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Lorente Bonde-Larsen et al.

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(54) **METHODS FOR THE PREPARATION OF DEOXYCHOLIC ACID, AND INTERMEDIATES USEFUL IN THE PREPARATION OF DEOXYCHOLIC ACID**

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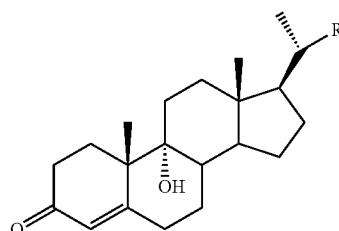
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(57) **ABSTRACT**

The present invention relates to new and improved processes for the preparation of deoxycholic acid (DCA) and pharmaceutically acceptable salts thereof, as well as to novel intermediates useful for the preparation of DCA and pharmaceutically acceptable salts thereof. The starting compounds are steroids, sterols or fermented phytosterols of vegetable origin, being of formula SM:



SM

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This patent is subject to a terminal disclaimer.

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C07J 9/00 (2006.01)

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C07J 41/00 (2006.01)

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(52) **U.S. Cl.**

CPC **C07J 9/005** (2013.01); **C07J 9/00** (2013.01); **C07J 41/0061** (2013.01); **C07J 71/0005** (2013.01); **C07J 31/006** (2013.01)

(58) **Field of Classification Search**

CPC **C07J 9/005**; **C07J 41/0061**; **C07J 71/0005**; **C07J 31/006**; **C07J 9/00**

See application file for complete search history.

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8 Claims, No Drawings

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METHODS FOR THE PREPARATION OF DEOXYCHOLIC ACID, AND INTERMEDIATES USEFUL IN THE PREPARATION OF DEOXYCHOLIC ACID

This application is a national stage application under 35 U.S.C. § 371 from PCT Application No. PCT/EP2017/063701, filed Jun. 6, 2017, which claims the benefit of European Patent Application Serial No. 16173095.7, filed Jun. 6, 2016, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to new and improved processes for the preparation of deoxycholic acid (DCA) and pharmaceutically acceptable salts thereof, as well as to novel intermediates useful for the preparation of DCA and pharmaceutically acceptable salts thereof.

BACKGROUND OF THE INVENTION

Deoxycholic acid (DCA) is a known drug compound. DCA has the CAS number [83-44-3], and is also known as deoxycholate, cholanoic acid, and 3 α ,12 β -dihydroxy-5 β -cholanoate. Pure DCA is a white to off-white crystalline powder.

DCA is one of the secondary bile acids, which are metabolic byproducts of intestinal bacteria.

Since its discovery DCA has been used in various fields of human medicine. In the human body DCA is used in the emulsification of fats for the absorption in the intestine. Also, when injected into submental fat, DCA helps destroying fat cells. In the United States DCA has been approved by the Food and Drug Administration (FDA) for reducing moderate-to-severe fat below the chin, and is marketed under the trademark Kybella®. Kybella® is produced by Kythera Biopharmaceuticals.

Recent patent applications describing DCAs fat-reducing properties include WO 2005/117900, WO 2005/112942, US 2005/0261258, US 2005/0267080, US 2006/127468 and US 2006/0154906.

Pharmaceutical preparations containing bile acids are commercially available at rather low costs, because bile acids are easily available from animal corpses, such as cows and sheep.

However, bile acids obtained from animal sources may contain pathogens, such as prions, or other harmful agents, such as toxins.

Bile acids from animal sources are typically purified in order to exclude impurities. In practice such purified compositions contain a mixture of bile acids. For example, commercially available compositions of DCA of animal origin contain some chenodeoxycholic acid and cholic acid.

Accordingly, bile acids, including DCA, obtained either synthetically or from plant sources, have recently gained increased interest since the above-mentioned problems associated with bile acids from animal origin can thereby be eliminated.

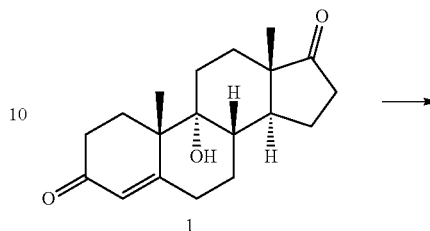
Thus, there is a need for novel and efficient synthetic routes for preparing bile acids, including DCA, where the starting compounds are steroids, sterols or fermented phytosterols of vegetable origin.

It is known to prepare DCA starting from phytosterols obtained by fermentation of a *Mycobacterium* strain. For

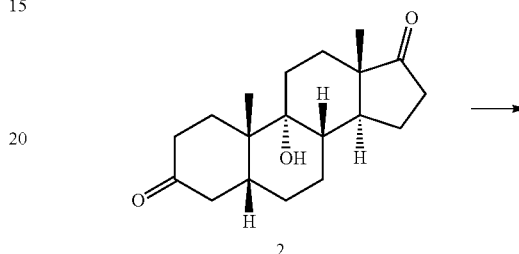
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example, WO 2008/157635 and WO 2013/044119 describe the synthesis of DCA from 9-hydroxy-4-androstene-3,17-dione:

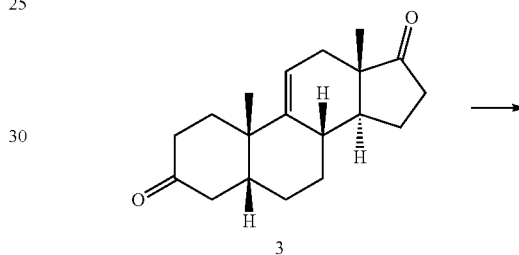
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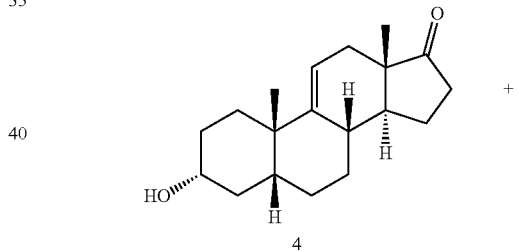
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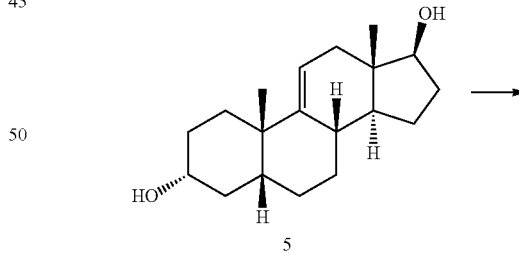
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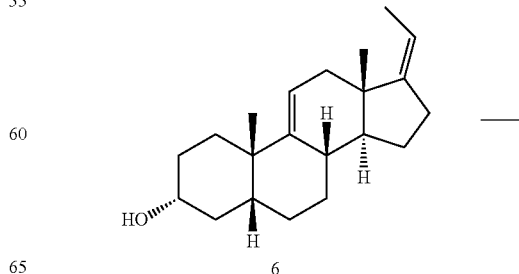
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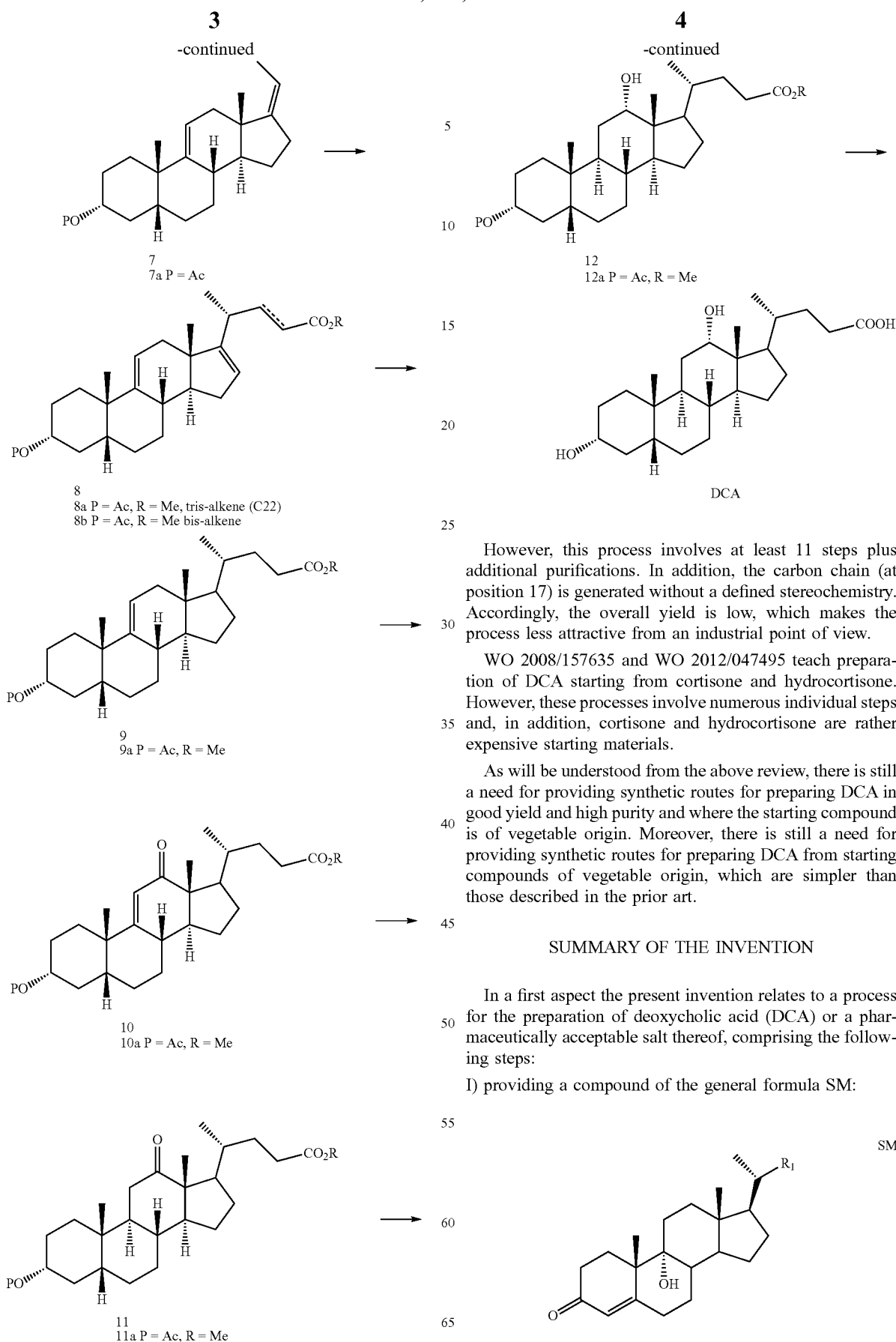
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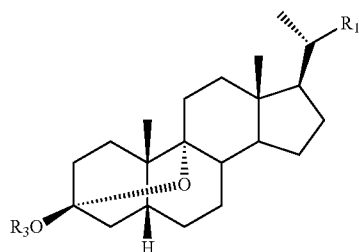


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II) reducing the compound of the general formula SM to obtain an intermediate of the general formula INT 1:

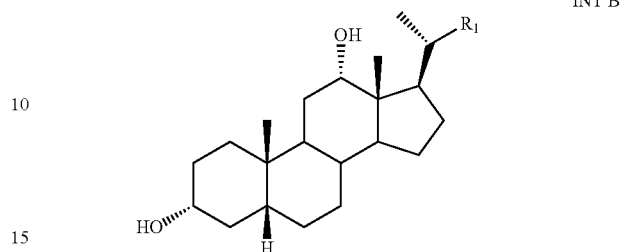


INT 1

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or
IVb) converting the intermediate of the general formula INT 2 into an intermediate with the general formula INT B:

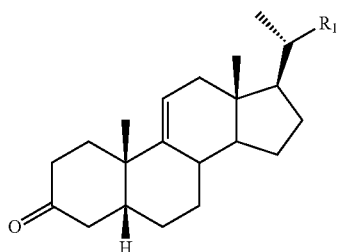


INT B

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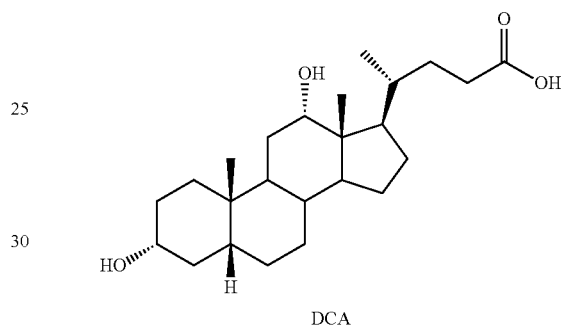
III) converting the intermediate of the general formula INT 1 into an intermediate of the general formula INT 2:



INT 2

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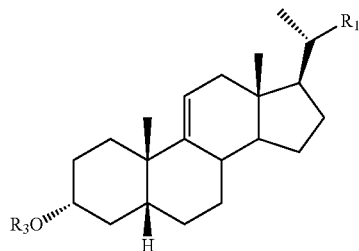
V) converting the intermediate of the general formula INT B into deoxycholic acid (DCA):



DCA

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IVa) reducing the intermediate of the general formula INT 2 into an intermediate of the general formula INT 3:



INT 3

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VI) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof, wherein

R_1 is COOR_2 , CH_2OH , CH_2OP , CH_2X , CH_2CHO , $\text{CH}_2\text{—CH}_2\text{—OH}$, $\text{CH}_2\text{—CH}_2\text{OP}$, or $\text{CH}_2\text{—CH}_2\text{X}$ or $\text{CH}_2\text{—CH}_2\text{—CHO}$;

R_2 is H or a linear or branched $\text{C}_1\text{—C}_6$ -alkyl group;

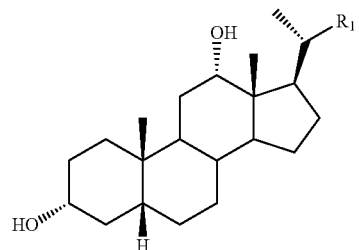
P is an alcohol protection group;

R_3 either P or R_2 ; and

X is a halogen atom.

In one embodiment step IVa) is carried out, and step IVb) is not carried out. In another embodiment step IVb) is carried out, and step IVa) is not carried out.

followed by converting the intermediate of the general formula INT 3 into an intermediate of the general formula INT B:



INT B

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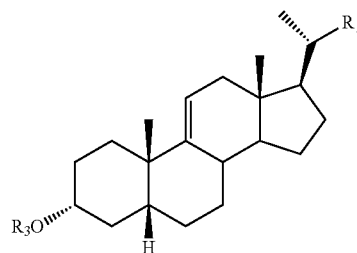
In another aspect the present invention relates to a process for the preparation of deoxycholic acid (DCA) or a pharmaceutically acceptable salt thereof, comprising the following steps:

I) providing a compound of the general formula INT 3:

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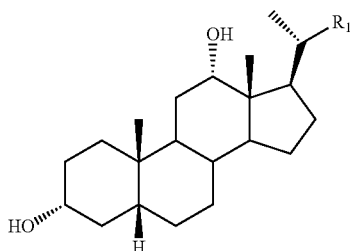
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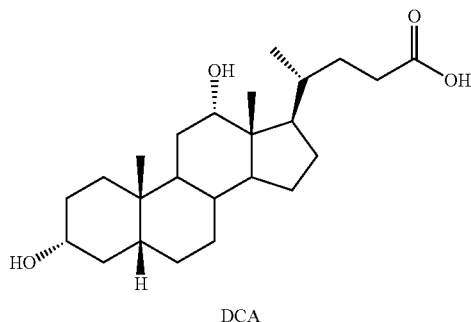
INT 3

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II) converting the intermediate of the general formula INT 3 into an intermediate of the general formula INT B:



III) converting the intermediate of the general formula INT B into deoxycholic acid (DCA):



IV) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof, wherein

R_1 is COOR_2 , CH_2OH , CH_2OP , CH_2X , CH_2CHO , $\text{CH}_2\text{—CH}_2\text{—OH}$, $\text{CH}_2\text{—CH}_2\text{OP}$, or $\text{CH}_2\text{—CH}_2\text{X}$ or $\text{CH}_2\text{—CH}_2\text{—CHO}$;

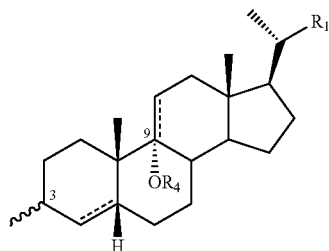
R_2 is H or a linear or branched $\text{C}_1\text{—C}_6\text{—alkyl}$ group;

P is an alcohol protection group;

R_3 either P or R_2 ; and

X is a halogen atom.

In another aspect the present invention relates to a compound of the general formula I



wherein

R_1 is COOR_2 , CH_2OP , CH_2X , CH_2CHO , $\text{CH}_2\text{—CH}_2\text{—OH}$, $\text{CH}_2\text{—CH}_2\text{OP}$ or $\text{CH}_2\text{—CH}_2\text{X}$;

R_2 is H or a linear or branched $\text{C}_1\text{—C}_6\text{—alkyl}$ group;

P is an alcohol protection group with the proviso that P is not Ac or Pv;

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X is a halogen atom;

===== is either a C—C bond or a C=C bond;

~~~~~ is either =O or ~~~~~ OR<sub>3</sub> where  $R_3$  is either P or  $R_2$ ;

OR<sub>4</sub> is either OH or  $R_4$  is the C<sub>3</sub> carbon in the A ring; and with the proviso that formula I is not

INT B 5

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Wherein R is H or Me

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Wherein R is H or Me

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I 50

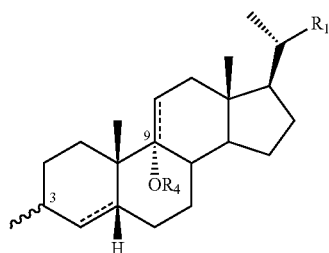
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In a still further aspect the present invention relates to the use of a compound the general formula I



wherein

$R_1$  is  $\text{COOR}_2$ ,  $\text{CH}_2\text{OH}$ ,  $\text{CH}_2\text{OP}$ ,  $\text{CH}_2\text{X}$ ,  $\text{CH}_2\text{CHO}$ ,  $\text{CH}_2\text{—CH}_2\text{—OH}$ ,  $\text{CH}_2\text{—CH}_2\text{OP}$ ,  $\text{CH}_2\text{—CH}_2\text{X}$  or  $\text{CH}_2\text{—CH}_2\text{—CHO}$ ;

$R_2$  is H or a linear or branched  $\text{C}_1\text{—C}_6\text{-alkyl}$  group;

P is an alcohol protection group;

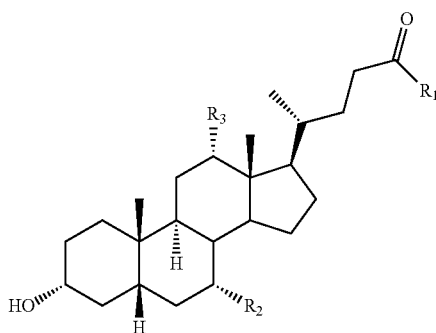
X is a halogen atom;

----- either a  $\text{C—C}$  bond or a  $\text{C=C}$  bond;

~~~~~ is either  $=\text{O}$  or  $\text{OR}_3$  where  $R_3$  is either P or  $R_2$ ; and

OR_4 is either OH or R_4 is the C_3 carbon in the A ring;

for the preparation of a compound of the general formula II or a pharmaceutically acceptable salt thereof.



wherein

R_1 is OH, $\text{NHCH}_2\text{CH}_2\text{SO}_3\text{H}$ or NHCH_2COOH ;

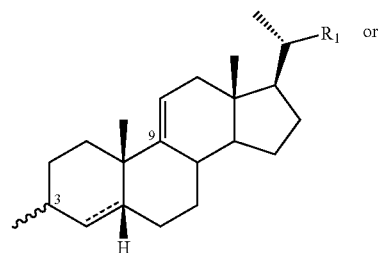
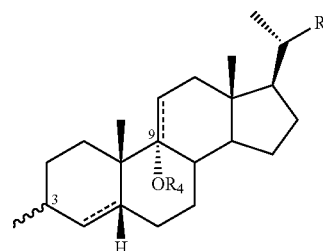
R_2 and R_3 is H or OH.

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DETAILED DESCRIPTION OF THE INVENTION

Definitions

In the present context the compound of the general formula I is to be understood as either a compound of formula Ia or Ib as shown below.



In the present context, the term “ $\text{C}_1\text{—C}_6\text{-alkyl}$ group” is intended to mean a linear or branched saturated carbon chain having from 1 to 6 carbon atoms. Specific examples of a $\text{C}_1\text{—C}_6\text{-alkyl}$ group are methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, n-pentyl, isopentyl, n-hexyl and iso-hexyl. Preferred examples include methyl, ethyl, n-propyl and isopropyl, in particular methyl and ethyl. Most preferably, the $\text{C}_1\text{—C}_6\text{-alkyl}$ group is methyl.

Herein, the term “ $\text{C}_1\text{—C}_6\text{-alkanol}$ ” means a linear or branched saturated alcohol having from 1 to 6 carbon atoms.

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Specific examples of a C₁-C₆-alcohol are methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, tert-butanol, n-pentanol, isopentanol, n-hexanol and iso-hexanol. Preferred examples includes methanol, ethanol, n-propanol and isopropanol, in particular methanol and ethanol. Most preferably, the C₁-C₆-alcohol is methanol.

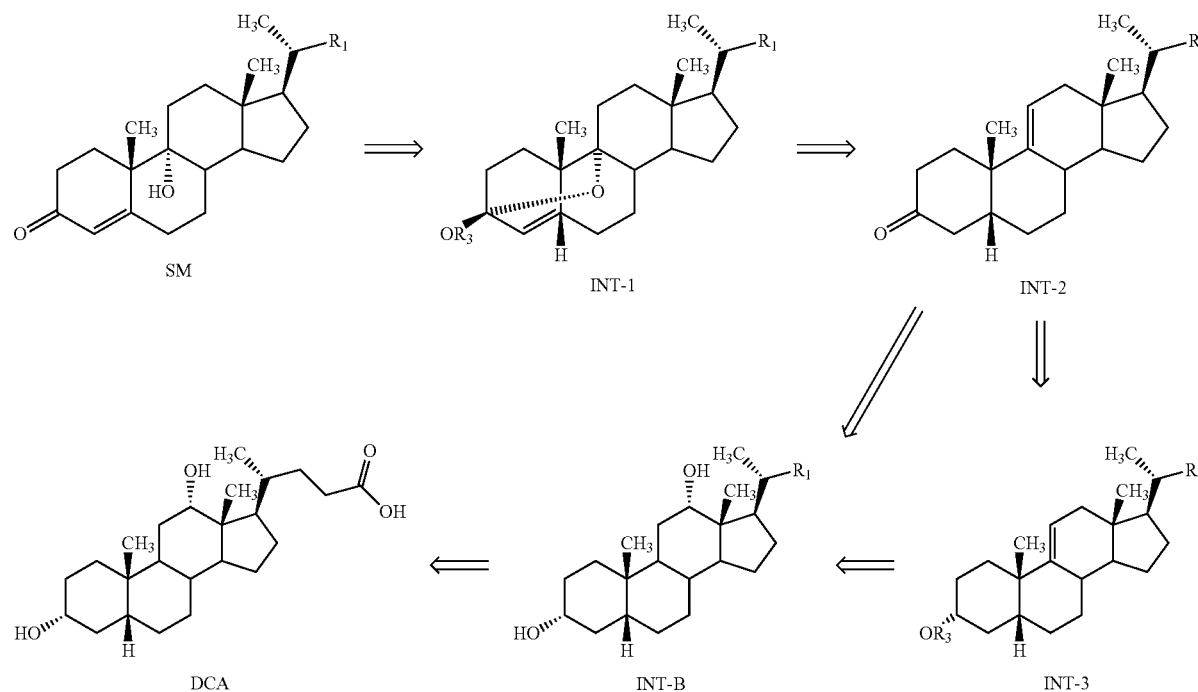
The term “leaving group” is intended to mean a molecular fragment that is capable of departing from a molecule with a pair of electrons in heterolytic bond cleavage. Specific examples of leaving groups include halides, such a chloride,

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salt, a calcium salt, a magnesium salt and the like, or salts with an organic base, such as a piperidine salt, a morpholine salt, a pyrrolidone salt, an arginine salt, a lysine salt and the like. In a preferred embodiment of the invention, the pharmaceutically acceptable salt is the sodium salt.

