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Semi-synthesis and anti-tumor evaluation of novel 25-hydroxyprotopanaxadiol derivatives as apoptosis inducing agents

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Supporting Information

Section A: Chemistry

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded (in KBr) on a FT-IR 1730 (PerkineElmer, USA). The ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV-300 spectrometer in a $CDCl_3$ solution using tetramethylsilane as the internal standard. Chemical shifts (δ) are reported in ppm, and coupling constants (J) are reported in Hz. Proton coupling patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), double doublet(dd), multipet (m) and broad (br). ESIMS were measured on a Bruker micro-TOF-Q mass spectrometer. The major chemicals for the synthesis of compounds were purchased from Sigma–Aldrich and Fluka and of analytical grade. Solvents were dried by refluxing with the appropriate drying agents and distilled before use. Silica gel (200–300 mesh, Qingdao city, China) was used for column Chromatography.

Section B: General procedure for semi-synthesis of compounds 1x-9x, 1y-6y and 1z-4z

The 25-OH-PPD (1 equiv, 46 mg, 0.1 mmol) was added to a solution of DCC (2 equiv, 25 mg), DMAP (2 equiv, 41 mg) or CDI (2 equiv, 21 mg, for compounds **5x**, **6x**, **7x** and **6y**) and N-Boc- leucine (1 equiv) in dry dichloromethane (50 mL) and the reaction mixtures were shaken for 24 h. The solvent was removed under reduced pressure to give a white solid. The white solid was dissolved in ethyl ether and washed with $NaHCO_3$ (5%), dried ($MgSO_4$) and concentrated under reduced pressure to give the crude product. The crude products were chromatographed by silica gel and eluted with petroleum ether/ethyl acetate (4:1, 2:1 and 1:1) to give the pure products **1z**, **1y** and **1x**. Using the same procedure described above for the other compounds.

(20R)-3β-O-(Boc-L-leucyl)-dammarane-12β, 20, 25-triol (**1x**). White solid; Yield: 71%; Mp: 245–248 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.87 (m, 1H, 3-CH), 4.27 (s, 1H, CH-CO), 3.48 (td, J = 12.8, 5.0 Hz, 1H, 12-CH), 1.43 (s, 18H), 1.21 (s, 9H), 1.12 (s, 3H), 0.98 (s, 3H), 0.90 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 38.5 (C-1), 23.7 (C-2), 82.0 (C-3), 38.1 (C-4), 56.1 (C-5), 18.3 (C-6), 34.8 (C-7), 39.9 (C-8), 50.0 (C-9), 37.1 (C-10), 31.4 (C-11), 71.2 (C-12), 49.3 (C-13), 51.7 (C-14), 31.1 (C-15), 26.5 (C-16), 50.2 (C-17), 16.3 (C-18), 16.7 (C-19), 74.3 (C-20), 23.1 (C-21), 42.2 (C-22), 18.3 (C-23), 44.3 (C-24), 70.8 (C-25), 29.6 (C-26), 29.5 (C-27), 28.2 (C-28), 15.8 (C-29), 17.3 (C-30), 173.3 (Leu-COO), 155.5 (Boc-CO-NH), 79.8 (Boc-C-CO), 48.5 (NH-CH), 34.7 (Leu-CH), 28.5 (Boc-3×CH_3), 24.9 (Leu-CH_2), 25.1, 23.8 (Leu-CH_3); IR $_{max}$ (cm^{-1}): 3394 (OH), 2960 (CH_3), 1734 (C=O), 1384 (CH_3). HR-ESIMS: m/z 714.5263 $[M+Na]^+$ (calcd for $C_{41}H_{73}NNaO_7$, 714.5279).

(20R)-3β-O-(Boc-L-isoleucyl)-dammarane-12β, 20, 25-triol (**2x**). White solid; Yield: 73%; Mp: 231–233 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.52 (dd, J = 10.9, 5.2 Hz, 1H, 3-CH), 3.84 (s, 1H, CH-CO), 3.59 (td, J = 10.3, 5.6 Hz, 1H, 12-CH), 1.35 (s, 9H), 1.43 (s, 12H), 1.15 (s, 6H), 0.98 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.87 (m, 3H); ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 38.7 (C-1), 26.5 (C-2), 82.2 (C-3), 38.0 (C-4), 51.7 (C-5), 18.3 (C-6), 34.7 (C-7), 39.6 (C-8), 56.1 (C-9), 38.2 (C-10), 31.5 (C-11), 71.2 (C-12), 48.5 (C-13), 51.8 (C-14), 34.8 (C-15), 23.9 (C-16), 50.1 (C-17), 16.3 (C-18), 16.8 (C-19), 74.3 (C-20), 22.0 (C-21), 44.3 (C-22), 18.3 (C-23), 43.1 (C-24), 71.0 (C-25), 29.5 (C-26), 31.1 (C-27), 28.2 (C-28), 16.0 (C-29), 16.9 (C-30), 172.2 (Ile-COO), 154.3 (Boc-CO-NH), 79.8 (Boc-C-CO), 60.1 (NH-CH), 38.0

(Ile-CH), 28.5 (Boc-3×CH_3), 24.6 (Ile-CH_2), 17.5, 15.7 (Ile-CH_3); IR $_{max}$ (cm^{-1}): 3398 (OH), 2965 (CH_3), 1734 (C=O), 1384 (CH_3). HR-ESIMS: m/z 714.5283 $[M+Na]^+$ (calcd for $C_{41}H_{73}NNaO_7$, 714.5279).

(20R)-3β-O-[Boc-L-glutamoyl(OBzl)]-dammarane-12β, 20, 25-triol (**3x**). White solid; Yield: 68%; Mp 208–210 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.44 (t, J = 10.9 Hz, 1H, 3-CH), 4.25 (s, 1H, CH-CO), 3.50 (td, J = 13.6, 6.4 Hz, 1H, 12-CH), 1.35 (s, 18H), 1.14 (s, 6H), 1.04 (s, 3H), 0.91 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 38.7 (C-1), 23.7 (C-2), 82.5 (C-3), 38.1 (C-4), 56.0 (C-5), 18.2 (C-6), 34.8 (C-7), 39.9 (C-8), 50.1 (C-9), 37.1 (C-10), 31.3 (C-11), 71.1 (C-12), 48.5 (C-13), 51.6 (C-14), 31.1 (C-15), 26.4 (C-16), 50.2 (C-17), 16.3 (C-18), 16.7 (C-19), 74.3 (C-20), 21.9 (C-21), 42.9 (C-22), 17.7 (C-23), 44.3 (C-24), 70.7 (C-25), 29.6 (C-26), 29.5 (C-27), 28.2 (C-28), 15.8 (C-29), 17.2 (C-30), 172.7, 172.0 (Glu-COO), 155.5 (Boc-CO-NH), 135.9, 128.7, 128.4, 128.3 (OBzl-Phe-CH), 77.4 (Boc-C-CO), 66.6 (Phe-CH_2), 55.9 (NH-CH), 28.4 (Boc-3×CH_3), 28.0, 26.3 (Glu-CH_2); IR $_{max}$ (cm^{-1}): 3392 (OH), 2962 (CH_3), 1733.8, 1732 (C=O), 1384 (CH_3). HR-ESIMS m/z 820.5343 $[M+Na]^+$ (calcd for $C_{47}H_{75}NNaO_9$, 820.5334).

(20R)-3β-O-(Boc-L-histidyl)-dammarane-12β, 20, 25-triol (**5x**). White solid; Yield: 57%; Mp: 202–204 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.53 (t, J = 8.9 Hz, 1H, 3-CH), 4.30 (s, 1H, CH-CO), 3.59 (td, J = 9.4, 4.9 Hz, 1H, 12-CH), 1.44 (s, 12H), 1.25 (s, 3H), 1.14 (s, 3H), 0.98 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.87 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 38.7 (C-1), 26.5 (C-2), 83.4 (C-3), 38.2 (C-4), 56.1 (C-5), 18.2 (C-6), 34.8 (C-7), 39.8 (C-8), 50.5 (C-9), 38.2 (C-10), 31.5 (C-11), 71.3 (C-12), 48.5 (C-13), 51.8 (C-14), 33.8 (C-15), 23.8 (C-16), 50.1 (C-17), 16.3 (C-18), 16.5 (C-19), 74.2 (C-20), 22.1 (C-21), 44.3 (C-22), 18.2 (C-23), 44.2 (C-24), 71.1 (C-25), 29.6 (C-26), 31.2 (C-27), 28.1 (C-28), 16.3 (C-29), 16.5 (C-30), 173.2 (His-COO), 156.0 (Boc-CO-NH), 135.5, 124.8, 117.3 (His-CH), 79.6 (Boc-C-CO), 56.1 (NH-CH), 28.3 (Boc-3×CH_3), 24.6 (His-CH_2); IR $_{max}$ (cm^{-1}): 3392 (OH), 2968 (CH_3), 1734 (C=O), 1383 (CH_3). HR-ESIMS m/z 738.5035 $[M+Na]^+$ (calcd for $C_{41}H_{69}N_3NaO_7$, 738.5028).

(20R)-3β-O-(Boc-L-tyrosyl)-dammarane-12β, 20, 25-triol (**6x**). White solid; Yield: 62%; Mp: 214–216 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.50 (dd, J = 10.2, 5.7 Hz, 1H, 3-CH), 4.35 (s, 1H, CH-CO), 3.60 (td, J = 10.3, 5.2 Hz, 1H, 12-CH), 2.05 (m, 1H), 1.44 (s, 6H), 1.41

(s, 6H), 1.14 (s, 6H), 0.98 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.84 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 38.7 (C-1), 26.5 (C-2), 82.4 (C-3), 38.2 (C-4), 54.7 (C-5), 18.3 (C-6), 34.7 (C-7), 39.6 (C-8), 50.1 (C-9), 38.2 (C-10), 31.5 (C-11), 71.4 (C-12), 48.9 (C-13), 51.8 (C-14), 33.8 (C-15), 23.7 (C-16), 51.0 (C-17), 16.3 (C-18), 16.5 (C-19), 74.5 (C-20), 22.0 (C-21), 44.4 (C-22), 18.3 (C-23), 44.2 (C-24), 70.9 (C-25), 29.3 (C-26), 31.7 (C-27), 28.4 (C-28), 16.0 (C-29), 16.9 (C-30), 173.1 (Tyr-COO), 156.0 (Boc-CO-NH), 130.6, 127.5, 121.3, 115.7 (Tyr-Phe-CH), 79.7 (Boc-C-CO), 56.0 (NH-CH), 38.0 (Tyr-CH_2), 28.3 (Boc-3$\times CH_3$); IR $_{max}$ (cm^{-1}): 3392 (OH), 2963 (CH_3), 1734 (C=O), 1382 (CH_3). HR-ESIMS m/z 764.5061 $[M+Na]^+$ (calcd for $C_{44}H_{71}NNaO_8$, 764.5070).

(20R)-3β-O-(Boc-L-seryl)-dammarane-12β, 20, 25-triol (**7x**). White solid; Yield: 66%; Mp: 235–237 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.51 (dd, J = 10.9, 5.4 Hz, 1H, 3-CH), 4.22 (s, 1H, CH-CO), 3.59 (td, J = 10.0, 4.9 Hz, 1H, 12-CH), 2.05 (m, 1H), 1.68 (s, 3H), 1.63 (s, 3H), 1.44 (s, 9H), 1.23 (s, 3H), 1.14 (s, 3H), 0.99 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 39.1 (C-1), 28.5 (C-2), 86.7 (C-3), 37.3 (C-4), 56.0 (C-5), 18.4 (C-6), 34.9 (C-7), 39.9 (C-8), 49.3 (C-9), 37.1 (C-10), 31.1 (C-11), 71.1 (C-12), 48.0 (C-13), 51.7 (C-14), 35.3 (C-15), 25.1 (C-16), 50.2 (C-17), 16.3 (C-18), 15.8 (C-19), 74.8 (C-20), 25.3 (C-21), 48.0 (C-22), 18.4 (C-23), 44.2 (C-24), 70.8 (C-25), 31.3 (C-26), 31.7 (C-27), 28.2 (C-28), 17.9 (C-29), 17.0 (C-30), 176.1 (Ser-COO), 151.5 (Boc-CO-NH), 79.0 (Boc-C-CO), 69.1 (Ser-C-OH), 53.5 (NH-CH), 28.2 (Boc-3$\times CH_3$); IR $_{max}$ (cm^{-1}): 3395 (OH), 2964 (CH_3), 1734 (C=O), 1384 (CH_3). HR-ESIMS m/z 688.4769 $[M+Na]^+$ (calcd for $C_{38}H_{67}NNaO_8$, 688.4758).

(20R)-3β-O-(Boc-L-threonyl)-dammarane-12β, 20, 25-triol (**8x**). White solid; Yield: 63%; Mp: 168–170 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.51 (dd, J = 11.2, 5.0 Hz, 1H, 3-CH), 3.59 (td, J = 10.4, 5.2 Hz, 1H, 12-CH), 2.05 (m, 1H), 1.44 (s, 6H), 1.22 (s, 6H), 1.12 (s, 3H), 0.99 (s, 6H), 0.88 (s, 12H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.7 (C-1), 26.4 (C-2), 83.6 (C-3), 38.0 (C-4), 55.4 (C-5), 18.2 (C-6), 34.8 (C-7), 39.9 (C-8), 50.1 (C-9), 38.2 (C-10), 31.4 (C-11), 71.2 (C-12), 48.5 (C-13), 51.7 (C-14), 33.8 (C-15), 23.6 (C-16), 51.0 (C-17), 16.3 (C-18), 16.7 (C-19), 74.3 (C-20), 21.9 (C-21), 44.3 (C-22), 18.2 (C-23), 44.2 (C-24), 70.8 (C-25), 29.5 (C-26), 31.4 (C-27), 28.4 (C-28), 15.8 (C-29), 17.2 (C-30), 170.5 (Thr-COO), 156.0 (Boc-CO-NH), 80.1 (Boc-C-CO), 67.8 (Thr-C-OH), 56.0 (NH-CH), 28.4 (Boc-3$\times CH_3$), 20.0

(Thr-CH_3); IR $_{max}$ (cm^{-1}): 3394 (OH), 2966 (CH_3), 1735 (C=O), 1384 (CH_3). HR-ESIMS m/z 702.4918 $[M+Na]^+$ (calcd for $C_{39}H_{69}NNaO_8$, 702.4927).

(20R)-12β-O-(Boc-L-leucyl)-dammarane-3β, 20, 25-triol (**1y**). White solid; Yield: 11%; Mp: 135–137 ℃; ^1H NMR (600 MHz, $CDCl_3$, δ, ppm): 4.40 (m, 1H, 12-CH), 3.65 (m, 1H, 3-CH), 1.70 (m, 12H), 1.40 (s, 18H), 0.88 (s, 6H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.2 (C-1), 23.9 (C-2), 78.9 (C-3), 38.0 (C-4), 56.1 (C-5), 18.3 (C-6), 34.9 (C-7), 39.9 (C-8), 48.7 (C-9), 37.2 (C-10), 31.4 (C-11), 77.0 (C-12), 49.2 (C-13), 51.8 (C-14), 31.1 (C-15), 26.7 (C-16), 50.1 (C-17), 15.9 (C-18), 16.3 (C-19), 74.9 (C-20), 22.1 (C-21), 43.3 (C-22), 18.3 (C-23), 44.7 (C-24), 70.9 (C-25), 29.8 (C-26), 30.3 (C-27), 28.2 (C-28), 15.8 (C-29), 17.5 (C-30), 172.1 (Leu-COO), 155.8 (Boc-CO-NH), 79.5 (Boc-C-CO), 52.1 (NH-CH), 38.0 (Leu-CH), 28.5 (Boc-3×CH_3), 25.1 (Leu-CH_2), 24.9, 23.1 (Leu-CH_3); IR $_{max}$ (cm^{-1}): 3390 (OH), 2965 (CH_3); IR $_{max}$ (cm^{-1}): 3394 (OH), 2965 (CH_3), 1732 (C=O), 1384 (CH_3). HR-ESIMS: m/z 714.5266 $[M+Na]^+$ (calcd for $C_{41}H_{73}NNaO_7$, 714.5279).

(20R)-12β-O-(Boc-L-isoleucyl)-dammarane-3β, 20, 25-triol (**2y**). White solid; Yield: 9%; Mp: 164–167 ℃; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.56 (dd, J = 10.5, 5.3 Hz, 1H, 12-CH), 3.79 (m, 1H, 3-CH), 1.44 (s, 18H), 1.37 (s, 3H), 1.13 (s, 6H), 1.09 (s, 3H), 0.99 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H), 0.88 (m, 6H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.7 (C-1), 26.5 (C-2), 79.7 (C-3), 39.9 (C-4), 56.1 (C-5), 18.3 (C-6), 34.8 (C-7), 39.9 (C-8), 50.1 (C-9), 38.1 (C-10), 31.1 (C-11), 76.7 (C-12), 49.2 (C-13), 51.7 (C-14), 34.8 (C-15), 23.6 (C-16), 51.0 (C-17), 16.3 (C-18), 16.6 (C-19), 74.9 (C-20), 23.6 (C-21), 43.2 (C-22), 18.3 (C-23), 46.4 (C-24), 70.8 (C-25), 28.6 (C-26), 31.5 (C-27), 28.3 (C-28), 16.3 (C-29), 17.5 (C-30), 172.9 (Ile-COO), 154.0 (Boc-CO-NH), 80.0 (Boc-C-CO), 60.1 (NH-CH), 38.1 (Ile-CH), 28.5 (Boc-3×CH_3), 24.8 (Ile-CH_2), 17.3, 15.8 (Ile-CH_3); IR $_{max}$ (cm^{-1}): 3390 (OH), 2963 (CH_3), 1732 (C=O), 1384 (CH_3). HR-ESIMS: m/z 714.5287 $[M+Na]^+$ (calcd for $C_{41}H_{73}NNaO_7$, 714.5279).

(20R)-12β-O-[Boc-L-glutamoyl(OBzl)]-dammarane-3β, 20, 25-triol (**3y**). White solid; Yield: 12%; Mp: 157–159 ℃; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.98 (m, 1H, 12-CH), 3.71 (m, 1H, 3-CH), 2.19 (s, 1H), 1.65 (s, 3H), 1.55 (m, 3H), 1.26 (s, 6H), 1.15 (s, 3H), 0.99 (s, 6H), 0.88 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.5 (C-1), 28.2 (C-2), 78.5 (C-3), 38.1 (C-4), 55.9 (C-5), 18.2 (C-6), 34.5 (C-7), 39.8 (C-8), 48.8 (C-9), 37.1 (C-

10), 28.2 (C-11), 76.4 (C-12), 45.6 (C-13), 52.8 (C-14), 32.0 (C-15), 26.9 (C-16), 50.0 (C-17), 16.1 (C-18), 16.7 (C-19), 73.8 (C-20), 23.7 (C-21), 43.0 (C-22), 17.9 (C-23), 44.5 (C-24), 71.1 (C-25), 29.3 (C-26), 29.5 (C-27), 28.3 (C-28), 15.7 (C-29), 17.7 (C-30), 172.7, 171.4 (Glu-COO), 155.5 (Boc-CO-NH), 135.9, 128.7, 128.3, 126.9 (OBzl-Phe-CH), 80.0 (Boc-C-CO), 66.9 (Phe-CH_2), 55.9 (NH-CH), 28.5 (Boc-3×CH_3), 25.9, 25.2 (Glu-CH_2); IR $_{max}$ (cm^{-1}): 3400 (OH), 2968 (CH_3), 1740 (C=O), 1384 (CH_3). HR-ESIMS m/z 820.5347 $[M+Na]^+$ (calcd for $C_{47}H_{75}NNaO_9$, 820.5334).

(20R)-12β-O-(Boc-L-histidyl)-dammarane-3β, 20, 25-triol (**5y**). White solid; Yield: 9%; Mp: 171–173 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.92 (td, J = 13.7, 6.7 Hz, 1H, 12-CH), 3.18 (dd, J = 10.9, 5.4 Hz, 1H, 3-CH), 1.40 (s, 6H), 1.19 (s, 6H), 1.08 (s, 3H), 0.99 (s, 3H), 0.96 (s, 6H), 0.95 (s, 3H), 0.85 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 38.7 (C-1), 26.5 (C-2), 78.8 (C-3), 38.2 (C-4), 56.1 (C-5), 18.3 (C-6), 34.8 (C-7), 39.9 (C-8), 50.5 (C-9), 38.2 (C-10), 31.5 (C-11), 77.4 (C-12), 48.7 (C-13), 51.8 (C-14), 33.8 (C-15), 23.8 (C-16), 50.1 (C-17), 16.3 (C-18), 16.5 (C-19), 74.6 (C-20), 22.1 (C-21), 44.3 (C-22), 18.2 (C-23), 44.2 (C-24), 70.9 (C-25), 29.6 (C-26), 31.1 (C-27), 28.0 (C-28), 16.3 (C-29), 16.5 (C-30), 173.1 (His-COO), 156.0 (Boc-CO-NH), 134.8, 124.5, 117.2 (His-CH), 74.9 (Boc-C-CO), 56.1 (NH-CH), 28.3 (Boc-3×CH_3), 24.6 (His-CH_2); IR $_{max}$ (cm^{-1}): 3392 (OH), 2968 (CH_3), 1734 (C=O), 1383 (CH_3). HR-ESIMS m/z 738.5038 $[M+Na]^+$ (calcd for $C_{41}H_{69}N_3NaO_7$, 738.5028).

(20R)-12β-O-(Boc-L-tyrosyl)-dammarane-3β, 20, 25-triol (**6y**). White solid; Yield: 12%; Mp: 168–171 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 5.12 (m, 1H, 12-CH), 4.78 (dd, J = 11.9, 4.8 Hz, 1H, 3-CH), 2.05 (m, 1H), 1.25 (s, 12H), 1.14 (s, 3H), 1.07 (s, 3H), 1.03 (s, 3H), 0.99 (s, 3H), 0.96 (s, 3H), 0.91 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 39.0 (C-1), 26.7 (C-2), 77.8 (C-3), 37.3 (C-4), 55.9 (C-5), 18.4 (C-6), 34.6 (C-7), 39.8 (C-8), 50.1 (C-9), 38.2 (C-10), 31.0 (C-11), 77.4 (C-12), 49.2 (C-13), 52.6 (C-14), 34.0 (C-15), 22.8 (C-16), 50.2 (C-17), 16.2 (C-18), 17.2 (C-19), 74.7 (C-20), 22.2 (C-21), 46.1 (C-22), 17.7 (C-23), 43.5 (C-24), 71.0 (C-25), 29.8 (C-26), 31.7 (C-27), 28.7 (C-28), 15.5 (C-29), 15.9 (C-30), 174.9 (Tyr-COO), 154.6 (Boc-CO-NH), 148.7, 135.4, 129.6, 122.0 (Tyr-Phe-CH), 78.8 (Boc-C-CO), 56.8 (NH-CH), 39.1 (Tyr-CH_2), 25.1 (Boc-3×CH_3); IR $_{max}$ (cm^{-1}): 3392 (OH), 2963 (CH_3), 1734 (C=O), 1382 (CH_3). HR-ESIMS m/z 764.5058 $[M+Na]^+$ (calcd for $C_{44}H_{71}NNaO_8$,

764.5070).

