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Synthesis of 3β,6α-dihydroxy-5α-cholan-23-one

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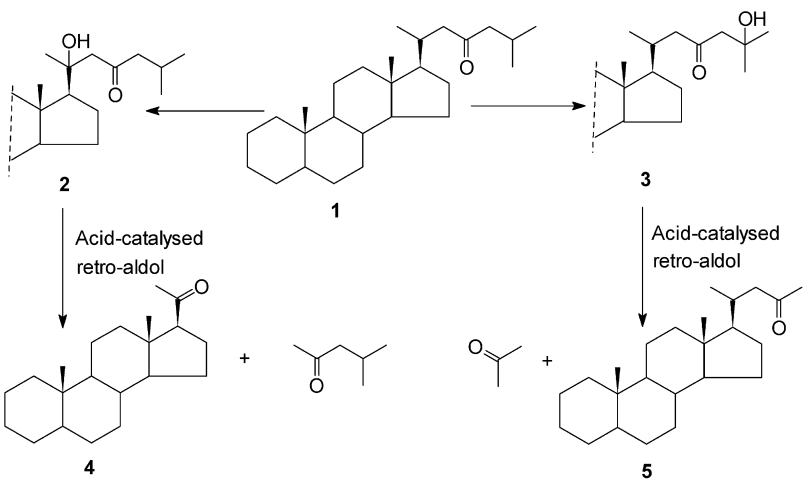
Abstract—Evidence for the presence of 3β,6α-dihydroxy-5α-chol-9(11)-en-23-one in the aglycone mixture from the starfish *Marthasterias glacialis* is provided by the synthesis of 3β,6α-dihydroxy-5α-cholan-23-one (**19**) and its identification in the hydrogenated aglycone mixture. The side-chain is constructed from the 23,24-dinorcholanol (**13**) by reaction of the 22-tosylate (**16**) with the acetylide anion, followed by hydration of the resulting 23-yne (**17**).

1. Introduction

23-Oxocholestane conjugates were first isolated from an Atlantic starfish more than 30 years ago.[1,2] Since then there have been numerous reports of the isolation and characterisation of related saponins from marine organisms which continue up to the present day.[3] The commonly observed hydroxylation of 23-oxocholestanes such as marthasterone **1** at tertiary positions in the side-chain (C-20 and C-25) leads to enones by dehydration and also provides an opportunity for degradation of the cholestane side-chain by retro-aldol cleavage (Scheme 1). Both of these reactions could occur in vivo or during the acid hydrolysis procedures normally used to obtain the aglycones. In particular, the 20-oxopregnane **4** and 23-oxocholane **5** can be expected to arise from the side-chain hydroxylated 23-oxocholestanes **2** and **3**. Evidence for the presence of pregnanes and cholanes in the aglycone mixture from the Atlantic starfish, *Marthasterias glacialis*, was originally adduced from GCMS studies,[4] and in this paper we discuss additional evidence for the cholanes from analytical studies and synthetic work.

2. Results and discussion

The synthesis of 23-oxocholanes from cholic acids did not look appealing, so a route involving the addition of a two-carbon fragment to the readily available bisnorcholanals was devised (Scheme 2) and carried out initially in the 4-en-3-one series. 22-Hydroxy-23,24-dinorchol-4-en-3-one (**7**)[5,6] was obtained in 68% yield by reduction of the commercially available 3-oxo-23,24-dinorchol-4-en-22-al (**6**) using one hydride equivalent of $NaBH_4$ at rt.



Scheme 1. Possible origin of 20-oxopregnane (**4**) and 23-oxocholane (**5**) from 23-oxocholestanes via acid-catalysed retro-aldol cleavage.

Keywords: starfish; steroid; dinorcholane; oxocholestane; NMR.
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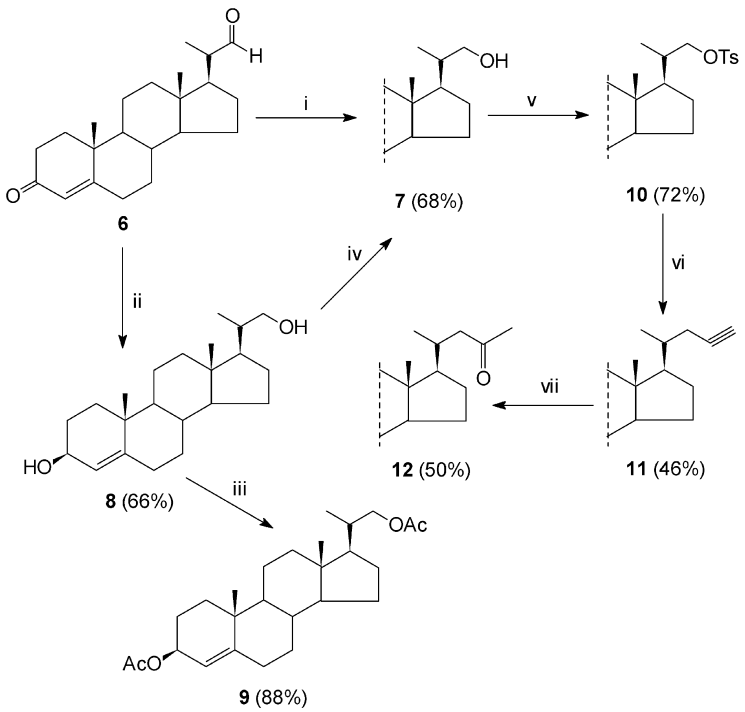


