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Cholic Acid as an Architectural Component in Biomimetic/Molecular Recognition Chemistry; Synthesis of "Cholaphanes" With Facial Differentiation of Functionality.

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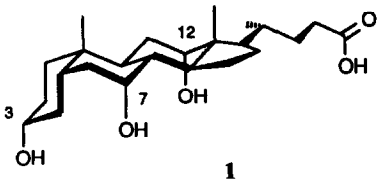
Abstract *Facially differentiated cholaphanes* (**2**) *were synthesized in good yields from cholic acid* (**1**) *Key steps were the selective 3,7 bis-O-acetylation of methyl cholate* (**3**) *the 12-O-benzylation of diacetate* **4**, *and the introduction of a 3β (p-aminomethyl)phenyl substituent using an arylmanganese reagent and employing the novel 'benzostabase"* N *protection methodology*

Introduction

The two preceding articles[1] have highlighted the value of cholic acid (**1**) as a starting material for the assembly of extended, preorganised molecular frameworks with applications in molecular recognition/biomimetic chemistry In addition to the size and rigidity common to all steroids, this inexpensive bile acid has a nicely-spaced array of functionality which facilitates its elaboration into complex architectures and allows for variable substitution within such structures We have already described how the (equatorial) C3-OH in **1** may be distinguished from the (axial) C7 and C12 hydroxyls, and used in conjunction with the carboxyl group to construct macrocyclic "cholaphane" frameworks [1-3] We now give details for the differential protection of the axial hydroxyls, and for the conversion of the resulting derivatives into facially-differentiated cholaphanes **2** [3] Included in this work is a modification of our original procedure for cholaphane synthesis, in which the bis(trimethylsilyl) *N*-protection used in the introduction of the aryl spacer unit is replaced by the novel "benzostabase" (BSB) methodology [4]



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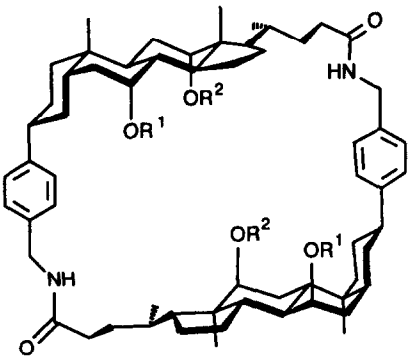
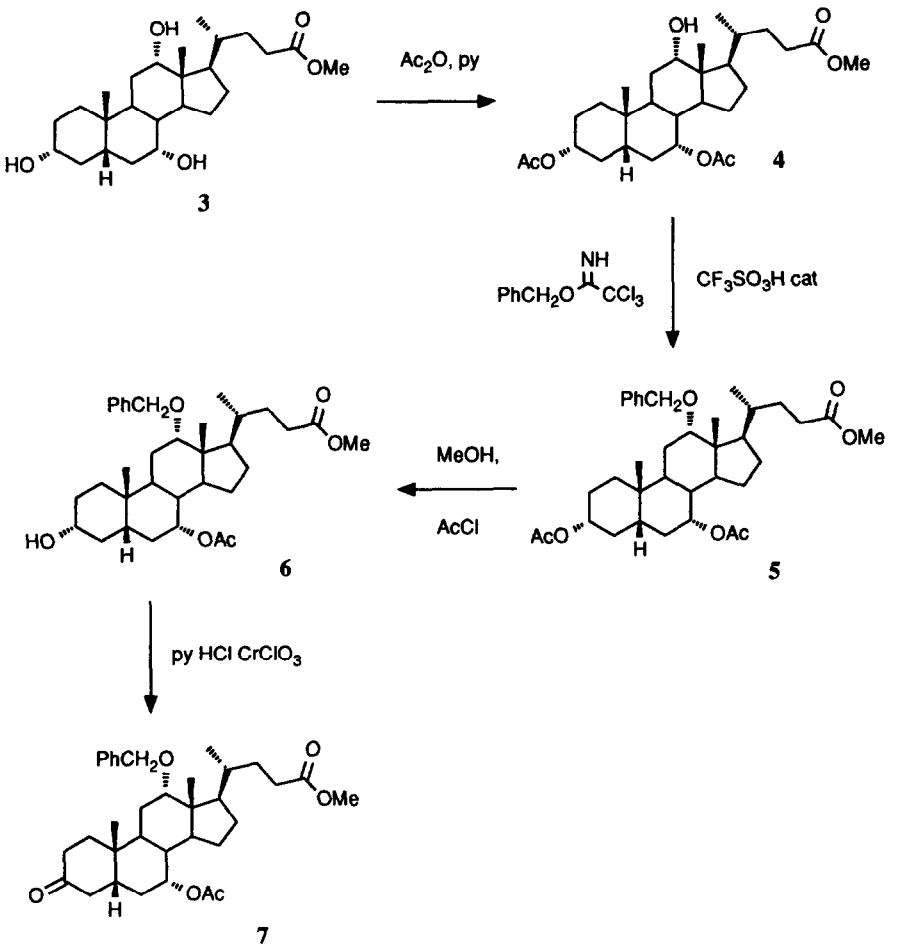


2a $R^1 = Ac$, $R^2 = CH_2Ph$
2b $R^1 = H$, $R^2 = CH_2Ph$
2c $R^1 = Ac$, $R^2 = H$

