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Synthesis and biological characterization of 1α,24,25-trihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (24-hydroxylated ED-71)

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Abstract

24-Hydroxylated derivatives were synthesized in 24(*R*) and 24(*S*) forms by the convergent method as analogs related to 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3. In the convergent synthesis, the A-ring fragment, synthesized from diethyl D-tartarate, and the C/D-ring fragments in 24(*R*) and 24(*S*) forms (vitamin D numbering), obtained from vitamin D_2 via the Inhoffen-Lythgoe diol, were coupled in moderate yields to give 1α,24(*R*),25-trihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 and 1α,24(*S*),25-trihydroxy-2β-(3-hydroxypropoxy)vitamin D_3. In preliminary biological evaluations, 24-hydroxylation of 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 caused weakened affinity to vitamin D binding protein in vitro and less calcemic activity in vivo compared to the parent compound. While the affinity to vitamin D receptor in 24*(R)* epimer was sustained, the affinity in 24(*S*) epimer was less than that of the parent compound.

Keywords: 1α,25-Dihydroxyvitamin D_3; 1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3; ED-71; 24-Hydroxylated ED-71; Sterol

1. Introduction

Various analogs of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] (**1**), a hormonally active sterol of native vitamin D_3, have been investigated in attempts to separate differentiation-induction and antiproliferation activities from calcemic activity, with the aim of obtaining useful analogs for the medical treatment of psoriasis, secondary hyperparathyroidism, cancer, etc [1]. There is also an intense interest in obtaining analogs more potent than 1α,25$(OH)_2D_3$ (**1**) or 1α-hydroxyvitamin D_3 (1αOHD_3) (**2**), a clinically important prodrug of **1,** in terms of regulatory effects in calcium and phosphorous metabolism, with the aim of treating bone diseases such as osteoporosis [2]. 1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71) (**3**) is an analog of 1α,25$(OH)_2D_3$ (**1**) bearing a hydroxypropoxy substituent at the 2β-position [2]. ED-71 (**3**) is characterized by high calcemic activity and long half-life in plasma arising from the strong affinity to vitamin D binding protein (DBP) [3]. Clinical trials of ED-71 (**3**) as a promising candidate for the treatment of osteoporosis have been conducted in Japan, based upon the preventive and therapeutic effects on bone mineral loss in osteoporosis model rats [4]. We recently reported that in the modification study of ED-71 (**3**) at the 2β-position, **3** was proved to be an optimized analog possessing preventive activity in pre-osteoporosis model rats, as well as 1α,25-dihydroxy-2β-(4-hydroxybutyl) vitamin D_3 (carbaED-71) (**4**) [5]. We also found that 1α,25-dihydroxy-26,27-dimethyl-2β-(3-hydroxypropoxy)vitamin D_3 (26,27-dimethyl ED-71) (**5**), obtained in our modification study of ED-71 (**3**) at the side chain, showed a more potent efficacy on spinal bone mineral density in ovariectomized rats compared to **3** [6]. These results prompted us to investigate further synthesis and biological evaluation of analogs of ED-71 (**3**) especially modified at the side chain. We have been also interested in the analogs of **3** having an oxidized side chain, taking postulated metabolites of ED-71 (**3**) into consideration. Thus, in this paper, we describe the synthesis of 24-hydroxylated ED-71 in both 24(*R*) and 24(*S*) forms, 1α,24(*R*),25-trihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 [(24*R*)OH-ED-71] (**6R**) and 1α,24(*S*), 25-trihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 [(24*S*)OH-ED-71] (**6S**), respectively, as a continuation of our modification study of ED-71 (**3**). Preliminary biological evaluations of the synthesized 24-hydroxylated

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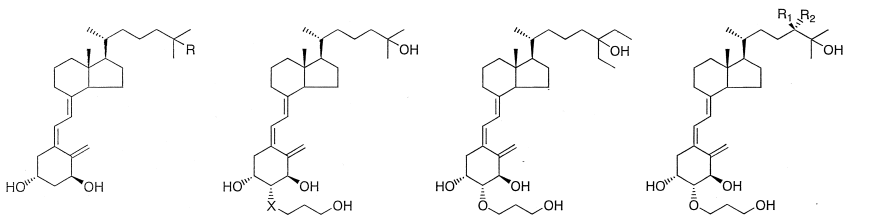

Fig. 1. Structures of active vitamin D_3 analogs.

ED-71 (**6R** and **6S**) in comparison with $1\alpha,25(OH)_2D_3$ (**1**) and ED-71 (**3**) are also described (Fig. 1).

2. Experimental

2.1. General

All melting points were taken on Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with JASCO DIP-140 polarimeter. Infrared (IR) spectra were obtained using JASCO FT/IR-5300, JEOL JIR-6000, and Hitachi 270–30 spectrophotometers. ^1H and ^{13}C NMR spectra were recorded on VARIAN Gemini-300, JEOL FX-200, and JNM-270EX spectrometers using $CDCl_3$ as a solvent. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane or calibrated from $CHCl_3$. Mass spectra (MS) were measured with JEOL JMS-HX-100, Shimadzu GCMS QP-1000, and Hitachi M1200H instruments. High resolution mass spectra (HRMS) were recorded on JEOL JMS-AX-500 and VG Auto Spec Q instruments. Ultra violet (UV) spectra were obtained with Shimadzu UV-240 spectrometer using EtOH as a solvent. All reactions were carried out under an atmosphere of argon unless otherwise noted. All extract were dried over $MgSO_4$ and evaporated under reduced pressure with a rotary evaporator. Chromatographic purification was carried out with Merck silica gel 60 (column) or Merck silica gel 60 PF_{254} (thin layer).

2.1.1. (2R,3S)-1,4-Dibenzyloxy-3-(3-hydroxypropoxy)butan-2-ol ***(12)***

A mixture of **11** [7] (22.2 g, 0.078 mol) and potassium *tert*-butoxide (*t*-BuOK) (29.82 g, 0.27 mol) in 1,3-propanediol (60 ml) was stirred at 120°C for 3 h. Excess 1,3-propanediol was removed by distillation. The residue was extracted with CH_2Cl_2. The extract was washed with H_2O and saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (1:1) gave **12** (24.2 g, 86%) as a colorless oil: IR (neat) 3448, 1496, 1453, 1364, 1098 cm^{-1}; ^1H NMR δ: 1.76 (q, 2H, J = 5.7 Hz), 2.70 (br s, 2H), 3.51–3.84 (m, 9H), 3.89 (br q, 1H, J = 5.0 Hz), 4.54 (s, 1H), 7.23–7.41 (m, 10H); HRMS calcd for $C_{21}H_{28}O_5$ (M^+): 360.1937; found: 360.1937; $[\alpha]_D$ +9.32 (*c* 1.03, $CHCl_3$).

2.1.2. (2R,3S)-1,4-Dibenzyloxy-3-(3-pivaloyloxypropoxy)butan-2-ol ***(13)***

To a stirred solution of **12** (11.36 g, 0.031 mol) in CH_2Cl_2 (500 ml), were added pyridine (20.42 ml, 0.252 mol) and pivaloyl chloride (15.48 ml, 0.126 mol). After being stirred at room temperature for 4 h, the mixture was extracted with CH_2Cl_2, washed with H_2O and saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (1:3) gave **13** (12.26 g, 88%) as a colorless oil: IR (neat) 3487, 1725, 1478, 1455, 1364, 1285, 1162, 1106 cm^{-1}; ^1H NMR δ: 1.85 (q, 2H, J = 6.3 Hz), 2.64 (br d, 1H, J = 5.2 Hz), 3.46–3.77 (m, 7H), 3.91 (br quint, 1H, J = 5.2 Hz), 4.13 (dt, 2H, J = 6.3, 1.6 Hz), 4.54 (s, 4H), 7.16–7.52 (m, 10H); HRMS calcd for $C_{26}H_{36}O_6$ (M^+): 444.2512; found: 444.2555; $[\alpha]_D$ +6.55 (*c* 1.19, $CHCl_3$).

