Supporting Information

Pyridazine Nucleobase in Peptide Nucleic Acids Improves Triple Helical Recognition of Cytosine Interruptions of Polypurine Tracts in Double-Stranded RNA

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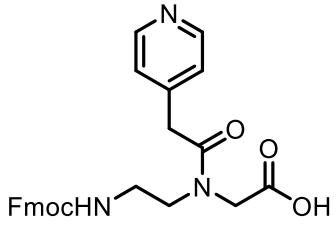
General Synthetic Procedures. Solvents and reagents were obtained from commercial suppliers and were used without further purification unless stated otherwise. THF and methylene chloride were dried by passing over activated alumina. The anhydrous reactions were carried out under an atmosphere of nitrogen using a Schlenk line or argon from a balloon. Analytical thin layer chromatography (TLC) was carried out using either Merck silica gel 60 F254 plates (0.2 mm) or Silacycle 60 Å silica gel F254 plates (0.25 mm) and visualization was aided by UV light, iodine, or $KMnO_4$ stain. Column chromatography was performed using either Merck Kieselgel 60 H or Silacycle P60 230−400 mesh silica gel or using flash chromatography system using CM modified silica (Agela Technologies). NMR spectra were obtained using Bruker AM 400 or 300 spectrometers with the chemical shift (δ) reported in parts per million (ppm) relative to TMS or to the solvent peak [dimethyl sulfoxide (DMSO)-d6 or $CDCl_3$] as a reference. High resolution mass spectrometry (HRMS) analyses using positive electrospray ionization (ESI+) were recorded on a Micromass quadrupole time-of-flight (Q-TOF) microinstrument.

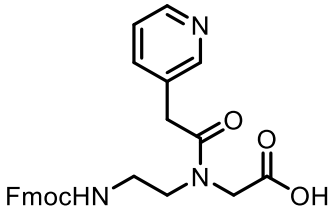
N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-(pyridin-4-yl)acetyl)glycine (1)



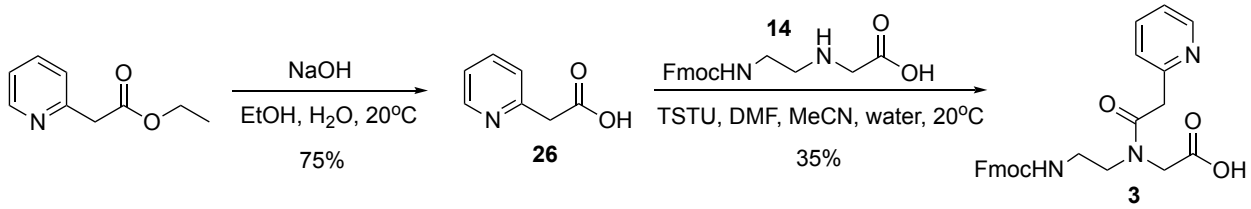
TSTU (262 mg, 0.87 mmol) was added under nitrogen to a solution of 2-(pyridin-4-yl)acetic acid HCl salt (SIgmaAldrich Cat.No. P65851) (137 mg, 0.79 mmol) and *i*-Pr_2NEt (275 µL, 2.00 mmol) in anhydrous DMF (4 mL). After 30 minutes at room temperature solution of N-Fmoc-Aeg-OH HCl salt (**14**, 297 mg, 0.79 mmol) and *i*-Pr_2NEt (137 µL, 0.79 mmol, 1 equiv.) in anhydrous DMF (2

mL) were added. The resulting solution was stirred for 12 h at room temperature, then acidified to pH 6-7 using a 1 M aqueous HCl solution and evaporated under reduced pressure. The crude product was purified by flash chromatography on CM-modified silica (Agela Technologies) using a linear gradient (0-8%) of CH_2Cl_2 in MeOH to afford the title compound as white foam (66 mg, 18% yield). R_f = 0.36 (20% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{26}H_{26}N_3O_5$, 460.1872; found 460.1870. ^{1}H NMR (400 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 8.44 (2H, s), 7.88 (2H, d, *J* = 7.5 Hz), 7.67 (2H, d, *J* = 7.4 Hz, 2H), 7.58 – 7.47 (1H, m), 7.40 (2H, t, *J* = 7.4 Hz), 7.36 – 7.13 (4H, m), 4.32 (1H, d, *J* = 6.8 Hz), 4.29 – 4.15 (2H, m), 4.03 – 3.84 (2H, m), 3.75 (1H, s), 3.62 (1H, s), 3.51 – 3.32 (2H, m), 3.18 (2H, q, *J* = 7.6, 6.1 Hz), 2.91 (1H, q, *J* = 7.3 Hz). ^{13}C NMR (101 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 170.64, 156.59, 149.76, 149.61, 145.63, 144.40, 144.34, 141.24, 141.18, 128.11, 128.08, 127.57, 125.72, 125.57, 125.22, 120.62, 120.57, 65.96, 52.85, 52.21, 48.70, 48.45, 47.55, 47.20, 41.07, 39.23, 39.05, 38.68, 38.62, 18.56.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-(pyridin-3-yl)acetyl)glycine (2)



TSTU (331 mg, 1.10 mmol) was added under nitrogen to a solution of 2-(pyridin-3-yl)acetic acid (FluoroChem Cat.No. 076680) (137 mg, 1.00 mmol) and *i*-Pr_2NEt (226 µL, 1.30 mmol, 1.3 equiv.) in anhydrous DMF (3.5 mL). After 30 minutes at room temperature solution of N-Fmoc-Aeg-OH HCl salt (**14**, 377 mg, 1.00 mmol) and *i*-Pr_2NEt (226 µL, 1.30 mmol, 1.3 equiv.) in anhydrous DMF (2 mL) were added. The resulting solution was stirred for 12 h at room temperature, then acidified to pH 6-7 using a 1 M aqueous HCl solution and evaporated under reduced pressure. The crude product was purified by flash chromatography on CM-modified silica (Agela Technologies) using a linear gradient (0-8%) of CH_2Cl_2 in MeOH to afford the title compound as white foam (67 mg, 15% yield). R_f = 0.40 (20% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{26}H_{26}N_3O_5$, 460.1872; found 460.1868. ^{1}H NMR (400 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 12.65 (1H, s), 8.40 (2H, d, *J* = 19.5 Hz), 7.88 (2H, d, *J* = 7.5 Hz), 7.67 (2H, d, *J* = 7.6 Hz), 7.63 – 7.53 (1H, m), 7.51 – 7.36 (3H, m), 7.31 (3H, td, *J* = 7.6, 2.7 Hz), 4.34 (1H, d, *J* = 6.7 Hz), 4.28 (1H, d, *J* = 7.0 Hz), 4.22 (1H, d, *J* = 6.6 Hz), 3.96 (1H, s), 3.76 (1H, s), 3.67 – 3.54 (2H, m), 3.45 (2H, t, *J* = 6.6 Hz), 3.22 (2H, q, *J* = 6.4 Hz), 3.12 (1H, ddd, *J* = 11.0, 7.6, 5.5 Hz). ^{13}C NMR (101 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 171.21, 170.70, 156.84, 150.74, 148.02, 144.38, 144.33, 141.24, 141.21, 137.61, 137.36, 128.11, 127.55, 125.63, 125.55, 120.62, 65.93, 53.87, 48.34, 48.04, 47.20, 42.14, 36.21.

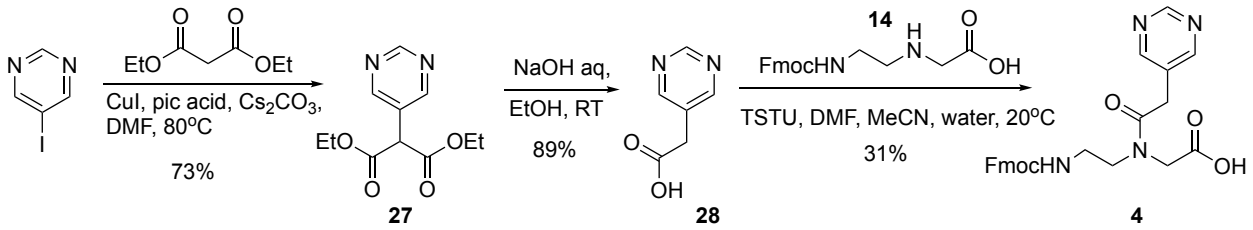


2-(Pyridin-2-yl)acetic acid (26)

NaOH (339 mg, 8.47 mmol, 2 equiv.) in water (3 mL) was added to a solution of ethyl 2-pyridylacetate (Fluorochem Cat.No. 2739-98-2) (700 mg, 4.23 mmol) in EtOH (10 mL). After 1 h at room temperature Amberlite was added till pH ~3-4, the mixture was filtered, solids were washed with a mixture of water and EtOH (1:1), and evaporated under reduced pressure to afford the title compound as a yellow solid (442 mg, 75% yield). ^{1}H NMR (300 MHz, DMSO-d6, ppm) δ: 13.0 – 12.2 (1H, br s), 8.48 (1H, ddd, *J* = 4.9, 1.9, 1.1 Hz), 7.76 (1H, td, *J* = 7.7, 1.9 Hz), 7.35 (1H, dt, *J* = 7.7, 1.1 Hz), 7.27 (1H, ddd, *J* = 7.7, 4.9, 1.1 Hz), 3.75 (2H, s). ^{13}C NMR (100.6 MHz, DMSO-d6, ppm) δ: 171.8, 157.6, 148.5, 136.7, 123.3, 121.0, 23.8.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-(pyridin-2-yl)acetyl)glycine (3)

TSTU (472 mg, 1.56 mmol) was added under argon to a solution of 2-(pyridin-2-yl)acetic acid (**26**) (215 mg, 1.56 mmol) and *i*-Pr_2NEt (540 µL, 3.13 mmol, 2 equiv.) in anhydrous THF (10 mL). After 1h at room temperature solution of N-Fmoc-Aeg-OH (**14**, 534 mg, 1.56 mmol) and *i*-Pr_2NEt (542 µL, 3.13 mmol, 2 equiv) in a mixture of H_2O/MeCN (1:1, 14 mL) were added. The resulting solution was stirred for 14 h at room temperature, then acidified to pH 2-3 using a 20% aqueous solution of citric acid, extracted with CH_2Cl_2 (3 × 15 mL). Organic phases were combined, extracted with saturated aqueous NaCl, dried with Na_2SO_4, evaporated under reduced pressure. The crude product was purified by reverse phase column chromatography using a linear gradient (0-60%) of MeCN in water to afford the title compound as an off-white solid (253 mg, 35% yield). HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ calcd. for $C_{26}H_{26}N_3O_5$ 460.1872; found 460.1875. ^{1}H NMR (400 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 13.6 – 11.9 (1H, br s), 8.47 – 8.40 (1H, m), 7.89 (2H, d, *J* = 7.6 Hz), 7.73 – 7.63 (3H, m), 7.47 – 7.19 (7H, m), 4.45 – 4.15 (4H, m), 3.96 (1H, s), 3.89 (1H, s), 3.74 (1H, s), 3.53 – 3.30 (2H, m), 3.25 – 3.09 (2H, m). ^{13}C NMR (100.6 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 170.8, 170.0, 156.3, 156.0, 155.8, 148.81, 148.75, 143.88, 143.85, 140.74, 140.71, 136.39, 136.36, 127.6, 127.1, 125.2, 125.1, 124.0, 123.9, 121.8, 120.1, 65.4, 48.0, 47.5, 46.7, 42.5, 41.7. IR (neat, cm^{-1}) 3334, 3066, 2940, 1717, 1653, 1646, 1539, 1260, 1208.



Diethyl 2-(pyrimidin-5-yl)malonate (27)

Diethyl malonate (4.42 mL, 29.1 mmol, 2 equiv.) was added under argon to a solution of 5-iodopyrimidine (3.00 g, 14.6 mmol), Cs_2CO_3 (10.1 g, 33.50 mmol, 2.3 equiv.), CuI (277 mg, 1.45 mmol, 0.1 equiv.) and picolinic acid (358 mg, 2.91 mmol, 0.2 equiv.) in anhydrous dioxane (40 mL). The reaction mixture was stirred at 80°C for 2 days, then cooled to room temperature, diluted with EtOAc (50 mL) and extracted with NH_4Cl (30 mL). The organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using a linear gradient (30-100%) of EtOAc in petrol ether to afford the title compound as a light yellow oil (2.53 g, 73% yield). R_f = 0.29 (hexanes/EtOAc, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 9.20 (1H, s), 8.81 (2H, s), 4.59 (1H, s), 4.34 – 4.18 (4H, m), 1.29 (3H, t, *J* = 7.1 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ: 166.7, 158.5, 157.6, 127.3, 62.8, 53.9, 14.1. HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ Calcd. for $C_{11}H_{15}N_2O_4$ 239.1032; Found 239.1034. IR (neat, cm^{-1}) 3049, 2984, 2940, 2909, 1748, 1563, 1315, 1244.

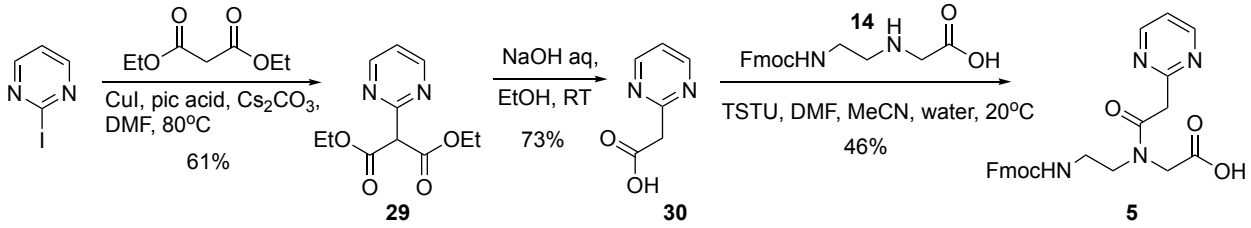
2-(Pyrimidin-5-yl)acetic acid (28)

3 M solution of NaOH in water (1.00 g, 25.0 mmol, 2.36 equiv.) was added to a solution of diethyl 2-(pyrimidin-5-yl)malonate (**27**, 2.52 g, 10.6 mmol) in EtOH (20 mL). After 3 days at room temperature Amberlite was added till pH = 3-4, the mixture was filtered, solids were washed with EtOH, evaporated under reduced pressure to afford the title compound as a yellow solid (1.30 g, 89% yield). ^{1}H NMR (300 MHz, DMSO-d6, ppm) δ: 12.9 – 12.3 (1H, br s), 9.07 (1H, s), 8.71 (2H, s), 3.70 (2H, s). ^{13}C NMR (100.6 MHz, DMSO-d6, ppm) δ: 171.8, 157.7, 156.8, 129.2, 35.0.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-(pyrimidin-5-yl)acetyl)glycine (4)

TSTU (1.20 g, 3.98 mmol, 1.1 equiv.) was added under argon to a solution of 2-(pyrimidin-5-yl)acetic acid (**28**, 500 mg, 3.62 mmol) and *i*-Pr_2NEt (0.82 ml, 4.71 mmol, 1.3 equiv.) in anhydrous THF (20 mL) and the reaction was stirred for 1h 45min (suspension) at room temperature followed by the addition of a solution of N-Fmoc-Aeg-OH (**14**, 1.23 g, 3.62 mmol) and *i*-Pr_2NEt (0.82 mL, 4.71 mmol, 1.3 equiv.) in mixture of H_2O/MeCN (15 mL, 1:1). After 20 h at room temperature, the reaction mixture was acidified to pH 3 using 20% aqueous solution of citric acid and extracted with EtOAc (3 × 50 mL). Organic phases were combined, dried with

Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by reverse phase column chromatography using a linear gradient (20-60%) of MeCN in water to afford the title compound as a white solid (530 mg, 31% yield). HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ calcd. for $C_{25}H_{25}N_4O_5$ 461.1825; found 461.1819. ^{1}H NMR (300 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 13.0 – 12.5 (1H, br s), 9.04 (1H, d, *J* = 5.2 Hz), 8.61 (2H, d, *J* = 5.9 Hz), 7.88 (2H, d, *J* = 7.5 Hz), 7.67 (d, *J* = 7.5 Hz, 2H), 7.46 – 7.27 (5H, m), 4.43 – 4.14 (4H, m), 3.97 (1H, s), 3.83 (1H, s), 3.67 (1H, s), 3.52 – 3.33 (2H, m), 3.26 – 3.08 (2H, m). ^{13}C NMR (100.6 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 170.6, 170.2, 169.6, 157.7, 157.6, 156.6, 156.5, 156.3, 156.1, 143.9, 143.8, 140.73, 140.71, 127.6, 127.0, 125.1, 125.0, 120.1, 65.4, 47.9, 47.7, 46.7, 38.1, 33.4, 33.1. IR (neat, cm^{-1}) 3347, 3064, 2949, 2496, 1700, 1653, 1246.



Diethyl 2-(pyrimidin-2-yl)malonate (29)

2-Iodopyridine (10.0 g, 48.5 mmol), Cs_2CO_3 (38.0 g, 117 mmol, 2.4 equiv.), CuI (1.85 g, 9.71 mmol, 0.2 equiv.) and picolinic acid (2.39 , 19.4 mmol, 0.4 equiv.) were placed into a dry flask under atmosphere of argon. DMF (75 mL) was added followed by diethyl malonate (14.7 mL, 97.1 mmol, 2 equiv.) and the reaction mixture was stirred at 80 °C for 16 h. The brown mixture was cooled to room temperature, filtered through a pad of celite, and celite was washed with EtOAc (4 × 30 mL). The mixture was extracted with NH_4Cl (70 mL), the organic layer was dried with Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using a linear gradient (20-50%) of EtOAc in petrol ether to afford the title compound as a yellow oil (7.12 g, 61% yield). R_f = 0.63 (hexanes/EtOAc, 1:1). HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ calcd. for $C_{11}H_{15}N_2O_4$ 239.1032; found 239.1040. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 8.75 (2H, d, *J* = 4.9 Hz), 7.25 (1H, t, *J* = 4.9 Hz), 5.10 (1H, s), 4.28 (4H, t, *J* = 7.1 Hz), 1.29 (6H, t, *J* = 7.1 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ: 166.7, 163.7, 157.6, 120.0, 62.2, 62.0, 14.1. IR (neat, cm^{-1}) 3473, 2984, 2941, 2907, 1756, 1568, 1423, 1308, 1255, 1179, 1153.

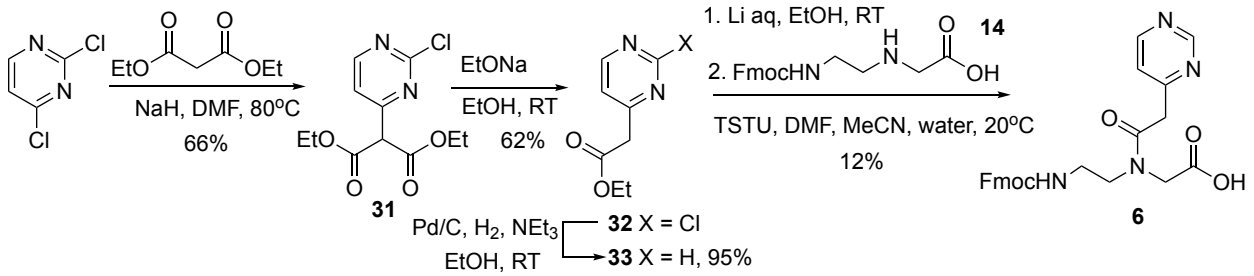
2-(Pyrimidin-2-yl)acetic acid (30)

NaOH (1.91 g, 47.85 mmol, 15 mL, 3 M in water, 3eq) in water was added to a solution of diethyl 2-(pyrimidin-2-yl)malonate (**29**, 3.80 g, 16.0 mmol) in EtOH (20 mL). The reaction mixture was stirred at room temperature for 22 h, then partly evaporated under reduced pressure, and acidified with 1M HCl. Water phase was separated and extracted with EtOAc (8 × 25 mL). All organic phases were combined, dried with Na_2SO_4, and evaporated under reduced

pressure to afford the title compound as a yellow solid (1.73 g, 73% yield), which was used in the next reaction without further purification.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-(pyrimidin-2-yl)acetyl)glycine (5)

TSTU (1.20 g, 3.98 mmol, 1.1 equiv.) was added to a solution of the 2-(pyrimidin-2-yl)acetic acid (500 mg, 3.62 mmol) and *i*-Pr_2NEt (0.81 mL, 4.70 mmol, 1.3 equiv) in anhydrous THF (20 mL). The reaction mixture was stirred for 4h (suspension) at room temperature followed by the addition of a solution of N-Fmoc-Aeg-OH (**14**, 1.20 g, 3.62 mmol, 1.0 equiv) and *i*-Pr_2NEt (0.81 mL, 4.70 mmol, 1.3 equiv.) in a H_2O/MeCN mixture (20 mL, 1:1). The resulting solution was stirred for 18 h at room temperature. The reaction mixture was acidified to pH 3-4 using a 20% aqueous solution of citric acid and extracted with EtOAc (3 × 50 mL). The organic phases were combined, dried with Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by reverse phase column chromatography using a linear gradient (0-45%) of MeCN in water to afford the title compound as an off-white foam (800 mg, 46% yield). HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ calcd. for $C_{25}H_{25}N_4O_5$ 461.1825; found 461.1831. ^{1}H NMR (400 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 13.2 – 12.3 (1H, br s), 8.71 (1H, d, *J* = 4.9 Hz), 8.70 (1H, d, *J* = 2.4 Hz) 7.88 (2H, d, *J* = 7.5 Hz), 7.67 (2H, d, *J* = 7.4 Hz), 7.44 – 7.24 (6H, m), 4.47 – 4.17 (4H, m), 4.05 (1H, s), 3.97 (1H, s), 3.90 (1H, s), 3.55 – 3.30 (2H, m), 3.25 – 3.07 (2H, m). ^{13}C NMR (100.6 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 171.2, 170.7, 169.6, 169.3, 165.4, 165.2, 157.2, 156.3, 143.88, 143.85, 140.73, 140.71, 127.6, 127.1, 125.2, 125.1, 120.1, 119.4, 65.4, 48.0, 47.5, 46.7, 46.4, 44.3, 43.5. IR (neat, cm^{-1}) 3316, 3049, 2945, 2507, 1706, 1565, 1231.



Diethyl 2-(2-chloropyrimidin-4-yl)malonate (31)

Diethylmalonate (7.13 mL, 47.0 mmol, 2 equiv) was added dropwise at 0 °C to a suspension of NaH (1.88 g, 47.0 mmol, 2 equiv) in anhydrous DMF (40 mL). The reaction mixture was stirred at room temperature till it become clear (15 min), then 2,4-dichloropyrimidine (3.50 g, 24.5 mmol) was added and mixture was stirred at 80 °C for 35 h. The mixture was diluted with EtOAc (60 mL) and saturated aqueous NH_4Cl (30 mL) was added. The water phase was separated and extracted with EtOAc (15 mL). The organic phases were combined, extracted with saturated aqueous NaCl (30 mL), dried with Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using a linear gradient (0-25%) of EtOAc in petrol ether to afford the title compound as a colorless oil (4.24 g, 66% yield). R_f = 0.40 (hexanes/EtOAc, 3:1). HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ calcd. for $C_{11}H_{14}ClN_2O_4$ 273.0642; found 273.0635. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 8.64 (1H, d, *J* = 5.1 Hz), 7.55 (1H, d, *J* = 5.1 Hz), 4.84 (1H, s), 4.31 – 4.20 (4H, m), 1.28 (6H, t, *J* = 7.1 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ: 165.8, 164.5, 161.2, 159.9, 119.8, 62.8, 59.9, 14.1. IR (neat, cm^{-1}) 3462, 2985, 2941, 2908, 1756, 1569, 1542, 1348, 1308.

Ethyl 2-(2-chloropyrimidin-4-yl)acetate (32)

A 21% EtONa solution in absolute EtOH (2.9 mL, 7.8 mmol, 0.5 eq) was added to a solution of diethyl 2-(2-chloropyrimidin-4-yl)malonate (**31**, 4.24 g, 15.5 mmol) in absolute EtOH (20 mL). The reaction mixture was stirred at 75 °C for 21 h. After cooling to room temperature, 1M HCl was added to adjust pH to 7. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using a linear gradient (10-30%) of EtOAc in petrol ether to afford the title compound as a yellow oil (1.94 g, 62% yield). R_f = 0.36 (hexanes/EtOAc, 3:1). HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ calcd. for $C_8H_{10}ClN_2O_2$ 201.0431; found 201.0429. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 8.59 (1H, d, *J* = 5.1 Hz), 7.33 (1H, d, *J* = 5.0 Hz), 4.21 (2H, q, *J* = 7.1 Hz), 3.82 (2H, s), 1.28 (3H, t, *J* = 7.1 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ: 168.7, 166.3, 161.4, 159.7, 120.0, 61.8, 43.1, 14.3. IR (neat, cm^{-1}) 3467, 2984, 2940, 2908, 2524, 1737, 1576, 1542, 1345, 1267, 1183.

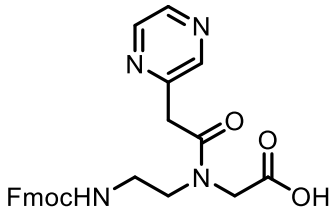
Ethyl 2-(pyrimidin-4-yl)acetate (33)

NEt_3 (0.8 mL, 6.0 mmol, 2 equiv.) and Pd/C (100 mg) were added to a solution of ethyl 2-(2-chloropyrimidin-4-yl)acetate (**32**, 600 mg, 2.99 mmol) in EtOH (7 mL). H_2 gas (1 atm) was bubbled through the reaction mixture for 2 h. The mixture was filtered through pad of celite, celite was washed with EtOH (3 × 3 mL), and the solutions were evaporated under reduced pressure to afford the title compound as a yellow oil that contained some residual NEt_3 (875 mg, 95% estimated yield). For analytical purpose product was purified by silica gel flash column chromatography using a linear gradient (30-50%) of EtOAc in petrol ether. Rf = 0.46 (hexanes/EtOAc, 1:1). HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ Calcd. for $C_8H_{11}N_2O_2$ 167.0821; found 167.0826. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 9.15 (1H, d, *J* = 1.4 Hz), 8.68 (1H, d, *J* = 5.2 Hz), 7.35 (1H, dd, *J* = 5.2, 1.4 Hz), 4.19 (2H, q, *J* = 7.1 Hz), 3.80 (2H, s), 1.26 (3H, t, *J* = 7.1 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ: 169.3, 162.9, 159.0, 157.2, 121.5, 61.6, 43.5, 14.2. IR (neat, cm^{-1}) 3461, 3043, 2983, 2940, 2908, 1739, 1582, 1389, 1258, 1184.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-(pyrimidin-4-yl)acetyl)glycine (6)

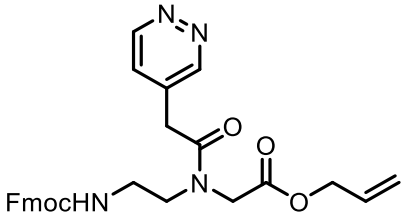
A solution of LiOH (159 mg, 6.60 mmol, 3 equiv.) in water (4 mL) was added to a solution of ethyl 2-(pyrimidin-4-yl)acetate (**33**, 2.20 mmol) in EtOH (8 mL). The reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure, the residual orange solid was dried in vacuum for 5 h and used directly in the next step without purification. 2-(Pyrimidin-4-yl)acetic acid Li salt (2.17 mmol) was suspended in DMF (8 mL) and N-methylmorpholine (0.478 mL, 4.34 mmol, 2 equiv.) was added. Then TSTU (654 mg, 2.17 mmol, 1 eq) was added and, after stirring for 1 h at room temperature, a solution of Fmoc-Aeg-OH (**14**, 0.739 g, 2.17 mmol, 1 equiv.) and *i*-Pr_2NEt (0.75 mL, 4.34 mmol, 2 equiv.) in a mixture of water and MeCN (15 mL, 1:1) was added. The reaction was stirred at room temperature overnight. The solvent was partially evaporated under reduced pressure and the residue was purified by reverse phase column chromatography using a linear gradient (0-60%) of MeCN in water to afford the title compound as a brown foam after lyophilized (115 mg, 11% yield). HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ calcd. for $C_{25}H_{25}N_4O_5$ 461.1825; found 461.1813. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) (mixture of rotamers, 3:2) δ 13.4 – 12.5 (1H, br s), 9.06 (0.6H, d, *J* = 1.4 Hz), 9.04 (0.4H, d, *J* = 1.4 Hz), 8.70 (0.6H, d, *J* = 5.2 Hz), 8.66 (0.4H, d, *J* = 5.2 Hz), 7.88 (2H, d, *J* = 7.5 Hz), 7.67 (2H, d, *J* = 7.5 Hz), 7.45 – 7.29 (6H, m), 4.35 – 4.13 (4H, m), 3.96 (1H, s), 3.92 (1H, s), 3.78 (1H, s), 3.48 (1H, t, *J* = 6.7 Hz), 3.35 (1H, t, *J* = 6.7 Hz), 3.23 (1H, q, *J* = 6.4 Hz), 3.13 (1H, q, *J* = 6.4 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 170.7, 169.4, 168.9, 164.6, 158.1, 158.0, 156.8, 156.6, 143.9, 143.8, 140.73, 140.69, 127.6, 127.1, 125.2, 125.1, 122.1, 121.9, 120.1, 65.4, 54.9, 48.0, 47.6, 46.7, 41.8, 41.2, 38.1. IR (Nujol, cm^{-1}) 3284, 2942, 2688, 2493, 2251, 1447, 1377.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-(pyrazin-2-yl)acetyl)glycine (7)



TSTU (720 mg, 2.39 mmol) was added under nitrogen to a solution of 2-(pyrazin-2-yl)acetic acid (FluoroChem Cat.No 040103) (300 mg, 2.17 mmol) and *i*-Pr_2NEt (490 µL, 2.82 mmol, 1.3 equiv.) in anhydrous DMF (11 mL). After stirring for 30 minutes at room temperature, a solution of N-Fmoc-Aeg-OH (HCl salt) (**14**, 818 mg, 2.17 mmol) and *i*-Pr_2NEt (490 µL, 2.82 mmol, 1.3 equiv.) in anhydrous DMF (4 mL) were added. The resulting solution was stirred for 12 hr at room temperature, then acidified to pH 6-7 with 1 M aqueous HCl solution, and evaporated under reduced pressure. The crude product was purified by flash chromatography on CM-modified silica (Agela Technologies) using a linear gradient (0-8%) of MeOH in CH_2Cl_2 to afford the title compound as a white foam (394 mg, 39% yield). R_f = 0.16 (20% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{25}H_{25}N_4O_5$, 461.1825; found 461.1818. ^{1}H NMR (400 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 12.78 (1H, s), 8.67 – 8.40 (3H, m), 7.89 (2H, d, *J* = 7.5 Hz), 7.68 (2H, d, *J* = 7.5 Hz), 7.56 – 7.38 (3H, m), 7.32 (3H, tdd, *J* = 7.5, 3.6, 1.2 Hz), 4.35 (1H, d, *J* = 6.8 Hz), 4.30 (1H, d, *J* = 6.2 Hz), 4.22 (1H, q, *J* = 6.6 Hz), 4.00 (2H, d, *J* = 4.9 Hz), 3.86 (1H, s), 3.56 (2H, dt, *J* = 21.7, 6.6 Hz), 3.37 (1H, t, *J* = 6.7 Hz), 3.27 (2H, q, *J* = 6.4 Hz), 3.12 (2H, dt, *J* = 14.7, 7.3 Hz). ^{13}C NMR (101 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 171.13, 169.98, 156.83, 146.31, 146.14, 144.38, 144.34, 144.30, 144.21, 143.10, 143.01, 141.24, 141.21, 128.10, 127.55, 125.64, 125.56, 120.60, 65.94, 53.78, 50.62, 48.43, 48.06, 47.22, 40.01, 12.66.

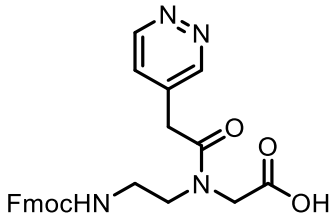
Allyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-(pyridazin-4-yl)acetyl)glycinate (19)



HATU (130 mg, 0.34 mmol) was added under nitrogen to a solution of sodium 2-(pyridazin-4-yl)acetate (FluoroChem Cat.No 464382) (**17**, 50 mg, 0.31 mmol) and *i*-Pr_2NEt (108 µL, 0.62 mmol, 2 equiv.) in anhydrous DMF (2 mL). After stirring for 30 minutes at room temperature, solid N-Fmoc-Aeg-O-Allyl HCl salt (**18**, 130 mg, 0.31 mmol) was added. The resulting solution was stirred for 12 h at room temperature, then concentrated under reduced pressure. The product mixture was redissolved in CH_2Cl_2 (30 mL) and washed with 5% aqueous $NaHCO_3$ (10 mL). The aqueous layer was back-extracted in CH_2Cl_2 (20 mL), the organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography using a linear gradient of MeOH (0-5%) in CH_2Cl_2 to

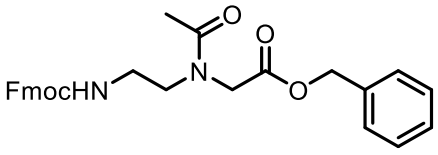
afford the title compound as yellow oil (46 mg, 29% yield). R_f = 0.25 (5% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{28}H_{29}N_4O_5$, 501.2138; found 501.2134. ^{1}H NMR (400 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 9.19 – 9.03 (2H, m), 7.89 (2H, d, *J* = 7.5 Hz), 7.68 (2H, d, *J* = 7.4 Hz), 7.55 – 7.37 (4H, m), 7.32 (2H, tdd, *J* = 7.5, 2.9, 1.1 Hz), 6.05 – 5.82 (1H, m), 5.42 – 5.16 (2H, m), 4.62 (2H, ddt, *J* = 26.9, 5.4, 1.5 Hz), 4.47 – 4.18 (3H, m), 4.11 (1H, s), 3.88 (1H, s), 3.74 (1H, s), 3.50 (1H, t, *J* = 6.5 Hz), 3.36 (1H, d, *J* = 17.8 Hz), 3.33 – 3.20 (1H, m), 3.20 – 3.10 (1H, m). ^{13}C NMR (101 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 169.72, 169.35, 156.86, 156.65, 153.79, 153.63, 151.52, 151.48, 144.38, 144.33, 141.25, 136.23, 132.79, 132.68, 128.11, 128.03, 127.79, 127.54, 125.61, 125.52, 120.62, 118.78, 118.36, 65.92, 65.34, 50.54, 48.52, 48.30, 47.21, 38.58, 36.12, 35.78.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-(pyridazin-4-yl)acetyl)glycine (8)



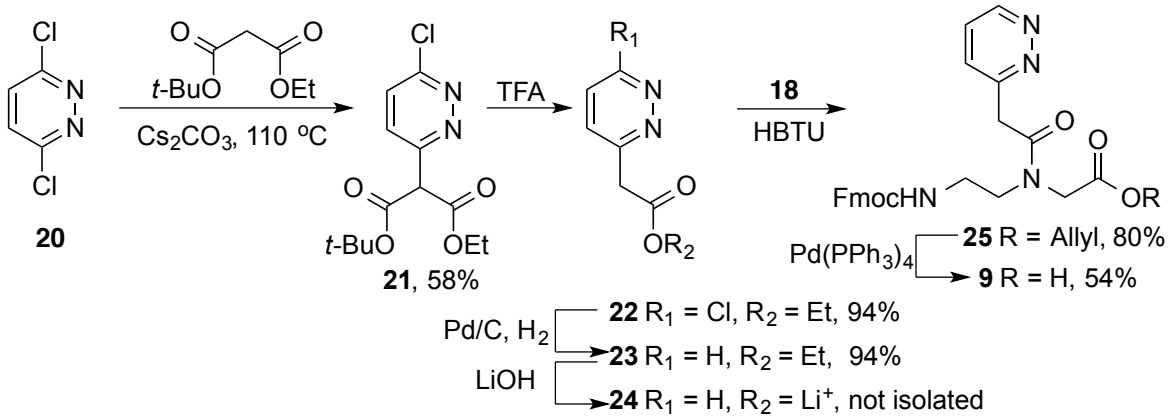
The allyl ester of pyridazin-4-yl monomer (**19**, 98 mg, 0.20 mmol) was dissolved in anhydrous THF (7 mL), $Pd(PPh_3)_4$ (9 mg, 0.01 mmol) was added followed by *N*-ethylaniline (50 µL, 0.34 mmol). The solution was stirred under nitrogen for 3 h at room temperature. After the reaction was complete, the solvent was removed under reduced pressure. The crude product was purified by flash chromatography using CM-modified silica gel (Agela Technologies) and a linear gradient (0-10%) of MeOH in CH_2Cl_2 to afford the title compound as a yellow oil (24 mg, 25% yield). R_f = 0.03 (20% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{25}H_{25}N_4O_5$, 461.1825; found 461.1816. ^{1}H NMR (400 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 12.78 (1H, s), 9.19 – 8.95 (2H, m), 7.89 (2H, d, *J* = 7.5 Hz), 7.68 (2H, d, *J* = 7.5 Hz), 7.50 (1H, dt, *J* = 5.1, 2.4 Hz), 7.42 (3H, t, *J* = 7.5 Hz), 7.37 – 7.23 (2H, m), 4.41 – 4.13 (4H, m), 3.99 (1H, s), 3.87 (1H, s), 3.72 (1H, s), 3.47 (2H, t, *J* = 6.5 Hz), 3.43 – 3.29 (3H, m). ^{13}C NMR (101 MHz, DMSO-d6, ppm) (mixture of rotamers) δ: 171.09, 169.52, 156.84, 153.82, 153.68, 151.50, 151.45, 144.38, 144.32, 141.25, 141.22, 136.38, 128.11, 128.05, 127.85, 127.55, 125.63, 125.54, 120.62, 65.94, 65.87, 48.40, 48.13, 47.21, 39.27, 36.11, 35.80.

Benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-acetylglycinate (16)



N-Fmoc-Aeg-O-Bn HCl salt (**15**, 2.00 mg, 4.29 mmol) was suspended in anhydrous CH_2Cl_2 (50 mL) followed by addition of *i*-Pr_2NEt (970 µL, 5.57 mmol, 1.3 equiv.). The solution became clear

over 5 min, and acetic anhydride (530 µL, 5.57 mmol, 1.3 equiv.) was added. The reaction mixture was stirred for 1 h at room temperature, concentrated under reduced pressure, and the residue was purified by silica gel chromatography using a linear gradient (0-3%) of MeOH CH_2Cl_2 to afford the title compound as transparent oil (2.02 g, 99% yield). R_f = 0.44 (5% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{28}H_{29}N_2O_5$, 473.2076; found 473.2075. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 7.88 (2H, dd, *J* = 7.7, 2.5 Hz), 7.67 (2H, dd, *J* = 7.5, 2.5 Hz), 7.51 – 7.28 (10H, m), 5.19 (1H, s), 5.13 (1H, s), 4.42 – 4.16 (3H, m), 4.06 (1H, s), 3.47 – 3.29 (2H, m), 3.27 – 3.07 (2H, s), 2.00 (2H, s), 1.91 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 170.82, 169.92, 156.76, 144.39, 144.34, 141.26, 141.23, 136.41, 128.96, 128.91, 128.70, 128.52, 128.30, 128.09, 127.54, 125.63, 125.53, 120.60, 66.89, 66.26, 65.82, 48.99, 47.87, 47.23, 39.27, 21.76, 21.12.



1-(*tert*-Butyl)-3-ethyl-2-(6-chloropyridazin-3-yl)malonate (21). *tert*-Butyl ethyl malonate (2.8 mL, 15 mmol) was added to a solution of 3,6-dichloropyridazine (**20**, 1.5 g, 10 mmol) in DMSO (3 mL). Then Cs_2CO_3 (6.5 g, 20 mmol) was added and the reaction mixture was kept at 110 ˚C for 1 h. The mixture was diluted with EtOAc (100 mL) and water (50 mL) and the aqueous phase was washed with EtOAc (2 × 100 mL). The organic phases were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography using a linear gradient (0-30%) of EtOAc in hexanes to afford the title compound as a yellow oil (1.7 g, 58% yield). R_f = 0.43 (25% EtOAc in hexanes). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{13}H_{17}N_2O_4ClNa$, 323.0775; found 323.0771.^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 7.76 (1H, d, *J* = 8.9 Hz), 7.51 (1H, d, *J* = 8.9 Hz), 5.10 (1H, s), 4.31 – 4.05 (2H, m), 1.40 (9H, s), 1.23 (3H, t, *J* = 7.1 Hz). ^{13}C NMR (101 MHz, $CDCl_3$, ppm) δ: 166.76, 165.41, 156.74, 155.94, 130.06, 128.28, 83.72, 62.38, 59.00, 27.76, 13.98.

Ethyl 2-(6-chloropyridazin-3-yl)acetate (22). TFA (6 mL) was added to a solution of 1-(tert-butyl) 3-ethyl 2-(6-chloropyridazin-3-yl)malonate (**21**, 1.6 g, 5.4 mmol) in CH_2Cl_2 (6 mL). The reaction was completed in 30 min at room temperature. The reaction mixture was concentrated under reduced pressure and purified by silica gel chromatography using a linear gradient (0-50%) of EtOAc in hexanes to afford the title compound as a pale yellow oil (1.0 g,

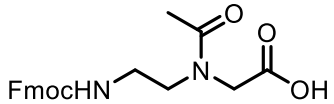
94% yield). R_f = 0.68 (5% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_8H_{10}N_2O_2Cl$, 201.0431; found 201.0433. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 7.55 (1H, d, *J* = 8.8 Hz), 7.48 (1H, d, *J* = 8.8 Hz), 4.14 (2H, q, *J* = 7.1 Hz), 4.00 (2H, s), 1.22 (3H, t, *J* = 7.2 Hz).^{13}C NMR (101 MHz, $CDCl_3$, ppm) δ: 169.39, 156.40, 155.97, 130.03, 128.31, 61.52, 40.84, 14.07.

Ethyl 2-(pyridazin-3-yl)acetate (23). NEt_3 (1.26 mL, 9.01 mmol) was added to a solution of ethyl 2-(6-chloropyridazin-3-yl)acetate (**22**, 909 mg, 4.53 mmol) in EtOH (12 mL). The mixture was purged with nitrogen followed by addition 10% Pd/C (182 mg). Hydrogen gas (1 atm) was bubbled through the mixture for 4 h at room temperature. Then reaction mixture was filtered through a pad of celite, the filtrate was concentrated, and the residue was purified by silica gel chromatography using a linear gradient (0-4%) of MeOH in CH_2Cl_2 to afford the title compound as a pale yellow oil (708 mg, 94% yield). R_f = 0.38 (5% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_8H_{11}N_2O_2$, 167.0821; found 167.0826. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 9.07 (1H, dd, *J* = 4.9, 1.8 Hz), 7.53 (1H, dd, *J* = 8.5, 1.7 Hz), 7.43 (1H, dd, *J* = 8.5, 4.9 Hz), 4.15 (2H, q, *J* = 7.2 Hz), 4.02 (2H, s), 1.22 (3H, t, *J* = 7.2 Hz).^{13}C NMR (101 MHz, $CDCl_3$, ppm) δ: 169.78, 157.16, 150.35, 127.45, 126.49, 61.35, 41.73, 14.08.

Allyl *N*-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl) amino)ethyl)-*N*-(2-(pyridazin-3-yl)acetyl)glycinate (24). A solution of LiOH (50 mg, 2.1 mmol) water (2.2 mL) was added to a solution of ethyl 2-(pyridazin-3-yl)acetate (**23**, 315 mg, 1.90 mmol) in EtOH (5.4 mL). After 1 h at room temperature, the mixture was concentrated under reduced pressure. The crude product was dried on high vacuum for 6 h and used directly in the next step without further purification. *i*-Pr_2NEt (430 µL, 2.47 mmol, 1.3 equiv.) was added under nitrogen to a solution of the crude lithium 2-(pyridazin-3-yl)acetate (1.90 mmol), HBTU (722 mg, 1.90 mmol), N-Fmoc-Aeg-O-Allyl HCl salt (**18**, 632 mg, 1.52 mmol) in anhydrous DMF (13 mL). The reaction mixture was stirred for 12 h at room temperature, and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with aqueous 5% $NaHCO_3$ (30 mL). The aqueous layer was back-extracted with CH_2Cl_2 (50 mL), the organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography using a linear gradient (0-5%) of MeOH in CH_2Cl_2 to afford the title compound as a yellow oil (757 mg, 80% yield). R_f = 0.27 (5% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{28}H_{29}N_4O_5$, 501.2138; found 501.2133. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) (mixture of rotamers) δ: 9.30 – 8.80 (1H, m), 7.74 (2H, dd, *J* = 7.6, 3.8 Hz), 7.64 – 7.49 (3H, m), 7.37 (3H, tt, *J* = 6.3, 3.2 Hz), 7.29 (2H, dt, *J* = 7.4, 1.7 Hz), 5.88 (1H, tq, *J* = 11.7, 5.8 Hz), 5.39 – 5.19 (2H, m), 4.62 (2H, dt, *J* = 6.0, 1.3 Hz), 4.47 – 4.29 (2H, m), 4.26 – 3.96 (4H, m), 3.76 – 3.50 (2H, m), 3.38 (2H, tt, *J* = 12.1, 5.8 Hz). ^{13}C NMR (101 MHz, $CDCl_3$, ppm) (mixture of rotamers) δ: 170.02, 169.63, 158.14, 156.61, 150.43, 150.35, 143.97, 143.86, 141.31, 131.43, 131.16, 127.88, 127.73, 127.71, 127.11, 127.07, 126.65, 126.55, 125.09, 119.99, 119.97, 119.63, 119.00, 66.82, 66.71, 66.58, 66.10, 51.15, 49.79, 49.02, 47.23, 41.41, 40.46, 39.44, 39.21.

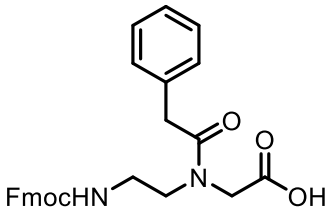
***N*-(2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl) amino)ethyl)-*N*-(2-(pyridazin-3-yl)acetyl)glycine (9).** $Pd(PPh_3)_4$ (37 mg, 0.03 mmol) and *N*-ethylaniline (173 µL, 1.38 mmol) were added sequentially to a solution of allyl ester of pyridazin-3-yl monomer (**24**, 407 mg, 0.81 mmol) in anhydrous THF (30 mL). The solution was stirred under nitrogen for 3 h at room temperature. After the reaction was complete, the mixture was acidified to pH 5-6 with 1 M aqueous HCl and solvent was removed under reduced pressure. The crude product was purified by C18 reverse phase flash chromatography using a linear gradient (0-80%) of MeCN in water to afford the title compound as a pale yellow foam (202 mg, 54% yield). R_f = 0.06 (20% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{25}H_{25}N_4O_5$, 461.1825; found 461.1827. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 9.07 (1H, dd, *J* = 4.4, 2.2 Hz), 7.88 (3H, d, *J* = 7.5 Hz), 7.68 (2H, d, *J* = 7.5 Hz), 7.62 – 7.49 (2H, m), 7.40 (2H, t, *J* = 7.4 Hz), 7.31 (2H, t, *J* = 7.4 Hz), 4.34 – 4.16 (3H, m), 4.04 (1H, d, *J* = 4.6 Hz), 3.97 (2H, d, *J* = 3.4 Hz), 3.81 (2H, d, *J* = 8.1 Hz), 3.56 – 3.47 (1H, m), 3.39 (2H, d, *J* = 6.2 Hz), 3.26 (1H, d, *J* = 6.3 Hz), 3.18 (2H, q, *J* = 6.2 Hz). ^{13}C NMR (101 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 170.59, 159.70, 156.57, 150.59, 144.42, 141.14, 128.72, 128.07, 127.63, 126.80, 125.83, 125.66, 120.59, 120.54, 66.04, 47.87, 47.18, 40.75, 39.07, 38.66.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-acetylglycine (10)

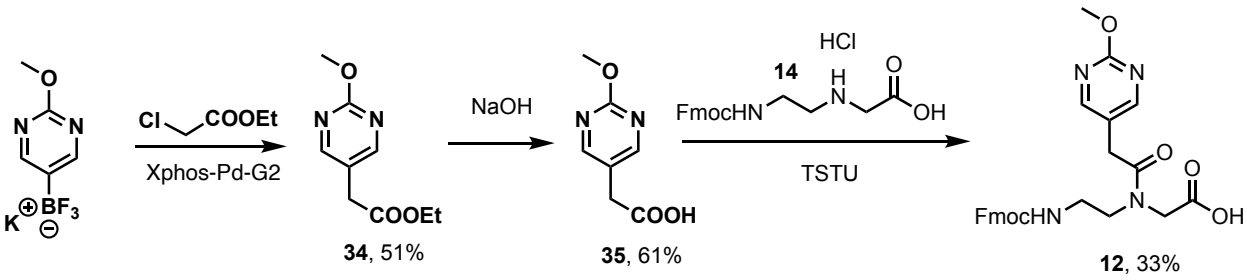


The benzyl ester of acetyl monomer (**16**, 1.70 g, 3.59 mmol) was dissolved in EtOH (10 mL). The reaction mixture was purged with nitrogen followed by addition Pd/C 10% (145 mg). Hydrogen gas (1 atm) was bubbled through the reaction mixture for 2 h at room temperature. The reaction mixture was filtered through a pad of celite, filtrate was concentrated, and the residue was purified by flash chromatography using CM-silica gel (Agela Technologies) and a linear gradient (0-5%) of MeOH in CH_2Cl_2 to afford the title compound as a white foam (1.25 g, 91% yield). R_f = 0.24 (20% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{21}H_{23}N_2O_5$, 383.1607; found 383.1596. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 12.69 (1H, s), 7.89 (2H, dt, *J* = 7.6, 0.9 Hz), 7.68 (2H, dd, *J* = 7.5, 3.2 Hz), 7.42 (2H, td, *J* = 7.5, 1.2 Hz), 7.34 (3H, td, *J* = 7.4, 1.2 Hz), 4.35 (1H, d, *J* = 6.8 Hz), 4.30 (1H, d, *J* = 7.0 Hz), 4.22 (1H, t, *J* = 6.8 Hz), 4.10 (1H, s), 3.92 (1H, s), 3.34 (3H, dt, *J* = 17.3, 6.6 Hz), 3.15 (2H, dq, *J* = 21.8, 6.3 Hz), 1.99 (2H, s), 1.91 (1H, s).^{13}C NMR (101 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 171.43, 170.60, 156.73, 144.39, 144.34, 141.25, 141.22, 128.10, 127.54, 125.65, 125.53, 120.60, 65.83, 51.08, 48.89, 47.57, 47.22, 46.66, 39.16, 38.74, 21.76, 21.19

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-phenylacetyl)glycine (11)



TSTU (240 mg, 0.80 mmol) was added under nitrogen to a solution of 2-phenylacetic acid (100 mg, 0.73 mmol) and *i*-Pr_2NEt (165 µL, 0.95 mmol, 1.3 equiv.) in anhydrous DMF (4.2 mL). After 30 minutes at room temperature, a solution of N-Fmoc-Aeg-OH HCl salt (**14**, 275 mg, 0.73 mmol) and *i*-Pr_2NEt (165 µL, 0.95 mmol, 1.3 equiv.) in anhydrous DMF (2 mL) were added. The resulting solution was stirred for 12 h at room temperature, then acidified to pH 6-7 using a 1 M aqueous HCl solution, and evaporated under reduced pressure. The crude product was purified by C18 reverse phase flash chromatography using a linear gradient (0-90%) of MeCN in water to afford the title compound as a white solid (125 mg, 37% yield). R_f = 0.41 (20% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{27}H_{27}N_2O_5$, 459.1920; found 459.1912. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 7.89 (2H, d, *J* = 7.5 Hz), 7.69 (2H, dd, *J* = 7.8, 2.8 Hz), 7.42 (3H, t, *J* = 7.4 Hz), 7.37 – 7.15 (7H, m), 4.37 (1H, d, *J* = 6.8 Hz), 4.30 (1H, d, *J* = 7.0 Hz), 4.23 (1H, t, *J* = 6.6 Hz), 4.17 (1H, s), 3.99 (1H, s), 3.73 (1H, s), 3.59 (1H, s), 3.41 (2H, dt, *J* = 21.5, 6.5 Hz), 3.28 – 3.11 (3H, m). ^{13}C NMR (101 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 171.36, 171.24, 156.85, 144.39, 144.35, 141.26, 141.22, 136.16, 129.78, 129.56, 128.66, 128.59, 128.11, 127.56, 126.81, 126.77, 125.65, 125.55, 120.62, 120.59, 65.94, 50.85, 48.40, 47.98, 47.23, 47.09, 39.28.



Ethyl 2-(2-methoxypyrimidin-5-yl)acetate (34)[1]

Potassium trifluoro(2-methoxypyrimidin-5-yl)borate (1.00 g, 4.17 mmol, 1.1 equiv.), Cs_2CO_3 (3.87 g, 11.91 mmol, 3 equiv.), Xphos-Pd-G2 (62 mg, 0.08 mmol, 0.02 equiv.) were added to the first flask and purged with nitrogen for 30 min. Ethyl 2-chloroacetate (420 µL, 3.97 mmol, 1 equiv.) was dissolved in THF (13 mL) and DI water (3 mL) in the second flask, mixture was purged with nitrogen for 20 min, then mixture was transferred to the first flask through

[1] Molander, G.A.; Traister, K.M.; Barcellos, T. Palladium-Catalyzed α-Arylation of 2-Chloroacetates and 2-Chloroacetamides. *J. Org. Chem.* **2013**, *78*, 4123-4131.

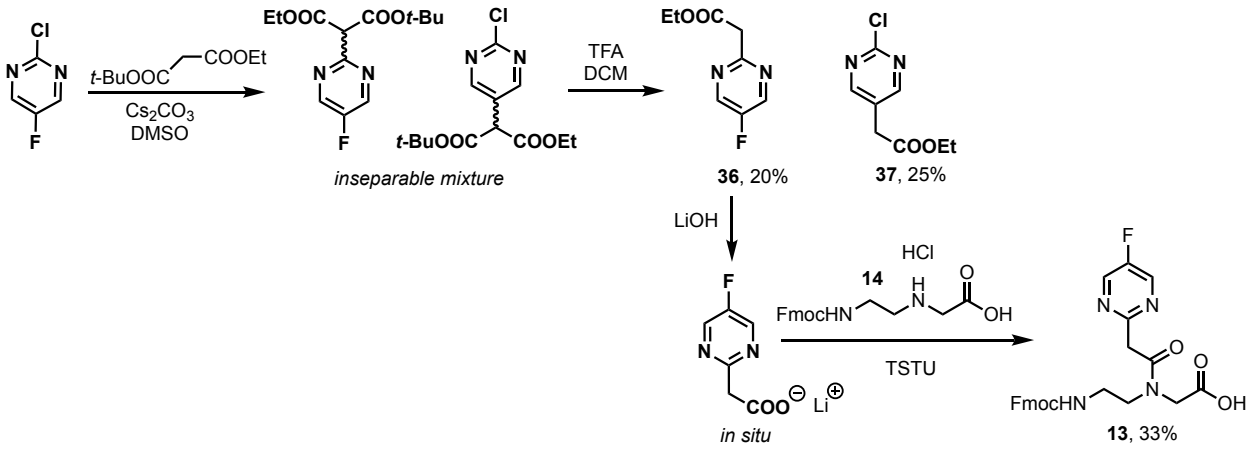
cannula. Reaction was refluxed under slow flow of nitrogen at 100˚C for 20 h. Reaction was brought to room temperature, diluted with DI water (30 mL) and EtOAc (50 mL), aqueous layer was washed three times with EtOAC (50 mL). Organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure. Crude product was purified by silica gel chromatography Hexanes/EtOAc = 0-50% to afford the title compound as pale yellow oil (420 mg, 51% yield). R_f = 0.45 (50% EtOAc in hexanes). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd for $C_9H_{13}N_2O_3$, 197.0926; found 197.0922. ^{1}H NMR (400 MHz, Chloroform-*d*, ppm) δ: 8.40 (s, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.96 (s, 3H), 3.50 (s, 2H), 1.22 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*, ppm) δ: 170.29, 164.94, 159.61, 120.96, 61.38, 54.90, 34.98, 14.10.

2-(2-methoxypyrimidin-5-yl)acetic acid (35)

1 M aqueous NaOH (10 mL) was added to a solution of ethyl 2-(2-methoxypyrimidin-5-yl)acetate (**34**, 420 mg, 2.14 mmol) in THF (10 mL). The reaction mixture was stirred for 2 h at room temperature and THF was evaporated under reduced pressure. The aqueous residual solution was brought to pH 5-6 with 1 M aqueous HCl resulting in precipitation. The precipitate was filtered and dried yielding the title compound as a white solid (218 mg, 61% yield). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_7H_9N_2O_3$, 169.0613; found 169.0607. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 12.57 (1H, s), 8.48 (2H, s), 3.90 (3H, s), 3.60 (2H, s). ^{13}C NMR (101 MHz, DMSO-d_6, ppm) δ: 172.65, 164.59, 160.45, 122.83, 54.97, 34.36.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-(2-methoxypyrimidin-5-yl)acetyl)glycine (12)

TSTU (316 mg, 1.05 mmol) was added under nitrogen to a solution of 2-(2-methoxypyrimidin-5-yl)acetic acid (**35**, 159 mg, 0.95 mmol) and *i*-Pr_2NEt (180 µL, 1.1 mmol, 1.1 equiv.) in anhydrous DMF (5.5 mL). After 30 minutes at room temperature, a solution of N-Fmoc-Aeg-OH HCl salt (**14**, 358 mg, 0.95 mmol) and *i*-Pr_2NEt (330 µL, 1.9 mmol, 2 equiv.) in anhydrous DMF (2.5 mL) were added. The resulting solution was stirred for 12 h at room temperature, then acidified to pH 6-7 using a 1 M aqueous HCl solution and evaporated under reduced pressure. The crude product was purified by flash chromatography using CM-modified silica (Agela Technologies) and a linear gradient (0-10%) of MeOH in CH_2Cl_2 to afford the title compound as white foam (156 mg, 33% yield). R_f = 0.16 (20% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{26}H_{27}N_4O_6$, 491.1931; found 491.1927. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 12.88 (1H, s), 8.39 (2H, d, *J* = 10.6 Hz), 7.89 (2H, d, *J* = 7.5 Hz), 7.68 (2H, d, *J* = 7.5 Hz), 7.42 (3H, q, *J* = 7.2 Hz), 7.32 (2H, tt, *J* = 7.4, 1.3 Hz), 4.34 (1H, d, *J* = 6.8 Hz), 4.27 (1H, d, *J* = 6.9 Hz), 4.25 – 4.19 (1H, m), 4.17 (1H, s), 3.96 (1H, s), 3.89 (2H, s), 3.87 (1H, s), 3.73 (1H, s), 3.57 (1H, s), 3.48 (1H, t, *J* = 6.5 Hz), 3.36 (1H, t, *J* = 6.5 Hz), 3.25 (1H, q, *J* = 6.4 Hz), 3.19 – 3.06 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 171.20, 170.45, 164.49, 160.37, 160.27, 156.82, 156.61, 144.39, 144.32, 141.24, 141.20, 128.09, 127.55, 125.67, 125.53, 120.62, 120.58, 65.92, 54.93, 54.87, 48.24, 47.43, 47.19, 38.59, 32.93, 32.60.



Ethyl 2-(2-chloropyrimidin-5-yl)acetate (36) and ethyl 2-(5-fluoropyrimidin-2-yl)acetate (37)

2-chloro-5-fluoropyrimidine (0.7 mL, 7.55 mmol) was dissolved in DMSO (2.5 mL) followed by addition of *tert*-butyl ethyl malonate (1.5 mL, 7.92 mmol). Then Cs_2CO_3 (4.9 g, 15 mmol) was added and reaction was kept at 110˚C for 1 h. The reaction mixture was diluted with EtOAc (100 mL) and water (50 mL), aqueous phase was washed with EtOAc (2 x 100 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product mixture was purified by silica gel chromatography using a linear gradient (0-30%) of EtOAc in Hexanes to afford useparable mixture of fluoro- and chloro-malonates that was dissolved in CH_2Cl_2 (10 mL) followed by addition of TFA (10 mL). The reaction mixture was kept at room temperature for 15 h and concentrated under reduced pressure. Crude products were purified by silica gel chromatography using a linear gradient (0-50%) of EtOAc in Hexanes to afford the title compounds as pale yellow oils.