The Synthetic Routes to DCA

The present inventors have provided new synthetic routes to DCA, which may be described by the following overall reaction scheme:



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bromide and iodide, and sulfonate esters, such as tosylate. In a preferred embodiment of the invention the leaving group is bromide.

When used herein, the term “alcohol protection group” means a molecule that can modify, and hence temporarily mask the characteristic chemistry of, an alcohol group. Specific examples of alcohol protection groups include trimethylsilyl ether (TMS), triethylsilyl ether (TES), triisopropylsilyl ether (TIPS), tert-butyldimethylsilyl ether (TBS, TBDMS), tert-butyldiphenylsilyl ether (TBDPS), acetyl (Ac, COCH₃), benzoyl (Bz), benzyl ether (Bn), 4-methoxybenzyl ether (PMB), 2-naphthylmethyl ether (Nap), methoxymethyl acetal (MOM), 2-methoxyethoxy-methyl ether (MEM), ethoxyethyl acetal (EE), methoxypropyl acetal (MOP), benzyloxymethyl acetal (BOM), tetrahydropyranyl acetal (THP), 2,2,2-trichloro-ethyl carbonate (Troc), methyl ether, dimethoxytrityl (DMT), methoxytrityl (MMT), methylthiomethyl ether, pivaloyl (Piv), tetrahydropyranyl (THP), triphenylmethyl (trityl, Tr), and tosyl (Ts). In a preferred embodiment of the invention the alcohol protection group is Ac, TBDMS and Ts, in particular Ac.

In the present context “Ac” means acetyl (COCH₃).

A “pharmaceutically acceptable salt” means that the salt is non-toxic and suitable for being administered to a mammal, in particular a human being. Examples of pharmaceutically acceptable salts include salts with a base, e.g. salts with an inorganic base, such as a sodium salt, a potassium

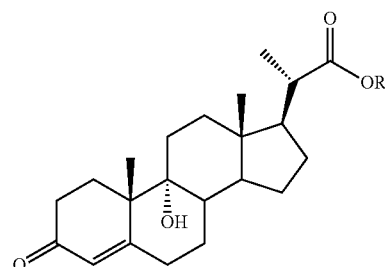
wherein R₁ and R₃ are as defined previously.

The individual process steps are disclosed in more detail infra.

Synthetic Route A

In a preferred embodiment of the invention, the process for the preparation of deoxycholic acid (DCA) or a pharmaceutically acceptable salt thereof, comprises the following steps:

- i) providing a compound of the general formula SM-a:



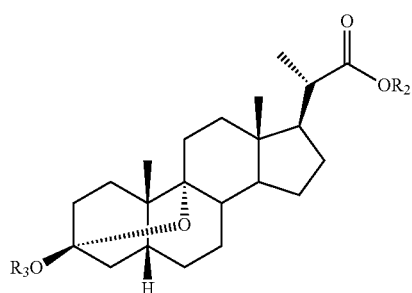
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ii) reducing the compound of the general formula SM-a to obtain an intermediate of the general formula Int A1:

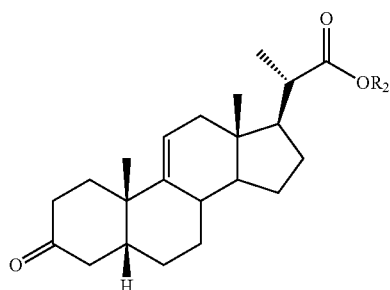


INT A1 5

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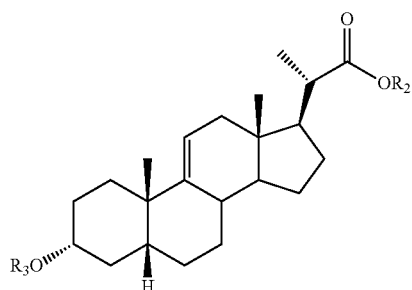
iii) converting the intermediate of the general formula Int A1 into an intermediate of the general formula Int A2:



INT A2 30

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iv) reducing the intermediate of the general formula Int A2 into an intermediate of the general formula Int A3:



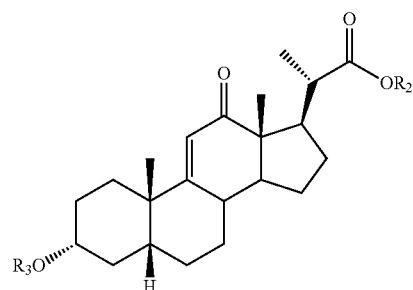
INT A3 55

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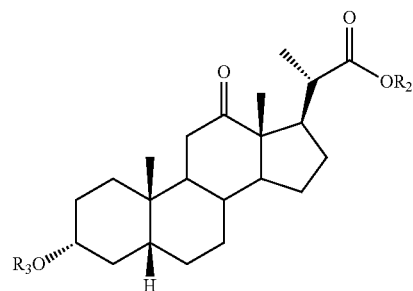
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v) oxidising the intermediate of the general formula Int A3 into an intermediate of the general formula Int A5:



Int A5

vi) reducing the intermediate of the general formula Int A5 into an intermediate of the general formula Int A6:



Int A6

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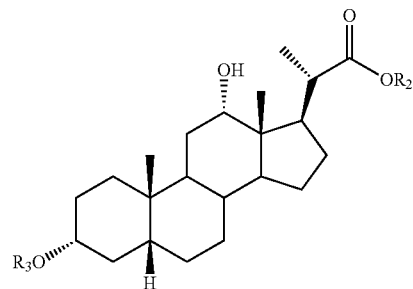
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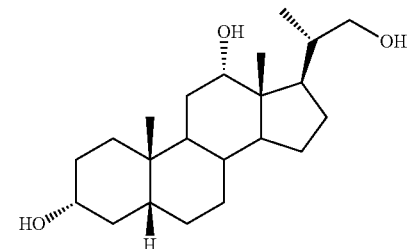
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viii) reducing the compound of the general formula Int A7 into an intermediate of the general formula Int A8:



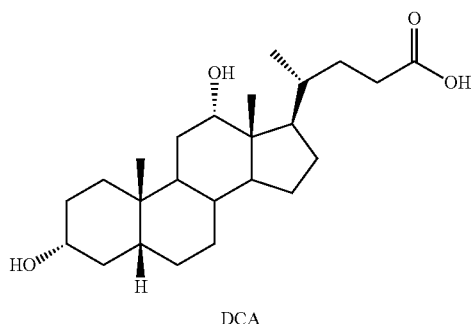
Int A7



Int A8

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ix) elongating the carbon chain of the compound of the general formula Int A8 to obtain deoxycholic acid (DCA):



x) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof,

wherein

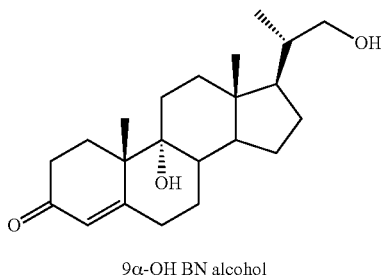
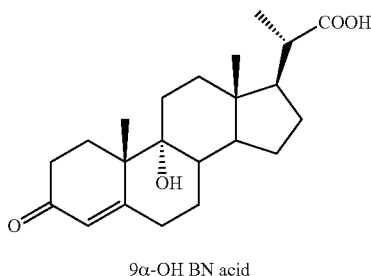
R_2 is H or a linear or branched C_1 - C_6 -alkyl group;

R_3 is H, R_2 or an alcohol protection group.

Step i)

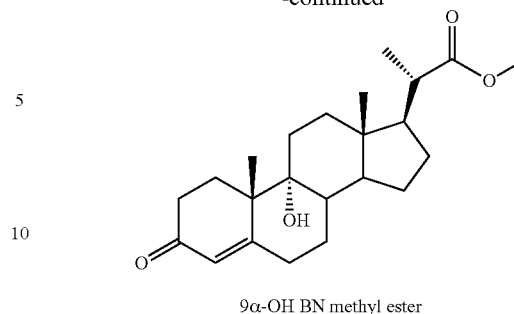
The starting compound, intermediate SM-a, may be obtained from (or easily prepared from compounds obtained from) fermentation products of *Mycobacterium fortuitum* in the presence of an appropriate carbon source.

For example, U.S. Pat. No. 4,029,549 shows the production of 9α -OH BN acid, 9α -OH BN alcohol and 9α -OH BN methyl ester by fermenting the microorganism *Mycobacterium fortuitum* NRRL B-8119 in the presence of either sitosterol (example 2) or cholesterol, stigmasterol or campesterol (example 3). The purification and isolation of 9α -OH BN acid is disclosed in example 5 of U.S. Pat. No. 4,029, 549.



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-continued



Accordingly, steps i) to vii) described in the "Synthetic route A" may be preceded by a step comprising cultivating a 9α -OH BN acid-producing microorganism in an aqueous nutrient medium under aerobic conditions in the presence of a carbon source. This applies mutatis mutandis to the other synthetic routes described herein, including "Synthetic route C", "Synthetic route D", "Synthetic route E" and "Synthetic route F".

The 9α -OH BN acid-producing microorganism may be selected from the group consisting of *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Microbacterium*, *Nocardia*, *Proaminobacter*, *Serratia*, *Streptomyces* and *Mycobacterium*. In a preferred embodiment of the invention the 9α -OH BN acid-producing microorganism is *Mycobacterium fortuitum*, in particular *Mycobacterium fortuitum*. In the most preferred embodiment of the invention the 9α -OH BN acid-producing microorganism is *Mycobacterium fortuitum* NRRL B-8119.

The carbon source may be a steroid, such as cholesterol, stigmasterol, campesterol and sitosterol, preferably sitosterol.

As will be understood, 9α -OH BN acid, 9α -OH BN alcohol and 9α -OH BN methyl ester may, if needed, easily be converted into compounds of the general formula SM-a by standard methods well known to the person skilled in organic chemistry.

Step ii)

Step ii) involves reducing the compound of the general formula SM-a to obtain an intermediate of the general formula Int A1.

The reaction is typically carried out by hydrogenation of SM-a in the presence of palladium on charcoal (Pd/C) at a temperature of 50-90° C., preferably around 70° C., for 1-24 hours, preferably 8-16 hours. Other transition metal catalysts may also be employed, such as Ni or Rh.

If R_3 is H, the reaction is preferably carried out in a polar aprotic solvent, such as N-methylpyrrolidone, tetrahydrofuran (THF), ethylacetate (EtOAc), acetone, dimethylformamide (DMF), acetonitrile or dimethyl sulfoxide (DMSO). In a preferred embodiment the polar aprotic solvent is DMF.

If R_3 is a C_1 - C_6 -alkyl group the reaction is carried out in the corresponding alcohol, i.e. the solvent is an C_1 - C_6 -alkanol. In a preferred embodiment of the invention R_3 is methyl and the solvent is methanol.

Step iii)

Step iii) involves converting the intermediate of the general formula Int A1 to obtain an intermediate of the general formula Int A2.

The skilled person will be aware of suitable oxidising agents, and examples include chromium oxide (CrO_3) and strong acids, such as HI, HBr, $HClO_4$, HCl, $HClO_3$, H_2SO_4 , HNO_3 , preferably HCl or H_2SO_4 , in particular H_2SO_4 . The

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reaction is typically carried out in a non-polar solvent, such as dichloromethane (DCM), at a temperature between 0 and 90° C.

Step iv)

Step iv) involves reducing the intermediate of the general formula Int A2 to obtain an intermediate of the general formula Int A3.

The skilled person will be aware of suitable reducing agents capable of reducing a ketone to a secondary alcohol. Preferably, the reducing agent is a metal hydride, such as LiAlH_4 , NaBH_4 , LiBH_4 or $\text{LiAlH}(\text{OtBu})_3$, in particular $\text{LiAlH}(\text{OtBu})_3$.

The reaction is typically carried out in a polar aprotic solvent, such as N-methylpyrrolidone, tetrahydrofuran (THF), ethylacetate (EtOAc), acetone, dimethylformamide (DMF), acetonitrile or dimethyl sulfoxide (DMSO), in particular THF, at a temperature between 0 to 20° C.

Step v)

Step v) involves oxidising the intermediate of the general formula Int A3 to obtain an intermediate of the general formula Int A5.

The skilled person will be aware of suitable oxidising agents for performing an allylic oxidation, and a preferred example include chromium oxide (CrO_3). Other suitable oxidising agents include tert-butyl hydroperoxide ($\text{t-BuO}_2\text{H}$), NaOCl , SeO_2 , pyridinium chlorochromate (PCC), BiCl_3 and V_2O_5 . The reaction is typically carried out in a polar solvent, such as AcOH, at a temperature between 0 and 90° C.

Step vi)

Step ii) involves reducing the intermediate of the general formula Int A5 to obtain an intermediate of the general formula Int A6.

The reaction is typically carried out by hydrogenation of Int A5 in the presence of palladium on charcoal (Pd/C) at a temperature of 50-90° C., preferably around 70° C., for 1-24 hours, preferably 8-16 hours. Other transition metal catalysts may also be employed, such as Ni or Rh.

The reaction is preferably carried out in a polar aprotic solvent, such as N-methylpyrrolidone, tetrahydrofuran (THF), ethylacetate (EtOAc), acetone, dimethylformamide (DMF), acetonitrile or dimethyl sulfoxide (DMSO). In a preferred embodiment the polar aprotic solvent is EtOAc.

Step viii)

Step viii) involves reducing the intermediate of the general formula Int A7 to obtain an intermediate of the general formula Int A8.

The skilled person will be aware of suitable reducing agents capable of reducing a carboxylic acid or an ester thereof to a primary alcohol. Preferably, the reducing agent is a metal hydride, such as LiAlH_4 , NaBH_4 , LiBH_4 or $\text{LiAlH}(\text{OtBu})_3$, in particular LiAlH_4 .

The reaction is typically carried out in a polar aprotic solvent, such as N-methylpyrrolidone, tetrahydrofuran (THF), ethylacetate (EtOAc), acetone, dimethylformamide (DMF), acetonitrile or dimethyl sulfoxide (DMSO), in particular THF, at a temperature between 0 to 50° C.

It should be noted that it is possible to elongate the carbon chain of the intermediate of the general formula Int A7 directly to obtain an intermediate of the general formula Int B2 in a similar way as described in step ix) infra. This may be done by a "Reformatsky reaction", i.e. by reacting Int A7 with $\text{Br}-\text{CH}_2-\text{COOR}_2$ in the presence of Zn in a suitable solvent.

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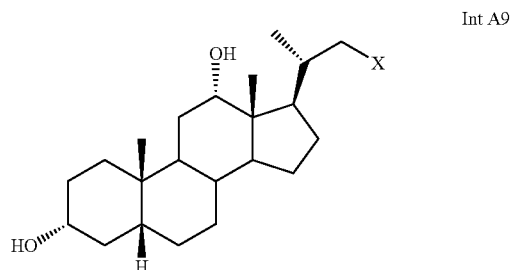
Step ix)

Step ix) involves elongating the carbon chain of the compound of the general formula Int A8 to obtain DCA.

Different synthetic routes are possible for elongating the carbon chain of Int A8 to obtain DCA:

One possible route for elongating the carbon chain of Int A8 to obtain DCA comprises the steps ix-a) and ix-b):

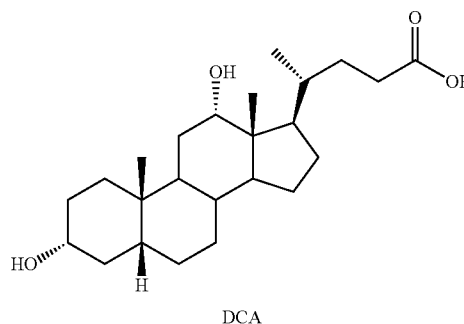
ix-a) halogenating the compound of the general formula Int A8 to obtain an intermediate of the general formula Int A9:



where X is halogen, preferably, Cl, Br or I, in particular Br, optionally acylating Int A9 with a dicarboxylic acid or an dicarboxylic acid derivative to obtain Int A9a.

where R_3 is $(\text{CH}_2)_n\text{COOH}$ with n being an integer from 0 to 11 included. Acylation of the alcohol in Int A9 can be achieved in a number of ways. For example Int A9 may be reacted with an acyl halide, an anhydride, an ester or condensed with a free carboxylic acid. Alternative Int A9 may be coupled with the carboxylic acid using as suitable coupling reagent known in the art such as DCC, DIC, EDAC.HCl, HATU, TBTU, BOP, PyBOP. The coupling may be performed in the presence of base.

ix-b) elongating the carbon chain of the compound of the general formula Int A9 to obtain DCA:



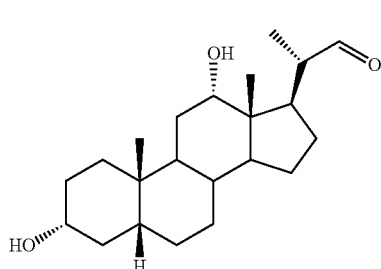
Halogenation of primary alcohols is well known to the person skilled in organic chemistry, and may be achieved in various ways. For example, the compound of the general formula Int A8 may be treated with HX, where X is Cl, Br or I, preferably HBr. Alternatively, the compound of the general formula Int A8 may be treated with CX_4 and triphenylphosphine (PPh_3), where X is Cl, Br or I, preferably Br. In a preferred embodiment of the invention Int A9 is obtained by treating Int A8 with CBr_4 and PPh_3 .

Elongation of the carbon chain of Int A9 to obtain DCA may be carried out using the so-called "Malonic ester synthesis" (see Morrison and Boyd, *Organic Chemistry*, 5th edition, 1987, pp. 1060-1063). In an embodiment of the invention Int A9 is treated with a malonate ester, preferably

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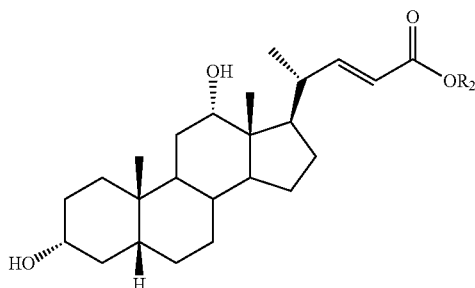
diethyl malonate, in the presence of a base, preferably NaH, and subsequently acidified to obtain DCA.

Another possible route for elongating the carbon chain of Int A8 to obtain DCA comprises the steps ix-c) to ix-e): ix-c) oxidising the compound of the general formula Int A8 to obtain an intermediate of the general formula Int B1:



Int B1

ix-d) elongating the carbon chain of the compound of the general formula Int B1 to obtain an intermediate of the general formula Int B2:



Int B2

where R₂ is a linear or branched C₁-C₆-alkyl group, ix-e) converting the compound of the general formula Int B2 into DCA.

With respect to step ix-c), oxidation of primary alcohols into aldehydes is well known to the person skilled in organic chemistry, and may be achieved in various ways. For example by chromium-based reagents, such as Collins reagent, PDC or PCC, or by catalytic TEMPO in presence of NaOCl.

Elongation of the carbon chain of Int B1 to Int B2 (step ix-d)) may be carried out using the so-called "Wittig reaction" (see Morrison and Boyd, *Organic Chemistry*, 5th edition, 1987, pp. 920-921). Alternatively, the carbon elongation step may be performed by "Horner-Emmons olefination", by "Peterson olefination", or by a "Reformatsky reaction", i.e. by reacting Int B1 with Br-CH₂-COOR₂ in the presence of Zn in a suitable solvent.

Conversion of Int B2 to DCA (step ix-e)) may be performed by hydrogenation of Int B2 followed by alkaline hydrolysis, or vice versa.

Step x)

The optional step x) involves converting DCA into a pharmaceutically acceptable salt of DCA.

Examples of pharmaceutically acceptable salts include salts with a base, e.g. salts with an inorganic base, such as a sodium salt, a potassium salt, a calcium salt, a magnesium

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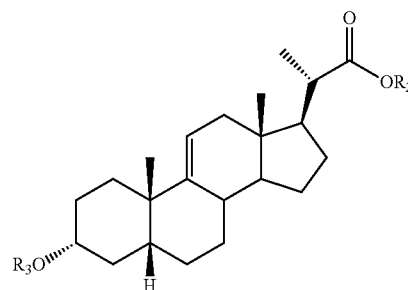
salt and the like, or salts with an organic base, such as a piperidine salt, a morpholine salt, a pyrrolidine salt, an arginine salt, a lysine salt and the like. In a preferred embodiment of the invention, the pharmaceutically acceptable salt is the sodium salt.

In a preferred embodiment of the invention the sodium salt of DCA is obtained by reacting DCA with NaOH.

Synthetic Route A'

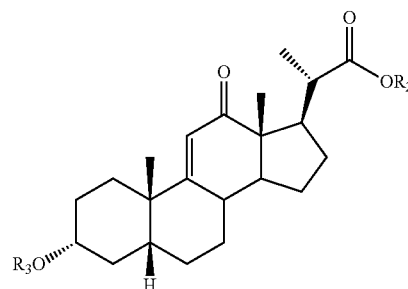
In another preferred embodiment of the invention, the process for the preparation of deoxycholic acid (DCA) or a pharmaceutically acceptable salt thereof, comprises the following steps:

i) providing a compound of the general formula



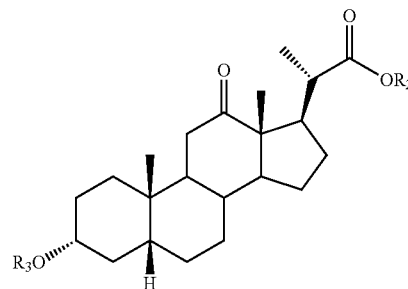
Int A3

ii) oxidising the intermediate of the general formula Int A3 into an intermediate of the general formula Int A5:



Int A5

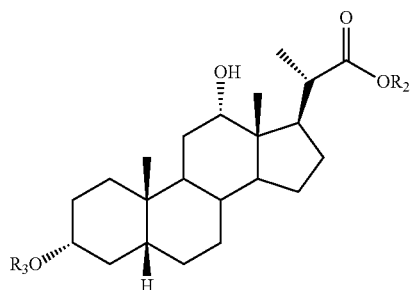
iii) reducing the intermediate of the general formula Int A5 into an intermediate of the general formula Int A6:



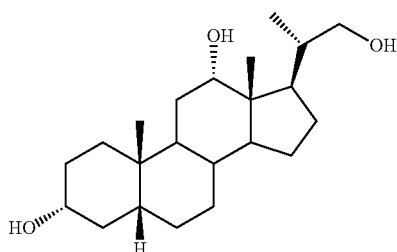
Int A6

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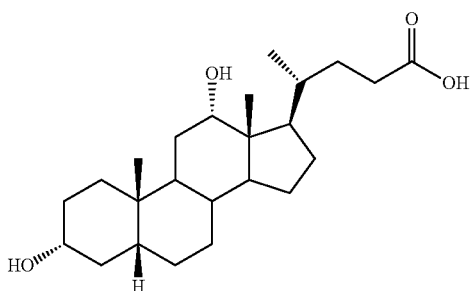
iv) reducing the intermediate of the general formula Int A6 into an intermediate of the general formula Int A7:



v) reducing the compound of the general formula Int A7 into an intermediate of the general formula Int A8:



vi) elongating the carbon chain of the compound of the general formula Int A8 to obtain deoxycholic acid (DCA):



vii) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof,

wherein

R_2 is H or a linear or branched C_1 - C_6 -alkyl group;

R_3 is H, R_2 or an alcohol protection group.

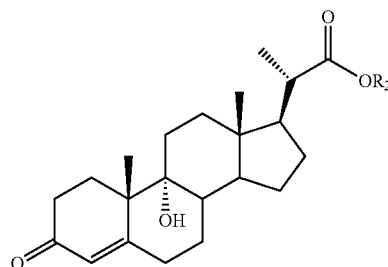
Steps ii) to vii) above corresponds exactly to steps v) to x) discussed in connection with "Synthetic Route A". The comments provided for steps v) to x) in connection with "Synthetic Route A" therefore apply mutatis mutandis to steps ii) to vii) of the "Synthetic Route A".

