Supporting Information

Amide Modifications in the Seed Region of the Guide Strand Improve the On-Target Specificity of Short Interfering RNA

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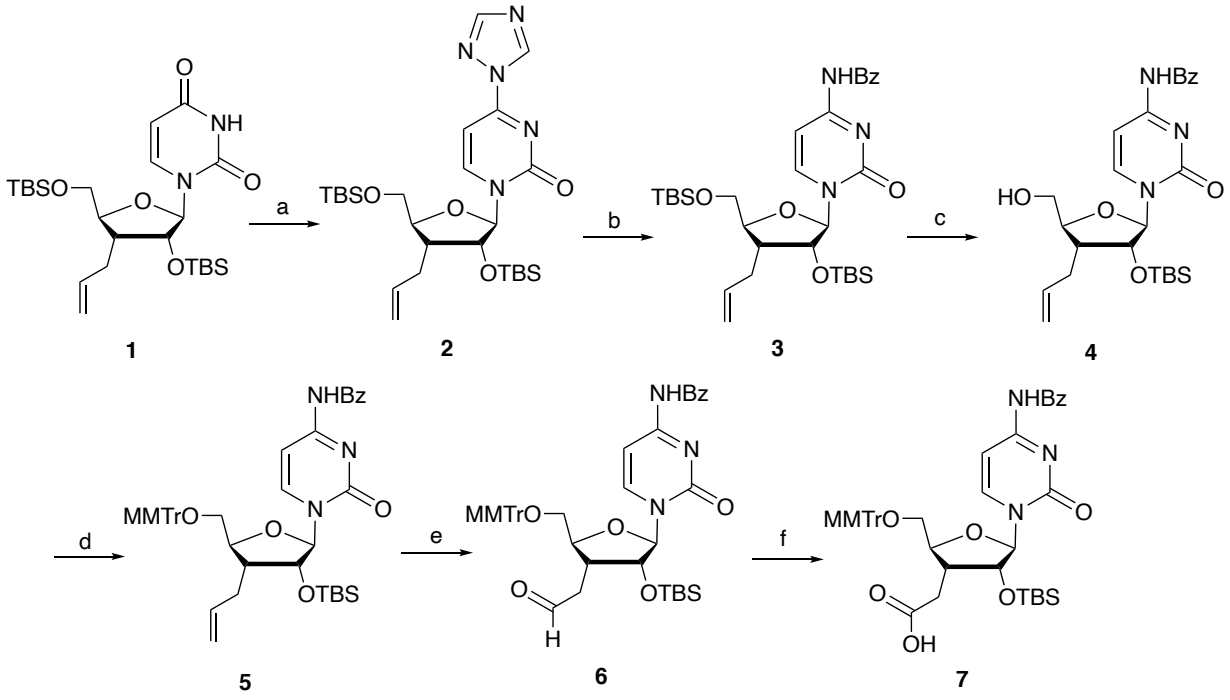
General synthetic procedures

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 molecular sieves in an MBRAUN solvent purification system. 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) was done using Silacycle 60 Å 10-12 µm silica gel F254 plates (0.25 mm), and visualization was aided by UV light, iodine, or ninhydrin stain. Column chromatography was done using Silacycle P60 230−400 mesh silica gel as the stationary phase unless otherwise noted. NMR spectra were obtained using a Bruker AM 400 spectrometer, with chemical shifts (δ) reported in parts per million (ppm) relative to TMS and the solvent peak [dimethyl sulfoxide (DMSO)-d6, CD_3CN, 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. 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 amide-linked dinucleotide phosphoramidites



Scheme S1. Synthesis of cytosine carboxylic acid (**7**). Steps: (a) phosphorus oxychloride, triethylamine, 1,2,4-triazole, MeCN, 0°C, 30 m, 81%. (b) benzamide, NaH, dioxane, rt 1.5 h, 74%. (c) 3:1 TFA/H_2O, THF, 0 °C, 2h, 90%. (d) 4-methoxytrityl chloride, pyridine, 0 °C, 17 h, 99%. (e) 4-methylmorpholine N-oxide/H_2O, 4% OsO_4 in H_2O, dioxane, rt, 26 h, 88%. (f) 2-methyl-2-butene, sodium chlorite, sodium phosphate, tert-butyl alcohol, H_2O, rt, 3 h, 69%.

4-(1H-1,2,4-triazol-1-yl)-3'-allyl-3'-deoxy-2',5'-O-bis(tert-butyldimethylsilyl)-2H-2-pyrimidin-2-one (2) A suspension of 1,2,4-triazole (3.96 g, 57.4 mmol) in acetonitrile (25 mL) was cooled to 0 °C, phosphorus oxychloride (1.26 mL, 13.5 mmol) was added dropwise, and the reaction mixture was stirred for 15 mins at 0 °C. Triethylamine (9.4 mL, 68 mmol) was then added and the reaction mixture was stirred at 0 °C for 30 minutes. Compound **1**[1] (838 mg, 1.69 mmol) was dried azeotropically with acetonitrile (3x10 mL), dissolved in acetonitrile (15 mL), cooled to 0 °C, and added dropwise to the reaction mixture at 0 °C. The mixture was stirred at 0 °C for 10 min and at room temperature for 18 hrs. The mixture was extracted with ethyl acetate (30 mL). The organic phase was separated, washed with water (10 mL), brine (10 mL),

dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified using flash silica gel chromatography (10-50% ethyl acetate in hexanes) to afford compound **2** as a white foam. Yield: 750 mg, 81%. R_f = 0.4 (30% ethyl acetate in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.78 (s, 1H), 8.68 (d, *J* = 7.5 Hz, 1H), 7.89 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.60 – 7.54 (m, 1H), 7.51 – 7.45 (m, 2H), 7.39 (s, 1H), 5.77 – 5.65 (m, 2H), 5.09 – 4.95 (m, 2H), 4.31 (d, *J* = 3.7 Hz, 1H), 4.20 (dd, *J* = 12.0, 1.8 Hz, 1H), 4.15 – 4.08 (m, 1H), 3.77 (dd, *J* = 12.1, 1.8 Hz, 1H), 2.34 (dddt, *J* = 14.3, 9.1, 6.2, 1.5 Hz, 1H), 2.11 (dddd, *J* = 10.5, 8.9, 5.0, 3.8 Hz, 1H), 2.04 – 1.94 (m, 1H), 0.94 (d, *J* = 11.6 Hz, 18H), 0.34 (s, 3H), 0.16 (d, *J* = 2.7 Hz, 6H), 0.13 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 162.2, 154.8, 145.5, 135.7, 133.4, 133.0, 128.9, 127.55, 116.4, 95.7, 92.3, 85.39, 77.6, 77.4, 77.0, 76.7, 61.3, 39.5, 28.3, 26.0, 25.9, 18.5, 18.2, -4.0, -5.4, -5.5, -5.7. MS-ESI (+): Mass calc. for $C_{26}H_{45}N_5O_4Si_2$ (M+H), 548.3; found, 548.3.

3′-Allyl-4-N-benzoyl-2′,5′-O-*bis*(*tert*-butyldimethylsilyl)-3′-deoxycytidine (3) Benzamide (620 mg, 5.12 mmol) was dissolved in dioxane (8 mL), after which 60 wt% NaH in oil (186 mg, 4.66 mmol) was added under an inert atmosphere of N_2 gas. The bubbly white paste was stirred at room temperature for 30 min. Compound **2** (623 mg, 1.14 mmol) in dioxane (10 mL) was added dropwise and the reaction mixture was stirred at room temperature for 1.5 hours, resulting in a faintly yellow suspension. The reaction mixture was brought to pH 7 with acetic acid (~100 µL) and partitioned between water and dichloromethane. The organic phase was dried over sodium sulfate, concentrated to 12 mL under reduced pressure and flushed through a plug of silica. The reaction mixture was concentrated, the crude product was redissolved in 10 % ethyl acetate in hexanes (15 mL), and the turbid solution was purified on an Isco CombiFlash Sg 100c chromatography system using a SiliCycle SiliaSep Flash Cartridge (40 g, 40-63 µm particle size, 60 Å pore size); solvent A: hexanes; solvent B: ethyl acetate; flow rate: 20 mL/min; equilibration: 10 % B; gradient: 10 % B for 30.1 min, 45 % B over 56.6 min; retention time: 49 min; to afford pure compound **3** as glassy crystals. Yield: 503 mg, 74 %. R_f = 0.5 (30 % ethyl acetate in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.68 (d, J = 7.5 Hz, 1H), 7.89 (dd, J = 8.3, 1.3 Hz, 2H), 7.60 – 7.54 (m, 1H), 7.51 – 7.45 (m, 2H), 7.39 (s, 1H), 5.77 – 5.65 (m, 2H), 5.09 – 4.95 (m, 2H), 4.31 (d, J = 3.7 Hz, 1H), 4.20 (dd, J = 12.0, 1.8 Hz, 1H), 4.15 – 4.08 (m, 1H), 3.77 (dd, J = 12.1, 1.8 Hz, 1H), 2.34 (dddt, J = 14.3, 9.1, 6.2, 1.5 Hz, 1H), 2.11 (dddd, J = 10.5, 8.9, 5.0, 3.8 Hz, 1H), 2.04 – 1.94 (m, 1H), 0.94 (d, J = 11.6 Hz, 18H), 0.34 (s, 3H), 0.16 (d, J = 2.7 Hz, 6H), 0.13 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$ δ 166.6, 162.1, 154.7, 145.5, 135.6, 133.3, 132.9, 128.9, 127.5, 116.4, 95.6, 92.3, 85.3, 77.6, 77.3, 77.0, 76.7, 61.2, 39.5, 28.3, 25.9, 25.9, 18.4, 18.1, - 3.9, -5.3, -5.4, -5.6. MS-ESI (+): Mass calc. for $C_{31}H_{49}N_3O_5Si_2$ (M+H), 600.3; found, 600.3.

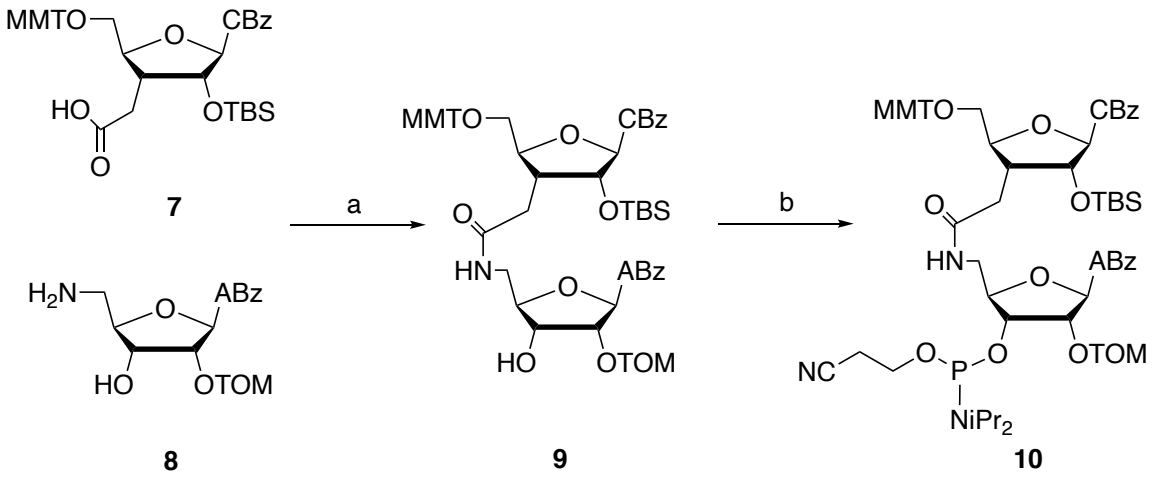
3′-Allyl-4-N-benzoyl-2′-O-*tert*-butyldimethylsilyl-3′-deoxycytidine (4) Compound **3** (185 mg, 0.308 mmol) was dissolved in tetrahydrofuran (10 mL) and chilled to °0 C. A pre-chilled solution of 3:1 trifluoroacetic acid/water (10 mL) was added. The reaction mixture was stirred at room temperature for 2 hours, before being neutralized with $NaHCO_3$. The crude product mixture was partially concentrated to remove tetrahydrofuran under reduced pressure, diluted with ethyl acetate (100 mL), washed with sat. aq. $NaHCO_3$ (50 mL), water (50 mL), and brine (50 mL). The organic phase was dried over sodium sulfate, adsorbed on 4 grams of Celite under reduced pressure and purified on an Isco CombiFlash Sg 100c chromatography system using a SiliCycle SiliaSep Flash Cartridge (40 g, 40-63 µm particle size, 60 Å pore size); solvent A: hexanes; solvent B: ethyl acetate; flow rate: 20 mL/min; equilibration: 10 % B; gradient: 50 % B over 60 min; retention time: 55 min; to afford compound **4** as white foam. Yield: 134 mg, 90 %. R_f = 0.11 (30 % ethyl acetate in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, J = 7.5 Hz, 1H), 7.87 – 7.80 (m, 2H), 7.59 – 7.50 (m, 1H), 7.44 (dd, J = 8.3, 7.0 Hz, 3H), 5.80 – 5.65 (m, 2H), 5.09 – 4.99 (m, 1H), 4.99 – 4.94 (m, 1H), 4.39 (d, J = 3.8 Hz, 1H), 4.23 – 4.05 (m, 2H), 3.82 (dd, J = 12.6, 3.0 Hz, 1H), 2.34 (dddt, J = 14.3, 8.1, 6.4, 1.4 Hz, 1H), 2.21 – 2.02 (m, 2H), 0.91 (s, 9H), 0.30 (s, 3H), 0.15 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 162.3, 155.1, 146.0, 135.7, 133.1, 133.0, 128.9, 127.5, 116.5, 95.9, 93.0, 85.6, 77.4, 77.3, 77.0, 76.7, 60.8, 39.9, 28.6, 25.9, 18.1, -3.9, -5.5. MS-ESI (+): Mass calc. for $C_{25}H_{35}N_3O_5Si$ (M+H), 486.2; found, 486.2.

3′-Allyl-4-N-benzoyl-2′-O-*tert*-butyldimethylsilyl-3′-deoxy-5′-O-methoxytritylcytidine (5) Compound **4** (401 mg, 0.826 mmol) was dried azeotropically with pyridine (3 x 5 mL), dissolved in pyridine (18 mL), and chilled to 0 °C. 4-Methoxytrityl chloride (1.28 g, 4.13 mmol) was added at 0 °C, giving a pale yellow and slightly turbid mixture. The reaction mixture was stirred at room temperature for 17 hours. The reaction mixture, now yellow-orange and no longer turbid, was concentrated under reduced pressure. The crude product was redissolved in ethyl acetate (50 mL) and washed with of water containing little brine (50 mL), water (50 mL), and brine (50 mL). The organic phase was dried over sodium sulfate, adsorbed on 8 grams of Celite under reduced pressure, and purified on an Isco CombiFlash Sg 100c chromatography system using a SiliCycle SiliaSep Flash Cartridge (40 g, 40-63 µm particle size, 60 Å pore size); solvent A: hexanes with 0.5 % NEt3; solvent B: ethyl acetate with 0.5 % NEt3; flow rate: 20 mL/min; equilibration: 15 % B; gradient: 15.2 % B for 18.2 min, 36 % B over 35.7 min; retention time: 34 min; to afford pure compound **5** as white foam. Yield: 621 mg, 99 %. R_f = 0.23 (30 % ethyl acetate in hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, J = 7.4 Hz, 1H), 7.89 (dd, J = 7.1, 1.8 Hz, 2H), 7.65 – 7.56

(m, 1H), 7.51 (dd, J = 8.3, 6.8 Hz, 2H), 7.48 – 7.42 (m, 4H), 7.40 – 7.25 (m, 9H), 7.20 (s, 1H), 6.93 – 6.87 (m, 2H), 5.79 (s, 1H), 5.66 (dddd, J = 17.5, 10.2, 7.4, 5.9 Hz, 1H), 5.01 (dq, J = 17.1, 1.6 Hz, 1H), 4.92 (dt, J = 10.2, 1.5 Hz, 1H), 4.37 (d, J = 3.5 Hz, 1H), 4.19 (dt, J = 10.6, 2.6 Hz, 1H), 3.83 (s, 3H), 3.79 – 3.72 (m, 1H), 3.34 (dd, J = 11.3, 2.8 Hz, 1H), 2.26 (dddd, J = 23.5, 13.3, 9.3, 6.3 Hz, 2H), 1.92 – 1.81 (m, 1H), 0.92 (s, 7H), 0.91 (s, 10H), 0.36 (s, 3H), 0.17 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.2, 158.9, 154.9, 145.5, 143.7, 143.7, 135.7, 135.1, 133.4, 133.1, 130.4, 129.1, 128.7, 128.6, 128.1, 127.6, 127.4, 116.5, 113.4, 96.1, 92.7, 87.4, 84.4, 77.6, 77.4, 77.1, 76.8, 61.6, 55.3, 40.9, 28.4, 26.0, 18.3, -3.8, -5.5. HRMS-ESI (+): Mass calc. for $C_{45}H_{51}N_3O_6Si$ (M+H), 758.3625; found, 758.3611.

4-N-benzoyl-2′-O-*tert*-butyldimethylsilyl-3′-CH$_2$CHO-3′-deoxy-5′-O-methoxytritylcytidine (6) Compound **5** (603 mg, 0.796 mmol) was dissolved in dioxane (10 mL). To this solution 4-methylmorpholine N-oxide from a 50 wt% solution in water (183 µL, 0.880 mmol) was added, followed by OsO_4 from a 4 wt% solution in water (97 µL, 0.015 mmol). The reaction mixture was protected from light and stirred for 26 hours at room temperature to affect conversion to a diol intermediate (not isolated; R_f = 0.6 in 10 % MeOH/dichloromethane, or 0.32 in 5 % MeOH/dichloromethane). 2,6-lutidine (187 µL, 1.61 mmol) was added to the reaction mixture, yielding a pale-yellow solution. Water (3.3 mL) was added bringing dioxane/water ratio to 3:1. Sodium metaperiodate (678 mg, 3.17 mmol) was added, yielding a white suspension. The reaction mixture was now vigorously stirred at room temperature for 2 hours, after which it was diluted with dichloromethane (60 mL) and water (15 mL). The organic phase was collected, and product was extracted with dichloromethane (3 x 60 mL). The dichloromethane portions were combined, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was redissolved in dichloromethane, adsorbed on 12 g of Celite, and purified on an Isco CombiFlash Sg 100c chromatography system using a SiliCycle SiliaSep Flash Cartridge (40 g, 40-63 µm particle size, 60 Å pore size); solvent A: hexanes; solvent B: ethyl acetate; flow rate: 40 mL/min; equilibration: 0 % B; gradient: 0 % B for 2 min, 70 % B over 40 min; retention time: 26 min; to afford compound **6** as off-white foam. Yield: 534 mg, 88%. R_f = 0.33 (5 % MeOH/dichloromethane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H), 8.69 (d, J = 7.5 Hz, 1H), 7.92 (d, J = 7.6 Hz, 2H), 7.62 (t, J = 7.4 Hz, 1H), 7.53 (t, J = 7.6 Hz, 2H), 7.44 (d, J = 7.4 Hz, 4H), 7.39 – 7.27 (m, 9H), 6.92 – 6.86 (m, 2H), 5.84 (s, 1H), 4.51 (d, J = 3.8 Hz, 1H), 4.17 – 4.07 (m, 2H), 3.84 (s, 3H), 3.29 (dd, J = 11.7, 2.8 Hz, 1H), 2.69 – 2.54 (m, 2H), 2.04 (s, 1H), 1.93 (dd, J = 18.0, 2.9 Hz, 1H), 0.88 (s, 9H), 0.33 (s, 3H), 0.07 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.3, 158.8, 143.5, 143.4, 134.9, 130.3, 129.0, 128.5, 128.4, 128.1, 127.4, 113.4, 87.6, 83.6, 77.3, 77.0, 76.7, 55.2, 38.6, 35.4, 25.9, 18.1, -4.18, -5.73. HRMS-ESI (+): Mass calc. for $C_{44}H_{49}N_3O_7Si$ (M+H), 760.3418; found, 760.3398.

4-N-benzoyl-2'-O-*tert*-butyldimethylsilyl-3'-CH_2COOH-3'-deoxy-5'-O-methoxytritylcytidine (7).
Compound **6** (534 mg, 0.703 mmol) was dissolved in tert-butyl alcohol (10 mL) with a few drops of water (warm slightly, as needed, if compound precipitates or does not dissolve). Water (2 mL) was added dropwise while stirring vigorously, giving a clear solution to which 2-methyl-2-butene (2M in tetrahydrofuran, 2.1 mL, 4.2 mmol) was added resulting in a pale-yellow turbid mixture. Monobasic sodium phosphate (210 mg, 1.75 mmol) was added, followed by the addition of sodium chlorite (267 mg, 2.95 mmol), at which point the slightly turbid reaction mixture turned bright yellow and briefly became warm. The reaction mixture was stirred at room temperature for 3 hours, then quenched with saturated aqueous sodium thiosulfate (3.7 mL), causing the mixture to become very turbid and viscous. The crude product mixture was frozen and lyophilized. The resulting residue was vigorously agitated with water (25 mL) and dichloromethane (125 mL). The organic phase was collected, and the aqueous phase was extracted with dichloromethane (125 mL). The combined dichloromethane portions were dried over sodium sulfate, filtered through Celite, and concentrated under reduced pressure. The crude product was redissolved in dichloromethane containing 0.5 % NEt_3 (8 mL), loaded onto a dry Yamazen size-"M" silica inject column and purified on an Isco CombiFlash Sg 100c chromatography system using a Yamazen Universal Premium Column (55 g, 25- 40 µm particle size, 60 Å pore size) solvent A: dichloromethane with 0.5 % NEt3; solvent B: 20 % MeOH/dichloromethane; flow rate: 20 mL/min; equilibration: 0 % B; gradient: 0 % B for 10 min, 30 % B over 30 min; retention time: 44 min), to afford monomer **7** (with approximately 1 eq of NEt_3) as white foam. Yield: 427 mg, 69 %. R_f = 0.58 (10 % MeOH/dichloromethane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, J = 7.5 Hz, 1H), 7.96 – 7.89 (m, 2H), 7.63 – 7.54 (m, 1H), 7.51 – 7.41 (m, 6H), 7.35 (td, J = 8.3, 7.8, 6.5 Hz, 6H), 7.32 – 7.23 (m, 3H), 6.93 – 6.84 (m, 2H), 5.82 (s, 1H), 4.57 (d, J = 3.1 Hz, 1H), 4.19 – 4.08 (m, 1H), 3.83 (s, 3H), 3.69 (dd, J = 11.6, 2.1 Hz, 1H), 3.33 (dd, J = 11.5, 3.6 Hz, 1H), 2.58 – 2.44 (m, 2H), 2.00 (dd, J = 13.6, 4.2 Hz, 1H), 0.90 (s, 9H), 0.31 (s, 3H), 0.09 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.3, 162.4, 158.8, 145.6, 143.9, 143.8, 135.2, 133.4, 133.1, 130.4, 128.9, 128.6, 128.5, 128.1, 127.9, 127.3, 127.3, 113.4, 93.1, 87.4, 83.7, 77.6, 77.4, 77.1, 76.8, 61.9, 55.3, 38.2, 29.8, 26.0, 18.2, - 4.1, -5.4. HRMS-ESI (+): Mass calc. for $C_{44}H_{49}N_3O_8Si$ (M+H), 776.3367; found, 776.3362.



