Steroids 77 (2012) 780–790

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

A new approach to the side chain formation of 24-alkyl-22-hydroxy steroids: Application to the preparation of early brassinolide biosynthetic precursors

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ARTICLE INFO

Article history:
Received 18 January 2012
Received in revised form 19 March 2012
Accepted 21 March 2012
Available online 2 April 2012

Keywords:
Brassinosteroids
Cathasterone
(22*S*)-22-Hydroxycampesterol
6-Deoxocathasterone
Deuterium labeling

ABSTRACT

A new synthetic route to 22*S*-hydroxy-24*R*-methyl steroids has been developed and applied for the preparation of cathasterone, (22*S*)-hydroxycampesterol, and 6-deoxocathasterone, which are precursors in the early stages of the biosynthesis of brassinolide. The construction of the steroid side chain with the correct stereochemistry at C-24 is based on the use of Claisen rearrangement. The introduction of the 22-hydroxyl group has been achieved by epoxidation of the Δ^{22}-double bond, nucleophilic opening of the intermediate mesyl epoxide with sodium sulfide, and desulfurization of the formed tetrahydrothiophenes with Raney nickel.

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1. Introduction

The 22-hydroxy steroids **1–3** are known biosynthetic precursors of the steroidal plant hormone brassinolide [1] (Fig. 1). These compounds and their deuterated analogs are useful tools for studies of the initial steps of the biosynthetic transformations of campesterol into brassinolide. A number of synthetic routes have been developed for the preparation of 24-alkyl-22-hydroxy steroids. One of the first was based on the coupling of the steroidal moiety with an appropriate C23–C28 fragment [2]. The use of racemic 2,3-dimethylbutylmagnesium bromide is an evident drawback of this method. Somewhat better results were obtained with a chiral C23–C28 fragment, prepared from commercial (*R*)-Roche ester [3]. The major problems of this approach were connected with the use of relatively expensive reagents and a rather lengthy synthetic sequence.

Among the large number of steroidal side chain syntheses, those making use of the Claisen rearrangement proved to be very effective and were repeatedly used for the preparation of Δ^{22}-24-alkyl steroids with a predefined stereochemistry at C-24 [4,5]. However, the subsequent transformation into the desired 24-alkyl-22-hydroxy derivatives (involving Δ^{22}-bond epoxidation followed by epoxide reduction) suffered from a low overall efficiency owing to the low regio- and stereoselectivity of these reactions [6]. The main task of the present work was to develop an improved synthetic route to 24-alkyl-22-hydroxy steroids, using products of the Claisen rearrangement as key intermediates.

2. Experimental

2.1. General

Melting points were recorded on a Boetius micro-melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker AVANCE 500 (Bruker Biospin, Rheinstetten, Germany) spectrometer in $CDCl_3$ operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts were determined relative to the residual solvent peaks ($CHCl_3$, δ = 7.26 for hydrogen atoms, δ = 77.00 for carbon atoms). Mass spectra were performed on a LCQ Fleet mass spectrometer (Thermo Electron Corporation, USA) with an APCI source. Spectra were collected in a positive ion mode and analyzed by Xcalibur software. Chemicals were purchased from Aldrich and Fluka and used as received. Reactions were monitored by TLC using aluminum or plastic sheets, silica gel 60 F_{254} precoated (Merck Art. 5715). Column chromatography was carried out on Kieselgel 60 (Merck Art. 7734).

Abbreviations: DMM, dimethoxymethane; Na_2EDTA, disodium ethylenediamine tetraacetate; MOM, methoxy methyl; $(DHQD)_2AQN$, hydroquinidine (anthraquinone-1,4-diyl) diether; brsm, based on recovered starting material; DMP, dimethoxy propane; DIPA, diisopropanolamine; PPTS, pyridinium *p*-toluenesulfonate; MCPBA, 3-chloroperoxybenzoic acid.

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http://dx.doi.org/10.1016/j.steroids.2012.03.010

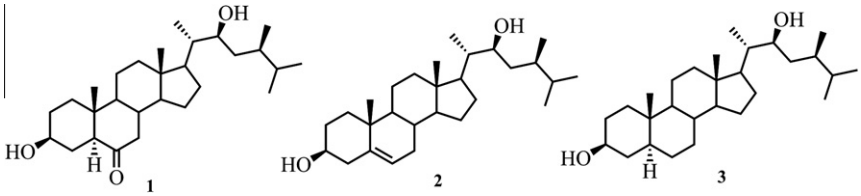


Fig. 1. Structures of early brassinolide biosynthetic precursors: cathasterone **1**, (22*S*)-22-hydroxy campesterol **2**, and 6-deoxocathasterone **3**.

2.2. Synthesis of compounds

2.2.1. Addition of lithium methyl acetylide to (20S)-6-(1,3-dioxolan-2-yl)-3α,5-cyclo-20-formyl-5α-pregnane (**5**)

Methylacetylene (20 mL, 0.32 mol) was condensed into THF (75 mL), which was a cooled to −65 °C. Then 1.6 M BuLi (57 mL, 91 mmol) was added at −65 °C. After keeping at this temperature for 10 min, a solution of *(20S)-6-(1,3-dioxolan-2-yl)-3α,5-cyclo-20-formyl-5α-pregnane* **5** (11.0 g, 29.6 mmol, prepared according to [7]) was added. The mixture was stirred at −65 °C for 3 h, and then NH_4Cl (10 g) was added. After warming to room temperature, the mixture was diluted with water (150 mL) and extracted with $CHCl_3$ (3 × 75 mL). The organic layer was dried (Na_2SO_4) and evaporated. The residue was chromatographed on SiO_2 (petroleum ether–EtOAc = 30:1⇒10:1) to give:

(22R)-6-(1,3-Dioxolan-2-yl)-3α,5-cyclo-26,27-bisnor-5α-cholest-23-yn-22-ol **6** (7.7 g, 63%) as an oil. ^{1}H NMR δ: 0.32 (t, J = 4.2 Hz, 1H, C4–H), 0.60 (dd, J = 8.1, 4.6 Hz, 1H, C4–H), 0.72 (s, 3H, C18–H), 1.00 (s, 3H, C19–H), 1.09 (d, J = 6.6 Hz, 3H, C21–H), 1.85 (d, J = 2.2 Hz, 3H, C25–H), 3.74 (q, J = 6.6 Hz, 1H, –OCH_2CH_2O–), 3.80–3.86 (m, 1H, –OCH_2CH_2O–), 3.89 (dt, J = 7.9, 6.3 Hz, 1H, –OCH_2CH_2O–), 4.02 (dt, J = 7.9, 6.0 Hz, 1H, –OCH_2CH_2O–), 4.43 (s, 1H, C22–H). ^{13}C NMR δ: 3.55, 7.24, 12.11, 13.14, 18.95, 22.60, 23.01, 24.15, 24.88, 27.57, 33.20, 34.14, 39.21, 39.84, 40.13, 42.29, 42.70, 45.57, 47.32, 51.79, 56.02, 64.59, 64.84, 65.63, 80.37, 81.11, 109.85. MS (APCI$^+$) m/z (%): 413 ([M+H]$^+$, 35), 395 ([M−H_2O+H]$^+$, 100).

(22S)-6-(1,3-Dioxolan-2-yl)-3α,5-cyclo-26,27-bisnor-5α-cholest-23-yn-22-ol (3.8 g, 31%) as an oil. ^{1}H NMR δ: 0.32 (t, J = 4.2 Hz, 1H, C4–H), 0.61 (ddd, J = 8.3, 4.6, 1.8 Hz, 1H, C4–H), 0.72 (s, 3H, C18–H), 1.00 (s, 3H, C19–H), 1.03 (d, J = 6.5 Hz, 1H, C21–H), 1.85 (d, J = 2.2 Hz, 3H, C25–H), 3.74 (q, J = 6.6 Hz, 1H, –OCH_2CH_2O–), 3.80–3.86 (m, 1H, –OCH_2CH_2O–), 3.89 (dt, J = 7.9, 6.3 Hz, 1H, –OCH_2CH_2O–), 4.02 (dt, J = 7.9, 6.0 Hz, 1H, –OCH_2CH_2O–), 4.42 (d, J = 16.9 Hz, 1H, C22–H).

2.2.2. (22S,23Z)-6-(1,3-Dioxolan-2-yl)-3α,5-cyclo-26,27-bisnor-5α-cholest-23-en-22-ol (**7**)

A mixture of propargyl alcohol **6** (6.0 g, 14.5 mmol), 5% Pd on $BaSO_4$ (0.9 g), quinoline (1.0 mL), ethanol (70 mL) and THF (10 mL) was stirred under H_2 until the gas adsorption had ceased. The catalyst was removed by filtration, the solvent was evaporated and the residue was chromatographed on SiO_2 (hexane–EtOAc = 40:1⇒10:1) to give allylic alcohol **7** (4.2 g, 70%) as an oil. ^{1}H NMR δ: 0.32 (t, J = 4.2 Hz, 1H, C4–H), 0.60 (dd, J = 8.2, 4.6 Hz, 1H, C4–H), 0.71 (s, 3H, C18–H), 0.95 (d, J = 6.3 Hz, 3H, C21–H), 1.00 (s, 3H, C19–H), 1.65 (dd, J = 6.6, 1.3 Hz, 1H, C25–H), 3.74 (q, J = 6.5 Hz, 1H, –OCH_2CH_2O–), 3.80–3.86 (m, 1H, –OCH_2CH_2O–), 3.90 (dt, J = 8.0, 6.3 Hz, 1H, –OCH_2CH_2O–), 4.02 (dt, J = 8.0, 6.0 Hz, 1H, –OCH_2CH_2O–), 4.52–4.61 (m, 1H, C22–H), 5.45–5.59 (m, 1H, C22– and C23–H). ^{13}C NMR δ: 7.25, 12.03, 12.31, 13.41, 18.95, 22.58, 23.04, 24.16, 24.88, 27.81, 33.19, 34.15, 39.21, 40.00, 40.17, 41.90, 42.75, 45.56, 47.34, 52.50, 56.11, 64.60, 64.84, 69.58, 109.87, 124.99, 133.13. MS (APCI$^+$) m/z (%): 415 ([M+H]$^+$, 24), 397 ([M−H_2O+H]$^+$, 100).

