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Preparation of (25*R*)- and (25*S*)-26-functionalized steroids as tools for biosynthetic studies of cholic acids

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Abstract

A new synthesis of both epimeric forms of 26-cholestanoic acids and 26-alcohols containing a 3β-hydroxy-Δ^5- or a Δ^4-3-keto-functionality in ring A is described starting from stigmasterol or (20*S*)-3β-acetoxy-pregn-5-en-20-carboxylic acid. The obtained compounds are useful as standards for studies of cholic acids. Construction of the side chain was achieved by linkage of steroidal 23-iodides to sulfones prepared from (2*R*)- and (2*S*)-3-hydroxy-2-methylpropanoates. Oxidation of intermediate 26-alcohols into the corresponding carboxylic acids ensuring preservation of stereochemistry at C-25 and functional groups in the cyclic part was achieved with sodium chlorite catalyzed by TEMPO and bleach.

Keywords: Cholic acids; Cholestenoic acids; Biosynthesis; 26-Hydroxycholesterol

1. Introduction

The past years have witnessed the growth in research of various aspects of bile acids [1–5]. Although the main intermediates of bile acid biosynthesis and metabolism were established quite a long time ago [6,7], many subtle details are still a matter of investigations. Deficiency of certain enzymes responsible for the transformation of cholesterol into bile acids may lead to accumulation of intermediate products having toxic effects and/or to the formation of unusual compounds, which are normally absent [8–12]. For instance, accumulation of 26-hydroxycholesterol in human atheroma may reflect reduced levels of oxysterol 7α-hydroxylase activity in human macrophages. The same enzyme determines the proportion of mono-, di-, and tri-hydroxy bile acids synthesized in the liver [13]. Liver dysfunction in Zellweger syndrome is attributed to overproduction and accumulation of cholestenoic acids [14]. Deficiency of 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase/isomerase results in chronic liver injury due to an overproduction or a low clearance of cholenoic acids [15–17]. 3-Oxo-Δ^4-steroid 5β-reductase deficiency may result in severe neonatal cholestasis associated with hypertyrosinemia [18,19].

An important part in the biosynthetic pathway is the transformation of C_{26}-cholestanoic acids by peroxisomal β-oxidation into the corresponding C_{24}-acids [20]. The availability of a set of differently functionalized C_{26}-cholestanoic acids is a necessary prerequisite for analytical determination of potential intermediates or metabolites [21–23]. We became interested in the stereoselective synthesis of both epimeric C_{26}-acids containing a 3β-hydroxy-Δ^5- or Δ^4-3-keto-functionality in ring A (cholestenoic acid derivatives like **1** and **2**) (Scheme 1). (25*R*)-Cholestenoic acid **1** itself is an intermediate compound for elimination of excessive cholesterol [24,25] and a signaling molecule for the regulation of

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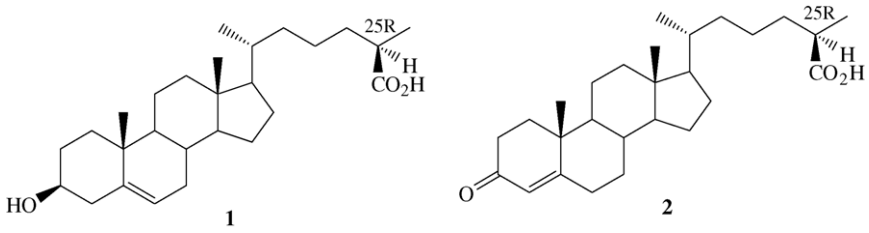


Scheme 1.

lipid metabolism via LXR_α [26]. C_{26}-acids with an enone function in ring A are not only intermediates of bile acid biosynthesis [27], but similar compounds of the stigmastane series were obtained from products of microbiological oxidation of β-sitosterol, e.g., with the genetically modified *Mycobacterium* sp. [28]. Earlier syntheses of a number of functionalized 26-cholestanoic acids should be mentioned in this connection [29–34], but the demand for new ones or the improved preparation of known compounds still remains.

2. Experimental

Melting points were taken on a Boetius micro-melting point apparatus and are uncorrected. IR spectra were recorded on an UR-20 spectrophotometer in KBr tablets. Optical rotations were measured on a JASCO J-20 polarimeter. ^{1}H and ^{13}C NMR spectra were taken on Bruker AC-200 (200 MHz for ^{1}H, 50 MHz for ^{13}C) and Bruker AVANCE 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) spectrometers using TMS as an internal standard in $CDCl_3$ (if not stated otherwise). The exact 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. (20*S*)-3β-Acetoxy-pregn-5-en-20-carboxylic acid was a gift from Steraloids Inc. (Newport, RI, USA). Reactions were monitored by TLC using aluminium or plastic sheets precoated with silica gel 60 F_{254} (Merck Art. 5715). Column chromatography was carried out on silica gel 60 (Merck Art. 7734).

2.1. 3β-Acetoxy-23,24-bisnorchol-5-en-22-ol (9)

$BH_3{\cdot}Me_2S$ (2 M solution in THF, 200 ml) was added dropwise to a solution of (20*S*)-3β-acetoxy-pregn-5-en-20-carboxylic acid 7 (130 g, 335 mmol) in THF (700 ml) at −78 °C under argon. The reaction mixture was then stirred at −78 °C for 30 min. After addition of methanol (150 ml), the reaction mixture was allowed to warm to room temperature, and the solvents were removed in vacuo. The residue was dissolved in EtOAc, and the solution was passed rapidly through a pad of SiO_2 with EtOAc as eluent. The filtrate was chromatographed on SiO_2 to give alcohol **9** (80 g, 64%). Mp 155–157 °C (EtOAc) (lit. mp 143–148 °C [35], 154–155 °C [36]). $[\alpha]_D = -56.4$ (c 0.004, $CHCl_3$). IR (cm^{-1}): 2955, 1755, 1490, 1460, 1390, 1380, 1280, 1050, 990. ^{1}H NMR δ: 0.70 (s, 3H, 18-H), 1.02 (s, 3H, 19-H), 2.04 (s, 3H, OAc), 3.36 (m, 2H, 22-H), 4.50–4.72 (m, 1H, 3-H), 5.37 (d, *J* = 4.5 Hz, 1H, 6-H). ^{13}C NMR δ: 11.9, 16.8, 19.3, 21.0, 21.4, 24.4, 27.7, 31.9, 36.6, 37.0, 38.1, 38.7, 39.6, 42.4, 50.0, 52.4, 56.3, 56.4, 67.9, 74.0, 122.6, 139.7, 170.6. HRMS calc. for $C_{22}H_{35}O$ ($[M - AcOH]^+$) 315.2688; found: 315.2687; EIMS *m/z*: 328 (12), 314 ($[M - AcOH]^+$, 100), 299 (10), 255 ($[M - AcOH–C_3H_7O$ (fission C-17/C-20)]$^+$, 5), 213 (6), 193 (12), 159 (5), 147 (12), 133 (7), 119 (7), 107 (10), 93 (7), 81 (9).

2.2. 6β-Methoxy-3α,5-cyclo-5α-23,24-bisnorcholan-22-ol (10)

Starting with *(20S)-6β-methoxy-3α,5-cyclo-5α-pregnan-20-carboxylic acid* **8** [48], the title compound was isolated as an oil according to the procedure described above in 95% yield. ^{1}H NMR δ: 0.38 (dd, *J* = 7.9, 4.9 Hz, 1H, 3-H), 0.61 (t, *J* = 4.8 Hz, 1H, 4-H), 0.69 (s, 3H, 18-H), 0.82 (d, *J* = 6.1 Hz, 3H, 21-H), 0.98 (s, 3H, 19-H), 2.73 (t, *J* = 2.4 Hz, 1H, 6-H), 3.28 (s, 3H, OMe), 3.29 (dd, *J* = 10.0, 7.5 Hz, 1H, 22-H), 3.60 (dd, *J* = 10.0, 3.0 Hz, 1H, 22-H). ^{13}C NMR δ: 12.1, 12.9, 16.5, 19.0, 21.2, 22.5, 24.0, 24.9, 27.5, 30.2, 33.1, 34.5, 35.0, 38.5, 39.9, 42.6, 43.1, 47.7, 52.4, 56.0, 56.3, 67.6, 82.1.

