The synthesis of A- and B-ring fluorinated analogues of cholesterol

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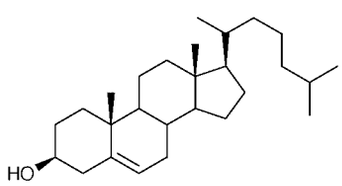
The synthesis of a number of mono- and difluorinated steroids with the potential to act as probes of the metabolism of cholesterol is described. 2α-Fluorocholestan-3-one **7**, 4-fluorocholest-5-en-3-one **11** and 6-fluorocholest-4-en-3-one **12** were synthesised from the appropriate silyl enol ethers using 1-fluoropyridinium triflate, and subsequently reduced to the corresponding alcohols, **8**, **13** and **14** respectively. A similar approach was used to synthesise 2,2-difluorocholestan-3-ol **16** starting from the monofluoro steroid **7**. To synthesise 4,4-difluorocholestan-3β-ol **26**, 3β-acetoxycholestan-4-one **25** was generated *via* an acid catalysed rearrangement of 4,5-epoxycholestan-3-one **20** and treated with DAST. Finally, a difluorocyclopropyl analogue of cholesterol **28** was synthesised using chlorodifluoroacetic acid.

Introduction

Atherosclerosis, the thickening of artery walls, ranks alongside cancer as one of the major causes of mortality in the western world.[1] As such, it remains a major target for novel therapies. It has long been known that excess cholesterol in the blood stream plays a large contributory role in the onset of atherosclerosis and compounds which reduce these levels are thus highly desirable. Although a great deal of effort has been put into understanding the metabolic pathways of cholesterol **1**, the exact



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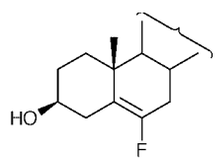
mechanisms of regulation, particularly at the genetic level, are still poorly understood. Therefore, compounds with the potential to interact with either cholesterol processing enzymes or the regulatory processes at a genetic level could prove to be of great interest.

The use of fluorine to influence the biological activity of compounds has been well studied,[2] due in large part to the steric similarity between fluorine and hydrogen,[3] coupled with fluorine's much larger electronegativity.

Fluorinated analogues of cholesterol would appear to have the desired characteristics to act as subtle probes of cholesterol metabolism, and an earlier series of compounds was synthesised and studied by this group.[4] These included 6-fluorocholesterol **2**, 6β- and 7β-fluorocholestan-3β-ol (**3a** and **4a**), and both 6,6- and 7,7-difluorocholestan-3β-ol (**3b** and **4b**). These compounds were screened for biological activity with regard to cholesterol biosynthesis, cholesterol esterification by acyl CoA:cholesterol acyl transferase (ACAT), and hydroxylation by cholesterol 7α-hydroxylase. The studies proved worthwhile with a number of different effects observed. Whilst none of the compounds affected cholesterol biosynthesis, 6β-fluoro-



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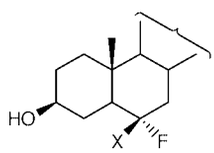
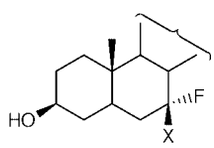


3a X=H
3b X=F



4a X=H
4b X=F

cholestan-3β-ol **2** and the two difluorinated steroids **3b** and **4b** were seen to act as substrates for the enzyme ACAT, and also stimulated cholesterol esterification in full cell assays. The most subtle effect observed was the effect of 6-fluorinated steroids on cholesterol 7α-hydroxylase in microsomal assays. Here, 6-fluorocholesterol **2** had no effect, whereas 6β-fluorocholestan-3β-ol **3a** was quite a potent inhibitor, suggesting that the orientation of the fluorine was very important in determining activity.

As a continuation of this work, it was proposed to synthesise a second series of fluorinated cholesterol analogues, again including a number of B-ring modified analogues, but also placing an emphasis on the A-ring of cholesterol. Ring A fluorinated analogues were of particular interest as the 3β-hydroxy group is likely to be a key site for enzyme recognition and the close proximity of fluorine might be expected to show substantial changes in behaviour.

Studies on the effects of a series of bile acids on cholesterol 7α-hydroxylase and sterol 27-hydroxylase regulation[5] have suggested that the hydroxy groups in steroids can be replaced by a ketone with little effect on enzyme activity. The 3-keto intermediates to the desired 3-hydroxy compounds were also therefore of interest to further investigate this. Finally, the effect of 5,6-cyclopropylcholesterol analogues on cholesterol

7α-hydroxylase has also been studied by this group[6] so incorporation of fluorine into this analogue was also investigated.

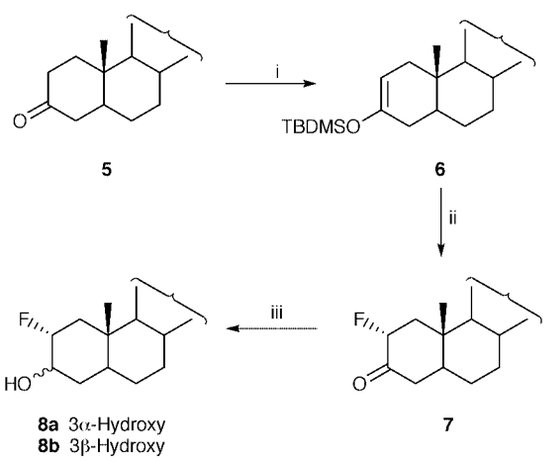
With these factors in mind, a number of monofluorinated and difluorinated steroids were chosen as synthetic targets, with a view to screening their effect on the metabolism of cholesterol. These were 2-fluorocholestan-3-ol **8**, 4-fluorocholest-5-en-3-ol **13**, 6-fluorocholest-4-en-3-ol **14**, 2,2-difluorocholestan-3-ol **16**, 4,4-difluorocholestan-3-ol **26** and difluorocyclopropa[5,6]cholesterol **28**.

Results

Synthesis of monofluorinated steroids

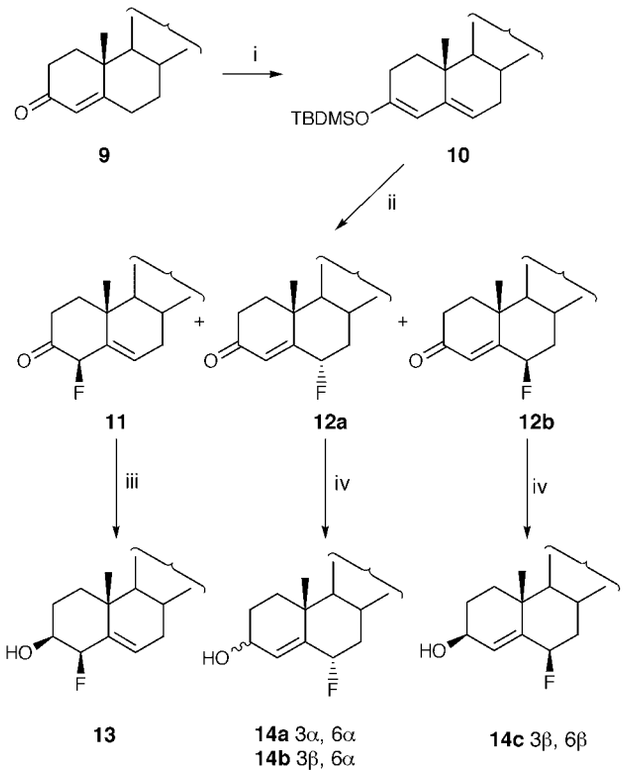
The simplest approach to the synthesis of the monofluorinated steroids would be fluorination of the relevant enolate anion using an electrophilic fluorinating agent such as 1-fluoropyridinium triflate. However, the work of Umemoto *et al.*[7] suggested that trapping the enolate as a silyl enol ether was likely to be the most effective approach.

To synthesise 2-fluorocholestan-3-ol **8** (Scheme 1), cholestan-3-one **5** was first treated with potassium hexamethyldisilazanide (KHMDSA) at −78 °C and the anion quenched with trimethylsilyl chloride. Unfortunately the resulting silyl enol ether was too unstable to isolate and purify. However, quenching of the anion with *tert*-butyldimethylsilyl chloride gave the corresponding silyl enol ether **6** in a 68% yield after purification. Reaction of **6** with 1-fluoropyridinium triflate yielded 2α-fluorocholestan-3-one **7**. Initial attempts resulted in low yields, but it was found that predrying the fluorinating agent at 80 °C and reduced pressure increased yields to 79%. Treatment of **7** with sodium borohydride in methanol gave a 1:1 mixture of 2α-fluorocholestan-3α-ol **8a** and 2α-fluorocholestan-3β-ol **8b**.



