

Supporting Material

Novel C-3 and C-20 Derived analogs of Betulinic acid as Potent Cytotoxic Agents: Design, Synthesis, *In Vitro* and *In Silico* Studies

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Compound 9a

^1H NMR (CDCl_3 , 400 MHz): δ 4.73 and 4.60 (1H each, s, H-29), 4.51 (1H, t, $J = 8.4$ Hz, H-3), 3.65 (2H each, t, $J = 5.2$ Hz, 2x CH_2O), 3.22 (2H, s, COCH_2NH), 3.00 (1H, m, H-19), 2.60 (2H each, t, $J = 5.2$ Hz, 2x $\text{CH}_2\text{N-}$), 2.24 and 1.63 (1H, each, m, H-22), 2.20 and 1.49 (1H each, m, H-16), 1.95 (2H, m, H-2), 1.69 (3H, s, H-30), 1.64 (1H, m, H-13), 1.61 (1H, m, H-18), 1.59 and 1.39 (1H each, m, H-6), 1.52 and 1.25 (1H each, m, H-15), 1.54 and 1.41 (1H each, m, H-7), 1.41 and 1.17 (1H each, m, H-21), 1.37 and 1.23 (1H each, m, H-12), 1.34 (1H, m, H-9), 1.15 and 1.07 (1H each, m, H-11), 1.62 and 1.02 (1H each, m, H-1), 0.97 (3H, s, H-26), 0.93 (3H, s, H-24), 0.84 (6H, each, s, H-27 and H-23), 0.82 (3H, s, H-25), 0.78 (1H, m, H-5); ^{13}C NMR (100 MHz, CDCl_3) δ 179.63, 169.89, 150.60, 109.50, 81.65, 66.59 (2x $\text{CH}_2\text{-O}$), 59.49, 56.20 (2x $\text{CH}_2\text{-N}$), 55.35, 53.05, 50.39, 49.17, 46.94, 42.40, 40.65, 38.30, 38.24, 37.77, 37.05, 34.19, 32.21, 30.55, 29.63, 27.96, 25.43, 23.73, 20.87, 19.24, 18.12, 16.48, 16.09, 15.89, 14.58. HRMS m/z calcd for $\text{C}_{36}\text{H}_{57}\text{NO}_5$ $[\text{M}+\text{H}]^+$ 583.0215, found 583.0226.

Compound 9b

^1H NMR (CDCl_3 , 400 MHz): δ 4.73 and 4.60 (1H, each, s, H-29), 4.52 (1H, t, $J = 8.4$ Hz, H-3), 3.00 (1H, ddd, $J = 4.8, 4.4, 4.3$ Hz, H-19), 3.2 (2H, m, COCH_2NH) 2.55 (4H, m, 2x $\text{CH}_2\text{CH}_2\text{N-}$), 2.26 and 1.63 (1H, each, m, H-22), 2.19 and 1.49 (1H each, m, H-16), 1.97 (2H, m, H-2), 1.69 (3H, s, H-30), 1.64 (1H, m, H-13), 1.62 (1H, m, H-18), 1.59 and 1.39 (1H each, m, H-6), 1.52 and 1.25 (1H each, m, H-15), 1.53 and 1.41 (1H each, m, H-7), 1.43 (4H, m, 2x $\text{CH}_2\text{CH}_2\text{CH}_2$) 1.41 and 1.19 (1H each, m, H-21), 1.38 (2H, m, $\text{CH}_2\text{CH}_2\text{CH}_2$) 1.37 and 1.24 (1H each, m, H-12), 1.34 (1H, m, H-9), 1.15 and 1.07 (1H each, m, H-11), 1.62 and 1.05 (1H each, m, H-1), 0.96 (3H, s, H-26), 0.93 (3H, s, H-24), 0.84 (6H, each, s, H-27 and H-23), 0.82 (3H, s, H-25), 0.77 (1H, m, H-5); ^{13}C NMR (125 MHz, CDCl_3) δ 180.68, 169.55, 150.77, 109.51, 81.37, 59.08, 56.30, 55.44, 53.33 (2x

CH), 50.43, 49.22, 46.98, 42.42, 40.67, 38.35, 38.29, 37.75, 37.13, 34.25, 32.37, 30.70, 29.74, 29.58, 28.04, 25.49, 25.08 (2x CH), 23.75, 23.61, 20.91, 19.40, 18.18, 16.58, 16.20, 16.03, 14.67. HRMS m/z calcd for $C_{37}H_{59}NO_4$ $[M+H]^+$ 532.0230, found 532.0241.

Compound 9c

1H NMR ($CDCl_3$, 400 MHz): δ 4.73 and 4.60 (1H, each, s, H-29), 4.52 (1H, t, J = 6.8 Hz, H-3), 3.2 (2H, s, $COCH_2NH$), 3.00 (1H, m, H-19), 2.85 (4H, m, 2x $CH_2CH_2N^-$), 2.70 (4H, m, 2x $CH_2CH_2S^-$), 2.26 and 1.63 (1H, each, m, H-22), 2.18 and 1.49 (1H each, m, H-16), 1.97 (2H, m, H-2), 1.69 (3H, s, H-30), 1.64 (1H, m, H-13), 1.62 (1H, m, H-18), 1.59 and 1.39 (1H each, m, H-6), 1.52 and 1.25 (1H each, m, H-15), 1.53 and 1.41 (1H each, m, H-7), 1.41 and 1.19 (1H each, m, H-21), 1.37 and 1.24 (1H each, m, H-12), 1.34 (1H, m, H-9), 1.15 and 1.07 (1H each, m, H-11), 1.62 and 1.05 (1H each, m, H-1), 0.97 (3H, s, H-26), 0.93 (3H, s, H-24), 0.84 (6H, each, s, H-27 and H-23), 0.83 (3H, s, H-25), 0.79 (1H, m, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 181.74, 169.96, 150.50, 109.71, 81.54, 60.05, 56.36, 55.37, 54.34 (2x CH), 50.37, 49.21, 46.97, 42.42, 40.67, 38.37, 38.35, 37.79, 37.09 (2x C), 34.21, 32.21, 30.60, 29.72, 28.07, 27.65 (2x CH), 25.43, 23.81, 20.87, 19.37, 18.16, 16.62, 16.19, 16.03, 14.68. HRMS m/z calcd for $C_{36}H_{57}NO_5$ $[M+H]^+$ 600.0145, found 600.0146.

Compound 9d

1H NMR ($CDCl_3$, 400 MHz): δ 4.72 and 4.59 (1H, each, s, H-29), 4.51 (1H, t, J = 8.0 Hz, H-3), 3.73 (1H, m, $CHOH$), 3.00 (1H, m, H-19), 2.88 (2H, m, $COCH_2N^-$), 2.45 and 1.92 (2H each, m, 2x $CH_2CH_2N^-$), 2.26 and 1.63 (1H, each, m, H-22), 2.19 and 1.49 (1H each, m, H-16), 1.95 (2H, m, H-2), 1.68 (3H, s, H-30), 1.64 (1H, m, H-13), 1.61 (1H, m, H-18), 1.59 and 1.39 (1H each, m, H-6), 1.52 and 1.25 (1H each, m, H-15), 1.54 and 1.41 (1H each, m, H-7), 1.41 and 1.17 (1H each, m, H-21), 1.37 and 1.23 (1H each, m, H-12), 1.34 (1H, m, H-9), 1.15 and 1.07 (1H each, m, H-11),

1.62 and 1.02 (1H each, m, H-1), 1.38 (4H, m, 2x CHCH₂CH₂) 0.95 (3H, s, H-26), 0.92 (3H, s, H-24), 0.83 (6H, each, s, H-27 and H-23), 0.81 (3H, s, H-25), 0.76 (1H, m, H-5); ¹³C NMR (100 MHz, CDCl₃) δ 180.19, 169.84, 150.60, 109.56, 81.58, 77.29, 66.83, 58.85, 56.24, 55.39, 50.39 (2x CH₂), 49.19, 46.95, 42.41, 41.25, 40.67, 38.28, 38.30, 37.77, 37.08, 34.22, 33.56 (2x CH₂), 32.24, 30.61, 29.69, 28.02, 25.44, 23.74, 20.89, 19.32, 18.15, 16.53, 16.07, 15.66, 14.64. HRMS *m/z*calcd for C₃₇H₅₉NO₅ [M+H]⁺ 598.3215, found 598.3246.

Compound 9e

¹H NMR (CDCl₃, 400 MHz): δ 4.73 and 4.60 (1H, each, s, H-29), 4.51 (1H, t, *J* = 8.4 Hz, H-3), 3.65 (2H, t, *J* = 5.2 Hz, CH₂OH), 3.00 (1H, m, H-19), 2.89 (1H, s, OH), 2.82 (2H, m, COCH₂NH), 2.29 (2H, t, *J* = 5.0 Hz, CH₂NH), 2.30 and 1.92 (1H each, m, 2x CH₂CH₂N-), 2.24 and 1.63 (1H, each, m, H-22), 2.20 and 1.49 (1H each, m, H-16), 1.95 (2H, m, H-2), 1.69 (3H, s, H-30), 1.64 (1H, m, H-13), 1.61 (1H, m, H-18), 1.59 and 1.39 (1H each, m, H-6), 1.52 and 1.25 (1H each, m, H-15), 1.54 and 1.41 (1H each, m, H-7), 1.41 and 1.17 (1H each, m, H-21), 1.37 and 1.23 (1H each, m, H-12), 1.34 (1H, m, H-9), 1.15 and 1.07 (1H each, m, H-11), 1.62 and 1.02 (1H each, m, H-1), 0.97 (3H, s, H-26), 0.93 (3H, s, H-24), 0.84 (6H, each, s, H-27 and H-23), 0.82 (3H, s, H-25), 0.78 (1H, m, H-5). ¹³C NMR (100 MHz, CDCl₃) δ 180.01, 171.56, 150.73, 109.47, 82.30, 60.28, 55.34, 50.67, 50.38, 49.39, 49.09, 46.96, 42.38, 40.61, 38.23, 38.16, 37.79, 37.11, 36.98, 34.15, 32.28, 30.56, 29.64, 27.90, 25.41, 23.66, 22.65, 20.86, 19.24, 18.10, 16.40, 16.11, 15.85, 14.57. HRMS *m/z*calcd for C₃₄H₅₅NO₅ [M+H]⁺ 558.3005, found 558.3006.

