New Procedures for Selectively Protected Cholic Acid Derivatives. Regioselective Protection of the 12α-OH Group, and t-Butyl Esterification of the Carboxyl Group

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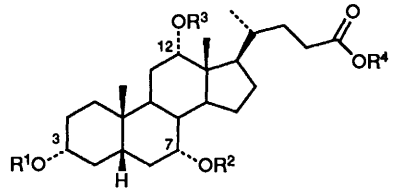
Effective procedures have been developed for the preparation of various selectively protected cholic acid derivatives. Treatment of cholic acid or methyl cholate with trifluoroacetic anhydride in THF, followed by partial deacylation under acidic conditions, leads to the 12α-trifluoroacetates (**10a**) and (**10m**), respectively. Trifluoroacetic anhydride may also be used as a condensing agent in the synthesis of t-butyl cholates. Particularly notable is the preparation of the ester (**10b**), which incorporates both these developments and is arguably the most efficient method yet for differentiating between positions 7 and 12 in the cholic acid nucleus.

The selective modification of the bile acids is important not only because of their biological significance[1,2] but also because their ready availability makes them attractive starting materials for other steroids.[3–7] Cholic acid (**1a**) is particularly inexpensive, and therefore of particular interest. In the course of work on the use of cholic acid as a building-block for functionalised macrocyclic host molecules ['cholaphanes,' *e.g.* compound (**15**)[8]] we have discovered a number of new methods for making

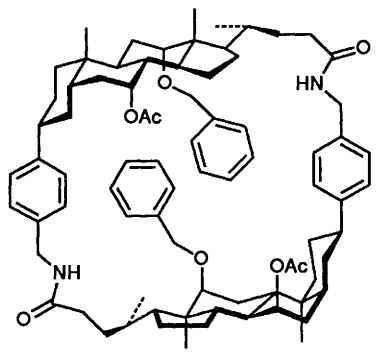


a $R^4 = H$
m $R^4 = Me$
b $R^4 = Bu^t$

	R^1	R^2	R^3
(1)	H	H	H
(2)	Ac	H	H
(3)	H	Ac	H
(4)	H	H	Ac
(5)	Ac	Ac	H
(6)	Ac	H	Ac
(7)	Ac	Ac	Ac
(8)	CF_3CO	H	H
(9)	H	CF_3CO	H
(10)	H	H	CF_3CO
(11)	CF_3CO	CF_3CO	H
(12)	CF_3CO	H	CF_3CO
(13)	CF_3CO	CF_3C	CF_3CO
(14)	Ac	H	CF_3CO

useful selectively protected derivatives. We now wish to communicate some of these procedures.

One of the major problems in the chemistry of cholic acid is the differentiation between the two axial hydroxyl groups at positions 7 and 12. Traditionally, two solutions have been available. Firstly, a number of reagents have been reported to selectively oxidise the 7-OH in the presence of the 12-OH



(15)

group.[9] However, recent work has cast doubt on the efficacy of these procedures,[10] and in any case they are inappropriate (or, at least inconvenient) if a change of oxidation state is not in itself required.† The alternative to oxidation, and until the present work the only practical method for the selective protection of one of these hydroxyls, is due to Fieser and Rajagopalan.[9] It involves the treatment of methyl cholate (**1m**) with acetic anhydride and pyridine to give the 3,7-diacetate (**5m**) in up to 70% crystallised yield without chromatography. If required, the acetyl group on the 3α-OH can be removed selectively with acidic methanol to give the monoacetate (**3m**).

As noted originally by Blickenstaff,[12] the selectivity of the 3,7-bis-acetylation is rather curious. It is expected, of course, that of the three hydroxyl groups the equatorial 3α-OH will be acetylated most rapidly. However, when the axial 7α and 12α hydroxyls are compared, it would appear that the 7α is the more hindered, principally due to the close approach of the 4α proton. Indeed, detailed investigations by Blickenstaff and co-workers confirmed that, in the absence of other functional groups, a 12α hydroxyl on a cholane nucleus is acetylated *ca.* 1.5 times faster than a 7α hydroxyl.[13] Their results suggested that the selective formation of 3,7-diacetate (**5m**) from methyl cholate (**1m**) was mainly due to a substantial (and unreciprocated) acceleration of the acetylation at the 7 position by a free 12α-OH.

