(hexane/AcOEt (15:1)) gave the alcohol **21** (92 mg, 81%) as a colorless oil.

$[\alpha]_D^{21}$ −0.9° (*c* 0.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.07 (3H, s), 0.09 (3H, s), 0.90 (9H, s), 1.04 (9H, s), 1.31–1.43 (2H, m), 1.53–1.62 (2H, m), 1.72 (1H, t, J = 6.3 Hz), 1.90 (1H, dt, J = 7.4, 13.8 Hz), 2.31 (1H, dt, J = 7.4, 13.8 Hz), 3.54 (2H, t, J = 5.6 Hz), 3.63 (2H, t, J = 6.3 Hz), 3.74–3.77 (1H, m), 4.98 (1H, d, J = 10.0 Hz), 5.00 (1H, d, J = 17.1 Hz), 5.73 (1H, ddt, J = 7.1, 10.0, 17.1 Hz), 7.35–7.44 (6H, m), 7.65–7.67 (4H, m). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −4.4, −4.4, 18.2, 19.3, 25.8, 25.9, 26.9, 30.7, 36.2, 34.3, 41.6, 63.7, 64.1, 115.9, 127.5, 129.5, 133.9, 135.5, 137.6. IR (neat, cm^{-1}) 3443, 3073, 2932, 2894, 2857, 1640, 1472, 1428, 1113, 1007, 704. LRMS (EI(+)) *m/z* 508 ($[M-H_2O]^+$), 495 ($[M-OMe]^+$), 467, 199. HRMS (EI(+)) calcd for $C_{31}H_{48}O_2Si_2$ ($[M-H_2O]^+$) 508.3187, found 508.3190.

3.5.11. 4-{(1*S*)-1-(*tert*-Butyldimethylsilyloxy)-2-(*p*-toluenesulfonyloxy)}ethyl-7-(*tert*-butyldiphenylsilyloxy) hept-1-ene. Under an Ar atmosphere, to a solution of **21** (95 mg, 0.18 mmol) in CH_2Cl_2 (2 mL) were added TsCl (38 mg, 0.20 mmol), Et_3N (62 μL, 0.45 mmol), and DMAP (44 mg, 0.36 mmol), and stirred at room temperature for 4 h. The reaction mixture was diluted with AcOEt (30 mL) and washed with water (3 mL) and brine (3 mL), dried ($MgSO_4$), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (15:1)) gave the tosylate (112 mg, 91%) as a colorless oil.

$[\alpha]_D^{21}$ +7.6° (*c* 0.3, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.01 (6H, s), 0.84 (9H, s), 1.03 (9H, s), 1.22–1.54 (5H, m), 1.90 (1H, dt, J = 7.0, 14.1 Hz), 2.16 (1H, dt, J = 7.0, 14.1 Hz), 2.42 (3H, s), 3.56 (1H, dd, J = 5.2, 10.0 Hz), 3.61 (1H, dd, J = 6.2, 10.0 Hz), 3.88 (2H, t, J = 6.3 Hz), 3.93–3.96 (1H, m), 4.95–4.99 (2H, m), 5.61–5.71 (1H, m), 7.31 (2H, d, J = 8.2 Hz), 7.36–7.45 (6H, m), 7.64–7.66 (4H, m), 7.77 (2H, d, J = 8.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −4.8, −4.2, 18.1, 19.2, 21.7, 25.2, 25.8, 26.9, 30.6, 34.3, 41.7, 63.9, 71.4, 71.7, 116.2, 127.5, 127.9, 129.5, 129.7, 133.9, 135.5, 137.1, 144.6. IR (neat, cm^{-1}): 2995, 2924, 2911, 2861, 1599, 1364, 1179, 1111, 704. LRMS (EI(+)) *m/z* 623 ($[M-t\text{-Bu}]^+$), 451, 427, 229. HRMS (EI(+)) calcd for $C_{34}H_{47}O_5Si_2S$ ($[M-t\text{-Bu}]^+$) 623.2682, found 623.2687.

3.5.12. (4*R*)-4-[(*S*)-Oxiranyl]hept-6-en-1-ol. Under an Ar atmosphere, to a solution of the tosylate prepared as above (92 mg, 0.14 mmol) in THF (1.4 mL) was added TBAF (1 M in THF, 1 mL, 1 mmol) and stirred at room temperature for 1.5 h. The mixture was diluted with AcOEt (20 mL) and washed with saturated aqueous NH_4Cl solution (2 mL), brine (2 mL), dried ($MgSO_4$), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (5:1 to 1:1)) gave the epoxide (16 mg, 75%) as a colorless oil.

$[\alpha]_D^{21}$ +6.4° (*c* 1.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 1.21–1.30 (1H, m), 1.53–1.59 (1H, m), 1.65–1.74 (2H, m), 2.09–2.17 (2H, m), 2.49 (1H, dd, J = 3.2, 4.6 Hz), 2.72–3.63 (2H, m), 3.63 (1H, dd, J = 2.8, 6.4 Hz), 3.67 (1H, dd, J = 2.8, 6.4 Hz), 5.02 (1H, br d, J = 10.5 Hz), 5.06 (1H, br d, J = 17.8 Hz), 5.78 (1H, ddt, J = 7.2, 10.4, 17.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 29.1, 30.0, 36.2, 41.3, 46.6, 56.0, 63.2, 116.5, 135.9. IR (neat, cm^{-1}) 1601, 1584, 1453. LRMS (EI(+)) *m/z* 156 (M^+), 125 ($[M-CH_2OH]^+$), 107. HRMS (EI(+)) calcd for $C_9H_{16}O_2$ (M^+), 156.1150, found 156.1152.

3.5.13. (4*R*,5*S*)-4-{3-(*tert*-Butyldimethylsilyloxy)propyl}-5,6-epoxyhex-1-ene (22). Under an Ar atmosphere, to a solution of the epoxy alcohol prepared as above (47.6 mg, 0.31 mmol) in CH_2Cl_2 (3 mL) were added Et_3N (86 mL, 0.62 mmol), TBSCl (93 mg, 0.62 mmol), and DMAP (38 mg, 0.31 mmol), and stirred at room temperature for 1 h. DMAP (38 mg, 0.31 mmol) was added and stirred at room temperature for 1 h. The mixture was diluted with AcOEt (50 mL), washed with water (5 mL), brine (5 mL), dried ($MgSO_4$), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (15:1)) gave the product **22** (71.6 mg, 85%) as a colorless oil.

$[\alpha]_D^{21}$ −0.6° (*c* 1.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.05 (6H, s), 0.89 (9H, s), 1.20–1.25 (1H, m), 1.47–1.53 (2H, m), 1.58–1.67(2H, m), 2.10 (1H, dd, J = 7.1, 14.0 Hz), 2.17 (1H, dd, J = 7.1, 14.0 Hz), 2.49 (1H, dd, J = 3.4, 4.4 Hz), 2.71–2.75 (2H, m), 3.61 (2H, t, J = 6.3 Hz), 5.01 (1H, br d, J = 11.2 Hz), 5.05 (1H, br d, J = 19.0 Hz), 5.78 (1H, ddt, J = 7.1, 10.3, 17.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −5.2, 0.1, 18.4, 26.0, 28.7, 32.1, 36.0, 46.5, 55.9, 63.2, 116.3, 136.1. IR (neat, cm^{-1}) 2953, 2930, 2901, 2859, 1640, 1255, 1101. LRMS (EI(+)) *m/z* 213 ($[M-t\text{-Bu}]^+$), 183, 101. HRMS (EI(+)) calcd for $C_{11}H_{21}O_2Si$ ($[M-t\text{-Bu}]^+$) 213.1311, found 213.1287.

3.5.14. (4*R*,5*R*)-5-{3-(*tert*-Butyldimethylsilyloxy)propyl}-1-(trimethylsilyl)oct-7-en-1-yn-4-ol. Under an Ar atmosphere, to a cooled (−78 °C) solution of TMS acetylene (49 μL, 0.35 mmol) in THF (2 mL) was added *n*-BuLi (1.5 M in hexane, 189 μL, 0.3 mmol) and stirred at the same temperature for 10 min. To the resulting lithium acetylide solution was added a solution of **22** (27.2 mg, 0.10 mmol) in THF (2 mL) via cannula, and then $BF_3{\cdot}OEt_2$ (14 mL, 0.11 mmol) was added. The mixture was stirred at the same temperature for 25 min. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution (10 mL), and the mixture was extracted with AcOEt (100 mL). The organic layer was washed with brine (10 mL), dried ($MgSO_4$), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (20:1)) gave enyne (26.3 mg, 71%) as a colorless oil.

$[\alpha]_D^{23}$ +0.9° (*c* 2.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.04 (6H, s), 0.15 (9H, s), 0.89 (9H, s), 1.31–1.40 (1H, m), 1.42–1.67 (4H, m), 2.04 (1H, dt, J = 7.1, 14.0 Hz), 2.22 (1H, dt, J = 7.1, 14.0 Hz), 2.40 (1H, dd, J = 7.3, 16.8 Hz), 2.45 (1H, dd, J = 5.4, 16.8 Hz), 3.59 (2H, t, J = 6.4 Hz), 3.72–3.77 (1H, m), 5.02 (1H, br d, J = 10.1 Hz), 5.06 (1H, br d, J = 17.4 Hz), 5.78 (1H,

ddt, $J = 7.1$, 10.1, 17.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −5.3, 0.0, 18.2, 24.5, 25.9, 26.0, 30.3, 34.5, 41.9, 63.2, 71.3, 87.3, 103.4, 116.2, 136.7. IR (neat, cm^{-1}) 2957, 2932, 2903, 2859, 2176, 1252, 1009, 845. LRMS (EI(+)) *m/z* 368 (M^+), 311 ([M−*t*-Bu]$^+$), 293 ([M−*t*-Bu−H_2O]$^+$), 219. HRMS (EI(+)) calcd for $C_{20}H_{40}O_2Si_2$ (M^+) 368.2567, found 368.2559.

3.5.15. (4*R*,5*R*)-5-{3-(*tert*-Butyldimethylsilyloxy)propyl}-oct-7-en-1-yn-4-ol. The enyne alcohol prepared as above (26.3 mg, 0.071 mmol) was dissolved in MeOH (500 mL) and to the solution was added K_2CO_3 (14.7 mg, 0.107 mmol). After stirred at room temperature for 3.5 h, the reaction mixture was diluted with saturated aqueous NH_4Cl solution (3 mL), and the mixture was extracted with AcOEt (30 mL). The organic layer was washed with brine (3 mL), dried ($MgSO_4$), and concentrated. Purification by silica gel column chromatography (PhMe/AcOEt (15:1)) gave the product (17.5 mg, 83%) as a colorless oil.

$[\alpha]_D^{22}$ −5.0° (*c* 1.4, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.05 (6H, s), 0.89 (9H, s), 1.36–1.42 (1H, m), 1.44–1.54 (2H, m), 1.59–1.68 (2H, m), 2.02–2.10 (3H, m), 2.23 (1H, dt, $J = 6.9$, 14.0 Hz), 2.40 (1H, t, $J = 2.7$ Hz), 2.41 (1H, dd, $J = 1.1$, 2.7 Hz), 3.60 (2H, t, $J = 6.3$ Hz), 3.75–3.80 (1H, m), 5.04 (1H, br d, $J = 10.0$ Hz), 5.07 (1H, br d, $J = 17.1$ Hz), 5.79 (1H, ddt, $J = 7.1$, 10.0, 17.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −5.2, 18.4, 24.6, 24.7, 26.0, 26.0, 30.3, 34.7, 42.0, 63.3, 70.6, 71.6, 81.3, 116.3, 136.7. IR (neat, cm^{-1}) 3422, 3306, 2934, 2859, 2116, 1638, 1256, 1098, 837, 700. LRMS (EI(+)) *m/z* 239 ([M−*t*-Bu]$^+$), 221([M−*t*-Bu−H_2O]$^+$), 147, 105. HRMS (EI(+)) calcd for $C_{13}H_{23}O_2Si$ ([M−*t*-Bu]$^+$) 239.1467, found 239.1465.

3.5.16. (4*R*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-4-{3-(*tert*-butyldimethylsilyloxy)propyl}oct-1-en-7-yne (23). Under an Ar atmosphere, to a cold (0 °C) solution of the alcohol prepared as above (17.5 mg, 0.059 mmol) in CH_2Cl_2 (600 mL) were added 2,6-lutidine (20 μL, 0.177 mmol) and TBSOTf (20 μL, 0.089 mmol), and stirred at the same temperature for 1 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution (3 mL), and the mixture was extracted with AcOEt (30 mL). The organic layer was washed with brine (3 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (100:1)) gave the product **23** (24 mg, quant.) as a colorless oil.

$[\alpha]_D^{21}$ −6.5° (*c* 1.9, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.05 (6H, s), 0.06 (3H, s), 0.08 (3H, s), 0.89 (9H, s), 0.89 (9H, s), 1.24–1.73 (6H, m), 1.99 (1H, dt, $J = 7.0$, 14.0 Hz), 2.21 (1H, dt, $J = 7.0$, 14.0 Hz), 2.29 (1H, ddd, $J = 2.7$, 6.4, 16.8 Hz), 2.36 (1H, ddd, $J = 2.7$, 6.4, 16.8 Hz), 3.59 (2H, t, $J = 6.3$ Hz), 3.87 (1H, ddd, $J = 3.2$, 6.4, 6.4 Hz), 5.03 (2H, dd, $J = 10.1$, 17.1 Hz), 5.77 (1H, ddt, $J = 7.0$, 10.1, 17.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −5.2, −4.6, −4.1, 18.1, 18.4, 24.4, 24.9, 25.9, 26.0, 31.0, 34.7, 42.5, 63.5, 69.8, 72.3, 82.1, 115.7, 137.7. IR (neat, cm^{-1}) 2957, 2930, 2890, 2857, 2161, 1507, 1254, 1099, 837, 708, 671. LRMS (EI(+)) *m/z* 410 (M^+), 353 ([M−*t*-Bu]$^+$), 221, 147. HRMS (EI(+)) calcd for $C_{23}H_{46}O_2Si_2$ (M^+) 410.3019, found 410.3028.

3.5.17. 2α-(3-Hydroxypropyl)-25-hydroxyvitamin D_3 (3). Under an Ar atmosphere, a mixture of A-ring enyne **23** (4.4 mg, 10.7 μmol), CD-ring bromoolefin **6**[12] (20.0 mg, 56.0 μmol), $Pd(PPh_3)_4$ (6.3 mg, 5.5 μmol), PhMe (500 μL), and Et_3N (1.0 mL) was stirred at 110 °C for 2 h. After cooled to room temperature, the mixture was diluted with Et_2O and filtered through Celite. The filtrate was diluted with AcOEt (20 mL), and washed with water (2× 1 mL), brine (1 mL), dried ($MgSO_4$), and concentrated. The residue was partially purified through silica gel pad (eluent: hexane/AcOEt (20:1)) to remove polar materials and dissolved in THF (50 μL). The TBAF solution (1 M in THF, 110 μL, 0.11 mmol) was added, and stirred at room temperature for 2 h. The mixture was partitioned between AcOEt (20 mL) and water (1 mL), and the organic layer was washed with water (1 mL) and brine (1 mL), dried ($MgSO_4$), and concentrated. Purification by preparative TLC (AcOEt) gave the product (1.7 mg, 35% for 2 steps) as a while amorphous.

