Aminooxy click chemistry (AOCC) as a tool for bis-homo and bis-hetero ligand conjugation to nucleic acids

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Supporting Information

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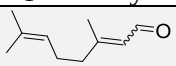
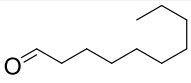
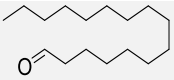
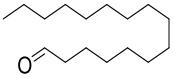
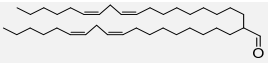
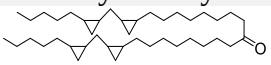
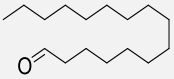
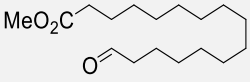
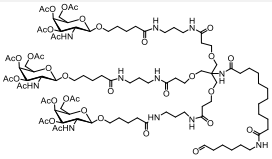
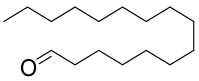
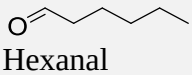
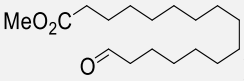
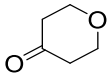
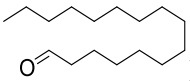
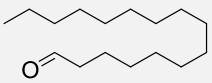
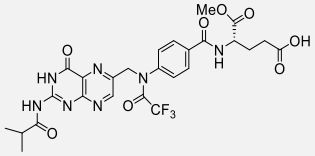
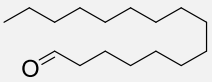
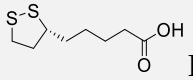
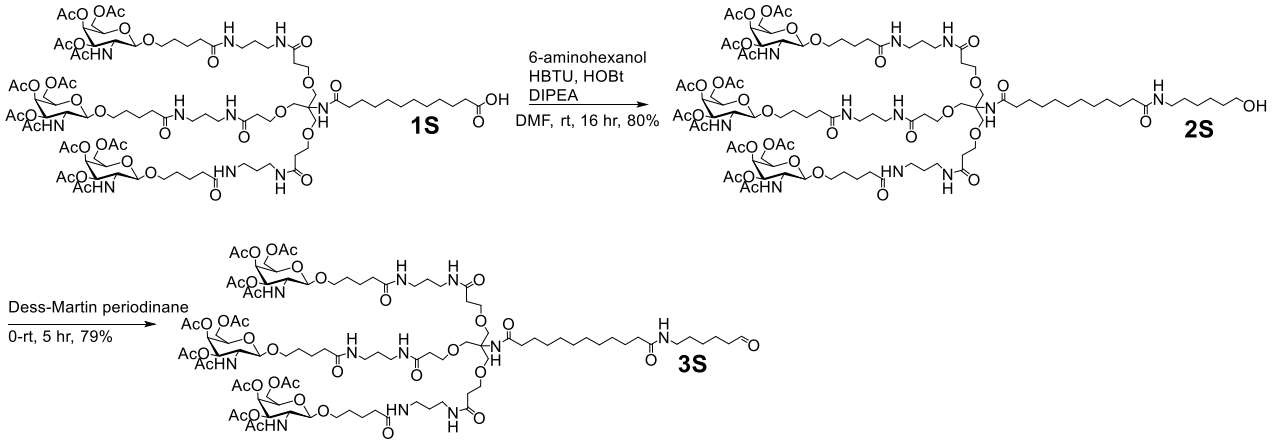
Table S1: Carbonyl compounds and acids used for AOCC modifications

Reagents used as ligands for AOCC		AOCC Conjugates	
R_1 carbonyls	R_2 carbonyls / acids	bis-homo	bis-hetero
 Citral		**5a**, **6a**, **7a**	
 Decanal		**5b**, **6b**, **7b**	
 Hexadecanal		**5c**, **6c**, **7c**, **12-15**	
 Hexadecanal	Formaldehyde (HCHO)		**16a**, **17a**, **18a**
 dilinoleyl aldehyde	Formaldehyde (HCHO)		**16b**, **17b**, **18b**
 cyclopropyl-dilinoleyl ketone	Formaldehyde (HCHO)		**16c**, **17c**, **18c**
 Hexadecanal	 Methyl-16-oxohexadecanoate		**16d**, **17d**, **18d**
 TriGalNAc-aldehyde	 Hexadecanal		**16e**, **17e**, **18e**
 Hexanal	 Methyl-16-oxohexadecanoate		**26**, **28**, **30**, **32**
 Tetrahydro-4H-pyran-4-one	 Hexadecanal		**27**, **29**, **31**, **33**
 Hexadecanal	 Folic acid		**20**, **22**, **24**
 Hexadecanal	 Lipoic acid		**21**, **23**, **25**

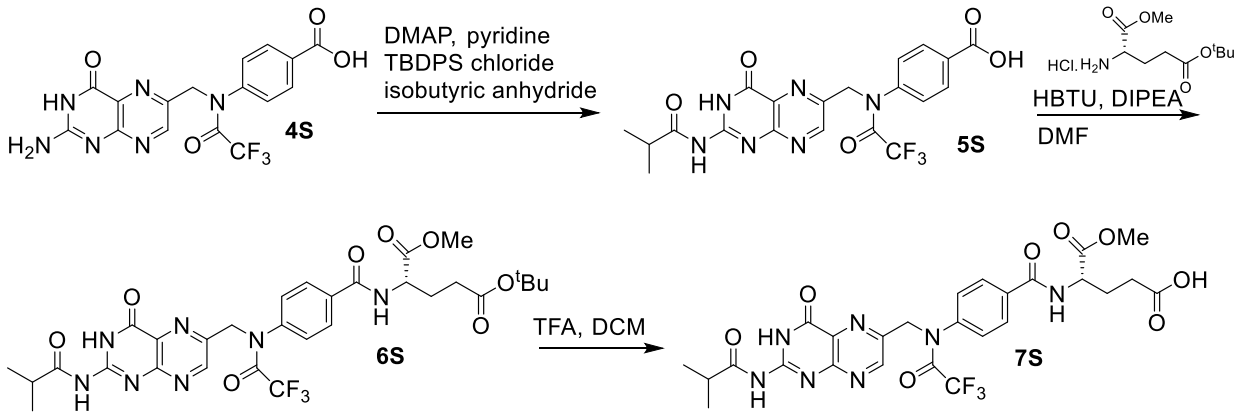
EXPERIMENTAL SECTION

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. 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 400, 500, and 600 MHz. ^{13}C NMR spectra were recorded at 101, 126 and 151 MHz. ^{31}P NMR spectra were recorded at 162, 202 and 243 MHz. ^{19}F NMR spectra were recorded at 565 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)[1]. 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).

Synthesis of building blocks:

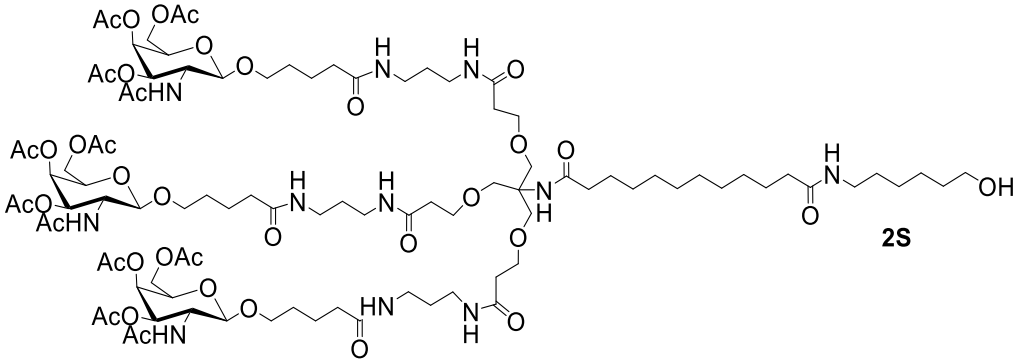


Scheme S1: Synthesis of TriGalNAc aldehyde (**3S**) from TriGalNAc acid.

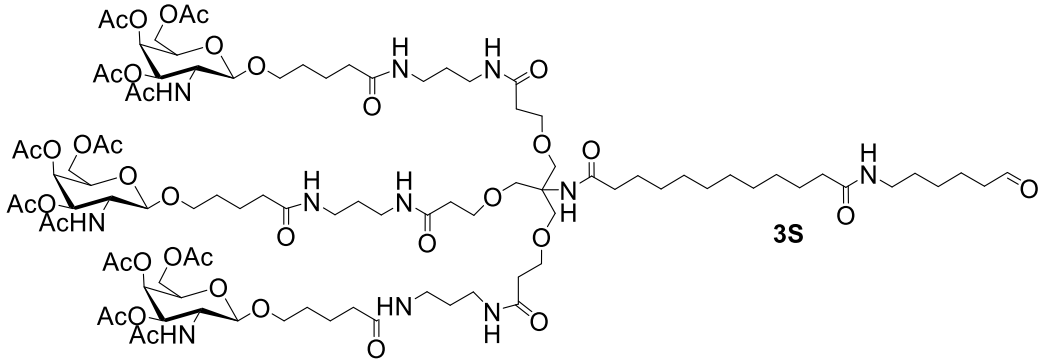


Scheme S2: Synthesis of partially protected Folic acid **7S**[2].

Synthesis and characterization of building blocks for AOCC

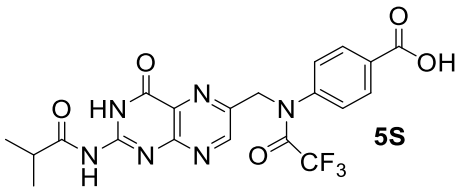


***[(3R,6R)-5-acetamido-6-[5-[3-[3-[3-[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]propylamino]-3-oxo-propoxy]-2-[[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]propylamino]-3-oxo-propoxy]methyl]-2-[[12-(6-hydroxyhexylamino)-12-oxo-dodecanoyl]amino]propoxy]propanoylamino]propylamino]-5-oxo-pentoxy]-3,4-diacetoxy-tetrahydropyran-2-yl]methyl acetate* (2S):** To a clear solution of TriGalNAc acid **1S**[3] (8.3 g, 4.14 mmol) in dry dimethylformamide (30 mL) was added HBTU (1.88 g, 4.96 mmol), HOBt (670.82 mg, 4.96 mmol) and DIPEA (1.60 g, 12.41 mmol, 2.16 mL) in single portions. Reaction mixture was stirred for 5 minutes and then was added 6-aminohexan-1-ol (969.67 mg, 8.27 mmol) slowly. Resulting mixture was stirred at 22 °C for 16 hr and then all volatile matter was removed under high vacuum pump. Residue was diluted with DCM (70 mL) and washed with NH_4Cl solution (3 x 30 mL), $NaHCO_3$ solution (3 x 40 mL), water (50 mL) and brine (2 x 50 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. Solid residue thus obtained, was again evaporated with DCM (20 mL) and kept for drying overnight at 22 °C to afford **2S** (6.97 g, 80% yield) as yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.88 – 7.82 (m, 6H), 7.74 (dt, J = 17.0, 5.7 Hz, 4H), 7.01 (s, 1H), 5.21 (d, J = 3.4 Hz, 3H), 4.96 (dd, J = 11.3, 3.4 Hz, 3H), 4.47 (d, J = 8.5 Hz, 3H), 4.03 – 4.00 (m, 8H), 3.87 (dt, J = 11.2, 8.9 Hz, 3H), 3.70 (dt, J = 9.8, 5.8 Hz, 3H), 3.53 (dd, J = 12.5, 6.2 Hz, 13H), 3.43 – 3.37 (m, 2H), 3.01 (dt, J = 14.9, 7.4 Hz, 14H), 2.27 (t, J = 6.4 Hz, 6H), 2.10 (s, 8H), 2.03 (q, J = 6.9 Hz, 9H), 1.99 (s, 10H), 1.89 (s, 8H), 1.77 (s, 9H), 1.52 – 1.32 (m, 30H), 1.21 (s, 12H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.5, 172.0, 171.9, 170.1, 170.1, 170.0, 169.7, 169.4, 162.4, 101.0, 70.5, 69.8, 68.7, 68.2, 67.3, 66.7, 61.5, 60.7, 59.5, 49.3, 38.4, 38.3, 36.4, 36.3, 36.0, 35.8, 35.5, 35.1, 32.5, 30.8, 29.4, 29.3, 29.0, 28.9, 28.8, 28.7, 28.7, 28.6, 26.4, 25.4, 25.4, 25.3, 22.8, 21.9, 20.6, 20.5, 20.5 ppm. MALDI mass calcd. for $C_{97}H_{161}N_{11}O_{39}Na$ [M + Na]+ 2128.38, found 2130.96.



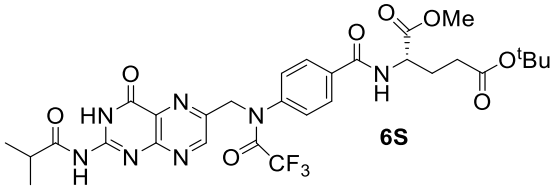
[(3R,6R)-5-acetamido-6-[5-[3-[3-[3-[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyltetrahydropyran-2-yl]oxypentanoylamino]propylamino]-3-oxo-propoxy]-2-[[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoyl amino]propylamino]-3-oxo-propoxy]methyl]-2-[[12-oxo-12-(6-oxohexylamino)dodecanoyl]

***amino]propoxy]propanoylamino]propylamino]-5-oxo-pentoxy]-3,4-diacetoxy-tetrahydropyran-2-yl]methyl acetate* (3S):** To a solution of **2S** (1.0 g, 474.98 µmol) in dry DCM (50 mL) at 0 °C, Dess-Martin periodinane (302.19 mg, 712.47 µmol) was added slowly and then stirred for 5 hr maintaining 0 °C. Reaction mixture was diluted with DCM (30 mL) and washed with 10% $NaHCO_3$ solution (50 mL) followed by 10% $Na_2S_2O_3$ solution (2 x 30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness to afford **3S** (0.79 g, 79% yield) as white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.65 (q, *J* = 2.0 Hz, 1H), 7.88 – 7.81 (m, 6H), 7.75 (dt, *J* = 11.4, 5.7 Hz, 4H), 7.01 (s, 1H), 5.21 (d, *J* = 3.4 Hz, 3H), 4.96 (dd, *J* = 11.3, 3.4 Hz, 3H), 4.47 (d, *J* = 8.5 Hz, 3H), 4.03 – 4.00 (m, 8H), 3.87 (dt, *J* = 11.2, 8.9 Hz, 3H), 3.70 (dt, *J* = 9.6, 5.8 Hz, 3H), 3.53 (dd, *J* = 12.5, 6.2 Hz, 14H), 3.40 (dt, *J* = 9.8, 6.3 Hz, 3H), 3.01 (dp, *J* = 13.1, 6.8 Hz, 15H), 2.40 (td, *J* = 7.3, 1.7 Hz, 2H), 2.27 (t, *J* = 6.4 Hz, 7H), 2.10 (s, 8H), 2.04 (t, *J* = 7.3 Hz, 8H), 1.99 (s, 9H), 1.89 (s, 9H), 1.77 (s, 9H), 1.54 – 1.40 (m, 21H), 1.21 (s, 15H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 203.5, 172.5, 172.0, 170.1, 170.1, 170.0, 169.7, 169.4, 101.0, 70.5, 69.8, 68.7, 68.2, 67.4, 66.7, 61.5, 59.5, 55.0, 49.4, 43.0, 38.3, 38.2, 36.4, 36.3, 36.0 35.9, 35.8, 35.5, 35.1, 29.4, 29.0, 28.9, 28.8, 28.7, 28.7, 28.6, 26.0, 25.4, 25.4, 22.8, 21.9, 21.3, 20.6, 20.5, 20.5 ppm. MALDI mass calcd. for $C_{97}H_{159}N_{11}O_{39}Na$ [M + Na]+ 2126.37, found 2128.87.



5S

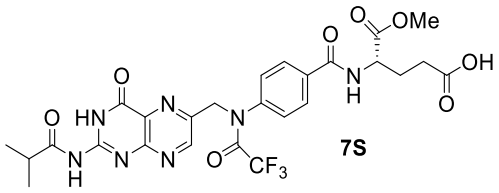
***4-(2,2,2-trifluoro-N-((2-isobutyramido-4-oxo-3,4-dihydropteridin-6-yl)methyl)acetamido) benzoic acid* (5S):** To a suspension of commercially available compound **4S**[4] (25 g, 61.2 mmol) and DMAP (11.25 g, 92 mmol) in anhydrous pyridine (400 mL), TBDPS chloride (42 g, 153 mmol) was added. The reaction mixture was stirred at room temperature for 30 hr after which isobutyric anhydride (14.6 g, 92 mmol) was added and the mixture was slightly warmed. An additional 60 mL of pyridine was also added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture became homogenous after which pyridine and other volatiles were concentrated in a rotary evaporator. The residue was stirred with EtOAc (1 L) and acetic acid (100 mL) and water (500 mL) for 24 hr. The thus obtained slurry was filtered, the residue was washed with water (500 mL), EtOAc (1 L) and dried to obtain the pure product **5S** as a white solid (26.1 g, 89%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 8.87 (s, 1H), 7.95 (d, J=8.6 Hz, 2H), 7.67 (d, J=8.6 Hz, 2H), 5.21 (s, 2H), 2.79-2.74 (m, 1H), 1.12 (d, J=6.83 Hz, 6H),^{13}C NMR (DMSO-d_6) δ =180.7, 166.5, 159.3, 149.9, 147.7, 142.7, 136.3, 134.5, 130.5, 129.2, 128.9, 127.5, 35.0, 33.1, 26.5, 18.9, 18.7. ^{19}FNMR (DMSO-d_6) δ = -64.32.



6S

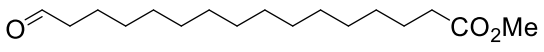
***5-(tert-butyl) 1-methyl (4-(2,2,2-trifluoro-N-((2-isobutyramido-4-oxo-3,4-dihydropteridin-6-yl)methyl)acetamido)benzoyl)-L-glutamate* (6S):** Compound **5S** (2.4 g, 5 mmol) was dissolved in anhydrous DMF (20 mL), HBTU (1.9 g, 1 eq.) followed by DIEA (1 mL, 5 eq.) were added and stirred for 20 minutes. To this reaction mixture the 5-(*tert*-butyl)-1-methyl-L-glutamate hydrochloride (1.2 g, 1 eq) was added as a solution in DMF (6 mL). Reaction was monitored by TLC (8% MeOH/DCM, PMA stain). TLC of the reaction mixture showed completion of the reaction. The reaction mixture was slowly poured in ice with vigorous stirring. The precipitated product was filtered to get the product **6S** as a white solid (Yield=2.85 g, 86%). ^{1}H NMR (DMSO-d_6,400 MHz) δ=12.33 (s, 1H), 11.94 (s, 1H), 8.88 (s, 1H), 8.82 (d, J=7.3 Hz, 1H), 7.90 (d, J=8.6 Hz, 2H), 7.68 (d, J=8.4 Hz, 2H), 5.22 (s, 2H), 4.46-

4.40 (m, 1H), 3.62 (s, 3H), 2.86-2.73 (m, 1H), 2.32 (t, J=7.4 Hz, 2H) 2.05-1.90 (m, 2H), 1.35 (m, 9H), 1.12 (d, J=6.8 Hz, 6H).[13]C NMR DMSO-d$_6$) δ = 180.8, 172.1, 171.5, 165.6, 159.1, 154.8, 150.0, 149.8, 147.7, 141.6, 134.2, 130.5, 128.7, 128.5, 117.5, 114.6, 79.8, 52.0, 51.9, 35.0, 31.2, 27.7, 25.7, 18.7 ppm.



7S

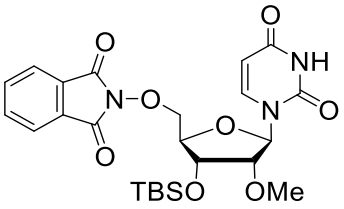
***(S)-5-methoxy-5-oxo-4-(4-(2,2,2-trifluoro-N-((2-isobutyramido-4-oxo-3,4-dihydropteridin-6-yl)methyl)acetamido)benzamido)pentanoic acid* (7S)[5]:** Compound **6S** (2 g, 2.9 mmol) was dissolved in 20mL of 50% TFA in DCM and the solution was stirred at room temperature for 30 min. after which the TLC showed the complete disappearance of the starting ester. The reaction mixture was concentrated, and the residue was crystallized from DCM : hexanes (2:3) and crystallized product was filtered off and dried to obtain the pure product **7S** (1.76 g, 96%) as off-white powder. [1]H NMR (600 MHz, DMSO-d_6) δ 12.36 (s, 1H), 11.97 (s, 1H), 8.91 (s, 1H), 8.88 (d, J = 7.3 Hz, 1H), 7.93 (d, J = 8.3 Hz, 2H), 7.71 (d, J = 8.2 Hz, 2H), 5.24 (s, 2H), 4.46 (ddd, J = 9.8, 7.3, 5.1 Hz, 1H), 3.65 (s, 3H), 2.84 – 2.72 (m, 1H), 2.37 (t, J = 7.4 Hz, 2H), 2.13 – 2.04 (m, 1H), 2.01 – 1.90 (m, 1H), 1.14 (d, J = 6.9 Hz, 6H) ppm. [13]C NMR (151 MHz, DMSO-d_6) δ 180.8, 173.8, 172.2, 165.8, 159.2, 156.1, 155.9, 155.6, 155.4, 154.8, 150.0, 149.9, 147.8, 141.8, 134.2, 130.6, 128.8, 128.5, 119.0, 117.1, 115.2, 113.2, 54.0, 52.1, 52.0, 35.0, 30.2, 25.7, 18.8 ppm. [19]F NMR (565 MHz, DMSO-d_6) δ -66.06 ppm.



Methyl-16-oxohexadecanoate

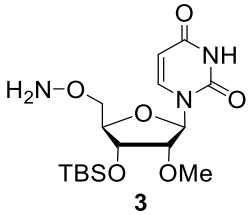
Methyl-16-oxohexadecanoate[6, 7] was synthesized following the literature procedure.
To a clear solution of commercially available oxacyclohexadecan-2-one (1.03 g, 4.16 mmol) in methanol (10 mL) was added hydrogen chloride (151.68 mg, 4.16 mmol, 189.59 µL) dropwise at 22 °C and stirred for 12 hr. All the volatile matters were evaporated, and the residue was crystallized from chilled diethylether (30 mL) to afford methyl-16-hydroxyhexadecanoate[8] (1.1 g, 3.84 mmol, 92% yield) as white solid which was used for next steps without further purification. [1]H NMR (400 MHz, $CDCl_3$) δ 3.64 (s, 3H), 3.61 (t, J = 6.6 Hz, 2H), 2.28 (t, J = 7.6 Hz, 2H), 1.99 (s, 1H), 1.65 – 1.49 (m, 3H), 1.24 (d, J = 9.6 Hz, 23H) ppm. [13]C NMR (101 MHz, $CDCl_3$) δ 174.5, 63.1, 51.5, 34.2, 32.9, 29.7, 29.7, 29.7, 29.7, 29.5, 29.4, 29.3, 29.2, 25.9, 25.0 ppm.

Dess-Martin Periodinane (2.02 g, 4.77 mmol) was dissolved in 20 mL of DCM and cooled to 0 °C in an ice bath. A solution of methyl-16-hydroxyhexadecanoate (0.91 g, 3.18 mmol) in 20 mL of DCM was added via a syringe to the mixture and the ice bath was removed. After stirring for 1 hr at room temperature the reaction was quenched with a solution of $NaHCO_3$ (15 mL) and sodium thiosulfate (15 mL) and stirred for 5 min. After separation, the organic phase was washed with water (30 mL), brine (30 mL) successively and then dried over anhydrous Na_2SO_4. The solvent was removed, and the crude product was purified by column chromatography (gradient: 0-10% EtOAc in hexane) to yield **methyl-16-oxohexadecanoate** (0.77 g, 85% yield) as a white solid. Compound was stored at -20 °C. [1]H NMR (500 MHz, $CDCl_3$) δ 9.74 (t, J = 1.9 Hz, 1H), 3.64 (s, 3H), 2.40 (td, J = 7.3, 1.9 Hz, 2H), 2.28 (t, J = 7.6 Hz, 2H), 1.60 (pd, J = 7.2, 5.1 Hz, 4H), 1.36 – 1.11 (m, 20H) ppm. [13]C NMR (101 MHz, $CDCl_3$) δ 203.3, 174.7, 51.8, 44.3, 34.5, 30.0, 30.0, 29.9, 29.8, 29.8, 29.7, 29.6, 29.6, 25.4, 22.5 ppm.



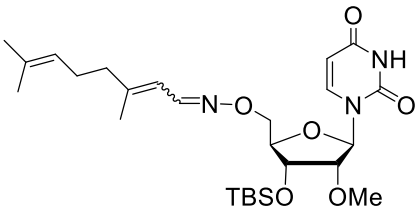
2

***2-[[(2R,5R)-3-[tert-butyl(dimethyl)silyl]oxy-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetrahydrofuran-2-yl]methoxy]isoindoline-1,3-dione* (2)[9]:** To a solution of **1**[10] (5 g, 13.42 mmol) in dimethylformamide (DMF) (60 mL) was added triphenylphosphine (4.82 g, 17.45 mmol) and N-hydroxyphthalimide (2.93 g, 17.45 mmol). To this resulting mixture, diethyl azodicarboxylate (DEAD) (3.20 g, 17.45 mmol, 3.35 mL) was added dropwise at 0 °C. The reaction mixture was stirred at 22 °C for 4 hr. The reaction mixture was then quenched with 10% aqueous $NaHCO_3$ (50 mL) and extracted with EtOAc (3 x 50 mL). Combined organic layer was dried over anhydrous Na_2SO_4, filtered and the filtrated was evaporated to dryness. The crude residue thus obtained was purified by flash chromatography (gradient: 0-40% EtOAc in hexane) to afford **2** (5.8 g, 83% yield) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.92 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.89 – 7.80 (m, 2H), 7.80 – 7.72 (m, 2H), 5.91 (d, *J* = 2.9 Hz, 1H), 5.77 (dd, *J* = 8.1, 2.1 Hz, 1H), 4.56 (dd, *J* = 10.4, 2.7 Hz, 1H), 4.50 – 4.41 (m, 2H), 4.23 (dt, *J* = 6.8, 2.4 Hz, 1H), 3.76 (dd, *J* = 4.9, 2.9 Hz, 1H), 3.53 (s, 3H), 0.90 (s, 9H), 0.14 (d, *J* = 8.3 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.9, 163.1, 150.6, 140.5, 134.8, 128.8, 123.8, 102.5, 88.4, 83.5, 81.9, 75.9, 69.2, 69.2, 58.5, 25.7, 18.2, -4.7, -4.9 ppm. HRMS calcd. for $C_{24}H_{32}N_3O_8Si$ $[M + H]^+$ 518.1959, found 518.1978.



3

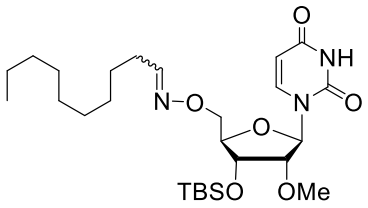
***1-((2R,3R,4R,5R)-5-((aminooxy)methyl)-4-((tert-butyldimethylsilyl)oxy)-3-methoxytetrahydro-furan-2-yl)pyrimidine-2,4(1H,3H)-dione* (3)**: To a solution of compound **2**[9] (2.0 g, 3.86 mmol) in DCM (25 mL) was added N-methylhydrazine (0.21 g, 4.64 mmol, 0.24 mL) at 0°C for 1 hr with stirring. TLC was checked which confirmed consumption of starting material. All the volatile matters were evaporated to dryness and the crude compound thus obtained was purified by column chromatography (gradient: 0-5% MeOH in DCM) to afford **3** (1.4 g, 94% yield) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.37 (brs, 1 H), 7.70 (d, *J* = 8.0 Hz, 1 H), 6.20 (brs, 1 H), 5.79 (d, *J* = 4.8 Hz, 1 H), 5.65 (d, *J* = 4.8 Hz, 1 H), 4.25 (dd, *J* = 4.8, 4.4 Hz, 1 H), 3.96 (dd, *J* = 8.4, 4.8 Hz, 1 H), 3.83 (dd, *J* = 5.2, 4.8 Hz, 1 H), 3.78 – 3.65 (m, 2 H), 3.32 (s, 3 H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 163.0, 150.4, 140.5, 102.1, 86.6, 82.4, 81.6, 74.5, 70.2, 57.5, 25.6, 25.5, 17.7, -4.9, -5.0. HRMS calcd. for $C_{16}H_{30}N_3O_6Si$ $[M + H]^+$ 388.1904, found 388.1909.



4a

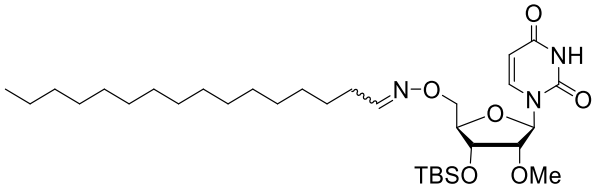
***1-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-5-[[[(2E)-3,7-dimethylocta-2,6-dienylidene]amino] oxymethyl]-3-methoxy-tetrahydrofuran-2-yl]pyrimidine-2,4-dione* (4a):** To a solution of **3** (0.6 g,

1.55 mmol) in dry DCM (20 mL), DIPEA (606.40 mg, 4.65 mmol, 817.25 µL) was added and stirred for 5 minutes. To the resulting solution, citral (294.64 mg, 1.86 mmol, 331.80 µL) was added in single portion and the reaction mixture was stirred for 16 hr at 25 °C. TLC showed consumption of starting material. All the volatile matters were removed, diluted with EtOAc (30 mL) and washed with water (30 mL) and brine (2 x 30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The residue thus obtained was purified flash column chromatography to afford **4a** (0.62 g, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.11 (s, 1H), 7.97 (dd, *J* = 12.6, 10.4 Hz, 1H), 7.83 – 7.71 (m, 1H), 7.33 – 7.29 (m, 0H), 6.30 (ddt, *J* = 9.2, 6.7, 1.5 Hz, 1H), 5.91 – 5.82 (m, 2H), 5.64 (dd, *J* = 8.2, 4.5 Hz, 1H), 5.56 (dd, *J* = 8.1, 7.1 Hz, 1H), 5.04 (dtdq, *J* = 6.9, 4.1, 2.8, 1.4 Hz, 1H), 4.57 – 4.39 (m, 1H), 4.33 – 4.12 (m, 4H), 3.62 (ddd, *J* = 15.5, 4.3, 2.0 Hz, 1H), 3.56 – 3.50 (m, 3H), 2.34 – 2.03 (m, 4H), 1.89 – 1.75 (m, 3H), 1.69 – 1.60 (m, 4H), 1.57 (dd, *J* = 3.8, 1.5 Hz, 4H), 0.88 (s, 9H), 0.07 (d, *J* = 1.6 Hz, 7H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.0, 164.0, 163.9, 163.9, 151.8, 151.6, 150.4, 150.4, 149.0, 148.9, 148.3, 148.1, 145.3, 145.0, 140.1, 140.1, 139.9, 139.8, 133.0, 132.9, 132.6, 132.4, 123.2, 123.0, 122.9, 122.8, 118.3, 117.3, 113.8, 112.9, 101.9, 101.9, 101.8, 88.6, 88.6, 88.1, 88.1, 84.0, 84.0, 83.8, 82.7, 82.6, 82.6, 77.5, 77.2, 76.8, 71.6, 71.5, 71.5, 69.5, 69.5, 69.4, 69.3, 58.5, 40.5, 40.1, 32.9, 32.7, 26.9, 26.8, 26.2, 26.1, 25.8, 25.7, 25.7, 24.7, 24.4, 18.2, 18.2, 17.8, 17.8, 17.4, 17.2, -4.6, -4.7, -4.8, -4.8, -4.8 ppm. HRMS calcd. for $C_{26}H_{44}N_3O_6Si$ [M + H]$^+$ 522.2999, found 522. 2994.



