained from plant origin (6β, 7α, 22E)-6-ethyl-7-hydroxy-3-oxo-4,22-choladien-24-oic acid ethyl ester (see Example 4).

Examples 15 to 17 – Conversion of (6 β , 5 β , 7 α)-6-ethyl-7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester to (3 α , 5 β , 6 α , 7 α)-6-ethyl-3,7-dihydroxy-cholan-24-oic acid

5 **Example 15 – Synthesis of (6 β , 5 β)-3,7-dioxo-6-ethyl-cholan-24-oic acid ethyl ester**



Method 1

10 A solution of Jones's reagent prepared from CrO₃ (1.10 g, 11 mmol) in H₂SO₄ (1.4 mL) and made to 5 mL with water was charged dropwise to a solution of (6 β , 5 β , 7 α)-6-ethyl-7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester (0.18 g, 0.40 mmol) in acetone (10 mL) until an orange colour persisted. The reaction mixture was quenched with IPA (1 mL), filtered through a 0.45 μ m nylon syringe filter and the filter
15 was washed with acetone (10 mL). The combined filtrate and wash was concentrated, the residue was dissolved in EtOAc (20 mL) and washed with water (2 x 10 mL). The aqueous phase was extracted with EtOAc (20 mL), the combined EtOAc phases were concentrated and the residue was dissolved and concentrated from toluene (20 mL) then acetone (20 mL) to give a clear oil containing (6 β , 5 β , 7 α)-
20 6-ethyl-7-hydroxy-3,7-dioxo-cholan-24-oic acid ethyl ester (185 mg).

¹H NMR (700 MHz, CDCl₃): δ = 4.12 (2H, q, *J* = 7.1), 2.42 (1H, t, *J* = 11.4), 2.38-2.17 (6H, m), 2.09-1.74 (9H, m), 1.68-1.11 (17H, m), 0.93 (3H, d, *J* = 6.5), 0.85 (3H, t, *J* = 7.4), 0.72 (3H, s). ¹³C NMR (100 MHz, CDCl₃): δ = 214.5, 211.4, 174.0, 60.1, 57.1, 55.1, 50.3, 48.4, 47.3, 44.9, 43.6, 43.1, 39.2, 35.8, 35.2 (\times 2), 34.9, 31.3, 30.9, 28.1, 24.6, 23.7, 23.4, 21.7, 18.3, 14.2, 12.6, 12.2. (IR) ν_{max} (cm⁻¹): 2950, 2872, 1709, 1461, 1377, 1304, 1250, 1177, 1097, 1034; HRMS (ESI-TOF) *m/z*: (M+H)⁺ calcd for C₂₈H₄₅O₄ 445.3318; found: 445.3312;

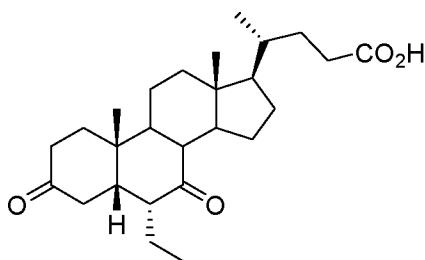
Method 2

30 To a solution of (6 β , 5 β , 7 α)-6-ethyl-7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester (41.0 g crude mass) in anhydrous CH₂Cl₂ (600 mL) at 0 °C was added solid DMP