2.3. Tosylation of (9)

2.3.1. Variant A

A mixture of alcohol **9** (21 g, 56 mmol), *p*-toluensulfonylchloride (19 g, 100 mmol), and pyridine (100 ml) was kept at room temperature for 12 h. Then, water (450 ml) was slowly added to the reaction mixture, and it was extracted with $CHCl_3$. The combined organic fractions were dried (Na_2SO_4), and the solvent was evaporated in vacuo. The residue was chromatographed on SiO_2 to give: (a) *3β-acetoxy-22-chloro-23,24-bisnorchol-5-ene* **13** (4.8 g, 22%). Mp 143–144 °C (hexane–EtOAc). IR (cm^{-1}): 2950, 1755, 1480, 1450, 1380, 1385, 1260, 1045. ^{1}H NMR δ: 0.71 (s, 3H, 18-H), 1.02 (s, 3H, 19-H), 1.10 (d, *J* = 6.5 Hz, 3H, 21-H), 2.04 (s, 3H, OAc), 2.32 (d, *J* = 8.0 Hz, 2H, 4-H), 3.42 (dd, *J* = 11.0, 6.0 Hz, 1H, 22-H), 3.60 (dd, *J* = 11.0, 2.5 Hz, 1H, 22-H), 4.61 (m, 1H, 3-H), 5.39 (d, *J* = 5.0 Hz, 1H, 6-H). ^{13}C NMR δ: 12.0, 17.6, 19.3, 21.0, 21.4, 24.2, 27.5, 27.7, 31.8, 31.9, 36.5, 37.0, 38.1, 38.3, 39.4, 42.3, 49.9, 52.4, 52.8, 56.3, 73.9, 122.5, 139.6, 170.5. HRMS calc. for $C_{24}H_{39}ClO_2$ (M + 2H) 394.2638; found: 394.2630;

EIMS *m/z*: 394 ([*M* + 2H]$^+$, 0.6), 332 ([*M* − AcOH]$^+$, 100), 317 ([*M* − AcOH–CH_3]$^+$, 12), 224 (11), 211 (14), 159 (6), 147 (14), 107 (12); (b) *3β-acetoxy-22-toluenesulfonyloxy-23,24-bisnorchol-5-ene* **11** (19.6 g, 57 %). Mp 126–128 °C (MeOH) (lit. mp 108–110 °C [36]). $[\alpha]_D = -10.1$ (c 0.005, $CHCl_3$). IR (cm^{-1}): 2950, 2915, 1750, 1615, 1375, 1260, 1200, 1190, 1110, 1045. ^{1}H NMR *δ*: 0.66 (s, 3H, 18-H), 1.00 (s, 3H, 19-H), 2.04 (s, 3H, OAc), 2.46 (s, 3H, OTs), 3.78 (dd, *J* = 9.5, 6.5 Hz, 1H, 22-H), 3.98 (dd, *J* = 9.5, 3 Hz, 1H, 22-H), 4.60 (m, 1H, 3-H), 5.37 (d, *J* = 4.0 Hz, 1H, 6-H), 7.34 (d, *J* = 8.0 Hz, 2H, OTs), 7.80 (d, *J* = 8.0 Hz, 2H, OTs). ^{13}C NMR *δ*: 11.8, 16.9, 19.3, 20.9, 21.4, 21.7, 24.3, 27.4, 27.7, 31.8, 36.2, 36.6, 37.00, 38.1, 39.4, 42.4, 49.9, 51.8, 56.3, 73.9, 75.6, 122.5, 127.9, 129.8, 133.1, 139.7, 144.6, 170.6. HRMS calc. for $C_{29}H_{41}O_3S$ (*M* − AcOH) 469.2776; found: 469.2788. EIMS *m/z*: 468 ([*M* − AcOH]$^+$, 100), 453 ([*M* − AcOH–CH_3]$^+$, 6), 296 ([*M* − AcOH–TsOH]$^+$, 24), 281 (16), 255 ([$C_{13}H_{18}O_3S$ (C/D ring fission) + H]$^+$, 7), 213 ([$C_{10}H_{13}O_3S$ (fission C-17/C-20)]$^+$, 11), 188 (10), 175 (13), 145 (18).

2.3.2. Variant B

A mixture of alcohol **9** (70 g, 0.19 mol), *p*-toluenesulfonylchloride (95 g, 0.5 mol), $CHCl_3$ (100 ml), and pyridine (70 ml) was kept at room temperature for 2 days. Then, water was added, and the mixture was extracted with $CHCl_3$. The combined chloroform fractions were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified by column chromatography on SiO_2 to give tosylate **11** (80 g, 81%).

*2.4. 6β-Methoxy-22-toluenesulfonyloxy-3α,5-cyclo-5α-23,24-bisnorcholane (**12**)*

The title compound was isolated as an oil in 76% yield starting with alcohol **10** according to the procedure described above for tosylation of alcohol **9**. ^{1}H NMR *δ*: 0.42 (dd, *J* = 7.9, 4.9 Hz, 1H, 3-H), 0.63 (t, *J* = 4.8 Hz, 1H, 4-H), 0.66 (s, 3H, 18-H), 0.97 (d, *J* = 6.7 Hz, 3H, 21-H), 1.00 (s, 3H, 19-H), 2.44 (s, 3H, OTs), 2.75 (t, *J* = 2.4 Hz, 1H, 6-H), 3.30 (s, 3H, OMe), 3.78 (dd, *J* = 9.2, 6.1 Hz, 1H, 22-H), 3.95 (dd, *J* = 9.2, 3.0 Hz, 1H, 22-H), 7.39 (d, 2H, *J* = 8.5 Hz, OTs), 7.77 (d, 2H, *J* = 8.5 Hz, OTs). ^{13}C NMR *δ*: 12.2, 13.0, 16.8, 19.2, 21.4, 21.6, 22.7, 24.1, 24.9, 27.5, 30.5, 33.3, 35.0, 35.2, 36.2, 39.9, 42.8, 43.3, 47.9, 51.9, 56.1, 56.5, 75.7, 82.3, 127.9, 129.7, 133.1, 144.5.

*2.5. 3β-Acetoxy-23,24-bisnorchol-5-en-22-nitrile (**14**)*

A mixture of tosylate **11** (80 g, 0.15 mol) and NaCN (11 g, 0.2274 mol) in DMF (400 ml) was heated at 80 °C for 5 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with $CHCl_3$. The combined organic fractions were partly evaporated in vacuo and filtered through a short column of SiO_2. The filtrate was evaporated in vacuo, and the residue was purified by column chromatography on SiO_2 to give nitrile **14** (56 g, 96%). Mp 180–182 °C (hexane–EtOAc) (lit. mp 186–187 °C [37], 179–181 °C [38]). IR (cm^{-1}): 2945, 2910, 1740, 1475, 1375, 1260, 1045. ^{1}H NMR *δ*: 0.68 (s, 3H, 18-H), 1.00 (s, 3H, 19-H), 1.15 (d, *J* = 6.5 Hz, 3H, 21-H), 2.02 (s, 3H, OAc), 4.58 (m, 1H, 3-H), 5.35 (d, *J* = 5 Hz, 1H, 6-H). ^{13}C NMR *δ*: 11.9, 19.7, 20.9, 21.4, 24.1, 24.8, 27.7, 28.0, 31.7, 33.6, 36.5, 36.9, 38.0, 39.3, 42.4, 49.8, 54.8, 56.4, 73.8, 119.0, 122.4, 139.6, 170.5. HRMS calc. for $C_{25}H_{39}NO_2$ (M + 2H) 385.2980; found: 385.2979. EIMS *m/z*: 385 ([*M* + 2H]$^+$, 0.4), 323 ([*M* − AcOH]$^+$, 100), 308 ([*M* − AcOH–CH_3]$^+$, 15), 215 (11), 202 (9), 145 (9), 121 (13), 107 (9), 91 (8).

*2.6. Synthesis of (**14**) from chloride (**13**)*

According to the procedure described above for the transformation of tosylate **11**, but under more severe conditions (100 °C, 10 h), the nitrile **14** was prepared from chloride **13** in 84% yield.

*2.7. 6β-Methoxy-3α,5-cyclo-5α-23,24-bisnorcholan-22-nitrile (**15**)*

The title compound was prepared in 89% yield as an oil starting from **12** according to the procedure described for the transformation of tosylate **11** to nitrile **14**. ^{1}H NMR *δ*: 0.43 (dd, *J* = 7.9, 4.9 Hz, 1H, 3-H), 0.64 (t, *J* = 4.7 Hz, 1H, 4-H), 0.73 (s, 3H, 18-H), 1.01 (s, 3H, 19-H), 1.15 (d, *J* = 6.7 Hz, 3H, 21-H), 2.20 (dd, *J* = 16.5, 6.7 Hz, 1H, 22-H), 2.32 (dd, *J* = 16.5, 4.3 Hz, 1H, 22-H), 2.73 (t, *J* = 2.4 Hz, 1H, 6-H), 3.32 (s, 3H, OMe). ^{13}C NMR *δ*: 12.3, 13.1, 19.2, 19.3, 21.4, 22.6, 24.0, 24.8, 24.9, 28.1, 30.5, 33.3, 33.5, 35.0, 35.1, 39.8, 42.8, 43.3, 47.8, 54.9, 56.2, 56.6, 82.3, 119.0.

*2.8. 3β-Hydroxy-23,24-bisnornorchol-5-en-22-carbaldehyde (**16**)*

DIBAL-H (1 M solution in toluene, 170 ml) was added dropwise to a stirred solution of nitrile **14** (28 g, 73 mmol) in absolute toluene (300 ml) at −78 °C under argon. After 30 min, EtOAc (50 ml) and water (100 ml) were added to the reaction mixture, and it was allowed to warm to room temperature. The reaction mixture was diluted with water (200 ml) and concentrated HCl (80 ml) and extracted with EtOAc. The combined organic fractions were washed with saturated Na_2CO_3 solution and water, dried (Na_2SO_4), and evaporated to give the crude aldehyde **16** (28 g) as an oil. IR (cm^{-1}): 2945, 1735, 1470, 1380, 1055. ^{1}H NMR *δ*: 0.72 (d, *J* = 6.0 Hz, 3H, 21-H), 1.02 (s, 3H, 18-H), 3.52 (m, 1H, 3-H), 5.35 (d, *J* = 5.0 Hz, 1H, 6-H), 9.76 (d, *J* = 2.0 Hz, 1H, C*H*O). ^{13}C NMR *δ*: 11.9, 19.4, 20.0, 21.0, 24.2, 28.5, 31.6, 31.8, 36.5, 37.2, 39.6, 42.3, 42.5, 50.0, 50.9, 55.8, 56.7, 71.7, 76.4, 77.2, 96.1, 121.5, 140.8, 203.5.

