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Synthesis of [26,27-$^2H_6$]brassinosteroids from 23,24-bisnorcholenic acid methyl ester

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Abstract

A number of hexadeuterated brassinosteroids (BS) containing a hydroxy group at C-22 or a 22*R*,23*R*-diol function were prepared starting from 23,24-bisnorcholenic acid methyl ester for biosynthetic studies. Synthesis of the cyclic part was accomplished via the initial hydroboration–oxidation of Δ^5-double bond. The key step in the synthesis of the side chain involved addition of (2*S*)-[3,4-$^2H_6$]2,3-dimethylbutylphenyl sulfone to the corresponding C-22 aldehydes.

Keywords: Brassinosteroids; 23,24-Bisnorcholenic acid; Labeled compounds; Brassinolide; Plant growth regulator; Phytohormone; Biosynthesis

1. Introduction

Knowledge of the subtle details of BS biosynthesis is essential for a better understanding of many aspects of the physiological action of these plant hormones [1–3]. As a prerequisite for successful biosynthetic studies, a broad spectrum of possible biosynthetic precursors is required [4]. Recently, we reported the synthesis of many BS bearing three [5] or six [6] deuteriums in the side chain. These compounds were used for the identification of new BS, such as 3-epibrassinolide [7] and secasterol [8], and elucidation of the biosynthetic route to 2,3-epoxybrassinosteroids via teasterone/typhasterol. To continue further biosynthetic studies, a number of new labeled compounds were necessary. Thus, the present work partially aimed at preparation of possible biosynthetic precursors of brassinolide (as hexadeuterated derivatives). The majority of syntheses concerning the steroidal side chain formation made use of 22-aldehydes as key intermediates, which, in turn, were prepared by ozonolysis of Δ^{22}-steroids. In this respect, another task of the present investigation was the evaluation of the synthetic potential of commercialy available 23,24-bisnorcholenic acid methyl ester 1 [methyl (20*S*)-3β-hydroxypregn-5-ene-20-carboxylate] as a starting material for preparation of BS. Until now, only one paper has described the synthesis of BS analogs with an ester function in the side chain from **1** [9].

2. Experimental

2.1. General

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 (400 MHz for ^1H, 100 MHz for ^{13}C) spectrometer using TMS as an internal standard in $CDCl_3$ (if not stated otherwise). Accurate mass measurements were carried out on a Micromass MasSpec mass spectrometer operating in the 70 eV-EI mode. Samples were introduced by direct probe for accurate mass measurement by peak matching. Chemicals were purchased from Aldrich, Fluka, and Steraloids chemical companies and were used as received. [$^2H_3$]Methyl iodide (99.5%) was supplied by Deutero Gmbh. Reactions were

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monitored by TLC using aluminium or plastic sheets precoated with silica gel 60 F_{254} (VWR 1.05554). Column chromatography was carried out on Kieselgel 60 (VWR 1.07734). Jones reagent refers to a solution of CrO_3 (26.7 g) in concentrated H_2SO_4 (23 ml) diluted to 100 ml with water.

2.2. (20S)-6β-Methoxy-3α,5-cyclo-5α-pregnane-20-methanol (**2**)

A mixture of ester **1** (5 g, 13.9 mmol) and TsCl (5 g, 26.2 mmol) in pyridine (60 ml) was kept at room temperature for 24 h. Water (400 ml) was then added, and the resulting precipitate was filtered off. After drying in air, the tosylate was dissolved in MeOH (100 ml) and pyridine (5 ml), and the mixture was heated under reflux for 2 h. Solvents were evaporated in vacuo to give an oil (5.1 g, i-steroid/**1a** = 10/1 according to ^{1}H NMR). The obtained product was dissolved in ether (25 ml) and added to a suspension of $LiAlH_4$ (6.4 g, 169 mmol) in ether (100 ml). The mixture was stirred at room temperature for 2 h, after which water (6.4 ml), 15% NaOH (6.4 ml), and again water (19.2 ml) were consecutively added. The obtained precipitate was filtered off, and the filtrate was evaporated to give an oily product. A solution of 1M BH_3 in THF (10 ml, 10 mmol) was added to this oil. The mixture was kept at room temperature for 14 h and then treated with 30% H_2O_2 (10 ml, 79 mmol) and a 17% solution of $NaHCO_3$ (15 ml, 38 mmol) for 30 min. The mixture was diluted with water (100 ml) and extracted with EtOAc (3 × 70 ml). The organic phase was dried (Na_2SO_4) and evaporated. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 4:1) to give alcohol **2** (3.62 g, 75%) as an oil. ^{1}H NMR δ: 0.74 (s, 3H, 18-H), 1.03 (s, 3H, 19-H), 1.05 (d, J = 6.6 Hz, 3H, 21-H), 2.77 (t, J = 2.6 Hz, ^{1}H, 6-H), 3.32 (s, 3H, OMe), 3.37 (dd, J = 10, 7 Hz, ^{1}H, 22-H), 3.64 (dd, J = 10, 3 Hz, ^{1}H, 22-H). ^{13}C NMR δ: 12.34, 13.09, 16.76, 19.30, 21.52, 22.77, 24.30, 24.98, 27.81, 30.54, 33.38, 35.09, 35.31, 38.78, 40.15, 42.89, 43.41, 48.04, 52.63, 56.27, 56.57, 68.04, 82.41. HRMS calc. for $C_{23}H_{38}O_3$: 346.2872; found: 346.2880. EI-MS$^{m/z}$ (%): 255 (10), 288 (25), 291 (100), 299 (12) [M–CH_3OH–CH_3]$^+$, 314 (89) [M–CH_3OH]$^{\bullet+}$, 331 (54) [M–CH_3]$^+$, 346 (49) [M]$^{\bullet+}$, 347 (10) [M + H]$^+$.

2.3. (20S)-6β-Methoxy-3α,5-cyclo-5α-pregnane-20-carbaldehyde (**3**)

DMSO (10.5 ml, 149 mmol) was added dropwise to a solution of $(COCl)_2$ (9.5 ml, 109 mmol) in CH_2Cl_2 (100 ml) at −80 to −72 °C under argon. The mixture was kept at −70 to −65 °C for 30 min, cooled to −75 °C, and alcohol **2** (3.6 g, 10.4 mmol) in CH_2Cl_2 (30 ml) was added slowly. The mixture was stirred at −70 °C for 1 h, and Et_3N (31 ml, 0.22 mol) was then added dropwise. After 30 min, the mixture was allowed to warm to 0 °C and then consecutively treated with NH_4Cl (2 g) and water (200 ml) while vigorous stirring. The aqueous phase was extracted with EtOAc (3 × 100 ml). The combined organic phases were dried (Na_2SO_4) and evaporated. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 4:1) to give aldehyde **3** (3.05 g, 85%) as an oil. ^{1}H NMR δ: 0.77 (s, 3H, 18-H), 1.03 (s, 3H, 19-H), 1.12 (d, J = 7 Hz, 3H, 21-H), 2.37 (m, 1H, 20-H), 2.78 (t, J = 2.9 Hz, 1H, 6-H), 3.33 (s, 3H, OMe), 9.58 (d, J = 3.3 Hz, 1H, –CHO). ^{13}C NMR δ: 12.63, 13.11, 13.43, 19.29, 21.48, 22.72, 24.56, 24.96, 27.15, 30.54, 33.39, 35.11, 35.26, 39.98, 43.42, 43.44, 48.07, 49.53, 51.24, 55.78, 56.59, 82.30, 205.18. HRMS calc. for $C_{23}H_{36}O_2$: 344.2715; found: 344.2720. EI-MS$^{m/z}$ (%): 286 (24), 289 (100), 312 (66) [M–CH_3OH]$^{\bullet+}$, 313 (18), 329 (61) [M–CH_3]$^+$, 330 (15) [M + H–CH_3]$^+$, 344 (52) [M]$^{\bullet+}$, 345 (12) [M + H]$^+$.

2.4. Hydroboration of (**1**)

A solution of 1 M BH_3·THF (15 ml, 15 mmol) was added to a solution of **1** (1.05 g, 2.91 mmol) in THF (20 ml). The mixture was kept at ambient temperature for 14 h and treated with 30% H_2O_2 (15 ml, 121 mmol) and a 25% solution of $NaHCO_3$ (15 ml, 121 mmol) for 30 min. After dilution with water, the desired product was extracted with EtOAc (3 × 100 ml). The combined extracts were dried (Na_2SO_4) and evaporated in vacuo. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 0:1) to give: (a) methyl (20*S*)-3β,6α-dihydroxy-5α-pregnane-20-carboxylate **4** (310 mg, 28%) as an oil. ^{1}H NMR (C_5D_5N) δ: 0.62 (s, 3H, 18-H), 0.89 (s, 3H, 19-H), 1.23 (d, J = 6.9 Hz, 3H, 21-H), 3.64–3.78 (m, 4H, 6-H and OMe), 3.93 (m, 1H, 3-H). ^{13}C NMR (C_5D_5N) δ: 12.29, 13.73, 17.32, 21.45, 24.50, 27.50, 32.38, 33.75, 34.68, 36.56, 38.04, 39.91, 42.68, 42.82, 51.23, 52.75, 53.46, 54.25, 56.12, 60.30, 68.64, 71.01, 176.96. HRMS calc. for $C_{23}H_{38}O_4$: 378.2770; found: 378.2774. EI-MS$^{m/z}$ (%): 161 (12), 213 (21), 231 (22), 246 (13), 264 (12), 301 (8), 327 (5), 342 (7) [M–$2H_2O$]$^{\bullet+}$, 345 (12) [M–H_2O–CH_3]$^+$, 360 (100) [M–H_2O]$^{\bullet+}$, 361 (26), 378 (3) [M]$^{\bullet+}$; (b) (20*S*)-3β,6α-dihydroxy-5α-pregnane-20-methanol **5** (640 mg, 63%) as an oil. ^{1}H NMR (C_5D_5N) δ: 0.70 (s, 3H, 18-H), 0.90 (s, 3H, 19-H), 1.30 (d, J = 6.5 Hz, 3H, 21-H), 3.55–3.63 (m, 1H, 22-H), 3.66–3.75 (m, 1H, 6-H), 3.88–3.98 (m, 2H, 3- and 22-H). ^{13}C NMR (C_5D_5N) δ: 12.38, 13.77, 17.56, 21.54, 24.67, 28.12, 32.39, 33.75, 34.76, 36.56, 38.06, 39.75, 40.16, 42.80, 42.90, 52.76, 53.21, 54.33, 56.38, 67.04, 68.73, 71.05. HRMS calc. for $C_{22}H_{38}O_3$: 350.2821; found: 350.2827. EI-MS$^{m/z}$ (%): 213 (19), 231 (38), 232 (28), 246 (11), 264 (10), 299 (6), 314 (7) [M–$2H_2O$]$^{\bullet+}$, 317 (10) [M–H_2O–CH_3]$^+$, 332 (100) [M–H_2O]$^{\bullet+}$, 333 (25), 350 (2) [M]$^{\bullet+}$.