Scheme 1 *Reagents and conditions*: i, KHMDSA, TBDMSCl, −78 °C; ii, 1-fluoropyridinium triflate, DCM, reflux; iii, $NaBH_4$, EtOH.

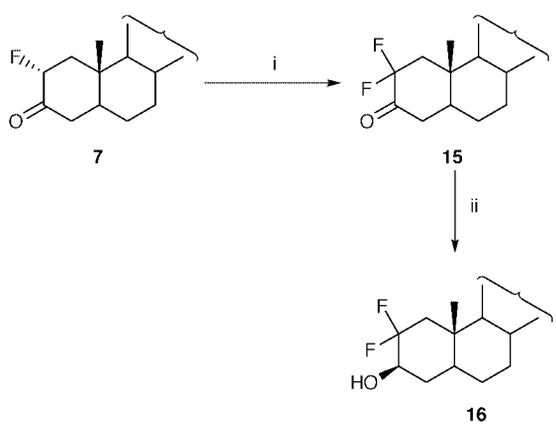
The synthesis of the 4- and 6-monofluorinated steroids utilised the same approach (Scheme 2), this time starting from cholest-4-en-3-one **9**. Treatment of **9** with KHMDSA at −20 °C and quenching of the anion with TBDMS chloride gave the silyl enol ether **10** which, on refluxing with dry 1-fluoropyridinium triflate, gave a mixture of 4β-fluorocholest-5-en-3-one **11**, 6α-fluorocholest-4-en-3-one **12a** and 6β-fluorocholest-4-en-3-one **12b**. These were separated by column chromatography and reduced to the 3-hydroxy steroids using sodium borohydride, with Luche conditions[8] being employed for the 6-fluorinated steroids. The 4β-fluorosteroid **11** and the 6β-fluorosteroid **12b** were both reduced exclusively to 3β-hydroxy steroids (**13** and **14c** respectively), whilst reduction of the 6α-fluorosteroid **12a** led to a 3:4 mixture of the 3α- and 3β-hydroxy steroids **14a** and **14b**.



Scheme 2 *Reagents and conditions*: i, KHMDSA, TBDMSCl, −20 °C; ii, 1-fluoropyridinium triflate, DCM; iii, $NaBH_4$, MeOH; iv, $NaBH_4$, MeOH, $CeCl_3$.

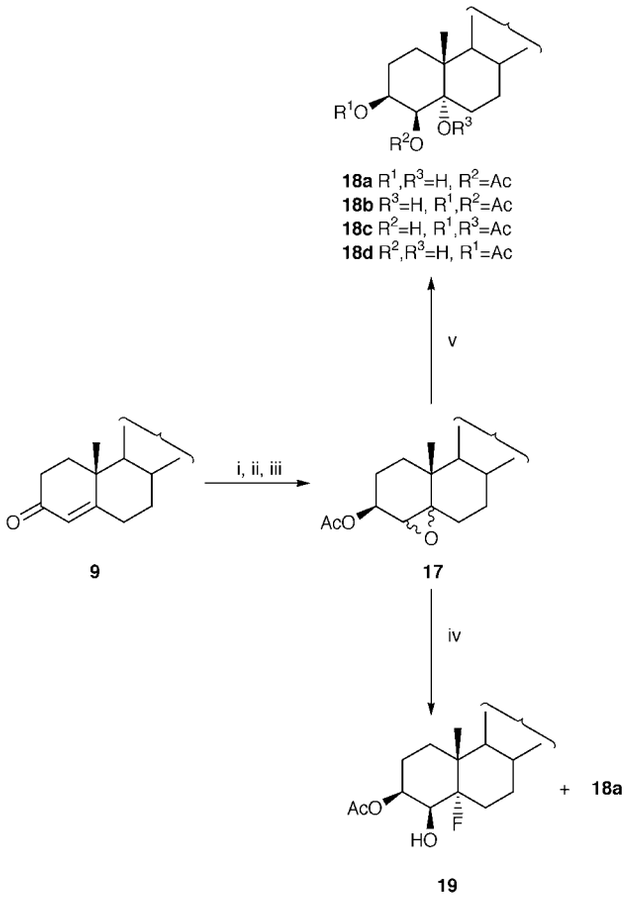
Synthesis of difluorinated steroids

A similar approach to that described above was used to synthesise the 2,2-difluorinated steroid **16** from the 2-monofluoro steroid **7**, based on a method described by Nakaso *et. al.*[9] (Scheme 3). Here, instead of trapping and isolating the enolate anion, it was generated using KHMDSA, stabilised with zinc chloride, and treated directly with *N*-fluorobenzenesulfonimide to give 2,2-difluorocholestan-3-one **15** in a 46% yield. Reduction of **15** with sodium borohydride gave the desired 2,2-difluorocholestan-3β-ol **16** in a 55% yield, with none of the 3α-epimer visible by either TLC or NMR.



Scheme 3 *Reagents and conditions*: i, KHMDSA, $ZnCl_2$, *N*-fluorobenzene sulfonimide; ii, $NaBH_4$, MeOH.

An electrophilic source of fluorine could not easily be utilised in the synthesis of the 4,4-difluorinated steroid **26**, as enolisation of 3-keto steroids occurs almost exclusively at the 2-position. It was therefore decided to generate 3β-acetoxycholestan-4-one **25** and fluorinate this using DAST.[10] The chosen route to **25** was the acid catalysed rearrangement of a 4,5-epoxy steroid.

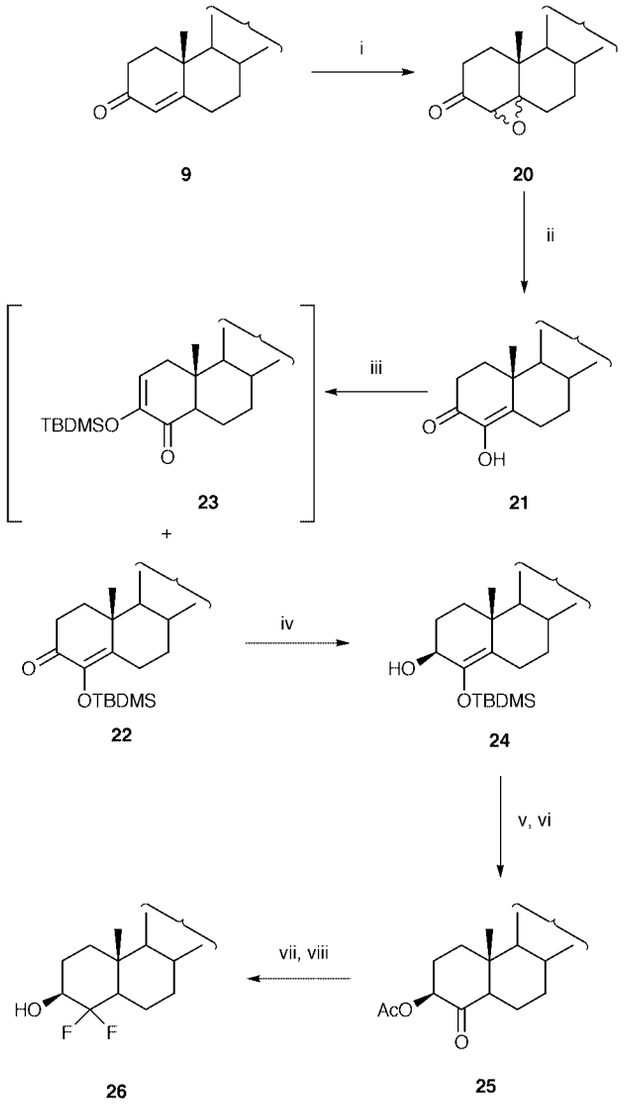


Scheme 4 *Reagents and conditions*: i, $LiAlH_4$, ether, RT; ii, Ac_2O, pyridine; iii, *m*CPBA, $CHCl_3$; iv, $BF_3{\cdot}OEt_2$, ether; v, AcOH, H_2SO_4.

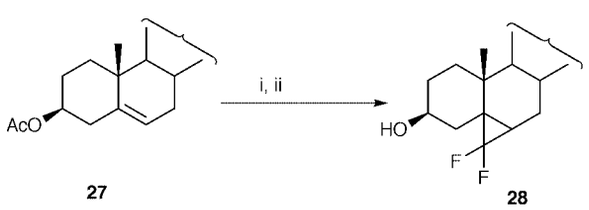
Initially (Scheme 4), cholest-4-en-3-one **9** was reduced using lithium aluminium hydride,[11] acetylated with acetic acid in pyridine[11] and epoxidised using *m*-chloroperoxybenzoic acid to give **17** as a mixture of the α- and β-epoxides. Reaction of the mixture of diastereomeric epoxides with boron trifluoride–diethyl ether yielded 3β,5α-dihydroxycholestan-4β-acetate **18a** and 5α-fluoro-4β-hydroxycholestan-3β-yl acetate **19** with none of the required 4-keto steroid isolated. Acetic acid and sulfuric acid also failed to catalyse the desired rearrangement, leading instead to a mixture of ring opened compounds, with either water or acetate acting as a nucleophile to give dihydroxy monoacetates and monohydroxy diacetates **18a**–**d**.

