Electronic Supplementary Information

Expanding the binding space of argonaute-2: incorporation of either *E* or *Z* isomers of 6′-vinylphosphonate at the 5′ end of the antisense strand improves RNAi activity

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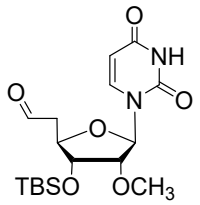
Contents

General conditions

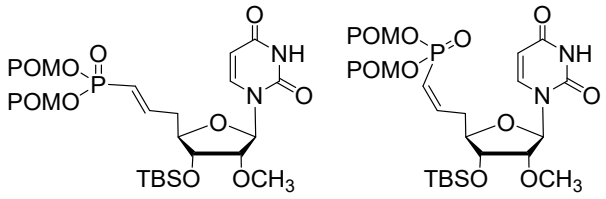
TLC was performed on Merck silica gel 60 plates coated with F254. Compounds were visualized under UV light (254 nm) or after spraying with the *p*-anisaldehyde staining solution followed by heating. Flash column chromatography was performed using a Teledyne ISCO Combi Flash system with pre-packed RediSep Teledyne ISCO silica gel cartridges and Prep-Achiral supercritical fluid chromatography. All moisture-sensitive reactions were carried out under anhydrous conditions using dry glassware, anhydrous solvents, and argon atmosphere. All commercially available reagents and solvents were purchased from Sigma-Aldrich unless otherwise stated and were used as received. ESI-MS spectra were recorded on a Waters QTof Premier instrument using the direct flow injection mode. ^{1}H NMR spectra were recorded at 6600 MHz. ^{13}C NMR spectra were recorded at 151 MHz. ^{31}P NMR spectra were recorded at 243 MHz. Chemical shifts are given in ppm referenced to the solvent residual peak (DMSO-d_6 – ^{1}H: δ at 2.50 ppm and ^{13}C δ at 39.5 ppm; $CDCl_3$ – ^{1}H: δ at 7.26 ppm and ^{13}C δ at 77.16 ppm; CD_3CN – ^{1}H: δ at 1.94 ppm and ^{13}C δ at 1.32 ppm respectively). Coupling constants are given in Hertz. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), septet (sept), broad signal (brs), or multiplet (m).

Syntheses of building blocks



2

2-((2R,3R,4R,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-methoxytetrahydrofuran-2-yl)acetaldehyde 2: To a clear solution of **1** synthesized as previously described[1] (1.0 g, 2.59 mmol) in DCM (30 mL) was added Dess-Martin periodinane (1.37 g, 3.23 mmol) at 0 °C. The resulting mixture was stirred for 2 h at 22 °C and then cooled to 0 °C. The reaction mixture was diluted with $NaHCO_3$ solution (25 mL). The organic layer was separated and washed with 5% sodium thiosulfate solution (25 mL), and the resulting organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated to dryness to afford aldehyde **2** (0.93 g, 99%) as a white foam, which was used for next step without further purification. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.40 (d, J = 2.1 Hz, 1H), 9.66 (t, J = 1.8 Hz, 1H), 7.66 (d, J = 8.1 Hz, 1H), 5.78 (d, J = 4.5 Hz, 1H), 5.66 (dd, J = 8.0, 2.1 Hz, 1H), 4.26 (dt, J = 7.4, 5.5 Hz, 1H), 4.18 (t, J = 5.2 Hz, 1H), 3.94 (t, J = 4.8 Hz, 1H), 3.33 (s, 3H), 2.87 – 2.79 (m, 2H), 0.88 (s, 9H), 0.10 (d, J = 5.7 Hz, 6H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 201.0, 163.0, 150.4, 141.2, 102.2, 87.7, 80.9, 78.2, 73.0, 57.5, 46.2, 25.6, 17.8, -4.8, -4.9 ppm.

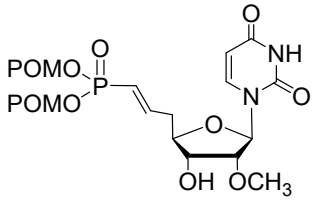


3 **4**

[[(E)-3-[(2R,3S,4R,5R)-5-[(1S)-2,4-bis(oxidanylidene)pyrimidin-1-yl]-3-[1,1-dimethylethyl (dimethyl)silyl]oxy-4-methoxy-tetrahydrofuran-2-yl]prop-1-enyl]-(2,2-dimethylpropanoy loxymethoxy)phosphoryl]oxymethyl 2,2-dimethylpropanoate 3 and (((Z)-3-((2R,3R,4R,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-methoxytetra hydrofuran-2-yl)prop-1-en-1-yl)phosphoryl)bis(oxy))bis(methylene)bis(2,2-dimethylpropa noate ***4****:* To a clear solution of [bis(2,2-dimethylpropanoyloxymethoxy) phosphorylmethyl-(2,2-dimethylpropanoyloxymethoxy)phosphoryl]oxymethyl-2,2-dimethylpropanoate (*bis*-POM VP reagent) (8.18 g, 12.94 mmol) in anhydrous THF (30 mL) was added sodium hydride (620.93 mg, 15.52 mmol, 60% purity) at -30 °C. After stirring for 10 min at 0 °C, the solution was cooled to -30 °C, and aldehyde dissolved in anhydrous THF (20 mL) was added. After stirring for 3 hr at 0 °C, the reaction was quenched with 10% ammonium chloride (30 mL). Ethyl acetate (EtOAc; 30 mL) was added, and organic layer was removed. The aqueous layer was washed with EtOAc (2 x 20 mL). The organic layers were combined and dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated to dryness. The crude compound was purified by flash column chromatography (gradient: 40-80% EtOAc in hexanes) to afford the *E*-isomer **3** (2.0 g, 56% yield) as a white foam and the *Z*-isomer **4** (0.4, 11% yield) as a white foam. The *Z*-isomer was the faster moving spot in TLC and eluted first during column chromatography.

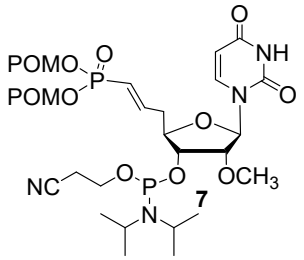
Data for **3**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.96 (d, J = 2.1 Hz, 1H), 7.25 (d, J = 8.2 Hz, 1H), 6.92 – 6.80 (m, 1H), 5.93 – 5.83 (m, 1H), 5.78 (dd, J = 8.1, 2.2 Hz, 1H), 5.71 – 5.64 (m, 5H), 4.06 (td, J = 8.1, 3.5 Hz, 1H), 3.90 (dd, J = 7.7, 5.4 Hz, 1H), 3.75 (dd, J = 5.4, 2.2 Hz, 1H), 3.51 (s, 3H), 2.76 – 2.60 (m, 1H), 2.50 – 2.42 (m, 1H), 1.22 (s, 19H), 0.91 (s, 9H), 0.10 (d, J = 6.6 Hz, 6H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.0, 163.3, 149.9, 149.4, 149.3, 140.4, 120.2, 119.0, 102.8, 90.6, 83.0, 81.6, 81.6, 81.0, 73.8, 58.6, 38.8, 36.9, 36.8, 26.9, 25.8, 18.2, -4.4, -4.7 ppm. ^{31}P NMR (243 MHz, $CDCl_3$) δ 17.19 ppm. HRMS (ESI$^+$) m/z calcd for $C_{30}H_{51}N_2O_{12}PSiNa$ $[M + Na]^+$ 713.2847, found 713.2839.

Data for **4**: ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.39 (d, *J* = 2.2 Hz, 1H), 7.67 (d, *J* = 8.1 Hz, 1H), 6.78 – 6.59 (m, 1H), 5.85 – 5.75 (m, 2H), 5.65 – 5.55 (m, 5H), 5.28 (d, *J* = 6.2 Hz, 1H), 3.96 (q, *J* = 5.6 Hz, 1H), 3.88 (dt, *J* = 8.7, 5.0 Hz, 1H), 3.83 (t, *J* = 5.0 Hz, 1H), 3.36 (s, 3H), 2.98 – 2.89 (m, 1H), 2.86 – 2.77 (m, 1H), 1.15 (d, *J* = 9.7 Hz, 18H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.1, 163.1, 163.0, 151.1, 151.0, 150.4, 140.8, 118.0, 116.8, 102.1, 87.0, 82.0, 81.7, 81.3, 81.3, 79.2, 71.5, 57.6, 38.2, 38.2, 34.0, 34.0, 26.5, 26.5 ppm. ^{31}P NMR (243 MHz, DMSO-d_6) δ 16.24 ppm. HRMS (ESI$^+$) m/z calcd for $C_{30}H_{52}N_2O_{12}PSi$ $[M + H]^+$ 691.3027, found 691.3029.

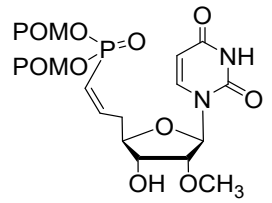


5

[[(E)-3-[(2R,3S,4R,5R)-5-[(1S)-2,4-bis(oxidanylidene)pyrimidin-1-yl]-3-hydroxy-4-methoxy-tetrahydrofuran-2-yl]prop-1-enyl]-(2,2-dimethylpropanoyloxymethoxy)phosphoryl]oxymethyl 2,2-dimethylpropanoate 5: A solution containing **3** (0.55 g, 796.19 μmol) in 20 mL of $HCOOH/H_2O$ (1:1, v/v) was stirred at 22 °C for 18 h. TLC analysis confirmed the formation of the product. The solution was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (gradient: 0-5% methanol in DCM) to afford **5** (0.38 g, 83% yield) as a white hygroscopic foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.39 (d, J = 2.2 Hz, 1H), 7.60 (d, J = 8.1 Hz, 1H), 6.80 – 6.57 (m, 1H), 6.03 – 5.92 (m, 1H), 5.80 – 5.74 (m, 1H), 5.64 (dd, J = 8.1, 2.1 Hz, 1H), 5.61 – 5.57 (m, 2H), 5.57 – 5.54 (m, 2H), 5.29 (d, J = 6.3 Hz, 1H), 3.94 (q, J = 5.7 Hz, 1H), 3.88 – 3.82 (m, 2H), 3.35 (s, 5H), 2.69 – 2.52 (m, 2H), 1.14 (d, J = 1.7 Hz, 18H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.0, 163.0, 150.5, 150.5, 150.4, 141.0, 119.2, 118.0, 102.2, 87.3, 81.5, 81.4, 81.4, 71.6, 57.7, 38.2, 37.1, 36.9, 26.5 ppm. ^{31}P NMR (243 MHz, DMSO-d_6) δ 17.77 ppm. HRMS (ESI^+) m/z calcd for $C_{24}H_{37}N_2O_{12}PNa$ $[M + Na]^+$ 599.1982, found 599.1976.

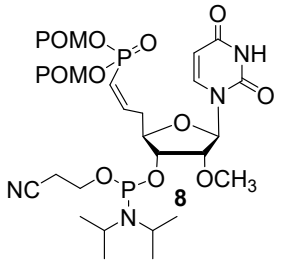


[[(E)-3-[(2R,3S,4R,5R)-3-[[bis(1-methylethyl)amino]-(2-cyanoethoxy)phosphanyl]oxy-5-[(1S)-2,4-bis(oxidanylidene)pyrimidin-1-yl]-4-methoxy-tetrahydrofuran-2-yl]prop-1-enyl]-(2,2-dimethylpropanoyloxymethoxy)phosphoryl]oxymethyl 2,2-dimethylpropanoate 7: To a clear solution of **5** (0.3 g, 520.36 μmol) and 5-(ethylthio)-1*H*-tetrazole (67.73 mg, 520.36 μmol) in anhydrous acetonitrile (ACN) (10 mL) was added 2-cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphorodiamidite (313.68 mg, 1.04 mmol, 330.54 μL). The reaction mixture was stirred at 22 °C for 2 h. TLC analysis confirmed formation of the product. The reaction mixture was filtered and concentrated, and the residue thus obtained was purified by flash column chromatography (gradient: 60-95% EtOAc in hexanes) to afford **7** (0.29 g, 71.0% yield) as a white foam. ^{1}H NMR (600 MHz, CD_3CN) δ 9.05 (s, 1H), 7.36 (ddt, *J* = 8.2, 3.9, 1.3 Hz, 1H), 6.85 – 6.69 (m, 1H), 6.02 – 5.86 (m, 1H), 5.80 (dd, *J* = 4.0, 1.8 Hz, 1H), 5.64 (dd, *J* = 8.1, 2.5 Hz, 1H), 5.58 (dt, *J* = 13.5, 2.7 Hz, 5H), 4.19 – 4.03 (m, 3H), 3.94 – 3.79 (m, 2H), 3.75 – 3.62 (m, 3H), 3.48 – 3.36 (m, 3H), 2.85 – 2.63 (m, 5H), 2.60 – 2.51 (m, 1H), 2.15 (d, *J* = 2.5 Hz, 1H), 1.95 – 1.93 (m, 2H), 1.24 – 1.15 (m, 43H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 177.6, 177.6, 163.8, 163.7, 151.2, 151.2, 151.1, 151.1, 150.8, 150.8, 141.3, 141.3, 120.9, 120.8, 119.7, 119.6, 119.6, 119.5, 103.2, 103.1, 89.8, 89.2, 82.9, 82.8, 82.6, 82.6, 82.3, 82.3, 82.1, 81.6, 81.5, 74.7, 74.6, 74.6, 74.5, 60.9, 59.8, 59.6, 59.2, 59.0, 58.9, 58.9, 58.8, 58.8, 58.1, 44.1, 44.1, 44.0, 44.0, 39.4, 38.0, 38.0, 37.8, 37.8, 27.1, 25.0, 24.9, 24.9, 24.9, 24.9, 24.8, 21.0, 20.9 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 149.71, 149.62, 17.36, 17.22 ppm. HRMS (ESI^+) m/z calcd for $C_{33}H_{54}N_4O_{13}P_2Na$ $[M + Na]^+$ 799.3060, found 799.3090.



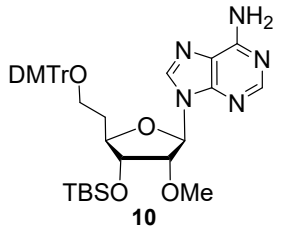
6

[[(E)-3-[(2R,3S,4R,5R)-5-[(1S)-2,4-bis(oxidanylidene)pyrimidin-1-yl]-3-hydroxy-4-methoxy-tetrahydrofuran-2-yl]prop-1-enyl]-(2,2-dimethylpropanoyloxymethoxy)phosphoryl]oxymethyl 2,2-dimethylpropanoate 6: A solution containing **4** (0.45 g, 651.43 μmol) in 10 mL of $HCOOH/H_2O$ (1:1, v/v) was stirred at 22 °C for 16 h. TLC analysis confirmed the formation of the product. The solution was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (gradient: 0-5% methanol in DCM) to afford **6** (0.31 g, 82% yield) as an off-white hygroscopic foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.39 (d, J = 2.2 Hz, 1H), 7.67 (d, J = 8.1 Hz, 1H), 6.78 – 6.59 (m, 1H), 5.85 – 5.75 (m, 2H), 5.65 – 5.55 (m, 5H), 5.28 (d, J = 6.2 Hz, 1H), 3.96 (q, J = 5.6 Hz, 1H), 3.88 (dt, J = 8.7, 5.0 Hz, 1H), 3.83 (t, J = 5.0 Hz, 1H), 3.36 (s, 3H), 2.98 – 2.89 (m, 1H), 2.86 – 2.77 (m, 1H), 1.15 (d, J = 9.7 Hz, 18H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.1, 163.1, 163.0, 151.1, 151.0, 150.4, 140.8, 118.0, 116.8, 102.1, 87.0, 82.0, 81.7, 81.3, 81.3, 79.2, 71.5, 57.6, 38.2, 38.2, 34.0, 34.0, 26.5, 26.5 ppm. ^{31}P NMR (243 MHz, DMSO-d_6) δ 16.24 ppm. HRMS (ESI^+) m/z calcd for $C_{24}H_{37}N_2O_{12}PNa$ $[M + Na]^+$ 599.1982, found 599.1976.



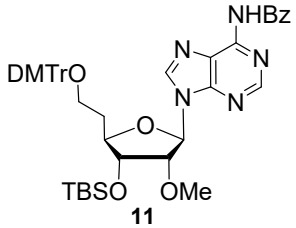
8

[[(E)-3-[(2R,3S,4R,5R)-3-[[bis(1-methylethyl)amino]-(2-cyanoethoxy)phosphanyl]oxy-5-[(1S)-2,4-bis(oxidanylidene)pyrimidin-1-yl]-4-methoxy-tetrahydrofuran-2-yl]prop-1-enyl]-(2,2-dimethylpropanoyloxymethoxy)phosphoryl]oxymethyl 2,2-dimethylpropanoate 8: To a clear solution of **6** (0.3 g, 520.36 μmol) and 5-(ethylthio)-1*H*-tetrazole (67.73 mg, 520.36 μmol) in anhydrous ACN (10 mL) was added 2-cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphorodiamidite (313.68 mg, 1.04 mmol, 330.54 μL). The reaction mixture was stirred at 22 °C for 2 h. TLC analysis confirmed formation of the product. The reaction mixture was filtered and concentrated, and the residue thus obtained was purified by flash column chromatography (gradient: 60-85% EtOAc in hexanes) to afford **8** (0.29 g, 73% yield) as a white hygroscopic foam. ^{1}H NMR (600 MHz, CD_3CN) δ 8.97 (s, 1H), 7.62 (d, J = 8.2 Hz, 1H), 6.76 – 6.56 (m, 1H), 5.84 (dd, J = 10.1, 4.5 Hz, 1H), 5.80 – 5.73 (m, 1H), 5.66 – 5.55 (m, 5H), 4.23 – 4.17 (m, 1H), 4.16 – 4.11 (m, 1H), 3.94 – 3.78 (m, 3H), 3.76 – 3.60 (m, 2H), 3.47 – 3.39 (m, 3H), 3.17 – 3.01 (m, 1H), 2.98 – 2.81 (m, 1H), 2.73 – 2.64 (m, 2H), 1.21 – 1.17 (m, 29H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 177.6, 177.6, 163.8, 163.7, 151.6, 151.4, 151.3, 151.3, 141.3, 141.3, 119.8, 119.7, 119.6, 119.5, 103.1, 103.0, 89.0, 88.4, 83.0, 83.0, 82.8, 82.5, 82.5, 82.5, 82.5, 82.4, 82.4, 82.3, 74.8, 74.7, 74.5, 74.4, 59.8, 59.7, 59.2, 59.1, 58.9, 58.9, 58.6, 58.6, 58.1, 44.1, 44.1, 44.1, 44.0, 39.4, 39.4, 35.2, 35.1, 27.1, 27.1, 25.0, 24.9, 24.9, 24.9, 24.9, 24.8, 21.0, 20.9 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 149.99, 149.71, 16.06, 15.98 ppm. HRMS (ESI^+) m/z calcd for $C_{33}H_{54}N_4O_{13}P_2Na$ $[M + Na]^+$ 799.3060, found 799.3071.



10

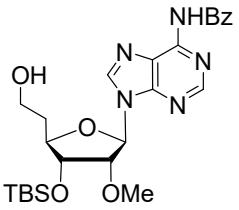
9-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-3-methoxy-5-[2-[(3-methoxyphenyl)-(4-methoxy phenyl)-phenyl-methoxy]ethyl]tetrahydrofuran-2-yl]purin-6-amine 10: To a clear solution of **9** synthesized as described previously[2] (2.0 g, 4.88 mmol) in dry pyridine (30 mL) was added 4,4′-dimethoxytrityl chloride (1.99 g, 5.86 mmol) in three portions. The reaction mixture was stirred for 16 h at 22 °C and then quenched with saturated $NaHCO_3$ solution (30 mL). After extraction with DCM (2 x 40 mL), the combined organic layers were washed with brine (40 mL) and with saturated NH_4Cl (25 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated to dryness. The crude compound was purified by flash column chromatography (gradient: 10-50% EtOAc in hexanes) to afford **10** (2.31 g, 66% yield) as a white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.36 (s, 1H), 8.14 (s, 1H), 7.36 – 7.31 (m, 4H), 7.26 (dd, J = 8.5, 7.0 Hz, 2H), 7.23 – 7.16 (m, 5H), 6.85 – 6.79 (m, 4H), 5.96 (d, J = 6.1 Hz, 1H), 4.66 (dd, J = 6.1, 4.6 Hz, 1H), 4.49 (dd, J = 4.6, 3.2 Hz, 1H), 4.07 – 4.02 (m, 1H), 3.71 (d, J = 1.7 Hz, 6H), 3.27 (s, 3H), 3.07 (ddd, J = 9.2, 6.8, 5.3 Hz, 1H), 3.01 – 2.94 (m, 1H), 2.09 – 1.99 (m, 1H), 0.91 (s, 9H), 0.09 (d, J = 1.8 Hz, 6H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.0, 156.1, 152.7, 149.3, 145.2, 140.1, 135.8, 135.8, 129.6, 129.5, 127.7, 127.6, 126.6, 119.3, 113.1, 85.5, 85.3, 82.5, 81.0, 73.3, 59.8, 59.8, 57.5, 55.0, 33.5, 25.7, 17.9, -4.7, -4.8 ppm. HRMS (ESI$^+$) m/z calcd for $C_{39}H_{50}N_5O_6Si$ $[M + H]^+$ 712.3530, found 712.3542.



11

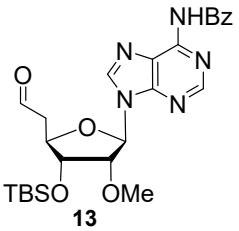
N-[9-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-3-methoxy-5-[2-[(3-methoxyphenyl)-(4-methoxy phenyl)-phenyl-methoxy]ethyl]tetrahydrofuran-2-yl]purin-6-yl]benzamide 11: To a solution of **10** (2.19 g, 3.08 mmol) in DCM (30 mL) was added pyridine (729.98 mg, 9.23 mmol, 746.40 µL) and 4-dimethylaminopyridine (37.58 mg, 307.62 µmol). The reaction mixture was cooled to 0 °C, and benzoyl chloride (1.08 g, 7.69 mmol, 892.69 µL) was added. The mixture was stirred at 22 °C for 1 h. TLC indicated that the starting material was consumed completely. The reaction mixture was quenched by addition methanol (1 mL), and then extracted with DCM (50 mL). The combined organic layers were washed with water (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. To a solution of this crude compound THF (30 mL) was added ammonium hydroxide, 28% NH_3 (215.64 mg, 6.15 mmol, 239.60 µL) at 0 °C. The mixture was stirred at 15 °C for 4 h. TLC indicated that the bis-benzoyl compound was consumed completely. All the volatile matters were removed under high vacuum. The residue was purified by flash column chromatography (0-10% methanol in DCM) to afford **11** (1.71 g, 68% yield) as a white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.26 (s, 1H), 8.75 (s, 1H), 8.72 (s, 1H), 8.08 – 8.03 (m, 2H), 7.68 – 7.62 (m, 1H), 7.56 (t, J = 7.7 Hz, 2H), 7.38 – 7.33 (m, 2H), 7.28 – 7.16 (m, 8H), 6.87 – 6.81 (m,

4H), 6.12 (d, *J* = 6.1 Hz, 1H), 4.72 (dd, *J* = 6.2, 4.5 Hz, 1H), 4.53 (dd, *J* = 4.5, 3.0 Hz, 1H), 4.11 (ddd, *J* = 8.4, 4.9, 3.0 Hz, 1H), 3.70 (s, 7H), 3.31 (s, 3H), 3.15 – 3.06 (m, 1H), 3.05 – 2.98 (m, 1H), 2.11 – 2.00 (m, 2H), 0.92 (s, 9H), 0.11 (s, 6H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.6, 158.0, 152.1, 151.8, 150.6, 145.2, 143.7, 135.8, 135.8, 133.3, 132.5, 129.6, 129.5, 128.5, 128.5, 127.8, 127.6, 126.6, 126.0, 113.1, 113.1, 85.5, 85.5, 82.8, 81.1, 73.2, 59.8, 59.8, 57.6, 55.0, 33.5, 25.7, 25.6, 17.8, -4.7, -4.8 ppm. HRMS (ESI$^+$) m/z calcd for $C_{46}H_{54}N_5O_7Si$ $[M + H]^+$ 816.3793, found 816.3786.



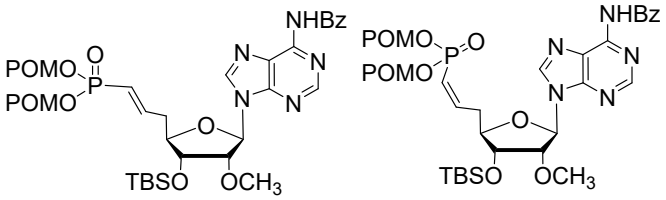
12

N-[9-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-5-(2-hydroxyethyl)-3-methoxy-tetrahydrofuran-2-yl]purin-6-yl]benzamide 12: To a solution of **11** (1.3 g, 1.59 mmol) in DCM (30 mL) were added dodecane-1-thiol (483.66 mg, 2.39 mmol, 572.38 µL) and trifluoroacetic acid (726.58 mg, 6.37 mmol, 490.93 µL). The mixture was stirred for 2 h at 22 °C. TLC showed complete consumption of starting material. The reaction mixture was washed with aq. $NaHCO_3$ (30 mL x 2) to bring the pH to 7 and diluted with DCM (30 mL). The organic layers were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under high vacuum. The residue was purified by flash column chromatography (gradient: 0-5% methanol in DCM) to afford **12** (0.72 g, 1.40 mmol, 88% yield) as a white hygroscopic foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.42 (s, 1H), 7.95 (s, 1H), 7.92 (s, 1H), 7.25 – 7.21 (m, 2H), 6.86 – 6.80 (m, 1H), 6.74 (t, *J* = 7.8 Hz, 2H), 5.29 (d, *J* = 5.7 Hz, 1H), 3.81 (dd, *J* = 5.8, 4.6 Hz, 1H), 3.74 (dd, *J* = 4.6, 3.6 Hz, 1H), 3.26 (ddd, *J* = 8.6, 5.1, 3.6 Hz, 1H), 2.74 – 2.67 (m, 1H), 2.64 (ddd, *J* = 10.6, 7.9, 5.8 Hz, 1H), 2.50 (s, 3H), 1.12 – 0.97 (m, 2H), 0.11 (s, 9H), -0.67 (d, *J* = 6.1 Hz, 6H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.7, 152.1, 151.8, 150.5, 143.7, 133.3, 132.5, 128.5, 128.5, 126.0, 85.5, 82.3, 81.4, 73.3, 57.6, 57.4, 54.9, 36.2, 25.7, 17.9, -4.7, -4.8 ppm. HRMS (ESI$^+$) m/z calcd for $C_{25}H_{36}N_5O_5Si$ $[M + H]^+$ 514.2486, found 514.2477.



13

N-[9-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-3-methoxy-5-(2-oxoethyl)tetrahydrofuran-2-yl]purin-6-yl]benzamide 13: To a clear solution of **12** (0.5 g, 973.41 µmol) in DCM (20 mL) at 0 °C was added Dess-Martin periodinane (516.08 mg, 1.22 mmol). The reaction mixture was stirred at 0 °C for 3 h and then quenched with saturated $NaHCO_3$ (15 mL) and 10% sodium thiosulfate solution (15 mL). This mixture was diluted with DCM (20 mL), and then the organic layer was separated, dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated to dryness to afford **13** (0.49 g, 98% yield) as a white foam that was used for the next step without further purification.

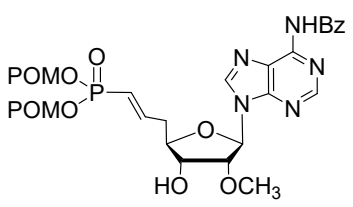


14 **15**

[[(E)-3-[(2R,5R)-5-(6-benzamidopurin-9-yl)-3-[tert-butyl(dimethyl)silyl]oxy-4-methoxy-tetra hydrofuran-2-yl]prop-1-enyl]-(2,2-dimethylpropanoyloxymethoxy)phosphoryl]oxymethyl-2,2-dimethylpropanoate 14 and [[(Z)-3-[(2R,5R)-5-(6-benzamidopurin-9-yl)-3-[tert-butyl(dimethyl)silyl]oxy-4-methoxy-tetrahydrofuran-2-yl]prop-1-enyl]-(2,2-dimethylpropan oyloxymethoxy)phosphoryl]oxymethyl 2,2-dimethylpropanoate 15: To a clear solution of *bis*-POM VP reagent (1.51 g, 2.39 mmol) in anhydrous THF (15 mL) at 0 °C was added sodium hydride (115 mg, 2.87 mmol, 60% purity). After stirring for 5 min at 0 °C, **13** (0.49 g, 957.70 µmol) dissolved in anhydrous THF (10 mL) was added. The reaction mixture was then stirred for 1.5 h at 22 °C. TLC confirmed consumption of starting material. The reaction was quenched with 10% NH_4Cl solution (30 mL). EtOAc (30 mL) was added to this mixture, and organic layer was separated after stirring. The aqueous layer was washed with EtOAc (2 x 20mL). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated to dryness. The crude compound thus obtained was purified by flash column chromatography (gradient: 40-80% EtOAc in hexanes) to afford the *E*-isomer **14** (210 mg, 27% yield) and the *Z*-isomer **15** (60 mg, 8% yield), both as white foams.

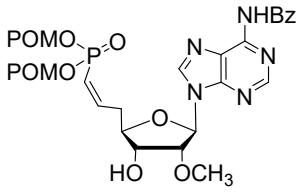
Data for **14**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.80 (s, 1H), 8.08 (s, 1H), 8.05 – 8.00 (m, 2H), 7.65 – 7.59 (m, 1H), 7.54 (t, *J* = 7.8 Hz, 3H), 6.91 – 6.79 (m, 1H), 6.00 (d, *J* = 3.8 Hz, 1H), 5.90 – 5.80 (m, 1H), 5.69 – 5.60 (m, 4H), 4.56 – 4.50 (m, 1H), 4.42 (t, *J* = 5.3 Hz, 1H), 4.16 (ddd, *J* = 8.5, 5.7, 4.2 Hz, 1H), 3.46 (d, *J* = 8.6 Hz, 3H), 2.77 – 2.56 (m, 2H), 1.19 (d, *J* = 1.2 Hz, 18H), 0.95 (d, *J* = 6.4 Hz, 9H), 0.15 (d, *J* = 3.3 Hz, 6H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.9, 176.9, 164.7, 152.8, 151.4, 149.9, 149.8, 149.7, 149.6, 142.6, 133.7, 133.0, 133.0, 129.0, 128.0, 124.2, 120.1, 118.8, 88.3, 82.5, 82.1, 81.6, 81.5, 73.9, 58.7, 38.8, 37.7, 37.5, 26.9, 25.8, 18.3, -4.4, -4.6 ppm. ^{31}P NMR (243 MHz, $CDCl_3$) δ 17.37 ppm. HRMS (ESI^+) m/z calcd for $C_{38}H_{57}N_5O_{11}PSi$ $[M + H]^+$ 818.3561, found 818.3570.

Data for **15**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.00 (s, 1H), 8.82 (s, 1H), 8.37 (s, 1H), 8.05 – 8.01 (m, 2H), 7.65 – 7.60 (m, 1H), 7.54 (t, *J* = 7.7 Hz, 2H), 6.77 – 6.49 (m, 1H), 6.11 (d, *J* = 4.6 Hz, 1H), 5.78 – 5.63 (m, 6H), 4.47 (t, *J* = 4.7 Hz, 1H), 4.32 (t, *J* = 4.6 Hz, 1H), 4.19 (dt, *J* = 9.0, 4.5 Hz, 1H), 3.47 (d, *J* = 0.8 Hz, 3H), 3.17 (dt, *J* = 15.9, 8.6 Hz, 1H), 3.10 – 2.99 (m, 1H), 1.23 – 1.16 (m, 19H), 0.94 (d, *J* = 0.8 Hz, 9H), 0.14 (d, *J* = 3.3 Hz, 6H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.9, 176.9, 164.6, 152.8, 151.6, 150.2, 150.1, 149.7, 142.6, 133.8, 133.0, 129.1, 128.0, 123.9, 119.0, 117.8, 87.6, 83.7, 82.5, 81.5, 81.5, 81.5, 81.4, 73.7, 58.6, 38.9, 38.9, 34.6, 34.5, 27.0, 27.0, 27.0, 26.9, 25.9, 18.3, -4.5, -4.6 ppm. ^{31}P NMR (243 MHz, $CDCl_3$) δ 16.11 ppm. HRMS (ESI^+) m/z calcd for $C_{38}H_{57}N_5O_{11}PSi$ $[M + H]^+$ 818.3561, found 818.3566.



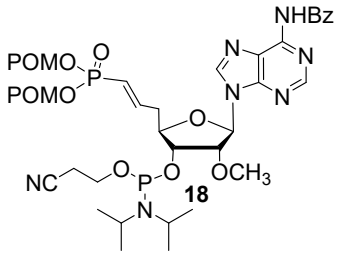
16

[[(E)-3-[(2R,5R)-5-(6-benzamidopurin-9-yl)-3-hydroxy-4-methoxy-tetrahydrofuran-2-yl]prop-1-enyl]-(2,2-dimethylpropanoyloxymethoxy)phosphoryl]oxymethyl 2,2-dimethylpropanoate 16: To a clear solution of **14** (0.3 g, 366.78 μmol) in formic acid (2 mL) was added water (2 mL), and the mixture was stirred at 22 °C for 12 h. All the volatile matters were then removed under high vacuum, and the residue was purified by flash column chromatography (gradient: 0-5% methanol in DCM) to afford **16** (0.16 g, 62% yield) as a hygroscopic foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.24 (s, 1H), 8.77 (d, J = 1.3 Hz, 1H), 8.70 (d, J = 1.2 Hz, 1H), 8.07 – 8.02 (m, 2H), 7.65 (ddt, J = 8.7, 7.2, 1.2 Hz, 1H), 7.59 – 7.53 (m, 2H), 6.86 – 6.59 (m, 1H), 6.13 (dd, J = 5.2, 1.2 Hz, 1H), 6.04 – 5.92 (m, 1H), 5.60 – 5.52 (m, 4H), 5.45 (d, J = 5.8 Hz, 1H), 4.52 (td, J = 5.1, 1.2 Hz, 1H), 4.35 (q, J = 5.0 Hz, 1H), 4.05 (dt, J = 9.2, 4.6 Hz, 1H), 3.37 (d, J = 1.1 Hz, 3H), 2.70 (qd, J = 15.6, 8.8 Hz, 2H), 1.09 (dd, J = 5.0, 1.2 Hz, 16H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.0, 165.6, 163.1, 152.0, 151.8, 150.6, 150.5, 150.5, 143.5, 133.3, 132.5, 128.5, 128.5, 126.0, 119.2, 118.0, 86.0, 82.7, 81.7, 81.4, 81.3, 71.6, 57.7, 38.1, 37.4, 37.3, 26.4 ppm. ^{31}P NMR (243 MHz, DMSO-d_6) δ 17.81 ppm. HRMS (ESI$^+$) m/z calcd for $C_{32}H_{43}N_5O_{11}P$ [M + H]$^+$ 704.2697, found 704.2677.