Results and Discussion

While it is relatively simple to distinguish chemically between the equatorial hydroxyl group at C3 of **1** and its axial counterparts at carbons 7 and 12, differentiation of the latter is, not surprisingly, less straightforward Fortunately, the problem had already been addressed by earlier generations of workers who were concerned with other aspects of steroid chemistry In particular, it had been found by Fieser and Rajagopalan that, in the acetylation of methyl cholate **3** with acetic anhydride and pyridine, rapid reaction at position 3 was followed by selective acylation of the 7-OH to give the 3,7-diacetate **4** in 70% yield (Scheme 1) [5,6] Our earlier work had established that acetylation would be a satisfactory protection method for the 7-OH, being compatible with the organomanganese methodology used to introduce the aryl spacer group at C3 It was only therefore necessary to identify a protecting group for the 12-OH which could be introduced in good yield, carried through the cholaphane synthesis and would be "orthogonal" to the 7-OAc with respect to conditions of removal

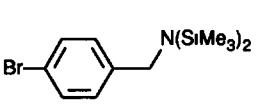
Although *O*-benzyl was an obvious candidate, it was not clear that it would be possible to alkylate a hindered hydroxyl group such as the 12α-OH in a molecule with several other functional groups Indeed, attempts to benzylate diacetate **4** using (a) sodium hydride, benzyl bromide and tetrabutylammonium iodide in THF,[7] (b) benzyl bromide and silver oxide in DMF[8] and (c) hexabutyldistannoxane followed by benzyl bromide and tetrabutylammonium bromide[9] all failed to give the desired product **5** However, the method of Bundle and co-workers,[10] employing benzyl trichloroacetimidate and trifluoromethanesulphonic acid, was more successful The conversion was not entirely clean due to the formation of non-polar byproducts, but careful optimisation resulted in a procedure giving **5** in 68% yield without recourse to chromatography (Scheme 1) Acid-catalysed 3-deacetylation to **6** and oxidation to ketone **7** proceeded uneventfully *via* the methodology established in the earlier work,[1] the overall yield of **7** from **4** being an acceptable 61%



Scheme 1

For the elaboration of ketone **7** into cholaphane **2a**, our initial studies again used methods previously developed for the synthesis of the "symmetrically-protected" (i e tetra-acetyl) analogue [1] Thus, *p*-bromo-*N*,*N*-bis(trimethylsilyl)benzylamine (**8**) was treated with lithium metal to give the corresponding aryllithium which, after conversion to an organomanganese reagent with MnI_2, added to the keto group of **7** with good yields and chemoselectivity Work-up of the reaction with trifluoroacetic anhydride (TFAA) resulted in *N*-desilylation, *N*-trifluoracetylation and elimination to give (presumably)[11] a mixture of regioisomeric alkenes **13** (*cf* Scheme 3), and further transformations (*vide infra*) led to **2a** While this work was successful, it was hampered by the fact that the lithiation step proved somewhat capricious unless freshly-prepared **8** was used Presuming that the difficulty was due to inhibition by Li-N species derived from desilylated reagent, we felt that a procedure based on a more stable analogue of **8** might be more convenient and reliable Our cholaphane programme had stimulated a general interest in methodology for

N-protection under strongly basic conditions, which had resulted in the development of the "benzostabase" (BSB) protecting group, as in **9**[4] As *N*BSB derivatives had proved quite easy to handle, we decided to apply our new method in the present work



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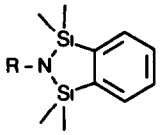
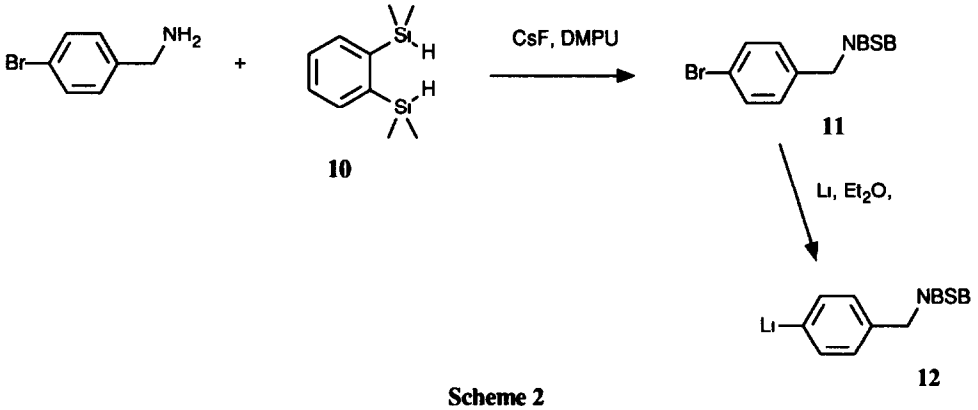