$[\alpha]_D^{18}$ +27.0° (*c* 0.04, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$, ppm) δ 0.54 (3H, s), 0.93 (3H, d, $J = 6.6$ Hz), 1.03–1.08 (1H, m), 1.19–1.20 (1H, m), 1.21 (6H, s), 1.23–1.33 (5H, m), 1.36–1.49 (9H, m), 1.51–1.73 (8H, m), 1.84–1.93 (2H, m), 1.96–2.02 (2H, m), 2.25 (1H, dd, $J = 8.8$, 13.1 Hz), 2.47 (1H, dd, $J = 4.5$, 13.7 Hz), 2.61 (1H, dd, $J = 4.0$, 13.1 Hz), 2.82 (1H, dd, $J = 3.5$, 12.1 Hz), 3.55–3.59 (1H, m), 3.67 (2H, br s), 4.83 (1H, s), 5.04 (1H, s), 6.02 (1H, d, $J = 11.3$ Hz), 6.22 (1H, d, $J = 11.3$ Hz). ^{13}C NMR (150 MHz, $CDCl_3$, ppm) δ 12.0, 18.8, 20.8, 22.2, 23.5, 27.7, 27.8, 29.0, 29.2, 29.4, 29.8, 36.1, 36.4, 37.9, 40.5, 44.4, 44.4, 44.6, 45.9, 56.4, 56.3, 56.5, 63.1, 71.2, 73.5, 113.0, 117.4, 121.9, 135.2, 142.4, 144.1. IR (film, cm^{-1}) 3374, 2951, 2928, 2897, 2851, 1674, 1615, 1555, 1458, 1053. LRMS (EI(+)) *m/z* 458 (M^+), 440 ([M−H_2O]$^+$), 341, 311. HRMS (EI(+)) calcd for $C_{30}H_{50}O_3$ (M^+) 458.3760, found 458.3758.

3.6. Synthesis of 1α- and 1β-hydroxymethyl-2-unsubstituted analogues (4a, 4b)

3.6.1. (2*R*,3*S*,5*S*,6*S*)-2-Benzyloxymethyl-5-(*tert*-butyldimethylsilyloxy)-6-methoxytetrahydropyran-3-ol (25). Under an Ar atmosphere, to a suspension of **24**[17] (93.1 mg, 0.245 mmol), MS3A (241.1 mg), and Et_3SiH (195 μL, 1.22 mmol) in CH_2Cl_2 (2.5 mL) was added TFA (95 μL, 1.23 mmol) and stirred at 0 °C, and gradually raised up to room temperature for 6 h. The reaction was quenched by the addition of saturated aqueous Na_2CO_3 solution (5 mL), and the mixture was filtered through Celite pad and the solid was washed with CH_2Cl_2 and water. Layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (5 mL). The combined organic layers were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (5:1)) gave the products **25** (69.6 mg, 76%,) as a colorless oil.

$[\alpha]_D^{18}$ +35.6° (*c* 0.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.05 (3H, s), 0.07 (3H, s), 0.89 (9H, s), 1.78 (1H, ddd, J = 2.5, 11.2, 12.8 Hz), 1.95 (1H, ddd, J = 3.4, 4.6, 12.8 Hz), 2.63 (1H, br s), 3.37 (3H, s), 3.62–3.74 (2H, m), 3.78 (1H, dd, J = 4.8, 9.2 Hz), 3.83 (1H, m), 3.97 (1H, ddd, J = 4.6, 9.0, 11.2 Hz), 4.39 (1H, s), 4.57 (1H, d, J = 11.8 Hz), 4.64 (1H, d, J = 11.8 Hz), 7.26–7.38 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −4.8, −4.8, 18.1, 25.8, 35.0, 54.7, 65.5, 68.4, 70.8, 72.2, 73.7, 100.3, 127.6, 127.7, 128.4, 137.7. IR (neat, cm^{-1}) 3445, 2930, 1464, 1256, 1132, 837, 735. LRMS (EI(+)) *m/z* 382 (M^+), 363 ([M−H_2O−H]$^+$), 351 ([M−OMe]$^+$), 333 ([M−H_2O−OMe]$^+$), 325 ([M−*t*-Bu]$^+$), 307 ([M−H_2O−*t*-Bu]$^+$), 293 ([M−*t*-Bu−MeOH]$^+$), 275, 257, 225, 203, 185, 159, 101, 91 (C_7H_7, bp). HRMS (EI(+)) calcd for $C_{20}H_{34}O_5Si$ (M^+) 382.2176, found 382.2175.

3.6.2. (2*R*,5*S*,6*S*)-2-Benzyloxymethyl-5-(*tert*-butyldimethylsilyloxy)-6-methoxytetrahydropyran-3-one. A mixture of alcohol **25** (3.02 g, 7.89 mmol), MS4A (6.49 g), NMO (1.36 g, 11.6 mmol), TPAP (136.4 mg, 0.388 mmol) in CH_2Cl_2 (75 mL) was stirred at room temperature for 0.5 h. The mixture was filtered through Celite, washed with CH_2Cl_2, and the solvent was removed under reduced pressure. Purification by silica gel column chromatography (hexane/AcOEt (15:1)) gave the ketone (2.64 g, 88%) as a colorless oil.

$[\alpha]_D^{19}$ +98.3° (*c* 1.1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.05 (3H, s), 0.07 (3H, s), 0.87 (9H, s), 2.56 (1H, dd, J = 6.1, 15.1 Hz), 2.74 (1H, dd, J = 4.4, 15.1 Hz), 3.49 (3H, s), 3.77 (1H, dd, J = 5.9, 10.8 Hz), 3.99 (1H, dd, J = 2.9, 10.8 Hz), 4.06 (1H, ddd, J = 3.0, 4.4, 6.1 Hz), 4.18 (1H, dd, J = 2.9, 5.9 Hz), 4.57 (1H, d, J = 12.2 Hz), 4.62 (1H, d, J = 12.2 Hz), 4.72 (1H, d, J = 3.0 Hz), 7.24–7.36 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −4.8, −4.8, 18.0, 25.7, 44.4, 55.6, 68.8, 70.5, 73.5, 75.0, 101.9, 127.5, 127.5, 128.2, 138.0, 206.5. IR (neat, cm^{-1}) 2930, 1732, 1254, 1109, 837. LRMS (EI(+)) *m/z* 380 (M^+), 349 ([M−OMe]$^+$), 323 ([M−*t*-Bu]$^+$), 291 ([M−*t*-Bu−MeOH]$^+$), 215, 201, 159, 145, 115, 101, 91 (C_7H_7), 89 (bp). HRMS (EI(+)) calcd for $C_{20}H_{32}O_5Si$ (M^+) 380.2019, found 380.2008.

3.6.3. (2*S*,3*S*,6*S*)-6-Benzyloxymethyl-3-(*tert*-butyldimethylsilyloxy)-2-methoxy-5-methylenetetrahydropyran (26). Under an Ar atmosphere, to a cold (−40 °C) mixture of activated Zn dust (3.75 g, 57.4 mmol), CH_2Br_2 (1.2 mL, 17.1 mmol) in THF (40 mL) was added $TiCl_4$ (1.3 mL, 11.9 mmol), and the mixture was stirred at 5 °C (cold room) for 4 d. The mixture was diluted with CH_2Cl_2 (20 mL), and a solution of ketone prepared as above (2.64 g, 6.94 mmol) in CH_2Cl_2 (25 mL) was added. The mixture was stirred at room temperature for 1 h. The reaction mixture was poured into a mixture of Et_2O (100 mL)–saturated aqueous $NaHCO_3$ solution (100 mL) and stirred vigorously for several minutes. Resulting mixture was filtered through Celite, washed with Et_2O and water, and the layers of the filtrate were separated. The organic layer was washed with water (50 mL), brine (50 mL), dried (Na_2SO_4), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (15:1)) gave the *exo*-methylene compound **26** (2.16 g, 82%) as a colorless oil.

$[\alpha]_D^{16}$ +73.1° (*c* 1.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.05 (3H, s), 0.06 (3H, s), 0.88 (9H, s), 2.25 (1H, dd, J = 6.2, 13.5 Hz), 2.54 (1H, dd, J = 4.4, 13.5 Hz), 3.43 (3H, s), 3.70 (1H, dd, J = 6.6, 14.2 Hz), 3.70–3.74 (1H, m), 3.75 (1H, dd, J = 4.6, 14.2 Hz), 4.37 (1H, apparent t, J = 5.4 Hz), 4.53 (1H, d, J = 2.4 Hz), 4.58 (1H, d, J = 12.2 Hz), 4.64 (1H, d, J = 12.2 Hz), 4.83 (1H, s), 4.86 (1H, t, J = 2.0 Hz), 7.24–7.38 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −4.7, 18.2, 25.8, 37.5, 55.2, 70.0, 70.7, 71.1, 73.3, 102.6, 109.9, 127.4, 127.5, 128.2, 138.2, 141.3. IR (neat, cm^{-1}) 2930, 1655, 1472, 1256, 1183, 1100, 837. LRMS (EI(+)) *m/z* 378 (M^+), 347 ([M−OMe]$^+$), 321 ([M−*t*-Bu]$^+$), 289 ([M−*t*-Bu−MeOH]$^+$), 257 ([M−$BnOCH_2$]$^+$), 210, 199, 153, 91 (C_7H_7, bp). HRMS (EI(+)) calcd for $C_{21}H_{34}O_4Si$ (M^+) 378.2226, found 378.2221.

3.6.4. Hydroboration of the *exo*-methylene compound (26). Under an Ar atmosphere, to a cold (0 °C) solution of *exo*-methylene compound **26** (2.16 g, 5.71 mmol) in THF (20 mL) was added BH_3·THF (1 M in THF, 11 mL, 11 mmol), and the mixture was stirred at the same temperature for 1.5 h. 1 N NaOH solution (10 mL) and 30% H_2O_2 solution (10 mL) were added, and the solution was stirred the same temperature for 1.5 h. The reaction was quenched by the addition of 10% aqueous $Na_2S_2O_3$ solution (50 mL), and the mixture was extracted with AcOEt (3× 250 mL). The combined organic layers were washed with 10% aqueous $Na_2S_2O_3$ solution (50 mL), brine (50 mL), dried (Na_2SO_4), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (8:1)) gave **27a** (less polar isomer, 1.64 g, 72%) and **27b** (more polar isomer, 185.7 mg, 8%) as colorless oils, respectively.

3.6.5. (2*S*,3*S*,5*S*,6*S*)-[2-Benzyloxymethyl-5-(*tert*-butyldimethylsilyloxy)-6-methoxytetrahydropyran-3-yl]methanol (27a). $[\alpha]_D^{20}$ +24.4° (*c* 1.3, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.10 (6H, s), 0.91 (9H, s), 1.70 (1H, m), 1.83 (1H, m), 2.14 (1H, ddd, J = 3.3, 5.9, 14.5 Hz), 3.38 (3H, s), 3.66 (1H, dt, J = 1.3, 3.3 Hz), 3.75 (1H, dd, J = 6.1, 10.1 Hz), 3.74–3.83 (2H, m), 4.15 (1H, dt, J = 3.2, 6.1 Hz), 4.48 (1H, s), 4.56 (1H, d, J = 11.8 Hz), 4.63 (1H, d, J = 11.8 Hz), 7.25–7.38 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −5.0, −4.9, 18.1, 25.8, 30.9, 35.2, 54.6, 62.9, 66.5, 68.3, 71.0, 73.5, 101.2, 127.6, 128.3, 138.0. IR (KBr, cm^{-1}) 3472, 2928, 1468, 1258, 1123, 1030, 862, 700. LRMS (EI(+)) *m/z* 396 (M^+), 379 ([M−OH]$^+$), 365 ([M−MeO]$^+$), 321 ([M−*t*-Bu−H_2O]$^+$), 307 ([M−*t*-Bu−MeOH]$^+$), 289 ([M−BnO]$^+$), 231, 101, 91 (C_7H_7, bp). HRMS (EI(+)) calcd for $C_{21}H_{36}O_5Si$ (M^+) 396.2332, found 396.2347.

3.6.6. (2*S*,3*R*,5*S*,6*S*)-[2-Benzyloxymethyl-5-(*tert*-butyldimethylsilyloxy)-6-methoxytetrahydropyran-3-yl]methanol (27b). $[\alpha]_D^{21}$ +34.3° (*c* 0.7, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.05 (6H, s), 0.89 (9H, s), 1.52 (1H, m), 1.68 (1H, ddd, J = 2.7, 13.1, 13.1 Hz), 2.16 (1H,

m), 2.75 (1H, br s), 3.36 (3H, s), 3.46 (1H, dd, $J = 6.6$, 11.7 Hz), 3.49 (1H, dd, $J = 4.0$, 11.7 Hz), 3.62–3.80 (4H, m), 4.43 (1H, s), 4.57 (1H, d, $J = 11.6$ Hz), 4.65 (1H, d, $J = 11.8$ Hz), 7.26–7.38 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −4.8, −4.7, 18.1, 25.9, 30.1, 35.9, 54.6, 65.1, 66.8, 71.1, 72.8, 73.6, 100.8, 127.7, 127.8, 128.4, 137.5. IR (neat, cm^{-1}) 3476, 2930, 1464, 1256, 1190, 1129, 1055, 1019, 835. LRMS (EI(+)) *m/z* 396 (M^+), 365 ([M−MeO]$^+$), 347 ([M−OH−MeOH]$^+$), 339 ([M−*t*-Bu]$^+$), 321 ([M−*t*-Bu−H_2O]$^+$), 307 ([M−*t*-Bu−MeOH]$^+$), 289 ([M−BnO]$^+$), 243, 101, 91 (C_7H_7, bp). HRMS (EI(+)) calcd for $C_{21}H_{36}O_5Si$ (M^+) 396.2332, found 396.2349.

3.6.7. Epimerization of 27a to 27b. A solution of **27a** (1.11 g, 2.80 mmol), NMO (485.2 mg, 4.14 mmol), and TPAP (57.8 mg, 0.164 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature for 6 h. NMO (297.2 mg, 2.54 mmol) and TPAP (19.2 mg, 54.6 μmol) were added, and the mixture was stirred at room temperature for another 4 h. TPAP (41.4 mg, 0.118 mmol) was added, and the mixture was further stirred at the same temperature for 12 h. NMO (241.3 mg, 2.06 mmol) was added, and the mixture was further stirred at the same temperature for 6 h. The mixture was washed with 10% aqueous $Na_2S_2O_3$ solution (50 mL), and aqueous layer was extracted with CH_2Cl_2 (50 mL). The combined organic layers were washed with 0.1 N HCl solution (50 mL), water (50 mL), brine (50 mL), dried (Na_2SO_4) and concentrated. The residue was dissolved in MeOH (20 mL), and K_2CO_3 (408 mg, 2.95 mmol) was added. The mixture was stirred at room temperature for 20 min, and $NaBH_4$ (175.0 mg, 4.62 mmol) was added. The mixture was further stirred at the same temperature for 10 min. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution (50 mL), and the mixture was extracted with AcOEt (2× 50 mL). The combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (8:1)) gave the epimerized **27b** (650.2 mg, 59%), accompanied by the starting material **27a** (15%).