17

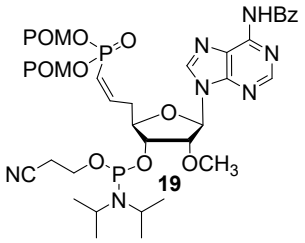
[[(Z)-3-[(2R,5R)-5-(6-benzamidopurin-9-yl)-3-hydroxy-4-methoxy-tetrahydrofuran-2-yl]prop-1-enyl]-(2,2-dimethylpropanoyloxymethoxy)phosphoryl]oxymethyl 2,2-dimethylpropanoate 17: To a clear solution of **15** (0.2 g, 244.52 μmol) in formic acid (1.5 mL) was added water (1.5 mL), and the mixture was stirred at 22 °C for 12 h. Volatile matters were removed under high vacuum, and residue was purified by flash column chromatography (gradient: 0-5% methanol in DCM) to afford **17** (0.12 g, 70% yield) as a hygroscopic foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.36 (s, 1H), 8.09 (s, 1H), 8.07 – 8.04 (m, 2H), 7.66 – 7.58 (m, 1H), 7.57 – 7.51 (m, 2H), 6.76 – 6.49 (m, 1H), 6.16 (d, J = 2.9 Hz, 1H), 5.86 – 5.77 (m, 1H), 5.74 – 5.64 (m, 4H), 4.39 – 4.31 (m, 2H), 4.19 (td, J = 6.7, 5.0 Hz, 1H), 3.60 (s, 3H), 3.17 (ddddd, J = 15.5, 8.4, 6.9, 2.8, 1.3 Hz, 1H), 3.05 (ddtd, J = 13.0, 6.9, 3.2, 1.6 Hz, 1H), 1.22 (s, 9H), 1.20 (s, 9H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.1, 177.0, 165.2, 163.8, 152.5, 151.7, 149.9, 149.8, 149.7, 142.0, 133.4, 133.1, 128.9, 128.3, 123.7, 119.7, 118.4, 87.4, 83.2, 82.5, 82.5, 81.6, 81.6, 81.6, 81.6, 72.2, 59.2, 38.9, 38.9, 33.6, 33.6, 27.0, 26.9 ppm. ^{31}P NMR (243 MHz, $CDCl_3$) δ 16.12 ppm. HRMS (ESI$^+$) m/z calcd for $C_{32}H_{43}N_5O_{11}P$ [M + H]$^+$ 704.2697, found 704.2701.



18

[[(E)-3-[(2R,5R)-5-(6-benzamidopurin-9-yl)-3-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-4-methoxy-tetrahydrofuran-2-yl]prop-1-enyl]-(2,2-dimethylpropanoyloxymethoxy)phosphoryl]oxymethyl-2,2-dimethylpropanoate 18: To a clear solution of **16** (0.2 g, 284.22 μmol) and 5-

(ethylthio)-1*H*-tetrazole (37.00 mg, 284.22 μmol) in anhydrous ACN (10 mL) was added 2-cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphorodiamidite (171.33 mg, 568.44 μmol). The reaction mixture was stirred at 22 °C for 1 h. TLC analysis confirmed formation of the product. The reaction mixture was filtered and concentrated, and the residue thus obtained was purified by flash column chromatography (gradient: 50-95% EtOAc in hexanes) to afford **18** (0.19 g, 74% yield) as a white foam. ^{1}H NMR (600 MHz, CD_3CN) δ 9.40 (s, 1H), 8.68 (s, 1H), 8.26 (d, *J* = 2.9 Hz, 1H), 8.00 (d, *J* = 7.7 Hz, 2H), 7.67 – 7.61 (m, 1H), 7.54 (t, *J* = 7.7 Hz, 2H), 6.86 – 6.71 (m, 1H), 6.08 (dd, *J* = 15.3, 4.4 Hz, 1H), 5.94 – 5.82 (m, 1H), 5.58 – 5.50 (m, 4H), 4.70 – 4.59 (m, 2H), 4.34 – 4.21 (m, 1H), 3.94 – 3.75 (m, 2H), 3.69 (dh, *J* = 10.2, 6.7 Hz, 2H), 3.51 – 3.34 (m, 3H), 2.88 – 2.68 (m, 4H), 1.25 – 1.20 (m, 14H), 1.13 (td, *J* = 3.1, 0.7 Hz, 18H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 177.5, 152.8, 151.3, 151.3, 151.1, 151.0, 151.0, 143.8, 143.8, 134.9, 133.6, 129.6, 129.1, 126.0, 126.0, 120.8, 120.7, 119.7, 119.6, 119.6, 119.4, 88.6, 88.3, 83.2, 82.7, 82.7, 82.6, 82.5, 82.5, 82.5, 82.5, 82.3, 82.2, 74.8, 74.7, 74.5, 74.4, 59.9, 59.8, 59.3, 59.1, 59.0, 59.0, 58.8, 58.8, 44.1, 44.1, 44.1, 44.0, 39.3, 38.2, 38.1, 27.0, 25.0, 25.0, 25.0, 24.9, 24.9, 24.9, 21.1, 21.0, 21.0, 21.0 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 149.97, 149.33, 17.43, 17.27 ppm. HRMS (ESI$^+$) m/z calcd for $C_{41}H_{60}N_7O_{12}P_2$ $[M + H]^+$ 904.3775, found 904.3786.



[[(Z)-3-[(2R,5R)-5-(6-benzamidopurin-9-yl)-3-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-4-methoxy-tetrahydrofuran-2-yl]prop-1-enyl]-(2,2-dimethylpropanoyloxymethoxy)phosphoryl]oxymethyl 2,2-dimethylpropanoate 19: To a clear solution of **17** (0.1 g, 142.11 μmol) and 5-(ethylthio)-1*H*-tetrazole (18.50 mg, 142.11 μmol) in anhydrous ACN was added 2-cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphorodiamidite (85.67 mg, 284.22 μmol). The reaction mixture was stirred at 22 °C for 1 h. TLC analysis confirmed formation of the product. The reaction mixture was filtered and concentrated, and the residue thus obtained was purified by flash column chromatography (gradient: 50-95% EtOAc in hexanes) to afford **19** (950 mg, 74% yield) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.99 (s, 1H), 8.82 (s, 1H), 8.37 (d, *J* = 14.2 Hz, 1H), 8.03 (dt, *J* = 7.1, 1.3 Hz, 2H), 7.65 – 7.59 (m, 1H), 7.54 (t, *J* = 7.5 Hz, 2H), 6.80 – 6.51 (m, 1H), 6.16 – 6.09 (m, 1H), 5.78 – 5.72 (m, 1H), 5.71 – 5.63 (m, 4H), 4.75 – 4.58 (m, 1H), 4.53 – 4.37 (m, 1H), 4.32 (ddt, *J* = 8.7, 6.1, 4.9 Hz, 1H), 4.00 – 3.87 (m, 2H), 3.80 – 3.63 (m, 3H), 3.53 – 3.41 (m, 3H), 3.25 – 3.01 (m, 2H), 2.83 – 2.64 (m, 2H), 1.27 – 1.16 (m, 32H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.9, 176.9, 164.6, 152.9, 152.9, 151.8, 151.7, 150.3, 149.6, 142.5, 142.5, 133.8, 132.9, 129.0 127.95, 123.8, 123.8, 119.3, 118.9, 118.1, 118.0, 117.8, 117.7, 87.4, 87.3, 83.1, 82.8, 82.0, 82.0, 81.6, 81.5, 81.5, 81.5, 81.4, 74.2, 74.1, 73.3, 73.2, 59.0, 58.9, 58.8, 58.5, 58.2, 58.0, 43.6, 43.5, 43.4, 38.9, 38.8, 34.7, 34.7, 34.5, 27.0, 26.9, 24.8, 24.8, 24.7, 20.5 ppm. ^{31}P NMR (243 MHz, $CDCl_3$) δ 150.94, 150.03, 16.08, 15.93 ppm. HRMS (ESI$^+$) m/z calcd for $C_{41}H_{60}N_7O_{12}P_2$ $[M + H]^+$ 904.3775, found 904.3765.

Syntheses of oligonucleotides

Syntheses of oligonucleotides for preparation of siRNAs targeting *Ttr* and *Sod1* mRNAs were performed on a ABI DNA/RNA synthesizer at scales between 1−10 µmol using commercially available 5′-O-(4,4′-dimethoxytrityl)-2′-fluoro- and 5′-O-(4,4′-dimethoxytrityl)-2′-O-methyl-3′-O-(2-cyanoethyl-*N,N*-diisopropyl)phosphoramidite monomers of uridine, 4-*N*-acetylcytidine, 6-*N*-benzoyladenosine, and 2-N-isobutyrylguanosine using standard solid-phase oligonucleotide synthesis protocols[1, 3]. The GalNAc ligand was introduced at the 3′ end of the sense strand of the siRNA using a functionalized solid support as described[4]. The (n − 1)-mer of the desired antisense strand was first synthesized on a solid-support under standard solid-phase synthesis conditions and the 5′-DMTr was removed. The VP-modified phosphoramidite **7** or **8** was then introduced at the 5′ terminus of the solid-support bound oligonucleotide using 0.25 M 5-(ethylthio)-1*H*-tetrazole in acetonitrile as phosphoramidite activator to obtain the desired 5′-phosphonate-modified, full-length antisense strand. The phosphite triester was converted into a phosphorothioate linkage with 0.1 M DDTT. No DMT removal step was required as VP-containing phosphoramidites lack the DMT group. After completion of synthesis, the 5′-phosphonate-modified oligonucleotides were deprotected in 30% ammonia solution containing 5% DEA (v/v) for 5 h at 60 °C. The crude oligonucleotides were purified by IEX-HPLC using a column packed with TSK-Gel Super Q-5PW support and a linear gradient of 15−45 % buffer B over 120 min with 5 mL/min flow rate (buffer A, 0.02 M Na_2HPO_4 in 15% acetonitrile, pH 8.5; buffer B, buffer A plus 1 M NaBr). All single strands were purified to >90% based on HPLC (260 nm)

For preparation of siRNAs targeting *ApoB*, sense strands were synthesized on solid support functionalized with GalNAc (Reference 4), and antisense strands were assembled on support functionalized with 2′-*O*-methyl-uridine (porosity 616 Å, loading 87 µmol/g; LGC Biosearch Technologies). The single strands were synthesized on K&A H-8-SE synthesizer. A solution of 0.5 M 5-(*S*-ethylthio)-1*H*-tetrazole in acetonitrile was used as the activator. The solutions of commercially available phosphoramidites and synthesized phosphoramidites were used at 0.1 M in anhydrous acetonitrile or DCM. The oxidizing reagent was 0.05 M I_2 in THF/pyridine/H_2O. A solution of 100 mM 3-amino-1,2,4-dithiazole-5-thione (TCI Chemicals) dissolved in acetonitrile-pyridine (2:3 *v/v*) was employed as sulfurizing agent. The detritylation reagent was 3% trichloroacetic acid in DCM. Waiting times for coupling, capping, oxidation, and sulfurization step were 450 s, 25 s, 80 s, and 300 s, respectively. After completion of the automated synthesis, the oligonucleotide was manually released from the solid support and deprotected using a solution of concentrated aqueous ammonia and 40% aqueous methylamine (1:1, v/v, both from Sigma Aldrich). Cleavage from solid support and quantitative deprotection was achieved using a solution of ammonia and ethanol (3:1, v/v). After filtration through a 0.45-µm nylon filter, oligonucleotides were purified by ion exchange HPLC using a Dionex DNA Pac100 column (9 x 250 mm; ThermoFisher). Appropriate gradients of mobile phase (buffer A, 20 mM Tris, pH 7.4, 20% acetonitrile; buffer B, 500 mM $NaClO_4$ in buffer A) were employed.

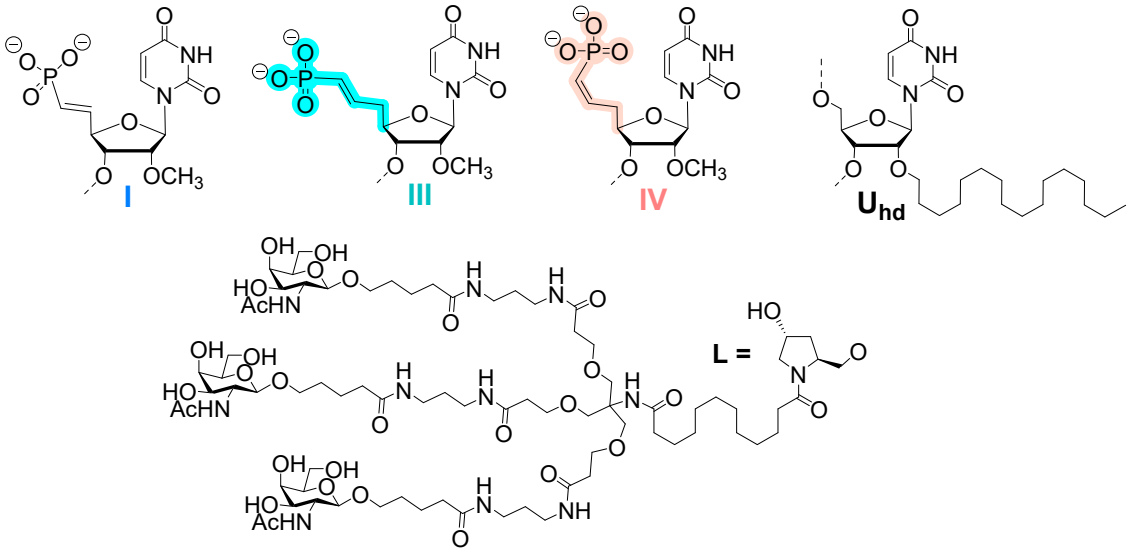
Oligonucleotides were desalted by size exclusion chromatography on an AKTA desaltification system using a HiPrep 26/10 packed with Sephadex G25 resin (GE Healthcare) with elution with sterile nuclease-free water at 10 mL/min. The isolated yields for the final oligonucleotides were calculated based on the ratios of measured to theoretical 260 nm optical density units. Extinction coefficients were calculated using the following extinction coefficients for each residue: A, 13.86;

T/U, 7.92; C, 6.57; and G, 10.53 $M^{-1}cm^{-1}$. The identities of modified oligonucleotides were verified by mass spectrometry (Table S1). Purities were evaluated by analytical reverse-phase HPLC. For reverse-phase HPLC, a C-18 column was used with a gradient of 2-29% buffer B (buffer A, 95 mM hexafluoroisopropanol, 16.3 mM TEA, 0.05 mM EDTA; buffer B: methanol) over 39 min. Equimolar amounts of complementary sense and antisense strands were annealed by heating in a water bath at 95 °C for 5 min and cooling to room temperature to obtain the desired siRNAs. The siRNA samples were analyzed for purity, endotoxin, and osmolality, and the observed values were within the allowed range for the concentration tested.

Table S1. Sequences and chemical modifications of sense and antisense strands of siRNAs targeting *Ttr, Sod1*, and *ApoB* mRNAs [a]

Entry	Sequence 5′-3′[a]	Antisense (AS) or sense (S) strand	Target	Mass (M-H)⁻	
				Cald.	Obsd.
ON1	u●*U*●auaGagcaagaAcAcuguu●u●u	AS	*Mouse Ttr*	7655.11	7656.32
ON2	**I**●*U*●auaGagcaagaAcAcuguu●u●u	AS		7731.11	7731.81
ON3	**III**●*U*●auaGagcaagaAcAcuguu●u●u	AS		7745.14	7745.80
ON4	**IV**●*U*●auaGagcaagaAcAcuguu●u●u	AS		7745.14	7745.78
ON5	a●a●caguGuUCUugcucuauaa**L**	S		8685.45	8685.62
ON6	u●*U*●uagAgUGaggaUuAaaaug●a●g	AS	*Rat Sod1*	7774.16	7772.64
ON7	**I**●*U*●uagAgUGaggaUuAaaaug●a●g	AS		7850.16	7849.69
ON8	**III**●*U*●uagAgUGaggaUuAaaaug●a●g	AS		7864.18	7865.32
ON9	**IV**●*U*●uagAgUGaggaUuAaaaug●a●g	AS		7864.18	7864.05
ON10	c●a●uuuU_{hd}AaUCCucacucua●a●a	S		7042.97	7043.34
ON11	u●*U*●cUuGuUcUgaaUgUcCaGg●g●u	AS	*Mouse ApoB*	7557.80	7557.50
ON12	**I**●*U*●cUuGuUcUgaaUgUcCaGg●g●u	AS		7633.80	7633.50
ON13	**III**●*U*●cUuGuUcUgaaUgUcCaGg●g●u	AS		7648.90	7647.10
ON14	**III***U*●cUuGuUcUgaaUgUcCaGg●g●u	AS		7632.80	7631.00
ON15	**IV**●*U*●cUuGuUcUgaaUgUcCaGg●g●u	AS		7648.90	7647.10
ON16	**IV***U*●cUuGuUcUgaaUgUcCaGg●g●u	AS		7632.80	7631.00
ON17	*C*●c●UgGaCaUUCaGaAcAaGaA**L**	S		8697.30	8696.10

[a] Chemical modifications: ●, PS linkage; lower case, 2′-OMe; italicized upper case, 2′-fluoro; **L**, trivalent-GalNAc; 2′-*O*-hexadecyl uridine; **I**, 5′-(E)-VP; **III**, 6′-(E)-VP; and **IV**, 6′-(Z)-VP.



In vitro assay

The *in vitro* gene silencing activities of siRNAs targeting *Ttr* and *ApoB* were evaluated in primary mouse hepatocytes. Cells were plated in 96-well format (20,000 cells/well) in hepatocyte plating medium (Primacyt). *Ttr*-targeting siRNAs were tested at 0.001, 0.01, and 0.1 nM, and *ApoB*-targeting siRNAs were tested at 200 and 20 nM. Each concentration was tested in quadruplicate. Cells were incubated 48 h at 37 ^{o}C and subsequently lysed. RNA was isolated using a Dynabeads mRNA Isolation Kit (Invitrogen). mRNA was quantified using the Quantigene Singleplex assay system (ThermoFisher), according to the manufacturer's protocol. cDNA synthesis was accomplished with the High-capacity cDNA Reverse Transcription Kit (Applied Biosystems). Probes for murine *Ttr*, *ApoB*, and *Gapdh* were obtained from ThermoFisher. Real-time PCR was done in an ABI 7900HT RT-PCR system (Applied Biosystems) using the ΔΔCt (RQ) assay. For each well, the target mRNA level was normalized to *Gapdh* mRNA level. The activity of a given siRNA is expressed as percent of target mRNA concentration in treated cells relative to the target mRNA concentration averaged across control wells treated with a non-targeting siRNA and PBS.

Table S2. Sequences and chemistries of siRNAs targeting *ApoB* mRNA[a]

Duplex	Sense strand (upper) and antisense strand (lower)[a] (5′-3′)
si-9 (Control)	*C*●c●*U*g*G*a*C*a*UU*C*a*G*a*A*c*A*a*G*a*A***L** u●*U*●c*U*u*G*u*U*c*U*gaa*U*g*U*c*C*a*G*g●g●u
si-10	*C*●c●*U*g*G*a*C*a*UU*C*a*G*a*A*c*A*a*G*a*A***L** **I**●*U*●c*U*u*G*u*U*c*U*gaa*U*g*U*c*C*a*G*g●g●u
si-11	*C*●c●*U*g*G*a*C*a*UU*C*a*G*a*A*c*A*a*G*a*A***L** **III**●*U*●c*U*u*G*u*U*c*U*gaa*U*g*U*c*C*a*G*g●g●u
si-12	*C*●c●*U*g*G*a*C*a*UU*C*a*G*a*A*c*A*a*G*a*A***L** **IV**●*U*●c*U*u*G*u*U*c*U*gaa*U*g*U*c*C*a*G*g●g●u
si-13	*C*●c●*U*g*G*a*C*a*UU*C*a*G*a*A*c*A*a*G*a*A***L** **III***U*●c*U*u*G*u*U*c*U*gaa*U*g*U*c*C*a*G*g●g●u
si-14	*C*●c●*U*g*G*a*C*a*UU*C*a*G*a*A*c*A*a*G*a*A***L** **IV***U*●c*U*u*G*u*U*c*U*gaa*U*g*U*c*C*a*G*g●g●u

[a]Chemical modifications: ●, PS linkage; lower case, 2′-OMe; italicized upper case, 2′-fluoro; **L**, trivalent-GalNAc; **I**, 5′-(E)-VP; **III**, 6′-(E)-VP; and **IV**, 6′-(Z)-VP.

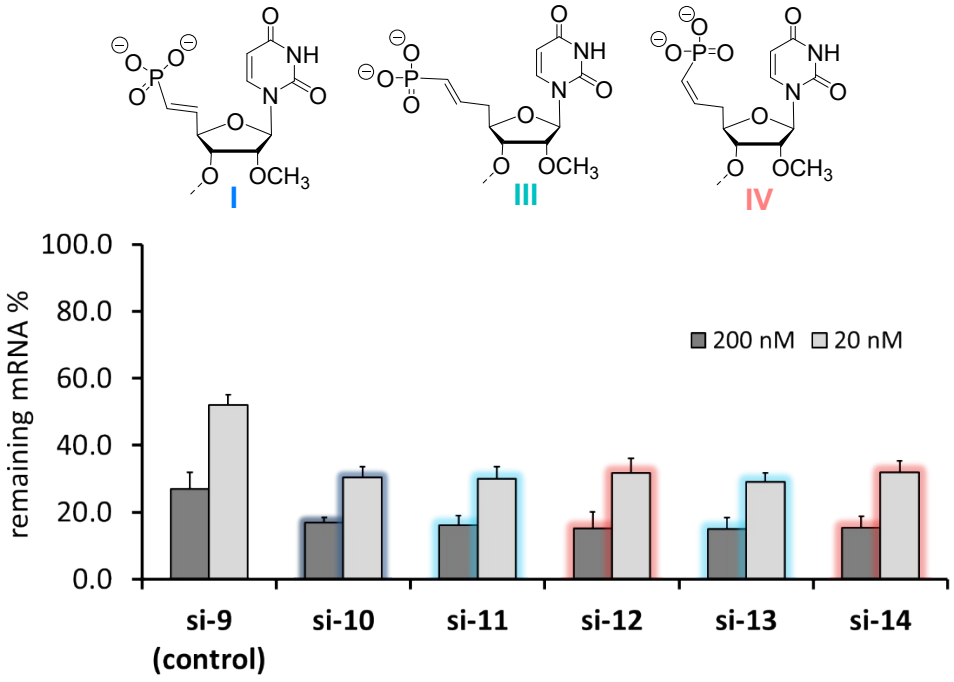


Figure S1: Percent *ApoB* mRNA remaining after treatment of primary mouse hepatocytes with 20 or 200 nM indicated siRNAs for 48 hours. The antisense strands of **si-10, si-11**, and **si -12** have two phosphorothioate linkages at the 5′ ends, whereas **si-13** and **si-14** have one. Levels of *ApoB*

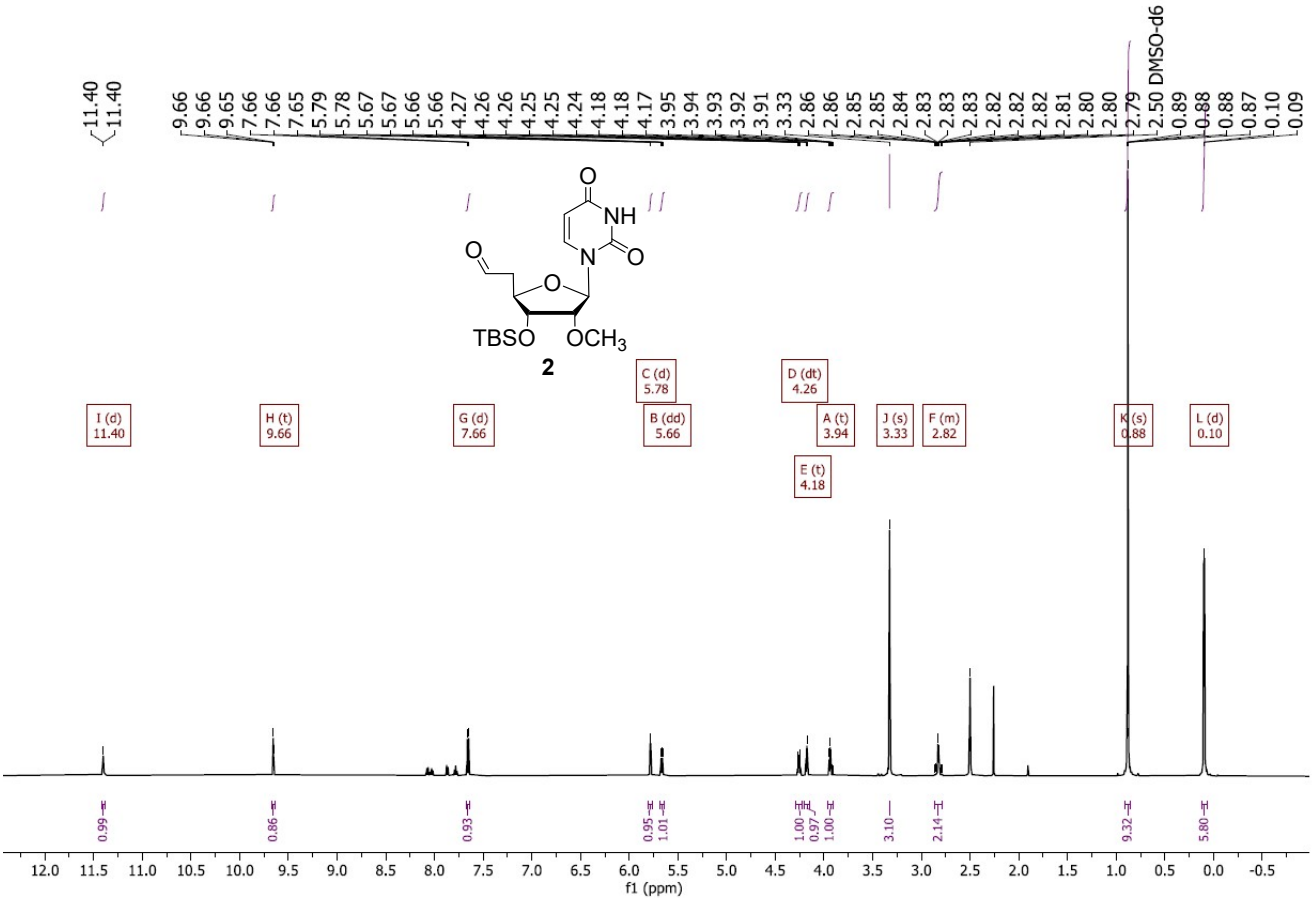
mRNA were quantified using RT-qPCR and were normalized to *ApoB* mRNA in cells treated with a non-targeting siRNA. Plotted are averages ± standard deviation (n=4).

In vivo assays

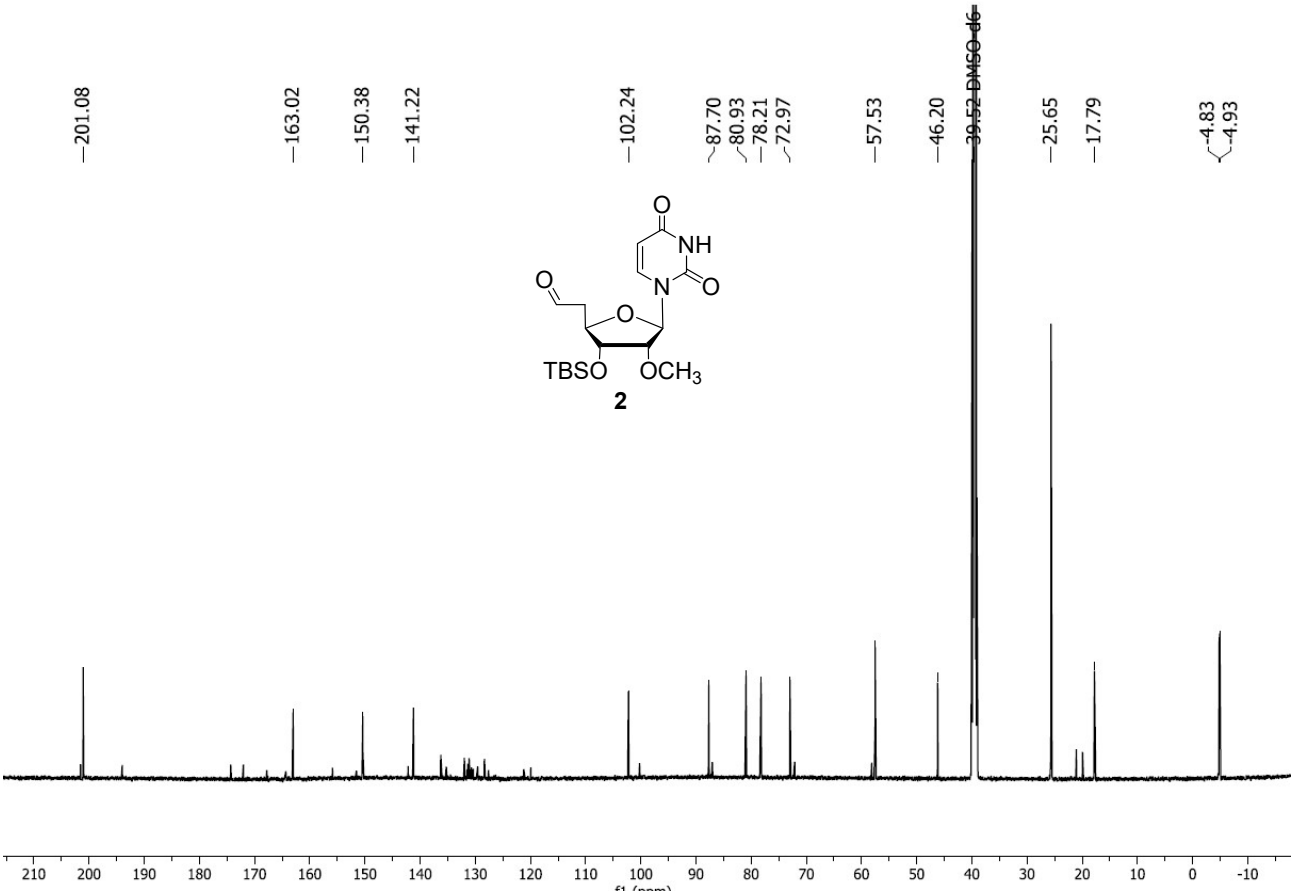
All studies were conducted using protocols consistent with local, state, and federal regulations, as applicable, and approved by the Institutional Animal Care and Use Committee at Alnylam Pharmaceuticals. For analyses in mice of *Ttr*-targeted siRNAs, mice were given a single subscapular subcutaneous injection of 0.3 mg/kg siRNA, prepared in an injection volume of 10 µL/g body weight in PBS. At the indicated time pre- or post-dosing, animals were anesthetized with isofluorane, and blood was obtained via retroorbital bleed. TTR protein was quantified by ELISA from serum isolated from whole blood. The ELISA was performed according to the manufacturer's protocol (ALPCO, 41-PALMS-E01) after a 3025-fold dilution of the serum samples. Data were normalized to prebleed TTR levels for each individual. All samples were assayed in duplicate, and each data point is the average of all the mice within each cohort (n = 3). Data were analyzed using a two-way ANOVA with a Tukey post-hoc test for multiple comparison in GraphPad Prism.

Female SD rats (Charles River Laboratories) of 11-12 weeks were used for analyses of *Sod1*-targeted siRNAs. Rats were given one intrathecal administration of 0.6 mg in 30 µL of artificial cerebrospinal fluid. There were 2-3 rats for each group. Rats were euthanized on day 14, and tissue samples were harvested and flash frozen. RNA isolation was performed on tissue lysate using the Chemagic 360 system (Perkin Elmer) and cDNA synthesis was performed using the SuperScript IV VILO cDNA kit (Invitrogen, catalog no. 11754050) in accordance with the manufacturer's protocol. *Sod1* mRNA levels were determined using qPCR on a LightCycler 480 system (Roche), and data were normalized to *PPIB* mRNA levels and are reported as a percentage of *Sod1* mRNA levels in the artificial cerebrospinal fluid control-treated rats.