2.1.3. (2R,3S)-1,2-O-Isopropylidene-3-(3-pivaloyloxypropoxy)butane-1,2,4-triol ***(14)***

To a stirred solution of **13** (12.26 g, 0.028 mol) in MeOH (200 ml), was added palladium hydroxide ($Pd(OH)_2$). The resulting mixture was stirred at room temperature under hydrogen atmosphere for 24 h. An insoluble material was removed by Celite filtration and the filtrate was evaporated. To a stirred solution of a colorless residue (7.29 g) in acetone (20 ml), were added 2,2-dimethoxypropane (3.2 ml, 0.026 mol) and *p*-toluenesulfonic acid (TsOH) (8.2 mg, 0.043 mmol). The mixture was stirred at room temperature for 4 h, extracted with AcOEt, washed with saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (1:2) gave **14** (4.79 g, 87%) as a colorless oil: IR (neat) 3499, 1725, 1480, 1460, 1398, 1371, 1286, 1256, 1213, 1163, 1075 cm^{-1}; ^1H NMR δ: 1.19 (s, 3H), 1.34 (s, 3H), 1.41 (s, 3H), 1.89 (quint, 2H, J = 6.3 Hz), 2.23 (br s, 1H), 3.34 (dt, 1H, J = 6.2, 4.2 Hz), 3.53–3.72 (m, 3H), 3.79 (br d, 1H, J = 12.0 Hz), 3.87 (dd, 1H, J = 8.0, 5.4 Hz), 4.02–4.18 (m, 3H), 4.25 (dt, 1H, J = 11.2, 6.1 Hz); HRMS calcd for $C_{15}H_{28}O_6$ (M^+): 304.1886; found: 304.1888; $[\alpha]_D$ +9.51 (*c* 1.03, $CHCl_3$).

2.1.4. (2R,3S,4RS)-1,2-O-Isopropylidene-4-pivaloyloxy-3-(3-pivaloyloxypropoxy)-5-hexene-1,2-diol ***(15)***

To a stirred solution of oxalyl chloride (5.85 ml, 0.067 mol) in CH_2Cl_2 (100 ml), was added dimethyl sulfoxide (9.44 ml, 0.133 mol) in CH_2Cl_2 (100 ml) at −60°C. The

mixture was stirred at −60°C for 15 min. To the stirred mixture, was added **14** (10.02 g, 0.033 mol) in CH_2Cl_2 (200 ml) at −60°C. The mixture was stirred at −60°C for 30 min. After addition of triethylamine (23.28 ml, 0.167 mol) at −60°C, the resulting mixture was allowed to be room temperature and the stirring was continued at room temperature for 30 min. After addition of NH_4Cl, the mixture was extracted with AcOEt, washed with saturated NaCl, dried, and evaporated to give a residue (10.71 g). To a stirred solution of the residue (10.71 g) in THF (200 ml), was added 0.97 M vinylmagnesium bromide in THF (102 ml, 0.099 mol) at −60°C. The mixture was stirred at −60°C for 2 h. After addition of NH_4Cl, the mixture was extracted with CH_2Cl_2, dried, and evaporated to give a residue (11.21 g). A mixture of the residue (11.21 g), triethylamine (18.54 ml, 0.133 mol), dimethylaminopyridine (10 mg, 0.825 mmol), pivaloyl chloride (8.25 ml, 0.067 mol) in CH_2Cl_2 (400 ml) was stirred at room temperature for 20 h. The mixture was extracted with CH_2Cl_2, washed with saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (1:10) gave **15** (7.89 g, 58%) as a colorless oil: IR (neat) 1728, 1480, 1460, 1373, 1284, 1214, 1160, 1058 cm^{-1}; ^1H NMR δ: 1.20 (s, 3H), 1.34 (s, 3H), 1.42 (s, 3H x 0.6), 1.43 (s, 3H x 0.4), 1.88 (quint, 0.4H, J = 6.3 Hz), 1.89 (quint, 0.6H, J = 6.3 Hz), 2.48 (d, 0.4H, J = 4.1 Hz), 2.65 (d, 0.6H, J = 8.2 Hz), 3.41 (dd, 1H, J = 6.4, 3.6 Hz), 3.45 (dd, 1H, J = 5.5, 4.3 Hz), 3.70 (m, 2H), 3.89 (t, 0.4H, J = 8.2 Hz), 3.90 (t, 0.6H, J = 8.2 Hz), 4.04 (t, 0.4H, J = 8.5 Hz), 4.05 (t, 0.6H, J = 8.5 Hz), 4.08−4.34 (m, 3H), 5.23 (dt, 0.4H, J = 3.0, 1.6 Hz), 5.25 (dt, 0.6H, J = 3.0, 1.6 Hz), 5.35 (dt, 0.4H, J = 8.9, 1.6 Hz), 5.38 (dt, 0.6H, J = 8.9, 1.6 Hz); HRMS calcd for $C_{22}H_{38}O_7$ (M^+): 414.2618; found: 414.2618.

*2.1.5. (2R,3S,4RS)-4-Pivaloyloxy-3-(3-pivaloyloxypropoxy)-5-hexene-1,2-diol **(16)***

A mixture of **15** (1.83 g, 4.4 mmol) and 1 M HCl (10 ml) in MeOH (10 ml) was stirred at room temperature for 10 h. The mixture was extracted with Et_2O, washed with H_2O and saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (1:1) gave **16** (1.67 g, 100%) as a colorless oil: IR (neat) 3448, 1731, 1480, 1285, 1170, 1108 cm^{-1}; ^1H NMR δ: 1.20 (s, 9H), 1.23 (s, 9H), 1.89 (quint, 2H, J = 6.0 Hz), 2.01 (br s, 0.6H), 2.50 (br s, 0.4H), 2.68 (br s, 0.4H), 2.95 (br s, 0.6H), 3.30–3.90 (m, 6H), 4.00−4.34 (m, 2H), 5.20–5.41 (m, 2H), 5.50 (m, 0.6H), 5.63 (m, 0.4H), 5.84−6.10 (m, 1H); HRMS calcd for $C_{19}H_{34}O_7$ (M^+): 374.2305; found: 374.2305.

*2.1.6. (2R,3S,4RS)-1,2-Epoxy-4-pivaloyloxy-3-(3-pivaloyloxypropoxy)-5-hexene **(17)***

A solution of **16** (1.08 *g*, 2.9 mmol) in benzene (30 ml) was azeotropically refluxed for 1 h. To the ice-cooled mixture of **16** in benzene, were added triphenyl phosphine (Ph_3P) (3.04 g, 11.6 mmol) and diethyl azodicarboxylate (DEAD) (1.83 ml, 11.6 mmol) [8]. The resulting mixture was refluxed for 7 days, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (1:10) gave **17** (692 mg, 77%) as a colorless oil: IR (neat) 1730, 1480, 1397, 1365, 1282, 1156, 1066 cm^{-1}; ^1H NMR δ: 1.20 (s, 9H), 1.23 (s, 9H), 1.85 (quint, 2H, J = 6.0 Hz), 2.70–2.82 (m, 2H), 2.97–3.06 (m, 1H), 3.38 (t, 0.4H, J = 4.2 Hz), 3.45 (t, 0.6H, J = 4.7 Hz), 3.51–3.73 (m, 2H), 4.12 (m, 2H), 5.23–5.48 (m, 3H), 5.84–5.98 (m, 1H); HRMS calcd for $C_{19}H_{32}O_6$ (M^+): 356.2199; found: 356.2238.

*2.1.7. (4R,5S,6R)-4,6-Di(tert-butyldimethylsilyloxy)-5-[3-(t-butyldimethylsilyloxy)propoxy]-7-octen-2-yne **(7)** and (4R,5S,6S)-4,6-Di(tert-butyldimethylsilyloxy)-5-[3-(tert-butyldimethylsilyloxy)propoxy]-7-octen-2-yne **(18)***