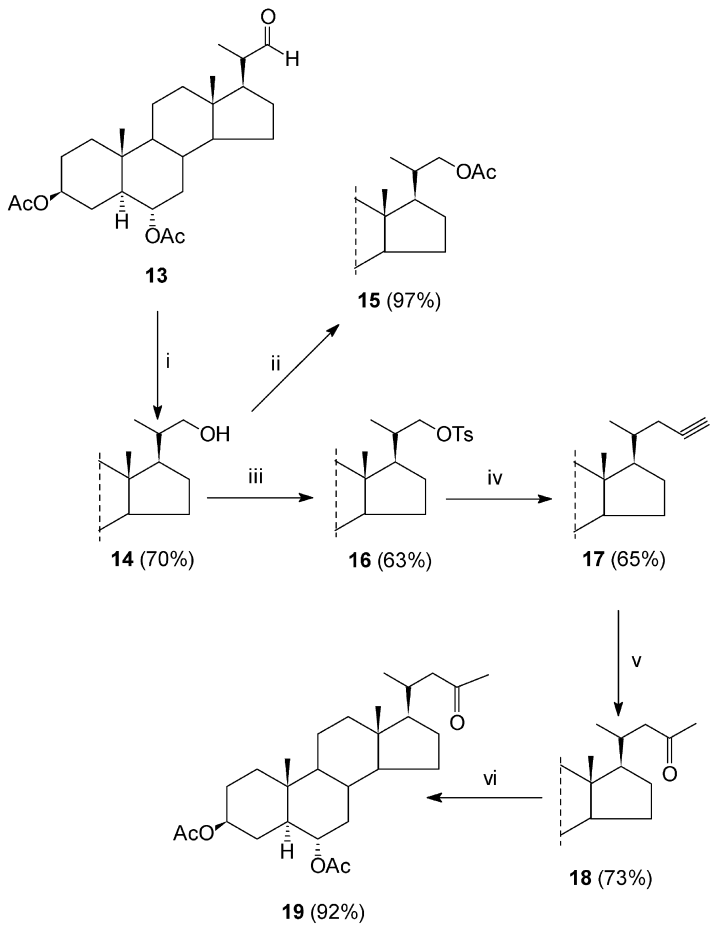
Scheme 2. (i) $NaBH_4$ (1 equiv.), EtOH, rt, 40 min. (ii) $NaBH_4$ (2 equiv.), EtOH, rt, 2 h. (iii) Ac_2O, Py, rt, 15 h. (iv) DDQ (1 equiv.), dioxane, rt, 24 h. (v) *p*-TsCl (4 equiv.) Py, rt, 36 h. (vi) LiC≡CH·EDA (13.6 equiv.), DMSO, 50°C, 3 h. (vii) AcOH, Hg-resin, H_2O, reflux, 5 h.

When two hydride equivalents of $NaBH_4$ were used for the reduction of the aldehyde **6**, the ketonic carbonyl group at C-3 was also reduced and 3β,22-dihydroxy-23,24-dinorchol-4-ene (**8**)[5] was obtained in 65% yield. Its ^1H NMR showed signals at δ_H 0.66 (s, 3H, CH_3), 1.01 (s, 3H, CH_3) and 1.00 (d, J=6.5 Hz, 3H, CH_3) for three methyl groups: 18-CH_3, 19-CH_3 and 21-CH_3, respectively. The resonances for the oxymethylene protons at C-22 were observed at δ_H 3.33 (dd, J=6.8, 10.3 Hz, 1H, OCH_2) and 3.59 (dd, J=3.1, 10.3 Hz, 1H, OCH_2) and that for the olefinic proton (at C-4) was at δ_H 5.24 (d, J=1.7 Hz, olefinic CH). The ^{13}C NMR spectrum showed signals for an olefinic methine (δ_C 124.3, C-4), an olefinic quaternary carbon (δ_C 148.7, C-5), an oxymethine (δ_C 69.1, C-3), and an oxymethylene group (δ_C 69.0, C-22). Acetylation of **8** with acetic anhydride in pyridine afforded 3β,22-diacetoxy-23,24-dinorchol-4-ene (**9**) in 88% yield. To confirm the identity of diol **8**, a selective allylic oxidation was performed using DDQ in dioxane at room temperature to produce 22-hydroxy-23,24-dinorchol-4-en-3-one (**7**) in 50% yield.

