

7-DIFLUORINATED STEROIDS FOR USE IN THE TREATMENT OF NEUROLOGICAL OR MITOCHONDRIAL DISEASES

The present invention relates to novel compounds which are of use in the treatment of neurodegenerative disorders and other conditions in which mitochondrial dysfunction is implicated and/or conditions in which modulating mitochondrial function is useful. In particular, the invention relates to bile acid derivatives, to pharmaceutical compositions containing them, process for preparing them and to the use of the compounds in the treatment or prevention of neurodegenerative and neuromuscular disorders and mitochondrial disease.

10 Background of the Invention

Neurodegenerative diseases are a group of disorders of the central nervous system (CNS) and include dementia (including Alzheimer's disease, vascular dementia, frontotemporal dementia (FTD) and dementia with Lewy bodies), Parkinson's disease, mild cognitive impairment, Huntington's disease, amyotrophic lateral sclerosis (motor neurone disease), multiple system atrophy (MSA), progressive supranuclear palsy (PSP) and Wilson's disease. The incidence of neurodegenerative disease increases with age and therefore such conditions are a growing problem in societies where the average age of the population is increasing. There is currently no cure for any of these diseases although there are some medications available which alleviate the symptoms of Parkinson's disease, some types of cognitive impairment and dementia.

Alzheimer's disease leads to progressive cognitive impairment and is characterised by the presence of extracellular neuritic plaques and intracellular neurofibrillary tangles. It is thought that mitochondrial dysfunction leads to the deposition of the β -amyloid proteins which are the major component of the neuritic plaques and to the formation of the neurofibrillary tangles. Alzheimer's disease may be either sporadic or familial, with the most common cause of familial Alzheimer's disease being mutations in the *PSEN1* gene, which encodes presenilin-1 (Kelleher and Shen, 2017) as well as the *PSEN2* (presenilin-2) and *APP* (amyloid precursor protein) genes (Petit *et al* (2022)). Presenilin-1 and 2 are associated with the mitochondrial membrane causing disease related pathology. Familial Alzheimer's disease is associated with early onset, in which symptoms may manifest in patients as young as 24 years of age. Sporadic Alzheimer's disease typically manifests much later, generally in patients over 65 years of age.

The symptoms of Parkinson's disease are resting tremor, bradykinesia and rigidity and these symptoms are caused by neurodegeneration and loss of dopaminergic neurons. There is a large body of evidence which suggests that there is a strong association between mitochondrial dysfunction and Parkinson's disease. A mild deficiency of mitochondrial electron

transport chain NADH dehydrogenase (complex I) activity has been found in the tissues of Parkinson's disease patients and a number of the proteins that are linked to the familial form of Parkinson's disease are either mitochondrial proteins or are associated with mitochondria.

- 5 Huntington's disease is an inherited progressive neurodegenerative disease and is characterised by motor impairment, personality changes and cognitive decline. The pathology of Huntington's disease provides evidence for a link with mitochondrial dysfunction.

- 10 Amyotrophic lateral sclerosis is also thought to be linked to mitochondrial dysfunction. This disease targets motor neurons in the CNS resulting in muscle weakness, atrophy and, death within 2-3 years of diagnosis. Phase 3 clinical trials have been conducted on TUDCA for ALS (Albanese et al, 2022).

- 15 Attempts have been made to find compounds which are capable of treating neurodegenerative disorders and several compounds have been developed which target mitochondria. For example, it is known that bile acids such as UDCA (ursodeoxycholic acid) exert a beneficial effect on mitochondrial dysfunction in tissue from certain patients suffering from Parkinson's disease, in particular in tissue from *parkin* mutant Parkinson's disease patients (Mortiboys, *et al* 2013) and LRRK2^{G2019S} mutant Parkinson's disease patients (Mortiboys *et al* 2015).
- 20 Furthermore it is known that bile acids such as UDCA exert a beneficial effect on fibroblasts from patients suffering from both sporadic Alzheimer's Disease and familial Alzheimer's Disease due to PSEN1 mutations (Bell *et al* 2018). Furthermore, additional studies have shown that UDCA is beneficial to cells from sporadic Parkinson's patients (Carling *et al* 2020). Furthermore, an innovative Phase 2a clinical trial testing UDCA in Parkinson's patients has
- 25 shown some positive effects of UDCA treatment in this small patient group (Payne et al, 2023).

- WO 2014/036379, WO 2015/061421 and WO 2016/145216 teach that bile acids may be of use in the treatment of neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis. WO 2015/061421 relates to
- 30 deuterated bile acids and WO 2016/145216 discloses fluorinated bile acids particularly bile acids fluorinated at the 3- and/or 7-positions, for example 7-fluoro, 3,7-difluoro and 3,3,7,7-tetrafluoro analogues of ursodeoxycholic acid. WO 2020/128514 relates to 2-fluorinated bile having mitochondrial rescue properties and WO 2023/233164 relates to further bile acid derivatives which also have mitochondrial rescue properties.

If a compound is to be used for the treatment of a neurodegenerative disorder it is, of course, necessary for it to enter the plasma, cross the blood brain barrier and remain in the CNS in sufficient amounts for activity.

In order for an orally administered drug to enter the CNS, it must first enter the plasma and this has proved problematic in the case of bile acids, even when the intrinsic activity of the compounds is relatively high. When taken orally, bile acids are transported from the gut to the liver where they are conjugated, especially with taurine or glycine residues, to form bile salts. The bile salts are then exported to the gall bladder and enter the enterohepatic circulation (Dawson, 2009). The result of the bile acids/salt entering the enterohepatic circulation is that only a small proportion of the administered compound enters the blood stream and is therefore available to cross the blood brain barrier.

The blood brain barrier is formed by a monolayer of endothelial cells joined at tight junctions and other elements including a basement membrane, glial cells, pericytes and neurons. The function of the blood brain barrier is to prevent harmful substances from entering the brain, while allowing access to oxygen and nutrients such as glucose.

The ability of a drug molecule to achieve an effective concentration in the CNS depends on a number of factors, which are discussed in Banks, 2009. Most drugs cross the CNS by transmembrane diffusion and this is favoured by low molecular weight and lipophilicity. The drug must be sufficiently lipid soluble to pass into the cell membrane but not so lipophilic that it remains in the blood brain barrier rather than passing through it or is taken up by peripheral tissues. Drugs can, of course, diffuse across the blood brain barrier in both directions and if the drug is to achieve an effective concentration in the brain, it is also preferable that the rate of passage from the plasma into the brain (influx) is higher than the rate of passage from the brain into the plasma (efflux) (Dolghih, 2013; Doan, 2002. Efflux may be enhanced by binding of the drug to P-glycoprotein (also known as MDR1), which is an active efflux transporter and brain penetration of a drug can be predicted by the efflux ratio, which is represented by the following equation:

$$\text{Efflux Ratio} = \frac{P_{\text{app (B-A)}}}{P_{\text{app (A-B)}}$$

Where $P_{\text{app (B-A)}}$ represents the permeability coefficient in the apical to basolateral (A-B) direction, representing transport of the compound from the plasma to the CNS, and $P_{\text{app(A-B)}}$ represents the permeability coefficient in the basolateral to apical (B-A) direction, representing efflux from the CNS. This is discussed in greater detail in Biological Example 3 below. For

effective CNS concentration, the efflux ratio should preferably be less than 2.5 (Doan, 2002), which is not the case with bile acids such as ursodeoxycholic acid (UDCA). In some cases, however, if a compound is particularly effective at passing through the blood brain barrier, it may achieve a pharmaceutically effective concentration in the CNS even if the efflux ratio is higher than the preferred value. However, more suitable compounds have lower efflux ratios.

Another relevant factor is the degree of protein binding, since this can prevent a drug from diffusing across the blood brain barrier (Banks, 2009). The problem of protein binding is particularly acute with bile acids, many of which are bound in plasma at high levels.

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Neuromuscular disorders include conditions such as muscular dystrophies, myopathies, neuromuscular junction diseases, motor neurone diseases, peripheral nerve diseases, mitochondrial diseases and ion channel diseases. Many of these diseases arise because of mitochondrial defects (Cantó-Santos, 2020) and therefore can be treated by compounds which are capable of rescuing dysfunctional mitochondria. When a bile acid derivative is intended for use in the treatment of neuromuscular diseases, the ability to cross the blood brain barrier is not so important as for compounds intended for the treatment of neurodegenerative diseases. However, it is still necessary to ensure that the bile acid derivative does not become trapped in the enterohepatic circulation and that the degree of binding to plasma proteins is not sufficiently high to prevent the compound from crossing the mitochondrial membrane.

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Mitochondrial diseases include Barth syndrome, chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS), Leigh syndrome, 3-methylglutaconic aciduria, deafness, encephalopathy, and Leigh-like syndrome (MEGDEL syndrome), myoclonic epilepsy and ragged-red fibers syndrome (MERRF syndrome), mitochondrial encephalomyopathy, lactic acidosis and stroke-like syndrome (MELAS), mitochondrial neurogastrointestinal encephalopathy (MNGIE) and Senger syndrome. These diseases can also be treated by compounds which are capable of rescuing dysfunctional mitochondria.

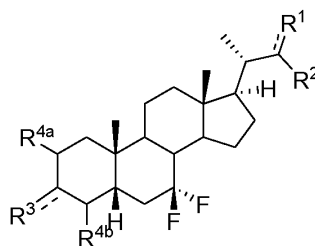
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It is therefore desirable to develop bile acid derivatives which are able to rescue dysfunctional mitochondria, which are not recirculated in the enterohepatic circulation, which do not bind in large quantities to plasma proteins and which cross the blood brain barrier and are retained in the CNS in an effective concentration.

30

35 **Summary of the Invention**

In a first aspect of the present invention there is provided a compound of formula (I):



(I)

wherein:

each --- is independently a single or a double bond

- 5 when --- connected to R^1 is a single bond, R^1 is selected from H, fluoro, chloro, OH, NH_2 , N_3 , C_{1-4} alkyl, $\text{O}(\text{C}_{1-4}$ alkyl), $\text{NH}(\text{C}_{1-4}$ alkyl), $\text{C}(\text{O})\text{OH}$, $\text{C}(\text{O})\text{NHR}^{1a}$ and $\text{NHC}(\text{O})\text{OR}^{1b}$;

wherein when R^1 is C_{1-4} alkyl or $\text{O}(\text{C}_{1-4}$ alkyl), it is optionally substituted with one or more substituents selected from fluoro, chloro, OH, NH_2 , N_3 , $\text{O}(\text{C}_{1-4}$ alkyl) and $\text{NH}(\text{C}_{1-4}$ alkyl);

- 10 wherein each of R^{1a} and R^{1b} is independently H or C_{1-4} alkyl optionally substituted with halo;

when --- connected to R^1 is a double bond, R^1 is selected from CHR^8 and O;

wherein R^8 is H or CH_3 ;

R^2 is selected from $\text{C}(\text{O})\text{OR}^5$, C_{1-6} alkyl, C_{2-6} alkenyl and $\text{C}(\text{O})\text{N}(\text{R}^{5a})(\text{R}^{5b})$;

- 15 wherein R^5 is H, C_{1-4} alkyl or benzyl;
each of R^{5a} and R^{5b} is H or C_{1-4} alkyl or R^{5a} and R^{5b} together with the nitrogen atom to which they are attached form a piperidine, pyrrolidine, piperazine or morpholine ring;
and

- wherein alkyl and alkenyl groups of R^2 are optionally substituted with one or more substituents selected from OH, NH_2 , N_3 , fluoro and chloro;

- 20 when --- connected to R^3 is a single bond, R^3 is OH;

when --- connected to R^3 is a double bond, R^3 is O;

each of R^{4a} and R^{4b} is independently H, F, Cl, C_{1-2} alkyl or C_{1-2} haloalkyl;
or a salt or solvate thereof.

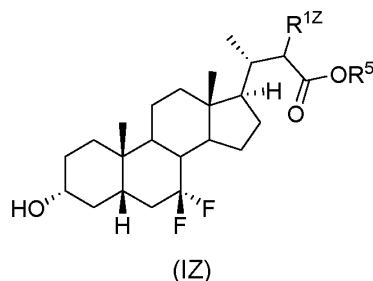
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Compounds of formula (I) include all isotopic variants but, in particular, isotopic variants in which:

in an alkyl group R^1 , one or more hydrogen atoms are present as ^2H (deuterium) or ^3H (tritium), especially deuterium; and/or

- 30 when --- connected to R^3 is a single bond, the hydrogen atom at the 3-position of the bile acid skeleton is replaced by ^2H (deuterium) or ^3H (tritium), especially deuterium.

In a second aspect of the invention, there is provided a compound of formula (IZ):



5 wherein:

R^{12} is selected from H, fluoro, chloro, OH, C_{1-4} alkyl and $O(C_{1-4}$ alkyl), wherein when R^{12} is C_{1-4} alkyl it is optionally substituted with one or more substituents selected from fluoro, chloro, OH and $O(C_{1-4}$ alkyl);

R^5 is H, C_{1-6} alkyl or benzyl;

10 or a salt or solvate thereof.

Compounds of formula (IZ) include all isotopic variants thereof.

Detailed Description of the Invention

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

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

25

In the present specification, references to “pharmaceutical use” refer to use for administration to a human or an animal, in particular a human or a mammal, for example a domesticated or livestock mammal, for the treatment or prophylaxis of a disease or medical condition. The term “pharmaceutical composition” refers to a composition which is suitable for pharmaceutical use and “pharmaceutically acceptable” refers to an agent which is suitable for use in a
30 pharmaceutical composition. Other similar terms should be construed accordingly.

In the present application, the term "C₁₋₆" alkyl refers to a straight or branched fully saturated hydrocarbon group having from 1 to 6 carbon atoms. The term encompasses methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *s*-butyl and *t*-butyl. Other alkyl groups, for example C₁₋₄ alkyl, C₁₋₃ alkyl, or C₁₋₂ alkyl are as defined above but contain different numbers of carbon atoms.

The term "C₁₋₆ alkylene" refers to a straight or branched fully saturated hydrocarbon chain having from one to 6 carbon atoms. The term encompasses -CH₂-, -CH₂CH₂-, -CH(CH₃)-CH₂-, -CH₂CH(CH₃)-, -CH₂CH₂CH₂-, -CH₂CH(CH₂CH₃)- and -CH₂CH(CH₂CH₃)CH₂-. Other alkylene groups, for example C₁₋₅ alkylene, C₁₋₄ alkylene, C₁₋₃ alkylene, or C₁₋₂ alkylene are as defined above but contain different numbers of carbon atoms.

The term "C₂₋₆ alkenyl" refers to a straight or branched hydrocarbon group having 2 to 6 carbon atoms and containing one or more carbon-carbon double bonds. Examples include -CH=CH₂, -CH₂CH=CH₂, -CH=CH-CH₃, -CH=C(CH₃)₂, -CH₂CH=CH-CH₃, and CH=CH-CH₂CH₃. Other alkenyl groups, for example C₂₋₄ alkenyl and C₂₋₃ alkenyl are as defined above but contain different numbers of carbon atoms.

The term "protected OH group" refers to a hydroxyl protected by any known protecting group. Examples of protected OH groups of this type include R¹⁸C(O)O, where R¹⁸ is C₁₋₆ alkyl or benzyl, especially methyl. Silyl ether protecting groups may also be used and OH can also be protected as an ether, for example a C₁₋₆ alkyl, benzyl or *p*-methoxybenzyl ether. Other suitable protecting groups for OH are well known to those of skill in the art (see e.g. Wuts, PGM and Greene, TW (2006) "Greene's Protective Groups in Organic Synthesis", 4th Edition, John Wiley & Sons, Inc., Hoboken, NJ, USA).

Salts of the compounds of formula (I) may be basic addition salts.

Any salts intended to be administered to a patient will be pharmaceutically acceptable but other salts may also be used during the synthesis of a pharmaceutically acceptable final product. Pharmaceutically acceptable salts are known to those of skill in the art and are summarised in Gupta *et al*, *Molecules*, **23**, 1719 (2018).

Pharmaceutically acceptable basic addition salts include sodium, potassium, calcium, aluminium, zinc, magnesium and other metal salts as well as choline, amine salts including triethylamine, *N,N*-diisopropylethylamine (DIPEA), diethanolamine, ethanolamine, ethyl diamine, meglumine and other well-known basic addition salts.

The compounds of formula (I) include all stereoisomers. In the compounds of the invention, the stereochemistry of the bile acid ring system is fixed and therefore the term "stereoisomers" as used herein refers only to stereoisomers of the R¹ and/or the R³ substituents in the
5 compounds of formula (I) and not to stereoisomers of the bile acid ring system.

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

As noted above, the invention provides a compound of formula (I) as defined above or a salt, solvate and/or isotopic variant thereof.
20

The compounds of formula (I) have significant advantages when compared to the prior art compounds such as ursodeoxycholic acid and the fluorinated derivatives described in WO 2016/145216.

25 Firstly, the compounds of formula (I) have a side chain which is one carbon atom shorter than that of ursodeoxycholic acid and the ursodeoxycholic acid derivatives described in WO 2016/145216. Surprisingly, it appears that this prevents recognition of the compounds by the enzymes which control conjugation of bile acids in the liver. As a result, bile salts of the compounds of formula (I) are not formed in the liver and the compounds of formula (I) do not
30 enter the enterohepatic circulation but pass to the bloodstream. This means that if a compound of formula (I) and the ursodeoxycholic acid derivatives described in WO 2016/145216 are administered at the same dose, a larger amount of the compound of formula (I) passes to the bloodstream. This is the case for both parenteral and oral administration and is demonstrated in the assay described in Biological Example 4, where a number of compounds of formula (I)
35 are compared with Comparative Compound A, a compound falling within the claim scope of WO 2016/145216.

Secondly, the 7,7-difluoro substitution pattern of the compounds of formula (I) leads to an unexpectedly low efflux ratio. Particularly preferred compounds of formula (I) retain acceptable permeability but the efflux ratio is significantly decreased compared to UDCA. For example, as shown in Biological Example 3 below, Compound 1 of Example 1 has an efflux ratio of 1.2, whereas UDCA has an efflux ratio of 3.6. Because of this, the achievable concentration in the CNS is significantly greater for compounds of formula (I) than for UDCA.

Thirdly, the compounds of formula (I) bind to proteins in lower amounts than UDCA or the compounds of WO 2016/145216. Thus for example, 89% of Compound 1 of Example 1 binds to plasma proteins but 99% of UDCA is protein-bound in plasma. This means that the unbound concentration in the blood is significantly higher, for the compounds of the invention than for UDCA meaning that penetration across the blood brain barrier is higher.

The above factors all lead to an unexpected increase in oral bioavailability of the compounds of formula (I) compared to either UDCA or the compounds of WO 2016/145216. Biological Example 1 describes a dose response assay for mitochondrial membrane potential (MMP) and mitochondrial morphology as represented by the percentage of long mitochondria. In these assays, Compound 1, which is a compound of formula (I) was compared with Comparative Compound A, which has the same difluoro substitution at the 7-position of the steroid ring system but has a side chain one carbon atom longer. The results showed that Compound 1 achieved a higher maximum MMP value than Comparative Compound A and a similar maximum for long mitochondria. However, there is likely to be a more significant difference in the *in vivo* activities of Compound 1 and Comparative Compound A than might have been predicted from their *in vitro* activity because levels in the CNS will be significantly higher for a compound of formula (I) than for compounds with a longer side chain.

In some suitable compounds of formula (I),  connected to R¹ is a single bond.

In some compounds of this type, R¹ is suitably selected from H, fluoro, chloro, OH, NH₂, N₃, C₁₋₄ alkyl, O(C₁₋₄ alkyl), NH(C₁₋₄ alkyl) and C(O)OH.

In some suitable compounds of this type, R¹ is H.

In other suitable compounds of this type, R¹ is C₁₋₄ alkyl which is unsubstituted or is substituted as described above. The C₁₋₄ alkyl group may be unsubstituted and, for example may be methyl or ethyl. Alternatively, the C₁₋₄ alkyl group may be substituted with one or more substituents selected from fluoro, chloro, OH, NH₂, N₃, and O(C₁₋₄ alkyl), especially fluoro,

chloro, OH, NH₂ and methoxy. In particularly suitable compounds of this type, R¹ is methyl, ethyl, or methyl substituted with one or more substituents selected from OH, methoxy, NH₂ or F. For example, R¹ is methyl, ethyl, CH₂OH, CH₂F, CHF₂, CH₂OCH₃ or CH₂NH₂. In some cases, the compound is an isotopic variant in which one or more of the hydrogen atoms of a substituted or unsubstituted alkyl group R¹ is replaced by ²H (deuterium, D) or ³H (tritium, T).
5 For example, R¹ may be CD₃.

In still other suitable compounds of this type, R¹ is fluoro, chloro or azido (N₃), especially fluoro or chloro.

10

In still other suitable compounds of this type, R¹ is OH, NH₂, O(C₁₋₄ alkyl) or NH(C₁₋₄ alkyl), suitably OH, methoxy, ethoxy, NH₂, methyl amino or dimethylamino. In some still more suitable compounds, R¹ is OH or methoxy and in other still more suitable compounds, R¹ is NH₂.

15 In still other suitable compounds of this type, R¹ is C(O)OH.

In still other suitable compounds in which  connected to R¹ is a single bond, R¹ is C(O)NHR^{1a} or NHC(O)OR^{1b}. Suitably, R^{1a} and R^{1b} are each independently selected from H and methyl.

20

In some suitable compounds of formula (I),  connected to R¹ is a double bond.

In some suitable compounds of this type, R¹ is CHR⁸, wherein R⁸ is as defined above but is still more suitably H.

25

In other suitable compounds of the type, R¹ is O.

In some suitable compounds of formula (I) R² is C(O)OR⁵.

30 As noted above, R⁵ may be H, C₁₋₆ alkyl or benzyl.

In particularly suitable compounds of formula (I) and formula (IZ), R⁵ is H such that R² in formula (I) is C(O)OH and C(O)OR⁵ in formula (IZ) is C(O)OH.

35 In other compounds of formula (I) and formula (IZ), R⁵ is a group R⁶, where R⁶ is C₁₋₆ alkyl or benzyl, and more suitably C₁₋₄ alkyl, for example methyl.

In other suitable compounds of formula (I), R^2 is C_{1-4} alkyl or C_{2-4} alkenyl wherein said alkyl and alkenyl groups are optionally substituted with one or more substituents selected from OH, NH_2 , N_3 , fluoro and chloro.

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More suitably in these compounds, R^2 is methyl, ethyl or ethenyl, any of which is optionally substituted with one or more substituents selected from OH, NH_2 , N_3 , fluoro and chloro, for example unsubstituted ethyl, unsubstituted ethenyl or methyl substituted with one or more substituents selected from OH, NH_2 , N_3 , fluoro and chloro.

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Still more suitably in these compounds, R^2 is unsubstituted ethyl, unsubstituted ethenyl, CH_2OH , CH_2NH_2 , CH_2N_3 , CH_2F or CHF_2 .

In still other suitable compounds, R^2 is $C(O)NR^{5a}R^{5b}$, where R^{5a} and R^{5b} are as defined above.

15 More suitably, R^{5a} and R^{5b} are each independently H, methyl or ethyl, for example H or methyl. In some still more suitable compounds, R^{5a} is H and R^{5b} is methyl.

In alternative compounds where R^2 is $C(O)NR^{5a}R^{5b}$, R^{5a} and R^{5b} together with the nitrogen atom to which they are attached form a piperidine, pyrrolidine, piperazine or morpholine ring, 20 more suitably a piperidine, piperazine or morpholine ring, for example a morpholine ring.

In some suitable compounds of the invention, each of R^{4a} and R^{4b} is independently H, F, Cl, methyl or methyl substituted with one or more fluoro substituents. More suitably, one of R^{4a} and R^{4b} is H and the other of R^{4a} and R^{4b} is H, F, Cl, methyl or methyl substituted with one or 25 more fluoro substituents.

In some suitable compounds, both R^{4a} and R^{4b} are H.

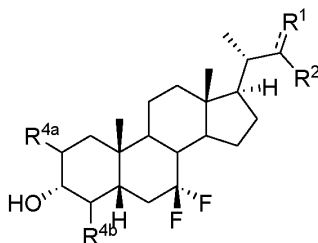
In some suitable compounds R^{4a} is H and R^{4b} is F, Cl, methyl or trifluoromethyl, more suitably 30 F or Cl and especially F.

In other suitable compounds R^{4b} is H and R^{4a} is F, Cl, methyl or trifluoromethyl, more suitably F or Cl and especially F.

35 In some suitable compounds of the invention,  connected to R^3 is a double bond, and R^3 is O.

More suitably, however,  connected to R³ is a single bond, and R³ is OH.

In particularly suitable compounds,  connected to R³ is a single bond, and R³ is α-OH such that the compound of formula (I) is a compound of formula (IY):



(IY)

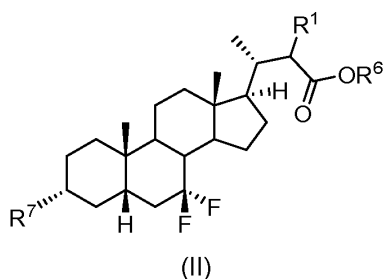
In compounds of formula (IY), R¹, R², R^{4a} and R^{4b} are as described above for compounds of formula (I).

- 10 Particularly suitable compounds of the invention include:
 - 7,7-difluoro 24-norlithocholic acid (Compound 1);
 - Methyl 3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-oate (Compound 1 Me ester)
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-22-methyl-cholanic acid (Compound 2);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-22-ethyl-cholanic acid (Compound 3);
- 15 3α-hydroxy-7,7-difluoro-5β-24-nor-22-fluoro-cholanic acid (Compound 4);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-22-hydroxy-cholanic acid (Compound 5);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-22-chloro-cholanic acid (Compound 6);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-22-(methyl-d₃)-cholanic acid (Compound 7);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-22-methoxy-cholanic acid (Compound 8);
- 20 3α-hydroxy-7,7-difluoro-5β-24-nor-22-keto-cholanic acid (Compound 9);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-22-azido-cholanic acid (Compound 10);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-22-amine-cholanic acid (Compound 11);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-ol (Compound 12);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-chol-22-ene (Compound 13);
- 25 3α-hydroxy-7,7-difluoro-5β-24-nor-cholane (Compound 14);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-azide (Compound 15);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-amine (Compound 16);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-22-hydroxymethyl-cholanic acid (Compound 17);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-22-fluoromethyl-cholanic acid (Compound 18);
- 30 3α-hydroxy-7,7-difluoro-5β-24-nor-22-di-fluoromethyl-cholanic acid (Compound 19);
 - 3α-hydroxy-7,7-difluoro-5β-24-nor-22-methylene-cholanic acid (Compound 20);

- 3 α -Hydroxy-7,7-difluoro-5 β -24-nor-22-methoxymethyl-cholanic acid (Compound 21);
 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-aminomethyl-cholanic acid (Compound 22);
 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-ol (Compound 23);
 3-keto-7,7-difluoro-5 β -24-nor-cholan-23,23-dicarboxylic acid (Compound 24);
 5 3-keto-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 25);
 3 β -Hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 26);
 2 β -fluoro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 27);
 2 β -fluoro-3 β -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 28);
 4 β -fluoro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 29);
 10 4 β -fluoro-3 β -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 30);
 4 α -chloro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 31);
 [²H]-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oic acid (Compound 32);
N-(3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oyl)-(morpholine) (Compound 33); and
N-(3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oyl)-(methylamine) (Compound 34);
 15 and salts and solvates of any one thereof.

Preparation of Compounds of the Invention

- Compounds of formula (I) in which  connected to R¹ is a single bond, R² is C(O)OR⁵, where
 R⁵ is H, R³ is α -OH and R^{4a} and R^{4b} are both H. Compound 1 may be prepared from a protected
 20 compound of formula (II):



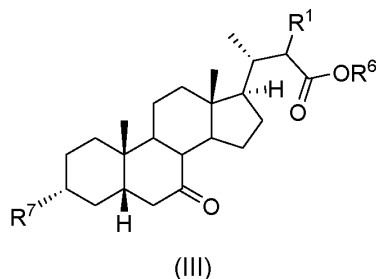
- wherein R¹ is as defined for formula (I), R⁶ is C₁₋₆ alkyl or benzyl and R⁷ is a protected OH
 group;
 25 by hydrolysis, particularly base hydrolysis, for example by treatment with a strong base such
 as sodium or potassium hydroxide in an alcoholic solvent such as methanol, ethanol or
 isopropanol.

Compounds of formula (II) are new and form an aspect of the invention.

30

Suitably, R⁷ is R¹⁸C(O)O, where R¹⁸ is C₁₋₆ alkyl or benzyl, especially methyl. These protecting
 groups can easily be removed by hydrolysis at the same time as the R⁶ group.

A compound of formula (II) may be prepared by fluorination of a compound of formula (III):



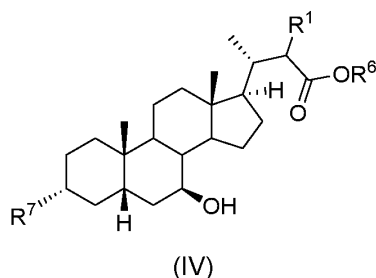
- 5 wherein R¹ is as defined for formula (I) and R⁶ and R⁷ are as defined for formula (II).

The fluorination may be carried out using a fluorinating agent such as diethylaminosulfur trifluoride (DAST), a DAST alternative, for example morpholino-DAST, SF₄ or Deoxo-Fluor® (bis(2-methoxyethyl)aminosulfur trifluoride solution).

10

Suitably, fluorination using DAST or a DAST derivative is conducted at elevated temperature, for example at about 30 °C to 70 °C, for example about 40 °C to 60 °C and typically at about 50 °C. Fluorination with SF₄ is generally conducted at low temperature, typically about -78 °C.

- 15 A compound of formula (III) may be prepared by oxidation of a compound of formula (IV):



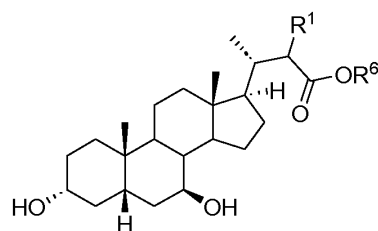
wherein R¹ is as defined for formula (I) and R⁶ and R⁷ are as defined for formula (II).

- 20 One suitable method is a Dess-Martin periodinane (1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol) oxidation, which may be carried out in a chlorinated solvent such as chloroform or dichloromethane at a temperature of about 15 to 25 °C, suitably at room temperature.

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

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

A compound of formula (IV) may be prepared by protecting an ester of formula (V):



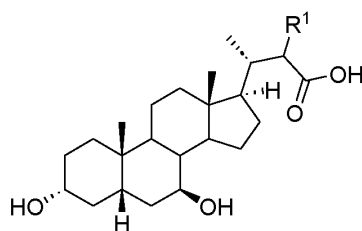
(V)

wherein R¹ is as defined for formula (I) and R⁶ is as defined for formula (II).

For example, when the protected OH group R⁷ of the compound of formula (IV), is R¹⁸C(O)O, the compound of formula (V) may be reacted with a carboxylic acid of formula R¹⁸C(O)OH, or its acid chloride or anhydride.

The reaction may be conducted in an organic solvent, for example a solvent such as tetrahydrofuran, at the reflux temperature of the solvent.

An ester of formula (V) may be prepared from a carboxylic acid of formula (VI):



(VI)

wherein R¹ is as defined for formula (I);

by reaction with an alcohol of formula (VII):

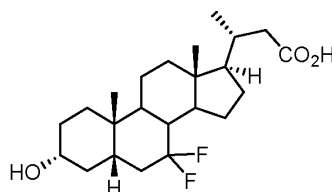


(VII)

where R⁶ is as defined for formula (II).

The reaction may be conducted at about 15 °C to 25 °C, for example at room temperature. Compounds of formulae (VI) and (VII) are known and are commercially available or may be prepared by methods known to those of skill in the art.

- 5 In compounds of formulae (II), (III), (IV), (V) and (VI) above, it is preferred that R^1 is H and therefore this method is particularly suitable for preparing compounds of formula (I) in which R^1 is H, for example Compound 1, which has the formula:

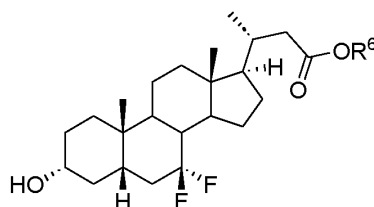


Compound 1;

- 10 In this case, the starting material of formula (VI) is nor-ursodeoxycholic acid.

Compounds of formula (I) in which R^1 is other than hydrogen may be prepared from compounds of formula (II) in which R^1 is hydrogen.

- 15 A compound of formula (II) in which R^1 is hydrogen and R^7 is $R^{18}C(O)O$, where R^{18} is C_{1-6} alkyl or benzyl, may be converted to a compound of formula (Ia), which is a compound of formula (I) in which R^2 is $C(O)OR^5$ and R^5 is a group R^6 :



(Ia)

- 20 wherein R^6 is as defined for formula (II);

by hydrolysis with a base such as an alkali metal alkoxide in an alcoholic solvent, for example with sodium or potassium methoxide in methanol. The reaction is suitably conducted at about 15 °C to 25 °C, for example at room temperature.

25

The hydrolysis is selective for the removal of the $R^{18}C(O)$ protecting group, while leaving the ester on the side chain intact.

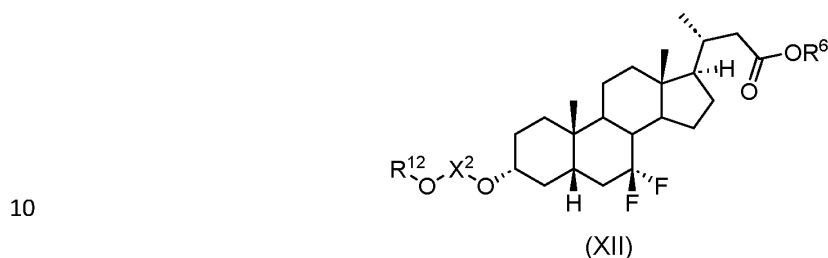
In some cases, transesterification may occur for example, hydrolysis of a compound of formula (II) in which R^6 is other than methyl with an alkali metal methoxide would result in the production

of a compound of formula (Ia) in which R⁶ is methyl.

A compound of formula (Ia) may be reacted with a compound of formula (XI) comprising an ether protecting group:



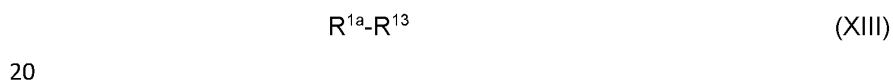
wherein R¹⁰ is halo, especially chloro or bromo, X² is C₁₋₆ alkylene and R¹² is C₁₋₆ alkyl; to give a protected compound of formula (XII):



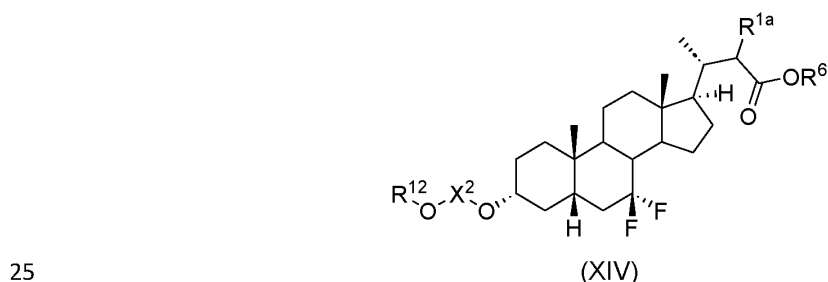
wherein R⁶ is as defined for formula (II) and R¹² and X² are as defined for formula (XI).

Suitably, the reaction is carried out under an inert atmosphere such as nitrogen, in mildly basic conditions, for example using N,N-diisopropylethylamine (DIPEA).

The compound of formula (XII) may be reacted with a compound of formula (XIII):



where R^{1a} is C₁₋₄ alkyl optionally substituted as defined above for R¹ and R¹³ is halo, for example chloro, bromo or iodo, especially iodo to give a compound of formula (XIV):



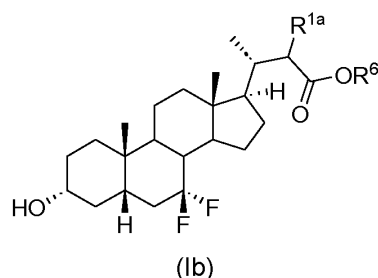
wherein R⁶ is as defined for formula (II) and R¹² and X² are as defined for formula (XI) and R^{1a} is C₁₋₄ alkyl optionally substituted as defined above for R¹.

The reaction is suitably carried out under strongly basic conditions, for example using lithium

diisopropylamide (LDA) in the presence of hexamethylphosphoric triamide (HMPT), hexamethylphosphoramide (HMPA), tris(N,N-tetramethylene)phosphonic acid triamide (tripyrrolidinophosphoric acid triamide; TPPA). The reaction is suitably conducted under an inert atmosphere, for example nitrogen.

5

The protecting group $R^{12}-O-X^2$ may be removed by reaction of the compound of formula (XIV) with an acid, for example hydrochloric acid to give a compound of formula (Ib):



10 wherein R^6 is as defined for formula (II) and R^{1a} is C_{1-4} alkyl.

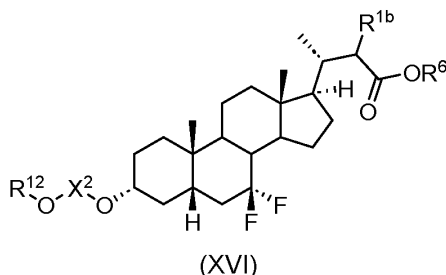
Compounds of formula (Ib) are compounds of formula (I) in which R^1 is C_{1-4} alkyl and R^2 is $C(O)OR^5$, where R^5 is C_{1-6} alkyl or benzyl.

15 Suitably, the reaction is conducted at a temperature of about 30 °C to 50 °C.

If a compound of formula (I) in which R^2 is $C(O)OH$ is required, the protecting group R^6 may be removed by reaction of the compound of formula (Ib) with a strong base, such as a sodium or potassium hydroxide, in an alcoholic such as methanol to give a product of formula (I) in which R^2 is $C(O)OH$ and R^1 is C_{1-4} alkyl. Suitably, the reaction is conducted at a temperature of about 50 °C to 70 °C.

20

A compound of formula (XII) may also be converted to compound of formula (XVI):

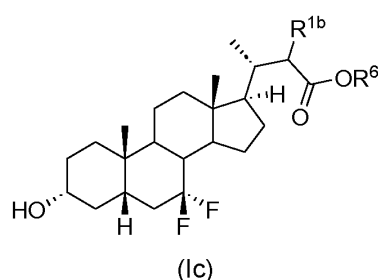


25

wherein R^6 is as defined for formula (II), R^{12} and X^2 are as defined for formula (XI) and R^{1b} is fluoro or chloro by reaction with a fluorinating agent such as N-fluorobenzenesulfonimide (NFSI) or a chlorinating agent such as N-chlorosuccinimide.

The reaction is suitably carried out under strongly basic conditions, for example using LDA in the presence of an agent such as hexamethylphosphorous triamide (HMPT) hexamethylphosphoramidate (HMPA) or tripyrrolidinophosphoric acid triamide (TPPA). Such agents enhance the rate and selectivity of lithiation reactions. The reaction is suitably carried out under an inert atmosphere such as nitrogen.

Removal of the protecting groups to give a compound of formula (I) may be carried out in two steps. Firstly, the compound of formula (XVI) may be reacted with an acid, for example hydrochloric acid to give a compound of formula (Ic):



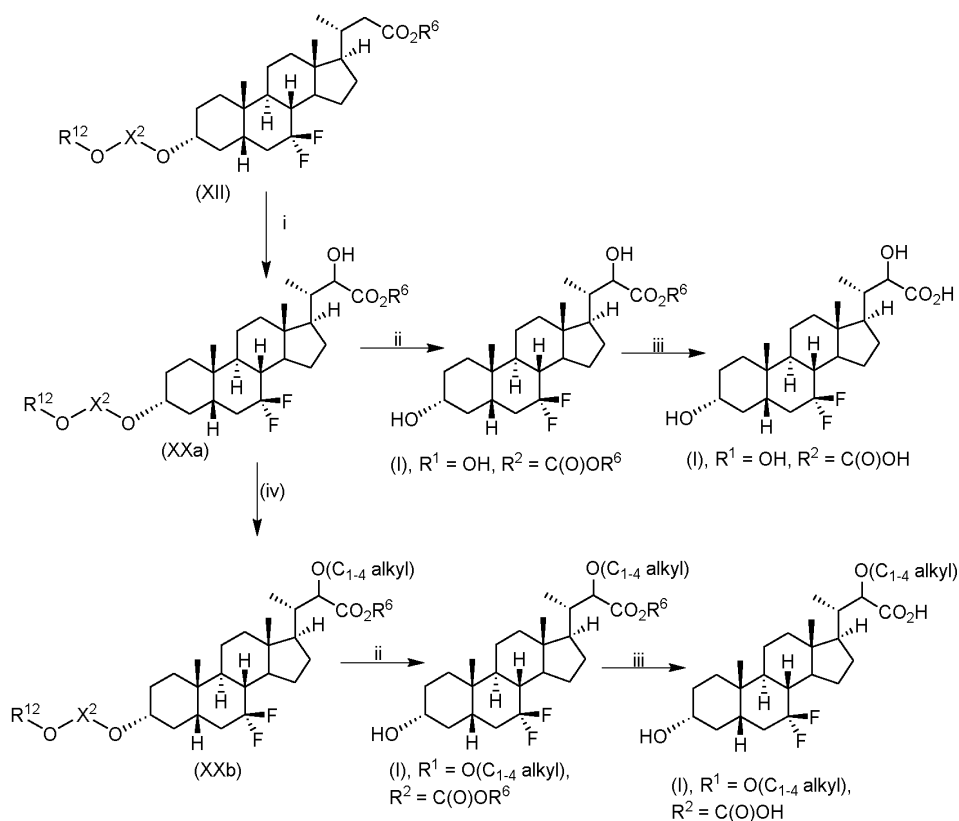
wherein R⁶ is as defined for formula (II) and R^{1b} is as defined for formula (XVI).

Compounds of formula (Ic) are compounds of formula (I) in which R¹ is fluoro or chloro and R² is C(O)OR⁵, where R⁵ is C₁₋₆ alkyl or benzyl.

Subsequently the protecting group R⁶ may be removed to give a product of formula (I) in which R¹ is fluoro or chloro and R² is C(O)OH by reaction of the compound of formula (XVII) with a strong base, such, for example sodium hydroxide, in an alcoholic such as methanol. Suitably, the reaction is conducted at a temperature of about 35 °C to 55 °C.