Compound 9f

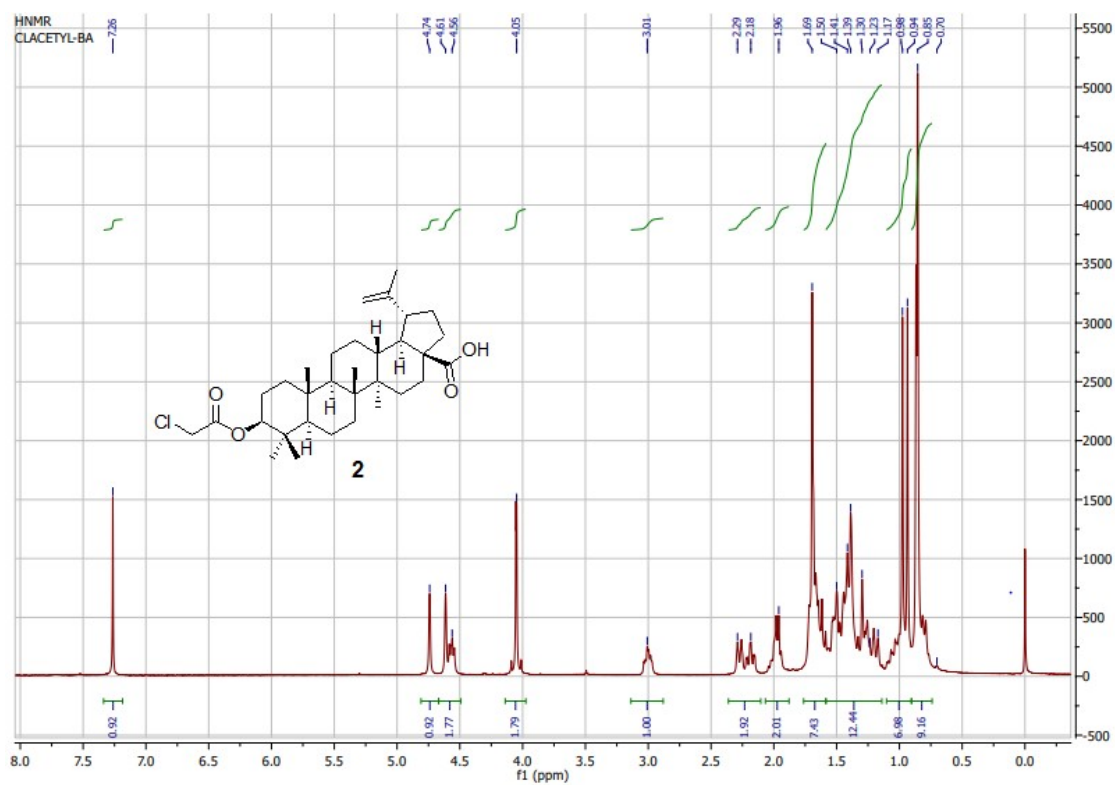
¹H NMR (CDCl₃, 400 MHz): δ 4.73 and 4.60 (1H, each, s, H-29), 4.54 (1H, t, *J* = 6.8 Hz, H-3), 3.5 (2H, d, *J* = 5.6 Hz, COCH₂NH), 3.01 (1H, ddd, *J* = 4.8, 4.4, 5.2 Hz, H-19), 2.69 (4H, m, 2x CH₂CH₂N-), 2.26 and 1.65 (1H, each, m, H-22), 2.20 and 1.51 (1H each, m, H-16), 1.97 (2H, m,

H-2), 1.8 (4H, m, 2x CH₂CH₂CH₂), 1.69 (3H, s, H-30), 1.64 (1H, m, H-13), 1.62 (1H, m, H-18), 1.59 and 1.39 (1H each, m, H-6), 1.52 and 1.25 (1H each, m, H-15), 1.53 and 1.41 (1H each, m, H-7), 1.41 and 1.19 (1H each, m, H-21), 1.37 and 1.24 (1H each, m, H-12), 1.34 (1H, m, H-9), 1.17 and 1.09 (1H each, m, H-11), 1.61 and 1.06 (1H each, m, H-1), 0.96 (3H, s, H-26), 0.93 (3H, s, H-24), 0.84 (6H, each, s, H-27 and H-23), 0.82 (3H, s, H-25), 0.78 (1H, m, H-5); ¹³C NMR (125 MHz, CDCl₃) δ 180.53, 169.58, 150.90, 109.43, 81.54, 56.35, 55.54, 55.44, 52.90 (2x CH₂), 50.43, 49.22, 46.97, 42.40, 40.65, 38.36, 38.23, 37.77, 37.19, 37.07, 34.24, 32.46, 30.25, 29.78, 28.01, 25.50, 23.82 (2x CH₂), 23.67, 20.92, 19.40, 18.16, 16.55, 16.20, 16.01, 14.65. HRMS *m/z* calcd for C₃₆H₅₇NO₄ [M+H]⁺ 568.3215, found 568.3217.

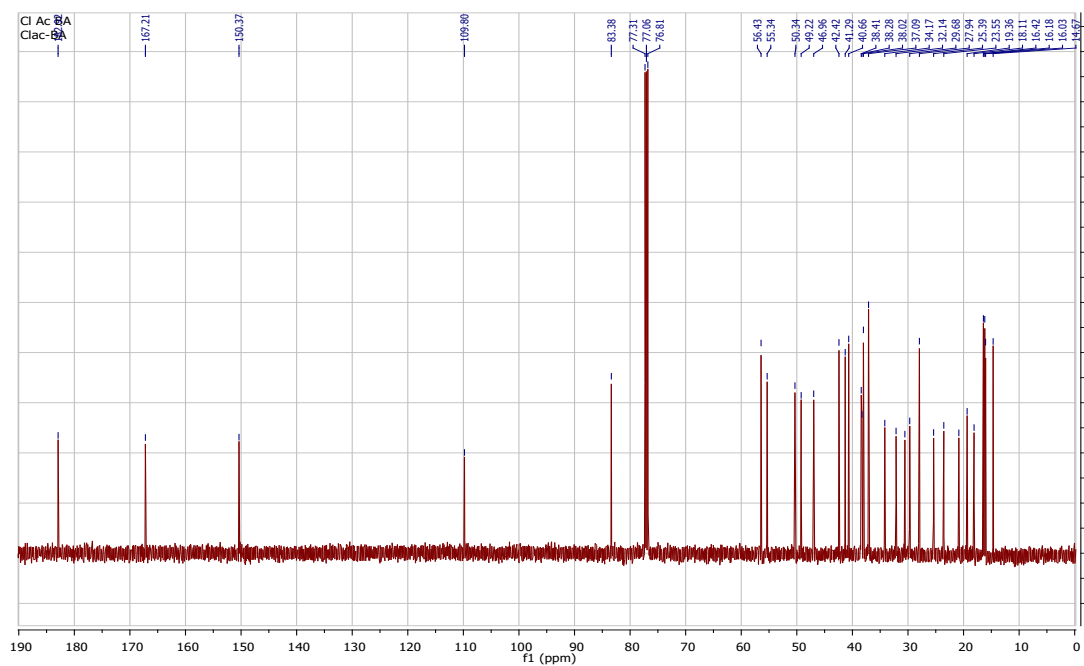
Compound 9g

¹H NMR (CDCl₃, 400 MHz): δ 4.73 and 4.60 (1H, each, s, H-29), 4.52 (1H, t, *J* = 6.8 Hz, H-3), 3.2 (2H, s, COCH₂NH), 3.00 (1H, m, H-19), 2.85 (4H, m, 2x CH₂CH₂N-), 2.70 (4H, m, 2x CH₂CH₂N-), 2.26 and 1.63 (1H, each, m, H-22), 2.18 and 1.49 (1H each, m, H-16), 1.97 (2H, m, H-2), 1.69 (3H, s, H-30), 1.64 (1H, m, H-13), 1.62 (1H, m, H-18), 1.59 and 1.39 (1H each, m, H-6), 1.52 and 1.25 (1H each, m, H-15), 1.53 and 1.41 (1H each, m, H-7), 1.41 and 1.19 (1H each, m, H-21), 1.37 and 1.24 (1H each, m, H-12), 1.34 (1H, m, H-9), 1.15 and 1.07 (1H each, m, H-11), 1.62 and 1.05 (1H each, m, H-1), 0.97 (3H, s, H-26), 0.93 (3H, s, H-24), 0.84 (6H, each, s, H-27 and H-23), 0.83 (3H, s, H-25), 0.79 (1H, m, H-5); ¹³C NMR (100 MHz, CDCl₃) δ 181.74, 169.96, 150.50, 109.71, 81.54, 60.05, 56.36, 55.37, 54.34 (2x CH), 50.37, 49.21, 46.97, 42.42, 40.67, 38.37, 38.35, 37.79, 37.09 (2x C), 34.21, 32.21, 30.60, 29.72, 28.07, 27.65 (2x CH), 25.43, 23.81, 20.87, 19.37, 18.16, 16.62, 16.19, 16.03, 14.68. HRMS *m/z* calcd for C₃₆H₅₈N₂O₄ [M+H]⁺ 583.4405, found 583.4426.

