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Synthesis of cytotoxic novel 9,11-secosterol analogs: Structure/activity studies

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ABSTRACT

In an effort to determine the pharmaceutical utility and the structural requirements for activity against tumor cell lines, 30 novel 9,11-secosterol analogues with different side chains and degrees of oxidation at C-9 were synthesized starting from hecogenin. Evaluation of the synthesized compounds for cytotoxicity against KB, HeLa and MCF-7 cell lines revealed that some important structural features are required for activity. The presence of a cholesterol-type side chain, which appears to play a major role in determining the biological activity, the existence of a ketone functional at C-9 is also crucial for anticancer activity whereas hydroxyl/ketone function at C-22 on the side chain did not increase cytotoxicity.

1. Introduction

Steroids isolated from various marine organisms (marine steroids) manifest diverse biological activities [1–5]. Some of them are extremely toxic against tumor cells [6–12] and show anti-inflammatory [13] and other effects [14,15]. It is therefore not surprising that marine steroids arouse considerable interest in not only chemists, but also in pharmacologists and physicians.

A large number of 9,11-secosterols have been isolated from marine sponge, many of which have been shown to exert a cytotoxic activity on human cancer cell lines in vitro, such as glaciasterol A (**1**) and B (**2**), isolated from *Aplysilla glacialis*, showed in vitro cytotoxic activity against murine leukemia L1210 and human breast cancer cell lines [16]. Blancasterol (**3**) isolated from *Pleraplysilla* species, showed in vitro cytotoxicity against L1210 murine leukemia, drug-sensitive MCF-7 human breast cancer, and drug-resistant MCF-7 Ad^r human breast cancer cell lines [17]. Three secosterols **4–6**, isolated from gorgonian *Pseudopterogorgia* sp. showed moderate inhibitory activity against various human protein kinase C enzymes [13,18]. Aplidiasterol A (**7a**) and B (**7b**) were isolated from the Mediterranean ascidian *Aplidium conicum* and exhibited cytotoxic activity against rat glioma (C6) and murine monocyte/macrophage (J774) tumor cells in vitro [19,20]. 24-Nor-9,11-seco-11-acetoxy-3β,6α-dihydroxycholesta-7,22-(*E*)-dien-9-one(**8**) isolated from the White Sea soft coral *Gersemia fruticosa* showed strong cytotoxic activity against human leukemia K562, human cervical cancer HeLa and Ehrlich ascites tumor cell lines [21–26]. These active compounds; structurally, all have a keto group at C-9 and a side chain like those usually found in 'normal' sterols, but difference residing in the A/B ring (*cis* or *trans* junction or Δ^5) and in the types and degree of oxygenation (Fig. 1).

In order to determine the role of the C-9 keto group and alkyl side chain of 9,11-secosterols in the anticancer activity, we have synthesized various types of 9,11-secosteroids with and without a keto group at C-9, containing three different sterol side chain: (i) cholesterol-like side chain (ii) acetyl side chain (iii) spiroketal moiety and evaluate the anticancer activity of these compounds and gain insight into structure–activity relationships (SARs).

2. Experimental

2.1. General

Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Varian Gemini 300 spectrophotometer and on 400 MHz and 100 MHz Brucker Advance DPX-400. Chemical shifts were recorded as δ values in ppm. Spectra were acquired in $CDCl_3$ unless otherwise stated. The peak due to residual $CHCl_3$ (7.26 ppm for ^{1}H and 77.23 ppm for ^{13}C) was used as the internal reference. Coupling constants (*J*) are given in Hz, and multiplicity is defined as follows: br = broad, s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, t = triplet, q = quartet, m = multiplet. Infrared (IR) spectra were recorded in cm^{-1} on a Perkin-Elmer 2000

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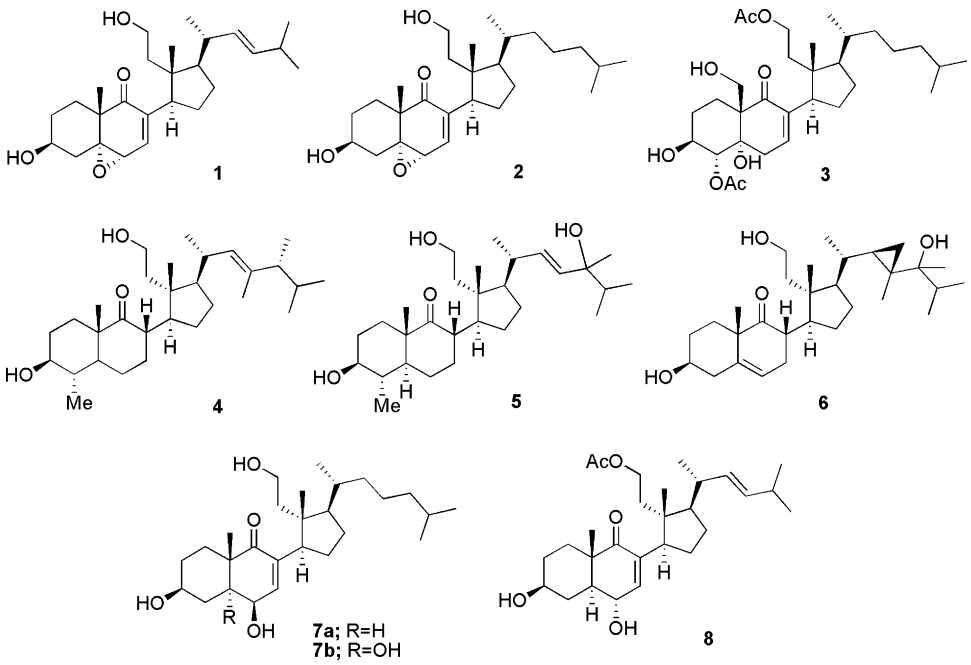


Fig. 1. Structure of some naturally occurring 9,11-seco sterols.

Fourier transform infrared spectrophotometer at the Chemistry Department, Faculty of Science, Kasetsart University. Samples were analyzed as KBr disks. Mass spectra (MS) was obtained on a GCMS-QP-5050A spectrometer in electron impact mode at 70 eV at the Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), Kasetsart University. Melting points (mp) were determined on a Fisher John apparatus and MEL-TEMP capillary melting point apparatus at the Chemistry Department, Kasetsart University and are reported uncorrected in °C.

2.2. General procedure for the synthesis of compounds **9b**; **11c**; **12c**; **26b**; **27b**; **40b**; **42b** and **48b**

A mixture of (**9a, 11b, 12b, 26a, 27a, 40a, 42a** or **48a**) (2 mmol), acetic anhydride (1 ml) and pyridine (2 ml) was stirred overnight at room temperature for 3 h. The reaction was quenched by addition of ice and stirred for 1 h, then extracted with dichloromethane. The organic phase was washed with 10% hydrochloric acid, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel.

2.2.1. $\Delta^{9(11)}$-22-isoallospirosten-3β-ol 3-acetate (**9b**)

8.80 g (19.3 mmol scale) in quantitative yield, mp 195–197 °C. FTIR (film) cm^{-1}: 2932, 1727, 1456, 1244. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.29 (1H, bs, H-11), 4.70–4.63 (1H, m, H-3), 4.41 (1H, dd, *J* = 7.6 and 14.8 Hz, H-16), 3.47 (1H, ddd, *J* = 1.9, 4.1 and 10.7 Hz, H-26), 3.37 (1H, t, *J* = 10.7 Hz, H-26), 2.12–1.22 (m, CH and CH_2), 2.01 (3H, s, $OCOCH_3$), 0.96 (3H, s, H-19), 0.80 (3H, d, *J* = 7.6 Hz, H-21), 0.79 (3H, d, *J* = 6.4 Hz, H-27) and 0.69 (3H, s, H-18). ^{13}C NMR δ: 170.63, 146.81, 116.19, 109.30, 80.79, 73.43, 66.91, 61.40, 53.72, 42.91, 42.15, 41.83, 38.81, 37.85, 35.75, 35.16, 34.15, 33.04, 32.85, 31.30, 30.30, 28.77, 28.28, 27.51, 21.42, 17.80, 17.11, 15.82 and 14.14. MS (DCI^+), *m*/*z*: 457 (M^++H, 100), 397 (51), 312 (13), 253 (14).

2.2.2. 3β,11-dihydroxy-9,11-seco-5α-22-isoallospirostan-9-one 3,11-diacetate (**11c**)

0.585 g (1.1 mmol scale) in quantitative yield, mp 43–45 °C. FTIR (KBr) cm^{-1}: 2951, 1737, 1242, 1030. ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.59–4.54 (1H, m, H-3), 4.35 (1H, dt, *J* = 6.8 and 8.8 Hz, H-16), 4.02 (2H, t, *J* = 7.4 Hz, H-11), 3.42–3.38 (1H, m, H-26), 3.30 (1H, t, *J* = 10.9 Hz, H-26), 2.77–2.70 (1H, m, H-8), 2.54–2.51 (1H, m, H-14), 2.12 (1H, t, *J* = 7.6 Hz, CH), 1.95 (s, 3H, $OCOC\underline{H}_3$), 1.94 (s, 3H, $OCOC\underline{H}_3$), 1.93–1.19 (m, CH and CH_2), 1.11 (3H, s, H-19), 0.90 (3H, d, *J* = 6.8 Hz, H-21), 0.98 (3H, s, H-18) and 0.72 (CH_3, d, *J* = 6.2 Hz, H-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 214.83, 171.00, 170.43, 108.35, 80.05, 72.49, 66.95, 61.99, 57.71, 47.91, 45.00, 44.03, 43.83, 43.63, 43.15, 40.58, 32.72, 32.30, 32.08, 31.51, 30.90, 30.28, 28.69, 27.90, 26.61, 21.30, 21.06, 18.54, 17.07, 15.61 and 14.36. MS (DCI^+), *m*/*z*: 532 (M^+, 100), 514 (11), 490 (4), 472 (3). Anal. Calcd. for $C_{31}H_{48}O_7$: C, 69.89; H, 9.08; found: C, 69.83; H, 9.10.

2.2.3. 3β,9β,11-trihydroxy-9,11-seco-5α-22-isoallospirostan-3,11-diacetate (**12c**)

0.55 g, (1.1 mmol scale), 89% yield, mp 134–135 °C. FTIR (KBr) cm^{-1}: 3553, 2949, 1743, 1716, 1243 and 1029. ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.66–4.57 (1H, m, H-3), 4.34–4.23 (2H, m, H-11), 4.01–3.95 (1H, m, H-16), 3.39 (1H, ddd, *J* = 1.7, 4.3 and 10.9 Hz, H-26), 3.31 (1H, t, *J* = 10.9 Hz, H-26), 2.71 (1H, d, *J* = 10.9 Hz, H-9), 2.28 (1H, dd, *J* = 5.8 and 14.2 Hz, H-9), 2.10 (1H, t, *J* = 9.2 Hz, CH), 1.96 (3H, s, $OCOC\underline{H}_3$), 1.95 (3H, s, $OCOC\underline{H}_3$), 1.95–1.04 (m, CH and CH_2), 0.91 (3H, d, *J* = 6.8 Hz, H-21), 0.83 (3H, s, H-19), 0.76 (3H, s, H-18) and 0.72 (3H, d, *J* = 6.4 Hz, H-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.48, 170.57, 108.98, 81.24, 79.76, 73.34, 67.01, 62.05, 56.24, 45.90, 43.03, 42.85, 42.07 39.08, 39.01, 36.78, 36.03, 33.12, 31.29, 31.09, 30.30, 29.18, 28.65, 27.72, 26.96, 21.37, 21.15, 19.57, 17.08, 14.16 and 10.58. MS (DCI^+), *m*/*z*: 535 (M^++H, 100), 517 (77), 499 (27), 456 (57), 439 (20). Anal. Calcd. for $C_{31}H_{50}O_7$: C, 69.63; H, 9.42; found: C, 69.39; H, 9.30.

2.2.4. $\Delta^{9(11)}$-24-norchlolest-3β-ol 3-acetate (**26b**)

0.30 g (0.81 mmol scale) 88% yield as a colourless gum which was used in the following step without further purification.

2.2.5. $\Delta^{9(11)}$-Chlolest-3β-ol 3-acetate (**27b**)

0.45 g (1.4 mmol scale), 81% yield as a colourless solid. FTIR (KBr) cm^{-1}: 2928, 1738, 1241. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.24 (1H, d, *J* = 5.8 Hz, H-11), 4.69–4.61 (1H, m, H-3), 2.13 (1H, dd, *J* = 6.6 and

15.4 Hz, CH), 2.00 (s, 3H, OCOC$\underline{H}_3$), 1.97–0.97 (m, CH and CH_2), 0.94 (3H, s, H-19), 0.85 (2×3H, 2×d, J = 6.6 Hz, H-26 and 27), 0.84 (3H, d, J = 6.6 Hz, H-21) and 0.58 (3H, s, H-18). MS (CDI$^+$), m/z 427 (M^+−H, 65), 369 (M^+−OAc, 100).

2.2.6. 3β,11-dihydroxy-9,11-seco-5α-chlolest-9,22-dione-3,11-diacetate (**40b**)

0.40 g (0.08 mmol scale), 93% yield, mp 59–60 °C. FTIR (KBr) cm^{-1}: 2959, 1735, 1707, 1248, 1033. ^{1}H NMR (acetone-d_6, 400 MHz) δ: 4.62–4.54 (1H, m, H-3), 4.15–4.05 (2H, m, H-11), 2.93 (1H, ddd, J = 3.3, 5.5 and 13.3 Hz, C$\underline{H}$), 2.62–2.52 (2H, m, C$\underline{H}_2$), 2.47–2.39 (1H, m, C$\underline{H}$), 2.05–2.03 (2H, m, C$\underline{H}_2$), 1.95 (3H, s, OCOC$\underline{H}_3$), 1.93 (3H, s, OCOC$\underline{H}_3$), 1.88–1.34 (m, CH and CH_2), 1.23 (3H, s, H-19), 1.13 (3H, d, J = 7.0 Hz H-21,), 0.87 (2×3H, 2×d, J = 6.6 Hz, H-26 and 27) and 0.77 (3H, s, H-18). ^{13}C NMR δ: 214.82, 213.69, 170.77, 170.19, 72.92, 61.20, 49.42, 48.49, 46.37, 45.72, 45.69, 43.47, 41.85, 40.01, 36.65, 33.39, 32.96, 32.54, 31.77, 28.42, 28.12, 27.36, 27.28, 23.18, 22.56, 22.54, 20.99, 20.82, 17.39, 16.50 and 15.80. MS (CDI$^+$), m/z: 517 (M^+−H, 3), 459 (M^+−OAc, 100), 331 (10). Anal. Calcd. for $C_{31}H_{50}O_6$: C, 71.78; H, 9.72; found: C, 71.56; H, 9.61.