Synthetic Route C

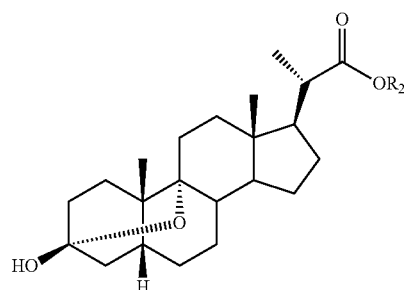
In another interesting embodiment of the invention, the process for the preparation of deoxycholic acid (DCA) or a pharmaceutically acceptable salt thereof, comprises the following steps:

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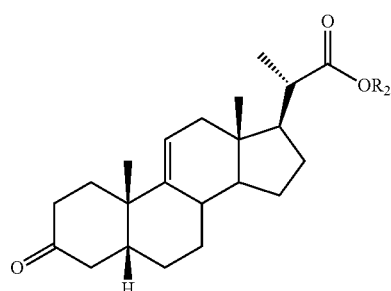
i) providing a compound of the general formula SM-a:



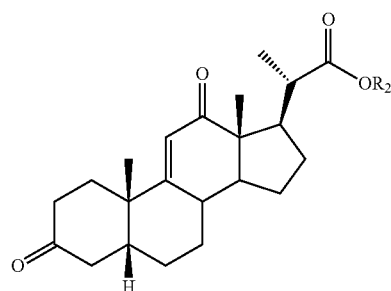
ii) reducing the compound of the general formula SM-a to obtain an intermediate of the general formula Int A1:



iii) converting the intermediate of the general formula Int A1 into an intermediate of the general formula Int A2:

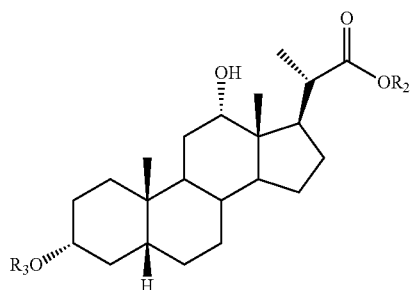


iv) oxidising the intermediate of the general formula Int 2A into an intermediate of the general formula Int C1:



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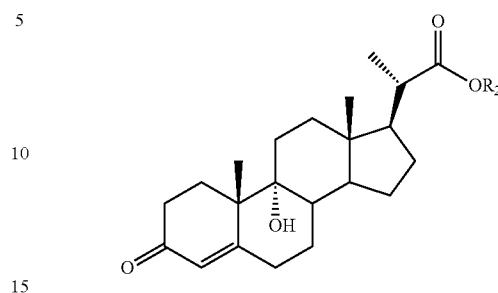
v) reducing the intermediate of the general formula Int C1 into an intermediate of the general formula Int C4:



Int C4 5

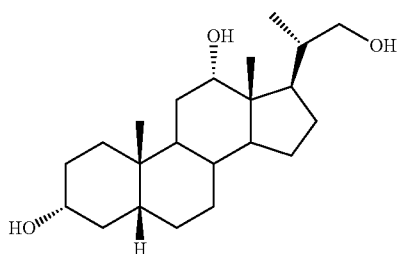
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i) providing a compound of the general formula SM-a:



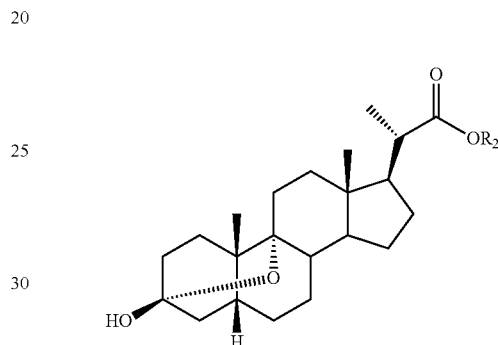
SM-a

vi) reducing the compound of the general formula Int C4 into an intermediate of the general formula Int A8:



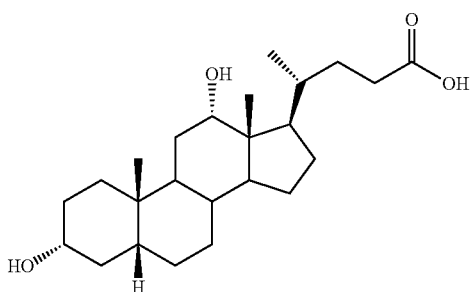
Int A8

ii) reducing the compound of the general formula SM-a to obtain an intermediate of the general formula Int A1:



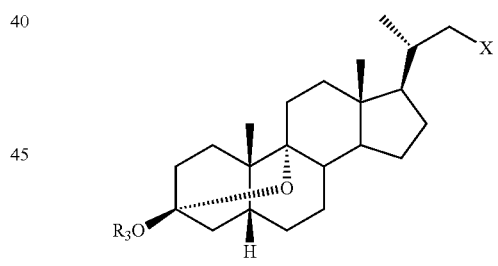
INT A1

vii) elongating the carbon chain of the compound of the general formula Int A8 to obtain deoxycholic acid (DCA):



DCA

iii) converting the intermediate of the general formula A1 into an intermediate of the general formula D1:



INT D1

viii) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof,

wherein

R_2 is H or a linear or branched C_1 - C_6 -alkyl group;

R_3 is H, R_2 or an alcohol protection group.

x) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof,

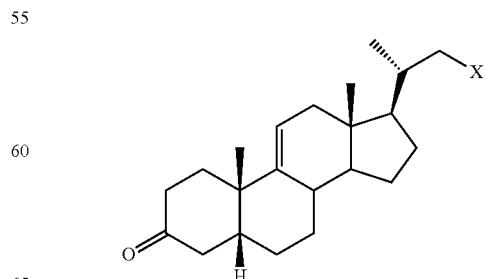
wherein

R_2 is H or a linear or branched C_1 - C_6 -alkyl group.

Synthetic Route D

In still another interesting embodiment of the invention, the process for the preparation of deoxycholic acid (DCA) or a pharmaceutically acceptable salt thereof, comprises the following steps:

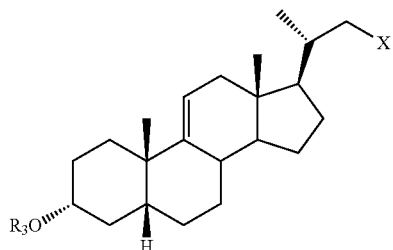
iv) oxidising the intermediate of the general formula Int D1 into an intermediate of the general formula Int D2:



INT D2

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v) reducing the intermediate of the general formula Int D2 into an intermediate of the general formula Int D3:

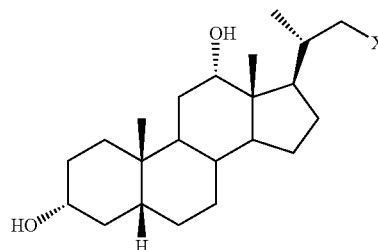


Int D3

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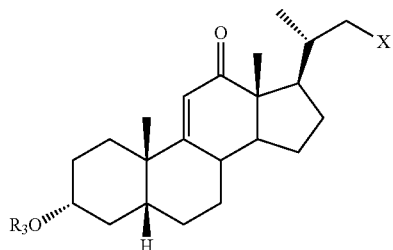
ix) hydrolysing the compound of the general formula Int D7 into an intermediate of the general formula Int D9:

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Int D9

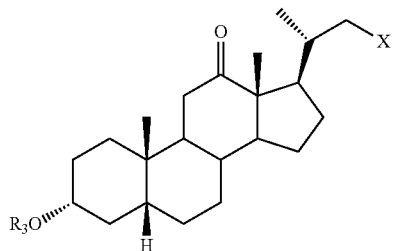
vi) oxidising the intermediate of the general formula Int D3 into an intermediate of the general formula Int D5:

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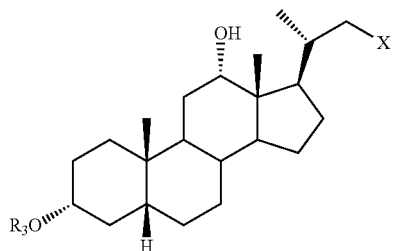
Int D5

vii) reducing the intermediate of the general formula Int D5 into an intermediate of the general formula Int D6:

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Int D6

viii) reducing the intermediate of the general formula Int D6 into an intermediate of the general formula Int D7:

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Int D7

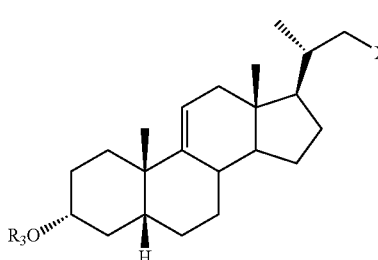
xi) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof, wherein
R₂ is H or a linear or branched C₁-C₆-alkyl group;
R₃ is H, R₂ or an alcohol protection group;
and X is a halogen atom.

Synthetic Route D'

In another interesting embodiment of the invention, the process for the preparation of deoxycholic acid (DCA) or a pharmaceutically acceptable salt thereof, comprises the following steps:

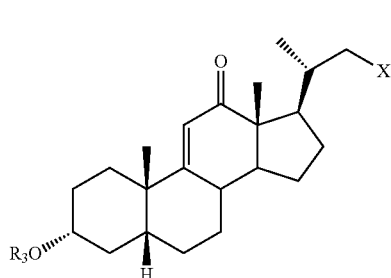
i) providing a compound of the general formula Int D3:

Int D3

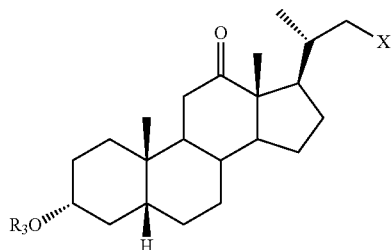


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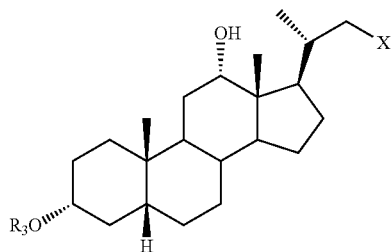
ii) oxidising the intermediate of the general formula Int D3 into an intermediate of the general formula Int D5:



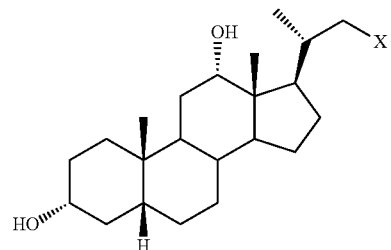
iii) reducing the intermediate of the general formula Int D5 into an intermediate of the general formula Int D6:



iv) reducing the intermediate of the general formula Int D6 into an intermediate of the general formula Int D7:

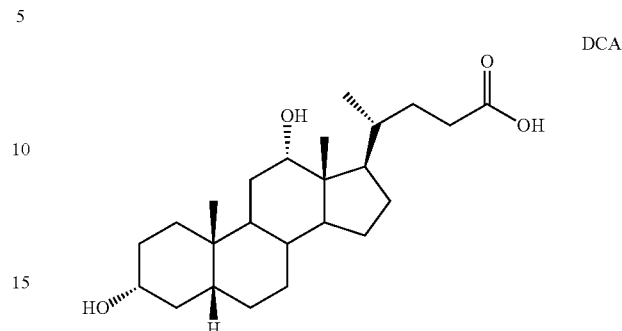


v) hydrolysing the compound of the general formula Int D7 into an intermediate of the general formula Int D9:



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vi) elongating the carbon chain of the compound of the general formula Int D9 to obtain an deoxycholic acid (DCA):



vii) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof,

wherein

R_2 is H or a linear or branched C_1 - C_6 -alkyl group;

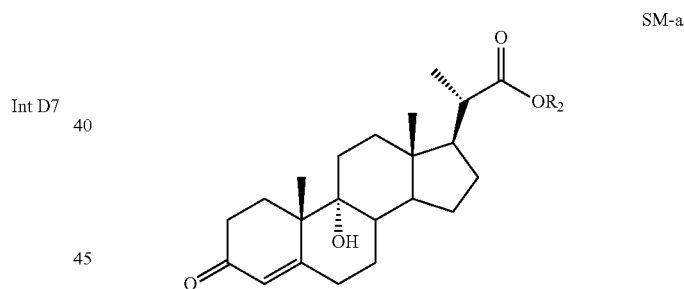
R_3 is H, R_2 or an alcohol protection group;

and X is a halogen atom.

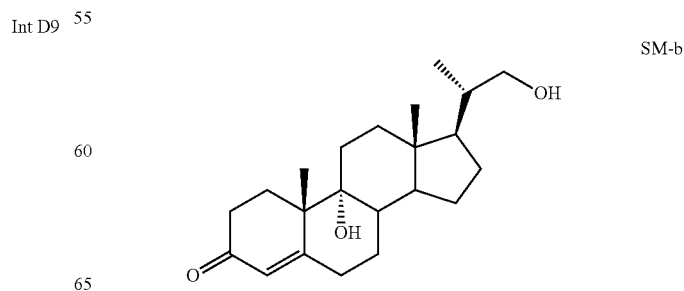
Synthetic Route E

In yet another interesting embodiment of the invention, the process for the preparation of deoxycholic acid (DCA) or a pharmaceutically acceptable salt thereof, comprises the following steps:

i) providing a compound of the general formula SM-a:

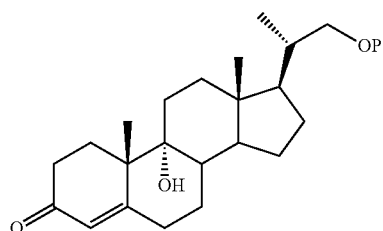


ii) reducing the compound of the general formula SM-a to obtain an intermediate of the general formula SM-b:

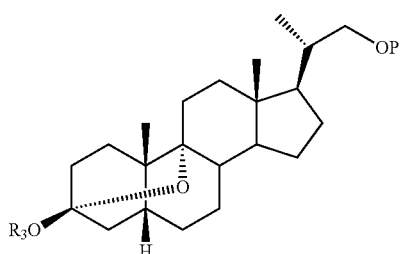


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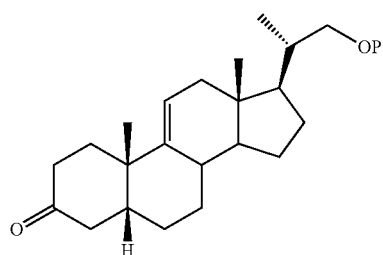
iii) protecting the alcohol group at position 22 to obtain an intermediate of the general formula Int E1:



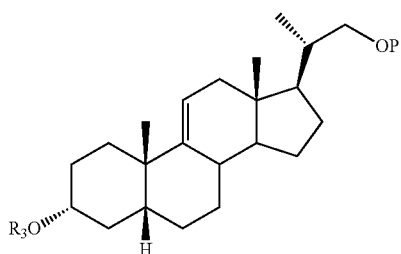
iv) converting the compound of the general formula Int E1 to obtain an intermediate of the general formula Int E2:



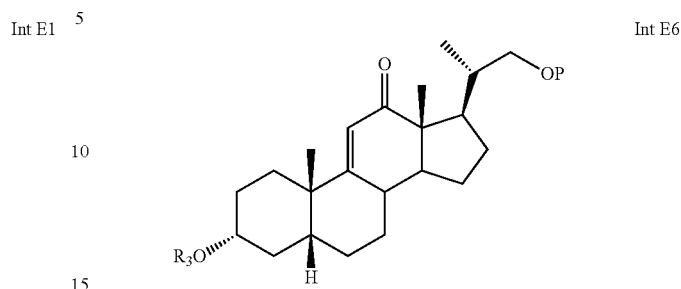
v) dehydration of the intermediate of the general formula Int E2 into an intermediate of the general formula Int E3:



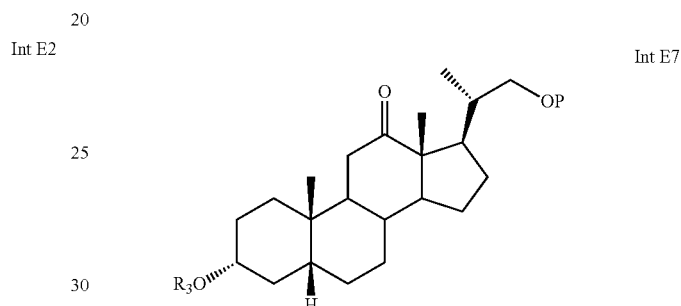
vi) reducing the intermediate of the general formula Int E3 into an intermediate of the general formula Int E5:

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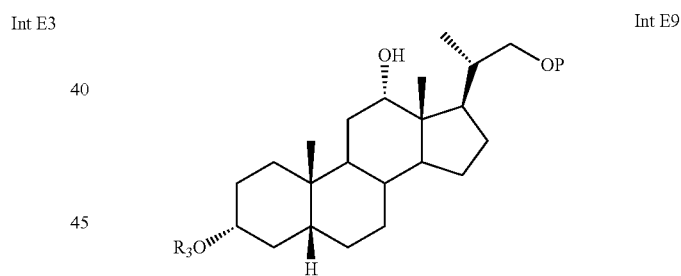
vii) oxidising the intermediate of the general formula Int E5 into an intermediate of the general formula Int E6:



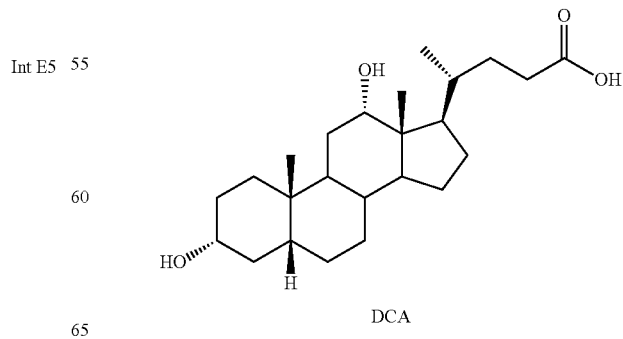
viii) reducing the intermediate of the general formula Int E6 into an intermediate of the general formula Int E7:



ix) reducing the intermediate of the general formula Int E7 into an intermediate of the general formula Int E9



x) elongating the carbon chain of the compound of the general formula Int E9 to obtain an deoxycholic acid (DCA):



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x) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof,

wherein

R_2 is H or a linear or branched C_1 - C_6 -alkyl group;

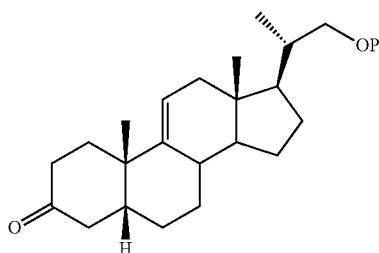
R_3 is H, R_2 or an alcohol protection group; and

P is an alcohol protection group.

Synthetic Route E'

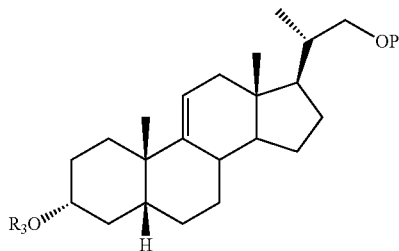
In another interesting embodiment of the invention, the process for the preparation of deoxycholic acid (DCA) or a pharmaceutically acceptable salt thereof, comprises the following steps:

i) providing a compound of the general formula Int E3:



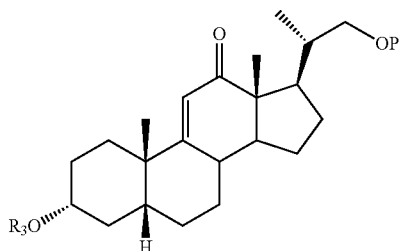
Int E3

ii) reducing the intermediate of the general formula Int E3 into an intermediate of the general formula Int E5:



Int E5

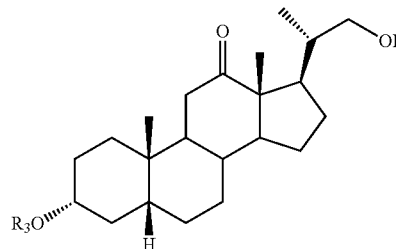
iii) oxidising the intermediate of the general formula Int E5 into an intermediate of the general formula Int E6:



Int E6

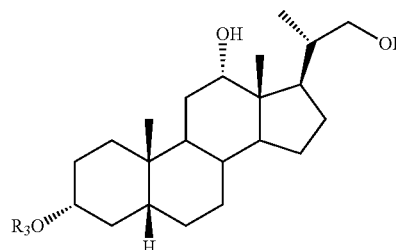
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iv) reducing the intermediate of the general formula Int E6 into an intermediate of the general formula Int E7:



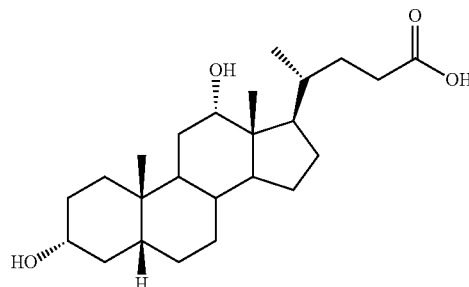
Int E7

v) reducing the intermediate of the general formula Int E7 into an intermediate of the general formula Int E9



Int E9

vi) elongating the carbon chain of the compound of the general formula Int E9 to obtain an deoxycholic acid (DCA):



DCA

vii) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof,

wherein

R_2 is H or a linear or branched C_1 - C_6 -alkyl group;

R_3 is H, R_2 or an alcohol protection group; and

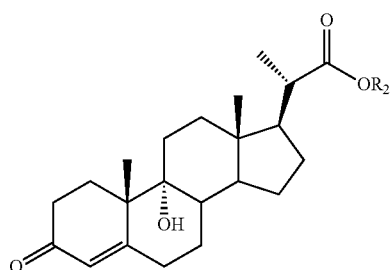
P is an alcohol protection group.