Compounds of formula (XII) may also be converted to compounds of formula (I) in which R¹ is OH or alkoxy as shown in Scheme 1 below. In Scheme 1, the products of steps (ii) are compounds of formula (I) in which R² is C(O)OR⁵, where R⁵ is a group R⁶ and is C₁₋₆ alkyl or benzyl, and the products of steps (iii) are compounds of formula (I) in which R² is C(O)OR⁵, where R⁵ is H. In these compounds of formula (I), R¹ is either OH or O(C₁₋₄) alkyl optionally substituted as described above for formula (I).

Scheme 1



Conditions:

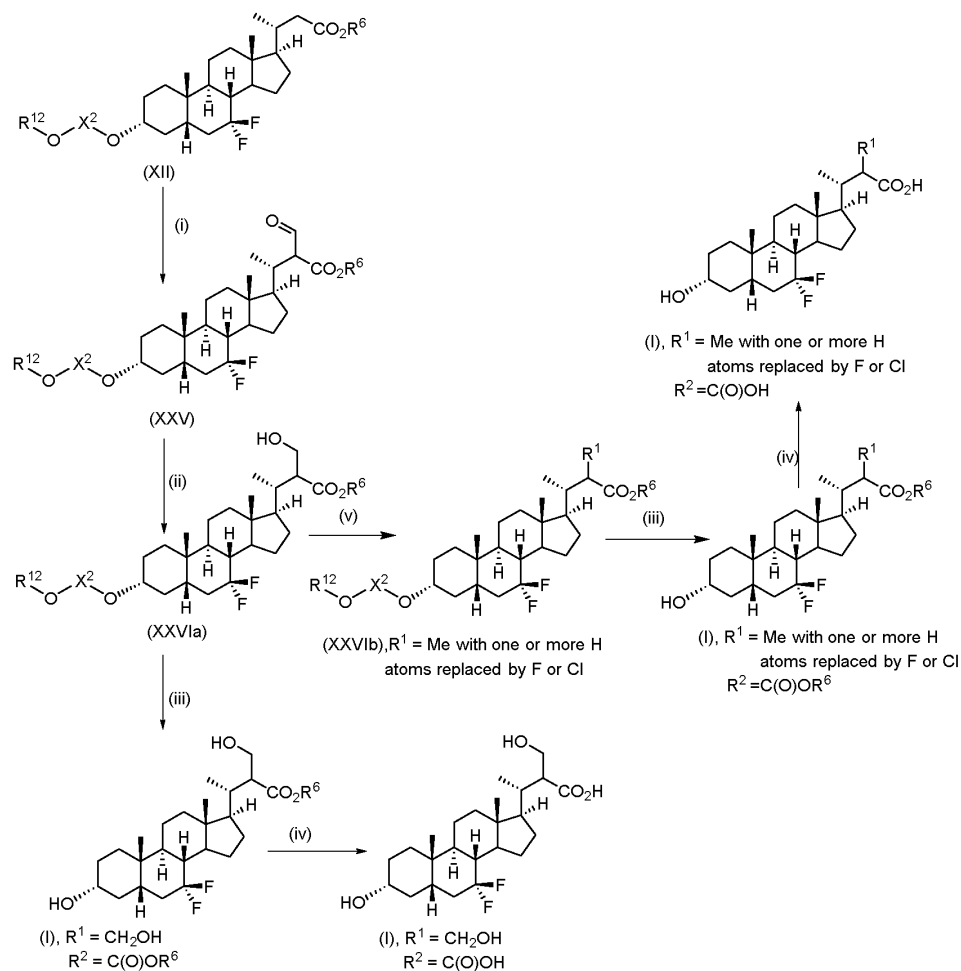
- (i) The compound of formula (XII) is oxidised, for example by treating with a base such as 1M lithium diisopropylamide (LDA) 1M in THF/hexanes, Davis reagent ((3-phenyl-2-(phenylsulfonyl)-1,2-oxaziridine or 2-(benzenesulfonyl)-3-phenyloxaziridine) and tris (N,N-tetramethylene)phosphonic acid triamide in tetrahydrofuran (THF) to give an OH substituted compound of formula (XXa) wherein R⁶ is as defined for formula (II) and R¹² and X² are as defined for formula (XI).
- (ii) Treatment of the compound of formula (XXa) or the compound of formula (XXb) with an acid such as HCl, suitably in an alcoholic solvent such as methanol and at a temperature of about 45 °C to give a compound of formula (I) in which R¹ is OH and R² is C(O)OR⁵, where R⁵ is a group R⁶, which is C₁₋₆ alkyl or benzyl or a compound of formula (I) in which R¹ is O(C₁₋₄ alkyl) optionally substituted as described above and R² is C(O)OR⁵, where R⁵ is a group R⁶, which is C₁₋₆ alkyl or benzyl.
- (iii) Hydrolysis of a compound of formula (I) in which R¹ is OH or O(C₁₋₄ alkyl) optionally substituted as described above and R² is C(O)OR⁵, where R⁵ is a group R⁶, which is C₁₋₆ alkyl or benzyl with a strong base such as sodium hydroxide, suitably in an alcoholic solvent such as methanol and at a temperature of about 60 °C to give a compound of formula (I) in which

R^2 is $C(O)OH$ and R^1 is OH or R^2 is $C(O)OH$ and R^1 is $O(C_{1-4} \text{ alkyl})$ optionally substituted as described above.

- (iv) Treatment of the compound of formula (XXa) with a compound of formula $R^{15}-R^{16}$, where R^{15} is C_{1-4} alkyl and R^{16} is halide, especially chloride, bromide or iodide, but particularly iodide to give a compound of formula (XXb) in which R^1 is a group OR^{15} , where R^{15} is C_{1-4} alkyl, optionally substituted as described above for formula (I) and R^6 , X^2 and R^{12} are as defined for formula (XX). The reaction may be conducted in a solvent such as THF under an inert atmosphere such as nitrogen and in the presence of a strong base such as NaHMDS.

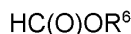
- 10 Compounds of formula (XII) may also be converted to compounds of formula (I) in which R^1 is C_{1-4} alkyl substituted with fluoro, chloro, OH or $O(C_{1-4} \text{ alkyl})$ as shown in Scheme 2 below.

Scheme 2



- 15 Conditions:

- (i) Treatment of the compound of formula (XII) with a base such as lithium diisopropylamide (LDA) and a formic acid ester of formula:



wherein R^6 is as defined for formula (II)

- 5 in a suitable solvent such as THF and in the presence of an activating agent such as TPPA, suitably under an inert atmosphere such as nitrogen; to yield a compound of formula (XXV) wherein R^6 , R^{12} and X^2 are as defined for formula (XII).

- 10 (ii) Reduction of the compound of formula (XXV), for example with a hydride reducing agent such as sodium borohydride in a suitable solvent such as THF and suitably in an inert atmosphere such as nitrogen, to give a compound of formula (XXVIa) wherein R^6 is as defined for formula (II) and R^{12} and X^2 are as defined for formula (XI).

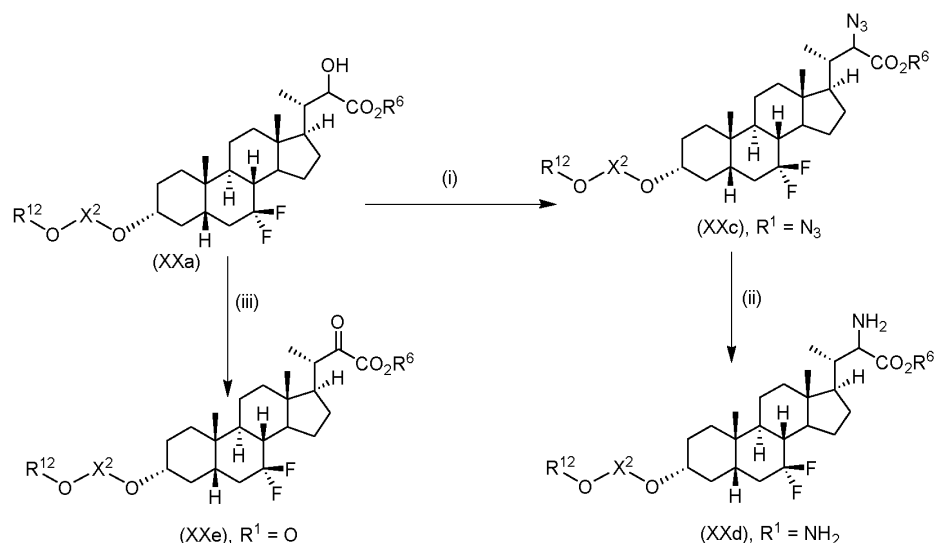
- 15 (iii) Treatment of the compound of formula (XXVIa) with an acid such as TFA suitably in a solvent such as dichloromethane or HCl, suitably in an alcoholic solvent such as methanol and at a temperature of about 45 °C to give a compound of formula (I) wherein R^6 is as defined for formula (XII) and R^1 CH_2OH and R^2 is C(O)OR^5 , where R^5 is C_{1-6} alkyl or benzyl; or treatment of the compound of formula (XXVIb) to give a compound of formula (I) in which R^1 is methyl in which one or more hydrogen atoms are replaced by F or Cl and R^2 is C(O)OR^5 , where R^5 is C_{1-6} alkyl or benzyl.

- 20 (iv) Treatment of the product of step (iii) with sodium hydroxide in MeOH at 60 °C to give a compound of formula (I) in which R^1 is CH_2OH and R^2 is C(O)OH or a compound of formula (I) in which R^1 is methyl in which one or more hydrogen atoms are replaced by F or Cl and R^2 is C(O)OH .

- 25 (v) Treatment of the compound of formula (XXVIa) with a fluorinating agent such as DAST in dichloromethane or a chlorinating agent such as carbon tetrachloride/triphenylphosphine to give a compound of formula (XXVIb) wherein R^6 , R^{12} and X^2 are as defined for formula (XII) and R^1 is methyl in which one or more hydrogen atoms are replaced by F or Cl.

- 30 In Scheme 2, the products of steps (iii) are compounds of formula (I) in which R^2 is C(O)OR^5 , where R^5 is a group R^6 and is C_{1-6} alkyl or benzyl, and the products of steps (iv) are compounds of formula (I) in which R^2 is C(O)OR^5 , where R^5 is H. In these compounds of formula (I), either R^1 is CH_2OH or methyl substituted with one or more F or Cl (e.g. CH_2F , CH_2Cl , CHF_2 or CF_3). Compounds of formula (XXa) of Scheme 1 may also be converted into compounds of formula
35 (XXc) in which R^1 is N_3 , (XXd) in which R^1 is NH_2 and (XXe) in which R^1 is connected to R^1 is a double bond and R^1 is O. This is shown in Scheme 3.

Scheme 3



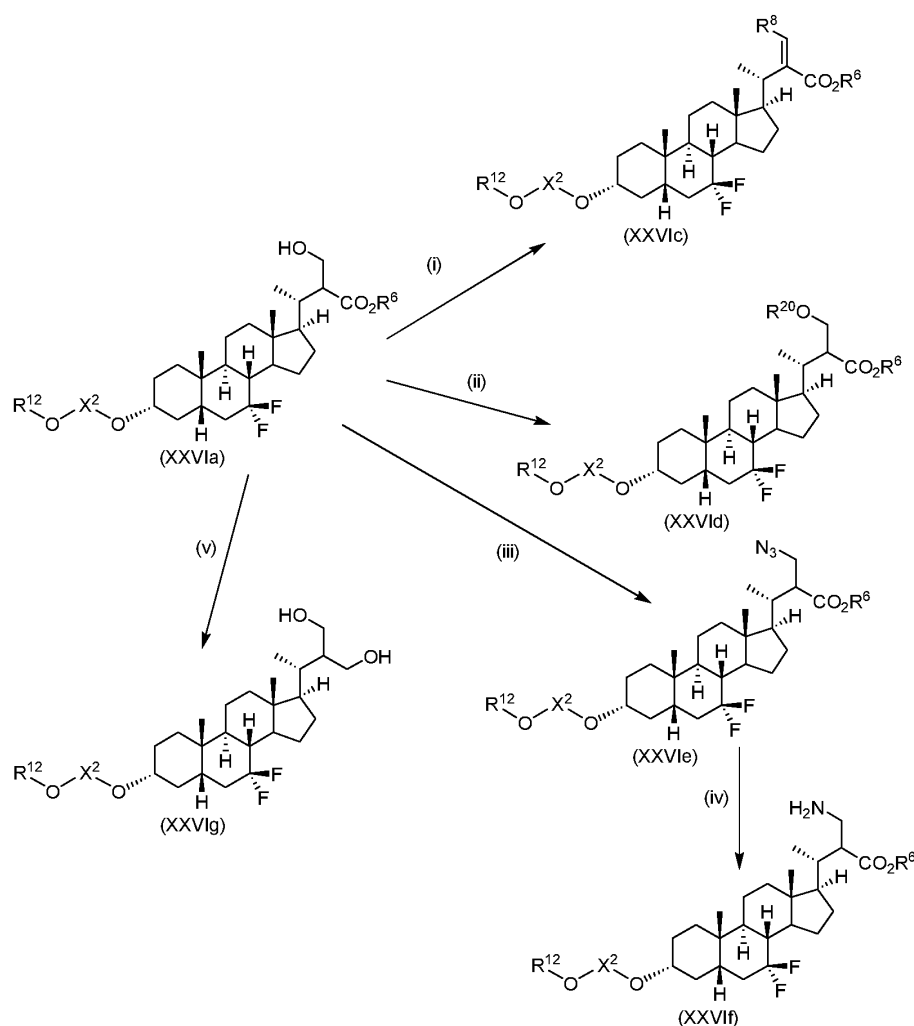
Conditions:

- (i) Treatment of the compound of formula (XXa) to replace OH with a leaving group, followed by replacement of the leaving group with azide. For example, the compound of formula (XXa) may be treated with methanesulfonyl chloride in the presence of a base such as triethylamine. Suitably, the reaction is carried out in a solvent such as dichloromethane under an inert atmosphere such as nitrogen and at reduced temperature, for example about -5 °C to 5 °C, typically about 0 °C. The product is then treated with an azide, typically sodium azide, in a solvent such as DMF in order to obtain compound (XXc), in which R¹ is N₃, R⁶ is as defined for formula (II) and R¹² and X² are as defined for formula (XI).
- (ii) Treatment of the compound of formula (XXc) with a reducing agent such as triphenylphosphine in the presence of water to give a compound of formula (XXd) in which R¹ is NH₂, R⁶ is as defined for formula (II) and R¹² and X² are as defined for formula (XI). Typically, the reaction is conducted in a solvent such as THF and at an elevated temperature, suitably the reflux temperature of the solvent.
- (iii) Oxidation of the compound of formula (XXa) with a suitable oxidising agent, for example Dess Martin periodinane to give a compound of formula (XXe) in which R¹ is O and R⁶ is as defined for formula (II) and R¹² and X² are as defined for formula (XI).

Compounds of formulae (XXc), (XXd) and (XXe) can be converted to compounds of formula (I) using the same method described above for compounds of formulae (XXa) and (XXb), i.e. by employing the procedures described for steps (ii) and (iii) of Scheme 1.

Compounds of formula (XXVIa) of Scheme 2 may also be converted into compounds of formula (XXVc), in which R^1 is a double bond and R^8 is H or CH_3 , compounds of formula (XXVId), (XXVle) and (XXVIf), in which R^1 is a single bond and R^1 is $\text{CH}_2\text{OR}^{20}$, where R^{20} is C_{1-4} alkyl, N_3 or NH_2 , or compound of formula (XXVIg) in which R^1 is a single bond, R^1 is CH_2OH and R^2 is CH_2OH . These reactions are shown in Scheme 4.

Scheme 4



10 Conditions:

- (i) Treatment of the compound of formula (XXVIa) to replace OH with a leaving group, for example by reaction with methanesulfonyl chloride, followed by reaction with rubidium fluoride. For example, the compound of formula (XXVIa) may be treated with methanesulfonyl chloride in the presence of a base such as triethylamine. Suitably, the

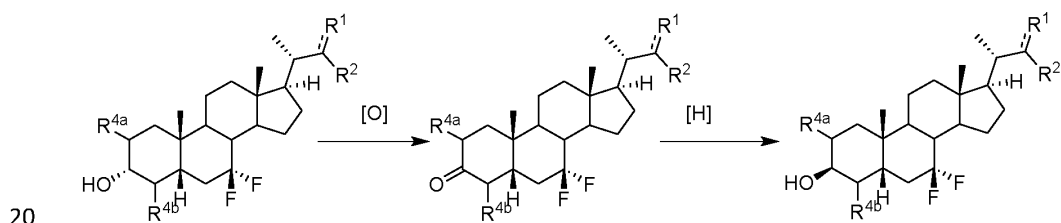
- reaction is carried out in a solvent such as dichloromethane under an inert atmosphere such as nitrogen and at reduced temperature, for example about -5 °C to 5 °C, typically about 0 °C. Conversion of the mesylate intermediate to an alkene may be achieved by treating a solution of the mesylate in a solvent such as DMF with Rubidium fluoride, suitably at elevated temperature, for example about 40 °C to 60 °C. The product is a compound of formula (XXVIc) in which R⁶ is as defined for formula (II) and R¹² and X² are as defined for formula (XI),  connected to R¹ is a double bond and R¹ is CHR⁸.
- (ii) Treatment of the compound of formula (XXVIa) with a compound R²⁰-R²¹, where R²⁰ is C₁₋₄ alkyl and R²¹ is a halide, for example chloride, bromide or iodide, but especially iodide. Suitably, the reaction is carried out in a solvent such as THF and in the presence of a base such as NaHMDS. The product is a compound of formula (XXVId), in which R⁶ is as defined for formula (II) and R¹² and X² are as defined for formula (XI),  connected to R¹ is a single bond and R¹ is CH₂OR²⁰, where R²⁰ is C₁₋₄ alkyl.
- (iii) Treatment of a compound of formula (XXVIa) to replace OH with a leaving group, for example by reaction with methanesulfonyl chloride, followed by reaction of the intermediate with an azide, for example sodium azide. For example, the compound of formula (XXVIa) may be treated with methanesulfonyl chloride in the presence of a base such as triethylamine. Suitably, the reaction is carried out in a solvent such as dichloromethane under an inert atmosphere such as nitrogen and at reduced temperature, for example about -5 °C to 5 °C, typically about 0 °C. Reaction of the mesylate intermediate with the azide may be carried out in a solvent such as DMF. The product is a compound of formula (XXVle) in which R⁶ is as defined for formula (II) and R¹² and X² are as defined for formula (XI) and R¹ is CH₂N₃.
- (iv) Treatment of the compound of formula (XXVle) with a reducing agent such as triphenylphosphine in the presence of water to give a compound of formula (XXVIf) in which R¹ is CH₂NH₂. Typically, the reaction is conducted in a solvent such as THF and at an elevated temperature, suitably the reflux temperature of the solvent.
- (v) Reduction of compound (XXVIa), for example with a hydride reducing agent such as lithium borohydride gives rise to a product of formula (XXVIg) in which R⁶ is as defined for formula (II) and R¹² and X² are as defined for formula (XI), R¹ is CH₂OH and R² is CH₂OH.

Compounds of formulae (XXVIc), (XXVId), (XXVle) and (XXVIf) can be converted to compounds of formula (I) by the same methods as used for the compounds of formulae (XXVIa) and (XXVIb), i.e. by employing the procedures described for steps (iii) and (iv) of Scheme 2. To convert a compound of formula (XXVIg) to a compound of formula (I), it may be treated with an acid according to step (iii) of Scheme 2.

As shown in Scheme 5, Compounds of formula (I) in which R^3 is a single bond and R^3 is α -OH can be converted to compounds of formula (I) in which R^3 is a double bond and R^3 is O by oxidation. Appropriate oxidising agents vary depending on the nature of R^1 and R^2 , but suitable oxidising agents include Jones reagent (chromium trioxide in aqueous sulfuric acid) and Dess Martin periodinane. When Jones reagent is used, the reaction is suitably conducted in a solvent such as acetone but when Dess Martin periodinane is used, a more suitable solvent is dichloromethane. In some cases, oxidation of the R^3 group may also result in oxidation of the R^1 and/or R^2 groups. For example, CH_2OH groups may be converted to $\text{C}(\text{O})\text{OH}$ as shown in Example 24 below. Other suitable oxidising agents are as described above for the oxidation of a compound of formula (IV) to give a compound of formula (III).

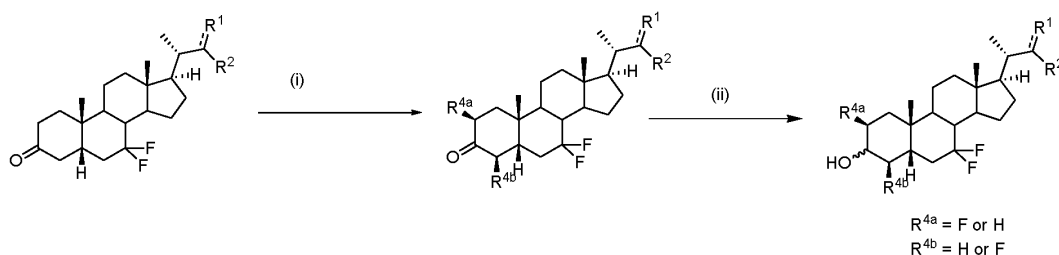
Compounds of formula (I) in which R^3 is a double bond and R^3 is O can be reduced to give compounds of formula (I) in which R^3 is a single bond and R^3 is β -OH, for example using a reducing agent such as potassium tri-sec-butylborohydride (K-selectride®) in a solvent such as tetrahydrofuran. Again, this is illustrated in Scheme 5.

Scheme 5



Compounds of formula (I) in which R^3 is a double bond, R^3 is O and R^{4a} and R^{4b} are both H can be converted into a mixture of isomers of compounds of formula (I) in which R^3 is a double bond, R^3 is O and R^{4a} and R^{4b} are either H or fluoro, chloro, C_{1-2} alkyl or C_{1-2} haloalkyl. These compounds of formula (I) can then be reduced to give compounds of formula (I) in which R^3 is a single bond and R^3 is α -OH or β -OH. This is shown in Scheme 6 and illustrated in Example 27 below.

Scheme 6



Conditions:

- (i) Conversion of the ketone to the silyl enol ether followed by reaction with an electrophilic agent. Fluorination can be achieved by reaction with an electrophilic fluorinating reagent such as 1-(chloromethyl)-4-fluoro-1,4-diazabicyclo[2.2.2]octane-1,4-diium ditetrafluoroborate (sold under the trade mark Selectfluor®). When R^{4a} or R^{4b} is Cl, the agent may be a chlorinating agent such as N-chlorosuccinimide or carbon tetrachloride/triphenylphosphine. For the preparation of compounds in which R^{4a} or R^{4b} is C_{1-2} alkyl or C_{1-2} haloalkyl, the reagent may be an alkyl or haloalkyl iodide or trimethane sulfonate. Preparation of the silyl enol ether may be achieved by treatment with trimethylsilyl trifluoromethanesulfonate under basic conditions, for example in the presence of a triethylamine, in a solvent such as dichloromethane and under an inert atmosphere, such as nitrogen. Reactions with an electrophilic reagent, e.g. fluorinations with Selectfluor® are suitably carried out under an inert atmosphere such as nitrogen and in a solvent such as acetonitrile. The product is a mixture of isomers of formula (I) in which one of R^{4a} and R^{4b} is F, Cl, C_{1-2} alkyl or C_{1-2} haloalkyl and the other of R^{4a} and R^{4b} is H. The isomers are suitably separated before the reduction step (ii), for example using column chromatography as described in Example 27. Di-substituted compounds may be prepared from the monosubstituted products by repeating step (i).
- (ii) Reduction of the separate isomers isolated after step (i), for example using a hydride reducing agent such as sodium borohydride. Such reductions are suitably carried out under an inert atmosphere such as nitrogen, in a solvent such as THF and at reduced temperature, for example about $-25\text{ }^{\circ}\text{C}$ to $-15\text{ }^{\circ}\text{C}$. The product is a mixture of isomers $3\alpha\text{-OH}$ and $3\beta\text{-OH}$ isomers, which can be separated as described in Example 27.

Compounds of formula (I) in which R^2 is $C(O)N(R^{5a})(R^{5b})$ can be prepared from compounds of formula (I) in which R^2 is $C(O)OH$ by reaction with an amine of formula (XXVII)



wherein R^{5a} and R^{5b} are as defined for formula (I).

- In some cases, the compound of formula (I) may be reacted with ethyl chloroformate under basic conditions, for example in the presence of triethylamine, to form an acid anhydride, which may then be reacted with the compound of formula (XXVII).

Suitably, both steps of the reaction are conducted at reduced temperature for example -5 °C to 5 °C, typically about 0 °C.

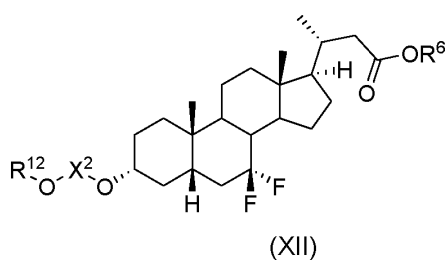
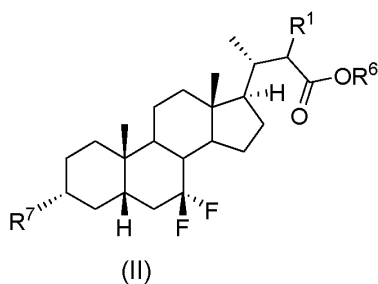
In reaction with the anhydride, the compound of formula (XXVII) may be in aqueous solution
5 under basic conditions, for example provided by sodium bicarbonate.

Alternatively, the reaction may be carried out under basic conditions, for example using triethylamine and in the presence of a coupling agent.

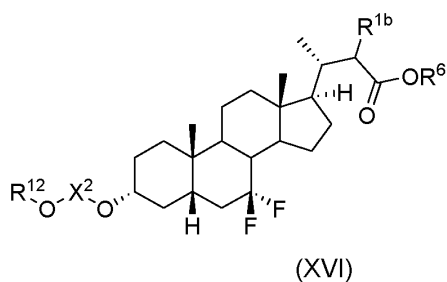
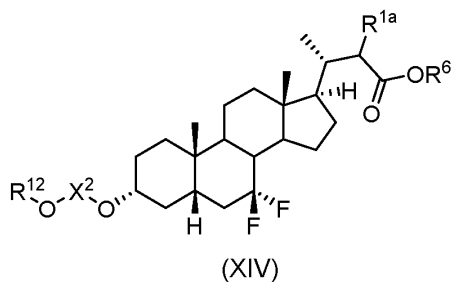
10 Suitable coupling reagents include known peptide coupling agents such as *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU), *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU), *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TATU), (benzotriazol-1-
15 yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) carbodiimides such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and triazoles such as 1-hydroxy-7-azabenzotriazole (HOAt) or hydroxybenzotriazole (HOBt); and chloroformates such as isobutyl chloroformate.

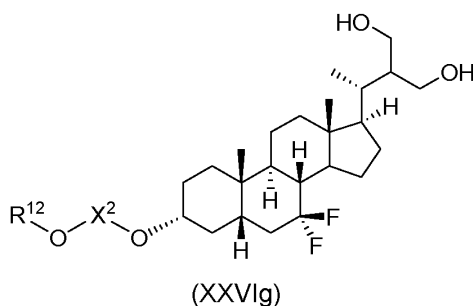
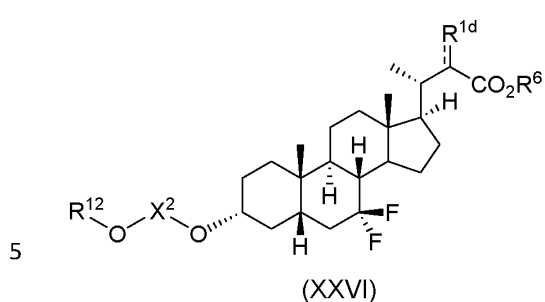
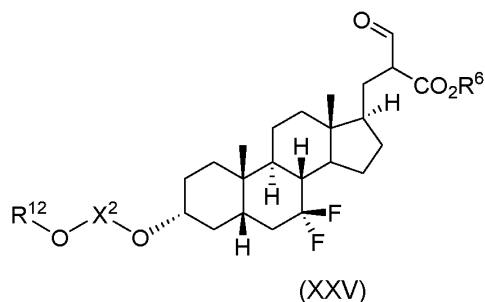
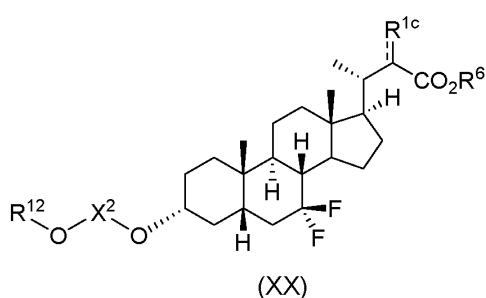
20

Compounds of formulae (II), (XII), (XIV), (XVI), (XX), (XXV), (XXVI) and (XXVIIg) are new and form a further aspect of the invention:



25





Wherein:

R^1 is as defined for formula (I);

R^{1a} is C_{1-4} alkyl optionally substituted as defined above for R^1 ;

10 R^{1b} is fluoro or chloro;

R^{1c} is OH, $O(C_{1-4} \text{ alkyl})$, N_3 , NH_2 or $=O$;

R^{1d} is CH_2OH , methyl in which one or more H is replaced by Cl or F, CH_2N_3 , CH_2NH_2 or $=CHR^8$;

R^6 is C_{1-6} alkyl or benzyl;

15 R^7 is a protected OH group, for example $R^{18}C(O)O$, where R^{18} is C_{1-6} alkyl or benzyl;

R^8 is H or CH_3 ;

R^{12} is C_{1-6} alkyl; and

X^2 is C_{1-6} alkylene.

20

Therapeutic Methods

The compounds of the invention are able to restore mitochondrial function. Surprisingly, it has been shown that they do not enter the enterohepatic recirculation, can cross the blood brain barrier in relatively high levels and bind to plasma proteins in lower levels than compounds
 25 such as UDCA, meaning that they have excellent bioavailability in comparison with known bile acid derivatives. They are therefore of use in the treatment or prevention of neurodegenerative disorders including Parkinson's disease, mild cognitive impairment, dementia (including

Alzheimer's disease, vascular dementia, dementia with Lewy bodies and frontotemporal dementia (FTD)), Huntington's disease, amyotrophic lateral sclerosis (motor neurone disease), progressive supranuclear palsy and Wilson's disease.

5 In the discussion below, references to compounds of formula (I) for use in medicine, the use of compounds of formula (I) in the preparation of a medicament, methods of treatment employing compounds of formula (I) and pharmaceutical compositions comprising compounds of formula (I) apply equally to the pharmaceutically acceptable salts and solvates of compounds of formula (I).

10

The compounds of formula (I) are also useful in treating or preventing conditions in which modulating mitochondrial function is advantageous, particularly neurodegenerative disorders such as Parkinson's disease, mild cognitive impairment, dementia (including Alzheimer's disease, vascular dementia, dementia with Lewy bodies and FTD), Huntington's disease, 15 amyotrophic lateral sclerosis (motor neurone disease), multiple system atrophy, progressive supranuclear palsy and Wilson's disease.

Other diseases and conditions in which modulating mitochondrial function is advantageous include neuromuscular diseases such as muscular dystrophies, myopathies, neuromuscular 20 junction (NMJ) diseases, motor neuron diseases, peripheral nerve diseases, mitochondrial diseases and ion channel diseases.

In a further aspect of the invention, there is provided a compound of formula (I) for use in medicine.

25

There is also provided:

- A compound of formula (I) for use in the treatment of a neurodegenerative disorder;
- A compound of formula (I) for use in the prevention of a neurodegenerative disorder;
- A compound of formula (I) for use in the treatment of a neuromuscular disorder;
- 30 • A compound of formula (I) for use in the prevention of a neuromuscular disorder.

The invention also provides:

- The use of a compound of formula (I) in the manufacture of a medicament for the treatment of a neurodegenerative disorder;
- 35 • The use of a compound of formula (I) in the manufacture of a medicament for the prevention of a neurodegenerative disorder;

- The use of a compound of formula (I) in the manufacture of a medicament for the treatment of a neuromuscular disorder;
- The use of a compound of formula (I) in the manufacture of a medicament for the prevention of a neuromuscular disorder.

5

The invention further provides:

- A method for the treatment of a neurodegenerative disorder, the method comprising administering to a patient in need of such treatment an effective amount of a compound of formula (I);
- 10 • A method for the prevention of a neurodegenerative disorder, the method comprising administering to a patient in need of such treatment an effective amount of a compound of formula (I);
- A method for the treatment of a neuromuscular disorder, the method comprising administering to a patient in need of such treatment an effective amount of a compound of formula (I);
- 15 • A method for the prevention of a neuromuscular disorder, the method comprising administering to a patient in need of such treatment an effective amount of a compound of formula (I).

20 Examples of neurodegenerative disorders which can be treated with the compounds of formula (I) include Parkinson's disease, mild cognitive impairment, dementia (including Alzheimer's disease, vascular dementia, dementia with Lewy bodies and FTD), Huntington's disease, amyotrophic lateral sclerosis (motor neurone disease), multiple system atrophy (MSA), progressive supranuclear palsy and Wilson's disease. Disorders which are particularly

25 suitable for treatment with the compounds of the present invention include Parkinson's disease, mild cognitive impairment, dementia (including Alzheimer's disease, vascular dementia, dementia with Lewy bodies and FTD), Huntington's disease and amyotrophic lateral sclerosis and especially Parkinson's disease, mild cognitive impairment and dementia (including Alzheimer's disease, vascular dementia, dementia with Lewy bodies and FTD).

30

It has been shown that the compounds of the invention are particularly suitable for the treatment of dementia, for example Alzheimer's disease, vascular dementia, dementia with Lewy bodies and FTD and especially Alzheimer's disease.

35 Examples of neuromuscular disorders which can be treated with the compounds of formula (I) include muscular dystrophies, myopathies, NMJ diseases, motor neuron diseases, peripheral nerve diseases, mitochondrial diseases and ion channel diseases.

Examples of muscular dystrophies include Becker muscular dystrophy (BMD), congenital muscular dystrophy (CMD), Duchenne muscular dystrophy (DMD), Emery-Dreifuss muscular dystrophy (EDMD), Fasiosca-pulohume-ra1 muscular dystrophy (FSHD), limb-girdle muscular dystrophy (LGMD), Oculopha-ryngeal MD (OPMD) and distal muscular dystrophy (DD).

5

Examples of myopathies include congenital myopathies, endocrine myopathies, inflammatory myopathies, metabolic myopathies, myofibrillar myopathies and scapula-peroneal myopathies.

Examples of NMJ diseases include congenital myasthenic syndromes (CMS), Lambert-Eaton myasthenic syndrome (LEMS) and myasthenia gravis (MG).

10

Examples of motor neuron diseases include amyotrophic lateral sclerosis (ALS, also included in the list of neurodegenerative disorders above since it arises from degeneration of nerve cells in the brain and spinal cord), spinal-bulbar muscular atrophy (SBMA) and spinal muscular atrophy (SMA).

15

Examples of peripheral nerve diseases include Charcot-Marie-Tooth disease (CMT) and giant axonal neuropathy (GAN).

Examples of mitochondrial diseases include mitochondrial myopathies and Friedreich's ataxia as well as Barth syndrome, chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS), Leigh syndrome, 3-methylglutaconic aciduria, deafness, encephalopathy, and Leigh-like syndrome (MEGDEL syndrome), myoclonic epilepsy and ragged-red fibers syndrome (MERRF syndrome), mitochondrial encephalomyopathy, lactic acidosis and stroke-like syndrome (MELAS), mitochondrial neurogastrointestinal encephalopathy (MNGIE) and Senger syndrome.

20

25

Examples of ion channel diseases include Andersen-Tawil syndrome, hyperkalemic periodic paralysis, hypokalemic periodic paralysis, myotonia congenita, paramyotonia congenita and potassium-aggravated myotonia.

30

Pharmaceutical Compositions

The compounds of formula (I) will generally be administered as part of a pharmaceutical composition.

35

Therefore, in a further aspect of the invention, there is provided a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof and a pharmaceutically acceptable excipient or carrier.

- 5 The composition may be formulated for administration by any route, for example parenteral, including intravenous, intramuscular, subcutaneous or intradermal; or oral, rectal, nasal, topical (including transdermal, eye drops, topical administration to the lung, buccal and sublingual) or vaginal administration.
- 10 More suitably, the composition is formulated for parenteral administration or for oral administration.

In some embodiments, the composition is formulated for oral administration.

- 15 In other embodiments, the composition is formulated for parenteral administration, especially intravenous administration.

- The composition may be prepared by bringing into association the above defined active agent with the carrier. In general, the formulations are prepared by uniformly and intimately bringing
20 into association the active agent with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product. The invention extends to methods for preparing a pharmaceutical composition comprising bringing a compound of formula (I) in conjunction or association with a pharmaceutically acceptable excipient or carrier.

- 25 Formulations for oral administration in the present invention may be presented as: discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active agent; as a powder or granules; as a solution or a suspension of the active agent in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water in oil liquid emulsion; or as a bolus etc.
- 30 In some cases, the compositions may be formulated for delayed, slow or controlled release of the compound of formula (I).

- For compositions for oral administration (e.g. tablets and capsules), the term "acceptable carrier" includes vehicles such as common excipients e.g. binding agents, for example syrup,
35 acacia, gelatin, sorbitol, tragacanth, polyvinylpyrrolidone (Povidone), methylcellulose, ethylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sucrose and starch; fillers and carriers, for example corn starch, gelatin, lactose, sucrose, microcrystalline

cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride and alginic acid; and lubricants such as magnesium stearate, sodium stearate and other metallic stearates, glycerol stearate, stearic acid, silicone fluid, talc waxes, oils and colloidal silica. Flavouring agents such as peppermint, oil of wintergreen, cherry flavouring and the like can also be used. It may be desirable to add a colouring agent to make the dosage form readily identifiable. Tablets may also be coated by methods well known in the art.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active agent in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active agent.

Other formulations suitable for oral administration include lozenges comprising the active agent in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active agent in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active agent in a suitable liquid carrier.

For topical application to the skin, compounds of formula (I) may be made up into a cream, ointment, jelly, solution or suspension etc. Cream or ointment formulations that may be used for the drug are conventional formulations well known in the art, for example, as described in standard text books of pharmaceuticals such as the British Pharmacopoeia.

Topical administration to the lung may be achieved by use of an aerosol formulation. Aerosol formulations typically comprise the active ingredient suspended or dissolved in a suitable aerosol propellant, such as a chlorofluorocarbon (CFC) or a hydrofluorocarbon (HFC). Suitable CFC propellants include trichloromonofluoromethane (propellant 11), dichlorotetrafluoromethane (propellant 114), and dichlorodifluoromethane (propellant 12). Suitable HFC propellants include tetrafluoroethane (HFC-134a) and heptafluoropropane (HFC-227). The propellant typically comprises 40%-99.5% e.g. 40%-90% by weight of the total inhalation composition. The formulation may comprise excipients including co-solvents (e.g. ethanol) and surfactants (e.g. lecithin, sorbitan trioleate and the like). Other possible excipients include polyethylene glycol, polyvinylpyrrolidone, glycerine and the like. Aerosol formulations are packaged in canisters and a suitable dose is delivered by means of a metering valve (e.g. as supplied by Bepak, Valois or 3M or alternatively by Aptar, Coster or Vari).

Topical administration to the lung may also be achieved by use of a non-pressurised formulation such as an aqueous solution or suspension. These may be administered by means of a nebuliser e.g. one that can be hand-held and portable or for home or hospital use (ie non-portable). The formulation may comprise excipients such as water, buffers, tonicity adjusting agents, pH adjusting agents, surfactants and co-solvents. Suspension liquid and aerosol formulations (whether pressurised or unpressurised) will typically contain the compound of the invention in finely divided form, for example with a D_{50} of 0.5-10 μm e.g. around 1-5 μm . Particle size distributions may be represented using D_{10} , D_{50} and D_{90} values. The D_{50} median value of particle size distributions is defined as the particle size in microns that divides the distribution in half. The measurement derived from laser diffraction is more accurately described as a volume distribution, and consequently the D_{50} value obtained using this procedure is more meaningfully referred to as a Dv_{50} value (median for a volume distribution). As used herein Dv values refer to particle size distributions measured using laser diffraction. Similarly, D_{10} and D_{90} values, used in the context of laser diffraction, are taken to mean Dv_{10} and Dv_{90} values and refer to the particle size whereby 10% of the distribution lies below the D_{10} value, and 90% of the distribution lies below the D_{90} value, respectively.

Topical administration to the lung may also be achieved by use of a dry-powder formulation. A dry powder formulation will contain the compound of the disclosure in finely divided form, typically with a mass mean diameter (MMAD) of 1-10 μm or a D_{50} of 0.5-10 μm e.g. around 1-5 μm . Powders of the compound of the invention in finely divided form may be prepared by a micronization process or similar size reduction process. Micronization may be performed using a jet mill such as those manufactured by Hosokawa Alpine. The resultant particle size distribution may be measured using laser diffraction (e.g. with a Malvern Mastersizer 2000S instrument). The formulation will typically contain a topically acceptable diluent such as lactose, glucose or mannitol (preferably lactose), usually of comparatively large particle size e.g. a mass mean diameter (MMAD) of 50 μm or more, e.g. 100 μm or more or a D_{50} of 40-150 μm . As used herein, the term "lactose" refers to a lactose-containing component, including α -lactose monohydrate, β -lactose monohydrate, α -lactose anhydrous, β -lactose anhydrous and amorphous lactose. Lactose components may be processed by micronization, sieving, milling, compression, agglomeration or spray drying. Commercially available forms of lactose in various forms are also encompassed, for example Lactohale[®] (inhalation grade lactose; DFE Pharma), InhaLac[®]70 (sieved lactose for dry powder inhaler; Meggle), Pharmatose[®] (DFE Pharma) and Respitose[®] (sieved inhalation grade lactose; DFE Pharma) products. In one embodiment, the lactose component is selected from the group consisting of α -lactose monohydrate, α -lactose anhydrous and amorphous lactose. Preferably, the lactose is α -lactose monohydrate.

Dry powder formulations may also contain other excipients. Thus in one embodiment a dry powder formulation according to the present disclosure comprises magnesium or calcium stearate. Such formulations may have superior chemical and/or physical stability especially when such formulations also contain lactose.

A dry powder formulation is typically delivered using a dry powder inhaler (DPI) device. Example dry powder delivery systems include SPINHALER®, DISKHALER®, TURBOHALER®, DISKUS®, SKYEHALER®, ACCUHALER® and CLICKHALER®. Further examples of dry powder delivery systems include ECLIPSE, NEXT, ROTAHALER, HANDIHALER, AEROLISER, CYCLOHALER, BREEZHALER/NEOHALER, MONODOSE, FLOWCAPS, TWINCAPS, X-CAPS, TURBOSPIN, ELPENHALER, MIATHALER, TWISTHALER, NOVOLIZER, PRESSAIR, ELLIPTA, ORIEL dry powder inhaler, MICRODOSE, PULVINAL, EASYHALER, ULTRAHALER, TAIFUN, PULMOJET, OMNIHALER, GYROHALER, TAPER, CONIX, XCELOVAIR and PROHALER.