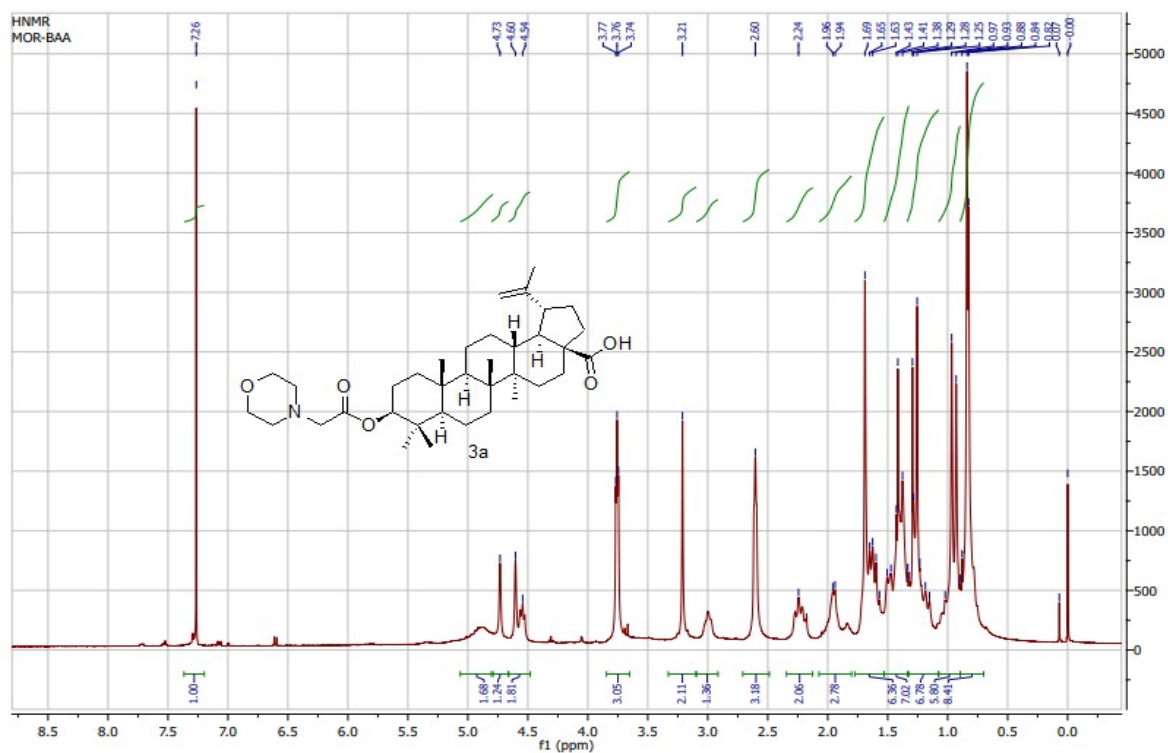
^1H -NMR spectrum (400MHz) of compound 8 in CDCl_3



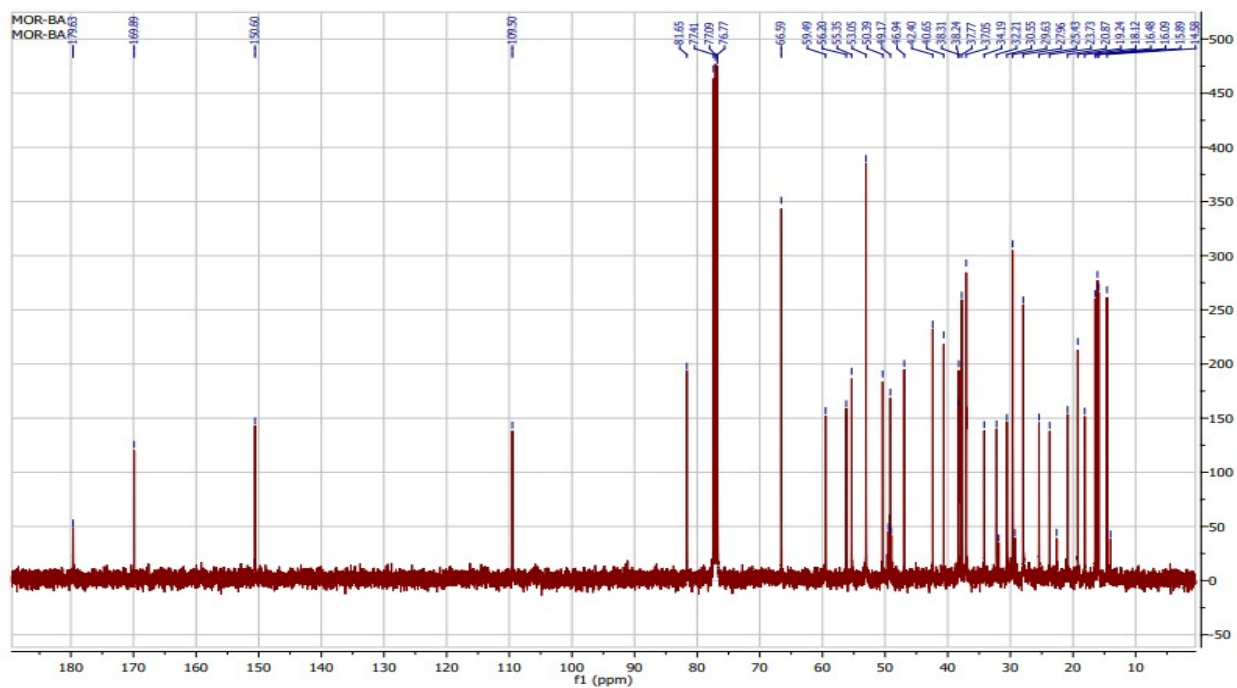
^{13}C -NMR spectrum (125MHz) of compound 8 in CDCl_3



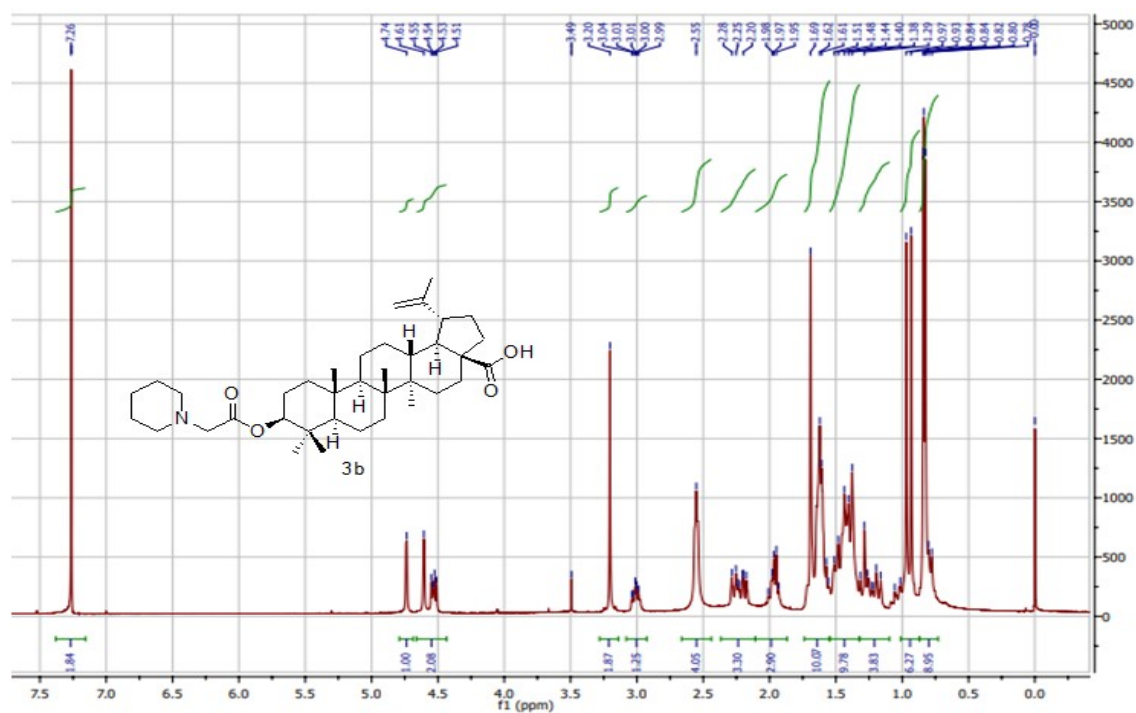
¹H-NMR spectrum (400MHz) of compound 9a in CDCl₃