† A modern addition to these procedures is the biochemical oxidation of cholic acid using enzymes which are selective for either the 7α or 12α hydroxyls.[11]

Table. Selected ^{1}H NMR data for trifluoroacetates (**8m**)–(**13m**).[a]

Compound	12-H t, *J* 3 Hz	7-H q, *J* 3 Hz	3-H m[b]	21-Me d, *J* 6 Hz	19-Me s	18-Me s	4-H$_\alpha$ q, *J* 13 Hz
(**8m**)	3.99	3.88	4.76	1.01	0.92	0.70	2.54
(**9m**)	3.98	5.11	3.45	n[c]	n	n	n
(**10m**)	5.33	3.90	3.45	0.80	0.90	0.79	n
(**11m**)	4.00	5.15	4.78	0.99	0.97	0.71	n
(**12m**)	5.32	3.91	4.78	0.83	0.93	0.80	2.49
(**13m**)	5.32	5.15	4.78	0.84	0.99	0.80	n

[a] Tris-trifluoroacetate (**13m**)[24] had been reported previously, and the 12-trifluoroacetate (**10m**) was prepared and characterised during the course of this work (see Experimental). The remaining compounds were not isolated, but spectra of mixtures containing varying proportions of the derivatives allowed confident assignments to be made. [b] Broad multiplet at 80 MHz. At 250 MHz, appears as tt, *J* 11.5, 4 Hz. [c] n = not assigned.

It appeared quite likely that this effect might be specific to the reaction with acetic anhydride–pyridine, and that other protection methods might be more sensitive to the steric difference between the 7α and 12α hydroxyl groups. Indeed, a literature survey uncovered a report by Schwartz *et al.*[14] which asserted that treatment of compound (**1b**) with Ac_2O in toluene, with KOAc as base, resulted in preferential 3,12-diacetylation [(**2m**):(**5m**):(**6m**):(**7m**) in the ratio 10.3:7.6:51:26.2]. Unfortunately, diacetate (**6m**) could only be isolated in 33% yield in a procedure which required chromatography, seriously limiting the usefulness of the method. It seemed that a practical, large-scale procedure for either a 12-protected or a 3,12-diprotected derivative of cholic acid, complementing the 3,7-diacetylation of Fieser and Rajagopalan, would be of clear value. Accordingly, we undertook to develop such a method.

As acetyl is the best-established OH protecting group in cholic acid chemistry, our initial investigations were aimed at an improved procedure for 3,12-diacetylation. In particular, methyl cholate (**1m**) was treated with acetic anhydride in the presence of a number of acidic catalysts. In accord with the results of Schwartz *et al.*, some selectivity was observed in favour of 3,12-*vs.* 3,7-diacetylation. Although we were unable to improve (or even to quite match) the selectivity obtained by the Czechoslovakian group, we did discover a method for facilitating the isolation of a 12-protected derivative. Thus, treatment of the crude product mixtures with MeOH–HCl (generated from MeOH–AcCl) not only deacetylated the 3α-OH (*vide supra*), but also showed some selectivity in favour of deacetylation at position 7. The end result was a procedure for the synthesis of 12-monoacetate (**4m**), a new selectively protected cholic acid derivative, in 29% yield from methyl cholate. Further details are given in the Experimental section.

Although perhaps of some value, this procedure has the same disadvantages as that of Schwartz *et al.* and, in addition, involves an inconveniently long reaction time. In search of an improved method, we investigated the use of a more reactive acylating agent, trifluoroacetic anhydride (TFAA). Trifluoroacetyl is not especially common as an OH protecting group, presumably because of its sensitivity to basic or nucleophilic conditions.[15] However, this lability is reduced in the case of hindered hydroxyl groups, and trifluoroacetylation has previously been found to be a useful method of blocking axial steroidal hydroxyls.[16,17] Thus, whereas equatorial trifluoroacetates in steroids are very readily hydrolysed by mild base (*e.g.* $NaHCO_3$–H_2O–MeOH,[16] or repeated washing of an ethereal solution with aqueous $NaHCO_3$[17]), axial examples survive such conditions.*

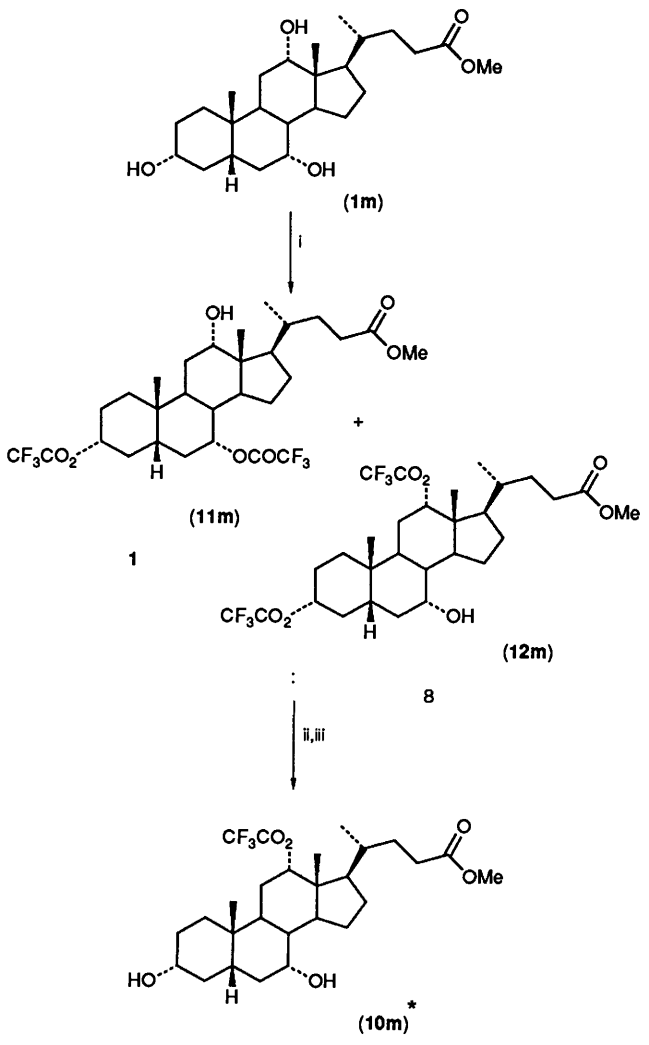
* It may be noted that axial acetates are correspondingly difficult to remove, to an extent which can prove problematical under some circumstances.[25,26]

