Synthesis of C-2 substituted Vitamin D derivatives having ringed side chains and biological evaluation, especially biological effect on bone by modification at C-2 position

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Supporting Information

6. Experimental Section

6.1. Synthesis

6.1.1. General

NMR spectra were measured using a JEOL AL-400 magnetic resonance spectrometer. Infrared spectra data were recorded on a JASCO FTIR-5300 spectrometer. Mass spectra were measured on a Shimadzu LC-MS-IT-TOF. Specific optical rotations were measured using a JASCO P-1030 polarimeter. Purification by flash column chromatography on silica gel was carried out using a Biotage FLASH system. Preparative thin layer chromatography was performed using Merck Kieselgel F^{254} plates. Reversed-phase HPLC was carried out on a Shimadzu LC-2010 system.

6.1.8.

(3*R*,4*S*,5*R*)-3,5-Bis-[(*tert*-butyldimethylsilyl)oxy]-4-[3-(hydroxy)-2,2-ethanopropyl]oxyoct-1-en

-7-yne (14)

To a solution of **13** (305 mg, 1.27 mmol) in pyridine (3 mL) was added pivaloyl chloride (0.24 mL, 1.9 mmol) at 0°C and it was stirred at the same temperature for 45 min. Saturated aq. $NaHCO_3$ and EtOAc were added to the reaction mixture and evaporated. The residue was diluted with EtOAc, washed with brine, and dried over $MgSO_4$, followed by filtration and evaporation. The residue was diluted with CH_2Cl_2, and to the reaction mixture were added 2,6-lutidine (0.7 ml, 6 mmol) and *t*-butyldimethylsilyl trifluoromethanesulfonate (1.15 mL, 5 mmol) at 0°C and stirred at the same temperature for 30 min. Dry MeOH (3 mL) was added to the reaction mixture at room temperature and stirred at the same temperature for 5 min. The reaction mixture was diluted with EtOAc, washed with brine and dried over $MgSO_4$, followed by filtration and evaporation. The residue was diluted with MeOH (5 mL), and sodium methoxide (270 mg, 5 mmol) was added to the solution at room temperature and refluxed for 1 h. Saturated aq. NH_4Cl was added and evaporated. The residue was diluted with EtOAc, washed with brine and dried over $MgSO_4$, followed by filtration and evaporation. The residue was purified by flash column chromatography on silica gel (hexane / EtOAc =95/5) to give **14** (267.8 mg, 0.57 mmol) in 45% yield. $[a]^{25}_D$ -4.7 (c 0.5, $CHCl_3$); IR (film, $CHCl_3$) 3018, 2932, 1219, 1086 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.95 (1H, ddd, *J* = 10.0, 17.3, 7.0 Hz), 5.28 (1H, dq, *J* = 17.3, 1.0 Hz), 5.21 (1H, dq, *J* = 10.3, 0.9 Hz), 4.30 (1H, ddt, *J* = 4.0, 7.0, 0.9 Hz), 3.90-3.85 (2H, m), 3.56 (1H, dd, *J* = 5.9, 10.0 Hz), 3.50-3.42 (3H, m), 3.25 (1H, t, *J* = 6.1 Hz), 2.53 (1H, ddd, *J* = 17.0, 6.7, 2.7 Hz), 2.37 (1H, ddd, *J* = 16.8, 3.0, 5.0 Hz), 2.00 (1H, t, *J* = 2.7 Hz), 0.91 (9H, s), 0.90 (9H, s), 0.58-0.45 (3H, m), 0.40-0.35 (1H, m), 0.11 (3H, s), 0.09 (0H, s), 0.09 (3H, s), 0.08 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 137.9, 117.1, 84.9, 81.0, 80.3, 74.6, 71.0, 70.6, 69.6, 27.2, 25.9, 25.8, 24.3, 18.2, 18.0, 9.2, 8.6, -3.9, -4.3, -4.4, -4.7; EI-LRMS *m/z* 491.2 (M+Na); EI-HRMS calcd for $C_{25}H_{48}O_4Si_2Na$ 491.2983, found 491.2972.