To a stirred solution of trimethylsilylacetylene (8.4 ml, 11.9 mmol), was added 1.59 M *n*-BuLi in hexane (35.2 ml, 56.0 mmol) at –78°C. The mixture was stirred at –78°C for 30 min. To the stirred mixture, was added borontrifluoride etherate (BF_3-OEt_2) and the stirring was continued at –78°C for 1 h. To the stirred mixture, was added **17** (4.24 g, 11.9 mmol) in THF (65 ml) and the stirring was continued at –78°C for 2 h. The mixture was quenched by saturated $NaHCO_3$ and H_2O, extracted with AcOEt, washed with saturated NaCl, dried, and evaporated to give a yellow oil (5.1 g), which was used without further purification. A mixture of the yellow oil (5.1 g) and 10 N NaOH (35 ml) in MeOH (80 ml) was stirred at room temperature for 1.5 h. The mixture was neutralized by conc. HCl under ice-cooling, azeotropically refluxed with toluene, extracted with THF, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt gave a colorless oil (1.73 g), which was used without further purification. To a stirred solution of the colorless oil (1.73 g) in CH_2Cl_2 (100 ml), were added triethylamine (11.3 ml, 0.4 mol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (10.7 ml, 40.4 mmol) at 0°C. The stirring was continued at 0°C for 1.5 h. After the addition of saturated $NaHCO_3$ and H_2O, the mixture was extracted with CH_2Cl_2, washed with saturated NaCl, dried, evaporated. Repetition of chromatography on silica gel [AcOEt-hexane (1:50)] gave **7** (2.50 g, 36%) and **18** (1.58 g, 24%), respectively, as colorless oils. **7**: IR (neat) 3314, 1468, 1254 cm^{-1}; ^1H NMR δ: 0.03 (s, 3H), 0.04 (s, 3H), 0.06 (s, 3H), 0.08 (s, 3H), 0.10 (s, 3H), 0.89−0.90 (m, 2H), 1.78 (quint, 2H, J = 6.0 Hz), 1.92 (t, 1H, J = 2.7 Hz), 2.46 (ddd, 1H, J = 3.0, 5.1, 17.1 Hz), 3.27 (dd, 1H, J = 2.1, 6.6 Hz), 3.60–3.82 (m, 4H), 3.98 (ddd, 1H, J = 1.8, 4.8, 7.2 Hz), 4.18 (t, 1H, J = 6.3 Hz), 5.14 (dt, 1H, J = 1.5, 10.5 Hz), 5.26 (dt, 1H, J = 1.5, 17.1 Hz), 5.89 (ddd, 1H, J = 6.0, 10.5, 17.4 Hz); ^{13}C NMR δ: 138.5, 116.0, 86.9, 83.2, 74.4, 72.1, 69.5, 60.5, 33.7, 26.1, 26.0, 23.3, 18.5, 18.4, −4.3, −4.4, −4.5, −5.2; HRMS calcd for $C_{28}H_{57}O_4Si_3$ (M^+-Me): 541.3565; Found 541.3547; $[\alpha]_D^{25}$ +15.4 (*c* 0.52, $CHCl_3$). **18**: IR (neat) 3314, 1468, 1254 cm^{-1}; ^1H NMR δ: 0.03–0.12 (m, 18H), 0.88 (s, 9H), 0.90 (s, 9H), 0.91 (s, 9H), 1.74 (quint, 2H, J = 6.3Hz), 1.93 (t, 1H, J = 2.7 Hz), 2.53 (ddd, 1H, J = 2.7, 5.7, 17.4 Hz), 3.36 (dd, 1H, J = 4.5, 5.7 Hz),

3.67 (t, 2H, J = 6.3 Hz), 3.74 (ddd, 2H, J = 3.0, 6.3, 12.9 Hz), 3.85 (dt, 1H, J = 4.2, 5.7 Hz), 4.27 (dd, 1H, J = 4.5, 6.0 Hz), 5.14 (dt, 1H, J = 1.5, 10.5 Hz), 5.26 (dt, 1H, J = 1.5, 17.1 Hz), 5.88 (ddd, 1H, J = 6.0, 10.2, 17.4 Hz); ^{13}C NMR δ: 138.2, 116.1, 85.7, 82.3, 74.4, 71.0, 69.8, 69.7, 60.3, 33.7, 26.1, 23.3, 18.5, 18.2, −4.2, −4.3, −4.4, −5.2; $[\alpha]_D^{25}$ –2.3 (*c* 0.80, $CHCl_3$).

*2.1.8. (1R,3aR,4S,7aR,1S)-4-Acetoxy-7a-methyl-1-[(S)-1-methyl-2-(phenylsulphonyl)ethyl]octahydro-1H-indene **(21)***

To an ice-cooled solution of **20** [9,10] (334 mg, 0.994 mmol), 4-dimethylaminopyridine (18 mg, 0.148 mmol) and pyridine (0.2 ml) in CH_2Cl_2 (20 ml), was added Ac_2O (0.188 ml, 1.99 mmol). The mixture was stirred at room temperature for 16 h, poured into diluted HCl, and extracted with CH_2Cl_2. The extract was washed with H_2O and saturated $NaHCO_3$, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (2:3) gave **21** (368 mg, 98%) as a colorless oil: IR (neat) 2945, 1735, 1310, 1250, 1150 cm^{-1}; ^{1}H NMR δ: 0.86 (s, 3H), 1.20 (d, 3H, J = 6.8 Hz), 2.03 (s, 3H), 2.84 (dd, 1H, J = 14.1, 9.8 Hz), 3.11 (d, 1H, J = 14.1 Hz), 5.12 (br s, 1H), 7.52–7.96 (m, 5H); MS m/z: 336 (M^+–Ac), 135 (100%); UV λmax 270, 263, 257, 217 nm.

*2.1.9. (1R,3aR,4S,7aR)-7a-Methyl-1-[(1S,2RS,4R)-4,5-dihydroxy-1,5-dimethyl-2-(phenylsulphonyl)hexyl]octahydro-1H-inden-4-ol **(23R)***

To a stirred solution of **21** (160 mg, 0.423 mmol) and (*R*)-3-methyl-1-(*p*-toluenesulphonyl)oxybutane-2,3-diol (**22R**) [11,12] (232 mg, 0.847 mmol) in THF (15 ml) at –20°C, was added 1.63 M *n*-BuLi in hexane (2.3 ml, 3.75 mmol). After being stirred at –20°C for 2 h, the reaction mixture was poured into saturated NH_4Cl and extracted with AcOEt. The extract was washed with saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with EtOH-CH_2Cl_2 (3:50) gave **23R** (131 mg, 71%) as a colorless foam: IR (neat) 3500 (br), 2935, 1300, 1280, 1135 cm^{-1}; ^{1}H NMR δ: 0.67 (s, 3H), 0.96 (d, 3H, J = 6.8 Hz), 1.20 (s, 3H), 1.26 (s, 3H), 3.51 (d, 1H, J = 10.2 Hz), 3.81 (br d, 1H, J = 11.7 Hz), 4.00 (br s, 1H), 7.52–7.94 (m, 5H); MS m/z: 439 (M^++1), 71 (100%); UV λmax 271, 263, 257, 216 nm.

*2.1.10. (1R,3aR,4S,7aR)-7a-Methyl-1-[(1S,2RS,4S)-4,5-dihydroxy-1,5-dimethyl-2-(phenylsulphonyl)hexyl]octahydro-1H-inden-4-ol **(23S)***

To a stirred solution of **21** (305 mg, 0.908 mmol) and (*S*)-3-methyl-1-(*p*-toluenesulphonyl)oxybutane-2,3-diol (**22S**) [12] (249 mg, 0.909 mmol) in THF (20 ml) at –20°C, was added 1.69 M *n*-BuLi in hexane (4.3 ml, 7.27 mmol). After being stirred at –20°C for 2 h, the reaction mixture was poured into saturated NH_4Cl and extracted with AcOEt. The extract was washed with saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (4:1) gave **23S** (110 mg, 28%) as a colorless oil: IR (neat) 3460 (br), 2925, 1280, 1135, 1075 cm^{-1}; ^{1}H NMR δ: 0.69 (s, 3H), 1.05 (d, 3H, J = 6.6 Hz, 3H), 1.20 (s, 3H), 1.25 (s, 3H), 3.35 (t, 1H, J = 5.1 Hz), 3.45 (br s, 1H), 4.01 (br s, 1H), 7.53–7.94 (m, 5H); MS m/z: 439 (M^++1), 60 (100%).

*2.1.11. (1R,3aR,4S,7aR)-1-[(1R,4R)-4,5-Dihydroxy-1,5-dimethylhexyl]-7a-methyl-octahydro-1H-inden-4-ol **(24R)***

Sodium amalgam (Na/Hg) (5%, 5.01 g, 10.9 mmol) was added to a solution of **23R** (104 mg, 0.237 mmol) in THF (5.5 ml) and MeOH (3.5 ml). The mixture was stirred at room temperature for 15 h. MeOH (5.4 ml) and H_2O (5.4 ml) were added and the mixture was stirred for 30 min. After removal of the excess Na/Hg by decantation, the collected solution was poured into saturated NH_4Cl and extracted with AcOEt. The extract was washed with saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with EtOH-CH_2Cl_2 (3:50) gave **24R** (68 mg, 97%) as a colorless foam: IR (neat) 3400 (br), 2930 cm^{-1}; ^{1}H NMR δ: 0.91 (d, 3H, J = 6.3 Hz), 0.94 (s, 3H), 1.21 (s, 3H), 1.24 (s, 3H), 3.33 (br t, 1H, J = 5.4 Hz), 4.08 (br s, 1H); MS m/z: 298 (M^+), 135 (100%).