Methyl cholate (**1m**) was treated with an excess of TFAA in a number of different solvents, to give mixtures of the trifluoroacetates (**8m**), (**11m**), (**12m**), and (**13m**). Analysis of the product mixtures was possible using ^{1}H NMR spectroscopy (see Table). As expected, trifluoroacetylation was much more rapid than the acetylation described above, and moreover we were pleased to find that the selectivity for the 12α-OH *versus* the 7α-OH was significantly enhanced. The best results were obtained in THF, the product ratio under optimal conditions (−35 to −40 °C, [TFAA] = 1.3M, 3 h) being (**8m**):(**11m**):(**12m**):(**13m**), ≈0:1:8:≈0. The two bis-trifluoroacetates proved difficult to separate, but again we found that acid-catalysed de-acylation improved matters. Thus, the crude product was treated with MeOH–HCl to give the mono-trifluoroacetates (**9m**) and (**10m**) in the ratio 1:10, from which mixture the 12-protected derivative (**10m**) could be isolated by crystallisation in 55% yield (Scheme 1).

We also found that, under the above trifluoroacetylation conditions, cholic acid can be converted directly to the 12-trifluoroacetate (**10a**) without the need for esterification of the carboxyl group. As shown in Scheme 2, treatment of cholic acid (**1a**) with TFAA lead to a mixture of partially trifluoroacetylated mixed anhydrides, principally (**16**) and (**17**). Aqueous workup, mild base hydrolysis and crystallisation gave the 12-trifluoroacetate (**10a**) in 65% yield.

The accessibility of the above mixed anhydrides suggested another development which appeared to be potentially valuable. Almost invariably, the carboxyl group in cholic acid has been protected as a methyl ester during synthetic modifications. However, there are many circumstances in which the acid-labile t-butyl ester group would have significant advantages. We had investigated the t-butyl esterification of cholic acid and found it to be less straightforward than might have been supposed. The best-known method, treatment with isobutene under acid catalysis,[18] led to an intractable mixture of products, and all the alternatives listed in the monograph of Greene[19] appeared unsuitable for molecules containing free hydroxyl groups. Attempts to transesterify methyl cholate (**1m**) with Bu^tOH–Bu^tOK (5 Å molecular sieve), following the method of Roelofsen *et al.*,[20] resulted in only partial conversion.

TFAA is well-known as a condensing agent for ester formation,[21] including t-butyl esters,[22] *via* mixed anhydrides such as (**16**) and (**17**). As expected, quenching of the cholic acid trifluoroacetylations with t-butanol proved to be an excellent method for t-butyl esterification. As shown in Scheme 2, this procedure allowed the isolation of the 12-protected ester (**10b**) in the highly satisfactory yield of 81%, or alternatively could be used in the preparation of t-butyl cholate (**1b**) in 90% yield. Neither procedure requires chromatography, and both are suitable for large-scale use. It is arguable that the preparation of ester (**10b**) is the most efficient practical method yet devised



Scheme 1. *Reagents and conditions:* i, TFAA (1.3M), THF, −35 to −40 °C, 3 h; ii, MeOH, AcCl; iii, crystallisation. * In 55% overall yield from compound (**1m**).

for differentiating positions 7 and 12 in the cholic acid nucleus.

It should be noted that, apart from being uniquely suitable for the case in hand, the use of TFAA for t-butyl esterification is unusually convenient and effective. It does not appear to be widely used, and perhaps deserves greater attention.

In the course of further work, we have performed a number of transformations on trifluoroacetates (**10a**) and (**10b**) which help to delineate the potential of these new derivatives (Scheme 2). Thus, trifluoroacetate (**10b**) was selectively acetylated at the 3-position to give the 3,12-diacylated derivative (**14b**), and the t-butyl ester cleaved with acid to provide the corresponding carboxylic acid (**14a**) in good yield. The more direct route to acid (**14a**) by acetylation of hydroxyacid (**10a**) with Ac_2O was lower-yielding and experimentally less convenient since, as has been previously observed,[23] acetylation of free bile acids leads to mixtures of partially *O*-acetylated anhydrides which can make product isolation awkward. Practically speaking, it has proved generally more efficient to handle cholic acid derivatives as the t-butyl esters which have convenient solubilities and chromatographic properties.