Synthetic Route F

In still another interesting embodiment of the invention, the process for the preparation of deoxycholic acid (DCA) or a pharmaceutically acceptable salt thereof, comprises the following steps:

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i) providing a compound of the general formula SM-a:

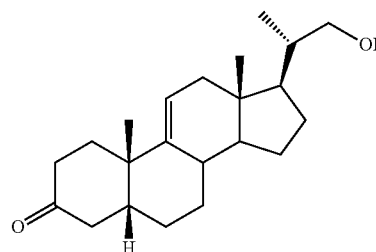


SM-a

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v) reducing the intermediate of the general formula Int F2 into an intermediate of the general formula Int E3:

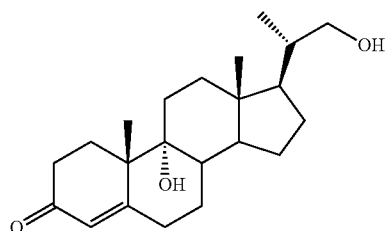


Int E3

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ii) reducing the compound of the general formula SM-a to obtain an intermediate of the general formula SM-b:

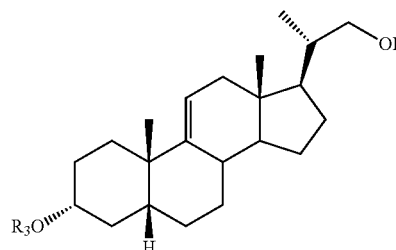


SM-b

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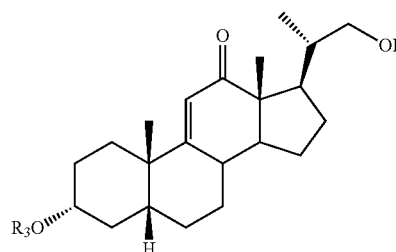
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vi) reducing the intermediate of the general formula Int E3 into an intermediate of the general formula Int E5:



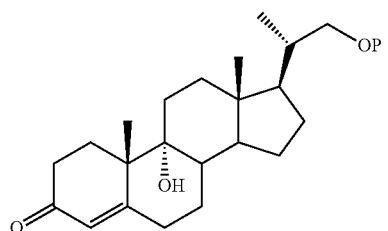
Int E5

vii) oxidising the intermediate of the general formula Int E5 into an intermediate of the general formula Int E6:



Int E6

iii) protecting the alcohol group at position 22 to obtain an intermediate of the general formula Int E1:

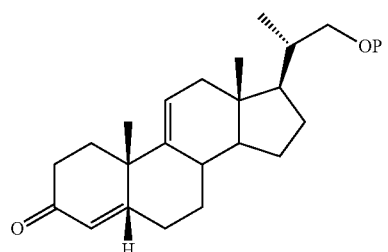


Int E1

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iv) converting the compound of the general formula Int E1 into an intermediate of the general formula Int F2:



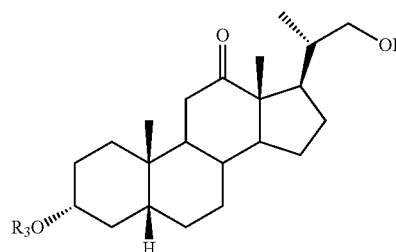
Int F2

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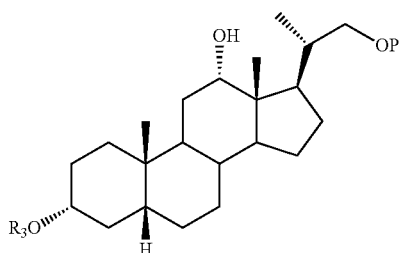
viii) reducing the intermediate of the general formula Int E6 into an intermediate of the general formula Int E7:



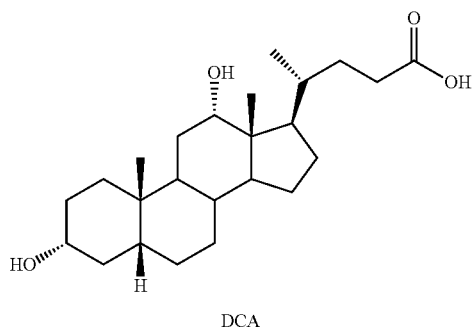
Int E7

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ix) reducing the intermediate of the general formula Int E7 into an intermediate of the general formula Int E9



x) elongating the carbon chain of the compound of the general formula Int E9 to obtain a deoxycholic acid (DCA):



x) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof, wherein

R_2 is H or a linear or branched C_1 - C_6 -alkyl group;

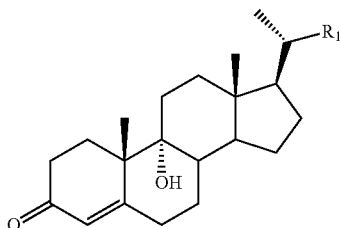
R_3 is H, R_2 or an alcohol protection group; and

P is an alcohol protection group.

The Intermediate Compounds

The Starting Compound—The Intermediate of the General Formula SM

In a further aspect, the present invention relates to a compound of the general formula SM



wherein

R_1 is $COOR_2$, CH_2OP , CH_2X , CH_2CHO , CH_2-CH_2-OH , CH_2-CH_2OP , CH_2-CH_2X or CH_2-CH_2-CHO ;

R_2 is a linear or branched C_1 - C_6 -alkyl group with the proviso that R_2 is not CH_3 ;

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P is an alcohol protection group with the proviso that P is not Ac; and

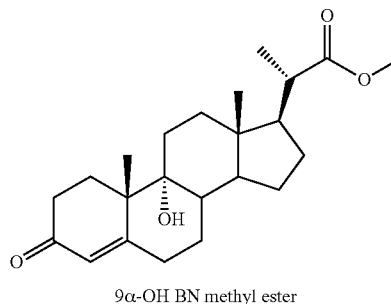
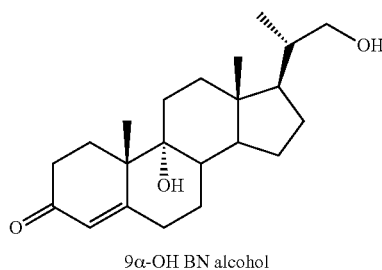
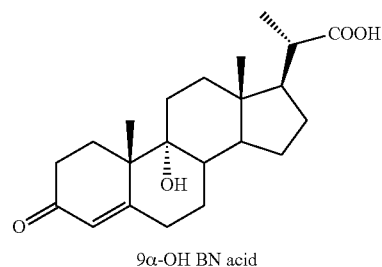
X is a halogen atom.

In a preferred embodiment of the invention, R_1 is $COOR_2$ or CH_2X where R_2 is selected from the group consisting of ethyl, n-propyl and iso-propyl, in particular ethyl, and X is selected from the group consisting of Cl, Br and I, in particular Br.

Accordingly, in one particular interesting embodiment of the invention R_1 is $COOC_2H_5$, and in another particular interesting embodiment of the invention R_2 is CH_2Br .

Such compounds can be obtained from (or easily prepared from compounds obtained from) fermentation products of *Mycobacterium fortuitum* in the presence of an appropriate carbon source.

For example, U.S. Pat. No. 4,029,549 shows the production of 9α -OH BN acid, 9α -OH BN alcohol and 9α -OH BN methyl ester by fermenting the microorganism *Mycobacterium fortuitum* NRRL B-8119 in the presence of either sitosterol (example 2) or cholesterol, stigmasterol or campesterol (example 3). The purification and isolation of 9α -OH BN acid is disclosed in example 5 of U.S. Pat. No. 4,029,549.



Accordingly, steps I) to VI) described herein may be preceded by a step comprising cultivating a 9α -OH BN acid-producing microorganism in an aqueous nutrient medium under aerobic conditions in the presence of a carbon source.

The 9α -OH BN acid-producing microorganism may be selected from the group consisting of *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Microbacterium*, *Nocardia*, *Proaminobacter*, *Serratia*, *Streptomyces* and *Mycobacter*

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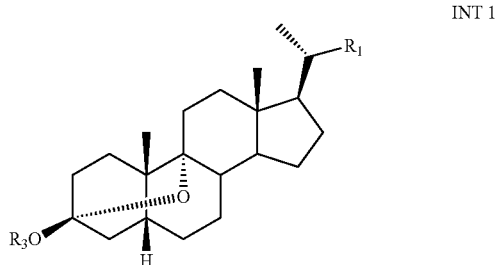
terium. In a preferred embodiment of the invention the 9 α -OH BN acid-producing microorganism is *Mycobacterium*, in particular *Mycobacterium fortuitum*. In the most preferred embodiment of the invention the 9 α -OH BN acid-producing microorganism is *Mycobacterium fortuitum* NRRL B-8119.

The carbon source may be a steroid, such as cholesterol, stigmasterol, campesterol and sitosterol, preferably sitosterol.

As will be understood, 9 α -OH BN acid, 9 α -OH BN alcohol and 9 α -OH BN methyl ester may, if needed, easily be converted into compounds of the general formula SM by standard methods well known to the person skilled in organic chemistry.

The Intermediate of the General Formula INT 1

In a still further aspect, the present invention relates to a compound of the general formula INT 1



wherein

R₁ is COOR₂, CH₂OH, CH₂OP, CH₂X, CH₂CHO, CH₂—CH₂—OH, CH₂—CH₂OP, or CH₂—CH₂X or CH₂—CH₂—CHO;

R₂ is H or a linear or branched C₁-C₆-alkyl group;

P is an alcohol protection group;

R₃ either P or R₂; and

X is a halogen atom.

In a preferred embodiment of the invention, R₁ is COOR₂, CH₂X, CH₂OH or CH₂OP where R₂ is H or selected from the group consisting of methyl, ethyl, n-propyl and isopropyl, in particular H or methyl, and X is selected from the group consisting of Cl, Br and I, in particular Br.

In a more preferred embodiment of the invention R₁ is COOR₂, CH₂X, CH₂OH or CH₂OP where R₂ is H or methyl, and X is Br.

In an even more preferred embodiment of the invention, R₁ is COOR₂ where R₂ is H or selected from the group consisting of methyl, ethyl, n-propyl and isopropyl, in particular H or methyl, and X is selected from the group consisting of Cl, Br and I, in particular Br.

Accordingly, in one particular interesting embodiment of the invention R₁ is COOH or COOCH₃ and R₃ is H or CH₃CO. Specific examples include embodiments where R₁ is COOH and R₃ is H, where R₁ is COOCH₃ and R₃ is H, where R₁ is COOH and R₃ is CH₃CO, and where R₁ is COOCH₃ and R₃ is CH₃CO.

In another highly preferred embodiment of the invention, R₁ is CH₂OH and R₃ is either H or CH₃CO.

In a further highly preferred embodiment of the invention, R₁ is CH₂X and R₃ is either H or CH₃CO, and X is selected from the group consisting of Cl, Br and I, in particular Br. Specific examples include embodiments where R₁ is CH₂Br and R₃ is H, and where R₁ is CH₂Br and R₃ is CH₃CO.

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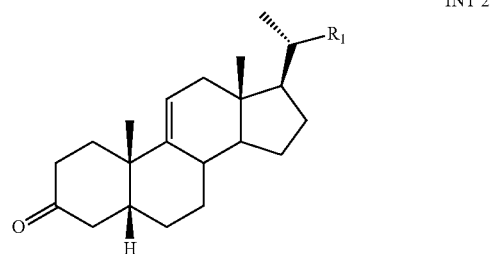
In a still further highly preferred embodiment of the invention R₁ is CH₂OP and R₃ is either H or CH₃CO, wherein P is selected from the group consisting of trimethylsilyl ether (TMS), triethylsilyl ether (TES), triisopropylsilyl ether (TIPS), tert-butyldimethylsilyl ether (TBS, TBDMS), tert-butyldiphenylsilyl ether (TBDPS), acetyl (Ac, COCH₃), benzoyl (Bz), benzyl ether (Bn), 4-methoxybenzyl ether (PMB), 2-naphthylmethyl ether (Nap), methoxymethyl acetal (MOM), 2-methoxyethoxy-methyl ether (MEM), ethoxyethyl acetal (EE), methoxypropyl acetal (MOP), benzyloxymethyl acetal (BOM), tetrahydropyranyl acetal (THP), 2,2,2-trichloro-ethyl carbonate (Troc), methyl ether, dimethoxytrityl (DMT), methoxytrityl (MMT), methylthiomethyl ether, pivaloyl (Piv), tetrahydropyranyl (THP), triphenylmethyl (trityl, Tr), and tosyl (Ts), in particular Ac, TBDMS and Ts.

Thus, specific embodiments include examples where R₁ is CH₂OAc and R₃ is H, where R₁ is CH₂OAc and R₃ is CH₃CO, where R₁ is CH₂OTBDMS and R₃ is H, where R₁ is CH₂OTBDMS and R₃ is CH₃CO, where R₁ is CH₂OTs and R₃ is H, and where R₁ is CH₂OTs and R₃ is CH₃CO.

Compounds of the general formula INT 1 may easily be prepared by reducing compounds of the general formula SM by methods well known to the person skilled in organic chemistry, as described herein.

The Intermediate of the General Formula INT 2

In a still further aspect, the present invention relates to a compound of the general formula INT 2



wherein

R₁ is COOR₂, CH₂OH, CH₂OP, CH₂X, CH₂CHO, CH₂—CH₂—OH, CH₂—CH₂OP, CH₂—CH₂X or CH₂—CH₂—CHO;

R₂ is H or a linear or branched C₁-C₆-alkyl group;

P is an alcohol protection group; and

X is a halogen atom.

In a preferred embodiment of the invention, R₁ is COOR₂, CH₂X, CH₂OH or CH₂OP where R₂ is H or selected from the group consisting of methyl, ethyl, n-propyl and isopropyl, in particular methyl, and X is selected from the group consisting of Cl, Br and I, in particular Br.

In a more preferred embodiment of the invention R₁ is COOR₂, CH₂X, CH₂OH or CH₂OP where R₂ is methyl, and X is Br.

Accordingly, in one particular interesting embodiment of the invention R₁ is COOCH₃. In another particular interesting embodiment of the invention R₁ is CH₂Br. In a further particular interesting embodiment of the invention R₁ is CH₂OH.

In a further highly preferred embodiment of the invention R₁ is CH₂OP, wherein P is selected from the group consisting of trimethylsilyl ether (TMS), triethylsilyl ether (TES),

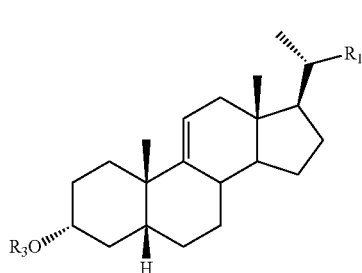
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triisopropylsilyl ether (TIPS), tert-butyldimethylsilyl ether (TBS, TBDMS), tert-butyldiphenylsilyl ether (TBDPS), acetyl (Ac, COCH₃), benzoyl (Bz), benzyl ether (Bn), 4-methoxybenzyl ether (PMB), 2-naphthylmethyl ether (Nap), methoxymethyl acetal (MOM), 2-methoxyethoxymethyl ether (MEM), ethoxyethyl acetal (EE), methoxypropyl acetal (MOP), benzyloxymethyl acetal (BOM), tetrahydropyranyl acetal (THP), 2,2,2-trichloro-ethyl carbonate (Troc), methyl ether, dimethoxytrityl (DMT), methoxytrityl (MMT), methylthiomethyl ether, pivaloyl (Piv), tetrahydropyranyl (THP), triphenylmethyl (trityl, Tr), and tosyl (Ts), in particular Ac, TBDMS and Ts. Thus, specific embodiments include examples where R₁ is CH₂OAc, where R₁ is CH₂OTBDMS and where R₁ is CH₂OTs.

Compounds of the general formula INT 2 may easily be prepared by oxidising compounds of the general formula INT 1 by methods well known to the person skilled in organic chemistry, as described herein.

The Intermediate of the General Formula INT 3

In an even further aspect, the present invention relates to a compound of the general formula INT 3



wherein

R₁ is COOR₂, CH₂OH, CH₂OP, CH₂X, CH₂CHO, CH₂—CH₂—OH, CH₂—CH₂OP, CH₂—CH₂X or CH₂—CH₂—CHO;

R₂ is H or a linear or branched C₁-C₆-alkyl group; P is an alcohol protection group;

R₃ is either P or R₂; and

X is a halogen atom;

with the proviso that R₁ is not CH₂—CH₂—OH when R₃ is H; R₁ is not CH₂—CH₂OAc when R₃ is Ac; and R₁ is not COOCH₃ when R₃ is Ac.

In a preferred embodiment of the invention, R₁ is COOR₂, CH₂X, CH₂OH or CH₂OP where R₂ is selected from the group consisting of ethyl, n-propyl and iso-propyl, in particular ethyl, and X is selected from the group consisting of Cl, Br and I, in particular Br.

In an interesting embodiment of the invention R₁ is COOCH₃ and R₃ is H.

In another interesting embodiment of the invention R₁ is CH₂X, wherein X is selected from the group consisting of Cl, Br and I, and R₃ is H or Ac. Particular examples are where X is Br and R₃ is H, and where X is Br and R₃ is Ac.

In another interesting embodiment of the invention R₁ is CH₂OP and R₃ is either H or CH₃CO, wherein P is selected from the group consisting of trimethylsilyl ether (TMS), triethylsilyl ether (TES), triisopropylsilyl ether (TIPS), tert-butyldimethylsilyl ether (TBS, TBDMS), tert-butyldiphenylsilyl ether (TBDPS), acetyl (Ac, COCH₃), benzoyl (Bz), benzyl ether (Bn), 4-methoxybenzyl ether (PMB), 2-naphthylmethyl ether (Nap), methoxymethyl acetal (MOM),

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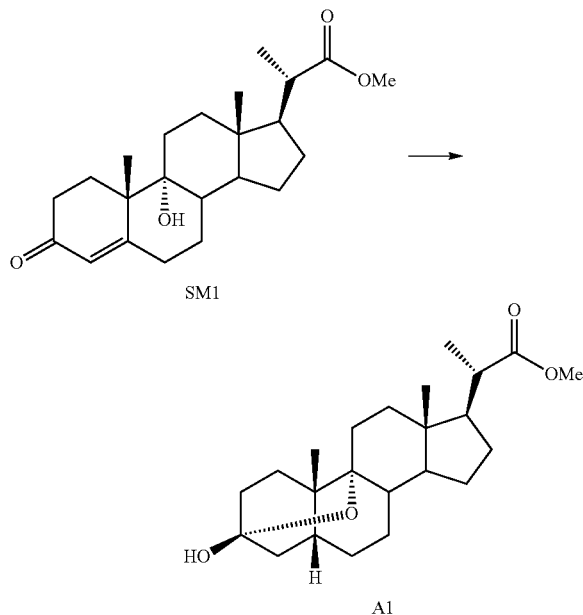
2-methoxyethoxy-methyl ether (MEM), ethoxyethyl acetal (EE), methoxypropyl acetal (MOP), benzyloxymethyl acetal (BOM), tetrahydropyranyl acetal (THP), 2,2,2-trichloro-ethyl carbonate (Troc), methyl ether, dimethoxytrityl (DMT), methoxytrityl (MMT), methylthiomethyl ether, pivaloyl (Piv), tetrahydropyranyl (THP), triphenylmethyl (trityl, Tr), and tosyl (Ts), in particular Ac, TBDMS and Ts. Thus, specific embodiments include examples where R₁ is CH₂OAc and R₃ is H, where R₁ is CH₂OAc and R₃ is CH₃CO, where R₁ is CH₂OTBDMS and R₃ is H, where R₁ is CH₂OTBDMS and R₃ is CH₃CO, where R₁ is CH₂OTs and R₃ is H, and where R₁ is CH₂OTs and R₃ is CH₃CO.

Compounds of the general formula INT 3 may easily be prepared by reducing compounds of the general formula INT 2 by methods well known to the person skilled in organic chemistry, as described herein.

As will be understood, the intermediates disclosed herein can be used for the preparation of DCA and pharmaceutically acceptable salts thereof. Since the synthetic routes described herein allow for introduction of an —OH group in position 12, it is contemplated that the same intermediates will also be suitable for preparing other bile acids, which include an —OH group in position 7. Specific examples of such bile salts include cholic acid, glycocholic acid, taurocholic acid, or a pharmaceutically acceptable salt thereof.

EXAMPLES

Example 1

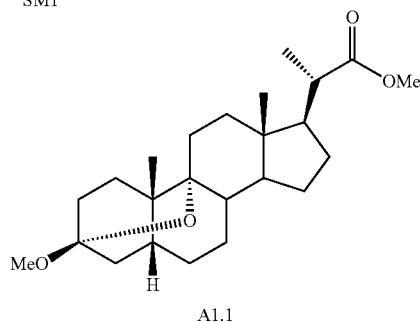
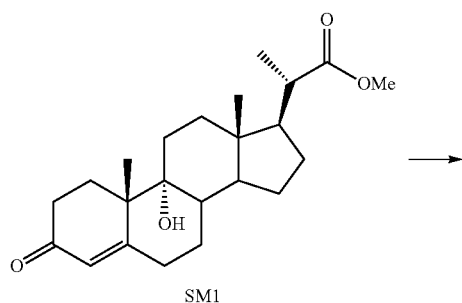


40 g of compound SM1 (106.80 mmol) was suspended in 150 ml of DMF, then 2.77 g of dry Pd/C 10% was added. The reaction mixture was stirred at 70° C. and hydrogenated (3.5 atm) overnight. The mixture was filtered through Celite®. Then, the mixture was poured over water forming a precipitate. The precipitate was filtered off as a white solid, washed with water and dried under vacuum, thereby yielding 39.3 g of compound A1.

¹H NMR (400 MHz, CDCl₃): δ 3.56 (s, 3H); 2.30 (m, 1H); 1.10 (d, 3H); 0.87 (s, 3H); 0.62 (s, 3H).

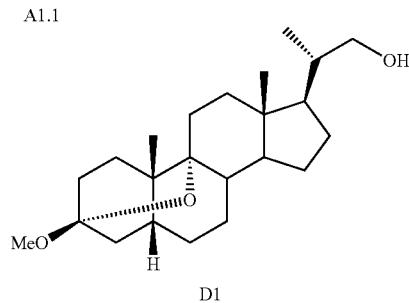
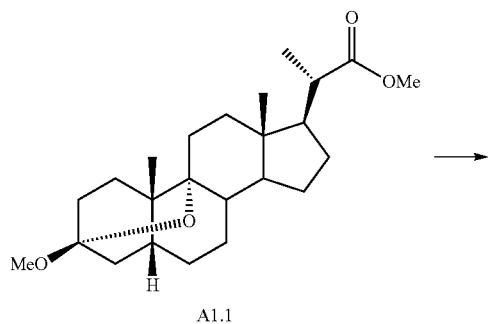
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Example 2



20 g of compound SM1 (53.40 mmol) was suspended in 150 ml of MeOH, then 1.4 g of dry Pd/C 10% was added. The reaction mixture was stirred at 70° C. and hydrogenated (1.0 atm) overnight. 1.0 g of p-TsOH (10% molar, 5.3 mmol) was added and stirred for 8 h. The mixture was filtered through Celite®. The solvent was evaporated under vacuum. The solid was recrystallized in 60 ml of EtOH. The solid was filtered off and dried under vacuum, yielding 18.8 g of compound A1.1.

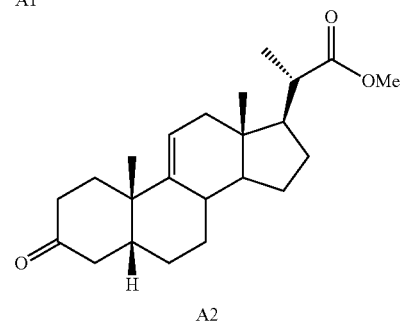
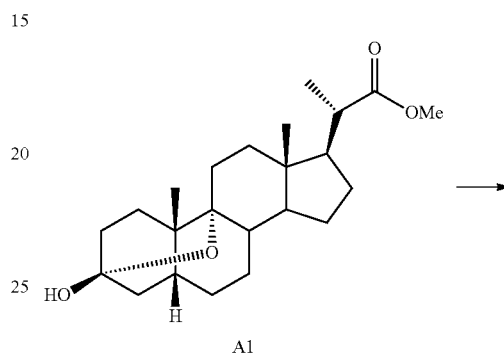
Example 3



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LiAlH₄ (1.88 g, 49.63 mmol, 1.3 eq.) and THF (20 ml) were mixed in an inert atmosphere. A mixture of A1.1 (14.0 g) and 40 ml of THF was added dropwise. The mixture was stirred overnight at room temperature until the reaction was completed. The mixture was then cooled at 0-5° C. and was quenched by dropwise addition of an aqueous solution of Na₂SO₄·10H₂O (16.20 g) and THF (50 ml). The precipitate was filtered off, the solvent was evaporated under reduced pressure. The solid was recrystallized in 150 ml EtOH. The solid was filtered off and dried under vacuum, thereby yielding 10.15 g of D1.

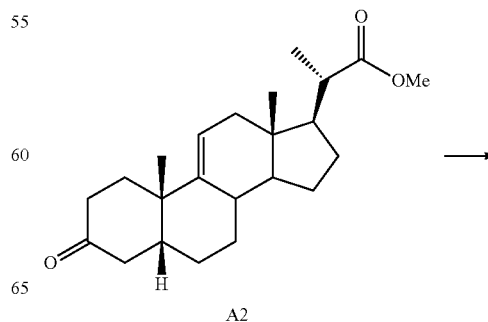
Example 4



39, 3 g of A1 (104.37 mmol) was dissolved in DCM (100 ml) and stirred at room temperature. Sulphuric acid (9.29 g, 0.9 eqv) was added at 10° C., then the mixture is stirred overnight. The reaction mixture was worked up by washing with water and NaHCO₃ and the organic layer was separated. The organic layer was concentrated followed by column chromatography to yield 28.8 g of A2.