3.6.8. (2*S*,3*S*,5*S*,6*S*)-2-Bromomethyl-5-(*tert*-butyldimethylsilyloxy)-6-methoxytetrahydropyran-3-ylmethyl pivalate (28a). A solution of alcohol **27a** (709.3 mg, 1.79 mmol), PivCl (330 μL, 2.68 mmol) in pyridine (9 mL) was added at room temperature for 2.5 h. The solvent was removed under reduced pressure, and the residue was partitioned between AcOEt (30 mL) and water (30 mL). The organic layer was washed with 1 N HCl solution (20 mL) and water (20 mL), and the aqueous layers were combined and extracted with AcOEt (20 mL). The combined organic layers were washed with saturated aqueous Na_2CO_3 solution (30 mL), brine (30 mL), dried (Na_2SO_4), and concentrated to give crude pivalate. The residue was dissolved in EtOH (5 mL), and $Pd(OH)_2$/C (20% dry basis, 27.0 mg) was added. The mixture was stirred under H_2 atmosphere at room temperature for 1 h. Insoluble materials were filtered off and the filtrate was concentrated. The residue was re-dissolved in EtOH (5 mL) and treated with $Pd(OH)_2$/C (20% dry basis, 40.5 mg) under H_2 atmosphere for 3.5 h. Insoluble material was filtered off, and the residue in EtOH (5 mL) was further treated with $Pd(OH)_2$/C (20% dry basis, 128.3 mg) under H_2 atmosphere for 3.5 h. Insoluble material was filtered off, concentrated, and the crude alcohol was dissolved in CH_2Cl_2 (10 mL). Under an Ar atmosphere, the solution was cooled to 0 °C, and Et_3N (750 μL, 5.38 mmol) and MsCl (210 μL, 2.71 mmol) were added. The mixture was stirred at the same temperature for 1 h, and the reaction was quenched by the addition of water (10 mL). Resulting mixture was extracted with AcOEt (2× 30 mL), and the organic layers were combined, washed with brine (20 mL), dried (Na_2SO_4), and concentrated to give crude mesylate. The crude mesylate was dissolved in TMU (10 mL), and LiBr (489.9 mg, 5.64 mmol) was added. The mixture was stirred under an Ar atmosphere at 80 °C for 7 h. After cooled to room temperature, the mixture was diluted with water (10 mL) and extracted with Et_2O (2× 20 mL). The combined organic layers were washed with water (10 mL), brine (10 mL), dried (Na_2SO_4), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (25:1 to 4:1 to 2:1)) gave the bromide **28a** (476.4 mg, 59% for four steps) as a colorless oil.

$[\alpha]_D^{22}$ +58.6° (*c* 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 0.06 (3H, s), 0.07 (3H, s), 0.91 (9H, s), 1.19 (9H, s), 1.72 (1H, m), 2.00–2.10 (2H, m), 3.46 (3H, s), 3.40–3.55 (2H, m), 3.61 (1H, m), 4.15 (1H, ddd, $J = 1.8$, 4.0, 9.0 Hz), 4.23 (1H, dd, $J = 3.2$, 11.9 Hz), 4.46 (1H, dd, $J = 8.6$, 11.9 Hz), 4.49 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −5.0, −4.8, 18.0, 25.8, 27.3, 30.7, 34.1, 35.4, 38.6, 55.0, 64.7, 66.1, 70.3, 102.0, 178.0. IR (neat, cm^{-1}) 2932, 1730, 1466, 1283, 1152, 1129, 1061, 1029, 837, 810, 777. LRMS (EI(+)) *m/z* 421 ([M(^{79}Br)−MeO]$^+$), 395 ([M(^{79}Br)−*t*-Bu]$^+$), 363 ([M(^{79}Br)−*t*-Bu−MeOH]$^+$), 293, 261, 211 (bp), 159. HRMS (EI(+)) calcd for $C_{18}H_{34}{}^{79}BrO_4Si$ ([M−MeO]$^+$) 421.1410, found 421.1418.

Compound **28b** could be prepared according to essentially the same manner (77% for four steps) as a colorless oil.

3.6.9. (2*S*,3*R*,5*S*,6*S*)-2-Bromomethyl-5-(*tert*-butyldimethylsilyloxy)-6-methoxytetrahydropyran-3-ylmethyl pivalate (28b). $[\alpha]_D^{22}$ +51.8° (*c* 1.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 0.06 (3H, s), 0.06 (3H, s), 0.90 (9H, s), 1.21 (9H, s), 1.63 (1H, ddd, $J = 3.2$, 3.2, 13.2 Hz), 1.80 (1H, ddd, $J = 2.7$, 13.2, 13.2 Hz), 2.37 (1H, m), 3.41 (3H, s), 3.50 (1H, dd, $J = 7.0$, 11.0 Hz), 3.67 (1H, dd, $J = 2.1$, 11.0 Hz), 3.70–3.78 (2H, m), 3.89 (1H, dd, $J = 5.2$, 11.7 Hz), 4.03 (1H, dd, $J = 4.6$, 11.7 Hz), 4.49 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −4.8, −4.7, 18.1, 25.8, 27.2, 30.1, 32.9, 34.9, 38.9, 54.8, 65.1, 66.4, 70.3, 100.7, 178.1. IR (neat, cm^{-1}) 2932, 1732, 1474, 1285, 1256, 1144, 1113, 1032, 837, 776. LRMS (EI(+)) *m/z* 421 ([M(^{79}Br)−MeO]$^+$), 395 ([M(^{79}Br)−*t*-Bu]$^+$), 363 ([M(^{79}Br)−*t*-Bu−MeOH]$^+$), 319, 293 (bp), 211, 159. HRMS (EI(+)) calcd for $C_{18}H_{34}{}^{79}BrO_4Si$ ([M−MeO]$^+$) 421.1410, found 421.1412.

3.6.10. (*R*)-2-[(*S*)-2-(*tert*-Butyldimethylsilyloxy)-3-hydroxypropyl]but-3-enyl pivalate (29a). A mixture of the bromide **28a** (595.3 mg, 1.31 mmol), activated Zn dust (2.18 g, 33.3 mmol), and $NaBH_3CN$ (615.5 mg, 9.79 mmol) in *n*-PrOH (5 mL)–H_2O (0.5 mL) was stirred at 80 °C for 6 h and then 100 °C for 6 h. After cooled to room temperature, the mixture was diluted with saturated aqueous NH_4Cl solution (20 mL), filtered through Celite, and washed with AcOEt and water. After layers were separated, the aqueous layer was extracted with AcOEt (20 mL), and organic layers were combined, washed with brine (20 mL), dried (Na_2SO_4), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (50:1 to 8:1 to 4:1)) gave ring opened product **29a** (337.0 mg, 75%) as a colorless oil.

$[\alpha]_D^{19}$ −14.5° (*c* 1.4, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.09 (6H, s), 0.90 (9H, s), 1.19 (9H, s), 1.49 (1H, ddd, J = 4.4, 9.6, 14.0 Hz), 1.69 (1H, ddd, J = 4.2, 8.2, 14.0 Hz), 1.88 (1H, br s), 2.54 (1H, m), 3.47 (1H, dd, J = 4.4, 11.1 Hz), 3.59 (1H, dd, J = 4.2, 11.1 Hz), 3.79 (1H, apparent dq, J = 8.2, 4.3 Hz), 3.94 (1H, dd, J = 6.4, 10.8 Hz), 4.01 (1H, dd, J = 6.8, 10.8 Hz), 5.08–5.16 (2H, m), 5.62 (1H, ddd, J = 8.5, 11.1, 16.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −4.4, −4.2, 18.1, 25.9, 27.2, 35.7, 38.8, 39.6, 66.9, 67.2, 70.7, 116.9, 138.5, 178.2. IR (neat, cm^{-1}) 3476, 2932, 1732, 1474, 1287, 1254, 1163, 837, 776. LRMS (EI(+)) *m/z* 313 ([M−CH_2OH]$^+$), 287 ([M−*t*-Bu]$^+$), 211, 185, 159, 117 (bp). HRMS (EI(+)) calcd for $C_{17}H_{33}O_3Si$ ([M−CH_2OH]$^+$) 313.2199, found 313.2193.

Compound **29b** could also be prepared according to essentially the same manner (61%) as a colorless oil.

3.6.11. (*S*)-2-[(*S*)-2-(*tert*-Butyldimethylsilyloxy)-3-hydroxypropyl]but-3-enyl pivalate (29b). $[\alpha]_D^{19}$ +26.0° (*c* 0.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.08 (6H, s), 0.90 (9H, s), 1.19 (9H, s), 1.49 (1H, ddd, J = 5.0, 9.3, 13.9 Hz), 1.69 (1H, ddd, J = 5.1, 8.5, 13.9 Hz), 1.90 (1H, br s), 2.41 (1H, m), 3.45 (1H, dd, J = 5.0, 11.3 Hz), 3.60 (1H, dd, J = 3.5, 11.3 Hz), 3.80 (1H, dddd, J = 3.5, 5.0, 5.0, 8.5 Hz), 3.94 (1H, dd, J = 5.6, 10.8 Hz), 4.01 (1H, dd, J = 7.2, 10.8 Hz), 5.05–5.14 (2H, m), 5.63 (1H, ddd, J = 8.8, 10.4, 16.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −4.5, −4.4, 18.1, 25.9, 27.2, 34.9, 38.8, 40.0, 65.5, 66.7, 70.5, 116.9, 138.4, 178.2. IR (neat, cm^{-1}) 3484, 2932, 1732, 1480, 1287, 1256, 1159, 837, 756. LRMS (EI(+)) *m/z* 313 ([M−CH_2OH]$^+$), 287 ([M−*t*-Bu]$^+$), 211, 185, 159, 117 (bp). HRMS (EI(+)) calcd for $C_{17}H_{33}O_3Si$ ([M−CH_2OH]$^+$) 313.2199, found 313.2200.

3.6.12. (*R*)-2-[(*S*)-2-(*tert*-Butyldimethylsilyloxy)-3-(4-toluenesulfonyloxy)propyl]but-3-enyl pivalate. Under an Ar atmosphere, a solution of alcohol **29a** (70.2 mg, 0.203 mmol), Et_3N (85 μL, 0.610 mmol), DMAP (24.7 mg, 0.202 mmol), TsCl (56.9 mg, 0.298 mmol) in CH_2Cl_2 (1 mL) was stirred at room temperature for 13 h. The reaction was quenched by the addition of water (5 mL), and the mixture was extracted with AcOEt (5 mL). The organic layer was washed with brine (5 mL), dried (Na_2SO_4), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (15:1)) gave tosylate (88.9 mg, 88%) as a colorless oil.

$[\alpha]_D^{21}$ −13.6° (*c* 0.3, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.00 (3H, s), 0.02 (3H, s), 0.84 (9H, s), 1.17 (9H, s), 1.38–1.47 (1H, m), 1.52 (1H, ddd, J = 3.6, 8.0, 13.6 Hz), 2.45 (3H, s), 2.48–2.59 (1H, m), 3.80–3.91 (3H, m), 3.87 (1H, dd, J = 6.4, 10.7 Hz), 3.97 (1H, dd, J = 6.2, 10.7 Hz), 5.03–5.14 (2H, m), 5.53 (1H, ddd, J = 8.4, 10.4, 17.2 Hz), 7.32–7.38 (2H, m), 7.78–7.81 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −4.6, −4.1, 18.0, 21.7, 25.8, 27.2, 35.7, 38.8, 39.3, 67.1, 67.9, 73.2, 117.5, 127.9, 129.8, 132.8, 137.9, 144.8, 178.1. IR (neat, cm^{-1}) 2930, 1727, 1480, 1352, 1285, 1175, 1130, 924, 835, 814, 777. LRMS (EI(+)) *m/z* 483 ([M−CH_3]$^+$), 441 ([M−*t*-Bu]$^+$), 329, 313 ([M−CH_2OTs]$^+$), 230 (bp), 211, 159. HRMS (EI(+)) calcd for $C_{24}H_{39}O_6SSi$ ([M−CH_3]$^+$) 483.2237, found 483.2238.

Tosylate from **29b** could be prepared as essentially the same manner (93%) as a colorless oil.

3.6.13. (*S*)-2-[(*S*)-2-(*tert*-Butyldimethylsilyloxy)-3-(4-toluenesulfonyloxy)propyl]but-3-enyl pivalate. $[\alpha]_D^{20}$ +14.4° (*c* 0.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.00 (3H, s), 0.02 (3H, s), 0.83 (9H, s), 1.17 (9H, s), 1.46 (1H, ddd, J = 5.2, 8.8, 13.9 Hz), 1.62 (1H, ddd, J = 6.3, 6.3, 13.9 Hz), 2.34–2.50 (1H, m), 2.45 (3H, s), 3.82–3.96 (3H, m), 3.90 (1H, dd, J = 5.4, 10.7 Hz), 3.95 (1H, dd, J = 6.8, 10.7 Hz), 4.98–5.08 (2H, m), 5.58 (1H, ddd, J = 8.6, 10.2, 17.0 Hz), 7.31–7.38 (2H, m), 7.76–7.82 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −4.8, −4.5, 18.0, 21.7, 25.7, 27.2, 35.4, 38.8, 39.4, 66.3, 68.0, 72.7, 117.0, 127.9, 129.7, 132.8, 138.2, 144.7, 178.1. IR (neat, cm^{-1}) 2934, 1730, 1460, 1366, 1285, 1179, 988, 839, 810, 781. LRMS (EI(+)) *m/z* 483 ([M−CH_3]$^+$), 441 ([M−*t*-Bu]$^+$), 339, 329, 313 ([M−CH_2OTs]$^+$), 229 (bp), 211, 159. HRMS (EI(+)) calcd for $C_{24}H_{39}O_6SSi$ ([M−CH_3]$^+$) 483.2237, found 483.2250.

3.6.14. (*R*)-2-[(*S*)-2-Oxiranylmethyl]but-3-enyl pivalate (30a). To a solution of tosylate (88.9 mg, 0.178 mmol) in THF (0.75 mL) was added TBAF (1 M in THF, 445 μL, 445 μmol), and the solution was stirred at room temperature for 3 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution (2 mL) and the mixture was extracted with AcOEt (2×2 mL). The combined organic layers were washed with brine (2 mL), dried (Na_2SO_4), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (15:1 to 4:1)) gave epoxide **30a** (30.1 mg, 80%) as a colorless oil.

$[\alpha]_D^{21}$ −22.9° (*c* 0.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 1.19 (9H, s), 1.55–1.68 (2H, m), 2.48 (1H, dd, J = 2.8, 5.0 Hz), 2.68 (1H, m), 2.78 (1H, dd, J = 4.4, 5.0 Hz), 2.96 (1H, m), 4.03 (1H, dd, J = 6.6, 10.7 Hz), 4.07 (1H, dd, J = 6.4, 10.7 Hz), 5.11–5.21 (2H, m), 5.69 (1H, ddd, J = 8.6, 10.2, 17.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 27.2, 34.5, 38.8, 41.2, 47.6, 50.4, 66.6, 117.1, 137.7, 178.2. IR (neat, cm^{-1}) 2975, 1730, 1482, 1285, 1157, 1038, 994, 926. LRMS (EI(+)) *m/z* 212 (M$^+$), 182 ([M−CH_2O]$^+$), 57 (*t*-Bu, bp). HRMS (EI(+)) calcd for $C_{12}H_{20}O_3$ (M$^+$) 212.1412, found 212.1412.

Compound **30b** could also be prepared essentially in the same manner (81%).

3.6.15. (*S*)-2-[(*S*)-2-Oxiranylmethyl]but-3-enyl pivalate (30b). $[\alpha]_D^{22}$ +5.6° (*c* 0.1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 1.19 (9H, s), 1.63 (1H, ddd, J = 6.0, 6.0, 14.1 Hz), 1.69 (1H, ddd, J = 6.0, 7.8, 14.1 Hz), 2.47 (1H, dd, J = 2.7, 5.0 Hz), 2.63 (1H, m), 2.77 (1H, m), 2.97 (1H, ddt, J = 2.7, 3.9, 6.0 Hz), 4.03 (1H, dd, J = 5.8, 11.0 Hz), 4.09 (1H, dd, J = 6.6, 11.0 Hz), 5.10–5.19 (2H, m), 5.75 (1H, ddd, J = 8.0, 10.4, 17.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 27.2, 34.3, 38.8, 41.0, 47.1, 50.5, 66.3, 116.6, 138.0, 178.2. IR (neat, cm^{-1}) 2934, 1730, 1482, 1285, 1157, 1036, 995, 924. LRMS (EI(+)) *m/z* 212 (M^+), 182 ([M−CH_2O]$^+$), 57 (*t*-Bu, bp). HRMS (EI(+)) calcd for $C_{12}H_{20}O_3$ (M^+) 212.1412, found 212.1422.