2.2.7. 22R-3β,22-dihydroxy-9(11)-chlolesten-3,22-diacetate (**42b**)

0.98 g (0.51 mmol scale), 90% yield as a colourless gum. FTIR (KBr) cm^{-1}: 2936, 1731, 1235, 1022. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.17 (1H, d, J = 5.6 Hz· H-11), 4.87 (1H, dt, J = 1.2 and 6.8 Hz, H-22), 4.64–4.56 (1H, m, H-3), 1.97 (3H, s, OCOC$\underline{H}_3$), 1.96 (3H, s, OCOC$\underline{H}_3$), 1.89–1.02 (m, CH and CH_2), 0.90 (3H, d, J = 6.8 Hz, CH_3), 0.89 (3H, s, H-19), 0.86 (2×3H, 2×d, J = 6.6 Hz, H-26 and 27) and 0.53 (3H, s, H-18). ^{13}C NMR δ: 171.53, 171.25, 147.52, 116.47, 77.14, 74.13, 54.16, 53.34, 43.68, 43.60, 41.48, 39.37, 38.26, 37.30, 35.79, 35.56, 34.82, 33.64, 30.57, 29.00, 28.84, 28.67, 28.16, 25.94, 23.30, 23.04, 22.05, 21.83, 18.56, 13.00 and 11.96. MS (CDI$^+$), m/z: 487 (M^++H, 98), 456 (30), 441 (100), 425 (43), 366 (26), 313 (49).

2.2.8. 3β-hydroxy-9(11)-androsten-17-one 3-acetate (**48b**)

Quantitative yield (0.51 mmol scale), colourless gum. FTIR (neat) cm^{-1}: 2924, 1737, 1735, 1029. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.32 (1H, bt, J = 1.8 Hz, H-11), 4.70–4.61 (1H, m, H-3), 2.42 (1H, t, J = 7.6 Hz, CH), 1.99 (3H, s, OCOC$\underline{H}_3$), 1.84–1.08 (m, CH and CH_2), 0.96 (3H, s, H-19), 0.79 (3H, s, H-18). ^{13}C NMR δ: 221.95, 170.64, 147.44, 115.12, 73.29, 57.17, 51.36, 44.85, 42.89, 36.32, 36.22, 35.83, 34.11, 33.26, 32.08, 28.09, 27.47, 22.76, 21.41, 17.96 and 13.85. MS (CDI$^+$), m/z: 329 (M^+−H, 61), 313 (100), 295 (65).

2.3. General procedure for the synthesis of compounds **10a**, **10b**, **21**, **28**, **29**, **30**, **39**, **43**, **44**, **49** and **51**

A solution of (**9a, 9b, 20a, 26a, 27a, 38, 42b, 48b** or **50**) (7.0 mmol) in 20% methanol/dichloromethane (100 ml) was cooled to −78 °C and ozone was bubbled through the solution with stirring. When the solution turned to blue, ozone addition was stopped. Nitrogen was passed through the solution until the blue colour was discharged. The reaction was quenched at −78 °C with PPh_3 (22.0 mmol), warmed to room temperature, stirred for 5 h, and concentrated under reduced pressure. The resulting yellow oil was purified by flash column chromatography (40% EtOAc/hexane) to give the corresponding keto aldehydes.

2.3.1. 3β-hydroxy-9,11-seco-22-isoallospirostan-9-one-11-al (**10a**)

2.3 g (7.2 mmol scale) 71% yield, colourless prisms (from dichloromethane-hexane), mp 198–201 °C. FTIR (KBr) cm^{-1}: 3539, 2938, 1707, 1053. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.84 (1H, t, J = 2.7 Hz, H-11), 4.46 (1H, dt, J = 6.7 and 8.8 Hz, H-16), 3.61–3.53 (1H, m, H-3), 3.50–3.46 (1H, m, H-26), 3.36 (1H, t, J = 11.1 Hz, H-26), 2.91–2.84 (1H, m, H-8), 2.73–2.67 (1H, m, H-14), 2.33 (1H, t, J = 8.6 Hz, CH), 2.28 (1H, dd, J = 2.3 and 5.1 Hz, CH), 2.11–1.26 (m, CH and CH_2), 1.17 (3H, s, H-19), 0.96 (3H, d, J = 6.8 Hz, H-21), 0.91 (3H, s, H-18) and 0.79 (3H, d, J = 6.4 Hz, H-27). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 215.60, 203.19, 108.31, 79.80, 70.29, 66.96, 58.96, 55.57, 48.14, 45.43, 44.97, 44.31, 43.84, 43.27, 36.60, 32.87, 32.45, 31.47, 30.96, 30.61, 30.20, 28.66, 28.04, 18.73, 17.05, 15.52 and 14.33. MS (DCI$^+$), m/z: 447 (M^++H, 100), 429 (67), 385 (18) and 279 (8). Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48; found: C, 72.67; H, 9.50.

2.3.2. 3β-Hydroxy-9,11-seco-22-isoallospirostan-9-one-11-al 3-acetate (**10b**)

1.8 g (5.5 mmol scale) 69% yield, colourless prisms, mp 132–133 °C. FTIR (KBr) cm^{-1}: 2926, 1731, 1698, 1261, 1056. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.76 (1H, t, J = 2.9 Hz, H-11), 4.59–4.51 (1H, m, H-3), 4.39 (1H, dt, J = 6.8 and 8.8 Hz, H-16), 3.39 (1H, ddd, J = 1.8, 4.2 and 10.9 Hz, H-26), 3.30 (1H, t, J = 11.0 Hz, H-26), 2.82–2.76 (1H, m, H-8), 2.66–2.60 (1H, m, H-14), 2.27 (1H, t, J = 8.5 Hz), 2.21 (1H, dd, J = 2.3 and 5.3 Hz), 1.96 (3H, s, OCOC$\underline{H}_3$), 1.89–1.16 (m, CH and CH_2), 1.12(3H, s, H-19), 0.89 (3H, d, J = 6.8 Hz, H-21), 0.84 (3H, s, H-18) and 0.72 (3H, d, J = 6.3 Hz, H-27). ^{13}C NMR ($CDCl_3$) δ: 215.09, 203.09, 170.42, 108.30, 79.74, 72.40, 66.95, 59.00, 55.58, 47.98, 45.13, 44.98, 44.27, 43.81, 43.26, 32.71, 32.66, 32.43, 31.47, 30.79, 30.21, 28.66, 27.91, 26.56, 21.27, 18.68, 17.04, 15.39 and 14.32. MS (DCI$^+$), m/z: 488 (M^+, 100), 470 (53), 427 (21). Anal. Calcd. for $C_{29}H_{44}O_6$: C, 71.28; H, 9.08; found: C, 70.94; H, 9.15.

2.3.3. 3β-Hydroxy-9,11-seco-5α-androst-9,20-dione-11-al-3-acetate (**21**)

597 mg (.1 mmol scale) 73% yield colourless gum which was used immediately without further purification.

2.3.4. 3β,11-dihydroxy-9,11-seco-5α-24-norchlolest-9-one 3-acetate (**28**) and 9,11-seco-5α-24-norchlolest-3β,9β,11-triol 3-acetate (**30**)

The aldehyde was reduced to give the corresponding alcohol **28** (0.38 g, 0.81 mmol), 39% yield colourless gum and small amount of **30**.

28: FTIR (neat) cm^{-1}: 3447, 2952, 1734, 1706, 1029. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.50–4.42 (1H, m, H-3), 3.48–3.36 (2H, m, H-11), 2.85–2.78 (1H, m, H-8), 2.44–2.38 (1H, m, H-11), 1.83 (3H, s, OCOC$\underline{H}_3$), 1.87–0.76 (m, CH and CH_2), 1.08 (3H, s, H-19), 0.86 (3H, d, J = 6.8 Hz, H-21), 0.75 (3H, d, J = 6.6 Hz, H-26 or 27), 0.74 (3H, d, J = 6.4 Hz, H-26 or 27) and 0.61 (3H, s, H-18). ^{13}C δ: 215.24, 170.21, 72.97, 58.39, 58.26, 50.33, 45.98, 45.72, 43.81, 42.45, 41.72, 36.63, 35.31, 33.57, 33.43, 32.49, 31.79, 30.24, 28.99, 28.53, 27.31, 26.41, 23.31, 22.59, 21.00, 19.69, 17.48 and 15.91. MS (CDI$^+$), m/z: 449 (M^++H, 9), 431 (M^+−OH, 100), 371 (8). Anal. Calcd. for $C_{28}H_{48}O_4$: C, 74.95; H, 10.78; found: C, 75.04; H, 10.87.

2.3.5. 3β,11-dihydroxy-9,11-seco-5α-chlolest-9-one 3-acetate (**29**) and 9,11-seco-5α-chlolest-3β,9β,11-triol 3-acetate (**31**)

0. 53 g, of **29** (.62 mmol scale) 48% yield as a colourless gum and 0.15 g of **31**, 14% yield, as a colourless gum.

29: FTIR (neat) cm^{-1}: 3447, 2952, 1735, 1707, 1242, 1029. ^{1}H NMR (acetone-d_6, 400 MHz) δ: 4.50–4.42 (1H, m, H-3), 3.48–3.37 (2H, m, H-11), 2.85–2.79 (1H, m, H-8), 2.43–2.38 (1H, m, H-14), 1.83 (3H, s, OCOC$\underline{H}_3$), 1.73–1.18 (m, CH and CH_2), 1.08 (s, 3H, H-19), 0.87 (3H, d, J = 6.8 Hz, H-21), 0.75 (d, 3H, J = 6.6 Hz, H-26 or 27), 0.74 (3H, d, J = 6.4 Hz, H-26 or 27) and 0.61 (3H, s, H-18). ^{13}C δ: 215.24, 170.20, 72.97, 58.40, 58.26, 50.42, 48.47, 46.00, 45.72, 43.81, 42.45, 41.77, 40.09, 39.89, 36.12, 35.01, 33.42, 32.50, 31.79, 28.53, 28.50, 27.31, 26.40, 25.08, 22.88, 22.70, 21.01, 19.68, 17.49 and 15.90. MS (CDI$^+$),

m/*z*: 462 (M^+, 7), 445 (M^+−OH, 100). Anal. Calcd. for $C_{29}H_{50}O_4$: C, 75.28; H, 10.89; found: C, 75.27; H, 10.85.

31: FTIR (neat) cm^{-1}: 3415, 2943, 1738, 1242, 1026. ^1H NMR (acetone-d_6, 400 MHz) δ: 4.53–4.43 (1H, m, H-3), 3.69–3.61 (1H, m, H-11), 3.55–3.44 (1H, m, H-11), 3.18 (1H, d, J = 5.1 Hz, H-9), 1.81 (3H, s, OCOC$\underline{H}_3$), 1.71–0.87 (m, CH and CH_2), 0.82 (3H, d, J = 6.8 Hz, H-21), 0.78 (3H, s, H-19), 0.74 (2×3H, 2×d, J = 6.6 Hz, H-26 and 27) and 0.64 (3H, s, H-18). ^{13}C δ: 170.22, 81.23, 76.41, 73.89, 58.53, 46.84, 46.59, 42.89, 41.38, 41.09, 39.85, 39.57, 39.27, 36.83, 35.41, 34.39, 33.64, 31.48, 28.55, 27.81, 27.29, 26.05, 25.69, 22.87, 22.72, 21.06, 18.19, 17.73, 16.19. MS (CDI$^+$), *m*/*z*: 405 (M^+−OAc, 28), 387 (405-H_2O, 100), 368 (387-H_2O, 21). Anal. Calcd. for $C_{29}H_{52}O_4$: C, 74.95; H, 11.28; found: C, 74.96; H, 11.30.

2.3.6. 3β-hydroxy-9,11-secochlolest-9,22-dione-11al-3-acetate (**39**)

0.21 g (1.2 mmol scale) 37% yield colourless solid, mp 110–112 °C. FTIR (KBr) cm^{-1}: 2959, 1734, 1709, 1242, 1031. ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.91 (1H, dd, J = 1.8 and 3.1 Hz, H-11), 4.67–4.61 (1H, m, H-3), 2.88–2.82 (1H, m, H-8), 2.78–2.72 (1H, m, H-14), 2.62–2.17 (m, CH and CH_2), 2.02 (s, 3H, OCOC$\underline{H}_3$), 1.93–1.26 (m, CH and CH_2), 1.19 (3H, s, H-19), 1.13 (3H, d, J = 7.0 Hz, H-21), 0.89 (2×3H, 2×d, J = 6.4 Hz, H-26 and 27) and 0.76 (3H, s, H-18). ^{13}C δ: 215.53, 214.08, 203.33, 170.49, 72.46, 51.17, 48.90, 48.27, 47.56, 45.30, 44.20, 43.14, 39.27, 38.92, 32.67, 32.58, 32.36, 30.76, 28.01, 27.60, 26.56, 26.34, 23.93, 22.37, 22.33, 21.28, 6.89, 16.40 and 15.36. MS (EI), *m*/*z*: 474 (M^+, 100%), 457 (55), 411 (39), 390 (40), 149 (42). Anal. Calcd. for $C_{29}H_{48}O_5$: C, 73.38; H, 9.77; found: C, 73.27; H, 9.76.

2.3.7. (22S)-3β,11,22-trihydroxy-9,11-secochlolest-9-one-11,22-hemiacetal 3-acetate (**43**)

0.54 g (016 mmol scale) 72% yield, mp 132–134 °C. FTIR (KBr) cm^{-1}: 3447, 2950, 1735, 1701, 1243, 1031. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.06 (1H, dd, J = 5.5 and 9.2 Hz, H-11), 4.62–4.54 (1H, m, H-3), 3.89 (1H, dd, J = 3.5 and 8.4 Hz, H-8), 2.89 (1H, ddd, J = 3.9, 5.5 and 13.3 Hz, H-22), 2.28 (1H, dt, J = 3.5 and 11.1 Hz, H-14), 1.87–1.28 (m, CH and CH_2), 1.94 (3H, s, OCOC$\underline{H}_3$), 1.87–1.28 (m, CH and CH_2), 1.21 (3H, s, H-19), 0.88 (3H, d, J = 7.2 Hz, H-21), 0.86 (3H, s, H-18), 0.85 (3H, d, J = 6.6 Hz, H-26 or 27) and 0.84 (3H, d, J = 6.6 Hz, H-26 or 27). ^{13}C δ: 214.88, 170.22, 94.24, 94.12, 73.70, 73.00, 57.83, 48.55, 46.09, 46.00, 45.76, 43.66, 39.21, 36.32, 33.45, 33.12, 32.48, 31.84, 28.66, 28.54, 27.34, 24.86, 22.88, 22.84, 22.56, 21.03, 15.93, 14.42 and 8.24. MS (DCI$^+$), *m*/*z*: 459 (M^+−OH, 77), 441 (100), 415 (52). Anal. ($C_{29}H_{48}O_3$) C, H: calcd., 73.07; 10.15%; found, 73.06; 10.08.

2.3.8. (22R)-3β,22-dihydroxy-9,11-secochlolest-9-one-11-al 3,22-diacetate (**44**)

0.10 g (.41 mmol scale) 47% yield, colourless gum which was used immediately in the next step.