2.9. 6β-Methoxy-3α,5-cyclo-5α-23,24-bisnorcholan-22-carbaldehyde (17)

The title compound was prepared in 82% yield as an oil starting from nitrile **15** according to the procedure described for the transformation of nitrile **14** into aldehyde **16**. ^{1}H NMR δ: 0.40 (dd, J = 7.9, 4.9 Hz, 1H, 3-H), 0.62 (t, J = 4.7 Hz, 1H, 4-H), 0.74 (s, 3H, 18-H), 0.99 (d, J = 6.5 Hz, 3H, 21-H), 1.00 (s, 3H, 19-H), 2.15 (dd, J = 13.4, 3.1 Hz, 1H, 22-H), 2.44 (dd, J = 13.4, 1.3 Hz, 1H, 22-H), 2.75 (t, J = 2.4 Hz, 1H, 6-H), 3.30 (s, 3H, OMe), 9.72 (dd, J = 3.1, 1.3 Hz, 1H, C*H*O). ^{13}C NMR δ: 12.3, 13.1, 19.3, 20.0, 21.5, 22.7, 24.1, 25.0, 28.6, 30.5, 31.9, 33.4, 35.1, 35.2, 40.1, 43.0, 43.4, 48.0, 50.9, 56.0, 56.5, 56.6, 82.3, 203.6.

2.10. 24-Norchol-5-en-3β,23-diol (18)

$LiAlH_4$ (5.7 g, 149.88 mmol) was added to a stirred solution of aldehyde **16** (28 g) in THF (400 ml), and the stirring was continued for 30 min. The excess of $LiAlH_4$ was decomposed with 15% NaOH aqueous solution, and the obtained precipitate was filtered off. The filtrate was evaporated, and the residue was purified by column chromatography on SiO_2 to give diol **18** (22 g, 87% from **14**). Mp 144–147 °C (MeOH). IR (cm^{-1}): 2945, 1480, 1390, 1065. ^{1}H NMR δ: 0.69 (s, 3H, 18-H), 0.96 (d, J = 6.5 Hz, 3H, 21-H), 1.01 (s, 3H, 19-H), 3.44–3.80 (m, 3H, 3α- and 23-H), 5.36 (d, J = 5.0 Hz, 1H, 6-H). ^{13}C NMR δ: 11.8, 18.9, 19.4, 21.1, 24.3, 28.4, 31.6, 31.9, 32.9, 36.5, 37.3, 39.0, 39.8, 42.3, 42.4, 50.1, 56.4, 56.8, 60.9, 71.8, 121.7, 140.8. HRMS calc. for $C_{23}H_{38}O_2$: 346.2874; found: 346.2872. EIMS *m*/*z*: 346 ([*M*]$^+$, 100), 331 ([$M - CH_3$]$^+$, 21), 328 ([$M - H_2O$]$^+$, 38), 313 ([$M - H_2O$–CH_3]$^+$, 21), 273 ([$M - C_4H_9O$ (fission C-17/C-20)]$^+$, 15), 261 (27), 255 ([$M - C_4H_9O$ (fission C-17/C-20)-H_2O]$^+$, 16), 235 (36), 213 (24), 161 (19), 145 (24), 107 (32), 81 (27).

2.11. 6β-Methoxy-3α,5-cyclo-5α-24-norcholan-23-ol (19)

The title compound was prepared in 86% yield as an oil starting from aldehyde **17** according to the procedure described for the transformation of aldehyde **16** into alcohol **18**. ^{1}H NMR δ: 0.42 (dd, J = 7.9, 4.9 Hz, 1H, 3-H), 0.63 (t, J = 4.7 Hz, 1H, 4-H), 0.72 (s, 3H, 18-H), 0.92 (d, J = 6.1 Hz, 3H, 21-H), 1.01 (s, 3H, 19-H), 2.76 (t, J = 2.4 Hz, 1H, 6-H), 3.31 (s, 3H, OMe), 3.65 (m, 2H, 23-H). ^{13}C NMR δ: 12.2, 13.0, 18.8, 19.3, 21.5, 22.7, 24.1, 24.9, 28.4, 30.4, 32.9, 33.3, 35.0, 35.2, 39.0, 40.2, 42.8, 43.3, 48.0, 56.5, 56.5, 60.9, 82.4.

2.12. Tosylation of (18)

A mixture of diol **18** (22 g, 64 mmol), $CHCl_3$ (200 ml), pyridine (10 ml), and *p*-toluenesulfonylchloride (20 g, 105 mmol) was kept for 2 days at room temperature. Then, the reaction mixture was diluted with water and extracted with EtOAc. The combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by column chromatography on SiO_2 to give: (a) 24-norchol-5-en-3β,23-diol 3β,23-ditosylate **20** (6.6 g, 16 %). Mp 119–120 °C (hexane–EtOAc). ^{1}H NMR δ: 0.61 (s, 3H, 18-H), 0.84 (d, J = 6.5 Hz, 3H, 21-H), 0.96 (s, 3H, 19-H), 2.46 (s, 6H, OTs), 4.08 (m, 2H, 23-H), 4.32 (m, 1H, 3-H), 5.30 (brs, 1H, 6-H), 7.34 (dd, J = 8.0, 2.0 Hz, 4H, OTs), 7.80 (d, J = 8.0 Hz, 4H, OTs). ^{13}C NMR δ: 11.7, 18.4, 19.1, 20.9, 21.6, 24.1, 28.0, 28.6, 31.7, 32.6, 34.8, 36.3, 36.8, 38.8, 39.5, 42.4, 49.8, 55.9, 56.5, 63.9, 68.9, 82.3, 123.4, 127.6, 127.8, 129.76, 129.80, 133.3, 134.7, 138.8, 144.4, 144.6. HRMS calc. for $C_{30}H_{42}O_3S$ (M – TsOH): 482.2855; found: 482.2859. EIMS *m*/*z*: 482 ([M – TsOH]$^+$, 100), 467 ([M – TsOH–CH_3]$^+$, 19), 361 (29), 310 ([M – 2TsOH]$^+$, 14), 295 ([M – 2TsOH–CH_3]$^+$, 16), 255 ([M – TsOH–$C_{11}H_{15}O_3S$ (fission C-17/C-20)]$^+$, 40), 213 (32), 189 (47), 172 ([TsOH]$^+$, 73), 145 (59), 107 (74), 91 ([$C_6H_4CH_3$]$^+$, 99); (b) 24-norchol-5-en-3β,23-diol 23-tosylate **21** (18.5 g, 58%). Mp 114–118 °C (MeOH–EtOAc). $[\alpha]_D = -33.4$ (c 0.004, $CHCl_3$). IR (cm^{-1}): 1050, 1060, 1105, 1180, 1195, 1365, 1470, 1605, 2945. ^{1}H NMR δ: 0.64 (s, 3H, 18-H), 0.84 (d, J = 6.5 Hz, 3H, 21-H), 0.98 (s, 3H, 19-H), 2.45 (s, 3H, OTs), 3.53 (m, 1H, 3-H), 4.08 (m, 2H, 23-H), 5.34 (d, J = 5.0 Hz, 1H, 6-H), 7.36 (d, J = 8.0 Hz, 2H, OTs), 7.80 (d, J = 8.0 Hz, 2H, OTs). ^{13}C NMR δ: 11.8, 18.4, 19.4, 21.0, 21.6, 24.2, 28.1, 31.6, 31.8, 32.6, 34.8, 36.4, 37.2, 39.7, 42.2, 42.4, 50.0, 55.9, 56.6, 69.0, 71.7, 121.5, 127.9, 129.8, 133.2, 140.8, 144.6. HRMS calc. for $C_{30}H_{42}O_3S$ ($M - H_2O$): 482.2839; found: 482.2855. EIMS *m*/*z*: 501 ([M + H]$^+$, 99), 482 ([$M - H_2O$]$^+$, 100), 467 ([$M - H_2O$–CH_3]$^+$, 24), 415 (18.5), 389 (31), 328 ([M – TsOH]$^+$, 21), 295 (26), 255 ([$M - H_2O$–$C_{11}H_{15}O_3S$ (fission C-17/C-20)]$^+$, 29), 246 (36), 213 (50), 172 ([TsOH]$^+$, 36), 159 (39), 145 (46).

2.13. 6β-Methoxy-3α,5-cyclo-5α-24-norcholan-23-ol 23-tosylate (22)

The title compound was prepared in 71% yield as an oil starting from alcohol **19** according to the procedure described for the tosylation of diol **18**. ^{1}H NMR δ: 0.42 (dd, J = 8.0, 4.9 Hz, 1H, 3-H), 0.63 (t, J = 4.8 Hz, 1H, 4-H), 0.66 (s, 3H, 18-H), 0.97 (d, J = 6.7 Hz, 3H, 21-H), 1.00 (s, 3H, 19-H), 2.44 (s, 3H, OTs), 2.75 (t, J = 2.4 Hz, 1H, 6-H), 3.31 (s, 3H, OMe), 4.05 (m, 2H, 22-H), 7.34 (d, J = 8.5 Hz, 2H, OTs), 7.78 (d, J = 8.5 Hz, 2H, OTs). ^{13}C NMR δ: 12.1, 13.0, 18.4, 19.2, 21.4, 21.8, 22.9, 24.0, 24.9, 28.1, 30.4, 32.8, 33.3, 34.8, 35.0, 35.2, 40.1, 42.8, 43.2, 47.9, 56.1, 56.4, 56.5, 67.0, 82.3, 127.9, 129.8, 133.3, 144.6.