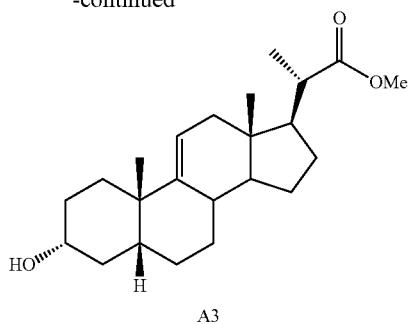
¹H NMR (400 MHz, CDCl₃): δ 5.50 (s, 1H); 3.57 (s, 3H); 1.15 (d, 3H); 1.09 (s, 3H); 0.59 (s, 3H).

Example 5



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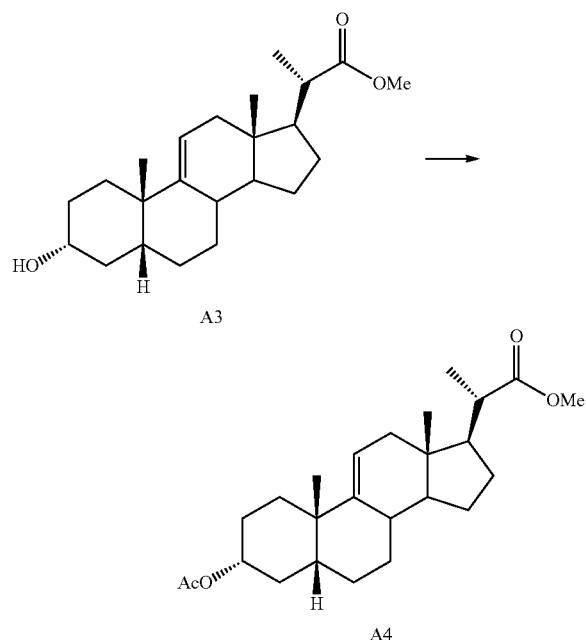
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28.6 g of A2 (79.87 mmol) was dissolved and stirred in THF (100 ml) under inert atmosphere. The solution was cooled at 0-5° C. and $\text{LiAlH}(\text{OtBu})_3$ (22.34 g, 1.1 eqv) was added slowly (exothermic reaction). The mixture was stirred at room temperature until the reaction was complete. The mixture was cooled at 0-5° C. and was hydrolyzed slowly with a solution of 1M HCl. The aqueous phase was extracted with EtOAc and the organic phase is washed with a solution of NaHCO_3 . The solvent was evaporated under reduced pressure, yielding 27.62 g of A3. ^1H NMR (400 MHz, CDCl_3): δ 0.59 (s, 3H); 1.05 (s, 3H); 1.18 (d, 3H); 2.43 (m, 2H); 3.65 (s, 3H); 3.65 (m, 1H); 5.32 (dd, 1H).

^1H NMR (400 MHz, CDCl_3): δ 5.29 (s, 1H); 3.57 (s, 3H); 3.39 (m, 1H); 1.11 (d, 3H); 1.01 (s, 3H); 0.55 (s, 3H).

Example 6



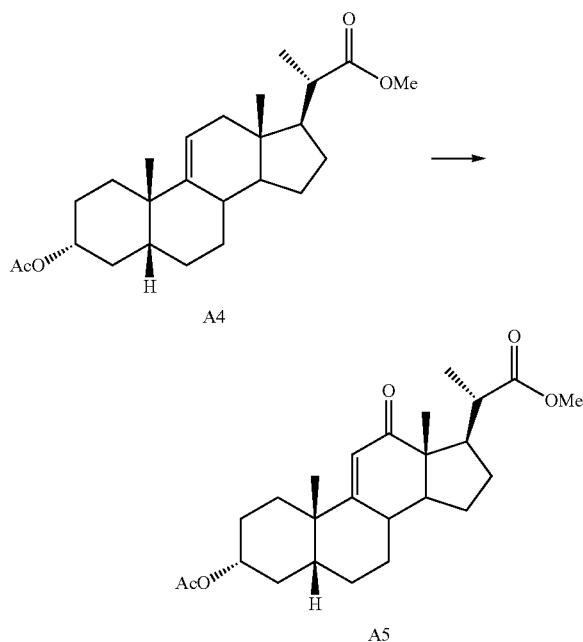
A3 27.62 g (76.61 mmol) (10 g) was dissolved in DCM (70 ml) at room temperature. Then, Triethylamine 50 ml (6.66 eqv), Acetic anhydride 8.85 g (3.33 eq) and DMAP (3.40 g) were added, keeping the temperature below 10° C. The mixture stirred until reaction was complete. The organic phase was concentrated under reduced pressure and the solid was suspended in 60 mL of DCM and then washed with a solution of 1M HCl. The solvent was evaporated under

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reduced pressure thereby yielding 32.29 g of A4. ^1H NMR (400 MHz, CDCl_3): δ 0.58 (s, 3H); 1.04 (s, 3H); 1.16 (d, 3H); 1.99 (s, 3H); 2.41 (m, 2H); 3.63 (s, 3H); 4.71 (m, 1H); 5.31 (dd, 1H).

^1H NMR (400 MHz, CDCl_3): δ 5.31 (s, 1H); 3.60 (s, 3H); 2.00 (m, 3H); 1.17 (d, 3H); 0.59 (s, 3H).

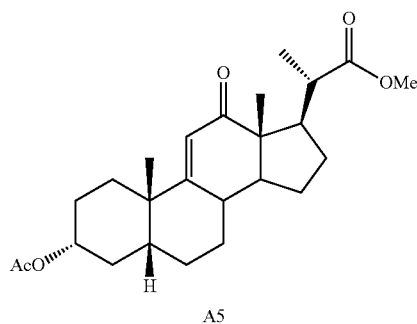
Example 7



27 g of compound A4 (64.5 mmol) was suspended in 300 ml of AcOH and then anhydrous CrO_3 (27.73 g, 4.30 eqv) was added. The suspension was heated at 60° C. The reaction mixture was stirred for 0.5 h until the reaction was complete. Then, the mixture was poured over 250 mL of water and a precipitate was formed. The organic phase was washed with water. The operation was repeated two more times. The organic phase were concentrated until an oily residue was obtained. The residue was purified on silica gel yielding 13.05 g of pure A5.

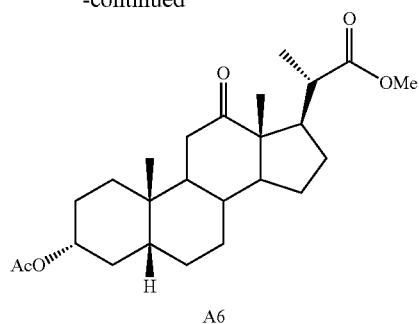
^1H NMR (400 MHz, CDCl_3): δ 0.84 (s, 3H); 1.17 (s, 3H); 1.30 (d, 3H); 1.98 (s, 3H); 3.63 (s, 3H); 4.72 (m, 1H); 5.72 (s, 1H).

Example 8



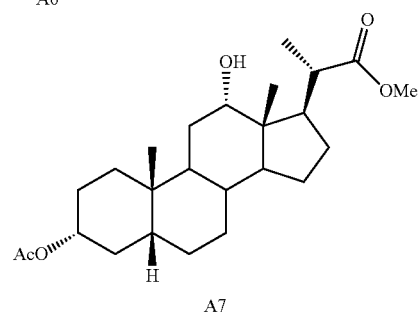
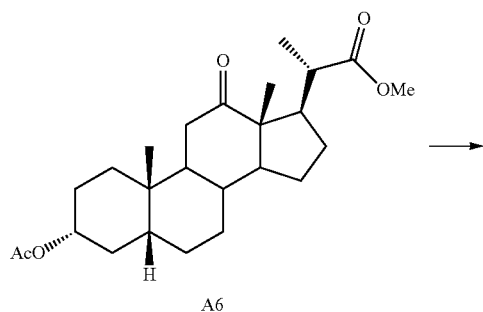
45

-continued



11 g of A5 (28.31 mmol) was dissolved in 65 ml of AcOEt followed by addition of 2.75 g dry Pd/C 10% (25% weight). The reaction mixture was stirred at 70° C. and hydrogenated (4.1 atm) overnight. The mixture was filtered through Celite® and the solvent was evaporated under vacuum, thereby yielding 11.02 g of A6 (a white solid. ¹H NMR (400 MHz, CDCl₃): δ 0.97 (s, 3H); 1.00 (s, 3H); 1.15 (d, 3H); 2.00 (s, 3H); 2.46 (m, 2H); 3.63 (s, 3H); 4.68 (m, 1H).

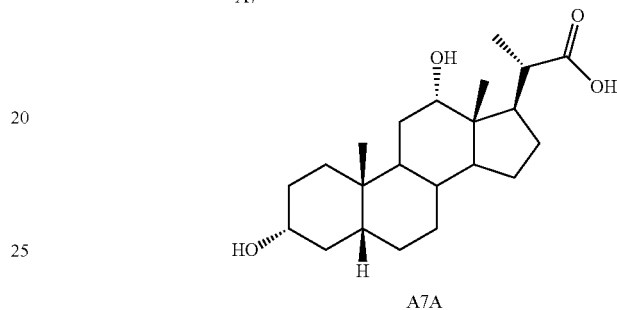
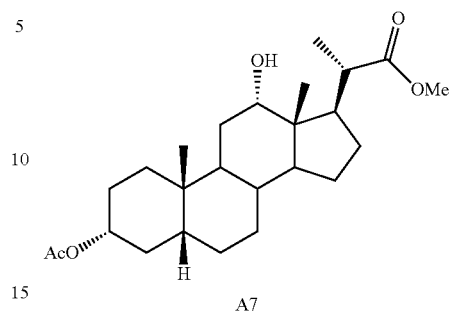
Example 9



11.02 g of A6 (26.30 mmol) was dissolved and stirred in THF (40 ml) under an inert atmosphere. The solution was cooled at 0-5° C. LiAlH(OtBu)₃ (1.5 eqv, 10.0 g, 39.45 mmol) was added dropwise (exothermic reaction). The mixture was stirred at room temperature until the reaction was complete. The mixture was cooled at 0-5° C. and was then quenched by adding an aqueous solution of 1M HCl. The aqueous phase was extracted with EtOAc and the organic phase was washed with a solution of NaHCO₃. The solvent was evaporated under reduced pressure, thereby yielding 11.16 g of A7. ¹H NMR (400 MHz, CDCl₃): δ 0.66 (s, 3H); 0.89 (s, 3H); 1.20 (d, 3H); 2.00 (s, 3H); 3.62 (s, 3H); 3.92 (m, 1H); 4.72 (m, 1H).

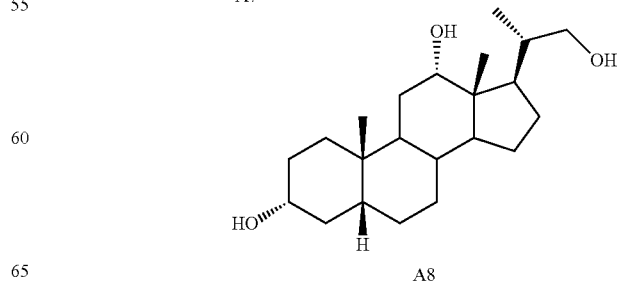
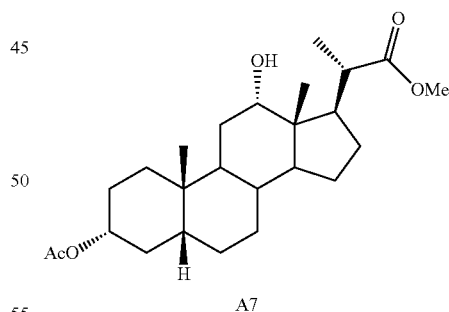
46

Example 10



3.00 g of A7 (7.13 mmol) was dissolved and stirred in a mixture of THF (30 ml) and MeOH (30 ml) under an inert atmosphere at room temperature. LiOH (4M, 30 ml) was added. The solution was heated at 60° C. The mixture was stirred until the reaction was complete (6 h). The mixture was cooled at room temperature. The solvent was evaporated and was quenched by adding an aqueous solution of HCl 2N until acidic pH. The precipitate was filtered off as a palid yellow solid, washed with water and EtOAc, and then dried under vacuum yielding 2.53 g of A7A.

Example 11

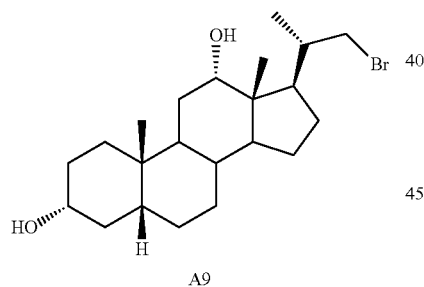
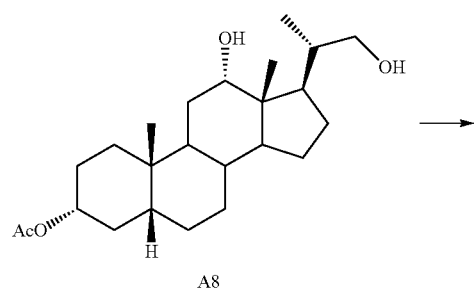


47

0.3 g of A7 (0.71 mmol) was dissolved and stirred in dry THF (7 ml) under an inert atmosphere. The solution was cooled at 0-5° C. LiAlH₄ (0.06 g, 1.49 mmol) was added dropwise (exothermic reaction). The mixture was stirred at room temperature until the reaction was completed. The mixture was cooled at 0-5° C. and was quenched by addition Na₂SO₄·10H₂O. The precipitate was filtered off and washed with THF. The solvent was evaporated under reduced pressure and the solid obtained was washed with EtOAc, thereby yielding 0.198 g of A8.

¹H NMR (400 MHz, CDCl₃): δ 3.95 (t, J=2.8 Hz, 1H), 3.56 (dd, J=10.5, 3.4 Hz, 1H), 3.50 (td, J=11.1, 5.5 Hz, 1H), 3.21 (dd, J=10.5, 7.7 Hz, 1H), 1.95-1.70 (m, 6H), 1.66-1.22 (m, 16H), 1.19-1.10 (m, 2H), 1.07 (d, J=6.6 Hz, 3H), 1.02-0.94 (m, 1H), 0.92 (s, 3H), 0.72 (s, 3H).

Example 12

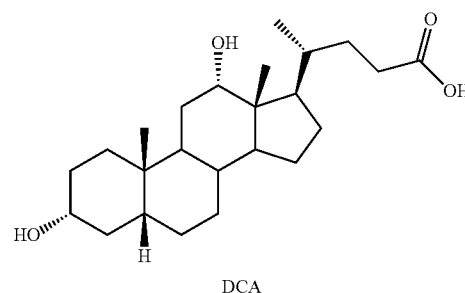
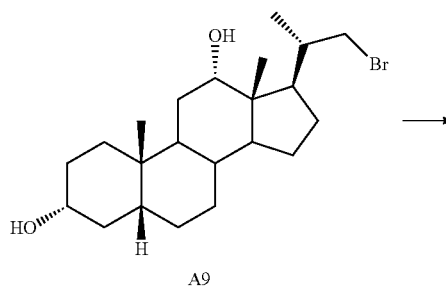


0.025 g of A8 (0.078 mmol) was dissolved and stirred in DCM (2 ml). CBr₄ (2.4 eqv, 0.062 g, 0.09 mmol) and triphenylphosphine (PPh₃, 2.5 eqv, 0.051 g, 2.5 mmol) was added. The solution was heated under reflux. The mixture was stirred until the reaction was completed. The mixture was cooled at room temperature. The residue was purified on silica gel yielding 0.05 g of A9.

¹H NMR (400 MHz, CDCl₃): δ 3.93 (t, J=2.8 Hz, 1H), 3.63-3.52 (m, 1H), 3.48 (dt, J=5.9, 2.9 Hz, 1H), 3.29 (dd, J=9.7, 6.5 Hz, 1H), 1.90-1.15 (m, 23H), 1.11 (d, J=6.5 Hz, 3H), 0.94 (ddd, J=12.6, 9.9, 2.5 Hz, 1H), 0.87 (s, 3H), 0.67 (s, 3H).

48

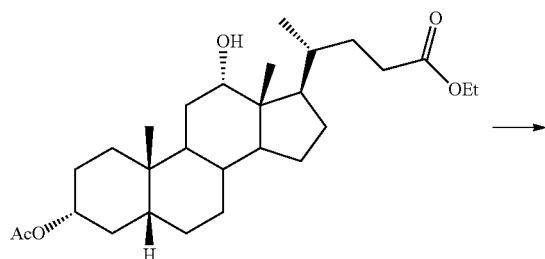
Example 13



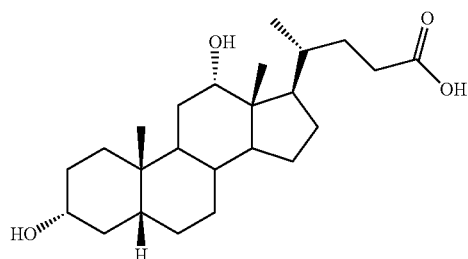
0.5 g of NaH 60% (12.5 mmol) was dissolved and stirred in dry DMF (10 ml) under an inert atmosphere. Diethyl malonate (2.0 g, 12.48 mmol) dissolved in 3.0 ml of DMF was added dropwise. The solution was heated and stirred until the mixture was turned clear. The mixture was cooled at 40° C. A9 (5.12 g, 12.4 mmol) dissolved in 3.0 ml of DMF was added. The solution was heated at 60° C. The mixture was quenched by addition of water (15 ml). The aqueous phase was extracted with EtOAc. The solvent was evaporated under reduced pressure and the residue was suspended in an aqueous solution of KOH 2.8 M (10.0 ml). The solution was heated under reflux. Water (10 ml) was added and the organic solvent was evaporated under reduced pressure. The aqueous phase was acidified by adding 2N HCl and was extracted with EtOAc. The solvent was evaporated under reduced pressure and the residue was suspended in a mixture of dioxane (5 ml) and 12N HCl (10 ml). The mixture was heated under reflux for 24 hours. The mixture was cooled at room temperature and was extracted with EtOAc. The organic phases were mixed and evaporated under reduced pressure. The residue was purified on silica gel thereby yielding 3.1 g of DCA.

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Example 14



B2.1

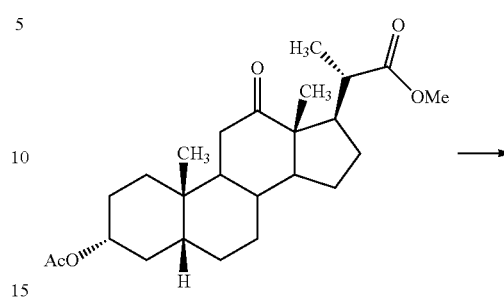


DCA

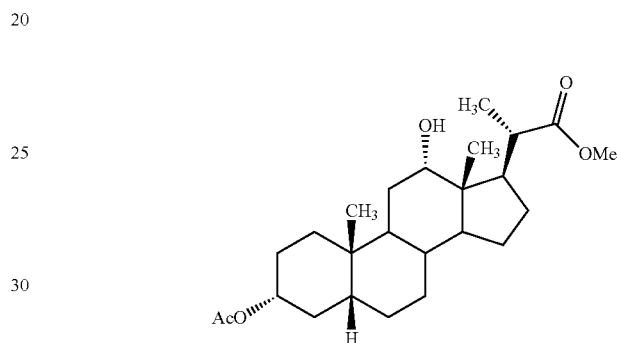
0.42 g of B2.1 (0.92 mmol) was dissolved and stirred in THF (8 ml). Water (8 ml) was added and stirred at room temperature. A solution of LiOH 4M (2.0 ml) was added. The mixture was heated at 50° C. and stirred overnight. The mixture was poured over water. The aqueous phase was extracted with EtOAc. The organic phase was washed with an aqueous solution of 2N HCl. The aqueous phase was extracted with EtOAc and the combined organic layers were evaporated under reduced pressure, thereby yielding 0.38 g of DCA. ¹H NMR (400 MHz, CDCl₃): δ 3.94 (t, J=2.7 Hz, 1H), 3.58-3.45 (m, 1H), 2.41-2.11 (m, 2H), 1.99-1.71 (m, 7H), 1.67-1.04 (m, 19H), 0.99 (d, J=6.4 Hz, 3H), 0.92 (s, 3H), 0.70 (s, 3H)

50

Example 15



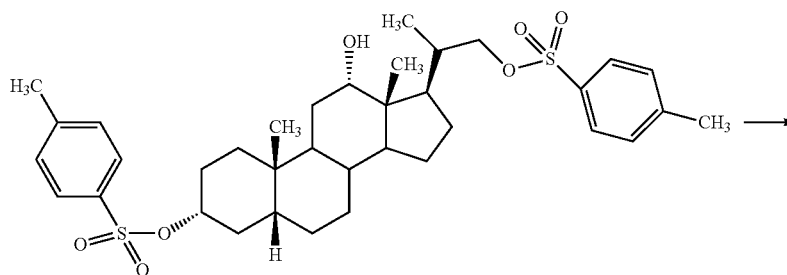
A6



A7

10.5 g of A6 (24.0 mmol) were dissolved and stirred in THF (137 ml) under inert atmosphere. The solution was cooled at -40° C. LiAl(OtBu)₃H (1.1 eq, 6.6 g, 26.0 mmol) was added dropwise (exothermic reaction). The mixture was stirred at -20° C. until the reaction was complete. The solvent was evaporated under reduced pressure then the mixture was cooled at 0/5° C. The solid was filtered off, washed with water and dried, affording 10.55 g of compound A7.

Example 16

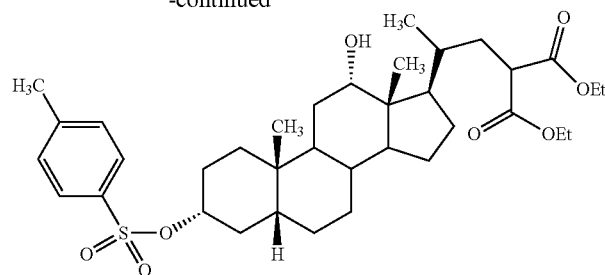


A9.1

51

52

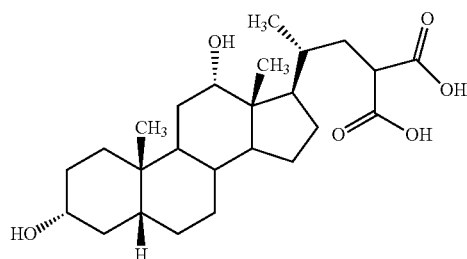
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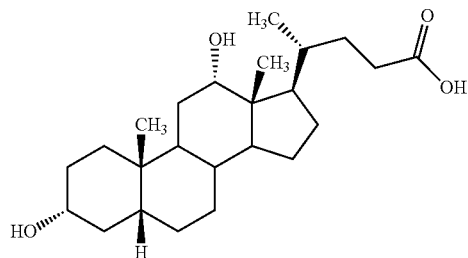
A10.1

1.6 g of NaH 60% (39.5 mmol) were dissolved and stirred in dry DMF (0.5 ml) under inert atmosphere. Diethyl malonate (6.2 ml, 4.02 mmol) dissolved in 0.5 ml of DMF were added dropwise. A suspension of the steroid intermediate (6.1 g, 9.8 mmol) in 1.0 ml DMF were added dropwise. The solution was heated and was stirred at 60° C. The mixture was quenched by adding water (15 ml). The aqueous phase was extracted with EtOAc. The solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel.