(20R)- 3β, 12β-O-di-(Boc-L-leucyl)-dammarane-20, 25-diol (**1z**). White solid; Yield: 6%; Mp: 163–166 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.95 (m, 1H, 12-CH), 4.24 (m, 1H, 3-CH), 2.05 (m, 1H), 1.63 (s, 3H), 1.41 (s, 18H), 1.18 (s, 3H), 1.11 (s, 3H), 0.93 (s, 6H), 0.84 (m, 6H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.5 (C-1), 23.7 (C-2), 81.7 (C-3), 38.1 (C-4), 55.9 (C-5), 18.3 (C-6), 34.8 (C-7), 39.7 (C-8), 47.8 (C-9), 37.3 (C-10), 32.1 (C-11), 75.8 (C-12), 52.1 (C-13), 51.8 (C-14), 30.9 (C-15), 26.4 (C-16), 49.8 (C-17), 16.1 (C-18), 16.6 (C-19), 73.7 (C-20), 21.6 (C-21), 42.2 (C-22), 17.9 (C-23), 44.7 (C-24), 71.0 (C-25), 29.8 (C-26), 29.3 (C-27), 27.2 (C-28), 15.7 (C-29), 17.8 (C-30), 173.2, 172.7 (Leu-COO), 156.1, 155.5 (Boc-CO-NH), 79.9, 79.7 (Boc-C-CO), 52.6, 45.8 (NH-CH), 38.0 (Leu-CH), 28.3 (Boc-3×CH_3), 24.9 (Leu-CH_2), 23.1, 22.0 (Leu-CH_3); IR $_{max}$ (cm^{-1}): 3390 (OH), 2965 (CH_3), 1734, 1731 (C=O), 1384 (CH_3). HR-ESIMS m/z 927.6638 $[M+Na]^+$ (calcd for $C_{52}H_{92}N_2NaO_{10}$, 927.6645).

(20R)-3β, 12β-O-di-(Boc-L-isoleucyl)-dammarane-20, 25-diol (**2z**). White solid; Yield: 7%; Mp: 159–162 ℃; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 4.92 (m, 1H, 12-CH), 4.51 (dd, J = 10.4, 4.8 Hz, 1H, 3-CH), 2.05 (m, 2H), 1.42 (s, 18H), 1.36 (s, 6H), 1.22 (s, 3H), 1.10 (s, 6H), 0.98 (s, 3H), 0.93 (s, 3H), 0.84 (s, 3H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.5 (C-1), 26.7 (C-2), 81.8 (C-3), 39.1 (C-4), 55.9 (C-5), 18.2 (C-6), 34.5 (C-7), 40.6 (C-8), 50.1 (C-9), 37.1 (C-10), 31.1 (C-11), 75.8 (C-12), 49.1 (C-13), 52.2 (C-14), 34.2 (C-15), 23.6 (C-16), 49.8 (C-17), 16.1 (C-18), 16.6 (C-19), 73.8 (C-20), 22.8 (C-21), 43.5 (C-22), 19.1 (C-23), 44.2 (C-24), 70.8 (C-25), 29.8 (C-26), 29.6 (C-27), 28.1 (C-28), 17.5 (C-29), 16.9 (C-30), 173.1, 172.6 (Ile-COO), 155.7, 155.0 (Boc-CO-NH), 79.8, 79.6 (Boc-C-CO), 60.1, 58.9 (NH-CH), 38.1, 37.1 (Ile-CH), 28.4 (Boc-3×CH_3), 25.1, 25.0 (Ile-CH_2), 17.8, 17.5, 14.2, 13.8 (Ile-CH_3); IR $_{max}$ (cm^{-1}): 3392 (OH), 2968 (CH_3), 1735, 1732 (C=O), 1380 (CH_3). HR-ESIMS m/z 927.6651 $[M+Na]^+$ (calcd for $C_{52}H_{92}N_2NaO_{10}$, 927.6645).

(20R)-3β, 12β-O-di-[Boc-L-glutamoyl(OBzl)]-dammarane-20, 25-diol (**3z**). White solid; Yield: 7%; Mp: 181–183 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.49 (dd, J = 11.2, 4.8 Hz, 1H, 3-CH), 4.05 (td, J = 11.6, 7.0 Hz, 1H, 12-CH), 2.05 (m, 2H), 1.41 (s, 18H), 1.22 (s, 6H), 1.09 (s, 6H), 0.98 (s, 3H), 0.84 (s, 9H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.4 (C-1), 28.1 (C-2), 82.3 (C-3), 38.1 (C-4), 55.9 (C-5), 18.2 (C-6), 34.5 (C-7), 39.7 (C-8), 59.7 (C-9), 37.2

(C-10), 28.3 (C-11), 76.5 (C-12), 45.5 (C-13), 52.8 (C-14), 32.1 (C-15), 27.4 (C-16), 50.3 (C-17), 16.1 (C-18), 16.8 (C-19), 73.7 (C-20), 23.7 (C-21), 42.9 (C-22), 18.1 (C-23), 44.2 (C-24), 71.2 (C-25), 29.8 (C-26), 29.5 (C-27), 28.2 (C-28), 15.6 (C-29), 17.7 (C-30), 172.9, 172.7, 171.9, 171.6 (Glu-COO), 156.1, 155.5 (Boc-CO-NH), 141.1, 135.8, 128.7, 128.5, 128.3, 128.2, 126.9, 126.6 (OBzl-Phe-CH), 80.1, 80.0 (Boc-C-CO), 66.6, 65.3 (Phe-CH_2), 55.9 (NH-CH), 28.4 (Boc-3×CH_3), 25.1, 22.6 (Glu-CH_2); IR $_{max}$ (cm^{-1}): 3395 (OH), 2856 (CH_3), 1734.5, 1732 (C=O), 1385 (CH_3). HR-ESIMS m/z 1117.6914 $[M+H]^+$ (calcd for $C_{64}H_{97}N_2O_{14}$, 1117.6934).

(20R)-3β, 12β-O-di-(Boc-L-alanyl)-dammarane-20, 25-diol (**4z**). White power; Yield: 7%; Mp: 156–159 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.93 (td, J = 10.9, 6.1 Hz, 1H, 12-CH), 4.55 (dd, J = 10.5, 5.3 Hz, 1H, 3-CH), 2.05 (m, 2H), 1.44 (s, 18H), 1.38 (s, 6H), 1.25 (s, 3H), 1.13 (s, 6H), 1.09 (s, 3H), 0.99 (s, 3H), 0.94 (s, 3H), 0.88 (s, 6H), 0.85 (m, 6H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.9 (C-1), 26.1 (C-2), 81.9 (C-3), 38.7 (C-4), 55.7 (C-5), 18.2 (C-6), 34.5 (C-7), 39.6 (C-8), 50.3 (C-9), 37.2 (C-10), 31.5 (C-11), 77.4 (C-12), 48.9 (C-13), 51.8 (C-14), 33.8 (C-15), 23.7 (C-16), 51.0 (C-17), 16.3 (C-18), 16.8 (C-19), 74.7 (C-20), 22.9 (C-21), 44.4 (C-22), 18.2 (C-23), 44.2 (C-24), 71.2 (C-25), 29.8 (C-26), 30.5 (C-27), 28.2 (C-28), 15.7 (C-29), 16.9 (C-30), 172.1, 171.7 (Ala-COO), 156.3, 155.8 (Boc-CO-NH), 79.9, 79.6 (Boc-C-CO), 60.1, 58.9 (NH-CH), 38.0, 37.7 (Ala-CH), 29.8, 28.4 (Boc-3×CH_3), 16.1, 15.6 (Ala-CH_3); IR $_{max}$ (cm^{-1}): 3396 (OH), 2968 (CH_3), 1735 (C=O), 1380 (CH_3). HR-ESIMS m/z 821.5895 $[M+H]^+$ (calcd for $C_{46}H_{81}N_2O_{10}$, 821.5891).

Section C: General procedure for deprotection compounds 1xt–5xt, 8xt, 9xt and 4yt

To a solution of the Boc-protected compounds **1x**–**5x**, **8x**, **9x** and **4y** (50 mg) in toluene (30 mL), silica gel (100 mg) was added. The mixture was allowed to stir at 115℃ for 6 h. After completion of the reaction (as monitored by TLC) the solution was removed under reduced pressure. The residue was washed with methanol until no target substance could be detected and concentrated under reduced pressure to give the crude product. The crude products were chromatographed using silica gel and eluted with ethyl acetate/methanol (10:1) to afford corresponding products **1xt**–**5xt**, **8xt**, **9xt** and **4yt**.

(20R)-3β-O-(L-leucyl)-dammarane-12β, 20, 25-triol (**1xt**). White solid; Yield: 84%; Mp: 214–216 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.23 (dd, J = 8.9, 5.2 Hz, 1H, 3-CH), 3.73 (td, J = 9.9, 5.0 Hz, 1H, 12-CH), 1.51 (s, 3H), 1.48 (s, 3H), 1.25 (s, 3H), 1.19 (s, 6H), 0.99 (s, 3H), 0.90 (s, 6H), 0.86 (s, 6H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.0 (C-1), 23.7 (C-2), 82.8 (C-3), 38.1 (C-4), 55.9 (C-5), 18.3 (C-6), 34.7 (C-7), 39.8 (C-8), 50.0 (C-9), 37.0 (C-10), 31.0 (C-11), 71.1 (C-12), 49.2 (C-13), 51.6 (C-14), 31.0 (C-15), 26.4 (C-16), 50.2 (C-17), 16.3 (C-18), 16.7 (C-19), 74.3 (C-20), 23.1 (C-21), 44.2 (C-22), 18.3 (C-23), 43.9 (C-24), 70.8 (C-25), 29.6 (C-26), 29.5 (C-27), 28.1 (C-28), 15.7 (C-29), 17.1 (C-30), 173.2 (Leu-COO), 53.2 (NH-CH), 38.0 (Leu-CH), 24.9 (Leu-CH_2), 23.1, 21.8 (Leu-CH_3); IR $_{max}$ (cm^{-1}): 3391 (OH), 2966 (CH_3), 1734 (C=O), 1384 (CH_3). HR-ESIMS m/z 614.4765 $[M+Na]^+$ (calcd for $C_{36}H_{65}NNaO_5$, 614.4754).

(20R)-3β-O-(L-isoleucyl)-dammarane-12β, 20, 25-triol (**2xt**). White power; Yield: 82%; Mp: 246–248 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.51 (m, 1H, 3-CH), 3.52 (m, 1H, 12-CH), 1.24 (s, 3H), 1.19 (s, 3H), 1.14 (s, 9H), 0.98 (s, 3H), 0.90 (s, 3H), 0.87 (s, 9H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.7 (C-1), 26.5 (C-2), 81.7 (C-3), 38.0 (C-4), 56.0 (C-5), 18.3 (C-6), 34.8 (C-7), 39.9 (C-8), 50.1 (C-9), 38.7 (C-10), 31.3 (C-11), 71.2 (C-12), 48.6 (C-13), 51.7 (C-14), 34.8 (C-15), 23.9 (C-16), 51.0 (C-17), 16.3 (C-18), 16.9 (C-19), 74.4 (C-20), 21.9 (C-21), 43.2 (C-22), 18.3 (C-23), 44.2 (C-24), 70.9 (C-25), 29.3 (C-26), 31.3 (C-27), 28.2 (C-28), 16.1 (C-29), 16.8 (C-30), 175.2 (Ile-COO), 56.1 (NH-CH), 38.0 (Ile-CH), 24.6 (Ile-CH_2), 15.8, 11.8 (Ile-CH_3); IR $_{max}$ (cm^{-1}): 3395 (OH), 2968 (CH_3), 1736 (C=O), 1382 (CH_3). HR-ESIMS m/z 614.4748 $[M+Na]^+$ (calcd for $C_{36}H_{65}NNaO_5$, 614.4754).

(20R)-3β-O-(L-glutamoyl)-dammarane-12β, 20, 25-triol (**3xt**). White solid; Yield: 80%; Mp: 204–207 ℃; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 4.56 (dd, J = 10.6, 4.4 Hz, 1H, 3-CH),

4.15 (m, 1H, 12-CH), 1.22 (s, 3H), 1.20 (s, 3H), 1.03 (s, 3H), 0.99 (s, 3H), 0.90 (s, 3H), 0.88 (m, 3H), 0.87 (s, 6H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.4 (C-1), 28.0 (C-2), 82.1 (C-3), 38.1 (C-4), 55.8 (C-5), 18.3 (C-6), 34.7 (C-7), 39.9 (C-8), 51.1 (C-9), 37.1 (C-10), 29.5 (C-11), 71.2 (C-12), 49.7 (C-13), 51.8 (C-14), 31.2 (C-15), 28.0 (C-16), 49.7 (C-17), 16.3 (C-18), 16.8 (C-19), 74.9 (C-20), 22.1 (C-21), 42.9 (C-22), 18.0 (C-23), 44.3 (C-24), 70.1 (C-25), 29.6 (C-26), 29.2 (C-27), 28.3 (C-28), 15.7 (C-29), 16.4 (C-30), 172.1, 166.1 (Glu-COO), 56.0 (NH-CH), 25.3, 23.9 (Glu-CH_2); IR $_{max}$ (cm^{-1}): 3393 (OH), 2962 (CH_3), 1734.6, 1733 (C=O), 1384 (CH_3). HR-ESIMS m/z 630.4344 $[M+Na]^+$ (calcd for $C_{35}H_{61}NNaO_7$, 630.4340).

(20R)-3β-O-(L-histidyl)-dammarane-12β, 20, 25-triol (**5xt**). White solid; Yield: 57%; Mp: 202–204 ℃; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 4.57 (dd, J = 10.7, 5.6 Hz, 1H, 3-CH), 3.62 (m, 1H, 12-CH), 1.45 (s, 3H), 1.23 (s, 6H), 1.14 (s, 3H), 0.99 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.88 (s, 6H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.7 (C-1), 27.5 (C-2), 81.7 (C-3), 38.9 (C-4), 56.1 (C-5), 18.3 (C-6), 34.8 (C-7), 39.9 (C-8), 50.1 (C-9), 38.7 (C-10), 31.3 (C-11), 74.2 (C-12), 48.6 (C-13), 51.7 (C-14), 34.8 (C-15), 23.9 (C-16), 51.1 (C-17), 16.3 (C-18), 16.8 (C-19), 74.4 (C-20), 22.0 (C-21), 44.2 (C-22), 18.3 (C-23), 44.2 (C-24), 70.8 (C-25), 29.6 (C-26), 31.1 (C-27), 28.6 (C-28), 16.3 (C-29), 17.5 (C-30), 175.2 (His-COO), 135.2, 124.8, 116.1 (His-CH), 59.8 (NH-CH), 25.1 (His-CH_2); IR $_{max}$ (cm^{-1}): 3395 (OH), 2968 (CH_3), 1732 (C=O), 1383 (CH_3). HR-ESIMS m/z 638.4508 $[M+Na]^+$ (calcd for $C_{36}H_{61}N_3NaO_5$, 638.4503).

(20R)-3β-O-(L-threonyl)-dammarane-12β, 20, 25-triol (**8xt**). White solid; Yield: 63%; Mp: 168–170 ℃; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.56 (t, J = 13.6, 6.9 Hz, 1H, 3-CH), 3.71 (td, J = 10.4, 5.2 Hz, 1H, 12-CH), 2.03 (m, 1H), 1.64 (s, 3H), 1.45 (s, 3H), 1.13 (s, 6H), 1.02 (s, 3H), 0.97 (s, 3H), 0.88 (s, 6H), 0.77 (s, 3H); ^{13}C NMR (600 MHz, $CDCl_3$, δ, ppm): 38.7 (C-1), 27.2 (C-2), 81.8 (C-3), 38.1 (C-4), 53.7 (C-5), 18.3 (C-6), 35.3 (C-7), 39.9 (C-8), 51.7 (C-9), 37.2 (C-10), 31.3 (C-11), 74.9 (C-12), 48.7 (C-13), 50.1 (C-14), 33.9 (C-15), 25.3 (C-16), 53.6 (C-17), 16.3 (C-18), 16.8 (C-19), 74.4 (C-20), 22.0 (C-21), 43.2 (C-22), 18.3 (C-23), 43.2 (C-24), 70.9 (C-25), 28.3 (C-26), 31.1 (C-27), 30.7 (C-28), 16.8 (C-29), 17.3 (C-30), 175.4 (Thr-COO), 70.6 (Thr-C-OH), 56.0 (NH-CH), 17.9 (Thr-CH_3); IR $_{max}$ (cm^{-1}): 3397 (OH), 2968 (CH_3), 1732 (C=O), 1384 (CH_3). HR-ESIMS m/z 602.4396 $[M+Na]^+$ (calcd for $C_{34}H_{61}NNaO_6$, 602.4391).

(20R)-3β-O-(L-arginyl)-dammarane-12β, 20, 25-triol (**9xt**). White solid; Yield: 78%; Mp: 205–207 ℃. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.73 (dd, J = 10.7, 5.2 Hz, 1H, 3-CH), 3.71 (s, 1H, CH-CO), 3.52 (m, 1H, 12-CH), 1.22 (s, 3H), 1.08 (s, 6H), 1.02 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H); ^{13}C NMR (600 MHz, C_5D_5N, δ, ppm): 38.9 (C-1), 26.7 (C-2), 81.6 (C-3), 38.5 (C-4), 50.6 (C-5), 18.7 (C-6), 35.3 (C-7), 40.3 (C-8), 44.4 (C-9), 37.5 (C-10), 30.3 (C-11), 71.1 (C-12), 43.1 (C-13), 49.6 (C-14), 31.8 (C-15), 23.2 (C-16), 45.6 (C-17), 16.3 (C-18), 16.4 (C-19), 73.7 (C-20), 22.9 (C-21), 44.1 (C-22), 18.8 (C-23), 45.9 (C-24), 70.1 (C-25), 28.2 (C-26), 30.5 (C-27), 27.0 (C-28), 16.2 (C-29), 17.2 (C-30), 174.6 (Arg-COO), 56.3 (Arg-NH-CH), 40.4 (NH-CH), 37.5, 32.2, 19.1 (Arg-CH_2). IR $_{max}$ (cm^{-1}): 3392 (OH), 2960 (CH_3), 1733 (C=O), 1379 (CH_3). HR-ESIMS m/z 657.4929 $[M+Na]^+$ (calcd for $C_{36}H_{66}N_4NaO_5$, 657.4925).

(20R)-12β-O-(L-alanyl)-dammarane-3β, 20, 25-triol (**4yt**). White solid; Yield: 72%; Mp: 217–219 ℃; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 5.02 (td, J = 10.8, 5.5 Hz, 1H, 12-CH), 3.69 (s, 1H, CH-CO), 3.19 (m, 1H, 3-CH), 1.35 (s, 3H), 1.26 (s, 3H), 1.22 (s, 3H), 1.06 (s, 3H), 1.02 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.88 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (600 MHz, C_5D_5N, δ, ppm): 39.5 (C-1), 27.1 (C-2), 78.4 (C-3), 38.2 (C-4), 50.1 (C-5), 18.1 (C-6), 34.5 (C-7), 39.8 (C-8), 44.5 (C-9), 37.1 (C-10), 29.6 (C-11), 81.2 (C-12), 43.1 (C-13), 49.6 (C-14), 31.4 (C-15), 23.5 (C-16), 45.7 (C-17), 16.2 (C-18), 16.2 (C-19), 73.7 (C-20), 22.5 (C-21), 41.2 (C-22), 17.8 (C-23), 41.6 (C-24), 70.1 (C-25), 29.5 (C-26), 29.2 (C-27), 28.7 (C-28), 15.7 (C-29), 18.1 (C-30), 177.0 (Ala-COO), 56.4 (NH-CH), 16.7 (Ala-CH_3); IR $_{max}$ (cm^{-1}): 3392 (OH), 2960 (CH_3), 1731.4 (C=O), 1380 (CH_3). HR-ESIMS m/z 572.4288 $[M+Na]^+$ (calcd for $C_{33}H_{59}NNaO_5$, 572.4285).

Section D: Cell viability assay

Cytotoxicities of these derivatives were evaluated on six different human tumor cell lines

(BGC-823 cell line, α-2 cell line, PC3 cell line , DU145 cell line and MDA-MB-231 cell line) using 3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium (MTT) assay. DMSO and MTT were purchased from Sigma Chemical Co., Ltd, USA. In short, 1×10^6 cells/well were seeded into 96-well plates; 24 h later, the cells were treated with serial dilutions of the compounds (0-100 μM) for another 48 h. 15 μL of MTT solution (5 mg/mL) was added to each well, and the tumor cells were incubated at 37 ℃ in a humidified atmosphere of 5% CO_2 air for 4 h. At the end of incubation, the growth medium was removed and replaced with 100 μL of DMSO (at room temperature). After agitating on a vortex for 10 minutes, the absorbance was determined at 490 nm as reference on a Bio-Rad (model 550) microplate reader to calculate 50% inhibition concentration (IC_{50}). The percentage of cell-growth inhibition was calculated as follows: cell death (%) = [(control)－(compound)]/(control)×100.