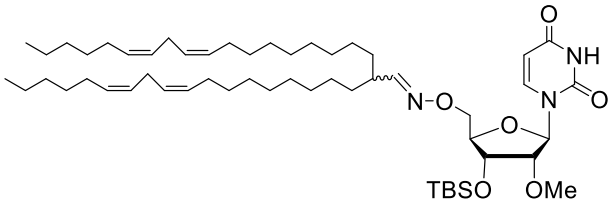
4b

1-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-5-[(decylideneamino)oxymethyl]-3-methoxy-tetra hydrofuran-2-yl]pyrimidine-2,4-dione (**4b)**: To a solution of **3** (0.2 g, 0.52 mmol) in dry DCM (20 mL), DIPEA (0.27 mL, 1.55 mmol,) was added and stirred for 5 minutes. To the resulting solution, decanal (0.16 g, 1.03 mmol) was added in single portion and the reaction mixture was stirred for 16 hr at 25 °C. All the volatile matters were removed when TLC showed consumption of starting material. Residue was diluted with EtOAc (30 mL) and washed with DI water (30 mL) and brine (2 x 30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The crude residue thus obtained was purified flash column chromatography to afford **4b** (0.26 g, 96% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.66 – 9.83 (m, 1H), 7.87 – 7.59 (m, 1H), 7.35 (t, *J* = 6.2 Hz, 0.5H), 6.67 (t, *J* = 5.4 Hz, 0.5H), 5.84 (dd, *J* = 6.5, 2.0 Hz, 1H), 5.63 (ddd, *J* = 13.2, 8.2, 1.5 Hz, 1H), 4.51 – 4.33 (m, 1H), 4.28 – 4.11 (m, 3H), 3.65 – 3.58 (m, 1H), 3.52 (d, *J* = 6.2 Hz, 3H), 2.25 (tt, *J* = 7.4, 5.3 Hz, 1H), 2.16 (dt, *J* = 7.8, 6.4 Hz, 1H), 1.49 – 1.39 (m, 2H), 1.33 – 1.16 (m, 12H), 0.85 (d, *J* = 14.5 Hz, 12H), 0.07 – 0.04 (m, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.0, 164.0, 152.6, 151.8, 150.4, 150.4, 140.0, 139.7, 101.9, 101.8, 88.5, 88.3, 83.9, 83.7, 82.5, 82.5, 71.4, 71.0, 69.4, 69.2, 60.4, 58.5, 58.4, 31.9, 31.8, 29.4, 29.4, 29.4, 29.4, 29.3, 29.3, 29.1, 26.6, 26.2, 26.0, 25.7, 25.7, 22.7, 18.1, 14.1, -4.7, -4.7, -4.9, -4.9 ppm. HRMS calcd. for $C_{26}H_{48}N_3O_6Si$ [M + H]$^+$ 526.3312, found 526.3314.



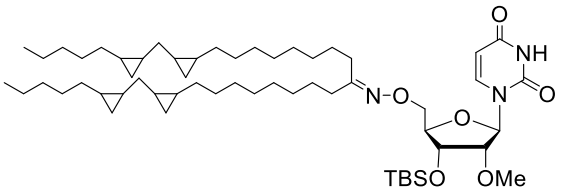
4c

Palmitaldehyde-O-(((2R,3R,4R,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-methoxytetrahydrofuran-2-yl)methyl) oxime **(4c)**: To a solution of **3** (2.00 g, 5.16 mmol) in DCM (50 mL) were added 1-hexadecanal (1.30 g, 5.42 mmol) and DIPEA (2.62 mL, 15.4 mmol). The reaction mixture was stirred for 1 hr at ambient temperature then concentrated. The crude material was purified by flash column chromatography (75% hexane in AcOEt) to give compound **4c** (2.21 g, 70% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (s, 1H), 7.82 – 7.71 (m, 1H), 7.38 (t, *J* = 6.2 Hz, 0.5H), 6.70 (t, *J* = 5.4 Hz, 0.5H), 5.87 (dd, *J* = 6.0, 2.2 Hz, 1H), 5.66 (dd, *J* = 13.0, 8.2 Hz, 1H), 4.54 – 4.36 (m, 1H), 4.29 – 4.14 (m, 3H), 3.67 – 3.60 (m, 1H), 3.55 (d, *J* = 5.9 Hz, 3H), 2.29 (tdd, *J* = 7.4, 5.4, 4.2 Hz, 1H), 2.23 – 2.15 (m, 1H), 1.56 – 1.39 (m, 2H), 1.25 (s, 25H), 0.89 (d, *J* = 14.8 Hz, 12H), 0.12 – 0.06 (m, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.4, 163.4, 152.8, 152.0, 150.2, 150.2, 140.1, 139.8, 102.0, 101.9, 88.5, 88.4, 84.0, 83.9, 82.8, 82.7, 71.6, 71.2, 69.6, 69.4, 58.6, 58.6, 32.1, 29.8, 29.8, 29.8, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.5, 29.4, 29.3, 26.8, 26.4, 26.2, 25.8, 22.8, 18.3, 14.2, -4.6, -4.6, -4.7, -4.8 ppm. HRMS calcd. for $C_{32}H_{60}N_3O_6Si$ [M + H]$^+$ 610.4251, found 610.4243.



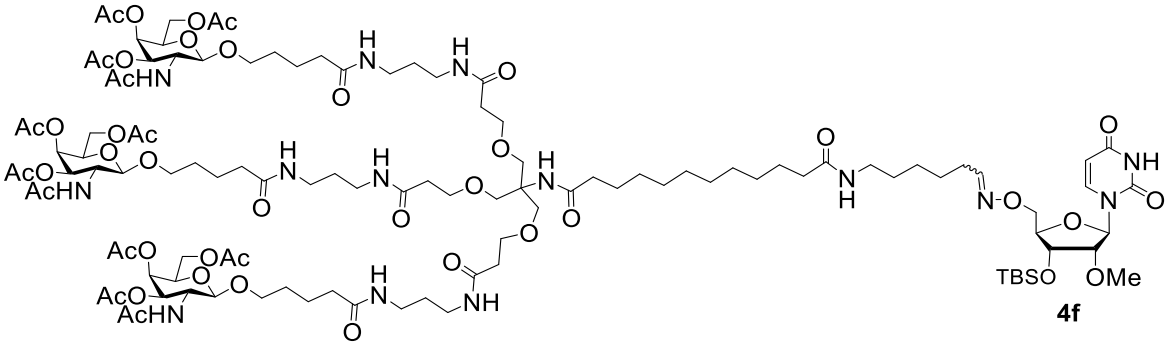
4d

1-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-3-methoxy-5-[[(E)-[(11Z,14Z)-2-[(9Z,12Z)-octadeca-9,12-dienyl]icosa-11,14-dienylidene]amino]oxymethyl]tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(4d):** To a solution of **3** (0.4 g, 1.03 mmol) in DCM (20 mL), (11Z,14Z)-2-[(9Z,12Z)-octadeca-9,12-dienyl]icosa-11,14-dienal (614.23 mg, 1.14 mmol) was added. To the resulting mixture, glacial acetic acid (1 mL) was added in single portion and stirred for 3 hr at 25 °C. Reaction mixture was diluted with DCM (20 mL) and water (30 mL) was added. Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and filtrate was evaporated to dryness. Crude compound was purified by flash column chromatography (gradient: 0-40% EtOAc in hexane) to afford **4d** (0.76 g, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 7.83 (d, *J* = 8.2 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 5.89 (dd, *J* = 14.9, 2.3 Hz, 1H), 5.66 (ddd, *J* = 8.2, 4.0, 1.6 Hz, 1H), 5.43 – 5.26 (m, 8H), 4.42 (dd, *J* = 12.1, 2.4 Hz, 1H), 4.27 – 4.13 (m, 3H), 3.64 (dd, *J* = 4.7, 2.2 Hz, 1H), 3.54 (d, *J* = 5.5 Hz, 3H), 2.82 – 2.68 (m, 4H), 2.20 (dt, *J* = 8.5, 5.3 Hz, 1H), 2.04 (q, *J* = 6.9 Hz, 8H), 1.48 – 1.21 (m, 44H), 0.89 (d, *J* = 7.9 Hz, 13H), 0.09 (d, *J* = 3.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5, 157.0, 155.9, 150.2, 140.1, 139.8, 130.3, 130.3, 130.2, 130.2, 130.2, 128.1, 128.1, 128.1, 128.0, 102.2, 101.9, 88.5, 88.3, 83.9, 83.0, 82.7, 77.5, 77.2, 76.8, 71.8, 71.1, 69.6, 69.5, 58.6, 58.6, 39.9, 36.5, 33.1, 33.1, 32.9, 31.7, 30.0, 29.9, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 27.4, 27.3, 27.2, 25.8, 25.8, 22.7, 18.3, 18.2, 14.2, -4.5, -4.7 ppm. HRMS calcd. for $C_{54}H_{96}N_3O_6Si$ [M + H]$^+$ 910.7068, found 910.7077.



4e

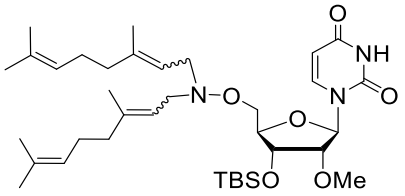
1-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-3-methoxy-5-[[[9-[2-[(2-pentylcyclopropyl)methyl] cyclopropyl]-1-[8-[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]octyl]nonylidene]amino] oxymethyl]tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(4e):** To a solution of **3** (0.69 g, 1.78 mmol) in DCM (20 mL), 1,17-bis[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]heptadecan-9-one (2.08 g, 3.56 mmol) was added. To the resulting mixture, glacial acetic acid (4 mL) was added in single portion and stirred for 4 hr at 25 °C. Reaction mixture was diluted with DCM (20 mL) and DI water (30 mL) was added. Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and filtrate was evaporated to dryness. Crude compound was purified by flash column chromatography (gradient: 0-40% EtOAc in hexane) to afford (1.4 g, 83% yield) **4e** as transparent gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.67 – 9.40 (m, 0H), 7.75 (d, *J* = 8.1 Hz, 1H), 5.90 (d, *J* = 2.4 Hz, 1H), 5.64 (dd, *J* = 8.1, 2.0 Hz, 1H), 4.42 (dd, *J* = 12.3, 2.3 Hz, 1H), 4.27 – 4.12 (m, 3H), 3.62 (dd, *J* = 4.8, 2.4 Hz, 1H), 3.54 (s, 3H), 2.41 – 2.32 (m, 2H), 2.30 – 2.21 (m, 2H), 2.19 – 2.11 (m, 2H), 1.53 – 1.24 (m, 54H), 1.20 – 0.96 (m, 5H), 0.89 (d, *J* = 8.1 Hz, 14H), 0.82 – 0.72 (m, 5H), 0.72 – 0.63 (m, 6H), 0.60 (td, *J* = 8.3, 4.1 Hz, 5H), 0.09 (d, *J* = 3.7 Hz, 6H), -0.29 (ddd, *J* = 11.1, 9.5, 5.1 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.6, 162.4, 150.3, 134.0, 137.9, 129.1, 128.3, 125.4, 102.0, 88.2, 84.0, 83.1, 77.4, 71.2, 69.6, 58.6, 42.9, 34.0, 32.0, 30.3, 30.3, 30.1, 30.0, 29.7, 29.7, 29.6, 29.6, 29.6, 29.6, 29.5, 29.4, 29.0, 29.0, 28.9, 28.8, 28.4, 28.2, 28.0, 26.8, 26.1, 25.8, 24.0, 22.8, 21.6, 18.3, 16.2, 16.1, 16.0, 16.0, 15.8, 15.8, 15.8, 14.2, 11.2, 11.0, -4.6, -4.7 ppm. HRMS calcd. for $C_{57}H_{102}N_3O_6Si$ $[M + H]^+$ 952.7538, found 952.7557.



4f

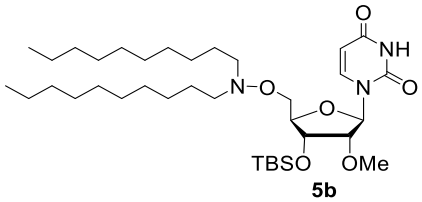
[(3R,6R)-5-acetamido-6-[5-[3-[3-[3-[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxy methyl)tetrahydropyran-2-yl]oxypentanoylamino]propylamino]-3-oxo-propoxy]-2-[[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino] propylamino]-3-oxo-propoxy]methyl]-2-[[12-[[(6Z)-6-[[(2R,5R)-3-[tert-butyl(dimethyl)silyl]oxy-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetrahydrofuran-2-yl]methoxyimino]hexyl]amino]-12-oxo-dodecanoyl]amino]propoxy]propanoylamino]propylamino]-5-oxo-pentoxy]-3,4-diacetoxy-tetrahydropyran-2-yl]methyl acetate **(4f):** To a clear solution of **3** (90.00 mg, 232.26 μmol) in DCM (20 mL), was added DIPEA (30.02 mg, 232.26 μmol, 40.45 μL) at 22°C. The resulting mixture was stirred for 5 minutes and then to this reaction mixture, added aldehyde **3S** (488.52 mg, 232.26 μmol) in a single portion. The reaction mixture was stirred for 18 hr and TLC was checked which showed consumption of starting materials. All the volatile matters were evaporated to dryness and the gummy residue obtained, was purified by flash column chromatography (gradient: 0-10% MeOH in DCM) to afford **4f** (0.41 g, 71% yield) as yellowish white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.42 (dd, *J* = 7.3, 2.2 Hz, 1H), 7.88 – 7.82 (m, 6H), 7.78 – 7.72 (m, 3H), 7.70 (d, *J* = 8.1 Hz, 0H), 7.65 (d, *J* = 8.1 Hz, 0H), 7.49 (t, *J* = 6.0 Hz, 0H), 7.01 (s, 1H), 6.81 (t, *J* = 5.3 Hz, 0H), 5.79 (dd, *J* = 7.9, 4.4 Hz, 1H),

5.71 – 5.56 (m, 1H), 5.21 (d, *J* = 3.4 Hz, 3H), 4.96 (dd, *J* = 11.3, 3.4 Hz, 3H), 4.47 (d, *J* = 8.5 Hz, 3H), 4.31 – 4.24 (m, 1H), 4.19 (dd, *J* = 12.3, 4.3 Hz, 1H), 4.15 – 4.07 (m, 3H), 4.05 – 3.96 (m, 10H), 3.92 – 3.83 (m, 4H), 3.70 (dt, *J* = 9.5, 5.8 Hz, 3H), 3.53 (dd, *J* = 12.6, 6.1 Hz, 13H), 3.40 (dt, *J* = 9.7, 6.3 Hz, 2H), 3.34 (s, 2H), 3.16 (d, *J* = 5.2 Hz, 6H), 3.02 (h, *J* = 7.0 Hz, 14H), 2.27 (t, *J* = 6.4 Hz, 7H), 2.10 (s, 9H), 2.03 (q, *J* = 7.4 Hz, 9H), 1.99 (s, 9H), 1.89 (s, 9H), 1.77 (s, 8H), 1.53 – 1.37 (m, 29H), 1.29 – 1.18 (m, 16H), 0.87 (d, *J* = 1.3 Hz, 11H), 0.08 (q, *J* = 3.4 Hz, 6H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.5, 172.0, 172.0, 170.1, 170.1, 170.0, 169.7, 169.4, 163.1, 152.6, 152.0, 150.5, 150.4, 140.3, 140.2, 102.1, 101.9, 101.0, 86.9, 86.7, 82.4, 82.2, 81.5, 81.3, 72.0, 71.8, 70.5, 69.9, 69.8, 69.7, 68.7, 68.2, 67.3, 66.7, 61.5, 59.5, 57.7, 57.6, 49.3, 48.6, 36.4, 36.3, 36.0, 35.9, 35.5, 35.1, 29.4, 29.0, 28.9, 28.9, 28.8, 28.8, 28.7, 28.6, 26.2, 26.0, 25.7, 25.6, 25.6, 25.4, 25.4, 25.4, 25.2, 22.8, 21.9, 20.6, 20.5, 20.5, 17.8, 17.8, -4.8, -4.8, -5.0, -5.1 ppm. MALDI calcd. for $C_{113}H_{186}N_{14}O_{44}SiNa$ $[M + Na]^+$ 2495.86; found 2498.76.



5a

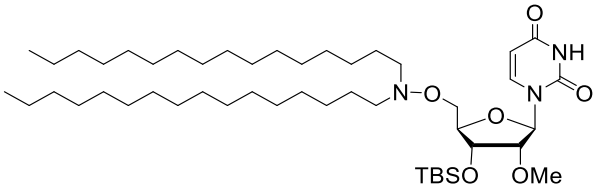
1-[(2R,5R)-5-[[bis[(2E)-3,7-dimethylocta-2,6-dienyl]amino]oxymethyl]-4-[tert-butyl(dimethyl) silyl]oxy-3-methoxy-tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(5a):** To a solution of **4a** (0.6 g, 1.15 mmol) in glacial acetic acid (5 mL), sodium cyanoborohydride (2.6 equiv) was added at 15 °C and stirred for 1 hr. To the clear solution, citral (357.29 mg, 2.30 mmol, 402.35 µL) was added and stirred for 0.5 h and continued stirring for 30 minutes at 20 °C. Second portion of sodium cyanoborohydride (2.6 equiv) was then added to this reaction mixture and stirred for 2 hr. TLC showed completion of reaction. After diluting the reaction mixture with DCM (20 mL), water (20 mL) was added, and organic layer was separated. DCM layer dried over anhydrous $CaCl_2$, filtered and filtrate was evaporated to dryness. Crude residue thus obtained was purified by flash column chromatography (gradient: 10-50% EtOAc in hexane) to afford **5a** (0.58 g, 76% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.00 – 7.91 (m, 1H), 5.92 (t, *J* = 2.5 Hz, 1H), 5.66 (dd, *J* = 8.1, 1.7 Hz, 1H), 5.33 – 5.25 (m, 2H), 5.07 (ddq, *J* = 8.5, 5.4, 1.5 Hz, 2H), 4.18 – 4.05 (m, 3H), 3.88 (ddd, *J* = 10.5, 6.1, 2.6 Hz, 1H), 3.61 (dd, *J* = 4.7, 2.9 Hz, 1H), 3.53 – 3.48 (m, 3H), 3.37 (q, *J* = 7.6 Hz, 4H), 2.12 – 1.97 (m, 8H), 1.73 (dd, *J* = 2.6, 1.4 Hz, 2H), 1.67 – 1.64 (m, 8H), 1.59 (dd, *J* = 6.3, 1.3 Hz, 6H), 0.90 (s, 9H), 0.09 (d, *J* = 6.8 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.6, 150.3, 140.4, 140.4, 139.8, 139.8, 139.7, 132.1, 131.8, 124.0, 124.0, 124.0, 120.3, 120.2, 119.4, 101.9, 101.8, 87.8, 84.0, 84.0, 84.0, 83.0, 83.0, 77.5, 77.2, 76.8, 71.2, 71.1, 70.0, 69.9, 58.4, 58.3, 55.5, 55.4, 55.3, 55.2, 39.9, 32.5, 26.6, 26.6, 26.6, 25.8, 25.8, 23.8, 23.7, 18.2, 17.8, 17.8, 16.7, 16.7, -4.5, -4.7 ppm. HRMS calcd. for $C_{36}H_{62}N_3O_6Si$ $[M + H]^+$ 660.4408, found 660.4403.



5b

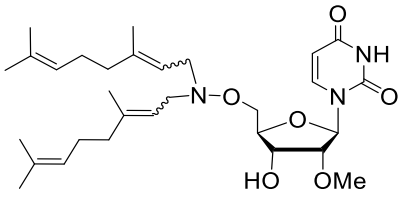
1-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-5-[(didecylamino)oxymethyl]-3-methoxy-tetrahydro furan-2-yl]pyrimidine-2,4-dione **(5b):** To a solution of **4b** (550.00 mg, 1.05 mmol) in acetic acid (3 mL) was added sodium cyanoborohydride (184 mg, 2.93 mmol) under 15 °C. The reaction mixture was stirred for 1 hr at 15 °C and decanal (500.41 mg, 3.14 mmol, 349.94 µL) in DCM (2 mL) was added. The stirring was continued for 30 min and additional amount of sodium cyanoborohydride (184

mg, 2.93 mmol) was added. The resulting mixture was stirred for another 2 hr and then diluted with DCM and washed with ice water. The organic layer was separated and concentrated. The crude material was purified by flash column chromatography (0-50% EtOAc in hexane) to afford **5b** (0.52 g, 74% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.21 (d, *J* = 7.4 Hz, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 5.91 (d, *J* = 2.3 Hz, 1H), 5.67 (dd, *J* = 8.2, 1.5 Hz, 1H), 4.16 (dd, *J* = 7.0, 4.9 Hz, 1H), 4.10 – 4.04 (m, 2H), 3.92 – 3.83 (m, 1H), 3.63 (d, *J* = 6.6 Hz, 2H), 3.60 (dd, *J* = 4.9, 2.4 Hz, 1H), 3.52 (s, 3H), 2.67 (td, *J* = 7.0, 2.3 Hz, 4H), 1.55 (dt, *J* = 14.5, 7.0 Hz, 6H), 1.34 – 1.22 (m, 43H), 0.90 (s, 9H), 0.87 (t, *J* = 6.8 Hz, 10H), 0.09 (d, *J* = 6.6 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.5, 150.3, 140.4, 101.7, 87.9, 84.1, 82.9, 77.5, 77.2, 76.8, 71.2, 69.64 63.2, 59.3, 58.3, 33.0, 32.0, 29.7, 29.7, 29.7, 29.6, 29.4, 27.6, 27.2, 25.9, 25.8, 22.8, 18.2, 14.2, -4.4, -4.7 ppm. HRMS calcd. for $C_{36}H_{70}N_3O_6Si$ $[M + H]^+$ 668.5034, found 668.5040.



5c

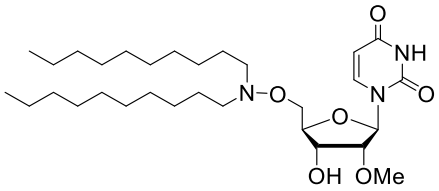
1-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-5-[(dihexadecylamino)oxymethyl]-3-methoxy-tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(5c)**: To a solution of **4c** (0.12 g, 196.75 µmol) in glacial acetic acid (0.3 mL) was added sodium cyanoborohydride (33.84 mg, 511.55 µmol) under 15 °C. The reaction mixture was stirred for 1 hr at 15 °C and hexadecanal (141.91 mg, 590.25 µmol) in DCM (0.2 mL) was added. The stirring was continued for 30 min and additional amount of sodium cyanoborohydride (33.84 mg, 511.55 µmol) was added. The resulting mixture was stirred for another 2 hr and then diluted with DCM (10 mL) and washed with ice water. The organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated. The crude material was purified by flash silica gel column chromatography (5% MeOH in DCM) to give **5c** (0.145 g, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 5.92 (d, *J* = 2.3 Hz, 1H), 5.67 (d, *J* = 8.1 Hz, 1H), 4.16 (dd, *J* = 6.9, 4.8 Hz, 1H), 4.12 – 4.04 (m, 2H), 3.90 – 3.84 (m, 1H), 3.68 – 3.57 (m, 1H), 3.52 (s, 3H), 2.73 – 2.62 (m, 4H), 1.53 (p, *J* = 6.9 Hz, 5H), 1.25 (bs, 60H), 0.89 (d, *J* = 11.9 Hz, 15H), 0.09 (d, *J* = 5.4 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.3, 163.5, 150.2, 140.5, 101.7, 87.90 84.1, 82.9, 71.2, 69.6, 63.3, 59.3, 58.3, 33.0, 32.1, 29.9, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 27.6, 27.3, 25.9, 25.8, 22.8, 20.7, 18.3, 14.3, -4.4, -4.7 ppm. HRMS calcd. for $C_{48}H_{94}N_3O_6Si$ $[M + H]^+$ 836.6912, found 836.6922.



6a

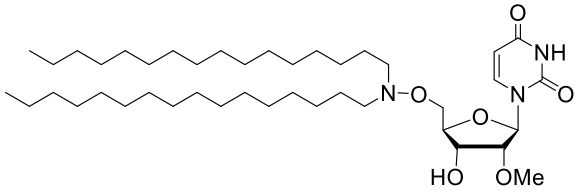
1-[(2R,5R)-5-[[bis[(2E)-3,7-dimethylocta-2,6-dienyl]amino]oxymethyl]-4-hydroxy-3-methoxy-tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(6a)**: To a solution of **5a** (0.5 g, 757.61 µmol) in THF (20 mL) at 25 °C, tetrabutylammonium fluoride (300.13 mg, 1.14 mmol) was added slowly in single portion and then stirred for 16 hr. Volatile matters were removed in high vacuum pump and crude residue thus obtained was purified by flash column chromatography (gradient: 10-60% EtOAc in hexane) to afford **6a** (0.31 g, 75% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.42 – 9.38 (m, 1H), 8.00 – 7.90 (m, 1H), 5.97 – 5.92 (m, 1H), 5.68 (dt, *J* = 8.1, 1.5 Hz, 1H), 5.35 – 5.28 (m, 2H), 5.12 – 5.05 (m, 2H), 4.21 – 4.08 (m, 2H), 4.03 (dq, *J* = 5.5, 2.7 Hz, 1H), 3.94 (ddd, *J* = 11.0, 5.1, 2.7 Hz, 1H), 3.74 (dd, *J* = 5.2, 2.4 Hz, 1H), 3.60 (s, 2H), 3.37 (t, *J* = 5.7 Hz, 4H), 2.71 (dd, *J* = 7.9, 3.1 Hz, 1H), 2.13 – 1.99 (m, 7H), 1.74 (d, *J* = 1.5 Hz, 2H), 1.71 – 1.64 (m, 10H), 1.60 (dd, *J* = 5.0, 1.3 Hz, 6H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.5, 163.5, 150.3, 140.2, 140.2, 139.9, 139.7, 139.7, 132.1, 131.8, 124.1,

124.0, 120.2, 119.5, 119.4, 102.0, 101.9, 87.4, 84.0, 83.9, 83.2, 71.3, 71.2, 68.8, 68.8, 60.5, 58.8, 55.9, 55.6, 39.8, 32.4, 26.6, 26.6, 26.5, 25.8, 25.8, 23.7, 23.7, 17.8, 16.7, 16.7 ppm. HRMS calcd. for $C_{30}H_{48}N_3O_6$ $[M + H]^+$ 546.3543, found 546.3548.



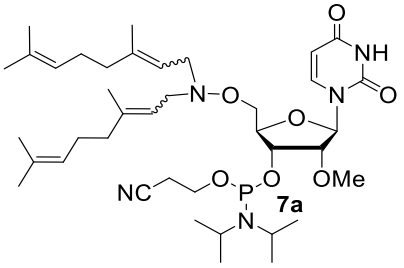
6b

1-[(2R,5R)-5-[(didecylamino)oxymethyl]-4-hydroxy-3-methoxy-tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(6b)**: To a clear solution of **5b** (0.51 g, 0.76 mmol) in tetrahydrofuran (10 mL), TBAF (0.24 g, 0.92 mmol) was slowly added and stirred at 20 °C for 16 hr. Volatile matters were evaporated when TLC showed completion of the reaction. The crude mass thus obtained was purified by flash column chromatography (gradient: 0-70% EtOAc in hexane) to afford **6b** (0.21 g, 50% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.00 (d, *J* = 8.1 Hz, 1H), 5.95 (d, *J* = 2.3 Hz, 1H), 5.68 (d, *J* = 8.1 Hz, 1H), 4.22 (td, *J* = 7.4, 5.1 Hz, 1H), 4.13 (dd, *J* = 11.0, 2.3 Hz, 1H), 4.03 (dt, *J* = 7.1, 2.5 Hz, 1H), 3.93 (dd, *J* = 11.0, 2.7 Hz, 1H), 3.74 (dd, *J* = 5.2, 2.3 Hz, 1H), 3.61 (s, 2H), 2.67 (qd, *J* = 9.6, 8.2, 3.5 Hz, 5H), 1.55 (p, *J* = 7.2 Hz, 4H), 1.36 – 1.18 (m, 29H), 0.88 (t, *J* = 6.9 Hz, 5H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.1, 150.2, 140.1, 101.9, 87.4, 83.9, 83.2, 71.3, 68.7, 59.5, 58.8, 32.0, 29.7, 29.7, 29.7, 29.5, 27.6, 27.3, 22.8, 14.2 ppm. HRMS calcd. for $C_{30}H_{56}N_3O_6$ $[M + H]^+$ 554.4169, found 554.4178.



6c

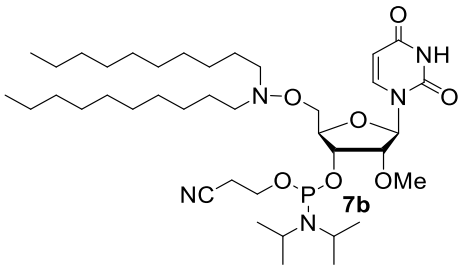
1-[(2R,5R)-5-[(dihexadecylamino)oxymethyl]-4-hydroxy-3-methoxy-tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(6c)**: To a solution of **5c** (0.12 g, 143.48 μmol) in THF (10 mL) at 25 °C, tetrabutylammonium fluoride, 1M in THF (56.84 mg, 215.22 μmol) was added slowly in single portion and then stirred for 12 hr. Volatile matters were removed in high vacuum pump and crude residue thus obtained was purified by flash column chromatography (gradient: 10-60% EtOAc in hexane) to afford **6c** (0.091 g, 88% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (d, *J* = 2.3 Hz, 1H), 7.99 (d, *J* = 8.2 Hz, 1H), 5.95 (d, *J* = 2.2 Hz, 1H), 5.68 (dd, *J* = 8.1, 2.2 Hz, 1H), 4.22 (td, *J* = 7.6, 5.2 Hz, 1H), 4.13 (dd, *J* = 11.0, 2.3 Hz, 1H), 4.03 (dt, *J* = 7.1, 2.5 Hz, 1H), 3.93 (dd, *J* = 11.0, 2.7 Hz, 1H), 3.74 (dd, *J* = 5.2, 2.3 Hz, 1H), 3.61 (s, 3H), 2.72 – 2.61 (m, 5H), 1.55 (p, *J* = 7.2 Hz, 5H), 1.25 (s, 55H), 0.88 (t, *J* = 6.9 Hz, 6H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.1, 150.2, 140.1, 101.9, 87.3, 83.9, 83.2, 71.3, 68.6, 59.5, 58.8, 32.1, 29.8, 29.8, 29.8, 29.8, 29.7, 29.5, 27.6, 27.2, 22.8, 14.3 ppm. HRMS calcd. for $C_{42}H_{80}N_3O_6$ $[M + H]^+$ 722.6047, found 722.6061.