3.6.16. (2*R*,4*S*)-2-Vinylhept-6-yne-1,4-diol (31a). To a cooled (−78 °C) solution of the epoxide **30a** (126.9 mg, 0.598 mmol) in THF (1 mL) was added a solution of TMS lithium acetylide (0.5 M in hexane/THF, prepared from TMS–acelylene and *n*-BuLi, 3.6 mL, 1.8 mmol) and $BF_3{\cdot}OEt_2$ (114 μL, 0.90 mmol), and stirred at the same temperature for 1.5 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution (5 mL), and the mixture was extracted with AcOEt (2× 5 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residue was dissolved in MeOH (2 mL) and cooled on ice-water bath. NaOMe (28% in MeOH, 345 μL, 1.79 mmol) was added and stirred at room temperature for 18 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl solution (3 mL), and the mixture was extracted with AcOEt (4× 5 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. Purification by silica gel column chromatography (AcOEt) gave the diol (72.8 mg, 79%) as a colorless oil.

$[\alpha]_D^{19}$ −18.8° (*c* 0.6, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 1.58 (1H, ddd, J = 3.3, 8.5, 14.2 Hz), 1.64 (1H, ddd, J = 5.5, 9.2, 14.2 Hz), 2.07 (1H, t, J = 2.7 Hz), 2.16 (2H, br s), 2.36 (1H, ddd, J = 2.7, 6.6, 16.8 Hz), 2.43 (1H, ddd, J = 2.7, 5.4, 16.8 Hz), 2.53 (1H, m), 3.56 (2H, d, J = 6.0 Hz), 3.84 (1H, dddd, J = 3.3, 5.4, 6.6, 9.2 Hz), 5.15–5.22 (2H, m), 5.65 (1H, ddd, J = 8.5, 10.5, 16.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 28.2, 38.1, 44.1, 66.1, 68.1, 71.0, 80.7, 117.3, 139.0. IR (neat, cm^{-1}) 3349, 3303, 3083, 2932, 2120, 1642, 1422, 1065, 1030, 924. LRMS (EI(+)) *m/z* 154 (M^+), 135 ([M−H_2O−H]$^+$), 115 ([M−C_3H_3]$^+$), 97 (bp). HRMS (EI(+)) calcd for $C_9H_{14}O_2$ (M^+) 154.0994, found 154.0997.

Diol from **30b** could also be prepared as in the same manner (80%) as a colorless oil.

3.6.17. (2*S*,4*S*)-2-Vinylhept-6-yne-1,4-diol (31b). $[\alpha]_D^{20}$ −1.5° (*c* 0.4, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 1.67 (1H, ddd, J = 7.6, 7.6, 14.2 Hz), 1.74 (1H, ddd, J = 4.9, 6.4, 14.2 Hz), 2.02 (2H, br s), 2.07 (1H, t, J = 2.7 Hz), 2.36 (1H, ddd, J = 2.7, 6.6, 16.6 Hz), 2.45 (1H, ddd, J = 2.7, 4.9, 16.6 Hz), 2.47 (1H, m), 3.56 (1H, dd, J = 6.6, 10.7 Hz), 3.63 (1H, dd, J = 6.0, 10.7 Hz), 3.92 (1H, dddd, J = 4.9, 4.9, 6.6, 7.6 Hz), 5.15–5.21 (2H, m), 5.74 (1H, ddd, J = 8.3, 9.7, 17.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 27.3, 37.5, 43.3, 65.3, 67.9, 71.0, 80.6, 117.1, 139.2. IR (neat, cm^{-1}) 3357, 3299, 3081, 2934, 2120, 1642, 1422, 1038, 918. LRMS (EI(+)) *m/z* 154 (M^+), 135 ([M−H_2O−H]$^+$), 115 ([M−C_3H_3]$^+$), 97 (bp). HRMS (EI(+)) calcd for $C_9H_{14}O_2$ (M^+) 154.0994, found 154.0998.

3.6.18. (3*R*,5*S*)-5-(*tert*-Butyldimethylsilyloxy)-3-(*tert*-butyldimethylsilyloxymethyl)oct-1-en-7-yne (32a). Under an Ar atmosphere, to a cooled (−78 °C) solution of diol (8.9 mg, 57.7 μmol), 2,6-lutidine (36 μL, 0.309 mmol) in CH_2Cl_2 (250 μL) was added TBSOTf (36 μL, 0.157 mmol), and the mixture was stirred at the same temperature for 2 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution (500 μL), and the mixture was extracted with AcOEt (2 mL). The organic layer was washed with brine (2 mL), dried (Na_2SO_4), and concentrated. Purification by silica gel column chromatography (hexane/AcOEt (50:1)) gave the bis-TBS ether **32a** (16.8 mg, 76%) as a colorless oil.

$[\alpha]_D^{21}$ −26.8° (*c* 1.1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.02 (6H, s), 0.06 (3H, s), 0.07 (3H, s), 0.89 (18H, s), 1.61 (1H, ddd, J = 3.4, 10.3, 13.8 Hz), 1.70 (1H, ddd, J = 3.7, 8.7, 13.8 Hz), 1.97 (1H, t, J = 2.7 Hz), 2.30 (1H, ddd, J = 2.7, 7.0, 16.6 Hz), 2.46 (1H, ddd, J = 2.7, 5.0, 16.6 Hz), 2.38 (1H, m), 3.47 (1H, dd, J = 6.6, 9.7 Hz), 3.52 (1H, dd, J = 6.0, 9.7 Hz), 3.83 (1H, dddd, J = 3.4, 5.0, 7.0, 8.7 Hz), 5.01–5.10 (2H, m), 5.63 (1H, ddd, J = 8.4, 9.6, 18.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −5.2, −5.2, −4.4, −4.1, 18.1, 18.4, 25.8, 25.9, 26.0, 28.3, 38.2, 42.8, 67.2, 68.9, 70.0, 81.5, 116.0, 139.9. IR (neat, cm^{-1}) 3316, 3079, 2932, 1472, 1256, 1100, 837, 776. LRMS (EI(+)) *m/z* 382 (M^+), 367 ([M−Me]$^+$), 343 ([M−C_3H_3]$^+$), 325 ([M−*t*-Bu]$^+$), 257, 211, 193, 147, 73 (bp). HRMS (EI(+)) calcd for $C_{21}H_{42}O_2Si_2$ (M^+) 382.2723, found 382.2724.

Compound **32b** could also be prepared as in the same manner (85%) as a colorless oil.

3.6.19. (3*S*,5*S*)-5-(*tert*-Butyldimethylsilyloxy)-3-(*tert*-butyldimethylsilyloxymethyl)oct-1-en-7-yne (32b). $[\alpha]_D^{22}$ +3.8° (*c* 0.7, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 0.03 (6H, s), 0.07 (3H, s), 0.08 (3H, s), 0.88 (9H, s), 0.89 (9H, s), 1.51 (1H, ddd, J = 5.7, 8.8, 13.7 Hz), 1.83 (1H, ddd, J = 5.6, 7.1, 13.7 Hz), 1.95 (1H, t, J = 2.7 Hz), 2.29 (1H, m), 2.30 (1H, ddd, J = 2.7, 5.7, 16.8 Hz), 2.37 (1H, ddd, J = 2.7, 5.7, 16.8 Hz), 3.51 (2H, d, J = 6.0 Hz), 3.86 (1H, dddd, J = 5.7, 5.7, 5.7, 7.1 Hz), 5.01–5.09 (2H, m), 5.69 (1H, ddd, J = 8.4, 10.4, 17.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ −5.3, −5.3, −4.5, −4.3, 18.2, 18.4, 25.8, 25.9, 26.0, 27.1, 38.1, 42.9, 66.5, 69.1, 69.9, 81.7, 115.5, 140.2. IR (neat, cm^{-1}) 3316, 3079, 2930, 2122, 1472, 1256, 1094, 810, 776. LRMS (EI(+)) *m/z* 382 (M^+), 367 ([M−Me]$^+$), 343 ([M−C_3H_3]$^+$), 325 ([M−*t*-Bu]$^+$), 257, 211, 193, 147, 73 (bp). HRMS (EI(+)) calcd for $C_{21}H_{42}O_2Si_2$ (M^+) 382.2723, found 382.2719.

3.6.20. 25-Hydroxy-1β-hydroxymethylvitamin D_3 (4a). Under an Ar atmosphere, a solution of A-ring enyne **32a** (14.8 mg, 38.7 μmol), CD-ring bromoolefin **6**[12] (58.0 mg, 0.163 mmol), $Pd(PPh_3)_4$ (5.0 mg, 4.3 μmol) in PhMe

(200 μL)–Et_3N (200 μL) was stirred at 80 °C for 2 h. After cooled to room temperature, the mixture was filtered through silica gel pad, washed with PhMe and AcOEt, and the filtrate was concentrated. The residue was dissolved in THF (250 μL) and cooled on ice-water bath. HF·py (50 μL) was added and the mixture was stirred at the same temperature for 1.5 h and then at room temperature for 30 min. The mixture was partitioned between AcOEt (1 mL) and saturated aqueous $NaHCO_3$ solution (1 mL), and the aqueous layer was extracted with AcOEt (2 mL). The combined organic layers were washed with brine (2 mL), dried (Na_2SO_4), and concentrated. Purification by silica gel column chromatography ($CHCl_3$/MeOH (40:1)) gave the product **4a** (6.5 mg, 39%) as a pale yellow powder.

$[\alpha]_D^{23}$ −22.3° (*c* 0.1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$, ppm) δ 0.54 (3H, s), 0.94 (3H, d, *J* = 6.7 Hz), 1.02–1.10 (1H, m), 1.22 (6H, s), 1.15–1.76 (17H, m), 1.83–1.92 (2H, m), 1.96–2.03 (2H, m), 2.13 (1H, dddd, *J* = 0.8, 3.7, 4.8, 13.5 Hz), 2.33 (1H, dd, *J* = 6.3, 13.3 Hz), 2.48 (1H, apparent tt, *J* = 5.7, 5.7 Hz), 2.59 (1H, dd, *J* = 3.7, 13.3 Hz), 2.79–2.86 (1H, m), 3.72 (1H, dd, *J* = 5.7, 10.9 Hz), 3.78 (1H, dd, *J* = 5.7, 10.9 Hz), 4.02 (1H, tt, *J* = 3.7, 6.3 Hz), 4.99 (1H, d, *J* = 1.7 Hz), 5.12 (1H, m), 6.00 (1H, d, *J* = 11.3 Hz), 6.29 (1H, d, *J* = 11.3 Hz). ^{13}C NMR (150 MHz, $CDCl_3$, ppm) δ 12.0, 18.8, 20.8, 22.3, 23.6, 27.6, 29.1, 29.2, 29.4, 36.1, 36.4, 37.3, 40.5, 44.2, 44.4, 45.9, 45.9, 56.3, 56.6, 66.4, 68.0, 71.1, 113.1, 117.0, 123.1, 135.0, 143.0, 146.0. IR (film, cm^{-1}) 3364, 2942, 1642, 1377, 1034, 911, 760. LRMS (EI(+)) *m/z* 430 (M^+), 412 ([M−H_2O]$^+$), 400([M−CH_2O]$^+$), 394 ([M−2×H_2O]$^+$), 381 ([M−H_2O−CH_2OH]$^+$), 363 ([M−2×H_2O−CH_2OH]$^+$), 135, 59 (bp). HRMS (EI(+)) calcd for $C_{28}H_{46}O_3$ (M^+) 430.3447, found 430.3440.

1α-Hydroxymethylated derivative (**4b**) could also be prepared as in the same manner (65%) as a white powder.

$[\alpha]_D^{26}$ +83.1° (*c* 0.2, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$, ppm) δ 0.52 (3H, s), 0.93 (3H, d, *J* = 6.6 Hz), 1.02–1.09 (1H, m), 1.22 (6H, s), 1.19–1.60 (15H, m), 1.62–1.74 (2H, m), 1.80 (1H, ddd, *J* = 6.3, 9.0, 12.9 Hz), 1.83–1.90 (1H, m), 1.90–1.96 (1H, m), 1.96–2.03 (2H, m), 2.27 (1H, dd, *J* = 8.1, 12.9 Hz), 2.60 (1H, dd *J* = 3.9, 12.9 Hz), 2.63 (1H, m), 2.78–2.84 (1H, m), 3.56–3.64 (2H, m), 4.01 (1H, m), 5.00 (1H, d, *J* = 2.4 Hz), 5.16 (1H, m), 5.95 (1H, d, *J* = 11.4 Hz), 6.32 (1H, d, *J* = 11.4 Hz). ^{13}C NMR (150 MHz, $CDCl_3$, ppm) δ 11.9, 18.8, 20.8, 22.2, 23.6, 27.7, 29.1, 29.2, 29.3, 36.1, 36.4, 37.4, 40.5, 44.4, 45.9, 46.2, 56.3, 56.5, 64.2, 67.0, 71.1, 113.9, 117.0, 123.7, 134.2, 143.3, 145.4. IR (film, cm^{-1}) 3312, 2944, 1658, 1632, 1468, 1044, 905, 751. LRMS (EI(+)) *m/z* 430 (M^+), 412 ([M−H_2O]$^+$), 400 ([M−CH_2O]$^+$), 394 ([M−2×H_2O]$^+$), 380 ([M−H_2O−CH_2OH−H]$^+$), 363 ([M−2×H_2O−CH_2OH]$^+$), 135 (bp). HRMS (EI(+)) calcd for $C_{28}H_{46}O_3$ (M^+) 430.3447, found 430.3449.

4. Reporter assays using luciferase as a reporter

Human breast cancer cell line MCF7 cells were grown at 37 °C in DMEM supplemented with 10% FBS and 1% P/S in an atmosphere of 95% air and 5% CO_2. Cells were collected, suspended in the DMEM supplemented with 5% FBS (stripped with dextran-coated charcoal) and 1% P/S without phenol red, and plated in 24-well plate (2.5×10^4 cells/well). Cells were incubated in CO_2 incubator at 37 °C overnight. Ligand stock solutions were prepared at various concentrations in DMSO (10^{-7} to 10^{-3} M). DMSO itself was used as vesicle. Plasmids used in our assays were as follows; receptor plasmids (pM(GAL4-hVDR(DEF)) for wild type hVDR, and pM(GAL4-hVDR(R274L)(DEF)) for mutant hVDR, the latter prepared by site-directed mutagenesis using QuikChange II XL Site-Directed Mutagenesis Kits (Stratagene)), reporter plasmid (17M2-G-Luc) and internal standard plasmid (pRL-CMV). Plasmids were diluted in OPTI-MEM medium at concentrations of 50 ng/well for receptor plasmid, 0.2 μg/well for reporter plasmid, and 2.5 ng/well for internal plasmid. Transfections were carried out by using TransFast reagent (Promega) according to the manufacturer's instruction. After 3–6 h of transfection, ligand stock solutions were added at the final concentrations of 10^{-10} to 10^{-6} M, and cells were further incubated overnight. Luciferase assays were performed by using Dual-Luciferase Reporter Assay System Kit (Promega). All experiments were carried out at least three times and data were shown as average ± SD.