6.1.10. (3*R*,4*S*,5*R*)-3,5-Bis-[(*tert*-butyldimethylsilyl)oxy]-5-[3-hydroxypropyl]oxyoct-1-en-7-yne

(15)

To a solution of **7c** (2.29 g, 4.11 mmol) in EtOH (20 mL) was added (+/-)camphor-10-sulfonic acid (954 mg, 4.11 mmol) at 0°C and it was stirred at the same temperature for 1 h. Saturated aq. $NaHCO_3$ was added to the reaction mixture, which was diluted with EtOAc, washed with H_2O, brine and dried over $MgSO_4$. After filtration and evaporation, the residue was purified by flash column chromatography on silica gel (hexane / EtOAc = 90 / 10) to give **15** (1.64 g, mmol) in 90% yield. $[\alpha]^{25}_D$ -4.82 (c 0.5, $CHCl_3$); IR (film, $CHCl_3$) 3020, 1217 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.92 (1H, ddd, *J* = 10.4, 17.2, 7.0 Hz), 5.26 (1H, dt, *J* = 17.2, 1.3 Hz), 5.21 (1H, dt, *J* = 10.4, 1.3 Hz), 4.29 (1H, ddt, *J* = 7.0, 4.0, 1.3 Hz), 3.87 (2H, dd, *J* = 6.6, 4.0 Hz), 3.81 (2H, dd, *J* = 10.6, 5.6 Hz), 3.75 (1H, br s), 3.46 (1H, dd, *J* = 5.6, 3.9 Hz), 3.01 (1H, s), 2.48 (2H, ddd, *J* = 17.0, 6.0, 3.0 Hz), 2.36 (2H, ddd, *J* = 17.0, 6.0, 3.0 Hz), 2.01 (1H, t, *J* = 2.7 Hz), 1.88-1.70 (2H, m), 0.91 (9H, s), 0.90 (9H, s), 0.10 (6H, s), 0.09 (3H, s), 0.07 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) δ:137.5, 117.0, 85.3, 80.9, 74.6, 72.7, 71.0, 70.5, 62.0, 32.0, 25.9, 25.8, 25.6, 24.3, 18.2, 18.0, -4.1, -4.4, -4.5, -4.8; EI-LRMS *m/z* 465.2 (M+Na); EI-HRMS calcd for $C_{23}H_{46}O_4Si_2Na$ 465.2827, found 365.2834.

6.2.2 (5*Z*,7*E*)-(1*S*,2*S*,3*R*)-2-(3-Hydroxypropyl)-20-[(2,2-dimethylcyclopentanone-(5*E*)-ylidene)] methyl-9,10-seco-5,7,10(19)-pregnatriene-1,3-diol (5b)

Under N_2 atmosphere, a solution of **6** (56.6 mg, 0.15 mmol), **7b** (97.4 mg, 0.18 mmol) and $Pd(PPh_3)_4$ (20 mg, 0.019 mmol) in toluene (1 mL) and Et_3N (1 mL) was stirred at 110°C for 2 h. The reaction mixture was evaporated and purified with PTLC (Merck Kiseigel plate Art. 113794 1 mm, the eluent was hexane/EtOAc = 92/8) to give a crude product (97.9 mg), which was dissolved in dry MeCN (1 mL) and CH_2Cl_2 (1 mL). To the solution was added 1 M H_2SO_4 in MeCN (1.2 mL, 1.2 mmol) at 0°C and it was stirred at the same temperature for 0.5 h. After the usual work up, the crude product (20.7 mg) was obtained with preparative TLC (Merck Kiseigel plate Art. 113794 1 mm, the eluent was CH_2Cl_2 / MeOH = 85 / 15). Further purification with reversed-phase HPLC