7a

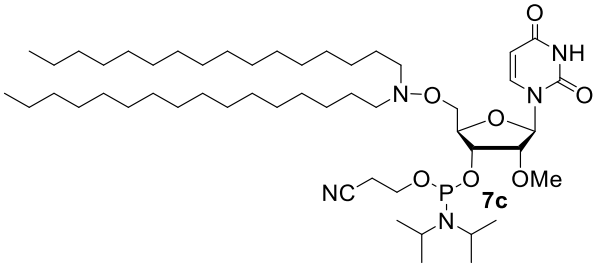
3-[[(2R,5R)-2-[[bis[(2E)-3,7-dimethylocta-2,6-dienyl]amino]oxymethyl]-5-(2,4-dioxopyrim- idin-1-yl)-4-methoxy-tetrahydrofuran-3-yl]oxy-(diisopropylamino)phosphanyl]oxypropane- nitrile **(7a)**:

To a clear solution of **6a** (0.3 g, 549.74 μmol) in DCM (10 mL), diisopropylethylamine (DIPEA) (358.83 mg, 2.75 mmol, 483.60 μL) and N-methylimidazole (NMI) (159.56 mg, 1.92 mmol, 154.92 μL) were added at 25 °C. To this reaction mixture, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (273.92 mg, 1.10 mmol, 258.42 μL) was added slowly after 5 minutes and stirred for 0.5 hr. Reaction mixture was diluted with DCM (20 mL) and quenched with 10% $NaHCO_3$ solution (20 mL). Organic layer was separated, dried on anhydrous Na_2SO_4, filtered and filtrate was evaporated to dryness. The crude compound was thus obtained was purified by flash column chromatography (gradient: 10-50% EtOAc in hexane) to afford **7a** (0.32 g, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H), 7.97 – 7.81 (m, 1H), 6.05 – 5.96 (m, 1H), 5.67 (dd, *J* = 8.2, 2.6 Hz, 1H), 5.36 – 5.25 (m, 1H), 5.08 (dddp, *J* = 5.5, 4.1, 2.8, 1.4 Hz, 2H), 4.31 (dddd, *J* = 17.1, 14.6, 8.4, 4.8 Hz, 1H), 4.17 – 4.04 (m, 1H), 3.97 – 3.76 (m, 3H), 3.73 – 3.55 (m, 1H), 3.54 – 3.45 (m, 3H), 3.45 – 3.34 (m, 4H), 2.73 – 2.57 (m, 2H), 2.05 (q, *J* = 8.0 Hz, 9H), 1.77 – 1.52 (m, 19H), 1.35 – 1.14 (m, 13H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.0, 163.0, 150.3, 150.3, 150.2, 140.3, 140.3, 140.2, 140.2, 140.1, 139.9, 139.8, 139.8, 139.7, 139.7, 139.7, 132.2, 131.9, 124.0, 124.0, 124.0, 123.9, 123.9, 120.4, 120.2, 119.5, 119.3, 117.8, 117.6, 102.3, 102.2, 102.2, 102.2, 102.1, 87.4, 87.3, 87.3, 87.2, 83.5, 83.5, 83.0, 83.0, 83.0, 82.8, 82.7, 82.6, 82.6, 82.6, 82.6, 71.8, 71.7, 71.7, 71.6, 71.3, 71.2, 70.6, 70.6, 70.5, 70.5, 59.0, 58.9, 58.9, 58.9, 58.8, 58.8, 58.7, 58.3, 58.3, 58.0, 57.9, 55.7, 55.5, 55.4, 55.3, 55.3, 53.6, 43.5, 43.5, 43.5, 43.4, 39.9, 32.6, 32.5, 32.5, 26.7, 26.7, 26.6, 26.6, 26.6, 25.8, 25.8, 24.8, 24.7, 24.7, 23.8, 23.8, 20.5, 20.5, 20.5, 17.8, 17.8, 17.8, 16.7, 16.7 ppm. ^{31}P NMR (162 MHz, $CDCl_3$) δ 152.28, 152.25, 152.12, 152.07, 152.00 ppm. HRMS calcd. for $C_{39}H_{65}N_5O_7P$ $[M + H]^+$ 746.4622, found 746.4630.

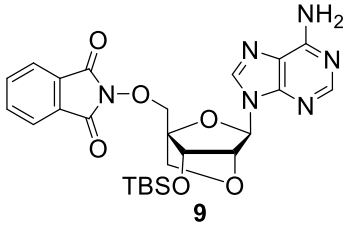


7b

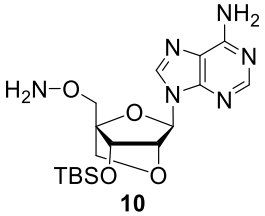
3-[[(2R,5R)-2-[(didecylamino)oxymethyl]-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetrahydro furan-3-yl]oxy-(diisopropylamino)phosphanyl]oxypropanenitrile **(7b)**: To a clear solution of **6b** (0.2 g, 0.36 mmol) in DCM (10 mL), was added DIPEA (0.28 g, 2.17 mmol, 0.38 mL) and NMI (0.104 g, 1.26 mmol, 0.10 mL) at 22 °C. The reaction mixture was stirred for 5 minutes and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.171 g, 0.72 mmol, 0.16 mL) was added in single portion. Stirring was continued for 1.25 hr at 22 °C after which the reaction mixture was diluted with DCM (20 mL) and saturated $NaHCO_3$ solution (20 mL) was added. Organic layer was washed with brine (30 mL), separated, dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated to dryness and the crude mass thus obtained, was purified by flash column chromatography to afford **7b** (0.22 g, 81% yield) as transparent gum. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.94 (dd, *J* = 27.9, 8.2 Hz, 1H), 5.99 (dd, *J* = 4.0, 2.3 Hz, 1H), 5.67 (dd, *J* = 8.2, 3.0 Hz, 1H), 4.37 – 4.24 (m, 1H), 4.19 (dt, *J* = 5.2, 2.5 Hz, 1H), 4.10 – 4.03 (m, 1H), 3.97 – 3.79 (m, 4H), 3.77 – 3.58 (m, 2H), 3.51 (d, *J* = 9.8 Hz, 3H), 2.75 – 2.56 (m, 6H), 1.62 – 1.48 (m, 5H), 1.34 – 1.13 (m, 48H), 0.88 (t, *J* = 6.9 Hz, 6H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.4, 163.3, 150.4, 150.3, 140.2, 140.1, 117.8, 117.5, 102.1, 101.9, 87.5, 87.5, 83.5, 83.5, 83.0, 83.0, 82.4, 82.4, 82.4, 82.3, 71.8, 71.5, 70.5, 70.4, 70.2, 70.1, 60.5, 59.3, 59.2, 58.8, 58.8, 58.8, 58.8, 58.7, 58.6, 58.5, 58.3, 58.3, 58.2, 58.1, 43.5, 43.4, 43.4, 43.3, 32.0, 29.8, 29.7, 29.7, 29.7, 29.7, 29.7, 29.4, 29.4, 27.6, 27.6, 27.2, 27.2, 24.8, 24.7, 24.7, 24.7, 22.8, 20.5, 20.5, 14.2 ppm. ^{31}P NMR (162 MHz, $CDCl_3$) δ 151.93, 151.89 ppm. HRMS calcd. for $C_{39}H_{73}N_5O_7P$ $[M + H]^+$ 754.5248, found 754.5247.



3-[[(2R,5R)-2-[(dihexadecylamino)oxymethyl]-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetra hydrofuran-3-yl]oxy-(diisopropylamino)phosphanyl]oxypropanenitrile **(7c)**: To a clear solution of **6c** (0.08 g, 110.79 µmol) in DCM (10 mL), DIPEA (72.32 mg, 553.95 µmol, 97.46 µL) and NMI (32.16 mg, 387.76 µmol, 31.22 µL) were added at 25 °C. To this reaction mixture, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (55.20 mg, 221.58 µmol, 52.08 µL) was added slowly after 5 minutes and stirred for 1 hr. Reaction mixture was diluted with DCM (20 mL) and quenched with 10% $NaHCO_3$ solution (20 mL). Organic layer was separated, dried on anhydrous Na_2SO_4, filtered and filtrate was evaporated to dryness. The crude compound was thus obtained was purified by flash column chromatography (gradient: 10-50% EtOAc in hexane) to afford **7c** (0.085 g, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.08 – 7.71 (m, 1H), 5.99 (t, *J* = 3.6 Hz, 1H), 5.67 (dd, *J* = 8.2, 2.7 Hz, 1H), 4.42 – 4.23 (m, 1H), 4.21 – 4.02 (m, 3H), 3.96 – 3.77 (m, 4H), 3.74 – 3.57 (m, 2H), 3.51 (d, *J* = 6.6 Hz, 3H), 2.65 (ddd, *J* = 17.1, 10.2, 6.2 Hz, 6H), 1.54 (q, *J* = 7.3 Hz, 4H), 1.35 – 1.14 (m, 63H), 0.91 – 0.82 (m, 6H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.0, 162.9, 150.2, 150.2, 140.3, 140.2, 117.8, 117.5, 102.1, 101.9, 87.6, 87.5, 83.6, 83.5, 83.0, 83.0, 82.5, 82.5, 71.8, 71.6, 70.7, 70.6, 70.3, 70.2, 59.3, 58.9, 58.8, 58.7, 58.3, 58.3, 58.2, 58.0, 43.5, 43.4, 43.4, 43.4, 32.1, 29.8, 29.8, 29.8, 29.8, 29.8, 29.7, 29.5, 27.7, 27.6, 27.3, 27.3, 24.8, 24.8, 24.8, 24.7, 24.7, 22.8, 20.6, 20.6, 20.5, 14.3 ppm. ^{31}P NMR (162 MHz, $CDCl_3$) δ 152.0, 151.98 ppm. HRMS calcd. for $C_{51}H_{97}N_5O_7P$ $[M + H]^+$ 922.7126, found 922.7106.

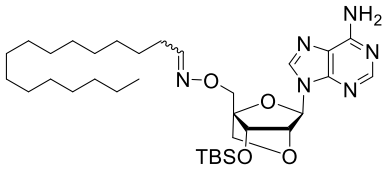


2-[[(4R,6R)-6-(6-aminopurin-9-yl)-7-[tert-butyl(dimethyl)silyl]oxy-2,5-dioxabicyclo[2.2.1]heptan-4-yl]methoxy]isoindoline-1,3-dione **(9):** Commercially available compound **8** (1.0 g, 2.54 mmol) was converted to compound **9** (1.12 g, 82% yield) following the synthetic procedure mentioned for compound **2**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (dd, *J* = 3.6, 1.3 Hz, 1H), 7.97 (s, 1H), 7.63 – 7.54 (m, 4H), 6.38 – 6.11 (m, 1H), 5.84 (d, *J* = 1.8 Hz, 1H), 4.66 (dd, *J* = 9.3, 2.2 Hz, 2H), 4.48 (dd, *J* = 11.9, 1.8 Hz, 1H), 4.39 (dd, *J* = 11.9, 1.9 Hz, 1H), 4.03 (dd, *J* = 7.8, 2.7 Hz, 1H), 3.93 (dd, *J* = 7.7, 2.5 Hz, 1H), 0.74 (d, *J* = 3.5 Hz, 9H), -0.00 (d, *J* = 2.3 Hz, 3H), -0.03 (d, *J* = 2.3 Hz, 3H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.1, 155.9, 155.8, 155.8, 153.1, 149.0, 149.0, 138.8, 138.8, 134.6, 128.7, 123.6, 120.0, 86.8, 86.4, 79.3, 72.7, 72.4, 72.3, 60.5, 25.6, 17.9, -4.7, -5.0 ppm. HRMS calcd. for $C_{25}H_{31}N_6O_6Si$ $[M + H]^+$ 539.2074, found 539.2067.



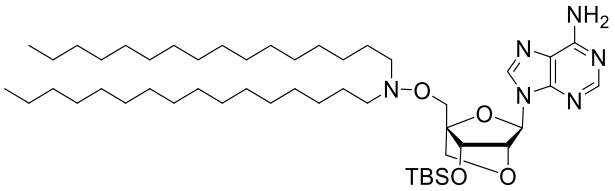
O-[[(4R,6R)-6-(6-aminopurin-9-yl)-7-[tert-butyl(dimethyl)silyl]oxy-2,5-dioxabicyclo[2.2.1]heptan-4-yl]methyl]hydroxylamine **(10):** To a solution of **9** (1.0 g, 1.86 mmol) in DCM (10 mL) at 0 °C, N-

methylhydrazine (83.47 mg, 1.86 mmol, 95.39 µL) was added in single portion and stirred for 1 hr. All volatile matters were evaporated to dryness and the crude product was purified by flash column chromatography (gradient: 0-10% MeOH in DCM) to afford **10** (0.61 g, 80% yield) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (s, 1H), 8.14 (s, 1H), 7.34 (s, 2H), 6.21 (s, 2H), 5.92 (s, 1H), 4.70 (s, 1H), 4.55 (s, 1H), 4.08 – 3.96 (m, 2H), 3.92 – 3.78 (m, 2H), 0.86 (s, 9H), 0.09 (d, *J* = 2.5 Hz, 6H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.1, 152.5, 148.5, 139.0, 119.0, 86.4, 85.5, 78.8, 72.7, 71.9, 71.5, 25.5, 17.6, -4.9, -5.1 ppm. HRMS calcd. for $C_{17}H_{29}N_6O_4Si$ $[M + H]^+$ 409.2020, found 409.2033.



11

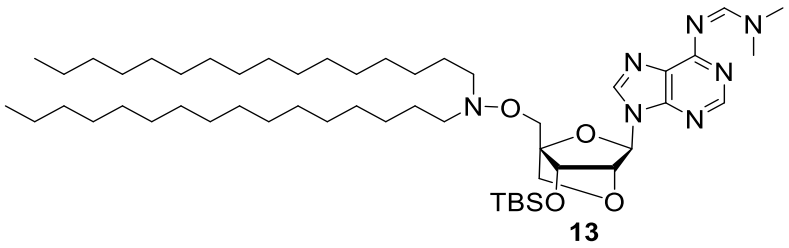
9-[(4R,6R)-7-[tert-butyl(dimethyl)silyl]oxy-4-[(hexadecylideneamino)oxymethyl]-2,5-dioxabicyclo[2.2.1]heptan-6-yl]purin-6-amine **(11):** To a solution of **10** (0.3 g, 734.35 µmol) in DCM (10 mL), DIPEA (191.73 mg, 1.47 mmol, 258.40 µL) was added at 25 °C. To this reaction mixture, hexadecanal (264.83 mg, 1.10 mmol) was added in single portion and resulting clear solution was stirred for 16 hr. The reaction mixture was diluted with DCM (20 mL), washed with water (10 mL), brine (20 x 2 mL) and organic layer was separated. DCM layer was dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The crude compound was purified by flash column chromatography (gradient: 10-60% EtOAc in hexane) to afford **11** (0.4 g, 633.98 µmol, 86% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (d, *J* = 11.3 Hz, 1H), 8.13 (d, *J* = 1.6 Hz, 1H), 7.43 (t, *J* = 6.0 Hz, 0.5H), 7.37 – 7.31 (m, 2H), 6.76 (t, *J* = 5.6 Hz, 0.5H), 5.93 (d, *J* = 1.5 Hz, 1H), 4.71 (d, *J* = 6.8 Hz, 1H), 4.58 (d, *J* = 1.6 Hz, 1H), 4.48 – 4.18 (m, 2H), 3.98 (dd, *J* = 8.0, 1.3 Hz, 1H), 3.80 (dd, *J* = 7.9, 3.3 Hz, 1H), 2.24 – 2.05 (m, 2H), 1.38 (dt, *J* = 14.3, 7.3 Hz, 2H), 1.21 (t, *J* = 6.1 Hz, 26H), 0.88 – 0.79 (m, 12H), 0.08 (dd, *J* = 2.8, 1.5 Hz, 6H) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.1, 152.5, 152.5, 152.2, 151.6, 148.4, 148.4, 138.8, 138.6, 119.0, 119.0, 86.5, 86.3, 85.6, 85.5, 78.8, 72.63, 72.4, 71.8, 71.7, 69.1, 68.9, 31.3, 29.0, 29.0, 28.9, 28.8, 28.8, 28.7, 28.6, 28.5, 28.3, 25.7, 25.4, 25.3, 25.1, 22.1, 17.6, 13.9, -4.9, -5.0, -5.2, -5.3 ppm. HRMS calcd. for $C_{33}H_{59}N_6O_4Si$ $[M + H]^+$ 631.4367, found 631.4390.



12

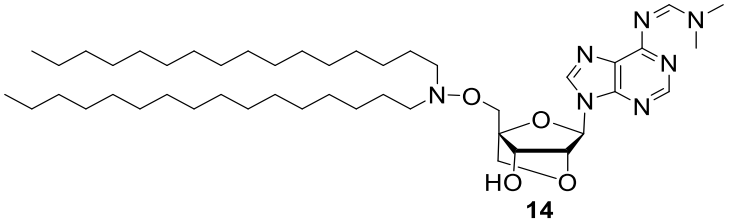
9-[(4R,6R)-7-[tert-butyl(dimethyl)silyl]oxy-4-[(dihexadecylamino)oxymethyl]-2,5-dioxabicyclo[2.2.1]heptan-6-yl]purin-6-amine **(12):** To a clear solution of **11** (0.25 g, 396.24 µmol) in acetic acid (7 mL) was added sodium cyanoborohydride (66.06 mg, 1.03 mmol) in single portion and stirred at 15 °C for 1 hr. Hexadecanal (95.26 mg, 396.24 µmol) was added to the reaction mixture and stirred further for 1 hr at 20 °C. Finally, second portion of sodium cyanoborohydride (66.06 mg, 1.03 mmol) was added to the resultant turbid mixture and stirred for 2 hr. Diluted the mixture with DCM (20 mL) and organic layer was washed with water (2 x 30 mL). DCM layer dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The crude residue was purified by flash column chromatography to afford **12** (0.15 g, 44% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (s, 1H), 8.03 (s, 1H), 6.29 (s, 2H), 5.95 (s, 1H), 4.68 (s, 1H), 4.32 (s, 1H), 4.11 – 4.04 (m, 2H), 4.03 – 3.90 (m, 2H), 2.66 (hept, *J* = 6.4 Hz, 4H), 1.54 (p, *J* = 7.3 Hz, 4H), 1.32 – 1.18 (m, 57H), 0.86 (d, *J* = 2.1 Hz, 16H), 0.04 (d, *J* = 12.4 Hz, 6H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.8, 153.0, 148.8, 138.8, 120.0,

87.0, 86.8, 79.1, 72.7, 72.3, 69.3, 59.4, 32.0, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 27.6, 27.3, 25.7, 22.8, 18.0, 14.2, -4.6, -5.0 ppm. HRMS calcd. for $C_{49}H_{93}N_6O_4Si$ $[M + H]^+$ 857.7028, found 857.7016.



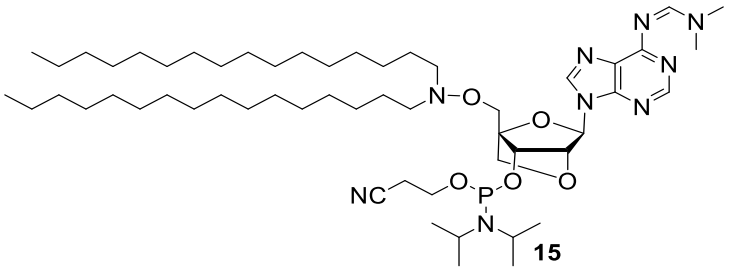
13

(Z)-N'-(9-((1R,3R,4R,7S)-7-((tert-butyldimethylsilyl)oxy)-1-(((dihexadecylamino)oxy)methyl)-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-9H-purin-6-yl)-N,N-dimethylformimidamide **(13)**: To a clear solution of **12** (0.4 g, 466.54 μmol) in dimethylformamide (5 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (88.71 mg, 699.81 μmol, 99.68 μL) in single portion and the reaction mixture was stirred at 65 °C for 4 hr. TLC was checked, and volatile matters was removed under high vacuum pump. Residue was dissolved in DCM (100 mL) and the organic layer was washed with brine (3 x 50 mL). DCM layer was then dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. Crude mass thus obtained, was purified by flash column chromatography (gradient: 30-80% EtOAc in hexane) to afford **13** (0.35 g, 82% yield) as white hygroscopic solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.94 (d, *J* = 2.1 Hz, 1H), 8.51 (s, 1H), 8.13 (s, 1H), 5.99 (s, 1H), 4.64 (s, 1H), 4.30 (s, 1H), 4.09 – 4.04 (m, 2H), 4.03 – 3.98 (m, 1H), 3.93 (dd, *J* = 7.7, 2.0 Hz, 1H), 3.25 (s, 3H), 3.20 (s, 3H), 2.66 (q, *J* = 6.7 Hz, 4H), 1.54 (t, *J* = 7.4 Hz, 4H), 1.23 (d, *J* = 5.0 Hz, 51H), 0.85 (dd, *J* = 8.7, 2.1 Hz, 14H), 0.00 (d, *J* = 9.1 Hz, 6H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.7, 158.1, 152.8, 150.5, 139.8, 126.6, 86.9, 86.7, 79.1, 72.7, 72.1, 69.1, 59.5, 41.4, 35.3, 32.0, 29.8, 29.8, 29.8, 29.7, 29.7, 29.7, 29.5, 27.6, 27.3, 25.7, 22.8, 18.0, 14.2, -4.6, -5.0 ppm. HRMS calcd. for $C_{52}H_{98}N_7O_4Si$ $[M + H]^+$ 912.7450, found 912.7438.



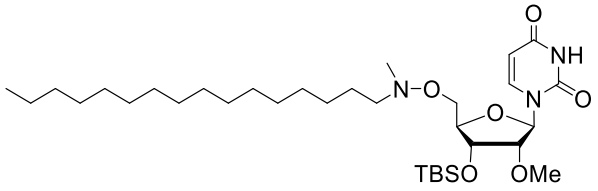
14

N'-[9-[(4R,6R)-4-[(dihexadecylamino)oxymethyl]-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-6-yl]purin-6-yl]-N,N-dimethyl-formamidine **(14):** To a clear solution of **13** (0.43 g, 471.26 μmol) in THF (10 mL) was added TBAF (160.18 mg, 612.63 μmol) in single portion and stirred for 4 hr at 22 °C. All the volatile matters were evaporated under high vacuum pump and the crude residue thus obtained, was purified by flash column chromatography (gradient: 0-5% MeOH in DCM) to afford **14** (0.32 g, 85% yield) as white hygroscopic solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.93 (s, 1H), 8.49 (s, 1H), 8.08 (s, 1H), 6.06 (s, 1H), 4.64 (s, 1H), 4.40 (d, *J* = 3.1 Hz, 1H), 4.18 – 4.13 (m, 3H), 4.01 (d, *J* = 8.1 Hz, 1H), 3.87 (d, *J* = 4.5 Hz, 1H), 3.26 (s, 3H), 3.21 (s, 3H), 2.71 (hept, *J* = 6.4 Hz, 4H), 1.56 (p, *J* = 7.4 Hz, 4H), 1.36 – 1.20 (m, 53H), 0.88 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.5, 158.2, 152.9, 150.6, 139.1, 126.5, 86.8, 86.4, 79.6, 72.2, 71.9, 68.8, 58.9, 41.6, 35.4, 32.1, 29.9, 29.8, 29.8, 29.8, 29.7, 29.5, 27.6, 27.1, 22.8, 14.3 ppm. HRMS calcd. for $C_{46}H_{84}N_7O_4$ $[M + H]^+$ 798.6585, found 798.6596.



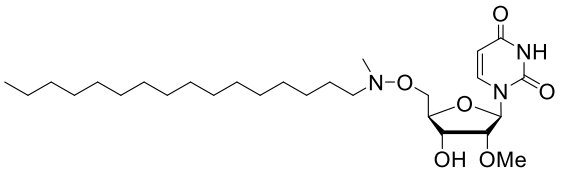
15

N'-[9-[(4R,6R)-7-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-4-[(dihexadecylamino)oxy methyl]-2,5-dioxabicyclo[2.2.1]heptan-6-yl]purin-6-yl]-N,N-dimethyl-formamidine **(15):** To a clear solution of **14** (0.289 g, 362.07 µmol) in DCM (10 mL) was added NMI (44.59 mg, 543.10 µmol, 43.29 µL) and DIPEA (233.97 mg, 1.81 mmol, 315.32 µL) in single portions. After stirring the reaction mixture for 5 minutes at 22 °C, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (171.39 mg, 724.14 µmol, 161.69 µL) was added and continued stirring for 1 hr and TLC was checked. Starting material was consumed and reaction mixture was diluted with DCM (15 mL). DCM layer was washed with 10% $NaHCO_3$ (2 x 25 mL) solution, and brine (30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and filtrate was evaporated at 36°C to afford crude compound which was purified by flash column chromatography (60-90% EtOAc in hexane) to afford **15** (0.3 g, 83% yield) as transparent gum. ^{1}H NMR (600 MHz, CD_3CN) δ 8.93 (s, 1H), 8.42 (d, *J* = 4.5 Hz, 1H), 8.09 (d, *J* = 7.0 Hz, 1H), 6.35 (s, 0H), 6.00 (d, *J* = 4.8 Hz, 1H), 4.79 – 4.54 (m, 1H), 4.48 – 4.32 (m, 1H), 4.13 – 4.00 (m, 6H), 3.83 – 3.70 (m, 3H), 3.63 – 3.43 (m, 4H), 3.19 (s, 4H), 2.76 (t, *J* = 6.0 Hz, 2H), 2.73 – 2.62 (m, 5H), 1.61 – 1.50 (m, 5H), 1.39 – 1.09 (m, 81H), 1.07 – 0.98 (m, 7H), 0.88 (t, *J* = 7.0 Hz, 6H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 171.4, 160.4, 159.0, 159.0, 153.2, 151.5, 151.5, 139.7, 139.7, 127.1, 127.1, 119.2, 118.9, 118.7, 87.3, 87.3, 87.3, 87.2, 87.1, 73.3, 73.1, 73.1, 72.5, 72.4, 69.7, 69.6, 60.8, 59.8, 59.7, 59.6, 59.4, 59.4, 59.3, 59.2, 59.0, 59.0, 55.1, 45.8, 45.8, 43.8, 43.9, 43.9, 43.9, 43.8, 43.8, 41.5, 35.2, 32.5, 30.3, 30.3, 30.2, 30.2, 30.2, 30.1, 30.1, 30.0, 28.0, 28.0, 27.7, 27.7, 24.8, 24.8, 24.8, 24.8, 24.7, 24.6, 24.5, 23.3, 23.1, 23.1, 23.1, 23.1, 21.1, 20.9, 20.9, 20.8, 20.8, 20.8, 20.5, 20.5, 14.5, 14.4 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 148.5, 148.4 ppm. HRMS calcd. for $C_{55}H_{101}N_9O_5P$ $[M + H]^+$ 998.7663, found 998.7681.



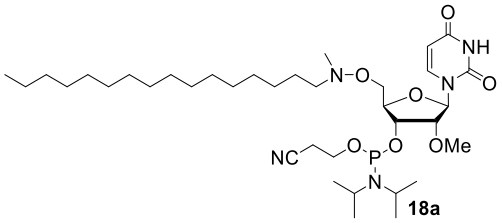
16a

1-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(((hexadecyl(methyl)amino)oxy)methyl)-3-methoxytetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione **(16a)**: To a solution of compound **4c** (2.00 g, 3.28 mmol) in AcOH (8 mL) was added $NaCNBH_3$ (247 mg, 3.94 mmol) under 15 ºC. The reaction mixture was stirred for 1 hr at 15 ºC and to the cold 30% formaldehyde solution (1.64 mL) was added. The stirring was continued for 30 min. and additional amount of $NaCNBH_3$ (2.47 mg, 3.94 mmol) was added in a similar manner. The resulting mixture was stirred for another 2 hr and then diluted with DCM and washed with ice water. The organic layer was separated and concentrated. The crude material was purified by flash column chromatography (75% hexane in AcOEt) to give compound **16a** (1.89 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.35 (brs, 1 H), 7.74 (d, *J* = 4.4 Hz, 1 H), 7.77 (d, *J* = 3.6 Hz, 1 H), 5.60 (dd, *J* =8.0, 2.0 Hz, 1 H), 4.14 (dd, *J* = 5.6, 5.2 Hz, 1 H), 3.95 – 3.70 (m, 4 H), 3.33 (s, 5 H), 2.49 (s, 3 H), 1.49 – 1.38 (m, 2 H), 1.31 – 1.13 (m, 26 H), 0.87 – 0.79 (m, 12 H), 0.06 (s, 6 H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 163.0, 150.3, 140.1, 101.6, 86.8, 81.9, 70.8, 69.9, 60.3, 57.5, 45.2, 31.3, 29.1, 29.0, 29.0, 29.0, 29.0, 28.7, 26.8, 26.6, 25.5, 22.1, 17.6, 13.8, -4.8, -5.3 ppm. HRMS calcd. for $C_{33}H_{64}N_3O_6Si$ $[M + H]^+$ 626.4564, found 626.4568.



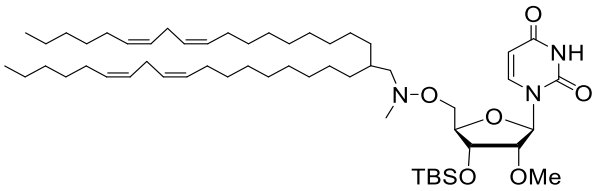
17a

1-((2R,3R,4R,5R)-5-(((hexadecyl(methyl)amino)oxy)methyl)-4-hydroxy-3-methoxytetrahydro-furan-2-yl)pyrimidine-2,4(1H,3H)-dione **(17a)**: To a solution of compound **16a** (1.80 g, 2.88 mmol) in anhydrous THF (30 mL) was added 1M TBAF in THF (5.72 mL) at ambient temperature. The reaction mixture was stirred for 30 min. and then diluted with CH_2Cl_2 and washed with saturated aqueous NH_4Cl solution. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude material was purified by flash column chromatography (50 % hexane in AcOEt) to give compound **17a** (1.06 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (s, 1H), 7.94 (d, *J* = 8.2 Hz, 1H), 5.94 (d, *J* = 2.2 Hz, 1H), 5.69 (dd, *J* = 8.2, 2.1 Hz, 1H), 4.19 (ddd, *J* = 8.2, 7.1, 5.2 Hz, 1H), 4.12 (dd, *J* = 11.0, 2.4 Hz, 1H), 4.06 (dt, *J* = 7.1, 2.6 Hz, 1H), 3.92 (dd, *J* = 11.0, 2.8 Hz, 1H), 3.75 (dd, *J* = 5.2, 2.3 Hz, 1H), 3.61 (s, 3H), 2.73 (d, *J* = 8.1 Hz, 1H), 2.65 (d, *J* = 7.6 Hz, 2H), 2.61 (s, 3H), 1.53 (p, *J* = 7.3 Hz, 2H), 1.25 (s, 26H), 0.92 – 0.83 (m, 3H). ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.2, 150.2, 140.1, 102.0, 87.5, 83.9, 83.1, 70.4, 68.8, 61.4, 58.8, 45.8, 32.1, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 27.6, 27.4, 22.8, 14.3 ppm. HRMS calcd. for $C_{27}H_{50}N_3O_6$ $[M + H]^+$ 512.3700, found 512.3694.