As an alternative route (Scheme 5), **9** was treated with hydrogen peroxide and sodium hydroxide[12] and the resulting epoxide **20** rearranged using a mixture of acetic and sulfuric acids. Reaction of the resulting diketone **21** with potassium *tert*-butoxide and TBDMS chloride gave a mixture of the required 3-keto-4-silyl 5-enol ether **22**, and the isomeric 4-keto-2-en-3-ol **23** in yields of 48 and 23% respectively. Enol ether **22** was reduced to alcohol **24** using lithium aluminium hydride at −78 °C, with higher temperatures leading to cleavage of the protecting group and reduction of the resulting 4-ketone. The silyl group of **24** was removed with TBAF to yield the corresponding keto alcohol **24a**, and the hydroxy group was acetylated with acetic acid in pyridine to give **25**. Treatment of **25** with neat DAST at 80 °C led to 4,4-difluorocholestan-3β-yl acetate **25a** in a 45% yield. Hydrolysis of the acetoxy group yielded **26** in a 71% yield.

The difluorocyclopropyl steroid **28** was synthesised from cholesteryl acetate **27** (Scheme 6). Treating **27** with the sodium salt of chlorodifluoroacetic acid at reflux in diglyme led to difluorocyclopropa[5,6]cholestan-3β-yl acetate **27a** in a 23% yield, which was subsequently hydrolysed to yield the required cholesterol analogue **28**.



Scheme 5 *Reagents and conditions*: i, H_2O_2, NaOH; ii, AcOH, H_2SO_4; iii, K_2CO_3, TBDMSCl; iv, $LiAlH_4$, −78 °C; v, TBAF; vi, Ac_2O, pyridine; vii, DAST, 80 °C; viii, Na_2CO_3, MeOH.



Scheme 6 *Reagents and conditions*: i, $ClF_2CCOONa$, Diglyme; ii, NaOH, EtOH.

Discussion

Determination of stereochemistries

The stereochemistry of the fluorine substituent in 2-fluorocholestan-3-one **7** was assigned as being α (equatorial) from the coupling constants between the C2 proton and the two C1 protons which were 12 and 7 Hz. Assuming ring A of the steroid adopts a chair conformation, the 12 Hz coupling suggests a transdiaxial arrangement between hydrogens on C1 and C2. This was confirmed by the appearance of an interaction on the NOESY spectrum between the axial C2 proton and the C19 methyl group which did not appear on the COSY spectrum. The coupling constants for the C3 protons of the 3-hydroxy steroids **8a** and **8b** could not be resolved, but the coupling from the axial C2 proton to the C3 proton was clear, being 9 Hz for

Table 1 Stereochemical outcomes of reductions of fluorinated steroid ketones

Steroid ketone	α-Hydroxy (%)[a]	β-Hydroxy (%)[a]
7	40	40
11	—	78
12a	24	32
12b	—	43
15	—	55

[a] Isolated yield.

the 3β-hydroxy steroid and 3 Hz for the 3α-hydroxy steroid (transdiaxial and axial–equatorial couplings respectively).

For the 6-fluorinated steroid ketones **12** and alcohols **14**, the stereochemistry of the fluorine substituents was again assigned from coupling constants. For **12a**, the C6 proton was seen to couple to the C7 protons with couplings of 14 and 6 Hz suggesting that the fluorine was α (equatorial), whereas 5 and 5 Hz couplings in **12b** suggested that the fluorine was β. The coupling from the C3 proton to the two C2 protons for the alcohols **14a**, **14b** and **14c** again clearly indicated which contained α- or β-hydroxy substitutuents.

The stereochemistry of the fluorine in 4-fluorinated steroid ketone **11** was determined to be β (axial) from a number of pieces of evidence. Firstly, the C4 proton showed two 2 Hz couplings, suggesting w-coupling to the C2 equatorial proton and possibly some coupling to the C6 vinyl proton. Also, the NOESY spectrum showed an interaction between the C4 and C6 protons, not present on the COSY spectrum, again indicating that the C4 proton was equatorial. Finally, no NOESY interaction was seen between the C4 proton and the C19 methyl group, which would be expected if the proton was axial.

The coupling between the C3 and C4 protons of the difluorosteroid alcohol **16** could not be resolved. To determine the stereochemistry of the hydroxy group at C3, the COSY spectrum was used to identify the signal due to the axial C5 proton. The C4 protons were initially identified from their coupling to the C3 proton, and the coupling between the C4 and the axial C5 proton was then easily observed. A NOESY interaction between the axial C5 and the C3 proton could be seen, indicating that the 3-hydroxy group was in the equatorial, β, position.

Stereochemistry of reductions of 3-keto steroids

In the sodium borohydride reduction of the various fluorinated 3-keto steroids prepared (Table 1), a number of interesting results were obtained. Steroids **11**, **12b** and **15** all gave, as expected,[13] the 3β-hydroxy steroid exclusively. However, both 2α-fluorocholestanone **7** and 6α-fluorocholestanone **12a** gave mixtures of 3α- and 3β-hydroxy steroids, an unusual result for the reduction of this type of steroid. The reason for predominant hydride attack from the α-face during sodium borohydride reductions of 3-keto steroids is generally given in terms of steric interactions in a late transition state.[14] For steroids with axial fluorines on the β-face, this effect is possibly enhanced by repulsion between the electron-rich fluorine and the incoming nucleophile. In the case of the two steroids with only an equatorial α-fluoro substituent, **7** and **12a**, the fluorine does not appear to be in a position to exert a similar effect, and it is possible that the strong electron withdrawing group is affecting the position of the transition state, reducing the steric influence of the ring substituents on the stereochemical outcome.

Reactions of 4,5-epoxy steroid 17

Attempts to convert **17** to a 4-keto steroid proved unsuccessful. Reaction with boron trifluoride led to two compounds: the 5-fluoro steroid **19**, formed from the β-epoxide by intermolecular delivery of fluorine from BF_3, and the 4-acetoxy steroid **18a**, presumably formed from the α-epoxide by ring walking of the acetate *via* an intermediate oxonium ion.[15]

Reaction of **17** with acetic acid and sulfuric acid led to a mixture of compounds. Ring opening by acetate gave the diacetoxy steroids **18b** and **18c**, whilst hydrolysis of the epoxide led to the dihydroxy steroid **18d**. Again, ring walking of the acetate of **18d** was observed, resulting in the 4β-acetoxy steroid **18a**. The stereochemistry and substitution patterns of **18a–d** were determined from their proton NMR spectra.

Mechanism of fluorination

The mechanism of electrophilic fluorination has been the focus of some debate.[7,16] The earliest examples, such as those using CF_3OF,[17] could be explained by an S_N2 type attack of a nucleophile on the electron-poor fluorine. However, with the N–F class of reagents, some results, such as those of Umemoto *et al.*,[7] suggest a single electron transfer mechanism.

The results reported here suggest that both mechanisms may be possible. The fluorination of silyl enol ether **6** to give the 2-fluoro steroid **7** by nucleophilic attack on the fluorinating agent might be expected to give exclusive β-fluorination due to the favourable introduction of an axial substituent. The exclusive α-fluorination observed could therefore indicate that a single electron transfer was involved. The formation of a charge transfer complex between the steroid π-bond and the pyridinium ring could then be the key factor in determining stereochemistry, with the β-face of the steroid being sterically less accessible due to the ring junction methyl group. Fluorination of the silyl enol ether **10** however, was not so clear cut. The 4-position was exclusively fluorinated on the β-face, and both α- and β-fluorinations were observed for the 6-position, suggesting that the mechanism may display some degree of nucleophilicity.

Biological activity

The series of fluorinated cholesterol analogues synthesised was screened for biological activity with regard to cholesterol biosynthesis, cholesterol esterification and 7α-hydroxylation of cholesterol.[18] These studies showed some interesting results; the 6-fluorinated steroid alcohols **14** were seen to increase esterification of cholesterol in whole cell assays; the fluorinated steroid ketone **11** was a mild inhibitor of esterification in rat liver microsomes and both **11** and its 3-hydroxy analogue **13** were inhibitors of cholesterol 7α-hydroxylase activity. These studies will be reported in more detail elsewhere.