(YMC-Pack ODS column, 30-250 mm, 10 mL/min, eluent A: MeCN/H_2O = 5/95, eluent B: MeCN/MeOH/H_2O = 59.5/40/0.5, eluent A/B = 27/73) gave **5b** (10.0 mg, 0.008 mmol) in 13% yield. $[\alpha]^{25}_D$ +160.3 (c 0.1 , $CHCl_3$); IR (film, $CHCl_3$) 3331, 2974, 2928, 2885, 1089, 1051 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.46-6.35 (2H, m), 5.99 (1H, d, *J* = 11.5 Hz), 5.27 (1H, d, *J* = 1.2 Hz), 4.99 (1H, d, *J* = 2.0 Hz), 4.38 (1H, t, *J* = 3.4 Hz), 3.94-3.87 (1H, m), 3.73-3.68 (2H, m), 2.84 (1H, dd, *J* = 12.2, 3.66 Hz), 2.7 (1H, dd, *J* = 13.5, 4.5 Hz), 2.53-2.47 (2H, m), 2.39-2.31 (1H, m), 2.25 (1H, dd, *J* = 12.9, 8.8 Hz), 2.05-1.96 (2H, m), 1.78-1.60 (13H, m), 1.56-1.44 (5H, m), 1.38 (1H, td, *J* = 12.87, 3.8 Hz), 1.18-1.10 (1H, m), 1.07 (3H, s), 1.05 (3H, s), 1.05 (3H, d, J = 7.0 Hz), 0.57 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 211.23, 146.50, 142.80, 142.54, 133.69, 132.90, 124.71, 117.08, 113.68, 73.63, 70.47, 62.91, 56.05, 55.90, 49.06, 46.05, 45.59, 44.28, 40.30, 37.17, 35.44, 30.21, 29.03, 26.82, 23.80, 23.44, 23.30, 22.81, 22.22, 19.25, 12.48; EI-LRMS *m/z* 519.2 (M+Na), 479.2 (M-H_2O+H), 461.2 (M-2H_2O+H); EI-HRMS calcd for $C_{32}H_{48}O_4Na$ (M+Na) 519.3445, found 519.3450.

6.2.3.

(5*Z*,7*E*)-(1*S*,2*S*,3*R*)-2-(3-Hydroxypropyloxy)-20-[(2,2-dimethylcyclopentanone-(5*E*)-ylidene)] methyl-9,10-seco-5,7,10(19)-pregnatrine-1,3-diol (5c)

Under N_2 atmosphere, a solution of **6** (40 mg, 0.105 mmol), **7c** (83 mg, 0.15 mmol) and $Pd(PPh_3)_4$ (20 mg, 0.019 mmol) in toluene (1 mL) and Et_3N (1 mL) was stirred at 110°C for 2 h. The reaction mixture was evaporated and purified with PTLC (Merck Kiseigel plate Art. 113794 1 mm, the eluent was hexane/EtOAc = 90/10) to give a crude product (86.0 mg), which was dissolved in dry CH_2Cl_2 (1 mL) and dry MeCN (1mL), and to the solution was added $LiBF_4$ (56 mg, 0.6 mmol) and 1 M H_2SO_4 in MeCN (60 μL, 0.06 mmol) at 0°C and it was stirred at room temperature for 0.5 h. After the usual work up, the crude product (28.0 mg) was obtained with preparative TLC (Merck Kiseigel plate Art. 113794 1mm, the eluent was EtOAc). Further purification with

reversed-phase HPLC (YMC-Pack ODS column, 30-250 mm, 10 mL/min, eluent A: MeCN/H_2O = 5/95, eluent B: MeCN/MeOH/H_2O = 59.5/40/0.5, eluent A/B = 15/85) gave **5c** (14.3 mg, 0.028 mmol) in 26%yield. $[\alpha]^{25}_D$ = +182.4 (c 0.1, $CHCl_3$); IR (film, $CHCl_3$): 3319, 2974, 2928, 2885, 1381, 1089, 1051 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.43-6.38 (2H, m), 6.01 (1H, d, *J* = 11.2 Hz), 5.38 (1H, d, *J* = 1.0 Hz), 5.08 (1H, d, *J* = 2.0 Hz), 4.45 (1H, d, *J* = 2.2 Hz), 4.10-4.01 (1H, m), 3.91-3.75 (4H, m), 3.38 (1H, dd, *J* = 7.4, 3.3 Hz), 2.83 (1H, dd, *J* = 11.8, 3.8 Hz), 2.68 (1H, dd, *J* = 13.7, 4.4 Hz), 2.63-2.32 (6H, m), 2.27-2.20 (1H, m), 2.05-1.95 (2H, m), 1.90-1.84 (2H, m), 1.80-1.69 (5H, m), 1.60-1.35 (6H, m), 1.14-1.09 (1H, m), 1.07 (3H, s), 1.06 (3H, s), 1.04 (3H, d, *J* = 6.8 Hz), 0.57 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 211.3, 144.2, 142.3, 142.6, 133.7, 131.8, 125.4, 117.3, 116.2, 71.9, 64.5, 68.5, 68.4, 61.3, 56.1, 55.9, 46.1, 45.6, 41.0, 40.3, 37.2, 35.4, 31.9, 31.0, 29.0, 26.9, 23.8, 23.4, 23.3, 22.2, 19.2, 12.5; EI-LRMS *m/z* 535.2 (M+Na), 513.2 (M+H), 495.2 (M-H_2O+H); EI-HRMS calcd for $C_{32}H_{48}O_5$ (M+H) 513.3575, found 513.3572.

