Supporting Information

2-Guanidyl Pyridine PNA Nucleobase for Triple-Helical Hoogsteen Recognition of Cytosine 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. Tetrahydrofuran (THF) and dichloromethane (DCM) were dried by passing over activated alumina. Pyridine and diisopropylethylamine (DIPEA) were dried by refluxing over CaH_2 followed by distillation. Reactions were carried out under an atmosphere of nitrogen using a Schlenk line unless otherwise states. Analytical thin layer chromatography (TLC) used Silacycle 60 Å silica gel F254 plates (0.25 mm) and visualization was aided by UV light, iodine, or ninhydrin stain. Column chromatography used Silacycle P60 230−400 mesh silica gel as the stationary phase. NMR spectra were obtained using Bruker AM 400 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. LC/MS analysis was performed using Shimadzu LCMS 2020 system with an ESI ionization source using a gradient of 0-100% MeCN/H_2O (0.1% formic acid) in 18 minutes. For compounds 1, 3, and 4, a MeCN/H_2O without formic acid was used to avoid Boc-deprotection. The column used for all small molecules was a Cosmosil 2.5 C18-MS-II, 120 Å, 2.5 µm, 2.0 x 50 mm.

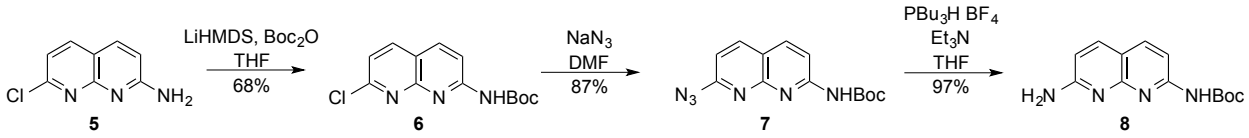
Synthesis of PNA monomers

5-Oxo-5-((6-((2,2,10,10-tetramethyl-4,8-dioxo-3,9-dioxa-5,7-diazaundecan-6-ylidene)amino)pyridin-2-yl)amino)pentanoic acid (1). 2,6-Diaminopyridine (109 mg, 1 mmol) was added to round bottom flask, purged with nitrogen gas, and dissolved in dry pyridine (2.5 mL, 0.4 M). Glutaric anhydride (110 mg, 0.96 mmol) was added and the solution was heated at 50 °C for 6 hours. The solution was cooled to room temperature, 1,3-di-Boc-2-trifluoromethylsulfonyl guanidine (391 mg, 1 mmol) was added, the vessel

was purged with nitrogen, and heated at 45 °C for 18 hours. Pyridine was partially removed by rotary evaporation and the residual oil was redissolved in dichloromethane (5 mL) and washed with 5% aqueous AcOH (2.5 mL) and deionized water (5 mL) to remove remaining pyridine. The organic layer was separated, dried over Na_2SO_4, and concentrated. The residue was purified via silica gel chromatography (0-8% MeOH/DCM) to afford the title compound **1** as a white foam (197 mg ,42%). R_f = 0.15 in 5% MeOH/DCM. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.55 (18H, s), 2.08 (2H, p, J = 6.8 Hz), 2.53 (2H, t, J = 6.3 Hz), 2.65 (2H, t, J = 7.6 Hz), 7.77 (1H, t, J = 8.2 Hz), 8.05 (2H, m), 9.71 (1H, s) 10.6-11.6 (2H, m). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.1, 28.1, 32.4, 35.9, 110.3, 112.4, 141.4, 148.4, 150.2, 153.0, 171.8, 188.7. Mass spec (ESI) m/z: $[M+H]^+$ calcd for $C_{21}H_{31}N_5O_7$ 466.5; found 466.2.

Benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-((6-((2,2,10,10-tetramethyl-4,8-dioxo-3,9-dioxa-5,7-diazaundecan-6-ylidene)amino)pyridin-2-yl)amino)pentanoyl)glycinate (3). 5-Oxo-5-((6-((2,2,10,10-tetramethyl-4,8-dioxo-3,9-dioxa-5,7-diazaundecan-6-ylidene)amino)pyridin-2-yl)amino)pentanoic acid **1** (250 mg, 0.54 mmol) and EDC·HCl (230 mg, 0.54 mmol) were added to a round bottom flask. The vessel was purged with nitrogen gas and charged with 1:1 mixture of dry dimethyl formamide (DMF)/pyridine (2.5 mL, 0.2M). The reaction mixture was stirred at room temperature until solids dissolve (~1 hour), cooled to 0°C, and PNA backbone **2**[1] (230 mg, 0.53 mmol) was added. The reaction mixture was allowed to warm up to room temperature and stirred for 6 hours. The reaction mixture was diluted with ethyl acetate and washed with 5% aqueous AcOH (5 mL) and deionized water (5 mL) to remove remaining pyridine. The organic layer was separated, dried over Na_2SO_4, and concentrated. The residue was purified via silica gel chromatography (50-75% ethyl acetate/hexanes) to afford the title compound **3** as a white foam (362 mg ,77%). R_f = 0.3 in 60% ethyl acetate/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.55 (18H, s), 2.04 (2.3H, m), 2.37 (2.7H, m), 2.48 (1.1H, t) 2.8-3.1 (0.5H, bm), 3.37 (1.8H, m), 3.55 (1.7H, m) 3.85 (0.3H, bs), 4.09-4.20 (2.8H, m), 4.40 (1.7H, d), 4.62 (0.2H, bs), 5.16-5.22 (2.0H, d), 5.57 (0.3H, t), 5.83 (0.5H, bs) 7.27-7.41 (9.1H, m), 7.57-7.60 (2.0H, d), 7.65 (0.9H, q, J = 8.3 Hz), 7.74 (2.0H, t, J = 7.6 Hz), 7.87 (1.1H, d, J = 7.9 Hz), 7.94 (0.7H, bs), 8.10 (1.0H, bs), 10.6 (0.9H, bs), 11.5 (0.9H, bs). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.4, 20.7, 24.7, 28.1, 31.3, 31.7, 36.1, 36.3, 36.7, 39.4, 47.2, 47.3, 47.9, 48.9, 49.2, 50.7, 66.7, 66.9, 67.3, 67.6, 80.1, 84.0, 109.5, 109.6, 111.5, 119.9, 120.0, 125.0, 125.1, 127.0, 127.1, 127.6, 127.7. 128.4, 128.5, 128.7, 128.7, 134.9, 135.2, 140.6, 141.3, 143.8, 144.0, 149.5, 152.8, 156.6, 156.7, 169.5, 170.2, 171.2, 173.1, 173.6. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **3** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{47}H_{55}N_7O_{10}$ 879.0; found 878.3.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-((6-((2,2,10,10-tetramethyl-4,8-dioxo-3,9-dioxa-5,7-diazaundecan-6-ylidene)amino)pyridin-2-yl)amino)pentanoyl)glycine (4). Prior to the reaction, dry THF was degassed by purging with nitrogen gas for 30 minutes. A round bottom flask was charged with benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-((6-((2,2,10,10-tetramethyl-4,8-dioxo-3,9-dioxa-5,7-diazaundecan-6-ylidene)amino)pyridin-2-yl)amino)pentanoyl)glycinate **3** (362 mg, 0.41 mmol) and 10% Pd/C (75 mg, 20 wt%) and purged with nitrogen gas. Degassed THF (1.25 mL, 0.33M) was added followed by purging the mixture with H_2 gas using a balloon for 3 minutes. The reaction was stirred at high speed under an H_2 atmosphere (balloon) for 3 hours. Once complete, the reaction mixture was diluted with methanol and filtered through a celite plug to remove the Pd/C. The solvent was removed via rotary evaporation to give pure **4** (316 mg, 95%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.54 (18H, m), 1.98-2.04 (2.3H, bs), 2.37-2.45 (3.8H, m), 3.41-3.58 (3.4H, m), 3.87-3.96 (1.8H, m), 4.16-4.23 (1.0H, m), 4.33 (1.4H, d), 4.43 (0.2H, bs), 4.57 (0.2H, bs), 6.40 (0.3H, bs), 6.52 (0.2H, bs), 6.69 (0.5H, bs), 7.01 (0.2H, bs), 7.23-7.31 (2.5H, m), 7.32-7.39 (2.1H, t, J = 7.4 Hz), 7.53-7.65 (3.1H, m), 7.73 (2.0H, d, J = 7.3 Hz), 7.85 (1.0H, d, J = 7.8 Hz), 8.06 (1H, d), 8.37-8.41 (0.7H, m), 8.53-8.64 (0.3H, m) 10.56 (0.8H, bs), 11.53 (0.8H, bs). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.6, 22.4, 22.6, 28.1, 31.6, 32.1, 36.1, 36.6, 39.2, 39.7, 44.6, 47.2, 47.2, 49.7, 51.1, 53.4, 66.7, 66.9, 80.1, 83.9, 109.7, 111.4, 119.9, 120.0, 125.1, 127.0, 127.7, 140.5, 141.2, 143.9, 144.1, 148.6, 149.7, 152.7, 156.7, 156.8, 171.4, 171.9, 173.5, 174.1, 175.0. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **4** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{40}H_{49}N_7O_{10}$ 788.8; found 788.3.



Tert-butyl (7-chloro-1,8-naphthyridin-2-yl)carbamate (6). In an oven dried round bottom flask, 7-chloro-1,8-naphthyridin-2-amine[1] **5** (250 mg, 1.39 mmol) was dissolved in THF (4.2 mL, 0.33 M). The flask was purged with nitrogen gas and cooled to -78 °C in a dry ice/acetone bath. 2 M NaHMDS in THF (1.39 mL, 2 eq) was added. After 5 minutes of stirring, a solution of Boc_2O (303 mg, 1 eq) in THF (1.0 mL)

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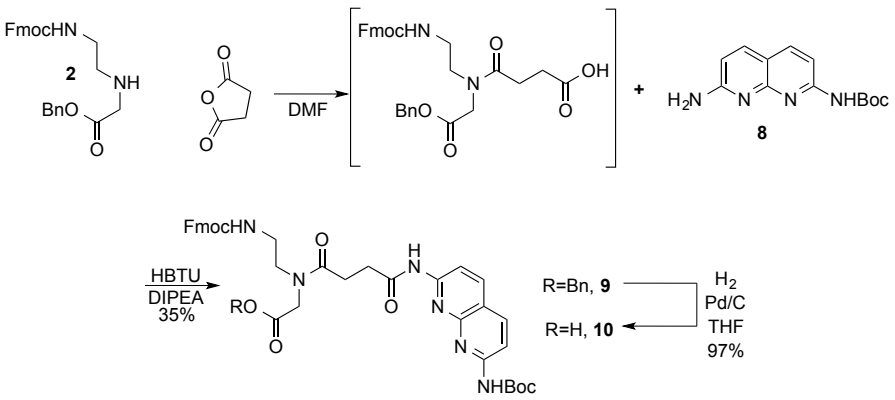
was added and the reaction mixture was stirred for 18 hours. The solution was partially concentrated and diluted with DCM. The organic layer was then washed with 5% aqueous AcOH (3 mL), saturated aqueous $NaHCO_3$ (3 mL), water (3 mL), and brine (3 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated. The resulting solid was purified via silica gel chromatography (10-50% ethyl acetate/hexanes) to afford the title compound **6** as a white solid (197 mg, 65%). R_f = 0.25, 20% ethyl acetate/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.58 (9H, s), 7.38 (1H, d, J = 8.3 Hz), 7.62 (1H, s), 8.06 (1H, d, J = 8.4 Hz), 8.15 (2H, d, J = 8.9 Hz), 8.37 (1H, d, J = 8.9 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 28.2, 82.2, 113.9, 118.6, 121.6, 138.7, 152.0, 154.2, 154.8. Mass spec (ESI) m/z: $[M+H]^+$ calcd for $C_{13}H_{14}ClN_3O_2$ 280.1 and 282.1; found 280.1 and 282.1.

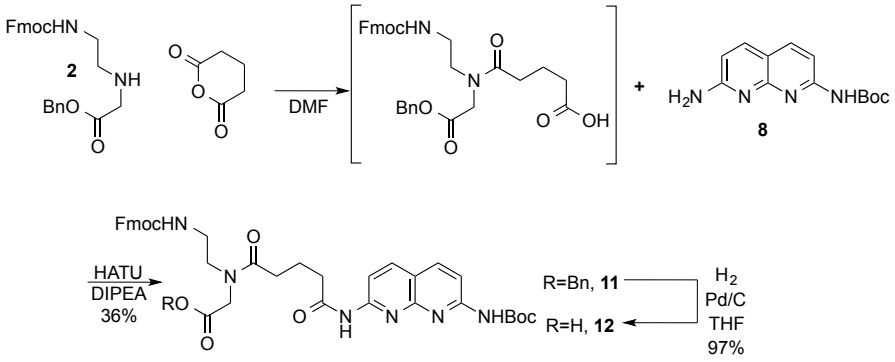
Tert-butyl (7-azido-1,8-naphthyridin-2-yl)carbamate (7). Tert-butyl (7-chloro-1,8-naphthyridin-2-yl)carbamate **6** (264 mg, 0.94 mmol) and sodium azide (67 mg, 1.03 mmol) were combined in a round bottom flask. DMF (2.3 mL, 0.4 M) was added to the flask and the reaction mixture was heated to 60 °C for 18 hours. After 18 hours, solvent was removed by rotary evaporation and the resulting oil was purified by silica gel column chromatography (20-50% ethyl acetate/hexanes) to afford the tittle compound **7** as a pale yellow solid (235 mg, 87%). R_f = 0.12 in 20% ethyl acetate/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.54 (9H, s), 7.88 (2H, q, J = 16.7, 9.2 Hz), 8.24 (1H, s), 8.31 (1H, d, J = 8.9 Hz), 8.46 (1H, d, J = 8.8 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 28.1, 82.5, 111.3, 113.4, 114.5, 132.0, 139.9, 140.6, 149.4, 151.7, 153.5. Mass spec (ESI) m/z: $[M+H]^+$ calcd for $C_{13}H_{14}N_6O_2$ 287.3; found 287.2.

Tert-butyl (7-amino-1,8-naphthyridin-2-yl)carbamate (8). Tert-butyl (7-azido-1,8-naphthyridin-2-yl)carbamate **7** (223 mg, 0.78 mmol) and tri-n-butylphosphonium tetrafluoroborate (253 mg, 0.87 mmol) were combined in a round bottom flask. The solids were dissolved in 9:1 mixture of THF/water (3 mL 0.25 M) followed by the addition of triethylamine (121 µL, 0.87 mmols). The reaction was heated to 65 °C for 6 hours. The reaction was diluted with methanol, filtered through a 3 cm celite plug, and concentrated. The compound was purified by silica gel chromatography (2-6% MeOH/DCM, containing 0.1% Et_3N) to afford the tittle compound **8** as a white solid (198 mg, 97%). R_f = 0.35 in 5% MeOH/DCM. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.59 (9H, s), 6.82 (1H, d, J = 8.7 Hz), 7.81 (1H, d, J = 8.8 Hz), 7.93 (2H, m), 8.45 (1H, bs). ^{13}C NMR (101 MHz, $CDCl_3$) δ:28.2, 82.0, 109.6, 111.4, 113.7, 138.5, 138.8, 152.6, 153.6, 154.0, 159.3. Mass spec (ESI) m/z: $[M+H]^+$ calcd for $C_{13}H_{16}N_4O_2$ 261.3; found 261.2.



Benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(4-((7-((tert-butoxycarbonyl)amino)-1,8-naphthyridin-2-yl)amino)-4-oxobutanoyl)glycinate (9). Benzyl (2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)glycinate **2** (233 mg, 0.5 mmol) and succinic anhydride (55 mg, 0.55 mmol) were combined in a round bottom flask. The flask was purged with nitrogen gas and cooled to 0 °C. DMF (2 mL, 0.25 M) added followed by slow addition of distilled triethylamine (153 µL, 1.1 mmol). The reaction mixture was stirred for 18 hours. HBTU (180 mg, 0.47 mmol) was added, and solution was stirred for 30 minutes. The reaction was cooled to 0 °C and tert-butyl (7-amino-1,8-naphthyridin-2-yl)carbamate **8** was added followed by slow addition of diisopropylethylamine (187 µL, 1.1 mmol). The reaction was warmed to room temperature and stirred for 18 hours. The reaction was diluted with ethyl acetate (5 mL) and washed with 5% aqueous AcOH (5 mL), water (5 mL), and brine (5 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated. The resulting oil was then purified by silica gel chromatography (50-100% ethyl acetate/hexanes then 1% MeOH/ethyl acetate) to afford the title compound **9** as a white solid (134 mg, 35%). R_f = 0.2 in 70% ethyl acetate/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39-1.45 (4.7H, s), 1.55-1.61 (6.5H, s), 2.63-2.73 (1.2H, m), 2.83 (1.6H, s), 2.91 (0.1H, s), 2.98 (0.1H, s), 3.07 (1.1H, m), 3.40 (0.9H, m), 3.45 (0.8H, q), 3.51 (0.2H, d, J = 5.4 Hz), 3.63 (1.4H, m), 3.73 (0.7H, t), 3.97 (0.4H, s), 4.14 (0.7H, s), 4.22-4.29 (1.1H, m), 4.34 (0.6H, s), 4.42 (0.7H, d, J = 7.1 Hz), 4.48 (0.7H, d, J = 7.5 Hz), 4.66 (0.4H, d, J = 5.5 Hz), 5.21 (2.0H, m), 7.18 (0.8H, t, J = 7.3 Hz), 7.25-7.38 (7.2H, m), 7.55 (0.7H, d, J = 7.0 Hz), 7.60 (1.2H, d, J = 7.4 Hz), 7.70-7.79 (2.2H, m), 7.91-8.06 (1.9H, m), 8.18-8.30 (1.3H, m), 8.39 (0.5H, t, J = 8.9 Hz), 8.77 (0.4H, bs), 9.71 (0.3H, bs), 10.05 (0.1H, bs). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 28.1, 28.2, 32.2, 32.3, 47.2, 47.3, 49.1, 49.3, 66.6, 67.3, 67.6, 81.7, 112.0, 112.2, 113.1, 120.0, 125.1, 125.3, 126.9, 127.1, 127.6, 127.7, 128.5, 128.7, 134.9, 138.5, 138.7, 141.3, 143.9, 152.3, 154.4, 154.7, 156.8, 169.2, 173.3. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **9** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{43}H_{44}N_6O_8$ 773.9; found 773.4.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(4-((7-((tert-butoxycarbonyl)amino)-1,8-naphthyridin-2-yl)amino)-4-oxobutanoyl)glycine (10). THF was degassed via bubbling nitrogen for 1 hour prior to reaction. Benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(4-((7-((tert-butoxycarbonyl)amino)-1,8-naphthyridin-2-yl)amino)-4-oxobutanoyl)glycinate **9** (130 mg, 0.17 mmol) and 10% Pd/C (24 mg, 20 wt%) were combined in a round bottom flask. The flask was purged with nitrogen gas and THF (1 mL, 0.17 M) was added via syringe. The flask was purged with hydrogen gas and the reaction mixture was stirred under a hydrogen atmosphere (balloon) for 4 hours. The reaction mixture was diluted with methanol (1 mL) and filtered through a 3 cm celite plug to remove Pd/C. After filtration, solvent was removed by rotary evaporation giving pure PNA monomer **10** as a white foam (112 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45-1.52 (9H, s), 2.29 (0.3H, s), 2.50 (0.2H, bs), 2.70-2.96 (2.7H, m), 2.99-3.13 (0.6H, bs), 3.21-3.39 (0.7H, s), 3.47 (1.1H, bs), 3.60 (1.0H, bs), 3.81-4.34 (3.2H, m), 4.58 (0.4H, m), 6.50-6.91 (0.5H, bs), 7.00 (0.2H, s), 7.19 (1.4H, m), 7.26-7.37 (1.14H, m), 7.46-7.57 (1.6H, m), 7.61-7.84 (3.2H, m), 8.02-8.22 (1.6H, m), 10.56 (0.5H, bs). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 28.2, 30.3, 31.9, 32.4, 39.2, 39.4, 47.1, 48.5, 49.6, 50.7, 53.4, 56.0, 66.7, 67.0, 81.6, 112.1, 113.2, 116.5, 119.8, 119.8, 125.1, 125.2, 125.5, 127.0, 127.6, 128.3, 135.8, 138.4, 141.1, 141.3, 143.9, 151.5, 152.4, 154.1, 154.6, 156.8, 172.6, 173.5, 173.7, 174.7. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **3** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{36}H_{38}N_6O_8$ 683.7; found 683.3.

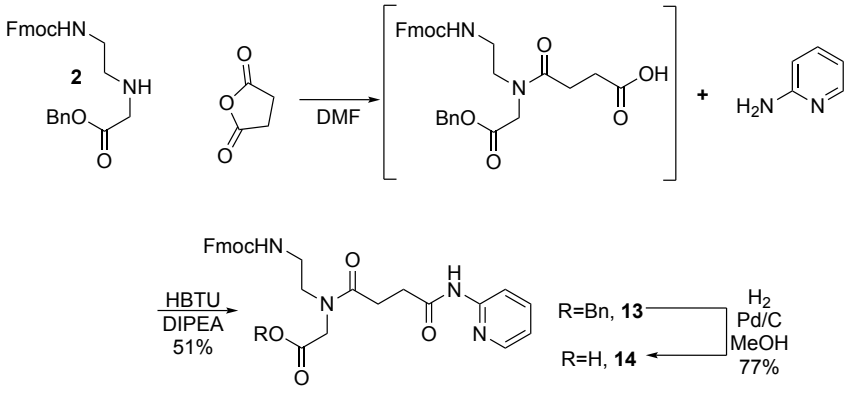


Benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-((7-((tert-butoxycarbonyl)amino)-1,8-naphthyridin-2-yl)amino)-5-oxopentanoyl)glycinate (11). Benzyl (2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)glycinate **2** (230 mg, 0.49 mmol) and glutaric anhydride (62 mg, 0.58 mmol) were combined in a round bottom flask. The flask was purged with nitrogen gas and cooled to 0° C. DMF (2 mL, 0.25 M) was added followed by slow addition of distilled diisopropylethylamine (83 µL,

0.48 mmol). The reaction was stirred for 18 hours. HATU (220 mg, 0.58 mmol) was added, and the solution was stirred for 30 minutes. The reaction was cooled to 0 °C and tert-butyl (7-amino-1,8-naphthyridin-2-yl)carbamate **8** was added followed by slow addition of diisopropylethylamine (187 µL, 0.96 mmol). The reaction was warmed to room temperature and stirred 18 hours. The reaction was diluted with ethyl acetate (5 mL) and washed with 5% aqueous AcOH (5 mL), water (5 mL), and brine (5 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated. The resulting oil was then purified by silica gel chromatography (50-100% ethyl acetate/hexanes then 1% MeOH/ethyl acetate) to afford the title compound **11** as a white solid (135 mg, 36%). R_f = 0.2 in 70% ethyl acetate/hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.53 (9H, m), 2.08 (2.1, m), 2.25-2.39 (1.0H, m), 2.40-2.58 (2.7H, m), 2.65 (0.6H, t), 3.04 (0.3H, bd), 3.26-3.53 (2.2H, m), 3.55-3.65 (1.5H, m), 3.80 (0.2H, bs), 3.89 (0.2H, bs), 4.11 (1.1H, s), 4.13-4.27 (1.6H, m), 4.40 (1.5H, t), 4.67 (0.3H, m), 5.05 (0.4H, s), 5.19 (2.1H, m), 7.28-7.42 (8.5H, m), 7.58 (2.5H, d, J = 7.4 Hz), 7.73 (1.9H, d, J = 7.4 Hz), 7.84-7.93 (0.3H, m), 7.96-8.10 (2.0H, m), 8.17-8.39 (2H, m), 8.59 (0.5H, bs), 8.84 (0.2H, bs), 8.90 (0.2H, bs), 9.04 (0.1H, bs). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.7, 28.2, 31.2, 32.6, 36.5, 39.4, 39.6, 47.2, 48.0, 49.0, 49.2, 50.9, 66.7, 66.9, 67.3, 67.6, 79.2, 81.8, 112.1, 113.1, 117.6, 119.9, 120.0, 125.0, 125.2, 127.0, 127.6, 127.7, 128.4, 128.5, 128.6, 128.7, 128.7, 129.0, 134.9, 135.2, 138.6, 138.8, 141.3, 143.8, 143.9, 152.2, 153.6, 153.8, 153.8, 153.9, 156.6, 170.2, 171.9, 173.1, 173.7. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **11** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{44}H_{46}N_6O_8$ 787.9; found 787.5.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-((7-((tert-butoxycarbonyl)amino)-1,8-naphthyridin-2-yl)amino)-5-oxopentanoyl)glycine (12). THF was degassed via bubbling nitrogen for 1 hour prior to reaction. Benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-((7-((tert-butoxycarbonyl)amino)-1,8-naphthyridin-2-yl)amino)-5-oxopentanoyl)glycinate **11** (135 mg, 0.17 mmol) and 10% Pd/C (25 mg, 20 wt%) were combined in a round bottom flask. The flask was purged with nitrogen gas then THF (0.5 mL, 0.34 M) was added via syringe. The flask was purged with hydrogen gas, and the reaction mixture was stirred under a hydrogen atmosphere (balloon) for 4 hours. The reaction mixture was diluted with methanol (0.5 mL) and filtered through a 3 cm celite plug to remove Pd/C. After filtration, solvent was removed by rotary evaporation giving pure PNA monomer **12** as a white foam (118 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.43-1.57 (9H, m), 1.84-2.24 (2.6H, m), 2.29 (0.4H, s), 2.42-2.72 (3.5H, m), 3.04 (0.5H, d), 3.21 (0.4H, bs), 3.36-3.67 (3.0H, m), 3.92-4.25 (2.9H, m), 4.31 (1.3H, m), 4.60 (0.5H, d), 6.23 (0.5H, bd), 6.67 (0.3H, bd), 7.00 (0.2H, s), 7.21 (1.2H, t), 7.26-7.41 (4.6H, m), 7.50

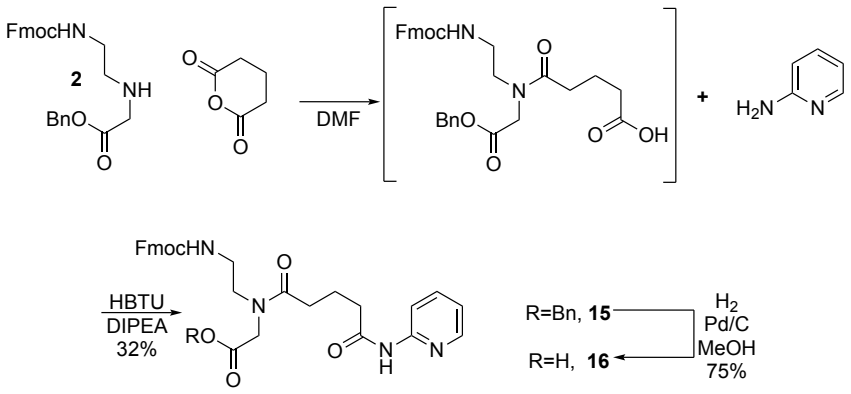
(1.7H, d), 7.59-7.77 (2.4H, m), 7.78-7.88 (0.8H, m), 7.89-7.98 (0.9H, m), 8.04 (0.3H, bs), 8.16 (1.0H, t), 8.27 (0.8H, bs), 10.39 (0.7H, bs). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.5, 28.2, 30.3, 31.2, 34.2, 36.3, 39.6, 47.2, 48.6, 49.5, 50.0, 52.0, 53.4, 66.7, 81.8, 82.0, 112.1, 112.3, 113.4, 113.6, 116.8, 119.8, 119.9, 125.0, 125.1, 125.5, 126.9, 127.5, 127.6, 135.8, 138.9, 139.1, 141.1, 143.8, 143.9, 152.3, 154.6, 154.8, 156.6, 156.8, 174.1. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **12** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{37}H_{40}N_6O_8$ 697.8; found 697.5.



Benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(4-oxo-4-(pyridin-2-ylamino) butanoyl)glycinate (13). Benzyl (2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)glycinate **2** (230 mg, 0.49 mmol) and succinic anhydride (62 mg, 0.62 mmol) were combined in a round bottom flask. The flask was purged with nitrogen gas and cooled to 0 °C. DMF (2 mL, 0.25 M) was added followed by slow addition of distilled diisopropylethylamine (104 µL, 0.6 mmol). The reaction mixture was stirred for 18 hours. HBTU (274 mg, 0. 72mmol) was added and the reaction mixture was stirred for 30 minutes. The reaction mixture was cooled to 0°C and 2-aminopyridine (68 mg, 0.72 mmol) was added followed by slow addition of distilled diisopropylethylamine (146 µL, 0.86 mmol). The reaction mixture was warmed to room temperature and stirred 18 hours. The reaction was diluted with ethyl acetate (5 mL) and washed with 5% aqueous AcOH (5 mL), water (5 mL), and brine (5 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated. The resulting oil was then purified by silica gel chromatography (50-100% ethyl acetate/hexanes then 1% MeOH/ethyl acetate) to afford the title compound **13** as a white solid (147 mg, 51%). R_f = 0.55 in ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.60 (0.8H, t), 2.77 (3.8H, m), 3.07 (0.3H, bs), 3.26 (0.3H, bs), 3.38 (1.7H, m), 3.57 (1.6H, bs), 3.87 (0.2H, bs), 4.14 (2.5H, m), 4.32 (0.6H, d, J = 7.2 Hz), 4.39 (0.8H, d, J = 7.0 Hz), 4.62 (0.3H, m), 5.16 (2.0H, m), 5.84 (0.3H, t), 6.01 (0.4H, t), 6.95 (0.8H, t, J = 6.0 Hz), 7.23-7.40 (8.5H, m), 7.57 (2.7H, m), 7.71 (1.8H, d, J = 7.6 Hz), 8.13

(0.9H, t, J = 8.4 Hz), 8.23 (0.9H, m), 8.95 (0.6H, m), 9.12 (0.3H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 27.7, 28.1, 28.4, 32.1, 32.3, 38.6, 39.1, 39.4, 47.2, 48.1, 49.1, 49.2, 50.7, 53.5, 66.7, 67.0, 67.2, 67.7, 114.1, 119.5, 119.9, 120.0, 125.1, 127.1, 127.7, 128.3, 128.5, 128.7, 128.7, 134.9, 135.2, 138.2, 141.3, 143.9, 144.0, 147.7, 147.7, 151.5, 151.6, 156.7 156.8, 169.3, 170.1, 170.9, 171.0, 172.7, 173.3. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **13** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{35}H_{34}N_4O_6$ 607.7; found 607.3.

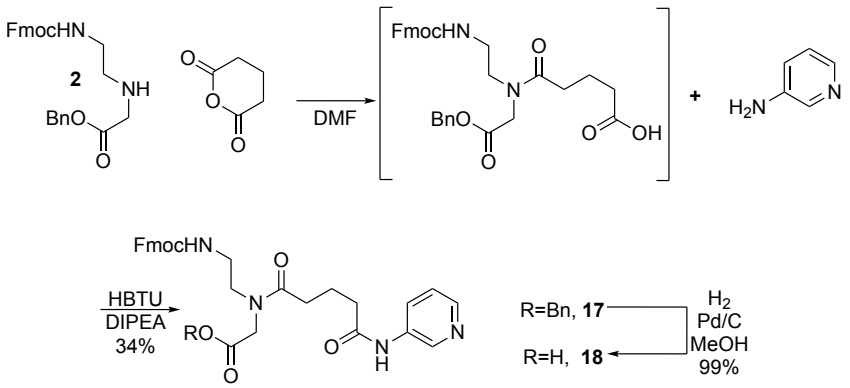
N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(4-oxo-4-(pyridin-2-ylamino)butanoyl) glycine (14). Methanol was degassed via bubbling nitrogen gas for 1 hour prior to reaction. Meanwhile, 10% Pd/C (19 mg, 40 wt%) and benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(4-oxo-4-(pyridin-2-ylamino)butanoyl)glycinate **13** (38 mg, 0.06 mmol) were combined in a round bottom flask. The flask was purged with nitrogen gas and degassed methanol (250 µL, 0.24 M) was added. The reaction mixture was purged with hydrogen gas and stirred under a hydrogen atmosphere (balloon) for 1.5 hours. The reaction mixture was diluted with methanol (250 µL) and filtered through a 3 cm celite plug to remove Pd/C. After filtration, the compound was adsorbed on celite and filtered through a 3 cm silica plug using DCM to 50% MeOH/DCM as eluent. The solvent was then removed, solids were redissolved in DCM, and filtered through 5 cm of celite in a pipette to remove residual silica. Solvent was removed via rotary evaporation affording the tittle compound **14** as a pale yellow waxy solid (24 mg, 77%). R_f = 0.3 in 20% MeOH/DCM. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.38-2.83 (3.7H, m), 3.00 (0.4H, m), 3.20 (0.2H, bs), 3.35 (1.7H, m), 3.50 (1.2H, m), 3.80 (0.3H, bs), 3.88-4.21 (2.2H, m), 4.31 (1.2H, m), 4.54 (0.1H, d), 4.99 (0.2H, s), 5.90 (0.1H, m), 6.05 (0.1H, bs), 6.19 (0.4H, m), 6.97 (1.0H, m), 7.23 (0.9H, m), 7.33 (2.8H, m), 7.54 (1.8H, d, J = 7.3 Hz), 7.69 (2.8H, m), 8.05 (0.9H, m), 8.21 (0.9H, d, J = 8.4 Hz), 10.41 (0.7H, m). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 30.3, 32.0, 32.2, 36.7, 39.5, 47.2, 49.4, 49.8, 66.8, 67.0, 115.1, 119.3, 119.9, 125.1, 127.0, 127.6, 127.7, 128.6, 129.0, 139.9, 141.2, 143.9, 145.2, 151.3, 156.7, 156.8, 171.9, 172.0, 172.7, 173.5, 175.0. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **14** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{28}H_{28}N_4O_6$ 517.6; found 517.3.



Benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-(pyridin-2-ylamino) pentanoyl)glycinate (15). Benzyl (2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)glycinate **2** (230 mg, 0.49 mmol) and glutaric anhydride (61 mg, 0.53 mmol) were combined in a round bottom flask. The flask was purged with nitrogen gas and cooled to 0 °C. DMF (2 mL, 0.25 M) was added followed by slow addition of distilled diisopropylethylamine (80 µL, 0.46 mmol). The reaction mixture was stirred for 18 hours. HBTU (219 mg, 0. 58 mmol) was added and the reaction mixture was stirred for 30 minutes. The reaction was cooled to 0°C and 2-aminopyridine (54 mg, 0.58 mmol) was added followed by slow addition of distilled diisopropylethylamine (125 µL, 0.72 mmol). The reaction was stirred 18 hours allowing for passive warming to room temperature. The reaction was diluted with ethyl acetate (5 mL) and washed with 5% aqueous AcOH (5 mL), water (5 mL), and brine (5 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated. The resulting oil was then purified by silica gel chromatography (50-100% ethyl acetate/hexanes then 1% MeOH/ethyl acetate) to afford the title compound **15** as a clear waxy solid (95 mg, 32%). R_f = 0.65 in 5% MeOH/ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.03 (2.6H, m), 2.14-2.33 (1.6H, m), 2.39 (1.7H, q, J = 6.0 Hz), 2.48 (1H, t, J = 6.6 Hz), 2.79 (0.5H, s), 3.33 (1.9H, m), 3.52 (1.5H, m), 3.81 (0.2H, bs), 4.12 (2.8H, m), 4.37 (1.4H, t, J = 5.8 Hz), 4.60 (0.2H, d), 5.14 (2.0H, m), 5.69 (0.3H, t), 5.89 (0.5H, t), 6.97 (0.8H, m), 7.23-7.39 (8.1H, m), 7.56 (1.8H, m), 7.63 (0.9H, q, J = 7.7 Hz), 7.72 (1.2H, d, J = 7.6 Hz), 8.19 (1.7H, m), 8.68 (0.8H, m). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 20.5, 20.8, 21.1, 28.4, 29.3, 31.3, 31.7, 31.8, 36.1, 36.2, 38.6, 39.4, 39.5, 47.2, 47.3, 47.8, 49.0, 49.2, 50.7, 56.0, 60.4, 66.7, 66.9, 67.3, 67.6, 114.1, 119.6, 119.9, 120.0, 125.0, 125.1, 127.1, 127.7, 127.7, 128.4, 128.5, 128.7, 128.7, 134.9, 135.2, 138.3, 141.3, 143.8, 144.0, 147.7, 151.5, 156.6, 156.7, 169.4, 170.2, 171.5, 173.3, 173.7. Because the NMR spectra were complicated by mixtures of rotamers, the

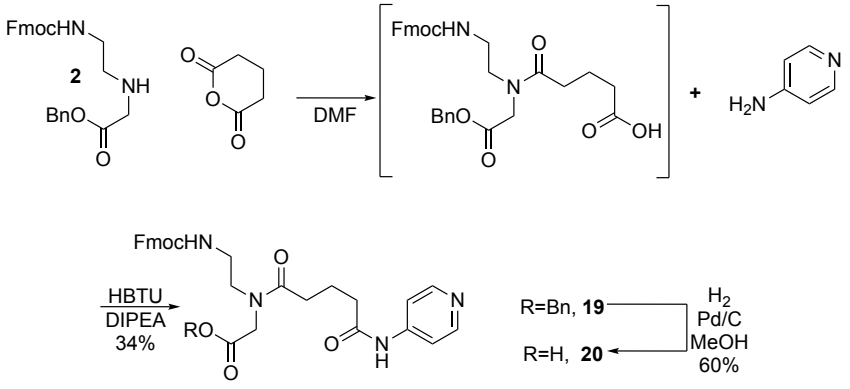
identity and purity of **15** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{36}H_{36}N_4O_6$ 621.7; found 621.3.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-(pyridin-2-ylamino)pentanoyl) glycine (16). Methanol was degassed via bubbling nitrogen gas for 1 hour prior to reaction. Meanwhile, 10% Pd/C (19 mg, 40 wt%) and benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-(pyridin-2-ylamino)pentanoyl)glycinate **15** were combined in a round bottom flask. The flask was purged with nitrogen gas and methanol (250 µL) and DMF (20 µL) were added. The reaction mixture was purged with hydrogen gas and stirred under a hydrogen atmosphere (balloon) for 1.5 hours. The reaction mixture was diluted with of methanol (270 µL) and filtered through a 3 cm celite plug to remove Pd/C. After filtration, the compound was adsorbed on celite and filtered through a 3 cm silica plug using DCM to 50% MeOH/DCM as eluent. The solvent was removed, solids were redissolved in DCM, and the solution was filtered through 5 cm of celite in a pipette to remove residual silica. Solvent was removed via rotary evaporation affording the tittle compound **16** as a pale yellow waxy solid (23 mg, 75%). R_f=0.3 in 20% MeOH/DCM. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.95 (3.0H, bs), 2.37 (4.4H, m), 3.10-3.59 (4.5H, m), 3.94 (2.1H, m), 4.12 (1.1H, m), 4.27 (1.5H, s), 4.45 (0.2H, bs), 6.08 (0.2H, m), 6.34 (0.6H, m), 6.90 (1.0H, m), 7.22 (1.7H, m), 7.30 (2.5H, m), 7.44-7.62 (3.2H, m), 7.67 (2.7H, m), 8.11 (2.1H, m), 9.94 (0.8H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.4, 20.5, 20.8, 21.1, 31.5, 31.9, 32.0, 36.2, 39.4, 47.2, 49.3, 66.8, 114.7, 114.9, 119.4, 119.9, 125.1, 125.2, 127.0, 127.7, 139.2, 141.2, 143.9, 144.0, 146.3, 151.5, 156.7, 156.9, 172.3, 173.7, 174.1, 174,9. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **16** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{29}H_{30}N_4O_6$ 531.6; found 531.3.



Benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-(pyridin-3-ylamino) pentanoyl)glycinate (17). Benzyl (2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)glycinate **2** (190 mg, 0.41 mmol) and glutaric anhydride (62 mg, 0.54 mmol) were combined in a round bottom flask. The flask was nitrogen purged and cooled to 0°C. DMF (2 mL, 0.2 M) added followed by slow addition of distilled diisopropylethylamine (78 µL, 0.45 mmol). The reaction was stirred for 18 hours, HBTU (154 mg, 0. 41 mmol) was added, and the reaction mixture was stirred 30 minutes. The reaction was cooled to 0° C and 3-aminopyridine (118 mg, 1.25 mmol) was added followed by slow addition of diisopropylethylamine (78 µL, 0.45 mmol). The reaction was warmed to room temperature and stirred for 18 hours. The reaction mixture was diluted with ethyl acetate (5 mL) and washed with 5% aqueous AcOH (5 mL), water (5 mL), and brine (5 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated. The resulting oil was then purified by silica gel chromatography (50-100% ethyl acetate/hexanes then 2% MeOH/ethyl acetate) to afford the title compound **17** as a clear waxy solid (86 mg, 34%) R_f = 0.25 in 5% MeOH/ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.00 (2.2H, m), 2.26-2.42 (2.5H, m), 2.47 (1.1H, t, J = 6.7 Hz), 2.89 (0.4H, m), 3.04 (0.3H, bd), 3.33 (1.9H, m), 3.47 (2.6H, m), 3.81 (0.4H, d, J = 15.9 Hz), 4.03-4.25 (2.6H, m), 4.37 (1.6H, m), 4.60 (0.3H, d, J = 35.5 Hz), 5.15 (2.0H, m), 5.72 (0.1H, d), 5.87 (0.2H, m), 6.07 (0.5H, m), 7.20 (1.1H, m), 7.23-7.41 (8.7H, m), 7.55 (1.9H, m), 7.72 (1.9H, d, J = 7.4 Hz), 8.16 (1.0H, d, J = 7.4 Hz), 8.27 (0.8H, bs), 8.64 (0.8H, bs), 9.13 (0.8H, s). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 19.0, 20.7, 21.1, 31.2, 31.8, 36.0, 36.1, 39.2, 39.4, 47.2, 47.2, 47.8, 48.9, 49.2, 50.4, 50.7, 66.7, 66.8, 67.3, 67.7, 120.0, 123.8, 125.0, 127.1, 127.8, 128.3, 128.5, 128.6, 128.7, 128.7, 134.8, 135.1, 141.0, 141.3, 143.8, 143.9, 144.5, 156.7, 156.8, 169.5, 170.1, 172.0, 173.7, 174.0. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **17** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{36}H_{36}N_4O_6$ 621.7; found 621.3.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-(pyridin-3-ylamino)pentanoyl) glycine (18). Methanol was degassed via bubbling nitrogen gas for 1 hour prior to reaction. Meanwhile, 10% Pd/C (19 mg, 40 wt%) and benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-(pyridin-3-ylamino)pentanoyl)glycinate **17** (104 mg, 0.17 mmol) were combined in a round bottom flask. The flask was purged with nitrogen gas and methanol (500 µL, 0.33 M) and DMF (40 µL) were added. The reaction mixture was purged with hydrogen gas and stirred under a hydrogen atmosphere (balloon) for 1.5 hours. The reaction mixture was diluted with methanol (540 µL) and filtered through a 3 cm celite plug to remove Pd/C. Solvent was removed by rotary evaporation affording the tittle compound **18** as a clear waxy solid (89 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.90 (2.6H, d), 2.32 (3.4H, m), 2.78 (1.1H, bs), 3.11-3.54 (3.0H, m), 3.95 (1.4H, m), 4.08 (0.7H, m), 4.24 (1.5H, m), 4.41 (0.2H, bs), 4.64 (0.5H, bs), 6.17 (0.2H, bs), 6.34 (0.4H, bs), 6.95 (0.2H, bs), 7.17 (2.1H, m), 7.27-7.41 (2.1H, m), 7.49 (2.0H, m), 7.69 (2.0H, m), 8.02-8.28 (1.9H, m), 8.50-8.72 (0.8H, m), 8.88 (0.1H, bs), 9.59 (0.2H, bs), 9.68 (0.4H, bs), 9.84 (0.5H, bs). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 19.6, 20.7, 21.0, 31.1, 31.9, 35.5, 35.9, 36.1, 39.3, 39.8, 47.1. 47.9, 49.2, 49.7, 51.7, 66.6, 66.8, 120.0, 124.3, 125.0, 125.1, 127.0, 127.3, 127.7, 127.9, 136.5, 139.1, 139.9, 141.2, 141.5, 142.1, 142.8, 143.8, 143.9, 156.7, 156.9, 157.2, 172.3, 172.5, 173.1, 173.5, 173.7, 173.8, 174.3. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **18** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{29}H_{30}N_4O_6$ 531.6; found 531.2.



Benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-(pyridin-4-ylamino)pentanoyl)glycinate (19). Benzyl (2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)glycinate **2** (190 mg, 0.41 mmol) and glutaric anhydride (62 mg, 0.54 mmol) were combined in a round bottom

flask. The flask was purged with nitrogen gas and cooled to 0°C. DMF (2 mL, 0.2 M) was added followed by slow addition of distilled diisopropylethylamine (78 µL, 0.45 mmol). The reaction was stirred for 18 hours, HBTU (154 mg, 0. 41 mmol) was added, and the reaction mixture was stirred 30 minutes. The reaction was cooled to 0°C and 4-aminopyridine (118 mg, 1.25 mmol) was added followed by slow addition of diisopropylethylamine (78 µL, 0.45 mmol). The reaction was warmed to room temperature and stirred 18 hours. The reaction was diluted with ethyl acetate (5 mL) and washed with 5% aqueous AcOH (5 mL), water (5 mL), and brine (5 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated. The resulting oil was then purified by silica gel chromatography (50-100% ethyl acetate/hexanes then 2% MeOH/ethyl acetate) to afford the title compound **19** as a clear waxy solid (86 mg, 34%) R_f = 0.25 in 5% MeOH/ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.89-2.12 (2.6H, m), 2.21-2.51 (3.3H, m), 2.83-3.11 (0.6H, m), 3.23-3.56 (3.7H, m), 3.83 (0.4H, bd, J = 14.8 Hz), 4.00-4.22 (2.3H, m), 4.39 (1.4H, m), 4.59 (0.4H, d, J = 40.2 Hz), 5.15 (2.0H, m), 5.61 (0.4H, m), 5.80 (0.5H, m), 7.23-7.41 (7.8H, m), 7.44-7.62 (3.7H, m), 7.71 (1.8H, d, J = 7.4 Hz), 8.41 (1.6H, bs), 9.09 (0.8H, m). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 18.9, 20.6, 21.0, 30.9, 31.0, 31.7, 36.3, 36.4, 39.3, 39.4, 47.2, 47.2, 47.9, 49.0, 49.3. 50.5, 50.7, 66.7, 66.8, 67.4, 67.7, 113.6, 120.0, 124.9, 125.0, 127.1, 127.8, 128.3, 128.6, 128.7, 128.8, 134.7, 135.0, 141.3, 143.7, 143.8, 145.8, 150.1, 156.6, 169.5, 170.1, 172.3, 173.7, 174.0. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **19** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{36}H_{36}N_4O_6$ 621.7; found 621.3.

N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-(pyridin-4-ylamino)pentanoyl) glycine (20). Methanol was degassed via bubbling nitrogen gas for 1 hour prior to reaction. Meanwhile, 10% Pd/C (19 mg, 40 wt%) and benzyl N-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-N-(5-oxo-5-(pyridin-4-ylamino)pentanoyl)glycinate **19** (43 mg, 0.07 mmol) were combined in a round bottom flask. The flask was purged with nitrogen gas and methanol (250 µL, 0.28 M) and DMF (20 µL) were added. The reaction mixture was purged with hydrogen gas and stirred under a hydrogen atmosphere (balloon) for 1.5 hours. The reaction mixture was diluted with methanol (270 µL) and filtered through a 3 cm celite plug to remove Pd/C. Solvent was then removed by rotary evaporation affording the tittle compound **20** as a clear waxy solid (22 mg, 60%). ^{1}H NMR (400 MHz, DMSO) δ: 1.79 (2.2H, p, J = 7.3 Hz), 2.24 (1.7H, t, J = 7.4 Hz), 2.36 (3.1H, m), 2.72 (0.3H, s), 2.88 (0.3H, s), 2.95 (0.3H, bs), 3.12 (2.0H, m), 3.22 (0.5H, m), 3.34 (1.8H, m), 3.54 (0.4H, bs), 3.80 (2.2H, m), 4.21 (1.8H, m), 4.30 (0.9H, d, J = 7.0 Hz),7.30 (2.0H, q, J = 7.3 Hz), 7.39 (2.5H, m), 7.57 (2.1H, m), 7.65 (2.3H, t, J = 8.0 Hz), 7.86 (2.2H, m), 8.37 (2.0H, m), 10.45 (1.0H, m). ^{13}C NMR (101 MHz, DMSO) δ: 20.9, 21.1, 31.2, 32.1, 36.1, 36.4, 38.8, 47.2, 47.4,

48.1, 52.6, 65.9, 110.2, 113.6, 113.6, 120.5, 120.6, 121.8, 125.5, 125.7, 127.5, 127.8, 128.0, 129.4, 137.9, 139.9, 141.1, 141.2, 144.3, 144.4, 146.3, 146.3, 150.7, 156.6, 156.7, 171.9, 172.3, 172.8, 173.0. Because the NMR spectra were complicated by mixtures of rotamers, the identity and purity of **12** was further confirmed with LCMS (ESI) m/z: $[M+H]^+$ calcd for $C_{29}H_{30}N_4O_6$ 531.6; found 531.2.