2.3.9. 3β,11-dihydroxy-9,11-secoandrost-9,17-dione 3-acetate (**49**)

The aldehyde was reduced to give the corresponding alcohol **49** (0.10 g, 0.47 mmol) 59% yield, colourless solid, mp 92–93 °C. FTIR (KBr) cm^{-1}: 3430, 2936, 1727, 1701, 1235, 1026. ^1H NMR (acetone-d_6, 400 MHz) δ: 4.51–4.43 (1H, m, H-11), 3.54–3.43 (2H, m, H-11), 3.08–3.02 (1H, m, H-8), 2.12–2.03 (1H, m, CH), 1.83 (3H, s, OCOC$\underline{H}_3$), 1.80–1.24 (m, CH and CH_2), 1.10 (3H, s, H-19), 0.67 (3H, s, H-18). ^{13}C δ: 222.13, 215.13, 170.24, 72.94, 58.92, 50.33, 46.44, 46.32, 46.01, 40.73, 40.29, 37.30, 33.42, 32.39, 31.63, 28.41, 27.31, 22.95, 21.04, 19.19 and 15.58. MS (CDI$^+$), *m*/*z*: 365 (M^++H, 60), 312 (69), 253 (100), 143 (77). Anal. Calcd. for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85; found: C, 69.22; H, 8.60.

2.3.10. 3β,11-dihydroxy-9,11-seco-5α-androst-9-one 3-acetate (**51**)

The aldehyde was reduced to give the corresponding alcohol **51** (31 mg, 0.38 mmol) 63% yield, as a colourless gum. FTIR (KBr) cm^{-1}: 3385, 2936, 1731, 1705, 1239, 1026. ^1H NMR (acetone-d_6, 400 MHz) δ: 4.62–4.54 (1H, m, H-3), 3.59–3.54 (1H, m, CH), 3.33–3.25 (1H, m, C$\underline{H}$), 2.91 (1H, dt, J = 5.7 and 13.3 Hz, CH), 2.24–2.18 (1H, m, C$\underline{H}$), 1.94 (3H, s, OCOC$\underline{H}_3$), 1.89–1.35 (m, CH and CH_2), 1.21 (3H, s, H-19), 0.78 (3H, s, H-18). ^{13}C δ: 215.41, 170.16, 72.91, 59.71, 48.50, 45.94, 45.39, 45.33, 45.27, 43.06, 39.84, 33.35, 32.86, 31.64, 28.50, 27.40, 27.24, 22.24, 21.71, 20.95 and 15.55. MS (CDI$^+$), *m*/*z*: 351 (M++H, 4), 333 (100), 273 (43). Anal. Calcd. for $C_{21}H_{32}O_5$: C, 71.96; H, 9.78; found: C, 71.81; H, 9.71.

2.4. General procedure for the synthesis of compounds **11a**, **11b**, **12a**, **12b**, **22**, **40a**, **41**, **45a** and **46a**

Sodium borohydride (69 mg, 1.2 mmol) was added to a solution of **10a, 10b, 21, 39** or **44** (1.8 mmol scale) in 30% ethanol/dichloromethane or methanol (70 ml) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C or room temperature, diluted with 10% acetic acid in water, and then concentrated under reduced pressure. The residue was diluted with ethyl acetate and washed with water, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The residue was purified by flash column chromatography.

2.4.1. 3β,11-dihydroxy-9,11-seco-22-isoallospirostan-9-one (**11a**) and 9,11-seco-22-isoallospiro-sten-3β,9β,11-triol (**12a**)

0.56 g of **11a** (1.8 mmol scale) 71% yield colourless prisms; mp 198–200 °C and 70 mg **12a** 9% yield colourless prisms; mp 196–197 °C.

11a: FTIR (KBr) cm^{-1}: 3293, 2952, 1707, 1060. ^1H NMR (acetone-d_6 + DMSO-d_6, 400 MHz) δ: 4.37–4.31 (1H, m, H-16), 3.57–3.49 (2H, m, H-11), 3.46–3.41 (1H, m, H-3), 3.43–3.39 (1H, m, H-26), 3.31 (1H, t, J = 10.7 Hz, H-26), 2.96–2.93 (1H, m, H-8), 2.57–2.51 (1H, m, H-14), 2.13 (1H, t, J = 8.6 Hz, CH), 1.80–1.37 (m, CH and CH_2), 1.17 (3H, s, H-19), 0.94 (3H, d, J = 6.8 Hz, H-21), 0.82 (3H, s, H-18) and 0.75 (3H, d, J = 6.4 Hz, H-27). ^{13}C NMR (acetone-d_6 + DMSO-d_6, 100 MHz) δ: 215.48, 108.57, 81.05, 70.24, 67.25, 59.41, 59.15, 48.79, 46.34, 46.24, 44.85, 44.60, 44.19, 44.00, 37.75, 33.14, 32.58, 32.29, 32.15, 31.50, 31.04, 29.50, 28.73, 18.89, 17.36, 15.96 and 14.76. MS (DCI$^+$), *m*/*z*: 449 (M^++H, 31), 411 (100), 389 (57), 373 (16) and 147 (35). Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89; found: C, 72.06; H, 9.85.

12a: FTIR (KBr) cm^{-1}: 3390, 2929 and 1055. ^1H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz) δ: 4.34 (1H, dd, J = 7.6 and 16.9 Hz H-16), 3.56–3.51 (1H, m, H-11), 3.45–3.38 (1H, m, H-11), 3.37–3.32 (1H, m, H-3), 3.35–3.28 (1H, ddd, J = 1.9, 4.3 and 11.1 Hz, H-26), 3.22 (1H, t, J = 10.9 Hz, H-26), 2.57 (1H, d, J = 10.5 Hz, H-9), 2.22 (1H, dd, J = 5.5 and 14.2 Hz, CH), 1.95 (1H, t, J = 9.2 Hz, CH), 1.72–0.85 (m, CH and CH_2), 0.73 (3H, d, J = 6.8 Hz, H-21), 0.65 (2×3H, s, H-18 and 19) and 0.67 (3H, d, J = 6.4 Hz, H-27). ^{13}C NMR (acetone-d_6 + DMSO-d_6, 100 MHz) δ: 108.51, 80.46, 79.40, 70.17, 66.53, 57.80, 56.25, 44.16, 43.50, 42.83, 42.47, 41.87, 38.79, 36.90, 35.96, 35.45, 30.85 (2C), 30.60, 29.88, 28.77, 28.45, 27.61, 20.12, 16.73, 13.80 and 10.40. MS (DCI$^+$), *m*/*z*: 451 (M^++H, 94), 433 (100), 415 (47), 307 (59) and 289 (57). Anal. Calcd. for $C_{27}H_{46}O_5$: C, 71.96; H, 10.29; found: C, 71.72; H, 10.14.

2.4.2. 3β,11-dihydroxy-9,11-seco-5α-22-isoallospirostan-9-one, 3-acetate (**11b**) and 3β,9β,11-trihydroxy-9,11-seco-5α-22-isoallospirostan-3-acetate (**12b**)

0.58 g of **11b** (1.2 mmol scale), 74% yield, colourless solid; mp: 67–68 °C and 06.0 g **12b** 8% yield, colourless solid, mp: 159–160 °C.

11b: FTIR (KBr) cm^{-1}: 3545, 2937, 1737, 1701, 1243, 1026. ^1H NMR (acetone-d_6, 400 MHz) δ: 4.62–4.54 (1H, m, H-3), 4.38–4.32 (1H, m, H-16), 3.56 (2H, t, J = 7.2 Hz, H-11), 3.43–3.39 (1H, m, H-26), 3.31 (1H, t, J = 10.9 Hz, H-26), 2.98–2.92 (1H, m, H-8), 2.55–2.53 (1H, m, H-14), 2.13 (1H, t, J = 8.6 Hz CH), 1.95 (3H, s, OCOC$\underline{H}_3$), 1.88–1.27 (m, CH and CH_2), 1.21 (3H, s, H-19), 0.94 (3H, d, J = 6.8 Hz, H-21), 0.83 (3H, s, H-18) and 0.76 (3H, d, J = 6.2 Hz, H-27). ^{13}C δ: 214.98, 170.33, 108.64, 81.11, 73.07, 67.35, 59.59, 59.27, 48.65, 46.40, 45.87, 44.88, 44.70, 44.28, 44.12, 33.53, 33.05, 32.66, 32.39, 31.91, 31.14, 29.22, 28.57, 27.43, 21.13, 18.97, 17.41, 15.86 and 14.82. MS (DCI$^+$), m/z: 490 (M^+, 100), 347 (12). Anal. Calcd. for $C_{29}H_{46}O_6$: C, 70.99; H, 9.45; found: C, 70.65; H, 9.25.

12b: FTIR (KBr) cm^{-1}: 3421, 2950, 1734, 1243 and 1056. ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.72–4.64 (1H, m, H-3), 4.35 (1H, dt, J = 7.6 and 9.2 Hz, H-16), 3.81–3.77 (1H, m, H-11), 3.68–3.63 (1H, m, H-11), 3.50–3.46 (1H, m, H-26), 3.39 (1H, t, J = 10.9 Hz, H-26), 2.83 (1H, d, J = 10.5 Hz, H-9), 2.36 (1H, dd, J = 5.5 and 14.2 Hz, CH), 2.16 (t, 1H, J = 9.0 Hz, CH), 2.01 (3H, s, OCOC$\underline{H}_3$), 1.92–1.05 (m, CH and CH_2), 0.92 (3H, d, J = 7.0 Hz, H-21), 0.86 (3H, s, H-19), 0.84 (3H, s, H-18) and 0.79 (3H, d, J = 6.2 Hz, H-27). ^{13}C δ: 170.62, 109.01, 81.14, 79.69, 73.36, 67.04, 59.00, 56.58, 44.74, 43.60, 43.32, 42.98, 41.95, 39.10, 35.93, 35.84, 33.11, 31.29 (2C), 30.34, 29.02, 28.67, 27.76, 26.96, 21.40, 20.53, 17.11, 14.18 and 10.62. MS (DCI$^+$), m/z: 492 (M^+, 100), 475 (21), 450 (37), 432 (39) and 415 (18). Anal. Calcd. for $C_{29}H_{48}O_6$: C, 70.70; H, 9.82; found: C, 70.83; H, 9.67.

2.4.3. 3β,11-dihydroxy-9,11-seco-5α-pregna-11,20-dihydropyran-9-one 3-acetate (**22**)

0.78 g (2.1 mmol scale) 42% yield, as a colourless solid, mp 114–116 °C. FTIR (KBr) cm^{-1}: 2949(CH), 1731 (C=C), 1711 (C=O), 1244 (C=C), 1030 (C–O). ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.54 (1H, m, H-3), 3.94 (1H, dd, J = 2.5 and 11.1 Hz, H-11), 3.76 (1H, t, J = 11.1 Hz, H-11), 2.79–2.72 (1H, m, H-8), 2.18–2.10 (1H, m, H-14), 1.86–1.24 (m, CH and CH_2), 1.95 (3H, s, OCOC$\underline{H}_3$), 1.60 (3H, s, H-21), 1.11 (3H, s, H-19) and 0.75 (3H, s, H-18). ^{13}C δ: 215.44, 170.49, 141.29, 115.03, 72.54, 62.34, 48.05, 47.63, 45.99, 45.72, 39.31, 36.32, 33.14, 32.75, 30.62, 28.19, 26.64, 25.86, 24.52, 21.30, 20.53, 16.58 and 15.26. MS (DCI$^+$), m/z: 375 (M^++H, 100). Anal. Calcd. for $C_{23}H_{34}O_5$: C, 73.76; H, 9.15; found: C, 73.63; H, 9.22.

2.4.4. 3β,11-dihydroxy-9,11-secochlolest-9,22-dione-3-acetate (**40a**) and 9,11-secochlolest-22-one-3β,9β,11-triol 3-acetate (**41**)

0.74 mg of **40a** (0.3 mmol scale), 49% yield, as a colourless solid, mp: 67–68 °C and 0.50 g of **41**, 33% yield, as a colourless solid, mp: 71–72 °C.

40a: FTIR (KBr), cm^{-1}: 3384, 2957, 1731, 1707, 1244, 1031. ^1H NMR (acetone-d_6, 400 MHz) δ: 4.61–4.54 (1H, m, H-3), 3.65–3.59 (1H, m, H-11), 3.57–3.50 (1H, m, H-11), 2.95 (1H, ddd, J = 2.9, 5.6 and 13.3 Hz, C$\underline{H}$), 2.59–2.50 (2H, m, C$\underline{H}_2$), 2.46–2.38 (1H, m, C$\underline{H}$), 2.06–2.03 (2H, m, C$\underline{H}_2$), 1.94 (3H, s, OCOC$\underline{H}_3$), 1.87–1.30 (m, CH and CH_2), 1.20 (3H, s, H-19), 1.13 (3H, d, J = 7.0 Hz, H-21,), 0.87 (2×3H, 2×d, J = 6.4 Hz, H-26 and 27) and 0.75 (3H, s, H-18). ^{13}C δ: 215.17, 213.98, 170.32, 73.07, 58.33, 49.60, 48.58, 46.93, 45.94, 45.76, 43.67, 42.13, 41.76, 40.08, 33.52, 33.11, 32.52, 31.89, 28.59, 28.24, 27.56, 27.41, 23.27, 22.68, 22.65, 21.10, 17.66, 16.79 and 16.01. MS (EI), m/z: 461 (M^+−15, 100%), 443 (43), 401 (42). Anal. Calcd. for $C_{29}H_{48}O_5$: C, 73.07; H, 10.15; found: C, 73.18; H, 10.07.

41: FTIR (KBr), cm^{-1}: 3384, 2957, 1731, 1244, 1031. ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.65–4.56 (1H, m, H-3), 3.87–3.81 (1H, m, H-11), 3.65–3.59 (1H, m, H-11), 2.80 (1H, d, J = 10.7 Hz, H-9), 2.45–2.34 (2H, m, H-23), 2.32–2.23 (1H, m, H-20), 1.81 (3H, s, OCOC$\underline{H}_3$), 1.73–1.07 (m, CH and CH_2), 0.99 (3H, d, J = 6.8 Hz, H-21), 0.79 (2×3H, 2×d, J = 6.4 Hz, H-26 and 27), 0.82 (3H, s, H-19) and 0.65 (3H, s, H-18). ^{13}C δ: 214.77, 170.65, 81.43, 73.39, 58.36, 49.07, 45.95, 45.34, 43.23, 42.02, 40.12, 39.95, 39.23, 35.97, 35.29, 33.12, 32.36, 28.82, 27.89, 27.66, 27.06, 26.96, 22.39, 22.36, 21.40, 21.22, 17.91, 16.63 and 10.67. MS (EI), m/z: 477 (M^+−H, 13), 460 (M^+−H_2O, 100), 442 (43), 401 (46), 383 (23). Anal. Calcd. for $C_{29}H_{50}O_4$: C, 72.76; H, 10.53; found: C, 72.85; H, 10.28.

2.4.5. $\Delta^{9(11)}$-Chlolest-3β,22-diol 3-acetate (**42a**)

225 mg (0.63 mmol scale), a mixture of 22-*S* and *R* of **42a** in the ratio 2:1, 81% yield.