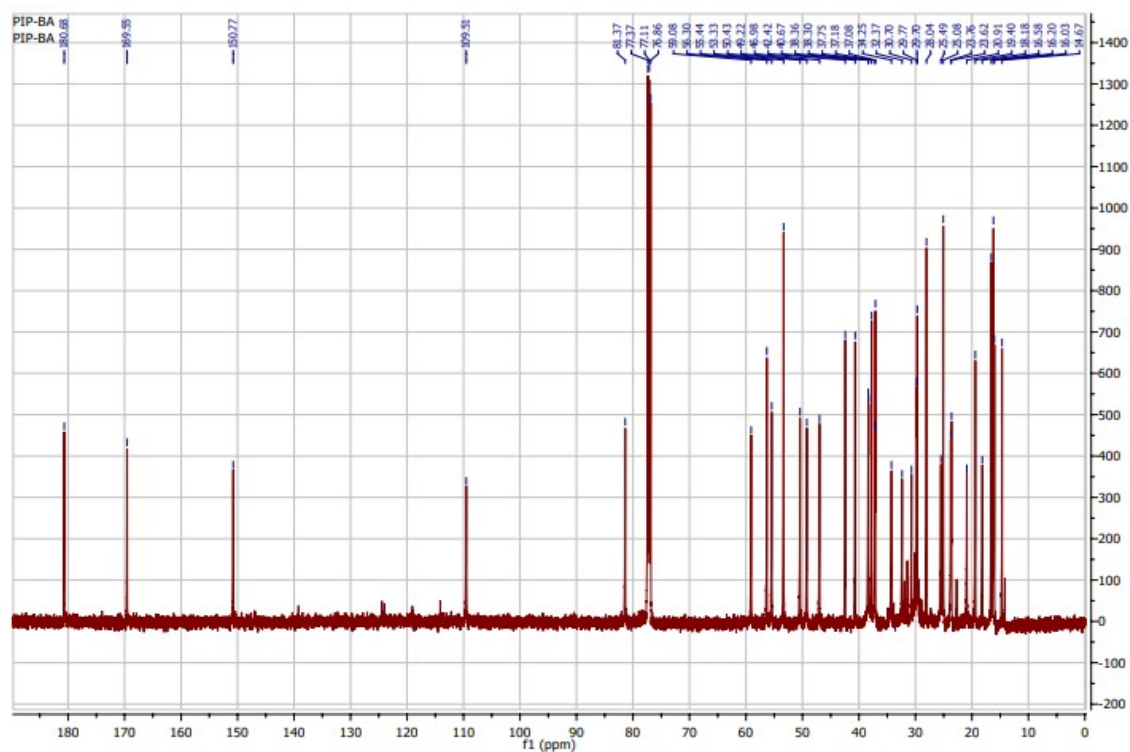
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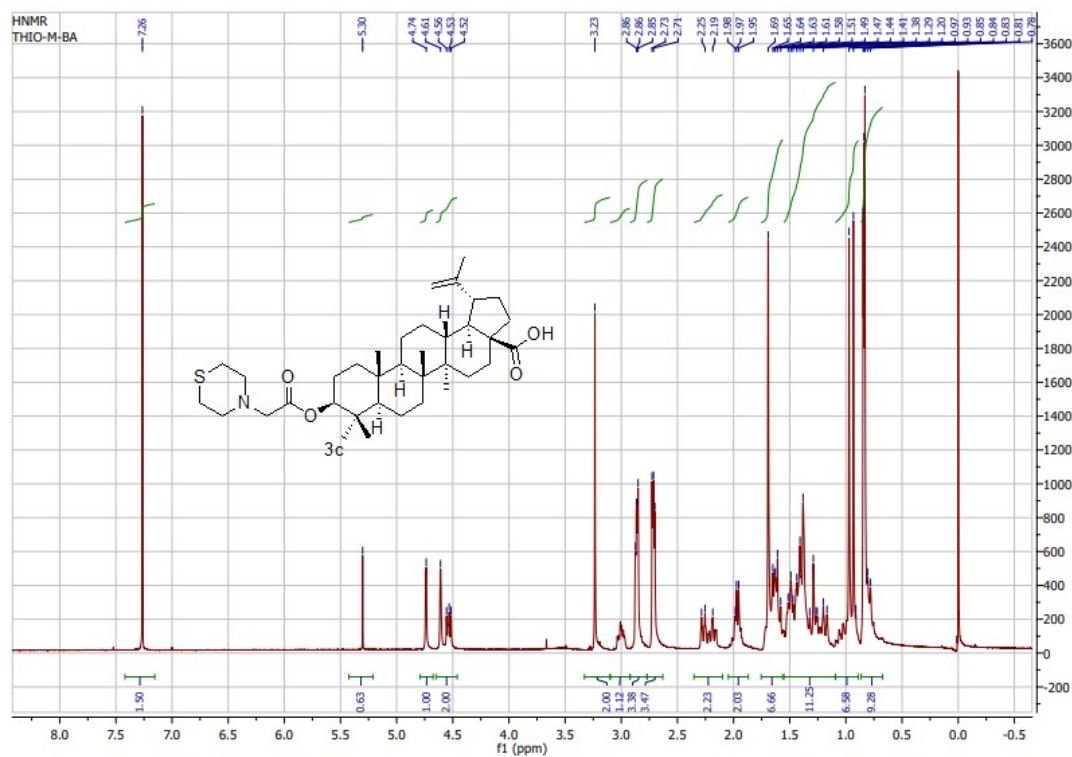
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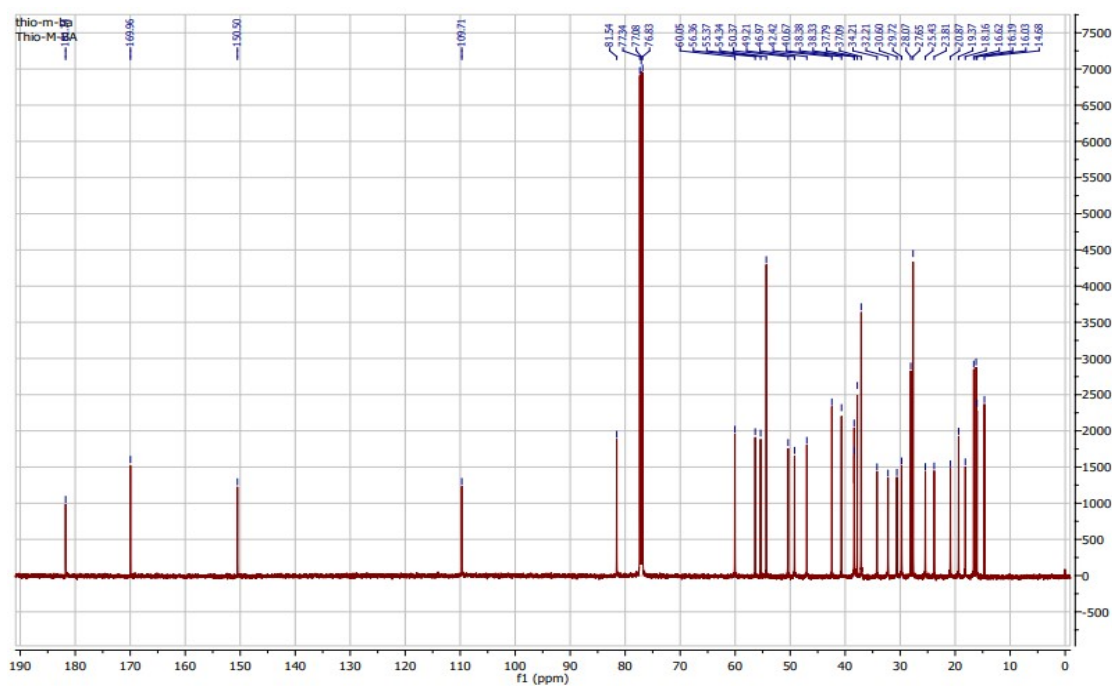
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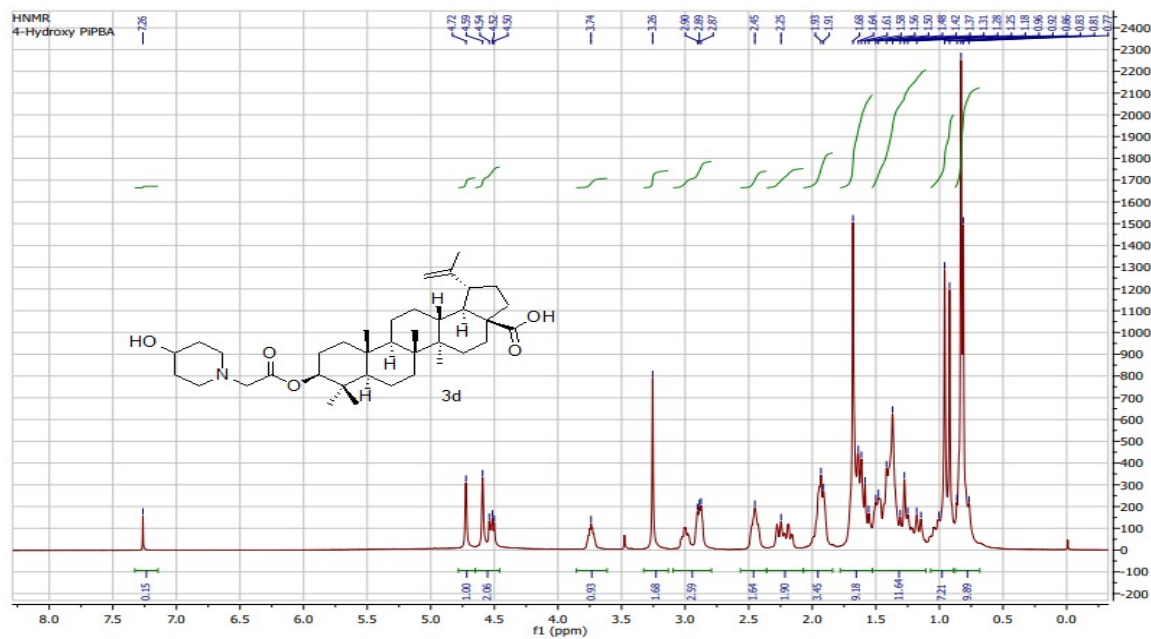
^1H -NMR spectrum (400MHz) of compound 9c in CDCl_3