Ethyl 2-(2-chloropyrimidin-5-yl)acetate 36: 383 mg, 25% yield over two steps. R_f = 0.32 (25% EtOAc in hexanes). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_8H_{10}N_2O_2Cl$, 201.0431; found 201.0430. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 8.60 (2H, s), 4.18 (2H, q, *J* = 7.2 Hz), 3.63 (2H, s), 1.26 (3H, t, *J* = 7.1 Hz).^{13}C NMR (101 MHz, $CDCl_3$, ppm) δ: 169.24, 160.07, 159.79, 126.84, 61.94, 35.00, 14.07.

Ethyl 2-(5-fluoropyrimidin-2-yl)acetate 37: 273 mg, 20% yield over two steps. R_f = 0.38 (25% EtOAc in hexanes). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_8H_{10}N_2O_2F$, 185.0726; found 185.0728. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 8.58 (2H, s), 4.22 (2H, q, *J* = 7.1 Hz), 4.04 (2H, s), 1.28 (3H, t, *J* = 7.1 Hz).^{13}C NMR (101 MHz, $CDCl_3$, ppm) δ: 169.45, 160.53, 160.48, 145.25, 145.05, 61.39, 44.33, 14.10.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(2-(5-fluoropyrimidin-2-yl)acetyl)glycine (13)

LiOH (14 mg, 0.60 mmol) in water (0.6 mL) was added to a solution of ethyl 2-(5-fluoropyrimidin-2-yl)acetate (**37**, 100 mg, 0.54 mmol) in EtOH (1.5 mL). The reaction mixture was stirred for 1 h at room temperature, concentrated under reduced pressure, and the residue

was dried on high vacuum for 6 h. The crude product was used in the next step without further purification. TSTU (177 mg, 0.59 mmol) was added under nitrogen to a solution of lithium 2-(5-fluoropyrimidin-2-yl)acetate (0.54 mmol) and *i*-Pr_2NEt (122 µL, 0.70 mmol, 1.3 equiv.) in anhydrous DMF (3 mL). After 30 minutes at room temperature, a solution of N-Fmoc-Aeg-OH HCl salt (**14**, 203 mg, 0.54 mmol) and *i*-Pr_2NEt (122 µL, 0.70 mmol, 1.3 equiv.) in anhydrous DMF (1.5 mL) were added. The resulting solution was stirred for 12 hr at room temperature, then acidified to pH 6-7 using a 1 M aqueous HCl solution, and evaporated under reduced pressure. The crude product was purified by C18 reverse phase flash chromatography (Agela Technologies) using a linear gradient (0-80%) of MeCN in water to afford the title compound as yellow oil (77 mg, 30% yield). R_f = 0.24 (20% MeOH in CH_2Cl_2). HRMS (ESI/TOF) *m/z*: $[M + H]^+$ calcd. for $C_{25}H_{24}N_4O_5F$, 479.1731; found 479.1725. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 8.74 (2H, d, *J* = 18.5 Hz), 7.87 (2H, d, *J* = 7.5 Hz), 7.65 (2H, d, *J* = 7.5 Hz), 7.39 (2H, t, *J* = 7.4 Hz), 7.30 (2H, t, *J* = 7.4 Hz), 4.30 (1H, d, *J* = 6.9 Hz), 4.27 – 4.11 (2H, m), 4.04 (1H, s), 3.92 (3H, d, *J* = 17.4 Hz), 3.56 – 3.44 (1H, m), 3.34 (1H, d, *J* = 6.5 Hz), 3.28 – 3.05 (3H, m). ^{13}C NMR (101 MHz, DMSO-d_6, ppm) (mixture of rotamers) δ: 174.86, 162.93, 161.31, 160.34, 149.12, 149.08, 145.95, 145.91, 132.82, 132.31, 130.47, 130.34, 125.31, 70.72, 51.95, 51.91, 48.46, 48.03, 43.83, 43.36.

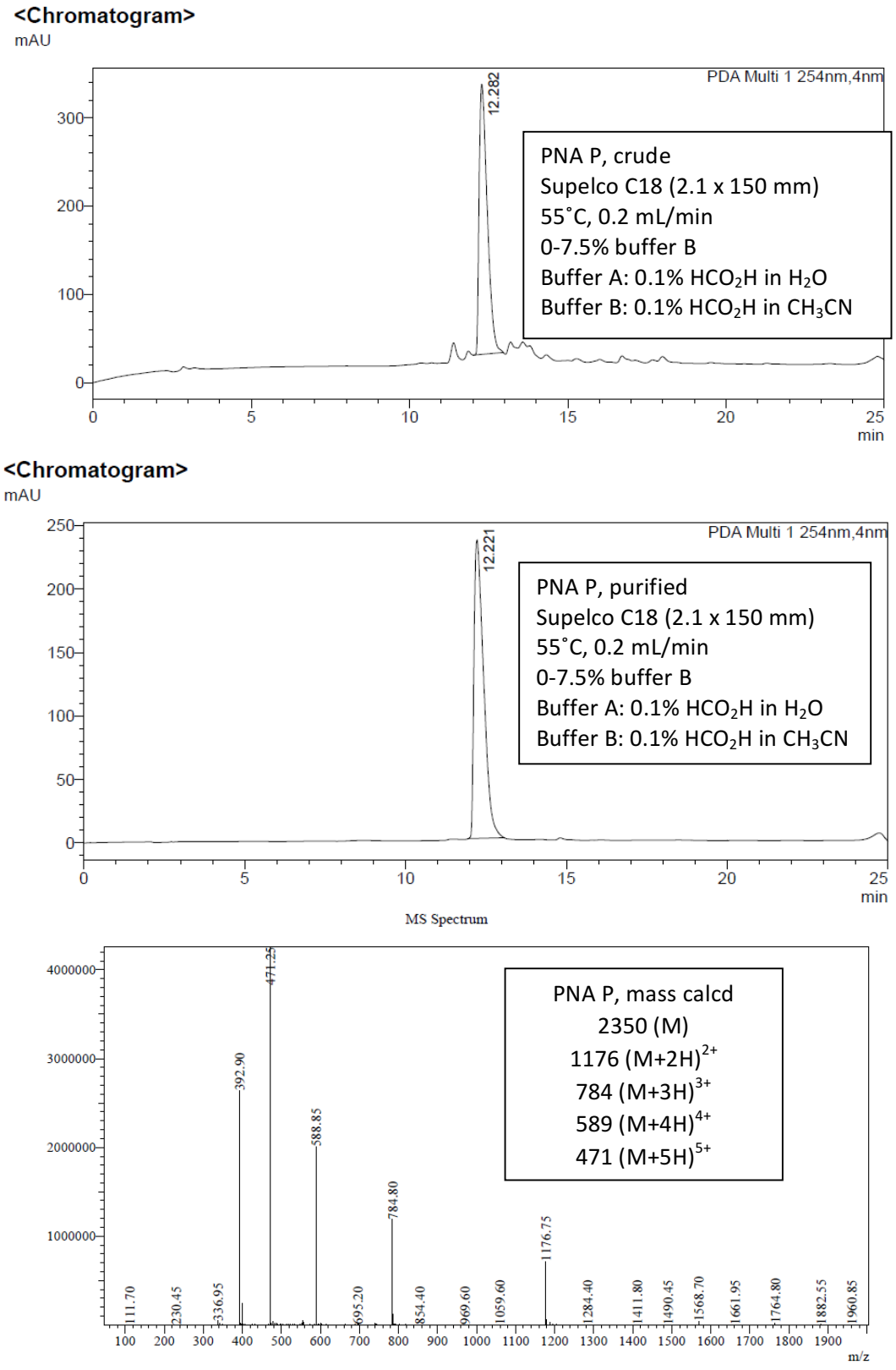


Figure S1. LCMS analysis of PNA P.

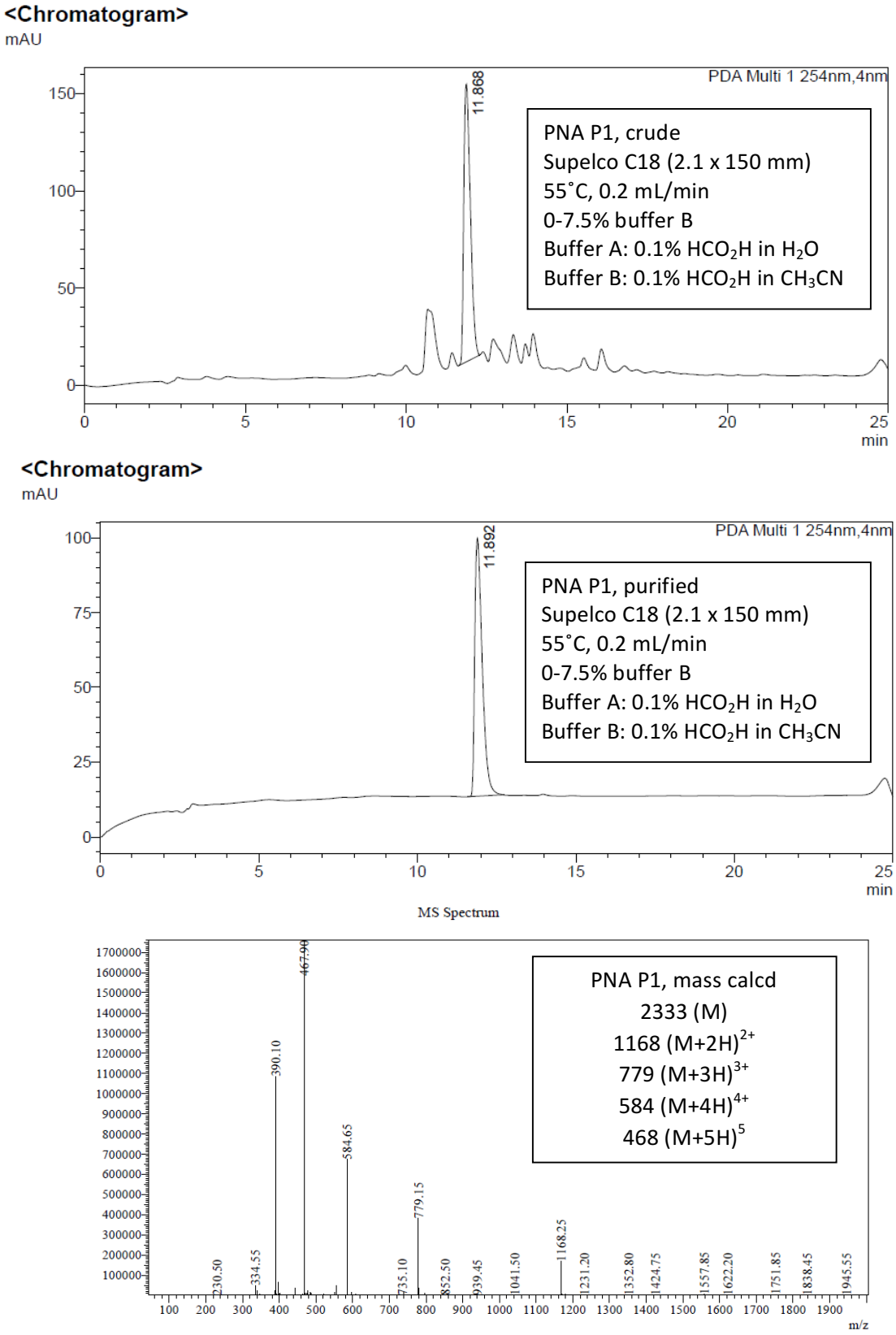


Figure S2. LCMS analysis of PNA P1.

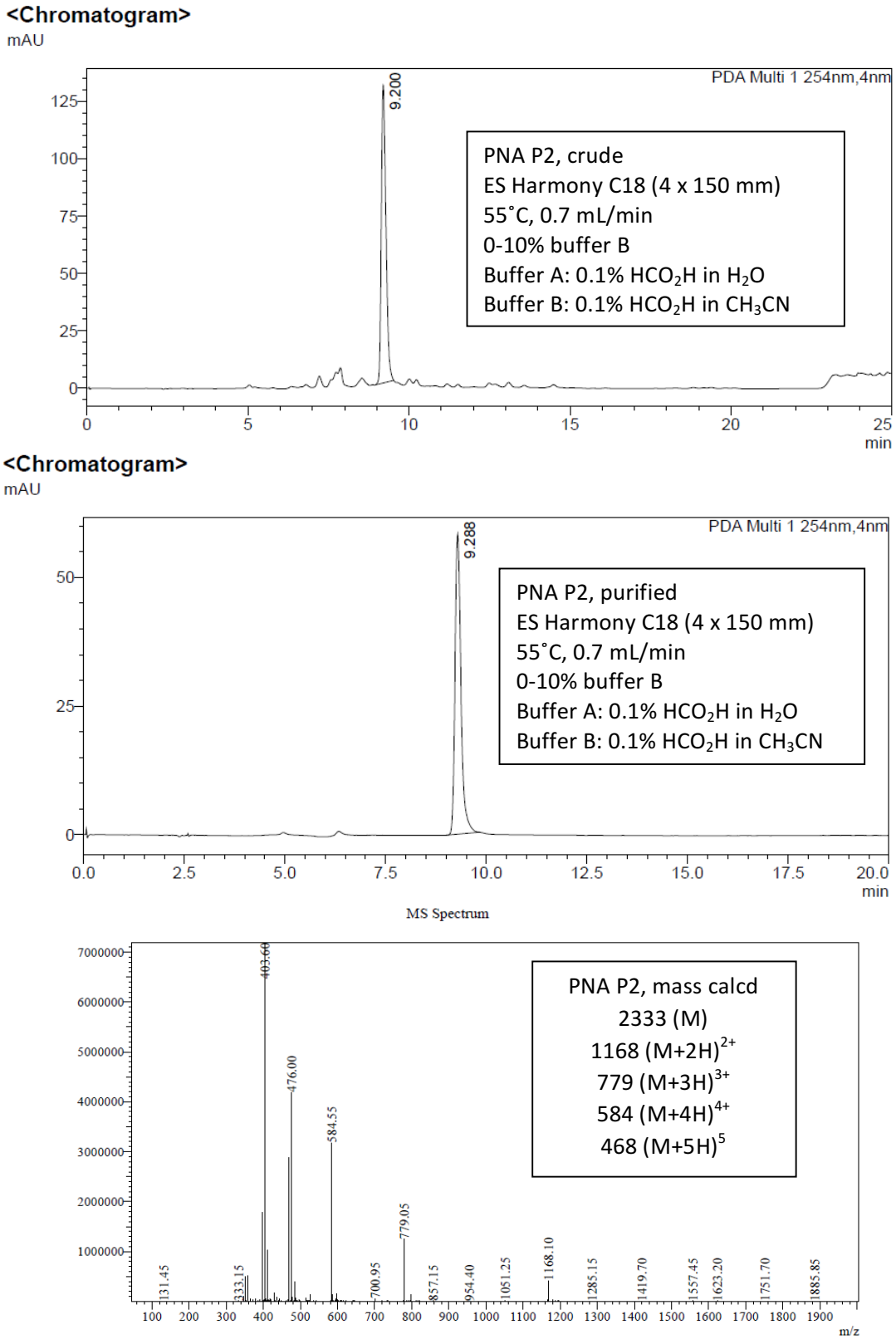


Figure S3. LCMS analysis of PNA P2.

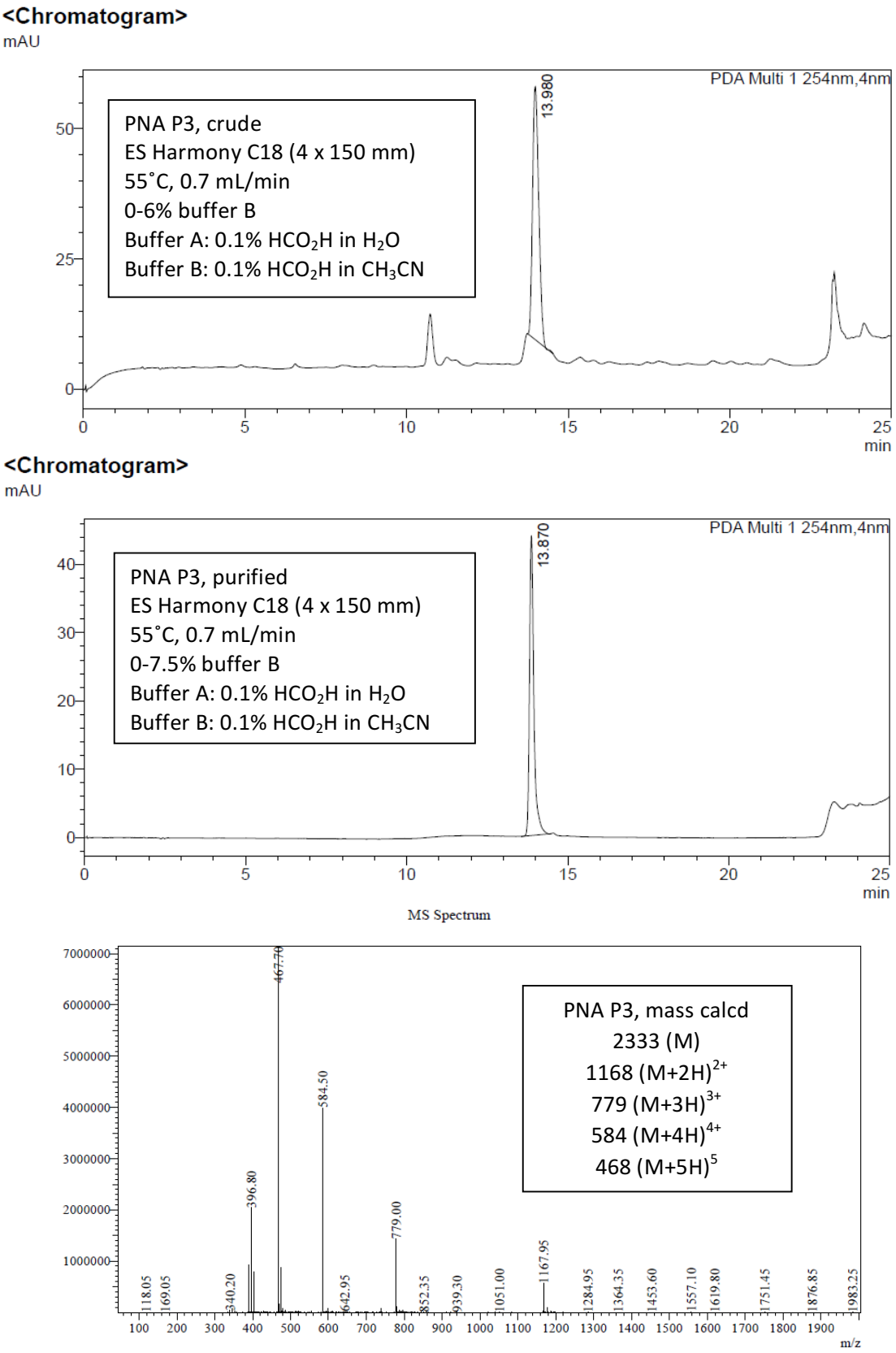


Figure S4. LCMS analysis of PNA P3.

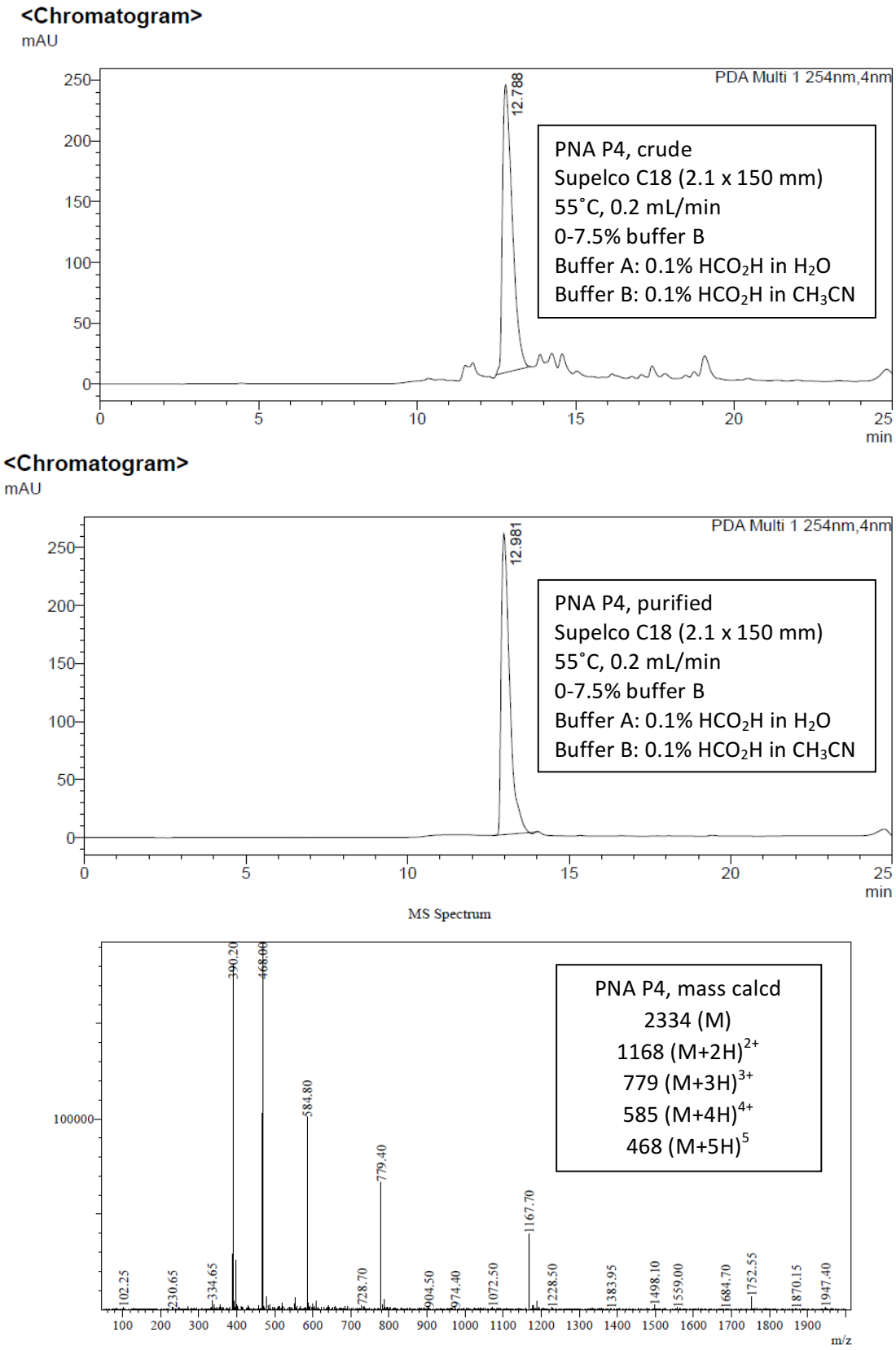


Figure S5. LCMS analysis of PNA P4.

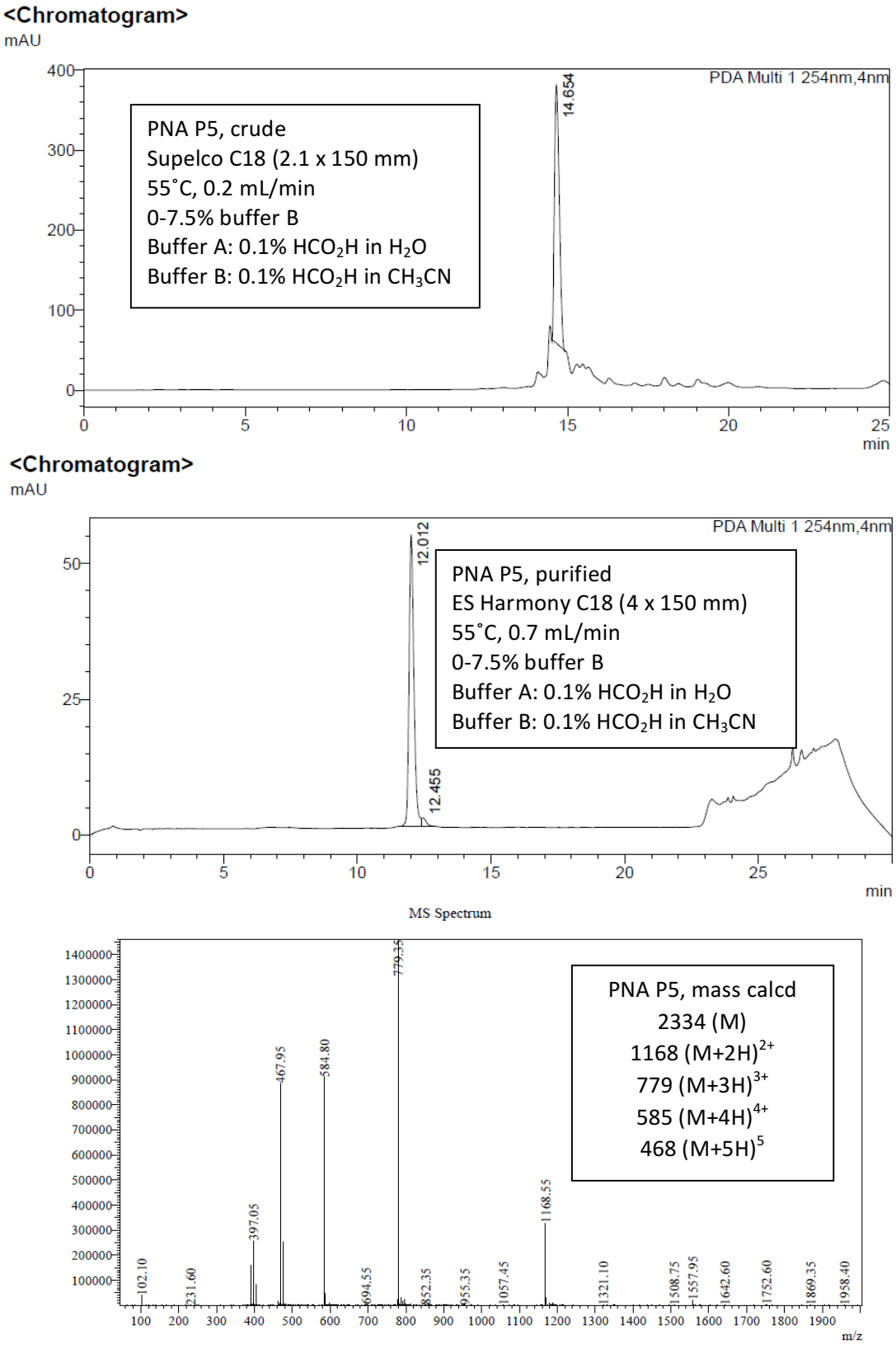


Figure S6. LCMS analysis of PNA P5.

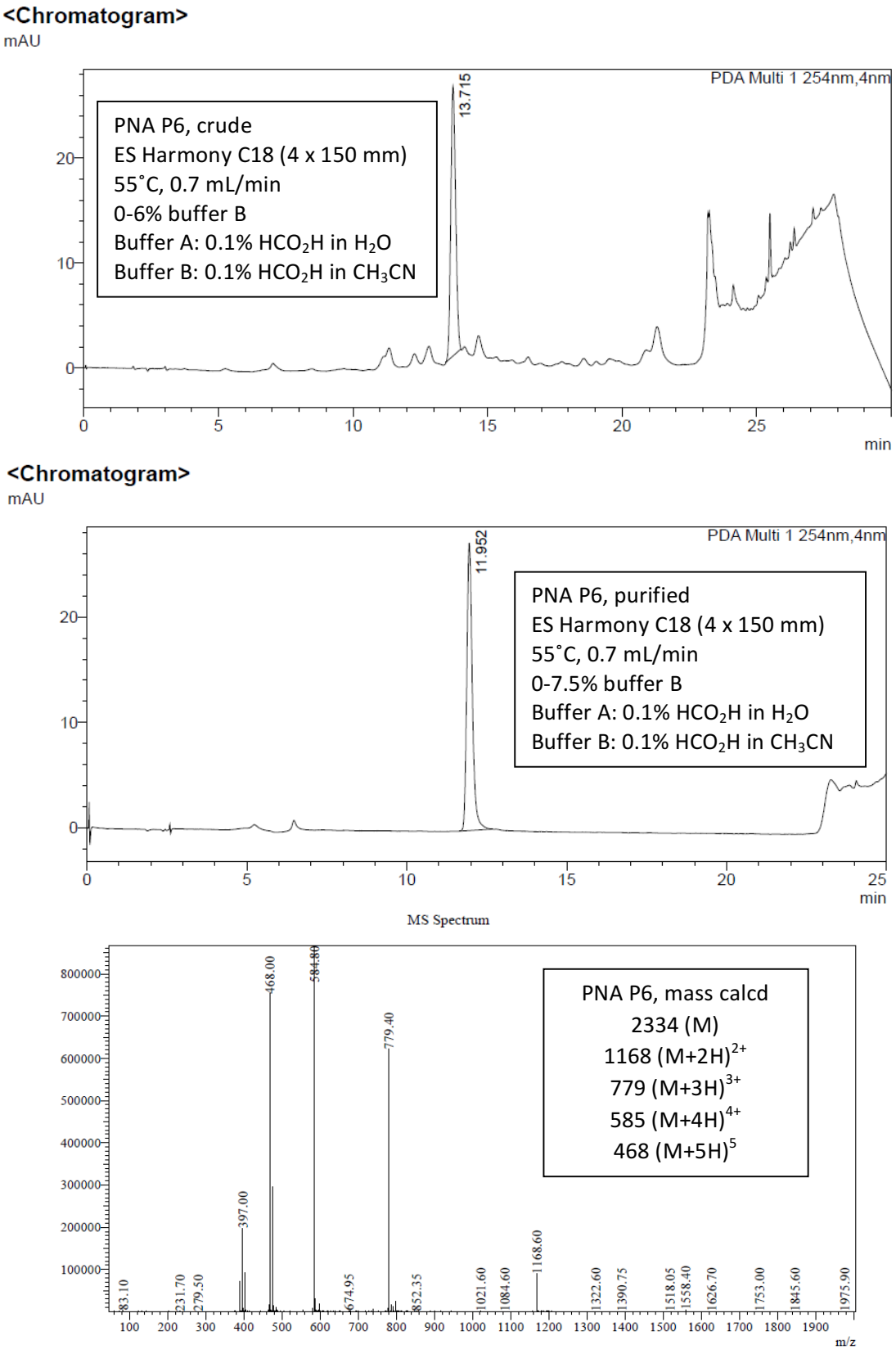


Figure S7. LCMS analysis of PNA P6.

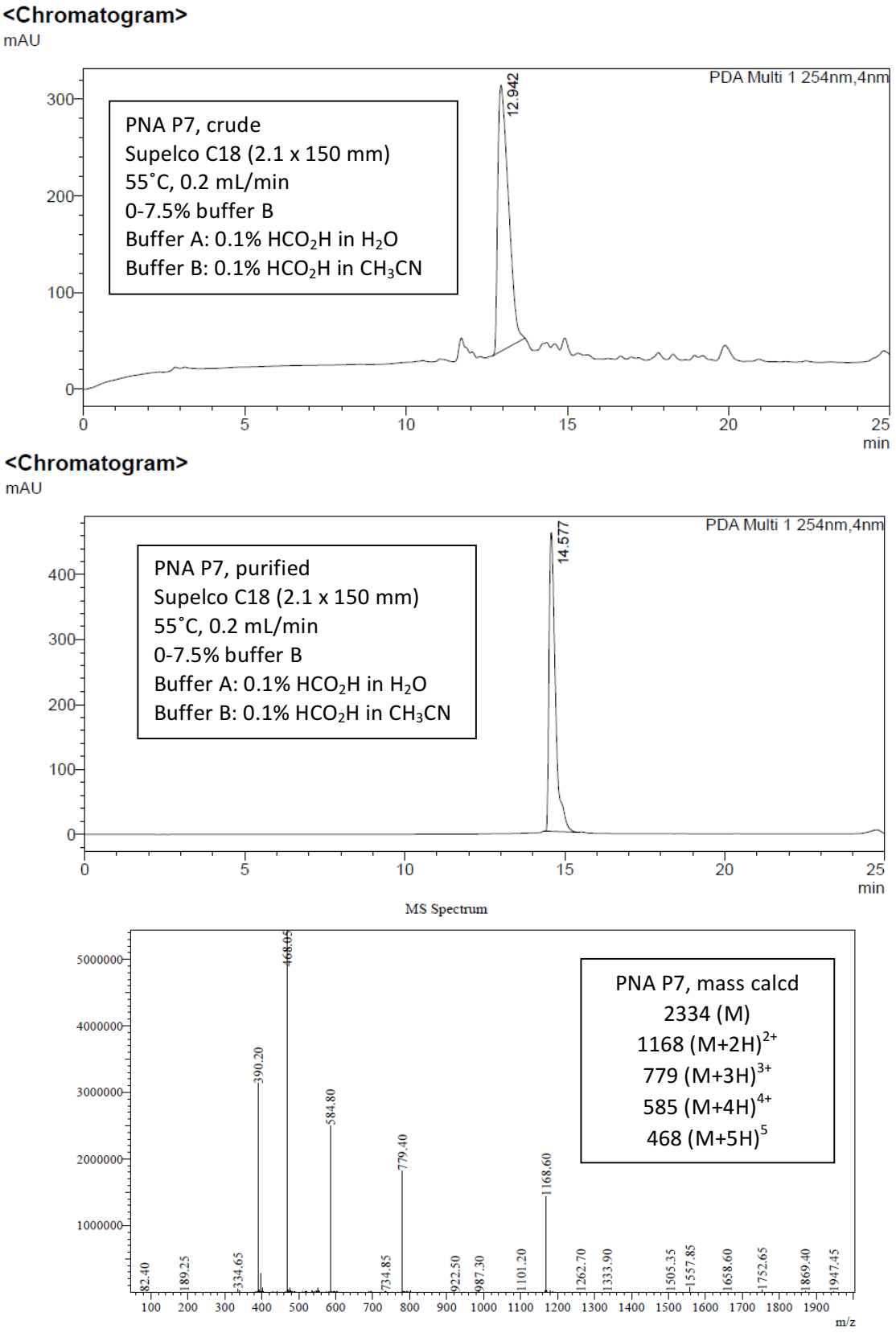


Figure S8. LCMS analysis of PNA P7.

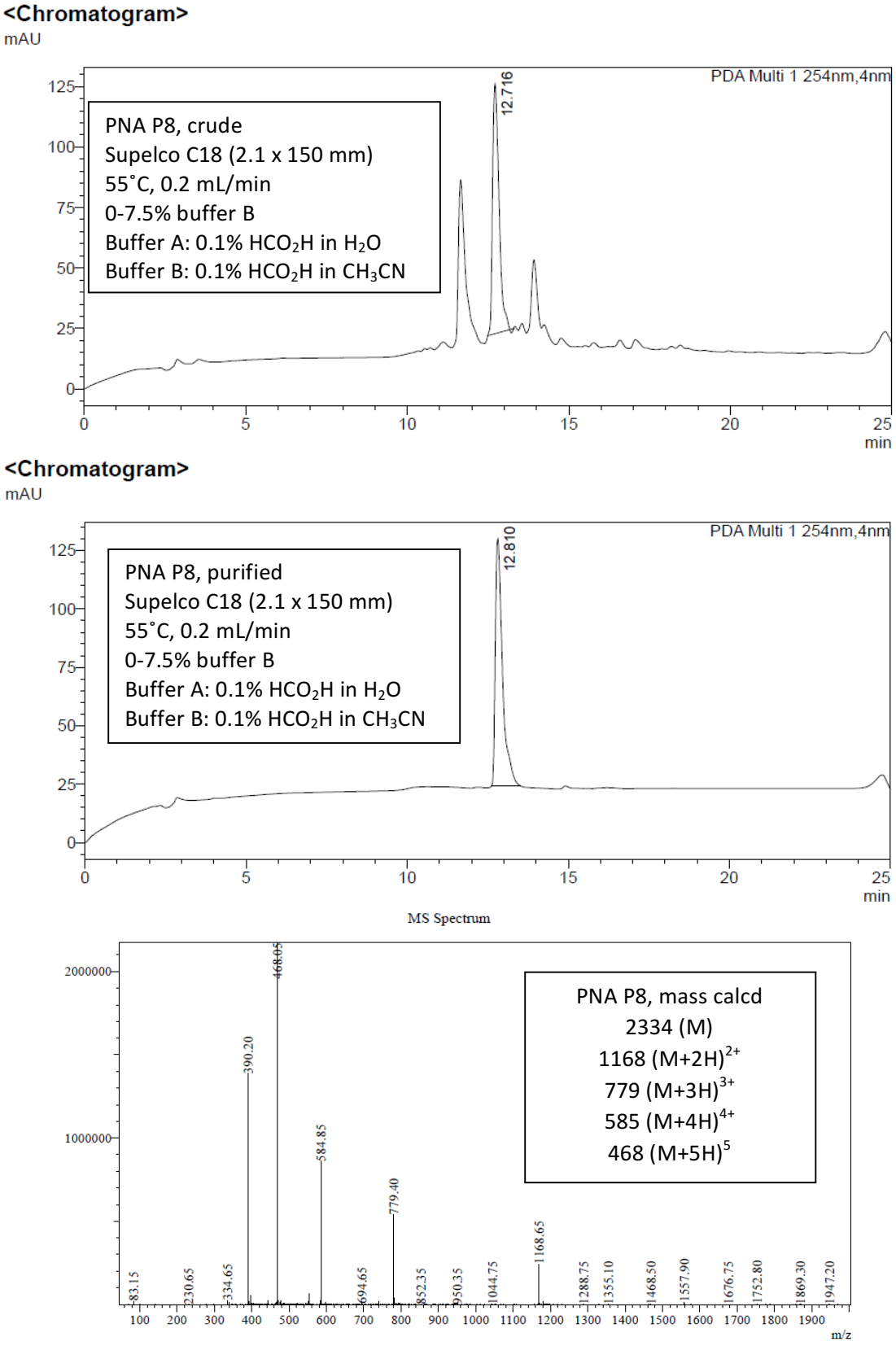


Figure S9. LCMS analysis of PNA P8.

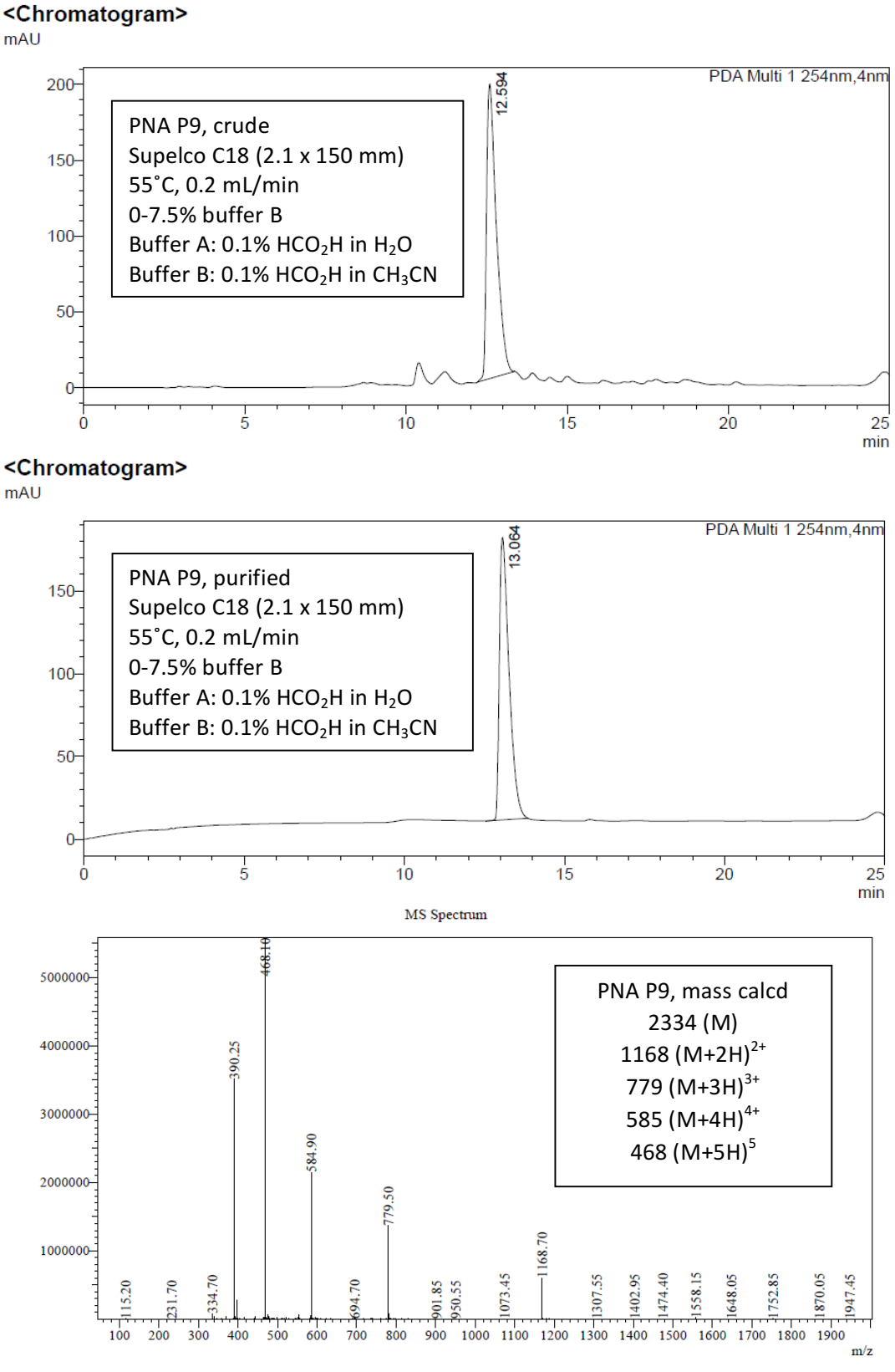


Figure S10. LCMS analysis of PNA P9.

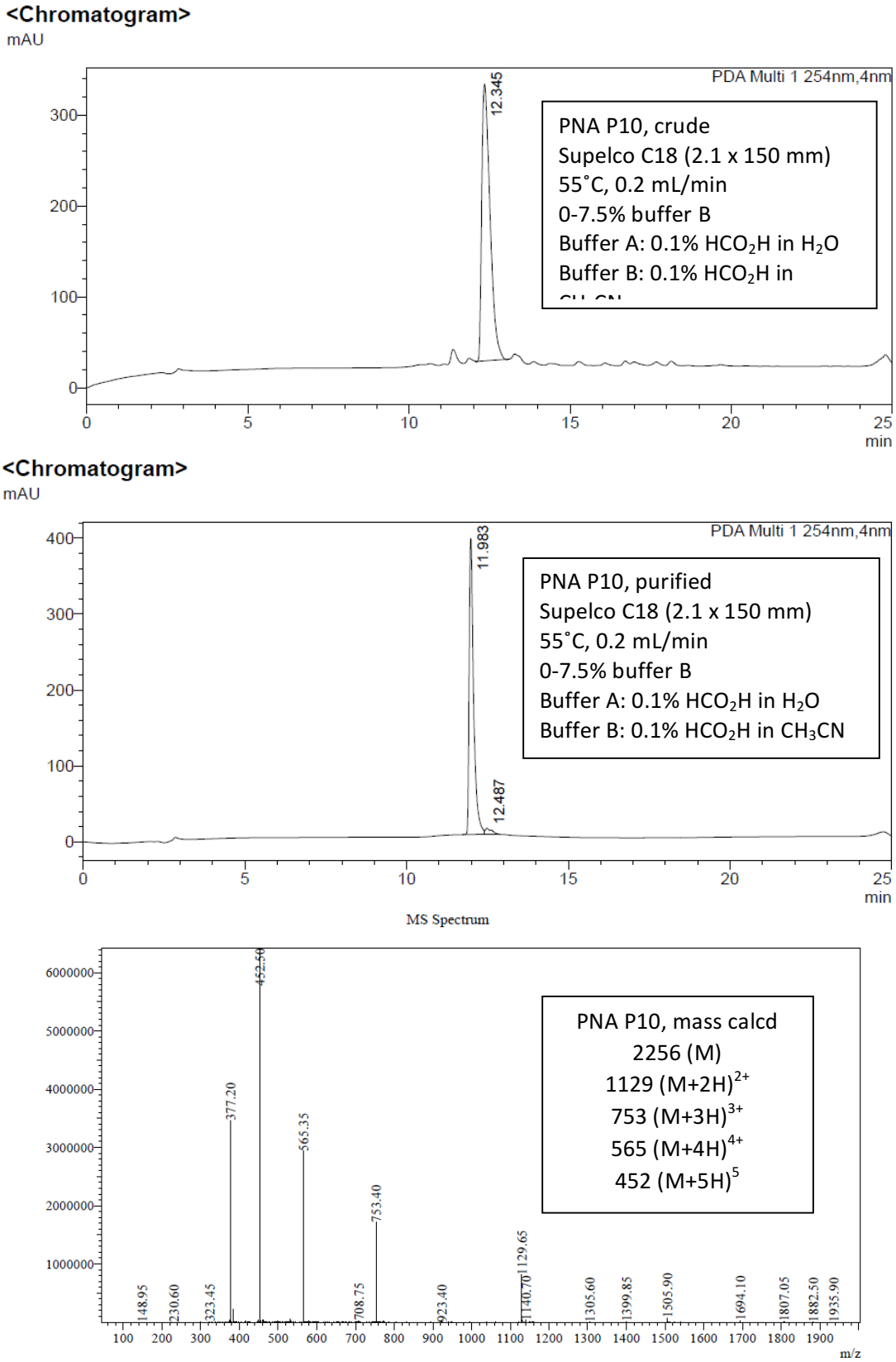


Figure S11. LCMS analysis of PNA P10.

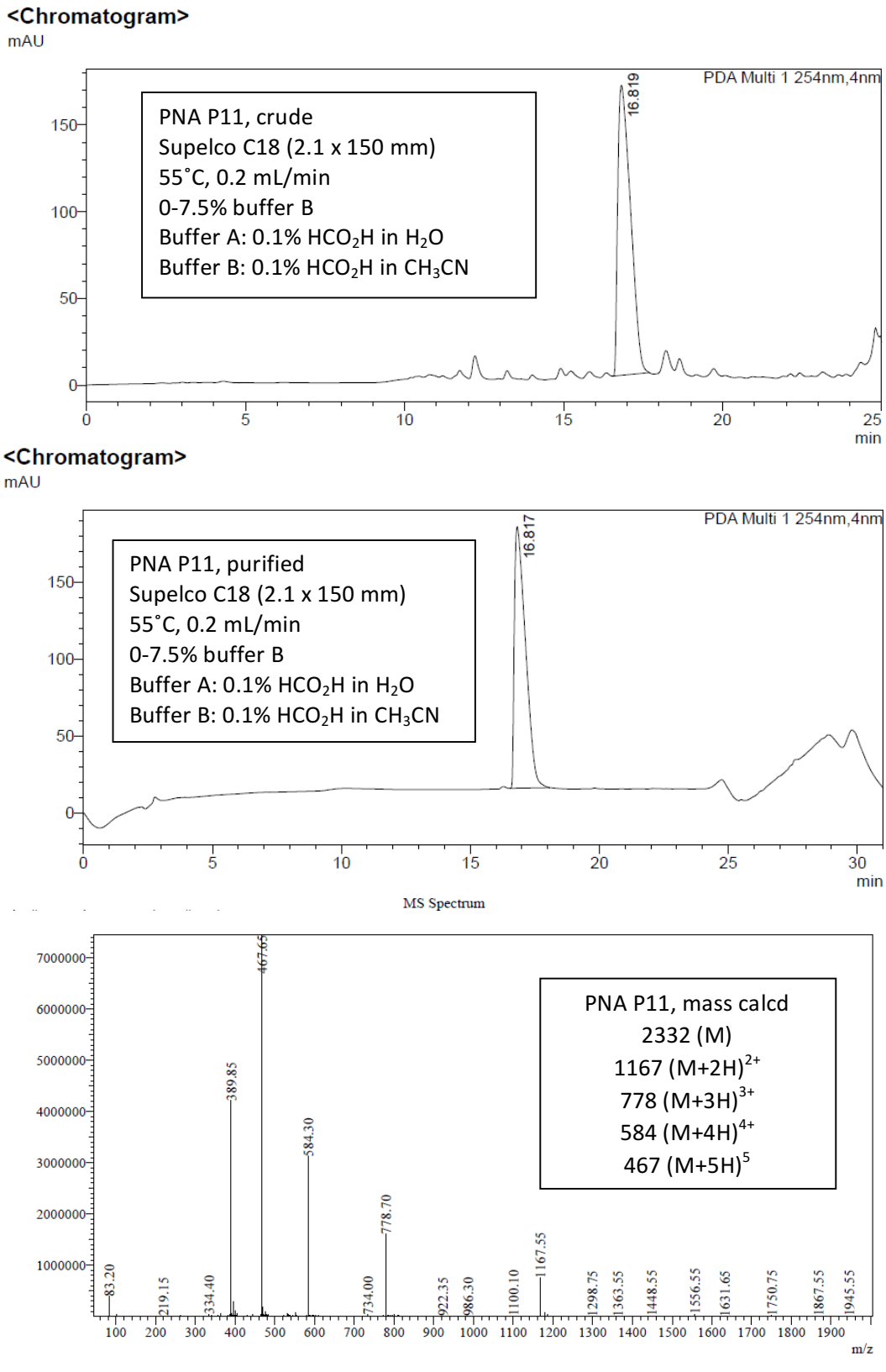


Figure S12. LCMS analysis of PNA P11.

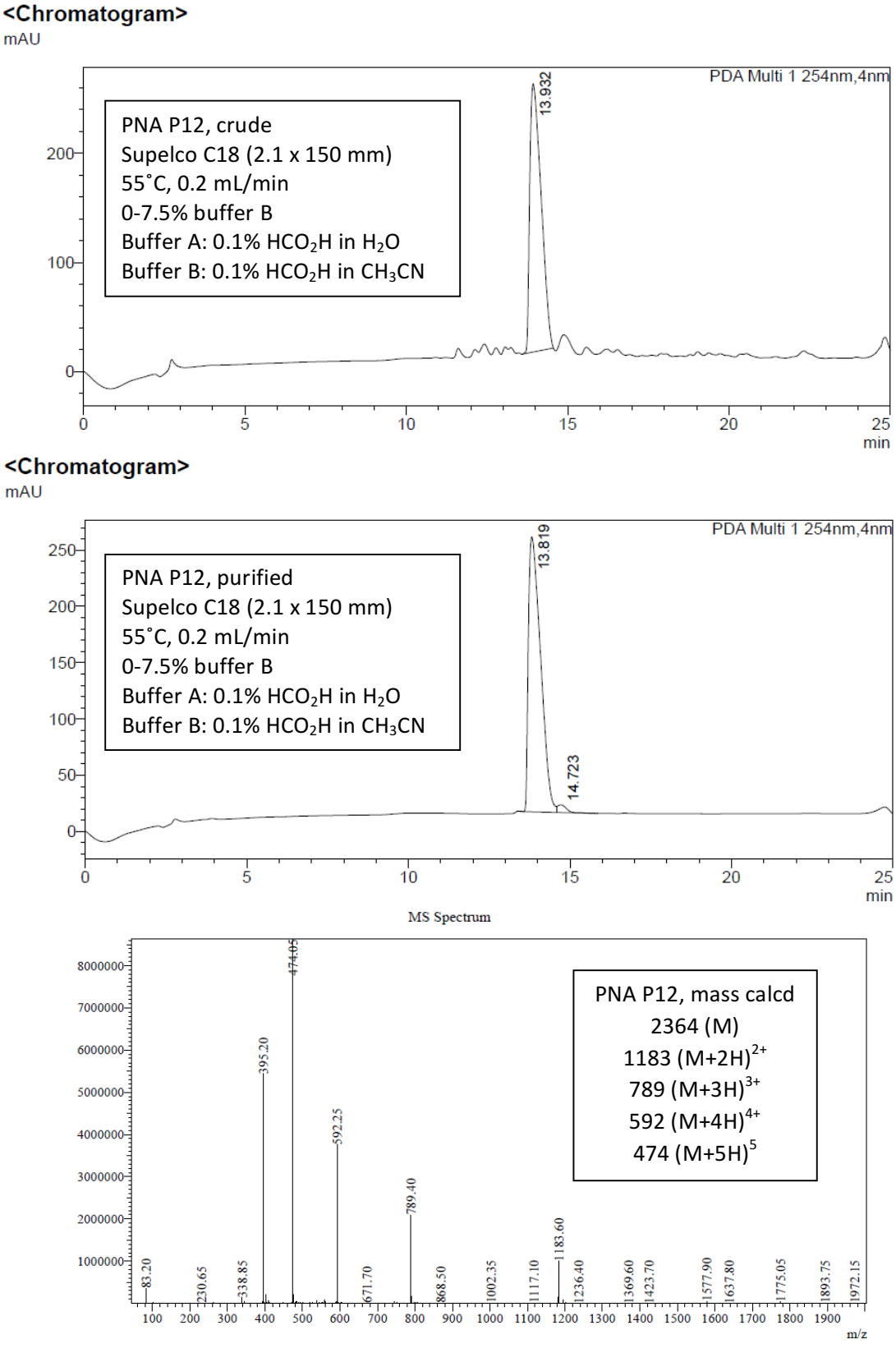


Figure S13. LCMS analysis of PNA P12.

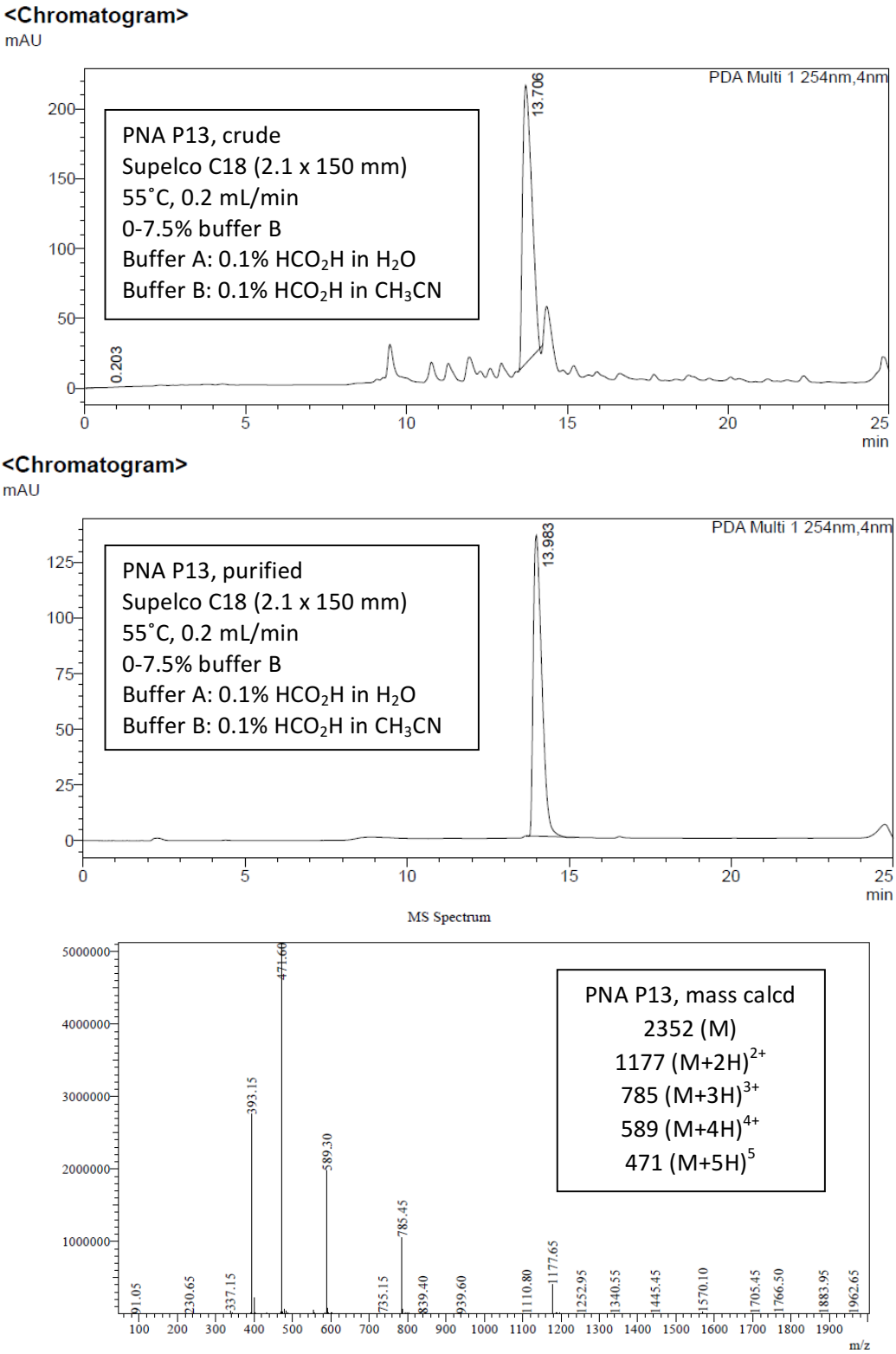


Figure S14. LCMS analysis of PNA P13.

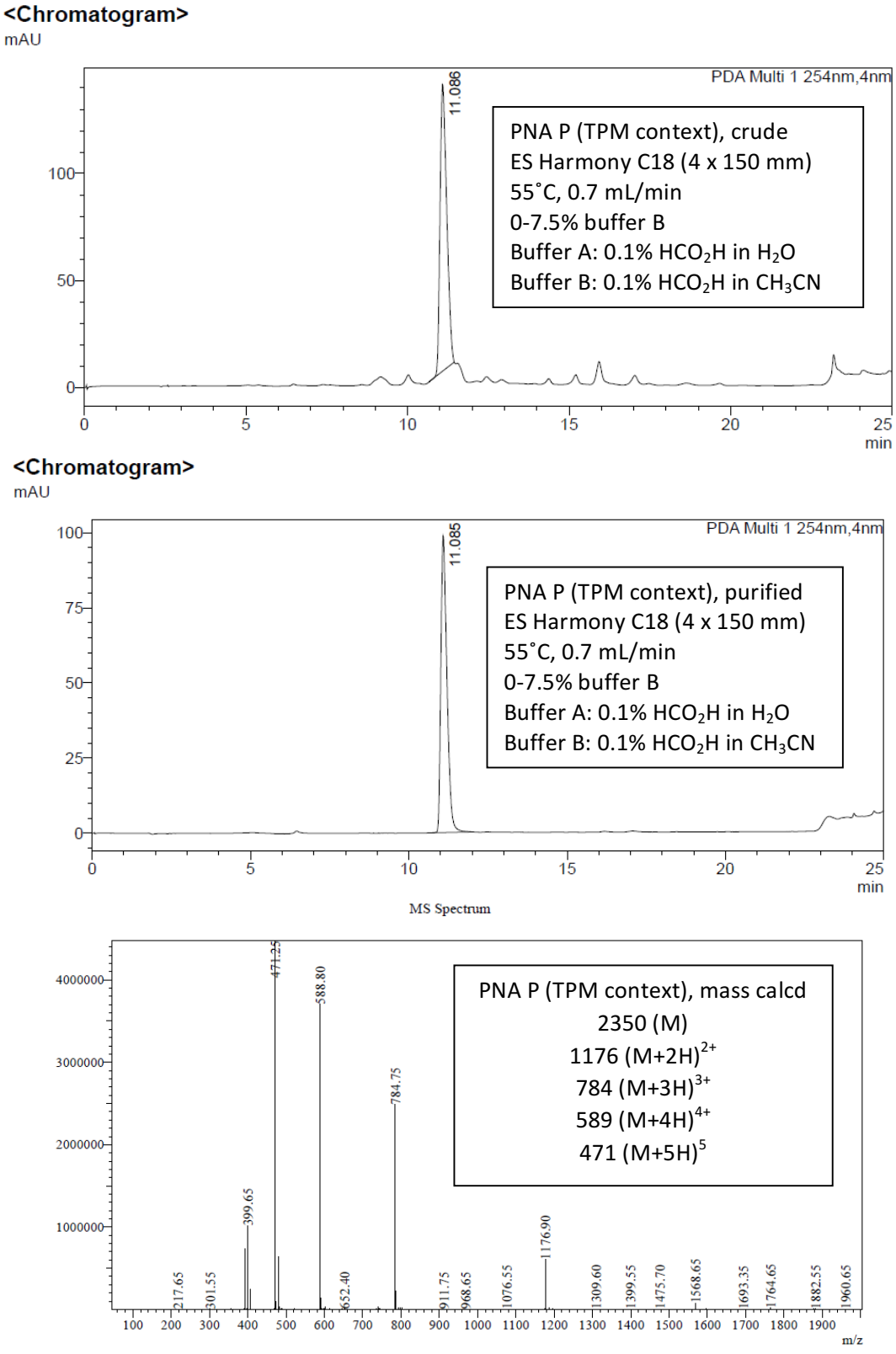
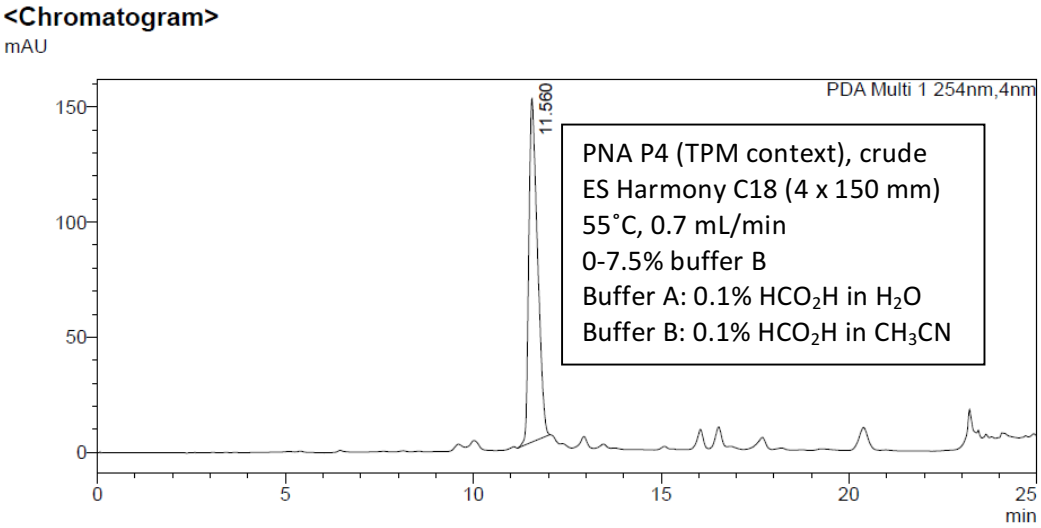


Figure S15. LCMS analysis of PNA_{TPM}.