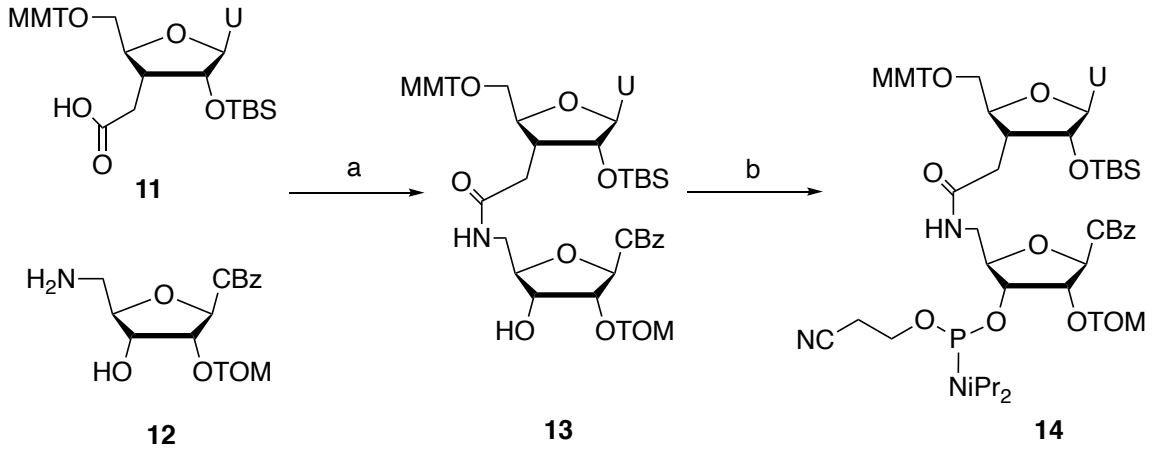
Scheme S2. Synthesis of amide-linked CaA dimer phosphoramidate (**10**). Steps: (a) HOBt, HBTU, N,N-diisopropylethylamine, DCM, rt, 23 h, 43% (b) 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphoro-diamidite, 4,5-dicyanoimidazole, DCM, rt, 4 h, 74%.

Amide-linked CaA dimer (9) Carboxylic acid **7** (as NEt_3 salt, 109 mg, 0.124 mmol) and HOBt (20 wt% water, 21 mg, 0.13 mmol) were co-evaporated with dry acetonitrile (2 x 5 mL). HBTU (52 mg, 0.14 mmol) was added followed by dichloromethane (8 mL), forming a suspension. N,N-diisopropylethylamine (40 µL, 0.23 mmol) was added and the reaction mixture was stirred at room temperature for 5 minutes. Amine **8**[2] (76 mg, 0.14 mmol) was added, and the reaction mixture was stirred at room temperature for 23 hours, after which it was partitioned between dichloromethane (50 mL) and brine (50 mL). The organic phase was collected and the crude product further extracted with dichloromethane (3 x 50 mL). The combined dichloromethane portions were dried over sodium sulfate and concentrated. The product was purified using reverse-phase column-chromatography on an Isco CombiFlash Sg 100c chromatography system using a SiliCycle SiliaSep C18 (17 %) Flash Cartridge reverse-phase column (25 g, 40-63 µm particle size, 60 Å pore size); solvent A: 25 % MeCN/water; solvent B: MeCN; flow rate: 15 mL/min; equilibration: 0 % B; gradient: 0 % B for 20 min, 100 % B over 140 min; retention time: 155 min) to afford amide-linked CaA dimer **9**. Yield: 70mg, 43%. R_f = 0.64 (5 % MeOH/dichloromethane). ^{1}H NMR (400 MHz, 2:1 $CD_3CN/CDCl_3$) δ 9.64 (s, 1H), 9.02 (s, 1H), 8.64 – 8.55 (m, 2H), 8.28 (s, 1H), 8.06 – 7.99 (m, 2H), 7.91 – 7.85 (m, 2H), 7.66 – 7.42 (m, 10H), 7.38 – 7.28 (m, 6H), 7.28 – 7.18 (m, 3H), 7.03 (dd, J = 7.2, 4.2 Hz, 1H), 6.90 – 6.82 (m, 2H), 6.08 (d, J = 6.3 Hz, 1H), 5.71 (s, 1H), 5.00 – 4.88 (m, 3H), 4.45 (d, J = 3.9 Hz, 1H), 4.28 (dt, J = 5.2, 2.8 Hz, 1H), 4.17 (dt, J = 6.7, 3.6 Hz, 1H), 4.10 (dt, J = 11.1, 2.7 Hz, 1H), 3.75 – 3.63 (m, 5H), 3.45 (d, J = 3.8 Hz, 1H), 3.43 – 3.27 (m, 2H), 2.69 – 2.59 (m, 1H), 2.42 (dd, J = 16.1, 9.5 Hz, 1H), 1.91 – 1.81 (m, 1H), 0.89 (dh, J = 5.0, 2.9 Hz, 21H), 0.85 (s, 9H), 0.22 (s, 3H). ^{13}C NMR (101 MHz, 2:1

$CD_3CN/CDCl_3$) δ 172.0, 163.6, 160.0, 152.9, 151.3, 146.2, 145.2, 145.0, 144.5, 136.3, 134.6, 134.0, 133.8, 131.5, 129.8, 129.8, 129.6, 129.5, 129.4, 129.2, 129.0, 128.3, 126.1, 118.2, 114.4, 93.7, 90.9, 88.9, 88.4, 85.1, 84.6, 80.2, 79.4, 79.1, 79.0, 78.7, 72.0, 62.7, 56.1, 42.2, 38.6, 30.9, 26.7, 19.0, 18.4, 12.7, 2.4, 2.2, 2.0, 1.8, 1.5, 1.3, 1.1, -3.6, -4.8. HRMS-ESI (+): Mass calc. for $C_{71}H_{87}N_9O_{12}Si_2$ (M+H), 1314.6091; found, 1314.6056.

Amide-linked CaA dimer phosphoramidite (10). Amide-linked CaA dimer **9** (69 mg, 0.052 mmol) was co-evaporated with acetonitrile (2 x 5 mL) and dissolved in dry dichloromethane (2 mL), followed by the dropwise addition of 2-cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite (100 µL, 0.32 mmol). The mixture was stirred at room temperature for 5 minutes. 4,5-dicyanoimidazole (7.2 mg, 0.053 mmol) was added and the reaction mixture was stirred under an inert argon atmosphere at room temperature for 4 hours. The mixture was diluted with dichloromethane (30 mL), washed with saturated aqueous $NaHCO_3$ (2 x 10 mL) and brine (10 mL), dried over sodium sulfate, adsorbed on 7 g of Celite, and purified on an Isco CombiFlash Sg 100c chromatography system using a SiliCycle SiliaSep C8 Monomeric Flash Cartridge reverse-phase column (12 g); solvent A: 50 % MeCN/Water; solvent B: MeCN; flow rate: 20 mL/min; equilibration: 0 % B; gradient: 0 % B for 12.9 min, 100 % B over 90 min; retention time: 90-95 min; to afford amide-linked CaA dimer phosphoramidite **10** as glassy solids. Yield: 59mg, 74 %. R_f = 0.90 (5 % MeOH/dichloromethane). ^{1}H NMR (400 MHz, CD_3CN) δ 8.55 (t, J = 7.2 Hz, 1H), 8.50 (s, 1H), 8.29 (d, J = 7.9 Hz, 1H), 8.00 (dt, J = 7.1, 1.3 Hz, 2H), 7.89 (ddd, J = 8.5, 3.0, 1.3 Hz, 2H), 7.59 (td, J = 7.2, 1.6 Hz, 2H), 7.52 – 7.43 (m, 8H), 7.35 – 7.28 (m, 6H), 7.22 (t, J = 7.1 Hz, 2H), 7.15 (dd, J = 15.5, 8.5 Hz, 1H), 6.91 – 6.82 (m, 2H), 6.07 (dd, J = 7.1, 2.9 Hz, 1H), 5.67 (s, 1H), 5.18 (ddd, J = 24.8, 7.2, 5.0 Hz, 1H), 4.93 (dd, J = 9.5, 5.1 Hz, 1H), 4.84 (dd, J = 16.2, 5.2 Hz, 1H), 4.55 – 4.42 (m, 2H), 4.32 (ddt, J = 43.2, 6.2, 2.4 Hz, 1H), 4.09 (d, J = 10.7 Hz, 1H), 3.92 – 3.75 (m, 2H), 3.71 (d, J = 1.5 Hz, 3H), 3.64 (dddd, J = 10.4, 9.0, 4.2, 2.4 Hz, 3H), 3.44 (dt, J = 14.5, 4.2 Hz, 1H), 3.33 (ddd, J = 11.7, 5.3, 3.4 Hz, 1H), 2.75 – 2.57 (m, 3H), 2.44 (ddd, J = 15.5, 9.5, 5.7 Hz, 1H), 1.90 (dd, J = 9.4, 4.2 Hz, 2H), 1.19 (dt, J = 11.8, 6.9 Hz, 12H), 0.85 (d, J = 1.4 Hz, 9H), 0.79 (d, J = 4.0 Hz, 21H), 0.19 (d, J = 3.3 Hz, 3H), -0.03 (d, J = 4.9 Hz, 3H). ^{13}C NMR (101 MHz, CD_3CN) δ 171.7, 171.7, 168.1, 166.6, 163.6, 159.8, 155.7, 152.7, 152.6, 151.3, 145.8, 145.0, 144.9, 144.9, 144.5, 144.4, 136.2, 135.0, 134.4, 133.7, 133.4, 131.2, 129.5, 129.5, 129.5, 129.3, 129.2, 129.2, 129.0, 128.9, 128.1, 126.2, 126.1, 119.5, 119.4, 118.2, 114.2, 96.7, 93.5, 89.9, 89.6, 88.5, 88.4, 88.1, 85.0, 84.9, 84.3, 78.7, 78.7, 77.3, 76.9, 76.8, 73.5, 73.3, 72.6, 72.4, 62.6, 62.6, 60.1, 60.0, 59.2, 59.0, 55.8, 44.2, 44.1, 44.0, 43.9, 42.1, 41.9, 38.5, 30.7, 26.4, 25.0, 25.0, 25.0, 25.0, 24.9, 24.9, 24.9, 24.8, 21.0, 21.0, 21.0, 20.9, 18.7, 18.0,

18.0, 12.5, 12.5, 1.9, 1.7, 1.5, 1.3, 1.1, 0.8, 0.6, -3.9, -3.9, -5.1, -5.1. 31P NMR (162 MHz, CD_3CN) δ 150.5, 149.8. HRMS-ESI (+): Mass calc. for $C_{80}H_{104}N_{11}O_{13}PSi_2$ (M+H), 1514.7169; found, 1514.7134.



Scheme S3. Synthesis of amide-linked UaC dimer phosphoramidite (**14**). Steps: (a) N,N-diisopropylethylamine, DCM, rt, 5 h, 28% (b) 2-cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite, 4,5-dicyanoimidazole, DCM, rt, 5 m, 29%.

Amide-linked UaC dimer (13). Carboxylic acid **11**[3] (as NEt_3 salt, 74 mg, 0.096 mmol), amine **12** (76 mg, 0.14 mmol), HBTU (44 mg, 0.11 mmol), and HOBt (20 wt% water, 17 mg, 0.10 mmol) were suspended in dichloromethane (2.5 mL) and stirred at room temperature for 5 minutes. N,N-Diisopropylethylamine (33 µL, 0.19 mmol) was added, and the suspension was stirred at room temperature for 5 hours. The reaction mixture was diluted with ethyl acetate (30 mL) and washed with $NaHCO_3$ (30 mL) and brine (2 x 30 mL). The organic phase was dried over sodium sulfate and concentrated under reduced pressure. The crude product was redissolved in dichloromethane (10 mL) containing 0.5 % NEt_3 and purified on an Isco CombiFlash Sg 100c chromatography system using a SiliCycle SiliaSep Flash Cartridge (40 g, 40-63 µm particle size, 60 Å pore size); solvent A: dichloromethane with 0.5 % NEt3; solvent B: 30 % MeCN and 2 % MeOH in dichloromethane; flow rate: 20 mL/min; equilibration: 0 % B; gradient: 0 % B for 2 min, 71 % B over 52.9 min; retention time: 27 min; to afford amide-linked UaC dimer **13** as glassy solids. Yield: 32 mg, 28 %. R_f = 0.31 (33 % MeCN and 2 % MeOH in dichloromethane). ^{1}H NMR (600 MHz, CD_3CN) δ 9.10 (s, 1H), 7.97 (t, J = 6.0 Hz, 3H), 7.94 (d, J = 8.1 Hz, 1H), 7.64 (t, J = 7.4 Hz, 1H), 7.52 (t, J = 7.6 Hz, 2H), 7.44 (d, J = 7.8 Hz, 4H), 7.35 – 7.29 (m, 6H), 7.29 – 7.23 (m, 2H), 6.88 (d, J = 8.2 Hz, 3H), 5.74 (d, J = 3.3 Hz, 1H), 5.64 (s, 1H), 5.14 (d, J = 8.1 Hz, 1H), 5.09 – 5.04 (m, 2H), 4.49 (d, J = 4.6 Hz, 1H), 4.44 (t, J = 4.2 Hz, 1H), 4.00 (tq, J = 10.9, 6.0, 4.6 Hz, 3H), 3.76 (s, 3H), 3.53 – 3.46 (m, 2H), 3.42 (dd, J = 13.5, 5.0 Hz, 2H),

3.34 (dd, J = 11.5, 3.7 Hz, 1H), 3.16 – 3.06 (m, 3H), 2.68 (tt, J = 9.6, 5.1 Hz, 1H), 2.44 (dd, J = 16.2, 8.2 Hz, 1H), 2.02 (dd, J = 16.1, 5.5 Hz, 1H), 1.81 (s, 1H), 1.80 – 1.74 (m, 2H), 1.06 – 1.01 (m, 21H), 0.88 (s, 9H), 0.16 (s, 3H), 0.06 (s, 3H). ^{13}C NMR (151 MHz, CD_3CN) δ 172.0, 164.0, 159.8, 151.4, 145.2, 145.1, 141.2, 135.9, 133.9, 131.4, 129.6, 129.4, 129.3, 129.1, 128.9, 128.9, 128.1, 128.1, 118.2, 114.1, 101.8, 93.0, 92.2, 90.8, 87.9, 84.3, 84.0, 80.7, 78.7, 71.3, 63.2, 55.9, 47.0, 47.0, 41.7, 39.3, 34.7, 31.6, 27.0, 27.0, 26.2, 23.0, 18.6, 18.1, 15.0, 12.8, 12.6, 1.7, 1.5, 1.4, 1.2, 1.1, 1.0, 0.8, -4.2, -5.0. HRMS-ESI (+): Mass calc. for $C_{71}H_{87}N_9O_{12}Si_2$ (M+H), 1187.5557; found, 1187.5543.

Amide-linked UaC dimer phosphoramidite (14). Amide-linked UaC dimer **13** (64 mg, 0.054 mmol) was co-evaporated with acetonitrile (3 x 2 mL), dissolved in dichloromethane (1 mL), followed by addition of 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphorodiamidite (110 µL, 0.035 mmol). The reaction mixture was stirred at room temperature for 5 min, then 4,5-dicyanoimidazole (9.5 mg, 0.081 mmol) was added. The reaction mixture was stirred under an inert argon atmosphere at room temperature overnight. The mixture was diluted with dichloromethane (30 mL), washed with saturated aqueous $NaHCO_3$ (2 x 10 mL), brine (10 mL), and dried over sodium sulfate. The reaction mixture was adsorbed on 5 g of Celite and purified on an Isco CombiFlash Sg 100c chromatography system using a SiliCycle SiliaSep C8 Monomeric Flash Cartridge reverse-phase column (12 g); solvent A: 50 % MeCN/Water; solvent B: MeCN; flow rate: 20 mL/min; equilibration: 0 % B; gradient: 0 % B for 10 min, 100 % B over 60 min; retention time: 62-65 min; to afford amide-linked UaC dimer phosphoramidite **14**. Yield: 22 mg, 0.016 mmol, 29 %. R_f = 0.58 (10 % MeOH/dichloromethane). ^{1}H NMR (400 MHz, CD_3CN) δ 9.25 (s, 1H), 8.01 – 7.87 (m, 4H), 7.68 – 7.59 (m, 1H), 7.52 (dd, J = 8.4, 7.1 Hz, 2H), 7.44 (dq, J = 8.2, 1.6 Hz, 4H), 7.37 – 7.28 (m, 6H), 7.28 – 7.21 (m, 2H), 6.97 – 6.84 (m, 3H), 5.82 (dd, J = 5.0, 1.1 Hz, 1H), 5.64 (t, J = 1.4 Hz, 1H), 5.14 (dd, J = 8.1, 3.8 Hz, 1H), 5.06 (dd, J = 13.4, 4.9 Hz, 1H), 4.96 (dd, J = 18.5, 4.9 Hz, 1H), 4.62 (dt, J = 7.0, 5.2 Hz, 1H), 4.52 – 4.45 (m, 1H), 4.38 – 4.26 (m, 1H), 4.26 – 4.11 (m, 1H), 4.04 – 3.96 (m, 1H), 3.87 – 3.78 (m, 1H), 3.75 (s, 3H), 3.69 – 3.58 (m, 2H), 3.55 – 3.41 (m, 3H), 3.37 – 3.28 (m, 1H), 2.73 – 2.60 (m, 3H), 2.44 (dt, J = 16.2, 8.1 Hz, 1H), 2.02 (ddd, J = 16.1, 7.9, 5.3 Hz, 1H), 1.29 – 1.13 (m, 12H), 1.11 – 0.94 (m, 21H), 0.89 (s, 9H), 0.17 (d, J = 1.6 Hz, 3H), 0.07 (d, J = 4.8 Hz, 3H). ^{13}C NMR (101 MHz, CD_3CN) δ 171.8, 171.8, 164.0, 159.8, 151.3, 147.7, 145.2, 145.2, 145.1, 141.2, 135.9, 134.3, 133.9, 131.4, 131.4, 129.6, 129.3, 129.3, 129.1, 128.9, 128.9, 128.1, 119.6, 119.4, 118.2, 114.1, 101.8, 97.9, 93.4, 93.0, 92.2, 92.2, 90.3, 90.0, 87.9, 84.2, 84.0, 83.7, 78.6, 77.9, 77.4, 77.3, 73.2, 73.1, 72.8, 72.6, 63.2, 63.2, 59.9, 59.7, 59.2, 59.0, 55.9, 44.2, 44.0, 43.9, 41.9, 41.8, 39.3, 31.6, 26.2, 25.0, 25.0, 24.9, 24.9, 24.8, 24.8, 21.0, 20.9, 20.9, 18.6, 18.2, 18.2, 12.7, 12.7,

1.9, 1.7, 1.4, 1.2, 1.0, 0.8, 0.6, -4.2, -4.2, -4.9, -5.0. 31P NMR (162 MHz, CD_3CN) δ 149.8, 149.8. HRMS-ESI (+): Mass calc. for $C_{72}H_{99}N_8O_{14}PSi_2$ (M+Na), 1409.6455; found, 1409.6436.

Synthesis and purification of amide-modified siRNAs

Amide-modified siRNAs were prepared on a 1 µmol scale using the standard 2'-O-TOM RNA phosphoramidite (Glen Research) synthesis protocol on an Expedite 8909 Nucleic Acid Synthesis System. A standard coupling time was used for all dimeric amide-linked phosphoramidites. Cleavage of oligoribonucleotides from solid support and deprotection of the heterocyclic amino groups was done by treating the solid support with a mixture of ethanolic methylamine and aqueous methylamine (1:1) solution at room temperature for 3 hours. The cleavage solution was freeze-dried and the residue was dissolved in DMSO (100 µL). Triethylamine trihydrofluoride (125 µL) was added and the reaction mixture was left for 24 hours at room temperature to remove the 2'-O-TBS and 2'-O-TOM protecting groups. The reaction mixtures were diluted with water (1275 µL) and desalted on a Sephadex C25 NAP column in accordance with the manufacturer's recommendations. The amide-modified oligoribonucleotides were analyzed and purified by reverse-phase HPLC using a Supelco Discovery Bio C18 column (300 Å, 3 µm, 4.6 x 150 mm) or in case of **G3** an Agilent Bio PLRP-S column (100 Å, 8 µm, 4.6 x 150 mm) and a linear gradient of acetonitrile in 0.1 M triethylammonium acetate buffer, pH 7.0, flow rate 1 mL/min. For specific gradient details, see Figures S1-S28. The fractions containing the target material were freeze-dried. The residue was dissolved in water (5 mL) and freeze-dried again three times to remove the bulk of triethylammonium salts. To completely convert the amide-modified RNAs into the sodium salt form, the samples were dissolved in phosphate buffer (0.5 mL of 20 mM sodium phosphate, 80 mM NaCl, 50 mM EDTA, pH 6.3) and desalted on a Sephadex C25 NAP column according to the manufacturer's recommendations. The identity of the amide-modified oligonucleotides was confirmed by MALDI-TOF mass spectrometry, details are given in Figure S1-S28 legends below.

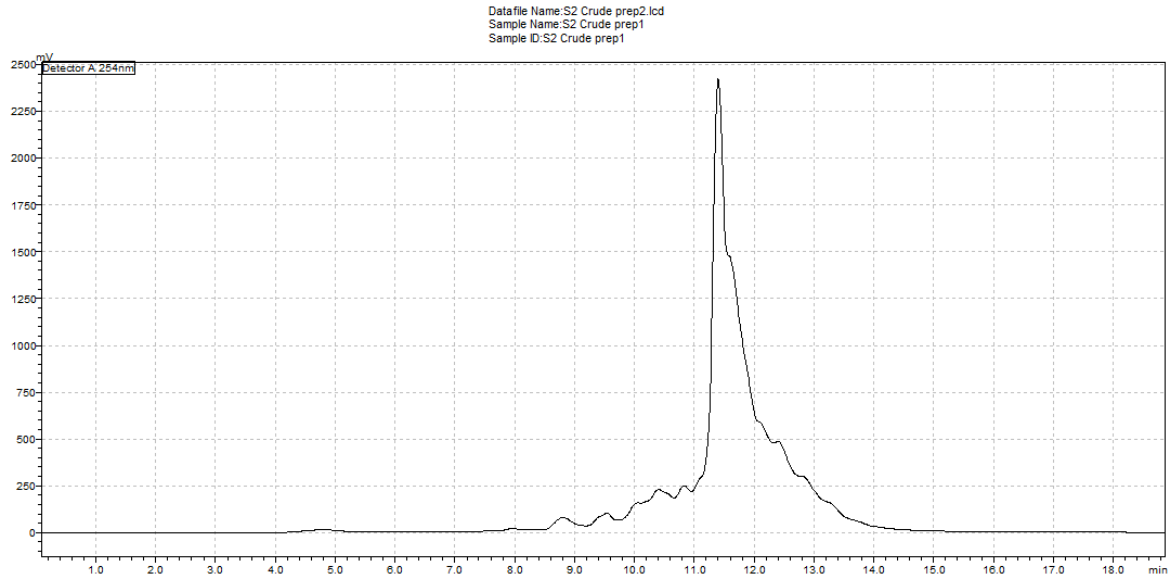


Figure S1. Reverse Phase HPLC trace of crude **G2**. Acetonitrile gradient: 0 min 3%, 20 min 20%.

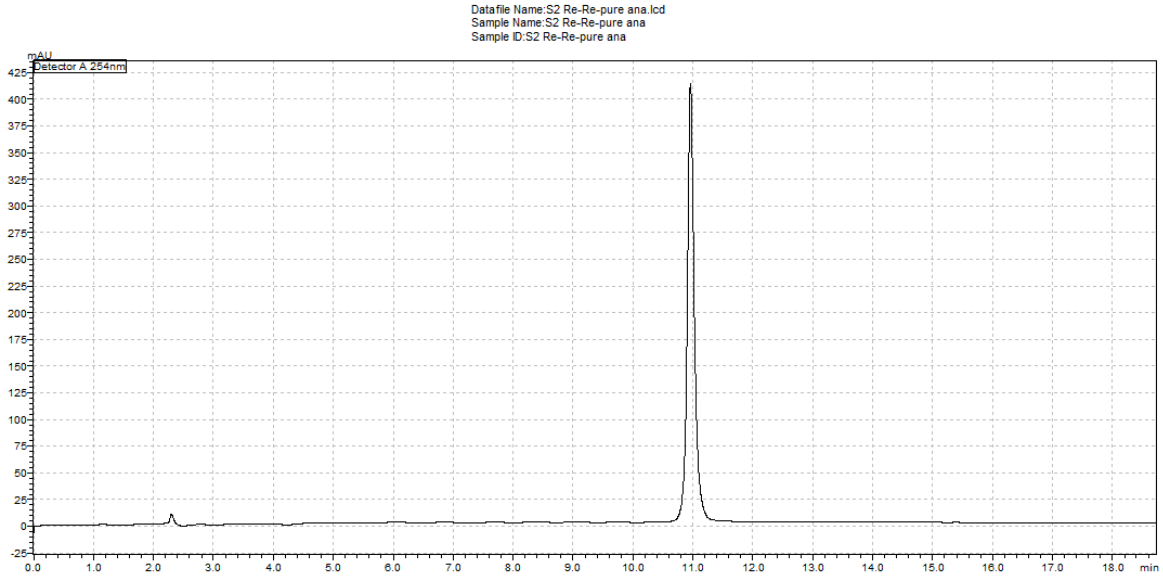


Figure S2. Reverse Phase HPLC trace of pure **G2**. Acetonitrile gradient: 0 min 3%, 20 min 15%. MALDI-TOF: calc. 6706; found 6708.