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References and notes

1. (a) *Vitamin D*, 2nd ed.; Feldman, D., Pike, J. W., Glorieux, F. H., Eds.; Elsevier Academic Press: New York, 2005; (b) Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocr. Rev.* **1995**, *16*, 200; (c) Zhu, G. D.; Okamura, W. H. *Chem. Rev.* **1995**, *95*, 1877; (d) Ettinger, R. A.; DeLuca, J. F. *Adv. Drug Res.* **1996**, *28*, 269.
2. (a) Evans, R. M. *Science* **1988**, *240*, 889; (b) Chambon, P. *Mol. Endocrinol.* **2005**, *19*, 1418.
3. Rochel, N.; Wurtz, J. M.; Mitschler, A.; Klahoz, B.; Moras, D. *Mol. Cell* **2000**, *5*, 173.
4. Malloy, P. J.; Pike, J. W.; Feldman, D. *Endocr. Rev.* **1999**, *20*, 156.
5. Kristjansson, K.; Rut, A. R.; Hewison, M.; O'Riordan, J. L.; Hughes, M. R. *J. Clin. Invest.* **1993**, *92*, 12.
6. (a) Swann, S. L.; Bergh, J. J.; Farach-Carson, M. C.; Koh, J. T. *Org. Lett.* **2002**, *4*, 3863; Non-seco steroid type analogues were reported by the same research group: (b) Swann, S. L.; Bergh, J. J.; Farach-Carson, M. C.; Ocasio, C. A.; Koh, J. T. *J. Am. Chem. Soc.* **2002**, *124*, 13795.
7. (a) Gardezi, S. A.; Nguyen, C.; Malloy, P. J.; Posner, G. H.; Feldman, D.; Peleg, S. *J. Biol. Chem.* **2001**, *276*, 29148; (b) At first, Posner et al. reported that the analogue has

1β,3α stereochemistry, but recently, they revised this stereochemistry was 1α,3β. See Ref. 21e.

8. (a) Suhara, Y.; Nihei, K.; Tanigawa, H.; Fujishima, T.; Konno, K.; Nakagawa, K.; Okano, T.; Takayama, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1129; (b) Suhara, Y.; Nihei, K.; Kurihara, M.; Kittaka, A.; Yamaguchi, K.; Fujishima, T.; Konno, K.; Miyata, N.; Takayama, H. *J. Org. Chem.* **2001**, *66*, 8760; (c) Saito, N.; Matsunaga, T.; Fujishima, T.; Anzai, M.; Saito, H.; Takenouchi, K.; Miura, D.; Ishizuka, S.; Takayama, H.; Kittaka, A. *Org. Biomol. Chem.* **2003**, *1*, 4396; (d) Yoshida, A.; Ono, K.; Suhara, Y.; Saito, N.; Takayama, H.; Kittaka, A. *Synlett* **2003**, 1175; (e) Saito, N.; Masuda, M.; Matsunaga, T.; Saito, H.; Anzai, M.; Takenouchi, K.; Miura, D.; Ishizuka, S.; Takimoto-Kamimura, M.; Kittaka, A. *Tetrahedron* **2004**, *60*, 7951; (f) Saito, N.; Matsunaga, T.; Saito, H.; Anzai, M.; Takenouchi, K.; Miura, D.; Ishizuka, S.; Takayama, H.; Kittaka, A. *Heterocycles* **2006**, *67*, 311.
9. For a recent review, see: Saito, N.; Honzawa, S.; Kittaka, A. *Curr. Top. Med. Chem.* **2006**, *6*, 1273.
10. (a) Kittaka, A.; Kurihara, M.; Peleg, S.; Suhara, Y.; Takayama, H. *Chem. Pharm. Bull.* **2003**, *51*, 357; Analogues which possess 2α-aromatic ring substituent were tested for the mutant VDR (Arg274Leu), and 2α-benzyl analogue was shown to have similar activity as the natural hormone, **1**: (b) Honzawa, S.; Hirasaka, K.; Yamamoto, Y.; Peleg, S.; Fujishima, T.; Kurihara, M.; Saito, N.; Kishimoto, S.; Sugiura, T.; Waku, K.; Takayama, H.; Kittaka, A. *Tetrahedron* **2005**, *61*, 11253.
11. Hourai, S.; Fujishima, T.; Kittaka, A.; Suhara, Y.; Takayama, H.; Rochel, N.; Moras, D. *J. Med. Chem.* **2006**, *49*, 5199.
12. Trost, B. M.; Dumas, J.; Villa, M. *J. Am. Chem. Soc.* **1992**, *114*, 9836.
13. (a) Suhara, Y.; Kittaka, A.; Kishimoto, S.; Carverley, M. J.; Fujishima, T.; Saito, N.; Sugiura, T.; Waku, K.; Takayama, H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3255; (b) Honzawa, S.; Suhara, Y.; Nihei, K.; Saito, N.; Kishimoto, S.; Fujishima, T.; Kurihara, M.; Sugiura, T.; Waku, K.; Takayama, H.; Kittaka, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3503; (c) Honzawa, S.; Yamamoto, Y.; Hirasaka, K.; Takayama, H.; Kittaka, A. *Heterocycles* **2003**, *61*, 327.
14. Wiggins, L. F. *Methods Carbohydr. Chem.* **1963**, *2*, 188.
15. DeNinno, M. P.; Etienne, J. B.; Duplantier, K. C. *Tetrahedron Lett.* **1995**, *36*, 669.
16. (a) Lombardo, L. *Org. Synth. Coll.* **2002**, *VIII*, 81; (b) Lombardo, L. *Tetrahedron Lett.* **1982**, *23*, 4293.
17. Saito, N.; Masuda, M.; Saito, H.; Takenouchi, K.; Ishizuka, S.; Namekawa, J.; Takimoto-Kamimura, M.; Kittaka, A. *Synthesis* **2005**, 2533.
18. (a) Takeyama, K.; Masuhiro, Y.; Fuse, H.; Endoh, H.; Murayama, A.; Kitanaka, S.; Suzawa, M.; Yanagisawa, J.; Kato, S. *Mol. Cell Biol.* **1999**, *19*, 1049; (b) Kodera, Y.; Takeyama, K.; Murayama, A.; Suzawa, M.; Masuhiro, Y.; Kato, S. *J. Biol. Chem.* **2000**, *275*, 33201.
19. Koh, J. T.; Biggins, J. B. *Curr. Top. Med. Chem.* **2005**, *5*, 413.
20. (a) Yamamoto, K.; Abe, D.; Yoshimoto, N.; Choi, M.; Yamagishi, K.; Tokiwa, H.; Shimizu, M.; Makishima, M.; Yamada, S. *J. Med. Chem.* **2006**, *49*, 1313; (b) Shimizu, M.; Yamamoto, K.; Mihori, M.; Iwasaki, Y.; Morizono, D.; Yamada, S. *J. Steroid Biochem. Mol. Biol.* **2004**, *89–90*, 75; (c) Choi, M.; Yamamoto, K.; Itoh, T.; Makishima, M.; Mangelsdorf, D. J.; Moras, D.; DeLuca, H. F.; Yamada, S. *Chem. Biol.* **2003**, *10*, 261.
21. (a) Reddy, C. D.; Patti, R.; Guttapalli, S.; Maris, J. M.; Yanamandra, N.; Rachamallu, A.; Sutton, L. N.; Phillips, P. C.; Posner, G. H. *J. Cell. Biochem.* **2006**, *97*, 198; (b) Peleg, S.; Khan, F.; Navone, N. M.; Cody, D. D.; Johnson, E. M.; Van Pelt, C. S.; Posner, G. H. *J. Steroid Biochem. Mol. Biol.* **2005**, *97*, 203; (c) Dixon, K. M.; Deo, S. S.; Wong, G.; Slater, M.; Norman, A. W.; Bishop, J. E.; Posner, G. H.; Ishizuka, S.; Halliday, G. M.; Reeve, V. E.; Mason, R. S. *J. Steroid Biochem. Mol. Biol.* **2005**, *97*, 137; (d) Hatcher, M. A.; Peleg, S.; Dolan, P.; Kensler, T. W.; Sarjeant, A.; Posner, G. H. *Bioorg. Med. Chem.* **2005**, *13*, 3964; (e) Posner, G. H.; Jeon, H. B.; Sarjeant, A.; Riccio, E. S.; Doppalapudi, R. S.; Kapetanovic, I. M.; Saha, U.; Dolan, P.; Kensler, T. W. *Steroids* **2004**, *69*, 757; (f) Posner, G. H.; Nelson, T. D.; Guyton, K. Z.; Kensler, T. W. *J. Med. Chem.* **1992**, *35*, 3280.
22. We synthesized 1-methyl analogues of **2a,b** and **4a,b** to confirm this hypothesis, and the results will be published elsewhere.

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Computational Study on Secondary Structure of Oligopeptides Containing α,α-Disubstituted α-Amino Acids

Masaaki Kurihara[1], Yukiko Sato[1], Fumiya Kaneko[1], Haruhiro Okuda[1], Masanobu Nagano[2], Yosuke Demizu[2], Mitsunobu Doi[3], Masakazu Tanaka[2], and Hiroshi Suemune[2]

[1]Division of Organic Chemistry, National Institute of Health Sciences, Tokyo 158-8501, Japan, [2]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan, [3]Osaka University of Pharmaceutical Sciences, Osaka 569-1094, Japan
e-mail:masaaki@nihs.go.jp

Computational simulation of the conformation of oligopeptides presents an interesting challenge to predict the conformation for the design of functionalized and bioactive molecules. Here we report computational study on conformation of oligopeptides containing cyclic α,α-disubstituted α-amino acids with side-chain chiral centers and also conformational search using various force fields and evaluation by MO calculations.

Keywords: α,α-disubstituted α-amino acid, conformational search, *MacroModel*, oligopeptide

Introduction

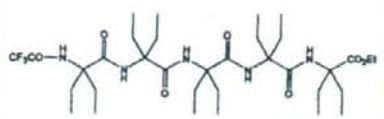
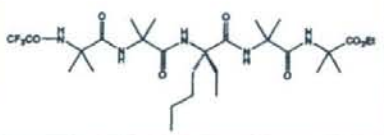
We have studied on computational simulation of proteins [1-2] and peptides. We have shown MCMM conformational search method and AMBER* force field using *MacroModel* is useful to predict secondary helical structures (α-helix, 3_{10}-helix) of oligopeptides prepared from α,α-disubstituted α-amino acids. Moreover, we have studied conformational analysis of oligopeptides containing chiral α,α-disubstituted α-amino acids to predict the helical screw sense of helical structures [3-7].

Fig. Helical structures of oligopeptides containing α,α-disubstituted α-amino acids.

• Results and Discussion

We calculated α,α-disubstituted peptide using MCMM conformational search with various force fields (AMBER*, MMFF, OPLS) and showed the results in table. In the case of using AMBER* force field the results were in agreement with those of x-ray and were most stable conformation evaluated by 3-21G level molecular orbital calculation. These results indicated that computational simulation using conformational search calculations with AMBER* force field is most useful for conformational analysis of oligopeptides containing α,α-disubstituted α-amino acids.

Table. Conformational search with various force fields.

	Global Minimum By *MacroModel* MCMM Conformational Search			X-ray
	AMBER*	MMFF	OPLS	
	3_{10}-helix	Random coil	Random coil	3_{10}-helix
	3-21G by *Spartan*			
	0 (kcal/mol)	+2.61	+14.24	
	(*P*)-3_{10} -helix	Random coil	Random coil	(*P*)-3_{10} -helix
	3-21G by *Spartan*			
	0 (kcal/mol)	+14.09	+22.84	

Acknowledgement

This work was supported in part by the Budget for Nuclear Research of the Ministry of Education, Culture, Sports, Science and Technology, based on the screening and counseling by the Atomic Energy Commission and by a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science.

References

1. Kurihara, M., Rouf, A. S. S., Kansui, H., Kagechika H., Okuda, H., N. Miyata, N, (2004) *Bioorg. Med. Chem. Lett.*, **14**, 4131-4134.
2. Hakamata, W., Sato, Y., Okuda, H., Honzawa, S., Saito, N., Kishimoto, S., Yamashita, A., Sugiura, T., Kittaka, A., Kurihara, M. (2008) *Bioorg. Med. Chem. Lett.*, **18**, 120-123.
3. Demizu, Y., Tanaka, M., Nagano, M., Kurihara, M., Doi, M., Maruyama, T., Suemune, H. (2007) *Chem. Pharm. Bull.*, **55**, 840-842.
4. Kurihara, M., Sato, Y., Hakamata, W., Okuda, H., Nagano, M., Hama, M., Demizu, Y., Doi, M., Tanaka, M., Suemune, H. (2006) *Peptide Science 2006*, 88.
5. Tanaka, M., Anan, K., Demizu, Y., Kurihara, M., Doi, M., Suemune, H. (2005) *J. Am. Chem. Soc.*, **127**,11570-11571.
6. Tanaka, M., Demizu, Y., Doi, M., Kurihara, M., Suemune, H. (2004) *Angew. Chem. Int. Ed.*, **43**, 5360-5363.
7. Tanaka, M., Nishimura, S., Oba, M., Demizu, Y., M. Kurihara, M. Suemune , H. (2003) *Chemistry Eur. J.*, **9**, 3082-3090.

Isolation and Structural Elucidation of Cyclopentynafil and *N*-Octylnortadalafil Found in a Dietary Supplement

Takashi HASEGAWA,[a] Kazunaga TAKAHASHI,[a] Masaaki SAITO,[a] Toshiyasu ISHII,[a] Tomoko NAGATA,[a] Masaaki KURIHARA,[b] Yuji HAISHIMA,[b] Yukihiro GODA,*[,b] and Nobuo KAWAHARA[b]

[a] *Chiba Prefectural Institute of Public Health; 666–2 Nitona-cho, Chuo-ku, Chiba, Chiba 260–8715, Japan: and* [b] *National Institute of Health Sciences; 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan.*
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A new sildenafil analogue, cyclopentynafil (1) and a new tadalafil analogue, *N*-octylnortadalafil (2) were isolated from a dietary supplement illegally marketed for erectile dysfunction. The structures of the sildenafil and tadalafil analogues were elucidated by using HPLC-photodiode array (PDA), LC-MS, high-resolution MS, NMR and circular dichroism (CD). These compounds were determined to be 5-[2-ethoxy-5-(4-cyclopentylpiperazin-1-ylsulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one and (6*R*,12a*R*)-2-octyl-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione, respectively. Recently, a large number of phosphodiesterase-5 (PDE-5) inhibitors, including their analogues, have been isolated from dietary supplements, while cyclopentynafil and *N*-octylnortadalafil are the first compounds reported to be new sildenafil and tadalafil analogues, respectively. Quantitative HPLC analysis showed that the contents of 1 and 2 in the product were about 130 mg/tablet (301 g/mg) and about 27 mg/tablet (64.1 g/mg), respectively.

Key words cyclopentynafil; *N*-octylnortadalafil; phosphodiesterase-5 inhibitor; LC-MS; NMR; erectile dysfunction

Recently, along with the rise in health consciousness, the consumption of dietary supplements has increased year by year. In Japan, some of these products are illegally advertised as effective for sexual enhancement. Consumers take these products without knowing that most are adulterated with synthetic compounds, such as sildenafil (Fig. 1), vardenafil and tadalafil (Fig. 1), all of which are known as active drug ingredients for the treatment of penile erectile dysfunction (ED).[1—3]

In our previous paper, we identified a new tadalafil analogue, chloropretadalafil,[4] which had been synthesized as a tadalafil precursor,[5] from a dietary supplement along with hydroxyhomosildenafil and aminotadalafil.