Tosylation of the alcohol **7** with toluene-*p*-sulfonyl chloride in pyridine gave the 22-tosyloxy-23,24-dinorchol-4-en-3-one (**10**)[5,7] in 72% yield. Selective displacement of the 22-tosyl group by the ethynyl group was achieved using a suspension of lithium acetylide/ethylenediamine complex in dry dimethylsulfoxide. No products of addition of the ethynyl group to the unsaturated ketone system were detected. The crude product was purified by preparative thin-layer chromatography (TLC) to give the 23-yne **11** as a colourless glass (46%, R_f 0.56, silica gel, benzene/EtOAc=3:1). Its IR spectrum showed stretching frequencies characteristic of the acetylenic C–H bond (3300 cm^{-1}) and the C≡C bond (2130 cm^{-1}), as well as the conjugated carbonyl group (1666 and 1620 cm^{-1}). Hydration of the triple bond using Hg-resin prepared from Zeo-Karb by Newman's method[8] as catalyst afforded chol-4-ene-3,23-dione (**12**) in 50% yield. The overall yield of the dione **12** from the bisnorcholenal **6** was 11%.

This route was then applied in the 3β,6α-dihydroxy-5α-cholane series for the synthesis of the starfish steroid (Scheme 3). Ethynylation of the bisnorcholane tosylate **16**[4] with an equimolar amount of lithium acetylide/ethanediamine complex in anhydrous dimethylsulfoxide gave the alkyne as before, but the crude product was found to consist of a mixture of partially and fully deacetylated material. It was found most convenient to reacetylate the crude product with acetic anhydride in pyridine before purification, when the alkyne **17** was obtained in 65% yield. Hydration of the alkyne using the resin catalyst as before gave the 23-oxocholane diacetate **18** in 73% yield, and subsequent hydrolysis using methanolic potassium hydroxide gave the crystalline 3β,6α-dihydroxy-5α-cholan-23-one (**19**), in 95% yield. In their mass spectra, both of the 23-oxocholanes **18** and **19** exhibited characteristic loss of acetone from the side-chain by McLafferty rearrangement[9] to afford the base peak. This was evident in the mass spectrum of diacetate **18** from both the parent ion and from the daughter ions formed by loss of acetic acid. In all cases the abundance of the molecular ion was low under electron impact.

The gas chromatographic retention time (t_R 3.56) and the R_f value on TLC of the synthetic compound **19** were identical to that of a minor component in a sample of the aglycone mixture from *M. glacialis* which had been subjected to prolonged hydrogenation over Pd/C. As shown previously, the 9(11)-double bond is reduced under these conditions[4] so this provides further evidence for the presence of



Scheme 3. (i) $NaBH_4$ (1 equiv.) EtOH, rt, 40 min. (ii) Ac_2O, Py, rt, 24 h. (iii) *p*-TsCl (3.4 equiv.), Py, rt, 44 h. (iv) LiC≡CH·EDA (13.9 equiv.), DMSO, 40°C, 2 h. (v) AcOH, Hg-resin, H_2O, reflux, 7 h. (vi) KOH (2 M), MeOH, rt, 27 h.

3β,6α-dihydroxy-5α-chol-9(11)-en-23-one in the original aglycone mixture. No evidence has been found for enzymic catalysis of the retro-aldol reaction leading to the C-24 sterol, but the work of Kitagawa[10] suggests that this type of side-chain cleavage takes place only during the acid hydrolysis used for the isolation of the aglycones.

3. Experimental

3.1. General

The steroid starting materials [stigmasterol, stigmastadieneone, and 3-oxo-22,23-dinorchol-4-en-22-al] were purchased from Sigma. Most chemicals and solvents were analytical grade and used without further purification. The purity of the products and the reaction time were supported by TLC performed on silica gel (Merck type 60) and visualised under UV illumination and/or by I_2 vapour. The gas chromatographic (GC) was conducted on 2 m×1.5 mm columns packed with 2.5% silicone on OV-1 (3%) at 250°C with a nitrogen flow rate of 16 ml min^{-1} on a Perkin–Elmer F-11 instrument. Quoted retention times are relative to pregnenolone (t_R 1.0). Mps were determined on a Kofler hot-stage apparatus and are uncorrected. Infrared spectra (wave numbers in cm^{-1}) were recorded Nicolet Avatar 320 or ATI Mattson Genesis Series FT-IR spectrometer as films or KBr discs. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Unity INOVA 400 MHz NMR spectrometer. Chemical shifts (δ) are reported in ppm downfield from TMS, using the middle resonance of $CDCl_3$ (7.25 ppm for ^1H and 77.23 ppm for ^{13}C) as an internal standard and coupling constants (J) in Hz. Data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, m=multiplet) coupling constant(s), integration and peak assignment. Elemental analyses were performed in Butterworth Laboratories Limited, Middlesex. The MS were recorded at the EPSRC Central Mass Spectrometry Service at Swansea.

