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Synthesis of putative metabolites of 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71)

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ABSTRACT

1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71), an analog of active vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] is under phase III clinical trials in Japan for the treatment of osteoporosis and bone fracture prevention. Since ED-71 has a substituent at the 2β-position of the A-ring, it is recognized that the metabolic pathway of ED-71 might be more complicated than 1,25$(OH)_2D_3$ because of metabolism at the 2β-position substituent in addition to the inherent metabolism of the side chain. To clarify the metabolism of hydroxypropoxy substituent of the 2β-positon and a combination of metabolism between side chain and 2β-positon, four putative metabolites of ED-71 have been prepared as authentic samples. The metabolites at the 2β-positon, the methyl ester derivative considered as an ester standard of the oxidized metabolite and the tetraol derivative as the truncated metabolite were synthesized from α-epoxide, a key intermediate of ED-71 synthesis. The combination metabolites between side chain and 2β-positon, the 24(*S*)- and 24(*R*)-pentaols were synthesized using Trost's convergent method.

1. Introduction

1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71, **1**) [1–5], an analog of active vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$, **2**] [6] containing a hydroxypropoxy substituent at the 2β-position of **2**, has potent effects on bone therapy. ED-71 is currently under phase III clinical trials in Japan as a promising candidate for the treatment of osteoporosis and bone fracture prevention [7] (Fig. 1). During the development of ED-71 (**1**), it was necessary to synthesize possible metabolites of **1** for pharmacokinetic and metabolic studies. It is well known that 1,25$(OH)_2D_3$ (**2**) is hydroxylated at the 24-position of the side chain as a first step of its metabolism to produce 24-hydroxylated 1,25$(OH)_2D_3$ [8–12]. On the assumption that hydroxylation pathway of ED-71 (**1**) and **2** would be similar, we, therefore, reported the synthesis of 24-hydroxylated ED-71 in 24(*S*) and 24(*R*) forms (**3** and **4**) in our previous paper [13]. In the case of 1,25$(OH)_2D_3$ (**2**), 24-hydroxylated 1,25$(OH)_2D_3$ is further hydroxylated at the 23- and 26-positions of the side chain and oxidized to a keto-alcohol, lactone (calcitriol lactone), or carboxylic acid (calcitroic acid) [8–12]. Since ED-71 (**1**) has a substituent at the 2β-position of the A-ring, it is recognized that the metabolic pathway of **1** might be more complicated than 1,25$(OH)_2D_3$ (**2**) because of metabolism at the 2β-position substituent in addition to the inherent metabolism of the side chain. In fact, in our preliminary metabolic studies of ED-71 (**1**),

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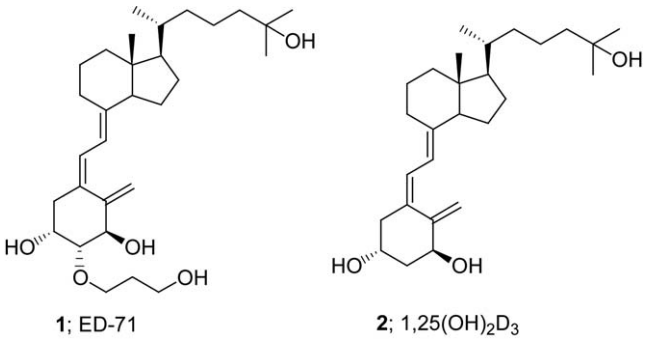

Fig. 1 – Structures of ED-71 and 1,25(OH)$_2$D$_3$.

24-hydroxylated ED-71 (**3** and **4**) were found as metabolites of **1** which were accompanied by several other metabolic associates that may be derived from metabolism of the hydroxypropoxy substituent of the 2β-position and a combination of metabolism between the side chain and the 2β-position. In order to assist in the confirmation and structure elucidation of metabolic derivatives of ED-71 (**1**), the synthesis of putative metabolites of **1** was undertaken.

In this paper, we describe the synthesis of postulated metabolites of ED-71 (**1**), namely the methyl ester derivative (**6**) as an ester standard of the oxidized metabolite (**5**) at the 2β-position, the tetraol derivative (**7**) as a truncated metabolite at the 2β-position, and pentaols (**8** and **9**) in 24(*S*) and 24 (*R*) forms that arise from the combination metabolism of the side chain and the 2β-position (Fig. 2).

2. Experimental

Optical rotations were measured with JASCO DIP-370 polarimeter. Infrared (IR) spectra were obtained using JASCO FT/IR-230 and HORIBA FT-730 spectrophotometers. ^{1}H and ^{13}C NMR spectra were recorded on VARIAN Gemini-300, VARIAN Unity Plus 500, and JEOL JNM-EX270 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-DX303 (electron ionization method (EI)), JEOL JMS-700N (EI), Shimadzu GCMS QP-1000 (EI), and Waters micromassZQ (electrospray ionization method (ESI)) instruments. High resolution mass spectra (HRMS) were recorded on JEOL JMS-AX500 (EI) and VG Auto Spec Q (ESI) instruments. Ultra violet (UV) spectra were obtained with Shimadzu UV-1600PC instrument using ethanol as a solvent. All reactions were carried out under an atmosphere of argon or nitrogen unless otherwise noted. All extracts 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} (preparative TLC).

2.1. 2β-(2-Acetyloxyethoxy)-1α,3β,25-trihydroxycholesta-5,7-diene (11)

To a mixture of **10** (306 mg, 0.643 mmol), 4-dimethylaminopyridine (DMAP) (10 mg), and pyridine (0.60 ml) in CH_2Cl_2 (30 ml), acetic anhydride (0.150 ml, 1.59 mmol) was added at 0 °C and the mixture was stirred at 0 °C for 1.5 h, poured into dilute hydrochloric acid, and extracted with CH_2Cl_2. The extract was washed with saturated $NaHCO_3$, dried, and evaporated. The residue was chromatographed on column (CH_2Cl_2/EtOH = 50/3) to give **11** (215 mg, 65%) with a recovery of **10** (105 mg, 34%); IR (neat): 3415 (br), 2930, 1740 cm^{-1}; ^{1}H NMR δ: 0.63 (3H, s), 0.96 (3H, d, J = 6.3 Hz), 1.05 (3H, s), 1.12 (6H, s), 2.07 (3H, s), 3.64–3.77 (2H, m), 3.84 (1H, brs), 3.89–4.00 (2H, m), 4.17–4.34 (2H, m), 5.33–5.41 (1H, m), 5.70 (1H, d, J = 3.6 Hz); UV λ_{max}: 293, 282, 271 nm; MS (EI (*m*/*z*)): 518 (M^+), 87 (100%).

2.2. 2β-(2-Acetyloxyethoxy)-1α,3β,25-tris(triethylsilyloxy)cholesta-5,7-diene (12)

To a mixture of **11** (260 mg, 0.502 mmol) and 2,6-lutidine (0.887 ml, 7.53 mmol) in CH_2Cl_2 (30 ml), triethylsilyl trifluoromethanesulfonate (TESOTf) (1.14 ml, 5.02 mmol) was added

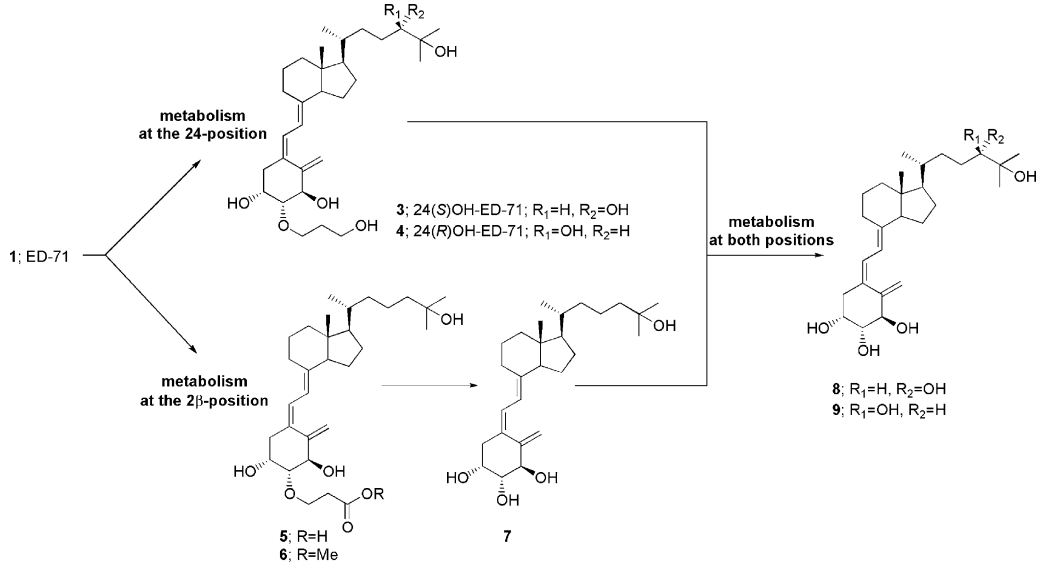

Fig. 2 – Putative metabolic pathway of ED-71.

at 0 °C and the mixture was stirred at 0 °C for 1.5 h and evaporated. The residue was chromatographed on column (hexane/AcOEt = 20/1) to give **12** (327 mg, 76%) as a colorless oil; IR (neat): 2950, 1750 cm^{-1}; ^1H NMR δ: 0.52–0.72 (21H, m), 0.88–1.03 (33H, m), 1.18 (6H, s), 2.04 (3H, s), 3.58 (1H, brs), 3.63–3.74 (1H, m), 3.75 (1H, d, J = 3.6 Hz), 3.94–4.11 (2H, m), 4.21 (2H, t, J = 5.0 Hz), 5.28–5.35 (1H, m), 5.57–5.62 (1H, m); UV λ_{max}: 293, 282, 271 nm; MS (EI (*m*/z)): 860 (M^+), 87 (100%).

2.3. *2β-(2-Hydroxyethoxy)-1α,3β,25-tris(triethylsilyloxy)cholesta-5,7-diene (13)*

To a solution of **12** (327 mg, 0.380 mmol) in THF (5 ml), 1N KOH in MeOH (10 ml) was added at room temperature and the mixture was stirred at room temperature for 1 h. To the mixture, acetic acid (0.15 ml) was added and the mixture was poured into water and extracted with hexane/AcOEt (1:1). The extract was washed with saturated $NaHCO_3$ and brine, dried, and evaporated. The residue was chromatographed on column (hexane/AcOEt = 12/1) to give **13** (176 mg, 56%) as a colorless oil; IR (neat): 3465 (br), 2955 cm^{-1}; ^1H NMR δ: 0.51–0.71 (21H, m), 0.89–1.07 (33H, m), 1.19 (6H, s), 3.48–3.71 (4H, m), 3.76 (1H, brs, J = 3.6 Hz), 3.82–3.91 (1H, m), 4.02–4.13 (1H, m), 5.30–5.36 (1H, m), 5.61 (1H, m); UV λ_{max}: 293, 282, 271 nm; MS (EI (*m*/z)): 818 (M^+), 75 (100%).

