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SYNTHESIS OF 28-HOMOBRASSINOSTEROIDS MODIFIED IN THE 26-POSITION

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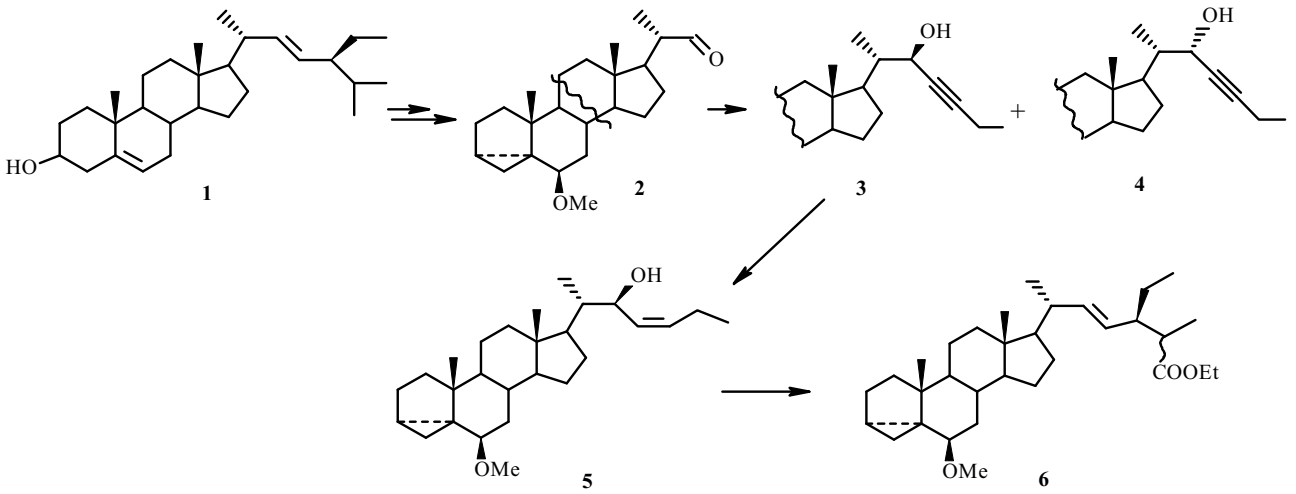
28-Homobrassinosteroids modified in the 26-position were synthesized from 22-hydroxy-23-ensteroids using Claisen rearrangement and subsequent cis-hydroxylation of the resulting Δ^{22}-derivative.

Key words: steroids, brassinosteroids, synthesis, spectral characteristics.

We have previously developed a synthetic method for brassinolide, 24-epi- and 28-norbrassinolide, and their 26-derivatives using Claisen rearrangement of the corresponding 22-allyl alcohols as the key step [1, 2]. This enabled the aforementioned brassinosteroids, their deuterated derivatives [3], and haptens containing a linker at the end of the brassinosteroid side chain [2] to be produced.

In continuation of research on the preparation and properties of 28-homobrassinosteroids, we developed a synthetic scheme for their derivatives with functional groups at the C-26 position of the carbon skeleton that are promising as terminal haptens and key intermediates for new physiologically active analogs of 28-homobrassinosteroids.

The starting compound was aldehyde **2**, which was prepared in three steps from stigmasterol by the literature method [4]. The synthesis included reaction of **2** with 1-butynyllithium, reduction of the resulting acetylenic alcohol to the allyl compound, and its Claisen rearrangement. 1-Butynyllithium was synthesized by reaction of a hexane solution of *n*-butyllithium with 1-butyne, which in turn was produced by refluxing sodium acetylenide with diethylsulfate in xylene [5].



Addition of 1-butynyllithium to **2** in THF solution at –60°C formed a mixture of two C-22 epimers of acetyleneic alcohols **3** and **4** in a 4:3 ratio in total yield 66%. Addition of methylacetylene to **2** occurred more stereoselectively, giving the corresponding 22-epimers in a 2:1 ratio [1].