2.14. 23-Iodo-24-norchol-5-en-3β-ol (23)

A mixture of tosylate **21** (18.5 g, 37 mmol), KI (53 g, 319 mmol), and acetone (200 ml) was refluxed for 20 h. After cooling, the reaction mixture was partly evaporated

in vacuo, diluted with water, and extracted with $CHCl_3$. The combined extracts were dried (Na_2SO_4), and the solvents were evaporated in vacuo. The residue was purified by column chromatography on SiO_2 to give iodide **23** (15 g, 89%). Mp 130–132 °C (MeOH). IR (cm^{-1}): 2945, 2910, 2870, 2855, 1645, 1480, 1390, 1230, 1200, 1065. ^1H NMR δ: 0.69 (s, 3H, 18-H), 0.93 (d, J = 6.0 Hz, 3H, 21-H), 1.00 (s, 3H, 19-H), 3.10 (m, 1H, 23-H), 3.31 (m, 1H, 23-H), 3.52 (m, 1H, 3-H), 5.36 (d, J = 5.0 Hz, 1H, 6-H). ^{13}C NMR δ: 5.1, 11.9, 17.9, 19.4, 21.0, 24.2, 28.1, 31.6, 31.8, 36.5, 36.9, 37.2, 39.7, 40.3, 42.3, 42.4, 50.0, 55.6, 56.7, 71.7, 121.6, 140.7. HRMS calc. for $C_{23}H_{37}OI$: 456.1889; found: 456.1891. EIMS *m/z*: 456 ([*M*]$^+$, 100), 442 (25), 438 ([$M - H_2O$]$^+$, 31), 423 ([$M - H_2O$–CH_3]$^+$, 22), 371 (21), 345 (27), 331 (7), 273 ([$M - C_4H_8I$ (fission C-17/C-20)]$^+$, 5), 255 ([$M - H_2O$–C_4H_8I (fission C-17/C-20)]$^+$, 8), 213 (11), 145 (15), 107 (19).

*2.15. 23-Iodo-6β-methoxy-3α,5-cyclo-5α-24-norcholane (**24**)*

The title compound was prepared in 98% yield starting from tosylate **22** according to the procedure described for the transformation of tosylate **21** into iodide **23**. Mp 93–95 °C (hexane–EtOAc) (lit. mp 104–105 °C [39]). IR (cm^{-1}): 2950, 2870, 1475, 1455, 1385, 1330, 1200, 1160, 1100, 1015. ^1H NMR δ: 0.44 (dd, J = 8.0, 5.5 Hz, 1H, 3-H), 0.66 (t, J = 4.5 Hz, 1H, 4-H), 0.74 (s, 3H, 18-H), 0.93 (d, J = 6.0 Hz, 3H, 21-H), 1.03 (s, 3H, 19-H), 2.78 (t, J = 2.7 Hz, 1H, 6-H), 3.02–3.38 (m, 2H, 23-H), 3.33 (s, 3H, OMe). ^{13}C NMR δ: 5.1, 12.2, 13.0, 17.8, 19.3, 21.4, 22.7, 24.1, 24.9, 28.2, 30.4, 33.3, 35.0, 35.2, 37.2, 40.2, 40.4, 42.9, 43.3, 47.9, 55.8, 56.4, 56.5, 82.3. HRMS calc. for $C_{24}H_{39}OI$: 470.2047; found: 470.2046. EIMS *m/z*: 470 ([*M*]$^+$, 70), 455 ([$M - CH_3$]$^+$, 57), 438 ([M – MeOH]$^+$, 100), 423 ([M – MeOH–CH_3]$^+$, 13), 415 (96), 317(11), 255 (7), 213 (8), 145 (18), 95 (22).

*2.16. (25R)-24-Phenylsulfonylcholest-5-en-3β,26-diol 26-(2′-tetrahydropyranyl) ether (**26**)*

A 2.5 M BuLi (11.4 ml, 28.5 mmol) solution was added to a stirred solution of $^iPr_2NH$ (4 ml, 28.5 mmol) in THF (15 ml) at −10 °C. The mixture was warmed to 0 °C and stirred for 5 min. A solution of *2-[(2R)-2-methyl-3-phenylsulfonylpropyloxy]tetrahydro-2H-pyran* **25** (5.1 g, 17.1 mmol) (prepared according to [40]) in THF (20 ml) was added to the obtained solution of $^iPr_2NLi$ at −78 °C. This was followed by the addition of a solution of steroidal iodide **23** (2.6 g, 5.7 mmol) in THF (15 ml) and HMPA (5.8 ml) over 10 min. After stirring at −78 to −65 °C for 20 min, the reaction mixture was allowed to warm to room temperature and left overnight. Then, saturated NH_4Cl was added, and the mixture was extracted with $CHCl_3$. The organic extracts were dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by column chromatography on SiO_2 to give sulfone **26** (2.9 g, 82%) as an oil. IR (cm^{-1}): 2945, 2875, 1460, 1390, 1310, 1210, 1150, 1085, 1070, 1040. ^1H NMR δ: 3.08–3.82 (m, 3H, 26-H and OTHP), 4.36–4.68 (m, 1H, OTHP), 5.35 (d, J = 4.5 Hz, 1H, 6-H), 7.58 (m, 3H, Ph), 7.90 (m, 2H, Ph). HRMS calc. for $C_{33}H_{50}O_4S$ ([M – THP + 1H]$^+$): 542.3430; found: 542.3448. EIMS *m/z*: 542 ([M – THP + 1H]$^+$, 22), 524 ([M – THPOH]$^+$, 76), 509 ([M – THPOH–CH_3]$^+$, 20), 431 (15), 383 ([M – THPOH–SO_2Ph]$^+$, 16), 352 ([$C_{19}H_{29}O_4S$ (fission C-17/C-20)-1H]$^+$, 7), 271 (20), 255 ([$M - C_{19}H_{29}O_4S$ (fission C-17/C-20)-H_2O]$^+$, 38), 225 (30), 213 (45), 143 ([SO_2Ph + 2H]$^+$, 56), 84 ([THP–H]$^+$, 64%).

*2.17. (25R)-6β-Methoxy-24-phenylsulfonyl-3α,5-cyclo-5α-cholestan-26-ol 26-(2′-tetrahydropyranyl) ether (**27**)*

The title compound was prepared in 78% yield as an oil from iodide **24** and sulfone **25** according to the procedure described above for the preparation of sulfone **26**. IR (cm^{-1}): 1030, 1080, 1145, 1200, 1300, 1380, 1445, 2875, 2945. ^1H NMR δ: 0.42 (dd, J = 8.0, 5.5 Hz, 1H, 4-H), 2.76 (s, 1H, 6-H), 3.30 (s, 3H, OMe), 3.36–3.80 (m, 4H, 26-H and OTHP), 4.34–4.66 (m, 1H, OTHP), 7.48–7.68 (m, 3H, Ph), 7.90 (m, 2H, Ph). HRMS calc. for $C_{39}H_{60}O_5S$ [*M*]$^+$: 640.4163; found: 640.4161. EIMS *m/z*: 640 ([*M*]$^+$, 12), 625 ([$M - CH_3$]$^+$, 9), 608 ([$M - CH_3OH$]$^+$, 6), 585 (16), 556 ([M – THP + H]$^+$, 16), 539 (10), 524 ([M – THP–CH_3O]$^+$, 35), 498 ([$M - SO_2Ph$–H]$^+$, 8), 383 (8), 255 ([$M - CH_3OH$–$C_{19}H_{29}O_4S$ (fission C-17/C-20)]$^+$, 21), 213 (12), 143 (12), 95 (15), 85 ([THP–H]$^+$, 100).

*2.18. (25S)-Cholest-5-en-3β,26-diol 26-(2′-tetrahydropyranyl) ether (**28**)*

A solution of sulfone **26** (2.9 g, 7.3 mmol) in THF (35 ml) was added to the blue colored mixture of liquid ammonia (150 ml), lithium (0.5 g, 73.17 mmol) and THF (30 ml) at −78 °C and stirred for 1.5 h. The excess of lithium was quenched by addition of NH_4Cl. When the reaction mixture became white, the cooling bath was removed, and the reaction mixture was allowed to warm to room temperature and left until the ammonia completely evaporated. Then, water was added to the residue, and the mixture was extracted with $CHCl_3$. The organic fractions were dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by column chromatography on SiO_2 to give ether **28** (1.5 g, 67%) as an oil. IR (cm^{-1}): 1040, 1065, 1085, 1130, 1210, 1360, 1385, 1450, 1475. ^1H NMR δ: 0.68 (s, 3H, 18-H), 1.02 (s, 3H, 19-H), 3.06–3.30 (m, 1H, 26-H), 3.42–3.68 (m, 3H, OTHP and 26-H), 3.80–3.94 (m, 1H, OTHP), 4.58 (d, J = 3.0 Hz, 1H, 3-H), 5.36 (d, J = 5.0 Hz, 1H, 6-H). HRMS calc. for $C_{32}H_{54}O_3$: 486.4073; found: 486.4067. EIMS *m/z*: 486 ([*M*]$^+$, 11), 468 ([$M - H_2O$]$^+$, 71), 402 ([M – THP + H]$^+$, 21), 384 ([M – THPOH]$^+$, 48), 366 ([M – THPOH–H_2O]$^+$, 21), 351 (12), 271 ([$M - C_{13}H_{25}O_2$ (fission C-17/C-27)-

2H]$^+$, 34), 255 (16), 213 ([$C_{13}H_{25}O_2$ (fission C-17/C-27)]$^+$, 19), 159 (21), 145 (23), 107 (23), 85 ([THP-H]$^+$, 100).