6.2.4

(5*Z*,7*E*)-(1*S*,2*S*,3*R*)-2-(2,2-Ethano-3-hydroxypropoxy)-20-[(2,2-dimethylcyclopentanone-(5*E*)-ylidene)]methyl-9,10-seco-5,7,10(19)-pregnatriene-1,3-diol (5d)

Under N_2 atmosphere, a solution of **6** (30 mg, 0.08 mmol), **7d** (35 mg, 0.06 mmol) and $Pd(PPh_3)_4$ (11 mg, 0.01 mmol) in toluene (1 mL) and Et_3N (1 mL) was stirred at 110°C for 3 h. The reaction mixture was evaporated and purified with PTLC (Merck Kiseigel plate Art. 113794 1 mm, the eluent was hexane/EtOAc = 90/10) to give a crude product (41.5 mg), which was dissolved in THF (0.6 mL). Tetrabutyl ammonium fluoride (1 M in THF, 0.4 mL, 0.4 mmol) was added to the solution, and the reaction mixture was stirred at 60°C for 1 h. After the usual work up, the crude product was purified with preparative TLC (Merck Kiseigel plate Art. 113794 1 mm, the eluent was hexane / EtOAc = 15/85). Further purification with reversed-phase HPLC (YMC-Pack ODS-AM column, 20-250 mm, 15 mL/min, eluent A: MeCN/H_2O = 5/95, eluent B: MeCN/MeOH/H_2O = 59.5/40/0.5,

eluent A/B = 20/80) gave **5d** (6.2 mg, 0.012 mmol) in 19% yield. $[\alpha]^{26}_{D}$ +83.32 (c 0.1, EtOH); IR (film, $CHCl_3$) 3346, 2974, 2885, 1089, 1051 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.43-6.38 (2H, m), 6.01 (1H, d, *J* = 11.5 Hz), 5.38 (1H, d, *J* = 1.0 Hz), 5.08 (1H, d, *J* = 1.7 Hz), 4.43 (1H, s), 4.07 (1H, td, *J* = 8.2, 4.7 Hz), 3.71 (1H, d, *J* = 11.0 Hz), 3.61 (1H, d, *J* = 11.0 Hz), 3.58 (1H, d, *J* = 11.0 Hz), 3.55 (1H, d, *J* = 11.00 Hz), 3.39 (1H, dd, *J* = 8.0, 3.5 Hz), 2.86-2.49 (7H, m), 2.40-2.32 (1H, m), 2.24 (1H, dd, *J* = 12.9, 9.3 Hz), 2.05-1.68 (8H, m), 1.60-1.35 (6H, m), 1.15-1.09 (2H, m), 1.07 (3H, s), 1.06 (3H, s), 1.04 (3H, d, *J* = 6.6 Hz), 0.57 (3H, s), 0.56-0.52 (4H, m). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 211.6, 144.5, 143.3, 142.9, 134.0, 132.1, 125.7, 117.7, 116.6, 85.0, 72.3, 69.4, 68.7, 56.4, 56.2, 46.4, 45.9, 41.3, 40.7, 37.5, 35.8, 29.4, 27.2, 24.1, 23.8, 23.6, 22.9, 22.5, 19.5, 12.8, 9.3, 9.1; EI-LRMS *m/z* 556.2 ($M+H_2O$), 539.3 (M+H); EI-HRMS calcd for $C_{34}H_{50}O_5Na$ (M+Na) 561.3550, found 561.3539.

