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SUPPORTING INFORMATION FOR

Environmentally benign peptide synthesis using liquid-assisted ball-milling: application to the synthesis of Leu-enkephalin

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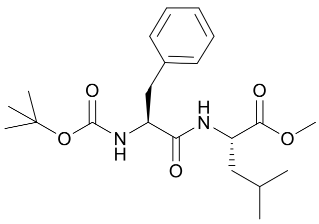
General: All reagents were purchased from Aldrich Chemical Co. and Isochem and used without further purification excepted for Boc-AA-NCA that were disolved in EtOAc and washed with saturated $NaHCO_3$ aqueous solution before use. The milling treatments were carried out in a Retsch Mixer Mill 200. ^{1}H NMR spectra were recorded on a Bruker Avance DPX 200 MHz spectrometer and are reported in ppm using solvent as an internal standard ($CDCl_3$ at 7.24 ppm). Data are reported as s = singlet, d = doublet, t = triplet, m = multiplet or overlap of non-equivalent resonances; coupling constant in Hz; integration. ^{13}C NMR spectra were recorded on a Bruker Avance AM 75 MHz spectrometer and are reported in ppm using solvent as an internal standard ($CDCl_3$ at 77.2 ppm). Mass spectra were obtained by LC-MS with ESI using a Water Alliance 2695 as LC, coupled to a Waters ZQ spectrometer with electrospray source, a simple quadrupole analyzer and a UV Waters 2489 detector. HRMS analyse was performed on a Q-Tof (Waters, ESI, 2001) spectrometer. Enantiomeric excess was measured using a

Beckman Coulter System Gold 126 Solvent Module HPLC machine and System Gold 168 Detector with 4.6 mm x 250 mm Daicel Chiralpak OD columns using *n*-hexane and 2-propanol as solvents.

General procedure using Boc-protected *α*-amino acid *N*-carboxyanhydrides (Boc-AA-NCA; named as general procedure A): The Boc-protected *α*-amino acid *N*-carboxyanhydride species (1 eq), *α*-amino ester hydrochloride (1 eq), $NaHCO_3$ (1 eq) and EtOAc (between 1.1 and 1.6 µL per mg of solid reactants depending on the reaction) were introduced in a 10 mL stainless steel grinding bowl with one stainless steel ball (10 mm diameter). The bowl was closed and placed 20 minutes within the mixer mill at 30 Hz. Then, the suspension was diluted with EtOAc and washed two times with aqueous saturated sodium carbonate solution and two times with 1N aqueous HCl solution. Then the organic layer was dried on $MgSO_4$, filtered, concentrated under *vacuo* and dried over P_2O_5 to obtain the final product.

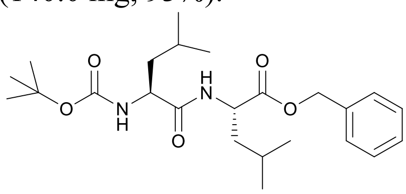
General procedure using Boc-protected *α*-amino acid hydroxysuccinimide esters (Boc-AA-OSu; named as general procedure B): The Boc-protected *α*-amino acid hydroxysuccinimide ester (1 eq), *α*-amino ester hydrochloride (1 eq), $NaHCO_3$ (1 eq) and EtOAc (between 1.1 and 1.6 µL per mg of solid reactants depending on the reaction) were introduced in a 10 mL stainless steel grinding bowl with one stainless steel ball (10 mm diameter). The bowl was closed and placed 20 minutes within the mixer mill at 30 Hz. Then, 2 mL of 1N aqueous NaOH solution was added and the reactor was put in the mixer mill for 5 min at 30 Hz. Afterwards, the suspension was diluted with EtOAc and washed two times with 1N aqueous NaOH solution and two times with 1N aqueous HCl solution. Then the organic layer was dried on $MgSO_4$, filtered, concentrated under *vacuo* and dried over P_2O_5 to obtain the final product.

Boc-Phe-Leu-OMe[1]
Following general procedure A, Boc-Phe-NCA (111.3 mg, 0.382 mmol), $NaHCO_3$ (32.1 mg, 0.382 mmol), EtOAc (300 µL) and HCl·H-Leu-OMe (69.4 mg, 0.382 mmol) were used. Boc-Phe-Leu-OMe was recovered as a white solid (142.6 mg, 95%).
Following general procedure, Boc-Phe-OSu (138.4 mg, 0.382 mmol), $NaHCO_3$ (32.1 mg, 0.382 mmol), EtOAc (300 µL) and HCl·H-Leu-OMe (69.4 mg, 0.382 mmol) were used. Boc-Phe-Leu-OMe was recovered (123.8 mg, 83 %) as a white solid.



^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.26–7.12 (m, 5H), 6.16 (d, J = 8.1 Hz, 1H), 4.98–4.81 (m, 1 H), 4.55–4.45 (m, 1H), 4.32–4.22 (dd, J = 6.8, 13.7 Hz, 1H), 3.62 (s, 3H), 3.00 (d, J = 6.8 Hz, 2H), 1.62–1.03 (m with singulet, 12H), 0.83 (apparent t, J = 5.7 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.0, 171.2, 155.5, 136.8, 129.5, 128.7, 127.0, 80.2, 55.7, 52.3, 50.9, 41.6, 38.3, 28.4, 24.8, 22.9, 22.0 ppm; MS (ESI): *m*/*z* 393.2 $[M+H]^+$.

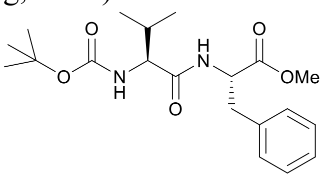
Boc-Leu-Leu-OBn[2]
Following general procedure A, Boc-Leu-NCA (88.8 mg, 0.345 mmol), $NaHCO_3$ (29.0 mg, 0.345 mmol), EtOAc (300 µL) and *p*-TsOH·H-Leu-OBn (135.8 mg, 0.345 mmol) were used. Boc-Leu-Leu-OBn was recovered as a white solid (140.0 mg, 93%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.33–7.22 (m, 5H), 6.48 (d, J = 8.4 Hz, 1H), 5.12 (d, J = 1.2 Hz, 2H), 4.90 (d, J = 8.6 Hz, 1H), 4.64–4.52 (m, 1H), 4.10–3.90 (m, 1H), 1.68–1.30 (m, 15H), 0.89–0.78 (m, 12H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.7, 172.5, 155.9, 135.6, 128.8, 128.6, 128.4, 80.2, 67.2, 53.1, 51.0, 41.6, 41.1, 28.5, 24.9, 24.8, 23.0, 22.3, 22.0 ppm; MS (ESI): *m*/*z* 435.2 [*M*+H]$^+$.

Boc-Val-Phe-OMe[3]

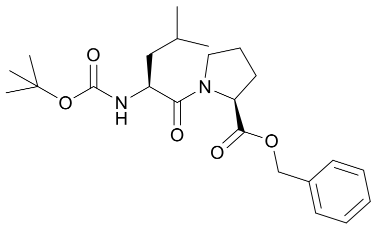
Following general procedure A, Boc-Val-NCA (96.3 mg, 0.396 mmol), $NaHCO_3$ (33.3 mg, 0.396 mmol), EtOAc (300 µL) and HCl·H-Phe-OMe (85.5 mg, 0.396 mmol) were used. Boc-Val-Phe-OMe was recovered as a white solid (126.3 mg, 84 %).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.25–7.00 (m, 5H), 6.44–6.33 (m, 1H), 5.07–4.92 (m, 1H), 4.85–4.73 (m, 1H), 3.89–3.78 (m, 1H), 3.63 (s, 3H), 3.07–2.99 (m, 2H), 2.09–1.92 (m, 1H), 1.37 (s, 9H), 0.88–0.74 (m, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.9, 171.5, 155.9, 135.9, 129.4, 128.8, 127.3, 80.0, 60.0, 53.3, 52.4, 38.1, 31.0, 28.5, 19.3, 17.8 ppm; MS (ESI): *m*/*z* 379.2 [*M*+H]$^+$.

Boc-Leu-Pro-OBn[4]

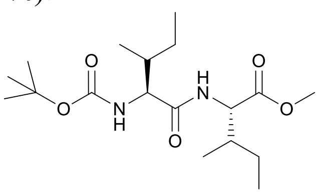
Following general procedure A, Boc-Leu-NCA (92.1 mg, 0.358 mmol), $NaHCO_3$ (30.1 mg, 0.358 mmol), EtOAc (300 µL) and HCl·H-Pro-OBn (86.6 mg, 0.358 mmol) were used. Boc-Leu-Pro-OBn was recovered as an oil (135.0 mg, 90%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.32–7.17 (m, 5H), 5.17–4.97 (m, 3H), 4.56–4.32 (m, 2H), 3.76–3.40 (m, 2H), 2.21–2.07 (m, 1H), 2.05–1.83 (m, 3H), 1.77–1.55 (m, 1H), 1.43–1.28 (m with singulet, 11H), 0.89 (d, J = 6.5 Hz, 3H), 0.83 (d, J = 6.5 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) (several conformers): δ = 172.1, 172.0, 155.9, 135.7, 128.7, 128.5, 128.3, 79.7, 67.1, 59.0, 50.5, 46.9, 42.1, 29.1, 28.5, 25.1, 24.7, 23.6, 21.9 ppm; MS (ESI): *m*/*z* 419.2 [*M*+H]$^+$.

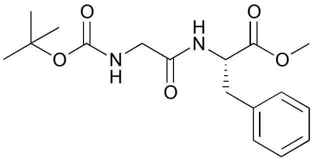
Boc-Ile-Ile-OMe[5]

Following general procedure A, Boc-Ile-NCA (107.5 mg, 0.418 mmol), $NaHCO_3$ (35.1 mg, 0.418 mmol), EtOAc (300 µL) and HCl·H-Ile-OMe (75.9 mg, 0.418 mmol) were used. Boc-Ile-Ile-OMe was recovered as a white solid (142.5 mg, 95%).



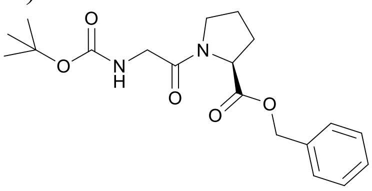
^{1}H NMR (200 MHz, $CDCl_3$): δ = 6.43 (d, J = 8.8 Hz, 1H), 5.06 (d, J = 8.6 Hz, 1H), 4.53 (dd, J = 4.8, 8.4 Hz, 1H), 3.90 (dd, J = 6.8, 8.6 Hz, 1H), 3.66 (s, 3H), 1.98–1.68 (m, 2H), 1.42–1.33 (m, 11H), 1.20–0.95 (m, 2H), 0.89–0.79 (m, 12H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.3, 171.7, 155.9, 80.0, 59.5, 56.6, 52.2, 38.0, 37.2, 28.5, 25.3, 25.0, 15.7, 15.6, 11.7, 11.5 ppm; MS (ESI): *m*/*z* 359.2 [*M*+H]$^+$.

Boc-Gly-Phe-OMe[6]
Following general procedure B, Boc-Gly-OSu (121.4 mg, 0.446 mmol), $NaHCO_3$ (37.5 mg, 0.446 mmol), EtOAc (300 μL) and HCl·H-Phe-OMe (96.2 mg, 0.446 mmol) were used. Boc-Gly-Phe-OMe was recovered as an oil (143.9 mg, 96%).



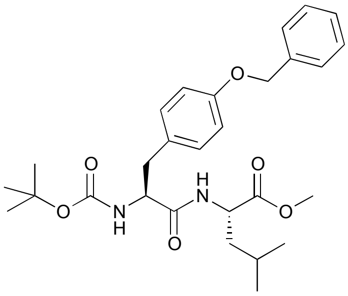
^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.25–6.99 (m, 5H), 6.82–6.72 (m, 1H), 5.37–5.27 (m, 1H), 4.84–4.73 (m, 1H), 3.72–3.63 (m, 2H), 3.61 (s, 3H), 3.02 (dd, J = 3.0, 5.6 Hz, 2H), 1.36 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.9, 169.4, 156.0, 135.8, 129.2, 128.6, 127.1, 80.1, 53.2, 52.3, 44.1, 37.9, 28.3 ppm; MS (ESI): m/z 337.1 $[M+H]^+$.

Boc-Gly-Pro-OBn
Following general procedure B, Boc-Gly-OSu (112.7 mg, 0.414 mmol), $NaHCO_3$ (35.8 mg, 0.414 mmol), EtOAc (300 μL) and HCl·H-Pro-OBn (100.0 mg, 0.414 mmol) were used. Boc-Gly-Pro-OBn was recovered as an oil (145.1 mg, 97%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.46–7.34 (m, 5H), 5.55–5.40 (m, 1H), 5.32–5.13 (m, 2H), 4.68–4.58 (m, 1H), 4.11–3.89 (m, 2H), 3.72–3.43 (m, 2H), 2.35–1.80 (m, 4H), 1.49 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) (major conformer): δ = 171.7, 167.7, 155.8, 135.6, 128.6, 128.3, 128.1, 79.6, 67.0, 59.0, 45.9, 43.0, 29.0, 28.4, 24.6 ppm; MS (ESI): m/z 363.1 $[M+H]^+$, HRMS: m/z: calcd for $C_{19}H_{27}N_2O_5$: 363.1920; found: 363.1938.

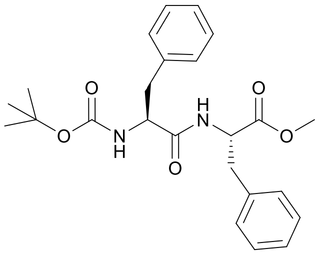
Boc-Tyr(Bn)-Leu-OMe[7]
Following general procedure B, Boc-Tyr(Bn)-OSu (141.0 mg, 0.301 mmol), $NaHCO_3$ (25.3 mg, 0.301 mmol), EtOAc (300 μL) and HCl·H-Leu-OMe (54.7 mg, 0.301 mmol) were ball-milled for 1 h. Boc-Tyr(Bn)-Leu-OMe was recovered as white solid (147.0 mg, 98%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.45–7.26 (m, 5H), 7.10 (d, J = 10.8 Hz, 2H), 6.87 (d, J = 10.5 Hz, 2H), 6.36 (d, J = 9.8 Hz, 1H), 5.13–4.95 (m, 3H), 4.63–4.47 (m, 1H), 4.39–4.22 (m, 1H), 3.66 (s, 3H), 2.99 (d, J = 8.0 Hz, 2H), 1.61–1.33 (m, 12H), 0.94–0.81 (m, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.0, 171.2, 157.9, 155.5, 137.1, 130.6, 128.9, 128.7, 128.1, 127.5, 115.1, 80.3, 70.1, 55.9, 52.4, 50.9, 41.7, 37.4, 28.4, 24.8, 22.9, 22.0 ppm; MS (ESI): m/z 499.2 $[M+H]^+$.

Boc-Phe-Phe-OMe[8]

Following general procedure B, Boc-Phe-OSu (127.6 mg, 0.352 mmol), $NaHCO_3$ (29.6 mg, 0.352 mmol), EtOAc (300 µL) and HCl·H-Phe-OMe (75.9 mg, 0.352 mmol) were used. Boc-Phe-Phe-OMe was recovered as a white solid (138.5 mg, 92%).

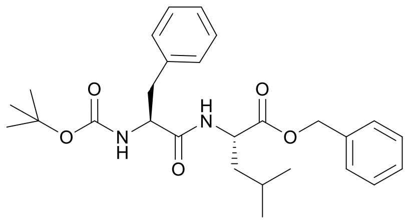


^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.26–7.04 (m, 8H), 6.95–6.85 (m, 2H), 6.30 (d, J = 7.6 Hz, 1H), 4.98–4.82 (m, 1H), 4.77–4.64 (m, 1H), 4.34–4.19 (m, 1H), 3.59 (s, 3H), 3.06–2.87 (m, 4H), 1.32 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.6, 171.0, 155.4, 136.7, 135.8, 129.5, 129.4, 128.8, 128.7, 127.3, 127.1, 80.4, 55.8, 53.5, 52.4, 38.5, 38.1, 28.4 ppm; MS (ESI): *m*/*z* 427.2 $[M+H]^+$.

Boc-Phe-Leu-OBn[9]

Following general procedure B, Boc-Phe-OSu (116.0 mg, 0.320 mmol), $NaHCO_3$ (26.9 mg, 0.320 mmol), EtOAc (300 µL) and *p*-TsOH·H-Leu-OBn (125.8 mg, 0.320 mmol) were used. Boc-Phe-Leu-OBn was recovered as a white solid (134.3 mg, 90%).

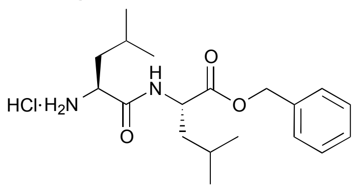
When realised on a 2.13 mmoles scale, Boc-Phe-OSu (771.9 mg, 2.13 mmol), $NaHCO_3$ (178.9 mg, 2.13 mmol), EtOAc (2000 µL) and *p*-TsOH·H-Leu-OBn (838.7 mg, 2.13 mmol) were introduced in a 10 mL stainless steel grinding bowl with one stainless steel ball (10 mm diameter). The bowl was closed and placed 2 h within the mixer mill at 30 Hz. Then, 2 mL of 1N aqueous NaOH solution was added and the reactor was put in the mixer mill for 5 min at 30 Hz. Afterwards, the suspension was diluted with EtOAc and washed two times with 1N aqueous NaOH solution and two times with 1N aqueous HCl solution. Then the organic layer was dried on $MgSO_4$, filtered, concentrated under *vacuo* and dried over P_2O_5 to obtain the final product. Boc-Phe-Leu-OBn was recovered as a white solid (796.5 mg, 80%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.29–7.06 (m, 10H), 6.41 (d, J = 8.1 Hz, 1H), 5.12–4.97 (m, 3H), 4.61–4.44 (m, 1H), 4.38–4.21 (m, 1H), 3.05–2.86 (m, 2H), 1.55–1.15 (m with singulet, 12H), 0.85–0.72 (m, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.4, 171.2, 155.6, 136.8, 135.5, 129.5, 128.7, 128.5, 128.3, 127.0, 80.3, 67.1, 55.7, 51.0, 41.6, 38.3, 28.4, 24.8, 22.9, 22.0 ppm; MS (ESI): *m*/*z* 469.2 $[M+H]^+$.

HCl·H-Leu-Leu-OBn

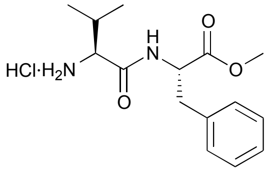
Boc-Leu-Leu-OBn (110.9 mg, 0.255 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Leu-Leu-OBn was recovered as a white solid (94.6 mg, >99%).



^{1}H NMR (200 MHz, D_2O): δ = 7.50–7.29 (m, 5H), 5.18–5.14 (m, 2H), 4.53–4.43 (m, 1H), 4.01–3.92 (m, 1H), 1.73–1.48 (m, 6H), 0.97–0.72 (m, 12H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 176.1, 172.9, 137.6, 131.40, 131.35, 131.0, 70.4, 54.4, 54.2, 42.4, 41.6, 32.2, 26.9, 26.2, 24.4, 24.1, 23.6, 23.3 ppm; MS (ESI): *m*/*z* 335.2 $[M+H]^+$, HRMS: m/z: calcd for $C_{19}H_{31}N_2O_3$: 335.2335; found: 335.2302.

HCl·H-Val-Phe-OMe[10]

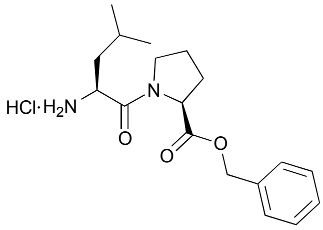
Boc-Val-Phe-OMe (90.9 mg, 0.240 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Val-Phe-OMe was recovered as a white solid (75.6 mg, >99%).



^{1}H NMR (200 MHz, D_2O): δ = 7.44–7.25 (m, 5H), 4.79–4.72 (m, 1H, merged with HDO peak), 3.81–3.76 (m, 1H), 3.71 (s, 3H), 3.29–3.03 (m, 2H), 2.29–2.12 (m, 1H), 1.04–0.96 (m, 6H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 173.2, 169.3, 136.3, 129.2, 128.9, 127.4, 58.3, 54.5, 53.0, 36.4, 30.1, 17.6, 16.7 ppm; MS (ESI): *m/z* 279.1 [*M*+H]$^+$.

HCl·H-Leu-Pro-OBn

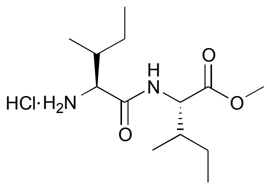
Boc-Leu-Pro-OBn (82.2 mg, 0.204 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Leu-Pro-OBn was recovered as a white solid (72.2 mg, >99%).



^{1}H NMR (200 MHz, D_2O): δ = 7.48–7.36 (m, 5H), 5.20 (d, J = 12.0 Hz, 1H), 5.12 (d, J = 12.0 Hz, 1H), 4.60–4.51 (m, 1H), 4.24 (t, J = 6.9 Hz, 1H), 3.77–3.52 (m, 2H), 2.40–2.20 (m, 1H), 2.11–1.93 (m, 3H), 1.76–1.47 (m, 3H), 0.90 (d, J = 5.4 Hz, 3H), 0.87 (d, J = 5.4 Hz, 3H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 173.4, 169.1, 135.1, 128.9, 128.6, 68.0, 59.9, 50.4, 47.6, 38.9, 28.7, 24.7, 23.8, 22.4, 20.4 ppm; MS (ESI): *m/z* 319.2 [*M*+H]$^+$, HRMS: m/z: calcd for $C_{18}H_{27}N_2O_3$: 319.2022; found: 319.2018.

HCl·H-Ile-Ile-OMe[5]

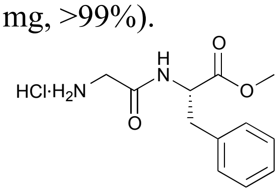
Boc-Ile-Ile-OMe (92.2 mg, 0.257 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Ile-Ile-OMe was recovered as a white solid (91.8 mg, >99%).



^{1}H NMR (200 MHz, D_2O): δ = 4.37 (d, J = 5.9 Hz, 1H), 3.92 (d, J = 5.7 Hz, 1H), 3.74 (s, 3H), 2.08–1.86 (m, 2H), 1.58–1.10 (m, 4H), 1.04–0.82 (m, 12H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 176.1, 172.2, 60.4, 60.1, 55.2, 39.1, 38.7, 27.4, 26.6, 17.3, 16.5, 13.09, 13.07 ppm; MS (ESI): *m/z* 259.1 [*M*+H]$^+$.

HCl·H-Gly-Phe-OMe[11]

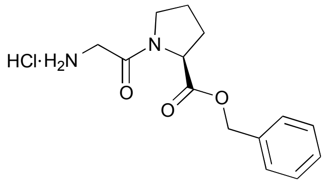
Boc-Gly-Phe-OMe (127.5 mg, 0.379 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Gly-Phe-OMe was recovered as a white solid (103.3 mg, >99%).



^{1}H NMR (200 MHz, D_2O): δ = 7.44–7.23 (m, 5H), 4.85–4.74 (m, 1H, merged with HDO peak), 3.87–3.67 (m, 5H), 3.30–2.98 (m, 2H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 173.4, 166.8, 136.4, 129.3, 128.9, 127.4, 54.3, 53.1, 40.3, 36.8 ppm; MS (ESI): *m*/*z* 237.2 $[M+H]^+$.

HCl·H-Gly-Pro-OBn

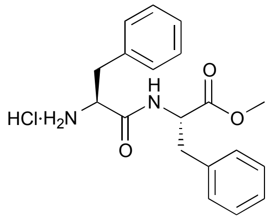
Boc-Gly-Pro-OBn (95.0 mg, 0.262 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Gly-Pro-OBn was recovered as a white solid (78.1 mg, >99%).



^{1}H NMR (200 MHz, D_2O): δ = 7.42 (s, 5H), 5.27–5.17 (m, 2H), 4.59–4.50 (m, 1H), 3.97–3.87 (m, 2H), 3.65–3.45 (m, 2H), 2.35–2.15 (m, 1H), 2.10–1.90 (m, 3H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 173.7, 165.7, 135.3, 129.0, 128.9, 128.4, 68.0, 59.6, 46.7, 40.4, 28.9, 24.3 ppm; MS (ESI): *m*/*z* 263.1 $[M+H]^+$, HRMS: m/z: calcd for $C_{14}H_{19}N_2O_3$: 263.1396; found: 263.1384.

HCl·H-Phe-Phe-OMe[12]

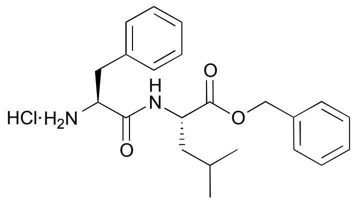
Boc-Phe-Phe-OMe (85.6 mg, 0.201 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Phe-Phe-OMe was recovered as a white solid (72.5 mg, >99%).



^{1}H NMR (200 MHz, D_2O): δ = 7.46–7.18 (m, 10H), 4.71 (dd, J = 6.4, 8.0 Hz, 1H), 4.18 (t, J = 7.1 Hz, 1H), 3.67 (s, 3H), 3.24–2.96 (m, 4H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 172.7, 168.8, 136.2, 133.6, 129.5, 129.23, 129.20, 128.9, 128.1, 127.4, 54.4, 54.2, 53.0, 36.8, 36.7 ppm; MS (ESI): *m*/*z* 327.1 $[M+H]^+$.

HCl·H-Phe-Leu-OBn

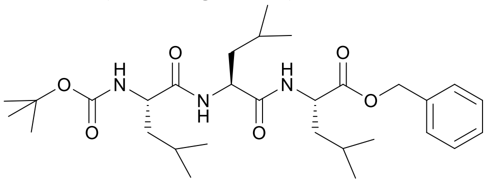
Boc-Phe-Leu-OBn (145.0 mg, 0.309 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Phe-Leu-OBn was recovered as a white solid (125.0 mg, >99%).



^{1}H NMR (200 MHz, D_2O): δ = 7.27–7.14 (m, 10H), 5.00 (s, 2H), 4.46–4.36 (m, 1H), 4.26 (t, J = 7.0 Hz, 1H), 3.16–2.94 (m, 2H), 1.60–1.38 (m, 3H), 0.83–0.73 (m, 6H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 170.7, 166.6, 132.9, 131.3, 127.3, 126.8, 126.5, 126.4, 126.1, 125.7, 65.3, 51.8, 49.5, 37.3, 34.6, 22.0, 19.6, 18.8 ppm; MS (ESI): *m*/*z* 369.2 $[M+H]^+$, HRMS: m/z: calcd for $C_{22}H_{29}N_2O_3$: 369.2178; found: 369.2173.

Boc-Leu-Leu-Leu-OBn

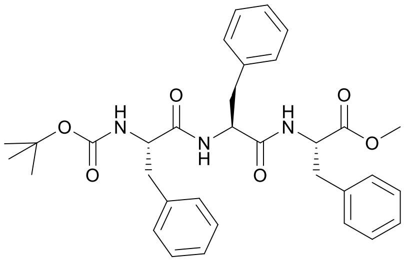
Following general procedure A, Boc-Leu-NCA (23.5 mg, 0.0913 mmol), $NaHCO_3$ (7.7 mg, 0.0913 mmol), EtOAc (100 µL) and HCl·H-Leu-Leu-OBn (33.9 mg, 0.0913 mmol) were used. Boc-Leu-Leu-Leu-OBn was recovered as a white solid (39.3 mg, 79%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.33–7.20 (m, 5H), 6.60–6.46 (m, 2H), 5.16 (d, J = 12.2 Hz, 1H), 5.08 (d, J = 12.2 Hz, 1H), 4.92 (d, J = 7.8 Hz, 1H), 4.64–4.48 (m, 1H), 4.44–4.31 (m, 1H), 4.08–3.89 (m, 1H), 1.67–1.30 (m, 18H), 0.91–0.73 (m, 18H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.7, 172.4, 171.5, 155.7, 135.4, 128.6, 128.4, 128.3, 80.2, 67.0, 53.0, 51.6, 50.9, 41.3, 40.9, 40.8, 28.3, 24.7, 24.6, 22.9, 22.8, 22.0, 21.8 ppm; MS (ESI): m/z 548.4 $[M+H]^+$, HRMS: m/z: calcd for $C_{30}H_{50}N_3O_6$: 548.3700; found: 548.3706.

Boc-Phe-Phe-Phe-OMe[13]

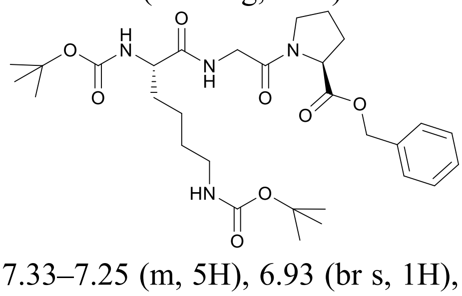
Following general procedure B, Boc-Phe-OSu (31.6 mg, 0.0872 mmol), $NaHCO_3$ (7.3 mg, 0.0872 mmol), EtOAc (100 µL) and HCl·H-Phe-Phe-OMe (31.6 mg, 0.0872 mmol) were used. Boc-Phe-Phe-Phe-OMe was recovered as a white solid (43.0 mg, 86%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.28–6.89 (m, 15H), 6.40 (d, J = 7.6 Hz, 1H), 6.17 (d, J = 7.4 Hz, 1H), 4.79 (d, J = 7.0 Hz, 1H), 4.69–4.57 (m, 1H), 4.49 (qd, J = 7.4 Hz, 1H), 4.30–4.16 (m, 1H), 3.59 (s, 3H), 3.05–2.76 (m, 6H), 1.30 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.4, 171.2, 170.1, 155.6, 136.6, 136.4, 135.9, 129.5, 129.4, 128.9, 128.83, 128.79, 127.33, 127.25, 115.2, 80.6, 55.8, 54.5, 53.7, 52.5, 38.2, 38.0, 28.4 ppm; MS (ESI): m/z 574.3 $[M+H]^+$.

Boc-Lys(Boc)-Gly-Pro-OBn

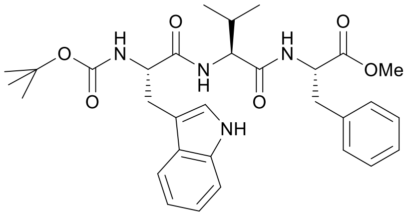
Following general procedure B, Boc-Lys(Boc)-OSu (37.5 mg, 0.085 mmol) $NaHCO_3$ (7.1 mg, 0.085 mmol), EtOAc (100 µL) and HCl·H-Gly-Pro-OBn (25.3 mg, 0.085 mmol) were used. Boc-Lys(Boc)-Gly-Pro-OBn was recovered as a white solid (39.7 mg, 79%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.33–7.25 (m, 5H), 6.93 (br s, 1H), 5.20–5.02 (m, 3H), 4.72–4.61 (m, 1H), 4.56–4.55 (m, 1H), 4.35–3.85 (m, 3H), 3.65–3.35 (m, 2H), 3.09–2.95 (m, 2H), 2.21–1.15 (m, 28H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) (major conformers): δ = 172.2, 171.6, 166.9, 156.2, 155.7, 135.5, 128.6, 128.4, 128.1, 80.0, 79.1, 67.0, 59.0, 54.4, 46.0, 42.0, 40.1, 32.5, 29.7, 29.1, 28.5, 28.3, 24.6, 22.6 ppm; MS (ESI): m/z 591.4 $[M+H]^+$, HRMS: m/z: calcd for $C_{30}H_{47}N_4O_8$: 591.3394; found: 591.3372.

Boc-Trp-Val-Phe-OMe

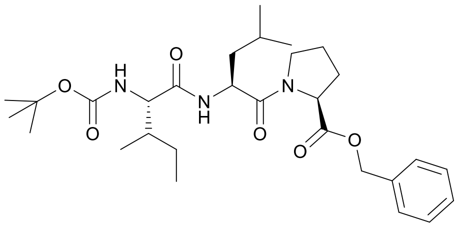
Following general procedure A, Boc-Trp-NCA (29.2 mg, 0.0885 mmol), $NaHCO_3$ (7.4 mg, 0.0885 mmol), EtOAc (100 μL) and HCl·H-Val-Phe-OMe (27.9 mg, 0.0885 mmol) were used. Boc-Trp-Val-Phe-OMe was recovered as a white solid (43.0 mg, 86%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.02 (s, 1H), 7.61 (d, J = 7.4 Hz, 1H), 7.30–6.86 (m, 9H), 6.20 (dd, J = 4.6, 7.6 Hz, 2H), 5.10 (d, J = 6.2 Hz, 1H), 4.67 (qd, J = 6.8 Hz, 1H), 4.44–4.32 (m, 1H), 4.09 (dd, J = 5.6, 8.0 Hz, 1H), 3.64 (s, 3H), 3.28–2.82 (m, 4H), 2.04–1.87 (m, 1H), 1.35 (s, 9H), 0.69 (d, J = 6.8 Hz, 3H), 0.56 (d, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.0, 170.5, 155.8, 136.5, 136.1, 129.4, 128.9, 127.4, 123.4, 122.6, 120.1, 119.1, 111.4, 110.8, 77.4, 58.7, 55.5, 53.4, 52.5, 38.0, 30.5, 28.5, 19.1, 17.5 ppm; MS (ESI): m/z 565.3 $[M+H]^+$, HRMS: m/z: calcd for $C_{31}H_{41}N_4O_6$: 565.3026; found: 565.3005.

Boc-Ile-Leu-Pro-OBn

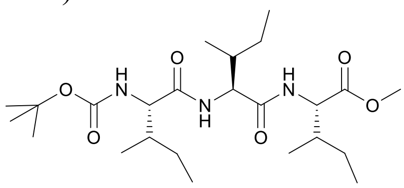
Following general procedure A, Boc-Ile-NCA (24.2 mg, 0.094 mmol), $NaHCO_3$ (7.9 mg, 0.094 mmol), EtOAc (100 μL) and HCl·H-Leu-Pro-OBn (33.3 mg, 0.094 mmol) were used. Boc-Ile-Leu-Pro-OBn was recovered as an oil (42.8 mg, 86%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.33–7.21 (m, 5H), 6.44 (d, J = 8.6 Hz, 1H), 5.17–4.92 (m, 3H), 4.80–4.45 (m, 2H), 3.95–3.81 (m, 1H), 3.77–3.63 (m, 1H), 3.59–3.43 (m, 1H), 2.30–0.74 (m, 31H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.9, 171.5, 171.0, 155.8, 135.7, 128.8, 128.5, 128.4, 80.0, 67.1, 59.5, 59.1, 49.1, 47.0, 42.1, 37.6, 29.2, 28.5, 25.1, 25.0, 24.7, 23.5, 22.0, 15.8, 11.6 ppm; MS (ESI): m/z 532.3 $[M+H]^+$, HRMS: m/z: calcd for $C_{29}H_{46}N_3O_6$: 532.3387; found: 532.3386.

Boc-Ile-Ile-Ile-OMe

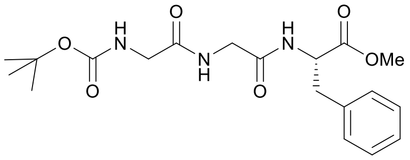
Following general procedure A, Boc-Ile-NCA (27.3 mg, 0.106 mmol), $NaHCO_3$ (8.9 mg, 0.106 mmol), EtOAc (100 μL) and HCl·H-Ile-Ile-OMe (31.2 mg, 0.106 mmol) were used. Boc-Ile-Ile-Ile-OMe was recovered as a white solid (43.2 mg, 86%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 6.66–6.51 (m, 2H), 5.15 (d, J = 8.4 Hz, 1H), 4.50 (dd, J = 5.0, 8.7 Hz, 1H), 4.33–4.23 (m, 1H), 3.92–3.83 (m, 1H), 3.66 (s, 3H), 1.90–1.68 (m, 4H), 1.55–0.73 (m, 32H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.2, 172.0, 171.1, 156.0, 80.1, 59.7, 58.0, 56.7, 52.3, 37.9, 37.2, 37.1, 28.5, 25.3, 25.1, 25.0, 15.7, 15.6, 15.5, 11.7, 11.5, 11.4 ppm; MS (ESI): m/z 472.4 $[M+H]^+$, HRMS: m/z: calcd for $C_{24}H_{46}N_3O_6$: 472.3387; found: 472.3380.

Boc-Gly-Gly-Phe-OMe[14]

Following general procedure B, Boc-Gly-OSu (34.6 mg, 0.127 mmol), $NaHCO_3$ (10.7 mg, 0.127 mmol), EtOAc (100 µL) and HCl·H-Gly-Phe-OMe (34.7 mg, 0.127 mmol) were used. Boc-Gly-Gly-Phe-OMe was recovered as a white solid (37.1 mg, 74%).

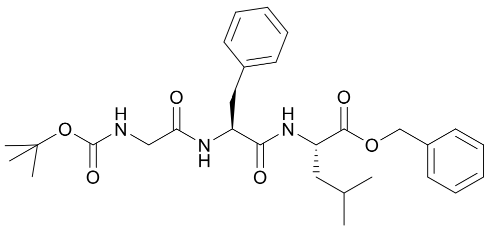


^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.26–6.92 (m, 6H), 6.85–6.77 (m, 1H), 5.35–5.26 (m, 1H), 4.82–4.70 (m, 1H), 3.88–3.68 (m, 4H), 3.63 (s, 3H), 3.13–2.91 (m, 2H), 1.38 (s, 9H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.0, 170.3, 168.7, 156.3, 136.0, 129.4, 128.8, 127.4, 80.6, 53.6, 52.6, 44.4, 43.0, 38.0, 28.5 ppm; MS (ESI): *m*/*z* 394.1 [*M*+H]$^+$.

Boc-Gly-Phe-Leu-OBn[9]

Following general procedure B, Boc-Gly-OSu (77.7 mg, 0.285 mmol), $NaHCO_3$ (23.9 mg, 0.285 mmol), EtOAc (300 µL) and HCl·H-Phe-Leu-OBn (115.4 mg, 0.285 mmol) were used. Boc-Gly-Phe-Leu-OBn was recovered as an oil (139.1 mg, 93%).