2.19. (25S)-6β-Methoxy-3α,5-cyclo-5α-cholestan-26-ol 26-(2′-tetrahydropyranyl) ether (29)

The title compound was prepared in 72% yield as an oil from **27** according to the procedure described above for the preparation of **28** from **26**. IR (cm^{-1}): 1040, 1085, 1105, 1125, 1190, 1210, 1280, 1330, 1360, 1385, 1460, 1475, 2875, 2945. ^{1}H NMR δ: 0.44 (dd, J = 8.5, 5.5 Hz, 1H, 3-H), 0.64 (t, J = 4.5 Hz, 1H, 4-H), 0.71 (s, 3H, 18-H), 1.04 (s, 3H, 19-H), 2.78 (brs, 1H, 6-H), 3.08–3.28 (m, 1H, 26-H), 3.33 (s, 3H, OMe), 3.44–3.68 (m, 3H, OTHP and 26-H), 3.80–3.94 (m, 1H, OTHP), 4.58 (m, 1H, 3-H). HRMS calc. for $C_{33}H_{56}O_3$: 500.4245; found: 500.4229. EIMS m/z: 500 ([M]$^+$, 41), 485 ([$M - CH_3$]$^+$, 30), 468 ([$M - CH_3OH$]$^+$, 50), 445 (43), 416 ([M − THP + H]$^+$, 6), 384 ([M − THP–CH_3O]$^+$, 15), 366 (7), 255 ([$M - C_{13}H_{25}O_2$ (fission C-17/C-20)-CH_3OH]$^+$, 12), 213 ([$C_{13}H_{25}O_2$ (fission C-17/C-20)]$^+$, 8), 95 (14), 85 ([THP–H]$^+$, 100).

2.20. (25S)-24-Phenylsulfonylcholest-5-en-3β,26-diol 26-(2′-tetrahydropyranyl) ether (31)

The title compound was prepared in 79% yield as an oil from iodide **23** and *2*-[(*2S*)-*2-methyl-3-phenylsulfonylpropyloxy*]*tetrahydro-2H-pyran* **30** as described above for the synthesis of **26** from **23** and **25**. ^{1}H NMR δ: 3.09–3.82 (m, 3H, 26-H and OTHP), 4.35–4.68 (m, 1H, OTHP), 5.36 (d, J = 4.5 Hz, 1H, 6-H), 7.58 (m, 3H, arom.), 7.90 (m, 2H, arom.).

2.21. (25R)-Cholest-5-en-3β,26-diol 26-(2′-tetrahydropyranyl) ether (32)

The title compound was prepared in 68% yield as an oil from sulfone **31** according to the procedure described above for the preparation of **28** from **26**. ^{1}H NMR δ: 0.69 (s, 3H, 18-H), 1.02 (s, 3H, 19-H), 3.08–3.28 (m, 1H, 26-H), 3.42–3.66 (m, 3H, OTHP and 26-H), 3.78–3.94 (m, 1H, OTHP), 4.58 (m, 1H, 3-H), 5.35 (d, J = 4.5 Hz, 1H, 6-H). HRMS calc. for $C_{32}H_{54}O_3$: 486.4073; found: 486.4080. EIMS m/z: 486 ([M]$^+$, 6), 468 ([$M - H_2O$]$^+$, 38), 402 ([M − THP + H]$^+$, 14), 384 ([M − THPOH]$^+$, 38), 366 ([M − THPOH–H_2O]$^+$, 18), 351 (6), 271 ([$M - C_{13}H_{25}O_2$ (fission C-17/C-27)-2H]$^+$, 18), 255 (13), 213 ([$C_{13}H_{25}O_2$ (fission C-17/C-27)]$^+$, 15), 159 (17), 145 (21), 107 (22), 85 ([THP–H]$^+$, 100).

2.22. (25S)-3β-Acetoxy-cholest-5-en-26-ol (33)

The mixture of compound **29** (3.2 g, 6.4 mmol) and acetic acid (30 ml) was refluxed for 1.5 h. After cooling down to room temperature, 37% HCl (2 ml) and water (1 ml) were added to the reaction mixture, and the resulting mixture was stirred at room temperature for 1 h. Then, the reaction mixture was neutralized with a saturated solution of Na_2CO_3 and extracted with EtOAc. The combined organic fractions were dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by column chromatography on SiO_2 to give acetate **33** (1.1 g, 39%). Mp 129–131 °C (MeOH). ^{1}H NMR δ: 0.61 (s, 3H, 18-H), 0.95 (s, 3H, 19-H), 1.98 (s, 3H, OAc), 2.25 (d, J = 8.0 Hz, 2H, 4-H), 3.40 (m, 2H, 26-H), 4.54 (m, 1H, 3-H), 5.32 (d, J = 4.5 Hz, 1H, 6-H). ^{13}C NMR δ: 12.3, 17.1, 19.1, 19.7, 21.4, 21.8, 23.9, 24.7, 28.2, 28.6, 32.3, 34.1, 36.2, 36.7, 38.5, 40.2, 42.7, 50.5, 56.3, 56.5, 57.1, 68.6, 68.8, 74.4, 123.0, 140.1, 170.9. HRMS calc. for $C_{29}H_{48}O_3$ ([M]$^+$): 444.3603; found: 444.3604. EIMS m/z: 384 ([M − AcOH]$^+$, 100), 370 ([$M - H_2O$–AcOH]$^+$, 47), 263 (8), 255 ([$C_{19}H_{28}$ (fission C-17/C-20)-AcOH]$^+$, 11), 213 (8), 159 (6.5), 147 (17), 133 (8), 105 (11), 81 (14).

2.23. (25R)-Cholest-5-en-3β,26-diol ((25R)-26-hydroxycholesterol) (34)

HCl (37%, 0.4 ml) was added to a stirred solution of tetrahydropyranyl ether **32** (200 mg, 0.41 mmol) in THF (1.5 ml) and MeOH (1.5 ml), and stirring was continued for 15 min. After addition of pyridine (0.4 ml), the reaction mixture was evaporated. The residue was dissolved in $CHCl_3$, washed with saturated $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated. The obtained oily product was purified by column chromatography on SiO_2 (toluene–EtOAc, 20:1 ⇒ 2:1) to give **34** (90 mg, 54%). Mp 172–174 °C (hexane–EtOAc) (lit. mp 169–170 °C [41], 177–178 °C [44], 175–177 °C [42]). IR (cm^{-1}): 2945, 2880, 1755, 1695, 1480, 1390, 1285, 1065. ^{1}H NMR δ: 0.68 (s, 3H, 18-H), 0.93 (d, J = 6.5 Hz, 3H, 21-H), 1.02 (s, 3H, 19-H), 3.36–3.63 (m, 3H, 3- and 26-H), 5.36 (d, J = 5.0 Hz, 1H, 6-H). ^{13}C NMR δ: 11.9, 16.5, 18.7, 19.4, 21.1, 23.4, 24.3, 28.3, 31.7, 31.9, 33.6, 35.7, 35.8, 36.2, 36.5, 37.3, 39.8, 42.3, 50.1, 56.2, 56.8, 68.5, 71.8, 121.7, 140.8. HRMS calc. for $C_{27}H_{46}O_2$: 402.34978; found: 402.34974. EIMS m/z: 402 ([M]$^+$, 100), 387 ([$M - CH_3$]$^+$, 20), 384 ([$M - H_2O$]$^+$, 50), 369 ([$M - H_2O$–CH_3]$^+$, 19), 317 (19), 291 (25), 273 ([$M - C_8H_{17}O$ (fission C-17/C-20)]$^+$, 10), 255 ([$M - C_8H_{17}O$ (fission C-17/C-20)-H_2O]$^+$, 12), 213 (12), 145 (14), 107 (13).

2.24. (25S)-Cholest-5-en-3β,26-diol((25S)-26-hydroxycholesterol) (41)

The title compound was isolated in 62% yield from tetrahydropyranyl ether **28** according to the procedure described above for the preparation of **34** from **32**. Mp 160–162 °C (MeOH) (lit. mp 171–174 °C [43]). $[\alpha]_D = -50$ (c 0.003, MeOH). IR (cm^{-1}): 2945, 2875, 1640, 1475, 1385, 1060. ^{1}H NMR ($CDCl_3$:CD_3OD) δ: 0.68 (s, 3H, 18-H), 0.92 (d, J = 7.0 Hz, 3H, 21-H), 1.03 (s, 3H, 19-H), 3.27–3.61 (m, 3H, 3- and 26-H), 5.34 (d, J = 4.0 Hz, 1H, 6-H). ^{13}C NMR ($CDCl_3$:CD_3OD) δ: 11.9, 16.7, 18.8, 19.4, 21.1, 23.5, 24.3, 28.3, 31.3, 32.0, 33.8, 35.8, 35.9, 36.3, 36.6, 37.3, 39.9, 42.0, 42.4, 50.2, 56.0, 56.2, 56.9, 67.9,

71.4, 121.6, 140.9. HRMS calc. for $C_{27}H_{46}O_2$: 402.3495; found: 402.3498. EIMS *m/z*: 402 ($[M]^+$, 100), 388 (34), 384 ($[M - H_2O]^+$, 48), 369 ($[M - H_2O–CH_3]^+$, 22), 317 (22), 291 (32), 273 ($[M - C_8H_{17}O$ (fission C-17/C-20)$]^+$, 17), 255 ($[M - C_8H_{17}O$ (fission C-17/C-20)-$H_2O]^+$, 18), 213 (18), 145 (14), 107 (13.5).