2.4. *PTAD adduct of 2β-(2-hydroxyethoxy)-1α,3β,25-tris(triethylsilyloxy)cholesta-5,7-diene (14)*

To a solution of **13** (173 mg, 0.211 mmol) in CH_2Cl_2 (5 ml), 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) (36.9 mg, 0.211 mmol) was added at room temperature and the mixture was stirred at room temperature for 30 min and evaporated. The residue was chromatographed on preparative TLC (hexane/AcOEt = 1/3) to give **14** (173 mg, 82%) as a yellow oil; IR (neat): 3580 (br), 2950, 1750, 1695 cm^{-1}; ^1H NMR δ: 0.51–0.82 (21H, m), 0.89–1.01 (30H, m), 1.05 (3H, s), 1.19 (6H, s), 3.00 (1H, dd, J = 13.9, 4.6 Hz), 3.58–3.71 (4H, m), 3.83–3.89 (2H, m), 3.91 (1H, d, J = 3.0 Hz), 4.90–4.99 (1H, m), 6.23 (1H, d, J = 8.3 Hz), 6.36 (1H, d, J = 8.3 Hz), 7.23–7.49 (5H, m); UV λ_{max}: 258, 218, 206 nm; MS (EI (*m*/z)): 818 ($[M - PTAD]^+$), 103 (100%).

2.5. *PTAD adduct of 2β-(2-iodoethoxy)-1α,3β,25-tris(triethylsilyloxy)cholesta-5,7-diene (15)*

To a mixture of **14** (173 mg, 0.174 mmol), triphenyl phosphine (Ph_3P) (123 mg, 0.469 ml), and imidazole (32 mg, 0.470 mmol) in CH_2Cl_2 (7 ml), iodide (75.2 mg, 0.296 mmol) was added at room temperature and the mixture was stirred at room temperature for 80 min, poured into 10% $Na_2S_2O_3$, and extracted with CH_2Cl_2. The extract was dried and evaporated. The residue was chromatographed on preparative TLC (hexane/AcOEt = 10/1) to give **15** (188 mg, 98%) as a colorless oil; IR (neat): 2980, 1765, 1715 cm^{-1}; ^1H NMR δ: 0.52–0.73 (18H, m), 0.81 (3H, s), 0.88–1.01 (30H, m), 1.07 (3H, s), 1.19 (6H, s), 2.97 (1H, dd, J = 13.9, 4.6 Hz), 3.20–3.34 (2H, m), 3.66 (1H, brt, J = 3.3 Hz), 3.69–3.81 (1H, m), 3.97 (1H, d, J = 3.3 Hz), 4.11–4.22 (1H, m), 4.86–4.96 (1H, m), 6.23 (1H, d, J = 8.3 Hz), 6.35 (1H, d, J = 8.3 Hz), 7.22–7.49 (5H, m); UV λ_{max}: 256, 204 nm; MS (EI (*m*/z)): 928 ($[M - PTAD]^+$), 75 (100%).

2.6. *PTAD adduct of 2β-(2-cyanoethoxy)-1α,3β,25-tris(triethylsilyloxy)cholesta-5,7-diene (16)*

To a solution of **15** (180 mg, 0.163 mmol) in dimethyl sulfoxide (DMSO) (26 ml) and THF (15 ml), a solution of sodium cyanide (NaCN) (8.0 mg, 0.163 mmol) in DMSO (8 ml) was added at room temperature. The mixture was stirred at 50 °C for 3.5 h, poured into ice, and extracted with hexane/AcOEt (10:1). The extract was washed with H_2O, dried, and evaporated. The residue was chromatographed on preparative TLC (hexane/AcOEt = 10/1) to give **16** (141 mg, 87%) as a colorless oil; IR (neat): 2955, 2250, 1750, 1700 cm^{-1}; ^1H NMR δ: 0.51–0.75 (18H, m), 0.80 (3H, s), 0.89–1.02 (30H, m), 1.05 (3H, s), 1.19 (6H, s), 2.98 (1H, dd, J = 13.9, 4.6 Hz), 3.66 (1H, t, J = 3.3 Hz), 3.71–3.79 (1H, m), 3.93 (1H, d, J = 3.0 Hz), 4.06–4.13 (1H, m), 4.90–4.98 (1H, m), 6.23 (1H, d, J = 8.3 Hz), 6.35 (1H, d, J = 8.3 Hz), 7.22–7.49 (5H, m); UV λ_{max}: 258, 204 nm; MS (EI (*m*/z)): 827 ($[M - PTAD]^+$), 75 (100%).

2.7. *PTAD adduct of 2β-(2-methoxycarbonylethyloxy)-1α,3β,25-trihydroxycholesta-5,7-diene (17)*

To a solution of **16** (180 mg, 0.163 mol) in Et_2O (6.6 ml), methanolic hydrochloric acid (concd. HCl/MeOH) (3.3 ml) was added at room temperature and the mixture was stirred at room temperature for 6.5 h and evaporated. The residue was chromatographed on preparative TLC (CH_2Cl_2/EtOH = 10/1) to give **17** (13 mg, 25%) as a colorless oil; IR (neat): 3450 (br), 2950, 1740, 1690 cm^{-1}; ^1H NMR δ: 0.80 (3H, s), 0.96 (3H, d, J = 6.6 Hz), 1.00 (3H, s), 1.21 (6H, s), 2.59 (2H, t, J = 5.6 Hz), 3.08–3.18 (1H, m), 3.70 (3H, s), 3.78–3.98 (4H, m), 6.20 (1H, d, J = 8.3 Hz), 6.40 (1H, d, J = 8.3 Hz), 7.25–7.42 (5H, m); MS (EI (*m*/z)): 518 ($[M - PTAD]^+$), 60 (100%).

2.8. *2β-(2-Methoxycarbonylethyloxy)-1α,3β,25-trihydroxycholesta-5,7-diene (18)*

A solution of **17** (18 mg, 0.026 mmol) in 1,3-dimethyl-2-imidazolidinone (DMI) (3 ml) was stirred at 140 °C for 1 h. The mixture was poured into H_2O and extracted with hexane/AcOEt (1:4). The extract was washed with H_2O, dried, and evaporated. The residue was chromatographed on preparative TLC (EtOH/AcOEt = 1/100) to give **18** (8 mg, 59%) as a white powder; IR (neat): 3420 (br), 2960, 1725 cm^{-1}; ^1H NMR δ: 0.63 (3H, s), 0.96 (3H, d, J = 6.6 Hz), 1.00 (3H, s), 1.20 (6H, s), 2.61 (2H, t, J = 5.8 Hz), 3.64–3.85 (4H, m), 3.74 (3H, s), 3.90–4.01 (1H, m), 5.31–5.39 (1H, m), 5.62–5.69 (1H, m); UV λ_{max}: 293, 282, 271 nm; MS (EI (*m*/z)): 518 (M^+), 60 (100%).

2.9. *2β-(2-Methoxycarbonylethoxy)-1α,3β,25-trihydroxy-9,10-secocholesta-5,7,10(19)-triene (6)*

A solution of **18** (8.0 mg, 0.015 mmol) in EtOH (200 ml) was irradiated using a 400 W high pressure mercury lamp with Vycor® filter at 0 °C for 95 s and the mixture was refluxed for 2 h and evaporated. The residue was chromatographed on preparative TLC (EtOH/AcOEt = 1/100) to give **6** (2.0 mg, 25%) as a colorless oil; ^1H NMR δ: 0.55 (3H, s), 0.93 (3H, d, J = 6.3 Hz), 1.22 (6H, s), 2.66 (2H, t, J = 5.1 Hz), 3.26 (1H, dd, J = 9.1, 2.8 Hz), 3.74–3.83 (1H, m), 3.92–4.02 (1H, m), 4.24–4.32 (2H, m), 5.08 (1H, s), 5.53 (1H, s), 6.06 (1H, d, J = 11.4 Hz), 6.36 (1H, d, J = 11.4 Hz); UV λ_{max}:

264 nm, λ_{min}: 229 nm; MS (EI (*m*/z)): 518 (M^+), 60 (100%); MS (ESI): 536($[M+NH_4]^+$), 1054 ($[2M+NH_4]^+$); HRMS (ESI) calcd for $C_{31}H_{54}NO_6$ [$(M+NH_4)^+$]: 536.3951, found: 536.3937.

2.10. *PTAD adduct of 3-acetoxy-1α,2α-epoxy-25-hydroxycholesta-5,7-diene (20)*

To a solution of **19** (100 mg, 0.171 mmol) in pyridine (3 ml), acetic anhydride (0.323 ml, 3.42 mmol) was added at room temperature and the mixture was stirred at 50 °C for 16 h and extracted with AcOEt. The extract was washed with 1N hydrochloric acid (HCl) and brine, dried, and evaporated. The residue was chromatographed on preparative TLC (CH_2Cl_2/EtOH = 10/1) to give **20** (88 mg, 82%) as a colorless oil; IR (neat): 3500 (br), 3000, 2900, 1760, 1700 cm^{-1}; ^1H NMR δ: 0.90 (3H, s), 0.95 (3H, d, J = 5.9 Hz), 1.10 (3H, s), 1.21 (6H, s), 2.10 (3H, s), 2.67–2.75 (1H, m), 3.35–3.44 (1H, m), 5.92–5.97 (1H, m), 6.20 (1H, d, J = 8.5 Hz), 6.45 (1H, d, J = 8.5 Hz), 7.27–7.47 (5H, m); MS (EI (*m*/z)): 454 ($[M-PTAD]^+$), 364 (100%).

2.11. *3β-Acetoxy-1α,2β,25-trihydroxycholesta-5,7-diene (22)*

To a solution of **20** (88 mg, 0.139 mmol) in THF (4 ml), boron trifluoride-diethyl etherate ($BF_3{\cdot}OEt_2$) (0.0159 ml, 0.139 mmol) was added at room temperature and the mixture was stirred at 60 °C for 16 h and extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on preparative TLC (CH_2Cl_2/EtOH = 10/1). The resultant **21** was dissolved in DMI (6.5 ml) and the mixture was stirred at 140 °C for 2 h and extracted with hexane/AcOEt (1:100). The extract was washed with H_2O, dried, and evaporated. The residue was chromatographed on preparative TLC (CH_2Cl_2/EtOH = 10/1) to give **22** (29 mg, 44%) as a colorless oil; IR (neat): 3450 (br), 2960, 2900, 1730, 1390, 1260, 1060, 930, 750 cm^{-1}; ^1H NMR δ: 0.62 (3H, s), 0.95 (3H, d, J = 5.9 Hz), 1.01 (3H, s), 1.22 (6H, s), 2.10 (3H, s), 3.77–3.84 (1H, m), 4.16–4.23 (1H, m), 5.11–5.19 (1H, m), 5.32–5.40 (1H, m), 5.65–5.73 (1H, m); UV λ_{max}: 293, 281, 270 nm; MS (EI (*m*/z)): 456 (M^+), 412 (100%).