2.2.3. (22E,24R,25R)- and (22E,24R,25S)-6-(1,3-Dioxolan-2-yl)-24-methyl-3α,5-cyclo-5α-cholest-22-en-26-oic acid ethyl esters [(25R)-**8a** and (25S)-**8a**]

A solution of allylic alcohol **7** (10.0 g, 20.1 mmol), triethyl orthopropionate (80 mL, 0.4 mol) and propionic acid (2 mL, 27 mmol) in benzene (250 mL) was refluxed under argon for 2 h. Then the solution was cooled to room temperature, diluted with water (100 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was dried (Na_2SO_4) and evaporated. The residue was chromatographed on SiO_2 (cyclohexane–EtOAc = 30:1⇒10:1) to give the ester **8a** (8.5 g, 75%, an oil) as a mixture of 25*R*- and 25*S*-isomers (ratio 5.4:1 based on the ratio of integral intensity peaks with δ 129.96 and 130.40 or 137.71 and 136.89). ^{1}H NMR: δ 0.31 (t, J = 4.2 Hz, 1H, C4–H), 0.60 (dd, J = 8.2, 4.6 Hz, 1H, C4–H), 0.71 (s, 3H, C18–H), 0.95 (d, J = 6.8 Hz, 3H), 0.99 (s, 3H, C19–H), 1.06 (d, J = 6.9 Hz, 3H), 1.24 (d, J = 7.1 Hz, 3H, C27–H), 3.73 (q, J = 6.6 Hz, 1H, –OCH_2-CH_2O–), 3.83 (q, J = 6.4 Hz, 1H, –OCH_2CH_2O–), 3.86–3.94 (m, 1H, –OCH_2CH_2O–), 4.01 (dt, J = 8.0, 6.0 Hz, 1H, –OCH_2CH_2O–), 4.04–4.17 (m, 2H, –OCH_2CH_3), 5.08 (dd, J = 15.1, 8.5 Hz, 1H, C22– or C23–H), 5.18–5.29 (m, 1H, C23– or C22–H). ^{13}C NMR δ: 7.25, 12.32, 13.85, 14.26, 15.04, 17.23, 18.95, 19.30, 20.65, 20.77, 22.55, 23.02, 24.12, 24.18, 24.88, 28.53, 28.73, 29.67, 33.21, 34.08, 39.23, 39.39, 39.98, 40.16, 40.26, 42.73, 45.14, 45.52, 45.58, 47.42, 55.83, 56.31, 59.95, 60.00, 64.59, 64.83, 77.25, 109.85, 109.93, 129.96, 130.40, 136.89, 137.71, 175.84, 176.30. MS (APCI$^+$) m/z (%): 499 ([M+H]$^+$, 100).

2.2.4. (22R,23R,24S,25R)-6-(1,3-Dioxolan-2-yl)-22-hydroxy-24-methyl-3α,5-cyclo-5α-cholestano-26,23-lactone (**9**)

To a stirred solution of isomeric mixture of **8a** (25*R*:25*S* = 5.4:1) in *t*-BuOH (18 mL) and water (18 mL), K_2CO_3 (0.79 g, 5.72 mmol), $MsNH_2$ (0.27 g, 2.84 mmol), $(DHQD)_2AQN$ (81 mg, 0.189 mmol), $K_3Fe(CN)_6$ (1.87 g, 5.68 mmol) and $K_2OsO_4{\cdot}2H_2O$ (7 mg, 0.019 mmol) were added. The reaction mixture was stirred at room temperature for 48 h, diluted with water (40 mL) and acidified to pH 6 with tartaric acid. The water layer was separated and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried (Na_2SO_4), concentrated *in vacuo* and the residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒1:1) to give lactone **9** (0.26 g, 49%) as white crystals. Mp 236–238 °C (MeCN). ^{1}H NMR δ: 0.33 (t, J = 4.2 Hz, 1H, C4–H), 0.61 (dd, J = 8.1, 4.6 Hz, 1H, C4–H), 0.74 (s, 3H, C18–H), 0.90 (d, J = 7.1 Hz, 3H, C28–H), 1.00 (d, J = 6.9 Hz, 3H, C21–H), 1.01 (s, 3H, C19–H), 1.17 (d, J = 7.2 Hz, 3H, C27–H), 2.50 (dp, J = 6.9, 4.2 Hz, 1H, C24–H), 2.86 (p, J = 7.1 Hz, 1H, C25–H), 3.75 (dd, J = 13.4, 6.5 Hz, 1H, –OCH_2CH_2O–), 3.81–3.87 (m, 2H, C22-H, –OCH_2CH_2O–), 3.90 (td, J = 7.9, 6.2 Hz, 1H, –OCH_2CH_2O–), 4.03 (td, J = 7.7, 6.2 Hz, 1H, –OCH_2CH_2O–), 4.34 (dd, J = 8.9, 4.3 Hz, 1H, C23–H). ^{13}C NMR δ: 7.25, 8.55, 9.56, 12.14, 12.56, 18.95, 22.58, 23.05, 23.91, 24.86, 27.43, 33.20, 34.19, 35.91, 36.17, 39.19, 40.06, 40.11, 41.59, 42.55, 45.54, 47.26, 52.03, 56.01, 64.62, 64.86,

71.37, 83.71, 109.83, 178.21. MS (APCI$^+$) *m/z* (%): 487 ([M+H]$^+$, 100), 469 ([M−H_2O+H]$^+$, 15).

*2.2.5. (22R,23R,24S,25R)-6-(1,3-Dioxolan-2-yl)-22-methoxymethyl-24-methyl-3α,5-cyclo-5α-cholestano-26,23-lactone (**10**)*

A 2 M solution of MOMCl in toluene (0.6 mL, 1.2 mmol) was added to a mixture of alcohol **9** (99 mg, 0.203 mmol), *N*,*N*-diisopropylethylamine (0.32 mL, 1.84 mmol) and Bu_4NI (15 mg, 0.041 mmol) in THF (1.5 mL). The reaction mixture was stirred at room temperature overnight and quenched with saturated $NaHCO_3$ (5 mL). The water layer was separated and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na_2SO_4), concentrated and the residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒3:1) to give ether **10** (91 mg, 85%) as an oil. ^{1}H NMR δ: 0.32 (t, *J* = 4.0 Hz, 1H, C4–H), 0.61 (dd, *J* = 8.2, 4.5 Hz, 1H, C4–H), 0.74 (s, 3H, C18–H), 0.89 (d, *J* = 6.9 Hz, 3H, C28–H), 0.98–1.02 (m, 6H, C19– and C21–H), 1.15 (d, *J* = 7.1 Hz, 3H, C27–H), 2.43 (td, *J* = 7.0, 4.3 Hz, 1H, C24–H), 2.78 (p, *J* = 7.0 Hz, 1H, C25–H), 3.43 (s, 3H, CH_3OCH$_2$O–), 3.66 (d, *J* = 9.5 Hz, 1H, C22–H), 3.74 (q, *J* = 6.6 Hz, 1H, –OCH$_2$CH_2O–), 3.84 (q, *J* = 6.3 Hz, 1H, –OCH$_2$CH_2O–), 3.87–3.93 (m, 1H, –OCH$_2$CH_2O–), 4.02 (ddd, *J* = 11.7, 6.6, 0.9 Hz, 1H, –OCH$_2$CH_2O–), 4.41 (dd, *J* = 9.4, 3.9 Hz, 1H, C23–H), 4.67 (d, *J* = 6.8 Hz, 1H, CH$_3$OCH_2O–), 4.87 (d, *J* = 6.8 Hz, 1H, CH$_3$OCH_2O–). ^{13}C NMR δ: 7.25, 8.24, 9.53, 12.05, 13.17, 18.93, 22.55, 23.05, 24.05, 24.86, 27.59, 33.22, 34.16, 36.36, 36.67, 39.20, 40.06, 40.26, 41.35, 42.65, 45.54, 47.29, 52.11, 56.12, 56.34, 64.61, 64.86, 77.97, 83.94, 97.89, 1i09.80, 178.51. MS (APCI$^+$) *m/z* (%): 531 ([M+H]$^+$, 25), 499 ([M−MeOH+H]$^+$, 100), 487 (M−CH_2CH_2O+H]$^+$, 26), 469 ([M−MeOH−CH_2O+H]$^+$ 39), 456 (15).

*2.2.6. (22R,23R,24S,25R)-6-(1,3-Dioxolan-2-yl)-22-methoxymethyl-24-methyl-3α,5-cyclo-5α-cholestan-23,26-diol (**11**)*

$LiAlH_4$ (8 mg, 0.21 mmol) was added to a stirred ice cooled solution of lactone **10** (35 mg, 0.066 mmol) in Et_2O (2 mL). The reaction mixture was stirred at room temperature for 30 min, quenched with water (30μL), filtered and the filter cake was washed thoroughly with EtOAc (3 × 5 mL). The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether–EtOAc = 9:1⇒1:1) to give diol **11** (30 mg, 88%) as an oil. ^{1}H NMR δ: 0.32 (t, *J* = 4.2 Hz, 1H, C4–H), 0.60 (dd, *J* = 8.1, 4.6 Hz, 1H, C4–H), 0.73 (s, 3H, C18–H), 0.82 (d, *J* = 7.1 Hz, 3H, C28–H), 0.90 (d, *J* = 7.0 Hz, 3H, C21– or C27–H), 0.92 (d, *J* = 7.4 Hz, 3H, C27– or C21–H), 0.99 (s, 3H, C19–H), 3.30–3.37 (m, 2H, C23– and C26–H), 3.43 (s, 3H, CH_3OCH$_2$O), 3.53 (dd, *J* = 11.3, 9.0 Hz, 1H, C26–H), 3.67 (dd, *J* = 8.3, 0.8 Hz, 1H, C22–H), 3.74 (dd, *J* = 13.4, 6.4 Hz, 1H, –OCH$_2$CH_2O–), 3.83 (dd, *J* = 12.5, 6.5 Hz, 1H, –OCH$_2$CH_2O–), 3.90 (td, *J* = 7.9, 6.3 Hz, 1H, –OCH$_2$CH_2O–), 4.02 (td, *J* = 7.8, 6.2 Hz, 1H, –OCH$_2$CH_2O–), 4.63 (d, *J* = 6.6 Hz, 1H, CH$_3$OCH_2O–), 4.76 (d, *J* = 6.5 Hz, 1H, CH$_3$OCH_2O–). ^{13}C NMR δ: 6.39, 7.24, 12.12, 12.68, 17.93, 18.93, 22.51, 23.01, 24.11, 24.86, 28.41, 33.18, 34.08, 37.75, 38.03, 39.16, 40.08, 40.12, 40.89, 42.63, 45.52, 47.30, 52.01, 55.89, 56.22, 63.83, 64.60, 64.87, 74.92, 88.24, 99.29, 109.79. MS (APCI$^+$) *m/z* (%): 535 ([M+H]$^+$, 13), 503 ([M−MeOH+H]$^+$, 64), 485 [M−H_2O+H]$^+$, 84), 473 ([M−MeOH−CH_2O+H]$^+$ 100), 455 ([M−MeOH–CH_2O–H_2O+H]$^+$ 91).

*2.2.7. (22R,23R,24S,25R)-6-(1,3-Dioxolan-2-yl)-26-methanesulfonyloxy-22-methoxymethyl-24-methyl-3α,5-cyclo-5α-cholestan-23-ol (**12**)*

MsCl (0.015 mL, 0.19 mmol) was added to a stirred ice cooled solution of diol **11** (25 mg, 0.048 mmol) and Et_3N (0.054 mL, 0.39 mmol) in THF (2 mL). The reaction mixture was stirred at room temperature overnight and quenched with saturated aqueous $NaHCO_3$ (2 mL). The water layer was separated and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na_2SO_4), concentrated and the residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒1:1) to give mesylate **12** (23 mg, 79%) as an oil. ^{1}H NMR δ: 0.32 (t, *J* = 4.2 Hz, 1H, C4–H), 0.60 (dd, *J* = 8.1, 4.6 Hz, 1H, C4–H), 0.73 (s, 3H, C18–H), 0.88 (d, *J* = 6.8 Hz, 1H, >CHCH_3), 0.89 (d, *J* = 6.4 Hz, 3H, >CHCH_3), 1.00 (s, 1H, C19–H), 1.11 (d, *J* = 6.8 Hz, 3H, >CHCH_3), 3.00 (s, 3H, OMs), 3.34 (d, *J* = 8.2 Hz, 1H, C23–H), 3.43 (s, 3H, CH_3O-CH$_2$O–), 3.71 (d, *J* = 8.2 Hz, 1H, C22–H), 3.74 (dd, *J* = 13.5, 6.6 Hz, 1H, –OCH$_2$CH_2O–), 3.82–3.86 (m, 1H, –OCH$_2$CH_2O–), 3.90 (dt, *J* = 7.9, 6.3 Hz, 1H, –OCH$_2$CH_2O–), 3.99–4.05 (m, 1H, –OCH$_2$CH_2O–), 4.18 (dd, *J* = 9.6, 6.4 Hz, 1H, C26–H), 4.39 (dd, *J* = 9.6, 3.9 Hz, 1H, C26–H), 4.64 (d, *J* = 6.5 Hz, 1H, CH$_3$OCH_2O–), 4.76 (d, *J* = 6.5 Hz, 1H, CH$_3$OCH_2O–). ^{13}C NMR δ: 7.24, 9.72, 12.13, 12.69, 15.50, 18.93, 22.51, 23.02, 24.11, 24.86, 28.40, 33.19, 34.09, 35.60, 36.27, 36.99, 37.50, 39.17, 40.12, 42.65, 45.54, 47.33, 52.03, 55.81, 56.25, 64.61, 64.87, 68.05, 71.81, 73.76, 88.49, 99.31, 109.79. MS (APCI$^+$) *m/z* (%): 613 ([M+H]$^+$, 9), 581 ([M−MeOH+H]$^+$, 100), 551 [M−MeOH−CH_2O+H]$^+$, 21).