Thus far, a large number of analogues of sildenafil, tadarafil and vardenafil have been reported,[6—23] while a new type of phosphodiesterase-5 (PDE-5) inhibitor, (*R*)-xanthoanthrafil, an anthranilic acid derivative, has been found in a dietary supplement advertising sexual enhancement for men.[24] (*R*)-Xanthoanthrafil was first synthesized as a candidate compound for the treatment of ED by Fujisawa Pharmaceutical Co., Ltd. (currently Astellas Pharma Inc., Tokyo, Japan),[25] and was reported as a PDE-5 inhibitor, FR226807, after the manufacturer discontinued the process of developing the drug for approval. Furthermore, another new type of PDE-5 inhibitor, thioquinapiperifil, an imidazoquinazoline derivative, was also detected in a dietary supplement.[26] This compound was first synthesized as KF31327 by Kyowa Hakko Kogyo Co., Ltd., and Hirose *et al.* reported that it was a more potent and selective PDE-5 inhibitor than sildenafil.[27—29]

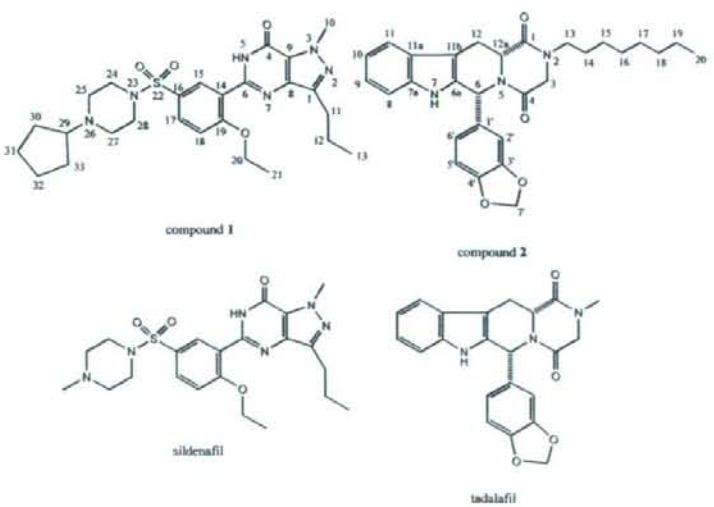

Fig. 1. Structures of Compounds **1**, **2** and Related Compounds

* To whom correspondence should be addressed. e-mail: goda@nihs.go.jp

In this paper, we report the analysis and structural elucidation of a new sildenafil analogue, cyclopentynafil and a new tadalafil analogue, *N*-octylnortadalafil, that were isolated from a dietary supplement illegally marketed for erectile dysfunction.

Experimental

Chemicals and Reagents HPLC-grade acetonitrile and all other reagents (analytical grade) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Sample The examined product was purchased as a processed food composed mainly of walnuts through the Internet and was composed of four pieces of ivory tablets (400 mg). The product properties were as follows: product name; Highperwalnup 2, producing company; Art Creation Co., Ltd., sales company; Ogawa Planning Co., Ltd., date of purchase; December 28, 2007.

Preparation of Sample Solution One tablet was finely powdered, and 100 mg of the powder was ultrasonically extracted in 10 ml of 70% methanol for 15 min. The extract was centrifuged at 1700×***g***. The supernatant was filtered through a 0.45 m filter. The filtrate was used for HPLC, and a portion of it was diluted 10-fold with methanol for liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis.

HPLC Analysis HPLC analysis was performed using a JASCO PU-2089 apparatus equipped with a photodiode array (PDA) detector model MD-2015 (JASCO Corporation, Tokyo, Japan). The sample solution was separated by using a TSK-GEL ODS-80Ts column (150×4.6 mm i.d., 5 m, Tosoh Co., Tokyo, Japan). The mobile phase was an acetonitrile/water/phosphoric acid (100 : 900 : 1) mixture solution containing 5 mmol/l sodium hexanesulfonate (eluent A) and an acetonitrile/water/phosphoric acid (900 : 100 : 1) mixture solution containing 5 mmol/l sodium hexanesulfonate (eluent B). The gradient elution was started at 90% eluent A, and was linearly decreased to 55% A in 25 min and to 10% A in 44—49 min. The flow rate of the mobile phase was set at 1.0 ml/min, and the injection volume was 20 l. The column temperature was maintained at 40 °C. The PDA detection wavelength was set from ultraviolet (UV) 200 to 400 nm, and max-plot chromatographic monitoring was performed (200—400 nm).

LC-ESI-MS Analysis LC-ESI-MS analysis was performed using a Waters alliance 2695 separation module and ZQ mass spectrometer (Waters Corporation, Milford, MA, U.S.A.). The sample solution was separated by using an Atlantis dC18 column (150×2.1 mm i.d., 3 m, Waters Corporation). The mobile phase was 0.1% formic acid aqueous solution (eluent A) and acetonitrile containing 0.1% formic acid (eluent B). The gradient elution began at 95% eluent A, and linearly decreased to 80% A in 15 min and to 20% A in 30—35 min. The flow rate of the mobile phase was set at 0.2 ml/min, and the injection volume was 10 l. The column temperature was maintained at 40 °C. ESI on both positive and negative modes was used for the analysis. The instrument parameters were as follows: source temperature, 120 °C; desolation temperature, 350 °C; capillary voltage, 3 kV; cone voltage, 30, 60 V (ESI positive), −60 V (ESI negative); and desolation gas flow, 600 l/h. The mass range of the spectra was *m/z* 100 to *m/z* 800.

Isolation of Compounds 1 and 2 Sample powder (300 mg) was dissolved in 20 ml water, and the solution was extracted with 40 ml diethyl ether for 10 min, three times. All of the diethyl ether layers were combined, dehydrated with anhydrous sodium sulfate for 1 h, and filtrated by filter paper. The filtrate was evaporated to dryness then reconstituted with 3 ml methanol. The methanol solution was centrifuged, and the precipitate was dried *in vacuo* to afford compound **1** (18.8 mg). The supernatant was applied to silica gel $60F_{254}$ TLC plates (20×10 cm with 1.0 mm thickness, Merck, Darmstadt, Germany) in a band. The plates were developed using a saturated tank with a hexane/ethyl acetate/acetic acid mixture (50 : 50 : 1) to a distance of about 7 cm. After air-drying, the plates were examined using UV light (wavelength: 254 nm). A band with an *Rf* value of 0.39 was scraped and dissolved in 120 ml of methanol. The methanol solution was filtered, and the filtrate was evaporated to dryness. The residue was reconstituted in 10 ml diethyl ether. This solution was filtered, and the filtrate was evaporated to dryness to afford compound **2** (4.4 mg).

Measurement of Accurate Mass The accurate mass of the target compound was measured by the LTQ OrbiTrap XL instrument (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) with the direct-infusion ESI positive-ion mode under the following conditions: solvent flow rate 5 l/min, sheath gas flow rate 20 arb, aux gas flow rate 10 arb, spray voltage 5 kV, capillary temperature 275 °C, capillary voltage 4 V, and tube lens 60 V. Tyrosine 1, 3, 6 standard was used as a mass calibrant of FT mass analyzer (resolution=100000), and tyrosine 3 standard was used as a lock mass ion (*m/z* 508.20783) during the measurement. Theoretical mass and delta value (mmu) were calculated by using the elemental composition tool of Xcalibur/Qual Browser software.

NMR Analysis $CDCl_3$ (99.96%) and CD_3OD (99.96%) were purchased from ISOTEC Inc., which is part of Sigma-Aldrich Inc. (St. Louis, MO, U.S.A.). The NMR spectra were obtained on an ECA-800 spectrometer (JEOL Ltd., Tokyo, Japan) equipped with HCNFG and CH5FG probes (JEOL Ltd.). The ^{1}H- and ^{13}C-NMR chemical shifts of compounds **1** and **2** were assigned by heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) and nuclear Overhauser effect (NOE) spectra.

Measurement of Circular Dichroism The circular dichroism (CD) spectra of compound **2** and tadalafil were measured by using a J-720 spectrophotometer (JASCO Corporation, Tokyo, Japan) with a quartz cell 10 mm in length. The concentration in methanol solution of compound **2** and tadalafil were 0.041 mmol/l and 0.044 mmol/l, respectively.

Docking Study of Compounds 1 and 2 with PDE-5 Docking models of compounds **1** and **2** bound to PDE-5 were constructed by conformational search simulation (Mixed MCMM/Low Mod). AMBER* was used as force field. Calculations were performed by *MacroModel* (ver. 8.1). 1UDT (PDB ID) and 1UDU, crystal structures of PDE-5 were used for docking models of compounds **1** and **2**, respectively.

Results and Discussion

In this study, we reported **1** and **2** as newly isolated compounds from an illegal dietary supplement. Figure 2A shows the HPLC chromatograms of an extract of the supplement. Two main peaks were detected in the extract, one at 20.9 min (compound **1**) and the other at 37.2 min (compound **2**). The PDA-sliced UV spectrum of **1** exhibited a quite similar profile ($_{max}$ nm: 218, 290, Fig. 2B) to that of sildenafil; however, **1** eluted at a later retention time (20.9 min) than sildenafil (18.3 min) under the same chromatographic conditions. Meanwhile, the PDA-sliced UV spectrum of **2** showed a quite similar profile ($_{max}$ nm: 281, Fig. 2C) to tadalafil, but **2** eluted at a later retention time (37.2 min) than tadalafil

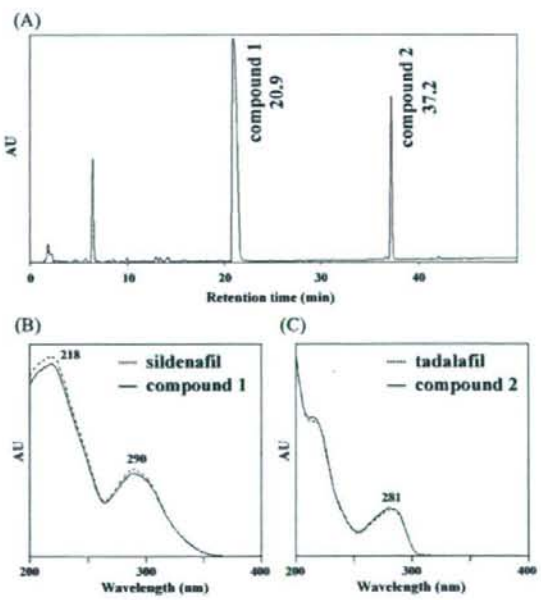

Fig. 2. (A) HPLC Chromatogram of the Sample Solution Monitored Max Plot (200—400 nm) and (B) the Overlaid UV Spectra of Sildenafil and Compound **1**, and (C) the Overlaid UV Spectra of Tadalafil and Compound **2**

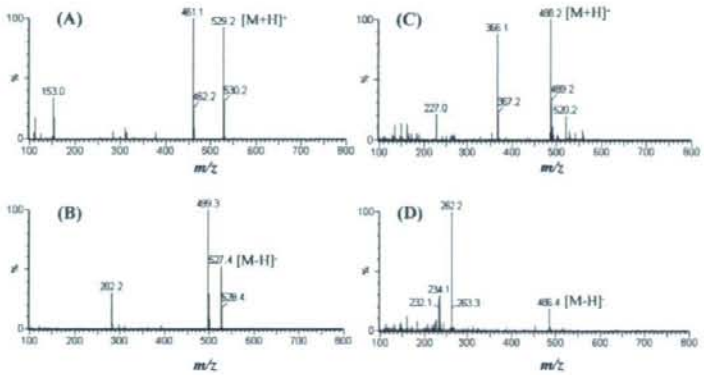

Fig. 3. Mass Spectra of Compounds **1** and **2** by LC-ESI-MS Analysis

(A) Compound **1** (positive, cone voltage: 60 V), (B) compound **1** (negative, cone voltage: −60 V), (C) compound **2** (positive, cone voltage: 30 V), (D) compound **2** (negative, cone voltage: −60 V).

(20.0 min). Furthermore, the peak of **1** exhibited major ion peaks at *m/z* 529 $[M+H]^+$ in the positive scan mode and at *m/z* 527 $[M-H]^-$ in the negative scan mode by LC-ESI-MS analysis (Figs. 3A, B). Also major ion peaks at *m/z* 488 $[M+H]^+$ and at *m/z* 486 $[M-H]^-$ were detected in the peak of **2** (Figs. 3C, D). These data strongly suggested that **1** is a sildenafil analogue and **2** a tadalafil analogue.

Compound **1** formed a colorless amorphous powder and was determined by accurate mass measurement to have the molecular formula $C_{26}H_{36}N_6O_4S$ with a quasimolecular ion at *m/z* 529.2590 (Calcd 529.2597) $[M+H]^+$. The ^{1}H-NMR spectrum of **1** exhibited 35 nonexchangeable protons, including a methyl signal at 4.28 (3H, s), an ethoxyl group of signals at 1.65 (3H, t, *J*=6.9 Hz), 4.37 (2H, q, *J*=6.9 Hz), a *n*-propanyl group of signals at 1.01 (3H, t, *J*=7.3 Hz), 1.86 (2H, sext, *J*=7.3 Hz), 2.92 (2H, t, *J*=7.3 Hz) and ABX-type aromatic proton signals at 7.14 (1H, d, *J*=8.7 Hz), 7.83 (1H, dd, *J*=2.3, 8.7 Hz), 8.83 (1H, d, *J*=2.3 Hz). The ^{13}C-NMR spectrum of **1** showed 3 methyls, 11 methylenes, including an oxygenated carbon (66.1), 4 methines including 3 aromatic carbons (113.0, 131.3, 131.8) and 7 aromatic quaternary carbons (121.1, 124.5, 128.6, 138.4, 146.4, 147.0, 159.3), and a carbonyl group (153.6). These signals are very similar to those of sildenafil (Table 1), except for the disappearance of an *N*-methyl group and the presence of a methine signal at 2.51 (1H, quint, *J*=7.6 Hz) and two sets of equivalent methylene signals at 1.53 (2H, m) and 1.63 (2H, m), and 1.28 (2H, m) and 1.83 (2H, m).

Interpretation of the ^{1}H–^{1}H COSY and HMQC spectra of **1** indicated the presence of a cyclopentyl group (Fig. 4). The connectivity of this group was deduced from the HMBC spectrum (Fig. 4). The methine proton at 2.51 (H-29) of the cyclopentyl group showed correlations to the methylene carbons at 51.2 (C-25, C-27) of sildenafil. These data determined the structure of **1** as 5-[2-ethoxy-5-(4-cyclopentylpiperazin-1-ylsulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one. The assignments of the ^{1}H- and ^{13}C-NMR signals of **1** are summarized in Table 1. Considering its properties, compound **1** is named as cyclopentynafil.

Compound **2** formed a colorless amorphous powder and was determined by accurate mass measurement to have the molecular formula $C_{29}H_{33}N_3O_4$ with a quasimolecular ion at *m/z* 510.2362 (Calcd 510.2363) $[M+Na]^+$. The ^{1}H-NMR spectrum of **2** exhibited 32 nonexchangeable protons, including a methyl signal at 0.89 (3H, t, *J*=7.3 Hz), a methylenedioxyl group signal at 5.85 (2H, d, *J*=6.9 Hz), ABX-type aromatic proton signals at 6.68 (1H, d, *J* =7.8 Hz), 6.78 (1H, d, *J*=1.9 Hz) and 6.79 (1H, dd, *J*=7.8, 1.9 Hz), and AB-type aromatic proton signals at 7.02 (1H, br t, *J*=7.8 Hz), 7.07 (1H, br t, *J*=8.2 Hz), 7.27 (1H, d, *J*= 8.2 Hz) and 7.52 (1H, d, *J*=7.8 Hz). The ^{13}C-NMR spectrum of **2** showed 1 methyl, 9 methylenes, including a methylenedioxy carbon (

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts of Compound **1** and Sildenafil in $CDCl_3$

Position	**1** (^{1}H[a)])	Sildenafil (^{1}H[a)])	**1** (^{13}C[b)])	Sildenafil (^{13}C[b)])
1			147.0	146.4
4			153.6	153.6
5	10.80 s	10.82 s		
6			146.4	147.0
8			138.4	138.4
9			124.5	124.5
10 (3H)	4.28 s	4.28 s	38.2	38.2
11 (2H)	2.92 t (7.3)	2.93 t (7.2)	27.8	27.7
12 (2H)	1.86 sext (7.3)	1.86 sext (7.2)	22.3	22.2
13 (3H)	1.01 t (7.3)	1.02 t (7.2)	14.0	14.0
14			121.1	121.1
15	8.83 d (2.3)	8.82 d (2.4)	131.3	131.2
16			128.6	129.0
17	7.83 dd (2.3, 8.7)	7.84 dd (2.4, 8.6)	131.8	131.7
18	7.14 d (8.7)	7.15 d (8.6)	113.0	113.0
19			159.3	159.3
20 (2H)	4.37 q (6.9)	4.37 q (6.9)	66.1	66.1
21 (3H)	1.65 t (6.9)	1.65 t (6.9)	14.6	14.5
24 (2H)	3.10 br s	3.11 br s	46.1	45.9
25 (2H)	2.59 br s	2.50 br s	51.2	54.0
27 (2H)	2.59 br s	2.50 br s	51.2	54.0
28 (2H)	3.10 br s	3.11 br s	46.1	45.9
29	2.51 quint (7.6)	2.28 (3H) s	66.8	45.7
30 (2H)	1.28 m, 1.83 m		30.4	
31 (2H)	1.53 m, 1.63 m		24.0	
32 (2H)	1.53 m, 1.63 m		24.0	
33 (2H)	1.28 m, 1.83 m		30.4	

a) Recorded in 800 MHz and *J* values (in Hz) in parentheses. *b*) Recorded in 200 MHz.