Experimental

General

^{1}H NMR were recorded on a Bruker WM-250 spectrometer. ^{13}C and ^{19}F NMR were recorded on a Bruker AMX 400. ^{1}H chemical shifts are given in ppm (δ values), relative to proton traces in solvent. ^{19}F chemical shifts are relative to $CFCl_3$. ^{13}C chemical shifts are relative to solvent. Diagnostic signals only are given in ^{1}H NMR. *J* values are given in Hz. NOESY NMRs were run using a standard Bruker NOESYTP program, with mixing times of 0.8 or 1.2 s and acquisition times of 0.128 s. IR spectra were recorded on a Nicolet Impact 400d FTIR Spectrometer. Mass spectra were recorded on a JEOL JMS AX505 Mass Spectrometer. Melting points were recorded on a Reichert hot stage/microscope and are uncorrected.

3-(*tert*-Butyldimethylsilyloxy)cholest-2-ene 6

Cholestan-3-one **5** (1 g, 2.6 mmol) was dissolved in dry ether (40 ml) and cooled to −78 °C under nitrogen. To this was added potassium hexamethyldisilazanide (3.1 mmol, 6.2 ml of 0.5 M solution in toluene), and the solution stirred for 2 hours. A solution of *tert*-butyldimethylsilyl chloride (0.76 g, 5 mmol) in

ether (5 ml) was added, the solution stirred for 4 hours, and allowed to warm to room temperature overnight. The solution was washed with water, the organic layer dried over magnesium sulfate, filtered, and the solvent removed under reduced pressure. Recrystallisation from ethanol yielded *3-(tert-butyldimethylsilyloxy)cholest-2-ene* **6** (886 mg, 68%); mp 153–155 °C (Found: C, 79.3; H, 12.0%. $C_{33}H_{60}OSi$ requires C, 79.1, H, 12.1%); ν_{max} (KBr disc)/cm^{-1} 2853–2947s (aliphatic CH), 1673m (C=C), 1471m, 1464m, 1252s, 1198s; δ_H (250 MHz, $CDCl_3$) 4.75 (1H, br d, *J* 6, HC=C), 0.92 (9H, s, $(CH_3)_3C$), 0.82–0.90 (9H, m, CH_3 C21/26/27), 0.75 (3H, s, CH_3 C19), 0.66 (3H, s, CH_3 C18), 0.08 (6H, s, H_3C-Si).

2α-Fluorocholestan-3-one 7

To a stirred solution of **6** (500 mg, 1 mmol) in dry dichloromethane (40 ml) under nitrogen, was added 1-fluoropyridinium triflate (497 mg, 2 mmol) and the solution heated under reflux for 5 hours. The solution was cooled, washed with water, and the organic layer dried over sodium sulfate, filtered and the solvent removed under reduced pressure. Flash chromatography using 20% ether in hexane gave **7** as a white solid (319 mg, 79%) which was pure by $^1H/^{19}F$ NMR; mp 168–169 °C (from ethanol) (lit.,[19] mp 173–174 °C) (HRMS (EI): found M^+, 404.3450. $C_{27}H_{45}OF$ requires *M*, 404.3454); ν_{max} (KBr Disc)/cm^{-1} 2858–2947s (aliphatic CH), 1742s (C=O), 1471m, 1383m, 1093w, 1030m; δ_H (400 MHz, $CDCl_3$) 5.02 (1H, ddd, $^2J_{HF}$ 48, $^3J_{HH}$ 12 and 7, HCF), 2.51 (1H, m), 2.34 (1H, m), 2.25 (1H, m), 2.01 (1H, m), 1.58 (1H, m), 1.09 (3H, s, CH_3 C19), 0.92 (3H, d, *J* 7, CH_3 C21), 0.85 (6H, d, *J* 7, CH_3 C26/27), 0.68 (3H, s, CH_3 C18); δ_C (100 MHz, $CDCl_3$) 205.0 (d, $^2J_{CF}$ 13, C=O), 91.0 (CF, d, $^1J_{CF}$ 190), 56.4, 56.3, 47.9, 46.0 (d, *J* 16), 43.6, 42.8, 39.9, 39.7, 39.0, 37.8 (d, *J* 10), 36.3, 36.0, 34.7, 31.7, 28.5, 28.4, 28.2, 24.4, 24.0, 23.0, 22.8, 21.9, 18.9, 13.0, 12.3; δ_F (377 MHz, $CDCl_3$) −194.48 (dddd, $^2J_{HF}$ 48, $^3J_{HF}$ 11 and 6, $^4J_{HF}$ 4).

2α-Fluorocholestan-3-ol 8a and 8b

To a solution of **7** (180 mg, 0.45 mmol) in ethanol (20 ml) under nitrogen, was added sodium borohydride (18 mg, 0.45 mmol) and the solution stirred for 1 hour. The solution was poured into cold (ice bath) hydrochloric acid (2 M) and extracted with ether. The combined organics were washed with water, dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. Flash chromatography using 20% ether in hexane yielded two white solids.

2α-Fluorocholestan-3α-ol **8a** (74 mg, 40%) R_f 0.26 [ether–hexane (1:4)]; mp 129–131 °C (HRMS (EI) found M^+, 406.3633. $C_{27}H_{47}OF$ requires *M*, 406.3611); ν_{max} (KBr disc)/cm^{-1} 3542s (OH), 2849–2956s (aliphatic C-H), 1466m, 1458m, 1382m, 1042m, 978m; δ_H (400 MHz, $CDCl_3$) 4.65 (1H, dddd, $^2J_{HF}$ 46, $^3J_{HH}$ 12, 5 and 3, HCF), 4.17 (1H, m, *H*COH), 0.92 (3H, d, *J* 6, CH_3 C21), 0.86 (6H, d, *J* 7, CH_3 C27/28), 0.80 (3H, s, CH_3 C19), 0.65 (3H, s, CH_3 C18); δ_C (100 MHz, $CDCl_3$) 92 (d, $^2J_{CF}$ 172, CF), 67.8 (d, $^3J_{CF}$ 17, COH), 56.5, 54.3, 42.8, 42.7, 40.1, 39.7, 38.2, 37.9 (d, *J* 16), 37.6 (d, *J* 7), 36.4, 36.0, 34.9, 33.7 (d, *J* 6), 32.0, 28.5, 28.2, 27.7, 24.4, 24.1, 23.0, 22.8, 21.2, 18.9, 12.6, 12.3; δ_F (376 MHz, $CDCl_3$) −187.42 (dddd, $^2J_{HF}$ 46, $^3J_{HF}$ 14, 7 and 7).

2α-Fluorocholestan-3β-ol **8b** (74 mg, 40%) R_f 0.19 [ether–hexane (1:4)]; mp 94–97 °C (HRMS (EI) found M^+, 406.3622. $C_{27}H_{47}OF$ requires *M*, 406.3611); ν_{max} (KBr disc)/cm^{-1} 2849–2931s (aliphatic C-H), 1467m, 1460w, 1380w, 1079m, 1050m, 1018m; δ_H (400 MHz, $CDCl_3$) 4.50 (1H, dddd, $^2J_{HF}$ 51, $^3J_{HH}$ 14, 9 and 5, HCF), 3.69 (1H, m, *H*COH), 0.92 (3H, d, *J* 7, CH_3 C21), 0.9 (9H, m, CH_3 C19/26/27), 0.65 (3H, s, CH_3 C18); δ_C (100 MHz, $CDCl_3$) 96 (d, $^1J_{CF}$ 170, CF), 74.3 (d, $^2J_{CF}$ 18, COH), 56.5 (d, *J* 3), 54.4, 44.6 (d, *J* 2), 42.8, 42.6 (d, *J* 15), 40.1, 39.7, 38.0 (d, *J* 10), 36.3, 35.9, 34.9, 34.8, 32.0, 29.9, 28.4, 28.2, 27.9, 24.4, 24.0, 23.0, 22.8, 21.6, 18.9, 13.5, 12.3; δ_F (377 MHz, $CDCl_3$) −187.0 (ddddd, $^2J_{HF}$ 51, $^3J_{HF}$ 13, 11 and 6, $^4J_{HF}$ 6).

3-(*tert*-Butyldimethylsilyloxy)cholesta-3,5-diene 10

Compound **10** was synthesised from cholest-4-en-3-one **9** according to the procedure for preparation of **6**. Recrystallisation from ethanol gave *3-(tert-butyldimethylsilyloxy)cholesta-3,5-diene* **10** (185 mg, 30%); mp 125–127 °C; ν_{max} (KBr disc)/cm^{-1} 2859–2858s (aliphatic C-H), 1660m (C=C); δ_H (250 MHz, $CDCl_3$) 5.30 (1H, m, HC=COSi), 4.78 (1H, m, HC=C C6), 0.93 (9H, s, $(H_3C)_3C$), 0.86–0.97 (12H, m, CH_3 C19/21/26/27), 0.69 (3H, s, CH_3 C18), 0.13 (6H, s, H_3CSi).