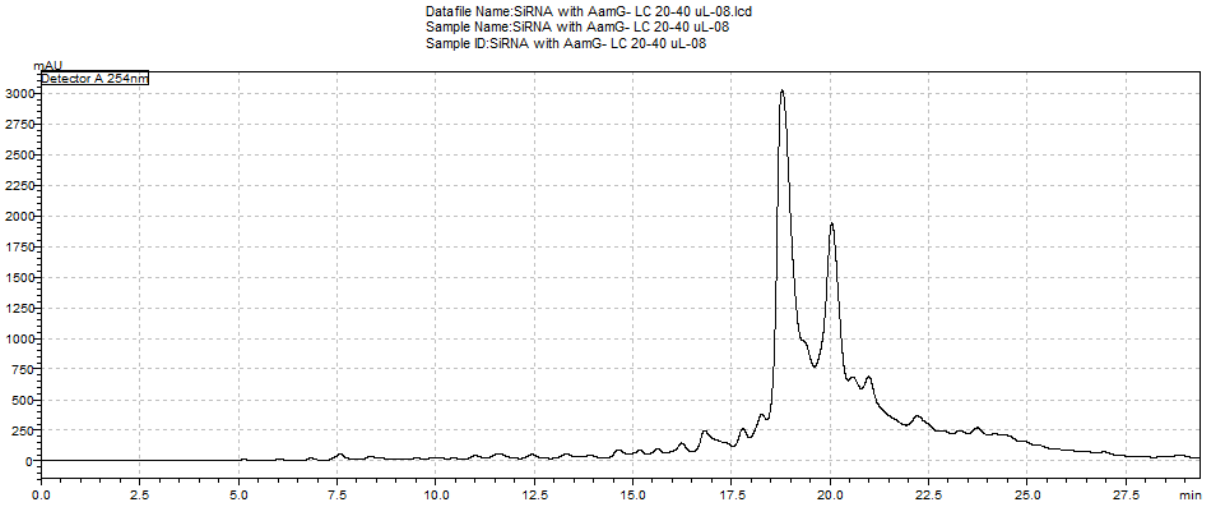


Figure S3. Reverse Phase HPLC trace of crude **G3**. Acetonitrile gradient: 0 min 4%, 30 min 14%.

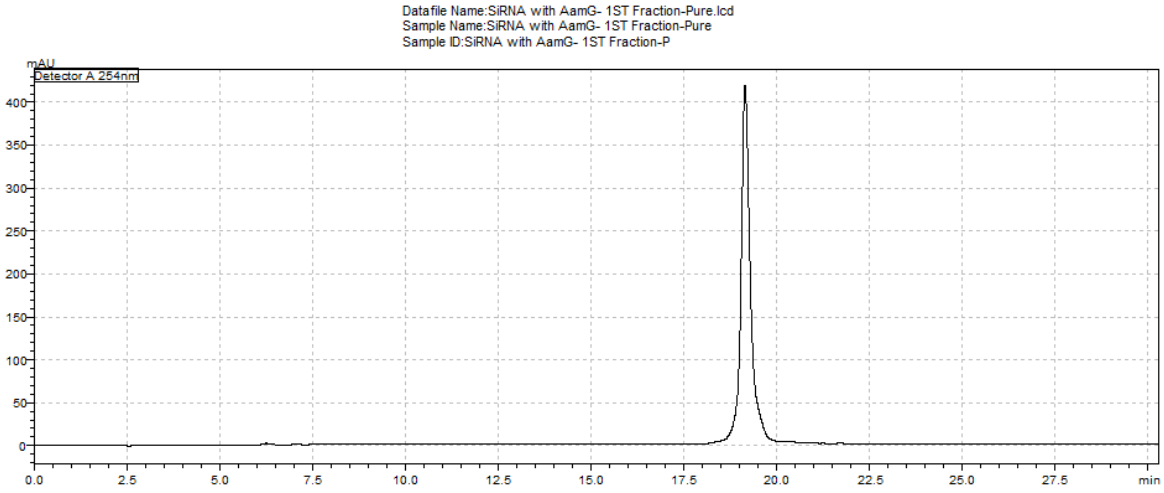


Figure S4. Reverse Phase HPLC trace of pure **G3**. Acetonitrile gradient: 0 min 4%, 30 min 14%. MALDI-TOF: calc. 6786; found 6786.

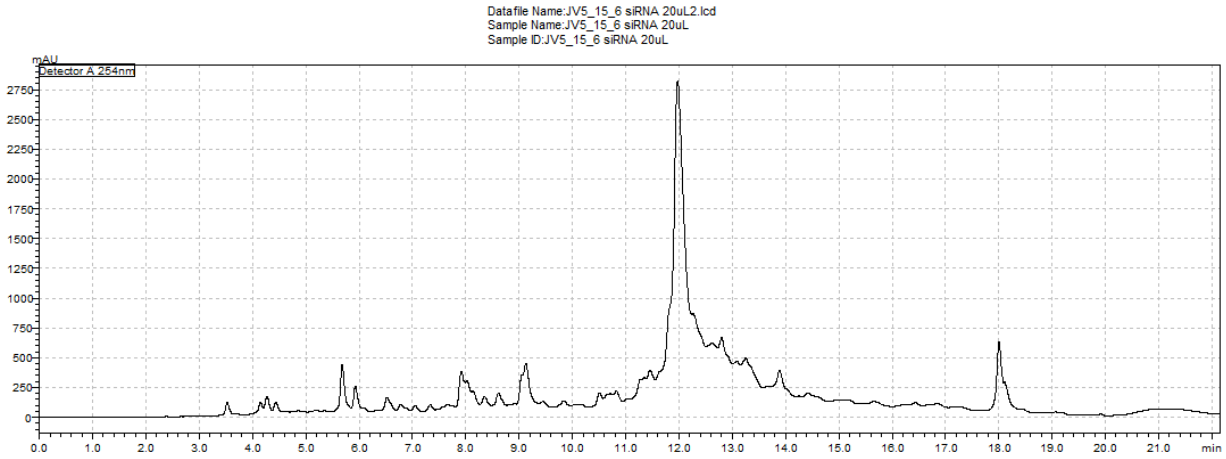


Figure S5. Reverse Phase HPLC trace of crude **G6**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%.

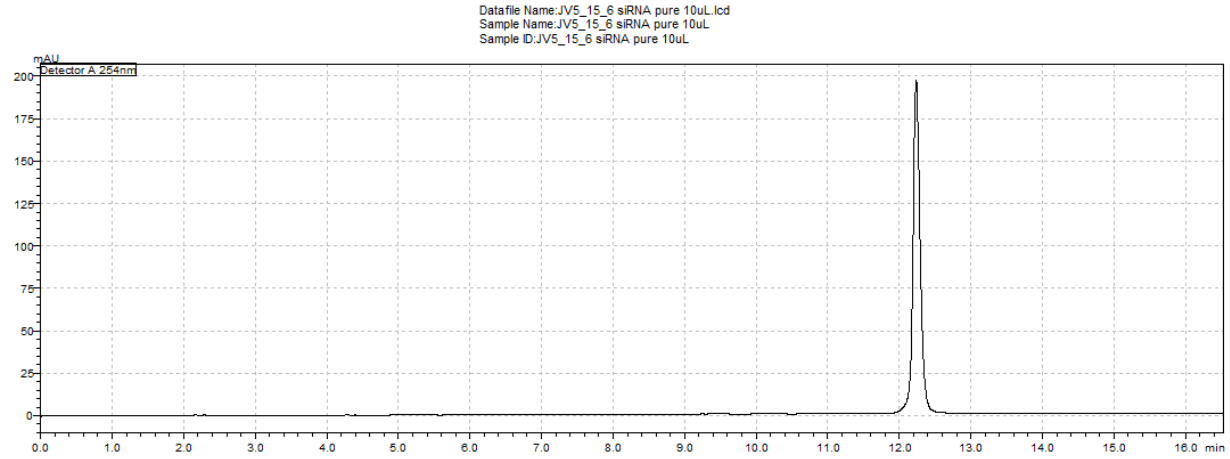


Figure S6. Reverse Phase HPLC trace of pure **G6**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%. MALDI-TOF: calc. 6706; found 6707.

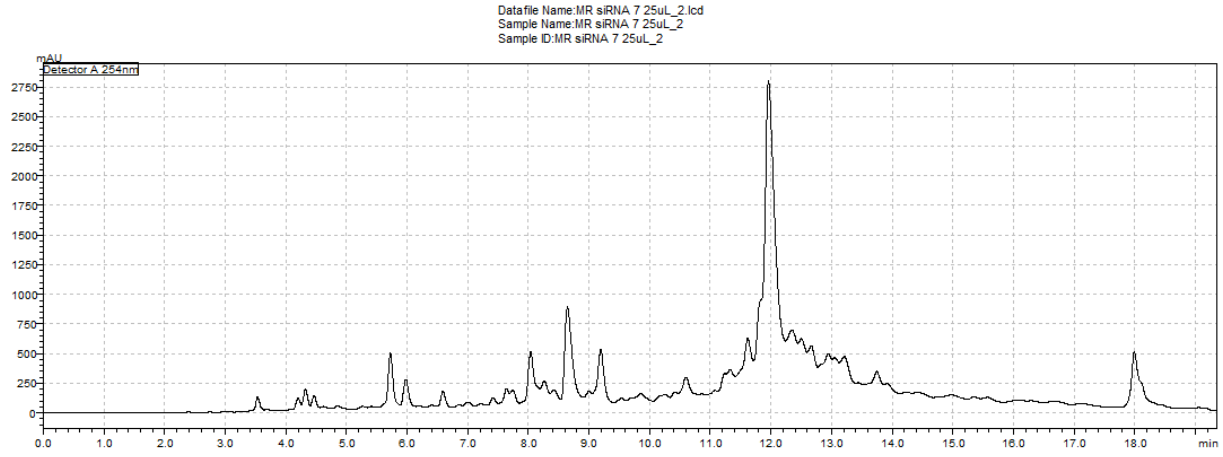


Figure S7. Reverse Phase HPLC trace of crude **G7**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%.

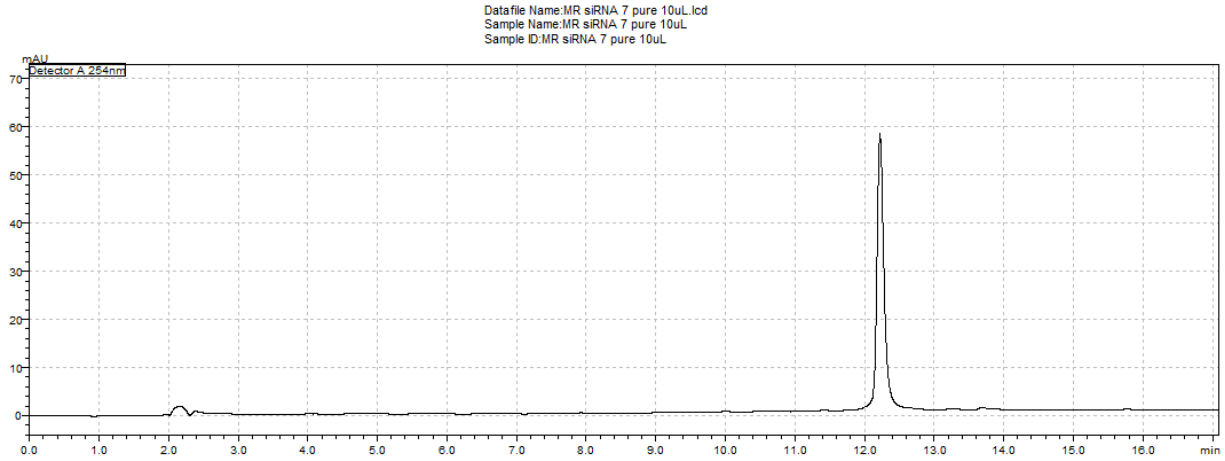


Figure S8. Reverse Phase HPLC trace of pure **G7**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%. MALDI-TOF: calc. 6706; found 6705.

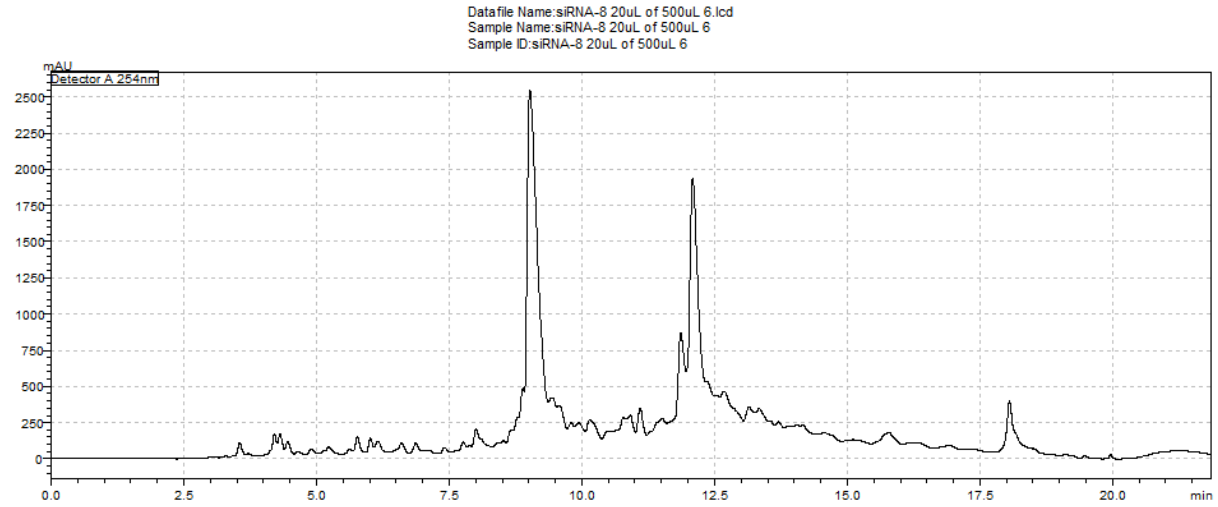


Figure S9. Reverse Phase HPLC trace of crude **G8**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%.

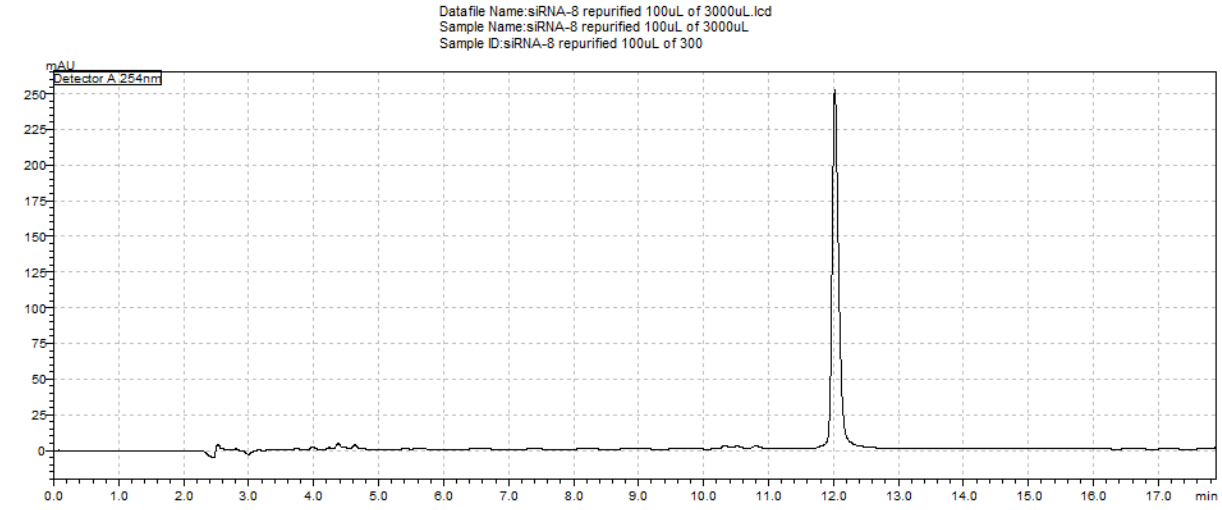


Figure S10. Reverse Phase HPLC trace of pure **G8**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%. MALDI-TOF: calc. 6706; found 6704.

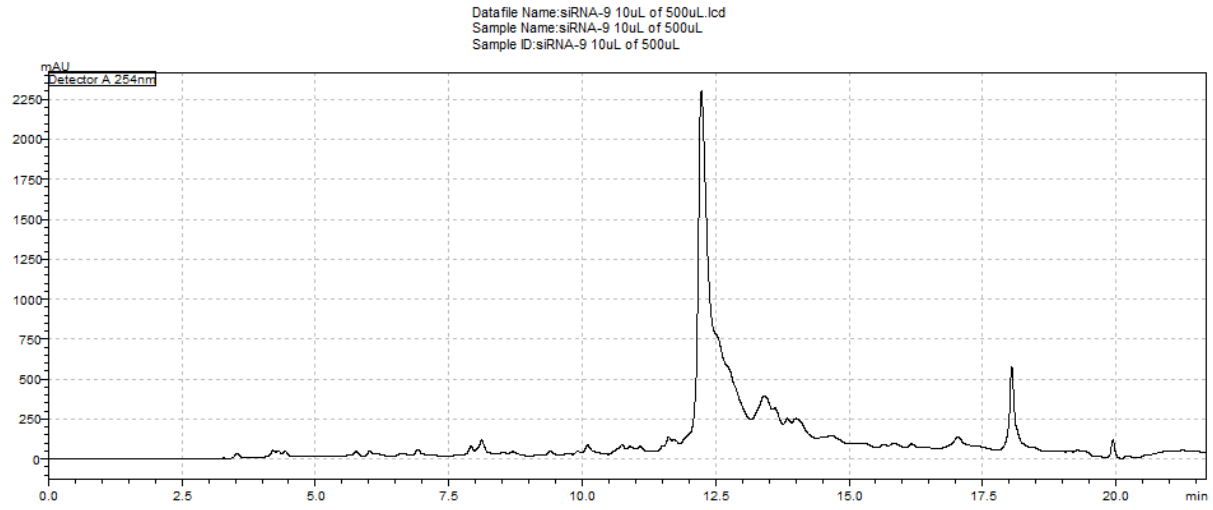


Figure S11. Reverse Phase HPLC trace of crude **G9**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%.

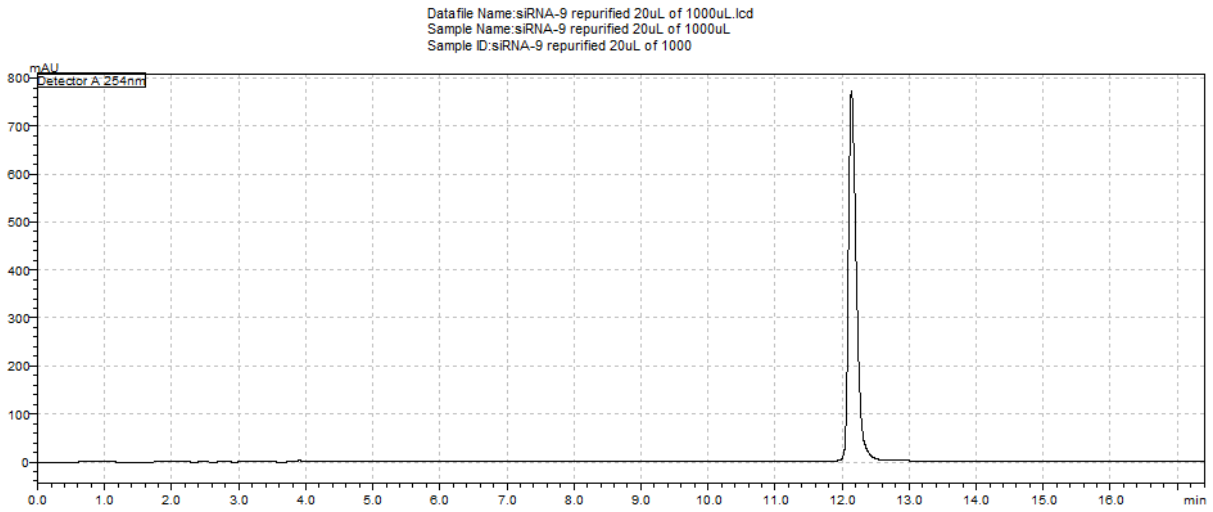


Figure S12. Reverse Phase HPLC trace of pure **G9**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%. MALDI-TOF: calc. 6706; found 6705.

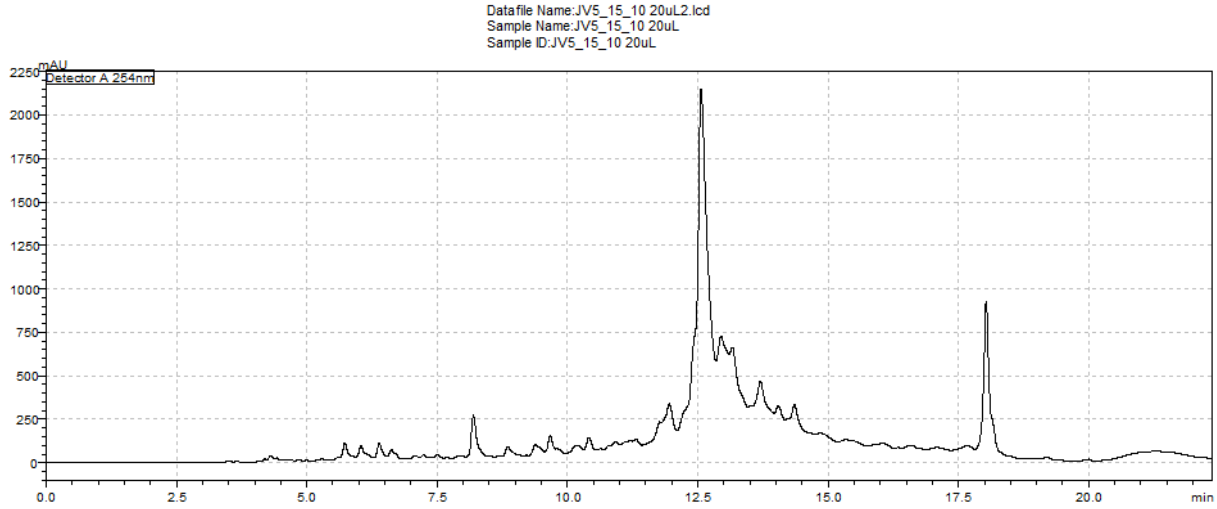


Figure S13. Reverse Phase HPLC trace of crude **G10**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%.

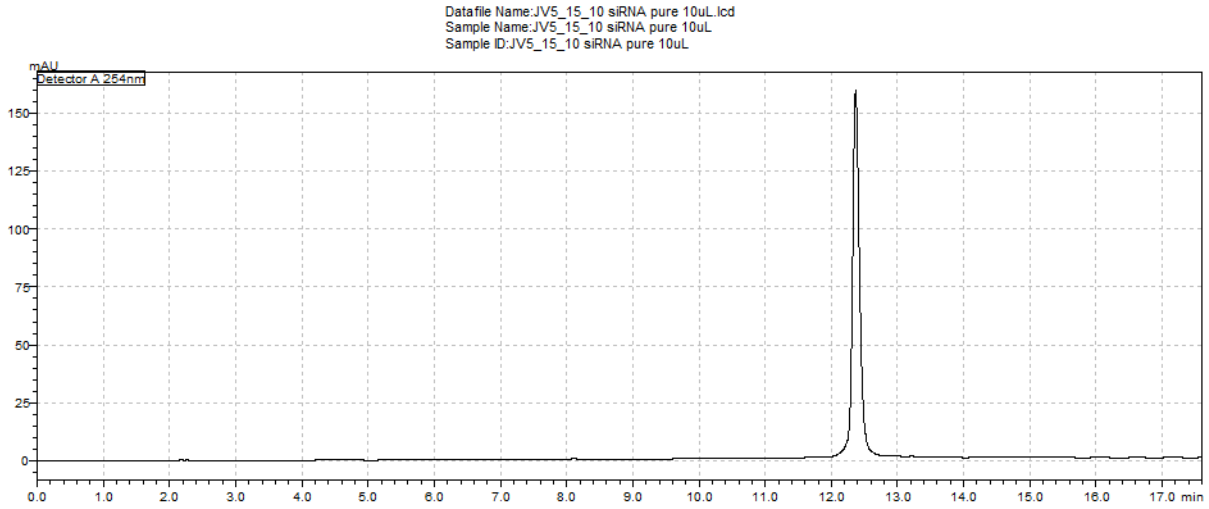


Figure S14. Reverse Phase HPLC trace of pure **G10**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%. MALDI-TOF: calc. 6706; found 6704.