3.2. Synthesis of chol-4-en-3,23-dione (12)

3.2.1. 22-Hydroxy-23,24-dinorchol-4-en-3-one (7). To a stirred ethanolic solution of 3-oxo-23,24-dinorchol-4-en-22-al **6** (200 mg, 0.60 mmol in 50 ml), $NaBH_4$ (23 mg, 0.60 mmol, 1 equiv.) was added (Scheme 1). Stirring was continued for 1 h at rt. When no starting material remained (checked by TLC), H_2O (40 ml) was added to the solution, and extracted with EtOAc (3×30 ml). The combined extracts were dried over $MgSO_4$ and concentrated in vacuo to give a slowly solidifying yellow oil (190 mg). The product was purified by column chromatography (neutral Al_2O_3, activity 1) using step gradient elution (petroleum-ether/EtOAc=75:25, 65:35) to yield the title compound **7** (134 mg, 68%) as a colourless solid, mp 134–136°C (lit.[5] mp 138–141°C). IR (KBr): ν 3427s (O–H),

2933s (C–H), 1673vs (C═O), 1617m (C═C), 1443m, 1230m and 1046m (alcoholic C–O) cm^{-1} (lit.[6] IR). ^1H NMR ($CDCl_3$): δ 0.69 (s, 3H, 18-CH_3), 1.08 (d, J=6.8 Hz, 3H, 21-CH_3), 1.14 (s, 3H, 19-CH_3), 3.33 (dd, J=6.8, 10.3 Hz, 1H, 22-OCH_2), 3.59 (dd, J=3.4, 10.6 Hz, 1H, 22-OCH_2), 5.68 (br s, 1H, 4-CH) (lit.[6] ^1H NMR). ^{13}C NMR ($CDCl_3$): δ 199.6 (C-3), 171.5 (C-5), 123.8 (C-4), 68.0 (C-22) 55.7 (C-17), 53.8 (C-14), 52.4 (C-9), 42.5 (C-13), 39.5 (C-12), 38.7 (C-10), 38.6 (C-20), 35.7 (C-8), 35.6 (C-1), 34.0 (C-2), 32.9 (C-6), 32.0 (C-7), 27.7 (C-16), 24.3 (C-15), 21.0 (C-11), 17.4 (C-19), 16.7 (C-21), 12.1 (C-18) (lit.[8] ^{13}C NMR: selected peaks).

3.2.2. 3β,22-Dihydroxy-23,24-dinorchol-4-ene (8). To a stirred solution of 3-oxo-23,24-dinorchol-4-en-22-al **6** (200 mg, 0.60 mmol) in EtOH (50 ml) at room temperature was added $NaBH_4$ (46 mg, 1.20 mmol, 2 equiv.) and stirring was continued for 2 h (Scheme 1). A pale yellow solution was formed. Then H_2O (20 ml) was added to the solution, and extracted with EtOAc (3×20 ml). The organic extracts were separated and dried over $MgSO_4$. Evaporation of the solvent gave the title compound **9** as a white amorphous solid (190 mg). Recrystallisation from EtOH gave a white solid (132 mg, 66%), mp 197–199°C (EtOH) (lit.[5] mp 196–205°C). IR (KBr): ν 3275br (O–H), 2940s (C–H), 2864 (C–H), 1659m (C═C), 1445m, 1361w, 1270m and 1059m (alcoholic C–O). ^1H NMR ($CDCl_3$): δ 0.66 (s, 3H, 18-Me), 1.00 (d, J=6.8 Hz, 3H, 21-Me), 1.01 (s, 3H, 19-Me), 3.33 (dd, J=6.8, 10.3 Hz, 1H, 22-OCH_2), 3.59 (dd, J=3.1, 10.3 Hz, 1H, 22-OCH_2), 4.11 (br t, J=1.7 Hz, 1H, 3-OCH), 5.24 (d, J=1.7 Hz, 1H, 4-CH). ^{13}C NMR ($CDCl_3$): δ 147.7 (C-5), 123.3 (C-4), 68.1 (C-3), 68.0 (C-22), 55.9 (C-17), 54.5 (C-14), 52.5 (C-9), 42.6 (C-13), 39.7 (C-12), 38.8 (C-10), 37.4 (C-20), 36.0 (C-8), 35.4 (C-1), 33.1 (C-6), 32.2 (C-7), 29.6 (C-2), 27.7 (C-16), 24.3 (C-15), 21.0 (C-11), 19.0 (C-19), 16.7 (C-21), 12.1 (C-18). MS (FAB) m/z: 333 $[M+H]^+$, 355 $[M+Na]^+$.

3.2.3. 3β,22-Diacetoxy-23,24-dinorchol-4-ene (9). Acetylation of **8** (50 mg, 0.15 mmol) with Ac_2O (0.5 ml) in pyridine (0.5 ml) at room temperature for 15 h, followed by addition of H_2O (1.0 ml) gave a colourless precipitate (Scheme 1). This was collected and dried in vacuo to give the title compound **9** (55 mg, 88%), mp 178–181°C. IR (KBr): ν 3470br (O–H), 2940s (C–H), 2865 (C–H), 1742vs (C═O), 1648m (C═C), 1446m, 1372m, 1239s (acetate C–O) and 1032m. ^1H NMR ($CDCl_3$): δ 0.66 (s, 3H, 18-Me), 0.96 (d, J=6.8 Hz, 3H, 21-Me), 1.02 (s, 3H, 19-Me), 2.01 (s, 6H, 3-OCO–Me and 22-OCO–Me), 3.72 (dd, J=7.5, 10.6 Hz, 1H, 22-OCH_2), 4.03 (dd, J=3.4, 10.6 Hz, 1H, 22-OCH_2), 5.18 (m, 2H, 3-OCH and 4-CH). ^{13}C NMR ($CDCl_3$): δ 171.4 (22-O*C*O–Me), 171.1 (3-O*C*O–Me), 149.5 (C-5), 119.1 (C-4), 70.9 (C-3), 69.5 (C-22), 55.8 (C-17), 54.2 (C-14), 52.8 (C-9), 42.7 (C-13), 39.6 (C-12), 37.8 (C-8), 37.3 (C-10), 35.9 (C-20), 35.1 (C-1), 32.9 (C-6), 32.2 (C-7), 27.7 (C-2), 25.1 (C-16), 24.3 (C-15), 21.5 (3-OCO–*Me*), 21.1 (22-OCO–*Me*), 21.0 (C-11), 18.8 (C-21), 17.1 (C-19), 12.1 (C-18). MS (FAB) m/z 417 $[M+H]^+$, 439 $[M+Na]^+$. HRMS (CI): found: $[M+NH_4]^+$, 434.3273. $C_{26}H_{44}NO_4$ requires 434.3270.