4β-Fluorocholest-5-en-3-one 11 and 6-fluorocholest-4-en-3-one 12a and 12b

Compounds **11**, **12a** and **12b** were synthesised from **10** according to the procedure for the preparation of **7**. Flash chromatography using 15% ether in hexane yielded three products.

4β-Fluorocholest-5-en-3-one **11** (120 mg, 38%) R_f 0.21 (ether–hexane (1:6); mp 84–88 °C (HRMS (EI) found: M^+, 402.3329. $C_{27}H_{43}OF$ requires *M*, 402.3298); ν_{max} (KBr disc)/cm^{-1} 2852–2928s (aliphatic CH), 1692s (C=O), 1628m (C=C), 1465m, 1377m, 1213m; δ_H (400 MHz, $CDCl_3$) 5.87 (1H, d, *J* 5, HC=C), 4.99 (1H, ddd, $^2J_{HF}$ 49, $^4J_{HH}$ 2 and 2, HCF), 1.31 (3H, s, CH_3 C19), 0.93 (3H, d, *J* 7, CH_3 C21), 0.87 (6H, d, *J* 5, CH_3 C26/27), 0.74 (3H, s, CH_3 C18); δ_C (100 MHz, $CDCl_3$) 200 (C=O), 162.36 (d, *J* 12, C=CH), 128.48 (d, *J* 9, HC=C), 93.74 (d, *J* 166, CF), 56.33, 56.0, 54.2, 53.5, 42.8, 39.7, 38.1, 37.7, 37.5, 37.1, 36.3, 32.1, 29.9, 28.3, 28.2, 24.3, 24.0, 23.0, 22.8, 21.1, 18.9, 18.6, 12.2.

6α-Fluorocholest-4-en-3-one **12a** (110 mg, 34%); R_f 0.24 (ether–hexane (1:6)) (HRMS (EI) found M^+, 402.3284. $C_{27}H_{43}OF$ requires *M*, 402.3298); ν_{max} (KBr disc)/cm^{-1} 2867–3025s (aliphatic C-H), 1685s (C=O), 1619m (C=C); δ_H (400 MHz, $CDCl_3$) 5.74 (1H, m, HC=C), 4.99 (1H, ddd, $^2J_{HF}$ 43, $^3J_{HH}$ 14 and 6, HCF), 1.01–0.89 (12H, m, CH_3 C19/21/26/27), 0.71 (3H, s, CH_3 C18).

6β-Fluorocholest-4-en-3-one **12b** (45 mg , 14%) R_f 0.32 (ether–hexane (1:6)); mp 53–56 °C (HRMS (FAB) found M + H^+, 403.3379. $C_{27}H_{44}OF$ requires *M* + *H*, 403.3376); ν_{max} (KBr disc)/cm^{-1} 2868–2953s (aliphatic C-H), 1699s (C=O), 1618m (C=C), 1466m, 1382m, 1250m, 879w; δ_H (400 MHz, $CDCl_3$) 5.81 (1H, m, HC=C), 4.96 (1H, ddd, $^2J_{HF}$ 42, $^3J_{HH}$ 5 and 5, HCF), 2.50 (1H, m), 1.26 (3H, s, CH_3 C19), 0.91 (3H, d, *J* 7, CH_3 C21), 0.86 (6H, d, *J* 7, CH_3 C26/27), 0.72 (3H, s, CH_3 C18); δ_C (100 MHz, $CDCl_3$) 193.2 (C=O), 174.4, 120.8, 88.3 (d, *J* 179, CF), 56.3, 56.0, 52.8, 42.9, 40.5 (d, *J* 6), 40.0, 39.8, 36.3, 35.9, 35.7, 34.0, 33.5, 29.9, 28.4, 28.2, 24.4, 24.0, 23.0, 22.8, 22.3, 22.2, 18.8, 12.3; δ_F (377 MHz, $CDCl_3$) −190.6 (ddd, $^2J_{HF}$ 42, $^3J_{HF}$ 23 and 18).

4β-Fluorocholest-5-en-3β-ol 13

Compound **13** was synthesised from ketone **11** according to the method used for preparation of **8**. Flash chromatography of the crude solid using 25% ether in hexane yielded *4β-fluorocholest-5-en-3β-ol* **13** as a white solid (38 mg, 78%); mp 110–111 °C (HRMS (EI) found: M^+, 404.3468. $C_{27}H_{45}OF$ requires *M*, 404.3454); ν_{max} (KBr disc)/cm^{-1} 3100–3500 (OH), 2860–2940s (aliphatic C-H), 1470m, 1383s, 1076m; δ_H (250 MHz, $CDCl_3$) 5.67 (1H, br d, *J* 5, HC=C), 4.85 (1H, ddd, $^2J_{HF}$ 50, $^4J_{HH}$ 2.5 and 2.5, HCF), 4.06 (1H, m, *H*COH), 1.14 (3H, s, CH_3 C19), 1.02 (3H, d, *J* 6, CH_3 C21), 0.90 (6H, d, *J* 7, CH_3 C26/27), 0.74 (3H, s, CH_3 C18).

6-Fluorocholest-4-en-3-ol 14a,b,c

The alcohols **14a**, **14b** and **14c** were synthesised from ketones **12a** and **12b** according to the method of Luche.[8] Reduction of **12a** yielded two compounds.

6β-Fluorocholest-4-en-3β-ol **14b** (34 mg, 32%) R_f 0.33 (ether–hexane (1:6)); mp 104–108 °C (HRMS (EI) found: M^+,

404.3465. $C_{27}H_{45}OF$ requires *M*, 404.3454); ν_{max} (KBr disc)/cm^{-1} 3560–3300 (OH), 2850–2950s (aliphatic C-H), 1466m, 1380m, 1047m; δ_H (400 MHz, $CDCl_3$) 5.10 (1H, d, *J* 7, HC=C), 4.56 (1H, m, HCF), 4.32 (1H, dd, *J* 16 and 7, *H*COH), 1.12 (3H, s, CH_3 C19), 0.91 (3H, d, *J* 6, CH_3 C21), 0.88 (6H, d, *J* 7, CH_3 C26/27), 0.69 (3H, s, CH_3 C18).

6α-Fluorocholest-4-en-3α-ol **14a** (24 mg, 24%) R_f 0.22 (ether–hexane (1:6)); mp 91–95 °C (HRMS (EI) found M$^+$, 404.3475. $C_{27}H_{45}OF$ requires *M*, 404.3454); ν_{max} (KBr disc)/cm^{-1} 3500–3300 (OH), 2860–2940s (aliphatic CH), 1466m, 1377m, 1063m, 1038w; δ_H (400 MHz, $CDCl_3$) 5.66 (1H, s, HC=C), 4.96 (1H, ddd, $^2J_{HF}$ 49, $^3J_{HH}$ 12 and 5, HCF), 4.24 (1H, m, *H*COH), 1.04 (3H, s, CH_3 C19), 0.91 (3H, d, *J* 6, CH_3 C21), 0.86 (6H, d, *J* 7, CH_3 C26/27), 0.68 (3H, s, CH_3 C18).

Reduction of **12b** yielded *6β-fluorocholest-4-en-3β-ol* **14c** (15 mg, 43%); mp 74–77 °C (HRMS (EI) found M$^+$, 404.3436. $C_{27}H_{45}OF$ requires *M*, 404.3454); ν_{max} (KBr disc)/cm^{-1} 3200–3400 (OH), 2860–2930s (aliphatic CH), 1472m, 1396m, 1077m; δ_H (400 MHz, $CDCl_3$) 5.25 (1H, m, HC=C), 4.85 (1H, ddd, $^2J_{HF}$ 51, $^3J_{HF}$ 3 and 3, HCF), 4.15 (1H, dd, *J* 13 and 7, *H*COH), 1.14 (3H, s, CH_3 C19), 0.91 (3H, d, *J* 7, CH_3 C21), 0.87 (6H, d, *J* 7, CH_3 C26/27), 0.68 (3H, s, CH_3 C18).