2.25. (25R)-Cholest-4-en-3-on-26-ol (**38**)

A mixture of alcohol **32** (0.4 g, 0.82 mmol), cyclohexanone (0.4 ml), and toluene (50 ml) was heated under nitrogen until about 10 ml of the solvents as an azeotropic mixture were distilled off to remove traces of water. Then, $Al(O^iPr)_3$ (168 mg, 0.82 mmol) was added, and the reaction mixture was refluxed for 2 h. After cooling to room temperature, methanol (2 ml) was added to the reaction mixture, and the solvents were evaporated in vacuo. The obtained tetrahydropyranyl ether **36** was dissolved in methanol (5 ml), and HCl (37%, 1.2 ml) was added dropwise to this solution. After 1 h, pyridine (0.6 ml) was added to the reaction mixture, and the solvents were concentrated in vacuo. The residue was mixed with a saturated solution of $NaHCO_3$ and extracted with $CHCl_3$. The combined organic fractions were dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by column chromatography on SiO_2 to give enone **38** (214 mg, 65%). Mp 124–126 °C (acetone) (lit. mp 129–131 °C [46]). IR (cm^{-1}): 2940, 1675, 1475, 1385, 1340, 1240, 1200, 1050. ^{1}H NMR δ: 0.71 (s, 3H, 18-H), 0.92 (d, J = 6.5 Hz, 6H, 21- and 27-H), 1.18 (s, 3H, 19-H), 3.46 (m, 1H, 26-H), 5.74 (s, 1H, 4-H). ^{13}C NMR δ: 11.9, 16.5, 17.3, 18.6, 21.0, 23.4, 24.1, 28.2, 32.0, 32.9, 33.5, 33.9, 35.56, 35.64, 35.8, 36.1, 38.6, 39.6, 42.4, 53.8, 55.8, 56.0, 68.4, 123.7, 171.8, 199.8. HRMS calc. for $C_{27}H_{44}O_2$: 400.3341; found: 400.3338. EIMS *m/z*: 400 ($[M]^+$, 100), 385 ($[M - CH_3]^+$, 5), 358 ($[M - CH_3–CO + H]^+$, 16), 277 (13), 229 (19), 124 (35).

2.26. (25S)-Cholest-4-en-3-on-26-ol (**43**)

The title compound was obtained from **28** in 63% yield as an oil according to the procedure described above for the synthesis of **38** from **32**. IR (cm^{-1}): 2940, 1670, 1470, 1385, 1340, 1245, 1210. ^{1}H NMR δ: 0.70 (s, 3H, 18-H), 1.19 (s, 3H, 19-H), 3.46 (m, 2H, 26-H), 5.73 (s, 1H, 4-H). ^{13}C NMR (125 MHz) δ: 17.3, 18.7, 21.0, 23.5, 24.2, 28.2, 32.1, 32.9, 33.7, 34.0, 35.6, 35.70, 35.74, 35.8, 36.2, 38.6, 39.6, 42.4, 53.8, 55.9, 56.1, 68.3, 123.7, 171.8, 199.6.

2.27. (25R)-3β-Acetoxy-cholest-5-en-26-ol (**37**)

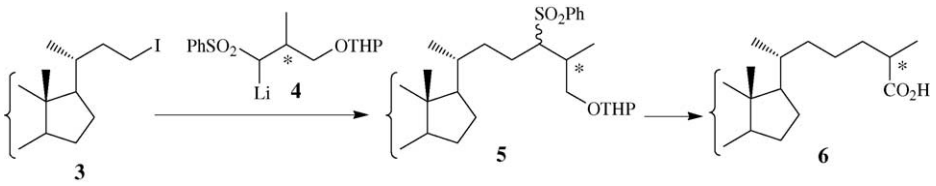
A mixture of alcohol **32** (2 g, 4.1 mol), pyridine (3 ml), Ac_2O (0.8 ml, 8.2 mmol), and DMAP (2 mg) was stirred at room temperature for 2 h. Then, the reaction mixture was treated with saturated $NaHCO_3$ solution and extracted with $CHCl_3$. The organic fractions were dried (Na_2SO_4) and evaporated in vacuo to give crude **35**. The crude product was dissolved in THF (25 ml), and MeOH (25 ml) and HCl (37%, 3 ml) were added. The mixture was stirred at room temperature for 30 min. After addition of pyridine (3.5 ml), the solvents were evaporated. The residue was dissolved in $CHCl_3$, washed with saturated $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated. The obtained product was purified by column chromatography on SiO_2 to give acetate **37** (1.6 g, 89%) as an oil. ^{1}H NMR δ: 0.62 (s, 3H, 18-H), 0.94 (s, 3H, 19-H), 2.00 (s, 3H, OAc), 2.26 (d, J = 8.0 Hz, 2H, 4-H), 3.90 (m, 2H, 26-H), 4.54 (m, 1H, 3-H), 5.32 (d, J = 4.5 Hz, 1H, 6-H). ^{13}C NMR δ: 12.3, 17.0, 19.1, 19.7, 21.8, 21.4, 23.7, 24.7, 28.2, 32.3, 33.1, 34.0, 36.1, 37.0, 37.4, 38.5, 40.1, 42.7, 50.5, 56.5, 57.1, 68.9, 73.4, 74.4, 123.0, 140.1, 170.9. HRMS calc. for $C_{29}H_{46}O_2$ ($[M - H_2O]^+$): 426.3484; found: 426.3498. EIMS *m/z*: 426 ($[M - H_2O]^+$), 384 ($[M - AcOH]^+$, 100), 370 ($[M - H_2O–AcOH]^+$, 9), 281 (7), 255 ($[C_{19}H_{28}$ (fission C-17/C-20)-$AcOH]^+$, 8), 231 (8), 181 (14), 147 (10), 131 (17), 119 (15), 107 (7.5), 81 (9.5).

2.28. (25S)-3β-Acetoxy-cholest-5-en-26-ol (**33**)

The title compound was prepared as an oil in 75% yield from **28** according to the procedure described above for the preparation of **37** from **32**.

2.29. (25R)-3β-Hydroxy-cholest-5-en-26-oic acid (**39**)

TEMPO (2,2,6,6-tetramethylpiperidine-*N*-oxyl, 50 mg, 0.32 mmol) and phosphate buffer (1.3 ml, pH 6.86) were added to a solution of alcohol **37** (1.6 g, 3.6 mmol) in a mixture of THF (20 ml) and CH_3CN (10 ml). Then, solutions of $NaClO_2$ (650 mg in 5 ml H_2O) and NaClO (0.2 ml in 2 ml H_2O) were added simultaneously at 35 °C to this mixture over 0.5 h. The reaction mixture was stirred at this temperature for 5 h, then cooled to room temperature, and mixed with a cold saturated solution of Na_2SO_3. After 15 min, an additional amount of phosphate buffer was added, and the reaction mixture was extracted with EtOAc. The combined organic fractions were dried (Na_2SO_4) and evaporated in vacuo. The obtained crude acetate was dissolved in THF (10 ml) and treated with a 3% solution of KOH in MeOH (13 ml) for 15 min at room temperature. Then, acetic acid (0.4 ml) and pyridine (five drops) were added, and the reaction mixture was filtered through a short pad of SiO_2. The filtrate was concentrated in vacuo and purified by column chromatography on SiO_2 to give acid **39** (820 mg, 55%). Mp 175–177 °C (MeOH) (lit. mp 168–170 °C [46]). $[\alpha]_D = -28$ (c 0.002, MeOH). IR (cm^{-1}): 1065, 1230, 1390, 1480, 1720, 2875, 2945. ^{1}H NMR (500 MHz) δ: 0.67 (s, 3H, 18-H), 0.90 (d, J = 6.4 Hz, 3H, 21-H), 1.00 (s, 3H, 19-H), 1.17 (d, J = 7.2 Hz, 3H, 27-H), 2.38–2.50 (m, 1H, 25-H), 3.53 (m, 1H, 3-H), 5.34 (brs, 1H, 6-H). ^{13}C NMR δ: 11.5, 16.4, 18.3, 19.0, 20.8, 23.3, 24.0, 27.9, 31.3, 31.6, 33.6, 35.3, 35.4, 36.2, 36.9, 38.9, 39.5, 41.9, 42.0, 49.8, 55.8, 56.4, 71.5, 121.4, 140.4, 181.7. HRMS calc. for $C_{26}H_{44}O$ ($[M - CO_2]^+$): 372.3392; found: 372.3398. EIMS *m/z*: 416 ($[M]^+$, 100), 398 (59), 331 (23), 305 (50), 273 (14),



Scheme 2.