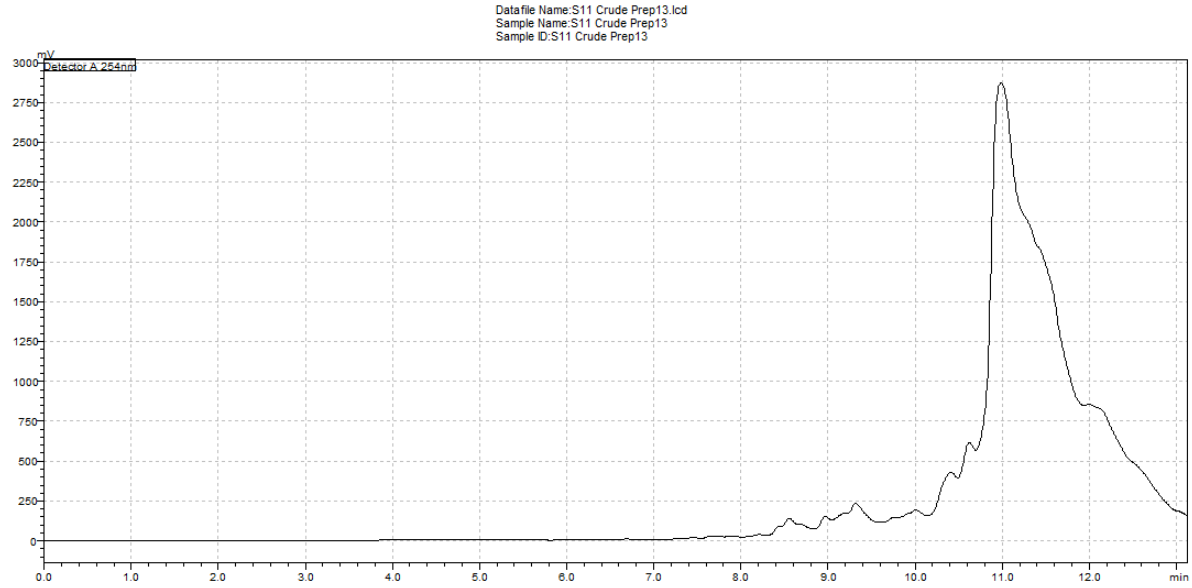


Figure S15. Reverse Phase HPLC trace of crude **G11**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%.

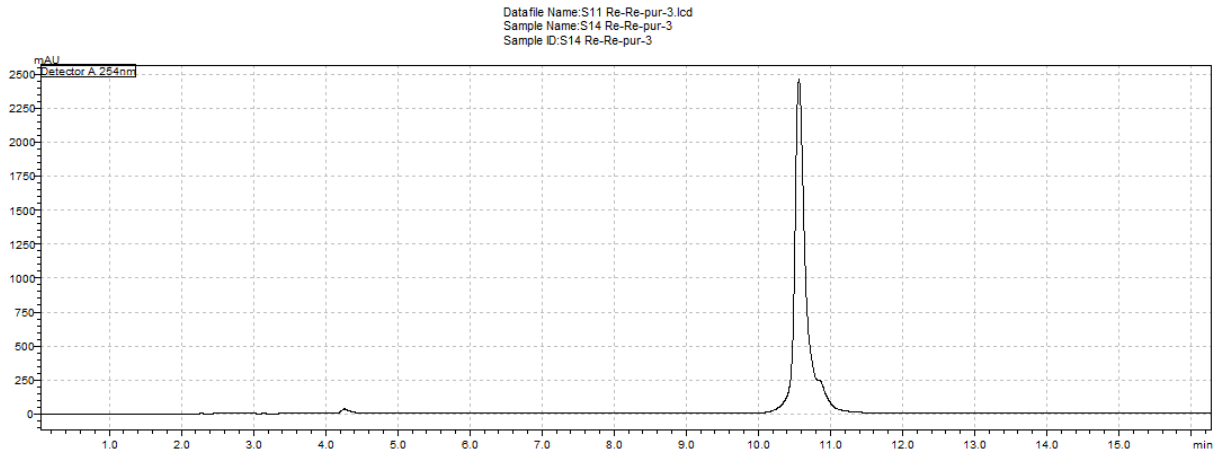


Figure S16. Reverse Phase HPLC trace of pure **G11**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%. MALDI-TOF: calc. 6706; found 6708.

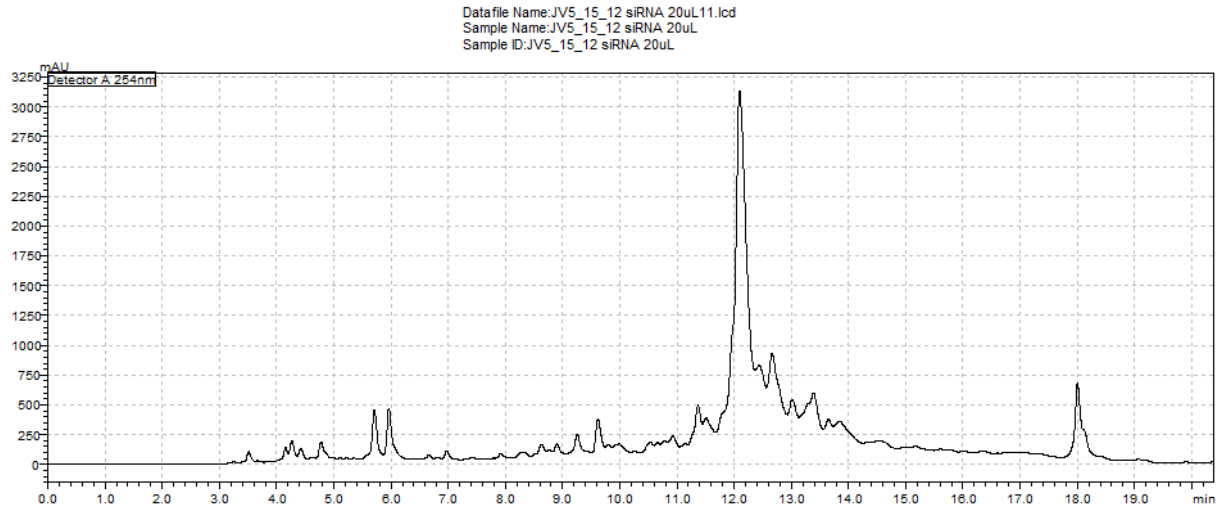


Figure S17. Reverse Phase HPLC trace of crude **G12**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%.

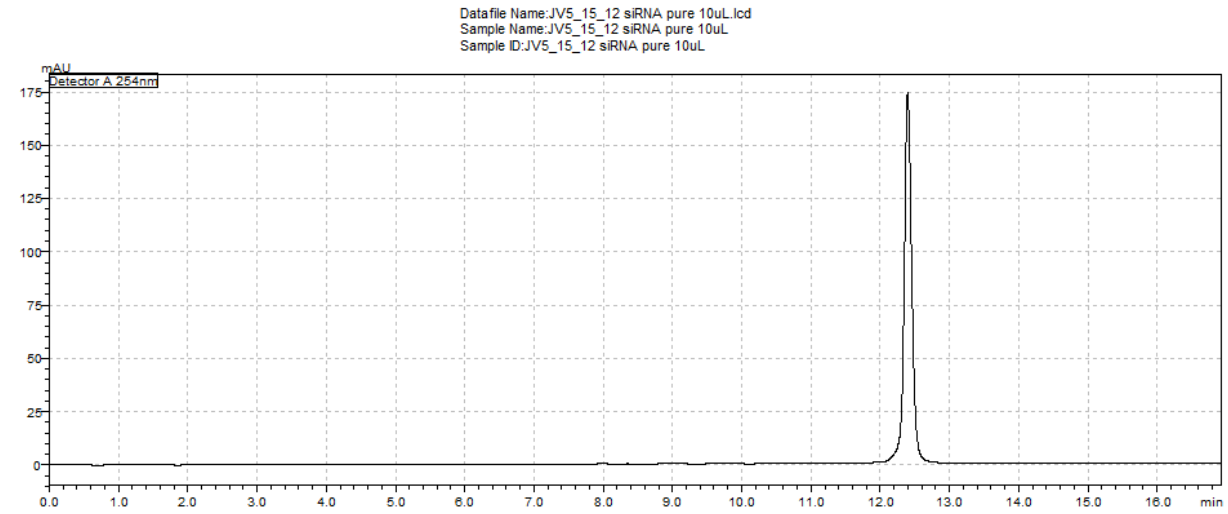


Figure S18. Reverse Phase HPLC trace of pure **G12**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%. MALDI-TOF: calc. 6706; found 6708.

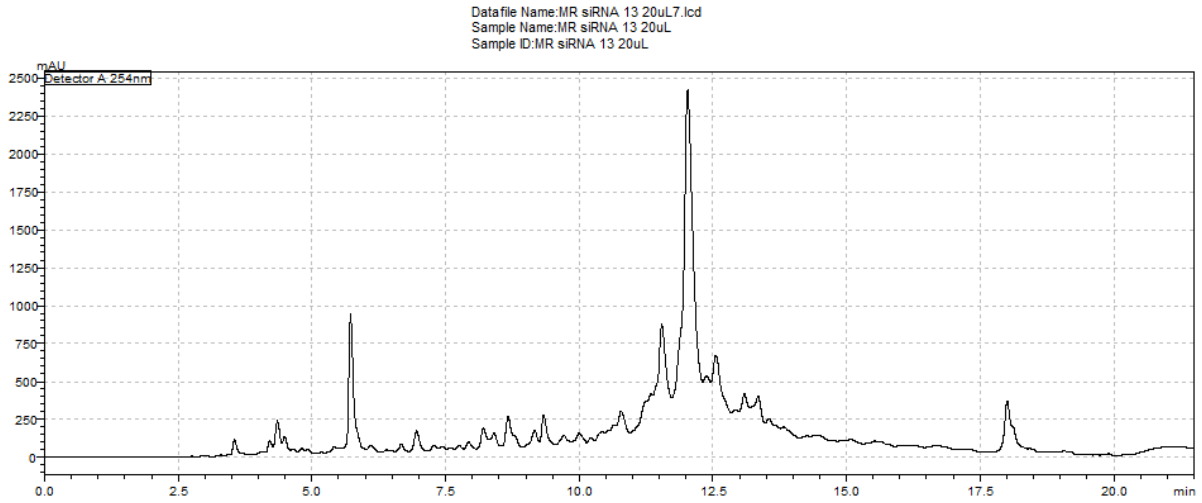


Figure S19. Reverse Phase HPLC trace of crude **G13**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%.

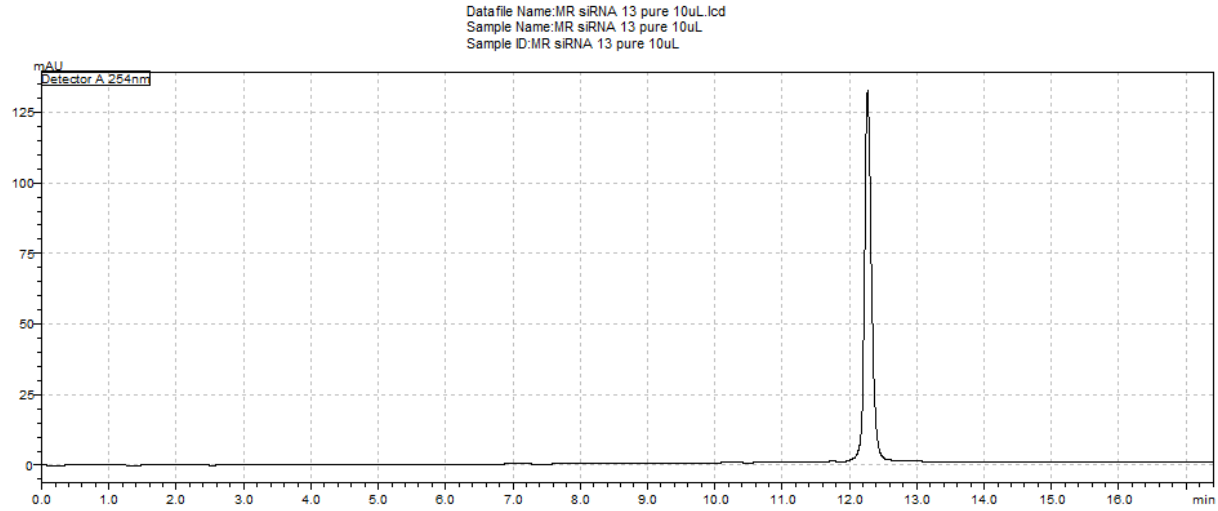


Figure S20. Reverse Phase HPLC trace of pure **G13**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%. MALDI-TOF: calc. 6706; found 6706.

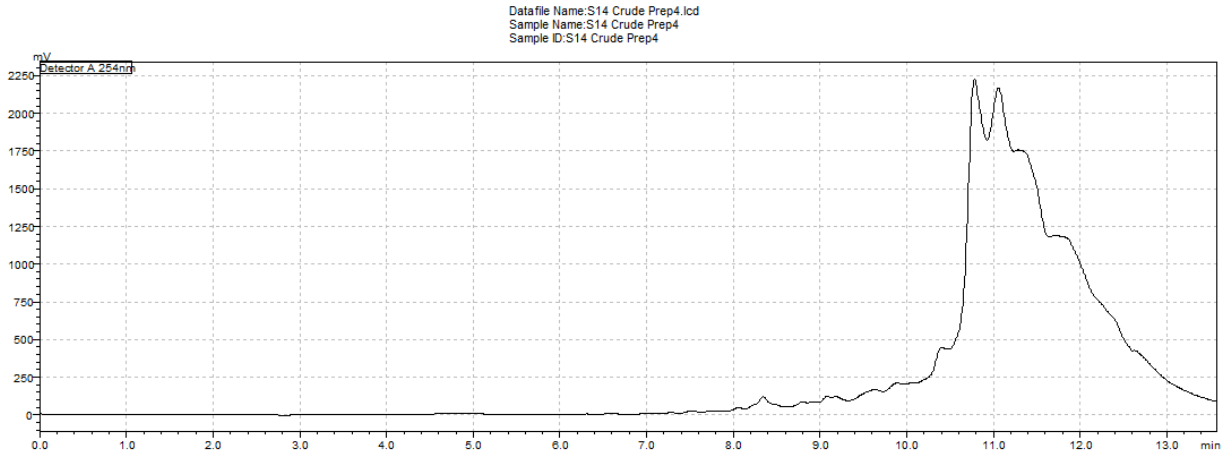


Figure S21. Reverse Phase HPLC trace of crude **G14**. Acetonitrile gradient: 0 min 3%, 20 min 20%.

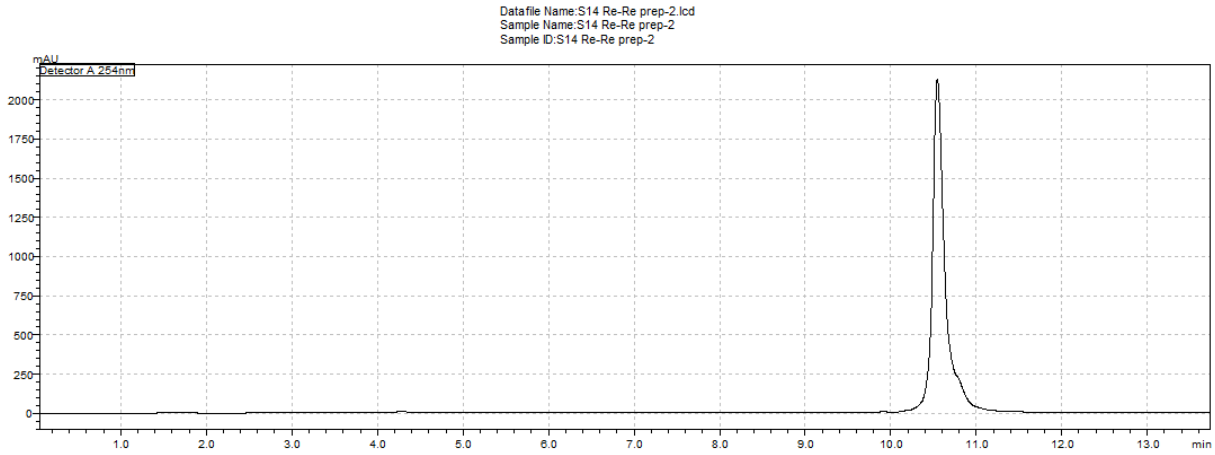


Figure S22. Reverse Phase HPLC trace of pure **G14**. Acetonitrile gradient: 0 min 3%, 20 min 15%. MALDI-TOF: calc. 6706; found 6708.

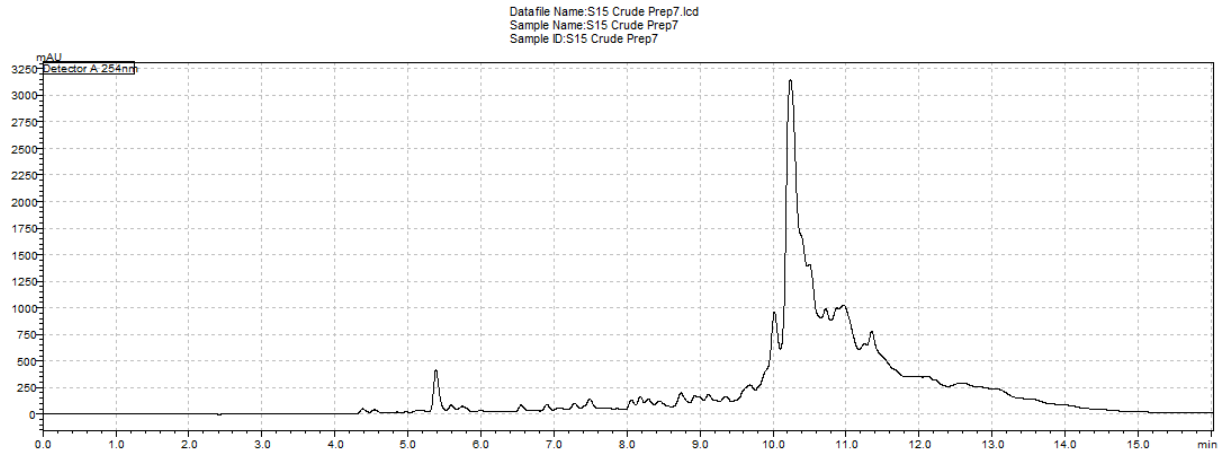


Figure S23. Reverse Phase HPLC trace of crude **G15**. Acetonitrile gradient: 0 min 3%, 20 min 15%.

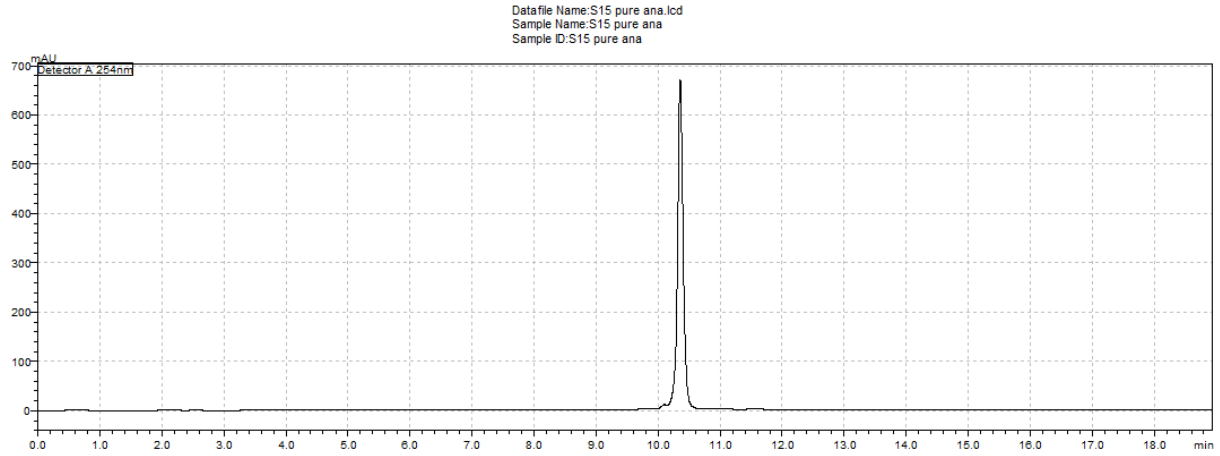


Figure S24. Reverse Phase HPLC trace of pure **G15**. Acetonitrile gradient: 0 min 3%, 20 min 15%. MALDI-TOF: calc. 6706; found 6708.

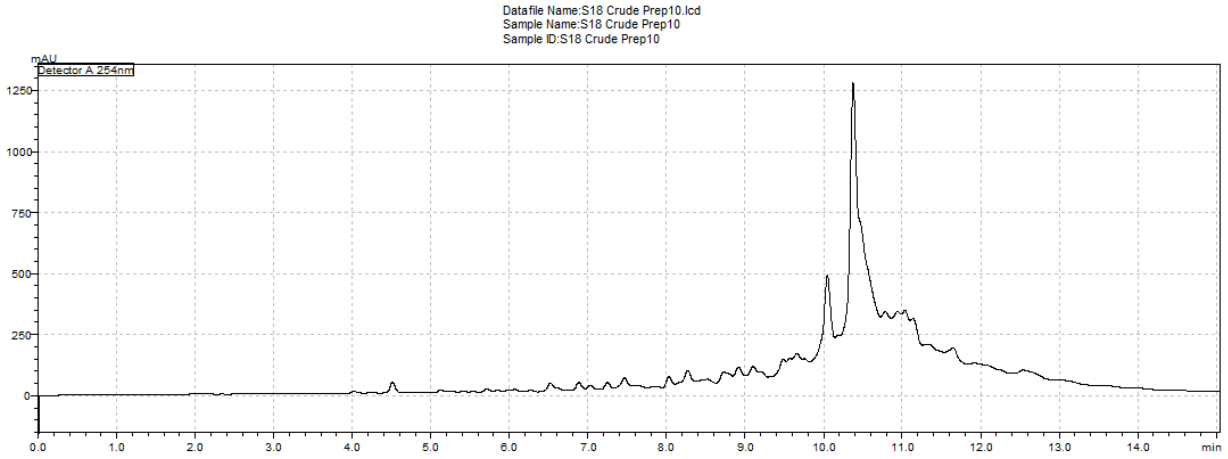


Figure S25. Reverse Phase HPLC trace of crude **G18**. Acetonitrile gradient: 0 min 4%, 20 min 15%.

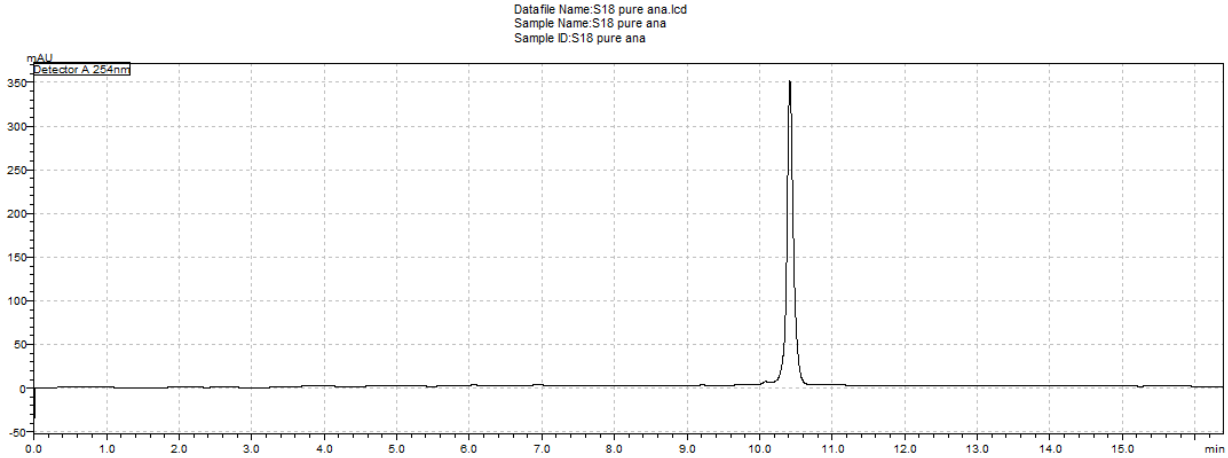


Figure S26. Reverse Phase HPLC trace of pure **G18**. Acetonitrile gradient: 0 min 3%, 20 min 15%. MALDI-TOF: calc. 6706; found 6708.