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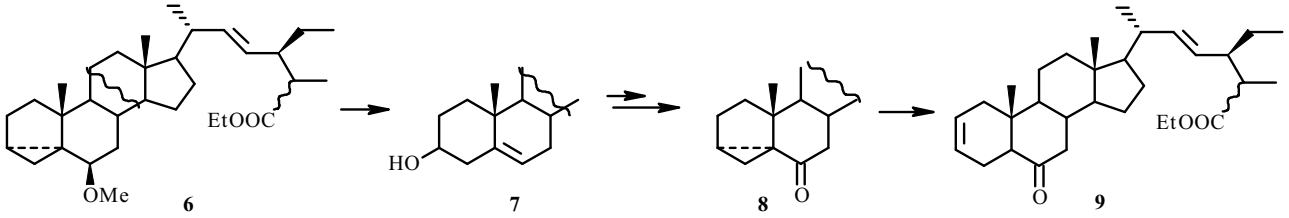
Formation of **3** and **4** was confirmed by the disappearance of stretching vibrations of the aldehyde and the appearance of vibrations of a hydroxyl (ν 3400 cm^{-1}) in the IR spectrum in addition to characteristic proton resonances for C-22 in PMR spectra at δ 4.48 and 4.45 ppm, respectively. The less polar epimer according to the literature [1, 6–8] is 22*R*-**3**; the more polar, 22*S*-**4**.

Acetylenic alcohol **3** was isolated by chromatography and reduced by hydrogen over a Lindlar catalyst in the presence of quinoline [7] to produce in 90% yield *cis*-23-en-22-ol **5**. This was confirmed by the appearance in the PMR spectrum of the hydrogenation product of a 2H multiplet for the olefinic protons at δ 5.51 ppm.

Further construction of the side chain was carried out using a Claisen rearrangement. Thus, refluxing **5** in benzene in the presence of triethylorthopropionate and propionic acid produced in 69% yield an epimeric mixture of esters **6**. The PMR spectrum of **6** exhibited a 2H quartet at δ 4.11 ppm for the ester methylene protons and a doublet for the C-27 methyl protons at δ 0.99 ppm. Resonances of the two vinyl protons were found as a multiplet at δ 5.21–5.29 ppm. The ^{13}C NMR spectrum showed characteristic resonances of the carboxyl C (δ 176.54 ppm). Stretching vibrations of the hydroxyl disappeared and a strong band for the ester carbonyl appeared at 1735 cm^{-1} in the IR spectrum of **6**. Separation of the mixture of 25-epimers was unsuccessful. Therefore, all subsequent transformations used the mixture of C-25 isomers.



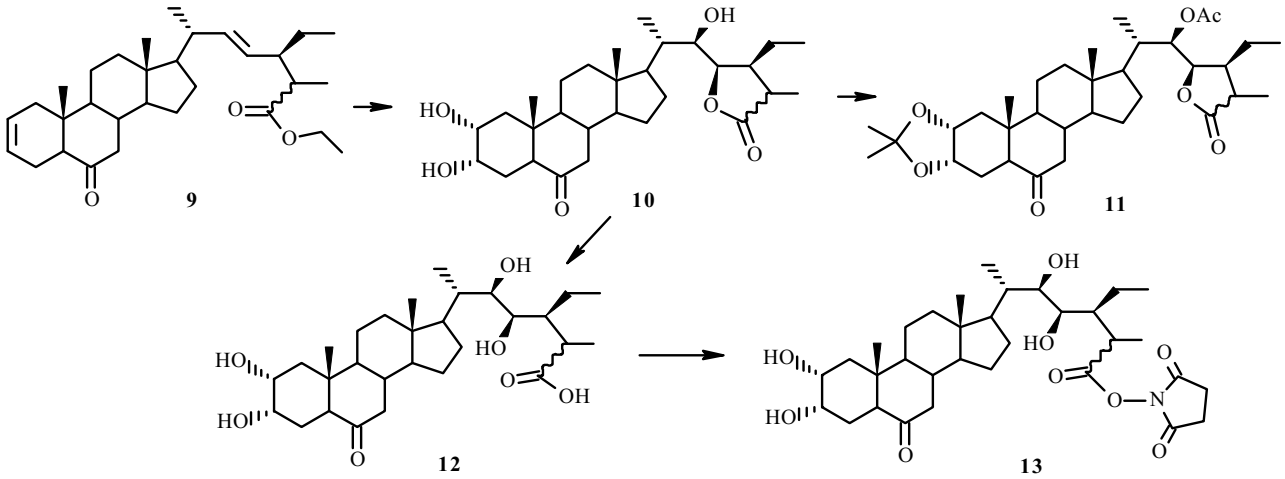
Functionalization of rings A and B of the steroid continued the scheme. Regeneration of the 3β-hydroxy-Δ^5-group by acid hydrolysis of **6** using *p*-toluenesulfonic acid in dioxane produced **7**. The PMR spectrum of **7** contained characteristic resonances such as a 1H multiplet at δ 3.52 ppm for the C-3 proton and a 1H multiplet for the C-6 vinyl proton (δ 5.35 ppm). Further transformation included mesylation of the 3β-hydroxy, isosteroid rearrangement in the presence of potassium acetate, and Jones oxidation of the 6-hydroxyl. This gave in 50% yield 3α,5-cycloketone **8**, the PMR spectrum of which lacked a resonance for the C-6 vinyl proton and the IR spectrum of which contained an absorption band for the ketone (ν 1690 cm^{-1}).

