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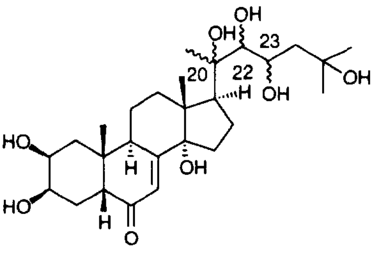
Synthesis and Structural Elucidation of a New Zooecdysteroid Gerardiasterone

**Masayoshi Tsubuki, Hironao Takada, Tadashi Katoh,
Shigehiro Miki and Toshio Honda***

Institute of Medicinal Chemistry, Hoshi University, Ebara 2-4-41, Shinagawa, Tokyo
142, Japan

Abstract: The first synthesis of a new zooecdysteroid gerardiasterone was achieved in a diastereoselective manner employing asymmetric dihydroxylation of the *E*-olefin **34** in the presence of DHQ-CLB as a chiral ligand. This synthesis unambiguously confirmed the structure of gerardiasterone as (20*R*,22*R*,23*S*)-2β,3β,14α,20,22,23,25-heptahydroxy-5β-cholest-7-en-6-one (**2a**).

A new zooecdysteroid gerardiasterone was isolated from the Mediterranean zoanthid *Gerardia savaglia* and the structure was proposed as a 23-hydroxylated ecdysteroid **1** as shown in Fig. 1.[1] Although its structure was inferred based on the spectroscopic data, especially by analysis of NMR spectra, the configuration on the side chain was not assigned yet. As a part of our continuing studies on the synthesis of physiologically active steroids with highly oxygenated side chain,[2] we focused our attention on the synthesis and structural elucidation of gerardiasterone. In this paper we report the stereoselective synthesis of 23-hydroxylated ecdysteroids **2a** and **3a**, thereby unambiguously confirming the configuration of gerardiasterone to be **2a**.[3]



Gerardiasterone (1)

Fig. 1

Due to three undefined stereocenters at the C-20, 22 and 23 positions, the structure of gerardiasterone (**1**) should be one of eight diastereomers **2a**-**9a** as shown in Fig. 2. Since most natural 20-hydroxylated ecdysteroids such as crustecdysone and makisterones have (20*R*) configuration,[4] we assumed the stereochemistry at the C-20 position for gerardiasterone (**1**) to be the same configuration as those and therefore regarded four (20*R*)-diastereomers **2a**-**5a** as the candidates for **1**. We first investigated a method for the stereoselective synthesis of model compounds **2b**-**5b** and the assignment of the stereochemistry on the side chain. Construction of the diol functionalities at the C-22 and 23 positions would be attained by a diastereoselective dihydroxylation[5] of 22*E*- and 22*Z*-olefins using chiral ligands.

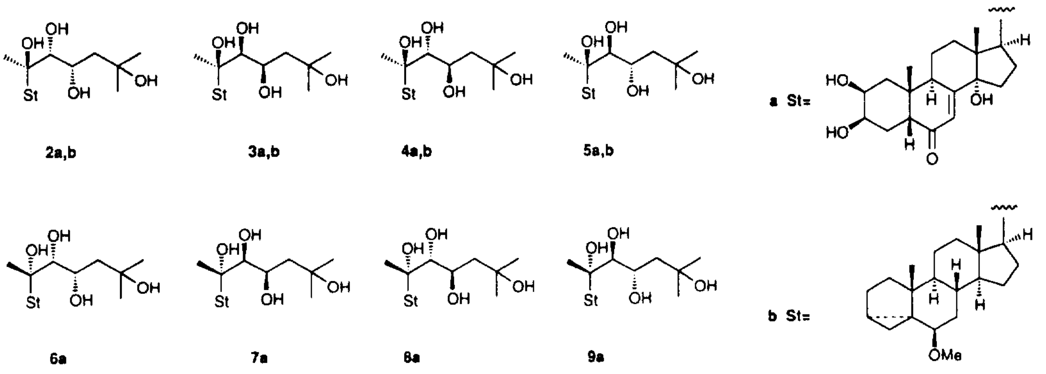
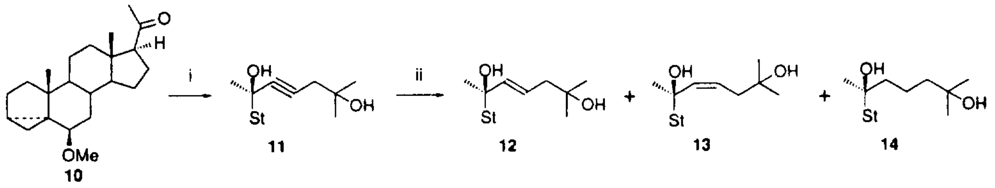


Fig. 2

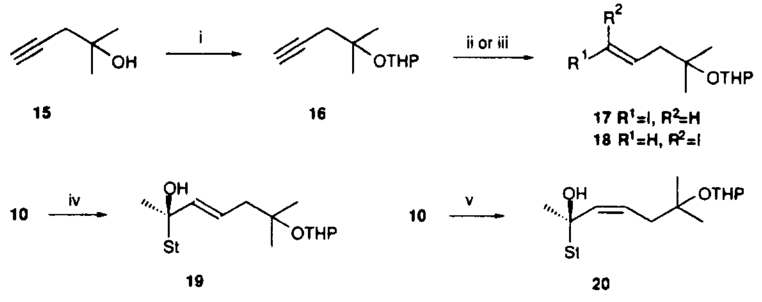
We embarked on the synthesis of *E*- and *Z*-olefins **12**, **13**, **19** and **20** as follows (Schemes 1 and 2). Grignard reaction of the ethynylmagnesium bromide, prepared from 2-methylpent-4-yn-2-ol (**15**)[6] and EtMgBr, to the ketone **10**[7] proceeded diastereoselectively to afford the (20*R*)-alcohol **11** and its epimer in a ratio of 81:19, respectively. Although partial reduction of the propargyl alcohol **11** to the *E*-allylic alcohol **12** was attempted under various conditions such as $LiAlH_4$ - NaOMe and Red-Al®, the desired product was not obtained at all. Interestingly, hydrogenation of **11** over Lindlar catalyst gave the Z-olefin **13** together with the *E*-olefin **12** and saturated product **14**.



Scheme 1 *Reagents and conditions:* i, EtMgBr, 2-methylpent-4yn-2-ol **15**, THF, 71%; ii, H_2, 5% Pd-$CaCO_3$, benzene, 16% (**12**), 57% (**13**), 9% (**14**)

We therefore synthesized the *E*- and Z-olefins **19** and **20** by the alkenylation of the ketone **10** (Scheme 2). Protection of the alcohol **15** gave the acetylenic ether **16** whose hydrostannation followed by substitution of the corresponding vinylstannane with iodine gave the *E*-vinyl iodide **17**.[8] *Z*-Vinyl iodide **18** was obtained by iodation of the terminal alkyne **16** followed by diimide reduction of the corresponding the iodoalkyne.[8] Addition of the alkenyllithium, prepared by lithiation[9] of **17** and **18** with *tert*-BuLi, to the ketone **10** gave the *E*- and Z-olefins **19** and **20** in 64 % and 75% yields, respectively.

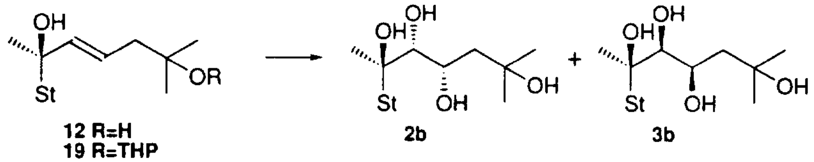
Dihydroxylation of the *E*- and *Z*-olefins **12**, **13**, **19** and **20** was investigated by osmylation in the presence of either chiral or achiral ligand and the results are shown in Tables 1 and 2. Since the reaction rate with



Scheme 2 *Reagents and conditions:* i, DHP, *p*-TsOH, CH_2Cl_2, 85%; ii, 1) *n*-Bu_3SnH, AIBN, 95%, 2) I_2, pyridine, CH_2Cl_2, 99% (**17**); iii,1) I_2, morpholine, benzene, 80%, 2) $KO_2CN{=}NCO_2K$, AcOH, MeOH, 90% (**18**); iv, **17**, *tert*-BuLi, THF, -78°C, 64%; v, **18**, *tert*-BuLi, THF, -78°C, 75%

a catalytic amount of osmium tetroxide and cooxidant was very sluggish, we used the stoichiometric amount of osmium tetroxide for the dihydroxylation. *E*-Olefins **12** and **19** were dihydroxylated by the use of an achiral ligand to give the tetraols **2b** and **3b** in a ratio of 76-85:24-15, respectively (Table 1, entries 1 and 4). The ratio in favor of the *anti,syn*-diastereomer **2b** could be increased to 91:9 through use of dihydroquinine *p*-chlorobenzoate (DHQ-CLB) (Table 1, entry 5). A complete reversal of selectivity was observed in the mismatched case when the reactions of **12** and **19** using dihydroqunidine *p*-chlorobenzoate (DHQD-CLB) were performed to give the *syn,syn*-diastereomer **3b** as a major product (Table 1, entries 3 and 6).