*2.2.8. (22E,24R)-6-(1,3-Dioxolan-2-yl)-26-methanesulfonyloxy-24-methyl-3α,5-cyclo-5α-cholest-22-ene (**13a**)*

A solution of ester **8a** (0.39 g, 0.78 mmol) in Et_2O (5 mL) was added to an ice cooled stirred suspension of $LiAlH_4$ (30 mg, 0.78 mmol) in Et_2O (2 mL). The reaction mixture was stirred at room temperature for 30 min, quenched at 0 °C with water (0.11 mL) and filtered. The filter cake was washed thoroughly with EtOAc (3 × 5 mL). The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether–EtOAc = 9:1⇒7:3) to give the 26-alcohol as an oil (0.34 g). It was dissolved, without further purification, in CH_2Cl_2 (7 mL), and Et_3N (0.5 mL, 3.6 mmol) and MsCl (0.1 mL, 1.3 mmol) were added at 0 °C. The reaction mixture was stirred at room temperature for 10 min and quenched with saturated aqueous $NaHCO_3$. The water layer was separated and extracted with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒7:3) to give mesylate **13a** (0.39 g, 94%) as an oil. ^{1}H NMR δ: 0.32 (t, *J* = 4.1 Hz, 1H, C4–H), 0.61 (dd, *J* = 8.1, 4.6 Hz, 1H, C4–H), 0.72 (s, 3H, C18–H), 0.90 (t, *J* = 6.7 Hz, 3H, >CHCH_3), 0.99 (d, *J* = 5.3 Hz, 3H, >CHCH_3), 1.00 (s, 1H, C19–H), 2.98, 2.99 (s, 3H, OMs), 3.74 (q, *J* = 6.6 Hz, 1H, –OCH$_2$-CH$_2$O–), 3.84 (dd, *J* = 12.5, 6.4 Hz, 1H, –OCH$_2$CH_2O–), 3.94–3.86 (m, 1H, –OCH$_2$CH_2O–), 4.06–3.96 (m, 2H, C26–H), 4.11 (dd, *J* = 9.4, 6.3 Hz, 1H, –OCH$_2$CH_2O–), 5.19–5.10 (m, 1H, C22– or C23–H), 5.28–5.20 (m, 1H, C23– or C22–H). ^{13}C NMR δ: 7.24, 12.34, 12.50, 13.91, 17.30, 18.57, 18.96, 20.76, 22.57, 23.01, 24.19, 24.88, 28.93, 29.68, 33.20, 34.07, 37.21, 37.66, 38.08, 38.56, 39.21, 39.95, 40.13, 40.34, 42.73, 45.58, 47.37, 55.70, 55.82, 56.27, 64.60, 64.84, 73.46, 73.57, 109.85, 128.67, 130.50, 137.50, 138.29. MS (APCI$^+$) *m/z* (%): 535 ([M+H]$^+$, 100).

*2.2.9. (24S)-6-(1,3-Dioxolan-2-yl)-26-methanesulfonyloxy-22,23-epoxy-24-methyl-3α,5-cyclo-5α-cholestane (**14a**)*

Variant A. $NaHCO_3$ (0.45 g, 5.5 mmol) and MCPBA (0.22 g, 0.982 mmol) were added sequentially to a stirred solution of olefin **13a** (99 mg, 0.185 mmol) in a mixture of CH_2Cl_2 (2 mL) and *i*-PrOH (2 mL). The resulting suspension was stirred at room temperature for 48 h, diluted with EtOAc (5 mL) and quenched with a 10% aqueous solution of Na_2CO_3 (5 mL). The water layer was separated and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒3:2) to give unreacted **8a** (8 mg, 8%) and a mixture (*RR*:*SS* = 70:30, based on relative intensity of signals at δ 2.75 and δ 2.78 attributed to epoxide proton of (22*R*,23*R*,25*R*/*S*) isomer, and signals at δ 2.39–2.54 attributed to epoxide proton of

(22*R*,23*R*,25*R/S*) isomer and two epoxide protons of (22*S*,23*S*,25*R/S*) isomer) of isomeric epoxides **14a** (85 mg, 84%, the yield was 91% based on recovered starting material). ^{1}H NMR δ: 0.31 (t, J = 4.2 Hz, 1H, C4–H), 0.60 (dd, J = 8.0, 4.6 Hz, 1H, C4–H), 0.69 (s, 3H, C18–H), 0.99 (s, 3H, C19–H), 2.39–2.54 (m, 1.3H, epoxide), 2.75 (dd, J = 5.6, 1.8 Hz, 0.14H, epoxide), 2.78 (dd, J = 5.0, 2.0 Hz, 0.56H, epoxide), 3.00 (s, 1H, OMs), 3.71–3.76 (m, 1H, $-OCH_2-CH_2O-$), 3.80–3.85 (m, 1H, $-OCH_2CH_2O-$), 3.86–3.91 (m, 1H, $-OCH_2CH_2O-$), 3.98–4.03 (m, 1H, $-OCH_2CH_2O-$), 4.07–4.14 (m, 1H, C26–H), 4.22–4.28 (m, 1H, C26–H). MS (APCI$^+$) m/z (%): 551 ([M+H]$^+$, 100), 533 ([M−H_2O+H]$^+$, 8), 455 ([M−MsOH+H]$^+$, 9).

Variant B. Olefin **13a** (30 mg, 0.056 mmol) and 1,1,1-trifluoroacetone (63 mg, 0.56 mmol) were dissolved in an ice cooled mixture of MeCN (0.2 mL), dimethoxymethane (DMM) (0.4 mL) and an aqueous 4×10^{-4}M solution of Na_2EDTA (0.2 mL). A mixture of Oxone (0.35 g, 0.56 mmol) and $NaHCO_3$ (0.15 g, 1.79 mmol) was added in portions to the vigorously stirred reaction mixture at 0 °C over 2 h. After stirring for 15 min, EtOAc (5 mL) and water (5 mL) were added, and the water layer was separated and extracted with EtOAc (2×3 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒3:2) to give isomeric mixture (*RR*:*SS* = 1:4, based on relative intensity of signals at δ 2.75 and δ 2.78 attributed to epoxide proton of (22*R*,23*R*,25*R/S*) isomer, and signals at δ 2.39–2.54 attributed to epoxide proton of (22*R*,23*R*,25*R/S*) isomer and two epoxide protons of (22*S*,23*S*,25*R/S*) isomer) of epoxides **14a** (24 mg, 78%). ^{1}H NMR δ: 0.31 (t, J = 4.2 Hz, 1H, C4–H), 0.60 (dd, J = 8.0, 4.6 Hz, 1H, C4–H), 0.69 (s, 3H, C18–H), 0.96 (d, J = 6.5 Hz, 1H, >CHC*H*$_3$), 0.99 (s, 3H, C19–H), 1.00 (d, J = 6.2 Hz, 1H, >CHC*H*$_3$), 1.08 (d, J = 7.0 Hz, 1H, >CHC*H*$_3$), 2.39–2.54 (m, 1.8H, epoxide), 2.75 (dd, J = 5.6, 1.8 Hz, 0.04H, epoxide), 3.01 (s, 3H, OMs), 2.78 (dd, J = 5.0, 2.0 Hz, 0.16H, epoxide), 3.69–3.77 (m, 1H, OCH_2CH_2O), 3.79–3.86 (m, 1H, OCH_2CH_2O), 3.86–3.92 (m, 1H, OCH_2CH_2O), 3.98–4.05 (m, 1H, OCH_2CH_2O), 4.13 (dd, J = 9.6, 6.9 Hz, 0.8H, H-26), 4.08–4.13 (m, 1H, 0.2H, H-26), 4.25 (dd, J = 9.6, 6.5 Hz, 0.8H, H-26), 4.25–4.29 (m, 1H, 0.2H, H-26).

2.2.10. (24S)-6β-Methoxy-26-methanesulfonyloxy-22,23-epoxy-24-methyl-3α,5-cyclo-5α-cholestane (**14b**)

Compound **14b** (75 mg, an isomeric mixture at C-22, C-23, and C-25 positions) was prepared in 73% yield (91% based on recovered starting material) as an oil from *(22E,24R)-6-(1,3-dioxolan-2-yl)-26-methanesulfonyloxy-24-methyl-3α,5-cyclo-5α-cholest-22-ene* **13b** [8] as described above for the preparation of epoxide **14a** (variant A). ^{1}H NMR δ: 0.42 (dd, J = 7.51, 5.10 Hz, 1H, C4–H), 0.59–0.65 (m, 1H, C4–H), 0.69 (s, 1H, C18–H), 1.00 (s, 1H, C19–H), 2.39–2.54 (m, 1.3H, epoxide), 2.73–2.80 (m, 1.7H, epoxide and C6–H), 3.00 (s, 3H, OMs), 3.31 (s, 3H, OMe), 4.05–4.15 (m, 1H, C26–H), 4.28–4.22 (m, 1H, C26–H). MS (APCI$^+$) m/z (%): 523 ([M+H]$^+$, 17), 491 ([M−MeOH+H]$^+$, 100), 473 ([M−MeOH−H_2O+H]$^+$, 82), 395 ([M−MeOH−MsOH+H]$^+$, 18), 371 (19).

2.2.11. (24S)-6-(1,3-Dioxolan-2-yl)-23,26-thio-24-methyl-3α,5-cyclo-5α-cholestan-22-ol (**15a**)

$Na_2S{\cdot}9H_2O$ (0.66 g, 2.75 mmol) was added to a solution of **14a** (0.19 g, 0.35 mmol) in DMF (11 mL). The reaction mixture was stirred at 85 °C for 5 h and evaporated under reduced pressure. The residue was diluted with water (20 mL) and extracted with EtOAc (3×10 mL). The combined organic layers were dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 20:1⇒4:1) to give compound **15a** (0.16 g, 94%). ^{1}H NMR signals of major isomer δ: 0.32 (t, J = 4.0 Hz, 1H, C4–H), 0.60 (dd, J = 7.9, 4.6 Hz, 1H, C4–H), 0.74 (s, 1H, C18–H), 0.83 (d, J = 6.83 Hz, 3H, >CHC*H*$_3$), 0.97 (d, J = 7.0 Hz, 3H, >CHC*H*$_3$), 0.99 (s, 1H, C19–H), 1.03 (d, J = 6.7 Hz, 3H, >CHC*H*$_3$), 2.48 (t, J = 10.0 Hz, 1H, C26–H), 2.78 (dd, J = 10.0, 6.3 Hz, 1H, C26–H), 2.93 (dd, J = 10.2, 1.9 Hz, 1H, C23–H), 3.47 (d, J = 10.2 Hz, 1H, C22–H), 3.70–3.78 (m, 1H, $-OCH_2CH_2O-$), 3.80–3.86 (m, 1H, $-OCH_2CH_2O-$), 3.86–3.92 (m, 1H, $-OCH_2CH_2O-$), 3.98–4.04 (m, 1H, $-OCH_2CH_2O-$). MS (APCI$^+$) m/z (%): 489 ([M+H]$^+$, 22), 471 ([M−H_2O+H]$^+$, 100).