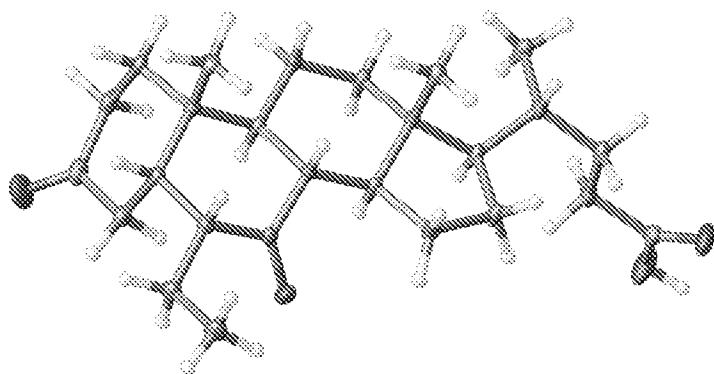
(34.0 g, 80.2 mmol) portion-wise over 20 mins (exothermic). The mixture was stirred at 0-5 °C for 2 h, then a further portion of DMP (4.0 g, 9.4 mmol) was added and reaction stirred at 0-5 °C for 1 h. The mixture was filtered through a GFA filter and the solid rinsed with CH₂Cl₂ (50 mL), the filtrate was stirred vigorously with 10% aq. 5 Na₂S₂O₃ and 2% aq. NaHCO₃ (100 mL) for 20 mins. The phases were separated and the aq. extracted with CH₂Cl₂ (2 × 100 mL). The combined organic extracts were washed with 1M NaOH (100 mL). The mixture was diluted with CH₂Cl₂ (300 mL) and phases separated. The organic layer was concentrated under reduced pressure and the residue (cloudy brown oil) was dissolved in TBME (600 mL) and washed with 1M 10 NaOH (100 mL) and NaCl (3 × 100 mL). The organic phase was concentrated *in vacuo* to give a dark yellow runny oil, crude mass 38.1 g. The oil was dissolved in EtOH (400 mL) and stirred with activated charcoal (10 g) at 50 °C, the mixture was then filtered, the charcoal rinsed with EtOH (200 mL) and the filtrate concentrated *in vacuo* to give (6β, 5β)-3,7-dioxo-6-ethyl-cholan-24-oic acid ethyl ester as a yellow oil 15 (35.9 g).

Method 3

A solution of (6β, 5β, 7α)-6-ethyl-7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester (218 mmol) in DMF (450 ml), CH₃CN (540 mL) and H₂O (90 mL) was charged into a 2 L 20 vessel and cooled to 9 °C, then AcOH (180 mL) was charged, followed by NaBr (4.1 g). A solution of sodium hypochlorite (~10.5% w/v, 450 mL) was added dropwise over 1.5 h, maintaining the internal temperature at 5–6 °C, then the mixture was stirred for 5 h at 7 °C. TLC of the reaction mixture indicated complete consumption of the starting material (IPC by TLC, eluent EtOAc/heptane 3:7, R_f for (6β, 5β, 7α)-6-ethyl- 25 7-hydroxy-3-oxo-cholan-24-oic acid ethyl ester = 0.34; (6β, 5β)-3,7-dioxo-6-ethyl-cholan-24-oic acid ethyl ester = 0.45). A solution of aq. 10% w/v Na₂SO₃ (360 mL) was charged dropwise with vigorous stirring, maintaining the internal temperature at 8–10 °C, then H₂O (270 mL) was added dropwise and the mixture stirred at 5 °C for 16 h. The solid was filtered and washed with H₂O (720 mL). The solid was then 30 dissolved in TBME (1.1 L) and subsequently washed with an aq. NaHCO₃ (300 mL) and 10% brine (300 mL). The organic phase was then stirred with activated charcoal (10 g) for 20 mins at 40 °C, treated with anhydrous MgSO₄ (5 g) and filtered via GFA filter paper, the filter cake was rinsed with TBME (50 mL) and the filtrate concentrated *in vacuo* to give (6β, 5β)-3,7-dioxo-6-ethyl-cholan-24-oic acid ethyl 35 ester as light brown oil which solidifies on standing (82.7 g).

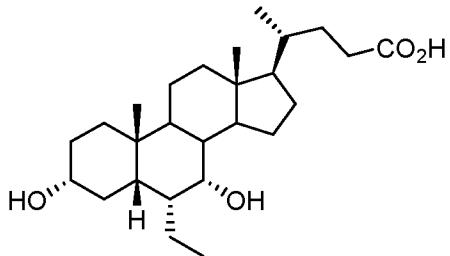
Example 16 – Synthesis of $(6\alpha, 5\beta)$ -3,7-dioxo-6-ethyl-cholan-24-oic acid

Into a 500 mL flask was charged 0.5 vol of 0.5 M NaOH (9 mL) followed by $(6\beta, 5\beta)$ -3,7-dioxo-6-ethyl-cholan-24-oic acid ethyl ester from Example 15 (18.00 g, 1 eq) and then IPA (180 mL, 10 vol) (the initial NaOH charge was to avoid the possibility of C3-ketal formation). The mixture was warmed to 60 ± 2 °C and held until a solution was obtained (10-15 mins). The remaining 0.5 M NaOH solution (171 mL, 9.5 vol) was charged over 20 mins and then the reaction was stirred for a further 3.5 h at 60 ± 2 °C. The IPA was removed under vacuum at 60 °C and then 2M HCl (8 mL) charged to pH 9. EtOAc was charged (90 mL, 5 vol) followed by 2M HCl (54 mL) to pH 1. Vigorous mixing was followed by phase separation. The aqueous phase was back extracted with additional EtOAc (90 mL, 5 vol) and then the combined organic phases were washed with water (54 mL, 3 vol), followed by three portions of 10% aq. NaCl (3 x 54 mL, 3 x 3 vol). The organic phase was treated with activated charcoal (100 mesh powder, 3.37 g, ~0.20 mass eq) for 12 mins and then filtered through GF/B. Concentration at 50 °C *in vacuo* gave $(6\alpha, 5\beta)$ -3,7-dioxo-6-ethyl-cholan-24-oic acid as a light yellow foam in quantitative yield.