(22*R*)-42a: As a colourless solid, mp 80–82 °C. FTIR (KBr) cm^{-1}: 3453, 2928, 1727, 1034. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.26 (1H, d, J = 5.8 Hz, H-11), 4.71–4.63 (1H, m, H-3), 3.61 (1H, dd, J = 2.9 and 8.2 Hz, H-22), 2.02 (3H, s, OCOC$\underline{H}_3$), 1.97–1.16 (m, CH and CH_2), 0.96 (3H, s, H-19), 0.91 (3H, d, J = 6.8 Hz, H-26 or 27), 0.90 (3H, d, J = 6.4 Hz, H-21), 0.89 (3H, d, J = 6.8 Hz, H-26 or 27) and 0.61 (3H, s, H-18). ^{13}C δ: 170.66, 146.78, 115.96, 74.07, 73.51, 53.32, 53.20, 43.02, 42.18, 41.74, 41.25, 37.63, 36.69, 36.10, 35.13, 34.17, 33.05, 28.37, 28.13, 27.62, 27.59, 27.51, 25.46, 22.92, 22.44, 21.41, 17.89, 12.01 and 11.47. MS (CDI$^+$), m/z: 443 (M^+−H, 9), 427 (M^+−OH, 60), 383 (28), 367 (100).

(22*S*)-42a, as colourless prisms, mp180–181 °C. FTIR (KBr) cm^{-1}: 3448, 2928, 1718, 1045. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.25 (1H, d, J = 5.8 Hz, H-11), 4.71–4.63 (1H, m, H-3), 3.63 (1H, brt, J = 5.85 Hz, H-22), 2.02 (s, 3H, OCOC$\underline{H}_3$), 1.96–1.06 (m, CH and CH_2), 0.96 (3H, s, H-19), 0.89 (2×3H, 2×d, J = 6.4 Hz, H-26 and 27), 0.89 (3H, d, J = 6.6 Hz, H-21) and 0.60 (3H, s, H-18). ^{13}C δ: 170.65, 146.78, 116.96, 73.88, 73.53, 53.54, 52.70, 42.98, 41.85, 40.86, 40.07, 37.64, 36.75, 35.67, 35.13, 34.19, 33.22, 33.00, 28.39, 28.18, 27.89, 27.52, 25.31, 22.68, 22.54, 21.44, 17.92, 11.43 and 11.11. MS (CDI$^+$), m/z: 443 (M^+−H, 9), 427 (60), 411 (28), 385 (35), 367 (100). Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.33; H, 10.90; found: C, 78.21; H, 10.90.

2.4.6. (22R)-3β,11,22-trihydroxy-9,11-secochlolest-9-one 3,22-diacetate (**45a**) and (22R)-3β,9β,11,22-tetrahydroxy-9,11-secochlolest-3,22-diacetate (**46a**)

0.35 g of **45a** (0.41 mmol scale), 36% yield and 0.28 g of **46a**, 29% yield which were used in the next step without further purification.

2.5. 3β-hydroxy-9,11-seco-5α-22-isoallospirosten-9-one-11-oxime 3-acetate (**13**)

A solution of **10b** (200 mg, 0.5 mmol) in methanol (9 ml) was added to a solution of hydroxylamine hydrochloride (82 mg, 2.2 mmol) in water (1 ml). The reaction mixture was stirred at room temperature for 3 h and then concentrated under reduce pressure. Purification by flash column chromatography (30% EtOAc/hexane) gave a mixture of *Z* and *E* of **13** in the ratio 3:2 (121 mg, 59% yield). FTIR (KBr) cm^{-1}: 3423, 2953, 1731, 1708, 1243 and 1058. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.40 (1H, t, J = 6.4 Hz, H-11), 6.87 (1H, t, J = 5.3 Hz, H-11), 4.61–4.52 (2×1H, 2×m, H-3), 4.39–4.33 (2×1H, 2×m, H-16), 3.43–3.38 (2×1H, 2×m, H-26), 3.30 (2×1H, 2×t, J = 11.1 Hz, H-26), 2.82–2.72 (2×1H, 2×m, H-8), 2.59–2.50 (1H, m, H-14), 2.51–1.13 (m, CH and CH_2), 1.95 (2×3H, 2×s, 2× OCOC$\underline{H}_3$), 1.11(3H, s, H-19), 1.10 (3H, s, H-19), 0.90 (3H, d, J = 6.8 Hz, H-21), 0.88 (3H, d, J = 6.8 Hz, H-21), 0.80 (3H, s, H-18), 0.77 (3H, s, H-18) and 0.71 (2×3H, 2×d, J = 6.4 Hz Me, H-27). ^{13}C δ: 215.07, 214.81, 170.51 (2C), 150.56 (2C), 108.48, 108.19, 80.05, 79.86, 72.51 (2C), 66.96 (2C), 58.35 (2C), 57.95 (2C), 47.99, 47.94, 45.14, 45.02, 44.44, 44.05, 43.92 (2C), 43.87, 43.82, 41.97 (2C), 36.76 (2C), 32.71, 32.51, 32.36, 32.27, 32.06 (2C), 31.54, 31.47, 30.87, 30.25, 28.70 (2C), 27.95, 27.92, 26.61 (2C), 21.31 (2C), 18.83, 18.61, 17.07 (2C), 15.61, 15.54, 14.39 and 14.25. MS (DCI$^+$), m/z: 504 (M^++H, 95), 485 (100). Anal. Calcd. for $C_{29}H_{45}NO_6$: C, 69.13; H, 9.04; N, 2.78; found: C, 69.15; H, 9.01; N, 2.78.

*2.6. 3β-Hydroxy-9,11-seco-5α-22-isoallospirosten-9-one-11-nitrile 3- acetate (**14**)*

A mixture of *Z* and *E* of **15** (70 mg, 0.1 mmol) and DDQ (150 mg, 0.7 mmol) in dioxane (5 ml) was refluxed for 30 min. The reaction mixture was filtered through neutral alumina, and then concentrated under reduce pressure. The residue was purified by flash column chromatography (10% EtOAc/hexane) to give **14** (24 mg, 36%) as a colourless solid; mp 155–157 °C. FTIR (KBr) cm^{-1}: 2926, 2238, 1736, 1697, 1259, 1055. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.67–4.59 (1H, m, H-3), 4.44 (1H, dt, *J* = 7.2 and 8.8 Hz, H-16), 3.46–3.45 (1H, m, H-26), 3.36 (1H, t, *J* = 10.9 Hz, H-26), 2.93–2.88 (1H, m, H-8), 2.61–2.54 (1H, m, H-14), 2.33 (2H, s, H-12), 2.25 (1H, t, *J* = 8.6 Hz, CH), 2.08–1.25 (m, CH and CH_2), 2.02 (3H, s, $OCOC\underline{H}_3$), 1.21 (3H, s, H-19), 1.03 (3H, d, *J* = 7.0 Hz, H-21), 0.95 (3H, s, H-18) and 0.78 (3H, d, *J* = 6.4 Hz, H-27). ^{13}C δ: 215.22, 170.47, 119.01, 108.51, 78.69, 72.40, 67.03, 59.67, 48.10, 45.76, 45.23, 44.81, 43.62, 42.81, 33.71, 32.97, 32.65, 31.61, 31.46, 30.75, 30.19, 28.64, 27.89, 26.56, 21.30, 17.90, 17.07, 15.26, and 14.18. MS (DCI$^+$), *m*/*z*: 486 (M^++H, 100), 468 (14). Anal. Calcd. for $C_{29}H_{43}NO_5$: C, 71.72; H, 8.92; N, 2.88; found: C, 71.53; H, 8.83; N, 2.98.

*2.7. General procedure for the synthesis of compounds **15** and **17***

A mixture of pyridine (0.6 ml), NH_4Cl (225 mg,), and acetic anhydride (6 ml) was added at once to **11b** or **12a** (3.4 mmol). The mixture was heated to 125–135 °C and kept at that temperature until TLC indicated the reaction to be complete (8 h). A mixture of acetic acid (3 ml), 1,2-dichloroethane (3 ml), and water (0.4 ml) was then added, and the mixture was cooled to 0 °C. A solution of CrO_3 (7.0 mmol) in water (1 ml) and acetic acid (0.5 ml) was cooled to 0 °C and then added dropwise to the reaction mixture and stirred for 1 h while the temperature was kept at 7–10 °C. A solution of NaCl (980 mg) in water (10 ml) and MeOH (0.5 ml) was then introduced and stirring continued for an additional 1 h. The reaction mixture was extracted with dichloromethane, and the combined organic layers were washed with water to remove residual chromium salts, then dried over anhydrous sodium sulphate and concentrated under reduce pressure. A solution of the residue in benzene (80 ml) was added with basic alumina (15 g), and stirring continued for 10 h. The reaction mixture was filtered, concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel.

*2.7.1. 3β,11-dihydroxy-9,11-seco-5α-pregna-16-en-9,20-dione 3,11-diacetate (**15**)*

Purification of the crude product by flash column chromatography (30% EtOAc/hexane) gave **15** (400 mg, 45%) as a colourless gum. FTIR (neat) cm^{-1}: 2946, 1735, 1660, 1241. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.71 (1H, t, *J* = 2.7 Hz, H-16), 4.68–4.61 (1H, m, H-3), 4.15–4.08 (1H, m, H-11), 3.96–3.89 (1H, m, H-11), 2.95–2.89 (1H, m, H-8), 2.82–2.76 (1H, m, H-14), 2.47 (1H, ddd, *J* = 3.1, 8.4 and 18.3 Hz, CH), 2.28 (3H, s, H-21), 2.02 (3H, s, $OCOC\underline{H}_3$), 1.97 (3H, s, $OCOC\underline{H}_3$), 1.86–1.31(m, CH and CH_2), 1.21 (3H, s, H-19) and 1.03 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 215.18, 196.73, 171.11, 170.46, 149.91, 144.09, 72.43, 62.42, 49.40, 48.04, 45.43, 45.16, 41.48, 34.90, 33.43, 32.64, 32.44, 30.71, 27.89, 27.64, 26.55, 21.27, 21.01, 20.82 and 15.39. MS (DCI$^+$), *m*/*z*: 373 (M^+−OAc, 100). Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39; found: C, 69.38; H, 8.29.

*2.7.2. 3β,11-dihydroxy-9,11-seco-5α-pregn-16-en-9,20-dione-3,11-diacetate (**17**)*

Purification of the crude product by flash column chromatography (30% EtOAc/hexane) gave **17** (660 mg, 49%). Crystallization from dichloromethane-hexane gave colourless prisms; mp 172–173 °C. FTIR (KBr) cm^{-1}: 2946, 1735, 1660, 1241, 1027. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.73 (1H, t, *J* = 2.7 Hz, H-16), 4.70–4.63 (1H, m, H-3), 4.50 (1H, d, *J* = 11.1 Hz, H-9), 4.03–3.97 (1H, m, H-11), 3.83–3.70 (1H, m, H-11), 2.37–2.33 (m, 2H, CH_2), 2.26 (3H, s, H-21), 2.18–2.10 (1H, m, CH), 2.08 (3H, s, $OCOC\underline{H}_3$), 2.01 (3H, s, $OCOC\underline{H}_3$), 1.99 (3H, s, $OCOC\underline{H}_3$), 1.95–1.16 (m, CH and CH_2), 1.12 (3H, s, H-19) and 0.97 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 196.71, 171.15, 170.98, 170.50, 149.69, 145.05, 81.08, 72.91, 61.92, 49.35, 43.27, 42.03, 38.73, 35.56, 35.36, 34.77, 32.70, 30.57, 28.12, 27.55, 27.33, 26.64, 21.34, 20.95, 20.82, 20.56 and 11.36. MS (DCI$^+$), *m*/*z*: 417 (M^+−OAc, 98), 357 (100), 297 (28). Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.04; H, 8.46; found: C, 68.11; H, 8.48.

*2.8. General procedure for the synthesis of compounds **16**, **18**, and **20a***

Method A: Raney nickel (Merck, for synthesis) (5 g) was washed with absolute ethanol and THF. To this mixture was added a solution of **15, 17** or **19** (554 mg, 1.28 mmol) in THF (20 ml). The mixture was sonicated for 2 h, filtered through silica and the residue was then rinsed with THF. The filtrate was concentrated and the residue was purified by flash column chromatography.

Method B: A solution of **15, 17** or **19** (80 mg, 0.18 mmol) in THF (10 ml) was treated with 10% palladium on charcoal (30 mg) under hydrogen gas (1 atm) for 30 min. The reaction mixture was filtered through celite and the residue was purified by flash column chromatography.

*2.8.1. 3β,11-dihydroxy-9,11-seco-5α-pregna-9,20-dione 3,11-diacetate (**16**)*

Method A: The residue was purified by flash column chromatography (20% EtOAc/hexane) to give 1:1 mixture of *R* and *S* isomer of **16** (516 mg, 93% yield) as a colourless gum.

Method B: The reaction mixture was filtered through celite, affording 1:1 mixture of *R* and *S* isomer of **16** (76 mg, 95%) as a colourless gum. FTIR (neat) cm^{-1}: 2947, 1736, 1701, 1242, 1030. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.68–4.60 (1H, m, H-3), 4.11–4.00 (2H, m, H-11), 2.81–2.76 (1H, m, H-8), 2.71–2.63 (1H, m, H-14), 2.59 (1H, t, *J* = 10 Hz, H-17), 2.18 (3H, s, H-21), 2.02 (s, 3H, $OCOC\underline{H}_3$), 2.00 (3H, s, $OCOC\underline{H}_3$), 1.93–1.38 (m, CH and CH_2), 1.19 (3H, s, H-19) and 1.01 (3H, s, H-18). ^{13}C δ: 215.04, 210.44, 171.00, 170.44, 72.46, 62.55, 61.85, 47.99, 45.94, 45.56, 45.11, 43.48, 36.00, 32.67, 31.87, 31.62, 30.80, 27.86, 26.57, 26.49, 26.13, 22.76, 21.28, 21.01 and 15.41. MS (CDI$^+$), *m*/*z*: 375 (M^+−OAc, 100%). Anal. Calcd. for $C_{25}H_{38}O_6$: C, 69.10; H, 8.81; found: C, 69.15; H, 8.90.

*2.8.2. 3β,11-dihydroxy-9,11-seco-5α-pregnane-9,20-dione-3,11-diacetate (**18**)*

Method A: The residue was purified by flash column chromatography (30% EtOAc/hexane) to give 1:1 mixture of *R* and *S* isomer of **18** (73 mg, 64% yield) as a colourless gum.