6.2.5

(5*Z*,7*E*)-(1*S*,2*S*,3*R*)-2-(3-hydroxy-3-methylbutyloxy)-20-[(2,2-dimethylcyclopentanone-(5*E*)-yli dene)]methyl-9,10-seco-5,7,10(19)-pregnatriene-1,3-diol (5e)

Under N_2 atmosphere, a solution of **6** (34 mg, 0.0897 mmol), **7e** (40.6 mg, 0.0747 mmol) and $Pd(PPh_3)_4$ (11mg, 0.01mmol) in toluene (1 mL) and Et_3N (1 mL) was stirred at 110°C for 3 h. The reaction mixture was evaporated and purified with PTLC (Merck Kiseigel plate Art. 113794 1 mm, the eluent was hexane/EtOAc = 95/5) to give a crude product (63.2 mg), which was dissolved in THF (1 mL). Tetrabutyl ammonium fluoride (1 M in THF, 0.6 mL, 0.6 mmol) was added to the solution and the reaction mixture was stirred at 60°C for 1 h. After the usual work up, the crude product was purified with preparative TLC (Merck Kiseigel plate Art. 113794 1 mm, the eluent was hexane / EtOAc = 25/75). Further purification with reversed-phase HPLC (YMC-Pack ODS-AM column, 20-250 mm, 15 mL/min, eluent A: $MeCN/H_2O$ = 5/95, eluent B: $MeCN/MeOH/H_2O$ = 59.5/40/0.5, eluent A/B = 28/72) gave **5e** (10.0 mg, 0.018 mmol) in 24% yield. $[\alpha]^{25}_{D}$ +87.2 (c 0.1,

EtOH); IR (film, $CHCl_3$) 3425, 3240, 2968, 2887, 1454, 1419, 1381, 1089, 1057, 1043 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.42-6.37 (2H, m), 6.00 (1H, d, *J* = 11. 2 Hz), 5.38 (1H, s), 5.08 (1H, d, *J* = 2.0 Hz), 4.44 (1H, s), 4.09-3.79 (3H, m), 3.39 (1H, dd, *J* = 7.2, 3.3 Hz), 2.83 (1H, dd, *J* = 12.3, 3.8 Hz), 2.71-2.32 (7H, m), 2.24 (1H, dd, *J* = 13.5, 8.7 Hz), 2.02-1.33 (20H, m), 1.29 (3H, s), 1.28 (3H, s), 1.16-1.08 (1H, m), 1.07 (3H, s), 1.06 (3H, s), 1.04 (3H, d, *J* = 6.83 Hz), 0.57 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 211.2, 144.3, 143.0, 142.6, 133.7, 131.8, 125.4, 117.3, 115.9, 84.8, 71.7, 70.8, 68.1, 67.4, 56.1, 55.6, 46.1, 45. 6, 41.6, 40.9, 40.3, 37.2, 35.4, 29.8, 29.5, 29.0, 26.9, 23.8, 23.4, 23.3, 22.2, 19.2, 12.4; EI-LRMS *m/z* 563.3 (M+Na), 523.2 (M-H_2O+H), 505.2 (M-2H_2O+H); EI-HRMS calcd for $C_{34}H_{52}O_5Na$ (M+Na) 563.3707, found 563.3708.

6.2.6

(5*Z*,7*E*)-(1*S*,2*S*,3*R*)-2-(3-cyanopropoxy)-20-[(2,2-dimethylcyclopentanone-(5*E*)-ylidene)]methyl -9,10-seco-5,7,10(19)-pregnatriene-1,3-diol (5f)

Under N_2 atmosphere, a solution of **6** (30 mg, 0.079 mmol), **7f** (39.3 mg, 0.087 mmol) and $Pd(PPh_3)_4$ (20 mg, 0.019 mmol) in toluene (1 mL) and Et_3N (1 mL) was stirred at 110°C for 1.5 h. The reaction mixture was evaporated and purified with PTLC (Merck Kiseigel plate Art. 113794 1 mm, the eluent was hexane/EtOAc = 92/8) to give a crude product (63.1 mg), which was dissolved in dry MeCN (1 mL) and toluene (1 mL). To the solution was added 1 M H_2SO_4 in MeCN (0.84 mL, 0.84 mmol) at 0°C and it was stirred at the same temperature for 0.5 h. After the work up, the crude product (20.7 mg) was obtained with preparative TLC (Merck Kiseigel plate Art. 113794 0.5 mm, the eluent was hexane / acetone = 67 / 33). Further purification with reversed-phase HPLC (YMC-Pack ODS column, 30-250 mm, 10 mL/min, eluent A: MeCN/H_2O = 5/95, eluent B: MeCN/MeOH/H_2O = 59.5/40/0.5, eluent A/B = 25/75) gave **5f** (13.9 mg, 0.026 mmol) in 33% yield. $[\alpha]^{25}_D$ +111.6 (c 0.1 , EtOH); IR (film, $CHCl_3$) 3352, 2974, 2928, 2885, 1089, 1049 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.44-6.38 (2H, m), 6.01 (1H, d, *J* = 11.2 Hz), 5.38 (1H, s), 5.09 (1H, d, *J* =