2.12. *1α,2β,3β,25-Tetahydroxycholesta-5,7-diene (23)*

To a solution of **22** (29 mg, 0.061 mmol) in THF (10 ml), lithium aluminum hydride ($LiAlH_4$) (2.3 mg, 0.122 mmol) was added at room temperature and the mixture was refluxed for 1 h and extracted with AcOEt. The extract was washed with 1N NaOH and brine, dried, and evaporated. The residue was chromatographed on preparative TLC (CH_2Cl_2/EtOH = 10/1) to give **23** (9 mg, 34%) as a white powder; IR (neat): 3450 (br), 2950, 2870, 1270, 1140, 1050, 820 cm^{-1}; ^1H NMR δ: 0.64 (3H, s), 0.96 (3H, d, J = 6.6 Hz), 1.22 (6H, s), 2.10 (3H, s), 3.75–4.10 (1H, m), 5.35–5.40 (1H, m), 5.70–5.75 (1H, m); UV λ_{max}: 294, 281, 271 nm; MS (EI (*m*/z)): 432 (M^+), 324 (100%).

2.13. *1α,2β,3β,25-Tetrahydroxy-9,10-secochoresta-5,7,10(19)-triene (7)*

A solution of **23** (150 mg, 0.20 mmol) in EtOH (230 ml) was irradiated using a 400 W high pressure mercury lamp with Vycor® filter at 0 °C for 130 s and the mixture was refluxed for 2 h and evaporated. The residue was chromatographed on preparative TLC (CH_2Cl_2/EtOH = 10/1) to give **7** (0.48 mg, 5%) as a white powder; ^1H NMR δ: 0.55 (3H, s), 0.94 (3H, d, J = 5.9 Hz), 1.22 (6H, s), 2.49–2.55 (2H, m), 2.78–2.83 (1H, m), 3.51 (1H, dd, J = 9.1, 2.8 Hz), 4.15–4.15 (2H, m), 5.09 (1H, s), 5.46 (1H, s), 6.02 (1H, d, J = 11.4 Hz), 6.36 (1H, d, J = 11.4 Hz); UV λ_{max}: 264 nm, λ_{min} 226 nm; MS (EI (*m*/z)): 432 (M^+), 324 (100%); MS (ESI): 450 ($[M+NH_4]^+$), 882($[2M+NH_4]^+$), 431 ($[M-H]^-$), 863 ($[2M-H]^-$); HRMS (ESI) calcd for $C_{27}H_{44}NaO_4$ [$(M+Na)^+$]: 455.3137, found: 455.3127.

2.14. *(1S,2S)-1,2-Bis((R)-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-dipivalate (25)*

To a solution of **24** (13.6 g, 51.8 mmol) in pyridine (52 ml), pivaloyl chloride (*tert*-BuCOCl) (16 ml, 129.5 mmol) and DMAP (0.63 g, 5.5 mmol) were added at room temperature and the mixture was refluxed for 24 h. To the mixture, MeOH (6.3 ml) was added and the mixture was filtrated through Celite® and evaporated. The residue was extracted with AcOEt. The extract was washed with cold H_2O, dried, and evaporated. The residue was recrystallized from AcOEt to give **25** (16 g, 72%) as colorless crystals; $[\alpha]_D^{20}$ + 33.8 (c 1.08, $CHCl_3$); IR (film) 1735, 1479, 1371, 1228, 1155, 1074 cm^{-1}; ^1H NMR δ: 1.23 (9H, s), 1.31 (3H, s), 1.38 (3H, s), 3.77 (1H, dd, J = 7.8, 6.3 Hz), 3.9 (1H, dd, J = 7.8, 6.3 Hz), 4.09 (1H, q, J = 6.3 Hz), 5.31 (1H, d, J = 6.0 Hz); ^{13}C NMR δ: 25.2, 26.5, 27.3, 39.1, 66.2, 71.4, 74.5, 109.4, 117.0; HRMS (EI) calcd for $C_{21}H_{35}O_8$ [$(M-Me)^+$]: 415.2332, found: 415.2312.

2.15. *(2R,3R,4S)-5-((R)-2,2-Dimethyl-1,3-dioxolan-4-yl)ethane-3,4-dipivaloxy-1,2-diol (26)*

To a solution of **25** (10.6 g, 24.6 mmol) in THF (100 ml), 1N HCl (98 ml) was added at 0 °C and the mixture was stirred at room temperature for 20 h. To the mixture, $NaHCO_3$ (10.5 g) was added to be neutralized. The mixture was evaporated. The residue was extracted with CH_2Cl_2. The extract was dried and evaporated. The residue was chromatographed on column ($CHCl_3$/MeOH = 20/1) to give **26** (5.3 g, 50%) and **27** (2.9 g, 34%) as a clear oil respectively with a recovery of **25** (1.5 g, 16%); $[\alpha]_D^{20}$ + 42.7 (c 1.01, $CHCl_3$); IR (film) 3498, 1745, 1481, 1371, 1174, 1070 cm^{-1}; ^1H NMR δ: 1.24 (9H, s), 1.25 (9H, s), 1.31 (3H, s), 1.40 (3H, s), 2.66 (1H, d, J = 6.3 Hz), 2.45 (2H, m), 3.6 (2H, m), 3.77 (1H, dd, J = 8.4, 6.0 Hz), 3.96 (1H, t, J = 8.4 Hz), 4.11 (1H, q, J = 6.3 Hz), 5.11 (1H, d, J = 9.6 Hz), 5.29 (1H, d, J = 7.2 Hz); ^{13}C NMR δ: 25.2, 26.6, 27.2, 39.2, 39.2, 62.6, 66.6, 69.1, 70.9, 71.6, 73.9, 109.6, 178.1, 179.0; HRMS (EI) calcd for $C_{18}H_{31}O_8$ [$(M-Me)^+$]: 375.2019, found: 375.2008.

2.16. *(1S,2S)-1-((R)-2-Ethoxy-1,3-dioxolan-4-yl)-2-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-dipivalate (28)*

To a solution of **26** (4.76 g, 12.2 mmol) in CH_2Cl_2 (61 ml), triethyl orthoformate ($HC(OEt)_3$) ((3.0 ml, 18.3 mmol) and *p*-toluenesulfonic acid monohydrate ($TsOH{\cdot}H_2O$) (16.2 mg, 0.085 mmol) were added at 0 °C and the mixture was stirred at room temperature for 3 h. To the mixture, triethylamine (NEt_3) (5.7 ml) was added and the mixture was extracted with CH_2Cl_2. The extract was washed with H_2O, dried, and

evaporated. The residue was chromatographed on column (hexane/AcOEt = 20/1) to give **28** (4.81 g, 88%) as a colorless oil; $[\alpha]_D^{19} + 38.8$ (c 1.05, $CHCl_3$); IR (film) 3506, 1729, 1479, 1376, 1277, 1151, 1066 cm^{-1}; ^1H NMR δ: 1.23 (9H, s), 1.24 (9H, s), 1.30 (3H, s), 1.39 (3H, s), 3.60 (3H, m), 3.77 (2H, m), 3.91 (4H, m), 4.08 (2H, m), 5.40 (2H, m), 5.78 (1H, s); ^{13}C NMR δ: 14.0, 14.9, 22.6, 25.1, 26.4, 27.0, 27.1, 31.5, 38.9, 60.4, 60.7, 65.2, 65.9, 66.1, 70.9, 71.3, 71.9, 73.7, 74.1, 74.3, 109.2, 109.4, 114.9, 115.4, 176.8; HRMS (EI) calcd for $C_{19}H_{31}O_9$ $[(M - C_3H_7)^+]$: 403.1968, found: 403.1963.

2.17. *(3S,4R)-4-((R)-2,2-Dimethyl-1,3-dioxolan-4-yl)-3,4-dipivaloxy-1-butene (29)*

A solution of **28** (4.53 g, 10.1 mmol) in acetic anhydride (51 ml) was refluxed for 16 h and the mixture was evaporated. To the residue, saturated $NaHCO_3$ was added to be neutralized. The mixture was extracted with CH_2Cl_2. The extract was dried and evaporated. The residue was chromatographed on column (hexane/AcOEt = 6/1) to give **29** (3.35 g, 93%) as a colorless oil; $[\alpha]_D^{21} + 50.0$ (c 1.05, $CHCl_3$); IR (film) 1737, 1479, 1371, 1276, 1139, 1064 cm^{-1}; ^1H NMR δ: 1.16 (9H, s), 1.20 (9H, s), 1.26 (3H, s), 1.34 (3H, s), 3.74 (1H, dd, J = 8.1, 6.6 Hz), 3.92 (1H, dd, J = 8.1, 6.3 Hz), 4.14 (1H, q, J = 6.3 Hz), 5.15 (1H, s), 5.44 (1H, m), 5.68 (1H, ddd, J = 15.9, 10.8, 5.4 Hz); ^{13}C NMR δ: 25.2, 26.6, 27.2, 38.9, 39.0, 66.1, 72.4, 72.5, 74.0, 109.3, 117.6, 132.6, 176.8, 177.1; HRMS (EI) calcd for $C_{19}H_{32}O_6$ (M^+): 356.2199, found: 356.2201.

2.18. *(2R,3S,4R)-3,4-Dipivaloxy-5-hexene-1,2-diol (30)*

To a solution of **29** (3.35 g, 9.4 mmol) in THF (37.6 ml), 1N HCl (37.6 ml) was added at room temperature and the mixture was stirred at room temperature for 44 h. To the mixture, saturated $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2. The extract was dried and evaporated. The residue was chromatographed on column ($CHCl_3$/MeOH = 15/1) to give **30** (2.73 g, 93%) as a colorless oil; $[\alpha]_D^{22} + 70.3$ (c 1.03, $CHCl_3$); IR (film) 3482, 1737, 1481, 1398, 1280, 1160 cm^{-1}; ^1H NMR δ: 1.21 (9H, s), 1.28 (9H, s), 2.36 (1H, dd, J = 9.6, 4.2 Hz), 3.37 (1H, d, J = 6.0 Hz), 3.48 (2H, m), 3.62 (1H, m), 4.95 (1H, d, J = 7.5 Hz), 5.23 (1H, dd, J = 10.5, 1.5 Hz), 5.32 (1H, dd, J = 16.8, 1.5 Hz), 5.70–5.80 (2H, m); ^{13}C NMR δ: 27.2, 27.2, 39.0, 39.2, 62.7, 69.3, 71.7, 72.5, 100.7, 117.4, 132.8, 178.1, 178.6; HRMS (EI) calcd for $C_{16}H_{28}O_6$ (M^+): 316.1886, found: 316.1880.