Example 17



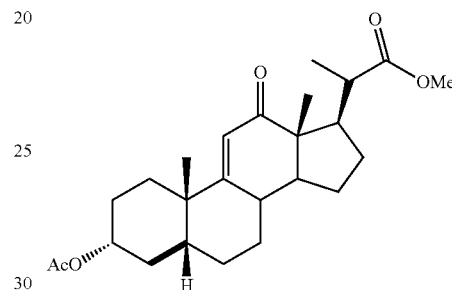
A11



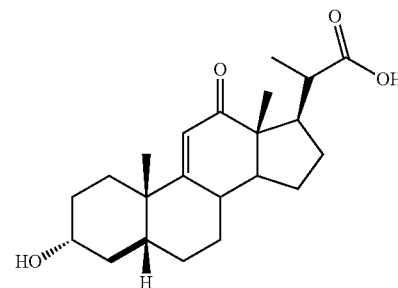
DCA

A11 (1.8 g, 4.12 mmol) were suspended in 108 ml of Xylene. The suspension was heated under reflux. The mixture was slowly cooled at 20/25° C. Water (54 ml) and EtOAc (270 ml) were added. The aqueous phase was cooled at 10° C. and acidified by adding HCl 2N. The mixture was stirred and the solid was filtered off, washed with water and dried, affording 1.04 g of Deoxycholic acid.

Example 18



A5



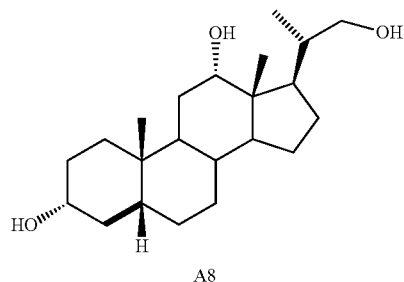
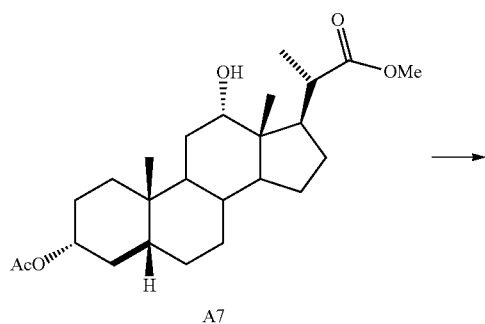
A5.1

4.00 g of A5 (9.6 mmol) was dissolved and stirred in MeOH (150 ml) under an inert atmosphere at room temperature. NaOH 20% (40 ml, 22 mmoles) was added. The solution was heated at reflux. The mixture was stirred until the reaction was complete (3 h). The mixture was cooled at room temperature. The solvent was evaporated and was quenched by adding an aqueous solution of HCl 6N until acidic pH. The precipitate was filtered off as a solid, washed with water and EtOH, and then dried under vacuum yielding 3.4 g (95%) of A5.1.

¹H-RMN (400 MHz, DMSO-d₆) δ=0.57 (s, 3H); 0.81 (s, 3H); 1.09 (d, J=6 Hz, 3H); 3.71 (m, 1H).

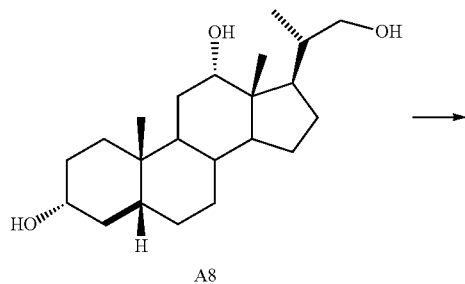
53

Example 19



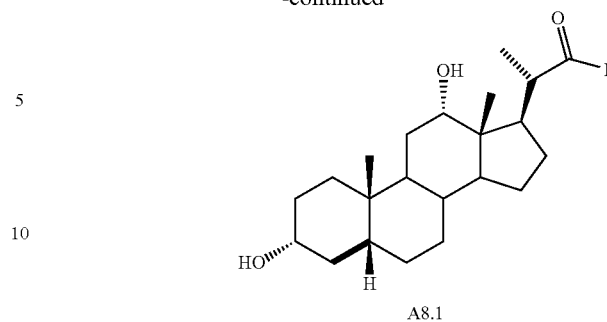
3.0 g of A7 (7.14 mmol) was dissolved and stirred in THF (75 ml) under inert atmosphere. The solution was cooled at 0-5° C. and LiAlH_4 (1.1 g, 28.6 mmol) was added slowly (exothermic reaction). The mixture was stirred at room temperature then heated under reflux until the reaction was complete. The mixture was cooled at room temperature and was hydrolyzed slowly with a solution of water (1.1 ml), NaOH (20%) 1.1 ml and water (3.3 ml). The white solid obtained was filtered off and was washed THF (150 ml). The organic phase was dried with anhydrous Magnesium sulfate. The solvent was evaporated under reduced pressure, yielding 2.3 g (92%) of A8.

Example 20



54

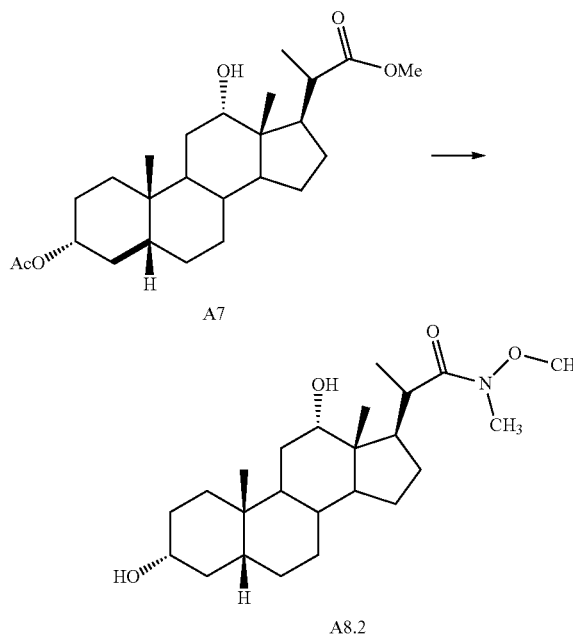
-continued



0.2 g of compound A8 (0.57 mmol) was suspended in a mixture of DCM (6.0 ml) and ACN (6.0 ml) under inert atmosphere and then Dess-Martin Periodinane reagent (0.24 g, 0.57 mmol) and 4-methylmorpholine 4-oxide (11 mg, 0.07 mmol) were added. The suspension was stirred at room temperature until the reaction was complete. Then, a solution of $\text{Na}_2\text{S}_2\text{O}_3$ (1M, 50 mL) was added. The aqueous phase was extracted with DCM (3×50 ml) and then was washed with brine. The organic phase were concentrated until a solid was obtained, yielding 0.25 g of A8.1.

^1H NMR (400 MHz, CDCl_3): δ 9.7 (d, 1H); 9.5 (d, 1H); 3.6 (m, 1H); 3.4 (bs, 1H); 2.3 (qd, $J=9$ Hz, 1H).

Example 21



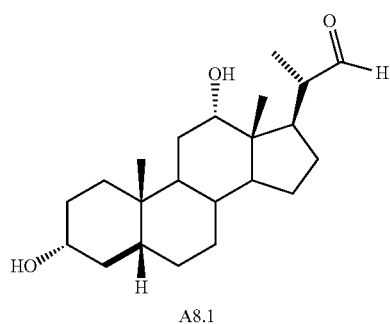
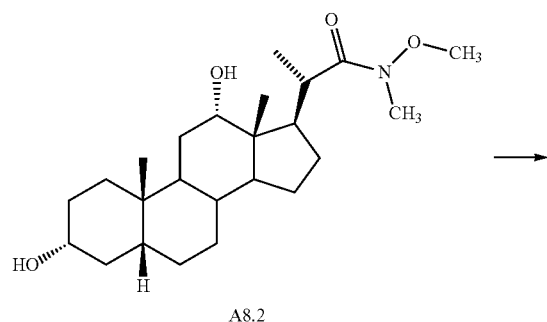
2.00 g of A7 (4.76 mmol) was dissolved and stirred in MeOH (100 ml) under inert atmosphere at room temperature. NaOH 20% (20 ml) was added. The solution was heated at 80° C. The mixture was stirred until the reaction was complete (3 h). The mixture was cooled at room temperature. The solvent was evaporated and the mixture was quenched by adding an aqueous solution of HCl 6N until acidic pH. The precipitate was filtered off as a solid, washed with water and MeOH, and then dried under vacuum yielding 1.4 g (80%) of acid intermediate.

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9.0 g of intermediate acid compound (25 mmol), DCC (6.2 g, 30 mmol), DMAP (3.7 g, 30 mmol) and N,O-dimethylhydroxylamine hydrochloride (4.9 g, 50 mmol) were dissolved in DCM (250 ml) under inert atmosphere. Et₃N (10 ml) was added and the suspension was stirred at room temperature until the reaction was complete. Then, the organic phase was washed with brine. The organic phase was concentrated until a solid was obtained. The solid was purified by column chromatography (AcOEt/Heptane), yielding 5.9 g of A8.2.

¹H NMR (400 MHz, CDCl₃): δ 3.9 (bs, 1H); 3.66 (s, 3H); 3.61 (m, 1H); 3.15 (s, 3H); 2.4 (q, J=9 Hz, 1H).

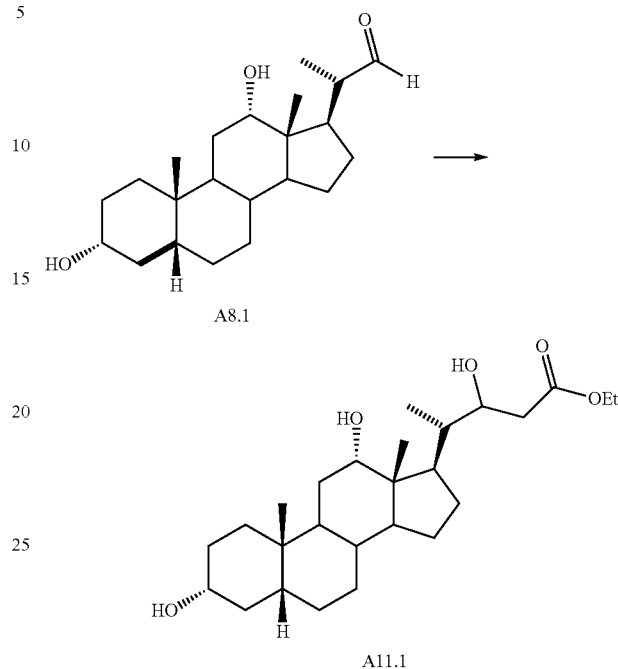
Example 22



2.5 g of A8.2 (6.14 mmol) was dissolved and stirred in THF (25 ml) under inert atmosphere. The solution was cooled at 0-5° C. and was added slowly (exothermic reaction) to a solution of LiAlH₄ (0.36 g, 9.2 mmol) in THF (75 ml). The mixture was stirred at 0-5° C. until the reaction was complete. The mixture was hydrolyzed slowly with a solution of water (0.25 ml), NaOH (20%) 0.25 ml and water (0.75 ml). The white solid obtained was filtered off and was washed THF (100 ml). The organic phase were concentrated and was purified by column chromatography (AcOEt/Heptane), yield 85% of A8.2.

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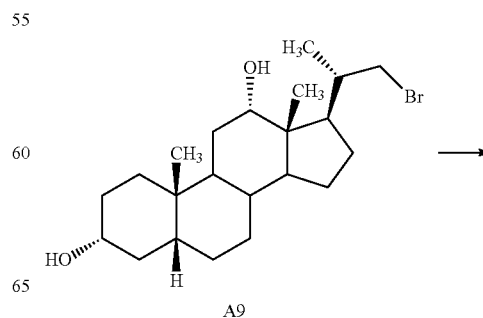
Example 23



0.26 g of Zn (4.1 mmol) was suspended in THF under inert atmosphere. Trimethylchlorosilane (0.1 mmol) were added. The suspension was stirred at heated under reflux 1 hour. Then, a solution of 0.2 g of A8.1 (0.57 mmol) and Ethyl Bromoacetate 0.3 ml (2.85 mmol) in THF (20 ml) were added. The mixture was stirred at heated under reflux until the reaction was complete. The mixture was cooled at room temperature. The solvent was evaporated and the mixture was quenched by adding an aqueous saturated solution of NH₄C₁ (50 ml). EtOAc (75 ml) was added. The organic phase was washed with an aqueous saturated solution of NaCl. The organic phase were concentrated and the solid was purified by column chromatography (EtOAc/Heptane).

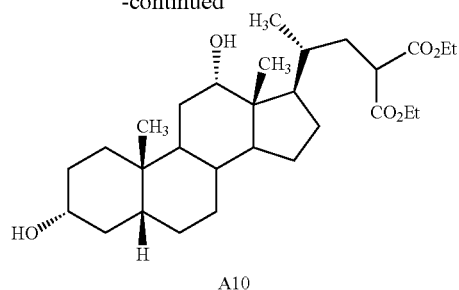
¹H NMR (400 MHz, CDCl₃): δ 4.35 (dt, J=9 Hz, J=3 Hz, 1H); 4.55 (q, J=7.5 Hz, 2H); 3.35 (m, 1H); 2.7 (dd, J=17 Hz, J=9 Hz, 1H); 2.75 (dd, J=17 Hz, J=3 Hz, 1H).

Example 24



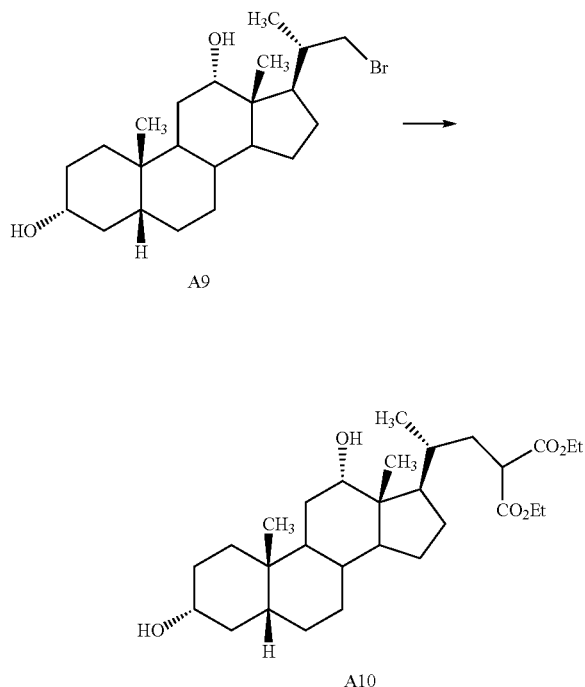
57

-continued



0.41 g of EtONa (0.60 mmol) was dissolved and stirred in EtOH (2 ml) under an inert atmosphere and was cooled at 0° C. Diethyl malonate (0.97 g, 0.60 mmol) was added to the mixture. The mixture was heated at room temperature and A9 (0.20 g, 0.48 mmol) were added. The solution was heated at 90° C. The mixture was quenched by addition of water (5 ml). The aqueous phase was extracted with EtOAc. The organic phases were evaporated under reduced pressure. The residue was purified on silica gel thereby yielding 0.12 g of A10.

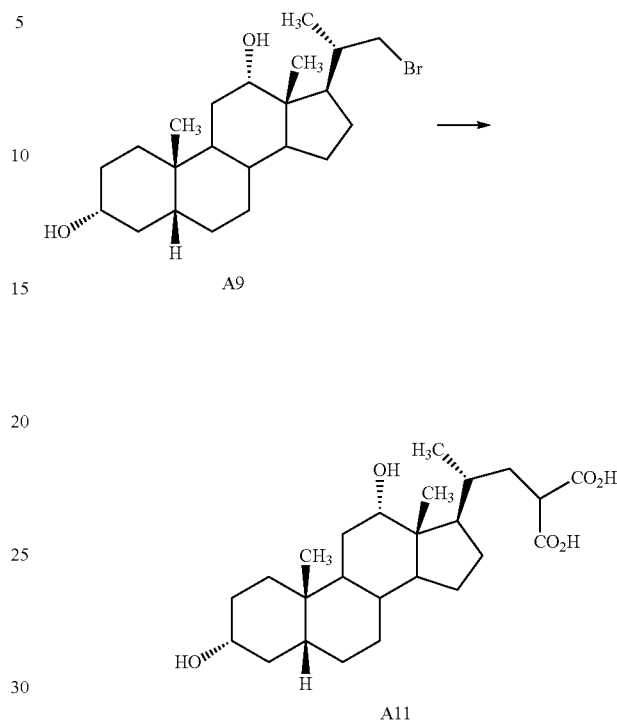
Example 25



0.15 g of NaH 60% (3.87 mmol) was dissolved and stirred in DMF (2 ml) under an inert atmosphere and was cooled at 0° C. Diethyl malonate (0.59 ml, 3.87 mmol) was added to the mixture. The mixture was heated at room temperature and A9 (0.40 g, 0.96 mmol) were added. The solution was heated at 60° C. The mixture was poured into a solution of NaCl 20% (30 ml). The aqueous phase was extracted with EtOAc. The organic phases were evaporated under reduced pressure. The residue was purified on silica gel thereby yielding 0.76 g of A10.

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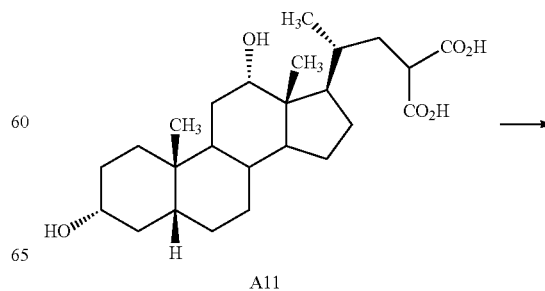
Example 26

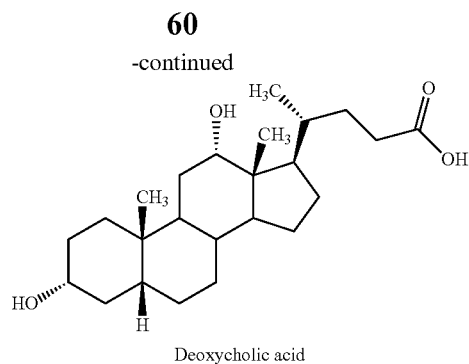
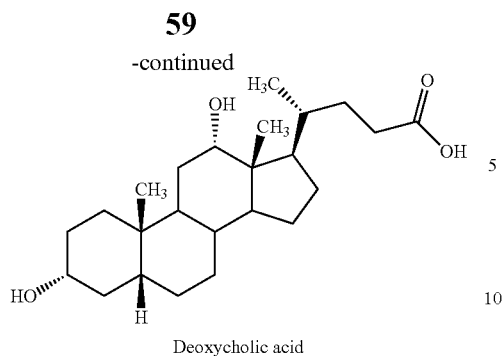


5.86 g of NaH 60% (146.4 mmol) was dissolved and stirred in dry DMF (75 ml) under an inert atmosphere cooled at 0° C. Diethyl malonate (23.4 g, 146.4 mmol) was added dropwise. The solution was stirred until the mixture was turned clear. A9 (15.13 g, 36.6 mmol) dissolved in 75.0 ml of DMF was added. The solution was heated at 60° C. The mixture was quenched with a solution of NaCl 20% (1200 ml). The aqueous phase was extracted with EtOAc. The solvent was concentrated under reduced pressure and the mixture was cooled at room temperature and stirred until solid was precipitated. The solid was filtered off and dried, yielding 25.3 g of A11.

¹H NMR (400 MHz, CDCl₃): δ 3.95-3.93 (m, 1H), 3.57-3.46 (m, 1H), 3.39 (dd, J=11.1, 3.5 Hz, 1H), 2.12 (t, J=11.4 Hz, 1H), 1.96-1.04 (m, 25H), 1.00 (d, J=7.5 Hz, 3H), 0.91 (s, 3H), 0.68 (s, 3H).

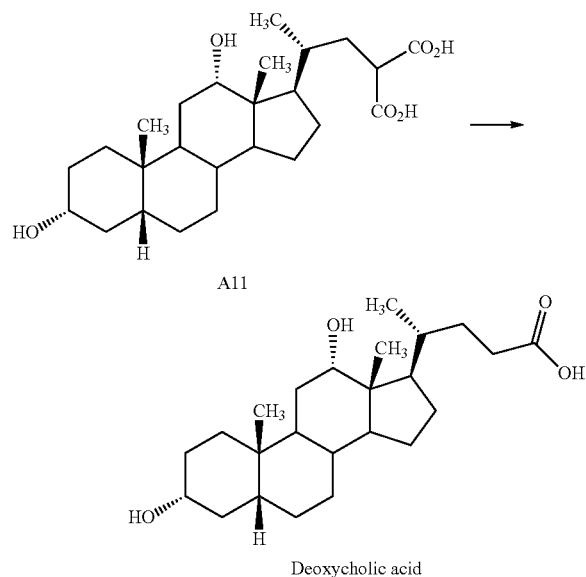
Example 27





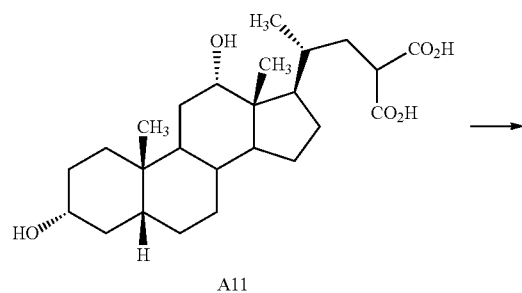
A11 (3.0 g, 0.76 mmol) were suspended in 90.0 ml of NaCl 20%. The suspension was heated at reflux for 60 hours. The mixture was cooled to room temperature. The solid was filtered off and dried, yielding 2.48 g of DCA.

Example 28



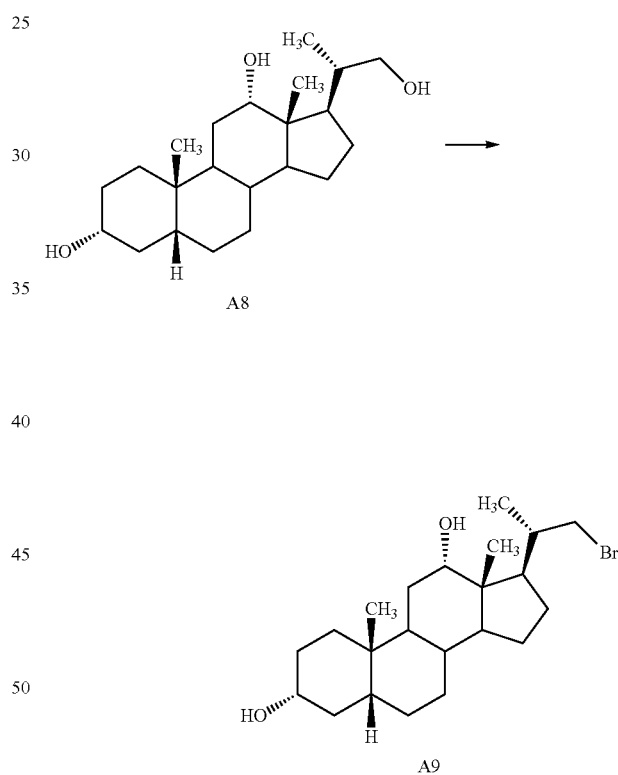
A11 (0.3 g, 0.076 mmol) were suspended in 9.0 ml of a solution of aqueous NaH_2PO_3 (pH 4.55). The suspension was heated at reflux for 70 hours. The mixture was cooled at room temperature. The solid was filtered off and dried, yielding 0.22 g of DCA.

Example 29



A11 (0.3 g, 0.076 mmol) were suspended in 9.0 ml of water in a pressure vessel and was closed. The suspension was heated at reflux for 80 hours. The mixture was cooled to room temperature. The solid was filtered off and dried, yielding 0.23 g of DCA.

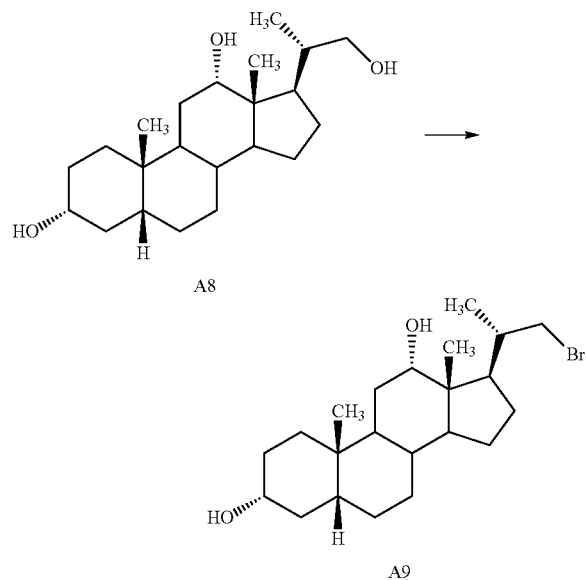
Example 30



0.05 g of A8 (0.14 mmol) was dissolved and stirred in ACN (1 ml), the mixture is cooled at 0°C . A solution of PPh_3Br_2 (0.105 g, 0.24 mmol) in ACN (1 ml) was added dropwise. The mixture was stirred until the reaction was completed. The mixture was cooled at room temperature. The residue was purified on silica gel yielding 0.04 g of A9.