2.5. Methyl (20S)-3,6-dioxo-5α-pregnane-20-carboxylate (**6**)

The dihydroxyester **4** (460 mg, 1.22 mmol) was dissolved in acetone (50 ml), and Jones reagent (5 ml) was added. The mixture was stirred for 15 min, ^{i}PrOH was added, and stirring was continued to quench any remaining Jones reagent. The reaction mixture was diluted with water (150 ml) and

extracted with EtOAc (3 × 75 ml). The extracts were dried (Na_2SO_4) and evaporated. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 2:1) to give 415 mg (91%) of the diketoester **6** as an oil. ^{1}H NMR δ: 0.71 (s, 3H, 18-H), 0.96 (s, 3H, 19-H), 1.20 (d, J = 6.8 Hz, 21-H), 3.65 (s, 3H, OMe). ^{13}C NMR δ: 12.20, 12.57, 17.09, 21.61, 24.04, 26.98, 36.96, 37.34, 37.94, 38.04, 39.13, 41.21, 42.33, 43.04, 46.49, 51.44, 52.78, 53.37, 56.17, 57.46, 177.10, 208.86, 211.20.

2.6. Methyl (20S)-3,6-(dioxolan-2-yl)-5α-pregnane-20-carboxylate (7)

Ethyleneglycol (1.2 ml, 21.6 mmol), triethylorthoformate (1.75 ml, 10.5 mmol), and TsOH (20 mg, 0.11 mmol) were consecutively added to a solution of **6** (390 mg, 1.04 mmol) in CH_2Cl_2 (20 ml). The mixture was kept at ambient temperature for 14 h, treated with Et_3N (0.5 ml), and followed by addition of water (100 ml). After stirring for 30 min, the organic phase was separated, and the aqueous phase was extracted with $CHCl_3$ (3 × 60 ml). The combined organic phases were dried (Na_2SO_4), and the solvents were evaporated in vacuo. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 4:1) to give **7** (351 mg, 73%) as an oil. ^{1}H NMR δ: 0.69 (s, 3H, 18-H), 0.95 (s, 3H, 19-H), 1.18 (d, J = 6.8 Hz, 21-H), 3.63 (s, 3H, OMe), 3.70–3.97 (m, 8H, dioxolane). ^{13}C NMR δ: 12.24, 13.54, 17.08, 21.00, 24.24, 27.11, 29.26, 31.05, 33.42, 36.86, 37.25, 39.59, 41.18, 42.49, 42.71, 49.57, 51.33, 52.91, 53.30, 55.61, 64.13, 64.20, 64.30, 65.43, 109.64, 109.68, 177.29. HRMS calc. for $C_{27}H_{42}O_6$: 462.2981; found: 462.2982. EI-MS$^{m/z}$ (%): 167 (8), 225 (13), 265 (100), 266 (21), 363 (13), 390 (74), 403 (8) $[M–CO_2CH_3]^+$, 462 (43) $[M]^{\bullet+}$, 463 (14) $[M + H]^+$.

2.7. (20S)-3,6-Dioxo-5α-pregnane-20-carboxylic acid (10)

Alcohol **5** was converted into the acid **10** using the procedure reported for the preparation of compound **6**. Compound **10** was isolated as an oil in 84% yield. ^{1}H NMR δ: 0.73 (s, 3H, 18-H), 0.96 (s, 3H, 19-H), 1.24 (d, J = 7.0 Hz, 21-H). ^{13}C NMR δ: 12.26, 12.60, 17.11, 21.68, 24.14, 27.17, 36.97, 37.35, 37.99, 38.15, 39.29, 41.23, 42.45, 43.17, 46.53, 52.56, 53.52, 56.31, 57.55, 208.64, 211.01.

2.8. (20S)-3,6-(Dioxolan-2-yl)-5α-pregnane-20-carboxylic acid (11)

Compound **10** was converted into the dioxolane derivative **11** using the procedure reported for the preparation of compound **7**. Compound **11** was isolated as an oil in 83% yield. ^{1}H NMR δ: 0.70 (s, 3H, 18-H), 0.95 (s, 3H, 19-H), 1.23 (d, J = 6.8 Hz, 3H, 21-H), 2.41 (dq, J = 6.8, 3.7 Hz, 20-H), 3.70–3.78 and 3.84–3.98 (m, 8H, dioxolane). ^{13}C NMR δ: 12.26, 13.54, 17.05, 21.07, 24.33, 27.30, 29.29, 31.10, 33.50, 36.93, 37.30, 39.72, 41.22, 42.47, 42.85, 49.62, 52.65, 53.40, 55.72, 64.14, 64.20, 64.30, 65.46, 109.72, 109.77, 181.82. HRMS calc. for $C_{26}H_{40}O_6$: 448.2719; found: 448.2709.

2.9. (20S)-3,6-(Dioxolan-2-yl)-5α-pregnane-20-methanol (8)

A solution of the ester **7** (400 mg, 0.86 mmol) in ether (20 ml) was added to a stirred suspension of $LiAlH_4$ (600 mg, 15.8 mmol) in ether (80 ml). The mixture was stirred at ambient temperature for 2 h and subsequently treated with water (0.6 ml), 15% NaOH (0.6 ml), and water (1.8 ml). The precipitate was filtered off, and the filtrate was dried (Na_2SO_4) and evaporated. The residue was chromatographed on SiO_2 with hexane–EtOAc (8:1 ⇒ 1:1) to give alcohol **8** (320 mg, 86%) as an oil. ^{1}H NMR δ: 0.70 (s, 3H, 18-H), 0.95 (s, 3H, 19-H), 1.04 (d, J = 6.6 Hz, 3H, 21-H), 3.35 (dd, J = 10.5, 6.8 Hz, 1H, 22-H), 3.62 (dd, J = 10.5, 3.2 Hz, 1H, 22-H), 3.70–3.78 and 3.85–3.97 (m, 8H, dioxolane). ^{13}C NMR δ: 12.18, 13.55, 16.75, 21.11, 24.35, 27.69, 29.32, 31.11, 33.52, 36.92, 37.33, 38.79, 39.77, 41.28, 42.80, 49.66, 52.64, 53.45, 55.85, 64.15, 64.20, 64.30, 65.45, 68.03, 109.77, 109.79. Using a similar procedure, alcohol **8** was prepared from acid **11** in 89% yield.

2.10. (20S)-3,6-(Dioxolan-2-yl)-5α-pregnane-20-carbaldehyde (9)

Alcohol **8** was converted into aldehyde **9** using the procedure reported for the preparation of compound **3**. Compound **9** was isolated as an oil in 89% yield. ^{1}H NMR δ: 0.73 (s, 3H, 18-H), 0.96 (s, 3H, 19-H), 1.12 (d, J = 7.0 Hz, 21-H), 2.35 (m, 1H, 20-H), 3.71–3.79 and 3.85–3.98 (m, 8H, dioxolane), 9.56 (d, 1H, J = 3.3 Hz, –C<u>H</u>O). ^{13}C NMR δ: 12.49, 13.46, 13.54, 21.05, 24.60, 27.00, 29.30, 31.10, 33.48, 37.33, 39.58, 41.26, 43.30, 49.44, 49.64, 51.19, 53.45, 55.37, 64.14, 64.21, 64.31, 65.46, 109.64, 109.69, 204.87. HRMS calc. for $C_{26}H_{40}O_5$432.2876; found: 432.2874. EI-MS$^{m/z}$ (%): 167 (16), 178 (10), 221 (70), 225 (12), 303 (8), 317 (10), 319 (9), 346 (100), 375 (12), 418 (55), 432 (2) $[M]^{\bullet+}$.

2.11. 2-[(2R)-2-Methyl-3-[$^2H_3$]methyl-3-phenylsulfonyl-[4-$^2H_3$]butyloxy]tetrahydro-2H-pyran (14)

A solution of 2.7 M BuLi (77.5 ml, 209 mmol) was added to a solution of 2-[(2*R*)-2-methyl-3-phenylsulfonylpropyloxy]tetrahydro-2H-pyran **12** prepared according to [5] (22.2 g, 74.4 mmol) in THF (235 ml) at −70 °C under argon. The mixture was warmed to −45 °C, kept at this temperature for 5 min, and CD_3I (10.3 ml, 165 mmol) was then added. The cooling bath was removed, and the mixture was allowed to warm to −10 °C. NH_4Cl (5 g) and water (300 ml) were added, and the mixture was extracted with EtOAc (3 × 100 ml). The combined organic phases were dried (Na_2SO_4) and evaporated. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 2:1) to give sulfone **14** (19.0 g, 76%). The ^{1}H and ^{13}C NMR

spectra of this product were identical with those obtained for this compound earlier [5].