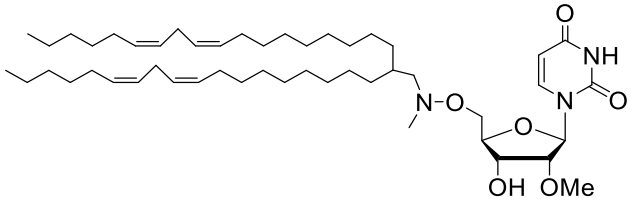
18a

2-cyanoethyl-((2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-(((hexadecyl (methyl)amino)oxy)methyl)-4-methoxytetrahydrofuran-3-yl)diisopropylphosphoramidite **(18a)**: To a solution of compound **17a** (1.06 g, 2.07 mmol) in anhydrous DCM (20 mL) were added DIPEA (1.05 mL, 6.21 mmol) and 2-cyanoethylchloro-*N*,*N*-diisopropylphosphoramidite (509 µL, 2.28 mmol) dropwisely. The reaction mixture was stirred for 3 hr at room temperature and then diluted with DCM. Quenched the reaction with saturated aq. $NaHCO_3$. Organic layer was separated and washed with brine. The solvent was removed in vacuo. The crude residue was purified via column chromatography on silica gel (20-60 % EtOAc in hexanes) to afford **18a** as colorless gum (1.26 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 7.92 (dd, *J* = 15.3, 8.2 Hz, 1H), 5.97 (d, *J* = 3.8 Hz, 1H), 5.68 (dd, *J* = 8.2, 1.2 Hz, 1H), 4.34 – 4.17 (m, 2H), 4.07 (ddd, *J* = 13.1, 10.9, 2.2 Hz, 1H), 3.96 – 3.69 (m, 4H), 3.64 (dqd, *J* = 13.6, 6.9, 3.5 Hz, 2H), 3.51 (d, *J* = 11.6 Hz, 3H), 2.73 – 2.57 (m, 6H), 1.52 (p, *J* = 7.4 Hz, 2H), 1.24 (s, 23H), 1.19 (ddd, *J* = 8.5, 6.7, 2.2 Hz, 12H), 0.87 (t, *J* = 6.7 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.3, 163.3, 163.3, 150.3, 150.3, 140.2, 140.1, 117.8, 117.6, 102.1, 102.0, 87.8, 87.6, 83.5, 83.5, 83.1, 83.0, 82.4, 82.3, 82.2, 82.1, 77.5, 76.8, 70.6, 70.5, 70.2, 70.1, 61.4, 61.3, 58.9, 58.8, 58.8, 58.7, 58.4, 58.3, 58.2, 58.0, 45.8, 45.7, 43.5, 43.5, 43.4, 43.4, 32.0, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 27.6, 27.6, 27.4, 24.8, 24.8, 24.8, 24.7, 24.7, 24.7, 22.8, 20.6, 20.5, 20.5, 20.5, 14.2 ppm. ^{31}P NMR (162 MHz, $CDCl_3$) δ 151.6, 151.4 ppm. HRMS calcd. for $C_{36}H_{67}N_5O_7P$ $[M + H]^+$ 712.4778, found 712.4769.



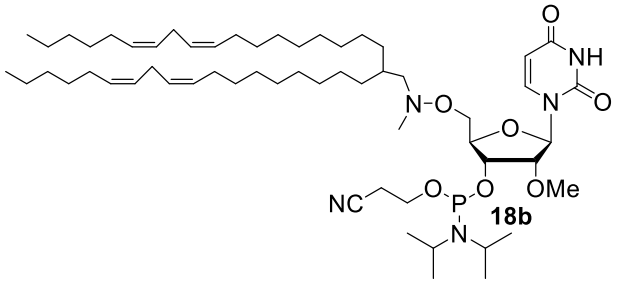
16b

1-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-3-methoxy-5-[[methyl-[(11Z,14Z)-2-[(9Z,12Z)-octadeca-9,12-dienyl]icosa-11,14-dienyl]amino]oxymethyl]tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(16b):** To a clear solution of **4d** (0.7 g, 768.87 umol) in glacial acetic acid (4 mL) at 15 °C was added sodium cyanoborohydride (128.18 mg, 2.00 mmol) in single portion and stirred for 1 hr. To this resulting reaction mixture, formaldehyde, 37% in aq. soln., (69.27 mg, 2.31 mmol, 64.14 uL) in DCM (1 mL) was added slowly and stirred for 0.5 hr. To this mixture was added sodium cyanoborohydride (128.18 mg, 2.00 mmol) and stirred for 2.5 hr at 15 °C. Reaction mixture was diluted with DCM (10 mL) and washed with brine (30 mL). Organic layer separated, dried over anhydrous Na_2SO_4, filtered and filtrate was evaporated to dryness. The crude residue thus obtained, was purified by flash column chromatography (gradient: 10-40% EtOAc in hexane) to afford **16b** (0.46 g, 65% yield) as transparent gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (d, *J* = 2.2 Hz, 1H), 7.99 (d, *J* = 8.1 Hz, 1H), 5.90 (d, *J* = 2.3 Hz, 1H), 5.68 (dd, *J* = 8.1, 2.1 Hz, 1H), 5.44 – 5.27 (m, 8H), 4.16 – 4.03 (m, 3H), 3.85 (dd, *J* = 10.9, 2.3 Hz, 1H), 3.60 (dd, *J* = 4.6, 2.3 Hz, 1H), 3.53 (s, 3H), 2.77 (t, *J* = 6.5 Hz, 4H), 2.58 (s, 3H), 2.51 (s, 2H), 2.05 (q, *J* = 6.8 Hz, 8H), 1.52 (d, *J* = 7.3 Hz, 1H), 1.40 – 1.22 (m, 30H), 0.90 (d, *J* = 8.3 Hz, 15H), 0.09 (d, *J* = 4.7 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.3, 150.2, 140.3, 130.3, 130.3, 130.3, 129.2, 128.3, 128.1, 128.1, 128.1, 125.4, 101.8, 88.0, 84.1, 82.7, 69.8, 69.6, 66.1, 58.4, 46.1, 36.0, 32.3, 32.2, 31.7, 30.3, 29.8, 29.8, 29.7, 29.5, 27.4, 27.3, 26.7, 26.6, 25.8, 25.8, 22.7, 18.2, 14.2, -4.4, -4.7 ppm. HRMS calcd. for $C_{55}H_{100}N_3O_6Si$ $[M + H]^+$ 926.7381, found 926.7360.

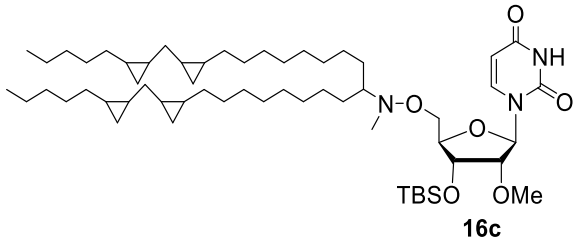


17b

1-[(2R,5R)-4-hydroxy-3-methoxy-5-[[methyl-[(11Z,14Z)-2-[(9Z,12Z)-octadeca-9,12-dienyl]icosa-11,14-dienyl]amino]oxymethyl]tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(17b):** To a solution of **16b** (0.42 g, 453.33 µmol) in THF (10 mL) at 25 °C , tetrabutylammonium fluoride, 1M in THF (119.73 mg, 453.33 µmol) was added slowly in single portion and then stirred for 12 hr. Volatile matters were removed in high vacuum pump and crude residue thus obtained was purified by flash column chromatography (gradient: 10-60% EtOAc in hexane) to afford **17b** (0.3 g, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (d, *J* = 2.2 Hz, 1H), 7.95 (d, *J* = 8.1 Hz, 1H), 5.95 (d, *J* = 2.3 Hz, 1H), 5.70 (dd, *J* = 8.1, 2.2 Hz, 1H), 5.44 – 5.27 (m, 8H), 4.18 (ddd, *J* = 8.1, 7.0, 5.2 Hz, 1H), 4.11 (dd, *J* = 11.0, 2.3 Hz, 1H), 4.05 (dt, *J* = 7.1, 2.5 Hz, 1H), 3.90 (dd, *J* = 11.1, 2.7 Hz, 1H), 3.74 (dd, *J* = 5.2, 2.3 Hz, 1H), 3.61 (s, 3H), 2.77 (t, *J* = 6.7 Hz, 4H), 2.67 (d, *J* = 8.1 Hz, 1H), 2.60 (s, 3H), 2.52 (d, *J* = 6.6 Hz, 2H), 2.05 (q, *J* = 6.8 Hz, 8H), 1.53 (s, 2H), 1.43 – 1.23 (m, 39H), 0.95 – 0.84 (m, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.2, 150.2, 140.1, 130.3, 130.3, 130.3, 128.1, 128.1, 128.1, 102.0, 87.4, 83.9, 83.1, 70.1, 68.8, 66.0, 58.8, 46.1, 35.9, 32.4, 32.2, 31.7, 30.3, 30.3, 29.8, 29.8, 29.7, 29.5, 29.5, 27.4, 27.3, 26.7, 26.6, 25.8, 22.7, 14.2 ppm. HRMS calcd. for $C_{49}H_{86}N_3O_6$ $[M + H]^+$ 812.6517, found 812.6537.

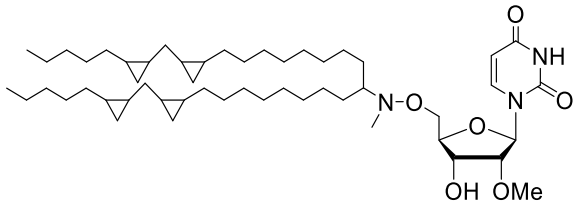


3-[(diisopropylamino)-[(2R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-2-[[methyl-[(11Z,14Z)-2-[(9Z,12Z)-octadeca-9,12-dienyl]icosa-11,14-dienyl]amino]oxymethyl]tetrahydrofuran-3-yl]oxy-phosphanyl]oxypropanenitrile **(18b):** To a clear solution of **17b** (0.27 g, 332.43 µmol) in DCM (10 mL), DIPEA (216.98 mg, 1.66 mmol, 292.43 µL) and NMI (96.49 mg, 1.16 mmol, 93.68 µL) were added at 25 °C. To this reaction mixture, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (165.64 mg, 664.85 µmol, 156.26 µL) was added slowly after 5 minutes and stirred for 1 hr. Reaction mixture was diluted with DCM (20 mL) and quenched with 10% $NaHCO_3$ solution (20 mL). Organic layer was separated, dried on anhydrous Na_2SO_4, filtered and filtrate was evaporated to dryness. The crude compound was thus obtained was purified by flash column chromatography (gradient: 10-50% EtOAc in hexane) to afford **18b** (0.28 g, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 1H), 7.92 (dd, *J* = 16.6, 8.2 Hz, 1H), 5.99 (dd, *J* = 4.0, 2.1 Hz, 1H), 5.69 (dd, *J* = 8.1, 2.0 Hz, 1H), 5.44 – 5.27 (m, 9H), 4.37 – 4.00 (m, 3H), 3.97 – 3.60 (m, 5H), 3.51 (d, *J* = 9.7 Hz, 3H), 2.77 (t, *J* = 6.5 Hz, 4H), 2.70 – 2.50 (m, 7H), 2.04 (dd, *J* = 7.7, 6.0 Hz, 8H), 1.60 – 1.49 (m, 2H), 1.43 – 1.15 (m, 44H), 0.95 – 0.84 (m, 6H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.0, 163.0, 150.2, 150.2, 140.3, 140.1, 130.3, 130.3, 130.3, 130.3, 128.1, 128.1, 128.1, 128.1, 128.0, 117.8, 117.6, 102.1, 102.0, 87.6, 87.4, 83.5, 83.5, 83.0, 83.0, 82.4, 82.4, 82.3, 82.3, 70.8, 70.7, 70.5, 70.3, 70.2, 70.1, 66.0, 58.9, 58.8, 58.8, 58.8, 58.3, 58.3, 58.1, 58.0, 53.6, 46.1, 46.1, 43.5, 43.4, 43.4, 43.4, 43.4, 36.0, 36.0, 32.4, 32.3, 32.2, 32.2, 31.7, 30.4, 30.4, 30.3, 29.8, 29.8, 29.8, 29.8, 29.5, 27.4, 27.3, 26.8, 26.6, 25.8, 24.8, 24.8, 24.8, 24.7, 24.7, 22.7, 20.6, 20.6, 20.5, 20.5, 14.2 ppm. ^{31}P NMR (162 MHz, $CDCl_3$) δ 148.6, 148.5 ppm. HRMS calcd. for $C_{58}H_{103}N_5O_7P$ $[M + H]^+$ 1012.7595, found 1012.7560.



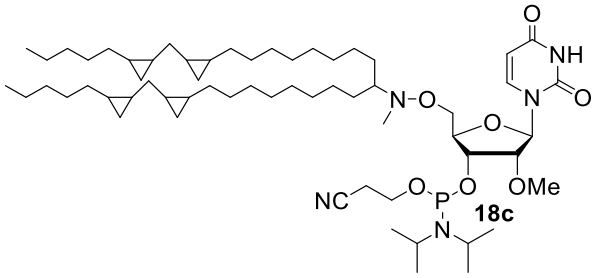
1-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-3-methoxy-5-[[methyl-[9-[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]-1-[8-[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]octyl] nonyl]amino]oxymethyl]tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(16c):**To a clear solution of **4e** (0.56 g, 587.92 µmol) in glacial acetic acid (10 mL) and DCM (3 mL) at 15 °C was added sodium cyanoborohydride (96.06 mg, 1.53 mmol) in single portion and stirred for 1 hr. To this resulting reaction mixture, formaldehyde, 37% in aq. soln. (190.84 mg, 2.35 mmol, 175.08 µL) was added slowly and stirred for 1 hr. To this mixture was added sodium cyanoborohydride (96.06 mg, 1.53 mmol) and stirred for 2 hr at 15 °C. Reaction mixture was diluted with DCM (20 mL) and washed with brine (30 mL). Organic layer separated, dried over anhydrous Na_2SO_4 , filtered and filtrate was evaporated to dryness. The crude residue thus obtained, was purified by flash column chromatography (gradient: 10-50% EtOAc in hexane) to afford **16c** (0.56 g, 98% yield) as transparent gum. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.98 (d, *J* = 8.1 Hz, 1H), 5.93 (d, *J* = 2.5 Hz, 1H), 5.68 (dd, *J* = 8.2, 2.0 Hz, 1H), 4.17 – 4.07 (m, 2H), 4.04 (dd, *J* = 11.1, 2.4 Hz, 1H), 3.81 (dd, *J* = 11.1, 2.5 Hz, 1H), 3.59 (dd, *J* = 4.8, 2.5 Hz, 1H), 3.52 (s, 3H), 2.57 (s, 4H), 1.56 – 1.45 (m, 2H), 1.46 – 1.23 (m, 50H), 1.21 – 1.07 (m, 3H), 1.03 (dt, *J* = 14.1, 7.9 Hz, 1H), 0.91 (s, 10H), 0.88 (d, *J* = 7.1 Hz, 3H), 0.78 (dtd, *J* =

15.5, 7.8, 2.2 Hz, 4H), 0.69 (dqd, *J* = 15.0, 7.0, 3.9 Hz, 4H), 0.61 (td, *J* = 8.4, 4.2 Hz, 4H), 0.10 (d, *J* = 7.0 Hz, 6H), -0.23 – -0.32 (m, 4H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.2, 150.1, 140.3, 101.9, 87.8, 84.2, 82.8, 69.9, 69.6, 66.7, 58.3, 40.2, 32.0, 30.4, 30.2, 30.2, 30.1, 30.0, 29.9, 29.9, 29.9, 29.8, 29.1, 29.0, 28.9, 28.9, 28.2, 28.0, 27.0, 26.9, 25.8, 22.9, 18.2, 16.2, 16.1, 16.1, 15.8, 15.8, 14.3, 11.2, 11.0, -4.40 -4.7 ppm. HRMS calcd. for $C_{58}H_{106}N_3O_6Si$ $[M + H]^+$ 968.7851, found 968.7839.



17c

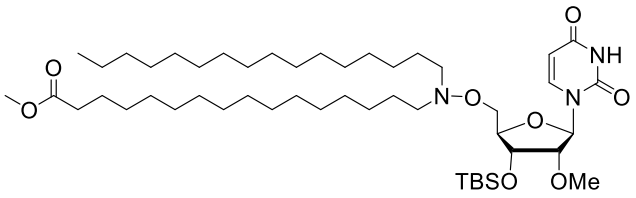
1-[(2R,5R)-4-hydroxy-3-methoxy-5-[[methyl-[9-[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]-1-[8-[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]octyl]nonyl]amino]oxymethyl]tetrahydro furan-2-yl]pyrimidine-2,4-dione **(17c):** To a clear solution of **16c** (0.55 g, 567.86 μmol) in THF (20 mL) at 22 °C, tetrabutylammonium fluoride, 1M in THF (193.01 mg, 738.22 μmol) was added slowly in single portion and then stirred for 3 hr. All the volatile matters were removed under high vacuum pump and the residue thus obtained was purified by flash column chromatography (gradient: 20-50% EtOAc in hexane) to afford **17c** (0.4 g, 82% yield) as white semi-solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 5.96 (d, *J* = 2.3 Hz, 1H), 5.69 (dd, *J* = 8.2, 2.2 Hz, 1H), 4.19 (td, *J* = 7.5, 5.2 Hz, 1H), 4.10 (dd, *J* = 11.1, 2.3 Hz, 1H), 4.05 (dt, *J* = 7.0, 2.4 Hz, 1H), 3.86 (dd, *J* = 11.2, 2.5 Hz, 1H), 3.73 (dd, *J* = 5.2, 2.3 Hz, 1H), 3.61 (s, 3H), 2.64 (d, *J* = 8.1 Hz, 1H), 2.58 (s, 3H), 2.54 (q, *J* = 5.4 Hz, 1H), 1.50 (tt, *J* = 14.1, 5.9 Hz, 2H), 1.39 (ddq, *J* = 9.1, 6.4, 3.4 Hz, 11H), 1.37 – 1.24 (m, 38H), 1.03 (dt, *J* = 14.2, 8.0 Hz, 1H), 0.94 – 0.85 (m, 6H), 0.83 – 0.74 (m, 4H), 0.69 (ptd, *J* = 8.6, 5.3, 3.0 Hz, 4H), 0.61 (td, *J* = 8.4, 4.1 Hz, 4H), -0.28 (dq, *J* = 17.1, 5.1 Hz, 4H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.9, 150.1, 140.1, 102.1, 87.2, 84.0, 83.1, 70.1, 68.7, 66.7, 58.8, 40.4, 32.0, 30.4, 30.2, 30.1, 30.0, 29.9, 29.8, 29.0, 29.0, 28.9, 28.9, 28.2, 28.0, 26.9, 26.8, 22.9, 16.2, 16.1, 16.0, 15.8, 15.8, 14.3, 11.2, 11.0 ppm. HRMS calcd. for $C_{52}H_{92}N_3O_6$ $[M + H]^+$ 854.6986, found 854.6977.



18c

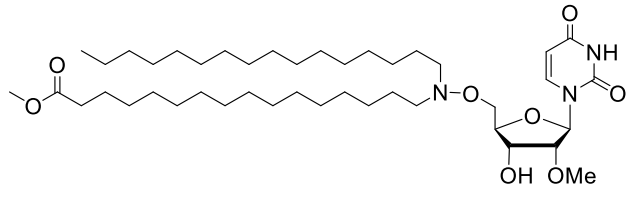
3-[(diisopropylamino)-[(2R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-2-[[methyl-[9-[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]-1-[8-[2-[(2-pentylcyclopropyl)methyl]cyclopropyl] octyl]nonyl]amino]oxymethyl]tetrahydrofuran-3-yl]oxy-phosphanyl]oxypropanenitrile **(18c):** To a clear solution of **17c** (0.4 g, 468.22 μmol) in DCM (10 mL) was added NMI (57.66 mg, 702.34 μmol, 55.98 μL) and DIPEA (302.57 mg, 2.34 mmol, 407.77 μL) in single portions. After stirring the reaction mixture for 5 minutes at 22 °C, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (221.64 mg, 936.45 μmol, 209.09 μL) was added and continued stirring for 1 hr and TLC was checked. Starting material was consumed and reaction mixture was diluted with DCM (15 mL). DCM layer was washed with 10% $NaHCO_3$ (2 x 25 mL) solution, and brine (30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and filtrate was evaporated at 36°C to afford crude compound which was purified by flash chromatography (60-100% EtOAc in hexane) to afford **18c** (0.41 g, 83% yield) as transparent gum. ^{1}H NMR (600 MHz, CD_3CN) δ 9.05 (s, 1H), 7.84 – 7.77 (m, 1H), 5.89 (dd, *J* = 13.3, 4.6 Hz, 1H), 5.60 (d, *J* = 8.3 Hz, 1H), 5.43 (s, 3H), 4.39 – 4.09 (m, 2H), 4.05 (q, *J* = 7.2 Hz, 1H), 3.95

(t, *J* = 9.2 Hz, 1H), 3.86 – 3.69 (m, 3H), 3.64 (s, 2H), 3.48 – 3.32 (m, 3H), 2.65 (dt, *J* = 13.2, 5.5 Hz, 2H), 2.55 (d, *J* = 11.8 Hz, 3H), 1.55 – 1.08 (m, 76H), 1.02 (dt, *J* = 15.0, 8.2 Hz, 1H), 0.87 (q, *J* = 8.2 Hz, 6H), 0.77 (dt, *J* = 14.1, 7.0 Hz, 4H), 0.68 (s, 6H), 0.58 (q, *J* = 7.5 Hz, 5H), -0.21 – -0.41 (m, 4H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 171.5, 163.7, 163.7, 151.2, 140.7, 140.7, 119.3, 119.2, 102.7, 102.6, 87.9, 87.5, 83.8, 83.3, 82.8, 71.8, 71.7, 71.4, 71.4, 71.3, 71.2, 67.0, 66.9, 60.8, 59.6, 59.5, 59.0, 59.0, 58.9, 58.9, 58.5, 58.5, 55.1, 44.0, 43.9, 43.9, 43.8, 40.4, 40.3, 32.5, 30.8, 30.5, 30.5, 30.5, 30.4, 30.2, 30.2, 30.2, 29.5, 29.3, 28.5, 28.4, 27.4, 27.3, 27.2, 25.0, 25.0, 24.9, 24.9, 24.8, 24.8, 23.3, 21.1, 21.0, 20.9, 20.9, 16.7, 16.6, 16.6, 16.3, 14.5, 14.4, 11.5, 11.4, 11.3 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 150.0 ppm. HRMS calcd. for $C_{61}H_{109}N_5O_7P$ [M + H]+ 1054.8065, found 1054.8014.



16d

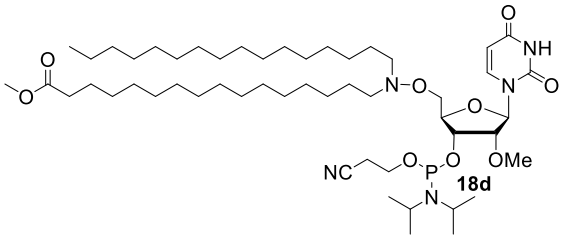
Methyl-16-[[(2R,5R)-3-[tert-butyl(dimethyl)silyl]oxy-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetrahydrofuran-2-yl]methoxy-hexadecyl-amino]hexadecanoate **(16d)**: To a clear solution of **4c** (1.5 g, 2.46 mmol) in dry DCM (20 mL) and acetic acid (10 mL) was added sodium cyanoborohydride (410.02 mg, 6.39 mmol) in single portion and the reaction mixture was stirred for 1.5 hr at 15 °C . To the resulting mixture was added methyl 16-oxohexadecanoate (1.05 g, 3.69 mmol) and stirring was continued for 1 hr. Reaction was again cooled to 15 °C and sodium cyanoborohydride (410.02 mg, 6.39 mmol) was added. After 2.5 hr TLC showed consumption of starting materials. Reaction mixture was diluted with DCM (25 mL) and quenched with water (30 mL). Layers were separated and aqueous layer was washed with DCM (20 mL). Combined DCM layer was washed with brine (2 x 30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The residue thus obtained was purified flash column chromatography (gradient: 10-40% EtOAc in hexane) to afford **16d** (1.95 g, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (d, *J* = 2.2 Hz, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 5.92 (d, *J* = 2.3 Hz, 1H), 5.68 (dd, *J* = 8.2, 2.1 Hz, 1H), 4.17 (dd, *J* = 6.9, 4.9 Hz, 1H), 4.12 – 4.03 (m, 2H), 3.92 – 3.82 (m, 1H), 3.67 (s, 6H), 3.65 – 3.59 (m, 1H), 3.53 (s, 3H), 2.73 – 2.62 (m, 4H), 2.30 (t, *J* = 7.6 Hz, 3H), 1.68 – 1.48 (m, 6H), 1.25 (s, 63H), 0.89 (d, *J* = 12.1 Hz, 12H), 0.10 (d, *J* = 5.4 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5, 163.4, 150.2, 140.3, 101.7, 87.8, 84.0, 82.9, 71.2, 69.6, 63.2, 59.3, 58.3, 51.6, 34.2, 32.9, 32.1, 29.8, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 27.6, 27.2, 25.9, 25.8, 25.1, 22.8, 18.2, 14.3, -4.4, -4.7 ppm. HRMS calcd. for $C_{49}H_{94}N_3O_8Si$ $[M + H]^+$ 880.6810, found 880.6799.



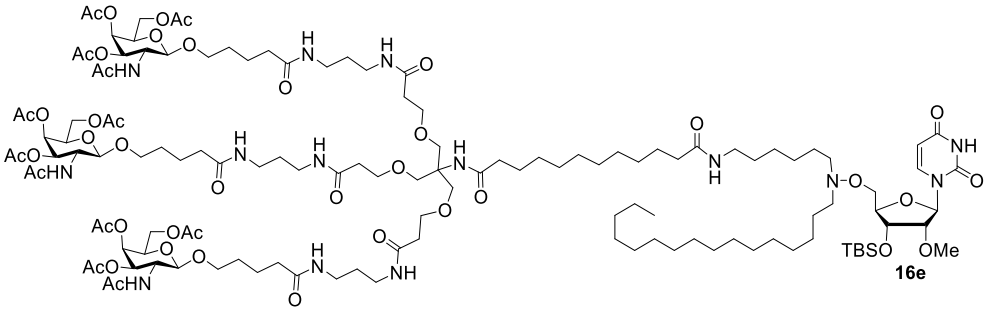
17d

Methyl-16-((((2R,3R,4R,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(2,4-dioxo-3,4-dihydropyrimid- in-1(2H)-yl)-4-methoxytetrahydrofuran-2-yl)methoxy)(hexadecyl)amino)hexadecanoate **(17d)**: To a clear solution of **16d** (1.9 g, 2.16 mmol) in THF (30 mL) at 22 °C, tetrabutylammonium fluoride (733.57 mg, 2.81 mmol) was added slowly in single portion and then stirred for 4 hr. All the volatile matters were removed under high vacuum pump and the residue thus obtained was purified by flash column chromatography (gradient: 20-60% EtOAc in hexane) to afford **17d** (1.35 g, 82% yield) as white semi-solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.00 (dd, *J* = 8.4, 2.1 Hz, 1H), 5.95 (d, *J* = 2.5 Hz, 1H), 5.71 – 5.65 (m, 1H), 4.22 (q, *J* = 6.8 Hz, 1H), 4.13 (dd, *J* = 11.1, 2.5 Hz, 1H), 4.03

(dd, *J* = 7.2, 2.5 Hz, 1H), 3.93 (dd, *J* = 11.1, 2.6 Hz, 1H), 3.74 (dd, *J* = 5.2, 2.5 Hz, 1H), 3.67 (d, *J* = 2.1 Hz, 3H), 3.61 (d *J* = 2.1 Hz, 3H), 2.67 (q, *J* = 7.2 Hz, 5H), 2.30 (td, *J* = 7.6, 2.2 Hz, 2H), 1.64 – 1.58 (m, 3H), 1.54 (q, *J* = 6.9 Hz, 5H), 1.35 – 1.22 (m, 43H), 0.88 (td, *J* = 7.1, 2.1 Hz, 3H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.5, 163.1, 150.1, 140.1, 101.9, 87.3, 83.9, 83.2, 71.3, 68.6, 59.5, 58.8, 51.6, 34.3, 32.1, 29.8, 29.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 27.6, 27.2, 25.1, 22.8, 14.3 ppm. HRMS calcd. for $C_{43}H_{80}N_3O_8$ $[M + H]^+$ 766.5945, found 766.5949.

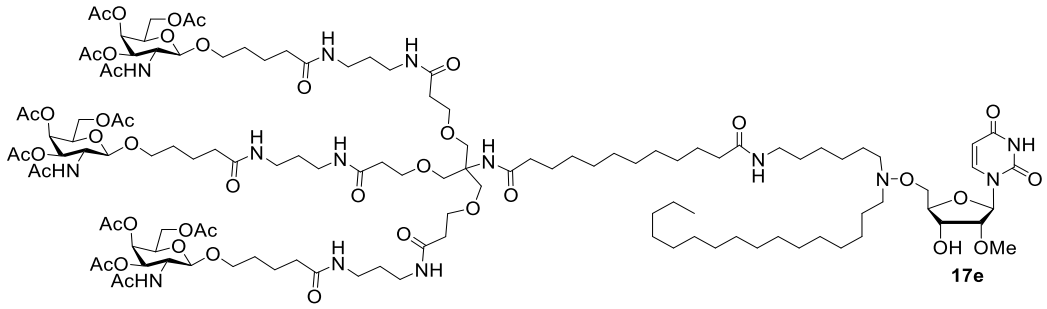


Methyl-16-[[(2R,5R)-3-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetrahydrofuran-2-yl]methoxy-hexadecyl-amino]hexadecanoate **(18d)**: To a clear solution of **17d** in DCM (30 mL) was added NMI (208.97 mg, 2.55 mmol, 202.89 µL) and DIPEA (1.10 g, 8.48 mmol, 1.48 mL) in single portions. After stirring the reaction mixture for 5 minutes at 22 °C, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (803.25 mg, 3.39 mmol, 757.78 µL) was added and continued stirring for 1 hr and TLC was checked. Starting material was consumed and reaction mixture was diluted with DCM (15 mL). DCM layer was washed with 10% $NaHCO_3$ (2 x 25 mL) solution, and brine (30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and filtrate was evaporated at 36°C to afforded crude compound which was purified by flash chromatography (60-100% EtOAc in hexane) to afford **18d** (1.31 g, 80% yield) as transparent gum. ^{1}H NMR (600 MHz, CD_3CN) δ 9.06 (s, 1H), 8.22 – 7.45 (m, 1H), 5.88 (dd, *J* = 12.7, 4.4 Hz, 1H), 5.60 (dd, *J* = 8.1, 1.4 Hz, 1H), 4.39 – 4.25 (m, 1H), 4.22 – 4.09 (m, 1H), 3.99 (td, *J* = 10.7, 2.6 Hz, 1H), 3.91 – 3.75 (m, 4H), 3.76 – 3.60 (m, 2H), 3.60 (s, 3H), 3.50 – 3.36 (m, 3H), 2.72 – 2.58 (m, 6H), 2.27 (t, *J* = 7.5 Hz, 2H), 1.61 – 1.46 (m, 7H), 1.38 – 1.25 (m, 49H), 1.19 (td, *J* = 6.5, 3.1 Hz, 13H), 0.88 (t, *J* = 7.0 Hz, 3H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 174.8, 163.9, 151.4, 141.0, 141.0, 119.5, 119.4, 102.5, 88.2, 87.9, 83.7, 83.7, 83.4, 83.3, 83.2, 83.2, 83.0, 83.0, 72.84, 72.7, 71.8, 71.7, 71.5, 71.4, 61.0, 59.8, 59.7, 59.7, 59.6, 59.3, 59.2, 59.0, 59.0, 58.6, 58.5, 55.3, 51.8, 44.1, 44.1, 44.0, 44.0, 34.5, 32.7, 30.4, 30.4, 30.4, 30.4, 30.3, 30.3, 30.3, 30.3, 30.2, 30.2, 30.1, 30.0, 29.8, 28.2, 28.1, 27.9, 25.7, 25.1, 25.0, 25.0, 24.9, 24.9, 24.9, 23.4, 21.0, 21.0, 14.5, 14.4 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 150.0, 149.8 ppm. HRMS calcd. for $C_{52}H_{97}N_5O_9P$ $[M + H]^+$ 966.7024, found 966.7043.