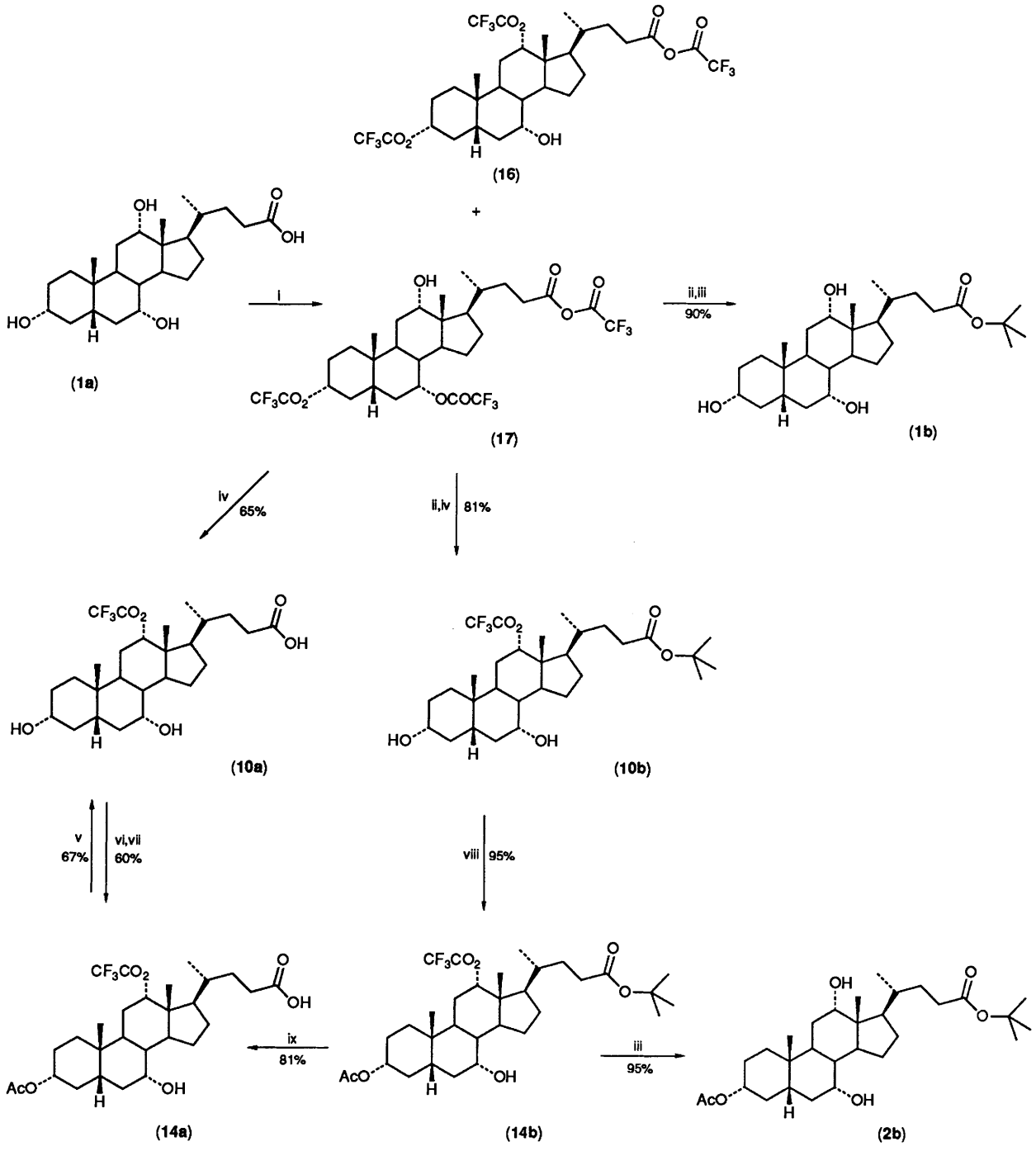
Finally, the conversions (**14a**) ⟶ (**10a**) and (**14b**) ⟶ (**2b**) illustrate the synthetic utility of the 12-*O*-trifluoroacetyl protecting group. It is stable under the acidic conditions used for removal of an equatorial 3-acetate, and in addition can be selectively removed when desired by mild basic hydrolysis.

Experimental

^1H NMR spectra were obtained in $CDCl_3$ as solvent with TMS as internal standard, using Bruker WP-80 (80 MHz) or WM-250 (250 MHz) spectrometers. IR spectra were recorded using a Perkin-Elmer 298 spectrometer. Mass spectra were obtained by positive ion FAB in *m*-nitrobenzyl alcohol matrices. Cholic acid was obtained from Lancaster Synthesis and used as received.