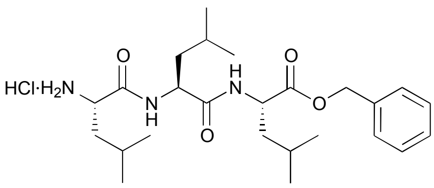
When realised on a 1.90 mmoles scale, Boc-Gly-OSu (517.3 mg, 1.90 mmol), $NaHCO_3$ (159.6 mg, 1.90 mmol), *t*BuOAc (2000 µL) and HCl·H-Phe-Leu-OBn (770.0 mg, 1.90 mmol) were introduced in a 10 mL stainless steel grinding bowl with one stainless steel ball (10 mm diameter). The bowl was closed and placed 40 min within the mixer mill at 30 Hz. Then, 2 mL of 1N aqueous NaOH solution was added and the reactor was put in the mixer mill for 5 min at 30 Hz. Afterwards, the suspension was diluted with EtOAc and washed two times with 1N aqueous NaOH solution and two times with 1N aqueous HCl solution. Then the organic layer was dried on $MgSO_4$, filtered, concentrated under *vacuo* and dried over P_2O_5 to obtain the final product. Boc-Gly-Phe-Leu-OBn was recovered as an oil (937.6 mg, 94%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.28–7.02 (m, 10H), 6.83 (d, J = 8.1 Hz, 1H), 6.59 (d, J = 8.0 Hz, 1H), 5.19 (t, J = 5.8 Hz, 1H), 5.00 (s, 2H), 4.64 (qd, J = 7.2 Hz, 1H), 4.52–4.40 (m, 1H), 3.68–3.60 (m, 2H), 2.98–2.91 (m, 2H), 1.53–1.27 (m with singulet, 12H), 0.81–0.69 (m, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.3, 170.7, 169.6, 156.2, 136.5, 135.6, 129.5, 128.8, 128.6, 128.4, 127.2, 80.5, 67.2, 54.3, 51.2, 44.4, 41.3, 38.3, 28.5, 24.9, 22.9, 22.1 ppm; MS (ESI): *m*/*z* 526.3 [*M*+H]$^+$.

HCl·H-Leu-Leu-Leu-OBn

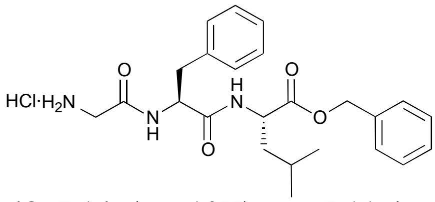
Boc-Leu-Leu-Leu-OBn (99.5 mg, 0.182 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Leu-Leu-Leu-OBn was recovered as a white solid (87.9 mg, >99%).



^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.48–7.37 (m, 5H), 5.24–5.14 (m, 2H), 4.52–4.37 (m, 2H), 4.03–3.94 (m, 1H), 1.77–1.45 (m, 9H), 0.98–0.79 (m, 18H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.2, 171.9, 168.2, 133.6, 127.2, 127.1, 126.7, 66.0, 50.5, 50.1, 49.8, 38.2, 38.1, 37.4, 22.6, 22.5, 22.2, 20.4, 20.0, 19.9, 19.7, 18.9 ppm; MS (ESI): *m*/*z* 448.2 [*M*+H]$^+$, HRMS: m/z: calcd for $C_{25}H_{42}N_3O_4$: 448.3175; found: 448.3166.

HCl·H-Gly-Phe-Leu-OBn

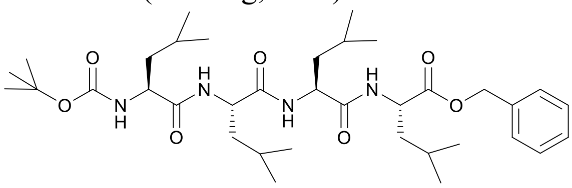
Boc-Gly-Phe-Leu-OBn (850.0 mg, 0.240 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Gly-Phe-Leu-OBn was recovered as a white solid (747.0 mg, >99%).



[1]H NMR (200 MHz, D_2O): δ = 7.48–7.14 (m, 10H), 5.11 (s, 2H), 4.68–4.58 (m, 1H), 4.40 (t, J = 6.8 Hz, 1H), 3.76 (d, J = 16.4 Hz, 1H), 3.66 (d, J = 16.4 Hz, 1H), 3.09–2.76 (m, 2H), 1.65–1.40 (m, 3H), 0.89–0.76 (m, 6H) ppm; [13]C NMR (75 MHz, D_2O): δ = 173.6, 172.8, 166.7, 136.0, 135.3, 129.2, 128.9, 128.8, 128.4, 127.3, 67.7, 54.9, 51.7, 40.3, 39.4, 37.3, 24.3, 22.0, 20.8 ppm; MS (ESI): *m/z* 426.3 $[M+H]^+$, HRMS: m/z: calcd for $C_{24}H_{32}N_3O_4$: 426.2393; found: 426.2392.

Boc-Leu-Leu-Leu-Leu-OBn

Following general procedure A, Boc-Leu-NCA (19.5 mg, 0.076 mmol), $NaHCO_3$ (6.4 mg, 0.076 mmol), EtOAc (100 µL) and HCl·H-Leu-Leu-Leu-OBn (36.6 mg, 0.076 mmol) were used. Boc-Leu-Leu-Leu-Leu-OBn was recovered as a white solid (47.8 mg, 96%).

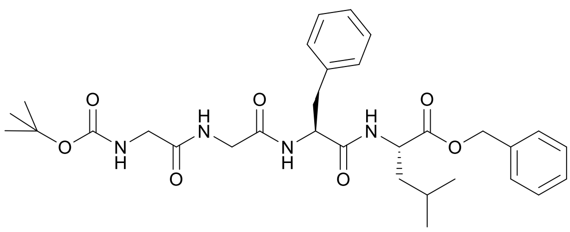


[1]H NMR (200 MHz, $CDCl_3$): δ = 7.32–7.15 (m, 5H), 6.84–6.72 (m, 2H), 6.66–6.54 (m, 1H), 5.15–4.86 (m, 3H), 4.62–4.25 (m, 3H), 4.05–3.87 (m, 1H), 1.75–1.15 (m, 21H), 0.95–0.65 (m, 24H) ppm; [13]C NMR (75 MHz, $CDCl_3$): δ = 173.1, 172.6, 171.94, 171.87, 156.3, 135.8, 128.7, 128.42, 128.36, 80.9, 67.1, 53.9, 52.4, 51.8, 51.1, 41.2, 41.0, 40.9, 40.7, 28.5, 25.04, 24.98, 24.92, 24.90, 23.19, 23.15, 22.2, 22.1, 22.0, 21.9 ppm; MS (ESI): *m/z* 661.3 $[M+H]^+$, HRMS: m/z: calcd for $C_{36}H_{61}N_4O_7$: 661.4540; found: 661.4576.

Boc-Gly-Gly-Phe-Leu-OBn

Following general procedure B, Boc-Gly-OSu (70.1 mg, 0.257 mmol), $NaHCO_3$ (21.6 mg, 0.257 mmol), EtOAc (300 µL) and HCl·H-Gly-Phe-Leu-OBn (118.7 mg, 0.257 mmol) were used. Boc-Gly-Gly-Phe-Leu-OBn was recovered as a white solid (143.4 mg, 96%).

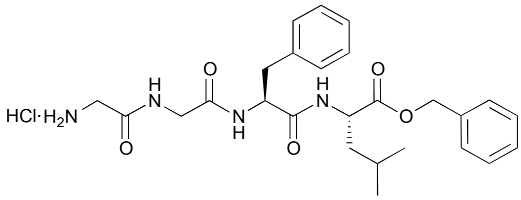
When realised on a 0.55 mmole scale, Boc-Gly-OSu (272.3 mg, 0.554 mmol), $NaHCO_3$ (46.5 mg, 0.554 mmol), *t*BuOAc (646 µL) and HCl·H-Gly-Phe-Leu-OBn (256.0 mg, 0.554 mmol) were introduced in a 10 mL stainless steel grinding bowl with one stainless steel ball (10 mm diameter). The bowl was closed and placed 40 min within the mixer mill at 30 Hz. Then, 2 mL of 1N aqueous NaOH solution was added and the reactor was put in the mixer mill for 5 min at 30 Hz. Afterwards, the suspension was diluted with EtOAc and washed two times with 1N aqueous NaOH solution and two times with 1N aqueous HCl solution. Then the organic layer was dried on $MgSO_4$, filtered, concentrated under *vacuo* and dried over P_2O_5 to obtain the final product. Boc-Gly-Gly-Phe-Leu-OBn was recovered as a white solid (291.2 mg, 90%).



[1]H NMR (200 MHz, $CDCl_3$): δ = 7.61–6.97 (m, 13H), 5.65–5.55 (m, 1H), 5.14–4.82 (m, 3H), 4.62–4.65 (m, 1H), 3.93–3.75 (m, 4H), 3.08–2.75 (m, 2H), 1.68–1.18 (m with singulet, 12H), 0.84–0.71 (m, 6H)

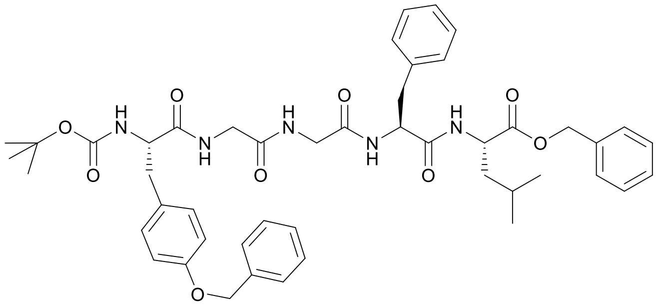
ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.6, 171.2, 169.9, 168.7, 156.3, 136.6, 135.6, 129.6, 128.8, 128.57, 128.56, 128.4, 127.0, 80.2, 67.2, 54.3, 51.2, 44.0, 43.2, 41.2, 39.0, 28.6, 25.0, 22.9, 22.1 ppm; MS (ESI): *m/z* 583.3 $[M+H]^+$, HRMS: m/z: calcd for $C_{31}H_{43}N_4O_7$: 583.3132; found: 583.3115.

HCl·H-Gly-Gly-Phe-Leu-OBn
Boc-Gly-Gly-Phe-Leu-OBn (333.7 mg, 0.572 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Gly-Gly-Phe-Leu-OBn was recovered as a white solid (297.1 mg, >99%).



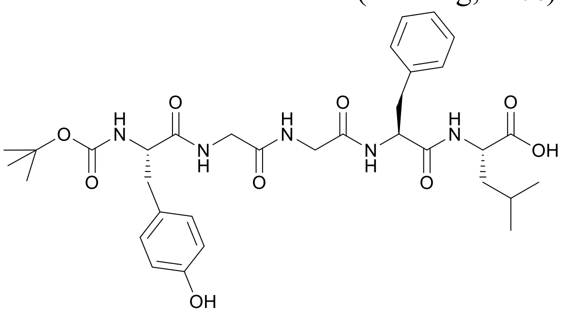
^{1}H NMR (200 MHz, D_2O): δ = 7.24–7.04 (m, 10H), 4.92 (s, 2H), 4.62 (t, J = 7.4 Hz, 1H), 4.41–4.31 (m, 1H), 3.92–3.70 (m, 4H), 3.03–2.75 (m, 2H), 1.55–1.35 (m, 3H), 0.79–0.62 (m, 6H) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 175.7, 175.0, 172.9, 170.0, 138.7, 137.8, 131.8, 131.1, 130.9, 130.7, 129.5, 69.7, 57.1, 53.9, 44.6, 12.9, 12.2, 40.0, 26.8, 24.6, 23.5 ppm; MS (ESI): *m/z* 483.3 $[M+H]^+$, HRMS: m/z: calcd for $C_{26}H_{35}N_4O_5$: 483.2607; found: 483.2625.

Boc-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn
Following general procedure B, Boc-Tyr(Bn)-OSu (28.0 mg, 0.060 mmol), $NaHCO_3$ (5.0 mg, 0.060 mmol), EtOAc (100 µL) and HCl·H-Gly-Phe-Leu-OBn (31.1 mg, 0.060 mmol) were ball-milled during 1 h. Boc-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn was recovered as a white solid (44.2 mg, 88%).



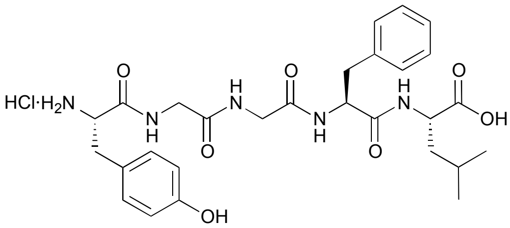
^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.60–6.96 (m, 21H), 6.83 (d, J = 7.2 Hz, 2H), 5.62–5.42 (m, 1H), 5.21–4.71 (m, 5H), 4.70–4.26 (m, 2H), 4.11–3.80 (m, 4H), 3.27–2.74 (m, 5H), 1.66–1.19 (m with singulet, 12H), 0.96–0.61 (m, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.6, 172.5, 171.1, 169.2, 168.6, 158.0, 156.1, 137.1, 136.8, 135.6, 130.5, 129.7, 128.8, 128.6, 128.4, 128.2, 127.0, 115.1, 80.4, 70.1, 67.2, 56.1, 54.4, 51.2, 43.5, 43.3, 41.3, 39.0, 38.1, 28.6, 25.0, 23.0, 22.2 ppm; MS (ESI): *m/z* 836.6 $[M+H]^+$, HRMS: m/z: calcd for $C_{47}H_{58}N_5O_9$: 836.4235; found: 836.4228.

Boc-Tyr(OH)-Gly-Gly-Phe-Leu-OH[15]
To a suspension of Boc-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn (30.0 mg, 0.0359 mmol) in 10 mL of EtOH, palladium on activated carbon (10% Pd, 3 mg) was added and the mixture was allowed to stir overnight under a H_2 atmosphere. The suspension was filtered through Celite and the filtrate was evaporated to give Boc-Tyr(OH)-Gly-Gly-Phe-Leu-OH as a white solid (18.2 mg, 77%).



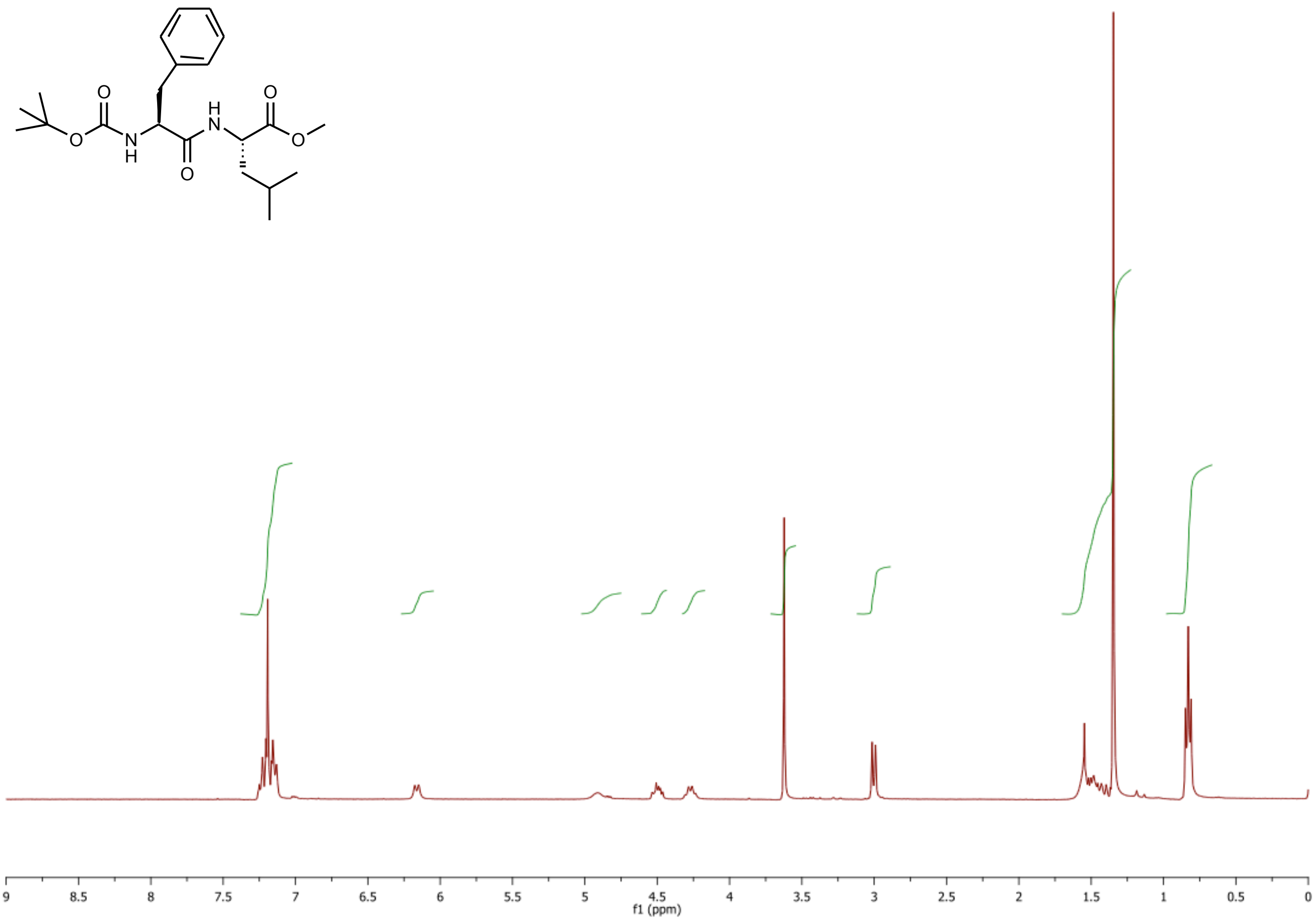
^{1}H NMR (200 MHz, MeOD): δ = 7.27–7.12 (m, 5H), 7.00 (d, J = 8.2 Hz, 2H), 6.66 (d, J = 8.2 Hz, 2H), 4.62 (dd, J = 4.8, 9.2 Hz, 1H), 4.45–4.32 (m, 1H), 4.20–4.07 (m, 1H), 3.90–3.55 (m, 4H), 3.33–2.61 (m, 4H), 1.75–1.52 (m, 2H), 1.41–1.15 (m with singulet, 10H), 0.88 (t, J = 6.6 Hz, 6H) ppm; ^{13}C NMR (75 MHz, MeOD): δ = 175.5, 173.5, 172.3, 171.3, 158.2, 157.5, 138.6, 131.5, 130.5, 129.6, 129.2, 127.9, 116.4, 81.0, 58.3, 56.0, 52.4, 43.9, 43.5, 41.8, 38.9, 38.1, 28.9, 26.1, 23.6, 22.1 ppm; MS (ESI): m/z 656.1[M+H]$^+$, HRMS: m/z: calcd for $C_{33}H_{46}N_5O_9$: 656.3296; found: 656.3279.

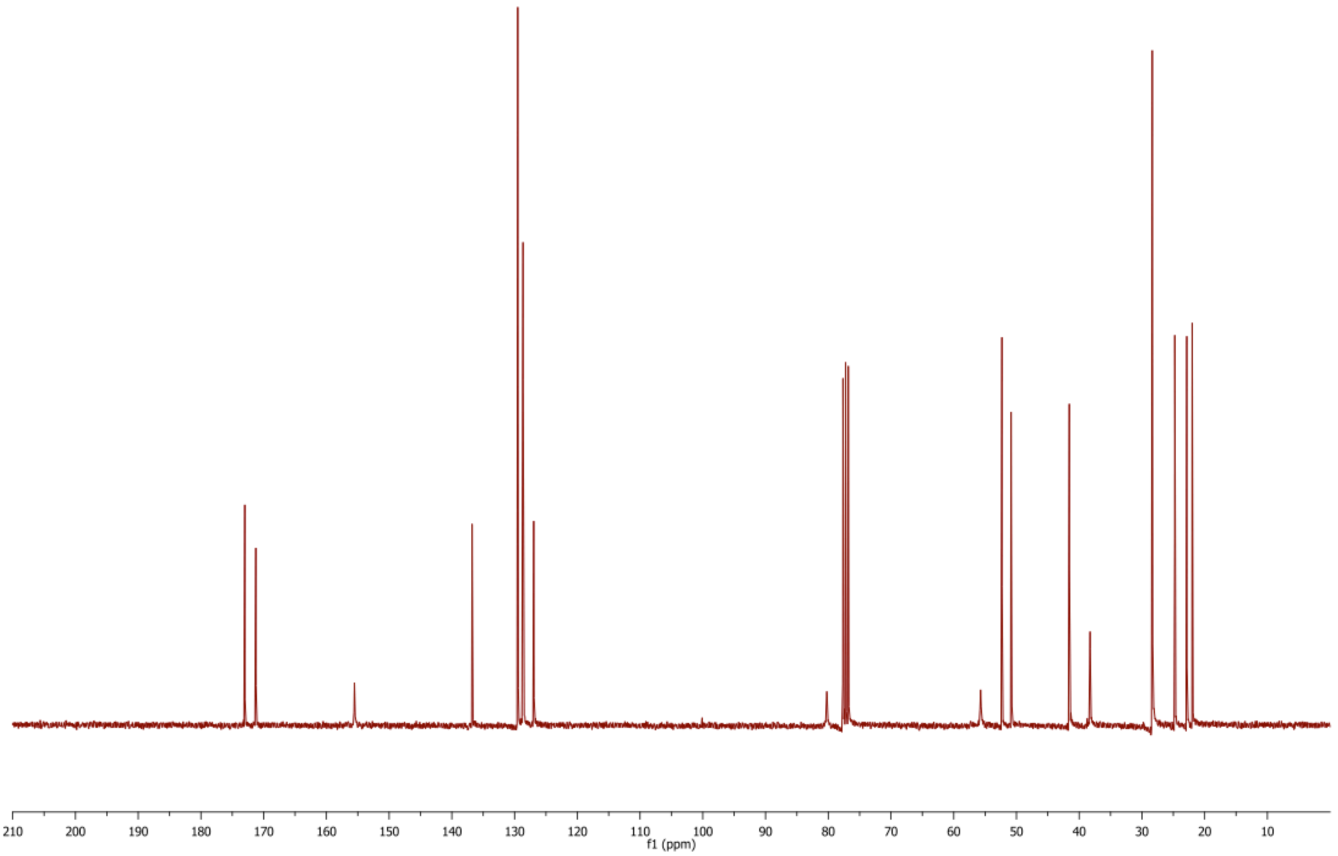
HCl·H-Tyr(OH)-Gly-Gly-Phe-Leu-OH (Leu-enkephalin HCl)[16]
Boc-Tyr(OH)-Gly-Gly-Phe-Leu-OH (17.2 mg, 0.026 mmol) was submitted to gaseous HCl for 2 h. HCl·H-Tyr(OH)-Gly-Gly-Phe-Leu-OH (Leu-enkephalin HCl) was recovered as a white solid (15.4 mg, >99%).



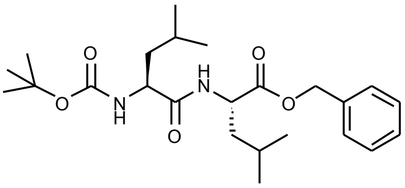
^{1}H NMR (400 MHz, DMSO): δ = 9.40 (s, 1H), 8.89–8.83 (m, 1H), 8.40–8.11 (m, 3H), 7.30–7.15 (m, 5H), 7.06 (d, J = 8.5 Hz, 2H), 6.71 (d, J = 8.5 Hz, 2H), 4.60–4.53 (m, 1H), 4.25–4.18 (m, 1H), 4.02–3.96 (m, 1H), 3.87–3.58 (m, 4H), 3.14–2.97 (m, 2H), 2.88–2.72 (m, 2H), 1.70–1.47 (m, 3H), 0.91 (d, J = 6.4, Hz, 6H), 0.85 (d, J = 6.4, Hz, 6H) ppm; ^{13}C NMR (400 MHz, DMSO): δ = 173.9, 171.2, 168.7, 168.3, 156.6, 137.8, 130.5, 129.3, 128.0, 126.3, 124.9, 115.3, 53.8, 50.3, 42.0, 41.6, 37.7, 36.1, 24.3, 22.9, 21.4 ppm; MS (ESI): m/z 556.1[M+H]$^+$.