[(3R,6R)-5-acetamido-6-[5-[3-[3-[3-[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]propylamino]-3-oxo-propoxy]-2-[[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoyl amino]propylamino]-3-oxo-propoxy]methyl]-2-[[12-[6-[[(2R,5R)-3-[tert-butyl(dimethyl)silyl]oxy-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetrahydrofuran-2-yl]methoxy-hexadecyl-amino]hexyl amino]-12-oxo-dodecanoyl]amino]propoxy]propanoylamino]propylamino]-5-oxo-pentoxy]-3,4-diacetoxy-tetrahydropyran-2-yl]methyl acetate **(16e):** To a clear solution of **4f** (2.0 g, 808.79 µmol)

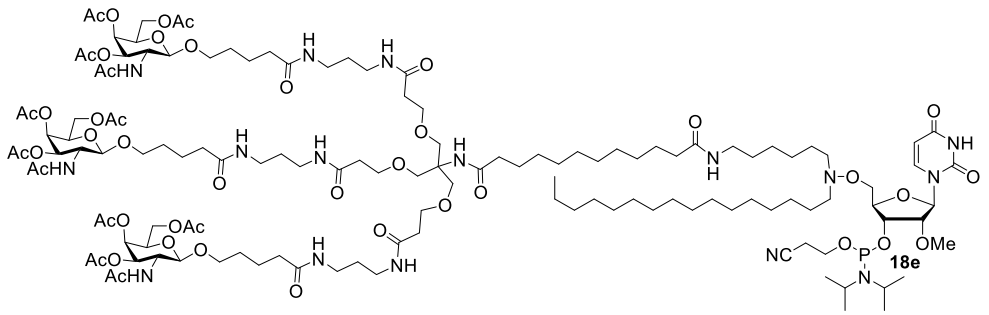
in dry DCM (20 mL) and acetic acid (10 mL) was added sodium cyanoborohydride (132.14 mg, 2.10 mmol) in single portion and the reaction mixture was stirred for 1.5 hr at 15 °C . To the resulting mixture was added palmitaldehyde (213.89 mg, 889.67 μmol) and stirring was continued for 1 hr. Reaction was again cooled to 15 °C and sodium cyanoborohydride (132.14 mg, 2.10 mmol) was added. After 2.5 hr TLC showed consumption of starting materials. Reaction mixture was diluted with DCM (25 mL) and quenched with water (30 mL). Layers were separated and aqueous layer was washed with DCM (20 mL). Combined DCM layer was washed with brine (2 x 30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The residue thus obtained was purified by flash column chromatography (gradient: 2-20% MeOH in DCM) to afford **16e** (1.52 g, 70% yield) as white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.38 (d, *J* = 2.2 Hz, 1H), 7.86 – 7.80 (m, 7H), 7.79 – 7.71 (m, 5H), 7.68 (t, *J* = 5.6 Hz, 1H), 6.98 (s, 1H), 5.79 (d, *J* = 3.9 Hz, 1H), 5.63 (dd, *J* = 8.1, 2.2 Hz, 1H), 5.21 (d, *J* = 3.4 Hz, 4H), 4.96 (dd, *J* = 11.3, 3.4 Hz, 3H), 4.48 (d, *J* = 8.5 Hz, 4H), 4.16 (t, *J* = 5.5 Hz, 1H), 4.09 (q, *J* = 5.2 Hz, 1H), 4.02 (h, *J* = 4.3 Hz, 11H), 3.92 (td, *J* = 5.1, 3.0 Hz, 1H), 3.90 – 3.82 (m, 5H), 3.78 (dd, *J* = 11.0, 4.8 Hz, 1H), 3.70 (dt, *J* = 9.7, 5.9 Hz, 4H), 3.53 (dd, *J* = 12.1, 5.7 Hz, 15H), 3.40 (dt, *J* = 9.8, 6.3 Hz, 4H), 3.35 (s, 3H), 3.17 (d, *J* = 5.2 Hz, 2H), 3.02 (tt, *J* = 13.0, 6.5 Hz, 17H), 2.65 – 2.59 (m, 5H), 2.27 (t, *J* = 6.4 Hz, 8H), 2.10 (s, 9H), 2.04 (t, *J* = 7.3 Hz, 9H), 1.99 (s, 9H), 1.89 (s, 9H), 1.77 (s, 11H), 1.53 – 1.40 (m, 29H), 1.31 – 1.19 (m, 40H), 0.86 (d, *J* = 13.4 Hz, 12H), 0.08 (d, *J* = 1.0 Hz, 6H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.5, 171.9, 171.9, 170.1, 170.0, 169.9, 169.6, 169.3, 163.1, 150.3, 140.2, 101.6, 101.0, 95.4, 86.9, 82.1, 81.8, 72.2, 70.5, 69.9, 69.8, 68.7, 68.2, 67.3, 66.7, 61.4, 59.5, 58.5, 58.4, 57.5, 49.3, 48.6, 38.3, 36.4, 36.3, 36.0, 35.9, 35.4, 35.0, 31.3, 29.3, 29.1, 29.0, 29.0, 29.0, 28.9, 28.9, 28.9, 28.9, 28.8, 28.7, 28.7, 28.7, 28.6, 26.8, 26.6, 26.5, 26.5, 26.4, 25.6, 25.4, 25.3, 22.8, 22.1, 21.8, 20.5, 20.5, 20.4, 17.7, 14.0, -4.7, -5.1 ppm. MALDI calcd. for $C_{129}H_{220}N_{14}O_{44}SiNa$ $[M + Na]^+$ 2722.31; found 2725.66.



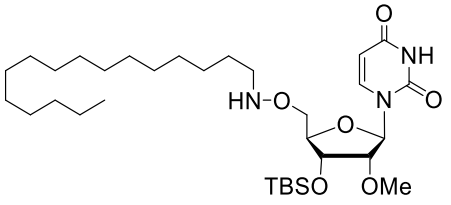
17e

[(3R,6R)-5-acetamido-6-[5-[3-[3-[3-[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]propylamino]-3-oxo-propoxy]-2-[[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoyl amino]propylamino]-3-oxo-propoxy]methyl]-2-[[12-[6-[[(2R,5R)-5-(2,4-dioxopyrimidin-1-yl)-3-hydroxy-4-methoxy-tetrahydrofuran-2-yl]methoxy-hexadecyl-amino]hexylamino]-12-oxo-dodecanoyl]amino]propoxy]propanoylamino]propylamino]-5-oxo-pentoxy]-3,4-diacetoxy-tetrahydropyran-2-yl]methyl acetate **(17e):** To a clear solution of **16e** (1.5 g, 555.71 μmol) in THF (25 mL) was added TBAF (188.88 mg, 722.42 μmol) in single portion and stirred for 12 hr at 22 °C. All the volatile matters were evaporated under high vacuum pump and the crude residue thus obtained, was purified by flash column chromatography (gradient: 5-20% MeOH in DCM) to afford **17e** (1.21 g, 84% yield) as white foam. ^{1}H NMR (600 MHz, CD_3CN) δ 9.52 (s, 1H), 7.83 (d, *J* = 8.2 Hz, 1H), 7.17 (t, *J* = 6.0 Hz, 2H), 6.99 (t, *J* = 5.9 Hz, 2H), 6.91 (d, *J* = 9.4 Hz, 2H), 6.64 (d, *J* = 6.5 Hz, 2H), 5.85 (d, *J* = 3.7 Hz, 1H), 5.61 (d, *J* = 8.2 Hz, 1H), 5.28 (dd, *J* = 3.5, 1.1 Hz, 3H), 5.03 (dd, *J* = 11.3, 3.4 Hz, 3H), 4.54 (d, *J* = 8.5 Hz, 3H), 4.15 (t, *J* = 5.3 Hz, 1H), 4.11 (dd, *J* = 11.3, 6.8 Hz, 3H), 4.05 (dd, *J* = 11.3, 6.1 Hz, 3H), 4.01 – 3.91 (m, 8H), 3.86 – 3.76 (m, 5H), 3.62 (d, *J* = 5.0 Hz, 13H), 3.52 – 3.44 (m, 6H), 3.18 (dq, *J* = 12.4, 6.5 Hz, 14H), 3.11 (q, *J* = 6.6 Hz, 2H), 2.69 – 2.64 (m, 3H), 2.34 (t, *J* = 5.9 Hz, 7H), 2.14 (td, *J* = 7.3, 1.8 Hz, 6H), 2.10 (s, 11H), 1.98 (s, 9H), 1.91 (s, 9H), 1.85 (s, 10H), 1.60 (p, *J* = 7.3 Hz, 13H), 1.55 – 1.48 (m, 13H), 1.44 (p, *J* = 6.9 Hz, 1H), 1.38 – 1.24 (m, 33H) ppm.

^{13}C NMR (151 MHz, CD_3CN) δ 174.5, 174.0, 174.0, 174.0, 174.0, 172.4, 172.4, 172.4, 171.4, 171.3, 171.3, 171.3, 171.2, 171.0, 164.1, 151.4, 141.2, 102.4, 102.2, 96.6, 88.1, 84.1, 83.8, 73.1, 71.6, 71.4, 70.1, 70.0, 69.9, 68.4, 67.9, 62.5, 62.5, 60.7, 59.9, 59.8, 58.8, 51.1, 39.7, 37.5, 37.5, 37.2, 37.1, 36.9, 36.5, 32.6, 30.4, 30.4, 30.4, 30.4, 30.4, 30.3, 30.3, 30.2, 30.2, 30.1, 30.1, 30.1, 30.0, 30.0, 29.8, 29.8, 29.8, 29.8, 29.5, 28.1, 27.8, 27.8, 27.7, 27.4, 26.6, 26.6, 24.3, 23.4, 23.2, 20.9, 20.9, 14.4, 13.8 ppm. MALDI calcd. for $C_{123}H_{206}N_{14}O_{44}Na$ $[M + Na]^+$ 2608.05; found 2611.20.

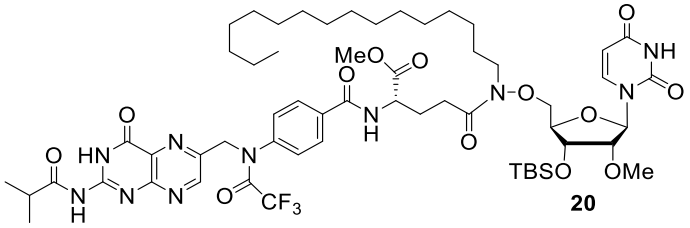


***[(3R,6R)-5-acetamido-6-[5-[3-[3-[3-[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoylamino]propylamino]-3-oxo-propoxy]-2-[[3-[3-[5-[(2R,5R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydropyran-2-yl]oxypentanoy lamino]propylamino]-3-oxo-propoxy]methyl]-2-[[12-[6-[[(2R,5R)-3-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetrahydrofuran-2-yl]methoxy-hexadecyl-amino]hexylamino]-12-oxo-dodecanoyl]amino]propoxy]propanoyl amino]propylamino]-5-oxo-pentoxy]-3,4-diacetoxy-tetrahydropyran-2-yl]methyl acetate* (18e):** To a clear solution of **17e** (1.0 g, 386.85 μmol) in DCM (20 mL) was added NMI (63.52 mg, 773.69 μmol, 61.67 μL) and DIPEA (249.98 mg, 1.93 mmol, 336.90 μL) in single portions. After stirring the reaction mixture for 5 minutes at 22 °C, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (183.12 mg, 773.69 μmol, 172.75 μL) was added and continued stirring for 1 hr and TLC was checked. Starting material was consumed and reaction mixture was diluted with DCM (15 mL). DCM layer was washed with 10% $NaHCO_3$ (25 x 2 mL) solution, and brine (30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and filtrate was evaporated at 36°C to afford crude gummy compound which was triturated with 1:1 hexane and diethylether (20 mL). The solid residue was then dissolved in methyl-*tert*-butylether (30 mL) and the organic layer was washed with 30% DMF in water (20 x 2 mL) followed by brine (30 x 3 mL). Organic layer was separated, dried over anhydrous Na_2SO_4 , filtered and the filtrated was evaporated to dryness. Residue was co-evaporated with diethyl ether and dried under high vacuum pump overnight to afford **18e** (0.78 g, 72% yield) as white foam. ^{1}H NMR (600 MHz, CD_3CN) δ 9.40 (s, 1H), 7.88 – 7.76 (m, 1H), 7.19 (q, *J* = 7.5 Hz, 3H), 7.05 – 6.88 (m, 7H), 6.61 (d, *J* = 17.9 Hz, 1H), 5.88 (dd, *J* = 13.4, 4.4 Hz, 1H), 5.61 (d, *J* = 8.2 Hz, 1H), 5.28 (dd, *J* = 3.4, 1.1 Hz, 4H), 5.03 (dd, *J* = 11.2, 3.3 Hz, 4H), 4.55 (d, *J* = 8.6 Hz, 4H), 4.41 – 4.23 (m, 1H), 4.14 – 4.02 (m, 8H), 4.02 – 3.93 (m, 8H), 3.90 – 3.77 (m, 4H), 3.70 – 3.60 (m, 16H), 3.52 – 3.44 (m, 5H), 3.18 (dd, *J* = 11.7, 6.1 Hz, 12H), 2.72 – 2.63 (m, 8H), 2.37 – 2.32 (m, 7H), 2.18 – 2.05 (m, 19H), 1.98 (s, 11H), 1.91 (s, 10H), 1.85 (s, 9H), 1.64 – 1.50 (m, 21H), 1.36 – 1.17 (m, 79H), 1.13 (s, 19H), 0.88 (t, *J* = 6.9 Hz, 3H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 174.4, 174.4, 174.0, 174.0, 173.8, 172.4, 172.4, 172.4, 171.3, 171.3, 171.3, 171.2, 171.0, 164.0, 163.2, 151.5, 141.1, 141.0, 119.6, 119.5, 102.6, 102.2, 88.3, 87.9, 83.6, 83.6, 83.3, 83.1, 83.0, 83.0, 73.1, 73.0, 72.8, 71.8, 71.7, 71.5, 71.4, 70.1, 69.9, 68.4, 67.9, 62.5, 60.7, 59.8, 59.7, 59.6, 59.3, 59.3, 59.2, 59.0, 59.0, 58.6, 58.6, 51.1, 49.5, 45.9, 45.8, 45.8, 45.7, 44.1, 44.1, 44.0, 44.0, 39.7, 37.5, 37.5, 37.2, 37.1, 36.9, 36.5, 32.6, 30.4, 30.4, 30.4, 30.4, 30.3, 30.3, 30.3, 30.3, 30.2, 30.2, 30.2, 30.1, 30.1, 30.1, 30.0, 30.0, 30.0, 30.0, 29.9, 29.8, 29.5, 28.2, 28.2, 27.9, 27.9, 27.9, 27.8, 27.6, 27.2, 26.7, 26.6, 25.1, 25.0, 25.0, 24.9, 24.9, 24.9, 24.8, 24.3, 23.4, 23.2, 21.0, 21.0, 21.0, 20.9, 20.9, 14.4 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 149.91, 149.87 ppm. MALDI calcd. for $C_{132}H_{223}N_{16}O_{45}PNa$ $[M + Na]^+$ 2808.26; found 2811.21.



19

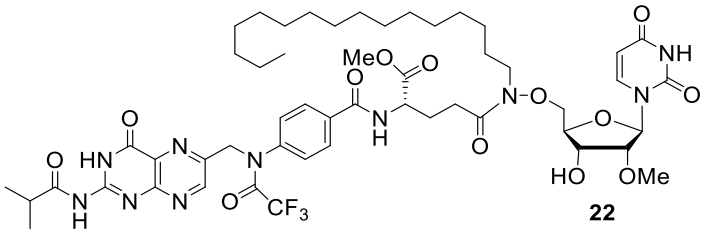
1-[(2R,5R)-4-[tert-butyl(dimethyl)silyl]oxy-5-[(hexadecylamino)oxymethyl]-3-methoxy-tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(19):** To a solution of **4c** (0.61 g, 1.00 mmol) in glacial acetic acid (10 mL), sodium cyanoborohydride (166.74 mg, 2.60 mmol) was added and stirred for 4 hr at 15 °C. Reaction mixture was diluted with DCM (20 mL) and the organic layer was washed with water (20 mL). Organic layer was dried over anhydrous Na_2SO_4, filtered, and the filtrate was evaporated to dryness. The crude compound was purified by flash column chromatography to afford **19** (0.52 g, 85% yield) as transparent gum. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.50 (s, 1H), 7.88 (d, *J* = 8.1 Hz, 1H), 5.88 (d, *J* = 2.1 Hz, 1H), 5.70 (d, *J* = 8.1 Hz, 1H), 4.17 (dd, *J* = 7.5, 4.8 Hz, 1H), 4.14 – 4.05 (m, 2H), 3.90 – 3.83 (m, 1H), 3.63 (dd, *J* = 4.8, 2.2 Hz, 1H), 3.54 (s, 3H), 2.95 (tt, *J* = 9.4, 4.7 Hz, 2H), 1.53 – 1.46 (m, 2H), 1.26 (s, 26H), 0.91 (s, 10H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.10 (d, *J* = 4.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.7, 163.7, 150.3, 140.2 102.0, 88.4, 84.0, 82.5, 77.5, 77.2, 76.8, 71.7, 69.6, 69.5, 58.6, 52.4, 32.0, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 27.4, 27.3, 25.8, 22.8, 18.3, 14.2, -4.5, -4.8 ppm. HRMS calcd. for $C_{32}H_{62}N_3O_6Si$ $[M + H]^+$ 612.4408, found 612.4423.



20

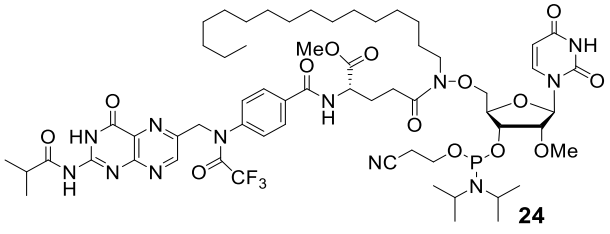
Methyl-(2S)-5-[[3-[tert-butyl(dimethyl)silyl]oxy-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetrahydrofuran-2-yl]methoxy-hexadecyl-amino]-2-[[4-[[2-(2-methylpropanoylamino)-4-oxo-3H-pteridin-6-yl]methyl-(2,2,2-trifluoroacetyl)amino]benzoyl]amino]-5-oxo-pentanoate **(20):** To a clear solution of (4S)-5-methoxy-4-[[4-[[2-(2-methylpropanoylamino)-4-oxo-3H-pteridin-6-yl]methyl-(2,2,2-trifluoroacetyl)amino]benzoyl]amino]-5-oxo-pentanoic acid (0.56 g, 901.02 μmol) in dimethylformamide (4 mL), were added N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (172.72 mg, 901.02 μmol) 1-hydroxy-7-azabenzotriazole tetrahydrate (187.56 mg, 901.02 μmol) and DIPEA (349.34 mg, 2.70 mmol, 470.81 μL) in single portions. After 5 minutes, **19** (551.36 mg, 901.02 μmol) was added and the resulting mixture was stirred for 10 hr at 25 °C. All the volatile matters were removed under high vacuum pump and the residue was diluted with DCM (30 mL), and water (20 mL). Organic layer was separated, washed with $NaHCO_3$ solution (20 mL), water (20 mL) and brine (30 x 3 mL). DCM layer was separated, dried on anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The crude mass obtained, was purified by flash column chromatography (gradient: 0-5% MeOH in DCM) to afford **20** (0.81 g, 666.43 μmol, 74% yield) as white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.54 (s, 1H), 10.14 (s, 1H), 9.99 (s, 1H), 8.96 (s, 1H), 7.85 – 7.80 (m, 2H), 7.63 – 7.47 (m, 2H), 7.42 (d, J = 8.2 Hz, 2H), 5.76 (s, 1H), 5.64 (dd, J = 8.3, 2.1 Hz, 1H), 5.29 (d, J = 11.8 Hz, 1H), 5.21 (d, J = 15.4 Hz, 1H), 4.74 – 4.67 (m, 1H), 4.20 – 4.13 (m, 2H), 4.08 (d, J = 11.5 Hz, 2H), 3.75 (s, 3H), 3.69 (s, 2H), 3.52 (s, 3H), 2.82 (s, 1H), 2.68 (s, 2H), 2.34 (d, J = 14.6 Hz, 1H), 2.21 – 2.12 (m, 1H), 1.59 (s, 2H), 1.30 – 1.22 (m, 35H), 0.90 (s, 10H), 0.87 (t, J = 7.0 Hz, 3H), 0.09 (d, J = 11.2 Hz, 6H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.5, 173.7, 173.5, 172.7, 171.4, 169.2, 165.9, 163.4, 159.6, 157.8, 157.5, 157.3, 157.0, 154.8, 151.3, 150.0, 149.9, 149.0, 148.8, 142.3, 141.9, 139.8, 134.5, 131.1, 130.7, 130.6, 129.0, 128.6, 128.2, 119.1, 117.1, 115.2, 113.3, 102.5, 89.4, 83.0, 81.0, 72.4, 69.7, 58.6, 58.4, 54.6, 53.6, 53.0, 53.0, 52.8, 46.1, 36.5, 32.0, 29.8, 29.8, 29.8,

29.8, 29.7, 29.7, 29.5, 29.5, 27.3, 26.9, 25.6, 22.8, 19.0, 19.0, 19.0, 19.0, 18.2, 14.3, 0.1, -4.4, -4.9 ppm. ^{19}F NMR (565 MHz, $CDCl_3$) δ -67.0 ppm. HRMS calcd. for $C_{58}H_{86}F_3N_{10}O_{13}Si$ $[M + H]^+$ 1215.6097, found 1215.6097.



22

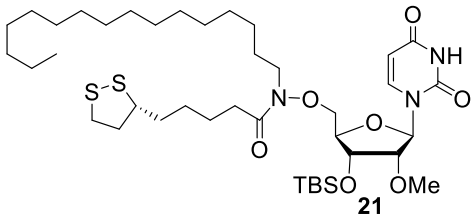
Methyl(2S)-5-[[5-(2,4-dioxopyrimidin-1-yl)-3-hydroxy-4-methoxy-tetrahydrofuran-2-yl]methoxy-hexadecyl-amino]-2-[[4-[[2-(2-methylpropanoylamino)-4-oxo-3H-pteridin-6-yl]methyl-(2,2,2-trifluoroacetyl)amino]benzoyl]amino]-5-oxo-pentanoate **(22):** To a clear solution of **20** (0.9 g, 740.48 μmol) in THF (20 mL) was added TBAF (251.69 mg, 962.62 μmol, 278.72 μL) in single portion and stirred for 12 hr at 22 °C. All the volatile matters were evaporated under high vacuum pump and the crude residue thus obtained, was purified by flash column chromatography (gradient: 5% MeOH in DCM) to afford **22** (0.55 g, 67% yield) as yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.35 (s, 1H), 11.97 (s, 1H), 11.38 (s, 1H), 8.90 (s, 1H), 8.87 (d, *J* = 7.3 Hz, 1H), 7.90 (d, *J* = 8.1 Hz, 2H), 7.69 (d, *J* = 8.1 Hz, 2H), 7.63 (s, 2H), 5.83 (d, *J* = 4.8 Hz, 1H), 5.61 (dt, *J* = 7.9, 1.6 Hz, 1H), 5.37 (d, *J* = 6.1 Hz, 1H), 5.27 – 5.18 (m, 2H), 4.44 (q, *J* = 7.2 Hz, 1H), 4.09 (t, *J* = 11.1 Hz, 3H), 4.04 – 3.95 (m, 2H), 3.81 (t, *J* = 5.0 Hz, 1H), 3.62 (s, 3H), 3.54 (qt, *J* = 13.9, 7.2 Hz, 2H), 3.34 (dd, *J* = 2.7, 1.3 Hz, 7H), 3.05 – 2.99 (m, 1H), 2.78 (hept, *J* = 6.9 Hz, 1H), 2.59 – 2.51 (m, 3H), 2.10 (dt, *J* = 14.2, 6.8 Hz, 1H), 1.96 (h, *J* = 7.3 Hz, 1H), 1.57 (td, *J* = 10.1, 6.1 Hz, 1H), 1.48 (hept, *J* = 6.9 Hz, 2H), 1.31 (h, *J* = 7.6 Hz, 1H), 1.24 – 1.11 (m, 42H), 0.91 (td, *J* = 7.3, 1.3 Hz, 1H), 0.86 – 0.81 (m, 3H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 180.8, 172.8, 172.4, 165.7, 162.9, 159.1, 156.1, 155.9, 155.6, 155.4, 154.8, 150.4, 150.0, 149.8, 147.8, 141.8, 140.2, 134.2, 130.6, 129.1, 128.7, 128.5, 119.0, 117.0, 115.1, 113.2, 102.1, 86.6, 81.7, 81.1, 79.2, 73.1, 68.6, 57.6, 54.0, 52.3, 51.9, 51.8, 44.3, 35.0, 31.3, 29.1, 29.0, 29.0, 28.9, 28.8, 28.7, 28.7, 28.1, 26.3, 26.1, 25.4, 25.1, 22.1, 19.4, 18.8, 13.9, 13.5 ppm. ^{19}F NMR (565 MHz, DMSO-d_6) δ -66.1 ppm. HRMS calcd. for $C_{52}H_{72}F_3N_{10}O_{13}$ $[M + H]^+$ 1101.5232, found 1101.5220.



24

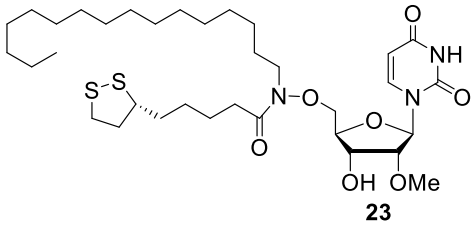
Methyl(2S)-5-[[3-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetrahydrofuran-2-yl]methoxy-hexadecyl-amino]-2-[[4-[[2-(2-methylpropanoylamino)-4-oxo-3H-pteridin-6-yl]methyl-(2,2,2-trifluoroacetyl)amino] benzoyl]amino]-5-oxo-pentanoate **(24)**: To a clear solution of **22** (0.31 g, 281.52 μmol) in DCM (20 mL) was added NMI (46.23 mg, 563.04 μmol, 44.88 μL) and DIPEA (181.92 mg, 1.41 mmol, 245.17 μL) in single portions. After stirring the reaction mixture for 5 minutes at 22 °C, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (133.26 mg, 563.04 μmol, 125.72 μL) was added and continued stirring for 1 hr and TLC was checked. Starting material was consumed and reaction mixture was diluted with DCM (15 mL). DCM layer was washed with 10% $NaHCO_3$ (2 x 25 mL) solution, and brine (30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and filtrate was evaporated at 36 °C to afford crude compound which was triturated with 1:1 diethylether-hexane mixture (30 mL) to afford off-white precipitate. This residue was dissolved in DCM (20 mL),

evaporated and co-evaporated with MTBE (20 mL) to afford **24** (0.33 g, 90% yield) as off-white foam. ^{1}H NMR (600 MHz, CD_3CN) δ 8.79 (d, *J* = 1.6 Hz, 1H), 7.87 – 7.75 (m, 3H), 7.60 – 7.32 (m, 3H), 5.89 – 5.74 (m, 1H), 5.62 – 5.56 (m, 1H), 5.17 (s, 2H), 4.57 – 4.20 (m, 2H), 4.18 – 3.96 (m, 4H), 3.91 – 3.60 (m, 9H), 3.55 – 3.38 (m, 5H), 2.76 – 2.62 (m, 4H), 2.25 – 2.01 (m, 10H), 1.55 (s, 2H), 1.28 – 1.15 (m, 56H), 0.87 (t, *J* = 7.0 Hz, 3H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 181.8, 173.3, 173.3, 173.2, 166.8, 166.7, 163.8, 163.7, 163.2, 160.7, 157.5, 157.3, 156.1, 151.2, 151.2, 151.0, 149.2, 143.2, 140.8, 138.9, 135.5, 132.1, 132.1, 130.0, 129.6, 129.3, 129.3, 119.7, 119.6, 116.4, 103.1, 73.4, 73.2, 73.1, 71.2, 71.1, 69.7, 59.7, 59.5, 59.2, 59.1, 59.1, 59.0, 59.0, 58.9, 58.6, 55.2, 54.0, 53.9, 52.8, 49.5, 46.0, 45.9, 44.1, 44.1, 44.0, 36.8, 36.5, 33.6, 32.6, 31.3, 30.4, 30.4, 30.3, 30.3, 30.3, 30.2, 30.2, 30.2, 30.1, 30.0, 30.0, 29.9, 29.6, 27.7, 27.4, 27.3, 27.2, 26.2, 25.0, 25.0, 25.0, 24.9, 24.9, 24.9, 24.8, 23.4, 23.2, 23.1, 23.1, 23.1, 21.0, 21.0, 21.0, 20.6, 20.6, 19.8, 19.2, 19.1, 14.4 ppm. ^{19}F NMR (565 MHz, CD_3CN) δ -67.69, -67.70 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 150.2, 149.9 ppm. HRMS calcd. for $C_{61}H_{89}F_3N_{12}O_{14}P$ $[M + H]^+$ 1301.6311, found 1301.6333.