3.2.4. 22-Hydroxy-23,24-dinorchol-4-en-3-one (7). To a stirred solution of **8** (80 mg, 0.24 mmol) in dioxane (3 ml) at room temperature was added dropwise a solution of DDQ (55 mg, 0.24 mmol, 1 equiv.) in dioxane (1 ml). After 24 h, a white precipitation was found (Scheme 1). The solid was filtered off, the orange solution was concentrated and washed several times with petroleum-ether (40–60°C) to yield a colourless solid (40 mg, 50%). The white solid was recrystallised from EtOH. The compound was identified as compound **7** by mixed melting point determination (134–136°C) and comparison of its IR and NMR data with that of the authentic material described above.

3.2.5. 22-Tosyloxy-23,24-dinorchol-4-en-3-one (10). *p*-Toluenesulfonyl chloride (175 mg, 0.92 mmol, 4 equiv.) was added to a stirred solution (1 ml) of **7** (75 mg, 0.23 mmol in dry pyridine) at 0°C The mixture was allowed to stand for 36 h at room temperature (Scheme 1). The reaction mixture was poured into cold H_2O. The resulting white precipitate was separated by filtration and washed with H_2O. It was then washed with ether and dried under reduced pressure at room temperature to give the title compound **10** (80 mg, 72%) as a white amorphous solid, mp 158–160°C (lit. mp[5] 169–172°C). IR (KBr): ν 2940s (C–H), 1672vs (C═O), 1627m (C═C), 1447m, 1360m, and 1176m (O–SO_2) cm^{-1} (lit.[7] IR). ^1H NMR ($CDCl_3$): δ 0.64 (s, 3H, 18-Me), 0.94 (d, J=6.8 Hz, 3H, 21-Me), 1.13 (s, 3H, 19-Me), 3.75 (dd, J=6.8, 9.5 Hz, 1H, 22-OCH_2), 3.93 (dd, J=3.1, 9.5 Hz, 1H, 22-OCH_2), 5.68 (s, 1H, 4-CH), 22-Tosyl: 7.74 (d, J=8.2 Hz, 2H, 2×Ph-*H*), 7.30 (d, J=8.2 Hz, 2H, 2×Ph-*H*), 2.41 (s, 3H, Ph-*Me*). ^{13}C NMR ($CDCl_3$): δ 199.5 (C-3), 171.3 (C-5), 123.9 (C-4), 75.5 (C-22), 55.5 (C-17), 53.7 (C-14), 51.8 (C-9), 42.5 (C-13), 39.3 (C-12), 38.6 (C-10), 36.2 (C-20), 35.7 (C-8), 35.6 (C-1), 34.0 (C-2), 32.9 (C-6), 32.0 (C-7), 27.4 (C-16), 24.1 (C-15), 21.0 (C-11), 17.4 (C-19), 16.7 (C-21), 12.1 (C-18), 22-OTs: 144.6 (C-1′), 133.2 (C-4′), 129.8 (C-2′ and C-6′), 127.9 (C-3′ and C-5′), 21.7 (C-7′). MS (FAB) m/z 485 $[M+H]^+$, 507 $[M+Na]^+$.