In one embodiment a compound of formula (I) is provided as a micronized dry powder formulation, for example comprising lactose of a suitable grade.

Thus, as an aspect of the invention there is provided a pharmaceutical composition comprising a compound of formula (I) in particulate form in combination with particulate lactose, said composition optionally comprising magnesium stearate.

In one embodiment a compound of formula (I) is provided as a micronized dry powder formulation, comprising lactose of a suitable grade and magnesium stearate, filled into a device such as DISKUS. Suitably, such a device is a multidose device, for example the formulation is filled into blisters for use in a multi-unit dose device such as DISKUS.

In another embodiment a compound of formula (I) is provided as a micronized dry powder formulation, for example comprising lactose of a suitable grade, filled into hard shell capsules for use in a single dose device such as AEROLISER.

In another embodiment a compound of formula (I) is provided as a micronized dry powder formulation, comprising lactose of a suitable grade and magnesium stearate, filled into hard shell capsules for use in a single dose device such as AEROLISER.

In another embodiment a compound of formula (I) is provided as a fine powder for use in an

inhalation dosage form wherein the powder is in fine particles with a D_{50} of 0.5-10 μm e.g. around 1-5 μm , that have been produced by a size reduction process other than jet mill micronisation e.g. spray drying, spray freezing, microfluidisation, high pressure homogenisation, super critical fluid crystallisation, ultrasonic crystallisation or combinations of these methods thereof, or other suitable particle formation methods known in the art that are used to produce fine particles with an aerodynamic particle size of 0.5-10 μm . The resultant particle size distribution may be measured using laser diffraction (e.g. with a Malvern Mastersizer 2000S instrument). The particles may either comprise the compound alone or in combination with suitable other excipients that may aid the processing. The resultant fine particles may form the final formulation for delivery to humans or may optionally be further formulated with other suitable excipients to facilitate delivery in an acceptable dosage form. The compound of the invention may also be administered rectally, for example in the form of suppositories or enemas, which include aqueous or oily solutions as well as suspensions and emulsions and foams. Such compositions are prepared following standard procedures, well known by those skilled in the art. For example, suppositories can be prepared by mixing the active ingredient with a conventional suppository base such as cocoa butter or other glycerides. In this case, the drug is mixed with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

Parenteral formulations will generally be sterile.

The medical practitioner, or other skilled person, will be able to determine a suitable dosage for the compound of formula (I), and hence the amount of the compound of the invention that should be included in any particular pharmaceutical formulation (whether in unit dosage form or otherwise).

Compounds of formula (I) may be used in combination with one or more other active agents which are useful in the treatment or prophylaxis of neurodegenerative disorders or neuromuscular disorders, for example the neurodegenerative and neuromuscular disorders described above.

Therefore, in a further aspect of the invention, there is provided a pharmaceutical composition as described above further comprising an additional active agent useful in the treatment or prophylaxis of neurodegenerative disorders or neuromuscular disorders, for example the neurodegenerative and neuromuscular disorders described above.

There is also provided a product comprising a compound of formula (I) and an additional active agent useful in the treatment or prevention of a neurodegenerative or neuromuscular disorder as a combined preparation for simultaneous, sequential or separate use in the treatment or prevention of a neurodegenerative disorder or neuromuscular disorder, for example the neurodegenerative and neuromuscular disorders as described above.

Figures and Examples

The invention will now be further described with reference to the following examples and to the drawings in which:

FIGURE 1 is a plot showing blood concentrations of Compound 1 following IV Administration to male C57 mice at 1.00 (mg/kg) where reference numbers 19, 20 and 21 each refer to an individual mouse.

FIGURE 2 is a plot showing blood concentrations of Comparative Compound A following IV Administration to male C57 mice at 1.00 (mg/kg) where reference numbers 13, 14 and 15 each refer to an individual mouse.

FIGURE 3 is a plot showing blood concentrations of Compound 1 following oral administration to male C57 mice at 3.00 (mg/kg) where reference numbers 22, 23 and 24 each refer to an individual mouse.

FIGURE 4 is a plot showing blood concentrations of Comparative Compound A following oral administration to male C57 mice at 3.00 (mg/kg) where reference numbers 16, 17 and 18 each refer to an individual mouse.

Abbreviations

CDCA	Chenodeoxycholic acid
DAST	Diethylaminosulfur trifluoride
DCM	Dichloromethane
DIPEA	<i>N,N</i> -diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethylsulfoxide

Eq	Equivalents
HMDS	Hexamethyldisilazane
HMPT	Hexamethylphosphorous triamide
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
IPA	Isopropyl alcohol
IR	Infrared
LDA	Lithium diisopropylamide
MeCN	Acetonitrile
MOMCl	Chloromethyl methyl ether
MS	Mass spectrometry
MsCl	Methanesulfonyl chloride
nor-UDCA	nor-ursodeoxycholic acid
NCS	N-chlorosuccinimide
NFSI	N-fluorobenzenesulfonimide
NMR	Nuclear magnetic resonance
Quant	Quantitative
TBAB	Tetrabutylammonium bromide
TBME	Tert butyl methyl ether
TFA	Trifluoroacetic acid
TFFA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSOTf	Trimethylsilyl trifluoromethane sulfonate (trimethylsilyl triflate)
TPPA	Tripyrrolidinophosphoric acid triamide
UDCA	Ursodeoxycholic acid

General Experimental Procedures

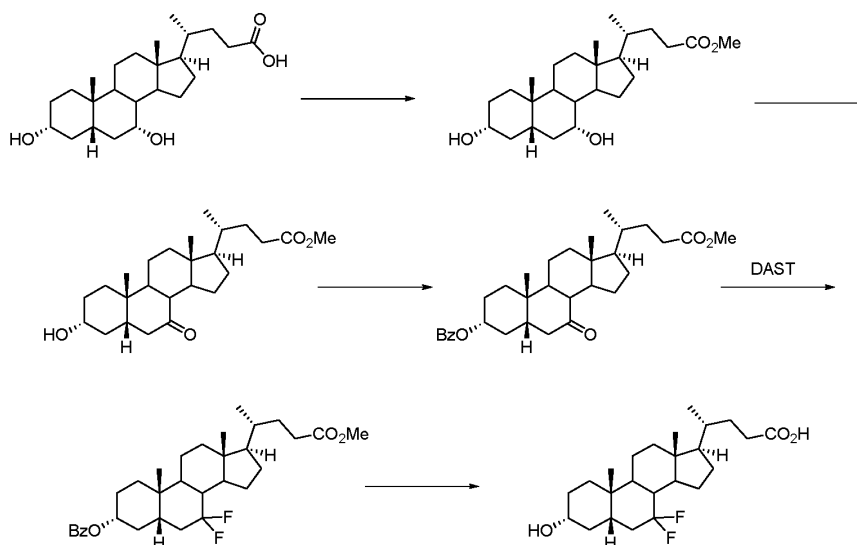
Proton (^1H), carbon (^{13}C) and fluorine (^{19}F) NMR-spectra were recorded on a Bruker AV400 spectrometer at 298K unless otherwise stated. Chemical shifts (δ) are quoted in parts per million (ppm) and coupling constants (J) in Hertz (Hz). Abbreviations indicating multiplicity are: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, hept = heptet, m = multiplet, br = broad, app. apparent. NMR spectra were referenced using residual solvent peaks set to δ 7.26 and 77.16 ppm (CDCl_3), or δ 3.31 and 49.00 ppm (CD_3OD), or δ 2.50 and 39.52 ppm (d_6 -DMSO), or δ 2.05 and 29.82 ppm (acetone- d_6).

Low resolution ES mass spectra were recorded on a WATERS ZMD single quadrupole system or an Agilent 1260 Infinity II, iQ MSD single quadrupole system. High resolution mass spectra were recorded on the Bruker Apex III FT-ICR-MS. Optical rotations were recorded on an OPTICAL ACTIVITY POLAAR 2001 polarimeter at 589 nm. All reactions were monitored by

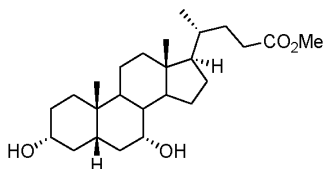
5 thin layer chromatography (TLC) using 0.2 mm silica gel (Merck Kieselgel 60 F₂₅₄) precoated aluminium or glass plates, using UV light, cerium ammonium molybdate stain, p-anisaldehyde, ninhydrin or potassium permanganate staining solution to visualize. Flash column chromatography was performed on a Biotage® Isolera automated flash system with Biotage® Sfar Columns. Preparative HPLC chromatography was performed on an Agilent 1260 II LC

10 system with preparative binary pump, using a multiple wavelength detector and a Phenomenex Prodigy ODS-3 100Å, 5μ, 250 x 21.2 mm column. Solvents for reactions and chromatography were analytical grade and were used as supplied unless otherwise stated.

15 **Comparative Example A – Synthesis of 7,7-difluoro lithocholic acid (Comparative Compound A)**



i. **Synthesis of methyl 3α,7α-dihydroxy-5β-cholan-24-oate**



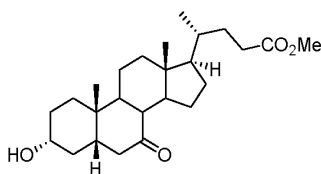
To a round-bottom flask were added CDCA (1 g, 2.55 mmol, 1 eq), MeOH (20 mL) and p-

20 toluenesulfonic acid monohydrate (50 mg, 0.26 mmol, 0.1 eq). The reaction mixture was stirred until the solution was homogeneous, and then sonicated at 30 °C for 2 h. The reaction mixture

was concentrated, the residue was dissolved in chloroform (30 mL), washed with saturated NaHCO_3 (2×10 mL), water (10 mL) and 10% NaCl (10 mL), dried over Na_2SO_4 , filtered and concentrated to afford the methyl ester quantitatively as gummy solid (95%), and used for next step without any purification.

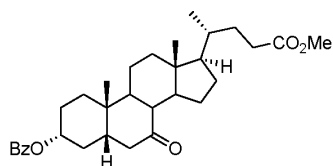
- 5 ^1H NMR (300 MHz, CDCl_3) δ 3.79-3.90 (m, 1H, H7), 3.66 (s, 3H, OCH_3), 3.37-3.55 (m, 1H, H3), 0.92 (d, $J = 6.4$ Hz, 3H, H21), 0.90 (s, 3H, H19), 0.66 (s, 3H, H18); ^{13}C NMR (75 MHz, CDCl_3) δ 174.7 (C24), 71.9, 68.5, 55.8, 51.4, 50.4, 42.6, 41.5, 39.9, 39.6, 39.4, 35.33, 35.31, 35.0, 34.6, 32.8, 30.97, 30.95, 30.6, 28.1, 23.7, 22.7, 20.5, 18.2, 11.7.

10 ii. **Synthesis of methyl 3 α -hydroxy-7-keto-5 β -cholan-24-oate**



- To a round-bottom flask were added methyl 3 α ,7 α -dihydroxy-5 β -cholan-24-oate from step i (3.59 g, 8.83 mmol, 1 eq), sodium bromide (0.057 eq), TBAB (3.30 eq) and a solution of MeOH/MeCOOH/ H_2O /AcOEt (v/v:3/1/0.25/6.5, 8.43 mL/mmol) and the reaction mixture was stirred till the solution was homogeneous. Sodium hypochlorite solution (11-14%, 1.1 eq) was added at 0 °C until the hypochlorite test (peroxide test paper) was positive. The reaction mixture was stirred at room temperature for 6 h. After TLC indicated the completion of the reaction, the reaction mixture was quenched with sodium bisulfite solution (3.3%) until the hypochlorite test was negative. Water was added and stirred for 15 min at room temperature.
- 15 The mixture was extracted with ethyl acetate (x3) and the combined organic phase washed with aqueous sodium bisulfite solution (3.3%), water and dried over anhydrous sodium sulphate, filtered and concentrated. The crude was purified by flash chromatography to afford the desired compound (acetone/petroleum ether 12:88-80:20) to afford (2.15 g, 60%) as a gummy solid.
- 20 ^1H NMR (300 MHz, CDCl_3) δ 3.65 (s, 3H, OCH_3), 3.52-3.63 (m, 1H, H3 β), 2.85 (dd, $J = 12.6$, 6.3 Hz, 1H, H6 β), 2.28-2.44 (m, 2H), 2.12-2.27 (m, 2H), 1.19 (s, 3H, H19), 0.91 (d, $J = 6.2$ Hz, 3H, H21), 0.64 (s, 3H, H18); ^{13}C NMR (75 MHz, CDCl_3) δ 212.0 (C7), 174.6 (C24), 70.8 (C3), 54.7, 51.4, 49.5, 48.9, 46.1, 45.4, 42.7, 42.6, 38.9, 37.4, 35.2, 35.1, 34.1, 31.0, 31.0, 29.8, 28.2, 24.8, 23.0, 21.6, 18.3, 12.0.

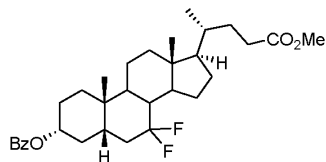
iii. Synthesis of methyl 3 α -benzoyloxy-7-keto-5 β -cholan-24-oate



To a round-bottom flask were added methyl 3 α -hydroxy-7-keto-5 β -cholan-24-oate from step ii (5 g, 12.4 mmol, 1.0 eq), toluene (50 mL), pyridine (1.6 mL) and BzCl (2.3 mL, 1.5 eq). The reaction mixture was stirred at ambient overnight. Upon completion indicated by TLC analysis, the reaction was quenched with H₂O and extracted with ethyl acetate. The organic layer was washed with aq. HCl (2.0 M), H₂O, 10% NaCl, and then dried over Na₂SO₄, filtered and concentrated. The crude was purified by flash chromatography (acetone/petroleum ether 8:92 - 20:80) to afford product (86%) as white solid. The crystals were obtained from recrystallisation in acetone/petroleum ether.

[α]_D -14.0 (c 0.7, CHCl₃, 20 °C); m.p. 136-138 °C (acetone/petroleum ether); R_f 0.22 (acetone/petroleum ether 10:90); IR (neat) 2940 (m), 2869 (m), 1736 (m), 1710 (s), 1445 (w), 1380 (w), 1274 (s), 1173 (w), 1112 (m), 756 (w), 711 (m) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.00 (dd, J = 8.4, 1.3 Hz, 2H, HAr(o)), 7.53 (tt, J = 7.3, 1.8 Hz, 1H, HAr(p)), 7.41 (t, J = 7.9 Hz, 2H, HAr(m)), 4.94 (tt, J = 11.4, 4.6 Hz, 1H, H3 β), 3.66 (s, 3H, OCH₃), 2.89 (dd, J = 12.4, 5.9 Hz, 1H, H6 β), 2.42 (t, J = 11.3 Hz, 1H, H8 β), 2.30-2.38 (m, 1H), 2.15-2.28 (m, 2H), 1.24 (s, 3H, H19), 0.92 (d, J = 6.3 Hz, 3H, H21), 0.67 (s, 3H, H18); ¹³C NMR (101 MHz, CDCl₃) δ 211.8 (C7), 174.6 (C24), 165.9 (PhCO), 132.8 (CAr(p)), 130.5 (CArCO), 129.5 (CAr(o)), 128.2 (CAr(m)), 73.4 (C3), 54.7 (CH), 51.4 (OCH₃), 49.5 (C8), 48.8 (CH), 45.9 (CH), 45.2 (C6), 42.8 (CH), 42.6 (C), 38.9 (CH₂), 35.17 (C), 35.16 (CH), 33.8 (CH₂), 33.2 (CH₂), 31.0 (CH₂), 30.9 (CH₂), 28.2 (CH₂), 26.1 (CH₂), 24.7 (CH₂), 23.0 (C19), 21.7 (CH₂), 18.3 (C21), 12.0 (C18); MS (ESI⁺) m/z 526.2 ([M+NH₄]⁺, 100%), 531.2 ([M+Na]⁺, 17%); HRMS(ESI⁺) for C₃₂H₄₄NaO₅ [M+Na]⁺ calcd 531.3081, found 531.3072 (1.7 ppm error).

iv. Synthesis of methyl 3 α -benzoyloxy-7,7-difluoro-5 β -cholan-24-oate



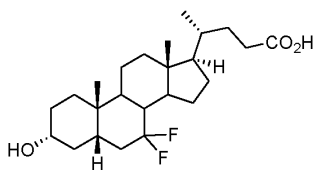
To a round-bottom flask were added methyl 3 α -benzoyloxy-7-keto-5 β -cholan-24-oate from step iii (1.36 g) and DAST (15 mL). The reaction mixture was heated at 80 °C overnight. Upon

completion monitored by TLC analysis, the reaction mixture was quenched with sat. aq. NaHCO₃ solution then extracted with ethyl acetate. The combined organic layer was washed with 10% NaCl, dried over Na₂SO₄, filtered, and concentrated. The crude was purified by flash chromatography (acetone/petroleum ether 4:96-8:92-20:80) and HPLC (acetone/hexane 5:95)

5 to afford the desired compound.

[α]_D + 30.4 (c 1.1, CHCl₃, 20 °C); m.p. 140-144 °C (acetone/hexane); R_f 0.29 (acetone/petroleum ether 10:90); IR (neat) 2940 (m), 2868 (w), 1740 (s), 1711 (s), 1455 (w), 1321 (w), 1275 (s), 1176 (m), 1141 (m), 1121 (s), 1065 (s), 1026 (m), 977 (m), 760 (s), 718 (s) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.02 (dd, *J* = 8.5, 1.5 Hz, 2H, HAr(o)), 7.51 (tt, *J* = 7.5, 1.4 Hz, 1H, HAr(p)), 7.40 (t, *J* = 7.5 Hz, 2H, HAr(m)), 4.87 (tt, *J* = 11.0, 4.5 Hz, 1H, H3β), 3.64 (s, 3H, OCH₃), 2.27-2.40 (m, 1H), 0.97 (s, 3H, H19), 0.92 (d, *J* = 6.5 Hz, 3H, H21), 0.66 (s, 3H, H18); ¹³C NMR (101 MHz, CDCl₃) 174.6 (C24), 166.0 (PhCO), 132.7 (CAr(p)), 130.7 (CArCO), 129.5 (CAr(o)), 128.2 (CAr(m)), 124.6 (t, *J* = 244.5 Hz, C7), 74.0 (C3), 55.0 (CH), 51.4 (OCH₃), 48.6 (d, *J* = 3.5 Hz, CH), 43.1 (C), 41.8 (dd, *J* = 22.9, 19.3 Hz, C8), 41.1 (d, *J* = 10.6 Hz, CH), 39.4 (CH₂), 37.1 (d, *J* = 9.0 Hz, CH), 36.1 (t, *J* = 23.7 Hz, C6), 35.3 (CH), 34.40 (CH₂), 34.36 (C), 33.1 (d, *J* = 4.4 Hz), 31.01 (CH₂), 30.98 (CH₂), 28.3 (CH₂), 26.5 (CH₂), 25.3 (d, *J* = 3.9 Hz, CH₂), 22.6 (C19), 20.9 (CH₂), 18.3 (C21), 11.8 (C18); ¹⁹F NMR (376 MHz, CDCl₃) δ -84.7 (br. d, *J* = 241.0 Hz, F7), -101.4 (dddd, *J* = 241.0, 38.1, 25.1, 14.7 Hz, F7); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) -84.7 (d, *J* = 241.0 Hz, F7), -101.4 (d, *J* = 241.0 Hz, F7); MS(ESI+) *m/z* 553.3 ([M+Na]⁺, 100%); HRMS (ESI+) for C₃₂H₄₄F₂NaO₄ [M+Na]⁺ calcd 553.3100, found 553.3101 (-0.2 ppm error).

v. Synthesis of 7,7-difluoro lithocholic acid (Comparative Compound A)



25 To a round-bottom flask were added methyl 3α-benzoyloxy-7,7-difluoro-5β-cholan-24-oate from step iv (40 mg), NaOH (200 mg) and MeOH (2 mL). The reaction mixture was stirred at rt overnight. Upon the completion indicated by TLC analysis, the solvent was firstly removed and then acidified with aq. 6 M HCl solution. The solution was further extracted with ethyl acetate. The combined organic layer was washed with 10% NaCl, dried over Na₂SO₄, filtered and concentrated. NMR analysis of the crude mixture indicated a quantitative conversion to the desired product. The crude was purified by flash chromatography (DCM/MeOH 97:3,

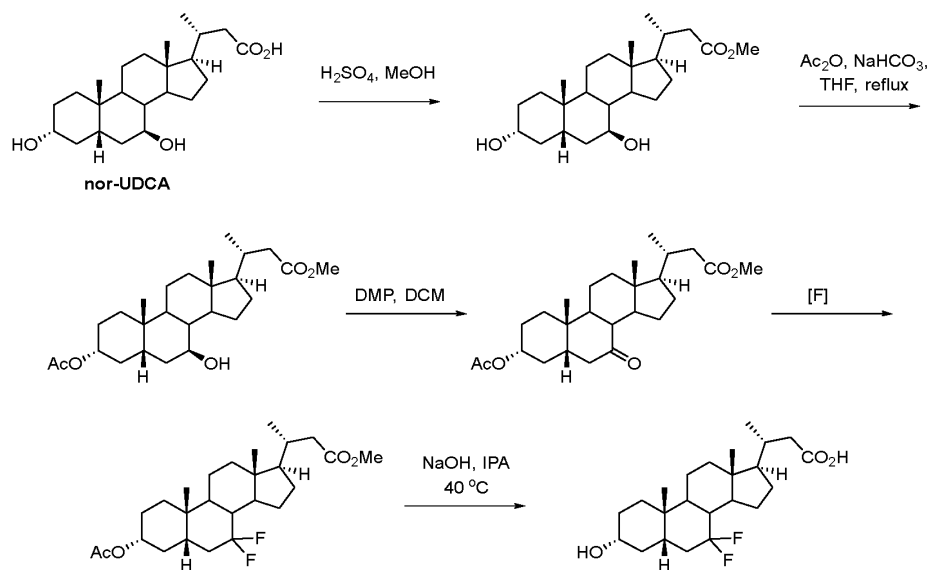
addition of 0.5% formic acid) to afford Comparative Compound A as white solid, recrystallised from neat MeOH.

IR (neat) 3361 (br. w), 2952 (s), 2927 (s), 2869 (s), 1708 (s), 1470 (m), 1455 (m), 1379 (m), 1072 (s), 978 (m), 916 (m), 732 (s) cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 3.47 (tt, $J = 11.0, 5.3$ Hz, 1H, H3 β), 2.29-2.45 (m, 1H), 0.98 (s, 3H, H19), 0.97 (d, $J = 6.9$ Hz, 3H, H21), 0.71 (s, 3H, H18); ^{13}C NMR (101 MHz, CDCl_3) δ 178.2 (C24), 126.0 (dd, $J = 244.5, 243.2$ Hz, C7), 72.1 (C3), 56.6, 50.2 (d, $J = 3.9$ Hz), 44.4, 43.3 (dd, $J = 23.3, 20.0$ Hz), 42.7 (d, $J = 10.6$ Hz), 41.0, 38.6 (d, $J = 9.0$ Hz), 38.2 (d, $J = 4.0$ Hz), 37.5 (t, $J = 23.5$ Hz), 36.8, 35.9, 35.5, 32.4, 32.1, 31.0, 29.4, 26.7 (d, $J = 3.9$ Hz), 23.2, 22.1, 19.0, 12.4; ^{19}F NMR (376 MHz, CDCl_3) 84.7 (br. d, $J = 241.0$ Hz, F7 β), -102.0 (dddd, $J = 241.0, 38.6, 25.1, 14.7$ Hz, F7 α); MS (ESI+) m/z 825.4 ($[\text{2M}+\text{H}]^+$, 46%); HRMS (ESI+) for $\text{C}_{24}\text{H}_{42}\text{F}_2\text{NO}_3$ $[\text{M}+\text{NH}_4]^+$ calcd 430.3127, found 430.3124 (0.7 ppm error).

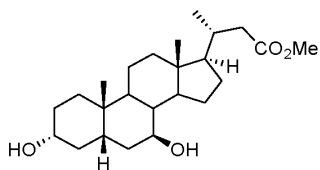
Example 1 – Synthesis of 7,7-difluoro 24-norlithocholic acid (Compound 1)

Compound 1 was synthesised as shown in Scheme 7

Scheme 7



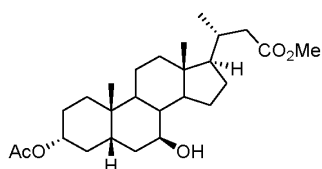
20 i. Methyl 3 α ,7 β -dihydroxy-5 β -24-nor-cholan-23-oate



To a suspension of nor-UDCA (40 g, 105.7 mmol) in MeOH (HPLC grade, 300 mL) was charged conc. sulfuric acid (~200 μ L). The reaction was stirred at ambient temperature for 3 days, then quenched with triethylamine and concentrated to dryness. The residue was dispersed in ethyl acetate (200 mL) and water (40 mL), stirred for 1 h then filtered, washed with water and dried. The filtrate was separated, the organic phase washed with water (40 mL), concentrated and combined with the isolated solid to afford nor-UDCA methyl ester as a white solid (41.57 g, quant).

^1H NMR (400 MHz, CDCl_3) δ 3.64 (s, 3H), 3.60-3.51 (m, 2H), 2.41 (dd J = 14.4, 3.2 Hz, 1H), 2.03-1.94 (m, 2H), 1.94-1.72 (m, 5H), 1.68-1.53 (m, 4H), 1.53-1.38 (m, 5H), 1.38-0.99 (m, 7H), 0.96 (d, J = 6.3 Hz, 3H), 0.92 (s, 3H), 0.69 (s, 3H).

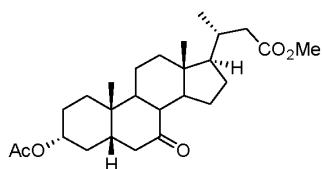
ii. **Methyl 3 α -acetylhydroxy-7 β -hydroxy-5 β -24-nor-cholan-23-oate**



To a solution of nor-UDCA methyl ester (24.13 g, 61.5 mmol) in THF (482 mL) at reflux was charged NaHCO_3 (30.1 g, 369 mmol), followed by the dropwise addition of acetic anhydride (29.1 mL, 307 mmol). The reaction mixture was stirred at reflux for 20 h and then cooled to ambient temperature, diluted with EtOAc (300 mL) and added slowly into a stirred solution of sat. NaHCO_3 . The organic phase was dried over MgSO_4 , concentrated *in vacuo* and purified by column chromatography (SiO_2 , heptane in ethyl acetate 0-50%) to afford the title compound (20.5 g, 76%).

^1H NMR (400 MHz, CD_3OD) δ 4.68-4.59 (m, 1H), 3.65 (s, 3H), 3.50-3.42 (m, 1H), 2.46 (dd, J = 14.5, 3.4 Hz, 1H), 2.07-1.98 (m, 2H), 2.00 (s, 3H), 1.95-1.79 (m, 5H), 1.74-1.64 (m, 3H), 1.59-1.05 (m, 13H), 1.07-0.68 (m, 6H), 0.75 (s, 3H).

iii. **Methyl 3 α -acetylhydroxy-7-keto-5 β -24-nor-cholan-23-oate**

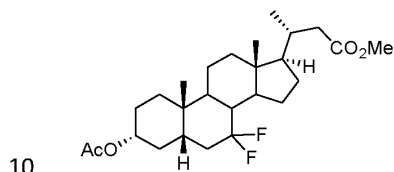


To a solution of methyl 3 α -acetylhydroxy-7 β -hydroxy-5 β -24-nor-cholan-23-oate from step ii (20 g, 46.0 mmol) in DCM (200 mL) was charged DMP (23.4 g, 55.2 mmol) portion-wise maintaining the temperature at $< 30^\circ\text{C}$. The reaction mixture was stirred at ambient temperature for 17 h then filtered and washed with DCM. The solution was stirred with sat.

NaHCO₃ (150 mL) for 1 h before charging 5% sodium thiosulfate (140 mL). The phases were separated and the organic phase was washed with water (2 x 200 mL) and 10% NaCl (200 mL), dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, acetone/DCM) to afford the title compound (18 g, 90%).

- 5 ¹H NMR (400 MHz, CDCl₃) δ 4.74-4.63 (m, 1H), 3.66 (s, 3H), 2.88-2.82 (m, 1H), 2.46-2.36 (m, 2H), 2.26-2.15 (m, 1H), 2.05-1.68 (m, 10H), 1.99 (s, 3H), 1.59-1.40 (m, 4H), 1.40-1.26 (m, 3H), 1.26-1.10 (m, 3H), 1.20 (s, 3H), 0.98 (d, *J* = 6.3 Hz, 3H), 0.69 (s, 3H).

Iv(a). Methyl 3α-acetylhydroxy-7,7-difluoro-5β-24-nor-cholan-23-oate



- To methyl 3α-acetylhydroxy-7-keto-5β-24-nor-cholan-23-oate from step iii (17.8 g, 41.1 mmol) was charged DAST (50 g). The reaction mixture was heated to 50 °C for 2 weeks, then diluted with DCM and quenched by slowly charging into 5% NaHCO₃ and stirring overnight. The phases were separated and the aqueous phase was extracted with DCM. The combined
15 extracts were washed with 5% NaHCO₃ and concentrated *in vacuo*. The resulting residue was purified by column chromatography (SiO₂, acetone/heptane), to afford the title compound (3.34 g, 18%).

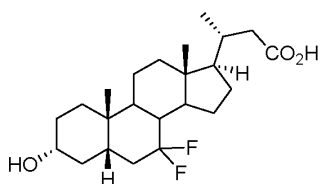
- ¹H NMR (400 MHz, CDCl₃) δ 4.69-4.58 (m, 1H), 3.66 (s, 3H), 2.44 (dd, *J* = 14.4, 3.2 Hz, 1H), 2.25-2.06 (m, 1H), 2.06-1.97 (m, 3H), 1.97-1.89 (m, 1H), 1.89-1.58 (m, 10H), 1.57-1.44 (m, 2H), 1.44-1.24 (m, 4H), 1.24-1.07 (m, 3H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.96 (s, 3H), 0.71 (s, 3H);
20 ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -84.5 (d, *J* = 240.8 Hz), -101.3 (d, *J* = 240.8 Hz).

- Iv(b) Methyl 3α-acetylhydroxy-7,7-difluoro-5β-24-nor-cholan-23-oate (alternative synthesis)**
25

- Methyl 3α-acetylhydroxy-7-keto-5β-24-nor-cholan-23-oate (15 g, 0.034 mol, 1 equiv.) and water (1.87 g, 0.1 mol, 3 equiv.) were placed in a 300 mL autoclave made of Hastelloy nickel alloy. The reaction vessel was cooled down by liquid nitrogen, and SF₄ (37.5 g, 0.34 mol, 10 equiv.) was condensed into a reaction vessel. The cooling bath was removed, and the mixture
30 was allowed to warm up to room temperature and stirred at this temperature for 24h. Then gaseous products were vented off into a trap with an aqueous solution of NaOH (1 M). The reaction vessel was again cooled and another portion of SF₄ (37.5 g, 0.34 mol, 10 equiv.) was condensed into autoclave. The cooling bath was removed, and the mixture was allowed to

warm up to room temperature and stirred at this temperature for additional 24h. Then gaseous products were vented off into a trap with an aqueous solution of NaOH (1 M) and residue was poured onto ice (200 g), extracted with DCM (200 ml), and an organic extract was washed with saturated aq. NaHCO₃ (150 mL). The organic layer was separated, dried over Na₂SO₄, filtered, and concentrated under reduced pressure to afford crude product. Crude product was purified by recrystallization from 220 ml of isopropyl alcohol to gave 11.6 g, 74% yield of material as slightly yellow solid.

v. **7,7-difluoro 24-norlithocholic acid (Compound 1)**



10

Methyl 3 α -acetylhydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate from step iv (0.2 g, 0.44 mmol) was suspended in IPA (2 mL), 1.0 M NaOH (1.45 mL) was charged and the mixture was heated at 40 °C for 18 h. The reaction mixture was concentrated and charged into 2.0 M H₂SO₄ (0.73 mL). the resulting solid was collected by filtration and purified by column chromatography (SiO₂, acetone/heptane) to afford the final compound as an off white solid (167 mg, 95%).

15

pK_a 4.1; ¹H NMR (400 MHz, CD₃OD) δ 3.52-3.43 (m, 1H), 2.46-2.40 (m, 1H), 2.30-2.12 (m, 1H), 2.06 (dt, *J* = 12.6, 3.2 Hz, 1H), 2.01-1.48 (m, 14H), 1.45-1.16 (m, 6H), 1.10 (td, *J* = 14.1, 2.9 Hz, 1H), 1.03 (d, *J* = 6.2 Hz, 3H), 0.99 (s, 3H), 0.75 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 177.4, 125.8 (t, *J* = 243.6 Hz), 71.9, 56.5, 50.1 (*J* = 4.2 Hz), 44.3, 43.1 (dd, *J* = 22.9, 19.3 Hz), 42.5 (br. d, *J* = 10.7 Hz), 42.5, 40.8, 38.5 (d, *J* = 9.0 Hz), 38.1 (d, *J* = 4.2 Hz), 37.3 (t, *J* = 23.0 Hz), 35.8, 35.4 (d, *J* = 1.2 Hz), 34.9, 30.9, 29.4 (d, *J* = 1.5 Hz), 26.5 (d, *J* = 4.3 Hz), 23.0, 22.0, 20.0, 12.2; ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.2 (d, *J* = 241.0 Hz), -102.4 (d, *J* = 241.0 Hz); MS (ESI⁻) *m/z* 397.3 [M-H]⁻.

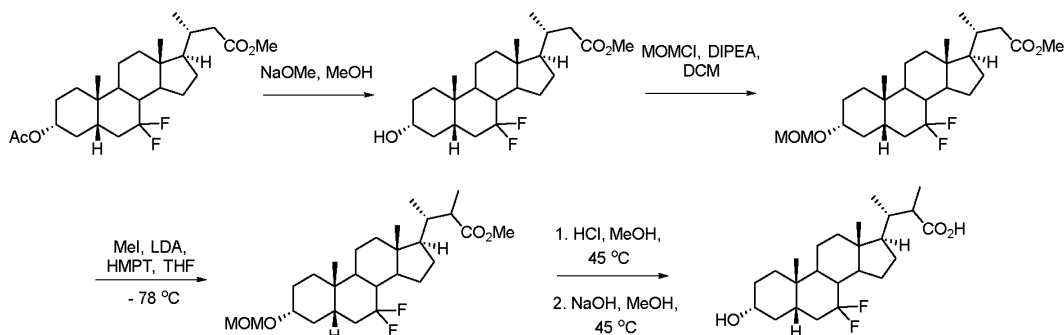
20

25 **Example 2 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-methyl-cholanic acid (Compound 2)**

Compound 2 was synthesised from methyl 3 α -acetylhydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate, i.e the product of Example 1, step iv, by the method shown in Scheme 8.

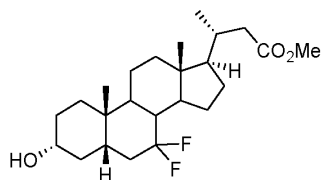
30

Scheme 8



5 i. **Methyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate (Compound 1 Me ester)**

The product of this step is the starting material for Compound 25 of Example 25 (3-keto).

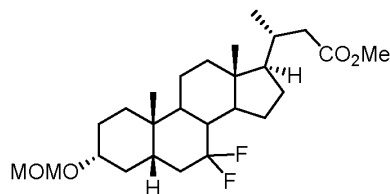


To a suspension of methyl 3 α -acetylhydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate (5.17 g, 11.4 mmol) in MeOH (100 mL) was charged 25 wt% NaOMe in MeOH to pH 12. The reaction mixture was stirred at ambient temperature for 18 h then quenched with Amberlyst 15 H⁺ resin, filtered and concentrated *in vacuo* to afford the title compound (4.59 g, quant.).

¹H NMR (400 MHz, CDCl₃) δ 3.66 (s, 3H), 3.59-3.49 (m, 1H), 2.44 (dd, *J* = 14.4, 3.1 Hz, 1H), 2.24-2.05 (m, 1H), 2.05-1.97 (m, 2H), 1.97-1.63 (m, 10H), 1.61-1.41 (m, 3H), 1.41-1.12 (m, 6H), 1.07 (td, *J* = 14.2, 3.2 Hz, 1H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.95 (s, 3H), 0.70 (s, 3H).

15 ii. **Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-oate**

The product of this step is the starting material for Compound 3 of Example 3, Compound 4 of Example 4, Compound 5 of Example 5, Compound 6 of Example 6, Compound 12 of Example 12, Compound 17 of Example 17.

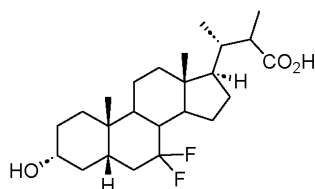


To a solution of methyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate from step i (4.69 g, 11.4 mmol) in DCM (20 mL) was charged DIPEA (6.34 mL, 36.4 mmol) dropwise, followed by MOMCl (1.64 mL, 21.6 mmol), which was washed in with additional DCM (30 mL). The reaction

mixture was stirred under nitrogen at ambient temperature for 4 days then quenched by the drop-wise addition of water (50 mL). After stirring for 1 h the phases were separated. The aqueous phase was extracted with DCM (2 x 15 mL) and the combined extracts were washed with 0.5 M HCl (3 x 15 mL, till pH < 5), 5% NaHCO₃ (25 mL), dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, 2-10% ethyl acetate in heptane) to afford the title compound as an off white solid (4.46, 86%).

¹H NMR (400 MHz, CDCl₃) δ 4.67 (s, 2H), 3.66 (s, 3H), 3.49-3.39 (m, 1H), 3.36 (s, 3H), 2.44 (dd, *J* = 14.4, 3.1 Hz, 1H), 2.24-2.05 (m, 1H), 2.05-1.97 (m, 2H), 1.96-1.88 (m, 1H), 1.88-1.67 (m, 9H), 1.59-1.43 (m, 3H), 1.40-1.23 (m, 4H), 1.22-1.10 (m, 2H), 1.05 (td, *J* = 14.2, 3.2 Hz, 1H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.95 (s, 3H), 0.70 (s, 3H).

iii. **3α-hydroxy-7,7-difluoro-5β-24-nor-22-methyl-cholanic acid (Compound 2)**



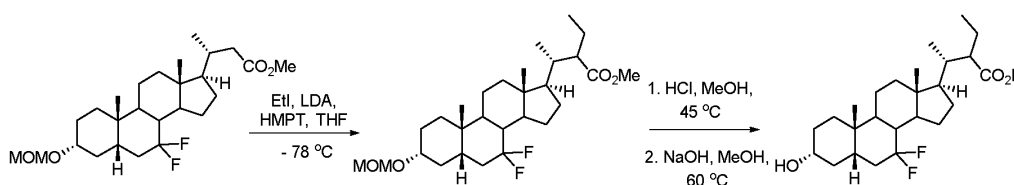
To a solution of methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-cholan-23-oate from step ii (0.2 g, 0.44 mmol) in THF (8 mL) was charged HMPT (40 μL, 0.22 mmol). The reaction mixture was cooled to -78 °C under nitrogen then LDA (1.0 M in THF/hexanes, 0.66 mL, 0.66 mmol) was charged. After stirring at -78 °C for 30 min MeI (41 μL, 0.66 mmol) in THF (1 mL) was charged and the solution was allowed to warm to ambient temperature whilst stirring under nitrogen. After 18 h the reaction was quenched by the addition of 1 M HCl (10 mL), stirred for 30 min then the phases were separated and the aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined extracts were washed with 5% NaHCO₃ (20 mL), 10% NaCl (20 mL) and concentrated *in vacuo*. The residue was dissolved in MeOH (14 mL), 2 M HCl (1.1 mL, 2.2 mmol) was charged and the reaction mixture was heated to 40 °C for 18 h, then 50% NaOH (0.24 mL, 4.4 mmol) was charged and the solution was heated at 40 °C for 5 days. The solution was concentrated *in vacuo*, water (15 mL) was charged the aqueous solution was extracted with TBME (15 mL). The aqueous phase was acidified with 2 M HCl, concentrated *in vacuo* and purified by column chromatography (SiO₂, 0-10% acetone in toluene) to afford the title compound as a single diastereomer an off white solid (88 mg, 48%). p*K*_a 4.9; ¹H NMR (400 MHz, CD₃OD) δ 3.52-3.43 (m, 1H), 2.58 (ddd, *J* = 14.1, 7.0, 3.0 Hz, 1H), 2.30-2.12 (m, 1H), 2.12-2.06 (m, 1H), 2.06-1.99 (m, 1H), 1.92-1.49 (m, 12H), 1.44-1.17 (m, 6H), 1.10 (td, *J* = 14.0, 2.9 Hz, 1H), 0.99 (s, 3H), 0.97 (d, *J* = 7.1 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.76 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 180.3, 125.9 (t, *J* = 241.6 Hz), 71.9, 53.9, 50.2 (d, *J* = 4.2 Hz), 44.2, 43.2 (dd, *J* = 23.4, 19.7 Hz), 43.1, 42.6 (br. d, *J* = 9.8 Hz), 40.9, 38.5,

38.5 (d, $J = 8.2$ Hz), 38.3 (dd, $J = 32.5, 4.2$ Hz), 37.4 (t, $J = 23.3$ Hz), 35.8, 35.4, 30.9, 29.0 (d, $J = 1.7$ Hz), 26.4 (d, $J = 4.3$ Hz), 23.0, 22.0, 14.9, 12.2, 8.9; ^{19}F $\{^1\text{H}\}$ NMR (376 MHz, CD_3OD) -85.2 (d, $J = 241.0$ Hz), -102.5 (d, $J = 241.0$ Hz); MS (ESI $^-$) m/z 411.3 $[\text{M}-\text{H}]^-$.

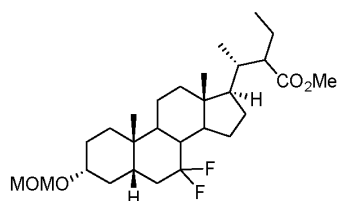
5 **Example 3 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-ethyl-cholan-23-oate (Compound 3)**

Compound 3 was synthesised from methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-oate, i.e. the product of Example 2, step ii according to Scheme 9.