^1H NMR (700 MHz, CDCl_3): δ = 2.74 (1H, dd, J = 12.8, 5.4), 2.47 (1H, t, J = 12.5), 2.43-0.90 (32H, m), 0.81 (3H, t, J = 7.4), 0.70 (3H, s). ^{13}C NMR (100 MHz, CDCl_3): δ = 212.1, 210.6, 179.4, 54.9, 52.4, 52.3, 50.0, 48.9, 43.7, 42.7, 38.9, 38.3, 36.7, 36.0, 35.5, 35.2, 30.9, 30.7, 28.2, 24.6, 22.9, 22.3, 18.6, 18.3, 12.1, 11.8. (IR) ν_{max} (cm^{-1}): 2939, 2873, 1706, 1458, 1382, 1284.8. HRMS (ESI-TOF) m/z : $(\text{M}+\text{H})^+$ calcd for $\text{C}_{26}\text{H}_{41}\text{O}_4$ 417.3005; found: 417.2997; mp = 71.2-75.9 °C

Example 17 – Synthesis of (3 α , 5 β , 6 α , 7 α)-6-ethyl-3,7-dihydroxy-cholan-24-oic acid



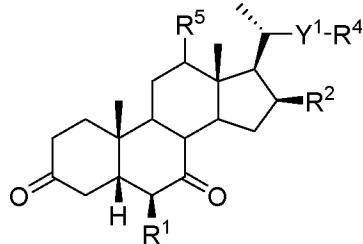
To a solution of crude (6 α , 5 β)-6-ethyl-3,7-dioxo-cholan-24-oic acid (21.7 g crude

5 mass) in H₂O (260 mL) and 50% NaOH (15.2 mL) at 90 °C was added, dropwise, a solution of NaBH₄ (4.4 g, 116.3 mmol) in aq. NaOH (prepared from 25 mL of H₂O and 0.8 mL 50% NaOH). The mixture was heated to reflux and stirred for 3 h. The mixture was then cooled to 60 °C and a 2M solution of HCl (200 mL) added dropwise with vigorous stirring. *n*BuOAc (100 mL) was then charged to the reaction flask and the mixture stirred for a further 20 mins. The phases were separated and the aqueous phase (pH = 1/2) extracted with *n*BuOAc (100 mL). The combined organic phases were washed with 2M HCl (50 mL) and 10% aq. NaCl (100 mL). The organic solvent was distilled off under reduced pressure at 70-80 °C. The residue (dense oil) was dissolved in *n*BuOAc (60 mL) at 70 °C and allowed to gradually cool to room 10 temperature, then stored at 6 °C for 2 h. The solid was collected *via* filtration, rinsed with cold *n*BuOAc (20 mL), then dried under vacuum at 70 °C for 5h to give (3 α , 5 β , 6 α , 7 α)-6-ethyl-3,7-dihydroxy-cholan-24-oic acid as a white solid (8.2 g).

15

CLAIMS

1. A compound of general formula (I):



(I)

5

wherein:

R¹ is C₁₋₄ alkyl optionally substituted with one or more substituents selected from halo, OR⁶ or NR⁶R⁷;

where each of R⁶ and R⁷ is independently selected from H or C₁₋₄ alkyl;

10 R² is H, halo or OH or a protected OH;

Y¹ is a bond or an alkylene linker group having from 1 to 20 carbon atoms and optionally substituted with one or more groups R³;

each R³ is independently halo, OR⁸ or NR⁸R⁹;

where each of R⁸ and R⁹ is independently selected from H or C₁₋₄ alkyl;