Synthesis of PNA oligomers

The PNAs used in this study (Figures 2 and 3) were synthesized on an Expedite 8909 synthesizer at 2 µmol scale using NovaSyn TG Sieber resin (Novabiochem) and following methods previously developed in our group.[2] Commercial PNA-T-monomer was purchased from Link Technologies. M monomer was synthesized using procedures previously described by our group.[3] For newly reported monomers as single modifications in PNA, manual coupling was employed using the same solutions prepared for the Expedite. First, the resin is washed twice with 1.0 mL dry DMF. Meanwhile, a solution of monomer is prepared in NMP at 0.2M concentration using 0.1 mL NMP, 0.1 mL base solution, and 0.1 mL activator solution. After washing the resin, half of monomer solution is loaded onto resin and left for 30 minutes with sporadic agitation using the two-syringe push/pull method. The resin is washed once with 1.0 mL DMF then the remaining monomer solution is loaded and reacted for 30 minutes. The resin is then washed with 1.0 mL DMF then 1.0 mL capping solution is added and left for 10 minutes with sporadic agitation. The resin is washed one final time with 1.0 mL DMF then returned to the Expedite for the remainder of the synthesis. PNAs containing multiple V-base modifications were performed entirely on the Expedite. PNAs were cleaved from the solid support using 0.6 mL of 20% m-cresol in TFA for 2 h using two-syringe pull-push method. Crude PNA (separated in three Eppendorf tubes, 200 µL in each) was precipitated by the addition of chilled diethyl ether (~1.0 mL) and incubation at -20 °C for 15 minutes followed by the centrifugation (15000 rpm, 30 minutes). Ethyl ether was then removed using a pipette and the PNA was washed once more with 1.0 mL ethyl ether and centrifuged (15000 rpm, 10 minutes). The ethyl ether was again decanted and the crude PNA, white solid, was dissolved in 1.25 mL 0.1% formic acid in water. Samples were then analyzed by LC/MS to confirm the synthesis, purified by HPLC, and finally reanalyzed by LC/MS to confirm their purity. Yields were quantified by absorbance at 260 nm using molar extinction coefficient of 8560 $M^{-1}cm^{-1}$ for T, 806 $M^{-1}cm^{-1}$ for M, 3770 $M^{-1}cm^{-1}$ for ^{DA}N and CR1, 3772 $M^{-1}cm^{-1}$ for CR2-5, and 4200 $M^{-1}cm^{-1}$ for V. The final three values were determined by acylation of respective heterocycles, removal of Boc protecting groups (if necessary), and subsequent

[2] N. Brodyagin, D. Hnedzko, J. A. MacKay, E. Rozners, *Methods Mol. Biol. (Totowa, NJ, U. S.)* **2020**, *2105*, 157-172.
[3] T. Zengeya, P. Gupta, E. Rozners, *Angew. Chem., Int. Ed.* **2012**, *51*, 12593-12596.

serial dilution in water with absorbance measurements at 260 nm. After PNA quantification, solutions were lyophilized and redissolved in nuclease-free DI water to a final concentration of 240 µM.

RNA Purification

RNA hairpins were purchased from Dharmacon. Prior to use, RNA was deprotected in accordance with manufacturer instruction and purified on reverse phase HPLC using a gradient of acetonitrile in 50 mM aqueous triethyl ammonium acetate buffer (pH 7.4). Post purification, a final injection of the collected material confirmed its purity. Absorbance at 260 nm was used to quantify the RNA. After, RNA was lyophilized and redissolved in nuclease-free water to a concentration of 240 µM.

Table S1: Molecular weights and deconvoluted masses of PNAs.

PNA(monomer)	Mass calcd	M/Z calcd (M+6, M+5, M+4…)	M/Z found (M+6, M+5, M+4…)
PNA1 X = ^{DA}N	2456.9	410.5, 492.4, 615.2, 820.0, 1229.5	410.6, 492.5, 615.3, 820.1, 1229.6
PNA1 X = CR1	2471.0	412.8, 495.2, 618.7, 824.6, 1236.5	412.9, 495.3, 618.8, 824.7, 1236.5
PNA1 X = CR2	2390.9	399.5, 479.2, 598.7, 798.0, 1196.4	399.6, 479.3, 598.8, 798.0, 1196.7
PNA1 X = CR3	2404.7	401.8, 481.9, 602.1, 802.6, 1203.4	401.9, 482.1, 602.3, 802.8, 1203.6
PNA1 X = CR4	2404.7	401.8, 481.9, 602.1, 802.6, 1203.4	402.0, 482.1, 602.4, 802.8, 1203.6
PNA1 X = CR5	2404.7	401.8, 481.9, 602.1, 802.6, 1203.4	401.9, 482.0, 602.3, 802.7, 1203.5
PNA1 X = V	2461.9	411.3, 493.4, 616.5, 821.6, 1232.0	411.4, 493.5, 616.6, 821.8, 1231.8
PNA2	2543.1	424.8, 509.6, 636.8, 848.7, 1272.5	425.0, 509.8, 636.9, 848.8, 1272.7
PNA3	2543.1	424.8, 509.6, 636.8, 848.7, 1272.5	424.8, 509.6, 636.8, 848.8, 1272.8
PNA4	2624.2	438.4. 525.8, 657.1, 875.7, 1313.1	438.5, 526.0, 657.1, 875.9, 1313.3

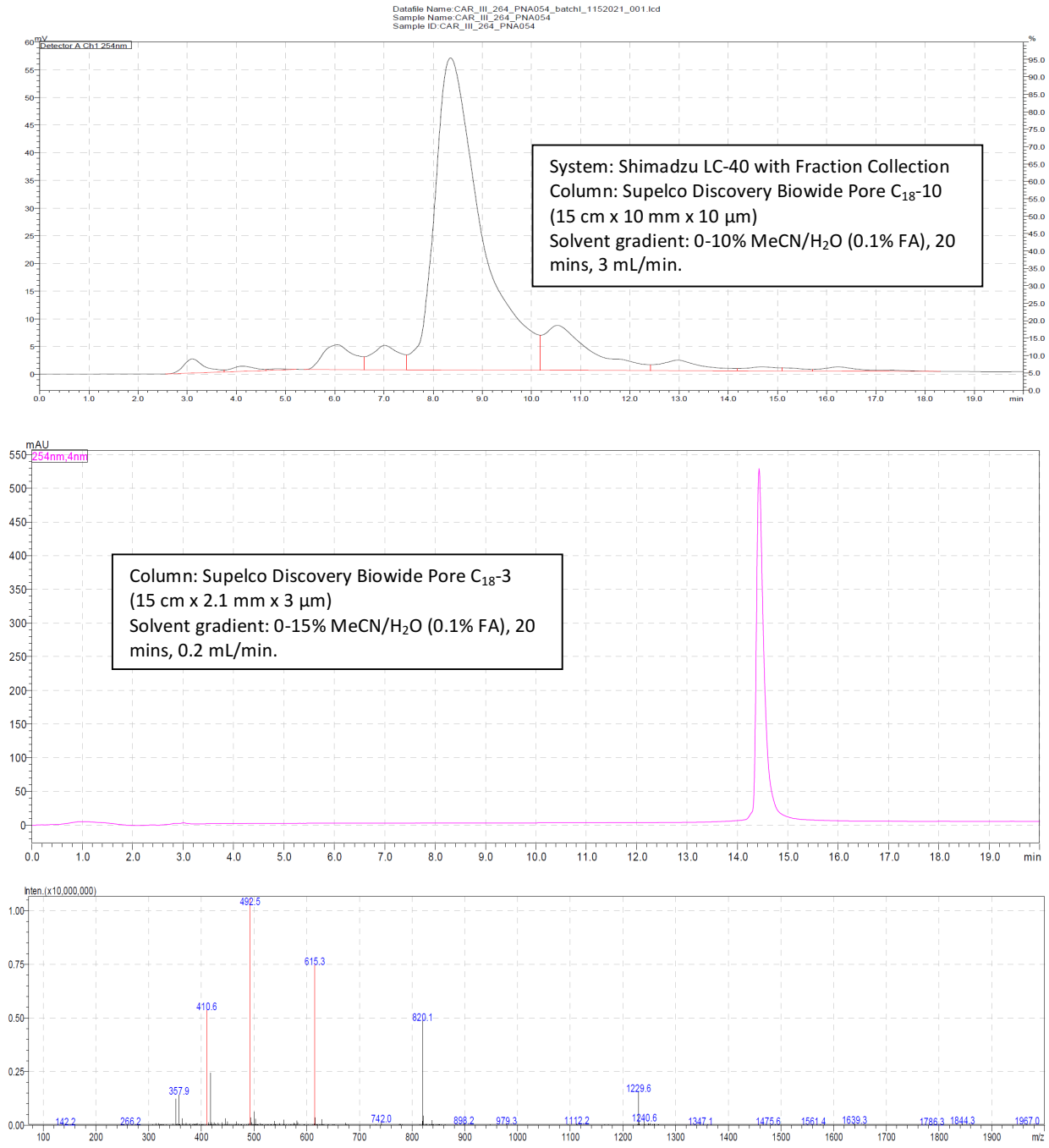


Figure S1. Crude HPLC (top) and purified LC/MS (middle/bottom) data of PNA1 X = ^{DA}N.

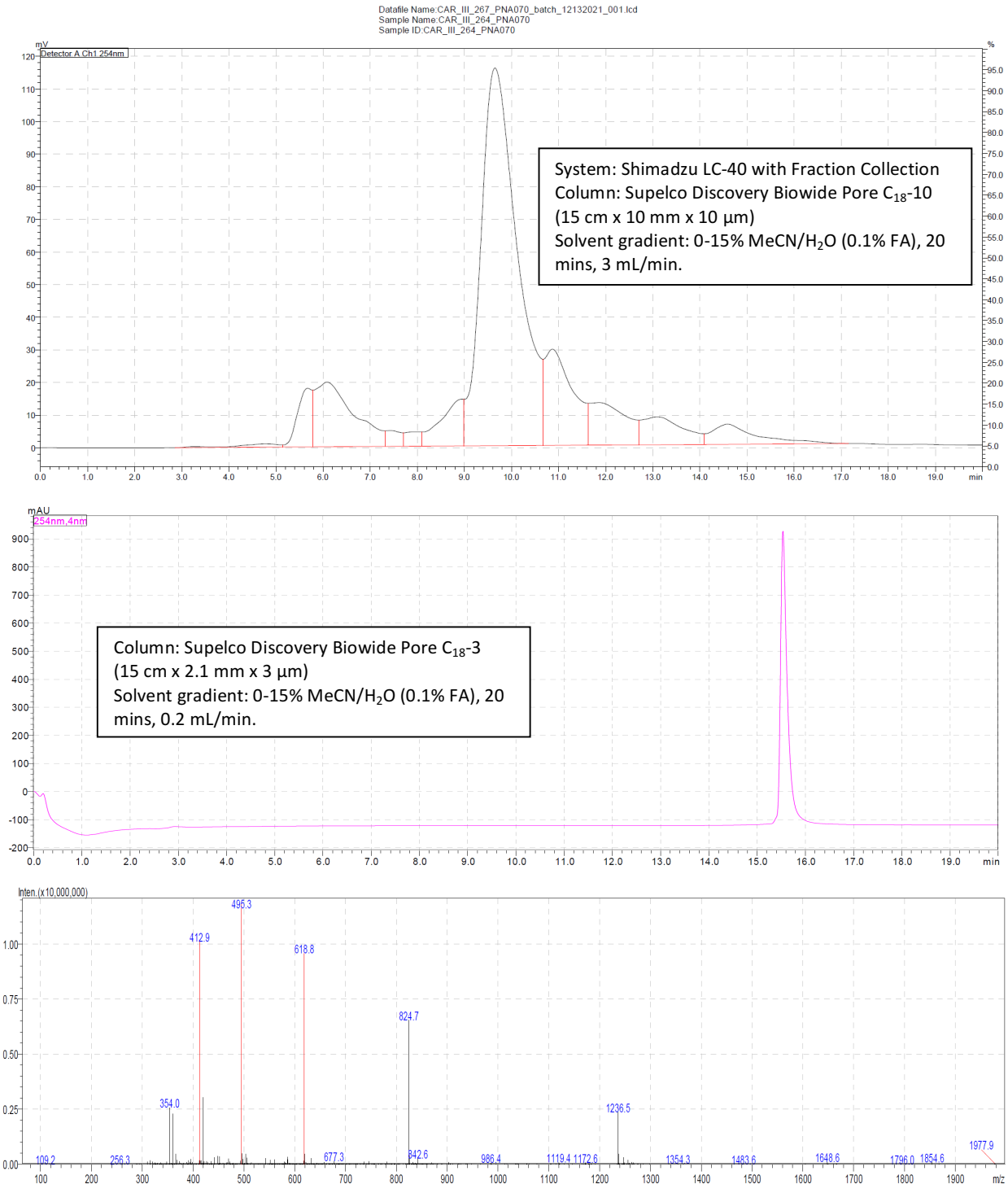


Figure S2. Crude HPLC (top) and purified LC/MS (middle/bottom) data of PNA1 X = CR1.

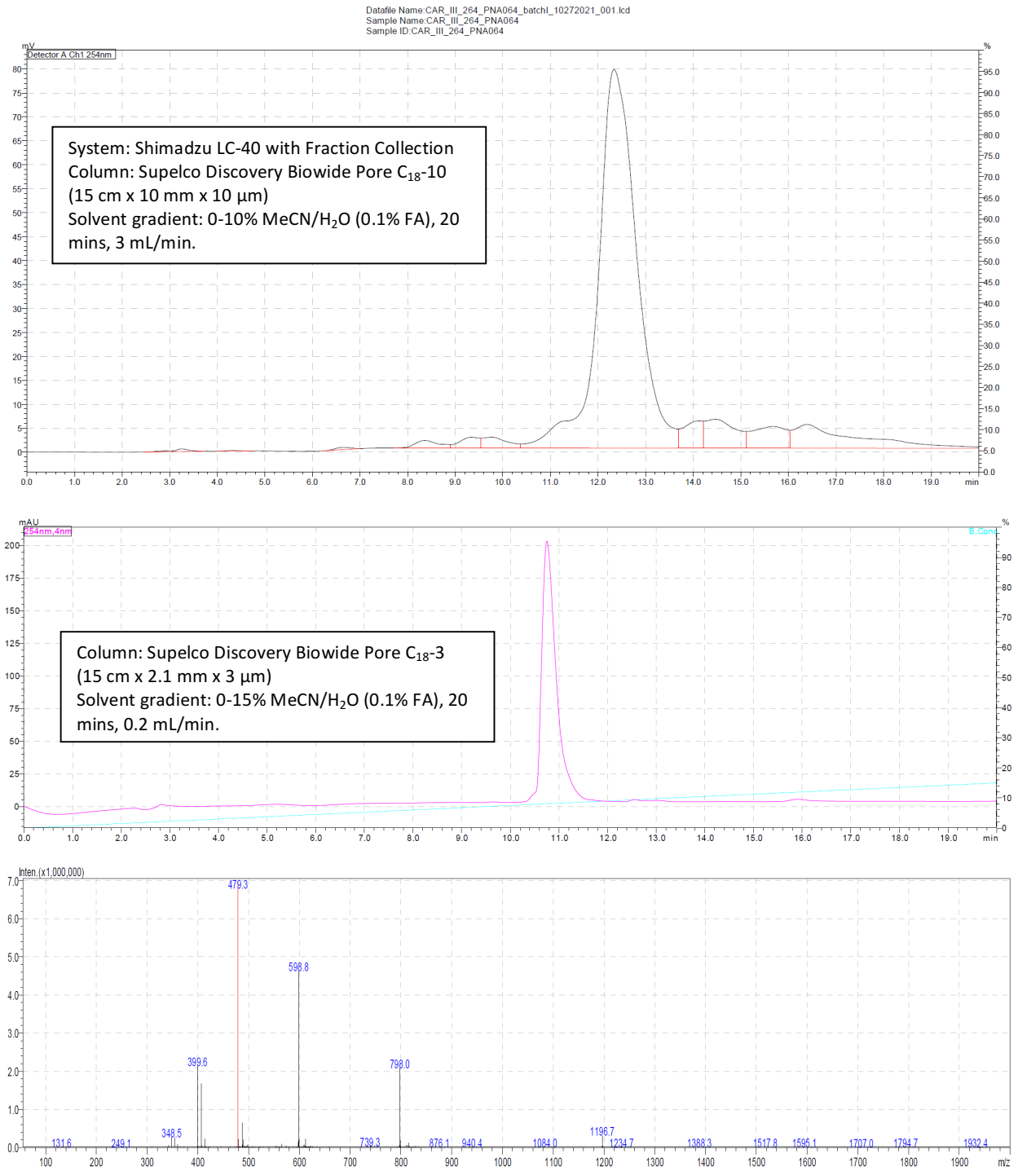


Figure S3. Crude HPLC (top) and purified LC/MS (middle/bottom) data of PNA1 X = CR2.

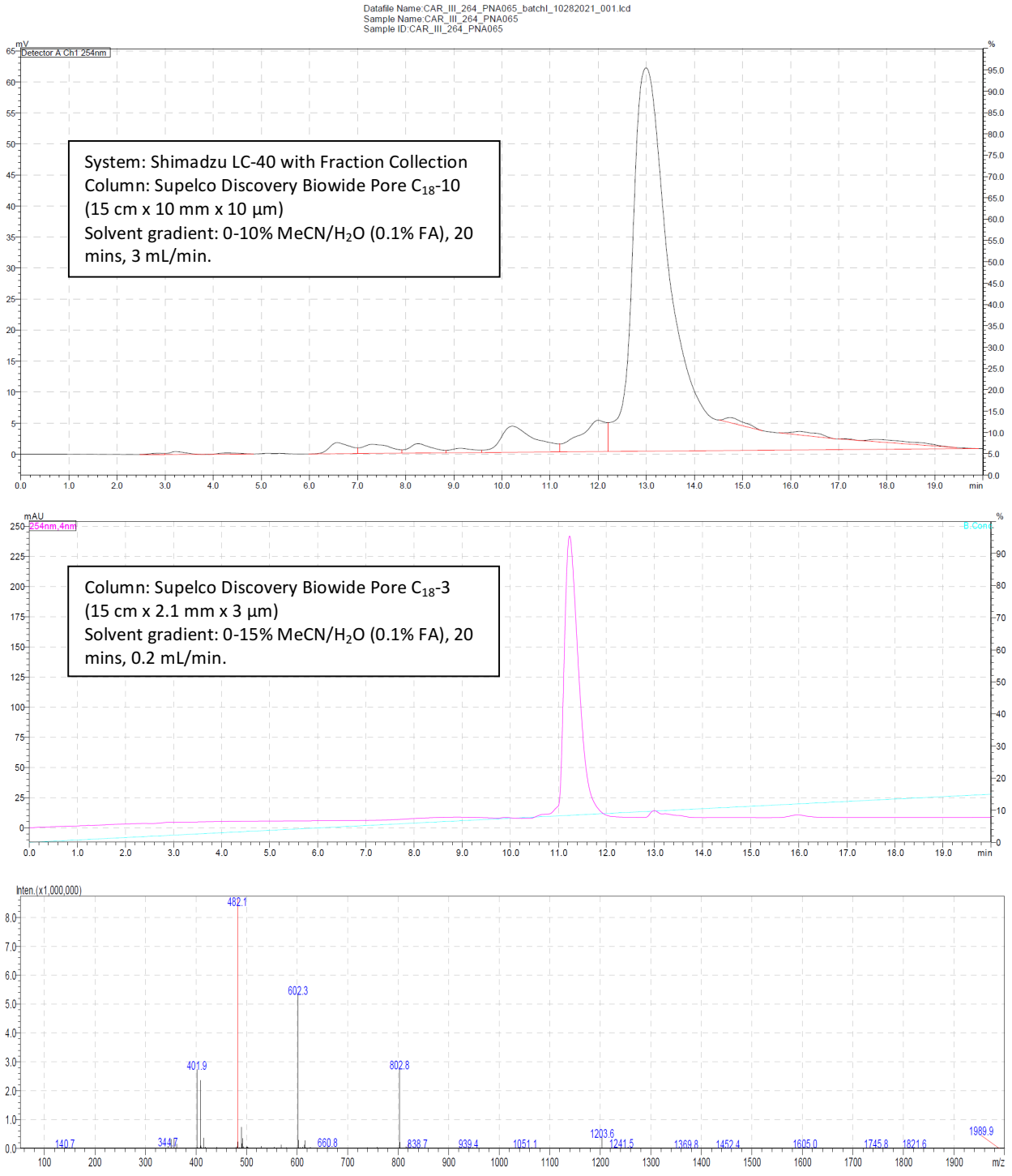


Figure S4. Crude HPLC (top) and purified LC/MS (middle/bottom) data of PNA1 X = CR3.

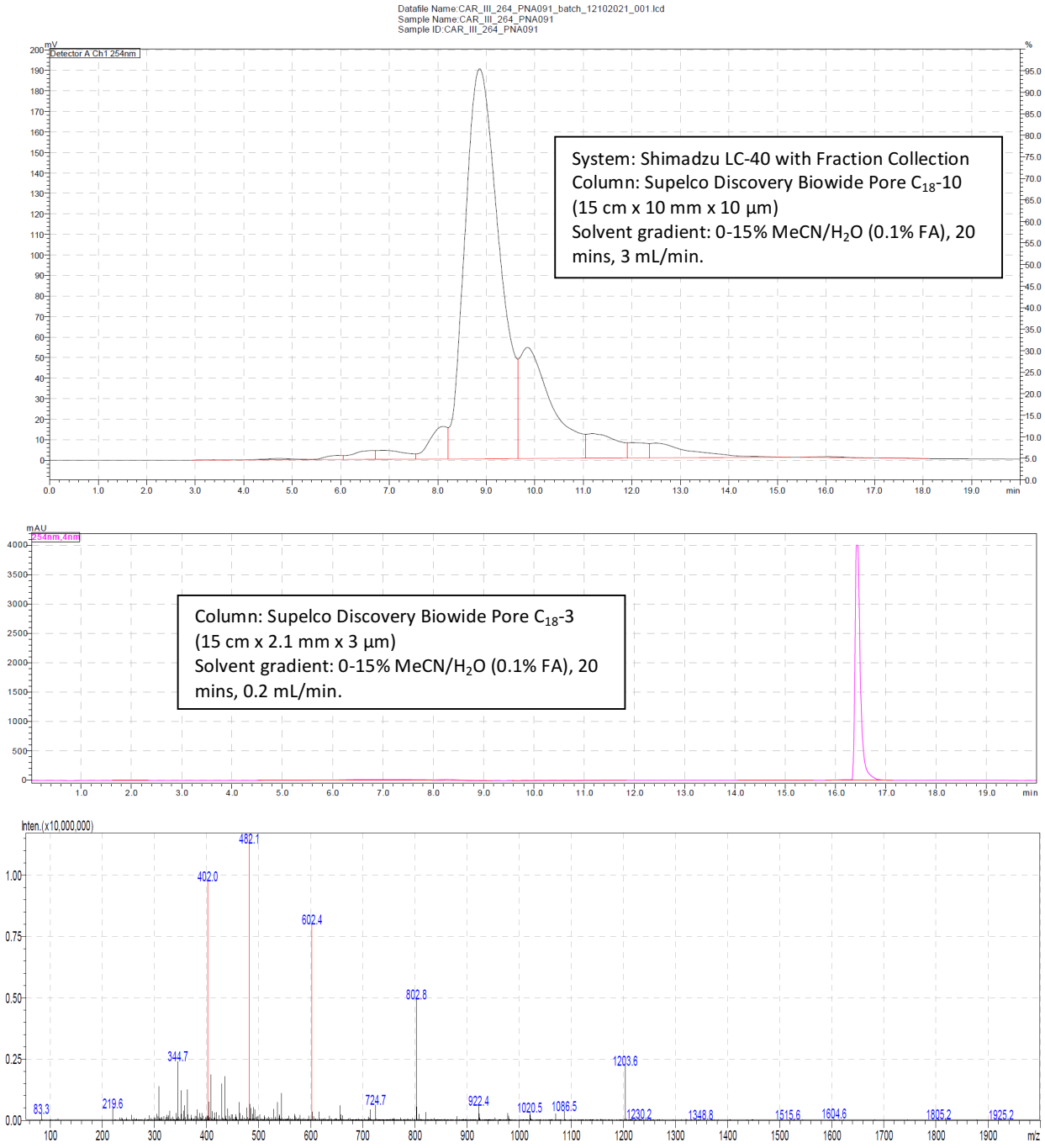


Figure S5. Crude HPLC (top) and purified LC/MS (middle/bottom) data of PNA1 X = CR4.

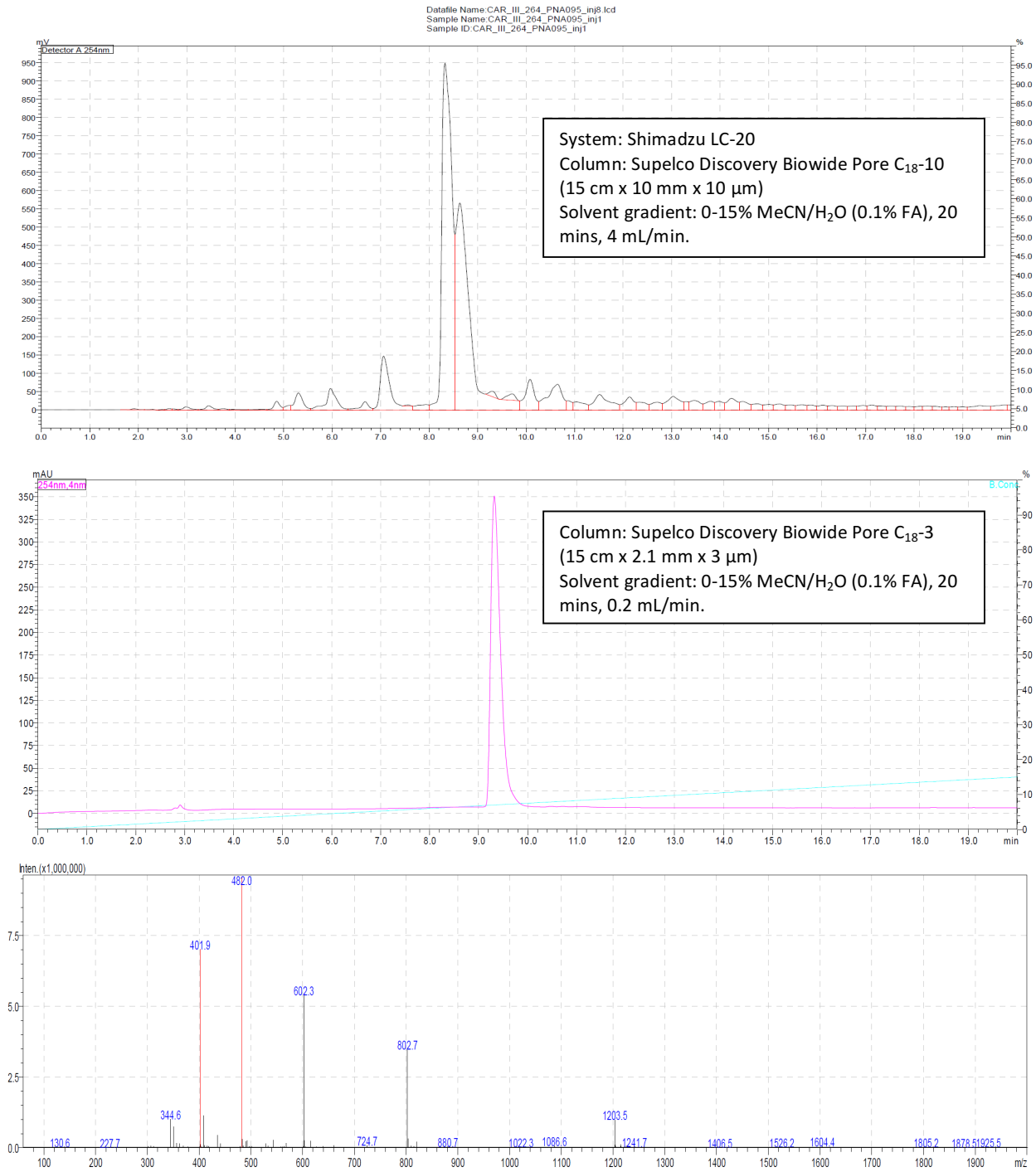


Figure S6. Crude HPLC (top) and purified LC/MS (middle/bottom) data of PNA1 X = CR5.

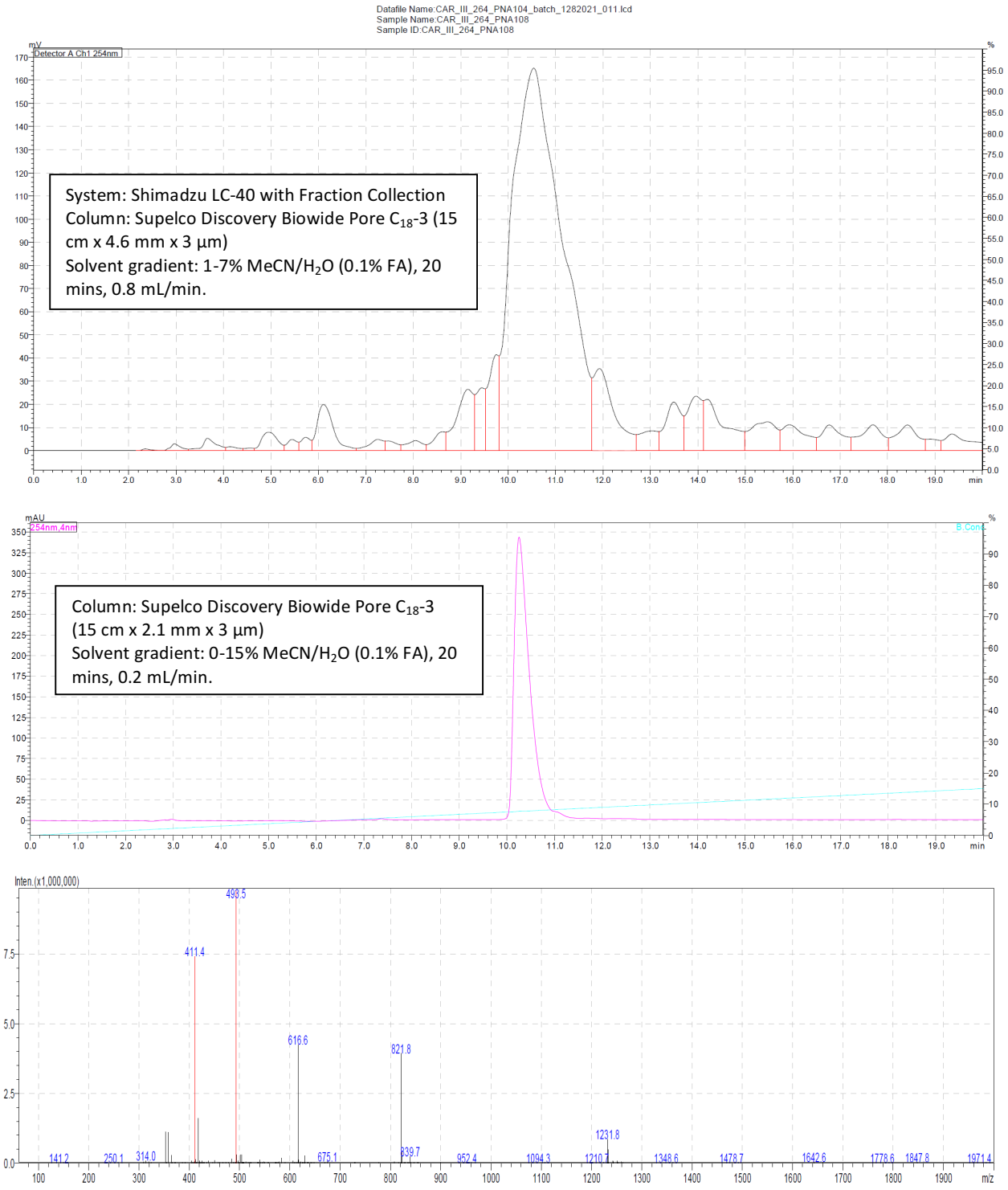


Figure S7. Crude HPLC (top) and purified LC/MS (middle/bottom) data of PNA1 X = V.

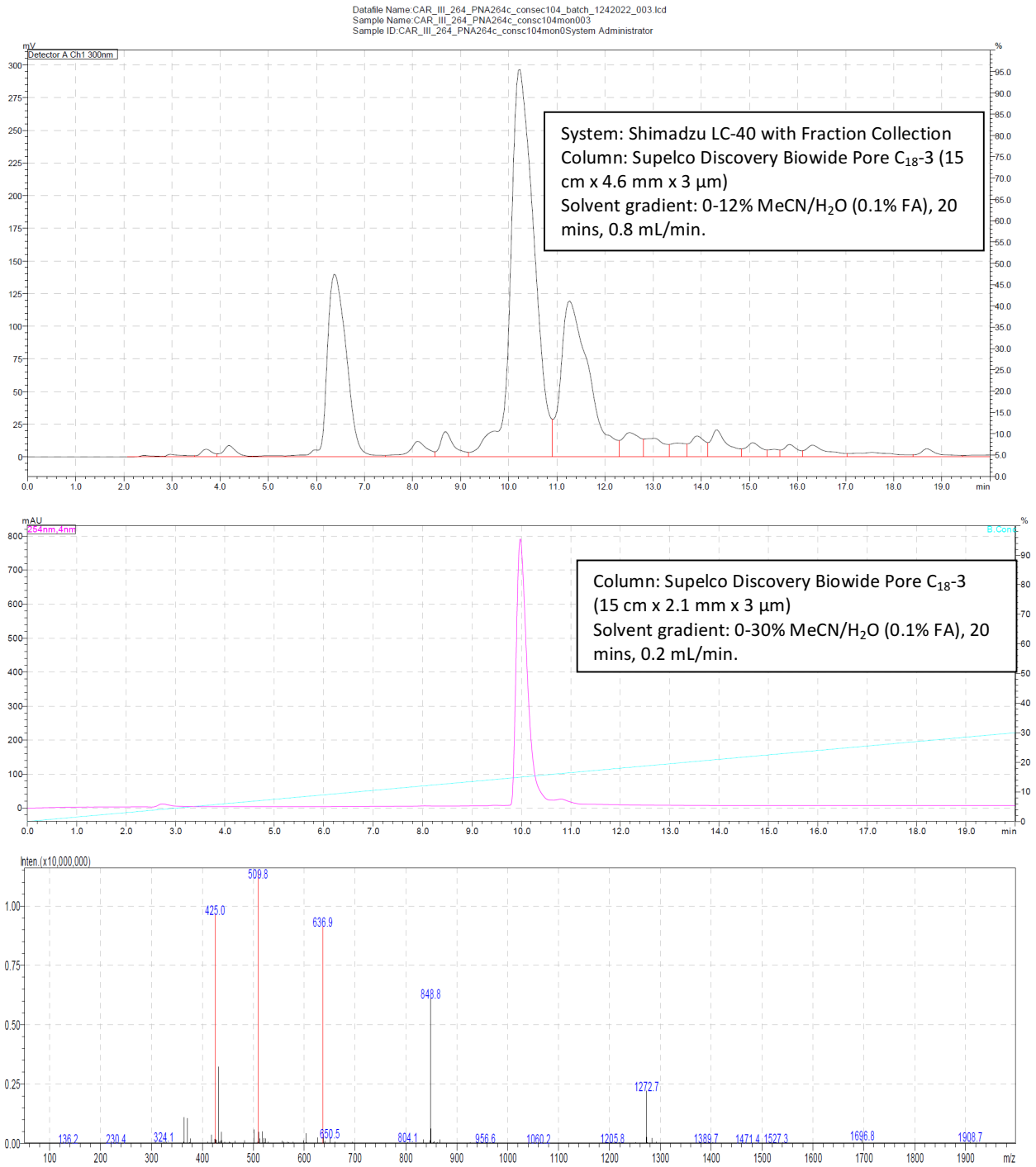


Figure S8. Crude HPLC (top) and purified LC/MS (middle/bottom) data of PNA2.

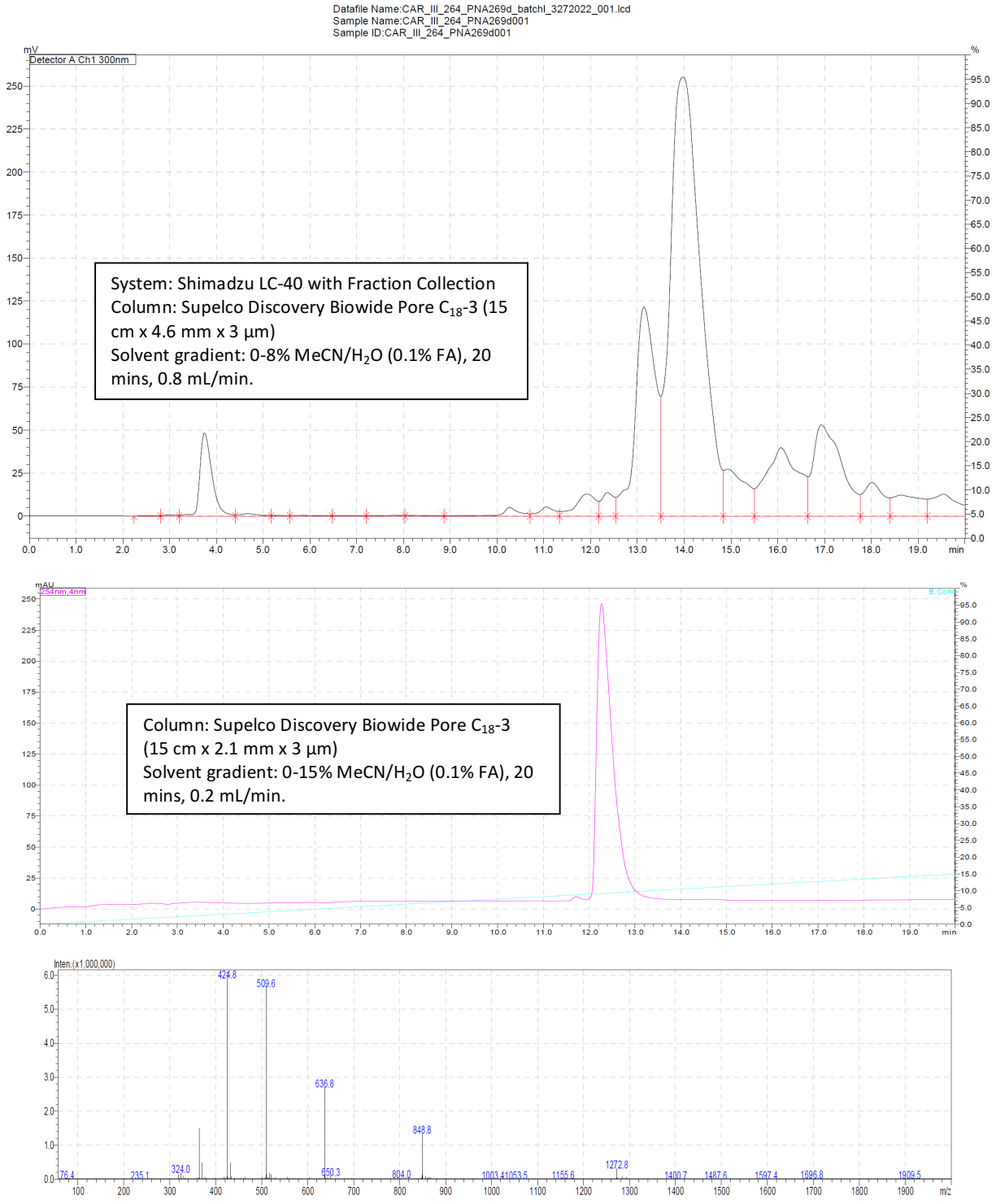


Figure S9. Crude HPLC (top) and purified LC/MS (middle/bottom) data of PNA3.

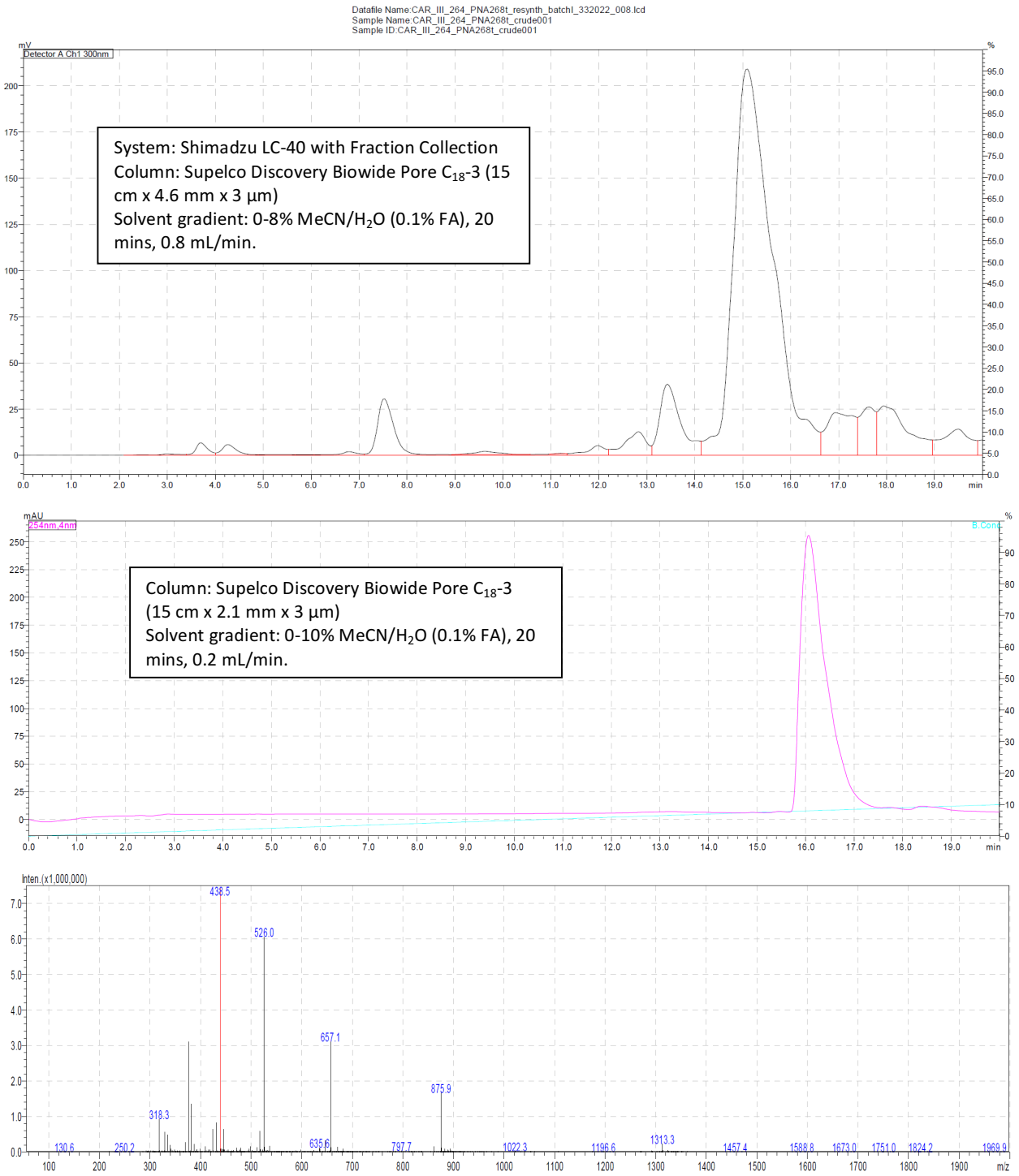


Figure S10. Crude HPLC (top) and purified LC/MS (middle/bottom) data of PNA4.

UV-melting experiments

UV-melting experiments were performed on a Shimadzu UV-2600 spectrophotometer equipped with TMSPC-8 temperature controllers. A temperature ramp rate of 1.0 °C was used in all cases. Absorbance was monitored at 300 nm for triplex-formation involving PNAs (18 µM). Experiments were performed in a buffer containing 50 mM potassium phosphate, 2 mM $MgCl_2$, 90 mM KCl, 10 mM NaCl at pH 7.4. Solutions were prepared as follows. Both PNA and RNA combined from 240 µM stock solutions in water, frozen, and lyophilized. Buffer (230 µL, 18 µM) is added and the pellet is dissolved by vortexing. The solution is then heated to 95 °C for 5 minutes and then snap-cooled to 4° C and left for 5 minutes. Samples were then melted three time, typically from 10°C to 95 °C. Five of the replicates were then used to determine average and standard deviation in melting temperature (T_m).

Table S2. Replicated Melting data for PNA1 X = ^{DA}N.

	HRP1 (G)	HRP2 (A)	HRP3 (C)	HRP4 (U)
Melting Temperatures (°C)	40.2	50.5	38.9	35.2
	41.2	50.3	38.7	35.2
	40.8	49.8	39.3	35.1
	40.5	51.3	38.7	35.6
	41.1	51.1	39.8	34.7
Average	40.8	50.6	39.1	35.2
Std. Dev	0.4	0.6	0.5	0.3

Table S3. Melting data for PNA1 X = CR1.

	HRP1 (G)	HRP2 (A)	HRP3 (C)	HRP4 (U)
Melting Temperatures (°C)	32.2	40.8	51.2	35.6
	31.5	40.8	51.1	35.7
	32.7	40.2	51.4	35.3
	32.2	40.4	50.7	35.8
	32.3	40.7	50.1	35.0
Average	32.2	40.6	50.9	35.5
Std. Dev	0.4	0.2	0.5	0.3

Table S4. Melting data for PNA1 X = CR2.

	HRP1 (G)	HRP2 (A)	HRP3 (C)	HRP4 (U)
Melting Temperatures (°C)	NM	55.5	39.9	34.0
	NM	55.9	40.2	34.3
	NM	55.3	40.2	33.4
	NM	55.4	40.6	34.4
	NM	55.3	40.5	33.7
Average	-	55.5	40.3	34.0
Std. Dev	-	0.3	0.3	0.4

*NM – no melting

Table S5. Melting data for PNA1 X = CR3.

	HRP1 (G)	HRP2 (A)	HRP3 (C)	HRP4 (U)
Melting Temperatures (°C)	30.8	39.1	51.6	34.6
	29.9	39.7	51.7	34.6
	30.6	39.1	51.3	34.2
	30.6	39.0	51.9	34.7
	29.6	38.8	51.0	34.6
Average	30.3	39.1	51.5	34.5
Std. Dev	0.5	0.3	0.3	0.2

Table S6. Melting data for PNA1 X = CR4.

	HRP1 (G)	HRP2 (A)	HRP3 (C)	HRP4 (U)
Melting Temperatures (°C)	32.0	36.5	51.3	34.2
	31.8	37.2	51.0	34.5
	31.3	37.0	51.4	34.6
	31.8	37.1	50.8	34.7
	31.9	37.8	51.6	34.6
Average	31.8	37.1	51.2	34.5
Std. Dev	0.3	0.5	0.3	0.2

Table S7. Melting data for PNA1 X = CR5.

	HRP1 (G)	HRP2 (A)	HRP3 (C)	HRP4 (U)
Melting Temperatures (°C)	32.8	37.7	52.7	35.3
	32.0	37.8	52.2	35.6
	32.4	37.9	52.1	35.8
	33.0	37.8	52.6	35.7
	32.7	37.8	52.7	36.0
Average	32.6	37.8	52.5	35.7
Std. Dev	0.4	0.1	0.3	0.3

Table S8. Melting data for PNA1 X = V.

	HRP1 (G)	HRP2 (A)	HRP3 (C)	HRP4 (U)
Melting Temperatures (°C)	35.8	42.3	60.1	36.5
	36.4	41.6	59.7	36.9
	36.3	42.3	60.8	37.1
	36.2	42.8	60.3	37.4
	36.4	42.6	60.4	37.0
Average	36.2	42.3	60.3	37.0
Std. Dev	0.3	0.5	0.4	0.3

Table S9. Melting data for PNA2.

	HRP5	HRP6	HRP7
Melting Temperatures (°C)	49.3	40.8	NM
	48.7	40.9	NM
	49.1	39.5	NM
	49.3	40.2	NM
	49.2	39.1	NM
Average	49.1	40.1	-
Std. Dev	0.2	0.8	-

Table S10. Melting data for PNA3.