2.19. *(R)-2-((1S,2R)-1,2-Dipivaloxy-3-butenyl)oxirane (31)*

To a solution of **30** (3.17 g, 10 mmol) in dioxane (140 ml), Ph_3P (7.78 g, 30 mmol) and diisopropyl azodicarboxylate (DIAD) (5.9 ml, 30 mmol) were added at room temperature and the mixture was refluxed for 23 h. Additionally, Ph_3P (5.33 g, 20 mmol) and DIAD (4.0 ml, 20 mmol) were added at room temperature and the mixture was refluxed for 22 h and evaporated. The residue was chromatographed on column (hexane/AcOEt = 30/1 to 10/1) to give **31** (2.19 g, 74%) as a colorless oil; $[\alpha]_D^{25} + 55.4$ (c 1.0, $CHCl_3$); IR (film) 1745, 1481, 1396, 1367, 1278, 1164, 1033 cm^{-1}; ^1H NMR δ: 1.19 (9H, s), 1.24 (9H, s), 2.74 (2H, m), 3.05 (1H, m), 4.99 (1H, t, J = 4.8 Hz), 5.28 (1H, dt, J = 10.8, 1.2 Hz), 5.35 (1H, dt, J = 16.8, 1.5 Hz), 5.54 (1H, tt, J = 4.8, 1.2 Hz), 5.79 (1H, ddd, J = 16.8, 10.8, 6.0 Hz); ^{13}C NMR δ: 27.0, 38.8, 38.9, 44.3, 49.4, 71.2, 72.7, 118.5, 132.0, 176.8, 176.9; HRMS (EI) calcd for $C_{16}H_{26}O_5$ (M^+): 298.1781, found: 298.1752.

2.20. *(4R,5R,6R)-5,6-Dipivaloxy-1-(trimethylsilyl)oct-7-en-1-yn-4-ol (32)*

To a solution of trimethylsilylacetylene (2.5 ml, 17.8 mmol) in THF (33 ml), *n*-butyl lithium (*n*-BuLi) (1.58 M in hexane; 10.8 ml, 17 mmol) was added at −78 °C and the mixture was stirred at −78 °C for 30 min. To the mixture, $BF_3{\cdot}OEt_2$ (2.2 ml, 17 mmol) was added at −78 °C and the mixture was stirred at −78 °C for 30 min. To the mixture, a solution of **31** (1.04 g, 3.5 mmol) in THF (97 ml) cooled to −70 °C was added at −78 °C for 20 min. The mixture was warmed to −30 °C, stirred at −30 °C for 3 h, quenched by addition of H_2O (1.0 ml), warmed to room temperature gradually, and evaporated. The residue was extracted with CH_2Cl_2. The extract was washed with H_2O, dried, and evaporated. The residue was chromatographed on column (hexane/AcOEt = 30/1 to 15/1) to give **32** (1.17 g, 85%) as a colorless oil; $[\alpha]_D^{24} + 61.0$ (c 1.03, $CHCl_3$); IR (film) 3517, 2177, 1737, 1479, 1278, 1141, 1034 cm^{-1}; ^1H NMR δ: 0.16 (9H, s), 1.19 (9H, s), 1.26 (9H, s), 2.22 (1H, dd, J = 17.1, 7.2 Hz), 2.31 (1H, dd, J = 16.8, 3.9 Hz), 2.65 (1H, d, J = 5.1 Hz), 3.57 (1H, m), 4.87 (1H, dd, J = 8.1, 2.7 Hz), 5.04 (1H, dd, J = 10.5, 1.5 Hz), 5.10 (1H, dd, J = 15.6, 1.5 Hz), 5.54 (1H, d, J = 1.5 Hz), 5.60 (1H, ddd, J = 15.3, 10.2, 4.8 Hz); ^{13}C NMR δ: −0.1, 25.0, 27.1, 27.2, 38.8, 39.1, 67.8, 71.8, 74.4, 88.0, 101.9, 117.1, 132.95, 177.0, 177.8; HRMS (EI) calcd for $C_{21}H_{36}O_5Si$ (M^+): 396.2332, found: 396.2342.

2.21. *(3R,4R,5R)-3,4,5-Tris(triethylsilyloxy)-oct-1-en-7-yne (33)*

To a solution of **32** (1.07 g, 2.69 mmol) in MeOH (11.7 ml), 1N NaOH (11.7 ml) was added at 0 °C and the mixture was stirred at room temperature for 6 h, neutralized by addition of 1N HCl, and evaporated. The residue was extracted with CH_2Cl_2. The extract was dried and evaporated. The residue was dissolved in CH_2Cl_2 (13.4 ml). To the solution, NEt_3 (3.0 ml, 21.5 mmol) and TESOTf (2.4 ml, 10.7 mmol) were added at −40 °C and the mixture was stirred at −40 °C for 11 h, quenched by addition of H_2O (1 ml), and extracted with Et_2O. The extract was washed with 1N NaOH, dried, and evaporated. The residue was chromatographed on column (hexane) to give **33** (1.25 g, 93%) as a colorless oil; $[\alpha]_D^{24} + 33.7$ (c 1.02, $CHCl_3$); IR (film) 3313, 1458, 1415, 1240, 1132, 1007 cm^{-1}; ^1H NMR δ: 0.61 (18H, m), 0.96 (27H, m), 1.90 (1H, t, J = 2.7 Hz), 2.26 (1H, ddd, J = 17.4, 8.4, 2.7 Hz), 2.43 (1H, dt, J = 17.4, 2.7 Hz), 3.72 (1H, d, J = 5.1 Hz), 4.0 (1H, dd, J = 8.1, 3.3 Hz), 4.09 (1H, t, J = 5.7 Hz), 5.13 (1H, d, J = 10.5 Hz), 5.23 (1H, dt, J = 16.8, 1.8 Hz), 5.88 (1H, ddd, J = 16.8, 10.5, 5.7 Hz); ^{13}C NMR δ: 4.9, 5.0, 5.1, 6.8, 6.9, 7.0, 23.1, 69.0, 71.6, 75.2, 80.3, 83.8, 115.6, 137.8; HRMS (EI) calcd for $C_{26}H_{54}O_5Si_3$ (M^+): 498.3381, found: 498.3378.

2.22. *(1R,3aR,4S,7aR)-7a-Methyl-1-[(1S)-1-methyl-1-(phenylsulfonyl)ethyl]octahydro-1H-inden-4-ol (36)*

To a solution of **35** (512 mg, 1.39 mmol) in acetone (9 ml), sodium iodide (NaI) (524 mg, 3.49 mmol) was added at room temperature and the mixture was refluxed for 2.5 h. The mixture was evaporated and the residue was dissolved in *N*,*N*-

dimethylformamide (DMF) (2.8 ml). To the solution, benzenesulfinic acid sodium salt dihydrate ($NaSO_2Ph{\cdot}2H_2O$) (114 mg, 0.7 mmol) was added at room temperature and the mixture was stirred at room temperature for 10 h. Additionally, $NaSO_2Ph{\cdot}2H_2O$ (114 mg, 0.7 mmol) was added at room temperature and the mixture was stirred at room temperature for 8 h and extracted with Et_2O. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on column (hexane/AcOEt = 10/1 to 4/1) to give **36** (399 mg, 78%) as a colorless oil; $[\alpha]_D^{25} + 48.2$ (c 1.0, $CHCl_3$); IR (film) 3535, 3062, 1704, 1583, 1450, 1298, 1143, 1078 cm^{-1}; ^1H NMR δ: 0.9 (3H, s), 1.17 (3H, d, J = 6.3 Hz), 1.20–1.8 (10H, m), 1.95 (1H, d, J = 13.8 Hz), 2.04 (1H, m), 2.84 (1H, dd, J = 14.1, 9.6 Hz), 3.14 (1H, dd, J = 14.1, 1.5 Hz), 4.05 (1H, d, J = 2.7 Hz), 7.54–7.68 (3H, m), 7.91 (2H, dt, J = 6.9, 1.5 Hz); ^{13}C NMR δ: 13.3, 17.3, 20.0, 22.4, 27.1, 32.0, 35.5, 40.2, 42.1, 52.5, 55.8, 61.9, 69.0, 127.9, 129.3, 129.4, 133.6, 140.3.

2.23. *(1R,3aR,4S,7aR)-7a-Methyl-1-[(1S)-1-methyl-1-(phenylsulfonyl)ethyl]-4-(triethylsilyloxy)octahydro-1H-indene (37)*

To a solution of **36** (1.67 g, 4.55 mmol) in DMF (9 ml), imidazole (0.93 g, 13.67 mmol) and triethylsilyl chloride (TESCl) (1.1 ml, 6.83 mmol) were added at room temperature and the mixture was stirred at room temperature for 4 h. To the mixture, saturated $NaHCO_3$ was added and the mixture was extracted with Et_2O. The extract was dried and evaporated. The residue was chromatographed on column (hexane/AcOEt = 8/1) to give **37** (2.17 g, 100%) as a colorless crystals; $[\alpha]_D^{24} + 54.0$ (c 1.01, $CHCl_3$); IR (film) 1701, 1454, 1307, 1238, 1151, 1081, 1018 cm^{-1}; ^1H NMR δ: 0.54 (6H, m), 0.86 (3H, s), 0.92 (9H, t, J = 8.1 Hz), 1.15 (3H, d, J = 6.6 Hz), 0.97–2.06 (12H, m), 3.03 (1H, dd, J = 14.1, 9.6 Hz), 3.35 (1H, d, J = 14.1 Hz), 4.19 (1H, d, J = 2.1 Hz), 7.54–7.67 (3H, m), 7.90 (2H, d, J = 7.2 Hz); ^{13}C NMR δ: 4.9, 7.0, 13.4, 17.6, 20.0, 22.8, 27.2, 32.0, 34.5, 40.6, 42.4, 53.0, 56.1, 62.0, 69.2, 127.9, 129.3, 133.5, 140.4.

2.24. *(1R,3aR,4S,7aR)-7a-Methyl-1-[(1S,2RS)-1,5-dimethyl-2-(phenylsulfonyl)-4-hexenyl)octahydro-1H-inden-4-ol (38)*

To a solution of **37** (2.17 g, 4.55 mmol) in THF (45 ml), *n*-BuLi (1.58 M in hexane; 3.75 ml, 5.9 mmol) was added at −40 °C and the mixture was stirred at −40 °C for 30 min. To the mixture, 4-bromo-2-methyl-2-butene (0.78 ml, 6.8 mmol) was added at −40 °C and the mixture was stirred at −40 °C for 4 h, quenched by addition of saturated $NaHCO_3$, warmed to room temperature, and extracted with Et_2O. The extract was dried and evaporated. The residue was dissolved in THF (5 ml). To the solution, 3N HCl (2.3 ml) was added at 0 °C and the mixture was stirred at room temperature for 12 h and extracted with CH_2Cl_2. The extract was dried and evaporated. The residue was chromatographed on column (hexane/AcOEt = 8/1 to 5/1) to give **38** (1.76 g, 96%); $[\alpha]_D^{24} + 14.9$ (c 1.47, $CHCl_3$); IR (film) 3540, 1668, 1448, 1378, 1297, 1143, 1079 cm^{-1}; ^1H NMR δ: 0.87 (3H, d, J = 4.8 Hz), 1.10 (3H, d, J = 6.9 Hz), 1.52 (3H, s), 1.54 (3H, s), 2.34–2.62 (2H, m), 3.06 (1H, m), 4.04 (1H, brs), 4.82 (1H, m), 7.51–7.61 (3H, m), 7.83–7.89 (2H, m); ^{13}C NMR δ: 13.2, 14.6, 17.5, 17.8, 22.4, 22.6, 25.6, 27.2, 33.6, 33.6, 33.9, 36.8, 40.4, 42.1, 42.2, 52.7, 54.3, 67.0, 69.1, 119.6, 121.3, 128.2, 128.6, 129.0, 129.1, 133.3; HRMS (EI) calcd for $C_{24}H_{36}O_3S$ (M^+): 404.2386, found: 404.2386.