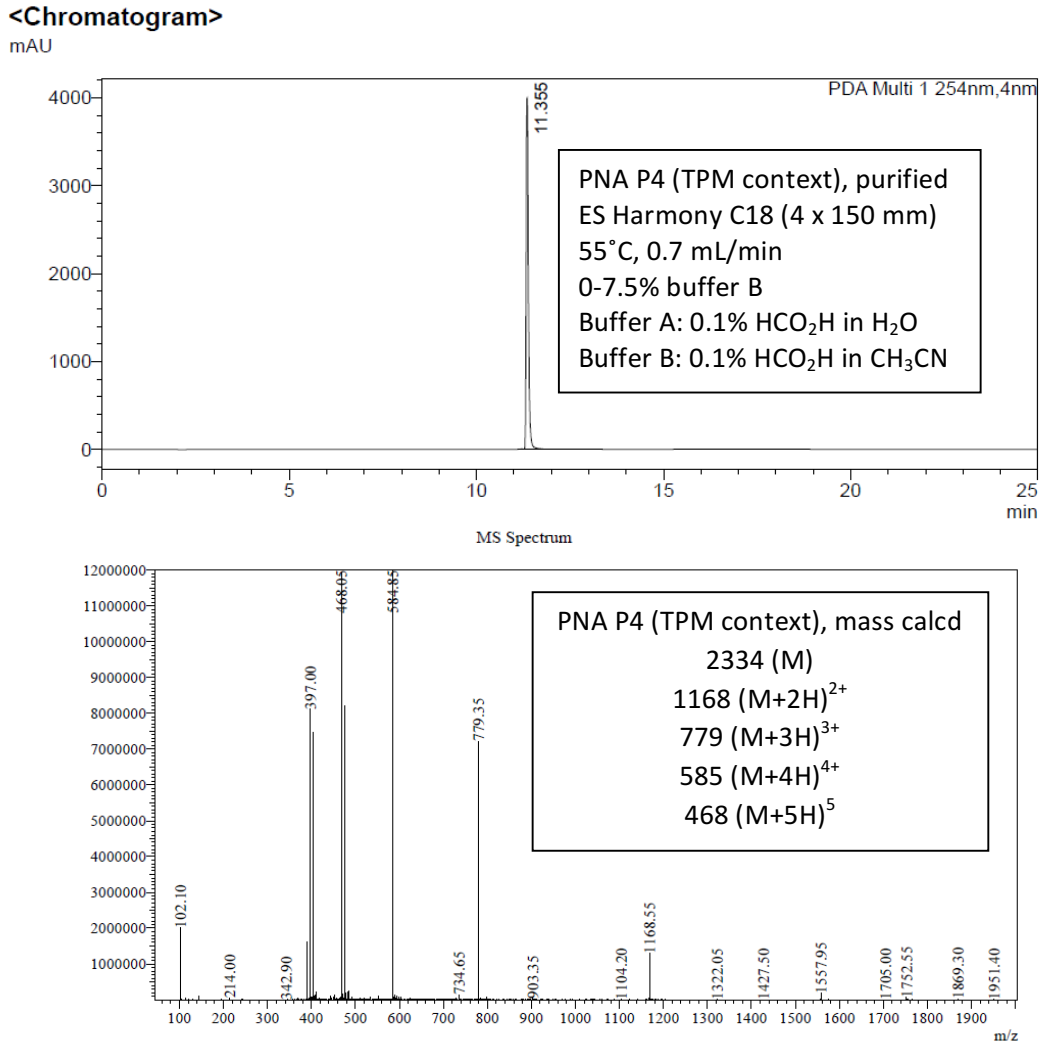


Figure S16. LCMS analysis of PNA_{TP4M}.

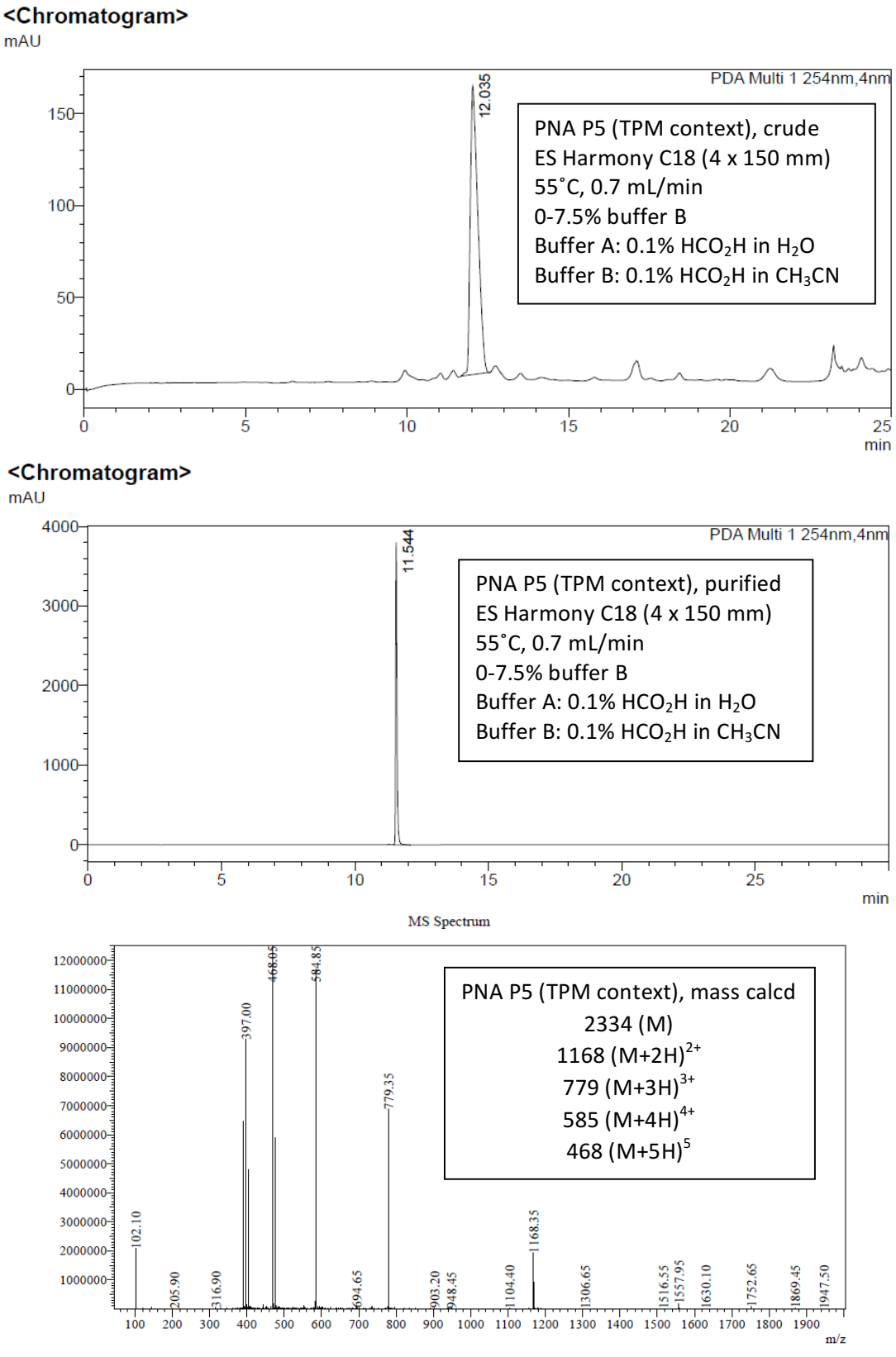


Figure S17. LCMS analysis of PNA_{TP5M}.

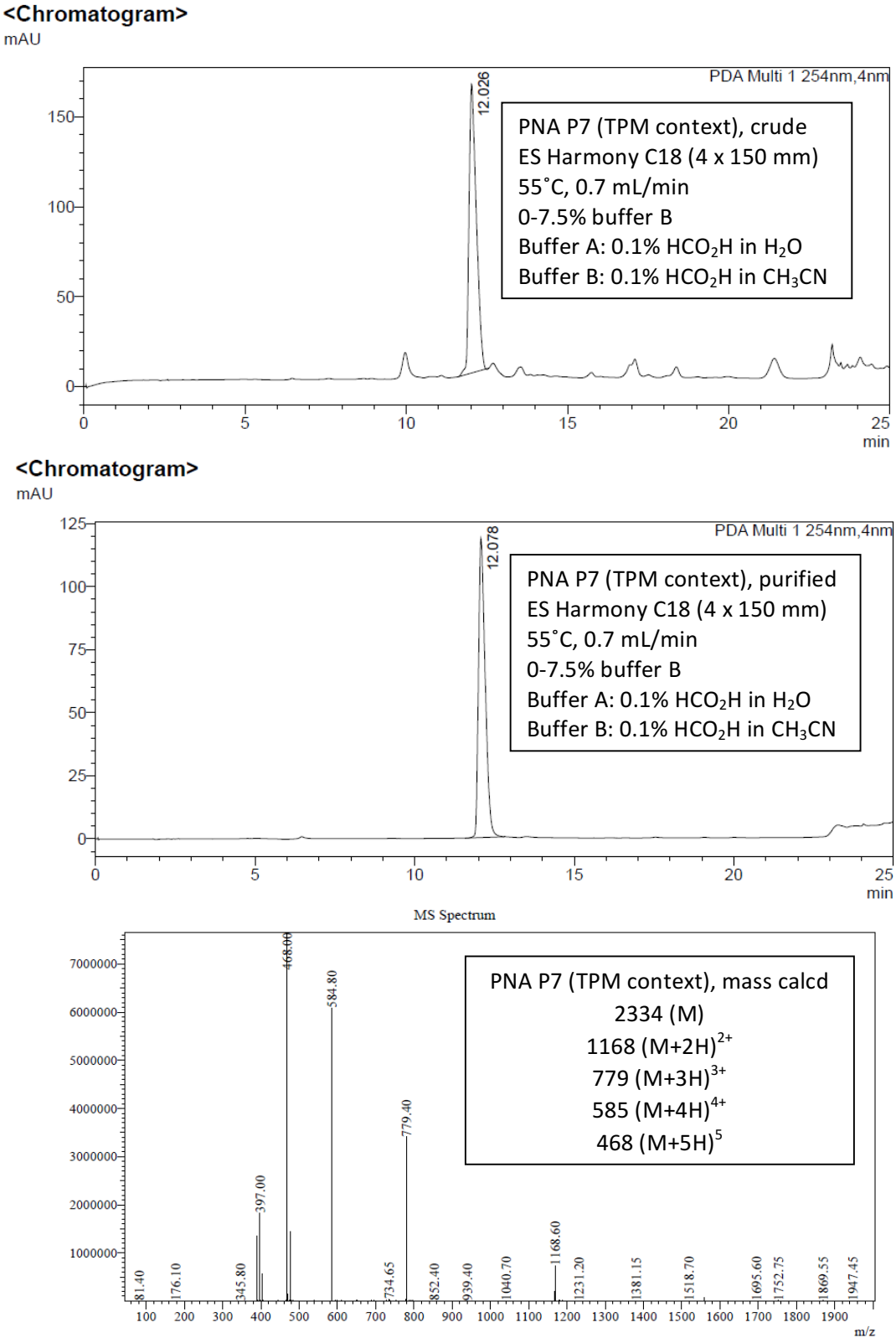


Figure S18. LCMS analysis of PNA_{TP7M}.

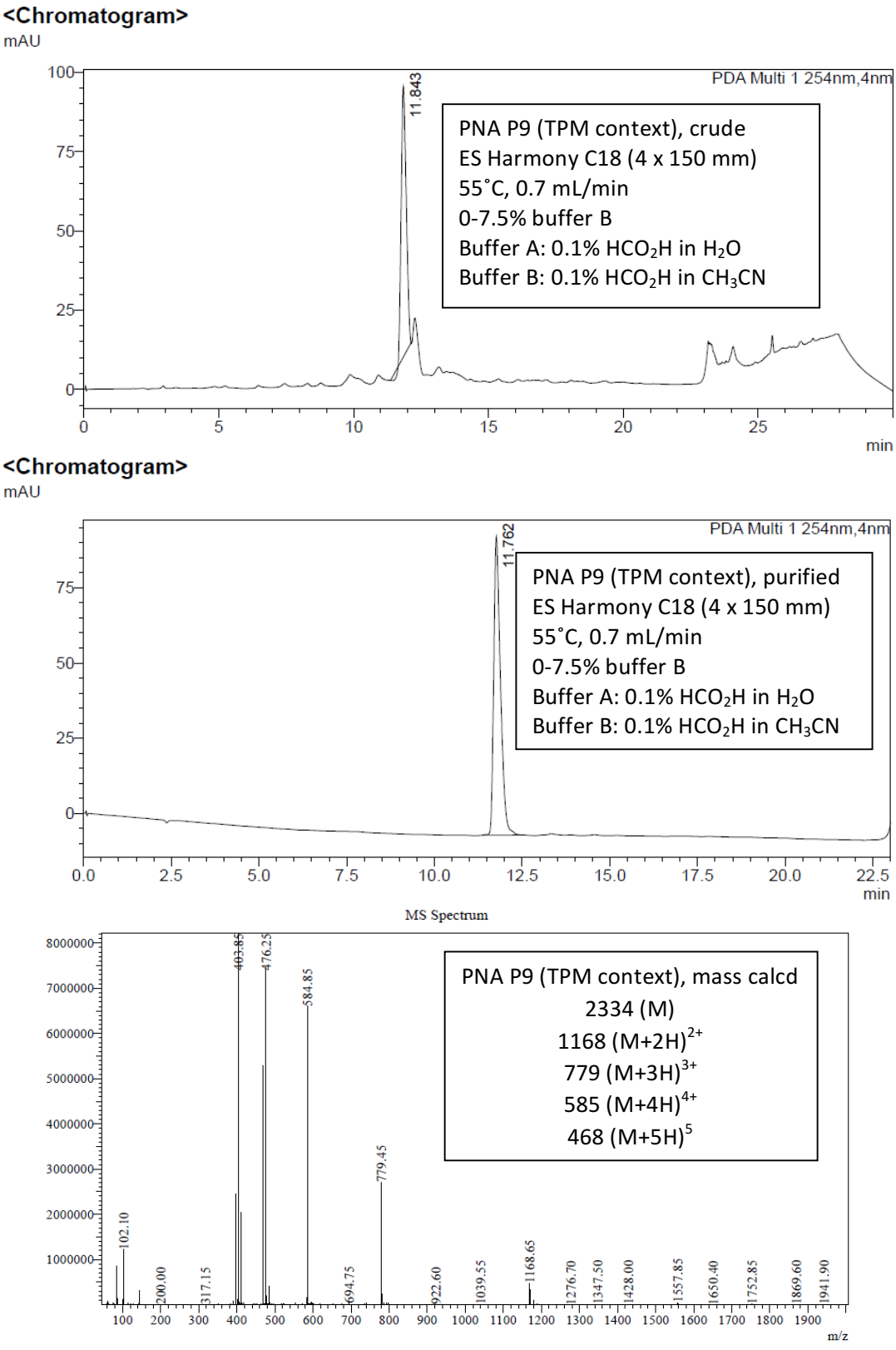


Figure S19. LCMS analysis of PNA_{TP9M}.

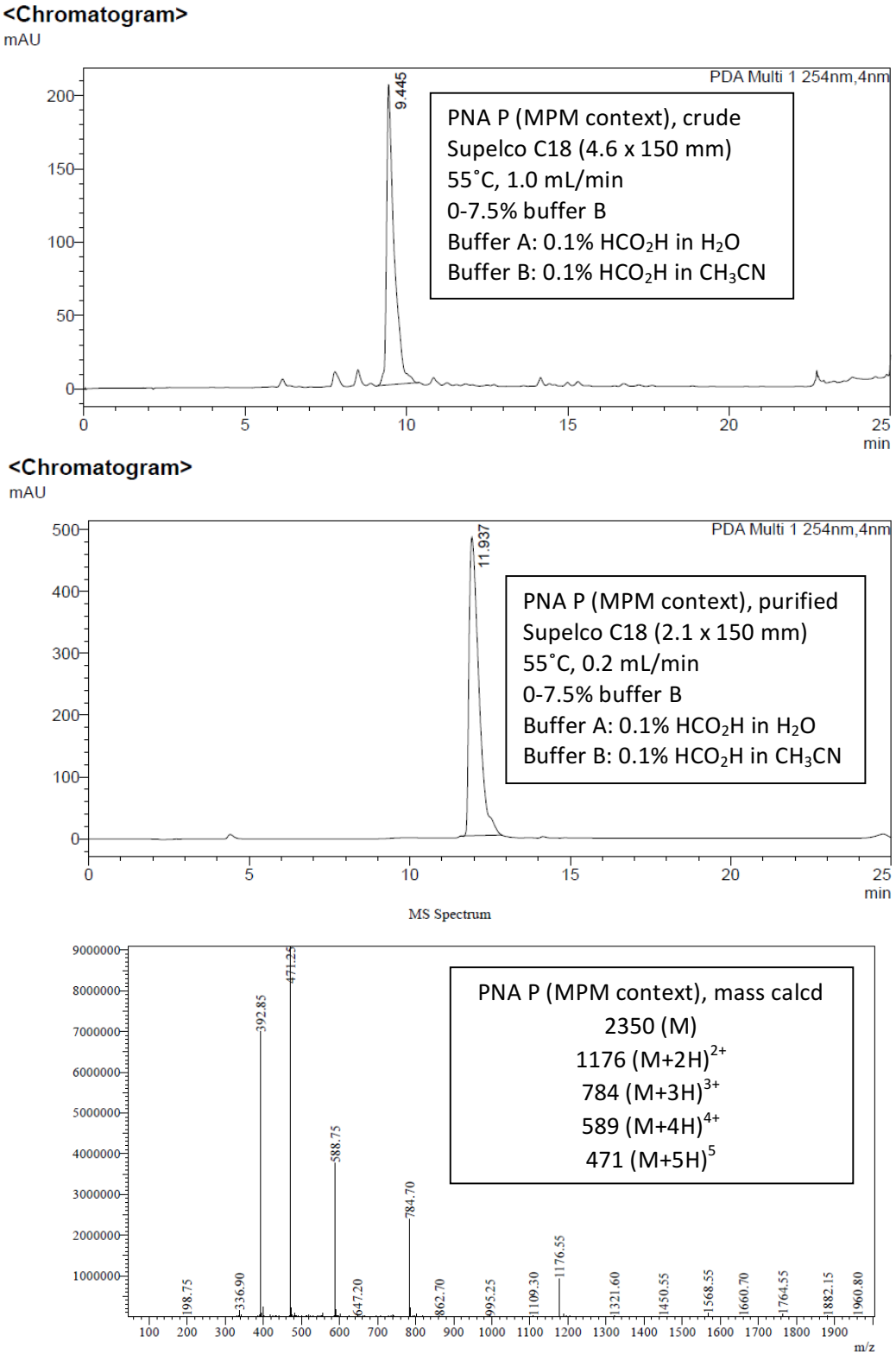
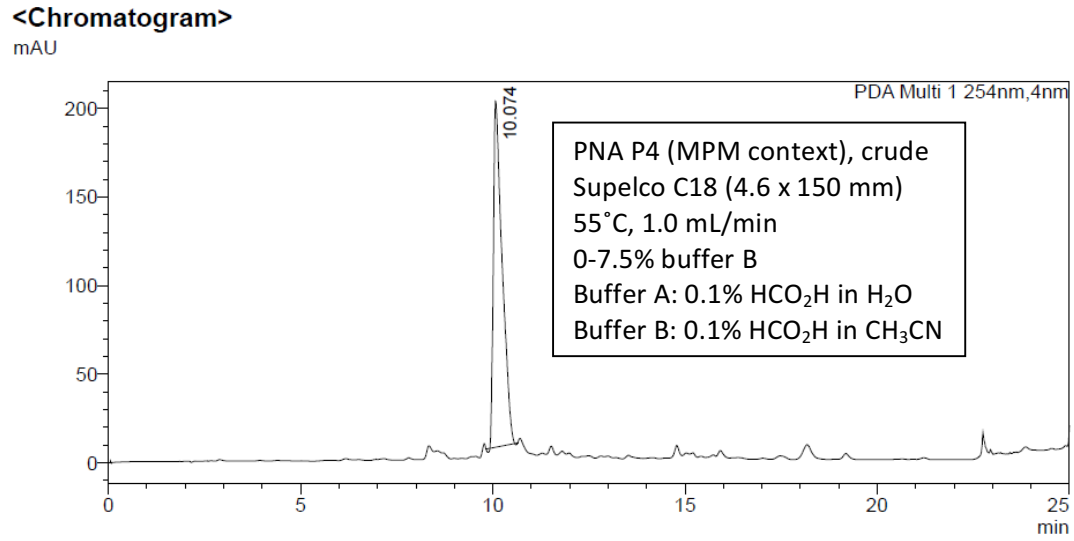


Figure S20. LCMS analysis of PNA_{MPM}.



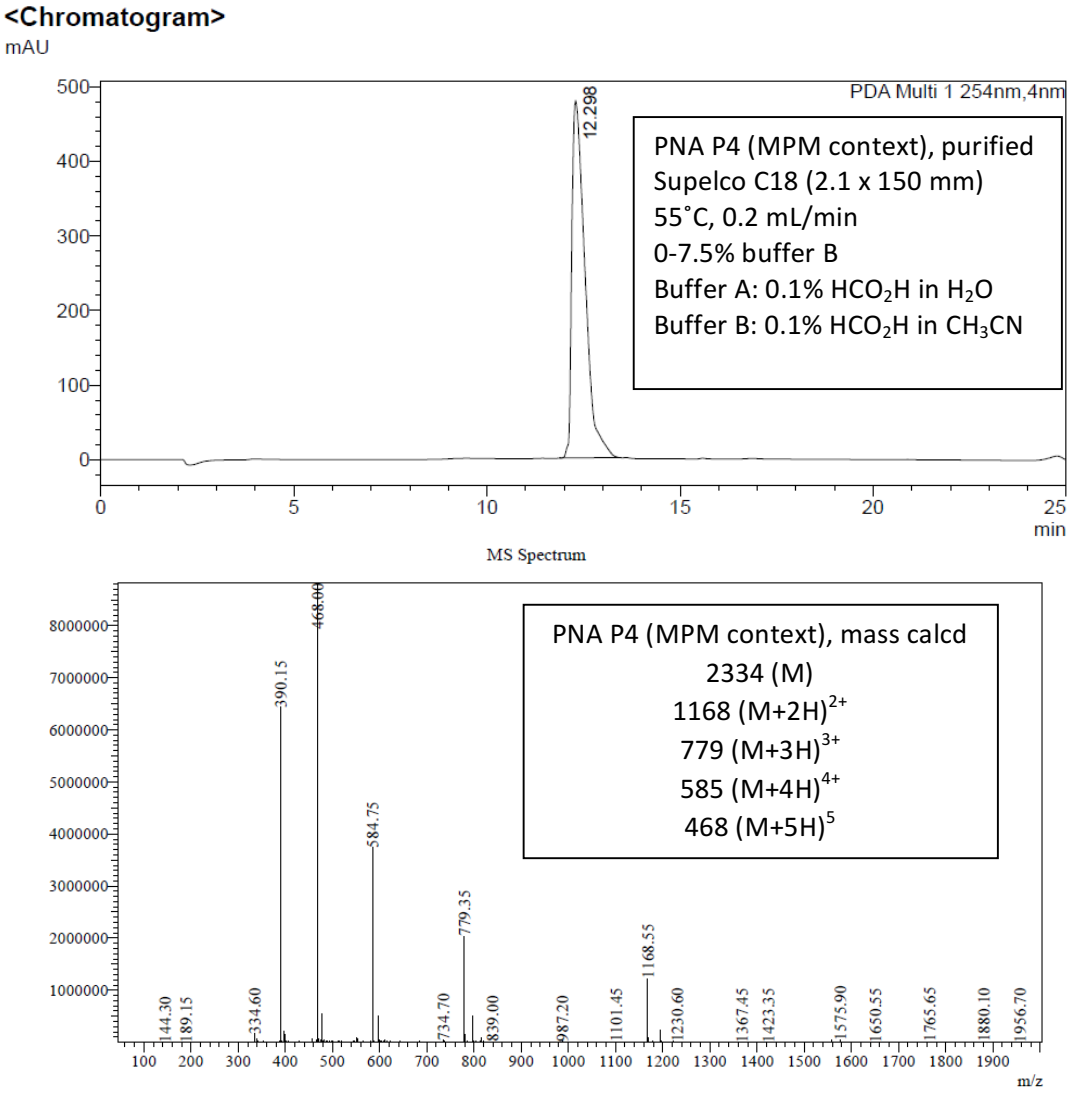


Figure S21. LCMS analysis of PNA_{MP4M}.

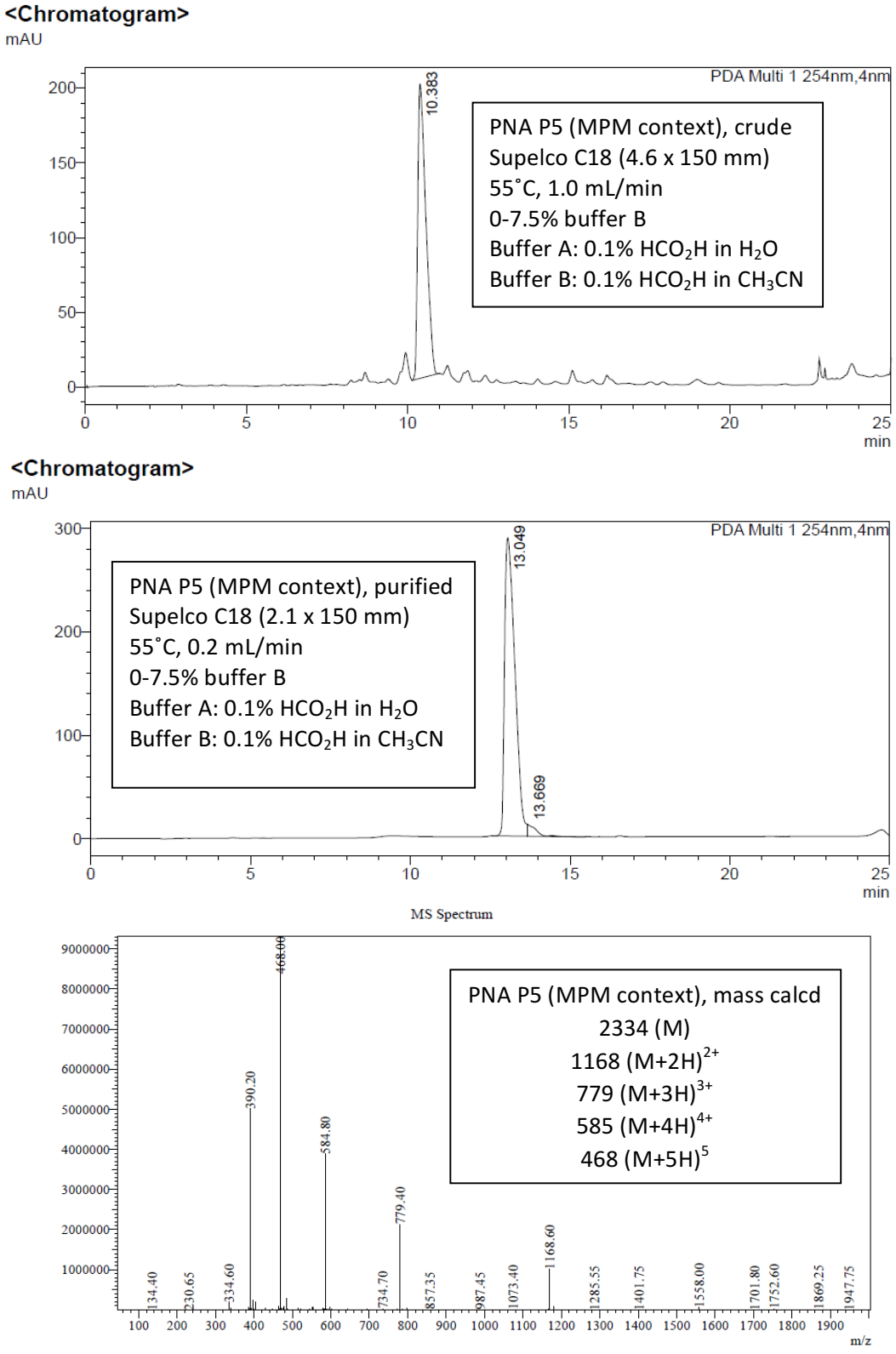


Figure S22. LCMS analysis of PNA_{MP5M}.

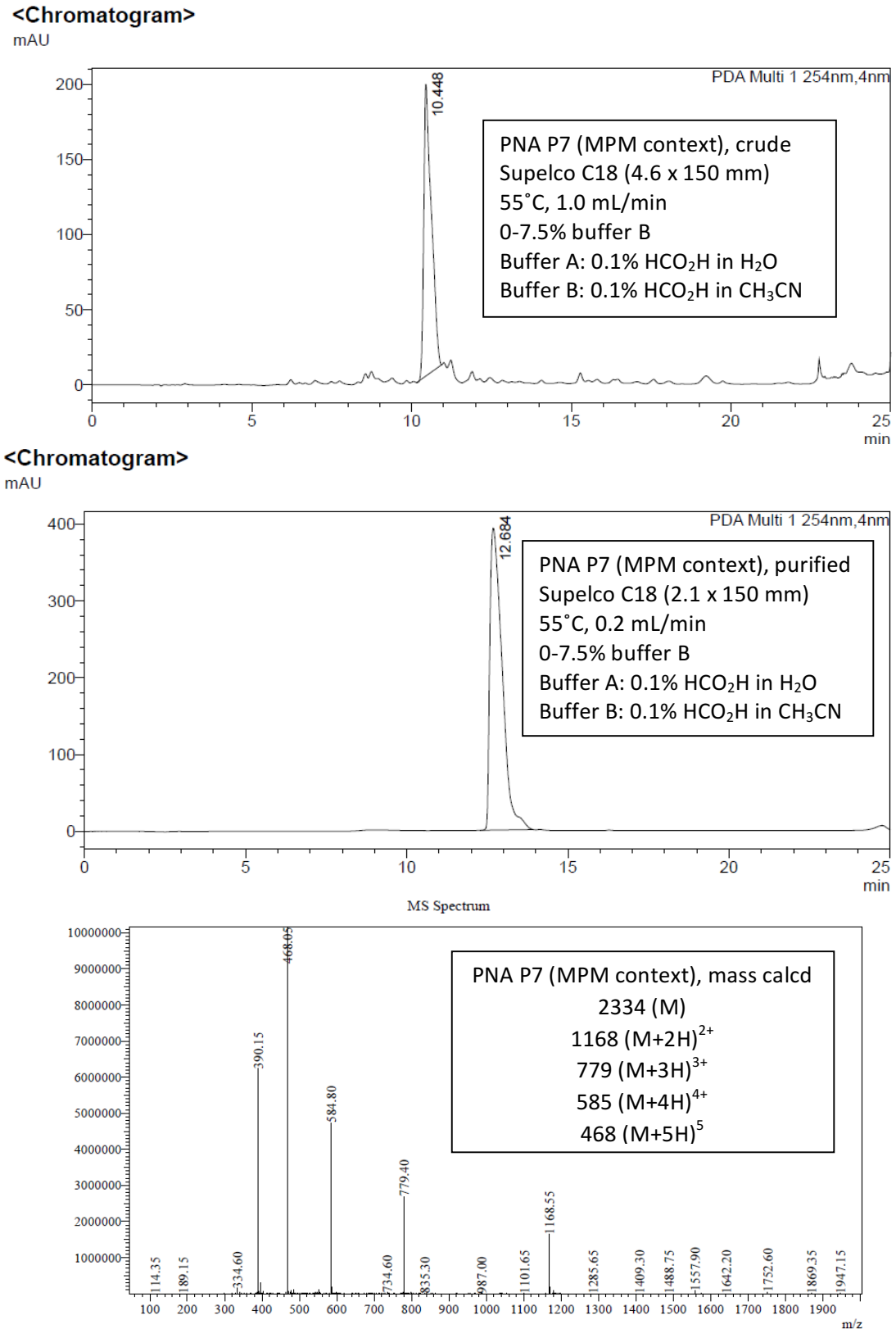


Figure S23. LCMS analysis of PNA_{MP7M}.

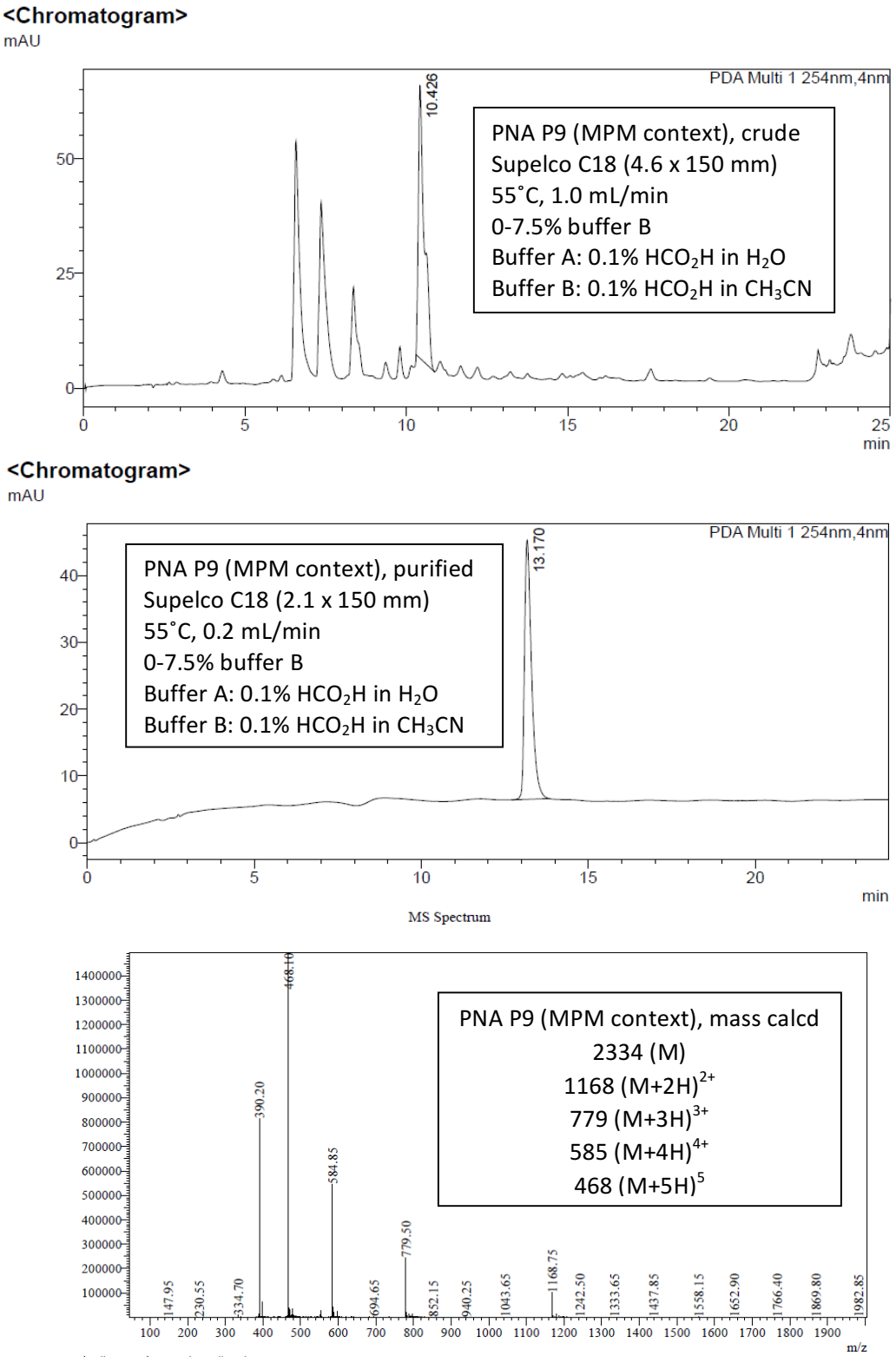


Figure S24. LCMS analysis of PNA_{MP9M}.

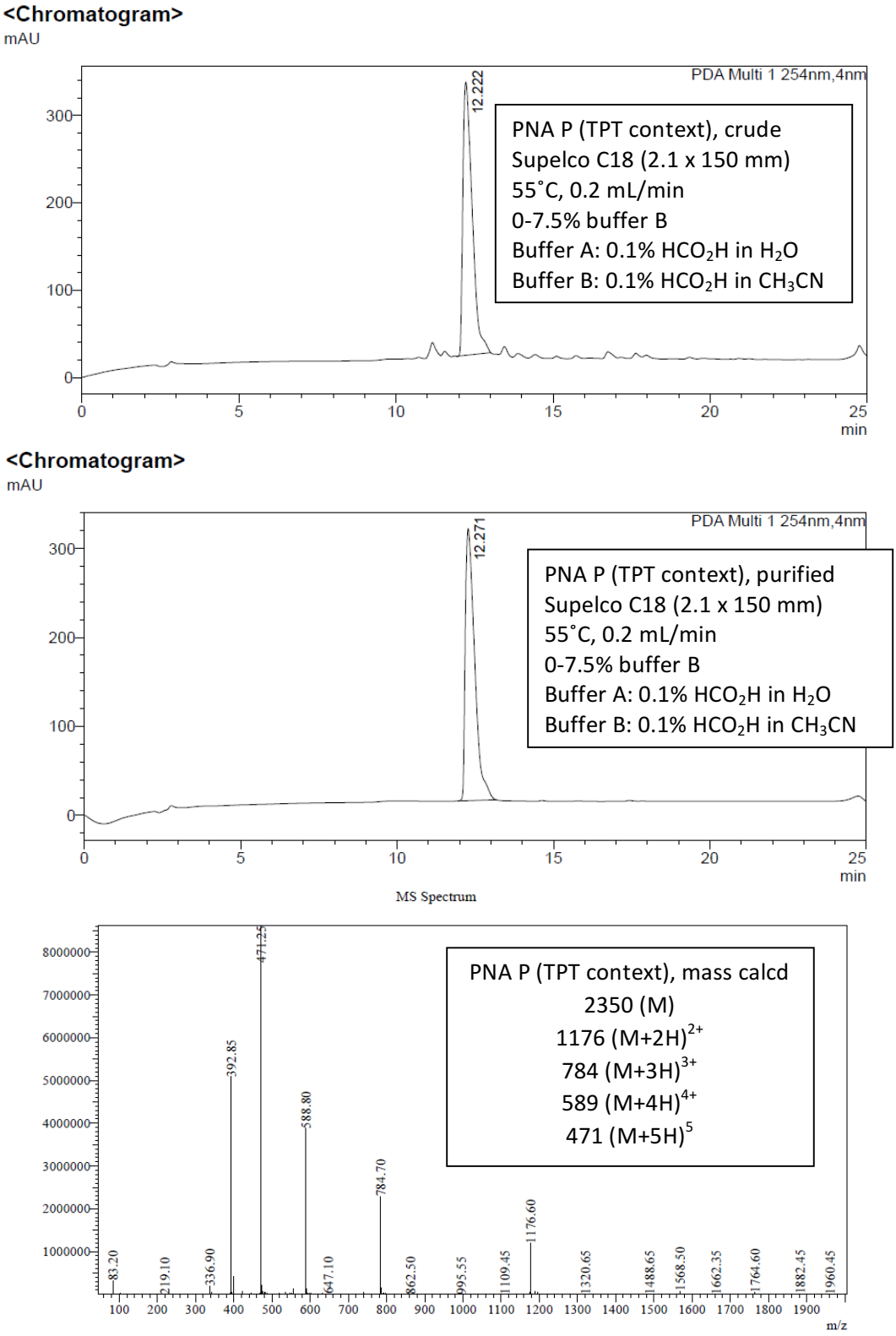


Figure S25. LCMS analysis of PNA_{TPT}.

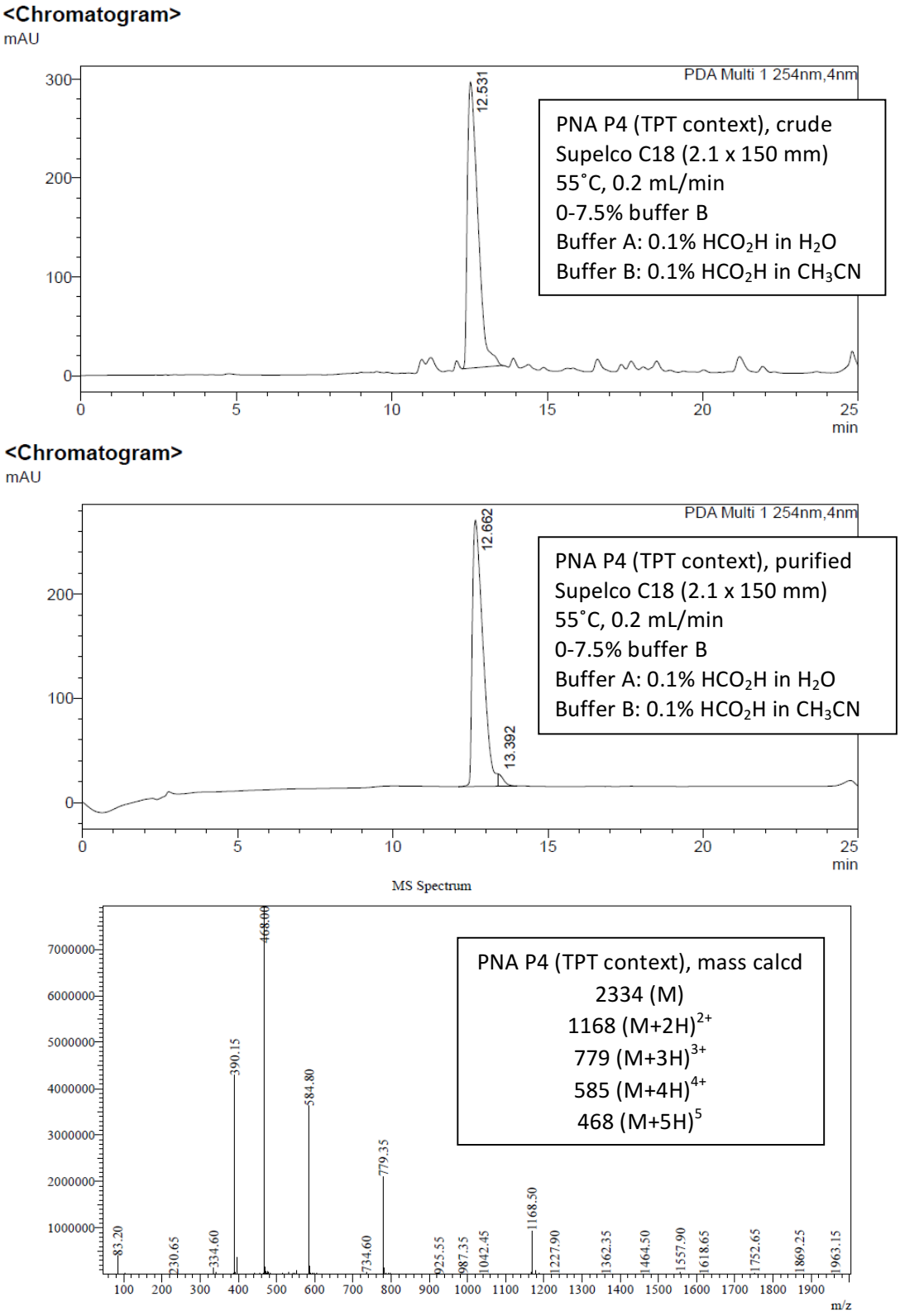


Figure S26. LCMS analysis of PNA_{TP4T}.

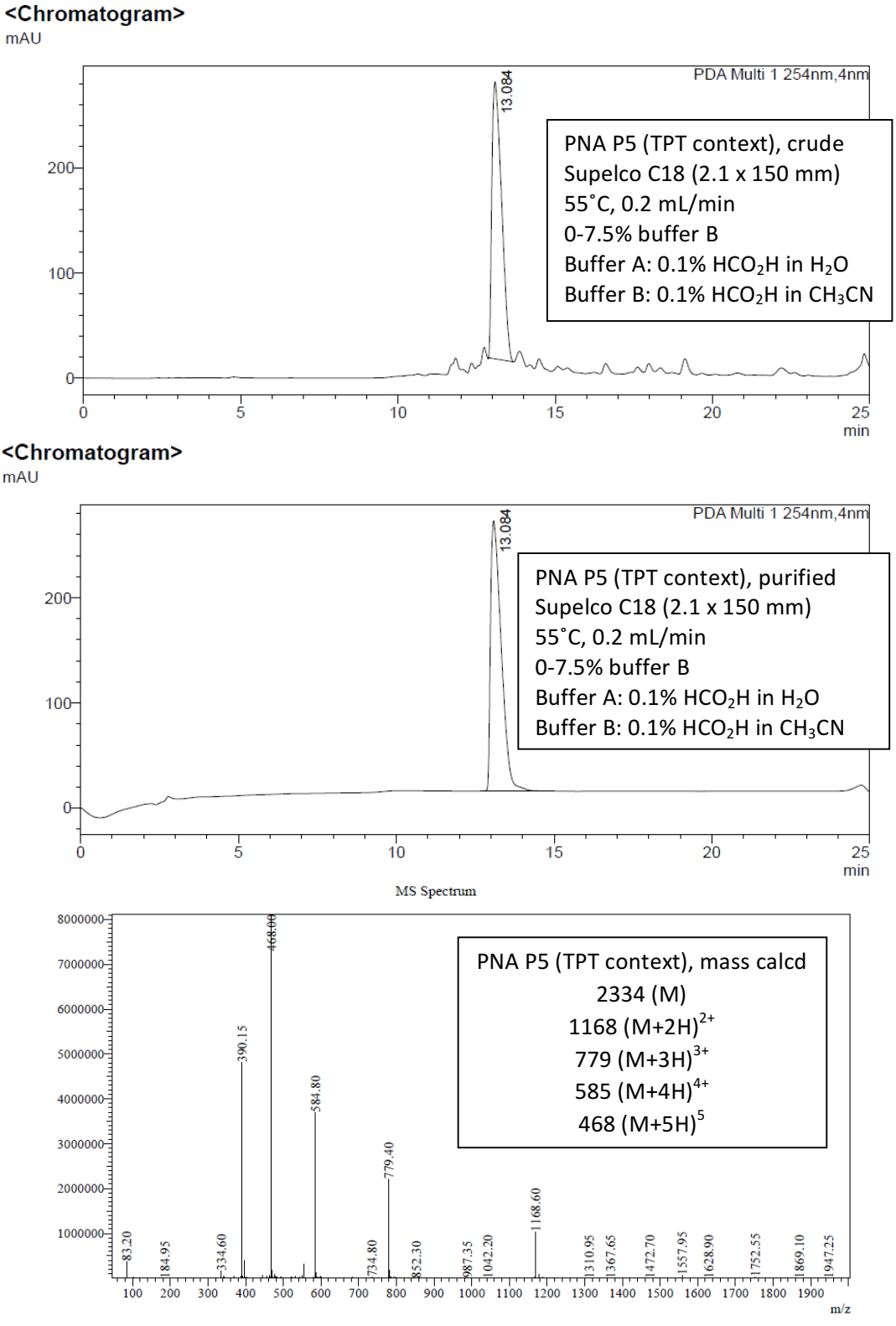


Figure S27. LCMS analysis of PNA_{TP5T}.

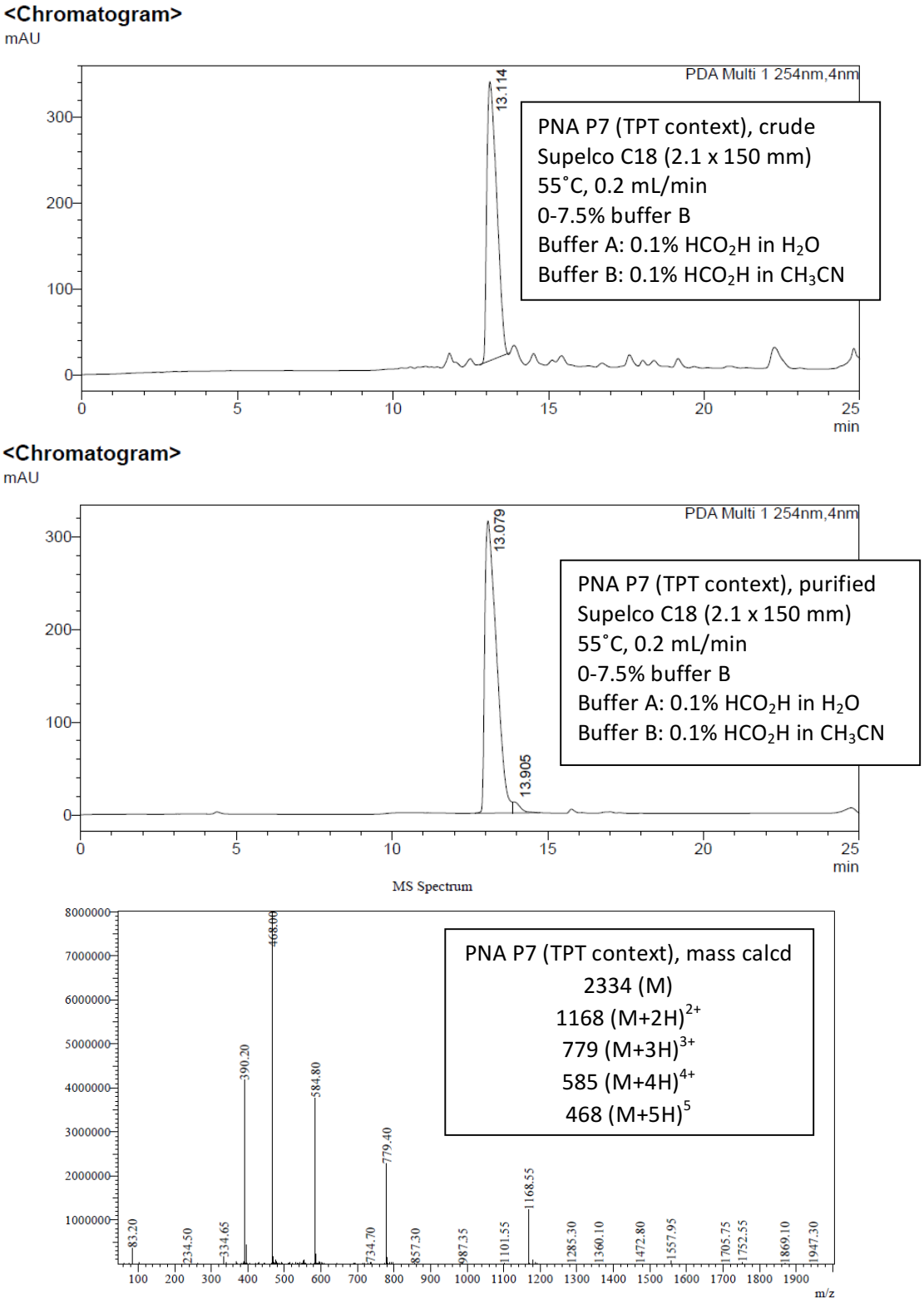


Figure S28. LCMS analysis of PNA_{TP7T}.

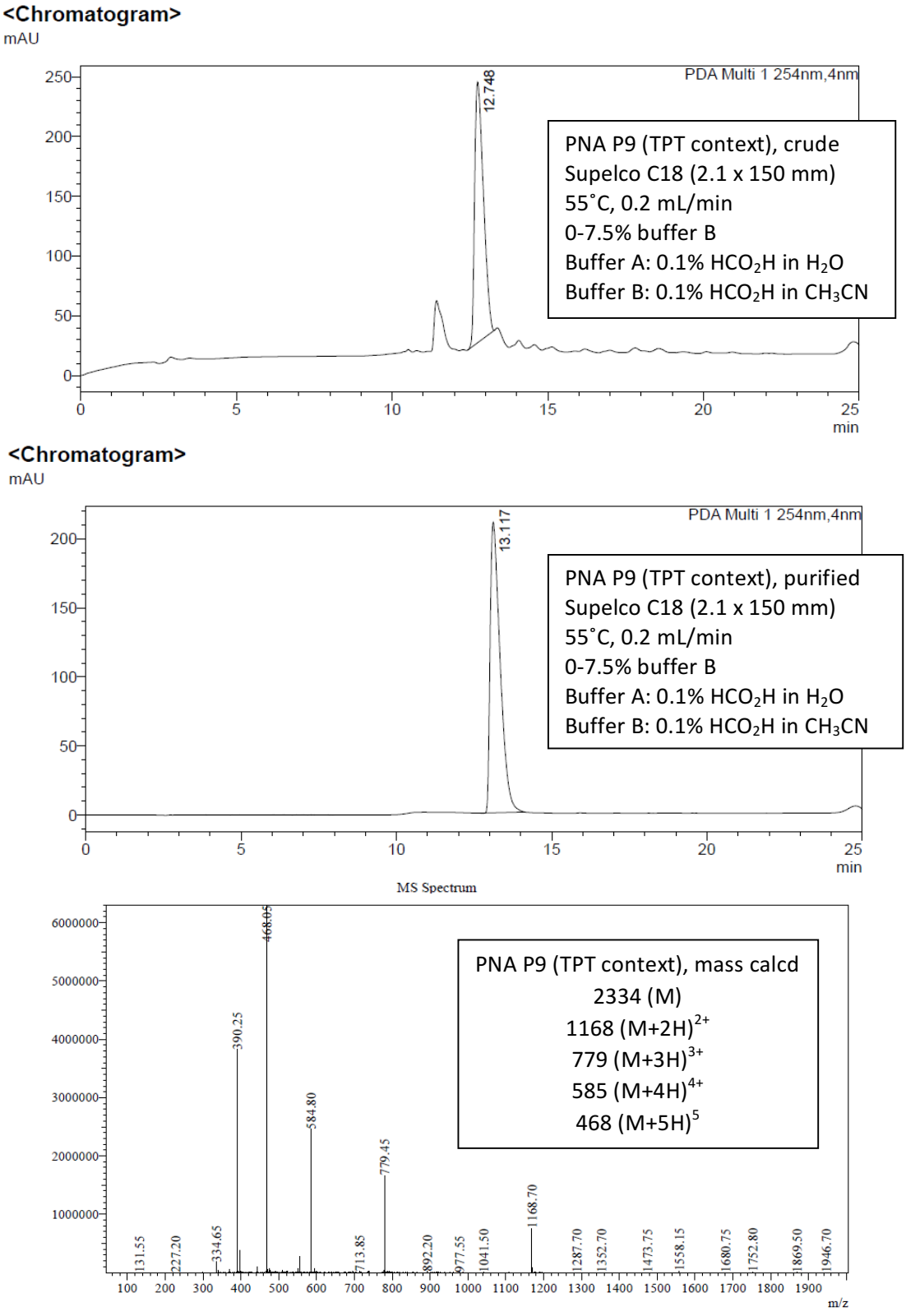


Figure S29. LCMS analysis of PNA_{TP9T}.

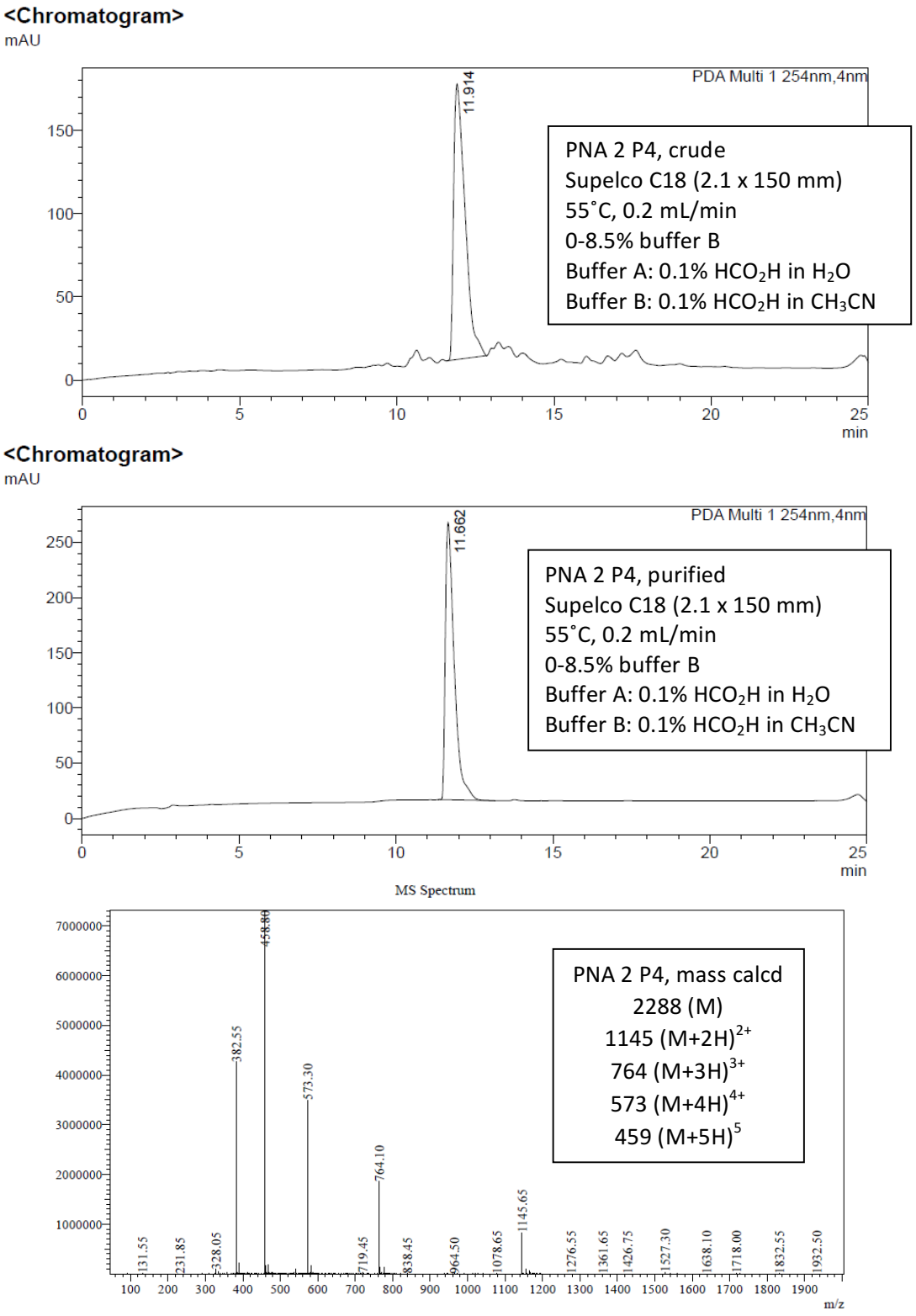


Figure S30. LCMS analysis of PNA 2_{P4}.