Method B: The reaction mixture was filtered through celite, affording 1:1 mixture of *R* and *S* isomer **18** (69 mg, 98%) as a colourless gum. FTIR (neat) cm^{-1}: 2943, 1727, 1701, 1239, 1026. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.66–4.60 (1H, m, H-3), 4.44 (1H, d, *J* = 11.1 Hz, H-9), 4.43 (1H, d, *J* = 11.1 Hz, H-9), 4.15–4.07 (2×2H, 2×m, H-11), 3.99–3.92 (1H, m, CH), 2.72 (1H, t, *J* = 9.2 Hz, CH), 2.62 (1H, dd, *J* = 6.4 and 8.6 Hz, CH), 2.12 (s, 3H, H-21), 2.11 (s, 3H, H-21), 2.11 (s, 3H, $OCOC\underline{H}_3$), 2.09 (s, 3H, $OCOC\underline{H}_3$), 2.03 (s, 3H, $OCOC\underline{H}_3$), 2.00 (s, 3H, $OCOC\underline{H}_3$), 1.85 (2×3H, 2×s, $OCOC\underline{H}_3$), 1.96–1.13 (m, CH and CH_2), 0.98 (s, 3H, $C\underline{H}_3$), 0.93 (3H, s, $C\underline{H}_3$), 0.92 (3H, s, $C\underline{H}_3$), and 0.77 (3H, s, $C\underline{H}_3$). ^{13}C δ: 210.69, 210.25, 171.08 (2C), 170.95 (2C), 170.45 (2C), 81.89, 77.21, 72.98, 72.89, 61.93, 61.23, 61.03, 58.35 (2C), 46.48, 46.27, 45.60, 45.01, 41.94 (2C), 41.85, 38.90, 38.56, 37.13, 35.58

(2C), 35.37 (2C), 34.22, 32.75, 32.70, 31.67, 31.61, 28.43, 27.81, 27.44, 27.41, 26.66, 26.19, 24.65, 22.88, 22.08, 22.03, 21.33 (2C), 21.05, 21.01, 20.94, 20.88, 18.08 and 11.34. MS (CDI$^+$), *m*/*z* (relative intensity): 419 (M^+−AcO, 59), 359 (100), 299 (27), 255 (14). Anal. Calcd. for $C_{27}H_{42}O_7$: C, 67.76; H, 8.84; found: C, 67.67; H, 8.78.

2.8.3. 3β-hydroxy-9(11)-allopregnene-20-one 3-acetate (**20a**)

Method A: The residue was purified by flash column chromatography (15% EtOAc/hexane) to give **20a** (459 mg, 86% yield) as a colourless solid; mp 128–131 °C.

Method B: The reaction mixture was filtered through celite and concentrated under reduced pressure. Purification by flash column chromatography (15% EtOAc/hexane) afforded **20a** (81 mg, 81% yield) as a colourless solid. FTIR (KBr) cm^{-1}; 2933, 1733, 1704, 1242, 1028. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.33 (1H, t, *J* = 2.7 Hz, H-11), 4.73–4.65 (1H, m, H-3), 2.61 (1H, t, *J* = 9.3 Hz, H-17), 2.27–1.24 (m, CH and CH_2), 2.15 (3H, s, H-21), 2.00 (3H, s, OCOC<u>H</u>$_3$), 0.93 (3H, s, H-19) and 0.52 (3H, s, H-18). ^{13}C δ: 209.93, 171.11, 147.78, 115.66, 73.80, 64.08, 54.19, 43.35, 43.00, 41.05, 38.19, 37.00, 35.56, 34.52, 33.35, 31.63, 28.66, 27.88, 25.93, 23.28, 21.86, 18.29, 13.41. MS (DCI$^+$), *m*/*z*: 358 (M^+, 10), 341 (46), 299 (46), 281 (100), 179 (70).

2.9. 3β-hydroxy-9(11)-allopregnene-20-one (**20b**)

A solution of **20a** (1 g, 2.8 mmol) in methanol (20 ml) was treated with a solution of potassium hydroxide (188 mg, 3.4 mmol) in H_2O (0.5 ml) and stirring continued for 3 h. The reaction mixture was concentrated, diluted with dichloromethane, washed with water, dried over anhydrous sodium sulphate, and concentrated. Purification by flash chromatography (30% EtOAc/hexane) afforded **20b** in quantitative yield as a colourless solid; mp 192–195 °C. FTIR (KBr) cm^{-1}: 2933, 1704, 1242, 1052. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.32–5.30 (1H, m, H-11), 3.61–3.56 (1H, m, H-3), 2.58 (1H, t, *J* = 9.4 Hz, H-17), 2.24–1.14 (m, CH and CH_2), 2.12 (3H, s, H-21), 0.93 (3H, s, H-19) and 0.54 (3H, s, H-18).^{13}C δ: 210.09, 147.78, 115.66, 71.10, 64.08, 54.19, 43.35, 43.00, 41.05, 38.19, 37.60, 35.56, 34.70, 32.10, 31.63, 28.66, 27.88, 25.93, 23.28, 21.86, 18.29. MS (DCI$^+$), *m*/*z*: 317 (M^++H, 16), 287 (100), 269 (67).

2.10. Δ$^{9(11)}$-Pregna-3β-ol-20,22-epoxy-20-methyl (**23**)

Sodium hydride (180 mg, 4.5 mmol) as a 60% mineral oil dispersion was placed in a reaction flask and washed twice with portions of petroleum ether with swirling. The hydride was then allowed to settle, and decanted in order to remove the mineral oil. A solution of trimethylsulfonium iodide (970 mg, 4.7 mmol) in DMSO (3 ml) was added to the reaction flask at 0 °C. After stirring for 30 min a solution of **20b** (300 mg, 0.95 mmol) in THF (5 ml) was added. Stirring was continued for 30 min at 0 °C and then for 2 h at room temperature, followed by removal of most of THF. The reaction mixture was diluted with dichloromethane and washed with water. The combined organic layers were dried over anhydrous sodium sulfate, and concentrated under reduce pressure. Purification by flash chromatography (30% EtOAc/hexane) gave **23** (263 mg, 84% yield) as a colourless solid, mp 144–146 °C. FTIR (KBr) cm^{-1}: 3415, 2913, 1045, 1015. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.26 (1H, d, *J* = 6.0 Hz, H-11), 3.60–3.52 (1H, m, H-3), 2.47 (1H, dd, *J* = 0.4 and 5.0 Hz, H-22), 2.30 (1H, d, *J* = 5.0 Hz, H-22), 2.04–1.01 (m, CH and CH_2), 1.36 (3H, s, H-21), 0.93 (3H, s, H-19) and 0.71 (3H, s, H-18). ^{13}C δ: 147.48, 115.38, 71.14, 56.15, 54.05, 53.70, 51.23, 43.23, 41.72, 41.49, 38.34, 37.75, 36.51, 35.43, 33.00, 31.58, 28.44, 24.90, 22.46, 22.08, 18.04 and 12.69. MS (CDI$^+$), *m*/*z*: 331 (M^++H, 37), 313 (38), 297 (100), 273 (36), 255 (48).

2.11. Δ$^{9(11),22}$-24-norchlolest-3β-ol (**24**)

Boron trifluoride etherate (0.1 ml) was added dropwise to a solution of **23** (240 mg, 0.73 mmol) in dichloromethane (10 ml) which was cooled in an ice bath. After stirring for 30 min, a solution of saturated sodium bicarbonate was added. The resulting mixture was stirred for further 30 min. The organic phase was then separated and the aqueous phase was extracted with methylene chloride. The combined organic extract was washed with saturated brine and dried over anhydrous sodium sulphate. The solvent was removed under vacuum and aldehyde compound obtained in quantitative yield, was used in the following step without further purification. A solution of *n*-BuLi (1.6 M in hexane, 3.8 ml, 6.0 mmol) was added dropwise over several minutes to a stirred suspension of isobutyl triphenylphosphonium bromide (2.4 g, 6.0 mmol) in dry THF (10 ml) at 0 °C. After stirring for 30 min a solution of aldehyde compound (670 mg, 2.0 mmol) in dry THF (10 ml) was added. Stirring was continued at room temperature for 16 h, followed by removal of most of the tetrahydrofuran. Water was added and the mixture was extracted with methylene chloride. The extract was dried over anhydrous sodium sulfate, and concentrated under reduce pressure. Purification by flash chromatography (20% ethyl acetate/hexane) obtained **24** (392 mg, 53% yield) as a colourless solid, mp 132–134 °C. FTIR (KBr) cm^{-1}: 3378, 2921, 1037. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.22–5.20 (1H, m, H-11), 4.97–4.89 (2×1H, m, H-22 and 23), 3.56–3.47 (1H, m, H-3), 2.55–2.53 (1H, m, H-20 or 25), 2.43–2.33 (1H, m, H-20 or 25), 2.15–1.05 (m, CH and CH_2), 0.94 (3H, d, *J* = 6.8 Hz, H-21), 0.93 (s, 3H, H-19), 0.91 (2×3H, 2×d, *J* = 6.6 Hz, H-26 and 27) and 0.57 (3H, s, H-18). ^{13}C δ: 147.90, 134.93, 134.66, 116.47, 71.82, 57.22, 56.86, 54.54, 43.89, 42.39, 39.02, 38.57, 37.36, 36.09, 35.24, 33.79, 32.24, 29.37, 29.17, 27.43, 26.00, 24.22, 23.80, 21.38, 18.70 and 12.37. MS (CDI$^+$), *m*/z: 370 (M^+, 23), 353 (M^+−OH, 100), 345 (10).

2.12. Δ$^{9(11)}$-24-norchlolest-3β-ol (**26a**)

A solution of **24** (148 mg, 0.4 mmol) in THF (10 ml) was treated with 10% palladium on carbon (50 mg, 0.05 mmol of palladium) under hydrogen atmosphere (1 atm) after stirring for 90 min, the reaction mixture was filtered through celite, and concentrated. Purification by flash column chromatography (20% ethyl acetate/hexane) afforded **26a** in quantitative yield as colourless gum. FTIR (KBr) cm^{-1}: 3373, 2928, 1041. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.19 (1H, d, *J* = 5.8 Hz, H-11), 3.55–3.47 (1H, m, H-3), 1.90–0.91 (m, CH and CH_2), 0.87 (3H, s, H-19) 0.83 (3H, d, *J* = 6.6 Hz, H-21), 0.80 (2×3H, 2×d, *J* = 6.2 Hz, H-26 and 27) and 0.52 (3H, s, H-18). ^{13}C δ: 147.13, 115.93, 71.17, 56.25, 53.76, 44.86, 43.25, 41.85, 40.92, 38.38, 38.21, 36.75, 35.73, 35.42, 53.27, 33.17, 31.61, 30.31, 28.53, 25.38, 23.08, 22.36, 18.69, 18.37, 18.04 and 11.49. MS (CDI$^+$), *m*/*z*: 371 (M^+−H, 12), 353 (100).

2.13. Δ$^{9(11),22}$-Chlolest-3β-ol (**25**)

Boron trifluoride etherate (0.1 ml) was added dropwise to a solution of **23** (240 mg, 0.73 mmol) in dichloromethane (10 ml) which was cooled in an ice bath. After stirring for 30 min, a solution of saturated sodium bicarbonate was added. The resulting mixture was stirred for further 30 min. The organic phase was then separated and the aqueous phase was extracted with methylene chloride. The combined organic extract was washed with saturated brine and dried over anhydrous sodium sulphate. The solvent was removed under vacuum and aldehyde (**24**) obtained in quantitative yield. The aldehyde was used in the next step without further purification. A solution of *n*-BuLi (1.6 M in hexane, 3 ml, 4.8 mmol) was added dropwise over several minutes to a stirred suspension of

isoamyl triphenylphosphonium bromide (1.9 g, 4.6 mmol) in dry THF (40 ml) at 0 °C. After stirring for 30 min a solution of aldehyde (760 mg, 2.3 mmol) in dry THF (10 ml) was added. Stirring was continued at room temperature for 5 h, followed by removal of most of the THF. Water was added and the mixture was extracted with dichloromethane. The extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure. Purification by flash chromatography (30% ethyl acetate/hexane) gave **25** (417 mg, 47%) as a colourless solid. FTIR (KBr) cm^{-1}: 3393, 2916, 1037. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.22–5.10 (2×1H, m, H-22 and 23), 5.12–5.09 (1H, m, H-11), 3.57–3.48 (1H, m, H-3), 2.15–0.96 (m, CH and CH_2), 0.84 (3H, d, J = 8.8 Hz, H-21), 0.82 (2×3H, 2×d, J = 6.8 Hz, H-26 and 27), 0.84 (3H, s, H-19) and 0.57 (3H, s, H-18). MS (CDI^+), m/z: 384 (M^+, 26), 367 (M^+−OH, 100), 271 (14).

2.14. $\Delta^{9(11)}$-Chlolest-3β-ol (**27a**)

A solution of **25** (543 mg, 1.4 mmol) in THF (10 ml) was treated with 10% palladium on charcoal (150 mg, 0.14 mmol of palladium) under hydrogen (1 atm). After stirring for 40 min, the reaction mixture was filtered through celite, and concentrated under reduced pressure to afforded crude of **27a** in quantitative yield which was used in the next step without further purification.

2.15. 9,11-secochlolest-3β,11-diol (**32**)

A mixture of **29** (90 g, 0.19 mmol), ethylene glycol (3 ml) and hydrazine hydrate (0.6 ml) was heated under reflux condition for 30 min. The reaction mixture was cooled at room temperature and treated with a solution of potassium hydroxide (100 mg, 714 mmol) in water (0.5 ml). The temperature was increased to 195–197 °C and refluxing continued for 1 h. After cooling, the mixture was neutralized with 10% hydrochloric acid, extracted with chloroform, washed with water and dried over anhydrous sodium sulphate. Removal of solvent under reduce pressure and purification by flash column (20% EtOAc/hexane) gave **32** (43 mg, 55%) as a colourless solid, mp 79–81 °C. FTIR (KBr) cm^{-1}: 3391, 2954. ^1H NMR ($CDCl_3$, 400 MHz) δ: 3.57–3.49 (3H, m, H-3 and H-11), 1.81–1.02 (m, CH and CH_2), 0.86 (3H, d, J = 6.8 Hz, H-21), 0.81 (3H, s, H-19), 0.79 (3H, d, J = 6.6 Hz, H-26 or 27), 0.78 (3H, d, J = 6.6 Hz, H-26 or 27) and 0.60 (3H, s, H-18). ^{13}C δ: 80.19, 71.17, 48.06, 46.34, 39.51, 38.14, 37.62, 35.62, 35.47, 35.16, 33.88, 32.98, 32.94, 31.03, 30.08, 28.55, 27.97, 25.51, 24.95, 24.92, 24.61, 22.76, 22.57, 19.29, 16.91, 16.27, and 7.90. MS (CDI^+), m/z: 405 (M^+−H, 19), 386 (28), 371 (100). Anal. Calcd. for $C_{27}H_{50}O_2$: C, 79.74; H, 12.39; found: C, 79.86; H, 12.62.

