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

Hiroshi Saitoh†*, Takayuki Chida†, Kenichiro Takagi†, Kyohei Horie†, Yoshiyuki Sawai‡, Yuko Nakamura†, Yoshifumi Harada†, Kazuya Takenouchi†, and Atsushi Kittaka‡

†Teijin Institute for Bio-Medical Research, Asahigaoka, Hino, Tokyo 191-8512, Japan
‡Faculty of Pharmaceutical Sciences, Teikyo University, Sagamihara, Kanagawa 252-5195, Japan

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 F254 plates. Reversed-phase HPLC was carried out on a Shimadzu LC-2010 system.

6.1.8.

(3R,4S,5R)-3,5-Bis-[(( tert-butyl dimethylsilyl) 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. NaHCO₃ and EtOAc were added to the reaction mixture and evaporated. The residue was diluted with EtOAc, washed with brine, and dried over MgSO₄, followed by filtration and evaporation. The residue was diluted with CH₂Cl₂, and to the reaction mixture were added 2,6-lutidine (0.7 ml, 6 mmol) and tert-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 MgSO₄, 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. NH₄Cl was added and evaporated. The residue was diluted with EtOAc, washed with brine and dried over MgSO₄, 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. [α]₂⁵° D -4.7 (c 0.5, CHCl₃); IR (film, CHCl₃) 3018, 2932, 1219, 1086 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) 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). ¹³C-NMR (100 MHz, CDCl₃) δ: 137.9, 117.1, 84.9, 81.0, 80.3, 74.6, 71.0, 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 C₂₅H₄₈O₄Si₂Na 491.2983, found 491.2972.

6.1.10. (3R,4S,5R)-3,5-Bis-[((tert-butyldimethylsilyl)oxy]-5-[3-hydroxypropyl]oxyoct-1-en-7-yne
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. NaHCO₃ was added to the reaction mixture, which was diluted with EtOAc, washed with H₂O, brine and dried over MgSO₄. 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, CHCl₃); IR (film, CHCl₃) 3020, 1217 cm⁻¹; \(^1\)H-NMR (400 MHz, CDCl₃) \(\delta\): 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, CDCl₃) \(\delta\):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 C₂₃H₄₆O₄Si₂Na 465.2827, found 365.2834.

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

Under N₂ atmosphere, a solution of 6 (56.6 mg, 0.15 mmol), 7b (97.4 mg, 0.18 mmol) and Pd(PPh₃)₄ (20 mg, 0.019 mmol) in toluene (1 mL) and Et₃N (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 CH₂Cl₂ (1 mL). To the solution was added 1 M H₂SO₄ 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 CH₂Cl₂ / MeOH = 85 / 15). Further purification with reversed-phase HPLC
(YMC-Pack ODS column, 30-250 mm, 10 mL/min, eluent A: MeCN/H2O = 5/95, eluent B: MeCN/MeOH/H2O = 59.5/40/0.5, eluent A/B = 27/73) gave 5b (10.0 mg, 0.008 mmol) in 13% yield. [α]25 D +160.3 (c 0.1, CHCl3); IR (film, CHCl3) 3331, 2974, 2928, 2885, 1089, 1051 cm⁻¹; 

1H-NMR (400 MHz, CDCl3) 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); 13C-NMR (100 MHz, CDCl3) 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-H2O+H), 461.2 (M-2H2O+H); EI-HRMS calcd for C32H48O4Na (M+Na) 519.3445, found 519.3450.

6.2.3.

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

Under N2 atmosphere, a solution of 6 (40 mg, 0.105 mmol), 7c (83 mg, 0.15 mmol) and Pd(PPh3)4 (20 mg, 0.019 mmol) in toluene (1 mL) and Et3N (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 CH2Cl2 (1 mL) and dry MeCN (1mL), and to the solution was added LiBF4 (56 mg, 0.6 mmol) and 1 M H2SO4 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/H2O = 5/95, eluent B: MeCN/MeOH/H2O = 59.5/40/0.5, eluent A/B = 15/85) gave 5c (14.3 mg, 0.028 mmol) in 26% yield. \([\alpha]_{25}^D = +182.4 \text{ (c 0.1, CHCl}_3\); IR (film, CHCl3): 3319, 2974, 2928, 2885, 1381, 1089, 1051 cm\(^{-1}\); \(^1\)H-NMR (400 MHz, CDCl3) \(\delta\): 6.43-6.38 (2H, m), 6.01 (1H, d, \(J = 11.2 \text{ Hz}\), 5.38 (1H, d, \(J = 1.0 \text{ Hz}\)), 5.08 (1H, d, \(J = 2.0 \text{ Hz}\)), 4.45 (1H, d, \(J = 2.2 \text{ Hz}\)), 4.10-4.01 (1H, m), 3.91-3.75 (4H, m), 3.38 (1H, dd, \(J = 7.4, 3.3 \text{ Hz}\)), 2.83 (1H, dd, \(J = 11.8, 3.8 \text{ Hz}\)), 2.68 (1H, dd, \(J = 13.7, 4.4 \text{ Hz}\)), 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, \(J = 6.8 \text{ Hz}\)), 0.57 (3H, s); \(^13\)C-NMR (100 MHz, CDCl3) \(\delta\): 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-H2O+H); EI-HRMS calcd for C\(_{32}\)H\(_{48}\)O\(_5\) (M+H) 513.3575, found 513.3572.