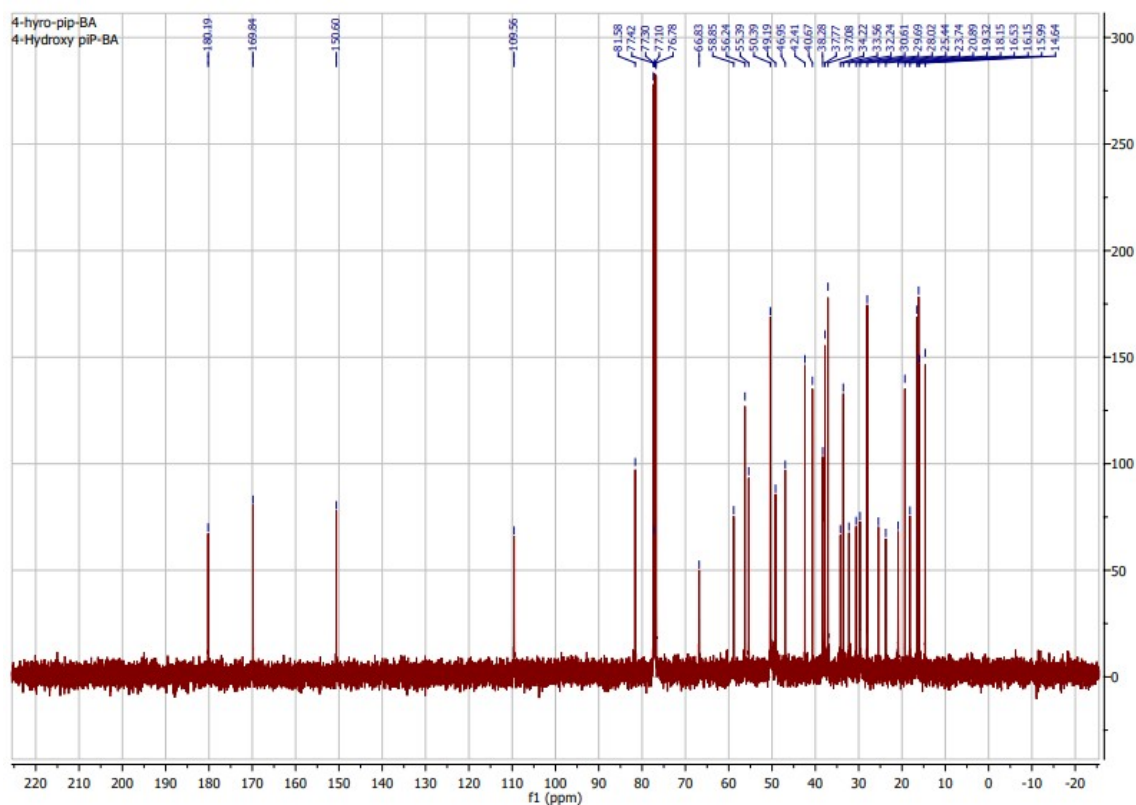
^{13}C -NMR spectrum (125MHz) of compound 3c in CDCl_3



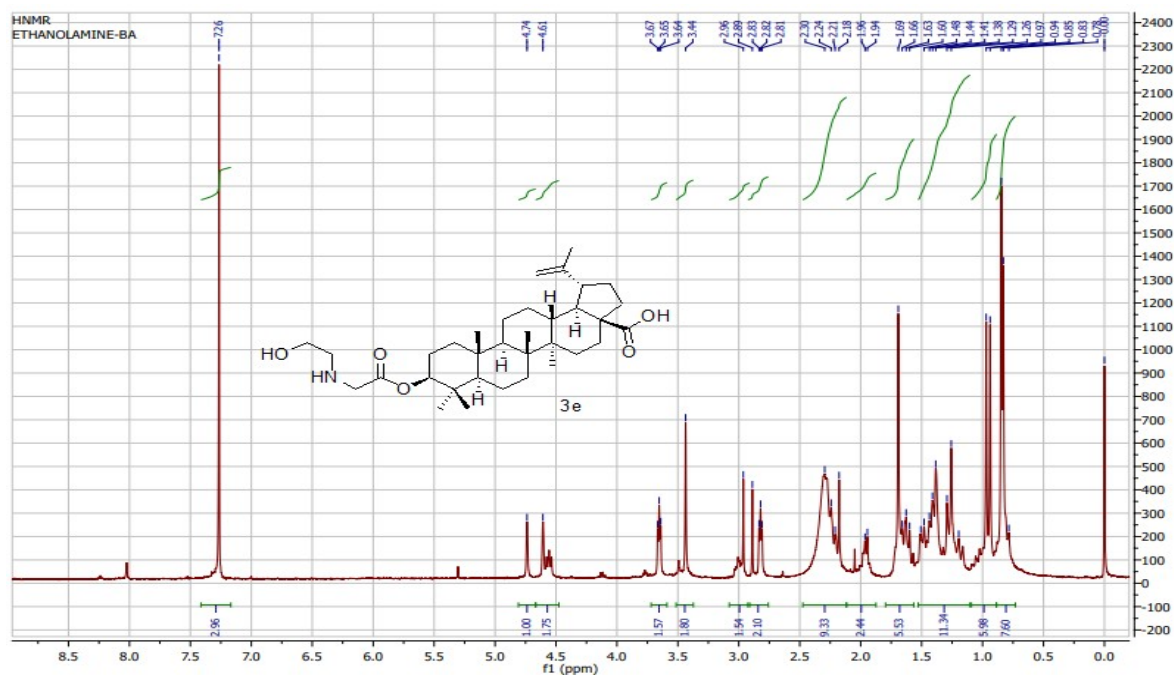
¹H-NMR spectrum (400MHz) of compound 9d in CDCl₃