10 **Scheme 9**

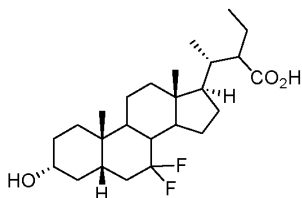


i. **Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-ethyl-cholan-23-oate**



- 15 To a solution of methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-oate (0.5 g, 1.10 mmol) and HMPT (95 μL , 0.52 mmol) in THF (15 mL) at -78°C under nitrogen was charged LDA (1.0 M in THF/hexanes, 1.64 mL, 1.64 mmol). After stirring for 30 min ethyl iodide (132 μL , 1.64 mmol) in THF (1 mL) was charged. The reaction mixture was allowed to warm to ambient temperature then stirred under nitrogen for 18 h. The mixture was partitioned between
- 20 1 M HCl (30 mL) and ethyl acetate (30 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined extracts were washed with 5% NaHCO_3 (30 mL) and 10% NaCl (30 mL), dried over MgSO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2 , 0-10% ethyl acetate in heptane) to afford the title compound as a single diastereomer (396 mg, 74%).
- 25 ^1H NMR (400 MHz, CDCl_3) δ 4.67 (s, 2H), 3.67 (s, 3H), 3.50-3.40 (m, 1H), 3.62 (s, 3H), 2.35 (dt, $J = 11.5, 2.4$ Hz, 1H), 2.25-2.05 (m, 1H), 1.96 (dt, $J = 12.5, 3.4$ Hz, 1H), 1.91-1.67 (m, 9H), 1.67-1.44 (m, 4H), 1.42-1.22 (m, 5H), 1.17 (td, $J = 12.9, 3.9$ Hz, 1H), 1.05 (td, $J = 14.3, 3.2$ Hz, 1H), 0.95 (s, 3H), 0.90-0.84 (m, 6H), 0.69 (s, 3H).

ii. **3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-ethyl-cholanic acid (Compound 3)**



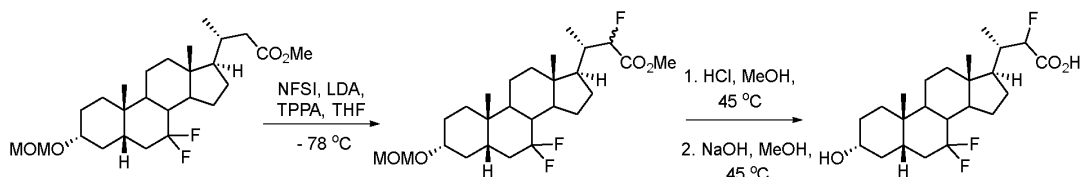
Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-ethyl-cholan-23-oate from step i (198 mg, 0.41 mmol) was dissolved in MeOH (15 mL), 2 M HCl (1.0 mL, 2.0 mmol) was charged and the reaction mixture was heated to 40 °C for 18 h, then 50% NaOH (0.2 mL, 4.1 mmol) was charged and the solution was heated at 60 °C for 7 days. The solution was concentrated *in vacuo*, water (15 mL) was charged the aqueous solution was extracted with TBME (15 mL). The aqueous phase was acidified with 2 M HCl, concentrated *in vacuo* and purified by column chromatography (SiO₂, acetone/toluene) to afford the title compound as one diastereomer as an off white solid (142 mg, 81%).

pK_a 4.9; ¹H NMR (400 MHz, CD₃OD) δ 3.53-3.43 (m, 1H), 2.32 (br. d t, *J* = 11.5, 2.3 Hz, 1H), 2.30-2.12 (m, 1H), 2.02 (br. dt, *J* = 12.6, 3.0 Hz, 1H), 1.98-1.90 (m, 1H), 1.89-1.48 (m, 13H), 1.48-1.17 (m, 7H), 1.14 (td, *J* = 14.1, 2.9 Hz, 1H), 0.98 (s, 3H), 0.92-0.89 (m, 6H), 0.74 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 179.5, 125.9 (t, *J* = 242.0 Hz), 71.9, 54.2, 51.7, 50.2 (d, *J* = 4.0 Hz), 44.2, 43.2 (dd, *J* = 23.0, 19.4 Hz), 42.5 (br. d, *J* = 10.6 Hz), 40.9, 39.4, 38.5 (d, *J* = 8.9 Hz), 38.1 (d, *J* = 4.1 Hz), 37.3 (t, *J* = 23.0 Hz), 35.8, 35.4 (d, *J* = 1.1 Hz), 30.9, 29.3, 26.4 (d, *J* = 4.2 Hz), 23.0, 22.0, 17.9, 15.3, 13.7, 12.2; ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.1 (d, *J* = 240.8 Hz), -102.4 (d, *J* = 240.8 Hz); MS (ESI⁻) *m/z* 425.3 [M-H]⁻.

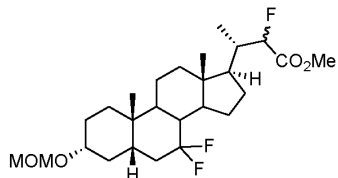
Example 4 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-fluoro-cholanic acid (Compound 4)

Compound 4 was synthesised from methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-oate, i.e. the product of Example 2, step ii according to Scheme 10.

Scheme 10



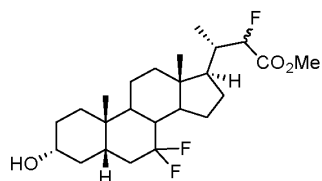
i. **Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-fluoro-cholan-23-oate**



To a solution of methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-oate (0.3 g, 0.66 mmol) and tripyrrolidinophosphoric acid triamide (TPPA, 75 μ L, 0.33 mmol) in THF (4.5 mL) at -78 $^{\circ}$ C under nitrogen was charged LDA (1.0 M in THF/hexanes, 0.99 mL, 0.99 mmol). After stirring for 30 min N-fluorobenzenesulfonimide (NFSI, 0.3 g, 0.99 mmol) in THF (1 mL) was charged. The reaction mixture was allowed to warm to ambient temperature then stirred under nitrogen for 18 h. The mixture was diluted with ethyl acetate (30 mL) and quenched with 5% NaHCO₃ (10 mL). The phases were separated, and the aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined extracts were washed with 10% NaCl (30 mL), dried over MgSO₄ and concentrated *in vacuo*. The crude residue was obtained as mixture of fluoro-diastereomers (89:11), used without further purification (370 mg).

¹H NMR (400 MHz, CDCl₃) δ 4.95 (dd, *J* = 48.9, 1.8 Hz, 1H, CHF major), 4.83 (dd, *J* = 47.6, 3.3 Hz, 1H, CHF minor), 4.65 (s, 2H), 3.78 (s, 3H), 3.48-3.37 (m, 1H), 3.34 (s, 3H), 2.25-2.05 (m, 1H), 2.05-1.62 (m, 13H), 1.62-1.22 (m, 8H), 0.97-0.91 (m, 6H), 0.69 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.4 (dd, *J* = 240.6, 8.5 Hz), -101.2 (dd, *J* = 240.6, 3.0 Hz), -208.7 (major), -191.0 (minor).

ii. **Methyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-fluoro-cholan-23-oate**

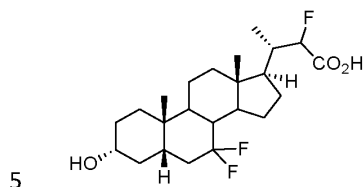


Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-fluoro-cholan-23-oate from step i (370 mg) was dissolved in MeOH (26 mL), 2 M HCl (1.0 mL, 2.0 mmol) was charged and the reaction mixture was heated to 40 $^{\circ}$ C for 18 h. After completion, the mixture was concentrated *in vacuo*, redissolve in water (10 mL) and extracted with EtOAc (3 x 20 mL). The combined extracts were washed with and 10% NaCl (30 mL), dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, acetone/heptane) to afford the title compound as a mixture of fluoro-diastereomers (96:4) (51 mg, 18%).

¹H NMR (400 MHz, CDCl₃) δ 4.97 (dd, *J* = 48.9, 1.8 Hz, 1H, CHF major), 4.90 (dd, *J* = 47.7, 3.4 Hz, 1H, CHF minor), 3.79 (s, 3H), 3.62-3.45 (m, 1H), 2.26-2.03 (m, 1H), 2.06-1.64 (m, 8H),

1.59-1.16 (m, 13H), 0.99-0.94 (m, 6H), 0.71 (s, 3H); ^{19}F $\{^1\text{H}\}$ NMR (376 MHz, CDCl_3) δ -84.4 (d, J = 240.6 Hz), -101.2 (d, J = 240.6 Hz), -208.7 (major), -191.0 (minor).

iii. **3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-fluoro-cholanic acid (Compound 4)**

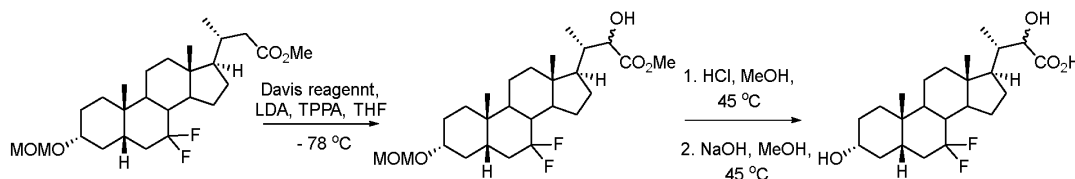


Methyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-fluoro-cholan-23-oate from step ii (51 mg, 0.12 mmol) was dissolved in MeOH (5 mL), 50% NaOH (0.14 mL, 1.78 mmol) was charged and the solution heated at 45 °C for 18 h. The solution was concentrated *in vacuo*. The crude residue was diluted with water (15 mL), acidified with 2 M HCl and extracted with EtOAc (3 x 30 mL).
 10 The combined extracts were washed with and 10% NaCl (30 mL), dried over MgSO_4 and concentrated *in vacuo*. The residue was purified by reverse phase column chromatography (C18, MeOH/ H_2O) to afford the title compound as one diastereomer (26 mg, 52%).
 pK_a 3.2; ^1H NMR (400 MHz, CD_3OD) δ 5.00-4.73 (m, 1H, CHF), 3.61-3.43 (m, 1H), 2.36-2.12 (m, 1H), 2.11-1.85 (m, 6H), 1.82-1.09 (m, 15H), 1.05-1.00 (m, 6H), 0.79 (s, 3H); ^{19}F $\{^1\text{H}\}$ NMR (376 MHz, CD_3OD) δ -85.2 (d, J = 240.9 Hz), -102.5 (d, J = 240.9 Hz), -203.8; MS (ESI $^-$) m/z 415.2 $[\text{M}-\text{H}]^-$.
 15

Example 5 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-hydroxy-cholanic acid (Compound 5)

20 Compound 5 was synthesised from methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-oate, i.e. the product of Example 2, step ii according to Scheme 11.

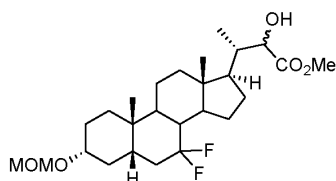
Scheme 11



25

i. **Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxy-cholan-23-oate**

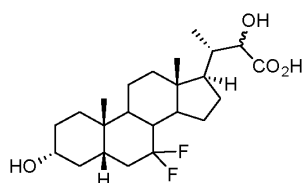
The product of this step is the starting material for Compound 8 of Example 8, Compound 9 of Example 9, Compound 10 of Example 10 and Compound 11 of Example 11.



To a solution of methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-cholan-23-oate (969 mg, 2.12 mmol) and tripyrrolidinophosphoric acid triamide (TPPA, 0.24 mL, 1.06 mmol) in THF (40 mL) at -78 °C under nitrogen was charged LDA (1.0 M in THF/hexanes, 10.6 mL, 10.6 mmol).
 5 After stirring for 30 min Davis reagent (2.77 g, 10.6 mmol) in THF (15 mL) was charged. The reaction mixture was allowed to warm to ambient temperature then stirred under nitrogen for 18 h. The mixture was partitioned between 1 M HCl (50 mL) and ethyl acetate (50 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (3 x 50 mL). The combined extracts were washed with 5% NaHCO₃ (50 mL) and 10% NaCl (50 mL), dried
 10 over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, 5-10% acetone in heptane) to afford the title compound (0.50 g, 50%) as a mixture of diastereomers (89:11).

¹H NMR (400 MHz, CDCl₃) δ 4.67 (s, 2H), 4.26-4.23 (m, 1H major), 4.21-4.18 (m, 1H minor), 3.80 (s, 3H minor), 3.78 (s, 3H major), 3.49-3.39 (m, 1H), 3.36 (s, 3H), 2.60 (d, *J* = 5.3 Hz, 1H),
 15 2.23-2.05 (m, 1H), 2.04-1.66 (m, 13H), 1.63-1.16 (m, 7H), 1.05 (td, *J* = 14.2, 3.1 Hz, 1H), 0.94 (s, 3H), 0.83 (d, *J* = 6.7 Hz, 3H), 0.70 (s, 3H major), 0.68 (s, 3H minor); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.4 (d, *J* = 240.2 Hz), -101.2 (d, *J* = 240.2 Hz).

ii. 3α-hydroxy-7,7-difluoro-5β-24-nor-22-hydroxy-cholanic acid (Compound 5)



20 Methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-hydroxy-cholan-23-oate from step i (50 mg, 0.11 mmol) was dissolved in MeOH (5 mL), 2 M HCl (0.26 mL, 0.53 mmol) was charged and the reaction mixture was heated to 40 °C for 18 h, then to 50 °C for 4 h. After this time additional 2 M HCl (0.13 mL, 0.26 mmol) was charged and the reaction mixture was heated at
 25 50 °C for a further 20 h. After completion, 50% NaOH (0.13 mL, 2.12 mmol) was charged and the solution heated at 50 °C for 24 h. The solution was concentrated *in vacuo*. The crude residue was diluted with water (10 mL), acidified with 2 M HCl and extracted with EtOAc (3 x 10 mL). The combined extracts were washed with 10% NaCl (10 mL), dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, 0-10% 7:2:1

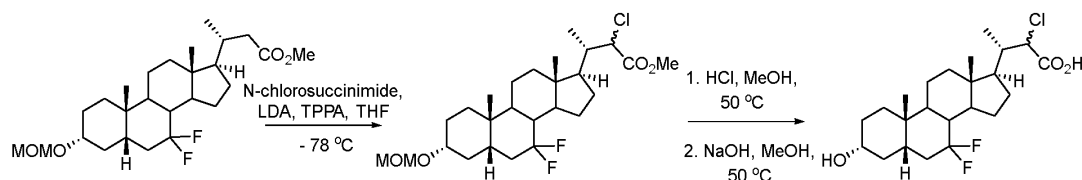
(ethyl acetate:MeOH:water) in ethyl acetate) to afford the title compound (27.2 mg, 60%) as a mixture of diastereomers (88:12).

pK_a 3.9; ¹H NMR (400 MHz, CD₃OD) δ 4.20 (d, *J* = 2.0 Hz, 1H major), 4.15 (d, *J* = 3.0 Hz, 1H minor), 3.52-3.42 (m, 1H), 2.30-2.12 (m, 1H), 2.08-1.49 (m, 15H), 1.48-1.19 (m, 5H), 1.10 (td, *J* = 14.1, 2.9 Hz, 1H), 1.02 (d, *J* = 6.8 Hz, 3H minor), 0.99 (s, 3H), 0.92 (d, *J* = 6.8 Hz, 3H major), 0.75 (s, 3H major), 0.74 (s, 3H minor); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.2 (d, *J* = 240.9 Hz), -102.4 (d, *J* = 240.9 Hz); MS (ESI⁺) *m/z* 413.3 [M-H].

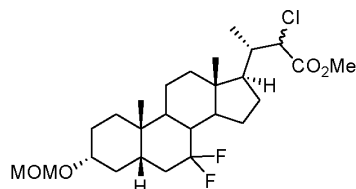
Example 6 – Synthesis of 3α-hydroxy-7,7-difluoro-5β-24-nor-22-chloro-cholanic acid (Compound 6)

Compound 6 was synthesised from methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-cholan-23-oate, i.e. the product of Example 2, step ii according to Scheme 12.

Scheme 12



i. Methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-chloro-cholan-23-oate

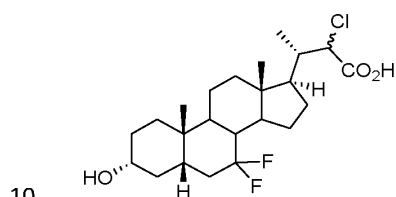


To a solution of methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-cholan-23-oate (250 mg, 0.55 mmol) and tripyrrolidinophosphoric acid triamide (TPPA, 63 μL, 0.27 mmol) in THF (8 mL) at -78 °C under nitrogen was charged LDA (1.0 M in THF/hexanes, 1.10 mL, 1.10 mmol). After stirring for 30 min N-chlorosuccinimide (150 mg, 1.10 mmol) in THF (2 mL) was charged. The reaction mixture was allowed to warm to ambient temperature then stirred under nitrogen for 18 h. Then the reaction was cooled to -78 °C and additional LDA (1.0 M in THF/hexanes, 0.55 mL, 0.55 mmol) was charged. After stirring for 40 minutes additional N-chlorosuccinimide (73 mg, 0.55 mmol). The reaction mixture was allowed to warm to ambient temperature then stirred under nitrogen for 18 h. The mixture was partitioned between 1 M HCl (30 mL) and ethyl acetate (30 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (3 x 30 mL). The combined extracts were washed with 5% NaHCO₃ (30 mL) and 10% NaCl (30 mL), dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by

column chromatography (SiO₂, 5-10% acetone in heptane) to afford the title compound (39 mg, 14%) as a 1:1 mixture of diastereomers and (49 mg, 18%) as a 92:8 mixture of diastereomers.

¹H NMR (400 MHz, CDCl₃) δ 4.66 (s, 2H), 4.54 (d, *J* = 2.2 Hz, 1H major), 4.49 (d, *J* = 3.2 Hz, 1H minor), 3.78 (s, 3H major), 3.77 (s, 3H minor), 3.49-3.39 (m, 1H), 3.36 (s, 3H), 2.25-2.06 (m, 2H), 2.05-1.66 (m, 10H), 1.65-1.46 (m, 4H), 1.45-1.12 (m, 5H), 1.06 (td, *J* = 14.2, 3.1 Hz, 1H), 0.78 (d, *J* = 6.5 Hz, 3H), 0.95 (s, 3H), 0.72 (s, 3H major), 0.69 (s, 3H minor).

ii. **3α-hydroxy-7,7-difluoro-5β-24-nor-22-chloro-cholanic acid (Compound 6)**



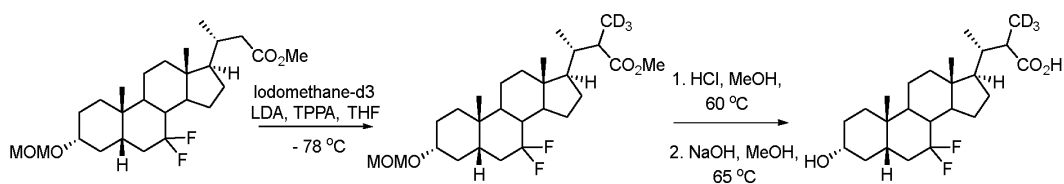
Methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-chloro-cholan-23-oate from step i (49 mg, 0.10 mmol) was dissolved in MeOH (5 mL), 2M HCl (0.25 mL, 0.5 mmol) was charged and the solution heated at 40 °C for 18 h, then heated at 50 °C and additional 2M HCl charged (0.13 mL, 0.25 mmol). After stirring at 50 °C for 22 h 50% NaOH (0.1 mL, 2 mmol) was charged and the heating continued for 22 h. The solution was concentrated *in vacuo* and the crude residue was diluted with water (10 mL), acidified with 2 M HCl and extracted with EtOAc (4 x 15 mL). The combined extracts were concentrated *in vacuo*. The residue was purified by reverse phase column chromatography (C18, MeOH/H₂O) to afford the title compound (42 mg, 97%) as a 96:4 mixture of diastereomers.

pK_a 3.1; ¹H NMR (400 MHz, CD₃OD) δ 4.54 (d, *J* = 1.8 Hz, 1H major), 4.49 (d, *J* = 3.1 Hz, 1H minor), 3.52-3.42 (m, 1H), 2.34-2.12 (m, 2H), 2.08-1.96 (m, 1H), 1.96-1.17 (m, 18H), 1.10 (td, *J* = 14.0, 2.8 Hz, 1H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.99 (s, 3H), 0.77 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 125.9 (t, *J* = 242.0 Hz), 71.9, 68.1, 53.7, 50.3 (d, *J* = 4.0 Hz), 44.0, 43.2 (dd, *J* = 23.0, 19.4 Hz), 42.6 (br. d, *J* = 9.9 Hz), 41.1, 40.8, 38.5 (d, *J* = 8.9 Hz), 38.1 (d, *J* = 4.1 Hz), 37.3 (t, *J* = 23.0 Hz), 35.8, 35.4 (d, *J* = 1.1 Hz), 30.9, 28.6, 26.3 (d, *J* = 4.3 Hz), 23.0, 22.0, 14.6, 12.5; ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.2 (d, *J* = 240.8 Hz), -102.5 (d, *J* = 240.8 Hz); MS (ESI⁻) *m/z* 431.2 [M-H]⁻.

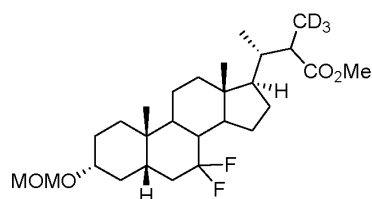
Example 7 – Synthesis of 3α-hydroxy-7,7-difluoro-5β-24-nor-22-(methyl-d₃)-cholanic acid (Compound 7)

Compound 7 was synthesised from methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-cholan-23-oate, i.e. the product of Example 2, step ii according to Scheme 13.

Scheme 13

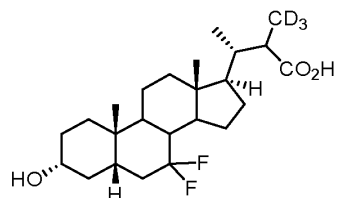


i. **Methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-(methyl-d₃)-cholan-23-oate**



To a solution of methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-cholan-23-oate (100 mg, 0.22 mmol) and tripyrrolidinophosphoric acid triamide (TPPA, 25 μL, 0.11 mmol) in THF (4 mL) at -78 °C under nitrogen was charged LDA (1.0 M in THF/hexanes, 0.33 mL, 0.33 mmol). After stirring for 30 min iodomethane-d₃ (20.4 μg, 0.33 mmol) in THF (0.5 mL) was charged. The reaction mixture was allowed to warm to ambient temperature then stirred under nitrogen for 18 h. Then the reaction was cooled to -78 °C and additional LDA (1.0 M in THF/hexanes, 0.33 mL, 0.33 mmol) was charged. After stirring for 30 minutes additional iodomethane-d₃ (20.4 μg, 0.33 mmol) in THF (0.5 mL) was charged. The reaction mixture was allowed to warm to ambient temperature then stirred under nitrogen for 2.5 h. The mixture was partitioned between 1 M HCl (15 mL) and ethyl acetate (15 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (3 x 15 mL). The combined extracts were washed with 5% NaHCO₃ (15 mL) and 10% NaCl (15 mL), dried over MgSO₄ and concentrated *in vacuo* to afford the title compound as a crude mixture (150 mg). The residue was continued on without purification.

ii. **3α-hydroxy-7,7-difluoro-5β-24-nor-22-(methyl-d₃)-cholan-23-oic acid (Compound 7)**



Methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-(methyl-d₃)-cholan-23-oate from step i (150 mg) was dissolved in MeOH (7 mL), 2M HCl (0.55 mL, 1.1 mmol) was charged and the

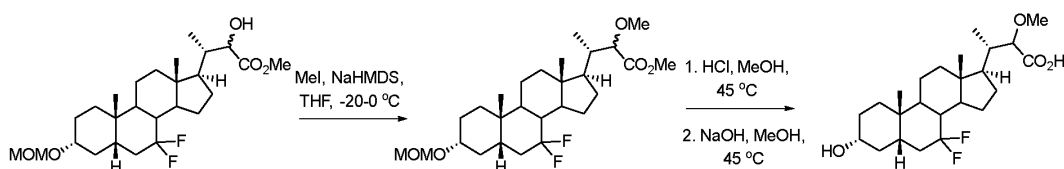
solution heated at 45 °C for 18 h, then heated at 60 °C and additional 2M HCl charged (0.25 mL, 0.50 mmol). After stirring at 60 °C for 22 h the solution was concentrated *in vacuo* and the crude residue was diluted with ethyl acetate (15 mL) and washed with 5% NaHCO₃ (15 mL). The aqueous phase was extracted with ethyl acetate (2 x 15 mL). The combined extracts were dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, 5-15% ethyl acetate in heptane) to afford the intermediate as a 85:15 mixture with methyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate (70 mg). The intermediate was dissolved in MeOH (5 mL), 50% NaOH (86 μ L, 1.63 mmol) was charged and the solution heated at 40 °C for 18 h, then heated at 65 °C and additional 50% NaOH charged (86 μ L, 1.63 mmol). After stirring at 50 °C for 22 h 50% NaOH (0.1 mL, 2 mmol) was charged and the heating continued for 44 h. The solution was concentrated *in vacuo* and the crude residue was diluted with water (5 mL), acidified with 2 M HCl and extracted with EtOAc (4 x 10 mL). The combined extracts were concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, 5-10% acetone in water) to afford the title compound as a single diastereomer (26 mg, 39%).

pK_a 4.9; ¹H NMR (400 MHz, CD₃OD) δ 3.53-3.43 (m, 1H), 2.58 (br. s, 1H), 2.31-1.98 (m, 3H), 1.93-1.49 (m, 11H), 1.46-1.17 (m, 6H), 1.10 (td, J = 14.4, 2.9 Hz, 1H), 0.98 (s, 3H), 0.88 (d, J = 6.8 Hz, 3H), 0.75 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 180.4, 125.9 (t, J = 241.8 Hz), 71.9, 53.9, 50.2 (d, J = 3.9 Hz), 44.2, 43.2 (dd, J = 23.0, 19.4 Hz), 42.9, 42.6 (br. d, J = 10.4 Hz), 40.9, 38.5 (d, J = 8.4 Hz), 38.5, 38.1 (d, J = 4.2 Hz), 37.3 (t, J = 23.5 Hz), 35.8, 35.4 (d, J = 1.1 Hz), 30.9, 29.0, 28.6, 26.4 (d, J = 4.2 Hz), 23.0, 22.0, 14.9, 12.2; ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.1 (d, J = 240.7 Hz), -102.4 (d, J = 240.7 Hz); MS (ESI⁺) m/z 414.3 [M-H]⁺.

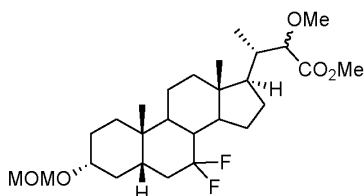
Example 8 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-methoxy-cholan-23-oic acid (Compound 8)

Compound 8 was synthesised from methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxy-cholan-23-oate, i.e. the product of Example 5, step i according to Scheme 14.

Scheme 14



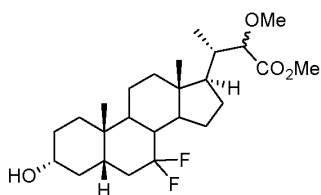
i. **Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-methoxy-cholan-23-oate**



To a solution of methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxy-cholan-23-oate (200 mg, 0.47 mmol) and methyl iodide (90 μ L, 0.71 mmol) in THF (8 mL) at -20 °C under nitrogen was charged NaHMDS (1.0 M in THF, 0.71 mL, 0.71 mmol) drop-wise. The reaction mixture was stirred at -20 °C for 2 h then warmed to 0 °C and stirred for 18 h. The mixture was quenched by the addition of 5% NaHCO₃ (10 mL). The mixture was extracted with ethyl acetate (2 x 20 mL). The combined extracts were washed with 10% NaCl (20 mL), dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, ethyl acetate/heptane) to afford the title compound as a mixture of diastereomers (80:20, 86 mg, 38%).

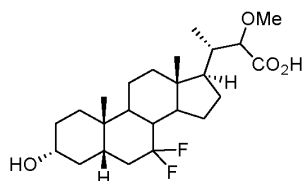
¹H NMR (400 MHz, CDCl₃) δ 4.67 (s, 2H major), 4.66 (s, 2H minor), 3.79 (d, *J* = 2.4 Hz, 1H major), 3.77 (d, *J* = 3.4 Hz, 1H minor), 3.75 (s, 3H minor), 3.75 (s, 3H major), 3.49-3.37 (m, 1H), 3.39 (s, 3H), 3.36 (s, 3H major), 3.36 (s, 3H minor), 2.25-2.05 (m, 1H), 2.01-1.67 (m, 12H), 1.63-1.16 (m, 8H), 1.05 (td, *J* = 14.3, 3.2 Hz, 1H), 0.98 (d, *J* = 6.8 Hz, 3H minor), 0.95 (s, 3H), 0.90 (d, *J* = 6.9 Hz, 3H major), 0.69 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.3 (d, *J* = 239.9 Hz), -101.1 (d, *J* = 239.9 Hz minor), -101.2 (d, *J* = 239.9 Hz major).

ii. **Methyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-methoxy-cholan-23-oate**



To a solution of methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-methoxy-cholan-23-oate from step i (86 mg, 0.18 mmol) in MeOH (5 mL) was charged 2M HCl (0.43 mL, 0.86 mmol). After stirring at 30 °C for 3 days the solution was concentrated *in vacuo* and the crude residue was diluted with ethyl acetate (10 mL) and washed with 5% NaHCO₃ (10 mL). The aqueous phase was extracted with ethyl acetate (2 x 10 mL). The combined extracts were washed with 10% NaCl, dried over MgSO₄ and concentrated *in vacuo* to afford the title compound as a mixture of diastereomers (70 mg, 85:15) which was continued on without any further purification.

iii. **3 α -Hydroxy-7,7-difluoro-5 β -24-nor-22-methoxy-cholan-23-oic acid (Compound 8)**

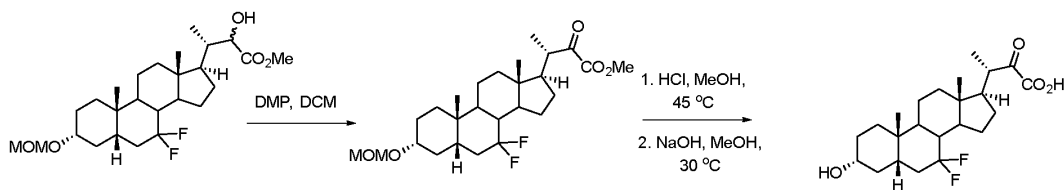


- Methyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-methoxy-cholan-23-oate from step ii (70 mg, 0.16 mmol) was dissolved in MeOH (5 mL), 50% NaOH (95 μ L, 2.37 mmol) was charged and the solution heated at 45 °C for 19 h. The solution was concentrated *in vacuo* and the crude residue was diluted with water (5 mL), acidified with 2 M HCl and extracted with EtOAc (2 x 10 mL). The combined extracts were concentrated *in vacuo*. The residue was purified by reverse phase column chromatography (C18, MeOH/H₂O) to afford the title compound as a single diastereomer (22 mg, 32%).
- pK_a 3.4; ¹H NMR (400 MHz, CD₃OD) δ 3.64 (br. s, 1H), 3.52-3.42 (m, 1H), 3.35 (br. s, 3H), 2.31-2.11 (m, 1H), 2.06-1.45 (m, 15H), 1.45-1.15 (m, 5H), 1.10 (td, *J* = 14.2, 3.0 Hz, 1H), 0.98 (s, 3H), 0.94 (d, *J* = 6.6 Hz, 3H), 0.73 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.2 (d, *J* = 240.8 Hz), -102.5 (d, *J* = 240.8 Hz); MS (ESI) *m/z* 427.2 [M-H]⁻.

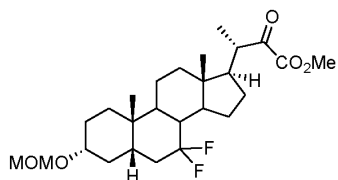
15 **Example 9 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-keto-cholan-23-oic acid (Compound 9)**

Compound 9 was synthesised from methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxy-cholan-23-oate, i.e. the product of Example 5, step i, according to Scheme 15.

20 **Scheme 15**



i. **Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-keto-cholan-23-oate**

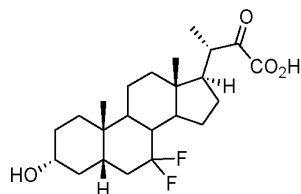


- 25 To a solution of methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxy-cholan-23-oate (100 mg, 0.21 mmol) in DCM (2 mL) was charged DMP (106 mg, 0.25 mmol). The reaction

was stirred at ambient temperature overnight and then quenched with $\text{Na}_2\text{S}_2\text{O}_3$. The phases were separated and the aqueous phase was extracted with DCM. The combined extracts were washed with 10% NaCl, dried over MgSO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2 , ethyl acetate/heptane) to afford the title compound (74 mg, 75%).

^1H NMR (400 MHz, CDCl_3) δ 4.67 (s, 2H), 3.86 (s, 3H), 3.50-3.39 (m, 1H), 3.36 (s, 3H), 3.26 (dq, J = 10.2, 6.9 Hz, 1H), 2.24-2.05 (m, 1H), 1.98-1.92 (m, 1H), 1.90-1.66 (m, 10H), 1.60-1.46 (m, 2H), 1.42-1.24 (m, 5H), 1.23-1.12 (m, 1H), 1.19, (d, J = 6.9 Hz, 3H), 1.06 (td, J = 14.3, 3.3 Hz, 1H), 0.95 (s, 3H), 0.73 (s, 3H).

ii. **3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-keto-cholanic acid (Compound 9)**



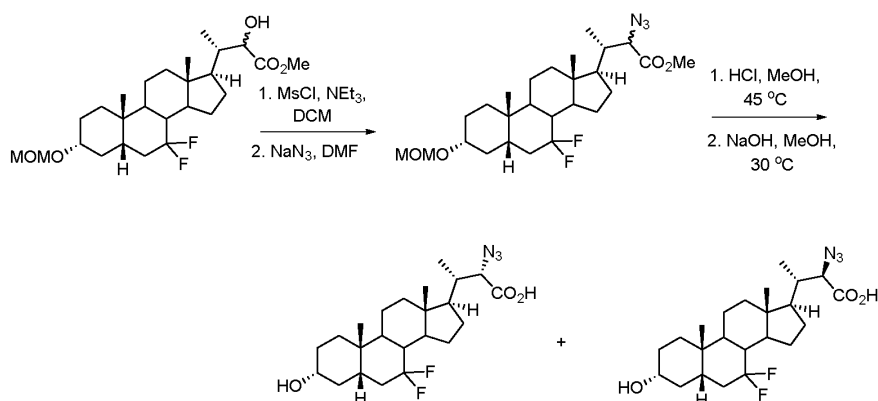
Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-keto-cholan-23-oate from step i (74 mg, 0.16 mmol) was dissolved in MeOH (2.6 mL). 2 M HCl (0.22 mL, 0.44 mmol) was charged and the reaction mixture was heated to 45 °C for 18 h. After this time additional 2 M HCl (0.20 mL, 0.40 mmol) was charged and the reaction mixture was heated at 45 °C for a further 21 h, then heated to 60 °C 5 h. After completion, 5 M NaOH (to pH 14) was charged and the solution was stirred at 30 °C for 5 days. The solution was concentrated *in vacuo*. The crude residue was diluted with water (10 mL), acidified with 2 M HCl and extracted with EtOAc (3 x 10 mL). The combined extracts were washed with 10% NaCl (10 mL), dried over MgSO_4 and concentrated *in vacuo*. Purification by preparative HPLC (C18, MeCN/ H_2O /0.1% formic acid) afforded the title compound (7.2 mg).

(Compound 9): pK_a 1.4; ^1H NMR (400 MHz, CDCl_3) δ 3.59-3.48 (m, 1H), 3.46-3.35 (m, 1H), 2.25-2.05 (m, 1H), 2.02-0.98 (m, 20H), 1.16, (d, J = 6.9 Hz, 3H), 0.93 (s, 3H), 0.67 (s, 3H); ^{19}F $\{^1\text{H}\}$ NMR (376 MHz, CDCl_3) δ -84.7 (d, J = 240.6 Hz), -101.1 (d, J = 240.6 Hz); MS (ESI $^-$) m/z 411.2 $[\text{M}-\text{H}]^-$.

Example 10 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-azido-cholanic acid (Compound 10)

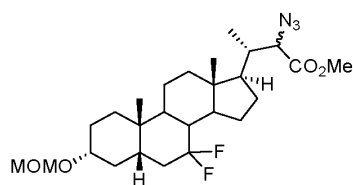
Compound 10 was synthesised from methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxy-cholan-23-oate, i.e. the product of Example 5, step i, according to Scheme 16.

Scheme 16



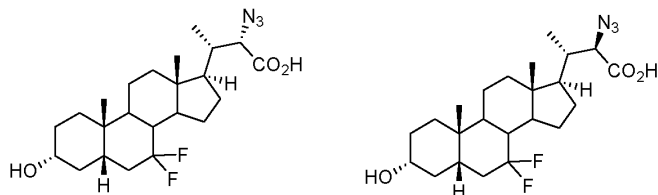
i. **Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-azido-cholan-23-oate**

- 5 The product of this step is the starting material for Compound 11 of Example 11.



- Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxy-cholan-23-oate (50 mg, 0.11 mmol) was dissolved in DCM (2.5 mL), cooled to 0 °C and then MsCl (12 μ L, 0.16 mmol) and Et₃N (74 μ L, 0.53 mmol) were charged. The reaction mixture was allowed to warm to ambient temperature, stirred for 18 h then additional MsCl (6 μ L) and Et₃N (37 μ L) was charged. Upon completion, indicated by TLC analysis, water (5 mL) was added and extracted with DCM (2 $\times$ 10 mL). The combined organic layer was washed with 5% NaHCO₃ solution (aq., 2 $\times$ 10 mL) and 10% NaCl (10 mL), dried over Na₂SO₄, filtered and concentrated to afford the mesylate intermediate. The residue was dissolved in DMF (2.5 mL) and charged into a flask containing NaN₃ (40 mg, 0.62 mmol). The reaction mixture was stirred at ambient temperature for 3 days, then heated to 50 °C for a further 2 days. After this time the reaction was cooled to ambient temperature, diluted with ethyl acetate and washed with 5% NaHCO₃. The aqueous phase was extracted with ethyl acetate and the combined extracts were washed with 10% NaCl and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, ethyl acetate/heptane) to afford the title compound as a 73:27 mix of diastereomers (52 mg). This was continued on without any further purification.

ii. **3 α -Hydroxy-7,7-difluoro-5 β -24-nor-22-azido-cholanic acid (Compounds 10a and 10b)**

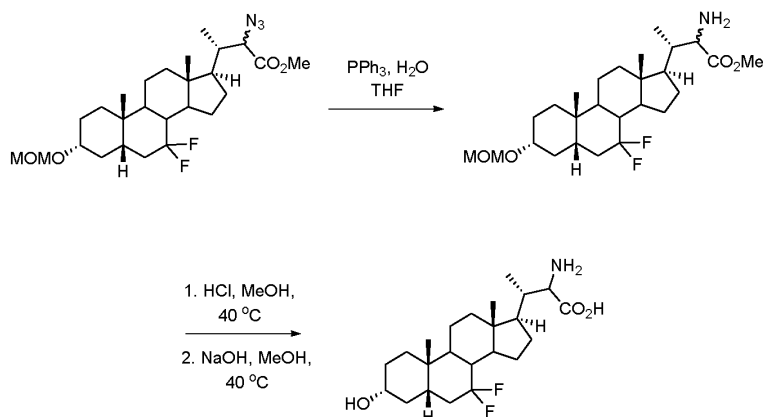


Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-azido-cholan-23-oate from step i (33 mg, 0.06 mmol) was dissolved in MeOH (5 mL). 2 M HCl (0.15 mL, 0.30 mmol) was charged and the reaction mixture was heated to 40 °C for 18 h. After completion, 2 M NaOH (to pH 14) was charged and the solution was stirred at 40 °C for 21 h. The solution was concentrated *in vacuo*. The crude residue was diluted with water, acidified with 2 M HCl and extracted with EtOAc (x3). The combined extracts were washed with 10% NaCl (10 mL), dried over MgSO₄ and concentrated *in vacuo*. Purification by preparative HPLC (C18, MeCN/H₂O/0.1% formic acid) afforded the title compound major diastereomer (9 mg) and minor diastereomer (5 mg). Major diastereomer (Compound 10a): pK_a 3.5; ¹H NMR (400 MHz, acetone-d₆) δ 4.36 (br. d *J* = 2.4 Hz, 1H), 3.51-3.42 (m, 1H), 2.31-2.10 (m, 2H), 2.08-1.22 (m, 19H), 1.10 (td, *J* = 14.2, 3.2 Hz, 1H), 1.00 (s, 3H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.76 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, acetone-d₆) δ -84.2 (d, *J* = 240.0 Hz), -101.5 (d, *J* = 240.0 Hz); MS (ESI⁻) *m/z* 438.1 [M-H]⁻. Minor diastereomer (Compound 10b): pK_a 3.5; ¹H NMR (400 MHz, acetone-d₆) δ 4.32 (br. d *J* = 2.9 Hz, 1H), 3.51-3.42 (m, 1H), 2.31-2.12 (m, 1H), 2.08-1.19 (m, 19H), 1.10 (td, *J* = 14.2, 3.2 Hz, 1H), 1.08 (d, *J* = 6.8 Hz, 3H), 0.99 (s, 3H), 0.75 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, acetone-d₆) δ -84.2 (d, *J* = 239.0 Hz), -101.5 (d, *J* = 239.0 Hz); MS (ESI⁻) *m/z* 438.3 [M-H]⁻.

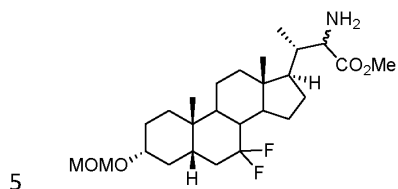
Example 11 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-amine-cholanic acid (Compound 11)

Compound 11 was synthesised from methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-azido-cholan-23-oate, i.e. the product of Example 10, step i, according to Scheme 17.