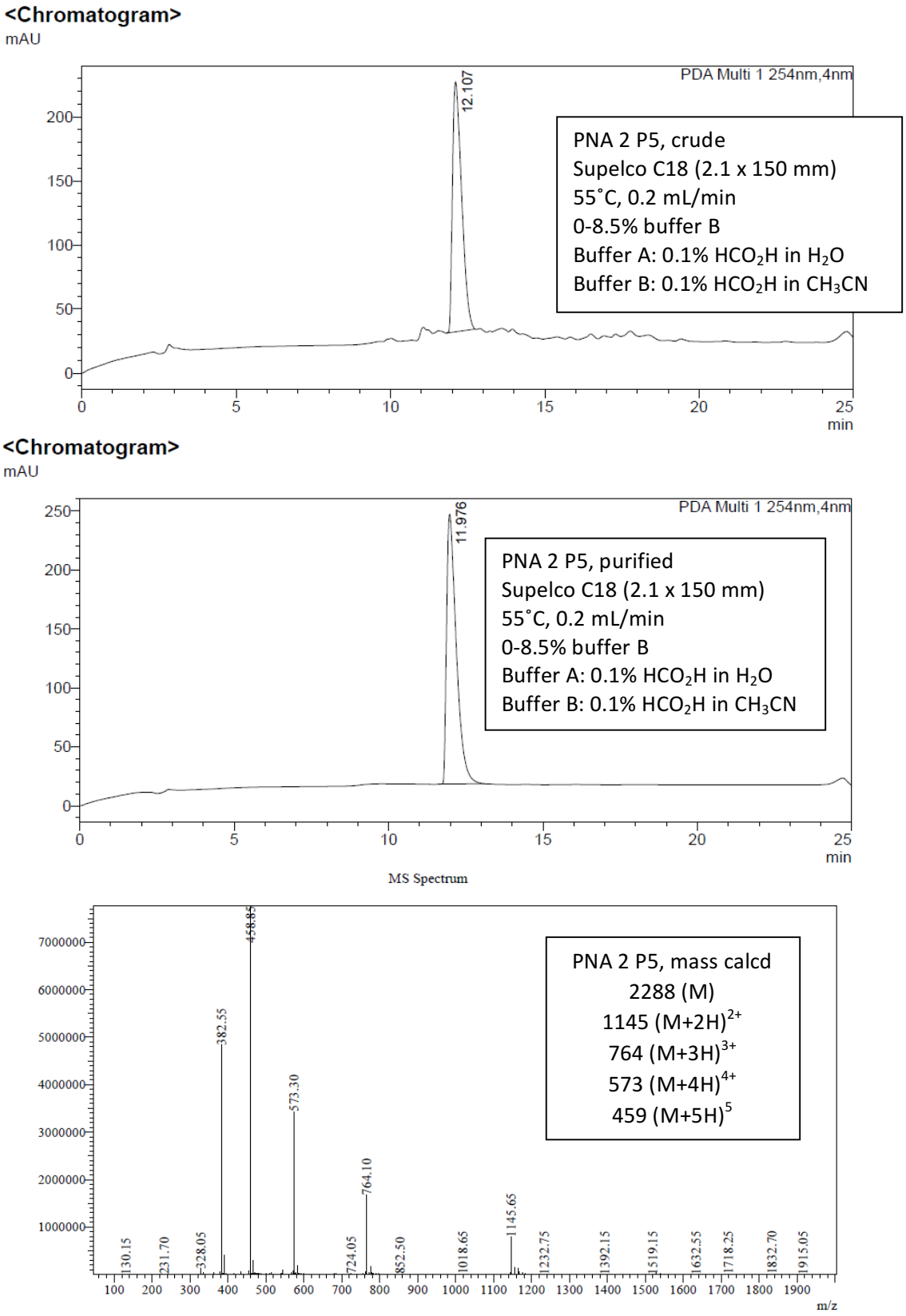


Figure 31. LCMS analysis of PNA 2_{P5}.

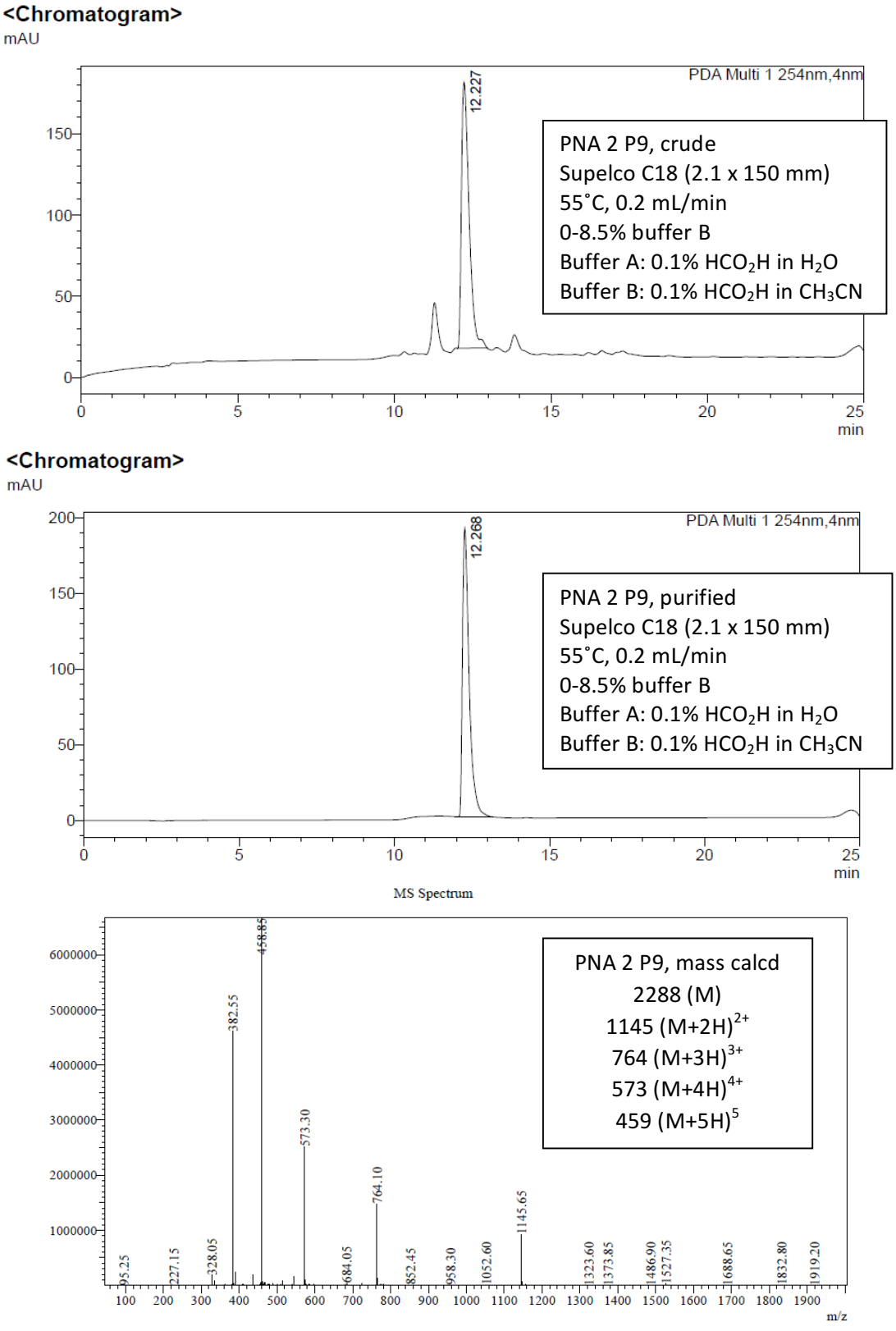


Figure S32. LCMS analysis of PNA 2_{P9}.

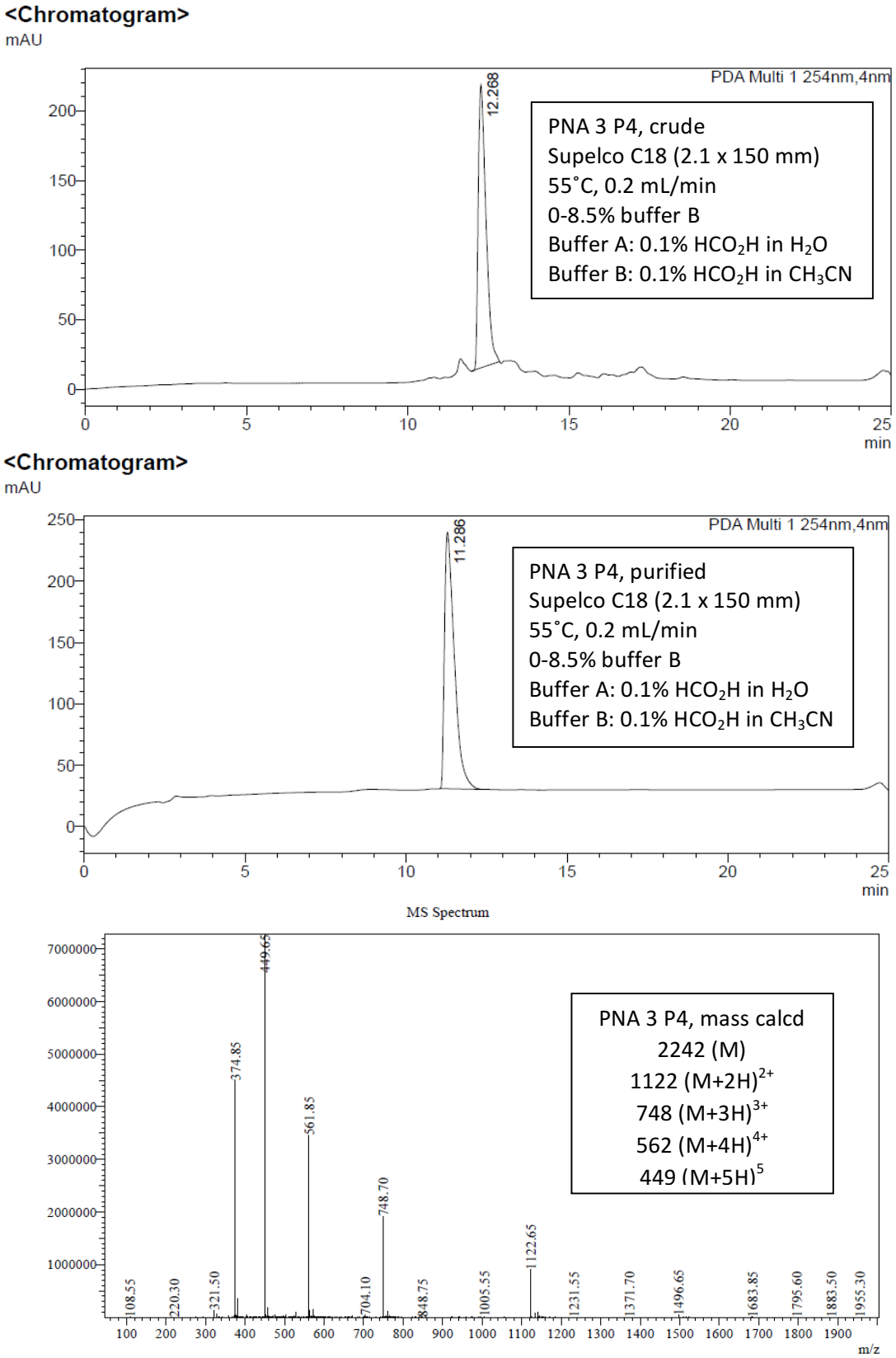


Figure S33. LCMS analysis of PNA 3_{P4}.

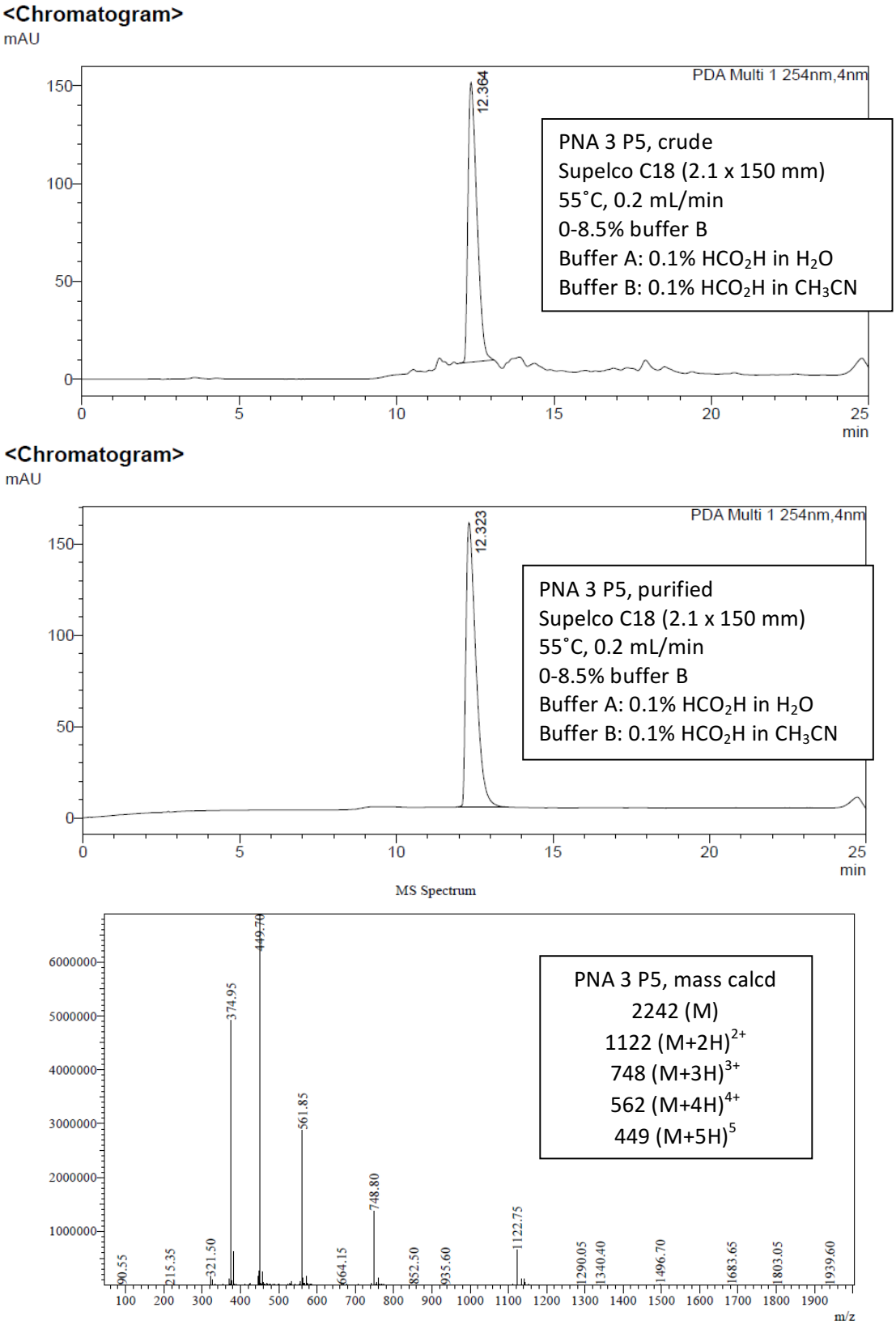


Figure S34. LCMS analysis of PNA 3_{P5}.

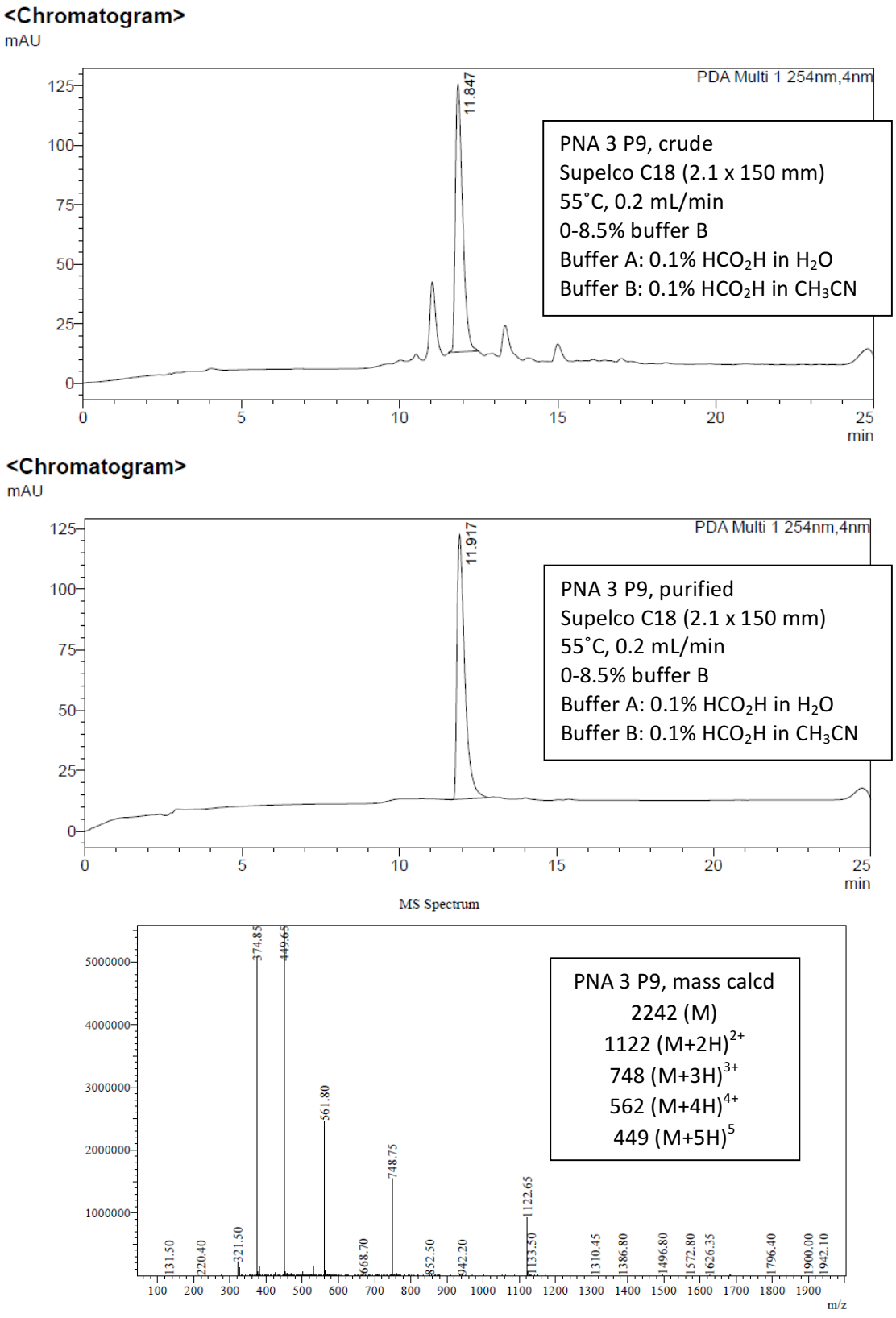


Figure S35. LCMS analysis of PNA 3_{P9}.

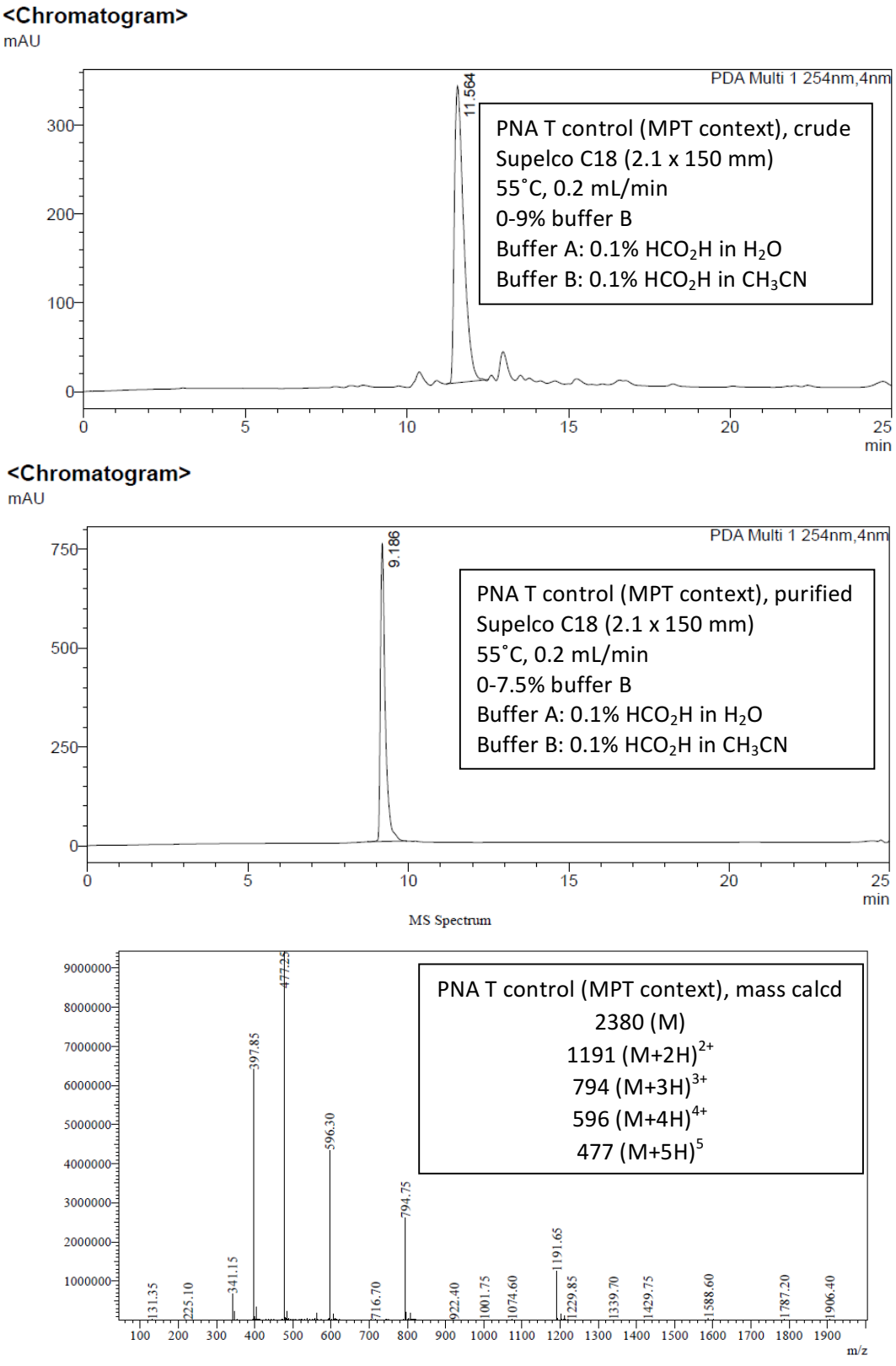


Figure S36. LCMS analysis of PNA T_{MPT}.

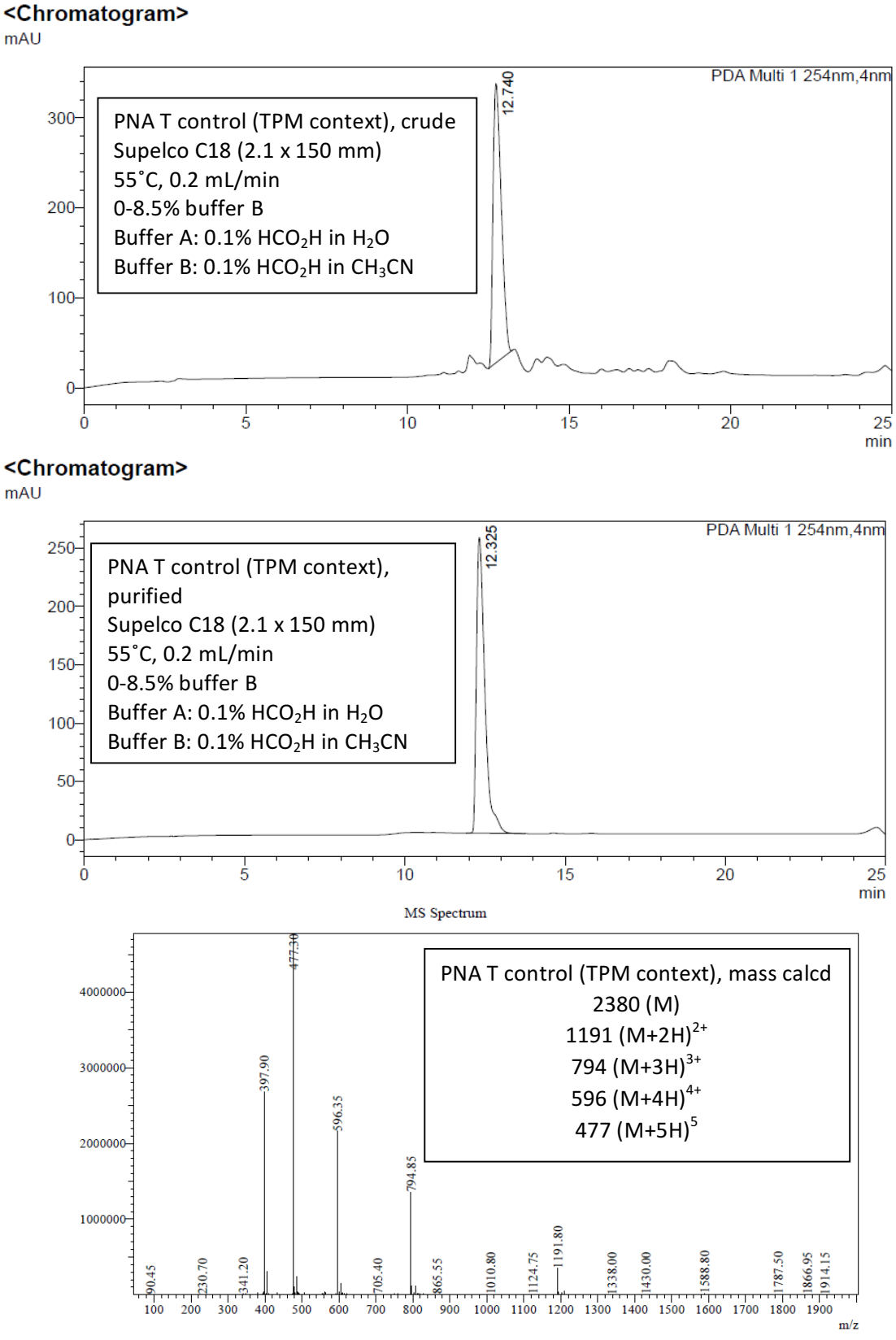


Figure S37. LCMS analysis of PNA T_{TPM}.

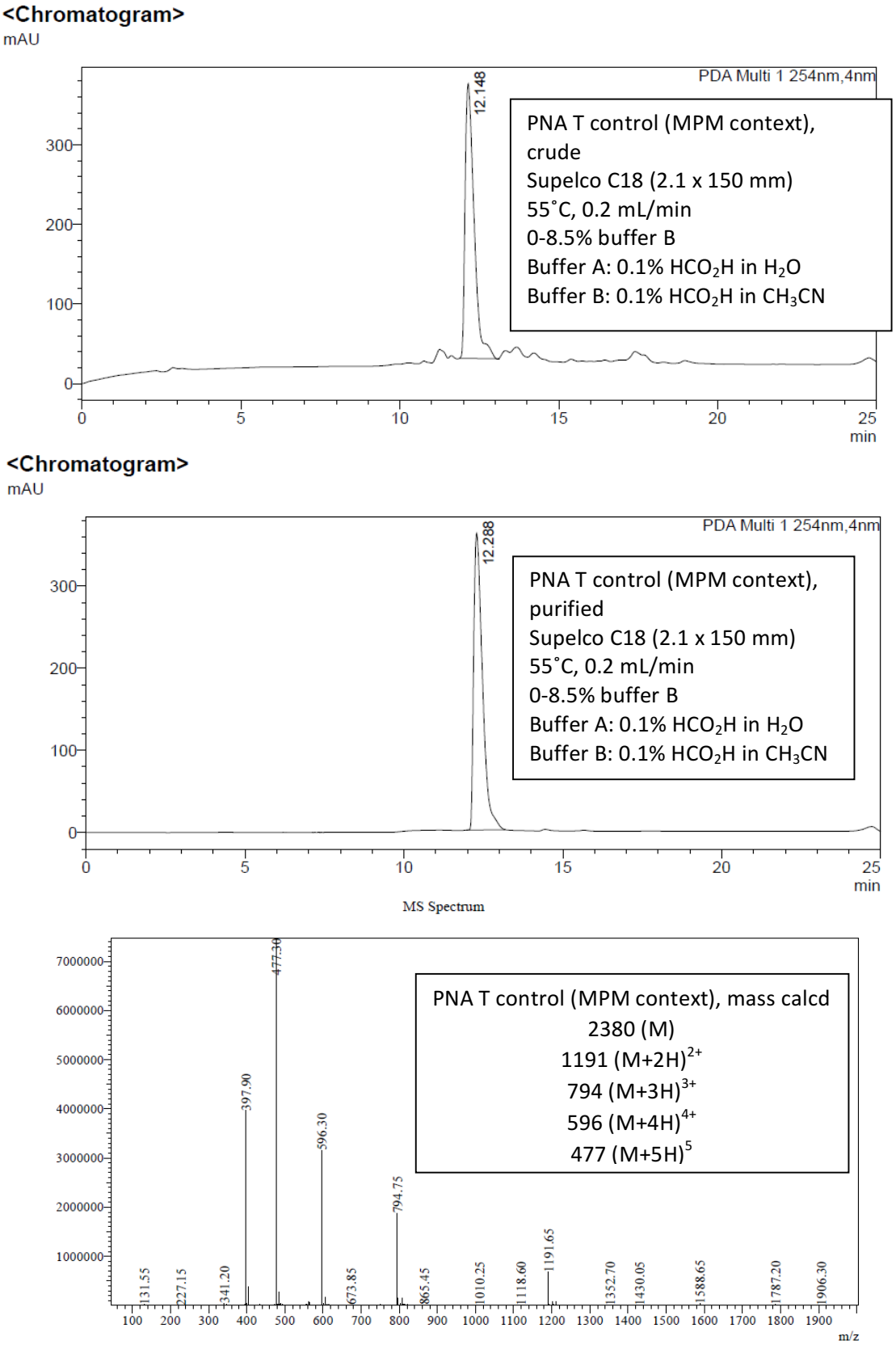


Figure S38. LCMS analysis of PNA T_{MPM}.

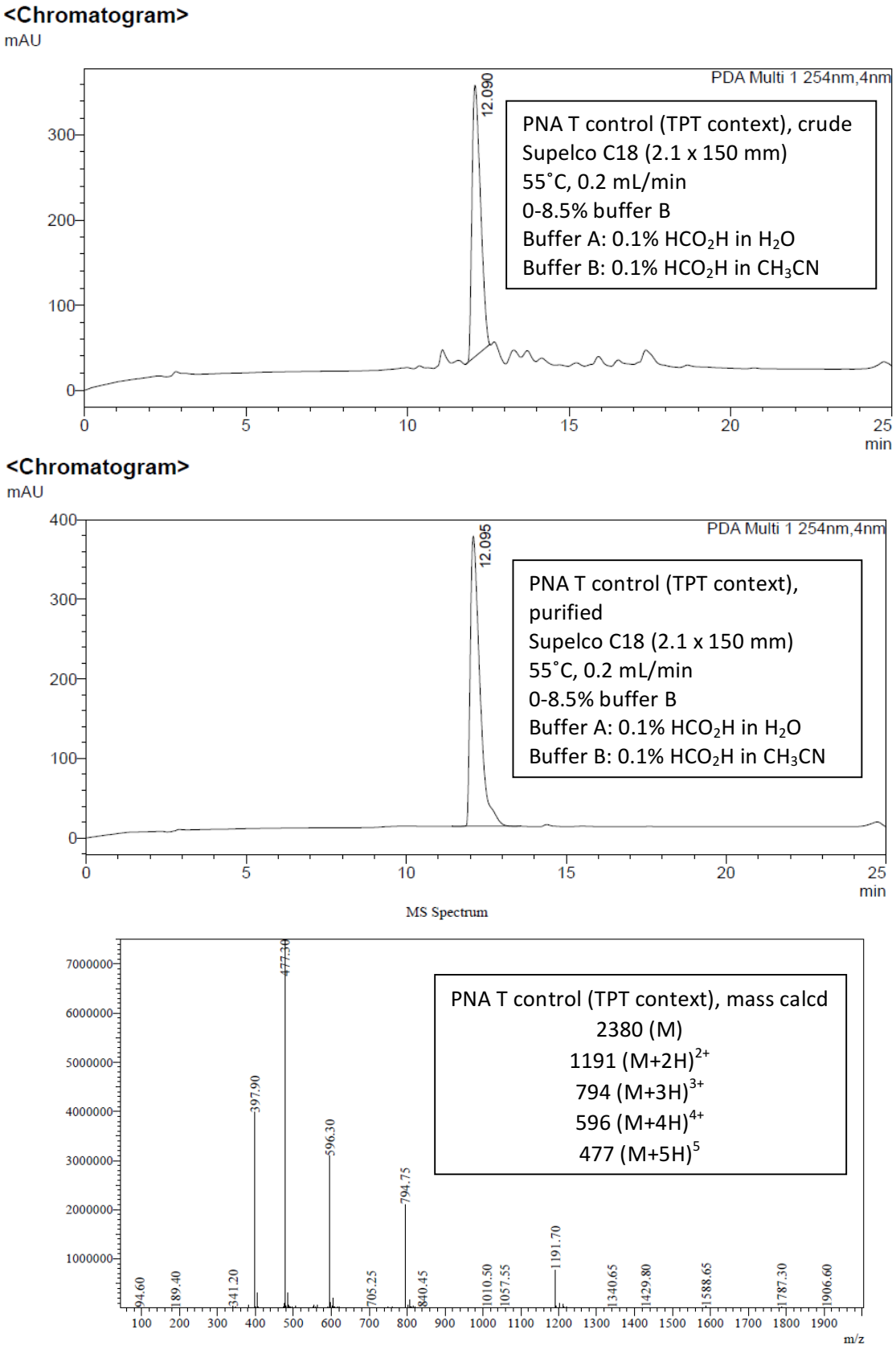


Figure S39. LCMS analysis of PNA T_{TPT}.

Table S1. LC/MS analysis of synthetic PNAs.

PNA	*Sequence*	*Mass calc.*	*Deconvoluted mass found $(M+2H)^{2+}$, $(M+3H)^{3+}$, $(M+4H)^{4+}$, $(M+5H)^{5+}$*
PNA P	H_2N – K MTM TMP TMM – $CONH_2$	2350	1176, 784, 589, 471
PNA P_1	H_2N – K MTM TMP_1 TMM – $CONH_2$	2333	1168, 779, 584, 468
PNA P_2	H_2N – K MTM TMP_2 TMM – $CONH_2$	2333	1168, 779, 584, 468
PNA P_3	H_2N – K MTM TMP_3 TMM – $CONH_2$	2333	1168, 779, 584, 468
PNA P_4	H_2N – K MTM TMP_4 TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA P_5	H_2N – K MTM TMP_5 TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA P_6	H_2N – K MTM TMP_6 TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA P_7	H_2N – K MTM TMP_7 TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA P_8	H_2N – K MTM TMP_8 TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA P_9	H_2N – K MTM TMP_9 TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA P_{10}	H_2N – K MTM TMP_{10} TMM – $CONH_2$	2256	1129, 753, 565, 452
PNA P_{11}	H_2N – K MTM TMP_{11} TMM – $CONH_2$	2332	1167, 778, 584, 467
PNA P_{12}	H_2N – K MTM TMP_{12} TMM – $CONH_2$	2364	1183, 789, 592, 474
PNA P_{13}	H_2N – K MTM TMP_{13} TMM – $CONH_2$	2352	1177, 785, 589, 471
PNA_{TPM}	H_2N – K MTM TPM TMM – $CONH_2$	2350	1176, 784, 589, 471
PNA_{TP4M}	H_2N – K MTM TP_4M TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA_{TP5M}	H_2N – K MTM TP_5M TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA_{TP7M}	H_2N – K MTM TP_7M TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA_{TP9M}	H_2N – K MTM TP_9M TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA_{MPM}	H_2N – K MTT MPM TMM – $CONH_2$	2350	1176, 784, 589, 471
PNA_{MP4M}	H_2N – K MTT MP_4M TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA_{MP5M}	H_2N – K MTT MP_5M TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA_{MP7M}	H_2N – K MTT MP_7M TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA_{MP9M}	H_2N – K MTT MP_9M TMM – $CONH_2$	2334	1168, 779, 585, 468
PNA_{TPT}	H_2N – K MTM TPT MMM – $CONH_2$	2350	1176, 784, 589, 471
PNA_{TP4T}	H_2N – K MTM TP_4T MMM – $CONH_2$	2334	1168, 779, 585, 468
PNA_{TP5T}	H_2N – K MTM TP_5T MMM – $CONH_2$	2334	1168, 779, 585, 468
PNA_{TP7T}	H_2N – K MTM TP_7T MMM – $CONH_2$	2334	1168, 779, 585, 468
PNA_{TP9T}	H_2N – K MTM TP_9T MMM – $CONH_2$	2334	1168, 779, 585, 468
PNA 2_{P4}	H_2N – K MTM M$\mathbf{P_4P_4}$ TMM – $CONH_2$	2288	1145, 764, 573, 459
PNA 2_{P5}	H_2N – K MTM M$\mathbf{P_5P_5}$ TMM – $CONH_2$	2288	1145, 764, 573, 459
PNA 2_{P9}	H_2N – K MTM M$\mathbf{P_9P_9}$ TMM – $CONH_2$	2288	1145, 764, 573, 459
PNA 3_{P4}	H_2N – K M$\mathbf{P_4}$M $\mathbf{P_4}$M$\mathbf{P_4}$ TMM – $CONH_2$	2242	1122, 748, 562, 449
PNA 3_{P5}	H_2N – K M$\mathbf{P_5}$M $\mathbf{P_5}$M$\mathbf{P_5}$ TMM – $CONH_2$	2242	1122, 748, 562, 449
PNA 3_{P9}	H_2N – K M$\mathbf{P_9}$M $\mathbf{P_9}$M$\mathbf{P_9}$ TMM – $CONH_2$	2242	1122, 748, 562, 449
PNA T_{MPT}	H_2N – MTM TMT TMM K – $CONH_2$	2380	1191, 794, 596, 477
PNA T_{TPM}	H_2N – K MTM TTM TMM – $CONH_2$	2380	1191, 794, 596, 477
PNA T_{MPM}	H_2N – K MTT MTM TMM – $CONH_2$	2380	1191, 794, 596, 477
PNA T_{TPT}	H_2N – K MTM TTT MMM – $CONH_2$	2380	1191, 794, 596, 477

Table S2. ITC results for Figure 3.

Name	K_D (M)	K_a (M^{-1})	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
PNA P vs HRP C 01	2.79E-07	3.6E+06	-35.3	-8.9	26.4
PNA P vs HRP C 02	2.76E-07	3.6E+06	-33.6	-9.0	24.7
PNA P vs HRP C 03	3.08E-07	3.2E+06	-32.5	-8.9	23.6
Average	**2.88E-07**	**3.5E+06**	**-33.8**	**-8.9**	**24.9**
St. Dev.	**1.44E-08**	**1.7E+05**	**1.2**	**0.0**	**1.2**
PNA P1 vs HRP C 01	4.11E-07	2.4E+06	-16.9	-8.7	8.2
PNA P1 vs HRP C 02	6.35E-07	1.6E+06	-20.0	-8.5	11.5
PNA P1 vs HRP C 03	4.51E-07	2.2E+06	-18.9	-8.7	10.3
Average	**4.99E-07**	**2.1E+06**	**-18.6**	**-8.6**	**10.0**
St. Dev.	**9.75E-08**	**3.6E+05**	**1.3**	**0.1**	**1.4**
PNA P2 vs HRP C 01	4.11E-07	2.4E+06	-29.0	-8.7	20.3
PNA P2 vs HRP C 02	3.02E-07	3.3E+06	-27.6	-8.9	18.7
Average	**3.57E-07**	**2.9E+06**	**-28.3**	**-8.8**	**19.5**
St. Dev.	**4.45E-08**	**3.6E+05**	**0.6**	**0.1**	**0.7**
PNA P3 vs HRP C 01	2.38E-07	4.2E+06	-27.0	-9.0	18.0
PNA P3 vs HRP C 02	3.45E-07	2.9E+06	-26.6	-8.8	17.8
PNA P3 vs HRP C 03	3.76E-07	2.7E+06	-27.6	-8.8	18.8
Average	**3.20E-07**	**3.3E+06**	**-27.1**	**-8.9**	**18.2**
St. Dev.	**5.91E-08**	**6.8E+05**	**0.4**	**0.1**	**0.4**
PNA P4 vs HRP C 01	1.34E-07	7.5E+06	-36.1	-9.4	26.7
PNA P4 vs HRP C 02	1.29E-07	7.8E+06	-32.1	-9.4	22.7
PNA P4 vs HRP C 03	1.89E-07	5.3E+06	-35.8	-9.2	26.6
Average	**1.51E-07**	**6.8E+06**	**-34.7**	**-9.3**	**25.3**
St. Dev.	**2.72E-08**	**1.1E+06**	**1.8**	**0.1**	**1.9**
PNA P5 vs HRP C 01	3.67E-07	2.7E+06	-47.2	-8.8	38.4
PNA P5 vs HRP C 02	2.01E-07	5.0E+06	-28.0	-9.1	18.9
PNA P5 vs HRP C 03	2.81E-07	3.6E+06	-26.2	-8.9	17.3
Average	**2.83E-07**	**3.8E+06**	**-33.8**	**-9.0**	**24.9**
St. Dev.	**6.78E-08**	**9.3E+05**	**9.5**	**0.1**	**9.6**
PNA P6 vs HRP C 01	2.37E-07	4.2E+06	-33.6	-9.0	24.6
PNA P6 vs HRP C 02	2.09E-07	4.8E+06	-31.9	-9.1	22.8
PNA P6 vs HRP C 03	2.39E-07	4.2E+06	-37.2	-9.0	28.1
Average	**2.28E-07**	**4.4E+06**	**-34.2**	**-9.1**	**25.2**
St. Dev.	**1.37E-08**	**2.8E+05**	**2.2**	**0.0**	**2.2**
PNA P7 vs HRP C 01	2.45E-07	4.1E+06	-40.1	-9.0	31.1
PNA P7 vs HRP C 02	4.50E-07	2.2E+06	-30.4	-8.7	21.7
PNA P7 vs HRP C 03	4.88E-07	2.0E+06	-35.8	-8.6	27.2
Average	**3.94E-07**	**2.8E+06**	**-35.4**	**-8.8**	**26.7**
St. Dev.	**1.07E-07**	**9.2E+05**	**4.0**	**0.2**	**3.9**

PNA P8 vs HRP C 01	4.63E-07	2.2E+06	-18.1	-8.6	9.4
PNA P8 vs HRP C 02	3.17E-07	3.2E+06	-19.0	-8.9	10.1
PNA P8 vs HRP C 03	4.94E-07	2.0E+06	-19.3	-8.6	10.7
Average	**4.25E-07**	**2.4E+06**	**-18.8**	**-8.7**	**10.1**
St. Dev.	**7.72E-08**	**5.0E+05**	**0.5**	**0.1**	**0.5**
PNA P9 vs HRP C 01	1.91E-07	5.2E+06	-48.9	-9.2	39.7
PNA P9 vs HRP C 02	1.36E-07	7.4E+06	-34.8	-9.4	25.5
PNA P9 vs HRP C 03	1.61E-07	6.2E+06	-49.3	-9.3	40.0
Average	**1.63E-07**	**6.3E+06**	**-44.3**	**-9.3**	**35.1**
St. Dev.	**2.25E-08**	**8.7E+05**	**6.7**	**0.1**	**6.8**
PNA P10 vs HRP C 01	2.61E-07	3.8E+06	-35.9	-9.0	26.9
PNA P10 vs HRP C 02	4.24E-07	2.4E+06	-40.6	-8.7	31.9
PNA P10 vs HRP C 03	2.85E-07	3.5E+06	-35.5	-8.9	26.5
Average	**3.23E-07**	**3.2E+06**	**-37.3**	**-8.9**	**28.4**
St. Dev.	**7.19E-08**	**6.3E+05**	**2.3**	**0.1**	**2.5**
PNA 11 vs HRP C 01	1.04E-06	9.6E+05	-39.8	-8.2	31.6
PNA 11 vs HRP C 02	1.09E-06	9.2E+05	-30.2	-8.1	22.0
PNA 11 vs HRP C 03	8.22E-07	1.2E+06	-30.8	-8.3	22.5
Average	**9.84E-07**	**1.0E+06**	**-33.6**	**-8.2**	**25.4**
St. Dev.	**1.16E-07**	**1.3E+05**	**4.4**	**0.1**	**4.4**
PNA P12 vs HRP C 01	9.66E-07	1.0E+06	-29.9	-8.2	21.7
PNA P12 vs HRP C 02	7.69E-07	1.3E+06	-33.7	-8.3	25.3
PNA P12 vs HRP C 03	5.42E-07	1.8E+06	-28.5	-8.6	19.9
Average	**7.59E-07**	**1.4E+06**	**-30.7**	**-8.4**	**22.3**
St. Dev.	**1.73E-07**	**3.4E+05**	**2.2**	**0.1**	**2.2**
PNA P13 vs HRP C 01	4.24E-07	2.4E+06	-28.5	-8.7	19.8
PNA P13 vs HRP C 02	3.06E-07	3.3E+06	-22.0	-8.9	13.1
PNA P13 vs HRP C 03	3.41E-07	2.9E+06	-21.9	-8.8	13.0
Average	**3.57E-07**	**2.9E+06**	**-24.1**	**-8.8**	**15.3**
St. Dev.	**4.95E-08**	**3.8E+05**	**3.1**	**0.1**	**3.2**

Table S3. ITC data, MPT sequence context.

Name	K_d (M)	K_a (M^{-1})	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
PNA P vs HRP MPT 01	9.23E-07	1.1E+06	-43.1	-8.2	34.8
PNA P vs HRP MPT 02	7.42E-07	1.3E+06	-41.3	-8.4	33.0
PNA P vs HRP MPT 03	7.18E-07	1.4E+06	-38.9	-8.4	30.5
Average	**7.94E-07**	**1.3E+06**	**-41.1**	**-8.3**	**32.8**
St. Dev.	**9.15E-08**	**1.4E+05**	**1.7**	**0.1**	**1.8**
PNA P4 vs HRP MPT 01	2.41E-07	4.1E+06	-40.2	-9	31.2
PNA P4 vs HRP MPT 02	2.36E-07	4.2E+06	-38.7	-9.1	29.6
PNA P4 vs HRP MPT 03	3.03E-07	3.3E+06	-40.9	-8.9	32
Average	**2.60E-07**	**3.9E+06**	**-39.9**	**-9**	**30.9**
St. Dev.	**3.05E-08**	**4.2E+05**	**0.9**	**0.1**	**1**
PNA P5 vs HRP MPT 01	4.25E-07	2.4E+06	-41.2	-8.7	32.5
PNA P5 vs HRP MPT 02	3.27E-07	3.1E+06	-36.0	-8.9	27.1
PNA P5 vs HRP MPT 03	3.38E-07	3.0E+06	-38.3	-8.8	29.5
Average	**3.63E-07**	**2.8E+06**	**-38.5**	**-8.8**	**29.7**
St. Dev.	**4.38E-08**	**3.1E+05**	**2.1**	**0.1**	**2.2**
PNA P7 vs HRP MPT 01	6.29E-07	1.6E+06	-37.8	-8.5	29.3
PNA P7 vs HRP MPT 02	5.59E-07	1.8E+06	-35.3	-8.5	26.8
PNA P7 vs HRP MPT 03	5.83E-07	1.7E+06	-37.8	-8.5	29.3
Average	**5.90E-07**	**1.7E+06**	**-37.0**	**-8.5**	**28.5**
St. Dev.	**2.90E-08**	**8.2E+04**	**1.2**	**0.0**	**1.2**
PNA P9 vs HRP MPT 01	1.41E-07	7.1E+06	-59.3	-9.4	49.9
PNA P9 vs HRP MPT 02	1.61E-07	6.2E+06	-42.4	-9.3	33.1
Average	**1.51E-07**	**6.7E+06**	**-50.9**	**-9.3**	**41.5**
St. Dev.	**1.00E-08**	**4.4E+05**	**8.5**	**0.0**	**8.4**

Table S4. ITC data, TPM sequence context.

Name	K_d (M)	K_a (M^{-1})	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
PNA TPM vs HRP TPM 01	1.21E-07	8.3E+06	-60.9	-9.5	51.4
PNA TPM vs HRP TPM 02	9.03E-08	1.1E+07	-58.0	-9.6	48.4
PNA TPM vs HRP TPM 03	9.41E-08	1.1E+07	-63.5	-9.6	53.9
Average	**1.02E-07**	**1.0E+07**	**-60.8**	**-9.6**	**51.2**
St. Dev.	**1.37E-08**	**1.2E+06**	**2.2**	**0.0**	**2.2**
PNA TP4M vs HRP TPM 01	5.19E-08	1.9E+07	-62.8	-9.9	52.9
PNA TP4M vs HRP TPM 02	5.54E-08	1.8E+07	-58.4	-9.9	48.5
PNA TP4M vs HRP TPM 03	5.95E-08	1.7E+07	-61.0	-9.8	51.2
Average	**5.56E-08**	**1.8E+07**	**-60.7**	**-9.9**	**50.9**
St. Dev.	**3.11E-09**	**1.0E+06**	**1.8**	**0.0**	**1.8**
PNA TP5M vs HRP TPM 01	8.71E-08	1.1E+07	-68.2	-9.7	58.5
PNA TP5M vs HRP TPM 02	8.41E-08	1.2E+07	-66.8	-9.7	57.1
PNA TP5M vs HRP TPM 03	7.94E-08	1.3E+07	-64.0	-9.7	54.3
Average	**8.35E-08**	**1.2E+07**	**-66.3**	**-9.7**	**56.6**
St. Dev.	**3.17E-09**	**4.6E+05**	**1.7**	**0.0**	**1.7**
PNA TP7M vs HRP TPM 01	7.30E-08	1.4E+07	-41.3	-9.7	31.6
PNA TP7M vs HRP TPM 02	8.51E-08	1.2E+07	-37.8	-9.7	28.1
PNA TP7M vs HRP TPM 03	8.22E-08	1.2E+07	-40.3	-9.7	30.6
Average	**8.01E-08**	**1.3E+07**	**-39.8**	**-9.7**	**30.1**
St. Dev.	**5.16E-09**	**8.4E+05**	**1.5**	**0.0**	**1.5**
PNA TP9M vs HRP TPM 01	5.09E-08	2.0E+07	-62.0	-9.9	52.1
PNA TP9M vs HRP TPM 02	5.26E-08	1.9E+07	-62.1	-9.9	52.2
PNA TP9M vs HRP TPM 03	6.52E-08	1.5E+07	-61.8	-9.8	52.0
Average	**5.62E-08**	**1.8E+07**	**-62.0**	**-9.9**	**52.1**
St. Dev.	**6.38E-09**	**1.9E+06**	**0.1**	**0.0**	**0.1**

Table S5. ITC data, MPM sequence context.

Name	K_d (M)	K_a (M^{-1})	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
PNA MPM vs HRP MPM 01	1.03E-07	9.7E+06	-65.4	-9.5	55.9
PNA MPM vs HRP MPM 02	1.06E-07	9.4E+06	-62.7	-9.5	53.2
PNA MPM vs HRP MPM 03	9.91E-08	1.0E+07	-62.8	-9.5	53.3
Average	**1.03E-07**	**9.7E+06**	**-63.6**	**-9.5**	**54.1**
St. Dev.	**2.82E-09**	**2.7E+05**	**1.2**	**0.0**	**1.2**
PNA MP4M vs HRP MPM 01	6.76E-08	1.5E+07	-65.5	-9.8	55.7
PNA MP4M vs HRP MPM 02	6.66E-08	1.5E+07	-66.0	-9.8	56.2
PNA MP4M vs HRP MPM 03	7.96E-08	1.3E+07	-65.5	-9.7	55.8
Average	**7.13E-08**	**1.4E+07**	**-65.7**	**-9.8**	**55.9**
St. Dev.	**5.91E-09**	**1.1E+06**	**0.2**	**0.0**	**0.2**
PNA MP5M vs HRP MPM 01	6.02E-08	1.7E+07	-64.2	-9.8	54.4
PNA MP5M vs HRP MPM 02	6.39E-08	1.6E+07	-66.3	-9.8	56.5
PNA MP5M vs HRP MPM 03	6.50E-08	1.5E+07	-64.6	-9.8	54.8
Average	**6.30E-08**	**1.6E+07**	**-65.0**	**-9.8**	**55.2**
St. Dev.	**2.05E-09**	**5.3E+05**	**0.9**	**0.0**	**0.9**
PNA MP7M vs HRP MPM 01	8.09E-08	1.2E+07	-55.6	-9.7	45.9
PNA MP7M vs HRP MPM 02	9.29E-08	1.1E+07	-57.0	-9.6	47.4
PNA MP7M vs HRP MPM 03	8.86E-08	1.1E+07	-59.6	-9.7	49.9
Average	**8.75E-08**	**1.1E+07**	**-57.4**	**-9.7**	**47.7**
St. Dev.	**4.96E-09**	**6.6E+05**	**1.7**	**0.0**	**1.6**
PNA MP9M vs HRP MPM 01	6.02E-08	1.7E+07	-50.2	-9.8	40.4
PNA MP9M vs HRP MPM 02	5.82E-08	1.7E+07	-54.6	-9.9	44.7
PNA MP9M vs HRP MPM 03	5.62E-08	1.8E+07	-52.7	-9.9	42.8
Average	**5.82E-08**	**1.7E+07**	**-52.5**	**-9.9**	**42.6**
St. Dev.	**1.63E-09**	**4.8E+05**	**1.8**	**0.0**	**1.8**

Table S6. ITC data, TPT sequence context.

Name	K_d (M)	K_a (M^{-1})	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
PNA TPT vs HRP TPT 01	2.88E-07	3.5E+06	-57.6	-8.9	48.7
PNA TPT vs HRP TPT 02	3.13E-07	3.2E+06	-55.4	-8.8	46.6
PNA TPT vs HRP TPT 03	2.70E-07	3.7E+06	-54.1	-9.0	45.1
Average	**2.90E-07**	**3.5E+06**	**-55.7**	**-8.9**	**46.8**
St. Dev.	**1.76E-08**	**2.1E+05**	**1.4**	**0.1**	**1.5**
PNA TP4T vs HRP TPT 01	7.07E-08	1.4E+07	-61.2	-9.8	51.4
PNA TP4T vs HRP TPT 02	7.62E-08	1.3E+07	-60.9	-9.7	51.2
PNA TP4T vs HRP TPT 03	7.13E-08	1.4E+07	-62.0	-9.8	52.2
Average	**7.27E-08**	**1.4E+07**	**-61.4**	**-9.8**	**51.6**
St. Dev.	**2.46E-09**	**4.6E+05**	**0.5**	**0.0**	**0.4**
PNA TP5T vs HRP TPT 01	2.73E-07	3.7E+06	-48.1	-9.0	39.1
PNA TP5T vs HRP TPT 02	3.61E-07	2.8E+06	-51.6	-8.8	42.8
PNA TP5T vs HRP TPT 03	2.82E-07	3.5E+06	-49.1	-8.9	40.2
Average	**3.05E-07**	**3.3E+06**	**-49.6**	**-8.9**	**40.7**
St. Dev.	**3.95E-08**	**4.0E+05**	**1.5**	**0.1**	**1.6**
PNA TP7T vs HRP TPT 01	2.71E-07	3.7E+06	-55.7	-9.0	46.7
PNA TP7T vs HRP TPT 02	2.24E-07	4.5E+06	-56.3	-9.0	47.3
PNA TP7T vs HRP TPT 03	3.08E-07	3.2E+06	-57.1	-8.9	48.2
Average	**2.68E-07**	**3.8E+06**	**-56.4**	**-9.0**	**47.4**
St. Dev.	**3.44E-08**	**5.0E+05**	**0.6**	**0.0**	**0.6**
PNA TP9T vs HRP TPT 01	5.40E-08	1.9E+07	-40.6	-9.9	30.7
PNA TP9T vs HRP TPT 02	5.50E-08	1.8E+07	-45.2	-9.9	35.3
PNA TP9T vs HRP TPT 03	5.81E-08	1.7E+07	-43.7	-9.9	33.8
Average	**5.57E-08**	**1.8E+07**	**-43.2**	**-9.9**	**33.3**
St. Dev.	**1.75E-09**	**5.5E+05**	**1.9**	**0.0**	**1.9**

Table S7. ITC data, P4 selectivity.

Name	K_d (M)	K_a (M^{-1})	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
PNA P4 vs HRP G 01	2.66E-06	3.8E+05	-38.5	-7.6	30.9
PNA P4 vs HRP G 02	2.98E-06	3.4E+05	-40.1	-7.6	32.5
PNA P4 vs HRP G 03	3.16E-06	3.2E+05	-41.8	-7.5	34.3
Average	**2.93E-06**	**3.4E+05**	**-40.1**	**-7.6**	**32.6**
St. Dev.	**2.07E-07**	**2.5E+04**	**1.3**	**0.0**	**1.4**
PNA P4 vs HRP U 01	1.19E-06	8.4E+05	-36.2	-8.1	28.1
PNA P4 vs HRP U 02	1.57E-06	6.4E+05	-39.0	-7.9	31.1
PNA P4 vs HRP U 03	1.13E-06	8.8E+05	-36.3	-8.1	28.2
Average	**1.30E-06**	**7.9E+05**	**-37.2**	**-8.0**	**29.1**
St. Dev.	**1.95E-07**	**1.1E+05**	**1.3**	**0.1**	**1.4**
PNA P4 vs HRP A 01	4.93E-07	2.0E+06	-47.5	-8.6	38.9
PNA P4 vs HRP A 02	5.50E-07	1.8E+06	-46.2	-8.5	37.7
PNA P4 vs HRP A 03	4.97E-07	2.0E+06	-45.0	-8.6	36.4
Average	**5.13E-07**	**2.0E+06**	**-46.2**	**-8.6**	**37.7**
St. Dev.	**2.60E-08**	**9.5E+04**	**1.0**	**0.0**	**1.0**

Table S8. ITC data, P5 selectivity.

Name	K_d (M)	K_a (M^{-1})	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
PNA P5 vs HRP G 01	2.78E-06	3.6E+05	-31.0	-7.6	23.4
PNA P5 vs HRP G 02	2.80E-06	3.6E+05	-35.5	-7.5	28.0
PNA P5 vs HRP G 03	2.34E-06	4.3E+05	-30.9	-7.7	23.2
Average	**2.64E-06**	**3.8E+05**	**-32.5**	**-7.6**	**24.9**
St. Dev.	**2.12E-07**	**3.3E+04**	**2.1**	**0.1**	**2.2**
PNA P5 vs HRP U 01	1.61E-06	6.2E+05	-31.1	-7.9	23.2
PNA P5 vs HRP U 02	9.76E-07	1.0E+06	-29.6	-8.2	21.4
PNA P5 vs HRP U 03	1.27E-06	7.9E+05	-35.3	-8.1	27.2
Average	**1.29E-06**	**8.1E+05**	**-32.0**	**-8.1**	**23.9**
St. Dev.	**2.59E-07**	**1.7E+05**	**2.4**	**0.1**	**2.4**
PNA P5 vs HRP A 01	8.14E-07	1.2E+06	-29.4	-8.3	21.1
PNA P5 vs HRP A 02	1.12E-06	8.9E+05	-33.3	-8.2	25.1
PNA P5 vs HRP A 03	9.30E-07	1.1E+06	-30.3	-8.3	22.0
Average	**9.55E-07**	**1.1E+06**	**-31.0**	**-8.3**	**22.7**
St. Dev.	**1.26E-07**	**1.4E+05**	**1.7**	**0.0**	**1.7**

Table S9. ITC parameters, P9 selectivity.