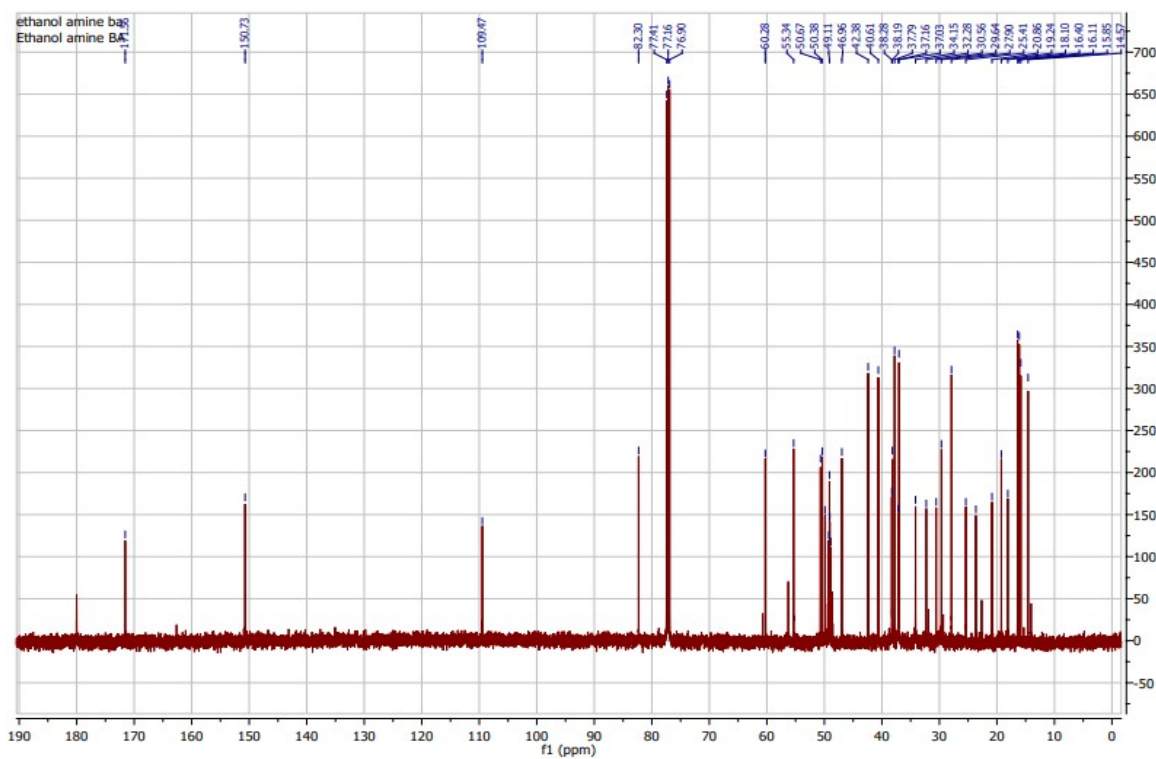
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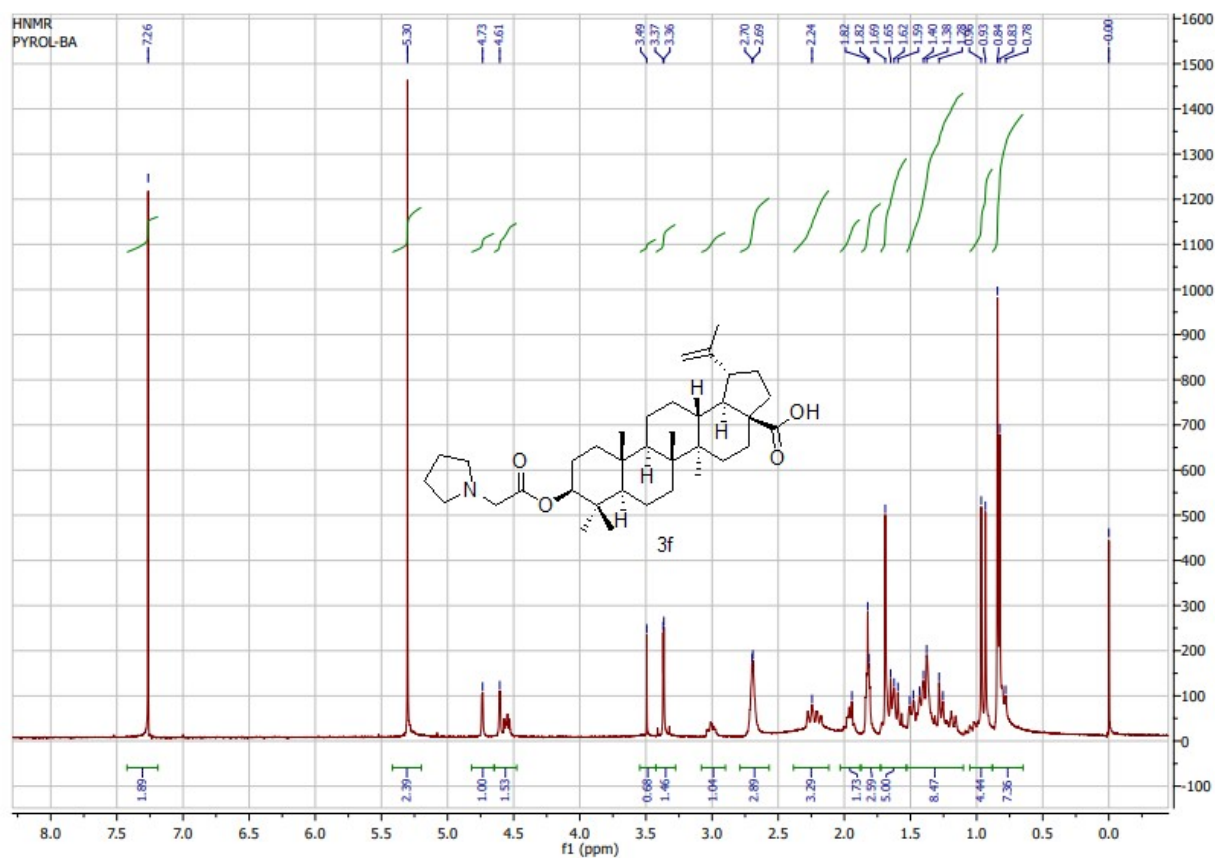
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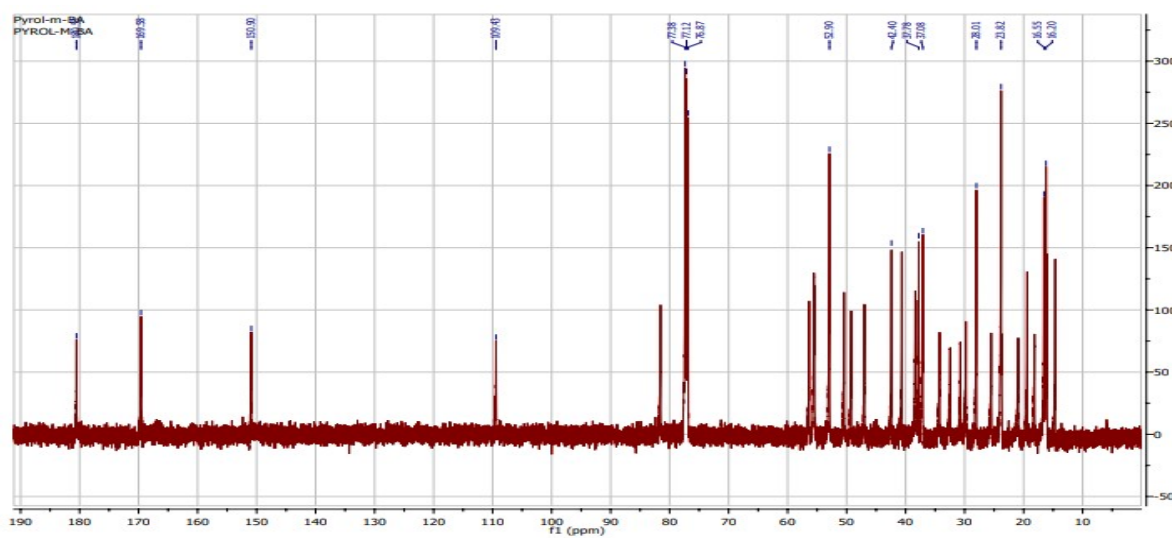
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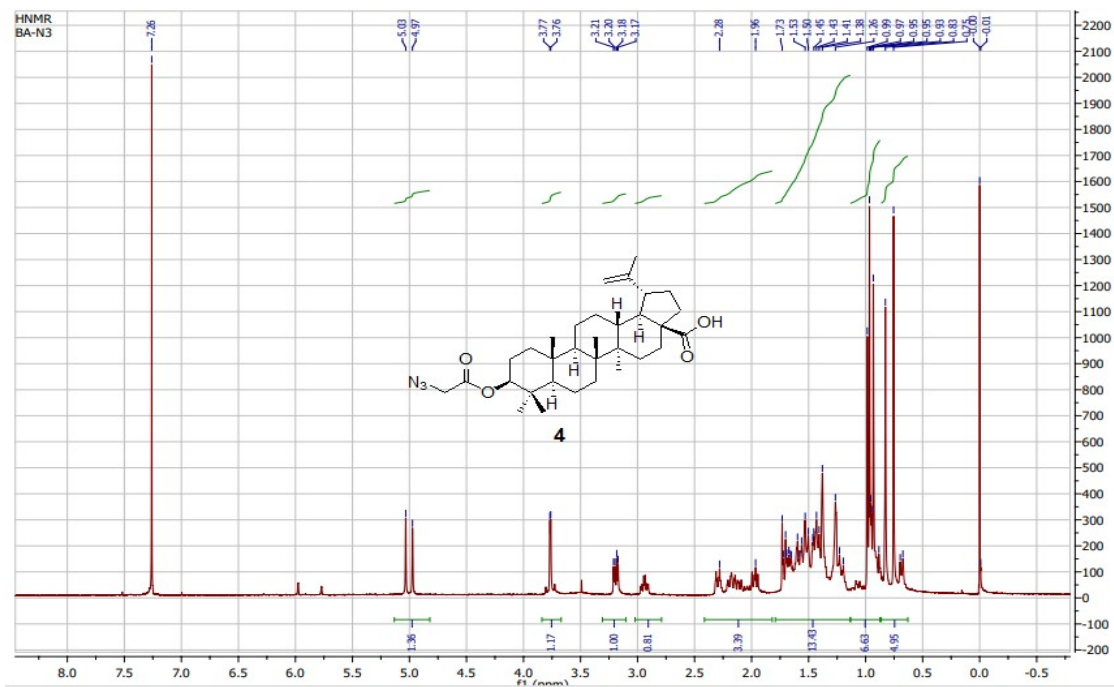
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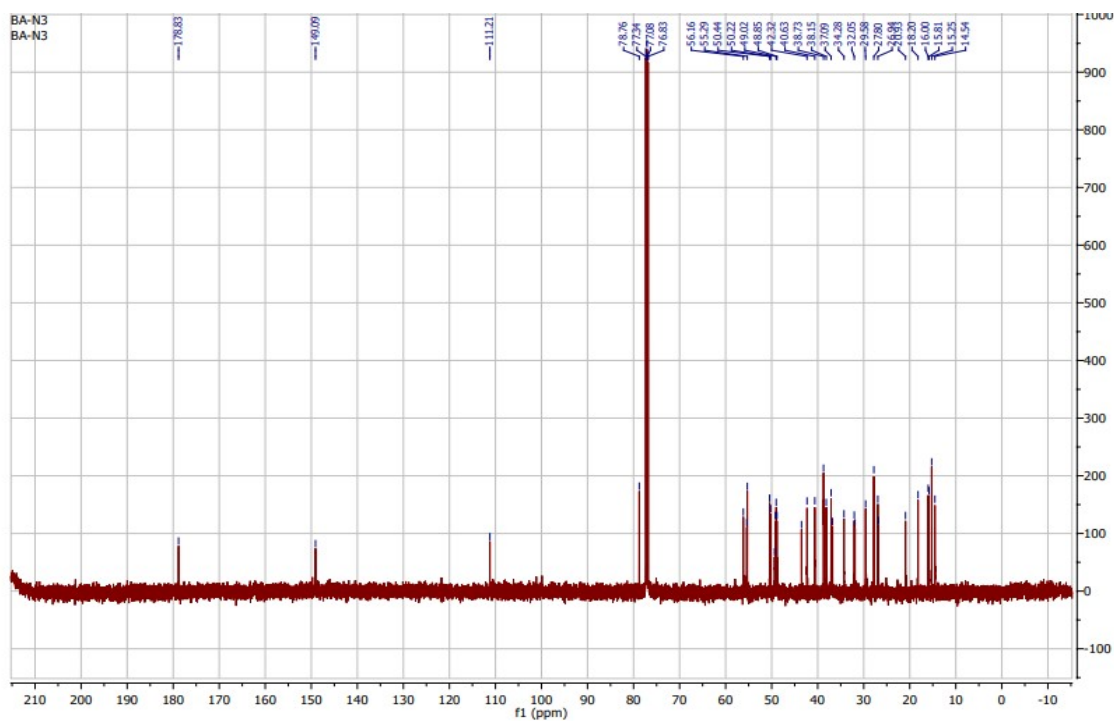
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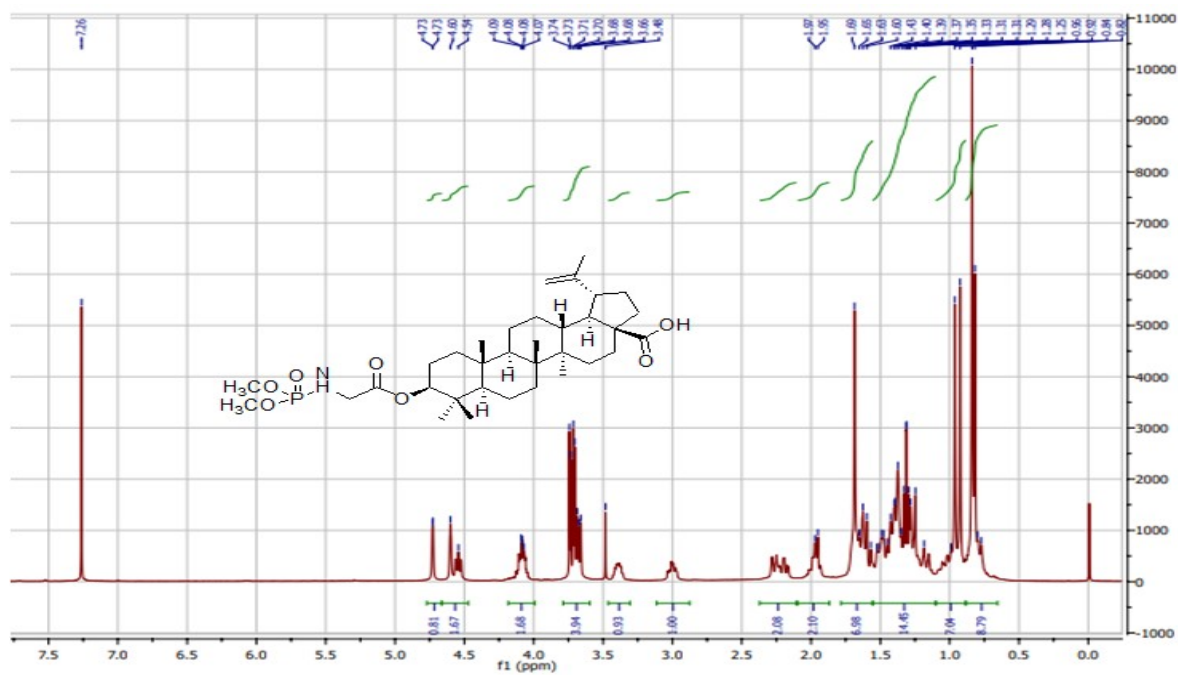
^1H -NMR spectrum (400MHz) of compound 10 in CDCl_3