Table 1 Dihydroxylation of the *E*-olefins **12** and **19** using osmium tetroxide[a]



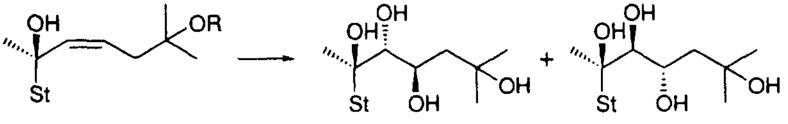
entry	substrate	ligand	yield (%)	ratio of products[b] 2b : 3b
1	**12**	pyridine	77	85 : 15
2	**12**	DHQ-CLB	83	82 : 18
3	**12**	DHQD-CLB	99	16 : 84
4	**19**	pyridine	85	76 : 24
5	**19**	DHQ-CLB	80	91 : 9
6	**19**	DHQD-CLB	87	13 : 87

[a] All reactions were run with stoichiometric amount of osmium tetroxide. After dihydroxylation of **19**, removal of the tetrahydropyranyl group with camphorsulfonic acid was carried out to give the tetraols **2b** and **3b**.

[b] Ratios were determined by ^1H NMR spectral analyses.

Dihydroxylation of the *Z*-olefins **13** and **20** by the use of an achiral ligand revealed the formation of the *anti,anti*-diastereomer **4b** to be intrinsically favored. (Table 2, entries 1 and 4). A 85:15 preference for the *syn,anti*-diastereomer **5b** in the reaction employing DHQD-CLB ligand was observed (Table 2, entry 3) whereas the reactions employing DHQ-CLB ligand did not display any useful level of diastereoselectivity (Table 2, entries 2 and 5). Although the stereochemistries of the tetraols **2b**-**5b** were predicted by the empirical mnemonic device,[5d] it was not possible to determine their structures based on their NMR spectral analyses at this stage. We thus carried out an alternative synthesis of the diastereomers in order to confirm the structures of the tetraols **2b**-**5b**.

Table 2 Dihydroxylation of the *Z*-olefins **13** and **20** using osmium tetroxide[a]



13 R=H
20 R=THP

4b

5b

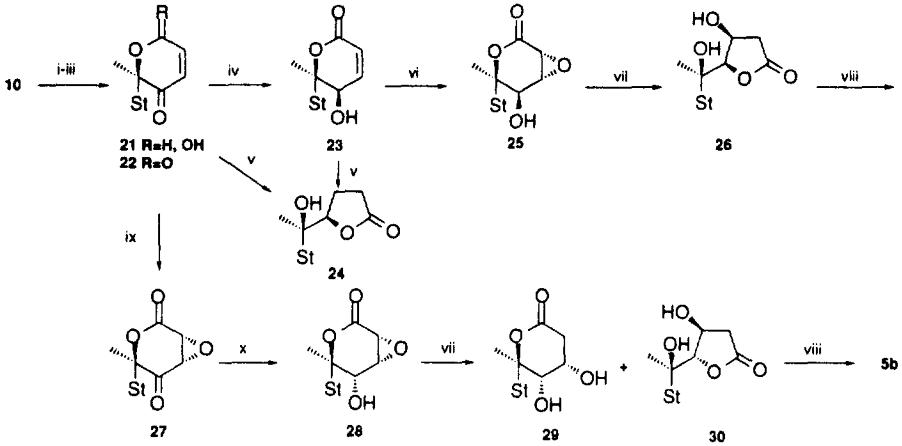
entry	substrate	ligand	yield (%)	ratio of products[b] **4b** : **5b**
1	**13**	pyridine	73	67 : 33
2	**13**	DHQ-CLB	89	57 : 43
3	**13**	DHQD-CLB	94	15 : 85
4	**20**	pyridine	96	67 : 33
5	**20**	DHQ-CLB	92	71 : 29
6	**20**	DHQD-CLB	84	35 : 65

[a] All reactions were run with stoichiometric amount of osmium tetroxide. After dihydroxylation of **20**, removal of the tetrahydropyranyl group with camphorsulfonic acid was carried out to give the tetraols **4b** and **5b**.

[b] Ratios were determined by ^1H NMR spectral analyses.

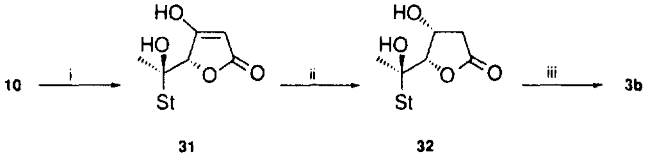
Synthesis of the tetraols **2b**-**5b** was achieved from the δ-lactone **22** as follows (Scheme 3). Enone **22** was prepared by successive addition of 2-lithiofuran to **10**, ring transformation of the furylcarbinol, and oxidation of the lactol **21** in 83% overall yield from **10** according to our previous mehtod.[2a,b] Reduction of **22** with $NaBH_4$ in the presence of $CeCl_3$[10] afforded the allyl alcohol **23** as a sole product. The stereochemistry at the C-22 position in **23** was assigned based on the fact that hydrogenation of both **22** and **23** over PtO_2 furnished the same (22*R*)-γ-lactone **24**, a key intermediate for the synthesis of crustecdysone.[2b] α,β-Unsaturated lactone **23** was treated with sodium hypochlorite[11] in pyridine to give the epoxide **25** as the only diastereomer. The observed selectivity in both the reduction and the epoxidation reactions would be explained by assuming that these reactions proceeded in the *anti* sense with respect to the steroidal nucleus at the C-20 position. Treatment of **25** with sodium phenylseleno(triisopropoxy)borate[12] brought about the regioselective ring opening of the epoxide followed by spontaneous transformation of the corresponding β,γ-dihydroxy-δ-lactone to the β-hydroxy-γ-lactone **26**. Grignard reaction of **26** with MeMgBr furnished the tetraol **2b**, whose spectroscopic data are identical with those obtained in the dihydroxylation of the *E*-olefins **12** and **19**.

Epoxidation of **22** with alkaline hydrogen peroxide proceeded with the same diastereoselectivity as above to give the epoxy ketone **27**, which was reduced with $NaBH_4$ to yield the *pseudo* equatorial alcohol **28** together with **25**. Reductive cleavage of the epoxide **28** with phenylseleno(triisopropoxy)borate gave the δ-lactone **29** and the γ-lactone **30** in a ratio of 1:1. Both compounds **29** and **30** were treated with MeMgBr to afford the tetraol **5b**, whose spectroscopic data are identical with those obtained in the dihydroxylation of the *Z*-olefins **13** and **20**.



Scheme 3 *Reagents and conditions:* i, 2-lithiofuran, THF, -78°C; ii, NBS, aq. THF; iii, PCC, NaOAc, CH_2Cl_2, 83% from **10**; iv, $NaBH_4$, $CeCl_3$, MeOH, 97%; v, H_2, PtO_2, AcOEt, 99% (from **22**), 99% (from **23**); vi, NaOCl, pyridine, 62%; vii, $(PhSe)_2$, $NaBH_4$, AcOH, *i*-PrOH, 63% (**26**), 53% (**29** : **30** = 1 : 1); viii, MeMgBr, THF, 25% (**2b**), 23% (**5b**); ix, 35% H_2O_2, 0.5M NaOH, THF, EtOH, 80%; x, $NaBH_4$, THF, -70° C, 87% (**28**), 7% (**25**)

Tetraol **3b** was also synthesized from **10** according to our previous method[2c] (Scheme 4). Addition of the dianion of tetronic acid to **10** proceeded *via* the chelation transition state to give **31**, which was hydrogenated over 5% Rh-Al_2O_3 to afford the γ-lactone **32** in 45% overall yield. Methylation of **32** furnished the tetraol **3b**, whose spectroscopic data are identical with those obtained in the dihydroxylation of the *E*-olefins **12** and **19**.