*2.1.12. (1R,3aR,4S,7aR)-1-[(1R,4S)-4,5-Dihydroxy-1,5-dimethylhexyl]-7a-methyl-octahydro-1H-inden-4-ol **(24S)***

Na/Hg (5%, 12.4 g, 27.0 mmol) was added to a solution of **23S** (216 mg, 0.493 mmol) in THF (12 ml) and MeOH (7.5 ml). The mixture was stirred at room temperature for 15 h. MeOH (5.4 ml) and H_2O (5.4 ml) were added and the mixture was stirred for 30 min. After removal of the excess Na/Hg by decantation, the collected solution was poured into saturated NH_4Cl and extracted with AcOEt. The extract was washed with saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with EtOH-CH_2Cl_2 (3:50) gave **24S** (100 mg, 68%) as a colorless oil: IR (neat) 3400 (br), 2930 cm^{-1}; ^{1}H NMR δ: 0.92 (d, 3H, J = 6.6 Hz), 0.93 (s, 3H), 1.16 (s, 3H), 1.21 (s, 3H), 3.27 (dd, 1H, J = 10.2, 2.0 Hz), 4.07 (br d, 1H, J = 2.6Hz); MS m/z: 280 (M^+- H_2O), 60 (100%).

*2.1.13. (1R,3aR,4S,7aR)-4-Acetoxy-7a-methyl-1-[(R)-1-methyl-3-[(R)-2,2,4,4-tetramethyl-1,3-dioxolan-5-yl]propyl]octahydro-1H-indene **(25R)***

To a solution of **24R** (86 mg, 0.289 mmol) and 2,2-dimethoxypropane (2.93 g, 28.1 mmol) in acetone (19.5 ml), was added TsOH (6.5 mg, 0.034 mmol). After being stirred at room temperature for 15 h, the reaction mixture was treated with $NaHCO_3$ (10 mg) and evaporated. The residue was extracted with CH_2Cl_2. The extract was washed with saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with EtOH-CH_2Cl_2 (1:25) gave a colorless residue (107 mg), which was used for the next reaction without further purification. The crude residue (107 mg) was dissolved in CH_2Cl_2 (10 ml) and pyridine (128 μl), 4-dimethylaminopyridine (7 mg, 0.058 mmol) and Ac_2O (120 μl, 1.27 mmol) were added. After being stirred

at room temperature for 4 h, the reaction mixture was poured into diluted HCl and extracted with CH_2Cl_2. The extract was washed with saturated $NaHCO_3$, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (3:17) gave **25R** (91 mg, 83%) as a colorless oil: IR (KBr) 3485 (br), 2920 cm^{-1}; ^1H NMR δ: 0.93 (d, 3H, J = 5.3 Hz), 0.95 (s, 3H), 1.10 (s, 3H), 1.25 (s, 3H), 1.33 (s, 3H), 1.42 (s, 3H), 3.64 (dd, 1H, J = 8.5, 3.7 Hz), 4.08 (br d, 1H, J = 2.4Hz); MS m/z: 338 (M^+), 323 (100%).

*2.1.14. (1R,3aR,4S,7aR)-4-Acetoxy-7a-methyl-1-[(R)-1-methyl-3-[(S)-2,2,4,4-tetramethyl-1,3-dioxolan-5-yl]propyl]octahydro-1H-indene **(25S)***

To a solution of **24S** (100 mg, 0.336 mmol) and 2,2-dimethoxypropane (3.80 g, 36.5 mmol) in acetone (23 ml), was added TsOH (15 mg, 0.079 mmol). After being stirred at room temperature for 15 h, the reaction mixture was treated with $NaHCO_3$ (10 mg) and evaporated. The residue was extracted with CH_2Cl_2. The extract was washed with saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with EtOH-CH_2Cl_2 (1:25) gave a colorless residue (76 mg), which was used without further purification. The crude residue (76 mg) was dissolved in CH_2Cl_2 (3 ml) and pyridine (500 μl), 4-dimethylaminopyridine (7 mg, 0.058 mmol) and Ac_2O (250 μl, 2.64 mmol) were added. After being stirred at room temperature for 4 h, the reaction mixture was poured into diluted HCl and extracted with CH_2Cl_2. The extract was washed with saturated $NaHCO_3$, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (3:17) gave **25S** (82 mg, 64%) as a pale yellow oil: IR (neat) 2930, 1735 cm^{-1}; ^1H NMR δ: 0.89 (s, 3H), 0.94 (d, 3H, J = 6.6 Hz), 1.10 (s, 3H), 1.25 (s, 3H), 1.33 (s, 3H), 1.41 (s, 3H), 2.04 (s, 3H), 3.61 (t, 1H, J = 6.3 Hz), 5.16 (br s, 1H); MS m/z: 365 (M^+– Me, 100%).

*2.1.15. (1R,3aR,4S,7aR)-4-Acetoxy-1-[(1R,4R)-4,5-dihydroxy-1,5-dimethylhexyl]-7a-methyloctahydro-1H-indene **(26R)***

A mixture of **25R** (46 mg, 0.121 mmol) and 1% I_2-MeOH (2 ml) was refluxed for 4.5 h. The reaction mixture was poured into 10% $Na_2S_2O_3$, evaporated, and extracted with AcOEt. The extract was washed with H_2O and saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (2:3) gave **26R** (25 mg, 61%) as a colorless oil: IR (neat) 3455 (br), 2930, 1735 cm^{-1}; ^1H NMR δ: 0.89 (s, 3H), 0.92 (d, 3H, J = 6.6 Hz), 1.16 (s, 3H), 1.21 (s, 3H), 2.04 (s, 3H), 3.32 (br t, 1H, J = 5.9 Hz), 5.14 (br s, 1H); MS m/z: 280 (M^+– Ac – H_2O), 59 (100%).

*2.1.16. (1R,3aR,4S,7aR)-4-Acetoxy-1-[(1R,4S)-4,5-dihydroxy-1,5-dimethylhexyl]-7a-methyloctahydro-1H-indene **(26S)***

A mixture of **25S** (34 mg, 0.090 mmol) and 1% I_2-MeOH (2 ml) was refluxed for 4.5 h. The reaction mixture was poured into 10% $Na_2S_2O_3$, evaporated, and extracted with AcOEt. The extract was washed with H_2O and saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (2:3) gave **26S** (25 mg, 83%) as a colorless oil: IR (neat) 3460 (br), 2935, 1730 cm^{-1}; ^1H NMR δ: 0.88 (s, 3H), 0.93 (d, 3H, J = 6.6 Hz), 1.16 (s, 3H), 1.22 (s, 3H), 2.04 (s, 3H), 3.23–3.32 (m, 1H)), 5.15 (br s, 1H); MS m/z: 340 (M^+), 135 (100%).

*2.1.17. (1R,3aR,4S,7aR)-4-Acetoxy-1-[(1R,4R)-4,5-di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-1H-indene **(27R)***

To an ice-cooled solution of **26R** (46 mg, 0.135 mmol) in CH_2Cl_2 (10 ml), were added 2,6-lutidine (355 μl, 3.03 mmol) and TBSOTf (466 μl ml, 2.03 mmol). After being stirred at room temperature for 1.5 h, the reaction mixture was poured into diluted HCl and extracted with CH_2Cl_2. The extract was washed with saturated $NaHCO_3$, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (3:47) gave **27R** (71 mg, 92%) as a colorless oil: IR (neat) 2945, 1745 cm^{-1}; ^1H NMR δ: 0.04 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.86 (s, 9H), 0.89 (s, 9H), 1.11 (s, 3H), 1.19 (s, 3H), 2.04 (s, 3H), 3.22 (br s, 1H), 5.15 (br s, 1H); MS m/z: 553 (M^+–Me), 173 (100%).