Section E: Determination of morphological changes of cells

DU145 cells were seeded at a concentration of 1×10^5 cells/ well in a six-well plate and

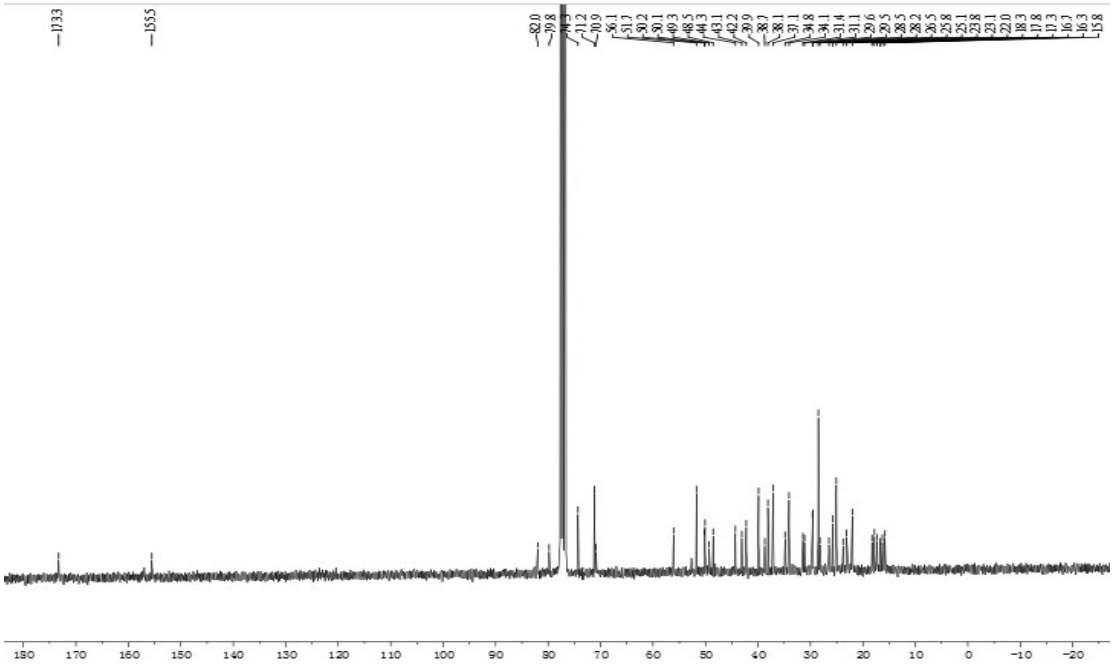
grown for 24 h. The cells were then treated with compound (**3xt** or **9xt)** for 12 h. Negative control cells were treated with 0.1% DMSO. Morphological changes of the cells were observed with an inverted fluorescence microscope. A similar experiment was also carried out in which the cells were fixed with paraformaldehyde for 30 min at 4 ℃ after they had been treated with compound (**3xt** or **9xt**) or 0.1% DMSO. After fixation, the cells were stained with 4', 6-diamidino-2-phenylindole (DAPI) in the dark for 30 min followed by another PBS wash. The stained cells were examined under a fluorescent microscope (IX51, OLYMPUS, Japan).

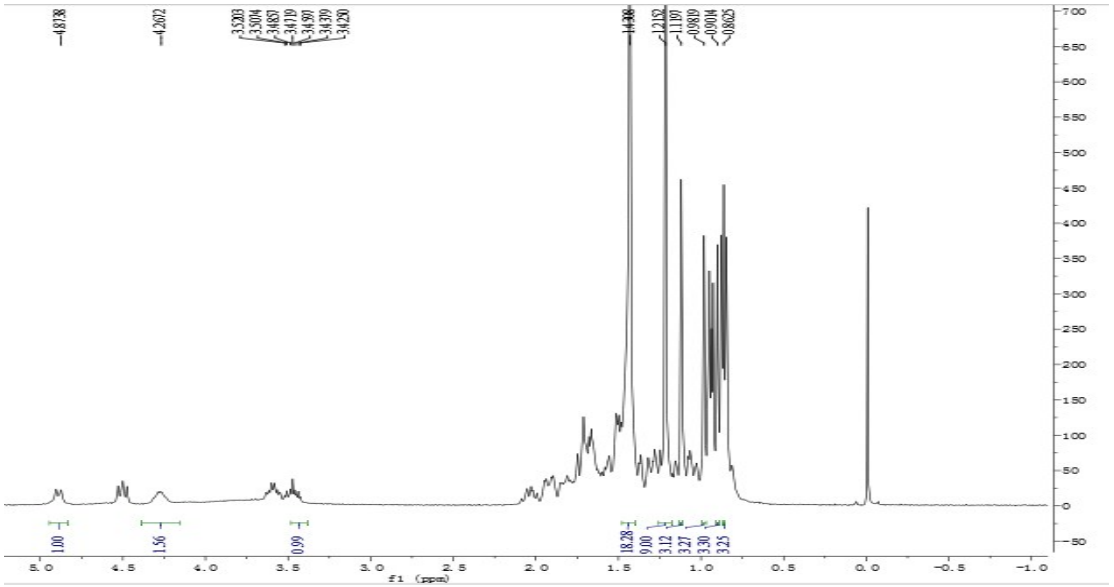
Section F: Cell cycle analysis by flow cytometry

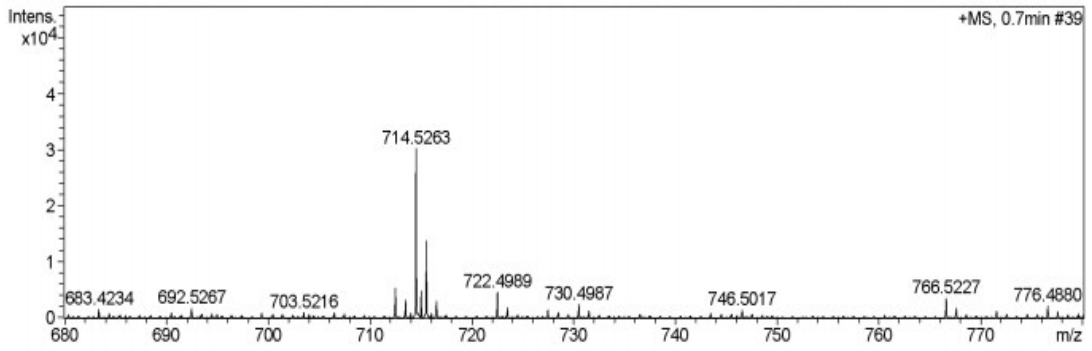
Treated and untreated cells were trypsinized, harvested, washed with PBS and then fixed with 75% ethanol for overnight at -20 ℃. The fixed cells were washed with PBS and stained with Propidium Iodide (PI) solution (50 μg/mL PI, 50 μg/mL RNase, 0.1% sodium citrate and 0.1% Triton X-100, pH8.0) for 4 h in the dark. Cell cycle distribution was analyzed using FACScan (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). Apoptotic cells with hypodiploid DNA content were measured by quantifying the subG1 peak in the cell cycle pattern.

Section G: Spectra

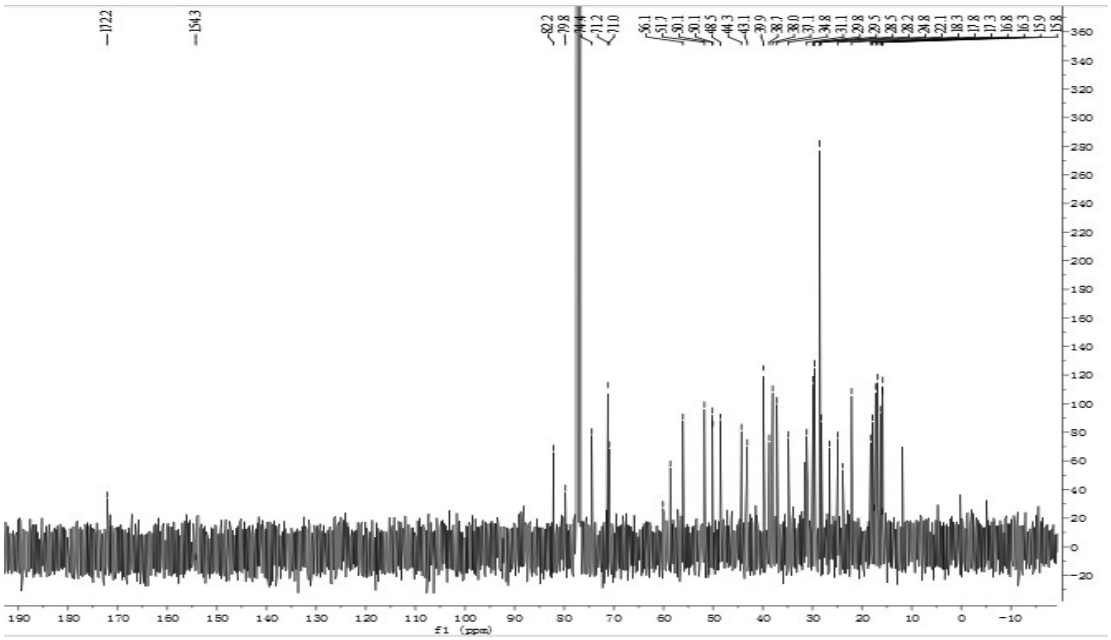
(20R)-3β-O-(Boc-L-leucyl)-dammarane-12β, 20, 25-triol (**1x**)

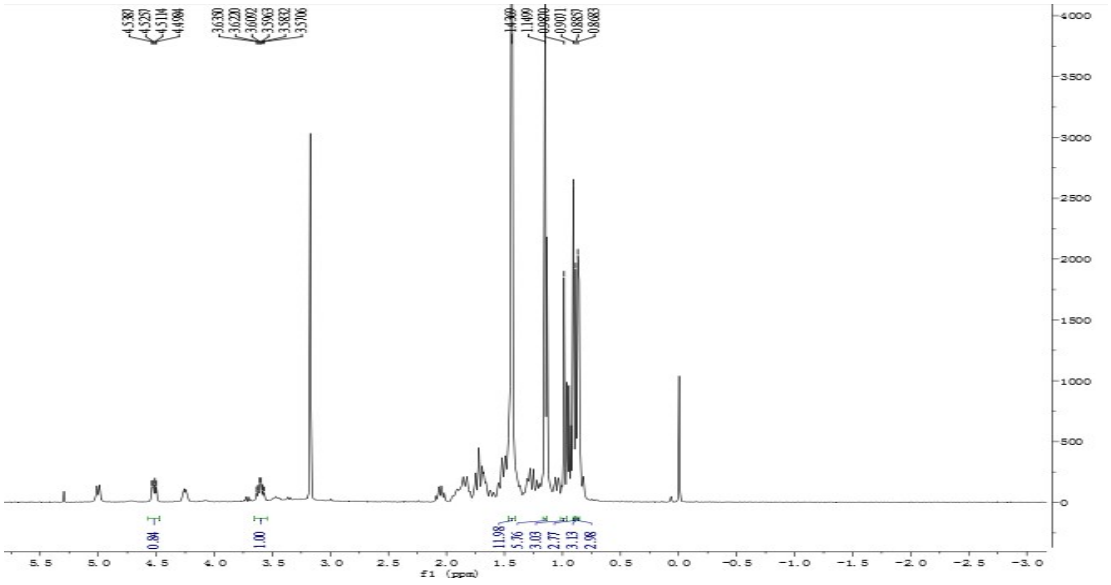


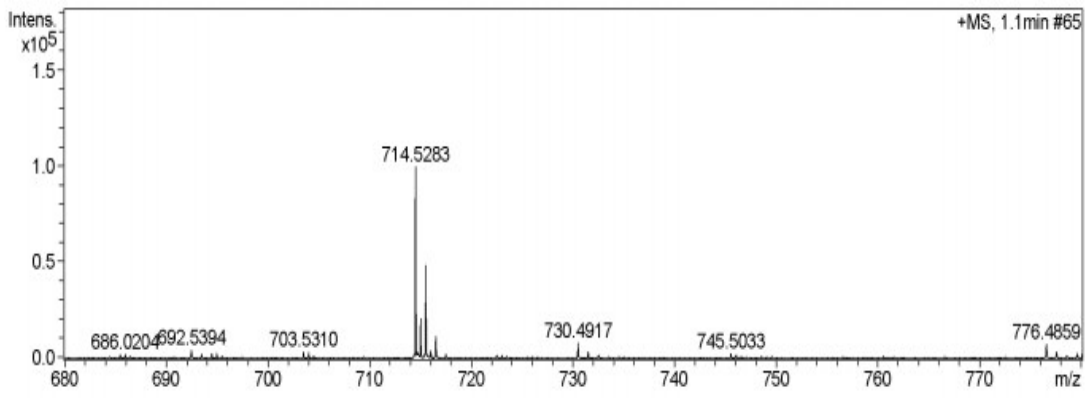




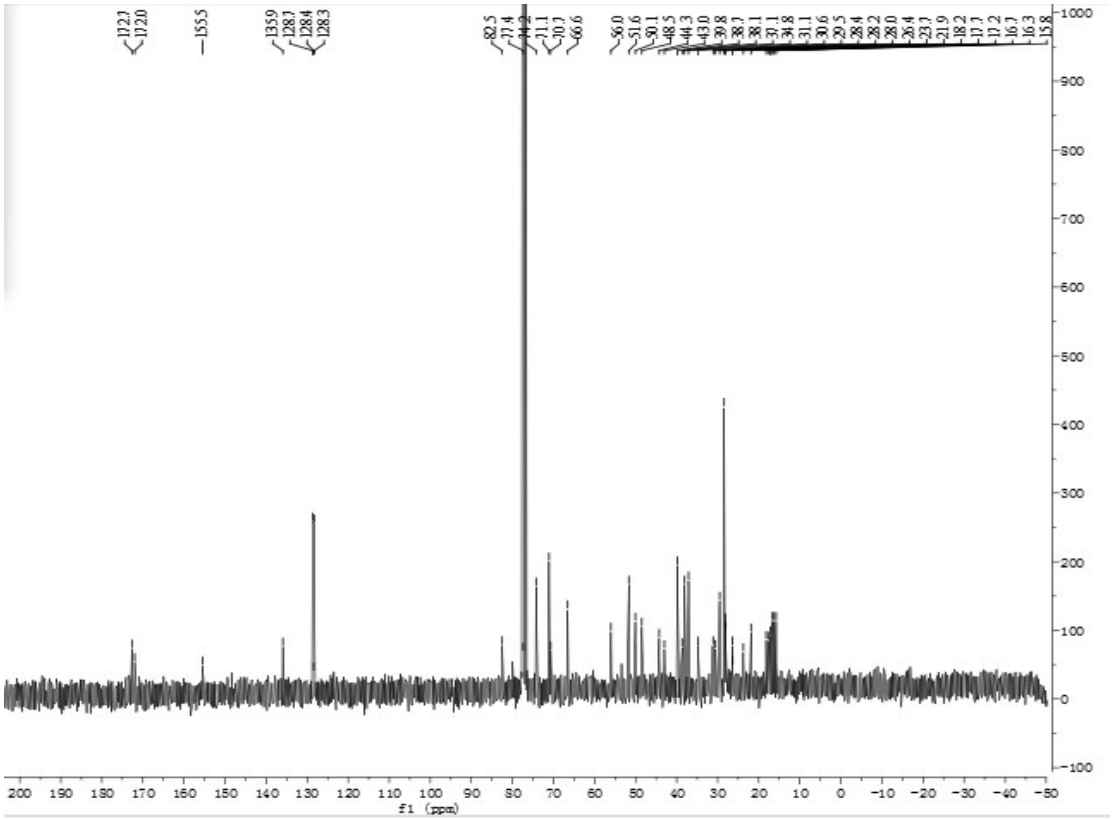
(20R)-3β-O-(Boc-L-isoleucyl)-dammarane-12β, 20, 25-triol (**2x**)

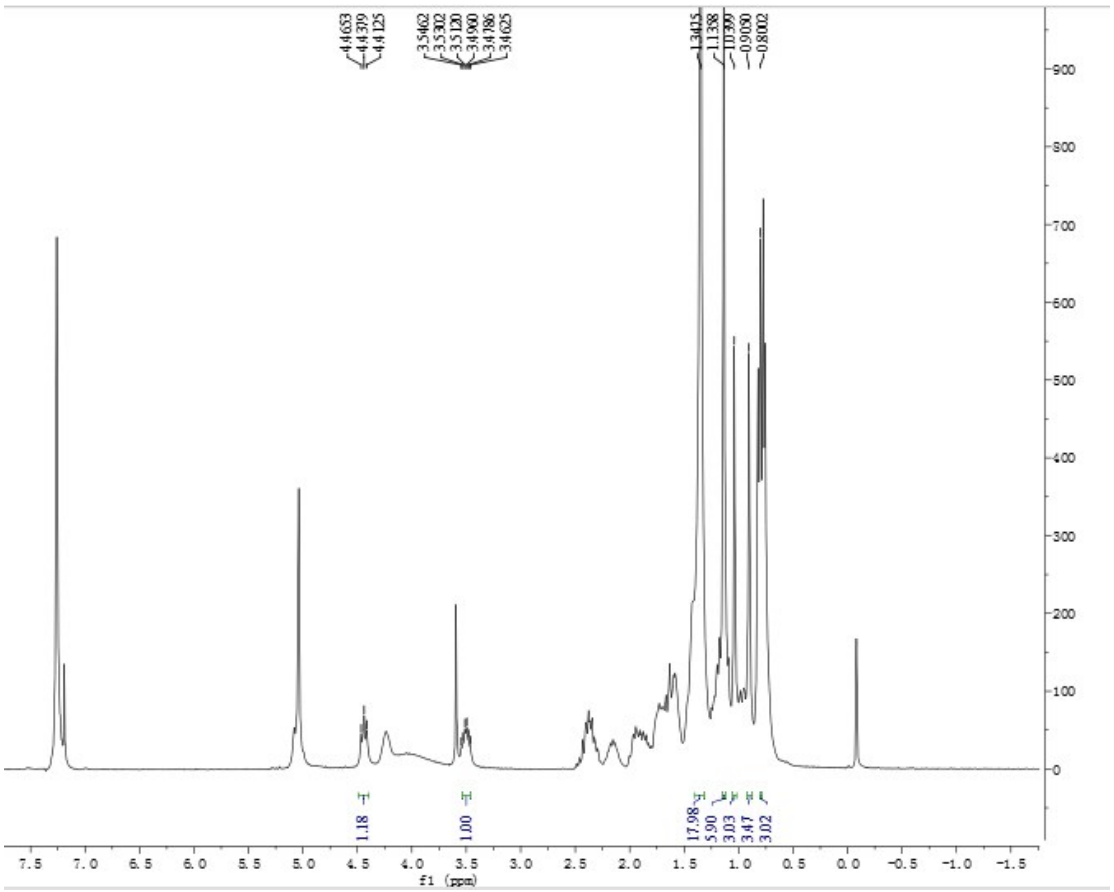




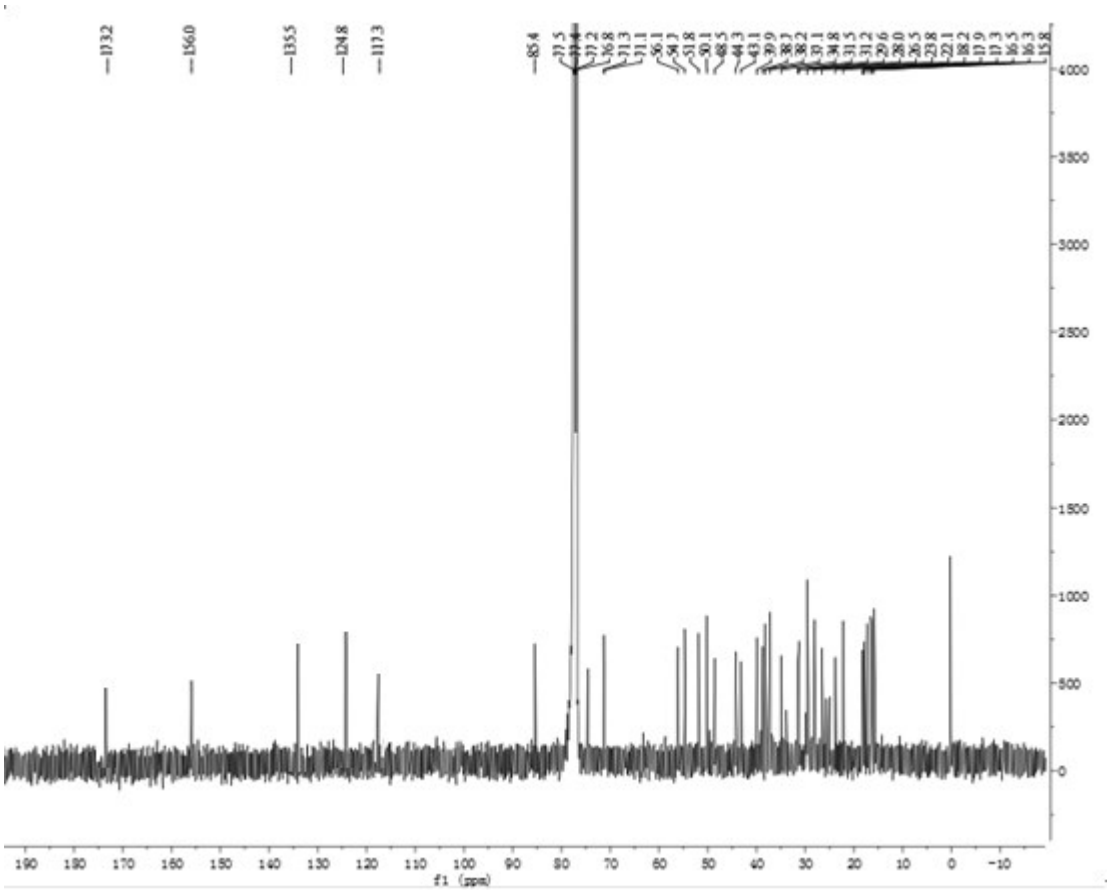


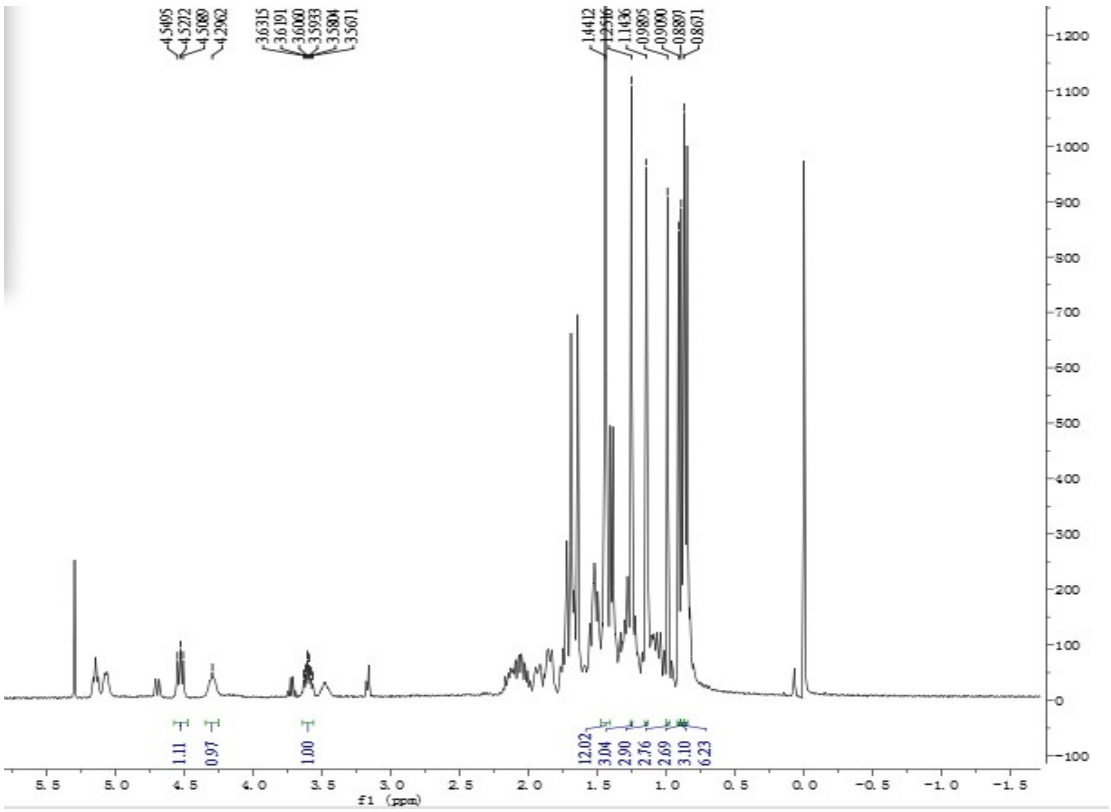
(20R)-3β-O-[Boc-L-glutamoyl(OBzl)]-dammarane-12β, 20, 25-triol (**3x**)