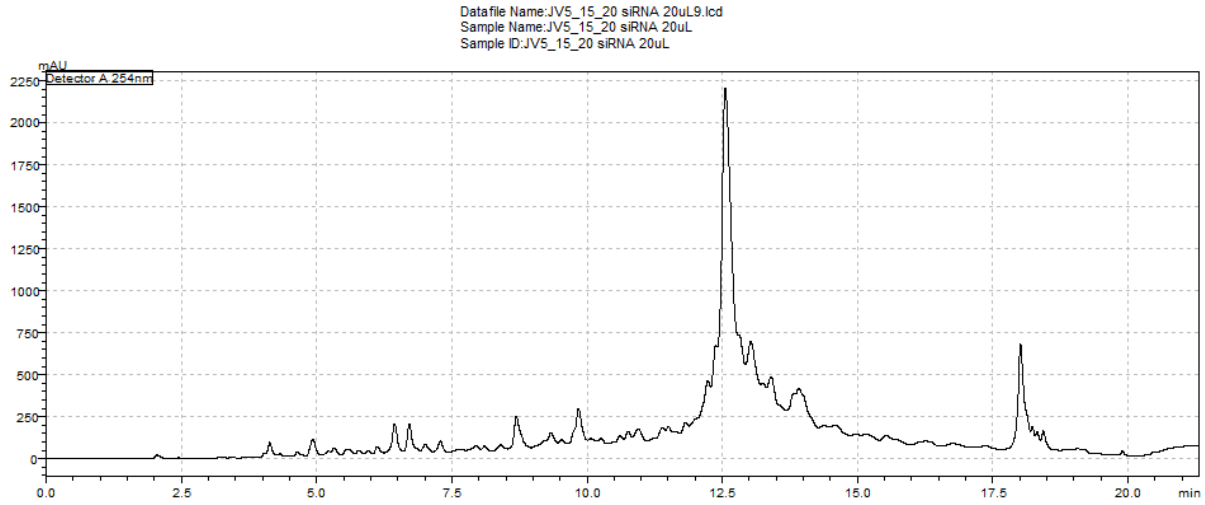


Figure S27. Reverse Phase HPLC trace of crude **G20**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%.

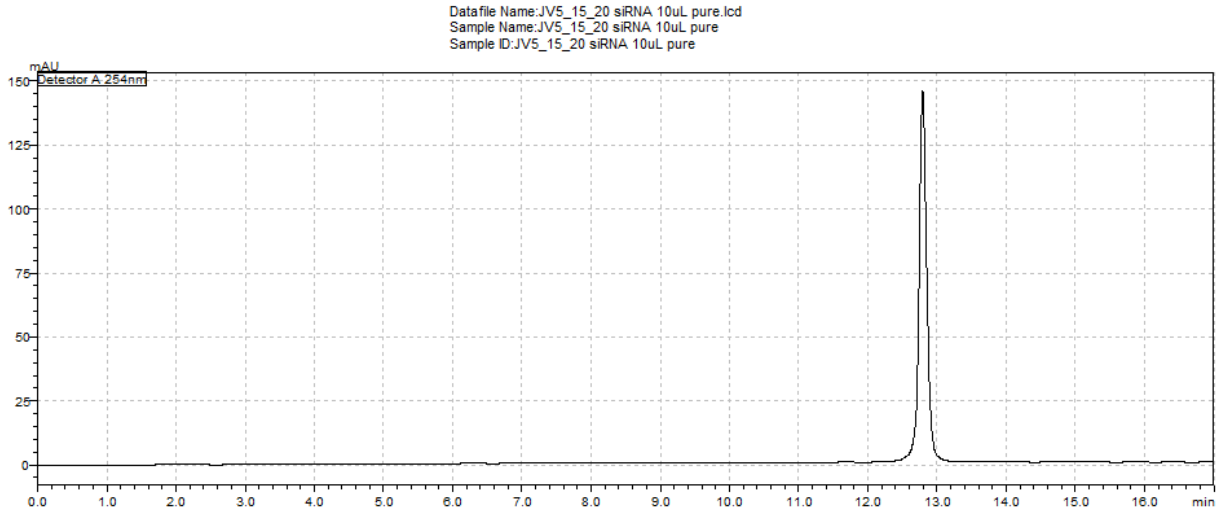


Figure S28. Reverse Phase HPLC trace of pure **G20**. Acetonitrile gradient: 0 min 4%, 15 min 8.8%. MALDI-TOF: calc. 6706; found 6706.

Cell Cultures

HeLa cells (ATCC) and were grown at 37 °C in humidified 5% CO_2 in complete growth media consisting of Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum (Corning) and 1% Penicillin-Streptomycin (10,000 U/mL, Gibco).

Luciferase assays

Plasmids were cloned into the psiCHECK2 vector (Promega). The plasmid containing the on-target sequence (PIK3CB) was generously donated by the Beal laboratory (UC Davis). Plasmids containing the off-target sequence (YY1, FADD) were cloned into psiCHECK2 as previously described.[4] Transfections were performed according to the manufacturer's protocol with Lipofectamine RNAiMAX (13778150, Invitrogen, Life Technologies). Cells were reverse transfected in white 96-well plates (655075, Greiner Bio-one) with complexes composed of 0.5 μL RNAiMAX, various amounts of siRNA, and either 20 ng (PIK3CB/FADD) or 30 ng (YY1) of plasmid. The transfection complexes were then overlaid with complete growth media and 40,000 cells/well for PIK3CB/FADD and 20,000 cells/well for YY1. The cells were harvested 22 hours post-transfection and the luciferase assay was conducted using the Dual-Glo Luciferase Assay System (E2940, Promega) with modifications as previously described,[5] using the GloMax 96 plate reader (E6521, Promega).

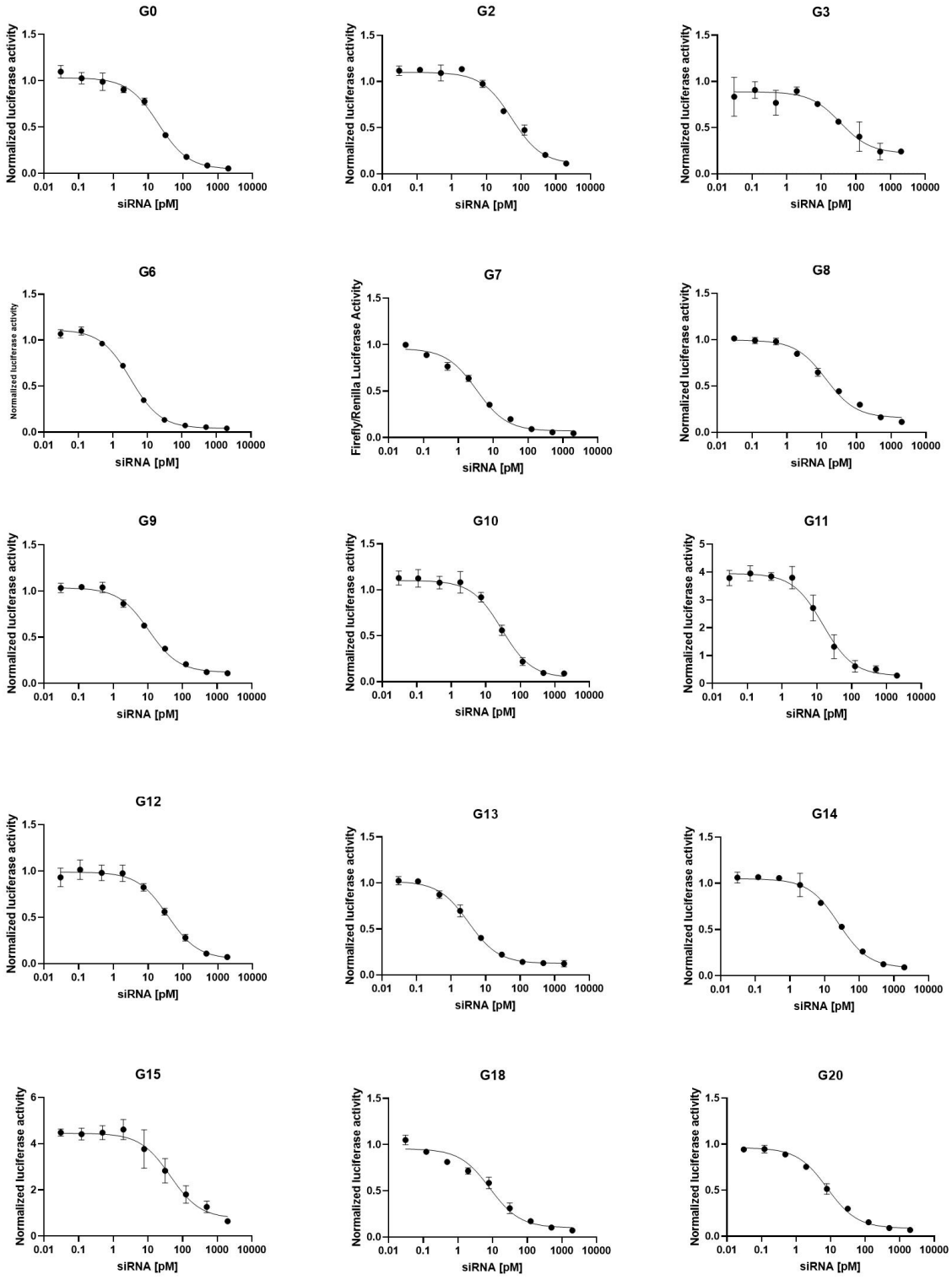


Figure S29. IC_{50} curves of dual luciferase assay of PIK3CB silencing by unmodified (G0) and AM1-modified (G2-G20) siRNAs.

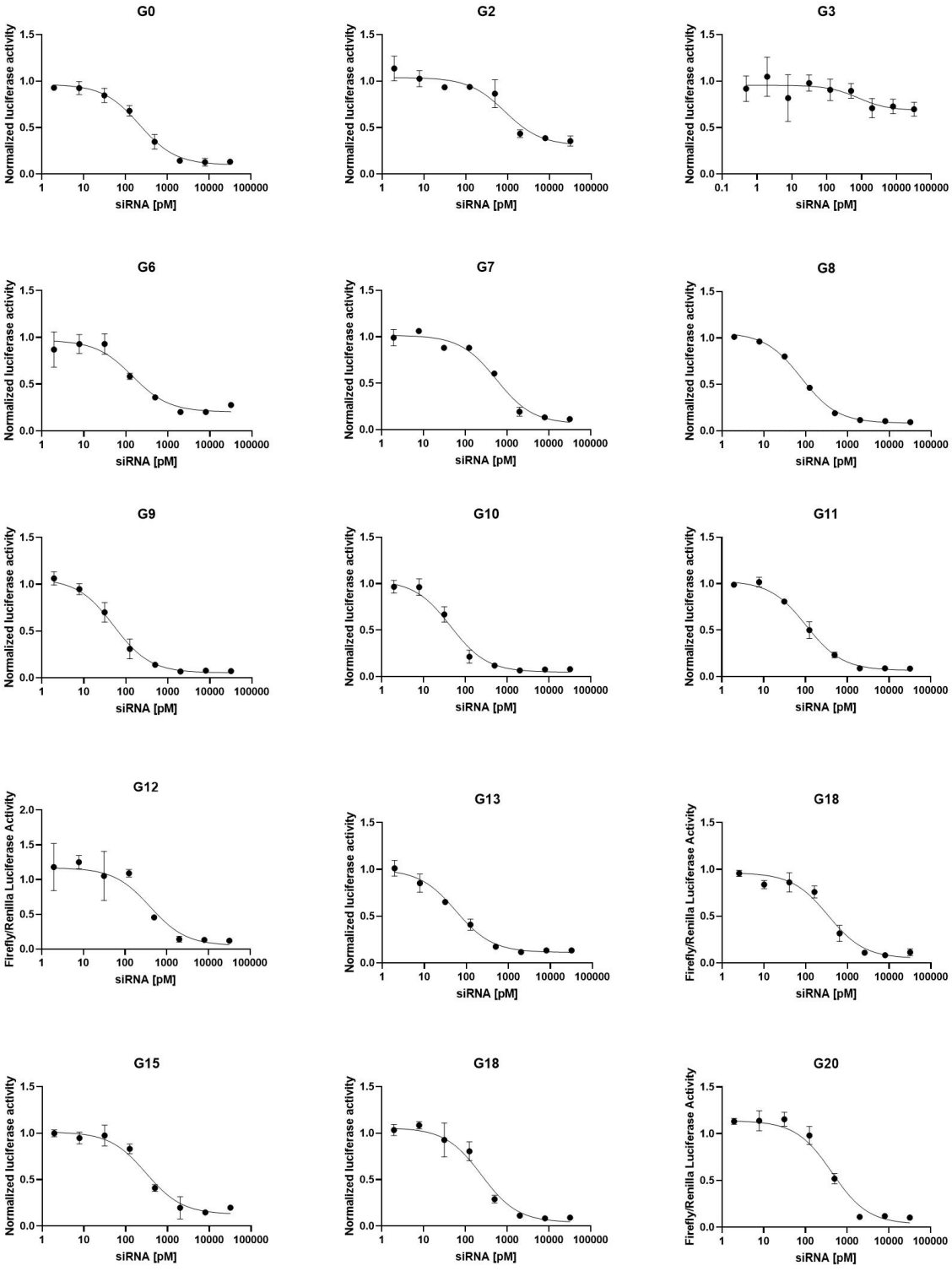


Figure S30. IC_{50} curves of dual luciferase assay of YY1 silencing by unmodified (G0) and AM1-modified (G2-G20) siRNAs.

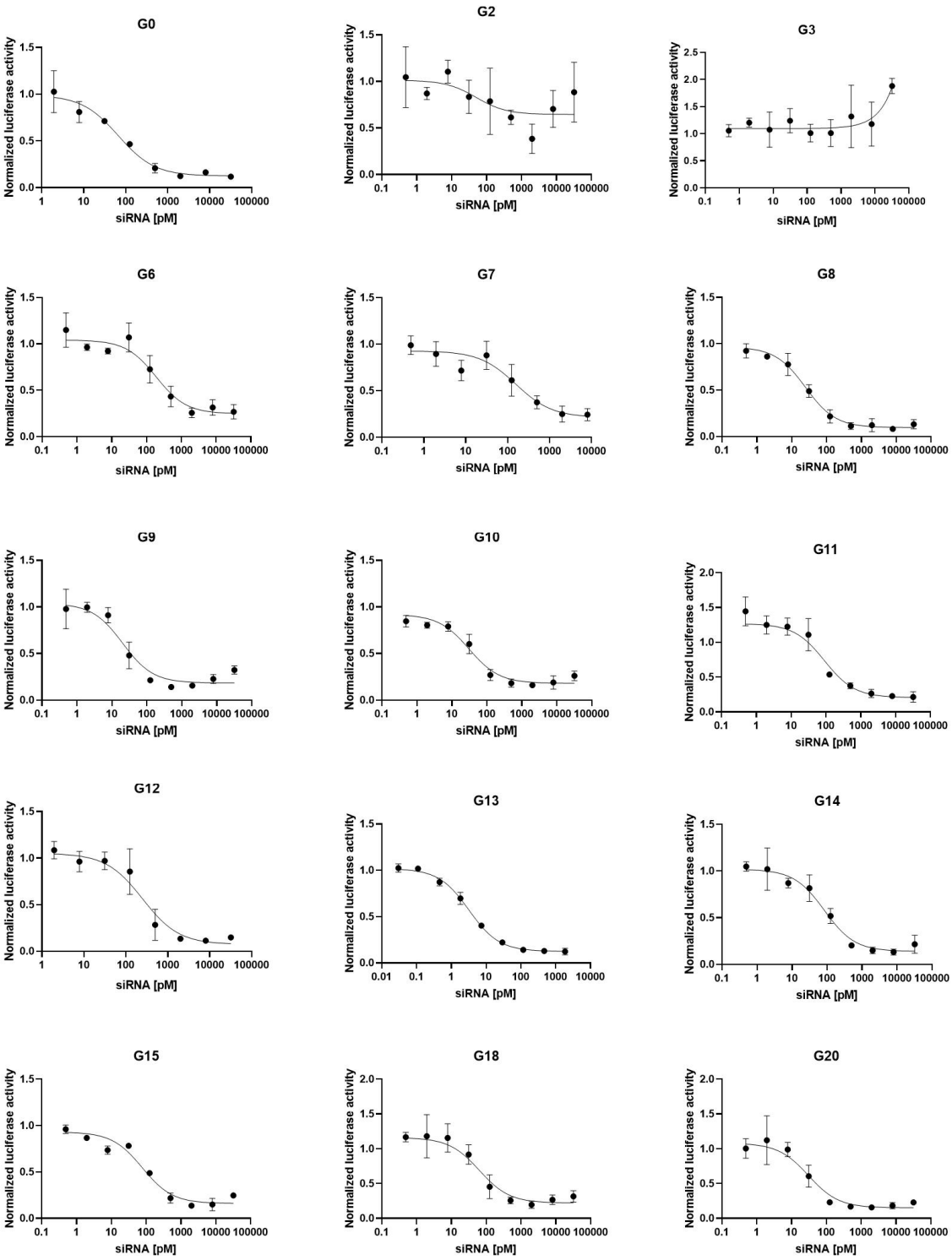


Figure S31. IC_{50} curves of dual luciferase assay of FADD silencing by unmodified (G0) and AM1-modified (G2-G20) siRNAs.

Table S1. Dual-luciferase assay IC_{50} values of unmodified (G0) and AM1-modified (G2-G20) siRNAs.

	PIK3CB		YY1		FADD	
Strand	AVG	STD	AVG	STD	AVG	STD
Unmod	25	5	130	60	89	19
G2	60	26	1000	500	ND*	
G3	36	6	ND*		ND*	
G6	3	1	180	49	118	49
G7	13	9	530	78	210	47
G8	16	5	91	27	30	10
G9	9	3	36	18	26	6
G10	4	0	53	16	52	27
G11	12	4	130	18	65	22
G12	5	5	300	89	91	18
G13	17	19	110	44	69	8
G14	20	5	360	43	67	25
G15	39	14	370	79	99	22
G18	7	2	310	58	50	15
G20	11	6	420	160	34	4

ND* - not determined because the expression did not go below 60% at the maximum concentration (20 nM)

UV Thermal Melting

UV thermal melting experiments were performed in 1x siRNA buffer (60 mM KCl, 60 mM HEPES, 0.2 mM $MgCl_2$, pH 7.4). The stock solutions of ssRNA (both unmodified and amide-modified) were made in 1x siRNA buffer. The ssRNA strands were annealed to form 2.0 µM siRNA duplexes by heating at 90 °C for 5 minutes then allowing to cool to room temperature for 30 minutes. The UV thermal melting experiments were carried out on a Shimadzu 1800 or 2600 UV-Vis spectrophotometers by measuring the absorbance over a range of 15 to 90 °C at 0.5 °C/min. The experiments were performed using an 8-series micro multi-cell (path length 10 mm). The melting temperatures (T_m) were obtained using Shimadzu Lab Solutions T_m Analysis software.

Table S2. UV thermal melting results (°C) of unmodified (G0) and AM1-modified siRNA duplexes.

Strand	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Average	Std dev	Δ Tm
Unmodified	64.4	64.3	64.2	64.4	64.3	64.2	64.3	0.1	0.0
G2	63.8	63.9	63.8	63.9	63.8	63.6	63.8	0.1	0.5
G3	65.1	65.2	64.8	65.3	65.2	64.9	65.1	0.2	0.8
G7	65.3	65.2	65.5	65.6	65.3		65.4	0.2	1.1
G8	64.5	64.3	64.3	64.4	64.3	64.3	64.4	0.1	0.1
G9	63.9	64.3	64.2	63.9	63.9	64	64.0	0.2	0.3
G10	63.9	63.6	63.5	63.8	63.8	63.7	63.7	0.1	0.6
G11	64.6	64.8	64.3	64.7	64.9	64.3	64.6	0.3	0.3
G12	65.4	65.2	65	65.6	65.3	65.1	65.3	0.2	1.0
G13	64.7	64.7	64.3	64.6	64.9		64.6	0.2	0.3
G14	64.1	64.1	63.7	64.5	64.2	63.9	64.1	0.3	0.2
G15	65.4	65.2	65	65.5	65.3	65	65.2	0.2	0.9
G18	64.9	64.9	64.9	65	65.2	65	65.0	0.1	0.7

References

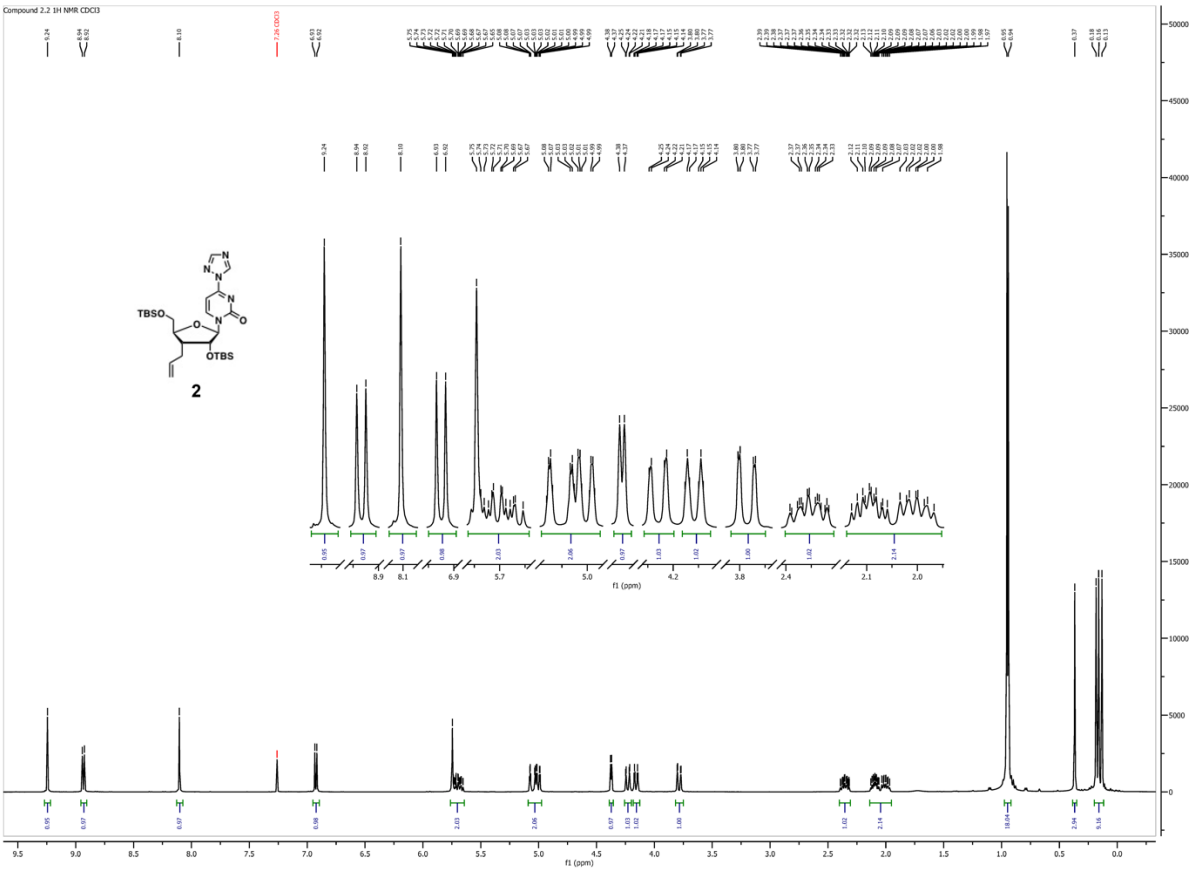
(1) Rozners, E.; Katkevica, D.; Bizdena, E.; Strömberg, R. Synthesis and Properties of RNA Analogs Having Amides as Interuridyl Linkages at Selected Positions. *J. Am. Chem. Soc.* **2003**, *125* (40), 12125-12136.