Name	K_d (M)	K_a (M^{-1})	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
PNA P9 vs HRP G 01	6.09E-07	1.6E+06	-49.4	-8.4	41.0
PNA P9 vs HRP G 02	7.70E-07	1.3E+06	-49.1	-8.4	40.7
PNA P9 vs HRP G 03	6.46E-07	1.5E+06	-44.8	-8.4	36.4
Average	**6.75E-07**	**1.5E+06**	**-47.8**	**-8.4**	**39.4**
St. Dev.	**6.89E-08**	**1.4E+05**	**2.1**	**0.0**	**2.1**
PNA P9 vs HRP U 01	6.69E-07	1.5E+06	-37.0	-8.4	28.6
PNA P9 vs HRP U 02	8.22E-07	1.2E+06	-37.7	-8.3	29.4
PNA P9 vs HRP U 03	8.90E-07	1.1E+06	-39.0	-8.3	30.7
Average	**7.94E-07**	**1.3E+06**	**-37.9**	**-8.3**	**29.6**
St. Dev.	**9.24E-08**	**1.6E+05**	**0.8**	**0.0**	**0.9**
PNA P9 vs HRP A 01	6.52E-07	1.5E+06	-46.7	-8.4	38.3
PNA P9 vs HRP A 02	8.10E-07	1.2E+06	-40.9	-8.3	32.6
PNA P9 vs HRP A 03	7.41E-07	1.3E+06	-43.3	-8.4	34.9
Average	**7.34E-07**	**1.4E+06**	**-43.6**	**-8.4**	**35.3**
St. Dev.	**6.47E-08**	**1.2E+05**	**2.4**	**0.0**	**2.3**

Table S10. ITC data, PNA2 and PNA3 P4, P5, P9.

Name	K_d (M)	K_a (M^{-1})	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
PNA 2 P4 vs HRP 2 01	1.11E-06	9.0E+05	-45.2	-8.1	37.0
PNA 2 P4 vs HRP 2 02	1.15E-06	8.7E+05	-46.5	-8.1	38.4
PNA 2 P4 vs HRP 2 03	1.10E-06	9.1E+05	-46.1	-8.1	38.0
Average	**1.12E-06**	**8.9E+05**	**-45.9**	**-8.1**	**37.8**
St. Dev.	**2.16E-08**	**1.7E+04**	**0.5**	**0.0**	**0.6**
PNA 2 P5 vs HRP 2 01	4.70E-06	2.1E+05	-45.3	-7.3	38.0
PNA 2 P5 vs HRP 2 02	4.61E-06	2.2E+05	-48.5	-7.3	41.2
PNA 2 P5 vs HRP 2 03	3.54E-06	2.8E+05	-43.7	-7.4	36.2
Average	**4.28E-06**	**2.4E+05**	**-45.8**	**-7.3**	**38.5**
St. Dev.	**5.27E-07**	**3.2E+04**	**2.0**	**0.1**	**2.1**
PNA 2 P9 vs HRP 2 01	7.27E-07	1.4E+06	-45.9	-8.4	37.5
PNA 2 P9 vs HRP 2 02	7.23E-07	1.4E+06	-41.2	-8.3	32.9
PNA 2 P9 vs HRP 2 03	7.92E-07	1.3E+06	-46.6	-8.3	38.3
Average	**7.47E-07**	**1.3E+06**	**-44.6**	**-8.3**	**36.2**
St. Dev.	**3.16E-08**	**5.5E+04**	**2.4**	**0.0**	**2.4**
PNA 3 P4 vs HRP 3 01	1.15E-06	8.7E+05	-38.7	-8.1	30.6
PNA 3 P4 vs HRP 3 02	1.39E-06	7.2E+05	-37.3	-8.0	29.3
PNA 3 P4 vs HRP 3 03	1.09E-06	9.2E+05	-36.9	-8.1	28.8
Average	**1.21E-06**	**8.4E+05**	**-37.6**	**-8.1**	**29.6**
St. Dev.	**1.30E-07**	**8.4E+04**	**0.8**	**0.1**	**0.8**
PNA 3 P5 vs HRP 3 01	6.94E-06	1.4E+05	-35.9	-7.0	28.9
PNA 3 P5 vs HRP 3 02	5.46E-06	1.8E+05	-37.8	-7.2	30.7
PNA 3 P5 vs HRP 3 03	4.44E-06	2.3E+05	-34.6	-7.3	27.3
Average	**5.61E-06**	**1.8E+05**	**-36.1**	**-7.2**	**29.0**
St. Dev.	**1.03E-06**	**3.3E+04**	**1.3**	**0.1**	**1.4**
PNA 3 P9 vs HRP 3 01	3.75E-07	2.7E+06	-36.9	-8.7	28.2
PNA 3 P9 vs HRP 3 02	3.21E-07	3.1E+06	-35.4	-8.9	26.5
PNA 3 P9 vs HRP 3 03	4.26E-07	2.3E+06	-36.5	-8.7	27.8
Average	**3.74E-07**	**2.7E+06**	**-36.3**	**-8.8**	**27.5**
St. Dev.	**4.29E-08**	**3.1E+05**	**0.6**	**0.1**	**0.7**

Table S11. ITC data, T controls.

Name	K_d (M)	K_a (M^{-1})	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)
PNA Tcon MPT vs HRP MPT 01	7.98E-08	1.3E+07	-42.4	-9.7	32.7
PNA Tcon MPT vs HRP MPT 02	8.34E-08	1.2E+07	-41.4	-9.7	31.7
PNA Tcon MPT vs HRP MPT 03	8.44E-08	1.2E+07	-40.4	-9.7	30.7
Average	**8.25E-08**	**1.2E+07**	**-41.4**	**-9.7**	**31.7**
St. Dev.	**1.98E-09**	**2.9E+05**	**0.8**	**0.0**	**0.8**
PNA Tcon TPM vs HRP TPM 01	4.28E-08	2.3E+07	-49.7	-10.0	39.7
PNA Tcon TPM vs HRP TPM 02	4.04E-08	2.5E+07	-44.1	-10.1	34.0
PNA Tcon TPM vs HRP TPM 03	3.91E-08	2.6E+07	-45.9	-10.1	35.8
Average	**4.08E-08**	**2.5E+07**	**-46.6**	**-10.1**	**36.5**
St. Dev.	**1.53E-09**	**9.1E+05**	**2.3**	**0.0**	**2.4**
PNA Tcon MPM vs HRP MPM 01	4.08E-08	2.5E+07	-46.9	-10.1	36.8
PNA Tcon MPM vs HRP MPM 02	5.33E-08	1.9E+07	-45.0	-9.9	35.1
PNA Tcon MPM vs HRP MPM 03	4.81E-08	2.1E+07	-47.9	-9.9	38.0
Average	**4.74E-08**	**2.1E+07**	**-46.6**	**-10.0**	**36.6**
St. Dev.	**5.13E-09**	**2.4E+06**	**1.2**	**0.1**	**1.2**
PNA Tcon TPT vs HRP TPT 01	3.35E-08	3.0E+07	-46.2	-10.2	36.0
PNA Tcon TPT vs HRP TPT 02	4.00E-08	2.5E+07	-48.2	-10.1	38.1
PNA Tcon TPT vs HRP TPT 03	3.38E-08	3.0E+07	-46.8	-10.2	36.6
Average	**3.58E-08**	**2.8E+07**	**-47.1**	**-10.2**	**36.9**
St. Dev.	**3.00E-09**	**2.2E+06**	**0.8**	**0.0**	**0.9**

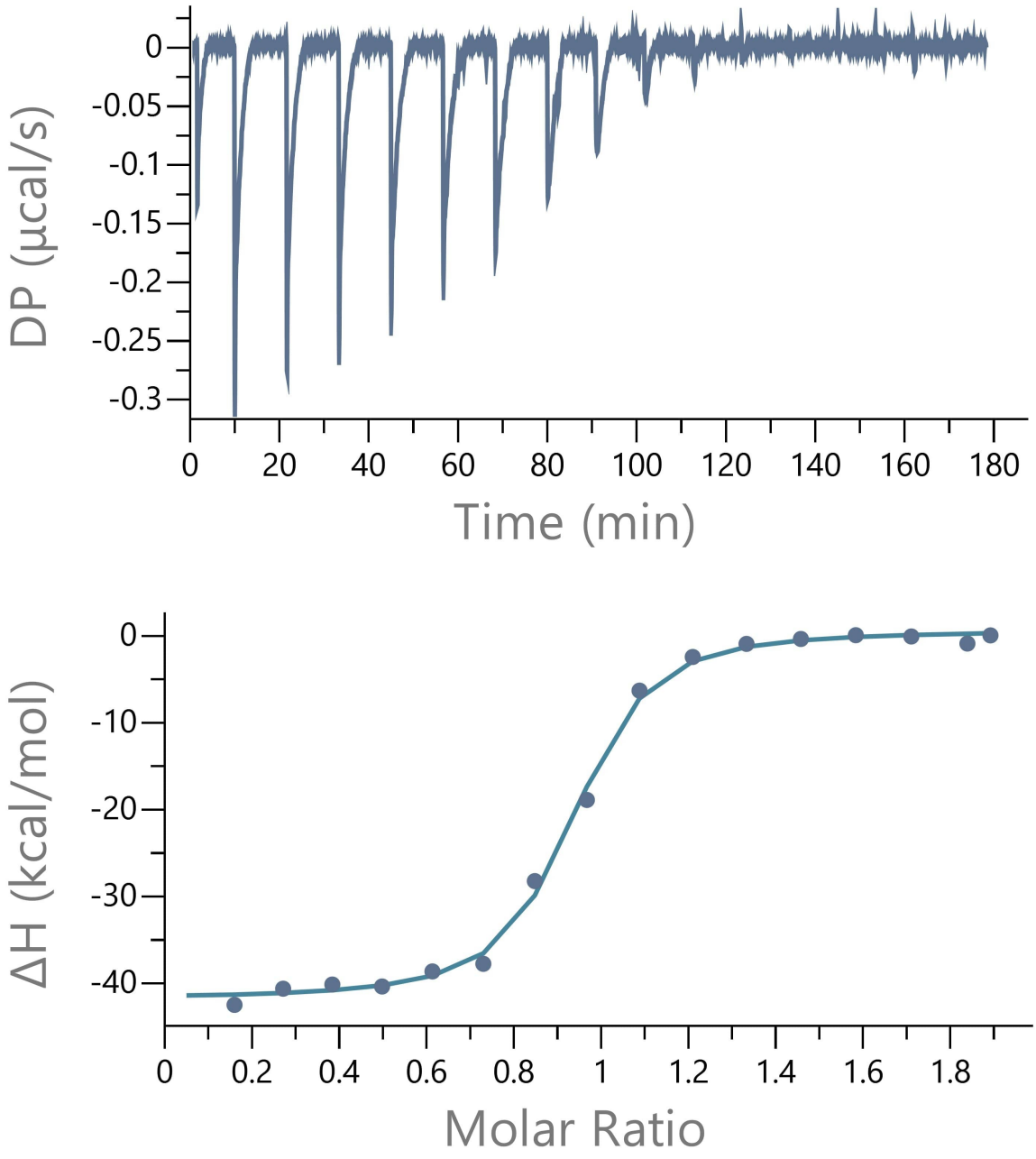


Figure S40. ITC titration of PNA_{MP9T} vs HRP_{MPT}.

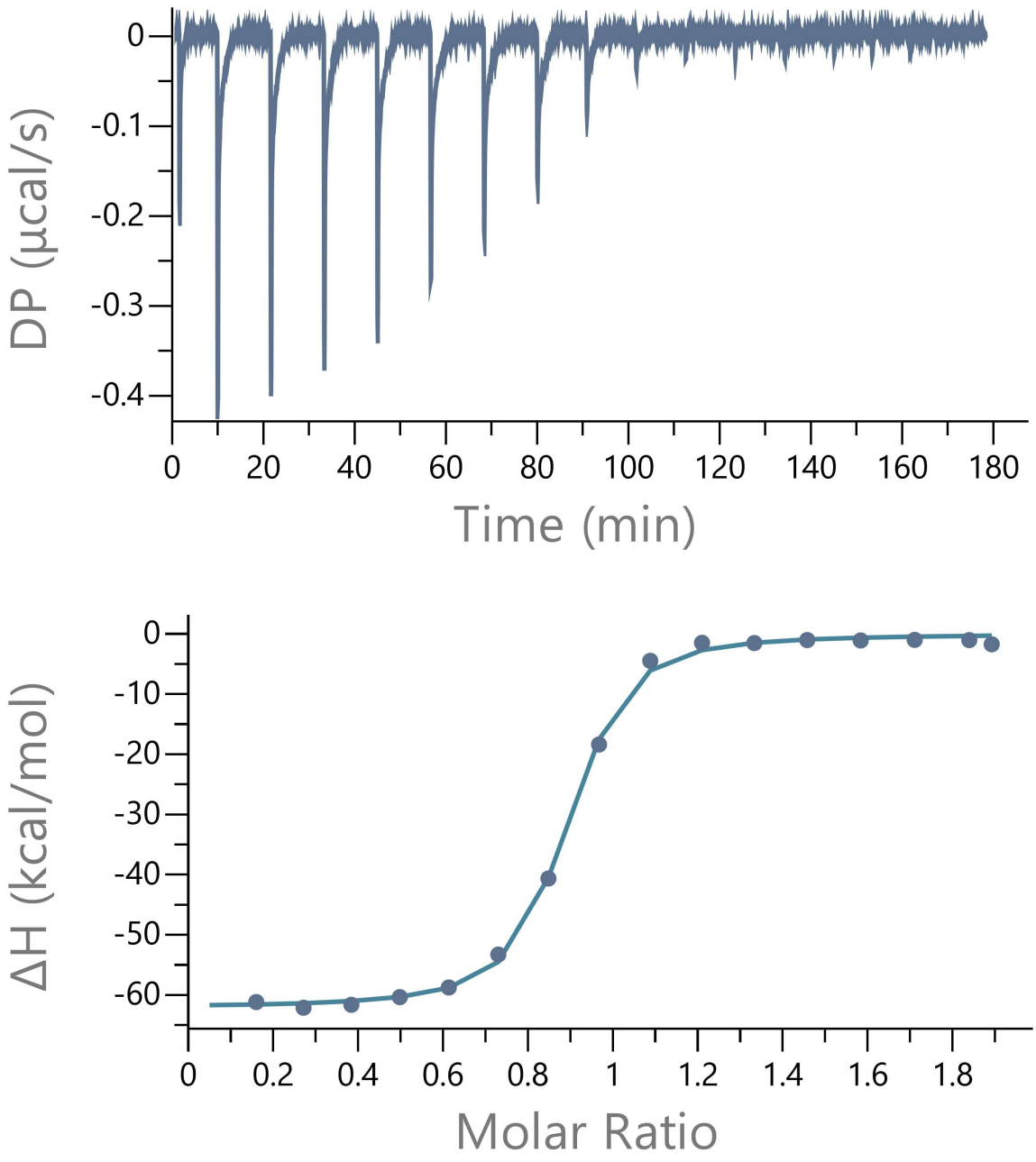


Figure S41. ITC titration of PNA_{TP9M} vs HRP_{TPM}.

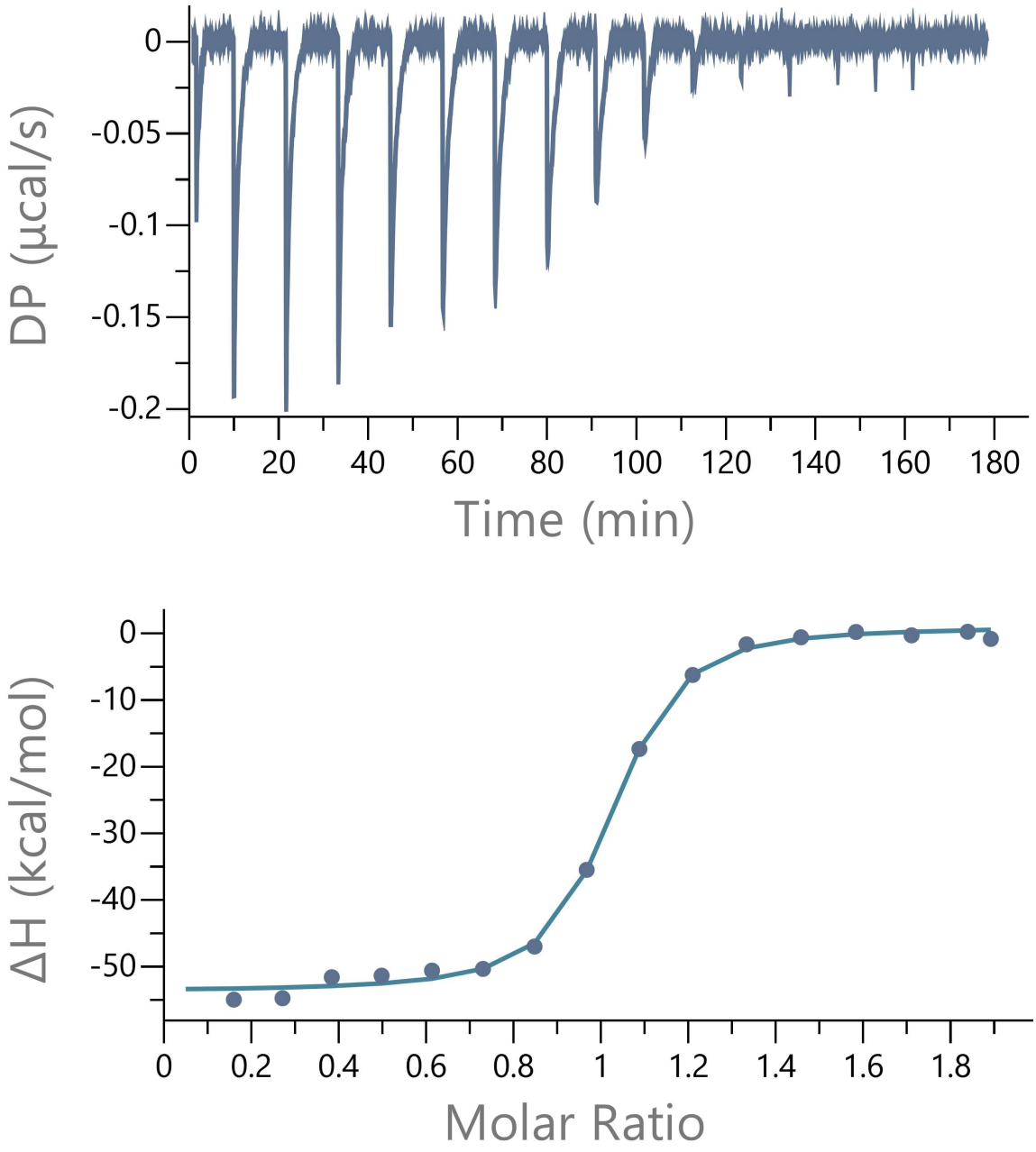


Figure S42. ITC titration of PNA_{MP9M} vs HRP_{MPM}.

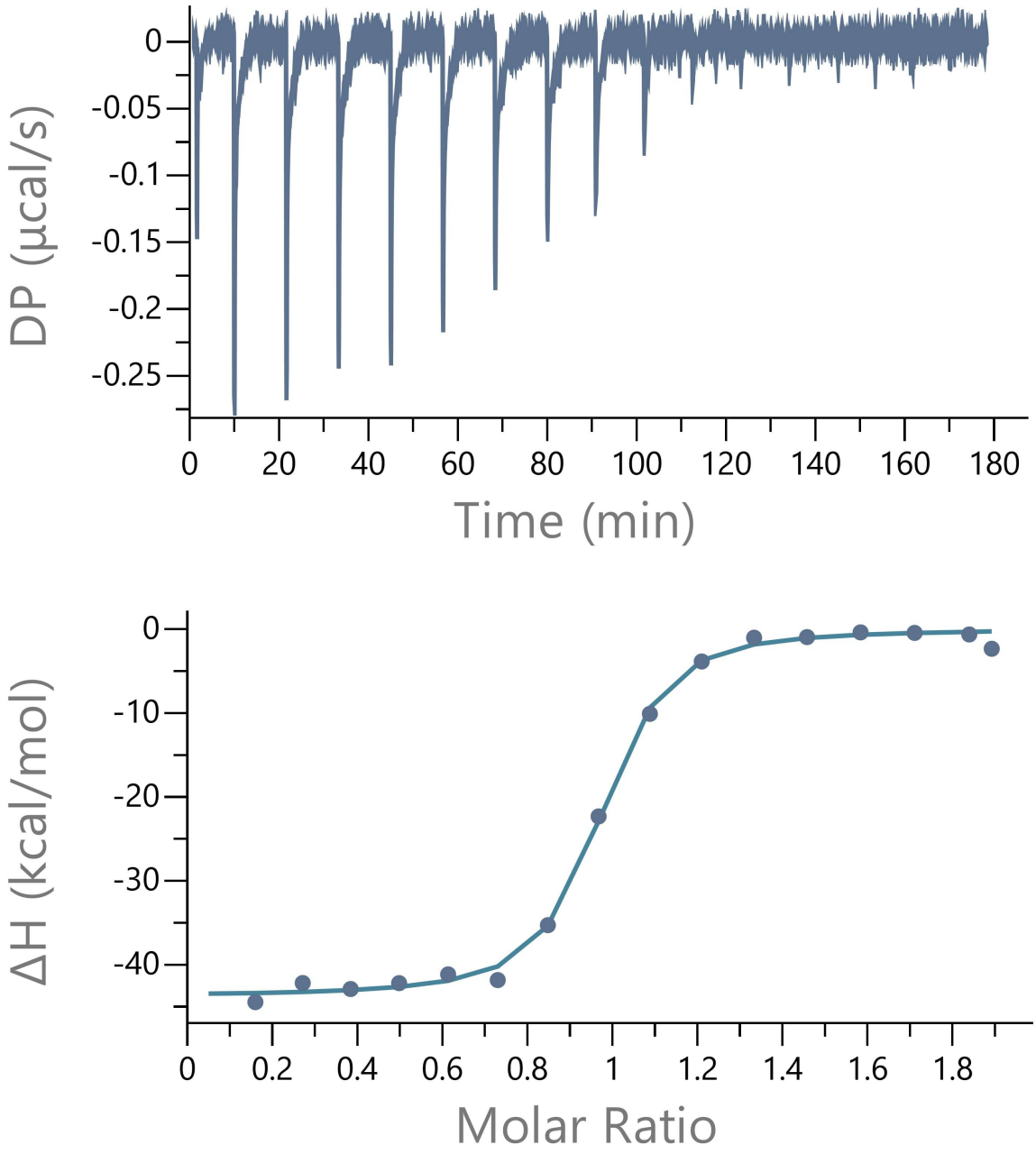


Figure S43. ITC titration of PNA_{TP9T} vs HRP_{TPT}.

Table S12. Melting temperatures of PNA Pn - HRP C triplexes.

Name	PNA P	PNA P1	PNA P2	PNA P3	PNA P4	PNA P5	PNA P6
	39.717	41.850	43.595	40.325	41.514	38.635	42.619
Melting	39.452	40.712	43.754	40.555	41.648	37.670	41.979
	39.413	41.464	42.296	40.571	41.996	37.823	42.113
temp. (˚C)	38.930	41.601	42.887	40.140	41.587	37.569	42.515
	40.101	41.585	43.232	41.210	41.823	37.632	42.642
Average	**39.5**	**41.4**	**43.2**	**40.6**	**41.7**	**37.9**	**42.4**
St. Dev.	**0.4**	**0.4**	**0.5**	**0.4**	**0.2**	**0.4**	**0.3**

Name	PNA P7	PNA P8	PNA P9	PNA P10	PNA P11	PNA P12	PNA P13
	39.347	40.699	48.769	49.759	39.230	41.506	40.140
Melting	39.155	39.387	48.403	48.613	39.458	41.946	40.075
	39.005	40.504	47.907	48.853	38.464	41.630	40.056
temp. (˚C)	39.250	39.460	48.245	48.681	39.321	41.314	40.140
	39.132	41.273	47.581	47.898	38.603	41.509	41.328
Average	**39.2**	**40.3**	**48.2**	**48.8**	**39.0**	**41.6**	**40.3**
St. Dev.	**0.1**	**0.7**	**0.4**	**0.6**	**0.4**	**0.2**	**0.5**

Table S13. Melting temperatures of PNA Pn - HRP MPT triplexes.

Name	PNA P	PNA P4	PNA P5	PNA P7	PNA P9
	42.495	44.311	41.040	42.769	48.268
Melting	42.391	44.001	42.014	42.137	48.581
	42.728	44.789	41.715	42.313	48.241
temp. (˚C)	42.417	44.490	41.440	43.822	48.697
	42.879	45.125	40.767	42.957	48.616
Average	**42.6**	**44.5**	**41.4**	**42.8**	**48.5**
St. Dev.	**0.2**	**0.4**	**0.4**	**0.6**	**0.2**

Table S14. Melting temperatures of PNA TPnM - HRP TPM triplexes.

Name	PNA TPM	PNA TP4M	PNA TP5M	PNA TP7M	PNA TP9M
	45.956	48.613	43.782	43.259	51.428
Melting	46.501	49.402	42.585	43.319	50.664
	45.296	49.488	42.871	44.689	50.775
temp. (˚C)	46.757	48.786	42.432	44.874	51.369
	45.748	49.173	43.226	44.237	50.534
Average	**46.1**	**49.1**	**43.0**	**44.1**	**51.0**
St. Dev.	**0.5**	**0.3**	**0.5**	**0.7**	**0.4**

Table S15. Melting temperatures of PNA MPnM - HRP MPM triplexes.

Name	PNA MPM	PNA MP4M	PNA MP5M	PNA MP7M	PNA MP9M
	49.093	49.489	47.407	48.962	49.904
Melting	49.300	50.383	48.476	48.885	49.538
	48.784	50.329	46.672	49.055	50.655
temp (˚C)	48.065	51.225	47.421	49.167	49.713
	47.686	51.020	48.127	49.113	49.224
Average	**48.6**	**50.5**	**47.6**	**49.0**	**49.8**
St. Dev.	**0.6**	**0.6**	**0.6**	**0.1**	**0.5**

Table S16. Melting temperatures of PNA TPnT - HRP TPT triplexes.

Name	PNA TPT	PNA TP4T	PNA TP5T	PNA TP7T	PNA TP9T
	38.161	43.479	35.384	37.244	48.444
Melting	38.441	42.958	34.929	37.49	48.571
	37.400	42.048	34.297	36.802	48.874
temp. (˚C)	37.942	43.252	35.402	36.354	48.083
	38.163	42.377	34.982	37.791	47.946
Average	**38.0**	**42.8**	**35.0**	**37.1**	**48.4**
St. Dev.	**0.3**	**0.5**	**0.4**	**0.5**	**0.3**

Table S17. Melting temperatures of PNA P4 triplexes, selectivity.

Name	PNA P4 vs HRP G	PNA P4 vs HRP U	PNA P4 vs HRP A
Melting temp. (˚C)	35.280	38.162	48.179
	34.462	37.828	47.379
	34.577	38.241	47.977
	34.577	38.271	47.862
	34.550	37.217	47.294
Average	**34.7**	**37.9**	**47.7**
St. Dev.	**0.3**	**0.4**	**0.3**

Table S18. Melting temperatures of PNA P5 triplexes, selectivity.

Name	PNA P5 vs HRP G	PNA P5 vs HRP U	PNA P5 vs HRP A
Melting temp. (˚C)	31.548	35.726	37.963
	31.612	35.469	38.063
	31.046	35.610	38.259
	32.142	35.583	39.601
	31.731	35.480	38.826
Average	**31.6**	**35.6**	**38.5**
St. Dev.	**0.4**	**0.1**	**0.6**

Table S19. Melting temperatures of PNA P9 triplexes, selectivity.

Name	PNA P9 vs HRP G	PNA P9 vs HRP U	PNA P9 vs HRP A
Melting Temp. (˚C)	36.816	36.576	36.377
	36.083	36.140	36.399
	36.261	36.241	36.680
	35.796	36.271	36.846
	36.159	36.774	35.989
Average	**36.2**	**36.4**	**36.5**
St. Dev.	**0.3**	**0.2**	**0.3**

Table S20. Melting temperatures of PNA 2 P4, P5, P9 – HRP 2 triplexes.

Name	PNA 2 P4	PNA 2 P5	PNA 2 P9
Melting temp. (˚C)	33.885	28.506	34.236
	34.028	27.839	34.222
	33.654	29.316	34.358
	34.025	28.822	34.666
	34.014	28.932	34.154
Average	**33.9**	**28.7**	**34.3**
St. Dev.	**0.1**	**0.5**	**0.2**

Table S21. Melting temperatures of PNA 3 P4, P5, P9 – HRP 3 triplexes.

Name	PNA 3 P4	PNA 3 P5	PNA 3 P9
Melting temp. (˚C)	36.959	23.305	39.464
	37.015	22.279	41.184
	37.999	23.000	41.713
	37.902	22.564	43.453
	37.783	23.986	41.602
Average	**37.5**	**23.0**	**41.5**
St. Dev.	**0.5**	**0.6**	**1.3**

Table S22. Melting temperatures of T control triplexes.

Name	T cont MTT	T cont TTM	T cont MTM	T cont TTT
Melting temp. (˚C)	70.130	76.893	77.206	71.043
	70.137	75.076	77.298	71.061
	70.420	74.924	76.425	70.263
	68.668	75.176	76.075	69.864
	68.639	75.359	76.405	70.215
Average	**69.6**	**75.5**	**76.7**	**70.5**
St. Dev.	**0.8**	**0.7**	**0.5**	**0.5**

Table S23. Comparison of ΔG_{25} obtained by Van't Hoff analysis of melting curves and ITC.

	UV melting (Van't Hoff) ΔG_{25} (kcal/mol)	ITC ΔG_{25} (kcal/mol)
PNA MP_9T - HRP_{MPT}	-12.6 ± 0.1	-9.3 ± 0.0
PNA TP_9M - HRP_{TPM}	-14.8 ± 0.1	-9.9 ± 0.0
PNA MP_9M - HRP_{MPM}	-13.5 ± 0.1	-9.9 ± 0.0
PNA TP_9T - HRP_{TPT}	-12.6 ± 0.1	-9.9 ± 0.0
PNA T cont MTT - HRP_{MPT}	-18.2 ± 0.1	-9.7 ± 0.0
PNA T cont TTM - HRP_{TPM}	-19.6 ± 0.5	-10.1 ± 0.0
PNA T cont MTM - HRP_{MPM}	-21.6 ± 0.5	-10.0 ± 0.1
PNA T cont TTT - HRP_{TPT}	-18.3 ± 0.3	-10.2 ± 0.0

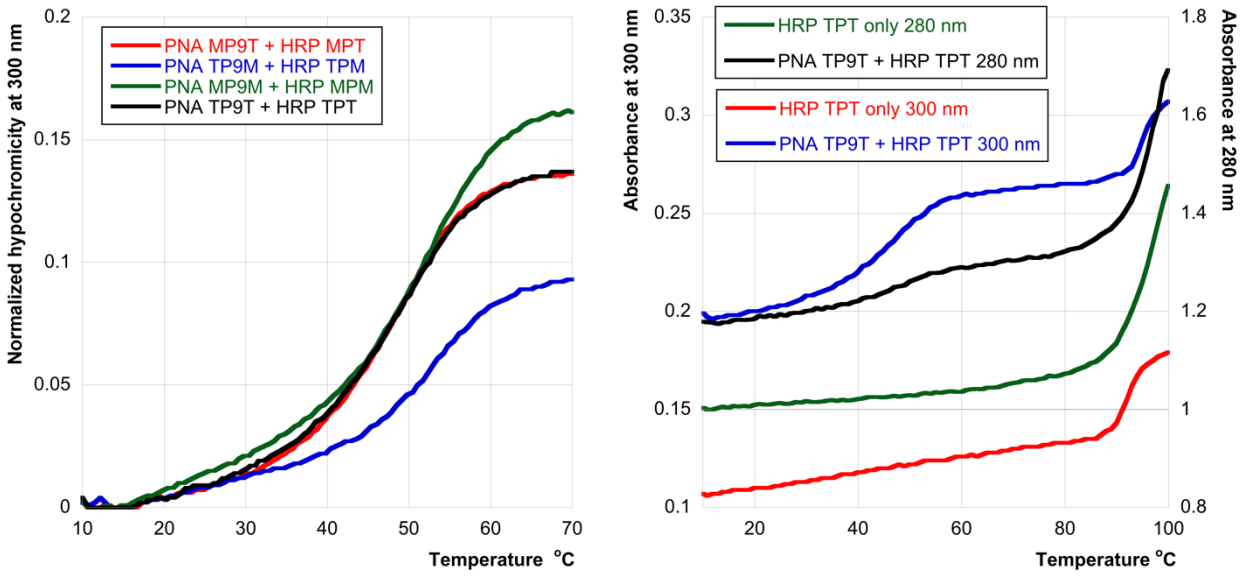


Figure S44. UV thermal melting curves of left: P_9-modified PNA binding different sequence contexts of dsRNA and right HRP TPT alone (green and red) and P_9-modified matched PNA TP9T complex with HRP TPT (black and blue).

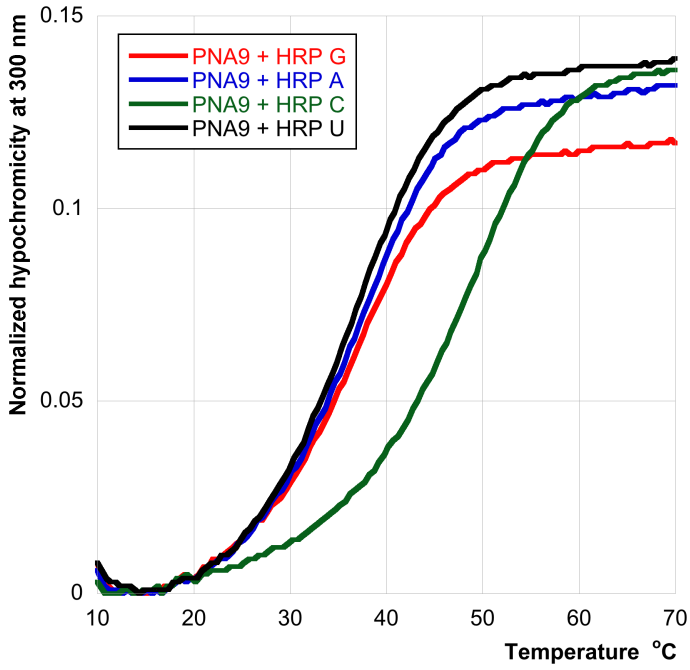


Figure S45. UV thermal melting curves of PNA9 binding matched (HRP C) and mismatched dsRNA.

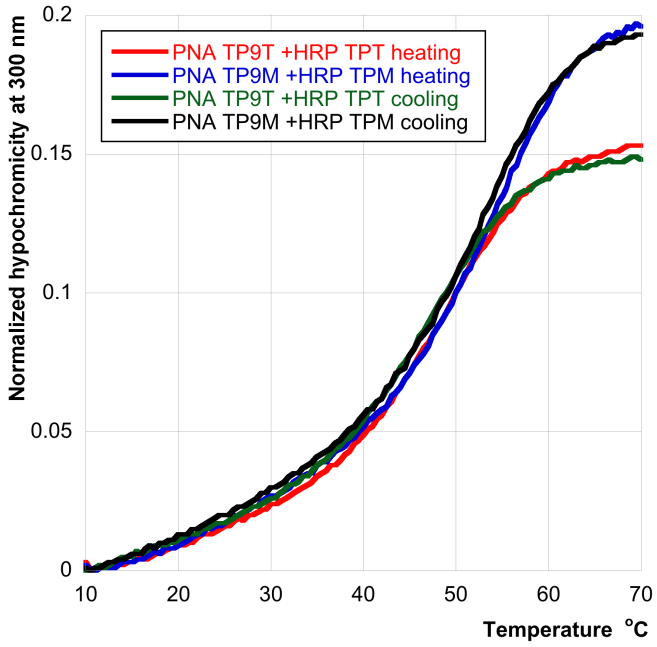
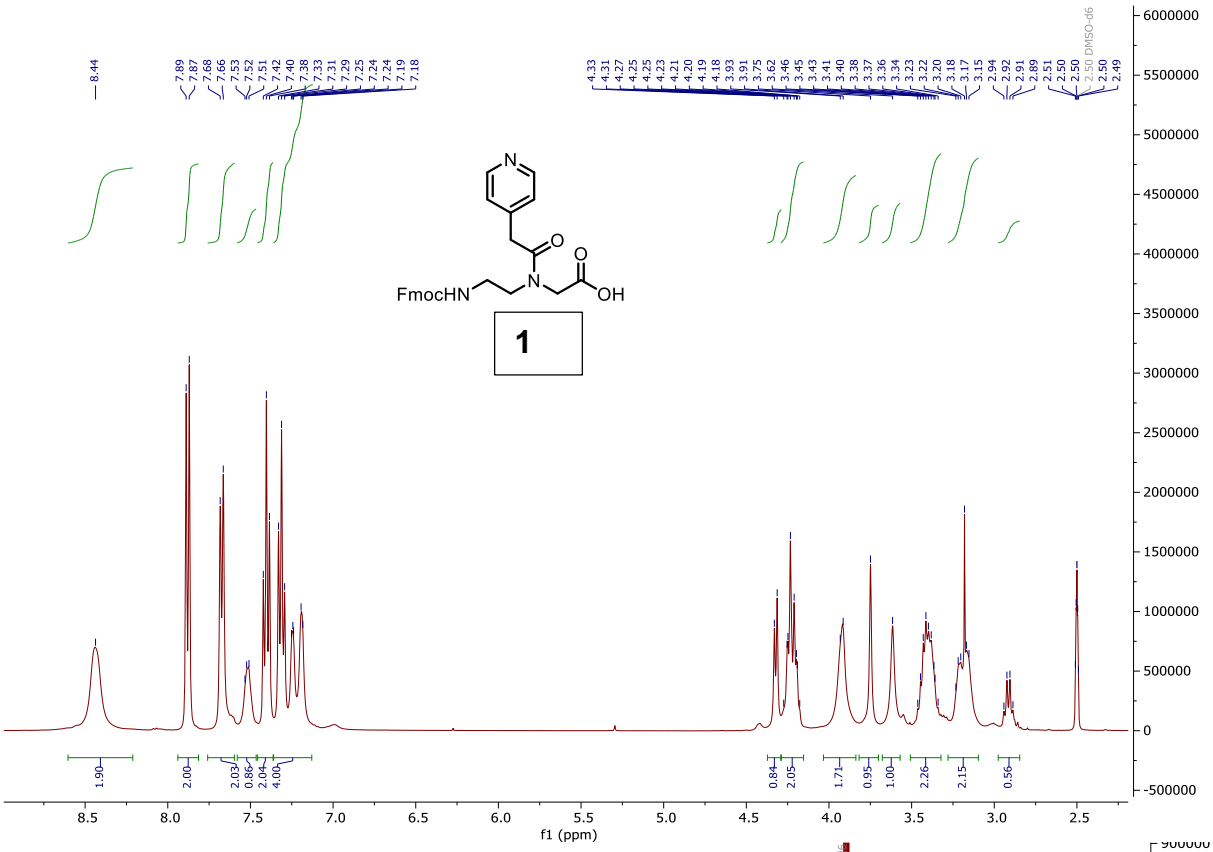


Figure S46. UV thermal melting curves of PNA TP9T and PNA TP9M binding their matched hairpins measured while heating (red and blue curves) or cooling (green and black curves) at 0.5 °C per minute. The results illustrate minimal hysteresis of ~ 1 °C.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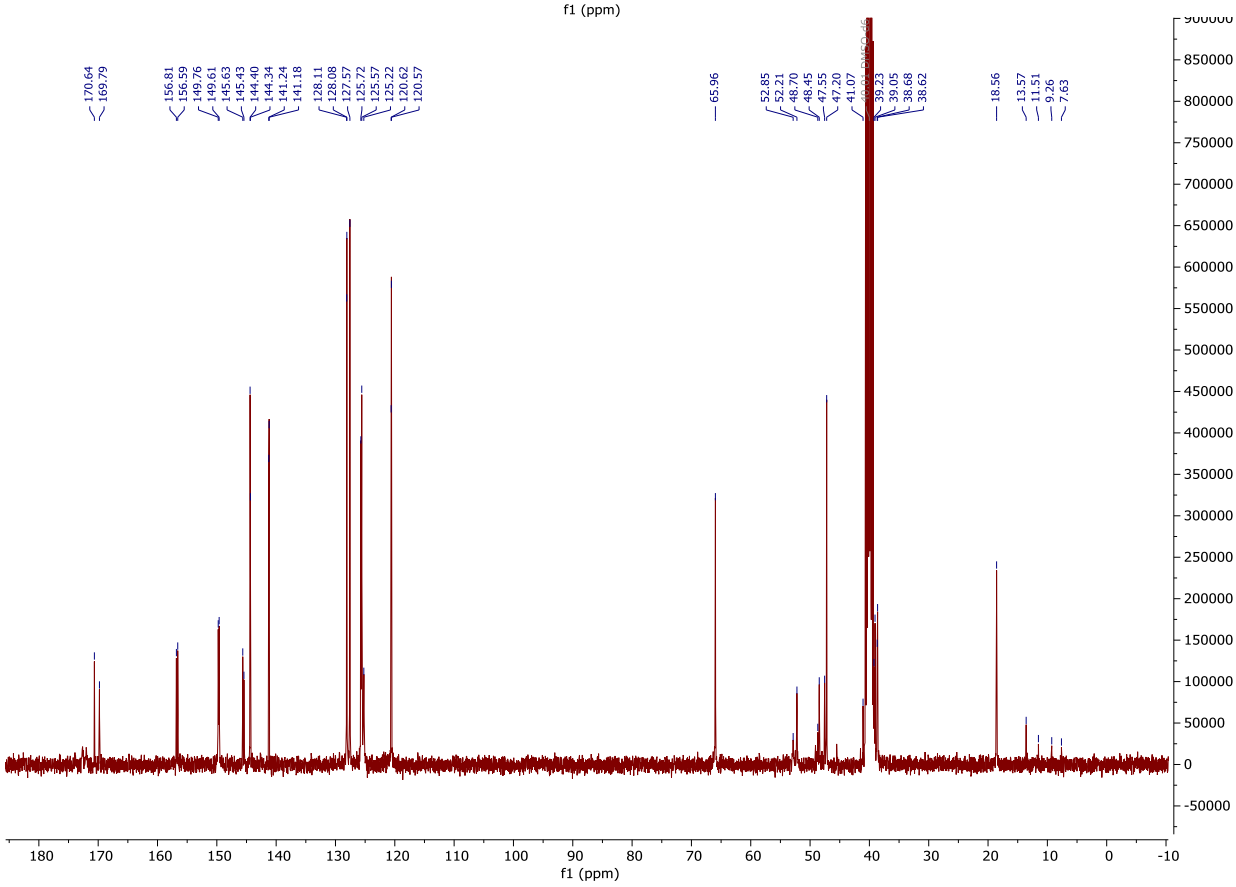
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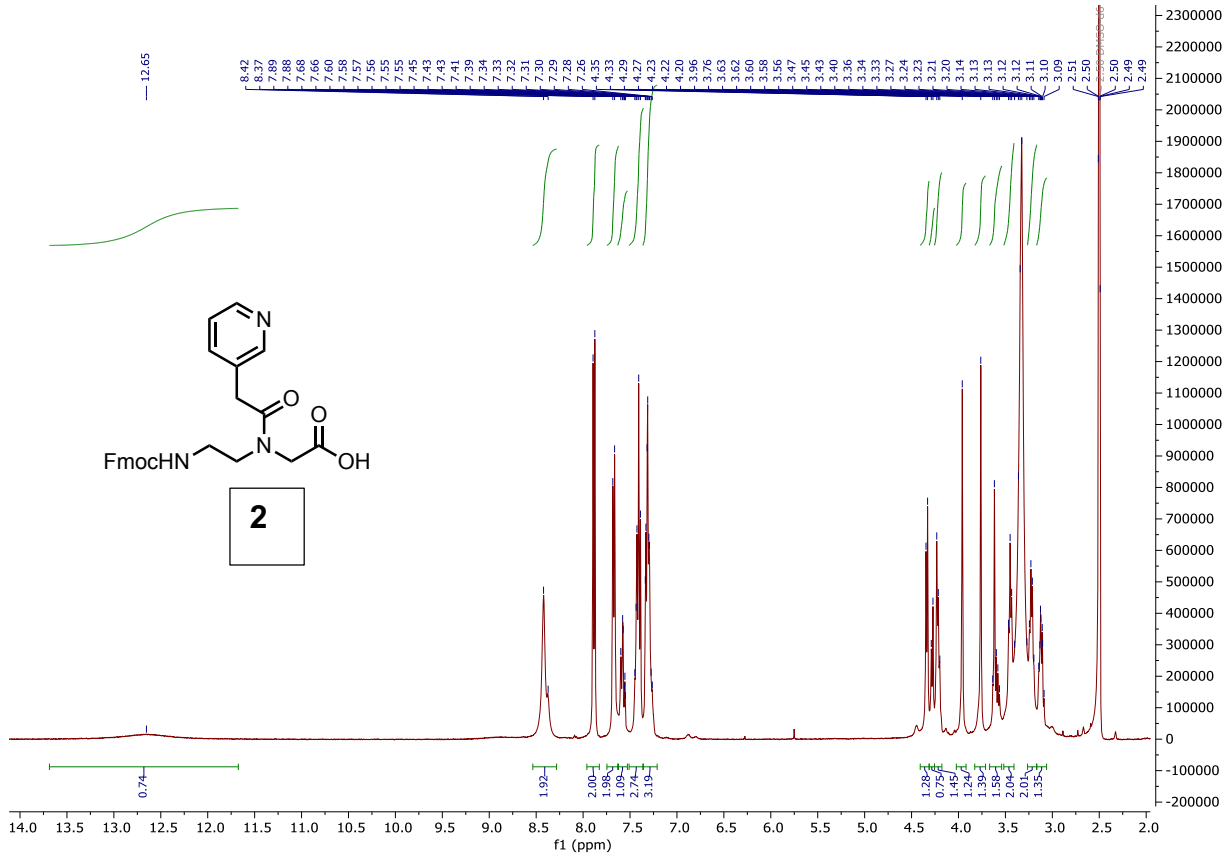
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O
N
OH
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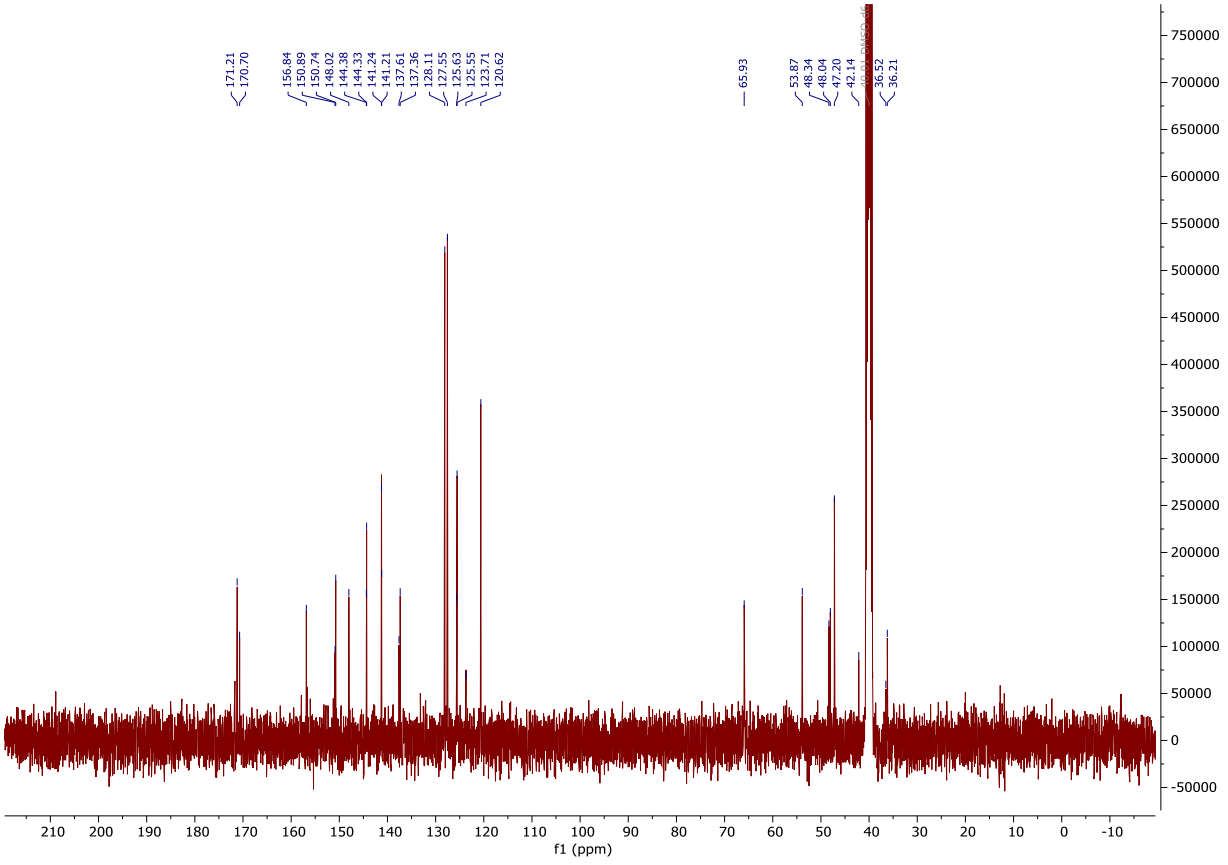
f1 (ppm)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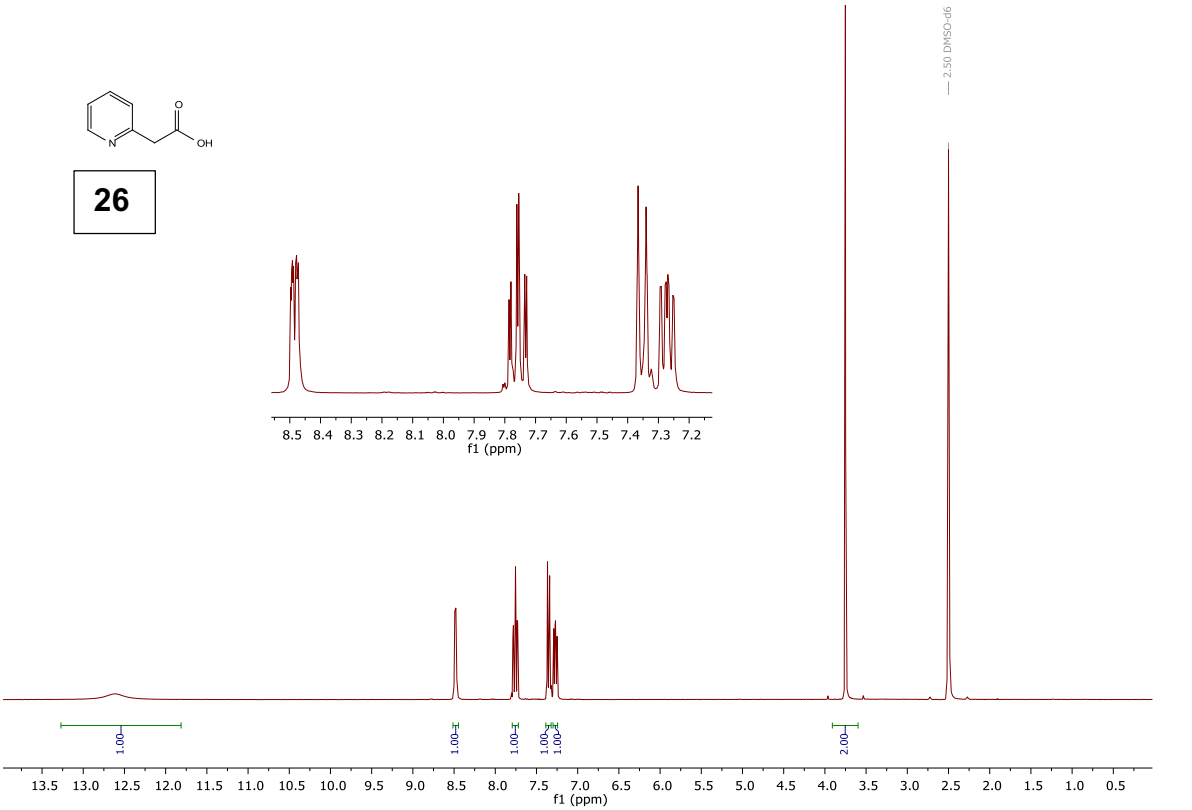
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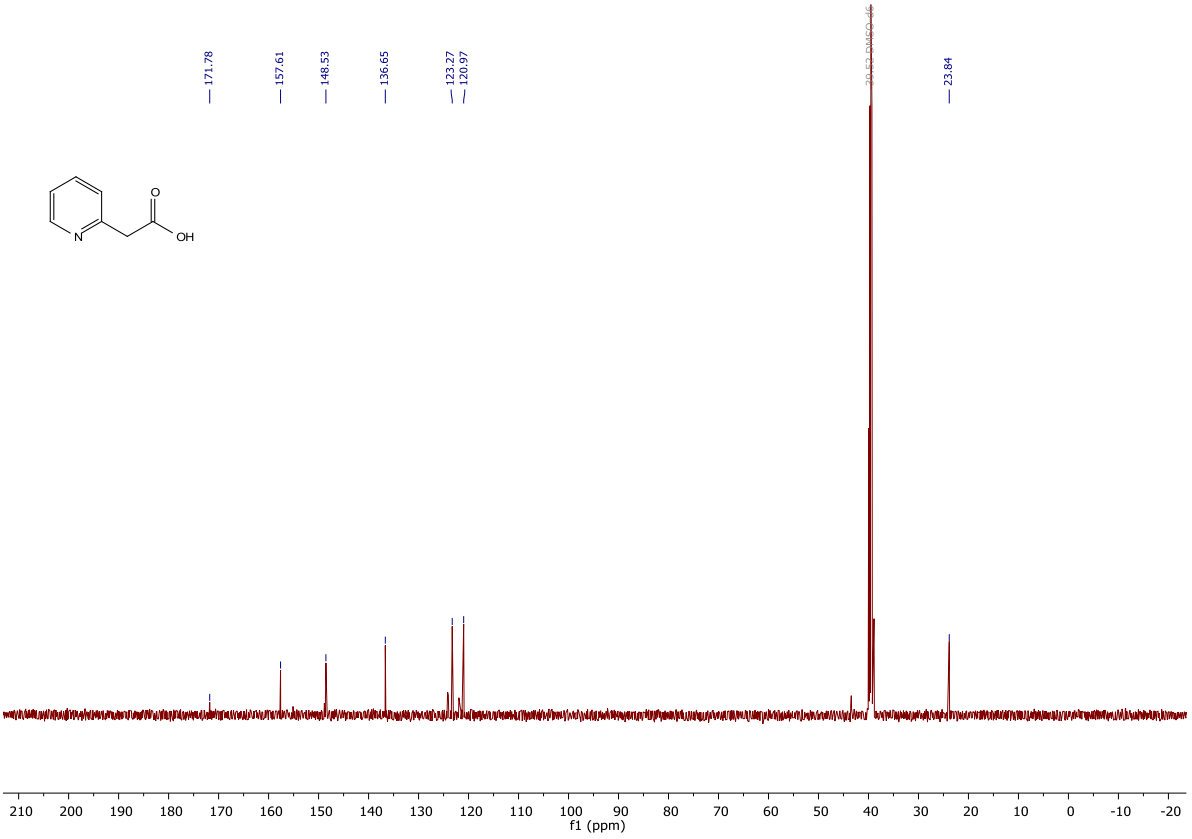
1.00
1.00
1.00
1.00
2.00
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)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136.65
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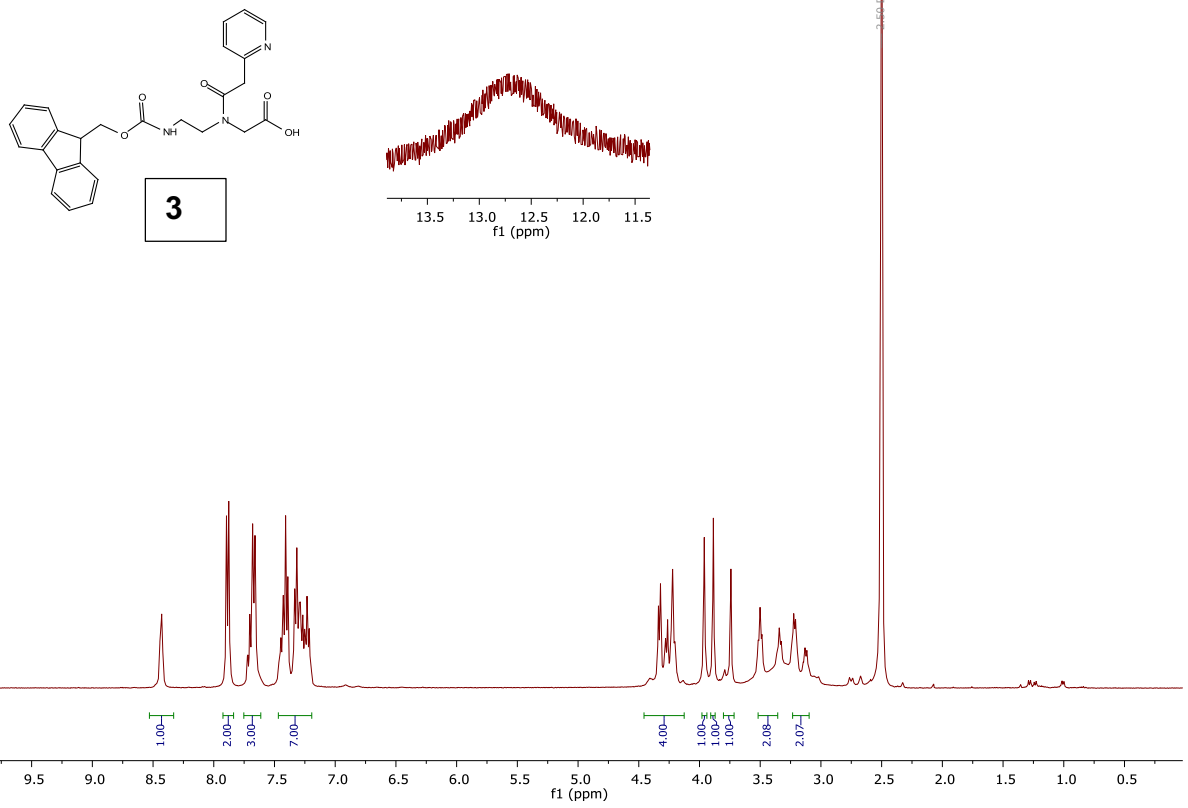
f1 (ppm)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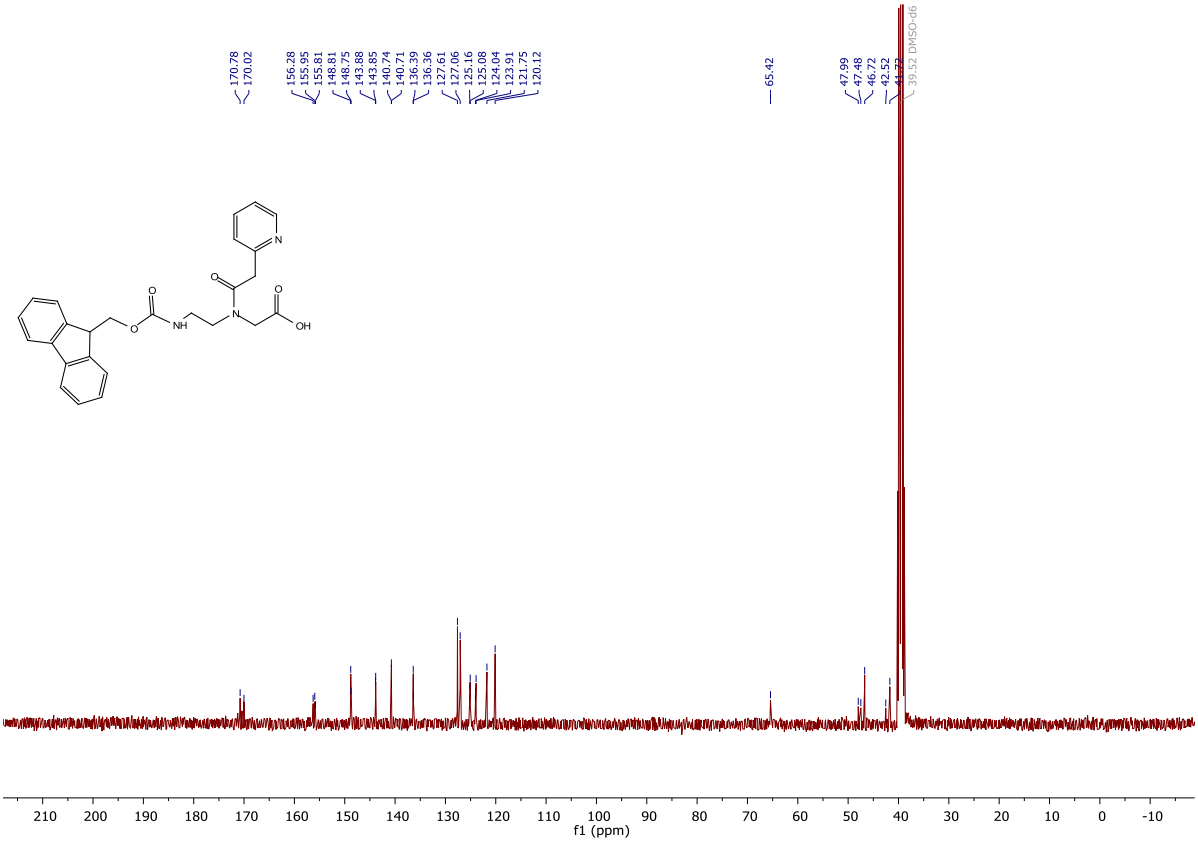
2.07
f1 (ppm)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127.46
125.16
125.08
124.04
123.91
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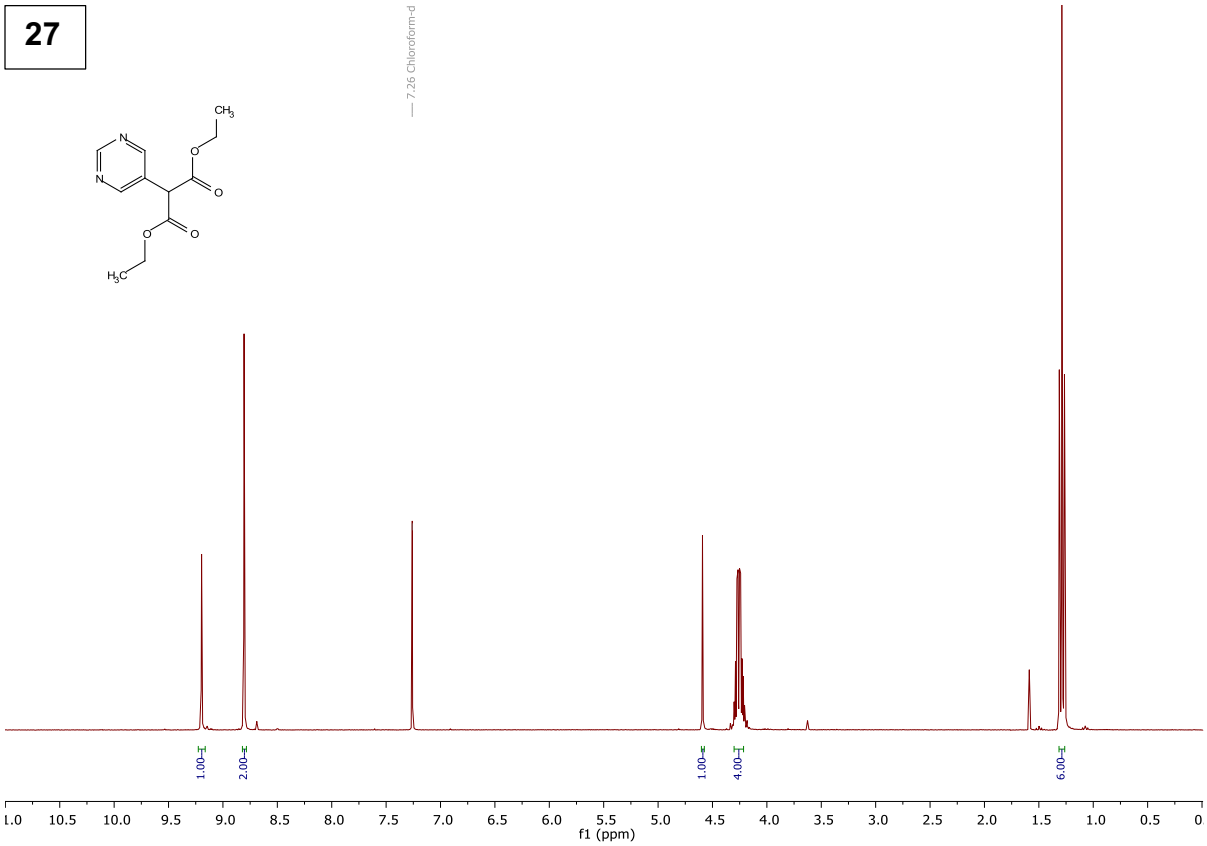
41.73
39.52 DMSO-d6
f1 (ppm)