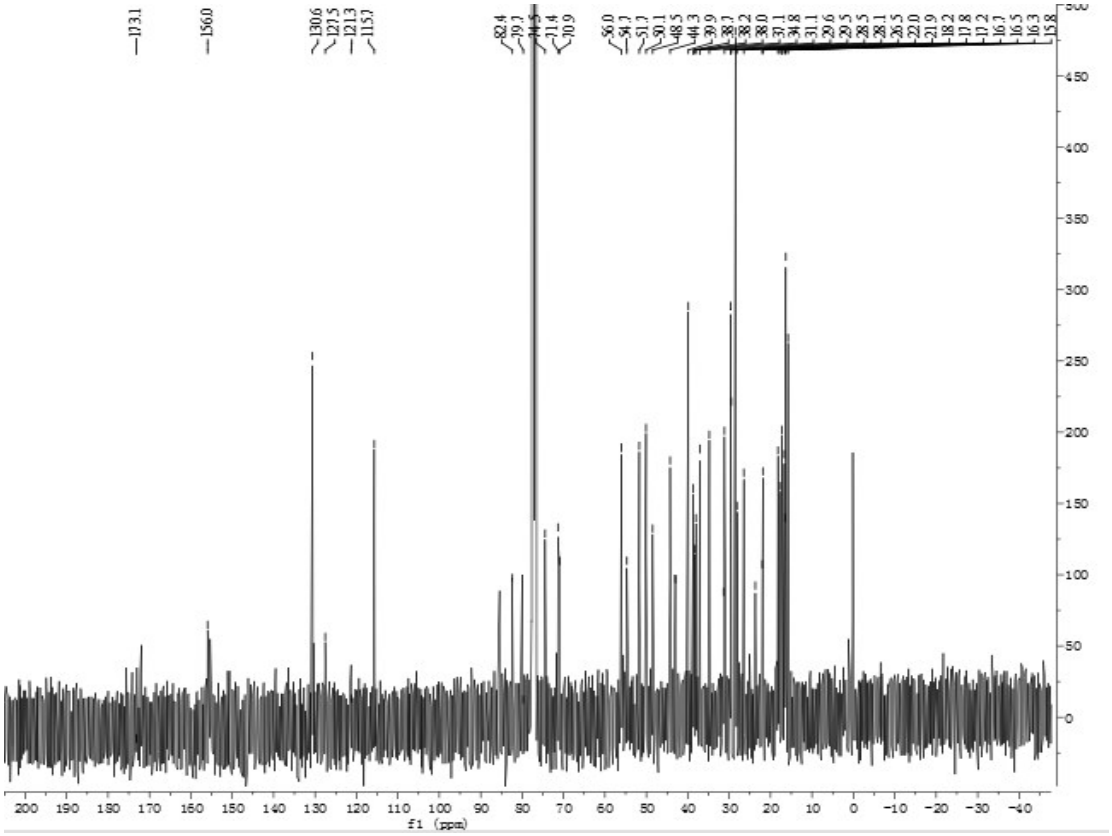


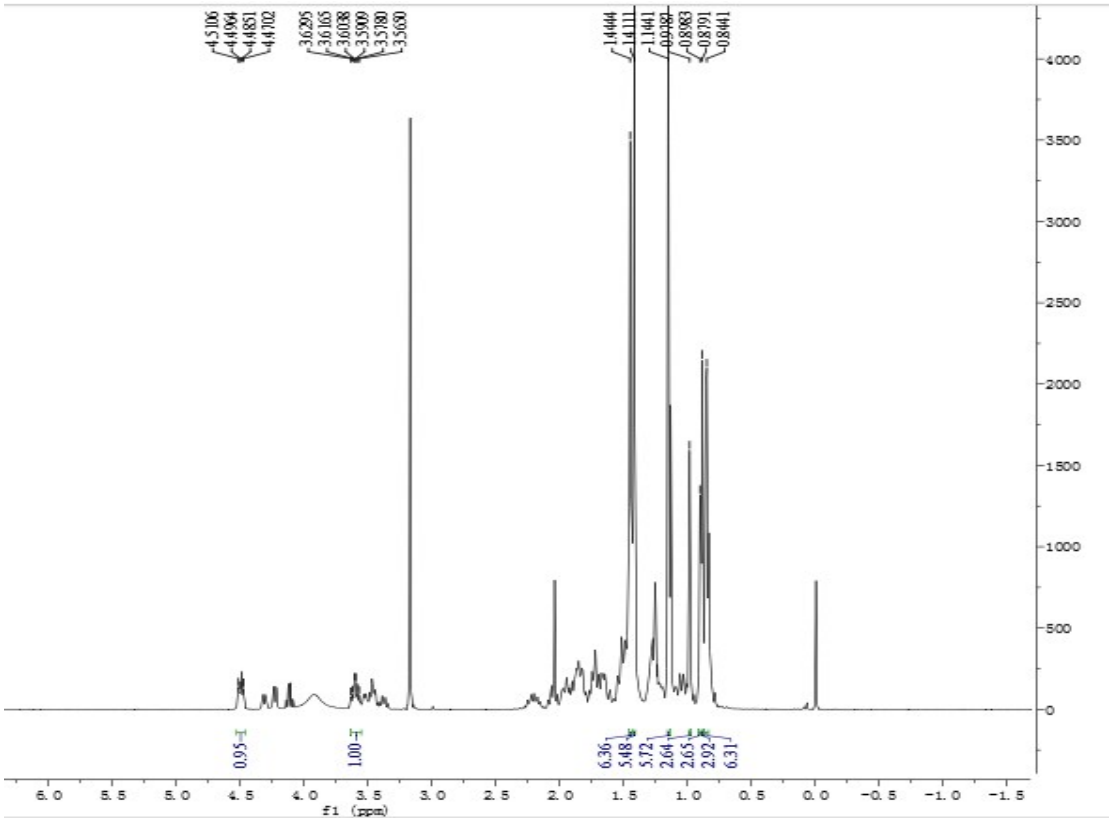
(20R)-3β-O-(Boc-L-histidyl)-dammarane-12β, 20, 25-triol (**5x**)



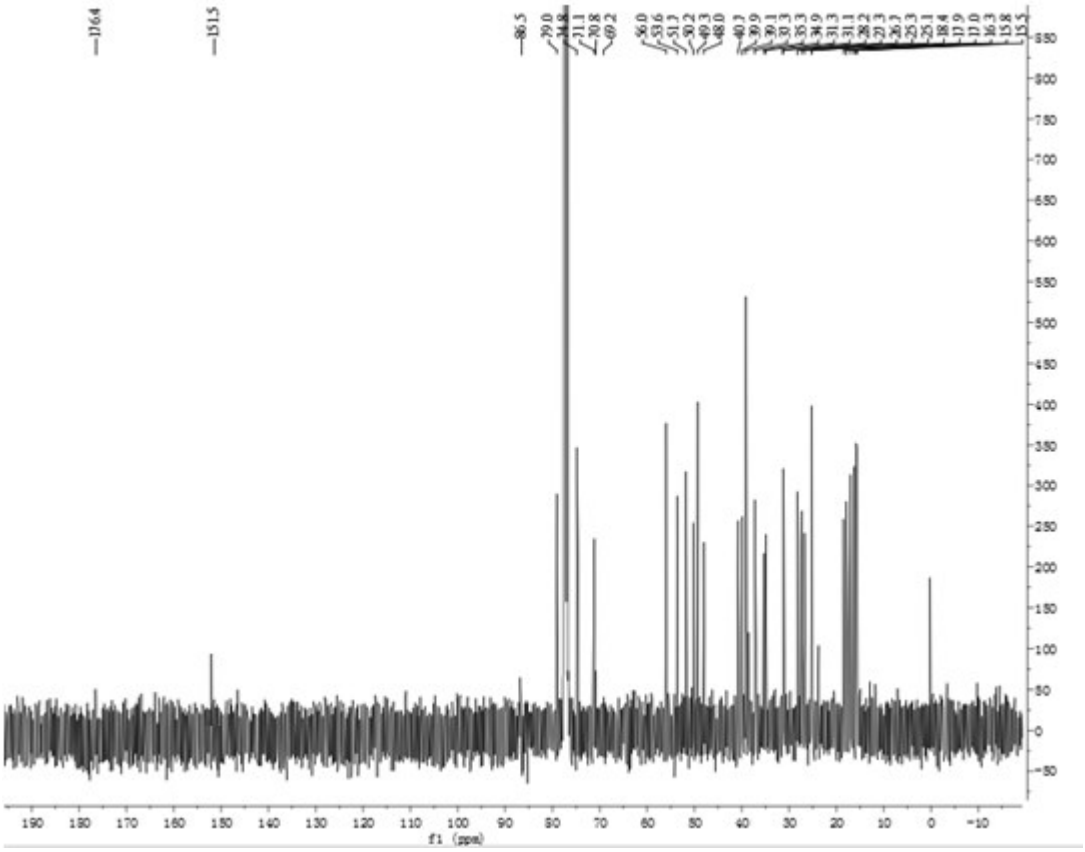


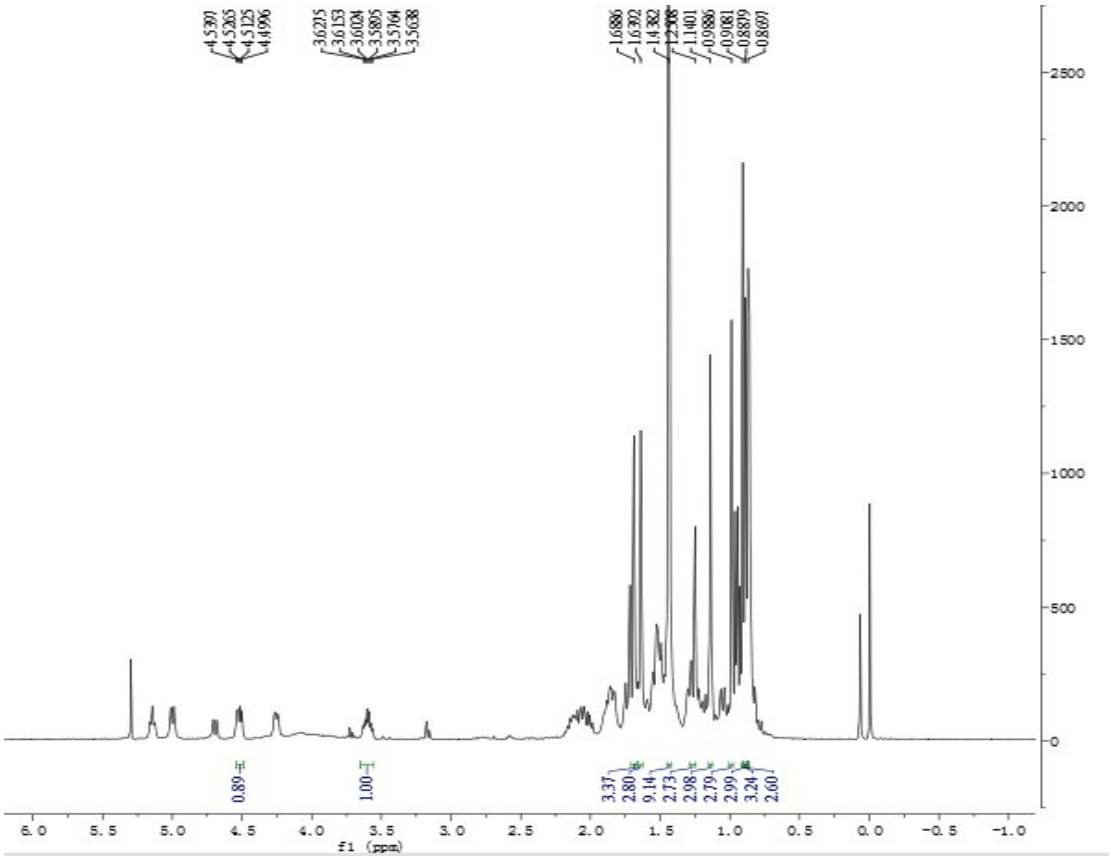
(20R)-3β-O-(Boc-L-tyrosyl)-dammarane-12β, 20, 25-triol (**6x**)



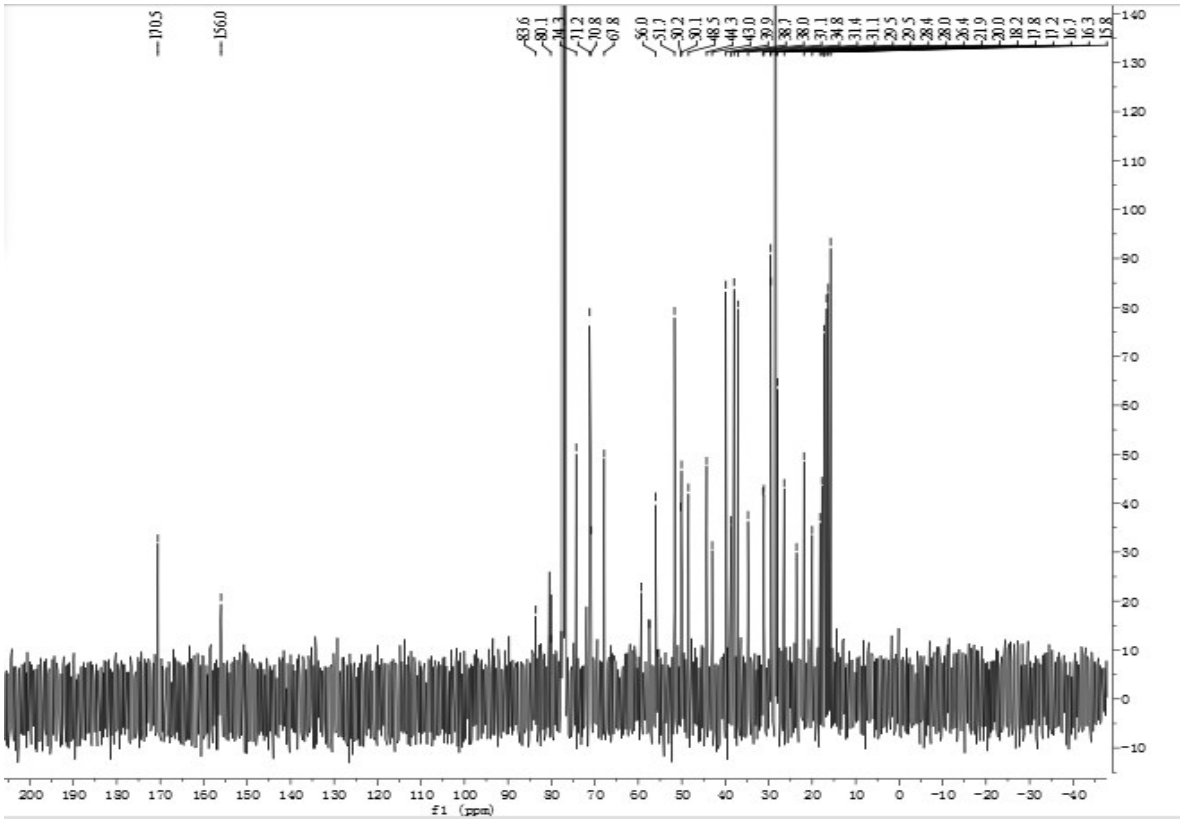


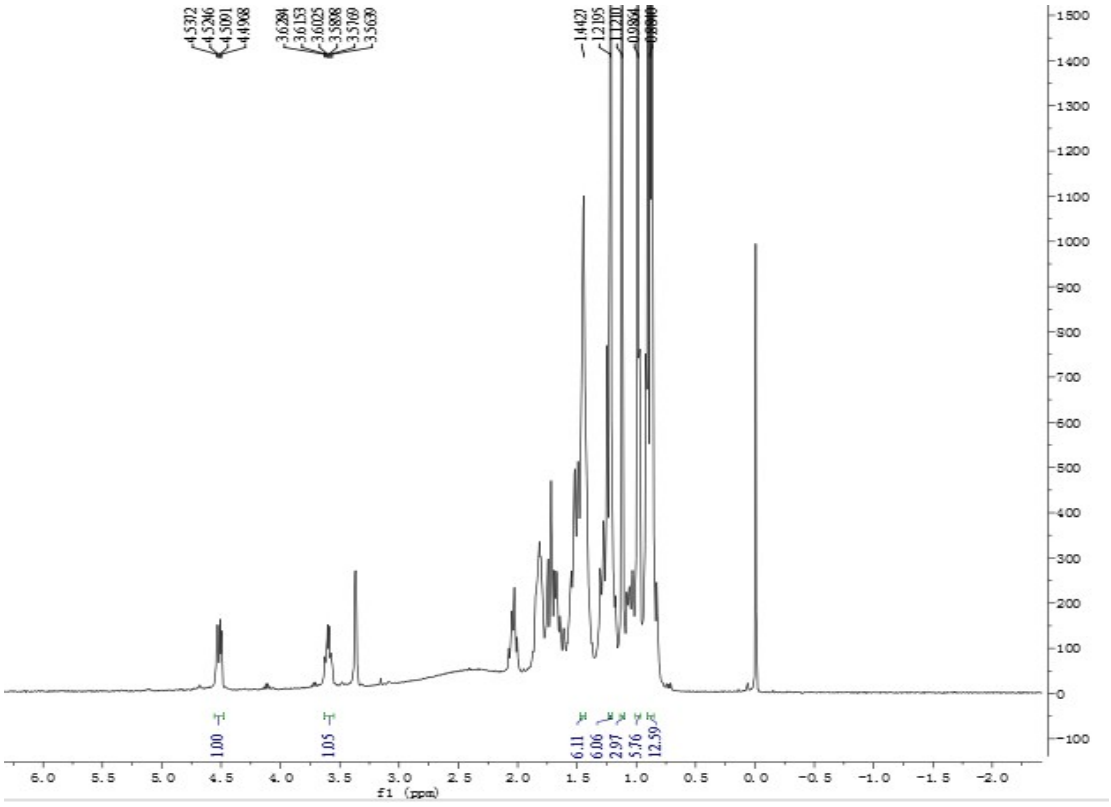
(20R)-3β-O-(Boc-L-seryl)-dammarane-12β, 20, 25-triol (**7x**)


176.4
151.5
86.5
79.0
74.6
71.1
70.8
69.2
56.0
53.6
51.7
50.2
49.3
48.0
40.3
39.9
39.1
37.3
35.3
34.9
31.3
31.1
28.2
27.3
26.7
25.3
25.1
18.4
17.9
17.0
16.3
15.8
15.2
f1 (ppm)

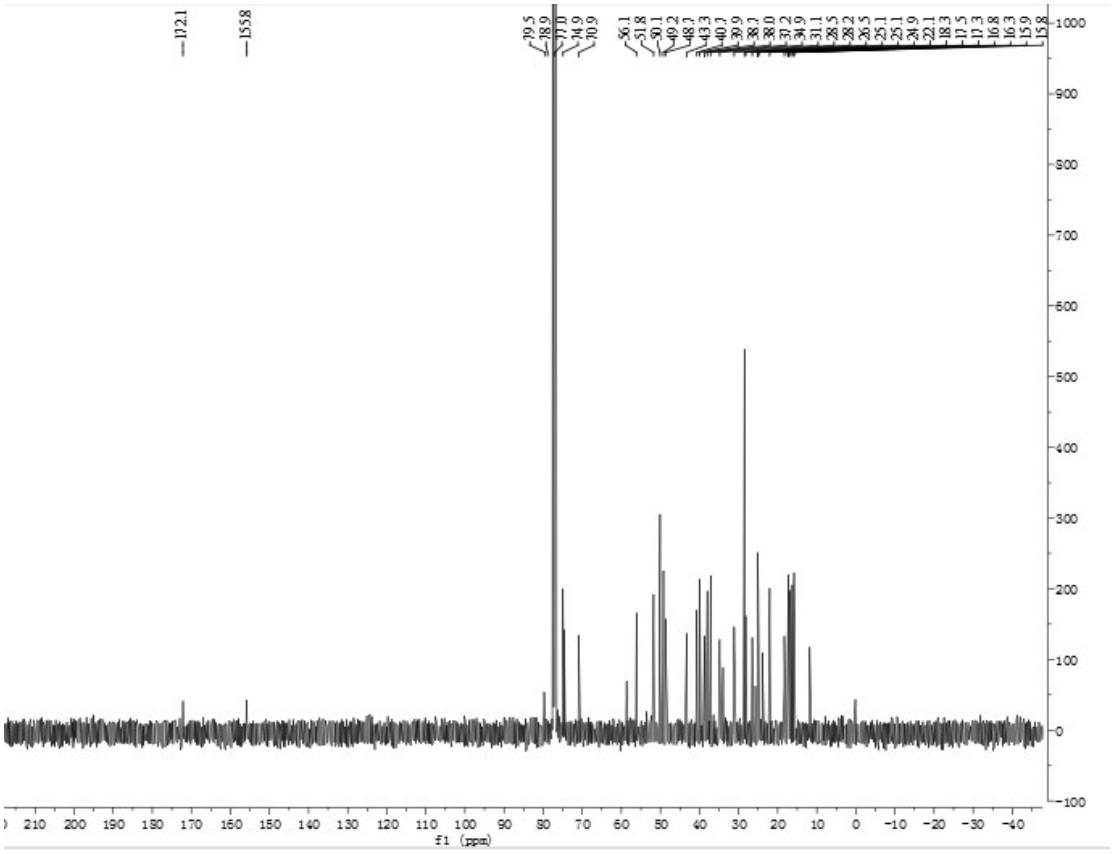

4.5301
4.5265
4.5125
4.4996
3.6225
3.6153
3.6024
3.5805
3.5764
3.5638
1.6886
1.6392
1.4382
1.2508
1.1401
0.9886
0.9081
0.8879
0.8697
0.89
1.00
3.37
2.80
9.14
2.73
2.98
2.79
2.99
3.24
2.60
f1 (ppm)