2.12. (24S)-[26,27-$^2H_6$]23-Phenylsulfonyl-24-methyl-3α,5-cyclo-5α-cholestan-22-ol (**18**)

A solution of 2.7 M BuLi in heptane (7 ml, 19 mmol) was added to a solution of (2*S*)-[3,4-$^2H_6$]2,3-dimethylbutylphenyl sulfone **17** (1.3 g, 5.6 mmol) in THF (40 ml), which was prepared according to [5], at −70 °C. The mixture was kept at −70 → −60° C for 30 min, then excess butyllithium was destroyed by adding diisopropylamine (5 ml, 35 mmol). The mixture was kept another 30 min and finally cooled to −70 °C. A solution of aldehyde **3** (2.05 g, 5.95 mmol) in THF (20 ml) was added over 1 h, and the cooling bath was removed. After room temperature was reached, NH_4Cl (2 g) and water (50 ml) were added. The crude product was extracted with EtOAc (3 × 30 ml), dried on Na_2SO_4, and the solvent was removed in vacuo. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 1:1) to give 2.69 g (83%) of sulfone **18** as an oil. ^{1}H NMR δ: 0.71 (s, 3H, 18-H), 1.01 (s, 3H, 19-H), 3.32 (s, 3H, OMe), 3.66 (m, 1H), 4.29–4.34 (m, 1H), 7.50–7.68 and 7.89–7.94 (m, 5H, Ph). ^{13}C NMR δ: 11.38, 12.25, 13.09, 13.53, 13.56, 15.90, 15.95, 19.28, 21.47, 21.54, 22.77, 24.03, 24.95, 27.86, 29.81, 30.60, 31.96, 33.36, 35.05, 35.23, 37.62, 40.20, 41.09, 42.68, 43.36, 47.94, 52.06, 56.39, 56.60, 60.55, 70.94, 71.20, 82.38, 127.66, 127.83, 127.88, 128.93, 129.08, 129.25, 133.00, 133.50.

2.13. (24S)-[26,27-$^2H_6$]24-Methyl-3α,5-cyclo-5α-cholestan-22-ene (**19**)

Pyridine (100 μl, 1.2 mmol), acetylchloride (88 μl, 1.2 mmol), and 4-(dimethylamino)-pyridine (5 mg, 41 μmol) were added to a solution of hydroxy sulfone **18** (600 mg, 1.04 mmol) in CH_2Cl_2 (30 ml). The mixture was kept at room temperature for 8 h and diluted with water (30 ml). The organic phase was decanted, and the aqueous layer was extracted with $CHCl_3$ (3 × 30 ml). The combined organic phases were dried (Na_2SO_4), and the solvents were removed in vacuo. The residue was dissolved in methanol (100 ml), and magnesium (1 g, 41 mmol) and mercury(II) chloride (400 mg, 1.5 mmol) were added. The mixture was stirred at 0 °C for 1 h and then filtered through SiO_2. The filtrate was diluted with water (50 ml) and extracted with hexane (3 × 50 ml). The combined hexane fractions were dried (Na_2SO_4) and the solvent was evaporated. The residue was chromatographed on SiO_2 with hexane–EtOAc (20:1 ⇒ 10:1) to give olefin **19** (210 mg, 48%) as an oil. ^{1}H NMR δ: 0.73 (s, 3H, 18-H), 0.91 (d, *J* = 6.6 Hz, 21- or 28-H), 1.00 (d, *J* = 7 Hz, 3H, 28- or 21-H), 1.03 (s, 3H, 19-H), 2.76 (t, *J* = 2.7 Hz, 1H, 6-H), 3.32 (s, 3H, OMe), 5.15–5.19 (m, 2H, 22- and 23-H). ^{13}C NMR δ: 12.49, 13.14, 17.94, 17.99, 19.32, 21.03, 21.60, 22.83, 24.29, 25.03, 28.89, 30.58, 32.81, 33.47, 35.17, 40.25, 40.31, 42.78, 43.00, 43.50, 48.23, 56.30, 56.57, 56.76, 82.54, 131.94, 136.12. HRMS calc. for $C_{29}H_{42}D_6O$: 418.4082; found: 418.4090. EI-MS$^{m/z}$ (%): 81 (46), 107 (36), 159 (31), 161 (23), 253 (31), 255 (100), 285 (17), 314 (23), 363 (78), 364 (77), 386 (68) [*M*-CH_3OH]$^{\bullet+}$, 387 (69), 403 (45) [*M*–CH_3]$^+$, 404 (48), 418 (91) [*M*]$^{\bullet+}$, 419 (98) [*M* + H]$^+$.

2.14. Hydroxylation of (**19**)

A mixture of **19** (120 mg), AD-mix-β (2 g), and $MeSO_2NH_2$ (182 mg) in *tert*-butanol–water (5:4, 18 ml) was stirred at ambient temperature for 14 days. A solution of $NaHSO_3$ (37%, 10 ml) was then added, and stirring was continued at 35 °C for 30 min. Solvents were removed in vacuo, and the residue was dissolved in water (40 ml) and extracted with $CHCl_3$ (3 × 30 ml). The combined organic extracts were dried (Na_2SO_4) and evaporated. The residue was chromatographed on SiO_2 with hexane–EtOAc (15:1 ⇒ 1:1) to give: a) (22*S*,23*S*,24*S*)-[26,27-$^2H_6$]6β-methoxy-24-methyl-3α,5-cyclo-5α-cholesta-22,23-diol **20** (29 mg, 22%). ^{1}H NMR δ: 0.75 (d, *J* = 6.4 Hz, 3H, 21- or 28-H), 0.76 (s, 3H, 18-H), 1.03 (s, 3H, 19-H), 1.04 (d, *J* = 7 Hz, 3H, 28- or 21-H), 2.77 (t, *J* = 2.7 Hz, 1H, 6-H), 3.32 (s, 3H, OMe), 3.46 (d, *J* = 9 Hz, 1H, 22-H), 3.67 (d, *J* = 4.4 Hz, 1H, 23-H). ^{13}C NMR δ: 10.22, 12.21, 13.15, 14.18, 19.28, 21.54, 21.61, 22.84, 24.48, 25.02, 26.15, 28.04, 30.59, 33.45, 35.17, 35.36, 40.37, 42.42, 43.40, 43.46, 48.13, 53.09, 56.26, 56.58, 71.43, 71.75, 82.47. HRMS calc. for $C_{29}H_{44}D_6O_3$: 452.4137; found: 452.4137. EI-MS$^{m/z}$ (%): 159 (48), 161 (39), 213 (40), 227 (25), 253 (29), 255 (45), 295 (52), 313 (100), 331 (13), 346 (30), 297 (24), 298 (28), 420 (28) [*M*-CH_3OH]$^{\bullet+}$, 421 (30) [*M* + H–CH_3OH]$^+$, 437 (13) [*M*–CH_3]$^+$, 438 (15), 452 (58) [*M*]$^{\bullet+}$, 453 (20) [*M* + H]$^+$; (b) (22*R*,23*R*,24*S*)-[26,27-$^2H_6$]6β-methoxy-24-methyl-3α,5-cyclo-5α-cholesta-22,23-diol **21** (67 mg, 51%). ^{1}H NMR δ: 0.74 (s, 3H, 18-H), 0.85 (d, *J* = 7 Hz, 3H, 21- or 28-H), 0.91 (d, *J* = 6.6 Hz, 3H, 28- or 21-H), 1.03 (s, 3H, 19-H), 2.77 (t, *J* = 2.9 Hz, 1H, 6-H), 3.33 (s, 3H, OMe), 3.57 (dd, *J* = 8, 1.1 Hz, 1H, 22-H), 3.70 (ddd, *J* = 8.4, 2.2, 1.1 Hz, 1H, 23-H). ^{13}C NMR δ: 10.07, 10.09, 11.97, 12.22, 13.15, 19.30, 21.56, 22.87, 24.11, 25.01, 26.98, 27.96, 30.43, 30.68, 33.45, 35.18, 37.03, 40.43, 42.72, 43.46, 48.10, 52.72, 56.53, 56.60, 73.61, 74.93, 82.50. HRMS calc. for $C_{29}H_{44}D_6O_3$: 452.4137; found: 452.4135. EI-MS$^{m/z}$ (%): 107 (25), 121 (25), 159 (18), 213 (16), 227 (20), 255 (25), 295 (48), 313 (100), 331 (11), 346 (33), 297 (27), 298 (28), 420 (17) [*M*–CH_3OH]$^{\bullet+}$, 421 (17), 437 (16) [*M*–CH_3]$^+$, 438 (16), 452 (74) [*M*]$^{\bullet+}$, 453 (38) [*M* + H]$^+$.