2.16. $\Delta^{9(11)}$-Furost-12,26-di(cycloethylenedithiolketal)-3β-ol 3-acetate (**34**)

A mixture of **33** (6.5 g, 13.8 mmol), 1,2-ethanedithiol (4.2 ml) and boron trifluoride etherate (5.1 ml) in dichloromethane (10 ml) was left at room temperature for 15 h. The reaction mixture was poured onto excess 5% sodium bicarbonate and extracted with diethyl ether. After removal of the excess 1,2-dithioethane, the residue was purified by flash column chromatography (hexane then 10% EtOAc/hexane) to give **34** (7.5 g, 87% yield). Crystallization from ethyl acetate gave 103 as colourless needles; mp 83–85 °C. HRMS (FAB^+) ($C_{33}H_{50}O_3S_4$) calcd., 623.2716; found 623.2721. FTIR (KBr) cm^{-1}: 2921, 1732, 1243, 1026. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.43 (1H, d, J = 1.4 Hz, H-11), 4.63–4.55 (1H, m, H-3), 4.46 (1H, d, J = 6.4 Hz, H-26), 4.33–4.27 (1H, m, H-16), 3.32–3.24 (4H, m, $C\underline{H}_2C\underline{H}_2$), 3.16–3.06 (4H, m, $C\underline{H}_2C\underline{H}_2$), 2.97–2.92 (1H, m, CH), 2.50 (1H, dd, J = 5.3 and 8.6 Hz, CH), 2.16–1.18 (m, CH and CH_2), 1.95 (3H, s, $OCOC\underline{H}_3$), 0.99 (3H, d, J = 6.6 Hz, H-21), 0.98 (3H, d, J = 6.6 Hz, H-27), 0.91 (3H, s, H-19) and 0.90 (3H, s, H-18). ^{13}C δ: 170.79, 143.57, 127.18, 100.47, 90.02, 83.05, 73.39, 60.68, 58.90, 54.66, 48.88, 43.00, 40.82, 40.43, 40.17, 39.68, 38.86, 38.82, 37.69, 36.50, 35.20, 34.15, 33.53, 33.07, 33.04, 31.39, 28.29, 27.36, 21.62, 19.33, 17.73, 17.39 and 16.06. MS (FAB^+), m/z: 623 (M^+, 100), 563 (11), 530 (21).

2.17. $\Delta^{9(11)}$-Furost-3β-ol 3-acetate (**35**)

Raney nickel (7 g) was stirred with absolute ethanol (30 ml) and to this mixture was added a solution of **34** (1 g, 1.6 mmol) in 50% diethyl ether–ethanol (10 ml). The mixture was heated under reflux condition for 19 h. The cooled mixture was filtered through silica and the residue was rinsed with ethanol. The filtrate was concentrated and then purified by flash column chromatography (10% EtOAc/hexane) to give **35** (434 mg, 61% yield) as a colourless gum. HRMS (FAB^+) ($C_{29}H_{46}O_3$–H) calcd., 441.3361; found 441.3369. FTIR (film) cm^{-1}: 2921, 1732, 1243, 1026. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.23 (1H, d, J = 5.5 Hz,H-11), 4.64–4.56 (1H, m, H-3), 4.28–4.23 (1H, m, H-16), 3.27–3.22 (1H, m, H-22), 2.09–1.11 (m, CH and CH_2), 1.96 (3H, s, $OCOC\underline{H}_3$), 0.93 (3H, d, J = 6.7 Hz, H-21), 0.90 (3H, s, H-19), 0.82 (3H, d, J = 6.6 Hz, H-26 or 27) and 0.81 (3H, d, J = 6.6 Hz, H-26 or 27) and 0.65 (3H, s, H-18). ^{13}C δ: 170.86, 147.07, 116.10, 90.49, 83.41, 73.66, 65.11, 54.43, 43.16, 41.44, 39.61, 38.53, 38.04, 36.38, 36.01, 35.39, 34.36, 33.51, 33.23, 31.59, 28.51, 28.47, 27.72, 22.78, 22.71, 21.65, 18.97, 18.03 and 16.18. MS (DCI^+), m/z: 442 (M^+, 50), 371 ($M^+-C_5H_{11}$, 100), 313 (80).

2.18. 3β-hydroxy-9(11)-chlolesten-16,22-dione- 3-acetate (**36**)

A solution of **35** (2.6 g, 5.9 mmol) and potassium dichromate (3.2 g) in glacial acetic acid (10 ml) was stirred at 70 °C for 6 h. The reaction was quenched by addition of water and the solution was extracted with dichloromethane. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, dried over anhydrous sodium sulphate, filtered and concentrated under reduce pressure. The residue was purified by flash column chromatography (10% EtOAc/hexane) to afford **36** (1.3 g, 48% yield). Crystallization from ethyl acetate-hexane gave **36** as colourless prisms; mp 154–155 °C. FTIR (KBr) cm^{-1}: 2926, 1734, 1245, 1031. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.27 (1H, d, J = 5.9 Hz, H-11), 4.64–4.58 (1H, m, H-3), 2.68–2.46 (5H, m, CH and CH_2), 2.31–1.24 (m, CH and CH_2), 1.96 (3H, s, $OCOC\underline{H}_3$), 0.98 (3H, d, J = 6.5 Hz, H-21), 0.91 (3H, s, H-19), 0.84 (2×3H, 2×d, J = 6.2 Hz, H-26 and 27) and 0.62 (3H, s, H-18). ^{13}C δ: 218.08, 214.46, 171.06, 147.39, 115.89, 73.68, 66.10, 48.18, 43.49, 43.22, 40.96, 40.76, 40.14, 38.47, 38.29, 36.03, 35.29, 34.45, 33.34, 32.60, 28.46, 28.06, 27.82, 22.86 (2C), 21.85, 18.35, 15.65 and 13.00. MS (ES), m/z: 457 (M^++H, 100). Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71; found: C, 76.24; H, 9.43.

2.19. $\Delta^{9(11)}$-22-oxochlolest-3β-ol 16,16-(cycloethylenedithiol ketal) 3β-acetate (**37**)

A mixture of **36** (87 mg, 0.2 mmol), 1,2-dithioethane (0.2 ml) and boron trifluoride etherate (0.5 ml) in acetic acid (1.5 ml) was left at room temperature for 3 h. The reaction mixture was poured onto excess 5% sodium bicarbonate and extracted with diethyl ether. After removal of the excess 1,2-dithioethane, the residue was purified by flash column chromatography (hexane then 10% EtOAc/hexane) to give **37** (89 mg, 87% yield). Crystallization from ethyl acetate gave **37** as colourless prisms, mp 186–187 °C. FTIR (KBr) cm^{-1}: 2924, 1732, 1244, 1028. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.27 (1H, t, J = 2.2 Hz, H-11), 4.72–4.64 (1H, m, H-3), 3.39–3.32 (1H, m, C$\underline{H}$), 3.22–3.15 (2H, m, $C\underline{H}_2$), 3.02–2.90 (1H, m, C$\underline{H}$), 2.74–2.59 (2H, m, $C\underline{H}_2$), 2.49–2.44 (2H, m, $C\underline{H}_2$), 2.19 (bs, 2H, $C\underline{H}_2$), 2.05 (3H, s, $OCOC\underline{H}_3$), 1.99–1.24 (m, CH and CH_2), 1.12 (3H, d, J = 6.9 Hz, H-21), 0.96 (3H, s, H-19), 0.90 (3H, d, J = 6.4 Hz, H-26 or 27), 0.89 (3H, d, J = 6.4 Hz, H-26 or 27) and 0.77 (3H, s, H-18). ^{13}C δ: 212.70, 171.05, 146.75, 115.91, 73.80, 71.89, 61.90, 55.04, 51.67, 48.25, 43.23, 42.59,

41.96, 41.45, 40.48, 38.10, 37.98, 35.55, 35.44, 34.48, 33.24, 32.90, 28.60, 28.09, 27.86, 22.95, 22.83, 21.86, 18.26, 17.69 and 12.98. MS (ES), *m*/*z*: 533 (M^++H, 100). Anal. Calcd. for $C_{31}H_{48}O_3S_2$: C, 69.88; H, 9.08; S, 12.03 found: C, 69.94; H, 9.25.

2.20. 3β-hydroxy-9(11)-chlolesten-22-one- 3-acetate (**38**)

Raney nickel (7 g) was stirred with absolute ethanol (50) and to this mixture was added a solution of **37** (950 mg, 1.8 mmol) in 50% diethyl ether–ethanol (20 ml). The mixture was heated at reflux condition for 5 h. The cooled mixture was filtered through silica and the residue was rinsed with ethanol. The filtrate was concentrated and then purified by flash column chromatography (10% ethyl acetate/hexane) to give **38** (720 mg, 91% yield). Crystallization from ethyl acetate-hexane gave **38** as colourless prisms; mp 110–111 °C. FTIR (KBr) cm^{-1}: 2954, 1734, 1712, 1242, 1029. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.27 (1H, bs, H-11), 4.71–4.65 (1H, m, H-3), 2.56–2.51 (1H, m, H-20), 2.49–2.33 (2H, m, H-23), 2.12–2.10 (2H, m, C$\underline{H}_2$), 2.04 (s, 3H, OCOC$\underline{H}_3$), 1.99–1.15 (m, CH and CH_2), 1.10 (3H, d, *J* = 6.9 Hz, H-21), 0.97 (3H, s, H-19), 0.90 (2×3H, 2×d, *J* = 6.5 Hz, H-26 and 27) and 0.63 (3H, s, H-18). ^{13}C δ: 214.07, 169.65, 145.92, 114.73, 72.46, 51.98, 51.21, 48.40, 41.93, 40.53, 40.12, 38.69, 36.66, 35.61, 34.12, 33.14, 31.97, 31.37, 27.32, 26.76, 26.69, 26.49, 24.57, 21.40, 21.37, 20.45, 16.91, 15.25 and 10.70. MS (EI), *m*/*z*: 442 (M^+, 22%), 367 (21), 314 (25), 213 (32), 99 (49). Anal. Calcd. for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47; found: C, 78.62; H, 10.51.

2.21. (22R)-3β,11,22-trihydroxy-9,11-secochlolest-9-one 3,22-diacetate (**45b**) and (22R)-9,11-secochlolest-3β,9β,11, 22-tetraol (**46b**)

A solution of **45a** (35 mg, 0.06 mmol) and in methanol (5 ml) was treated with a solution of potassium hydroxide (40 mg) in MeOH (1 ml), then stirred continuously for 30 min. The reaction mixture was dried over anhydrous sodium sulphate, and concentrated. Purification by flash chromatography (20% EtOAc/dicholomethane) afforded **45b** (25 mg, 96% yield) as a colourless solid with mp 123–124 °C. By the same method, the starting **46a** (28 mg, 0.05 mmol) was converted to **46b** (20 mg, 91% yield) as a colourless solid, mp 100–101 °C.

45b: FTIR (KBr) cm^{-1}: 3440, 2931, 1698, 1037. ^1H NMR (acetone-d_6, 400 MHz) δ: 3.64 (2H, d, *J* = 4.9 Hz, H-11), 3.55–3.50 (1H, m, H-3), 3.49–3.41 (1H, m, H-22), 2.96–2.91 (1H, m, CH), 2.48 (1H, dt, *J* = 10.7 and 3.1 Hz, CH), 1.67–1.23 (m, CH and CH_2), 1.17 (3H, s, H-19), 0.86 (3H, d, *J* = 6.6 Hz, H-26 or 27), 0.85 (3H, d, *J* = 6.6 Hz, H-26 or 27), 0.83 (3H, d, *J* = 6.6 Hz, H-21) and 0.72 (3H, s, H-18). ^{13}C δ: 215.83, 70.22, 58.50, 50.53, 48.67, 46.18, 46.11, 44.14, 42.57, 42.08, 42.07, 39.79, 38.40, 37.63, 37.58, 32.99, 32.60, 32.03, 31.40, 25.53, 24.12, 23.37, 22.76, 22.66, 17.98, 17.84 and 15.93.MS (CDI$^+$), *m*/*z*: 420 (M^+−OH, 4), 420 (M^+−2OH, 100), 385 (420−OH, 8). Anal. Calcd. for $C_{27}H_{48}O_4$: C, 74.26; H, 11.08; found: C, 74.49; H, 10.97.

46b: FTIR (KBr) cm^{-1}: 3403, 2951, 1040. ^1H NMR (acetone-d_6, 400 MHz) δ: 3.80–3.77 (1H, m, H-3), 3.60–3.58 (2H, m, H-11), 3.49–3.41 (m, 1H, H-22), 3.22 (1H, d, *J* = 4.7 Hz, H-9), 2.96–2.91 (dd, 1H, *J* = 8.8 and 11.9 Hz, CH), 1.67–1.23 (m, CH and CH_2), 0.94 (3H, d, *J* = 6.4 Hz, H-21), 0.87 (3H, d, *J* = 6.6 Hz, H-26 or 27), 0.87 (3H, d, *J* = 6.6 Hz, H-26 or 27), 0.85 (3H, s, H-19) and 0.73 (3H, s, H-18). ^{13}C δ: 81.53, 73.29, 70.95, 58.09, 57.95, 46.78, 46.61, 43.80, 43.20, 43.12, 41.10, 40.03, 38.32, 38.27, 37.23, 36.96, 36.37, 31.89, 29.53, 28.94, 28.65, 28.25, 27.64, 23.14, 22.65, 21.18 and 17.81. MS (CDI$^+$), *m*/*z*: 369 (M^+−C_5H_{11}, 100), 257 (18). Anal. Calcd. for $C_{27}H_{50}O_4$: C, 73.92; H, 11.49; found: C, 74.17; H, 11.17.

2.22. 3β-hydroxy-Δ$^{9(11)}$-androst-17-one (**48a**)

A mixture of **19** (1 g, 2.8 mmol), pyridine (1 ml), 95% ethanol (8 ml) and hydroxylamine hydrochloride (360 mg) was refluxed for 30 min and then cooled in ice. The precipitate was collected, washed with hot water and dried, giving oxime **47** (950 mg). The oxime was dissolved in anhydrous pyridine (2 ml), cooled at 0 °C and solution of *p*-acetamidobenzenesulfonyl chloride (1.8 g) in anhydrous pyridine (5 ml) was added with stirring, the temperature being maintained below 5 °C. After stirring for 2 h at 10 °C and additional 2 h at room temperature, the mixture was poured into ice and conc. sulfuric acid and left in the refrigerator overnight. The solid was collected, washed with hot water and dried. Saponification was effected by refluxing with 2.5% methanolic potassium hydroxide for 30 min. The mixture was neutralized with 10% HCl, extracted with CH_2Cl_2 and the combined organic layer was washed with water, dried over anh. sodium sulphate and then concentrated under reduce pressure. Purification by flash column (20% EtOAc/hexane) furnished **48a** (590 mg, 73% yield) as a colourless solid, mp 161–163 °C.

47: FTIR (KBr) cm^{-1}: 3378, 2927, 1701, 1272, 1031. ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.04 (1H, dd, *J* = 1.9 and 3.3 Hz, H-16), 5.30 (1H, d, *J* = 6.1 Hz, H-11), 4.70–4.61 (1H, m, H-3), 2.43 (1H, dd, *J* = 5.8 and 15.6 Hz CH,), 2.33–2.09 (m, CH and CH_2), 2.00 (3H, s, C$\underline{H}_3$), 1.99 (3H, s, H-21), 2.44–1.22 (m, CH and CH_2), 0.96 (3H, s, H-19), 0.82 (3H, s, H-18). ^{13}C δ: 170.73, 153.87, 150.13, 147.42, 132.45, 116.62, 73.55, 54.20, 45.04, 43.00, 38.47, 37.88, 35.08, 34.62, 34.19, 32.74, 32.36, 28.22, 27.52, 21.44, 17.96, 15.43 and 11.23. MS (CDI$^+$), *m*/*z*: 372 (M^++H, 100), 354 (9), 312 (32).