≡ R-NBSB

9

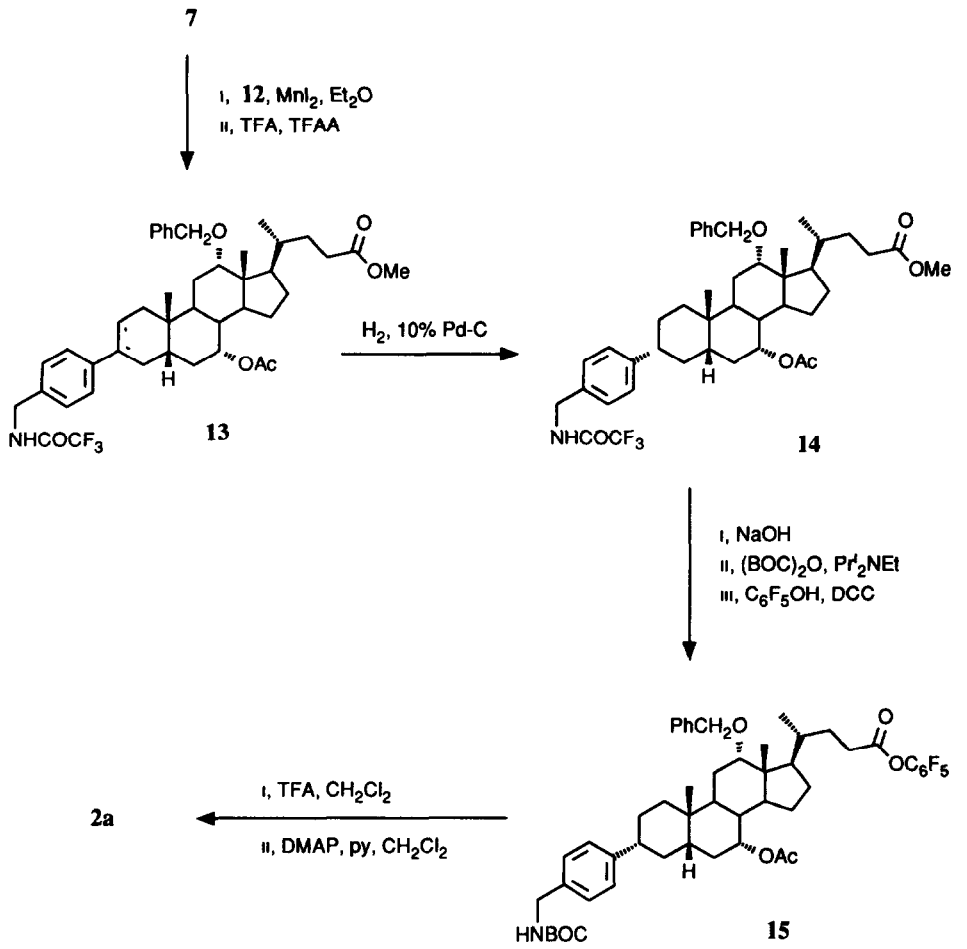
p-Bromobenzylamine was prepared from *p*-bromobenzyl bromide *via* the Gabriel synthesis,[12] and treated with 1,2-bis(dimethylsilyl)benzene (**10**) and caesium fluoride in DMPU[13] The crystalline BSB derivative **11** was isolated in 96% yield Unlike the bis(trimethylsilyl) derivative (**8**), compound **11** was found to be shelf-stable and to react smoothly and reliably with lithium metal to give the corresponding aryllithium **12** (Scheme 2)



10 **11** **12**

Scheme 2

The pathway from **7** to **2a**, employing **12** as aryl donor, is summarised in Scheme 3 Aside from its ease of formation the BSB-protected reagent behaved exactly like its bis(trimethylsilyl) analogue, reacting with **7** to give alkene(s) **13** in *ca* 90% yield Hydrogenation over Pd/C could be accomplished without significant cleavage of the *O*-benzyl protecting group, giving selectively the α-arylated steroid **14** in 75% overall yield from **7** Although we were not able to avoid the use of chromatography during these procedures, it was possible to obtain 18 g of **14** in a single batch without the sacrifice of undue amounts of silica gel (see Experimental) Deprotection of the amino and carboxyl functions was followed by *N*-reprotection with di-*tert*-butyl pyrocarbonate, then carboxyl activation with pentafluorophenol and DCC, giving the macrocycle precursor **15** in 83% yield



Scheme 3

For the cyclodimerisation we made another modification to our earlier methodology [1] In the synthesis of the tetra-acetyl analogue of **2a**, we had treated the corresponding precursor with TFA to replace NHBOC with NH_3^+, then deprotonated with K_2HPO_4 in $CHCl_3$/DMF at high dilution so that the amino and pentafluorophenyl ester groups could react with each other The procedure gave a 65% yield of crystalline cyclodimer, which seemed to be very satisfactory considering the size of the ring formed (≥38 atoms) However, during the course of the present work we developed a variant which gave even better results After treatment of **15** with TFA as described above, the *N*-protonated pentafluorophenyl ester was dissolved in dichloromethane and added slowly to a solution of DMAP and pyridine in a large volume of the same solvent Cholaphane **2a** was isolated in the remarkable yield of 90%, after crystallisation

Finally, as the main aim of this work was to synthesize cholaphanes capable of selective modification at either face, it was necessary to demonstrate that the 12-*O*-benzyl and 7-*O*-acetyl protecting groups could be removed independently without disturbing the rest of the molecule Thus, treatment of **2a** with ethanolic KOH resulted in deacetylation to give diol-diether **2b**, while hydrogenolysis of **2a** over 10% Pd/C gave diol-diacetate **2c** Both conversions took place cleanly and in good yield (*ca* 90% or greater) Compound **2b** proved useful in later work concerning the binding of carbohydrate derivatives in organic solvents [14] Although less active than the fully deprotected tetraol, it had greater solubility in organic solvents and was more amenable to study by e g solution IR

In conclusion, we have shown that the facially-differentiated cholaphanes **2a-c** may be prepared from cholic acid in substantial quantities and very acceptable yields (the overall yield of **2a** from diacetate **4** may be calculated to be 34%) This work illustrates the synthetic control possible in the construction of receptor molecules from cholic acid, and should facilitate the preparation of a range of cholaphanes with binding and/or catalytic functionality