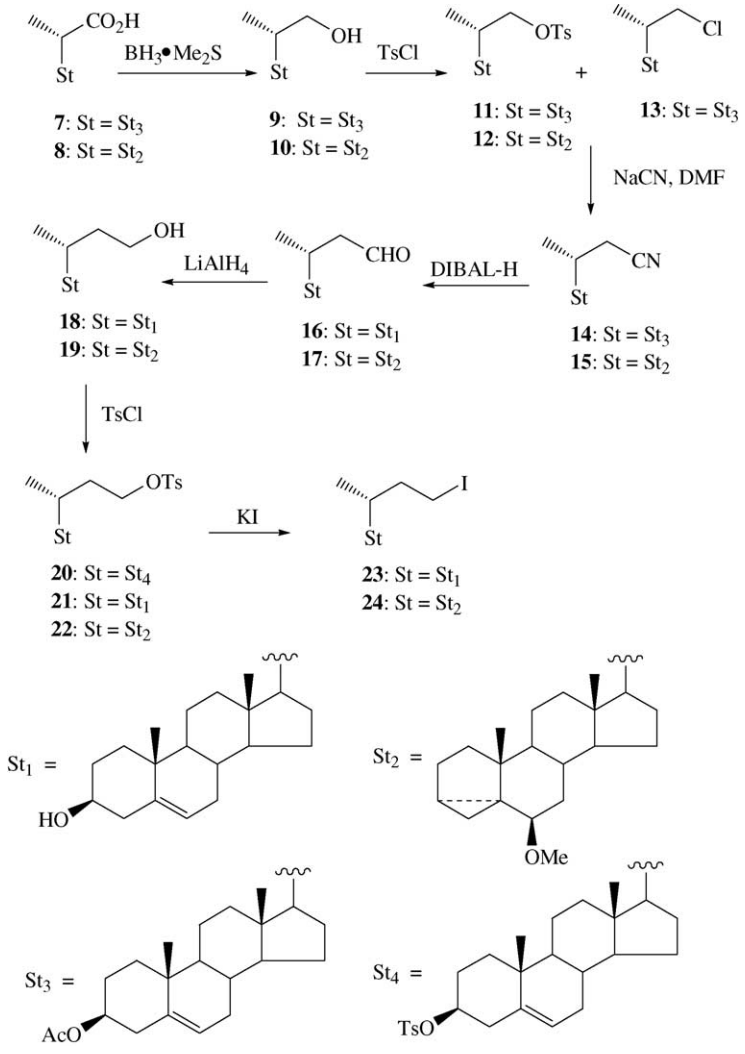
255 ([$C_{19}H_{28}$ (fission C-17/C-20)-H_2O]$^+$, 19), 213 (23), 159 (16), 145 (21), 107 (24), 81 (21).

2.30. (25S)-3β-Hydroxy-cholest-5-en-26-oic acid (42)

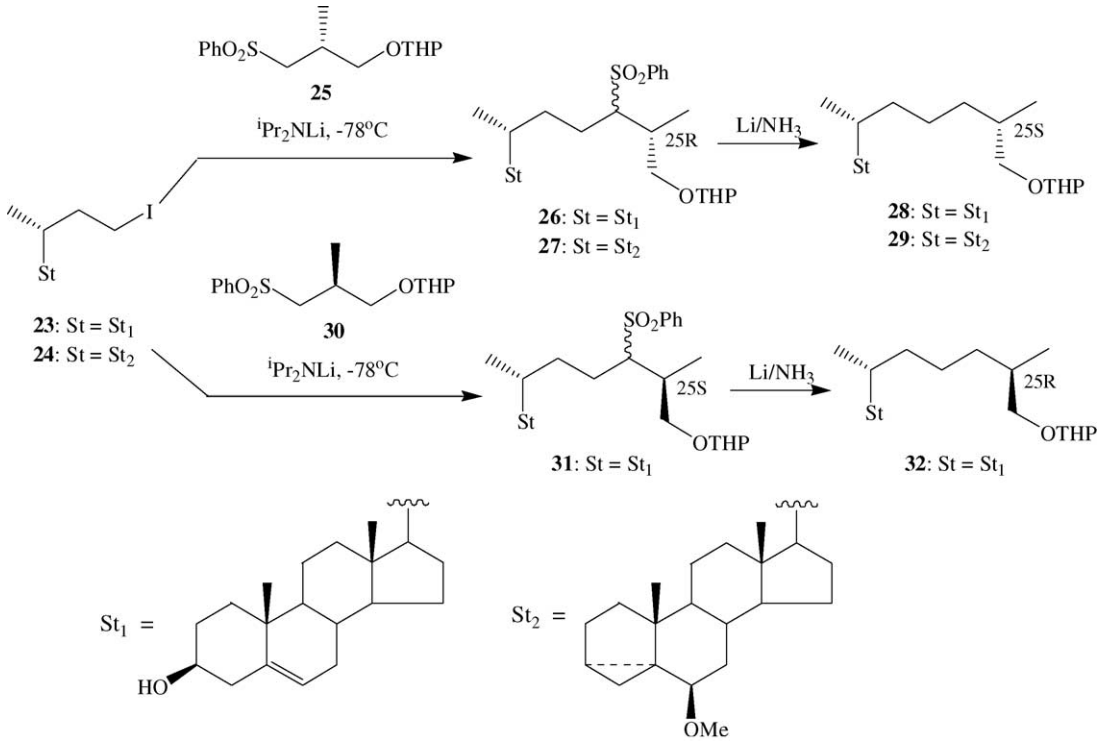
The title compound was obtained from 33 in 48% yield by the procedure described above for the synthesis of compound **39**. Mp 157–160 °C (MeOH). IR (cm^{-1}): 1030, 1060, 1200, 1245, 1390, 1480, 1725, 2875, 2950. ^{1}H NMR δ: 0.66 (s, 3H, 18-H), 0.92 (d, J = 6.5 Hz, 3H, 21-H), 1.02 (s, 3H, 19-H), 1.19 (d, J = 7.2 Hz, 3H, 27-H), 2.46 (m, 1H, 25-H), 3.51 (m, 1H, 3-H), 5.36 (d, J = 5.0 Hz, 1H, 6-H). ^{13}C NMR δ: 12.2, 17.4, 19.0, 19.7, 21.4, 24.1, 24.6, 28.6, 32.3, 32.0, 34.4, 36.0, 36.9, 37.6, 39.6, 40.1, 42.7, 50.5, 56.5, 57.1, 72.2, 122.1, 141.1, 181.8. HRMS calc. for $C_{25}H_{41}O$ ([$M - C_2H_3O_2$]$^+$): 357.3157; found: 357.3161. EIMS m/z: 416 ([M]$^+$, 100), 398 (60), 383 (28), 331 (25), 305 (48), 291 (11), 273 (14), 255 ([$C_{19}H_{28}$ (fission C-17/C-20)-H_2O]$^+$, 20), 213 (24), 159 (15), 145 (25), 140 (34), 107 (23), 81 (20).

2.31. (25R)-Cholest-4-en-3-on-26-oic acid (40)

TEMPO (6 mg, 0.04 mmol) and phosphate buffer (2 ml, pH 6.86) were added to a solution of alcohol **38** (0.16 g,



Scheme 3.



Scheme 4.

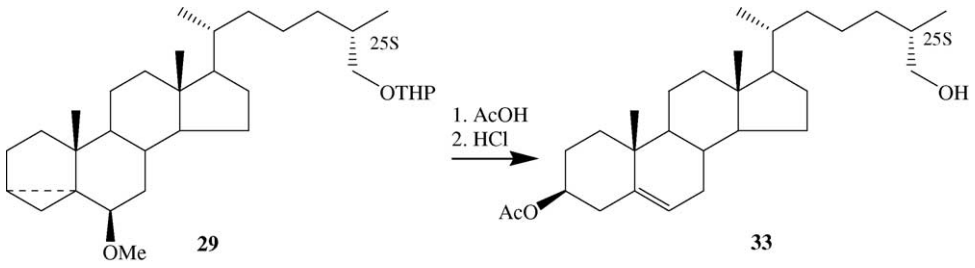
0.40 mmol) in a mixture of THF (2 ml) and CH_3CN (1 ml). Then, solutions of $NaClO_2$ (70 mg in 0.5 ml H_2O) and NaClO (1.3 ml of water solution with 2–3% of active Cl) were added simultaneously to this mixture at 35 °C. The reaction mixture was stirred at this temperature for 5 h, then cooled to room temperature, and mixed with a cold saturated solution of Na_2SO_3. After 15 min, an additional amount of phosphate buffer was added, and the reaction mixture was extracted with $CHCl_3$. The combined organic fractions were dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by column chromatography on SiO_2 to give acid **40** (80 mg, 48%). Mp 147–148 °C (hexane–EtOAc). ^{1}H NMR δ: 0.70 (s, 3H, 18-H), 0.90 (d, J = 6.4 Hz, 3H, 21-H), 1.20 (s, 3H, 19-H), 1.20 (d, J = 7.2 Hz, 3H, 27-H), 5.73 (s, 1H, 4-H). HRMS calc. for $C_{26}H_{41}O$ ($[M - COOH]^+$): 369.3156; found: 369.3157. EIMS m/z: 414 ($[M]^+$, 100), 400 (15), 369 ($[M - COOH]^+$), 291 (16), 271 ($[C_{19}H_{27}O$ (fission C-17/C-20)$]^+$, 11), 229 (43), 147 (16), 124 (67), 107 (15), 95 (19).