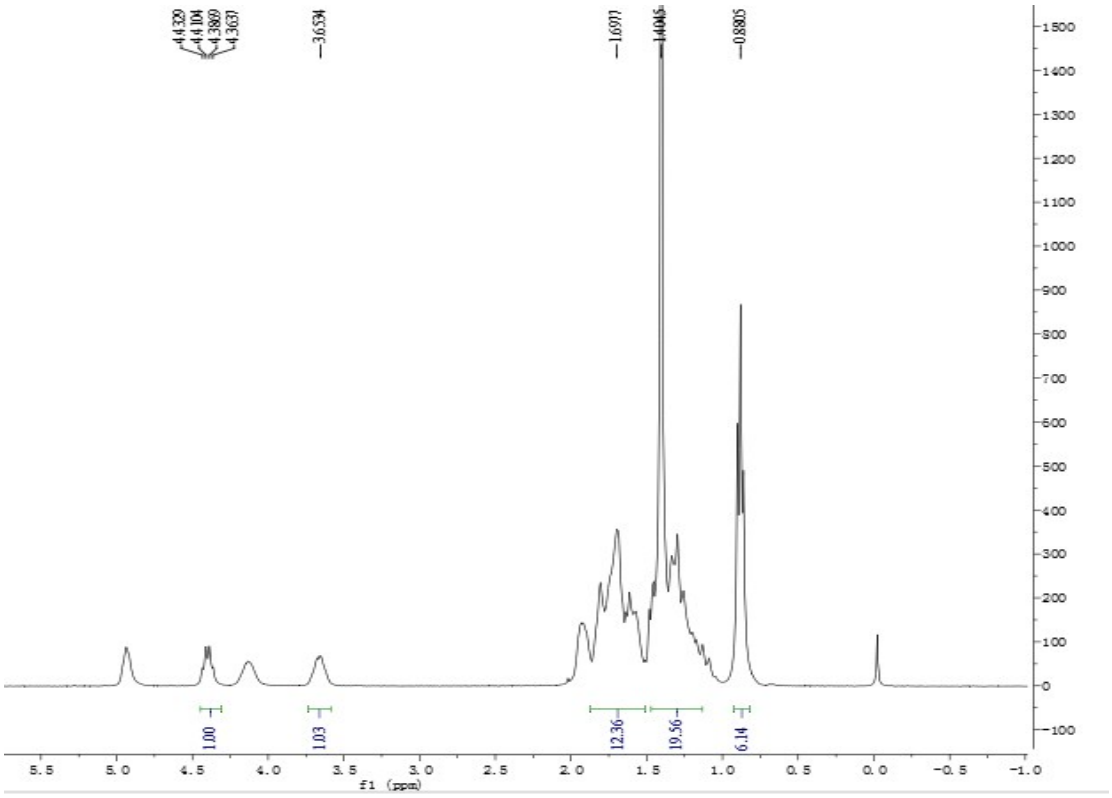
(20R)-3β-O-(Boc-L-threonyl)-dammarane-12β, 20, 25-triol (**8x**)



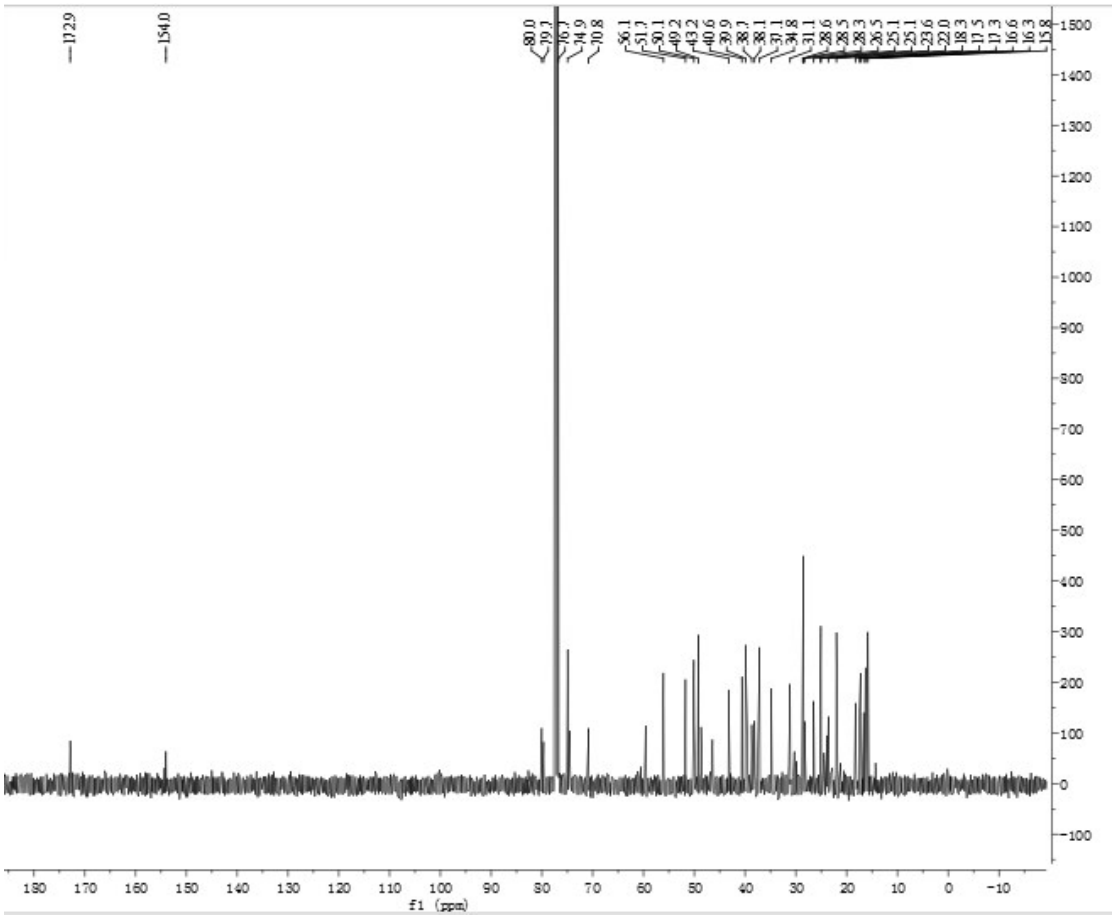


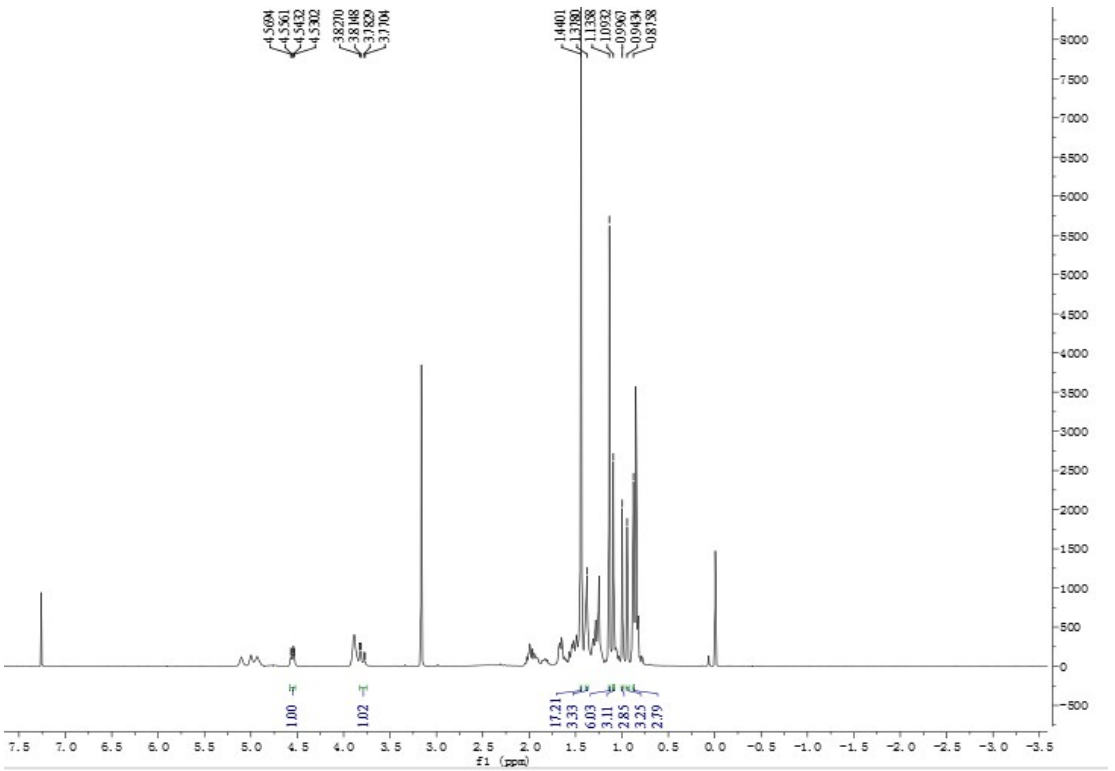
(20R)-12β-O-(Boc-L-leucyl)-dammarane-3β, 20, 25-triol (**1y**)



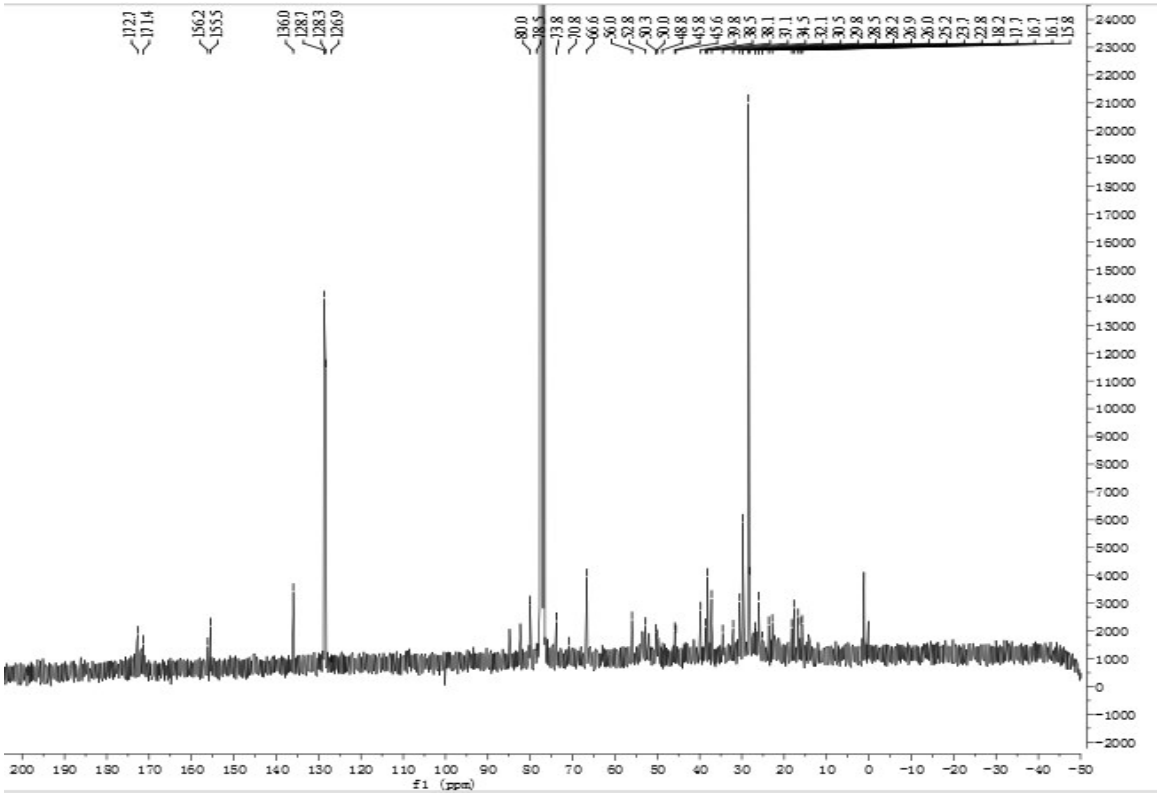


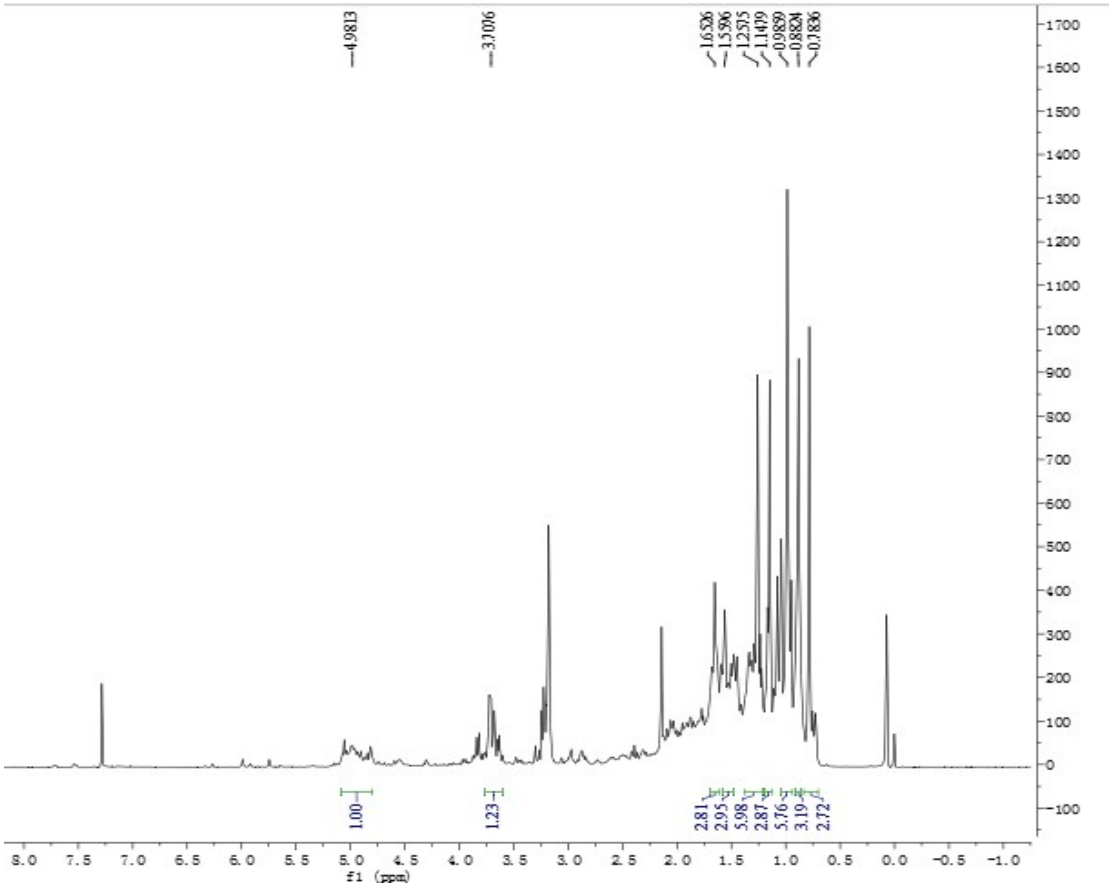
***(20R)-12β-O-(Boc-L-isoleucyl)-dammarane-3β, 20, 25-triol* (2y)**



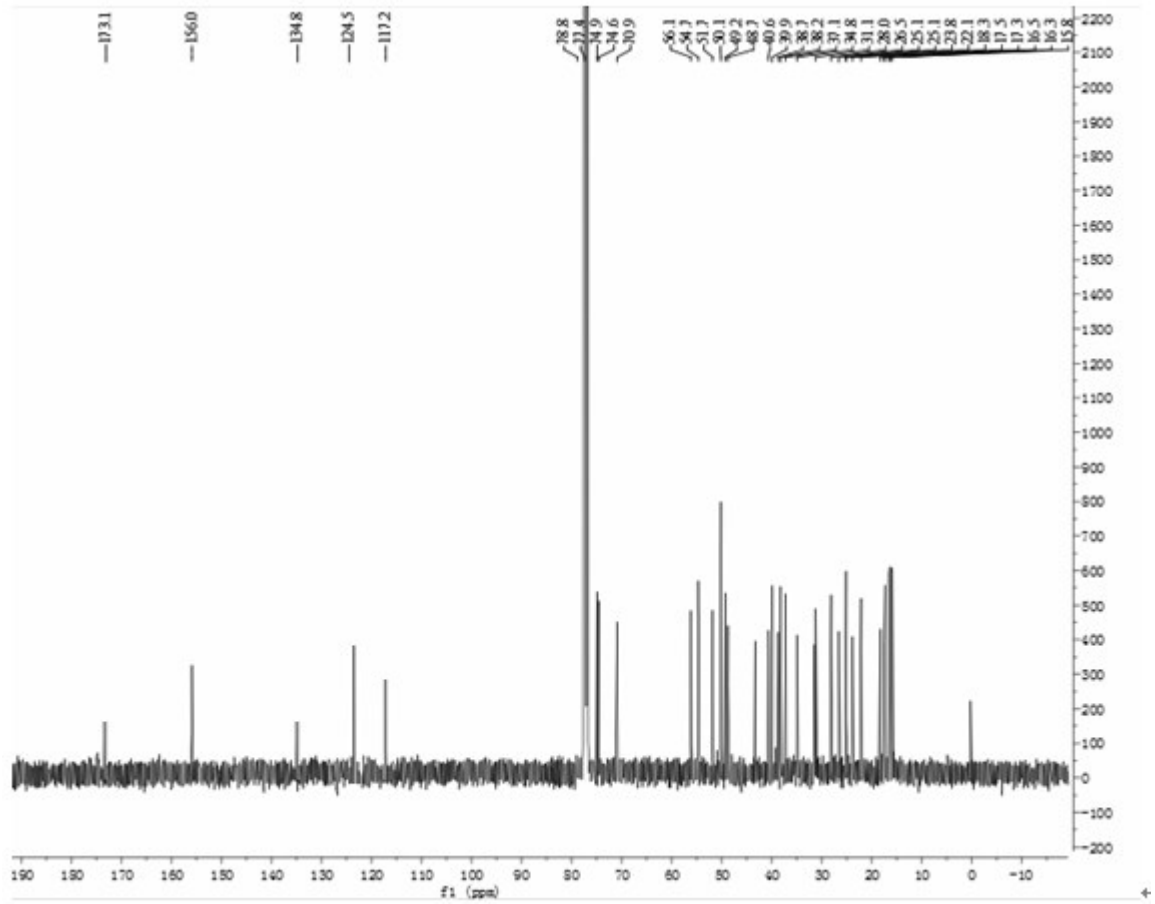


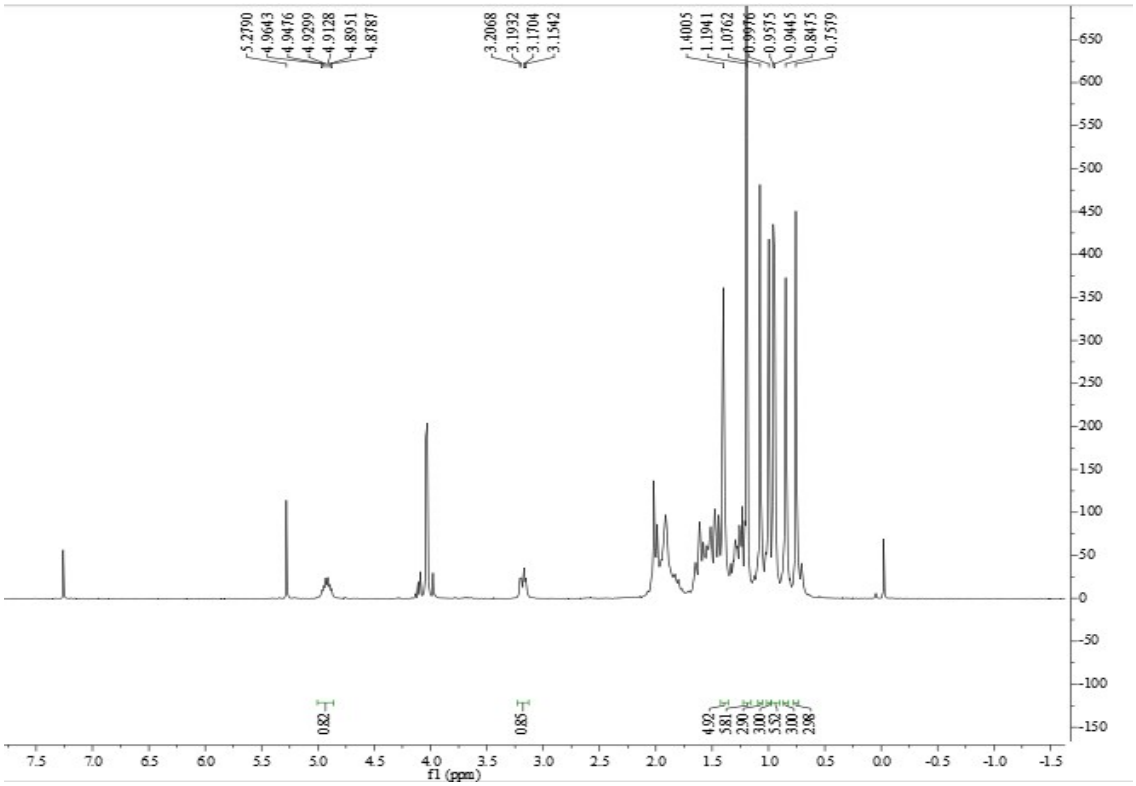
(20R)-12β-O-[Boc-L-glutamoyl(OBzl)]-dammarane-3β, 20, 25-triol (**3y**)