21

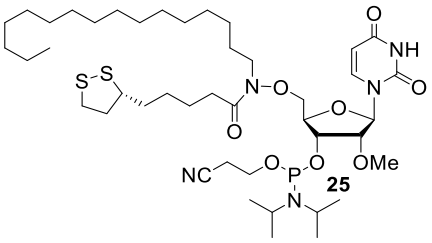
N-[[(2R,5R)-3-[tert-butyl(dimethyl)silyl]oxy-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetrahydrofuran-2-yl]methoxy]-5-(dithiolan-3-yl)-N-hexadecyl-pentanamide **(21)**: To a clear solution of (±)-1,2-dithiolane-3-pentanoic acid (454.16 mg, 2.16 mmol) in DMF (10 mL), were added 1-hydroxy-7-azabenzotriazole tetrahydrate (458.21 mg, 2.16 mmol), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (435.28 mg, 2.16 mmol) and DIPEA (469.34 mg, 3.60 mmol, 632.53 μL) in single portions. After 5 minutes, **19** (1.1 g, 1.80 mmol) was added and the resulting mixture was stirred for 8 hr at 25 °C. Reaction mixture was diluted with EtOAc (30 mL), and cold water (20 mL). Organic layer was separated, washed with $NaHCO_3$ solution (20 mL), water (20 mL) and brine (30 x 3 mL). EtOAc layer was separated, dried on anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The crude mass obtained, was purified by flash column chromatography to afford **21** (0.95 g, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.30 (s, 1H), 7.59 (s, 1H), 5.86 (t, *J* = 1.8 Hz, 1H), 5.72 (d, *J* = 8.1 Hz, 1H), 4.23 – 4.12 (m, 2H), 4.10 – 4.00 (m, 1H), 3.73 – 3.67 (m, 1H), 3.67 – 3.49 (m, 5H), 3.22 – 3.04 (m, 2H), 2.49 – 2.30 (m, 3H), 1.96 – 1.82 (m, 1H), 1.75 – 1.55 (m, 4H), 1.51 – 1.37 (m, 2H), 1.33 – 1.21 (m, 28H), 0.91 (s, 9H), 0.89 – 0.84 (m, 3H), 0.11 (d, *J* = 6.1 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.17, 150.04, 140.05, 102.51, 89.19, 83.27, 81.34, 72.22, 69.80, 58.37, 56.52, 56.50, 40.37, 38.63, 34.90, 34.87, 32.06, 29.83, 29.82, 29.79, 29.71, 29.49, 29.46, 29.17, 29.15, 26.91, 25.80, 24.48, 22.82, 18.24, 14.25, -4.27, -4.73 ppm. HRMS calcd. for $C_{40}H_{74}N_3O_7S_2Si$ $[M + H]^+$ 800.4737, found 800.4751.



23

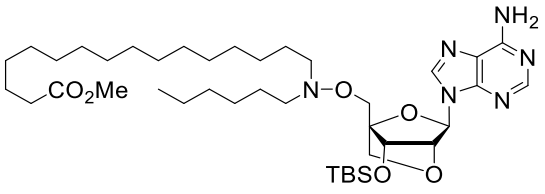
N-[[(2R,5R)-5-(2,4-dioxopyrimidin-1-yl)-3-hydroxy-4-methoxy-tetrahydrofuran-2-yl]methoxy]-5-(dithiolan-3-yl)-N-hexadecyl-pentanamide **(23):** To a solution of **21** (0.85 g, 1.06 mmol) in THF (10 mL) at 25 °C, tetrabutylammonium fluoride, 1M in THF (280.52 mg, 1.06 mmol, 1.06 mL) was added slowly in single portion and then stirred for 4 hr. Volatile matters were removed in high vacuum pump and crude residue thus obtained was purified by flash column chromatography (gradient: 10-60% EtOAc in hexane) to afford **23** (0.52 g, 71% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H), 7.61

(s, 1H), 5.92 (s, 1H), 5.72 (dd, *J* = 8.2, 1.8 Hz, 1H), 4.31 (dt, *J* = 10.4, 2.0 Hz, 1H), 4.24 (s, 1H), 4.14 – 4.01 (m, 2H), 3.81 (dd, *J* = 5.4, 1.7 Hz, 1H), 3.65 (s, 3H), 3.61 – 3.51 (m, 2H), 3.23 – 3.06 (m, 2H), 2.78 (s, 1H), 2.45 (tt, *J* = 15.3, 7.7 Hz, 3H), 1.97 – 1.84 (m, 1H), 1.82 – 1.46 (m, 7H), 1.56 – 1.40 (m, 2H), 1.25 (s, 26H), 0.88 (t, *J* = 6.8 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.9, 150.0, 139.4, 102.5, 88.2, 83.3, 81.5, 71.9, 68.5, 59.0, 56.6, 56.5, 40.4, 38.6, 34.8, 34.8, 32.4, 32.1, 29.8, 29.8, 29.7, 29.5, 29.4, 29.2, 29.1, 26.9, 24.5, 22.8, 14.3 ppm. HRMS calcd. for $C_{34}H_{60}N_3O_7S_2$ $[M + H]^+$ 686.3873, found 686.3855.



25

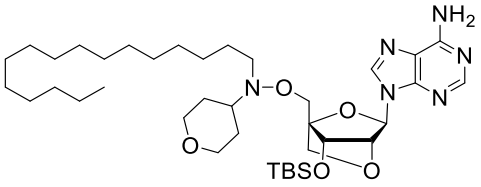
N-[[(2R,5R)-3-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-5-(2,4-dioxopyrimidin-1-yl)-4-methoxy-tetrahydrofuran-2-yl]methoxy]-5-(dithiolan-3-yl)-N-hexadecyl-pentanamide **(25):** To a clear solution of **23** (0.35 g, 510.22 μmol) in DCM (15 mL), DIPEA (333.04 mg, 2.55 mmol, 448.84 μL) and NMI (148.09 mg, 1.79 mmol, 143.78 μL) were added at 22 °C. To this reaction mixture, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (254.23 mg, 1.02 mmol, 239.84 μL) was added slowly after 5 minutes and stirred for 1 hr. Reaction mixture was diluted with DCM (10 mL) and quenched with 10% $NaHCO_3$ solution (20 mL). Organic layer was separated, dried on anhydrous Na_2SO_4, filtered and filtrate was evaporated to dryness. The crude compound was thus obtained was purified by flash column chromatography (gradient: 10-50% EtOAc in hexane) to afford **25** (0.205 g, 45% yield) as transparent yellow gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 7.58 (s, 1H), 5.99 – 5.86 (m, 1H), 5.78 – 5.65 (m, 1H), 4.44 – 4.31 (m, 2H), 4.27 – 4.18 (m, 2H), 4.13 – 3.98 (m, 1H), 3.96 – 3.85 (m, 2H), 3.83 – 3.50 (m, 10H), 3.23 – 3.06 (m, 2H), 2.65 (dt, *J* = 12.5, 6.2 Hz, 2H), 2.52 – 2.34 (m, 3H), 1.97 – 1.84 (m, 1H), 1.78 – 1.56 (m, 2H), 1.53 – 1.39 (m, 2H), 1.36 – 1.16 (m, 43H), 0.92 – 0.83 (m, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.0, 162.9, 150.1, 140.0, 117.8, 117.0, 102.7, 102.6, 82.9, 72.5, 70.3, 70.2, 60.5, 59.0, 58.5, 58.3, 58.3, 58.1, 57.9, 57.7, 56.6, 56.5, 45.5, 45.4, 43.6, 43.6, 43.5, 43.4, 40.4, 38.6, 34.9, 34.9, 32.0, 29.8, 29.8, 29.7, 29.5, 29.5, 29.2, 29.1, 26.9, 26.9, 24.9, 24.8, 24.7, 24.7, 24.7, 24.5, 23.1, 23.1, 23.0, 23.0, 22.8, 20.7, 20.6, 20.6, 20.3, 20.2, 14.3, 14.2 ppm. ^{31}P NMR (162 MHz, $CDCl_3$) δ 150.8 ppm. HRMS calcd. for $C_{43}H_{77}N_5O_8PS_2$ $[M + H]^+$ 886.4951, found 886.4966.



26

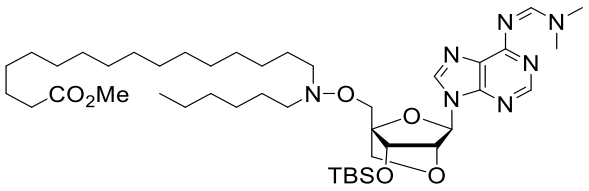
Methyl-16-[[(4R,6R)-6-(6-aminopurin-9-yl)-7-[tert-butyl(dimethyl)silyl]oxy-2,5-dioxabicyclo[2.2.1]heptan-4-yl]methoxy-hexyl-amino]hexadecanoate **(26)**: To a solution of **10** (0.7 g, 1.71 mmol) in DCM (13.87 mL)was added Hexanal (171.62 mg, 1.71 mmol, 205.78 μL) at 22 °C. The resulting clear solution was stirred for 3 hr. To this reaction mixture was added sodium cyanoborohydride (279.95 mg, 4.46 mmol) in portions. Reaction mixture was stirred for 1 hr and methyl-16-oxohexadecanoate[6, 7] (487.37 mg, 1.71 mmol, 501.94 μL) was added into it. Stirring continude for 1 hr and then second batch of sodium cyanoborohydride (279.95 mg, 4.46 mmol) was added. Reaction mixture was diluted with DCM (20 mL) after 3 hr. Orgainc layer was washed with water (10 mL), brine (2 x 20 mL) and organic layer was separated. DCM layer was dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The crude compound was

purified by column chromatography (gradient: 10-50 % EtOAc in hexane) to afford **26** (0.6 g, 46% yield) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.03 (s, 1H), 5.96 (s, 1H), 5.71 (s, 2H), 4.70 (s, 1H), 4.35 (s, 1H), 4.10 – 4.07 (m, 2H), 4.02 (d, *J* = 10.8 Hz, 1H), 3.96 (d, *J* = 7.7 Hz, 1H), 3.66 (s, 3H), 2.67 (hept, *J* = 6.3 Hz, 4H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.66 – 1.51 (m, 6H), 1.35 – 1.22 (m, 30H), 0.88 (s, 12H), 0.07 (s, 3H), 0.04 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5, 155.5, 153.2, 149.0, 138.9, 120.2, 87.0, 86.8, 79.3, 72.7, 69.4, 59.5, 51.6, 34.3, 31.9, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.4, 29.3, 27.6, 27.3, 27.3, 25.7, 25.1, 22.7, 18.0, 14.2, -4.5, -5.0 ppm. HRMS calcd. for $C_{40}H_{73}N_6O_6Si$ $[M + H]^+$ 761.5361, found 761.5358.



27

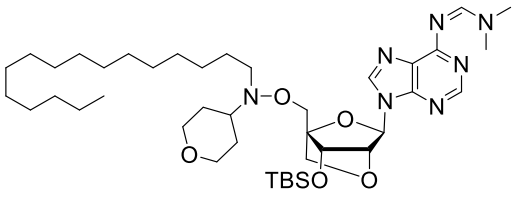
***9-[(4R,6R)-7-[tert-butyl(dimethyl)silyl]oxy-4-[[hexadecyl(tetrahydropyran-4-yl)amino]oxy methyl]-2,5-dioxabicyclo[2.2.1]heptan-6-yl]purin-6-amine* (27):** To a clear solution of **10** (0.8 g, 1.96 mmol) in a mixture of acetic acid (10 mL) and DCM (5 mL) was added tetrahydro-4H-pyran-4-one (196.06 mg, 1.96 mmol, 181.54 µL) and stirred for 3 hr at 22 °C. To this reaction mixture was added sodium cyanoborohydride (319.95 mg, 5.09 mmol). Reaction mixture was stirred for 2 hr and then hexadecanal (706.21 mg, 2.94 mmol) was added in single portion at 15 °C. After stirring the mixture for 1 hr, a second batch of sodium cyanoborohydride (319.95 mg, 5.09 mmol) was added and kept stirring for 9 hr. Reaction mixture was diluted with DCM (20 mL), organic layer was washed with water (20 mL) and brine (2 x 30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. The crude thus obtained was purified by flash column chromatography (gradient: 20-70% EtOAc in hexane) to afford **27** (0.7 g, 50% yield) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.05 (s, 1H), 6.03 (s, 2H), 5.97 (d, *J* = 0.7 Hz, 1H), 4.72 (s, 1H), 4.36 (s, 1H), 4.11 – 3.98 (m, 5H), 3.95 (d, *J* = 7.7 Hz, 1H), 3.72 (s, 2H), 3.37 (td, *J* = 11.9, 2.1 Hz, 2H), 2.87 – 2.68 (m, 3H), 1.81 (s, 3H), 1.73 – 1.55 (m, 4H), 1.25 (d, *J* = 3.6 Hz, 24H), 0.88 (s, 12H), 0.06 (d, *J* = 13.8 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.55, 155.59, 153.07, 152.07, 148.89, 138.88, 132.82, 132.59, 129.70, 127.24, 125.24, 124.77, 120.08, 87.09, 86.85, 79.07, 72.67, 72.19, 70.23, 67.14, 67.10, 62.82, 55.11, 37.70, 32.07, 29.85, 29.82, 29.81, 29.77, 29.72, 29.51, 27.65, 27.05, 25.70, 22.84, 17.99, 14.28, 0.15, -4.45, -4.95 ppm. HRMS calcd. for $C_{38}H_{69}N_6O_5Si$ $[M + H]^+$ 717.5099, found 717.5095.



28

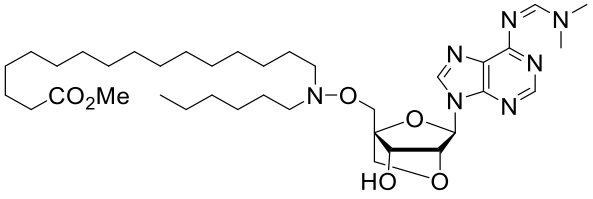
***Methyl-16-[[(4R,6R)-7-[tert-butyl(dimethyl)silyl]oxy-6-[6-[(Z)-dimethylaminomethylene amino]purin-9-yl]-2,5-dioxabicyclo[2.2.1]heptan-4-yl]methoxy-hexyl-amino]hexadecanoate* (28)**: To a clear solution of **26** (0.4 g, 525.54 µmol) in dimethylformamide was added *N*,*N*-dimethylformamide dimethyl acetal (93.94 mg, 788.31 µmol, 105.55 µL) in single portion and the reaction mixture was stirred at 65 °C for 4 hr. TLC was checked, and volatile matters was removed under high vacuum pump. Residue was dissolved in DCM (100 mL) and the organic layer was washed with brine (3 x 50 mL). DCM layer was then dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. Crude mass thus obtained, was purified by flash column chromatography (gradient: 30-80% EtOAc in hexane) to afford **28** (0.38 g, 89% yield) as transparent gum. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.95 (d, *J* = 2.1 Hz, 1H), 8.52 (d, *J* = 2.0 Hz, 1H), 8.13 (d, *J* = 2.0 Hz, 1H), 6.00

(d, *J* = 2.0 Hz, 1H), 4.66 (d, *J* = 2.1 Hz, 1H), 4.31 (d, *J* = 2.0 Hz, 1H), 4.10 – 4.05 (m, 2H), 4.02 (dd, *J* = 10.9, 2.0 Hz, 1H), 3.95 (dd, *J* = 7.7, 2.1 Hz, 1H), 3.66 (d, *J* = 2.0 Hz, 3H), 3.27 (d, *J* = 2.0 Hz, 3H), 3.21 (d, *J* = 2.1 Hz, 3H), 2.67 (q, *J* = 6.5 Hz, 4H), 2.29 (td, *J* = 7.6, 2.1 Hz, 2H), 1.61 (t, *J* = 7.1 Hz, 2H), 1.54 (q, *J* = 7.5 Hz, 4H), 1.34 – 1.22 (m, 29H), 0.90 – 0.84 (m, 12H), 0.05 – -0.08 (m, 6H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.5, 159.7, 158.1, 152.8, 150.5, 139.8, 126.6, 86.9, 86.8, 79.2, 72.7, 72.1, 69.2, 59.5, 51.6, 41.4, 35.3, 34.3, 31.9, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.4, 29.3, 27.6, 27.3, 27.3, 25.7, 25.1, 22.8, 18.0, 14.2, -4.6, -5.0 ppm. HRMS calcd. for $C_{43}H_{78}N_7O_6Si$ $[M + H]^+$ 816.5783, found 816.5756.



29

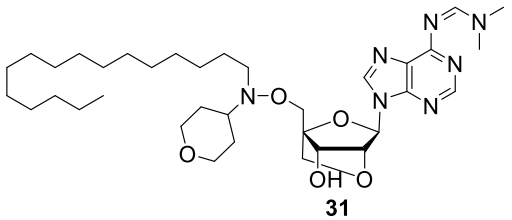
N'-[9-[(4R,6R)-7-[tert-butyl(dimethyl)silyl]oxy-4-[[hexadecyl(tetrahydropyran-4-yl)amino]oxymethyl]-2,5-dioxabicyclo[2.2.1]heptan-6-yl]purin-6-yl]-N,N-dimethyl-formamidine **(29):** To a clear solution of **27** (0.7 g, 976.20 μmol) in DMF (5 mL) was added dimethylformamide dimethyl acetal (116.32 mg, 976.20 μmol, 130.70 μL) in single portion and the reaction mixture was stirred at 65 °C for 10 hr. TLC was checked, and volatile matters was removed under high vacuum pump. Residue was dissolved in DCM (50 mL) and the organic layer was washed with brine (3 x 30 mL). DCM layer was then dried over anhydrous Na_2SO_4, filtered and the filtrate was evaporated to dryness. Crude mass thus obtained, was purified by flash column chromatography (gradient: 0-5% MeOH in DCM) to afford **29** (0.64 g, 85% yield) as transparent gum. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.40 (s, 1H), 8.26 (s, 1H), 5.98 (s, 1H), 4.63 (s, 1H), 4.59 (s, 1H), 4.10 (d, *J* = 11.0 Hz, 1H), 3.97 – 3.91 (m, 2H), 3.83 (dd, *J* = 9.6, 5.9 Hz, 3H), 3.27 – 3.19 (m, 5H), 3.13 (s, 3H), 2.79 – 2.60 (m, 3H), 1.70 (d, *J* = 15.3 Hz, 2H), 1.54 – 1.41 (m, 5H), 1.30 – 1.18 (m, 27H), 0.85 (d, *J* = 6.1 Hz, 12H), 0.07 (s, 6H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.3, 158.0, 151.9, 150.4, 140.4, 125.6, 86.1, 85.5, 78.7, 72.2, 71.7, 70.5, 66.0, 62.0, 54.2, 40.7, 34.6, 31.3, 29.0, 29.0, 29.0, 28.9, 28.9, 28.7, 26.9, 26.3, 25.4, 22.1, 17.5, 14.0, -4.8, -5.3 ppm. HRMS calcd. for $C_{41}H_{74}N_7O_5Si$ $[M + H]^+$ 772.5521, found 772.5514.



30

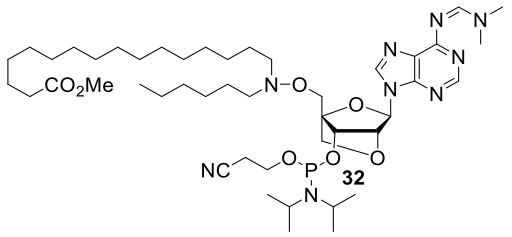
Methy-16-[[(4R,6R)-6-[6-[(Z)-dimethylaminomethyleneamino]purin-9-yl]-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-4-yl]methoxy-hexyl-amino]hexadecanoate **(30):** To a clear solution of **28** (0.37 g, 453.32 μmol) in THF (25 mL) was added TBAF (154.08 mg, 589.32 μmol, 170.63 μL) in single portion and stirred for 4 hr at 22 °C. All the volatile matters were evaporated under high vacuum pump and the crude residue thus obtained, was purified by flash column chromatography (gradient: 0-5% MeOH in DCM) to afford **30** (0.28 g, 88% yield) as transparent gum. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.42 (s, 1H), 8.25 (s, 1H), 5.94 (s, 1H), 5.78 (d, *J* = 4.4 Hz, 1H), 4.42 (s, 1H), 4.29 (d, *J* = 4.4 Hz, 1H), 4.15 (d, *J* = 11.2 Hz, 1H), 3.99 (d, *J* = 11.1 Hz, 1H), 3.95 (d, *J* = 7.8 Hz, 1H), 3.82 (d, *J* = 7.8 Hz, 1H), 3.57 (s, 3H), 3.20 (s, 3H), 3.13 (s, 3H), 2.67 – 2.55 (m, 5H), 2.27 (t, *J* = 7.4 Hz, 2H), 1.49 (pd, *J* = 7.5, 2.8 Hz, 7H), 1.31 – 1.17 (m, 35H), 0.83 (t, *J* = 6.8 Hz, 3H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.4, 159.2, 158.0, 152.1, 150.5, 139.5, 125.6, 86.2, 85.2, 79.2, 79.0, 71.4, 70.7,

69.7, 58.5, 58.4, 51.1, 40.7, 34.5, 33.3, 31.2, 29.0, 29.0, 28.9, 28.9, 28.9, 28.7, 28.4, 26.8, 26.6, 26.5, 26.4, 24.4, 22.1, 13.9 ppm. HRMS calcd. for $C_{37}H_{64}N_7O_6$ $[M + H]^+$ 702.4918, found 702.4937.



31

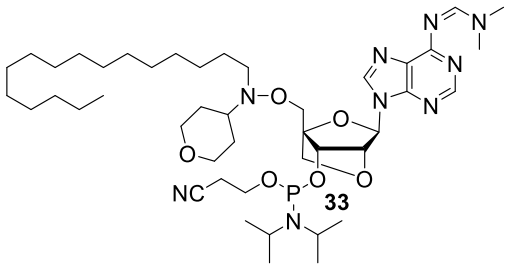
N'-[9-[(4R,6R)-4-[[hexadecyl(tetrahydropyran-4-yl)amino]oxymethyl]-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptan-6-yl]purin-6-yl]-N,N-dimethyl-formamidine **(31):** To a clear solution of **29** (0.19 g, 246.07 μmol) in THF (5 mL) was added TBAF (83.64 mg, 319.89 μmol) in single portion and stirred for 10 hr at 22 °C. All the volatile matters were evaporated under high vacuum pump and the crude residue thus obtained, was purified by flash column chromatography (gradient: 0-7% MeOH in DCM) to afford **31** (0.126 g, 78% yield) as white foam. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.42 (s, 1H), 8.24 (s, 1H), 5.94 (s, 1H), 5.79 (d, J = 4.4 Hz, 1H), 4.42 (s, 1H), 4.28 (d, J = 4.5 Hz, 1H), 4.13 (d, J = 11.0 Hz, 1H), 4.02 – 3.93 (m, 2H), 3.89 – 3.79 (m, 4H), 3.26 (td, J = 11.9, 2.2 Hz, 2H), 3.20 (s, 3H), 3.13 (s, 3H), 2.77 (tt, J = 11.1, 3.8 Hz, 1H), 2.68 (hept, J = 6.5 Hz, 2H), 1.77 – 1.70 (m, 2H), 1.58 – 1.43 (m, 5H), 1.30 – 1.10 (m, 17H), 0.85 (t, J = 7.0 Hz, 3H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.2, 158.0, 152.1, 150.4, 139.4, 125.6, 86.2, 85.3, 78.9, 71.4, 70.5, 70.4, 66.1, 66.0, 61.8, 54.1, 40.7, 34.6, 31.3, 29.0, 29.0, 29.0, 29.0, 28.9, 28.9, 28.7, 26.9, 26.1, 22.1, 14.0 ppm. HRMS calcd. for $C_{35}H_{60}N_7O_6$ $[M + H]^+$ 658.4656, found 658.4641.



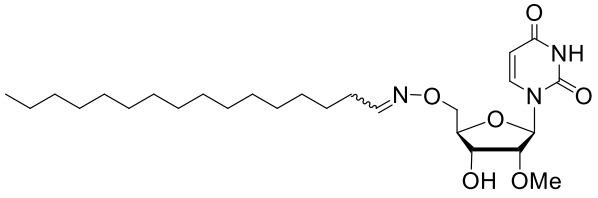
32

Methyl-16-[[(4R,6R)-7-[2-cyanoethoxy(dimethylamino)phosphanyl]oxy-6-[6-[(Z)-dimethyl aminomethyleneamino]purin-9-yl]-2,5-dioxabicyclo[2.2.1]heptan-4-yl]methoxy-hexyl-amino] hexadecanoate **(32):** To a clear solution of **30** (0.28 g, 398.90 μmol) in DCM (15 mL) was added NMI (65.50 mg, 797.79 μmol, 63.59 μL) and DIPEA (257.77 mg, 1.99 mmol, 347.39 μL) in single portions. After stirring the reaction mixture for 5 minutes at 22 °C, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (188.82 mg, 797.79 μmol) was added and continued stirring for 1 hr and TLC was checked. Starting material was consumed and reaction mixture was diluted with DCM (15 mL). DCM layer was washed with 10% $NaHCO_3$ (2 x 20 mL) solution, and brine (20 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and filtrate was evaporated at 36°C to afford crude compound which was purified by flash chromatography (0-3% MeOH in DCM containing 3% TEA) to afford **32** (0.29 g, 80% yield) as white foam. ^{1}H NMR (600 MHz, CD_3CN) δ 8.90 (s, 1H), 8.40 (d, J = 3.5 Hz, 1H), 8.07 (d, J = 6.6 Hz, 1H), 5.99 (d, J = 5.4 Hz, 1H), 5.45 (s, 1H), 4.80 – 4.55 (m, 1H), 4.45 – 4.37 (m, 1H), 4.17 (dd, J = 11.2, 5.8 Hz, 1H), 4.12 – 3.99 (m, 2H), 3.92 (dd, J = 7.9, 3.5 Hz, 1H), 3.83 – 3.69 (m, 2H), 3.60 – 3.48 (m, 5H), 3.17 (d, J = 11.2 Hz, 6H), 2.82 – 2.45 (m, 10H), 2.27 (t, J = 7.5 Hz, 2H), 1.61 – 1.49 (m, 8H), 1.35 – 1.20 (m, 42H), 1.13 (dd, J = 6.8, 2.3 Hz, 7H), 1.03 (d, J = 6.7 Hz, 3H), 1.01 – 0.94 (m, 9H), 0.87 (dt, J = 7.1, 3.4 Hz, 3H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 174.8, 160.6, 159.0, 159.0, 153.3, 151.8, 151.7, 139.9, 139.8, 127.3, 127.3, 119.2, 87.5, 87.4, 87.4, 87.4, 87.3, 87.3, 79.4, 79.4, 73.4, 73.4, 73.3, 72.7, 72.7, 70.1, 69.9, 60.0, 59.9, 59.9, 59.7, 59.6, 59.4, 59.1, 59.1, 55.3, 51.8, 49.1, 47.0, 46.0, 45.9, 44.1, 44.0, 44.0, 43.9, 41.5, 39.4, 35.2, 34.5, 32.5, 30.3, 30.3, 30.3, 30.3, 30.2, 30.2, 30.0, 29.8, 28.1, 28.1, 27.9, 27.9, 27.8, 27.8, 25.7, 24.8, 24.8, 24.7, 24.6, 24.6, 23.4, 23.4, 23.2, 23.1, 23.1, 23.1, 21.0, 21.0, 20.1, 20.9, 20.9, 20.6, 20.6, 14.4, 12.2

ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 148.43, 148.37 ppm. HRMS calcd. for $C_{46}H_{81}N_9O_7P$ $[M + H]^+$ 902.5997, found 902.5977.



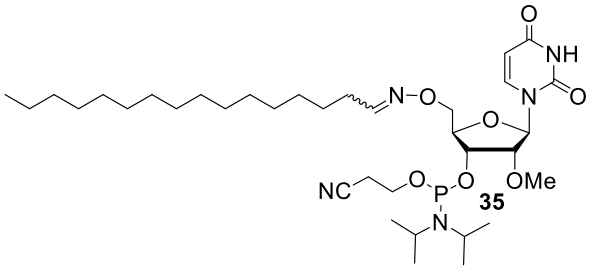
N'-[9-[(4R,6R)-7-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-4-[[hexadecyl(tetrahydro pyran-4-yl)amino]oxymethyl]-2,5-dioxabicyclo[2.2.1]heptan-6-yl]purin-6-yl]-N,N-dimethyl-formamidine **(33):** To a clear solution of **31** (0.2 g, 304.00 μmol) in DCM (20 mL) was added NMI (49.92 mg, 608.01 μmol, 48.46 μL) and DIPEA (196.45 mg, 1.52 mmol, 264.75 μL) in single portions. After stirring the reaction mixture for 5 minutes at 22 °C, 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (143.90 mg, 608.01 μmol, 135.76 μL) was added and continued stirring for 1 hr and TLC was checked. Starting material was consumed and reaction mixture was diluted with DCM (15 mL). DCM layer was washed with 10% $NaHCO_3$ (2 x 25 mL) solution, and brine (30 mL). Organic layer was separated, dried over anhydrous Na_2SO_4, filtered and filtrate was evaporated at 36°C to afford crude compound which was purified by flash chromatography (0-3% MeOH in DCM containing 3% TEA) to afford **33** (0.211 g, 81% yield) as white foam. ^{1}H NMR (600 MHz, CD_3CN) δ 8.90 (s, 1H), 8.41 (d, *J* = 3.4 Hz, 1H), 8.08 (d, *J* = 5.3 Hz, 1H), 5.99 (s, 1H), 4.77 – 4.64 (m, 1H), 4.46 – 4.35 (m, 1H), 4.18 – 3.99 (m, 4H), 3.91 (tt, *J* = 10.7, 4.5 Hz, 4H), 3.83 – 3.68 (m, 2H), 3.62 – 3.44 (m, 3H), 3.31 (tdt, *J* = 12.1, 8.2, 2.2 Hz, 2H), 3.18 (s, 3H), 3.16 (s, 3H), 2.85 – 2.70 (m, 4H), 2.60 (t, *J* = 6.0 Hz, 1H), 2.55 – 2.44 (m, 2H), 1.79 (s, 1H), 1.58 (qdd, *J* = 15.2, 8.3, 3.2 Hz, 2H), 1.34 – 1.20 (m, 37H), 1.13 (dd, *J* = 6.8, 1.6 Hz, 7H), 1.04 – 0.94 (m, 8H), 0.88 (t, *J* = 7.0 Hz, 3H) ppm. ^{13}C NMR (151 MHz, CD_3CN) δ 160.6, 159.0, 159.0, 153.3, 151.8, 151.7, 139.8, 127.3, 127.3, 119.3, 119.3, 87.6, 87.5, 87.5, 87.5, 87.4, 87.3, 79.4, 79.4, 79.3, 79.3, 73.4, 73.2, 73.2, 72.6, 72.5, 70.8, 70.7, 67.5, 67.4, 67.4, 63.4, 63.3, 59.9, 59.7, 59.5, 59.4, 59.1, 59.1, 55.5, 55.5, 55.3, 49.5, 46.0, 45.9, 44.1, 44.0, 44.0, 43.9, 41.5, 35.2, 32.6, 30.4, 30.4, 30.3, 30.3, 30.3, 30.2, 30.1 28.2, 28.1, 27.6, 27.5, 27.2, 24.8, 24.8, 24.7, 24.6, 24.6, 23.4, 23.2, 23.1, 23.1, 23.1, 21.0, 21.0, 21.0, 21.0, 14.4 ppm. ^{31}P NMR (243 MHz, CD_3CN) δ 148.31, 148.27 ppm. HRMS calcd. for $C_{44}H_{77}N_9O_6P$ $[M + H]^+$ 858.5734, found 858.5741.