1.5 Hz), 4.43 (1H, t, *J* = 3.9 Hz), 4.11-4.07 (1H, m), 3.87-3.67 (2H, m), 3.39 (1H, dd, *J* = 7.6, 3.2 Hz), 2.84 (1H, dd, *J* = 12.2, 3.9 Hz), 2.68 (1H, dd, *J* = 13.7, 4.4 Hz), 2.54-2.50 (4H, m), 2.39-2.22 (2H, m), 2.13-1.95 (6H, m), 1.76-1.68 (3H, m), 1.54-1.10 (6H, m), 1.07 (3H, s), 1.06 (3H, s), 1.05 (3H, d, *J* = 7.0 Hz), 0.58 (3H, s); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 211.2, 144.1, 143.2, 142.5, 133.7, 131.5, 126. 6, 119.6, 117.3, 116.1, 84.5, 71.8, 68.2, 67.6, 56.1, 55.9, 46.1, 44.6, 40.9, 40.3, 37.2, 35.4, 29.1, 26.9, 25.8, 23.8, 23.4, 23.3, 22.2, 19.2, 14.3, 12.5; EI-LRMS *m/z* 544.2 (M+Na), 539.3 (M+H_2O), 522.3 (M+H), 504.2 (M-H_2O+H); EI-HRMS calcd for $C_{33}H_{47}NO_4Na$ (M+Na) 544.3397, found 544.3397.

6.2.7

(5*Z*,7*E*)-(1*S*,2*S*,3*R*)-2-(2-cyano-2,2-ethanoethoxy)-20-[(2,2-dimethylcyclopentanone-(5E)-ylide ne)]methyl-9,10-seco-5,7,10(19)-pregnatriene-1,3-diol (5g)

Under N_2 atmosphere, a solution of **6** (32 mg, 0.085 mmol), **7g** (30 mg, 0.065 mmol) and $Pd(PPh_3)_4$ (11 mg, 0.01 mmol) in toluene (1 mL) and Et_3N (1 mL) was stirred at 110°C for 3 h. The reaction mixture was evaporated and purified with PTLC (Merck Kiseigel plate Art. 113794 1 mm, the eluent was hexane/EtOAc = 90/10) to give a crude product (40.2 mg), which was dissolved in THF (1 mL). Tetrabutyl ammonium fluoride (1 M in THF, 0.34 mL, 0.34 mmol) was added to the solution, and the reaction mixture was stirred at 60°C for 1 h. After the work up, the crude product was purified with preparative TLC (Merck Kiseigel plate Art. 113794 1 mm, the eluent was hexane / EtOAc = 25/75). Further purification with reversed-phase HPLC (YMC-Pack ODS-AM column, 20-250 mm, 15 mL/min, eluent A: MeCN/H_2O = 5/95, eluent B: MeCN/MeOH/H_2O = 59.5/40/0.5, eluent A/B = 25/75) gave **5g** (6.5 mg, 0.012 mmol) in 19% yield. $[\alpha]^{25}_D$ +126.7 (c 0.1, EtOH); IR (film, $CHCl_3$) 3285, 2974, 2928, 2885, 1381, 1089, 1051 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.44-6.38 (2H, m), 6.01 (1H, d, *J* = 11.2 Hz), 5.39 (1H, s), 5.10 (1H, d, *J* = 1.7 Hz), 4.41 (1H, t, *J* = 3.9 Hz), 4.15-4.08 (1H, m), 3.67-3.62 (2H, m), 3.46 (1H, dd, *J* = 7.3, 3.2 Hz), 2.84 (1H,