15 and

R⁴ is C(O)OR¹⁰, OC(O)R¹⁰, C(O)NR¹⁰R¹¹, OR¹⁰, OSi(R¹³)₃, S(O)R¹⁰, SO₂R¹⁰, OSO₂R¹⁰, SO₃R¹⁰, or OSO₃R¹⁰;

where each R¹⁰ and R¹¹ is independently:

a. hydrogen or

20 b. C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, C₂₋₂₀ alkynyl, -O-C₁₋₂₀ alkyl, -O-C₂₋₂₀ alkenyl or -O-C₂₋₂₀ alkynyl, any of which is optionally substituted with one or more substituents selected from halo, NO₂, CN, OR¹⁹, SR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂, or a 6- to 14- membered aryl or 5 to 14-membered heteroaryl group, either of which is optionally substituted with C₁₋₆ alkyl, C₁₋₆ haloalkyl, halo, NO₂, CN, OR¹⁹, SR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂; or

25 c. a 6- to 14- membered aryl or 5 to 14-membered heteroaryl group either of which is optionally substituted with one or more substituents selected from C₁₋₆ alkyl, C₁₋₆ haloalkyl, halo, NO₂, CN, OR¹⁹, SR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂;

30 d. a polyethylene glycol residue;

each R¹⁹ is independently selected from H, C₁₋₆ alkyl, C₁₋₆ haloalkyl, or a 6- to 14- membered aryl or 5 to 14-membered

heteroaryl group either of which is optionally substituted with halo, C₁₋₆ alkyl or C₁₋₆ haloalkyl;

each R¹³ is independently

5 a. C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl or C₂₋₂₀ alkynyl optionally substituted with one or more substituents selected from halo, NO₂, CN, OR¹⁹, SR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂, a 6- to 14- membered aryl or 5 to 14-membered heteroaryl group, either of which is optionally substituted with C₁₋₆ alkyl, C₁₋₆ haloalkyl, halo, NO₂, CN, OR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂; or

10 b. a 6- to 14- membered aryl or 5 to 14-membered heteroaryl group optionally substituted with one or more substituents selected from C₁₋₆ alkyl, C₁₋₆ haloalkyl, halo, NO₂, CN, OR¹⁹, SR¹⁹, SO₂R¹⁹, SO₃R¹⁹ or N(R¹⁹)₂;

each R¹⁹ is independently selected from H, C₁₋₆ alkyl or C₁₋₆ haloalkyl;

R⁵ is H or OH or a protected OH;

15 or a salt or an isotopic variant thereof.

2. A compound according to claim 1 wherein R¹ is ethyl.

3. A compound according to claim 1 or claim 2 wherein Y¹ is an alkylene linker group having from 1 to 8 carbon atoms and optionally substituted with one or more groups R³, wherein R³ is as defined in claim 1.

4. A compound according to any one of claims 1 to 3 wherein, independently or in any combination:

25 Y¹ is a bond or an alkylene group having 1 to 3 carbon atoms and is optionally substituted with one or two R³ groups;

R⁴ is C(O)OR¹⁰, SO₃R¹⁰, or OSO₃R¹⁰, where R¹⁰ is as defined above but is more suitably H, C₁₋₆ alkyl or benzyl;

R⁵ is H or OH.

30

5. A compound according to claim 4 wherein, independently or in any combination:

R¹ is ethyl; and/or

R² is H; and/or

35 Y¹ is a bond, -CH₂- or -CH₂CH₂-; and/or

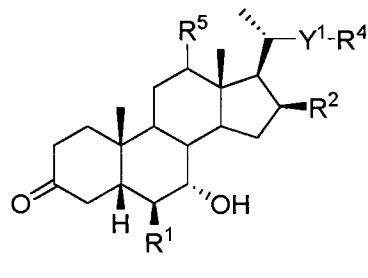
R⁴ is C(O)OR¹⁰, where R¹⁰ is H, C₁₋₆ alkyl or benzyl; and/or

R⁵ is H.

6. A compound according to claim 1 which is
 $(6\beta, 5\beta)$ -3,7-dioxo-6-ethyl-cholan-24-oic acid
 or a C_{1-6} alkyl or benzyl ester, especially the methyl or ethyl, thereof or salt thereof.

5 7. A process for the preparation of a compound according to any one of claims 1 to 6 comprising

A. oxidation of a compound of general formula (II):



10 wherein Y^1 , R^1 , R^2 , R^4 and R^5 are as defined in claim 1; or

B. conversion of a compound of general formula (I) to another compound of general formula (I).

15 8. A process according to claim 7 wherein the oxidation is carried out using:

a Dess-Martin periodinane (1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol) oxidation; or

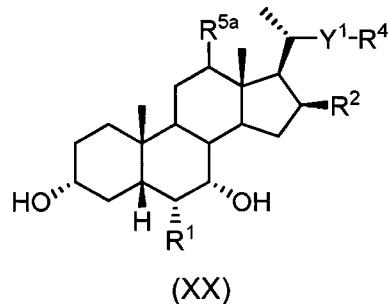
a hypochlorite, for example sodium hypochlorite, under acidic conditions; or

a Jones reaction using sodium dichromate or chromic trioxide in dilute sulfuric acid.