48a: FTIR (KBr) cm^{-1}: 3453, 2924, 1736, 1029. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.33 (1H, t, *J* = 1.9 Hz, H-11), 3.60–3.53 (1H, m, H-3), 2.42 (1H, t, *J* = 8.0 Hz, CH), 2.20–0.97 (m, CH and CH_2), 0.94 (3H, s, H-19), 0.79 (3H, s, H-18). ^{13}C δ: 222.04, 147.76, 114.90, 70.95, 54.44, 51.43, 49.07, 43.08, 38.23, 38.07, 36.31, 35.43, 33.25, 32.19, 31.52, 28.19, 22.76, 18.07 and 13.45. MS (CDI$^+$), *m*/*z*: 289 (M^++H, 3), 279 (21), 255 (100).

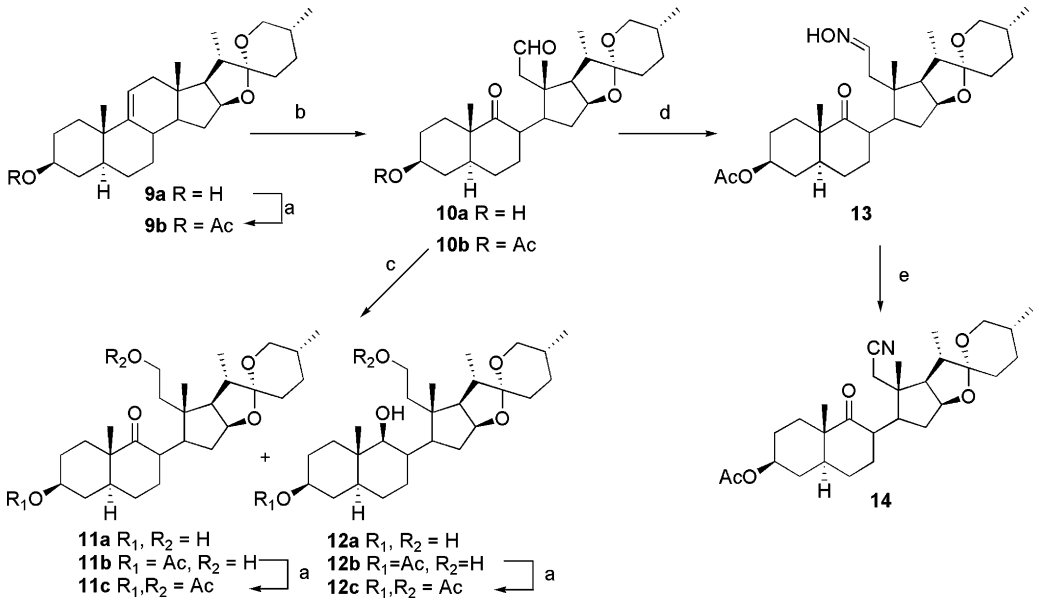
2.23. Δ$^{9(11)}$-Androst-3β-ol 3-acetate (**50**)

A mixture of **48b** (300 mg, 0.91 mmol), 1,2-dithioethane (1 ml) and boron trifluoride etherate (1 ml) in dichlormethane (5 ml) was left at room temperature for 15 h. The reaction mixture was poured onto excess 5% sodium bicarbonate and extracted with diethyl ether. After removal of the excess 1,2-dithioethane, the residue was purified by flash column chromatography (hexane then 10% ethyl acetate/hexane) to give Δ$^{9(11)}$-androst-3β-ol-17-one-17,17-(cycloethylene dithiolketal) 3-acetate (313 mg, 85% yield). Raney nickel (3 g) was stirred with absolute ethanol (5 ml) and to this mixture was added a solution of Δ$^{9(11)}$-22-oxochlolest-3β-ol 16,16-(cycloethylenedithiolketal) 3β-acetate (313 mg, 0.69 mmol) in 30% diethyl ether/ethanol (10 ml). The mixture was refluxed for 1 h. The cooled mixture was filtered through silica and the residue was rinsed with ethanol. The filtrate was concentrated and the residue was purified by flash column chromatography (10% EtOAc/hexane) giving **50** (150 mg, 69% yield) as a colourless gum. FTIR (neat) cm^{-1}: 2948, 1731, 1242. ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.29 (1H, dt, *J* = 6.0 Hz, H-11), 4.69–4.61 (1H, m, H-3), 3.28–3.23 (2H, m, C$\underline{H}_2$), 2.01 (s, 3H, OCOC$\underline{H}_3$), 1.96–1.00 (m, CH and CH_2), 0.94 (3H, s, H-19), 0.63(3H, s, H-18). ^{13}C δ: 170.63, 147.37, 116.22, 73.53, 52.02, 43.01, 40.39, 40.03, 39.37, 37.81, 36.92, 35.26, 34.23, 33.44, 28.48, 27.55, 26.68, 21.43, 21.05, 17.93 and 17.44. MS (CDI$^+$), *m*/*z*: 315 (M^+−H, 100).

3. Results and discussion

3.1. Chemistry

The preparation of 9,11-secosterol containing spiroketal ring **10**–**14** from an intermediate, 9,11-dehydrotigogenin (**9a**) is shown



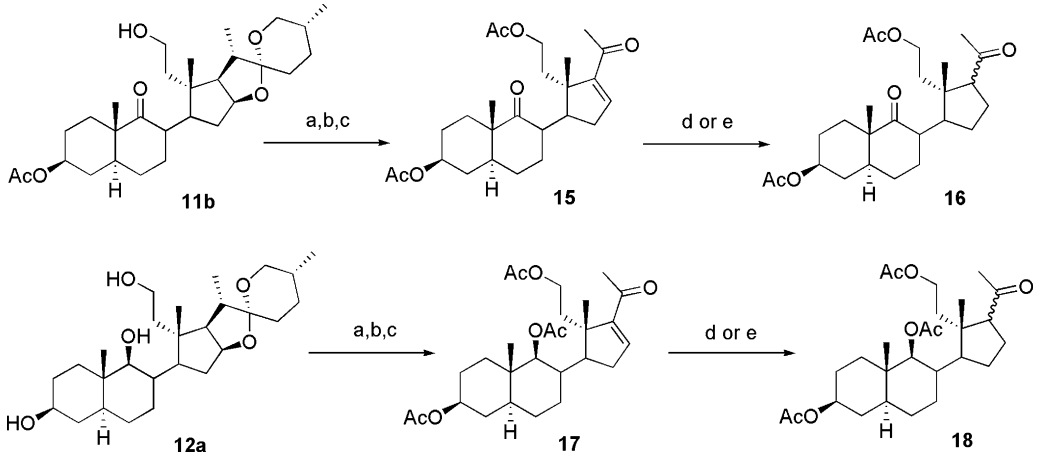
Scheme 1. Reagents and conditions: (a) Ac_2O, py, rt; (b) O_3, CH_2Cl_2, −78 °C then PPh_3; (c) $NaBH_4$, 30% EtOH in CH_2Cl_2, 0 °C; (d) NH_2OH, EtOH (59%); (e) DDQ, dioxane (36%).

in Scheme 1. Compound **9a** was synthesized from commercially available hecogenin acetate [27]. Ozonolysis of **9a** and **9b** in CH_2Cl_2 at −78 °C after reductive work-up with PPh_3 and column chromatography, gave the pure keto-aldehyde **10a** and **10b** in 71 and 69% yield, respectively. Reduction of **10a** and **10b** with $NaBH_4$ in 30% ethanol/CH_2Cl_2 at 0 °C proceeded predominantly at the sterically less hindered formyl group to afford **11a** and **11b**, as anticipated with the minor formation of the corresponding 9β-triol, **12a** and 9β-diol **12b**. Protection of the primary C-11 hydroxyl group as acetate proceeds chemoselectively with Ac_2O and pyridine at 0 °C to provide diacetate **11c** and **12c** in quantitative yield.

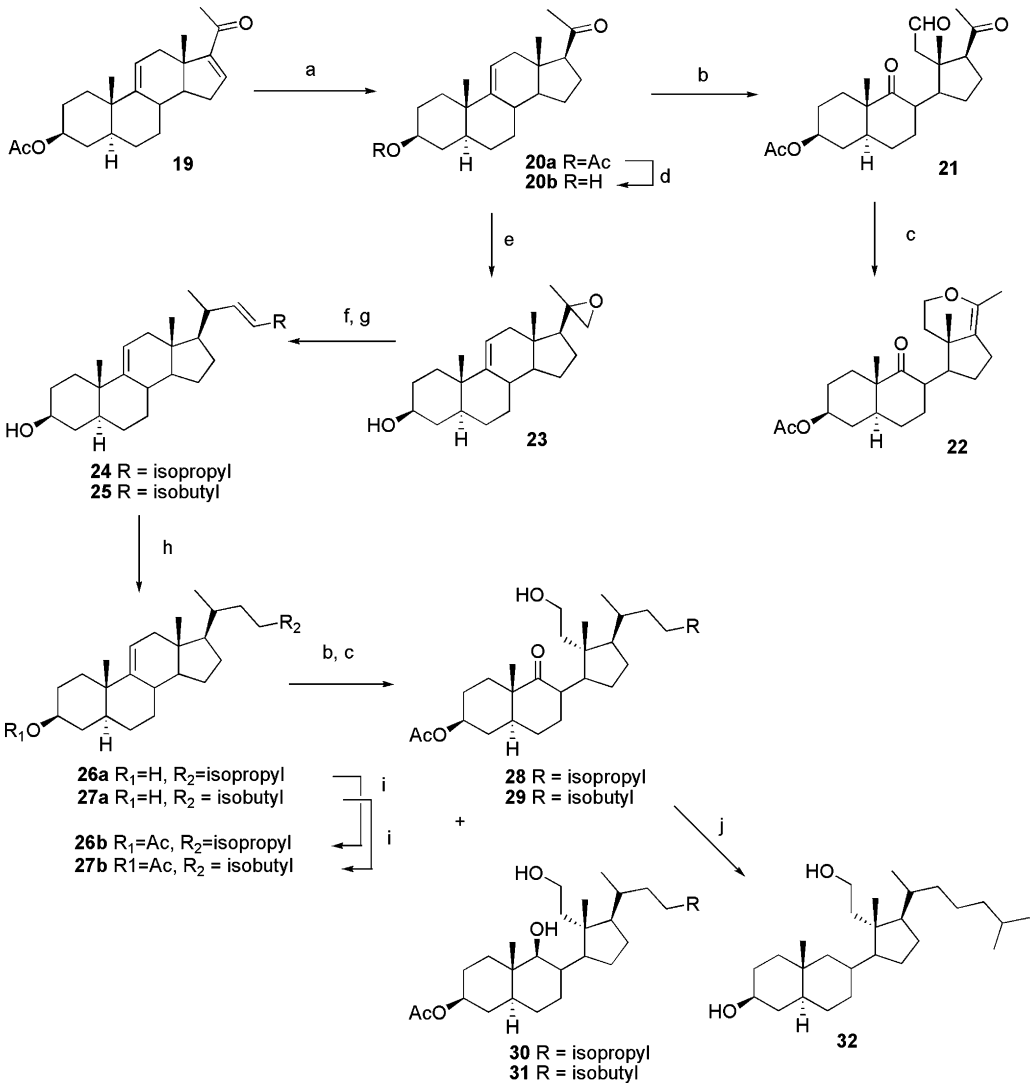
The preparation of 11-oxime and cyano analogs (**13** and **14**) were achieved by treatment of **10b** with hydroxyamine in a mixture of MeOH and H_2O to give the 11-oxime **13** which upon treatment with DDQ in dioxane provided keto nitrile **14** in 36% yield.

The synthesis of 9,11-secosterol containing acetyl side chain **15**–**18** was achieved in two steps from spiro ketal intermediate **11b** and **12a** (Scheme 2). Transformation of the spiro ketal moiety in **11b** to acetyl side chain in **15** was performed in one pot preparation by the method developed by Mićović et al. [28] and modified by Fuchs et al. [29]. Hydrogenation of both **15** and **17** either using Pd/C or Raney nickel [30] as catalyst provided **16** and **18**, respectively.

The synthesis of 9,11-secosterol containing cholesterol-like side chain with different degrees of oxidation at C-9 (**28**–**32**) is shown in Scheme 3. Compound **19** was prepared from compound **9a** by the method described in our previous work [27]. Selective hydrogenation of **19** (Scheme 2) using either Pd/C or Raney nickel as catalyst proceeded predominantly at the sterically less hindered double bond at C-16, 17 to afford **20a** in 64% yield. Ozonolysis of **20a** in CH_2Cl_2 at −78 °C gave 11-formyl secosterol **21**. Surprisingly when **21** was selectively reduced at 11-formyl group with $NaBH_4$ at 0 °C, no corresponding hydroxy compound was observed instead only dehydropyran compound **22** was obtained. It is quite clear that the formation of **22** resulted from nucleophilic attacked of the 11-hydroxy to the 20-keto side chain under reduction condition. After removal of acetate with alcoholic KOH and upon treatment with sulphur ylide [31,32] generated from trimethyl sulphonium iodide and sodium hydride, 20-ketosterol **20b** was converted to epoxide **23** in 84% yield. Lewis acid rearrangement of epoxide **23** with $BF_3{\cdot}Et_2O$ at 0 °C afforded corresponding aldehyde, which upon treatment with the appropriate alkyl phosphorus ylide provided unsaturated side chain sterol **24** and **25**. Selective hydrogenation at unsaturated side chain was achieved by using Pd/C catalyst in ethanol at atmospheric pressure and room temperature. The reac-



Scheme 2. Reagents and conditions: (a) Ac_2O, py, NH_4Cl; (b) CrO_3, AcOH; (c) Al_2O_3, benzene, (d) Raney Ni, THF; (e) H_2, Pd/C, THF.