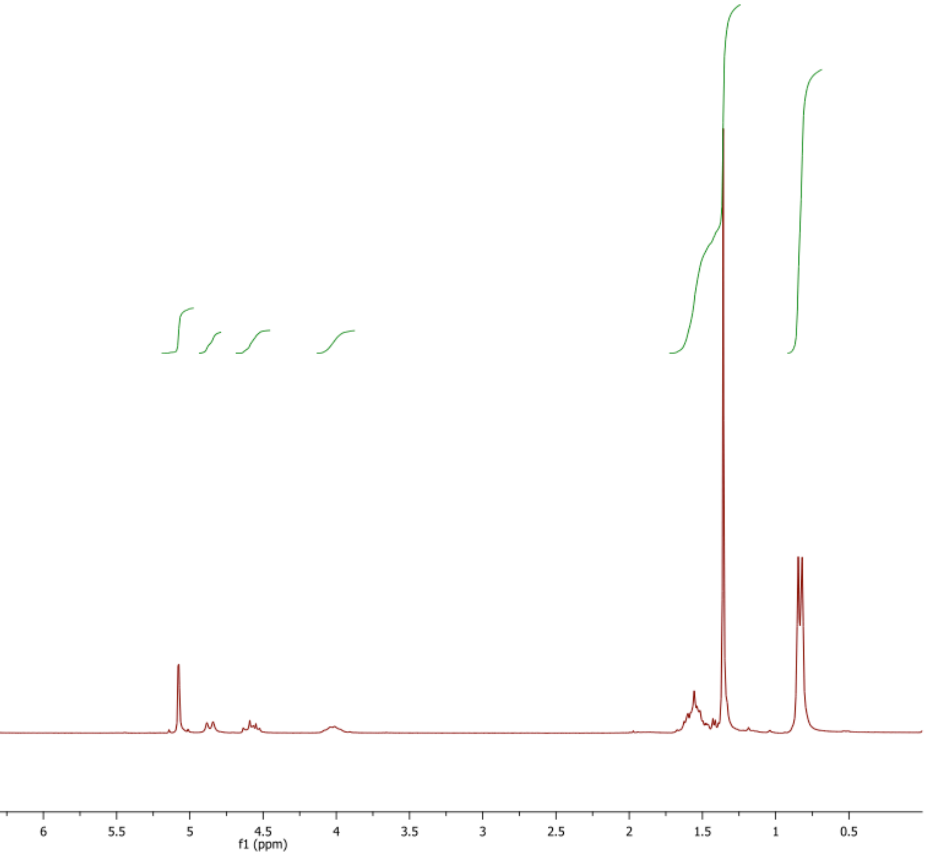
Boc-Phe-Leu-OMe

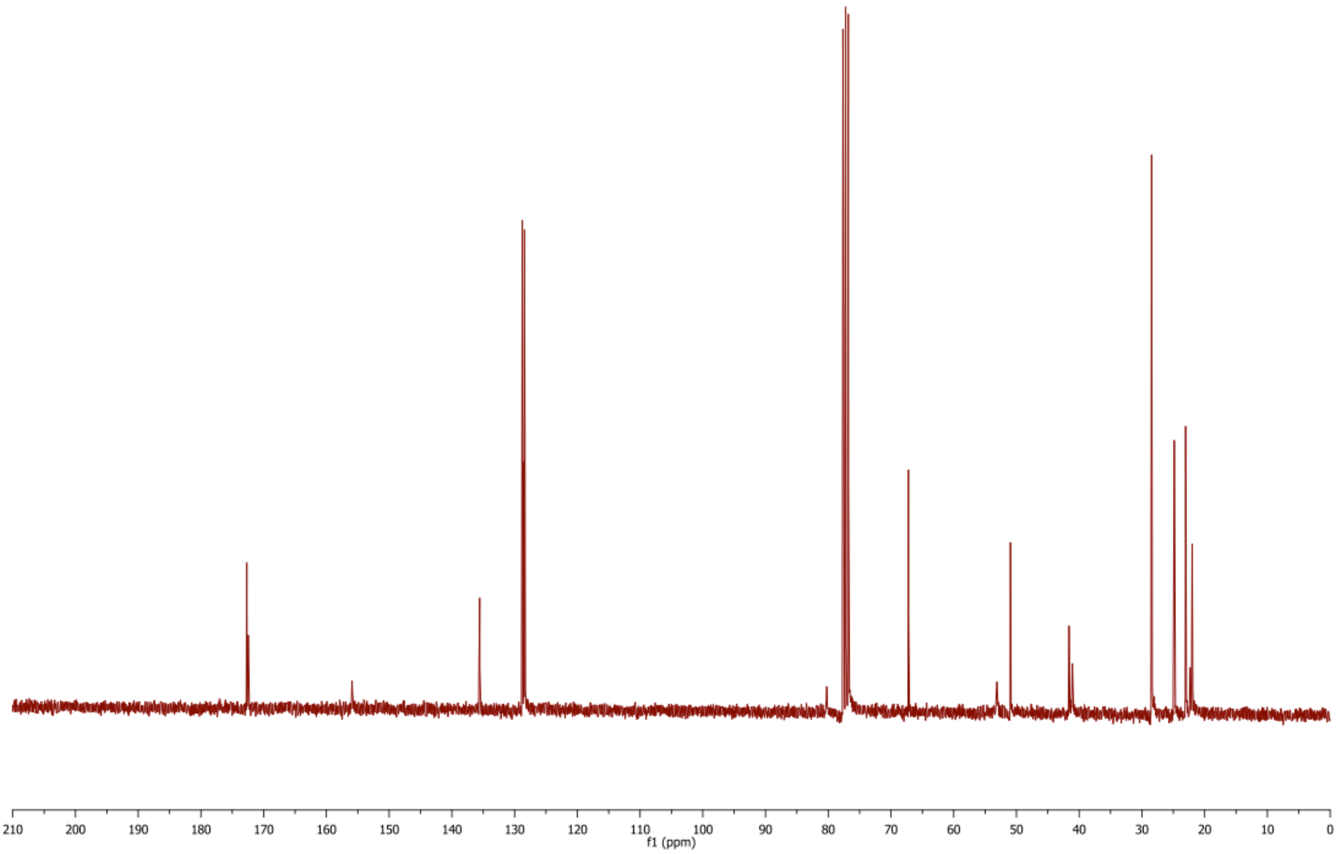




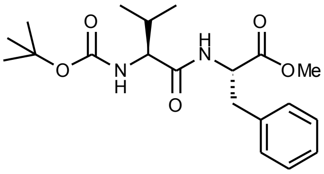
Boc-Leu-Leu-OBn

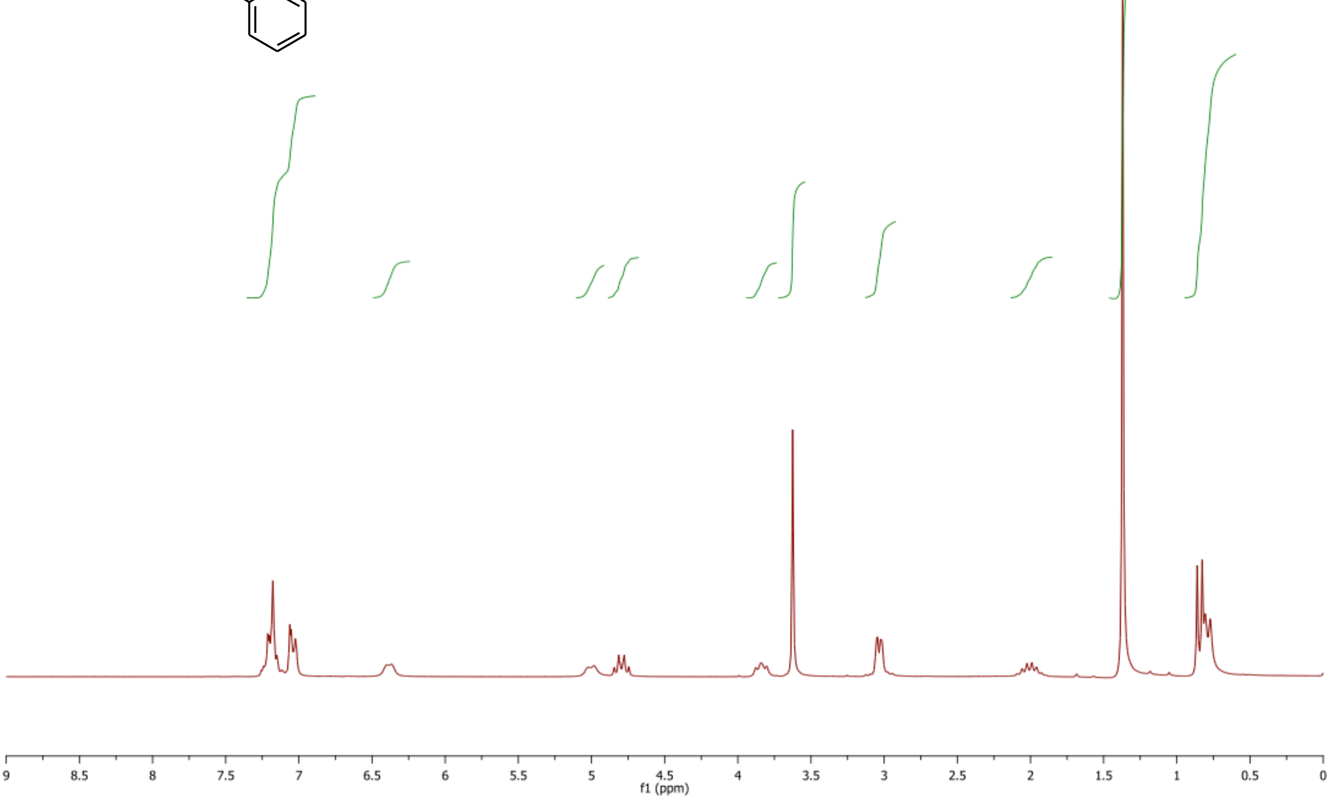


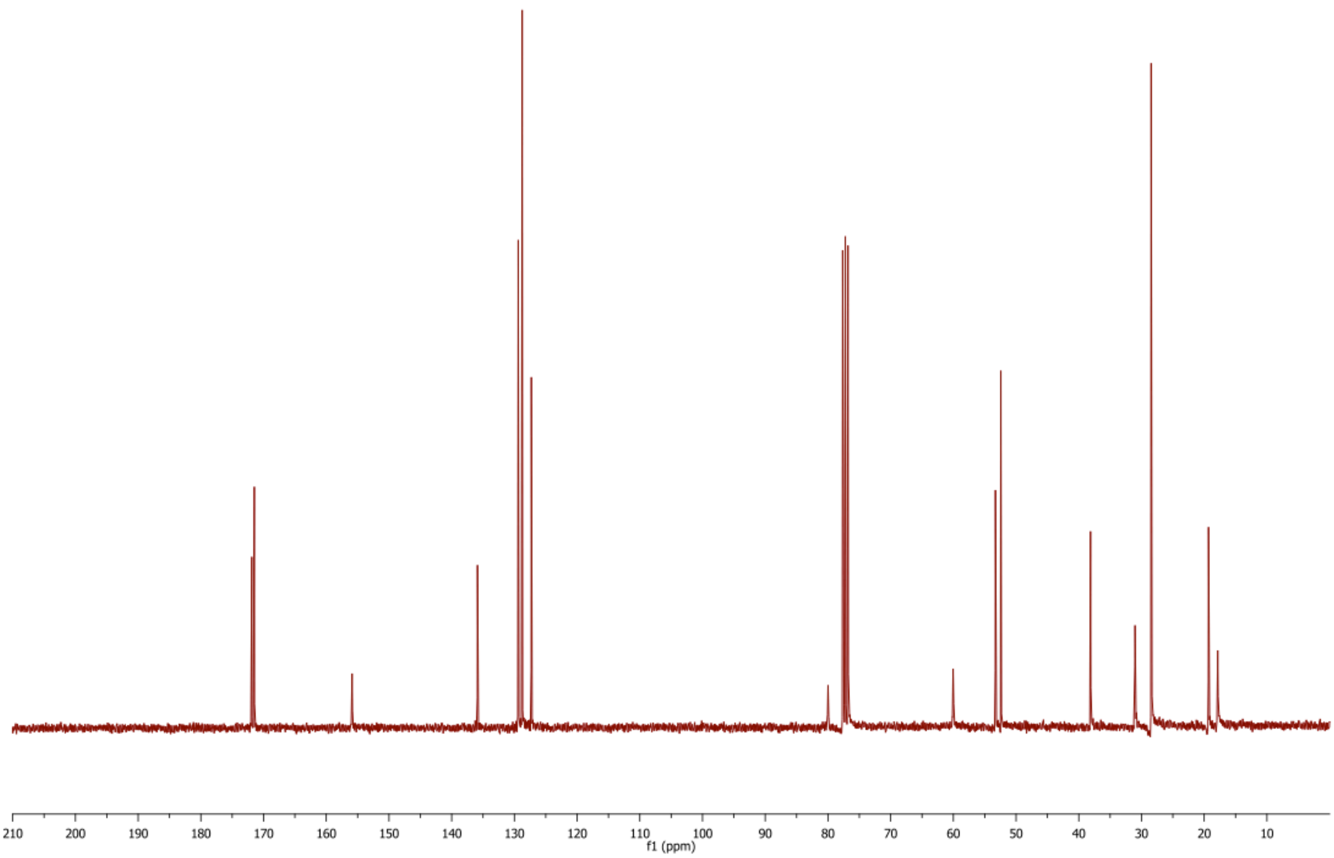




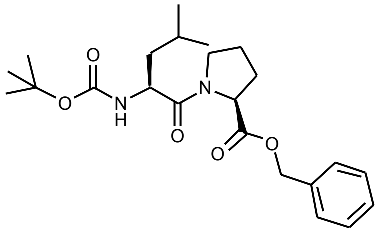
Boc-Val-Phe-OMe

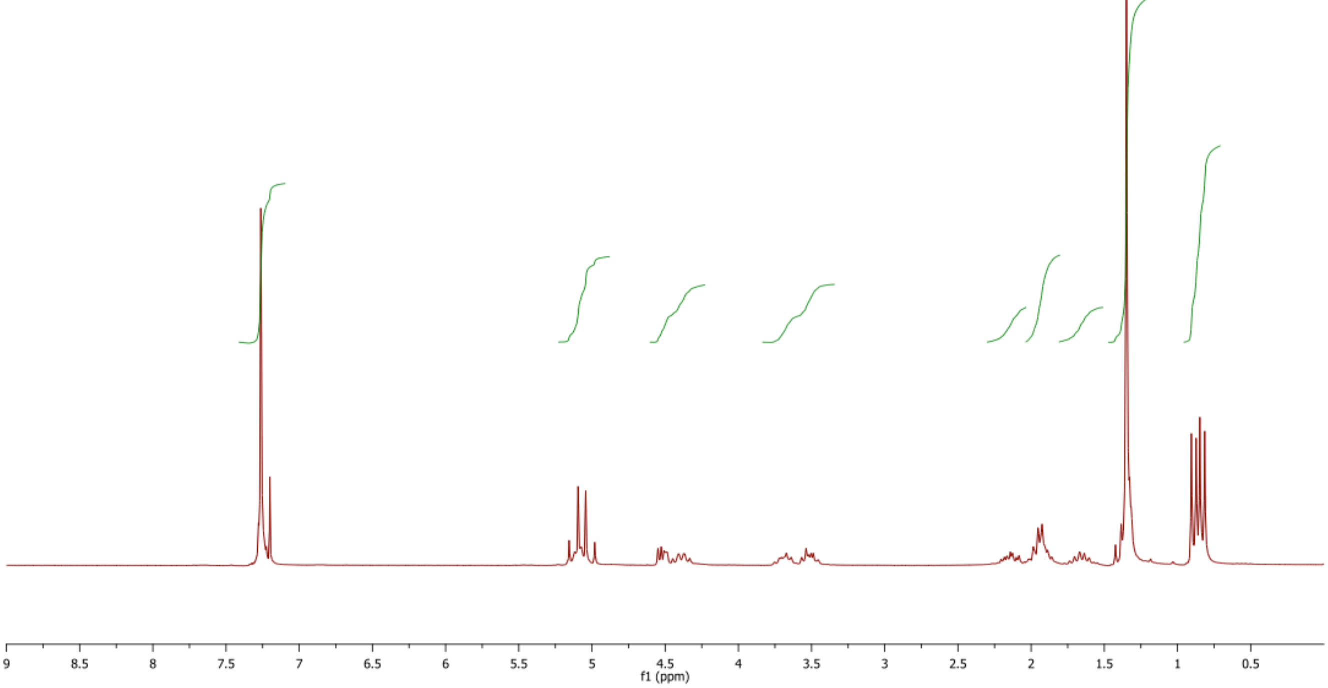


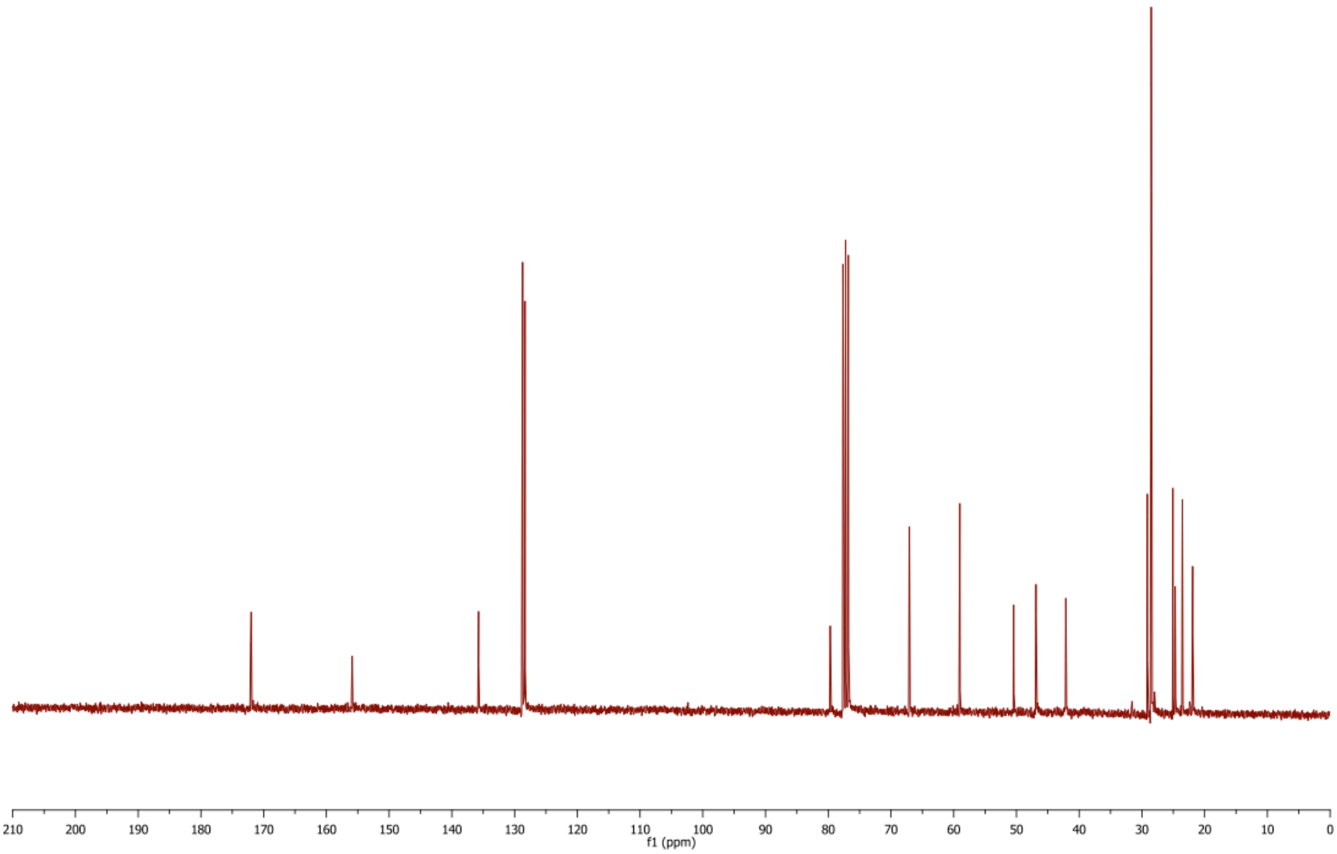




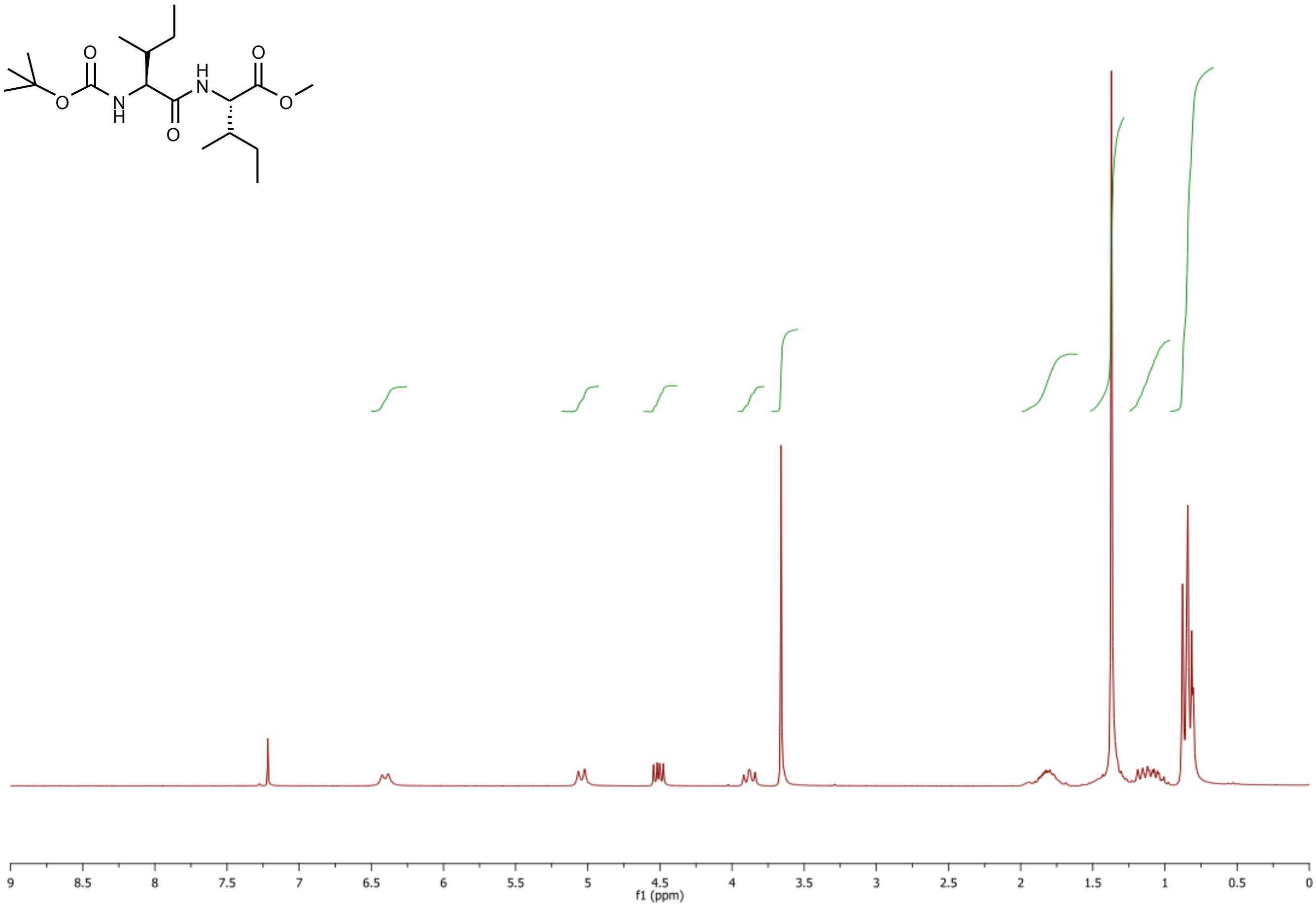
Boc-Leu-Pro-OBn

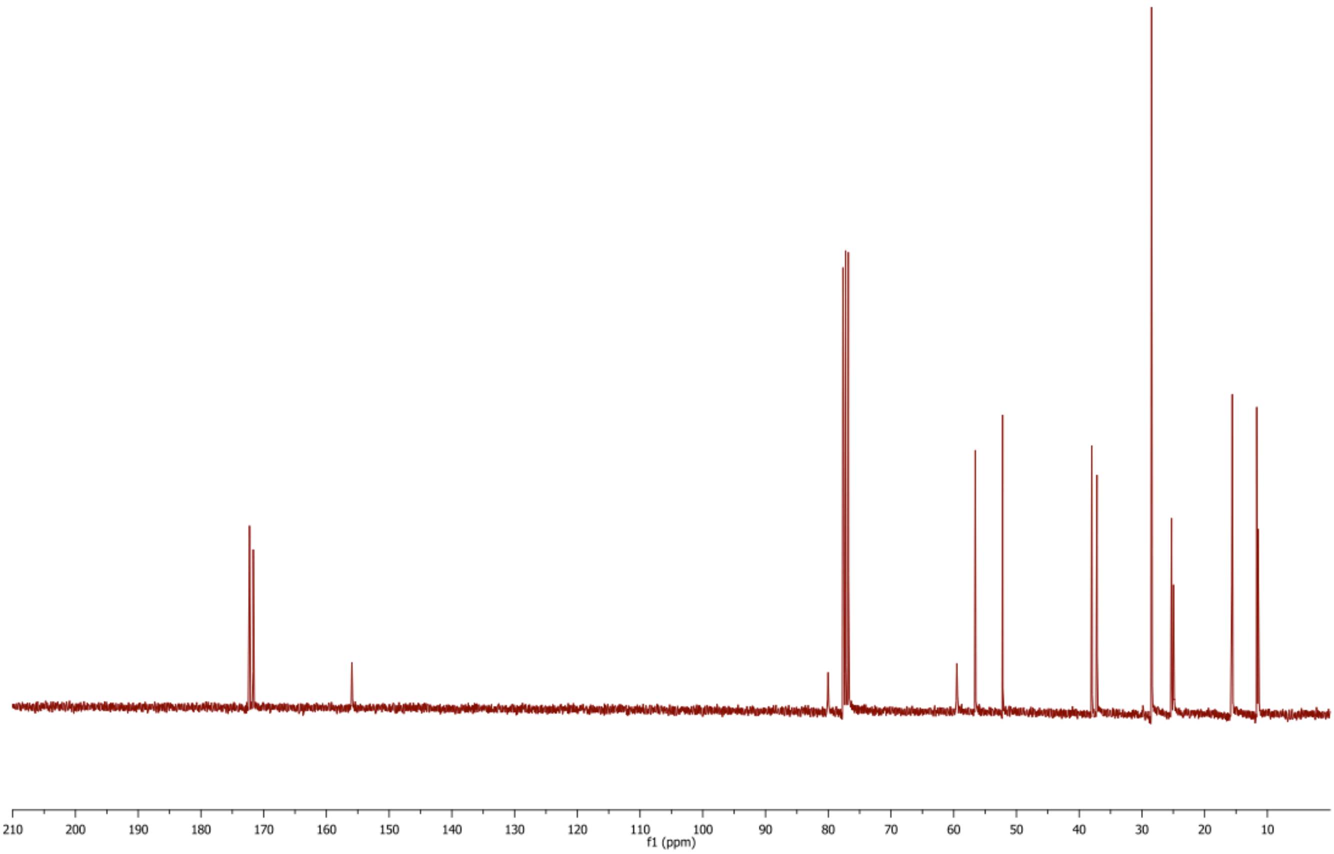




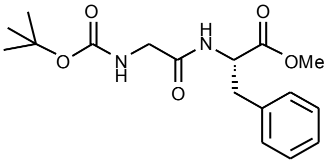


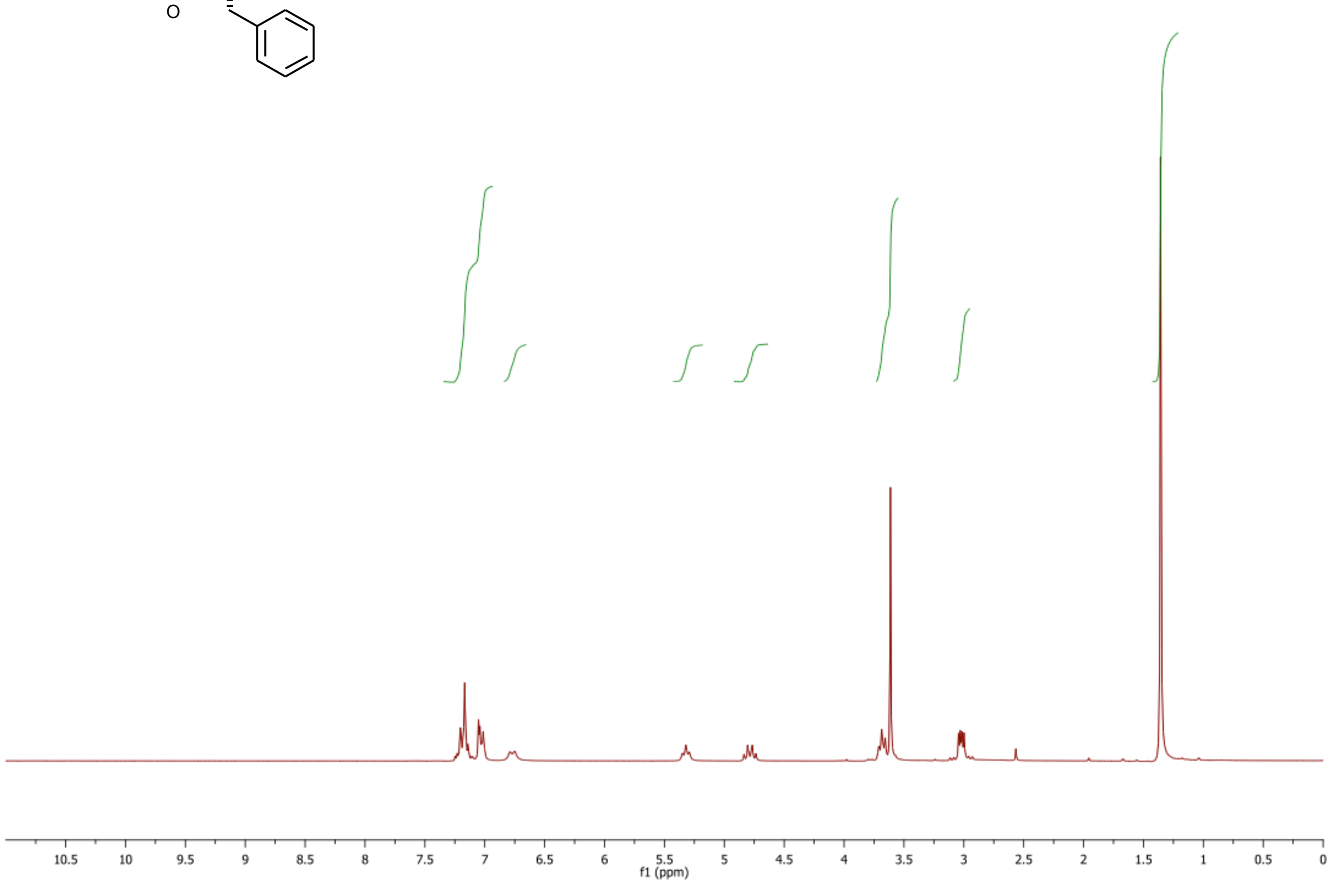
Boc-Ile-Ile-OMe

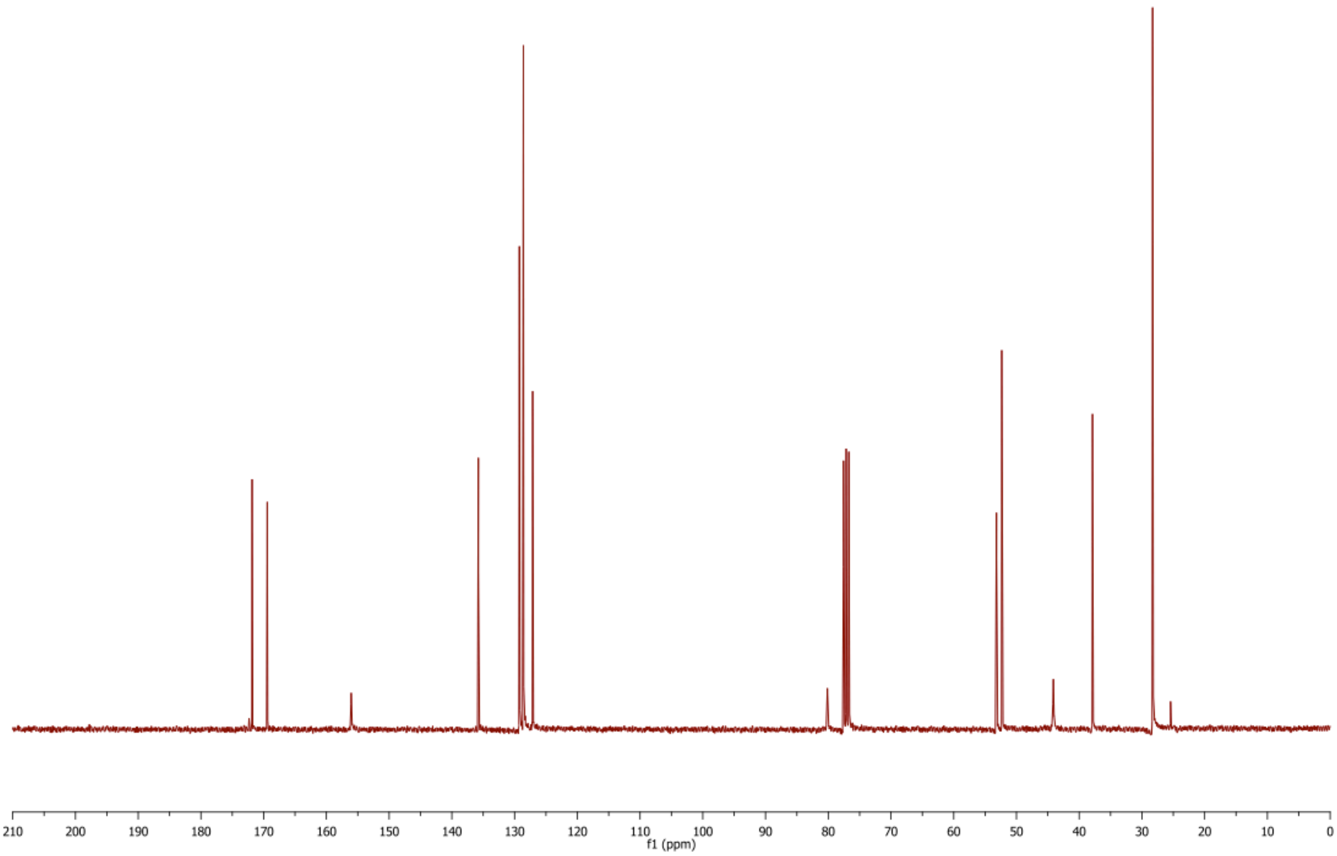




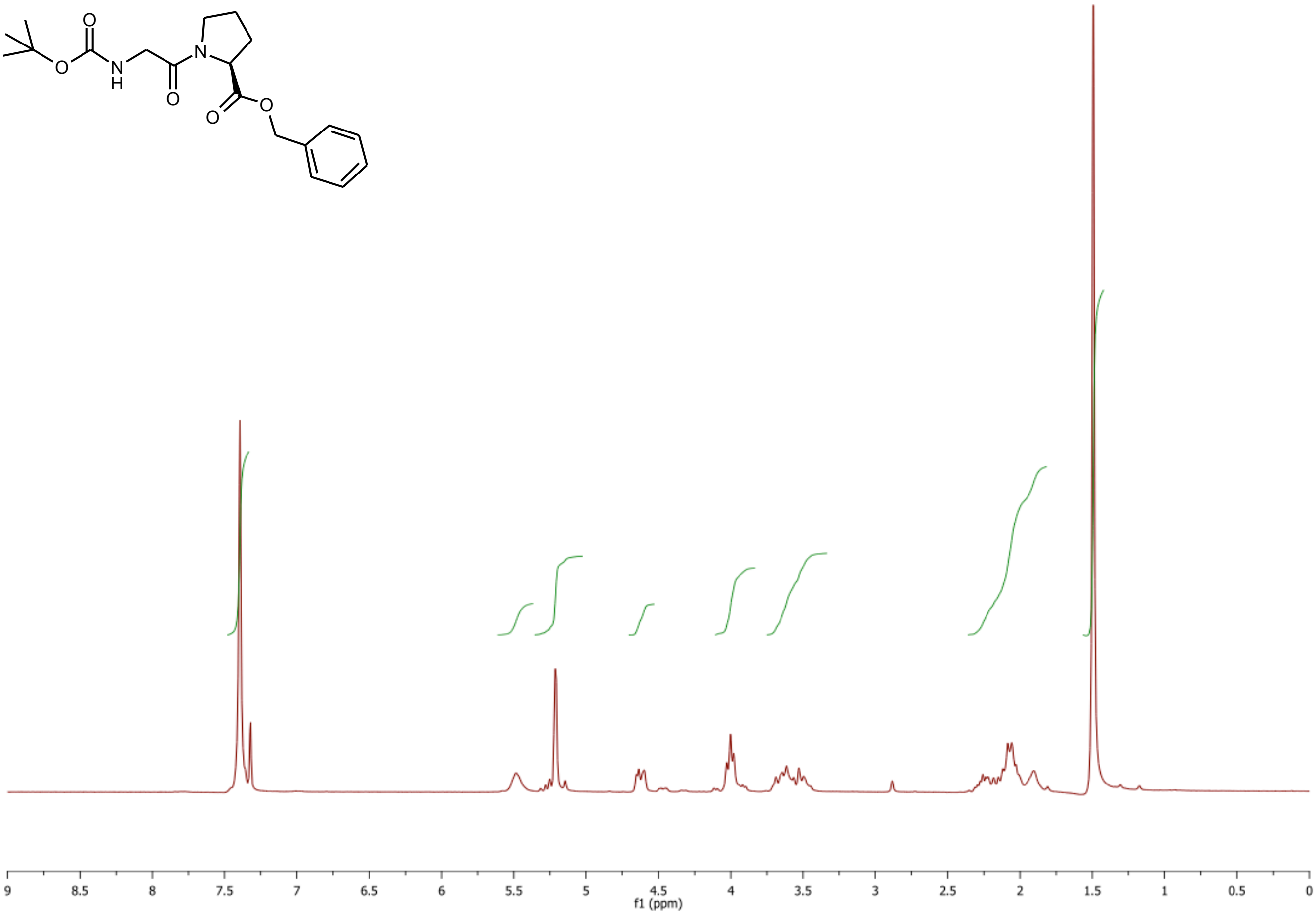
Boc-Gly-Phe-OMe

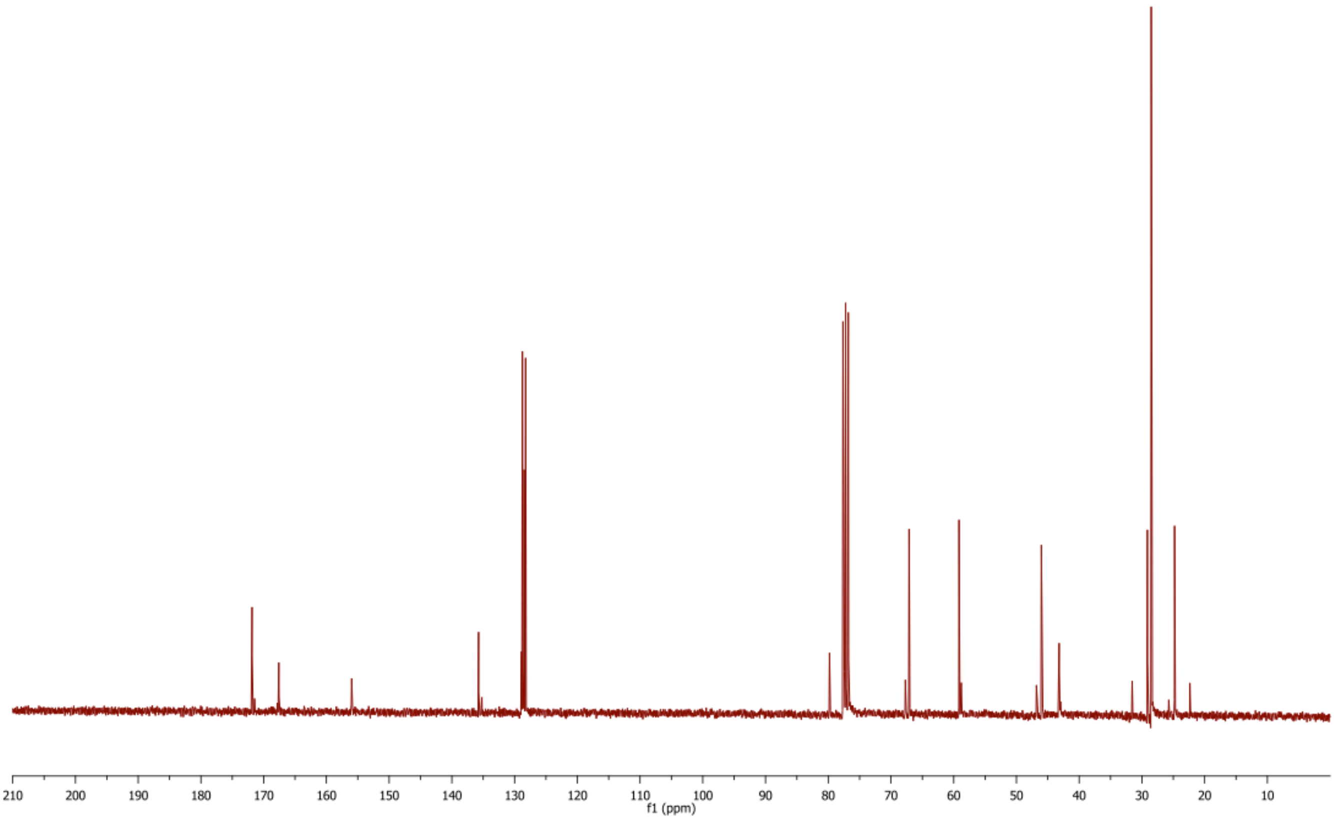




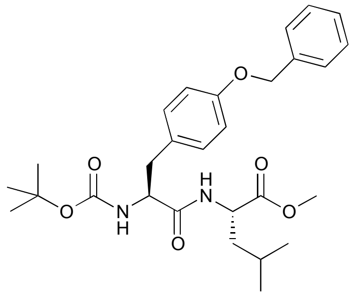


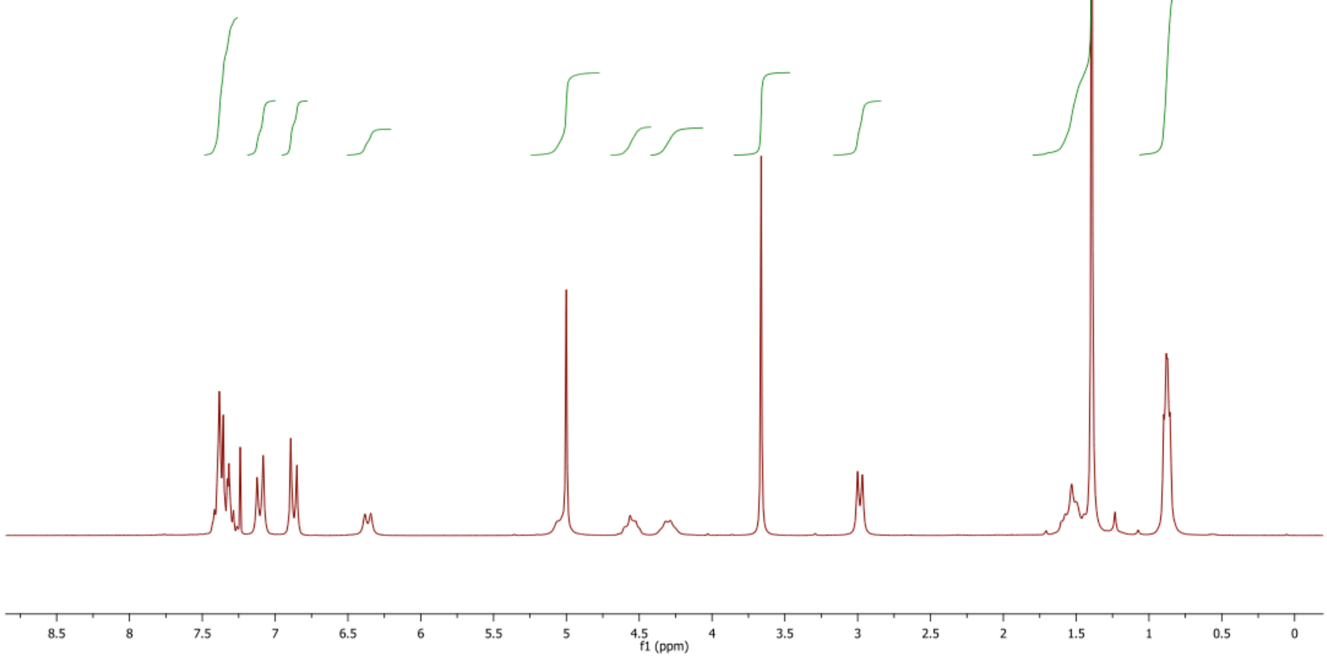
Boc-Gly-Pro-OBn

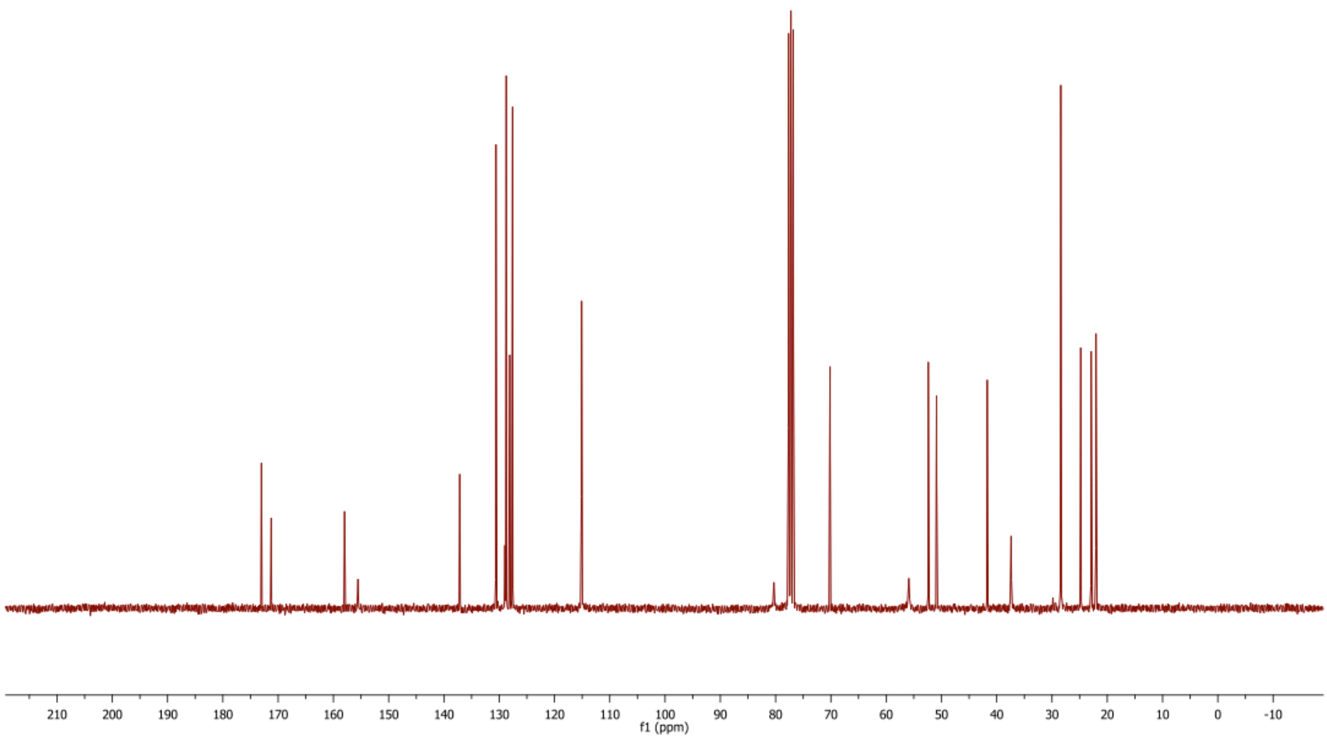




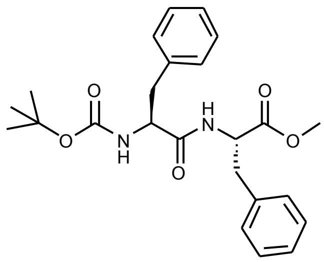
Boc-Tyr(Bn)-Leu-OMe

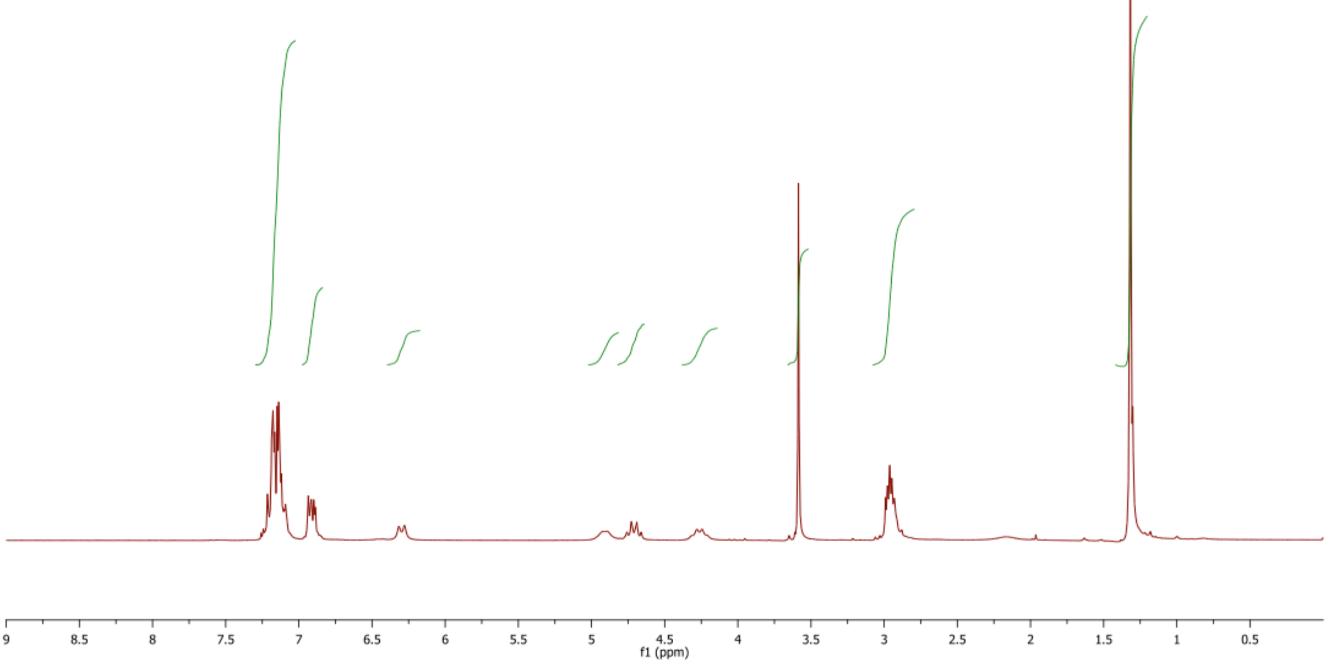


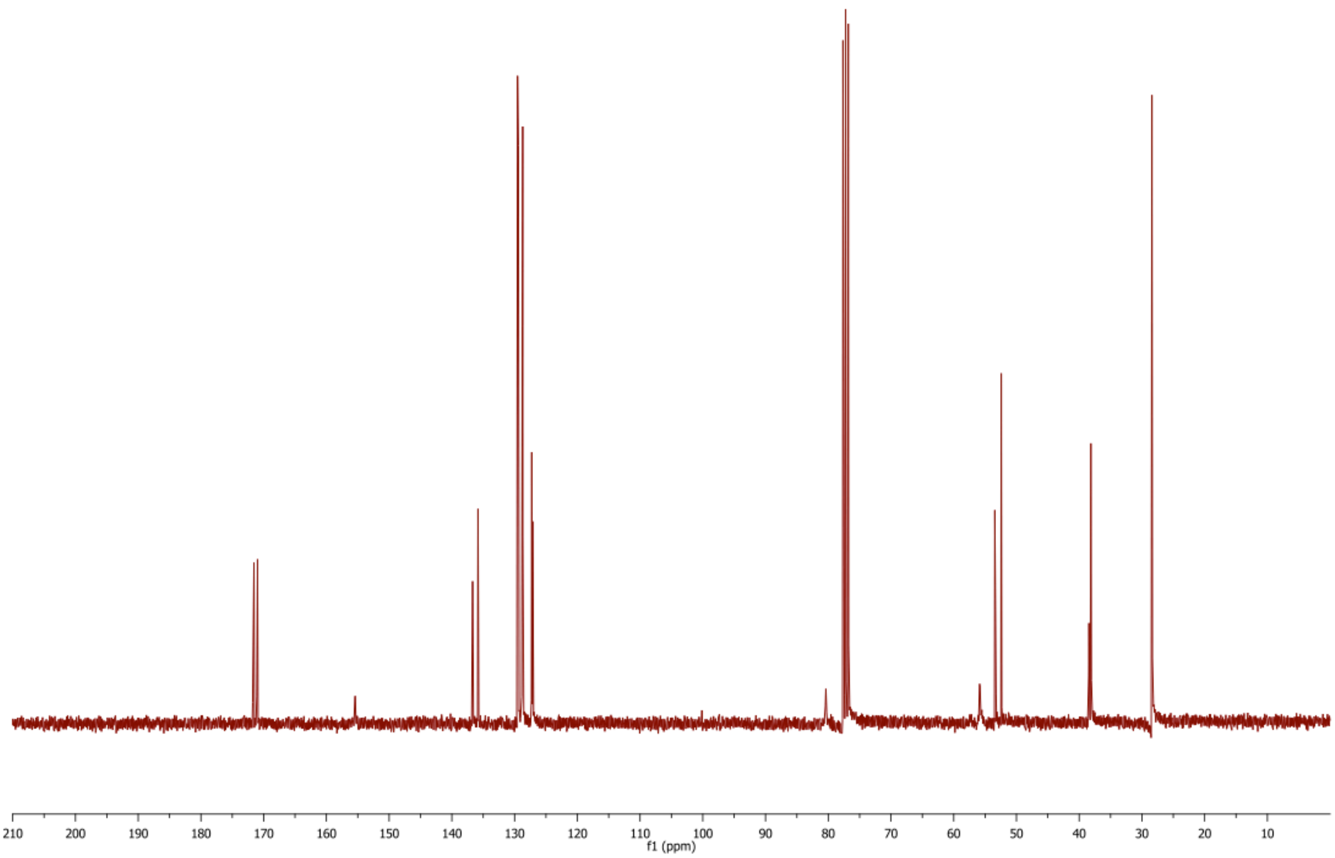




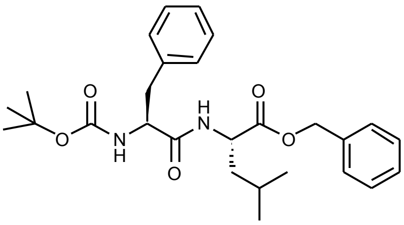
Boc-Phe-Phe-OMe

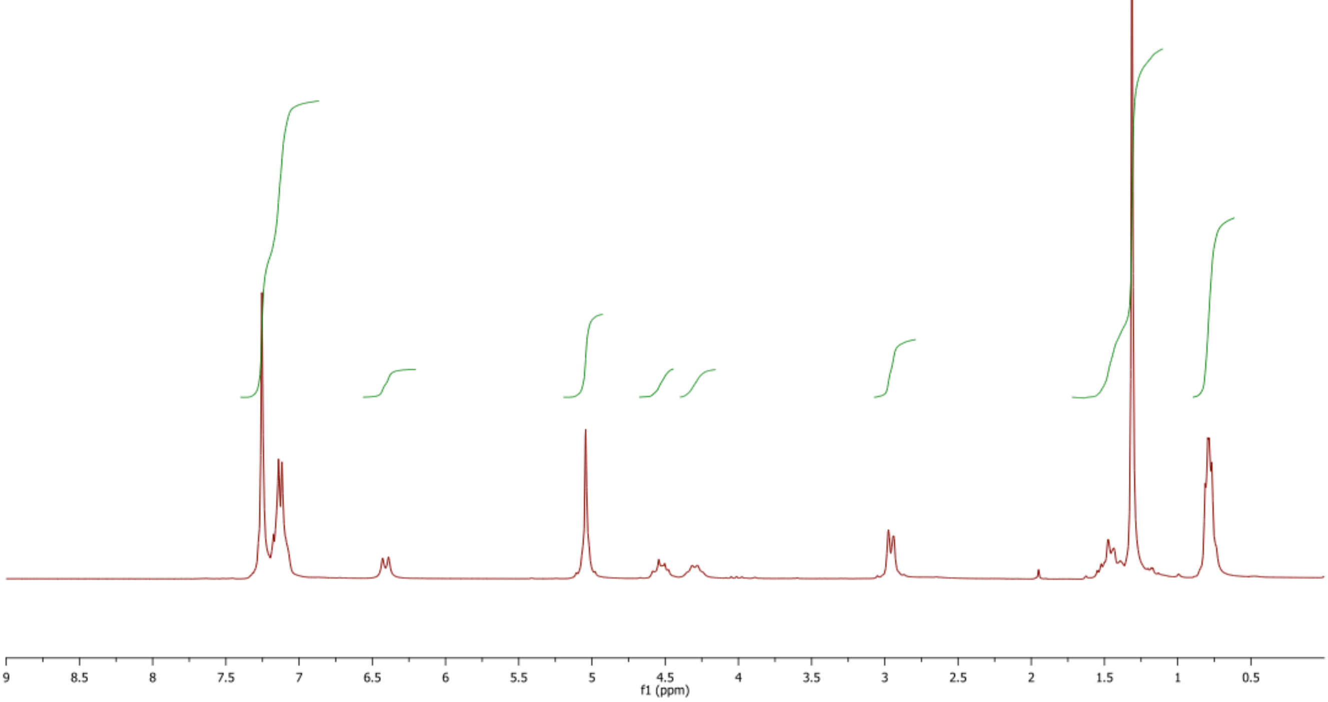


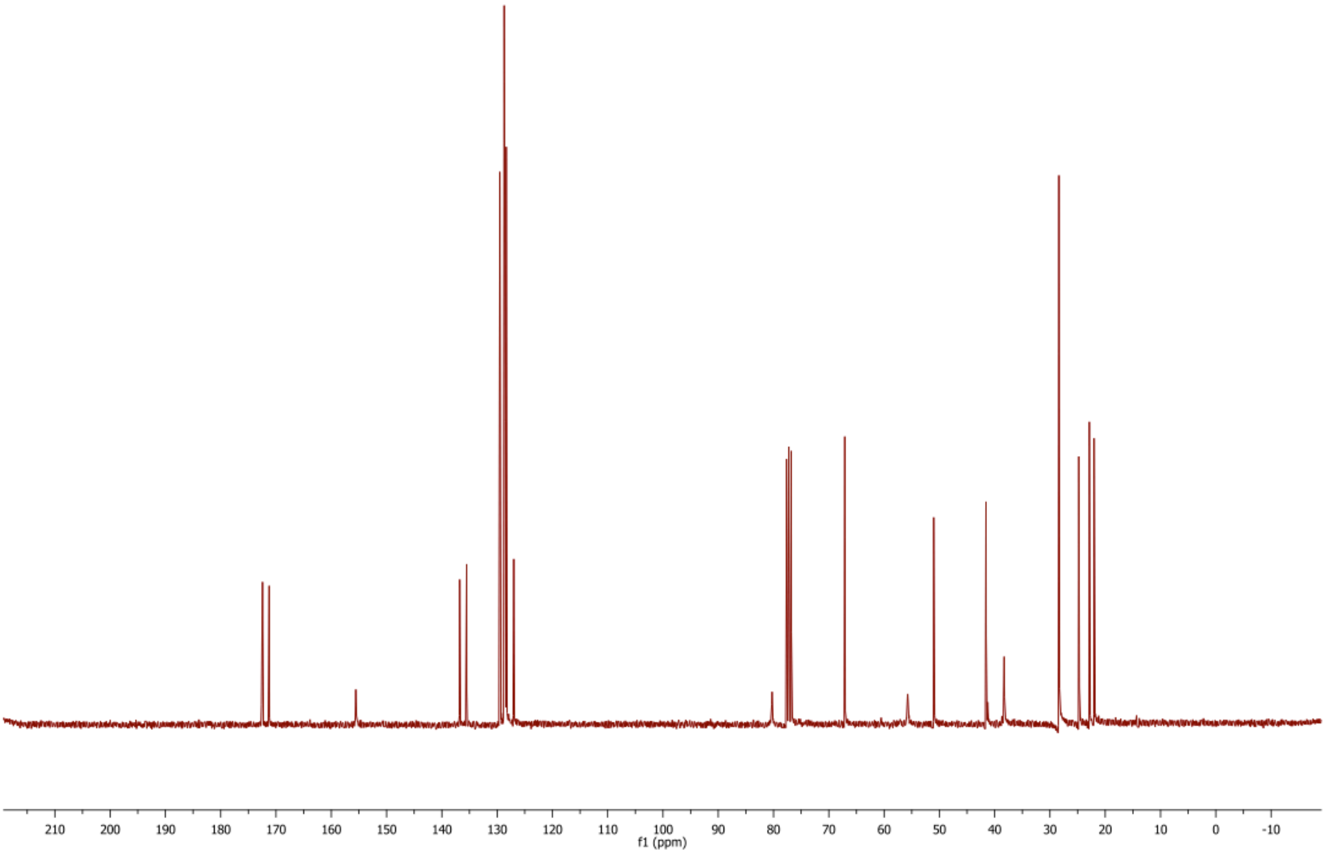




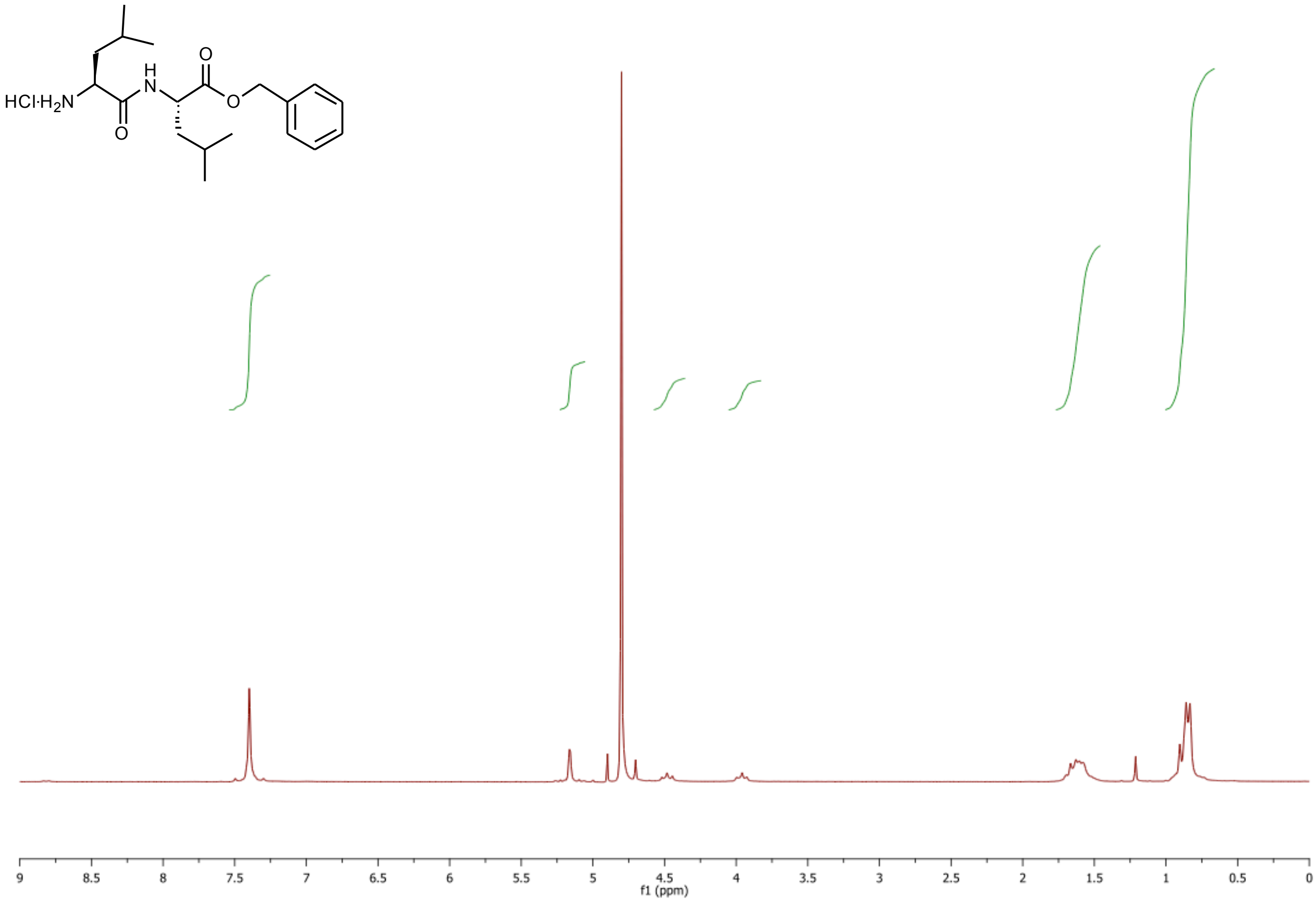
Boc-Phe-Leu-OBn

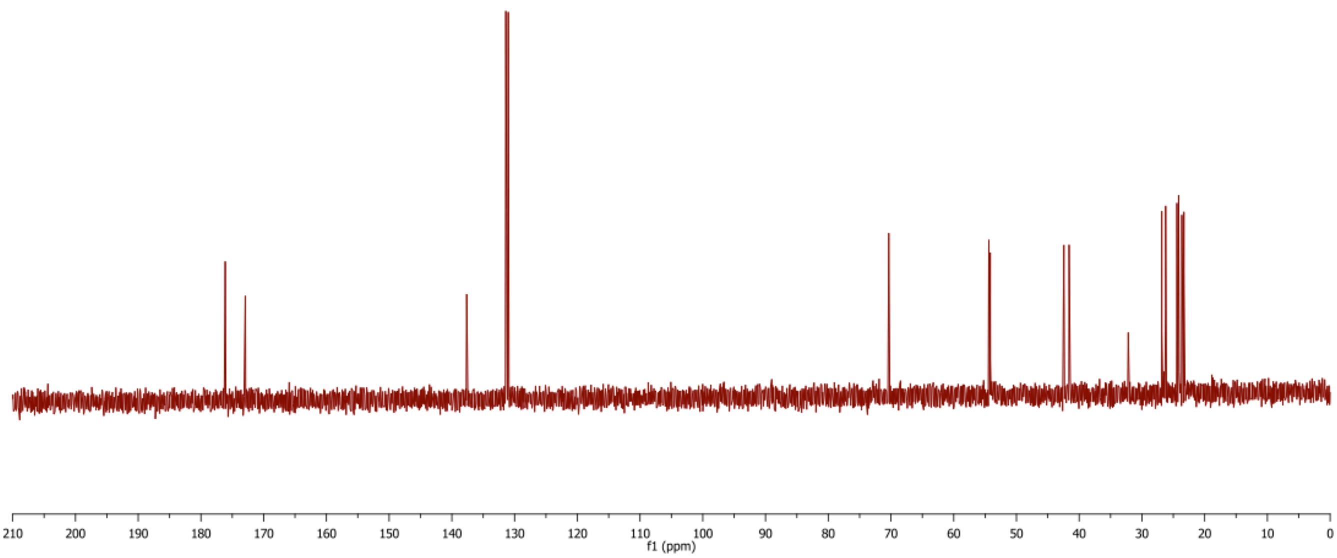




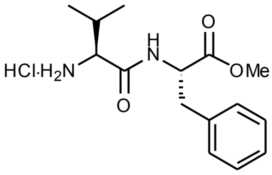


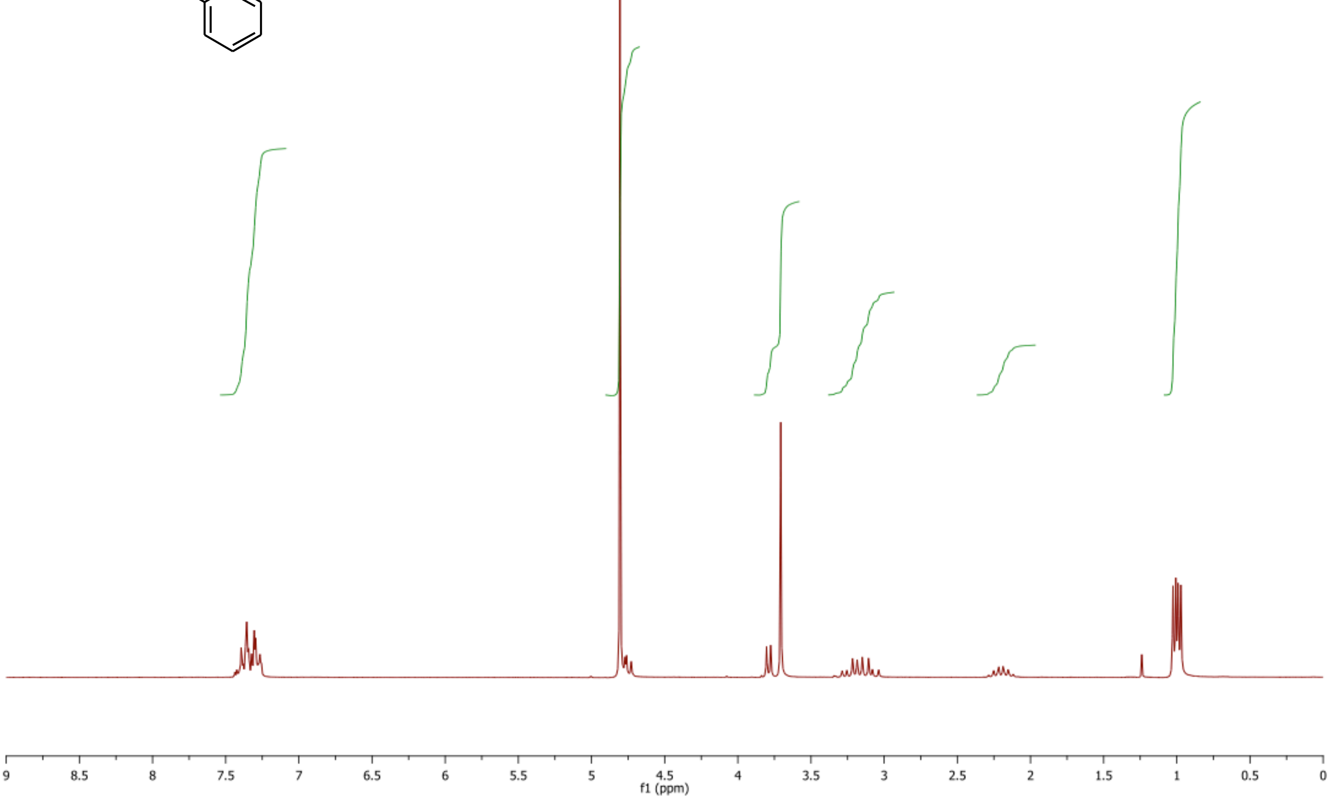
HCl·H-Leu-Leu-OBn

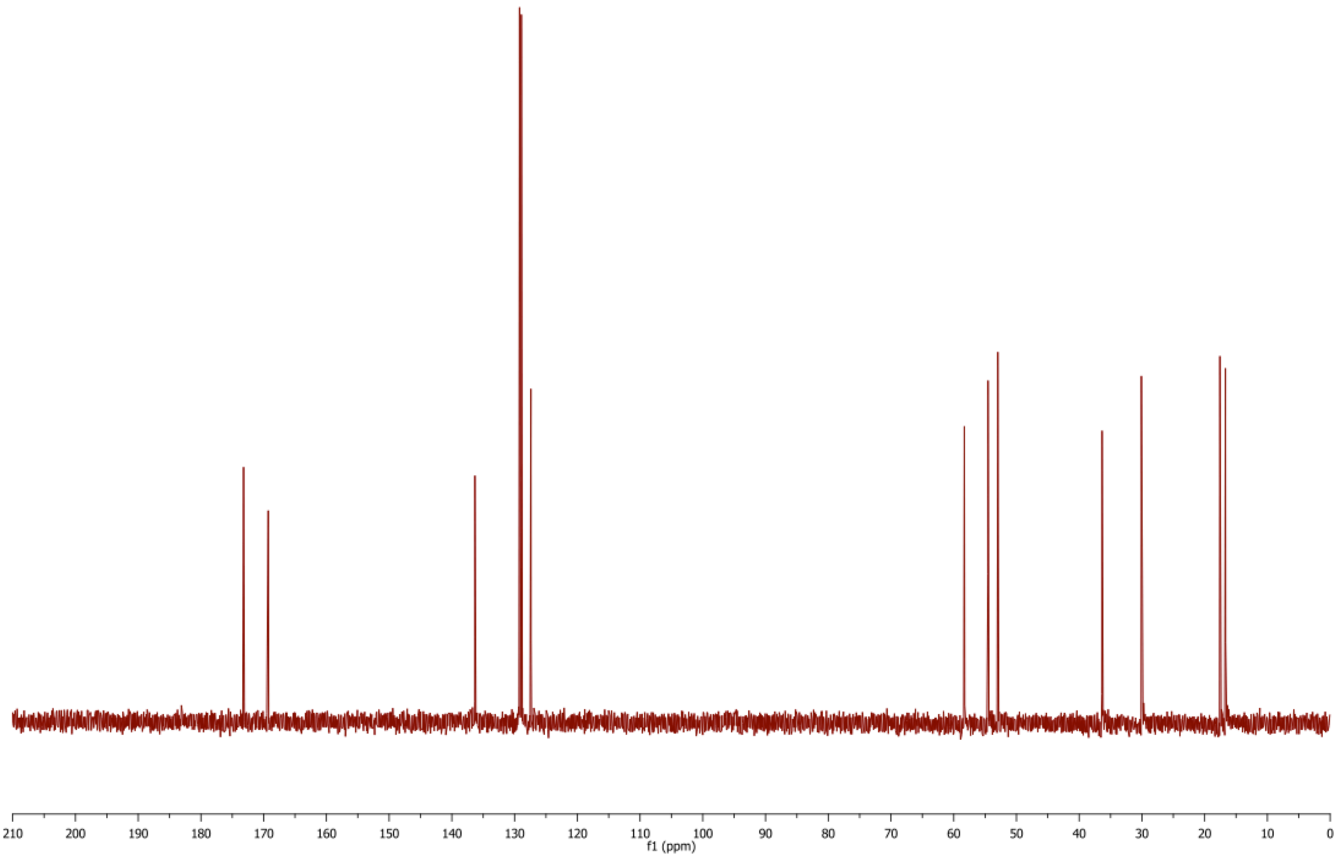




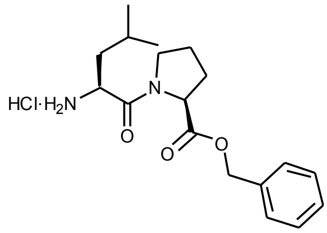
HCl·H-Val-Phe-OMe

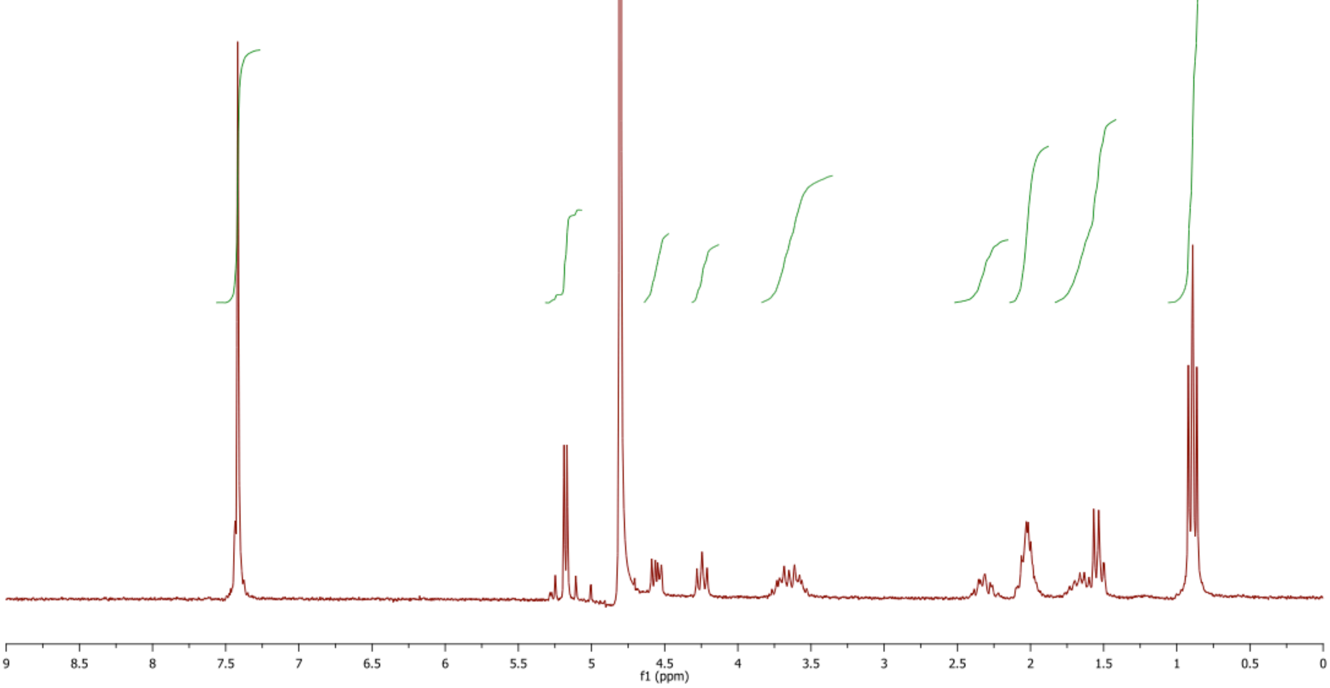


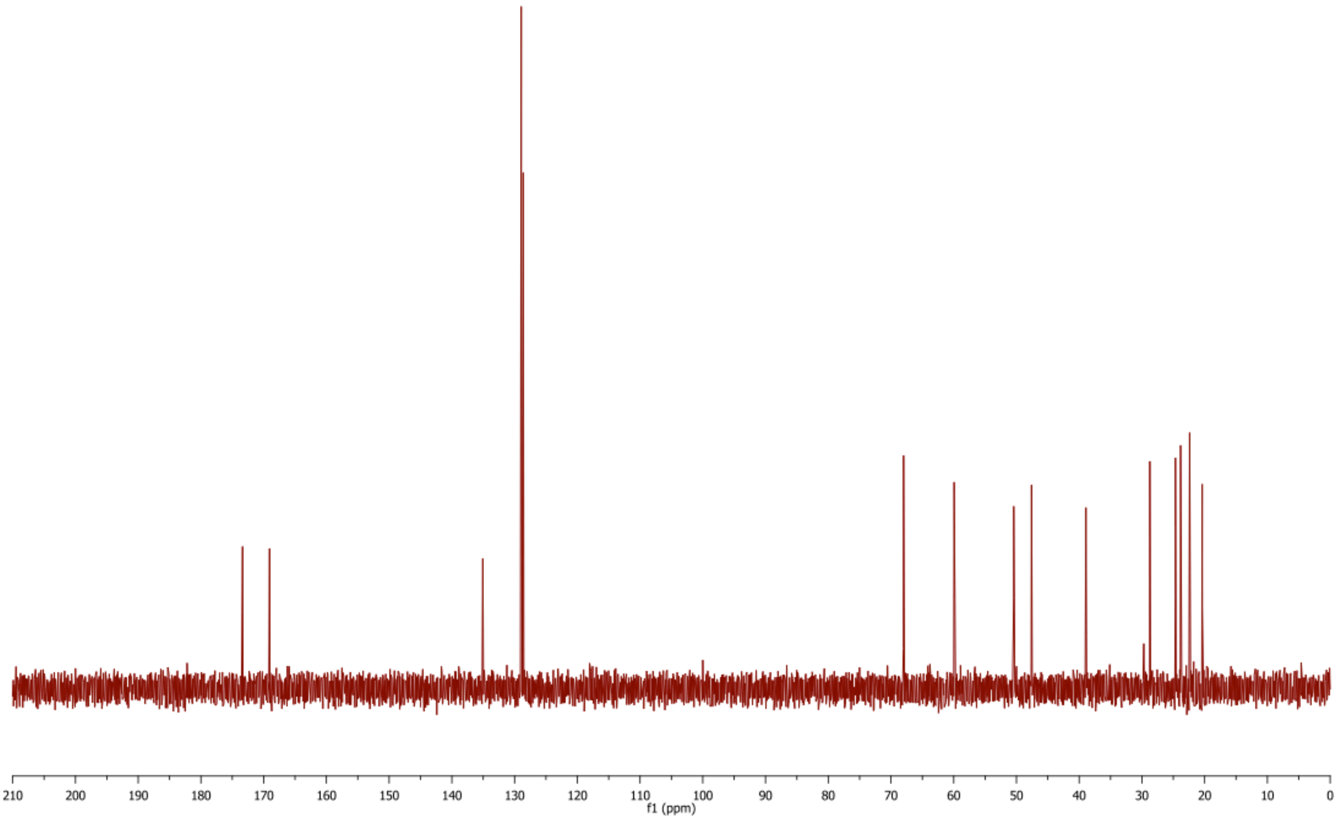




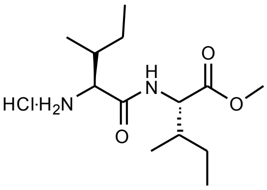
HCl·H-Leu-Pro-OBn

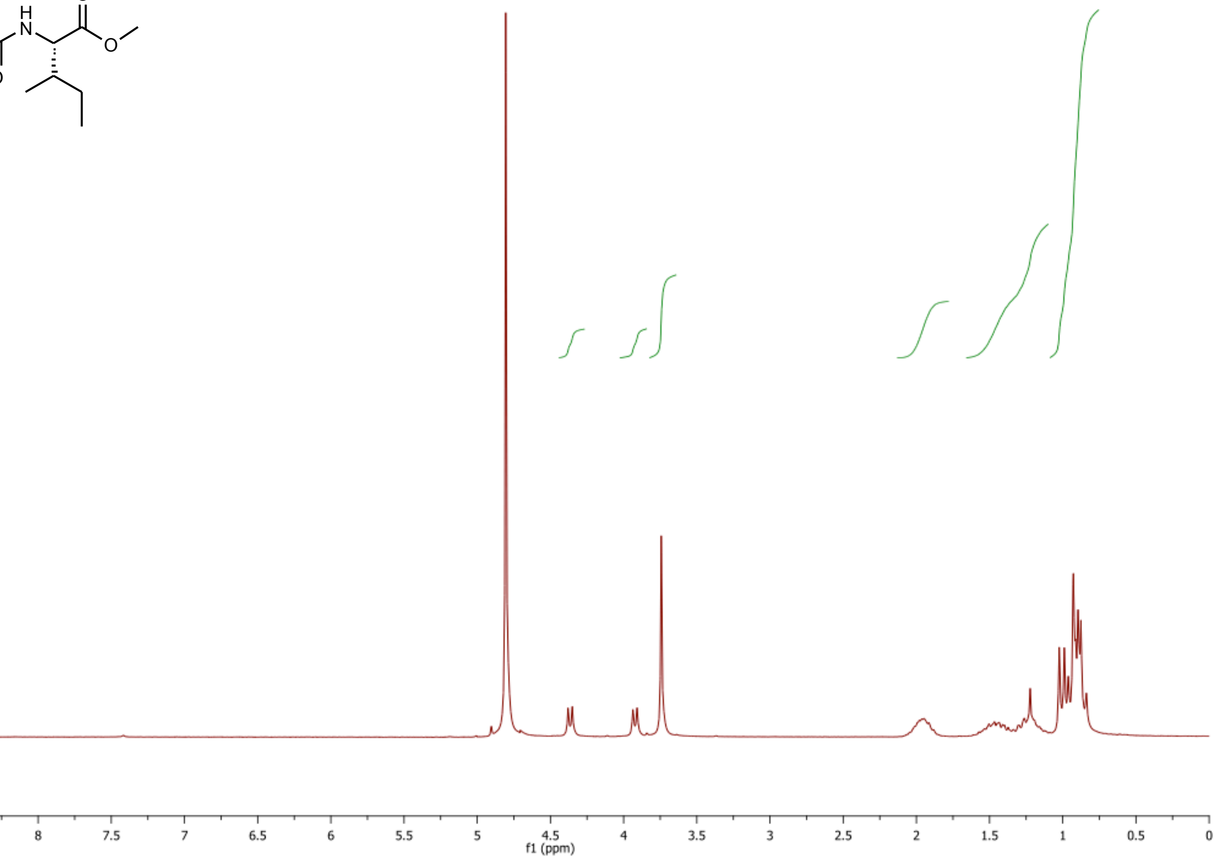


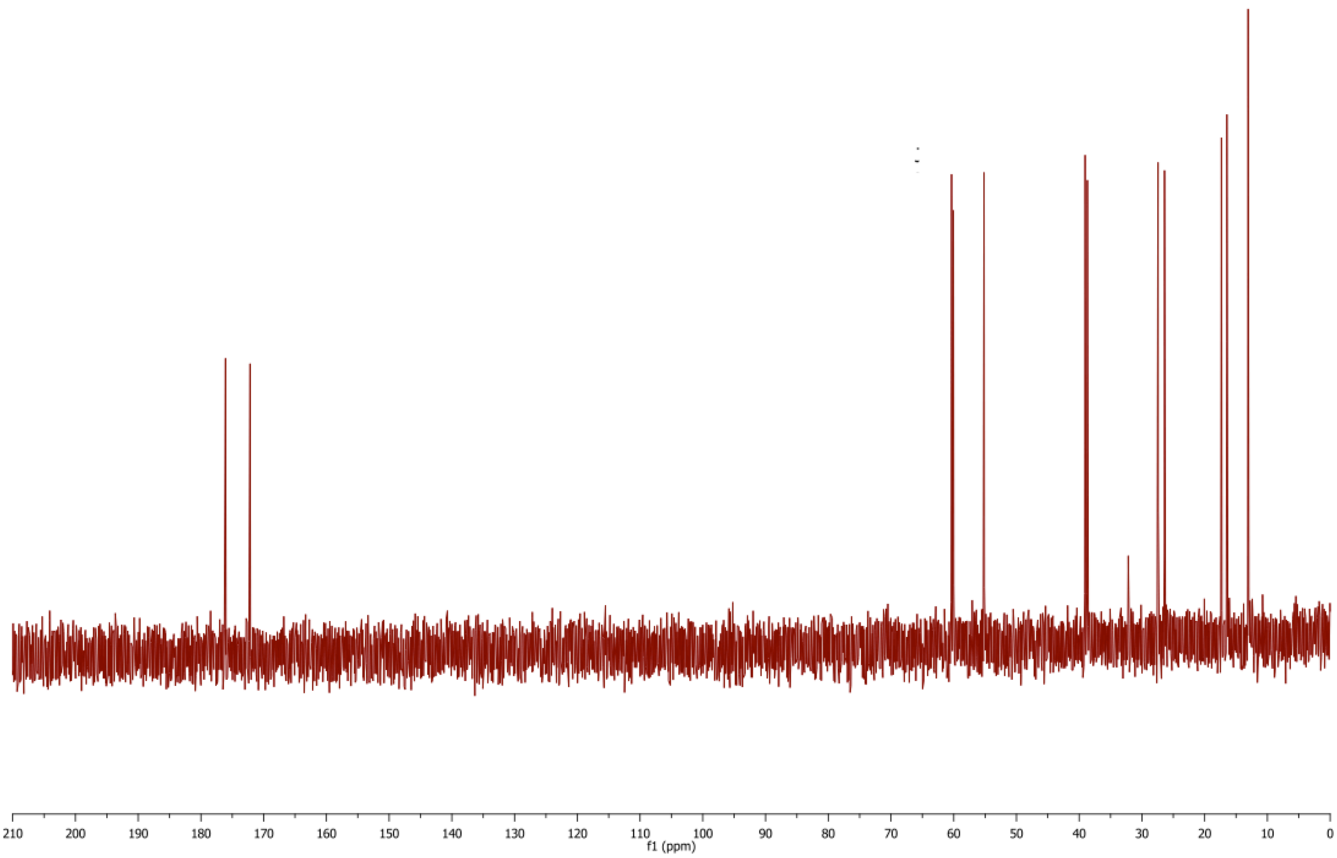




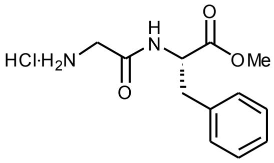
HCl·H-Ile-Ile-OMe

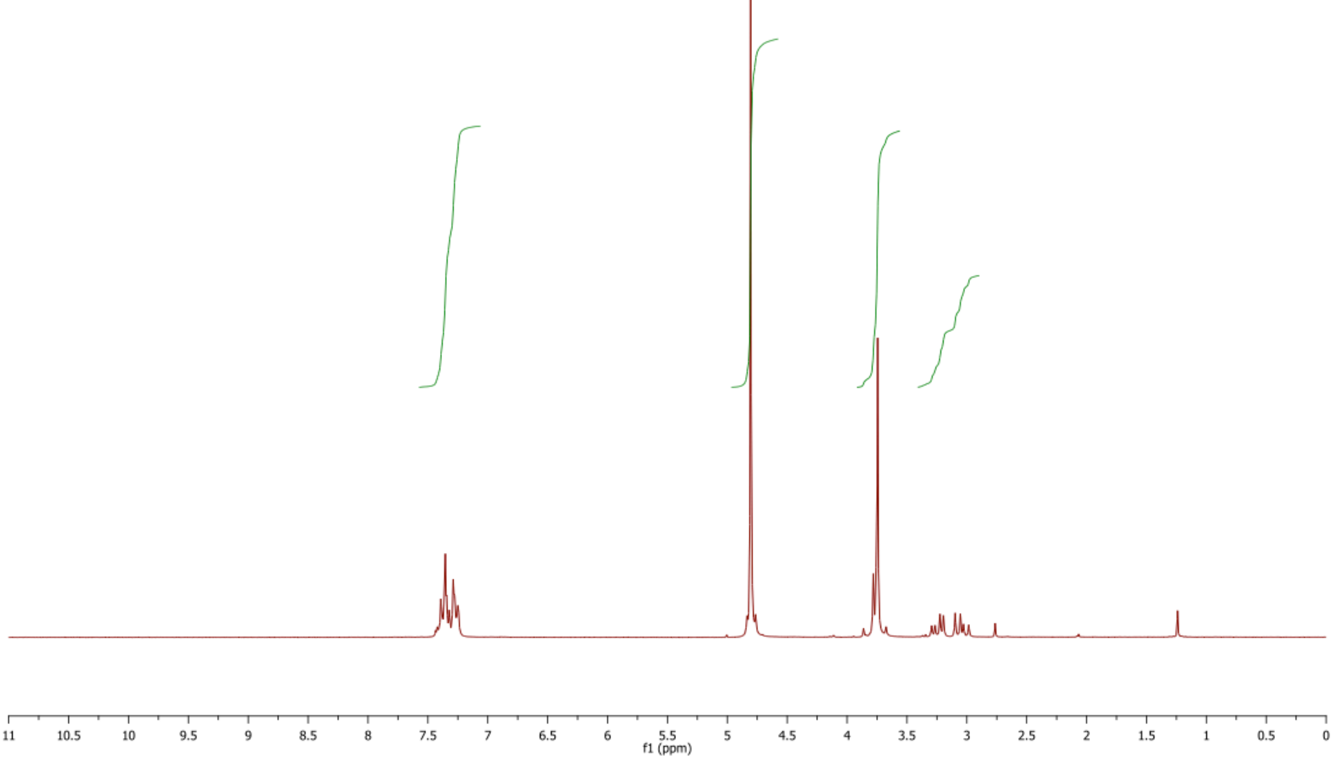


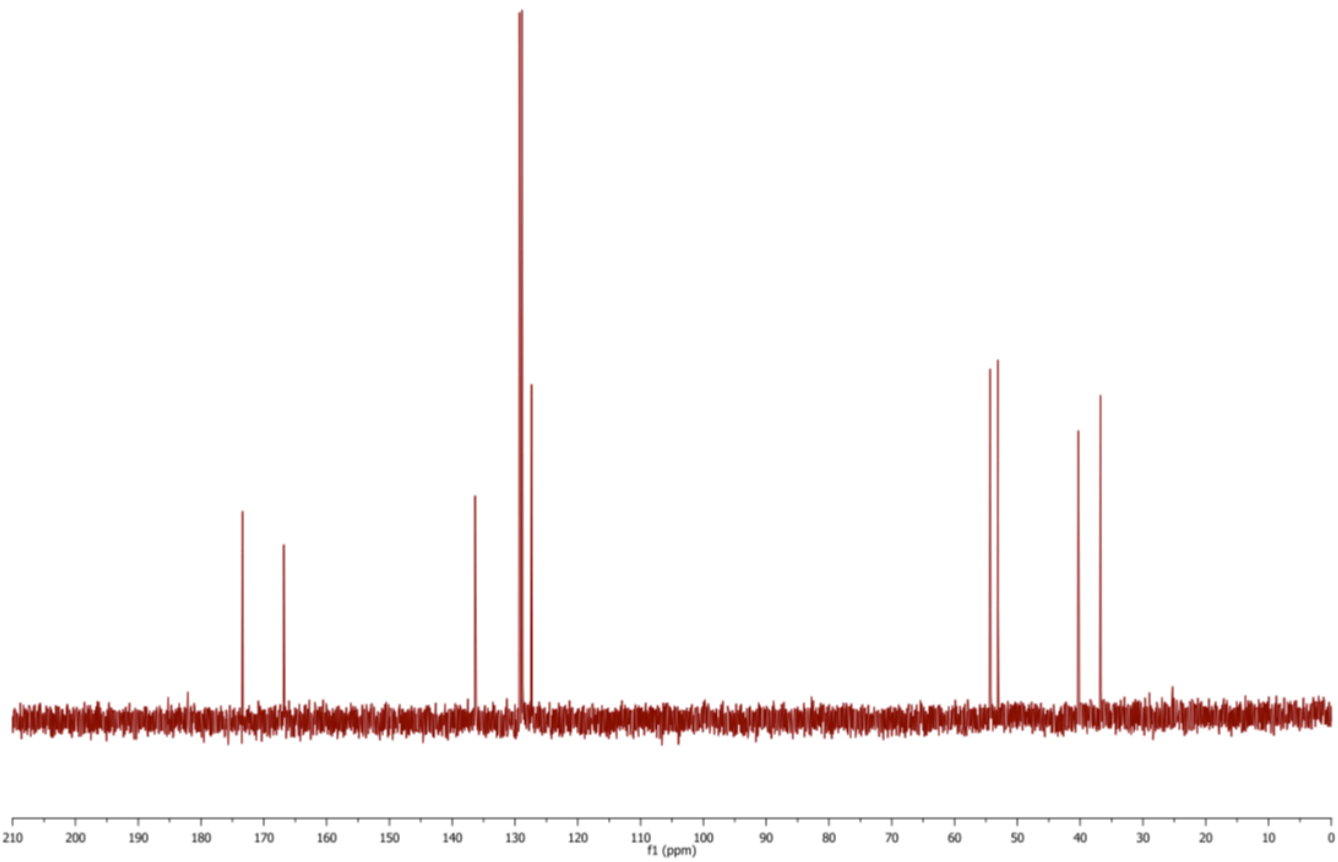




HCl·H-Gly-Phe-OMe