*2.1.18. (1R,3aR,4S,7aR)-4-Acetoxy-1-[(1R,4S)-4,5-di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-1H-indene **(27S)***

To an ice-cooled solution of **26S** (31 mg, 0.091 mmol) in CH_2Cl_2 (2 ml), were added 2,6-lutidine (64 μl, 0.55 mmol) and TBSOTf (84 μl ml, 0.37 mmol). After being stirred at room temperature for 1.5 h, the reaction mixture was poured into diluted HCl and extracted with CH_2Cl_2. The extract was washed with saturated $NaHCO_3$, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (3:47) gave **27S** (47 mg, 91%) as a colorless oil: IR (neat) 2945, 1735 cm^{-1}; ^1H NMR δ: 0.04 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.85 (s, 9H), 0.88 (s, 9H), 1.10 (s, 3H), 1.19 (s, 3H), 2.04 (s, 3H), 3.18 (dd, 1H, J = 7.4, 2.3 Hz), 5.15 (br s, 1H); MS m/z: 553 (M^+–Me), 173 (100%).

*2.1.19. (1R,3aR,4S,7aR)-1-[(1R,4R)-4,5-Di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-1H-inden-4-ol **(28R)***

To an ice-cooled solution of lithium aluminum hydride ($LiAlH_4$) (20 mg, 0.528 mmol) in THF (1 ml), was added dropwise a solution of **27R** (71 mg, 0.125 mmol) in THF (2 ml). After being stirred with cooling in an ice bath for 1.5 h, the reaction mixture was quenched with 1M NaOH and extracted with AcOEt. The extract was washed with saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (3:22) gave **28R** (63 mg, 96%) as a colorless oil: IR (neat) 3425 (br), 2945 cm^{-1}; ^1H NMR δ: 0.03 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.85 (s, 9H), 0.89 (s, 9H), 1.11 (s, 3H), 1.18 (s, 3H), 3.22 (br s, 1H), 4.08 (br s, 1H); MS m/z: 511 (M^+–Me), 173 (100%).

*2.1.20. (1R,3aR,4S,7aR)-1-[(1R,4S)-4,5-Di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-1H-inden-4-ol **(28S)***

To an ice-cooled solution of $LiAlH_4$ (6.3 mg, 0.166 mmol) in THF (1 ml), was added dropwise a solution of **27S** (47 mg, 0.083 mmol) in THF (2 ml). After being stirred with cooling in an ice bath for 1.5 h, the reaction mixture was quenched with 1M NaOH and extracted with AcOEt. The extract was washed with saturated NaCl, dried, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (3:22) gave **28S** (44 mg, 100%) as a colorless oil: IR (neat) 3430 (br), 2920 cm^{-1}; ^1H NMR δ: 0.04 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.85 (s, 9H), 0.89 (s, 9H), 0.92 (d, 3H, J = 6.3 Hz), 1.11 (s, 3H), 1.19 (s, 3H), 3.18 (dd, 1H, J = 7.6, 2.3 Hz), 4.00 (br s, 1H); MS m/z: 511 (M^+–Me), 173 (100%).

*2.1.21. (1R,3aR,7aR)-1-[(1R,4R)-4,5-Di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-4H-inden-4-one **(29R)***

A mixture of **28R** (15.4 mg, 0.029 mmol), *N*-methylmorpholine *N*-oxide (NMO) (7.0 mg, 0.059 mmol), and 4A molecular sieves (14.0 mg) in CH_2Cl_2 (2 ml) was stirred at room temperature for 1 h and then tetrapropylammonium perruthenate (n-Pr_4NRuO_4) (0.8 mg, 0.0023 mmol) was added. After being stirred at room temperature for 2.5 h, the mixture was filtered through Celite, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (1:5) gave **29R** (14.8 mg, 96%) as a colorless oil: IR (neat) 1716, 1460, 1252 cm^{-1}; ^1H NMR δ: 0.04 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.63 (s, 3H), 0.85 (s, 9H), 0.89 (s, 9H), 0.94 (d, 3H, J = 5.7 Hz), 1.11 (s, 3H), 1.18 (s, 3H), 1.20–2.34 (m, 16H), 2.45 (dd, 1H, J = 7.5, 11.1 Hz), 3.18–3.24 (m, 1H); ^{13}C NMR δ: –3.1, –1.9, –1.8, 12.6, 18.2, 18.3, 18.9, 19.2, 23.6, 24.2, 25.9, 26.2, 27.6, 29.0, 29.6, 33.8, 36.3, 39.1, 41.1, 50.0, 56.7, 62.1, 76.4, 81.0, 212.2; MS m/z: 509 (M^+–Me), 173 (100%); HRMS calcd for $C_{29}H_{57}O_3Si_2$ (M^+–Me): 509.3846, found: 509.3845; $[\alpha]_D^{23}$ +17.0° (*c* 0.83, $CHCl_3$).

*2.1.22. (1R,3aR,7aR)-1-[(1R,4S)-4,5-Di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-4H-inden-4-one **(29S)***

A mixture of **28S** (23.7 mg, 0.044 mmol), NMO (7.8 mg, 0.066 mmol), and 4A molecular sieves (19.0 mg) in CH_2Cl_2 (3 ml) was stirred at room temperature for 1 h and then n-Pr_4NRuO_4 (0.8 mg, 0.0023 mmol) was added. After being stirred at room temperature for 5 h, the mixture was filtered through Celite, evaporated, and chromatographed on silica gel. Elution with AcOEt-hexane (1:5) gave **29S** (23.7 mg, 100%) as a colorless oil: IR (neat) 1716, 1467, 1252 cm^{-1}; ^1H NMR δ: 0.04 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.63 (s, 3H), 0.85 (s, 9H), 0.88 (s, 9H), 0.94 (d, 3H, J = 6.3 Hz), 1.10 (s, 3H), 1.19 (s, 3H), 1.20–2.28 (m, 16H), 2.43 (dd, 1H, J = 7.5, 11.1 Hz), 3.18 (dd, 1H, J = 2.4, 7.5 Hz); ^{13}C NMR δ: −3.7, –3.1, –1.9, –1.8, 12.5, 18.2, 18.3, 18.8, 19.2, 23.2, 24.2, 26.0, 26.2, 27.8, 29.0, 29.7, 34.1, 36.2, 39.1, 41.1, 50.0, 56.8, 62.1, 76.4, 81.2, 212.2; MS m/z: 509 (M^+–Me), 467 (M^+–57), 173 (100%); HRMS calcd for $C_{29}H_{57}O_3Si_2$ (M^+–Me): 509.3846, found: 509.3852; $[\alpha]_D^{24}$ –5.3° (*c* 1.185, $CHCl_3$).

*2.1.23. (1R,3aR,7aR)-4-[(E)-Bromomethylene]-1-[(1R,4R)-4,5-di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-4H-inden **(9R)***

To a stirred solution of (bromomethylene)triphenylphosphonium bromide ($Ph_3PCH_2Br_2$) (130.5 mg, 0.30 mmol) in THF (1 ml), was added 1 M sodium hexamethyldisilazide ($NaN(TMS)_2$) in THF (290 μl, 0.29 mmol) at −60°C. The resulting mixture was stirred at −60°C for 1 h. After addition of **9R** (19.6 mg, 0.037 mmol) in THF (0.3 ml) at −60°C, the mixture was stirred at room temperature for 1 h. The mixture was diluted with hexane and filtered with silica gel. The filtrate was evaporated. The residue was purified by preparative TLC developed with hexane to give **9R** (11.0 mg, 49%) as an yellow oil: IR (neat) 1467, 1253 cm^{-1}; ^1H NMR δ: 0.04 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.56 (s, 3H), 0.85 (s, 9H), 0.89 (s, 9H), 0.92 (d, 3H, J = 6.0 Hz), 1.11 (s, 3H), 1.18 (s, 3H), 1.20–2.04 (m, 16H), 2.82–2.91 (m, 1H), 3.18–3.24 (m, 1H), 5.64 (s, 1H); ^{13}C NMR δ: −3.9, –3.1, –1.9, –1.8, 18.2, 18.3, 19.0, 22.2, 22.7, 23.6, 26.0, 26.2, 27.7, 29.1, 29.6, 31.2, 33.9, 36.8, 40.0, 45.6, 55.9, 56.0, 76.4, 81.1, 97.4, 145.3; MS m/z: 585 (M^+–Me), 543 (M^+–57), 73; HRMS calcd for $C_{30}H_{58}O_2Si_2Br$ (M^+–Me): 585.3150, found: 585.3134; $[\alpha]_D^{21}$ –106.18° (*c* 0.55, $CHCl_3$).