2.15. (22R,23R,24S)-[26,27-$^2H_6$]24-Methylcholest-5-en-3β,22,23-triol (**22**)

A mixture of alcohol **21** (50 mg, 0.11 mmol) and TsOH (5 mg, 0.028 mmol) in dioxane–water (4 ml, 4:1) was kept at 80 °C for 3 h, after which Et_3N (0.1 ml, 0.7 mmol) was

added, and the solvents were removed in vacuo. The residue was chromatographed on SiO_2 with hexane–EtOAc (8:1 ⇒ 1:1) to give compound **22** (43 mg, 88%). ^{1}H NMR (C_5D_5N) δ: 0.81 (s, 3H, 18-H), 1.06 (s, 3H, 19-H), 1.16 (d, J = 6.6 Hz, 21- or 28-H), 1.27 (d, J = 6.6 Hz, 3H, 28- or 21-H), 3.86 (m, 1H, 3-H), 3.99 (dd, J = 8.4, 1.1 Hz, 22-H), 4.14 (m, 1H, 23-H), 5.40–5.44 (m, 1H, 6-H). ^{13}C NMR (C_5D_5N) δ: 10.90, 10.92, 12.00, 12.84, 19.67, 21.47, 24.53, 28.42, 30.89, 32.27, 32.65, 36.92, 37.84, 38.15, 40.34, 41.05, 42.41, 43.52, 50.55, 53.11, 57.16, 71.30, 73.11, 74.41, 121.24, 141.99. HRMS calc. for $C_{28}H_{42}D_6O_3$: 438.3980; found: 438.3982. EI-MS$^{m/z}$ (%): 159 (23), 213 (20), 255 (30), 273 (15), 295 (31), 313 (70), 332 (100), 361 (3), 421 (3) $[M + H–H_2O]^+$, 438 (26) $[M]^{\bullet+}$

*2.16. (22R,23R,24S)-[26,27-$^2H_6$]24-Methylcholesta-3β,22,23-triol (**23**)*

A solution of **22** (33 mg, 75 μmol) in ethanol (15 ml) was hydrogenated over 5% Pd/C under H_2 for 12 h. The reaction mixture was filtered through SiO_2, and the solvent was evaporated. The residue was chromatographed on SiO_2 with hexane–EtOAc (8:1 ⇒ 2:1) to give **23** (32 mg, 97%) as an oil. ^{1}H NMR δ: 0.67 (s, 3H, 18-H), 0.81 (s, 3H, 19-H), 0.84 (d, J = 7 Hz, 3H, 21- or 28-H), 0.89 (d, J = 6.2 Hz, 3H, 28- or 21-H), 3.54–3.64 (m, 2H, 3- and 22-H), 3.72 (m, 1H, 23-H). ^{13}C NMR δ: 10.08, 10.10, 11.87, 11.99, 12.32, 21.27, 24.06, 27.83, 28.68, 31.50, 31.98, 35.56, 36.84, 36.96, 38.19, 40.04, 42.44, 44.78, 52.49, 54.25, 56.37, 71.33, 73.46, 74.88. HRMS calc. for $C_{28}H_{44}D_6O_3$: 440.4137; found: 440.4133. EI-MS$^{m/z}$ (%): 161 (15), 234 (26), 257 (41), 273 (15), 297 (23), 315 (63), 334 (100), 345 (3), 364 (2), 440 (6) $[M]^{\bullet+}$.

*2.17. (24R)-[26,27-$^2H_6$]6β-Methoxy-23-phenylsulfonyl-24-methyl-3α,5-cyclo-5α-cholestan-22-one (**24**)*

Ketone **24** was obtained from the alcohol **18** via Swern oxidation as described above for preparation of aldehyde **3**. Compound **24** was isolated as an oil in 87% yield. ^{1}H NMR δ: 0.98–1.03 (m, 6H, 18- and 19-H), 3.31, 3.32 (s, 3H, OMe), 4.35–4.40 (m, 1H, 23-H), 7.49–7.95 (m, 5H, Ph). ^{13}C NMR δ: 12.19, 12.61, 13.15, 15.56, 15.95, 16.00, 19.26, 19.30, 21.52, 21.59, 22.73, 22.86, 24.44, 24.58, 24.98, 25.02, 28.22, 30.57, 32.01, 33.44, 33.71, 33.73, 33.84, 35.13, 40.09, 43.43, 48.05, 48.11, 50.95, 51.14, 55.90, 56.58, 60.67, 82.39, 127.93, 128.79, 128.83, 129.26, 129.61, 129.95, 133.48, 133.75.

*2.18. (24R)-[26,27-$^2H_6$]6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholestan-22-one (**25**)*

Aluminum foil was cut into small strips and treated with 15% NaOH for 15 min. Then it was washed twice with water, then ethanol, and submerged for 10 min twice in a 0.5% aqueous mercury(II) chloride solution. The resulting aluminum amalgam was added to a solution of sulfone **24** (1.3 g, 2.26 mmol) in EtOH (50 ml). The mixture was stirred at ambient temperature for 15 h, filtered through a short pad of SiO_2 and the solvent was evaporated in vacuo. The residue was chromatographed on SiO_2 (15:1 ⇒ 4:1) to give 673 mg (68%) of ketone **25** as an oil. ^{1}H NMR δ: 0.74 (s, 3H, 18-H), 0.80 (d, J = 6.6 Hz, 3H, 28-H), 1.03 (s, 3H, 19-H), 1.08 (d, J = 7 Hz, 3H, 21-H), 2.23 (dd, J = 17, 9.2 Hz, 1H), 2.36 (m, 1H), 2.50 (m, 1H), 2.76 (t, J = 2.9 Hz, 1H, 6-H), 3.32 (s, 3H, OMe). ^{13}C NMR δ: 12.50, 13.15, 16.35, 19.29, 21.56, 22.79, 24.49, 25.01, 27.75, 27.80, 30.59, 33.46, 35.18, 35.36, 40.24, 42.97, 43.48, 46.69, 46.72, 48.14, 49.75, 49.79, 52.08, 55.99, 56.59, 82.45, 214.23. HRMS calc. for $C_{29}H_{42}D_6O_2$: 434.4031; found: 434.4023. EI-MS$^{m/z}$ (%): 119 (60), 120 (46), 213 (17), 255 (17), 283 (39), 327 (15), 379 (84), 380 (89), 385 (20), 402 (77) $[M\text{-}CH_3OH]^{\bullet+}$, 403 (82), 417 (11), 419 (54) $[M–CH_3]^+$, 420 (56), 434 (92) $[M]^{\bullet+}$, 435 (100) $[M + H]^+$.

*2.19. Reduction of the ketone (**25**)*

A solution of the ketone **25** (350 mg, 0.81 mmol) in ether (10 ml) was added to a stirred suspension of $LiAlH_4$ (350 mg, 9.22 mmol) in ether (20 ml). The stirring was continued at room temperature for 3 h, after which water (0.35 ml), 15% NaOH (0.35 ml), and water (1.05 ml) were consecutively added. The precipitate was filtered off, and the filtrate was dried (Na_2SO_4) and evaporated. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 1:1) to give: (a) (22*R*,24*R*)-[26,27-$^2H_6$]6β-methoxy-24-methyl-3α,5-cyclo-5α-cholestan-22-ol **26** (11%) as an oil. ^{1}H NMR δ: 0.74 (s, 3H, 18-H), 0.82 (d, J = 6.8 Hz, 3H, 21- or 28-H), 0.92 (d, J = 6.6 Hz, 3H, 28- or 21-H), 1.02 (s, 3H, 19-H), 2.77 (t, J = 2.9 Hz, 1H, 6-H), 3.32 (s, 3H, OMe), 3.74 (ddd, J = 11, 3, 1.2 Hz, 1H, 22-H). ^{13}C NMR δ: 12.28, 13.08, 14.97, 19.29, 21.49, 22.80, 24.29, 24.98, 27.40, 30.53, 33.36, 34.09, 34.12, 35.07, 35.28, 40.28, 42.40, 43.07, 43.39, 48.07, 53.24, 56.11, 56.56, 70.98, 82.38. HRMS calc. for $C_{29}H_{44}D_6O_2$: 436.4187; found: 434.4194. EI-MS$^{m/z}$ (%): 213 (16), 255 (10), 261 (12), 284 (34), 364 (14), 381 (48), 382 (46), 404 (42) $[M–CH_3OH]^{\bullet+}$, 405 (39), 419 (12) $[M + H–H_2O]^+$, 421 (30) $[M–CH_3]^+$, 422 (32), 436 (100) $[M]^{\bullet+}$, 437 (52) $[M + H]^+$; (b) (22*S*,24*R*)-[26,27-$^2H_6$]6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholestan-22-ol **27** (77%) as an oil. ^{1}H NMR δ: 0.74 (s, 3H, 18-H), 0.83 (d, J = 6.6 Hz, 3H, 21- or 28-H), 0.89 (d, J = 6.2 Hz, 3H, 28- or 21-H), 2.78 (t, J = 2.6 Hz, 1H, 6-H), 3.33 (s, 3H, OMe), 3.77 (t, J = 6.6 Hz, 1H, 22-H). ^{13}C NMR δ: 11.25, 12.22, 13.11, 15.80, 15.84, 19.30, 21.48, 22.82, 24.14, 24.98, 27.92, 30.59, 31.59, 33.37, 35.12, 35.26, 39.41, 40.31, 42.73, 43.40, 48.01, 52.73, 52.79, 56.46, 56.60, 71.73, 82.43. HRMS calc. for $C_{29}H_{44}D_6O_2$: 436.4187; found: 436.4193. EI-MS$^{m/z}$ (%): 213 (21), 255 (17), 261 (20), 284 (52), 364 (23), 381 (85), 382 (87), 404 (84) $[M–CH_3OH]^{\bullet+}$, 405 (84), 419 (20) $[M + H–H_2O]^+$, 421 (53) $[M–CH_3]^+$, 422 (55), 436 (94) $[M]^{\bullet+}$, 437 (100) $[M + H]^+$.

2.20. (22R,24R)-[26,27-$^2H_6$]24-Methylcholest-5-en-3β,22-diol (**28**)

Synthesis of the triol **28** was performed from **26** according to the procedure reported for the preparation of compound **22**. Diol **28** was isolated as an oil in 82% yield. ^{1}H NMR δ: 0.71 (s, 3H, 18-H), 0.82 (d, J = 6.6 Hz, 3H, 21- or 28-H), 0.92 (d, J = 6.6 Hz, 3H, 28- or 21-H), 1.01 (s, 3H, 19-H), 3.52 (m, 1H, 3-H), 3.74 (ddd, 1H, J = 11, 3.3, 1.1 Hz, 22-H), 5.33–5.37 (m, 1H, 6-H). ^{13}C NMR δ: 11.86, 12.27, 19.35, 21.06, 24.37, 27.27, 31.63, 31.87, 31.92, 34.07, 34.10, 34.48, 36.48, 37.24, 39.74, 42.27, 42.32, 42.60, 50.14, 53.05, 56.30, 70.98, 71.72, 71.83, 121.55, 140.80. HRMS calc. for $C_{28}H_{42}D_6O_2$: 422.4031; found: 434.4029. EI-MS$^{m/z}$ (%): 191 (45), 213 (36), 217 (32), 229 (19), 255 (14), 269 (32), 284 (53), 287 (32), 302 (100), 303 (26), 313 (9), 372 (8), 405 (30) $[M + H–H_2O]^+$, 422 (35) $[M]^{\bullet+}$, 423 (38) $[M + H]^+$.