	HRP5	HRP6	HRP7
Melting Temperatures (°C)	29.6	42.7	NM
	30.5	42.5	NM
	30.2	42.5	NM
	29.6	43.2	NM
	30.2	41.8	NM
Average	30.0	42.5	-
Std. Dev	0.4	0.5	-

Isothermal Titration Calorimetry

Isothermal titration calorimetry experiments were done on a MicroCal ITC200 instrument at 25 °C in 50 mM potassium phosphate buffer (pH 7.4) containing 2 mM $MgCl_2$, 90 mM KCl, 10 mM NaCl. In a typical experiment 2.45 µL aliquots of PNA solution were sequentially injected from a 40 µL rotating syringe (750 rmp) into 200 µL of RNA or DNA hairpin solution. Standard concentration of PNA was 75 µM and stand concentration of RNA was 10 µM. Experiments which deviate from these values have concentrations listed on their figures. Results were analyzed using MicroCal PEAQ-ITC software.

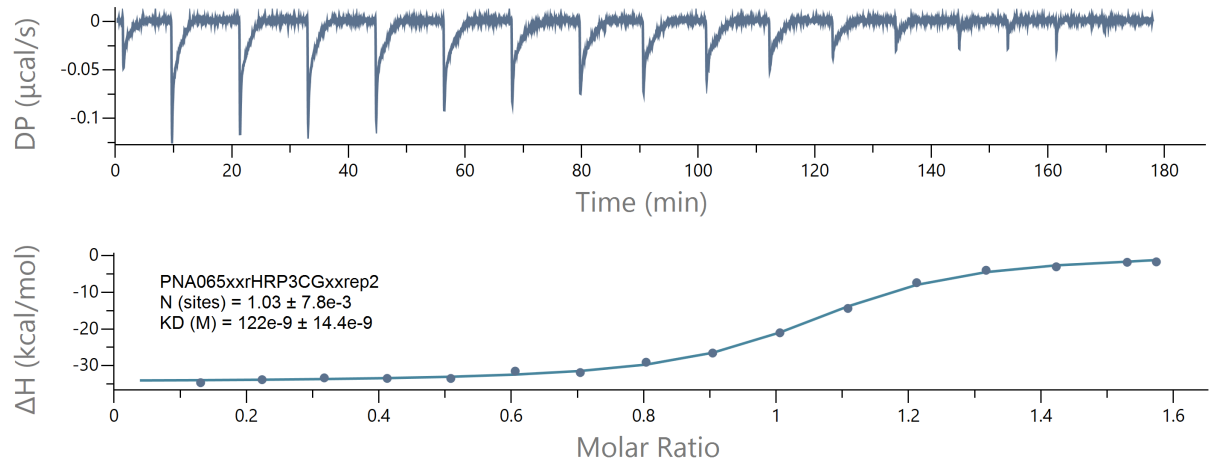


Figure S11. ITC experiment of PNA1 X = CR3 vs HRP3.

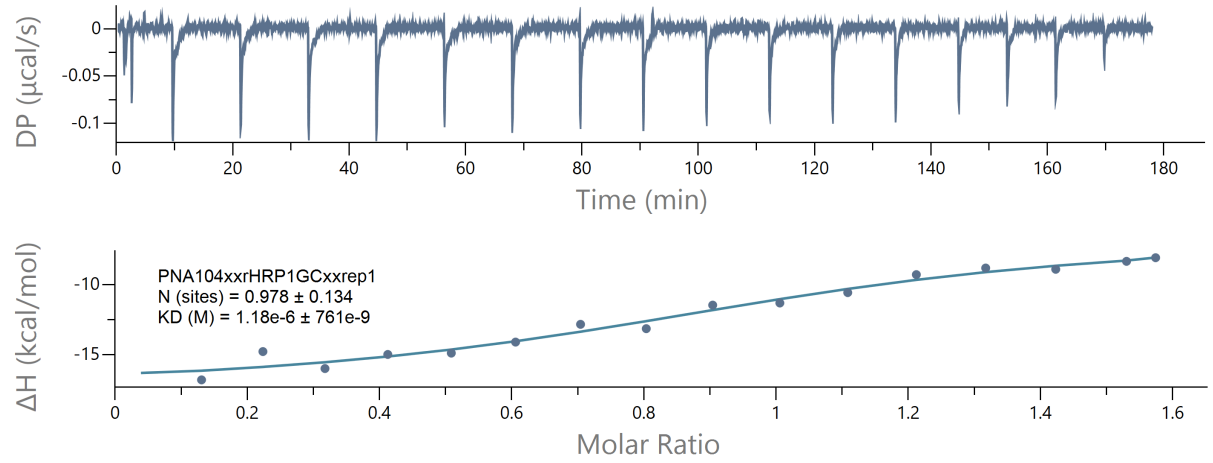


Figure S12. ITC experiment of PNA1 X = V vs HRP1.

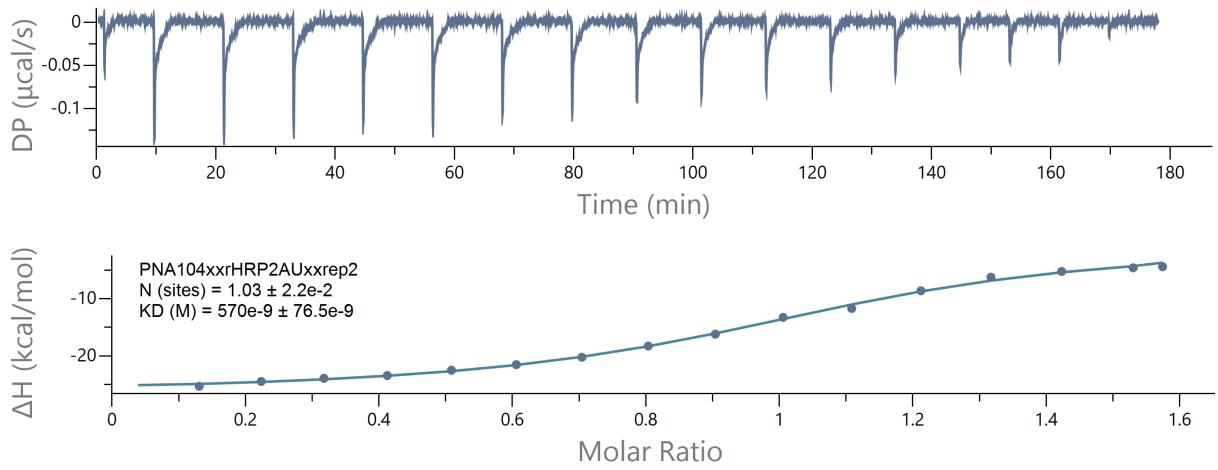


Figure S13.: ITC experiment of PNA1 X = V vs HRP2.

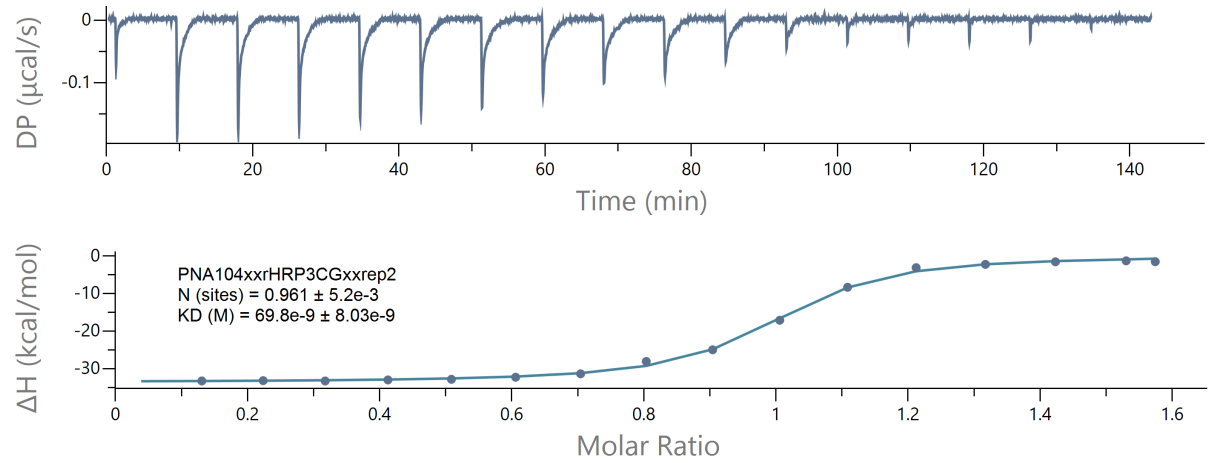


Figure S14. ITC experiment of PNA1 X = V vs HRP3.

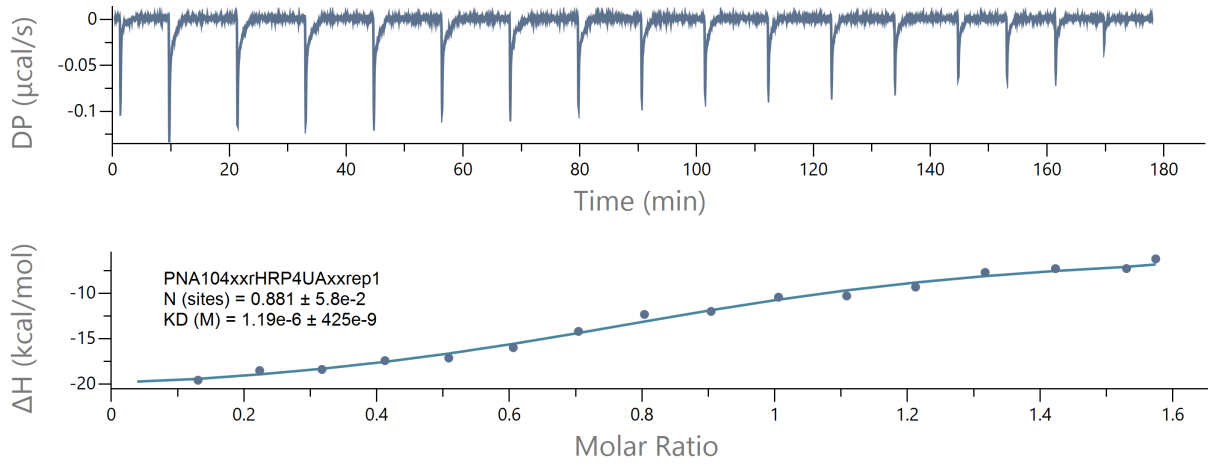


Figure S15. ITC experiment of PNA1 X = V vs HRP4.

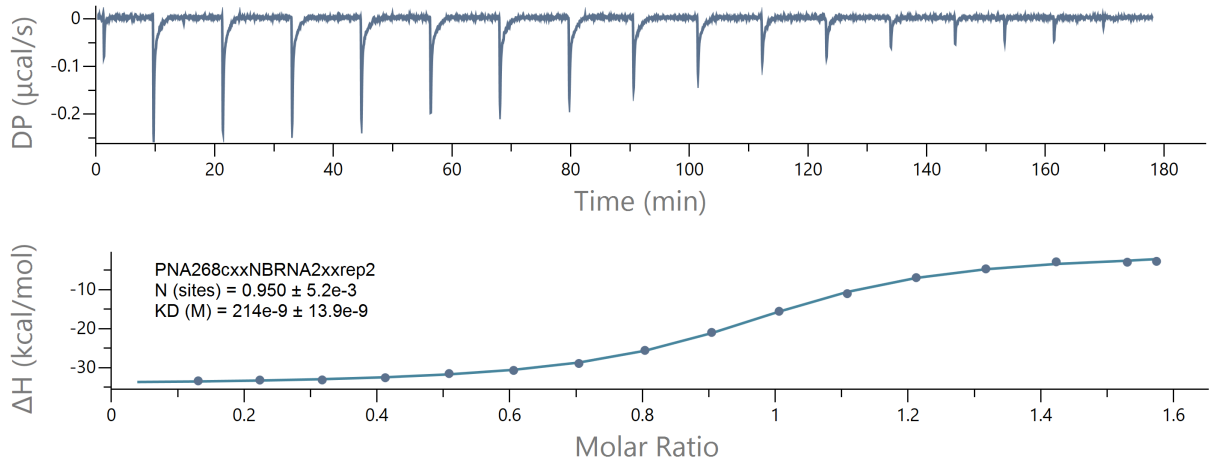


Figure S16. ITC experiment of PNA2 vs HRP5.

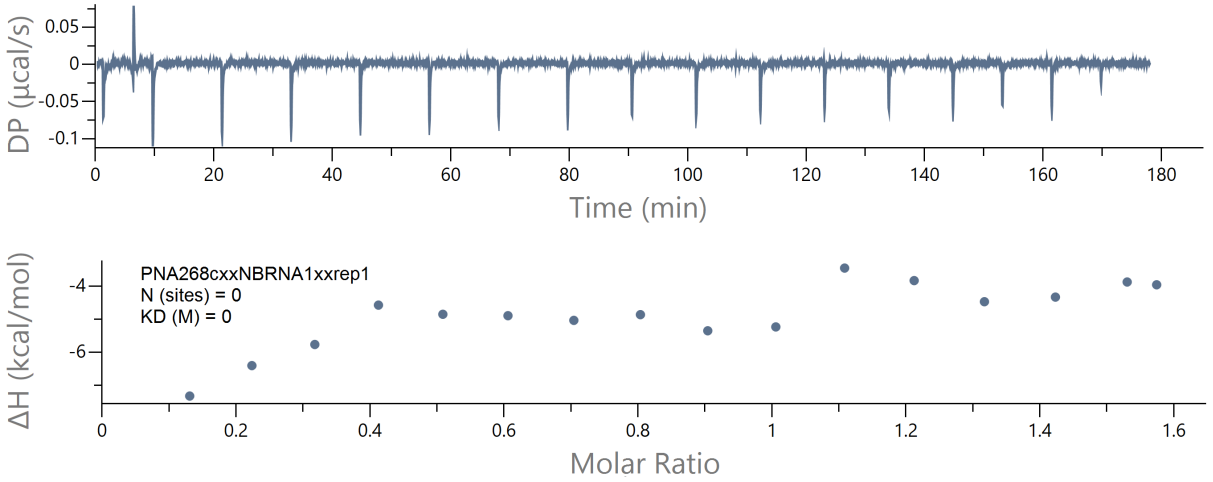


Figure S17. ITC experiment of PNA2 vs HRP7.

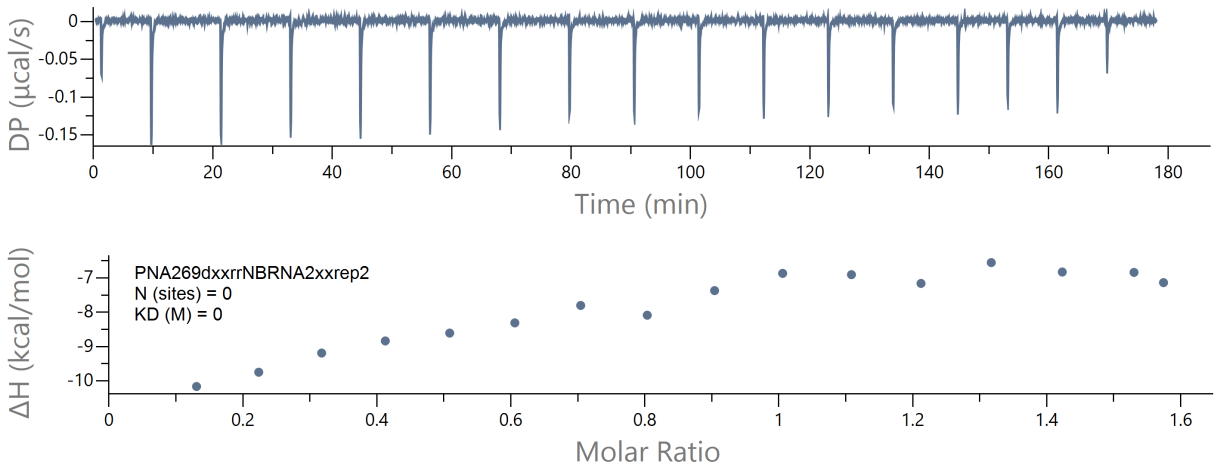


Figure S18. ITC experiment of PNA3 vs HRP5.

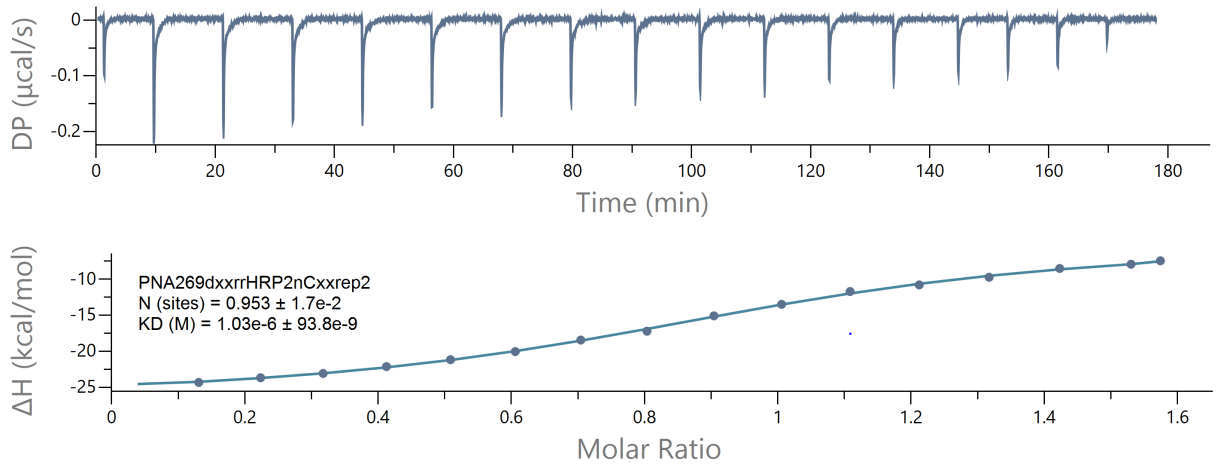


Figure S19. ITC experiment of PNA3 vs HRP6.

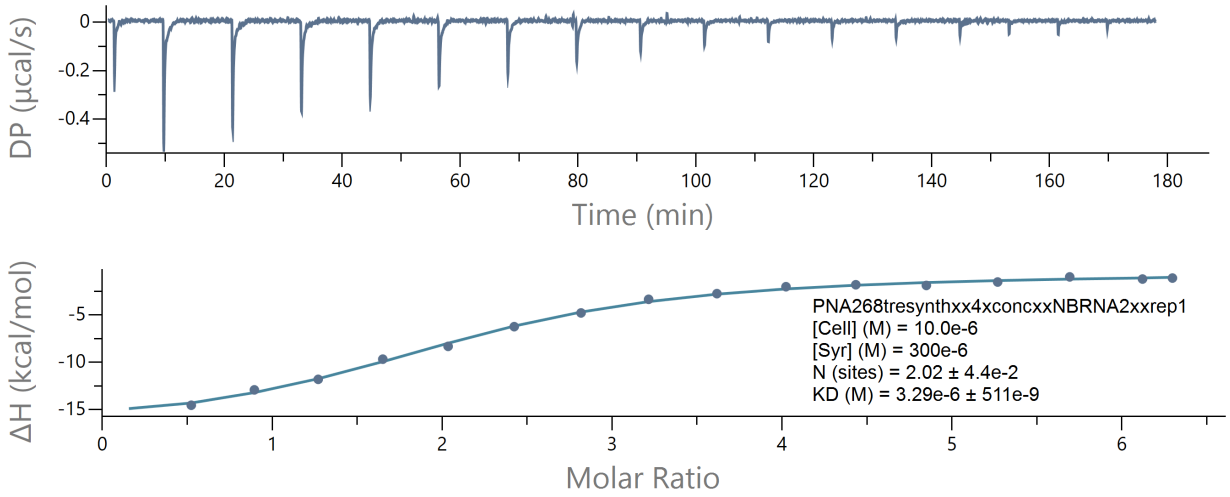


Figure S20. ITC experiment of PNA4 vs HRP5.

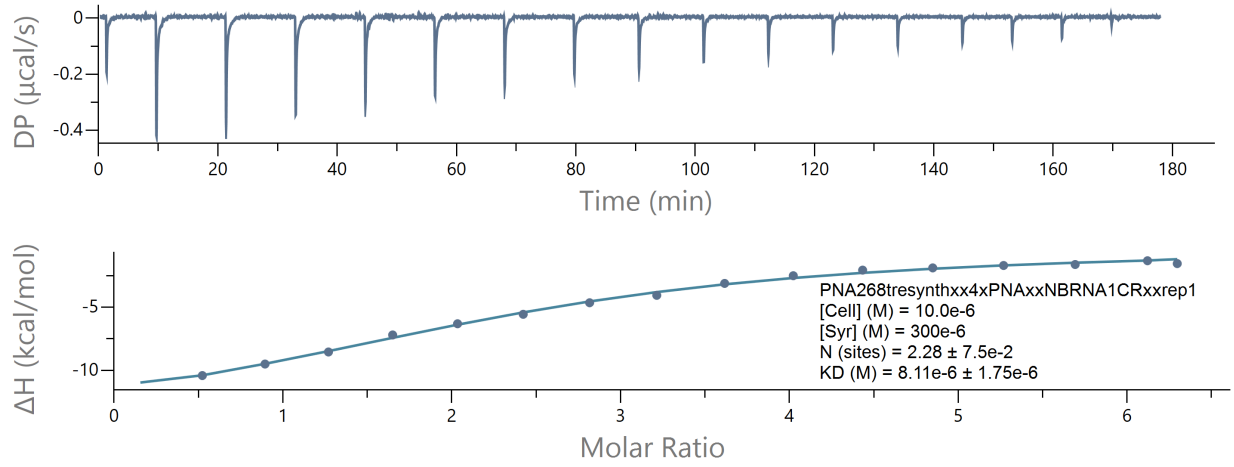


Figure S21. ITC experiment of PNA4 vs HRP7.

Table S11. PNA1 X = CR3 binding affinity and thermodynamic data obtained by ITC

Name	Kd (M)	Ka(M^{-1})	ΔH(kcal/mol)	-TΔS(kcal/mol)	N
PNA1 X = CR3 vs HRP3 01	1.3E-07	7.8E+06	-34.6	25.2	1.0
PNA1 X = CR3 vs HRP3 02	1.2E-07	8.2E+06	-34.7	25.2	1.0
PNA1 X = CR3 vs HRP3 03	1.3E-07	7.7E+06	-33.4	24.0	0.9
Average	1.3E-07	7.9E+06	-34.2	24.8	1.0
Std. Dev.	4.0E-09	2.6E+05	0.7	0.7	0.06

Table S12. PNA1 X = V binding affinity and thermodynamic data obtained by ITC

Name	Kd (M)	Ka(M^{-1})	ΔH(kcal/mol)	-TΔS(kcal/mol)	N
PNA1 X = V vs HRP1 01	1.2E-06	8.5E+05	-11.0	2.9	1.0
PNA1 X = V vs HRP1 02	9.4E-07	1.1E+06	-9.1	0.9	1.2
PNA1 X = V vs HRP1 03	7.9E-07	1.3E+06	-11.7	3.3	0.9
Average	9.7E-07	1.1E+06	-10.6	2.4	1.0
Std. Dev.	2.0E-07	2.1E+05	1.3	1.3	0.13
PNA1 X = V vs HRP2 01	5.8E-07	1.7E+06	-33.4	24.9	1.0
PNA1 X = V vs HRP2 02	5.7E-07	1.8E+06	-25.8	17.3	1.0
PNA1 X = V vs HRP2 03	6.6E-07	1.5E+06	-21.5	13.0	1.0
Average	6.0E-07	1.7E+06	-26.9	18.4	1.0
Std. Dev.	5.0E-08	1.3E+05	6.0	6.0	0.0
PNA1 X = V vs HRP3 01	7.0E-08	1.4E+07	-33.5	23.8	1.0
PNA1 X = V vs HRP3 02	7.0E-08	1.4E+07	-36.3	26.5	0.9
PNA1 X = V vs HRP3 03	7.2E-08	1.4E+07	-39.3	29.5	0.9
Average	7.1E-08	1.4E+07	-36.4	26.6	0.9
Std. Dev.	1.4E-09	2.8E+05	2.9	2.9	0.05
PNA1 X = V vs HRP4 01	1.2E-06	8.4E+05	-16.9	8.8	0.9
PNA1 X = V vs HRP4 02	8.7E-07	1.1E+06	-14.1	5.8	0.9
PNA1 X = V vs HRP4 03	1.2E-06	8.9E+05	-16.8	8.7	0.9
Average	1.1E-06	9.6E+05	-15.9	7.7	0.9
Std. Dev.	1.7E-07	1.7E+05	1.6	1.7	0.02

Table S13. PNA2 binding affinity and thermodynamic data obtained by ITC

Name	Kd (M)	Ka(M^{-1})	ΔH(kcal/mol)	-TΔS(kcal/mol)	N
PNA2 vs HRP5 01	2.1E-07	4.9E+06	-29.3	20.2	1.0
PNA2 vs HRP5 02	2.1E-07	4.7E+06	-33.9	24.8	1.0
PNA2 vs HRP5 03	2.1E-07	4.7E+06	-33.4	24.3	0.9
Average	2.1E-07	4.8E+06	-32.2	23.1	0.9
Std. Dev.	4.7E-09	1.1E+05	2.5	2.5	0.05

Table S14. PNA3 binding affinity and thermodynamic data obtained by ITC

Name	Kd (M)	Ka(M^{-1})	ΔH(kcal/mol)	-TΔS(kcal/mol)	N
PNA3 vs HRP6 01	9.1E-07	1.1E+06	-20.7	12.5	1.0
PNA3 vs HRP6 02	1.0E-06	9.7E+05	-21.9	13.8	1.0
PNA3 vs HRP6 03	1.1E-06	9.1E+05	-22.8	14.6	1.1
Average	1.0E-06	1.0E+06	-21.8	13.6	1.0
Std. Dev.	9.8E-08	9.9E+04	1.0	1.1	0.07

***Ab initio* calculations**

To obtain insights in possible H-bonding interactions of nucleobases in Figure 2, we optimized geometry of triplets formed by CR1 (same as ^{DA}N) and CR3 (same as CR2) with C-G base pair using *ab initio* calculations at the B3LYP/6-31G+(d,p) level of theory. The results (Figure S22A) showed that the CR1 nucleobase formed two H-bonds, one between the C=O of linker and -NH_2 of cytosine and the second, somewhat longer (2.11 Å) between -NH_2 of CR1 and N7 of guanosine. Most notably, ^{DA}N was tilted out of plane of C-G duplex by 12° angle. In contrast, the optimized geometries of CR3•C-G triplet converged on being planar (Figure S22B). Steric interactions between pyridine (H6) and guanosine (C=O) shifted CR3 towards cytidine and the PNA backbone resulting in only a weak interaction between pyridine N in CR3 and -NH_2 of cytidine observed at the distance of 2.42 Å and 127° angle.

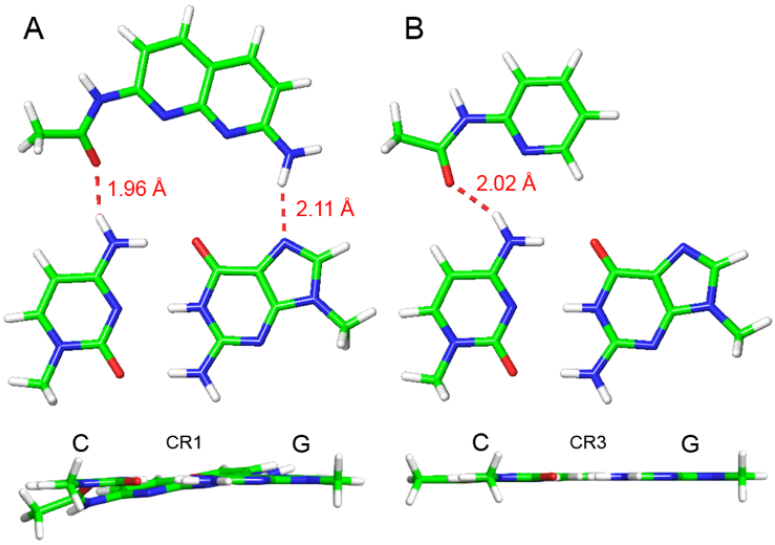


Figure S22. Geometry optimization of (A) CR1•C-G and (B) CR3•C-G triplets using B3LYP/6-31G+(d, p). Top view shows H-bond distances and side view shows planarity of extended system.

Molecular Dynamics

Molecular dynamics simulations were carried out with Maestro Version 12.6.144, Release 2020-4. PNA-dsRNA triple helix, constructed in our previous NMR structural studies,[4] was chosen as starting structure for the computational studies on modified PNA complexes with RNA. The sequence of interest was built by extending RNA and PNA strands to match the structure of PNA1-HRP3 triplex. The RNA hairpin loop

[4] Kotikam, V.; Kennedy, S. D.; MacKay, J. A.; Rozners, E. *Chem. Eur. J.* **2019**, *25*, 4367-4372.

and lysine were added from Maestro templates. Next, nucleobases were changed by hand to match the desired sequence. All extensions were achieved by copying existing pairs or triples to keep initial geometry and distances. Sequence was adjusted in the same way by copying nucleobases from the core strand, except for the V base, which was added manually according to optimized geometry from quantum calculations. Designed dsRNA-PNA triple helix was subjected to 5 ns molecular dynamics in MacroModel, with applying SHAKE to all bonds. Sampled structure with lowest energy was minimized applying AMBER* force field. For the 50 ns molecular dynamics simulation carried out in Desmond, minimized structure was solvated with explicit TIP4PD water molecules, in a orthorhombic box of 195088 $Å^3$ with 19 atoms of sodium added as counterions and mimicking 0.15 M NaCl solution. The final system is made by about 17800 atoms. Amber* force field was applied to all atoms.

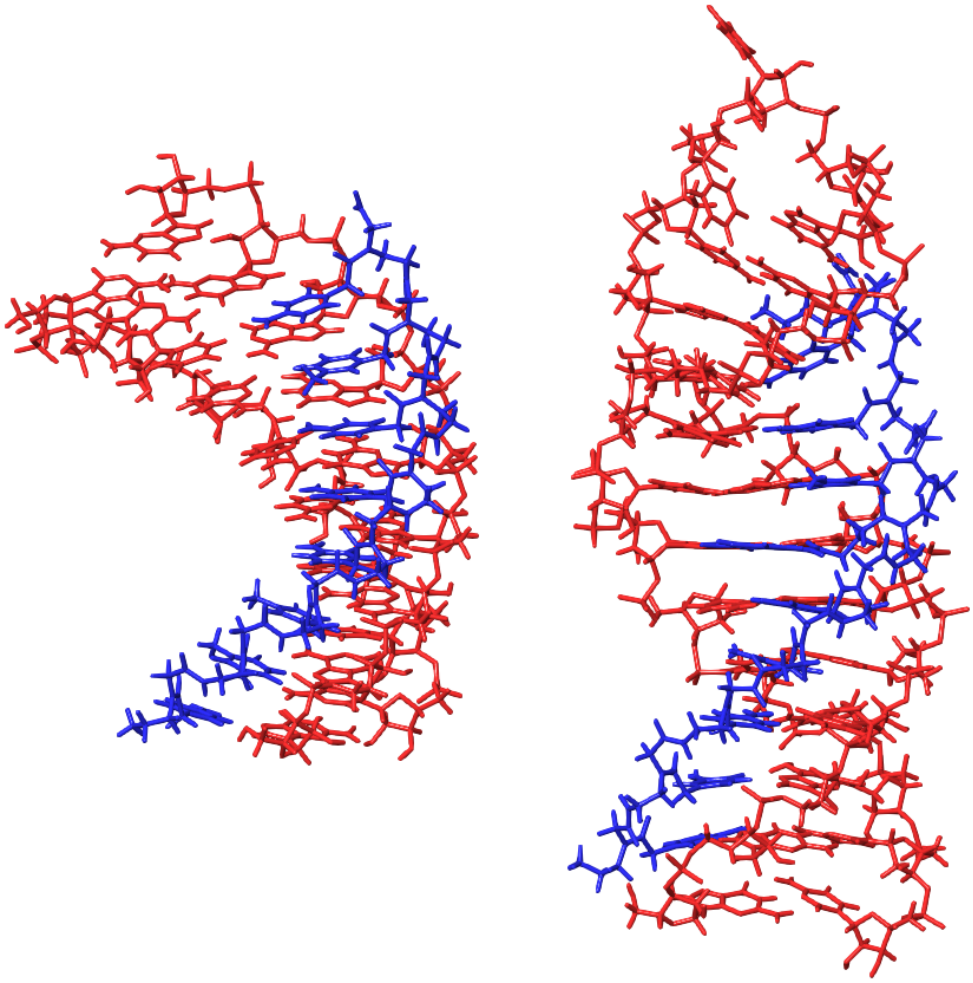


Figure 23. Triple helix derived from our previous NMR structural studies[4] (on the left) and structure used in MD simulation (on the right). RNA is colored in red and PNA in blue.

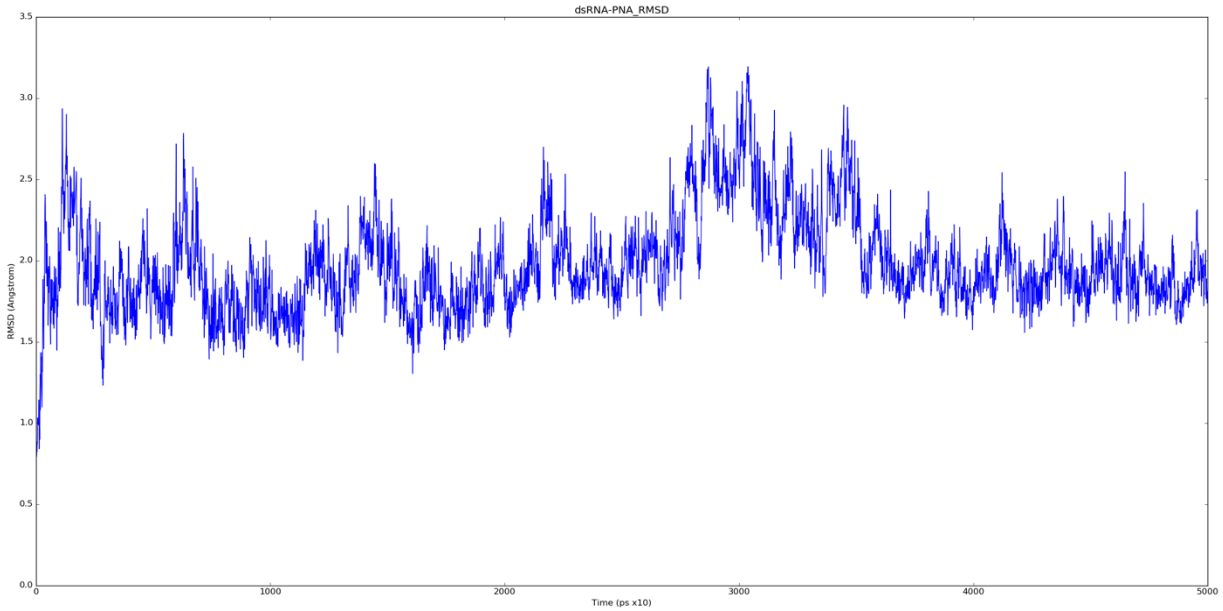


Figure S24. RMSD plot along with the molecular dynamics time for the whole dsRNA-PNA triple helix.

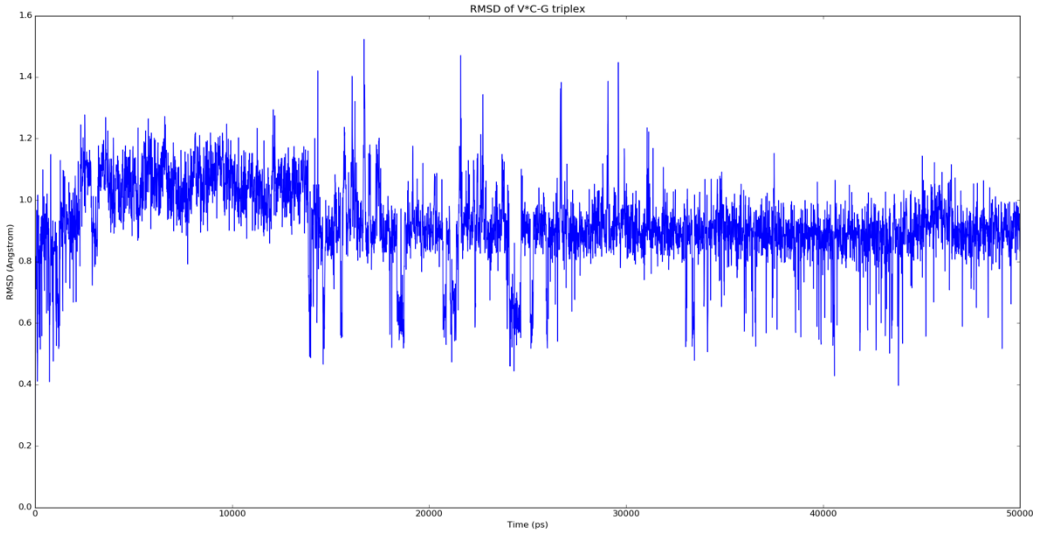


Figure S25. RMSD plot along with the molecular dynamics time for the V•C-G base triplex.

Table S15. Cartesian coordinates and total energies for geometry optimized base triplets CR1•C-G, Cr3•C-G, and V•C-G obtained at B3LYP/6-31G+(d,p) level.

Geometry optimized base triple ^{DA}N*C-G

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	2.689518	6.273285	0.336996
2	7	0	4.820532	-3.483439	-0.142699
3	6	0	3.812507	-4.402351	-0.182042
4	6	0	2.505720	-4.027031	-0.204818
5	6	0	2.227431	-2.620151	-0.187013
6	7	0	0.970422	-2.163451	-0.210763
7	7	0	3.225414	-1.716208	-0.147992
8	6	0	4.522461	-2.110274	-0.122561
9	8	0	5.472876	-1.297465	-0.081494
10	6	0	6.229096	-3.892721	-0.116254
11	7	0	1.879473	5.066057	0.219555
12	6	0	0.503060	4.986756	0.162386
13	7	0	0.056095	3.754464	0.053546
14	6	0	1.201357	2.974178	0.036959
15	6	0	1.373712	1.562087	-0.059221
16	8	0	0.491716	0.685084	-0.156565
17	7	0	2.727350	1.196412	-0.033580
18	6	0	3.787648	2.071491	0.069104
19	7	0	5.020777	1.528289	0.072777

20	7	0	3.636535	3.390107	0.158618
21	6	0	2.342865	3.775521	0.139400
22	6	0	-2.452306	-5.314499	0.700869
23	6	0	-2.673847	-3.882300	0.256081
24	8	0	-1.763204	-3.213304	-0.233977
25	7	0	-3.950955	-3.411607	0.453418
26	6	0	-4.259904	0.153903	0.015207
27	7	0	-3.705908	-1.074132	0.227417
28	6	0	-4.497813	-2.130586	0.213380
29	6	0	-5.903407	-2.075398	0.010669
30	6	0	-6.476838	-0.840200	-0.191220
31	6	0	-5.665315	0.314199	-0.199292
32	6	0	-6.155269	1.635394	-0.403796
33	7	0	-3.401034	1.206608	0.031736
34	6	0	-5.287457	2.689632	-0.388420
35	6	0	-3.888905	2.433634	-0.162088
36	7	0	-3.023883	3.480657	-0.127151
37	1	0	3.314280	6.220707	1.231381
38	1	0	4.115800	-5.442726	-0.194347
39	1	0	1.713601	-4.763480	-0.236972
40	1	0	0.799674	-1.153087	-0.193391
41	1	0	0.168261	-2.787151	-0.221254
42	1	0	6.755327	-3.479586	-0.978877
43	1	0	-0.110463	5.876250	0.206996
44	1	0	2.920666	0.176834	-0.086801
45	1	0	5.177934	0.514864	0.016230
46	1	0	5.806155	2.154582	0.161494
47	1	0	-3.377285	-5.863883	0.889728
48	1	0	-1.869411	-5.834608	-0.062311
49	1	0	-4.627618	-4.106766	0.738895
50	1	0	-6.497467	-2.982929	0.008900
51	1	0	-7.547489	-0.747090	-0.351653
52	1	0	3.328017	6.387441	-0.542150
53	1	0	2.023510	7.132962	0.412883

54	1	0	6.708330	-3.529753	0.795143
55	1	0	6.275779	-4.980954	-0.145761
56	1	0	-1.862222	-5.302268	1.623457
57	1	0	-5.630516	3.707732	-0.541426
58	1	0	-7.217439	1.792234	-0.571294
59	1	0	-3.353847	4.405622	-0.355735
60	1	0	-2.014274	3.346831	-0.056967

E(RB3LYP) = -1697.68295268 Hartree

Geometry optimized base triple Cr2(3)*C-G

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-6.802456	-2.401654	0.022506
2	7	0	0.257784	4.671646	-0.012094
3	6	0	1.581913	4.336728	0.003430
4	6	0	1.989360	3.040348	0.012704
5	6	0	0.967740	2.032138	0.004486
6	7	0	1.287928	0.732947	0.009476
7	7	0	-0.338412	2.365767	-0.008485
8	6	0	-0.722306	3.666306	-0.016207
9	8	0	-1.925199	4.014162	-0.027374
10	6	0	-0.178423	6.072027	-0.023350
11	7	0	-5.344765	-2.436958	0.013756
12	6	0	-4.534608	-3.556965	0.011253
13	7	0	-3.251319	-3.274663	0.004150
14	6	0	-3.207183	-1.889335	0.001946
15	6	0	-2.103819	-0.985580	-0.004208
16	8	0	-0.884548	-1.255396	-0.009408
17	7	0	-2.522721	0.353660	-0.003806

18	6	0	-3.834622	0.778649	0.002010
19	7	0	-4.039982	2.110215	0.002829
20	7	0	-4.869922	-0.056254	0.007959
21	6	0	-4.500676	-1.354759	0.007875
22	6	0	6.658257	0.774831	0.029325
23	6	0	5.317333	0.066166	0.030283
24	8	0	4.264704	0.707064	0.055765
25	7	0	5.394918	-1.304925	-0.000139
26	6	0	2.173734	-2.898015	-0.026042
27	7	0	3.101083	-1.921302	-0.016056
28	6	0	4.384286	-2.288958	-0.009675
29	6	0	4.801279	-3.634219	-0.014164
30	6	0	3.829513	-4.625922	-0.025681
31	6	0	2.479848	-4.256355	-0.031481
32	1	0	-7.162660	-1.886202	0.915923
33	1	0	2.282127	5.163977	0.007908
34	1	0	3.037063	2.771945	0.025109
35	1	0	0.542307	0.033855	0.001944
36	1	0	2.260011	0.425825	0.017909
37	1	0	-0.776389	6.271376	-0.914823
38	1	0	-4.960547	-4.551157	0.014861
39	1	0	-1.765507	1.064629	-0.007029
40	1	0	-3.269157	2.789997	-0.009810
41	1	0	-4.995369	2.432929	0.001356
42	1	0	7.516527	0.099984	0.021644
43	1	0	6.707932	1.423057	-0.850342
44	1	0	6.334264	-1.678876	-0.011288
45	1	0	5.856990	-3.886610	-0.008482
46	1	0	4.120736	-5.671685	-0.029496
47	1	0	1.688842	-4.998217	-0.039992
48	1	0	-7.173179	-1.884130	-0.865352
49	1	0	-7.175037	-3.426253	0.023510
50	1	0	-0.783651	6.284105	0.860352
51	1	0	0.703867	6.711561	-0.024457

52	1	0	6.714830	1.413304	0.915420
53	1	0	1.139180	-2.563561	-0.029923

E(RB3LYP) = -1472.60730525 Hartree

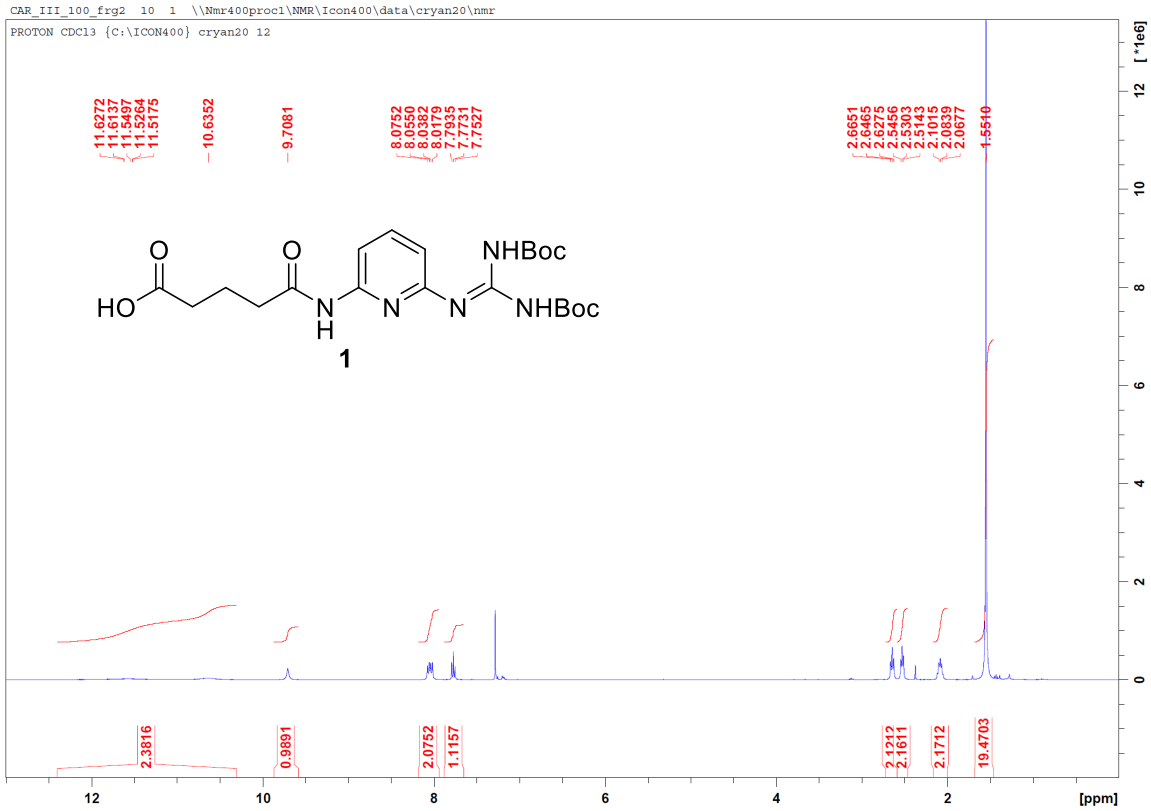
Geometry optimized base triple V*C-G

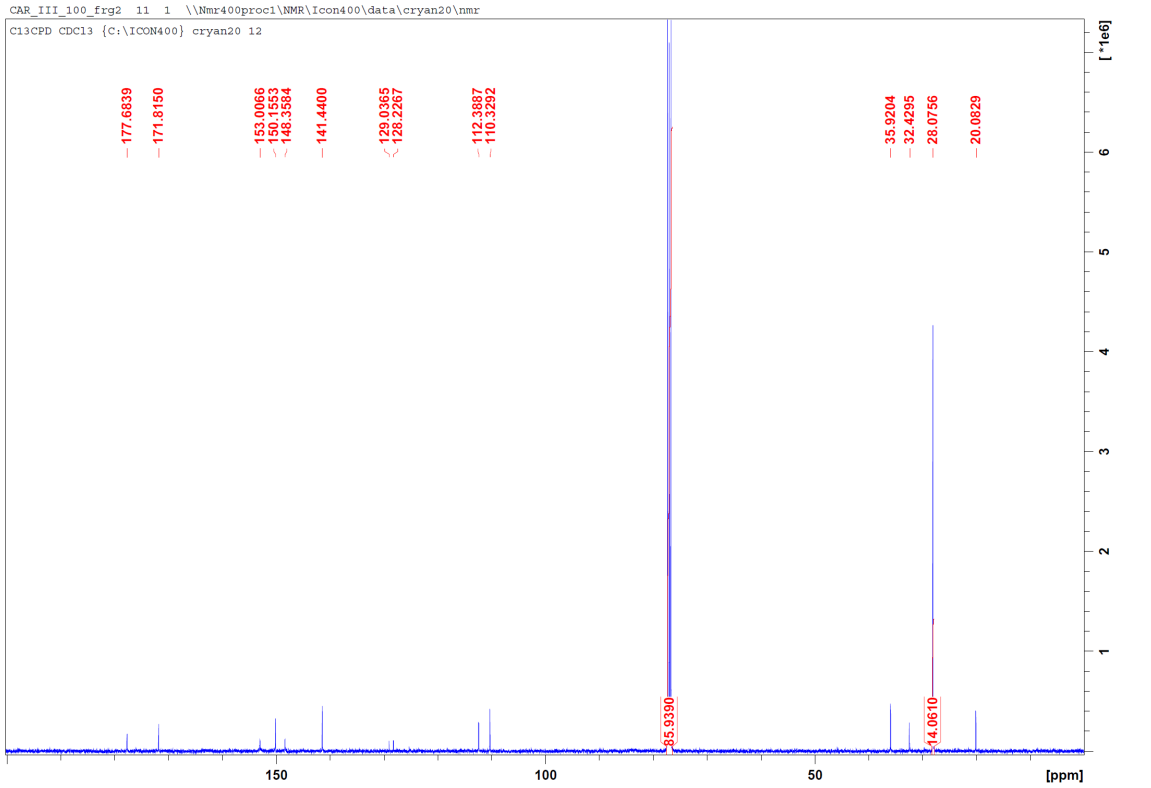
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-3.798528	-5.808714	0.042843
2	7	0	-3.990482	4.193249	0.102896
3	6	0	-2.824454	4.901166	0.092952
4	6	0	-1.614332	4.282993	0.029930
5	6	0	-1.614287	2.851226	-0.025002
6	7	0	-0.472419	2.155490	-0.086564
7	7	0	-2.767591	2.155034	-0.017736
8	6	0	-3.964521	2.789478	0.046257
9	8	0	-5.053203	2.173445	0.057371
10	6	0	-5.292725	4.865329	0.175712
11	7	0	-2.779547	-4.764536	0.001191
12	6	0	-1.411514	-4.936894	-0.028612
13	7	0	-0.745134	-3.802638	-0.063634
14	6	0	-1.729147	-2.827287	-0.056241
15	6	0	-1.640978	-1.412578	-0.083230
16	8	0	-0.600721	-0.711920	-0.126782
17	7	0	-2.888026	-0.794002	-0.056292