*2.1.24. (1R,3aR,7aR)-4-[(E)-Bromomethylene]-1-[(1R,4S)-4,5-di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-4H-inden **(9S)***

To a stirred solution of $Ph_3PCH_2Br_2$ (159.3 mg, 0.37 mmol) in THF (0.9 ml), was added 1 M $NaN(TMS)_2$ in THF (355 μl, 0.36 mmol) at −60°C. The resulting mixture was stirred at −60°C for 1 h. After addition of **9S** (23.9 mg, 0.046 mmol) in THF (0.3 ml) at −60°C, the mixture was stirred at room temperature for 1 h. The mixture was diluted with hexane and filtered with silica gel. The filtrate was evaporated. The residue was purified by preparative TLC developed with hexane to give **9S** (15.6 mg, 57%) as an yellow oil: IR (neat) 1466, 1252 cm^{-1}; ^1H NMR δ: 0.04 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.56 (s, 3H), 0.85 (s, 9H), 0.88 (s, 9H), 0.92 (d, 3H, J = 6.0 Hz), 1.10 (s, 3H), 1.19 (s, 3H), 1.00–2.16 (m, 16H), 2.82–2.91 (m, 1H), 3.18 (dd, 1H, J = 7.5, 2.1 Hz), 5.64 (s, 1H); ^{13}C NMR δ: −3.7, –3.1, –1.9, –1.8, 11.9, 18.2, 18.3, 18.9, 22.1, 22.7, 23.2, 25.9, 26.2, 27.9, 29.0, 29.7, 31.2, 34.2, 36.7, 40.0, 45.6, 55.9, 56.0, 76.4, 81.2, 97.4, 145.3; MS m/z: 585 (M^+–Me), 543 (M^+–57), 73; HRMS calcd for $C_{27}H_{52}O_2Si_2Br$ (M^+–57): 543.2690, found: 543.2692; $[\alpha]_D^{23}$ +47.31° (*c* 0.78, $CHCl_3$).

*2.1.25. (5Z,7E)-(1R,2R,3R,24R)-1,3,24-Tris(tert-butyldimethylsilyloxy)-2-(3-tert-butyldimethylsilyloxypropoxy)-9,10-secocholesta-5,7,10(19)-triene **(30R)***

A mixture of triphenyphosphine (PPh_3) (2.0 mg, 7.6 μmol), tris(dibenzylideneacetonedipalladium (0)-chloro-

form adduct ($(dba)_3Pd_2$-$CHCl_3$) (1.8 mg, 1.7 μmol), and triethylamine (0.3 ml) in toluene (0.2 ml) was stirred at room temperature for 10 min. To the stirred mixture, were added **7** (7.9 mg, 0.014 mmol) and **9R** (11.0 mg, 0.018 mmol) in toluene (0.2 ml) at room temperature. The resulting mixture was refluxed for 6h, diluted with hexane, filtered with silica gel, and evaporated. The residue was purified by preparative TLC developed with hexane-benzene (2:1) to give **30R** (5.5 mg, 36%) as a colorless oil: IR (neat) 1467, 1252 cm^{-1}; ^1H NMR δ: 0.02–0.10 (m, 30H), 0.52 (s, 3H), 0.82–0.94 (m, 48H), 1.18 (s, 3H), 1.20–2.05 (m, 18H), 2.23 (br dd, 1H, J = 12.6, 3.3 Hz), 2.46 (br dd, 1H, J = 12.9, 7.8 Hz), 2.81 (br d 1H, J = 12.3 Hz), 3.22 (m, 2H), 3.58–3.76 (m, 4H), 4.16–4.26 (m, 2H), 4.98 (br s, 1H), 5.26 (br s, 1H), 6.00 (d, 1H, J = 10.8 Hz), 6.22 (d, 1H, J = 10.5Hz); $[\alpha]_D^{23}$ +25.8° (*c* 0.28, $CHCl_3$).

2.1.26. (5Z,7E)-(1R,2R,3R,24S)-1,3,24-Tris(tert-butyldimethylsilyloxy)-2-(3-tert-butyldimethylsilyloxypropoxy)-9,10-secocholesta-5,7,10(19)-triene ***(30S)***

A mixture of PPh_3 (2.4 mg, 9.2 μmol), $(dba)_3Pd_2$-$CHCl_3$ (1.7 mg, 1.6 μmol), and triethylamine (0.3 ml) in toluene (0.2 ml) was stirred at room temperature for 10 min. To the stirred mixture, were added **7** (9.6 mg, 0.017 mmol) and **9S** (15.6 mg, 0.026 mmol) in toluene (0.2 ml) at room temperature. The resulting mixture was refluxed for 4 h, diluted with hexane, filtered with silica gel, and evaporated. The residue was purified by preparative TLC developed with hexane-benzene (2:1) to give **30S** (6,2 mg, 34%) as a colorless oil: IR (neat) 1467, 1252 cm^{-1}; ^1H NMR δ: 0.02–0.10 (m, 30H), 0.52 (s, 3H), 0.82–0.94 (m, 48H), 1.11 (s, 3H), 1.19 (s, 3H), 1.20–2.05 (m, 18H), 2.22 (br dd, 1H, J = 12.6, 3.8 Hz), 2.46 (br dd, 1H, J = 11.6, 8.1 Hz), 2.81 (br d, 1H, J = 13.5 Hz), 3.16–3.24 (m, 2H), 3.58–3.76 (m, 4H), 4.16–4.26 (m, 2H), 4.98 (br s, 1H), 5.26 (br s, 1H), 6.00 (d, 1H, J = 10.8 Hz), 6.22 (d, 1H, J = 10.5 Hz); $[\alpha]_D^{23}$ +10.7° (*c* 0.31, $CHCl_3$).

2.1.27. (5Z,7E)-(1R,2R,3R,24R)-2-(3-Hydroxypropoxy)-9,10-secocholesta-5,7,10(19)-triene-1,3,24.25-tetraol ***(6R)***

A mixture of **30R** (3.3 mg, 3.06 μmol) and 1 M tetrabutylammonium fluoride (TBAF) in THF (153 μl, 153 μmol) in toluene (0.5 ml) was stirred at 105°C for 2 h. The mixture was extracted with AcOEt, washed with H_2O and saturated NaCl, dried and evaporated. The residue was purified by preparative TLC developed with CH_2Cl_2-EtOH (5:1) to give **6R** (1.02 mg, 66%) as a colorless oil: IR (neat) 3400, 2947, 2927, 2871, 1377, 1105, 1070 cm^{-1}; ^1H NMR δ: 0.56 (s, 3H), 0.94 (d, 3H, J = 5.9 Hz), 1.16 (s, 3H), 1.22 (s, 3H), 2.42 (br d, 1H, J = 14.5 Hz), 2.55 (dd, 1H, J = 14.5, 4.0 Hz), 2.76–2.87 (m, 1H), 3.27 (dd, 1H, J = 8.8, 2.8 Hz), 3.30–3.37 (m, 1H), 3.68–3.78 (m, 1H), 3.80–4.00 (m, 3H), 4.21–4.36 (m, 2H), 5.08 (br s, 1H), 5.50 (br s, 1H), 6.04 (d, 1H, J = 11.2 Hz), 6.36 (d, 1H, J = 11.2 Hz); UV λmax 264 nm; $[\alpha]_D^{24}$ −23° (*c* 0.07, EtOH).

2.1.28. (5Z,7E)-(1R,2R,3R,24S)-2-(3-Hydroxypropoxy)-9,10-secocholesta-5,7,10(19)-triene-1,3,24.25-tetraol ***(6S)***

A mixture of **30S** (5.1 mg, 4.73 μmol) and 1 M TBAF in THF (237 μl, 237 μmol) in toluene (0.5 ml) was stirred at 105°C for 2 h. The mixture was extracted with AcOEt, washed with H_2O and saturated NaCl, dried and evaporated. The residue was purified by preparative TLC developed with CH_2Cl_2-EtOH (5:1) to give **6S** (1.45 mg, 60%) as a colorless oil: IR (neat) 3400, 2947, 2929, 2873, 1378, 1107, 1072 cm^{-1}; ^1H NMR δ: 0.55 (s, 3H), 0.95 (d, 3H, J = 6.3 Hz), 1.16 (s, 3H), 1.22 (s, 3H), 2.42 (br d, 1H, J = 13.9 Hz), 2.55 (dd, 1H, J = 14.5, 4.0 Hz), 2.75–2.87 (m, 1H), 3.20–3.37 (m, 2H), 3.68–3.78 (m, 1H), 3.80–3.99 (m, 3H), 4.20–4.36 (m, 2H), 5.08 (br s, 1H), 5.50 (br s, 1H), 6.04 (d, 1H, J = 11.2 Hz), 6.36 (d, 1H, J = 11.2 Hz); UV λmax 264 nm; $[\alpha]_D^{24}$ −49° (*c* 0.07, EtOH).