2.21. (22S,24R)-[26,27-$^2H_6$]22-Acetoxy-6β-methoxy-24-methyl-3α,5-cyclo-5α-cholestane (**29**)

A mixture of alcohol **27** (190 mg, 0.44 mmol) and 4-(dimethylamino)pyridine (10 mg, 0.08 mmol) in pyridine (2 ml) and acetic anhydride (1 ml) was kept at room temperature for 12 h. Water (20 ml) was then added, and the mixture was stirred for 30 min. The product was taken up in EtOAc (3 × 20 ml), the organic phases were dried (Na_2SO_4), and the solvent was evaporated in vacuo. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 2:1) to give acetate **29** (191 mg, 92%) as an oil. ^{1}H NMR δ: 0.73 (s, 3H, 18-H), 0.84 (d, J = 6.2 Hz, 3H, 21- or 28-H), 0.96 (d, J = 6.6 Hz, 3H, 28- or 21-H), 1.02 (s, 3H, 19-H), 2.02 (s, 3H, OAc), 2.76 (t, J = 2.9 Hz, 1H, 6-H), 3.32 (s, 3H, OMe), 5.06 (m, 1H, 22-H). ^{13}C NMR δ: 12.06, 12.54, 13.07, 15.54, 19.29, 21.30, 21.52, 22.81, 24.13, 24.98, 28.25, 30.56, 31.72, 33.39, 34.98, 35.32, 35.77, 38.23, 38.25, 40.29, 42.70, 43.38, 48.04, 52.71, 56.47, 56.58, 75.02, 82.42, 170.78.

2.22. (22S,24R)-[26,27-$^2H_6$]22-Acetoxy-24-methylcholest-5-en-3β-ol (**30**)

Synthesis of the hydroxyacetate **30** was performed from **29** according to the procedure reported for the preparation of compound **28**. The hydroxyacetate **30** was isolated as an oil in 91% yield. ^{1}H NMR δ: 0.69 (s, 3H, 18-H), 0.84 (d, J = 6.6 Hz, 3H, 21- or 28-H), 0.96 (d, J = 7 Hz, 3H, 28- or 21-H), 1.01 (s, 3H, 19-H), 2.03 (s, 3H, OAc), 3.53 (m, 1H, 3-H), 5.01 (m, 1H, 22-H), 5.35 (m, 1H, 6-H). ^{13}C NMR δ: 11.67, 12.60, 19.41, 21.09, 21.35, 24.24, 28.15, 31.64, 31.84, 31.94, 34.99, 35.74, 36.49, 37.26, 38.18, 38.20, 39.74, 42.25, 42.29, 50.10, 52.53, 56.64, 71.74, 75.01, 121.67, 140.72, 170.85. HRMS calc. for $C_{30}H_{44}D_6O_3$: 464.4137; found: 464.4156. EI-MS$^{m/z}$ (%): 107 (36), 145 (40), 159 (31), 213 (37), 272 (100), 293 (27), 299 (50), 372 (25), 389 (29), 404 (92) $[M–AcOH]^{\bullet+}$, 405 (95) $[M–AcO]^+$, 446 (24) $[M–H_2O]^{\bullet+}$, 447 (32) $[M + H–H_2O]^+$, 464 (74) $[M]^{\bullet+}$, 465 (52) $[M + H]^+$.

2.23. (22S,24R)-[26,27-$^2H_6$]24-Methylcholest-5-en-3β,22-diol (**31**)

The hydroxyacetate **30** (25 mg, 52 μmol) was treated with a 10% solution of NaOH (3 ml) at 70 °C for 15 min, and then, the mixture was neutralized with 2 M HCl, and the solvents were evaporated in vacuo. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 1:1) to give diol **31** (21 mg, 95%) as an oil. ^{1}H NMR δ: 0.70 (s, 3H, 18-H), 0.81 (d, J = 6.2 Hz, 3H, 21- or 28-H), 0.90 (d, J = 6.2 Hz, 3H, 28- or 21-H), 1.01 (s, 3H, 19-H), 3.52 (m, 1H, 3-H), 3.77 (t, J = 6.6 Hz, 1H, 22-H), 5.35 (m, 1H, 6-H). ^{13}C NMR δ: 11.27, 11.80, 15.77, 15.81, 19.42, 21.11, 24.22, 27.83, 31.66, 31.86, 31.95, 35.13, 36.49, 37.24, 39.38, 39.78, 42.26, 42.29, 42.37, 50.07, 52.55, 56.68, 71.72, 71.78, 121.64, 140.78. HRMS calc. for $C_{28}H_{42}D_6O_2$: 422.4031; found: 422.4036. EI-MS$^{m/z}$ (%): 95 (23), 107 (21), 215 (13), 217 (9), 233 (15), 248 (10), 274 (100), 285 (8), 301 (50), 391 (10), 406 (37), 407 (40) $[M–CH_3]^+$, 422 (5) $[M]^{\bullet+}$.

2.24. (22S,24R)-[26,27-$^2H_6$]22-Acetoxy-24-methylcholestan-3β-ol (**32**)

Synthesis of the hydroxyacetate **32** was performed from **30** according to the procedure reported for the preparation of the triol **23**. Hydroxyacetate **32** was isolated as an oil in 90% yield. ^{1}H NMR δ: 0.66 (s, 3H, 18-H), 0.80 (s, 3H, 19-H), 0.83 (dd, J = 6.6, 0.7 Hz, 3H, 21- or 28-H), 0.94 (d, J = 6.6 Hz, 3H, 28- or 21-H), 2.03 (s, 3H, OAc), 3.59 (m, 1H, 3-H), 5.05 (m, 1H, 22-H). ^{13}C NMR δ: 11.88, 12.33, 12.52, 15.47, 15.51, 21.26, 21.33, 24.16, 28.16, 28.69, 31.49, 31.68, 32.01, 34.97, 35.44, 35.53, 35.70, 37.01, 38.18, 40.00, 42.51, 44.83, 52.63, 54.33, 56.39, 71.32, 74.99, 170.83.

2.25. (22S,24R)-[26,27-$^2H_6$]24-Methylcholestan-3β,22-diol (**33**)

Synthesis of the diol **33** was performed from **32** according to the procedure reported for the preparation of compound **31**. Diol **33** was isolated as an oil in 93% yield. ^{1}H NMR δ: 0.67 (s, 3H, 18-H), 0.80 (s, 3H, 19-H), 0.82 (d, J = 6.2 Hz, 3H, 21- or 28-H), 0.88 (d, J = 6.2 Hz, 3H, 28- or 21-H), 3.59 (m, 1H, 3-H), 3.77 (t, J = 6.6 Hz, 1H, 22-H). ^{13}C NMR δ: 11.21, 12.02, 12.33, 15.77, 15.80, 21.28, 24.15, 27.84, 28.71, 31.52, 32.04, 35.24, 35.46, 35.56, 36.99, 38.20, 39.33, 39.37, 40.04, 42.54, 44.82, 52.67, 54.31, 56.43, 71.34, 71.69. HRMS calc. for $C_{28}H_{38}D_6O_3$: 424.4187; found: 424.4185. EI-MS$^{m/z}$ (%): 165 (26), 215 (35), 217 (23), 234 (94), 248 (25), 257 (13), 271 (19), 286 (34), 289 (20), 304 (100), 315 (9), 333 (6), 406 (7) $[M–H_2O]^{\bullet+}$, 407 (8) $[M + H–H_2O]^+$, 424 (53) $[M]^{\bullet+}$.

2.26. (22S,24R)-[26,27-$^2H_6$]24-Methylcholest-4-en-3,6-dion-22-ol (**34**)

The Jones reagent (0.5 ml) was added dropwise to a solution of hydroxyacetate **30** (20 mg, 42 μmol) in acetone. The mixture was stirred for 15 min; then, ^{i}PrOH (2 ml) was added, and stirring was continued for another 15 min. HCl (1 ml, 37%) was added, and the mixture was kept for 1 h, diluted with water (25 ml), and extracted with $CHCl_3$ (3 × 20 ml). The combined extracts were dried (Na_2SO_4) and concentrated. The crude product was dissolved in 10% NaOH in MeOH (3 ml), and the mixture was refluxed for 20 min. Afterwards, it was neutralized by 2 M HCl, and the solvents were removed in vacuo. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 2:1) to give diketone **34** (4.7 mg, 26%) as an oil. ^{1}H NMR δ: 0.74 (s, 3H, 18-H), 0.83 (d, J = 6.6 Hz, 3H, 21- or 28-H), 0.91 (d, J = 6.6 Hz, 3H, 28- or 21-H), 1.17 (s, 3H, 19-H), 3.77 (t, J = 7.3 Hz, 1H, 22-H), 6.17 (br. s, 1H, 4-H). ^{13}C NMR δ: 11.25, 11.84, 15.83, 17.54, 20.90, 23.91, 27.62, 29.70, 33.97, 34.26, 35.29, 35.51, 39.13, 39.34, 39.47, 39.50, 42.45, 46.76, 50.90, 52.35, 56.46, 71.57, 125.50, 161.00, 199.46, 202.27. HRMS Calc. for $C_{28}H_{38}D_6O_3$434.3667; Found: 434.3669. EI-MS$^{m/z}$ (%): 163 (34), 243 (24), 257 (14), 272 (13), 286 (39), 296 (22), 299 (28), 314 (100), 315 (46), 316 (47), 330 (6), 343 (11), 434 (2) $[M]^{\bullet+}$, 435 (3) $[M + H]^+$.