Scheme 4 *Reagents and conditions:* i, tetronic acid, LDA, THF, -78°C; ii, H_2, 5% Rh-Al_2O_3, AcOEt, 45% from **10**; iii, MeMgBr, THF, 78%

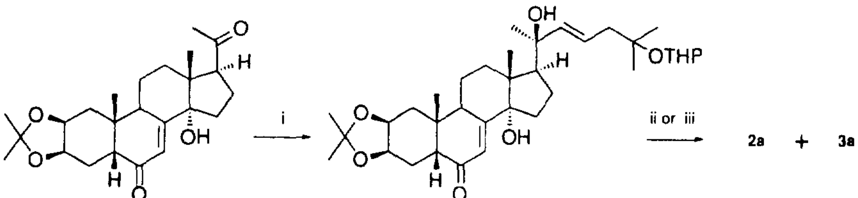
Table 3 ^1H NMR spectral data of gerardiasterone (**1**) and the tetraols **2b**-**5b**[a]

	21-H_3	26-, 27-H_3		22-H	23-H
1[b]	1.77	1.44	1.47	3.75 (br s, W/2=4 Hz)	4.80 (br d, *J*= 9.7 Hz)
2b	1.72	1.51	1.58	3.71 (br s, W/2=4 Hz)	4.78 (br d, *J*= 8.6 Hz)
3b	1.81	1.41	1.48	3.67 (br s, W/2=4 Hz)	5.01 (br d, *J*= 9.8 Hz)
4b	1.75	1.48	1.57	3.88 (d, *J* = 6.7 Hz)	4.60 (d t, *J*= 6.7 and 1.7 Hz)
5b	1.81	1.42	1.48	3.69 (t, *J* = 7.9 Hz)	4.67 (t, *J*= 7.9 Hz)

[a] ^1H NMR spectra were obtained for solutions in C_5D_5N. [b] See ref. 1.

Therefore, the structures of the tetraols **2b**-**5b** produced by the dihydroxylation of the olefins **12**, **13**, **19** and **20** were confirmed as Fig. 2 by their alternative synthesis. ^{1}H NMR spectral data of gerardiasterone **1** and the tetraols **2b**-**5b** are shown in Table 3. The 22- and 23-H signals of the 22, 23-*syn* diols **2b** and **3b** are close to those of gerardiasterone, while those of the 22, 23-*anti* diastereomers **4b** and **5b** are quite different. Thus, we deduced gerardiasterone to be either **2a** or **3a**.

Employing a diastereoselective dihydroxylation, synthesis of ecdysteroids **2a** and **3a** was carried out as follows (Scheme 5). Addition of the alkenyllithium, prepared from **17**, to the ketone **33**[13] afforded the *E*-olefin **34** in 75% yield. Dihydroxylation of **34** with stoichiometric amount of osmium tetroxide in the presence of either chiral ligand or achiral ligand followed by removal of the all protecting groups furnished **2a** and **3a**. The reaction of **34** using DHQ-CLB revealed that the diastereoselectivity was enhanced relative to the case with an achiral ligand (entry 1). A 79:21 preference for the *syn,syn*-diastereomer **3a** in the reaction employing DHQD-CLB was observed. ^{1}H NMR spectral data of gerardiasterone **1** and the ecdysteroids **2a** and **3a** as shown in Table 4 indicated that the *anti,syn*-diastereomer **2a** was identified with gerardiasterone by comparison of the resonances especially for 18-H$_3$, 21-H$_3$ and 23-H. Furthermore the physical properties including the specific rotation of **2a** are good accordance with those of gerardiasterone.



33 **34**

entry	ligand	yield (%)	ratio of products 2a : 3a
1	pyridine	38	79 : 21
2	DHQ-CLB	65	95 : 5
3	DHQD-CLB	54	21 : 79

Scheme 5 *Reagents and conditions:* i, **17**, *tert*-BuLi, -78°C, 0.5 h, 75%; ii, 1) OsO_4, DHQ-CLB, *tert*-BuOH, then $NaHSO_3$, pyridine, H_2O; 2) CSA, MeOH, 65% (**2a** from **34**); iii, 1) OsO_4, DHQD-CLB, *tert*-BuOH, then $NaHSO_3$, pyridine, H_2O; 2) CSA, MeOH, 54% (**3a** from **34**)

Table 4 ^{1}H NMR spectral data of gerardiasterone (**1**) and the ecdysteroids **2a** and **3a**[a]

	18-H$_3$	19-H$_3$	21-H$_3$	22-H	23-H	26-, 27-H$_3$	
1[b]	1.14	1.09	1.77	3.75 (br s, W/2=4 Hz)	4.80 (br d, *J*= 9.7 Hz)	1.44	1.47
2a	1.13	1.08	1.77	3.74 (br s, W/2=5 Hz)	4.78 (br d, *J*= 8.6 Hz)	1.41	1.46
3a	1.20	1.08	1.85	3.72 (br s, W/2=4 Hz)	5.05 (br d, *J*= 9.8 Hz)	1.41	1.48

[a] ^{1}H NMR spectra were obtained for solutions in C_5D_5N. [b] See ref. 1.

Thus, the structure of gerardiasterone is elucidated to be (20*R*,22*R*,23*S*)-2β,3β,14α,20,22,23,25-heptahydroxy-5β-cholest-7-en-6-one (**2a**) , namely (23*S*)-23-hydroxycrustecdysone.

EXPERIMENTAL

Melting points were measured with a Yanagimoto MP apparatus and are uncorrected. IR spectra were recorded on a Hitachi 260-10 spectrophotometer. ^{1}H NMR spectra were obtained on a JEOL GSX 270 instrument, and chemical shifts are reported in ppm on the δ scale from internal tetramethylsilane. J values are given in Hz. Mass spectra were measured with a JEOL JMS D-300 spectrometer.

(20*R*)-6β-Methoxy-3α,5-cyclo-5α-cholest-22-yne-22,25-diol (11). To a stirred solution of the ethynylmagnesium bromide, prepared by treatment of 2-methylpent-4-yn-2-ol (**15**)[6] (5.7 g, 58 mmol) and EtMgBr (3.0 M in Et_2O, 40 mL, 120 mmol) in dry THF (100 mL), was added a solution of the ketone **10**[7] (5.0 g, 15 mmol) in dry THF (60 mL) at -78°C under argon, and the solution was allowed to warm to room temperature. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (10:1, v/v) afforded a solid, which was recrystallized from hexane-AcOEt to give the alcohol **11** (4.57 g, 71%) as a colorless amorphous, mp 97-98°C. IR ($CHCl_3$) 3580, 2250 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.03 (6H, s, 18-H_3 and 19-H_3), 1.30 (6H, s, 26-H_3 and 27-H_3), 1.49 (3H, s, 21-H_3), 2.40 (2H, s, 24-H_2), 2.77 (1H, t, *J*=2.4 Hz, 6-H), 3.33 (3H, s, OMe). HRMS m/z: Calcd for $C_{28}H_{44}O_3$, 428.3290. Found 428.3293. Anal. Calcd for $C_{28}H_{44}O_3$: C, 78.45; H, 10.35. Found: C, 78.51; H, 10.62.

Further elution with the same solvent gave the epimer (1.08 g, 17%) as a colorless oil. IR ($CHCl_3$) 3580, 2250 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.30 (6H, s, 26-H_3 and 27-H_3), 1.51 (3H, s, 21-H_3), 2.40 (2H, s, 24-H_2), 2.78 (1H, t, *J*=2.4 Hz, 6-H), 3.33 (3H, s, OMe). HRMS m/z: Calcd for $C_{28}H_{44}O_3$, 428.3290. Found 428.3287.