2.2.12. (24S)-6β-Methoxy-23,26-thio-24-methyl-3α,5-cyclo-5α-cholestan-22-ol (**15b**)

The title compound (165 mg, an isomeric mixture at C-22, C-23, and C-25 positions) was prepared in 97% yield as an oil from epoxide **14b** as described above for the preparation of tetrahydrothiophene **15a**. ^{1}H NMR signals of major isomer δ: 0.42 (dd, J = 7.9, 5.0 Hz, 1H, C4–H), 0.60–0.66 (t, J = 4.4 Hz, 1H, C4–H), 0.74 (s, 3H, C18–H), 0.83 (d, J = 6.84 Hz, 1H, >CHC*H*$_3$), 0.96 (d, J = 7.02 Hz, 1H, >CHC*H*$_3$), 1.01 (s, 3H, C19–H), 1.02 (d, J = 6.8 Hz, 3H, >CHC*H*$_3$), 2.47 (t, J = 10.1 Hz, 1H, C26–H), 2.74–2.80 (m, 2H, C6– and C26–H), 2.92 (dd, J = 10.3, 2.1 Hz, 1H, C23–H), 3.31 (s, 3H, OMe), 3.48 (d, J = 10.2 Hz, 1H, C22–H). MS (APCI$^+$) m/z (%): 443 ([M−H_2O+H]$^+$, 100), 429 ([M−MeOH+H]$^+$, 20), 411 ([M−H_2O−MeOH+H]$^+$, 43).

2.2.13. Reduction of tetrahydrothiophene (**15a**) with Raney nickel

A solution of tetrahydrothiophene **15a** (82.6 mg, 0.169 mmol) in EtOH (6 mL) was added via syringe to a stirred suspension of a large excess of freshly prepared Raney-Ni (W-7) in EtOH (3 mL) under a hydrogen atmosphere. The reaction mixture was stirred for 12 h under hydrogen atmosphere and then the liquid was decanted. The catalyst was washed with EtOH (3×5 mL) and the combined organic phases were evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 20:1⇒4:1) to give an inseparable mixture of **16a** and **17a** (**16a**:**17a** = 2:1, 66.1 mg, 85%) as an oil. ^{1}H NMR δ: 0.32 (t, J = 4.2 Hz, 1H, C4–H), 0.60 (dd, J = 8.0, 4.6 Hz, 1H, C4–H), 0.72 and 0.73 (s, 1H, C18–H), 0.80 (d, J = 6.8 Hz, 3H, >CHC*H*$_3$), 0.82 (d, J = 6.5 Hz, 3H, >CHC*H*$_3$), 0.86 (d, J = 6.6 Hz, 3H, >CHC*H*$_3$), 0.88 (d, J = 6.3 Hz, 3H, >CHC*H*$_3$), 1.00 (s, 3H, C19–H), 3.69–3.78 (m, 2H, $-OCH_2CH_2O-$ and C22–H), 3.81–3.86 (m, 1H, $-OCH_2CH_2O-$), 3.87–3.92 (m, 1H, OCH_2CH_2O-), 3.99–4.04 (m, 1H, $-OCH_2CH_2O-$). ^{13}C NMR δ: 7.24, 11.20, 12.09, 12.17, 12.24, 14.89, 15.75, 17.80, 18.30, 18.95, 19.90, 19.97, 22.53, 22.57, 23.05, 24.09, 24.24, 24.87, 27.29, 27.75, 27.80, 29.67, 31.98, 33.19, 33.39, 34.04, 34.11, 34.14, 34.50, 35.28, 39.20, 39.27, 39.35, 40.05, 40.08, 40.16, 42.33, 42.72, 43.08, 45.56, 47.35, 47.41, 52.60, 53.11, 55.79, 56.10, 56.15, 64.60, 64.84, 70.89, 71.60, 109.83, 109.88. MS (APCI$^+$) m/z (%): 459 ([M+H]$^+$, 26), 441 ([M−H_2O+H]$^+$, 100), 397 ([M−OHCH$_2$CH$_2$OH+H]$^+$, 8).

2.2.14. Reduction of tetrahydrothiophene (**15b**) with Raney nickel

The reaction was carried out as described above for the reduction of tetrahydrothiophene **15a**. The obtained mixture of isomeric alcohols **16b** and **17b** was separated by column chromatography on silica gel (petroleum ether–EtOAc = 20:1⇒3:1) to afford:

(22S,24R)-6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholestan-22-ol (**16b**) as an oil (60 mg, 52%). ^{1}H NMR δ: 0.43 (dd, J = 8.1, 5.1 Hz, 1H, C4–H), 0.64 (t, J = 4.3 Hz, 1H, C4–H), 0.72 (s, 3H, C18–H), 0.80 (d, J = 6.8 Hz, 3H, >CHC*H*$_3$), 0.82 (d, J = 6.7 Hz, 3H, >CHC*H*$_3$), 0.86 (d, J = 6.1 Hz, 3H, >CHC*H*$_3$), 0.88 (d, J = 4.9 Hz, 3H, >CHC*H*$_3$), 1.02 (s, 3H, C19–H), 2.75–2.78 (m, 1H, C6–H), 3.32 (s, 3H, OMe), 3.77 (t, J = 6.9 Hz, 1H, C22–H). ^{13}C NMR δ: 11.19, 12.16, 13.05, 15.75, 17.80, 19.25, 19.96, 21.41, 22.76, 24.08, 24.92, 27.86, 30.52, 31.99, 33.31, 35.06, 35.16, 35.27, 39.28, 39.32, 40.24, 42.66, 43.33, 47.93, 52.64, 56.39, 56.55, 71.65, 82.36. MS (APCI$^+$) m/z (%): 399 ([M−MeOH+H]$^+$, 7), 381 ([M−H_2O−MeOH+H]$^+$, 100).

(22R,24R)-6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholestan-22-ol (**17b**) as an oil (39 mg, 27%). ^{1}H NMR δ: 0.43 (dd, J = 8.1, 5.1 Hz, 1H, C4–H), 0.65 (t, J = 4.5 Hz, 1H, C4–H), 0.74 (s, 3H, C18–H), 0.82 (d, J = 6.7 Hz, 3H, >CHC*H*$_3$), 0.83 (d, J = 6.4 Hz, 3H, >CHC*H*$_3$), 0.87 (d,

J = 6.4 Hz, 3H, >CHCH_3), 0.91 (d, J = 6.8 Hz, 3H, >CHCH_3), 1.02 (s, 3H, C19–H), 2.77 (m, 1H, C6–H), 3.32 (s, 3H, OMe), 3.74 (dd, J = 10.6, 2.6 Hz, 1H, C22–H). ^{13}C NMR δ: 12.26 (×2), 13.05, 14.92, 18.32, 19.27, 19.90, 21.46, 22.78, 24.27, 24.95, 27.37, 30.50, 33.33, 33.41, 34.07, 34.53, 35.03, 35.24, 40.24, 42.36, 43.04, 43.36, 48.03, 53.21, 56.07, 56.55, 70.97, 82.35. MS (APCI$^+$) m/z (%): 399 ([M−MeOH+H]$^+$, 8), 381 ([M−H_2O−MeOH+H]$^+$, 100).

2.2.15. (24R)-6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholestan-22-one (**18**)

A solution of Dess-Martin periodinane in CH_2Cl_2 (15%, 0.94 mL, 140 mg, 0.33 mmol) was added to a stirred solution of alcohol **17b** (115 mg, 0.267 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h and quenched with a mixture of saturated aqueous solutions of $NaHCO_3$ (2.5 mL) and $Na_2S_2O_3$ (2.5 mL). The water layer was separated and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography on silica gel (ethyl acetate-petrol ether) to give ketone **18** as an oil (85 mg, 74%). ^{1}H NMR δ: 0.42 (dd, J = 8.1, 5.0 Hz, 1H, C4–H), 0.64 (t, J = 4.4 Hz, 1H, C4–H), 0.72 (s, 3H, C18–H), 0.78 (d, J = 6.8 Hz, 3H, >CHCH_3), 0.81 (d, J = 6.8 Hz, 3H, >CHCH_3), 0.85 (d, J = 6.8 Hz, 3H, >CHCH_3), 1.01 (s, 3H, C19–H), 1.06 (d, J = 6.9 Hz, 3H, >CHCH_3), 2.23 (dd, J = 16.9, 9.3 Hz, 1H), 2.35 (dd, J = 17.0, 4.1 Hz, 1H), 2.49 (dq, J = 10.4, 6.9 Hz, 1H), 2.76 (t, J = 2.9 Hz, 1H, C6–H), 3.31 (s, 3H, OMe). ^{13}C NMR δ: 12.43, 13.06, 15.85, 16.27, 18.33, 19.25, 19.83, 21.41, 22.68, 24.39, 24.91, 27.68, 30.45, 32.06, 33.32, 33.49, 35.04, 35.16, 40.07, 42.85, 43.34, 46.59, 47.96, 49.70, 51.86, 55.81, 56.54, 82.30, 214.39. MS (APCI$^+$) m/z (%): 429 ([M+H]$^+$, 70), 397 ([M−MeOH+H]$^+$, 100), 379 ([M−MeOH−H_2O+H]$^+$, 23), 282 (22), 255 (13).

2.2.16. (22S,24R)-6β-Methoxy-24-methyl-3α,5-cyclo-5α-cholestan-22-ol (**16b**)

A solution of ketone **18** (95 mg, 0.22 mmol) in Et_2O (1 mL) was added to an ice cooled stirred suspension of $LiAlH_4$ (17 mg, 0.45 mmol) in Et_2O (2 mL). The reaction mixture was stirred at room temperature for 30 min, quenched with water (0.07 mL), and filtered. The filter cake was washed thoroughly with EtOAc (3 × 5 mL). The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether/EtOAc) to give an oily product (78 mg, 82%). The NMR data were identical with those described above for alcohol **16b**.

2.2.17. Acid catalyzed hydrolysis of a mixture of dioxolanes **16a** and **17a**

PPTS (16 mg) was added to a solution of the dioxolanes **16a** and **17a** (159 mg, 0.347 mmol) in 80% aqueous acetone (6 mL). After 3 h the solvents were evaporated under reduced pressure and the remaining residue was subjected to column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒3:2) to give:

(22S,24R)-24-Methyl-3α,5-cyclo-5α-cholestan-6-on-22-ol (22*S*-**19**) (89 mg, 62%) as white crystals. Mp 179–182 °C (MeCN). ^{1}H NMR δ: 0.72 (s, 3H, C18–H), 0.80 (d, J = 6.9 Hz, 3H, >CHCH_3), 0.82 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.86 (d, J = 6.9 Hz, 3H, >, >CHCH_3), 0.89 (d, J = 6.7 Hz, 3H, >CHCH_3), 1.00 (s, 3H, C19–H), 3.76 (td, J = 6.8, 1.7 Hz, 1H, C22–H). ^{13}C NMR δ: 11.21, 11.62, 11.94, 15.75, 17.79, 19.66, 19.95, 22.84, 23.96, 25.86, 27.72, 31.99, 33.42, 34.79, 35.29 (×2), 39.31, 39.35, 39.67, 42.59, 44.73, 45.99, 46.28, 46.71, 52.40, 56.84, 71.52, 209.67. MS (APCI$^+$) m/z (%): 415 ([M+H]$^+$, 100), 397([M−H_2O]$^+$, 17).