2.27. (22S,24R)-[26,27-$^2H_6$]24-Methylcholestan-3-on-22-ol (**35**)

Hydroxyacetate **32** was converted int compound **35** via Jones oxidation followed by saponification according to procedures described for the preparation of **34**. Compound **35** was isolated as an oil in 74% yield. ^{1}H NMR δ: 0.70 (s, 3H, 18-H), 0.83 (d, J = 6.6 Hz, 3H, 21- or 28-H), 0.87 (d, J = 6.6 Hz, 3H, 28- or 21-H), 1.01 (s, 3H, 19-H), 3.77 (t, J = 7 Hz, 1H, 22-H). ^{13}C NMR δ: 11.23, 11.47, 12.01, 15.77, 15.81, 21.47, 24.16, 27.82, 28.94, 31.57, 31.67, 35.25, 35.45, 35.63, 38.18, 38.52, 39.39, 39.90, 42.53, 44.71, 46.65, 52.64, 53.74, 56.21, 71.62, 212.17. HRMS calc. for $C_{28}H_{42}D_6O_2$: 422.4031; found: 422.4021. EI-MS$^{m/z}$ (%): 163 (25), 203 (16), 217 (22), 232 (98), 246 (25), 271 (7), 273 (8), 287 (19), 302 (100), 313 (8), 316 (5), 331 (6), 421 (34), 422 (24) $[M]^{\bullet+}$.

2.28. (3R,22S,24R)-[26,27-$^2H_6$]24-Methylcholestan-3,22-diol (**36**)

A mixture of hydroxyacetate **32** (40 mg, 85 μmol) and MsCl (0.2 ml, 2.6 mmol) in pyridine (2 ml) was kept at room temperature for 3 h, diluted with water (20 ml), and extracted with $CHCl_3$ (3 × 20 ml). The combined extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was dissolved in DMF (5 ml), and KO_2 (230 mg, 3.2 mmol) and 18-crown-6 (1.0 g, 3.8 mmol) were added. The mixture was stirred at room temperature for 10 h, diluted with water (25 ml), and extracted with $CHCl_3$ (3 × 20 ml). The combined extracts were dried (Na_2SO_4), and the residue after removal of the solvent was dissolved in 10% NaOH in MeOH (3 ml). The mixture was refluxed for 15 min, cooled to room temperature, and neutralized with 2 M HCl. After evaporation of the solvent, the residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 1:1) to give diol 36 (23 mg, 63%) as an oil. ^{1}H NMR δ: 0.67 (s, 3H, 18-H), 0.78 (s, 3H, 19-H), 0.82 (d, J = 6.6 Hz, 3H, 21- or 28-H), 0.88 (d, J = 6.2 Hz, 3H, 28- or 21-H), 3.77 (t, J = 6.6 Hz, 1H, 22-H), 4.04 (m, 1H, W/2 = 7 Hz, 3-H). ^{13}C NMR δ: 11.19, 11.22, 12.03, 15.80, 20.81, 24.11, 27.83, 28.56, 29.02, 31.97, 32.18, 35.56, 35.90, 36.07, 39.11, 39.30, 39.32, 39.39, 40.05, 42.54, 52.65, 54.29, 56.48, 66.60, 70.64, 71.69. EI-MS$^{m/z}$ (%): 165 (31), 215 (42), 217 (28), 234 (93), 248 (28), 257 (15), 271 (25), 286 (56), 289 (23), 304 (100), 315 (13), 333 (4), 389 (5), 406 (12) $[M–H_2O]^{\bullet+}$, 407 (14) $[M + H–H_2O]^+$, 424 (2) $[M]^{\bullet+}$, 425 (2) $[M + H]^+$.

2.29. (24S)-[26,27-$^2H_6$]3,6-(Dioxolan-2-yl)-23-phenylsulfonyl-24-methyl-5α-cholestan-22-ol (**37**)

Synthesis of the hydroxysulfone **37** was performed from aldehyde **9** according to the procedure reported for the preparation of the compound **18**. Hydroxysulfone **37** was isolated as an oil in 78% yield. ^{1}H NMR δ: 0.67 (s, 3H, 18-H), 0.94 (s, 3H, 19-H), 3.02 (t, J = 4.5 Hz), 3.65 (dd, J = 9.5, 2 Hz), 3.74–3.80 and 3.86–3.96 (m, 8H, dioxolane), 4.30 (dd, J = 9.5, 4.3 Hz), 7.50–7.62 and 7.88–7.92 (m, 5H, Ph). ^{13}C NMR δ: ^{13}C NMR δ: 11.68, 12.30, 13.55, 21.13, 24.10, 27.12, 29.24, 29.28, 29.30, 31.12, 31.16, 33.55, 36.89, 36.92, 37.33, 37.71, 38.99, 39.86, 39.98, 41.17, 42.59, 49.61, 52.08, 52.93, 53.35, 53.38, 53.44, 55.63, 56.01, 56.07, 64.12, 64.14, 64.18, 64.30, 64.41, 65.45, 65.47, 65.92, 70.83, 70.96, 71.21, 109.68, 109.71, 109.74, 109.77, 123.73, 127.69, 128.10, 128.50, 128.89, 129.01, 129.07, 129.32, 132.92, 133.23, 133.44, 135.94, 149.87. HRMS calc. for $C_{38}H_{52}D_6O_7S$: 664.4280; found: 664.4281. EI-MS$^{m/z}$ (%): 99 (100), 207 (10), 225 (15), 235 (23), 360 (18), 404 (35), 432 (11), 452 (9), 467 (11), 468 (11), 524 (7), 593 (14), 664 (12) $[M]^{\bullet+}$, 665 (15) $[M + H]^+$.

2.30. (24S)-[26,27-$^2H_6$]3,6-(Dioxolan-2-yl)-23-phenylsulfonyl-24-methyl-5α-cholestan-22-one (**38**)

Alcohol **37** was converted into ketone **38** using the procedure reported for the preparation of compound **3**. Compound **38** was isolated as an oil in 89% yield. ^{1}H NMR δ: 0.75 (s, 3H, 18-H), 0.96 (s, 3H, 19-H), 3.74–3.80 and 3.87–3.97 (m, 8H, dioxolane), 4.36 (m, 1H, 23-H), 7.51–7.85 (m, 5H, Ph). ^{13}C NMR δ: 11.71, 12.27, 13.56, 15.08, 21.14, 24.57, 27.06, 27.55, 29.30, 31.12, 33.48, 36.91, 37.34, 37.99, 39.95, 41.25, 42.81, 49.63, 51.27, 51.31, 52.05, 53.40, 55.53, 64.14, 64.22, 64.32, 65.48, 109.74, 128.79, 129.96, 134.02, 204.86, 204.89. HRMS calc. for $C_{38}H_{50}D_6O_7S$: 662.4123; found: 662.4139. EI-MS$^{m/z}$ (%): 99 (100), 225 (18), 303 (6), 325 (9), 403 (18),

450 (11), 465 (39), 466 (41), 473 (6), 522 (8), 591 (36), 662 (26) $[M]^{\bullet+}$, 663 (32) $[M + H]^+$.

2.31. (24S)-[26,27-$^2H_6$]3,6-(Dioxolan-2-yl)-24-methyl-5α-cholestan-22-one (39)

The reduction of **38** was carried out as described above for the preparation of **25**. Ketone **39** was isolated as an oil in 70% yield. ^{1}H NMR δ: 0.70 (s, 3H, 18-H), 0.95 (s, 3H, 19-H), 2.36 (dd, J = 17, 4 Hz), 2.48 (m, 1H), 3.72–3.78 and 3.86–3.98 (m, 8H, dioxolane).

2.32. (22S,24R)-[26,27-$^2H_6$]3,6-(Dioxolan-2-yl)-24-methyl-5α-cholestan-22-ol (40)

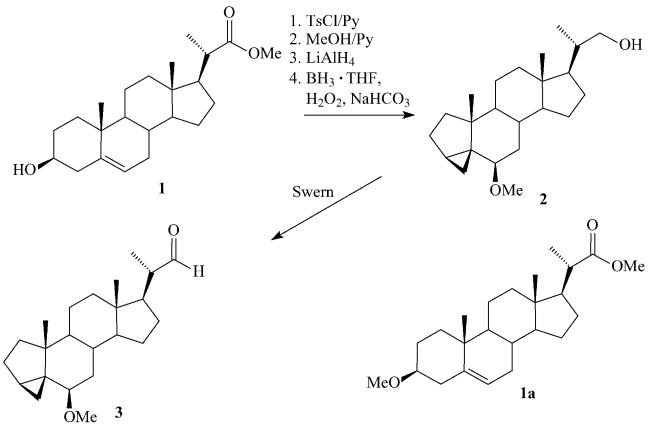
Alcohol **40** was prepared from ketone **39** according to the procedure described for the reduction of **25**. Compound **40** was obtained as an oil in 83% yield. ^{1}H NMR δ: 0.69 (s, 3H, 18-H), 0.83 (d, J = 6.2 Hz, 3H, 21- or 28-H), 0.87 (d, J = 6.2 Hz, 3H, 28- or 21-H), 3.72–4.98 (m, 9H, dioxolane and 22-H). ^{13}C NMR δ: 11.19, 11.97, 13.51, 15.74, 15.77, 21.03, 24.09, 27.74, 29.22, 31.00, 31.51, 33.43, 35.20, 36.80, 37.20, 39.26, 39.29, 39.35, 39.79, 41.16, 42.52, 49.52, 52.55, 53.26, 55.91, 64.08, 64.14, 64.25, 65.40, 71.63, 109.69, 109.71. HRMS calc. for $C_{32}H_{48}D_6O_5$: 524.4348; found: 524.4344. EI-MS$^{m/z}$ (%): 99 (100), 207 (6), 225 (19), 327 (44), 328 (42), 452 (45), 453 (49), 481 (6), 524 (34) $[M]^{\bullet+}$, 525 (36) $[M + H]^+$.