3.2.6. Chol-4-en-23-yn-3-one (11). A solution of tosylate **10** (79 mg, 0.16 mmol) in dry DMSO (2 ml) was added dropwise to a stirred suspension of lithium acetylide/ethylenediamine complex (200 mg, 2.17 mmol, 13.6 equiv.), in dry DMSO (0.5 ml) (Scheme 1). The brown solution was heated to 40°C for 5 min and then at 50°C for 3 h. Dilute HCl (2 ml) was cautiously added to the cooled mixture (vigorous exothermic reaction occurred), followed by H_2O (5 ml). The aqueous layer was decanted from the gummy product, which was washed with H_2O to remove DMSO, before being dissolved in $CHCl_3$ (10 ml, washed with H_2O, and dried ($MgSO_4$). The pale brown gum (61 mg), obtained by evaporation of the $CHCl_3$, was purified by preparative TLC (R_f 0.56) eluted with a mixture (3:1) of benzene and EtOAc to give the 23-yne **11** (25 mg, 46%) as a colourless glass. IR (KBr): ν 3270, 1660s (C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.73 (s, 3H, 18-CH_3), 0.85 (d, J=6.5 Hz, 3H, 21-CH_3), 1.21 (s, 3H, 19-CH_3), 2.15 (s, 1H, 23-C≡CH), 5.74 (s, 1H, 4-CH). ^{13}C NMR ($CDCl_3$): δ 200.7 (C-3), 172.2 (C-5), 124.8 (C-4), 84.3 (C-23), 70.3 (C-24), 56.8 (C-17), 55.9 (C-14), 54.8 (C-9), 43.5 (C-13), 39.6 (C-12), 38.5 (C-10), 36.7 (C-20), 36.3 (C-8), 35.8 (C-1), 35.0 (C-2), 33.9 (C-6), 33.2 (C-7), 29.0 (C-16), 26.6 (C-22), 25.2 (C-15), 22.0 (C-11), 19.6 (C-21), 18.4 (C-19), 13.1 (C-18). MS (EI): m/z 338 (M^+ 18%), 271 (M−C_5H_7, 11), 229 (M−C_8H_{13},

10), 215 (11), 159 (11), 148 (10), 147 (18), 131 (13), 124 (100). HRMS (EI): found: M^+, 338.2613. $C_{24}H_{34}O$ requires 338.2609.

3.2.7. Chol-4-ene-3,23-dione (12). A solution of 23-yn-3-one **11** (22 mg, 0.065 mmol) in acetic acid (1.0 ml) and water (0.1 ml) containing Hg-resin (190 mg) prepared from Zeo-Karb 225[6] was heated at reflux for 5 h (Scheme 1). The Hg-resin was removed by filtration and washed with EtOH. The filtrates were evaporated in vacuo to give the crude product (22 mg), which was purified by preparative TLC (R_f 0.47) eluting with a mixture (3:1) of benzene/EtOAc to give 3,23-dione **12** (11.5 mg, 50%) as colourless needles, mp 156–163°C, raised upon recrystallisation from EtOH to mp 165–167°C. UV: λ_{max} 240 nm (ε 16800). IR (KBr): ν 1705s (C=O), 1665s (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.77 (s, 3H, 18-CH_3), 0.98 (d, J=6.5 Hz, 3H, 21-CH_3), 1.19 (s, 3H, 19-CH_3), 2.12 (s, 3H, 23-CO–CH_3), 5.73 (m, 1H, 4-CH). ^{13}C NMR ($CDCl_3$): δ 209.8 (C-23), 200.7 (C-3), 172.2 (C-5), 124.8 (C-4), 56.9 (C-17), 56.0 (C-14), 54.8 (C-9), 43.7 (C-13), 39.6 (C-12), 38.5 (C-10), 36.9 (C-20), 36.4 (C-8), 35.8 (C-1), 35.0 (C-2), 33.9 (C-6), 33.6 (C-22), 33.2 (C-7), 29.3 (C-24), 29.1 (C-16), 25.3 (C-15), 22.0 (C-11), 17.6 (C-19), 14.6 (C-21), 13.1 (C-18). MS (EI): 356 (M^+, 16%), 299 (32), 298 (100), 283 (18), 229 (14), 175 (25), 174 (10), 124 (70), 85 (50), 83 (90). HRMS (EI): found: M^+, 356.2717. $C_{24}H_{36}O_2$ requires 356.2715.

3.3. Synthesis of 3β,6α-dihydroxy-5α-cholan-23-one (19)

3.3.1. 3β,6α-Diacetoxy-22-hydroxy-23,24-dinor-5α-cholane (14). Borohydride reduction (Scheme 2) of 3β,6α-diacetoxy-23,24-dinor-5α-cholan-22-al **13** (200 mg, 0.46 mmol) was performed as for compound **6**. After the usual work-up the title compound **14** (140 mg, 70%) was obtained as a white amorphous solid, mp 152–154°C, raised upon recrystallisation from EtOH/EtOAc to mp 176–177°C (lit.[4] mp 173–174°C). IR (film): ν 3533m (O–H), 2936s (C–H), 1731vs (C=O), 1471m, 1444m, 1377m, 1247vs (ester C–O), 1160m and 1029m (alcoholic C–O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.63 (s, 3H, 18-CH_3), 0.84 (s, 3H, 19-CH_3), 0.99 (d, J=6.5 Hz, 3H, 21-CH_3), 1.98 (s, 6H, 3-OCO–Me and 6-OCO–Me), 3.32 (dd, J=3.4, 10.9 Hz, 1H, 22-OCH_2), 3.58 (dd, J=6.8, 10.3 Hz, 1H, 22-OCH_2), 4.62 (m, 2H, 3-OCH and 6-OCH). ^{13}C NMR ($CDCl_3$): δ 171.9 (6-O*C*OCH$_3$), 171.6 (3-O*C*OCH$_3$), 74.2 (C-3), 73.3 (C-6), 69.0 (C-22), 56.9 (C-17), 54.5 (C-14), 53.5 (C-9), 49.5 (C-5), 43.7 (C-13), 40.6 (C-12), 38.7 (C-10), 37.9 (C-20), 37.6 (C-8), 35.2 (C-1), 35.1 (C-2), 29.4 (C-4), 28.7 (C-7), 28.2 (C-16), 25.2 (C-15), 22.5 (3-CO*C*H$_3$), 22.3 (6-CO*C*H$_3$), 22.1 (C-11), 17.7 (C-19), 14.3 (C-21), 13.1 (C-18). HRMS (CI): found: $[M+NH_4]^+$, 452.3379. $C_{26}H_{46}NO_5$ requires 452.3376.