27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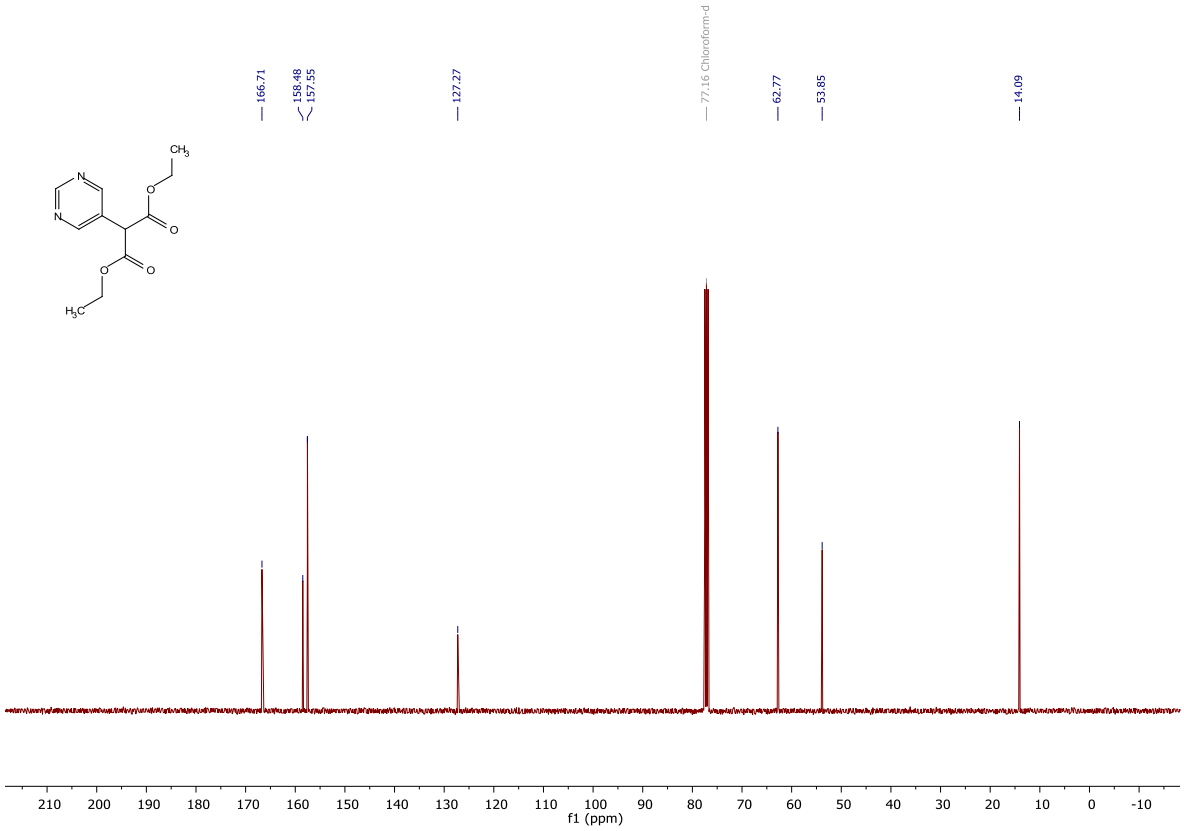
4.00
6.00
f1 (ppm)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14.09
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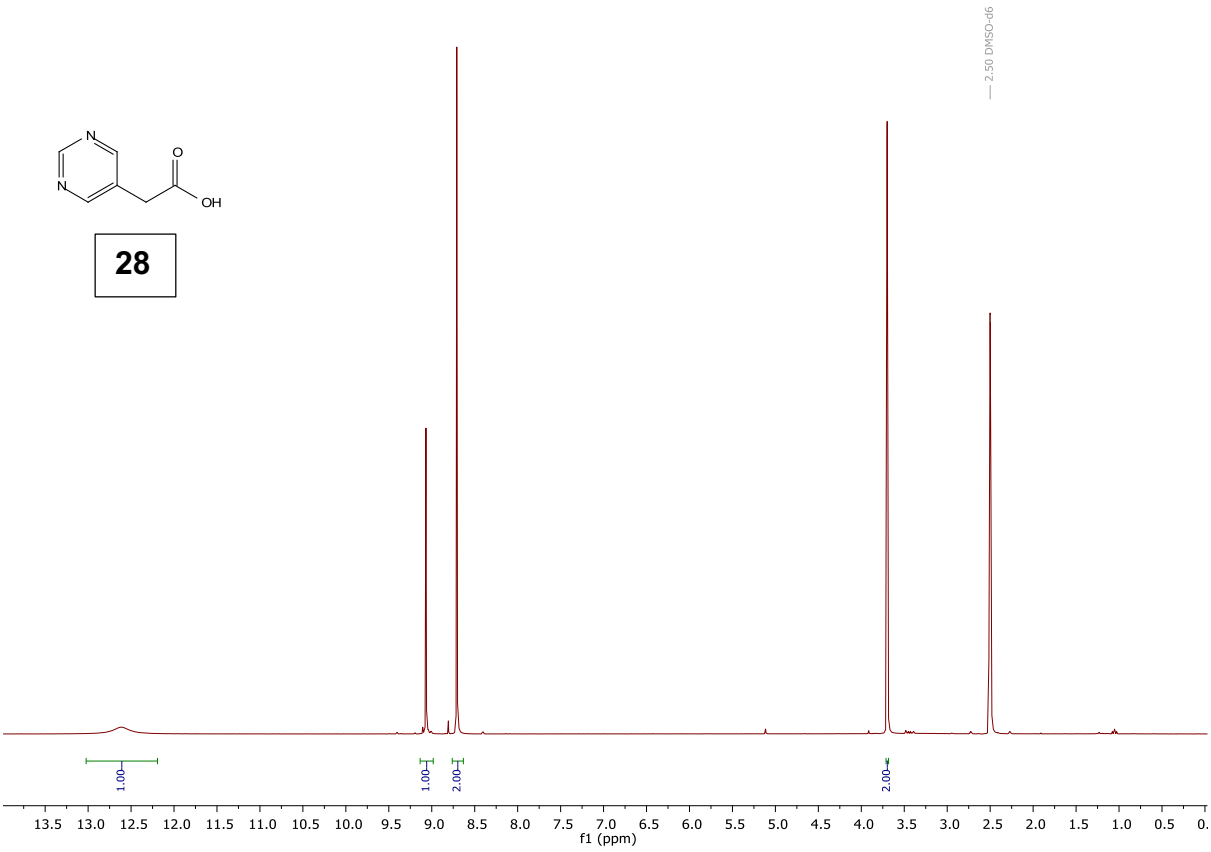
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O
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1.00
2.00
2.00
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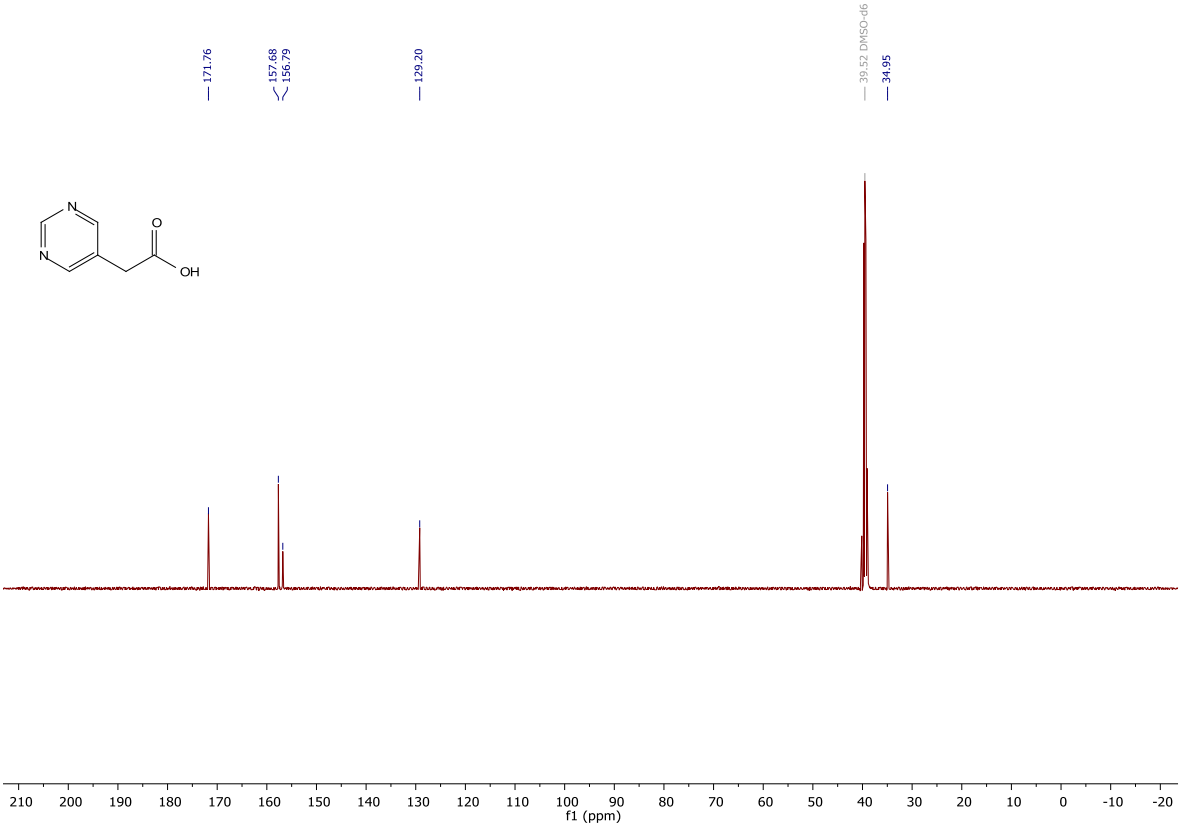
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O
OH
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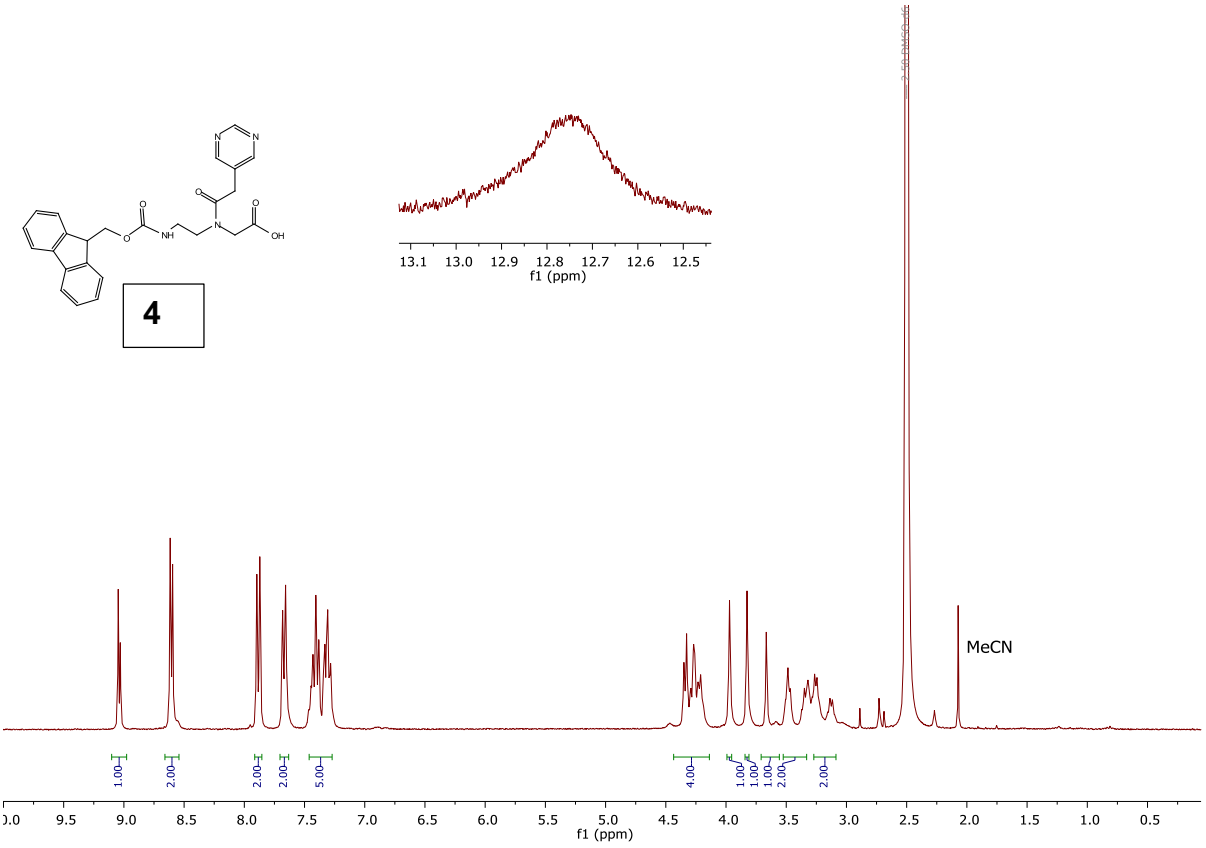
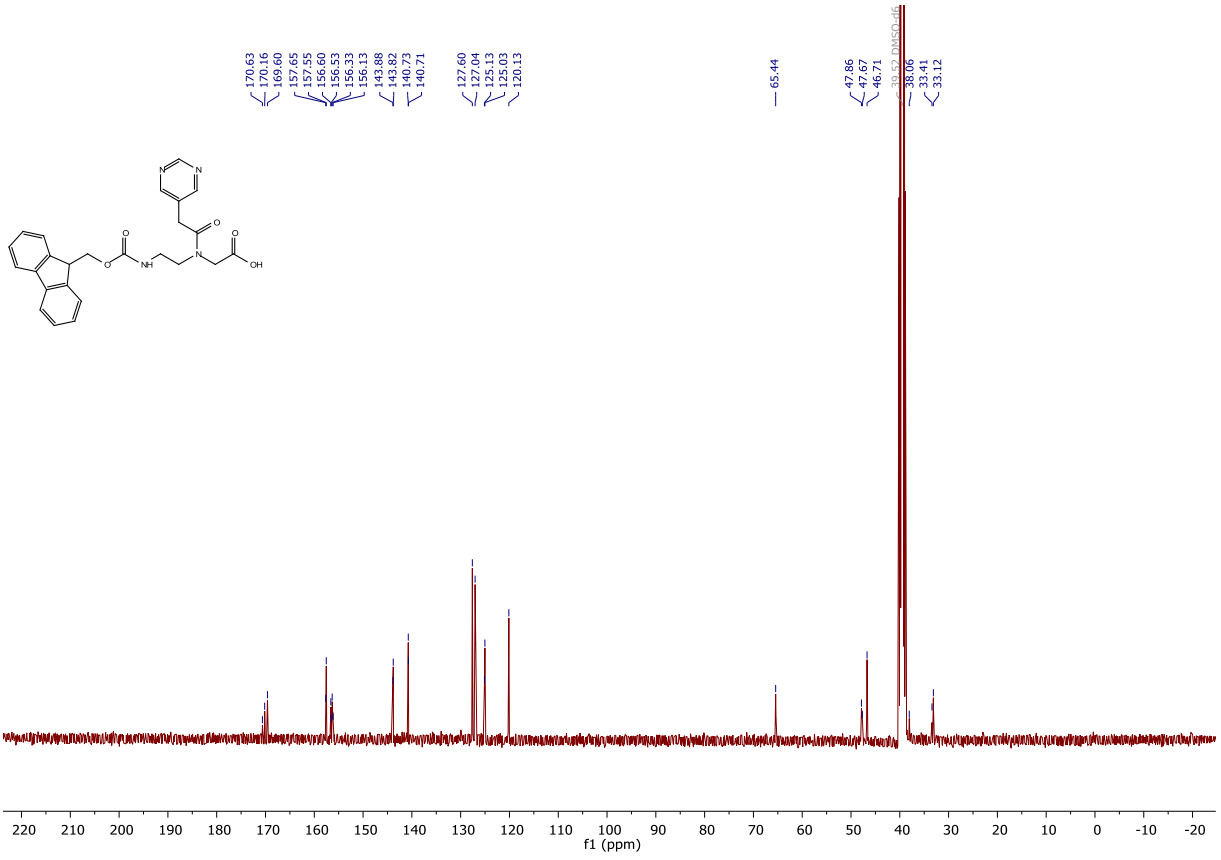
f1 (ppm)


4
MeCN
f1 (ppm)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127.60
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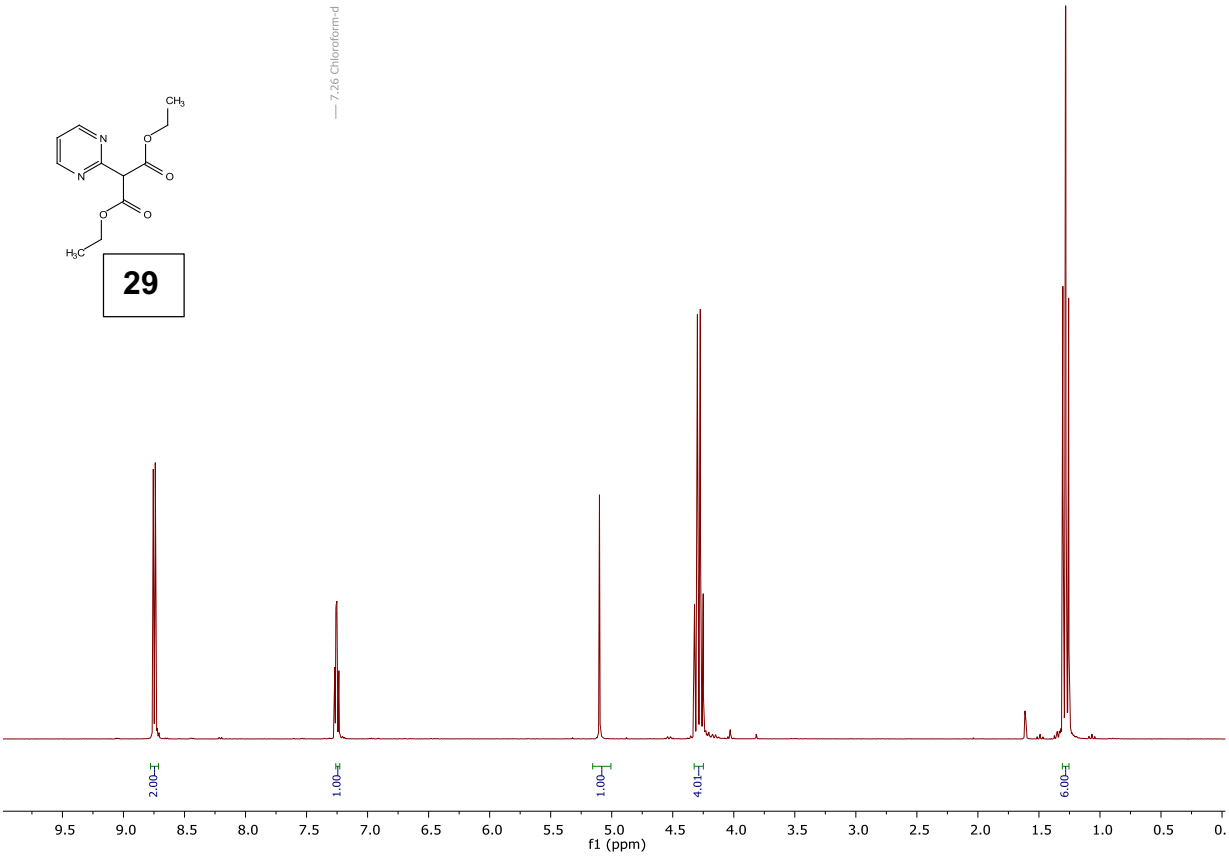
33.12
f1 (ppm)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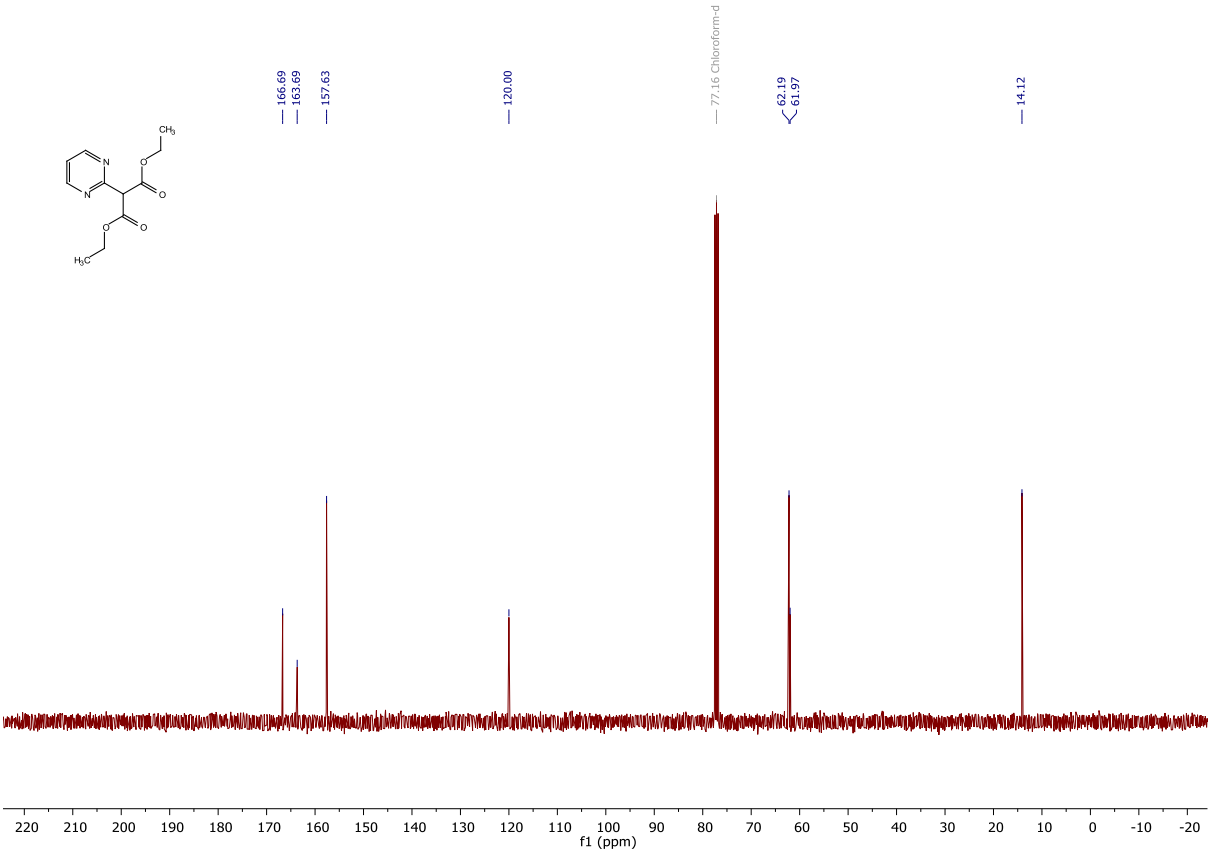
1.00
1.00
4.01
6.00
f1 (ppm)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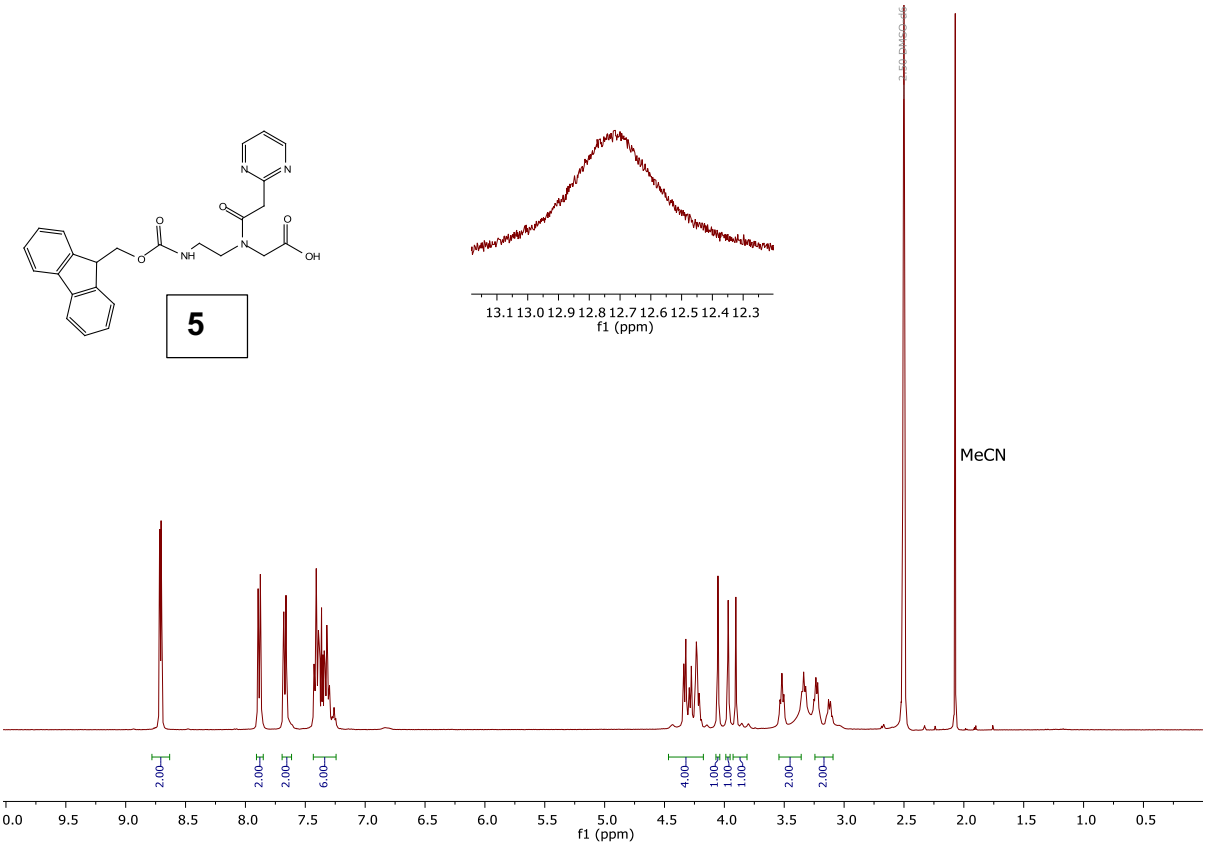
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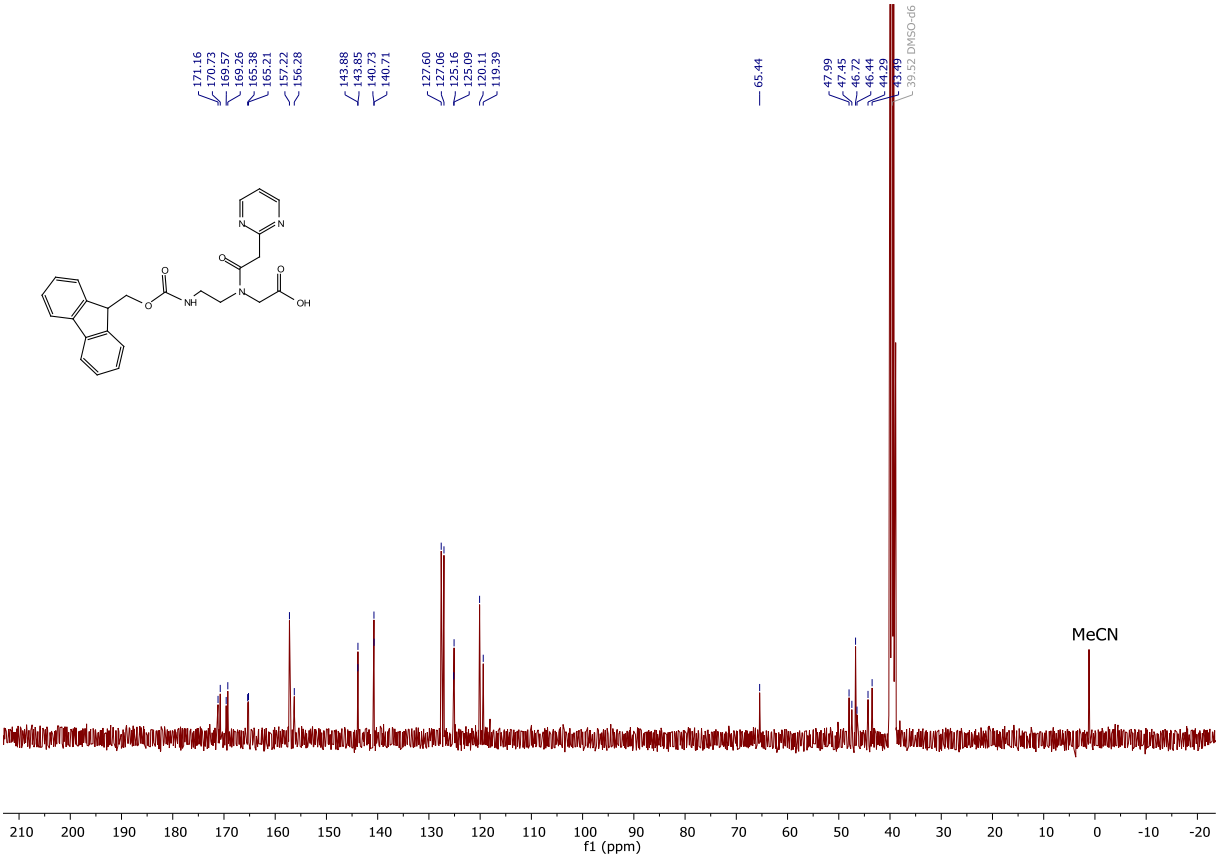
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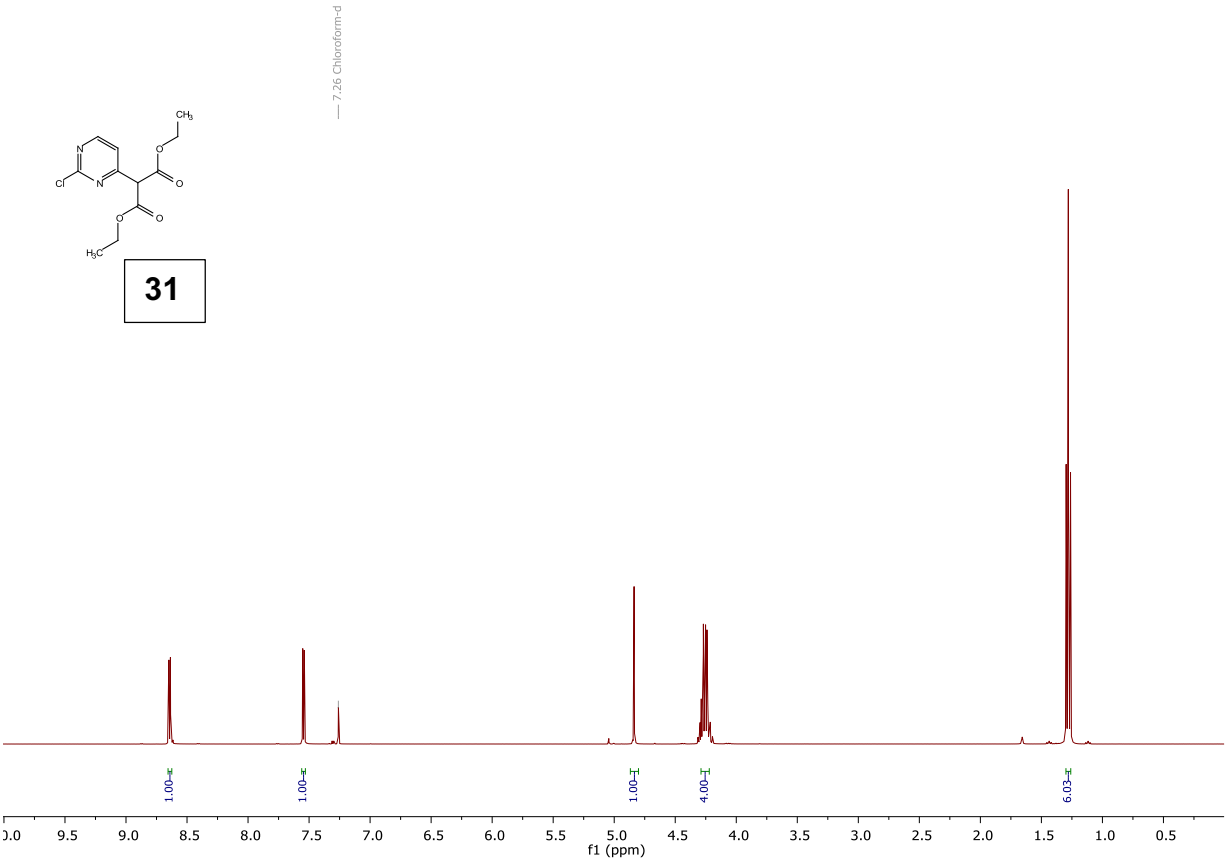
MeCN
f1 (ppm)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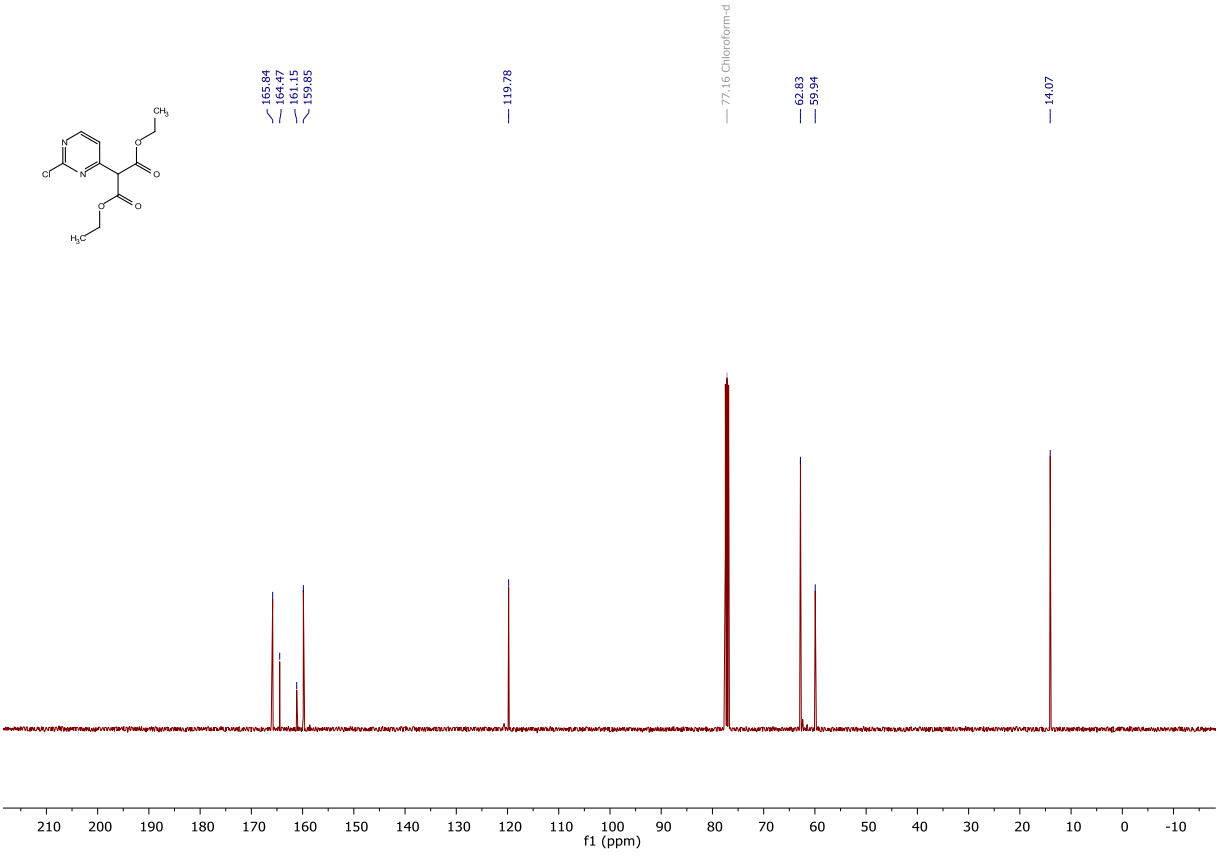
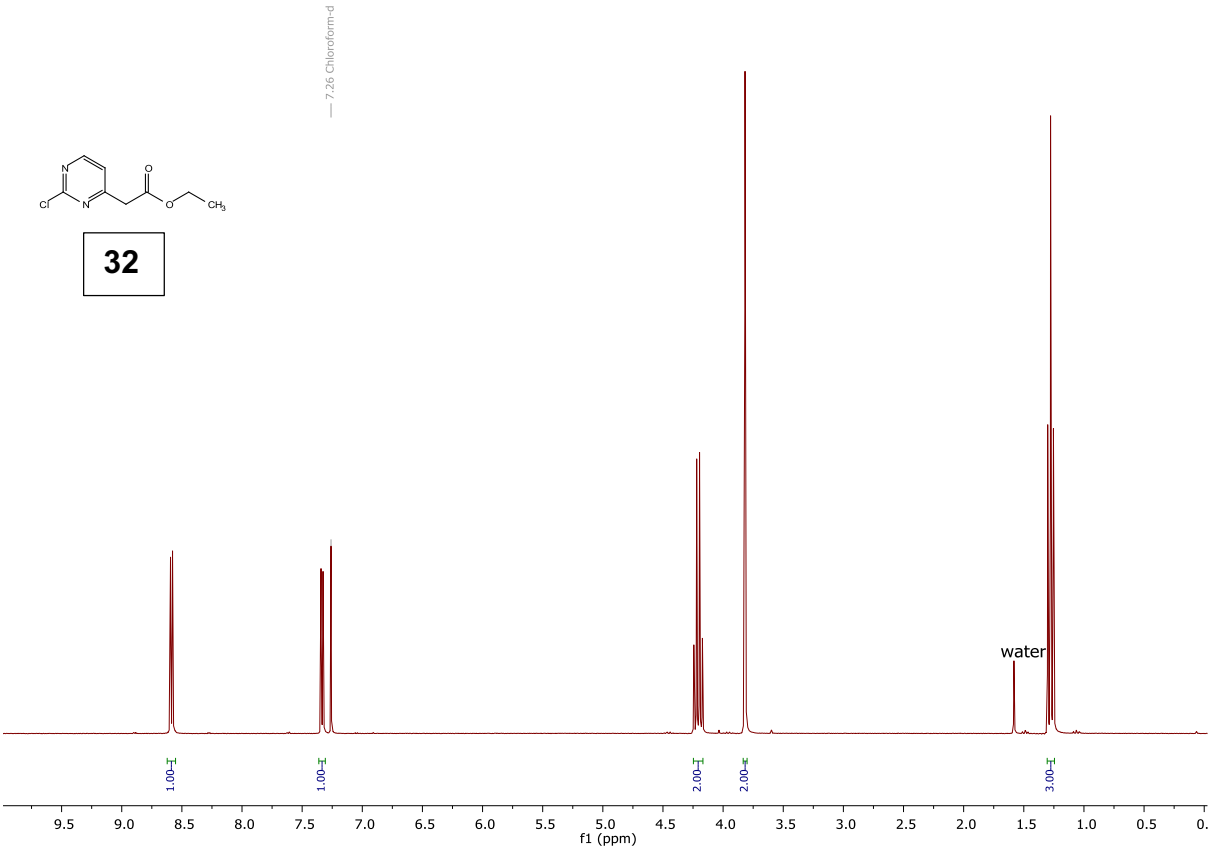
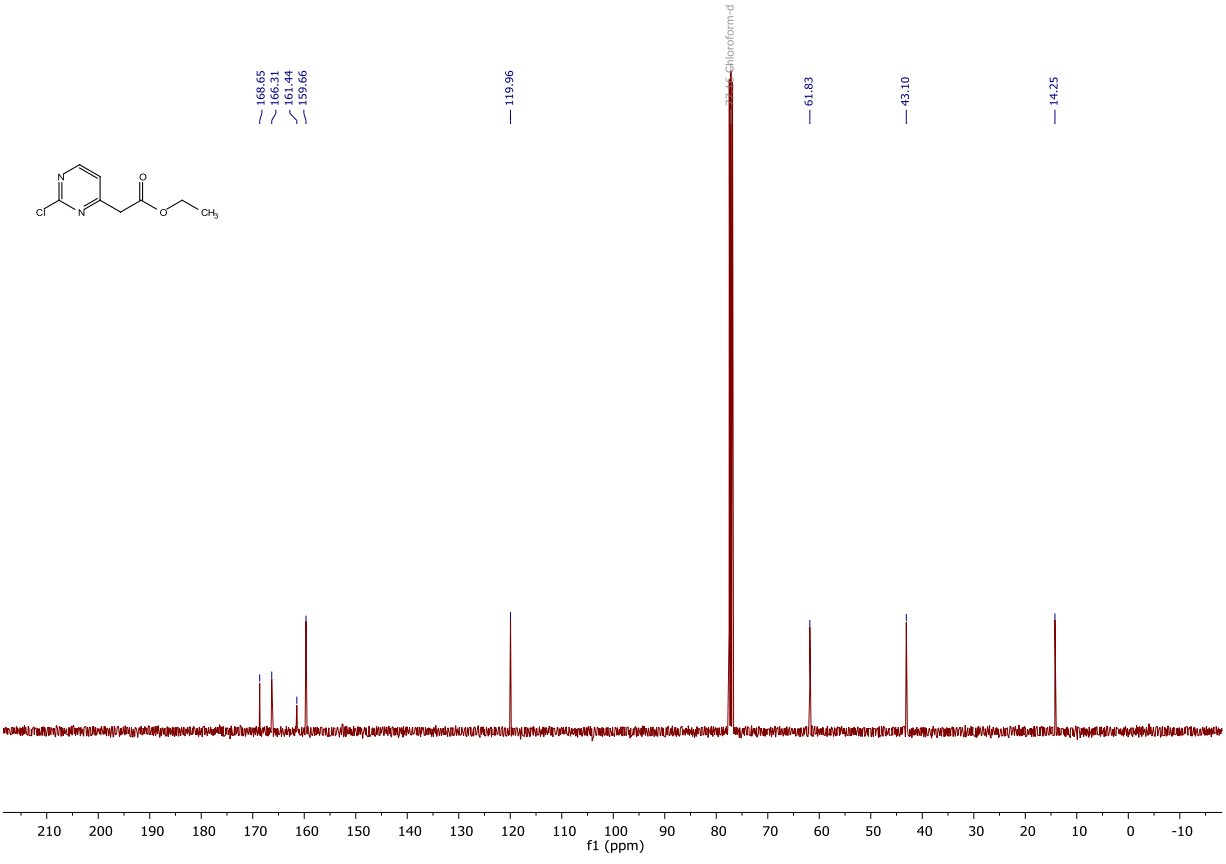
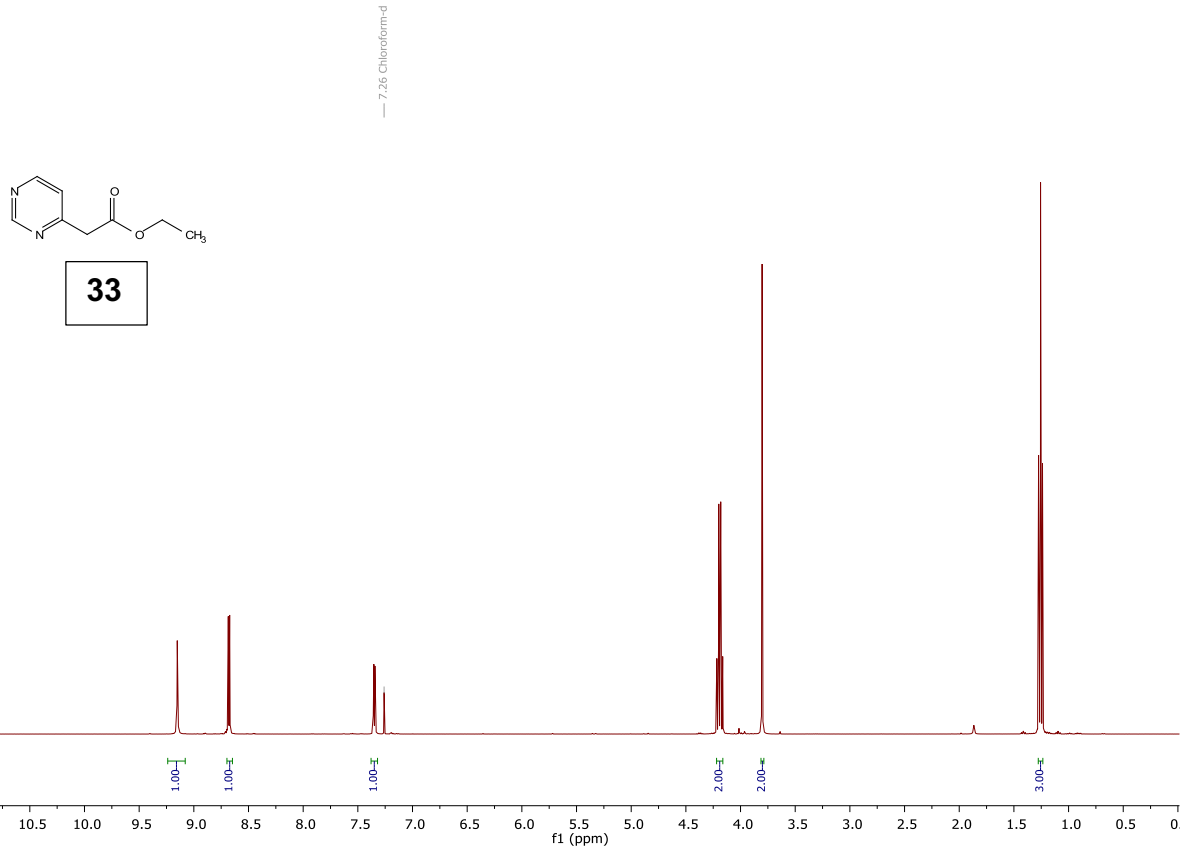
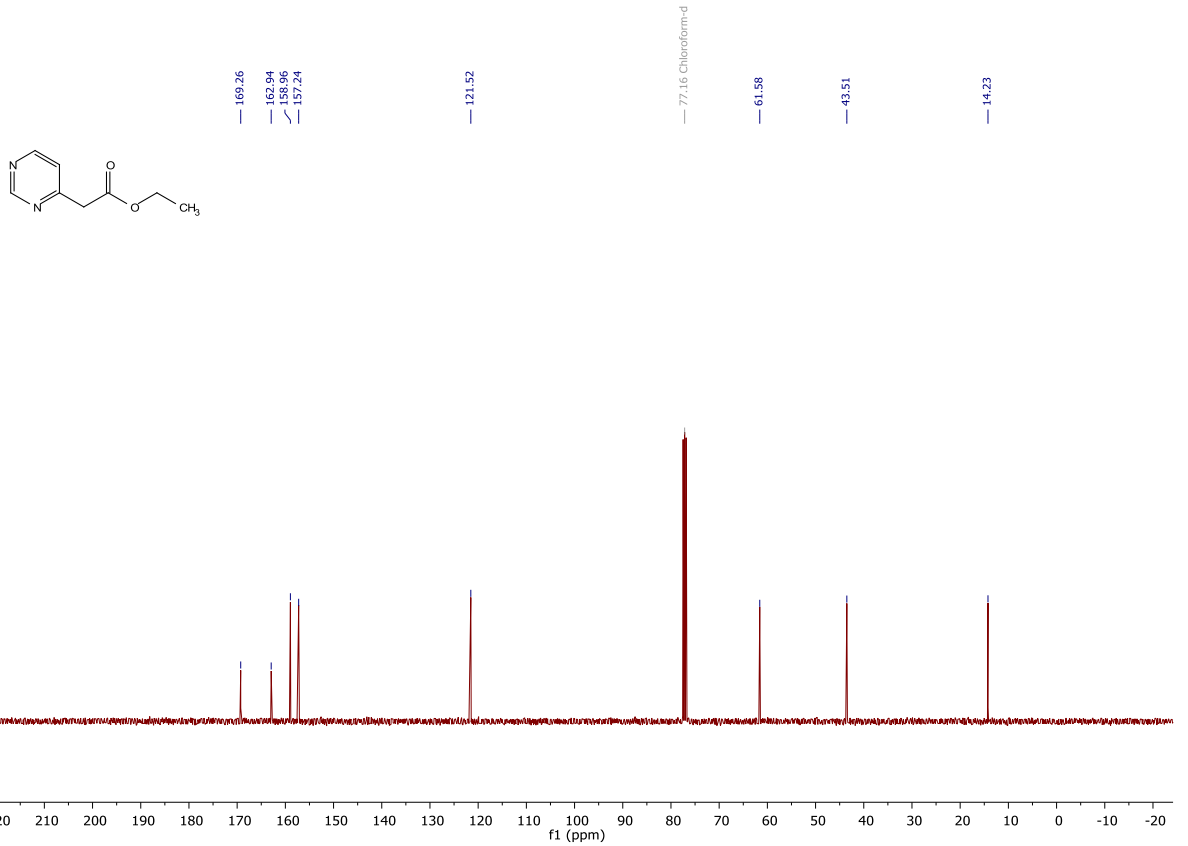
6.03
f1 (ppm)

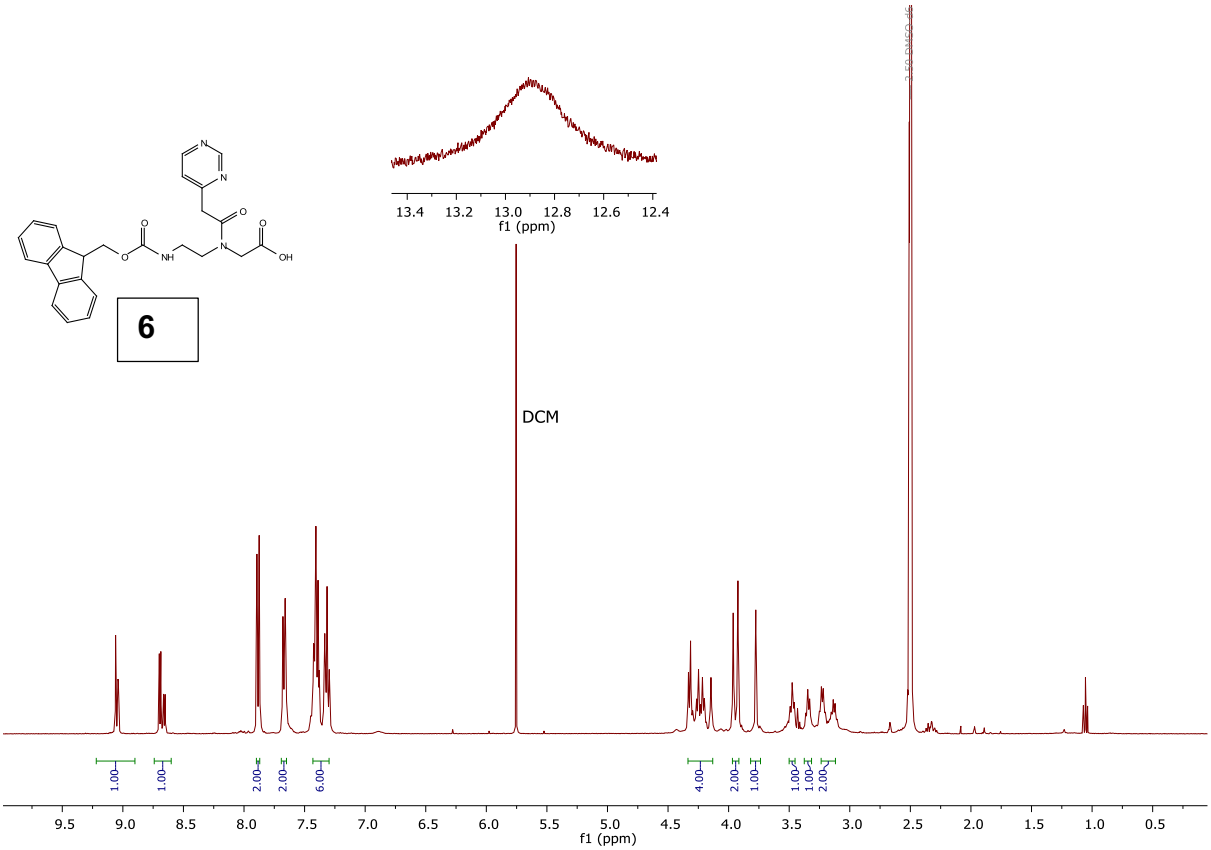

165.84
164.47
161.15
159.85
119.78
77.16 Chloroform-d
62.83
59.94
14.07
f1 (ppm)

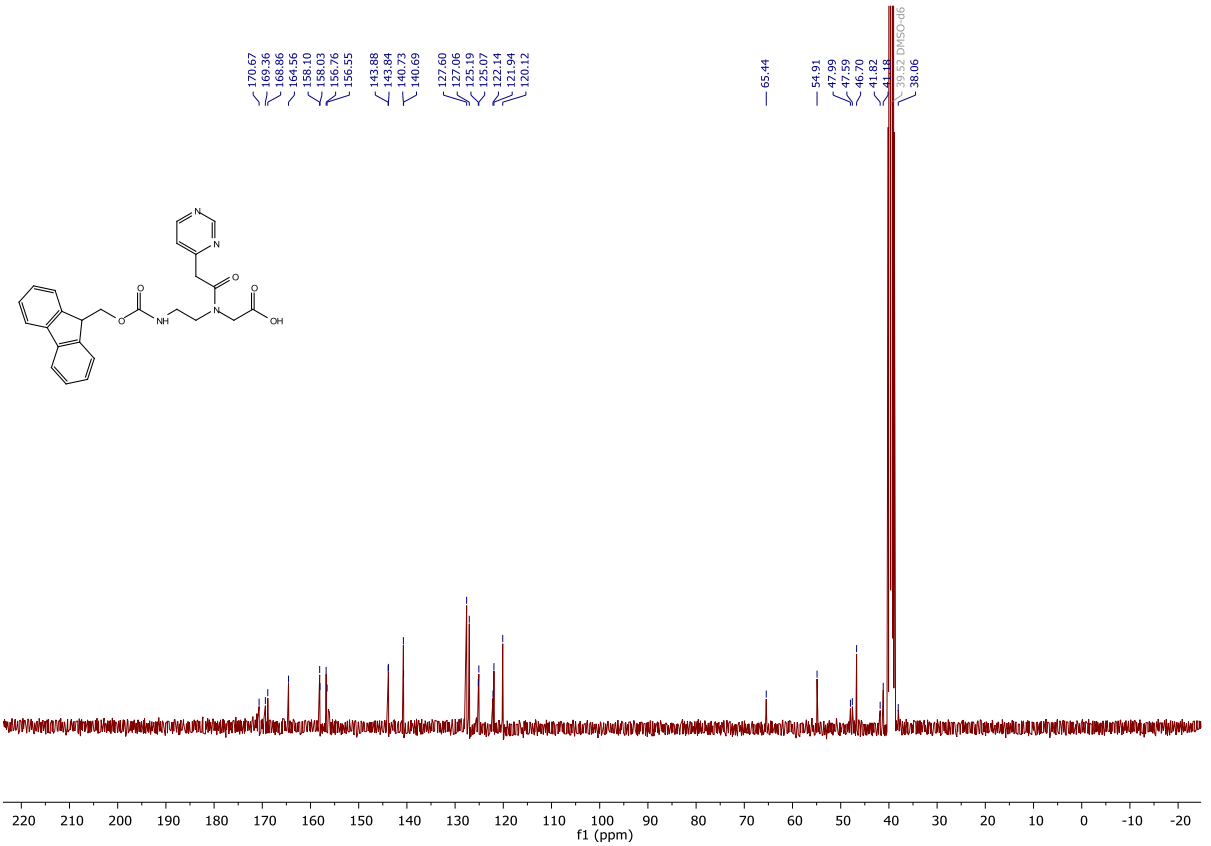

7.26 Chloroform-d
N
Cl
N
O
O
CH3
32
water
1.00
1.00
2.00
2.00
3.00
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)