(22R,24R)-24-Methyl-3α,5-cyclo-5α-cholestan-6-on-22-ol (22*R*-**19**) (47 mg, 33%) as an oil. ^{1}H NMR δ: 0.73 (s, 3H, C18–H), 0.81 (d, J = 6.6 Hz, 3H, >CHCH_3), 0.83 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.87 (d, J = 6.5 Hz, 3H, >CHCH_3), 0.92 (d, J = 6.7 Hz, 3H, >CHCH_3), 1.00 (s, 3H, C19–H), 3.67–3.78 (m, 1H, C22–H). ^{13}C NMR δ: 11.65, 12.05, 12.27, 14.90, 18.31, 19.65, 19.88, 22.83, 24.12, 25.86, 27.18, 33.38, 33.42, 34.04, 34.48, 34.74, 35.37, 39.64, 42.20, 42.96, 44.73, 46.04, 46.27, 46.68, 52.96, 56.49, 70.81, 209.67. MS (APCI$^+$) m/z (%): 415 ([M+H]$^+$, 100), 397([M−H_2O]$^+$, 17).

2.2.18. (22S,24R)-22-Acetoxy-24-methyl-3α,5-cyclo-5α-cholestan-6-one (**20**)

Ac_2O (0.024 mL, 0.25 mmol) was added to a stirred solution of alcohol **19** (54 mg, 0.13 mmol) and DMAP (3 mg, 0.03 mmol) in pyridine (1.3 mL). The reaction mixture was stirred at room temperature overnight, and then evaporated under reduced pressure. The residue was distributed between EtOAc (5 mL) and H_2O (5 mL). The aqueous layer was separated and extracted with EtOAc (3 × 3 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 20:1⇒9:1) to give acetate **20** (59 mg, 99%) as an oil. ^{1}H NMR δ: 0.71 (s, 3H, C18–H), 0.79 (d, J = 6.9 Hz, 3H, >CHCH_3), 0.82 (d, J = 6.6 Hz, 3H, >CHCH_3), 0.84 (d, J = 6.6 Hz, 3H, >CHCH_3), 0.95 (d, J = 6.9 Hz, 3H, >CHCH_3), 0.99 (s, 3H, C19–H), 2.02 (s, 3H, OAc), 5.04 (t, J = 7.1 Hz, 1H, C22–H). ^{13}C NMR δ: 11.59, 11.80, 12.56, 15.44, 17.91, 19.64, 19.83, 21.28, 22.83, 23.95, 25.85, 28.03, 32.07, 33.41, 34.75, 35.00, 35.26, 35.63, 38.10, 39.61, 42.57, 44.66, 45.98, 46.24, 46.68, 52.40, 56.80, 74.80, 170.72, 209.59. MS (APCI$^+$) m/z (%): 457 ([M+H]$^+$, 100), 397 ([M−AcOH+H]$^+$, 21).

2.2.19. (22S,24R)-3β,22-Diacetoxy-24-methyl-5α-cholestan-6-one (**21**)

An aqueous solution of H_2SO_4 (15%, 0.015 mL) was added to a solution of cyclopropyl ketone **20** (58 mg, 0.126 mmol) in AcOH (4 mL). The reaction mixture was stirred at 110 °C for 3 h and the volatiles were evaporated under reduced pressure. The residue was diluted with EtOAc (5 mL) and H_2O (5 mL). The water layer was separated and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒7:3) to give diacetate **21** (47 mg, 72%) as white crystals. Mp 213–215 °C (MeCN). ^{1}H NMR δ: 0.65 (s, 3H, C18–H), 0.75 (s, 3H, C19–H), 0.78 (d, J = 6.9 Hz, 3H, >CHCH_3), 0.82 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.83 (d, J = 6.9 Hz, 3H, >CHCH_3), 0.94 (d, J = 6.7 Hz, 3H, >CHCH_3), 2.01 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.29 (dd, J = 13.2, 4.5 Hz, 1H, C5–H), 4.65 (tt, J = 11.7, 4.7 Hz, 1H, C3–H), 5.03 (t, J = 7.0 Hz, 1H, C22–H). ^{13}C NMR δ: 11.78, 12.51, 12.99, 15.43, 17.89, 19.82, 21.27, 21.30, 21.41, 23.85, 26.05, 26.76, 27.90, 32.06, 35.00, 35.63, 36.36, 37.87, 38.08, 39.33, 40.85, 42.83, 46.51, 52.42, 53.74, 56.40, 56.48, 72.75, 74.74, 170.55, 170.70, 210.21. MS (APCI$^+$) m/z (%): 517 ([M+H]$^+$, 7), 457 ([M−AcOH+H]$^+$, 65), 397 ([M−2AcOH+H]$^+$, 100).

2.2.20. (22S,24R)-24-Methyl-5α-cholestan-6-one-3β,22-diol (**1**)/cathasterone/

A solution of diacetate **21** (47 mg, 0.09 mmol) in methanolic KOH (5%, 7 mL) was stirred at 45 °C for 10 h. The solvent was evaporated under reduced pressure and the residue was distributed between EtOAc (5 mL) and H_2O (5 mL). The water layer was separated and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 4:1⇒1:2) to give cathasterone **1** (37 mg, 76%). Mp 175–177 °C (EtOAc). Lit. [9] mp 176–177.5 °C, lit. [6] mp 188–190 °C. The ^{1}H and ^{13}C NMR data agreed with the literature values [6,9]. MS (APCI$^+$) m/z (%): 433 ([M+H]$^+$, 31), 415 ([M−H_2O+H]$^+$, 82), 397 ([M−2H_2O+H]$^+$, 100), 379 ([M−3H_2O+H]$^+$, 20).

*2.2.21. (22S,24R)-24-Methylcholest-5-en-22-ol (**2**)/(22S)-22-hydroxycampesterol/*

A mixture of ether **16b** (90 mg, 0.209 mmol), $TsOH{\cdot}H_2O$ (9 mg), dioxane (9 mL) and H_2O (3 mL) was stirred at 75 °C for 3 h. The solvents were evaporated under reduced pressure. The residue was diluted with water (10 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 4:1⇒1:1) to give (22S)-22-hydroxycampesterol **2** (70 mg, 80%). Mp 187–190 °C (EtOAc). Lit. [6] mp 193–195 °C. ^{1}H NMR δ: 0.71 (s, 3H, C18–H), 0.82 (d, J = 6.8 Hz, 3H, >CHCH_3), 0.85 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.89 (d, J = 6.9 Hz, 3H, >CHCH_3), 0.91 (d, J = 6.4 Hz, 3H, >CHCH_3), 1.02 (s, 3H, 19-H), 3.52 (tt, J = 11.0, 4.5 Hz, 1H, C3–H), 3.77 (t, J = 6.7 Hz, 1H, C22–H), 5.35 (dd, J = 5.1, 2.5 Hz, 1H, C5–H). ^{13}C NMR δ: 11.36, 11.83, 15.92, 17.89, 19.41, 19.99, 21.21, 24.28, 27.86, 31.81, 31.94, 32.12, 32.16, 35.66, 36.60, 37.38, 39.65 (×2), 39.96, 42.39, 42.44, 50.31, 52.80, 56.83, 71.84, 71.93, 121.61, 140.93. MS (APCI$^+$) m/z (%): 399 ([M−H_2O+H]$^+$, 64), 381 ([M−2H_2O+H]$^+$, 100).

*2.2.22. (22S,24R)-24-Methyl-5α-cholestan-22-ol (**3**)/6-deoxocathasterone/*

A mixture of 10% Pd/C (10 mg), alkene **2** (37 mg, 0.089 mmol) and EtOH (4 mL) was stirred under hydrogen atmosphere overnight and then filtered. The filter cake was washed thoroughly with EtOH. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether–EtOAc = 4:1⇒1:1) to give 6-deoxocathasterone **3** (35 mg, 95%). Mp 188–191 °C (EtOAc). Lit. [6] mp 200–202 °C. ^{1}H NMR δ: 0.66 (s, 3H, C18–H), 0.79 (s, 3H, C19–H), 0.80 (d, J = 6.8 Hz, 3H, >CHCH_3), 0.81 (d, J = 7.8 Hz, 3H, >CHCH_3), 0.86 (d, J = 6.5 Hz, 3H, >CHCH_3), 3.58 (tt, J = 10.7, 4.8 Hz, 1H, C3–H), 3.75 (t, J = 6.8 Hz, 1H, C22–H). ^{13}C NMR δ: 11.16, 11.98, 12.30, 15.74, 17.80, 19.96, 21.24, 24.11, 27.80, 28.66, 31.46, 31.99 (×2), 35.25, 35.41, 35.50, 36.95, 38.14, 39.26, 39.31, 39.99, 42.49, 44.78, 52.60, 54.27, 56.39, 71.29, 71.63. MS (APCI$^+$) m/z (%): 401 ([M−H_2O+H]$^+$, 23), 383 ([M−2H_2O+H]$^+$, 100).

*2.2.23. (24S)-[26,26-$^2H_2$]-6-(1,3-Dioxolan-2-yl)-26-methanesulfonyloxy-22,23-epoxy-24-methyl-3α,5-cyclo-5α-cholestane (**22**)*

A solution of ester **8a** (0.82 g, 1.64 mmol) in Et_2O (6 mL) was added to an ice cooled stirred suspension of $LiAlD_4$ (138 mg, 3.29 mmol) in Et_2O (4 mL). The reaction mixture was stirred at room temperature for 30 min, quenched at 0 °C with water (0.47 mL) and filtered. The filter cake was washed thoroughly with EtOAc (3 × 10 mL). The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether–EtOAc = 4:1⇒3:1) to give *(24R)-[26,26-$^2H_2$]-6-(1,3-dioxolan-2-yl)-24-methyl-3α,5-cyclo-5α-cholest-22-en-26-ol* (0.69 g, 92%) as an oil.

This compound was mesylated as described above for the preparation of compound **13a**, providing *[26,26-$^2H_2$]-6-(1,3-dioxolan-2-yl)-26-methanesulfonyloxy-24-methyl-3α,5-cyclo-5α-cholest-22-ene* in 99% yield as an oil.

This compound was dissolved in CH_2Cl_2 (10 mL), then $NaHCO_3$ (1.23 g, 14.7 mmol) and MCPBA (0.58 g, 2.61 mmol) were added sequentially to a stirred solution. The resulting suspension was stirred at room temperature for 3 h and quenched with 10% aqueous solution of Na_2CO_3 (20 mL). The water layer was separated and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒3:2) to give an isomeric mixture (*RR*:*SS* = 70:30) of epoxides **22** (0.57 g, 70%). ^{1}H NMR δ: 0.32 (t, J = 4.1 Hz, 1H, C4–H), 0.60 (dd, J = 8.1, 4.6 Hz, 1H, C4–H), 0.69 (s, 3H, C18–H), 0.99 (s, 3H, C19–H), 2.54–2.39 (m, 1.3H, epoxide), 2.75 (dd, J = 5.6, 2.2 Hz, 0.14H, epoxide), 2.78 (dd, J = 5.1, 2.2 Hz, 0.56H, epoxide), 3.01 (s, 3H, OMs), 3.76–3.71 (m, 1H, –OCH_2CH_2O–), 3.85–3.81 (m, 1H, –OCH_2CH_2O–), 3.92–3.86 (m, 1H, –OCH_2CH_2O–), 4.04–3.98 (m, 1H, –OCH_2CH_2O–). MS (APCI$^+$) m/z (%): 553 ([M+H]$^+$, 18), 509 ([M−CH_2CH_2O+H]$^+$, 100).