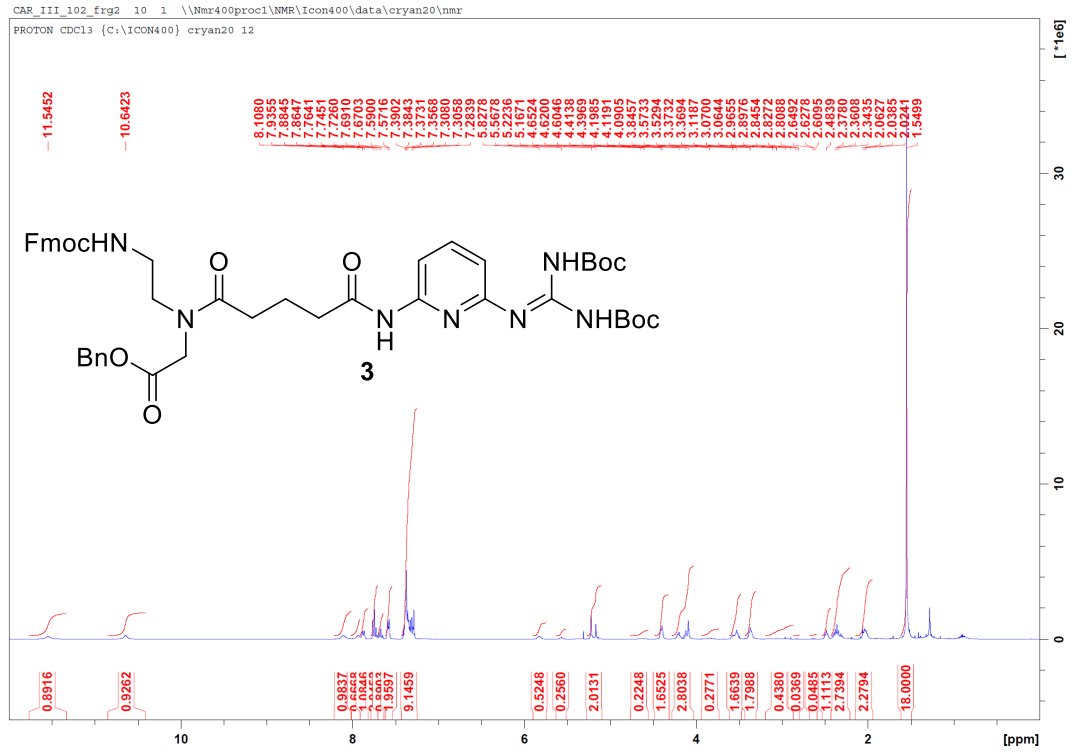
18	6	0	-4.096846	-1.459235	-0.013476
19	7	0	-5.202875	-0.693427	0.002159
20	7	0	-4.195460	-2.786844	0.006365
21	6	0	-2.999952	-3.408612	-0.015110
22	6	0	3.318162	4.307322	-0.229479
23	6	0	3.513054	2.808032	-0.133904
24	8	0	2.549159	2.039214	-0.103661
25	7	0	4.829052	2.404435	-0.082338
26	6	0	5.086542	-1.158687	0.080188
27	7	0	4.563066	0.065131	-0.006545
28	6	0	5.383323	1.117775	0.013023
29	6	0	6.776001	0.991364	0.118458
30	6	0	7.303829	-0.295363	0.202889
31	6	0	6.461016	-1.404819	0.185504
32	7	0	4.197450	-2.248981	0.061100
33	6	0	2.830713	-2.243262	-0.004952
34	7	0	2.147196	-1.113631	-0.076491
35	7	0	2.206209	-3.426906	0.002191
36	1	0	-4.400004	-5.710061	0.949109
37	1	0	-2.921259	5.979555	0.138214
38	1	0	-0.694978	4.853667	0.023409
39	1	0	-0.513006	1.133866	-0.110673
40	1	0	0.443107	2.590612	-0.085785
41	1	0	-5.893575	4.613821	-0.700310
42	1	0	-0.970435	-5.923974	-0.021619
43	1	0	-2.877251	0.247223	-0.057241
44	1	0	-5.158991	0.332688	0.020166
45	1	0	-6.094798	-1.160719	0.060001
46	1	0	4.250961	4.872686	-0.274621
47	1	0	2.726051	4.524600	-1.122849
48	1	0	5.511452	3.150103	-0.104283
49	1	0	7.418144	1.865044	0.134511
50	1	0	8.376765	-0.434408	0.284541
51	1	0	6.851403	-2.414104	0.252359

52	1	0	4.633389	-3.159786	0.114545
53	1	0	1.119251	-1.120042	-0.104159
54	1	0	1.177395	-3.501821	-0.030630
55	1	0	2.732354	-4.284878	0.077221
56	1	0	2.661253	-0.229085	-0.080230
57	1	0	-4.447807	-5.733980	-0.832158
58	1	0	-3.302052	-6.778943	0.042450
59	1	0	-5.827887	4.548651	1.073019
60	1	0	-5.126893	5.941681	0.209044
61	1	0	2.743340	4.639669	0.639919

E(RB3LYP) = -1677.30121913 Hartree


CAR_III_100_frg2 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 12
11.6272
11.6137
11.5497
11.5264
11.5175
10.6352
9.7081
8.0752
8.0550
8.0382
8.0179
7.7935
7.7731
7.7527
2.6651
2.6465
2.6275
2.5456
2.5303
2.5143
2.1015
2.0839
2.0677
1.5510
HO
O
O
N
H
N
N
NHBoc
NHBoc
1
2.3816
0.9891
2.0752
1.1157
2.1212
2.1611
2.1712
19.4703
[*1e6]
[ppm]


CAR_III_100_frg2 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDCl3 {C:\ICON400} cryan20 12
177.6839
171.8150
153.0066
150.1553
148.3584
141.4400
129.0365
128.2267
112.3887
110.3292
35.9204
32.4295
28.0756
20.0829
85.9390
14.0610
[*1e6]
[ppm]


CAR_III_102_frg2 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
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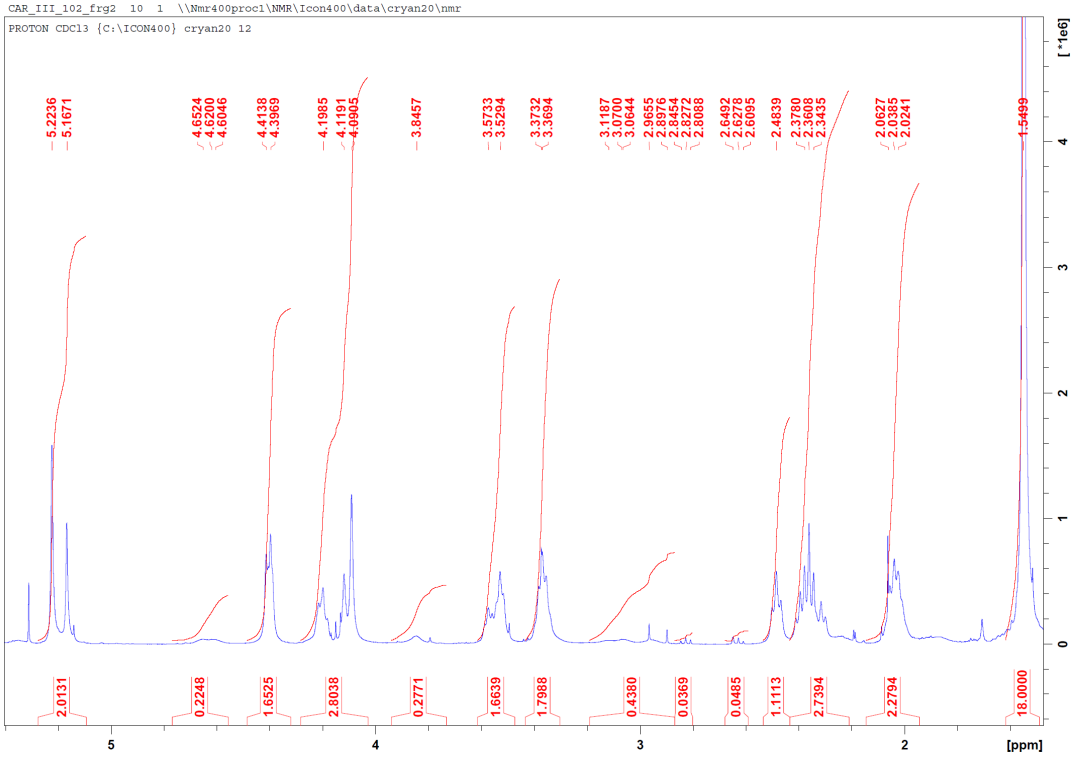
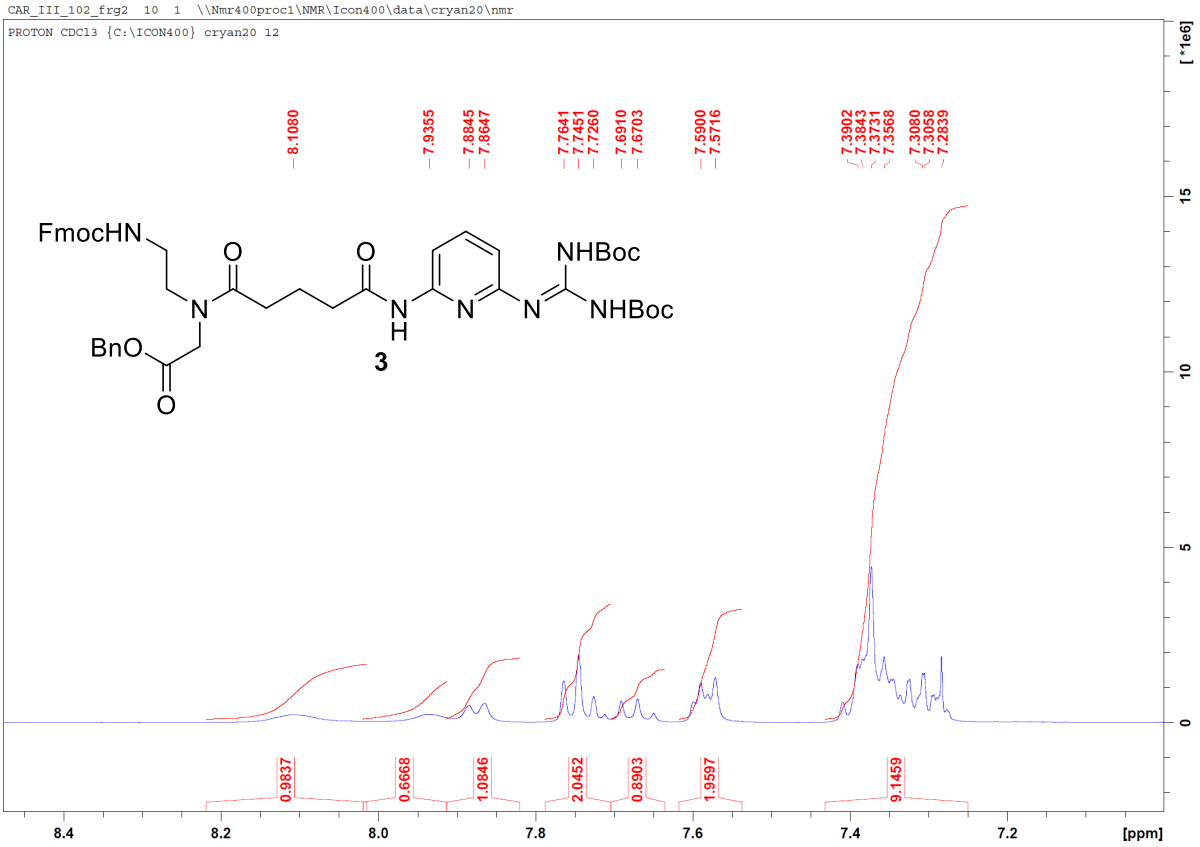
FmocHN
BnO
NHBoc
NHBoc
3
[*1e6]
[ppm]


CAR_III_102_frg2 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 12
[*1e6]
[ppm]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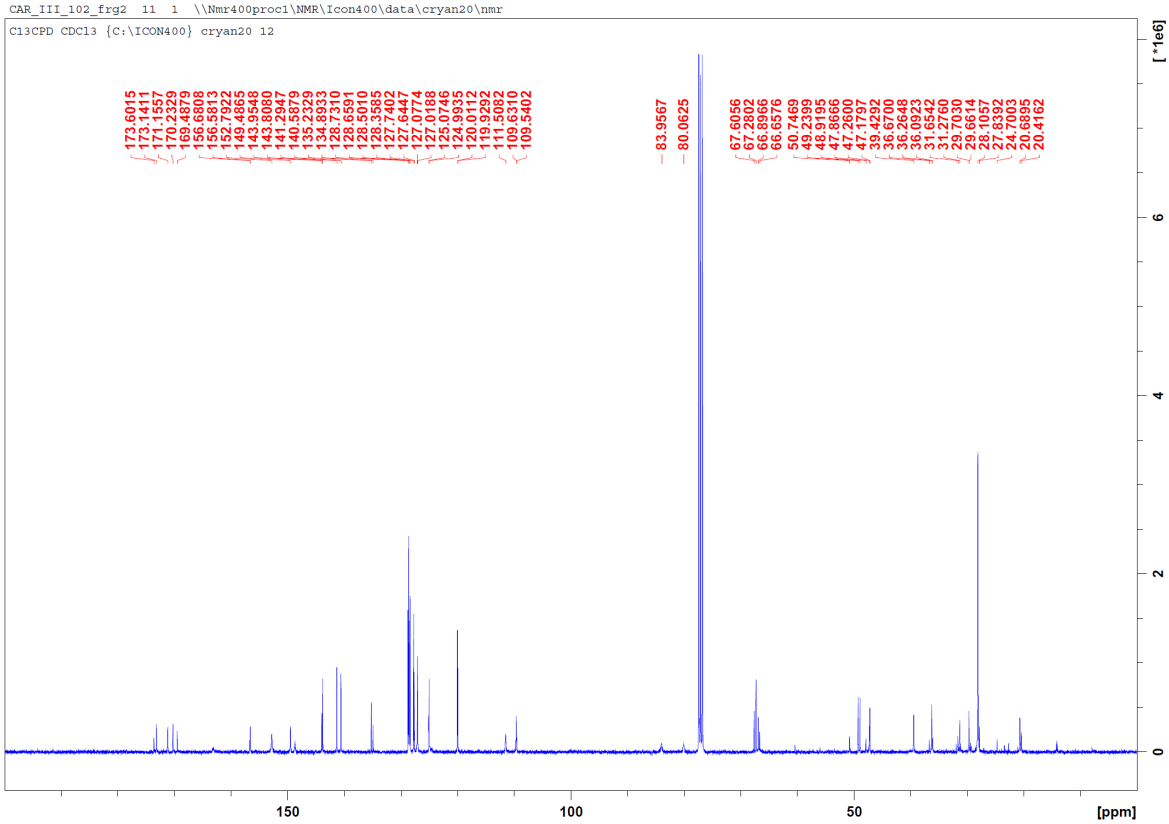
PROTON CDCl3 {C:\ICON400} cryan20 12
8.1080
7.9355
7.8845
7.8647
7.7641
7.7451
7.7260
7.6910
7.6703
7.5900
7.5716
7.3902
7.3843
7.3731
7.3568
7.3080
7.3058
7.2839
FmocHN
O
O
N
N
H
N
N
NHBoc
NHBoc
BnO
O
3
0.9837
0.6668
1.0846
2.0452
0.8903
1.9597
9.1459
[*1e6]
15
10
5
0
8.4
8.2
8.0
7.8
7.6
7.4
7.2
[ppm]


CAR_III_102_frg2 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDCl3 {C:\ICON400} cryan20 12
173.6015
173.1411
171.1557
170.2329
169.4879
156.6808
156.5813
152.7922
149.4865
143.9548
143.8080
141.2947
140.5879
135.2329
134.8933
128.7310
128.6591
128.5010
128.3585
127.7402
127.6447
127.0774
127.0188
125.0746
124.9935
120.0112
119.9292
111.5082
109.6310
109.5402
83.9567
80.0625
67.6056
67.2802
66.8966
66.6576
50.7469
49.2399
48.9195
47.8666
47.2600
47.1797
46.4792
38.4700
36.2644
36.0923
31.6542
31.2760
29.7030
29.6614
28.1057
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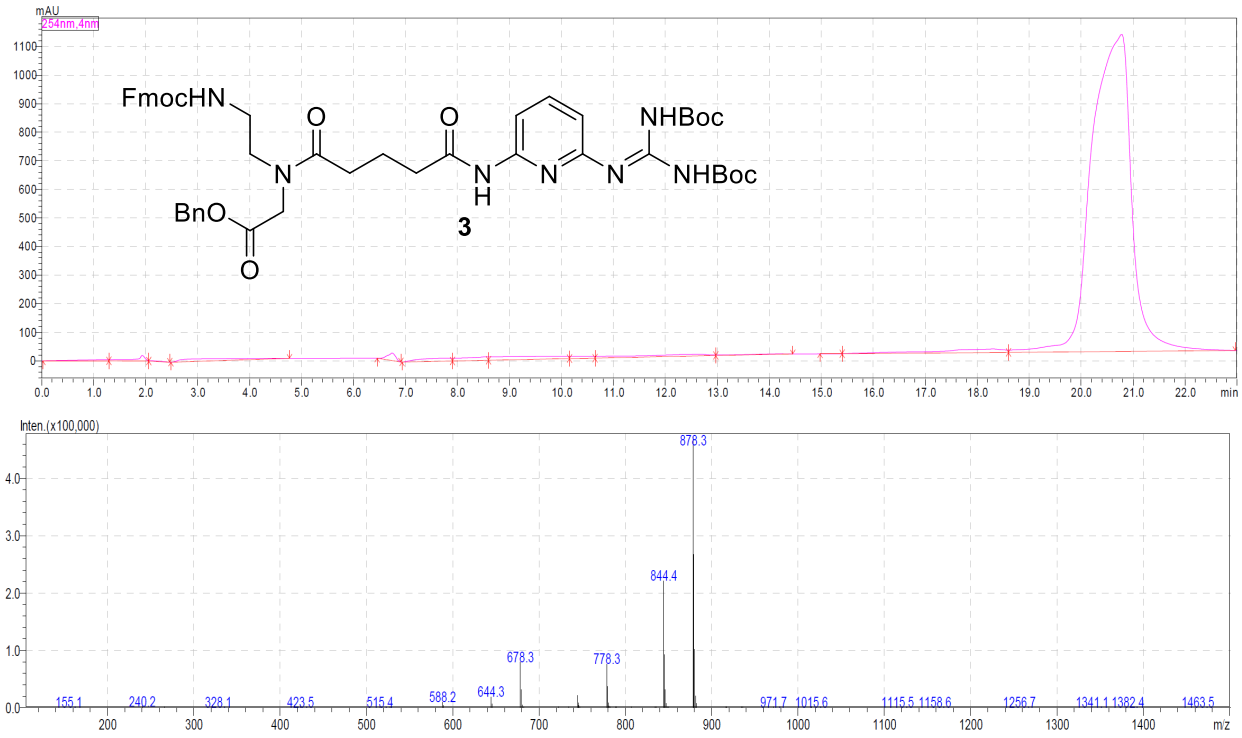
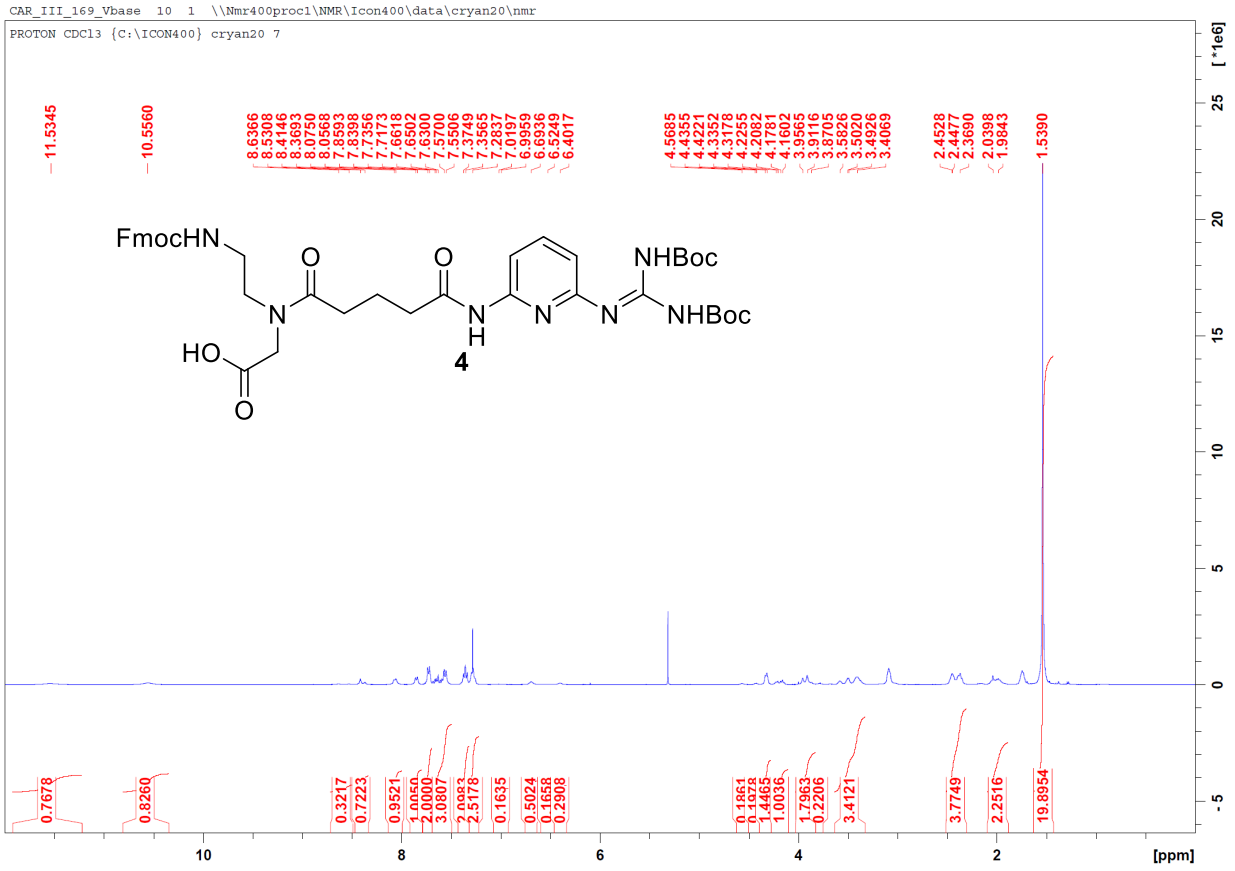
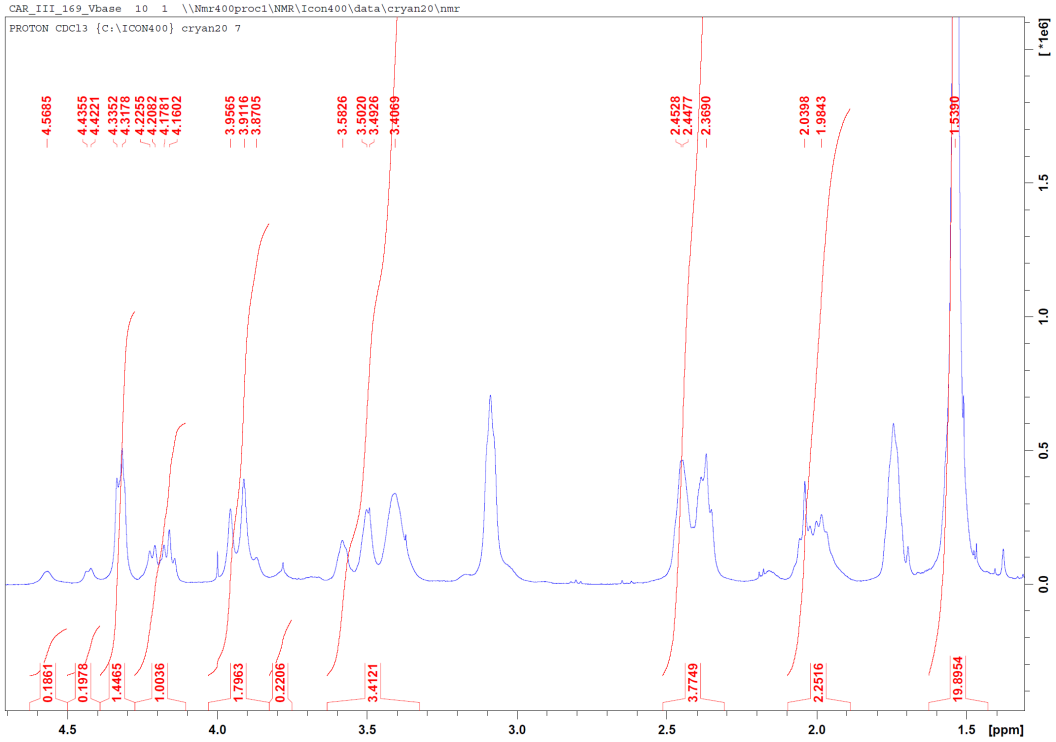
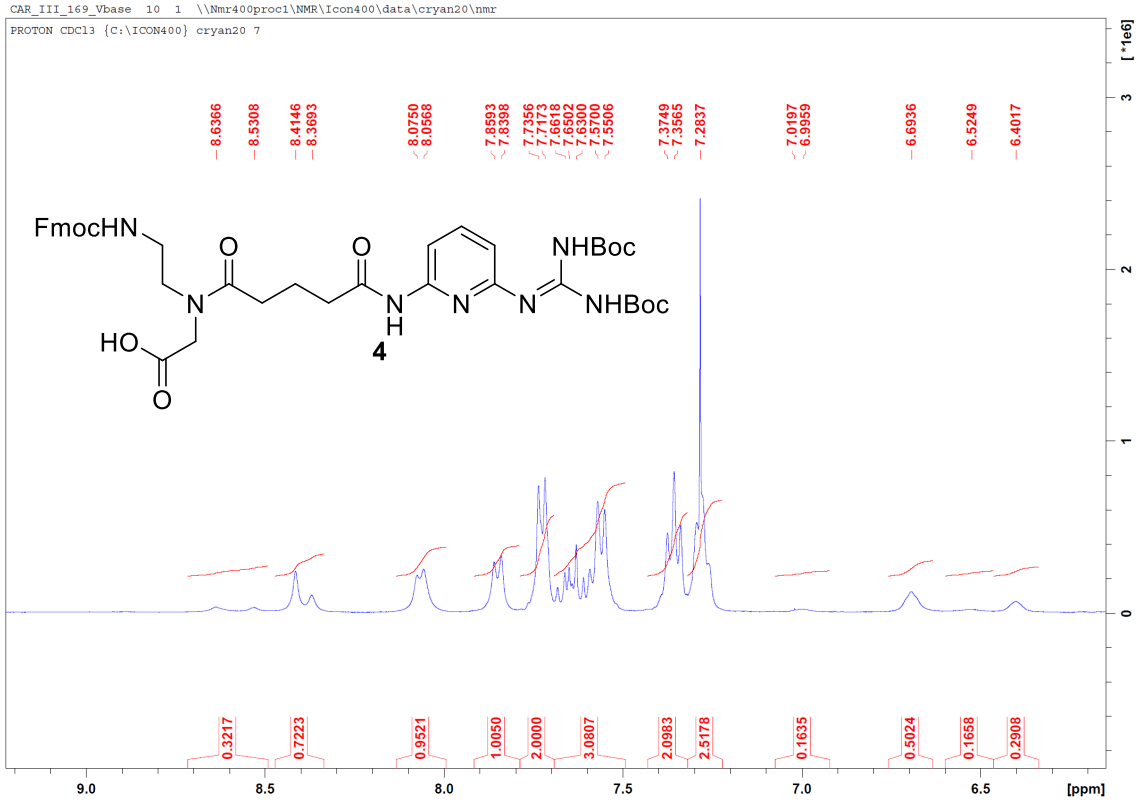
24.7003
20.6895
20.4162
[*1e6]
6
4
2
0
150
100
50
[ppm]

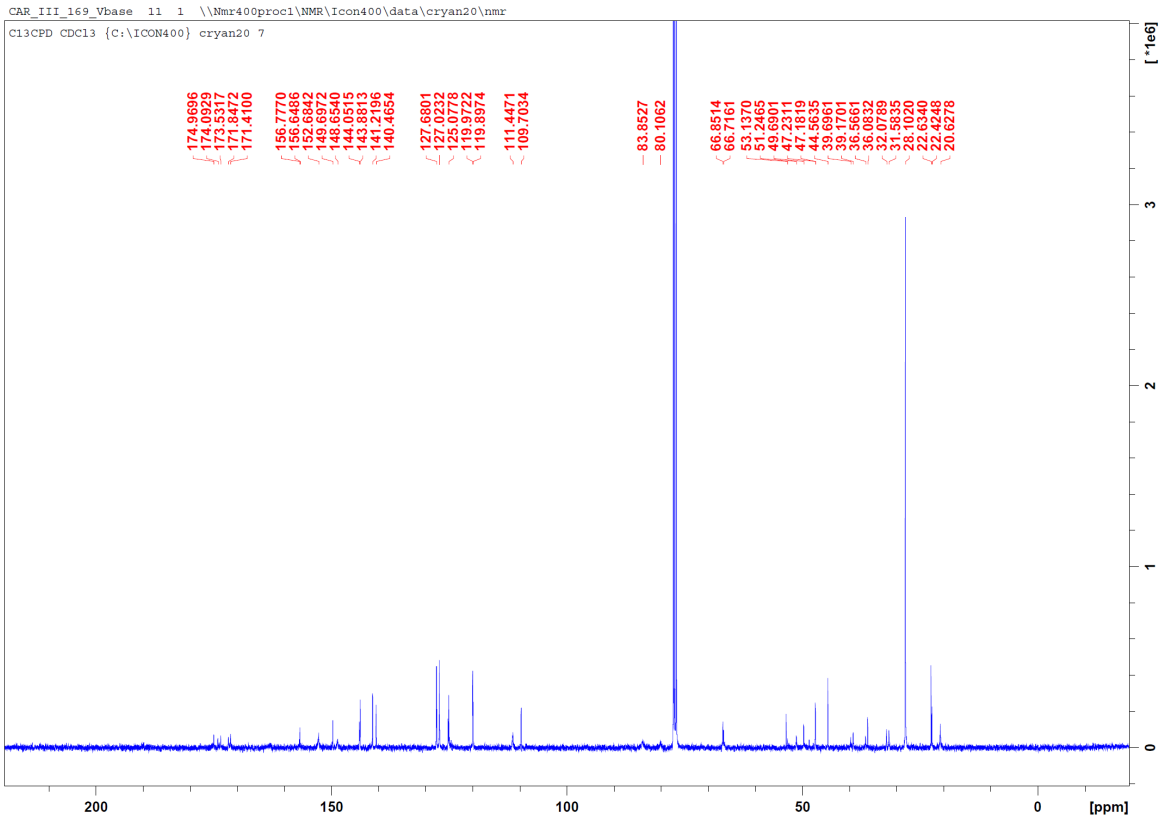


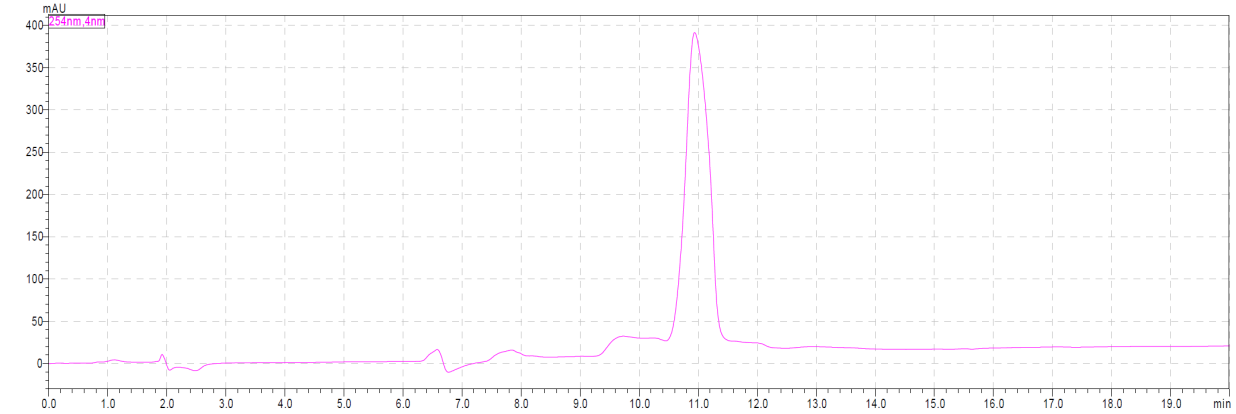
Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O, 18 mins, 100% ACN, 5 mins, 0.2 mL/min.

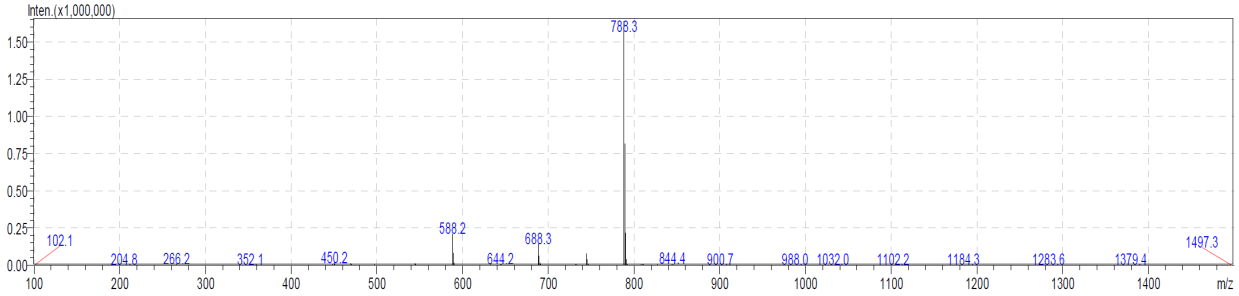

CAR_III_169_Vbase 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 7
FmocHN
HO
NHBoc
NHBoc
4
[ppm]


CAR_III_169_Vbase 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 7
[ppm]

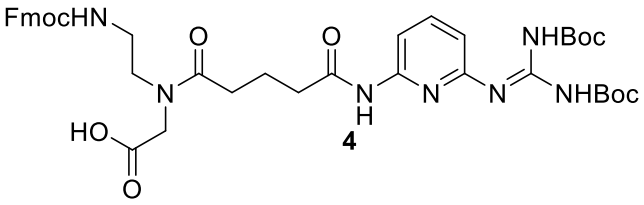

CAR_III_169_Vbase 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 7
8.6366
8.5308
8.4146
8.3693
8.0750
8.0568
7.8593
7.8398
7.7356
7.7173
7.6618
7.6502
7.6300
7.5700
7.5506
7.3749
7.3565
7.2837
7.0197
6.9959
6.6936
6.5249
6.4017
FmocHN
O
O
NHBoc
N
N
H
N
N
NHBoc
HO
O
4
0.3217
0.7223
0.9521
1.0050
2.0000
3.0807
2.0983
2.5178
0.1635
0.5024
0.1658
0.2908
9.0
8.5
8.0
7.5
7.0
6.5
[ppm]
[*1e6]


CAR_III_169_Vbase 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDCl3 {C:\ICON400} cryan20 7
174.9696
174.0929
173.5317
171.8472
171.4100
156.7770
156.6486
152.6842
149.6972
148.6540
144.0515
143.8813
141.2196
140.4654
127.6801
127.0232
125.0778
119.9722
119.8974
111.4471
109.7034
83.8527
80.1062
66.8514
66.7161
53.1370
51.2465
49.6901
47.2311
47.1819
44.5635
39.1761
36.5661
36.0832
32.0789
31.5835
28.1020
22.6340
22.4248
20.6278
200
150
100
50
0
[ppm]
[*1e6]

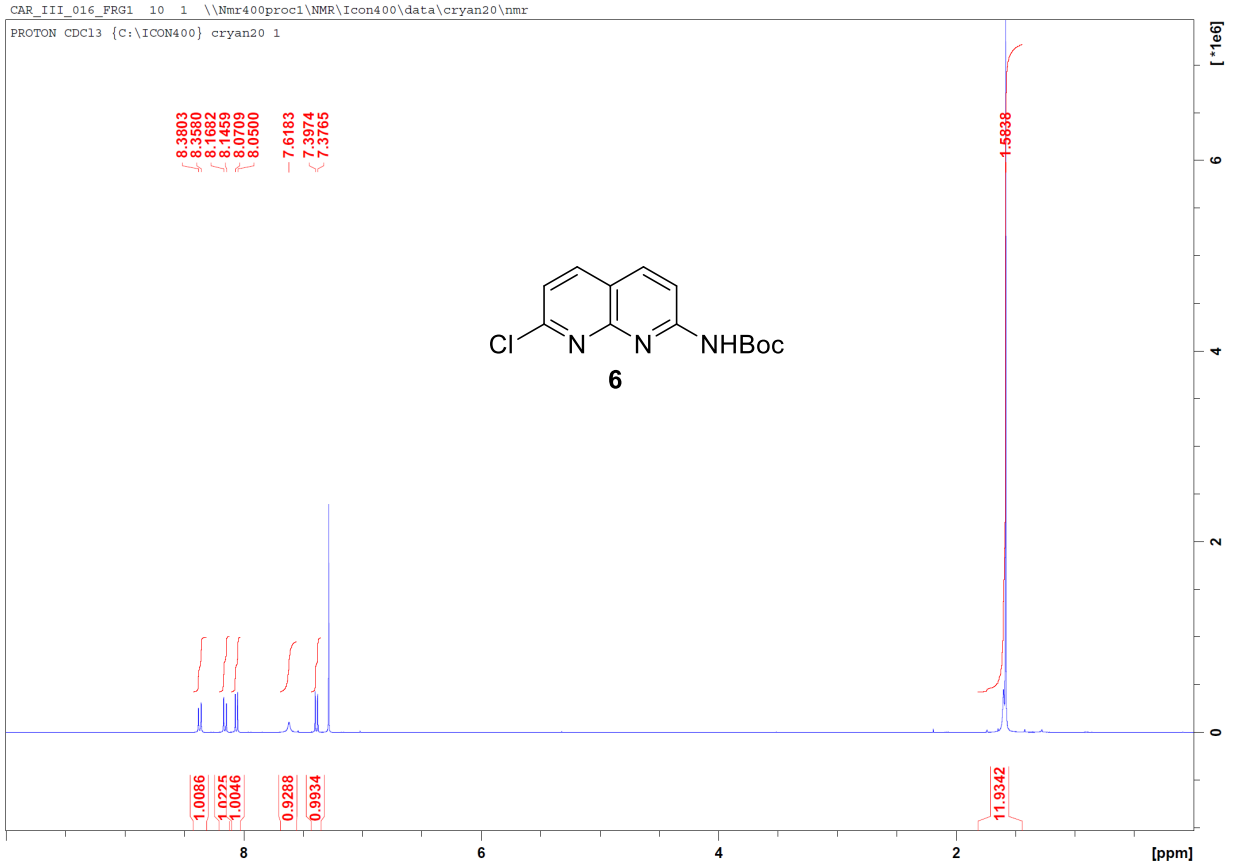


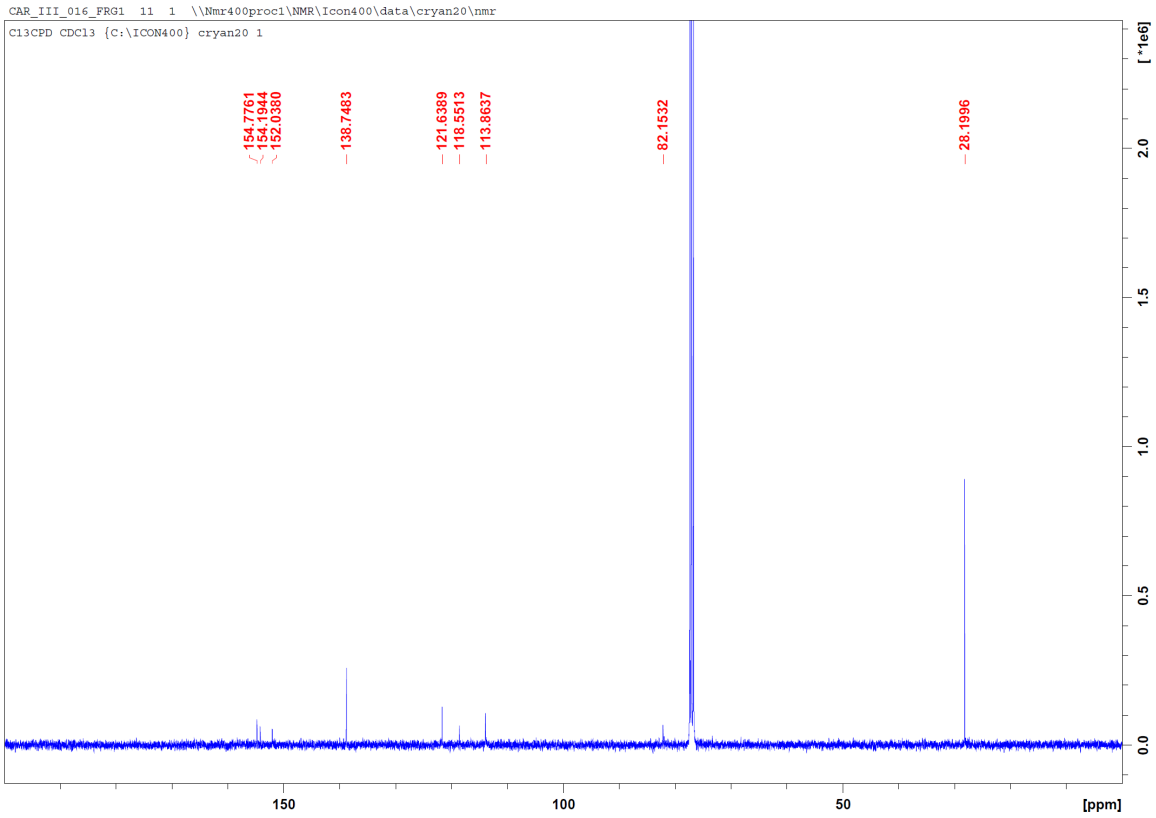


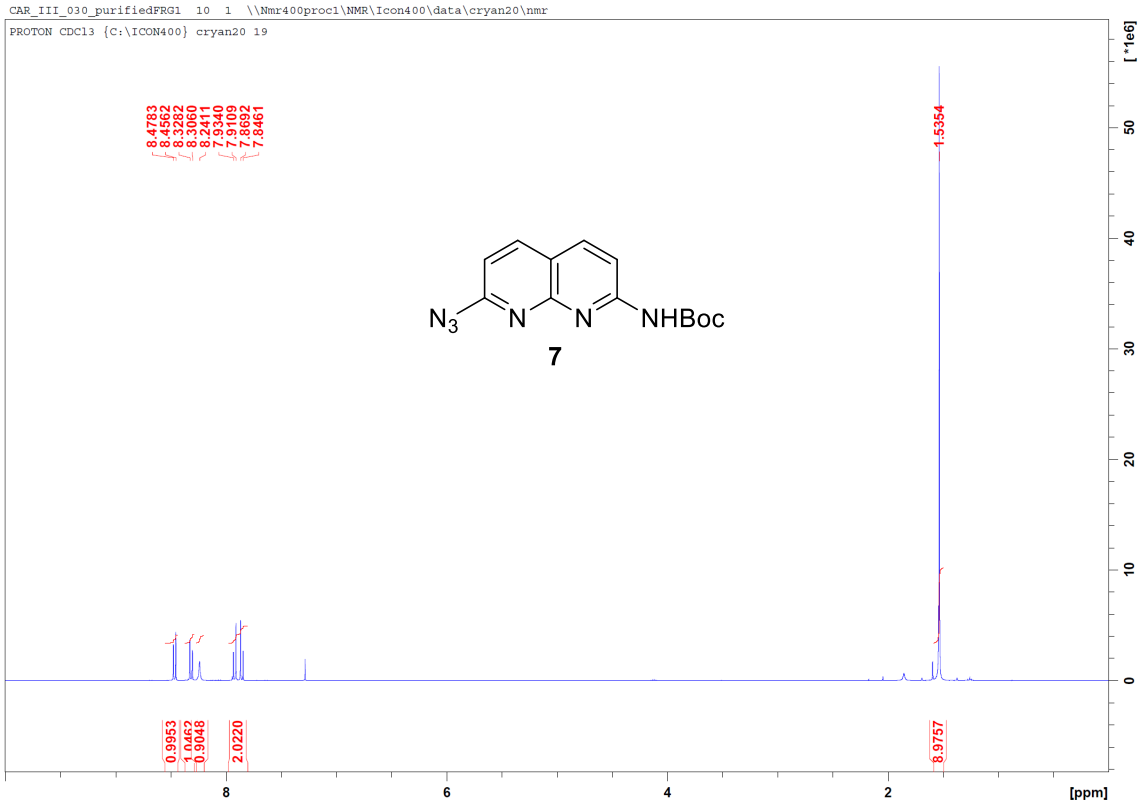
Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O, 18 mins, 0.2 mL/min.