2.1.29. Vitamin D receptor (VDR) binding assay [13]

Chick embryonic intestinal 1α,25$(OH)_2D_3$ receptor (Yamasa Shoyu Co., Tokyo, Japan) was incubated at 4°C for 3 h with 4.67 nCi of [^{3}H]1α,25$(OH)_2D_3$ and various concentration of analogs (**1, 3, 6R,** and **6S**). Bound and free forms of [^{3}H]1α,25$(OH)_2D_3$ were separated by addition of dextran-charcoal and centrifugation. The radioactivity of the receptor-bound [^{3}H]1α,25$(OH)_2D_3$ was measured with a PACKARD TRI-CARB 2700TR.

2.1.30. Vitamin D binding protein (DBP) binding assay [14,15]

The binding affinity of analogs (**1, 3, 6R,** and **6S**), with rat DBP was performed according to Revelle et al. [14,15].

2.1.31. Determination of ionized plasma calcium levels

Mice (BALB/c, male, 9 weeks of age) were i.v. given vehicle (EtOH/saline = 1/99) and 10 μg/kg of analogs (**1, 3, 6R,** and **6S**). Their ionized plasma calcium levels at 24 h and 48 h after a single i.v. injection were determined by automatic Ca^{2+}/pH analyzer (The 634 Ca^{2+}/pH Analyzer, CIBA-CORNING, Tokyo, Japan). The results (mmol/l) are expressed as the mean ± S.D. The statistical significance of the differences between the vehicle and the experimental groups was analyzed by the use of Student's *t* test.

3. Results

3.1. Synthesis

To synthesize 24-hydroxylated ED-71 (**6**), we adopted the convergent method, in which the A-ring fragment (**7**) and the C/D-ring fragments (**9**) in 24(*R*) and 24(*S*) forms (vitamin D numbering) are coupled to produce a triene system of vitamin D_3 structure [16]. While the A-ring fragment (**7**) was synthesized from diethyl D-tartarate (**8**), the C/D-ring fragments (**9**) were prepared from vitamin D_2 (**10**) in 24(*R*) and 24(*S*) forms, respectively, as shown in the retrosynthetic pathway in Fig. 2.

First, we undertook the synthesis of the A-ring fragment (**7**). The epoxide (**11**), obtained from diethyl D-tartarate (**8**) by a

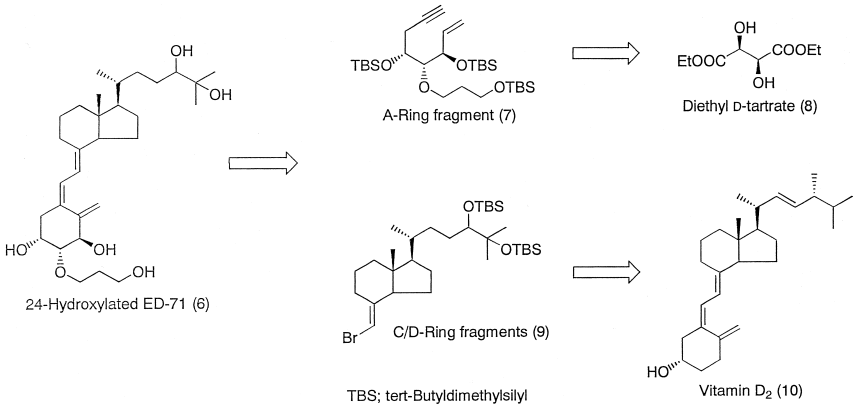

Fig. 2. Retrosynthesis of 24-hydroxylated ED-71.

known 5-step sequence [7], was cleaved by 1,3-propandiol in the presence of potassium *t*-BuOK at 120°C to give the alcohol (**12**) in 86% yield. After the protection of the introduced primary hydroxy part as the pivalate (**13**) in 88% yield, the cleavage of the benzyl ether in **13** and the subsequent protection of the resulting 1,2-diol moiety as the acetonide gave the alcohol (**14**) in 87% overall yield. The Swern oxidation of **14** and the Grignard reaction toward the resulting aldehyde afforded the dipivalate (**15**) as an epimeric mixture (R/S = 3/2) after the protection of the hydroxy part as the pivalate, although the stereoselectivity in the Grignard reaction was not able to be realized. Without separation of the epimeric mixture at this stage, acetonide moiety in **15** was cleaved quantitatively to give the diol (**16**). By the Mitsunobu reaction [8], **16** was converted to the epoxide (**17**) in 77% yield. The subsequent introduction of the acetylene unit was successfully carried out based upon the regioselective epoxide cleavage in **17** to provide the A-ring fragment (**7**) in 36% yield after separation of the accompanied (*S*)-epimer (**18**) (24%) by column chromatography (Fig. 3).

Next, we performed the synthesis of the C/D-ring fragments in both 24(*R*) and 24(*S*) series, respectively. The Inhoffen-Lythgoe diol (**19**), prepared from vitamin D_2 (**10**) by the ozonolysis [17], was converted to the sulfone (**21**) via **20** by known methodology [9,10]. The sulfone (**21**) was alkylated by both (*R*)- and (*S*)-2,3-dihydroxy-3-methylbutyl *p*-toluenenesulfonate (**22R** and **22S**) [13,14] to afford the alcohol (**23R**) in 71% yield and the alcohol (**23S**) in 28% yield, respectively. The (*R*)- and (*S*)-alcohol (**23R** and **23S**) were converted to the C/D ring fragments (**9R** and **9S**) as follows;

1. Desulfurization of **23R** and **23S** with Na/Hg giving **24R** and **24S** in 97% and 68% yields, respectively
2. Acetonide formation of **24R** and **24S** with 2,2-dimethoxypropane and the subsequent acetylation giving **25R** and **25S** in 83% and 64% yields, respectively
3. Deprotection of acetonide parts in **25R** and **25S** with iodine in methanol giving **26R** and **26S** in 61% and 83% yields, respectively
4. Silylation of **26R** and **26S** with TBSOTf giving **27R** and **27S** in 92% and 91% yields, respectively
5. Reductive cleavage of the acetyl parts in **27R** and **27S** with $LiAlH_4$ giving **28R** and **28S** in 96% and 100% yields, respectively

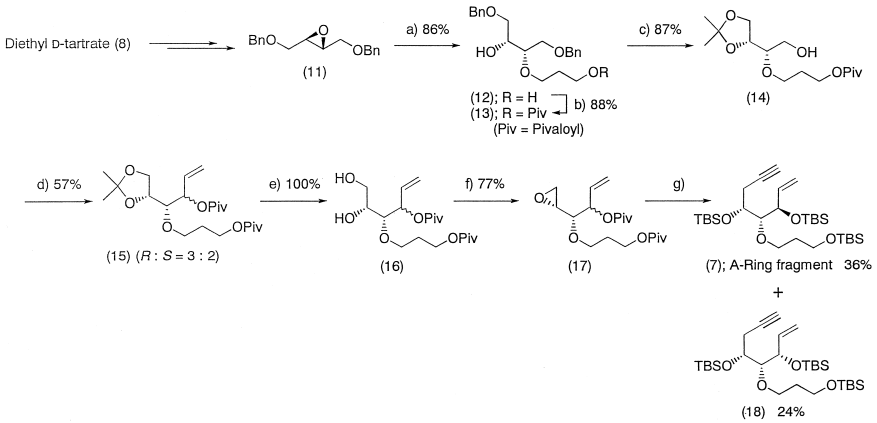

Fig. 3. Synthesis of the A-ring part. a) $HO(CH_2)_3OH$/*t*-BuOK; b) *t*-BuCOCl/pyridine; c) 1) H_2/$Pd(OH)_2$, 2) $Me_2C(OMe)_2$/TsOH, d); 1) Swern oxidation, 2) CH_2 = $CHMgBr$, 3) *t*-BuCOCl/Et_3N; e) 1M HCl; f) Ph_3P/DEAD; g) 1) LiC ≡ CTMS/BF_3-OEt_2, 2) 10N NaOH, 3) TBSOTf/Et_3N, 4) separation.