Hydrogenation of the alkyne 11. A suspension of the alkyne **11** (1.0 g, 2.3 mmol) and 5% Pd-$CaCO_3$ (0.8 g) in dry benzene (20 mL) was shaken in a hydrogen atmosphere for 15 h. After filtration of the catalyst, the filtrate was evaporated off to give a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (5:1, v/v) afforded the (20*S*,22*Z*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-ene-20,25-diol (**13**) (570 mg, 57%) as a colorless oil. IR ($CHCl_3$) 3600, 3420 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.25 (6H, s, 26-H_3 and 27-H_3), 1.37 (3H, s, 21-H_3), 2.47 (1H, dd, *J*=8.5 Hz and 13.4 Hz, 24-$\underline{H}$H), 2.59 (1H, dd, *J*=9.2 Hz and 13.4 Hz, 24-H$\underline{H}$), 2.77 (1H, t, *J*=2.4 Hz, 6-H), 3.33 (3H, s, OMe), 5.36 (1H, ddd, *J*=8.5, 9.2 and 12.2 Hz, 23-H), 5.63 (1H, d, *J*=12.2 Hz, 22-H). Anal. Calcd for $C_{28}H_{46}O_3$: C, 78.09; H, 10.77. Found: C, 78.12; H, 10.94.

Further elution with the same solvent gave (20*S*,22*E*)-6β-methoxy-3α,5-cyclo-5α-cholest-22-ene-20,25-diol (**12**) (165 mg, 16%) as a colorless oil. IR ($CHCl_3$) 3600, 3420 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.21 (6H, s, 26-H_3 and 27-H_3), 1.34 (3H, s, 21-H_3), 2.19 (2H, d, *J*=5.5 Hz, 24-H_2), 2.77 (1H, t, *J*=2.4 Hz, 6-H), 3.33 (3H, s, OMe), 5.60 (1H, dd, *J*=5.5 and 15.3 Hz, 23-H), 5.68 (1H, d, *J*=15.3 Hz, 22-H). Anal. Calcd for $C_{28}H_{46}O_3$: C, 78.09; H, 10.77. Found: C, 78.51; H, 10.80.

Further elution with the same solvent gave (20*S*)-6β-methoxy-3α,5-cyclo-5α-cholestane-20,25-diol (**14**) (90 mg, 9%) as a colorless oil. IR ($CHCl_3$) 3600, 3420 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.22 (6H, s, 26-H_3 and 27-H_3), 1.29 (3H, s, 21-H_3), 2.78 (1H, t, *J*=2.4 Hz, 6-H), 3.33 (3H, s, OMe).

4-Methyl-4-tetrahydropyranyloxypent-1-yne (16). A mixture of **15** (2.08 g, 21.2 mmol), dihydropyran (2.14 g, 25.4 mmol) and *p*-TsOH (5 mg) in dry CH_2Cl_2 (2 mL) was stirred for 12 h. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture was extracted with Et_2O. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a colorless oil. Distillation of the oil afforded the ether **16** (3.27 g, 85%) as a colorless oil, bp 110°C/50 mmHg. IR ($CHCl_3$) 2120 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.34 and 1.36 (each 3H, each s, 4-Me and 5-H_3), 2.00 (1H, t, *J*=3.0 Hz, 1-H), 2.40 and 2.49 (each 1H, each dd, *J*=3.0 and 16.5 Hz, 3-H_2), 3.4-3.5 and 3.9-4.0 (each 1H, each m, CH_2O), 4.80 (1H, br s, OCHO).

(*E*)-1-Iodo-4-methyl-4-tetrahydropyranyloxypent-1-ene (17). A mixture of **16** (3.59 g, 19.7 mmol), tributyltin hydride (5.8 mL, 21.7 mmol) and azoisobutyronitrile (90 mg, 0.55 mmol) was heated at 120°C for 12 h. The mixture was purified by column chromatography on silica gel. Elution with hexane-AcOEt (97:3, v/v) afforded (*E*)-1-tributylstannyl-4-methyl-4-tetrahydropyranyloxypent-1-ene (8.8 g, 95%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.20 and 1.22 (each 3H, each s, 4-Me and 5-H_3), 2.37 (2H, d, *J*=6.7 Hz, 3-H_2), 3.4-3.5 and 3.9-4.0 (each 1H, each m, CH_2O), 4.78 (1H, br s, OCHO), 5.9-6.05 (2H, m, 1-H and 2-H). To a stirred solution of the above vinylstannane (8.8 g, 18.6 mmol) and pyridine (1.95 mL, 24.2 mmol) in dry CH_2Cl_2 (90 mL) was added iodine (7.08 g, 27.9 mmol) at -78°C and the mixture was stirred at the same temperature for 1h. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture was extracted with CH_2Cl_2. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (95:5, v/v) afforded the vinyl iodide **17** (5.76 g, 99%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.19 and 1.22 (each 3H, each s, 4-Me and 5-H_3), 2.26 (2H, d, *J*=6.7 Hz, 3-H_2), 3.4-3.5 and 3.9-4.0 (each 1H, each m, CH_2O), 4.74 (1H, br s, OCHO), 6.03 (1H, d, *J*=14.7 Hz, 1-H), 6.59 (1H, dt, *J*=6.7 and 14.7 Hz, 2-H). Anal. Calcd for $C_{11}H_{19}I$: C, 42.60; H, 6.18. Found: C, 42.55; H, 6.34.

(*Z*)-1-Iodo-4-methyl-4-tetrahydropyranyloxypent-1-ene (18). To a stirred solution of the dark orange iodomorpholino complex, prepared by treatment of morpholine (2 mL, 23 mmol) with iodine (1.98 g, 7.8 mmol) in benzene (30 mL) at 45°C, was added a solution of **16** (944 mg, 5.2 mmol) in benzene (2 mL) at 45°C and the mixture was stirred at the same temperature for 24 h. Water was added to the mixture and the mixture was extracted with benzene. The extract was washed with saturated aqueous sodium thiosulfate solution and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (95:5, v/v) afforded 1-iodo-4-methyl-4-tetrahydropyranyloxypent-1-yne (1.27 g, 80%) as colorless oil. ^1H NMR ($CDCl_3$) δ 1.32 and 1.33 (each 3H, each s, 4-Me and 5-H_3), 2.56 and 2.66 (each 1H, each d, *J*=16.5 Hz, 3-H_2), 3.4-3.5 and 3.9-4.0 (each 1H, each m, CH_2O), 4.79 (1H, br s, OCHO).

Freshly prepared dipotassium azodicarboxylate (808 mg, 4.2 mmol) was added to a stirred solution of the above iodoacetylene (1.27 g, 4.2 mmol) in pyridine (0.8 mL) and MeOH (5 mL). Then, AcOH (0.46 mL) was added and the mixture was stirred for 18 h. The mixture was filtered and the filtrate was slowly hydrolyzed with ice-cold 5% HCl, and extracted with Et_2O. The extract was washed with saturated aqueous sodium thiosulfate solution and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane afforded **18** (1.16 g, 90%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.24 and 1.27 (each 3H, each s, 4-Me and 5-H_3), 2.3-2.5 (2H, m, 3-H_2), 3.4-3.5 and 3.9-4.0 (each 1H, each m, CH_2O), 4.75 (1H, br s, OCHO), 6.3-6.4 (2H, m, 1-H and 2-H). HRMS m/z: Calcd for C_6H_9I, 207.9748. Found 207.9748.

(20*S*,22*E*)-6β-Methoxy-25-tetrahydropyranyloxy-3α,5-cyclo-5α-cholest-22-en-20-ol (19). To a stirred solution of the alkenyllithium, prepared by treatment of **17** (607 mg, 1.96 mmol) with *tert*-BuLi (1.49 M in pentane, 2.63 mL, 3.9 mmol) in dry Et_2O (5 mL) at -78°C, was added a solution of the ketone **10** (323 mg, 0.98 mmol) in dry THF (5 mL) at -78°C under argon, and the solution was allowed to warm to room temperature. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (96:4, v/v) afforded the recovered ketone (27 mg) and **19** (323 mg, 70% based on the recovery of the starting material) as a colorless oil. IR ($CHCl_3$) 3420 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.86 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.19 and 1.20 (each 3H, each s, 26-H_3 and 27-H_3), 1.32 (3H, s, 21-H_3), 2.24 (2H, d, *J*=5.5 Hz, 24-H_2), 2.76 (1H, t, *J*=2.4 Hz, 6-H), 3.33 (3H, s, OMe), 3.4-3.5 and 3.9-4.0 (each 1H, each m, CH_2O), 4.76 (1H, br s, OCHO), 5.5-5.65 (2H, m, 22-H and 23-H). Anal. Calcd for $C_{33}H_{54}O_4 \cdot 0.2H_2O$: C, 76.46; H, 10.54. Found: C, 76.11; H, 10.66.