Refluxing **8** with pyridinium bromide in DMF produced in 70% yield rearrangement product Δ^2-6-ketone **9**, the PMR spectrum of which showed resonances for the two vinyl protons on C-2 and C-3 (δ 5.56 and 5.66 ppm) and the ^{13}C NMR spectrum of which exhibited a characteristic resonance for C-6 at δ 212.14.

The conclusion of the scheme should have been *cis*-hydroxylation of the double bonds of **9**. Considering that the 24-ethyl substituent reduces the stereoselectivity of hydroxylation of the Δ^{22}-bond, we decided to use an oxidizing mixture based on potassium osmate with a chiral hydroquinidine catalyst because it is known to be optimal for producing the desired 22*R*,23*R*-isomer [9]. The unexpected reaction product was γ-lactone **10**, formation of which could be explained by saponification of the ester under the basic reactions conditions and subsequent intramolecular cyclization upon acid work up during isolation of the product. The IR spectrum of **10** contained a strong band for hydroxyl stretching vibrations (ν 3400 cm^{-1}) and a band for ketone stretching vibrations at ν 1765 that was characteristic of γ-lactones. It lacked absorption bands for an ester. The PMR spectrum showed resonances for methine protons at δ 3.57 (H-22), 3.75 (H-2), and 4.04 (H-3) that were characteristic of 28-homocastasterone [10, 11]. The resonance of the C-23 proton was shifted to weak field (δ 4.38 ppm vs. 3.72 for 28-homocastasterone).

Transformations of the C-2, C-3, and C-22 hydroxyls provided proof that cyclic **10** had formed. Thus, treatment with acetone in the presence of *p*-toluenesulfonic acid formed the 2,3-isopropylidenedioxy derivative whereas further acetylation of the 22-hydroxyl (treatment with acetic anhydride in pyridine for 12 h at room temperature) gave 22-acetoxy-2,3-isopropylidenedioxy derivative **11**, the formation of which indicated that the starting molecule had one vicinal diol group and one secondary hydroxyl.

Opening of lactone **10** by methanolic KOH with subsequent careful acidification with HCl solution (0.1 N) until the pH was 6-7 formed acid **12**. The PMR spectrum of **12** was missing the multiplet at δ 4.38 ppm and showed a 1H multiplet at δ 3.67 ppm for the C-23 proton that was characteristic of 22*R*,23*R*-dihydroxybrassinosteroids. The spectrum also contained a weak peak for the carboxylic-acid proton at δ 10.73 ppm.