Experimental Section

General ^{1}H NMR spectra were recorded on Bruker WP 80 (80 MHz), Jeol JNM 270 (270 MHz) or Bruker MSL 300 (300 MHz) spectrometers TMS was used as the internal standard ^{13}C NMR spectra were recorded on the JNM 270 or MSL 300 instruments, using $CDCl_3$ as internal standard IR spectra were recorded on Perkin-Elmer 298 or 883 spectrophotometers Analytical thin layer chromatography (TLC) was performed on aluminium sheets coated with silica gel 60 (0 2 mm layer thickness) Steroidal compounds were visualised by charring over a flame Silica gel 60, 400-230 mesh (Merck) was used for flash chromatography Reactions involving sonication were performed in a B & T laboratory ultrasonic cleaning bath 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) was dried by distillation from calcium hydride at reduced pressure onto 4Å molecular sieves The dichloromethane used in the macrocyclisation was dried by distillation from calcium hydride Lithium wire of 98% purity (sodium content ~1%) was obtained from Aldrich MnI_2 was prepared from ground Mn flakes and I_2 according to the published procedure [15]

Methyl 7α-Acetoxy-12α-benzyloxy-3-oxocholan-24-oate (**7**) The diacetate **4**[5] (10 10 g, 19 93 mmols) was dried by evaporation of a solution in toluene, then dissolved in a minimum volume of carbon tetrachloride (*ca* 100 ml) Benzyl trichloroacetimidate (freshly distilled, 5 82 g, 39 88 mmols) was added, followed by cyclohexane (100 ml) The mixture was stirred under argon at room temperature for approximately 36 hours, during which time trifluoromethanesulphonic acid (0 3 ml) was added in three equal portions, the first being used to start the reaction and the others to restart it when analysis by TLC implied that it was no longer proceeding When all the starting material had been consumed, the reaction was quenched by addition of pyridine (*ca* 0 5 ml) Evaporation gave an oily white solid which was triturated with further carbon tetrachloride, causing the product to dissolve but leaving the byproduct 1,1,1-trichloroacetamide as a solid After removal of the latter by filtration, the solvent was evaporated to give an oil. Crystallisation from methanol at -20 °C (several crops collected over a period of days, with reduction of mother liquors after each filtration) gave the benzyl ether **5** (8 1 g, 68%) ^{1}H NMR (300 MHz,

$CDCl_3$) δ 7 36 (m, 5 H, Ar), 4 90 (br q, 1 H, 7β-H), 4 65, 4 37 (ABq, J = 12 Hz, 2 H, OCH_2Ph), 4 53 (m, 1 H, 3β-H), 3 70 (br s, 1 H, 12β-H), 3 66 (s, 3 H, CO_2Me), 2 06 (s, 3 H, OAc), 2 02 (s, 3 H, OAc), 0 93 (s, 3 H, 19-Me), 0 91 (d, J = 6 Hz, 3 H, 21-Me), 0 71 (s, 3 H, 18-Me) A portion of this material (5 0 g, 8 4 mmols) was dissolved in methanol, and acetyl chloride (1 ml) was added dropwise The mixture was stirred overnight at room temperature, after which analysis by TLC implied that all starting material had been consumed Evaporation gave an oil which was dissolved in dichloromethane, washed with dilute sodium hydroxide and dried The dichloromethane was removed *in vacuo* and the residue crystallised from ether to give alcohol **6** (4 4 g, 94 4%) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7 34 (m, 5 H, Ar), 4 89 (br q, 1 H, 7β-H), 4 64, 4 37 (ABq, J = 12 Hz, 2 H, OCH_2Ph), 3 70 (br s, 1 H, 12β-H), 3 66 (s, 3 H, CO_2Me), 3 40 (m, 1 H, 3β-H), 2 07 (s, 3 H, OAc), 0 92 (s, 3 H, 19-Me), 0 89 (d, 3 H, 21-Me), 0 70 (s, 3 H, 18-Me) A portion of this material (4 0 g, 7 21 mmols) was dissolved in dry dichloromethane (50 ml), to which were added pyridinium chlorochromate (4 0 g, 19 mmols) and activated 4Å molecular sieves (*ca* 0 2 g) The mixture was stirred under argon for 4 hours, then diluted with ether (150 ml) and stirred for a further half hour The resultant suspension was passed through a silica plug which was washed with further volumes of ether (3 × 50 ml) The washings were combined and the ether removed *in vacuo* leaving an oil which was crystallised from hexane and ether yielding ketone **7** (3 8 g, 96%) m p 92-94 °C, IR (film from $CHCl_3$) 1710 br (C=O) cm^{-1}, ^{1}H NMR (80 MHz, $CDCl_3$) δ 7 33 (m, 5 H, Ar), 4 98 (br q, 1 H, 7β-H), 4 55, 4 49 (ABq, J = 12 Hz, 2 H, OCH_2Ph), 3 75 (br t, 1 H, 12β-H), 3 66 (s, 3 H, CO_2Me), 2 97 (br t, J = 14 Hz, 1 H, 4α-H), 2 05 (s, 3 H, OAc), 1 02 (s, 3 H, 19-Me), 0 93 (d, J = 6 Hz, 3 H, 21-Me), 0 74 (3 H, s, 18-Me) Anal Calcd for $C_{34}H_{48}O_6$ C, 73 88, H, 8 75 Found C, 73 60, H, 9 00