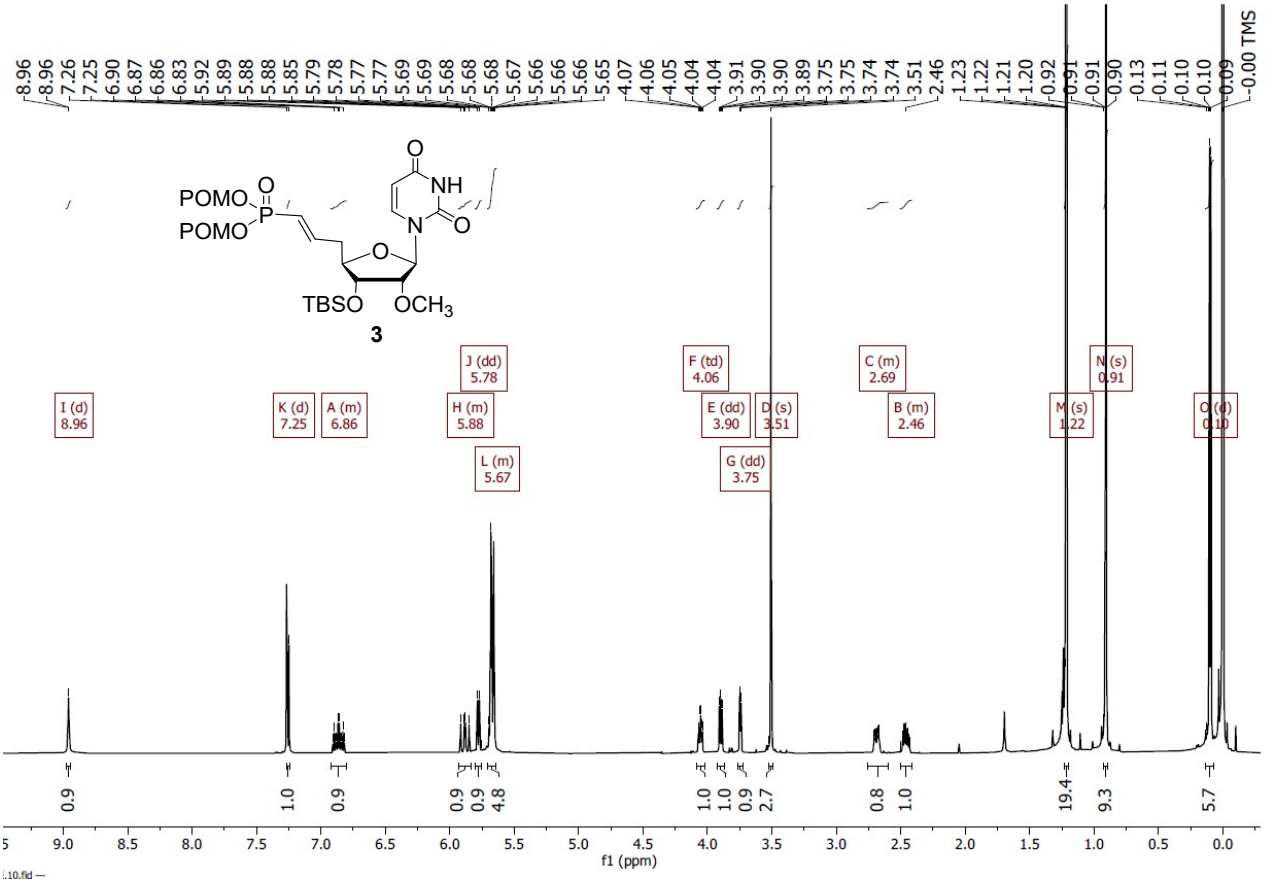
^{1}H, ^{13}C, and ^{31}P NMR data for new compounds



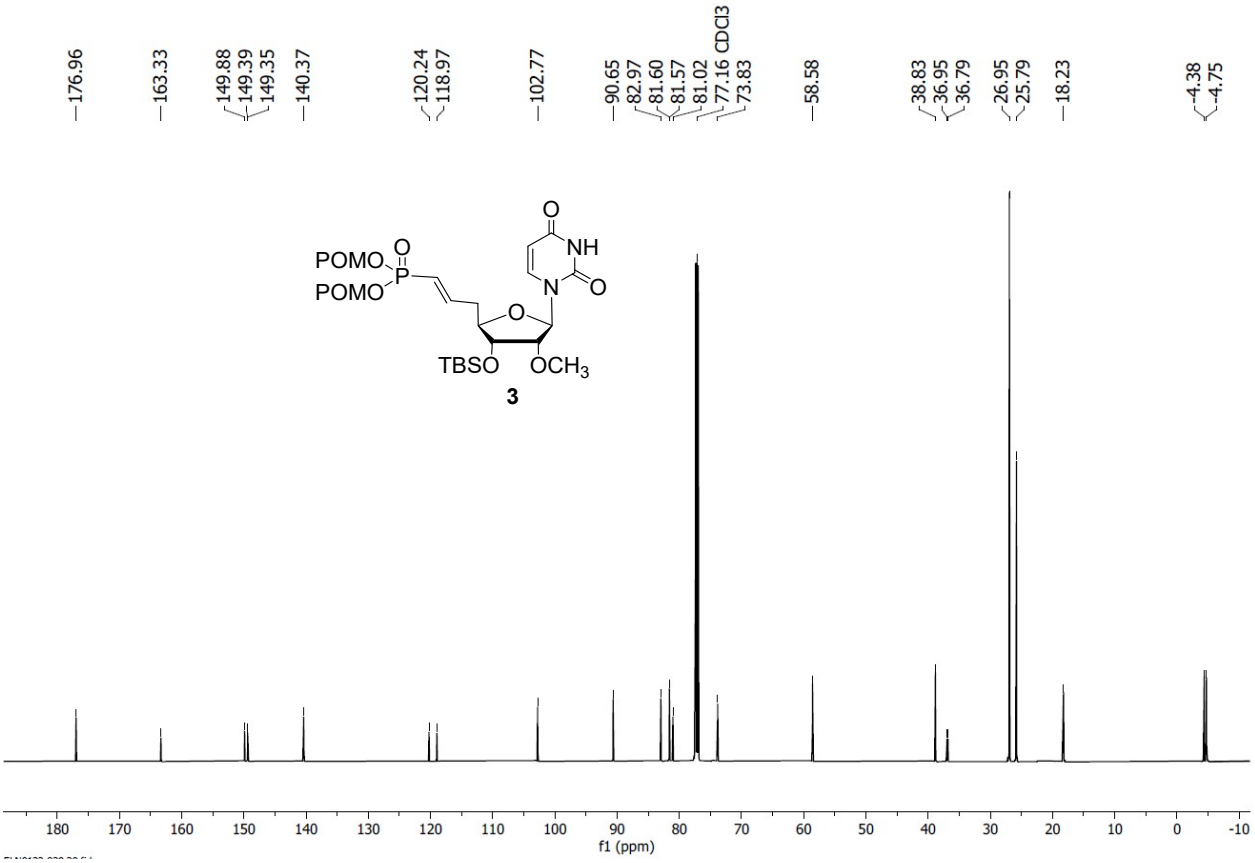
^{1}H NMR (600 MHz, DMSO-d_6) of compoud **2**



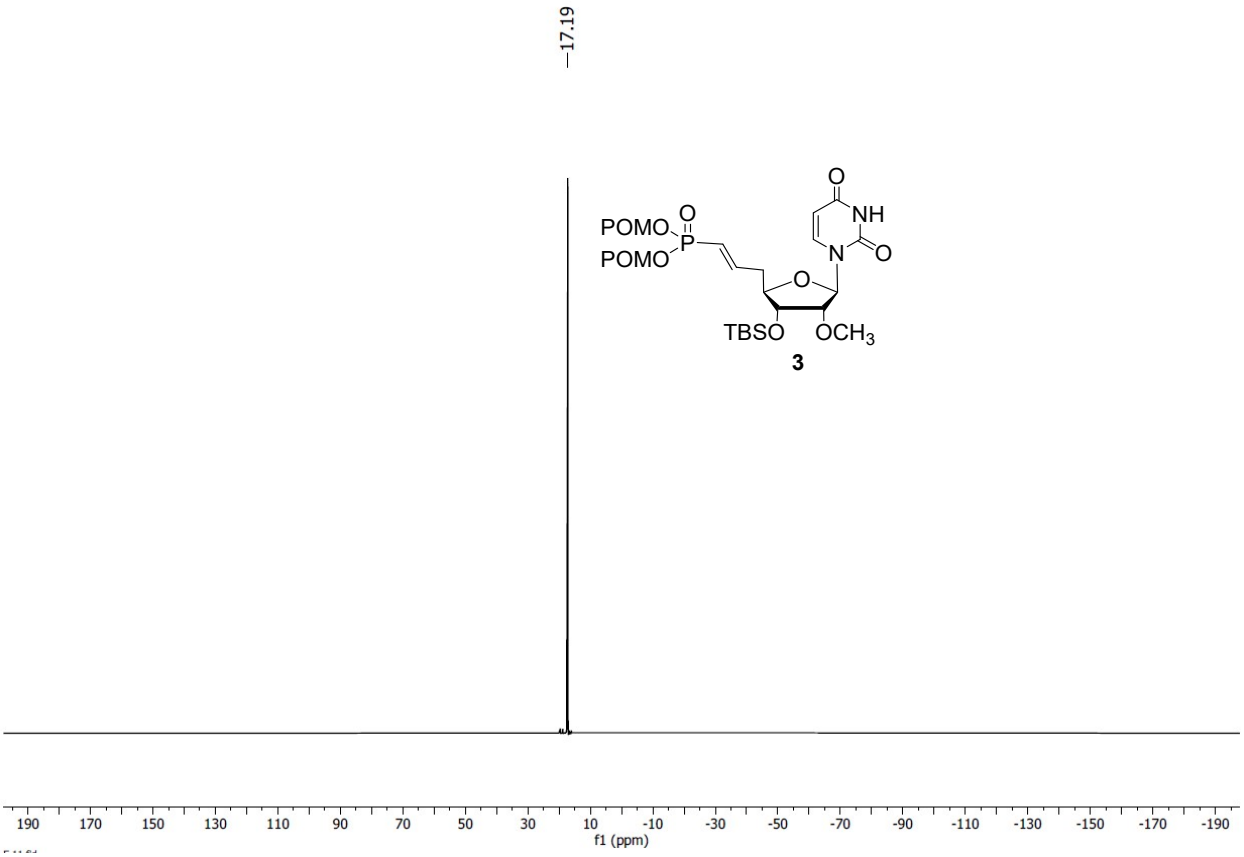
^{13}C NMR (151 MHz, DMSO-d_6) of compoud **2**



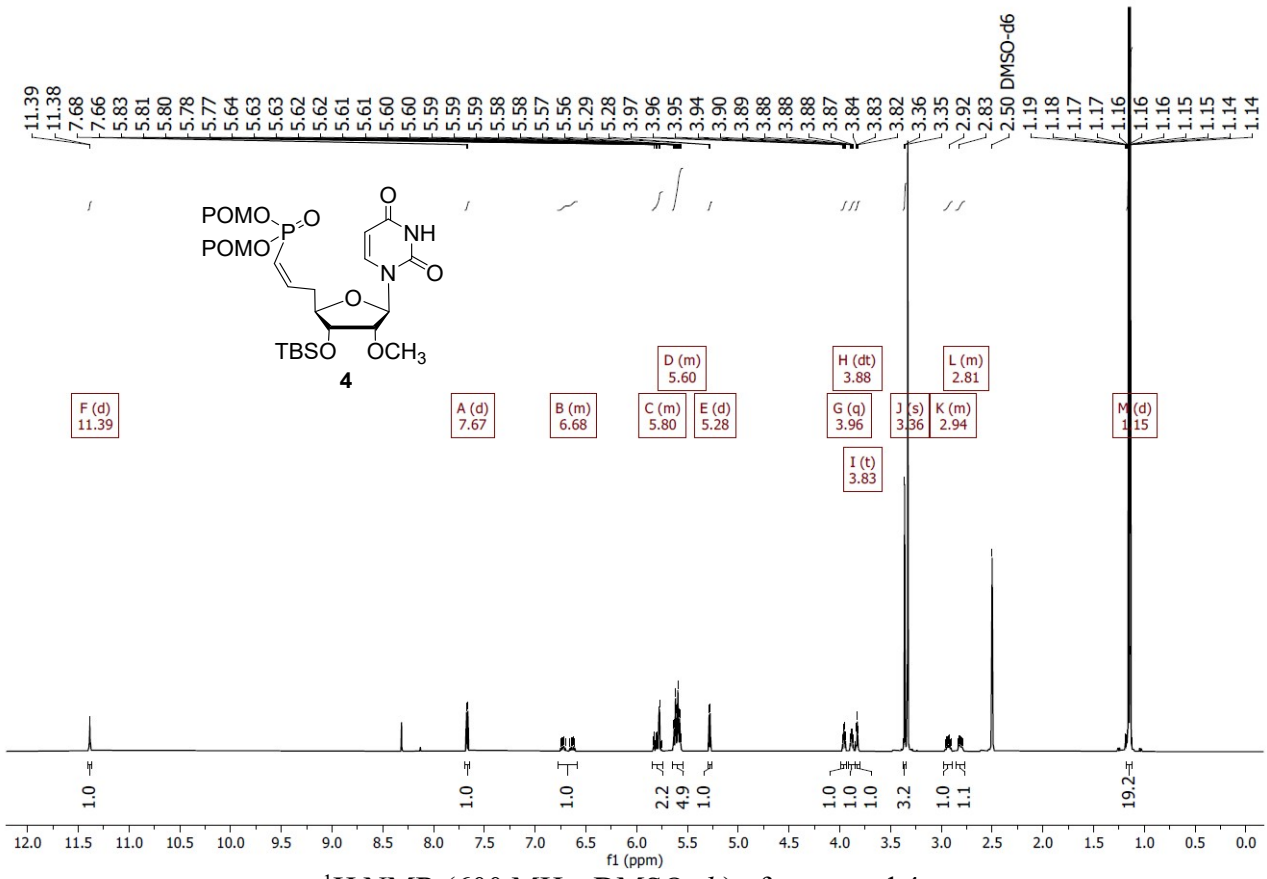
^{1}H NMR (600 MHz, $CDCl_3$) of compoud **3**



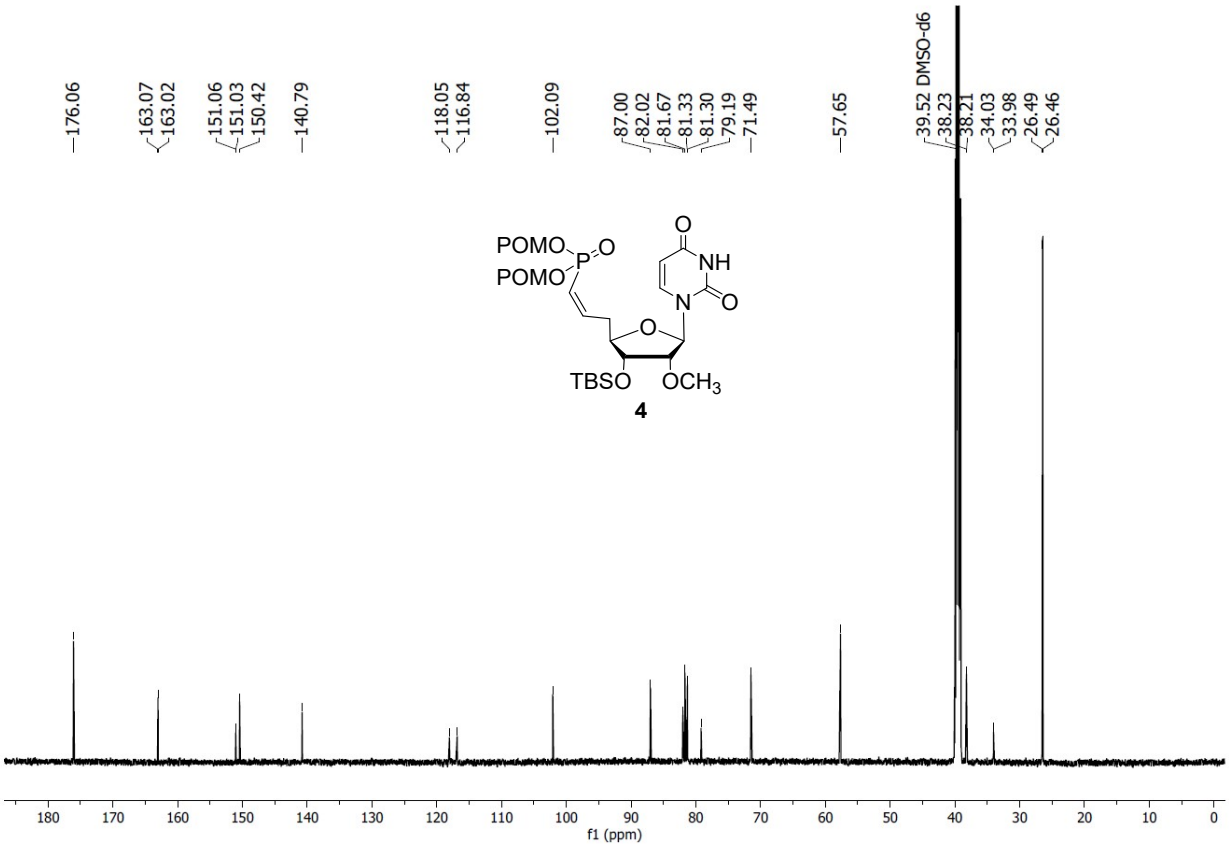
^{13}C NMR (151 MHz, $CDCl_3$) of compound **3**



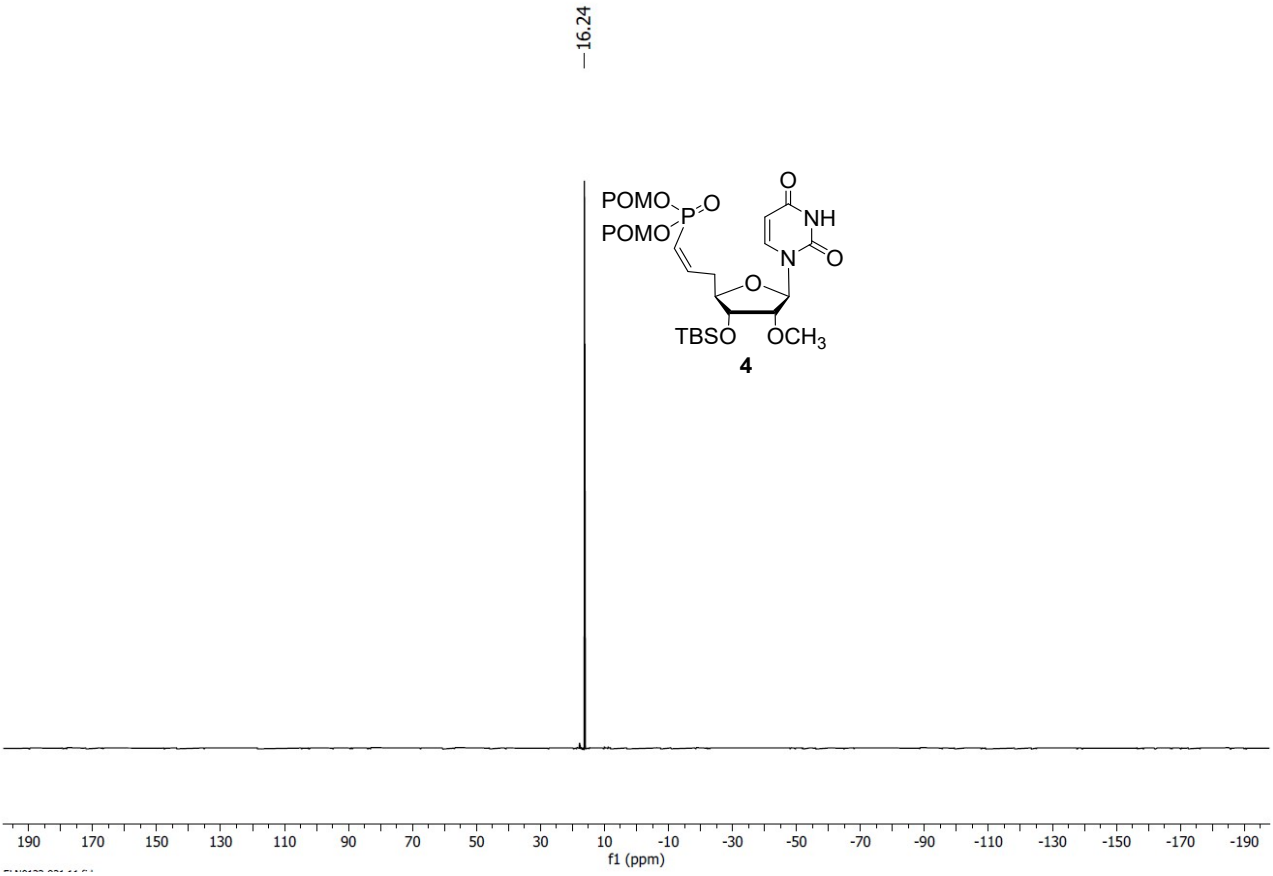
^{31}P NMR (243 MHz, $CDCl_3$) of compoud **3**



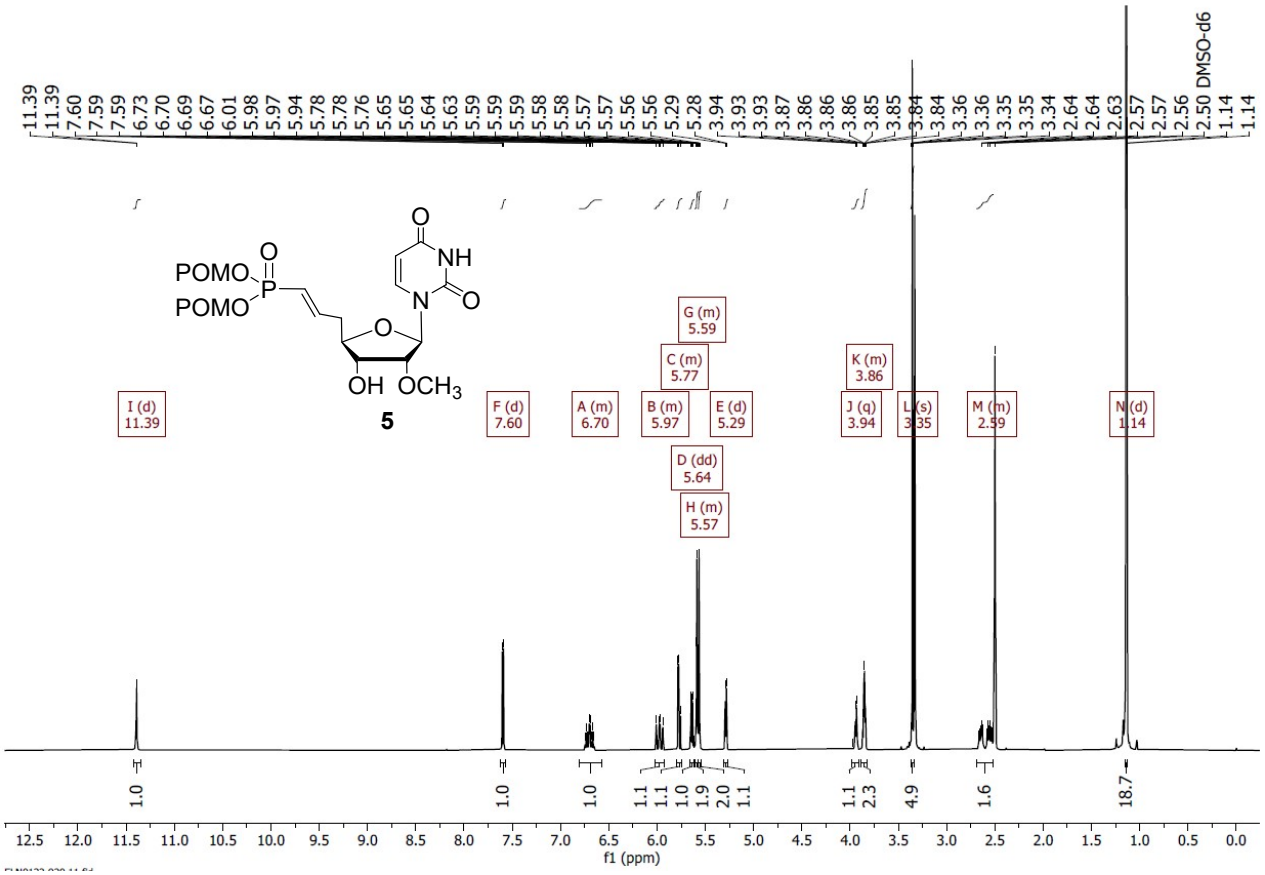
^{1}H NMR (600 MHz, DMSO-d_6) of compoud **4**



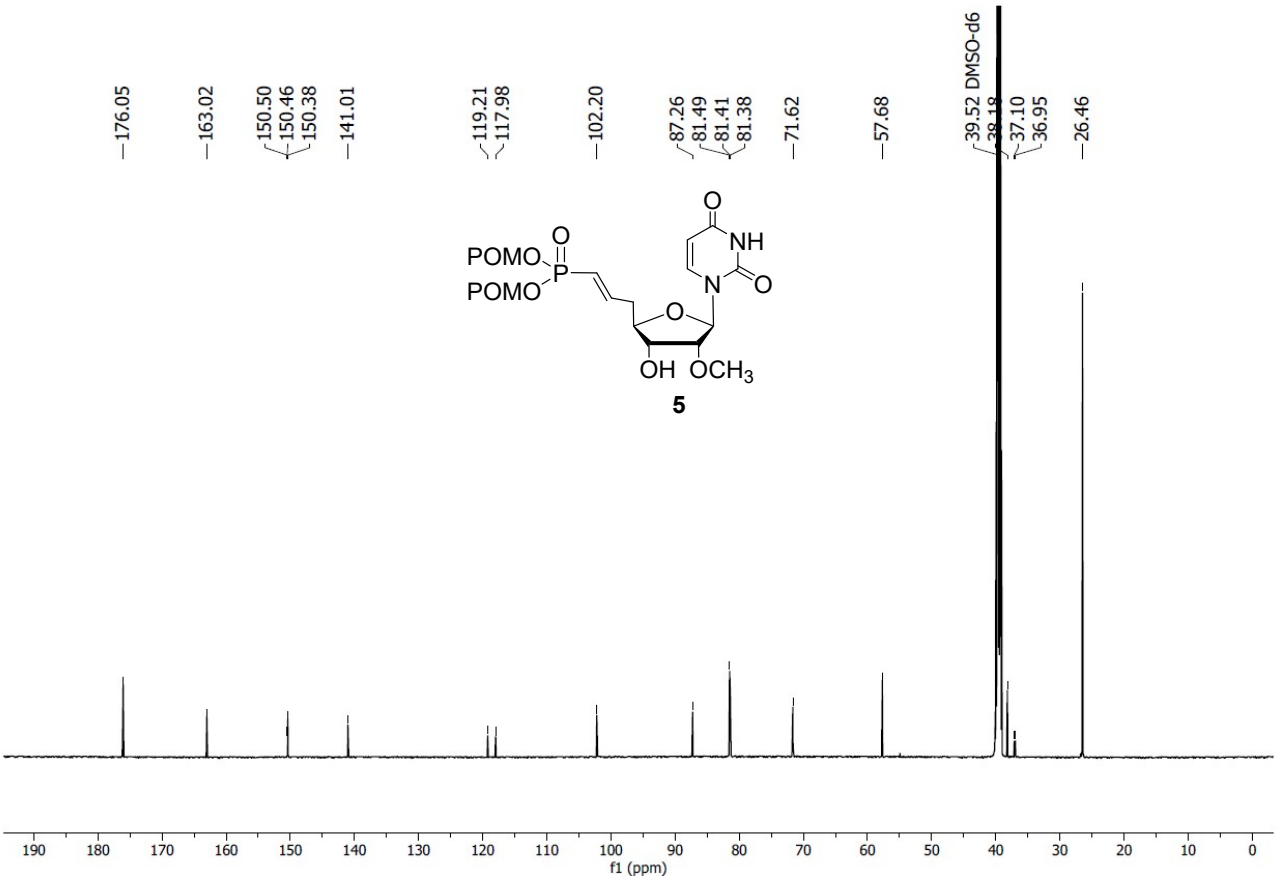
^{13}C NMR (151 MHz, DMSO-d_6) of compound **4**



^{31}P NMR (243 MHz, DMSO-d_6) of compoud **4**



^{1}H NMR (600 MHz, DMSO-d_6) of compound **5**



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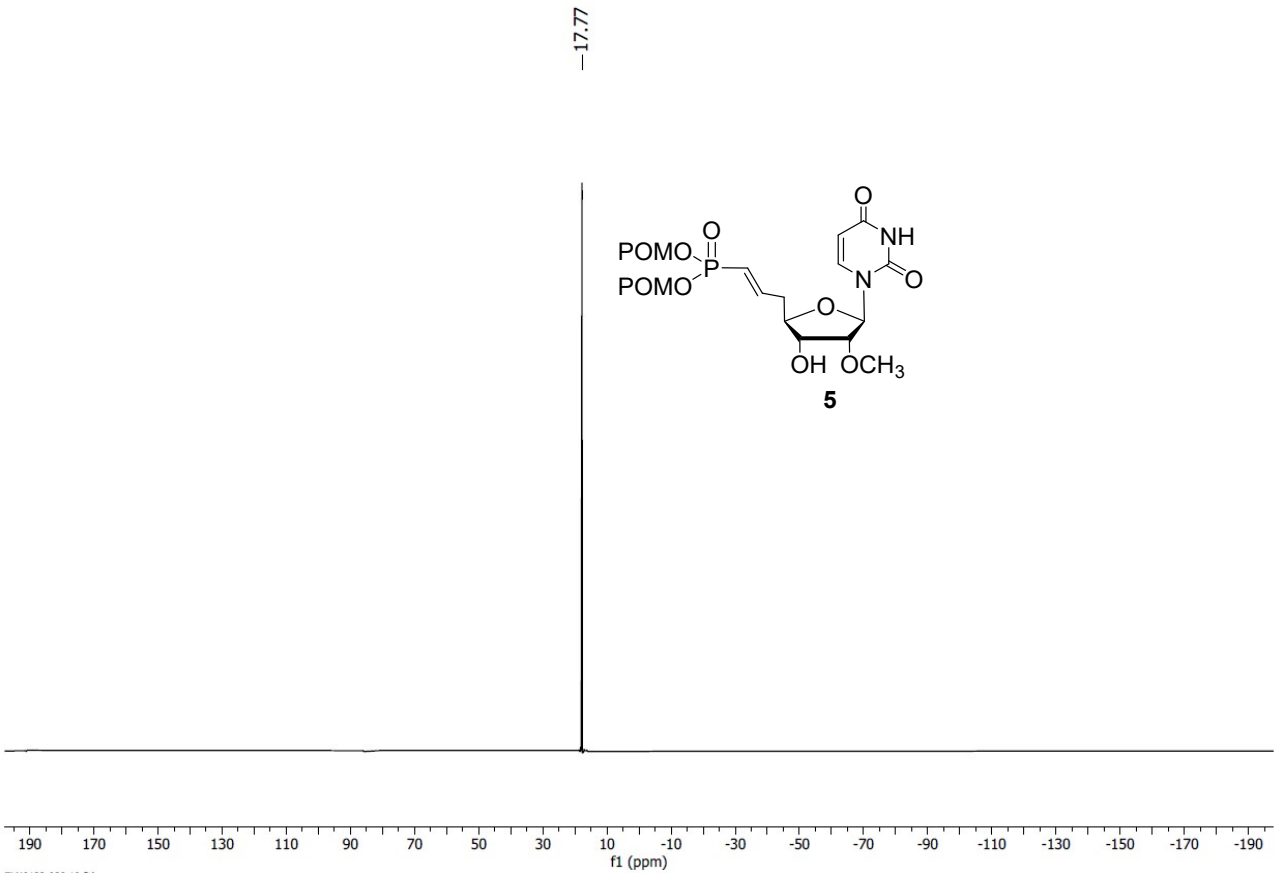

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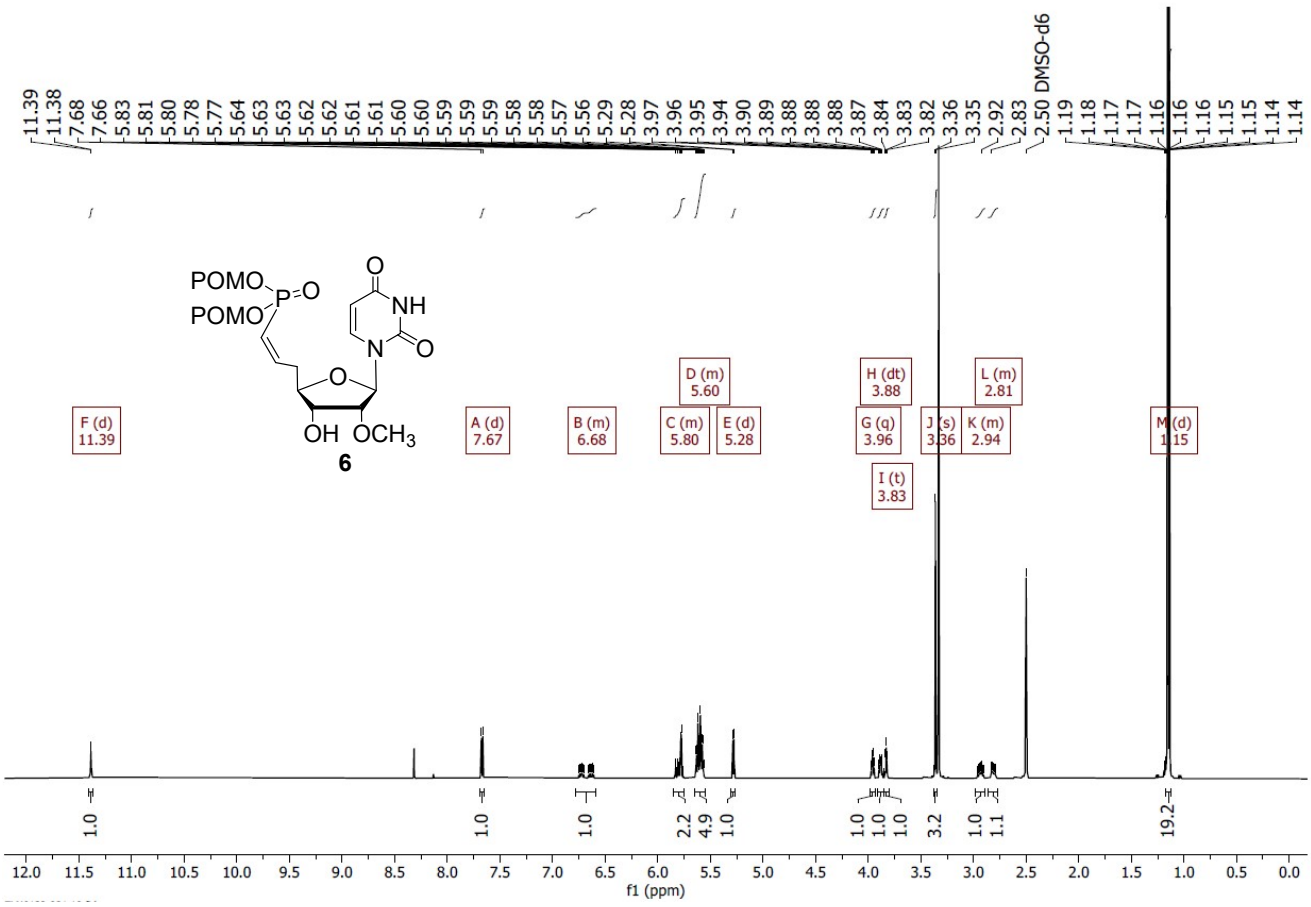
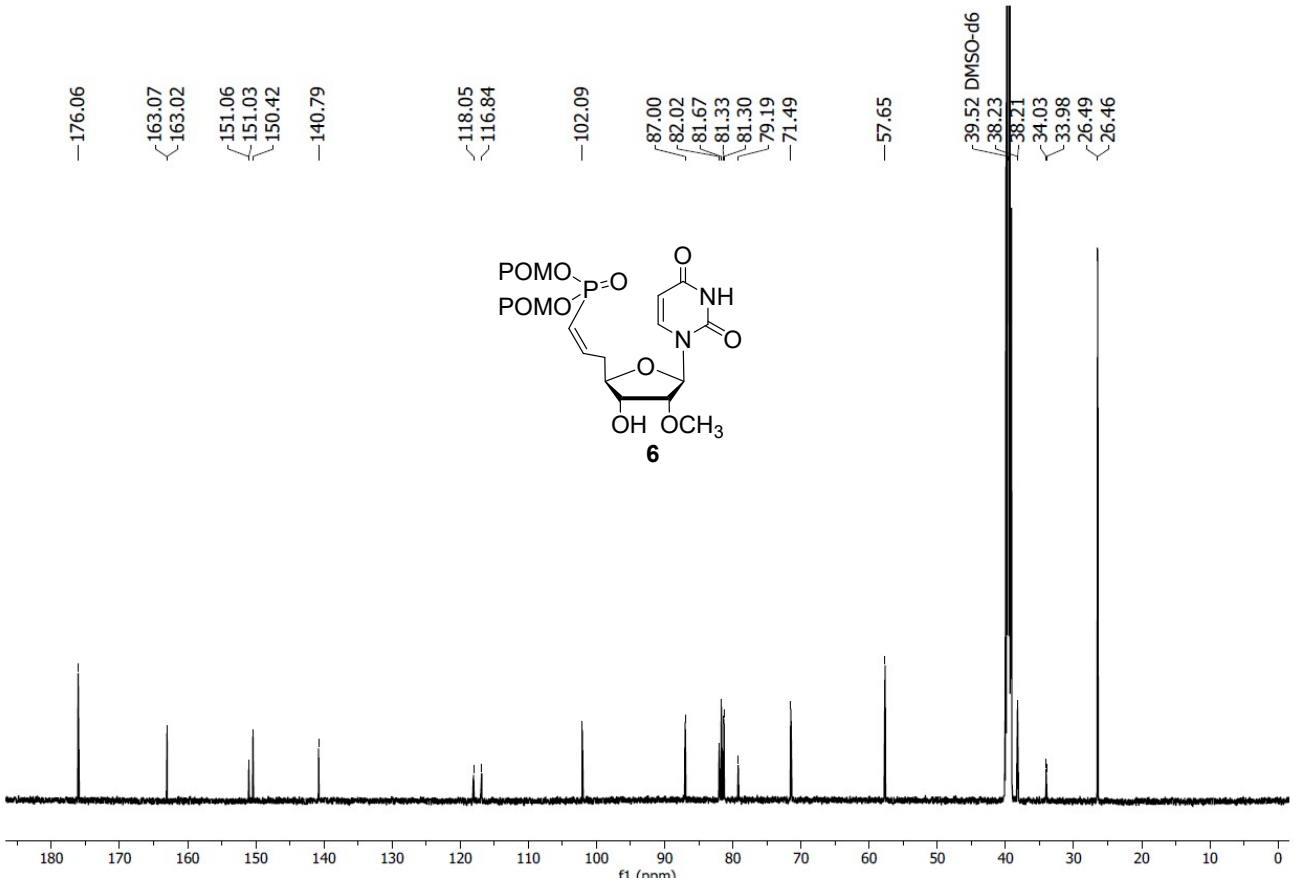