Thus, 26-modified 28-homobrassinosteroids were prepared for the first time. Compound **12** is promising as a hapten for fabricating immunochemical tests for brassinosteroid determination. This was confirmed by the synthesis of succinimide activated ester **13** by the method published previously by us [12]. The structure of **13** was confirmed by the appearance in the PMR spectrum of resonances for methylene protons of the succinimide ring as two 2H broad singlets (δ 2.58 and 2.66 ppm) and an absorption band for the carbonyl of the cyclic imide in the IR spectrum (ν 1755 cm^{-1}).

EXPERIMENTAL

Melting points were determined on a Kofler block. PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD on a Bruker A-500 instrument (operating frequency 500 MHz) with TMS internal standard. IR spectra were obtained (in films or KBr disks) on a UR-20 instrument. Mass spectra were measured on a LCQ^{TM} FLEET instrument with an ion trap (Thermo Electron Corp.) using He as the ionizing gas and N_2 as the auxiliary gas. The course of reactions was monitored by TLC on Merck plates (Kieselgel 60 F_{254}). Chromatographic separation of reaction mixtures were performed over silica gel 40/60 (Kieselgel 60, Merck).

6β-Methoxy-3α,5-cyclo-23,24-dinor-5α-cholan-22-al (2). Stigmasterol (10 g, **1**) was dissolved in pyridine (100 mL), treated with *p*-toluenesulfonylchloride (10 g), and left for 1 d at room temperature. After the reaction was finished, the mixture was diluted with water. The resulting crystals were filtered off and washed with water. The resulting tosylate was dissolved in pyridine (40 mL), treated with MeOH (200 mL), refluxed for 3 h, and evaporated. The solid was separated over a column of silica gel (petroleum ether:EtOAc eluent, 20:1) to isolate an oily compound (8 g) that was dissolved in a mixture of MeOH (160 mL) and $CHCl_3$ (160 mL), treated with pyridine (2 mL) and $NaHCO_3$ (1.6 g), cooled to –60°C, purged with ozone for 3 h and then oxygen to remove the ozone, treated with dimethylsulfide (40 mL), left overnight, and evaporated. The solid was separated over a column of silica gel (petroleum ether:EtOAc eluent, 20:1) to afford aldehyde **2** (6.5 g, 79%). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.44 (1H, m, H-3), 0.76 (3H, s, CH_3-18), 1.02 (3H, s, CH_3-19), 1.11 (3H, d, J = 7, CH_3-21), 2.36 (1H, m, H-20), 2.76 (1H, m, H-6), 3.32 (3H, s, $COCH_3$), 9.57 (1H, d, J = 3.2, H-22).

Reaction of 1-Butynyllithium with 2. A suspension of sodium acetylenide (10 g, 0.0375 mol, 18%) in xylene was treated with diethylsulfate (2.5 mL, 0.0187 mol) and heated to 90°C on an oil bath with vigorous stirring. The released butyne-1 was condensed in dry THF (10 mL) at –60°C for 2 h. The resulting solution of butyne-1 in THF at –60°C was treated with a solution of *n*-BuLi (2.5 mL, 1.8 M, 4.5 mmol), stirred for 30 min, warmed to 0°C, cooled to –70°C, stirred, and treated dropwise over 30 min with a solution of **2** (1 g, 3 mmol) in anhydrous THF (10 mL). After the reaction was finished, an excess of NH_4Cl was added. The mixture was diluted with water and extracted with $CHCl_3$. The extract was evaporated. The solid was separated over a column of silica gel (petroleum ether:EtOAc eluent, 40:1) to afford **3** (470 mg, 39%) and **4** (330 mg, 27%).