1,2-*Bis(dimethylsilyl)benzene* (**10**) [16] Magnesium (23 3 g, 959 mmol, 2 26 equiv) was overlaid with dry THF (100 ml) under an atmosphere of argon Chlorodimethylsilane (91 28 g, 965 mmol, 2 27 equiv) was added in three portions over 1 h, while 1,2-dibromobenzene (100 g, 424 mmol, 1 equiv) in dry THF was introduced dropwise at such a rate as to maintain reflux (1 5 h) When the addition was complete, the mixture was heated under reflux for 2 5 h then cooled to room temperature Hexane (1 L) and aqueous HCl (1 18 M, 1 L) were added, and the mixture stirred until the salts had dissolved and the organic layer appeared clear The organic layer was separated and the aqueous layer extracted with hexane (150 ml) The combined organic extracts were dried ($MgSO_4$) and the solvent evaporated to give a brown liquid Vacuum distillation through a Vigreaux column (10 cm) gave the silane **10** as a colourless oil (62 61 g, 326 mmol, 77%) b p 90-112 °C at 15 mm Hg (lit [17] 93 °C at 10 mm Hg), IR (liquid film) 2149 (Si-H), 1261, 1250, 1147, 1124, 1058, 1037, 890, 834, 769, 744, 671 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7 58-7 54, 7 35-7 31 (2 m, 2 x 2 H, Ar), 4 69 (septet, J = 3 7 Hz, 2 H, SiH), 0 36 (d, J = 3 7 Hz, 12 H, $SiCH_3$), ^{13}C NMR (75 47 MHz, $CDCl_3$) δ 144 31, 134 28, 128 39, -2 62 ($SiCH_3$)

p-*Bromobenzylamine* [18] *p*-Bromobenzyl bromide (102 50 g, 410 mmol) and potassium phthalimide (86 43 g, 467 mmol) in DMF (0 5 L) were stirred with cooling on a water bath at room temperature for 23 h Removal of solvent on a rotary evaporator gave the *N*-alkylated phthalimide as a white solid IR (nujol mull) 1883, 1776, 1490, 1471, 1431, 1396, 1328, 1109, 1072, 1011, 961, 927, 791, 728, 712 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$-CD_3OD, 1 1) δ 7 87-7 76 (m, 4 H, *o*-disubstituted Ar), 7 44, 7 30 (ABq, J = 8 3 Hz, 4 H, *p*-disubstituted Ar), 4 79 (s, 2 H, CH_2), ^{13}C NMR (75 47 MHz, $CDCl_3$-CD_3OD, 1 1) δ 167 56 (*C*=O), 134 83, 133 66, 131 20, 130 99, 122 65, 120 98, 40 08 (CH_2) This material was stirred under reflux with

hydrazine monohydrate (90 ml, 1 86 mol) in ethanol (1 L) and water (300 ml) for 16 h under an atmosphere of nitrogen, to give a yellow solution The solvent was removed on a rotary evaporator and the residual solid partitioned between ether (1 2 L) and aqueous NaOH (4 88 M, 1 L), minimising atmospheric exposure, to give a colourless organic layer and a yellow aqueous layer The organic layer under nitrogen was dried ($MgSO_4$), decanted and the solvent evaporated to give a yellow oil (83 5 g) Distillation at reduced pressure gave the amine (59 03 g, 317 mmol, 77%) as a colourless oil, b p 125 °C at 15 mm Hg (lit [12] b p 118-119 °C at 10 mm Hg) which solidifed on standing ^{1}H NMR (300 MHz, $CDCl_3$) δ 7 43, 7 18 (ABq, J = 8 4 Hz, 4 H, Ar), 3 81 (s, 2 H, CH_2), 1 48 (s, 2 H, NH_2), ^{13}C NMR (75 47 MHz, $CDCl_3$) δ 142 00 (C-4), 131 34, 128 66, 120 28 (C-1), 45 63 (CH_2)

2-*(*p*-Bromobenzyl)*-1,1,3,3-*tetramethyl*-1,3-*disilaisoindoline* (**11**) Caesium fluoride (6 23 g, 41 mmol) was added to a solution of *p*-bromobenzylamine (41 88 g, 222 mmol) and 1,2-bis(dimethylsilyl)benzene (**10**) (53 84 g, 277 mmol) in dry DMPU (75 ml) under argon, and the mixture was stirred at 120 °C for 18 h The cooled reaction mixture was partitioned between hexane-ether (1 1, 1 L) and aqueous NaOH (0 25 M, 500 ml) The organic layer was washed with water (2 x 400 ml), dried ($MgSO_4$), filtered and the solvent evaporated to give an oil (98 g) which solidified upon standing at RT Distillation under reduced pressure gave the disilaisoindoline as a colourless oil (79 9 g, 212 mmol, 96%) b p 142-172 °C at 0 4 mm Hg, which solidifed upon standing at RT m p 65-68 °C, IR (nujol mull) 1247, 1125, 1098, 1068, 1054, 1007, 871, 833, 746, 721 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7 56-7 53, 7 41-7 38 (2 m, 2 x 2 H, *o*-disubstituted Ar), 7 42, 7 20 (ABq, J = 8 5 Hz, 4 H, *p*-disubstituted Ar), 4 13 (s, 2 H, ArCH_2), 0 18 (s, 12 H, SiCH_3), ^{13}C NMR (75 47 MHz, $CDCl_3$) δ 146 73, 141 77, 134 01, 131 13, 129 42, 128 58, 120 26, 45 36 (ArCH_2), 0 29 (SiCH_3) This material was used without further purification in the subsequent reactions Recrystallisation from hexane gave an analytical sample Anal Calcd for $C_{17}H_{22}NBrSi$ C, 54 24, H, 5 89, N, 3 72, Br, 21 23 Found C, 54 34, H, 6 07, N, 4 01, Br, 21 62