Methyl 12α-Acetoxy-3α,7α-dihydroxy-5β-cholan-24-oate (**4m**).—A solution of methyl cholate (1.0 g, 2.20 mmol of steroid with 1 MeOH of crystallization) in Ac_2O (50 ml) was left at room temperature for 1 month, and then poured onto crushed ice (200 g) and stirred for 2 h. Et_2O (150 ml) was then added. The organic layer was separated and washed with 2M aqueous NaOH until neutral, then washed with water and dried ($MgSO_4$). The solvent was removed under reduced pressure. Analysis by ^1H NMR spectroscopy indicated that the acetate derivatives (**2m**), (**5m**), (**6m**), and (**7m**) were present in the ratio ≈0:26:64:10. The crude product was dissolved in dry MeOH (10 ml) and acetyl chloride (0.5 ml) was added with stirring. After 3 h at room temperature, the solution was diluted with Et_2O (75 ml), washed until neutral with 5% aqueous $NaHCO_3$, and dried ($MgSO_4$). Evaporation of the solvent followed by flash chromatography with hexane–EtOAc (7:2) as eluant, gave a mixture (680 mg) containing the 7 and 12-monoacetates (**3m**) and (**4m**) in the ratio 1:4 (analysis by ^1H NMR spectroscopy). Two crystallisations from MeOH–H_2O (1:1) gave the 12-*monoacetate* (**4m**) as hydrated needles (300 mg, 29%) m.p. >102 °C. (The upper m.p. limit was indistinct, the melt remaining turbid up to *ca.* 120 °C.) An analytical sample was prepared by drying at 50 °C/0.5 mmHg for 2 h (the crystals became opaque) (Found: C, 68.3; H, 9.6. $C_{27}H_{44}O_6{\cdot}H_2O$ requires C, 68.47; H, 9.58%); ν_{max}(Nujol) 3 450, 3 300 (OH), 1 740 (C=O), and 1 250 cm^{-1}; δ_H(80 MHz) 5.10 (1 H, t, *J* 3 Hz, 12-H), 3.88 (1 H, q, *J* 3 Hz, 7-H), 3.66 (3 H, s, OMe), 3.50 (1 H, m, 3-H), 2.09 (3 H, s, COMe), 0.89 (3 H, s, 19-Me), 0.82 (3 H, d, *J* 6 Hz, 21-Me), and 0.74 (3 H, s, 18-Me).

Methyl 3α,7α-Dihydroxy-12α-trifluoroacetoxy-5β-cholan-24-oate (**10m**).—Trifluoroacetic anhydride (6.6 ml, 47.1 mmol) was added in one portion to a stirred solution of methyl cholate (1.0 g, 2.57 mmol) in dry THF (30 ml) at −40 °C. The cooling bath was maintained at −40 (±4) °C for 3 h after which the reaction was quenched with MeOH (10 ml), and while still cold, partitioned between Et_2O (100 ml) and 5% aqueous $NaHCO_3$ (100 ml). After gas evolution had ceased, the organic layer was washed with aqueous H_2SO_4 (0.5M; 50 ml), H_2O (50 ml), and dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give a white foam (1.4 g). ^1H NMR analysis indicated that the bis-trifluoroacetates (**11m**) and (**12m**) were present in the ratio 1:8. The crude product in dry MeOH (10 ml) was cooled in ice and treated with acetyl chloride (0.5 ml). After 4 h at 0 °C, Et_2O (75 ml) was added, and the mixture worked up as above to give a white foam (1.15 g). ^1H NMR analysis indicated that compounds (**9m**) and (**10m**) were present in the ratio 1:10. Two crystallisations from hexane–dichloromethane gave mono-trifluoroacetate (**10m**) as a white powder (0.678 g, 55%), m.p. 78–83 °C (Found: C, 62.7; H, 8.01. $C_{27}H_{41}O_6F_3$ requires C, 62.53; H, 7.97%); ν_{max}(Nujol) 3 400 br, 1 780 ($COCF_3$), 1 740 (CO_2Me), 1 220, and 1 165 cm^{-1}; δ_H(80 MHz) 5.33 (1 H, t, *J* 3 Hz, 12-H), 3.90 (1 H, q, *J* 3 Hz, 7-H), 3.68 (3 H, s, OMe), 3.45 (1 H, m, 3-H), 0.90 (3 H, s, 19-Me), 0.80 (3 H, d, *J* 6 Hz, 21-Me), and 0.79 (3 H, s, 18-Me).



Scheme 2. *Reagents and conditions:* i, TFAA, THF; ii, Bu^{t}OH; iii, NH_3 aq.; iv, $NaHCO_3$ aq., MeOH, THF; v, HCl aq., MeCN; vi, Ac_2O; vii, pH 7 buffer; viii, Ac_2O, py; ix, TFA.