(22*R*)-6β-Methoxy-3α,5-cyclo-27-nor-5α-cholest-23-yn-22-ol (3). IR spectrum (film, ν, cm^{-1}): 3400 (OH). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.42 (1H, m, H), 0.72 (3H, s, CH_3-18), 1.01 (3H, s, CH_3-19), 1.10 (3H, d, J = 7, CH_3-21), 1.13 (3H, t, J = 8, CH_3-26), 2.21 (2H, q, J = 7, H-25), 2.76 (1H, m, H-6), 3.32 (3H, s, $COCH_3$), 4.48 (1H br.s, 22-H).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 12.36 q, 13.22 t, 14.28 q, 19.44 q, 21.63 q, 22.85 t, 24.29 t, 25.10 t, 27.78 t, 29.53 t, 30.70 q, 32.08 t, 33.49 t, 35.17 t, 35.38 s, 40.15 t, 42.17 d, 42.82 s, 43.51 s, 48.07 d, 51.86 d, 56.44 d, 56.71 d, 65.50 d, 73.27 s, 82.50 d, 85.13 s.

(22*S*)-6β-Methoxy-3α,5-cyclo-27-nor-5α-cholest-23-yn-22-ol (4). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.42 (1H, m, H-3), 0.72 (3H, s, CH_3-18), 1.01 (3H, s, CH_3-19), 1.10 (3H, d, J = 7, CH_3-21), 1.13 (3H, t, J = 8, CH_3-26), 2.21 (2H, q, J = 7, H-25), 2.76 (1H, H-6), 3.32 (3H, s, $COCH_3$), 4.45 (1H, br.s, H-22).

(22*R*,23*Z*)-6β-Methoxy-3α,5-cyclo-27-nor-5α-cholest-23-en-22-ol (5). Acetylenic alcohol **3** (100 mg, 0.25 mmol), Lindlar catalyst (16 mg), and quinoline (0.1 mL) were dissolved in EtOAc (16 mL). The resulting suspension was stirred under H_2 for 2.5 h until H_2 absorption was complete. The catalyst was filtered off. The solution was evaporated. The solid was chromatographed over a column of silica gel (petroleum ether:EtOAc eluent, 20:1) to afford **5** (90 mg, 90%). IR spectrum (film, ν, cm^{-1}): 3400 (OH). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.41–0.44 (1H, m, H-3), 0.73 (3H, s, CH_3-18), 0.88 (3H, t, J = 9, CH_3-26), 0.90 (6H, d, J = 6.5, CH_3-21), 1.01 (3H, s, CH_3-19), 2.76 (1H, m, H-6), 3.31 (3H, s, OCH_3), 4.26 (1H, m, H-22), 5.51 (2H, m, H-23, H-24).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 11.94, 12.33, 13.22, 19.42, 21.59, 22.89, 24.31, 25.089, 28.00, 29.86, 30.70, 32.08, 33.47, 35.19, 35.35, 40.28, 41.06, 42.80, 43.50, 48.08, 52.68, 56.50, 56.71, 74.36, 82.52, 113.73, 141.33.

(22*E*,24*S*)-6β-Methoxy-3α,5-cyclo-24-ethyl-5α-cholest-22-en-26-oic Acid Ethyl Ester (6). A solution of **5** (100 mg, 0.26 mmol) in benzene (8 mL) was treated with triethylorthopropionate (0.8 mL, 4.08 mmol) and propionic acid (0.02 mL, 0.26 mmol) and refluxed under Ar for 2 h. After the reaction was finished, the mixture was treated with saturated Na_2CO_3, extracted with EtOAc, and washed with water. The organic layer was evaporated. The solid was chromatographed over a column of silica gel (petroleum ether:EtOAc eluent, 20:1) to afford **6** (85 mg, 69%). IR spectrum (film, ν, cm^{-1}): 1735 (C=O). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, CH_3-18), 0.99 (3H, d, J = 6.4, CH_3-21), 1.01 (3H, s, CH_3-19), 1.11 (3H, d, J = 7, CH_3-27), 1.25 (3H, t, J = 7, CH_3-29), 2.76 (1H, m, H-6), 3.31 (3H, s, OCH_3), 4.11 (2H, q, J = 7, OEt), 5.21–5.29 (2H, m, H-22, H-23).

^{13}C NMR spectrum (δ, ppm): 12.57, 13.20, 14.41, 16.64, 19.42, 20.77, 20.80, 21.61, 22.88, 24.28, 24.31, 25.10, 28.73, 30.60, 33.48, 35.17, 35.41, 36.85, 39.95, 39.99, 40.26, 40.29, 42.83, 43.51, 48.16, 55.99, 56.71, 60.25, 82.53, 124.01, 139.76, 176.54.