20

9. A process according to claim 7 or claim 8 for the preparation of a compound of general formula (I) in which R^4 is $C(O)OR^{10}$, wherein R^{10} is as defined in claim 1 from a compound of general formula (II) where R^4 is also $C(O)OR^{10}$.

25 10. A process for the preparation of a compound of general formula (XX):



wherein R^1 , R^4 and Y^1 are as defined for general formula (I) in claim 1;

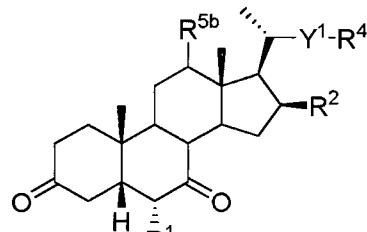
R² is H, halo or OH; and

R^{5a} is H or OH;

the process comprising:

i. epimerisation of a compound according to any one of claims 1 to 6 to give a

5 compound of general formula (XXI):



(XXI)

wherein R¹, R⁴ and Y¹ are as defined for general formula (I) in claim 1; and

R² is H, halo or OH or a protected OH group which is stable under basic conditions;

10 and

R^{5b} is H or OH or a protected OH group which is stable under basic conditions; and

ii. reduction of the compound of general formula (XXI) using a suitable reducing agent and, where R² and/or R^{5b} is a protected OH, removal of the protecting group(s), to give a compound of general formula (XX) as defined above, wherein

15 removal of the protecting group can take place before or after the reduction; and optionally

iii. conversion of a compound of general formula (XX) to another compound of

20 general formula (XX).

11. A process according to claim 10 wherein, in the epimerization reaction of step

(i), the compound of general formula (I) is dissolved in an alcoholic solvent, optionally mixed with water and contacted with a base.

25

12. A process according to claim 11 wherein the base is sodium or potassium hydroxide or a sodium or potassium alkoxide, typically an ethoxide.

13. A process wherein, in the compound of general formula (I), R⁴ is C(O)OR¹⁰,

30 where R¹⁰ is C₁₋₆ alkyl or benzyl and wherein the base is sodium or potassium hydroxide, such that the epimerization reaction is accompanied by hydrolysis to give a compound of general formula (XXI) in which R⁴ is C(O)OH.

14. A process according to any one of claims 10 to 13 wherein, in the compound of general formula (I), R² and/or R⁵ is a group OC(O)OR¹⁴, where R¹⁴ is C₁₋₆ alkyl or benzyl ; and wherein the epimerisation step yields a compound of general formula (XXI) in which R² and/or R^{5b} is OH.

5

15. A process according to any one of claims 10 to 13 wherein, in the compound of general formula (I), R² and/or R⁵ is a protected OH which is stable under basic conditions and the process further comprises the step of removing the protecting group before or after step (ii).

10

16. A process according to any one of claims 10 to 15 wherein, in step (ii), the reducing agent is typically a hydride, such as sodium borohydride.

17. A process according to any one of claims 10 to 16 for the preparation of a compound of general formula (XX) in which R¹ is ethyl, R² and R^{5a} are both H, Y¹ is -CH₂CH₂-, and R⁴ is C(O)OH.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/053518

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07J9/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07J

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

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

EPO-Internal, BEILSTEIN Data, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2006/122977 A2 (ERREGIERRE SPA [IT]; FERRARI MASSIMO [IT]; PELLICCIARI ROBERTO [IT]) 23 November 2006 (2006-11-23) cited in the application example 1 -----	1-17
A	US 2014/148428 A1 (PRUZANSKI MARK [US] ET AL) 29 May 2014 (2014-05-29) example 1 -----	1-17
A	WO 2010/059859 A1 (INTERCEPT PHARMACEUTICALS INC [US]; PELLICCIARI ROBERTO [IT]) 27 May 2010 (2010-05-27) page 24 ----- -/-	1-17



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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- "P" document published prior to the international filing date but later than the priority date claimed

"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2016	11/02/2016
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Watchorn, Peter

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/053518

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2015/053518

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