*2.2.24. (24S)-[26,26-$^2H_2$]-6-(1,3-Dioxolan-2-yl)-23,26-thio-24-methyl-3α,5-cyclo-5α-cholestan-22-ol (**23**)*

The title compound was prepared in 97% (141 mg) yield as an oil from epoxide **22** as described above for the preparation of tetrahydrothiophene **15a**. ^{1}H NMR signals of the major isomer δ: 0.32 (t, J = 3.9 Hz, 1H, C4–H), 0.60 (dd, J = 8.0, 4.7 Hz, 1H, C4–H), 0.74 (s, 3H, C18–H), 0.83 (d, J = 6.8 Hz, 3H, >CHCH_3), 0.97 (d, J = 7.03 Hz, 3H, >CHCH_3), 1.00 (s, 3H, C19–H), 1.03 (d, J = 6.7 Hz, 3H, >CHCH_3), 2.93 (dd, J = 10.2, 1.9 Hz, 1H, C23–H), 3.48 (d, J = 10.1 Hz, 1H, C22–H), 3.77–3.71 (m, 1H, –OCH_2CH_2O), 3.86–3.81 (m, 1H, –OCH_2CH_2O–), 3.93–3.87 (m, 1H, –OCH_2CH_2O–), 4.05–3.99 (m, 1H, –OCH_2CH_2O–). MS (APCI$^+$) m/z (%): 491 ([M+H]$^+$, 25), 473 ([M−H_2O+H]$^+$, 100).

*2.2.25. (24R)-[26,26-$^2H_2$]-6-(1,3-Dioxolan-2-yl)-24-methyl-3α,5-cyclo-5α-cholestan-22-ol (**24**)*

The title compound was prepared from tetrahydrothiophene **23** as described above for the preparation of alcohols **16a** and **17a**. An inseparable mixture of C-22 epimers was obtained in 78% (65 mg) yield. ^{1}H NMR signals of the major isomer δ: 0.31 (t, J = 4.0 Hz, 1H, C4–H), 0.60 (dd, J = 7.7, 4.7 Hz, 1H, C4–H), 0.72 (s, 1H, C18–H), 0.81 (d, J = 6.5 Hz, 3H, >CHCH_3), 0.86 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.87 (d, J = 6.2 Hz, 3H, >CHCH_3), 0.99 (s, 3H, C19–H), 3.77–3.70 (m, 2H, –OCH_2CH_2O– and C22–H), 3.85–3.81 (m, 1H, –OCH_2CH_2O–), 3.92–3.87 (m, 1H, –OCH_2CH_2O–), 4.04–3.99 (m, 1H, –OCH_2CH_2O–). ^{13}C NMR δ: 7.24, 11.20, 12.08, 12.16, 12.23, 14.89, 15.75, 17.19 (p, J = 19 Hz), 17.75, 18.94, 19.85, 19.92, 22.53, 22.56, 23.04, 24.08, 24.24, 24.87, 27.28, 27.74, 27.79, 29.66, 31.82, 33.19, 33.23, 34.04, 34.11, 34.14, 34.46, 35.25, 39.19, 39.27, 39.35, 40.05, 40.08, 40.15, 42.32, 42.71, 43.07, 45.55, 47.34, 47.40, 52.59, 53.10, 55.79, 56.10, 56.15, 64.59, 64.84, 70.88, 71.59, 109.83, 109.87. MS (APCI$^+$) m/z (%): 461 ([M+H]$^+$, 22), 443 ([M−H_2O+H]$^+$, 100).

*2.2.26. (24R)-1-$^2H_2$]-6-(1,3-Dioxolan-2-yl)-24-methyl-3α,5-cyclo-5α-cholestan-22-one (**25**)*

The title compound was prepared in 94% (60 mg) yield as an oil from alcohols **16a** and **17a** as described above for the preparation of ketone **18**. ^{1}H NMR δ: 0.32 (t, J = 4.2 Hz, 1H, C4–H), 0.60 (dd, J = 8.1, 4.5 Hz, 1H, C4–H), 0.73 (s, 3H, C18–H), 0.78 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.85 (d, J = 6.9 Hz, 3H, >CHCH_3), 1.00 (s, 3H, C19–H), 1.07 (d, J = 7.0 Hz, 3H, >CHCH_3), 2.23 (dd, J = 17.0, 9.3 Hz, 1H), 2.35 (dd, J = 17.0, 4.0 Hz, 1H), 2.44–2.53 (m, 1H), 3.73 (q, J = 6.6 Hz, 1H, –OCH_2CH_2O–), 3.83 (q, J = 6.4 Hz, 1H, –OCH_2CH_2O–), 3.86–3.92 (m, 1H, –OCH_2CH_2O–), 4.01 (dt, J = 7.7, 6.0 Hz, 1H, –OCH_2CH_2O–). ^{13}C NMR δ: 7.26, 12.36, 15.87, 16.28, 18.94, 19.80, 22.56, 22.98, 24.40, 24.87, 27.61, 31.92, 33.23, 33.47, 34.07, 39.21, 39.93, 40.16, 42.91, 45.57, 46.47, 47.38, 49.74, 51.84, 55.59, 64.61, 64.85, 109.78, 214.24. MS (APCI$^+$) m/z (%): 459 ([M+H]$^+$, 100).

*2.2.27. (24R)-[22,26,26-$^2H_3$]-6-(1,3-Dioxolan-2-yl)-24-methyl-3α,5-cyclo-5α-cholestan-22-ol (**26**)*

A solution of ketone **25** (123 mg, 0.269 mmol) in Et_2O (1.5 mL) was added to an ice cooled stirred suspension of $LiAlD_4$ (11 mg, 0.26 mmol) in Et_2O (0.5 mL). The reaction mixture was stirred at room temperature for 30 min, then quenched with water (0.04 mL), and filtered. The filter cake was washed thoroughly with

EtOAc (3 × 5 mL). The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether–EtOAc = 9:1⇒3:1) to give an inseparable mixture of alcohols **26** (115 mg, 93%) as an oil. ^{1}H NMR signals of major isomer δ: 0.32 (t, J = 4.3 Hz, 1H, C4–H), 0.61 (dd, J = 8.2, 4.6 Hz, 1H, C4–H), 0.73 (s, 3H, C18–H), 0.82 (d, J = 6.6 Hz, 3H, >CHCH_3), 0.87 (d, J = 6.6 Hz, 3H, >CHCH_3), 0.88 (d, J = 5.3 Hz, 3H, >CHCH_3), 1.00 (s, 3H, C19–H), 3.74 (q, J = 6.6 Hz, 1H, –OCH$_2$CH$_2$O–), 3.84 (q, J = 6.3 Hz, 1H, –OCH$_2$CH$_2$O–), 3.90 (dt, J = 8.1, 6.3 Hz, 1H, –OCH$_2$CH$_2$O–), 4.02 (dt, J = 7.9, 6.0 Hz, 1H, –OCH$_2$CH$_2$O–). ^{13}C NMR δ: 7.25, 11.19, 12.10, 15.76, 17.20 (p, J = 18.9 Hz), 17.76, 18.95, 19.93, 22.58, 23.05, 24.09, 24.88, 27.80, 29.68, 31.84, 33.20, 34.15, 35.24, 39.20, 39.23, 40.09, 40.17, 42.72, 45.56, 47.36, 52.60, 56.16, 64.61, 64.85, 71.18 (t, J = 21 Hz), 109.88. MS (APCI$^+$) m/z (%): 462 ([M+H]$^+$, 29), 444 ([M−H$_2$O]$^+$, 100).

2.2.28. (22S,24R)-[22,26,26-$^2H_3$]-24-methyl-3α,5-cyclo-5α-cholestan-6-on-22-ol (**27**)

PPTS (10 mg) was added to a solution of compound **26** (95 mg, 0.207 mmol) in 90% aqueous acetone (10 mL). After 3 h the solvents were evaporated under reduced pressure and residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒2:1) to give compound **27** (72 mg, 83%) as white crystals. Mp178–181 °C (MeCN). ^{1}H NMR δ: 0.72 (s, 3H, C18–H), 0.81 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.86 (d, J = 6.8 Hz, 3H, >CHCH_3), 0.89 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.99 (s, 3H, C19–H), 2.42 (d, J = 12.9 Hz, 1H). ^{13}C NMR δ:11.18, 11.61, 11.93, 15.73, 17.19 (t, J = 19 Hz), 17.75, 19.65, 19.89, 22.83, 23.95, 25.85, 27.70, 29.65, 31.82, 33.41, 34.78, 35.21, 35.29, 39.16, 39.25, 39.66, 42.57, 44.72, 45.98, 46.26, 46.70, 52.38, 56.84, 71.07 (t, J = 21 Hz), 209.65. MS (APCI$^+$) m/z (%): 418 ([M+H]$^+$, 100), 400 ([M−H$_2$O+H]$^+$, 8).

2.2.29. (22S,24R)-[22,26,26-$^2H_3$]-22-Acetoxy-24-methyl-3α,5-cyclo-5α-cholestan-6-one (**28**)

The title compound was prepared in 99% (59 mg) yield as an oil from alcohol **27** as described above for the preparation of acetate **20**. ^{1}H NMR δ: 0.71 (s, 1H, C18–H), 0.82 (d, J = 6.3 Hz, 3H, >CHCH_3), 0.83 (d, J = 6.4 Hz, 3H, >CHCH_3), 0.95 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.98 (s, 1H, C19–H), 2.01 (s, 1H, OAc). ^{13}C NMR δ: 11.56, 11.79, 12.51, 15.43, 17.29 (p, J = 18.9 Hz), 17.85, 19.62, 19.77, 21.25, 22.82, 23.93, 25.84, 28.01, 31.91, 33.40, 34.74, 34.94, 35.22, 35.54, 37.98, 39.60, 42.55, 44.64, 45.97, 46.22, 46.66, 52.38, 56.79, 74.44 (t, J = 21 Hz), 170.68, 209.53. MS (APCI$^+$) m/z (%): 460 ([M+H]$^+$, 100), 400 ([M−AcOH+H]$^+$, 13).

2.2.30. (22S,24R)-[22,26,26-$^2H_3$]-3β,22-Diacetoxy-24-methyl-5α-cholestan-6-one (**29**)

The title compound was prepared in 72% (47 mg) yield from cyclopropyl ketone **28** as described above for the preparation of acetate **21**. Mp 214–216 °C (white crystals from MeCN). ^{1}H NMR δ: 0.65 (s, 3H, C18–H), 0.75 (s, 3H, C19–H), 0.82 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.83 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.94 (d, J = 6.7 Hz, 3H, >CHCH_3), 2.005 (s, 3H, OAc), 2.013 (s, 3H, OAc), 2.24 (dd, J = 12.7, 2.9 Hz, 1H), 2.29 (dd, J = 13.3, 4.5 Hz, 1H, C5–H), 4.65 (tt, J = 11.7, 4.7 Hz, 1H, C3–H). ^{13}C NMR δ: 11.77, 12.48, 12.98, 15.42, 17.29 (p, J = 19.0 Hz), 17.85, 19.77, 21.26, 21.30, 21.40, 23.84, 26.04, 26.76, 27.89, 31.90, 34.94, 35.54, 36.35, 37.86, 37.95, 39.32, 40.84, 42.81, 46.50, 52.40, 53.73, 56.38, 56.47, 72.74, 74.38 (t, J = 21 Hz), 170.54, 170.68, 210.19. MS (APCI$^+$) m/z (%): 520 ([M+H]$^+$, 11), 460 ([M−AcOH+H]$^+$, 83), 400 ([M−2AcOH+H]$^+$, 100).