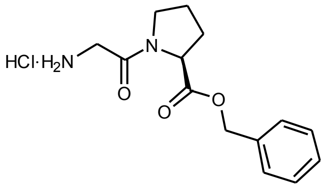


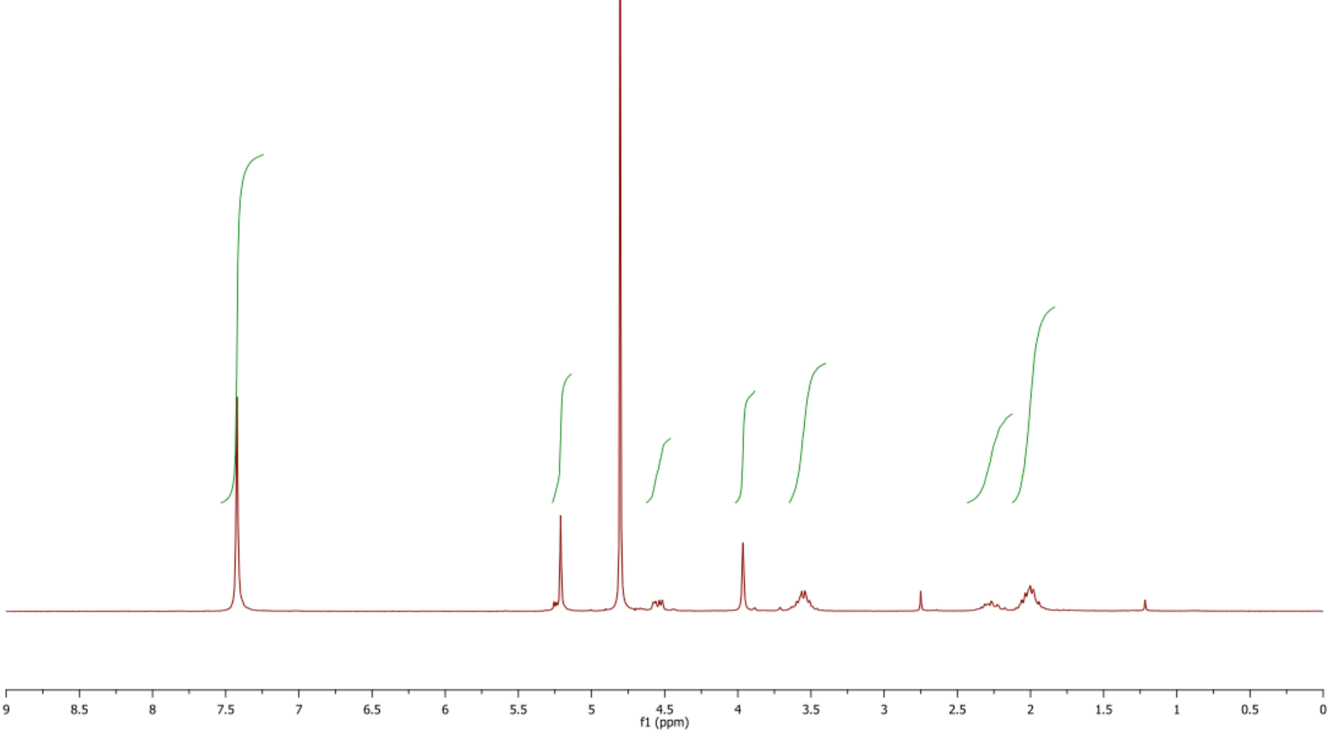


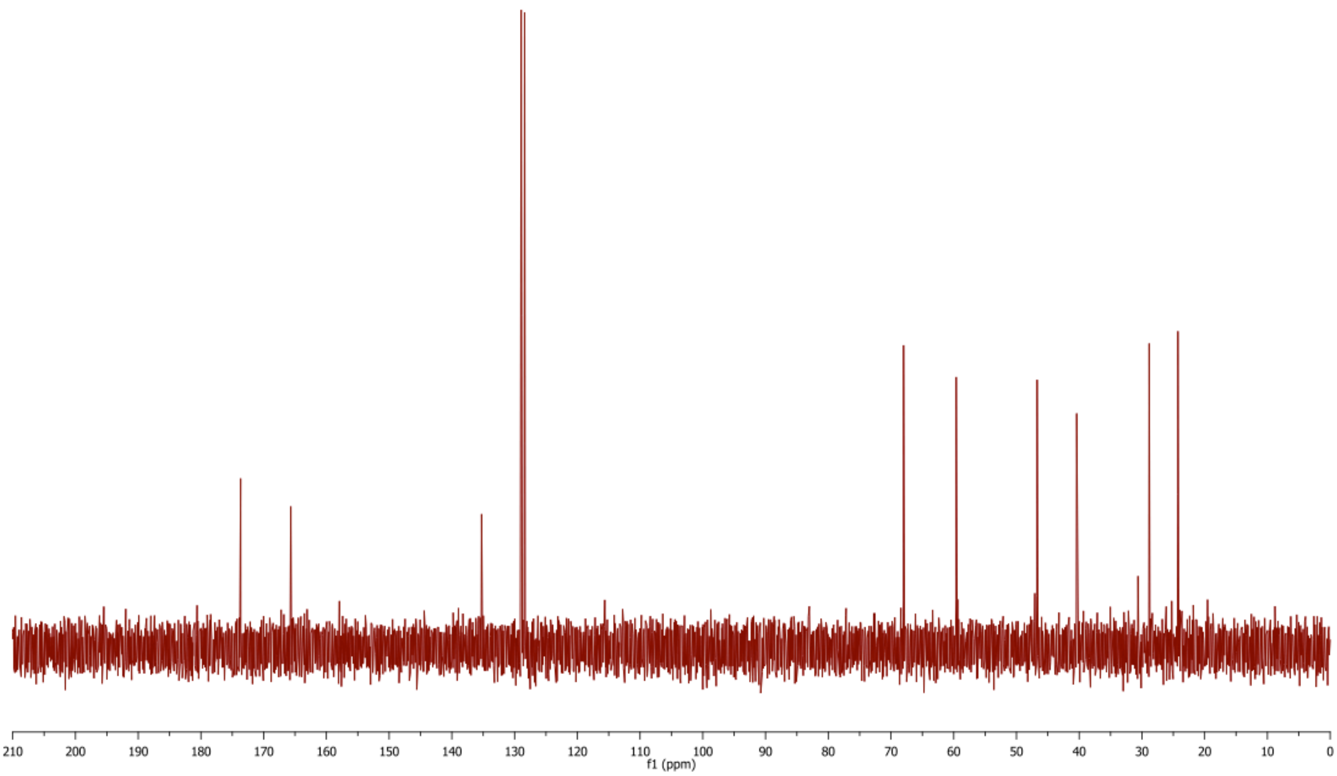


Electronic Supplementary Material (ESI) for Green Chemistry

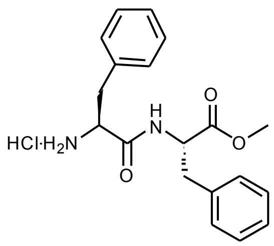
HCl·H-Gly-Pro-OBn

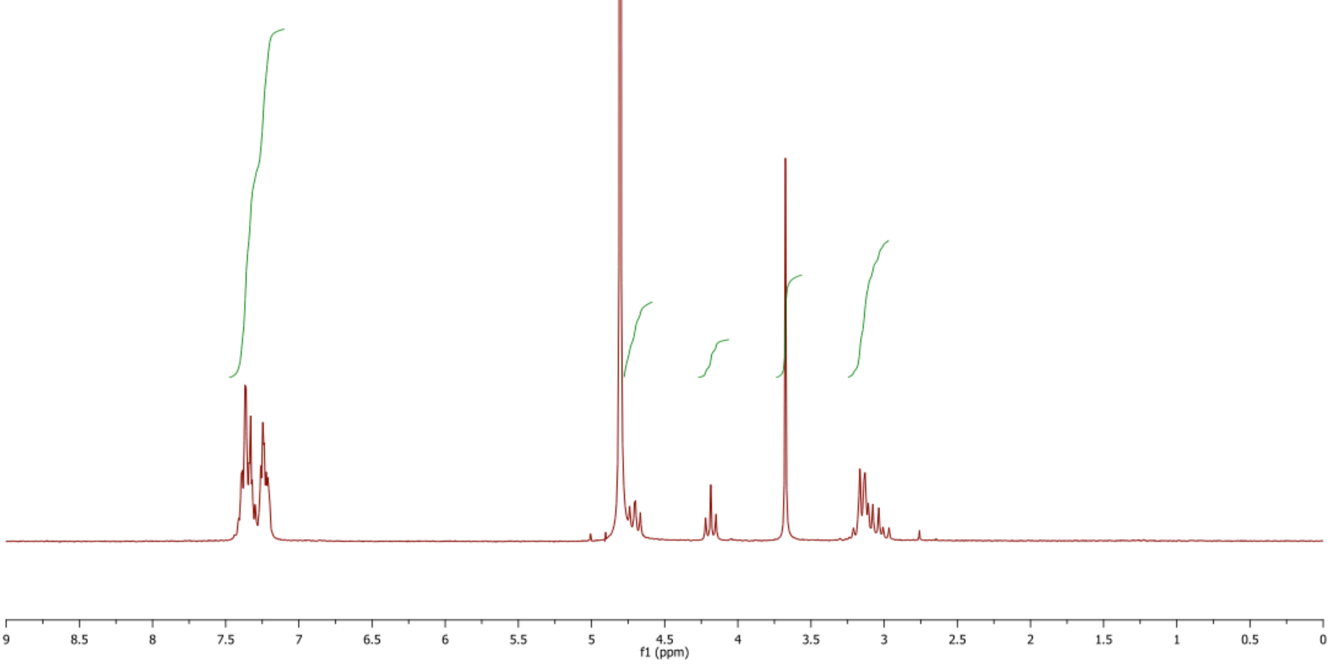


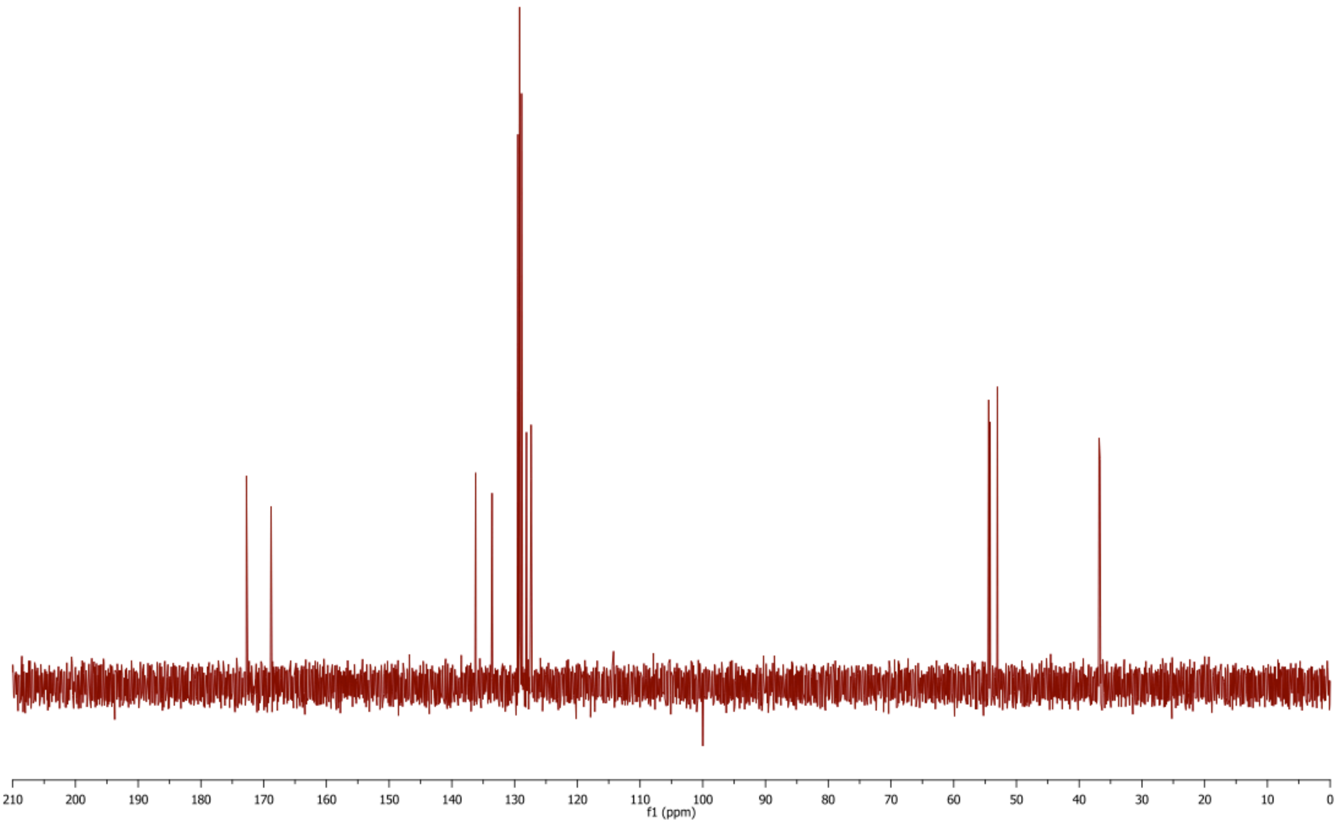




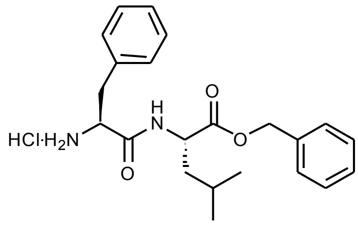
HCl·H-Phe-Phe-OMe

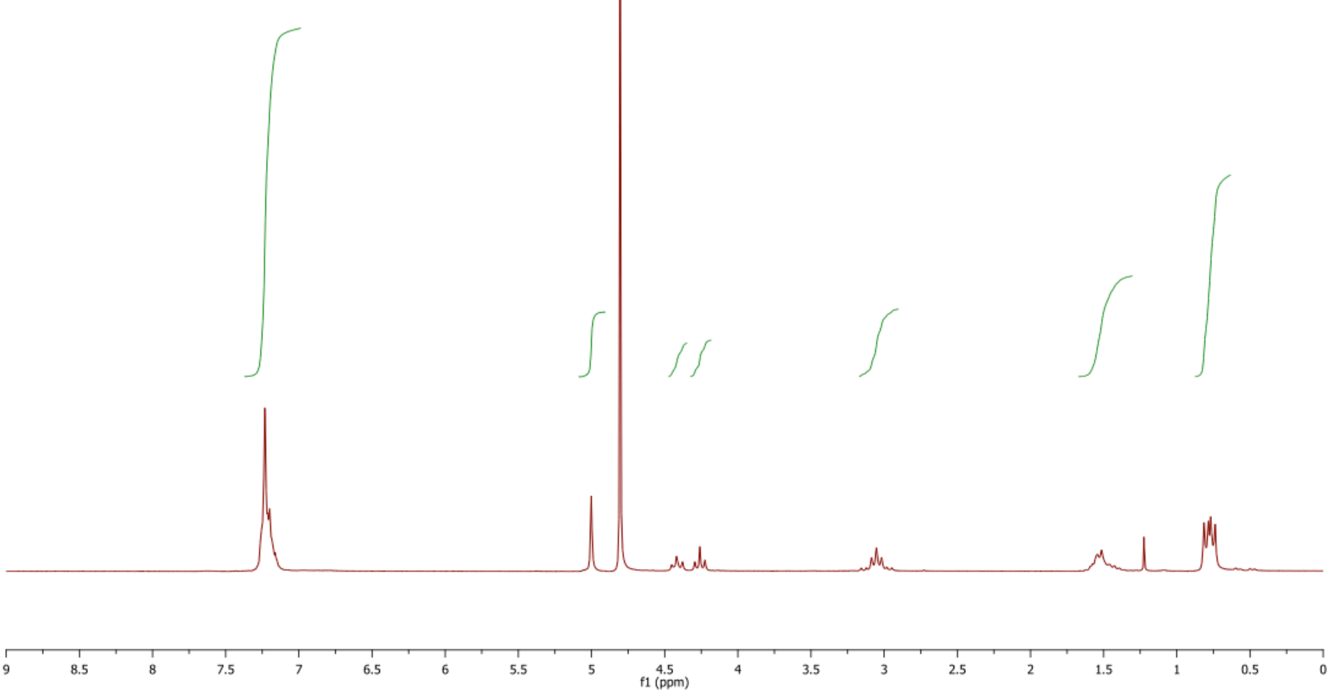


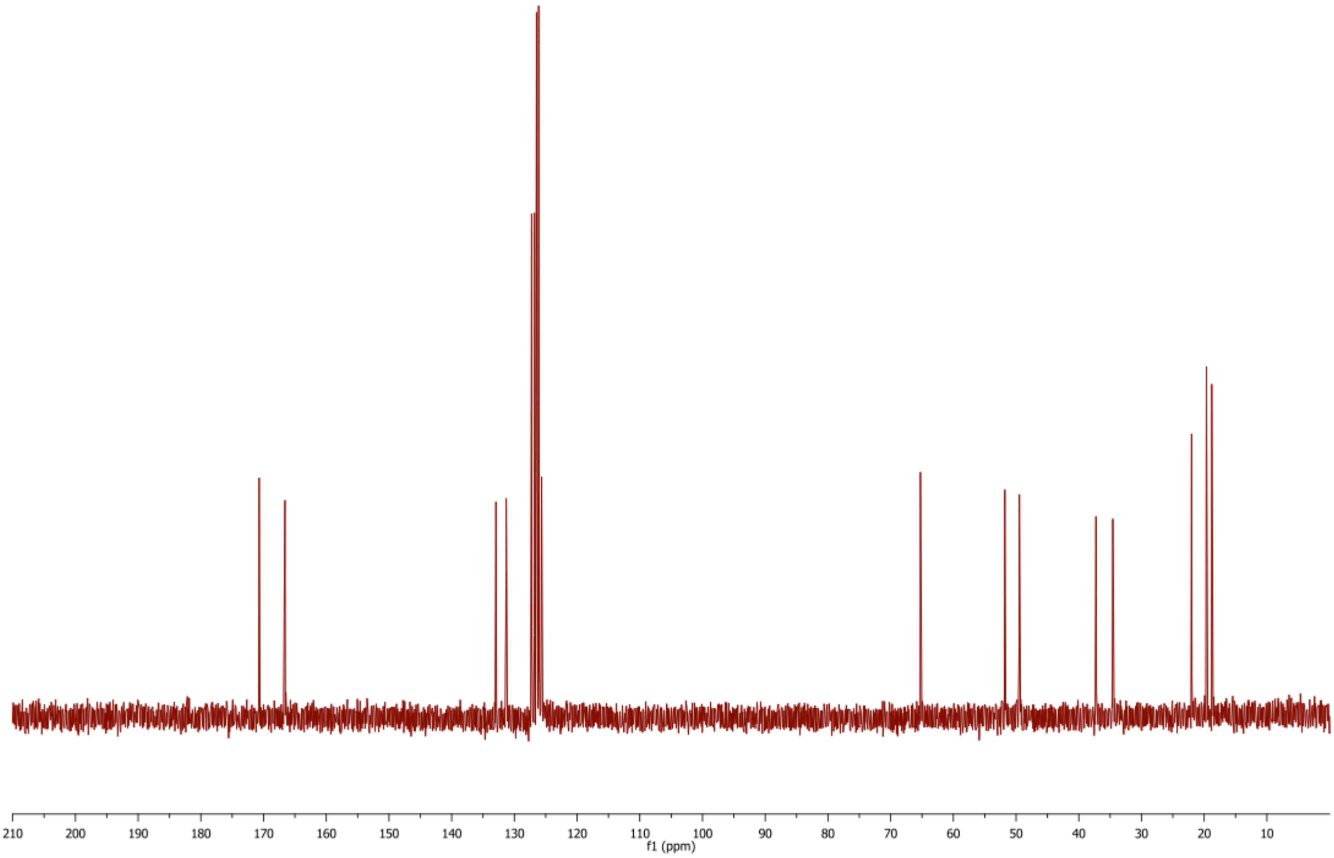




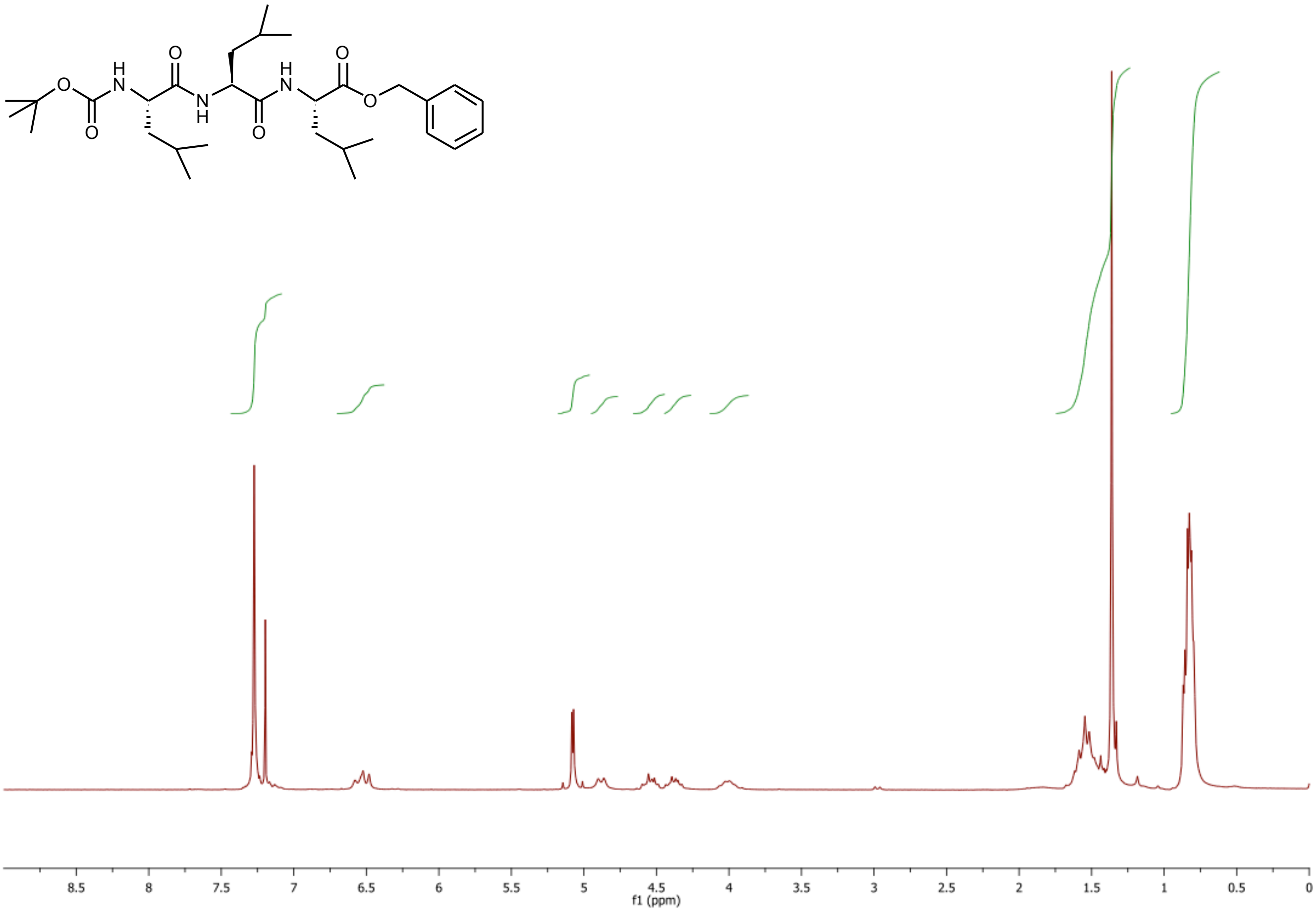
HCl·H-Phe-Leu-OBn

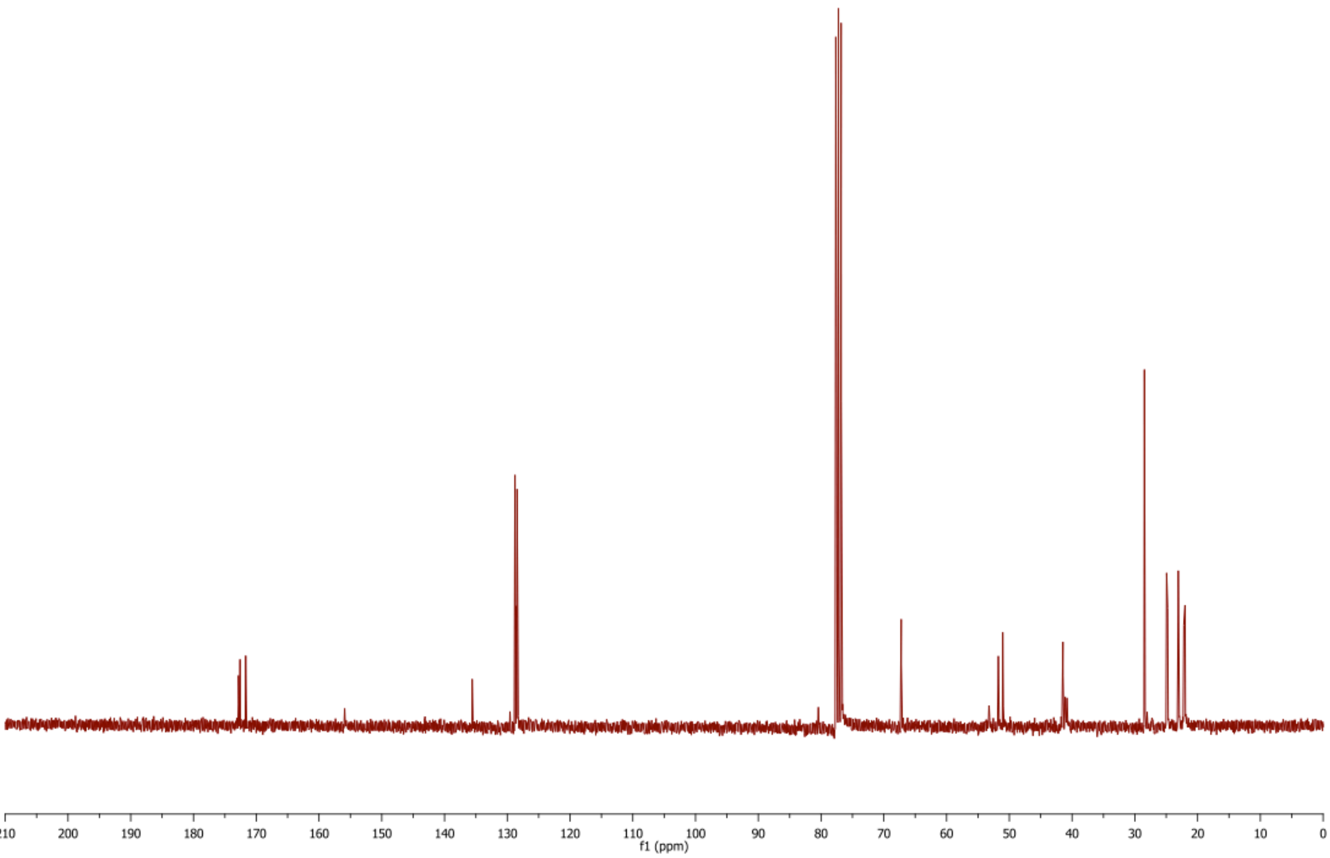




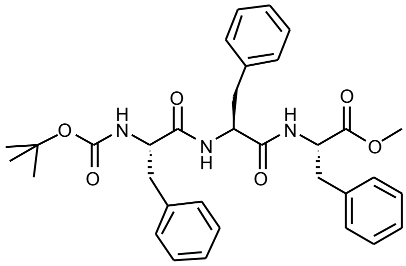


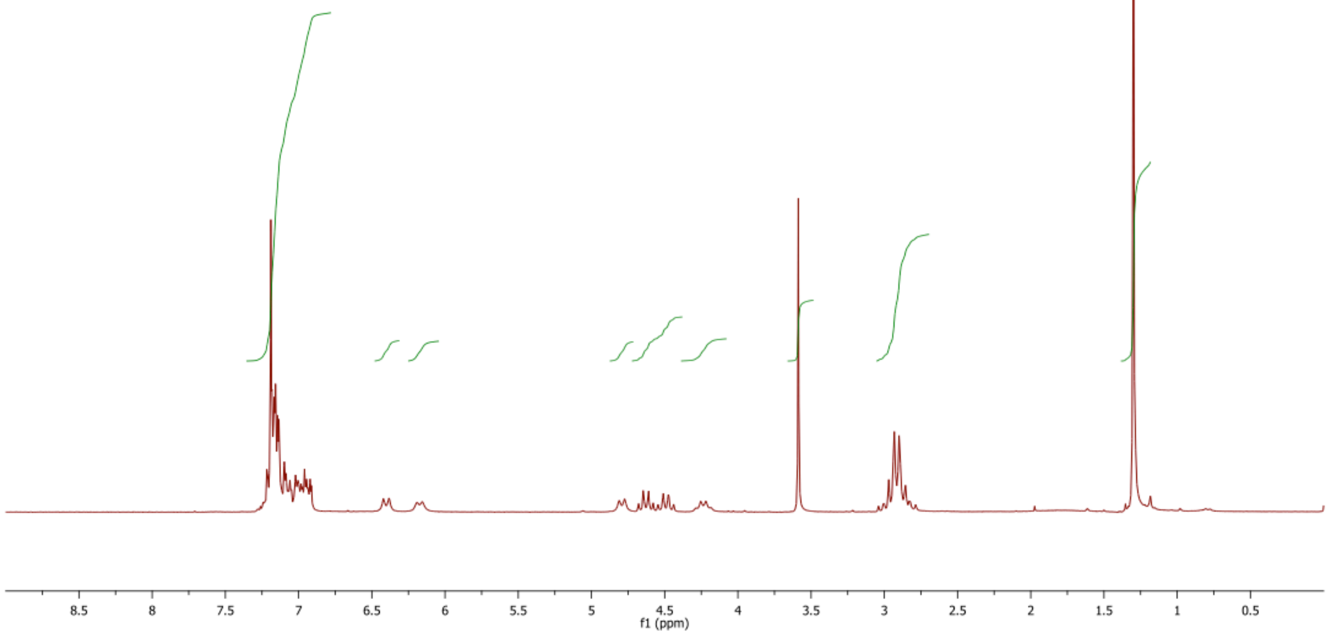
Boc-Leu-Leu-Leu-OBn

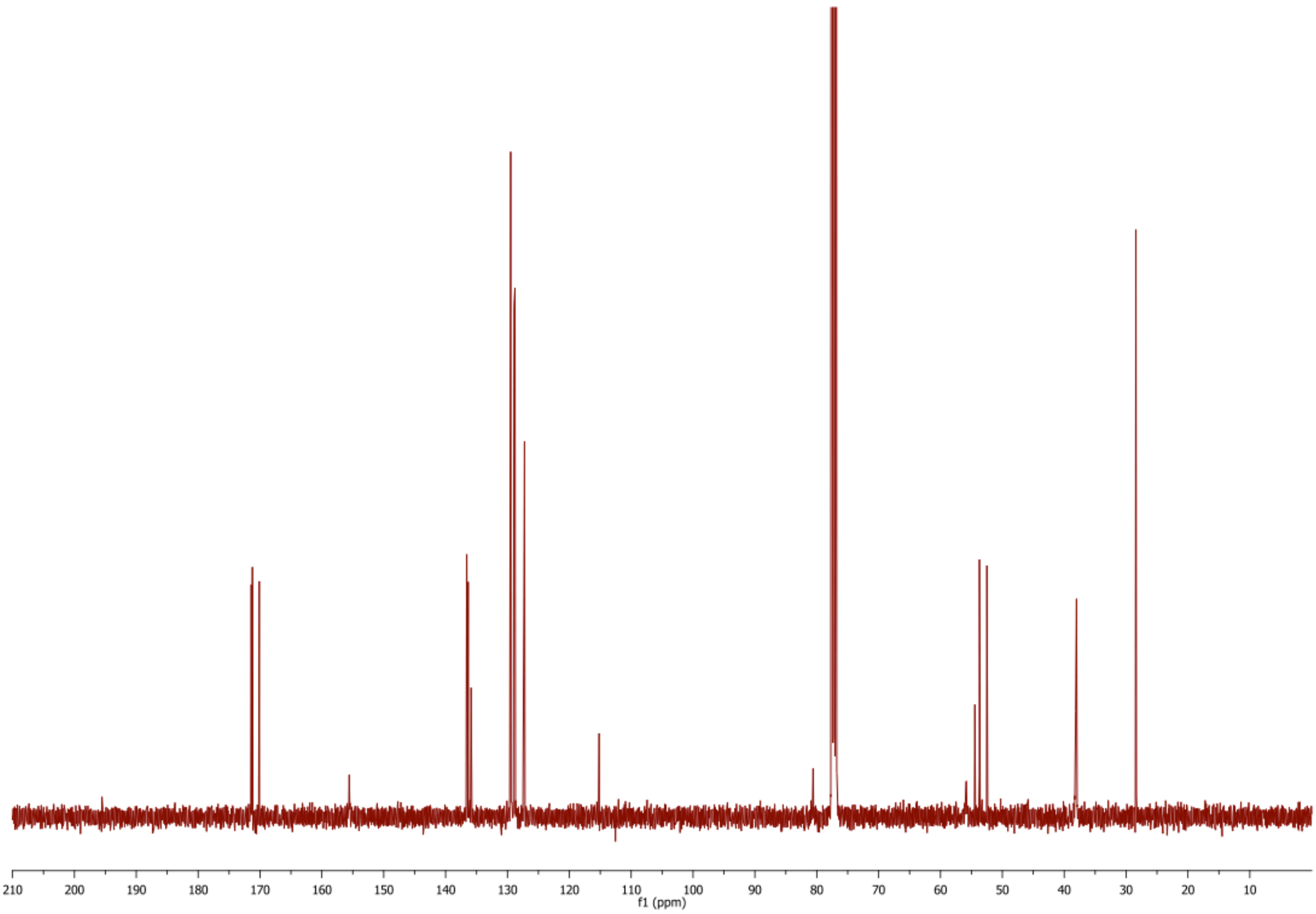




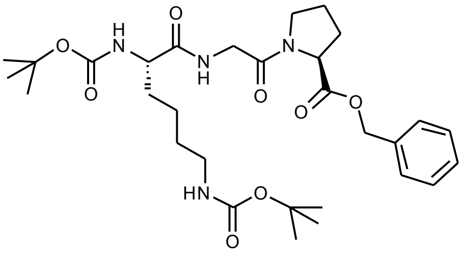
Boc-Phe-Phe-Phe-OMe

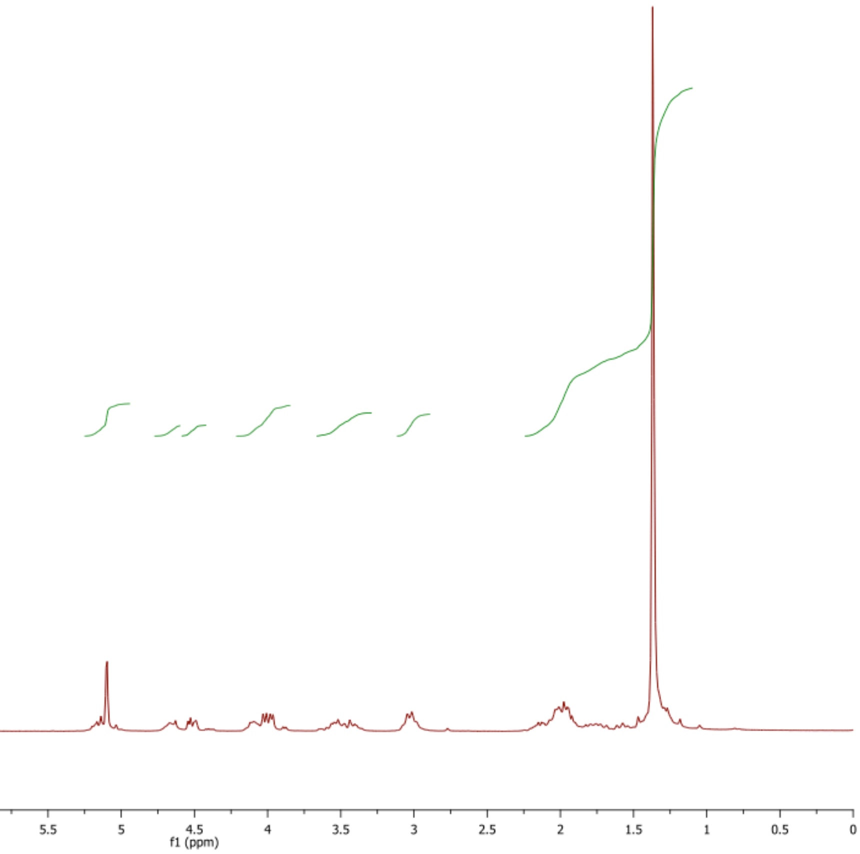


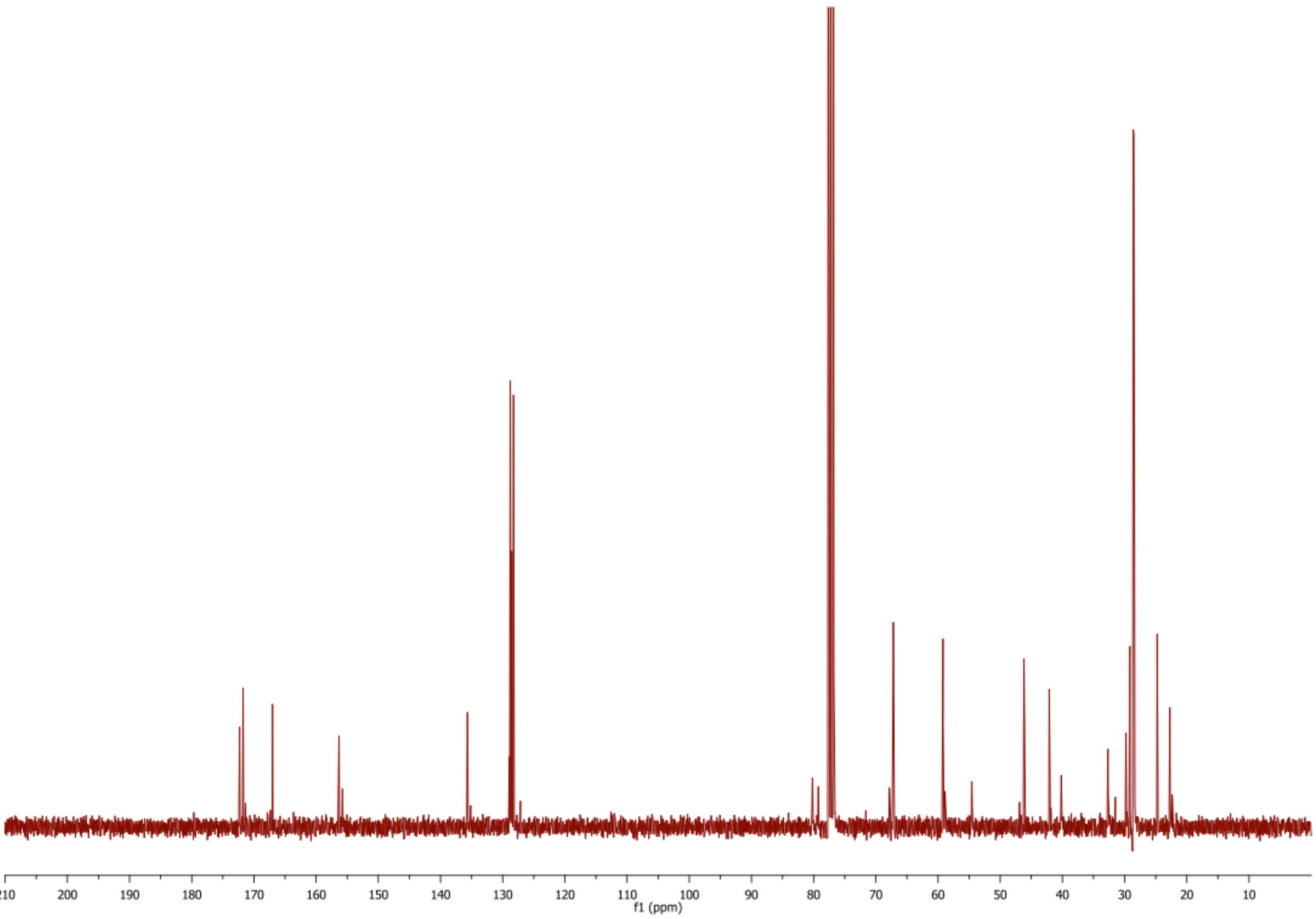




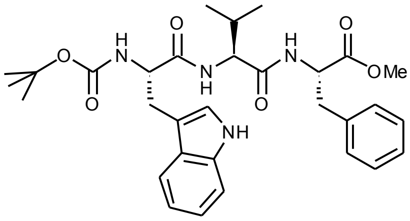
Boc-Lys(Boc)-Gly-Pro-OBn

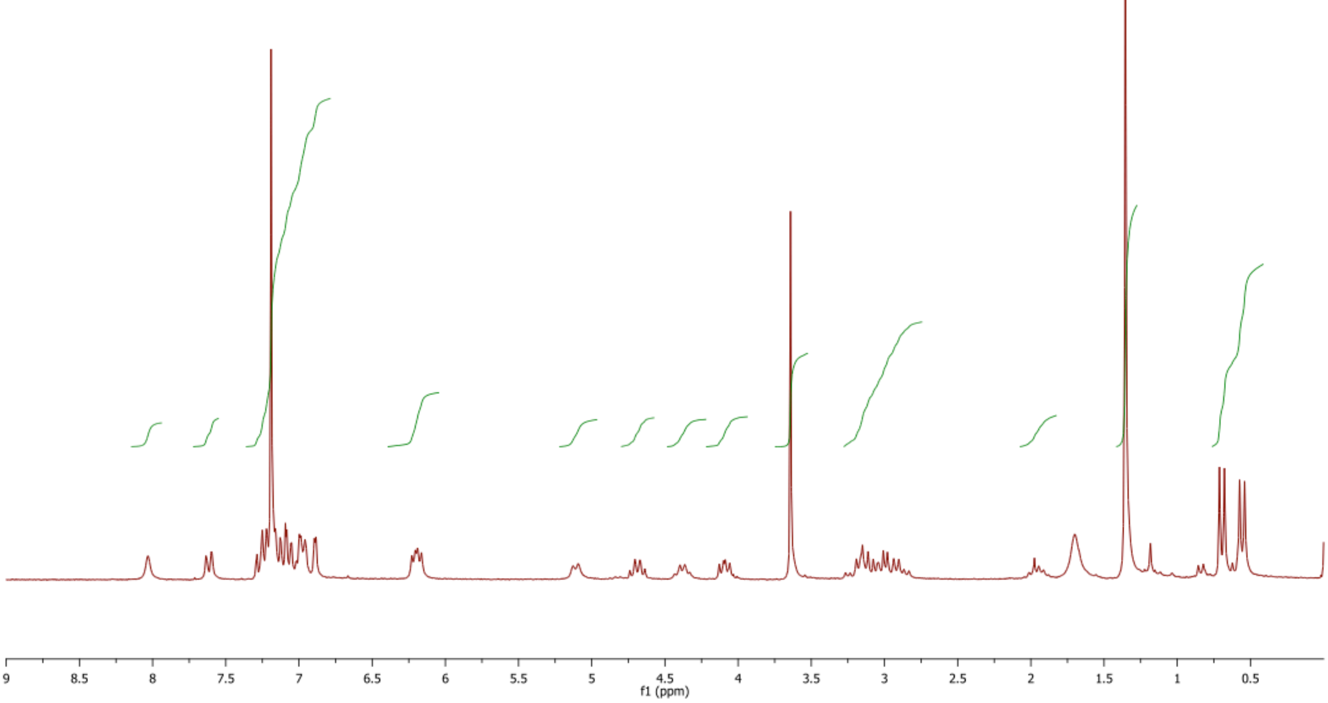


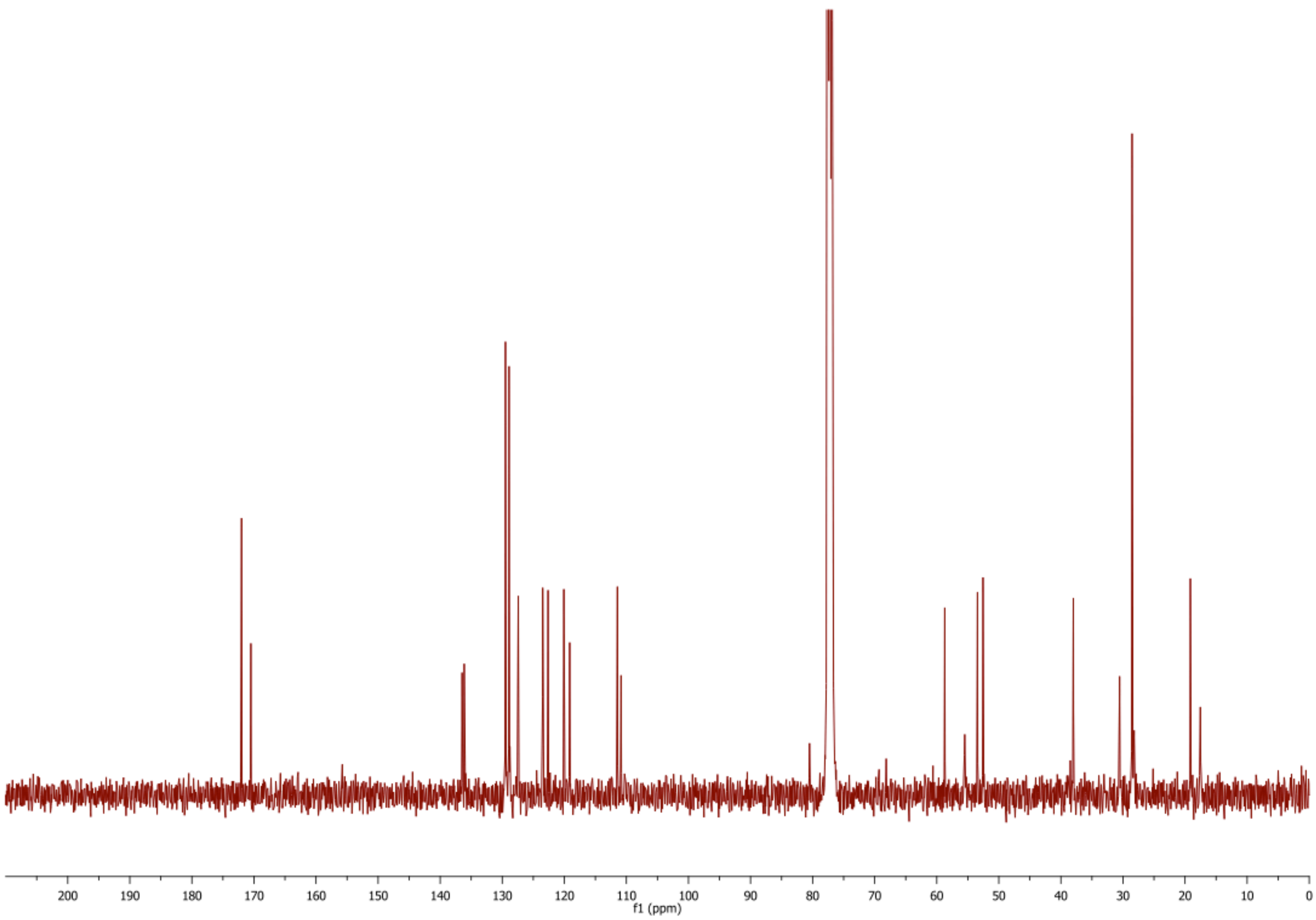




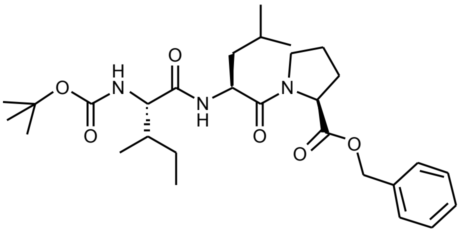
Boc-Trp-Val-Phe-OMe

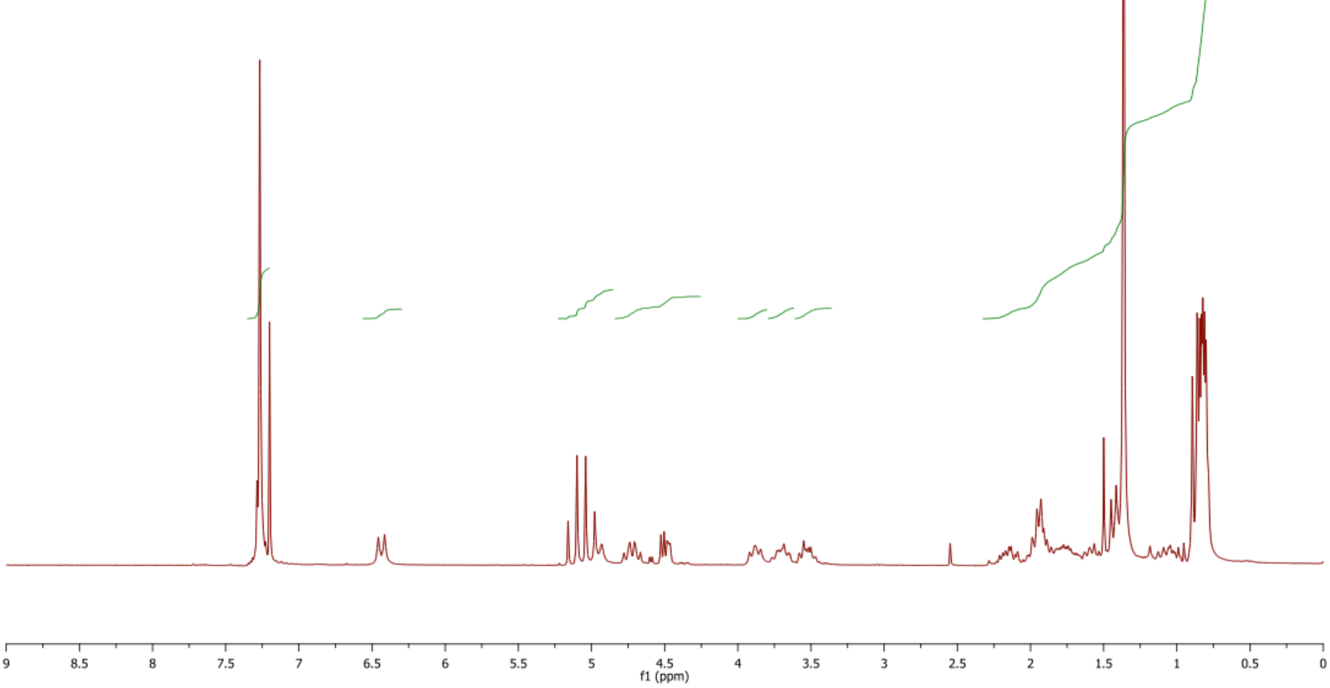


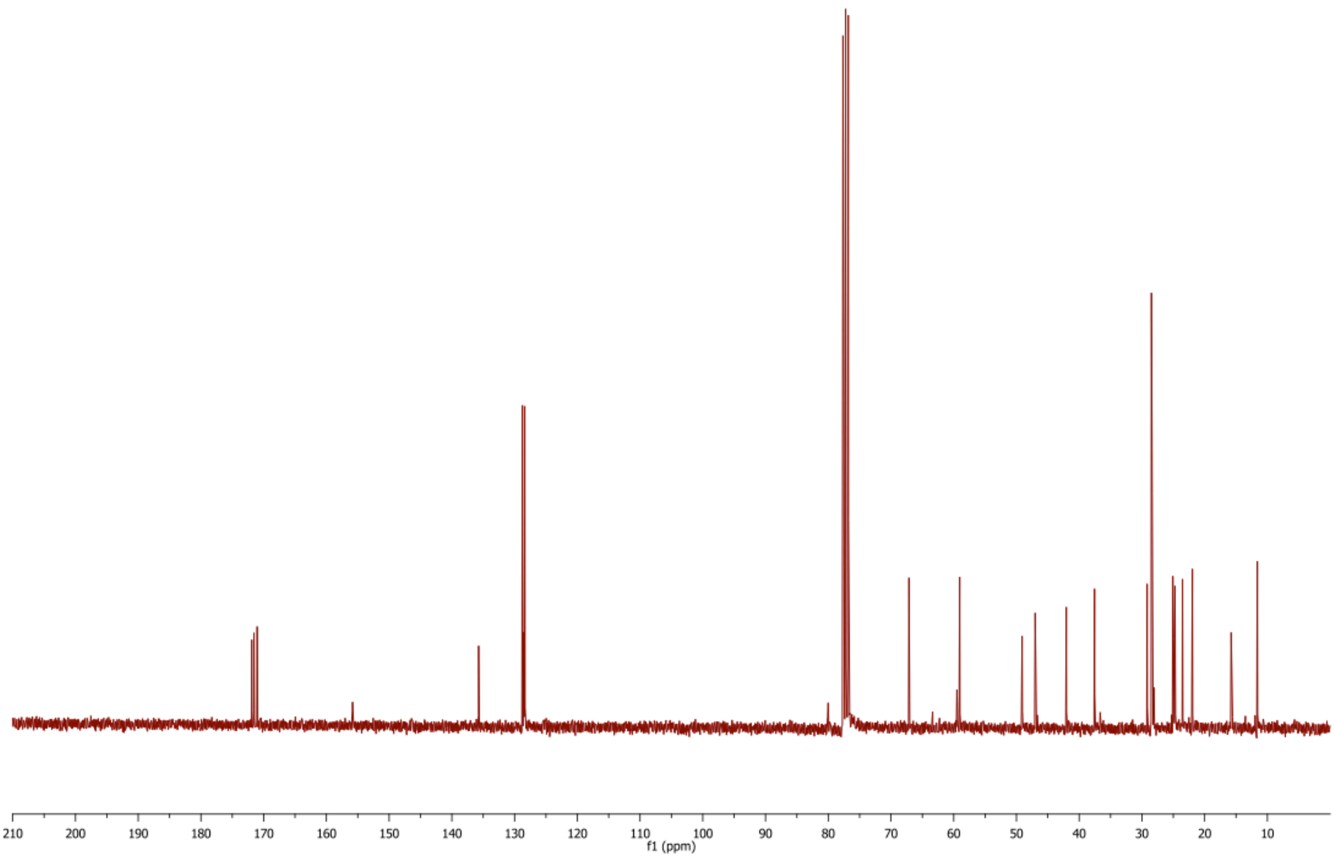




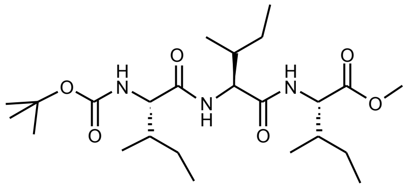
Boc-Ile-Leu-Pro-OBn

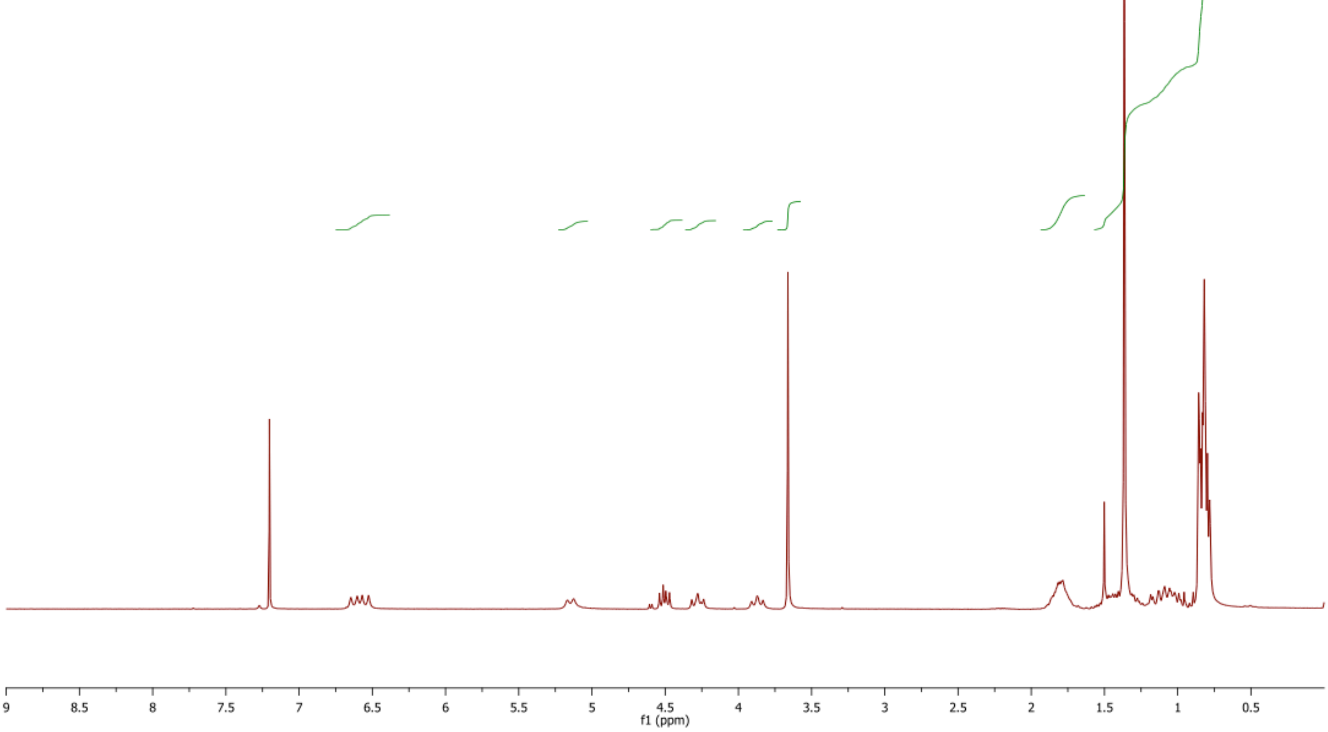


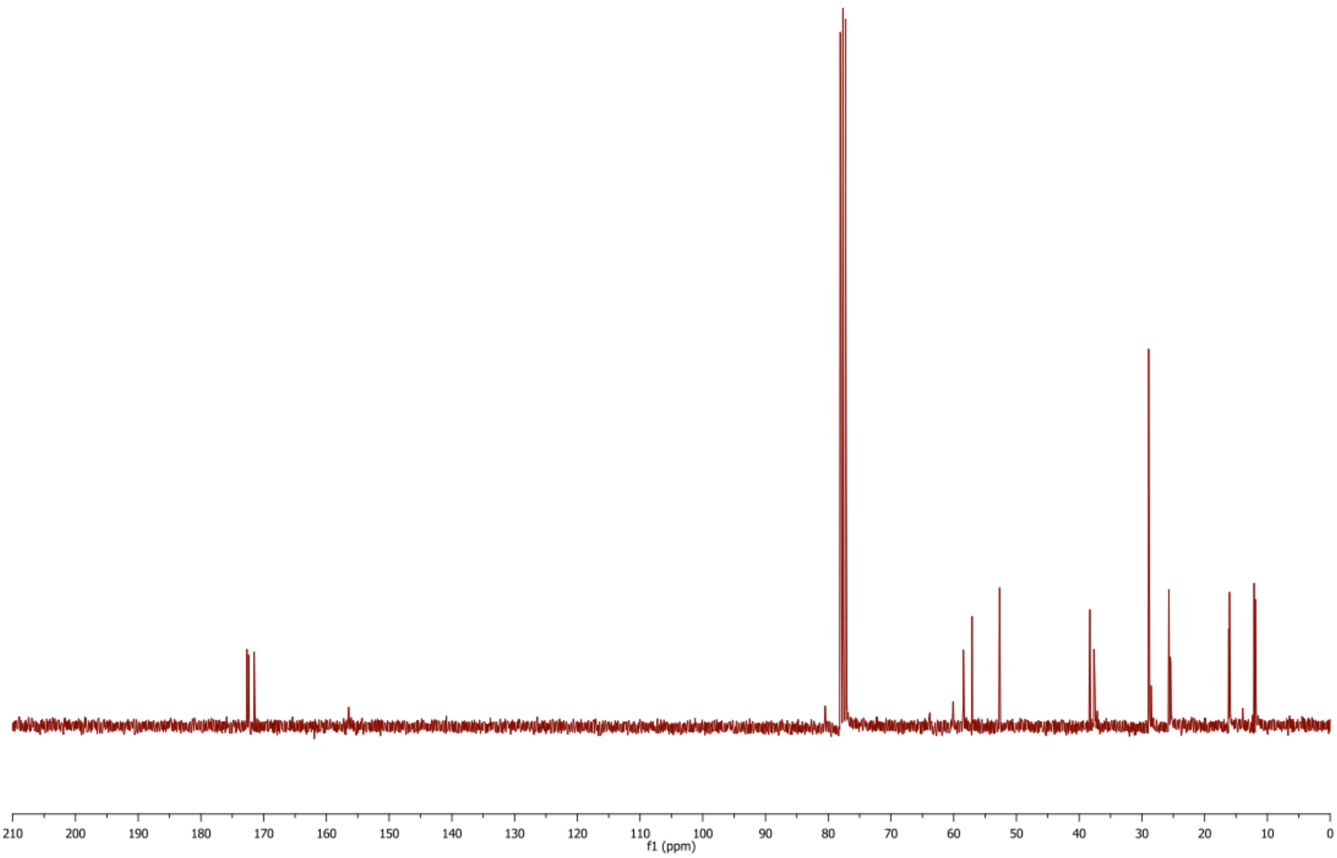




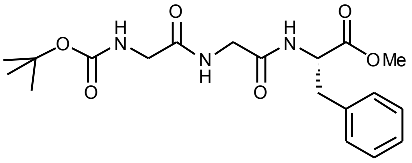
Boc-Ile-Ile-Ile-OMe

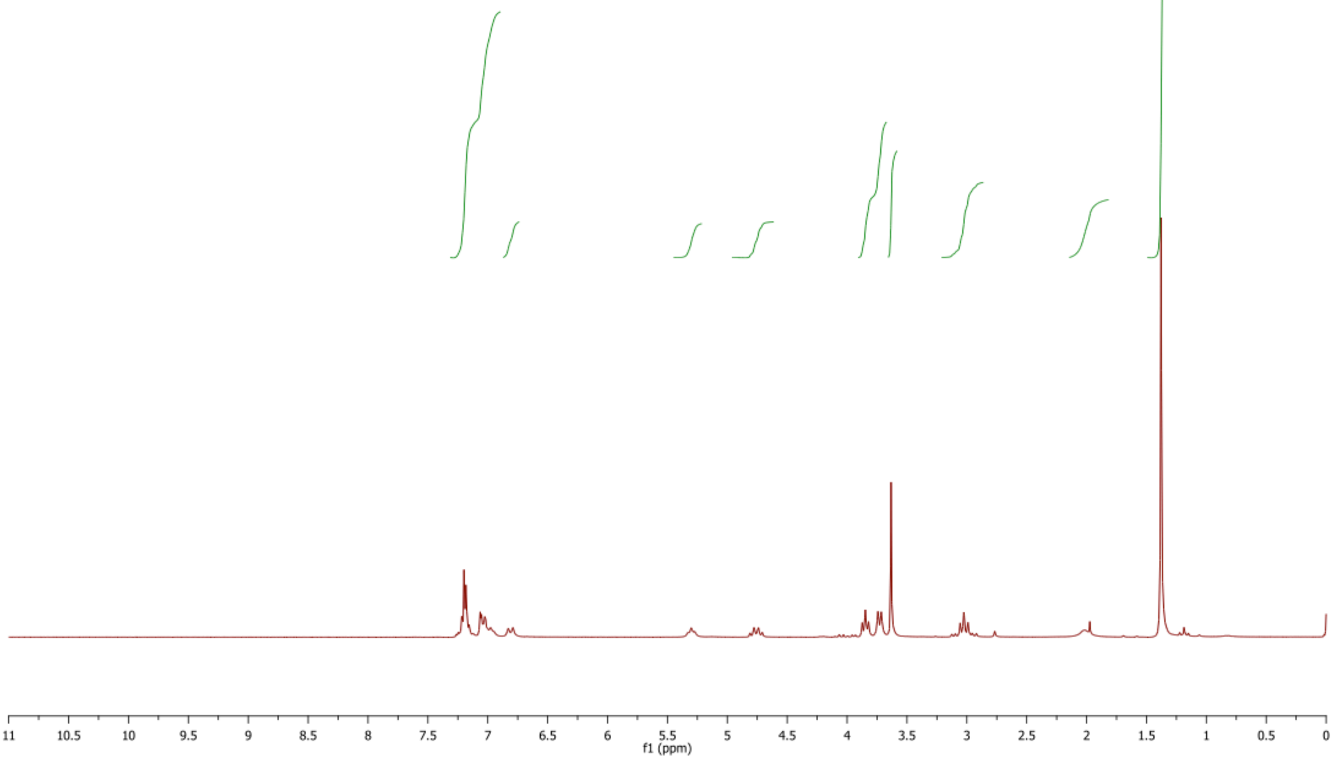


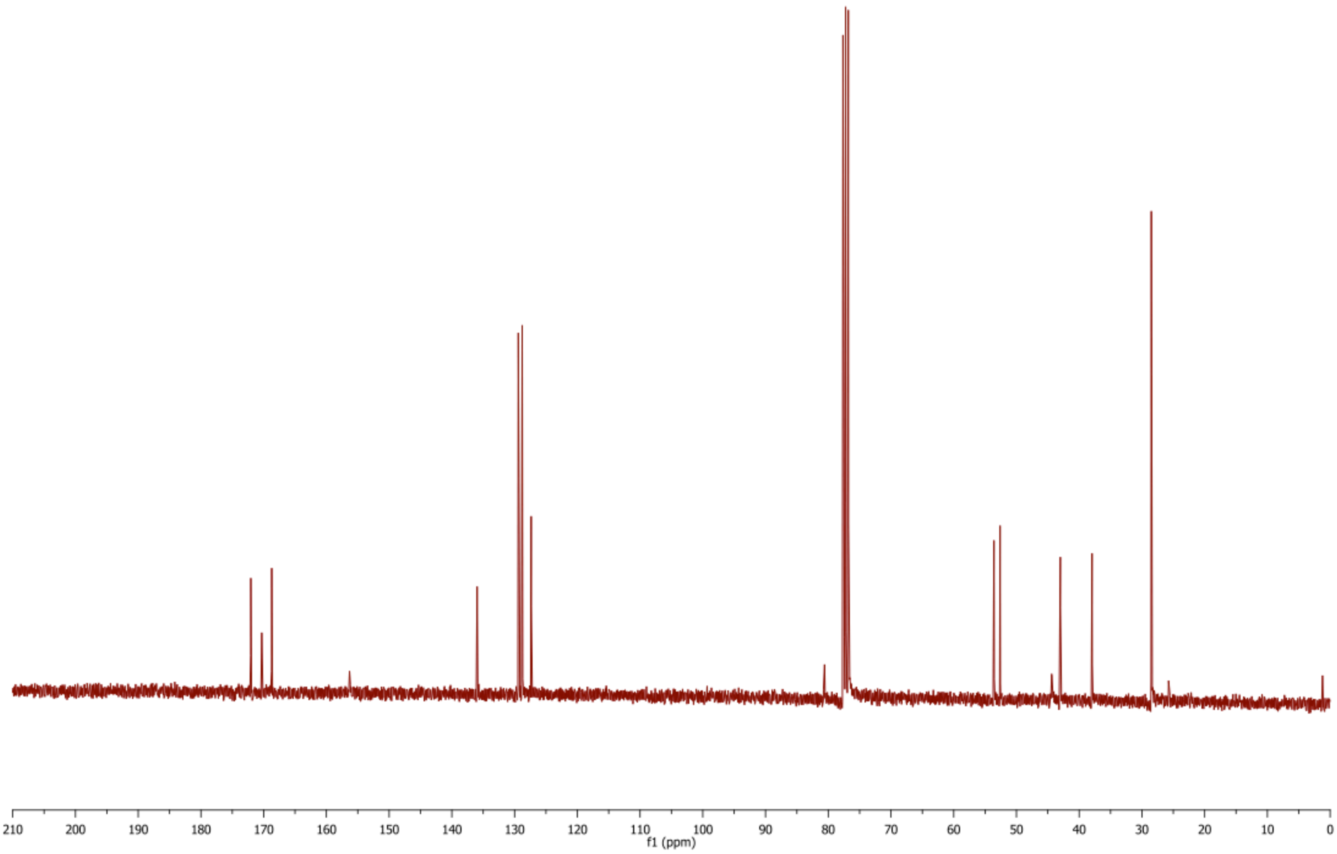




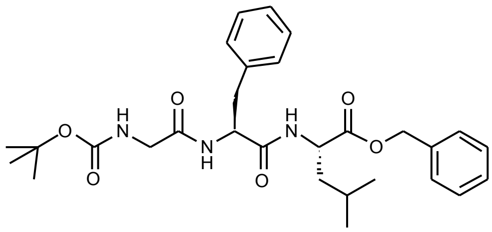
Boc-Gly-Gly-Phe-OMe

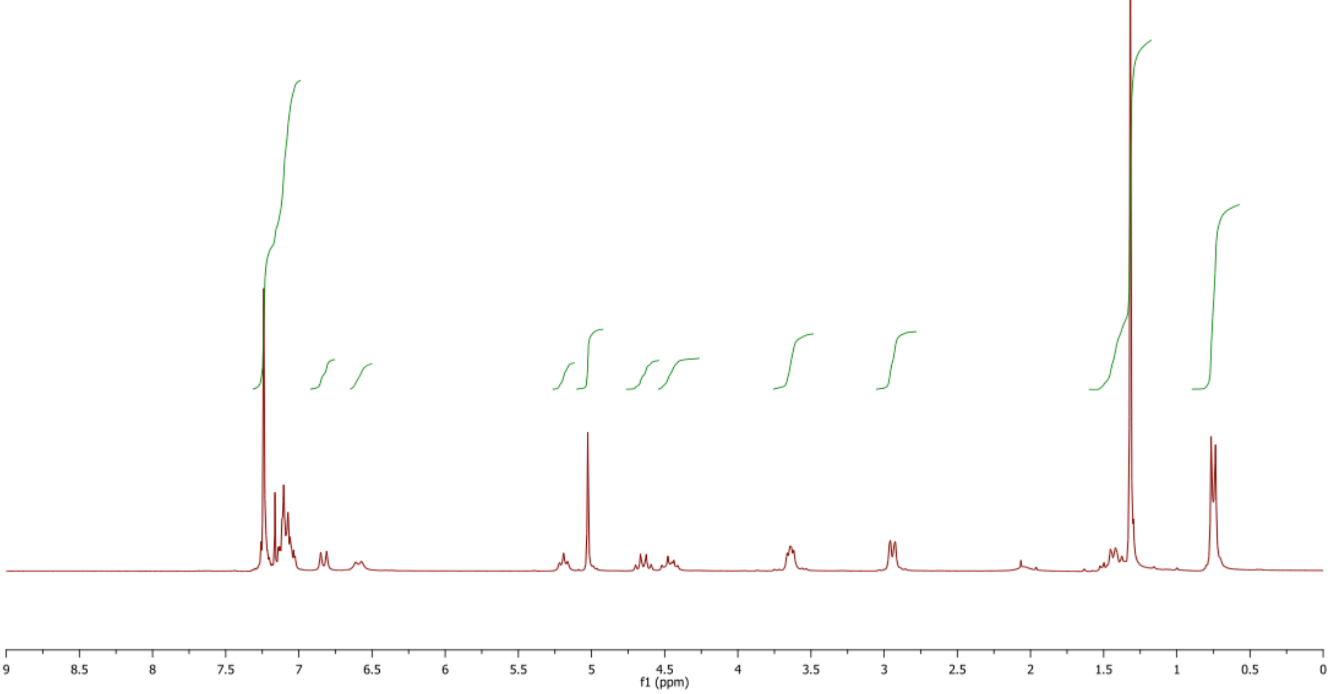


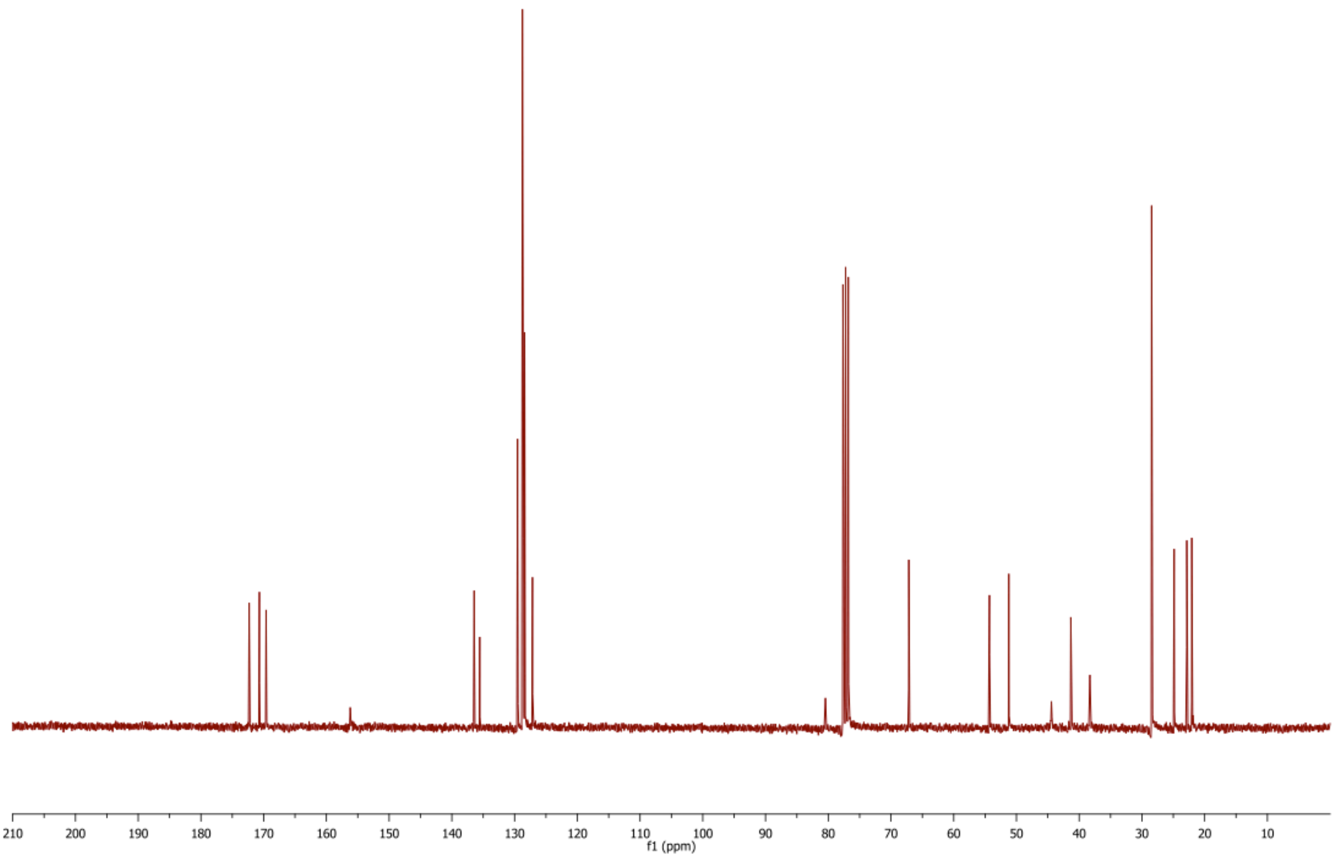




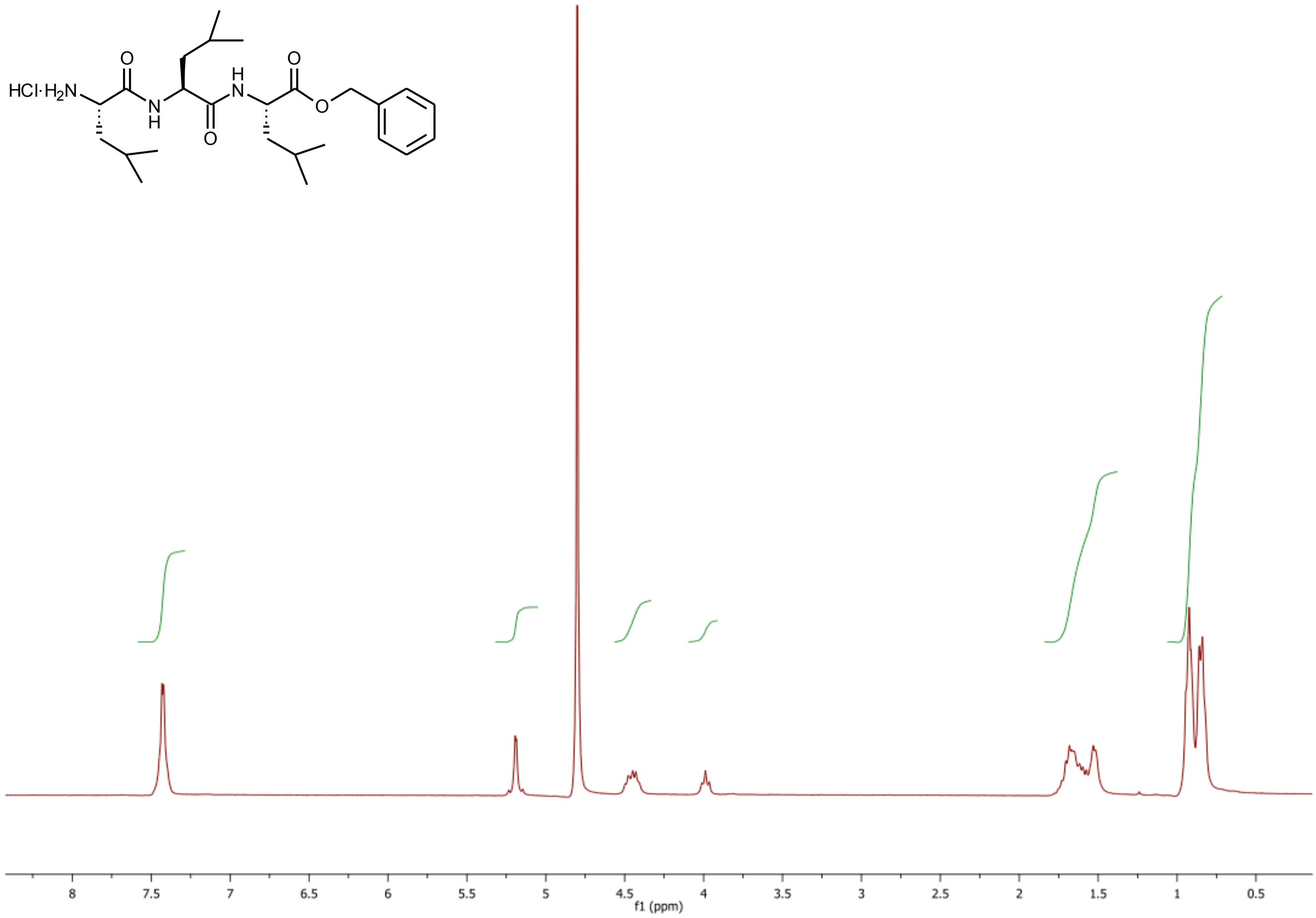
Boc-Gly-Phe-Leu-OBn

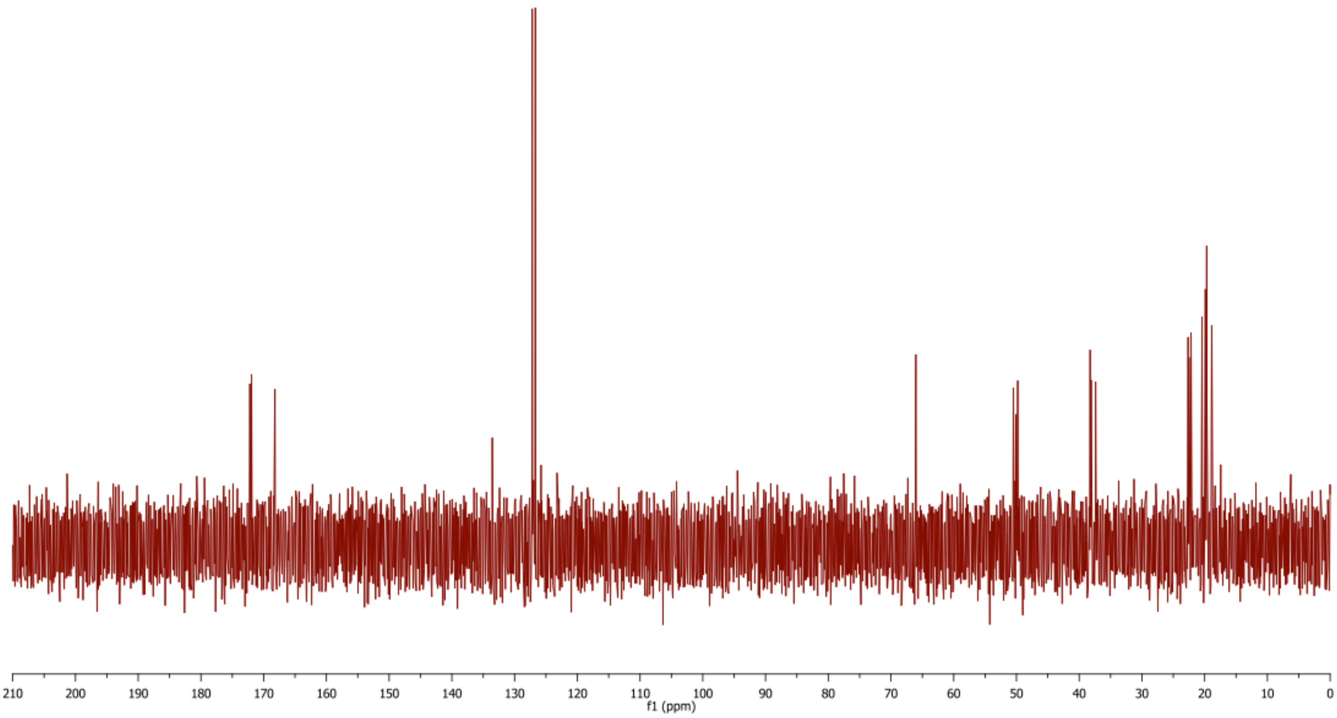




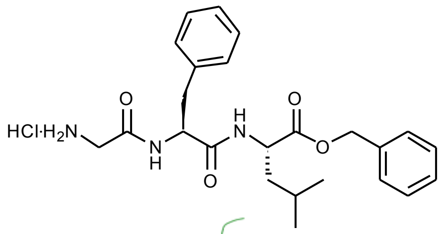


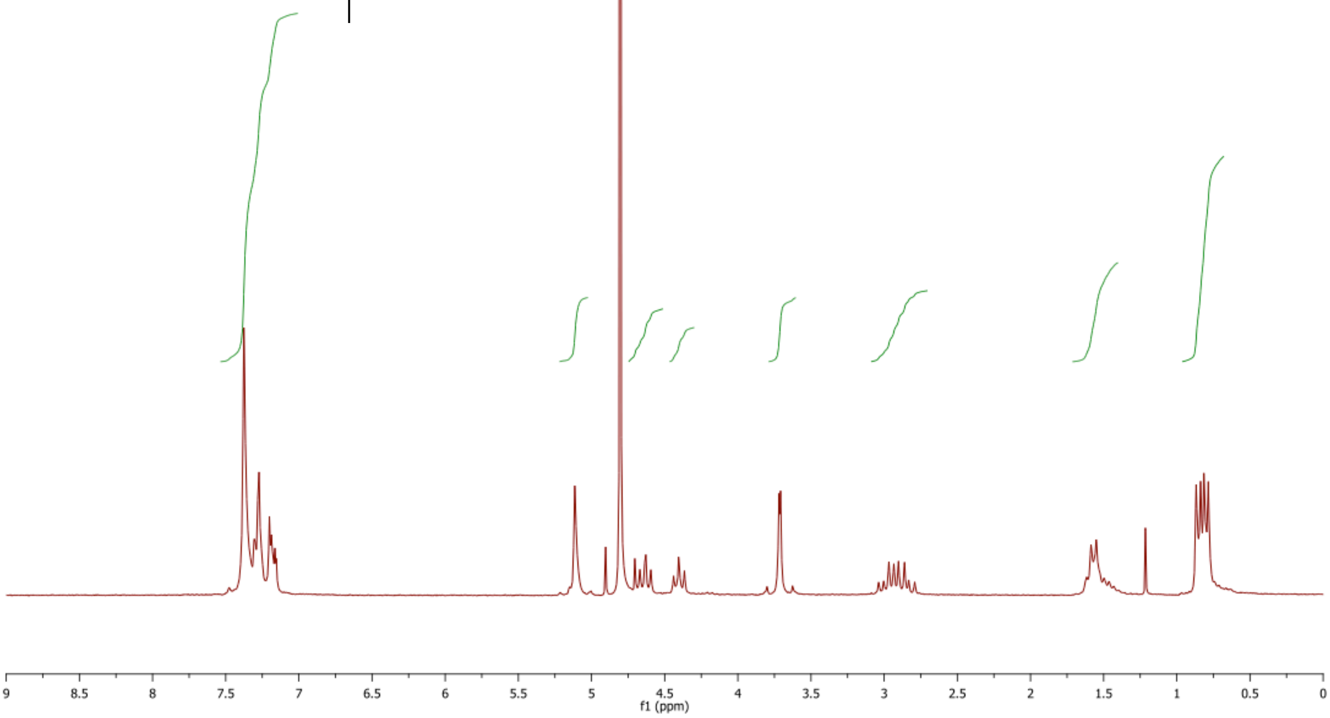
HCl·H-Leu-Leu-Leu-OBn

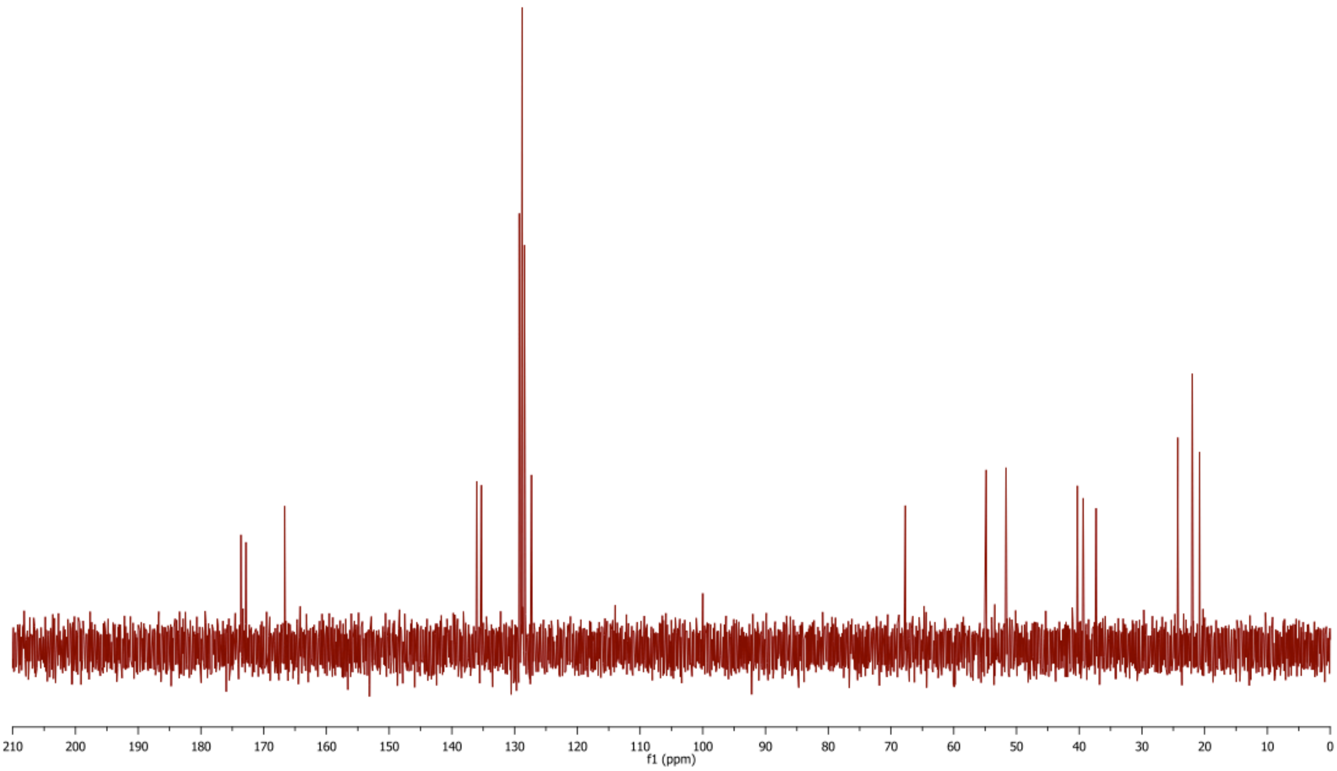




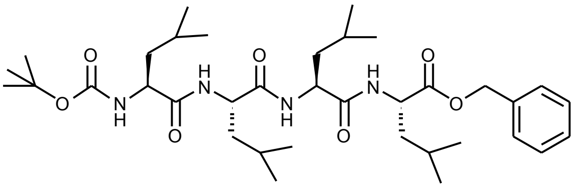