2,2-Difluorocholestan-3-one 15

To a suspension of zinc chloride (167 mg, 1.2 mmol) in dry THF under nitrogen, was added **7** (230 mg, 0.57 mmol) and the suspension cooled to −78 °C. To this was added potassium hexamethyldisilazanide (1.8 ml, 0.5 M in toluene) and *N*-fluorobenzenesulfonimide (215 mg, 0.68 mmol). The mixture was stirred for 2 hours, warmed to room temperature and poured into saturated sodium bicarbonate. This was extracted with ethyl acetate and the organic layer dried over sodium sulfate, filtered and the solvent removed under reduced pressure. Flash chromatography on neutralised alumina using 30% ether in hexane yielded **15** as a white solid (110 mg, 46%); mp 112–114 °C (HRMS (FAB) found M$^+$, 422.3364. $C_{27}H_{44}OF_2$ requires *M*, 422.3360); ν_{max} (KBr disc)/cm^{-1} 2860 2930s (aliphatic C-H), 1757s (C=O), 1637m, 1458m, 1467m, 1073s; δ_H (400 MHz, $CDCl_3$) 3.74 (1H, m, *H*COH), 2.72 (1H, m), 2.51 (1H, m), 2.22 (1H, m), 0.85–0.89 (9H, m, CH_3 C21/26/27), 0.67 (3H, s, CH_3 C18); δ_C (100 MHz, $CDCl_3$) 198 (dd, $^2J_{CF}$ 23 and 28, C=O), 115 (dd, $^1J_{CF}$ 256 and 256, F_2C), 66.1, 56.4, 56.2, 54.7, 48.1 (dd, *J* 22 and 22), 47.1, 43.3 (dd, *J* 20 and 20), 42.8, 42.1, 39.9, 39.7, 36.3, 36.0, 34.7, 31.6, 28.4, 28.3, 24.4, 24.0, 23.0, 22.8, 21.8, 18.9, 15.5, 12.3; δ_F (377 MHz, $CDCl_3$) −98.5 (ddd, $^2J_{FF}$ 265, $^3J_{HF}$ 37 and 17), −115.9 (d, $^2J_{FF}$ 265).

2,2-Difluorocholestan-3β-ol 16

Compound **16** was synthesised from **15**, according to the procedure for preparation of **8**. Flash chromatography using 20% ether in hexane yielded **16** as a white solid (33 mg, 55%); mp 115–116 °C (HRMS (FAB) found: M$^+$, 424.3519. $C_{27}H_{46}OF_2$ requires *M*, 424.3517); ν_{max} (KBr disc)/cm^{-1} 3250–3550 (OH), 2830–2950s (aliphatic C-H), 1602w, 1467w, 1383m, 1110s; δ_H (400 MHz, $CDCl_3$) 3.74 (1H, m, *H*COH), 2.24 (1H, m), 2.00 (2H, m), 0.91 (3H, d, *J* 5, CH_3 C21), 0.90 (3H, s, CH_3 C19), 0.85 (6H, dd, *J* 7 and 2, CH_3 C26/27), 0.66 (3H, s, CH_3 C18); δ_C (100 MHz, $CDCl_3$) 123 (dd, *J* 243 and 246, CF_2), 72.4 (dd, *J* 22 and 22, COH), 56.5, 55.0, 45.3 (dd, *J* 22 and 22), 44.8, 42.8, 40.0, 39.7, 37.4 (d, *J* 8), 36.4, 36.0, 34.8, 34.5 (d, *J* 7), 31.9, 28.4, 28.3, 27.7, 24.4, 24.1, 23.0, 22.8, 21.6, 18.9, 13.1, 13.0, 12.3; δ_F (377 MHz, $CDCl_3$) −99.6 (dddd, $^2J_{FF}$ 238, $^3J_{HF}$ 10, 5 and 5), 113.9 (dddd, $^2J_{FF}$ 238, $^3J_{HF}$ 47, 17 and 13).

4,5-Epoxycholestan-3β-yl acetate 17

Cholest-4-en-3β-yl acetate was prepared from **9** according to the method of Collins.[11] To a solution of cholest-4-en-3β-yl acetate (735 mg, 1.7 mmol) in chloroform (17 ml) under nitrogen at 0 °C, was added a solution of *m*-chloroperoxybenzoic acid (672 mg, 3.9 mmol) in chloroform (17 ml) over 10 minutes. The solution was warmed to room temperature and stirred for 3 hours, washed with water, dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. Flash chromatography using 15% ether in hexane yielded 4,5-epoxycholestan-3β-yl acetate **17** as a mixture of the 4α,5α and 4β,5β diastereomers (587 mg, 77%).

Acetate **17** (diastereomer 1); δ_H (250 MHz, $CDCl_3$) 4.96 (1H, dd, *J* 8 and 8, CH-OCO), 2.87 (1H, s, CH-O), 2.03 (3H, s, CH_3COO), 1.12 (3H, s, CH_3 C19), 0.91 (9H, m, CH_3 C21/26/27), 0.68 (3H, s, CH_3 C18); *m/z* (EI) 444 (M$^+$).

Acetate **17** (diastereomer 2); δ_H (250 MHz, $CDCl_3$) 5.13 (1H, m, CH-OCO), 3.15 (1H, m, CH-O), 2.05 (3H, s, CH_3COO), 1.19 (3H, s, CH_3 C19), 0.91 (9H, m, CH_3 C21/26/27), 0.68 (3H, s, CH_3 C18); *m/z* (EI) 444 (M$^+$).

Ring opening of 17 with BF_3–diethyl ether

To a solution of **17** (87 mg, 0.2 mmol) in dry ether (5 ml) under nitrogen, was added boron trifluoride–diethyl ether (0.1 ml) and the solution stirred overnight at room temperature. The solution was washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. Flash chromatography using 40% ether in hexane gave two products.

3β,5α-Dihydroxycholestan-4β-yl acetate **18a** (25 mg, 29%) R_f 0.04 (ether–hexane (2:3)); mp 163–165 °C; ν_{max} (KBr disc)/cm^{-1} 2867–2950s (aliphatic C-H), 1725s (C=O), 1638w, 1466m, 1376s, 1255s, 1051s; δ_H (250 MHz, $CDCl_3$) 4.95 (1H, d, *J* 4, HC-OCO), 4.28 (1H, ddd, *J* 12, 5 and 4, *H*COH), 2.13 (3H, s, CH_3-COO), 1.11 (3H, s, CH_3 C19), 0.91 (9H, m, CH_3 C21/26/27), 0.65 (3H, s, CH_3 C18); *m/z* (EI) 444 [(M$^+$) 8%], 384 (60), 332 (100).

5α-Fluoro-4β-hydroxycholestan-3β-yl acetate **19** (40 mg, 43%) R_f 0.36 (ether–hexane (2:3)); ν_{max} ($CHCl_3$)/cm^{-1} 2950–2840s (aliphatic C-H), 1740s (C=O), 1606w, 1478w, 1383m, 1038m; δ_H (250 MHz, $CDCl_3$) 5.09 (1H, m, HC-OCO), 3.81 (1H, m), 2.09 (3H, s, CH_3-COO), 1.14 (3H, s, CH_3 C19), 0.98 (9H, m, CH_3 C21/26/27), 0.66 (3H, s, CH_3 C18); δ_C (100 MHz, $CDCl_3$) 170.1, 101, 99, 74.4, 74.0, 72.6, 56.4, 56.2, 46.9, 42.9, 40.0, 39.7, 38.8, 38.6, 36.4, 31.1, 30.1, 29.9, 28.5, 28.2, 26.0, 24.3, 24.1, 23.0, 22.8, 21.9, 21.5, 18.8, 15.6, 15.5, 12.3; δ_F (376 MHz, $CDCl_3$) −66.6 (d, $^3J_{HF}$ 43); *m/z* (EI) 464 (65%), 404 (75), 309 (100).

Ring opening of 17 with acetic acid–sulfuric acid

To a solution of **17** (80 mg, 0.18 mmol) in acetic acid (1 ml) under nitrogen, was added concentrated sulfuric acid (1 drop). The solution was stirred overnight, poured onto ice (10 g) and allowed to warm to room temperature. The resulting suspension was extracted with dichloromethane and the organic extracts washed with water, dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. Flash chromatography using 40% ether in hexane yielded four compounds.

3β,5α-Dihydroxycholestan-4β-yl acetate **18a** (11 mg, 13%); data as above.

5α-Hydroxycholestane-3β,4β-diyl diacetate **18b** (30 mg, 32%); mp 159–161 °C; ν_{max} ($CHCl_3$)/cm^{-1} 2950–2880s (aliphatic C-H), 1753s (C=O), 1487m, 1377s, 1038m; δ_H (250 MHz, $CDCl_3$) 5.43 (1H, ddd, *J* 12, 5 and 4, CH-CO), 5.01 (1H, d, *J* 3, CH-OCO), 2.07 (3H, s, CH_3-COO), 1.97 (3H, s, CH_3-COO), 0.91 (9H, m, CH_3 C21/26/27), 0.64 (3H, s, CH_3 18); *m/z* (EI) 504 [(M$^+$) 10%], 444 (20), 426 (30), 384 (92), 332 (100).