^{1}H NMR (600 MHz, DMSO-d_6) of compound **6**



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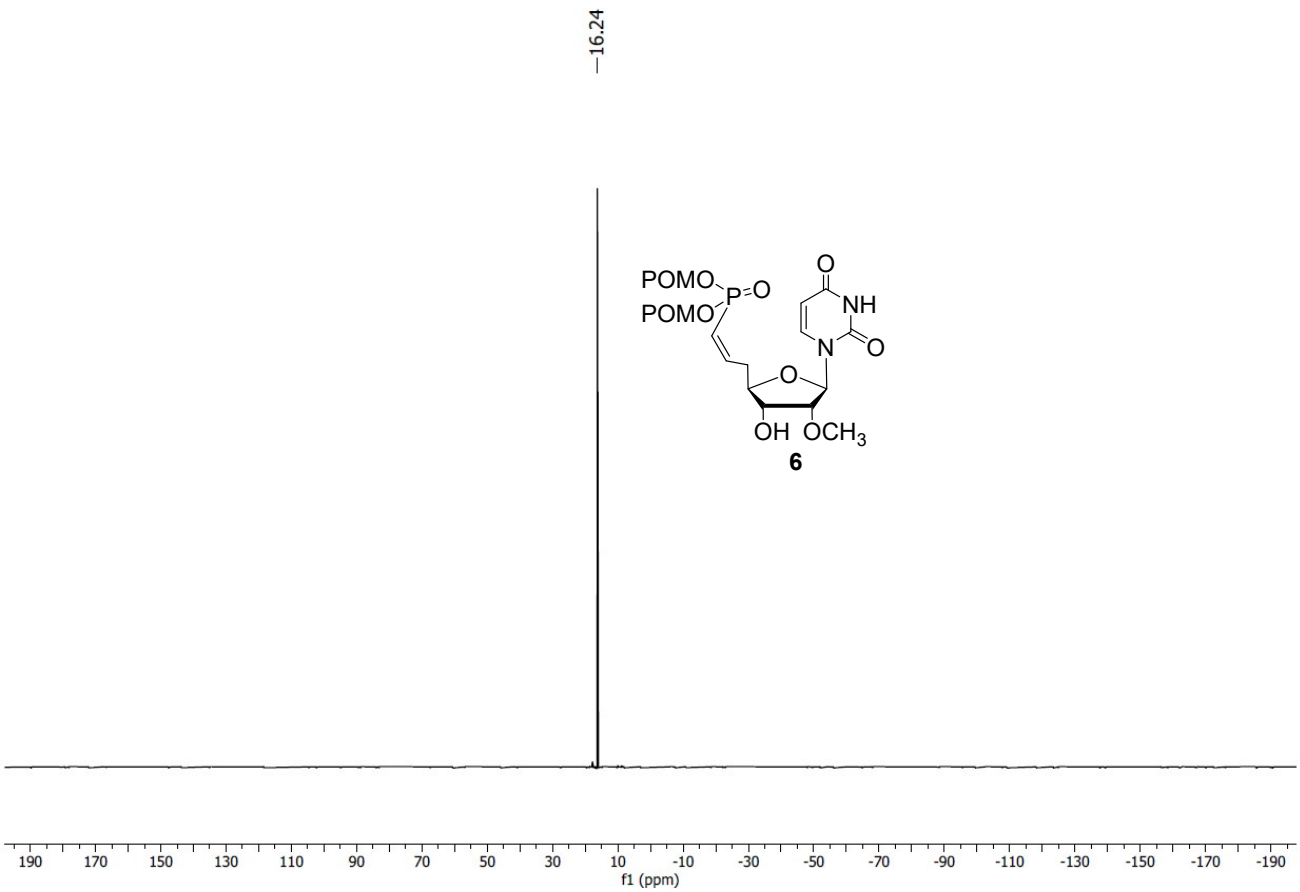
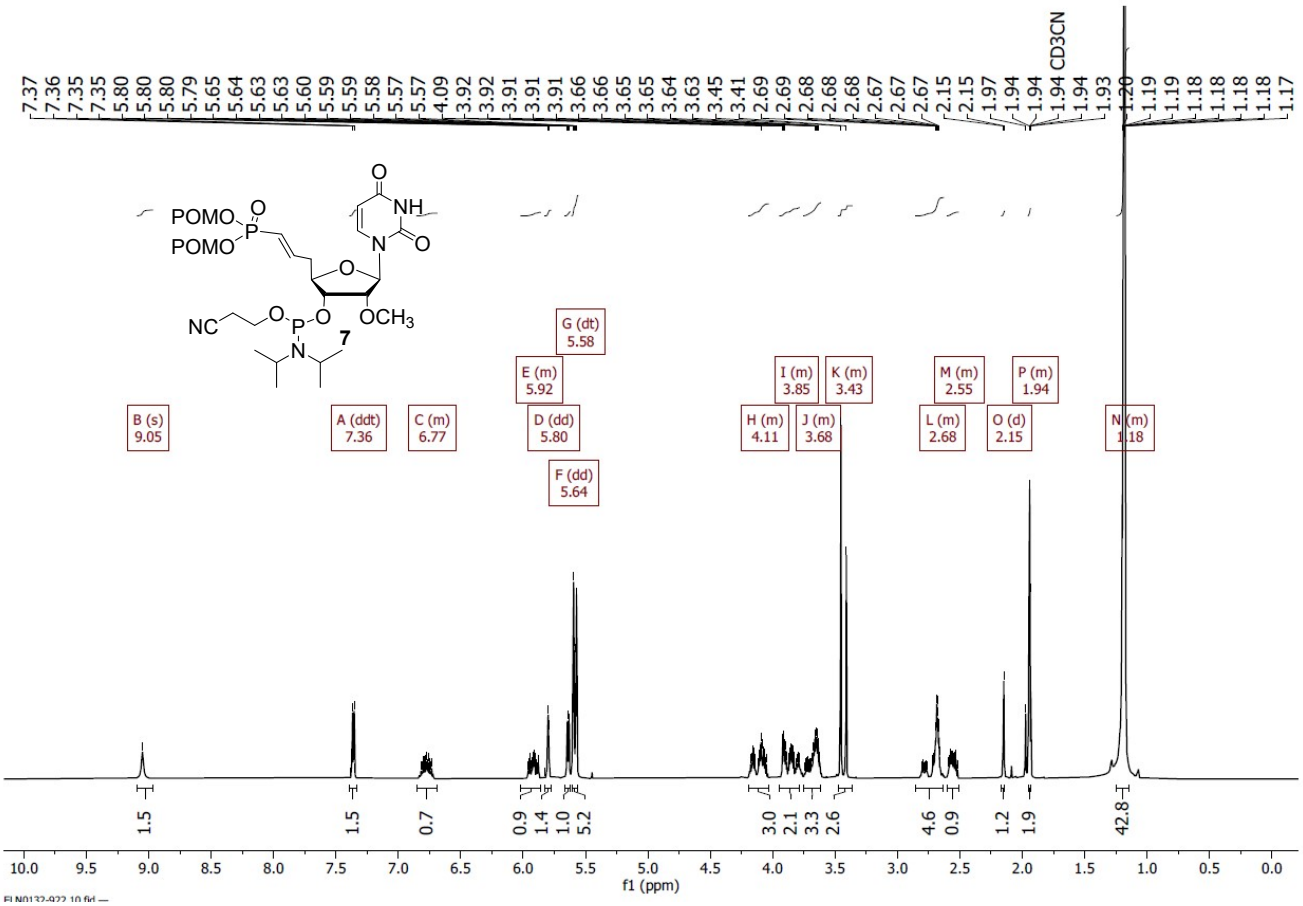
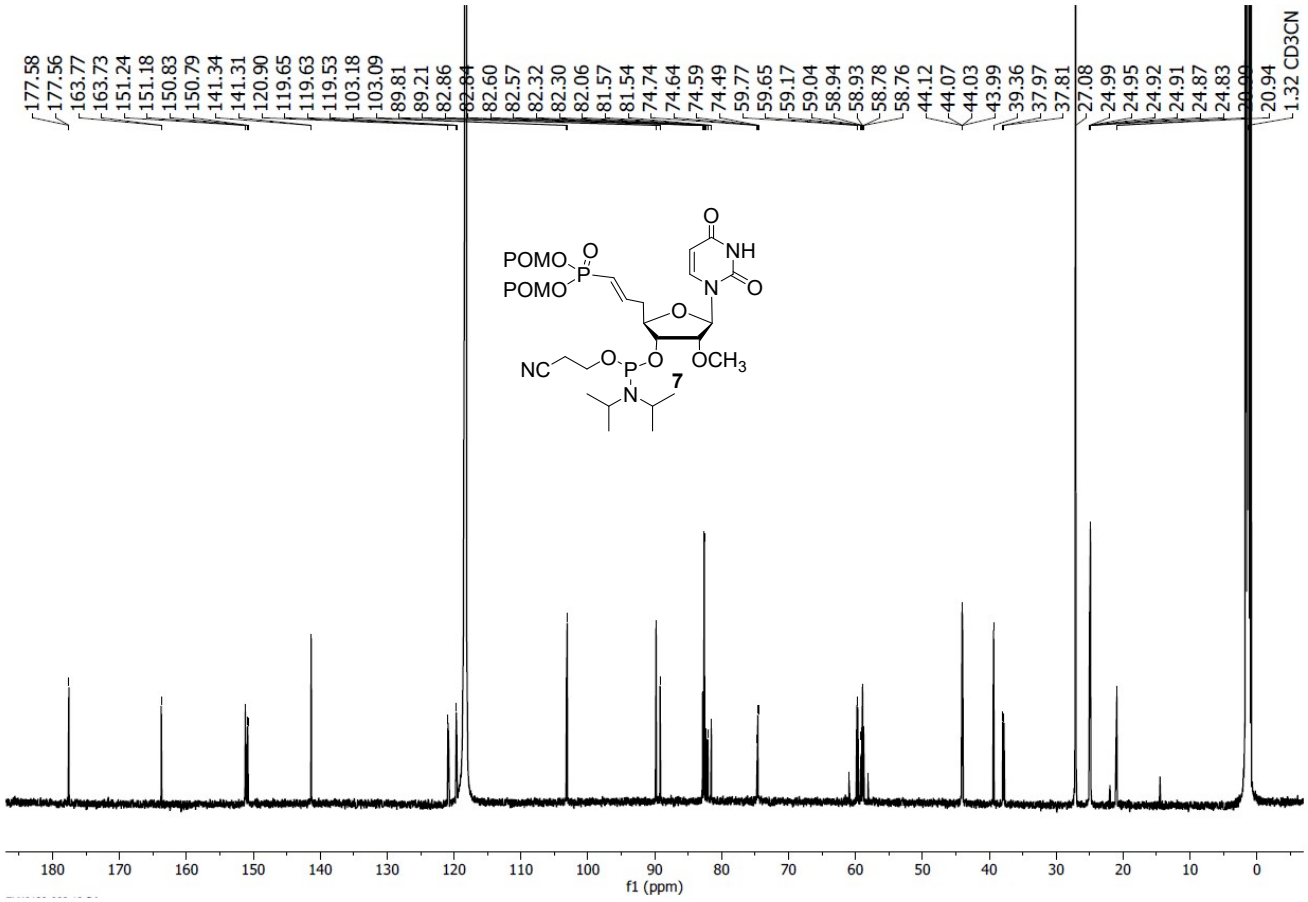


^{31}P NMR (243 MHz, DMSO-d_6) of compound **6**



^{1}H NMR (600 MHz, CD_3CN) of compound **7**



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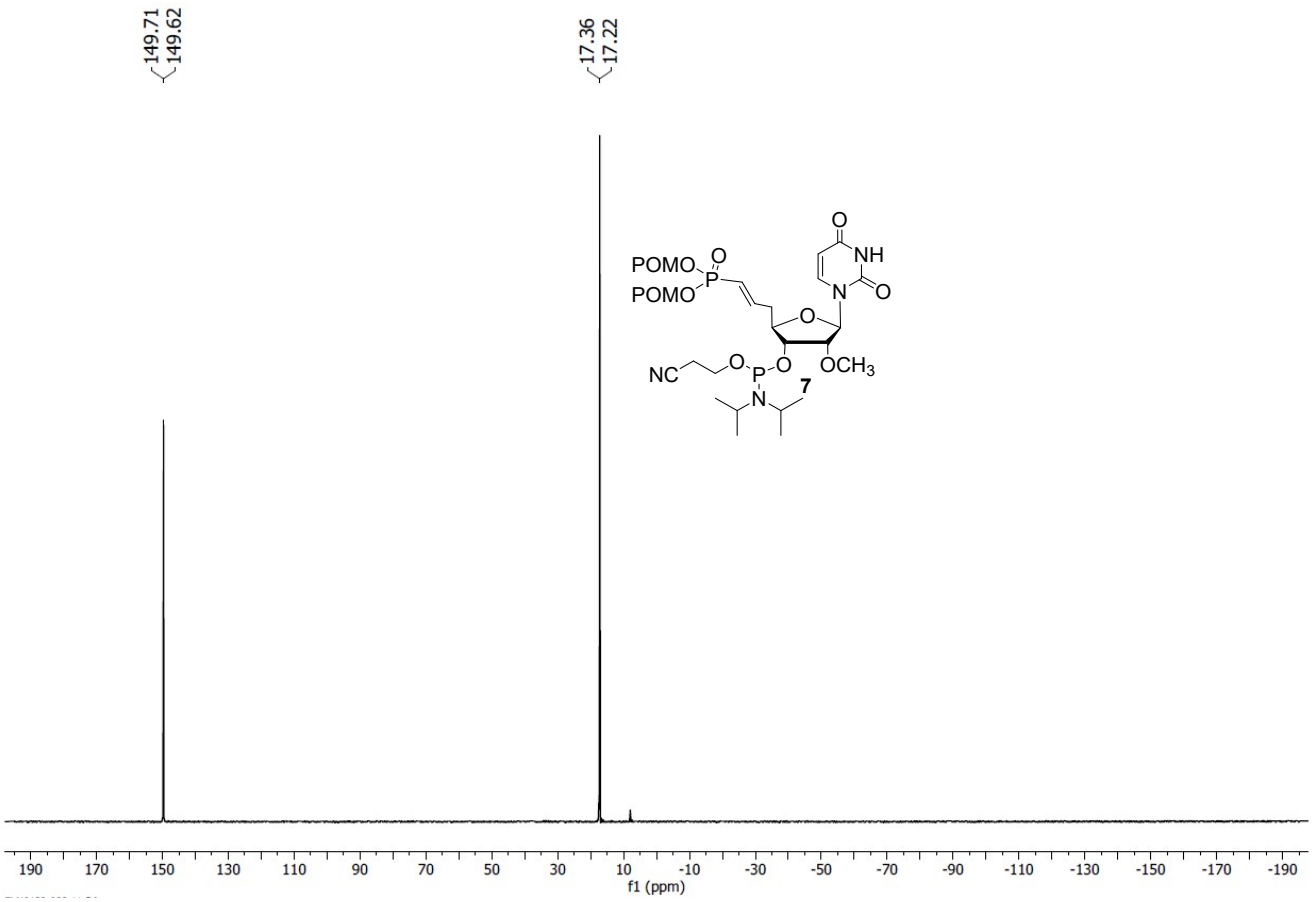

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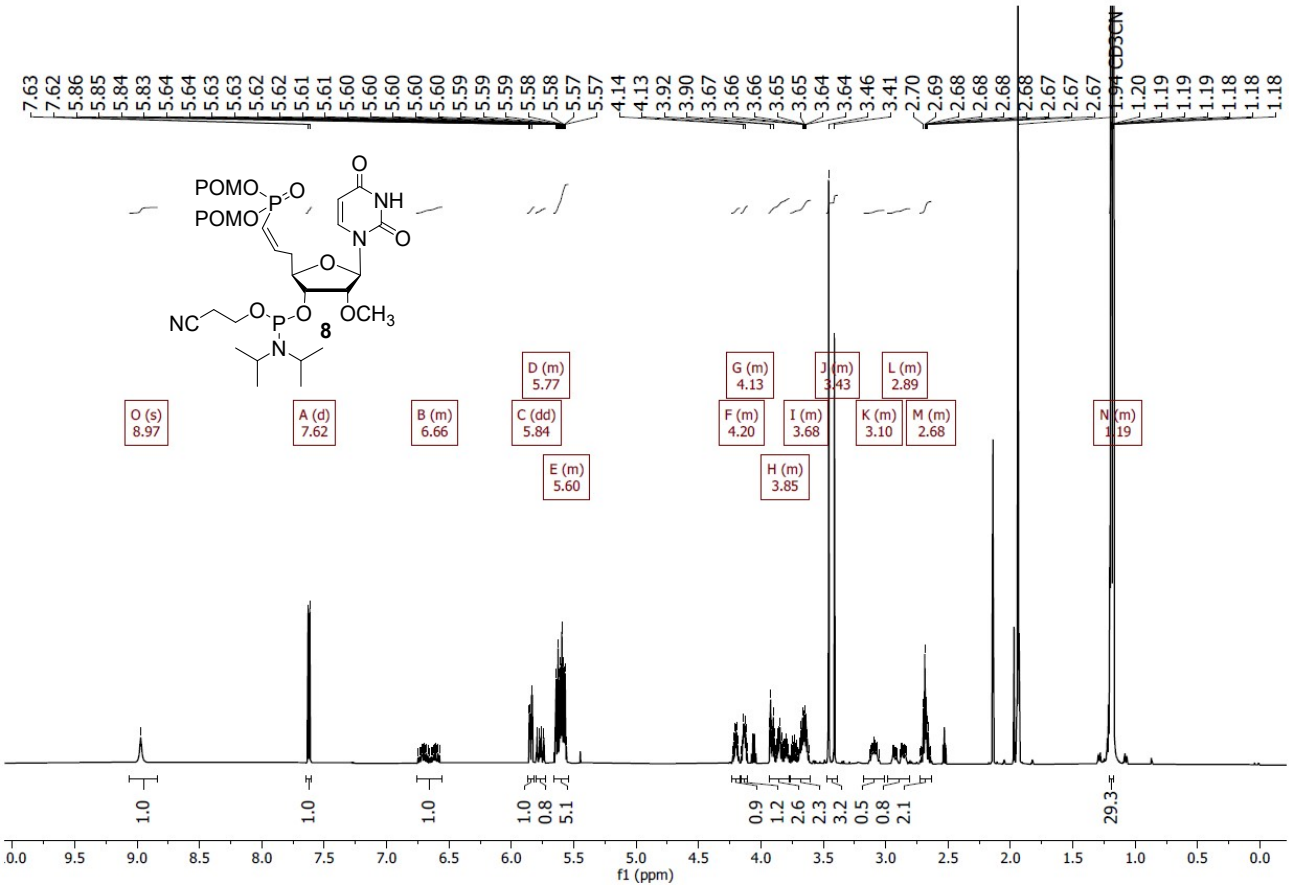

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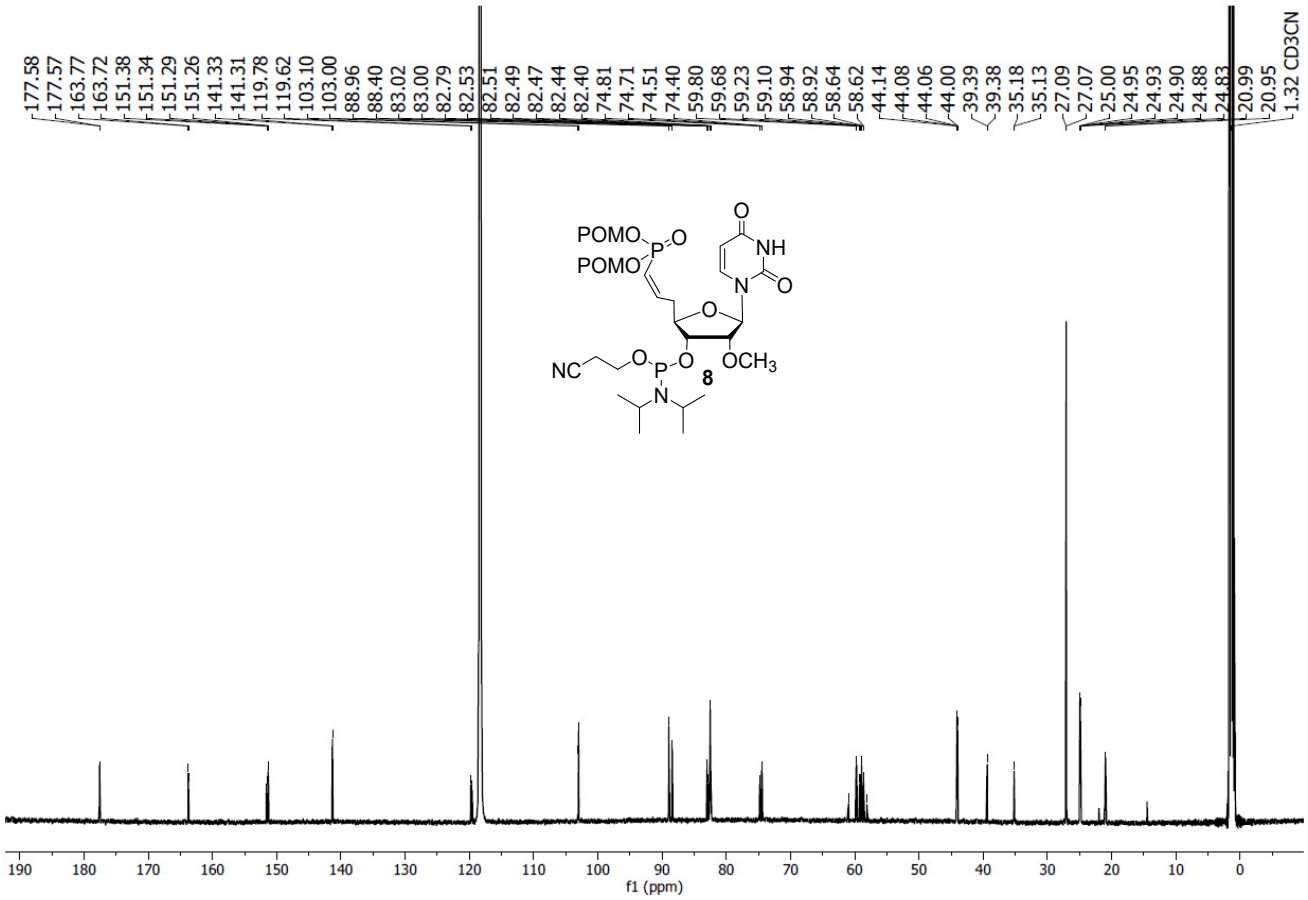

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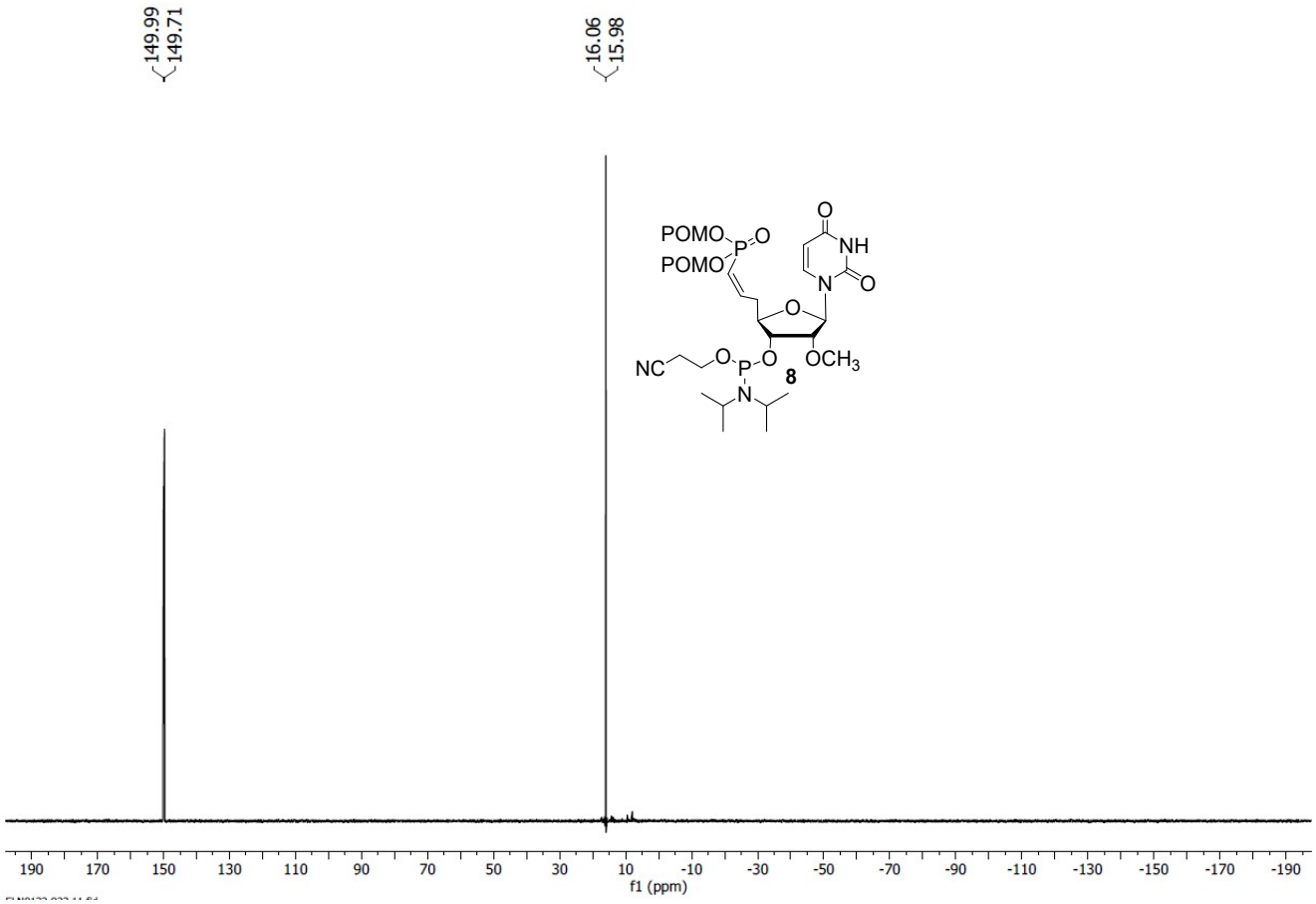

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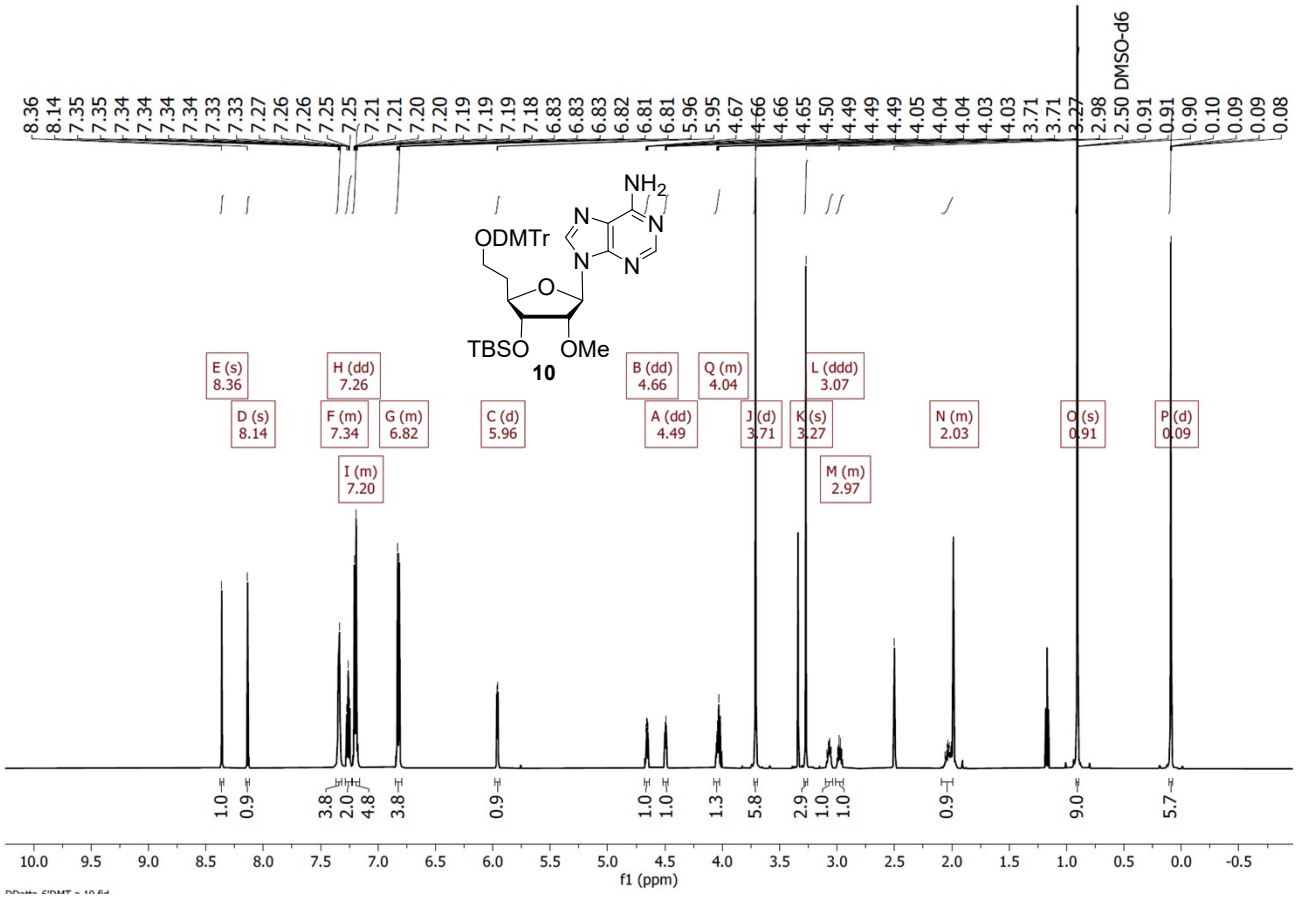
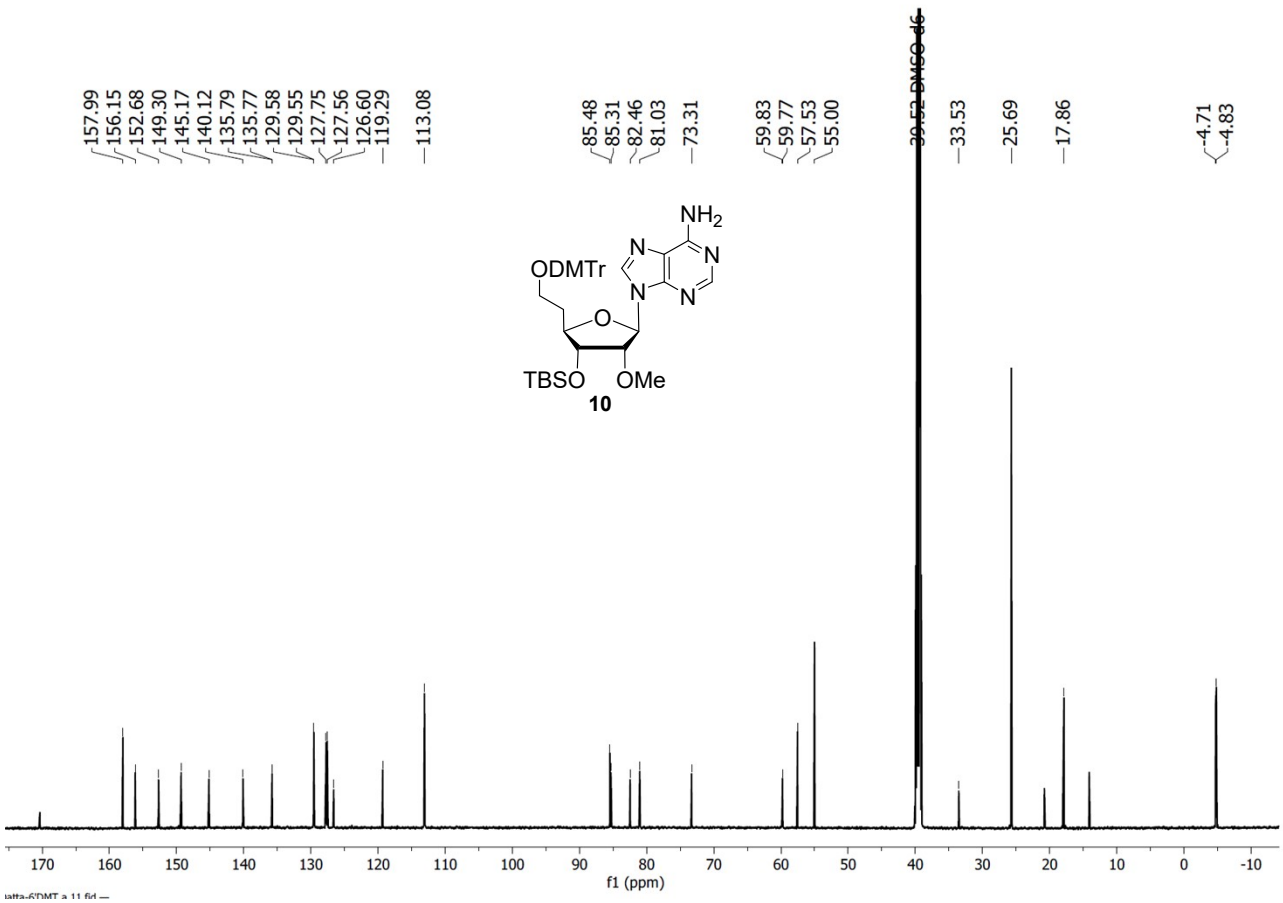