4


CAR_III_016_FRG1 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 1
8.3803
8.3580
8.1682
8.1459
8.0709
8.0500
7.6183
7.3974
7.3765
1.5838
Cl
N
N
NHBoc
6
1.0086
1.0225
1.0046
0.9288
0.9934
11.9342
8
6
4
2
[ppm]
[*1e6]


CAR_III_016_FRG1 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDCl3 {C:\ICON400} cryan20 1
154.7761
154.1944
152.0380
138.7483
121.6389
118.5513
113.8637
82.1532
28.1996
150
100
50
[ppm]
[*1e6]


CAR_III_030_purifiedFRG1 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 19
8.4783
8.4562
8.3282
8.3060
8.2411
7.9340
7.9109
7.8692
7.8461
1.5354
N3
N
N
NHBoc
7
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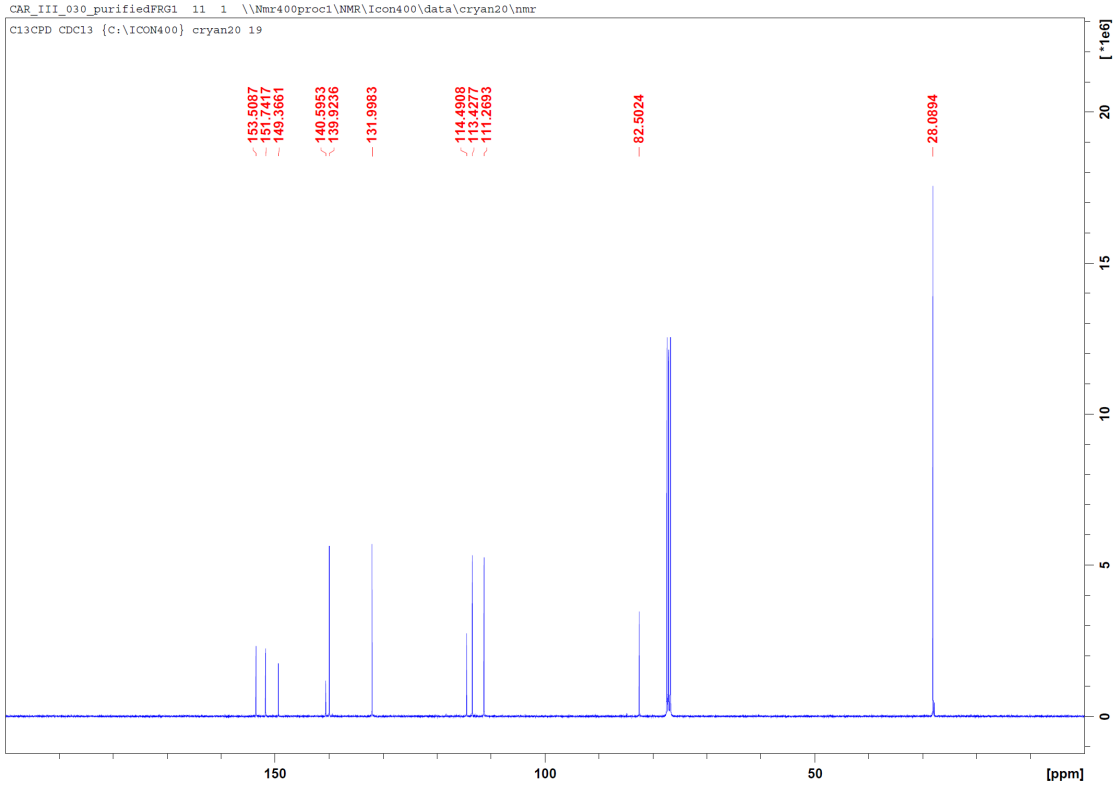
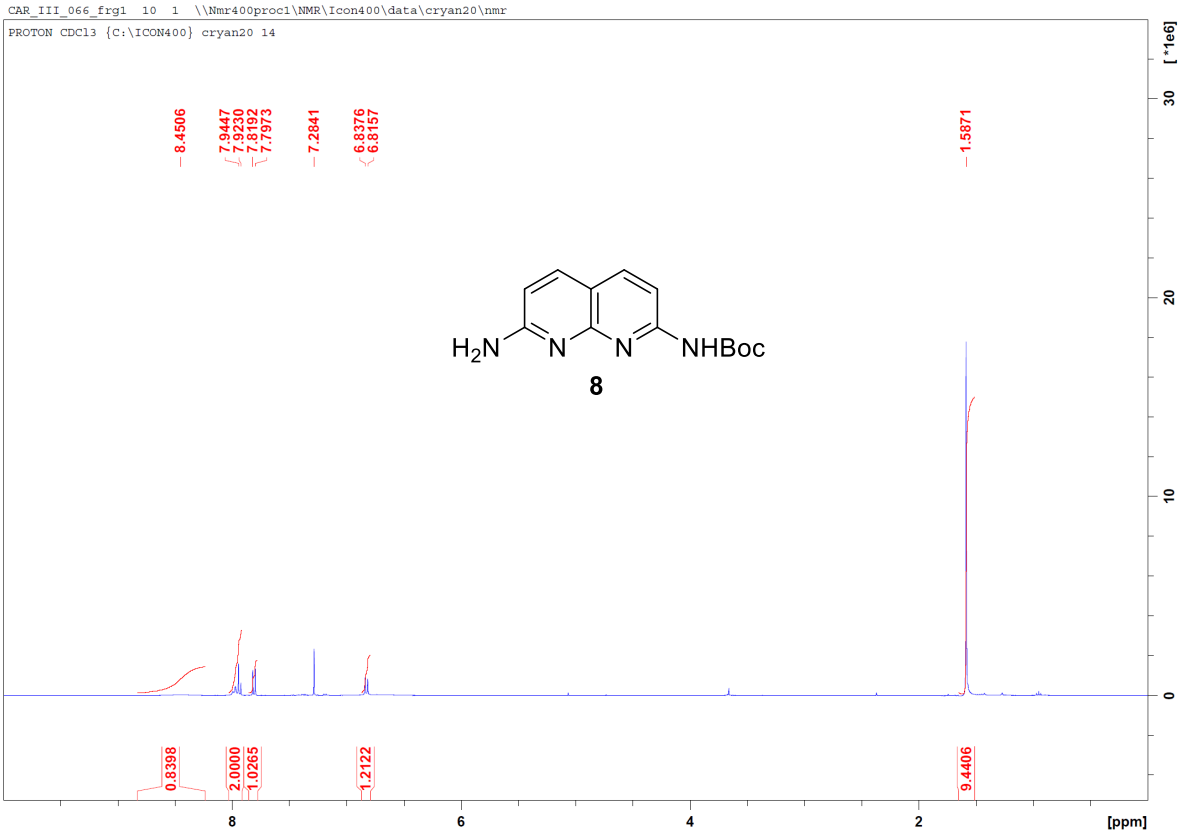
1.0462
0.9048
2.0220
8.9757
8
6
4
2
[ppm]
[*1e6]


CAR_III_030_purifiedFRG1 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDCl3 {C:\ICON400} cryan20 19
153.5087
151.7417
149.3661
140.5953
139.9236
131.9983
114.4908
113.4277
111.2693
82.5024
28.0894
150
100
50
[ppm]
[*1e6]


CAR_III_066_frg1 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 14
8.4506
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7.9230
7.8192
7.7973
7.2841
6.8376
6.8157
1.5871
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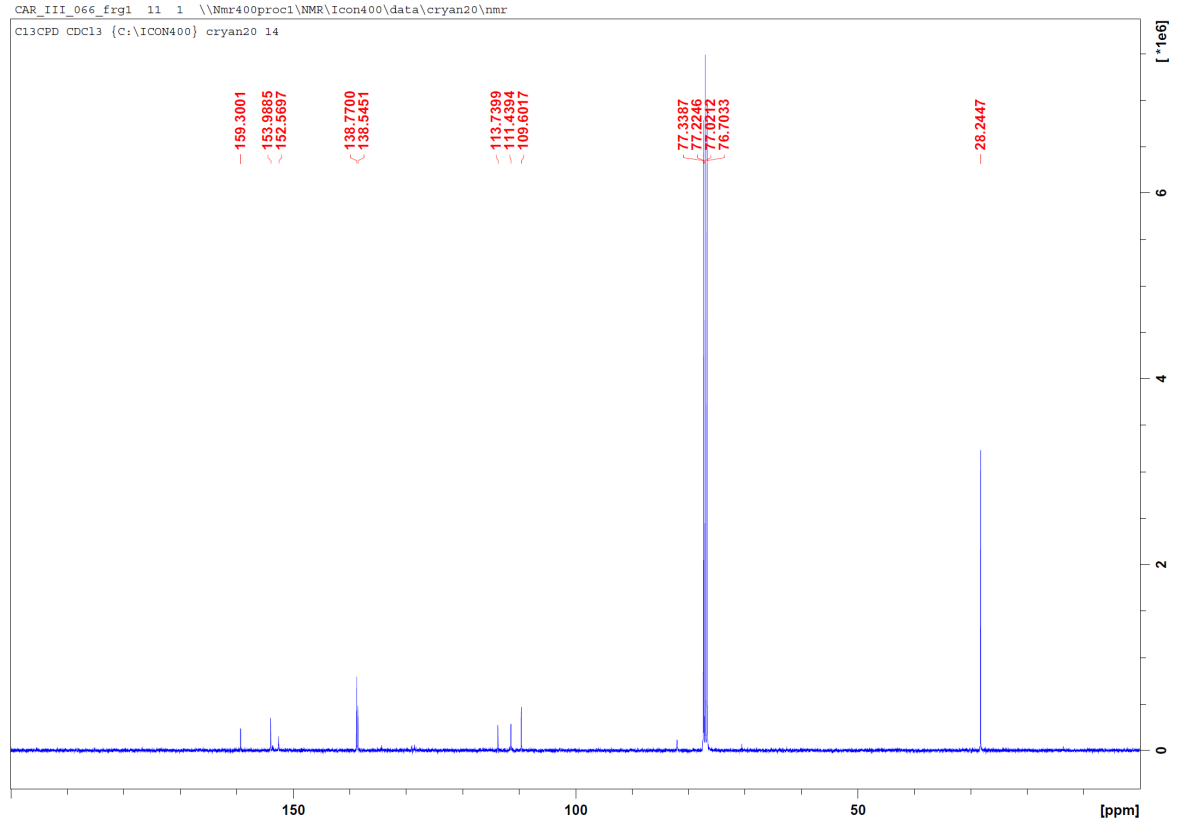
N
NHBoc
8
0.8398
2.0000
1.0265
1.2122
9.4406
[*1e6]
[ppm]


CAR_III_066_frg1 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDCl3 {C:\ICON400} cryan20 14
159.3001
153.9885
152.5697
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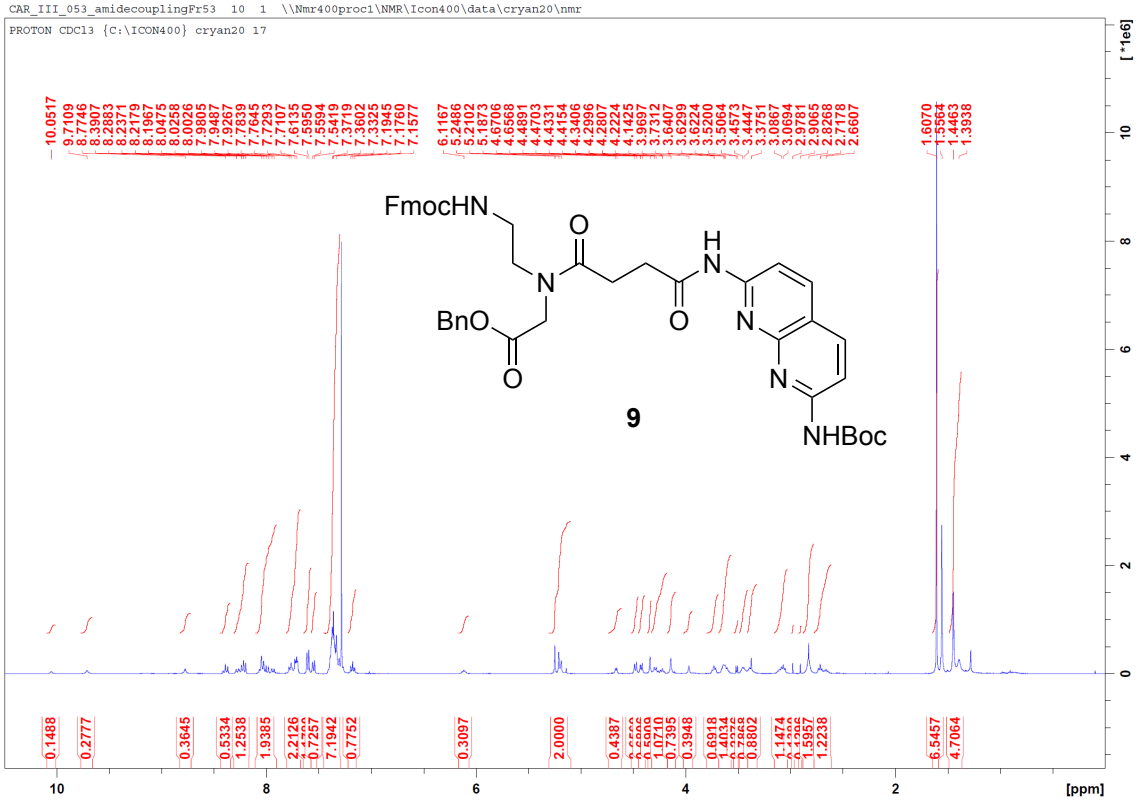
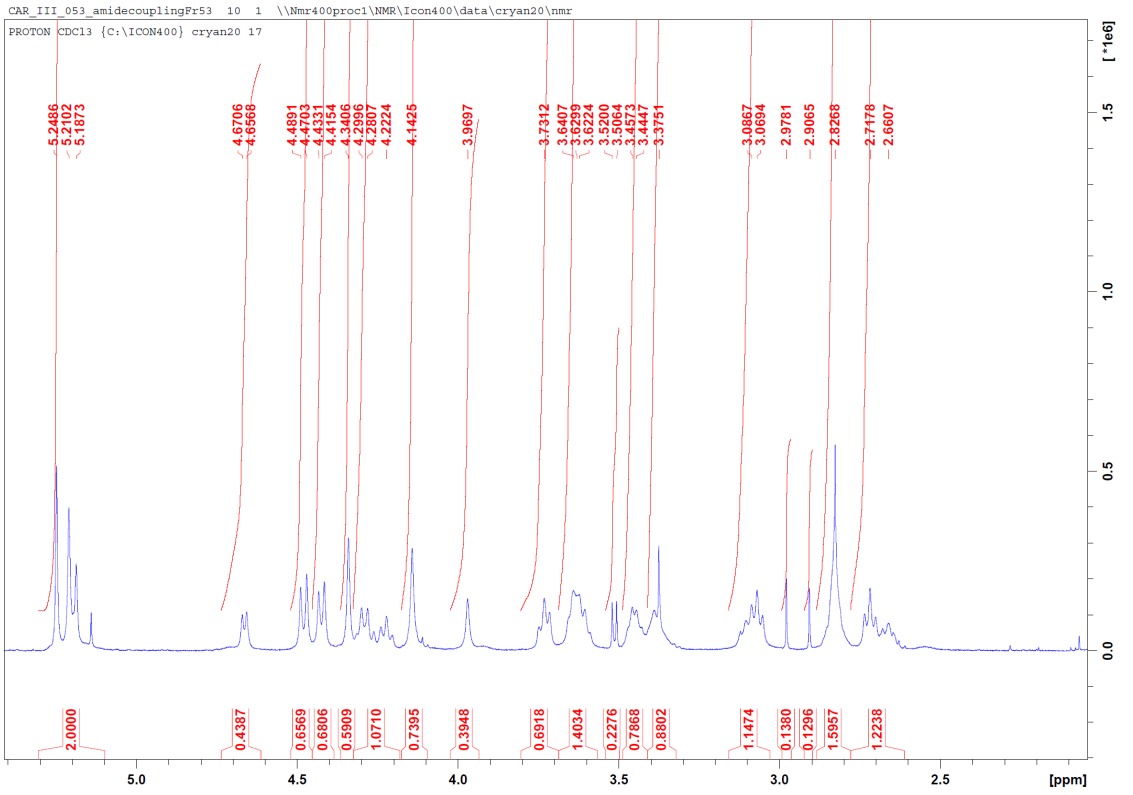
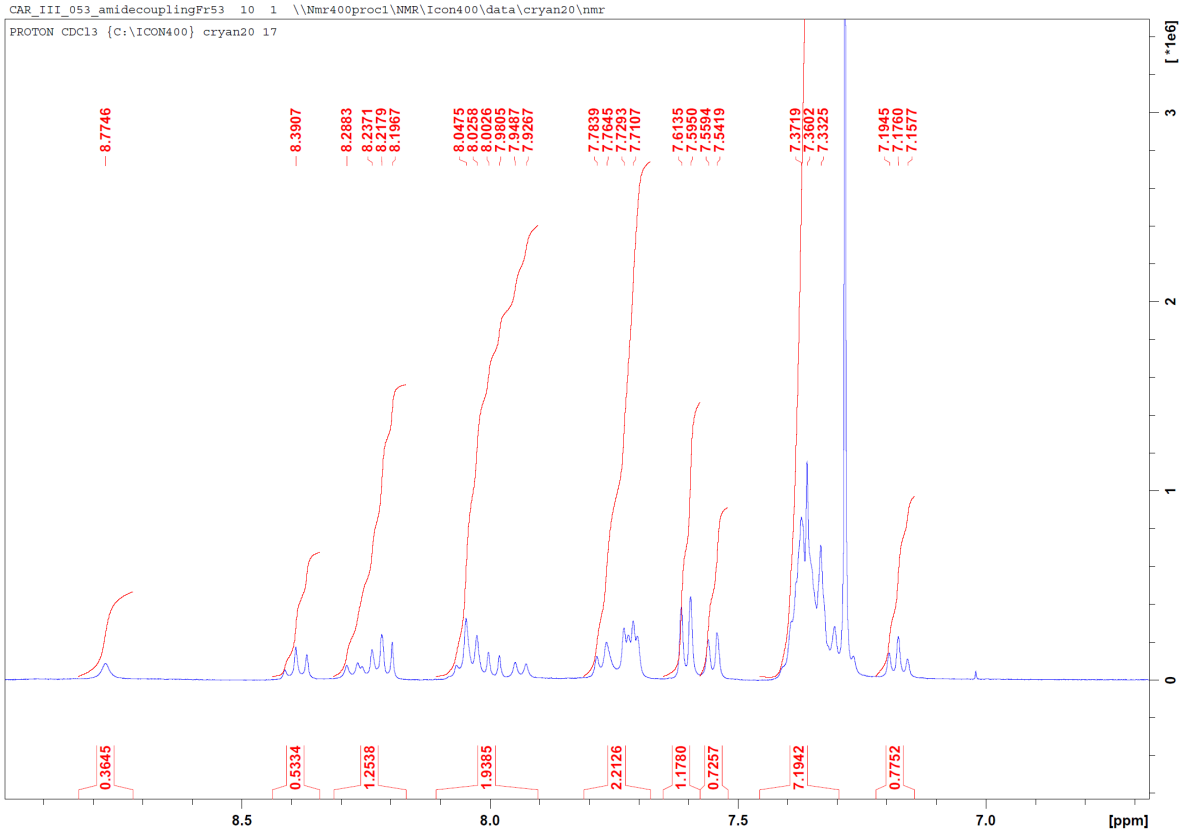
138.5451
113.7399
111.4394
109.6017
77.3387
77.2246
77.0212
76.7033
28.2447
[*1e6]
[ppm]


CAR_III_053_amidecouplingFr53 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 17
FmocHN
BnO
NHBoc
9
[ppm]
[*1e6]


CAR_III_053_amidecouplingFr53 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 17
[ppm]
[*1e6]


CAR_III_053_amidecouplingFr53 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 17
8.7746
8.3907
8.2883
8.2371
8.2179
8.1967
8.0475
8.0258
8.0026
7.9805
7.9487
7.9267
7.7839
7.7645
7.7293
7.7107
7.6135
7.5950
7.5594
7.5419
7.3719
7.3602
7.3325
7.1945
7.1760
7.1577
0.3645
0.5334
1.2538
1.9385
2.2126
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0.7257
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0.7752
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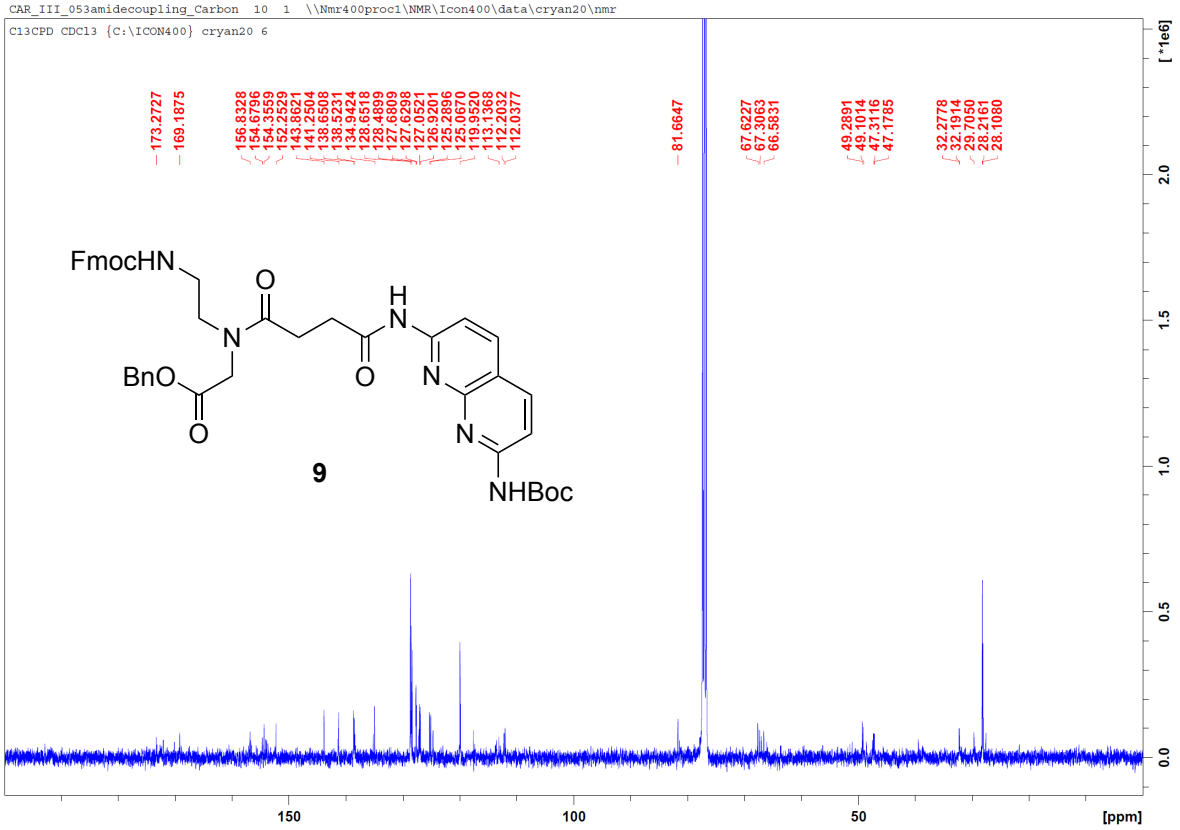
8.0
7.5
7.0
[ppm]
[*1e6]


CAR_III_053amidecoupling_Carbon 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDCl3 {C:\ICON400} cryan20 6
173.2727
169.1875
156.8328
154.6796
154.3559
152.2529
143.8621
141.2504
138.6508
138.5231
134.9424
128.6518
128.4899
127.6809
127.6298
127.0521
126.9201
125.2896
125.0670
119.9520
113.1368
112.2032
112.0377
81.6647
67.6227
67.3063
66.5831
49.2891
49.1014
47.3116
47.1785
32.2778
32.1914
29.7050
28.2161
28.1080
FmocHN
O
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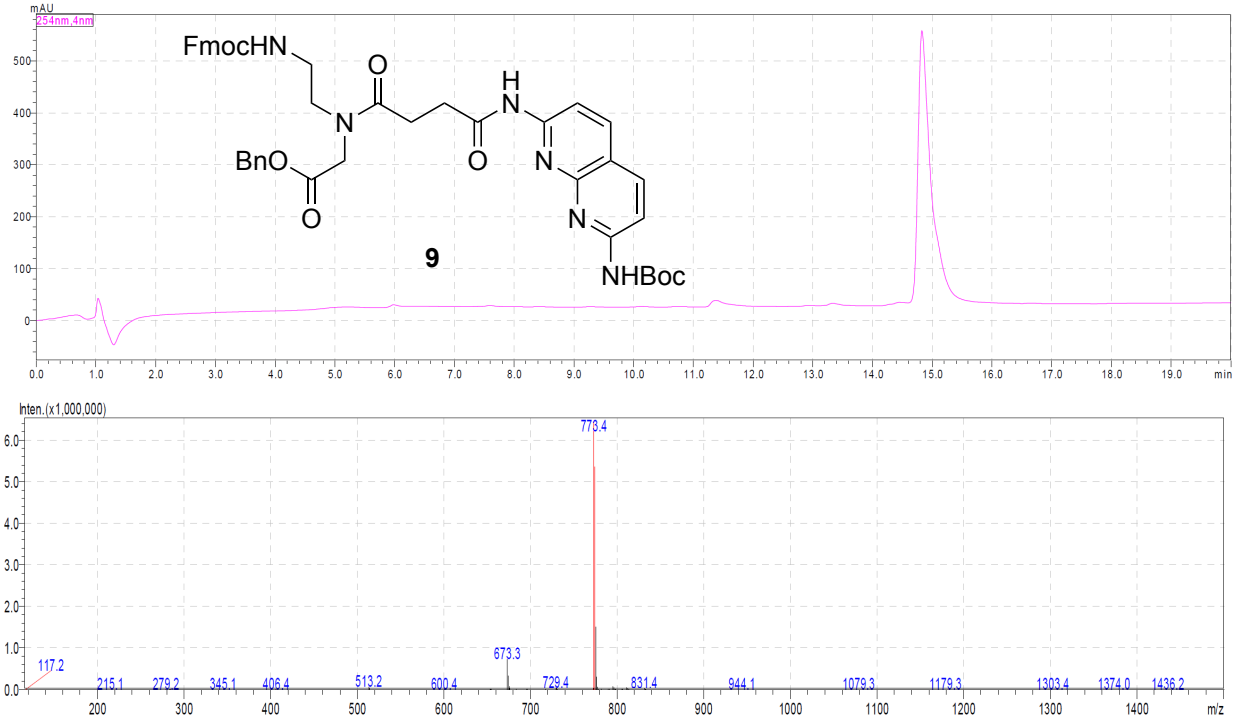
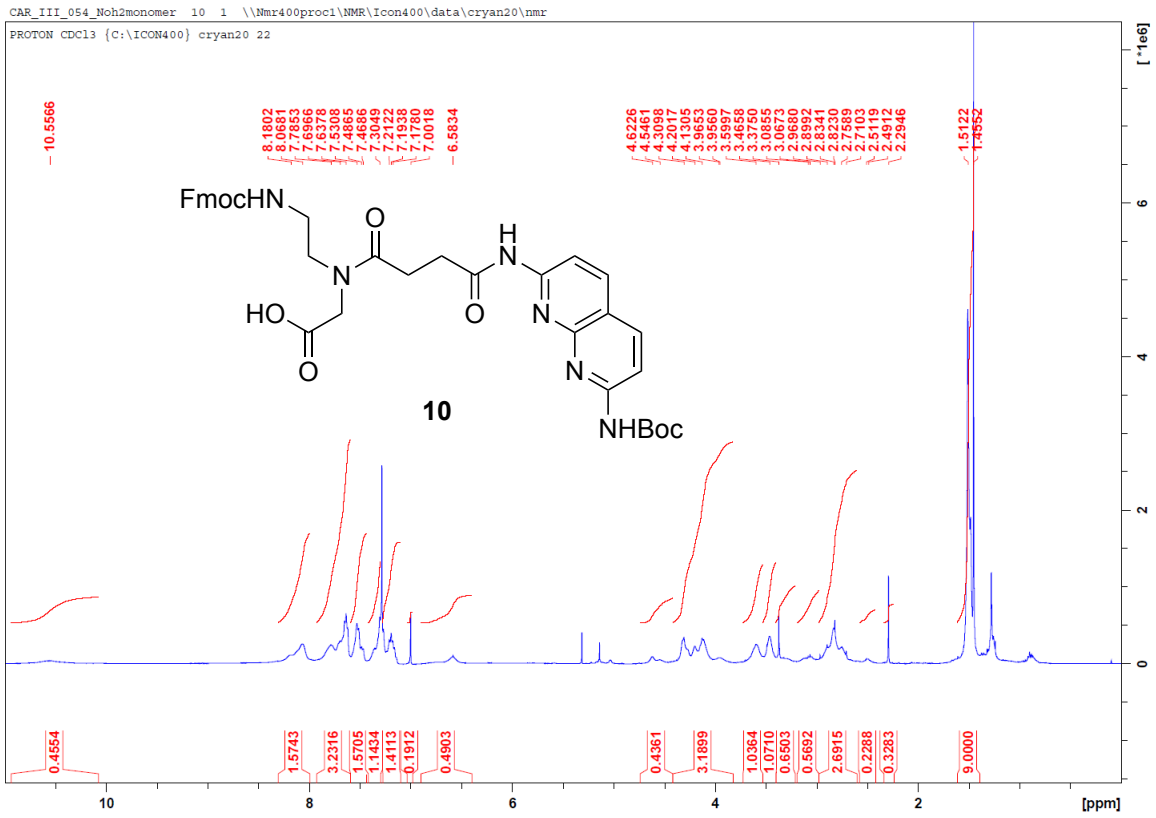
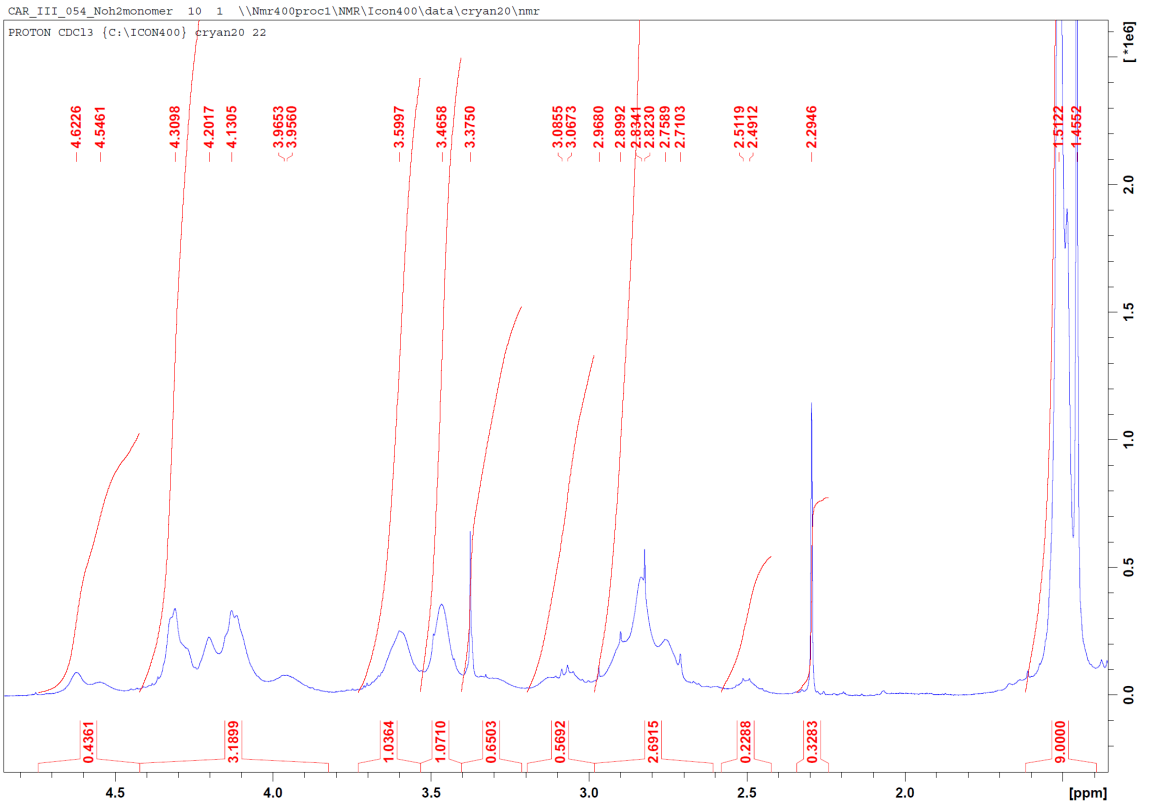
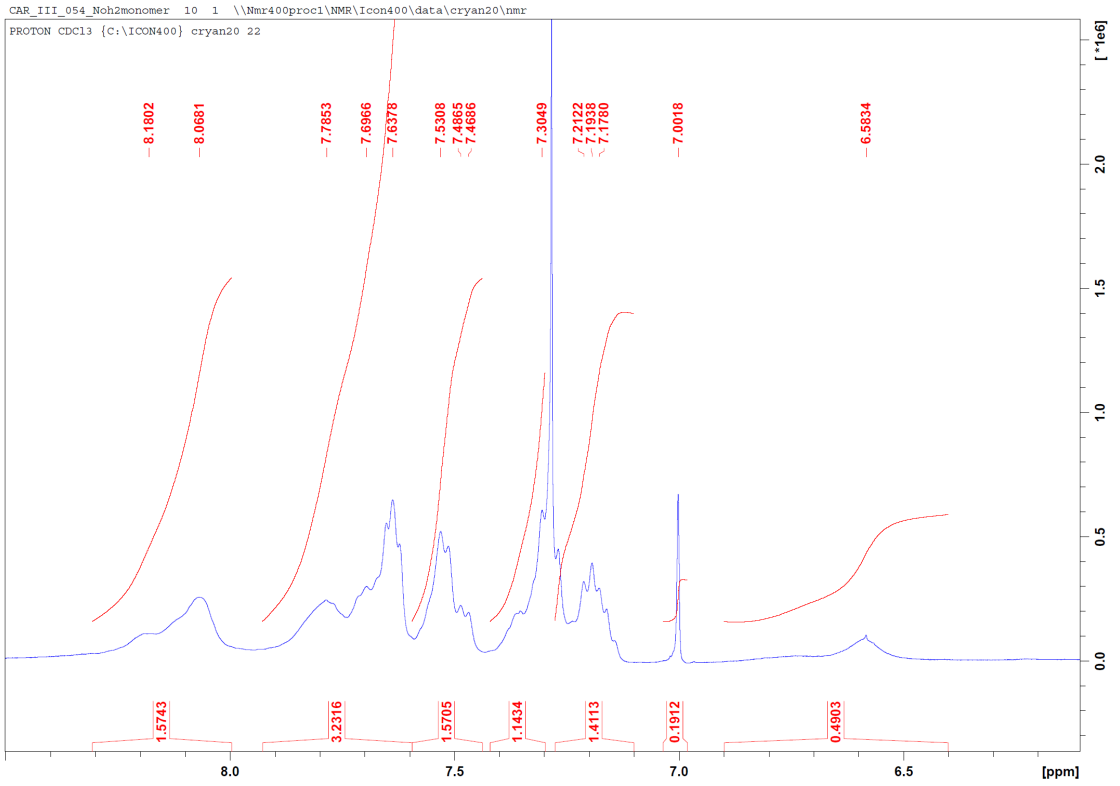
N
BnO
O
O
N
N
NHBoc
9
150
100
50
[ppm]
[*1e6]

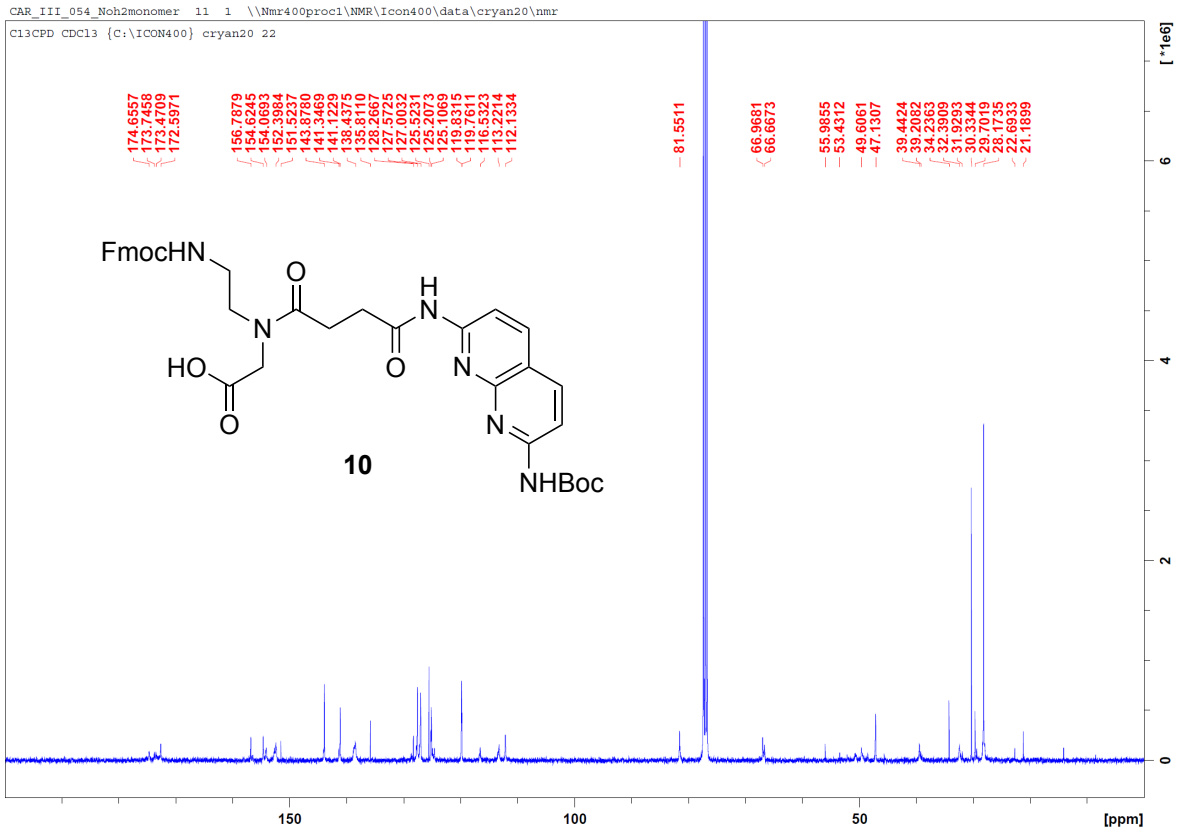


Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.


CAR_III_054_Noh2monomer 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 22
FmocHN
HO
10
NHBoc
[*1e6]
[ppm]


CAR_III_054_Noh2monomer 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 22
[*1e6]
[ppm]


CAR_III_054_Noh2monomer 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 22
8.1802
8.0681
7.7853
7.6966
7.6378
7.5308
7.4865
7.4686
7.3049
7.2122
7.1938
7.1780
7.0018
6.5834
1.5743
3.2316
1.5705
1.1434
1.4113
0.1912
0.4903
8.0
7.5
7.0
6.5
[ppm]
[*1e6]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173.4709
172.5971
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154.6245
154.0693
152.3864
151.5237
143.8780
141.3469
141.1229
138.4375
135.8610
129.2567
127.7025
127.0232
125.6231
125.2073
125.1069
119.8315
119.7611
116.5323
113.2214
112.1334
81.5511
66.9681
66.6673
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53.4312
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47.1307
39.4424
39.2082
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32.3909
31.9293
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29.7019
28.1735
22.6933
21.1899
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H
N
N
HO
O
O
N
N
NHBoc
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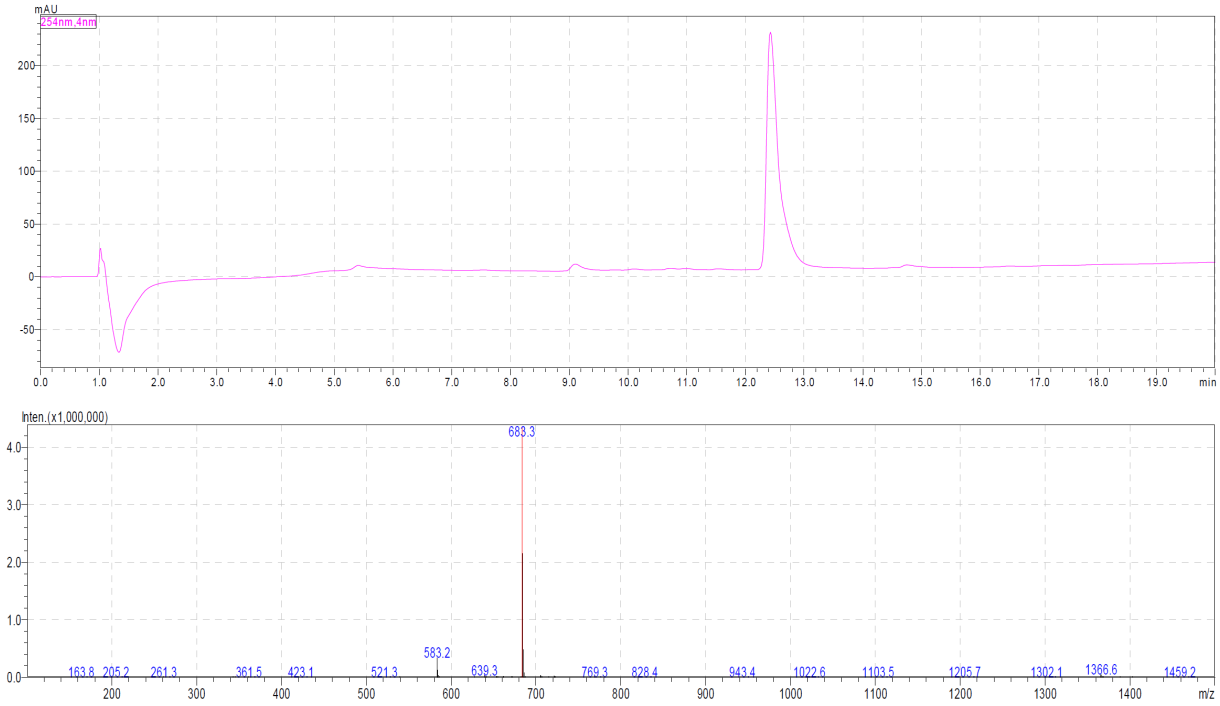
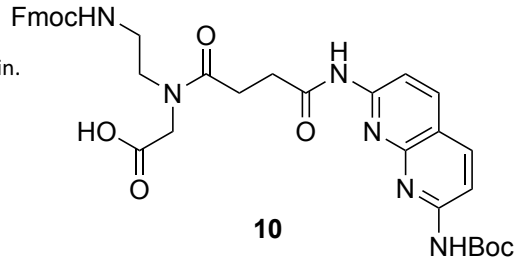
100
50
[ppm]
[*1e6]



Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.



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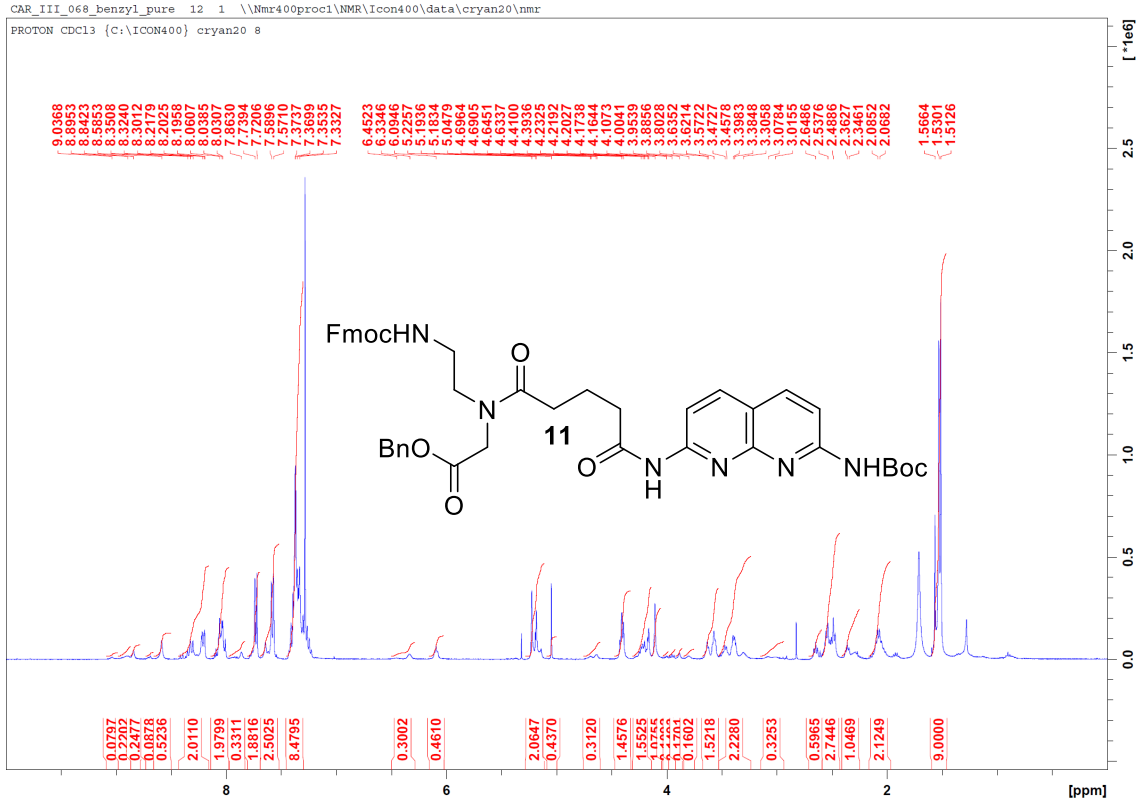
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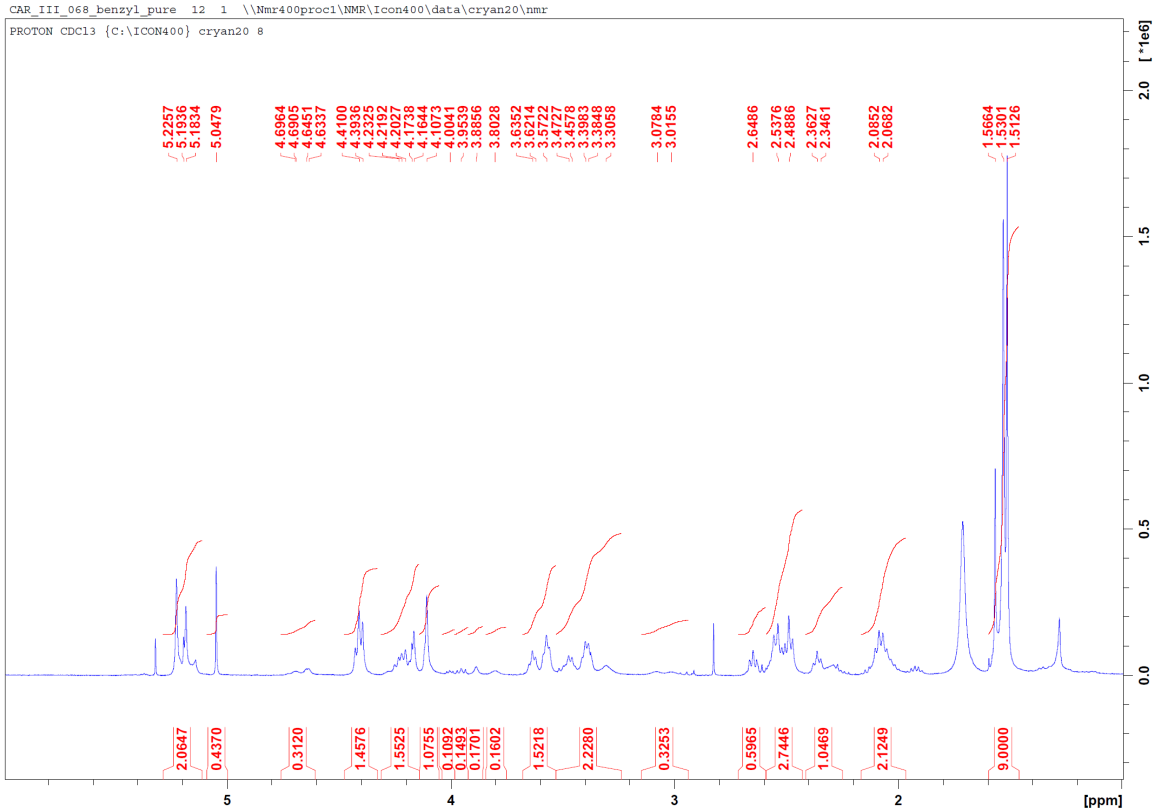
BnO
11
NHBoc
[*1e6]
[ppm]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PROTON CDCl3 {C:\ICON400} cryan20 8
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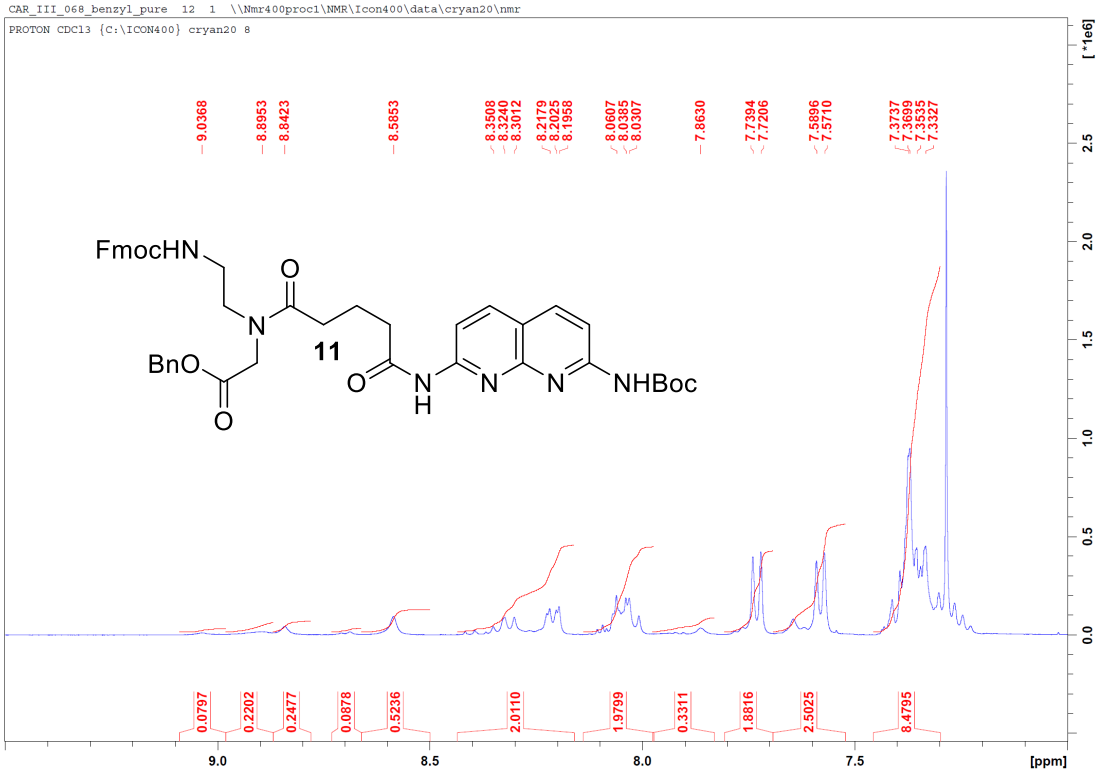
[ppm]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8.3012
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8.1958
8.0607
8.0385
8.0307
7.8630
7.7394
7.7206
7.5896
7.5710
7.3737
7.3699
7.3555
7.3327
FmocHN
BnO
11
NHBoc
0.0797
0.2202
0.2477
0.0878
0.5236
2.0110
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2.5025
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8.5
8.0
7.5
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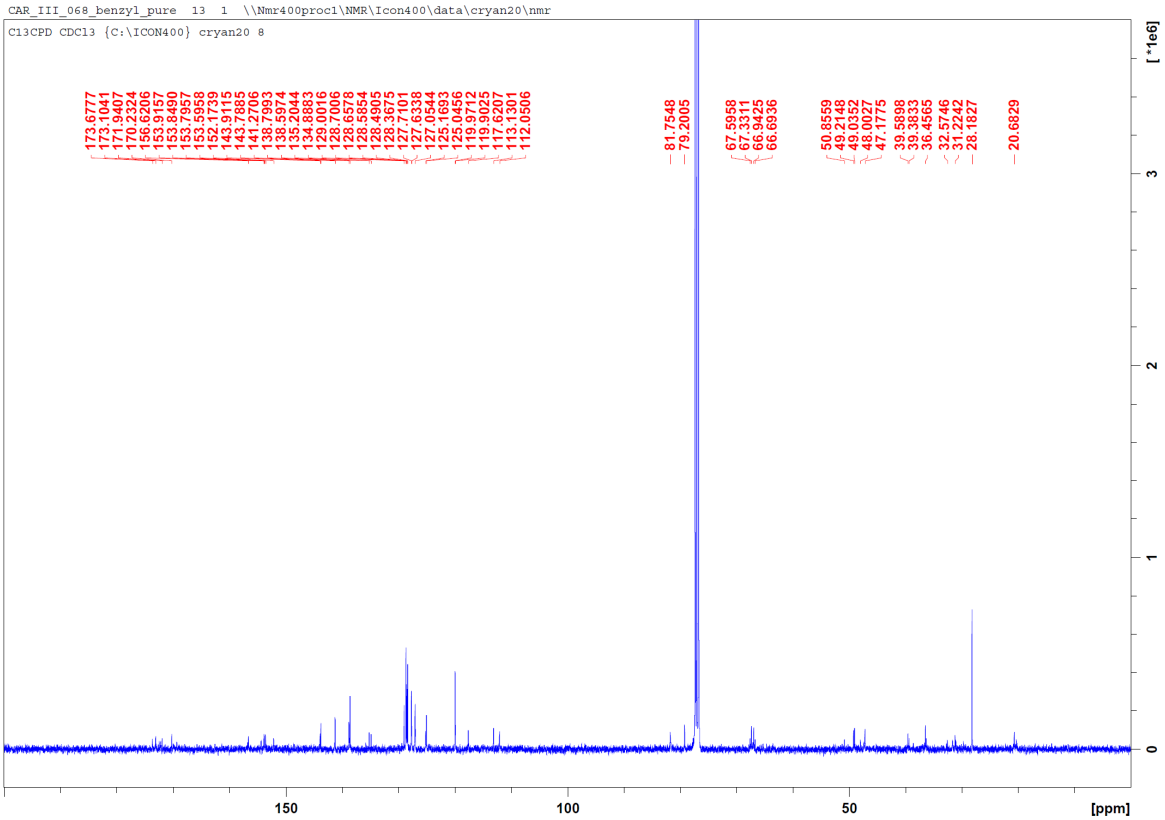
[*1e6]