4β-Hydroxycholestane-3β,5α-diyl diacetate **18c** (9 mg, 11%); δ_H (400 MHz, $CDCl_3$) 4.95 (1H, ddd, *J* 12, 5 and 4, HCOAc), 4.74 (1H, d, *J* 3, *H*COH), 2.61 (1H, m), 2.16 (6H, 2s, 2 × H_3CCO_2), 1.29 (3H, s, CH_3 C19), 0.97 (3H, d, *J* 5, CH_3 C21), 0.91 (6H, d, *J* 7, CH_3 C26/27), 0.69 (3H, s, CH_3 C18); *m/z* (EI) 444 (60%), 402 (35), 384 (100).

4β,5α-Dihydroxycholestan-3β-yl acetate **18d** (10 mg, 12%); mp 123–125 °C; δ_H (400 MHz, $CDCl_3$) 5.31 (1H, ddd, *J* 12, 5 and 4, HCOAc), 3.65 (1H, d, *J* 3, *H*COH), 2.09 (3H, s, CH_3CO_2), 0.90 (3H, d, *J* 6, CH_3 C21), 0.86 (6H, d, *J* 7, CH_3 C26/27), 0.66 (3H, s, CH_3 C18); *m*/*z* (EI) 444 (10%), 402 (15), 384 (60), 332 (100).

4,5-Epoxycholestan-3-one 20

To a solution of **9** (1 g, 2.6 mmol) in dichloromethane–methanol (100 ml, 1:1) under nitrogen, was added, dropwise, aqueous hydrogen peroxide (4 ml, 27.5%). The solution was stirred for 30 minutes, cooled to 0 °C, then aqueous sodium hydroxide (4 ml, 4 M) was added dropwise and the solution stirred for 12 hours. This was neutralised with hydrochloric acid (1 M) and water (100 ml) was added. The solution was extracted with dichloromethane and the combined organics dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. Flash chromatography using 10% ether in hexane yielded **20** as a mixture of the two diastereomers (2:1) (0.62 g, 58%);[20] δ_H (250 MHz, $CDCl_3$) 3.04 and 2.98 (2H, 2 s, HC-O), 0.92–0.85 (9H, m CH_3 C21/26/27), 0.70 and 0.68 (6H, 2 s, CH_3 C18); *m*/*z* (EI) 400 [(M^+) 63%].

Cholestane-3,4-dione 21

To a mixture of glacial acetic acid (5 ml) and concentrated sulfuric acid (0.1 ml) under nitrogen, was added **20** (1.3 g, 3.22 mmol) in portions, and the resulting suspension stirred for 4 hours, after which time it was poured into cold water (15 ml, ice bath). Filtration at reduced pressure yielded **21** (1.233 g, 95%); mp 145–147 °C (from MeOH) (lit.,[11] 146–148 °C); ν_{max} (KBr disc)/cm^{-1} 3413–3385s (enol OH), 2950–2850s (aliphatic C-H), 1670s (C=O), 1630m (C=O), 1382s, 1164m; δ_H (400 MHz, $CDCl_3$) 6.14 (1H, s, HOC=C C4), 3.00 (1H, m), 2.50 (2H, m), 1.18 (3H, s, CH_3 C19), 1.01 (3H, d, *J* 6, CH_3 C21), 0.88 (6H, d, *J* 7, CH_3, C26/27), 0.71 (3H, s, CH_3 C18); δ_C (100 MHz $CDCl_3$) 193 (C=O), 141 and 140 (*C*=*C*-OH), 56.4, 56.2, 54.0, 42.1, 40.0, 39.3, 37.0, 36.3, 36.0, 35.4, 34.9, 32.1, 31.0, 28.4, 28.2, 24.4, 24.0, 23.3, 23.0, 22.7, 21.2, 19.0, 17.2, 12.3.

4-(*tert*-Butyldimethylsilyloxy)cholest-4-en-3-one 22 and 3-(*tert*-butyldimethylsilyloxy)cholest-2-en-4-one 23

To a solution of **21** (1.23 g, 3.08 mmol) in dry THF (40 ml) at 0 °C under nitrogen, was added potassium *tert*-butoxide (370 mg, 3.3 mmol) and the reaction was stirred for 10 minutes then warmed to room temperature. *tert*-Butyldimethylsilyl chloride (1.6 g, 10.6 mmol) was added and the solution stirred for 20 minutes, after which time water was added. This was extracted with dichloromethane and the combined organics dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. Flash chromatography using 10% ether in hexane yielded a mixture of two compounds (900 mg). Recrystallisation from methanol yielded **23** as a white solid (225 mg, 14%); mp 162–164 °C (Found: C 76.8; H, 11.6%. $C_{33}H_{58}O_2Si$ requires C, 77.0; H, 11.3%); ν_{max} ($CHCl_3$)/cm^{-1} 2934–2869s (aliphatic C-H), 1681s (C=O), 1470w, 1383m, 1252m, 1110s; δ_H (400 MHz, $CDCl_3$) 5.96 (1H, dd, *J* 3 and 7, HC=C), 2.33 (1H, m), 2.22 (2H, m), 1.99 (2H, m), 0.94 (9H, s, $C(CH_3)_3$), 0.93 (3H, d, *J* 7, CH_3 C21), 0.86 (9H, m, CH_3 C19/26/27), 0.66 (3H, s, CH_3 C18), 0.14 (3H, s, Si-CH_3), 0.13 (3H, s, Si-CH_3); δ_C (100 MHz $CDCl_3$) 197.2 (C=O), 147.0 (O*C*=C), 124.4 (*C*=CO), 56.5, 56.4, 56.3, 54.3, 42.8, 40.7, 40.0, 39.7, 39.2, 36.4, 36.0, 35.0, 31.2, 28.4, 28.2, 26.0, 24.3, 24.0, 23.3, 22.8, 21.1, 20.9, 18.9, 18.6, 13.5, 12.2, −4.4 (C-Si), −4.5 (C-Si).

Evaporation of the mother liquor yielded **22** as a white solid (675 mg, 43%); mp 88–90 °C; ν_{max} ($CHCl_3$)/cm^{-1} 2950–2868s (aliphatic C-H), 1672s (C=O), 1469w, 1380w, 1250w, 1166m, 1111m; δ_H (400 MHz, $CDCl_3$) 3.13 (1H, m), 2.40 (2H, m), 0.93 (9H, s, $(CH_3)_3$), 0.90 (3H, d, *J* 7, CH_3 C21), 0.87 (9H, m, CH_3 19/26/27), 0.71 (3H, s, CH_3 C18), 0.14 (3H, s, Si-CH_3), 0.12 (3H, s, Si-CH_3); *m*/*z* (EI) 514 [(M^+) 2%], 499 (8), 457 (100).

4-(*tert*-Butyldimethylsilyloxy)cholest-4-en-3β-ol 24

To lithium aluminium hydride (20 mg, 0.53 mmol) in dry ether (10 ml) at −78 °C under nitrogen was added, *via* cannula, **22** (300 mg, 0.58 mmol) in dry ether (10 ml) and the reaction stirred for 1 hour. Two further portions of lithium aluminium hydride (20 mg, 0.53 mmol) were added, with 30 minutes between each addition. After a further hour, the reaction was quenched with water, allowed to warm to room temperature and neutralised with hydrochloric acid (1 M). The mixture was extracted with ether and the combined organics dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. Flash chromatography using 5% ether in hexane yielded **24** (140 mg, 51%); mp 43–44 °C; ν_{max} ($CHCl_3$)/cm^{-1} 2950–2859s (aliphatic CH), 1468m, 1382w, 1256m, 1157m; δ_H (400 MHz, $CDCl_3$) 4.04 (1H, m, *H*COH), 2.67 (1H, m), 1.99 (2H, m), 1.06 (3H, s, CH_3 C19), 0.98 (9H, s, $(CH_3)_3$), 0.91–0.86 (9H, m, CH_3 C21/26/27), 0.68 (3H, s, CH_3 C18), 0.18 (3H, s, H_3C-Si), 0.15 (3H, s, H_3C-Si); *m*/*z* (EI) 516 [(M^+) 5%], 459 (100), 332 (90).

3β-Hydroxycholestan-4-one 24a

To **24** (100 mg, 0.19 mmol) in dry THF (3 ml) under nitrogen, was added, dropwise, tetra-*n*-butylammonium fluoride (0.6 ml, 1 M in THF). The solution was stirred for 30 minutes and quenched by addition of water and extracted with ether. The combined organics were dried over sodium sulfate, filtered, and the solvent removed under reduced pressure to give 3β-hydroxycholestan-4-one **24a** (72 mg, 94%); mp 130–131 °C (lit.,[21] 115–121 °C) (HRMS (EI) found: M^+, 402.3500. $C_{27}H_{46}O_2$ requires *M*, 402.3498); ν_{max} ($CHCl_3$)/cm^{-1} 3590–3285w (OH), 2950–2869s (aliphatic C-H), 1710m (C=O), 1665m (enol C=C), 1636m, 1467m, 1386m; δ_H (400 MHz, $CDCl_3$) 6.07 (1H, s, HO-C), 4.11 (1H, m, *H*COH), 3.01 (1H, m), 1.18 (3H, s, CH_3 C19), 0.92–0.69 (9H, m, CH_3 C21/26/27), 0.66 (3H, s, CH_3 C18).