Scheme 17

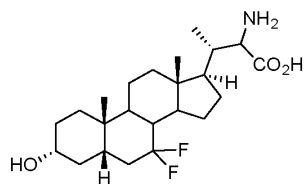


i. **Methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-amine-cholan-23-oate**



Methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-azido-cholan-23-oate (35 mg, 0.07 mmol) was dissolved in THF (1 mL), PPh₃ (51 mg, 0.20 mmol) and H₂O (10.5 μL, 0.59 mmol) were charged. The reaction mixture was heated at reflux for 2.5 h then concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, DCM/MeOH with 0.1% NEt₃) to afford the title compound as a 90:10 mix of diastereomers (22 mg). This was continued on without any further purification.

ii. **3α-Hydroxy-7,7-difluoro-5β-24-nor-22-amine-cholanic acid (Compound 11)**



15 Methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-amine-cholan-23-oate from step i (22 mg, 0.05 mmol) was dissolved in MeOH (3 mL). 2 M HCl (0.10 mL, 0.20 mmol) was charged and the reaction mixture was heated to 40 °C for 22 h. After completion, 2 M NaOH (to pH 14) was charged and the solution was stirred at 40 °C for 18 h. The solution was concentrated *in vacuo*. The crude residue was diluted with water, acidified with 2 M HCl and extracted with EtOAc (x3). The aqueous phase was saturated with NaCl and extracted with THF. The combined extracts were dried over MgSO₄ and concentrated *in vacuo*. Purification by

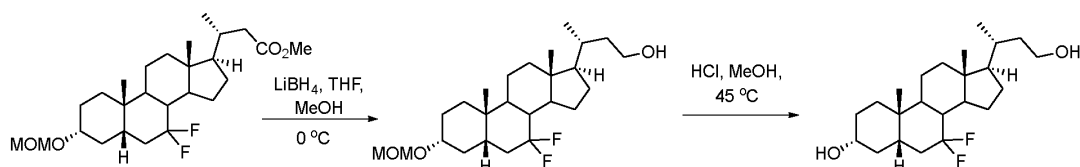
preparative HPLC (C18, MeCN/H₂O/0.1% formic acid) afforded the title compound as one diastereomer (1.3 mg).

pK_a 2.4 (acidic); pK_a 8.8 (basic); ¹H NMR (400 MHz, CD₃OD) δ 3.56 (br. s 1H), 3.51-3.42 (m, 1H), 2.30-2.10 (m, 2H), 2.06-1.99 (m, 1H), 1.92-1.20 (m, 18H), 1.15-1.02 (m, 4H), 0.98 (s, 3H),
 5 0.75 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -84.2 (d, *J* = 240.0 Hz), -101.5 (d, *J* = 240.0 Hz); MS (ESI⁺) *m/z* 414.2 [M+H]⁺.

Example 12 – Synthesis of 3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-ol (Compound 12)

10 Compound 12 was synthesised from methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-cholan-23-oate, i.e. the product of Example 2, step ii according to Scheme 18.

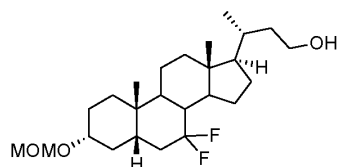
Scheme 18



15

i. 3α-Methoxymethoxy-7,7-difluoro-5β-24-nor-cholan-23-ol

The product of this step is the starting material for Compound 13 of Example 13 and Compound 15 of Example 15.

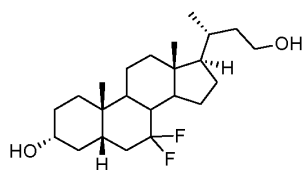


20 To a solution of methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-cholan-23-oate (300 mg, 0.66 mmol) in dry THF (5 mL) was charged dry MeOH (186 μL, 4.6 mmol). The solution was cooled to 0 °C then LiBH₄ (0.1 g, 4.6 mmol) was charged portion-wise. The reaction was stirred between 0 - 5 °C for 19 h and then quenched by the addition of 5% NH₄Cl (10 mL) and allowed to warm to ambient temperature. The mixture was extracted with EtOAc and the organic phase
 25 was washed with 5% NH₄Cl, 10% NaHCO₃ and 10% NaCl, dried over MgSO₄ and concentrated *in vacuo* to afford the title compound as a white solid (quant.). The material was continued on without any purification.

¹H NMR (400 MHz, CDCl₃) δ 4.67 (s, 2H), 3.76-3.59 (m, 2H), 3.50-3.40 (m, 1H), 3.36 (s, 3H), 2.24-2.06 (m, 1H), 2.06-1.98 (m, 1H), 1.95-1.66 (m, 9H), 1.68-1.42 (m, 9H), 1.38-1.02-1.01 (m, 4H), 0.96 (d, *J* = 6.7 Hz, 3H), 0.95 (s, 3H), 0.68 (s, 3H).

30

ii. **3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-ol (Compound 12)**

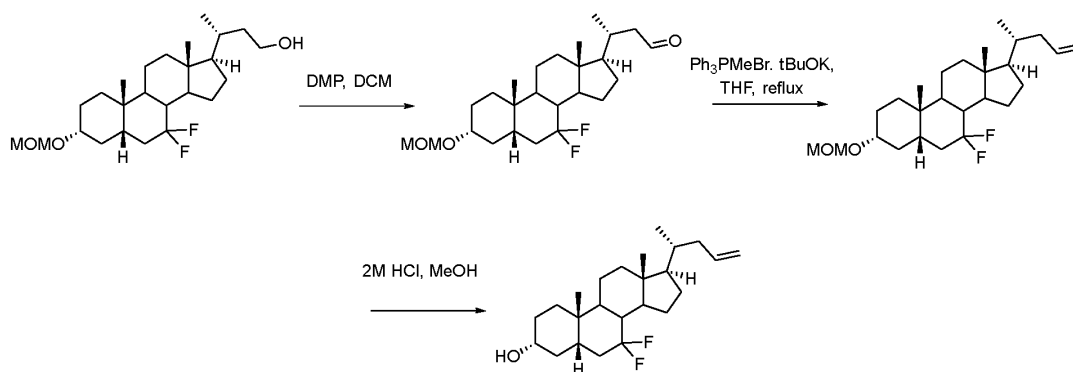


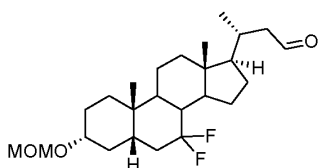
- To a solution of 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-ol from step i (53 mg, 0.12 mmol) in MeOH (2 mL) was charged 2M HCl (0.3 mL). The solution stirred at ambient temperature for 24 h, then heated to 45 °C for 2 days. The solution was concentrated *in vacuo* extracted with ethyl acetate (not soluble), the aqueous phase was saturated with solid NaCl and then extracted with THF. The organic phase was concentrated *in vacuo* and purified by reverse phase column chromatography (C18, MeOH/H₂O) to afford the title compound (9.6 mg, 21%).
- pK_a 13.0; ¹H NMR (400 MHz, CD₃OD) δ : 3.67-3.43 (m, 3H), 2.30-2.11 (m, 1H), 2.10-2.04 (m, 1H), 1.97-1.45 (m, 15H), 1.44-1.05 (m, 7H), 0.98 (d, *J* = 6.5 Hz, 3H), 0.98 (s, 3H), 0.73 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.2 (d, *J* = 240.7 Hz), -102.5 (d, *J* = 240.7 Hz); MS (ESI⁺) *m/z* 407.2 [M+Na]⁺.

15 **Example 13 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-chol-22-ene (Compound 13)**

Compound 13 was synthesised from 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-ol, i.e. the product of Example 12, step i, according to Scheme 19.

20 **Scheme 19**

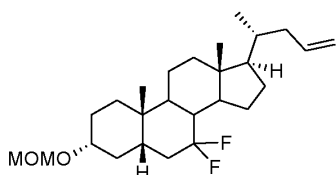


i. **3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholanal**

To 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-ol (250 mg, 0.60 mmol) in DCM (5 mL) was charged DMP (512 mg, 1.21 mmol) in two portions. The reaction mixture was stirred at ambient temperature for 4 h then quenched with 5% NaHCO₃ (aq) (30 mL). After stirring for 30 min the mixture was filtered and the filter cake washed with DCM. The filtrate was separated and the organic phase was concentrated *in vacuo* to afford the crude title compound which was continued on without any purification (210 mg, 82%).

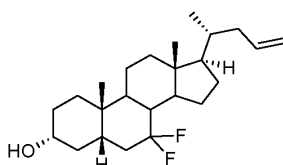
10 ii. **3 α -Methoxymethoxy-7,7-difluoro-5 β -24-nor-chol-22-ene**

The product of this step is the starting material for Compound 14 of Example 14.



To 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholanal from step i (210 mg, 0.49 mmol) in anhydrous THF (5 mL) at 0 °C was charged KO^tBu (221 mg, 1.97 mmol) in anhydrous THF (5 mL), followed by Ph₃PMeBr (703 mg, 1.97 mmol). The reaction mixture was allowed to warm to ambient temperature then stirred for 20 h and quenched with sat. NH₄Cl (aq) (10 mL). After stirring for 10 min phases were separated and the organic phase was concentrated *in vacuo*. The crude residue was purified by column chromatography (SiO₂, ethyl acetate/heptane) to afford the title compound (130 mg, 63%).

20 ¹H NMR (400 MHz, CDCl₃) δ 5.83-5.71 (m, 1H), 5.02-4.96 (m, 2H), 4.67 (s, 2H), 3.49-3.40 (m, 1H), 3.36 (s, 3H), 2.24-1.01 (m, 24H), 0.95 (s, 3H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.68 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.3 (d, *J* = 240.1 Hz), -101.1 (d, *J* = 240.1 Hz).

ii. **3 α -Hydroxy-7,7-difluoro-5 β -24-nor-chol-22-ene (Compound 13)**

25 To 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-chol-22-ene from step ii (60 mg, 0.14 mmol) in MeOH (5 mL) was charged 2 M HCl (353 μ L, 7.06 mmol). The reaction mixture stirred at 40

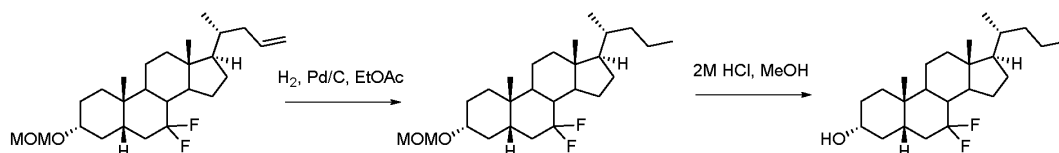
°C for 20 h, then cooled to ambient temperature, neutralised with 5% NaHCO₃ and TBME (20 mL) was charged. The phases were separated and the organic phase was dried over MgSO₄ and concentrated *in vacuo*. The crude residue was purified by column chromatography (SiO₂, ethyl acetate/heptane) to afford the title compound (9 mg, 17%).

- 5 ¹H NMR (400 MHz, CDCl₃) δ 5.83-5.71 (m, 1H), 5.02-4.96 (m, 2H), 3.59-3.50 (m, 1H), 2.24-1.01 (m, 24H), 0.95 (s, 3H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.68 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.3 (d, *J* = 240.1 Hz), -101.1 (d, *J* = 240.1 Hz).

Example 14 – Synthesis of 3α-hydroxy-7,7-difluoro-5β-24-nor-cholane (Compound 14)

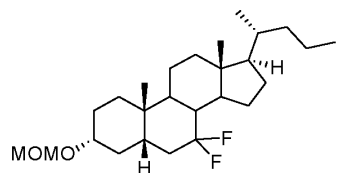
- 10 Compound 14 was synthesised from 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-chol-22-ene, i.e. the product of Example 13, step ii, according to Scheme 20.

Scheme 20



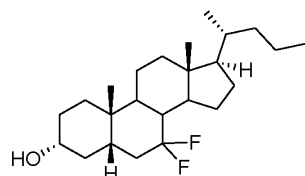
15

i. 3α-Methoxymethoxy-7,7-difluoro-5β-24-nor-cholane



- 3α-Methoxymethoxy-7,7-difluoro-5β-24-nor-chol-22-ene (65 mg, 0.15 mmol) in ethyl acetate (2 mL) was charged to a suspension of 10% Pd/C in ethyl acetate (3 mL) under nitrogen. The reaction vessel was purged with nitrogen (x3) and then purged with H₂ (x3), left under an H₂ atmosphere and stirred at ambient temperature for 18 h. The suspension was filtered, washed with ethyl acetate and the filtrate was concentrated *in vacuo* to afford the title compound (40 mg, 61%) which was continued on without purification.

25 ii. 3α-Hydroxy-7,7-difluoro-5β-24-nor-cholane (Compound 14)



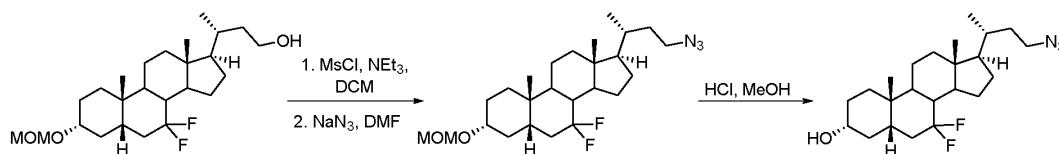
To 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholane from step i (40 mg, 0.09 mmol) in MeOH (3 mL) was charged 2 M HCl (234 μ L, 0.47 mmol). The reaction mixture stirred at 40 °C for 20 h, then additional 2 M HCl (234 μ L, 0.47 mmol) was charged and the reaction mixture was stirred at 40 °C for 24 h, followed by heating to 50 °C for 24 h. After this time the reaction was cooled to ambient temperature, neutralised with 5% NaHCO₃ and TBME (20 mL) was charged. The phases were separated and the organic phase was dried over MgSO₄ and concentrated *in vacuo* to afford the title compound (40 mg, Quant.).

¹H NMR (400 MHz, CDCl₃) δ 3.59-3.50 (m, 1H), 2.24-2.06 (m, 1H), 2.05-1.97 (m, 1H), 1.92-0.99 (m, 24H), 0.95 (s, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.86 (t, 7.0 Hz, 3H), 0.66 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 124.8 (t, *J* = 243.2 Hz), 71.5, 55.5, 48.8 (d, *J* = 3.9 Hz), 43.2, 42.0 (dd, *J* = 22.9, 19.2 Hz), 41.4 (d, *J* = 10.6 Hz), 39.7, 38.5, 37.5 (d, 4.3 Hz), 37.2 (d, *J* = 9.0 Hz), 36.4 (t, *J* = 23.2 Hz), 35.7, 34.9 (d, 1.4 Hz), 34.4 (d, *J* = 1.5 Hz), 30.4, 28.6 (d, *J* = 1.9 Hz), 25.5 (d, *J* = 4.1 Hz), 22.8, 21.1, 19.4, 18.9, 14.7, 11.9; ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.3 (d, *J* = 240.1 Hz), -101.2 (d, *J* = 240.1 Hz).

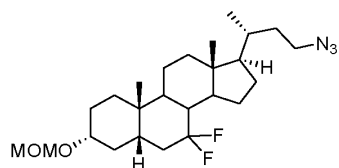
Example 15 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-azide (Compound 15)

Compound 15 was synthesised from 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-ol i.e. the product of Example 12, step i, according to Scheme 21.

Scheme 21



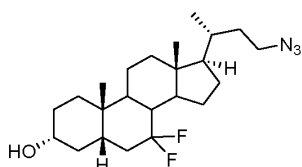
i. 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-azide



The starting material 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-ol (500 mg, 1.21 mmol) was dissolved in DCM (7.5 mL) and Et₃N (0.84 mL, 6.03 mmol) then cooled to 0 °C. MsCl (107 μ L, 1.82 mmol) was then charged, the reaction mixture was allowed to warm to ambient temperature and stirred for 18. Upon completion, indicated by TLC analysis, water (20 mL) was added and extracted with DCM (2 $\times$ 10 mL). The combined organic layer was washed

with 5% NaHCO₃ solution (20 mL), 10% NaCl (20 mL), then dried over MgSO₄, filtered and concentrated to afford the mesylate intermediate. The residue (100 mg, 0.2 mmol) was dissolved in DMF (5 mL) and charged into a flask containing NaN₃ (43 mg, 0.66 mmol). The reaction mixture was stirred at 50 °C for 3 days. After this time the reaction was cooled to ambient temperature, diluted with TBME and washed with 5% NaHCO₃. The aqueous phase was back extracted with TBME and the combined extracts were washed with 10% NaCl and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, ethyl acetate/heptane) to afford the title compound (72 mg, 79%). This was continued on without any further purification.

10 ii. **3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-azide (Compound 15)**



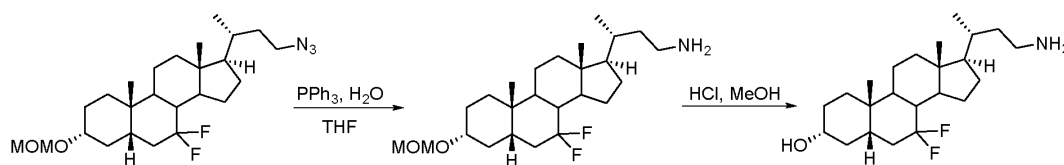
To a solution of 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-azide from step i (35 mg, 0.077 mmol) in MeOH (5 mL) was charged 2M HCl (0.2 mL). After stirring at 40 °C for 3 days the solution was concentrated *in vacuo* to afford the title compound (29 mg, 92%).

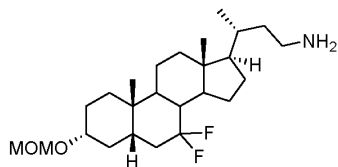
15 pK_a 14.3; ¹H NMR (400 MHz, acetone-d₆) 3.54-3.37 (m, 2H), 3.31-3.24 (m, 1H), 2.26-1.99 (m, 4H), 1.94-1.04 (m, 20H), 0.98 (d, *J* = 5.8 Hz, 3H), 0.97 (s, 3H), 0.70 (s, 3H). ¹⁹F {¹H} NMR (376 MHz, acetone-d₆) δ -84.1 (d, *J* = 240.1 Hz), -101.4 (d, *J* = 240.1 Hz); MS (ESI⁺) *m/z* 432.2 [M+Na]⁺.

20 **Example 16 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-amine (Compound 16)**

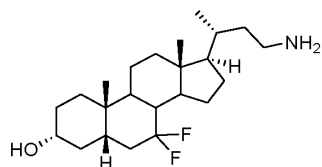
Compound 16 was synthesised from 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-azide i.e. the product of Example 15, step i, according to Scheme 22.

25 **Scheme 22**



i. **3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-amine**

- 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-azide (35 mg, 0.08 mmol) was dissolved in THF (1 mL), PPh₃ (60 mg, 0.23 mmol) and H₂O (12.4 μ L, 0.69 mmol) were charged. The reaction mixture was stirred at reflux for 18 h then concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, 7:2:1 (ethyl acetate: MeOH: H₂O) in ethyl acetate) to afford the title compound (18 mg, 55%). This was continued on without any further purification.

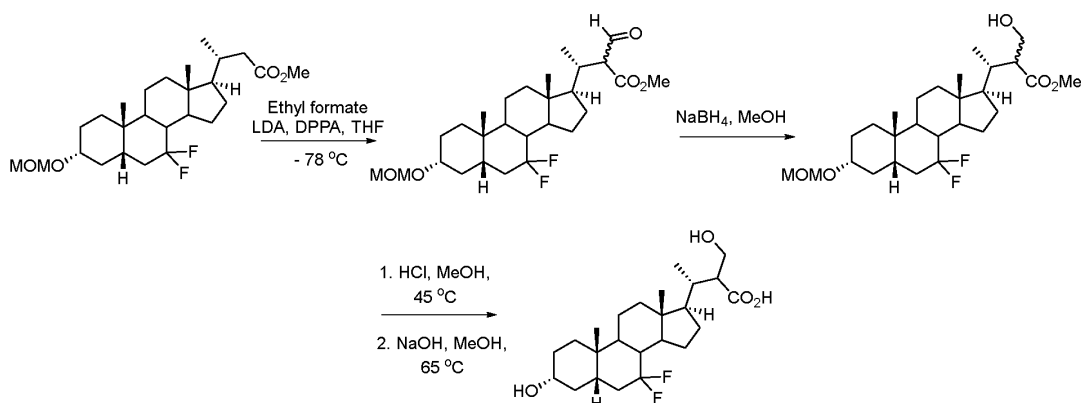
10 ii. **3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-amine (Compound 16)**

- To a solution of 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-amine from step i (18 mg, 0.042 mmol) in MeOH (2 mL) was charged 2M HCl (0.2 mL). After stirring at 40 °C for 2 days the solution was concentrated *in vacuo* to afford the title compound (16 mg, quant.).
- 15 pK_a 14.3 (acidic); pK_a 10.2 (basic); ¹H NMR (400 MHz, CD₃OD) 3.53-3.44 (m, 1H), 3.03-2.97 (m, 1H), 2.90-2.84 (m, 1H), 2.30-2.04 (m, 2H), 1.97-1.07 (m, 22H), 1.03 (d, *J* = 6.5 Hz, 3H), 0.98 (s, 3H), 0.74 (s, 3H). ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -86.5 (d, *J* = 240.7 Hz), -103.7 (d, *J* = 240.7 Hz); MS (ESI⁺) *m/z* 384.2 [M+H]⁺.

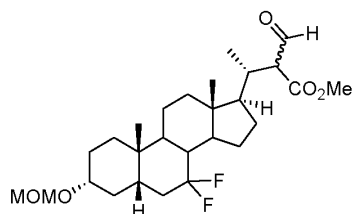
20 **Example 17 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholanic acid (Compound 17)**

Compound 17 was synthesised from methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-oate, i.e. the product of Example 2, step ii according to Scheme 23.

Scheme 23



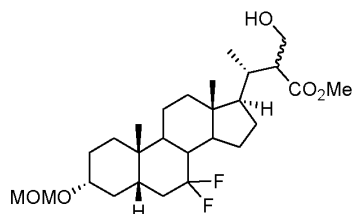
i. **Methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-formyl-cholan-23-oate**



- 5 To a solution of methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-cholan-23-oate (500 mg, 1.1 mmol) and tripyrrolidinophosphoric acid triamide (TPPA, 0.13 mL, 0.05 mmol) in THF (20 mL) at -78 °C under nitrogen was charged LDA (1.0 M in THF/hexanes, 5.48 mL, 5.48 mmol). After stirring for 30 min ethyl formate (0.88 mL, 11.0 mmol) was charged dropwise. The reaction mixture was allowed to warm to ambient temperature then stirred under nitrogen for
- 10 18 h. The mixture was partitioned between 1 M HCl (50 mL) and ethyl acetate (50 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (3 x 50 mL). The combined extracts were washed with 5% NaHCO₃ (50 mL) and 10% NaCl (50 mL), dried over MgSO₄ and concentrated *in vacuo* to afford the title compound as a crude mixture (0.87g). The residue was continued on without purification.

15

ii. **Methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-hydroxymethyl-cholan-23-oate**



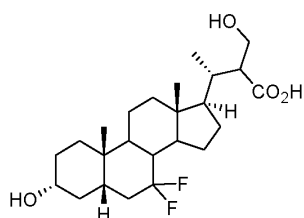
- Methyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-formyl-cholan-23-oate from step i (0.3 g, 0.62 mmol) was dissolved in MeOH (12 mL) and cooled to 0 °C under nitrogen. Sodium
- 20

borohydride (47 mg, 1.2 mmol) was charged in one portion then the reaction was stirred at 0 °C for 5 minutes and then allowed to warm to ambient temperature and stirred for 18 h. The reaction was quenched by the drop wise addition of 1 M HCl to pH 6. The mixture was concentrated *in vacuo*, diluted with ethyl acetate (15 mL) and washed with NaHCO₃ (to pH 8).

- 5 The aqueous phase was extracted with ethyl acetate (15 mL) and the combined extracts were washed with 10% NaCl, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, 10-40% ethyl acetate in heptane) to afford the crude intermediate as a 6:4 mixture of diastereomers (256 mg, 85%), which was continued on without any further purification.

10

iii. **3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholanic acid (Compound 17)**



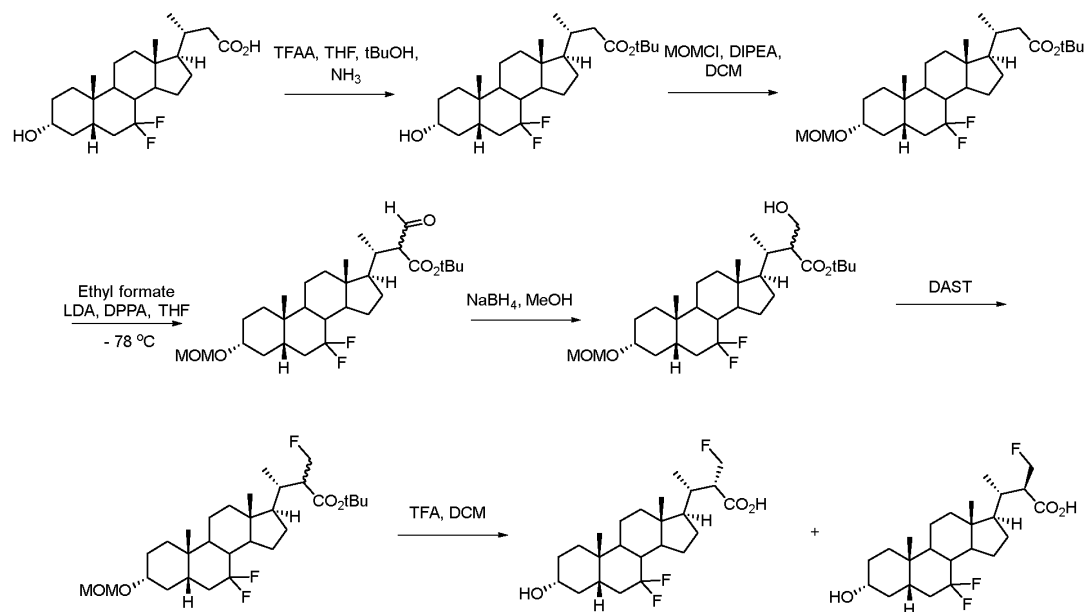
- Methyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-oate from step ii (100 mg, 0.21 mmol) was dissolved in MeOH (3.5 mL). 2 M HCl (0.5 mL, 1.0 mmol) was charged and the reaction mixture was heated to 45 °C for 19 h. After this time additional 2 M HCl (0.20 mL, 0.40 mmol) was charged and the reaction mixture was heated at 45 °C for a further 7.5 h. After completion, 50% NaOH (to pH 14) was charged and the solution was stirred at 45 °C for 3 days. The solution was concentrated *in vacuo*. The crude residue was diluted with water (10 mL), acidified with 2 M HCl and extracted with EtOAc (3 x 10 mL). The combined extracts were washed with 10% NaCl (10 mL), dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (7:2:1 (ethyl acetate: MeOH: H₂O) in ethyl acetate) to afford the title compound as a single diastereomer (19 mg, 22%) and as a 54:46 diastereomeric mixture (58 mg, 66%),
- 25 pK_a 4.6; ¹H NMR (400 MHz, CD₃OD) δ 3.80 (dd, *J* = 10.8, 9.6 Hz, 1H), 3.56 (dd, *J* = 10.8, 3.9 Hz, 1H), 3.52-3.42 (m, 1H), 2.44 (dt, 9.6, 3.9 Hz, 1H), 2.30-2.11 (m, 2H), 2.05-1.98 (m, 1H), 1.95-1.45 (m, 13H), 1.44-1.15 (m, 5H), 1.09 (td, *J* = 14.2, 3.1 Hz, 1H), 0.98 (s, 3H), 0.93 (d, *J* = 7.0 Hz, 3H), 0.74 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 183.3, 125.9 (t, *J* = 244.1 Hz), 71.9, 60.5, 54.3, 53.3, 50.3 (d, *J* = 3.8 Hz), 44.3, 43.2 (dd, *J* = 23.0, 19.4 Hz), 42.6 (br. d, *J* = 10.7 Hz), 40.9, 38.5 (d, *J* = 8.8 Hz), 38.1 (d, *J* = 4.0 Hz), 37.9, 37.4 (t, *J* = 23.3 Hz), 35.8, 35.4 (br. s), 30.9, 29.0, 26.5 (d, *J* = 3.9 Hz), 23.0, 22.0, 16.6, 12.0; ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.1 (d, *J* = 240.8 Hz), -102.4 (d, *J* = 240.8 Hz); MS (ESI⁺) *m/z* 427.3 [M-H]⁺.
- 30

Example 18 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-fluoromethyl-cholanic acid (Compounds 18a and 18b)

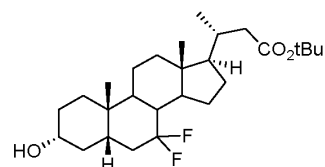
Compound 18 was synthesised from 7,7-difluoro 24-norlithocholic acid (Compound 1), according to Scheme 24.

5

Scheme 24



i. *t*-Butyl 3 α ,7 β -dihydroxy-5 β -24-nor-cholan-23-oate



10

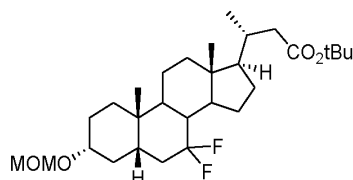
To a suspension of 7,7-difluoro 24-norlithocholic acid (4.31 g, 11.2 mmol) in dry THF (47 mL) at 0 °C was charged TFAA (10.11 mL, 40.2 mmol) dropwise, maintaining the temperature at < 25 °C. The reaction mixture was warmed to ambient temperature and stirred for 2 h then tBuOH (35.2 mL, 370 mmol) was added dropwise maintaining the temperature at < 25 °C. After stirring at ambient temperature for 20 h conc. ammonia (15 mL) was charged dropwise (maintaining temperature < 15 °C). After 3 days the reaction was quenched by the addition of 5% NaHCO₃ (50 mL). Ethyl acetate (150 mL) and 10% NaCl (25 mL) was charged. The phases were separated and the aqueous phase was extracted with ethyl acetate (x3). The combined extracts were washed with 10% NaCl and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, 0-100% acetone in toluene) to afford the title compound (3.30 g, 65%).

20

^1H NMR (400 MHz, CDCl_3) δ 3.59-3.49 (m, 1H), 2.38-2.29 (m, 1H), 2.24-2.06 (m, 1H), 2.00 (dt, $J = 12.4, 3.5$ Hz, 1H), 1.95-1.63 (m, 11H), 1.63-1.41 (m, 3H), 1.44 (s, 9H), 1.41-1.12 (m, 6H), 1.07 (td, $J = 14.4, 3.2$ Hz, 1H), 0.99 (d, $J = 6.0$ Hz, 3H), 0.95 (s, 3H), 0.70 (s, 3H); ^{19}F $\{^1\text{H}\}$ NMR (376 MHz, CDCl_3) δ -83.4 (d, $J = 240.8$ Hz), -101.2 (d, $J = 240.8$ Hz).

5

ii. ***t*-Butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-oate**



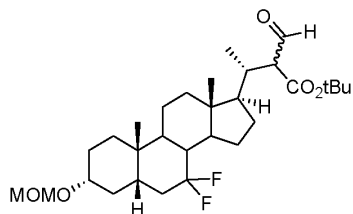
To a solution of *t*-Butyl 3 α ,7 β -dihydroxy-5 β -24-nor-cholan-23-oate from step i (3.30 g, 7.49 mmol) in DCM (33 mL) was charged DIPEA (3.20 mL, 24.0 mmol) dropwise, followed by MOMCl (1.05 mL, 14.2 mmol). The reaction mixture was stirred under nitrogen at ambient temperature for 4 h then quenched by the drop-wise addition of water (33 mL). After stirring for 18 h the phases were separated. The aqueous phase was extracted with DCM (2 x 15 mL) and the combined extracts were washed with 0.5 M HCl (3 x 15 mL, till pH < 5), 5% NaHCO_3 (15 mL), dried over MgSO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2 , 0-40% ethyl acetate in heptane) to afford the title compound (3.10 g, 83%).

^1H NMR (400 MHz, CDCl_3) δ 4.66 (s, 2H), 3.48-3.39 (m, 1H), 3.36 (s, 3H), 2.38-2.29 (m, 1H), 2.24-2.06 (m, 1H), 1.99 (dt, $J = 12.5, 3.4$ Hz, 1H), 1.93-1.66 (m, 8H), 1.58-1.41 (m, 3H), 1.43 (s, 9H), 1.38-1.21 (m, 8H), 1.20-1.10 (m, 1H), 1.04 (td, $J = 14.4, 3.4$ Hz, 1H), 0.98 (d, $J = 6.3$ Hz, 3H), 0.93 (s, 3H), 0.69 (s, 3H); ^{19}F $\{^1\text{H}\}$ NMR (376 MHz, CDCl_3) δ -84.3 (d, $J = 240.4$ Hz), -101.1 (d, $J = 240.4$ Hz).

20

iii. ***t*-Butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-formyl-cholan-23-oate**

The product of this step is the starting material for Compound 19 of Example 19.



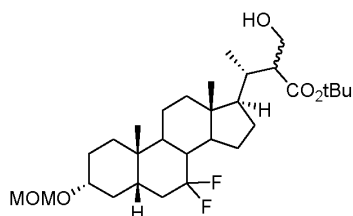
25

To a solution of *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-cholan-23-oate from step ii (3.08 g, 6.18 mmol) and tripyrrolidinophosphoric acid triamide (TPPA, 0.71 mL, 3.09 mmol) in THF (20 mL) at -78 °C under nitrogen was charged LDA (1.0 M in THF/hexanes, 30.8 mL, 30.8 mmol). After stirring for 30 min ethyl formate (4.97 mL, 61.8 mmol) was charged dropwise. The

reaction mixture was allowed to warm to ambient temperature then stirred under nitrogen for 18 h. The mixture was partitioned between 1 M HCl (60 mL) and ethyl acetate (50 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (2 x 40 mL). The combined extracts were washed with 5% NaHCO₃ (30 mL) and 10% NaCl (30 mL), dried
 5 over MgSO₄ and concentrated *in vacuo* to afford the title compound as a crude mixture (Quant.). The residue was continued on without purification.

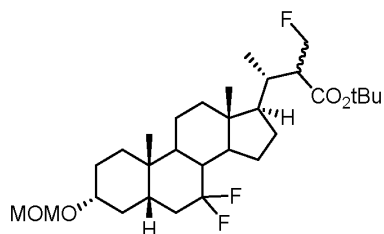
iv. ***t*-Butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-oate**

10 The product of this step is the starting material for Compound 20 of Example 20, Compound 21 of Example 21, Compound 22 of Example 22 and Compound 23 of Example 23.



The *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-formyl-cholan-23-oate from step iii (3.25 g, 6.18 mmol) was dissolved in MeOH (28 mL) and cooled to 0 °C under nitrogen. Sodium
 15 borohydride (0.7 g, 18.5 mmol) was charged in one portion then the reaction was stirred at 0 °C for 5 minutes and then allowed to warm to ambient temperature and stirred for 18 h. The reaction was quenched by the drop wise addition of 2 M HCl to pH < 5. Ethyl acetate (70 mL) was charged, followed by water (10 mL). The phases were separated and the organic phase was washed with 5% NaHCO₃ (to pH 8), dried over MgSO₄ and concentrated *in vacuo*. The
 20 residue was purified by column chromatography (SiO₂, 0-40% ethyl acetate in heptane) to afford the title compound as a crude 43:57 mixture of diastereomers (2.87 g, 88%), which was continued on without any further purification.

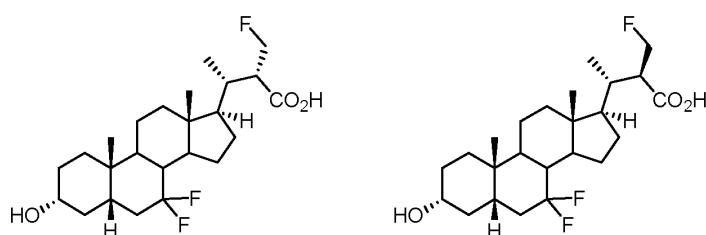
25 v. ***t*-Butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-fluoromethyl-cholan-23-oate**



The *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-oate from step iv (200 mg, 0.38 mmol) was dissolved in DCM (3 mL) and DAST (75 μ L, 0.57 mmol)

was charged. The reaction was stirred at ambient temperature for 4 h and then diluted with DCM and transferred into rapidly stirring solution of 5% NaHCO₃ and stirred for 30 min. The phases were separated and the aqueous phase was extracted with DCM. The combined extracts were washed with 10% NaCl, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, ethyl acetate/heptane) to afford the title compound as a crude inseparable mixture (17 mg), which was continued on without any further purification.

vi. **3 α -Hydroxy-7,7-difluoro-5 β -24-nor-22-fluoromethyl-cholanic acid (Compound 18)**



To a solution of *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-fluoromethyl-cholan-23-oate from step v (75 mg, 0.14 mmol) in DCM (5 mL) at 0 °C was charged TFA (0.2 mL). The reaction was allowed to warm to ambient temperature and stirred for 18 h. The reaction mixture was concentrated *in vacuo*, co-evaporated with water, then dissolved in MeOH and stirred with Na₂CO₃ (150 mg) for 3 h. The mixture was filtered and concentrated, acidified with 2 M HCl and then extracted into ethyl acetate and concentrated *in vacuo* to afford the title compound as a 7:3 mixture of diastereomers (36 mg). Purification by preparative HPLC (C18, MeCN/H₂O/0.1% formic acid) afforded the title compound major diastereomer (4.9 mg) and minor diastereomer (2 mg).

Major diastereomer (Compound 18a): pK_a 4.8; ¹H NMR (400 MHz, (CD₃)₂CO) δ 4.79 (ddd, *J* = 46.9, 9.3, 7.9 Hz, 1H), 4.66 (ddd, *J* = 46.9, 9.3, 4.3 Hz, 1H), 4.57-4.41 (m, 1H), 2.13-1.18 (m, 22H), 1.10 (td, *J* = 14.2, 3.0 Hz, 1H), 1.00 (s, 3H), 0.99 (d, *J* = 9.0 Hz, 3H), 0.76 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, (CD₃)₂CO) δ -84.2 (d, *J* = 240.1 Hz), -101.5 (d, *J* = 240.1 Hz), -224.3; MS (ESI⁻) *m/z* 429.2 [M-H]⁻.

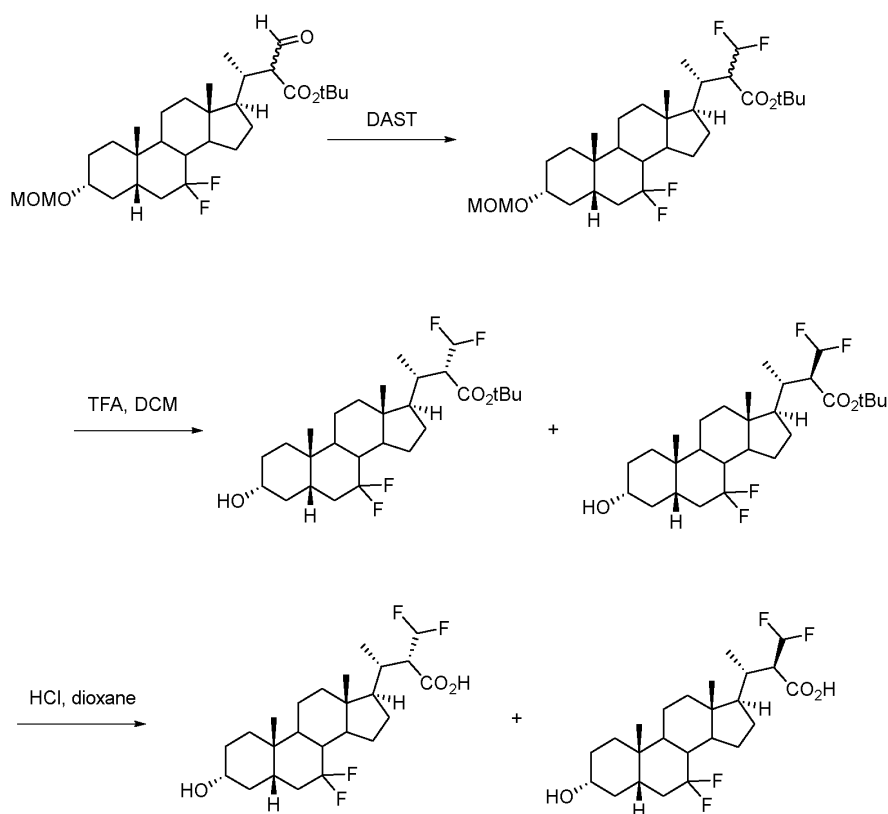
Minor diastereomer (Compound 18b): pK_a 4.8; ¹H NMR (400 MHz, (CD₃)₂CO) δ 4.73 (ddd, *J* = 47.0, 8.9, 7.8 Hz, 1H), 4.48 (ddd, *J* = 47.0, 8.9, 6.7 Hz, 1H), 4.57-4.41 (m, 1H), 2.30-1.17 (m, 22H), 1.10 (td, *J* = 14.2, 3.0 Hz, 1H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.99 (s, 3H), 0.75 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, (CD₃)₂CO) δ -84.1 (d, *J* = 240.1 Hz), -101.6 (d, *J* = 240.1 Hz), -222.5; MS (ESI⁻) *m/z* 429.2 [M-H]⁻.

Example 19 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-di-fluoromethyl-cholanic acid (Compound 19)

Compound 19 was synthesised from *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-formyl-cholan-23-oate i.e. the product of Example 18, step iii, according to Scheme 25.