*2.32. (25S)-Cholest-4-en-3-on-26-oic acid **(44)***

The title compound was obtained from alcohol **43** in 51% yield by the procedure described above for the synthesis of compound **40**. Mp 172–175 °C (EtOAc). ^{1}H NMR (500 MHz) δ: 0.70 (s, 3H, 18-H), 0.90 (d, J = 6.4 Hz, 3H, 21-H), 1.20 (s, 3H, 19-H), 2.22–2.50 (m, 3H, 2- and 25-H), 5.73 (s, 1H, 4-H). ^{13}C NMR (125 MHz) δ: 18.6, 21.0, 23.7, 24.2, 28.2, 32.1, 33.0, 33.9, 34.0, 35.61, 35.64, 35.71, 35.73, 38.6, 39.3, 39.6, 42.4, 53.8, 55.9, 56.1, 123.8, 171.8, 181.9, 199.7.

3. Results and discussion

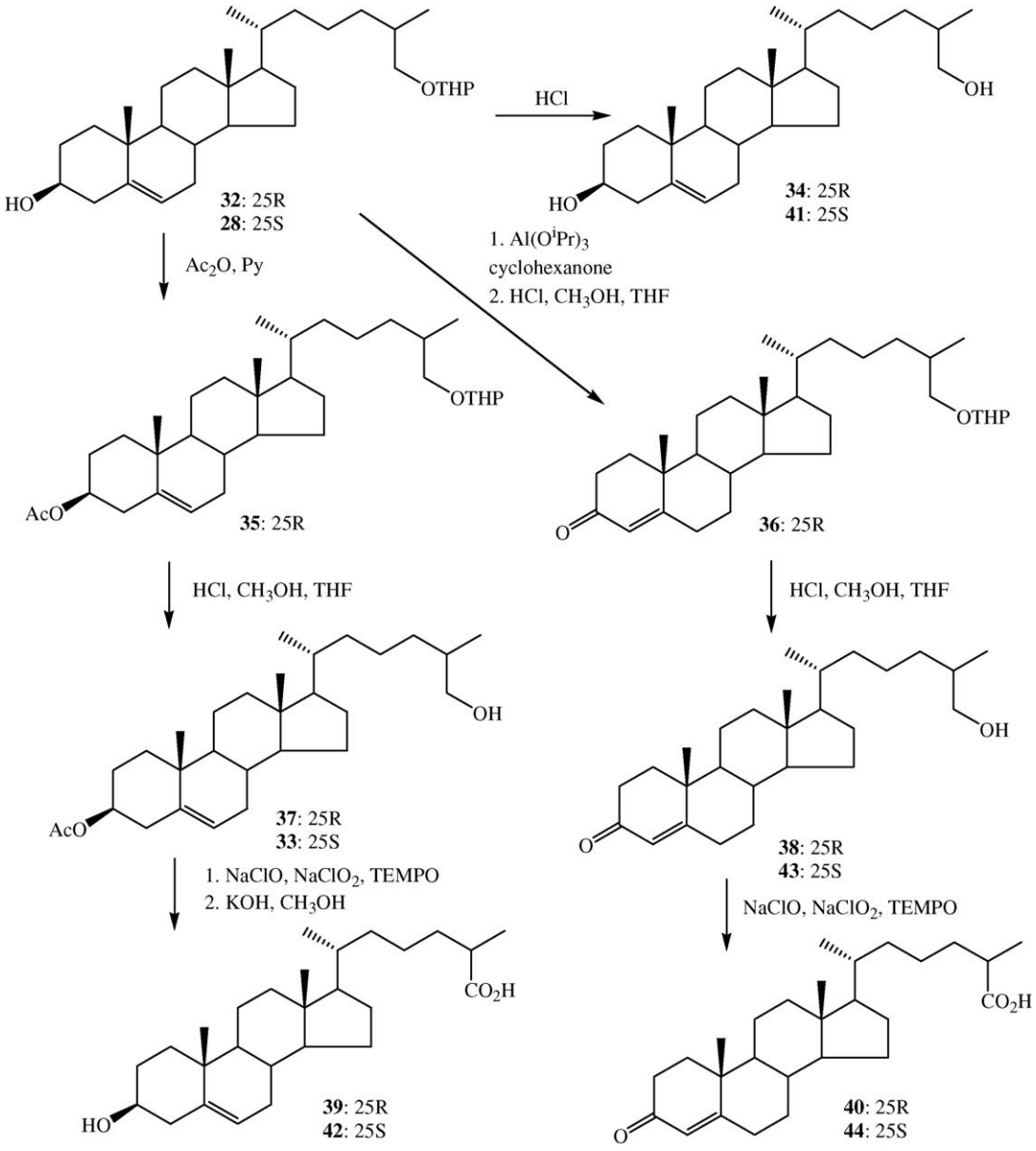
Synthetic studies on 26-functionalized steroids have a long history, and early attempts of their stereoselective preparation were made in the 1950's [44]. There are several common approaches to the 26-functionalized steroids with a chiral center



Scheme 5.

at C-25. The simplest variant is the use of the appropriate starting material where this stereocenter is already present. A few steps are necessary to get the desired functionality, but in practice, only one epimer can be obtained in such a way (e.g., (25*R*)-26-hydroxy cholesterol from diosgenin [45]). Access to both diastereomers is possible via the corresponding achiral precursors (e.g., 25-enes [46]). Most syntheses of 26-functionalized steroids are based on transformation of derivatives with a preformed carbon side chain skeleton. In our opinion, better results can be achieved by using a convergent scheme when a low-molecular building block is attached to a steroidal part (Scheme 2). Similar methodology was used for the preparation of related deuterium labeled compounds [47].

Preparation of the desired compounds requires formation of both the side chain and the cyclic part. There are at least two possibilities to achieve the final goal with respect to formation of the cyclic part, and we decided to study both of them (Scheme 3). The first one makes use of the commercially available acid **7**, and the second one relies on the acid **8** [48] available in a few steps from stigmasterol. First stages in both cases were essentially the same and were directed to the one-carbon homologation of the side chain and introduction of the 23-iodine. Reduction of acids **7** and **8** with borane proceeded smoothly. Even in the case of **7**, which had a Δ^5-double bond, the desired reduction product **9** was isolated in a reasonable (64%) yield. Tosylation of the primary alcohol **9** by treatment with TsCl in pyridine gave poor results because of formation



Scheme 6.

of a considerable amount (up to 22%) of 22-chloride **13** along with the desired tosylate **11** (57%). Both **11** and **13** could be used for preparation of nitrile **14** at the next stage, but for practical considerations, formation of **13** should be avoided. In this respect, tosylation of alcohols **9** and **10** in $CHCl_3$ in the presence of pyridine proved to be more efficient. The hydride reductions of nitriles **14** and **15** with DIBAL-H and then, aldehydes **16** and **17** with $LiAlH_4$ proceeded smoothly and gave the expected 23-alcohols **18** and **19**.

Synthesis of 23-iodides **23** and **24** was carried out by tosylation of **18** and **19** followed by nucleophilic substitution of the corresponding tosylates with KI. There was no problem with **19** which contained only one hydroxyl group. Tosylation of **18** proceeded with the formation of di- and monotosylates **20** and **21**, but the primary hydroxyl group was more reactive, and the desired monotosylate **21** could be obtained in up to 58% yield.

Reaction of the anions derived from sulfones **25** and **30** with iodides **23** and **24** gave the corresponding sulfones **26**, **27**, and **31** (Scheme 4). The latter ones were subjected to desulfurization with lithium in liquid ammonia to give **28**, **29**, and **32**. Until this point in the synthetic route, the use of 3α,5-cyclo derivatives was more preferable (**8** ⇒ **29**, 17% total yield) than similar transformation of 3β-hydroxy (acetoxy) steroids (**7** ⇒ **28**, 12% total yield). However, the necessity to regenerate the cyclic part in the first case changed this preference to the opposite. Transformation of **29** into **33** proceeded only in 39% yield (Scheme 5).

Subsequent transformations of **28** and **32** into the desired compounds were rather straightforward. Acid hydrolysis of **32** led to (25*R*)-hydroxycholesterol **34** (Scheme 6). Acetylation of **32** followed by acid hydrolysis of intermediate **35** gave 26-alcohol **37**. Its oxidation into the carboxylic acid had to be done under conditions that ensured preservation of the stereochemistry at C-25 and the functional groups in the cyclic part.

The use of TEMPO-catalyzed oxidation with $NaClO_2$ as the stoichiometric oxidant [49] solved all of these problems, and the desired (25*R*)-cholestenoic acid was isolated after saponification of the intermediate 3β-acetate. Oppenauer oxidation of **32** afforded the enone **36**, which on deprotection of the 26-hydroxy group, was oxidized under similar conditions as **40**. When compound **28** was used in a similar sequence of reactions, a set of corresponding (25*S*)-derivatives **41–44** was prepared.

In conclusion, a new synthesis of both (25*R*)- and (25*S*)-epimers of 26-hydroxycholesterol, 26-cholestenoic acid, and the corresponding Δ^4-3-keto derivatives was accomplished. The obtained steroids are useful standards for studying the biosynthesis of cholic acids.

Acknowledgments

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from either (25*R*)- or (25*S*)-3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid by rat liver homogenate. J Biochem 1984;96:1103–7.
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