(2) Selvam, C.; Thomas, S.; Abbott, J.; Kennedy, S. D.; Rozners, E. Amides as Excellent Mimics of Phosphate Linkages in RNA. *Angew. Chem., Int. Ed.* **2011**, *50*, 2068-2070.

(3) Mutisya, D.; Selvam, C.; Lunstad, B. D.; Pallan, P. S.; Haas, A.; Leake, D.; Egli, M.; Rozners, E. Amides are excellent mimics of phosphate internucleoside linkages and are well tolerated in short interfering RNAs. *Nucleic Acids Res.* **2014**, *42*, 6542-6551.

(4) Suter, S. R.; Sheu-Gruttadauria, J.; Schirle, N. T.; Valenzuela, R.; Ball-Jones, A. A.; Onizuka, K.; MacRae, I. J.; Beal, P. A. Structure-Guided Control of siRNA Off-Target Effects. *J. Am. Chem. Soc.* **2016**, *138*, 8667-8669.

(5) Gebert, L. F. R.; Rebhan, M. A. E.; Crivelli, S. E. M.; Denzler, R.; Stoffel, M.; Hall, J. Miravirsen (SPC3649) can inhibit the biogenesis of miR-122. *Nucleic Acids Res.* **2014**, *42*, 609-621.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OTBS
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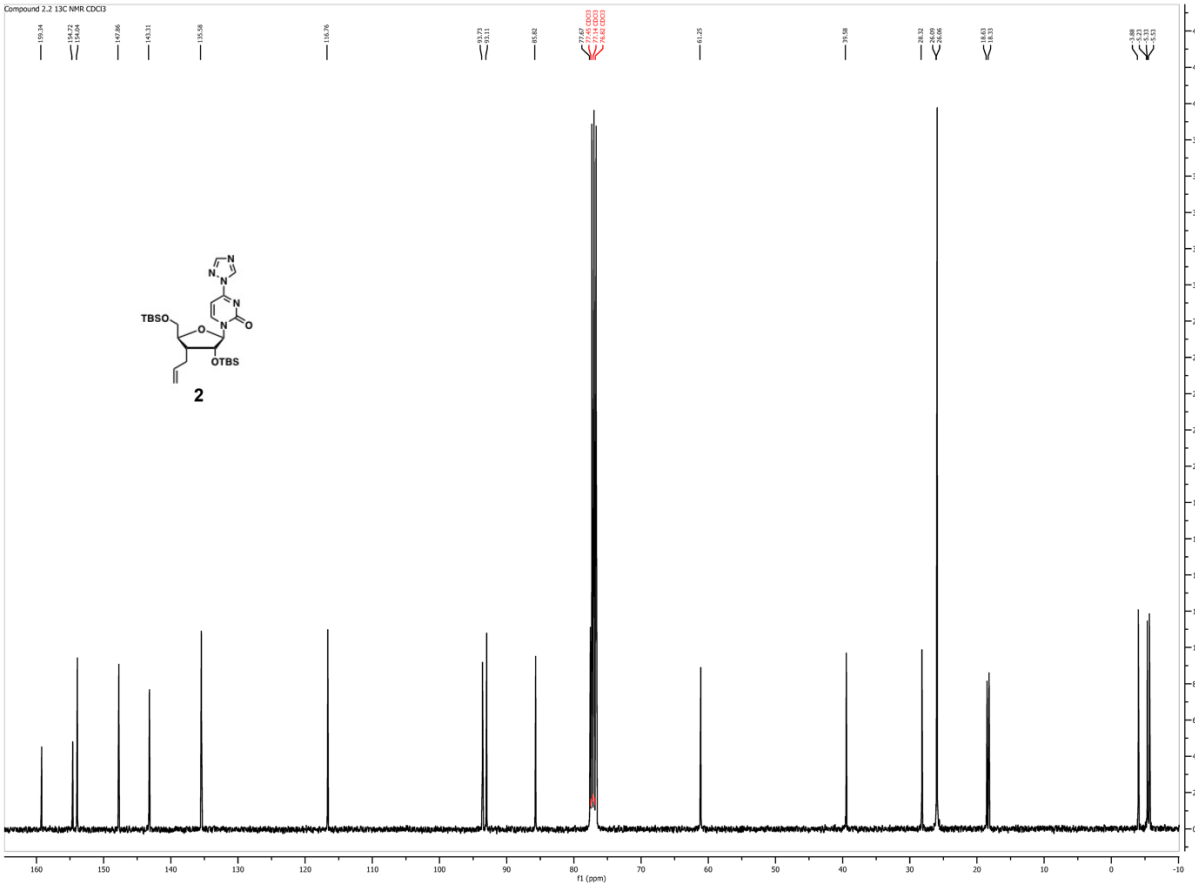