HCl·H-Gly-Phe-Leu-OBn

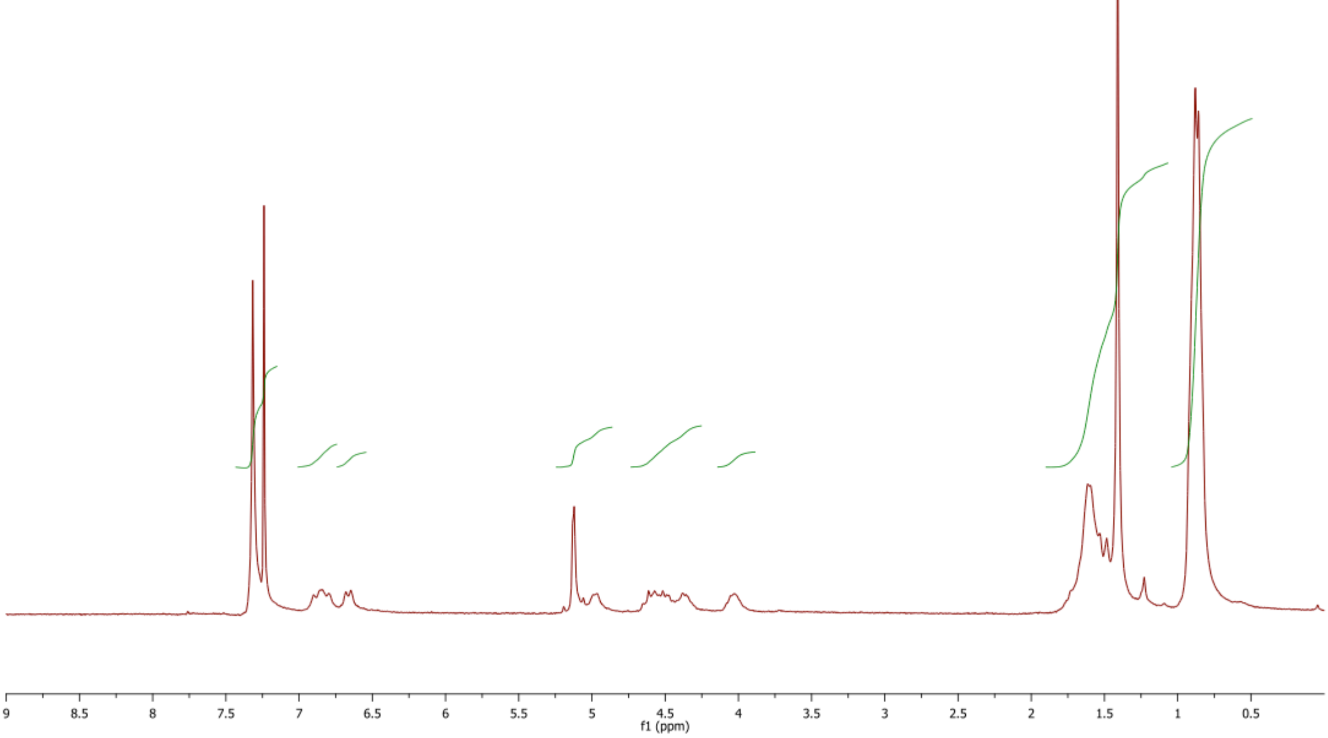


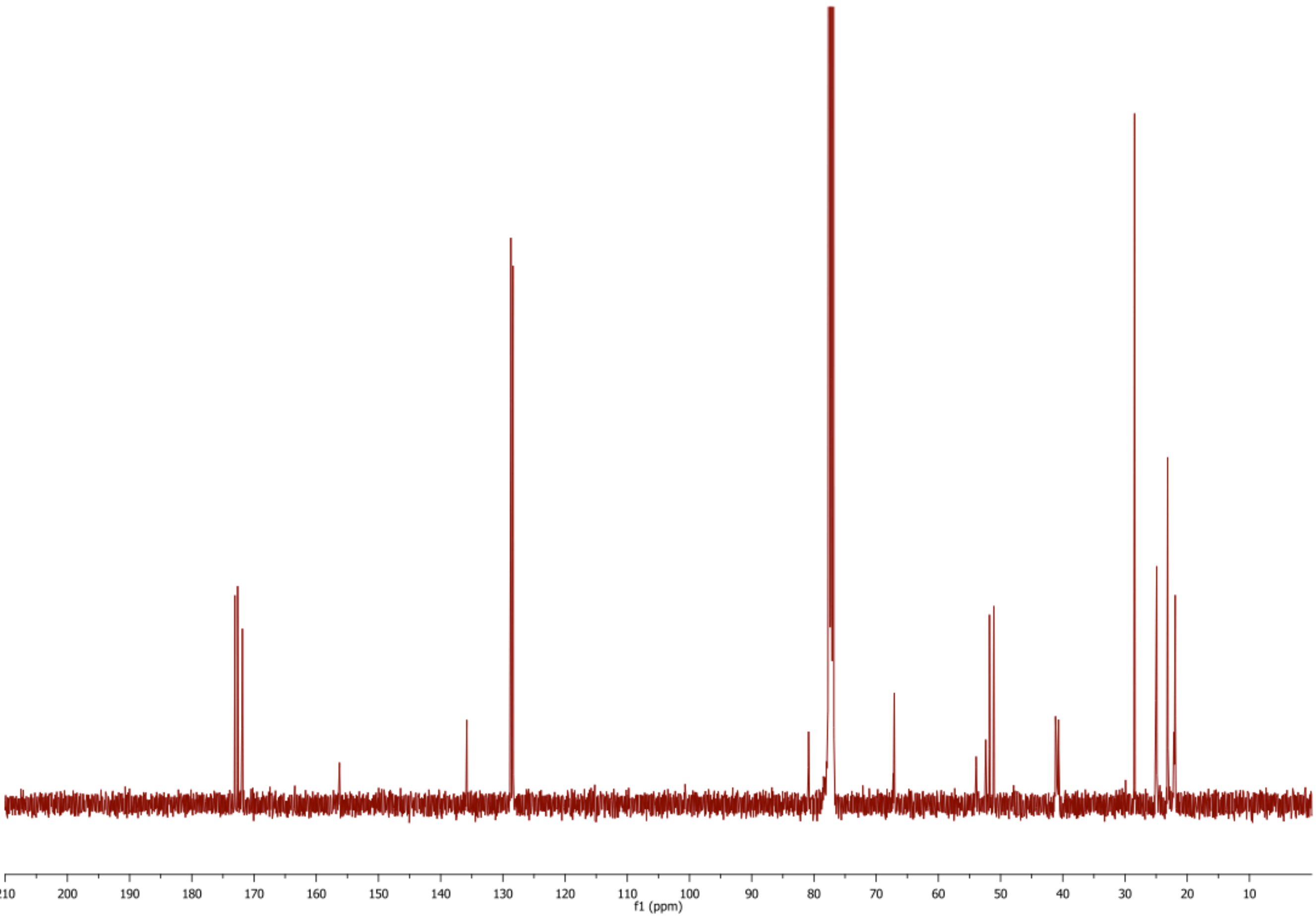




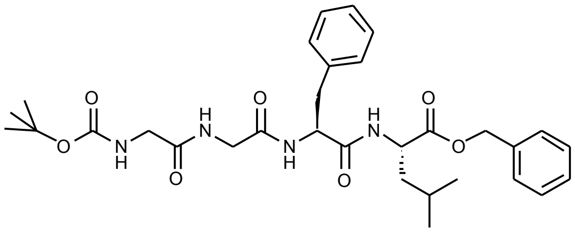
Boc-Leu-Leu-Leu-Leu-OBn

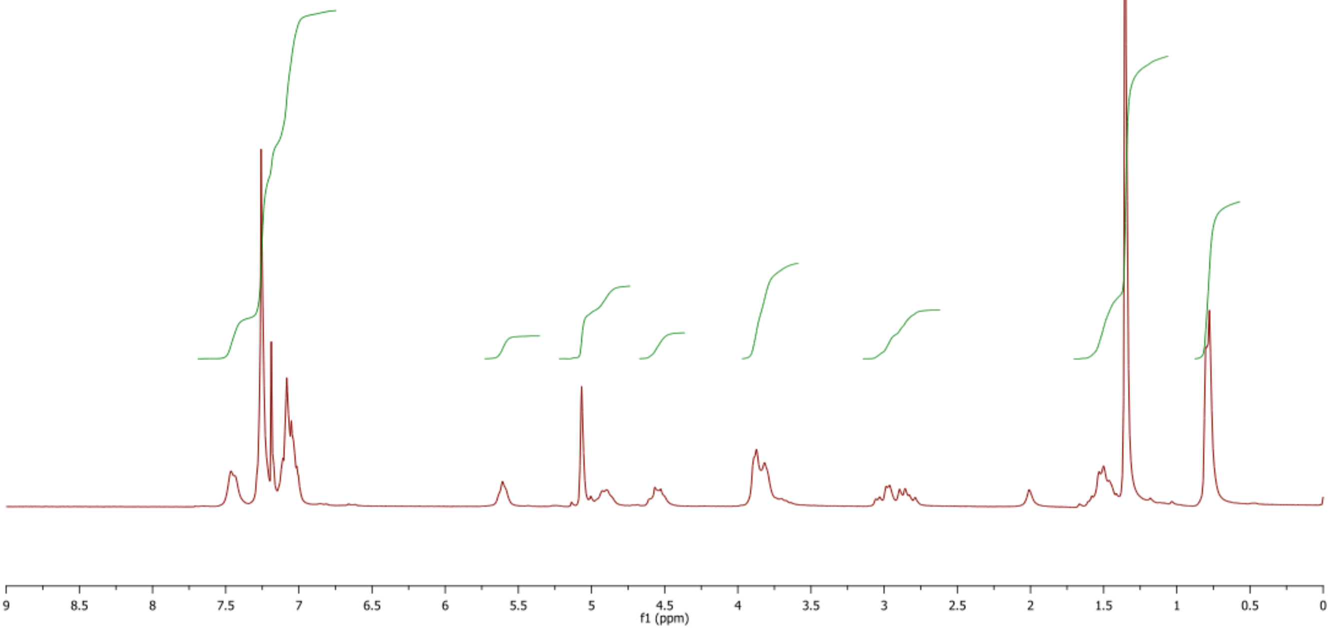


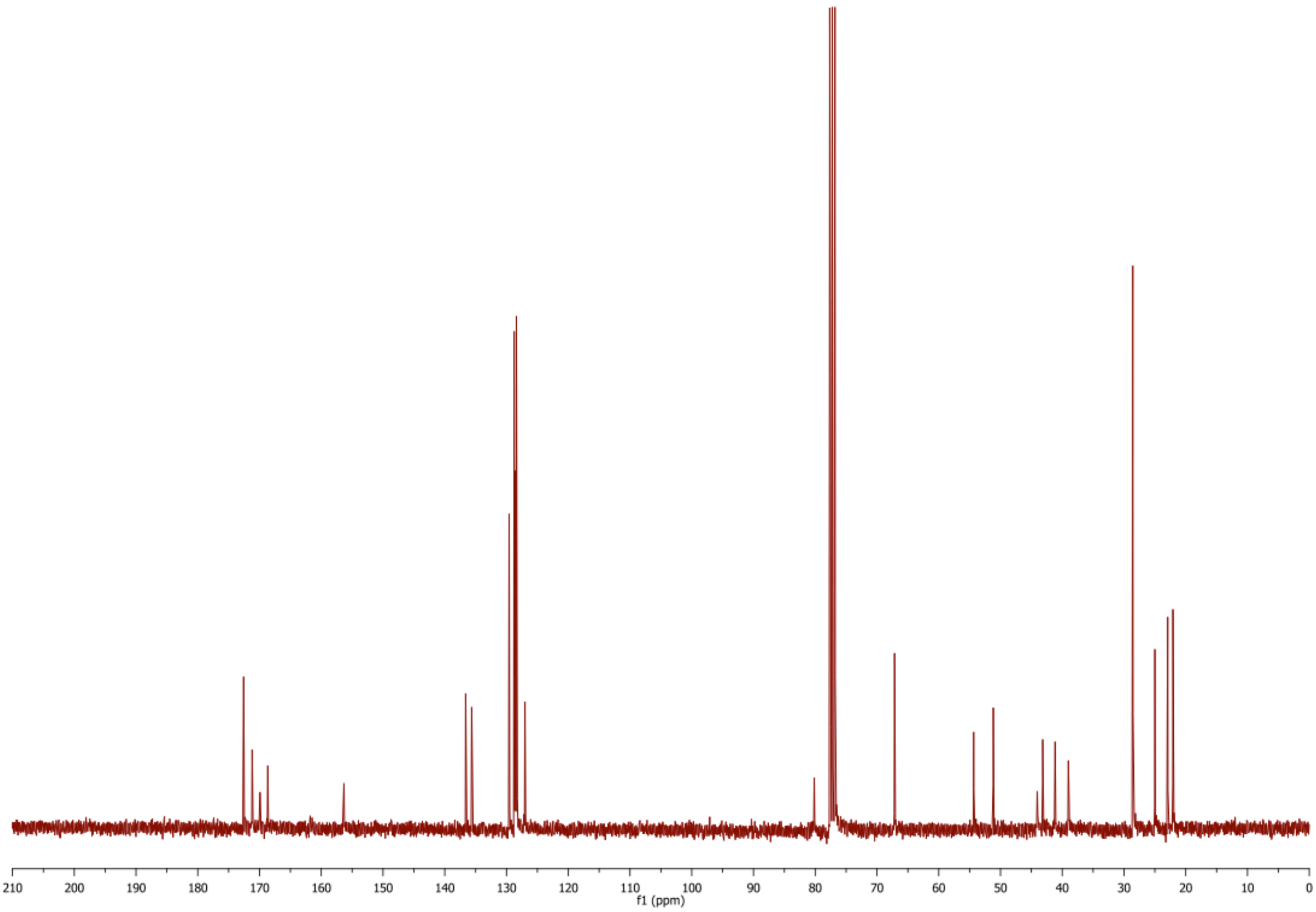




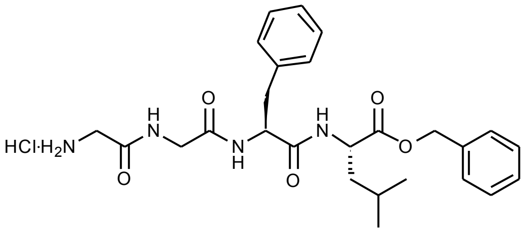
Boc-Gly-Gly-Phe-Leu-OBn

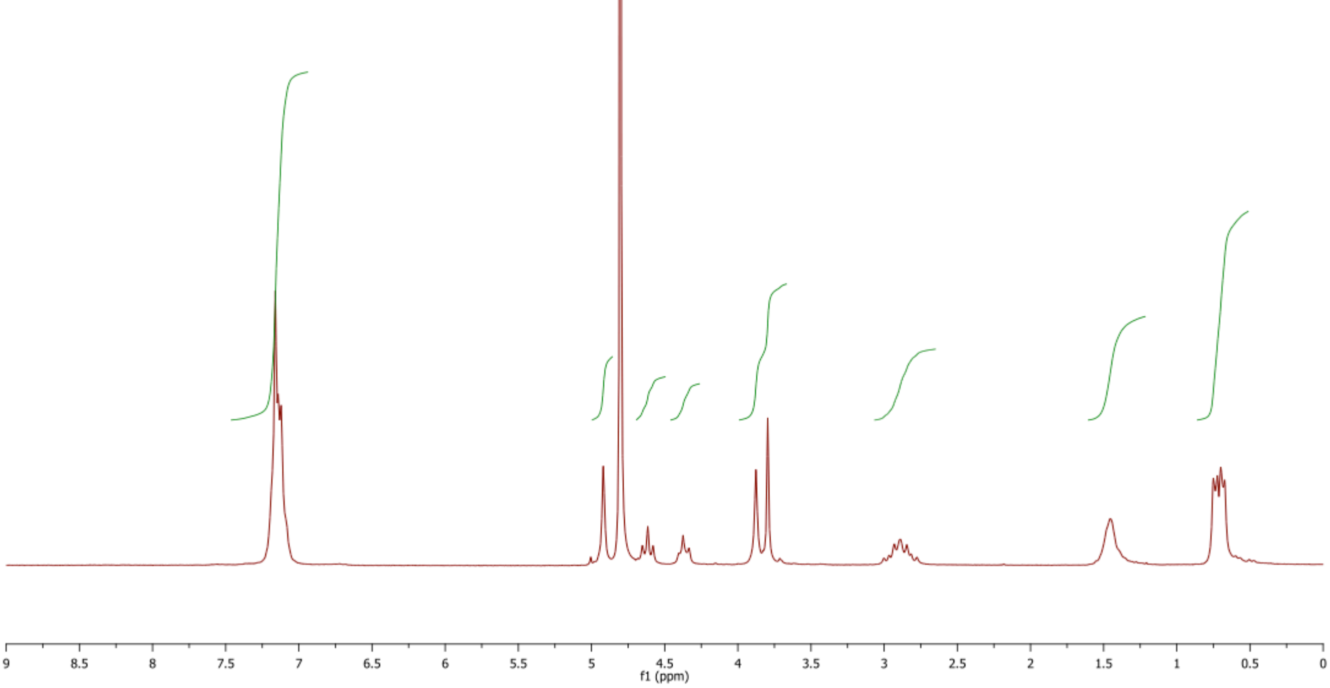


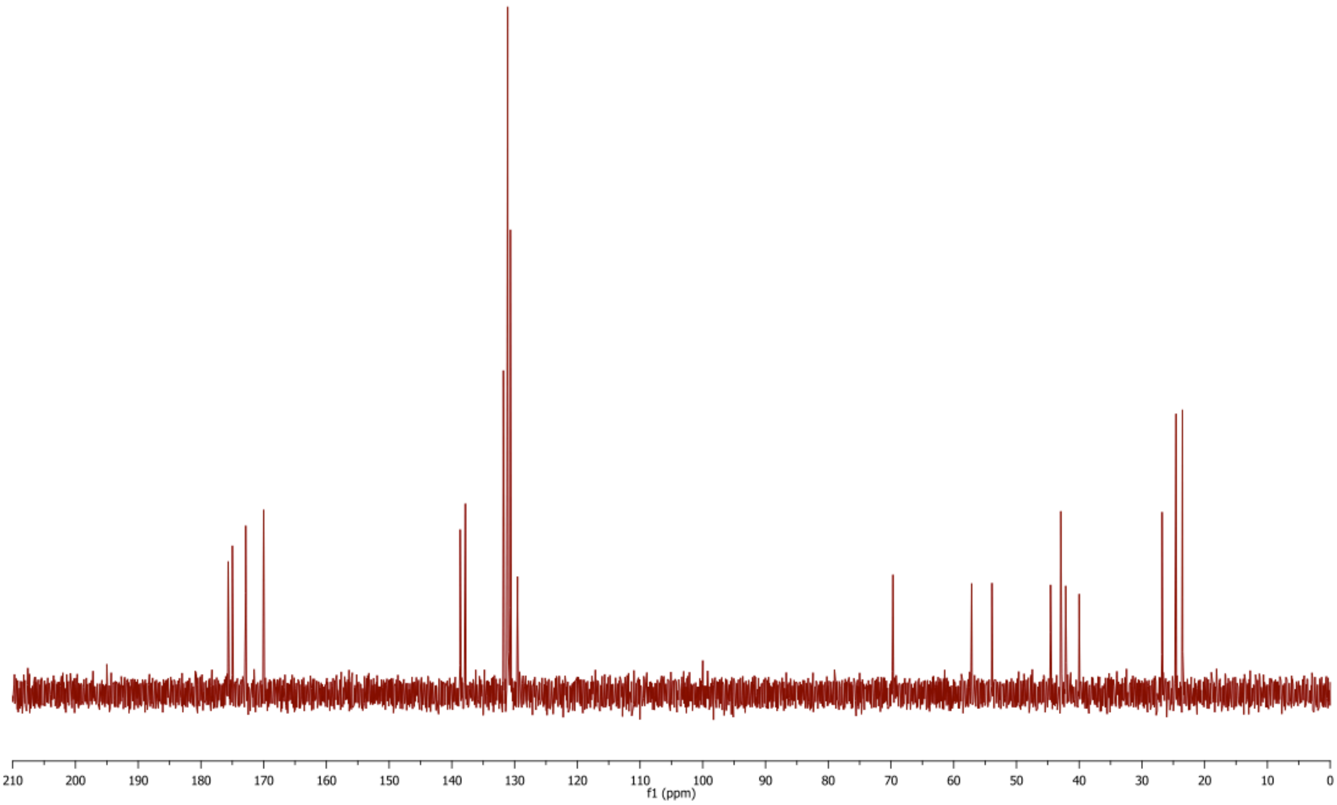




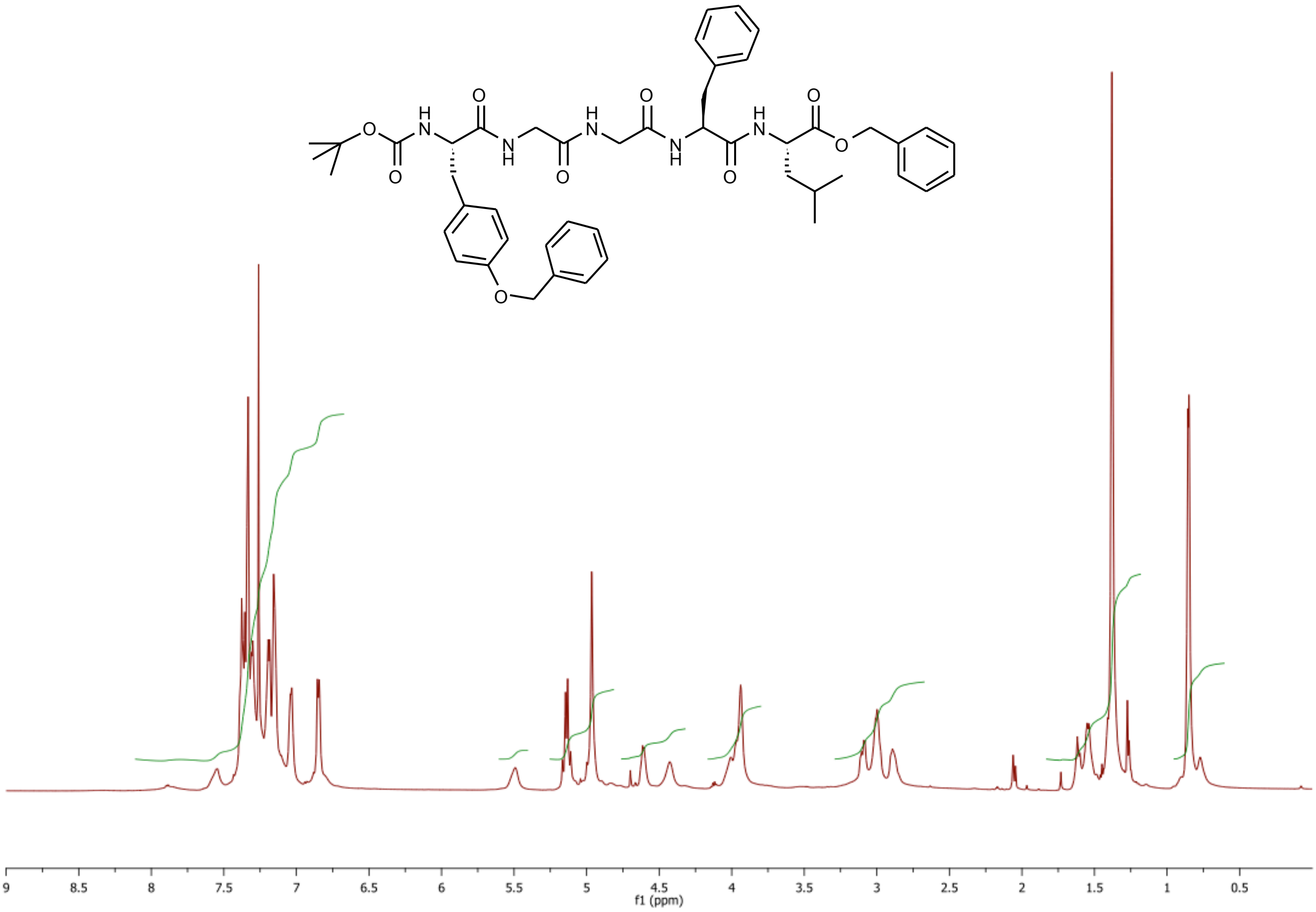
HCl·H-Gly-Gly-Phe-Leu-OBn

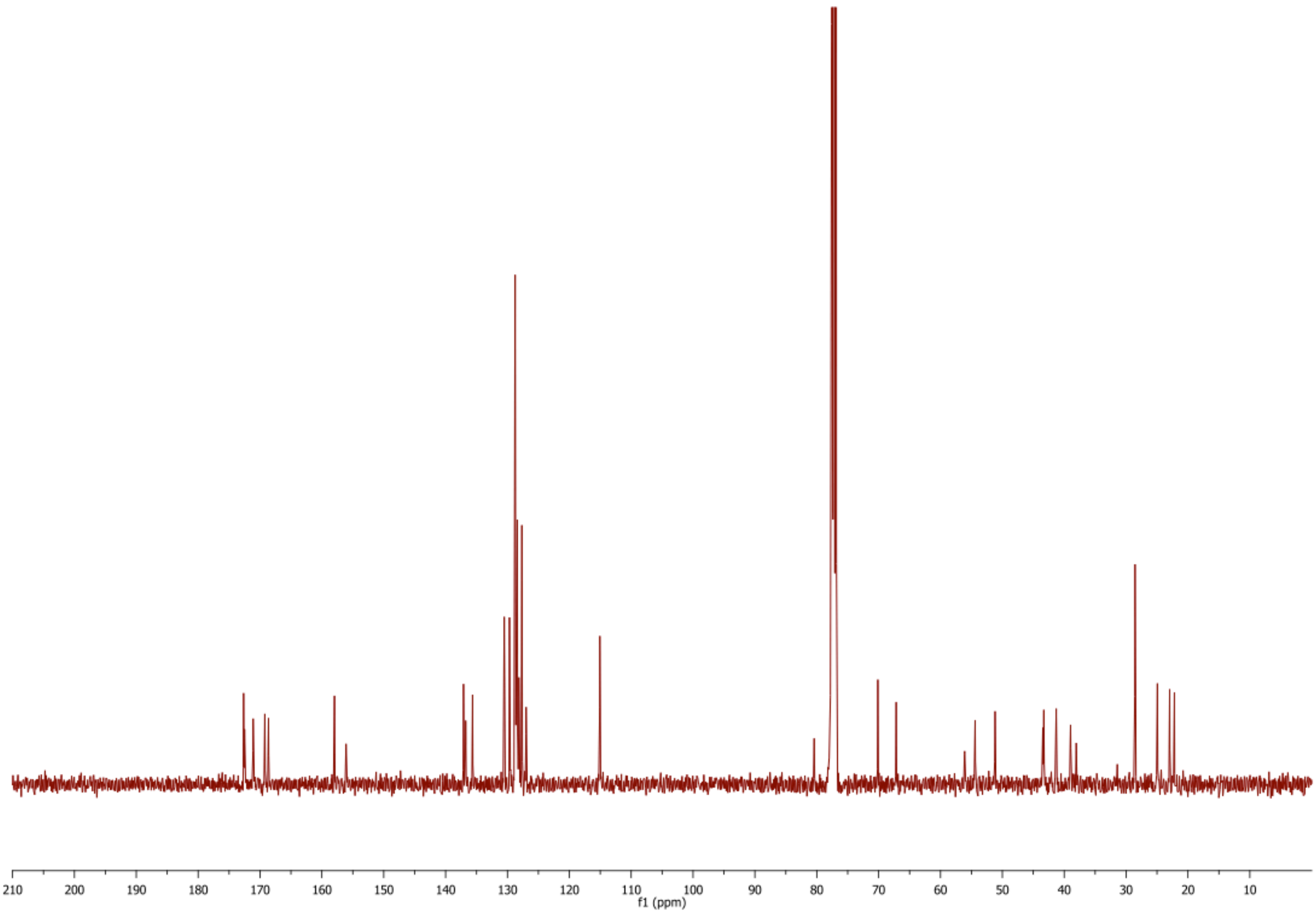




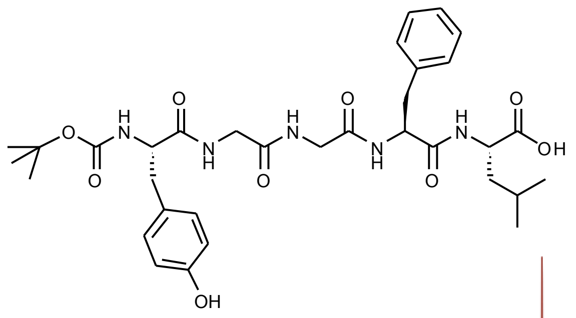


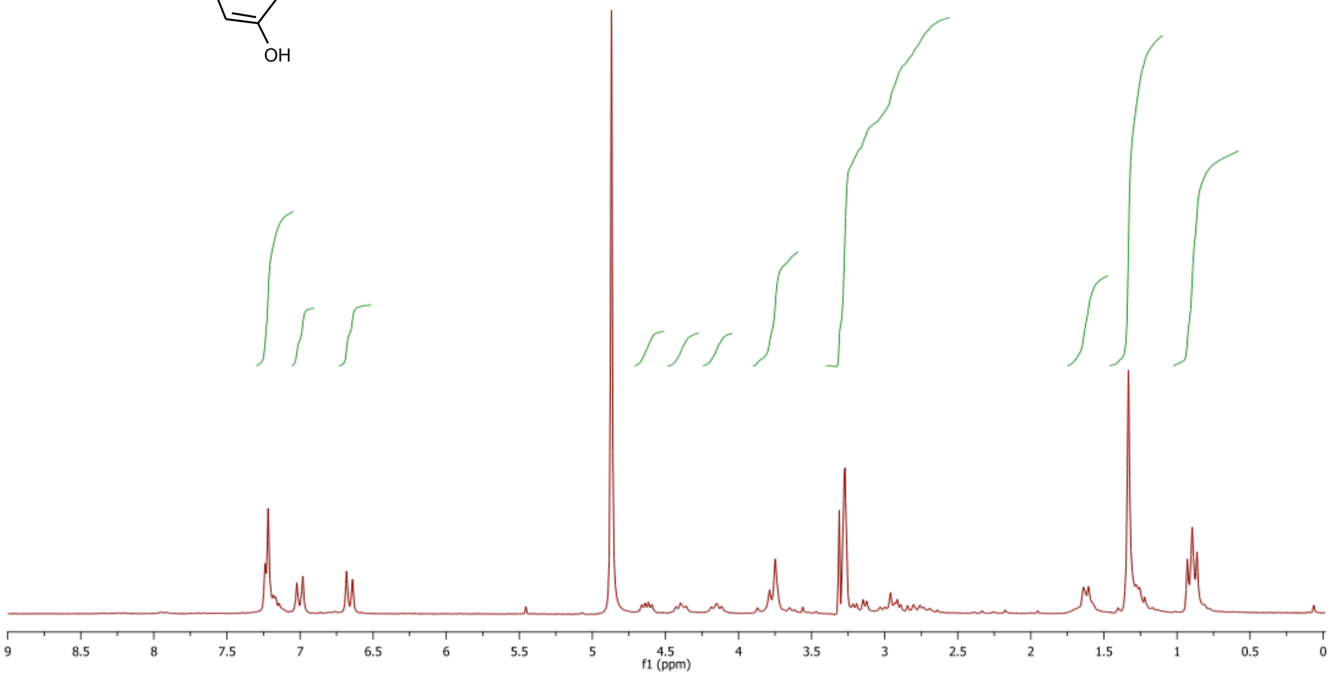
Boc-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn

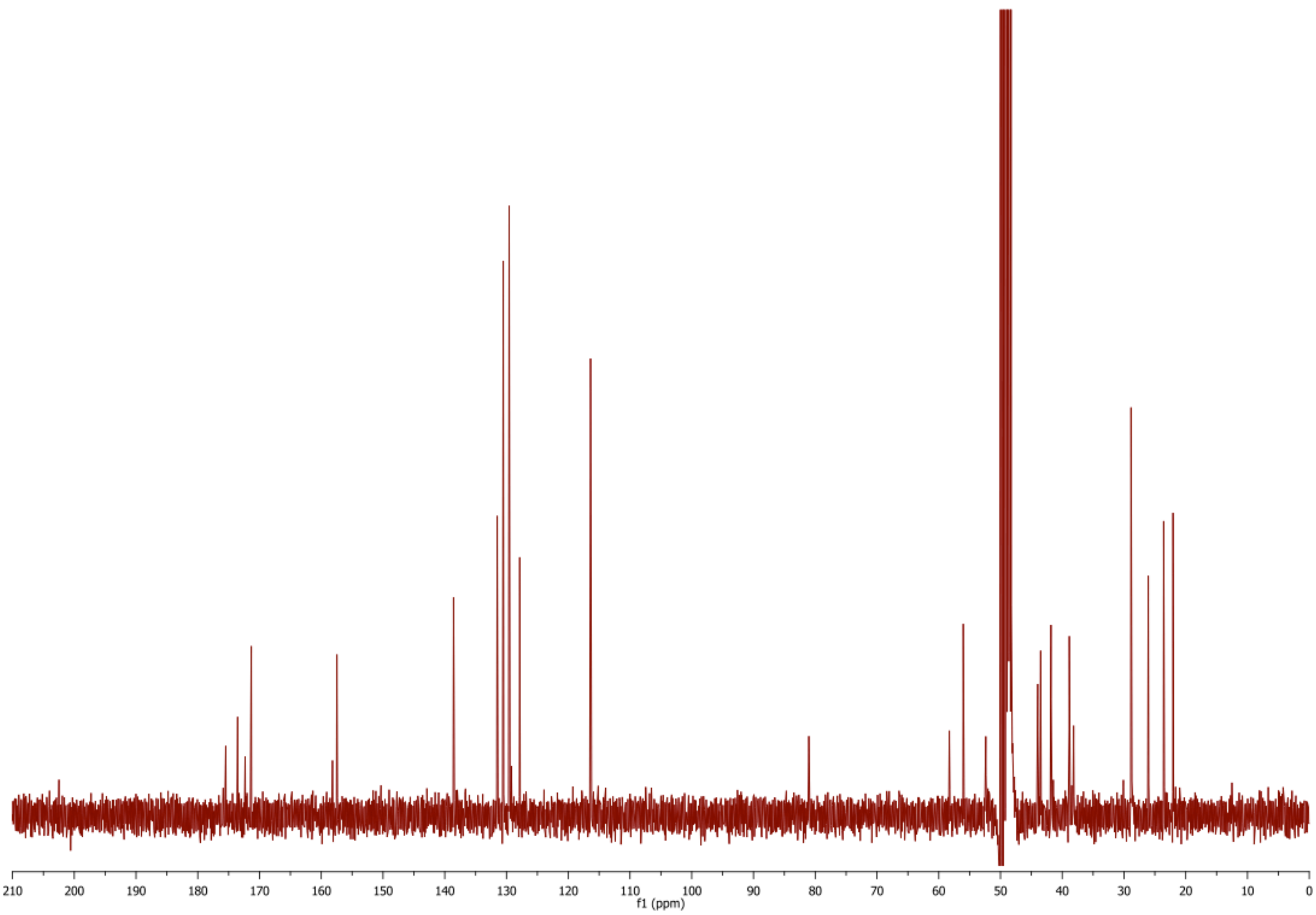




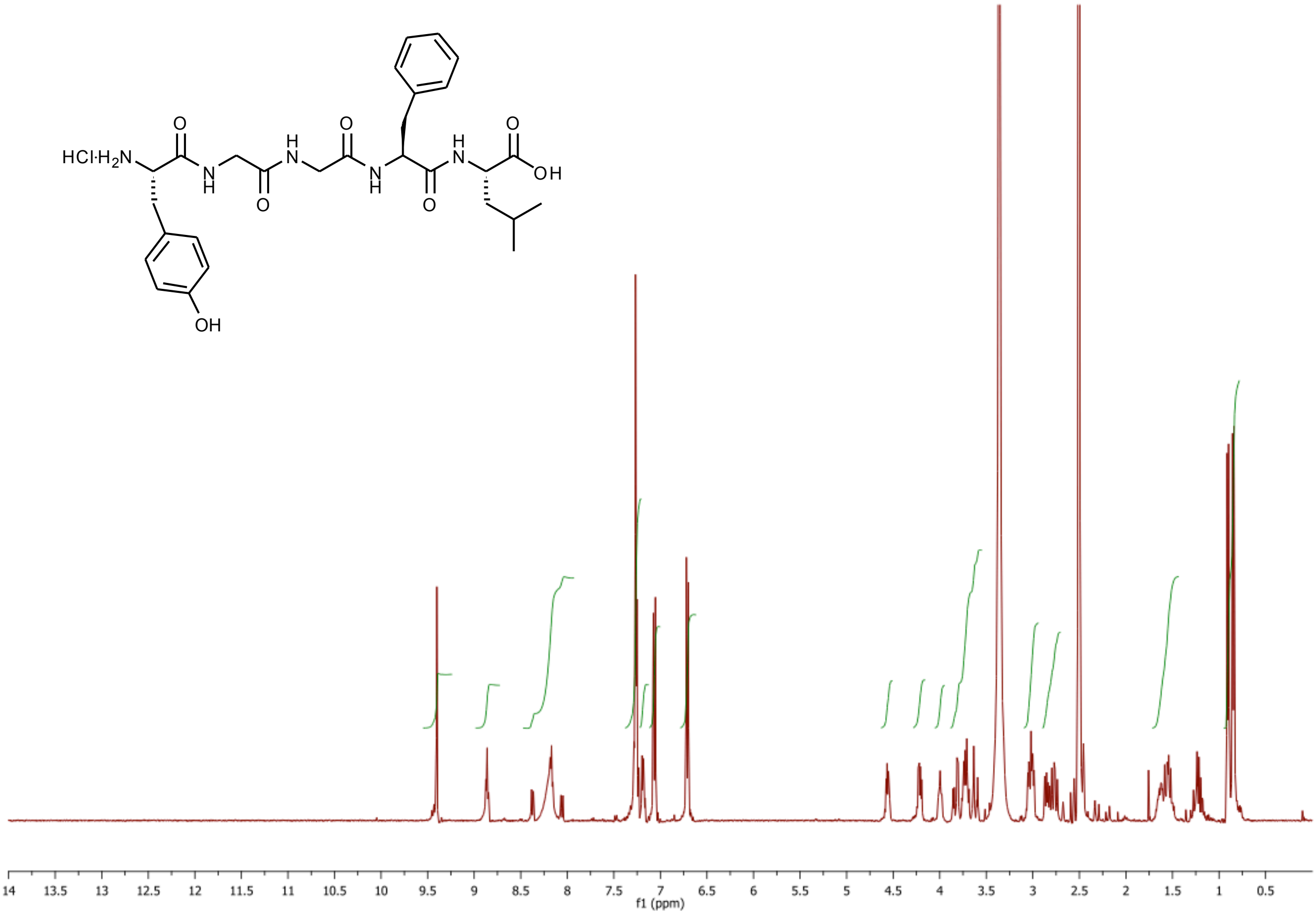
Boc-Tyr(OH)-Gly-Gly-Phe-Leu-OH

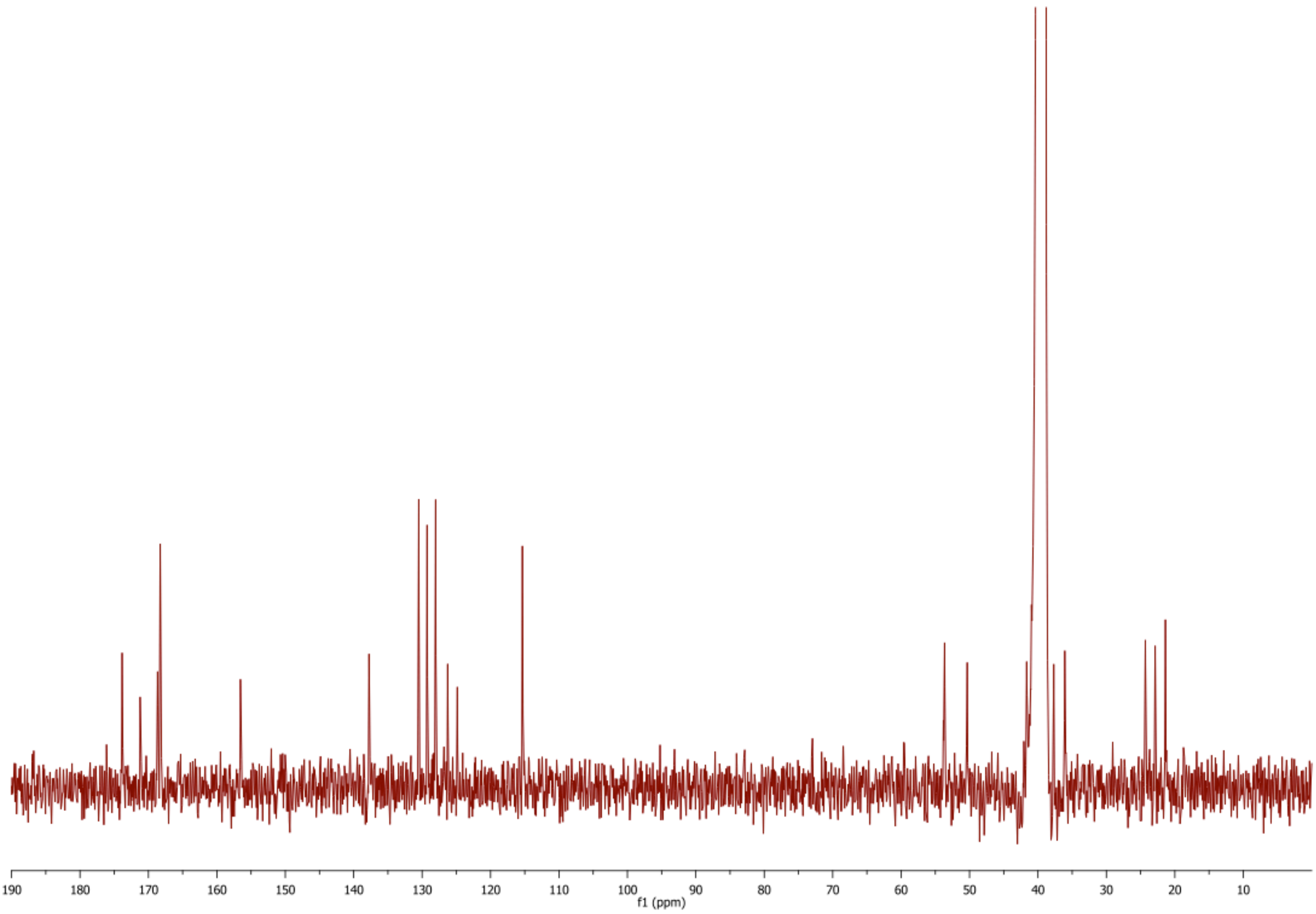






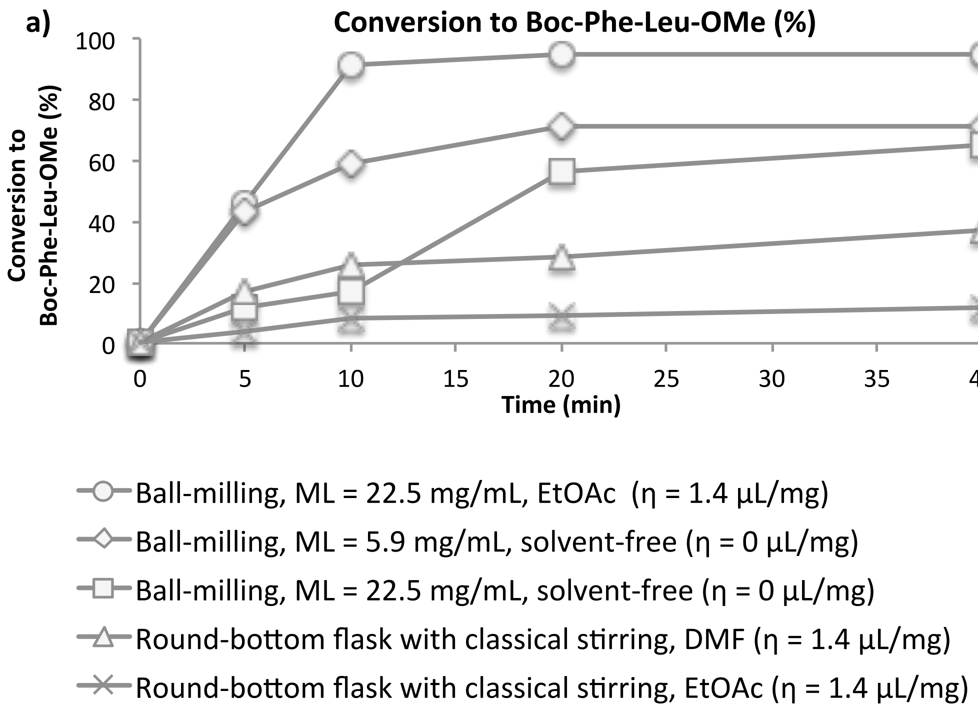
HCl·H-Tyr(OH)-Gly-Gly-Phe-Leu-OH





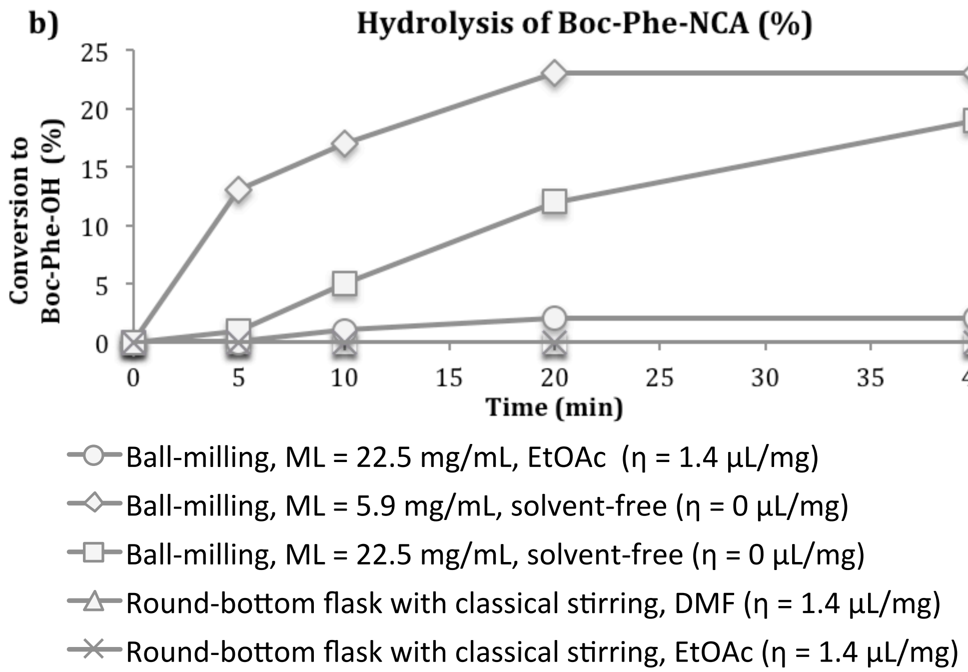
Kinetic study

a) Conversion to Boc-Phe-Leu-OMe



Time	*5*	*10*	*20*	*40*
Ball-milling, ML = 22.5 mg/mL, EtOAc (η = 1.4 µL/mg)	46	91	95	95
Ball-milling, ML = 5.9 mg/mL, solvent-free (η = 0 µL/mg)	43	59	71	71
Ball-milling, ML = 22.5 mg/mL, solvent-free (η = 0 µL/mg)	12	17	56	65
Round-bottom flask with classical stirring, DMF (η = 1.4 µL/mg)	17	26	28	37
Round-bottom flask with classical stirring, EtOAc (η = 1.4 µL/mg)	4	8	9	12

b) Hydrolysis of Boc-Phe-NCA



Time	*5*	*10*	*20*	*40*
Ball-milling, ML = 22.5 mg/mL, EtOAc (η = 1.4 µL/mg)	0	1	2	2
Ball-milling, ML = 5.9 mg/mL, solvent-free (η = 0 µL/mg)	13	17	23	23
Ball-milling, ML = 22.5 mg/mL, solvent-free (η = 0 µL/mg)	1	5	12	19
Round-bottom flask with classical stirring, DMF (η = 1.4 µL/mg)	0	0	0	0
Round-bottom flask with classical stirring, EtOAc (η = 1.4 µL/mg)	0	0	0	0

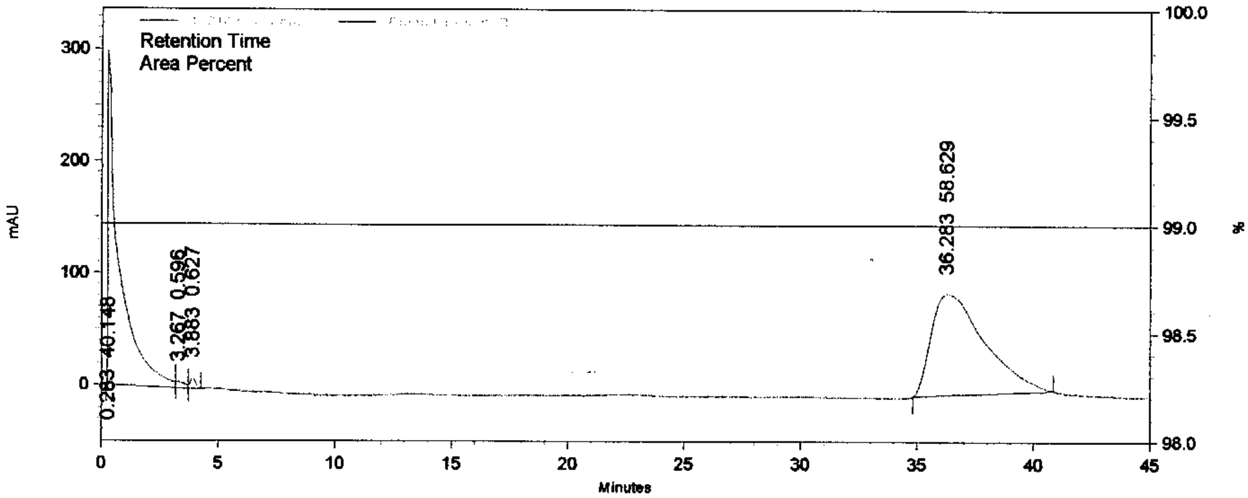
Electronic Supplementary Material (ESI) for Green Chemistry