CAR_III_068_benzyl_pure 13 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDCl3 {C:\ICON400} cryan20 8
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128.7096
128.5854
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127.0544
125.1693
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119.9025
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79.2005
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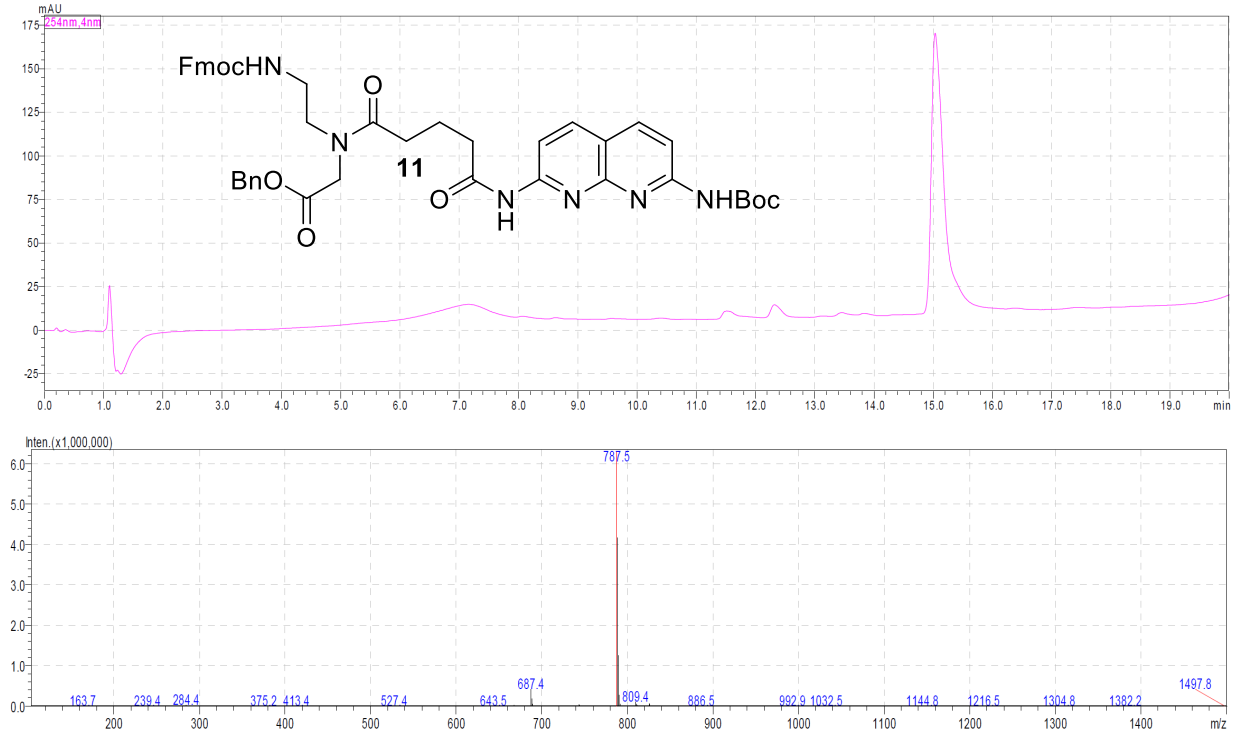
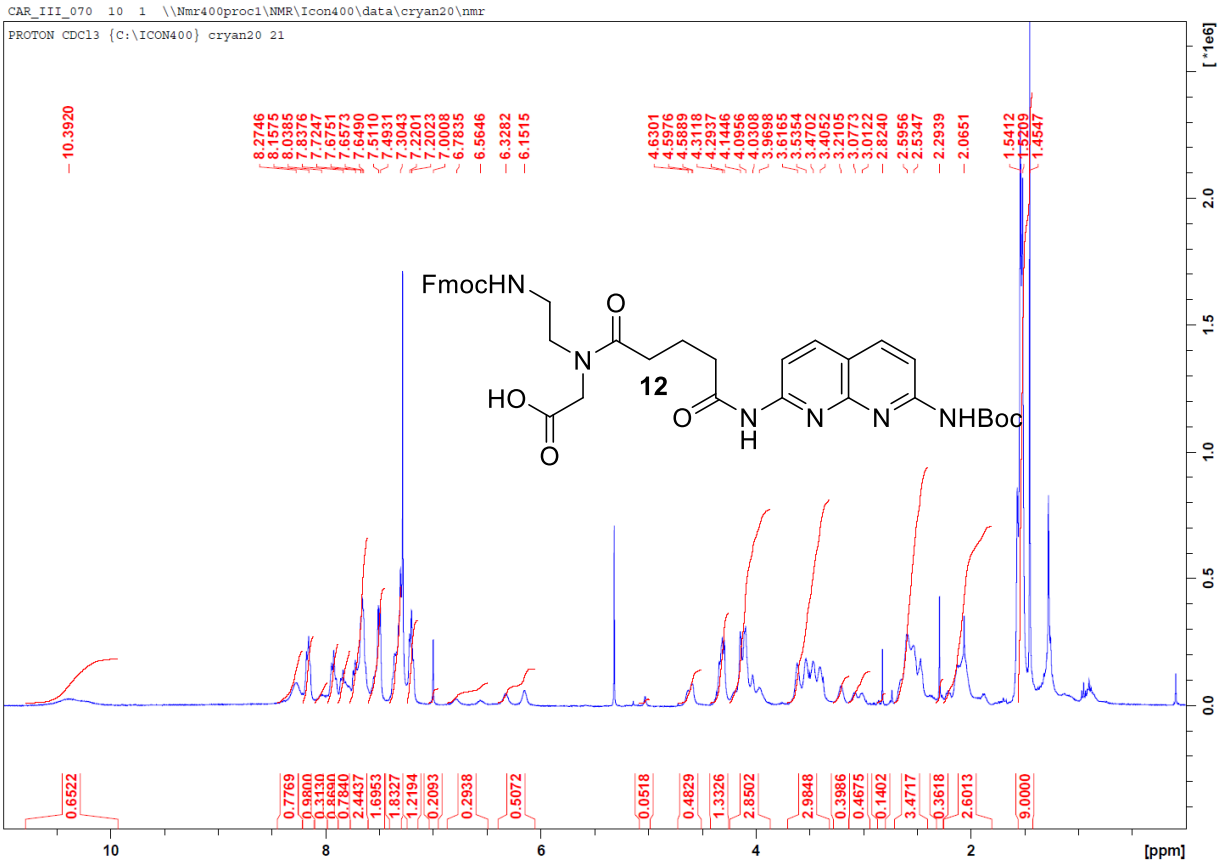
[ppm]
[*1e6]



Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.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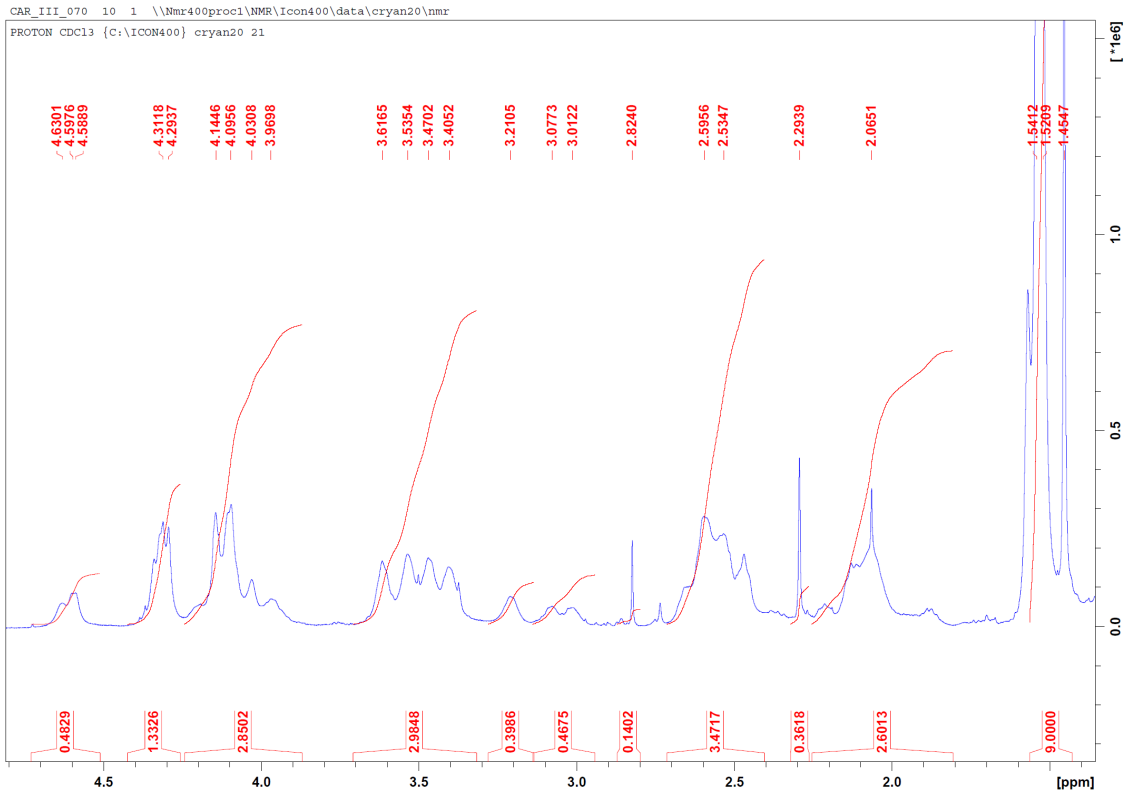
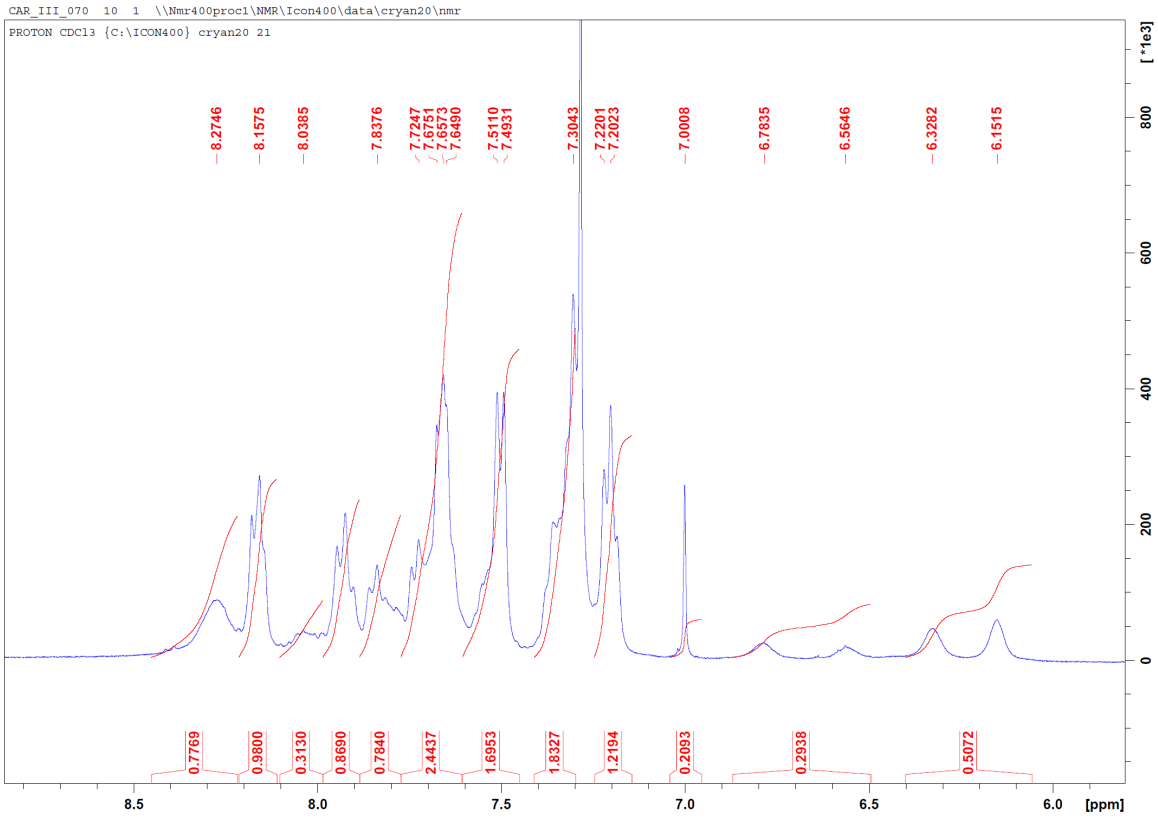
HO
12
NHBoc
[*1e6]
[ppm]


CAR_III_070 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 21
[*1e6]
[ppm]


CAR_III_070 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 21
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8.0385
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7.7247
7.6751
7.6573
7.6490
7.5110
7.4931
7.3043
7.2201
7.2023
7.0008
6.7835
6.5646
6.3282
6.1515
0.7769
0.9800
0.3130
0.8690
0.7840
2.4437
1.6953
1.8327
1.2194
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6.0
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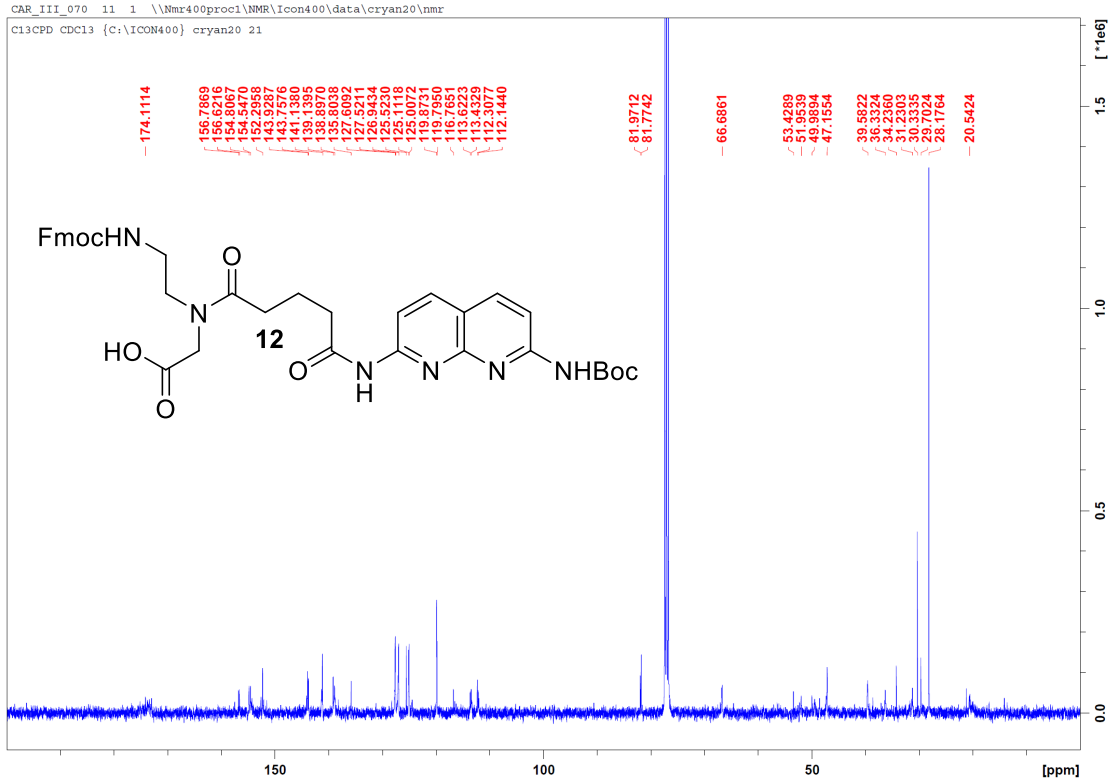
CAR_III_070 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDCl3 {C:\ICON400} cryan20 21
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143.7576
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139.1395
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112.1440
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FmocHN
HO
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NHBoc
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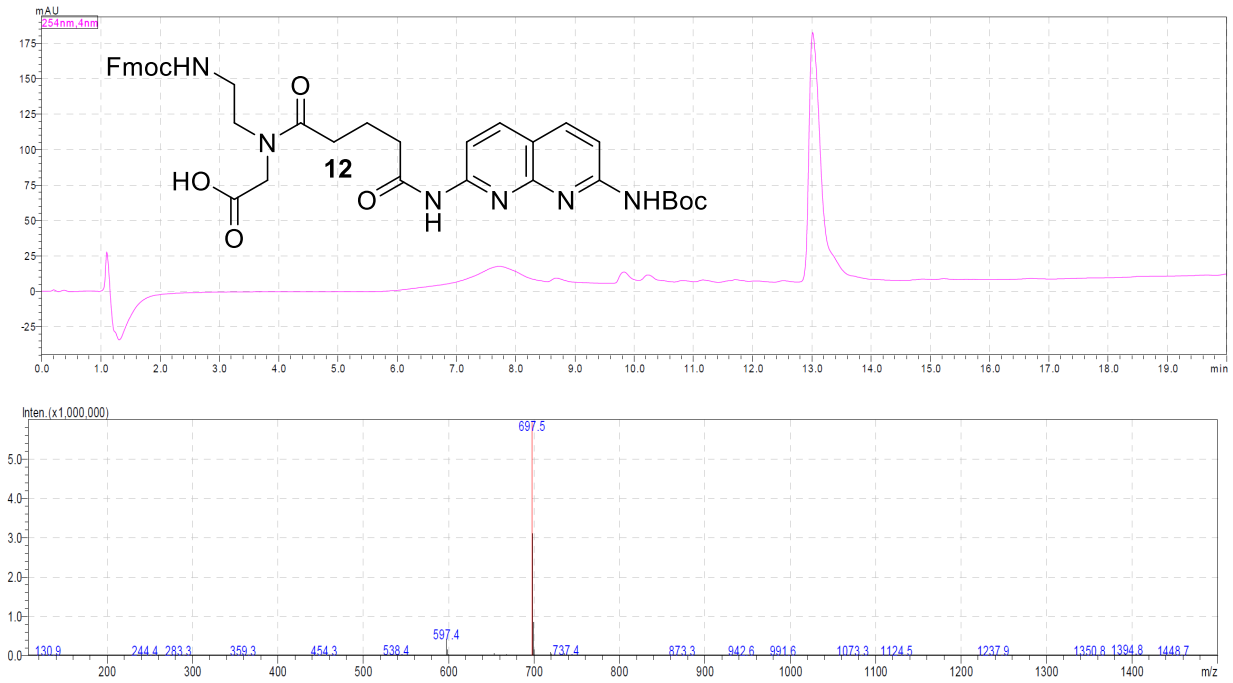
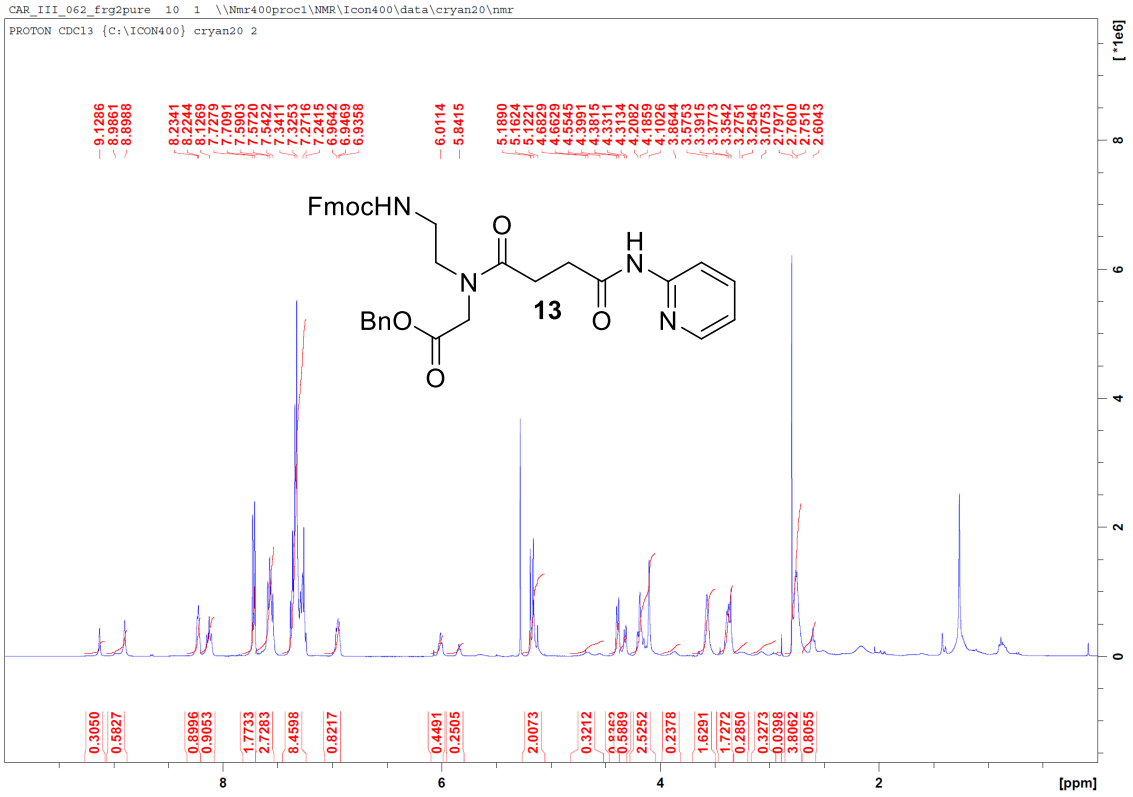
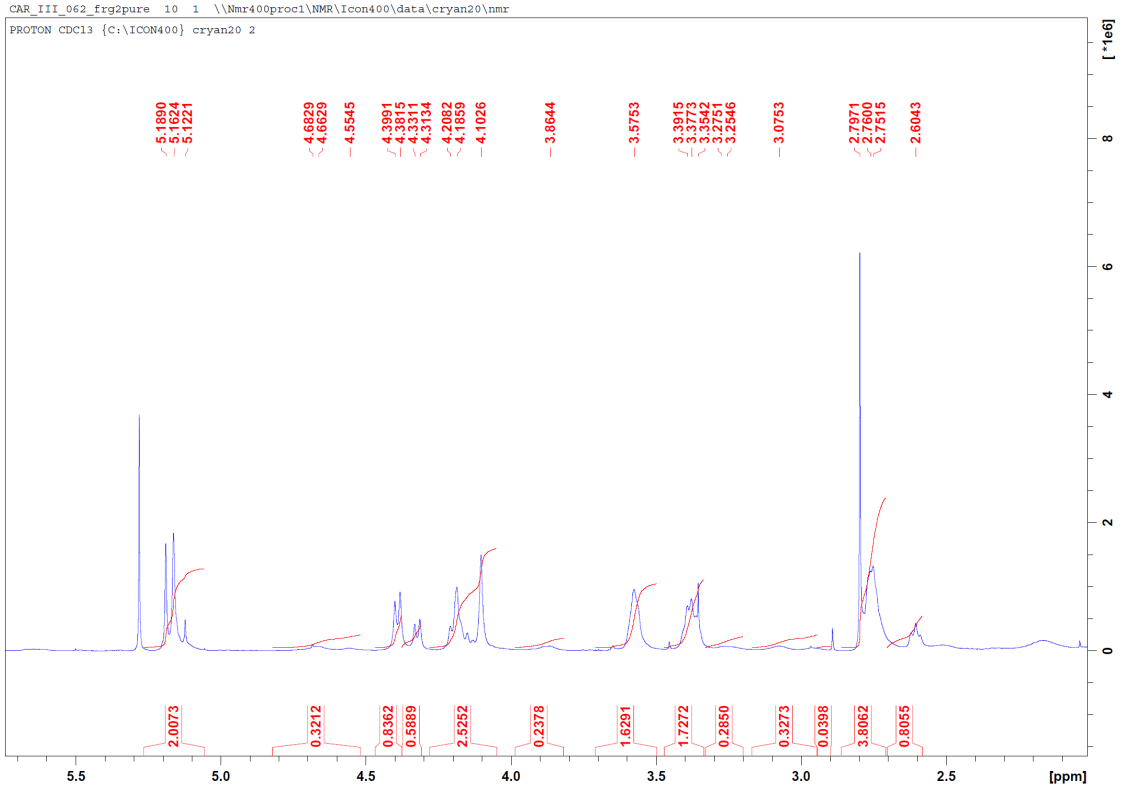
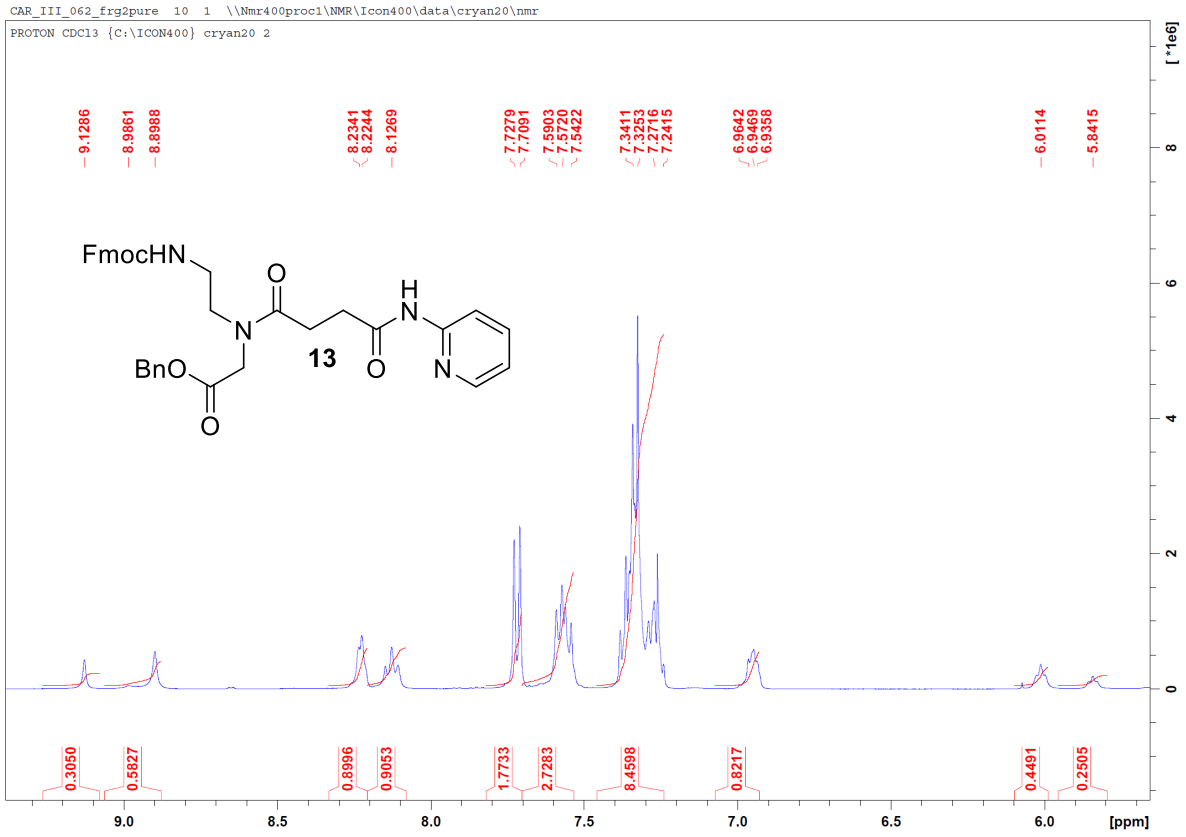
100
50
[ppm]
[*1e6]



Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.


CAR_III_062_frg2pure 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 2
FmocHN
BnO
13
[*1e6]
[ppm]


CAR_III_062_frg2pure 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 2
[*1e6]
[ppm]


CAR_III_062_frg2pure 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 2
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7.5903
7.5720
7.5422
7.3411
7.3253
7.2716
7.2415
6.9642
6.9469
6.9358
6.0114
5.8415
FmocHN
BnO
13
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0.9053
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2.7283
8.4598
0.8217
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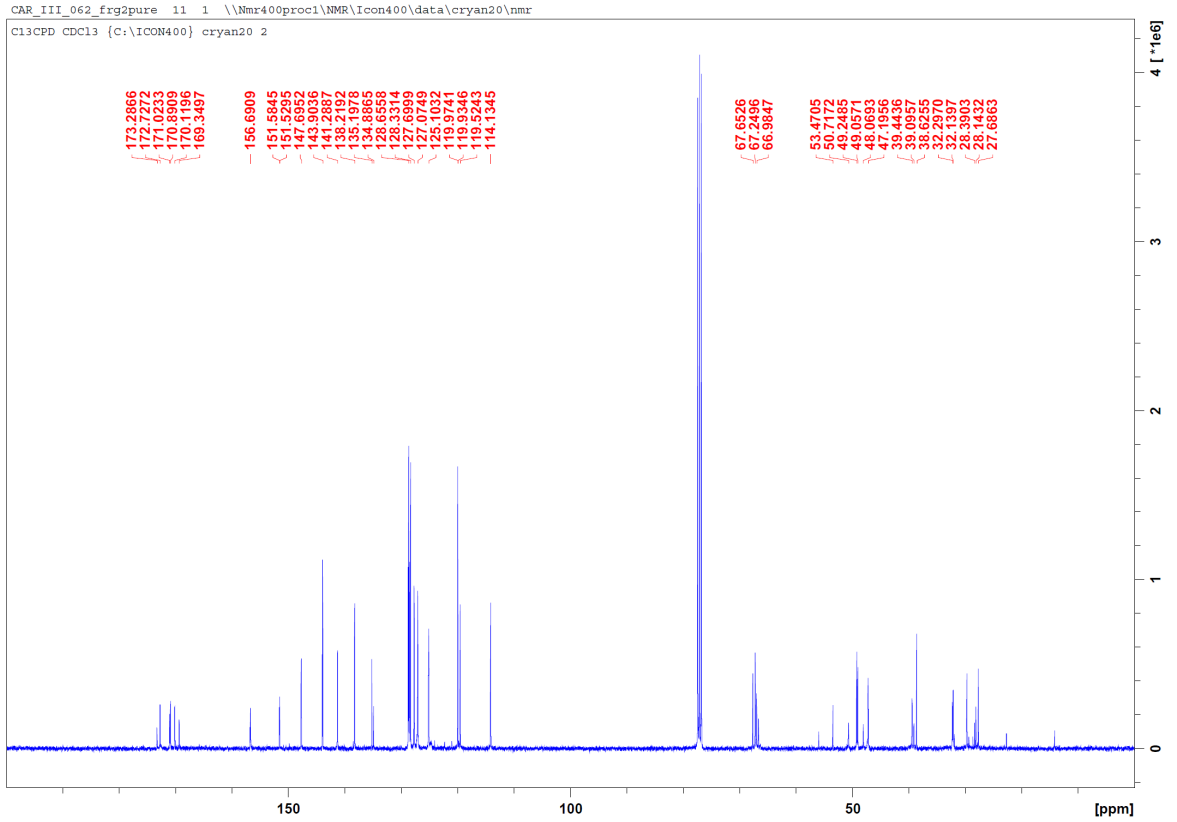
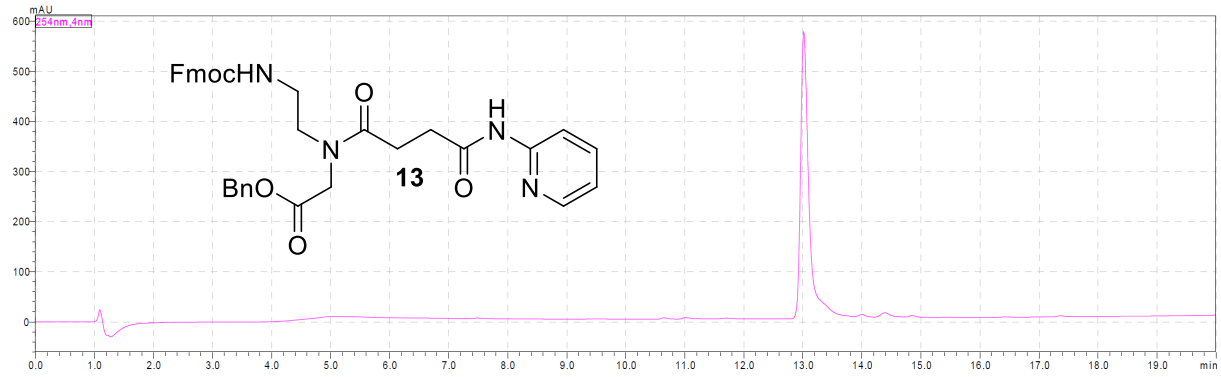
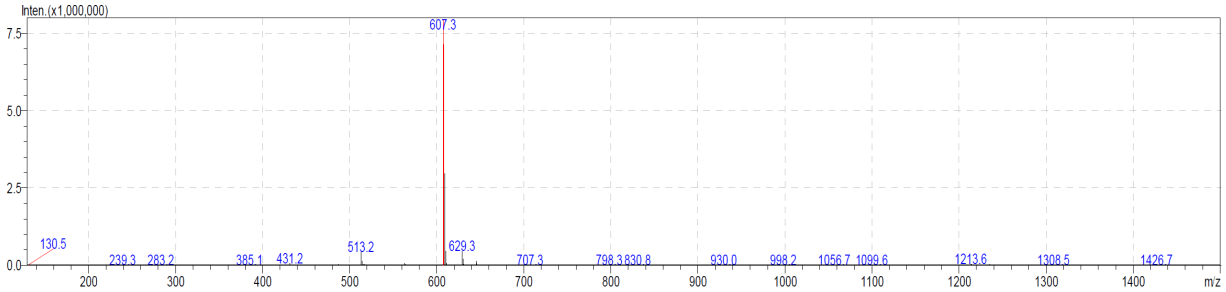
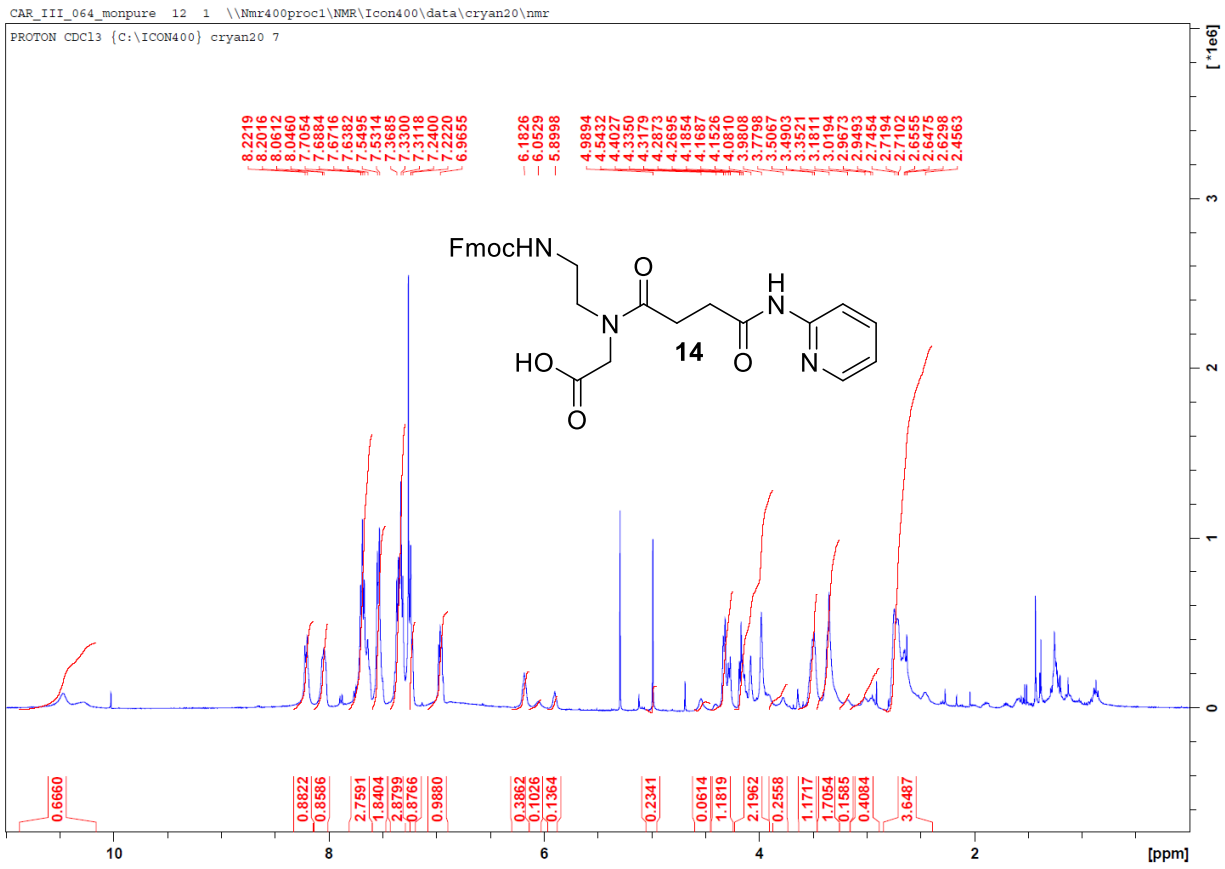
8.0
7.5
7.0
6.5
6.0
[ppm]
[*1e6]







Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.