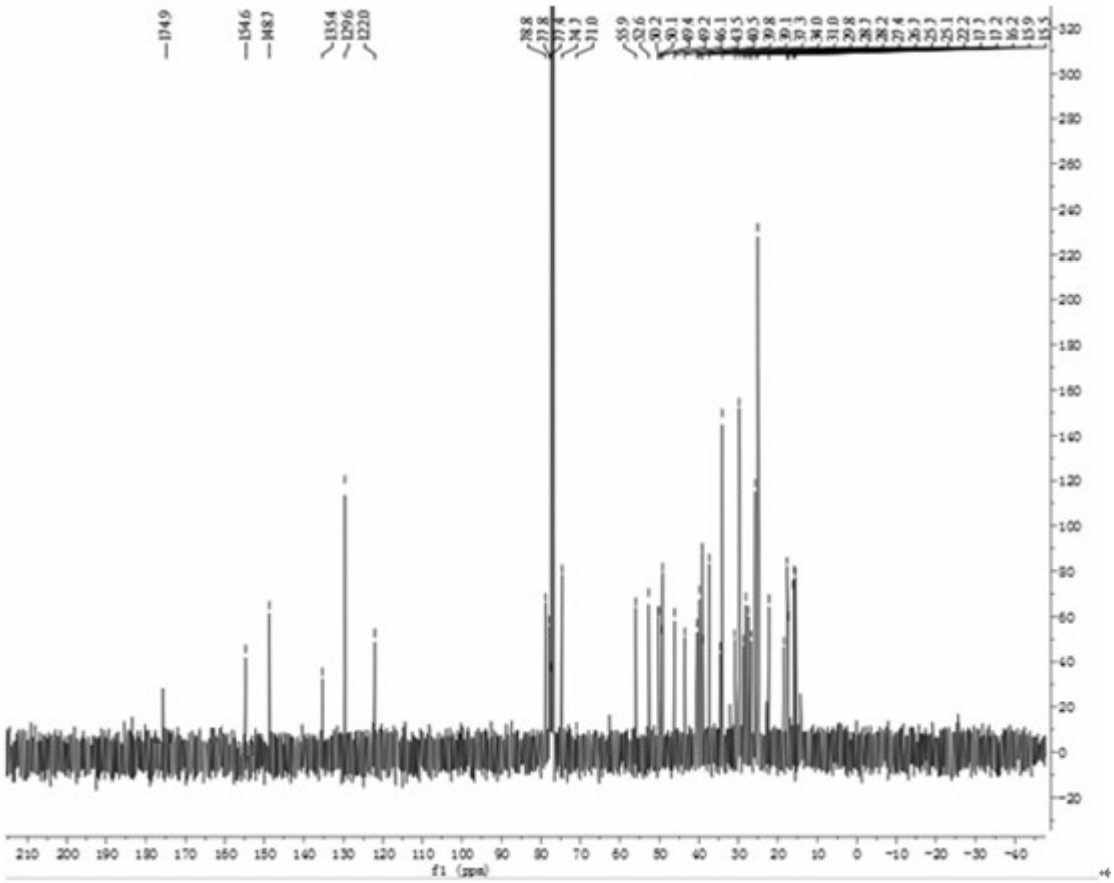


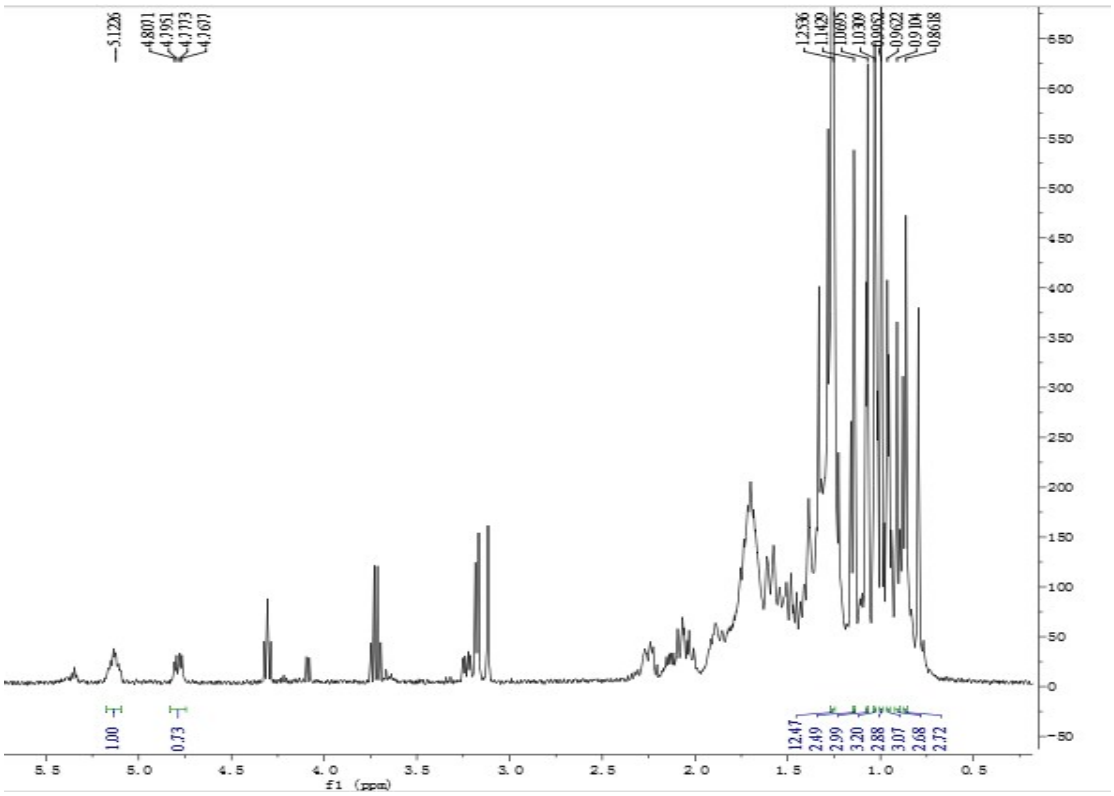
(20R)-12β-O-(Boc-L-histidyl)-dammarane-3β, 20, 25-triol (**5y**)



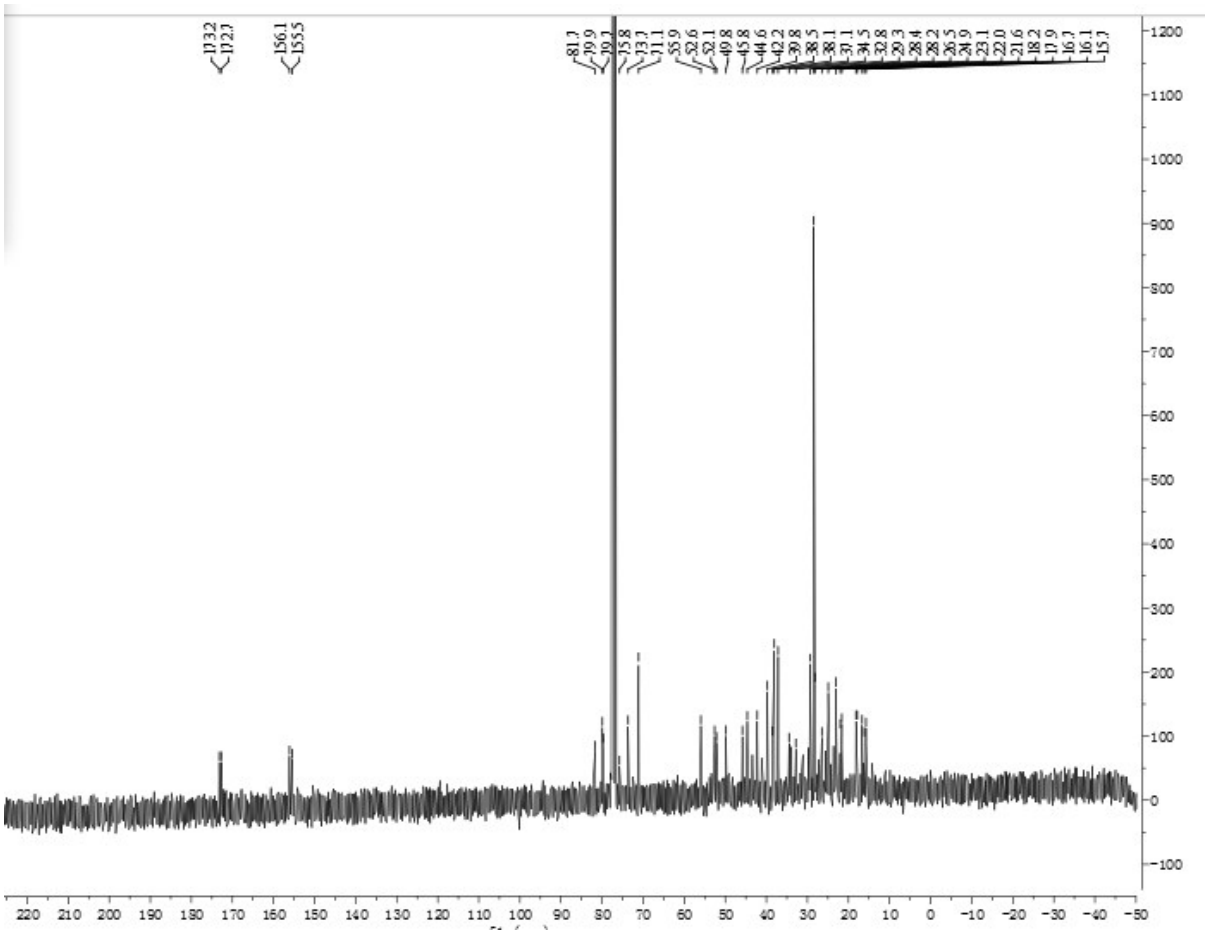


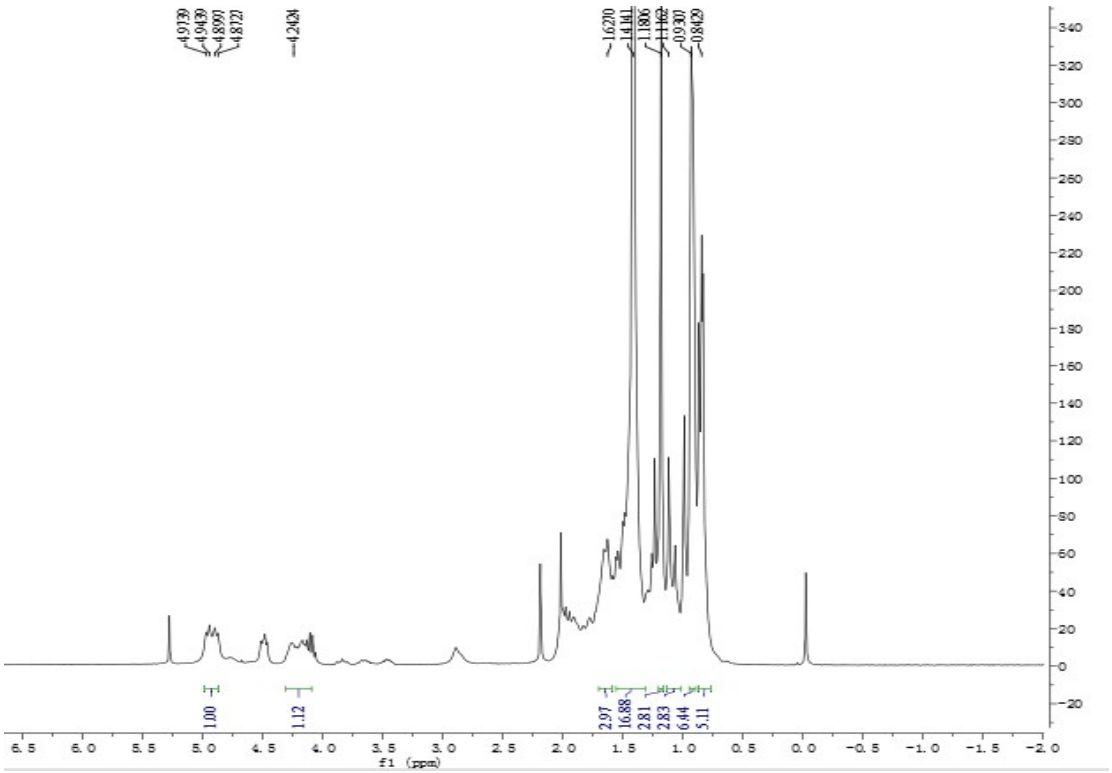
(20R)-12β-O-(Boc-L-tyrosyl)-dammarane-3β, 20, 25-triol (**6y**)



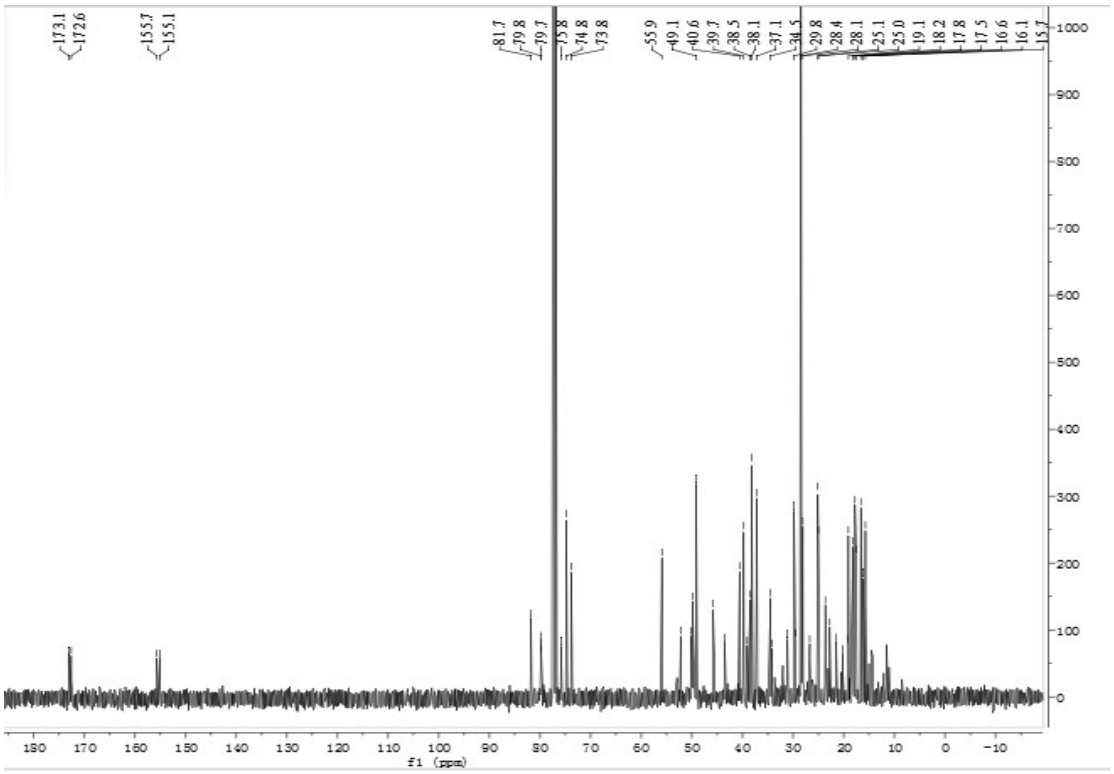


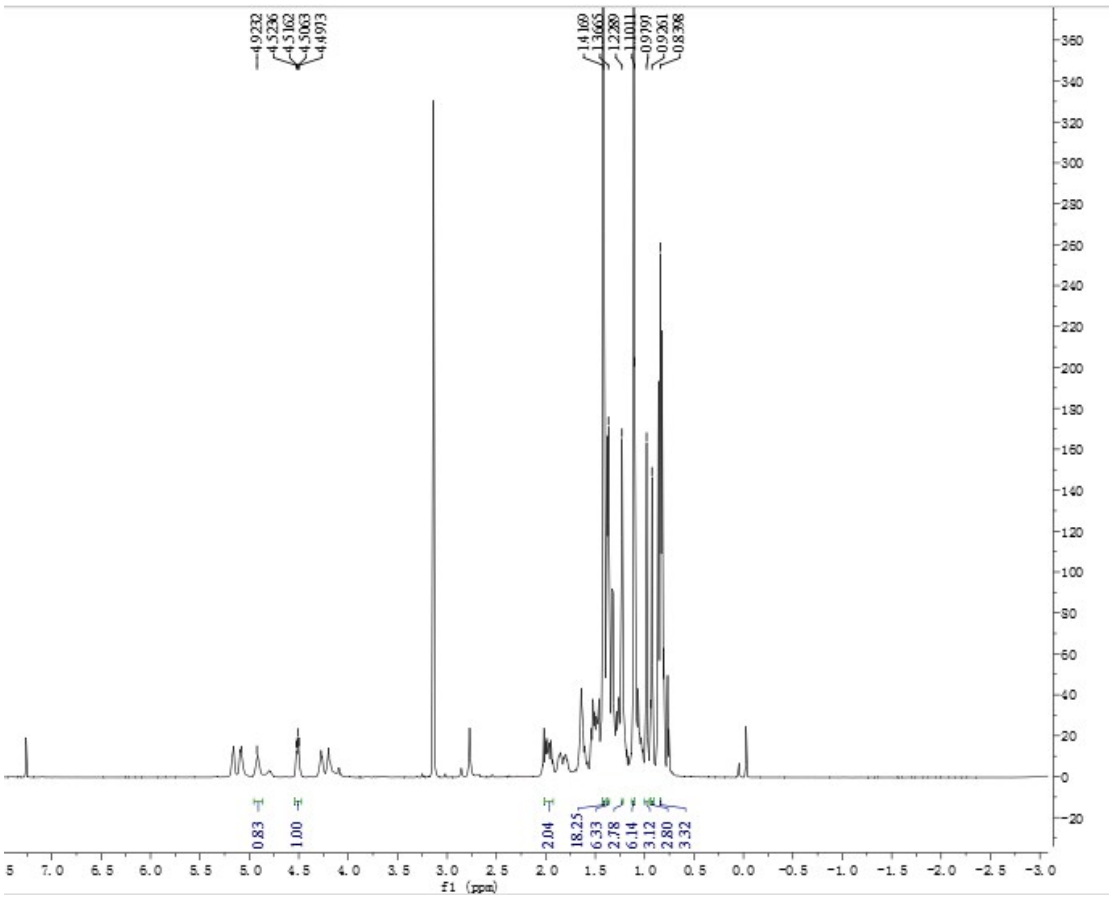
(20R)- 3β, 12β-O-di-(Boc-L-leucyl)-dammarane-20, 25-diol (**1z**)



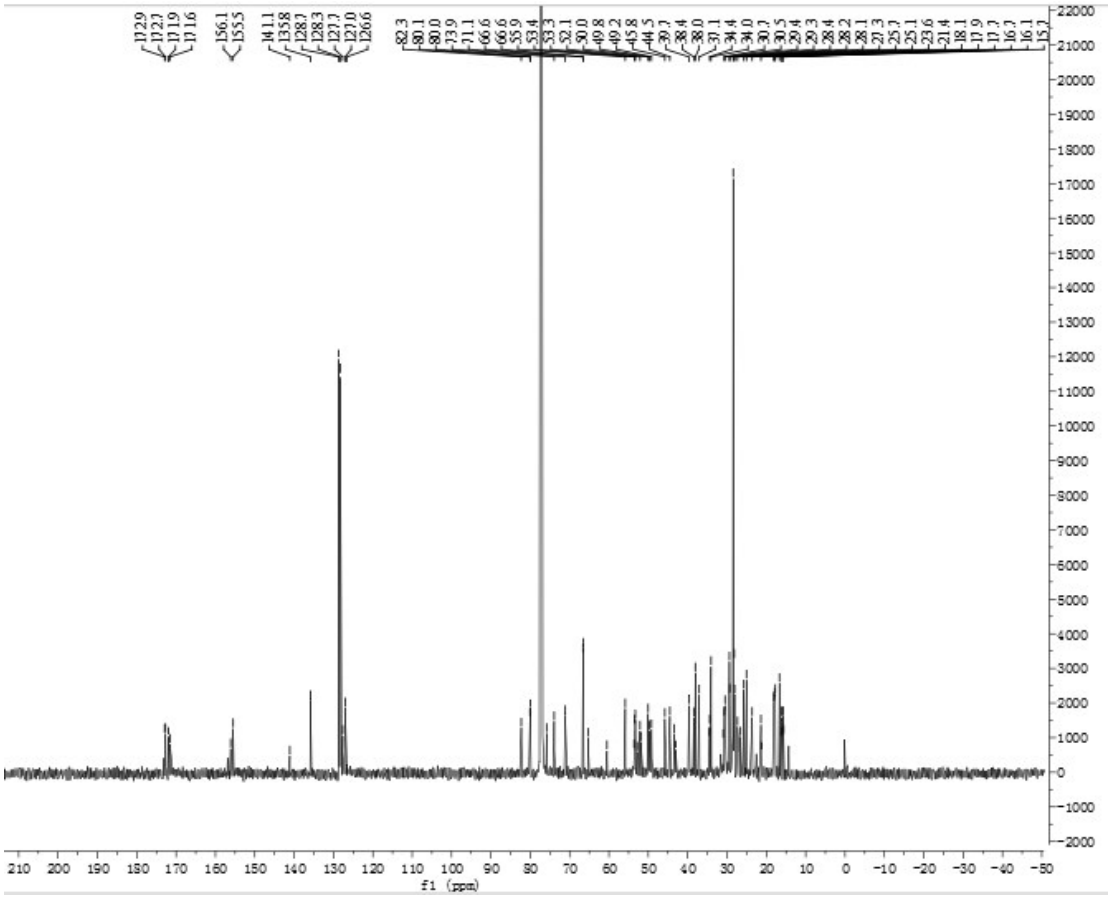


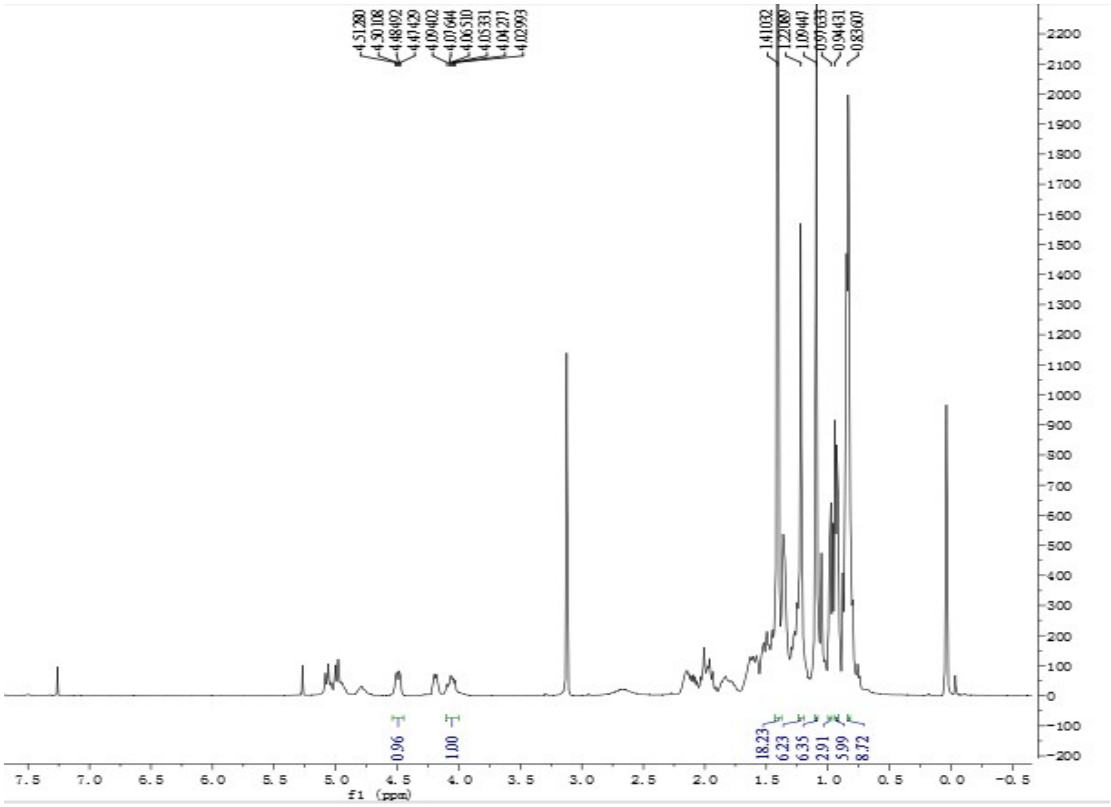
***(20R)-3β, 12β-O-di-(Boc-L-isoleucyl)-dammarane-20, 25-diol* (2z)**