General procedure for the coupling of Boc-amino acids with amino ester hydrochlorides under classical coupling conditions: To a solution of a Boc-(D or D,L)-Phe-OH (265 mg, 1 mmol, 1 eq.) in 5 mL of DMF were added successively HCl·H-Leu-OMe (181.6 mg, 1 mmol, 1 eq.), BOP (440 mg, 1.1 mmol, 1.1 eq.) and DIEA (0.435 mL, 2.5 mmol, 2.5 eq.). The resulting mixture was stirred for 3 h at room temperature and then concentrated under reduced pressure. The residue was diluted in EtOAc and the solution was neutralized with 10% aqueous solution of $KHSO_4$, washed with saturated aqueous solution of $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and evaporated to yield the dipeptide.

Chiral HPLC analysis of Boc-(D,L)-Phe-Leu-OMe, Boc-D-Phe-Leu-OMe and Boc-L-Phe-Leu-OMe:

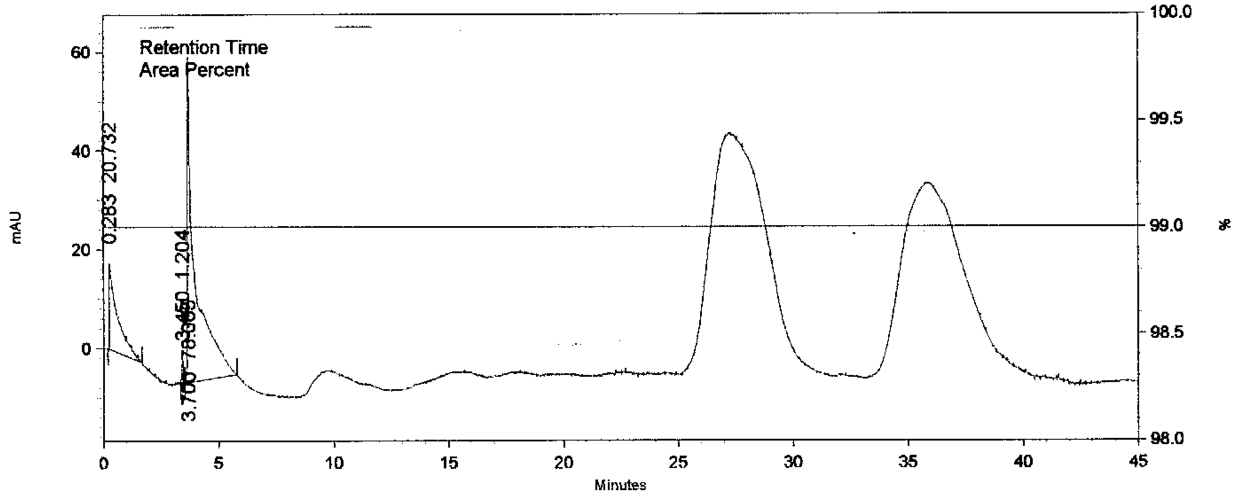
Boc-D-Phe-Leu-OMe prepared under classical conditions
Coupling of Boc-D-Phe-OH with HCl·H-Leu-OMe under the classical coupling conditions described above afforded the dipeptide Boc-D-Phe-Leu-OMe (383.1 mg, 98 %) as a white solid.

HPLC Chiralcel-OD (Hexane : *i*-PrOH) = (99 : 1), flow : 1mL/min



Boc-D,L-Phe-Leu-OMe prepared under classical conditions
Coupling of Boc-D,L-Phe-OH with HCl·H-Leu-OMe under the classical coupling conditions described above afforded the dipeptide Boc-D-Phe-Leu-OMe (335.2 mg, 85 %) as a white solid.

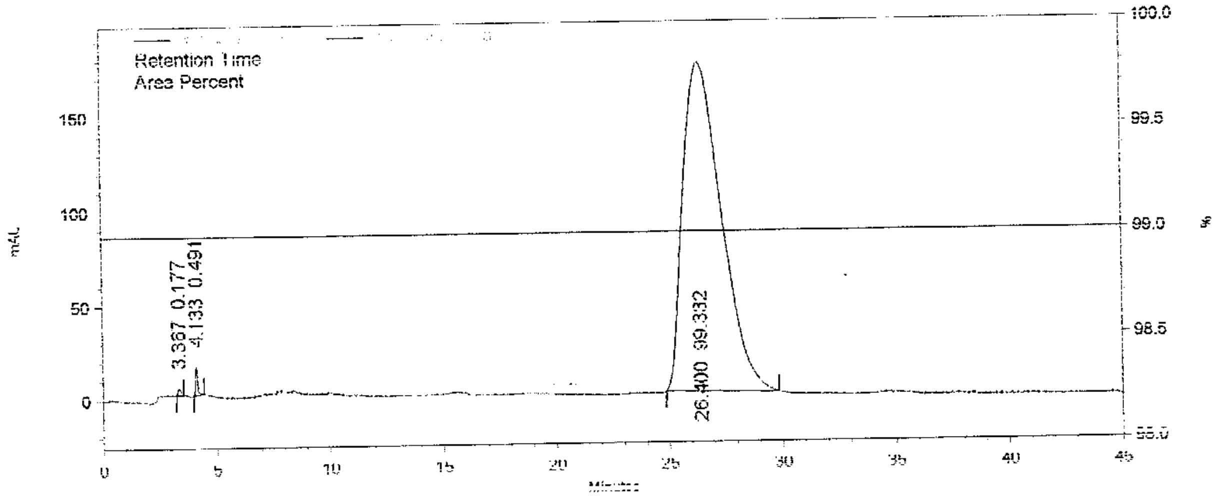
HPLC Chiralcel-OD (Hexane : *i*-PrOH) = (99 : 1), flow : 1mL/min



Electronic Supplementary Material (ESI) for Green Chemistry

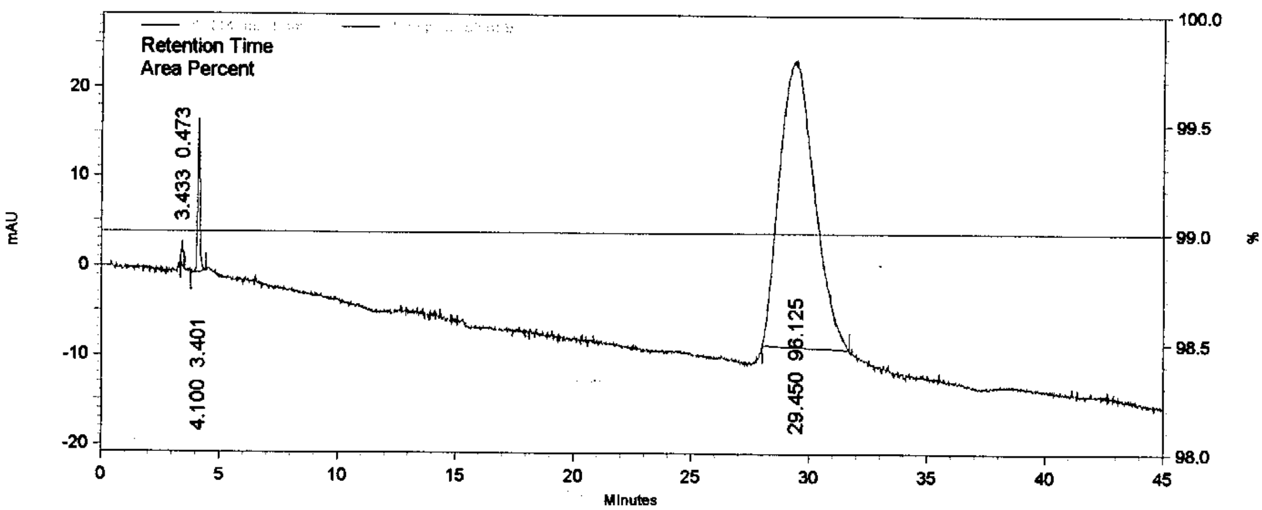
Boc-L-Phe-Leu-OMe prepared under ball-milling conditions from Boc-Phe-NCA
Coupling of Boc-Phe-NCA (37.1 mg, 0.127 mmol, 1 eq.) with HCl·H-Leu-OMe (21.1 mg, 0.127 mmol, 1 eq.) under ball-milling conditions described on page S2 afforded the dipeptide Boc-Phe-Leu-OMe (43.0 mg, 86 %) as a white solid.

HPLC Chiralcel-OD (Hexane : *i*-PrOH) = (99 : 1), flow : 1mL/min