2.25. *A 4:1 mixture of (1R,3aR,4S,7aR)-7a-methyl-1-[(1R)-1,5-dimethyl-4-hexenyl]octahydro-1H-inden-4-ol and (1R,3aR,4S,7aR)-7a-methyl-1-[(1R,2E)-1,5-dimethyl-2,4-hexadienyl]octahydro-1H-inden-4-ol (39)*

To a solution of **38** (842 mg, 2.08 mmol) in THF/MeOH (3:2) (42 ml), 5% sodium mercury amalgam (Na/Hg) (12.6 g, 27.4 mmol) was added at room temperature and the mixture was stirred at room temperature for 9 h and sonicated for 1.5 h. To the mixture, 50% MeOH solution (11 ml) was added and the mixture was stirred for 1 h, filtrated through Celite®, and evaporated. To the residue, saturated NH_4Cl was added and the mixture was extracted with AcOEt. The extract was dried and evaporated. The residue was chromatographed on column (hexane/AcOEt = 20/1 to 10/1) to give **39** (394 mg, 72%) as a mixture of ene/diene = 4/1; IR (film) 3831, 3735, 3671, 3610, 3201, 2130, 1701, 1523, 1459 cm^{-1}; ^1H NMR δ: 0.90–2.1 (37H, m), 4.07 (1.3H, brs), 5.08 (0.8H, t, J = 6.9 Hz), 5.38 (0.2H, dd, J = 15.9, 8.1 Hz), 5.74 (0.2H, d, J = 10.5 Hz), 6.14 (0.2H, dd, J = 15.9, 10.5 Hz); ^{13}C NMR δ: 13.6, 13.8, 17.5, 17.7, 18.3, 18.5, 20.5, 22.6, 24.8, 25.9, 26.0, 27.2, 33.6, 35.2, 36.0, 40.4, 41.9, 52.7, 56.7, 69.5, 125.2, 131.0.

2.26. *A 4:1 mixture of (1R,3aR,4S,7aR)-4-acetoxy-7a-methyl-1-[(1R)-1,5-dimethyl-4-hexenyl]octahydro-1H-indene and (1R,3aR,4S,7aR)-4-acetoxy-7a-methyl-1-[(1R,2E)-1,5-dimethyl-2,4-hexadienyl]octahydro-1H-indene (40)*

To a solution of **39** (255 mg, 0.753 mmol) in pyridine (3.8 ml), acetic anhydride (0.13 ml, 1.09 mmol) and DMAP (9.0 mg, 0.07 mmol) were added at 0 °C and the mixture was stirred at room temperature for 20 h and extracted with Et_2O. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on column (benzene) to give **40** (212 mg, 92%) as a mixture of ene/diene = 4/1; ^1H NMR δ: 0.87 (3H, s), 0.91 (3H, d, J = 6.6 Hz), 1.0–1.52 (12H, m), 1.59 (3H, s), 1.67 (3H, s), 1.71–2.01 (5H, m), 2.03 (3H, s), 5.07 (0.8H, t, J = 6.9 Hz), 5.13 (1H, brs), 5.37 (0.2H, dd, J = 14.4, 8.7 Hz), 5.73 (0.2H, d, J = 9.9 Hz), 6.13 (0.2H, dd, J = 14.4, 10.5 Hz); ^{13}C NMR δ: 13.1, 17.7, 18.0, 18.6, 21.5, 22.7, 24.7, 25.8, 27.2, 30.6, 35.3, 35.9, 40.1, 42.1, 51.4, 56.5, 71.5, 125.2, 131.1, 171.0.

2.27. *(1R,3aR,4S,7aR)-4-Acetoxy-1-[(1R,4S)-4,5-dihydroxy-1,5-dimethylhexyl]-7a-methyloctahydro-1H-indene (41)*

To a solution of **40** (46.4 mg, 0.151 mmol) in 50% *tert*-BuOH (1.8 ml) solution, methanesulfonamide ($MeSO_2NH_2$) (14.3 mg, 0.151 mmol) and the commercially available asymmetric osmium tetroxide dihydroxylation reagent, AD-mix-α (Aldrich, 0.5% OsO_4; 154 mg, 0.003 mmol) were added at 0 °C and the mixture was stirred at 2 °C for 3 days. To the mixture, saturated $Na_2S_2O_3$ was added and the mixture was extracted with AcOEt. The extract was dried and evaporated. The residue was chromatographed on column ($CHCl_3$/MeOH = 20/1). The

resultant was dissolved in EtOH (5.0 ml) and hydrogenated with 10% Pd/C (12 mg) at room temperature for 10 h. The mixture was filtrated through Celite® and evaporated to give **41** (49.8 mg, 96%); $[\alpha]_D^{25} + 21.7$ (*c* 1.03, $CHCl_3$); IR (film) 3448, 1735, 1448, 1378, 1297, 1143, 1079 cm^{-1}; ^1H NMR δ: 0.88 (3H, s), 0.93 (3H, d, J = 6.6 Hz), 1.16 (3H, s), 1.21 (3H, s), 2.04 (3H, s), 3.27 (1H, d, J = 6.6 Hz), 5.14 (1H, brs); ^{13}C NMR δ: 13.0, 17.9, 18.7, 21.3, 22.6, 23.1, 26.5, 27.0, 28.2, 30.4, 33.0, 35.5, 39.9, 41.9, 51.2, 56.2, 71.3, 73.1, 79.4, 170.7; HRMS (EI) calcd for $C_{20}H_{36}O_4$ (M^+): 340.2613, found: 340.2613.

2.28. *(1R,3aR,4S,7aR)-4-Acetoxy-1-[(1R,4S)-4,5-di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-1H-indene (42)*

To a solution of **41** (60 mg, 0.176 mmol) in CH_2Cl_2 (2.0 ml), 2,6-lutidine (0.13 ml, 1.10 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) (0.17 ml, 0.74 mmol) were added at 0 °C and the mixture was stirred at 0 °C for 1.5 h. To the mixture, saturated $NaHCO_3$ was added and the mixture was extracted with Et_2O. The extract was washed with saturated NH_4Cl, dried, and evaporated. The residue was chromatographed on column (hexane/AcOEt = 50/1) to give **42** (95 mg, 95%) as a colorless oil; $[\alpha]_D^{21} + 6.3$ (*c* 1.07, $CHCl_3$); IR (film) 1735 cm^{-1}; ^1H NMR δ: 0.04 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.85 (9H, s), 0.88 (9H, s), 1.10 (3H, s), 1.20 (3H, s), 2.04 (3H, s), 3.18 (1H, dd, J = 7.5, 2.4 Hz), 5.15 (1H, brs); ^{13}C NMR δ: 5.8, 6.3, 6.6, 6.9, 7.0, 7.0, 7.3, 13.1, 18.1, 18.7, 21.5, 22.9, 23.6, 27.3, 28.8, 29.0, 30.7, 34.1, 36.1, 40.2, 42.1, 51.5, 56.6, 71.6, 76.3, 81.7, 170.8; MS (EI (*m*/*z*)): 553 [$(M - Me)^+$], 173 (100%).

2.29. *(1R,3aR,4S,7aR)-1-[(1R,4S)-4,5-di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-1H-inden-4-ol (43)*

To a solution of **42** (260 mg, 0.458 mmol) in THF (5.0 ml), $LiAlH_4$ (26.1 mg, 0.687 mmol) was added at 0 °C and the mixture was stirred at 0 °C for 1.5 h. To the mixture, 1N NaOH was added and the mixture was extracted with AcOEt. The extract was washed with saturated NH_4Cl, dried, and evaporated. The residue was chromatographed on column (hexane/AcOEt = 40/1) to give **43** (241 mg, 100%) as a colorless oil; $[\alpha]_D^{21} + 6.3$ (*c* 1.07, $CHCl_3$); IR (film) 3430 cm^{-1}; ^1H NMR δ: 0.04 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.85 (9H, s), 0.89 (9H, s), 0.92 (3H, d, J = 6.3 Hz), 1.11 (3H, s), 1.19 (3H, s), 3.18 (1H, dd, J = 7.6, 2.3 Hz), 4.00 (1H, brs); ^{13}C NMR δ: 5.8, 6.3, 6.6, 6.9, 7.9, 7.0, 7.3, 13.1, 18.1, 18.7, 21.5, 22.9, 23.6, 27.3, 28.8, 29.0, 30.7, 34.1, 36.1, 40.2, 42.1, 51.5, 56.6, 71.6, 76.3, 81.7, 170.8; HRMS (EI) calcd for $C_{30}H_{62}O_3Si_2$ (M^+): 526.4237, found: 526.4247.

2.30. *(1R,3aR,7aR)-1-[(1R,4S)-4,5-Di(tert-butyldimethylsilanyloxy)-1,5-dimethylhexyl]-7a-methyl-octahydro-1H-inden-4-one (44)*

To a solution of **43** (59.0 mg, 0.112 mmol) in CH_2Cl_2 (7.5 ml), *N*-methylmorpholine-*N*-oxide (NMO) (23.6 mg, 0.201 mmol) and 4 Å molecular sieves (50 mg) were added at room temperature and the mixture was stirred at room temperature for 1 h. To the mixture, tetrapropylammonium perruthenate (TPAP) (2.7 mg, 0.0078 mmol) was added at room temperature and the mixture was stirred at room temperature for 4 h, filtrated through Celite® and evaporated. The residue was chromatographed on column (hexane/AcOEt = 5/1) to give **44** (55.9 mg, 95 %); $[\alpha]_D^{23} - 5.7$ (*c* 1.85, $CHCl_3$); IR (film) 1716, 1467, 1252 cm^{-1}; ^1H NMR δ: 0.04 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.63 (3H, s), 0.85 (9H, s), 0.88 (9H, s), 0.94 (3H, d, J = 6.3 Hz), 1.10 (3H, s), 1.19 (3H, s), 1.20–2.28 (16H, m), 2.43 (1H, dd, J = 11.1, 7.5 Hz), 3.18 (1H, dd, J = 7.5, 2.4 Hz); ^{13}C NMR δ: −3.7, −3.1, −1.9, 12.5, 18.8, 19.2, 23.1, 24.2, 25.9, 26.2, 27.8, 28.9, 29.7, 34.0, 36.2, 39.1, 41.1, 50.0, 62.1, 81.3, 212.3; HRMS (EI) calcd for $C_{30}H_{60}O_3Si_2$ (M^+): 524.4081, found: 524.4066.