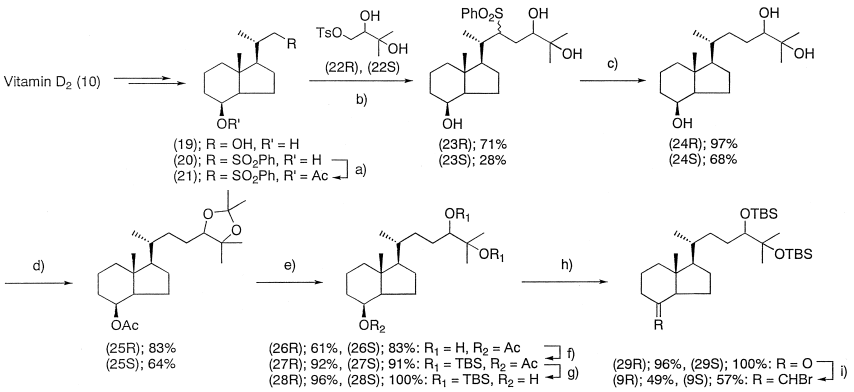

Fig. 4. Synthesis of the C/D-ring parts. a) Ac_2O/pyridine; b)*n*-BuLi; c) Na/Hg; d) 1) $Me_2C(OMe)_2$/TsOH, 2) Ac_2O/pyridine; e) I_2/MeOH; f) TBSOTf/lutidine; g) $LiAlH_4$; h) TPAP/NMO; i) $Ph_3PCH_2Br_2$/$NaN(TMS)_2$.

6. Oxidation of the hydroxy parts in **28R** and **28S** with NMO and tetra n-Pr_4NRuO_4 giving **29R** and **29S** in 96% and 100% yields, respectively
7. Wittig reaction of **29R** and **29S** with $Ph_3PCH_2Br_2$ in the presence of $NaN(TMS)_2$ giving **9R** and **9S** in 49% and 57% yields, respectively (Fig. 4).

Having the A-ring fragment (**7**) and the C/D-ring fragments (**9R** and **9S**), we performed the coupling reaction in the presence of PPh_3 and $(dba)_3Pd_2$-$CHCl_3$ following the Trost methodology [16], to give **30R** and **30S** in 34% and 36% yields, respectively, which were deprotected by TBAF to (24*R*)OH-ED-71 (**6R**) and (24*S*)OH-ED-71 (**6S**) in 60% and 66% yields, respectively (Fig. 5).

3.2. Biological evaluation

The results of preliminary biological evaluations of synthesized (24*R*)OH-ED-71 (**6R**) and (24*S*)OH-ED-71 (**6S**) in comparison with 1α,25$(OH)_2D_3$ (**1**) and ED-71 (**3**) are summarized in Table 1. The binding potency to rat DBP [14,15] in both (24*R*)OH-ED-71 (**6R**) and (24*S*)OH-ED-71 (**6S**) was less than that of ED-71 (**3**). The binding affinity to chick VDR [13] in (24*R*)OH-ED-71 (**6R**) was comparable to ED-71 (**3**). On the other hand, that of (24*S*)OH-ED-71 (**6S**) showed 1/10 affinity compared to ED-71 (**3**). At the dose of 10 μg/kg of each analog, plasma ionized calcium levels in BALB/c mice increased significantly at 24 and 48 h after i.v. administration compared to vehicle group. ED-71 (**3**) showed the most potent increasing activity of iozined calcium among analogs tested (vehicle: 1.32 $\pm$ 0.02 vs. ED-71 (**3**): 1.63 $\pm$ 0.02 mmol/l; $P <$ 0.001, vehicle: 1.31 $\pm$ 0.03 vs. ED-71 (**3**): 2.23 $\pm$ 0.11 mmol/l; $P < 0.001$). Although 1α,25$(OH)_2D_3$ (**1**), (24*R*)OH-ED-71 (**6R**), and (24*S*)OH-ED-71 (**6S**) showed tendency of increase in plasma calcium levels compared to vehicle, the changes were less than those of ED-71

4. Discussion

As analogs related to ED-71 (**3**), (24*R*)OH-ED-71 (**6R**) and (24*S*)OH-ED-71 (**6S**) were synthesized by the convergent method based upon the reaction between the A-ring fragment (**7**) obtained from diethyl D-tartarate (**8**) and the C/D-ring fragments (**9R** and **9S**) prepared from vitamin D_2 (**10**) via the Inhoffen-Lythgoe diol (**19**). 24-Hydroxylation of ED-71 (**3**) caused weakened affinity to DBP in vitro and less calcemic activity in vivo compared to **3.** The weakened calcemic characters of 24-hydroxylated analogs (**6R** and **6S**) might derive from the weak affinity to DBP, taking the high calcemic character of ED-71 (**3**) based on its strong affinity to DBP into

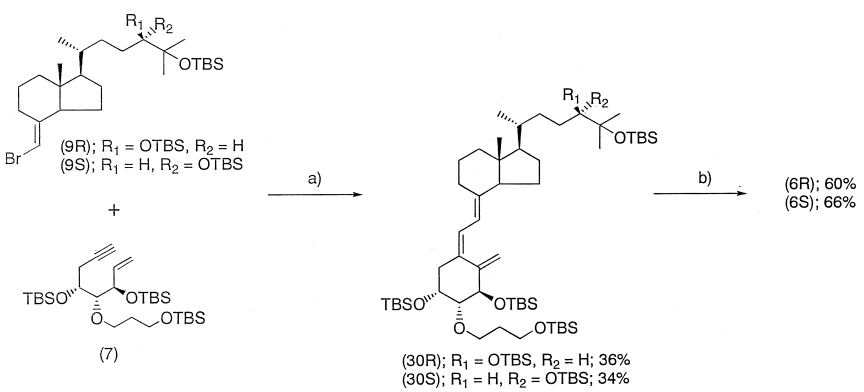

Fig. 5. Coupling the A-ring part with C/D-ring parts. a) $(dba)_3Pd_2CHCl_3/PPh_3$; b) TBAF.

Table 1
Biological characters of 24-hydroxylated ED-71

	VDR	DBP	Ca (24 h)	Ca (48 h)
Vehicle			1.32 ± 0.02 (n = 4)	1.31 ± 0.03 (n = 5)
1α,25(OH)$_2$D$_3$ (**1**)	1.00	1.00	1.59 ± 0.04 (n = 4)*	1.46 ± 0.05 (n = 4)*
ED-71 (**3**)	0.13	2.7	1.63 ± 0.02 (n = 5)*	2.23 ± 0.11 (n = 5)*
(24*R*)OH-ED-71 (**6R**)	0.10	0.3	1.48 ± 0.04 (n = 4)*	1.45 ± 0.03 (n = 4)*
(24*S*)OH-ED-71 (**6S**)	0.01	0.1	1.49 ± 0.05 (n = 4)**	1.44 ± 0.06 (n = 4)**

VDR: Affinity to chick intestinal vitamin D receptor.
DBP: Affinity to rat vitamin D binding protein.
Ca: Ionized calcium levels (mmol/l) after 10 μg/kg of analogs intravenous administration to BALB/c mice. * $P < 0.001$ vs. vehicle group; ** $P < 0.01$ vs. vehicle group.

consideration. While the affinity to VDR in (24*R*)OH-ED-71 (**6R**) was sustained, the affinity in (24*S*)OH-ED-71 (**6S**) was less than ED-71 (**3**). The observed effect of (24*R*)- and (24*S*)-hydroxylation of ED-71 (**3**) on the affinity to VDR corresponds well to the reported cases of the 24-hydroxylation of 1α,25(OH)$_2$D$_3$ (**1**) [18,19] and 1αOHD$_3$ (**2**) [13]. The preventive and therapeutic effects of (24*R*)OH-ED-71 (**6R**) and (24*S*)OH-ED-71 (**6S**) on bone mineral loss in ovariectomized rats are highly interested and under investigation. When ED-71 (**3**) was orally administered to rats, (24*R*)OH-ED-71 (**6R**) and (24*S*)OH-ED-71 (**6S**) were found in rat plasma as metabolites of **3** with accompanying several other metabolites. The detailed metabolic pathway and metabolites of ED-71 (**3**) will be reported elsewhere.

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