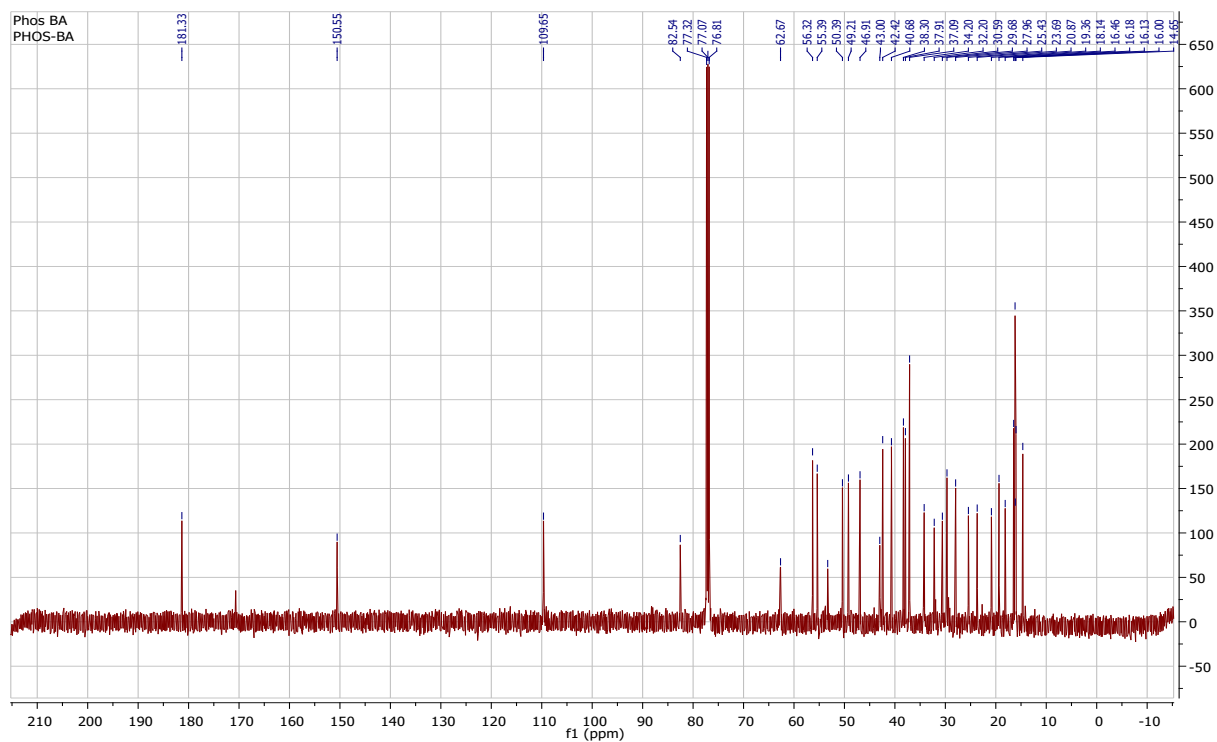
^{13}C -NMR spectrum (125MHz) of compound 10 in CDCl_3



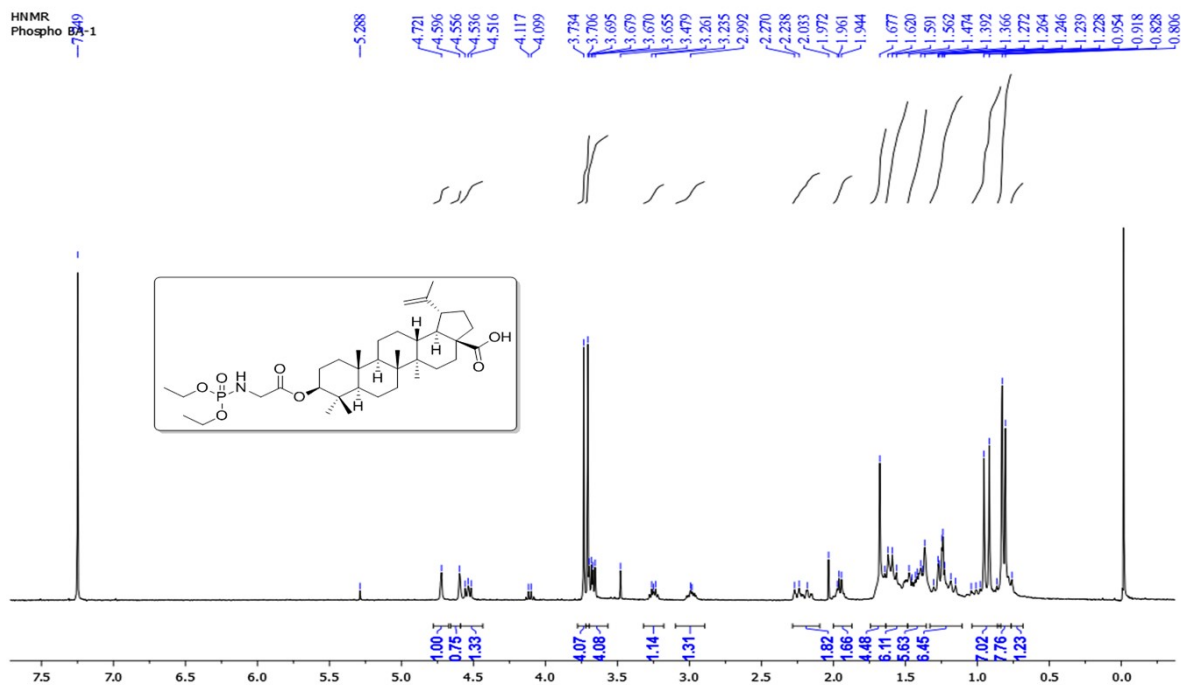
¹H-NMR spectrum (400MHz) of compound 11a in CDCl₃