CAR_III_064_monpure 12 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 7
FmocHN
HO
14
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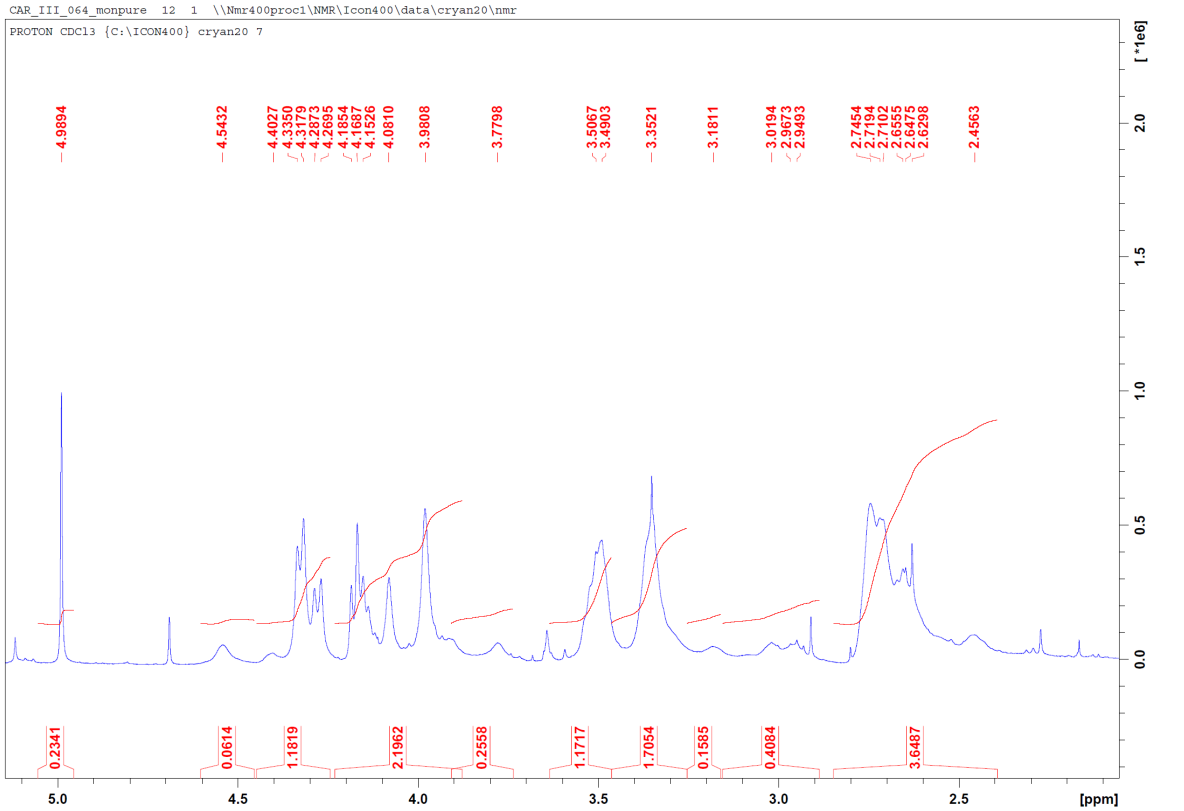
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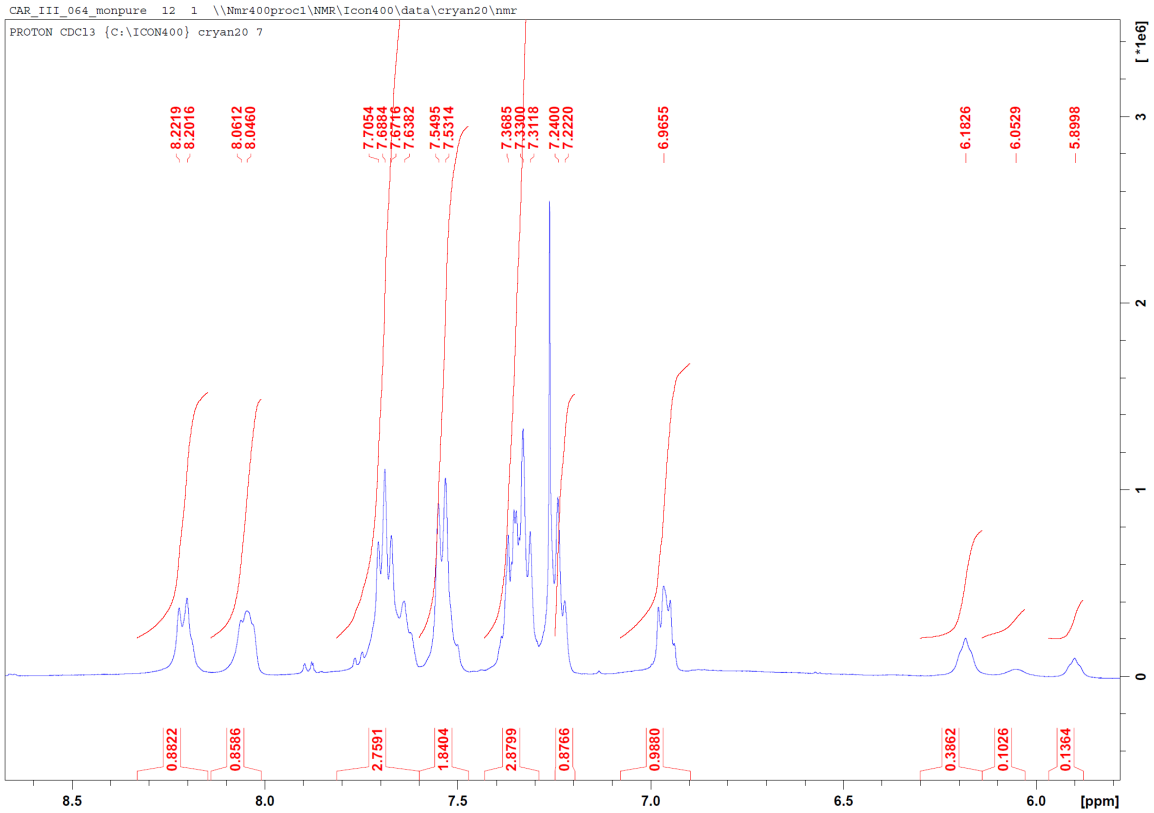
PROTON CDCl3 {C:\ICON400} cryan20 7
[*1e6]
[ppm]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7.3300
7.3118
7.2400
7.2220
6.9655
6.1826
6.0529
5.8998
0.8822
0.8586
2.7591
1.8404
2.8799
0.8766
0.9880
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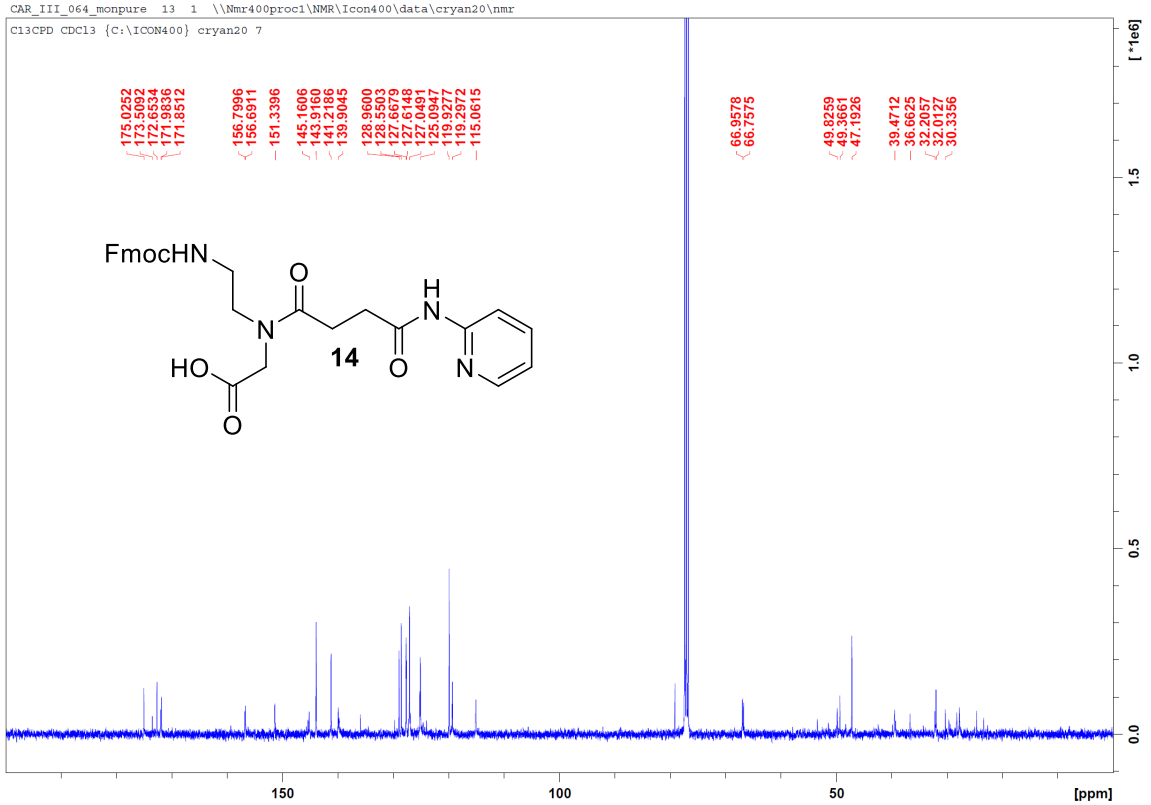
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173.5092
172.6534
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156.7996
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49.3661
47.1926
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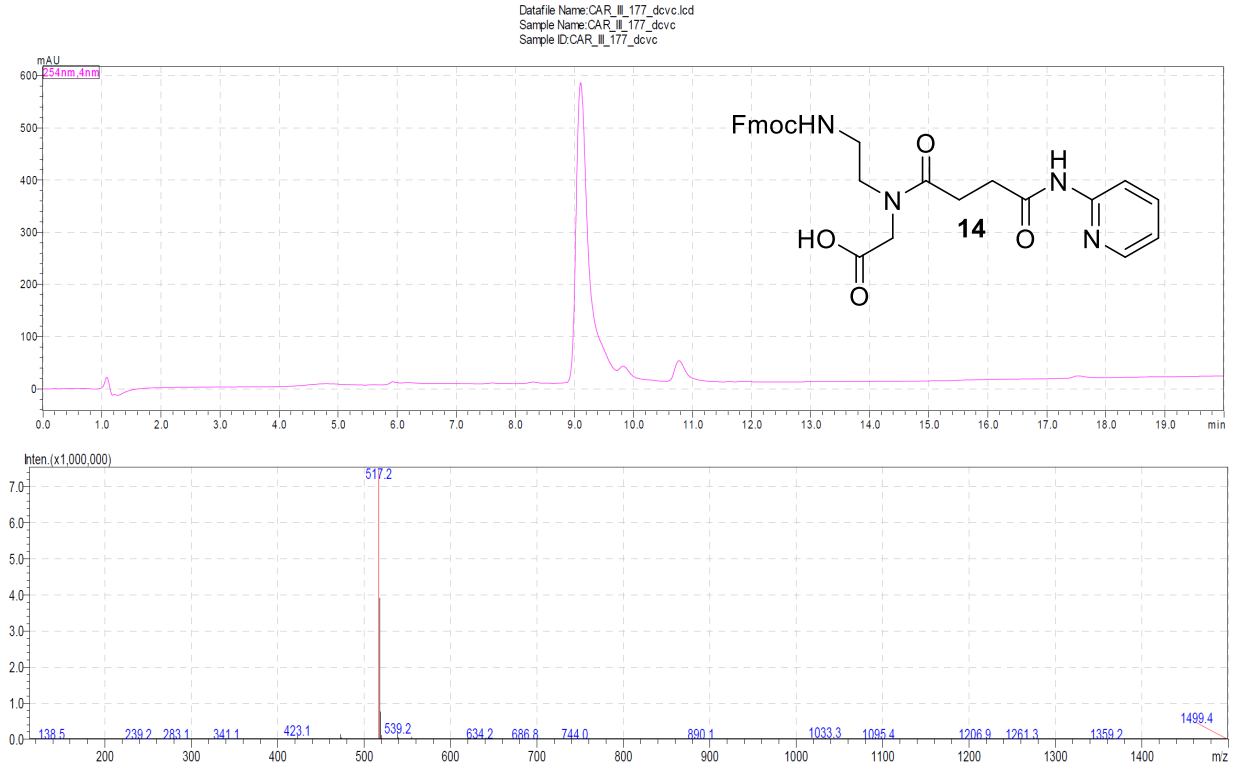
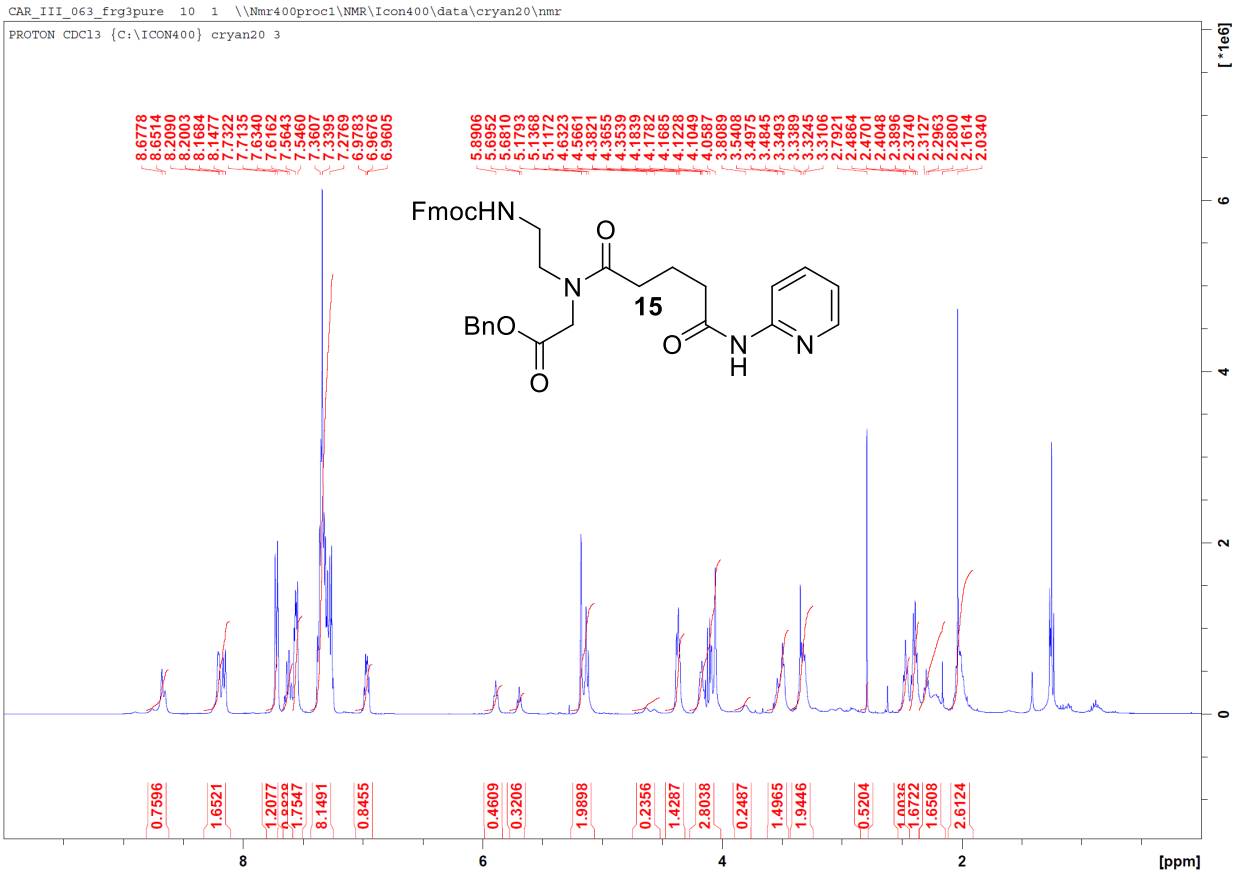
FmocHN
HO
14
150
100
50
[ppm]
[*1e6]



Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.


CAR_III_063_frg3pure 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
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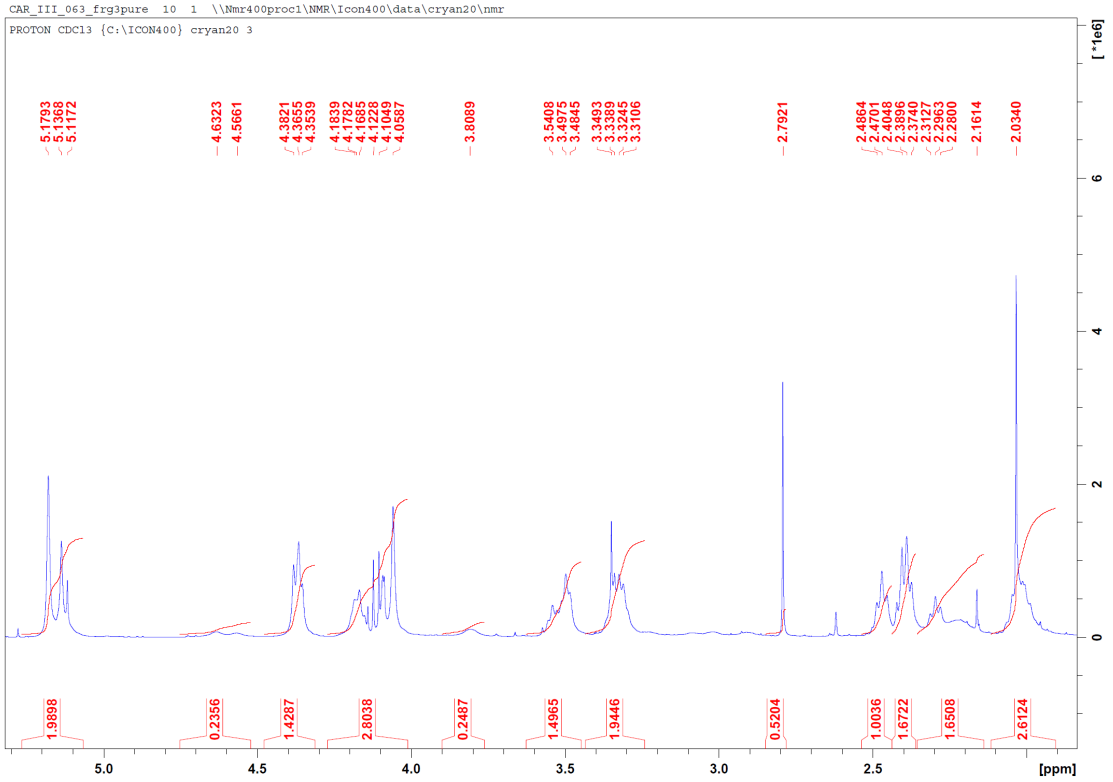
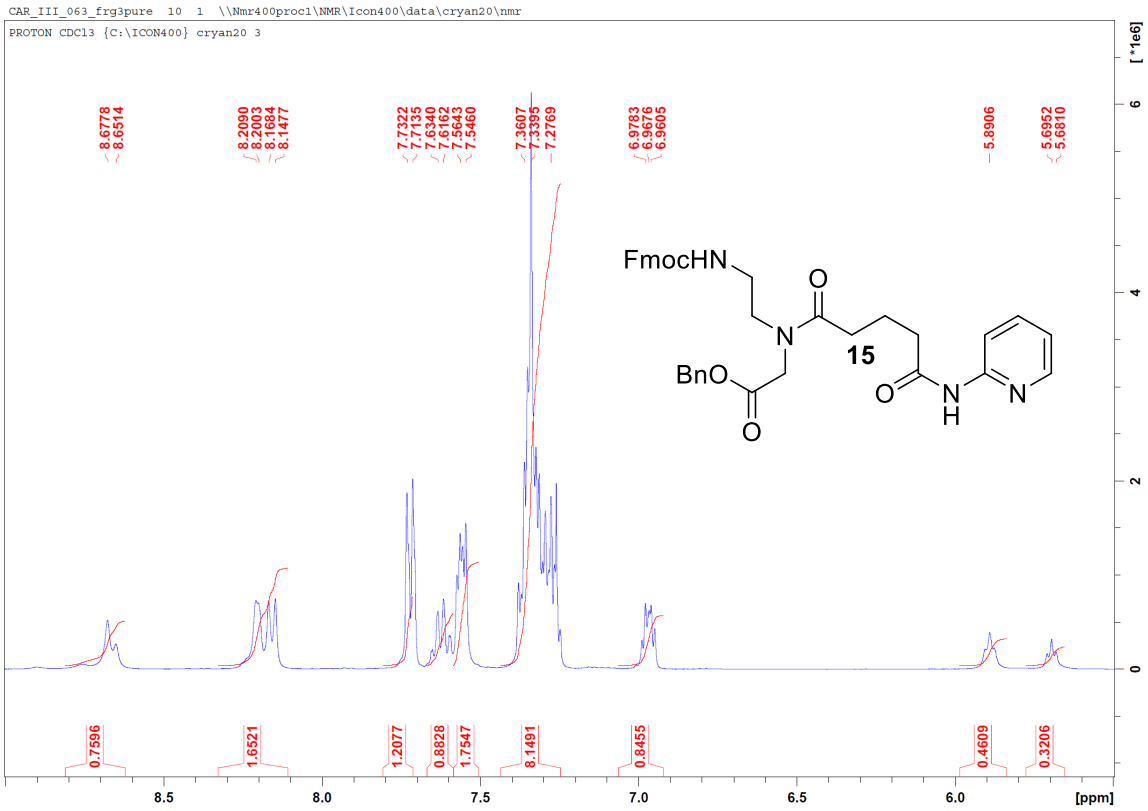
FmocHN
BnO
15
[*1e6]
[ppm]


CAR_III_063_frg3pure 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 3
[*1e6]
[ppm]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PROTON CDCl3 {C:\ICON400} cryan20 3
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7.7135
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FmocHN
BnO
O
N
O
15
O
N
H
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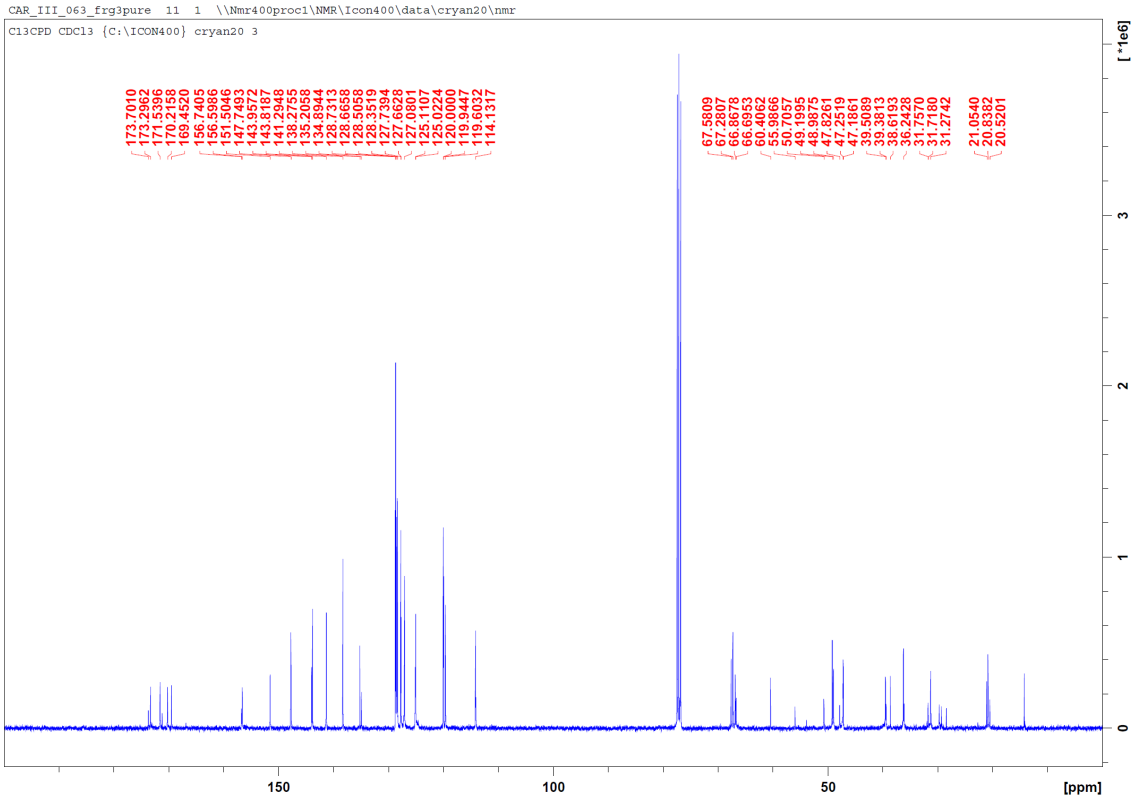
[*1e6]


CAR_III_063_frg3pure 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDCl3 {C:\ICON400} cryan20 3
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173.2962
171.5396
170.2158
169.4520
156.7405
156.5896
151.5046
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143.9572
143.8187
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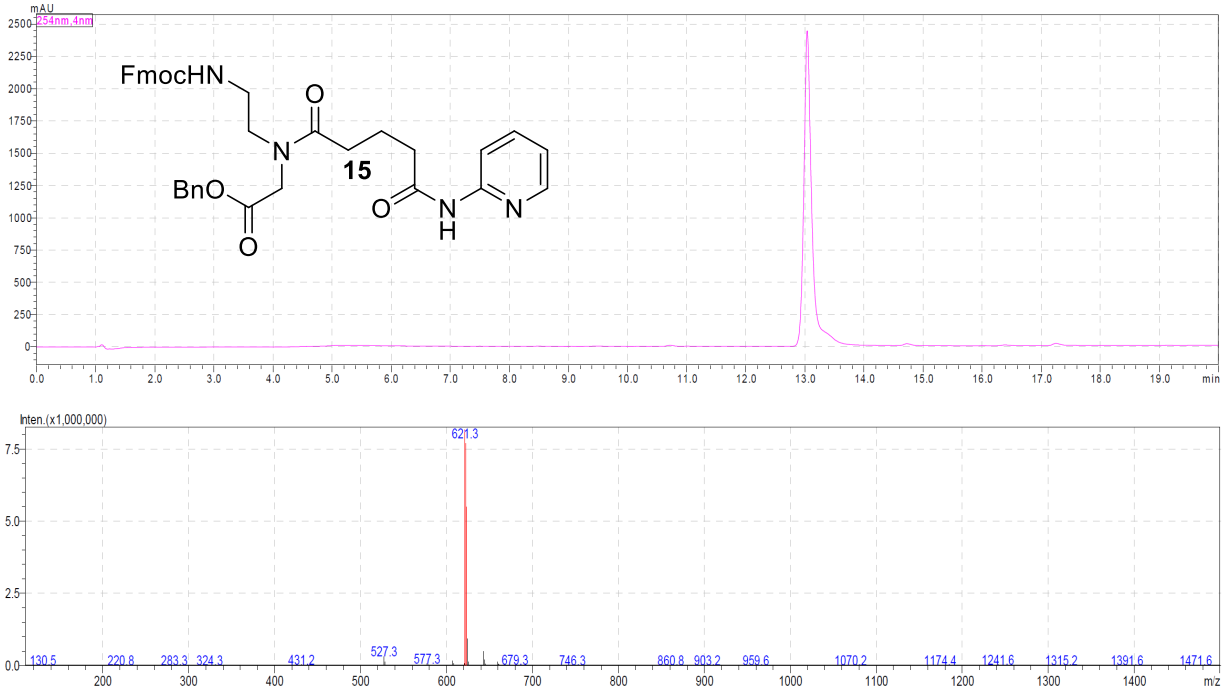
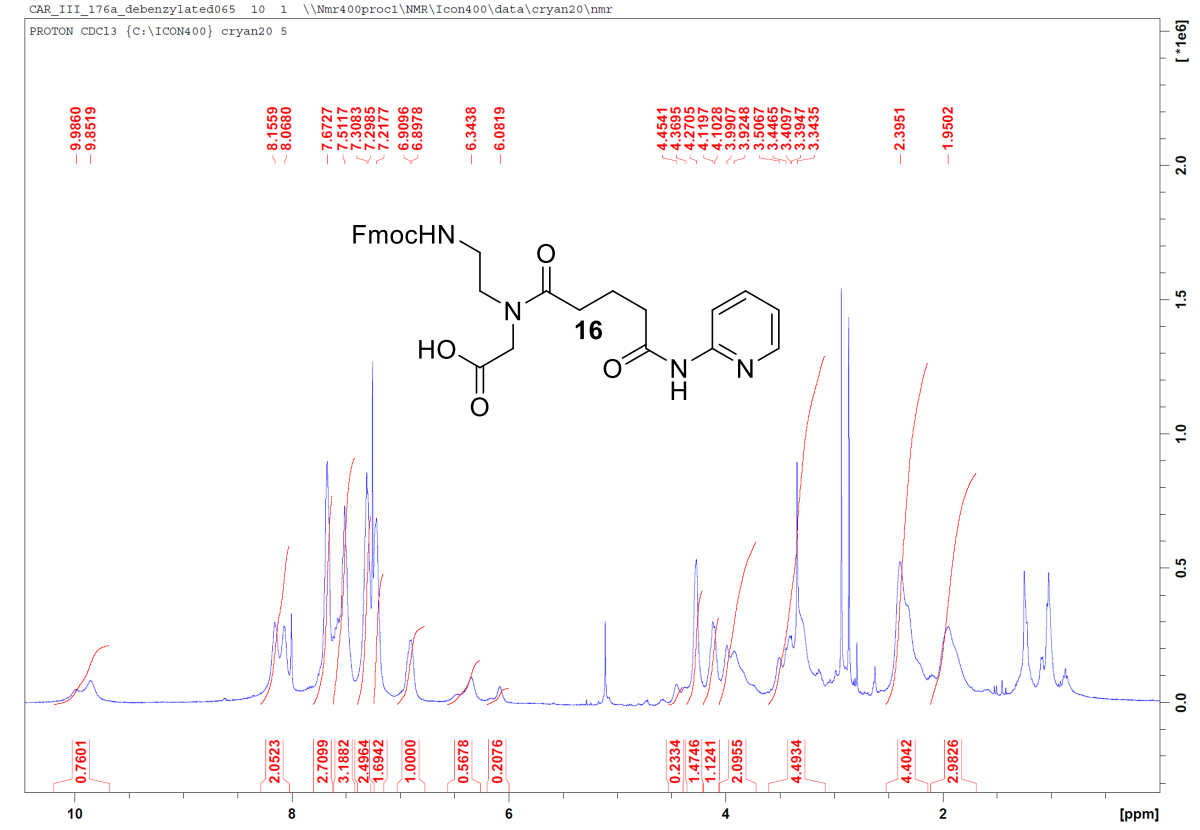


Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.


CAR_III_176a_debenzylated065 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
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8.0680
7.6727
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7.3083
7.2985
7.2177
6.9096
6.8978
6.3438
6.0819
4.4541
4.3695
4.2705
4.1197
4.1028
3.9907
3.9248
3.5067
3.4465
3.4097
3.3947
3.3435
2.3951
1.9502
FmocHN
HO
O
N
16
O
N
H
N
0.7601
2.0523
2.7099
3.1882
2.4964
1.6942
1.0000
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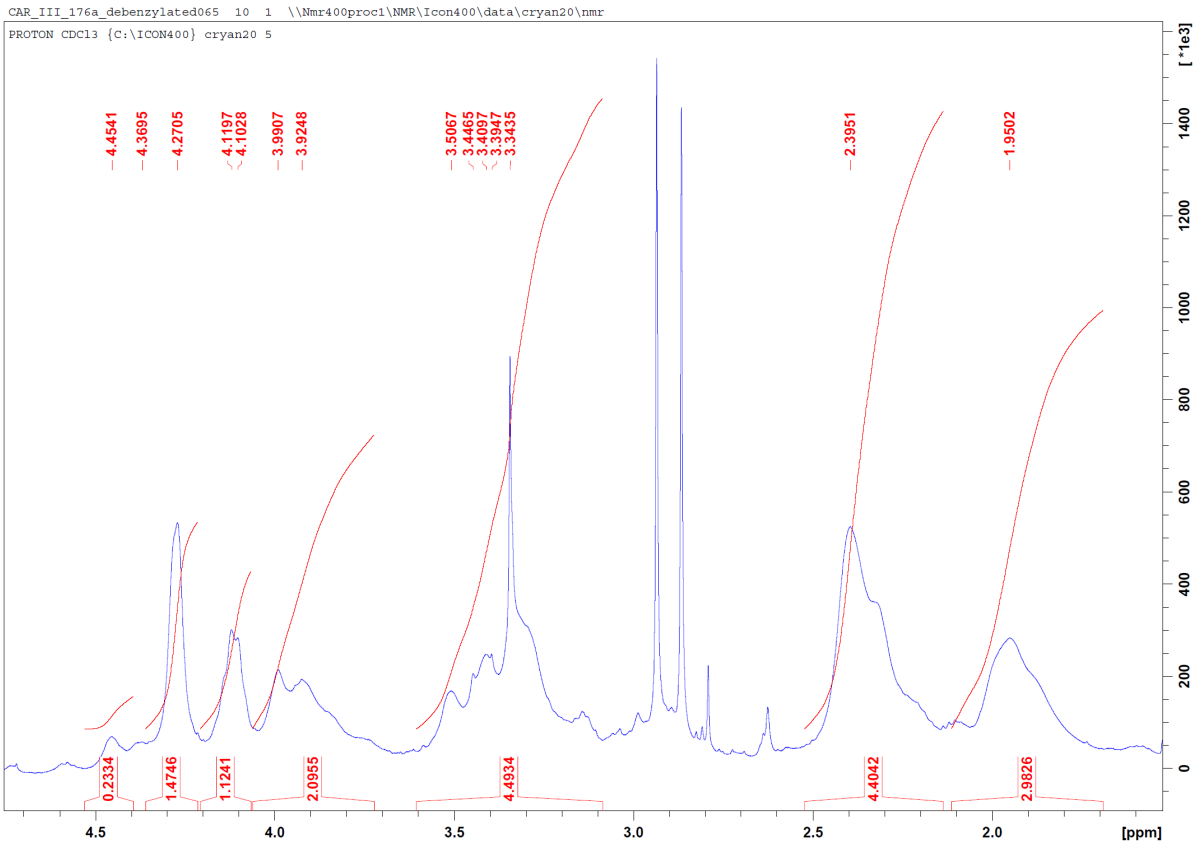
4.4934
4.4042
2.9826
[*1e6]
[ppm]


CAR_III_176a_debenzylated065 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 5
4.4541
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4.1028
3.9907
3.9248
3.5067
3.4465
3.4097
3.3947
3.3435
2.3951
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4.4042
2.9826
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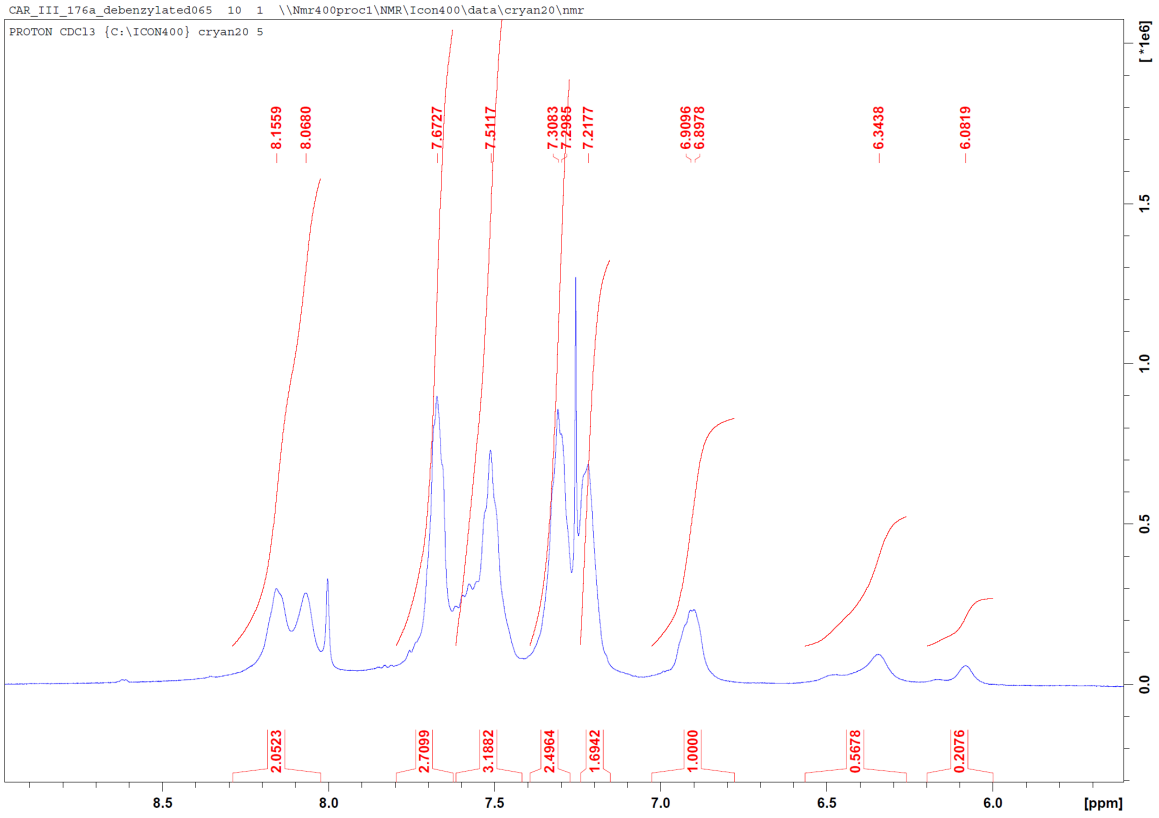
CAR_III_176a_debenzylated065 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 5
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8.0680
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7.2177
6.9096
6.8978
6.3438
6.0819
2.0523
2.7099
3.1882
2.4964
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6.0
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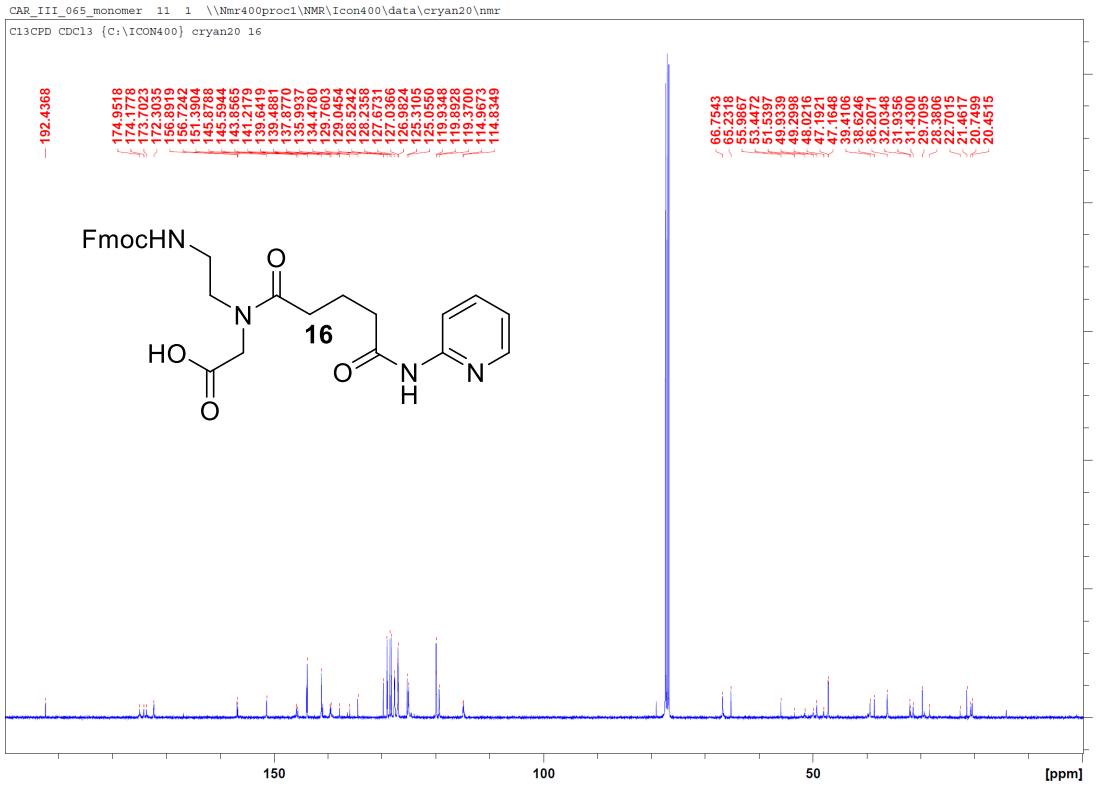
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173.7023
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53.4472
51.5397
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36.2071
32.0348
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31.4300
29.7095
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HO
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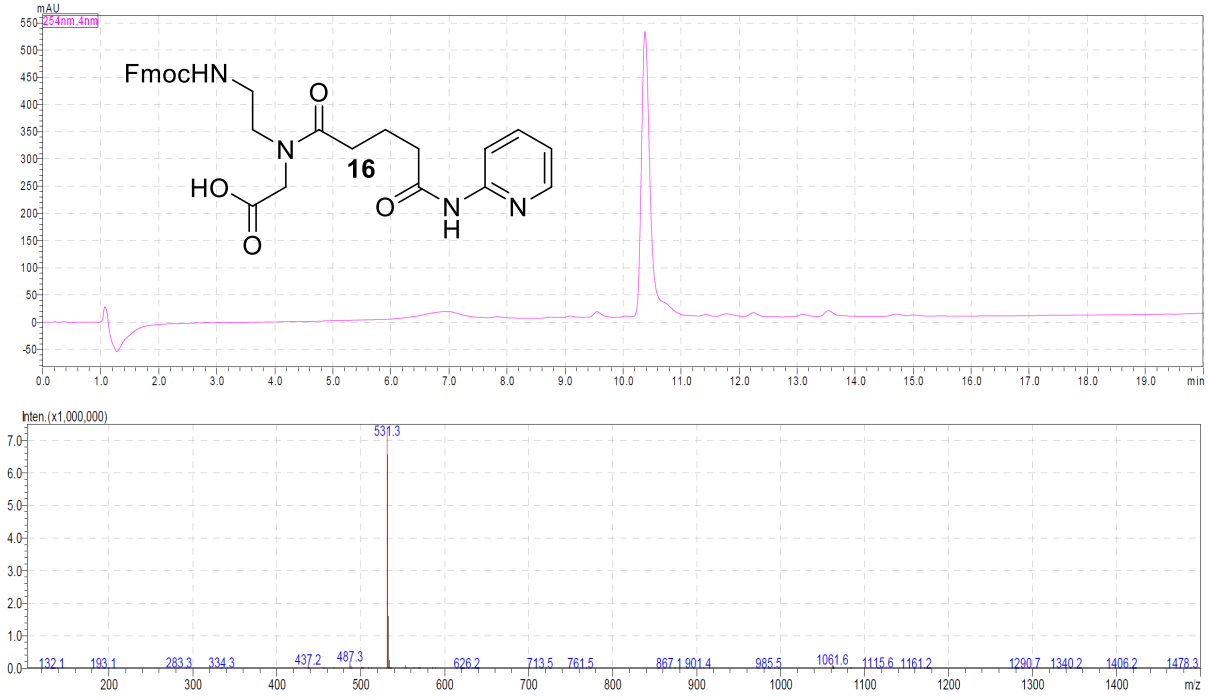
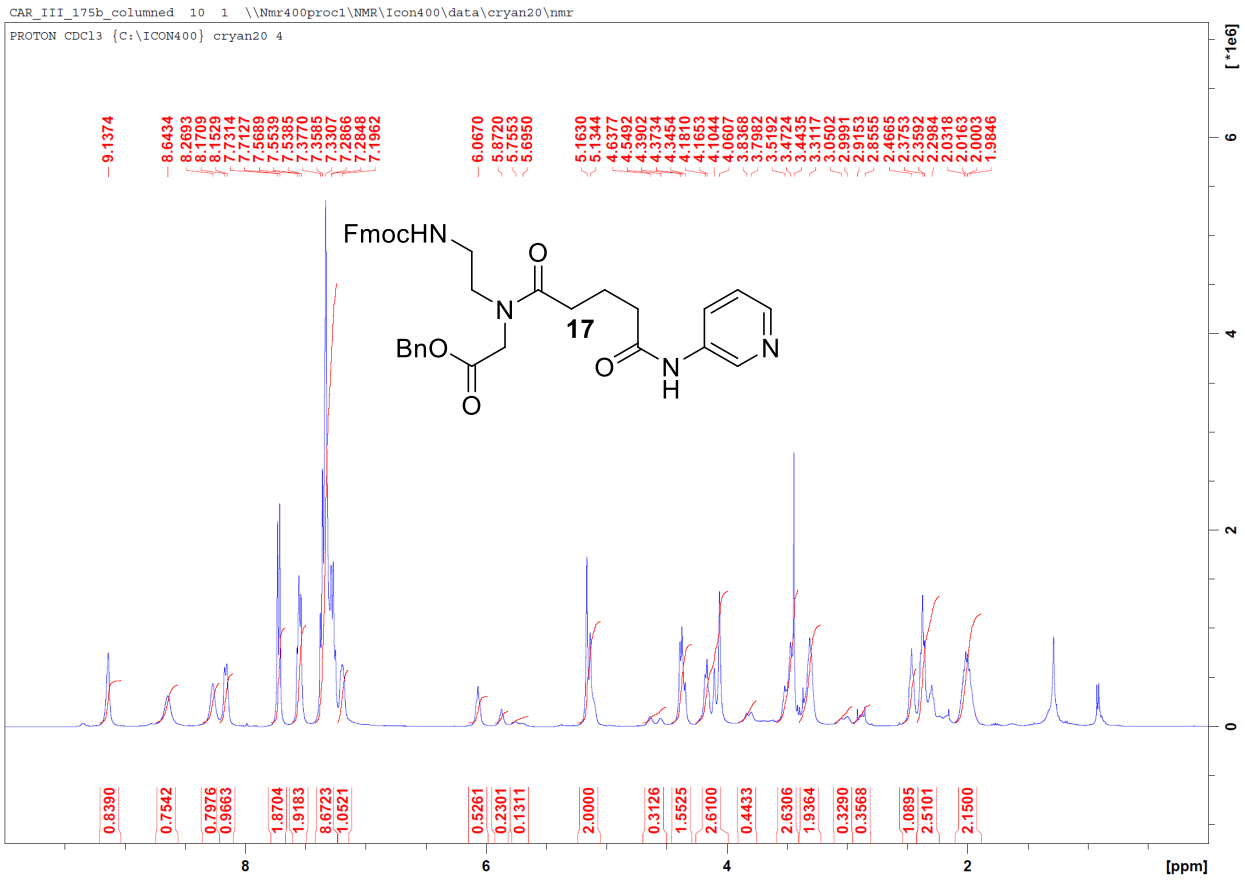


Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.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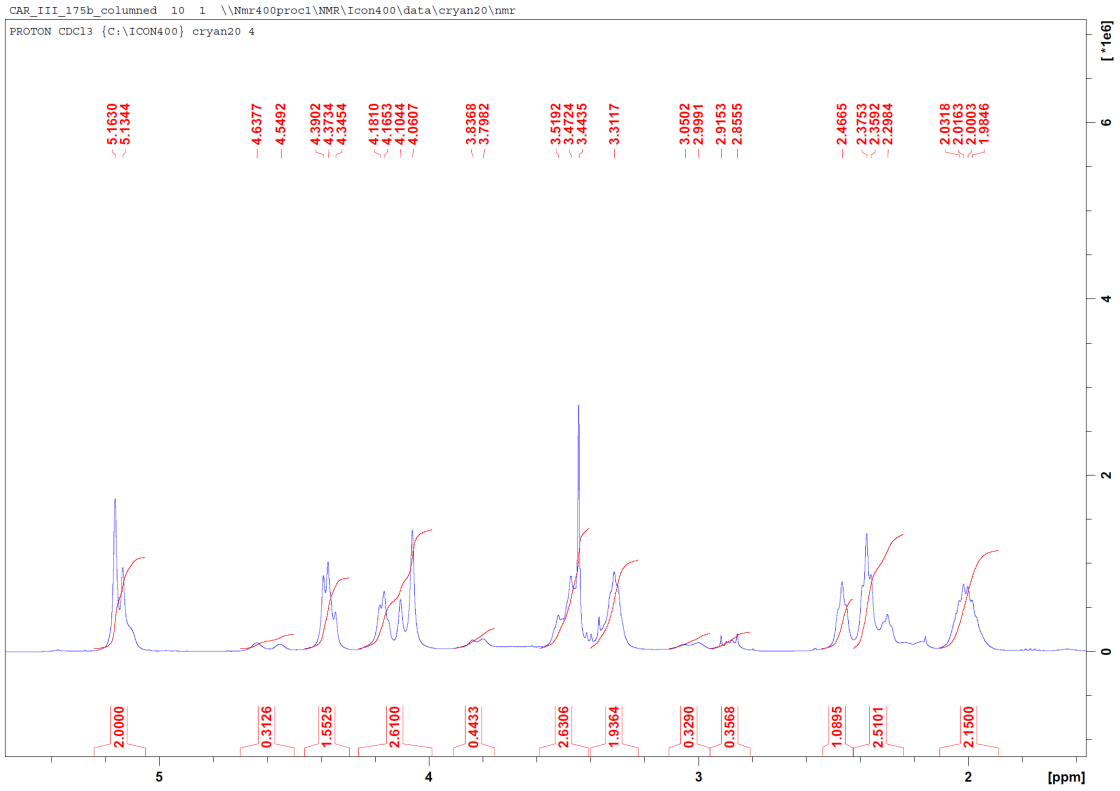
FmocHN
BnO
17
[*1e6]
[ppm]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PROTON CDCl3 {C:\ICON400} cryan20 4
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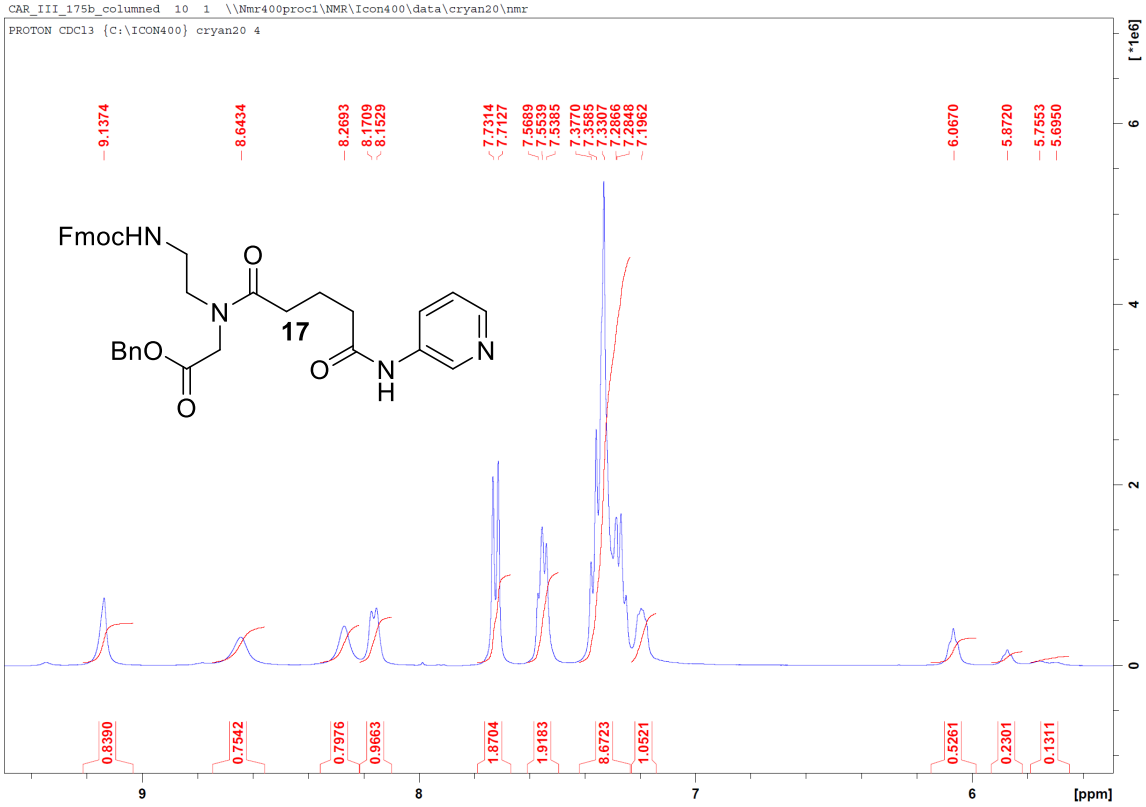
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7.5689
7.5539
7.5385
7.3770
7.3585
7.3307
7.2866
7.2848
7.1962
6.0670
5.8720
5.7553
5.6950
FmocHN
BnO
17
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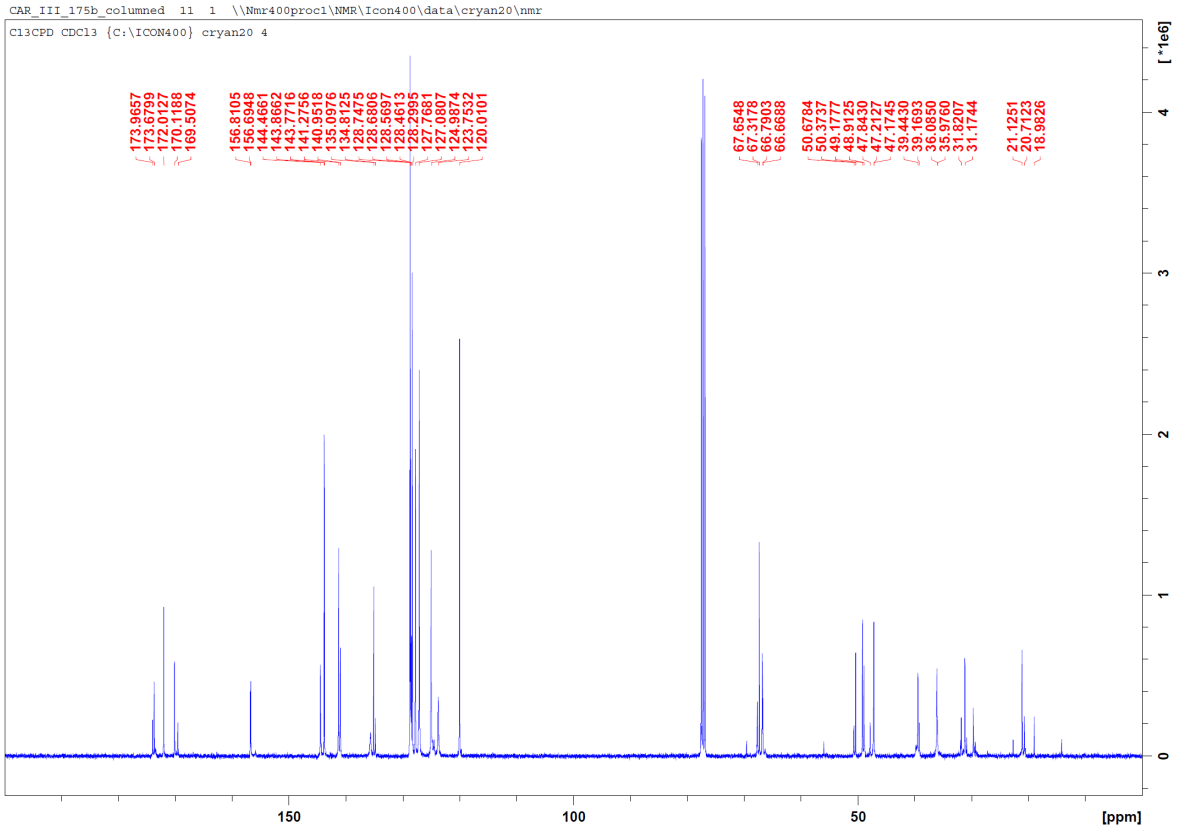
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C13CPD CDCl3 {C:\ICON400} cryan20 4
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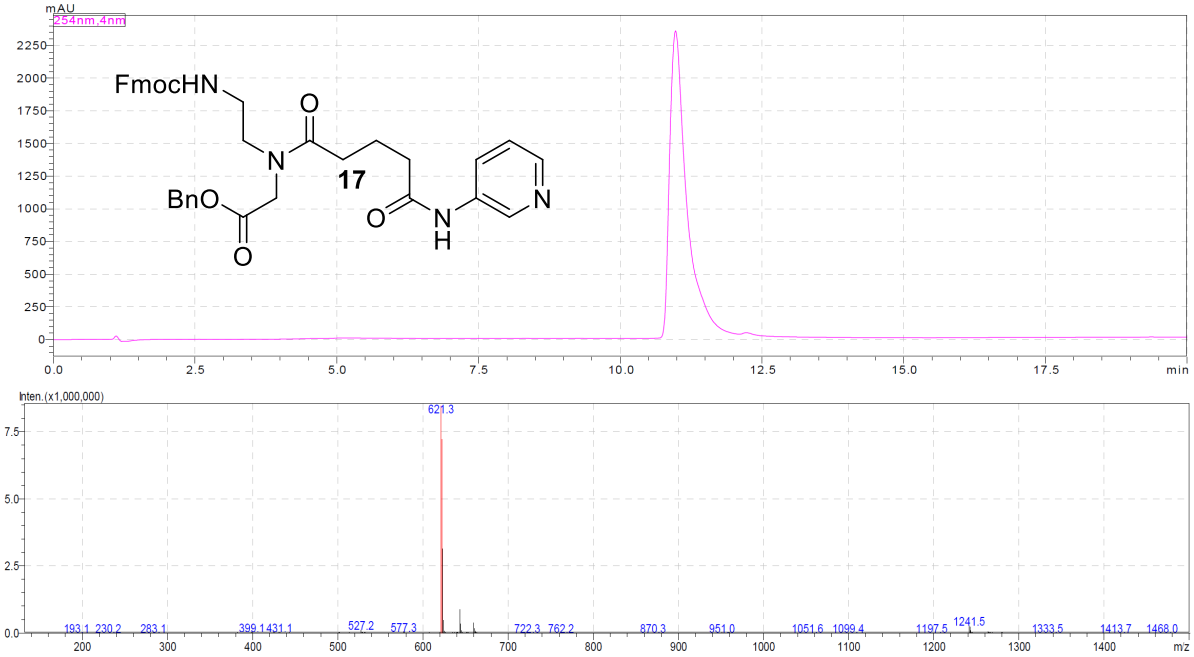
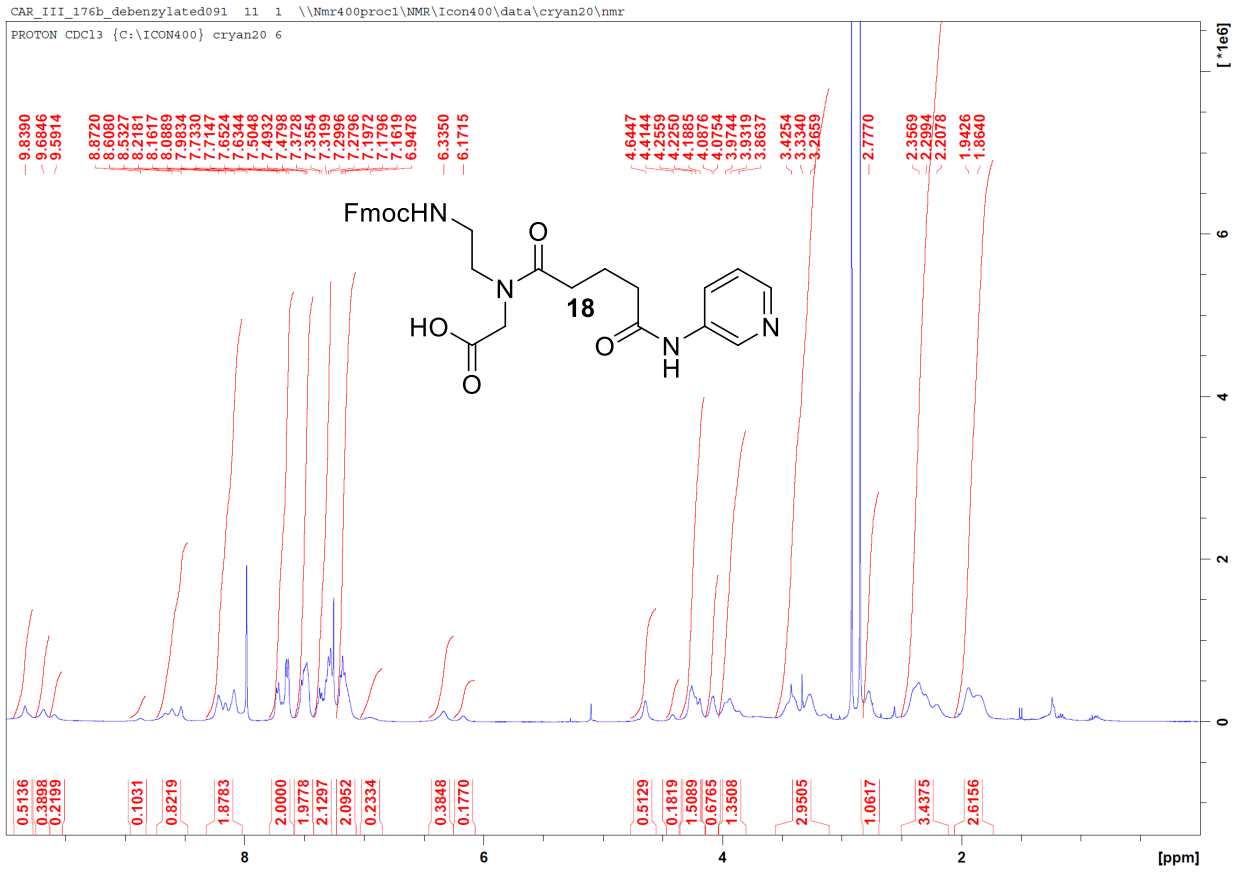


Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.