3α,7α-Dihydroxy-12α-trifluoroacetoxy-5β-cholan-24-oic Acid (**10a**).—Trifluoroacetic anhydride (63 ml, 0.446 mol) was added to a stirred, refrigerated solution of cholic acid (10.0 g, 0.0244 mol) in dry THF (250 ml) at a rate such that the temperature of the solution remained below −40 °C (*ca.* 15 min addition time). The reaction mixture was maintained at between −35 and −40 °C for 4 h, monitoring the extent of acylation by TLC [hexane–acetone, 3:2; R_F (**8a**) 0.3, R_F (**11a**) and R_F (**12a**) 0.4, R_F (**13a**) 0.35], and then while still cold, poured into Et_2O (400 ml) and ice–water (100 ml). The organic layer was washed with water (200 ml), then washed cautiously with saturated aqueous $NaHCO_3$ until the aqueous layer was basic (3 × 200 ml). After further washings with brine (200 ml), aqueous H_2SO_4 (1M; 200 ml), and brine (200 ml), the organic layer was dried ($MgSO_4$), and evaporated to a foam (15.7 g). ^1H NMR analysis at this stage indicated a mixture of bis-trifluoroacetates (**11a**) and (**12a**) in the ratio 1:9 (±1). Saturated aqueous $NaHCO_3$ (65 ml) was added to a stirred solution of the above crude product in MeOH (150 ml) and THF (150 ml) and the milky suspension was stirred at room temperature. After 1 h another portion of saturated aqueous $NaHCO_3$ (10 ml) was added, and the mixture was stirred until completion of the reaction (4 h) as monitored by TLC. The mixture was poured into Et_2O (500 ml) and aqueous H_2SO_4 (0.6M; 250 ml), and the organic layer

was washed with water (2 × 100 ml), dried ($MgSO_4$), and evaporated to a foam (14.41 g). Crystallisation from acetonitrile–water afforded the *trifluoroacetate* (**10a**) (8.02 g, 65% from cholic acid).

Recrystallisation of a sample of (**10a**) from acetonitrile–water gave colourless needles m.p. 206–208 °C which, after drying for 5 h at 0.05 mmHg (room temperature) was analysed as the hemihydrate (Found: C, 60.6; H, 7.9. $C_{26}H_{39}O_6F_3 \cdot 0.5H_2O$ requires C, 60.8; H, 7.8%); $[\alpha]_D$ + 70° (*c* 0.8 in EtOH); ν_{max}(Nujol) 3 400 (OH), 1 775 ($COCF_3$), and 1 730 cm^{-1} (CO_2H); δ_H(250 MHz) 5.33 (1 H, t, *J* Hz, 12-H), 3.90 (1 H, q, *J* 3 Hz, 7-H), 3.45 (1 H, m, tt, *J* 11.5, 4 Hz, 3-H), 0.90 (3 H, s, 19-Me), 0.80 (3 H, d, *J* 6 Hz, 21-Me), and 0.79 (3 H, s, 18-Me); *m/z* 505.278 (MH^+), 469, 373, and 355.

t-(Butyl 3α,7α-*Dihydroxy*-12α-*trifluoroacetoxy*-5β-*cholan*-24-*oate* (**10b**).—Trifluoroacetic anhydride (63 ml, 0.446 mol) was added to a stirred, refrigerated solution of cholic acid (10.0 g, 0.0244 mol) in dry THF (250 ml) at a rate such that the temperature of the solution remained below −45 °C (*ca.* 15 min addition time). The reaction mixture was maintained at between 35 and −40 °C for 4 h, with monitoring of the extent of acylation by TLC (*vide supra*), and was then cooled to −55 °C. Dry t-butanol (100 ml) was added at a rate such that the temperature remained below −40 °C. The cooling bath was removed, and the mixture was left overnight at room temperature before being partitioned between Et_2O (250 ml) and ice–water (250 ml). The organic layer was washed with ice-cold aqueous NaOH (2M; 150 ml) followed by saturated aqueous $NaHCO_3$ (150 ml), then dried ($MgSO_4$), and evaporated to give a foam (17 g; contained some t-butanol). ^1H NMR analysis at this stage indicated a mixture of bis-trifluoroacetates (**11b**) and (**12b**) in the ratio 1:9(±1). Saturated aqueous $NaHCO_3$ (75 ml) was added to a stirred solution of the above crude product in MeOH (150 ml) and THF (150 ml) at ice-bath temperature, the ice-bath was removed, and the milky suspension was stirred at room temperature. After 1 h another portion of saturated aqueous $NaHCO_3$ (20 ml) was added, and the mixture was stirred until completion of the reaction (4 h) as monitored by TLC [hexane–ethyl acetate, 1:1, R_F (**10b**) 0.35]. The mixture was poured into Et_2O (500 ml) and ice–water (300 ml), and the organic layer was washed with water (300 ml) and aqueous phosphate buffer (pH 7; 1M; 200 ml), then dried ($MgSO_4$), and evaporated to a foam (14.6 g). The crude product was dissolved in refluxing hexane (400 ml), filtered while hot, and on standing overnight deposited *trifluoroacetate* (**10b**) (10.0 g, 81% from cholic acid) as fine silky needles, m.p. 122.5–124 °C, regioisomerically pure by ^1H NMR spectroscopy (250 MHz). Recrystallization from hexane afforded an analytical sample, m.p. 123–125 °C (Found: C, 64.3; H, 8.7. $C_{30}H_{47}O_6F_3$ requires C, 64.27; H, 8.45%); $[\alpha]_D$ +57° (*c* 0.4 in $CHCl_3$); ν_{max}(Nujol) 3 400 (OH), 1 780 ($COCF_3$), and 1 730 cm^{-1} (CO_2Bu^t); δ_H(250 MHz) 5.33 (1 H, t, *J* 3 Hz, 12-H), 3.90 (1 H, q, *J* 3 Hz, 7-H), 3.45 (1 H, m, tt, *J* 11.5, 4 Hz, 3-H), 1.44 (9 H, *s*, t-butyl), 0.90 (3 H, *s*, 19-Me), 0.80 (3 H, d, *J* 6 Hz, 21-Me), and 0.79 (3 H, *s*, 18-Me); *m/z* 561 (MH^+), 541, 524, 505, 485, 469, 429, 411, 391, 373, and 355.