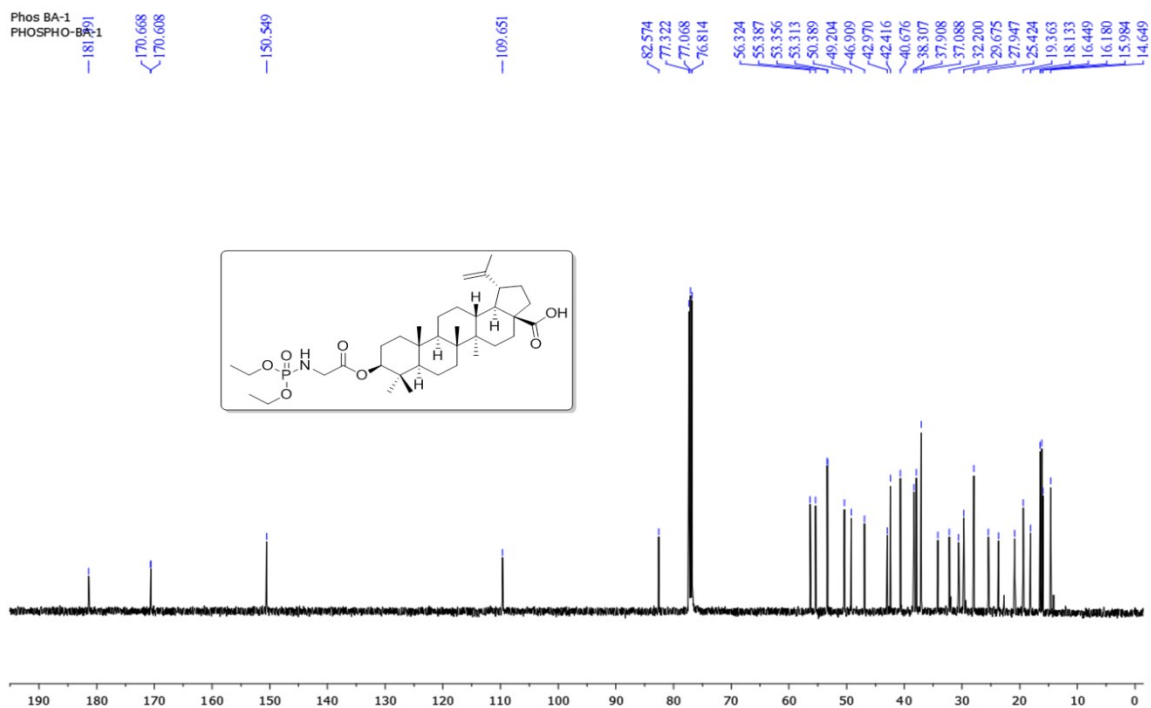
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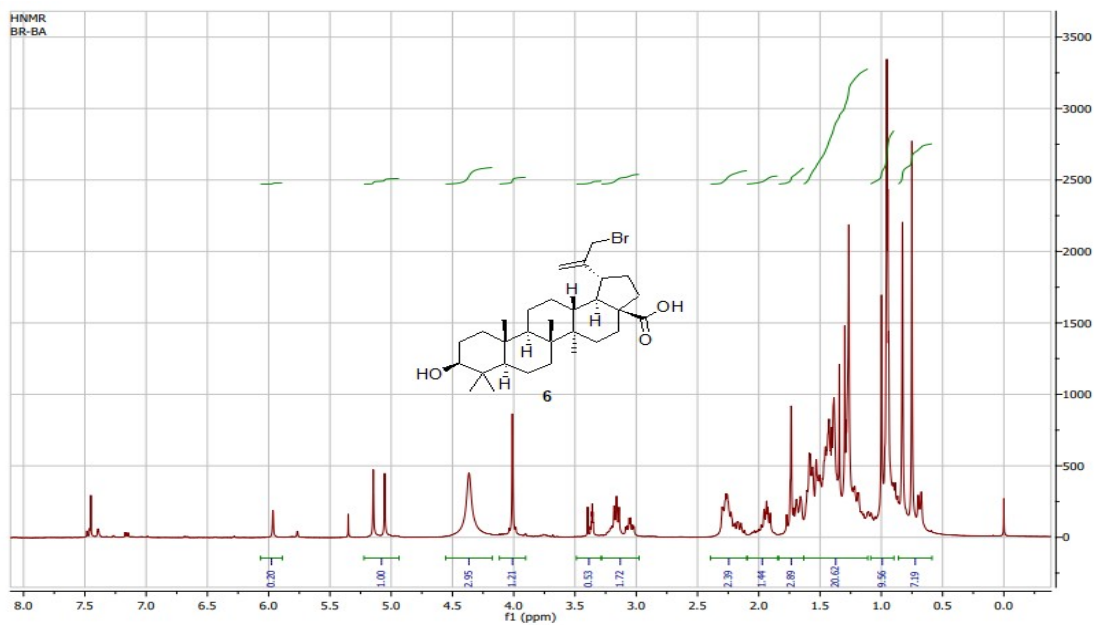
¹H-NMR spectrum (400MHz) of compound 11b in CDCl₃



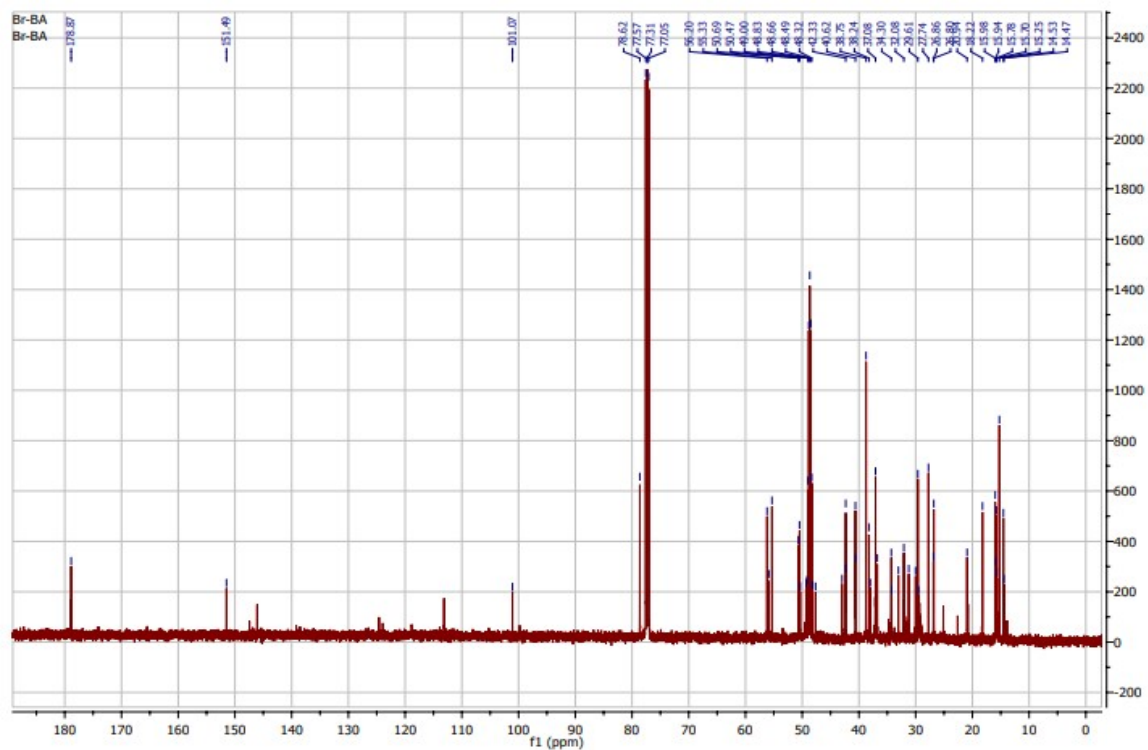
¹³C-NMR spectrum (125MHz) of compound 11b in CDCl₃



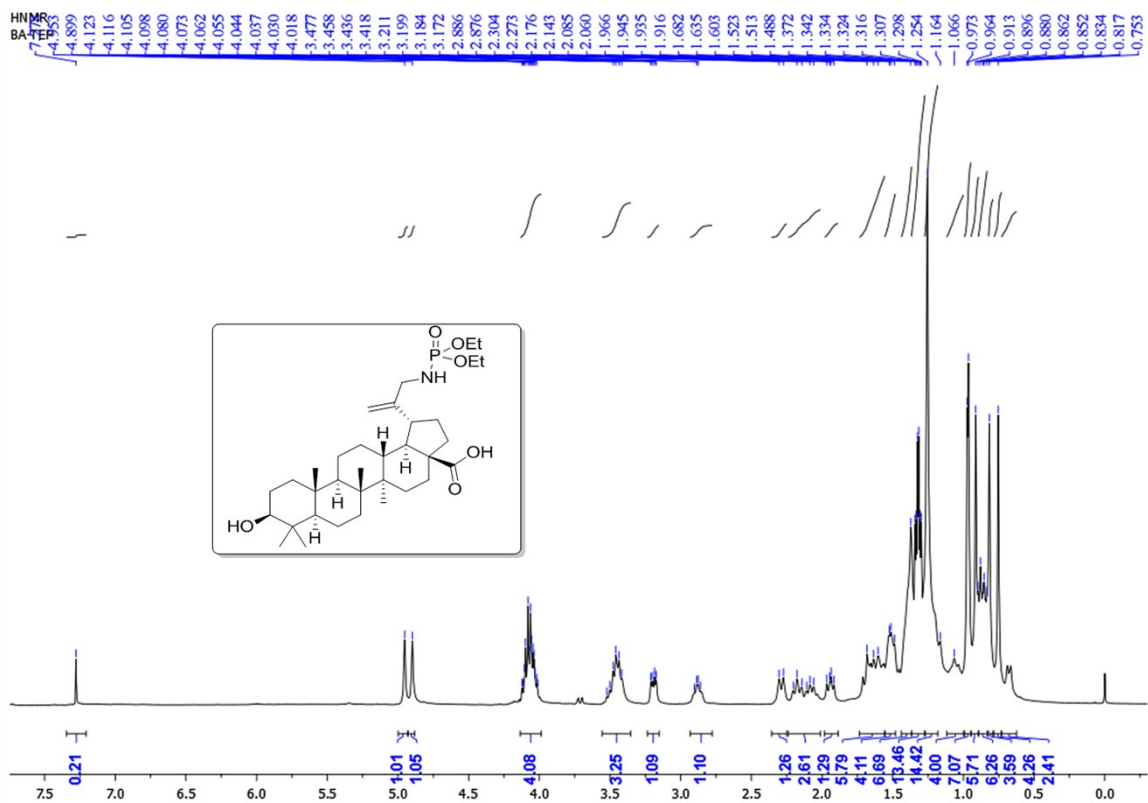
^1H -NMR spectrum (400MHz) of compound 12 in CDCl_3



^{13}C -NMR spectrum (125MHz) of compound 12 in CDCl_3



¹H-NMR spectrum (400MHz) of compound 14b in CDCl₃



^{13}C -NMR spectrum (125MHz) of compound 14b in CDCl_3