dd, *J* = 12.1, 3.8 Hz), 2.72 (1H, dd, *J* = 13.8, 4.5 Hz), 2.54-2.47 (2H, m), 2.42-2.22 (4H, m), 2.00-1.96 (2H, m), 1.76-1.68 (6H, m), 1.61-1.33 (8H, m), 1.17-1.08 (1H, m), 1.07 (3H, s), 1.06 (3H, s), 1.05 (3H, d, J = 8.0 Hz), 1.00 (2H, q, *J* = 4.0 Hz), 0.58 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 211.2, 144.1, 143.2, 142.5, 133.7, 131.5, 125.6, 119.6, 117.2, 116.1, 84.5, 71.8, 68.2, 67.6, 56.1, 55.9, 46.1, 46.0, 40.9, 40.3, 37.2, 35.4, 29.1, 26.9, 25.8, 23.8, 23.5, 23.3, 22.2, 19.2, 14.4, 12.4; EI-LRMS *m/z* 556.2 (M+Na), 551.3 (M+H_2O), 534.3 (M+H), 516.2 (M-H_2O+H); EI-HRMS calcd for $C_{34}H_{47}NO_4Na$ (M+Na) 556.3397, found 556.3406.

6.2.8

(5*Z*,7*E*)-(1*S*,2*S*,3*R*)-2-(2-ethoxycarbonylethoxy)-20-[(2,2-dimethylcyclopentanone-(5*E*)-ylidene)]methyl-9,10-seco-5,7,10(19)-pregnatriene-1,3-diol (5h)

Under N_2 atmosphere, a solution of **6** (57.9 mg, 0.153 mmol), **7h** (56.9 mg, 0.117 mmol) and $Pd(PPh_3)_4$ (20 mg, 0.019 mmol) in toluene (1 mL) and Et_3N (1 mL) was stirred at 110°C for 2.5 h. The reaction mixture was evaporated and purified with PTLC (Merck Kiseigel plate Art. 113794 1 mm, the eluent was hexane/EtOAc = 86/14) to give a crude product (71.2 mg), which was dissolved in dry MeCN (1 mL) and toluene (1 mL). To the solution was added 1 M H_2SO_4 in MeCN (1.0 mL, 1.0 mmol) at 0°C and it was stirred at the same temperature for 0.5 h. After the work up, the crude product (32.8 mg) was obtained with preparative TLC (Merck Kiseigel plate Art. 113794 0.5 mm, the eluent was hexane / acetone = 67 / 33). Then, 8.8 mg of the crude product was purified with reversed-phase HPLC (YMC-Pack ODS column, 30-250 mm, 10 mL/min, eluent A: MeCN/H_2O = 5/95, eluent B: MeCN/MeOH/H_2O = 59.5/40/0.5, eluent A/B = 20/80) gave **5h** (4.3 mg, 0.0078 mmol) in 25% yield. $[\alpha]^{25}_D$ +76.1 (c 0.1, EtOH); IR (film, $CHCl_3$) 3375, 3288, 2974, 2928, 2891, 1381, 1089, 1049 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.44-6.37 (2H, m), 6.01 (1H, d, *J* = 11.2 Hz), 5.4 (1H, d, *J* = 2.0 Hz), 5.09 (1H, d, *J* = 2.0 Hz), 4.39 (1H, d, *J* = 2.7 Hz), 4.18 (2H, q, *J* = 7.2 Hz), 3.95-3.90 (1H, m), 3.78-3.72 (1H, m), 3.70-3.64 (3H, m), 2.84 (1H, dd, *J* = 4.0, 12.0

Hz), 2.68 (1H, dd, *J* = 13.7, 4.4 Hz), 2.64-2.55 (2H, m), 2.53-2.46 (3H, m), 2.39-2.31 (1H, m), 2.18 (1H, t, *J* = 11.1 Hz), 2.02-1.96 (2H, m), 1.80-1.67 (6H, m), 1.56-1.34 (6H, m), 1.27 (3H, t, *J* = 8.0 Hz), 1.14-1.10 (1H, m), 1.07 (3H, s), 1.07 (3H, s), 1.06 (3H, s), 1.04 (4H, d, *J* = 6.6 Hz), 0.57 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 211.3, 172.4, 143.3, 142.9, 142. 6, 136.7, 131.9, 125.1, 117.8, 117.5, 78.6, 74.4, 74.7, 65.1, 60.9, 56.1, 55.9, 46.0, 40.6, 40.4, 38.9, 37.3, 35.4, 35.0, 29.0, 27.0, 23.8, 23.4, 23.3, 22.2, 19.2, 14.2, 12.5; EI-LRMS *m/z* 577.2 (M+Na), 555.2 (M+H), 537.2 (M-H_2O+H), 519.2 (M-2H_2O+H); EI-HRMS calcd for $C_{34}H_{50}O_6Na$ (M+Na) 577.3500, found 577.3495.