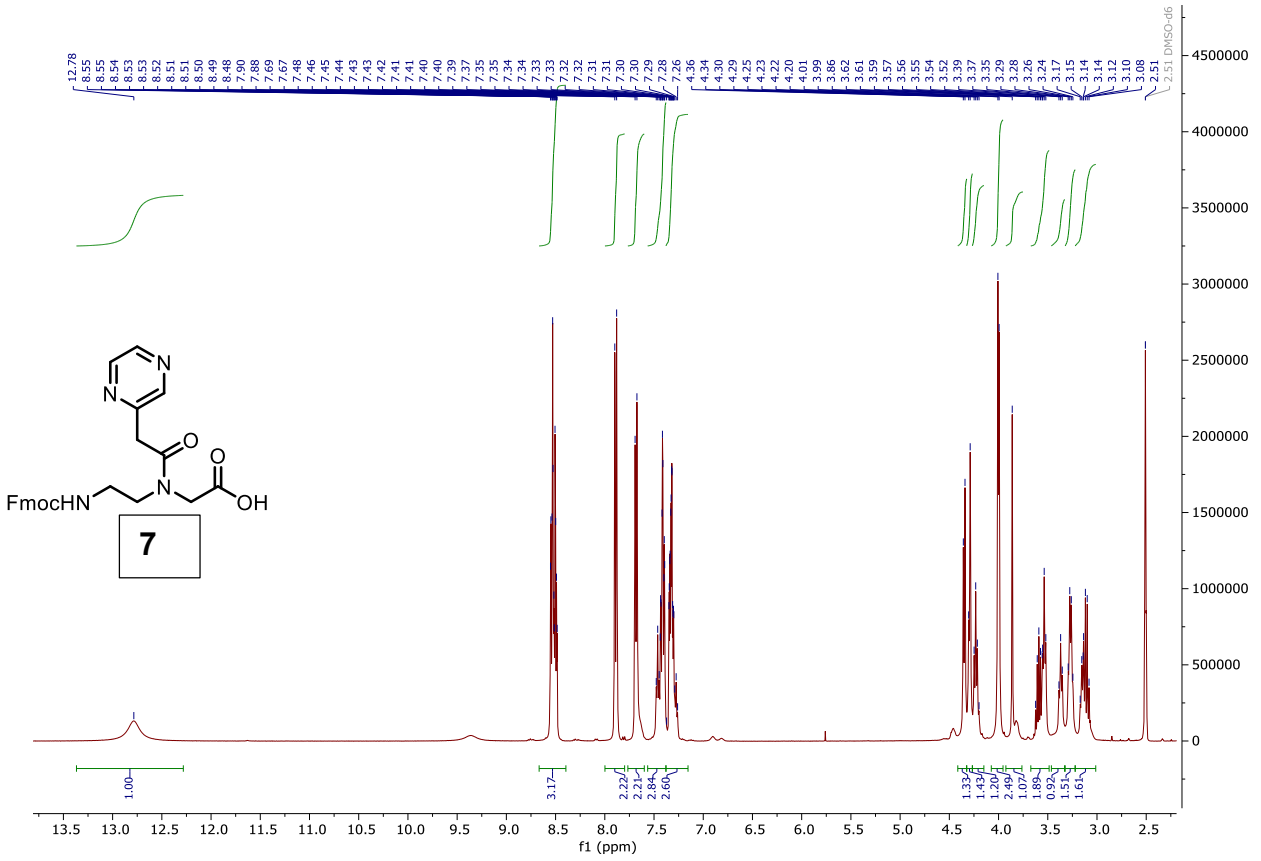

168.65
166.31
161.44
159.66
119.96
77.16 Chloroform-d
61.83
43.10
14.25
N
Cl
N
O
O
CH3
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

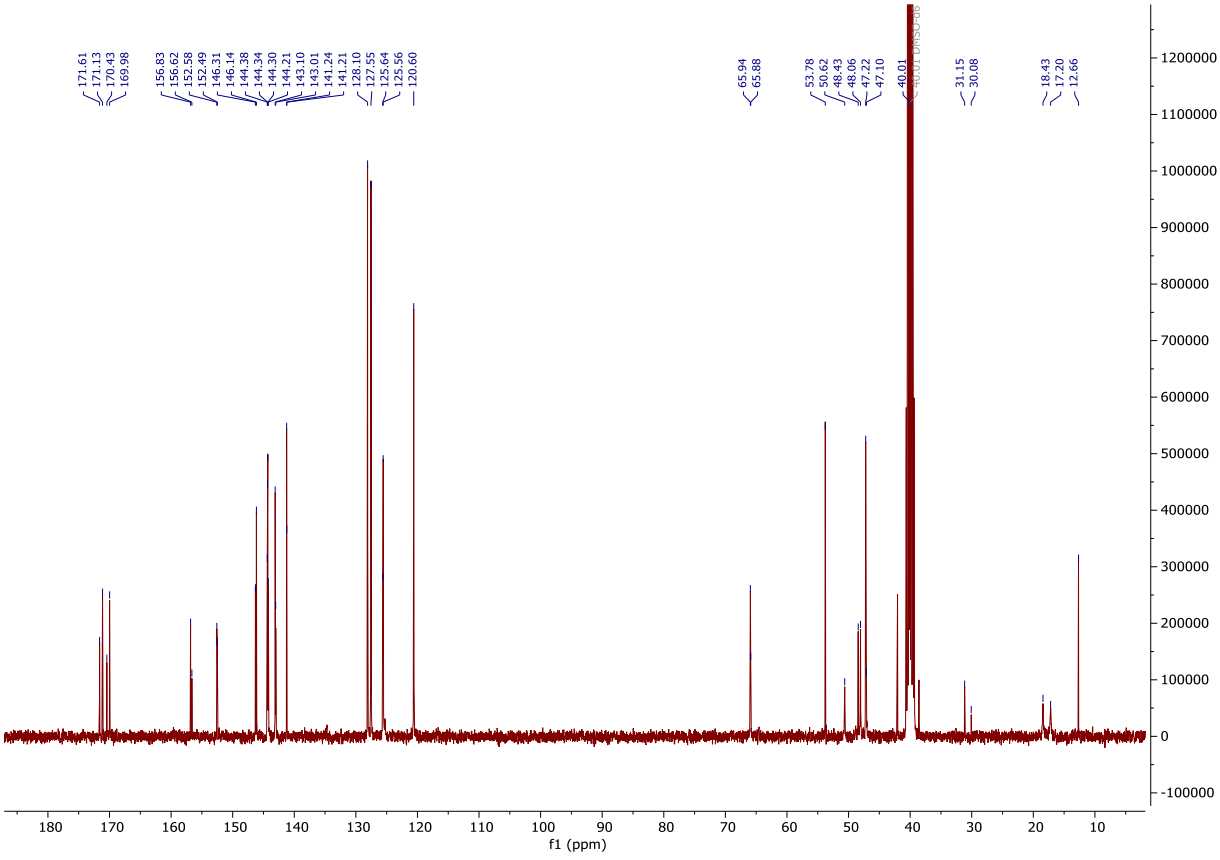

7.26 Chloroform-d
33
1.00
1.00
1.00
2.00
2.00
3.00
f1 (ppm)

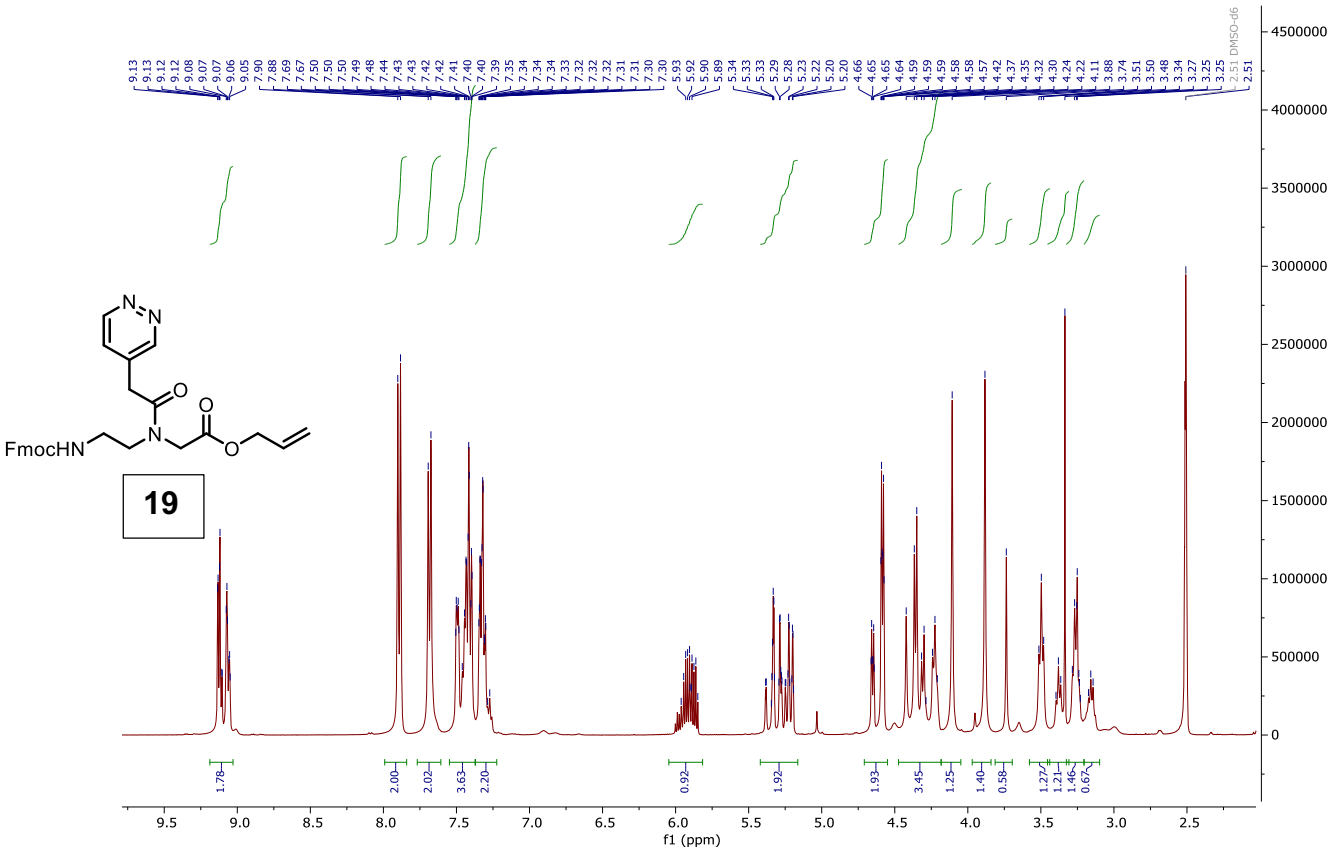

169.26
162.94
158.96
157.24
121.52
77.16 Chloroform-d
61.58
43.51
14.23
f1 (ppm)

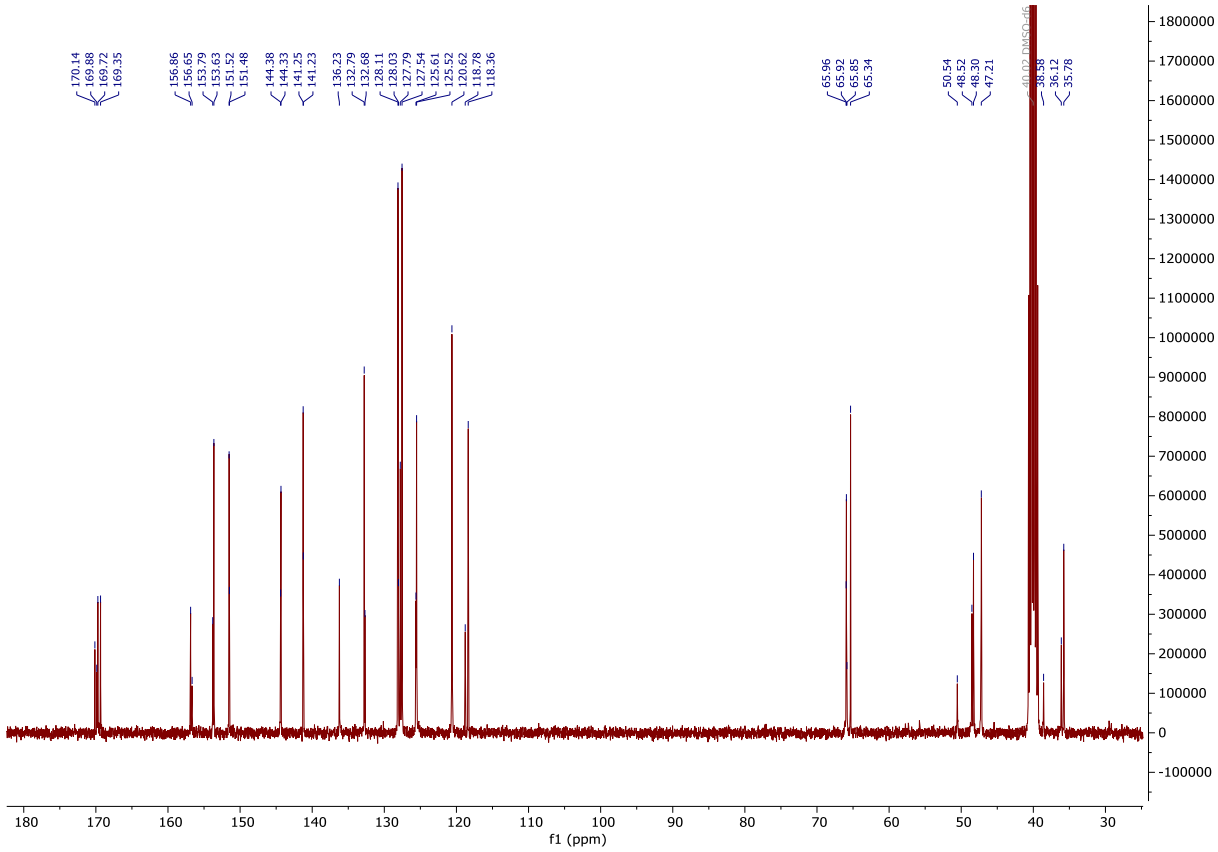

6
DCM
f1 (ppm)

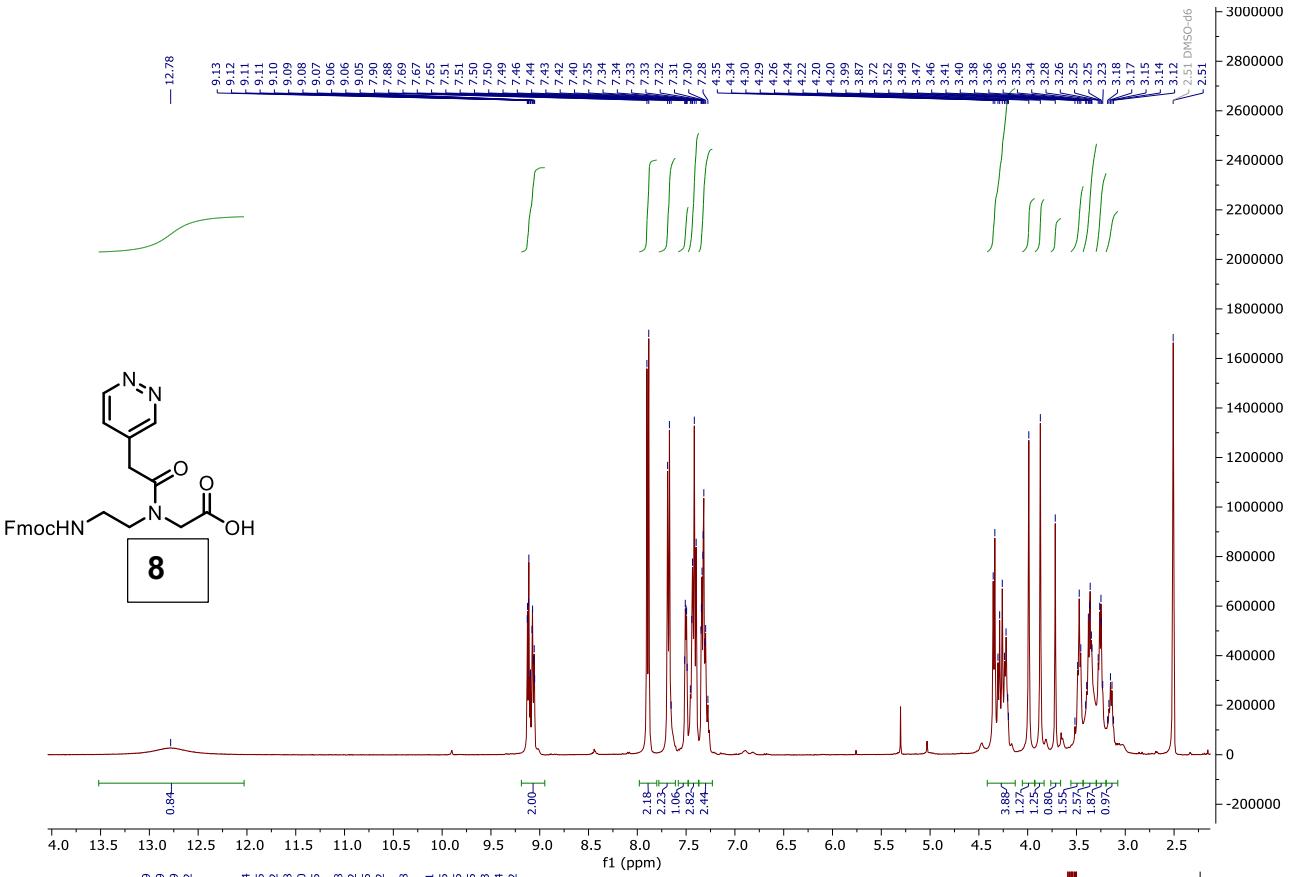

170.67
169.36
168.86
164.56
158.10
158.03
156.76
156.55
143.88
143.84
140.73
140.69
127.60
127.06
125.19
125.07
122.14
121.94
120.12
65.44
54.91
47.99
47.59
46.70
41.82
41.18
39.52 DMSO-d6
38.06
f1 (ppm)

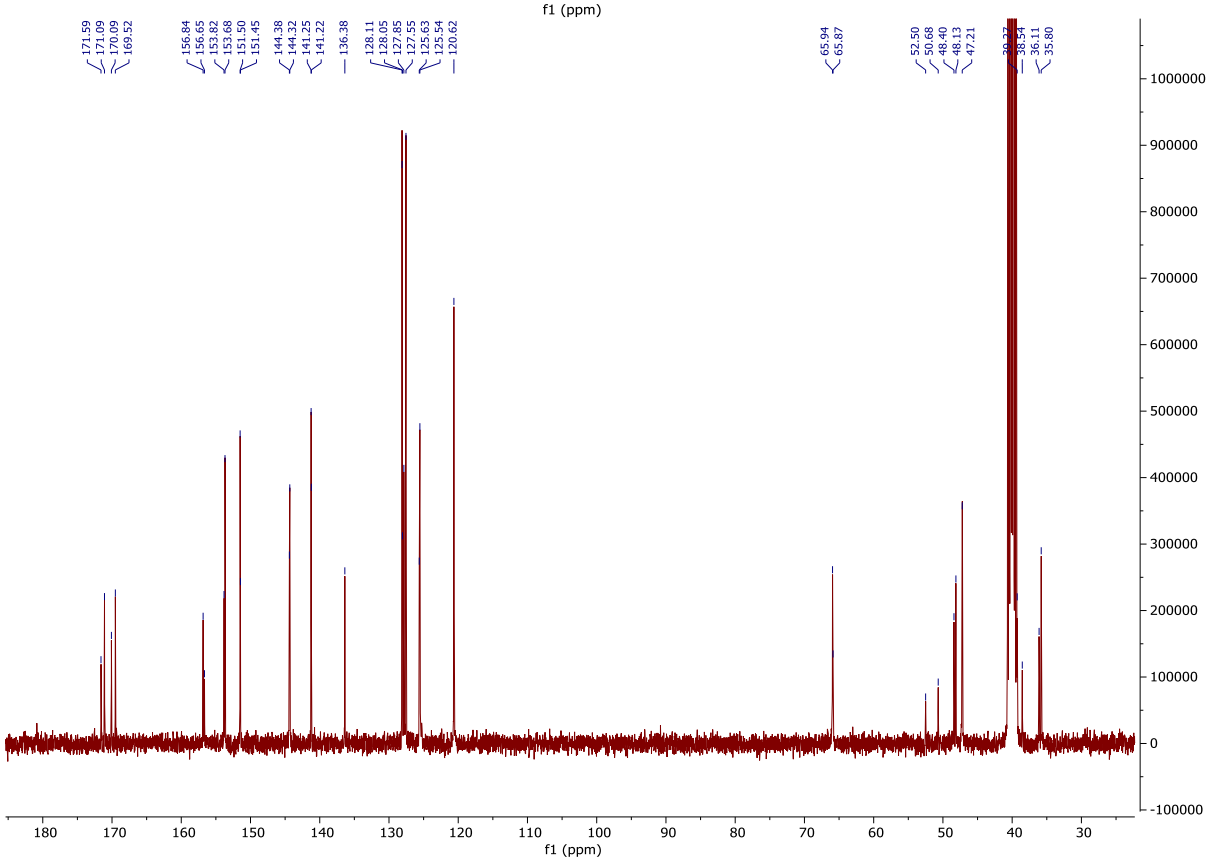

FmocHN
OH
7
f1 (ppm)

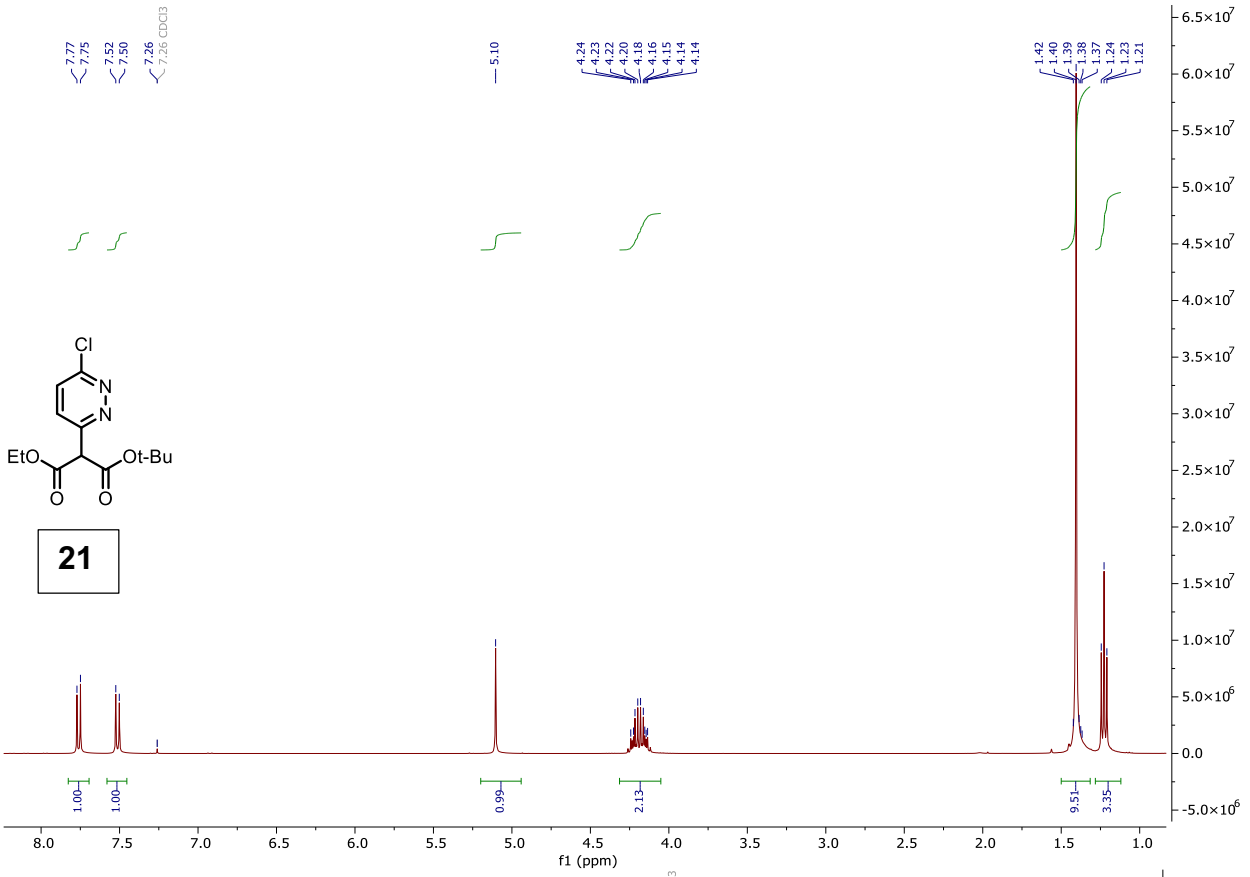

f1 (ppm)

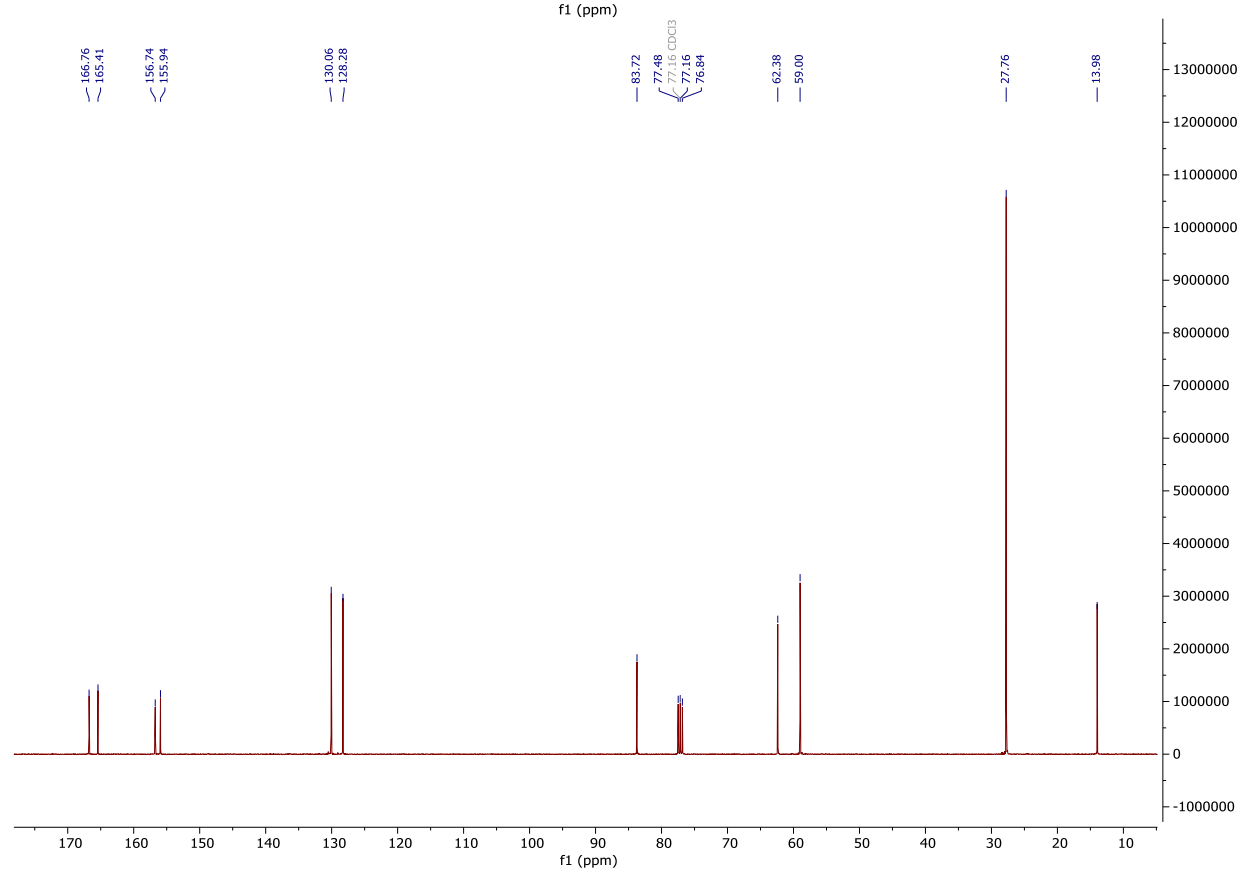

FmocHN
19
f1 (ppm)

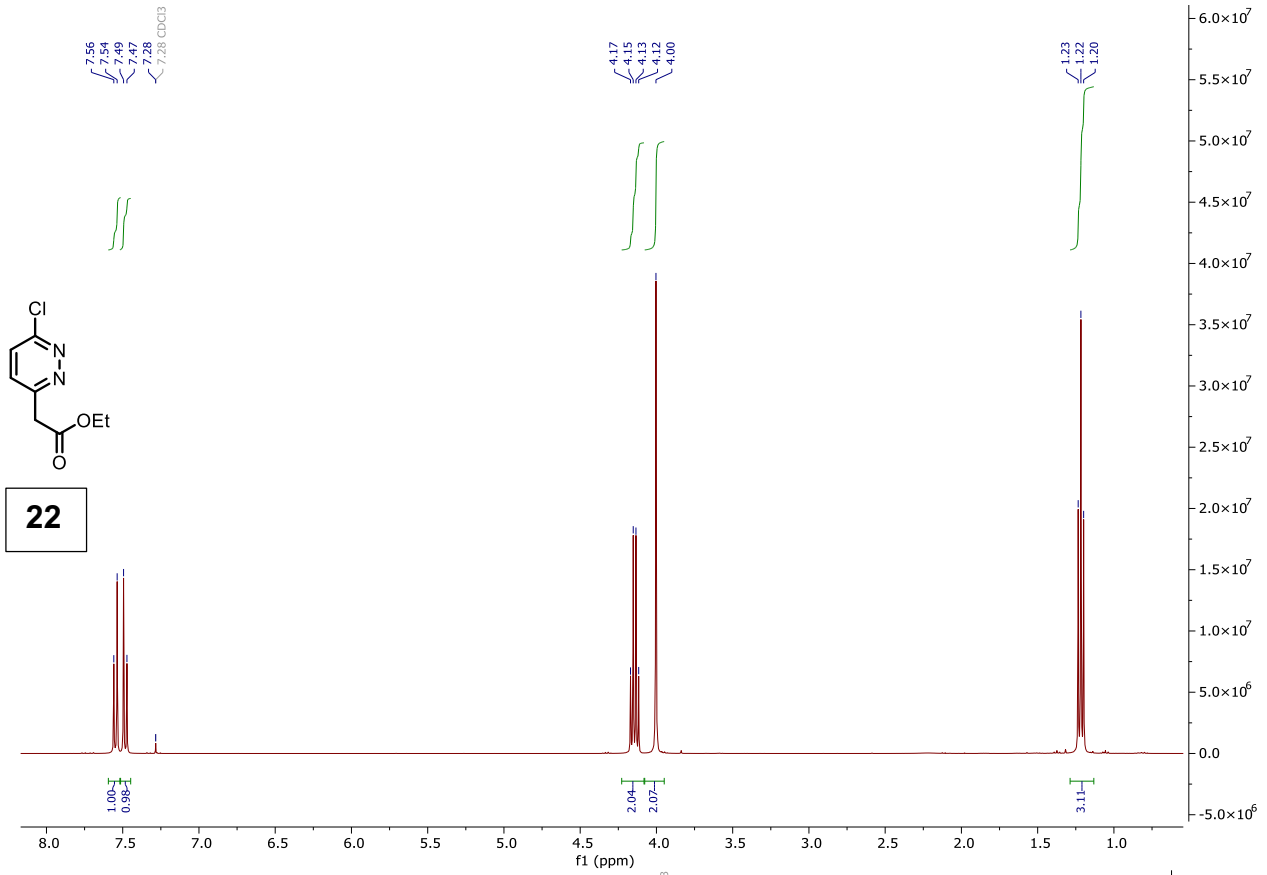

f1 (ppm)

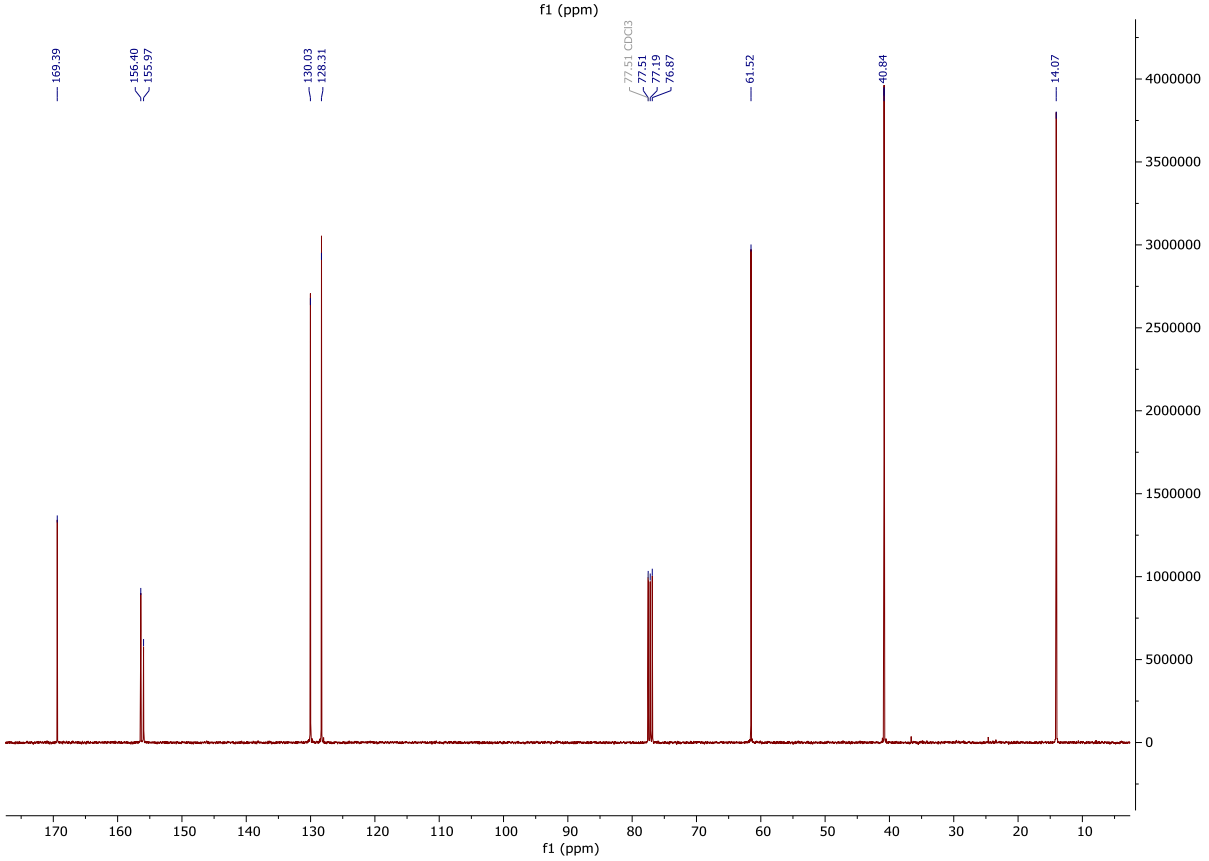

FmocHN
OH
8
f1 (ppm)

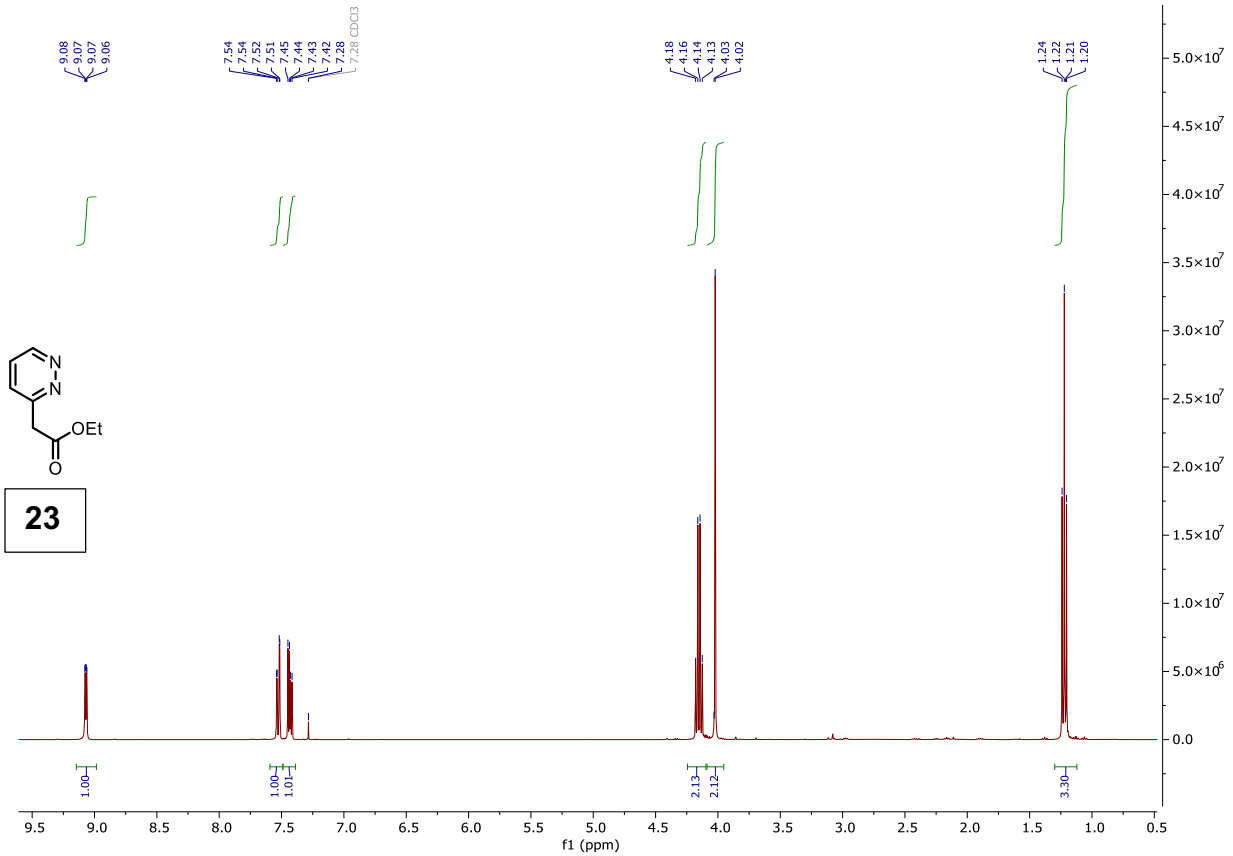

f1 (ppm)

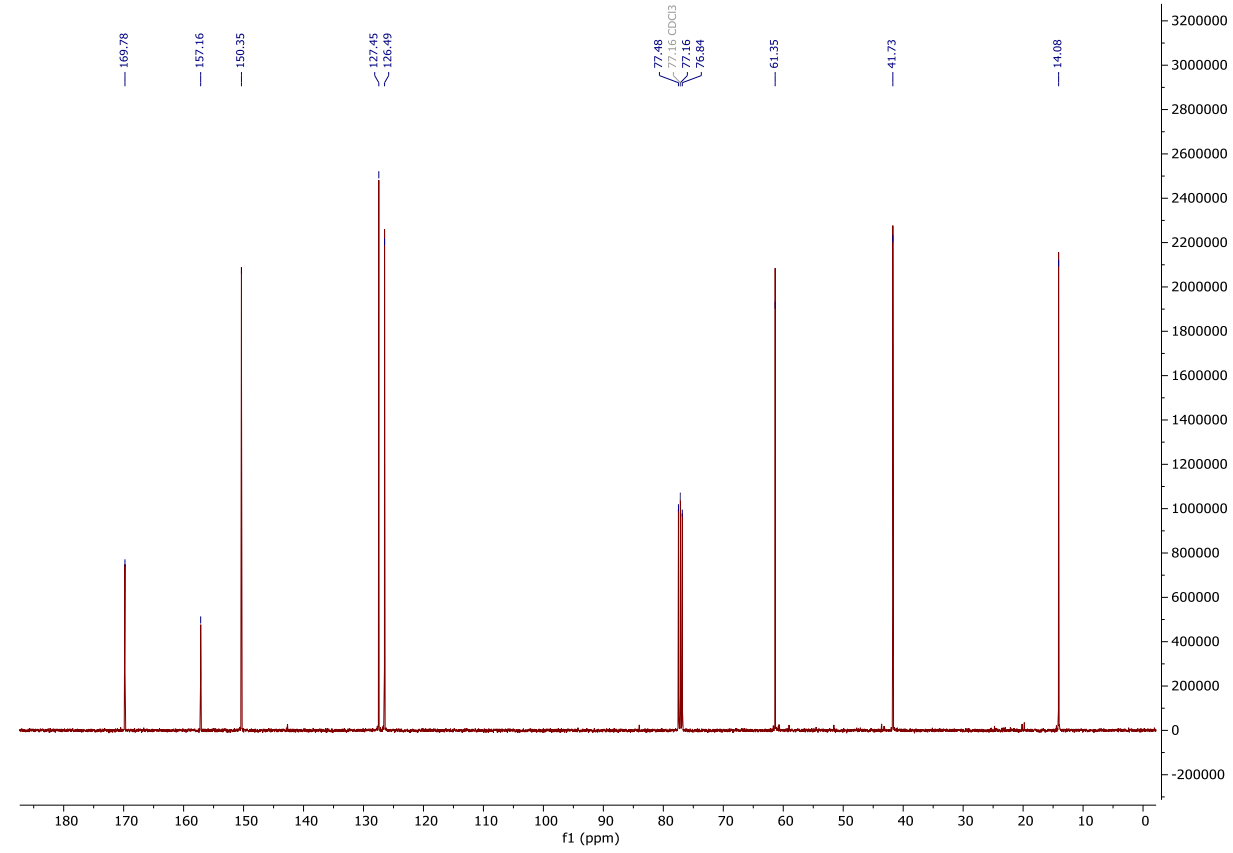

7.77
7.75
7.52
7.50
7.26
7.26 CDCl3
5.10
4.24
4.23
4.22
4.20
4.18
4.16
4.15
4.14
4.14
1.42
1.40
1.39
1.38
1.37
1.24
1.23
1.21
Cl
N
N
EtO
Ot-Bu
O
O
21
1.00
1.00
0.99
2.13
9.51
3.35
f1 (ppm)

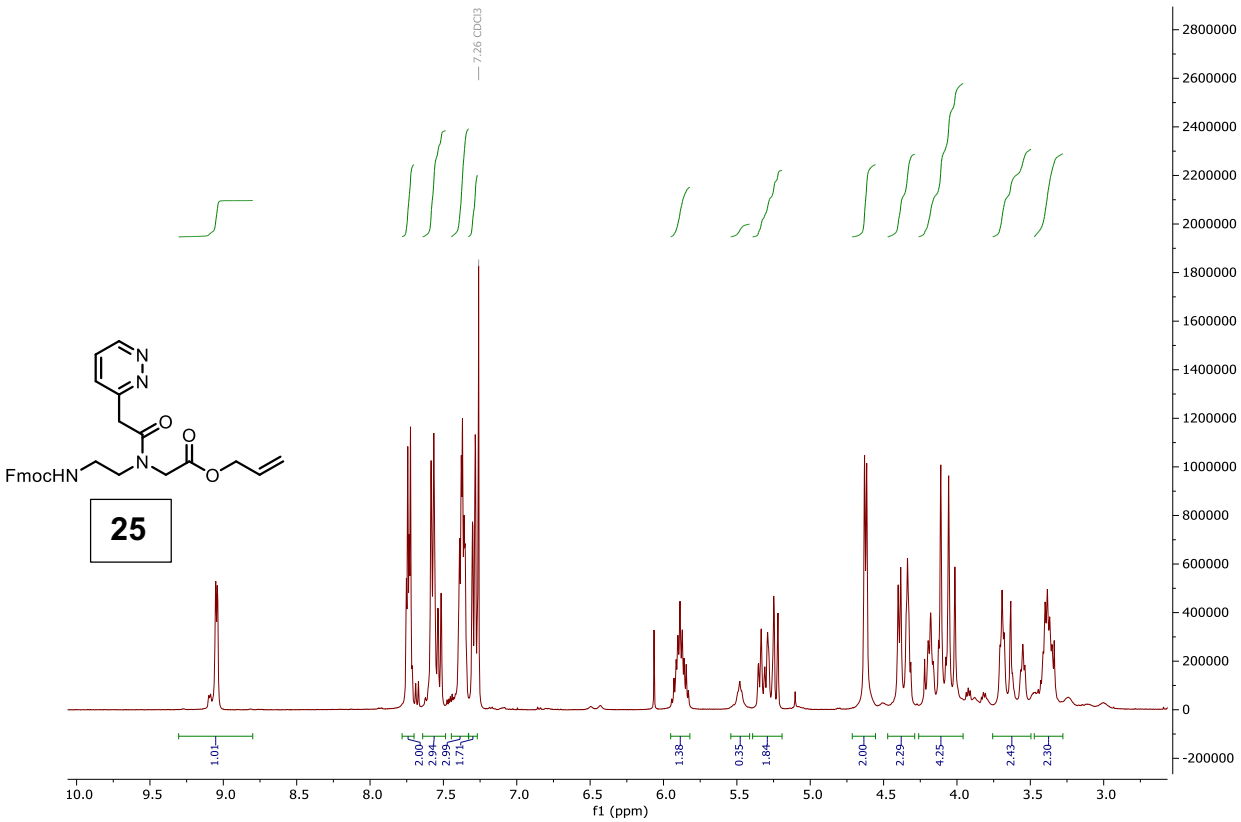

166.76
165.41
156.74
155.94
130.06
128.28
83.72
77.48
77.16 CDCl3
77.16
76.84
62.38
59.00
27.76
13.98
f1 (ppm)

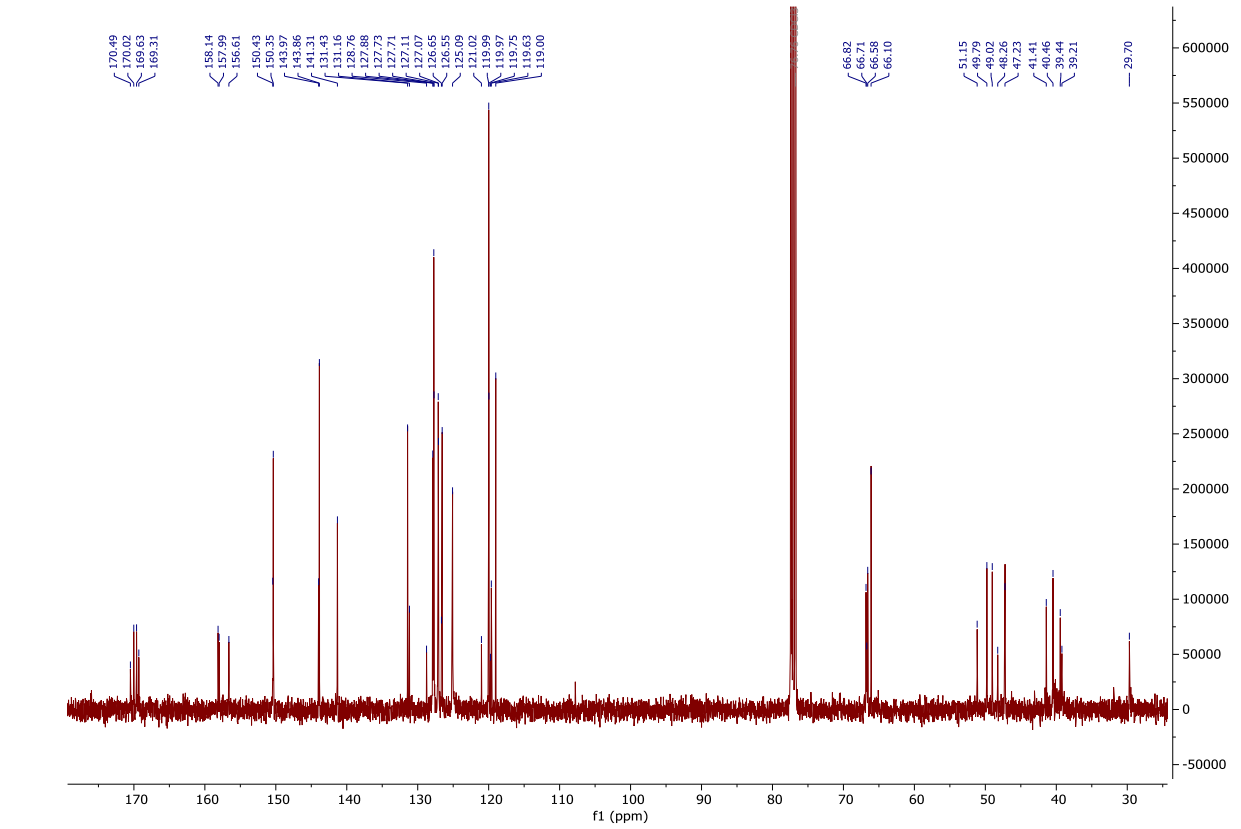

7.56
7.54
7.49
7.47
7.28
7.28 CDCl3
4.17
4.15
4.13
4.12
4.00
1.23
1.22
1.20
Cl
N
N
OEt
O
22
1.00
0.98
2.04
2.07
3.11
f1 (ppm)

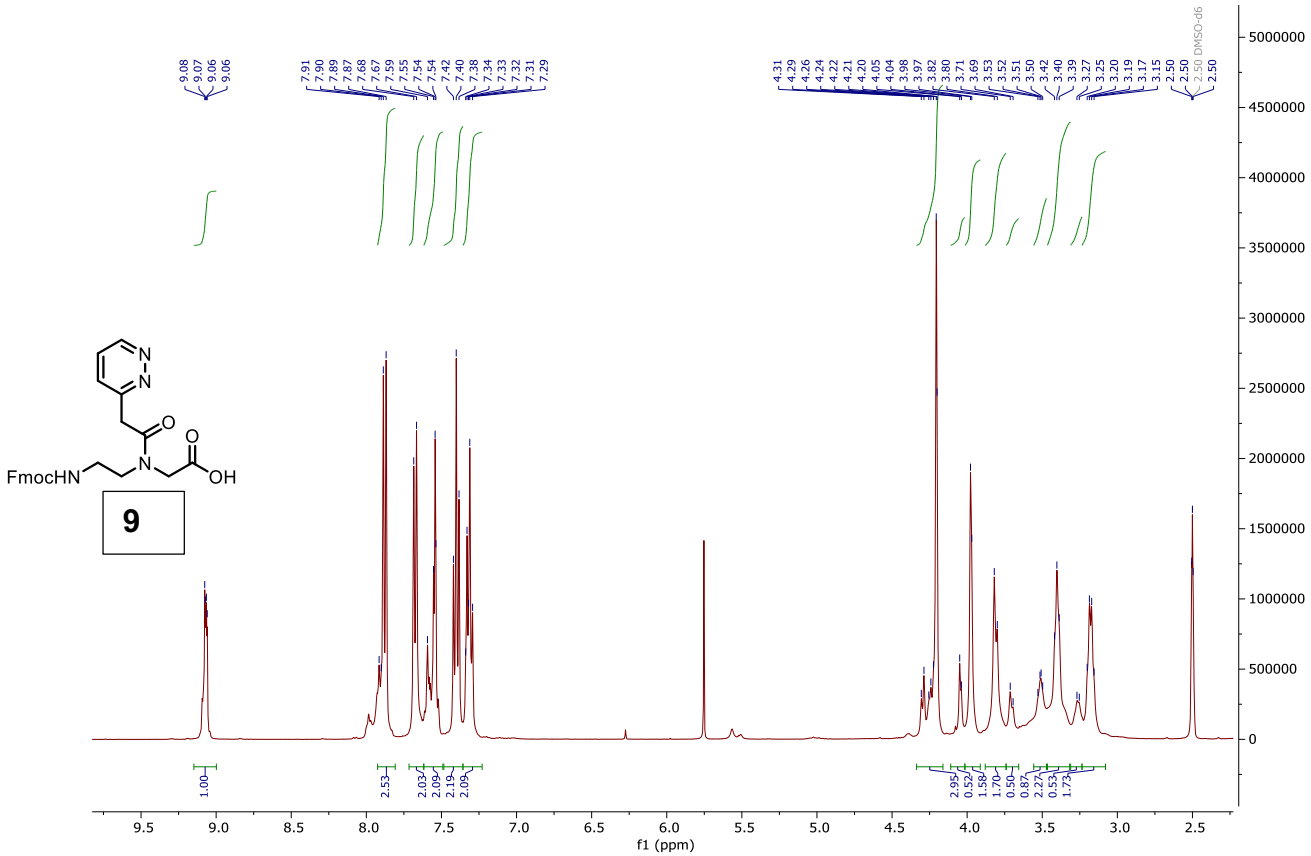

169.39
156.40
155.97
130.03
128.31
77.51 CDCl3
77.51
77.19
76.87
61.52
40.84
14.07
f1 (ppm)

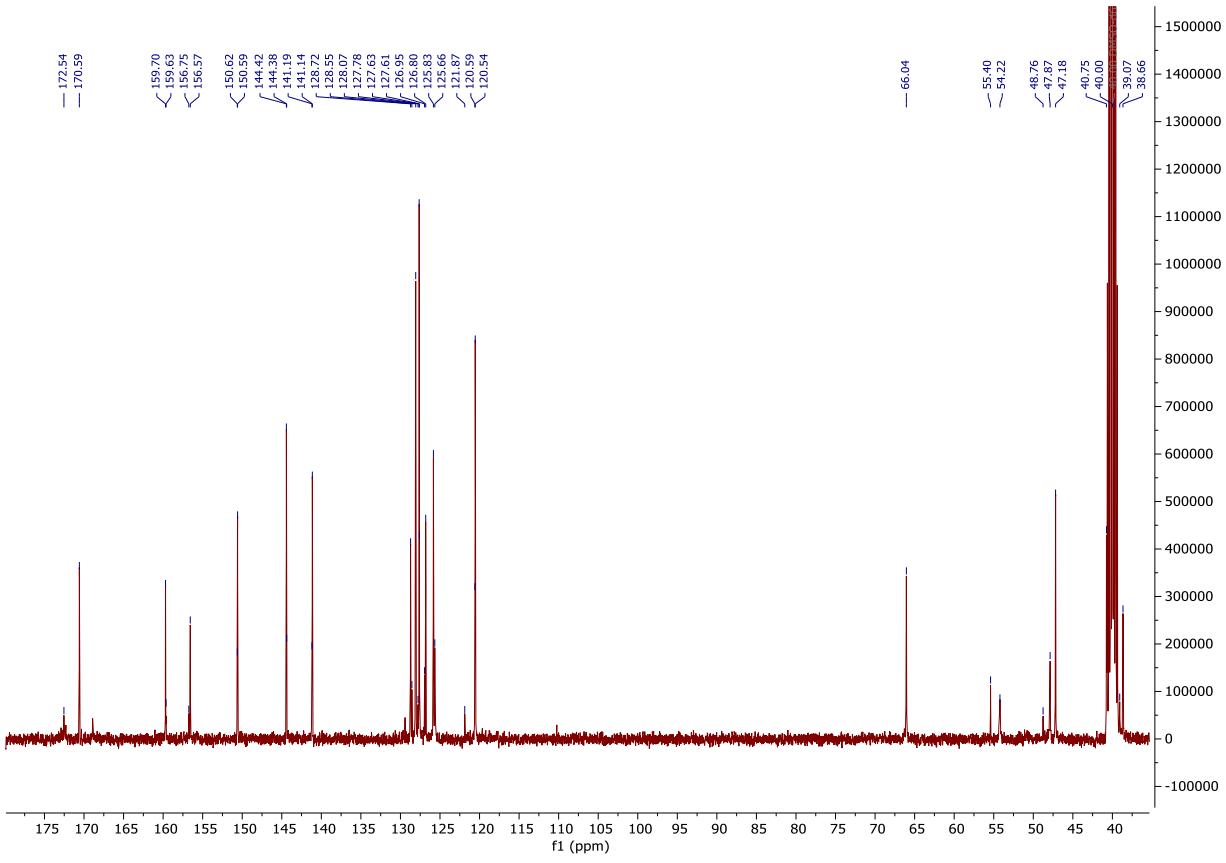

9.08
9.07
9.07
9.06
7.54
7.54
7.52
7.51
7.45
7.44
7.43
7.42
7.28
7.28 CDCl3
4.18
4.16
4.14
4.13
4.03
4.02
1.24
1.22
1.21
1.20
N
N
OEt
O
23
1.00
1.00
1.01
2.13
2.12
3.30
f1 (ppm)

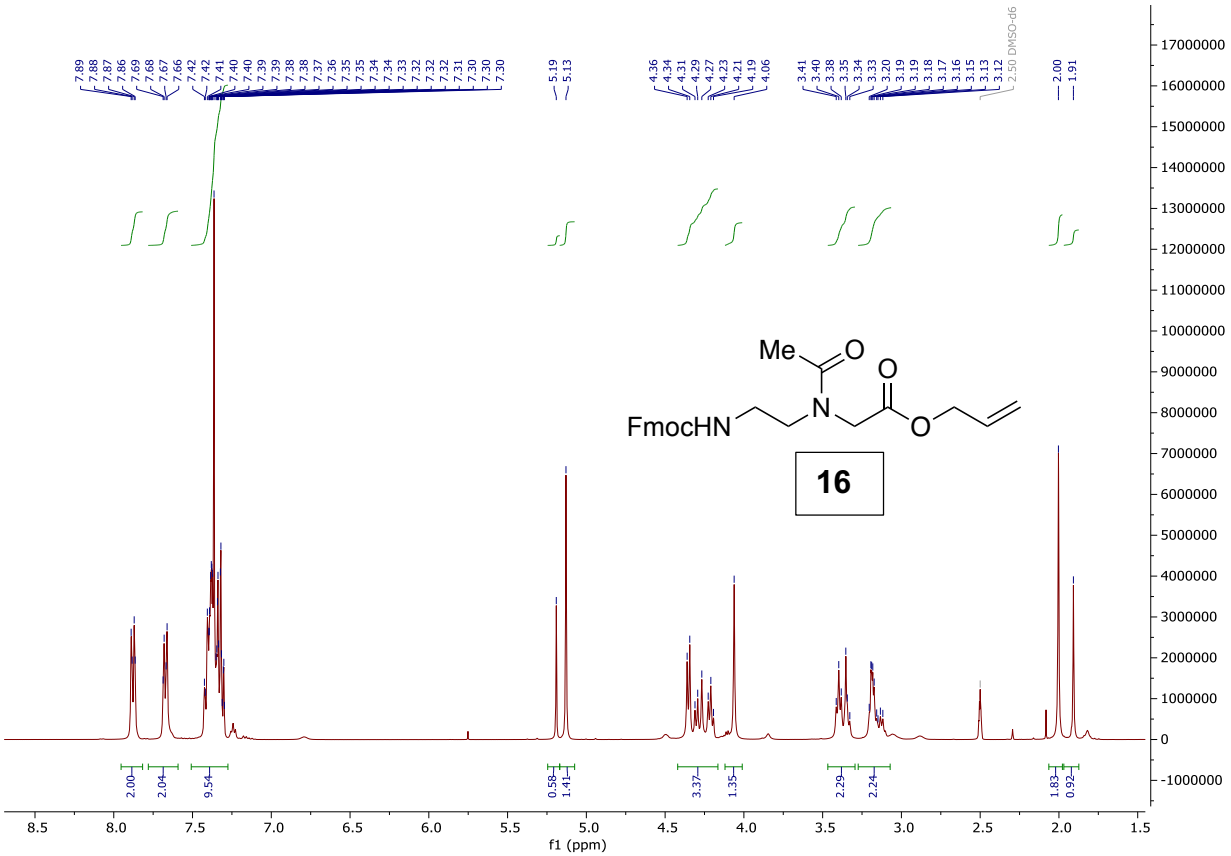

169.78
157.16
150.35
127.45
126.49
77.48
77.16 CDCl3
77.16
76.84
61.35
41.73
14.08
f1 (ppm)