34

1-[(2R,5R)-5-[(hexadecylideneamino)oxymethyl]-4-hydroxy-3-methoxy-tetrahydrofuran-2-yl]pyrimidine-2,4-dione **(34):** To a solution of **4c** (1 g, 1.64 mmol) in THF (15 mL) at 25 °C, tetrabutylammonium fluoride (433.02 mg, 1.64 mmol) was added slowly in single portion and then stirred for 12 hr. Volatile matters were removed in high vacuum pump and crude residue thus obtained was purified by flash column chromatography (gradient: 20-60% EtOAc in hexane) to afford **34** (0.75 g, 92% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.04 (d, *J* = 10.5 Hz, 1H), 7.73 (dd, *J* = 19.8, 8.1 Hz, 1H), 7.40 (t, *J* = 6.2 Hz, 0.5H), 6.71 (t, *J* = 5.4 Hz, 0.5H), 5.93 (t, *J* = 2.3 Hz, 1H), 5.68 (ddd, *J* = 11.0, 8.1, 1.7 Hz, 1H), 4.48 (ddd, *J* = 36.9, 12.5, 2.3 Hz, 1H), 4.32 (ddd, *J* = 31.7, 12.6, 3.1 Hz, 1H), 4.21 (tdd, *J* = 7.9, 5.2, 2.3 Hz, 1H), 4.12 (ddt, *J* = 7.8, 5.6, 2.7 Hz, 1H), 3.75 (ddd, *J* = 9.2, 5.2, 2.2 Hz, 1H),

3.62 (d, *J* = 5.5 Hz, 3H), 2.72 (dd, *J* = 8.3, 5.1 Hz, 1H), 2.30 (td, *J* = 7.6, 5.4 Hz, 1H), 2.22 – 2.13 (m, 1H), 1.47 (q, *J* = 7.2 Hz, 2H), 1.25 (s, 25H), 0.87 (t, *J* = 6.9 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.2, 163.1, 153.1, 152.3, 150.2, 150.1, 139.8, 139.6, 102.2, 102.2, 87.8, 87.7, 83.9, 83.7, 83.2, 83.2, 72.1, 71.8, 68.9, 68.7, 58.9, 58.9, 32.1, 29.8, 29.8, 29.8, 29.7, 29.6, 29.6, 29.6, 29.5, 29.4, 29.3, 26.8, 26.3, 26.1, 22.8, 14.2 ppm. HRMS calcd. for $C_{26}H_{46}N_3O_6$ $[M + H]^+$ 496.3387, found 496.3389.



3-[(diisopropylamino)-[(2R,5R)-5-(2,4-dioxopyrimidin-1-yl)-2-[(hexadecylideneamino)oxy methyl]-4-methoxy-tetrahydrofuran-3-yl]oxy-phosphanyl]oxypropanenitrile **(35)**: To a clear solution of **34** (0.7 g, 1.41 mmol) in DCM (15 mL), DIPEA (0.92 g, 7.06 mmol, 1.24 mL) and NMI (0.41 g, 4.94 mmol, 0.40 mL) were added at 25 °C. To this reaction mixture, was added 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.704 g, 2.82 mmol, 0.66 mL) slowly after 5 minutes and stirred for 1.5 h. Reaction mixture was diluted with DCM (20 mL) and quenched with 10% $NaHCO_3$ solution (20 mL). Organic layer was separated, dried on anhydrous Na_2SO_4, filtered and filtrate was evaporated to dryness. The crude compound was thus obtained was purified by flash column chromatography (gradient: 10-50% EtOAc in hexane) to afford **35** (0.75 g, 76% yield) as transparent gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H), 7.72 (ddd, *J* = 15.7, 8.2, 5.7 Hz, 1H), 7.41 (dt, *J* = 10.9, 6.2 Hz, 0.5H), 6.71 (dt, *J* = 7.1, 5.3 Hz, 0.5H), 5.97 (dd, *J* = 6.5, 3.3 Hz, 1H), 5.77 – 5.48 (m, 1H), 4.56 – 4.18 (m, 4H), 4.00 – 3.70 (m, 3H), 3.68 – 3.59 (m, 2H), 3.55 (d, *J* = 4.0 Hz, 1.6H), 3.51 (s, 1.4H), 2.74 – 2.55 (m, 2H), 2.38 – 2.14 (m, 2H), 1.48 (qd, *J* = 7.2, 4.7, 2.9 Hz, 2H), 1.33 – 1.13 (m, 38H), 0.95 – 0.69 (m, 3H) ppm. ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.5, 163.5, 153.0, 153.0, 152.2, 150.4, 150.4, 150.3, 150.3, 140.0, 139.9, 139.7, 139.6, 117.8, 117.8, 117.6, 102.3, 102.3, 102.2, 88.0, 87.9, 87.9, 87.7, 83.3, 83.3, 83.1, 83.1, 83.0, 82.9, 82.5, 82.5, 82.5, 82.4, 82.3, 82.2, 72.3, 72.0, 71.9, 71.7, 59.0, 59.0, 58.9, 58.9, 58.7, 58.7, 58.7, 58.5, 58.4, 58.4, 58.1, 58.0, 53.5, 43.5, 43.5, 43.4, 43.4, 43.3, 43.3, 32.0, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.6, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.4, 29.3, 29.3, 26.8, 26.7, 26.3, 26.3, 26.2, 26.2, 24.7, 24.7, 24.7, 24.7, 24.6, 22.8, 20.5, 20.5, 20.5, 20.5, 20.4, 20.4, 14.2 ppm. ^{31}P NMR (162 MHz, $CDCl_3$) δ 151.54, 151.3, 150.9 ppm. HRMS calcd. for $C_{35}H_{63}N_5O_7P$ $[M + H]^+$ 696.4465, found 696.4449.

Synthesis of AOCC modified oligonucleotides from AOCC-conjugate building blocks and AOCC precursors

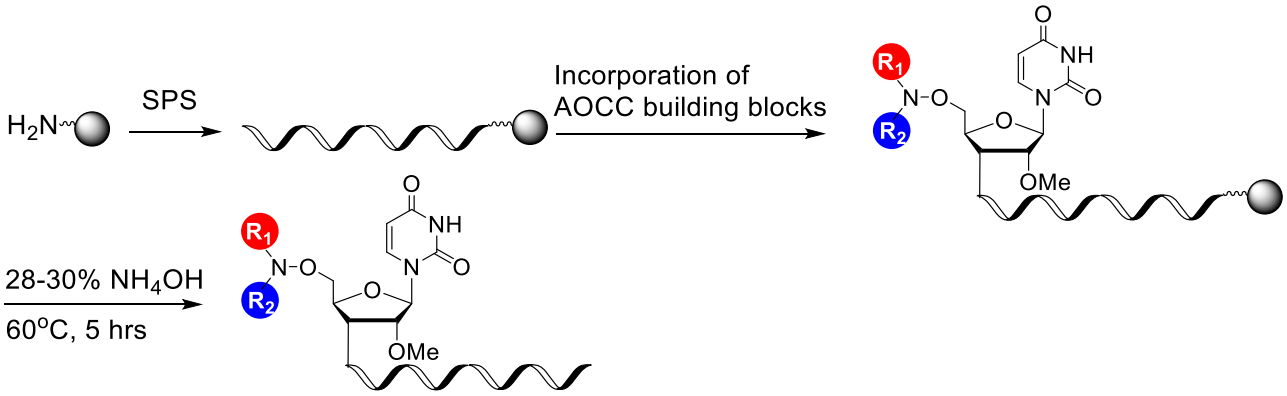


Figure S1: Incorporation of AOCC modified amidites at the 5′-end of oligonucleotides. (SPS: Solid-Phase Synthesis)

Oligonucleotide Synthesis: Oligonucleotides were synthesized on either an ABI-394 at 1-µmol scale using universal supports or a K&A H-8-SE at 40-µmol scale using universal supports. A solution of 0.25 M 5-(*S*-ethylthio)-1*H*-tetrazole in acetonitrile (CH_3CN) was used as the activator. The solutions of commercially available phosphoramidites and synthesized phosphoramidities were used at 0.15 M in anhydrous CH_3CN or CH_2Cl_2.The oxidizing reagent was 0.02 M I_2 in THF/pyridine/H_2O. *N*,*N*-Dimethyl-N′-(3-thioxo-3H-1,2,4-dithiazol-5-yl)methanimidamide (DDTT), 0.1 M in pyridine, was used as the sulfurizing reagent. The detritylation reagent was 3% dichloroacetic acid in CH_2Cl_2. Waiting time for coupling, capping, oxidation, and sulfurization step are 450s, 25s, 80s and 300s respectively. After completion of the automated synthesis, the oligonucleotide was manually released from support and deprotected using 28-30% ammonium hydroxide solution at 60 °C for 5h. The oligonucleotide containing V (5′-folate-C16) was pre-treated using 1 M aqueous piperidine at room temperature for 24 hr followed by ammonium hydroxide solution at 55 °C for 6 hr. After filtration through a 0.45-µm nylon filter, oligonucleotides were purified by ion exchange and/or reverse phase column chromatography. For ion exchange, preparative HPLC custom packed with TSKGel SuperQ-5PW(20) (Sigma) using an appropriate gradient of mobile phase (buffer A: 20 mM sodium phosphate, 15% CH_3CN, pH 8.5; buffer B: 1 M NaBr, 20 mM sodium phosphate, 15% CH_3CN, pH 8.5) and desalted using size-exclusion chromatography using a custom packed with Sephadex G25 (GE Healthcare) and water as an eluent. For reverse phase, preparative HPLC (prep RP-HPLC, Agilent, ZORBAX 300SB-C18 5µm 9.4x250mm) using an appropriate gradient of mobile phase (buffer A: 50 mM TEAA, 3% CH_3CN; buffer B 50 mM TEAA, 80% CH_3CN) and desalted using size-exclusion chromatography using a custom packed with Sephadex G25 (GE Healthcare) and water as an eluent. Triethyl ammonium cation was displaced by Na with an excess of 0.1M AcONa solution, and a second desalting process. Oligonucleotides were then quantified by measuring the absorbance at 260 nm. 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 purity and identity of modified ONs were verified by analytical reRP-HPLC chromatography and mass spectrometry, respectively.

Table S2: Sequences and mass spectroscopy characterization of target sequence conjugates using AOCC-conjugated building blocks

Target	Sense Strand	Sequence 5′-3′	Mass (M-H)⁻		
			Chemistry	Calcd.	Obs.
SOD1	ON1	I●a●uuuu*Aa**UCC*ucacucua●a●a	oxime	7072.99	7071.22
SOD1	ON2	II●a●uuuu*Aa**UCC*ucacucua●a●a	bis-homo	7131.12	7129.65
SOD1	ON3	III●a●uuuu*Aa**UCC*ucacucua●a●a	bis-hetero	7263.31	7261.77
SOD1	ON4	IV●a●uuuu*Aa**UCC*ucacucua●a●a	bis-hetero	7431.65	7429.90
βcat	ON5	V●a●*Cu**Gu**Ug**GAU*uG*aU*uC*gA*●a●*A*	bis-hetero	7589.3	7588.07
Apo-B	ON6	VI●g●*Ug**Ac**Aa**AU*A*uG**gG**cAu**C*●a●*A*	bis-hetero	8920.0	8916.50

Uppercase italicized and lowercase letters represent 2′-F and 2′-OMe nucleosides, respectively. Phosphorothioate linkages are indicated by the "●" symbol. AOCC-modified building blocks in the context of 2′-OMe-uridine are I (5′-C16 oxime), II (5′-bis-C10), III (5′-lipoic acid-C16), IV (5′-cyclopropyldilinoleyl-Me), V (5′-folate-C16), and VI (5′-triGalNAc-C16).

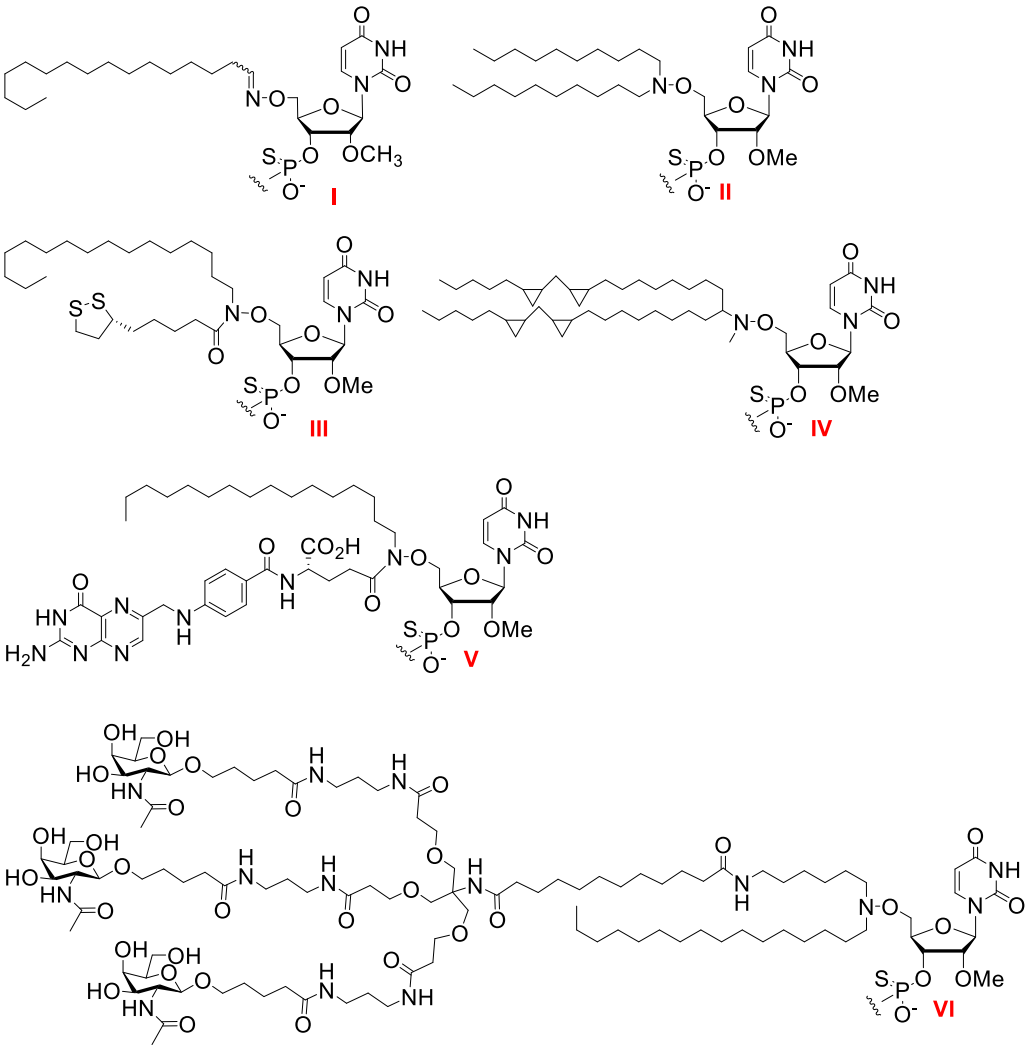


Figure S2: AOCC conjugates incorporated at the 5′-end of sense strands

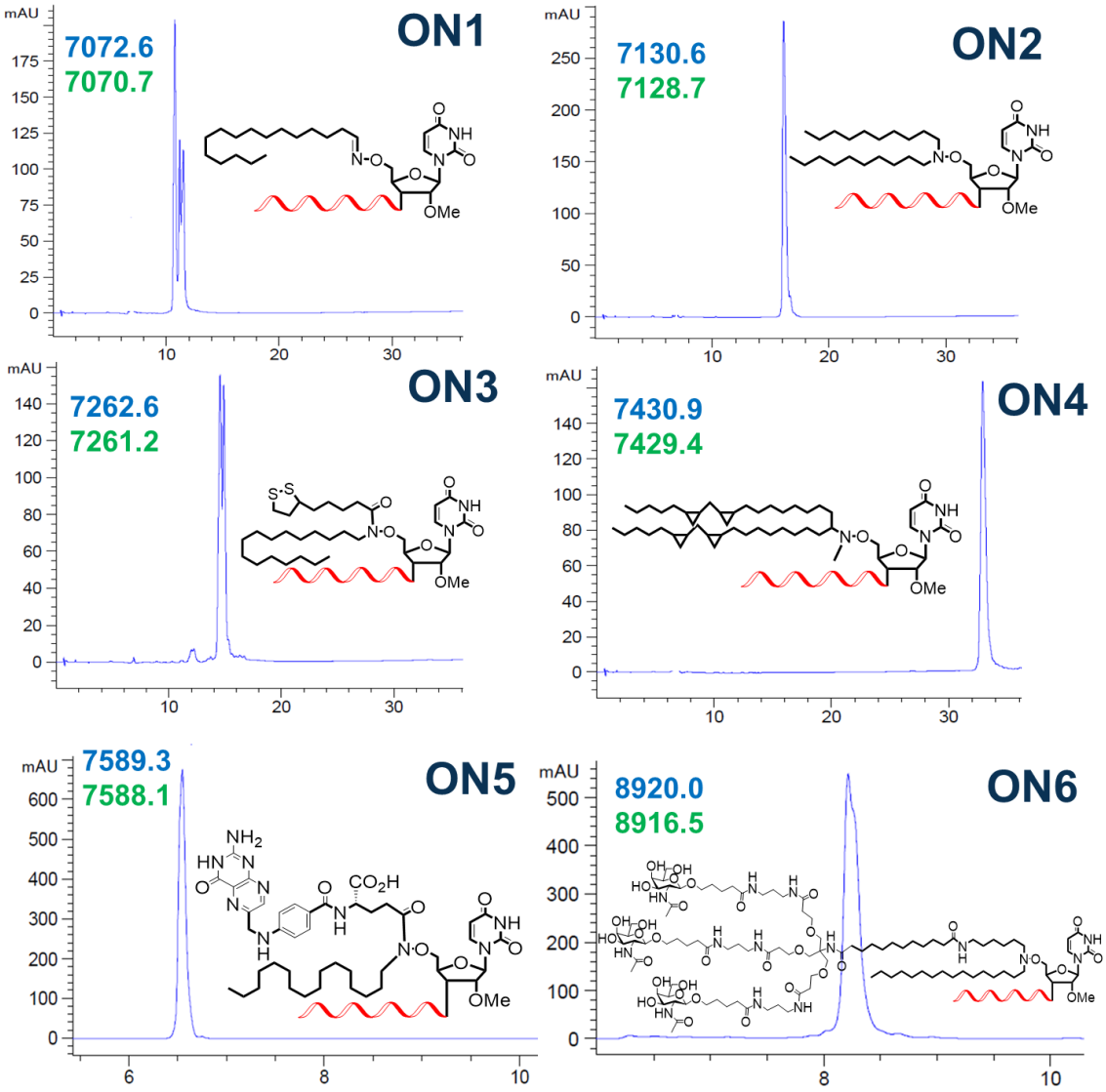
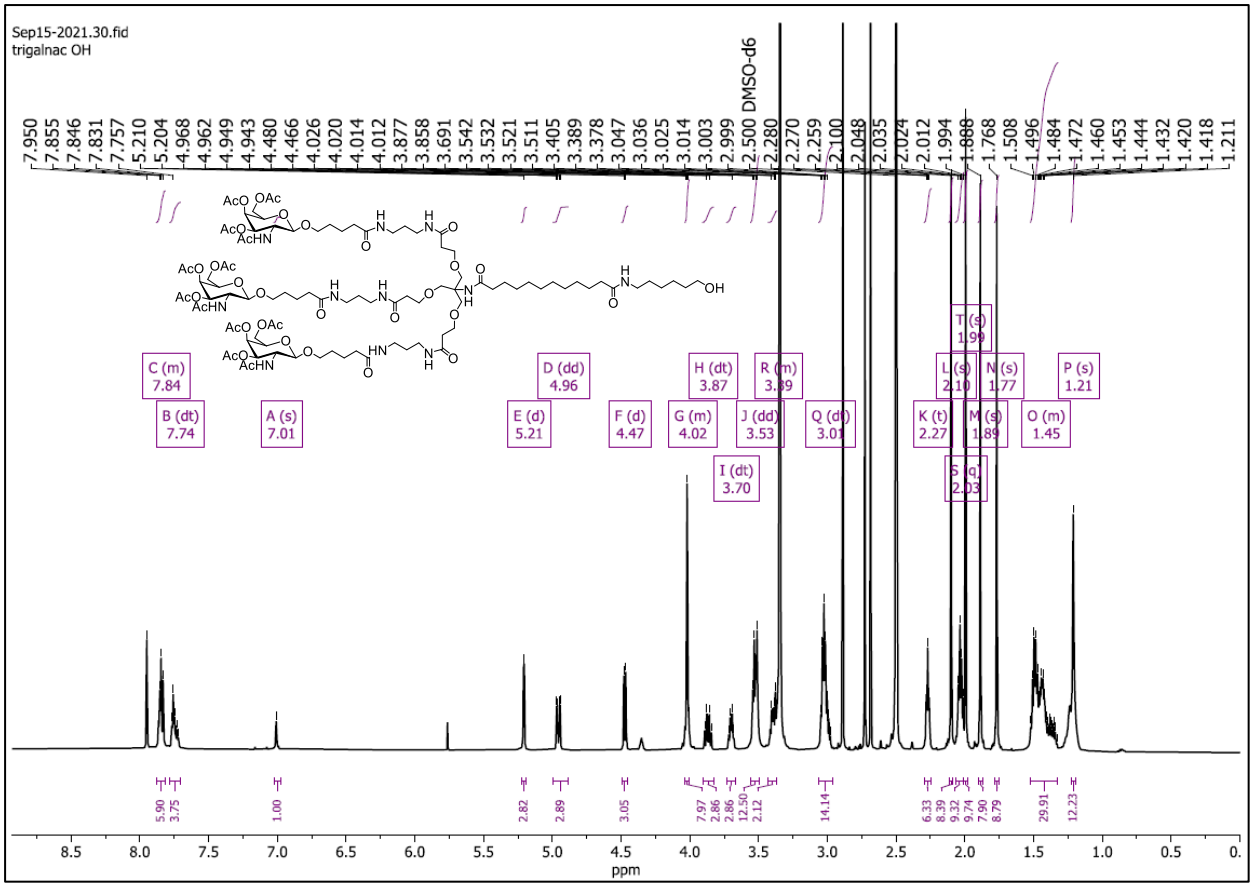


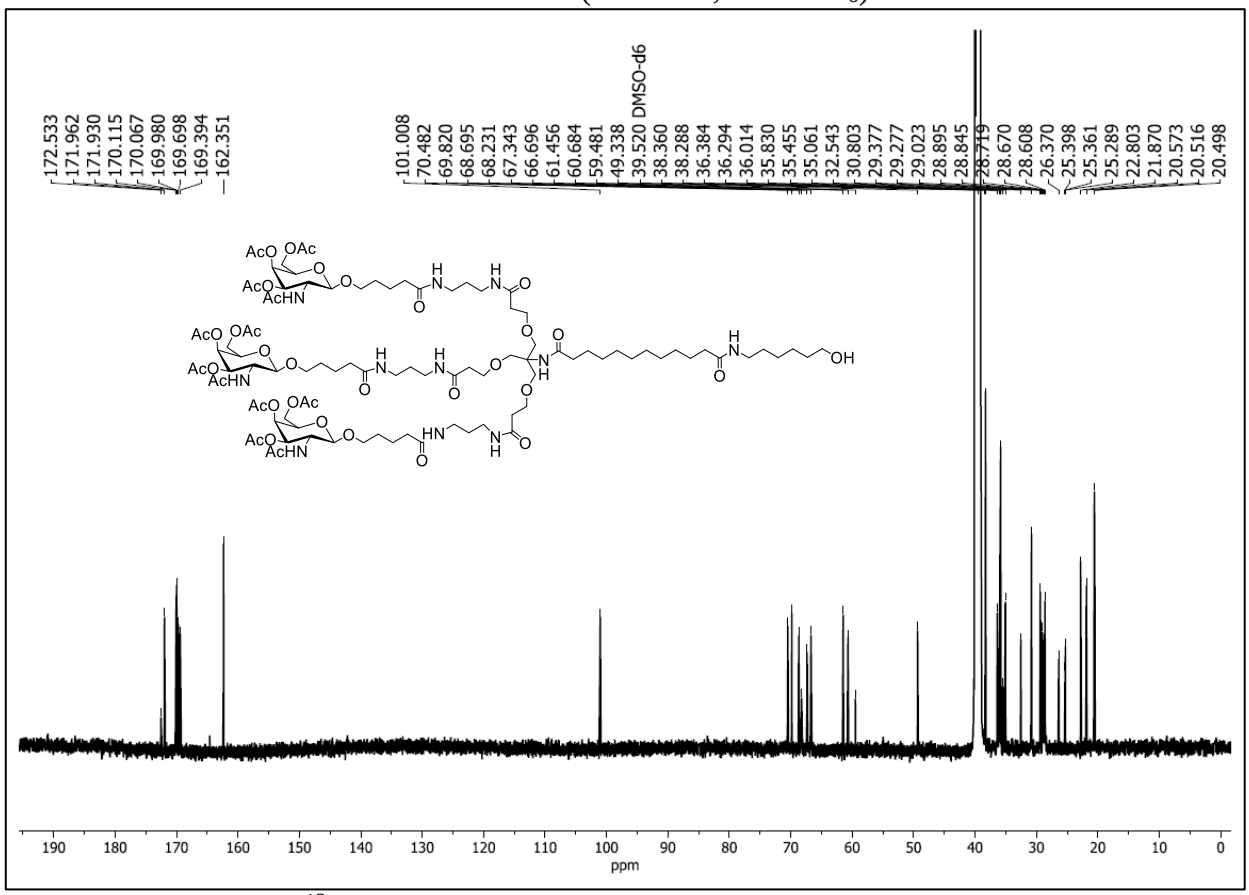
Figure S3: HPLC profiles of AOCC-modified oligonucleotides with calculated (blue) and observed (green) masses for of AOCC-modified oligonucleotides ON1-ON6 respectively. The multiplicity of peaks is due to diastereomeric chiral phosphorothioates and/or oxime rotomers.

HPLC Conditions:

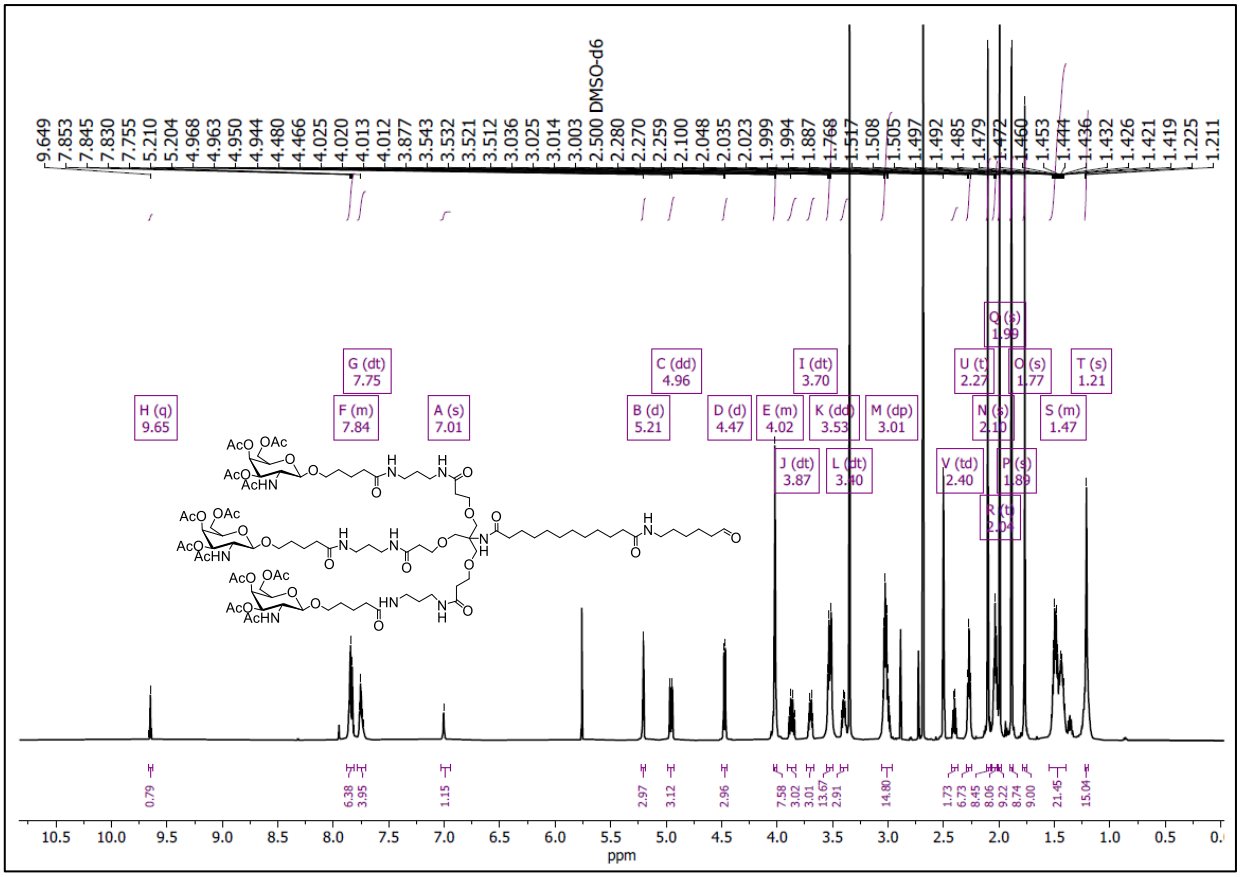
For **ON1-ON4** buffer A: 95mM Hexafluoroisopropanol, 16.3mM TEA, 0.05mM EDTA; buffer B: MeOH gradient 35 to 70% B for 31 min. For **ON5** and **ON6**: Buffer A – 16mM TEA, 200mM HFIP; Buffer B – MeOH; 9.6 min gradient going from 0-60% Buffer B; column heated to 75 °C.