3.3.2. 3β,6α,22-Triacetoxy-23,24-dinor-5α-cholane (15). Acetylation of the alcohol **14** (30 mg, 0.069 mmol) using Ac_2O (0.5 ml) in pyridine as before gave the triacetate as a colourless gum (R_f 0.75, from a mixture (3:1) of benzene and ethyl acetate as eluent, 32 mg, 97%) (Scheme 2). ^1H NMR ($CDCl_3$): δ 0.63 (s, 3H, 18-CH_3), 0.84 (s, 3H, 19-CH_3), 0.96 (d, J=6.8 Hz, 3H, 21-CH_3), 1.98 (s, 6H, 3-OCO–Me and 6-OCO–Me), 2.00 (s, 3H, 22-O*C*OCH$_3$), 3.72 (dd, J=7.5, 10.6 Hz, 1H, 22-OCH_2), 4.02 (dd, J=3.4, 10.6 Hz, 1H, 22-OCH_2), 4.62 (m, 2H, 3-OCH and 6-OCH). ^{13}C NMR ($CDCl_3$): δ 172.4 (22-O*C*OCH$_3$), 171.9 (6-O*C*OCH$_3$), 171.6 (3-O*C*OCH$_3$), 74.1 (C-3), 73.3 (C-6), 70.5 (C-22), 56.8 (C-17), 54.5 (C-14), 53.8 (C-9), 49.5 (C-5), 43.8 (C-13), 40.5 (C-12), 38.6 (C-10), 37.9 (C-20), 37.6 (C-8), 36.8 (C-1), 35.1 (C-2), 29.3 (C-4), 28.6 (C-16), 28.2 (C-7), 25.2 (C-15), 22.5 (3-OCO*C*H$_3$ and 6-OCO*C*H$_3$), 22.3 (22-OCO*C*H$_3$), 22.1 (C-11), 18.1 (C-19), 14.3 (C-21), 13.1 (C-18). MS (ESI): m/z 499 $[M+Na]^+$ and 975 $[2M+Na]^+$. HRMS (CI): found: $[M+NH_4]^+$, 494.3477. $C_{28}H_{48}NO_6$ requires 494.3482.

3.3.3. 3β,6α-Diacetoxy-22-tosyloxy-23,24-dinor-5α-cholane (16). A solution of the alcohol **14** (100 mg, 0.23 mmol) and *p*-TsCl (150 mg, 0.79 mmol, 3.4 equiv.) in dry pyridine (0.8 ml) was left at room temperature for 44 h (Scheme 2). Water (2 ml) was added and the resulting gummy precipitate was separated and washed with H_2O and then dried in vacuo to give the title compound **16** (85 mg, 63%). The crude product was used directly for ethynylation.

3.3.4. 3β,6α-Diacetoxy-5α-cholan-23-yne (17). A solution of the tosylate **16** (80 mg, 0.14 mmol) in dry DMSO (1.5 ml) was added dropwise to a stirred suspension of lithium acetylide/ethylenediamine complex (180 mg, 1.95 mmol, 13.9 equiv.) in dry DMSO (0.5 ml). The resulting brown solution was warmed to 40°C for 2 h, before being cooled in iced water and treated dropwise with dilute HCl (2 ml) followed by water (5 ml). The crude product, isolated by extraction with chloroform, was a brown gum (54 mg), the NMR spectrum of which showed no tosyl group but indicated partial deacetylation. It was therefore reacetylated by dissolution in pyridine (1 ml) containing acetic anhydride (0.3 ml) for 18 h at 20°C. Addition of water (0.5 ml) followed by evaporation gave a brown gum (62 mg), which was purified by preparative TLC (R_f 0.63) and eluted with a mixture (3:1) of benzene and EtOAc to give the 23-yne **17** (39 mg, 65%) as a colourless gum. ^1H NMR ($CDCl_3$): δ 0.62 (s, 3H, 18-CH_3), 0.84 (s, 3H, 19-CH_3), 1.02 (d, J=6.5 Hz, 3H, 21-CH_3), 1.98 (s, 6H, 3-OCOCH_3 and 6-OCOCH_3), 2.01 (s, 1H, 23-C≡CH), 4.63 (m, 2H, 3-OCH and 6-OCH). ^{13}C NMR ($CDCl_3$): δ 171.9 (6-O*C*OCH$_3$), 171.6 (3-O*C*OCH$_3$), 84.4 (C-23), 74.1 (C-3), 73.3 (C-6), 70.2 (C-24), 57.0 (C-17), 55.9 (C-14), 54.5 (C-9), 49.5 (C-5), 43.6 (C-13), 40.5 (C-12), 38.6 (C-10), 37.9 (C-20), 37.6 (C-8), 36.2 (C-1), 35.1 (C-2), 29.4 (C-4), 29.0 (C-7), 28.2 (C-16), 26.6 (C-22), 25.1 (C-15), 22.5 (3-CO*C*H$_3$), 22.3 (6-CO*C*H$_3$), 22.1 (C-11), 20.0 (C-19), 14.3 (C-21), 13.1 (C-18). MS (ESI): m/z 465 $[M+Na]^+$ and 907 $[2M+Na]^+$. MS (EI): m/z 382 (M−60, 10%), 323 (35%), 322 (100%), 228 (30%), 215 (22%), 214 (25%), 213 (45%). HRMS (CI): Found: $[M+NH_4]^+$, 460.3429. $C_{28}H_{46}NO_4$ requires 460.3427.