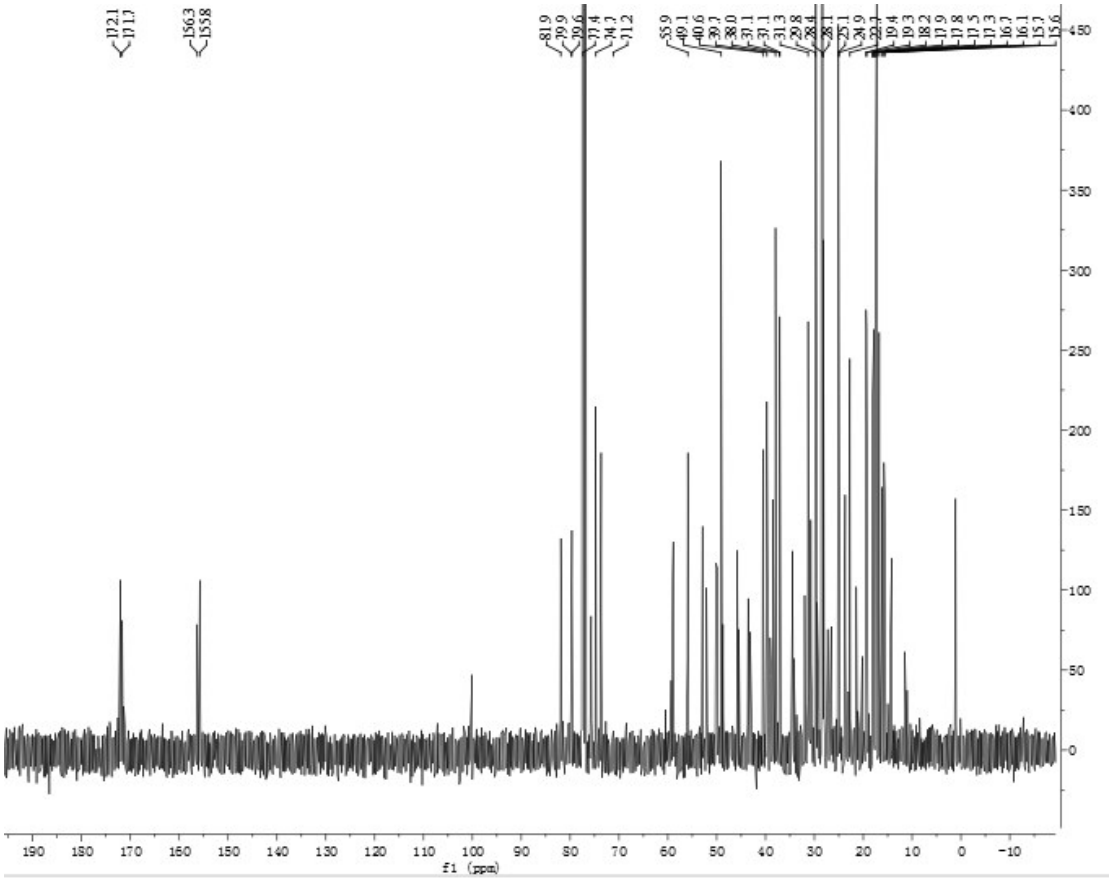


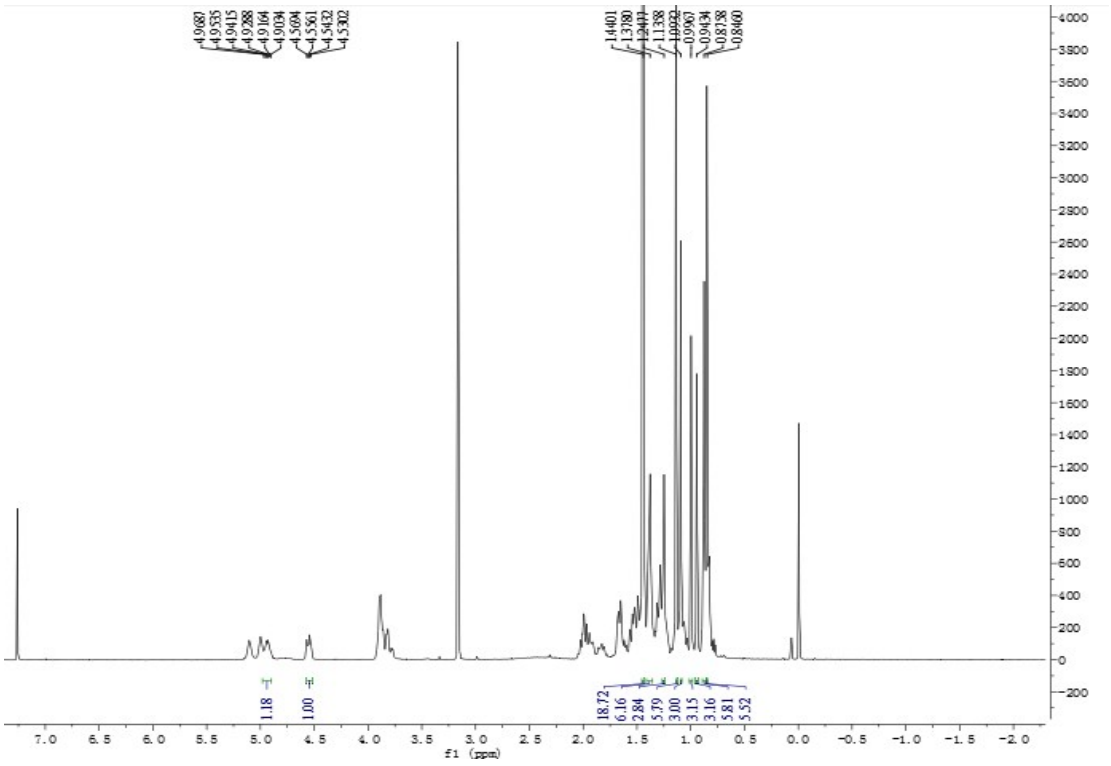
(20R)-3β, 12β-O-di-[Boc-L-glutamoyl(OBzl)]-dammarane-20, 25-diol (**3z**)



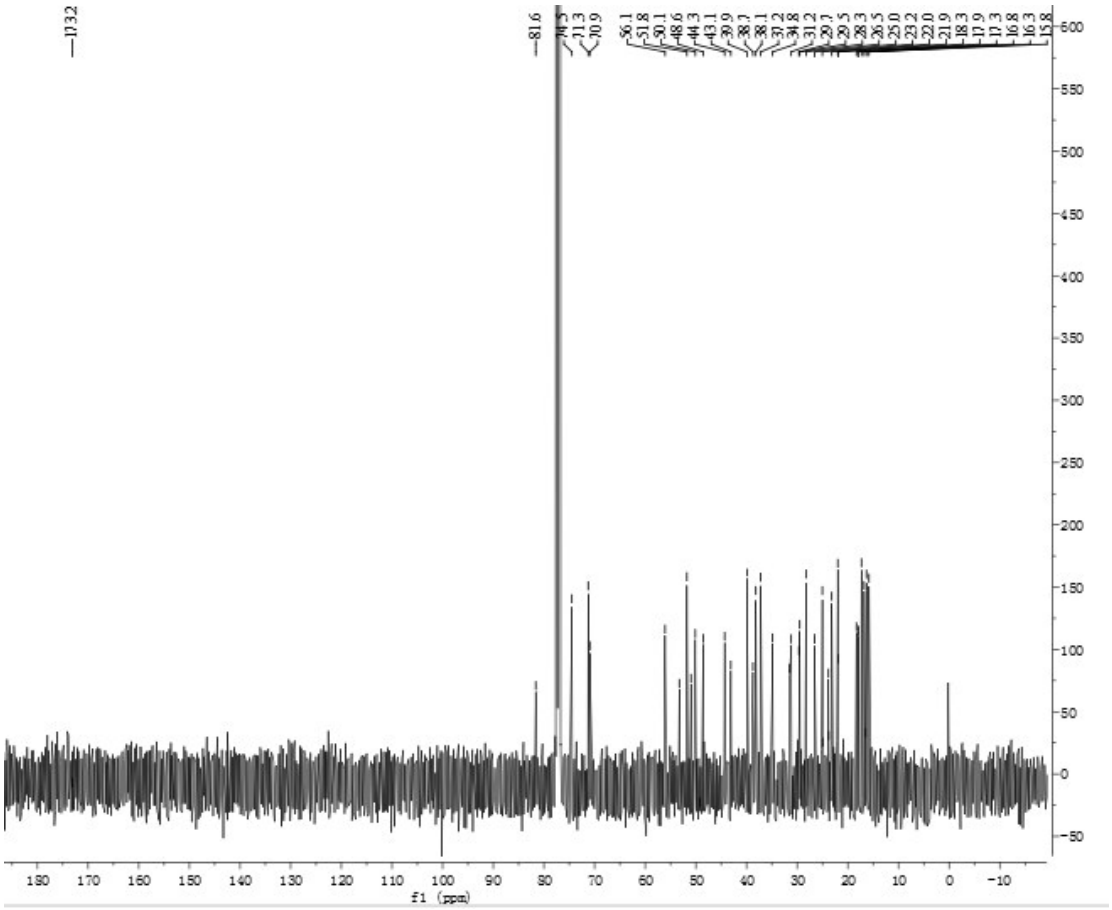


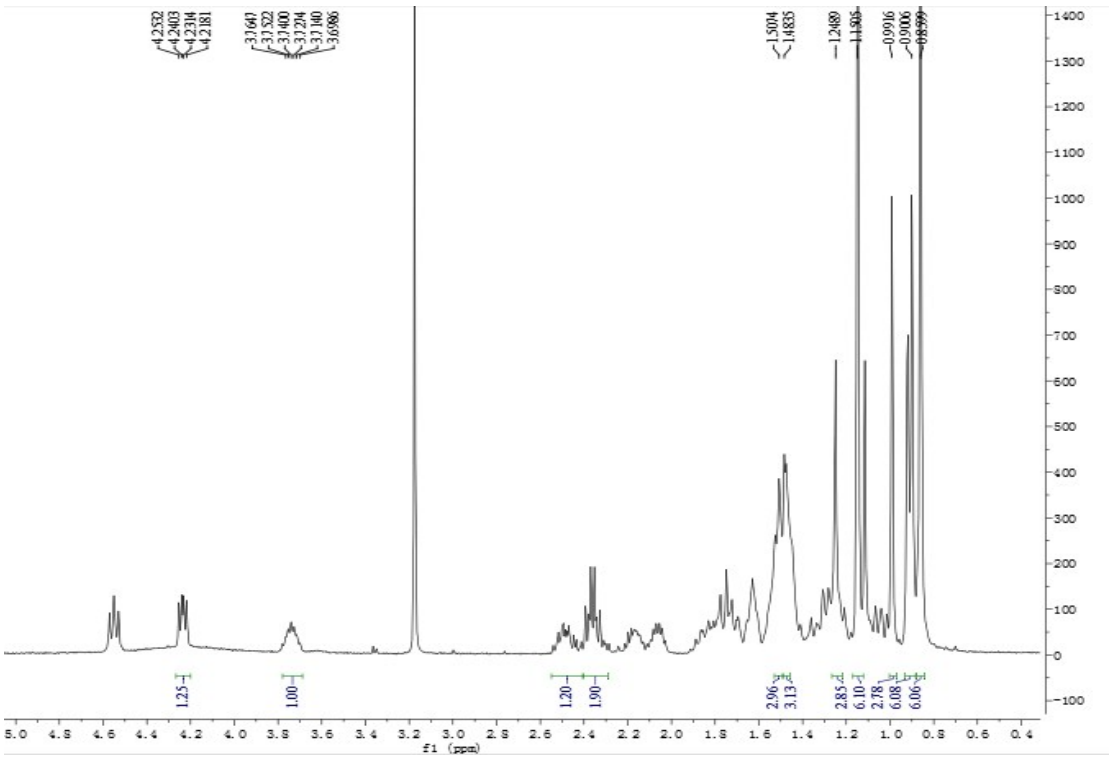
(20R)-3β, 12β-O-di-(Boc-L-alanyl)-dammarane-20, 25-diol (**4z**)



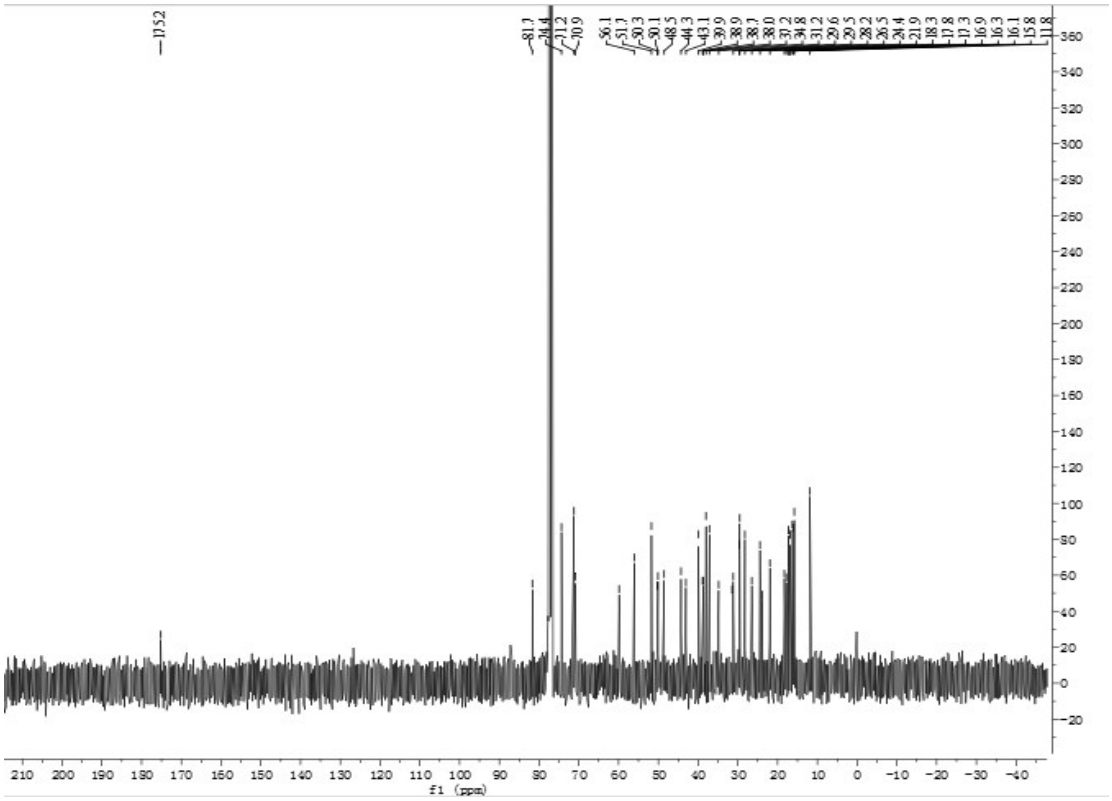


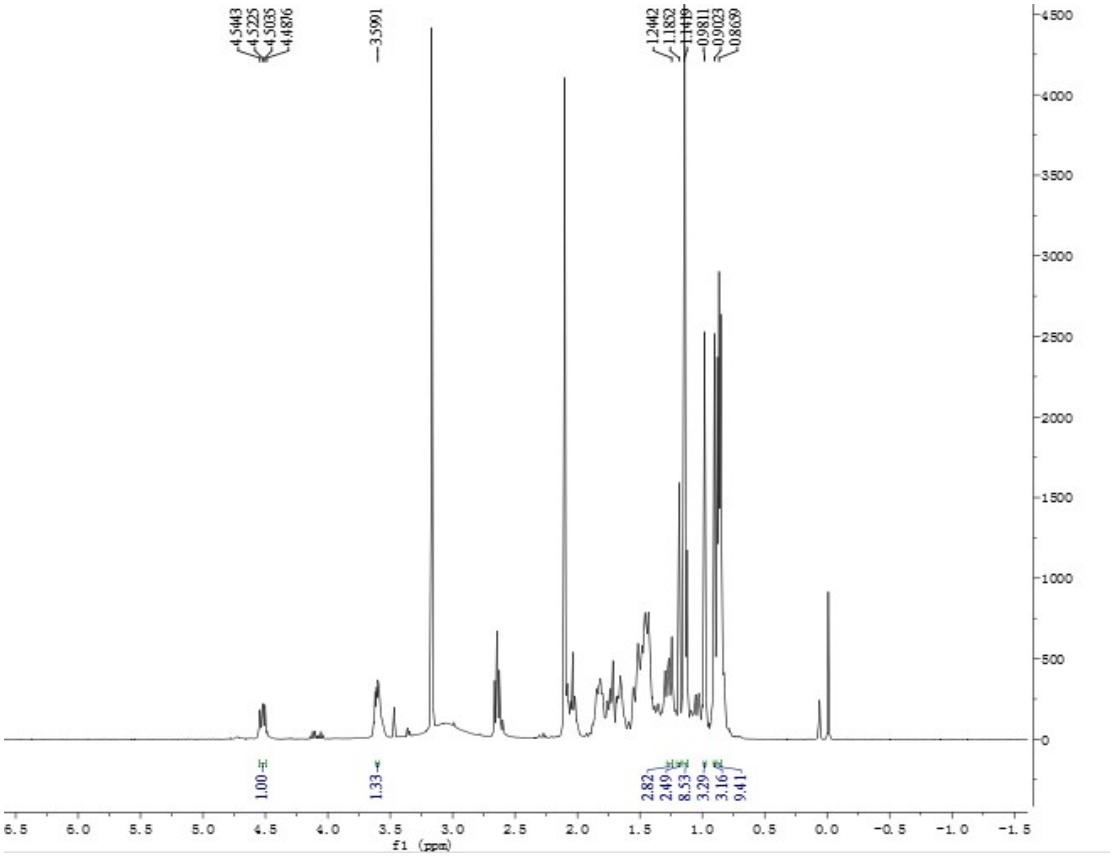
(20R)-3β-O-(L-leucyl)-dammarane-12β, 20, 25-triol **(1xt)**