3β-Acetoxycholestan-4-one 25

To **24a** (180 mg, 0.45 mmol) in dry pyridine (5 ml) was added acetic anhydride (1 ml) and the reaction stirred overnight with a calcium chloride drying tube attached. The mixture was poured into water, extracted with ethyl acetate and the combined organics washed with aqueous sodium hydrogen carbonate (10%), aqueous potassium hydrogen sulfate (10%) and aqueous sodium chloride (10%), then dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. Recrystallisation from methanol yielded **25** as a white solid (105 mg, 51%); mp 118–120 °C (lit.,[21] 114–116 °C) (HRMS (EI) found: M^+, 444.3589. $C_{29}H_{48}O_3$ requires *M*, 444.3604); ν_{max} ($CHCl_3$)/cm^{-1} 2950–2860s (aliphatic CH), 1740s (C=O), 1723s (C=O), 1379m, 1242m; δ_H (400 MHz, $CDCl_3$) 5.15 (1H, dd, *J* 12 and 7, HCOAc), 2.16 (3H, s, H_3CCO_2), 0.90 (3H, d, *J* 6, CH_3 C21), 0.87 (6H, d, *J* 7, CH_3 C26/27), 0.78 (3H, s, CH_3 C19), 0.66 (3H, s, CH_3 C18); *m*/*z* (EI) 444 [(M^+) 100%], 402 (52), 384 (44).

4,4-Difluorocholestan-3β-yl acetate 25a

To **25** (40 mg, 0.09 mmol) under nitrogen, was added diethylaminosulfur trifluoride (0.3 ml, 2.27 mmol) and the solution stirred at 80 °C for 1 hour then cooled to 0 °C. The reaction was quenched by dropwise addition of water, and the solution extracted with dichloromethane. The combined organics were washed with saturated sodium hydrogen carbonate and water, dried over sodium sulfate, filtered, and the solvent removed

under reduced pressure. Flash chromatography using 10% ether in hexane yielded 4,4-difluorocholestan-3β-yl acetate **25a** as a white solid (20 mg, 45%); mp 108–110 °C (HRMS (EI) found: M^+, 466.3640. $C_{29}H_{48}O_2F_2$ requires *M*, 466.3622); ν_{max} ($CHCl_3$)/cm^{-1} 3029–2860s (aliphatic C-H), 1730s (C=O), 1602m, 1375m, 1245s; δ_H (400 MHz, $CDCl_3$) 5.49 (1H, m, HCOAc), 2.78 (1H, m, $HCCF_2$), 2.09 (3H, s, H_3CO_2), 1.12 (3H, s, CH_3 C19), 0.94 (3H, d, *J* 7, CH_3 C21), 0.89 (6H, d, *J* 7, 2 × CH_3 C26/27), 0.68 (3H, s, CH_3 C18); *m*/*z* (EI) 466 [(M^+) 29%], 386 (100).

4,4-Difluorocholestan-3β-ol 26

A solution of **25a** (20 mg, 0.04 mmol) in methanolic sodium bicarbonate (0.2%, 10 ml) was heated to reflux for 45 minutes then cooled to room temperature. The solvent was removed under reduced pressure then water and ethyl acetate added. The layers were separated and the aqueous layer extracted with further ethyl acetate. The combined organics were washed with water, dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. Flash chromatography using 15% ether in hexane yielded **26** (12 mg, 71%); mp 93–95 °C (HRMS (EI) found: M^+, 424.3483. $C_{27}H_{46}OF_2$ requires *M*, 424.3517); ν_{max} ($CHCl_3$)/cm^{-1} 2980–2870s (aliphatic C-H), 1710m, 1383m, 1110s; δ_H (400 MHz, $CDCl_3$) 4.33 (1H, m, *H*COH), 2.71 (1H, m, HC-CF_2), 1.10 (3H, s, CH_3 C19), 0.97 (3H, d, *J* 6, CH_3 C21), 0.88 (6H, dd, *J* 7 and 2, CH_3 C26/27), 0.68 (3H, s, CH_3 C18); δ_C (100 MHz, $CDCl_3$) 153.0, 150.4, 123.7, 123.6, 66.1, 66.0, 56.4, 56.3, 54.3, 54.3, 42.7, 40.0, 39.7, 37.7, 37.7, 36.4, 36.0, 35.6, 34.0, 34.0, 31.8, 29.9, 28.4, 28.2, 28.0, 27.9, 24.4, 24.1, 23.0, 22.8, 21.4, 21.0, 20.9, 19.1, 19.1, 18.9, 15.5, 12.2.

3′,3′-Difluoro-5,6-dihydro-3′*H*-cyclopropa[5,6]cholestan-3-yl acetate 27a

To a solution of sodium hydroxide (2.95 g, 0.073 mol) in methanol (35 ml) at 0 °C was added, dropwise, chlorodifluoroacetic acid (9.6 g, 0.073 mol) in methanol (15 ml). The solution was stirred for 30 minutes and the methanol removed under reduced pressure. The resulting salt was ground to a powder and dried under high vacuum for 2 hours at room temperature to give the sodium salt of chlorodifluoroacetic acid.

A solution of cholesteryl acetate **27** (2 g, 4.48 mmol) and chlorodifluoroacetic acid sodium salt (2.8 g, 14.8 mmol) in diglyme (100 ml) under nitrogen, was heated to reflux for 30 minutes. The solution was cooled and a further batch of chlorodifluoroacetic acid sodium salt (2.8 g) added. The solution was again heated to reflux for thirty minutes, and a further 2 batches of chlorodifluoroacetic acid sodium salt (2.28 g) were added. The solution was cooled to room temperature and extracted with brine and ether. The brine was washed with ether, and the combined organic layers dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. Purification by flash chromatography using 5% ether in hexane yielded 3′,3′-difluoro-5,6-dihydro-3′*H*-cyclopropa-[5,6]cholestan-3-yl acetate **27a** (392 mg, 23%), as a clear viscous oil; ν_{max} (KBr disc)/cm^{-1} 2840–2960s (aliphatic C-H), 1753s (C=O), 1446s, 1383s, 1026w; δ_H (250 MHz, $CDCl_3$) 5.0 (1H, m, HCOCO), 2.03 (3H, s, H_3CCO_2), 1.06 (3H, m, CH_3 C19), 0.88 (3H, d, *J* 2, CH_3 C21), 0.86 (6H, m, CH_3 C26/27), 0.65 (3H, s, CH_3 C18); δ_C (100 MHz, $CDCl_3$) 171.6 (C=O), 118.0 (dd, $^1J_{CF}$ 290 and 290, CF_2), 69.0 (H_3C-O), 58.2, 57.0, 56.4, 56.0, 46.1, 42.5, 40.1, 39.7, 36.3, 35.9, 34.9, 33.3, 30.9 (t, $^2J_{CF}$ 7, H*C*-CF_2), 29.1 (t, *J* 9, *C*-CF_2), 28.9, 28.4, 26.2, 25.0, 24.4, 24.2, 23.0, 22.8, 22.0, 21.5, 21.0, 18.9, 12.2; δ_F (377 MHz, $CDCl_3$) 142.7 (1F, d, $^2J_{FF}$ 160), 129.7 (1F, dd, $^2J_{FF}$ 160, $^3J_{HF}$ 15).

3′,3′-Difluoro-5,6-dihydro-3′*H*-cyclopropa[5,6]cholestan-3β-ol 28

To a solution of **27a** (120 mg, 0.25 mmol) in ethanol (6 ml) was added sodium hydroxide (10 mg, 0.25 mmol) in water (4 ml). The solution was heated to reflux for 90 minutes, then cooled and extracted with ether. The combined organic extracts were dried over sodium sulfate, filtered, and the solvent removed under reduced pressure. Purification by flash chromatography yielded **28** as a white solid (65 mg, 60%); mp 117–120 °C (HRMS (EI) found: M^+, 436.3507. $C_{28}H_{46}OF_2$ requires *M*, 436.3517); ν_{max} (KBr disc)/cm^{-1} 2880–2950s (aliphatic C-H), 1460m, 1383w, 1083w; δ_H (250 MHz, $CDCl_3$) 4.0 (1H, m, *H*COH), 0.86–0.91 (9H, m, CH_3 C21/26/27), 0.66 (3H, s, CH_3 C18); δ_F (377 MHz, $CDCl_3$) −130.0 (dd, $^2J_{FF}$ 160, $^3J_{HF}$ 15), −142.8 (d, $^2J_{FF}$ 160).

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