2.33. (22S,24R)-[26,27-$^2H_6$]24-Methyl-5α-cholestan-3,6-dion-22-ol (41)

Hydrochloric acid (0.5 ml, 6 mmol) was added to a solution of **40** (90 mg, 0.17 mmol) in acetone (50 ml). The mixture was kept at 60 °C for 1 h, treated with Et_3N, and the solvent was evaporated in vacuo. The residue was chromatographed on SiO_2 with hexane–EtOAc (10:1 ⇒ 1:2) to give diketone **41** (58 mg, 77%) as an oil. ^{1}H NMR δ: 0.71 (s, 3H, 18-H), 0.83 (d, J = 7.3 Hz, 3 H, 21- or 28-H), 0.90 (d, J = 6.6 Hz, 3H, 28- or 21-H), 3.77 (dt, J = 6.6, 0.7 Hz, 1H, 22-H). ^{13}C NMR δ: 11.23, 11.97, 12.57, 15.79, 15.82, 21.69, 23.94, 27.64, 31.56, 35.28, 36.98, 37.37, 38.07, 39.34, 39.38, 39.49, 41.26, 42.92, 46.58, 52.49, 53.40, 56.52, 57.47, 71.56, 209.10, 211.26. HRMS calc. for $C_{22}H_{33}O_3$: 345.2430; found: 345.2430. EI-MS$^{m/z}$ (%): 138 (26), 163 (11), 168 (11), 223 (10), 245 (13), 246 (12), 260 (10), 287 (33), 299 (9), 301 (7), 316 (100), 345 (5) $[M–C_7H_7D_6]^+$.

3. Results and discussion

There are two main strategies for transformation of the sterol's cyclic part (Δ^5-3β-alcohols) into that characteristic of brassinosteroids [1]. The most widely used approach involves *i*-steroidal rearrangement to afford 3α,5-cyclo derivatives, which allow easy introduction of the required functionality. Alternatively, hydroboration–oxidation of the Δ^5-double bond was employed in many syntheses of BS.

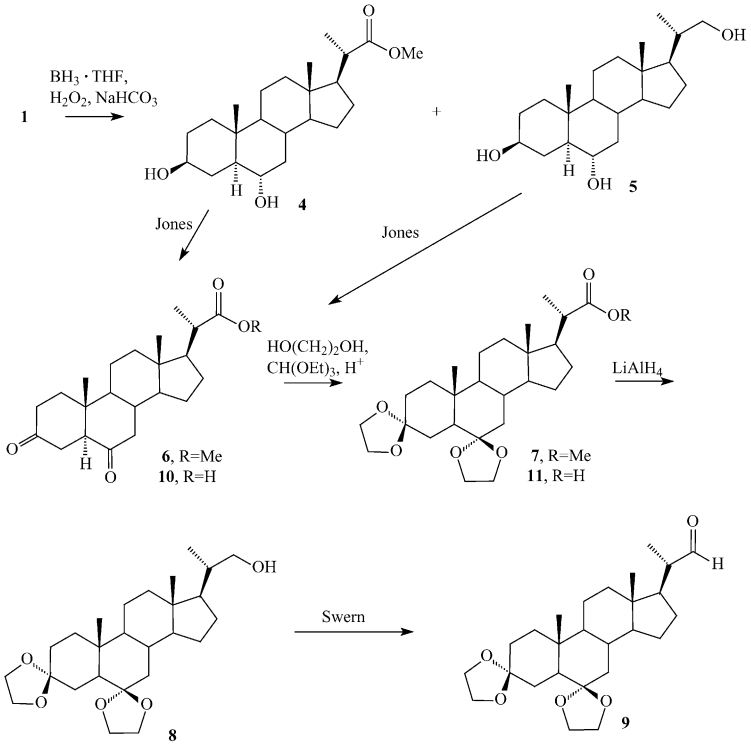


Scheme 1.

We experienced no difficulties with the realization of the first approach with ester **1** (Scheme 1). Aldehyde **3** was obtained in three steps from **1** in 63% total yield. An additional treatment of the crude mixture (consisting of 3α,5-cyclo derivative and 3β-methoxy ether **1a** [10]) with BH_3 was found to be desirable because large-scale separation of **3** and the by-product derived from **1a** proved to be rather difficult.

Accomplishment of the alternative strategy proved to be less straightforward. Hydroboration–oxidation of **1** gave a mixture of diol **4** and triol **5** (Scheme 2). To avoid isomerization at C-20 in **4**, the oxidation step was performed in the presence of $NaHCO_3$ instead of NaOH which is normally used for this purpose. Attempts to achieve the formation of diol **4** as the only product by reducing the amount of BH_3 gave poor results: both alcohols **4** and **5** were isolated from the reaction mixture as before in addition to unchanged **1**. However, the alcohols **4** and **5** were easily separable and could be transformed into aldehyde **9** using essentially the same protocol. Treatment of **4** with Jones reagent gave diketone **6**, whereas similar treatment of **5** proceeded with simultaneous oxidation at C-22 to afford the diketoacid **10**. Protection of the keto groups in **6** and **10** gave the expected bisdioxolane derivatives **7** and **11**, which on hydride reduction produced alcohol **8**. Swern oxidation of the latter resulted in the required aldehyde **9** in 49% total yield from **1**.

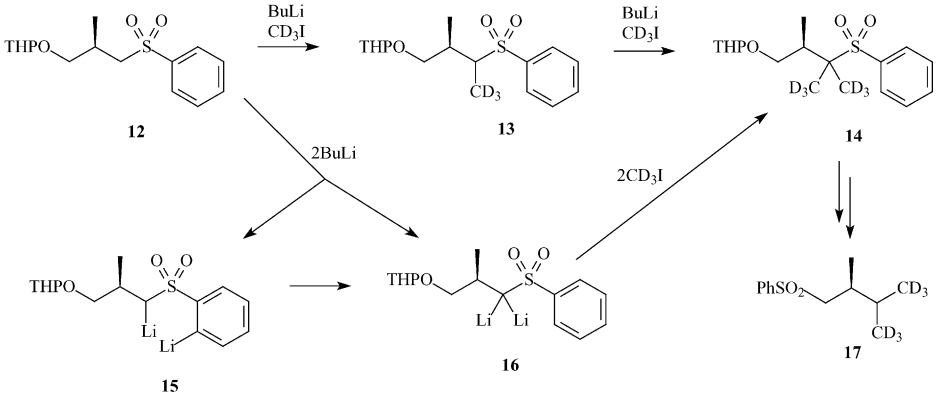
For biosynthetic studies, 24α-methyl derivatives containing 22α,23α-dihydroxy- (fully formed BS side chain) or 22α-monohydroxy fragments (side chain of some BS intermediates) were necessary. Both series of compounds could be prepared by addition of lithium salts of the phenyl sulfones to the corresponding C-22 aldehydes [5,11–13]. One of the problems encountered in the course of our previous investigations on BS side chain construction was the relatively low efficiency of the dialkylation procedure (**12** to **13** and



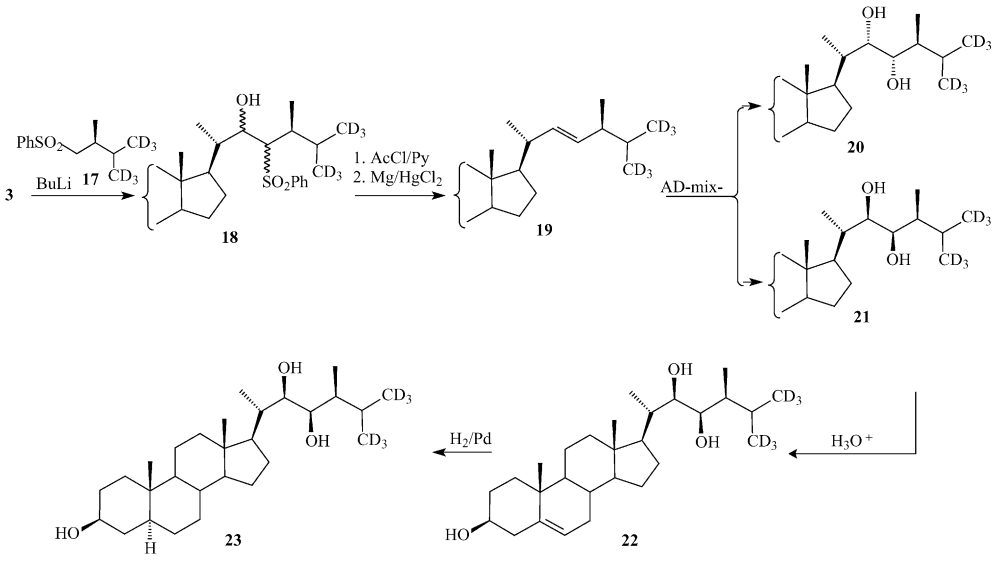
Scheme 2.

13 to **14**) in the course of preparation of phenyl sulfone **17** (Scheme 3). The usual protocol for α,α′-dialkylation of sulfones in the case of intermediate sulfone 14 synthesis would imply repeated treatment of the reaction mixture with BuLi and CD_3I [5,13]. It was supposed that compound **14** could be obtained from **12** more efficiently via the dianion **16**. Such thermodynamic anions are formed from sulfones and 2 eqv. of BuLi along with the kinetic dianions, like **15** [14]. However, the latter should be transformed into the thermodynamic dianion **16** prior to the alkylation. After many attempts with different temperatures, it was found that the optimal conditions for dianion **16** formation required keeping the mixture at −40 °C for 5–15 min. Subsequent single treatment of the reaction mixture with CD_3I led to the dialkylated product **14** in up to 76% yield. This compound was then transformed into the desired sulfone according to the previously published procedure [5]. The incorporation rate of deuterium atoms were calculated as described in [15] on the basis of MS data of (2*S*)-2,3-dimethylbutylphenyl sulfone **17**: 6-deuterated, 97.92%; 5-deuterated, 1.89%; 4-deuterated, 0.19%; 3-deuterated, not detected; 2-deuterated, not detected; 1-deuterated, not detected; non-deuterated, not detected.