^{1}H NMR (600 MHz, DMSO-d_6) of compound **10**



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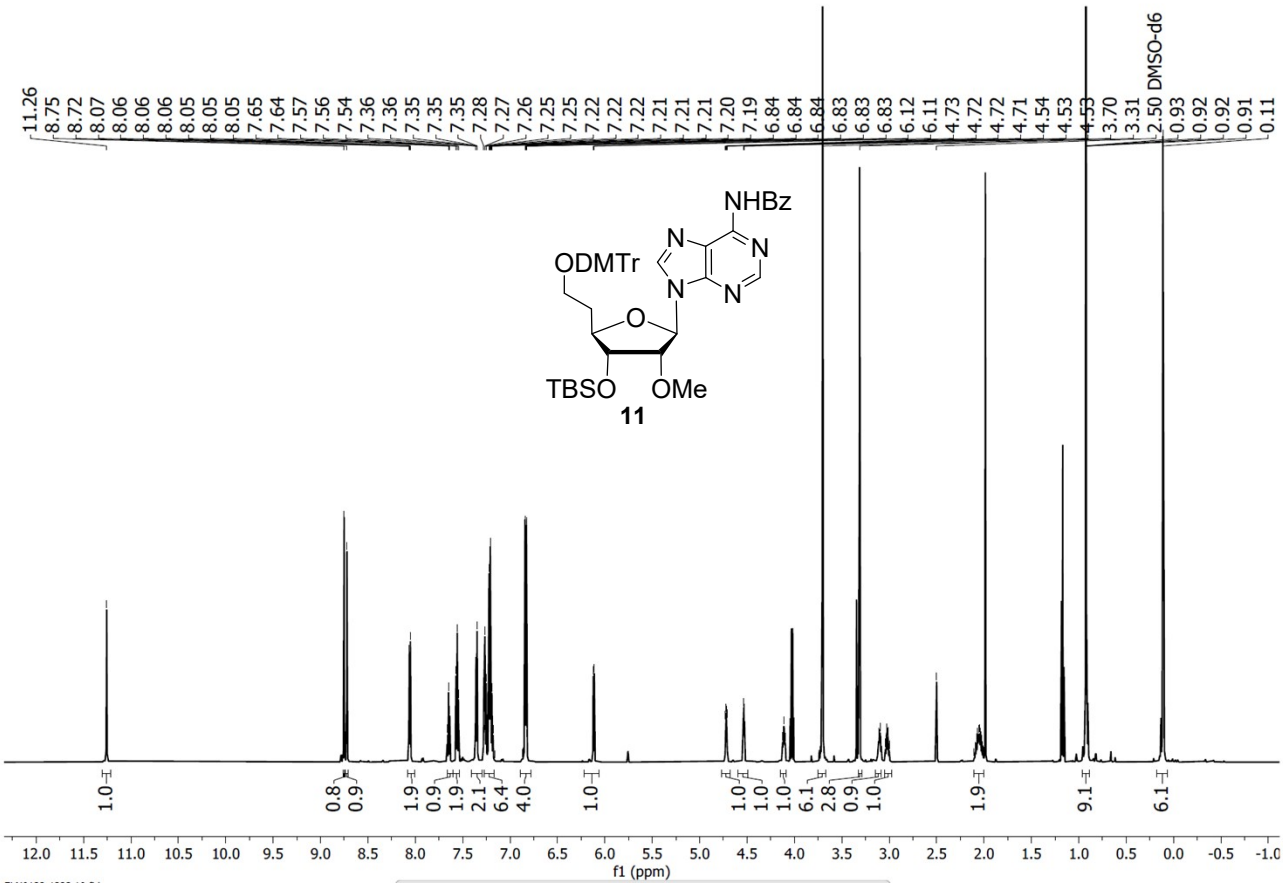
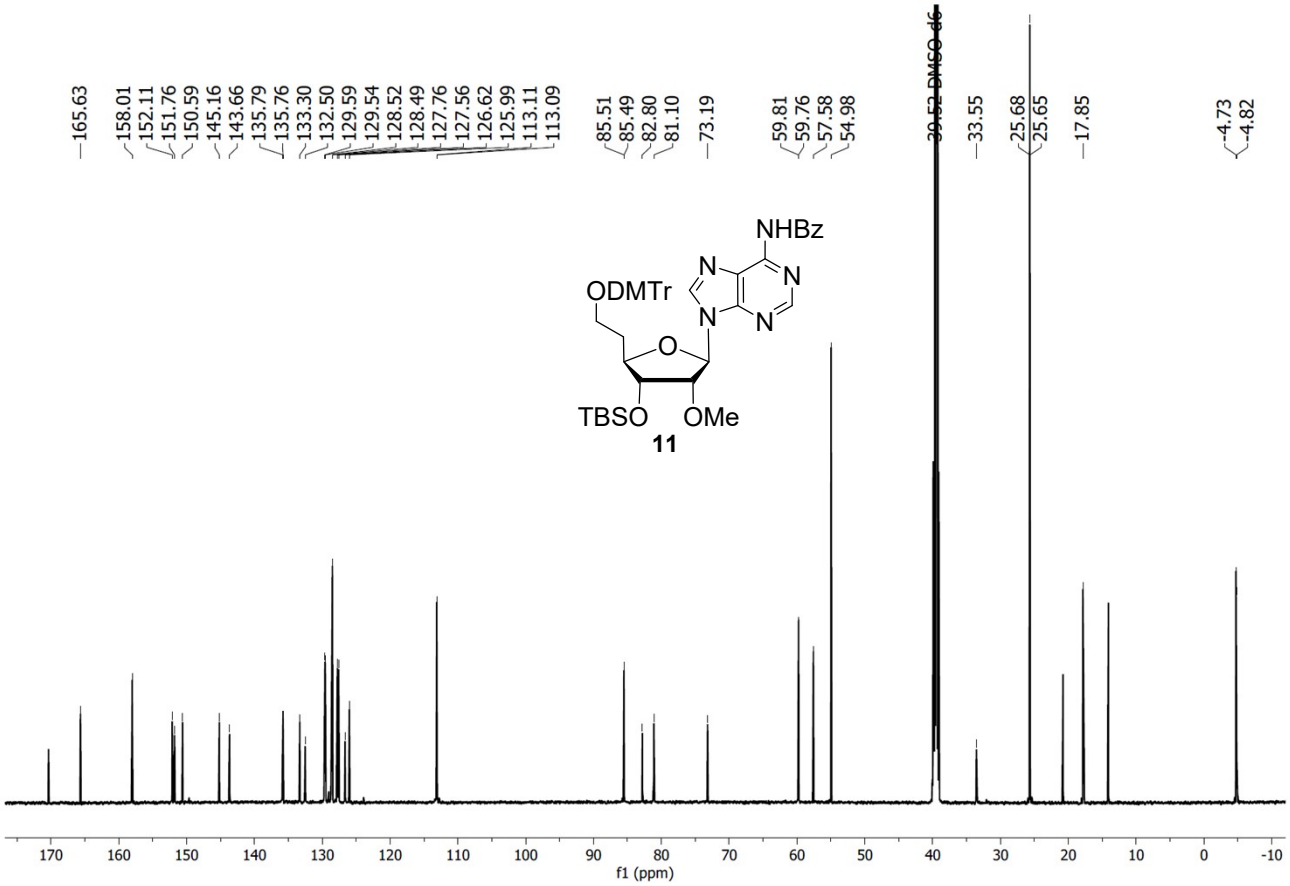


^{1}H NMR (600 MHz, DMSO-d_6) of compoud **11**



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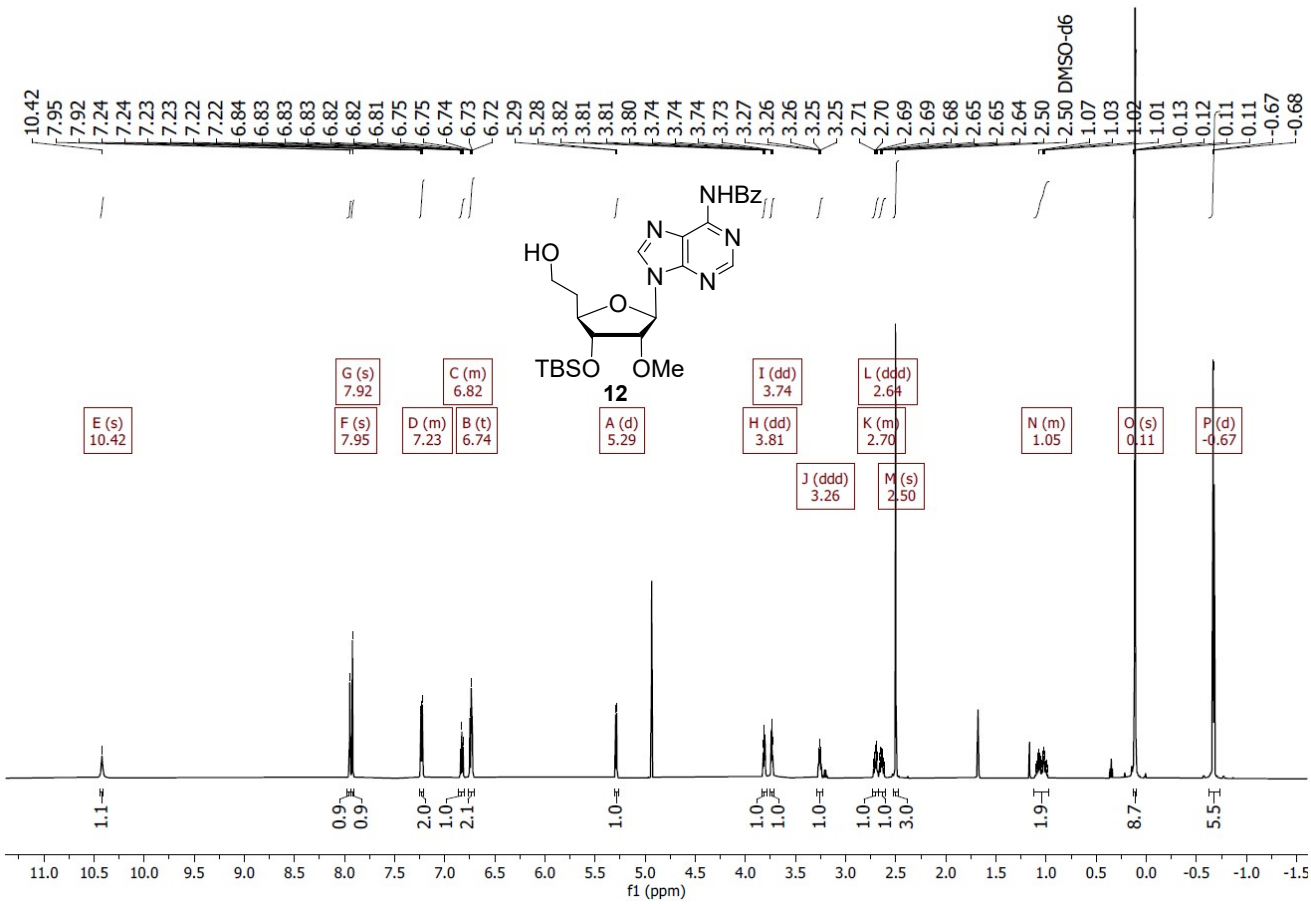

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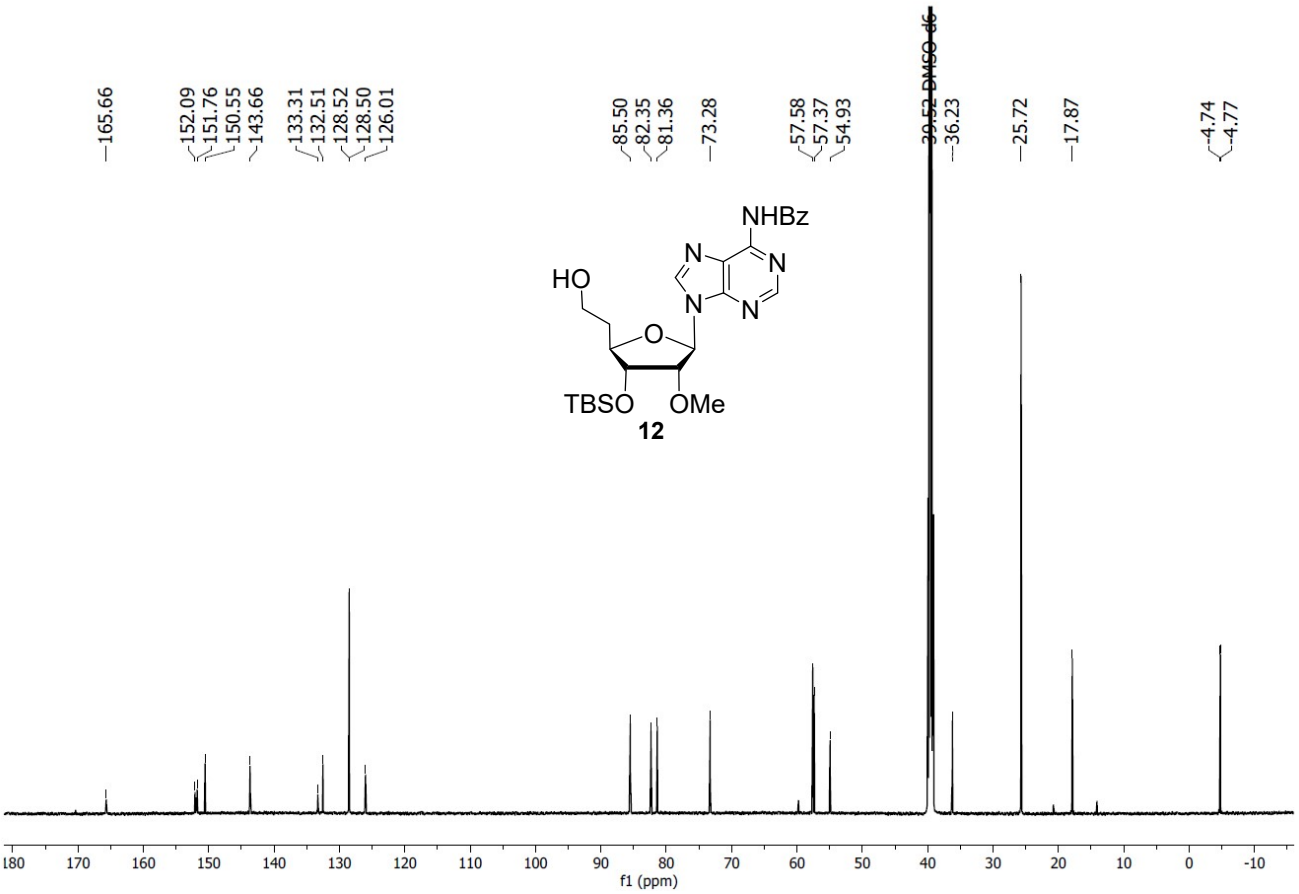

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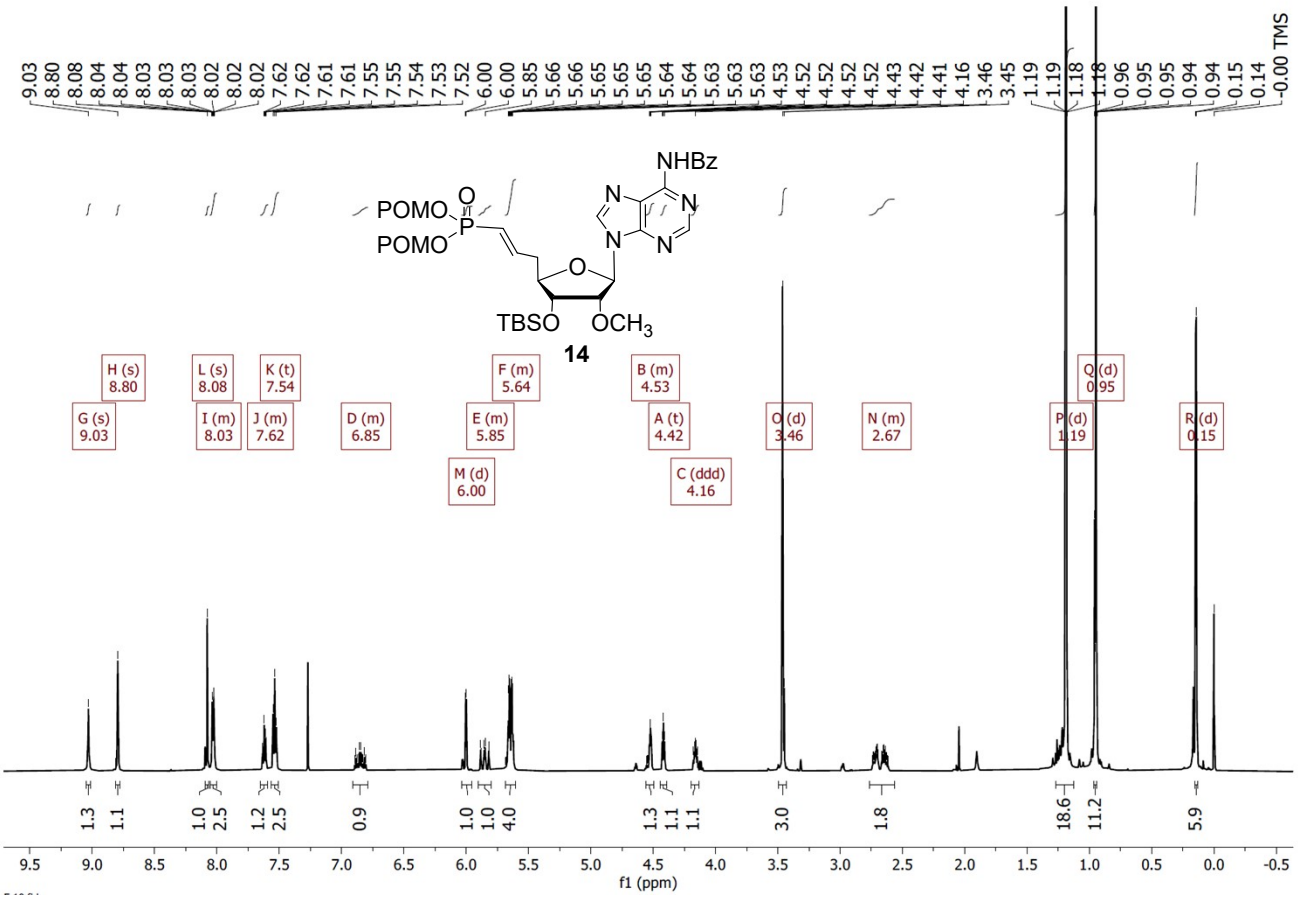
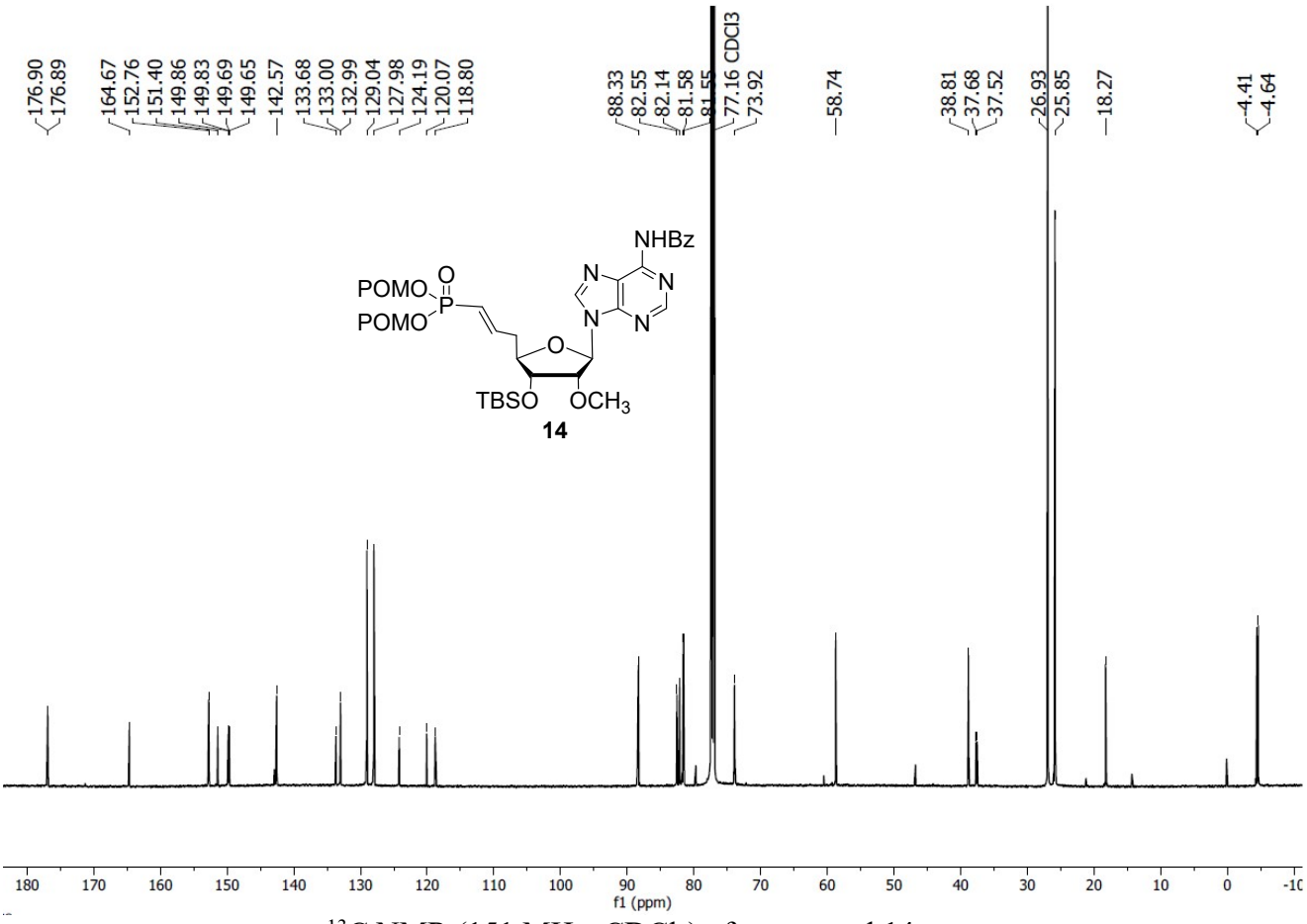
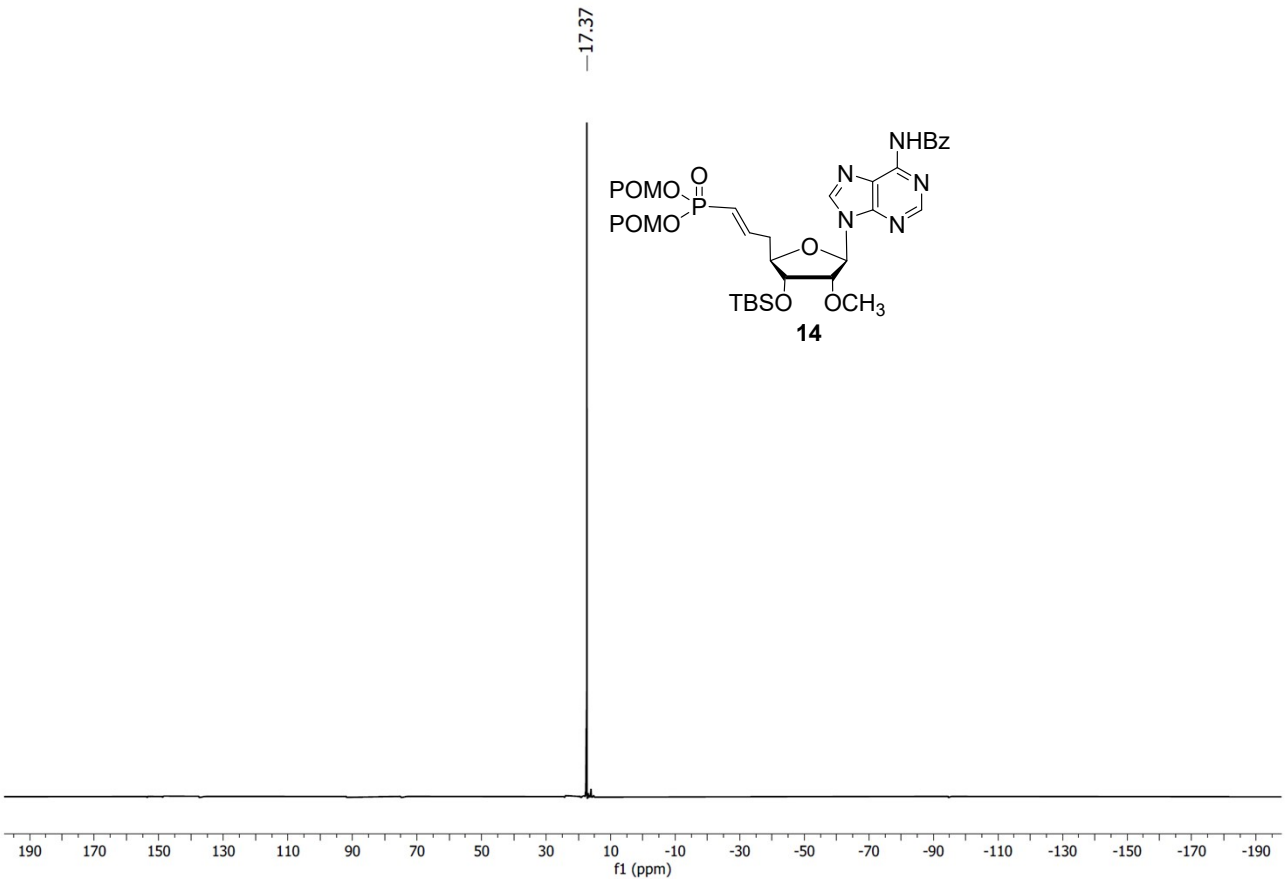


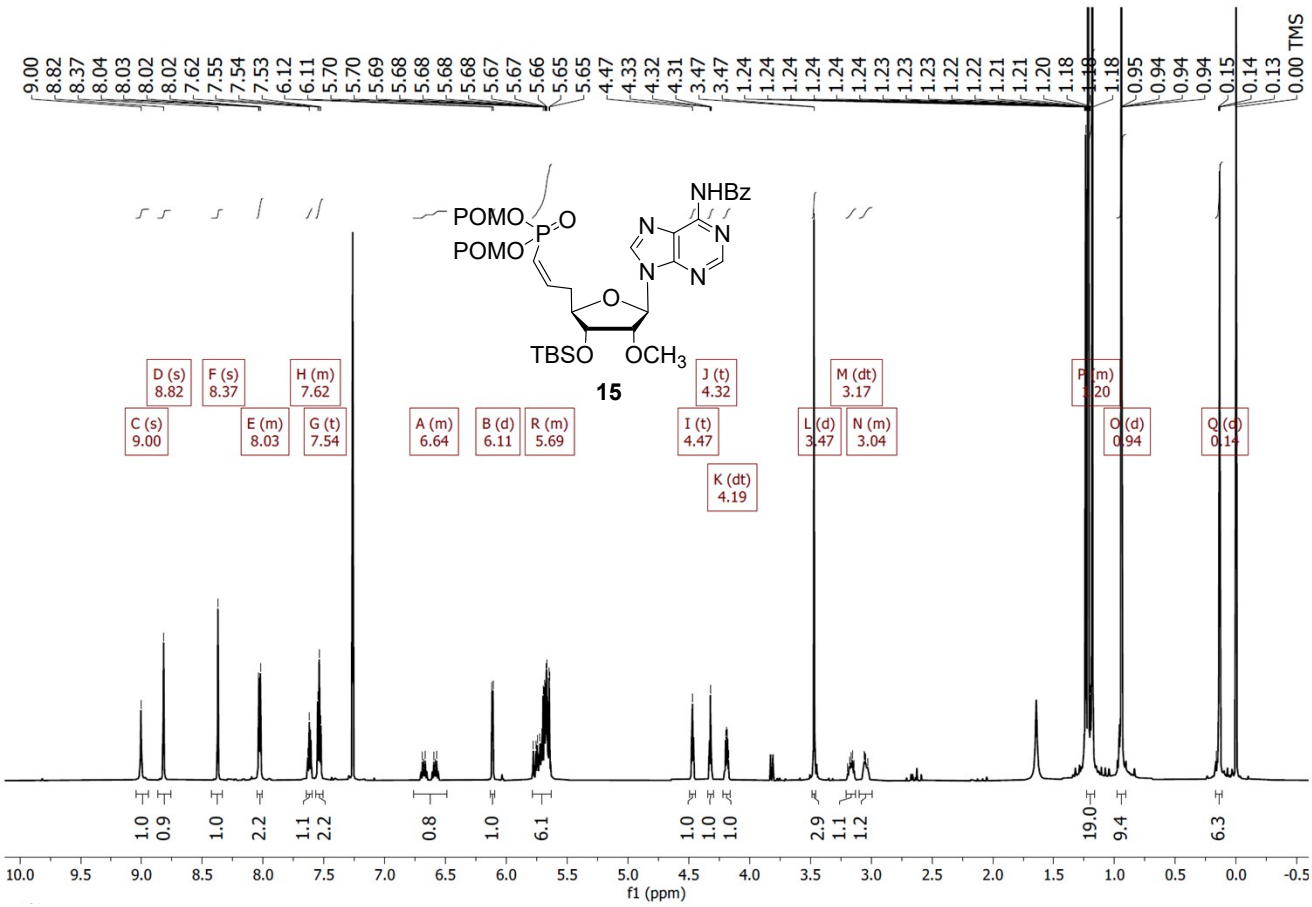
^{1}H NMR (600 MHz, $CDCl_3$) of compoud **14**



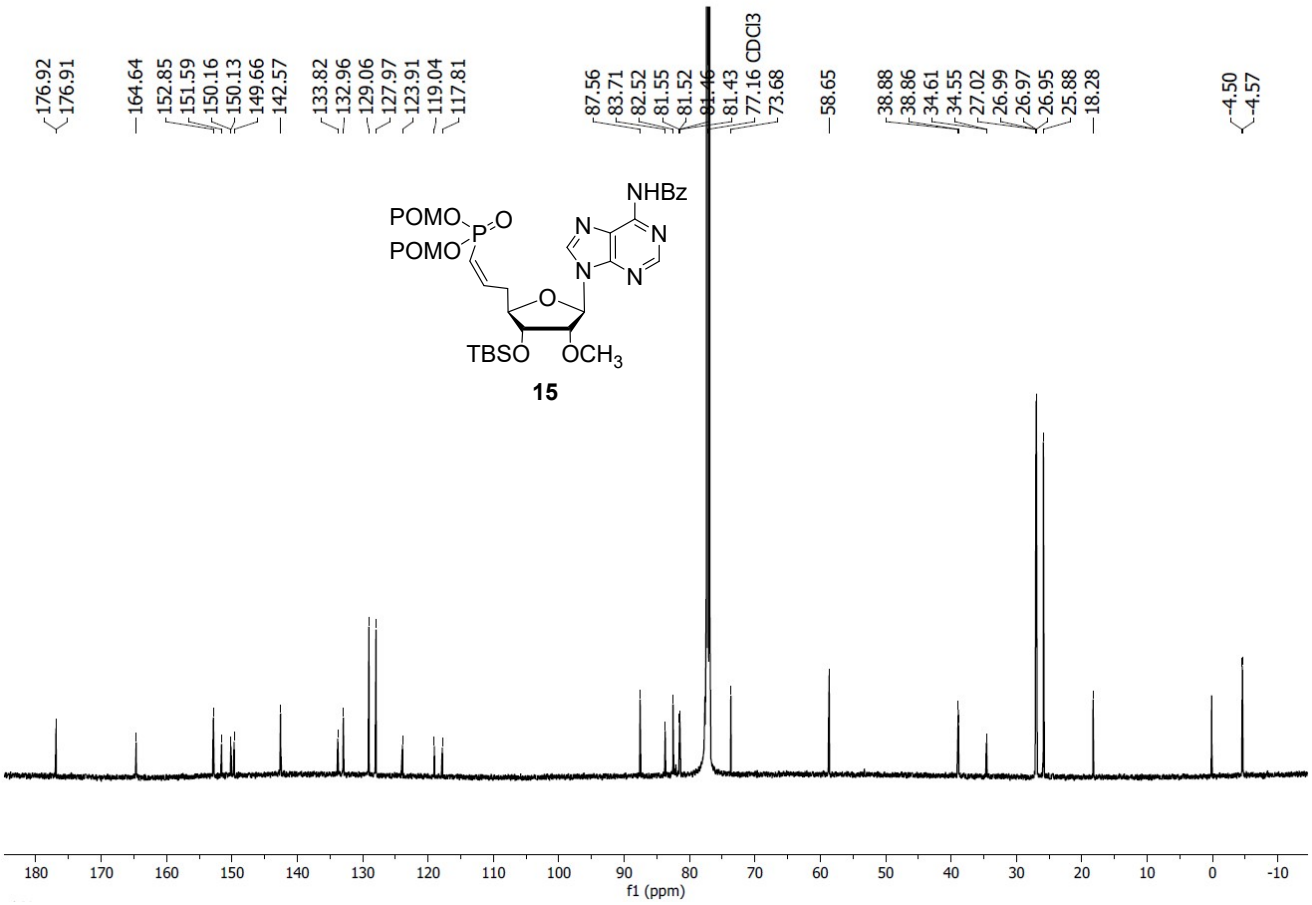
^{13}C NMR (151 MHz, $CDCl_3$) of compound **14**



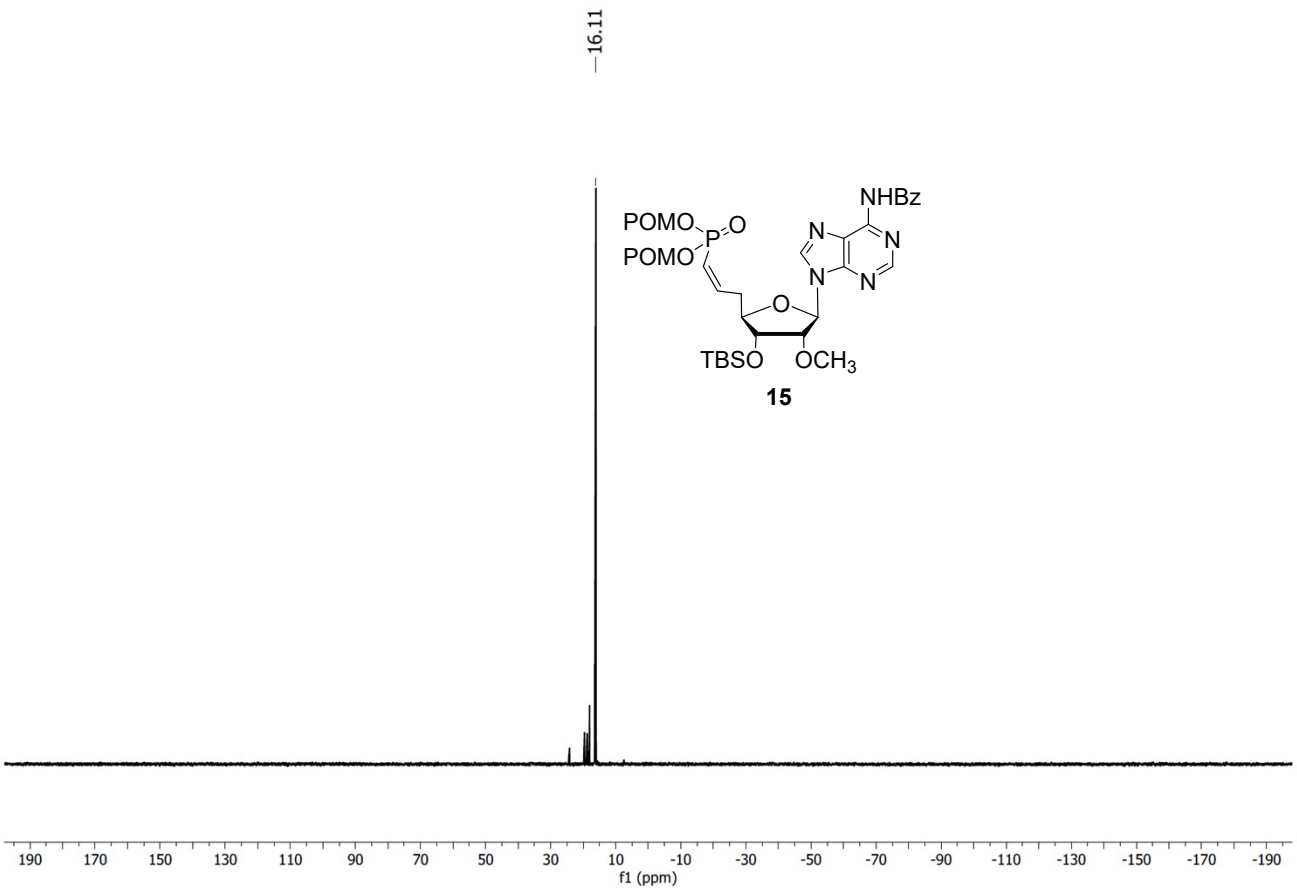
^{31}P NMR (243 MHz, $CDCl_3$) of compoud **14**