2.31. *(1R,3aR,7aR)-4-[(E)-Bromomethylene]-1-((1R,4S)-4,5-di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyl-octahydro-1H-inden (45)*

To a degassed solution of (bromomethyl)triphenylphosphonium bromide ($Ph_3PCH_2Br_2$) (400 mg, 0.917 mmol) in THF (2.2 ml), sodium hexamethyldisilazide (NaHMDS) (1.0 M in THF; 0.9 ml, 0.9 mmol) was added at −60 °C and the mixture was stirred at −60 °C for 1 h. To the mixture, a degassed solution of **44** (55.9 mg, 0.106 mmol) in THF (0.7 ml) was added at −60 °C. The mixture was warmed to room temperature gradually and stirred at room temperature for 1 h. To the mixture, saturated NH_4Cl was added at 0 °C and the mixture was extracted with AcOEt. The extract was dried and evaporated. The residue was chromatographed on column (hexane/benzene = 100/1 to 10/1) to give **45** (35 mg, 55%) as a yellow oil; $[\alpha]_D^{23} + 50.1$ (*c* 0.83, $CHCl_3$); IR (film) 1466, 1252 cm^{-1}; ^1H NMR δ: 0.04 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.56 (3H, s), 0.85 (9H, s), 0.88 (9H, s), 0.92 (3H, d, J = 6.0 Hz), 1.10 (3H, s), 1.18 (3H, s), 1.22–1.36 (5H, m), 1.4–1.74 (7H, m), 1.86–2.4 (4H, m), 2.86 (1H, m), 3.17 (1H, dd, J = 7.5, 2.4 Hz), 5.64 (1H, s); ^{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; HRMS (EI) calcd for $C_{30}H_{58}O_2Si_2Br$ [$(M - Me)^+$]: 587.3128, found: 587.3140.

2.32. *(1R,3aR,4S,7aR)-4-Acetoxy-1-[(1R,4R)-4,5-dihydroxy-1,5-dimethylhexyl]-7a-methyloctahydro-1H-indene (46)*

To a solution of **40** (50 mg, 0.164 mmol) in 50% *tert*-BuOH (2.0 ml) solution, $MeSO_2NH_2$ (15.5 mg, 0.164 mmol) and AD-mix-β (Aldrich, 0.5% OsO_4; 167 mg, 0.0032 mmol) were added at 0 °C and the mixture was stirred at 2 °C for 3 days. To the mixture, saturated $Na_2S_2O_3$ was added and the mixture was extracted with AcOEt. The extract was dried and evaporated. The residue was chromatographed on column ($CHCl_3$/MeOH = 20/1). The resultant was dissolved in EtOH (5.0 ml) and hydrogenated with 10% Pd/C (15 mg) at room temperature for 10 h. The mixture was filtrated through Celite® and evaporated to give **26** (53 mg, 95%); IR (film) 3455, 1735 cm^{-1}; ^1H NMR δ: 0.89 (3H, s), 0.92 (3H, d, J = 6.6 Hz), 1.16 (3H, s), 1.21 (3H, s), 2.04 (3H, s), 3.32 (1H, t, J = 5.9 Hz), 5.14 (1H, brs); MS (EI (*m*/*z*)): 280 [$(M - Ac\text{-}H_2O)^+$], 59 (100%).

2.33. *(1R,3aR,4S,7aR)-4-Acetoxy-1-[(1R,4R)-4,5-di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-1H-indene (47)*

Following the same experimental procedure as for **42**, **47** (130 mg, 97%) was obtained as a colorless oil from **46** (80 mg, 0.235 mmol); $[\alpha]_D^{21} + 6.3$ (*c* 1.07, $CHCl_3$); IR (film) 1745 cm^{-1}; ^1H NMR δ: 0.04 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.86 (9H, s), 0.89 (9H, s), 1.10 (3H, s), 1.19 (3H, s), 2.04 (3H, s), 3.22 (1H, brs), 5.15 (1H, brs); ^{13}C NMR δ: 5.8, 6.3, 6.6, 6.9, 7.0, 7.0, 7.3, 13.1, 18.1, 18.7, 21.5, 22.9, 23.6, 27.3, 28.8, 29.0, 30.7, 34.1, 36.1, 40.2, 42.1, 51.5, 56.6, 71.6, 76.3, 81.7, 170.8; MS (EI (*m*/z)): 553 [$(M - Me)^+$], 173 (100%).

2.34. *(1R,3aR,4S,7aR)-1-[(1R,4R)-4,5-di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyloctahydro-1H-inden-4-ol (48)*

Following the same experimental procedure as for **43**, **48** (120 mg, 93%) was obtained from **47** (140 mg, 0.246 mmol); $[\alpha]_D^{21} + 6.3$ (*c* 1.07, $CHCl_3$); IR (film) 3425, cm^{-1}; ^1H NMR δ: 0.03 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.85 (9H, s), 0.89 (9H, s), 1.11 (3H, s), 1.18 (3H, s), 3.22 (1H, brs), 4.08 (1H, brs); ^{13}C NMR δ: 5.8, 6.3, 6.6, 6.9, 7.9, 7.0, 7.3, 13.1, 18.1, 18.7, 21.5, 22.9, 23.6, 27.3, 28.8, 29.0, 30.7, 34.1, 36.1, 40.2, 42.1, 51.5, 56.6, 71.6, 76.3, 81.7, 170.8; MS (EI (*m*/z)): 511 [$(M - Me)^+$], 173 (100%).

2.35. *(1R,3aR,7aR)-1-[(1R,4R)-4,5-Di(tert-butyldimethylsilanyloxy)-1,5-dimethylhexyl]-7a-methyl-octahydro-1H-inden-4-one (49)*

Following the same experimental procedure as for **44**, **49** (273 mg, 100%) was obtained as a colorless oil from **48** (274 mg, 0.521 mmol); $[\alpha]_D^{25} + 16.2$ (*c* 1.02, $CHCl_3$); IR (film) 1716, 1460, 1252 cm^{-1}; ^1H NMR δ: 0.03 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.63 (3H, s), 0.85 (9H, s), 0.88 (9H, s), 0.94 (3H, d, J = 6.0 Hz), 1.11 (3H, s), 1.18 (3H,s), 1.20–2.28 (16H, m), 2.43 (1H, dd, J = 7.5, 11.1 Hz), 3.20 (1H, m); ^{13}C NMR δ: −3.7, −3.1, −1.9, −1.8, 12.5, 18.8, 18.3, 18.9, 19.2, 23.1, 24.2, 25.9, 26.2, 27.8, 28.9, 29.7, 34.0, 36.2, 39.1, 41.1, 50.0, 62.1, 81.3, 212.2; HRMS (EI) calcd for $C_{30}H_{60}O_3Si_2$ [$(M - Me)^+$]: 509.8846, found: 509.8848.

2.36. *(1R,3aR,7aR)-4-[(E)-Bromomethylene]-1-((1R,4R)-4,5-di(tert-butyldimethylsilyloxy)-1,5-dimethylhexyl]-7a-methyl-octahydro-1H-inden (50)*

Following the same experimental procedure as for **45**, **50** (96.1 mg, 34%) was obtained as a colorless oil from **49** (250 mg, 0.476 mmol); $[\alpha]_D^{23} + 65.7$ (*c* 1.06, $CHCl_3$); IR (film) 1467, 1253 cm^{-1}; ^1H NMR δ: 0.04 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.56 (3H, s), 0.85 (9H, s), 0.89 (9H, s), 0.92 (3H, d, J = 6.0 Hz), 1.11 (3H, s), 1.18 (3H, s), 1.22–1.36 (5H, m), 1.40–1.74 (7H, m), 1.86–2.40 (4H, m), 2.86 (1H, m), 3.21 (1H, m), 5.64 (1H, s); ^{13}C NMR δ: −3.9, −3.1, −1.9, −1.8, 11.9, 18.3, 19.0, 22.1, 22.7, 23.6, 26.0, 26.2, 27.7, 29.0, 29.6, 31.2, 33.9, 36.8, 40.0, 45.6, 55.9, 56.0, 76.4, 81.1, 97.4, 145.3; HRMS (EI) calcd for $C_{27}H_{52}O_2Si_2Br$ [$(M - C_4H_9)^+$]: 543.2690, found: 543.2692.

2.37. *[(R)-6-{(E,3R,3aR)-7-(Bromomethylene)-octahydro-3a-methyl-1H-inden-3-yl}-2-methylheptan-2-yloxy]trimethylsilane (52)*

Following the same experimental procedure as for **50**, **52** (12 mg, 25%) was obtained as a colorless oil from **51** (40 mg, 0.11 mmol); IR (neat) 2950 (br), 1247, 1043, 839 cm^{-1}; ^1H NMR δ: 0.10 (9H, s), 0.56 (3H, s), 0.93 (3H, d, J = 6.3 Hz), 1.20 (6H, s), 1.21–1.70 (14H, m), 1.83–2.05 (4H, m), 2.83–2.89 (1H, m), 5.64 (1H, s); MS (EI (*m*/z)) 429(M^+).

2.38. *1α,2β,3β-Tris(triethylsilyloxy)-24S,25-di(tert-butyldimethylsilyloxy)-9,10-secochoresta-5,7,10(19)-triene (53)*

To a solution of **33** (14.8 mg, 0.025 mmol) and **45** (9.0 mg, 0.018 mmol) in toluene (0.4 ml) and NEt_3 (0.25 ml), tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$) (5.7 mg, 0.005 mmol) was added at room temperature and the mixture was stirred at room temperature for 15 min and then refluxed for 1 h. After cooling to room temperature, the mixture was diluted with Et_2O, passed through small amount of silica gel, and evaporated. The residue was chromatographed on preparative TLC (hexane/benzene = 20/1) to give **53** (4.9 mg, 27%) as a yellow oil; $[\alpha]_D^{22} + 44.3$ (*c* 1.28, $CHCl_3$); IR (neat) 3831, 3737, 1687, 1525, 1461, 1255, 1097 cm^{-1}; ^1H NMR δ: 0.53–0.67 (15H, m), 0.86–0.99 (36H, m) 1.11 (3H, s), 1.19 (3H, s), 2.15 (1H, dd, J = 9.6, 3.3 Hz), 2.62 (1H, m), 2.84 (1H, d, J = 8.7 Hz), 3.18 (1H, dd, J = 5.7, 1.8 Hz), 3.75 (1H, brs), 4.02 (1H, d, J = 3.3 Hz), 4.11 (1H, m), 4.99 (1H, brs), 5.12 (1H, brs), 6.04 (1H, d, J = 8.7 Hz), 6.26 (1H, d, J = 6.3 Hz); ^{13}C NMR δ: −3.7, −3.1, −1.9, 4.9, 5.0, 5.2, 5.9, 6.8, 6.9, 7.0, 7.0, 12.0, 18.3, 18.9,22.2, 23.2, 23.7, 25.9, 26.2, 28.0, 29.0, 29.7, 34.2, 36.8, 40.4, 40.8, 45.9, 56.5, 56.7, 69.5, 76.5, 81.3, 116.5, 118.1, 123.3, 134.7, 141.3.