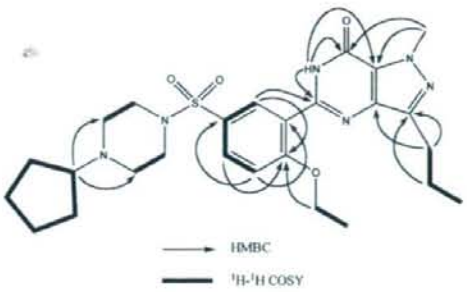

Fig. 4. ^{1}H–^{1}H and Major Long-Range ^{1}H–^{13}C Correlations of Compound **1**

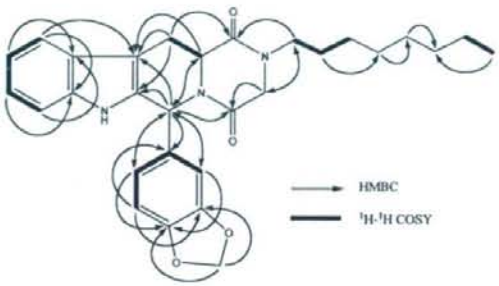

Fig. 5. ^{1}H–^{1}H and Major Long-Range ^{1}H–^{13}C Correlations of Compound **2**

102.4), 9 methines including 7 aromatic carbons (108.2, 109.0, 111.2, 118.9, 120.3, 121.1, 122.8) and 7 aromatic quaternary carbons (106.1, 127.4, 134.6, 137.6, 138.3, 148.3, 149.2), and 2 carbonyl groups (169.1, 169.6). These signals are very similar to those of tadalafil (Table 2), except for the disappearance of an *N*-methyl group and the presence of 7 methylene signals, including an *N*-methylene group signal at 3.49 (2H, br t, J=7.4 Hz) and a methyl signal at 0.89 (3H, t, J=7.3 Hz).

Interpretation of the ^{1}H–^{1}H COSY and HMQC spectra of **2** indicated the presence of an *N*-octyl group (Fig. 5). The connectivity of an *N*-octyl group was deduced from the HMBC spectrum (Fig. 5). The methylene proton at 3.49 (H-13) of the *N*-octyl group showed correlations to the carbonyl carbon at 169.1 (C-1) and a methylene carbon at 51.2 (C-3) of tadalafil. Also, methylene protons at 3.94 and 4.24 (H_2-3) showed correlations to the methylene carbon at 47.2 (C-13). These data determined the planar structure of **2**, as shown in Fig. 5.

The relative configuration between two methine protons at C-6 and C-12a was established a *cis* configuration by the NOE experiment. Furthermore, the CD spectrum of **2** is superimposable with that of tadalafil (Fig. 6), and it is clear that the absolute stereochemistry of two methine protons at C-6 and C-12a are the same as that of tadalafil. These results enabled us to elucidate the structure of **2** as (6*R*,12a*R*)-6-(1,3-benzodioxol-5-yl)-2-octyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′ : 1,6]pyrdo[3,4-*b*]indol-1,4-dione. The assignments of the ^{1}H- and ^{13}C-NMR signals of **2** are summarized in Table 2. Considering its properties, compound **2** is designated as *N*-octylnortadalafil.

Table 2. ^{1}H- and ^{13}C-NMR Chemical Shifts of Compound **2** and Tadalafil in CD_3OD

Position	**2** (^{1}H[a])	Tadalafil (^{1}H[a])	**2** (^{13}C[b])	Tadalafil (^{13}C[b])
1			169.1	168.9
3	4.24 br d (17.4)	4.20 br d (17.0)	51.2	52.9
	3.94 d (17.4)	3.97 d (17.0)		
4			169.6	169.0
6	6.24 s	6.17 s	57.5	58.0
6a			134.6	134.7
7a			138.3	138.4
8	7.27 d (8.2)	7.25 d (8.3)	111.2	112.2
9	7.07 br t (8.2)	7.06 br t (8.3)	122.8	122.7
10	7.02 br t (7.8)	7.02 br t (7.8)	120.3	120.3
11	7.52 d (7.8)	7.51 d (7.8)	118.9	118.9
11a			127.4	127.4
11b			106.1	106.3
12	3.62 dd (15.6, 5.1)	3.66 dd (15.6, 4.6)	24.2	24.7
	3.12 br dd (15.6, 11.5)	3.11 br dd (15.6, 11.9)		
12a	4.44 br dd (11.5, 5.1)	4.40 br dd (11.9, 4.6)	57.4	57.6
13 (2H)	3.49 br t (7.4)	3.03 (3H) s	47.2	33.8
14 (2H)	1.59 m		27.9	
15 (2H)	1.30 m		27.8	
16 (2H)	1.26 m		30.4	
17 (2H)	1.32 m		30.4	
18 (2H)	1.28 m		33.0	
19 (2H)	1.31 m		23.7	
20 (3H)	0.89 t (7.3)		14.4	
1′			137.6	137.7
2′	6.78 d (1.9)	6.80 d (1.4)	108.2	108.3
3′			149.2	149.1
4′			148.3	148.2
5′	6.68 d (7.8)	6.68 d (8.2)	109.0	108.9
6′	6.79 dd (7.8, 1.9)	6.82 dd (8.2, 1.4)	121.1	121.3
7′ (2H)	5.85 d (6.9)	5.84 d (9.1)	102.4	102.4

a) Recorded in 800 MHz and J values (in Hz) in parentheses. *b*) Recorded in 200 MHz.

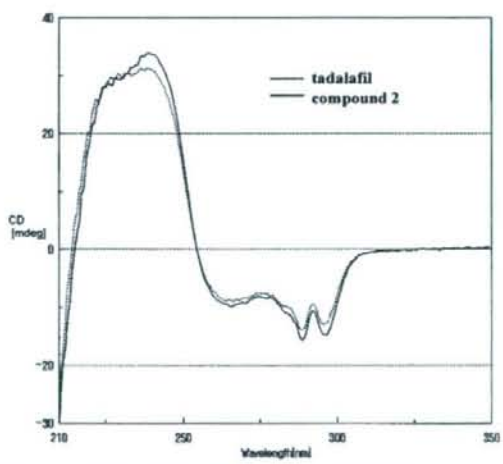

Fig. 6. Overlaid CD Spectra of Tadalafil and Compound **2**

Furthermore, quantitative analyses of **1** and **2** in the supplement product were determined by HPLC. The contents of **1** and **2** in the product were about 130 mg/tablet (301 g/mg) and about 27 mg/tablet (64.1 g/mg), respectively.

Finally, we calculated to make docking models of **1** and **2** bound to PDE-5. Compounds **1** and **2** were well fitted to the cavity of PDE-5 like sildenafil and tadarafil, respectively.

Therefore, both compounds are expected to have inhibitory activities against PDE-5.

In conclusion, a new sildenafil analogue, cyclopentynafil and a new tadalafil analogue, *N*-octylnortadalafil were isolated from a dietary supplement illegally marketed in Japan for erectile dysfunction. Their structures were elucidated by using HPLC-PDA, LC-MS, high-resolution MS, NMR and CD. Recently, Toque *et al.* synthesized a new cyclohexyl type of sildenafil analogue and its IC_{50} value as PDE-5 inhibitor was almost same as sildenafil,[30] whereas cyclopentynafil and *N*-octylnortadalafil are the first compounds reported to be new sildenafil and tadalafil analogues, respectively, and their inhibitory activities against PDE-5 are expected by docking study. Thus, tremendous risk is faced by patients who unknowingly look to dietary supplements, which are adulterated with such analogues for ED treatment.

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References

1) Ministry of Health, Labour and Welfare of Japan, websites: ⟨http://www.mhlw.go.jp/kinkyu/diet/other/050623-1.html⟩
2) Moriyasu T., Shigeoka S., Kishimoto K., Ishikawa F., Nakajima J., Kamimura H., Yasuda I., *Yakugaku Zasshi*, **121**, 765—769 (2001).
3) Zhu X., Xiao S., Chen B., Zhang F., Yao S., Wan Z., Yang D., Han H., *J. Chromatogr. A*, **1066**, 89—95 (2005).
4) Hasegawa T., Saijo M., Ishii T., Nagata T., Haishima, Y., Kawahara N., Goda Y., *J. Food Hyg. Soc. Jpn.*, **49**, 311—315 (2008).
5) Jefferson D. R., Natarajan S., Ganesan A., *Synlett*, **2004**, 1428—1430 (2004).
6) Venhuis B. J., Barends D. M., Zwaagstra M. E., De Kaste D., "RIVM Report 370030001/2007," National Institute for Public Health and the Environment, the Netherlands, 2007.
7) Shin M. H., Hong M. K., Kim W. S., Lee Y. J., Jeoung Y. C., *Food Addit. Contam.*, **20**, 793—796 (2003).
8) Shin C., Hong M., Kim D., Lim Y., *Magn. Reson. Chem.*, **42**, 1060—1062 (2004).
9) Park H. J., Jeong H. K., Chang M. I., Im M. H., Jeong J. Y., Choi D. M., Park K., Hong M. K., Youm J., Han S. B., *Food Addit. Contam.*, **24**, 122—129 (2007).
10) Blok-Tip L., Zomer B., Bakker F., Hartog K. D., Hamzink M., ten Hove J., Vredenbregt M., de Kaste D., *Food Addit. Contam.*, **21**, 737—748 (2004).
11) Hou P., Zou P., Low M. Y., Chan E., Koh H. L., *Food Addit. Contam.*, **23**, 870—875 (2006).
12) Zou P., Hou P., Low M.-Y., Koh H. L., *Food Addit. Contam.*, **23**, 446—451 (2006).
13) Zou P., Hou P., Oh S. S., Low M. Y., Koh H. L., *Rapid Commun. Mass Spectrom.*, **20**, 3488—3490 (2006).
14) Gratz S. R., Flurer, C. L., Wolnik K. A., *J. Pharm. Biomed. Anal.*, **36**, 525—533 (2004).
15) Gratz S. R., Gamble B. M., Flurer R. A., *Rapid Commun. Mass Spectrom.*, **20**, 2317—2327 (2006).
16) Lai K. C., Liu Y. C., Tseng M. C., Lin J. H., *J. Food Drug Anal.*, **14**, 19—23 (2006).
17) Reepmeyer J. C., Woodruff J. T., *J. Chromatogr. A*, **1125**, 67—75 (2006).
18) Reepmeyer J. C., Woodruff J. T., d'Avignon D. A., *J. Pharm. Biomed. Anal.*, **43**, 1615—1621 (2007).
19) Reepmeyer J. C., Woodruff J. T., *J. Pharm. Biomed. Anal.*, **44**, 887—893 (2007).
20) Lam Y. H., Poon W. T., Lai C. K., Chan A.Y. W., Mak T. W. L., *J. Pharm. Biomed. Anal.*, **46**, 804—807 (2008).
21) Zou P., Hou P., Oh S. S., Chong Y. M., Bloodworth B. C., Low M. Y., Koh H. L., *J. Pharm. Biomed. Anal.*, **47**, 279—284 (2008).
22) Cho E. Y., Chung S. H., Kim J. H., Kim D. K., Jin C., *J. Appl. Pharmacol.*, **11**, 232—237 (2003).
23) Kim J. H., Kim Y., Choi K., Kim D. H., Nam G., Seo J. H., WO 2002102802 (2002).
24) Kumasaka K., Kawahara N., Doi K., Kojima T., Goda Y., *Chem. Pharm. Bull.*, **56**, 227—230 (2008).
25) Hosogai N., Hamada K., Tomita M., Nagashima A., Takahashi T., Sekizawa T., Mizutani T., Urano Y., Kuroda A., Sawada K., Ozaki T., Seki J., Goto T., *Eur. J. Pharm.*, **428**, 295—302 (2001).
26) Uchiyama N., Saisho K., Kikura-Hanajiri R., Haishima Y., Goda Y., *Chem. Pharm. Bull.*, **56**, 1331—1334 (2008).
27) Fujino K., Takami H., Atsumi T., Ogasa T., Mohri S., Kasai M., *Org. Process Res. Dev.*, **5**, 426—433 (2001).
28) Onoda Y., Nomoto Y., Ohno T., Yamada K., Ichimura M., WO9808848 (1998).
29) Hirose R., Okumura H., Yoshimatsu A., Irie J, Onoda Y., Nomoto Y., Takai H., Ohno T., Ichimura M., *Eur. J. Pharm.*, **431**, 17—24 (2001).
30) Toque F. H. A., Priviero F. B. M., Teixeira C. E., Perissutti E., Fiorino F., Severino B., Frecentese F., Lorenzetti R., Baracat J., Santagada V., Caliendo G., Antunes E., De Nucci G., *J. Med. Chem.*, **51**, 2807—2815 (2008).

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Letter

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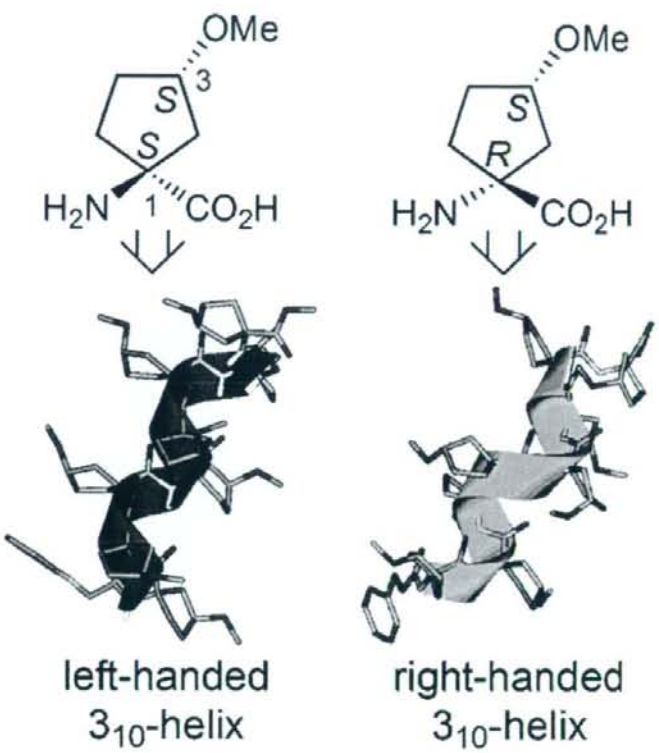

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Helical-Screw Directions of Diastereoisomeric Cyclic α-Amino Acid Oligomers

Masanobu Nagano,[†] Masakazu Tanaka,*[,†,‡] Mitsunobu Doi,[§] Yosuke Demizu,[||] Masaaki Kurihara,[||] and Hiroshi Suemune*[,†]

Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan, Osaka University of Pharmaceutical Sciences, Osaka 569-1094, Japan, and Division of Organic Chemistry, National Institute of Health Sciences, Tokyo 158-8501, Japan

matanaka@nagasaki-u.ac.jp

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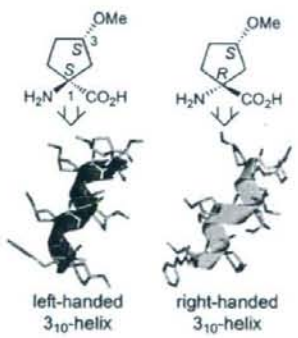
ABSTRACT



Two series of homooligomers composed of diastereoisomeric cyclic α-amino acids having two chiral centers at the α-carbon and the side chain were synthesized, and their preferred secondary structures were studied in solution and in the crystal state. The oligomers are a new class of helical-foldamers possessing two kinds of chiral centers on the helical backbone and at the lateral surface of the helix.