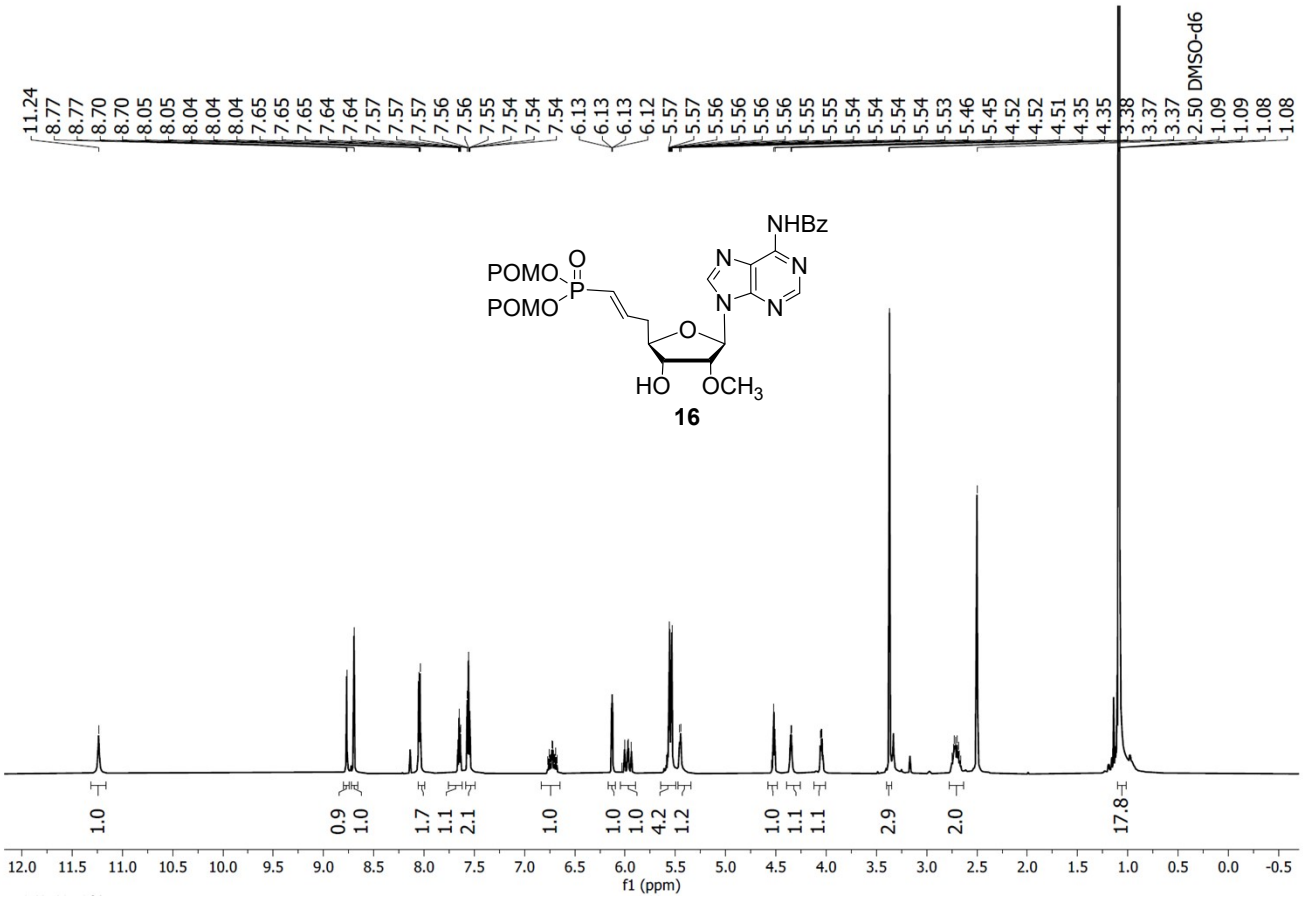
^{1}H NMR (600 MHz, $CDCl_3$) of compoud **15**



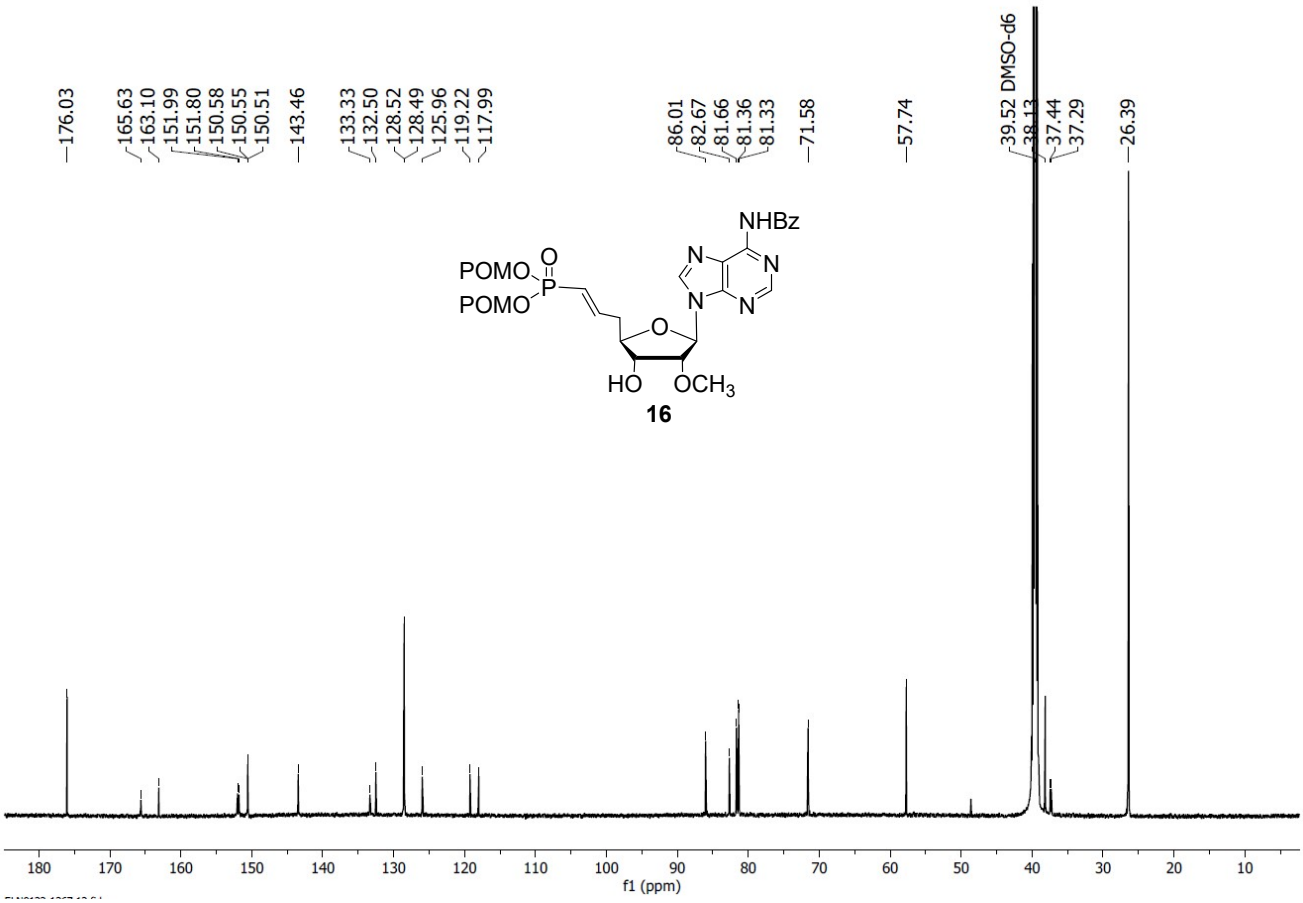
^{13}C NMR (151 MHz, $CDCl_3$) of compound **15**



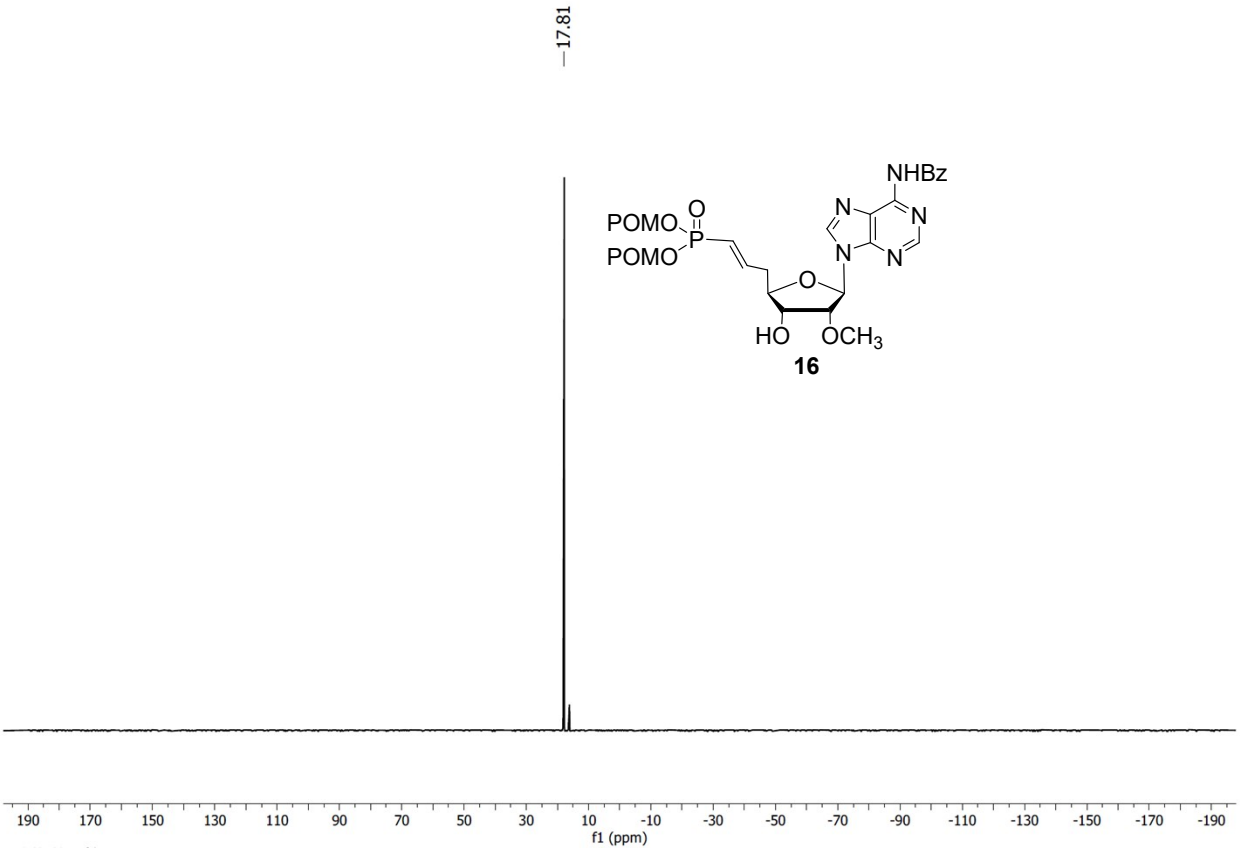
^{31}P NMR (243 MHz, $CDCl_3$) of compoud **15**



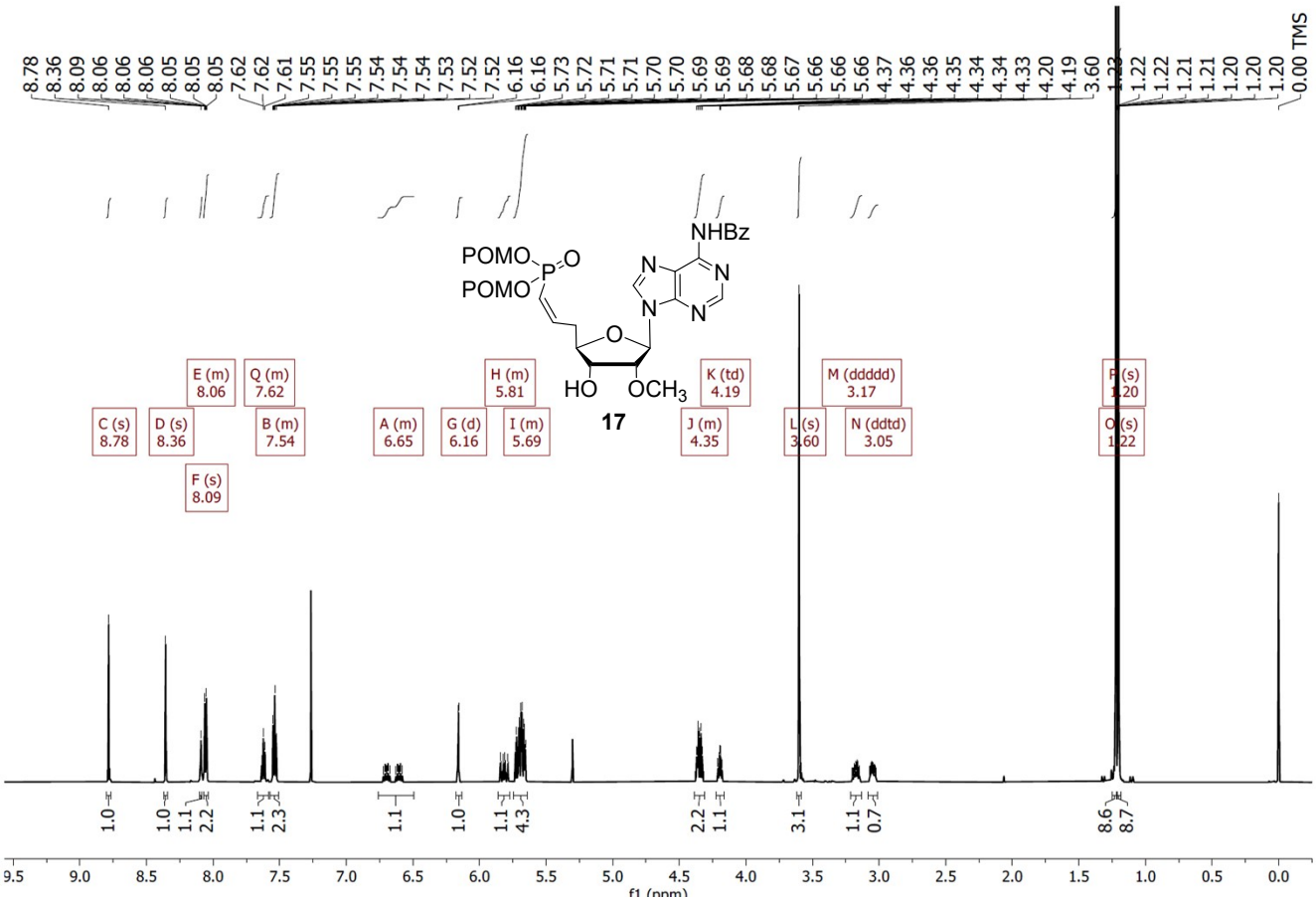
^{1}H NMR (600 MHz, DMSO-d_6) of compoud **16**



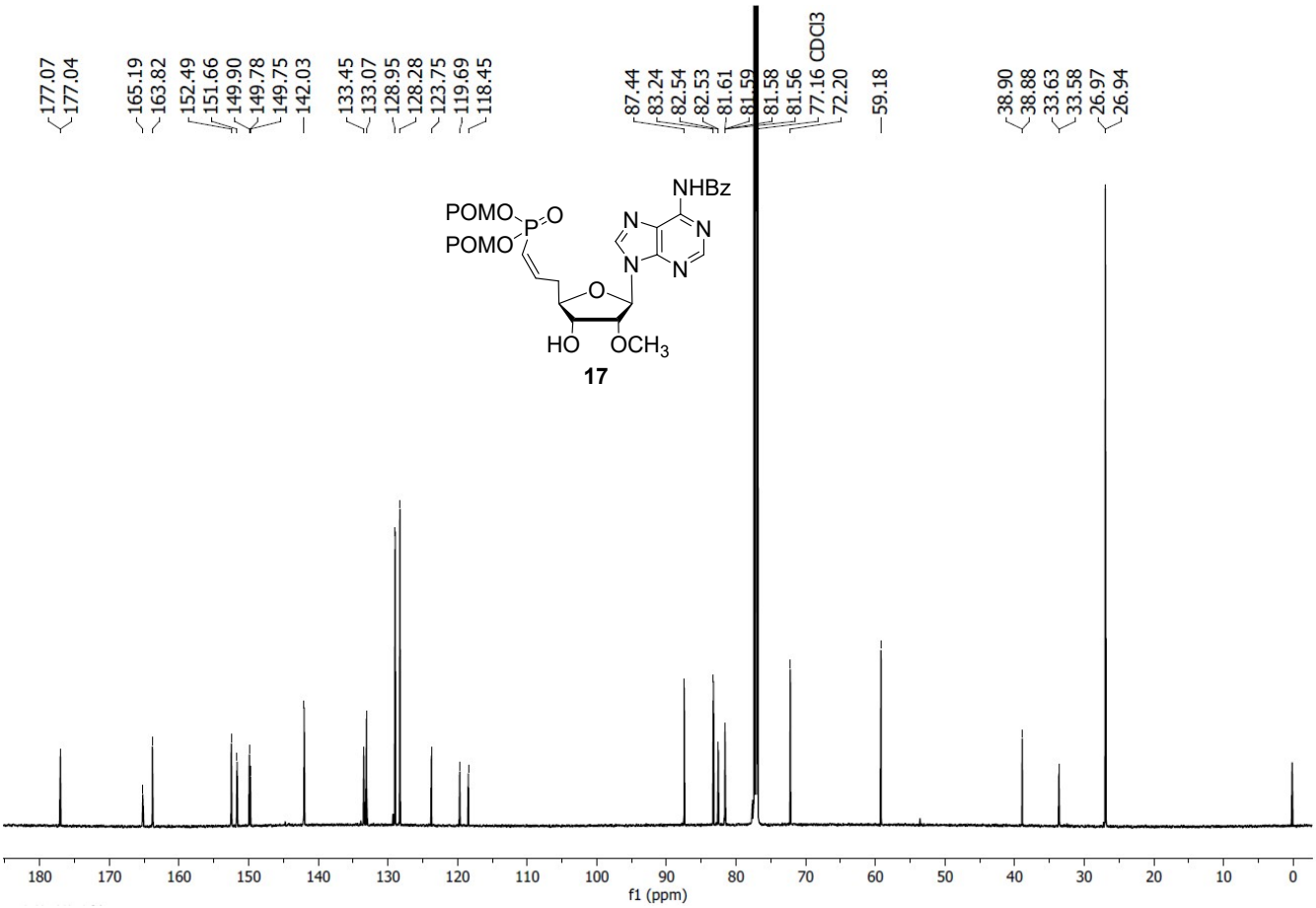
^{13}C NMR (151 MHz, DMSO-d_6) of compound **16**



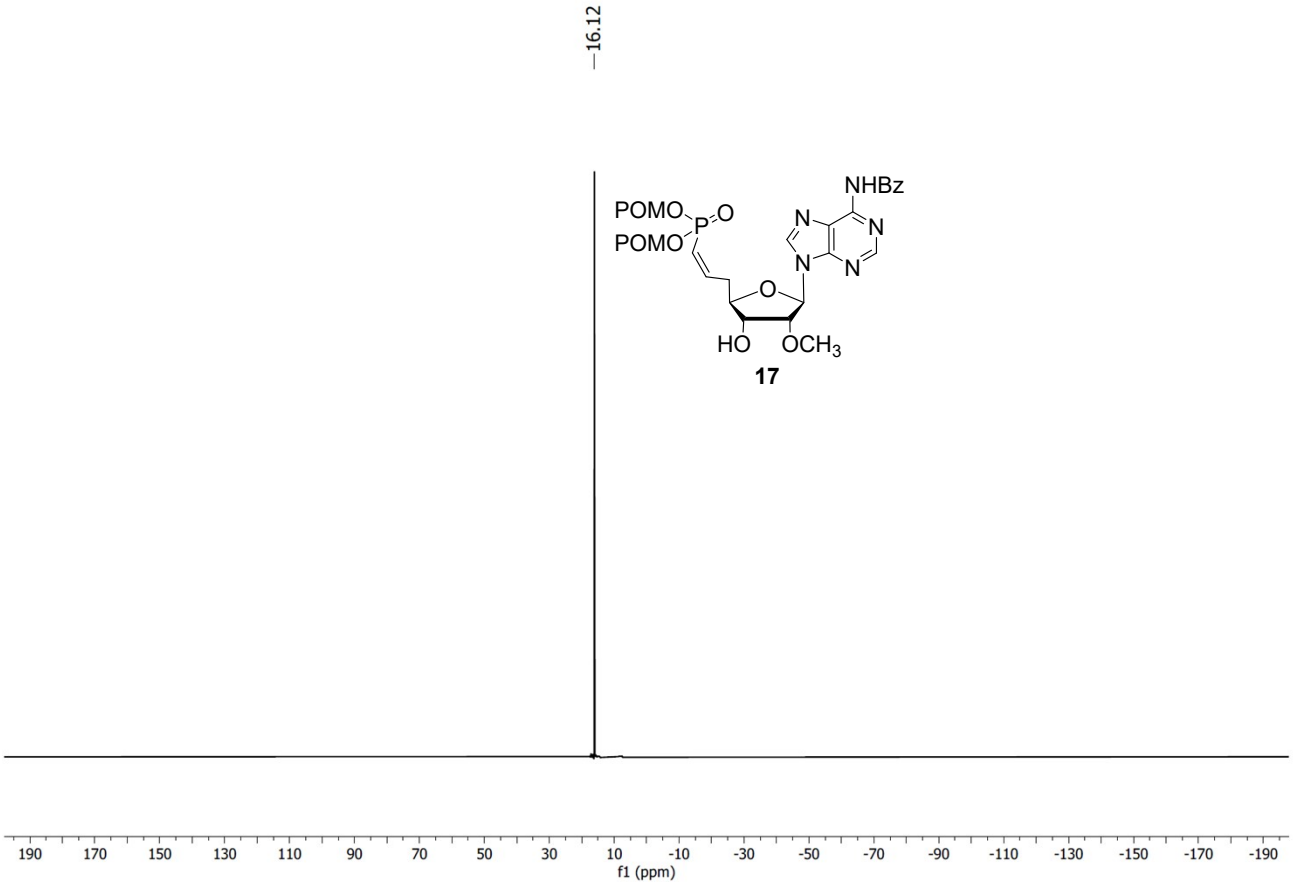
^{31}P NMR (243 MHz, DMSO-d_6) of compoud **16**



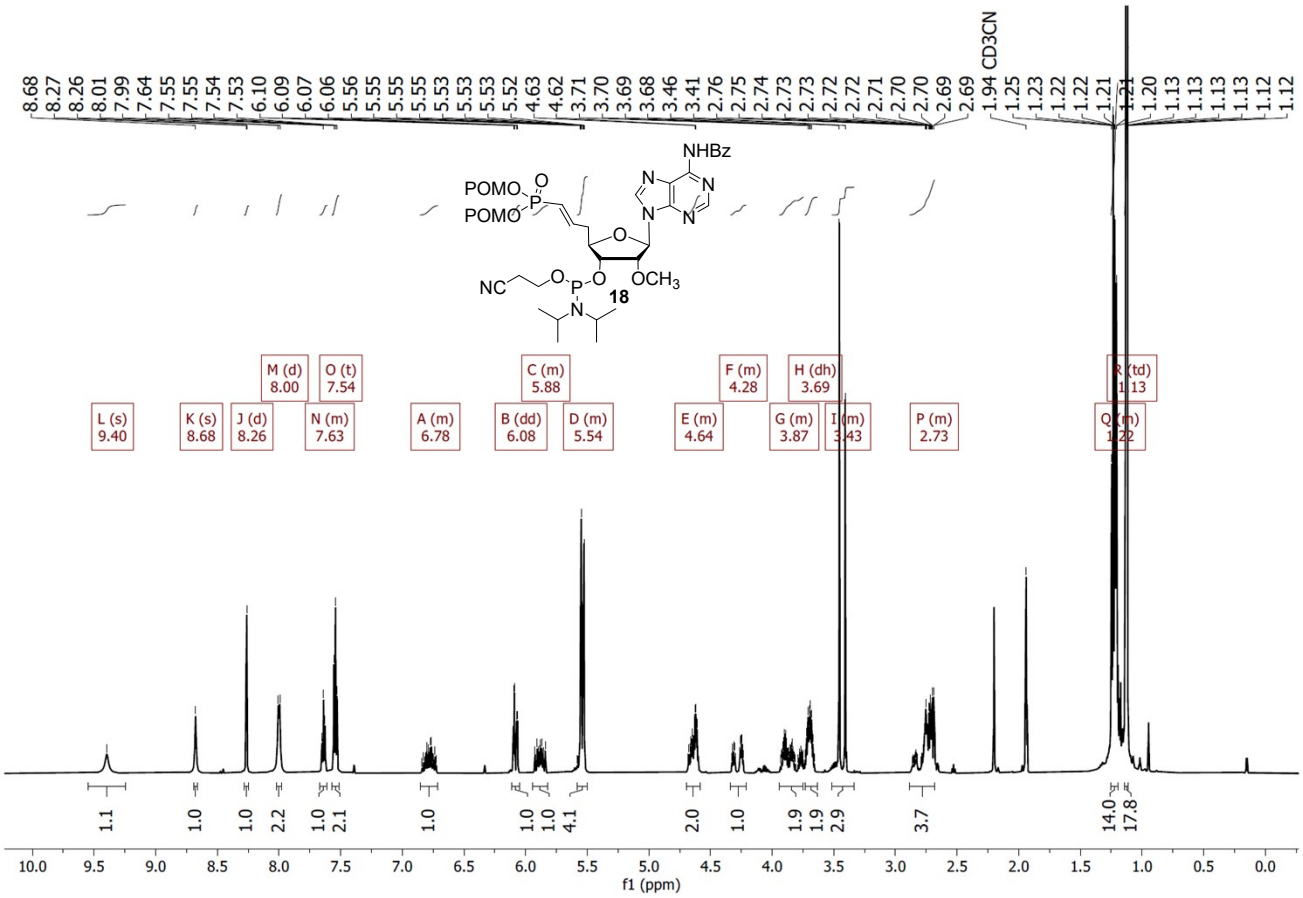
^{1}H NMR (600 MHz, $CDCl_3$) of compoud **17**



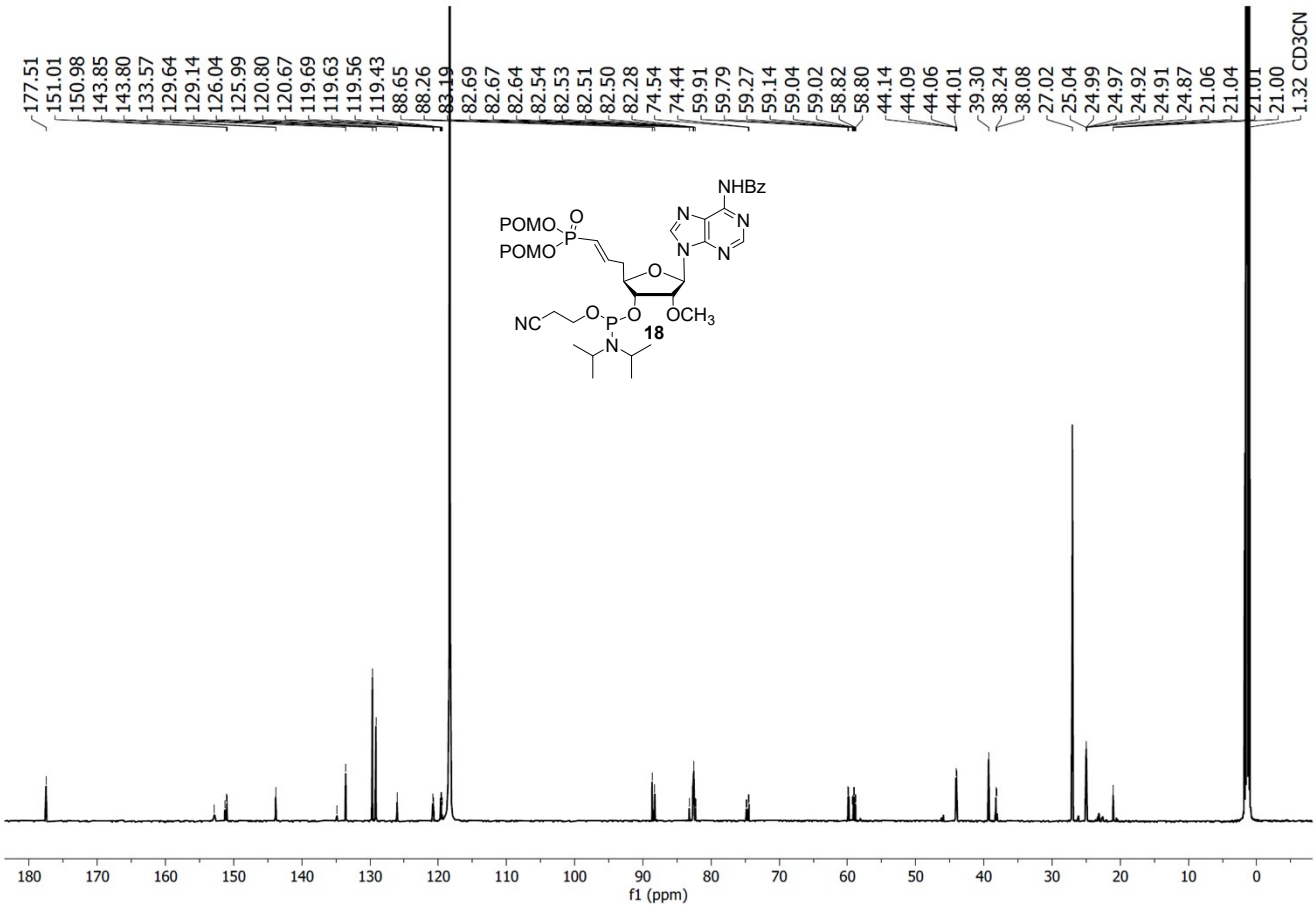
^{13}C NMR (151 MHz, $CDCl_3$) of compound **17**



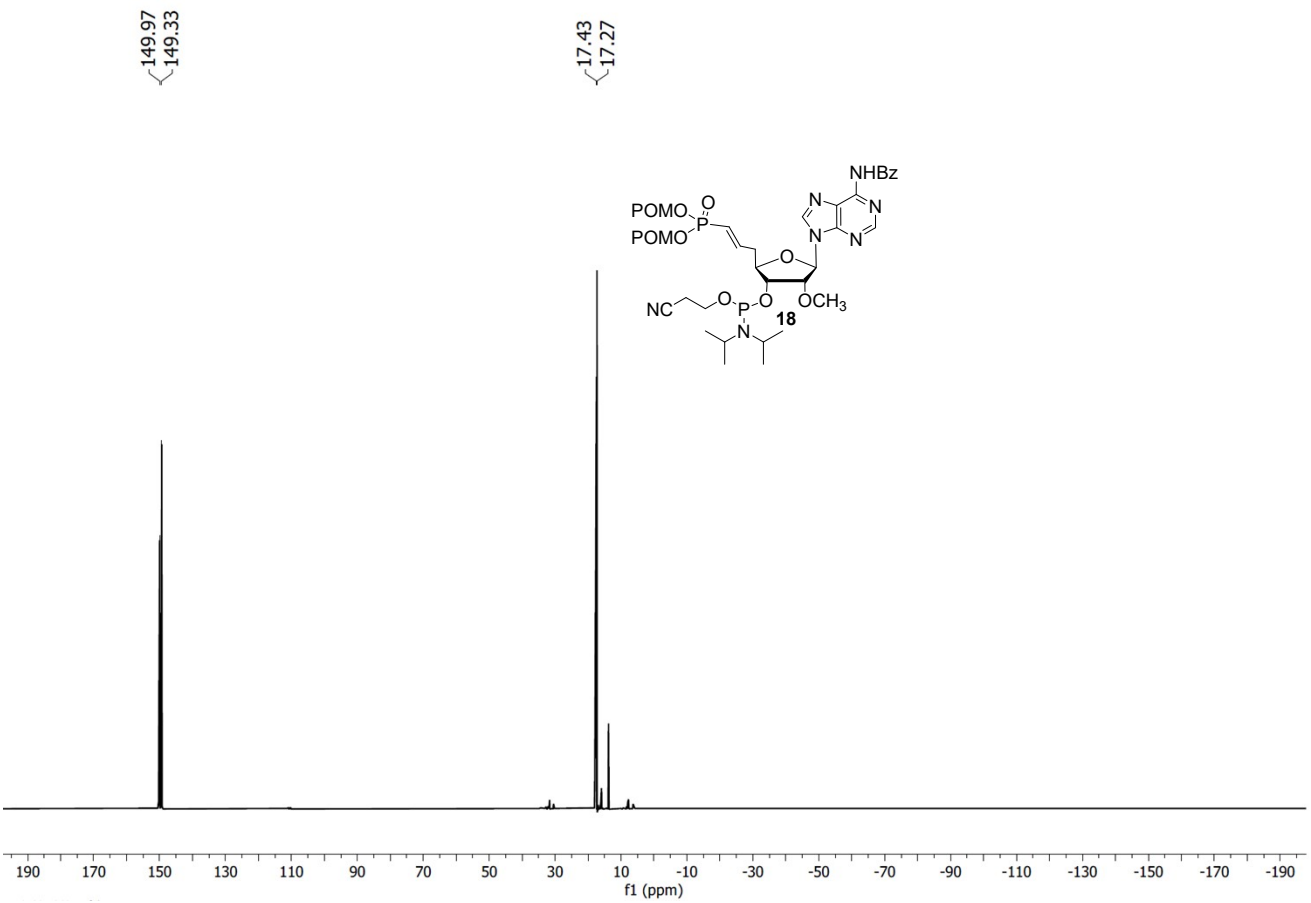
^{31}P NMR (243 MHz, $CDCl_3$) of compoud **17**