3.3.5. 3β,6α-Diacetoxy-5α-cholan-23-one (18). A solution of the above alkyne **17** (38 mg, 0.08 mmol) in acetic acid (2 ml) and water (0.2 ml) containing Hg-resin[6] (274 mg) was heated under reflux with stirring for 7 h (Scheme 3). The resin was filtered off and washed with ethanol. Evaporation of the filtrate and washings gave an oil (42 mg), which was purified by preparative TLC (R_f 0.50) eluted with a mixture (3:1) of benzene and EtOAc to give the 23-one **18** (28 mg, 73%) as a colourless oil. IR (KBr): ν

1725vs (acetate C═O), 1705vs (ketonic C═O), 1365m, 1235s (acetate C–O), 1020m, 955m and 895m cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.69 (s, 3H, 18-CH_3), 0.99 (d, J=6.8 Hz, 3H, 21-CH_3), 2.03 (s, 6H, 3-OCOCH_3 and 6-OCOCH_3), 2.11 (s, 3H, 23-COCH_3), 4.63 (m, 2H, 3-OCH and 6-OCH). ^{13}C NMR ($CDCl_3$): δ 210.6 (C-23), 171.9 (6-O*C*OCH$_3$), 171.6 (3-O*C*OCH$_3$), 74.2 (C-3), 73.4 (C-6), 56.9 (C-17), 54.6 (C-14), 53.5 (C-9), 49.6 (C-5), 43.8 (C-13), 40.6 (C-12), 38.6 (C-10), 38.2 (C-20), 37.8 (C-8), 35.3 (C-1), 35.1 (C-2), 30.9 (C-7), 29.5 (C-24), 29.4 (C-4), 28.7 (C-7), 28.2 (C-16), 25.3 (C-15), 22.4 (3-CO*C*H$_3$), 22.4 (6-CO*C*H$_3$), 22.1 (C-11), 20.8 (C-19), 14.7 (C-21), 13.1 (C-18). MS (EI): m/z 460 (M^+, 1%), 402 (M−Me_2CO, 4%), 400 (M−AcOH, 3%), 342 (13), 340 (7), 282 (7), 213 (15), 161 (35), 43 (100). HRMS (EI): found: M^+, 460.3191. $C_{28}H_{44}O_5$ requires 460.3188.

3.3.6. 3β,6α-Dihydroxy-5α-cholan-23-one (19). A solution of the diacetate **18** (20 mg) in methanolic KOH (1.5 M; 2.0 ml) was kept at room temperature under nitrogen for 27 h (Scheme 3). After evaporation to dryness and addition of H_2O, the dihydroxyketone **19** was extracted with $CHCl_3$, dried ($MgSO_4$) and rotary evaporated to obtain a colourless solid (15 mg, 92%), mp 181–186°C, raised upon recrystallisation from EtOH/EtOAc to mp 212–215°C. ^1H NMR ($CDCl_3$): δ 0.66 (s, 3H, 18-CH_3), 0.78 (s, 3H, 19-CH_3), 0.88 (d, J=6.2 Hz, 3H, 21-CH_3), 2.08 (s, 3H, 24-CH_3), 3.54 (m, 1H, 3-OCH), 3.38 (m, 1H, 6-OCH). ^{13}C NMR ($CDCl_3$): δ 210.4 (C-23), 72.3 (C-3), 70.5 (C-6), 57.2 (C-17), 54.8 (C-14), 52.7 (C-9), 51.9 (C-5), 43.7 (C-13), 42.6 (C-12), 40.7 (C-10), 38.3 (C-20), 37.3 (C-8), 35.3 (C-1), 33.7 (C-22), 33.3 (C-2), 32.1 (C-4), 31.7 (C-16), 30.7 (C-7), 29.4 (C-24), 25.2 (C-15), 22.2 (C-11), 20.7 (C-19), 14.5 (C-21), 13.1 (C-18). MS (EI): m/z 376 (M^+, 1.5%), 358 (7), 319 (16), 318 (100), 303 (19), 285 (11), 213 (15) 161 (18). HRMS (EI): found: M^+ and M−58, 376.2976 and 318.2556. $C_{24}H_{40}O_3$ and $C_{21}H_{34}O_2$ require 376.2977 and 318.2558.

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