(22*E*,24*S*)-3β-Hydroxy-24-ethyl-5α-cholest-5,22-dien-26-oic Acid Ethyl Ester (7). A mixture of **6** (200 mg, 0.4 mmol), *p*-TsOH (23 mg, 0.13 mmol), water (0.3 mL), and dioxane (5 mL) was heated at 70°C for 3 h, treated with pyridine, and evaporated. The solid was chromatographed over a column of silica gel (petroleum ether:EtOAc eluent, 10:1) to afford **7** (126 mg, 66%). IR spectrum (film, ν, cm^{-1}): 3440 (OH), 1735 (C=O). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, CH_3-18), 0.99 (3H, d, J = 6.5, CH_3-27), 1.04 (3H, s, CH_3-19), 1.15 (3H, d, J = 7, CH_3-21), 3.52 (1H, m, H-3), 4.11 (2H, q, J = 7, OEt), 5.22–5.31 (2H, m, H-22, H-23), 5.35 (1H, m, H-6).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 12.17,14.39, 16.63, 19.51, 20.80, 21.18, 24.40, 24.37, 28.67, 31.75, 32.00, 36.63, 36.74, 36.82, 37.38, 39.77, 39.93, 39.99, 40.22, 42.39, 50.24, 55.82, 56.93, 60.29, 71.89, 121.79, 124.05, 124.17, 139.69, 140.88, 176.57.

(22*E*,24*S*)-3α,5-Cyclo-24-ethyl-5α-cholest-22-en-6-on-26-oic Acid Ethyl Ester (8). Compound **7** (220 mg, 0.468 mmol) dissolved in CH_2Cl_2 (3 mL) was treated with triethylamine (0.15 mL), cooled to –5°C, treated with a solution of MsCl (107 mg, 0.936 mmol) in CH_2Cl_2 (2 mL), stirred at –5°C for 25 min, treated with MeOH (0.01 mL), worked up with water and saturated Na_2CO_3 solution, and extracted with CH_2Cl_2. The extract was evaporated. The resulting mesylate was dissolved in acetone (50 mL), treated with KOAc (250 mg) and water (4 mL), refluxed for 3 d, oxidized with Jones reagent (1 mL), treated with isopropanol, passed over a layer of silica gel, and evaporated. The solid was chromatographed over a column of silica gel (petroleum ether:EtOAc eluent, 20:1) to afford **8** (110 mg, 50%). IR spectrum (film, ν, cm^{-1}): 1735 (C=O), 1700 (C=O). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.72 (3H, s, CH_3-18), 1.01 (6H, m, CH_3-19,27), 1.18 (3H, d, J = 7, CH_3-21), 4.12 (2H, q, J = 7, OEt), 5.26–5.29 (2H, m, H-22,23).

(22*E*,24*S*)-24-Ethyl-5α-cholest-2,22-dien-6-on-26-oic Acid Ethyl Ester (9). Ketone **8** (100 mg, 0.21 mmol) in DMF (1.3 mL) was treated with Py·HBr (95 mg, 0.6 mmol), refluxed for 1 h under Ar, worked up with water, and extracted with $CHCl_3$. The extract was evaporated. The solid was chromatographed over a column of silica gel (petroleum ether:EtOAc eluent, 15:1) to afford **9** (70 mg, 70%). IR spectrum (film, ν, cm^{-1}): 1735 (C=O), 1700 (C=O). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.66 (3H, s, CH_3-18), 0.69 (3H, s, CH_3-19), 0.99 (3H, d, J = 6.4, CH_3-27), 1.10 (3H, d, J = 7, CH_3-21), 4.10 (2H, q, J = 7, OEt), 5.25 (2H, m, H-22,23), 5.56 (1H, m, H-2), 5.66 (1H, m, H-3).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 12.24, 13.63, 14.41, 16.63, 20.77, 21.21, 21.83, 24.05, 28.07, 28.42, 28.48, 36.71, 36.79, 37.80, 39.47, 39.88, 39.93, 40.15, 42.83, 47.10, 53.51, 53.94, 55.74, 56.92, 60.25, 124.45, 124.64, 125.05, 139.34, 176.44, 212.13.