Methyl 7α-*Acetoxy*-12α-*benzyloxy*-3α-*[*p*-(trifluoroacetylaminomethyl)phenyl]-cholan*-24-*oate* (**14**) The disilaisoindoline **11** (36 21 g, 96 19 mmol) was overlaid with dry ether (300 ml) Lithium wire (64 cm, 45 mg cm^{-1}, 415 mmol), was cut into 2-5 mm pieces, and added to the mixture Stirring was initiated After 1 min a purple colour appeared in the solution, and after 5 min the solvent began to reflux The mixture was stirred for a total of 1 h and then sonicated for 1 5 h The resulting organolithium solution was added to MnI_2 (30 0 g, 97 20 mmol) in dry ether (90 ml) and the mixture stirred with an overhead mechanical stirrer at room temperature for 15 min to give a greenish-brown solution A solution of ketone **7** (17 83 g, 32 26 mmol) in dry ether (250 ml) was added by syringe over 15 min with efficient stirring After stirring for 15 min the resultant insoluble materials were triturated with a glass rod and the mixture further stirred for 3 h to give a brown insoluble gum and a black solution The solution was decanted and discarded and the gum in dry ether (200 ml) was treated with trifluoroacetic acid (TFA) (30 ml, 389 mmol) and trifluoroacetic anhydride (60 ml, 425 mmol) The mixture was stirred overnight after which the volatiles were evaporated under reduced pressure, and the residue in ether (1 L) was washed with aqueous HCl (2 M, 3 x 300 ml) and aqueous NaOH (2 M, 2 x 400 ml) The organic phase was dried ($MgSO_4$), and the solvent removed on a rotary evaporator to give an orange oil (46 g) This material was divided into two portions for chromatography, which was performed on a column of silica gel 16 cm in length and 7 5 cm in diameter, using hexane-EtOAc (5 2) as eluant After the first batch had been purified, the silica gel was regenerated by eluting with methanol

followed by hexane-EtOAc (5 2). The alkene(s) **13** (R_f = 0 3) were isolated as a yellow foam (*ca* 22 g) ^{1}H NMR (80 MHz, $CDCl_3$) δ 7 4-7 0 (m, 9 H, ArH), 6 7 (br s, 1 H, NH), 5 75 (br s, 1 H, alkene-H), 4 82 (br s, 1 H, 7β-H), 4 72, 4 41 (ABq, *J* = 12 Hz, 2 H, OCH_2Ph), 4 50 (d, *J* = 6 Hz, 2 H, $NHCH_2$), 3 73 (br s, 1 H, 12β-H), 3 62 (s, 3 H, OMe), 1 71 (s, 3 H, OAc), 1 05 (s, 3 H, 19-Me), 0 91 (d, *J* = 6 Hz, 3 H, 21-Me), 0 65 (s, 3 H, 18-Me) This material was dissolved in EtOAc (400 ml) and treated with activated charcoal (20 g) [20] The solution was filtered through a plug of SiO_2,[20] eluting with further EtOAc, and the resulting solution (600 ml) hydrogenated over Pd/C (10%, 2 5 g) absorbing H_2 (*ca* 0 76 L at STP, *ca* 33 9 mmol) over 3 5 h The course of the hydrogenation was conveniently monitored by NMR, observing the disappearance of the alkene 7β-OAc signal at 1 71 ppm) Passage of the mixture through a plug of SiO_2 followed by evaporation of the solvent and recrystallisation from hexane-EtOAc gave the steroid **14** (17 97 g, 24 29 mmol, 75%) as white crystals m p 164-165 °C, IR (5% solution in $CHCl_3$) 3440 (NH), 1720 (br, C=O), 1600, 1520 (br) cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7 43-7 29 (m, 5 H, OCH_2Ph), 7 15, 7 08 (ABq, *J* = 8 2 Hz, 4 H, NCH_2Ar) 6 72 (br s, 1 H, NH), 4 90 (br s, 1 H, 7-βH), 4 64, 4 38 (ABq, *J* = 11 5 Hz, 2 H, OCH_2Ar), 4 51, 4 47 (ABX, J_{gem} = 14 2 Hz, J_{NH} = 5 8 Hz and 5 4 Hz, 2 H, CH_2NH), 3 74 (s, 1 H, 12-βH), 3 64 (s, 3 H, CO_2Me), 1 97 (s, 3 H, OAc), 1 00 (s, 3 H, C-19 Me), 0 94 (d, *J* = 6 0 Hz, 3 H, C-21 Me), 0 73 (s, 3 H, C-18 Me) ^{13}C NMR (75 47 MHz, $CDCl_3$) δ 174 67 (C-24), 170 43 ($C(O)CH_3$), 157 23 (q, $C(O)CF_3$), 147 83, 139 46, 133 21, 128 22, 127 27, 127 15, 127 09, 117 75 (q, $C(O)CF_3$), 79 92 (C-12), 71 25 (C-7), 69 56 (OCH_2Ph), 51 39 (OCH_3), 46 51 (C-13), 46 15, 45 29, 43 59, 42 88, 42 63, 38 13, 37 38 (2 x CH_2), 35 05, 34 50 (C-10), 31 52, 30 89 (2 x CH_2), 29 03, 28 30, 27 33, 23 13 (C-19), 23 05, 22 51, 21 53 ($C(O)CH_3$), 17 63 (C-21), 12 49 (C-18) An analytical sample was obtained by recrystallisation from hexane-dichloromethane m p 165-169 5 °C Anal Calcd for $C_{43}H_{56}NO_6F_3$ C, 69 80, H, 7 63, N, 1 89 Found C, 69 47, H, 7 63, N, 1 73