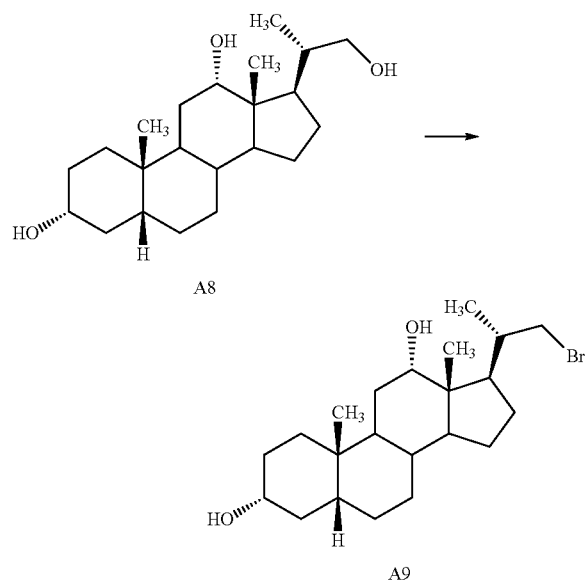
61

Example 31



0.05 g of A8 (0.14 mmol) was dissolved and stirred in DCM (2 ml), under an inert atmosphere and cooled to -40°C . 0.4 ml of a solution of TPP (0.077 g, 1.75 eq) in DCM (8 ml) was added dropwise. 0.4 ml of a solution of Br_2 (0.04 g, 1.75 eq) in DCM (8 ml) was added dropwise. The mixture was stirred until the reaction was completed. The mixture was allowed to reach room temperature. The residue was purified on silica gel yielding 0.05 g of A9.

Example 32

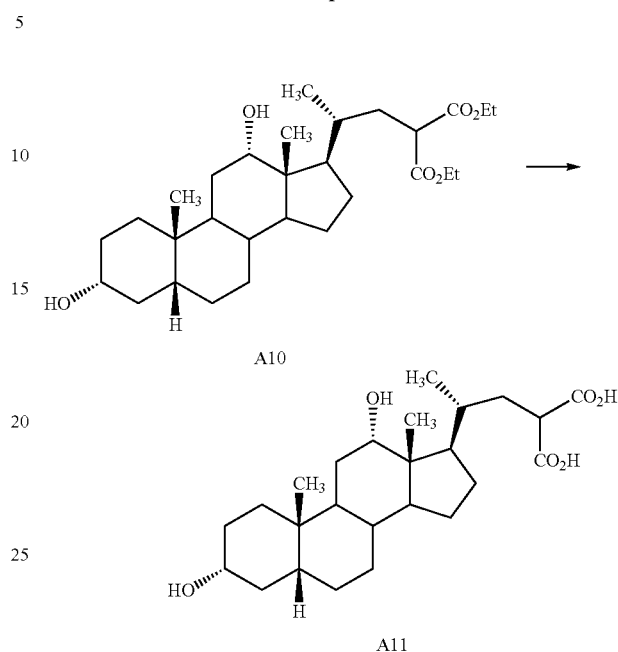


0.05 g of A8 (0.14 mmol) was dissolved and stirred in ACN (1 ml), and cooled to 0°C . A solution of PPh_3Br_2 (0.105 g, 0.24 mmol) in ACN (1 ml) was added dropwise. The mixture was stirred until the reaction was completed.

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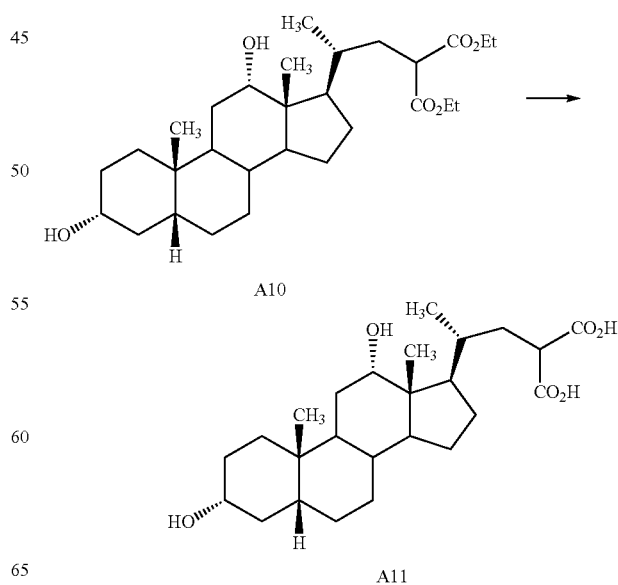
Example 33

The mixture was warmed to room temperature. The residue was purified on silica gel yielding 0.04 g of A9.



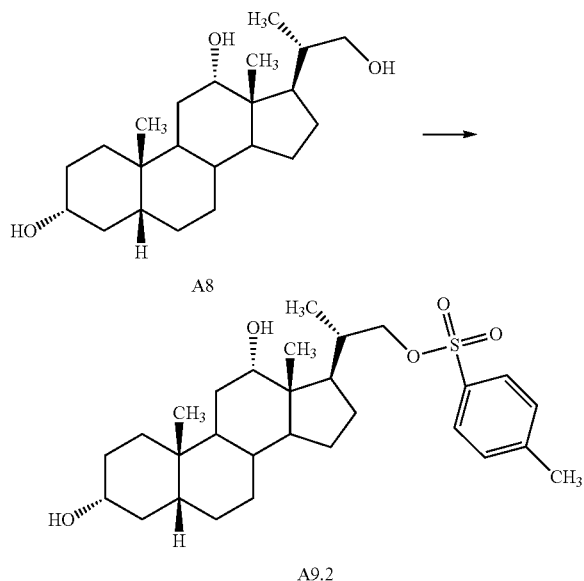
5.0 g of A10 (10.15 mmol) was dissolved in EtOH (25 ml) and stirred at room temperature. NaOH 4M (40 ml) was added and the mixture was stirred. The organic solvent was concentrated under reduced pressure. Water (30 ml) was added dropwise and a solid was obtained. The aqueous phase was washed with DCM (150 ml). The aqueous phase was acidified with HCl 2 N (until pH 1). The mixture was stirred at room temperature and a solid was obtained. The solid was filtered off and dried, yielding 3.5 g of A11.

Example 34



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5.0 g of A10 (10.15 mmol) was dissolved in EtOH (25 ml) and stirred at room temperature. LiOH 4M (40 ml) was added and the mixture was stirred at 40° C. until the reaction was completed. The organic solvent was concentrated under reduced pressure. Water (500 ml) and DCM (150 ml) was added. The aqueous phase was separated and was acidified with HCl 2 N (until pH 1). The mixture was stirred at room temperature and a solid was obtained. The solid was filtered off and dried, yielding 4.3 g of A11.

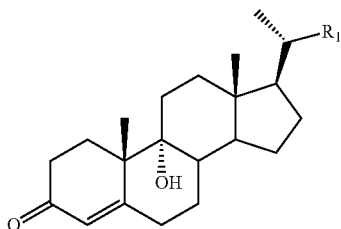
Example 35

0.05 g of A8 (0.14 mmol) was dissolved and stirred in DCM (1 ml), the mixture is cooled at 0° C. TsCl (0.05 g, 0.28 mmol) and DMAP (0.03 g, 0.28 mmol) were added. The suspension was stirred until the reaction was completed. The mixture was allowed to reach room temperature. The residue was purified on silica gel yielding 0.04 g of A9.2.

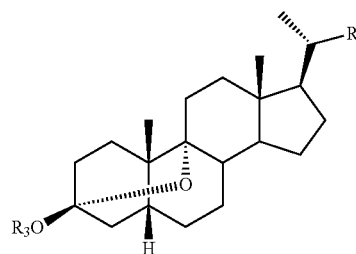
EMBODIMENTS OF THE INVENTION

A. A process for the preparation of deoxycholic acid (DCA) or a pharmaceutically acceptable salt thereof, comprising the following steps:

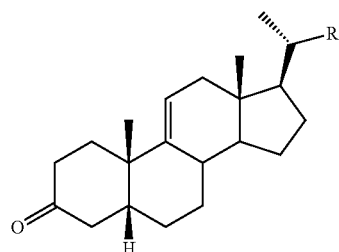
I) providing a compound of the general formula SM:

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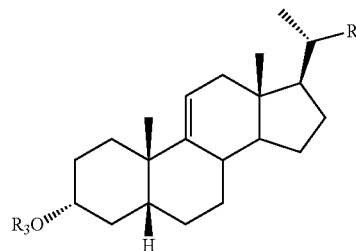
II) reducing the compound of the general formula SM to obtain an intermediate of the general formula INT 1:



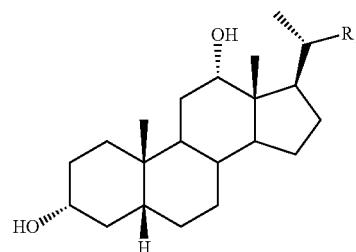
III) converting the intermediate of the general formula INT 1 into an intermediate of the general formula INT 2:



IVa) reducing the intermediate of the general formula INT 2 into an intermediate of the general formula INT 3:



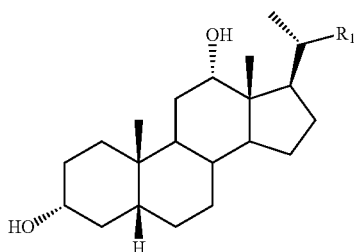
followed by converting the intermediate of the general formula INT 3 into an intermediate of the general formula INT B:



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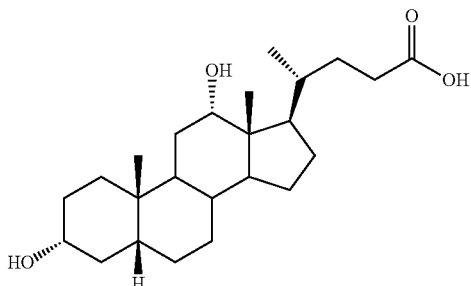
or

IVb) converting the intermediate of the general formula INT 2 into an intermediate with the general formula INT B:



5
INT B

V) converting the intermediate of the general formula INT B into deoxycholic acid (DCA):



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DCA

VI) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof,

wherein

R_1 is COOR_2 , CH_2OH , CH_2OP , CH_2X , CH_2CHO , $\text{CH}_2\text{—CH}_2\text{—OH}$, $\text{CH}_2\text{—CH}_2\text{OP}$, or $\text{CH}_2\text{—CH}_2\text{X}$ or $\text{CH}_2\text{—CH}_2\text{—CHO}$;

R_2 is H or a linear or branched $\text{C}_1\text{—C}_6\text{—alkyl}$ group;

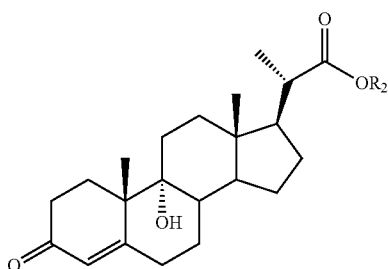
P is an alcohol protection group;

R_3 either P or R_2 ; and

X is a halogen atom.

B. The process according to embodiment A, comprising the following steps:

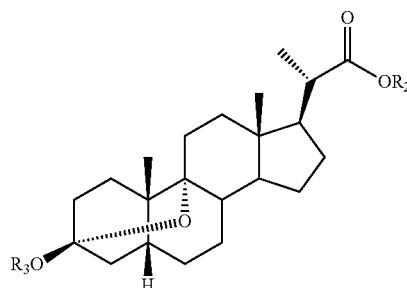
i) providing a compound of the general formula SM-a:



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SM-a

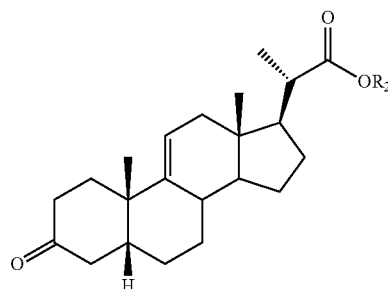
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ii) reducing the compound of the general formula SM-a to obtain an intermediate of the general formula Int A1:



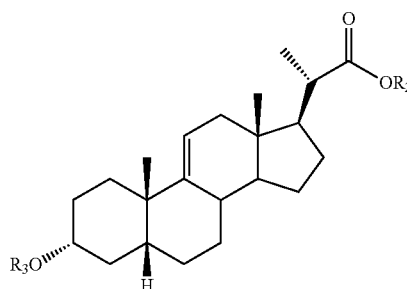
Int A1

iii) converting the intermediate of the general formula Int A1 into an intermediate of the general formula Int A2:



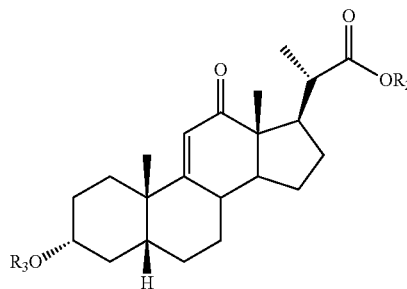
Int A2

iv) reducing the intermediate of the general formula Int A2 into an intermediate of the general formula Int A3:



Int A3

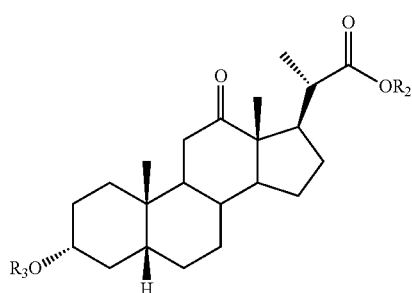
v) oxidising the intermediate of the general formula Int A3 into an intermediate of the general formula Int A5:



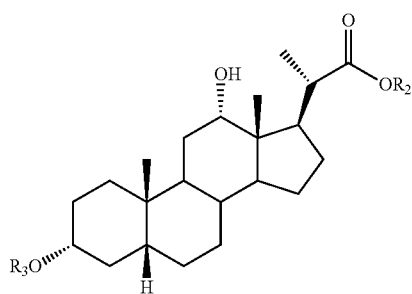
Int A5

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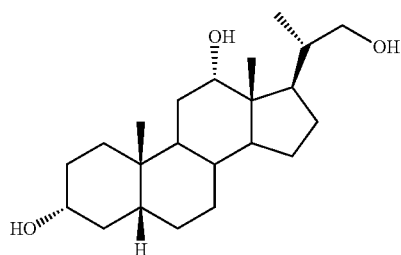
vi) reducing the intermediate of the general formula Int A5 into an intermediate of the general formula Int A6:



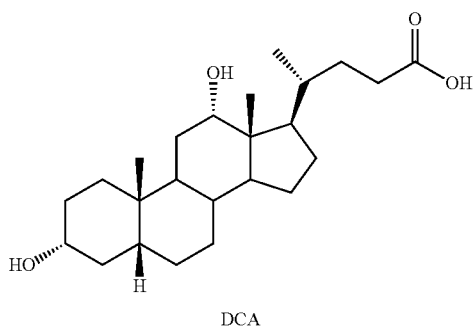
vii) reducing the intermediate of the general formula Int A6 into an intermediate of the general formula Int A7:



viii) reducing the compound of the general formula Int A7 into an intermediate of the general formula Int A8:



ix) elongating the carbon chain of the compound of the general formula Int A8 to obtain deoxycholic acid (DCA):



x) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof,

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wherein

R_2 is H or a linear or branched C_1 - C_6 -alkyl group;

R_3 is H, R_2 or an alcohol protection group.

C. The process according to embodiment A or B, wherein R_2 is selected from the group consisting of methyl, ethyl, n-propyl and isopropyl.

D. The process according to embodiment C, wherein R_2 is methyl or ethyl.

E. The process according to embodiment D, wherein R_2 is methyl.

F. The process according to any of the preceding embodiments, wherein R_3 is selected from the group consisting of trimethylsilyl ether (TMS), triethylsilyl ether (TES), triisopropylsilyl ether (TIPS), tert-butyldimethylsilyl ether (TBS, TBDMS), tert-butyldiphenylsilyl ether (TBDPS), acetyl (Ac, $COCH_3$), benzoyl (Bz), benzyl ether (Bn), 4-methoxybenzyl ether (PMB), 2-naphthylmethyl ether (Nap), methoxymethyl acetal (MOM), 2-methoxyethoxy-methyl ether (MEM), ethoxyethyl acetal (EE), methoxypropyl acetal (MOP), benzyloxymethyl acetal (BOM), tetrahydropyranyl acetal (THP), 2,2,2-trichloro-ethyl carbonate (Troc), methyl ether, dimethoxytrityl (DMT), methoxytrityl (MMT), methylthiomethyl ether, pivaloyl (Piv), tetrahydropyranyl (THP), triphenylmethyl (trityl, Tr), and tosyl (Ts).

G. The process according to embodiment F, wherein R_3 is selected from the group consisting of Ac, TBDMS and Ts.

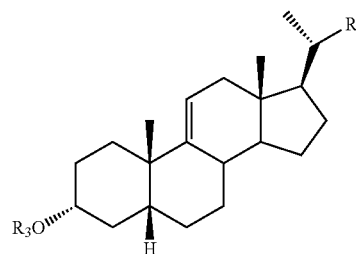
H. The process according to embodiment G, wherein R_3 is Ac.

I. The process according to embodiment A or B, wherein R_2 is methyl and R_3 is Ac.

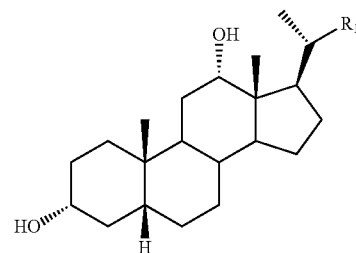
J. The process according to embodiment J, wherein R_3 is R_2 , and R_2 is as defined in any of embodiments B-D.

K. A process for the preparation of deoxycholic acid (DCA) or a pharmaceutically acceptable salt thereof, comprising the following steps:

I) providing a compound of the general formula INT 3:

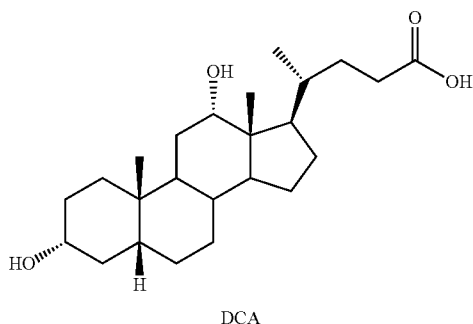


II) converting the intermediate of the general formula INT 3 into an intermediate of the general formula INT B:



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III) converting the intermediate of the general formula INT B into deoxycholic acid (DCA):



IV) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof,

wherein

R_1 is COOR_2 , CH_2OH , CH_2OP , CH_2X , CH_2CHO , $\text{CH}_2\text{—CH}_2\text{—OH}$, $\text{CH}_2\text{—CH}_2\text{OP}$, or $\text{CH}_2\text{—CH}_2\text{X}$ or $\text{CH}_2\text{—CH}_2\text{—CHO}$;

R_2 is H or a linear or branched $\text{C}_1\text{—C}_6$ -alkyl group;

P is an alcohol protection group;

R_3 either P or R_2 ; and

X is a halogen atom.

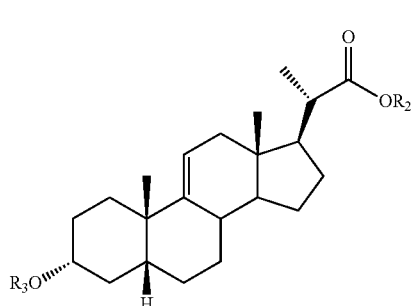
L. The process according to embodiment K, wherein INT 3 is provided from INT 2 as defined in step IVa) of embodiment A.

M. The process according to embodiment L, wherein INT 2 is provided from INT 1 as defined in step III) of embodiment A.

N. The process according to embodiment M, wherein INT 1 is obtained from SM as defined in step II) of embodiment A.

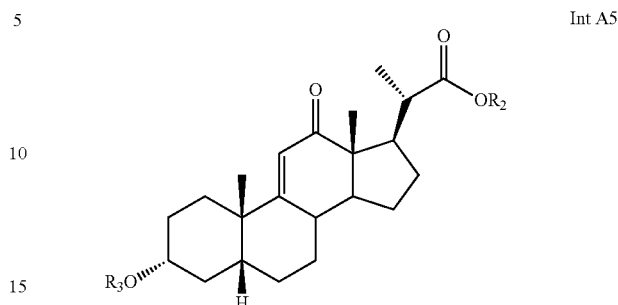
O. The process according to embodiment K, comprising the following steps:

i) providing a compound of the general formula Int A3:

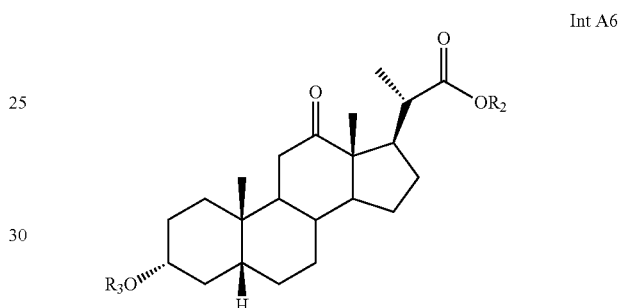


70

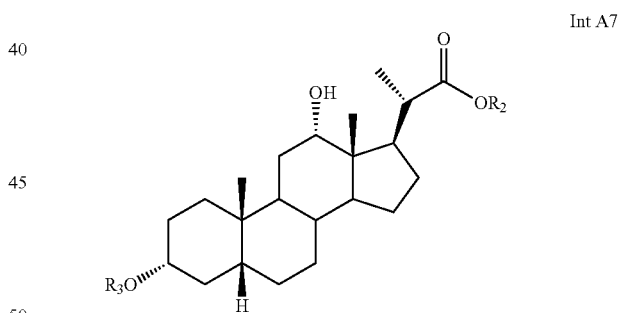
ii) oxidising the intermediate of the general formula Int A3 into an intermediate of the general formula Int A5:



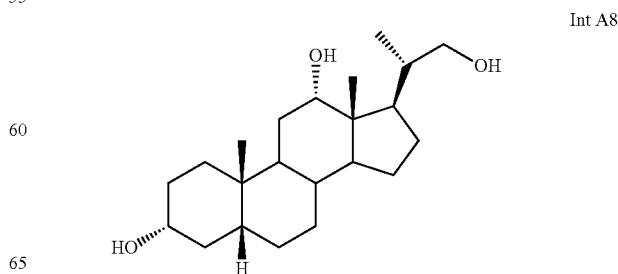
iii) reducing the intermediate of the general formula Int A5 into an intermediate of the general formula Int A6:



iv) reducing the intermediate of the general formula Int A6 into an intermediate of the general formula Int A7:

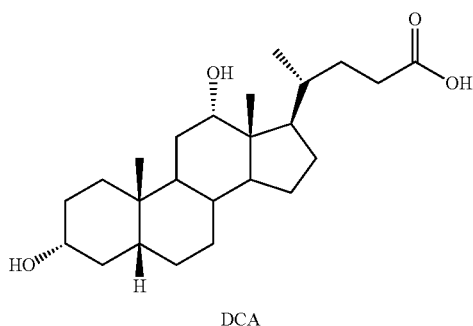


v) reducing the compound of the general formula Int A7 into an intermediate of the general formula Int A8:



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vi) elongating the carbon chain of the compound of the general formula Int A8 to obtain deoxycholic acid (DCA):



vii) optionally converting deoxycholic acid to a pharmaceutically acceptable salt thereof, wherein

R_2 is H or a linear or branched C_1 - C_6 -alkyl group;

R_3 is H, R_2 or an alcohol protection group.