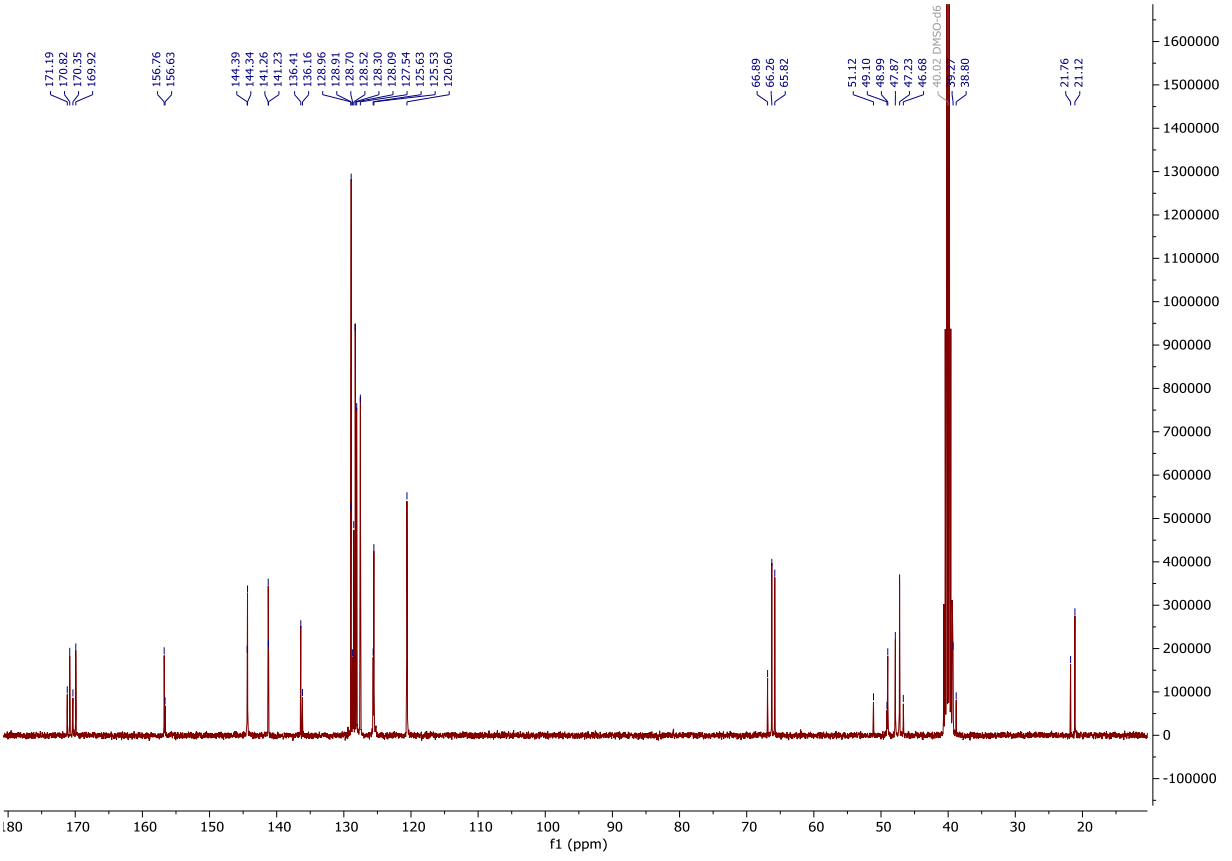

FmocHN
25
7.26 CDCl3
1.01
2.00
2.94
2.99
1.71
1.38
0.35
1.84
2.00
2.29
4.25
2.43
2.30
f1 (ppm)

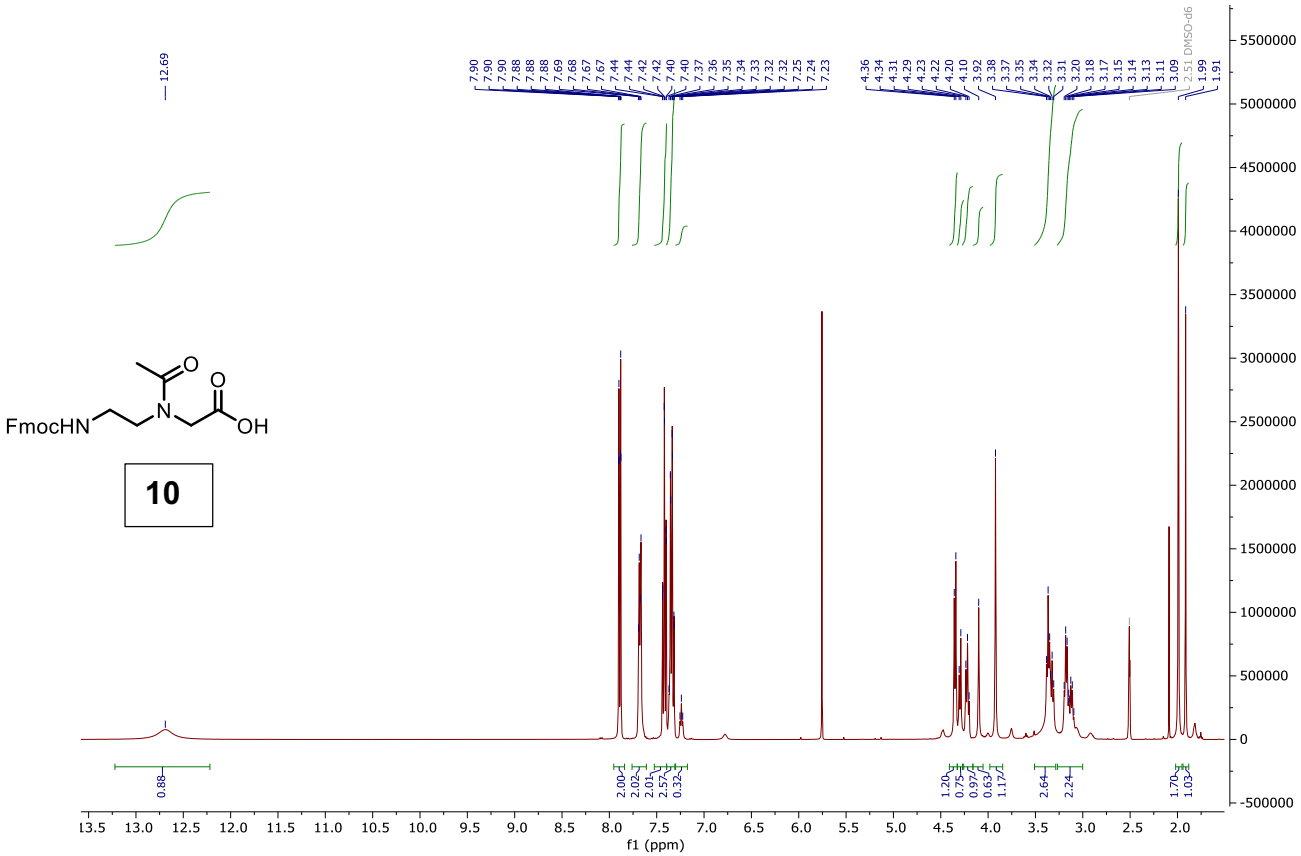

170.49
170.02
169.63
169.31
158.14
157.99
156.61
150.43
150.35
143.97
143.86
141.31
131.43
131.16
128.76
127.88
127.73
127.71
127.11
127.07
126.65
126.55
125.09
121.02
119.99
119.97
119.75
119.63
119.00
66.82
66.71
66.58
66.10
51.15
49.79
49.02
48.26
47.23
41.41
40.46
39.44
39.21
29.70
f1 (ppm)


FmocHN
OH
9
f1 (ppm)

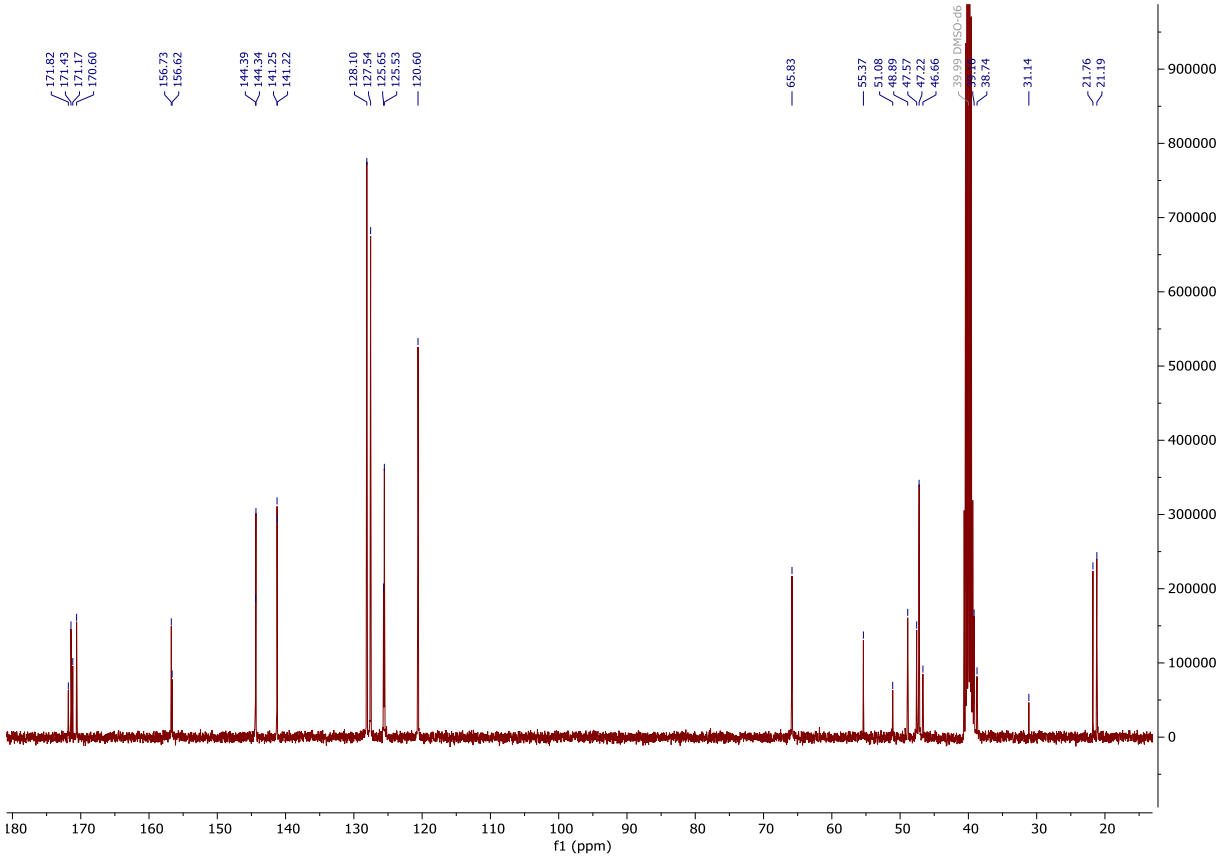

f1 (ppm)

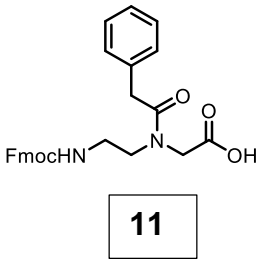

Me
O
O
N
FmocHN
O
16
f1 (ppm)


f1 (ppm)

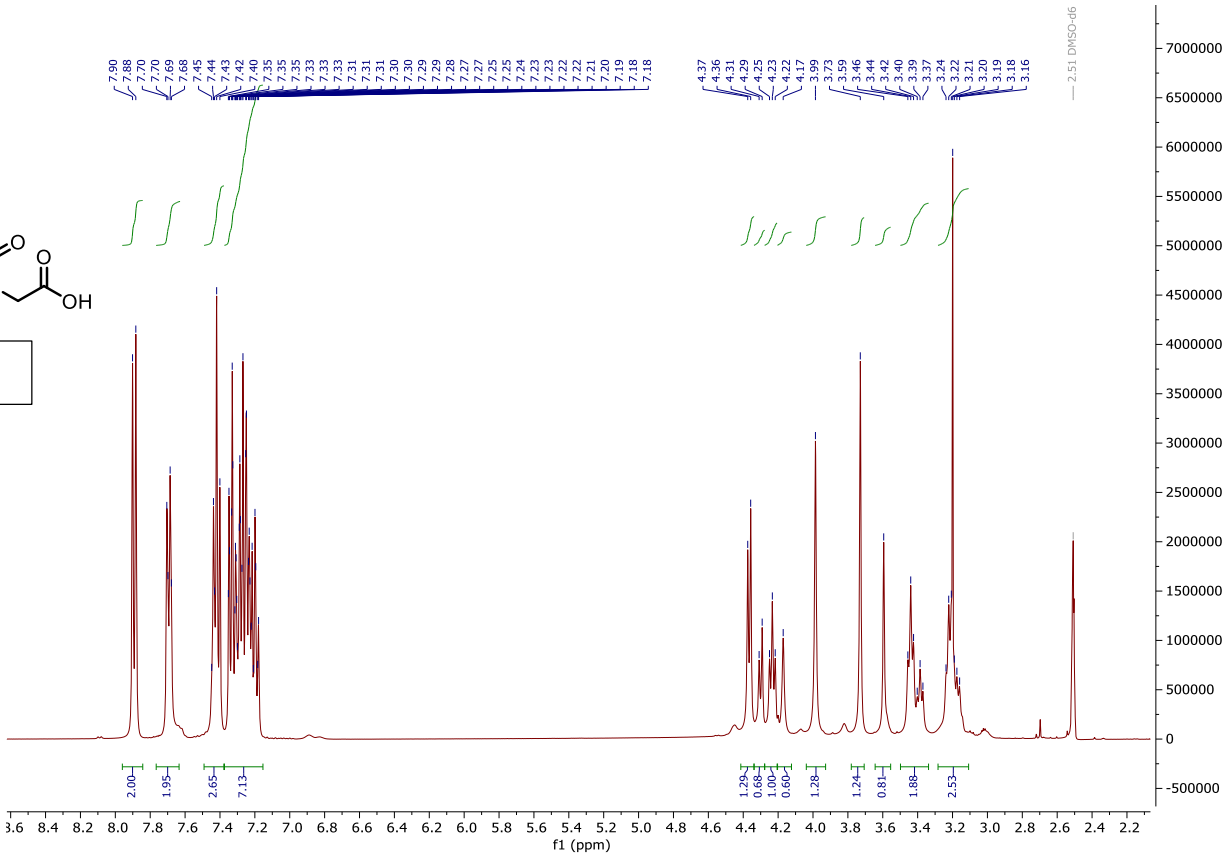


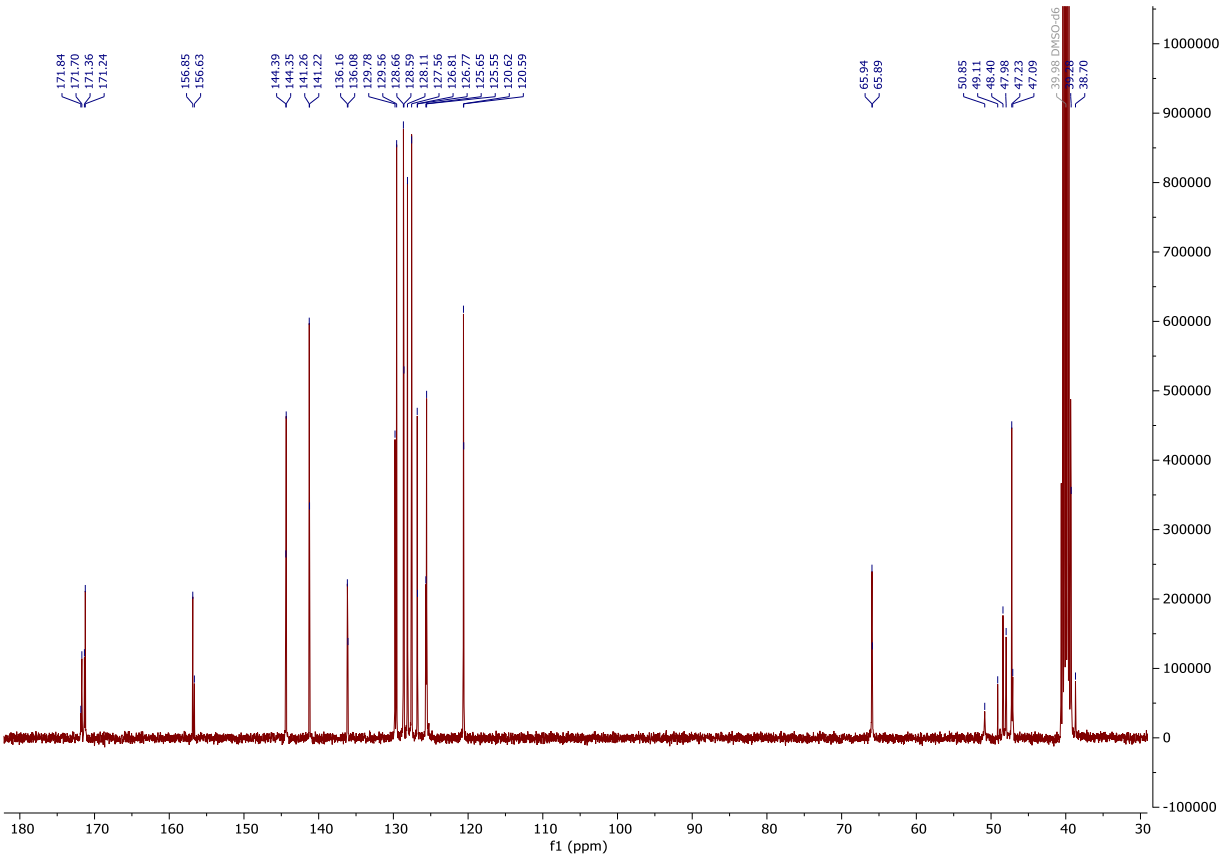
10

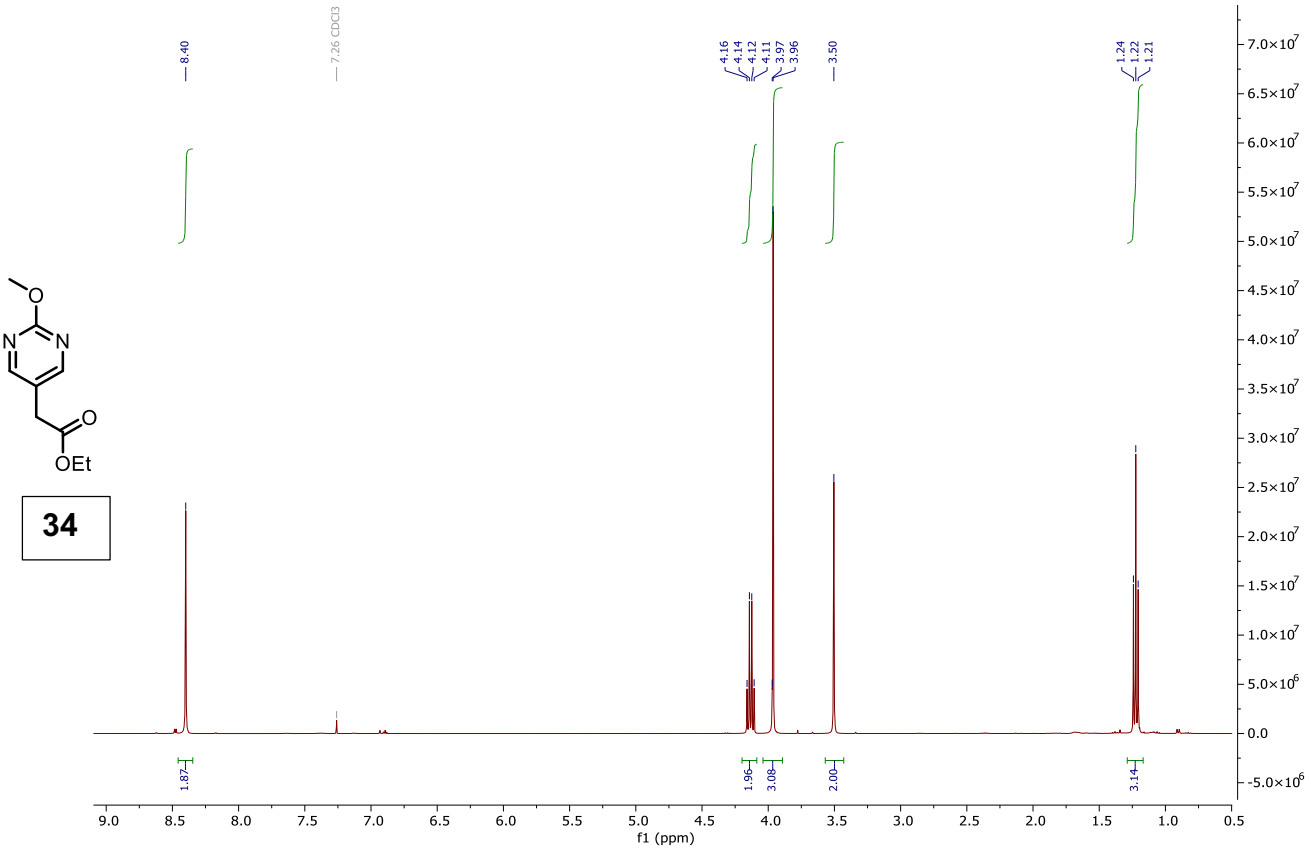


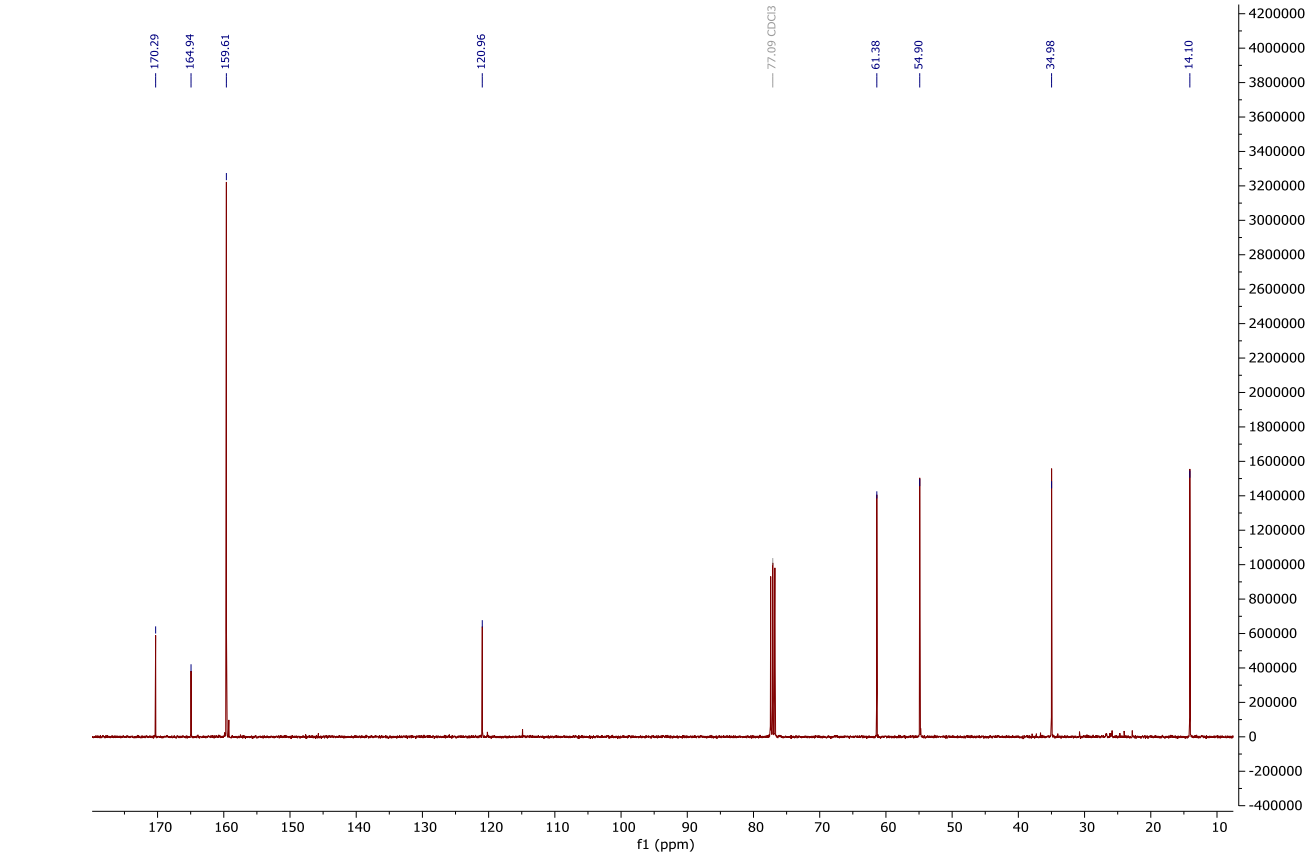


11

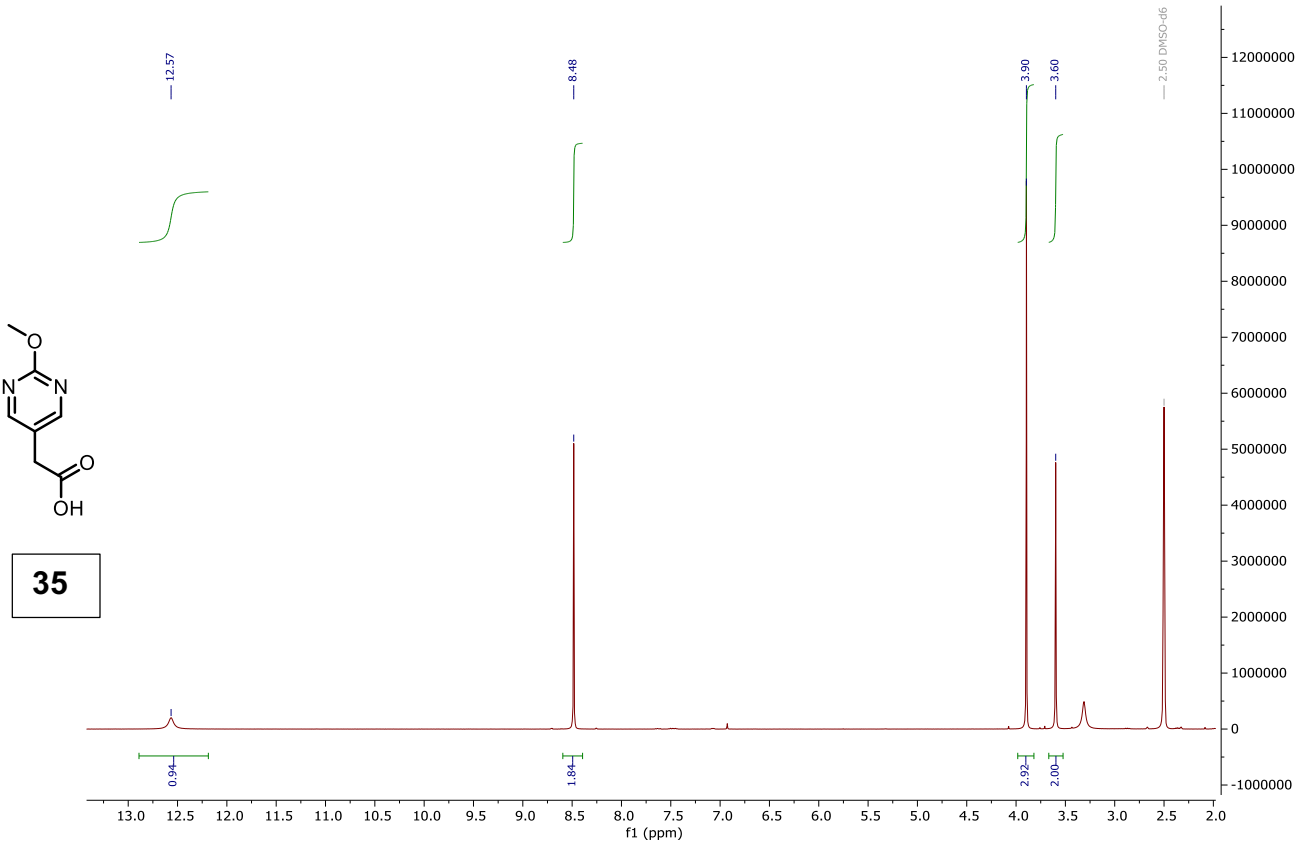




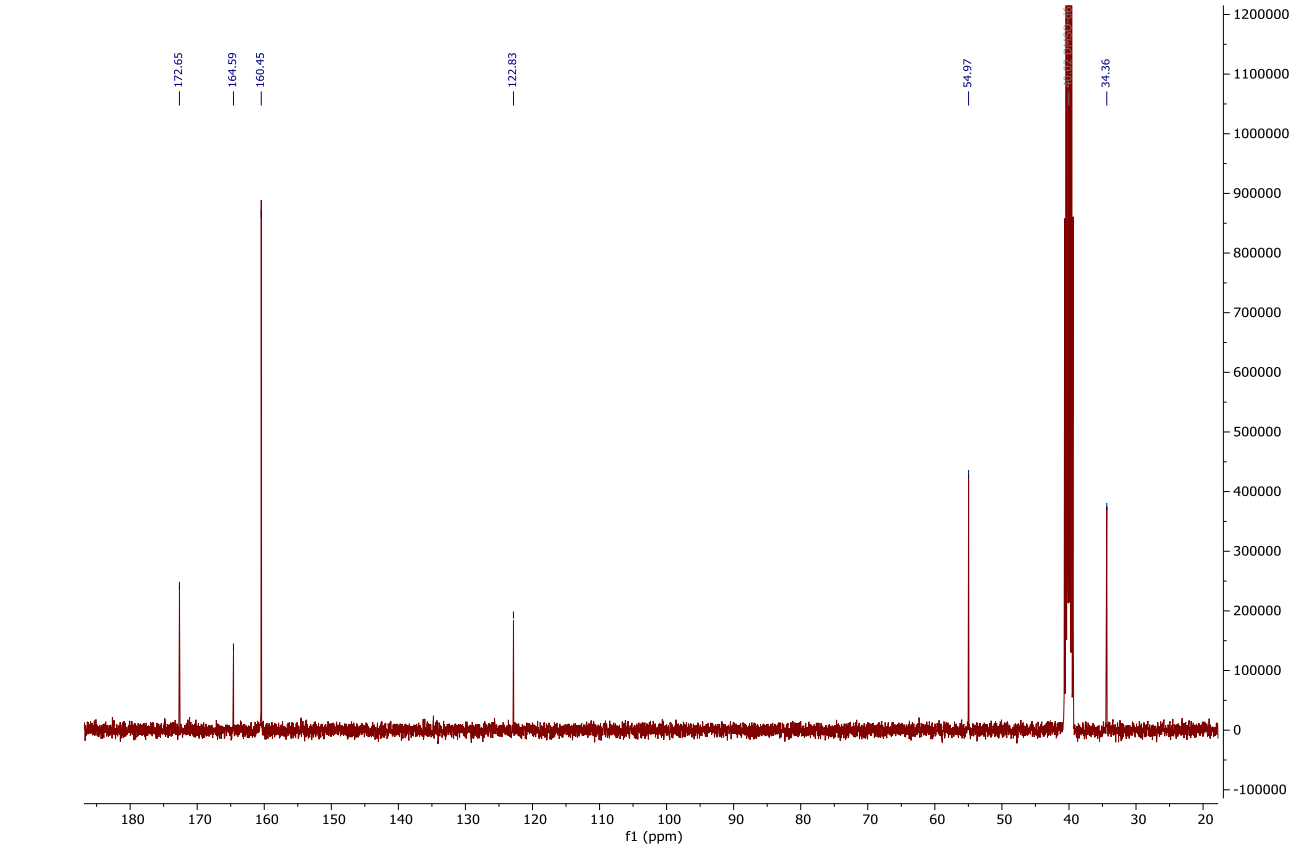

8.40
7.26 CDCl3
4.16
4.14
4.12
4.11
3.97
3.96
3.50
1.24
1.22
1.21
1.87
1.96
3.08
2.00
3.14
f1 (ppm)

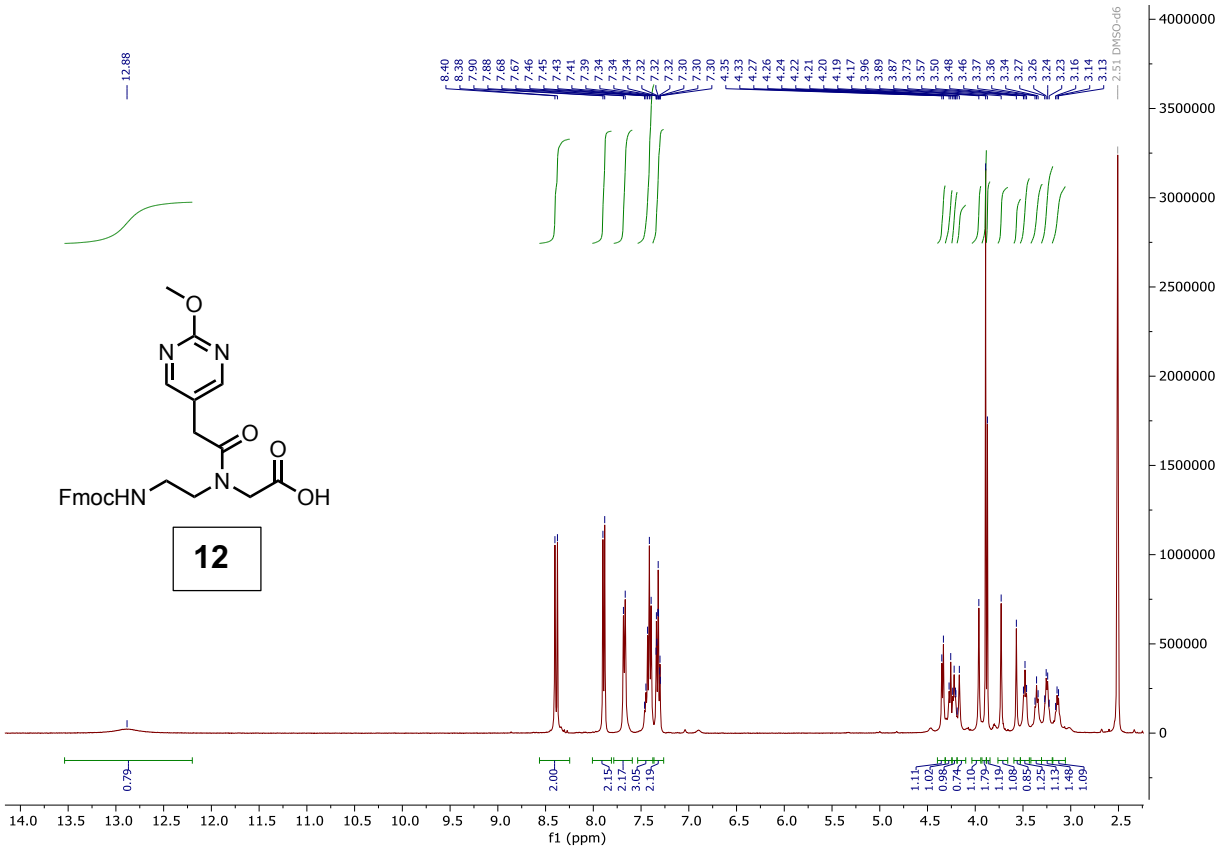

170.29
164.94
159.61
120.96
77.09 CDCl3
61.38
54.90
34.98
14.10
f1 (ppm)

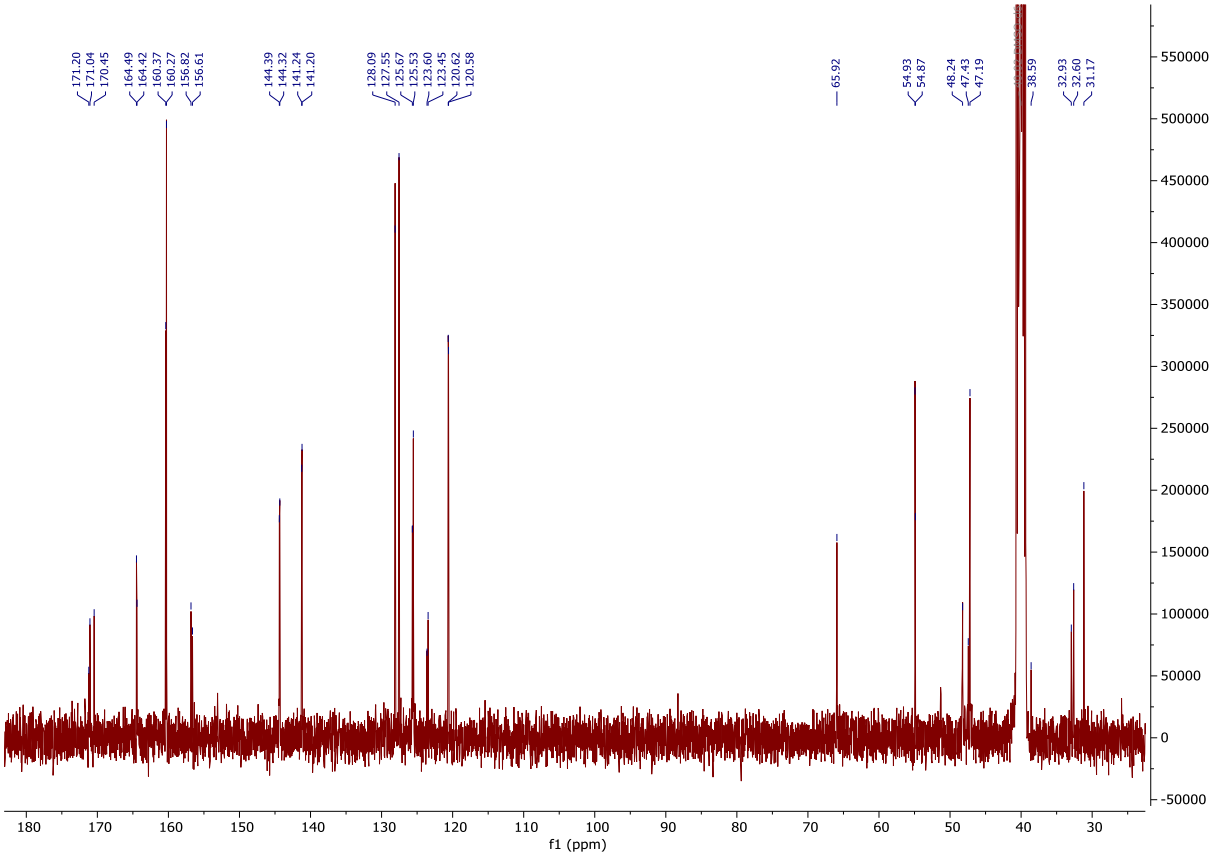
34

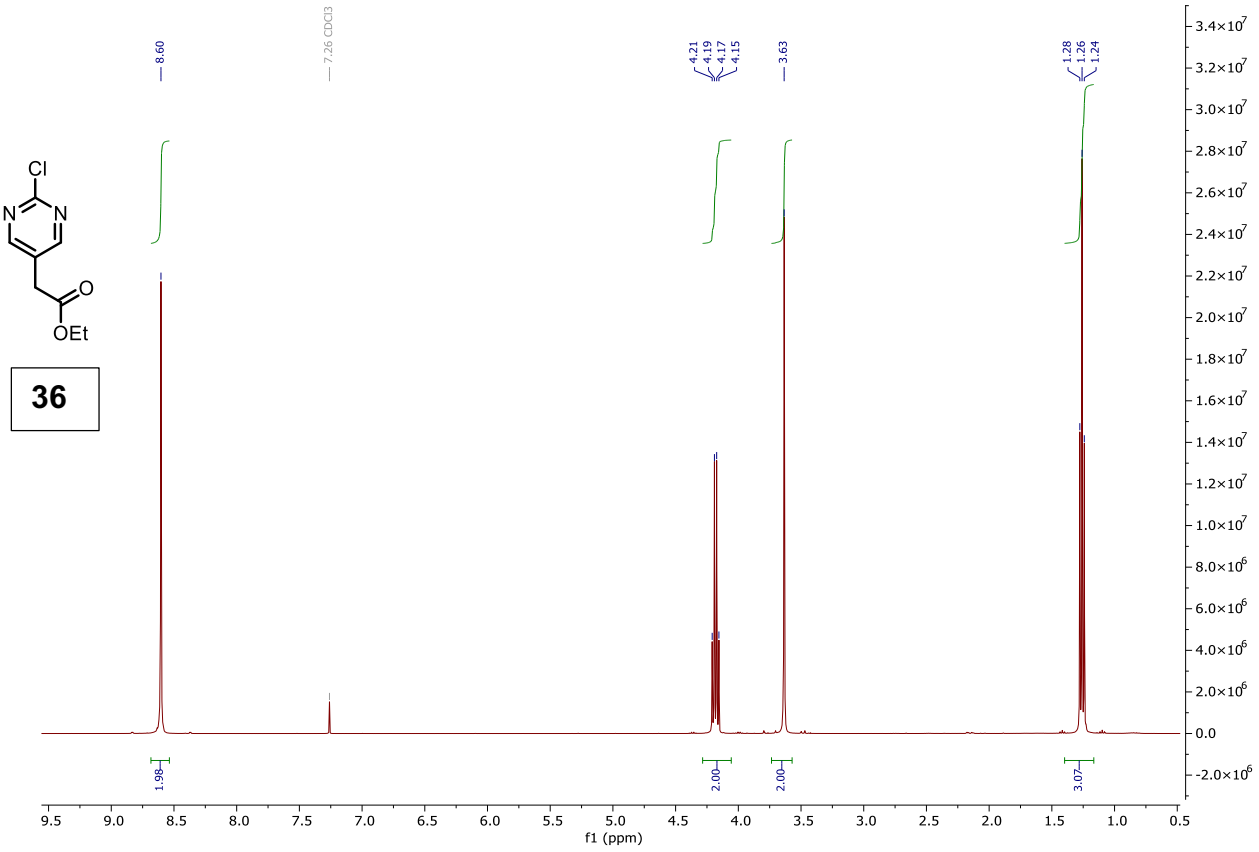

12.57
8.48
3.90
3.60
2.50 DMSO-d6
0.94
1.84
2.92
2.00
f1 (ppm)

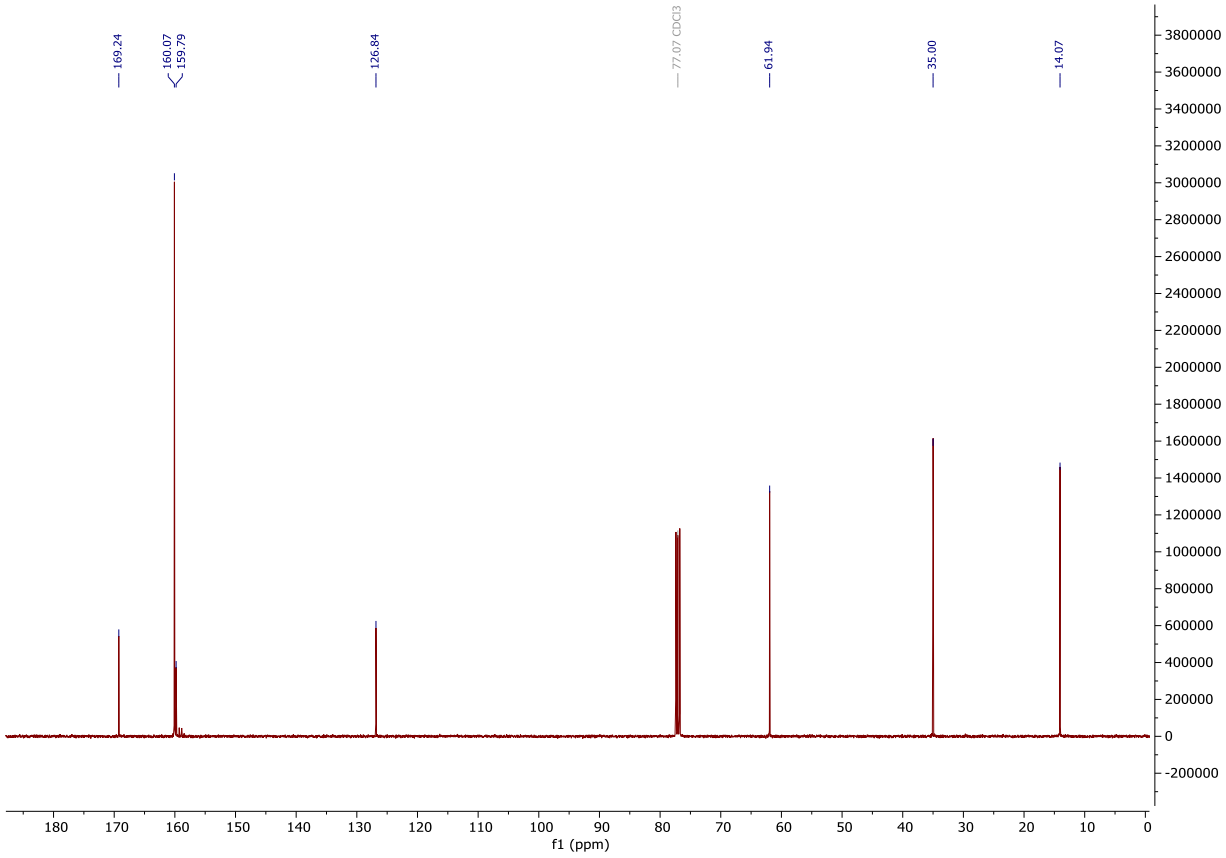
35

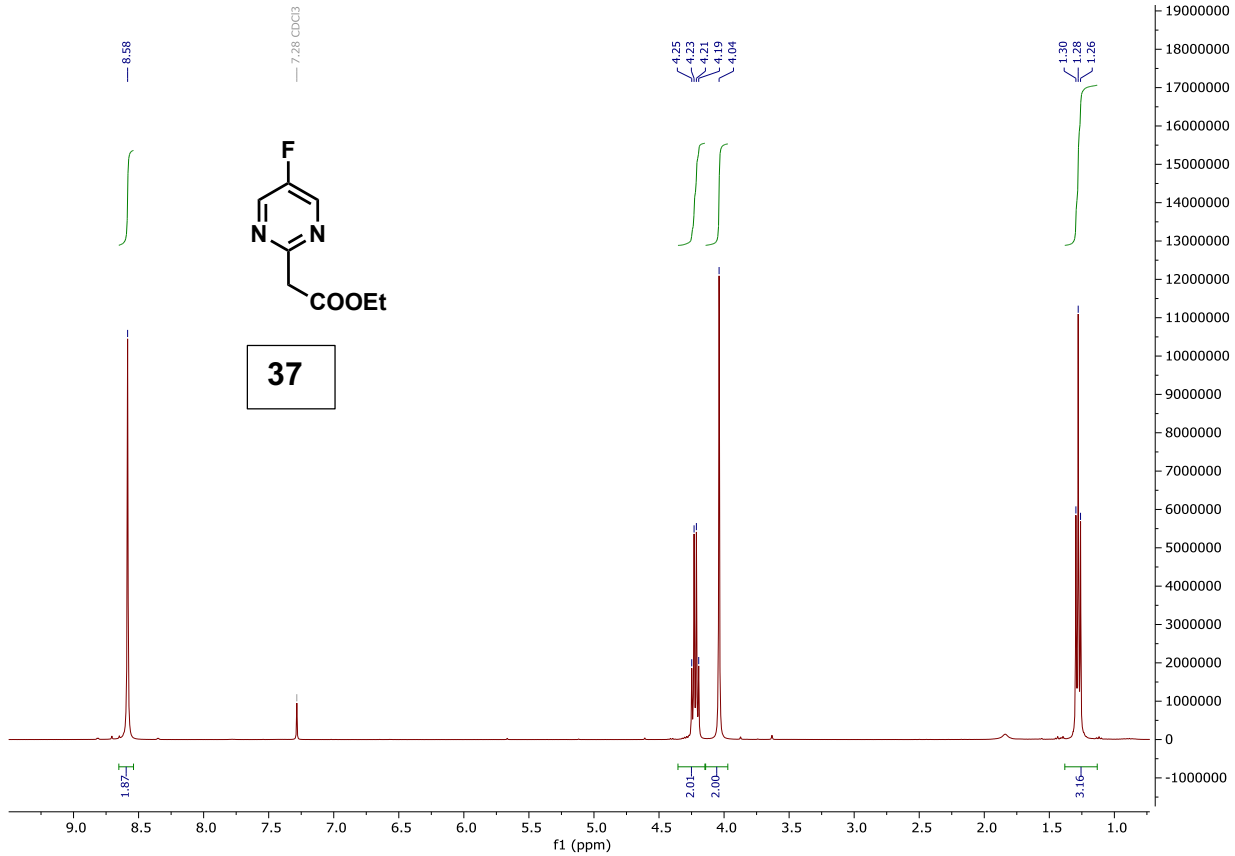

172.65
164.59
160.45
122.83
54.97
39.52
34.36
f1 (ppm)

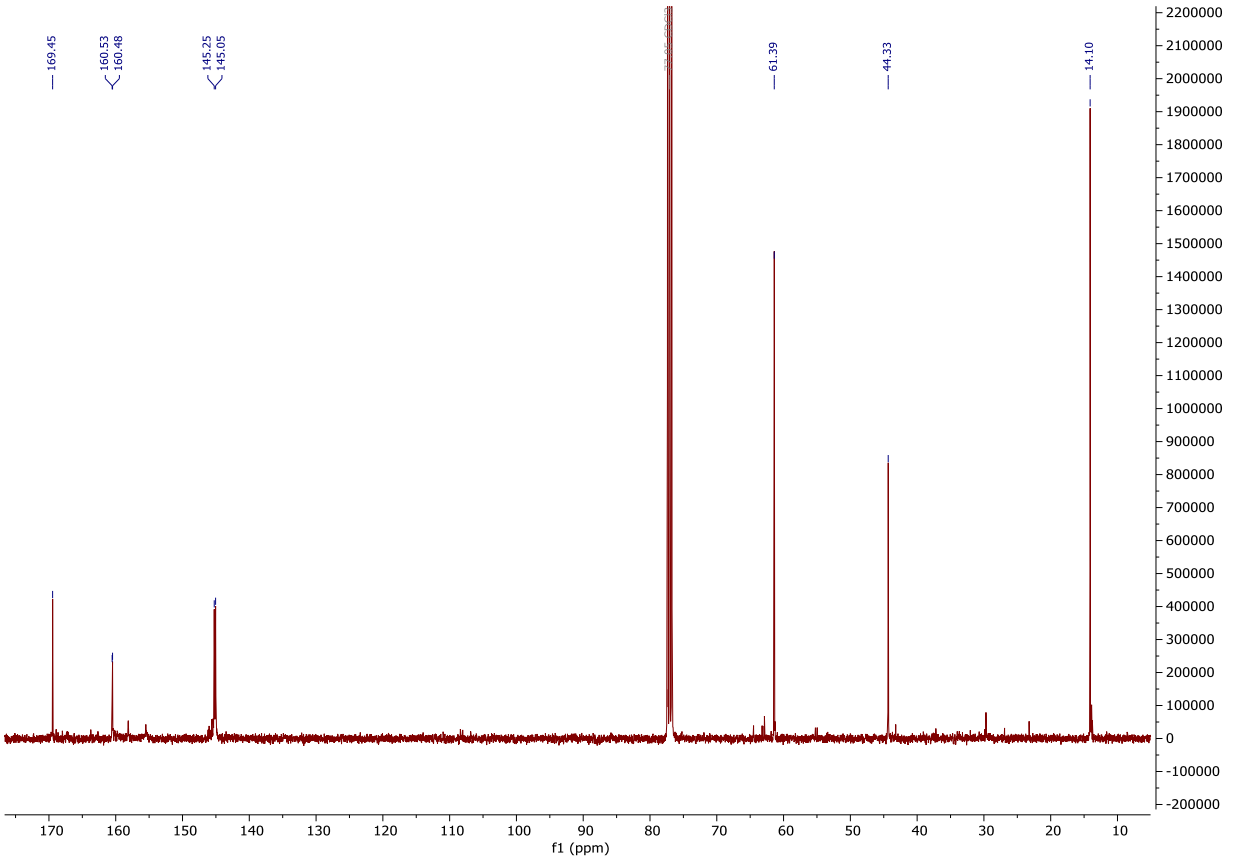

12.88
2.51 DMSO-d6
FmocHN
OH
12
f1 (ppm)

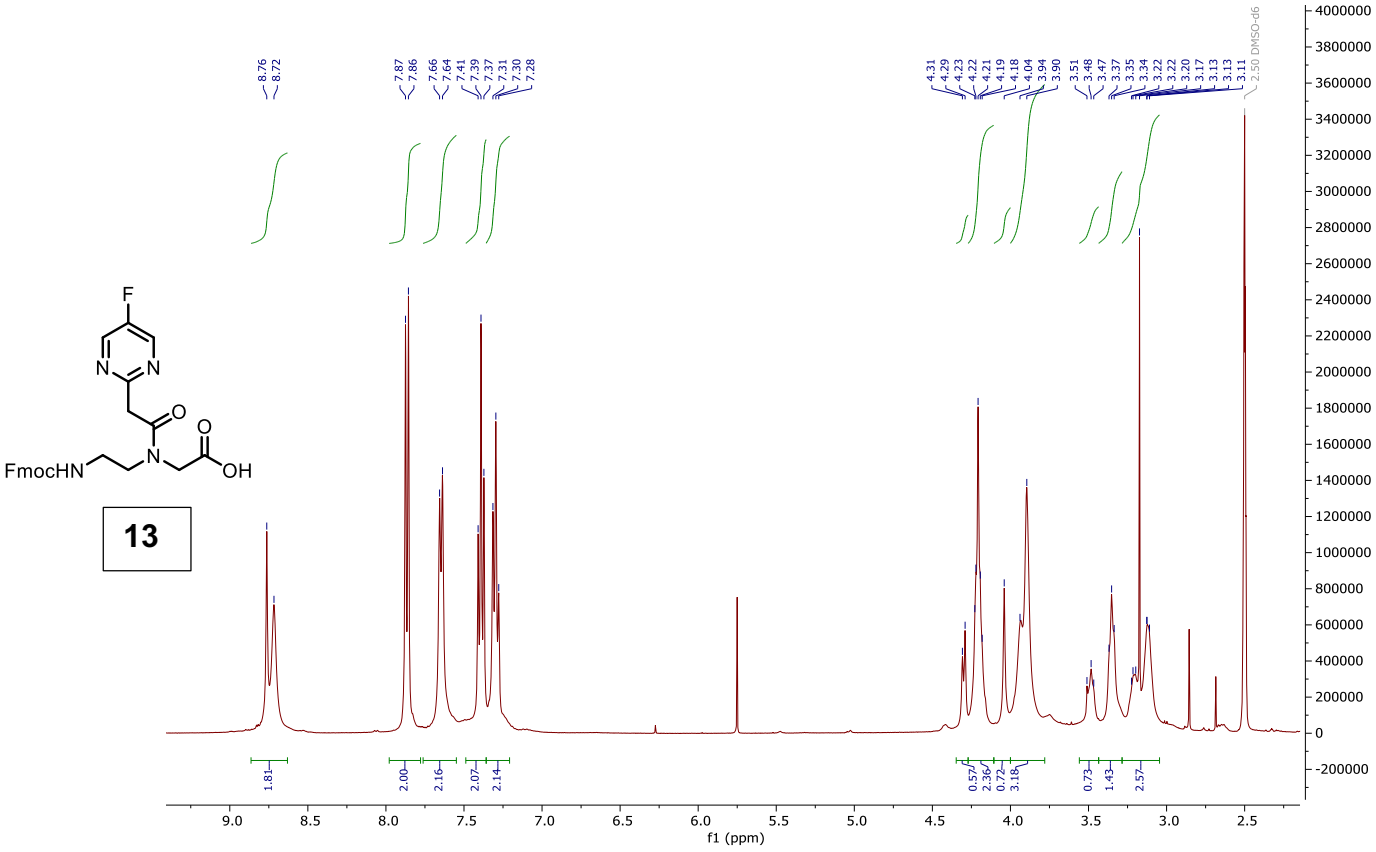

f1 (ppm)

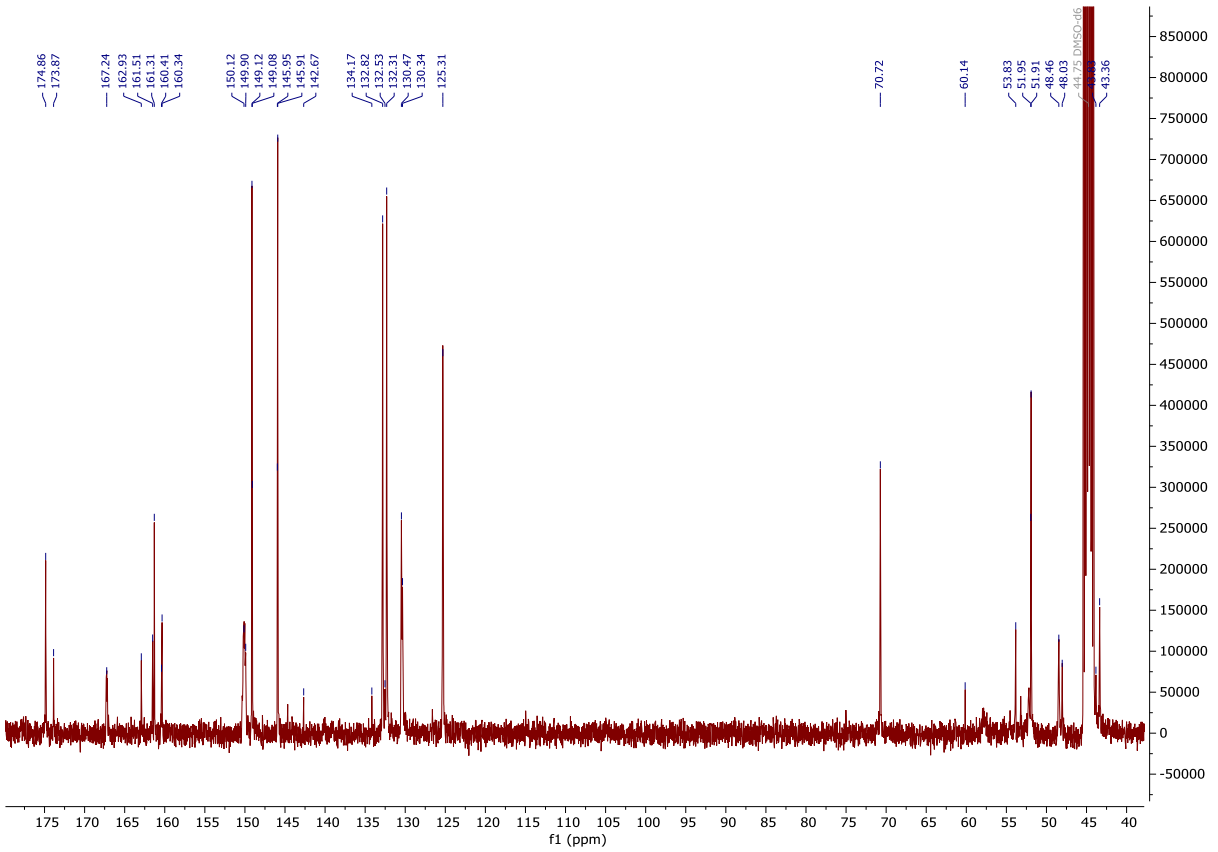

Cl
N
N
O
OEt
36
8.60
7.26 CDCl3
4.21
4.19
4.17
4.15
3.63
1.28
1.26
1.24
1.98
2.00
2.00
3.07
f1 (ppm)


169.24
160.07
159.79
126.84
77.07 CDCl3
61.94
35.00
14.07
f1 (ppm)


8.58
7.28 CDCl3
4.25
4.23
4.21
4.19
4.04
1.30
1.28
1.26
F
N
N
COOEt
37
1.87
2.01
2.00
3.16
f1 (ppm)


169.45
160.53
160.48
145.25
145.05
77.16
77.00
76.85
61.39
44.33
14.10
f1 (ppm)


F
N
N
O
O
N
FmocHN
OH
13
8.76
8.72
7.87
7.86
7.66
7.64
7.41
7.39
7.37
7.31
7.30
7.28
4.31
4.29
4.23
4.22
4.21
4.19
4.18
4.04
3.94
3.90
3.51
3.48
3.47
3.37
3.35
3.34
3.22
3.22
3.20
3.17
3.13
3.13
3.11
2.50 DMSO-d6
1.81
2.00
2.16
2.07
2.14
0.57
2.36
0.72
3.18
0.73
1.43
2.57
f1 (ppm)


174.86
173.87
167.24
162.93
161.51
161.31
160.41
160.34
150.12
149.90
149.12
149.08
145.95
145.91
142.67
134.17
132.82
132.53
132.31
130.47
130.34
125.31
70.72
60.14
53.83
51.95
51.91
48.46
48.03
44.75 DMSO-d6
43.36
f1 (ppm)