2.39. *1α,2β,3β-Tris(triethylsilyloxy)-24R,25-di(tert-butyldimethylsilyloxy)-9,10-secochoresta-5,7,10(19)-triene (54)*

Following the same experimental procedure as for **53**, **54** (25.2 mg, 23%) was obtained as a yellow oil from **33** (96.0 mg, 0.159 mmol) and **50** (55.0 mg, 0.110 mmol); $[\alpha]_D^{21} + 81.2$ (*c* 1.26, $CHCl_3$); IR (film) 1685, 1461, 1373, 1247, 1093, 1008 cm^{-1}; ^1H NMR δ: 0.53–0.65 (15H, m), 0.85–0.99 (36H, m) 1.11 (3H, s), 1.18 (3H, s), 2.15 (1H, dd, J = 9.6, 3.3 Hz), 2.62 (1H, m), 2.84 (1H, d, J = 11.1 Hz), 3.22 (1H, brs), 3.75 (1H, brs), 4.03 (1H, d, J = 4.2 Hz), 4.12 (1H, m), 4.99 (1H, brs), 5.12 (1H, brs), 6.05 (1H, d, J = 11.4 Hz), 6.27 (1H, d, J = 11.4 Hz); ^{13}C NMR δ: −3.9, −3.1, −1.9, −1.9, 4.9, 5.0, 5.2, 6.9, 7.0, 7.0, 12.0, 18.2, 18.3, 19.1, 22.2, 23.6, 26.0, 26.2, 27.9, 29.0, 29.2, 29.6, 34.0, 36.9, 40.3, 40.7, 45.9, 56.5, 56.6, 69.5, 76.3, 76.4, 81.2, 118.1, 123.3, 134.7, 141.3, 144.9.

2.40. *1α,2β,3β-Tris(tert-butyldimethylsilyloxy)-25(trimethylsilyloxy)-9,10-secochoresta-5,7,10(19)-triene (55)*

Following the same experimental procedure as for **53**, **55** (3.5 mg, 17%) was obtained as a colorless oil from **33** (12.0 mg, 0.024 mmol) and **52** (12 mg, 0.024 mmol); IR (neat): 2980 (br), 1080 cm^{-1}; ^1H NMR δ: 0.10 (9H, s), 0.50–0.68 (30H, m), 0.91–1.00

(21H, m), 1.20 (6H, s), 2.57–2.66 (1H, m), 2.79–2.88 (1H, m), 3.70–3.78 (1H, m), 4.02 (1H, d, J = 4.4 Hz), 4.08–4.15 (1H, m), 4.99 (1H, s), 5.12 (1H, s), 6.03 (1H, d, J = 11.5 Hz), 6.25 (1H, d, J = 11.5 Hz); MS (EI (m/z)): 847 (M^+).

2.41. 1α,2β,3β,24(S),25-Pentahydroxy-9,10-secochoresta-5,7,10(19)-triene (8)

To a solution of **53** (12.5 mg, 0.020 mmol) in toluene (1 ml) and THF (1.1 ml), tetrabutylammonium fluoride (TBAF) (1.0 M in THF; 0.12 ml, 0.12 mmol) was added at room temperature and the mixture was stirred at room temperature for 17 h, at 50 °C for 7 h, and at 70 °C for 5 h. The mixture was evaporated. The residue was chromatographed on reverse-phase preparative TLC (CH_3CN/H_2O = 3/1) and the resultant was purified by HPLC (ODS-M80, 5.0 ml/s, UV detect; 265 nm, $MeCN/H_2O$ = 1/1) to give **8** (3.9 mg, 70%) as a colorless powder; $[\alpha]_D^{24}$ − 37.3 (c 0.3, CH_3OH); ^{1}H NMR δ: 0.46 (3H, s), 0.86 (3H, d, J = 6.4 Hz), 1.05 (3H, s), 1.10 (3H, s), 2.38 (1H, brs), 2.75 (1H, d, J = 10.0 Hz), 3.14 (1H, d, J = 10.0 Hz), 3.28 (1H, brs), 3.33 (1H, d, J = 2.8 Hz), 3.40 (2H, d, J = 9.2 Hz), 4.02 (1H, brs), 4.10 (1H, d, J = 8.8 Hz), 4.98 (1H, s), 5.38 (1H, s), 5.95 (1H, d, J = 11.2 Hz), 6.24 (1H, d, J = 11.2 Hz); UV λ_{max}: 265 nm, λ_{min}: 229 nm; MS (ESI): 466 ($[M + NH_4]^+$), 447 ($[M - H]^-$); HRMS (ESI) calcd for $C_{27}H_{48}NO_5$ $[(M + NH_4)^+]$: 466.3532, found: 466.3510.

2.42. 1α,2β,3β,24(R),25-Pentahydroxy-9,10-secochoresta-5,7,10(19)-triene (9)

Following the same experimental procedure as for **8**, **9** (5.2 mg, 50%) was obtained as a colorless powder from **54** (24.0 mg, 0.023 mmol); $[\alpha]_D^{24}$ − 13.8 (c 0.52, MeOH); IR (film) 3363, 1644, 1124 cm^{-1}; ^{1}H NMR δ: 0.38 (3H, s), 0.76 (3H, d, J = 6.4 Hz), 0.92 (3H, s), 0.95 (3H, s), 1.12 (6H, m), 1.29 (6H, m), 1.48 (2H, m), 1.70–1.87 (3H, m), 2.18 (1H, m), 2.24 (1H, m), 2.65 (1H, m), 3.00 (1H, d, J = 8.8 Hz), 3.29 (1H, dd, J = 8.4, 3.2 Hz), 3.83 (1H, m), 3.94 (1H, d, J = 8.0 Hz), 4.78 (1H, brs), 5.20 (1H, brs), 5.86 (1H, d, J = 11.2 Hz), 6.09 (1H, d, J = 11.2 Hz); UV λ_{max}: 265 nm, λ_{min}: 229; MS (ESI): 471 ($[M + Na]^+$), 447 ($[M - H]^-$); HRMS (EI) calcd for $C_{27}H_{44}O_5$ (M^+): 448.3189, found: 448.3199.

2.43. 1α,2β,3β,25-Tetrahydroxy-9,10-secochoresta-5,7,10(19)-triene (7)

Following the same experimental procedure as for **8**, **7** (0.9 mg, 50%) was obtained as a white powder from **55** (3.5 mg, 0.004 mmol).

3. Results and discussion

3.1. Synthesis of the oxidized metabolite, methyl ester derivative (6), and the truncated metabolite, tetraol derivative (7), at the 2β-position

First, we undertook the synthesis of methyl ester derivative (**6**) as an ester standard of the oxidized metabolite, carboxylic acid (**5**), which in turn was derived from the oxidation of the hydroxypropoxy substituent at the 2β-position. After several unsuccessful trials, methyl ester derivative (**18**) was prepared from known hydroxyethoxy derivative (**10**) [4]. The hydroxyl group in the hydroxyethoxy moiety of **10** was acetylated to give **11** in 65% yield with recovery of **10** in 34% yield. The rest of three hydroxyl groups of **11** were triethylsilylated with triethylsilyl trifluoromethanesulfonate (TESOTf) to afford **12** in 76% yield and the acetyl group in **12** was hydrolized to furnish alcohol (**13**) in 56% yield. The acid-delicate 5,7-diene moiety of **13** was protected as 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) adduct (**14**) in 82%. The alcohol (**14**) was converted to carboxylic acid methyl ester (**17**) as follows: (i) iodination of **14** with iodine and triphenyl phosphine (Ph_3P) produced **15** in 98% yield, (ii) cyanation of **15** with sodium cyanide (NaCN) afforded **16** in 87% yield, and (iii) methanolysis of **16** with methanolic hydrochloric acid (concd. HCl/MeOH) gave **17** in 25% yield. The ester (**17**) was submitted to retrocycloaddition in 1,3-dimethyl-2-imidazolidione (DMI) at 140 °C to generate 5,7-diene (**18**) in 59% yield. Finally, irradiation of **18** at 0 °C using high pressure mercury lamp (400 W, Vycor filter), followed by thermal isomerization in boiling ethanol gave rise to methyl ester derivative (**6**) in 25% yield (Fig. 3). The synthesis has been also disclosed in our patent [14].

Next, we conducted the synthesis of the truncated metabolite, tetraol (**7**). Although **7** and its derivatives have been synthesized in a highly effective parallel synthesis on solid phase [15], we adopted an alternative approach using PTAD adduct of α-epoxide (**19**), a key intermediate in a large synthesis of ED-71 [1]. After acetylation of the 3β-hydroxyl group of α-epoxide (**19**), the α-epoxy ring in **20** was cleaved in the presence of boron triflioride diethyl etherate ($BF_3{\cdot}OEt_2$) in THF at 60 °C to give 1,2-diol (**21**) which was converted to 5,7-diene (**22**) by thermal retrocycloaddition in a 44% overall yield from **20**. After deacetylation of **22**, 5,7-diene (**23**) was irradiated and thermally isomerized to the tetraol (**7**) in 5% yield. Tetraol (**7**) obtained in the abovementioned linear method (Fig. 4) was

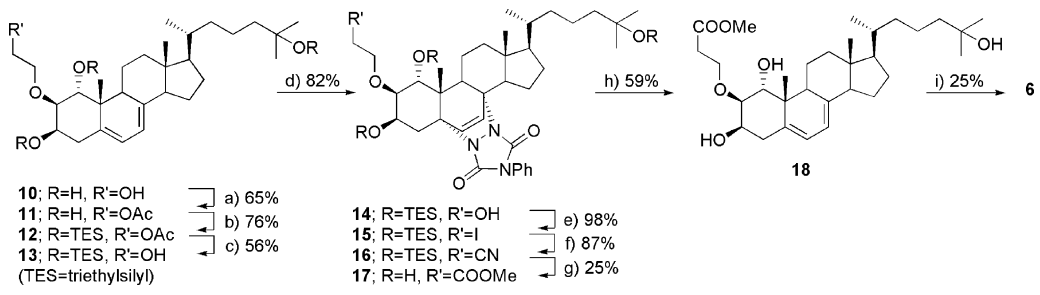

Fig. 3 – Synthesis of methyl ester derivative (6): (a) Ac_2O, pyridine, DMAP; (b) TESOTf, 2,6-lutidine; (c) KOH; (d) PTAD; (e) I_2, imidazole, Ph_3P; (f) NaCN; (g) concd. HCl/MeOH; (h) DMI, 140 °C; (i) (1) $h\nu$, (2) Δ.

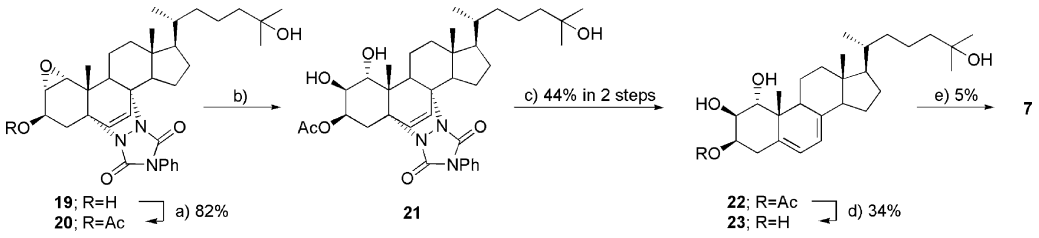

Fig. 4 – Synthesis of tetraol derivative (7): (a) Ac_2O, pyridine; (b) $BF_3{\cdot}OEt_2$; (c) DMI, 140 °C; (d) $LiAlH_4$; (e) (1) $h\nu$, (2) Δ.

completely identical with material prepared by the convergent method described below (Fig. 7).