(20*S*,22*Z*)-6β-Methoxy-25-tetrahydropyranyloxy-3α,5-cyclo-5α-cholest-22-en-20-ol (20). To a stirred solution of the alkenyllithium, prepared by treatment of **18** (550 mg, 1.77 mmol) with *tert*-BuLi (1.57 M in pentane, 2.2 mL, 3.45 mmol) in dry Et_2O (5 mL) at -78°C, was added a solution of the ketone **10** (240 mg, 0.73 mmol) in dry THF (3 mL) at -78°C under argon, and the solution was allowed to warm to room temperature. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (95:5, v/v) afforded the recovered ketone (26 mg) and **20** (280 mg, 84% based on the recovery of the starting material) as a colorless oil. IR ($CHCl_3$) 3430 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.88 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.24 and 1.29 (each 3H, each s, 26-H_3 and 27-H_3), 1.36 and 1.37 (each 1.5H, each s, 21-H_3), 2.59 (2H, d, *J*=8.5 Hz, 24-H_2), 2.77 (1H, t, *J*=2.4 Hz, 6-H), 3.32 (3H, s, OMe), 3.4-3.5 and 3.9-4.0 (each 1H, each m, CH_2O), 4.79 (1H, br s, OCHO), 5.3-5.4 (1H, m, 23-H), 5.57 (1H, d, *J*=11.6 Hz, 22-H). Anal. Calcd for $C_{33}H_{54}O_4$: C, 76.99; H, 10.57. Found: C, 76.98; H, 10.66.

Dihydroxylation of the olefins 12 and 13 with OsO_4 and pyridine.

a) From the *E*-olefin 12. To a stirred solution of **12** (80 mg, 0.18 mmol) and pyridine (0.03 mL) in dry Et_2O (1 mL) was added OsO_4 (48 mg, 0.19 mmol) at room temperature, and the mixture was stirred for 15 h at the same temperature. Evaporation of the solvent gave a residue, which was dissolved in pyridine (4 mL) and water (3 mL). Sodium bisulfite (250 mg, 2.4 mmol) was added to the mixture, and the reaction mixture was stirred for 2 h. The mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (50:50, v/v) afforded an inseparable mixture of (20*R*,22*R*,23*S*)-6β-methoxy-3α,5-cyclo-5α-cholestane-20,22,23,25-tetraol (**2b**) and (20*R*,22*S*,23*R*)-6β-methoxy-3α,5-cyclo-5α-cholestane-20,22,23,25-tetraol (**3b**) in a ratio of 85:15 as a colorless oil (66 mg, 77%). IR ($CHCl_3$) 3450 cm^{-1}. HRMS m/z: Calcd for $C_{28}H_{47}O_5$, 463.3424. Found 463.3430. For **2b**: ^1H NMR ($CDCl_3$) δ 0.84 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.29 and 1.31 (each 3H, each s, 26-H_3 and 27-H_3), 1.36 (3H, s, 21-H_3), 2.78 (1H, t, *J*=2.4 Hz, 6-H), 3.20 (1H, s, 22-H), 3.33 (3H, s, OMe), 4.32 (1H, d, *J*=11.0 Hz, 23-H). For **3b**: ^1H NMR ($CDCl_3$) δ 0.87 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.30 and 1.32 (each 3H, each s, 26-H_3 and 27-H_3), 1.37 (3H, s, 21-H_3), 2.78 (1H, t, *J*=2.4 Hz, 6-H), 3.09 (1H, br s, 22-H), 3.33 (3H, s, OMe), 4.46 (1H, d, *J*=12.2 Hz, 23-H).

b) From the Z-olefin 13. To a stirred solution of **13** (570 mg, 1.33 mmol) and pyridine (0.23 mL) in dry Et_2O (7 mL) was added OsO_4 (342 mg, 1.35 mmol) at room temperature, and the mixture was stirred for 15 h at the same temperature. Evaporation of the solvent gave a residue, which was dissolved in pyridine (30 mL) and water (20 mL). Sodium bisulfite (1.78 mg, 17 mmol) was added to the mixture, and the reaction mixture was stirred for 2 h. The mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (80:20, v/v) afforded a solid, which was recrystallized from hexane-$CHCl_3$ to give (20*R*,22*S*,23*S*)-6β-methoxy-3α,5-cyclo-5α-cholestane-20,22,23,25-tetraol (**5b**) (151 mg, 24%) as a colorless amorphous, mp 130-131°C. IR ($CHCl_3$) 3450 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.95 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.31 and 1.36 (each 3H, each s, 26-H_3 and 27-H_3), 1.41 (3H, s, 21-H_3), 2.78 (1H, t, *J*=3.0 Hz, 6-H), 3.26 (1H, d, *J*=8.5 Hz, 22-H), 3.33 (3H, s, OMe), 4.13 (1H, dt, *J*=3.1 and 8.5 Hz, 23-H). ^1H NMR (pyridine-d_5) δ 1.14 (3H, s, 18-H_3), 1.22 (3H, s, 19-H_3), 1.42 and 1.44 (each 3H, each s, 26-H_3 and 27-H_3), 1.81 (3H, s, 21-H_3), 2.73 (1H, br s, 6-H), 3.29 (3H, s, OMe), 3.69 (1H, t, *J*=7.9 Hz, 22-H), 4.67 (1H, t, *J*=7.9 Hz, 23-H). Anal. Calcd for $C_{28}H_{48}O_5$: C, 72.37; H, 10.41. Found: C, 72.08; H, 10.50.
Further elution with the same solvent gave (20*R*,22*R*,23*R*)-6β-methoxy-3α,5-cyclo-5α-cholestane-20,22,23,25-tetraol (**4b**) (301 mg, 49%) as a colorless foam, mp 61-62°C. IR ($CHCl_3$) 3450 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.89 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.29 and 1.31 (each 3H, each s, 26-H_3 and 27-H_3), 1.35 (3H, s, 21-H_3), 2.78 (1H, br s, 6-H), 3.33 (3H, s, OMe), 3.47 (1H, d, *J*=5.5 Hz, 22-H), 4.05-4.1 (1H, m, 23-H). ^1H NMR (pyridine-d_5) δ 1.17 (3H, s, 18-H_3), 1.22 (3H, s, 19-H_3), 1.48 and 1.57 (each 3H, each s, 26-H_3 and 27-H_3), 1.75 (3H, s, 21-H_3), 2.74 (1H, br s, 6-H), 3.30 (3H, s, OMe), 3.88 (1H, t, *J*=6.7 Hz, 22-H), 4.60 (1H, dt, *J*=1.7 and 6.7 Hz, 23-H). Anal. Calcd for $C_{28}H_{48}O_5$: C, 72.37; H, 10.41. Found: C, 72.76; H, 10.62.

Dihydroxylation of the olefins 19 and 20 with OsO_4 and pyridine.

a) From the *E*-olefin 19. Dihydroxylation of **19** (110 mg, 0.21 mmol) with OsO_4 (65 mg, 0.26 mmol) and pyridine (1 mL) was performed as above to afford the crude products. A mixture of the products and a catalytic amount of camphorsulfonic acid in MeOH (3 mL) was stirred for 0.5 h. The product was isolated by AcOEt extraction. Purification of the product by column chromatography on silica gel using hexane-AcOEt (50:50, v/v) as eluent gave an inseparable mixture of **2b** and **3b** (84.1 mg, 85%) in a ratio of 76:24.

b) From the *Z*-olefin 20. Dihydroxylation of **20** (18.5mg, 0.036 mmol) with OsO_4 (14 mg, 0.055 mmol) and pyridine (0.5 mL) was performed as above to afford the crude products. A mixture of the products and a catalytic amount of camphorsulfonic acid in MeOH (0.5 mL) was stirred for 0.5 h. The product was isolated by AcOEt extraction. Purification of the product by column chromatography on silica gel using hexane-AcOEt (80:20, v/v) as eluent gave a mixture of **4b** and **5b** (16 mg, 96%) in a ratio of 67:33.