2.2.31. (22S,24R)-[22,26,26-$^2H_3$]-24-Methyl-5α-cholestan-6-one-3β,22-diol (**30**)/[22,26,26-$^2H_3$]-cathasterone/

The title compound was prepared in 79% (37 mg) yield from diacetate **29** as described above for the preparation of cathasterone **1**. Mp 179–182 °C (white crystals from MeOH). ^{1}H NMR δ: 067 (s, 3H, C18–H), 0.74 (s, 3H, C19–H), 0.81 (d, J = 6.7 Hz, 3H, >CHCH_3), 0.86 (d, J = 7.0 Hz, 3H, >CHCH_3), 0.87 (d, J = 6.9 Hz, 3H, >CHCH_3), 2.30 (dd, J = 13.2, 4.5 Hz, 1H, C5–H), 3.56 (tt, J = 11.1, 4.7 Hz, 1H, C3–H). ^{13}C NMR δ: 11.15, 11.93, 13.12, 15.76, 17.20 (p, J = 19.0 Hz), 17.76, 19.90, 21.51, 23.88, 27.60, 29.99, 30.65, 31.84, 35.26, 36.63, 37.93, 39.15, 39.32, 39.47, 40.91, 42.85, 46.64, 52.45, 53.84, 56.63, 56.74, 70.59, 71.13 (t, J = 21 Hz), 210.84. MS 186 (APCI$^+$) m/z (%): 436 ([M+H]$^+$, 30), 418 [M−H$_2$O+H]$^+$, 55), 400 ([M−2H$_2$O+H]$^+$, 100), 382 ([M−3H$_2$O+H]$^+$, 15).

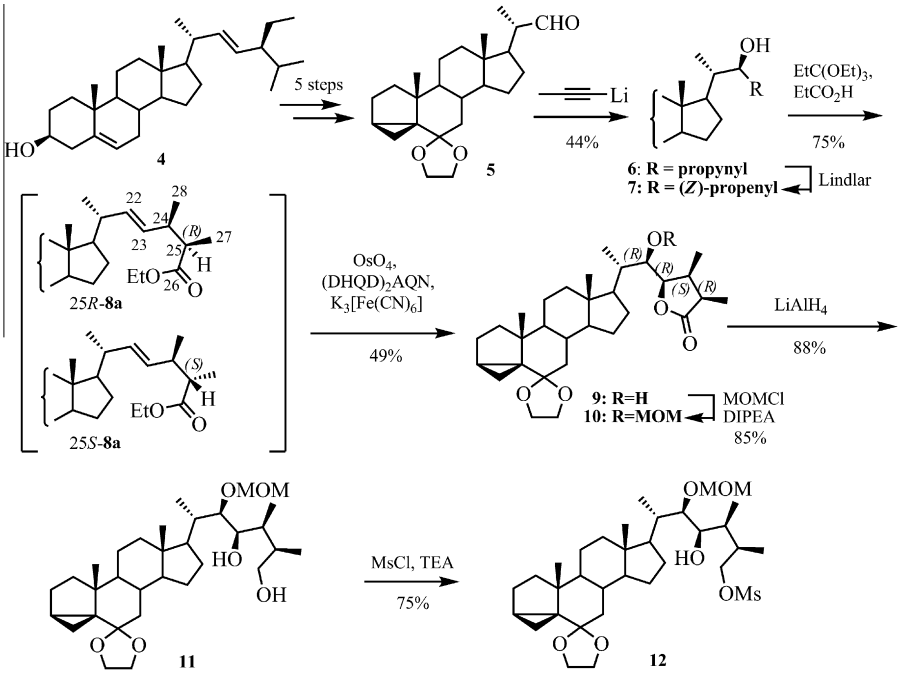
2.2.32. Oxidation of (22E,24R)-6-(1,3-dioxolan-2-yl)-26-methanesulfonyloxy-24-methyl-3α,5-cyclo-5α-cholest-22-ene (**13b**) with methyl(trifluoromethyl)dioxirane

(22E,24R)-6-(1,3-Dioxolan-2-yl)-26-methanesulfonyloxy-24-methyl-3α,5-cyclo-5α-cholest-22-ene **13b** [8] (30 mg, 0.059 mmol) and 1,1,1-trifluoroacetone (63 mg, 0.56 mmol) were dissolved in an ice cooled mixture of MeCN (0.2 mL), DMM (0.4 mL) and an aqueous 4 × 10^{-4}M solution of Na$_2$EDTA (0.2 mL). A mixture of Oxone (0.35 g, 0.56 mmol) and NaHCO$_3$ (0.15 g, 1.79 mmol) was added in portions to this vigorously stirred solution at 0 °C over 2 h. After stirring for another 15 min, EtOAc (5 mL) and water (5 mL) were added, and the water layer was separated and extracted with EtOAc (2 × 5 mL). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒1:1) to give epoxides **14b** (9 mg, 30%) as a mixture of isomers (22*R*,23*R*:22*S*,23*S* = 1:4). ^{1}H NMR δ: 0.43 (dd, J = 7.7, 5.1 Hz, 1H, C4–H), 0.67–0.60 (m, 1H, C4–H), 0.70 (s, 3H, C18–H), 0.97 (d, J = 6.3 Hz, 3H, >CHCH_3), 1.00 (d, J = 7.0 Hz, 3H, >CHCH_3), 1.02 (s, 3H, C19–H), 1.08 (d, J = 7.0 Hz, 3H, >CHCH_3), 2.39–2.55 (m, 1.8H, epoxide), 2.74–2.80 (m, 1.2H, C6–H and epoxide), 3.01 (s, 3H, OMs), 3.32 (s, 3H, OMe), 4.09–4.16 (m, 1H, C26–H), 4.23–4.31 (m, 1H, C26–H).

Further elution gave *(24S)-26-methanesulfonyloxy-22,23-epoxy-24-methyl-3α,5-cyclo-5α-cholestan-6-one* (***31***) (13 mg, 43%) as an oil. ^{13}C NMR signals of major isomer δ: 0.70 (s, 3H, C18–H), 0.98 (d, J = 6.6 Hz, 3H, >CHCH_3), 1.00 (s, 3H, C19–H), 1.01 (d, J = 6.0 Hz, 3H, >CHCH_3), 1.08 (d, J = 7.0 Hz, 3H, >CHCH_3), 2.43 (dd, J = 7.1, 2.1 Hz, 1H, epoxide), 2.49 (dd, J = 8.8, 2.1 Hz, 1H, epoxide), 3.01 (s, 3H, OMs), 4.14 (dd, J = 9.6, 6.8 Hz, 1H, C26–H), 4.25 (dd, J = 9.6, 6.5 Hz, 1H, C26–H). ^{13}C NMR δ: 11.65, 11.75, 12.12, 12.19, 12.55, 12.98, 14.61, 16.13, 19.65, 22.81, 24.24, 25.87, 26.93, 33.45, 34.73, 35.30, 36.30, 37.05, 37.34, 37.50, 38.15, 39.53, 43.03, 44.71, 46.03, 46.09, 46.28, 46.70, 53.50, 55.92, 56.49, 57.90, 59.46, 61.84, 62.58, 73.42, 209.48. MS (APCI$^+$) m/z (%): 507 ([M+H]$^+$, 100), 489 ([M−H$_2$O+H]$^+$, 9), 411 ([M−MsOH+H]$^+$, 67).

2.2.33. Oxidation of the ether (**16b**) with methyl(trifluoromethyl)dioxirane

A mixture of Oxone (0.92 g, 1.5 mmol) and NaHCO$_3$ (0.4 g, 4.8 mmol) was added portionwise over 4 h to an ice cooled vigorously stirred solution of ether **16b** (60 mg, 0.139 mmol) and 1,1,1-trifluoroacetone (0.17 g, 1.04 mmol) in a mixture of DMM (1 mL), MeCN (0.5 mL) and 4 × 10^{-4} M aqueous Na$_2$EDTA (0.6 mL). The reaction mixture was stirred at 0 °C for 1 h, diluted with water (10 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc = 9:1⇒3:2) to give starting ether **16b** (10 mg, 17%) and ketone **19** (37 mg, 64%, 77% based on recovered starting material).

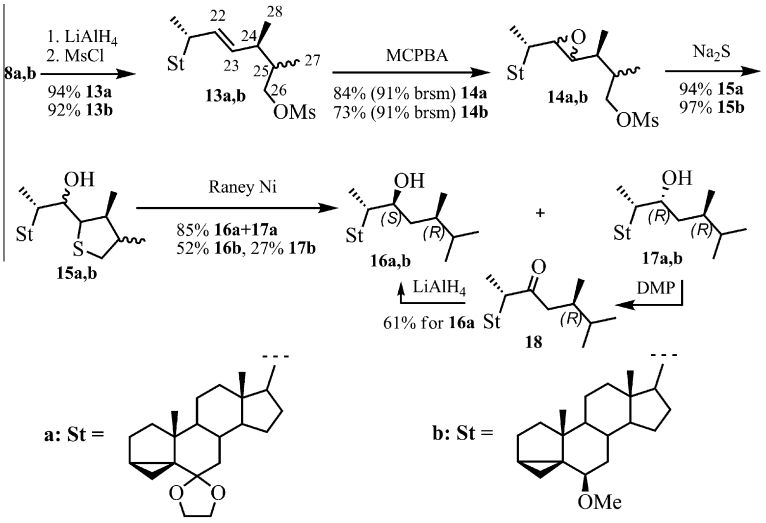


Scheme 1.

3. Results and discussion

The ester **8** was easily synthesized from stigmasterol **4** via the known aldehyde **5** [10] according to described procedures [8,11], in 8 steps and in 20% overall yield. Compound **8** already contains a methyl group at C-24, and the stereochemistry of C-24 is defined by the Claisen rearrangement of the propionate of allyl alcohol **7** (Scheme 1). The C-22 and C-23 hydroxyl groups were introduced by Sharpless asymmetric dihydroxylation [12]. Under the reaction conditions the C-23 hydroxyl group reacted with the ester group [13,14], which led to the formation of hydroxy lactone **9**, thus allowing selective manipulations at the 22- and 23-position. Protection of the free hydroxyl group in **9** was the most obvious way to continue. The use of the TBS protecting group failed because of its instability in a later stage under reduction conditions. Finally the 22-hydroxyl group was protected as a MOM ether.

It should be noted that the hydroxy lactone **9** was isolated as a single isomer starting from the inseparable C-25 diastereomeric mixture **8a** (for the meaning of a and b see Scheme 2). The formation of a 4:1 diastereomeric mixture of such esters was earlier shown by us [8]. At that time the preferred (*R*)-configuration at C-25 was assumed, based on analysis of the possibility of only one chair like transition state for the Claisen rearrangement. The experimental results obtained in this study are in agreement with the previous suggestions [8]. The ratio of 25*R*-**8a** (precursor of the lactone **9**) to its 25*S*-**8a** epimer was evaluated to be 5.4:1 based on the ratio of integral intensity peaks with δ 129.96 and 130.40 or 137.71 and 136.89. The assignment of the stereochemistry at C-24 became apparent from the NOESY spectrum of MOM ether **10**, which clearly showed a NOE correlation between the 27- and 28-methyl groups. This indicates a syn orientation of both methyl groups, confirming the stereochemistry at C-25 as being (*R*).