(22*R*,23*R*,24*S*)-2α,3α,22-Trihydroxy-24-ethyl-5α-cholest-6-on-26-oic Acid γ-Lactone (10). A mixture of **9** (80 mg, 0.17 mmol), $K_3[Fe(CN)_6]$ (339 mg, 1.03 mmol), K_2CO_3 (142 mg, 1.03 mmol), methanesulfonamide (49 mg, 0.52 mmol), $(DHQD)_2AQN$ (5 mg, 0.0057 mmol), and $K_2OsO_4 \cdot 2H_2O$ (1.3 mg, 0.0034 mmol) was dissolved in a *t*-BuOH:H_2O mixture (1:1), stirred for 4 d, extracted with EtOAc, acidified with HCl solution (0.1 N) until the pH was 6, and extracted with EtOAc. The combined extract was dried over anhydrous Na_2SO_4 and evaporated. The solid was chromatographed over silica gel (petroleum ether:EtOAc eluent, 1:3) to afford **10** (50 mg, 58%), mp 230–232°C (MeOH). IR spectrum (KBr, ν, cm^{-1}): 3400 (OH), 1765 (C=O), 1710 (C=O). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.67 (3H, s, CH_3-18), 0.74 (3H, CH_3-19), 0.98 (3H, d, J = 7, CH_3-21), 3.57 (1H, m, H-22), 3.75 (1H, m, H-2), 4.04 (1H, s, H-3), 4.38 (1H, m, H-23).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 12.01, 12.50, 13.68, 15.11, 21.33, 23.93, 26.46, 27.99, 32.38, 33.58, 34.65, 35.88, 37.04, 37.54, 37.82, 39.50, 40.28, 42.69, 42.85, 46.80, 50.86, 51.75, 51.90, 53.71, 56.68, 68.42, 76.12, 179.03, 212.18.

(22*R*,23*R*,24*S*)-22-Acetoxy-2α,3α-isopropylidendioxy-24-ethyl-5α-cholest-6-on-26-oic Acid γ-Lactone (11). A solution of **10** (20 mg, 0.04 mmol) in acetone (2 mL) was treated with *p*-toluenesulfonic acid (5 mg) and stirred for 1 d. The acetone was evaporated. The solid was dissolved in EtOAc, washed with Na_2CO_3 solution and water, and dried over Na_2SO_4. The solvent was evaporated. The solid was dissolved in a mixture of pyridine (1 mL) and acetic anhydride (0.3 mL) and left overnight. The mixture was evaporated. The solid was separated over a column of silica gel (petroleum ether:EtOAc eluent, 10:1) to afford **11** (13 mg, 57%). IR spectrum (film, ν, cm^{-1}): 1765 (C=O), 1740 (C=O), 1710 (C=O). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.64 (6H, s, CH_3-18,19), 0.99 (3H, d, J = 7, CH_3-21), 1.23–1.26 (6H, m, CH_3-27,29), 1.32 [3H, s, $C(CH_3)_2$], 1.47 [3H, s, $C(CH_3)_2$], 2.09 (3H, s, $COCH_3$), 4.08 (1H, m, H-2), 4.25 (1H, s, H-3), 4.34 (1H, m, H-23), 5.01 (1H, m, H-22).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 11.79 q, 12.78 q, 13.43 q, 15.16 q, 16.19 q, 21.07 q, 21.20 t, 22.64 t, 23.97 t, 26.64 q, 28.51 t, 28.72 q, 33.87 t, 33.99 d, 35.63 d, 37.15 d, 37.60 d, 39.35 t, 41.26 t, 42.55 s, 42.83 s, 46.84 t, 51.56 d, 52.12 d, 53.35 d, 56.66 d, 72.24 d, 72.40 d, 76.56 d, 78.41 d, 108.04 s, 170.41 s, 178.51 s, 211.38 s.