Boc-L-Phe-Leu-OMe prepared under ball-milling conditions from Boc-Phe-OSu
Coupling of Boc-Phe-OSu (138.4 mg, 0.382 mmol, 1 eq.) with HCl·H-Leu-OMe (69.4 mg, 0.382 mmol, 1 eq.) under ball-milling conditions described on page S2 afforded the dipeptide Boc-Phe-Leu-OMe (123.8 mg, 83 %) as a white solid.

HPLC Chiralcel-OD (Hexane : *i*-PrOH) = (99 : 1), flow : 1mL/min



EcoScale calculation[17]

An EcoScale evaluation has been done for production of 10 mmoles of Boc-Tyr(Bn)-Leu-OMe starting from Boc-Tyr(Bn)-OSu, HCl·H-Leu-OMe and $NaHCO_3$ as the reagents and EtOAc as the grinding auxiliary, under ball-mill induced mixing.

	Details	Penalty point	
1. Yield	98%	1	
2. Price of reagents (to obtain 10 mmol of dipeptide)			
	$NaHCO_3$ (0.858 g)	0	(1 kg = 5.5 USD)
	Boc-Tyr(Bn)-OSu (4.8 g)	5	(5 g = 96.4 USD)
	HCl·H-Leu-OMe (1.8 g)	0	(10 g = 29.0 USD)
	EtOAc (10.2 mL)	0	(5 L = 14.0 USD)
3. Safety			
	EtOAc	5	(F)
4. Technical Setup			
	Ball-mill	2	
5. Temperature/time			
	Room temperature, < 1h	0	
6. Workup and purification			
	Liquid-liquid extraction	3	

The sum of all penalty points is 16, which gives total score of **84** on the Ecoscale (an excellent synthesis).

Ecoscale evaluation for production of 10 mmoles of Boc-Tyr(Bn)-Leu-OMe starting from Boc-Tyr(Bn)-OSu, Et_3N and HCl·H-Leu-OMe as the reagents and DMF as the solvent, under classical agitation.[7]

1. Yield	96%	2	
2. Price of reaction reagents (to obtain 10 mmol of dipeptide)			
	Et_3N (1.4 mL)	0	(100 mL = 23.0 USD)
	Boc-Tyr(Bn)-OSu (4.8 g)	5	(5 g = 96.4 USD)
	HCl·H-Leu-OMe (1.9 g)	0	(10 g = 29.0 USD)
	DMF (17.1 mL)	0	(2.5 L = 6.3 USD)
3. Safety			
	Et_3N	10	(F, T)
	DMF	10	(F, T)
4. Technical Setup			
	Common setup	0	
5. Temperature/time			
	Room temperature, < 24h	1	
6. Workup and purification			
	Removal of solvent with bp > 150°C	2	
	Crystallization and filtration	1	

The sum of all penalty points is 31, which gives total score of **69** on the Ecoscale (an acceptable synthesis).