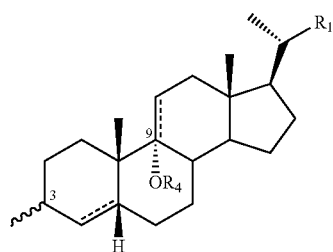
P. The process according to embodiment O, wherein INT A3 is provided from INT A2 as defined in step iv) of embodiment B.

Q. The process according to embodiment P, wherein INT A2 is provided from INT A1 as defined in step iii) of embodiment B.

R. The process according to embodiment Q, wherein INT A1 is provided from SM-a as defined in step ii) of embodiment B.

S. The process according to any of embodiments K-R, wherein R_2 and R_3 are defined in any of embodiments B-J.

T. A compound of the general formula I



wherein

R_1 is $COOR_2$, CH_2OH , CH_2OP , CH_2X , CH_2CHO , CH_2-CH_2-OH , CH_2-CH_2OP , CH_2-CH_2X or CH_2-CH_2-CHO ;

R_2 is H or a linear or branched C_1 - C_6 -alkyl group;

P is an alcohol protection group;

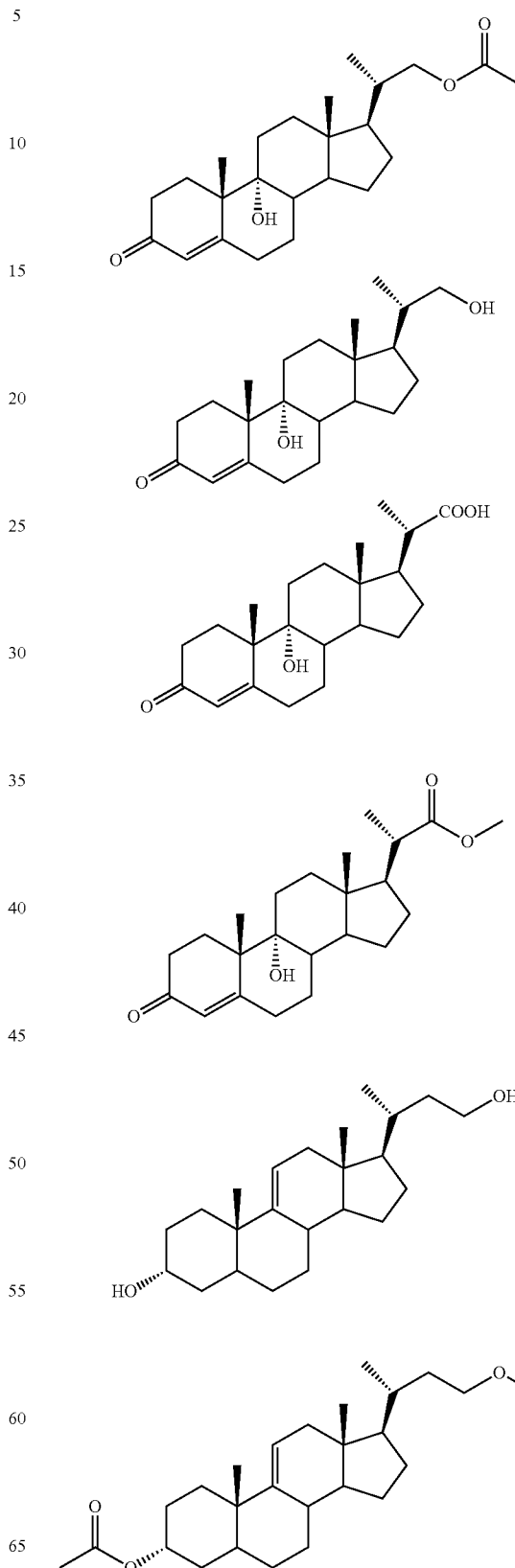
X is a halogen atom;

----- is either a C—C bond or a C=C bond;

~~~~~ is either =O or OR<sub>3</sub> where  $R_3$  is either P or  $R_2$ ;

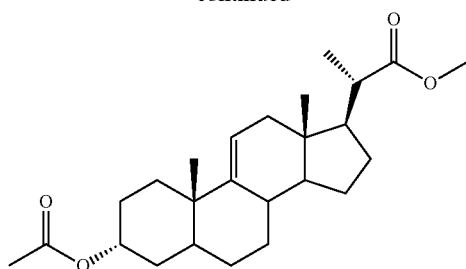
72

$OR_4$  is either OH or  $R_4$  is the  $C_3$  carbon in the A ring; and with the proviso that formula I is not

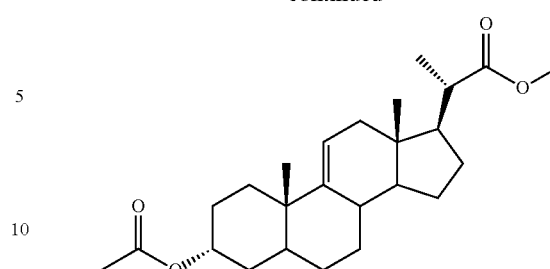


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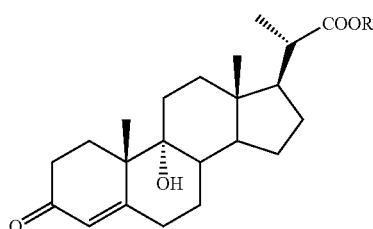
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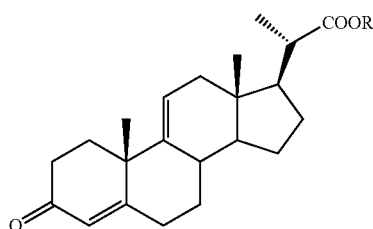
T1. The compound of embodiment T, wherein  
 $R_1$  is  $\text{COOR}_2$ ,  $\text{CH}_2\text{OP}$ ,  $\text{CH}_2\text{X}$ ,  $\text{CH}_2\text{CHO}$ ,  $\text{CH}_2\text{—CH}_2\text{—OH}$ ,  
 $\text{CH}_2\text{—CH}_2\text{OP}$  or  $\text{CH}_2\text{—CH}_2\text{X}$ ;

P is an alcohol protection group with the proviso that P is not  
 Ac or Pv;

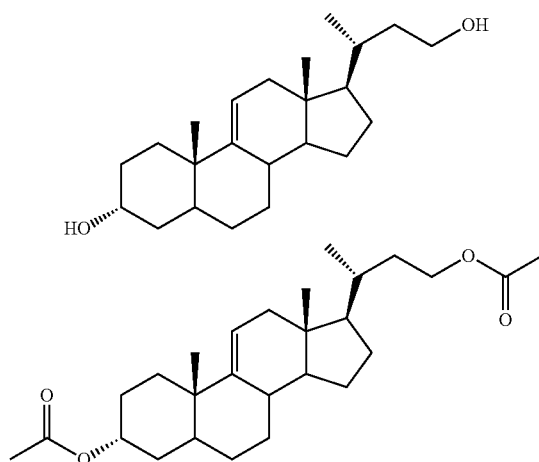
with the proviso that formula I is not



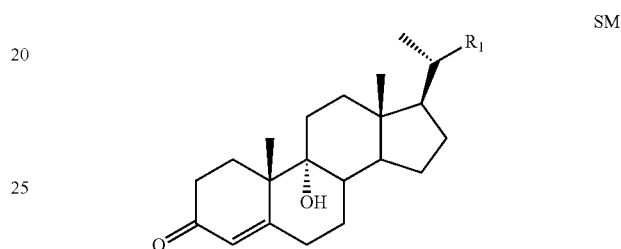
Wherein R is H or Me



Wherein R is H or Me



U. The compound according to embodiment T or T1 having  
 the general formula SM



wherein

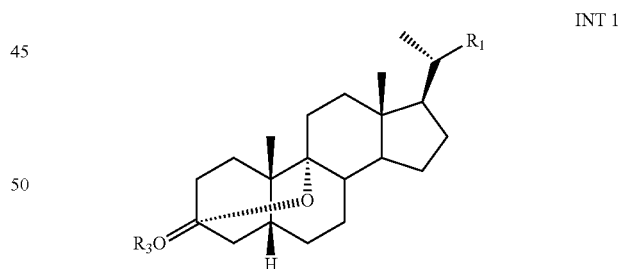
$R_1$  is  $\text{COOR}_2$ ,  $\text{CH}_2\text{OP}$ ,  $\text{CH}_2\text{X}$ ,  $\text{CH}_2\text{CHO}$ ,  $\text{CH}_2\text{—CH}_2\text{—OH}$ ,  
 $\text{CH}_2\text{—CH}_2\text{OP}$ , or  $\text{CH}_2\text{—CH}_2\text{X}$

$R_2$  is a linear or branched  $\text{C}_1\text{—C}_6\text{—alkyl}$  group with the proviso  
 that  $R_2$  is not  $\text{CH}_3$ ;

P is an alcohol protection group with the proviso that P is not  
 Ac; and

X is a halogen atom.

V. The compound according to embodiment T or T1 having  
 the general formula INT 1



wherein

$R_1$  is  $\text{COOR}_2$ ,  $\text{CH}_2\text{OH}$ ,  $\text{CH}_2\text{OP}$ ,  $\text{CH}_2\text{X}$ ,  $\text{CH}_2\text{CHO}$ ,  $\text{CH}_2\text{—}$   
 $\text{CH}_2\text{—OH}$ ,  $\text{CH}_2\text{—CH}_2\text{OP}$ ,  $\text{CH}_2\text{—CH}_2\text{X}$  or  $\text{CH}_2\text{—CH}_2\text{—}$   
 $\text{CHO}$ ;

$R_2$  is H or a linear or branched  $\text{C}_1\text{—C}_6\text{—alkyl}$  group;

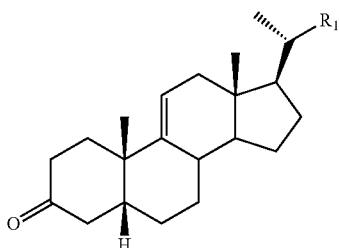
P is an alcohol protection group;

$R_3$  is either P or  $R_2$ ; and

X is a halogen atom.

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W. The compound according to embodiment T or T1 having the general formula INT 2



wherein

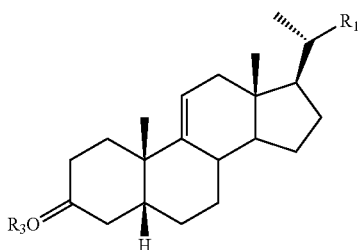
$R_1$  is  $\text{COOR}_2$ ,  $\text{CH}_2\text{OP}$ ,  $\text{CH}_2\text{X}$ ,  $\text{CH}_2\text{CHO}$ ,  $\text{CH}_2\text{—CH}_2\text{—OH}$ ,  $\text{CH}_2\text{—CH}_2\text{OP}$  or  $\text{CH}_2\text{—CH}_2\text{X}$ ;

$R_2$  is H or a linear or branched  $\text{C}_1\text{—C}_6\text{—alkyl}$  group;

P is an alcohol protection group; and

X is a halogen atom.

X. The compound according to embodiment T or T1 having the general formula INT 3



wherein

$R_1$  is  $\text{COOR}_2$ ,  $\text{CH}_2\text{OP}$ ,  $\text{CH}_2\text{X}$ ,  $\text{CH}_2\text{CHO}$ ,  $\text{CH}_2\text{—CH}_2\text{—OH}$ ,  $\text{CH}_2\text{—CH}_2\text{OP}$  or  $\text{CH}_2\text{—CH}_2\text{X}$ ;

$R_2$  is H or a linear or branched  $\text{C}_1\text{—C}_6\text{—alkyl}$  group;

P is an alcohol protection group;

$R_3$  is either P or  $R_2$ ; and

X is a halogen atom.

Y. The compound according to any of embodiments U-X, wherein  $R_1$  is  $\text{COOR}_2$ ,  $\text{CH}_2\text{OP}$  or  $\text{CH}_2\text{X}$ , where

$R_2$  is H or a linear or branched  $\text{C}_1\text{—C}_6\text{—alkyl}$  group;

P is an alcohol protection group;

$R_3$  is either P or  $R_2$ ; and

X is a halogen atom.

Z. The compound according to any of embodiments U-Y, wherein  $R_2$  is selected from the group consisting of methyl, ethyl, n-propyl and isopropyl.

AA. The compound according to embodiment Z, wherein  $R_2$  is methyl or ethyl.

AB. The compound according to embodiment AA, wherein  $R_2$  is methyl.

AC. The compound according to embodiment AA, wherein  $R_2$  is ethyl.

AD. The compound according to any of embodiments U-Y, wherein  $R_2$  is H.

AE. The compound according to any of embodiments U-Y, wherein X is selected from the group consisting of Cl, Br and I.

AF. The compound according to embodiment AE, wherein X is Br.

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AG. The compound according to any of embodiments U-Y, wherein P is selected from the group consisting of trimethylsilyl ether (TMS), triethylsilyl ether (TES), triisopropylsilyl ether (TIPS), tert-butyldimethylsilyl ether (TBS, TBDMS), tert-butyldiphenylsilyl ether (TBDPS), acetyl (Ac,  $\text{COCH}_3$ ), benzoyl (Bz), benzyl ether (Bn), 4-methoxybenzyl ether (PMB), 2-naphthylmethyl ether (Nap), methoxymethyl acetal (MOM), 2-methoxyethoxy-methyl ether (MEM), ethoxyethyl acetal (EE), methoxypropyl acetal (MOP), benzyloxymethyl acetal (BOM), tetrahydropyranyl acetal (THP), 2,2,2-trichloro-ethyl carbonate (Troc), methyl ether, dimethoxytrityl (DMT), methoxytrityl (MMT), methylthiomethyl ether, pivaloyl (Piv), tetrahydropyranyl (THP), triphenylmethyl (trityl, Tr), and tosyl (Ts).

AH. The compound according to embodiment AG, wherein P is selected from the group consisting of Ac, TBDMS and Ts.

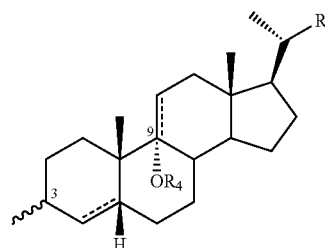
AI. The compound according to any of embodiments U-AH, wherein  $R_3$  is H.

AJ. The compound according to any of embodiments U-AH, wherein  $R_3$  is  $R_2$ , and  $R_2$  is as defined in any of embodiments Z-AC.

AK. The compound according to any of embodiments U-AH, wherein  $R_3$  is P, and P is as defined in embodiment AG or AH.

AL. The compound according to embodiment AK, wherein  $R_3$  is Ac.

AM. Use of a compound of the general formula I



wherein

$R_1$  is  $\text{COOR}_2$ ,  $\text{CH}_2\text{OH}$ ,  $\text{CH}_2\text{OP}$ ,  $\text{CH}_2\text{X}$ ,  $\text{CH}_2\text{CHO}$ ,  $\text{CH}_2\text{—CH}_2\text{—OH}$ ,  $\text{CH}_2\text{—CH}_2\text{OP}$ ,  $\text{CH}_2\text{—CH}_2\text{X}$  or  $\text{CH}_2\text{—CH}_2\text{—CHO}$ ;

$R_2$  is H or a linear or branched  $\text{C}_1\text{—C}_6\text{—alkyl}$  group;

P is an alcohol protection group;

X is a halogen atom;

----- either a  $\text{C—C}$  bond or a  $\text{C=C}$  bond;

~~~~~ is either  $=\text{O}$  or  $\text{OR}_3$  where  $R_3$  is either P or  $R_2$ ; and

OR_4 is either OH or R_4 is the C_3 carbon in the A ring;

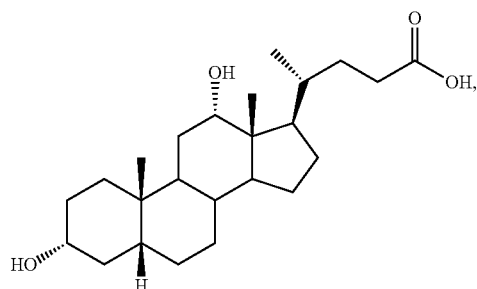
for the preparation of deoxycholic acid (DCA), cholic acid, glycocholic acid, taurocholic acid, or a pharmaceutically acceptable salt thereof.

AN. Use according to embodiment AM, wherein the compound of the general formula I is as defined in any of embodiments U-AL.

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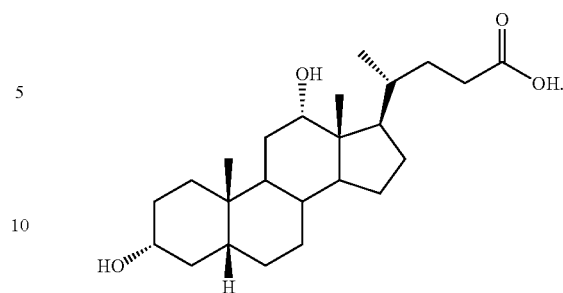
The invention claimed is:

1. A method of making deoxycholic acid or a pharmaceutically acceptable salt thereof of formula (DCA)



DCA

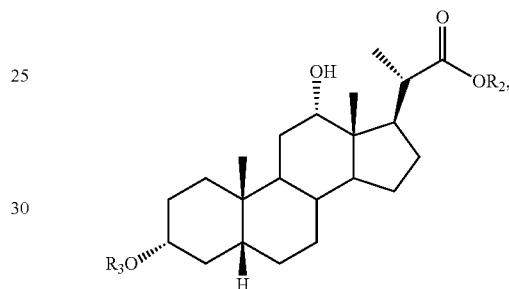
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DCA

2. The method according to claim 1, wherein said providing a compound of formula Int A8 comprises:
providing a compound of formula Int A7:

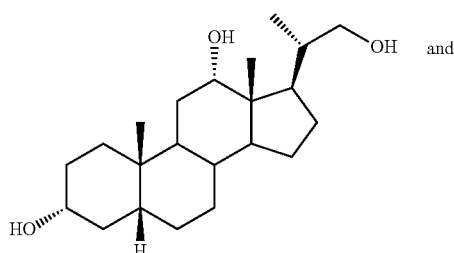
Int A7



Int A8

said method comprising:

providing a compound of formula Int A8:



and

wherein

R_2 is H or a linear or branched C_1 - C_6 alkyl group;

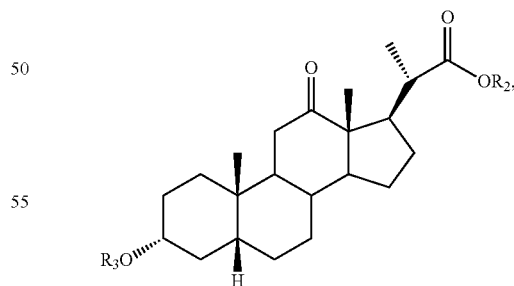
R_3 is H, R_2 , or an alcohol protection group; and

reducing the compound of formula Int A7 to the compound of formula Int A8.

3. The method according to claim 2, wherein said providing a compound of formula Int A7 comprises:

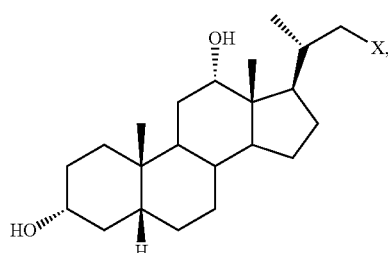
providing a compound of formula Int A6:

Int A6



Int A9

converting the primary alcohol in the formula Int A8 into a leaving group (X) to obtain an intermediate of formula Int A9:



wherein X is halogen, O-tosyl, or OP, wherein P is an alcohol protecting group; and

elongating the carbon chain of the compound of the formula Int A9 to obtain DCA:

wherein

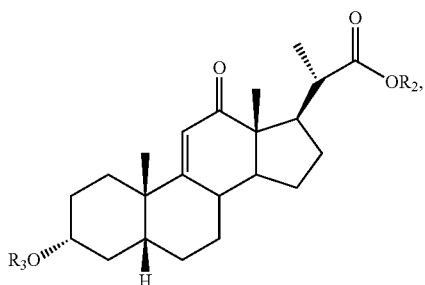
R_2 is H or a linear or branched C_1 - C_6 alkyl group;

R_3 is H, R_2 , or an alcohol protection group; and

converting reducing the compound of formula Int A6 to the compound of formula Int A7.

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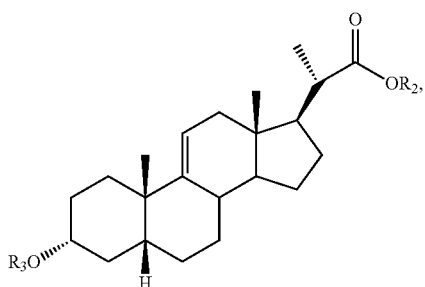
4. The method according to claim 3, wherein said providing a compound of formula Int A6 comprises:
providing a compound of formula Int A5:



wherein

R_2 is H or a linear or branched C_1 - C_6 alkyl group;
 R_3 is H, R_2 , or an alcohol protection group; and
reducing the compound of formula Int A5 to the compound of formula Int A6.

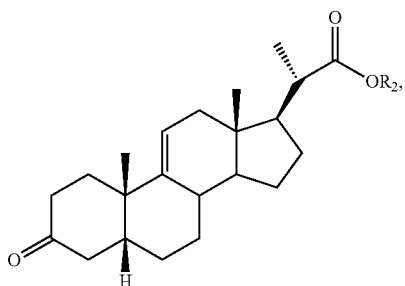
5. The method according to claim 4, wherein said providing a compound of formula Int A5 comprises:
providing a compound of formula Int A3:



wherein

R_2 is H or a linear or branched C_1 - C_6 alkyl group;
 R_3 is H, R_2 , or an alcohol protection group; and
oxidizing the compound of formula Int A3 to the compound of formula Int A5.

6. The method according to claim 5, wherein said providing a compound of formula Int A3 comprises:
providing a compound of formula Int A2:



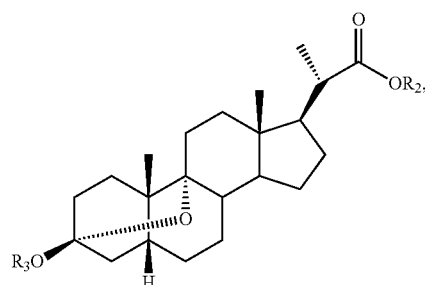
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wherein

R_2 is H or a linear or branched C_1 - C_6 alkyl group; and
reducing the compound of formula Int A2 to the compound of formula Int A3.

7. The method according to claim 6, wherein said providing a compound of formula Int A2 comprises:

providing a compound of formula Int A1:

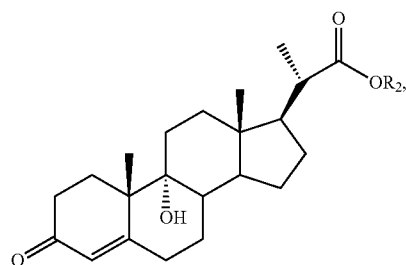


wherein

R_2 is H or a linear or branched C_1 - C_6 alkyl group;
 R_3 is H, R_2 , or an alcohol protection group; and
oxidizing the compound of formula Int A1 to the compound of formula Int A2.

8. The method according to claim 7, wherein said providing a compound of formula Int A1 comprises:

providing a compound of formula SM-a:



wherein

R_2 is H or a linear or branched C_1 - C_6 alkyl group; and
reducing the compound of formula SM-a to the compound of formula of Int A1.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 11,117,925 B2
APPLICATION NO. : 15/579298
DATED : September 14, 2021
INVENTOR(S) : Antonio Lorente Bonde-Larsen et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

Claim 3 Column 78, Line 66, delete “converting reducing” and insert --reducing--

Signed and Sealed this
Thirtieth Day of November, 2021



Drew Hirshfeld
*Performing the Functions and Duties of the
Under Secretary of Commerce for Intellectual Property and
Director of the United States Patent and Trademark Office*