(22*R*,23*R*,24*S*)-2α,3α,22,23-Tetrahydroxy-24-ethyl-5α-cholest-6-on-26-oic Acid (12). KOH (50 mg) was dissolved in H_2O (1 mL) and treated with **10** (10 mg) in MeOH (0.5 mL). The mixture was heated on a water bath for 1 h and evaporated to one half the volume. The resulting suspension was acidified with HCl solution (10%) until the pH was ≈ 6 and extracted with EtOAc. The extract was dried over anhydrous Na_2SO_4 and evaporated to afford **12** (4 mg, 40%), mp 174–176°C (MeOH). PMR spectrum ($CD_3OD/CDCl_3$, δ, ppm, J/Hz): 0.75 (3H, s, CH_3-18), 0.79 (3H, s, CH_3-19), 0.95 (3H, d, J = 7, CH_3-21), 1.15 (3H, d, J = 7, CH_3-25), 1.16 (3H, t, J = 7, CH_3-29), 3.59 (1H, m, H-22), 3.67–3.70 (2H, m, H-2,23), 3.98 (1H, s, H-3).

^{13}C NMR spectrum ($CD_3OD/CDCl_3$, δ, ppm): 11.0 q, 11.3 q, 12.5 q, 15.9 q, 16.9 q, 18.4 t, 20.0 t, 21.0 t, 21.6 t, 22.9 t, 22.9 d, 23.5 d, 26.4 d, 27.3 d, 29.4 t, 29.5 t, 31.7 s, 38.1 s, 39.6 t, 42.6 d, 53.7 d, 56.4 d, 56.5 d, 68.2 d, 76.8 d, 67.8 d, 71.3 d, 179.1 s, 214.0 s. Mass spectrum (*m*/*z*, I_{rel}, %): 549.28 (100) $[M^+ + MeCN]^+$, 507.27 (90) $[M]^+$, 493.18 (57), 479.06 (50), 465.07 (33), 369.31 (70).

(22*R*,23*R*,24*S*)-26-[(*N*-Hydroxysuccinimidyl)acetyl]2α,3α,22,23-tetrahydroxy-24-ethyl-5α-cholest-6-one (13). Acid **12** (15 mg, 0.029 mmol) and *N*-hydroxysuccinimide (4 mg, 0.035 mmol) were dissolved in anhydrous dioxane (1 mL), cooled to 5°C, stirred, treated with a solution of dicyclohexylcarbodiimide (10 mg) in anhydrous dioxane (0.5 mL), and held for 30 min at 5°C and for 20 h at room temperature. The precipitate of dicyclohexylurea was filtered off. The filtrate was evaporated. The solid was dissolved in EtOAc. The additional precipitate of dicyclohexylurea was filtered off. The filtrate was evaporated. The solid was chromatographed over a column of silica gel (EtOAc eluent) to afford **13** (10 mg, 57%). IR spectrum (film, ν, cm^{-1}): 3450 (OH), 1755 (C=O), 1730 (C=O), 1215. PMR spectrum ($CD_3OD/CDCl_3$, δ, ppm, J/Hz): 0.73 (3H, s, CH_3-18), 0.76 (3H, s, CH_3-19), 0.97 (3H, d, J = 6.5, CH_3-21), 1.21 (3H, t, J = 7, CH_3-29), 1.24 (3H, d, J = 7, CH_3-27), 2.58 (2H, br.s, –COC$\underline{H}_2$$CH_2$CO–), 2.66 (2H, br.s, –$COCH_2C\underline{H}_2$CO–), 3.52 (1H, m, H-22), 3.65–3.70 (2H, m, H-2,23), 3.94 (1H, s, H-3). Mass spectrum (*m*/*z*, I_{rel}, %): 606.68 (10) $[M]^+$, 578.16 (14), 561.02 (23), 535.11 (24), 508.15 (10), 476.96 (100), 458.90 (51), 445.18 (52), 427.02 (66).

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