6.2.4

\((5Z,7E)-(1S,2S,3R)-2-(2,2-Ethano-3-hydroxypropoxy)-20-[(2,2-dimethylcyclopentanone-(5E)-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\(_3\)N (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/H2O = 5/95, eluent B: MeCN/MeOH/H2O = 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$_3$) $\delta$: 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$_3$) $\delta$: 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$_2$O), 539.3 (M+H); EI-HRMS calcd for C$_{34}$H$_{50}$O$_5$Na (M+Na) 561.3550, found 561.3539.

6.2.5

(5Z,7E)-(1S,2S,3R)-2-(3-hydroxy-3-methylbutyloxy)-20-[(2,2-dimethylcyclopentanone-(5E))-ylidene)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$_3$N (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$_2$O = 5/95, eluent B: MeCN/MeOH/H$_2$O = 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, CHCl₃) 3425, 3240, 2968, 2887, 1454, 1419, 1381, 1089, 1057, 1043 cm⁻¹; 
¹H-NMR (400 MHz, CDCl₃) 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). 
¹³C-NMR (100 MHz, CDCl₃) 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-H₂O+H), 505.2 (M-2H₂O+H); EI-HRMS calcld for C₃₄H₅₂O₅Na (M+Na) 563.3707, found 563.3708.

6.2.6

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

Under N₂ atmosphere, a solution of 6 (30 mg, 0.079 mmol), 7f (39.3 mg, 0.087 mmol) and Pd(PPh₃)₄ (20 mg, 0.019 mmol) in toluene (1 mL) and Et₃N (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 H₂SO₄ 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/H₂O = 5/95, eluent B: MeCN/MeOH/H₂O = 59.5/40/0.5, eluent A/B = 25/75) gave 5f (13.9 mg, 0.026 mmol) in 33% yield. [
[α]D
25
]+111.6 (c 0.1 , EtOH); IR (film, CHCl₃) 3352, 2974, 2928, 2885, 1089, 1049 cm⁻¹; 
¹H-NMR (400 MHz, CDCl₃) 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$_3$) $\delta$: 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$_2$O), 522.3 (M+H), 504.2 (M-H$_2$O+H); EI-HRMS calcd for C$_{33}$H$_{47}$NO$_4$Na (M+Na) 544.3397, found 544.3397.

6.2.7

(5Z,7E)-(1S,2S,3R)-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$_3$N (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$_2$O = 5/95, eluent B: MeCN/MeOH/H$_2$O = 59.5/40/0.5, eluent A/B = 25/75) gave 5g (6.5 mg, 0.012 mmol) in 19% yield. [α]$_{25}^D$ +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$_3$) $\delta$: 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, \( J = 12.1, 3.8 \text{ Hz} \), 2.72 (1H, dd, \( J = 13.8, 4.5 \text{ Hz} \)), 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, \( J = 8.0 \text{ Hz} \)), 1.00 (2H, q, \( J = 4.0 \text{ Hz} \)), 0.58 (3H, s). \(^{13}\text{C-NMR} (100 \text{ MHz, CDCl}_3) \delta:\)
\[
211.2, 144.1, 143.2, 142.5, 133.7, 131.5, 125.6, 119.6, 117.2, 116.1, 84.5, 71.8, 68.2, 67.6, 56.1, 55.9, 46.1, 46.0, 40.9, 40.3, 37.2, 35.4, 29.1, 26.9, 25.8, 23.8, 23.5, 23.3, 22.2, 19.2, 14.4, 12.4; \]
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\(_4\)Na (M+Na) 556.3397, found 556.3406.

6.2.8

\((5Z,7E)-(1S,2S,3R)-2-(2-ethoxycarbonylethoxy)-20-[(2,2-dimethylcyclopentanone-(5E)-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\(_3\)N (1 mL) was stirred at 110\(^\circ\)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\(_2\)SO\(_4\) in MeCN (1.0 mL, 1.0 mmol) at 0\(^\circ\)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\(_2\)O = 5/95, eluent B: MeCN/MeOH/H\(_2\)O = 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\(^{-1}\); \(^1\text{H-NMR} (400 \text{ MHz, CDCl}_3) \delta:\) 6.44-6.37 (2H, m), 6.01 (1H, d, \( J = 11.2 \text{ Hz} \)), 5.4 (1H, d, \( J = 2.0 \text{ Hz} \)), 5.09 (1H, d, \( J = 2.0 \text{ Hz} \)), 4.39 (1H, d, \( J = 2.7 \text{ Hz} \)), 4.18 (2H, q, \( J = 7.2 \text{ Hz} \)), 3.95-3.90 (1H, m), 3.78-3.72 (1H, m), 3.70-3.64 (3H, m), 2.84 (1H, dd, \( J = 4.0, 12.0 \text{ Hz} \)).
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$_3$) $\delta$: 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-$\text{H}_2\text{O}$+H), 519.2 (M-2$\text{H}_2\text{O}$+H); EI-HRMS calcd for C$_{34}$H$_{50}$O$_6$Na (M+Na) 577.3500, found 577.3495.