CAR_III_176b_debenzylated091 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
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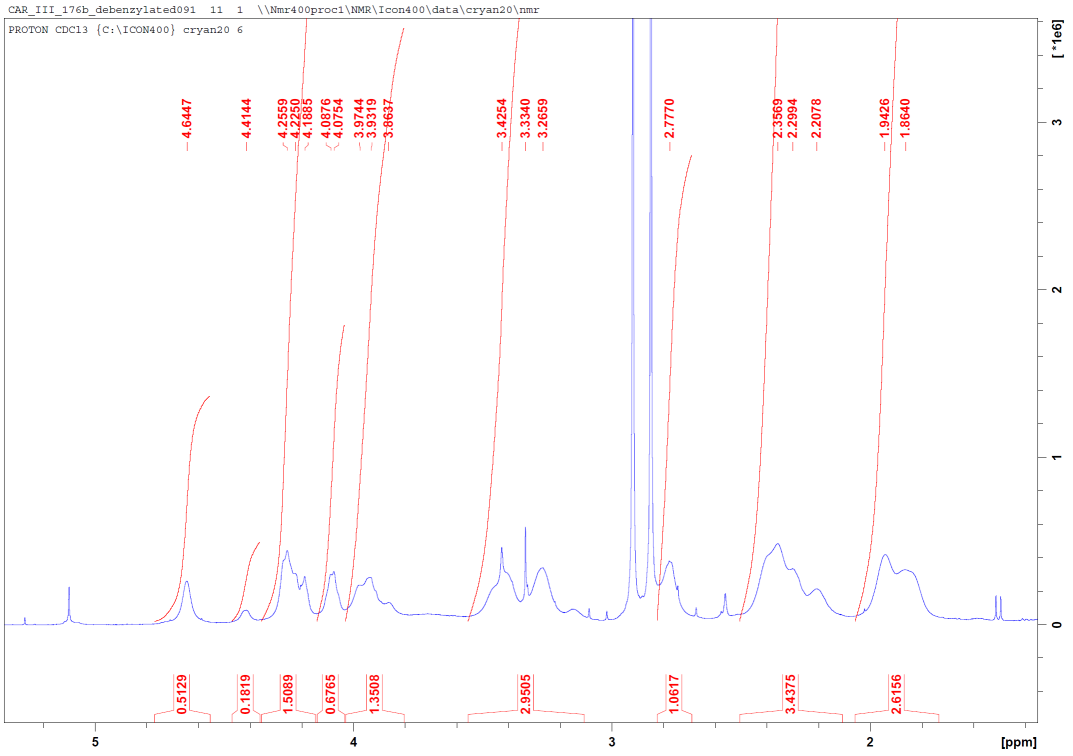
HO
O
N
O
18
O
N
H
N
[*1e6]
[ppm]


CAR_III_176b_debenzylated091 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
PROTON CDCl3 {C:\ICON400} cryan20 6
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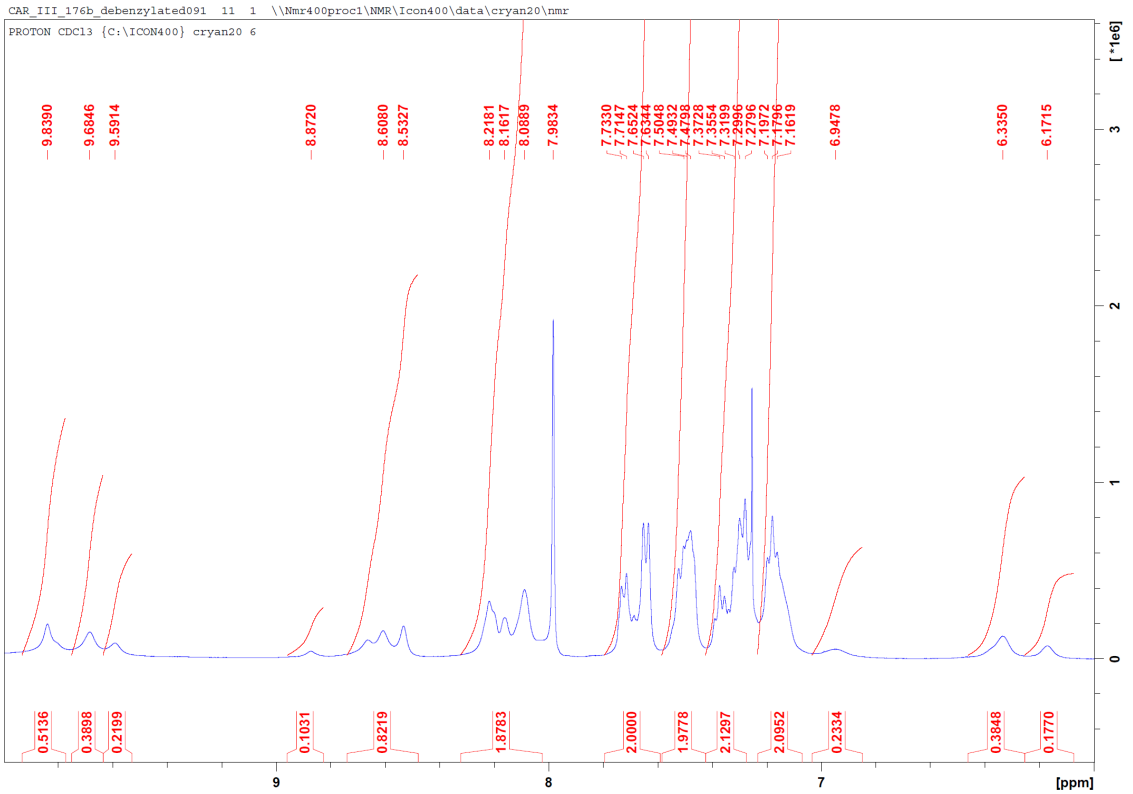
CAR_III_176b_debenzylated091 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
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9.5914
8.8720
8.6080
8.5327
8.2181
8.1617
8.0889
7.9834
7.7330
7.7147
7.6524
7.6344
7.5048
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7.2796
7.1972
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7.1619
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0.1031
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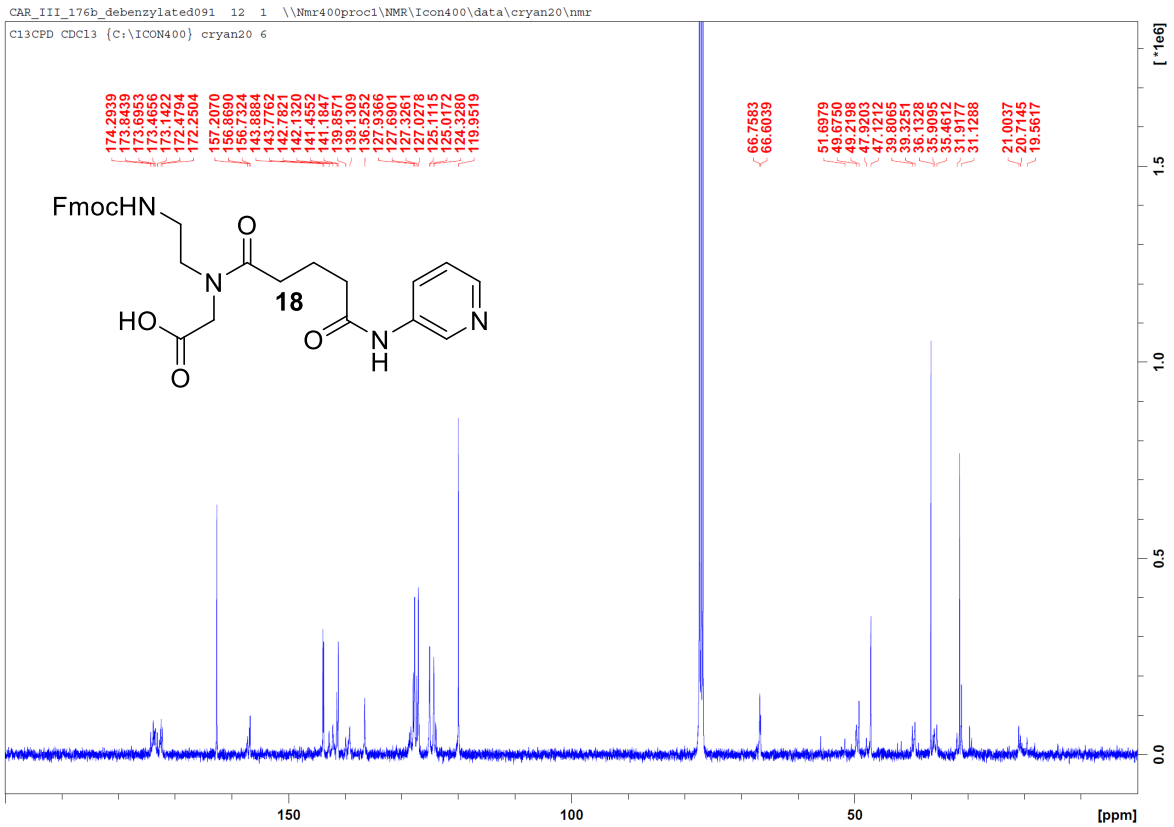
CAR_III_176b_debenzylated091 12 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDC13 {C:\ICON400} cryan20 6
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173.6953
173.4656
173.1422
172.4794
172.2504
157.2070
156.8690
156.7324
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142.1320
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141.4547
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139.1309
136.5252
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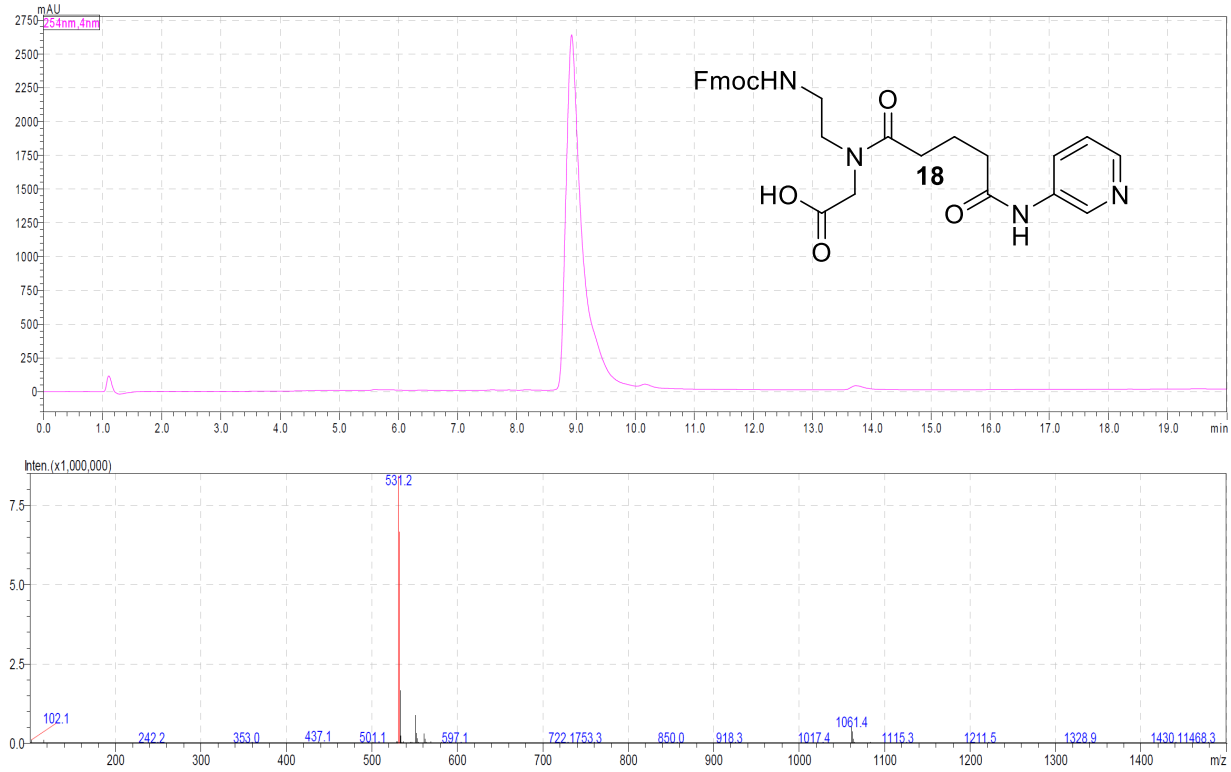
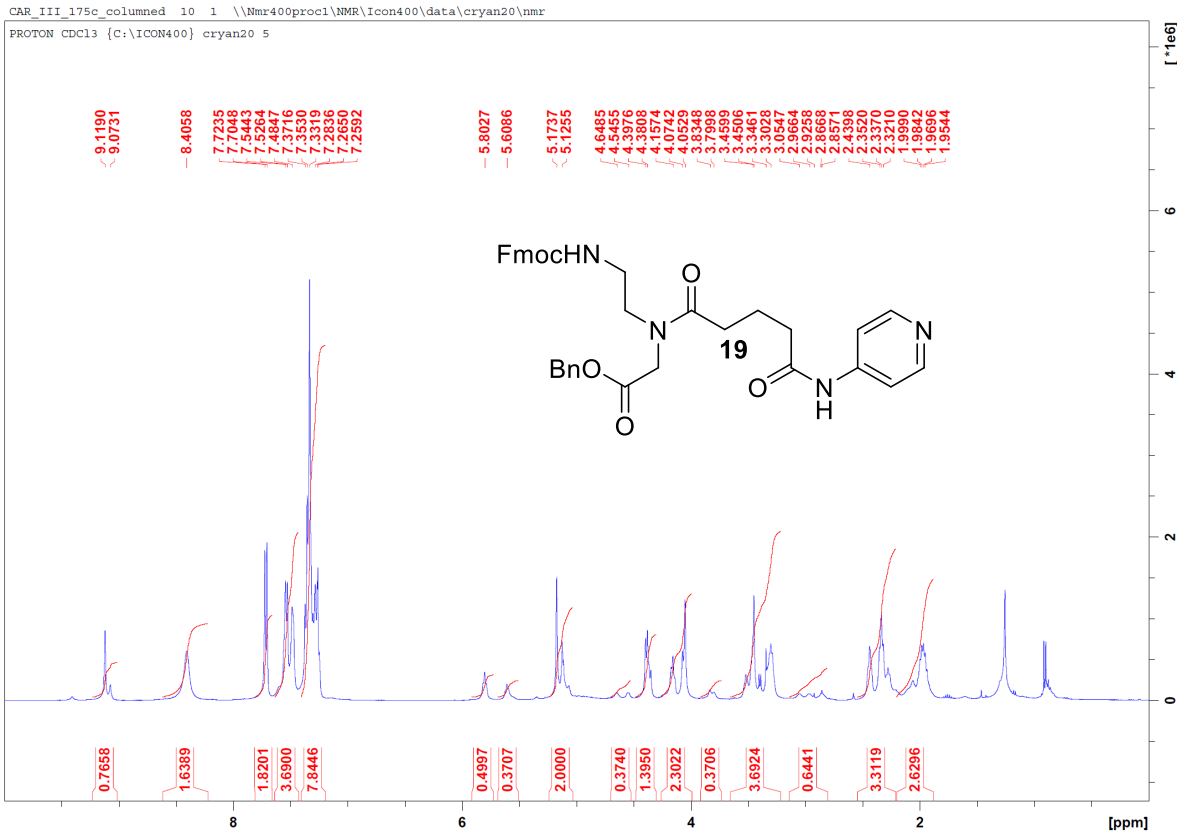


Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.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7.5443
7.5264
7.4847
7.3716
7.3530
7.3319
7.2836
7.2650
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5.1737
5.1255
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O
N
H
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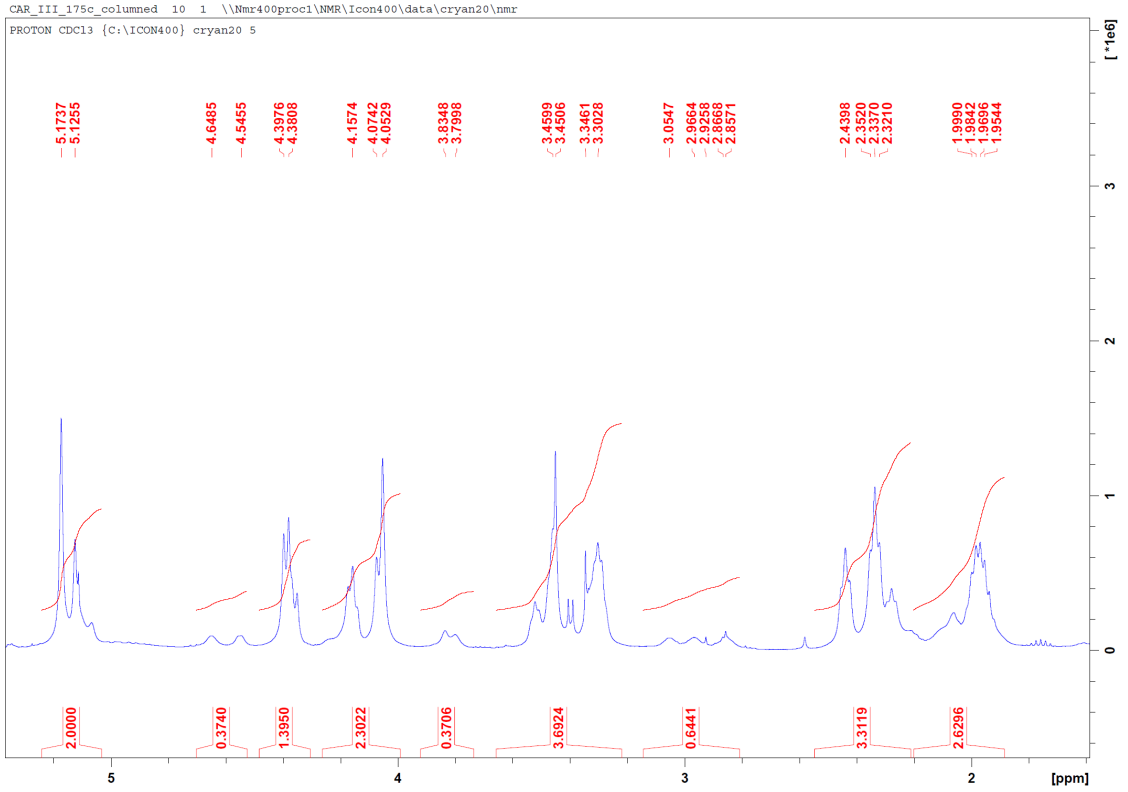
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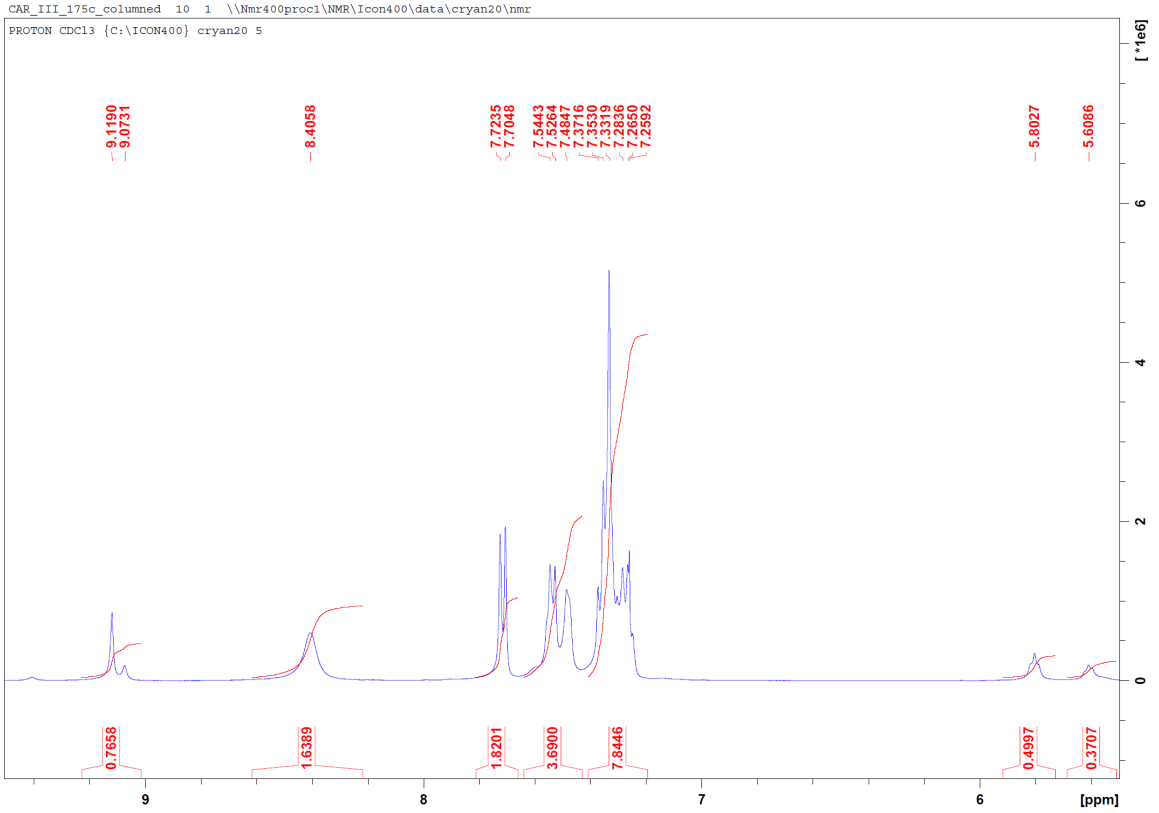
CAR_III_175c_columned 10 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
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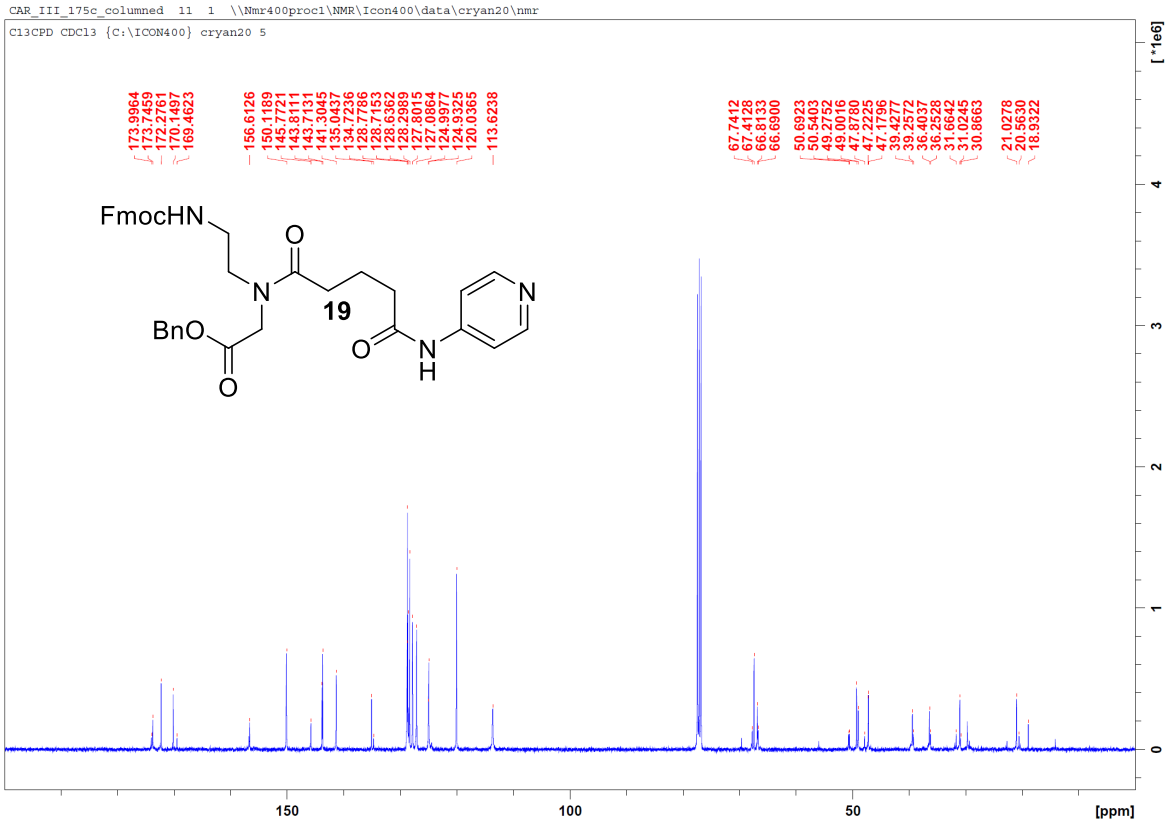
[ppm]


CAR_III_175c_columned 11 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD CDCl3 {C:\ICON400} cryan20 5
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BnO
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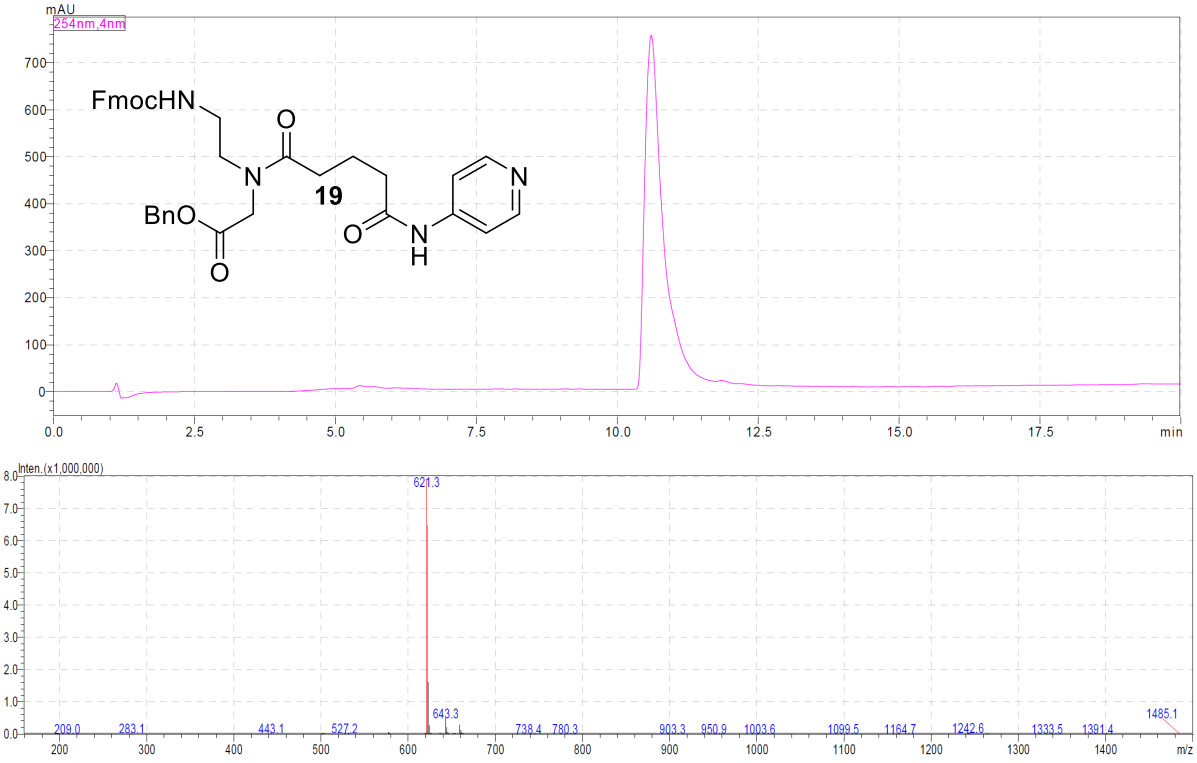
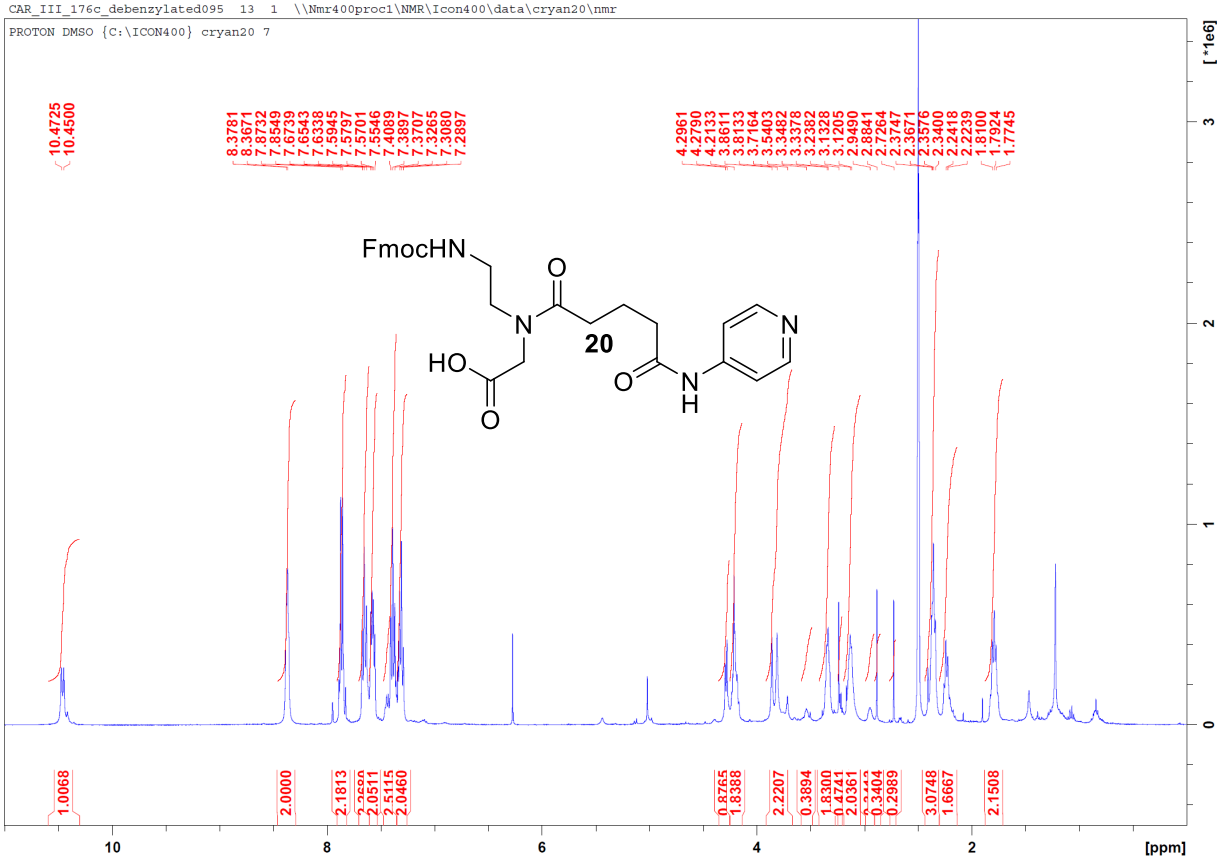


Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.


CAR_III_176c_debenzylated095 13 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
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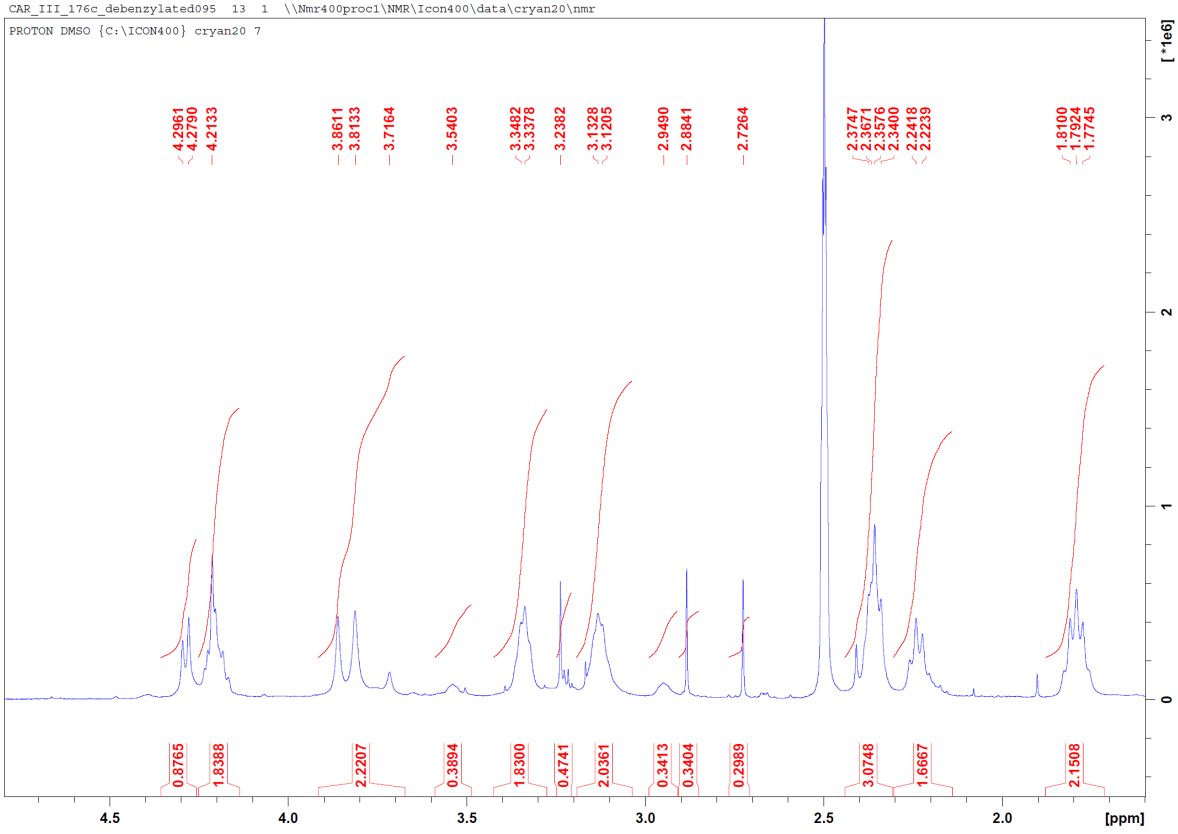
FmocHN
HO
20
[*1e6]
[ppm]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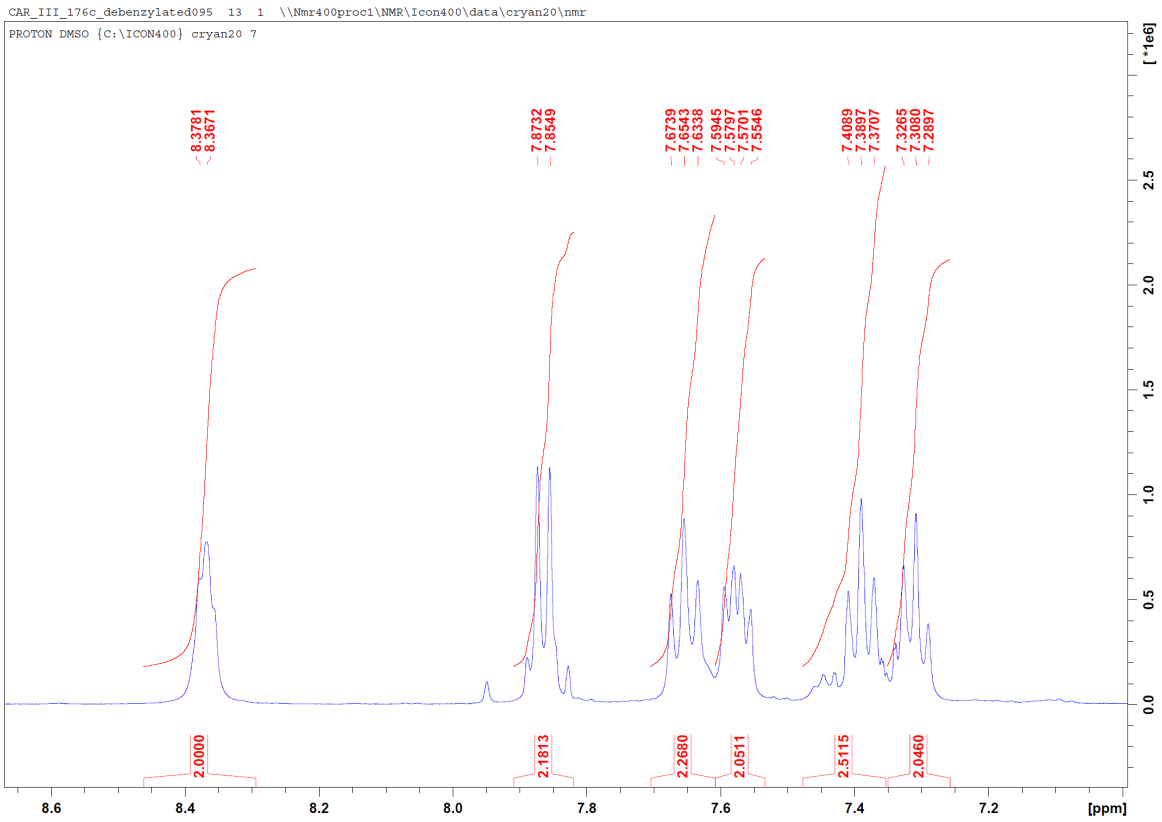
PROTON DMSO {C:\ICON400} cryan20 7
[*1e6]
[ppm]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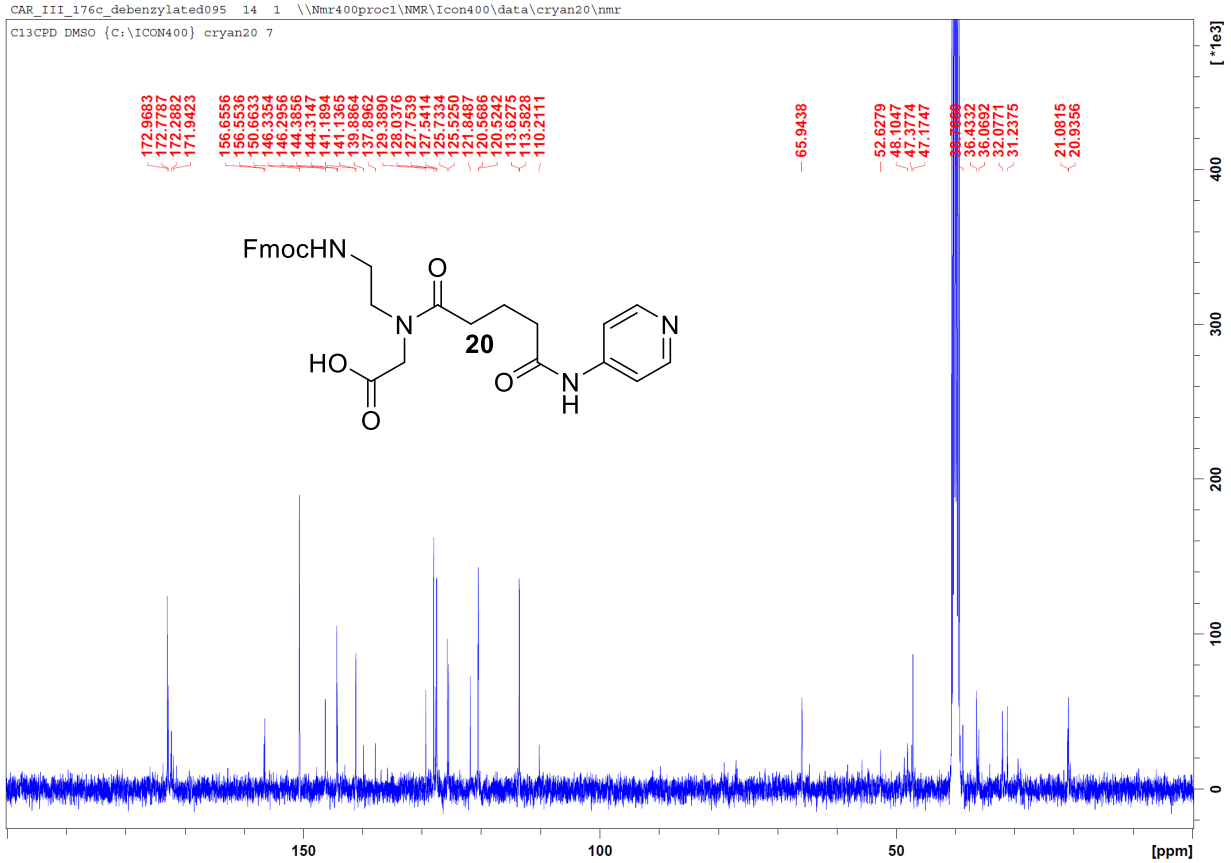
CAR_III_176c_debenzylated095 14 1 \\Nmr400proc1\NMR\Icon400\data\cryan20\nmr
C13CPD DMSO {C:\ICON400} cryan20 7
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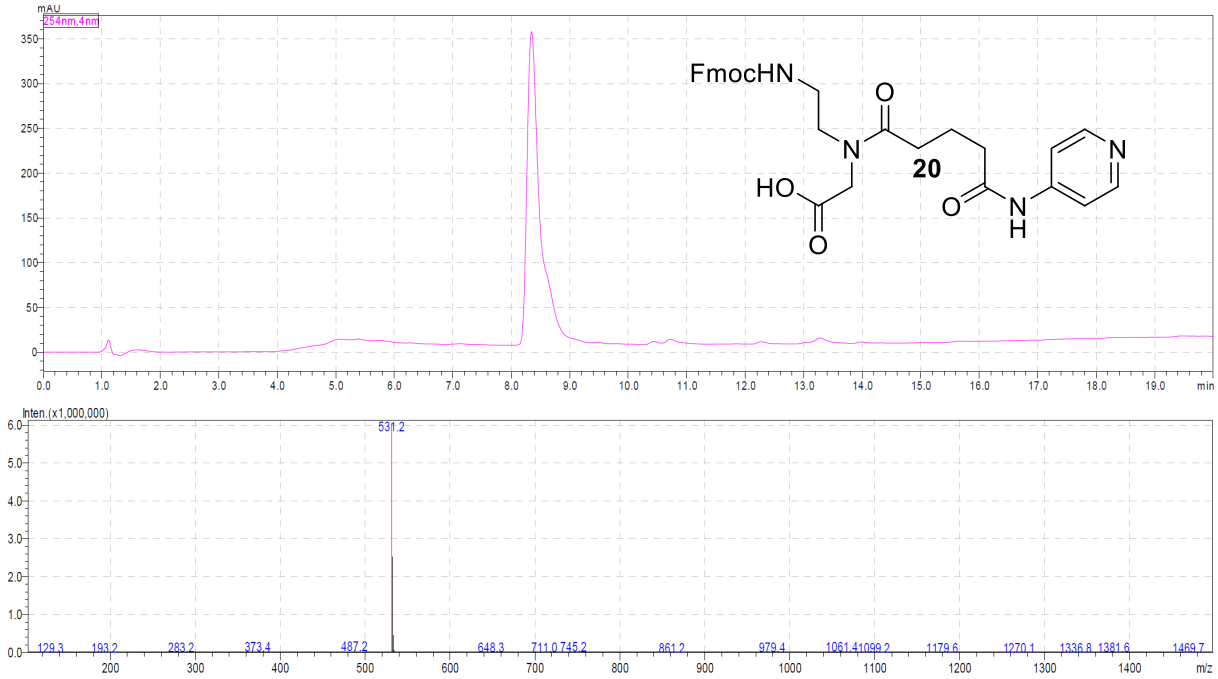
47.1747
36.4332
36.0692
32.0771
31.2375
21.0815
20.9356
FmocHN
O
N
HO
O
20
O
N
H
N
[*1e3]
[ppm]



Column: Cosmosil 2.5 C_{18}-MS-II, 120 Å 2.5 µm, 2.0 x 50 mm.
Solvent gradient: 0-100% ACN/H_2O (0.1% formic acid), 18 mins, 0.2 mL/min.