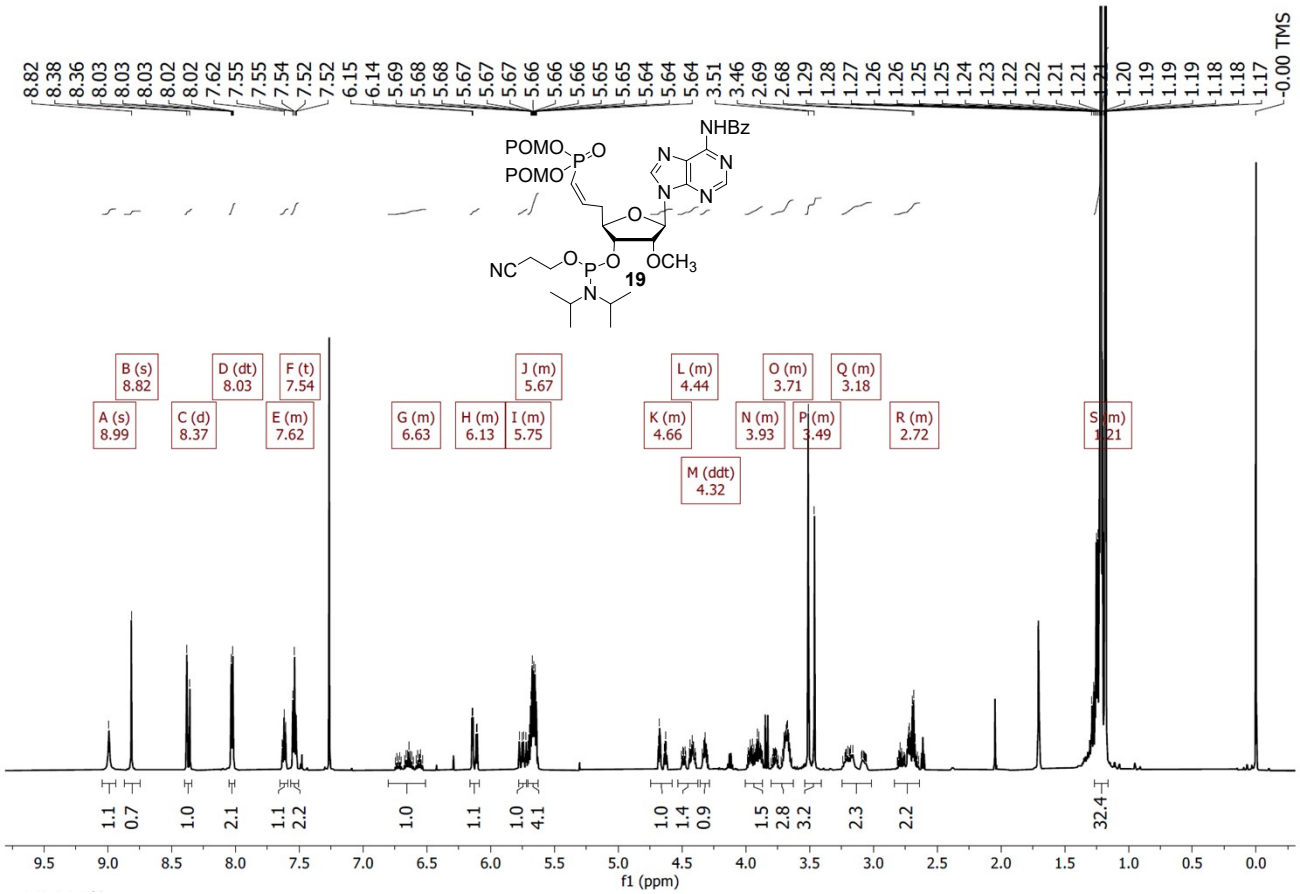
^{1}H NMR (600 MHz, CD_3CN) of compound **18**



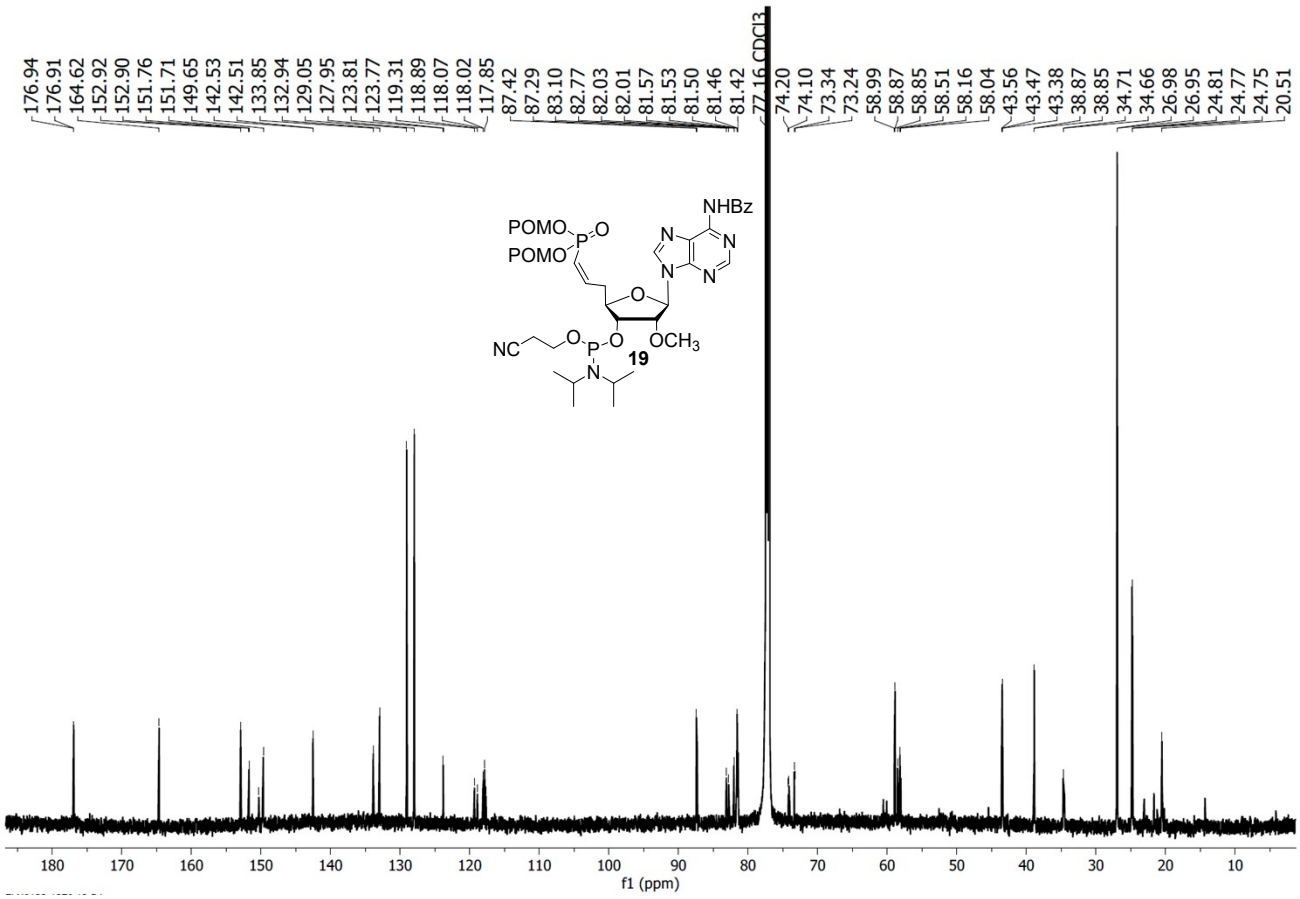
^{13}C NMR (151 MHz, CD_3CN) of compound **18**



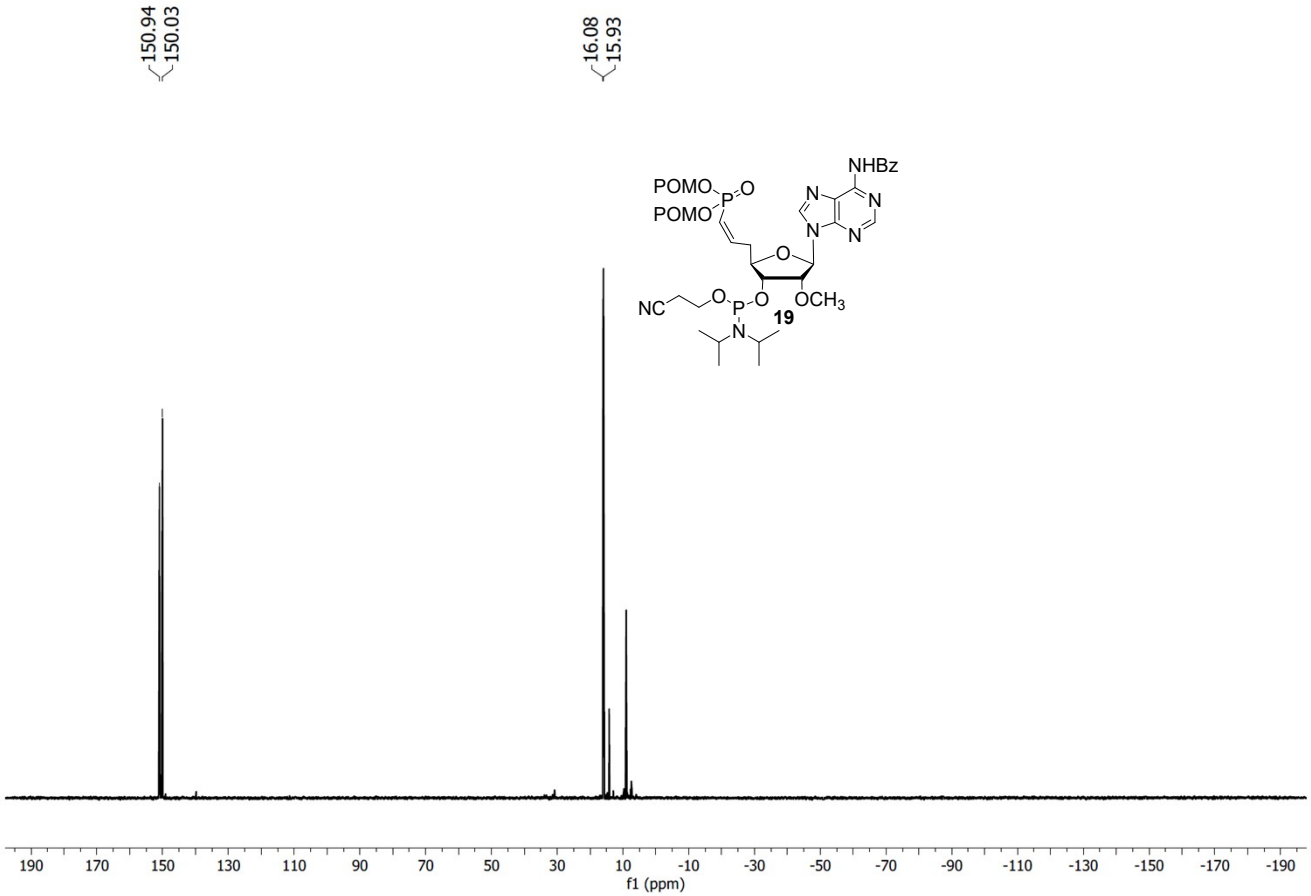
^{31}P NMR (243 MHz, CD_3CN) of compound **18**



^{1}H NMR (600 MHz, $CDCl_3$) of compoud **19**

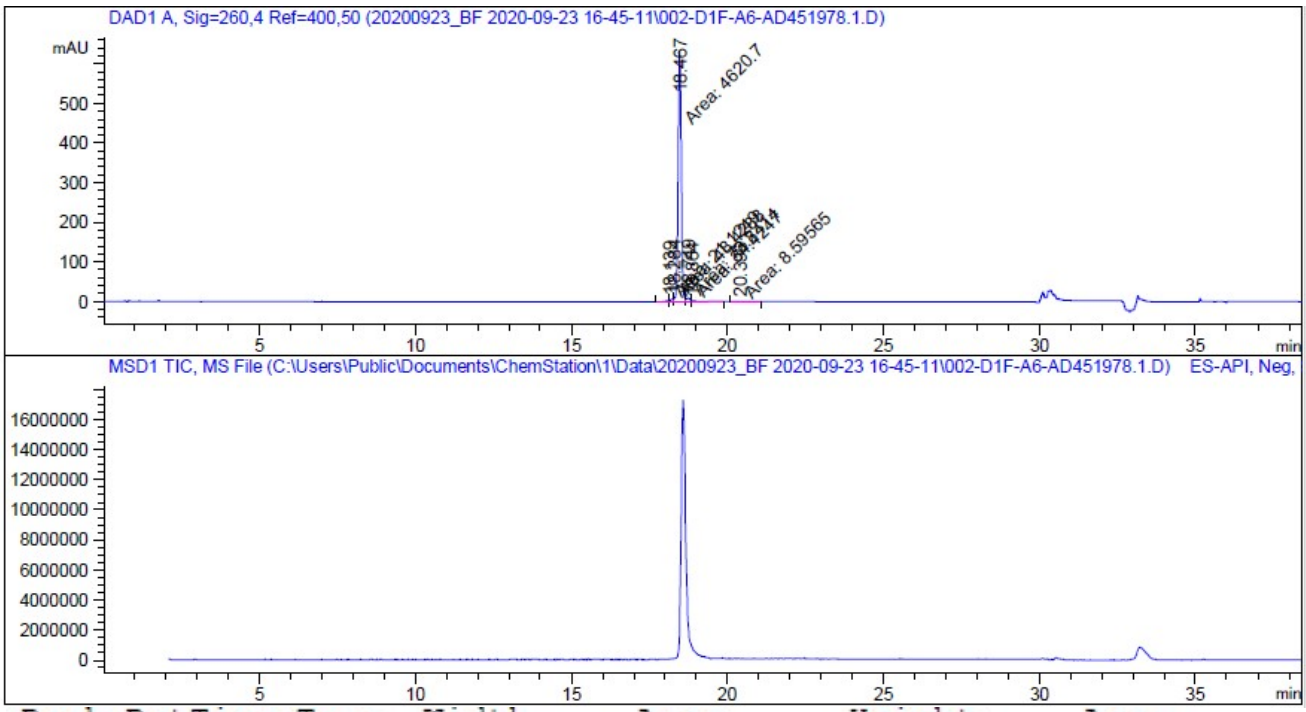


^{13}C NMR (151 MHz, $CDCl_3$) of compound **19**



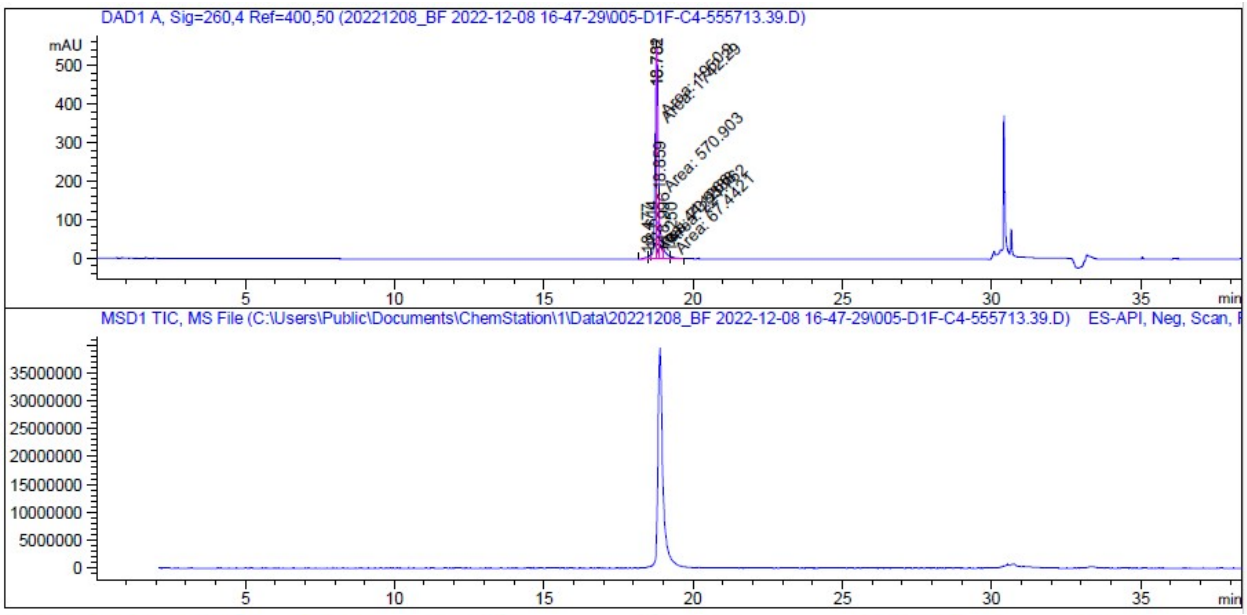
^{31}P NMR (243 MHz, $CDCl_3$) of compoud **19**

LCMS profiles of oligonucleotides



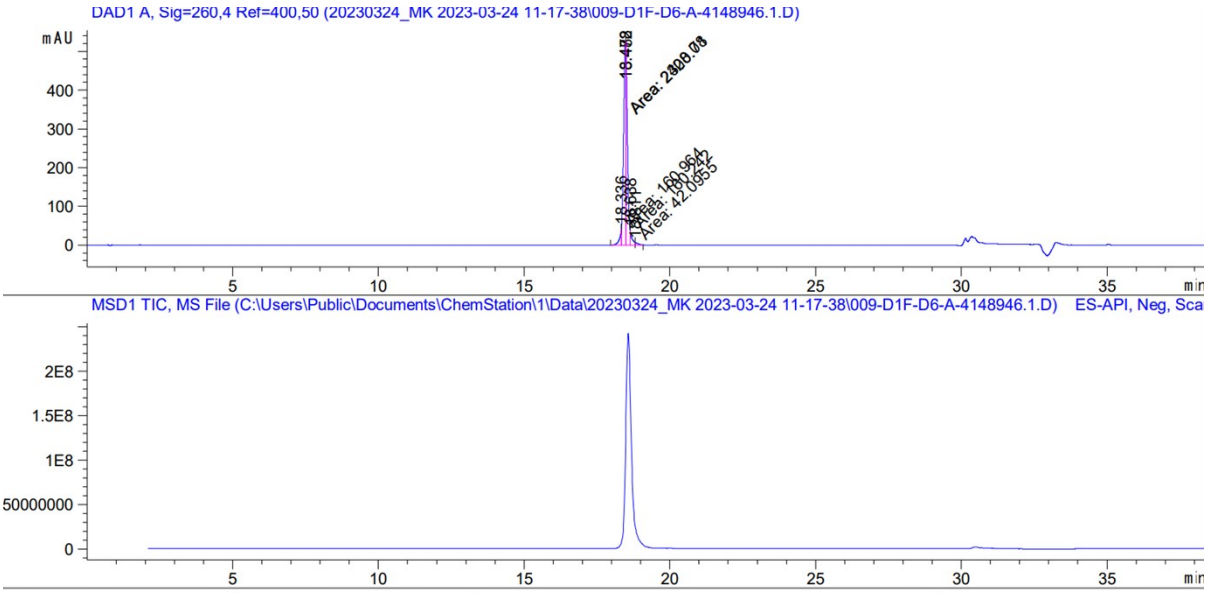
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.139	MF	0.1144	21.12487	3.07740	0.4395
2	18.284	MF	0.1010	48.42877	7.99512	1.0074
3	18.467	MF	0.1217	4620.69727	632.55286	96.1221
4	18.749	MF	0.1382	73.84138	8.90624	1.5361
5	18.864	FM	0.2410	34.42470	2.38096	0.7161
6	20.396	MM	0.2940	8.59565	4.87358e-1	0.1788

LCMS profile of **ON1**



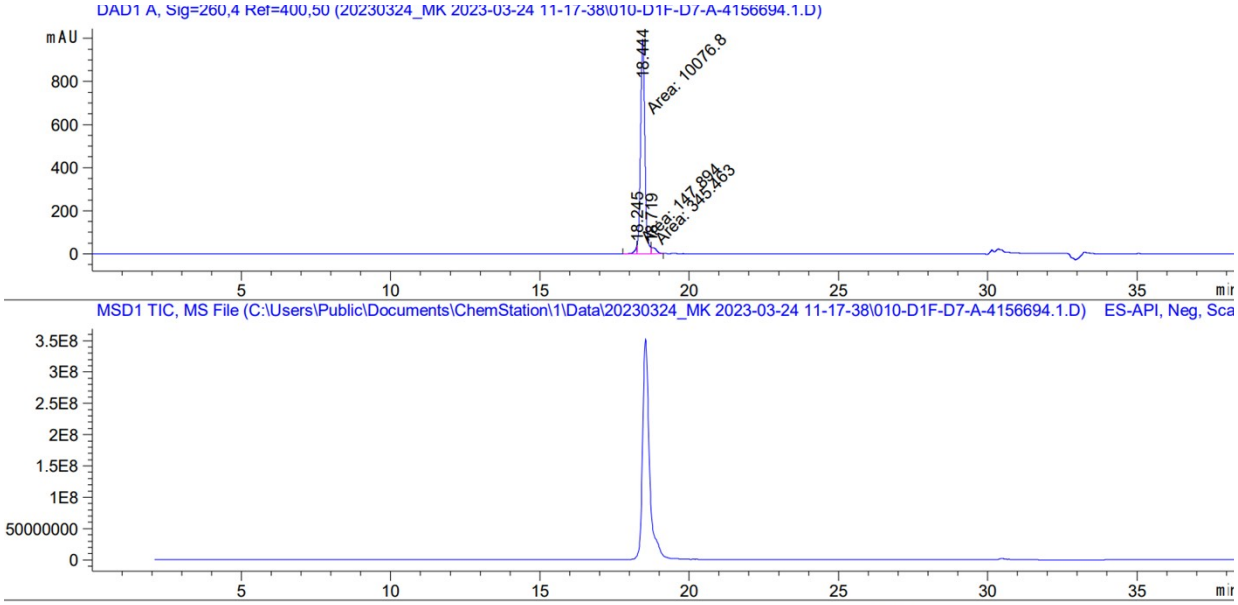
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.477	MF	0.1471	44.12663	5.00026	0.9452
2	18.614	MF	0.0957	70.93881	12.35623	1.5196
3	18.781	MF	0.0592	1950.90247	549.20850	41.7898
4	18.782	FM	0.0528	1742.29260	549.52917	37.3212
5	18.859	FM	0.0574	570.90283	165.76703	12.2292
6	18.996	FM	0.1159	221.76181	31.88197	4.7503
7	19.250	FM	0.1769	67.44212	6.35579	1.4447

LCMS profile of **ON2**



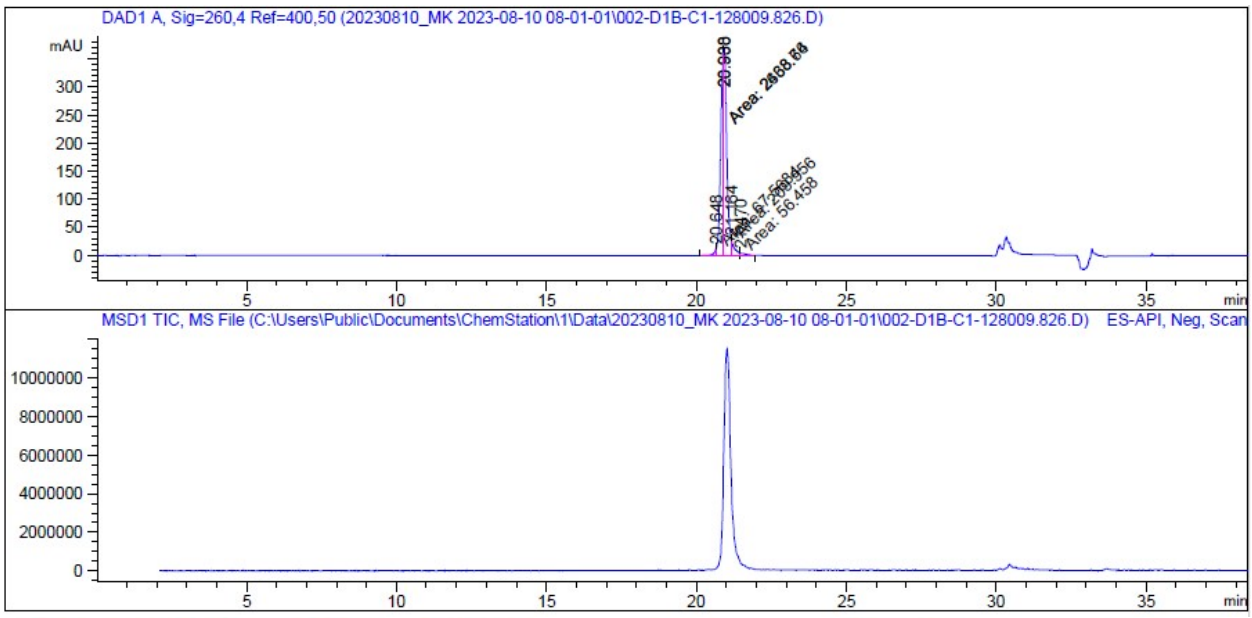
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.336	MF	0.0647	160.96371	41.46965	3.1610
2	18.478	MF	0.0768	2428.08228	526.71533	47.6833
3	18.482	FM	0.0729	2300.71484	525.77032	45.1821
4	18.638	MF	0.0622	160.24207	36.88239	3.1469
5	18.811	FM	0.1097	42.09549	6.39765	0.8267

LCMS profile of **ON3**



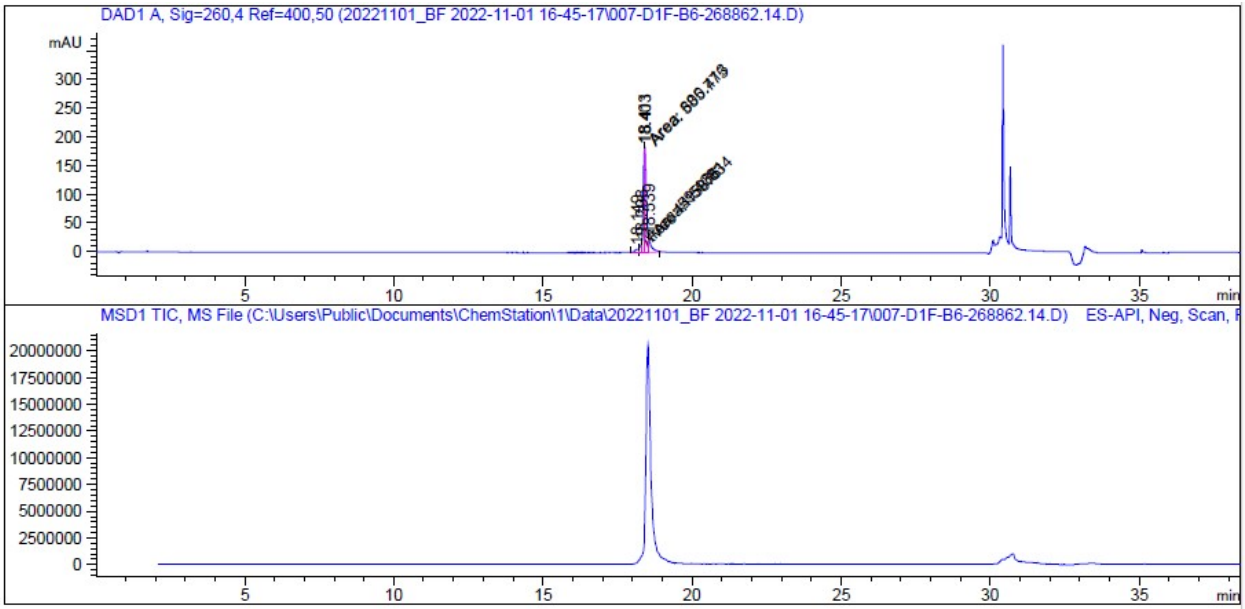
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.245	MF	0.0709	147.89392	34.78245	1.3992
2	18.444	FM	0.1688	1.00768e4	994.90497	95.3326
3	18.719	FM	0.1980	345.46283	29.07518	3.2683

LCMS profile of **ON4**



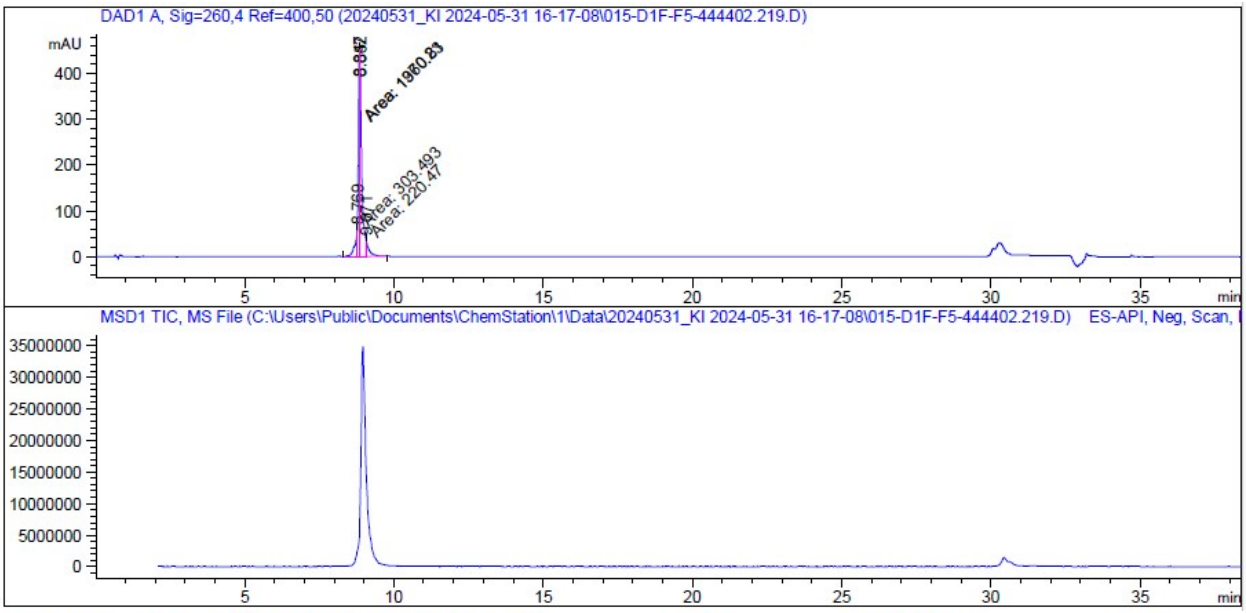
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	20.648	MF	0.0971	67.56838	11.59617	1.2417
2	20.908	MF	0.1120	2438.76416	362.86066	44.8188
3	20.930	FM	0.1201	2668.64160	370.23245	49.0434
4	21.164	FM	0.1268	209.95596	27.60480	3.8585
5	21.470	FM	0.2153	56.45798	4.37113	1.0376

LCMS profile of **ON5**



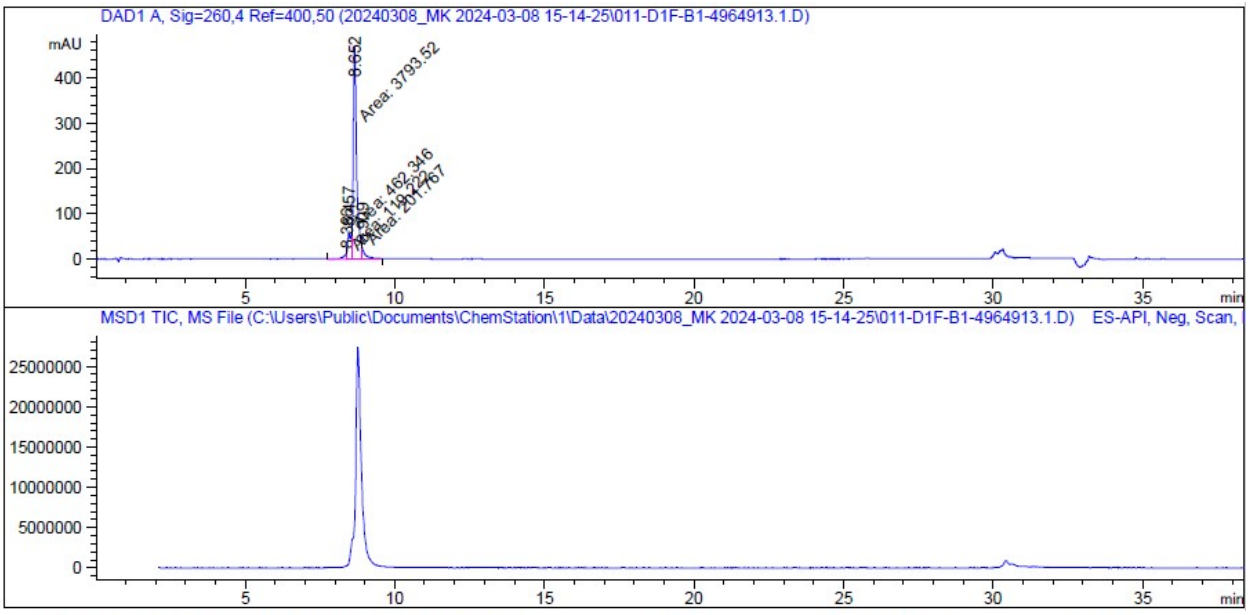
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.149	MF	0.1351	44.59863	5.50390	2.7045
2	18.298	MF	0.0689	59.43812	14.38445	3.6044
3	18.403	MF	0.0539	585.77515	180.99913	35.5218
4	18.411	FM	0.0727	800.41278	183.46268	48.5376
5	18.539	FM	0.1120	158.83356	23.62879	9.6318