General procedure for the dihydroxylation of the olefins 12, 13, 19 and 20 with chiral ligands. To a stirred solution of the olefin (0.05 mmol) and chiral ligand (DHQ-CLB or DHQD-CLB) (0.055 mmol) in *tert*-BuOH (0.5 mL) was added OsO_4 (0.055 mmol) at room temperature and the mixture was stirred until the starting material was consumed (usually 1-3 h). A solution of sodium bisulfite (0.5 mmol) in water (0.5 mL) was added to the mixture and the reaction mixture was stirred until the black insoluble material precipitated. After filtration of the inorganic material, the filtrate was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (50:50, v/v) afforded a mixture of dihydroxylated tetraols. In case of **19** and **20**, the crude product obtained by the dihydroxylation was further treated with a catalytic amount of camphorsulfonic acid in MeOH (0.5 mL). Workup described above afforded a mixture of the tetraols. The ratio of the tetraols was determined by the analysis of their ^1H NMR spectra and the results are shown in Tables 1 and 2.

(20*R*,23*Z*)-20,25-Epoxy-25-hydroxy-6β-methoxy-3α,5-cyclo-25-homo-5α-chol-23-en-22-one (21). To a stirred solution of 2-lithiofuran, prepared by treatment of furan (5.5 mL, 75 mmol) with *n*-BuLi (1.5 M in hexane, 46 mL, 69 mmol) in dry THF (60 mL) at 0°C, was added a solution of the ketone **10** (9.9 g, 30 mmol) in dry THF (100 mL) at -78°C under argon, and the mixture was stirred for 2 h at the same temperature. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the crude (20*R*)-furylcarbinol (11.94 g), which was used for the next reaction without further purification due to its instability.

NBS (5.34 g, 30 mmol) was added portionwise to a stirred solution of the crude product in THF-water (4:1 v/v, 150 mL) at 0°C over 1 h. After quenching the reaction by addition of aqueous 10% KI solution and then aqueous sodium thiosulfate solution, the solution was concentrated to give an oil, which was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (80:20, v/v) afforded an epimeric mixture (*ca* 1:1) of the lactol **21** (11.91 g, 96%) as a colorless glass. IR ($CHCl_3$) 3595, 3360, 1690

cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.85 and 0.87 (each 1.5H, each s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.45 and 1.58 (each 1.5H, each s, 21-H_3), 2.79 (1H, br s, 6-H), 3.33 (3H, s, OMe), 5.70 (1H, br s, 25-H), 5.99 and 6.04 (each 0.5H, each br d, *J*=10.4 Hz, 23-H), 6.85 and 6.86 (each 0.5H, each br d, *J*=10.4 Hz, 24-H). Anal. Calcd for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 74.83; H, 9.28.

(20*R*,23*Z*)-6β-Methoxy-22-oxo-3α,5-cyclo-25-homo-5α-chol-23-eno-25,20-lactone (22). To a stirred solution of **21** (1 g, 2.4 mmol) and anhydrous NaOAc (396 mg, 4.8 mmol) in dry CH_2Cl_2 (25 mL) was added portionwise PCC (1.04 g, 4.8 mmol) at 0°C, and the mixture was stirred for 1.5 h at room temperature. Et_2O (100 mL) was added to the mixture and the inorganic material was filtered off. The filtrate was evaporated to give a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (85:15, v/v) afforded a solid, which was recrystallized from AcOEt to give the lactone **22** (861 mg, 87%) as a colorless needles, mp 195-196°C. IR ($CHCl_3$) 1725, 1700 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.90 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.65 (3H, s, 21-H_3), 2.77 (1H, d, *J*=2.4 Hz, 6-H), 3.32 (3H, s, OMe), 6.67 (1H, d, *J*=9.8 Hz, 23-H), 6.87 (1H, d, *J*=9.8 Hz, 24-H). Anal. Calcd for $C_{26}H_{36}O_4$: C, 75.69; H, 8.80. Found: C, 75.82; H, 8.99.

(20*R*,22*R*,23*Z*)-22-Hydroxy-6β-methoxy-3α,5-cyclo-25-homo-5α-chol-23-eno-25,20-lactone (23). To a stirred solution of **22** (150 mg, 0.36 mmol) and $CeCl_3$ solution (0.4 M in MeOH, 0.91 mL, 0.36 mmol) was added portionwise $NaBH_4$ (13.8 mg, 0.36 mmol) at -70°C, and the mixture was stirred for 0.5 h at the same temperature. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (75:25, v/v) afforded the allyl alcohol **23** (146 mg, 97%) as a colorless glass. IR ($CHCl_3$) 3350, 1680 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.87 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.45 (3H, s, 21-H_3), 2.79 (1H, d, *J*=2.4 Hz, 6-H), 3.33 (3H, s, OMe), 4.00 (1H, dd, *J*=5.5 and 9.2 Hz, 22-H), 6.03 (1H, d, *J*=9.8 Hz, 24-H), 6.92 (1H, dd, *J*=5.5 and 9.8 Hz, 23-H). Anal. Calcd for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 75.07; H, 9.38.

(20*R*,22*R*)-20-Hydroxy-6β-methoxy-3α,5-cyclo-25-homo-5α-cholano-25,22-lactone (24).
a) From the enone 22: A suspension of **22** (10 mg, 0.024 mmol) and PtO_2 (10 mg) in AcOEt (2 mL) was shaken in a hydrogen atmosphere for 1 h. After filtration of the catalyst, the filtrate was evaporated off to give a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (75:25, v/v) afforded the γ-lactone **24** (10 mg, 99%) as a colorless glass. IR ($CHCl_3$) 3570, 1760 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.89 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.22 (3H, s, 21-H_3), 2.77 (1H, d, *J*=2.4 Hz, 6-H), 3.33 (3H, s, OMe), 4.46 (1H, t, *J*=7.6 Hz, 22-H). Anal. Calcd for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68. Found: C, 75.21; H, 9.81.
b) From the allyl alcohol 23: Hydrogenation of the **23** (15 mg, 0.036 mmol) over PtO_2 (10 mg) in AcOEt (2 mL) was performed as the method **a** to give the same γ-lactone **24** (15 mg, 99%) as above.

(20*R*,22*R*,23*R*,24*R*)-23,24-Epoxy-22-hydroxy-6β-methoxy-3α,5-cyclo-25-homo-5α-cholano-25,20-lactone (25). To a stirred solution of **23** (66 mg, 0.16 mmol) in pyridine (3 mL) was added dropwise NaOCl solution (5.25% in water, 0.53 mL, 0.37 mmol) over 10 min at -10°C, and the mixture was stirred for 0.5 h at the same temperature. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (70:30, v/v) afforded the epoxide **25** (32.1 mg, 62% based on the recovery of the starting material (16.6 mg)) as a colorless glass. IR ($CHCl_3$) 3400, 1705 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.84 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.56 (3H, s, 21-H_3), 2.78 (1H, d, *J*=2.4 Hz, 6-H), 3.33 (3H, s, OMe), 3.56 (1H, d, *J*=3.7 Hz, 24-H), 3.70 (1H, dd, *J*=3.1 and 3.7 Hz, 23-H), 4.11 (1H, t, *J*=3.1 Hz, 22-H). HRMS m/z: Calcd for $C_{26}H_{38}O_5$, 430.2718. Found 430.2718.

(20*R*,22*R*,23*S*)-20,23-Dihydroxy-6β-methoxy-3α,5-cyclo-25-homo-5α-cholano-25,22-lactone (26). To a stirred solution of diphenyl diselenide (28.6 mg, 0.09 mmol) in *i*-PrOH (0.5 mL) was added portionwise $NaBH_4$ (7 mg, 0.18 mmol) at room temperature, and after 3 min AcOH (1.8 μL, 0.03 mmol) was added. After 5 min, a solution of the epoxy lactone **25** (13.2 mg, 0.03 mmol) in *i*-PrOH (0.5 mL) was added to the mixture at 0°C. The reaction mixture was stirred for 40 min at the same temperature. The mixture was diluted with AcOEt and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (60:40, v/v) afforded the γ-lactone **26** (8.4 mg, 63%) as a colorless glass. IR ($CHCl_3$) 3400, 1775 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.82 (3H, s, 18-H_3), 1.05 (3H, s, 19-H_3), 1.46 (3H, s, 21-H_3), 2.62 and 2.68 (each 1H, each d, *J*=16.2 Hz, 24-H_2), 2.78 (1H, d, *J*=2.4 Hz, 6-H), 3.33 (3H, s, OMe), 4.18 (1H, d, *J*=3.1 Hz, 22-H), 4.69 (1H, br s, 23-H). HRMS m/z: Calcd for $C_{26}H_{40}O_5$, 432.2876. Found 432.2889.