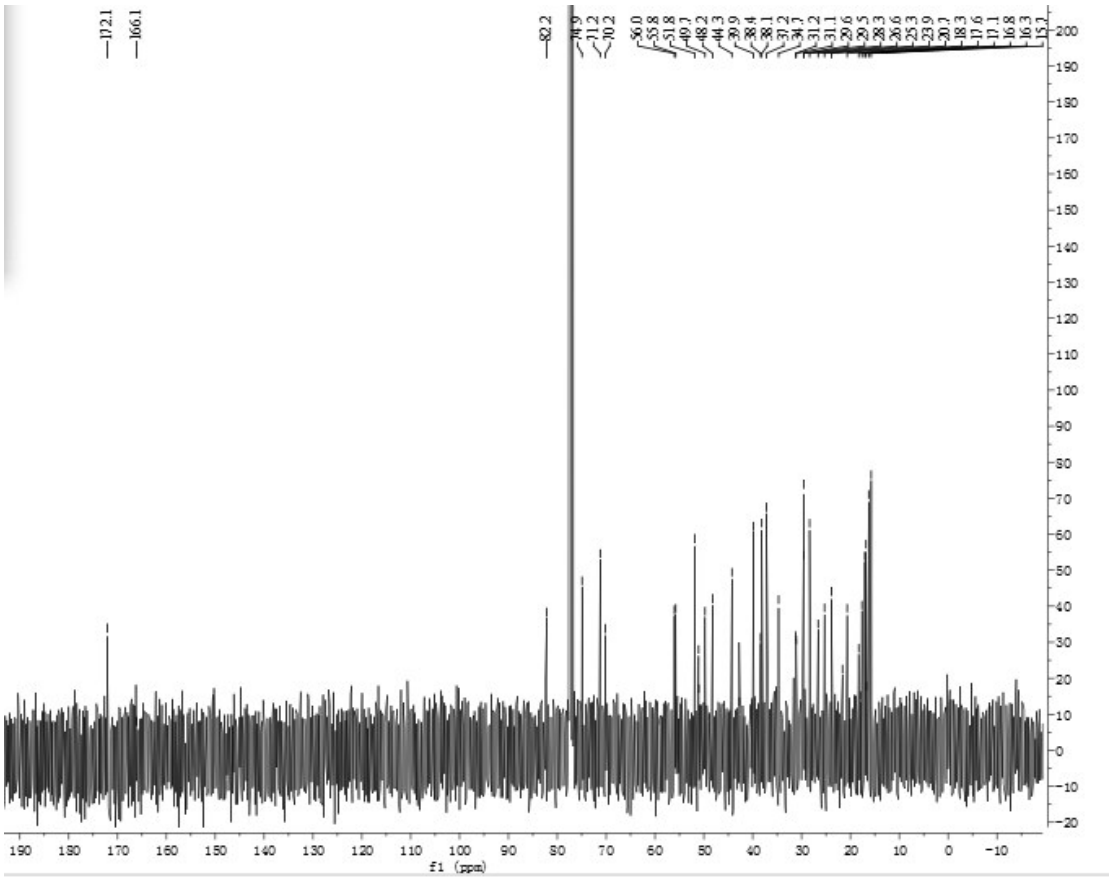


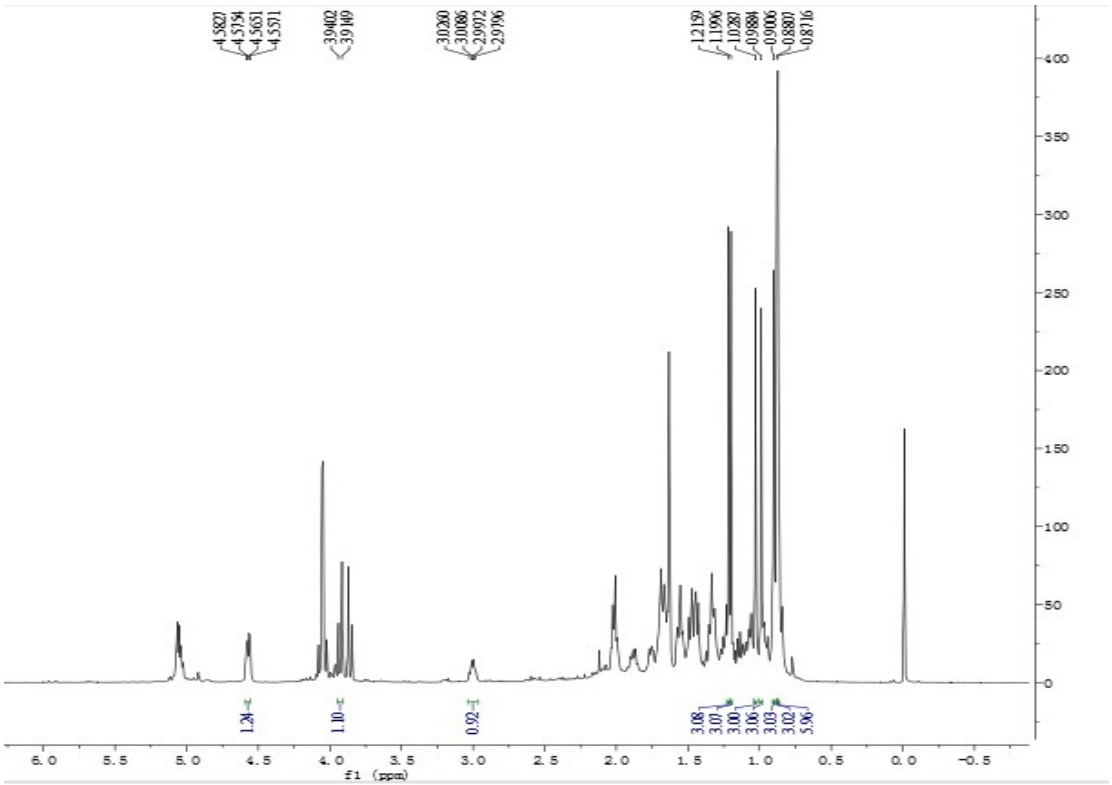
(20R)-3β-O-(L-isoleucyl)-dammarane-12β, 20, 25-triol (**2xt**)



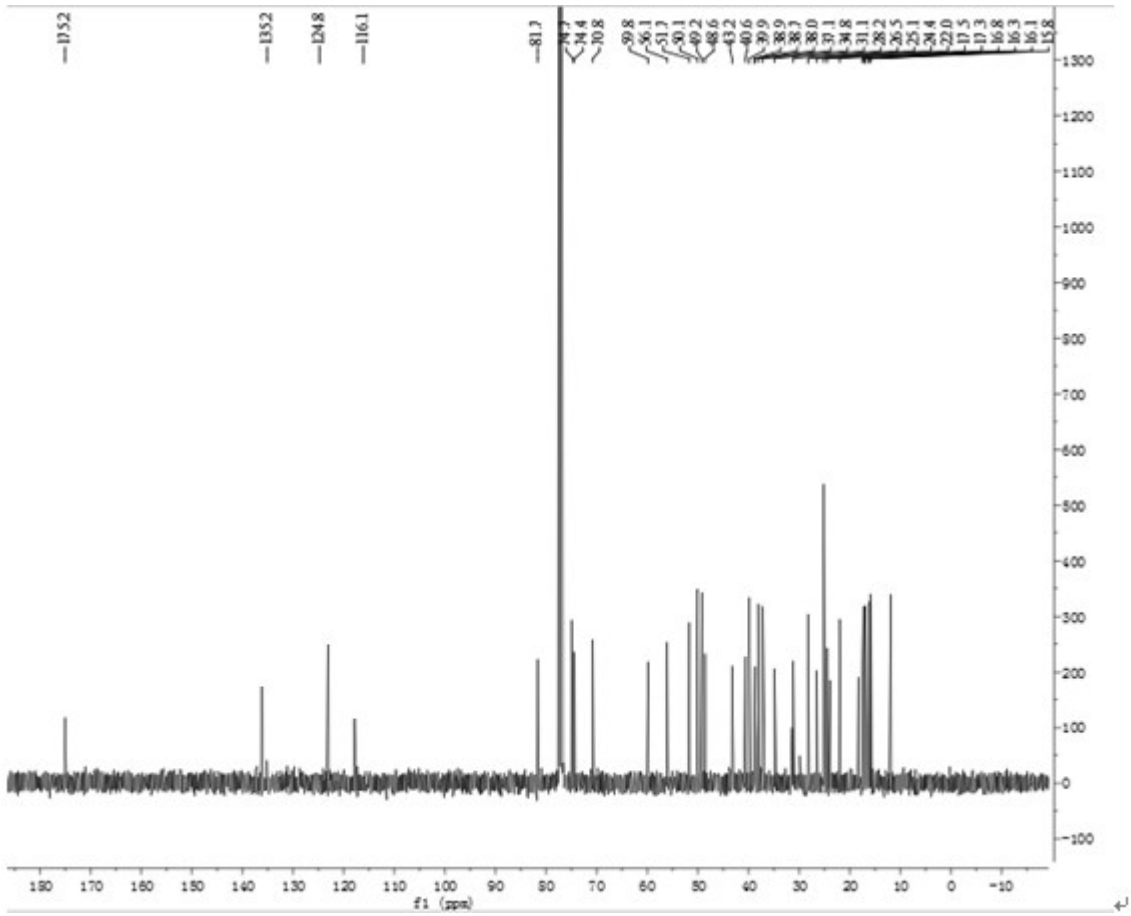


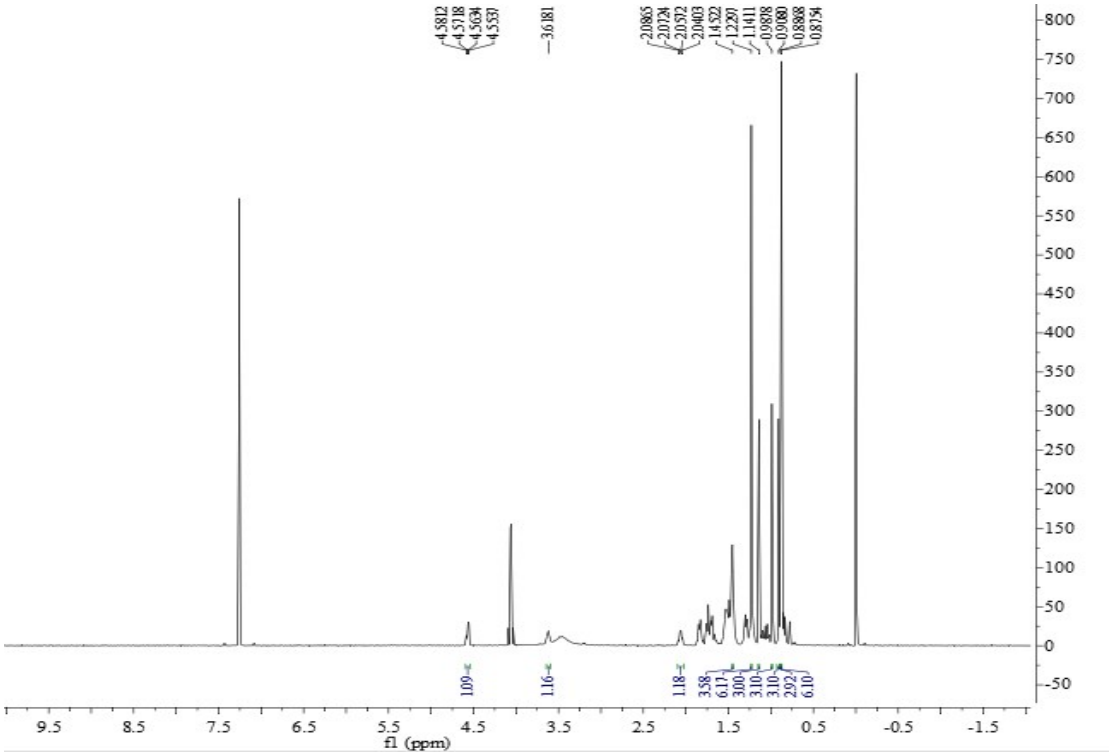
(20R)-3β-O-(L-glutamoyl)-dammarane-12β, 20, 25-triol (**3xt**)



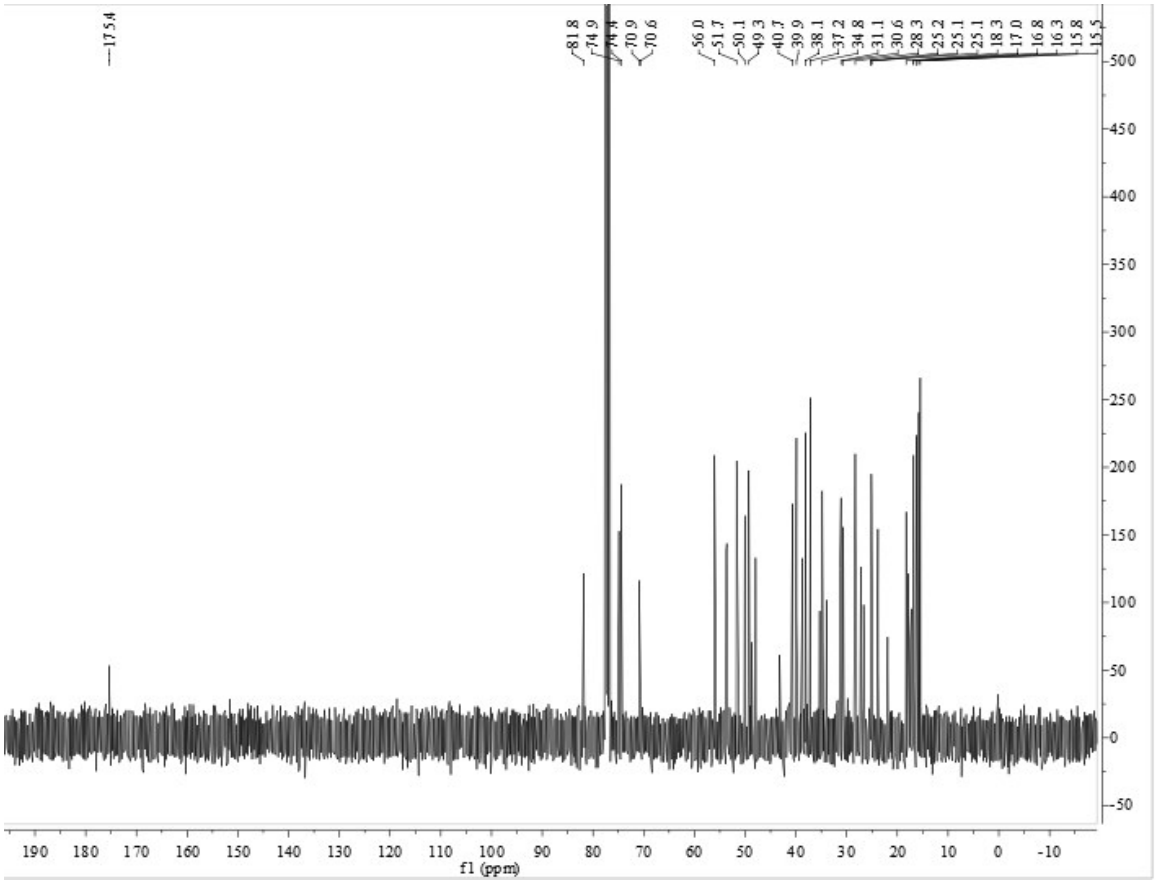


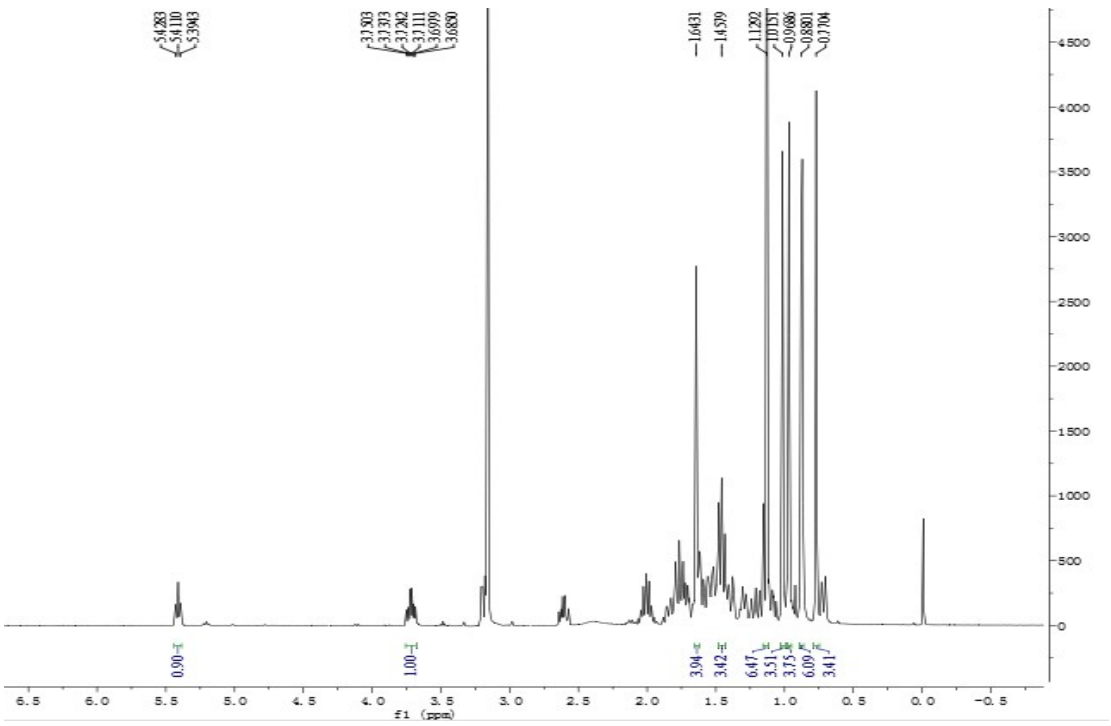
(20R)-3β-O-(L-histidyl)-dammarane-12β, 20, 25-triol (**5xt**)



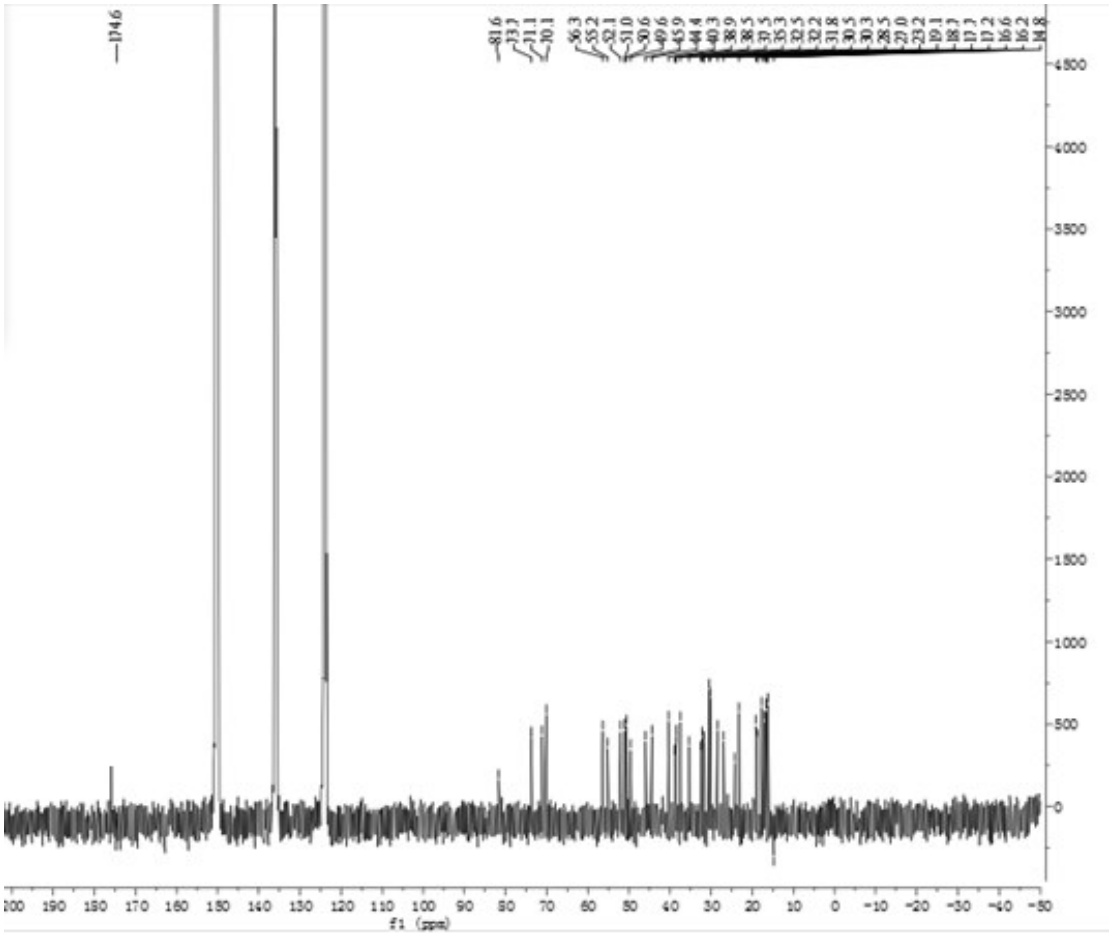


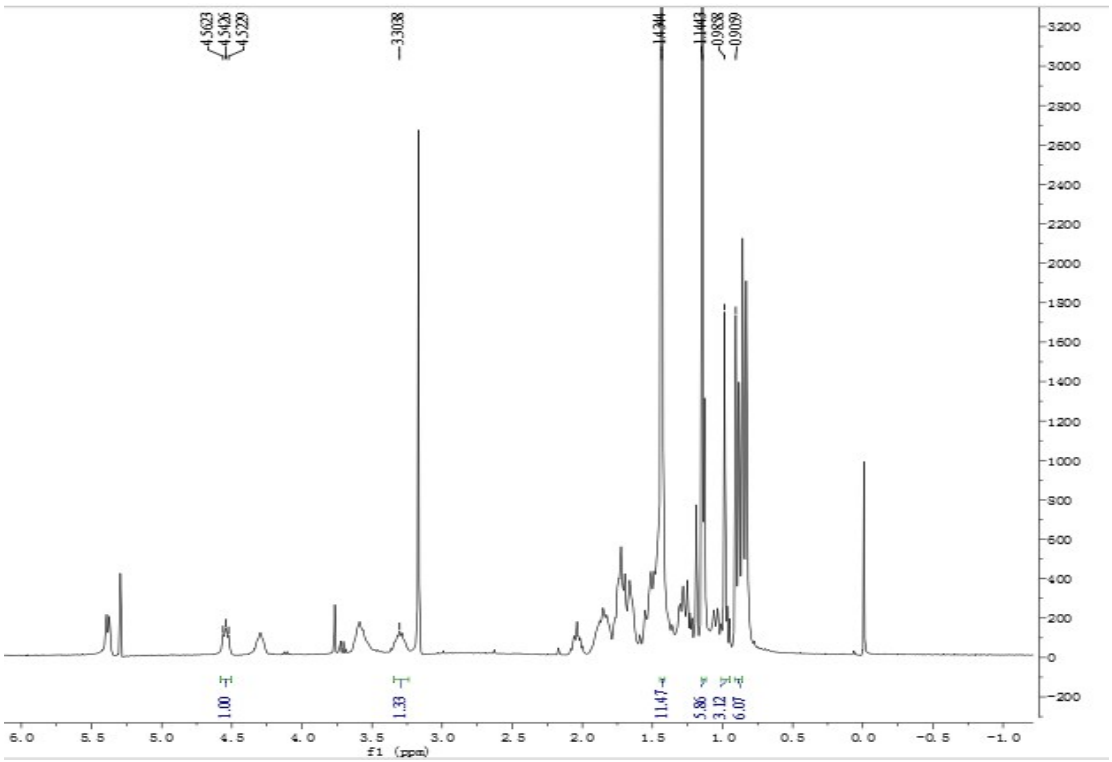
(20R)-3β-O-(L-threonyl)-dammarane-12β, 20, 25-triol (**8xt**)





(20R)-3β-O-(L-arginyl)-dammarane-12β, 20, 25-triol (**9xt**)





***(20R)-12β-O-(L-alanyl)-dammarane-3β, 20, 25-triol* (4yt)**