The standard Julia olefination protocol involves acetylation of hydroxy sulfones followed by reduction of intermediate acetoxy sulfones with dissolving metals [16]. For Δ^{22}-steroids, yields vary from poor to acceptable [17], but the reduction step is somewhat cumbersome for practical use because of the necessity to work with sodium amalgam. An



Scheme 3.

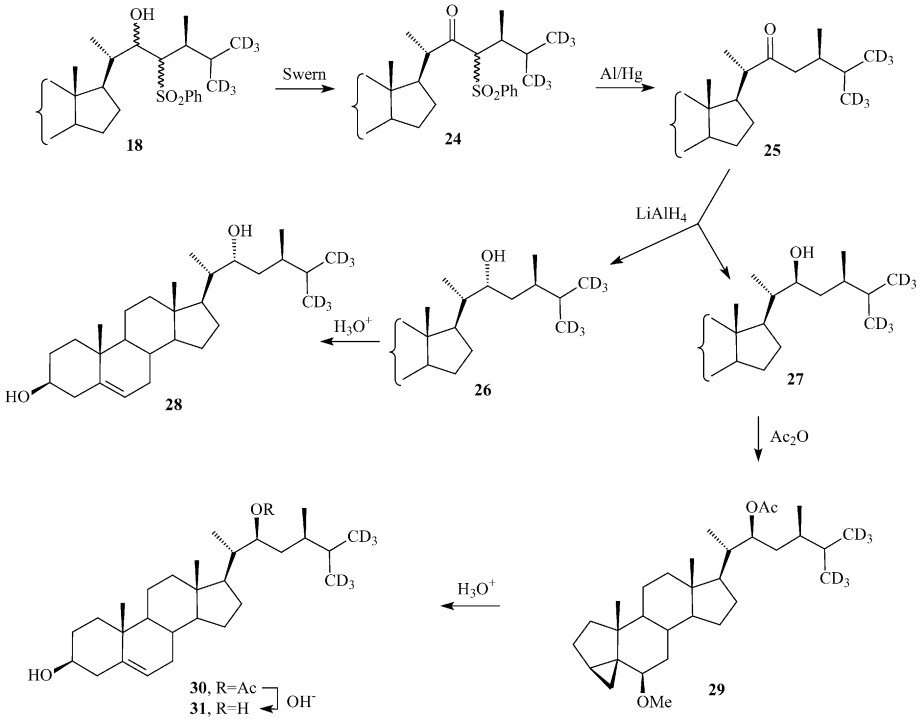


Scheme 4.

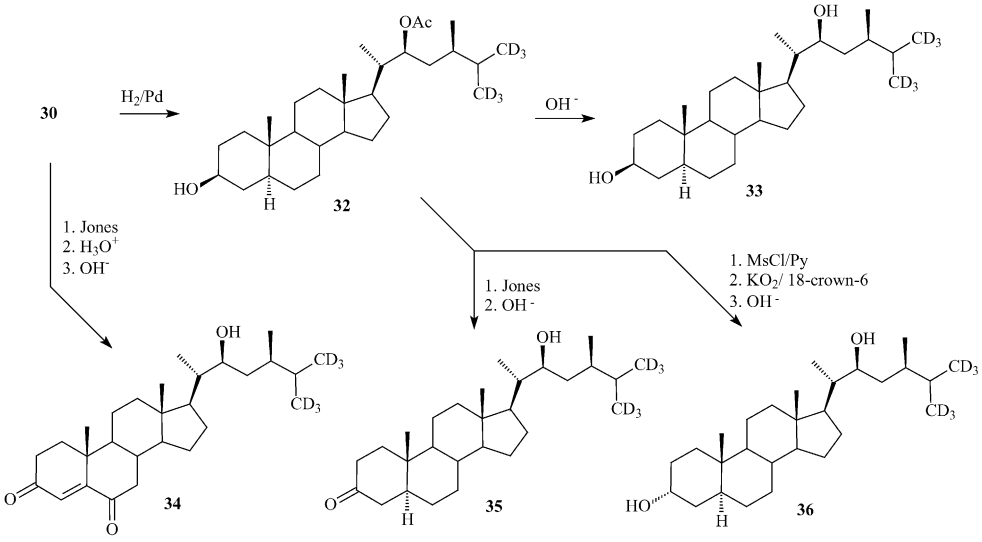
attempt to overcome this difficulty was undertaken by use of magnesium amalgam [18]. However, this replacement produced no increase in efficiency; the transformation of **18** into Δ^{22}-olefin **19** was performed in 48% yield (Scheme 4). Sharpless hydroxylation of **19** led to diol **21** as a major isomer along with the unnatural 22*S*,23*S*-diol **20**. The stereochemical assignments were based on the comparison of ^{1}H NMR signal pattern of **21** and **22** to those for non-deuterated compounds prepared earlier [12] and literature data on Sharpless hydroxylation of Δ^{22}-steroids [1].

Acid-catalyzed opening of the cyclopropane ring in **21** gave compound **22**. The latter contains the side chain of brassinolide and the cyclic part of campesterol from which brassinolide is biosynthesized in plants. The hydrogenation of **22** resulted in triol **23**, 6-deoxoteasterone, which is another intermediate in brassinosteroid biosynthesis [19].

Synthesis of derivatives containing a 22α-monohydroxy fragment was achieved according to the previously published method [6] involving Swern oxidation of **18**, desulfurization



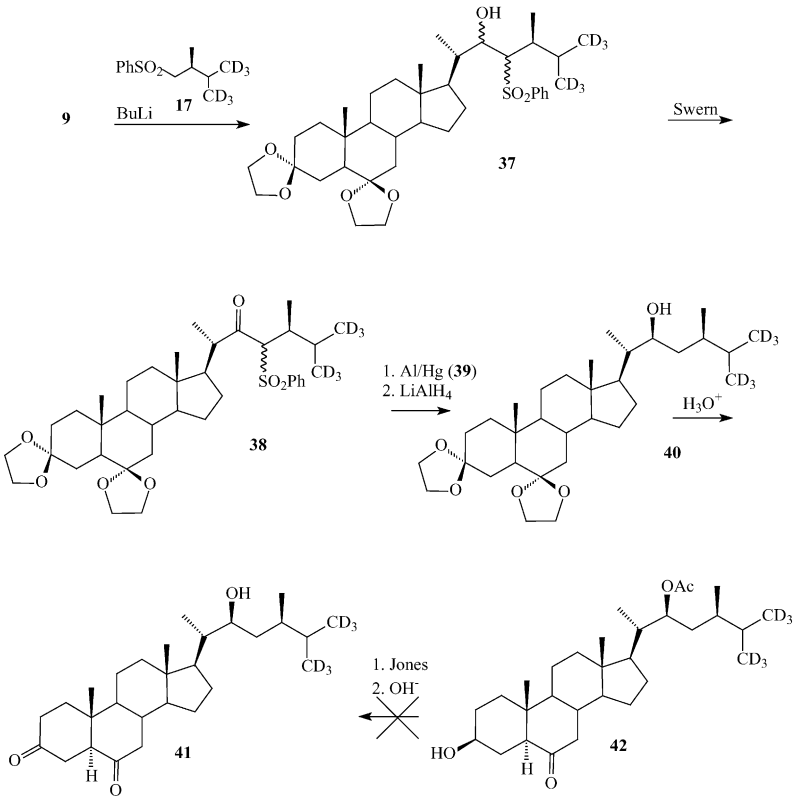
Scheme 5.



Scheme 6.

of **24**, and hydride reduction of **25** (Scheme 5). Special attention was paid to the purity of 22-ketones **24** and **25** containing a stereocenter at C-20, which is potentially prone to isomerization. However, our attempts to detect admixtures of any epimeric byproducts in **24** and **25** (^{1}H and ^{13}C NMR) gave no results. Evidently, the reaction conditions used for Swern oxidation of **18**, desulfurization of **24**, and hydride reduction of **25** were mild enough to avoid izomerization at C-20.

Stereochemistry at C-22 was assigned at the base of literature data [17,20], according to which reduction of 23-ketones proceeds with formation of 22α-alcohols as main isomers. Although steroids without a 23-hydroxy group in the side chain and bearing complete functions characteristic of BS in the cyclic part (2α-hydroxy, 3α-hydroxy, trans junction of A/B rings) have never been found in plants until now, the possible existence of compounds, such as natural BS or their



Scheme 7.

precursors/metabolites cannot be excluded. In this respect, compounds like **28** may be interesting both in the search for new BS and possible common points in the biosynthesis of BS, ecdysteroids [21] and oxysterols [22].

The same holds for the endione **34** (Scheme 6). Steroids with such a structural fragment in their AB-cycles are well-known as constituents of plants [23–25] and were prepared also as possible biosynthetic precursors of brassinolide [26]. 3,22-Difunctionalized derivatives **31**, **33**, **35** and **36** were obtained from the hydroxy acetate **30** according to a rather simple sequence of reactions. Recently, such compounds were found in cultured *Catharanthus roseus* cells and in *Arabidopsis* seedlings as brassinolide precursors of the novel subpathway via early C-22 oxidation [27].

The last part of the present investigation was connected with preparation of compound **41**, which bears a cyclic part of 3-oxoteasterone (Scheme 7). In our previous work [5] on 23-dehydroxy brassinosteroids synthesis, we had in our hands, cathasterone 23-acetate **42**, which could give the desired product in two steps via Jones oxidation followed by removal of the acetoxy group. However, attempts to carry out the last step gave only a complex mixture of compounds. The use of aldehyde **9** for this purpose solved the problem. The construction of the side chain was achieved by addition of the anion derived from sulfone **17** followed by Swern oxidation and the reduction steps. Acid-catalyzed deprotection of the bis-dioxolane **40** smoothly resulted in 3-oxocathasterone **41**.

Thus, a number of hexadeuterated biosynthetic precursors of brassinolide (known and suspected) have been prepared starting from 23,24-bisnorcholenic acid methyl ester for relevant studies.

Acknowledgements

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