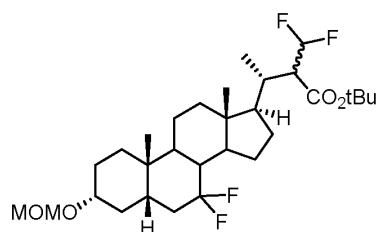
5

Scheme 25



i. ***t*-Butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-di-fluoromethyl-cholan-23-oate**

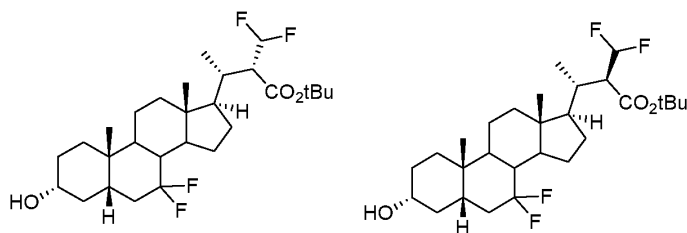
10



t-Butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-formyl-cholan-23-oate (150 mg, 0.29 mmol) was dissolved in DCM (2.3 mL) and DAST (380 μ L, 2.85 mmol) was charged. The reaction mixture was stirred at ambient temperature for 18 h and then diluted with DCM and

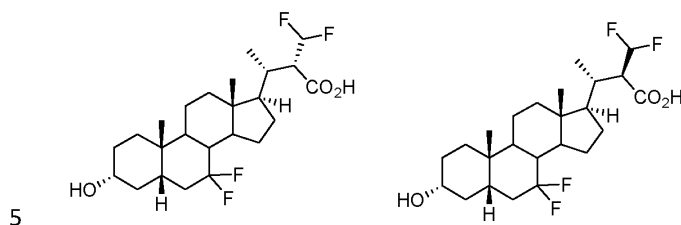
transferred in to rapidly stirring solution of 5% NaHCO₃ and stirred for 30 min. The phases were separated and the aqueous phase was extracted with DCM. The combined extracts were washed with 10% NaCl, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, 10-20% acetone in heptane) to afford the title compound as a crude inseparable mixture (134 mg), which was continued on without any further purification.

ii. ***t*-Butyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-di-fluoromethyl-cholan-23-oate**



- To a solution of *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-di-fluoromethyl-cholan-23-oate from step i (134 mg, 0.24 mmol) in DCM (3 mL) at 0 °C was charged TFA (56 μ L, 0.73 mmol). The reaction mixture was stirred at ambient temperature for 6 days then concentrated *in vacuo* and co-evaporated with water. The residue was dissolved in MeOH and stirred with 5% NaHCO₃ for 2 h. The mixture was extracted with ethyl acetate (x2). The combined extracts were washed with 10% NaCl and concentrated *in vacuo*. The crude residue was purified by column chromatography (SiO₂, ethyl acetate/heptane/0.1% AcOH) to afford the title compound as a mixture of diastereomers (24 mg). Purification by preparative HPLC (C18, MeCN/H₂O/0.1% formic acid) afforded the title compound major diastereomer (2.6 mg) and minor diastereomer (1.3 mg).
- Major diastereomer: ¹H NMR (400 MHz, CDCl₃) δ 6.03 (ddd, *J* = 57.2, 55.6, 7.4 Hz, 1H), 3.63-3.54 (m, 1H), 2.88-2.78 (m, 1H), 2.28-1.67 (m, 9H), 1.65-1.09 (m, 13H), 1.52 (s, 9H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.98 (s, 3H), 0.73 (s, 3H) ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.4 (d, *J* = 241.0 Hz), -101.1 (d, *J* = 241.0 Hz), -120.3 (d, *J* = 285.5 Hz), -125.5 (d, *J* = 285.5 Hz).
- Minor diastereomer: ¹H NMR (400 MHz, CDCl₃) δ 6.06 (ddd, *J* = 56.9, 55.3, 7.0 Hz, 1H), 3.61-3.49 (m, 1H), 2.87-2.76 (m, 1H), 2.25-1.62 (m, 11H), 1.62-1.02 (m, 11H), 1.47 (s, 9H), 1.14 (d, *J* = 7.0 Hz, 3H), 0.96 (s, 3H), 0.69 (s, 3H) ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.4 (d, *J* = 241.3 Hz), -101.1 (d, *J* = 241.3 Hz), -116.2 (d, *J* = 288.7 Hz), -122.7 (d, *J* = 288.7 Hz).

iii. **3 α -Hydroxy-7,7-difluoro-5 β -24-nor-22-di-fluoromethyl-cholanoic acid**
(Compounds 19a and 19b)



To *t*-Butyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-di-fluoromethyl-cholan-23-oate from step ii (2.1 mg major diastereomer, 0.9 mg minor diastereomer, reacted on separately) was charged 4 M HCl in dioxane (0.1 mL). The reaction mixture was stirred for 2 days until complete by TLC then concentrated *in vacuo* to afford the title compound major diastereomer (1 mg) and minor diastereomer (1.3 mg).

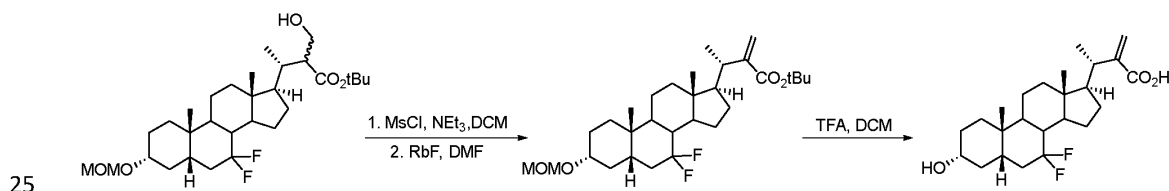
Major diastereomer (Compound 19a): pK_a 4.5; ¹H NMR (400 MHz, CD₃OD) δ 6.09 (td, *J* = 56.3, 7.3 Hz, 1H), 3.52-3.41 (m, 1H), 2.90-2.77 (m, 1H), 2.30-1.04 (m 24H), 1.07 (d, *J* = 6.8 Hz, 3H), 0.98 (s, 3H), 0.75 (s, 3H) ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.2 (d, *J* = 240.9 Hz), -102.5 (d, *J* = 240.9 Hz), -121.6 (d, *J* = 285.3 Hz), -126.6 (d, *J* = 285.3 Hz); MS (ESI⁻) *m/z* 447.2 [M-H]⁻.

Minor diastereomer (Compound 19b): pK_a 4.5; MS (ESI⁻) *m/z* 447.2 [M-H]⁻.

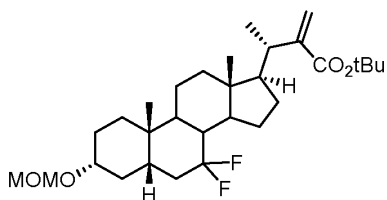
Example 20 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-methylene-cholanic acid (Compound 20)

Compound 20 was synthesised from *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-oate i.e. the product of Example 18, step iv, according to Scheme 26.

Scheme 26

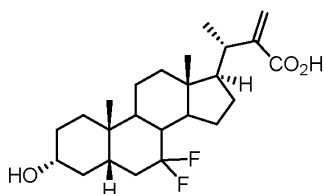


i. ***t*-Butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-methylenemethyl-cholan-23-oate**



t-Butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-oate (200 mg, 0.38 mmol) was dissolved in DCM (10 mL) and cooled to 0 °C under nitrogen. Triethylamine (0.263 mL, 1.89 mmol) and methanesulfonyl chloride (0.044 mL, 0.57 mmol) were charged then the reaction was stirred at 0 °C for 5 minutes and then allowed to warm to ambient temperature and stirred for 18 h. The reaction was monitored by TLC, further triethylamine (0.130 mL) and methanesulfonyl chloride (0.022 mL) was added and stirred at room temperature for a further 18 h. Upon the completion indicated by TLC analysis, water (5 mL) was added and extracted with DCM (2 $\times$ 10 mL). The combined organic layer was washed with 5% NaHCO₃ solution (aq., 2 $\times$ 10 mL) and 10% NaCl (10 mL), dried over Na₂SO₄, filtered and concentrated to afford the mesylate intermediate. The residue was dissolved in DMF (5 mL), RbF (0.099 g, 0.95 mmol) was charged and the reaction mixture heated to 50 °C for 4 days. The mixture was diluted with ethyl acetate (5 mL) and quenched with 5% NaHCO₃ (5 mL). The phases were separated, and the aqueous phase was extracted with ethyl acetate (3 $\times$ 20 mL). The combined extracts were washed with 10% NaCl (30 mL), dried over MgSO₄ and concentrated *in vacuo*. The resulting residue was purified by column chromatography (SiO₂, ethyl acetate/heptane), to afford the title compound (20 mg, 10%) which was continued on without any further purification.

ii. **3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-methylene-cholanic acid (Compound 20)**



To a solution of *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-methylenemethyl-cholan-23-oate from step i (82 mg, 0.16 mmol) in DCM (3 mL) at 0 °C was charged TFA (0.24 mL, 1.4 mmol). The reaction mixture was stirred at ambient temperature for 18 h then concentrated *in vacuo* and co-evaporated with water. The residue was dissolved in MeOH and stirred with Na₂CO₃ (150 mg) for 2 h, filtered and concentrated *in vacuo*. The crude residue

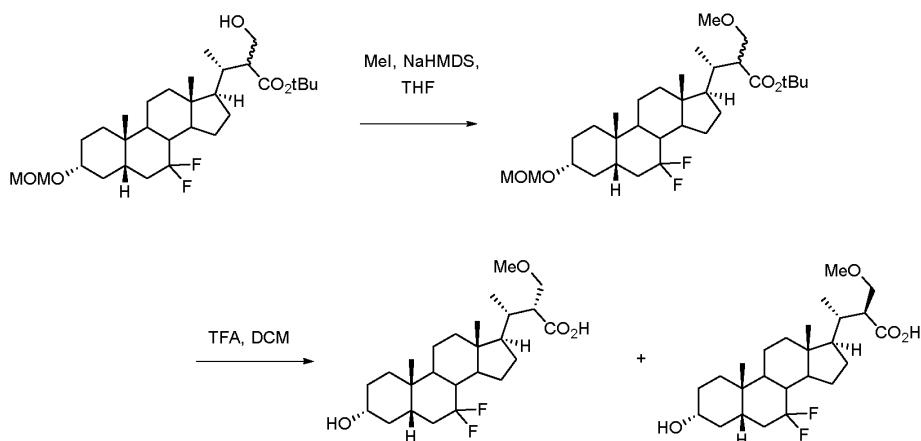
was purified by column chromatography (SiO₂, ethyl acetate/heptane/0.1% AcOH) to afford the title compound as a colourless solid (20 mg, 34%).

pK_a 3.9; ¹H NMR (400 MHz, CD₃OD) δ 6.15 (d, *J* = 1.3 Hz, 1H), 5.58 (d, *J* = 1.0 Hz, 1H), 3.52-3.44 (m, 1H), 2.72-2.64 (m, 1H), 2.30-2.11 (m, 1H), 2.11-2.04 (m, 1H), 1.91-1.06 (m, 19H),
 5 1.15 (d, *J* = 6.8 Hz, 3H), 0.99 (s, 3H), 0.76 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) -85.2 (d, *J* = 241.1 Hz), -102.4 (d, *J* = 241.1 Hz); MS (ESI⁺) *m/z* 409.2 ([*M*-H]⁺).

Example 21 – Synthesis of 3α-hydroxy-7,7-difluoro-5β-24-nor-22-methoxymethyl-cholanic acid (Compounds 21a and 21b)

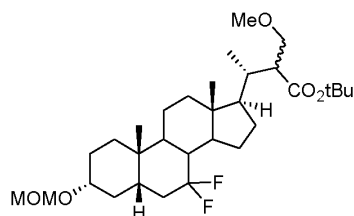
- 10 Compound 21 was synthesised from *t*-butyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-hydroxymethyl-cholan-23-oate i.e. the product of Example 18, step iv, according to Scheme 27.

Scheme 27



15

i. *t*-Butyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-methoxymethyl-cholan-23-oate

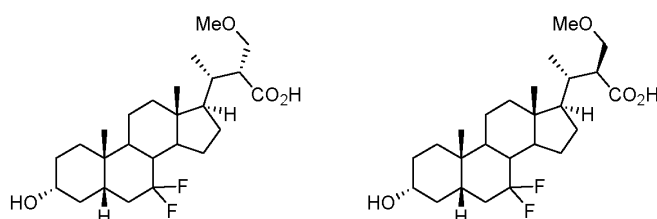


- 20 To a solution of *t*-butyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-hydroxymethyl-cholan-23-oate (240 mg, 0.45 mmol) and methyl iodide (283 μL, 4.54 mmol) in THF (12 mL) at -20 °C under nitrogen was charged NaHMDS (1.0 M in THF, 4.08 mL, 4.08 mmol) drop-wise. The reaction mixture was allowed to warm to ambient temperature and stirred for 18 h. After this

time the reaction was incomplete by ^1H NMR, NaH (170 mg, 4.24 mmol) followed by MeI (140 μL , 2.27 mmol) was charged and the mixture was stirred at ambient temperature for 1.5 h. The reaction was quenched by the addition of 5% NaHCO_3 (20 mL) and extracted with ethyl acetate (2 x 20 mL). The combined extracts were concentrated *in vacuo* and the residue was purified by column chromatography (SiO_2 , ethyl acetate/heptane) to afford the title compound as a mixture of diastereomers (1:1, 90mg, 37%).

^1H NMR (400 MHz, CDCl_3) δ 4.67 (s, 2H), 3.67-3.59 (m, 1H), 3.49-3.42 (m, 1H), 3.42-3.34 (m, 1H), 3.36 (s, 3H), 3.33 (s, 3H, diastereomer 1), 3.32 (s, 3H, diastereomer 2), 2.70 (td, $J = 7.2$, 2.7 Hz, diastereomer 1), 2.60 (dt, $J = 9.2$, 3.2 Hz, diastereomer 2), 2.25-1.66 (m, 12H), 1.60-0.99 (m, 10H), 1.48 (s, 9H, diastereomer 1), 1.45 (s, 9H, diastereomer 2), 0.95 (s, 3H, diastereomer 1), 0.95 (s, 3H, diastereomer 2), 0.94 (d, $J = 7.6$ Hz, 3H, diastereomer 1), 0.95 (d, $J = 7.0$ Hz, 3H, diastereomer 2), 0.69 (s, 3H, diastereomer 1), 0.68 (s, 3H, diastereomer 2); ^{19}F $\{^1\text{H}\}$ NMR (376 MHz, CDCl_3) δ -84.3 (d, $J = 240.3$ Hz, diastereomer 1), -84.3 (d, $J = 240.1$, diastereomer 2), -100.9 (d, $J = 240.1$ Hz, diastereomer 2), -101.1 (d, $J = 240.3$ Hz diastereomer 1).

ii. **3 α -Hydroxy-7,7-difluoro-5 β -24-nor-22-methoxymethyl-cholanic acid (Compound 21)**



To a solution of *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-methoxymethyl-cholan-23-oate from step i (90 mg, 0.17 mmol) in DCM (10 mL) at 0 $^\circ\text{C}$ was charged TFA (0.27 mL). The reaction was allowed to warm to ambient temperature and stirred for 17 h. The reaction mixture was concentrated *in vacuo* and co-evaporated with water. The residue was dissolved in MeOH and stirred with Na_2CO_3 (150 mg) for 3 h. The mixture was filtered and concentrated, acidified with 2 M HCl and then extracted into ethyl acetate and concentrated *in vacuo* to afford the title compound as a crude mixture. The crude residue was purified by preparative HPLC (C18, MeCN/ H_2O /0.1% formic acid) afforded the title compound major diastereomer (10.8 mg) and minor diastereomer (9.3 mg).

Major diastereomer (Compound 21a): pK_a 4.5; ^1H NMR (400 MHz, CD_3OD) δ 3.70 (t, $J = 9.3$ Hz, 1H), 3.50 (dd, $J = 9.3$, 3.4 Hz, 1H), 3.54-3.45 (m, 1H), 3.34 (s, 3H), 2.74-2.66 (m, 1H), 2.32-2.14 (m, 1H), 2.07-1.20 (m, 20H), 1.12 (td, $J = 14.1$, 3.1 Hz, 1H), 1.00 (s, 3H), 0.98 (d, $J = 7.0$

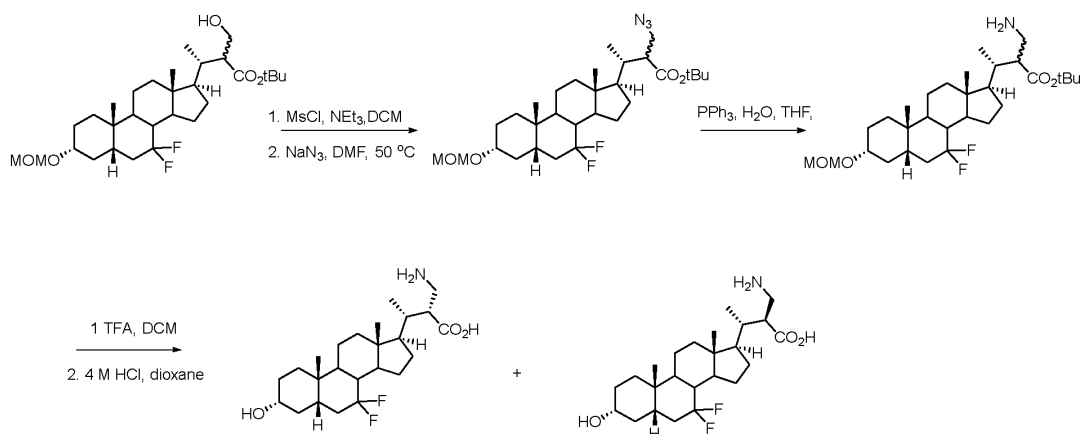
Hz, 3H), 0.76 (s, 3H); ^{19}F $\{^1\text{H}\}$ NMR (376 MHz, CD_3OD) δ -85.2 (d, J = 240.9 Hz), -102.5 (d, J = 240.9 Hz); MS (ESI $^-$) m/z 441.2 $[\text{M}-\text{H}]^-$.

Minor diastereomer (Compound 21b): pK_a 4.5; ^1H NMR (400 MHz, CD_3OD) δ 3.67 (dd, J = 9.4, 8.0 Hz, 1H), 3.52-3.43 (m, 1H), 3.41 (dd, J = 9.4, 6.5 Hz, 1H), 3.32 (s, 3H), 2.80-2.74 (m, 1H),
 5 2.30-2.01 (m, 3H), 1.89-1.15 (m, 18H), 1.10 (td, J = 13.9, 3.6 Hz, 1H), 1.00-0.96 (m, 6H), 0.73 (s, 3H); ^{19}F $\{^1\text{H}\}$ NMR (376 MHz, CD_3OD) δ -85.2 (d, J = 240.9 Hz), -102.5 (d, J = 240.9 Hz); MS (ESI $^-$) m/z 441.3 $[\text{M}-\text{H}]^-$.

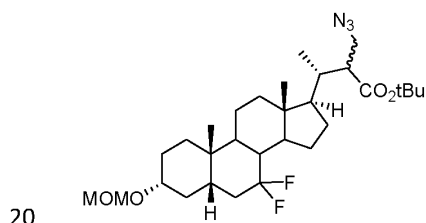
Example 22 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-aminomethyl-cholanic acid (Compounds 22a and 22b)

Compound 22 was synthesised from *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-oate i.e. the product of Example 18, step iv, according to Scheme 28.

15 Scheme 28



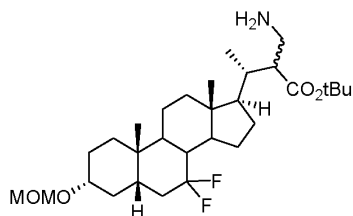
i. *t*-Butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-azidomethyl-cholan-23-oate



t-Butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-oate (200 mg, 0.38 mmol) was dissolved in DCM (10 mL) and cooled to 0 °C under nitrogen. Triethylamine (0.263 mL, 1.89 mmol) and methanesulfonyl chloride (0.044 mL, 0.57 mmol)

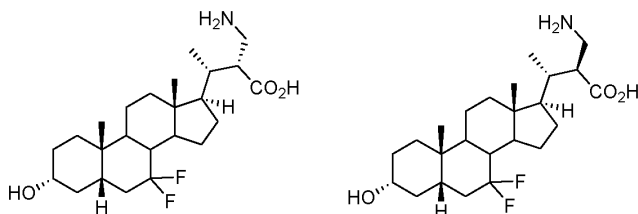
were charged then the reaction was stirred at 0 °C for 5 minutes and then allowed to warm to ambient temperature and stirred for 18 h. The reaction was monitored by TLC, further triethylamine (0.130 mL) and methanesulfonyl chloride (0.022 mL) was added and stirred at room temperature for a further 18 h. Upon the completion indicated by TLC analysis, water (5 mL) was added and extracted with DCM (2 × 10 mL). The combined organic layer was washed with 5% NaHCO₃ solution (aq., 2 × 10 mL) and 10% NaCl (10 mL), dried over Na₂SO₄, filtered and concentrated to afford the mesylate intermediate. A portion of the residue (114 mg, 0.17 mmol) was dissolved in DMF (5 mL) and transferred into a flask containing NaN₃ (30 mg, 0.46 mmol) and the reaction mixture heated to 50 °C for 4 days. After this time the reaction was cooled to ambient temperature, diluted with ethyl acetate and washed with 5% NaHCO₃. The aqueous phase was extracted with ethyl acetate and the combined extracts were washed with 10% NaCl and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, ethyl acetate/heptane) to afford the title compound as a 1:1 mix of diastereomers contaminated with the eliminated product *t*-butyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-methylene-cholan-23-oate (44 mg total mass). This was continued on without any further purification.

ii. ***t*-Butyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-aminomethyl-cholan-23-oate**



t-Butyl 3α-methoxymethoxy-7,7-difluoro-5β-24-nor-22-azidomethyl-cholan-23-oate from step i (44 mg, 0.08 mmol) was dissolved in THF (1 mL) and PPh₃ (62.5 mg, 0.24 mmol) and H₂O (12.8 μL, 0.71 mmol) were charged. The reaction mixture heated to reflux for 18 h then concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, DCM/MeOH with 0.1% NEt₃) to afford the title compound as a 6:4 mix of diastereomers (22 mg). This was continued on without any further purification.

iii. **3 α -Hydroxy-7,7-difluoro-5 β -24-nor-22-aminomethyl-cholanic acid (Compound 22)**



To a solution of *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-aminomethyl-cholan-23-oate from step ii (22 mg, 0.04 mmol) in DCM (3 mL) was charged TFA (0.1 mL). The reaction mixture was stirred at ambient temperature for 20 h then concentrated *in vacuo* and co-evaporated with water. The residue was dissolved in MeOH and stirred with Na₂CO₃ (100 mg) for 2 h, filtered and concentrated *in vacuo*. The crude residue was purified by preparative HPLC (C18, MeCN/H₂O, 0.1% formic acid) to afford the *t*-butyl ester intermediates major diastereomer (3.9 mg), minor diastereomer (2.6 mg). The *t*-butyl esters were reacted on separately, dissolve in 4 M HCl in dioxane (0.1 mL) and stirred at ambient temperature for 2 days until complete by TLC. After this time they were concentrated *in vacuo* to afford the major diastereomer (3.5 mg) and minor diastereomer (2.3 mg).

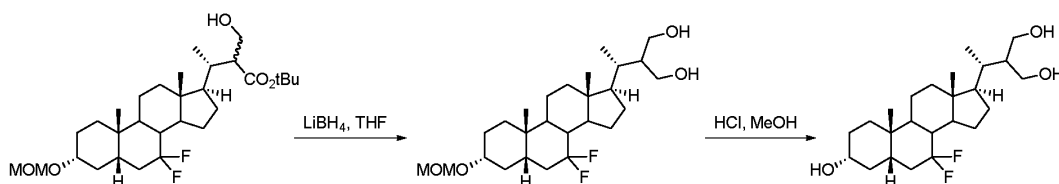
Major diastereomer (Compound 22a): pK_a 3.8 (acidic), 8.7 (basic); ¹H NMR (400 MHz, CD₃OD) δ 3.52-3.43 (m, 1H), 3.35-3.28 (m, 1H), 2.93 (dd, *J* = 12.8, 4.3 Hz, 1H), 2.84-2.78 (m, 1H), 2.32-1.99 (m, 3H), 1.92-1.16 (m, 18H), 1.11 (td, *J* = 14.1, 2.9 Hz, 1H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.98 (s, 3H), 0.75 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) -85.2 (d, *J* = 240.9 Hz), -102.5 (d, *J* = 240.9 Hz); MS (ESI⁺) *m/z* 428.2 ([M+H]⁺).

Minor diastereomer (Compound 22b): pK_a 3.8 (acidic), 8.7 (basic); ¹H NMR (400 MHz, CD₃OD) δ 3.52-3.44 (m, 1H), 3.18 (t, *J* = 12.3 Hz, 1H), 3.00 (dd, *J* = 12.8, 2.4 Hz, 1H), 2.82-2.74 (m, 1H), 2.33-2.13 (m, 2H), 2.06-1.19 (m, 19H), 1.11 (td, *J* = 14.0, 3.2 Hz, 1H), 0.99 (s, 3H), 0.93 (d, *J* = 6.9 Hz, 3H), 0.77 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) -85.2 (d, *J* = 240.7 Hz), -102.5 (d, *J* = 240.7 Hz); MS (ESI⁺) *m/z* 428.2 ([M+H]⁺).

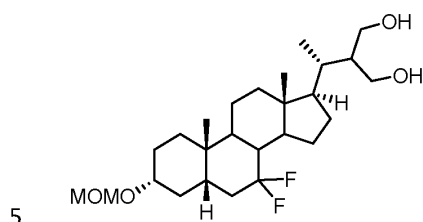
25 **Example 23 – Synthesis of 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-ol (Compound 23)**

Compound 23 was synthesised from *t*-butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-oate i.e. the product of Example 18, step iv, according to Scheme 29.

Scheme 29



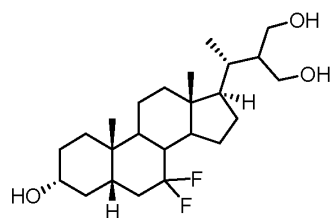
i. **3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-ol**



10 *t*-Butyl 3 α -methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-oate (350 mg, 0.66 mmol) was dissolved in anhydrous THF (5 mL), then MeOH (0.16 mL) was added and the resulting solution cooled to 5 °C under nitrogen. LiBH₄ (87 mg, 3.97 mmol) was charged portion-wise then stirred at ambient temperature for 18 h. The reaction was then quenched by the drop-wise addition of 5% NH₄Cl (10 mL), extracted with EtOAc (2 × 10 mL) and the combined organic phase was washed with 5% NH₄Cl (10 mL), brine (10 mL), dried over Na₂SO₄ and concentrated *in vacuo*. The resulting residue was purified by column chromatography (SiO₂, ethyl acetate/heptane), to afford the title compound (160 mg, 53%) which was continued on without any further purification.

15 ii. **3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-ol (Compound 23)**

The product of this step is the starting material for Compound 24 of Example 24.



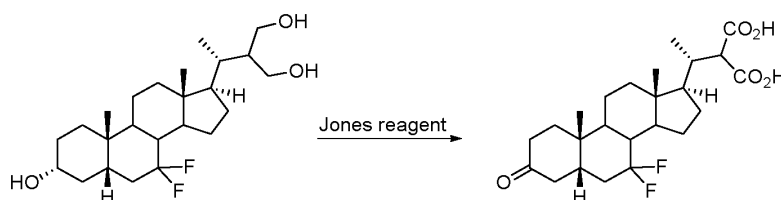
20 3 α -Methoxymethoxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-ol from step i (160 mg, 0.35 mmol) was dissolved in MeOH (5 mL), 2 M HCl (0.8 mL, 5 vol) was charged and the reaction mixture was heated to 45 °C for 18 h. After cooling to ambient, the mixture was diluted with ethyl acetate (10 mL) and quenched with 5% NaHCO₃ (10 mL). The phases were separated and the organic was concentrated *in vacuo* and purified by column chromatography (SiO₂, ethyl acetate/heptane) to afford the title compound as an off white solid (11 mg, 8%).

pK_a 10.1; ¹H NMR (400 MHz, CD₃OD) δ 3.75 (dd, *J* = 10.7, 5.6 Hz, 1H), 3.69 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.52-3.43 (m, 2H), 3.37-3.32 (m, 1H), 2.29-2.12 (m, 1H), 2.04 (dt, *J* = 12.3, 3.2 Hz, 1H), 1.99-1.06 (m, 21H), 0.98 (s, 3H), 0.86 (d, *J* = 7.0 Hz, 3H), 0.73 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) -85.1 (d, *J* = 241.2 Hz), -102.5 (d, *J* = 241.2 Hz); MS (ESI⁻) *m/z* 395.2 ([M-H₂O-H]⁻).

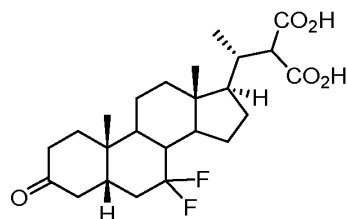
Example 24 – Synthesis of 3-keto-7,7-difluoro-5β-24-nor-cholan-23,23-dicarboxylic acid (Compound 24)

Compound 24 was synthesised from 3α-hydroxy-7,7-difluoro-5β-24-nor-22-hydroxymethyl-cholan-23-ol i.e. the product of Example 23, step ii, according to Scheme 30.

Scheme 30



i. **3-keto-7,7-difluoro-5β-24-nor-cholan-23,23-dicarboxylic acid (Compound 24)**

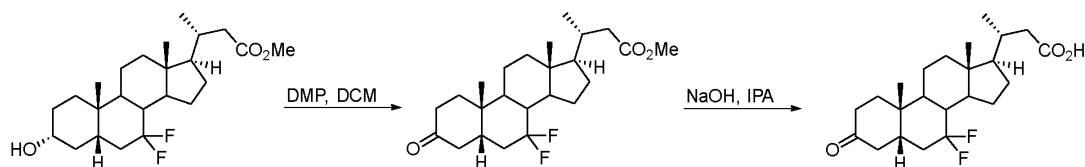


To a suspension of 3α-hydroxy-7,7-difluoro-5β-24-nor-22-hydroxymethyl-cholan-23-ol (50 mg, 0.12 mmol) in acetone (10 mL) was charged Jones reagent (2 M in H₂SO₄, 140 μL, 0.28 mmol). The reaction was stirred at ambient temperature for 5 h, then quenched with IPA (10 mL). The solution was diluted with ethyl acetate (5 mL) and washed with 10 % NaCl (9 mL) dried over MgSO₄ and concentrated *in vacuo*. The residue was passed through a silica plug (SiO₂, acetone/chloroform), then purified by preparative HPLC (C18, MeCN/H₂O/0.1% formic acid) afforded the title compound (4 mg, 8%).

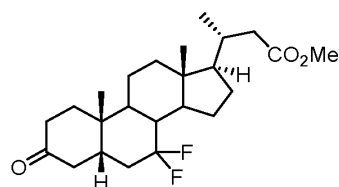
pK_a 3.1; ¹H NMR (400 MHz, acetone-d₆) δ 3.53 (d, *J* = 3.4 Hz, 1H), 2.72 (t, *J* = 14.3 Hz, 1H), 2.51-2.42 (m, 1H), 2.34-2.18 (m, 2H), 2.11-1.75 (m, 9H), 1.67-1.29 (m, 9H), 1.19 (d, *J* = 6.8 Hz, 3H), 1.12 (s, 3H), 0.81 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, acetone-d₆) δ -84.2 (d, *J* = 242.5 Hz), -101.7 (d, *J* = 242.5 Hz); MS (ESI⁻) *m/z* 439.2 [M-H]⁻.

Example 25 – Synthesis of 3-keto-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 25)

Compound 25 was synthesised from methyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate i.e. the product of Example 2, step i, according to Scheme 31.

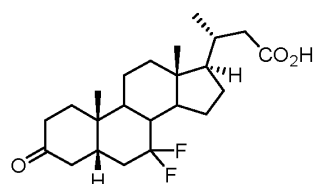
5 Scheme 31**i. Methyl 3-keto-7,7-difluoro-5 β -24-nor-cholan-23-oate**

The product of this step is the starting material for Compound 26 of Example 26, Compounds 27, 28, 29 and Compound 30 of Example 27 and Compound 31 of Example 28 and Compound 32 of Example 29.



To a solution of starting material methyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate (1.4 g, 3.39 mmol) in DCM (20 mL) was charged DMP (2.16 g, 5.09 mmol) portion-wise maintaining the temperature at < 30 °C. The reaction mixture was stirred at ambient temperature for 18 h then filtered and washed with DCM. The organic was washed with Na₂S₂O₃ (100 mL), 5% NaHCO₃ (100 mL), 10% NaCl (100 mL), dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, ethyl acetate/toluene) to afford the title compound (1.35 g, 97%).

¹H NMR (400 MHz, CDCl₃) δ 3.66 (s, 3H), 2.72 (t, *J* = 14.8 Hz, 1H), 2.44 (dd, *J* = 14.4, 3.1 Hz, 1H), 2.35-1.75 (m, 14H), 1.58-1.14 (m, 8H), 1.06 (s, 3H), 0.99 (d, *J* = 6.3 Hz, 3H), 0.74 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.4 (d, *J* = 244.4 Hz), -101.4 (d, *J* = 244.4 Hz).

ii. 3-keto-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 25)

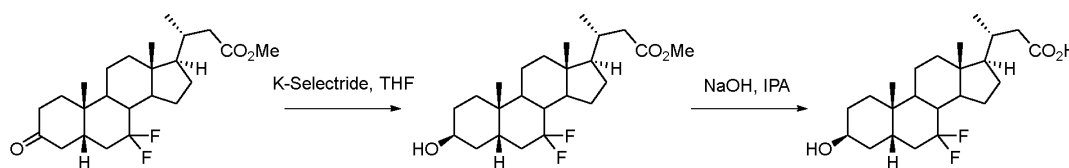
Methyl 3-keto-7,7-difluoro-5 β -24-nor-cholan-23-oate from step i (50 mg, 0.12 mmol) was dissolved in IPA (2 mL). 1 M NaOH (0.24 mL, 0.24 mmol) was charged and the reaction mixture was heated to 45 °C for 18 h. Upon the completion indicated by TLC analysis, the solution was concentrated *in vacuo*. The crude residue was diluted with water, acidified with 1 M HCl and extracted with EtOAc (x3). The combined extracts were washed with 10% NaCl (10 mL) dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, acetone/toluene) to afford the title compound (44 mg, 92%).

pK_a 4.0; ¹H NMR (400 MHz, CDCl₃) δ 2.70 (t, *J* = 14.7 Hz, 1H), 2.47 (dd, *J* = 15.1, 3.2 Hz, 1H), 2.33-1.15 (m, 22H), 1.04-1.02 (m, 6H), 0.73 (s, 3H; ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.4 (d, *J* = 244.0 Hz), -101.4 (d, *J* = 244.0 Hz). MS (ESI⁺) *m/z* 395.2 [M-H]⁺.

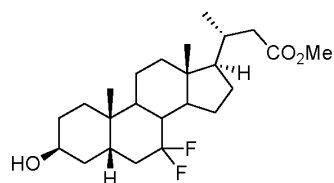
Example 26 – Synthesis of 3 β -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 26)

Compound 26 was synthesised from methyl 3-keto-7,7-difluoro-5 β -24-nor-cholan-23-oate i.e. the product of Example 25, step i, according to Scheme 32.

Scheme 32



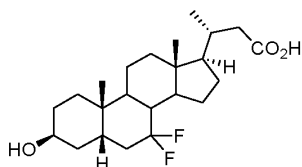
20 i. Methyl 3 β -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate



To a solution of methyl 3-keto-7,7-difluoro-5 β -24-nor-cholan-23-oate (100 mg, 0.24 mmol) in anhydrous THF (2 mL) at -78 °C, under nitrogen was charged K-selectride® (1 M in THF, 0.48 mL, 0.48 mmol) dropwise. The reaction mixture was allowed to warm to ambient temperature and stirred for 1.5 h. The reaction was quenched by the addition of 2 M HCl (1 mL) dropwise, stirred for 1 h and then ethyl acetate was charged. Additional HCl was charged to aid solubility, then the phases were separated and the aqueous phase was re-extracted with ethyl acetate. The combined extracts were washed with 5% NaHCO₃, then 10% NaCl, dried over MgSO₄ and concentrated *in vacuo*. The crude residue was purified by column chromatography (SiO₂, MeCN/toluene) to afford the title compound as a white solid (46 mg, 46%).

^1H NMR (400 MHz, CDCl_3) δ 4.09-4.06 (m, 1H), 3.65 (s, 3H), 2.43 (dd, $J = 14.3, 3.1$ Hz, 1H), 2.27-2.09 (m, 1H), 2.05-1.08 (m, 22H), 0.98 (d, $J = 6.3$ Hz, 3H), 0.98 (s, 3H), 0.70 (s, 3H); ^{19}F $\{^1\text{H}\}$ NMR (376 MHz, CDCl_3) δ -83.8 (d, $J = 239.9$ Hz), -101.1 (d, $J = 239.9$ Hz).

5 ii. **3 β -Hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 26)**



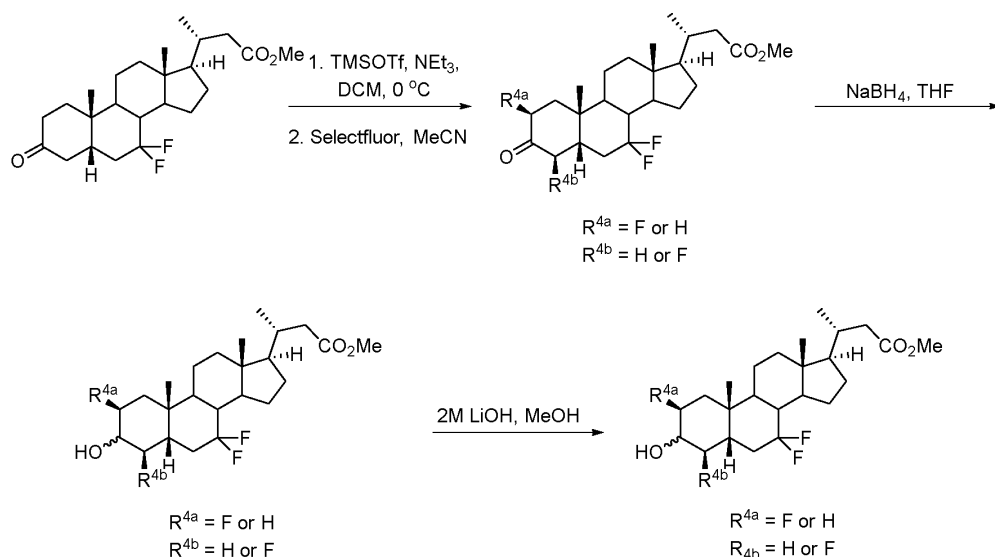
To a solution of methyl 3 β -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate from step i (46 mg, 0.11 mmol) in IPA (2 mL) and MeOH (0.5 mL) was charged 1 M NaOH (1 mL, 1.0 mmol). The reaction mixture was stirred at ambient temperature for 18 h. Upon the completion indicated by TLC analysis, the solution was concentrated *in vacuo*. The crude residue was diluted with water, acidified with 1 M HCl and extracted with EtOAc (x3). The combined extracts were washed with 10% NaCl (10 mL) dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (SiO_2 , acetone/DCM) to afford the title compound (22 mg, 50%).

15 pK_a 4.0; ^1H NMR (400 MHz, CD_3OD) δ 4.01-3.98 (m, 1H), 2.46-2.40 (m, 1H), 2.33-2.13 (m, 1H), 2.09-1.14 (m, 22H), 1.06 (d, $J = 6.2$ Hz, 3H), 1.01 (s, 3H), 0.75 (s, 3H); ^{19}F $\{^1\text{H}\}$ NMR (376 MHz, CD_3OD) δ -84.6 (d, $J = 240.6$ Hz), -102.3 (d, $J = 240.6$ Hz).

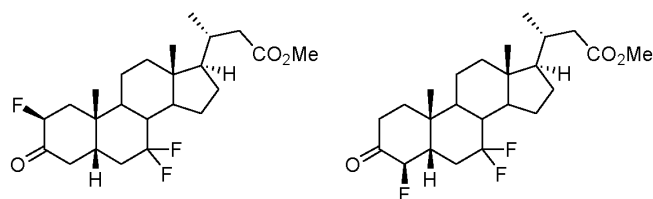
20 **Example 27 – Synthesis of 2 β -fluoro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 27), 2 β -fluoro-3 β -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 28), 4 β -fluoro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 29) and 4 β -fluoro-3 β -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 30)**

Compounds 27, 28, 29 and 30 were synthesised from methyl 3-keto-7,7-difluoro-5 β -24-nor-cholan-23-oate, i.e. the product of Example 25, step i according to Scheme 33.

Scheme 33



i. **Methyl 2β-fluoro-3-keto-7,7-difluoro-5β-24-nor-cholan-23-oate and methyl 4β-fluoro-3-keto-7,7-difluoro-5β-24-nor-cholan-23-oate**



5

Methyl 3-keto-7,7-difluoro-5β-24-nor-cholan-23-oate (410 mg, 1.0 mmol) was dissolved in anhydrous DCM (6 mL) under nitrogen and cooled to 0 °C. Triethylamine (0.28 mL, 2.0 mmol) was charged followed by TMSOTf (0.2 mL, 1.1 mmol) maintaining the temperature ca. 0 °C. The reaction mixture was stirred for 1 h at 0 °C then gradually warmed to ambient temperature. After 2 h at ambient the reaction was monitored by TLC analysis, further triethylamine (0.1 mL) was charged followed by TMSOTf (0.07 mL) and stirred for 2 h until complete by TLC. The reaction was diluted with DCM (5 mL) and quenched by the addition of 5% NaHCO₃ (aq) (15 mL) extracted with DCM (3 × 10 mL) and the combined organic phase was washed with 10% NaCl (10 mL), dried over Na₂SO₄ and concentrated *in vacuo* to afford the crude intermediate which was continued through without any purification. The intermediate was dissolved in anhydrous MeCN (7 mL) under nitrogen, transferred to a flask containing Selectfluor (709 mg, 2.0 mmol) and stirred for 18 h at ambient. The reaction was diluted with ethyl acetate, quenched by the addition of 5% NaHCO₃ (aq) (30 mL) and stirred for 1 h. The phases were separated and the aqueous back extracted with ethyl acetate. The combined extracts were washed with 10% NaCl (10 mL) dried over Na₂SO₄ and concentrated *in vacuo*. The residue

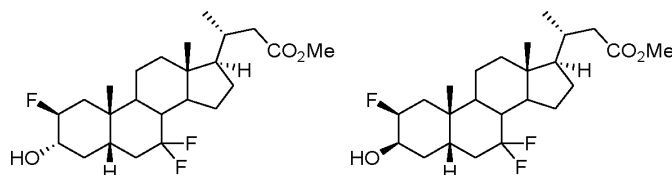
20

was purified by column chromatography (SiO₂, ethyl acetate/heptane) to afford the 2-fluoro compound (85 mg, 20%) and 4-fluoro compound (260 mg, 61%).

2-Fluoro: ¹H NMR (400 MHz, CDCl₃) δ 4.95 (ddd, *J* = 48.7, 13.5, 6.0 Hz, 1H), 3.67 (s, 3H), 2.83 (t, *J* = 14.3 Hz, 1H), 2.54-1.56 (m, 15H), 1.48-1.15 (m, 6H), 1.12 (s, 3H), 1.01 (d, *J* = 7.1 Hz, 3H), 0.75 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -85.0 (d, *J* = 246.1 Hz), -101.4 (d, *J* = 246.1 Hz), -195.1 (s).