(20*R*,22*R*,23*S*)-6β-Methoxy-3α,5-cyclo-5α-cholestane-20,22,23,25-tetraol (2b). To a stirred solution of **26** (7.4 mg, 0.017 mmol) in dry THF (1 mL) was added MeMgBr (3 M in Et_2O, 0.13mL, 0.39 mmol) at -78°C under argon, and the mixture was allowed to warm to room temperature. The reaction mixture was stirred for 15 h at the same temperature. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (60:40, v/v) afforded the tetraol **2b** (2 mg, 25%) as a colorless glass. Spectroscopic data (IR, ^1H NMR) of the compound were identical with those described in the dihydroxylation of the *E*-olefin **12**. ^1H NMR (pyridine-d_5) δ 1.12 (3H, s, 18-H_3), 1.16 (3H, s, 19-H_3), 1.51 and 1.58 (each 3H, each s, 26-H_3 and 27-H_3), 1.72 (3H, s, 21-H_3), 2.74 (1H, br s, 6-H), 3.31 (3H, s, OMe), 3.71 (1H, br s, W/2=4 Hz, 22-H), 4.78 (1H, br d, *J*=8.6 Hz, 23-H). Anal. Calcd for $C_{28}H_{48}O_5$: C, 72.37; H, 10.41. Found: C, 72.04; H, 10.64.

(20*R*,23*S*,24*R*)-23,24-Epoxy-6β-methoxy-22-oxo-3α,5-cyclo-25-homo-5α-cholano-25,20-lactone (27). To a stirred solution of **22** (80 mg, 0.19 mmol) and H_2O_2 (35% in water, 0.1 mL, 1.0 mmol) in

THF-MeOH (10:1 v/v, 4.5 mL) was added dropwise NaOH solution (0.5M in water, 0.2 mL, 0.1 mmol) at -70°C, and the mixture was allowed to warm to room temperature over 1 h. The mixture was diluted with AcOEt, and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (92:8, v/v) afforded a solid, which was recrystallized from hexane-AcOEt to give the epoxide **27** (66.5 mg, 80%) as colorless prisms, mp 161-162°C. IR ($CHCl_3$) 1725 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.91 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.71 (3H, s, 21-H_3), 2.77 (1H, d, *J*=2.4 Hz, 6-H), 3.32 (3H, s, OMe), 3.70 (1H, d, *J*=3.7 Hz, 24-H), 3.93 (1H, d, *J*=3.7 Hz, 23-H). HRMS m/z: Calcd for $C_{26}H_{36}O_5$, 428.2552. Found 428.2557. Anal. Calcd for $C_{26}H_{36}O_5$: C, 72.86; H, 8.47. Found: C, 72.96; H, 8.54.

(20*R*,22*S*,23*R*,24*R*)-23,24-Epoxy-22-hydroxy-6β-methoxy-3α,5-cyclo-25-homo-5α-cholano-25,20-lactone (28). To a stirred solution of **27** (199 mg, 0.46 mmol) in CH_2Cl_2-MeOH (1:1 v/v, 3 mL) was added portionwise $NaBH_4$ (18 mg, 0.47 mmol) at -5°C, and the mixture was stirred for 0.5 h at the same temperature. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (75:25, v/v) afforded the alcohol **25** (13.4 mg, 7%), which was also obtained by the epoxidation of the α,β-unsaturated lactone **23.** Further elution with the same solvent gave a solid, which was recrystallized from hexane-AcOEt to afford the alcohol **28** (173.7 mg, 87%) as colorless plates, mp 222-223°C. IR ($CHCl_3$) 36000, 3395, 1725 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.90 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.68 (3H, s, 21-H_3), 2.77 (1H, d, *J*=2.4 Hz, 6-H), 3.32 (3H, s, OMe), 3.66 (1H, d, *J*=4.3 Hz, 24-H), 3.69 (1H, dd, *J*=1.8 and 4.3 Hz, 23-H), 4.17 (1H, d, *J*=1.8 Hz, 22-H). Anal. Calcd for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.77; H, 9.10.

Reductive cleavage of the epoxy lactone 28. Reductive cleavage of **28** (83 mg, 0.19 mmol) with sodium phenylseleno(triisppropoxy)borate, prepared by treatment of diphenyl diselenide (181 mg, 0.58 mmol) with $NaBH_4$ (44 mg, 1.16 mmol) in AcOH (11 µL, 0.17 mmol) and *i*-PrOH (1.5 mL), was performed as the preparation of **26**. The product was isolated by AcOEt extraction and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (55:45, v/v) afforded (20*R*,22*S*,23*S*)-20,23-dihydroxy-6β-methoxy-3α,5-cyclo-25-homo-5α-cholano-25,22-lactone (**30**) (21.5 mg, 26%) as a colorless glass. IR ($CHCl_3$) 3405, 1790 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.93 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.29 (3H, s, 21-H_3), 2.58 (1H, dd, *J*=6.7 and 18.3 Hz, 24-$\underline{H}$H), 2.79 (1H, d, *J*=2.4 Hz, 6-H), 2.87 (1H, dd, *J*=7.9 and 18.3 Hz, 24-H$\underline{H}$), 3.33 (3H, s, OMe), 4.16 (1H, d, *J*=4.9 Hz, 22-H), 4.6-4.7 (1H, m, 23-H). HRMS m/z: Calcd for $C_{26}H_{40}O_5$, 432.2876. Found 432.2872.

Further elution with the same solvent gave (20*R*,22*S*,23*S*)-22,23-dihydroxy-6β-methoxy-3α,5-cyclo-25-homo-5α-cholano-25,20-lactone (**29**) (22.8 mg, 27%) as a colorless glass. IR ($CHCl_3$) 3450, 1725 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.89 (3H, s, 18-H_3), 1.02 (3H, s, 19-H_3), 1.58 (3H, s, 21-H_3), 2.68 (1H, dd, *J*=7.3 and 17.7 Hz, 24-$\underline{H}$H), 2.78 (1H, d, *J*=2.4 Hz, 6-H), 2.81 (1H, dd, *J*=7.3 and 17.7 Hz, 24-H$\underline{H}$), 3.33 (3H, s, OMe), 3.92

(1H, d, *J*=3.7 Hz, 22-H), 4.35 (1H, dt, *J*=3.7 and 7.3 Hz, 23-H). HRMS m/z: Calcd for $C_{26}H_{40}O_5$, 432.2876. Found 432.2879.

(20*R*,22*S*,23*S*)-6β-Methoxy-3α,5-cyclo-5α-cholestane-20,22,23,25-tetraol (5b).
a) From the δ-lactone 29: Addition of MeMgBr (3 M in Et_2O, 0.53 mL, 1.59 mmol) to **29** (22.8 mg, 0.053 mmol) in dry THF (1 mL) was performed as the preparation of **2b**. The product was isolated by AcOEt extraction and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (80:20, v/v) afforded the recovered starting material (5.6 mg) and the tetraol **5b** (1.6 mg, 7%). Spectroscopic data (IR, ^1H NMR) of the compound were identical with those described in the dihydroxylation of the *Z*-olefin **13**.
b) From the γ-lactone 30: Addition of MeMgBr (3 M in Et_2O, 0.55 mL, 1.65 mmol) to **30** (22.4 mg, 0.052 mmol) in dry THF (1 mL) was performed as the preparation of **2b**. The product was isolated by AcOEt extraction and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (80:20, v/v) afforded the recovered starting material (2.1 mg) and the same tetraol **5b** (5 mg, 23% base on the recovery of the starting material) as above.