t-Butyl 3α,7α,12α-*Trihydroxy*-5β-*cholan*-24-*oate* (*t-Butyl Cholate*) (**1b**).—Trifluoroacetic anhydride (20 ml, 90.14 mmol) was added over 1 min to a stirred solution of cholic acid (5.0 g, 12.3 mmol) in dry THF (100 ml) at ice-bath temperature. The ice-bath was removed, and after 80 min the solution was re-cooled and dry t-butanol (30 ml) was added. After 7 h at room temperature, aqueous NH_3 (20 ml; 35% w/w) was added with cooling, keeping the temperature below 20 °C, and the solution was left for 12 h at 0 °C. Another portion of aqueous NH_3 (10 ml) was added and after a further 4 h at room temperature the mixture was partitioned between Et_2O (200 ml) and water (100 ml) [the progress of trifluoroacetate hydrolysis is conveniently monitored by TLC with EtOAc as eluant, R_F (**1b**) 0.4]. The organic layer was washed with aqueous NaOH (1M; 100 ml), and water (2 × 100 ml), then dried ($MgSO_4$), and evaporated to a foam. Crystallization from acetonitrile (40 ml) afforded *t-butyl ester* (**1b**) (5.13 g, 90%), pure by TLC and NMR spectroscopy, m.p. 96–110 °C. Recrystallization from hexane gave an analytical sample, m.p. 114–116 °C (Found: C, 72.1; H, 10.3. $C_{28}H_{48}O_5$ requires C, 72,37; H, 10.41%); ν_{max}(Nujol) 3 350 (OH), 1 730 cm^{-1} (C=O); δ_H(250 MHz) 3.98 (1 H, t, *J* 3 Hz, 12-H), 3.87 (1 H, q, *J* 3 Hz, 7-H), 3.46 (1 H, m, tt, *J* 11.5, 4 Hz, 3-H), 1.44 (9 H, s, t-butyl), 0.97 (3 H, d, *J* 6 Hz, 21-Me), 0.89 (3 H, s, 19-Me), and 0.68 (3 H, s, 18-Me).

t-Butyl 3α-*Acetoxy*-7α-*hydroxy*-12α-*trifluoroacetoxy*-5β-*cholan*-24-*oate* (**14b**).—A solution of trifluoroacetate (**10b**) (2.5 g, 4.46 mmol), Ac_2O (2 ml, 21.2 mmol) and pyridine (2 ml, 24.7 mmol) in dichloromethane (10 ml) was left at room temperature for 33 h, and was then partitioned between Et_2O (50 ml) and aqueous H_2SO_4 (6M; 50 ml). The organic layer was washed with water (50 ml), saturated aqueous $NaHCO_3$ (50 ml), and aqueous phosphate buffer (pH 7; 1M; 50 ml), then dried and evaporated to a foam (2.63 g). Chromatography on silica gel with hexane–EtOAc (3:1) as eluant afforded the *acetyl derivative* (**14b**) (2.54 g, 95%) as an oil (Found: C, 64.0; H, 7.9. $C_{32}H_{49}O_7F_9$ requires C, 63.77; H, 8.19); ν_{max}(neat) 3 500 (OH), 1 780 ($COCF_3$), and 1 740 (cm^{-1} (CO_2Bu^t); δ_H(250 MHz) 5.32 (1 H, t, *J* 3 Hz, 12-H), 4.52 (1 H, m, tt, *J* 11.5, 4 Hz, 3-H), 3.85 (1 H, q, *J* 3 Hz, 7-H), 2.02 (3 H, s, 3-OAc), 1.44 (9 H, s, t-butyl), 0.91 (3 H, s, 19-Me), 0.83 (3 H, d, *J* 6 Hz, 21-Me), and 0.80 (3 H, s, 18-Me); *m/z* 601.329 ($M - H^+$), 541, 485, 415, and 355.