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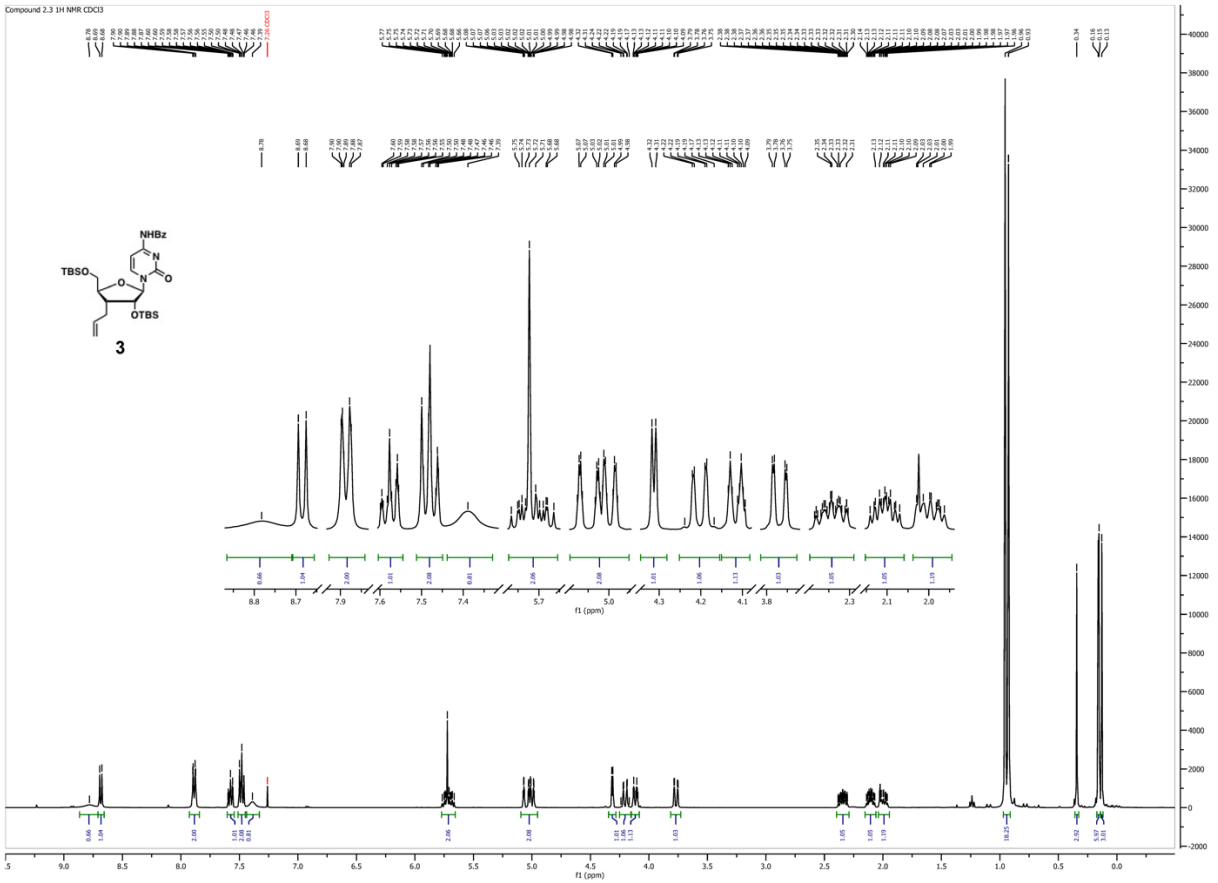
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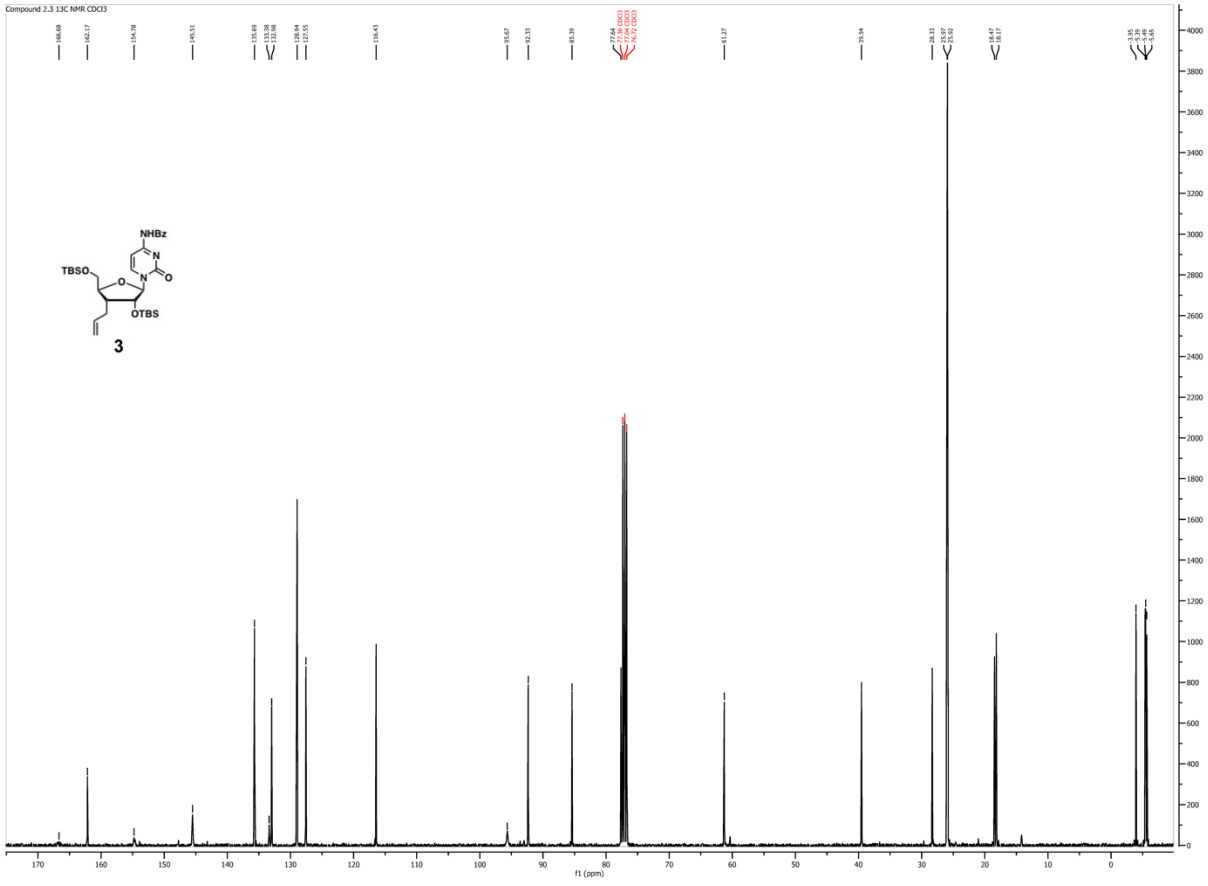
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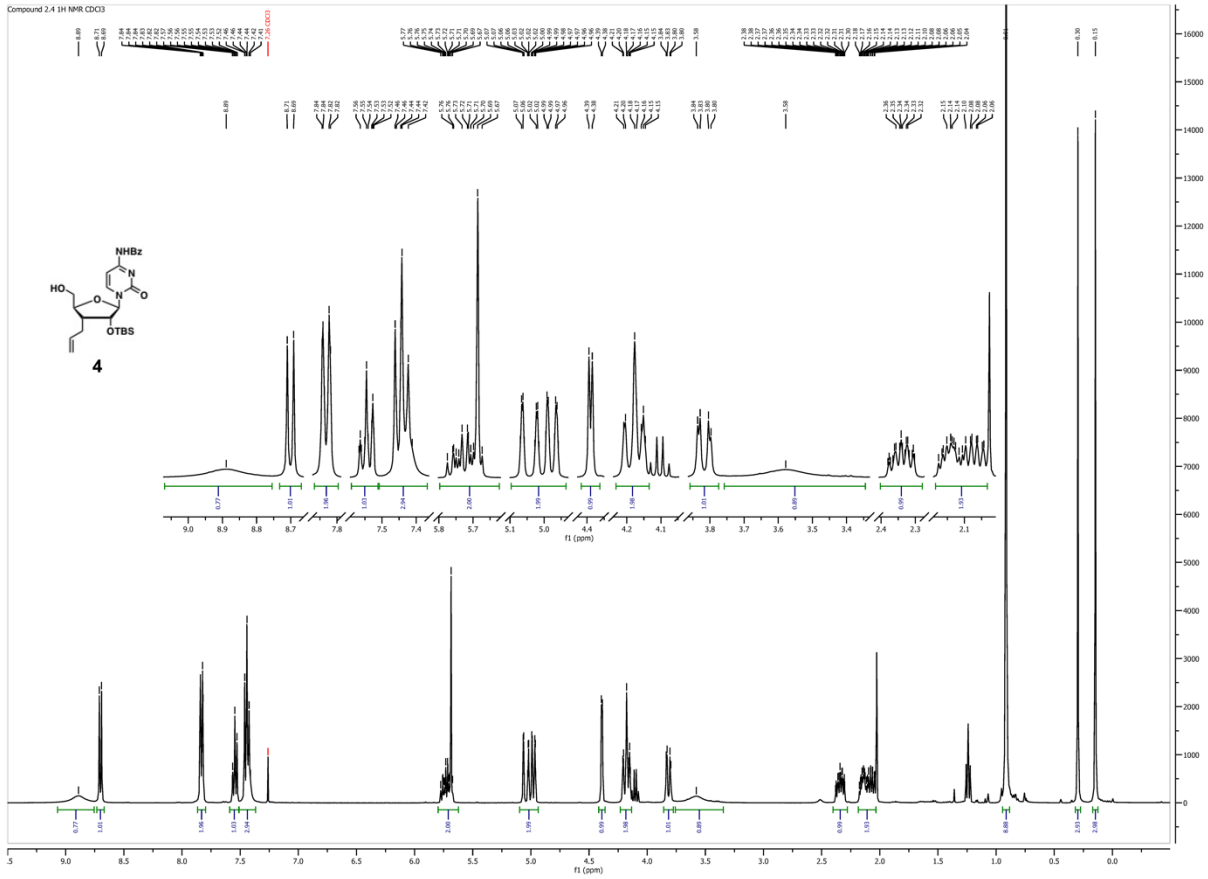
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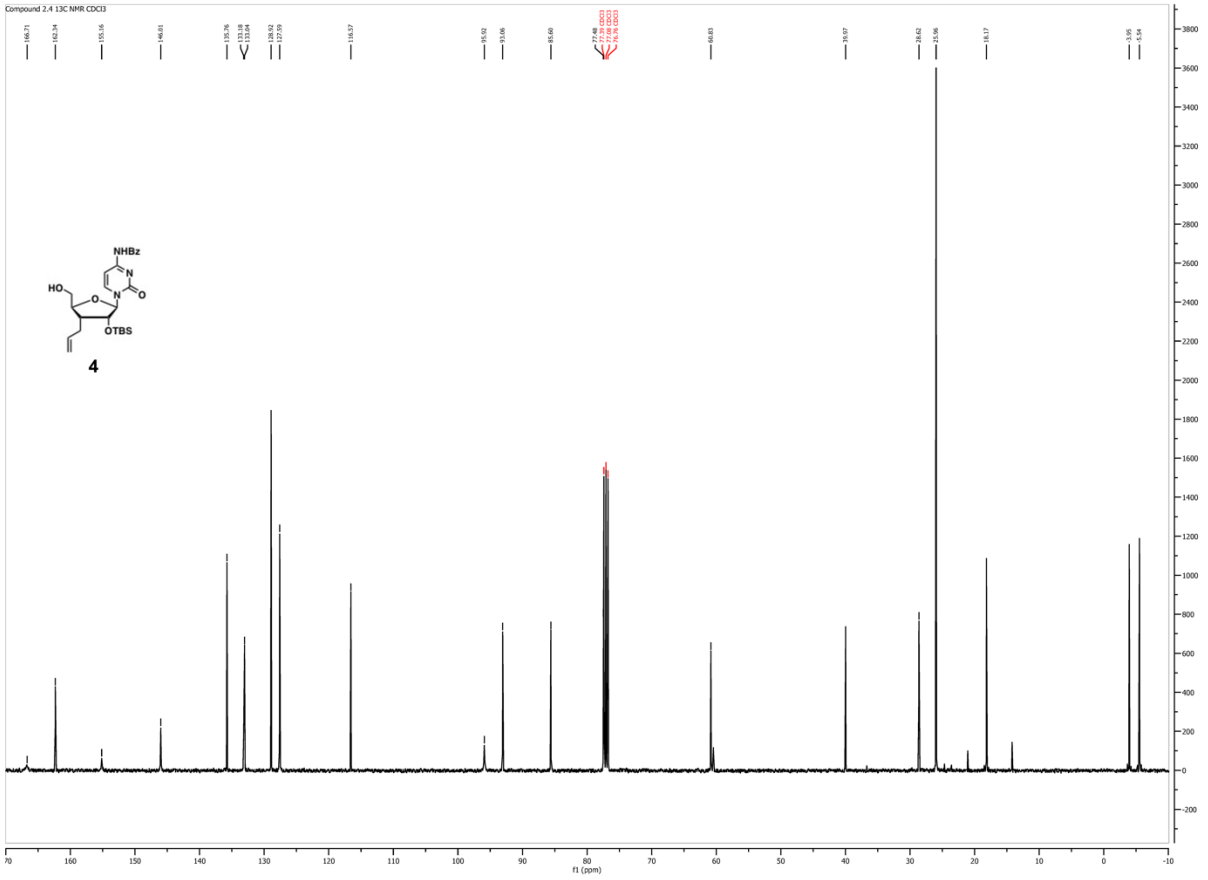
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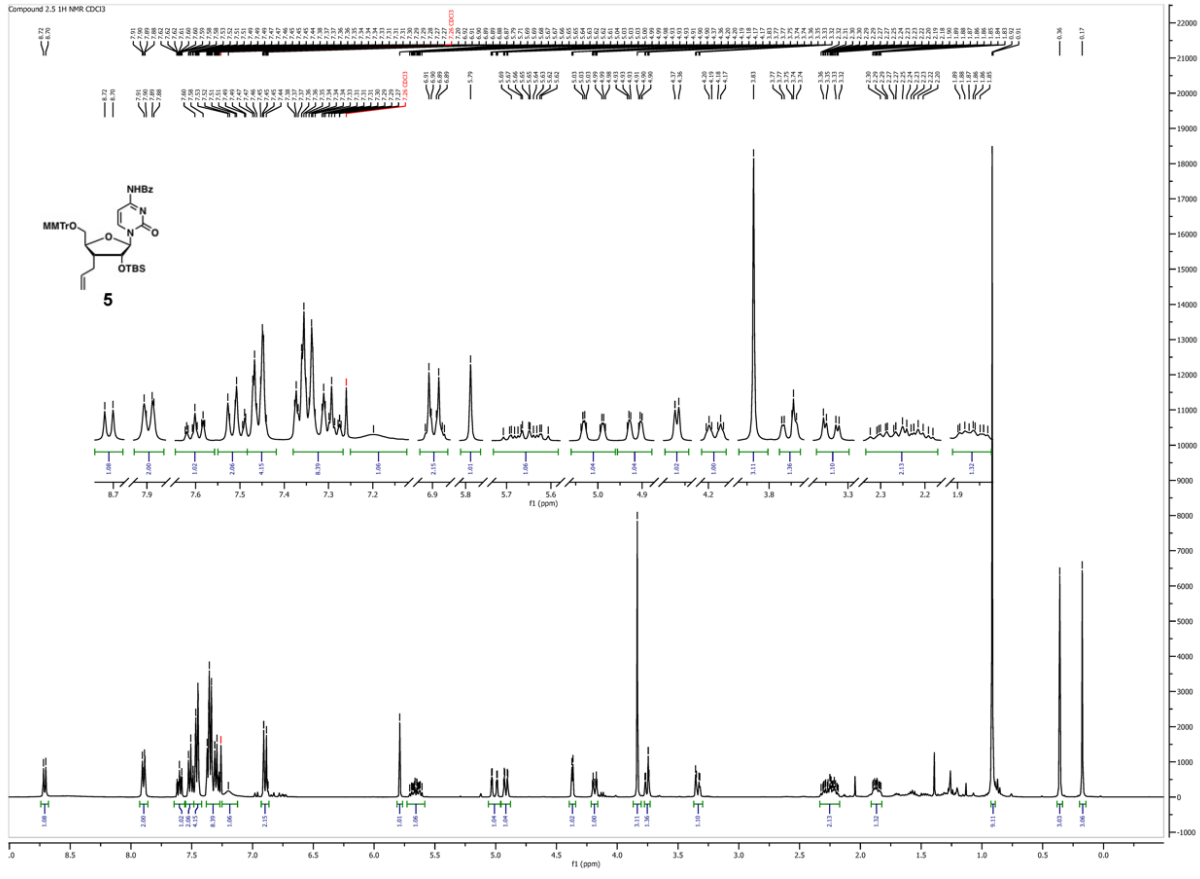
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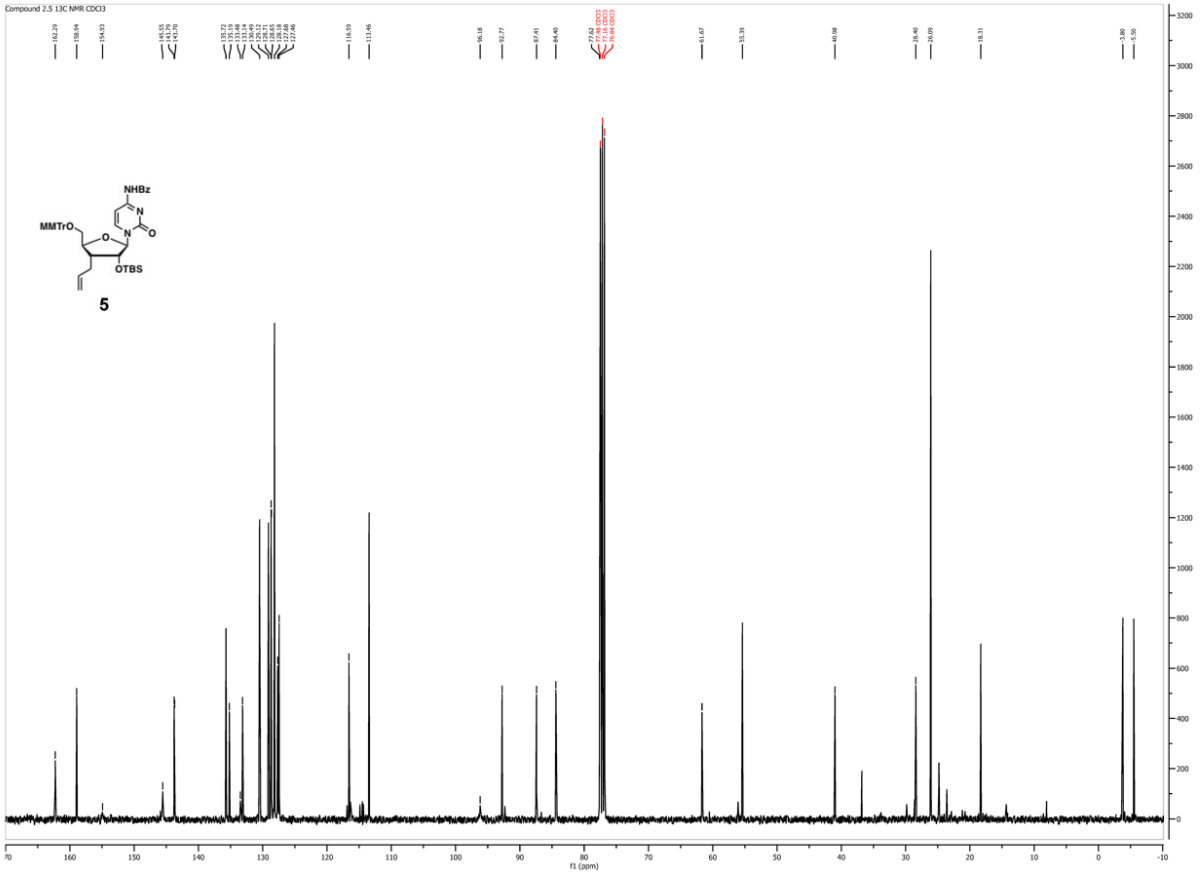
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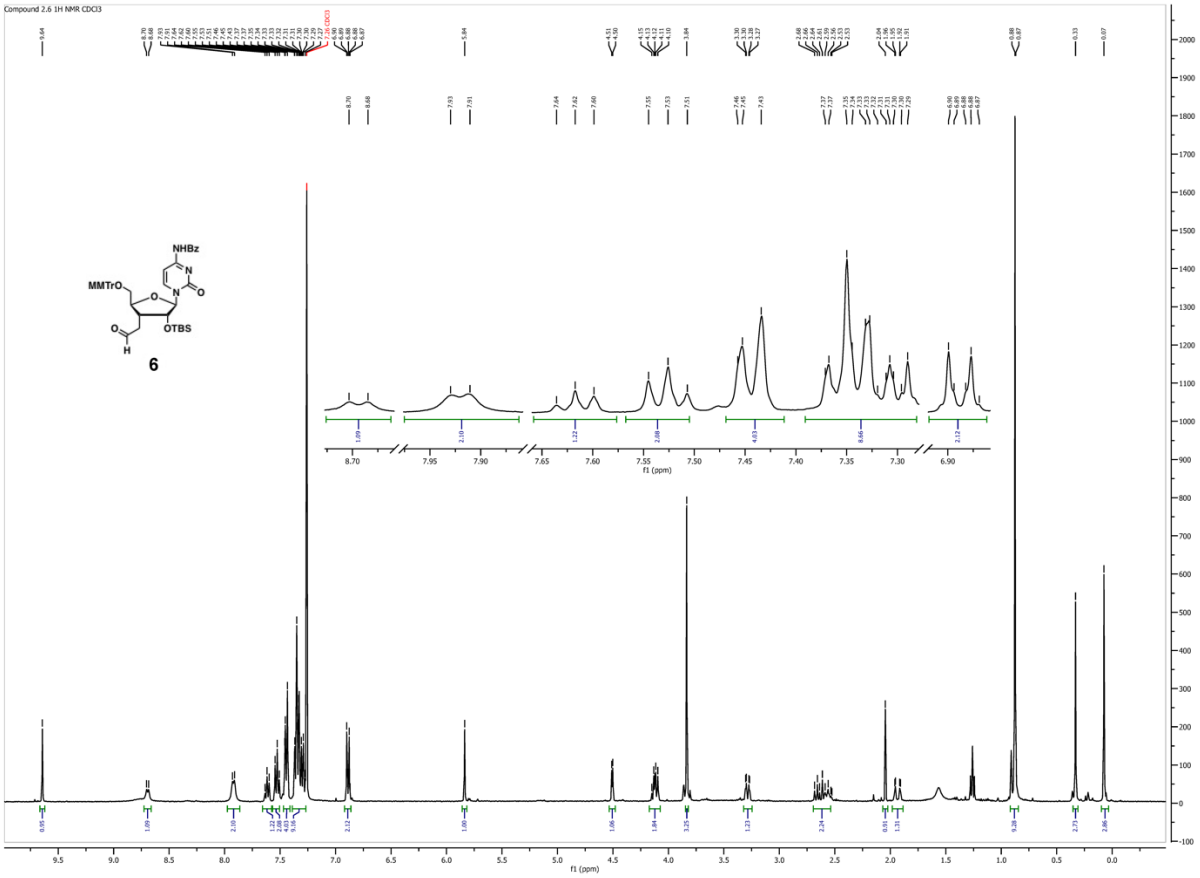
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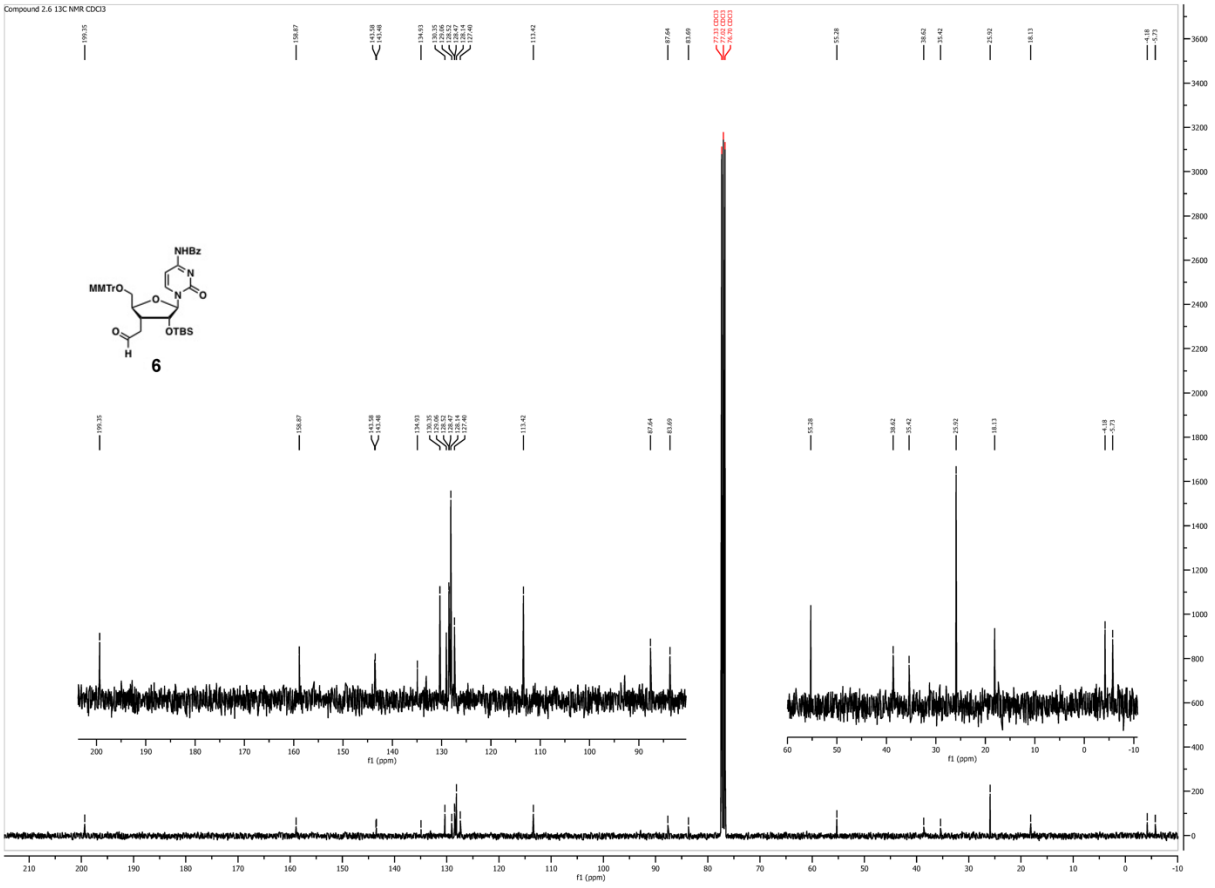
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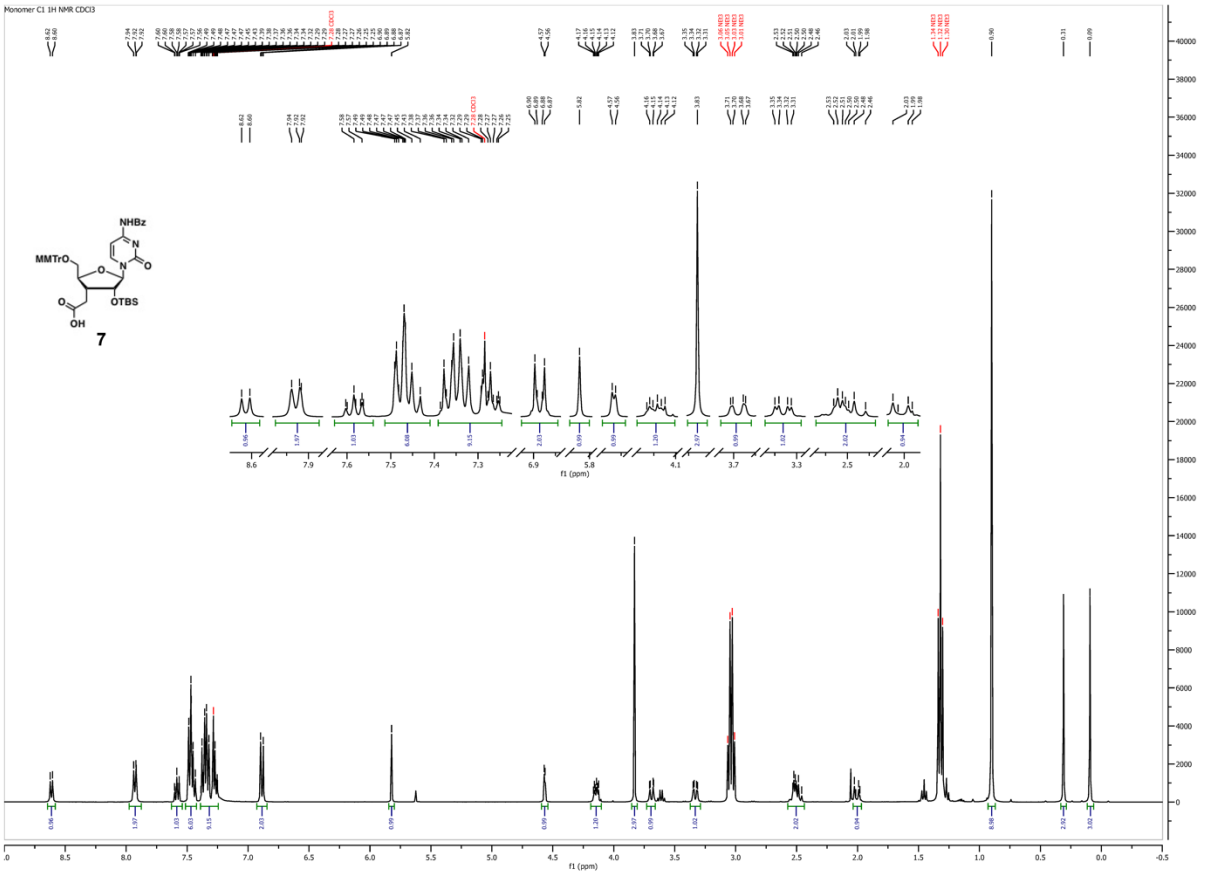
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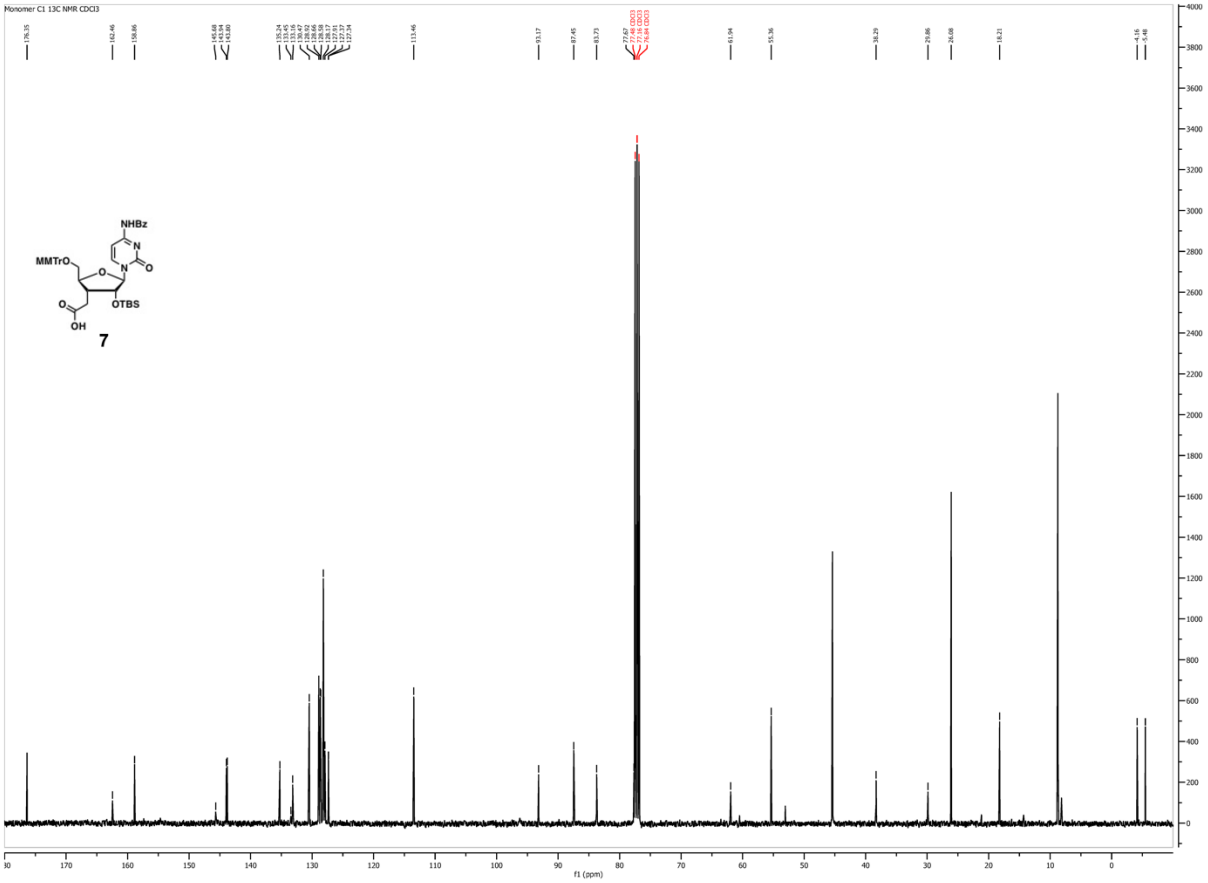
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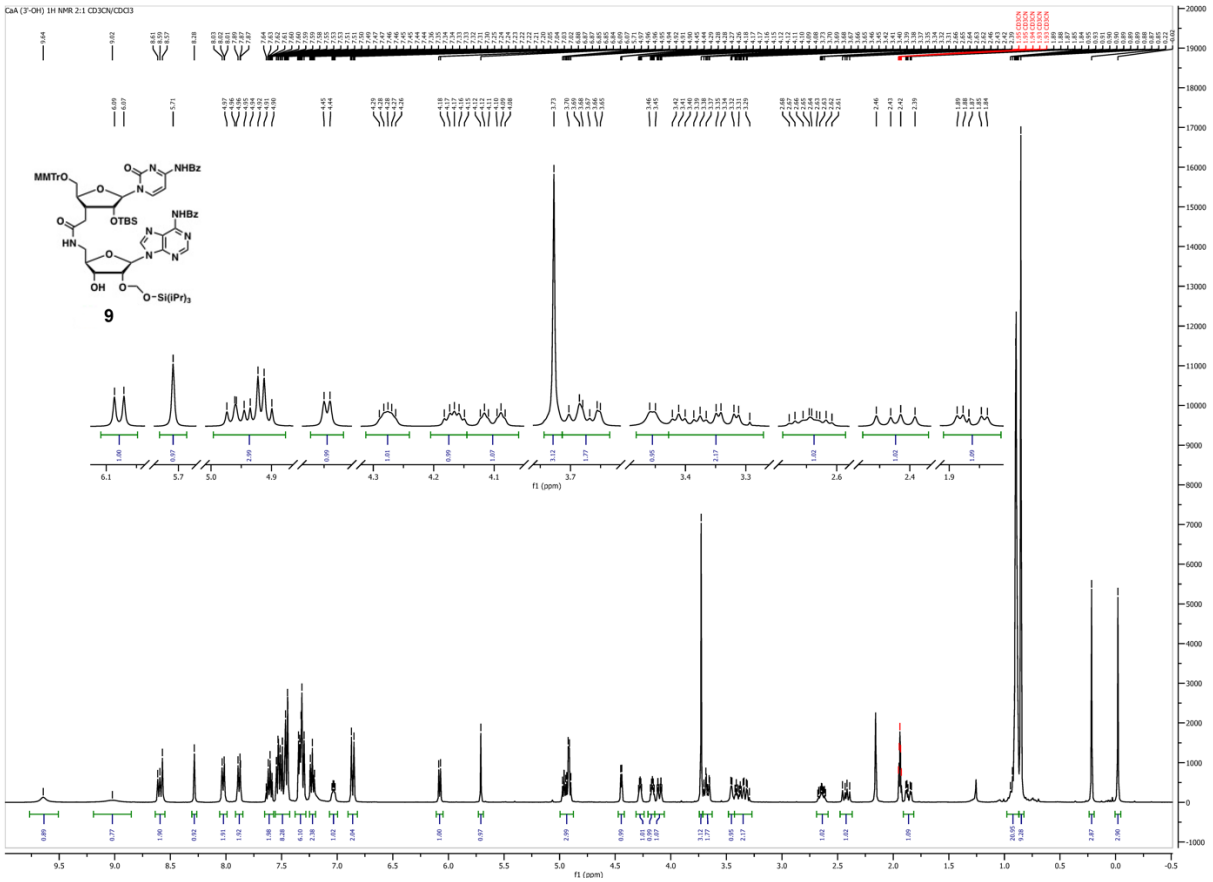