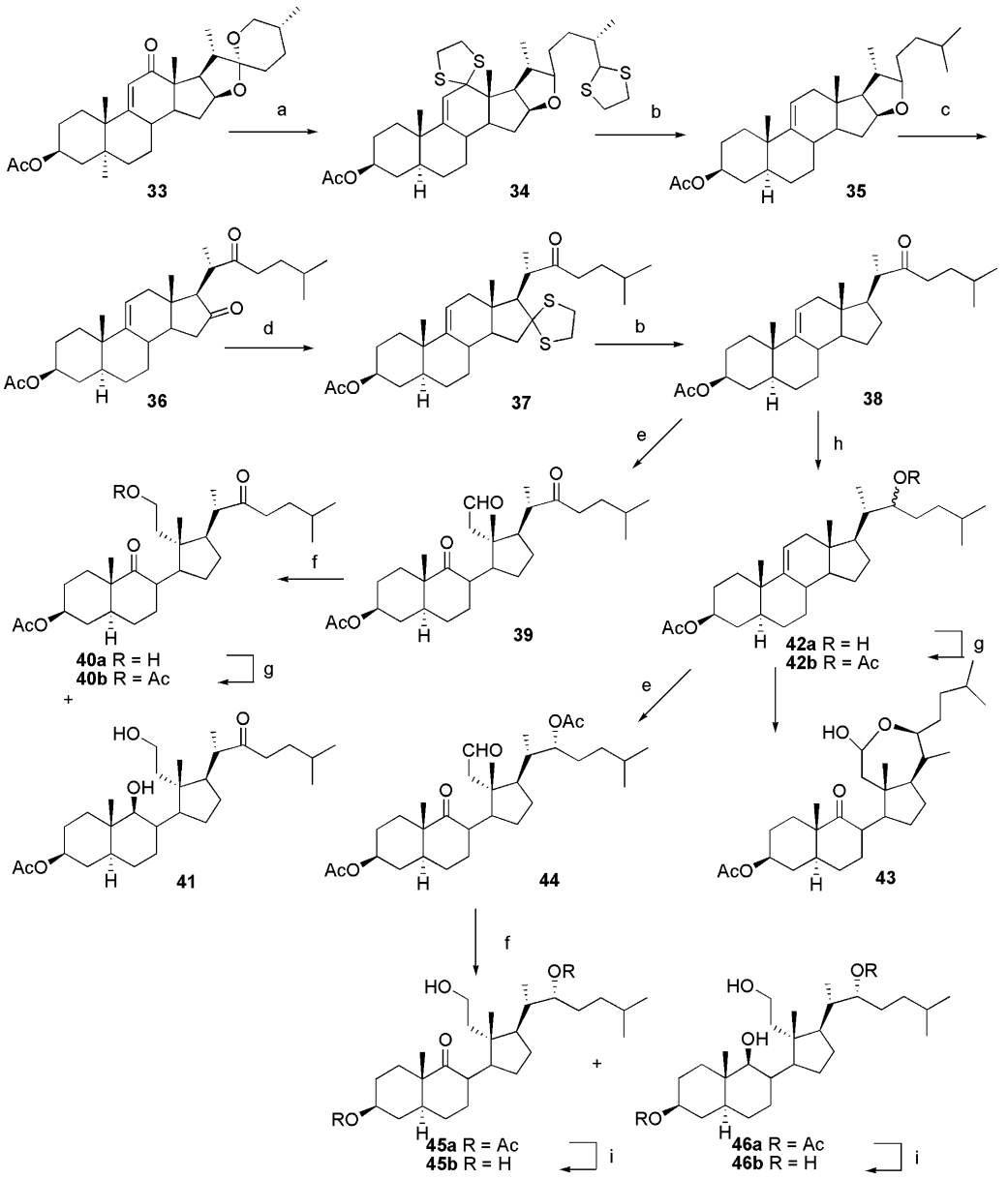


Scheme 3. Reagents and conditions; steps (a) as in Scheme 2, (b) O_3, CH_2Cl_2, −78 °C then PPh_3; (c) $NaBH_4$, 30% EtOH in CH_2Cl_2, 0 °C; (d) KOH, MeOH; (e) $(CH_3)_3SI$, NaH, THF, DMSO; (f) $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C. (g) RCH_2PPh_3Br, NaH; (h) H_2, Pd/C, rt, (i) Ac_2O, py; (j) NH_2NH_2, ethylene glycol and then KOH.

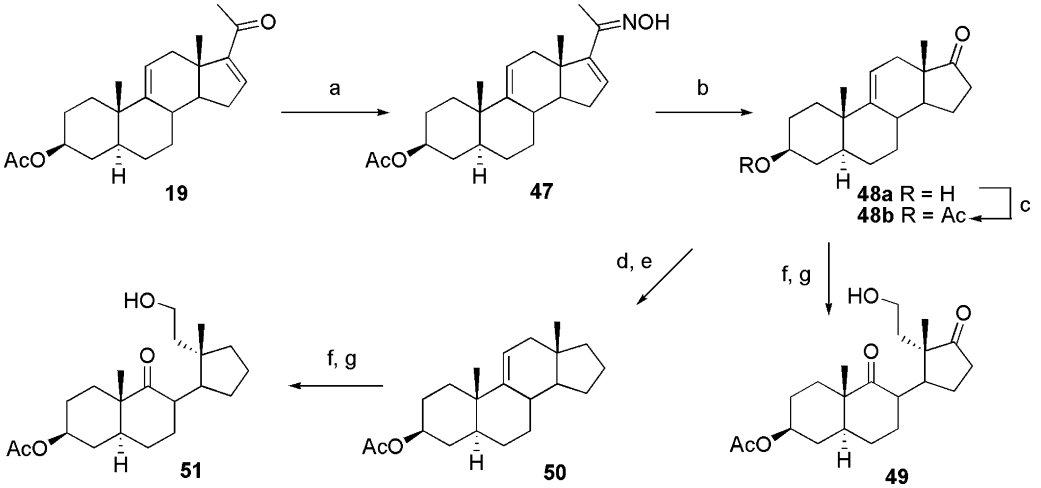
tion provided cholesterol-like side chain of $\Delta^{9,11}$-24-nor-**26a** and $\Delta^{9,11}$-sterol **27a** which upon acetylation with Ac_2O gave acetate derivatives **26b** and **27b**. Ozonolytic cleavage of the $\Delta^{9(11)}$ double bond of **26b** and **27b** was performed in CH_2Cl_2 at −78 °C to provide the corresponding keto-aldehyde which upon reduction with $NaBH_4$ in 30% ethanol/CH_2Cl_2 provided 11-hydroxy secosterol **28** and **29** as major products, together with a minor products 9,11-diol **30** and **31**. Wolff-Kishner reduction of **29** led to the formation of secosteroid diol **32**.

The synthesis of 9,11-secosterol containing cholesterol-like side chain with functional group (**39**–**46**) is outlined in Scheme 4. The 22-keto sterol **38** was considered as a precursor of our target molecules. Compound **38** was synthesized from dehydrohecogenin acetate (**33**) by the method described by Djerassi et al. [33]. Oxidative ring opening of the tetrahydrofuran moiety in **35** to a diketones (**36**) was accomplished by the method described in our previous work [34]. Removal of 16-keto moiety in **36** was achieved by selective thioketalisation by treatment with 1,2-ethanedithiol in the presence of $BF_3{\cdot}OEt_2$ in acetic acid at room temperature to provide expected thioketal **37** and then desulfurization with Raney nickel to give 22-koto sterol-acetate **38** [35]. Ozonolysis of **38** in CH_2Cl_2 at −78 °C gave 11-formyl secosterol **39**. Reduction of **39** with $NaBH_4$ in 30% ethanol/CH_2Cl_2 provided the corresponding 11-hydroxy secosterol **40a** as a major product, together with a minor product 9,11-diol **41**. Acetylation of **40a** with Ac_2O gave diacetate **40b** in 93% yield. Reduction of 22-keto moiety of **38** with $NaBH_4$ in MeOH gave a high yield of the (22*S*)–hydroxy and (*22R*)–hydroxy-**42a** in a ratio 2:1. The configuration of the chiral center at C-22 was established by the modified Mosher method [36], through chemical derivatization of (*22S*)–hydroxy-**42a** with (*R*)-and (*S*)-MTPA[2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid]. Oxidative cleavage of 9,11-double bond of (22*S*)-hydroxy-**42a** by ozonolysis resulted in the formation of 7-membered hemiketal **43** [37]. In order to avoid hemiketal formation, (22*R*)-hydroxy of **42a** was protected as acetate **42b**, and subsequently ozonolysis gave 11-formyl secosterol diacetate **44**. $NaBH_4$ reduction of **44** gave a mixture of 11-hydroxy secosterol acetate **45a** and corresponding 9,11-diol **46a**. Removal of acetate in **45a** and **46a** with alcoholic KOH gave the corresponding triol **45b** and tetraol **46b** in nearly quantitative yield.

In order to investigate the influence of the absence of a side chain, 9,11-secosterol lacking a side chain in steroidal skeleton was synthesized from 9,16-didehydropregnenolone acetate (**19**) by the method described by Rosenkranz et al. [38] as shown in Scheme 5. Thus compound **19** was converted into $\Delta^{9,11}$androstanol (**48a**) by treatment with hydroxylamine hydrochloride and subse-



Scheme 4. Reagents and conditions: (a) ethanedithiol, $BF_3{\cdot}OEt_2$, CH_2Cl_2 (87%), (b) Raney Ni, EtOH, reflux (c) $K_2Cr_2O_7$, AcOH, 70 °C (48%); (d) ethanedithiol, $BF_3{\cdot}OEt_2$, AcOH (81%); (e) O_3, CH_2Cl_2, −78 °C, then PPh_3, (f) $NaBH_4$, 30% EtOH in CH_2Cl_2, 0 °C; (g) Ac_2O, py (90%); (h) $NaBH_4$ in MeOH (8l%), (i) KOH in MeOH.



Scheme 5. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, EtOH (91%); (b) p-acetamidobenzenesulphonyl chloride, py then KOH (73%); (c) Ac_2O, py; (d) ethanedithiol, $BF_3{\cdot}OEt_2$, CH_2Cl_2 (85%); (e) Raney Ni, EtOH, reflux (69%); (f) O_3, CH_2Cl_2, −78 °C, then PPh_3; (g) $NaBH_4$, 30% EtOH in CH_2Cl_2, 0 °C.

Table 1
Cytotoxicity of 30 novel 9,11-secosterols against human carcinoma cell lines (KB, HeLa, and MCF-7).

Compound	Cancer cell lines, IC_{50} (μg/ml)[a]			Vero cell, IC_{50} (μg/ml)[a]
	KB	HeLa	MCF-7	
10a	>100	66.0 ± 2.3	52.2 ± 2.5	>100
10b	>100	>100	>100	>100
11a	>100	>100	>100	>100
11b	>100	>100	>100	>100
11c	32.0 ± 6.7	61.8 ± 10.4	58.8 ± 4.8	56.7 ± 8.2
12a	59.2 ± 8.0	43.2 ± 5.9	50.8 ± 4.9	79.5 ± 7.1
12b	>100	>100	>100	80.0 ± 5.5
12c	50.3 ± 0.5	38.3 ± 10.2	44.2 ± 8.0	>100
13	>100	>100	>100	>100
14	>100	>100	>100	>100
15	56.8 ± 10.3	65.0 ± 3.2	50.2 ± 3.3	53.8 ± 8.3
16	44.3 ± 2.2	59.2 ± 2.0	50.0 ± 5.5	45.2 ± 0.4
17	>100	>100	>100	>100
18	>100	>100	>100	>100
22	>100	>100	>100	>100
28	16.5 ± 4.2	17.5 ± 0.8	56.7 ± 2.6	42.2 ± 2.0
29	17.8 ± 0.4	5.0 ± 0.5	5.4 ± 0.2	5.8 ± 1.4
31	70.8 ± 0.2	49.7 ± 4.5	13.2 ± 1.5	14.7 ± 0.5
32	>100	55.3 ± 0.5	59.2 ± 1.0	54.6 ± 1.5
38	>100	>100	>100	>100
39	3.5 ± 0.6	5.6 ± 0.5	14.8 ± 3.8	11.7 ± 5.8
40a	17 ± 2.4	5.5 ± 0.6	18.7 ± 2.3	>100
40b	1.9 ± 0.2	4.8 ± 1.0	16.8 ± 1.0	11.0 ± 4.3
41	30.0 ± 4.8	13.8 ± 0.8	15.5 ± 0.8	18.2 ± 3.1
42a	>100	>100	>100	>100
43	3.3 ± 0.4	9.5 ± 4.4	21.8 ± 1.9	5.8 ± 1.1
45b	15.0 ± 1.1	4.8 ± 0.9	19.7 ± 1.4	7.1 ± 2.6
46b	25.8 ± 4.1	16.2 ± 0.8	18.7 ± 1.0	37.2 ± 8.3
49	>100	>100	>100	>100
51	>100	>100	>100	>100
Adriamycin[b]	0.018	0.18	0.22	38

[a] The results are the average mean of six replicate determinations ± SD.
[b] Used as reference KB = human epidermoid carcinoma, HeLa = human cervical carcinoma, MCF-7 = human breast cancer, Vero cell line = African green monkey kidney cell.

quently underwent Beckman rearrangement upon reaction with *p*-acetamidobenzenesulfonyl chloride in pyridine. Acetylation of **48a** provided acetate **48b** from which the 17-keto group was removed by thioketalisation and then desulfurization giving **50**. Conversion of **48b** and **50** into the 9,11-secosterols analogs **49** and **51** were performed by the application of the methodology already discussed in Scheme 1.

3.2. Biological activity

All compounds prepareed as described above were subjected to in vitro cytotoxic evaluation against KB (human epidermoid carcinoma), HeLa (human cervical carcinoma) and MCF-7 (human breast carcinoma) cell lines as well as the normal Vero cell line employing the MTT colorimetric method [39]. Adriamycin which exhibits cytotoxicity against KB, HeLa and MCF-7 cell lines, was used as reference. The results are summarized in Table 1.

From the data shown in Table 1, all 9,11-secosterol compounds containing spiroketal skeleton (**10–14**) were found inactive to all the tumor cells tested (IC_{50} value > 30 μg/ml). Similar results were found for the secosterol compound containing acetyl side chain at C-17 (**15–18**) and dihydropyran (**22**) (IC_{50} > 100 μg/ml for all cell lines). However, the cytotoxicity data of secosterol possesing cholesterol side chain at C-17 (**29**) showed significant cytotoxicity activity (IC_{50} value 17.8 ± 0.4, 5.0 ± 0.5 and 5.4 ± 0.2 μg/ml for KB, HeLa and MCF-7, respectively). The above results indicated that the cholesterol side chain must be present to retain cytotoxic activity. Introduction of keto and hydroxyl group on the side chain generated compounds **40a** and **45b**, displayed similar cytotoxicity potency as **29** for HeLa and KB but less potent for MCF-7 cell lines. When chain length of the cholesterol side chain in **29** was reduced to lower alkyl homologue (**28**), the cytotoxicity was significantly decreased (3- and 10-fold) for HeLa and MCF-7, respectively, but similar to KB tested cell. The importance of the presence of a cholesterol-like side chain (compound **29, 40a** and **45b**) is illustrated by the lack of cytotoxicity in compound **49** and **51** which have no side chain at C-17 (IC_{50} > 100 μg/ml). Compound **39**, **40a** and **40b** showed similar cytotoxic activity against HeLa and MCF-7 cell lines indicated that functionality at C-11 is not responsible for the observed activity.

Changing from keto at C-9 of 9,11-secosterol to a hydroxyl group resulted in loss of the activity by a factor of ~2–4 fold for KB and HeLa cell lines (see IC_{50} value of pairs **29/31, 40a/41** and **45b/46b**). When this ketone group was removed to generate **32**, no cytocoxicity was observed for all tested cell lines (IC_{50} value > 50 μg/ml for all cell lines).

4. Conclusions

In summary, we have described the chemical synthesis of a new series of 9,11-secosterols and have found some important features needed for cytotoxicity. These first-ever structure/cytotoxicity investigations have demonstrated that one important feature is the presence of a cholesterol-type side chain, which appears to play a major role in determining the biological activity. The existence of ketone functionality at C-9 was also crucial for cytotoxic activity. Finally functionality at C-11 is not responsible for the observed activity.

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