Scheme 2.

The lactone **10** was reduced with $LiAlH_4$ to give diol **11**. It was treated with an excess of MsCl, but unexpectedly the secondary hydroxyl group at C-23 was left intact. The search for an appropriate protocol for the preparation of the 23,26-dimesylate, using various solvents and catalysts [15], gave no results. Either the starting compound **11** was isolated or, under more severe conditions, cyclization took place with formation of the corresponding tetrahydrofuran. These results prohibited the simultaneous removal of both hydroxyl groups via dimesylate reduction. A successful implementation of this approach would require additional chemical steps thus making the whole synthetic sequence less attractive.

However this approach deserves consideration as a starting point for the preparation of brassinosteroid metabolites with a chiral center at C-25 [16].

Better results for the preparation of 22-hydroxy steroids were obtained by using the tetrahydrothiophene derivatives **15a,b** as intermediates (Scheme 2). The synthesis of cathasterone **1** started from ester **8a**, and the 6-deoxo- and Δ^5-derivatives **3** and **2** were obtained starting from ester **8b** [8]. A lithium aluminum hydride reduction of the esters **8a,b** followed by mesylation of the intermediate alcohols gave the bishomoallylic mesylates **13a,b**. The search for optimal epoxidation conditions of the Δ^{22}-double bond was carried out with compound **13a**. Its epoxidation with MCPBA in the presence of $NaHCO_3$ in methylene chloride led to a 2:1 mixture of isomers in favor of the 22*R*,23*R*-epimer **14a** in 70% yield. The stereochemical course of this epoxidation was assessed by chemical correlation with the alcohols 22*R*-**16a** and 22*S*-**16a** (vide infra). Attempts to increase the stereoselectivity by using different epoxidation conditions were unsuccessful. A solution of dimethyldioxirane in acetone [17] and Shi's catalyst [18,19] gave no reaction at all. Epoxidation with *in situ* generated methyl(trifluoromethyl)dioxirane [20] gave the undesired 22*S*,23*S*-epoxide **14a** as the main isomer. Ultimately it was found that epoxidation with MCPBA with isopropanol as a co-solvent gave a better yield of the epoxides **14a** (up to 91% based on the recovered starting material **13a**), but the 2:1 (22*R*,23*R*):(22*S*,23*S*)-isomeric ratio remained unchanged.

Compounds **14a** and **14b** produced a mixture of isomeric tetrahydrothiophenes **15a,b** with good regioselectivity when reacted with sodium sulfide. The desulfurization [21] of **15a,b** was smoothly achieved with Raney nickel to give mixtures of the 22*S* and 22*R* alcohols **16a** and **17a**. Their ratio depended on the epoxidation method used for the preparation of mesyl epoxides **13a,b**. The proportion of the desired **16a,b** could be increased by oxidation of the 22*R*-alcohols **17a,b** into corresponding 22-ketones followed by hydride reduction. The alcohols **16b** and **17b** could be separated easily by column chromatography, but we failed to do the same with the dioxolane derivatives **16a** and **16b**.

Fortunately, this problem was solved by separation of the isomeric alcohols after removal of the dioxolane protecting group. The pure 22*S*-alcohol **19** with the desired side chain could be isolated in 62% yield from the isomeric mixture of **16a** and **17a** by chromatography (Scheme 3). Compound **19** was further transformed into cathasterone **1** by standard methods, involving acid-catalyzed cyclopropane ring opening [22] of the acetate protected derivative **20**.

Regeneration of the AB-cycles by treatment of **16b** with acid led in one step to (22*S*)-hydroxycampesterol **2** (Scheme 4). Hydrogenation of **2** over Pd catalyst gave 6-deoxocathasterone **3**, which is another biosynthetic precursor of brassinolide [23,24].

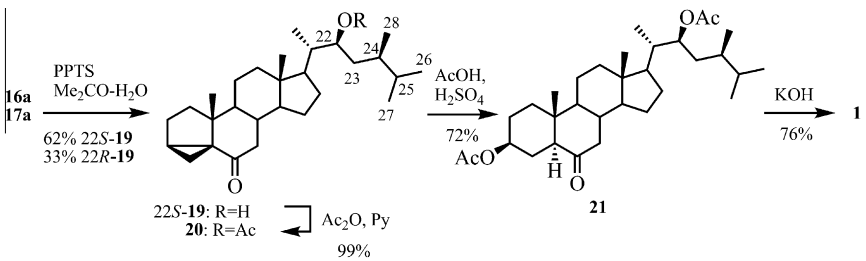
Modern techniques in detection, identification and quantification of brassinolide precursors are based on the use of the corresponding labeled derivatives as internal standards [25,26]. These compounds have to meet some requirements [27]. At least three deuterium atoms should be present in the molecule to avoid possible interferences with the peaks [M+1] and [M+2] belonging to natural ^{13}C and ^{18}O containing species. We have already reported the synthesis of labeled brassinosteroids containing three [28] or six [29] deuterium atoms at the end of the side chain. The present synthetic route to 22-hydroxysteroids provides an obvious method for the introduction of two deuterium atoms at C-26 via the reduction of the ester **8a** with $LiAlD_4$ (Scheme 5). The third deuterium atom can in principle be introduced at C-26 by hydrogenation of tetrahydrothiophene **23** over Raney nickel that is prepared in deuterated water. The main concern with such an approach is that it could not guarantee a sufficient isotopic purity level for the final compound. Labeling at C-22 by reduction of the ketone **25** with $LiAlD_4$ seems to be a more reliable and predictable alternative. The remaining steps of the synthesis of triple-deuterated cathasterone **30** were performed as described above for the preparation of its non-labeled congener **1**.

While optimizing the preparation of 22,23-epoxides, we had observed that the epoxidation of the Δ^{22}-olefin **13b** with methyl(trifluoromethyl)dioxirane yielded not only the expected product **14b**, but also the 6-ketone **31** (Scheme 6). Oxidation of steroidal ethers is known [30], but this direct reaction has never been used for the transformation of 3α,5-cyclo-6β-methoxy derivatives into the corresponding 6-ketones. Such a reaction would be highly profitable for the chemistry of brassinosteroids instead of the four-step reaction sequence that is in use for that purpose now [28,31,32].

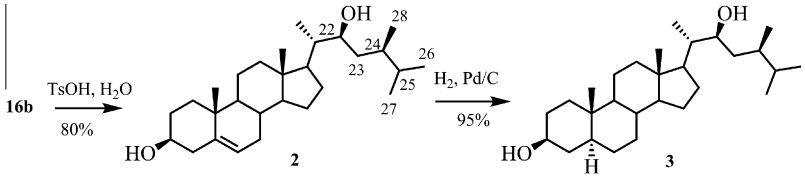
To check the synthetic utility of this method, we studied the oxidation of the less functionalized ether **16b** (Scheme 7). Its reaction with *in situ* generated methyl(trifluoromethyl)dioxirane proceeded smoothly to give 6-ketone **32** in a reasonable 64% yield (77% based on recovered starting material).

4. Conclusion

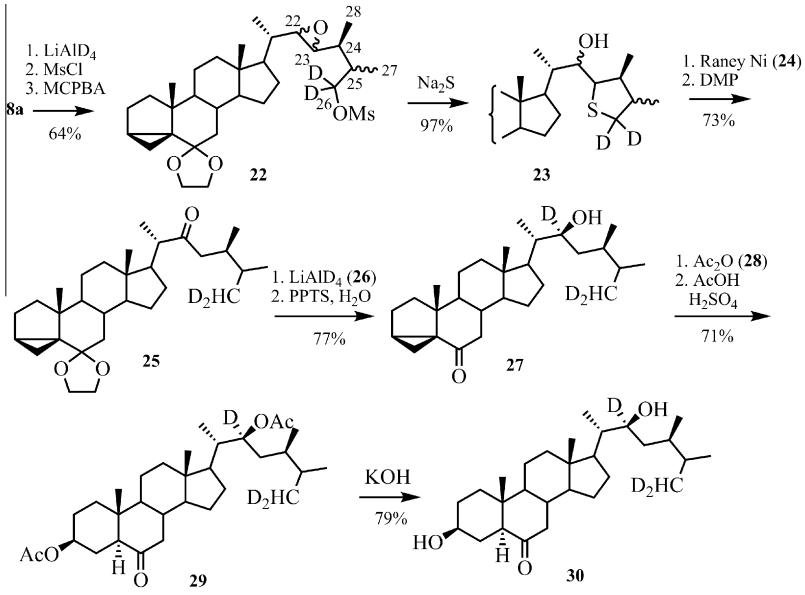
In summary, we have successfully developed a new method for the synthesis of 22*S*-hydroxy-22*R*-methyl steroids. Construction of the side chain skeleton with the correct stereochemistry at C-24 was based on the use of a Claisen rearrangement. After Sharpless dihydroxylation of the obtained C-25 isomeric mixture of Δ^{22}-26-esters, a (25*R*)-26,23-lactone was isolated as the major product. This is a possible synthetic intermediate for the



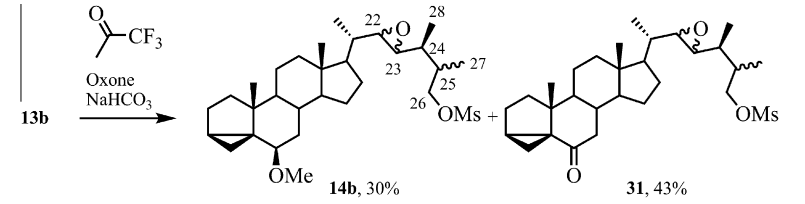
Scheme 3.



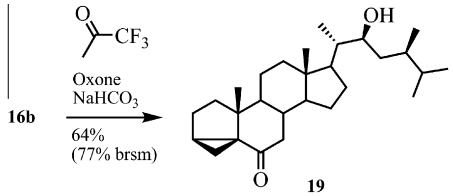
Scheme 4.



Scheme 5.



Scheme 6.



Scheme 7.

preparation of brassinosteroid metabolites with a chiral center at C-25, although attempts to use it for the introduction of the required 22-hydroxyl group did not give the expected results. The successful functionalization at C-22 was achieved by Δ^{22}-double bond epoxidation, nucleophilic epoxide ring opening with sodium sulfide of the intermediate mesyl epoxide, and desulfurization of the formed tetrahydrothiophenes with Raney nickel. The new methodology was applied to the preparation of cathasterone **1**, (22*S*)-hydroxycampesterol **2**, and 6-deoxocathasterone **3** which are biosynthetic precursors in the early stages of the brassinolide biosynthesis. A possibility to prepare the corresponding labeled 22-hydroxy steroids was demonstrated by the synthesis of [22,26,26-$^2H_3$]-cathasterone **30** containing three deuterium atoms in positions that did not allow isotopic exchange. In addition, a new method for the synthesis of steroidal 6-ketones was developed based on the oxidation of the 6-methyl ether with methyl(trifluoromethyl)dioxirane.

Acknowledgment

We thank Prof. Ae de Groot (Wageningen) for helpful discussions and linguistic support in the preparation of this manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2012.03.010.

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