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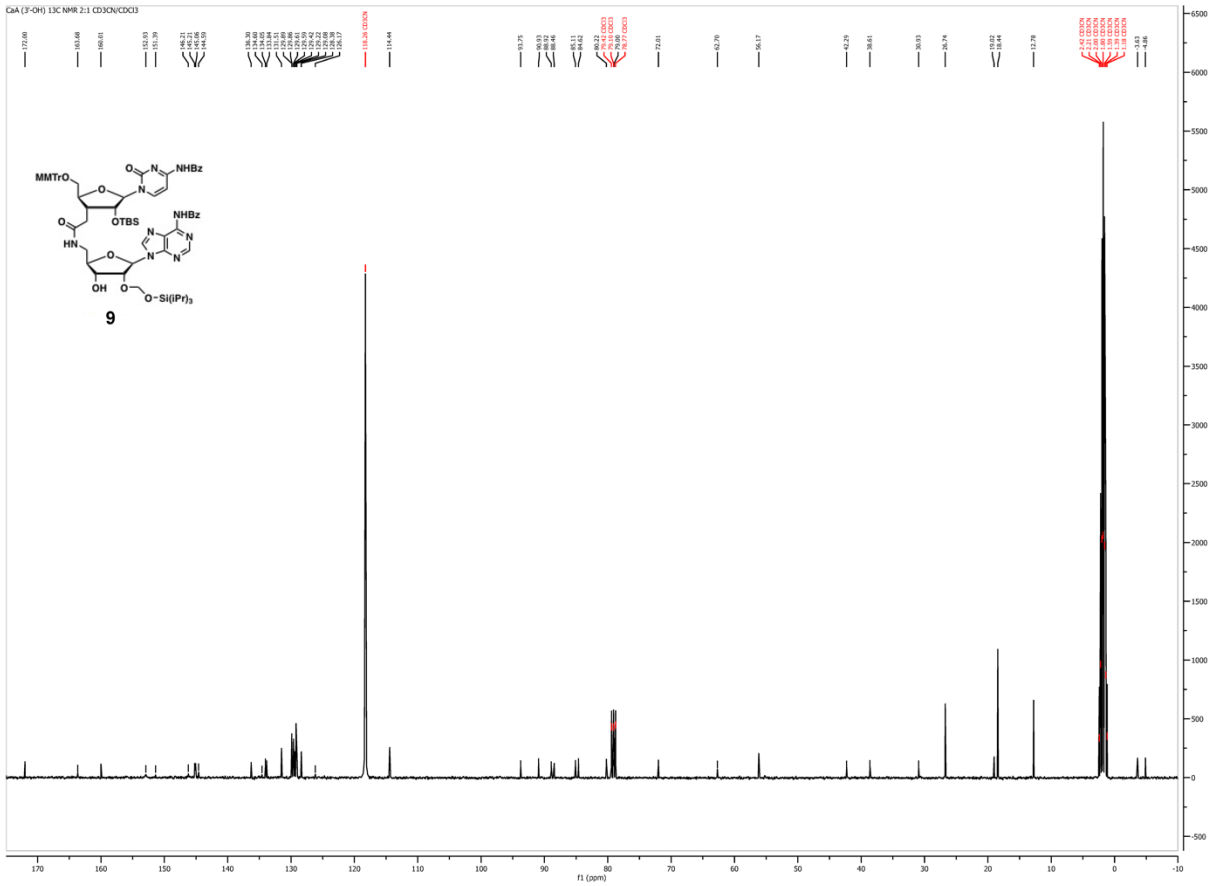
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HN
OH
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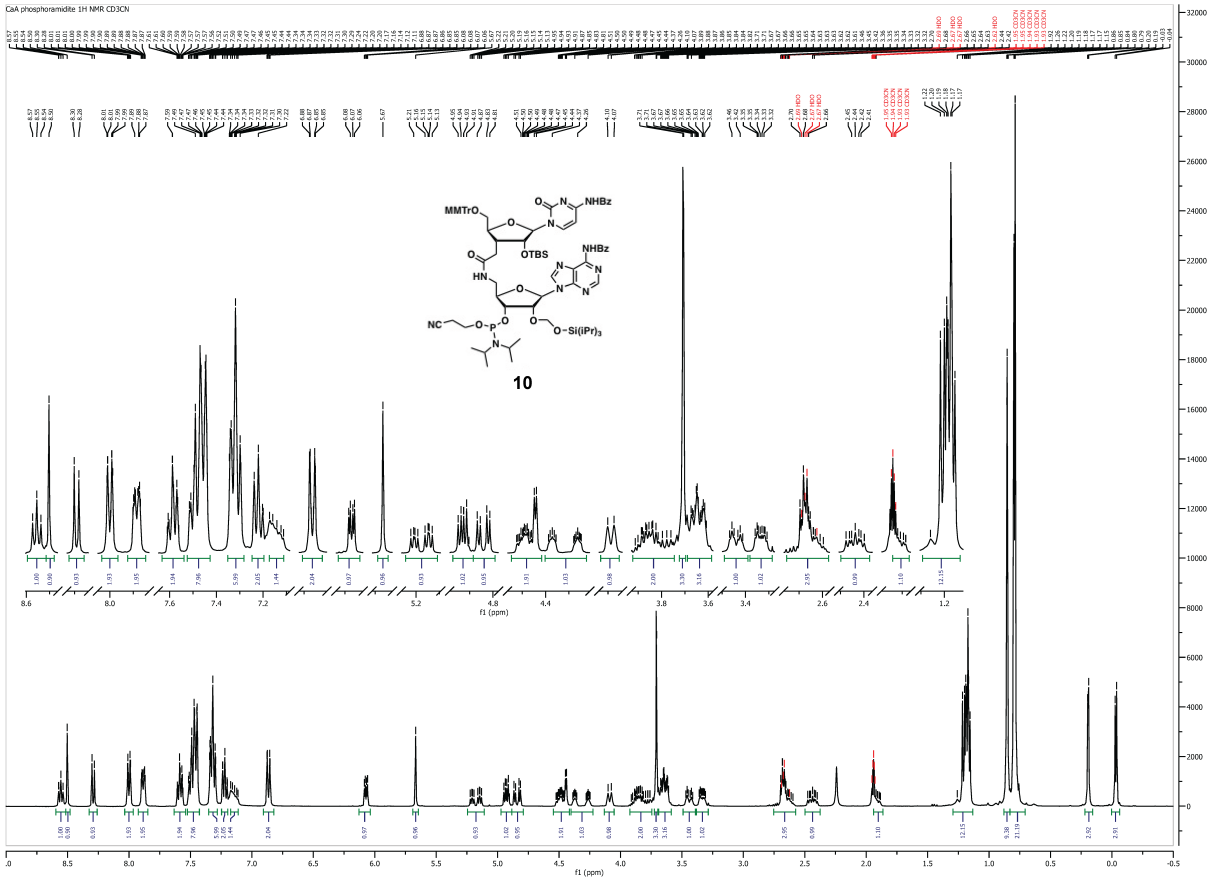
f1 (ppm)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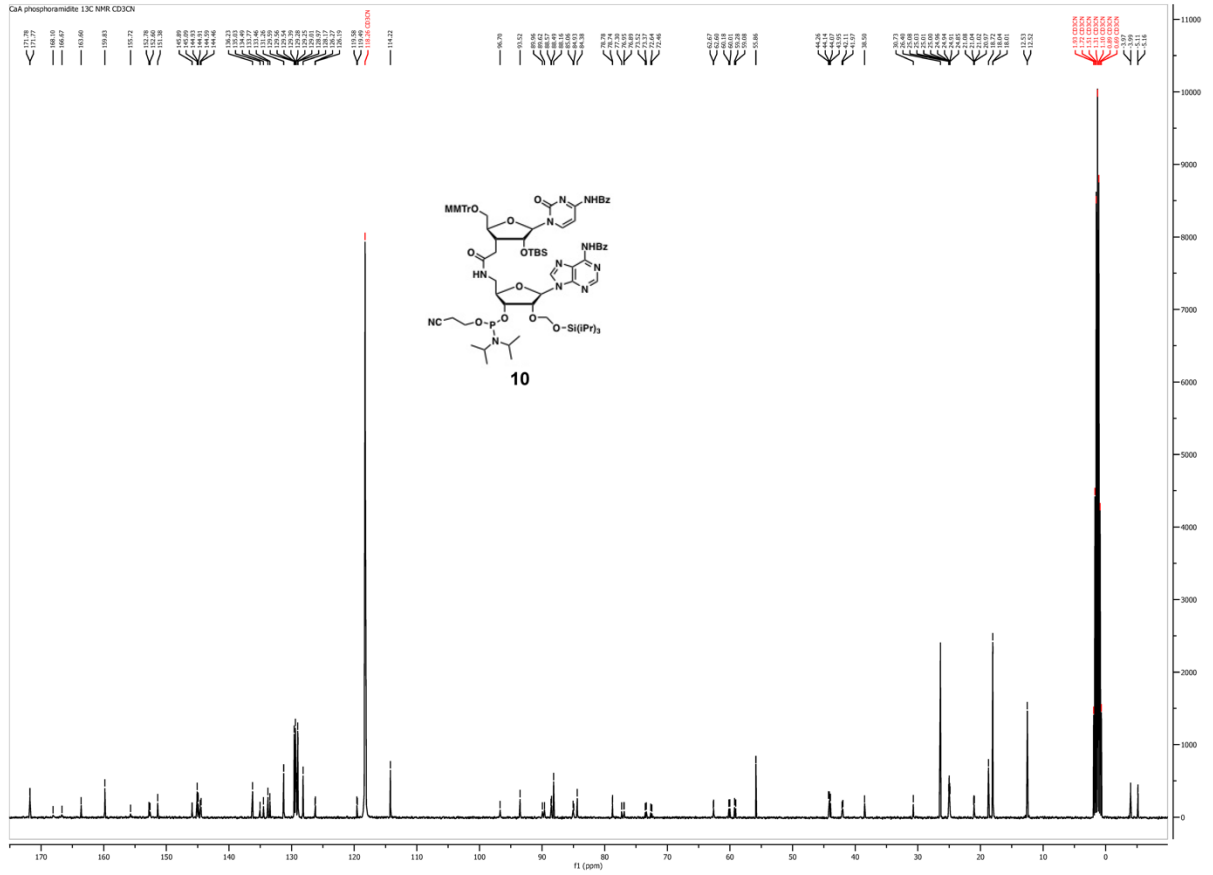
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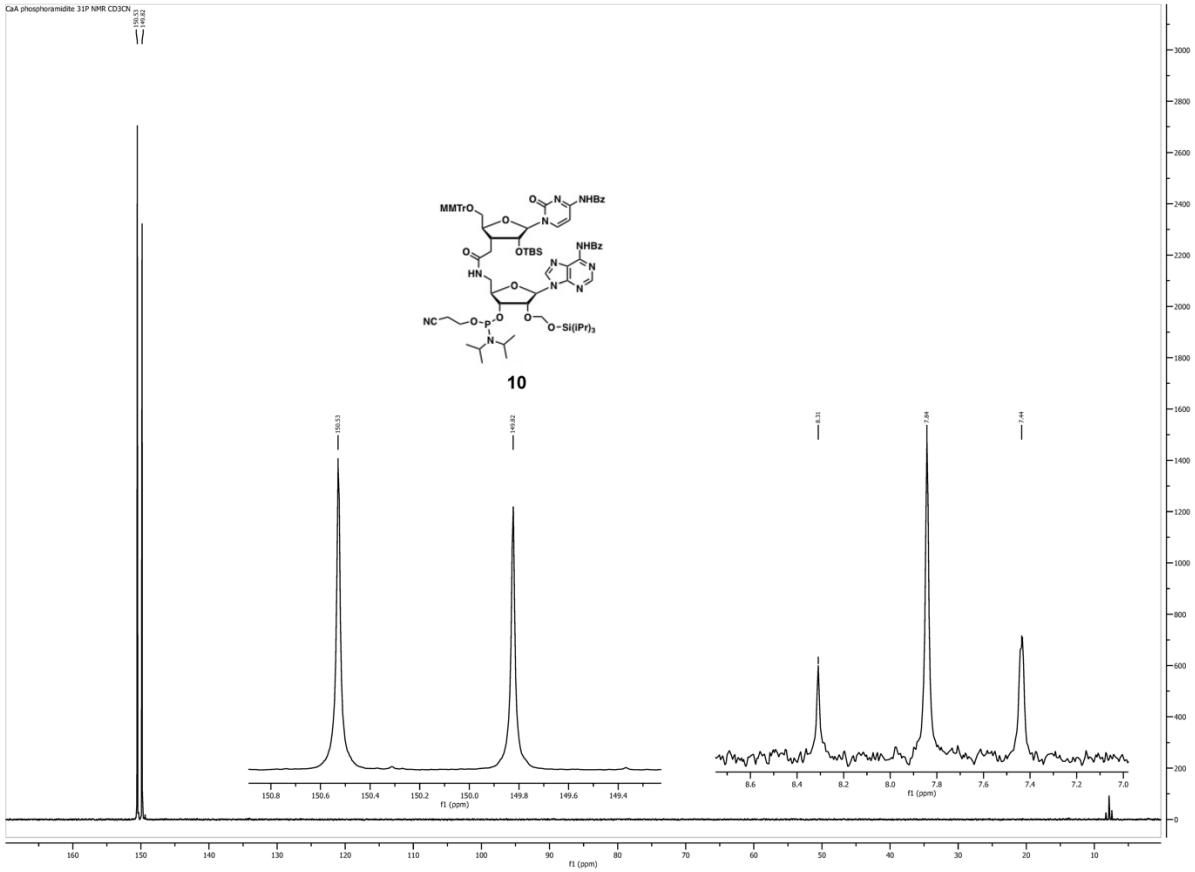
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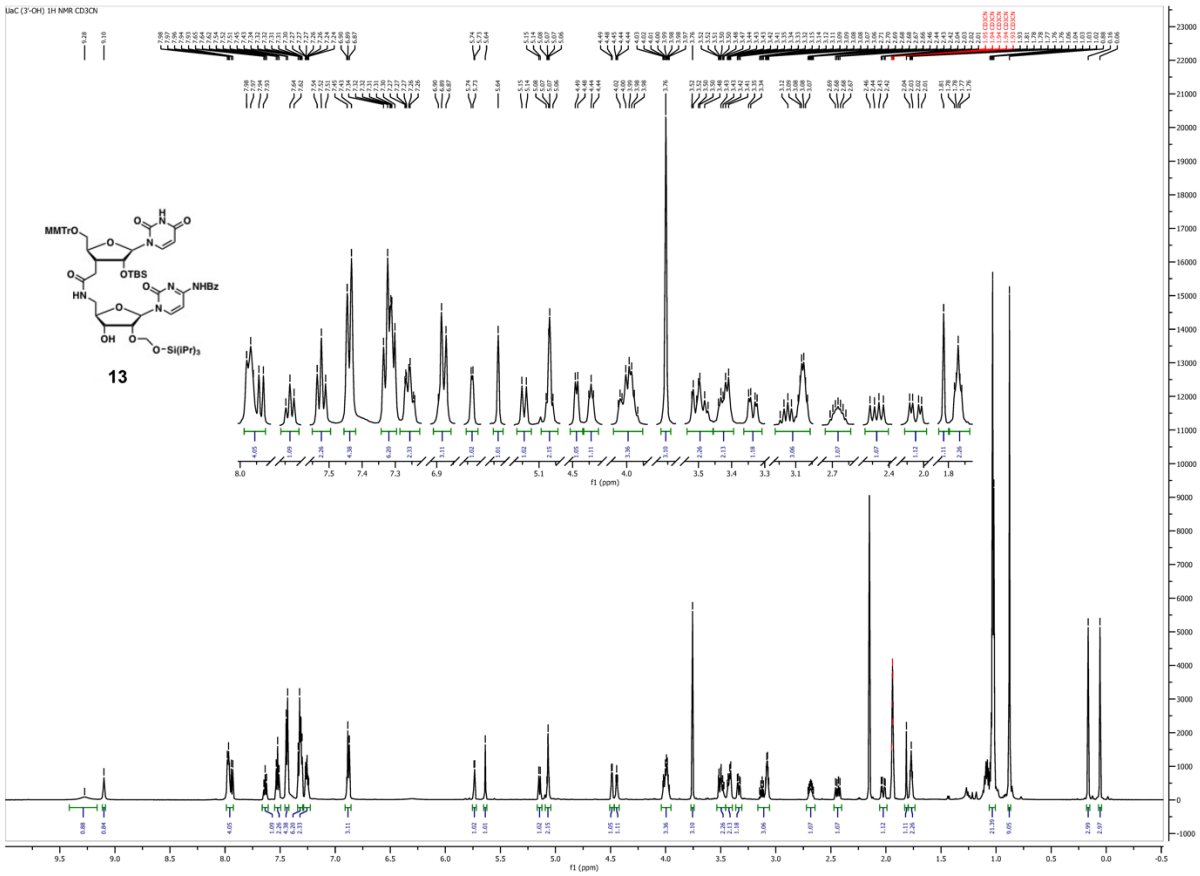
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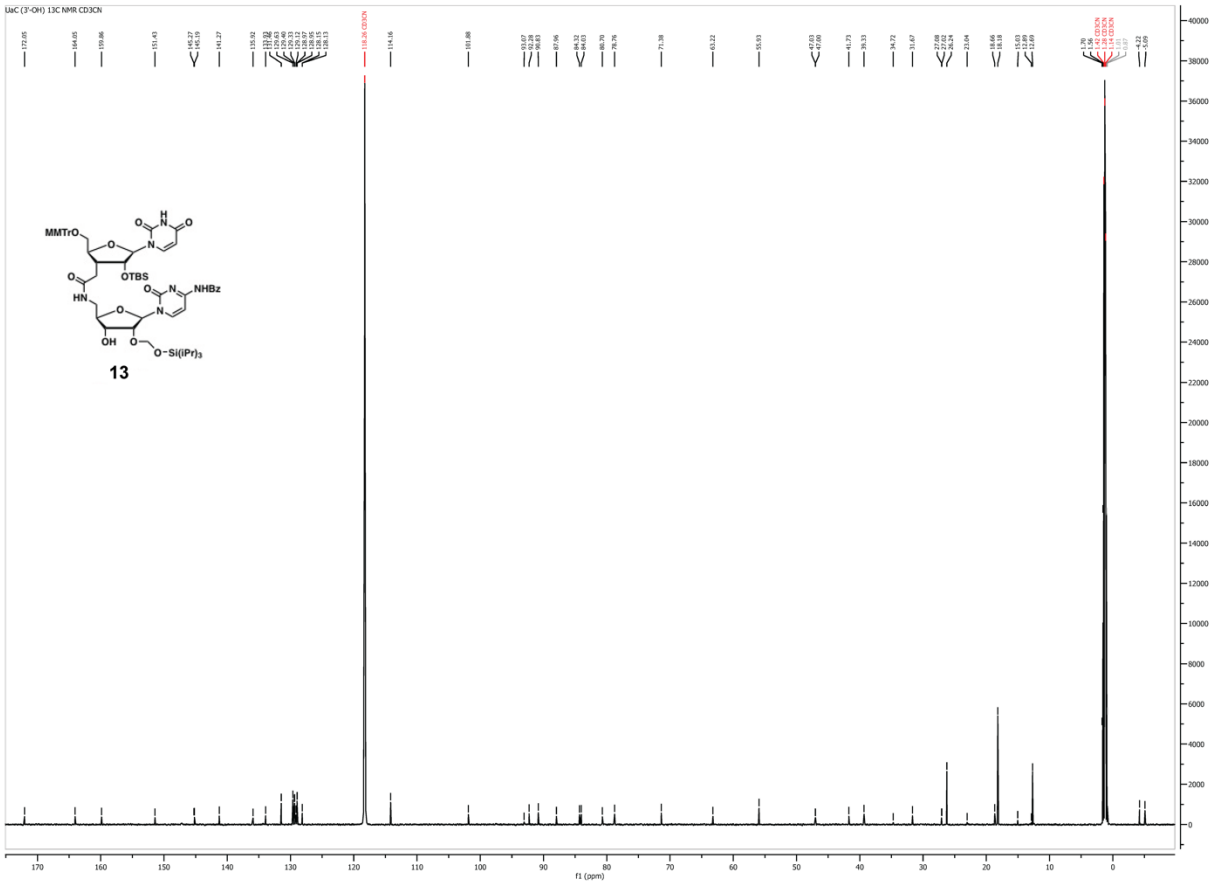
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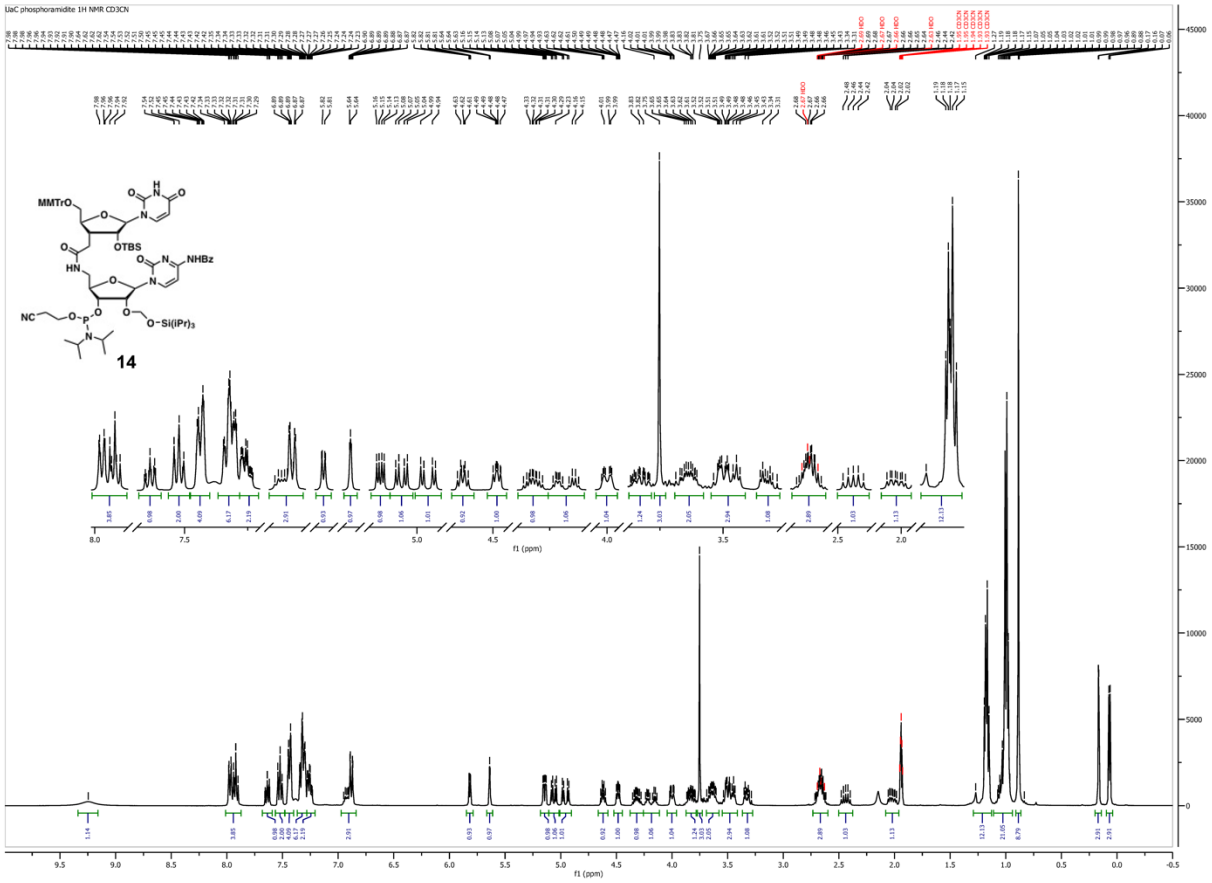
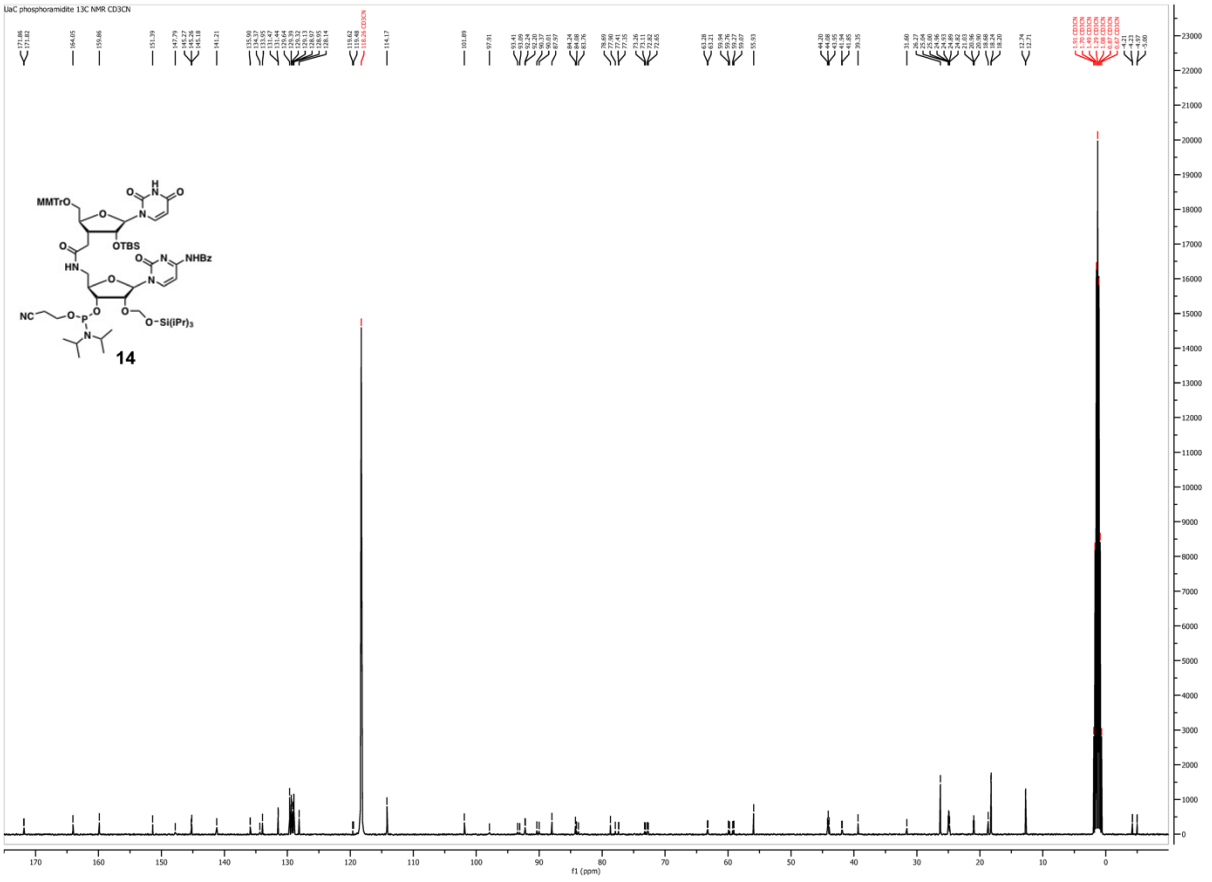
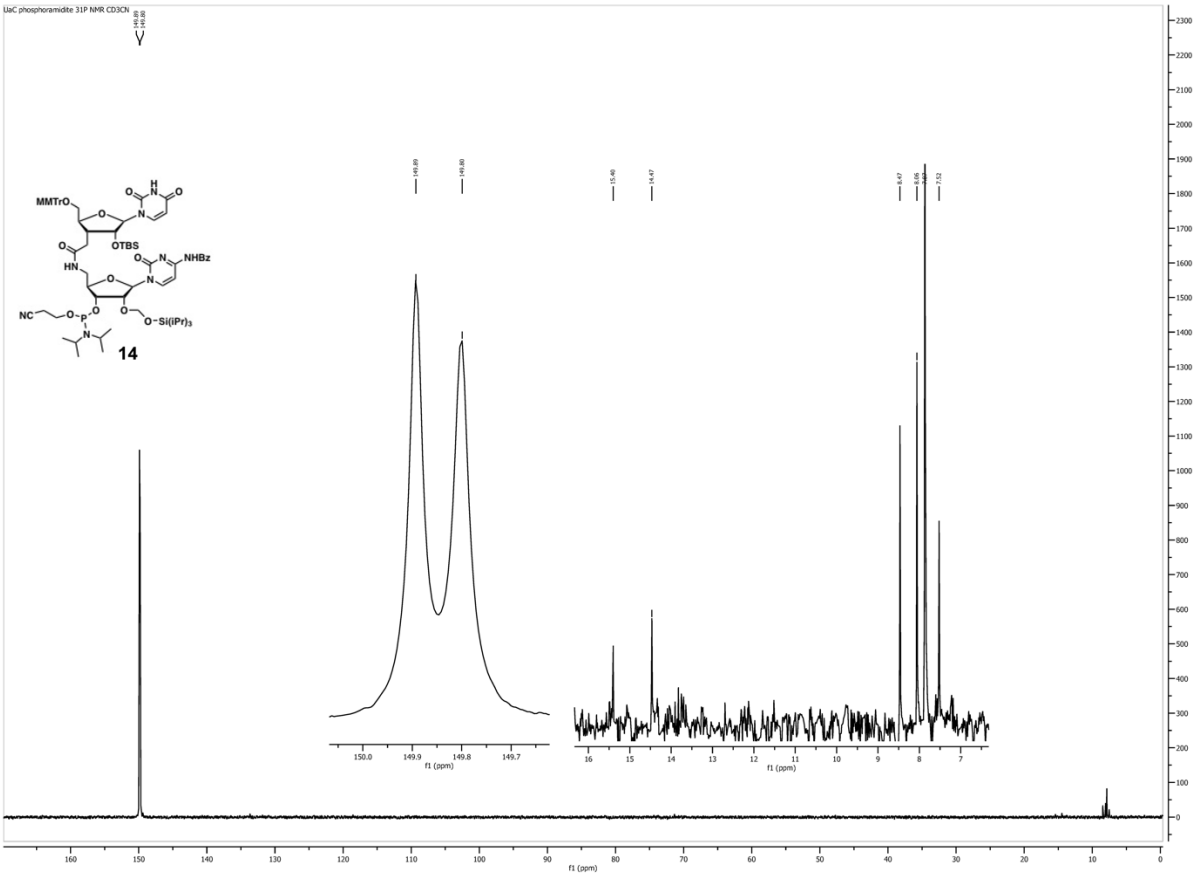
13
f1 (ppm)


UaC phosphoramidite 1H NMR CD3CN
MMTrO
OTBS
NHBz
NC
O-Si(iPr)3
14
f1 (ppm)


UaC phosphoramidite 13C NMR CD3CN
MMTrO
OTBS
NHBz
NC
O-Si(iPr)3
14
f1 (ppm)


LiaC phosphoramidite 31P NMR CD3CN
149.89
149.80
15.40
14.47
8.47
8.06
8.03
7.52
MMTrO
OTBS
HN
NHBz
NC
O-Si(iPr)3
14
f1 (ppm)