(20*R*,22*S*,23*R*)-20,23-Dihydroxy-6β-methoxy-3α,5-cyclo-25-homo-5α-cholano-25,22-lactone (32). To a stirred solution of the dianion of tetronic acid, prepared by treatment of tetronic acid (210 mg, 2.1 mmol) with lithium diisopropylamide (4.2 mmol) in dry THF (18 mL) at 0°C, was added a solution of the ketone **10** (70 mg, 0.21 mmol) in dry THF (8 mL) at -78°C under argon, and the mixture was stirred for 1 h at the same temperature. After quenching the reaction by addition of saturated aqueous ammonium chloride solution, the mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the crude adduct **31** (140 mg),which was used for the next reaction without further purification.
A suspension of the above compound (140 mg) and 5% Rh-Al_2O_3 (140 mg) in AcOEt (4 mL) was shaken in a hydrogen atmosphere for 16 h. After filtration of the catalyst, the filtrate was evaporated off to give a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (5:1, v/v) afforded the β-hydroxy-γ-lactone **32** (40 mg, 45% from **10**) as a colorless glass. IR ($CHCl_3$) 3300, 1760 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.89 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.52 (3H, s, 21-H_3), 2.6-2.75 (2H, m, 24-H_2), 2.79 (1H, d, *J*=2.4 Hz, 6-H), 3.33 (3H, s, OMe), 4.23 (1H, d *J*=3.7 Hz, 22-H), 4.78 (1H, br s, 23-H). HRMS m/z: Calcd for $C_{26}H_{40}O_5$, 432.2876. Found 432.2877.

(20*R*,22*S*,23*R*)-6β-Methoxy-3α,5-cyclo-5α-cholestane-20,22,23,25-tetraol (3b). Addition of MeMgBr (3 M in Et_2O, 1.54 mL, 4.6 mmol) to **32** (200 mg, 0.0.46 mmol) in dry THF (12 mL) was performed as the preparation of **2b**. The product was isolated by AcOEt extraction and the organic layer was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (80:20, v/v) afforded the tetraol **3b** (167 mg, 78%) as a colorless glass. Spectroscopic data (IR, ^{1}H NMR) of the compound were identical with those described in the dihydroxylation of the *E*-olefin **12**. ^{1}H NMR (pyridine-d$_5$) δ 1.16 (3H, s, 18-H$_3$), 1.19 (3H, s, 19-H$_3$), 1.41 and 1.48 (each 3H, each s, 26-H$_3$ and 27-H$_3$), 1.81 (3H, s, 21-H$_3$), 2.75 (1H, br s, 6-H), 3.31 (3H, s, OMe), 3.67 (1H, br s, W/2=4 Hz, 22-H), 5.01 (1H, br d, *J*=9.8 Hz, 23-H). Anal. Calcd for $C_{28}H_{48}O_5$: C, 72.37; H, 10.41. Found: C, 72.27; H, 10.55.

(20*S*,22*E*)-14α,20-Dihydroxy-2β,3β-isopropylidenedioxy-25-tetrahydropyranyloxy-5β-cholesta-7,22-dien-6-one (34). Addition of the alkenyllithium, prepared by treatment of **17** (515 mg, 1.49 mmol) with *tert*-BuLi (1.57 M in pentane, 1.9 mL, 2.98 mmol) in dry Et$_2$O (7 mL) at -78°C, to the ketone **33**[13] (134 mg, 0.33 mmol) in dry THF (20 mL) was performed as the preparation of **19**. The product was isolated by AcOEt extraction and the organic layer was washed with brine and dried over anhydrous Na$_2$SO$_4$. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel. Elution with hexane-AcOEt (60:40, v/v) afforded the *E*-olefin **34** (146 mg, 75%) as a colorless glass. IR (CHCl$_3$) 3460, 1675 cm^{-1}. ^{1}H NMR (CDCl$_3$) δ 0.80 (3H, s, 18-H$_3$), 0.98 (3H, s, 19-H$_3$), 1.19, 1.21, 1.33, 1.35 and 1.49 (each 3H, each s, 21-H$_3$, 26-H$_3$, 27-H$_3$ and Me$_2$C), 3.4-3.5 and 3.9-4.0 (each 1H, each m, CH$_2$O), 4.2-4.3 (2H, m, 2-H and 3-H), 4.75 (1H, br s, OCHO), 5.61 (2H, br s, 22-H and 23-H), 5.85 and 5.83 (each 0.5H, each d, *J*=2.4 Hz, 7-H). FABMS (glycerine and sodium iodide) 609 (M$^+$+Na).

(20*R*,22*R*,23*S*)-2β,3β,14α,20,22,23,25-Heptahydroxy-5β-cholest-7-en-6-one (2a) (Gerardiasterone). To a stirred solution of **34** (25 mg, 0.043 mmol) and DHQ-CLB (25.7 mg, 0.055 mmol) in *tert*-BuOH (0.5 mL) was added OsO$_4$ (13 mg, 0.051 mmol) at room temperature, and the mixture was stirred for 1 h at the same temperature. Evaporation of the solvent gave a residue, which was dissolved in pyridine-water (1:1 v/v, 1 mL). Sodium bisulfite (44 mg, 0.42 mmol) was added to the mixture, and the reaction mixture was stirred for 2 h. The mixture was extracted with AcOEt. The extract was washed with brine and dried over anhydrous Na$_2$SO$_4$. Evaporation of the solvent gave a residue, which was used for the next reaction without any further purification.

To a stirred solution of the above compound (50.8 mg) in MeOH (0.8 mL) was added camphorsulfonic acid (16 mg, 0.064 mmol) at room temperature, and the mixture was stirred for 1 h at the same temperature. Evaporation of the solvent gave a colorless oil. ^{1}H NMR spectra of the crude product showed that the ratio of **2a** and **3a** was 95:5, respectively. The crude oil was purified by column chromatography on silica gel. Elution with AcOEt-MeOH (95:5, v/v) afforded the heptaol **2a** (13.7 mg, 65% from **34**) as a colorless solid, mp 140-143°C (lit.,[1] 143-146°C). $[\alpha]_D^{26}$ +57.8 (c 0.1, MeOH){lit.,[1] $[\alpha]_D^{22}$ +52.3 (c 0.35, MeOH)}. ^{1}H NMR (pyridine-d$_5$) δ 1.08 (3H, s, 19-H$_3$), 1.13 (3H, s, 18-H$_3$), 1.41 and 1.46 (each 3H, each s, 26-H$_3$ and 27-H$_3$), 1.77 (3H, s, 21-H$_3$), 3.74 (1H, br s, W/2=5 Hz, 22-H), 4.17 (1H, m, W/2=21 Hz, 2-H), 4.24 (1H, br s, W/2=8 Hz, 3-H), 4.78 (1H, br d, *J*=8.6 Hz, 23-H), 6.26 (1H, br s, 7-H).

(20*R*,22*S*,23*R*)-2β,3β,14α,20,22,23,25-Heptahydroxy-5β-cholest-7-en-6-one (3a). Dihydroxylation of **34** (24 mg, 0.041 mmol) with OsO$_4$ (12.5 mg, 0.049 mmol) and DHQ-CLB (24.7 mg, 0.053 mmol) in *tert*-BuOH (0.5 mL) was performed as above. Acid treatment of the dihydroxylated product gave a colorless oil. ^{1}H NMR spectra of the crude product showed that the ratio of **2a** and **3a** was 21:79, respectively. The crude oil was purified by column chromatography on silica gel. Elution with AcOEt-MeOH (94:6, v/v) afforded the heptaol **3a** (11 mg, 54% from **34**) as a colorless solid, mp 147-148°C. $[\alpha]_D^{24}$ +73.3 (c 0.06, MeOH). ^{1}H NMR (pyridine-d$_5$) δ 1.08 (3H, s, 19-H$_3$), 1.20 (3H, s, 18-H$_3$), 1.41 and 1.48 (each 3H, each s, 26-H$_3$ and 27-H$_3$), 1.85 (3H, s, 21-H$_3$), 3.72 (1H, br s, W/2=4 Hz, 22-H), 4.14 (1H, m, W/2=22 Hz, 2-H), 4.23 (1H, br s, W/2=8 Hz, 3-H), 5.05 (1H, br d, *J*=9.8 Hz, 23-H), 6.25 (1H, d, *J*=2.4 Hz, 7-H). FABMS (glycerine) 497 (M$^+$+1).

Dihydroxylation of the *E*-olefin 34 with OsO$_4$ and pyridine. Dihydroxylation of **34** (71.7 mg, 0.12 mmol) with OsO$_4$ (46.5 mg, 0.18 mmol) and pyridine (0.04 mL) in Et$_2$O (7 mL) was performed as above. Acid treatment of the dihydroxylated product gave the crude product, which was passed through a short column of silica gel using AcOEt-MeOH (95:5, v/v) as eluent to give a mixture of **2a** and **3a** (22.9 mg, 38%). ^{1}H NMR spectrum of the mixture showed that the ratio of **2a** and **3a** was 79:21.

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