Pentafluorophenyl 7α-Acetoxy-12α-benzyloxy-3α-[p-(tert-butoxycarbonylaminomethyl)phenyl]cholan-24-oate (**15**) Aqueous NaOH (1 05 M, 3 5 ml, 3 68 mmol) was added to a solution of **14** (650 mg, 0 88 mmol) in THF (8 ml) and MeOH (4 ml) and the mixture stirred for 3 h at room temperature After neutralization with 2 M aqueous HCl, TLC in $CHCl_3$-MeOH (10 1) showed one major component, R_f = 0 2 The volatiles were removed by evaporation under reduced pressure, and the residue dissolved in a mixture of THF (10 ml), H_2O (5 ml) and di-isopropylethylamine (1 ml) Di-*tert*-butyl pyrocarbonate (220 µl, 1 42 mmol) was added, and after stirring overnight TLC in $CHCl_3$-MeOH (10 1) showed one major component, R_f = 0 6 The organic solvents were removed by evaporation under reduced pressure, and the residue partitioned between CH_2Cl_2 and 1 M aqueous H_3PO_4 After re-extraction of the aqueous layer with CH_2Cl_2, the combined organic layers were dried, and concentrated to *ca* 10 ml Di-cyclohexylcarbodiimide (DCC) (200 mg, 0 97 mmol) and pentafluorophenol (PFPOH) (200 mg, 1 08 mmol) were added After stirring overnight, the resulting suspension was filtered and the precipitate washed with CH_2Cl_2 (2 x 2 ml) The filtrates were diluted with CH_2Cl_2, washed with ice-cold 5% aqueous $NaHCO_3$, dried, and the solvent removed by evaporation under reduced pressure Chromatography in CH_2Cl_2-Et_2O (50,1) afforded PFP ester **15** as a colourless oil (635 mg, 83%) IR (neat) 3450 (NH), 1775 (PFP ester C=O), 1705, 1600 cm^{-1}, ^{1}H NMR (80 MHz, $CDCl_3$) δ 7 5-7 2 (5 H, m, OCH_2Ph), 7 15, 7 07 (ABq, *J* = 8 Hz, 4 H, 3α-Ar), 4 9 (br s, 1 H, 7β-H), 4 9 - 4 5 (br, 1 H, NH), 4 62, 4 42 (ABq, *J* = 12 Hz, 2 H, OCH_2Ph), 4 28 (d, *J* = 6 Hz, 2 H, CH_2NH), 3 76 (br s, 1 H, 12β-H), 2 8 - 2 55 (m, 2 H), 1 97 (s, 3 H, OAc), 1 47 (s, 9 H, Bu^t), 1 0 (s, 3 H, 19-Me), 0 76, (s, 3 H, 18-Me) This material was used in the following experiment without further purification

Macrocycle **2a.** The PFP ester **15** (9 16 g, 10 22 mmol) in dry CH_2Cl_2 (150 ml) was treated with TFA (15 ml) and the solution stirred at room temperature for 2 5 h. TLC (hexane-EtOAc, 3 1) indicated that cleavage of the *N-tert*-butoxycarbonyl group was complete The volatiles were removed on a rotary evaporator and the residual orange oil evacuated to 0 5 mm Hg for 30 min at room temperature The oil in dry CH_2Cl_2 (400 ml) was added dropwise to dry CH_2Cl_2 (4 L) containing DMAP (5 0 g, 45 39 mmol) and dry pyridine (6 ml, 74 18 mmol) over 6 h The homogenous yellow solution was stirred at room temperature for 1 d, after which analysis by TLC ($CHCl_3$-EtOAc, 3 1) showed macrocycle **2a** (R_f = 0 5) accompanied by a trace of baseline material DCC (3 80 g, 18 42 mmol) was added and the reaction stirred at room temperature for 21 h. TLC indicated that the baseline compounds had been replaced by faster-running material (R_f = 0 85) [21] The solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ (800 ml), washed with aq H_2SO_4 (2 M, 150 ml) and dried ($MgSO_4$) Evaporation gave a semi-crystalline oil which was purified by chromatography in $CHCl_3$-EtOAc (3 1), the crude product being applied to the column as a solution in warm $CHCl_3$ The resulting white solid (ca 8 g), which was contaminated by pentafluorophenol, was recrystallised from $CHCl_3$-ether to give macrocycle **2a** (5 62 g, 4 59 mmol, 90%, after drying at 60 °C/0 5 mm Hg for 2 d)· m.p >275 °C (decomp), MS (FAB, nitrobenzyl alcohol matrix) 1223 (MH^+), 1245 (MNa^+), IR (1% in $CHCl_3$) 3450 (NH), 1725 (ester C=O), 1665 (amide C=O), 1600 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ 7 35 - 7 1 (10 H, m, 2 x OCH_2Ph), 7 10, 7 08 (ABq, J = 8 8 Hz, 8 H, 2 x 3α-Ar), 5 7 (br t, 2 H, 2 x NH), 4 9 (2 H, br s, 2 x 7β-H), 4 57, 4 36 (4 H, ABq, J = 11 7 Hz, 2 x OCH_2Ph), 4 47, 4 41 (ABX, J_{gem} = 15 0 Hz, J_{NH} = 5 7 and 5 0 Hz, 4 H, 2 x CH_2NH), 3 71 (br s, 2 H, 2 x 12β-H), 1 94 (s, 6 H, 2 x OAc), 0.99 (d, J = 7 Hz, 6 H, 2 x 21-Me), 0 97 (s, 6 H, 2 x 19-Me), 0 75 (s, 6 H, 2 x 18-Me), ^{13}C NMR (68 MHz, $CDCl_3$) δ 173 5 (CONH), 170 2 ($COCH_3$), 147 0, 139 4, 136 0, 128 3, 127 2, 2 x 127 1 (8 x Ar), 80 0 (OCH_2Ph), 71 4 (C-7), 69 8 (C-12), 46 8, 46 0, 44 7, 43 6, 2 x 42 9, 38 2, 37 5, 37 3, 36 2, 34 5, 34 1, 33 0, 31 5, 29 3, 28 9, 28 0, 23 3, 23 1, 21 5 ($COCH_3$), 17 8 (C-21), 12 6 (C-18) (Two steroidal backbone resonances not detected or degenerate) An analytical sample was prepared by drying for 1 d at 100°C at 0 5 mm Hg Anal Calcd for $C_{80}H_{106}O_8N_2$· C, 78 52, H, 8 73, N, 2 29 Found C, 78 60, H, 8 96, N, 2 35