3α-*Acetoxy*-7α-*hydroxy*-12α-*trifluoroacetoxy*-5β-*cholan*-24-*oic Acid* (**14a**).—Trifluoroacetic acid (15 ml) was added to a stirred solution of t-butyl ester (**14b**) (2.43 g, 4.04 mmol) in dry dichloromethane (15 ml) at ice-bath temperature. The ice-bath was removed, and after 45 min the reaction mixture was partitioned between dichloromethane (100 ml) and water (100 ml). The organic layer was washed with water (2 × 100 ml), dried ($MgSO_4$), and evaporated to a foam (2.27 g). Crystallization from hexane–Et_2O afforded *acid* (**14a**) (1.79 g, 81%) m.p. 91–98 °C (Found: C, 61.7; H, 7.8. $C_{28}H_{41}O_7F_3$ requires C, 61.53; H, 7.56); ν_{max}(Nujol) 3 400 (OH), 1 780 ($COCF_3$), 1 730, and 1 710 cm^{-1} (C=O); δ_H(250 MHz) 5.32 (1 H, t, *J* 3 Hz, 12-H), 4.52 (1 H, m, tt, *J* 11.5, 4 Hz, 3-H), 3.85 (1 H, q, *J* 3 Hz, 7-H), 2.02 (3 H, s, 3-OAc), 0.91 (3 H, s, 19-Me), 0.83 (3 H, d, *J* 6 Hz, 21-Me), and 0.80 (3 H, s, 18-Me); *m/z* 545 ($M - H^+$), 485, 468, 455, 431, 415, 371, and 355.

Conversion of 3α,7α-*Dihydroxy*-12α-*trifluoroacetoxy*-5β-*cholan*-24-*oic Acid* (**10a**) *to* 3α-*Acetoxy*-7α-*hydroxy*-12α-*trifluoroacetoxy*-5β-*cholan*-24-*oic Acid* (**14a**).—A solution of hydroxy acid (**10a**) (3.0 g, 5.49 mmol) in Ac_2O (30 ml) and $CHCl_3$ (15 ml) was maintained at 35–40 °C for 40 h, and then the volatile components were evaporated at 50 °C/0.1 mmHg. The residue was dissolved in acetonitrile (50 ml), aqueous phosphate buffer (pH 7; 1M; 20 ml) was added and the mixture was stirred at 50 °C for 4 h. Most of the acetonitrile was evaporated under reduced pressure and the residue partitioned between Et_2O (150 ml) and aqueous H_2SO_4 (6M; 50 ml). The organic layer was washed with brine (50 ml), dried ($MgSO_4$), and evaporated under reduced pressure. Chromatography on silica gel, with hexane–acetone–acetic acid (3:2:0.02) as eluant, followed by crystallization from hexane–Et_2O, afforded the acetyl derivative (**14a**) (1.8 g, 60%), identical with the material prepared above.

Conversion of 3α-*Acetoxy*-7α-*hydroxy*-12α-*trifluoroacetoxy*-

5β-cholan-24-oic Acid (**14a**) *to* *3α,7α-Dihydroxy-12α-trifluoroacetoxy-5β-cholan-24-oic Acid* (**10a**).—Aqueous HCl (0.5 ml; 35% w/w) was added to a stirred solution of acetate (**14a**) (50 mg, 91.6 μmol) in acetonitrile (1 ml). After 5 h at room temperature the reaction mixture was partitioned between dichloromethane (20 ml) and aqueous phosphate buffer (pH 7; 1M; 20 ml), and the organic layer was washed with more buffer (20 ml), dried ($MgSO_4$), and evaporated to an oil. Crystallization from EtOAc–Et_2O followed by recrystallization from acetonitrile–water afforded pure (**10a**) (31 mg, 67%), identical with the material prepared above.

t-Butyl 3α-Acetoxy7α-12α-dihydroxy-5β-cholan-24-oate (**2b**).—Aqueous ammonia (0.1 ml; 35% w/w) was added to a stirred solution of trifluoroacetate (**14b**) (50 mg, 83 μmol) in THF (1.0 ml) and MeOH (1.0 ml). After 1 h the reaction mixture was partitioned between Et_2O (20 ml) and aqueous phosphate buffer (pH 7; 1M; 20 ml), and the organic layer was dried ($MgSO_4$), and solvent removed under reduced pressure. Chromatography in hexane–EtOAc (3:2) gave *acetate* (**2b**) (40 mg, 95%) as an oil which slowly crystallized. Crystallization from hexane–Et_2O afforded an analytical sample, m.p. 160–161 °C (Found: C, 70.9; H, 10.1. $C_{30}H_{50}O_6$ requires C, 71.11; H, 9.95); ν_{max}(Nujol) 3 400 (OH), and 1 730 cm^{-1} (C=O); δ_H(250 MHz) 4.58 (1 H, m, tt, *J* 11.5, 4 Hz, 3-H), 3.99 (1 H, t, *J* 3 Hz, 12-H), 3.85 (1 H, q, *J* 3 Hz, 7-H), 2.01 (3 H, s, 3-OAc), 1.44 (9 H, s, t-butyl), 0.98 (3 H, d, *J* 6 Hz, 21-Me), 0.91 (3 H, s, 19-Me), and 0.70 (3 H, s, 18-Me).

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