Ecoscale evaluation for production of 10 mmoles of Boc-Tyr(Bn)-Leu-OMe starting from Boc-Tyr(Bn)-OH, EtN(*i*Pr)$_2$, BOP reagent and HCl·H-Leu-OMe as the reagents and DMF as the solvent, under classical agitation.[18]

1. Yield	96%	2	
2. Price of reaction reagents (to obtain 10 mmol of dipeptide)			
	EtN(*i*Pr)$_2$ (12.6 mL)	0	(100 mL = 37.1 USD)
	BOP reagent (4.6 g)	5	(5 g = 56.9 USD)
	Boc-Tyr(Bn)-OH (3.9g)	3	(5 g = 25.40 euros)
	HCl·H-Leu-OMe (1.9g)	0	(10 g = 37.60 euros)
	DMF (52 mL)	0	(2.5 L = 4.76 euros)
3. Safety			
	EtN(*i*Pr)$_2$	10	(F, T)
	BOP reagent	10	(E)
	DMF	10	(F,T)
4. Technical Setup			
	Common setup	0	
5. Temperature/time			
	Room temperature, < 24h	1	
6. Workup and purification			
	Crystallization and filtration	1	

The sum of all penalty points is 42, which gives total score of **58** on the Ecoscale (an acceptable synthesis).

Milling-load calculations

The milling load (ML) can be defined as the sum of the mass of the reactants per free volume (V_f) in the jar. The free volume (V_f) is the volume of the jar minus the volume of the ball (V_{ball}). The milling load is expressed in mg/mL.

For the production of Boc-Phe-Leu-OMe from Boc-Phe-NCA, HCl·H-Leu-OMe and $NaHCO_3$, realised on a 0.10 mmol scale :

$V_f = V_{ball\ mill} - V_{ball}$

$V_f = V_{ball\ mill} - (4/3)\pi r^3$

$V_f = 10 - (4/3)\pi 0.5^3$

$V_f = 10 - 0.524$

$V_f = 9.476$ mL

$ML = (m_{Boc\text{-}Phe\text{-}NCA} + m_{HCl\cdot H\text{-}Leu\text{-}OMe} + m_{NaHCO3})/V_f$

$ML = (29.1 + 18.2 + 8.4)/9.476$

ML = 5.89 mg/mL

For the production of Boc-Phe-Leu-OMe from Boc-Phe-NCA, HCl·H-Leu-OMe and $NaHCO_3$, realised on a 0.382 mmol scale :

$ML = (m_{Boc\text{-}Phe\text{-}NCA} + m_{HCl\cdot H\text{-}Leu\text{-}OMe} + m_{NaHCO3})/V_f$

$ML = (111.3 + 69.4 + 32.1)/9.476$

ML = 22.5 mg/mL

References

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