4-Fluoro: ¹H NMR (400 MHz, CDCl₃) δ 5.13 (dd, *J* = 47.2, 11.7 Hz, 1H), 3.67 (s, 3H), 2.47-1.55 (m, 15H), 1.53-1.15 (m, 7H), 1.11 (s, 3H), 1.00 (d, *J* = 7.1 Hz, 3H), 0.75 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -85.5 (d, *J* = 248.2 Hz), -103.0 (d, *J* = 248.2 Hz), -200.5 (s).

ii. a. **Methyl 2β-fluoro-3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-oate and methyl 2β-fluoro-3β-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-oate**

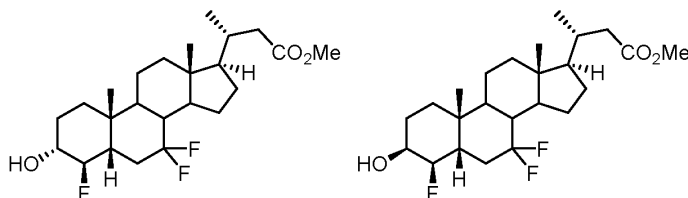


Methyl 2β-fluoro-3-keto-7,7-difluoro-5β-24-nor-cholan-23-oate from step i (85 mg, 0.19 mmol) was dissolved in anhydrous THF (6 mL) under nitrogen and cooled to -20 °C. NaBH₄ (7.3 mg, 0.19 mmol) was charged and stirred at -20 °C for 18 h. The reaction was warmed to ambient temperature, diluted with ethyl acetate and quenched by the addition of 2M HCl (aq) (10 mL). The phases were separated and the aqueous back extracted with ethyl acetate (10 mL). The combined extracts were washed with 5% NaHCO₃ (10 mL), 10% NaCl (10 mL), dried over Na₂SO₄ and concentrated *in vacuo* to afford the crude material as a mixture of isomers (~7:3 ratio; 3α:3β). The mixture was combined with other crude batches for purification by column chromatography (SiO₂, acetone/heptane) to afford the 3α-hydroxy compound (50 mg, 29%) and 3β-hydroxy compound (32 mg, 19%).

3α-hydroxy-2β-fluoro: ¹H NMR (400 MHz, CDCl₃) δ 4.46-4.24 (m, 1H), 3.67 (s, 3H), 3.70-3.59 (m, 1H), 2.44 (dd, *J* = 14.3, 3.4 Hz, 1H), 2.26-1.14 (m, 21H), 1.03 (s, 3H), 0.99 (d, *J* = 6.2 Hz, 3H), 0.71 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -85.1 (d, *J* = 241.7 Hz), -101.3 (d, *J* = 241.7 Hz), -187.7 (s).

3β-hydroxy-2β-fluoro: ¹H NMR (400 MHz, CDCl₃) δ 4.70-4.41 (m, 1H), 4.20-4.14 (m, 1H), 3.66 (s, 3H), 2.43 (dd, *J* = 14.6, 3.3 Hz, 1H), 2.23-1.11 (m, 21H), 1.05 (s, 3H), 0.99 (d, *J* = 6.3 Hz, 3H), 0.71 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.7 (d, *J* = 241.8 Hz), -101.6 (d, *J* = 241.8 Hz), -187.9 (s).

ii. b. Methyl 4 β -fluoro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate and methyl 4 β -fluoro-3 β -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate

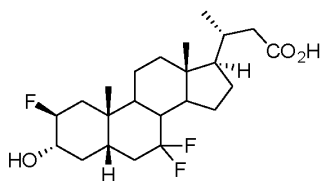


Methyl 4 β -fluoro-3-keto-7,7-difluoro-5 β -24-nor-cholan-23-oate from step i (260 mg, 0.59 mmol) was dissolved in anhydrous THF (18 mL) under nitrogen and cooled to -20 °C. NaBH₄ (22 mg, 0.59 mmol) was charged and stirred at -20 °C for 18 h. The reaction was warmed to ambient temperature, diluted with ethyl acetate and quenched by the addition of 2M HCl (aq) (20 mL). The phases were separated and the aqueous back extracted with ethyl acetate (20 mL). The combined extracts were washed with 5% NaHCO₃ (20 mL), 10% NaCl (20 mL), dried over Na₂SO₄ and concentrated *in vacuo* to afford the crude material as a mixture of isomers (~7:3 ratio; 3 α :3 β). The crude mixture was purified by column chromatography (SiO₂, ethyl acetate/heptane) to afford the 3 α -hydroxy compound (120 mg, 47%) and 3 β -hydroxy compound (68 mg, 27%).

3 α -hydroxy-4 β -fluoro: ¹H NMR (400 MHz, CDCl₃) δ 4.57 (ddd, *J* = 50.8, 19.9, 8.2 Hz, 1H), 3.67 (s, 3H), 3.68-3.57 (m, 1H), 2.47-2.32 (m, 3H), 2.06-1.10 (m, 19H), 1.02 (s, 3H), 0.99 (d, *J* = 6.2 Hz, 3H), 0.71 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -85.5 (d, *J* = 244.0 Hz), -102.9 (d, *J* = 244.0 Hz), -193.7 (s).

3 β -hydroxy-4 β -fluoro: ¹H NMR (400 MHz, CDCl₃) δ 4.73 (ddd, *J* = 46.3, 11.4, 3.6 Hz, 1H), 4.22-4.16 (m, 1H), 3.66 (s, 3H), 2.44 (dd, *J* = 14.4, 3.1, 1H), 2.36-1.08 (m, 21H), 1.04 (s, 3H), 0.98 (d, *J* = 6.1 Hz, 3H), 0.71 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -85.2 (d, *J* = 244.2 Hz), -103.1 (d, *J* = 244.2 Hz), -194.2 (s).

iii. a. 2 β -Fluoro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholanoic acid (Compound 27)

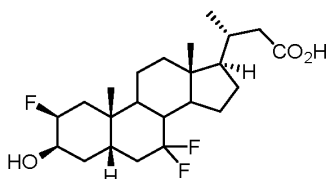


Methyl 2 β -fluoro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate from step ii. a (50 mg, 0.12 mmol) was dissolved in MeOH (5 mL). 2 M LiOH (1 mL) was charged and the reaction mixture was stirred at 40 °C for 3 days. Upon the completion indicated by TLC analysis, the solution was concentrated *in vacuo*. The crude residue was diluted with water, acidified with 1 M HCl

and extracted with EtOAc (x3). The combined extracts were washed with 10% NaCl (10 mL) dried over MgSO₄ and concentrated *in vacuo* to afford the title compound (30 mg, 62%).

pK_a 4.0; ¹H NMR (400 MHz, CD₃OD) 4.37-4.18 (m, 1H), 3.60-3.48 (m, 1H), 2.43 (d, *J* = 12.1 Hz, 1H), 2.27-1.19 (m, 21H), 1.05 (s, 3H), 1.03 (d, *J* = 6.4 Hz, 3H), 0.75 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -81.7 (d, *J* = 242.3 Hz), -98.3 (d, *J* = 242.3 Hz), -183.1 (s); MS (ESI⁻) *m/z* 415.2 [M-H]⁻.

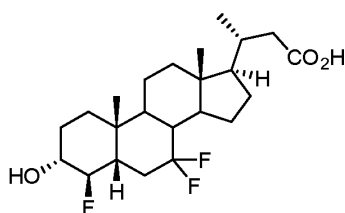
iii. b. 2β-Fluoro-3β-hydroxy-7,7-difluoro-5β-24-nor-cholanoic acid (Compound 28)



Methyl 2β-fluoro-3β-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-oate from step ii. a (32 mg, 0.07 mmol) was dissolved in MeOH (5 mL). 2 M LiOH (1 mL) was charged and the reaction mixture was stirred at 40 °C for 3 days. Upon the completion indicated by TLC analysis, the solution was concentrated *in vacuo*. The crude residue was diluted with water, acidified with 1 M HCl and extracted with EtOAc (x3). The combined extracts were washed with 10% NaCl (10 mL) dried over MgSO₄ and concentrated *in vacuo* to afford the title compound (13 mg, 42%).

pK_a 4.0; ¹H NMR (400 MHz, CDCl₃) 4.54 (dddd, *J* = 47.3, 12.5, 4.8, 3.0, 1H), 4.21-4.15 (m, 1H), 2.49 (dd, *J* = 14.8, 3.2 Hz, 1H), 2.25-1.15 (m, 21H), 1.06-1.04 (m, 6H), 0.72 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.7 (d, *J* = 242.0 Hz), -101.6 (d, *J* = 242.0 Hz), -187.9 (s); MS (ESI⁻) *m/z* 415.2 [M-H]⁻.

iii. c. 4β-Fluoro-3α-hydroxy-7,7-difluoro-5β-24-nor-cholanoic acid (Compound 29)

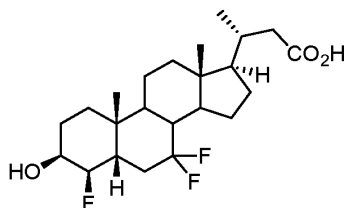


Methyl 4β-fluoro-3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-oate from step ii. b (120 mg, 0.28 mmol) was dissolved in MeOH (10 mL). 2 M LiOH (2 mL) was charged and the reaction mixture was stirred at 40 °C for 3 days. Upon the completion indicated by TLC analysis, the solution was concentrated *in vacuo*. The crude residue was diluted with water, acidified with 1 M HCl and extracted with EtOAc (x3). The combined extracts were washed with 10% NaCl (10 mL) dried over MgSO₄ and concentrated *in vacuo* to afford the crude compound. The residue

was purified by column chromatography (SiO₂, ethyl acetate/toluene + 0.1% acetic acid) to afford the title compound (37 mg, 32%).

pK_a 4.0; ¹H NMR (400 MHz, CDCl₃) 4.57 (ddd, *J* = 50.5, 19.8, 9.7 Hz, 1H), 3.67-3.56 (m, 1H), 2.49 (dd, *J* = 14.9, 2.9 Hz, 1H), 2.10-1.14 (m, 21H), 1.04 (d, *J* = 6.2 Hz, 3H), 1.02 (s, 3H), 0.71 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -85.5 (d, *J* = 245.0 Hz), -102.9 (d, *J* = 245.0 Hz), -193.7 (s); MS (ESI⁻) *m/z* 415.2 [M-H]⁻.

iii. d. **4β-Fluoro-3β-hydroxy-7,7-difluoro-5β-24-nor-cholanoic acid (Compound 30)**

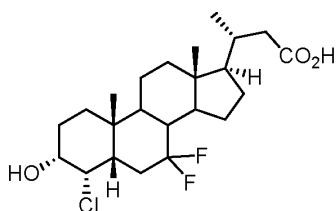


Methyl 4β-fluoro-3β-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-oate from step ii. b (68 mg, 0.16 mmol) was dissolved in MeOH (5 mL). 2 M LiOH (1 mL) was charged and the reaction mixture was stirred at 40 °C for 3 days. Upon the completion indicated by TLC analysis, the solution was concentrated *in vacuo*. The crude residue was diluted with water, acidified with 1 M HCl and extracted with EtOAc (x3). The combined extracts were washed with 10% NaCl (10 mL) dried over MgSO₄ and concentrated *in vacuo* to afford the crude compound (64 mg, quant.). The residue (30 mg) was purified by preparative HPLC (C18, MeCN/H₂O/0.1% FA) to afford the title compound (13 mg).

pK_a 4.0; ¹H NMR (400 MHz, CDCl₃) 4.73 (ddd, *J* = 45.7, 11.7, 2.9 Hz, 1H), 4.22-4.15 (m, 1H), 2.44-2.27 (m, 2H), 2.11-1.12 (m, 20H), 1.04 (s, 3H), 1.02 (d, *J* = 5.2 Hz, 3H), 0.71 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -85.2 (d, *J* = 244.1 Hz), -103.1 (d, *J* = 244.1 Hz), -194.1 (s); MS (ESI⁻) *m/z* 415.2 [M-H]⁻.

Example 28 – Synthesis of 4α-chloro-3α-hydroxy-7,7-difluoro-5β-24-nor-cholanic acid (Compound 31)

Compound 31 was synthesised from methyl 3-keto-7,7-difluoro-5β-24-nor-cholan-23-oate, i.e. the product of Example 25, step i using the method outlined in Scheme 33 but using N-chlorosuccinimide as opposed to the fluorinating reagent Selectfluor.



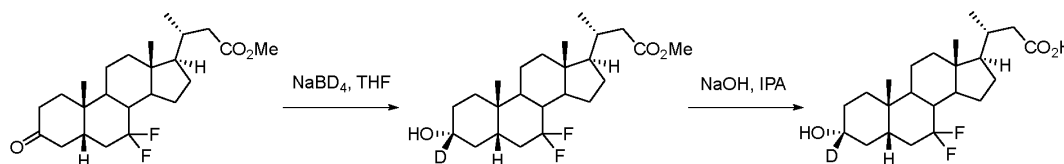
Methyl 4-chloro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate (82 mg, 0.18 mmol) was dissolved in MeOH (0.5 mL). 2 M LiOH (91 μ L, 1.03 mmol) was charged and the reaction mixture was stirred at 40 °C until complete by TLC analysis. Upon completion the solution was concentrated *in vacuo*. The crude residue was diluted with water, acidified with 2 M HCl and
 5 extracted with EtOAc (x3). The combined extracts were washed with 5% NaHCO₃ (5 mL) dried over MgSO₄ and concentrated *in vacuo* to afford the crude material. The crude mixture was purified by column chromatography (SiO₂, ethyl acetate/heptane/0.1% AcOH) to afford the title compound (5.0 mg, 6%).

pK_a 4.0; ¹H NMR (400 MHz, CD₃OD) 3.9 (dd, *J* = 11.7, 9.6 Hz, 1H), 3.4 (td, *J* = 9.6, 5.0 Hz, 1H), 2.5 (dd, *J* = 13.5, 3.3 Hz, 1H), 2.33 (dd, *J* = 11.4, 2.1 Hz, 1H), 2.08 – 1.00 (m, 20H), 0.96 (s, 3H), 0.92 (d, *J* = 6.24 Hz, 3H), 0.64 (s, 3H). ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -86.4 (d, *J* = 244.8 Hz), -106.3 (d, *J* = 244.8 Hz); MS (ESI⁻) *m/z* 431.2 [M-H]⁻.

Example 29 – Synthesis of [²H]-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oic acid (Compound 32)

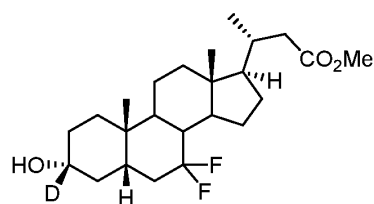
15 Compound 32 was synthesised from methyl 3-keto-7,7-difluoro-5 β -24-nor-cholan-23-oate i.e. the product of Example 25, step i, according to Scheme 34.

Scheme 34



20

i. Methyl 3-[²H]-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate

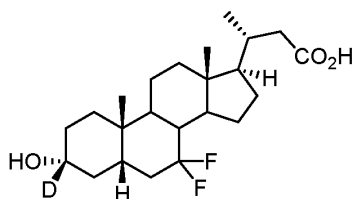


To a solution of methyl 3-keto-7,7-difluoro-5 β -24-nor-cholan-23-oate (100 mg, 0.24 mmol) in anhydrous THF (2 mL) and MeOD (2 mL) at 0 °C, under nitrogen was charged NaBD₄
 25 (0.48 mmol, 2 eq). The reaction mixture was allowed to warm to ambient temperature and stirred for 1 h. The reaction was quenched by the addition of 2 M HCl (2 mL) dropwise, stirred for 1 h and then ethyl acetate was charged. The phases were separated and the aqueous phase was re-extracted with ethyl acetate. The combined extracts were washed with 5% NaHCO₃, then 10% NaCl, dried over MgSO₄ and concentrated *in vacuo*. The crude residue

was purified by column chromatography (SiO₂, MeCN/toluene) to afford the title compound as a white solid (74 mg, 73%).

¹H NMR (400 MHz, CDCl₃) δ 3.59 (s, 3H), 2.37 (dd, *J* = 14.4, 3.1 Hz, 1H), 2.16-2.00 (m, 1H), 1.93-0.96 (m, 22H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.88 (s, 3H), 0.64 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -85.14 (d, *J* = 240.9 Hz), -102.4 (d, *J* = 240.9 Hz).

ii. **3-[²H]-3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-oic acid (Compound 32)**



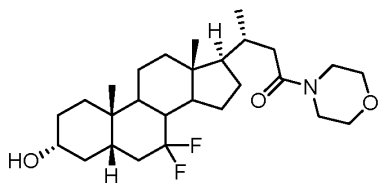
To a solution of Methyl 3-[²H]-3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-oate from step i (74 mg, 0.18 mmol) in IPA (2 mL) was charged 1 M NaOH (1 mL). The reaction mixture was stirred at ambient temperature for 4 d. Upon the completion indicated by TLC analysis, the IPA was removed *in vacuo*. The resulting slurry was acidified with 2 M HCl and extracted with EtOAc. The combined extracts were washed with 10% NaCl (2 x 10 mL) dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, acetone/DCM) to afford the title compound (19 mg, 27%).

pK_a 4.0; ¹H NMR (400 MHz, CD₃OD) δ 4.01-3.98 (m, 1H), 2.46-2.40 (m, 1H), 2.33-2.13 (m, 1H), 2.09-1.14 (m, 22H), 1.06 (d, *J* = 6.2 Hz, 3H), 1.01 (s, 3H), 0.75 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -84.6 (d, *J* = 240.6 Hz), -102.3 (d, *J* = 240.6 Hz).

20 **General Procedure for the formation of 7,7-difluoro 24-norlithocholic acid conjugates**

Compound 1 (1 equiv.) was dissolved in THF (25 vol) and cooled to 0 °C. Ethyl chloroformate (1.2 eq) was added followed by triethylamine (1.2 eq) and the reaction stirred for 1 h at 0 °C. After complete conversion of the starting material by TLC a solution of amine (1.5 eq) and NaHCO₃ (1.5 eq) in H₂O (25 vol) is added in one portion then stirred 0 °C for 2 h or until complete by TLC. Upon completion the mixture is concentrated under reduced pressure, diluted with H₂O and acidified to pH<2 with 2M HCl (aq.). The suspension was extracted (ethyl acetate or THF; x3); the organic layers were combined and washed with 10% NaCl, dried over magnesium sulphate and concentrated to afford a crude residue. The crude material was purified via column chromatography.

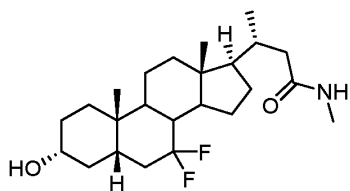
Example 30 – Synthesis of *N*-(3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oyl)-(morpholine) (Compound 33)



Using the general procedure 7,7-difluoro 24-norlithocholic acid (60 mg, 0.12 mmol) was conjugated and purified by column chromatography (SiO₂, 7:2:1 (ethyl acetate: MeOH: H₂O) in ethyl acetate) to yield the title compound (16.2 mg, 29%).

pK_a 14.3; ¹H NMR (400 MHz, CDCl₃) δ 3.67-3.59 (m, 6H), 3.58-3.51 (m, 1H), 3.49-3.45 (m, 2H), 2.39-2.34 (m, 1H), 2.16-1.02 (m, 23H), 0.98 (d, *J* = 5.9 Hz, 3H), 0.94 (s, 3H), 0.71 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.4 (d, *J* = 240.5 Hz), -101.2 (d, *J* = 240.5 Hz).

Example 31 – Synthesis of *N*-(3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oyl)-(methylamine) (Compound 34)



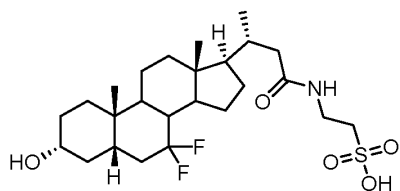
Using the general procedure 7,7-difluoro 24-norlithocholic acid (60 mg, 0.12 mmol) was conjugated and purified by column chromatography (SiO₂, 7:2:1 (ethyl acetate: MeOH: H₂O) in ethyl acetate) to yield the title compound (28.3 mg, 57%).

pK_a 12.5; ¹H NMR (400 MHz, CDCl₃) δ 5.42 (d, *J* = 3.6 Hz, 1H), 3.58-3.50 (m, 1H), 2.80 (d, *J* = 4.9 Hz, 3H), 2.40-2.35 (m, 1H), 2.23-1.03 (m, 23H), 0.97 (d, *J* = 6.5 Hz, 3H), 0.95 (s, 3H), 0.71 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CDCl₃) δ -84.4 (d, *J* = 240.1 Hz), -101.2 (d, *J* = 240.1 Hz).

Comparative Example 32 – Preparation of Comparative Compounds B to G

The General Procedure for the formation of conjugates was used for the synthesis of the following compounds.

***N*-(3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-amide)-ethylsulfonic acid
(Comparative Compound B)**

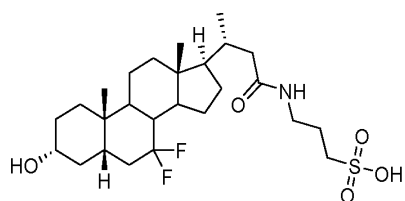


Using the general procedure 7,7-difluoro 24-norlithocholic acid (35 mg, 0.09 mmol) was
5 conjugated to yield Comparative Compound B as a white powder (15.6 mg, 34%).

^1H NMR (400 MHz, DMSO- d_6) δ 7.66 (t, J = 5.5 Hz, 1H), 6.82 (br. s, 1H), 3.28-3.18 (m, 2H),
3.10 (qd, J = 7.3, 4.8 Hz, 1H), 2.58-2.52 (m, 2H), 2.20-2.06 (m, 1H), 1.95 (d, J = 12.3 Hz, 1H),
1.87-1.20 (m, 15H), 1.20-0.96 (m, 7H), 0.91 (s, 3H), 0.87 (d, J = 6.4 Hz, 3H), 0.66 (s, 3H); ^{19}F
{ ^1H } NMR (376 MHz, DMSO- d_6) δ -82.6 (d, J = 237.6 Hz), -99.9 (d, J = 237.6 Hz).

10

***N*-(3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-amide)-propylsulfonic acid
(Comparative Compound C)**

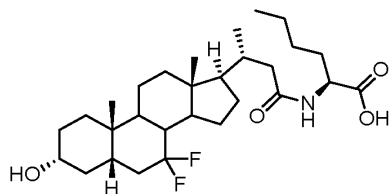


Using the general procedure 7,7-difluoro 24-norlithocholic acid (35 mg, 0.09 mmol) was
15 conjugated to yield Comparative Compound C as a white powder (29.1 mg, 62%).

^1H NMR (400 MHz, CD $_3$ OD) δ 3.53-3.42 (m, 1H), 3.41-3.33 (m, 2H), 3.24 (t, J = 6.7 Hz, 1H),
2.94-2.80 (m, 3H), 2.50-2.37 (m, 1H), 2.29-2.11 (m, 1H), 2.11-1.06 (m, 22H), 1.01-0.96 (m,
6H), 0.76 (s, 3H); ^{19}F { ^1H } NMR (376 MHz, CD $_3$ OD) δ -85.1 (d, J = 241.0 Hz), -102.4 (d, J =
241.0 Hz).

20

***N*-(3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-amide)-((*S*)-hexanoic acid
(Comparative Compound D)**

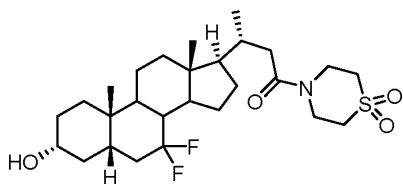


Using the general procedure 7,7-difluoro 24-norlithocholic acid (35 mg, 0.09 mmol) was
25 conjugated to yield Comparative Compound D as a white powder (19.3 mg, 42%).

¹H NMR (400 MHz, CD₃OD) δ 4.38 (dd, *J* = 9.0, 4.9 Hz, 1H), 4.14-4.05 (m, 1H), 3.55-3.42 (m, 1H), 2.43-2.21 (m, 1H), 2.31-2.11 (m, 1H), 2.10-2.03 (m, 1H), 2.04-1.49 (m, 10H), 1.47-1.09 (m, 15H), 1.05 (d, *J* = 5.6 Hz, 3H), 1.02 (s, 3H), 1.00-0.93 (m, 4H), 0.79 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.2 (dd, *J* = 240.6, 12.6 Hz), -102.4 (dd, *J* = 240.6, 15.1 Hz).

5

***N*-(3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-oyl)-((1,1)-dioxidothiomorpholine)
(Comparative Compound E)**



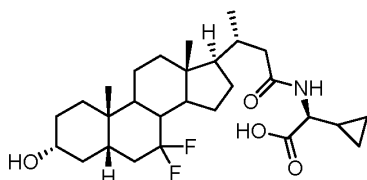
Using the general procedure 7,7-difluoro 24-norlithocholic acid (35 mg, 0.09 mmol) was conjugated to yield Comparative Compound E as a white powder (7.4 mg, 16%).

10

¹H NMR (400 MHz, CD₃OD) δ 4.09-3.98 (m, 4H), 3.53-3.43 (m, 1H), 3.18 (br. d, *J* = 21.0 Hz, 4H), 2.58 (dd, *J* = 15.2, 3.2 Hz, 1H), 2.38-1.51 (m, 15H), 1.51-1.09 (m, 8H), 1.02-0.97 (m, 6H), 0.81 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.2 (d, *J* = 241.0 Hz), -102.4 (d, *J* = 240.9 Hz).

15

***N*-(3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-amide)-((S)-2-cyclopropylacetic acid)
(Comparative Compound F)**



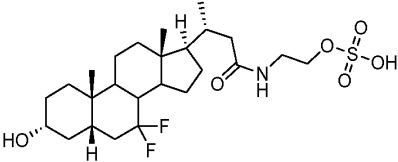
Using the general procedure 7,7-difluoro 24-norlithocholic acid (35 mg, 0.09 mmol) was conjugated to yield Comparative Compound F as a white powder (4.5 mg, 10%).

20

¹H NMR (400 MHz, CD₃OD) δ 3.71-3.66 (m, 1H), 3.52-3.42 (m, 1H), 2.38-2.05 (m, 3H), 1.93-1.24 (m, 15H), 1.16-1.02 (m, 3H), 0.97 (d, *J* = 5.8 Hz, 3H), 0.95 (s, 3H), 0.90-0.74 (m, 4H), 0.71 (s, 3H), 0.66-0.39 (m, 3H), 0.38-0.24 (m, 1H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.2 (dd, *J* = 241.0, 7.0 Hz), -102.4 (dd, *J* = 241.0, 7.0 Hz).

25

N-(3α-hydroxy-7,7-difluoro-5β-24-nor-cholan-23-oyl)-(2-aminoethyl hydrogen sulfate)
(Comparative Compound G)



Using the general procedure 7,7-difluoro 24-norlithocholic acid (35 mg, 0.09 mmol) was conjugated to yield Comparative Compound G as a white powder (18.2 mg, 39%).

¹H NMR (400 MHz, CD₃OD) δ 3.51-3.43 (m, 1H), 3.47 (t, *J* = 5.5 Hz, 2H), 3.38 (t, *J* = 5.6 Hz, 2H), 2.38 (dd, *J* = 13.2, 3.2 Hz, 1H), 2.31-2.09 (m, 1H), 2.09-2.03 (m, 1H), 1.97-1.47 (m, 9H), 1.44-1.02 (m, 12H), 1.02-0.93 (m, 6H), 0.75 (s, 3H); ¹⁹F {¹H} NMR (376 MHz, CD₃OD) δ -85.2 (d, *J* = 241.0 Hz), -102.4 (d, *J* = 241.0 Hz).

Biological Examples

Biological Example 1 – Biological Activity of Compounds in Alzheimer’s Disease Cells

Cell Methods

Cell lines used were obtained from the Coriell cell repository (4 individual PSEN1 mutant patient lines, PSEN1A, PSEN1B and PSEN1D and PSEN1E), and all show reductions in mitochondrial membrane potential (MMP; 25% reduction), increased percentage of long mitochondria (5% change) and a more fused mitochondrial network. Characterisation of these lines is described in Bell et al, 2018. Details of the mutations are provided below.

PSEN1 Mutation	Lines	Average Age of Onset	Notes	References
PRO264LEU	PSEN1A	Mid 40s - Mid 50s	High level of clinical variability, often atypical of AD Abolishes recognition site for cyclophilin B, required for maturation of PSEN1 and can lead to PSEN1 aggregation	Campion (1995), Raux (2005), Jacquemont (2002), Dumanchin (2006), Ben-Gedalya (2015)
Intron 4 deletion	PSEN1B	30s – Early 40s	Death in 40s Affects Notch signalling, can lead to premature neurogenesis	Tysoe (19980; De Jonghe (1999), Arber (2019; 2021)
ALA246GLU	PSEN1D PSEN1E	Early 50s	Accumulation of progranulin in the brain	Gliebus (2009), Podvin (2021),

			Causes disruption to endosomes May cause more aggressive tau pathology Can lead to premature neuronal differentiation Affects calcium dynamics Most well studied in patient derived cells and transgenic mouse models	Hook (2023), Yang (2017), Vanova (2023), Nelson (2007)
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When confluent, cells were plated into 1536 well plates, at a density of 200 cells per 5µl of media per well. Cells were drugged 24 hours after plating, using the Echo® 550 Liquid Handling System (Labcyte). This is an automated system, which uses acoustic droplet ejection, by which sound waves are used to move very small volumes of liquid without any physical contact. This increases precision and accuracy, whilst also decreasing the chance of contamination (Q. Guo et al., 2021). For the primary screen, cells were drugged with each compound at 2 concentrations (100nm and 1µM) in triplicate technical repeats, repeated twice on one 1536 well plate for two different cell lines.

To carry out the assay, cells were incubated for one hour with a working solution of 80nM tetramethylrhodamine, methyl ester (TMRM; Invitrogen), 1µM MitoTracker Green (Invitrogen) and 10µM Hoechst (Sigma) in phenol red free minimum essential media (MEM; Gibco by ThermoFisher Scientific). Immediately before imaging, wells were washed with 5µl MEM. Cells were imaged using the Opera Phenix® (Revvity). A total of 5 fields of view, approximately 20 cells per well, were imaged per well for 1536 well plates, using the using the Cy3 channel (excitation 645/30; emission 705/72), AlexaFluor 488 (excitation 488/emission 525) and the DAPI channel (excitation 350/50; emission 455/50). Exposure time was optimised for each plate.

Images were analysed using Harmony® software (Revvity), to obtain a range of mitochondrial parameters. Images were segmented to show the nuclei, cells, and individual mitochondria. As a quality control measure, any well with a nuclei count of less than 12 was excluded. As a functional parameter, mitochondrial membrane potential, based on the intensity of TMRM staining and normalised to cell area, was assessed. Furthermore, morphological parameters were calculated including the percentage of long mitochondria.

For dose response data, a 9-point dose response was carried out, including the following concentrations: 1nm, 3nm, 10nm, 30nm, 100nm, 300nm, 1µM, 3µM and 10µM. Each condition was repeated in four technical repeats.

The assay and analysis were carried out as previously described using the Opera Phenix® and Harmony® analysis software. EC₅₀ values were calculated using GraphPad prism software using nonlinear regression analysis.

5

Compound 1 and Comparative Compound A were tested in cell lines PSEN1A, PSEN1B, PSEN1D and PSEN1E; Comparative Compounds B to G were tested in cell lines PSEN1A and PSEN1B; and the remaining compounds were tested in cell lines PSEN1A, PSEN1D and PSEN1E.

10

Results

The results from the primary screen and the dose response are set out in Table 1 below. In Table 1, mean values in the primary screen over the cell lines tested are provided for each compound for both MMP and Long Mitochondria data. The columns headed “Activity” represent the EC₅₀ in the dose response assays for MMP and Long Mitochondria. In Table 1, the activity codes, AA, A1 and NA are used and the meanings for the activity codes are as follows:

15

- AA Consistent activity 2 or 3 cell lines
- A1 Active in one cell line or active in multiple cell lines but at only 1 or 2 concentrations
- 20 NA Not active

Table 1 – Primary Screen and Dose Response in MMP and Long Mitochondria Assays

Compound	MMP		Long Mitochondria	
	Mean Max %	Activity	Mean Max %	Activity
	Inc		Change	
A	15.4	AA	4.35	AA
B	3.2	NA	2.3	NA
C	3.1	NA	1.47	NA
D	-2.5	NA	3.57	NA
E	8.4	NA	2.27	NA
F	-0.03	NA	1.3	NA
G	1.7	NA	3.17	NA
1	40	AA	5.41	AA
1 methyl ester	9.37	NA	3.53	AA

Compound	MMP		Long Mitochondria	
	Mean Max %	Activity	Mean Max %	Activity
	Inc		Change	
2	15.4	A1	1.92	AA
4	22.77	A1	2.89	AA
3	14.97	AA	3.14	AA
5	28.3	AA	3.38	
6	17.77		3.44	AA
7	13.1	A1	1.72	A1
8	13.07	AA	3.53	AA
9	18.7	A1	9.1	AA
10a	12.23	A1	9.2	AA
10b	0.73	NA	7.6	AA
11	6.6	NA	7.4	AA
12	13.87	AA	4.7	AA
13	2.67	NA	3.77	AA
14	14.77	A1	6.8	AA
15	7.5	NA	4.07	AA
16	1.33	NA	3.33	A1
17	3.17	NA	1.54	A1
18a	6.47	NA	3.5	A1
18b	4.03	NA	4.1	A1
19a	29.7	AA	9.5	AA
19b	13.5		10.27	AA
20	17.53	A1	3.97	A1
21a	8.63	NA	11.03	AA
21b	6.37	NA	6.53	AA
22a	18.13	A1	9.5	AA
22b	24.3	A1	7	AA
23	-4.7	NA	5.1	AA
24	19.4	A1	8.2	AA
25	-0.57	NA	3.3	A1
26	3.29	NA	4.27	AA
27	12.73	A1	8.27	AA
28	12.37		7.7	AA

Compound	MMP		Long Mitochondria	
	Mean Max %	Activity	Mean Max %	Activity
	Inc		Change	
29	17.67	A1	6.1	AA
30	14.03	A1	8.23	AA
32	14.37	A1	8.13	AA
33	11.97	A1	3.5	A1
34	19.2	A1	4.27	AA

In the primary screen, % MMP is an indication of mitochondrial function. If mitochondrial function is lower than control levels, the mitochondria will function less efficiently. A substantial reduction in MMP may mean that the mitochondria are sufficiently dysfunctional to cause cell damage. Since the cell lines used in the assay show a reduction in MMP of 25%, an increase in MMP of 25% will restore the MMP in the PSEN1 mutant patient cell lines to that of controls.

The % long mitochondria is a measurement of mitochondrial morphology. Healthy mitochondria vary in size and shape depending on their environment and a mixture of different morphologies is found in healthy cells. The increase in the percentage of long mitochondria in the PSEN1 mutant patient lines indicates that the balance of different mitochondrial morphologies has been upset. Since the cell lines used show a 5% increase in long mitochondria, a reduction of 5% in % long mitochondria in will restore the patient phenotype to that of controls.

The results of the primary screen indicate that treatment with Comparative Compound A or with many of the Compounds of the invention elevates MMP to levels approaching those in controls, indicating that mitochondrial function is significantly improved. Treatment with Compound 1 is particularly effective. Treatment with Comparative Compounds B to G does not elevate MMP to such levels and so these compounds do not significantly improve mitochondrial function.

In the primary screen, Compound A and the compounds of the invention appear to restore the balance of mitochondrial morphology, whereas Comparative Compounds B to G do not, restore the balance mitochondrial morphology to any great extent.

In the concentration response assay, the results for MMP show that Comparative Compound A and a number of the compounds of the invention gave a response of AA. Compounds 1, 3,

5, 8, 9b, 12 and 19a were all particularly active in the concentration response assay and were consistently active in 2 or 3 of the cell lines tested.

5 The dose response assay results for long mitochondria show that Compound A and the majority of the compounds of the invention have an activity rating of AA. The remaining compounds of the invention have an activity rating of A1, indicating that either they are active in only one of the Alzheimer's disease cell lines tested or that they are active in multiple cell lines but at only one or two concentrations.

10 It is important to consider both the MMP and the long mitochondria data, since some compounds which do not appear to be active in the MMP screen may be highly active in the long mitochondria screen. This is an indication that these compounds will be effective in restoring mitochondrial morphology, which, in turn, is an indication that they are likely to have a positive effect on mitochondrial membrane potential but over a different timescale or a
15 different dose to that tested.

It is worth noting, however, that Comparative Compounds B to G are not active in the primary screen either for MMP or for long mitochondria. This is an indication that the nature of the R² substituent has considerable influence on the activity of the compounds.

20

Biological Example 2 – Biological Activity in Parkinson's Disease

Parkinson's Disease Cell Methods

Fibroblasts from sporadic PD patients were grown as described in Carling et al, 2020.

25

ATP Protocol

The ATP protocol is generally as described in Mortiboys *et al* 2008. Briefly, fibroblasts were cultured as and plated into white 384 well plates at a concentration of 5000 cells per well in 50 µl of media volume. The plates are left overnight in an incubator to allow the fibroblasts to
30 adhere to the plate surface. The following morning the Glucose based medium is replaced with 25µl of Galactose based medium. The plates were then spiked with the compounds using a ECHO 550 liquid handling system. The wells were dosed to provide an 8-point concentration range of 1-10000nM of compound. After dosing the wells are topped up with a further 25µl of Galactose based medium and then left in an incubator for 24 hours. Following this incubation,
35 the medium is removed from the plate and the wells are washed twice with sterile PBS. The wells are filled with 25µl of Sterile PBS followed by 12.5µl of Lysis solution from the ATPlite™ Luminescence ATP detection assay system (Perkin Elmer), including 16 cell free wells to use

as blank controls. The plate is then placed on a rotary shaker for 5 mins at 700 rpm. Following the shaking 12.5µl of ATP substrate solution (Perkin Elmer) is added to each well and a further 5 min of shaking. The plate is then placed in darkness for 10 minutes prior to reading. Using a PHERAStar® plate reader, luminescence intensity is recorded. The Results are shown in Table 3.

Table 3 – Dose Response in Parkinson’s Disease Fibroblasts

Compound	ATP levels		
	Min	Max	EC ₅₀ nM
1	75	139.4	17.9

The data in sporadic Parkinson’s Disease patient fibroblasts suggest Compound 1 to have an excellent profile increasing mitochondrial function in these cells with high potency. This effect is similar to other reported bile acid derivatives, for example the compounds described in WO2020/128514 and PCT/GB2023/051450, performing better than UDCA in the same cells and assays.

Biological Example 3 – MDCK-MDR1 Permeability Assay

A. Assay 1

Compounds of the invention, along with comparative Compound A and UDCA were tested in an MDCK-MDR1 permeability assay to investigate CNS permeability and efflux ratio.

MDCK-MDR1 cells are derived from Madin Darby canine kidney (MDCK) cells and have been transfected with the *MDR1* gene, which encodes the efflux protein, P-glycoprotein (P-gp).

MDR1-MDCK cells were seeded into 24 well Transwell™ plates and cultured for 3 days to form a confluent monolayer . The test compound was prepared at a concentration of 10 µM in Hanks’ Balanced Salt Solution containing 25 mM HEPES and loaded into the donor compartments of the Transwell™ plates bearing the cell monolayers (pH 7.4 for both donor and receiver compartments). Lucifer Yellow was added to the apical buffer in all wells to assess integrity of the cell monolayer. Duplicate wells were prepared and incubated at 37°C in a CO₂ incubator. Samples were removed at times 0 and 60 minutes and the test compound analysed by LC-MS/MS. Concentrations of Lucifer Yellow in the samples were measured using a fluorescence plate reader. The apparent permeability (P_{app}) values of the test compound were determined both in the apical to basolateral (A-B) direction, representing transport of the compound from the plasma to the CNS, and in the basolateral to apical (B-A) direction,

representing efflux from the CNS. The permeability coefficient P_{app} in each direction may be calculated from the following equation.

$$P_{app} = \left(\frac{dQ/dt}{C_0 \times A} \right)$$

- 5 where
- dQ/dt is the rate of permeation of the drug across the cells;
- C_0 is the concentration of the test compound at time zero and A is the area of the cell monolayer; and
- A is the area of the cell monolayer.

- 10 The efflux ratio was calculated from using the permeability coefficients in each direction:

$$\text{Efflux Ratio} = \frac{P_{app \text{ (B-A)}}}{P_{app \text{ (A-B)}}$$

Percent recovery data was also reported.

- 15 The assay was conducted using two replicates for each of the A-B and B-A measurements and the % recovery of the compounds was also calculated in order to ensure the validity of the results.

The results for Compound 1, Comparative Compound A and UDCA are presented in Table 4.

20

Table 4

Compound	$P_{app \text{ (A-B)}}$ Mean	$P_{app \text{ (B-A)}}$ Mean	Mean Efflux Ratio	Recovery A-B	Recovery B-A
Compound 1	49.6	59	1.2	100	81
Compound 2	51.0	44	0.9	98	94
Comparative Compound A	58.4	63	1.1	104	87
UDCA	20.6	75	3.6	96	92

As noted above, for an effective concentration of the compound in the CNS to be achieved, the efflux ratio should preferably be less than 2.5 (Doan, 2002), The results indicate that while

- 25 Compound 1, Compound 2 and Comparative Compound A all have an acceptable efflux ratio,

the efflux ratio for UDCA is too high for it to be present in a pharmaceutically effective concentration in the CNS.

B. Assay 2

- 5 A further assay was carried out. The results are not directly comparable with Assay 1 because a different assay protocol was used.

Cell Culture

- MDR1-MDCK I cells were seeded onto polycarbonate membrane (PC) in 96-well Corning
10 insert systems at 4.44×10^5 cells/ mL and cultured for 4-7 days for confluent cell monolayer formation.

Experimental Procedures

- The transport buffer in the study was HBSS with 10.0 mM HEPES at pH 7.40 ± 0.05 . Test
15 compounds were tested at 2.00 μ M bi-directionally in duplicate. Digoxin was tested at 10.0 μ M bi-directionally in duplicate, while nadolol and metoprolol were tested at 2.00 μ M in A to B direction in duplicate. Final DMSO concentration was adjusted to less than 1%. The plate was incubated for 1.5 hours in CO₂ incubator at $37 \pm 1^\circ\text{C}$, with 5% CO₂ at saturated humidity without shaking. And all samples after mixed with acetonitrile containing internal standard were
20 centrifuged at $3220 \times g$ for 10 min. For all samples, 150 μ L supernatant solution was diluted with 150 μ L ultra-pure water for LC-MS/MS analysis. In addition, the efflux ratio of each compound was also determined. Test and reference compounds were quantified by LC-MS/MS analysis based on the peak area ratio of analyte/IS.

- 25 After transport assay, Lucifer yellow rejection assay was applied to determine the cell monolayer integrity. Buffers are removed from both apical and basolateral chambers, followed by the addition of 75 μ L of 100 μ M lucifer yellow in transport buffer and 250 μ L transport buffer in apical and basolateral chambers, respectively. The plate was incubated for 30 minutes at 37°C with 5% CO₂ and 95% relative humidity without shaking. After 30 minutes incubation, 20
30 μ L of lucifer yellow samples were taken from the apical sides, followed by the addition of 60 μ L of Transport Buffer, and then 80 μ L of lucifer yellow samples were taken from the basolateral sides. The relative fluorescence unit (RFU) of lucifer yellow was measured at 425/528 nm (excitation/emission) with a microplate reader.