3.2. *Synthesis of pentaols (8 and 9) arising from the combination metabolism of the side chain and the 2β-position*

To synthesize pentaols (**8** and **9**) in 24(*S*) and 24(*R*) forms arising from the combination metabolism between the side chain and the 2β-position, we adopted a convergent approach. In the convergent synthesis, the A-ring fragment (**33**) is coupled with the C/D-ring fragments (**45** and **50**) for the construction of the triene system of the vitamin D_3 structures. First, we undertook the synthesis of the A-ring fragment (**33**) (Fig. 5). Diol (**24**), obtained from D-mannitol [16], was protected as the pivalate ester (**25**) in 72% yield. This ester was subjected to hydrolysis with 1N hydrochloric acid in THF at 20 °C for 12 hours to give diol (**26**) in 50% yield accompanied by tetraol (**27**) in 34% and recovery of **25** in 16%, which were easily separated by silica gel column chromatography. Diol (**26**) was then converted to orthoester (**28**) in 88% yield with triethyl orthoformate ($HC(OEt)_3$) and *p*-toluenesulfonic acid monohydrate ($TsOH{\cdot}H_2O$) in CH_2Cl_2. Upon treatment of orthester (**28**) with boiling acetic anhydride for 14 h, terminal olefin formation took place cleanly to produce olefin (**29**) in 93% yield [17–21]. Deketalization of **29** afforded diol (**30**) in 93% yield. Subsequent Mitsunobu reaction of **30** with diisopropyl azodicarboxylate (DIAD) and Ph_3P in dioxane gave epoxide (**31**) in 74% yield. Epoxide (**31**) was treated with lithio trimethylsilysacetylene in the presence of $BF_3{\cdot}OEt_2$ at −78 °C to provide 1,7-eneyne (**32**) in 85% yield. Finally, A-ring fragment (**33**) was obtained from **32** by hydrolysis and subsequent triethylsilyl ether formation in 93% yield.

Next, we performed the synthesis of the C/D-ring fragment (**45**) from the Inhoffen-Lythgoe diol (**34**) [22,23]. Selective tosylation of the primary alcohol gave tosylate (**35**) in 91% yield. The tosylate (**35**) was converted to sulfone (**36**) in 78 % yield in known procedure [24]. The secondary alchol of **36** was protected by triethylsilyl group to afford **37**, quantitatively. Alkylation of **37** with 4-bromo-2-methyl-2-butene in the presence of *n*-BuLi and subsequent desilylation gave **38** in 96% yield. Reductive desulfonylation with sodium mercury amalgam (Na/Hg) produced an inseparable ene/diene mixture (4/1) (**39**) in 72% yield [25]. After protection of the hydroxy group of **39** by acetyl group, the ene/diene mixture (4/1) (**40**) was treated with the commercially available reagent for sharpless asymmetric dihydroxylation, AD-mix-α followed by catalytic hydrogenation to afford diol (**41**) as a sole product in 96% yield [26]. Since the asymmetric dihydroxylation occurred predominantly at the trisubstituted olefin, subsequent catalytic hydrogenation gave the requisite diol as a sole product. Protection of two hydroxy groups in **41** by *tert*-butyldimethylsilyl (TBS) group gave **42** in 95% yield and deprotection of acetyl group in **42** with lithium aluminum hydride ($LiAlH_4$) furnished **43** quantitatively. The alcohol (**43**) was oxidized with tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine-*N*-oxide (NMO) to give ketone (**44**) in 95% yield. Wittig reaction of **44** with (bromomethyl)triphenylphosphonium bromide ($Ph_3PCH_2Br_2$) in the presence of sodium hexamethyldisilazide (NaHMDS) produced **45** in 55% yield. The similar reaction of **40** with AD-mix-β gave rise to the C/D-ring fragment (**50**) in the 24(*R*)-series (Fig. 6). This synthetic route represents

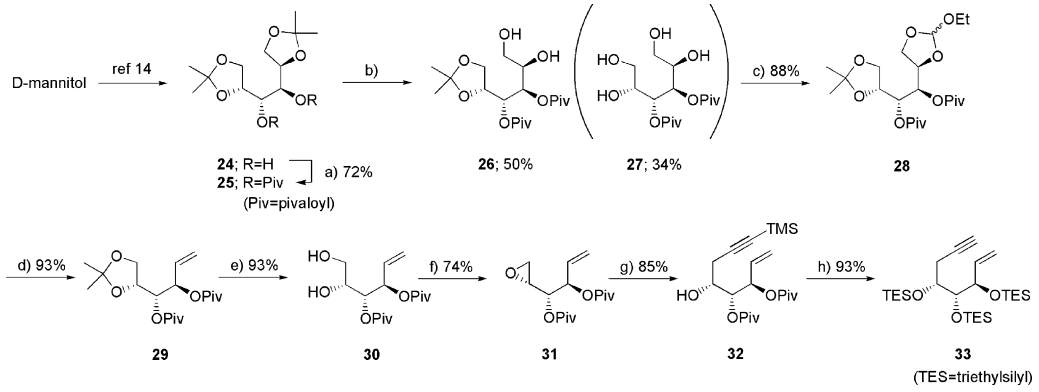

Fig. 5 – Synthesis of the A-ring fragment: (a) t-BuCOCl, pyridine, DMAP; (b) 1N HCl; (c) $HC(OEt)_3$, $TsOH{\cdot}H_2O$; (d) Ac_2O; (e) 1N HCl; (f) Ph_3P, DIAD; (g) LiC≡CTMS, $BF_3{\cdot}OEt_2$; (h) (1) 1N NaOH, (2) TESOTf, Et_3N.

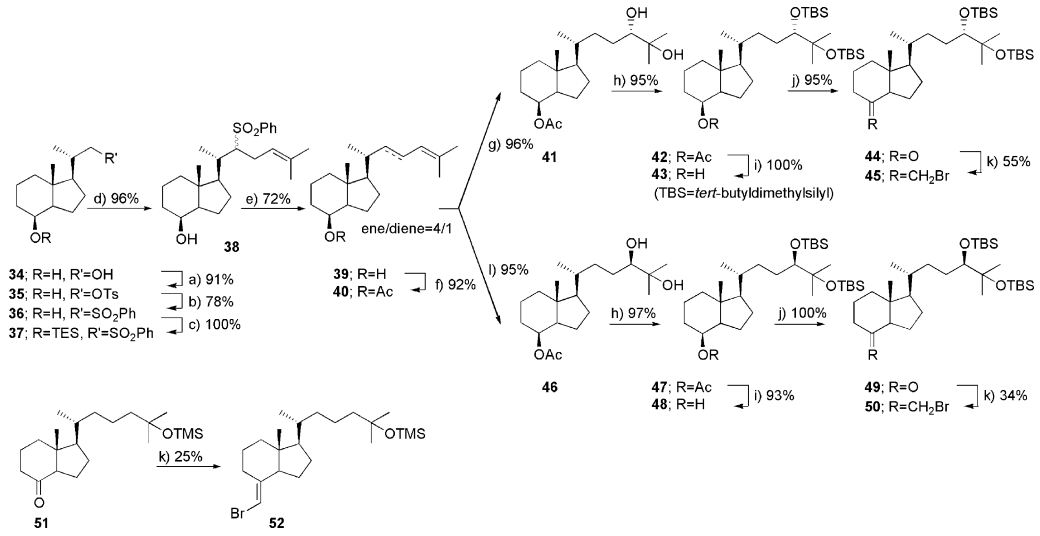

Fig. 6 – Synthesis of the C/D-ring fragments: (a) TsCl, pyridine, DMAP; (b) (1) NaI, (2) $NaSO_2Ph{\cdot}2H_2O$; (c) TESCl, imidazole; (d) (1) *n*-BuLi, $(CH_3)_2C{=}CHCH_2Br$, (2) 3N HCl; (e) Na/Hg; (f) Ac_2O, pyridine, DMAP; (g) (1) AD-mix-α, $Me_3SO_2NH_2$, (2) Pd-C, H_2; (h) TBSOTf, 2,6-lutidine; (i) $LiAlH_4$; (j) TPAP, NMO; (k) $Ph_3PCH_2Br_2$, NaHMDS; (l) (1) AD-mix-β, $Me_3SO_2NH_2$, (2) Pd-C, H_2.

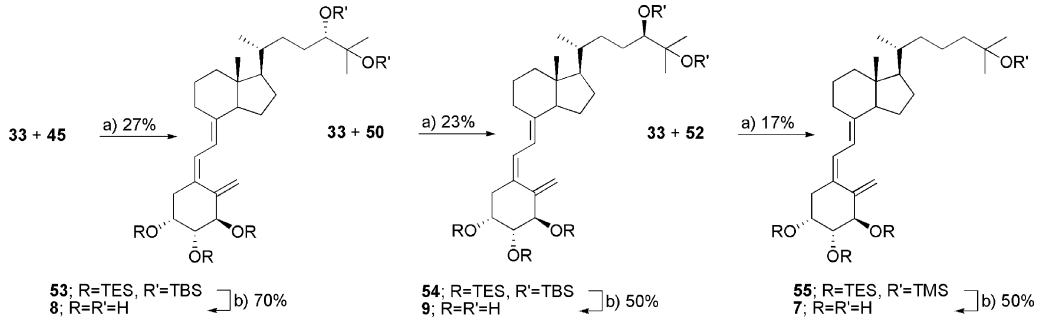

Fig. 7 – Coupling the A-ring fragment with C/D-ring fragments: (a) $Pd(PPh_3)_4$, Et_3N; (b) TBAF.

a novel versatile approach for preparation of 24-hydroxylated C/D-ring fragments in 24(*S*) and 24(*R*) series in good overall yields for the synthesis of vitamin D_3 analogs.

Having the A-ring fragment (**33**) and the C/D-ring fragments (**45** and **50**), we conducted the fragment coupling reaction in the presence of tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$) following Trost's methodology [27]. This gave rise to (**53**) and (**54**) in 27% and 23% yields, respectively, which were then deprotected by tetrabutylammonium fluoride (TBAF) to afford 24(*S*)OH-pentaol (**8**) and 24(*R*)OH-pentaol (**9**) in 70% and 50% yields, respectively.

Finally, tetraol (**7**) was also prepared by desilylation of **55**, which was also obtained in a convergent manner by coupling A-ring fragment (**33**) and C/D-ring fragment (**52**). This reaction sequence delivered **7** that was identical to authentic **7** produced in the aforementioned linear synthesis (Fig. 7).

4. Conclusion

Four putative metabolites of ED-71 (**1**) have been synthesized, namely methyl ester derivative (**6**), tetraol derivative (**7**), 24(*S*)-pentaol (**8**), and 24(*R*)-pentaol (**9**). These metabolites are now used as authentic samples for metabolic studies of ED-71 (**1**). The detailed metabolic pathway and metabolites of ED-71 (**1**) will be reported elsewhere.

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