Macrocycle **2b.** A suspension of **2a** (75 mg, 0 061 mmol), 1 M ethanolic KOH (1 ml) and EtOH (1 ml) was stirred at 80 °C for 5 d Further ethanolic KOH was added (0 5 ml) and heating continued for 4 d, after which TLC in $CHCl_3$-EtOAc (3 2) showed one major component, R_f = 0 3 The yellow suspension was neutralized with 2 M aqueous HCl, solvents removed by evaporation under reduced pressure, and the residue partitioned between $CHCl_3$ (10 ml) and H_2O (10 ml) The aqueous layer was extracted with $CHCl_3$ (2 x 10 ml), and the combined organic layers dried, and evaporated to a yellow solid Chromatography in $CHCl_3$-EtOAc (3 2) afforded a white solid which was crystallized from CH_2Cl_2 to give **2b** as small prisms (67 5 mg, after drying at 50 °C, 0 5 mmHg for 30 min) m.p >275 °C (decomp), MS (FAB, nitrobenzyl alcohol matrix) 1139 (MH^+), 1161 (MNa^+), IR (1% in $CHCl_3$) 3620 (OH), 3450 (NH), 1660 (amide C=O), 1600 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ 7 15 - 7 2 (m, 10 H, 2 x OCH_2Ph), 7 2 (br s, 8 H, 2 x 3α-Ar), 5 61 (br t, 2 H, 2 x NH), 4 51, 4 28 (*ABX*, J_{gem} = 14 7 Hz, J_{NH} = 5 9 and 4 8 Hz, 4 H, 2 x CH_2NH), 3 81, (br s, 2 H, 2 x 7β-H), 3 71 (br s, 2 H, 2 x 12β-H), 0 99 (d, J = 6 2 Hz, 6 H, 2 x 21-Me), 0 96 (s, 6 H, 2 x 19-Me), 0 75 (s, 6 H, 2 x 18-Me), ^{13}C NMR (68 MHz, $CDCl_3$) δ 173 6 (CONH), 147 1, 135 6, 2 x 128 4, 127 7, 3 x 127 3 (8 x Ar), 80 3 (OCH_2Ph), 70 14 (C-12), 68 43 (C-7), 46 7, 45 9, 44 8, 2 x 43 4, 39 8, 39 0, 37 6, 36 0, 34 8, 34 5, 33 8, 32 8, 28 1, 27 8, 27 6, 23 3, 22 9, 17 8 (C-21), 12 7 (C-18) (One steroidal backbone resonance not

detected or degenerate) Anal Calcd for the hydrate $C_{76}H_{104}O_7N_2$ C, 78 85, H, 9 05, N, 2 42 Found C, 78 35, H, 9 09, N, 2 51

Macrocycle **2c.** Macrocycle **2a** (48 mg, 0 039 mmol) and 10% Pd-C (45 mg) in a mixture of EtOAc (3 ml) and EtOH (1 ml) was stirred under an atmosphere of H_2 for 40 h The catalyst was filtered off, the solvents removed by evaporation under reduced pressure, and the residue dissolved in $CHCl_3$ (25 ml) Slow evaporation at room temperature (to a volume of *ca* 4 ml) caused **2c** to precipitate as small prisms (45 mg) ^{1}H NMR (270 MHz, $CDCl_3$) δ 7 18 (br s, 8 H, Ar), 5 7 (br t, 2 H, 2 x NH), 4 91 (br s, 2 H, 2 x 7β-H), 4 65, 4 24 (ABX, J_{gem} = 15 Hz, J_{NH} = 6 6 and 4 3 Hz, 4 H, 2 x C*H*$_2$NH), 4 0 (br s, 2 H, 2 x 12β-H), 2 03 (s, 6 H, 2 x OAc), 0 98 (s, 6 H, 2 x 19-Me), 0 72 (s, 6 H, 2 x 18-Me), ^{13}C NMR (68 MHz, 10% CD_3OD in $CDCl_3$) δ 164 5 (CONH), 170 6 (*C*OCH$_3$), 135 9, 126 9, 2 x 126 8 (Ar), 72 5 (C-12), 71 6 (C-7), 46 5, 44 6, 42 8, 2 x 42 5, 38 0, 37 3, 36 7, 35 9, 34 4, 33 7, 32 7, 31 4, 29 3, 28 6, 28 4, 27 4, 2 x 22 9, 21 1 (*C*OCH$_3$), 17 2 (C-21), 12 3 (C-18) (One steroidal backbone resonance not detected or degenerate) Characterisation was completed by acetylation with acetic anhydride and DMAP in pyridine, giving the tetra-acetoxycholaphane reported in the preceding papers [1]

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20 Omission of these purification steps sometimes led to slow and incomplete hydrogenation

21 The intention of adding the DCC was to reactivate any carboxyl groups produced by reaction with adventitious water and thus allow the macrocyclisation to proceed to completion While this may have occurred to come extent, it was found that the DCC also activated the trifluoroacetate salts leading to *N*-trifluoracetylation Thus, investigation of the faster-running material from this and similar experiments showed that the major component was pentafluorophenyl 7α-acetoxy-12α-benzyloxy-3α-[*p*-(trifluoroacetylaminomethyl)phenyl]-cholan-24-oate