35 Data Analysis

The apparent permeability coefficient Papp (cm/s) was calculated using the equation:

$$P_{app} = \frac{\left(\frac{dC_r}{dt}\right) \times V_r}{(A \times C_0)}$$

where:

$\frac{dC_r}{dt}$ is the cumulative concentration of compound in the receiver chamber as a function of time ($\mu\text{M/s}$);

5 V_r is the solution volume in the receiver chamber (0.075 mL on the apical side, 0.25 mL on the basolateral side);

A is the surface area for the transport, i.e. 0.143 cm^2 for the area of the monolayer;

C_0 is the initial concentration in the donor chamber (μM).

10 The efflux ratio was calculated using the equation:

$$\text{Efflux Ratio} = \frac{P_{app(B-A)}}{P_{app(A-B)}}$$

% recovery was calculated using the equation:

$$\% \text{ Solution Recovery} = 100 \times \frac{[(V_r \times C_r) + (V_d \times C_d)]}{(V_d \times C_0)}$$

15 where:

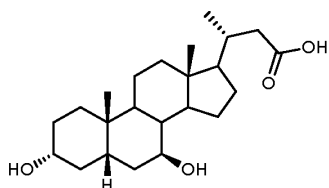
V_r and C_0 are defined as above;

V_d is the volume in the donor chambers (0.075 mL on the apical side, 0.25 mL on the basolateral side);

C_d and C_r are the final concentrations of transport compound in donor and receiver chambers,

20 respectively.

In this assay, compounds of the invention were compared with nor UDCA, which has the structure:



25 This is similar to Compound 1 of the invention but has a $7\beta\text{-OH}$ substituent rather than the 7,7-difluoro substitution pattern seen in the compounds of the invention. The mean efflux ratio was determined for each test compound, where efflux ratio is defined as:
and the results are shown below in Table 5.

30 In this assay, the most suitable compounds have an efflux ratio of less than 4.0

Table 5

Compound	P _{app} (A-B) Mean	P _{app} (B-A) Mean	Mean Efflux Ratio	Recovery A-B	Recovery B-A
Nor UDCA	1.1	15.5	14.2	110	112.6
3	5.22	18.1	3.46	55.6	63.6
4	0.398	9.43	23.7	80.7	93.6
5	0.111	2.04	18.3	78.9	92.6
6	<0.36	10.8	>30.1	<82%	91.9
7	11.4	27.4	2.4	80.2	88.6
17	0.342	23.7	69.3	78.2	92.2
26	9.08	34.3	3.78	72.4	83.7
32	9.64	36.2	3.75	74.7	88.8

Although Compounds 4, 5, 6 and 17 have high efflux ratios, the data set out in Biological Example 5 demonstrate that they are able to achieve high concentrations in plasma in comparison with Comparative Compound A.

Biological Example 4 - Phamacokinetic Data (Plasma)

10 The test compositions were prepared by dissolving the test compounds in an amount of 20% w/v in a solvent comprising DMSO and HP-β-CD in a volume ratio of 5/95.

The test compositions were administered to male C57 mice. In a first leg, each test composition was administered intravenously in an amount of 1.00 mg/kg to three mice and blood sampling was carried out at 5 minutes (0.0833 hours), 0.25 hours, 1 hour, 2 hours, 4 hours, 7 hours and 24 hours after administration for Compound 1 and Compound A and 0.25 hours, 0.5 hours, 1 hour, 2 hours, 4 hours, 8 hours and 24 hours after administration for the other test compounds. In a second leg, each test composition was administered orally in an amount of 3.00 mg/kg to three mice and blood sampling was carried out at 0.25 hours, 0.5 hours, 1 hour, 2 hours, 4 hours, 7 hours and 24 hours after administration for Compound 1 and Compound A and 0.25 hours, 0.5 hours, 1 hour, 2 hours, 4 hours, 8 hours and 24 hours after administration for the other test compounds. The concentration of each test compound was determined at each sampling timepoint.

The three mice used for IV administration of Comparative Compound A were given reference numbers 13, 14 and 15 and the three mice used for oral administration of Comparative Compound A were given reference numbers 16, 17 and 18.

- 5 The three mice used for IV administration of Compound 1 were given reference numbers 19, 20 and 21 and the three mice used for oral administration of Compound 1 were given reference numbers 22, 24 and 24.

- 10 Tables 6 and 7 shows the results for all compounds after IV administration and Figures 1 and 2 each illustrate the results for three individual mice treated with Compound 1 (Figure 1) or Comparative Compound A (Figure 2).

- 15 Tables 8 and 9 show the results for all compounds following oral administration and Figures 3 and 4 each illustrate the results for three individual mice treated respectively with Compound 1 (Figure 3) and Comparative Compound A (Figure 4).

Results for Test Compounds administered by IV bolus (0.2 mg/mL; nominal dose 1.0 mg/kg)

Table 6 – Mean Plasma Concentration

Time (h)	Mean Plasma concn. (ng/mL)									
	Compound A	Compound 1	Compound 3	Compound 4	Compound 5	Compound 6	Compound 7	Compound 17		
0.083	789 ± 217	4205 ± 237	5044 ± 367	3754 ± 192	1424 ± 75.0	1998 ± 119	4177 ± 219	1396 ± 109		
0.25	234 ± 64.1	3582 ± 626	1927 ± 175	2775 ± 49.4	836 ± 29.6	1237 ± 97.1	2178 ± 123	955 ± 101		
0.5			750 ± 219	2357 ± 294	622 ± 24.4	1009 ± 46.4	1219 ± 322	628 ± 141		
1	17.1 ± 4.52	2950 ± 396	120 ± 72.5	1965 ± 95.9	402 ± 72.0	862 ± 67.5	381 ± 136	234 ± 99.0		
2	15.5 ± 11.7	2422 ± 287	256 ± 153	1277 ± 126	167 ± 20.7	594 ± 37.6	198 ± 61.5	69.2 ± 9.20		
4	BLQ	1237 ± 176	180 ± 8.0	697 ± 115	56.6 ± 11.3	310 ± 34.0	201 ± 32.2	56.6 ± 15.1		
8 (7)*	(BLQ)	(840 ± 33.5)	143 ± 40.5	597 ± 45.7	51.0 ± 16.1	179 ± 33.0	194 ± 34.0	46.8 ± 11.7		
24	BLQ	255 ± 46.1	46.2 ± (SD not done)	154 ± 12.7	5.04 ± 0.618	26.3 ± 4.79	53.8 ± 14.0	7.13 ± 3.22		

* Concentrations of Compound A and Compound 1 measured at 7 hours, not 8 hours.

BLQ = below limit of quantification

Table 7 – Half-life and AUC

	Comp A	Comp 1	Comp 3	Comp 4	Comp 5	Comp 6	Comp 7	Comp 17
T _{1/2} (h)	0.176	6.30 ± 0.588	8.05 ± 2.99	8.98 ± 1.13	5.43 ± 1.01	5.66 ± 0.164	9.84 ± 1.47	6.25 ± 0.737
AUC _{0-inf} (ng.h/mL)	501	23397 ± 1464	4454 ± 1112	15908 ± 683	1792 ± 120	5230 ± 460	5679 ± 490	1583 ± 332

Results for Test Compounds administered per Oral (0.3 mg/mL; nominal dose 3.0 mg/kg)

Table 8 – Mean Plasma Concentration

Time (h)	Mean Plasma concn. (ng/mL)										
	Compound A	Compound 1	Compound 3	Compound 4	Compound 5	Compound 6	Compound 7	Compound 17			
0.083	477	4553 ± 778	1592 ± 484	131 ± 18.2	38.1 ± 23.1	121 ± 32.5	2980 ± 499	325 ± 135			
0.25	398	4804 ± 849	1777 ± 307	522 ± 189	115 ± 54.5	313 ± 63.2	3974 ± 385	646 ± 25.4			
0.5			977 ± 169	1683 ± 346	469 ± 31.9	759 ± 213	2775 ± 353	1233 ± 464			
1	251	7406 ± 720	273 ± 80.7	2104 ± 643	671 ± 21.0	1254 ± 469	761 ± 68.0	541 ± 214			
2	58.5	6312 ± 686	94.0 ± 10.3	1403 ± 474	370 ± 26.4	880 ± 390	260 ± 114	125 ± 133			
4	34.6	3634 ± 507	181 ± 36.4	854 ± 366	97.6 ± 22.2	309 ± 98.0	438 ± 92.9	70.4 ± 46.9			
8 (7)*	(62.4)	(2226 ± 157)	212 ± 138	1229 ± 688	75.4 ± 11.4	309 ± 87.8	730 ± 137	107 ± 44.1			
24	7.71	609 ± 72.3r	151 ± 11.4	168 ± 9 3	ND	24.8 ± 7.87	105 ± 7.31	3.05 ± 0.280			

* Concentrations of Compound A and Compound 1 measured at 7 hours, not 8 hours.
ND = not done

Table 9 – Half-life and AUC

	Comp A	Comp 1	Comp 3	Comp 4	Comp 5	Comp 6	Comp 7	Comp 17
T _{1/2} (h)	ND	7.53 ± 0.337	20.1 ± 6.24	7.41 ± 1.17	2.78 ± 0.466	4.86	8.28	3.98 ± 0.771
AUC _{0-inf} (ng.h/mL)	ND	57753 ± 3998	9401 ± 864	19731 ± 9346	1930 ± 326	6031 ± 1875	12154 ± 1172	2021 ± 664
Bioavailability (%)	ND	82.3 ± 5.7	43.8	41.3	35.9	38.4	71.3	42.4

ND = not done

It can be seen from Figures 1 to 4 and Tables 6 and 8 that at all timepoints sampled, the plasma concentration of Compounds 1, 3, 4, 5, 6, 7 and 17 is higher than that of Comparative Compound A when administered intravenously and at most timepoints sampled, the plasma concentration for these compounds is also higher than that of Comparative Compound A following oral administration.

Following IV administration of Comparative Compound A to Mice 13, 14 and 15, half-life was measured at 0.176 hours and AUC_{0-inf} was 207 ng.h/mL. Clearance was measured at 80.6 mL/min/kg.

In contrast, following IV administration of Compound 1, half-life was measured at 6.30 ± 0.588 hours and AUC_{0-inf} was 23397 ± 1464 ng.hr/mL. Clearance was measured at 0.714 ± 0.0464 mL/min/kg. Half-life and AUC_{0-inf} were also considerably higher for all the other example compounds than for Comparative Compound A.

Following PO administration of Compound A at 3.00 mg/kg to Mice 16, 17 and 18, a C_{max} of 497 ng/mL was measured at 0.250 h, C_{last} 7.71 ng/mL, and AUC_{0-t} of 1140 ng.hr/mL. Due to the irregularity of the PO profiles, $t_{1/2}$ and associated parameters could not be determined for Compound A.

Following PO administration of Compound 1 at 3.00 mg/kg a C_{max} of 7543 ± 522 ng/mL was measured at 1.00 h, C_{last} 609 ± 72.3 ng/mL, half-life 7.53 ± 0.337 and AUC_{inf} of 57753 ± 3998 ng.hr/mL. Bioavailability calculated using AUC_{0-inf} was measured at 82.3 ± 5.70 %.

Due to animal 17's concentrations being significantly lower than the other animals in the PO dose group and the consistency of the other animals profiles and data, animal 17 has been excluded from averages and PK calculations.

Compounds 3, 4, 5, 6, 7 and 17 also demonstrated significantly longer half-life and greater AUC_{0-inf} than Compound A when administered by IV bolus or orally.

These results clearly demonstrate the improved plasma half-life and bioavailability of Compound 1 and the other example compounds compared with Comparative Compound A. It is believed that this improvement arises because the shorter side chain of the compounds of the invention prevents recognition of the compounds by the enzymes which control conjugation of bile acids in the liver, leading to an inability of compounds of the invention to form salts or conjugates, which means that the compound passes to the blood rather than entering the

enterohepatic circulation. The significance of this is that compounds can only pass from the plasma through the blood brain barrier into the CNS, if they reach the blood. Compounds such as Comparative Compound A which are trapped to a significant extent in the enterohepatic circulation attain only low concentrations in the plasma and are therefore cannot cross the blood brain barrier in significant amounts.

Biological Example 5 – Pharmacokinetic data (CNS)

Preparation of Test Compositions

For each compound, test compositions 1 to 3 were prepared by the compound in amounts of 0.3 mg/mL, 1 mg/mL or 3 mg/mL in a solvent comprising:

- a) DMSO and
- b) HP- β -CD in water (20% w/v)

in a volume ratio a/b of 5/95.

The test compositions were administered orally to three groups of six male C57 mice at doses of 3.0 (G01), 10.0 (G02) and 30.0 (G03) mg/kg. For each group, levels of the compound were measured in plasma, brain and cerebrospinal fluid in three mice at 0.25 hours and in the other three mice at 0.5 hours.

The results for Compounds 1, 2 and are shown in Tables 10 and 11 below. For comparison, results for UDCA and nor UDCA are provided in Tables 12 and 13.

Table 10 – Results for Compound 1

Group	Time (h)	Mean Plasma concn. (ng/mL)	Mean Brain concn. (ng/g)	Mean CSF concn. (ng/mL)	Brain/Plasma ratio	CSF Plasma ratio
G01	0.25	10864 $\pm$ 1989	254 $\pm$ 19.2	27.8 $\pm$ 7.33	0.0237 $\pm$ 0.00287	0.00257 $\pm$ 0.000635
	0.5	13203 $\pm$ 1419	325 $\pm$ 40.5	41.4 $\pm$ 0.907	0.0248 $\pm$ 0.00452	0.00317 $\pm$ 0.000393
G02	0.25	31248 $\pm$ 2131	817 $\pm$ 52.0	94.7 $\pm$ 13.3	0.0262 $\pm$ 0.000241	0.00302 $\pm$ 0.000226
	0.5	39385 $\pm$ 5978	1207 $\pm$ 333	126 $\pm$ 25.5	0.0303 $\pm$ 0.00422	0.00318 $\pm$ 0.000198
G03	0.25	98605 $\pm$ 5151	3049 $\pm$ 208	283 $\pm$ 14.2	0.0310 $\pm$ 0.00325	0.00288 $\pm$ 0.000160

Group	Time (h)	Mean Plasma concn. (ng/mL)	Mean Brain concn. (ng/g)	Mean CSF concn. (ng/mL)	Brain/Plasma ratio	CSF Plasma ratio
	0.5	106350 ± 16632	3845 ± 730	374 ± 93.3	0.0367 ± 0.00916	0.00358 ± 0.00115

Table 11 – Results for Compound 2

Group	Time (h)	Mean Plasma concn. (ng/mL)	Mean Brain concn. (ng/g)	Mean CSF concn. (ng/mL)	Brain/Plasma ratio	CSF/ Plasma ratio
G01	0.25	2719 ± 321	152 ± 23.5	21.0 ± 8.66	0.0562 ± 0.00862	0.00797 ± 0.00405
	0.5	2587 ± 1937	102 ± 66.3	11.7 (SD not done)	0.0415 ± 0.00664	0.00507 (SD not done)
G02	0.25	11396 ± 2793	454 ± 155	38.0 (SD not done)	0.0393 ± 0.00376	0.00370 (SD not done)
	0.5	8338 ± 2996	299 ± 46.5	17.8 (SD not done)	0.0377 ± 0.00835	0.00189 (SD not done)
G03	0.25	39958 ± 10852	1636 ± 508	143 ± 108	0.0407 ± 0.00295	0.00342 ± 0.00217
	0.5	27808 ± 9904	1326 ± 221	55.7 ± 18.9	0.0514 ± 0.0192	0.00231 ± 0.00130

5

Table 12 – Results for nor UDCA

Group	Time (h)	Mean Plasma concn. (ng/mL)	Mean Brain concn. (ng/g)	Mean CSF concn. (ng/mL)	Brain/Plasma ratio	CSF/ Plasma ratio
G01	0.25	2008 ± 285	28.0 ± (SD not done)	5.58 ± 0.681	0.0149 ± (SD not done)	0.00281 ± 0.000484
	0.5	4895 ± 957	50.0 ± 13.0	9.89 ± 3.29	0.0102 ± 0.00129	0.00202 ± 0.000514
G02	0.25	9734 ± 501	102 ± 11.2	18.6 ± 5.28	0.0104 ± 0.000689	0.00191 ± 0.000538
	0.5	16701 ± 4928	153 ± 76.3	23.9 ± 5.50	0.00930 ± 0.00377	0.00150 ± 0.000498
G03	0.25	19990 ± 2662	203 ± 43.1	45.8 ± 7.27	0.0101 ± 0.00112	0.00230 ± 0.000314

Group	Time (h)	Mean Plasma concn. (ng/mL)	Mean Brain concn. (ng/g)	Mean CSF concn. (ng/mL)	Brain/Plasma ratio	CSF/ Plasma ratio
	0.5	34518 ± 4730	384 ± 41.4	118 ± 25.8	0.0112 ± 0.000529	0.00351 ± 0.00118

Table 13 – Results for UDCA

Group	Time (h)	Mean Plasma concn. (ng/mL)	Mean Brain concn. (ng/g)	Mean CSF concn. (ng/mL)	Brain/Plasma ratio	CSF/ Plasma ratio
G01	0.25	1675 ± 2504	BQL	BQL	not done	not done
	0.5	117 ± 26.3	BQL	BQL	not done	not done
G02	0.25	168 ± 40.1	BQL	BQL	not done	not done
	0.5	219 ± 96.4	BQL	BQL	not done	not done
G03	0.25	540 ± 216	BQL	BQL	not done	not done
	0.5	393 ± 131	BQL	BQL	not done	not done

Comparing Tables 12 and 13 shows that shortening side chain from UDCA to nor UDCA leads to a significant increase in plasma concentrations. It seems likely that this is because UDCA is trapped in the enterohepatic circulation whereas nor UDCA is not.

Comparing the results presented in Table 10 with those in Table 12 shows that Compound 1 achieves significantly higher brain/plasma and CSF/plasma ratios than nor UDCA. These two compounds are similar in structure except that in nor UDCA, the bile acid skeleton has OH at the 7-position, whereas in Compound 1, the bile acid skeleton has a 7,7-difluoro substitution pattern. This is confirmation that, as predicted by the efflux ratio determined in Biological Example 3, the 7,7-difluoro substitution has a significant effect on the ability of the molecule to achieve an effective concentration in the CNS. The plasma concentration achieved for nor UDCA is, in any case, lower than that achieved for Compound 1 and this, combined with the less favourable efflux ratio for nor UDCA leads to significantly lower concentrations of nor UDCA in the brain and CSF than can be achieved for Compound 1. As shown in Table 11, Compound 2 achieves slightly lower brain and CSF concentrations than Compound 1, although the levels are still significantly higher than those for nor UDCA. The brain/plasma and CSF/plasma ratios achieved by Compound 2 are similar to those achieved by Compound 1.

The results presented in Tables 10 and 11 show that, although the levels of Compounds 1 and 2 in the plasma were considerably higher than the levels in the brain and the CSF, both compounds were detected in tissue homogenates from the brain and the CSF at 15 minutes

and 30 minutes after oral dosing for all three doses given. The amounts detected in the brain homogenates were sufficient for pharmacological activity.

In summary, Biological Examples 1 and 2 demonstrate that the compounds of the invention
5 are active in cells from both Alzheimer's disease and Parkinson's disease patients. The compounds of the invention and Comparative Compound A are active in one or both MMP and long mitochondria in cells from Alzheimer's disease patients.

Biological Example 3 shows that UDCA has a high efflux ratio and consequently would not
10 be expected to remain in the CNS in sufficient quantities for it to be active. This is not the case for either Comparative Compound A or Compound 1.

However, although Comparative Compound A has a similar efflux ratio to Compound 1, Biological Example 4 demonstrates that it becomes trapped in the enterohepatic circulation
15 and is therefore not present in the plasma in sufficient levels for an effective amount of the compound to be available to enter the CNS. On the other hand, the bioavailability of Compounds 1, 3 to 7 and 17 is significantly greater than that of Comparative Compound A and they enter in the plasma in sufficient concentrations for them to be available to cross the blood brain barrier. The results for UDCA and nor UDCA support the theory that the longer
20 side chain present in UDCA and Compound A results in entrapment of the compounds in the enterohepatic circulation, whereas this is avoided by nor UDCA and Compounds 1 and 2, all of which have a shorter side chain.

Biological Example 5 demonstrates that Compounds 1 and 2 do indeed cross the blood brain
25 barrier and can both be found in both brain tissue and in CSF 15 minutes and 30 minutes after oral dosing in mice. This is not the case for nor UDCA even though the plasma concentrations achieved are comparable to those for Compounds 1 and 2. The inventors speculate that this is because nor UDCA lacks the 7,7-difluoro substitution pattern present in the compounds of the invention.

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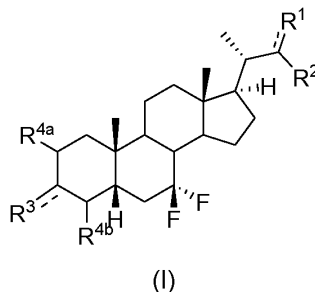
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CLAIMS

1. A compound of formula (I):



5 wherein:

each --- is independently a single or a double bond

when --- connected to R^1 is a single bond, R^1 is selected from H, fluoro, chloro, OH, NH_2 , N_3 , C_{1-4} alkyl, $\text{O}(\text{C}_{1-4}$ alkyl), $\text{NH}(\text{C}_{1-4}$ alkyl), $\text{C}(\text{O})\text{OH}$, $\text{C}(\text{O})\text{NHR}^{1a}$ and $\text{NHC}(\text{O})\text{OR}^{1b}$;

10 wherein when R^1 is C_{1-4} alkyl or $\text{O}(\text{C}_{1-4}$ alkyl), it is optionally substituted with one or more substituents selected from fluoro, chloro, OH, NH_2 , N_3 , $\text{O}(\text{C}_{1-4}$ alkyl) and $\text{NH}(\text{C}_{1-4}$ alkyl);

wherein each of R^{1a} and R^{1b} is independently H or C_{1-4} alkyl optionally substituted with halo;

when --- connected to R^1 is a double bond, R^1 is selected from CHR^8 and O;

15 wherein R^8 is H or CH_3 ;

R^2 is selected from $\text{C}(\text{O})\text{OR}^5$, C_{1-6} alkyl, C_{2-6} alkenyl and $\text{C}(\text{O})\text{N}(\text{R}^{5a})(\text{R}^{5b})$;

wherein R^5 is H, C_{1-4} alkyl or benzyl;

each of R^{5a} and R^{5b} is H or C_{1-4} alkyl or R^{5a} and R^{5b} together with the nitrogen atom to which they are attached form a piperidine, pyrrolidine, piperazine or morpholine ring;

20 and

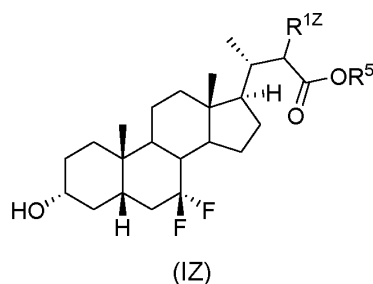
wherein alkyl and alkenyl groups of R^2 are optionally substituted with one or more substituents selected from OH, NH_2 , N_3 , fluoro and chloro;

when --- connected to R^3 is a single bond, R^3 is OH;

when --- connected to R^3 is a double bond, R^3 is O;

25 each of R^{4a} and R^{4b} is independently H, F, Cl, C_{1-2} alkyl or C_{1-2} haloalkyl; or a salt or solvate thereof.

2. A compound or a salt or solvate thereof according to claim 1 which is a compound of formula (IZ):



wherein:

R^{1Z} is selected from H, fluoro, chloro, OH, C₁₋₄ alkyl and O(C₁₋₄ alkyl), wherein when R^{1Z} is C₁₋₄ alkyl it is optionally substituted with one or more substituents selected from fluoro, chloro, OH and O(C₁₋₄ alkyl);

R⁵ is H, C₁₋₆ alkyl or benzyl;
or a salt or solvate thereof.

10 3. A compound or a salt or solvate thereof according to claim 1 which is an isotopic variant
in which:

in an alkyl group R¹, one or more hydrogen atoms are present as ²H (deuterium) or ³H (tritium), especially deuterium; and/or

when  connected to R³ is a single bond, the hydrogen atom at the 3-position of the bile acid skeleton is replaced by ²H (deuterium) or ³H (tritium), especially deuterium.

4. A compound or a salt or solvate thereof according to claim 1 or claim 3 wherein  connected to R¹ is a single bond.

20 5. A compound or a salt or solvate thereof according to claim 4 wherein R¹ is selected from H, fluoro, chloro, OH, NH₂, N₃, C₁₋₄ alkyl, O(C₁₋₄ alkyl), NH(C₁₋₄ alkyl) and C(O)OH.

6. A compound or a salt or solvate thereof according to claim 5 wherein R¹ is H.

25 7. A compound or a salt or solvate thereof according to claim 4 wherein R¹ is C₁₋₄ alkyl which is unsubstituted or substituted with one or more substituents selected from fluoro, chloro, OH, NH₂, N₃, and O(C₁₋₄ alkyl), especially fluoro, chloro, OH, NH₂ and methoxy.

8. A compound or a salt or solvate thereof according to claim 7 wherein R¹ is methyl,
ethyl, CH₂OH, CH₂F, CHF₂, CH₂OCH₃, CH₂NH₂ or CD₃.

9. A compound or a salt or solvate thereof according to claim 4 wherein:

R¹ is fluoro, chloro or azido (N₃); or

R¹ is OH, NH₂, O(C₁₋₄ alkyl) or NH(C₁₋₄ alkyl); or

R¹ is C(O)OH; or

R¹ is C(O)NHR^{1a} or NHC(O)OR^{1b} wherein R^{1a} and R^{1b} are each independently selected from

5 H and methyl.

10. A compound or a salt or solvate thereof according to claim 1 or claim 3 wherein  connected to R¹ is a double bond.

10 11. A compound or a salt or solvate thereof according to claim 10 wherein:

R¹ is CHR⁸, wherein R⁸ is H; or

R¹ is O.

12. A compound or a salt or solvate thereof according to any one of claims 1 or 3 to 11,
15 wherein R² is C(O)OR⁵, wherein R⁵ is as defined in claim 1.

13. A compound or a salt or solvate thereof according to claim 2 or claim 12, wherein R⁵ is H.

20 14. A compound or a salt or solvate thereof according to any one of claims 1 or 3 to 11, wherein R² is C₁₋₄ alkyl or C₂₋₄ alkenyl wherein said alkyl and alkenyl groups are optionally substituted with one or more substituents selected from OH, NH₂, N₃, fluoro and chloro.

15. A compound or a salt or solvate thereof according to claim 14 wherein R² is
25 unsubstituted ethyl, unsubstituted ethenyl, CH₂OH, CH₂NH₂, CH₂N₃, CH₂F or CHF₂.

16. A compound or a salt or solvate thereof according to any one of claims 1 to 3 or 11, wherein R² is C(O)NR^{5a}R^{5b}, where R^{5a} and R^{5b} are as defined in claim 1.

30 17. A compound or a salt or solvate thereof according to any one of claims 1 or 3 to 16 wherein each of R^{4a} and R^{4b} is independently H, F, Cl, methyl or methyl substituted with one or more fluoro substituents.

18. A compound or a salt or solvate thereof according to claim 17 wherein:

35 both R^{4a} and R^{4b} are H; or

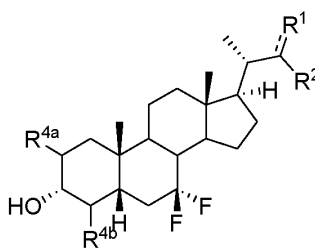
R^{4a} is H and R^{4b} is F; or

R^{4b} is H and R^{4a} is F.

19. A compound or a salt or solvate thereof according to any one of claims 1 or 3 to 18 wherein $\text{---}\text{C}=\text{C}\text{---}$ connected to R^3 is a double bond, and R^3 is O.

5 20. A compound or a salt or solvate thereof according to any one of claims 1 or 3 to 18 wherein $\text{---}\text{C}\text{---}$ connected to R^3 is a single bond, and R^3 is OH.

21. A compound or a salt or solvate thereof according to any one of claims 1 to 18 which is a compound of formula (IY):



(IY)

wherein in compounds of formula (IY), R^1 , R^2 , R^{4a} and R^{4b} are as defined in claim 1.

22. A compound according to claim 1 selected from:

- 15 7,7-difluoro 24-norlithocholic acid (Compound 1);
Methyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oate (Compound 1 Me ester)
3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-methyl-cholanic acid (Compound 2);
3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-ethyl-cholanic acid (Compound 3);
3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-fluoro-cholanic acid (Compound 4);
20 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-hydroxy-cholanic acid (Compound 5);
3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-chloro-cholanic acid (Compound 6);
3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-(methyl- d_3)-cholanic acid (Compound 7);
3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-methoxy-cholanic acid (Compound 8);
3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-keto-cholanic acid (Compound 9);
25 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-azido-cholanic acid (Compound 10);
3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-amine-cholanic acid (Compound 11);
3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-ol (Compound 12);
3 α -hydroxy-7,7-difluoro-5 β -24-nor-chol-22-ene (Compound 13);
3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholane (Compound 14);
30 3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-azide (Compound 15);
3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-amine (Compound 16);

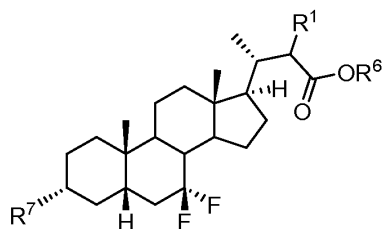
- 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholanic acid (Compound 17);
 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-fluoromethyl-cholanic acid (Compound 18);
 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-di-fluoromethyl-cholanic acid (Compound 19);
t-Butyl 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-di-fluoromethyl-cholan-23-oate (Compound 19 *t*-
 5 butyl ester)
 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-methylene-cholanic acid (Compound 20);
 3 α -Hydroxy-7,7-difluoro-5 β -24-nor-22-methoxymethyl-cholanic acid (Compound 21);
 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-aminomethyl-cholanic acid (Compound 22);
 3 α -hydroxy-7,7-difluoro-5 β -24-nor-22-hydroxymethyl-cholan-23-ol (Compound 23);
 10 3-keto-7,7-difluoro-5 β -24-nor-cholan-23,23-dicarboxylic acid (Compound 24);
 3-keto-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 25);
 3 β -Hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 26);
 2 β -fluoro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 27);
 2 β -fluoro-3 β -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 28);
 15 4 β -fluoro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 29);
 4 β -fluoro-3 β -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 30);
 4 α -chloro-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholanic acid (Compound 31);
 [²H]-3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oic acid (Compound 32);
N-(3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oyl)-(morpholine) (Compound 33); and
 20 *N*-(3 α -hydroxy-7,7-difluoro-5 β -24-nor-cholan-23-oyl)-(methylamine) (Compound 34);
 or a salt or solvate of any one thereof.

23. A process for the preparation of a compound according to claim 1 or a salt or solvate thereof, the process comprising:

25

A. for a compound of formula (I) in which  connected to R¹ is a single bond, R² is C(O)OR⁵, where R⁵ is H, R³ is α -OH and R^{4a} and R^{4b} are both H:

hydrolysis of a protected compound of formula (II):

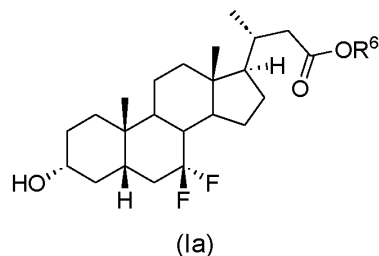


(II)

30

wherein R¹ is as defined for formula (I), R⁶ is C₁₋₆ alkyl or benzyl and R⁷ is a protected OH group;

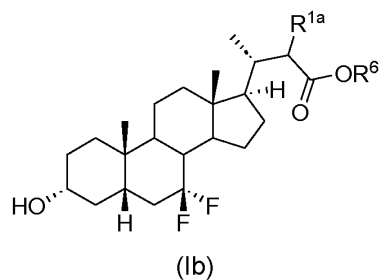
B. for a compound of formula (Ia), which is a compound of formula (I) in which R^2 is $C(O)OR^5$ and R^5 is a group R^6 :



5 wherein R^6 is as defined for formula (II);

hydrolysis of a compound of formula (II) with an alkali metal alkoxide in an alcoholic solvent;

C. for a compound of formula (Ib), which is a compound of formula (I) in which R^1 is C_{1-4} alkyl and R^2 is $C(O)OR^5$, where R^5 is C_{1-6} alkyl or benzyl:

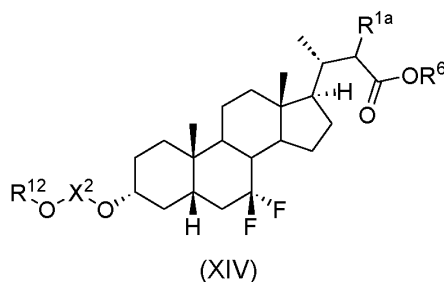


10

wherein R^6 is as defined for formula (II) and R^{1a} is C_{1-4} alkyl;

reaction of a compound of a compound of formula (XIV):

15

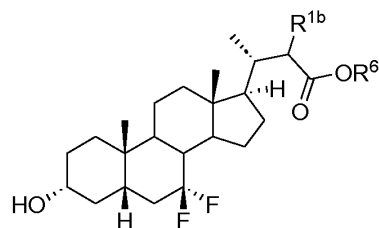


wherein X^2 is C_{1-6} alkylene, R^{12} is C_{1-6} alkyl, R^6 is as defined for formula (II) and R^{1a} is C_{1-4} alkyl optionally substituted as defined in claim 1 for R^1 ;

20

with an acid to remove protecting group $R^{12}-O-X^2$;

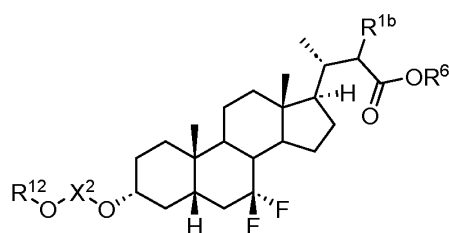
D. for a compound of formula (Ic):



(Ic)

wherein R^6 is as defined for formula (II) and R^{1b} is fluoro or chloro;

- 5 reaction of a compound of formula (XVI):

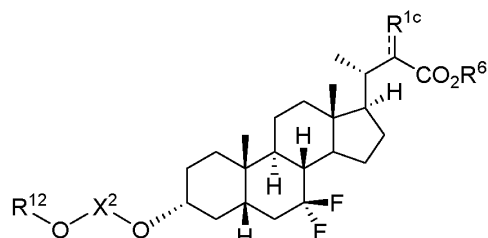


(XVI)

wherein X^2 is C_{1-6} alkylene, R^{12} is C_{1-6} alkyl, R^6 is as defined for formula (II) and R^{1b} is fluoro or chloro by reaction with a fluorinating agent or a chlorinating agent;

10

- E. for a compound of formula (I) in which R^1 is OH or alkoxy
i. reaction of a compound of formula (XX):



(XX)

- 15 wherein X^2 is C_{1-6} alkylene, R^{12} is C_{1-6} alkyl, R^6 is as defined for formula (II) and R^{1c} is OH, $O(C_{1-4}$ alkyl), N_3 , NH_2 or $=O$;

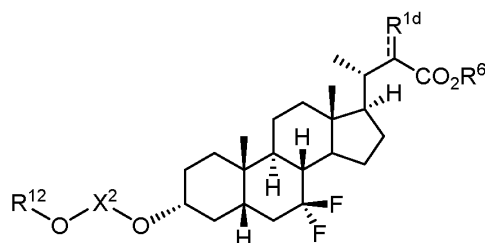
with an acid to give a compound of formula (I) in which R^2 is $C(O)OR^6$, wherein R^6 is as defined for formula (II); and optionally

- 20 ii. hydrolysis of the product of step i. to give a compound of formula (I) in which R^2 is $C(O)OH$;

F. For a compound of formula (I) in which R^1 is CH_2OH , methyl in which one or more H is replaced by Cl or F, CH_2N_3 , CH_2NH_2 or $=CHR^8$;

reaction of a compound of formula (XXVI):

5

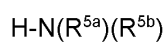


(XXVI)

wherein X^2 is C_{1-6} alkylene, R^{12} is C_{1-6} alkyl and R^6 is as defined for formula (II) and R^{1d} is CH_2OH , methyl in which one or more H is replaced by Cl or F, CH_2N_3 , CH_2NH_2 or $=CHR^8$; or

10 G. for a compound of formula (I) in which R^2 is $C(O)N(R^{5a})(R^{5b})$;

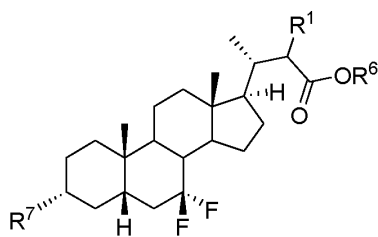
reacting a compound of formula (I) in which R^2 is $C(O)OH$ by reaction with an amine of formula (XXVII)



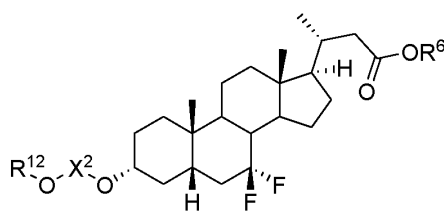
(XXVII)

15 wherein R^{5a} and R^{5b} are as defined in claim 1.

24. A compound of (II), (XII), (XIV), (XVI), (XX), (XXV), (XXVI) or (XXVIg):

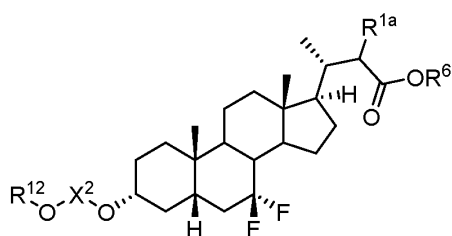


(II)

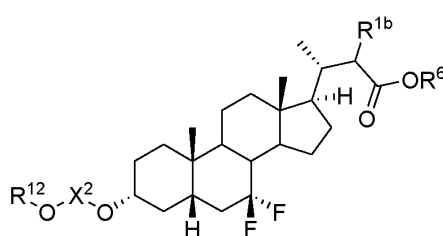


(XII)

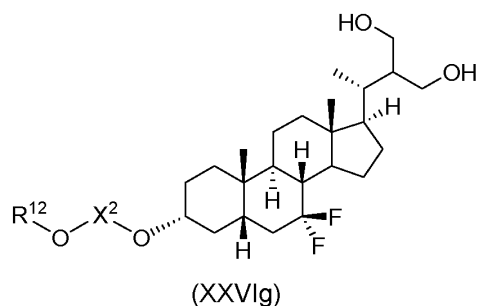
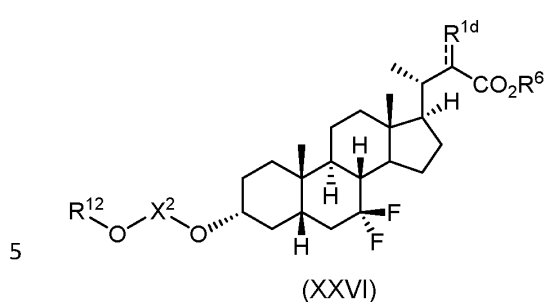
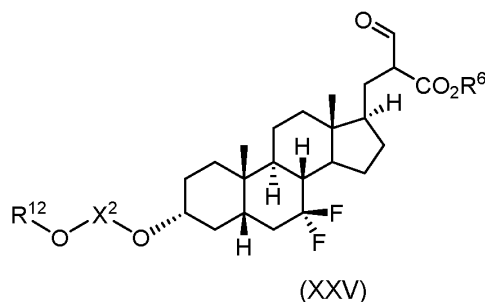
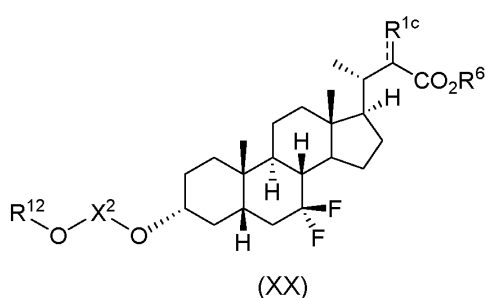
20



(XIV)



(XVI)



wherein:

R^1 is as defined for formula (I);

R^{1a} is C_{1-4} alkyl optionally substituted as defined above for R^1 ;

10 R^{1b} is fluoro or chloro;

R^{1c} is OH, $O(C_{1-4} \text{ alkyl})$, N_3 , NH_2 or $=O$;

R^{1d} is CH_2OH , methyl in which one or more H is replaced by Cl or F, CH_2N_3 , CH_2NH_2 or $=CHR^8$;

R^6 is C_{1-6} alkyl or benzyl;

15 R^7 is a protected OH group, for example $R^{18}C(O)O$, where R^{18} is C_{1-6} alkyl or benzyl;

R^8 is H or CH_3 ;

R^{12} is C_{1-6} alkyl; and

X^2 is C_{1-6} alkylene.

20 25. A compound or salt or solvate thereof according to any one of claims 1 to 22 for use in medicine.

26. A compound or salt or solvate thereof according to any one of claims 1 to 22 for use in the treatment or prevention of a neurodegenerative disorder, a neuromuscular disorder or
25 a mitochondrial disease.

27. The use of compound or salt or solvate thereof according to any one of claims 1 to 22

in the manufacture of a medicament for the treatment or prevention of a neurodegenerative disorder, a neuromuscular disorder or a mitochondrial disease.

28. A method for the treatment or prevention of a neurodegenerative disorder, a
5 neuromuscular disorder or a mitochondrial disease, the method comprising administering to a patient in need of such treatment an effective amount of a compound or salt or solvate thereof according to any one of claims 1 to 22.
29. A compound for use, a use or a method wherein:
10 the neurodegenerative disorder is selected from Parkinson's disease, mild cognitive impairment, dementia (including Alzheimer's disease, vascular dementia, dementia with Lewy bodies and FTD), Huntington's disease, amyotrophic lateral sclerosis (motor neurone disease), multiple system atrophy (MSA), progressive supranuclear palsy and Wilson's disease;
- 15 30. A pharmaceutical composition comprising a compound or salt or solvate thereof according to any one of claims 1 to 22 and a pharmaceutically acceptable excipient or carrier.
31. A composition according to claim 30 which is formulated for parenteral administration or for oral administration.
20
32. A pharmaceutical composition according to claim 31 or claim 32 further comprising an additional active agent useful in the treatment or prophylaxis of neurodegenerative disorders or neuromuscular disorders.
- 25 33. A product comprising a compound of formula (I) and an additional active agent useful in the treatment or prevention of a neurodegenerative or neuromuscular disorder as a combined preparation for simultaneous, sequential or separate use in the treatment or prevention of a neurodegenerative disorder or neuromuscular disorder.