LCMS profile of **ON6**



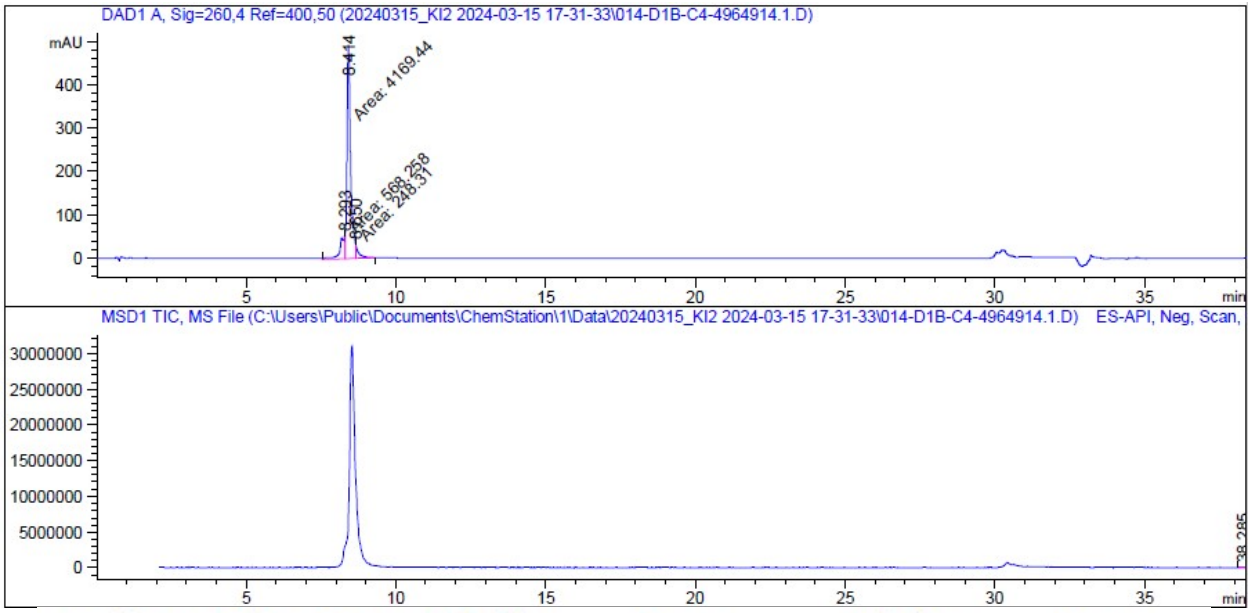
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.769	MF	0.0861	303.49307	58.75495	7.8727
2	8.847	MF	0.0498	1370.23035	458.90942	35.5442
3	8.852	FM	0.0717	1960.80884	455.60782	50.8640
4	9.071	FM	0.1070	220.47028	34.33929	5.7191

LCMS profile of **ON7**



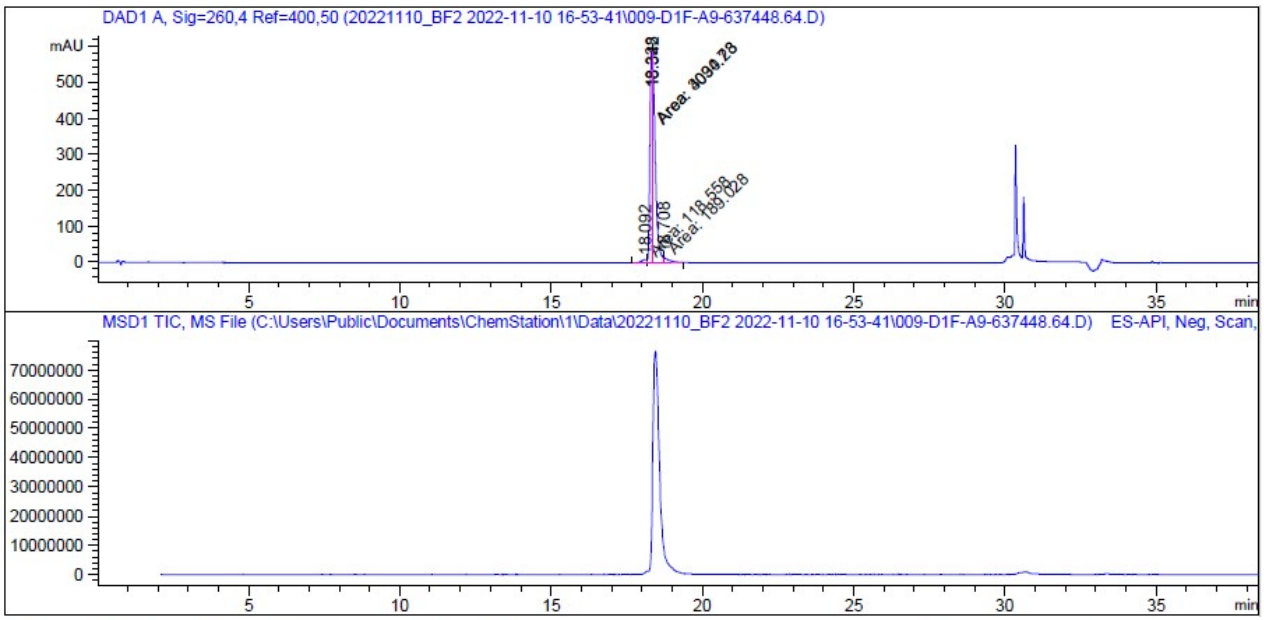
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.386	MF	0.1494	119.22225	13.30217	2.6049
2	8.457	FM	0.1252	462.34650	61.56461	10.1018
3	8.652	MF	0.1342	3793.51685	470.95563	82.8848
4	8.909	FM	0.1391	201.76714	24.17240	4.4084

LCMS profile of **ON8**



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.293	MF	0.1814	568.25812	52.21454	11.3970
2	8.414	MF	0.1413	4169.44434	491.82159	83.6228
3	8.650	FM	0.1226	248.30951	33.75040	4.9801

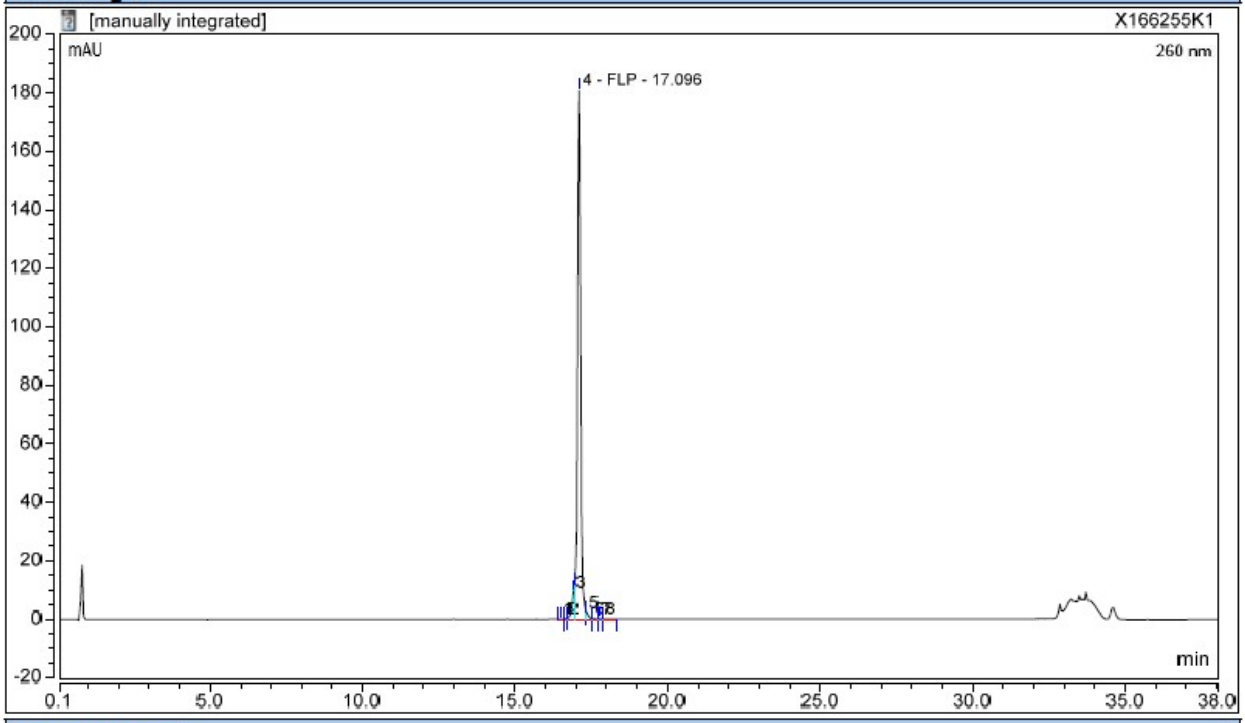
LCMS profile of **ON9**



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.092	MF	0.2233	118.55791	8.84803	1.5951
2	18.328	MF	0.0862	3094.77832	598.41614	41.6379
3	18.342	FM	0.1136	4030.23486	591.36383	54.2238
4	18.708	FM	0.2067	189.02788	15.24283	2.5432

LCMS profile of **ON10**

Chromatogram

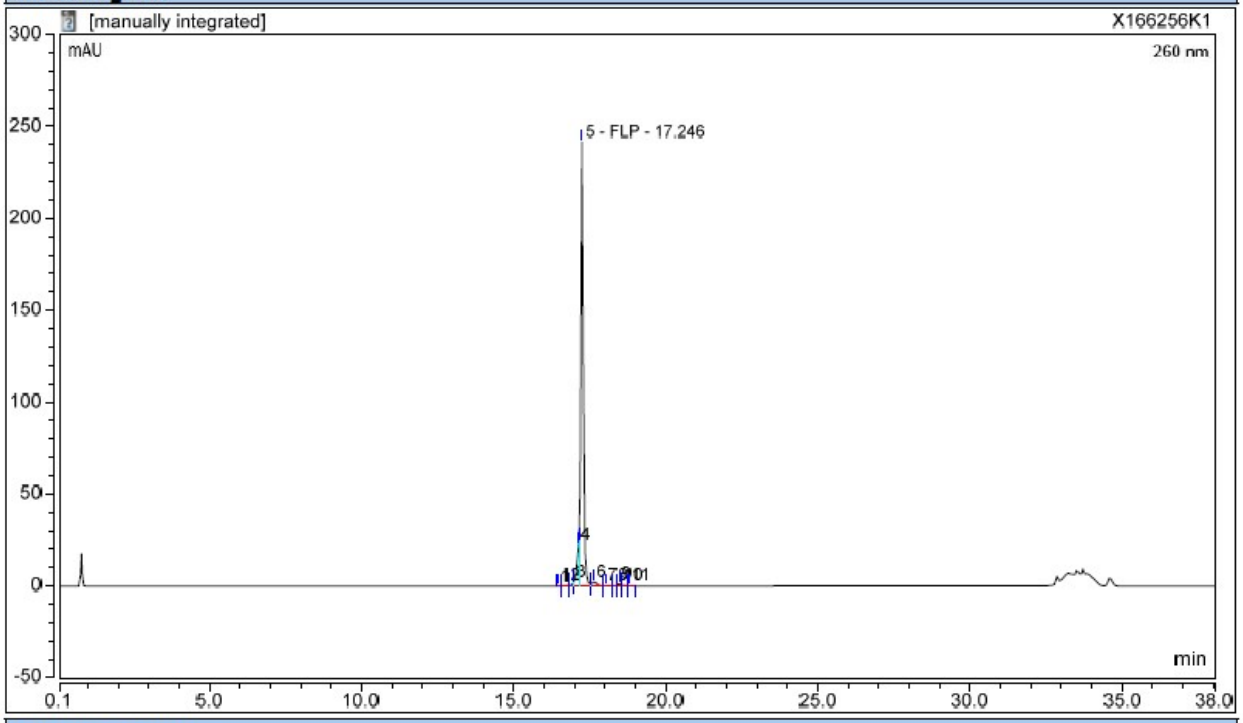


Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Peak Width min	Res. (USP) to FLP
1		16.50	0.008	0.075	0.0	n.a.	n.a.
2		16.69	0.026	0.299	0.1	n.a.	n.a.
3		16.91	0.972	9.257	4.3	n.a.	n.a.
4	FLP	17.10	21.454	180.755	94.5	0.18	0.00
5		17.34	0.162	2.391	0.7	n.a.	n.a.
6		17.54	0.026	0.229	0.1	n.a.	n.a.
7		17.77	0.026	0.212	0.1	n.a.	n.a.
8		17.88	0.018	0.097	0.1	n.a.	n.a.
Total:			**22.692**	**193.315**	**100.00**	**0.18**	

LCMS profile of **ON11**

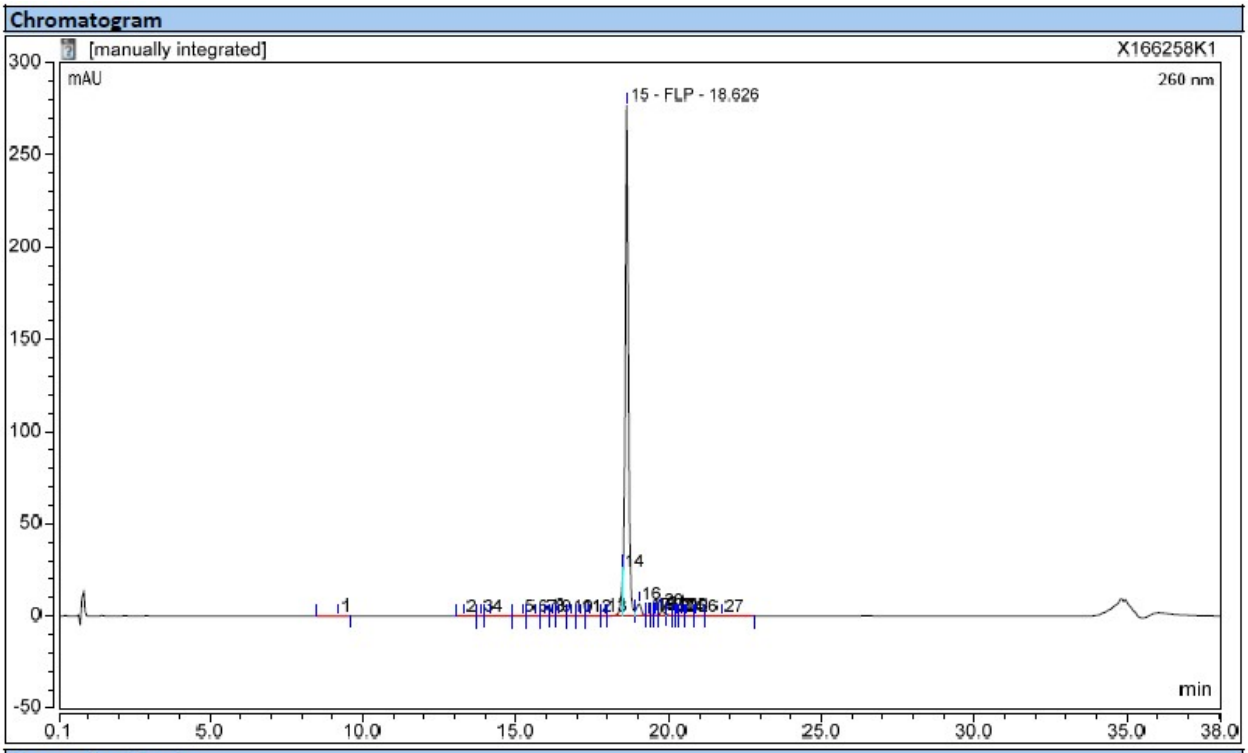
Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Peak Width min	Res. (USP) to FLP
1		16.46	0.007	0.060	0.0	n.a.	n.a.
2		16.79	0.038	0.328	0.1	n.a.	n.a.
3		16.97	0.154	1.822	0.6	n.a.	n.a.
4		17.13	1.867	22.586	7.0	n.a.	n.a.
5	FLP	17.25	23.997	241.428	89.8	0.15	0.00
6		17.65	0.404	2.059	1.5	0.34	0.02
7		18.02	0.040	0.185	0.2	0.28	0.04
8		18.37	0.021	0.187	0.1	n.a.	n.a.
9		18.47	0.144	1.337	0.5	0.17	0.08
10		18.57	0.042	0.385	0.2	n.a.	n.a.
11		18.81	0.018	0.137	0.1	0.13	0.11
Total:			**26.733**	**270.514**	**100.00**	**1.07**	

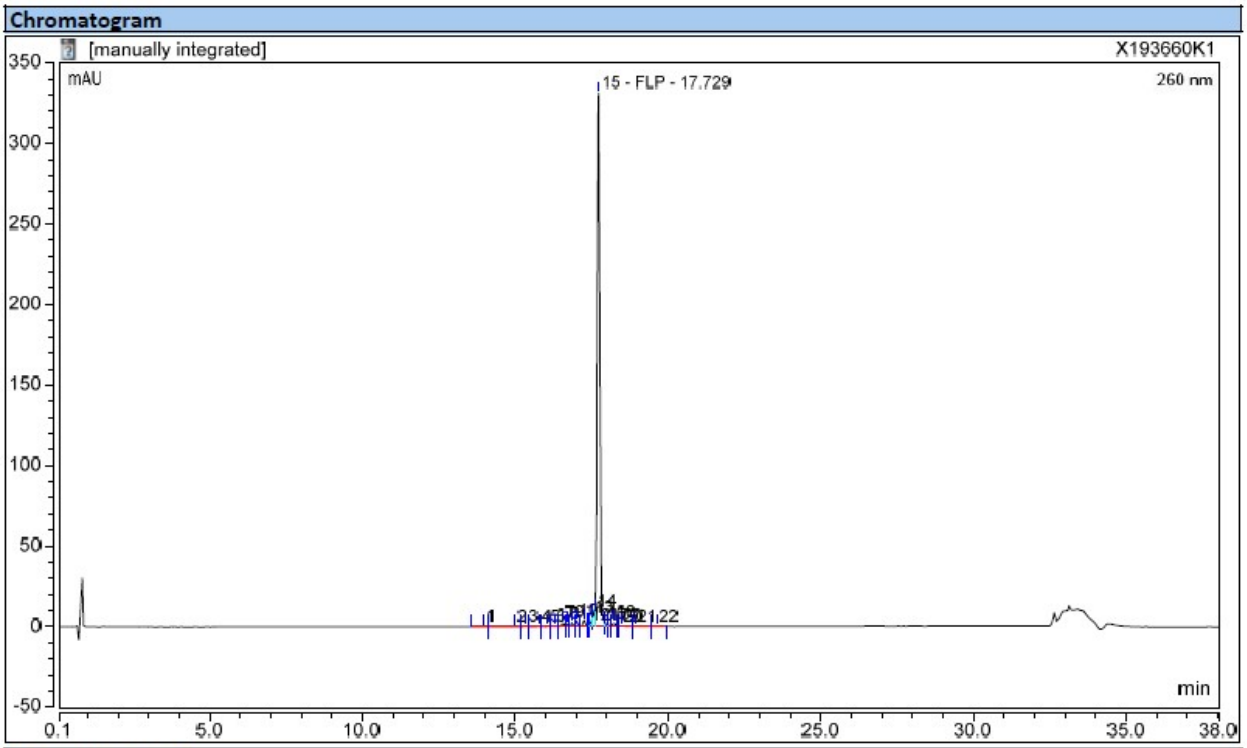
LCMS profile of **ON12**



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Peak Width min	Res. (USP) to FLP
1		9.20	0.016	0.066	0.0	0.14	0.58
2		13.32	0.011	0.055	0.0	0.11	0.36
3		13.87	0.009	0.055	0.0	0.12	0.31
4		14.17	0.024	0.061	0.1	0.09	0.32
5		15.22	0.010	0.061	0.0	n.a.	n.a.
6		15.66	0.005	0.027	0.0	0.10	0.21
7		15.95	0.004	0.021	0.0	0.08	0.21
8		16.20	0.019	0.147	0.0	n.a.	n.a.
9		16.39	0.014	0.075	0.0	n.a.	n.a.
10		16.80	0.009	0.050	0.0	0.06	0.15
11		17.11	0.008	0.051	0.0	0.00	0.16
12		17.41	0.022	0.058	0.1	0.06	0.10
13		17.92	0.015	0.103	0.0	0.19	0.04
14		18.50	1.734	24.133	4.6	n.a.	n.a.
15	FLP	18.63	33.564	276.905	89.1	0.18	0.00
16		19.03	1.273	6.732	3.4	0.31	0.02
17		19.34	0.062	0.452	0.2	n.a.	n.a.
18		19.49	0.049	0.522	0.1	n.a.	n.a.
19		19.57	0.074	0.585	0.2	0.40	0.03
20		19.79	0.458	3.349	1.2	0.21	0.06
21		19.90	0.121	1.094	0.3	n.a.	n.a.
22		20.09	0.027	0.246	0.1	n.a.	n.a.
23		20.28	0.022	0.209	0.1	n.a.	n.a.
24		20.43	0.027	0.176	0.1	0.26	0.08
25		20.56	0.024	0.104	0.1	n.a.	n.a.
26		20.90	0.012	0.054	0.0	n.a.	n.a.
27		21.74	0.036	0.054	0.1	0.21	0.16
Total:			**37.650**	**315.446**	**100.00**	**2.54**	

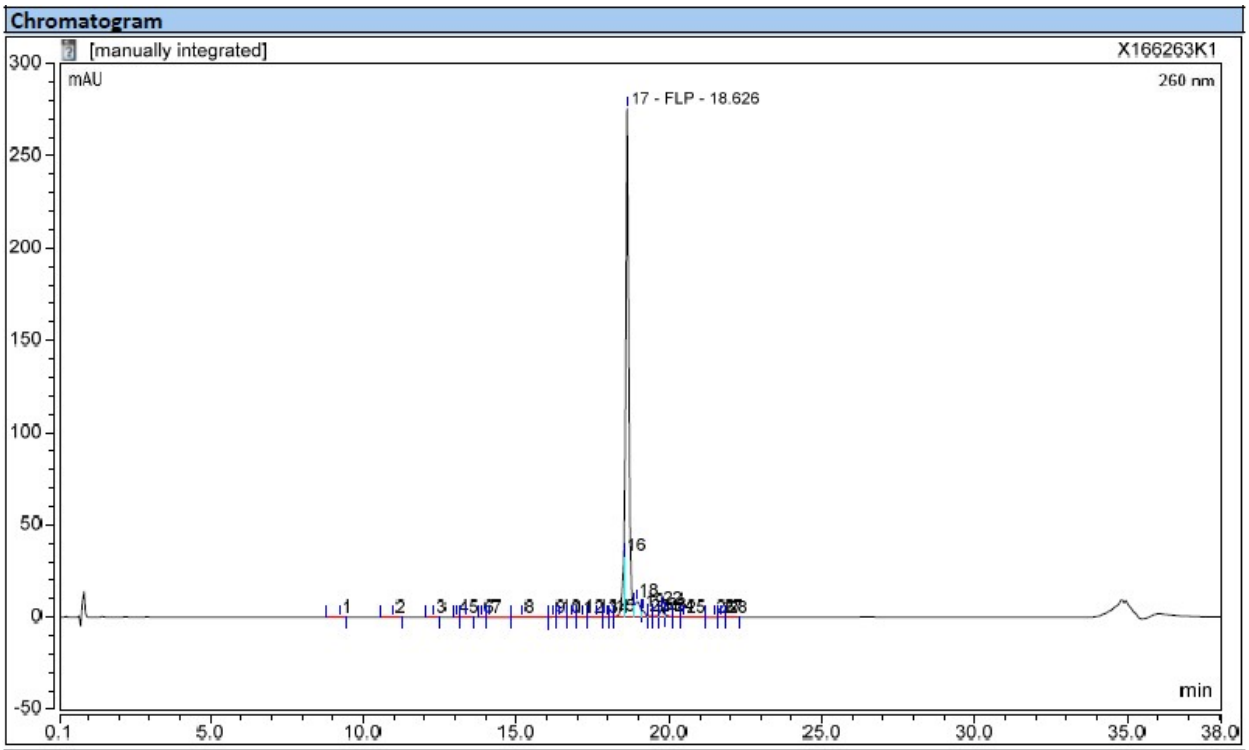
LCMS profile of **ON13**



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Peak Width min	Res. (USP) to FLP
1		13.97	0.026	0.084	0.1	0.10	0.29
2		14.99	0.028	0.049	0.1	0.07	0.24
3		15.36	0.024	0.227	0.1	0.15	0.16
4		15.79	0.022	0.087	0.1	0.15	0.13
5		16.05	0.019	0.093	0.1	0.24	0.09
6		16.28	0.046	0.325	0.1	0.16	0.09
7		16.57	0.199	2.327	0.5	0.13	0.08
8		16.70	0.148	1.791	0.4	0.13	0.07
9		16.87	0.203	2.419	0.6	0.13	0.06
10		17.03	0.294	3.335	0.8	0.13	0.05
11		17.26	0.341	3.790	0.9	0.13	0.03
12		17.42	0.064	1.090	0.2	n.a.	n.a.
13		17.54	0.374	5.923	1.0	n.a.	n.a.
14		17.60	0.484	9.942	1.3	n.a.	n.a.
15	FLP	17.73	33.848	331.081	92.1	0.15	0.00
16		17.93	0.142	2.272	0.4	n.a.	n.a.
17		18.03	0.084	0.999	0.2	n.a.	n.a.
18		18.24	0.251	2.611	0.7	0.15	0.03
19		18.33	0.015	0.340	0.0	n.a.	n.a.
20		18.47	0.092	0.329	0.2	n.a.	n.a.
21		18.93	0.037	0.091	0.1	0.16	0.08
22		19.63	0.017	0.067	0.0	0.10	0.15
Total:			**36.759**	**369.272**	**100.00**	**2.10**	

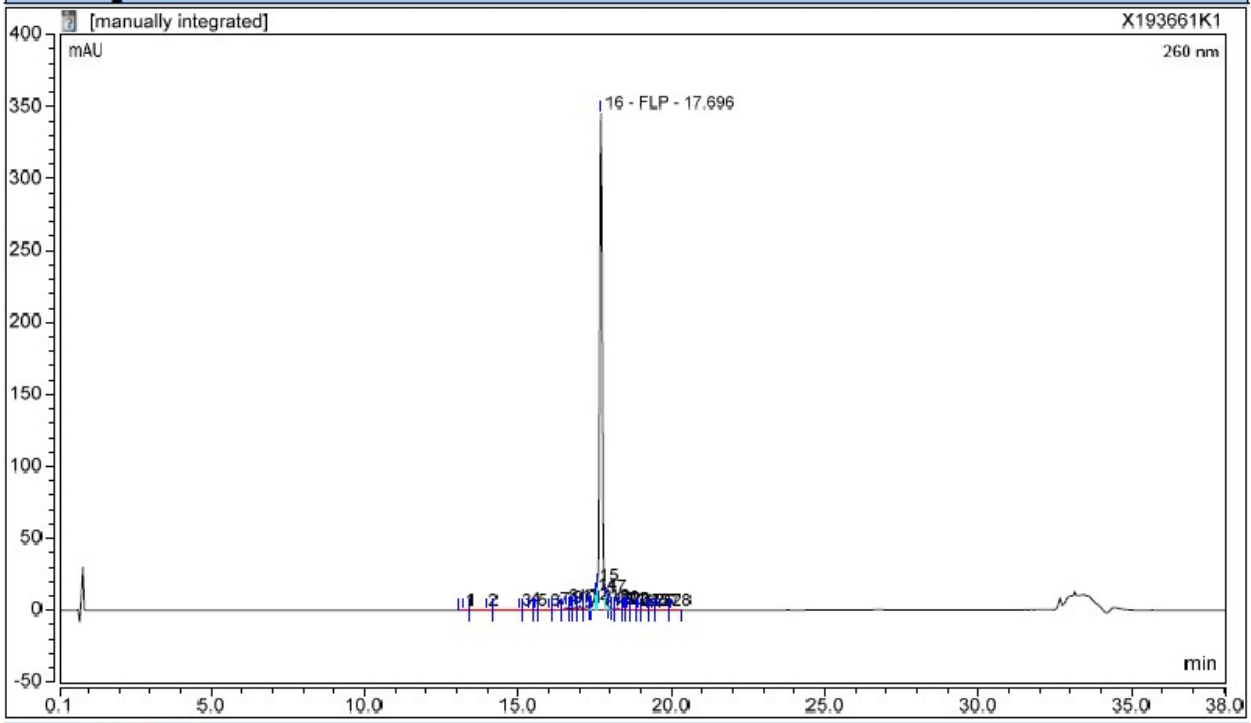
LCMS profile of **ON14**



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Peak Width min	Res. (USP) to FLP
1		9.22	0.023	0.102	0.1	0.15	0.56
2		10.97	0.014	0.060	0.0	0.18	0.42
3		12.31	0.012	0.075	0.0	n.a.	n.a.
4		13.07	0.011	0.138	0.0	0.14	0.35
5		13.36	0.012	0.073	0.0	0.14	0.32
6		13.88	0.010	0.072	0.0	n.a.	n.a.
7		14.13	0.019	0.059	0.0	0.12	0.30
8		15.19	0.025	0.083	0.1	0.19	0.18
9		16.21	0.025	0.214	0.1	0.23	0.12
10		16.41	0.021	0.116	0.1	0.14	0.14
11		16.80	0.010	0.059	0.0	0.09	0.13
12		17.17	0.012	0.070	0.0	0.15	0.09
13		17.62	0.028	0.069	0.1	0.21	0.05
14		17.98	0.016	0.107	0.0	0.23	0.03
15		18.18	0.018	0.225	0.0	n.a.	n.a.
16		18.52	2.047	33.253	5.3	n.a.	n.a.
17	FLP	18.63	33.201	275.640	85.3	0.18	0.00
18		18.97	1.861	8.671	4.8	0.51	0.01
19		19.15	0.393	3.552	1.0	n.a.	n.a.
20		19.30	0.097	0.750	0.2	n.a.	n.a.
21		19.64	0.088	0.746	0.2	n.a.	n.a.
22		19.74	0.621	4.486	1.6	0.23	0.05
23		19.86	0.181	1.446	0.5	n.a.	n.a.
24		20.11	0.055	0.298	0.1	n.a.	n.a.
25		20.49	0.071	0.149	0.2	n.a.	n.a.
26		21.47	0.030	0.135	0.1	0.60	0.07
27		21.68	0.020	0.132	0.1	0.24	0.14
28		21.85	0.007	0.035	0.0	n.a.	n.a.
Total:			**38.929**	**330.817**	**100.00**	**3.74**	

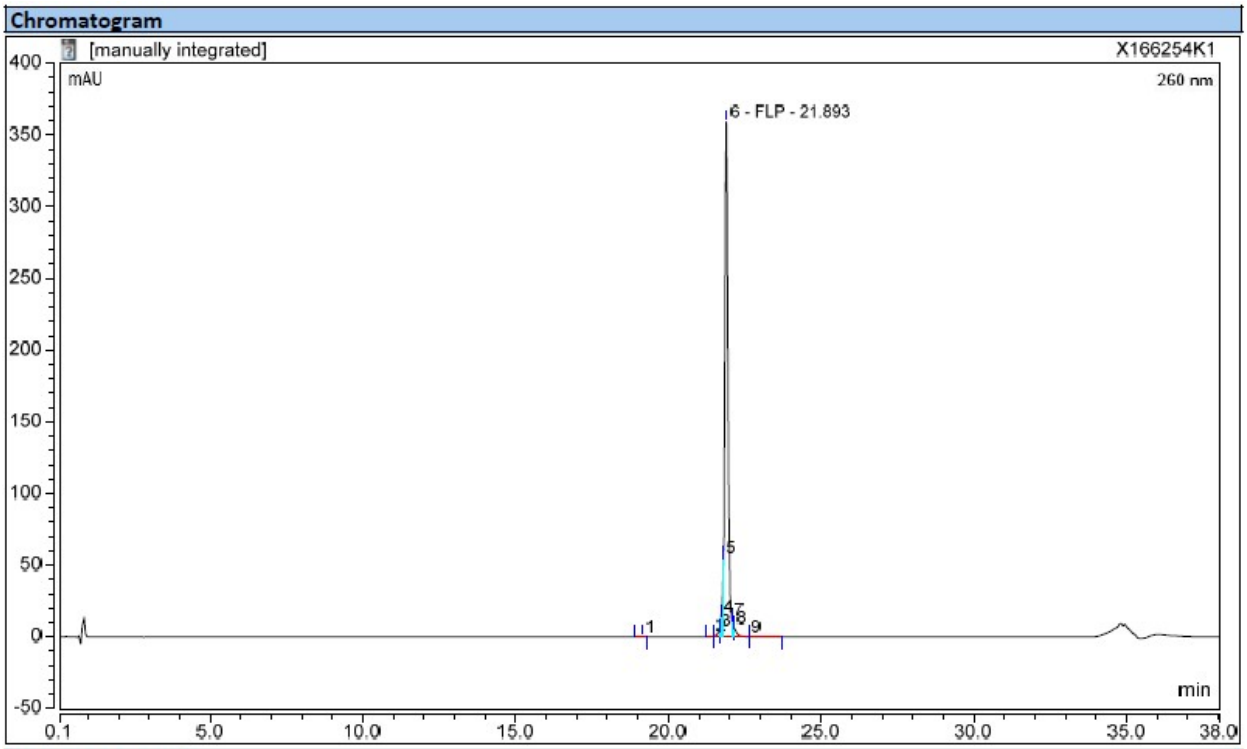
LCMS profile of **ON15**



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Peak Width min	Res. (USP) to FLP
1		13.20	0.005	0.028	0.0	0.08	0.39
2		13.95	0.026	0.074	0.1	0.11	0.28
3		15.05	0.021	0.053	0.1	0.05	0.25
4		15.35	0.032	0.271	0.1	0.14	0.16
5		15.55	0.008	0.085	0.0	0.17	0.13
6		15.98	0.032	0.088	0.1	0.34	0.07
7		16.29	0.049	0.369	0.1	0.16	0.09
8		16.57	0.201	2.400	0.5	0.13	0.08
9		16.71	0.145	1.769	0.4	0.13	0.07
10		16.86	0.191	2.245	0.5	0.13	0.06
11		17.01	0.283	3.297	0.7	0.13	0.05
12		17.25	0.344	3.695	0.9	0.14	0.03
13		17.39	0.107	1.849	0.3	n.a.	n.a.
14		17.53	0.801	10.554	2.1	n.a.	n.a.
15		17.59	0.875	16.904	2.2	n.a.	n.a.
16	FLP	17.70	34.837	345.136	89.2	0.16	0.00
17		17.83	0.435	9.730	1.1	n.a.	n.a.
18		17.91	0.256	3.207	0.7	n.a.	n.a.
19		18.03	0.088	1.110	0.2	n.a.	n.a.
20		18.25	0.157	1.401	0.4	0.16	0.03
21		18.46	0.042	0.364	0.1	0.41	0.03
22		18.55	0.037	0.336	0.1	0.32	0.04
23		18.63	0.026	0.171	0.1	n.a.	n.a.
24		18.92	0.011	0.074	0.0	0.15	0.08
25		19.21	0.014	0.067	0.0	0.15	0.10
26		19.37	0.005	0.036	0.0	0.11	0.13
27		19.60	0.011	0.046	0.0	0.11	0.14
28		19.94	0.003	0.029	0.0	0.08	0.19
Total:			**39.041**	**405.389**	**100.00**	**3.35**	

LCMS profile of **ON16**



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Peak Width min	Res. (USP) to FLP
1		19.17	0.029	0.252	0.1	0.17	0.15
2		21.49	0.044	0.469	0.1	n.a.	n.a.
3		21.66	0.294	4.062	0.6	n.a.	n.a.
4		21.74	0.650	14.143	1.4	n.a.	n.a.
5		21.81	2.190	54.873	4.7	n.a.	n.a.
6	FLP	21.89	41.954	359.293	90.1	0.18	0.00
7		22.07	0.666	11.468	1.4	n.a.	n.a.
8		22.15	0.680	5.969	1.5	n.a.	n.a.
9		22.63	0.043	0.145	0.1	n.a.	n.a.
Total:			**46.550**	**450.674**	**100.00**	**0.35**	

LCMS profile of **ON17**

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