Right-handed α-helical-screw structures in proteins are believed to result from the L-(S)-chiral α-carbon atoms on the peptide backbone.[1] Recently, Toniolo's group and we independently reported that the helical-screw sense of peptide-oligomers can be controlled without a chiral center on the peptide backbone, but by chiral centers at the side chain.[2] However, so far, little attention has been paid to how both chiral centers on the peptide backbone and at the side chain influence the secondary structures of their oligomers.[3] Herein, we designed two diastereoisomeric cyclic amino

[†] Kyushu University.
[‡] Nagasaki University.
[§] Osaka University of Pharmaceutical Sciences.
[||] National Institute of Health Sciences.

(1) Branden, C.; Tooze, J. Introduction to Protein Structure; Garland: New York, 1991; pp 1–31.

(2) (a) Tanaka, M.; Demizu, Y.; Doi, M.; Kurihara, M.; Suemune, H. Angew. Chem., Int. Ed. 2004, 43, 5360–5363. (b) Tanaka, M.; Anan, K.; Demizu, Y.; Kurihara, M.; Doi, M.; Suemune, H. J. Am. Chem. Soc. 2005, 127, 11570–11571. (c) Royo, S.; Borggraeve, W. M. D.; Peggion, C.; Formaggio, F.; Crisma, M.; Jiménez, A. I.; Cativiela, C.; Toniolo, C. J. Am. Chem. Soc. 2005, 127, 2036–2037.

(3) (a) Jiménez, A. I.; Cativiela, C.; Aubry, A.; Marraud, M. J. Am. Chem. Soc. 1998, 120, 9452–9459. (b) Sharma, G.; Reddy, K. R.; Krishna, P. R.; Sankar, A. R.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. Angew. Chem., Int. Ed. 2004, 43, 3961–3965. (c) Andreetto, E.; Peggion, C.; Crisma, M.; Toniolo, C. Biopolymers (Peptide Sci.) 2006, 84, 490–501. (d) Kümin, M.; Sonntag, L.-S.; Wennemers, H. J. Am. Chem. Soc. 2007, 129, 466–467.

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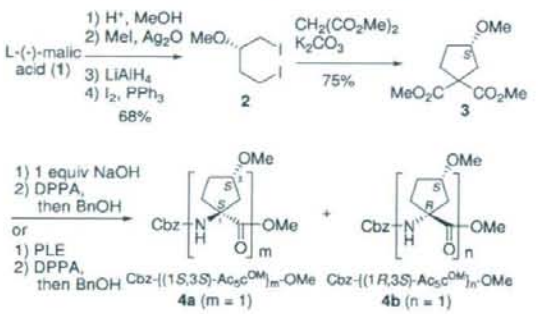
acids,[4] (1S,3S)- and (1R,3S)-1-amino-3-(methoxy)cyclopentanecarboxylic acids (Ac_5c^{OM}), constructed their homooligomers having chiral centers both on the peptide-backbone and at the side chain, and revealed their unique helical structures with high resolution analyses.

Two cyclic amino acids (1S,3S)-Ac_5c^{OM} and (1R,3S)-Ac_5c^{OM} were synthesized starting from L-(−)-malic acid 1 (Scheme 1). After esterification of 1, the secondary alcohol

Scheme 1. Synthesis of (1S,3S)- and (1R,3S)-Ac_5c^{OM}



was converted to a methyl ether. Then, reduction of diester followed by iodination of alcohol gave a diiodide 2. Dialkylation of dimethyl malonate with 2 afforded a cyclopentane diester 3. Monohydrolysis of 3 with an alkaline solution, followed by Curtius rearrangement with diphenylphosphoryl azide (DPPA), produced two separable diastereoisomers (1S,3S)-Ac_5c^{OM} (4a) and (1R,3S)-Ac_5c^{OM} (4b) in a ratio of 3:1 in 85% yield.[5] Meanwhile, hydrolysis of 3 with pig liver esterase (PLE) followed by Curtius rearrangement could change the diastereoselectiviy (4a:4b = 1:2; 84% yield). The stereochemistry of products was unambiguously assigned by the X-ray crystallographic analyses of their oligomers.[6]

Oligomers Cbz-{(1S,3S)-Ac_5c^{OM}}$_m$-OMe {m = 2 (5a), 4 (7a), 6 (8a), 8 (9a), 10 (10a)} and Cbz-{(1R,3S)-Ac_5c^{OM}}$_n$-OMe {n = 2 (5b), 3 (6b), 4 (7b), 6 (8b), 8 (9b), 10 (10b)} were generally prepared by the coupling between N-terminal-free oligomers and N-protected dipeptide acid via solution-phase methods. It should be noted that elongation of N-terminal-free (1R,3S)-Ac_5c^{OM} dimer to a tetramer 7b did not work well because diketopiperazine was formed as a byproduct. Thus, the (1R,3S)-tetramer was prepared via a trimer 6b, which was derived from an N-terminal-free amino acid and the dipeptide acid.[6]

The FT-IR absorption spectra of both (1S,3S)- and (1R,3S)-Ac_5c^{OM} oligomers in $CDCl_3$ solution showed weak bands in the region 3420−3430 cm^{-1} [free (solvated) peptide NH groups] and strong bands at 3320−3380 cm^{-1} (intramolecularly H-bonded peptide NH groups). The latter bands observed at 3380 cm^{-1} in 5a and at 3376 cm^{-1} in 5b shift to lower wavenumbers (3320 cm^{-1} in 10a and 3320 cm^{-1} in 10b), respectively, and the relative intensities increase with elongation of the peptide length. These IR spectra are very similar to those of helical $Ac_{(n)}c$ oligomers.[7]

In the ROESY ^{1}H NMR spectra, the hexamers 8a and 8b showed a complete series of sequential d_{NN} cross-peaks of NOEs from the N-terminal NH(1) to the C-terminal NH(6), respectively.[6] These correlations are characteristic for the helical structure, albeit that those of longer oligomers only gave a partial series of sequential d_{NN} cross-peaks. Addition of DMSO or the free-radical TEMPO in the ^{1}H NMR spectroscopy indicated that the two NH signals [NH(1) and NH(2)] at the N-terminus of (1R,3S)-oligomers 8b and 9b are sensitive (solvent-exposed NH group), respectively, suggesting that the two NH groups are not intramolecularly H-bonded, and formation of a helical structure.[2,6,7] The experiments of (1S,3S)-oligomers 8a and 9a did not give clear results because the relevant NH peaks overlapped.[6]

The CD spectra of tetramers 7 and hexamers 8 in 2,2,2-trifluoroethanol (TFE) solution, both (1S,3S)- and (1R,3S)-Ac_5c^{OM}, did not show characteristic maxima (208 and 222 nm) for the helical structure. These spectra may suggest the presence of both right-handed (P) and left-handed (M) helices or disordered structures. Elongation of oligomer length changed the shapes of CD spectra, and positive maxima at 208 and 222 nm were observed in the (1S,3S)-octamer 9a and decamer 10a while negative maxima were seen in the (1R,3S)-9b and 10b. These CD spectra suggest that the dominant conformation of (1S,3S)-9a and 10a is a left-handed (M) helix and that of (1R,3S)-9b and 10b is a right-handed (P) helix. The CD spectra of (1S,3S)- and (1R,3S)-oligomers showed a pseudosymmetric shape, strongly indicating that the CD curves are the result of approximately enantiomeric global chain helicity (Figure 1).

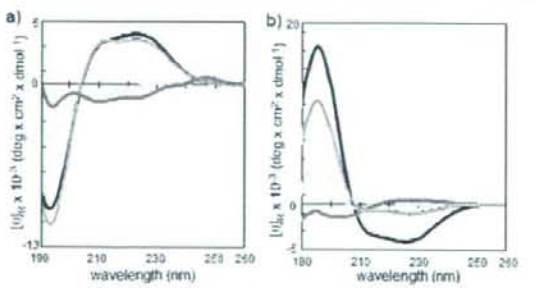


Figure 1. CD spectra of (1S,3S)- and (1R,3S)-oligomers in TFE solution (100 μM). (a) 7−10a: Cbz-{(1S,3S)-Ac_5c^{OM}}$_m$-OMe (m = 4, 6, 8, 10). (b) 7−10b: Cbz-{(1R,3S)-Ac_5c^{OM}}$_n$-OMe (n = 4, 6, 8, 10). Tetramer 7 (green), hexamer 8 (yellow), octamer 9 (blue), and decamer 10 (red).

The X-ray analysis of (1S,3S)-Ac_5c^{OM} hexamer 8a having 12 chiral centers showed both diastereomeric right-handed

(4) (a) Cativiela, C.; D.-de-Villegas, M. D. Tetrahedron: Asymmetry 2000, 11, 645–732. (b) Kotha, S. Acc. Chem. Res. 2003, 36, 342–351.

(5) Nagano, M.; Demizu, Y.; Tanaka, M.; Kurihara, M.; Doi, M.; Suemune, H. Peptide Science 2005; The Japanese Peptide Society: Osaka, Japan, 2006; pp 345−346.

(6) See the Supporting Information.

(P) and left-handed (M) 3_{10}-helices (Figure 2). In contrast, (1S,3S)-octamer 9a crystallized exclusively to left-handed

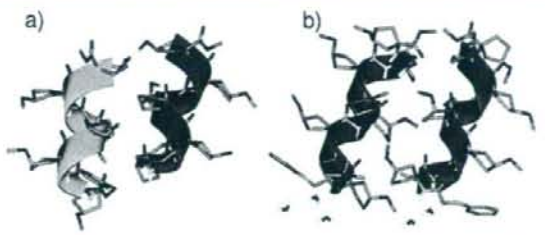


Figure 2. X-ray crystallographic analysis of Cbz-{(1S,3S)-Ac_5c^{OM}}$_m$-OMe {(a) 8a: m = 6, (b) 9a: m = 8}. Red: left-handedness (M), blue: right-handedness (P).

(M) 3_{10}-helices, while in the asymmetric unit there were two crystallographically independent (M) 3_{10}-helical conformers. The diastereomeric (1R,3S)-Ac_5c^{OM} hexamer 8b showed two crystallographically independent right-handed (P) 3_{10}-helices. The (1R,3S)-octamer 9b also showed two conformers, both (P) 3_{10}-helices but in the one conformer the signs of ϕ, ψ torsion angles at the C-terminal residue were opposite. There, six consecutive intramolecular H-bonds of the i←i+3 type (i = 0~5) were found, respectively.[6,8]

In the crystal state, the (1S,3S)-hexamer 8a having 12 chiral centers at the α-carbon and the side chain assumed both (P) and (M) 3_{10}-helices,[2b] while the (1R,3S)-hexamer 8b formed only (P) helices, probably influenced by the crystal packing force. By the elongation of peptide length, i.e., by the increase of chiral centers, both (1S,3S)- and (1R,3S)-oligomers were controlled to form one-handed helical-screw structures in solution and in the crystal state; i.e., the (1S,3S)-octamer 9a formed (M) helices and the (1R,3S)-octamer 9b formed (P) helices. Molecular mechanics/ab initio (RHF/3-21G*) calculations of (1S,3S)-9a produced a (M) 3_{10}-helix as a global minimum-energy (GME) conformation, and those of (1R,3S)-9b gave a (P) 3_{10}-helix as a GME conformation.[6]

At first, we considered each effect of the α-carbon and the side-chain chiral centers or match/mismatch of chiral centers on one-handed helical-screw direction. However, the

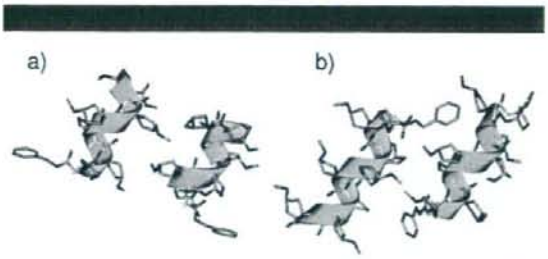


Figure 3. X-ray crystallographic analysis of Cbz-{(1R,3S)-Ac_5c^{OM}}$_n$-OMe [(a) 8b: n = 6, (b) 9b: m = 8].

Ac_5c^{OM} possess a unique chiral structure; i.e., transfer of the MeO-substituent on the γ-positions of cyclopentane in (1S,3S)-4a results in formation of enantiomer (1R,3R) or diastereomer (1R,3S) because the chirality of the α-carbon (C1) is changed (Figure 4). Thus, the helical-screw structures

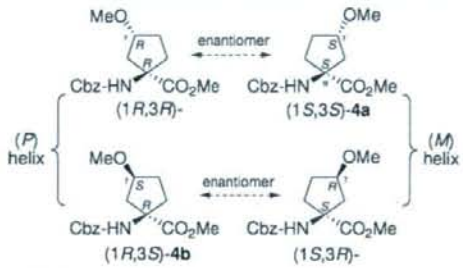


Figure 4. Unique chiral structure of Ac_5c^{OM} and helical-screw direction of their oligomers.

might be controlled by the whole chiral cyclopentane amino acid structure.

In summary, we synthesized two series of the diastereomeric Ac_5c^{OM} oligomers that are a new class of helical-foldamers possessing two kinds of chiral centers on the helical backbone and at the lateral surface of the helix[9] and revealed their preferred helical conformations in solution and in the crystal state. These results might be useful for design of foldamer catalysts and lead compounds, which are currently under investigation in our group.

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Supporting Information Available: Experimental section, spectroscopic data of new compounds, crystallographic details (CIF), and molecular calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) (a) Santini, A.; Barone, V.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Fraternali, F.; Lelj, F.; Pavone, V.; Pedone, C.; Crisma, M.; Bonora, G. M.; Toniolo, C. Int. J. Biol. Macromol. 1988, 10, 292–299. (b) Crisma, M.; Bonora, G. M.; Toniolo, C.; Benedetti, E.; Bavoso, A.; Di Blasio, B.; Pavone, V.; Pedone, C. Int. J. Biol. Macromol. 1988, 10, 300–304.

(8) In some oligomers, inversions of signs or distortions of ϕ, ψ torsion angles at the C-terminal residue are observed. See the Supporting Information.

(9) (a) Gellman, S. H. Acc. Chem. Res. 1998, 31, 173–180. (b) Seebach, D.; Beck, A. K.; Bierbaum, D. J. Chem. Biodiversity 2004, 1, 1111–1239. (c) Wu, C. W.; Kirshenbaum, K.; Sanborn, T. J.; Patch, J. A.; Huang, K.; Dill, K. A.; Zuckermann, R. N.; Barron, A. E. J. Am. Chem. Soc. 2003, 125, 13525–13530. (d) Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C.; Broxterman, Q. B.; Kaptein, B. Biopolymers (Peptide Sci.) 2004, 76, 162–176. (e) Toniolo, C.; Formaggio, F.; Kaptein, B.; Broxterman, Q. B. Synlett 2006, 1295–1310. (f) Tanaka, M. Chem. Pharm. Bull. 2007, 55, 349–358. (g) Maity, P.; König, B. Biopolymers (Peptide Sci.) 2008, 90, 8–27.