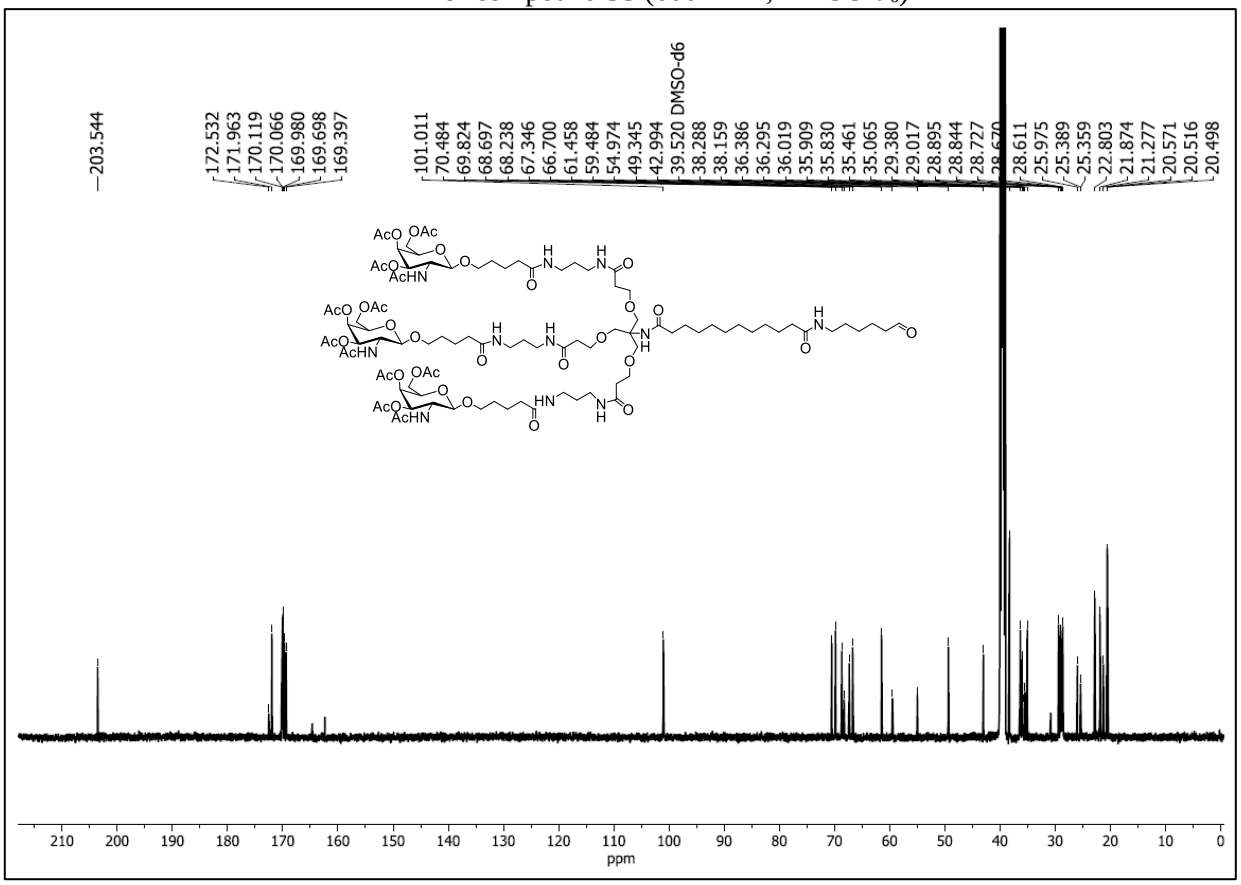
^{1}H NMR of **2S** (600 MHz, DMSO-d_6)



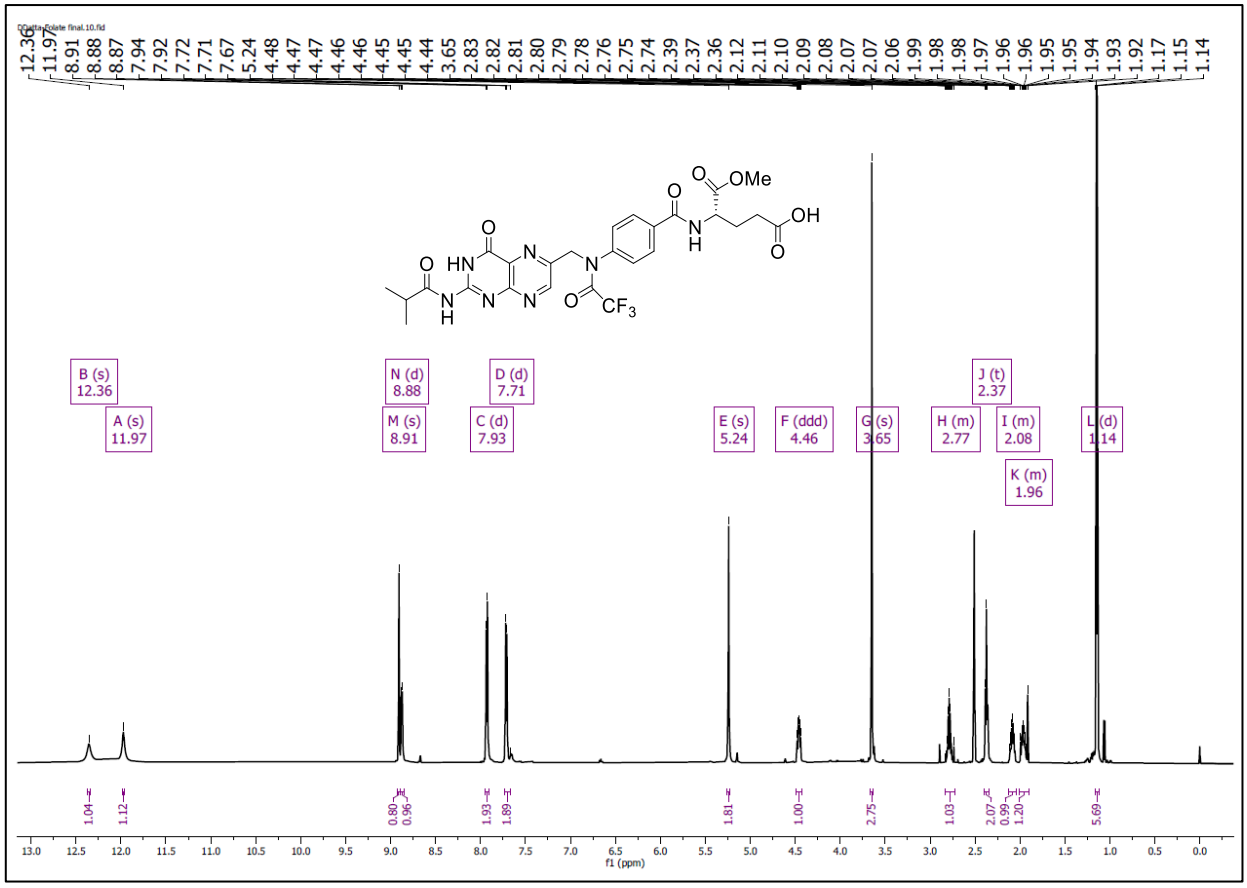
^{13}C NMR of compound **2S** (151 MHz, DMSO-d_6)



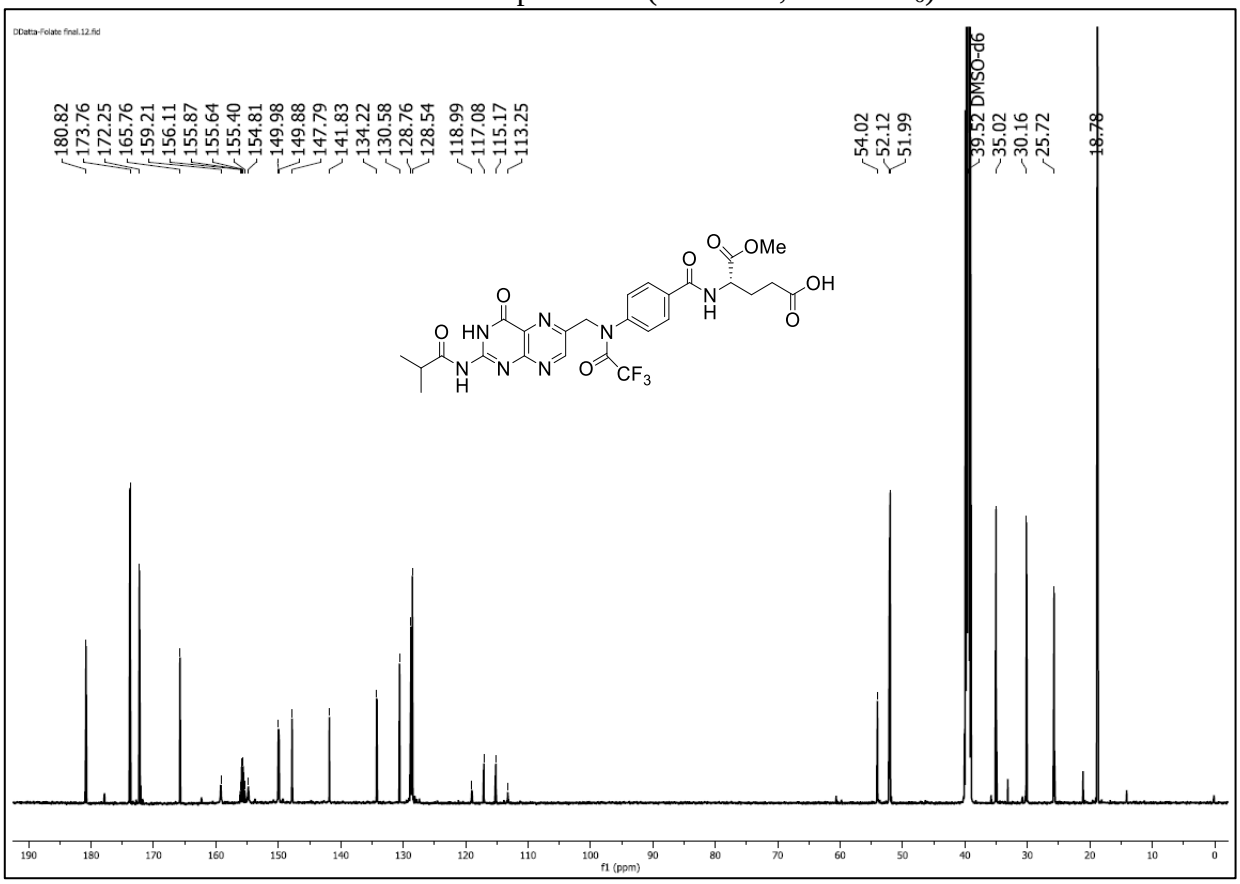
^{1}H NMR of compound **3S** (600 MHz, DMSO-d_6)



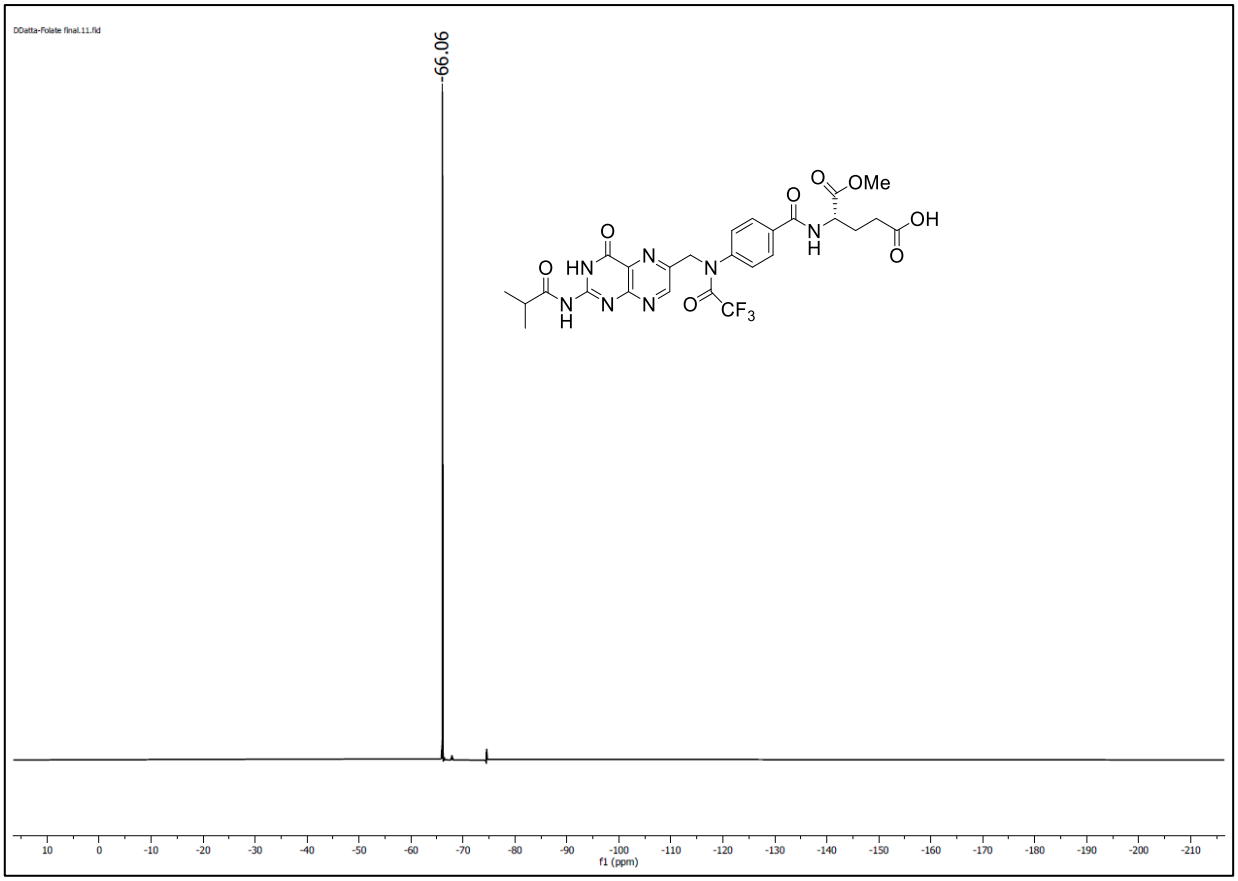
^{13}C NMR of compound **3S** (151 MHz, DMSO-d_6)



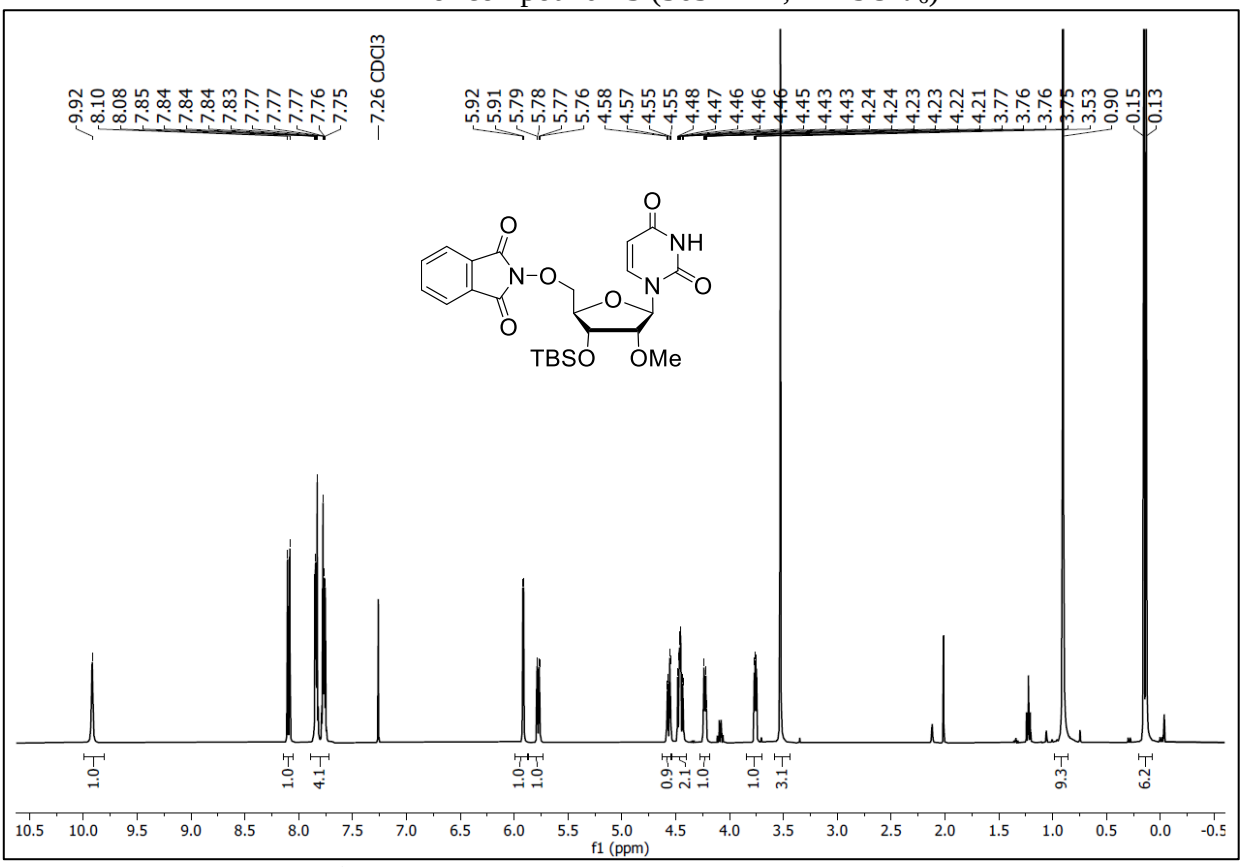
^{1}H NMR of compound **7S** (600 MHz, DMSO-d_6)



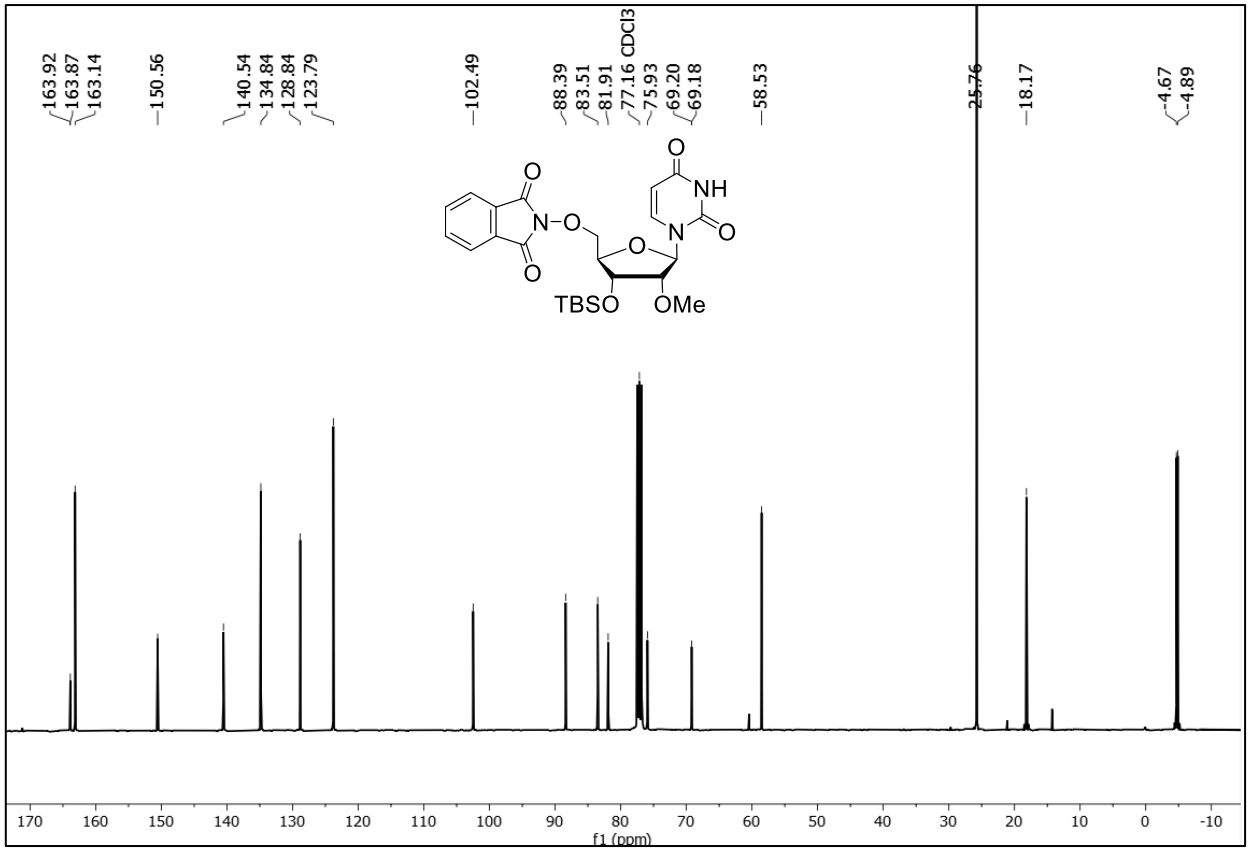
^{13}C NMR of compound **7S** (151 MHz, DMSO-d_6)



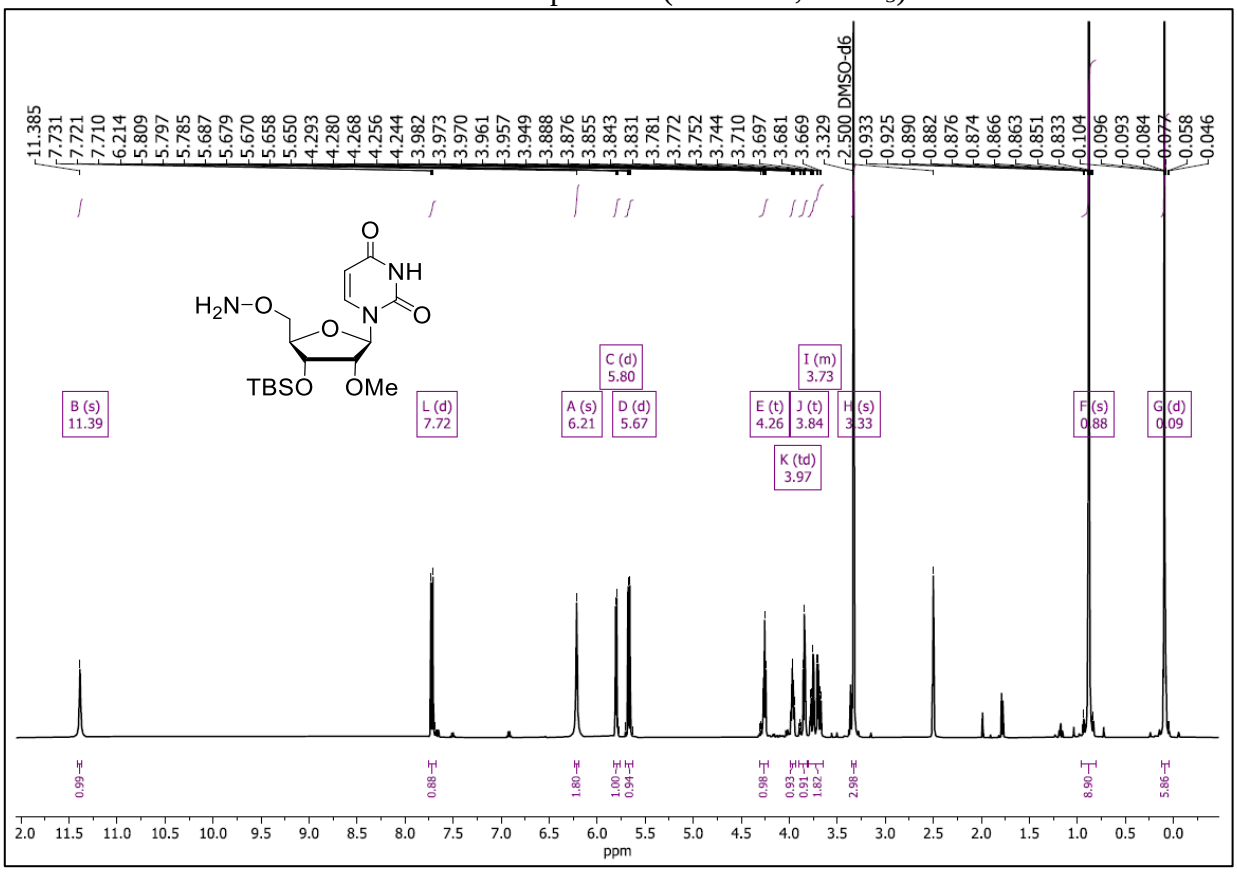
^{19}F NMR of compound **7S** (565 MHz, DMSO-d_6)



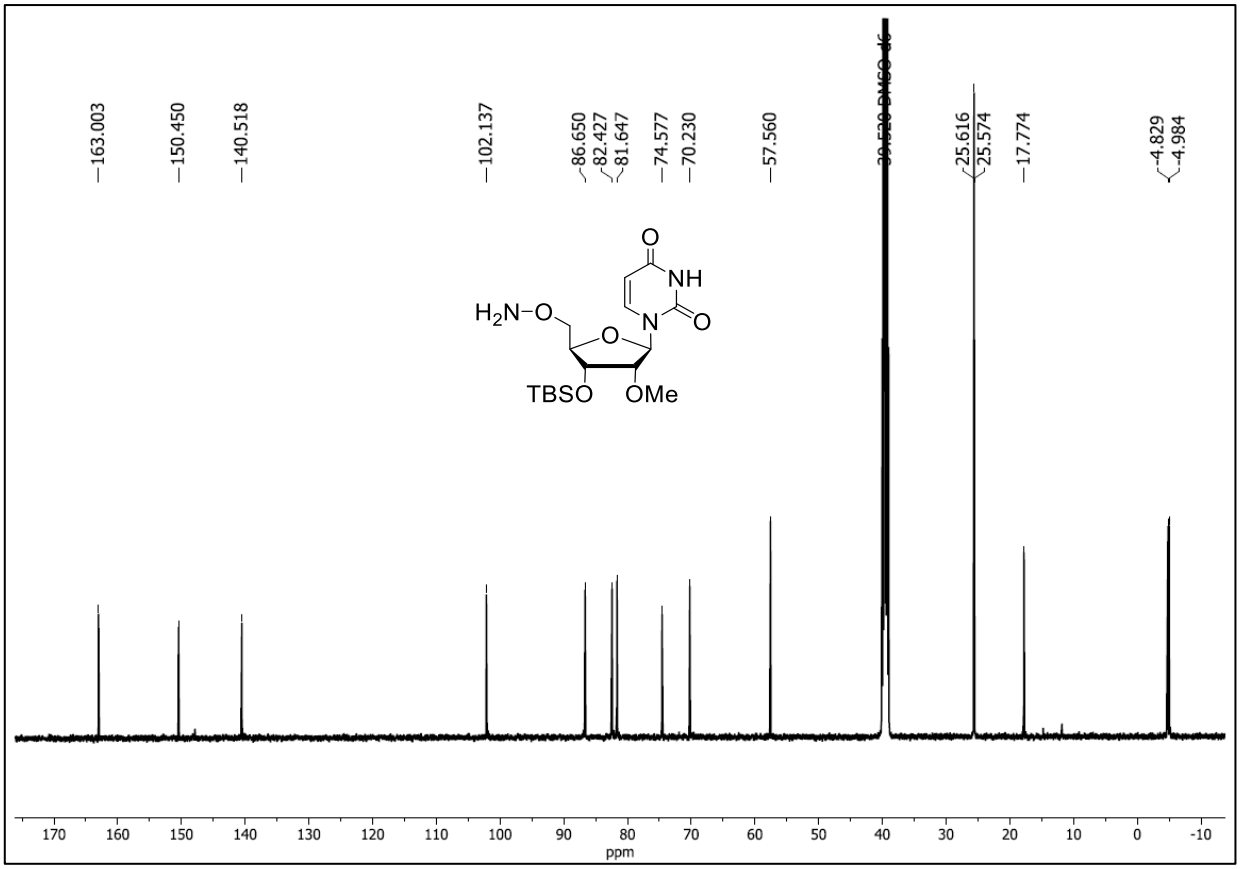
^{1}H NMR of compound **2** ($CDCl_3$, 400 MHz)



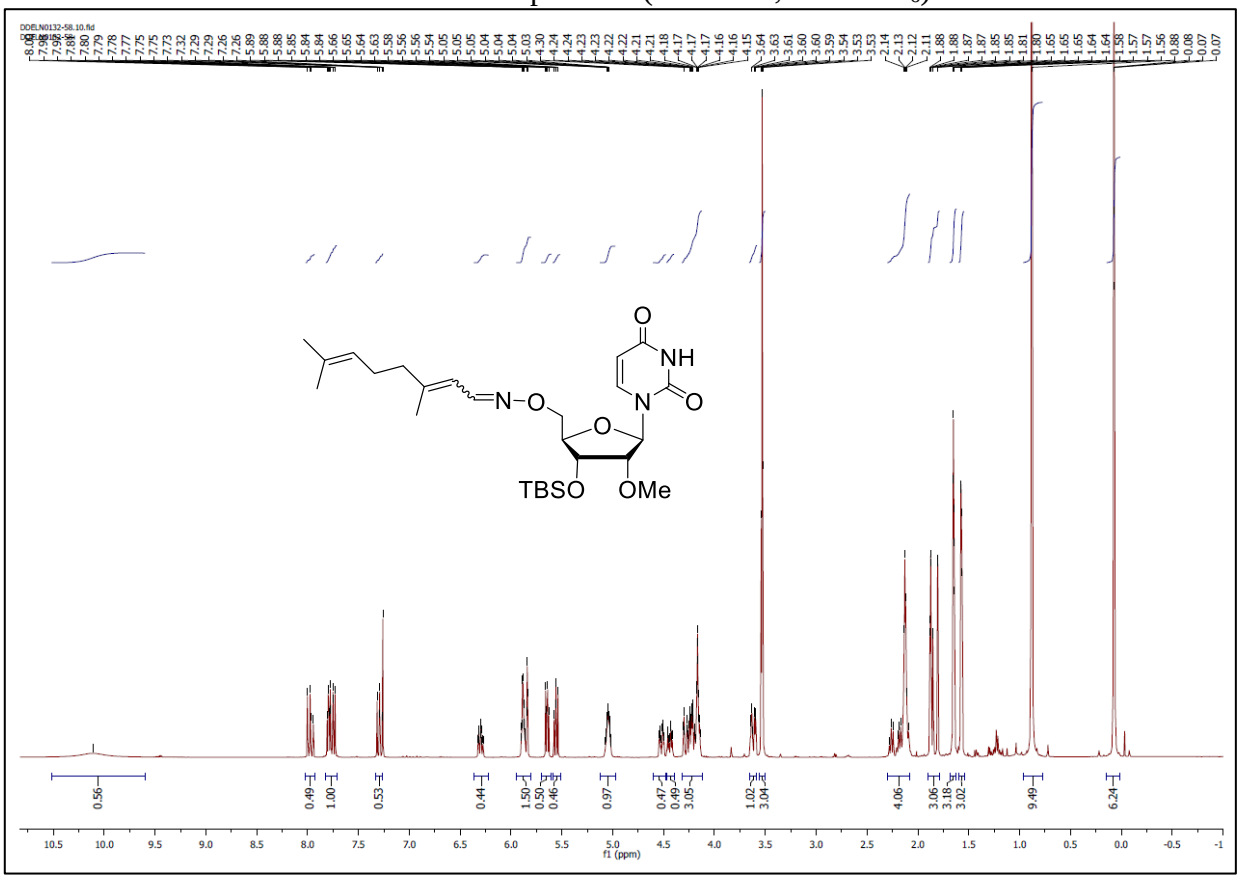
^{13}C NMR of compound **2** (101 MHz, $CDCl_3$)



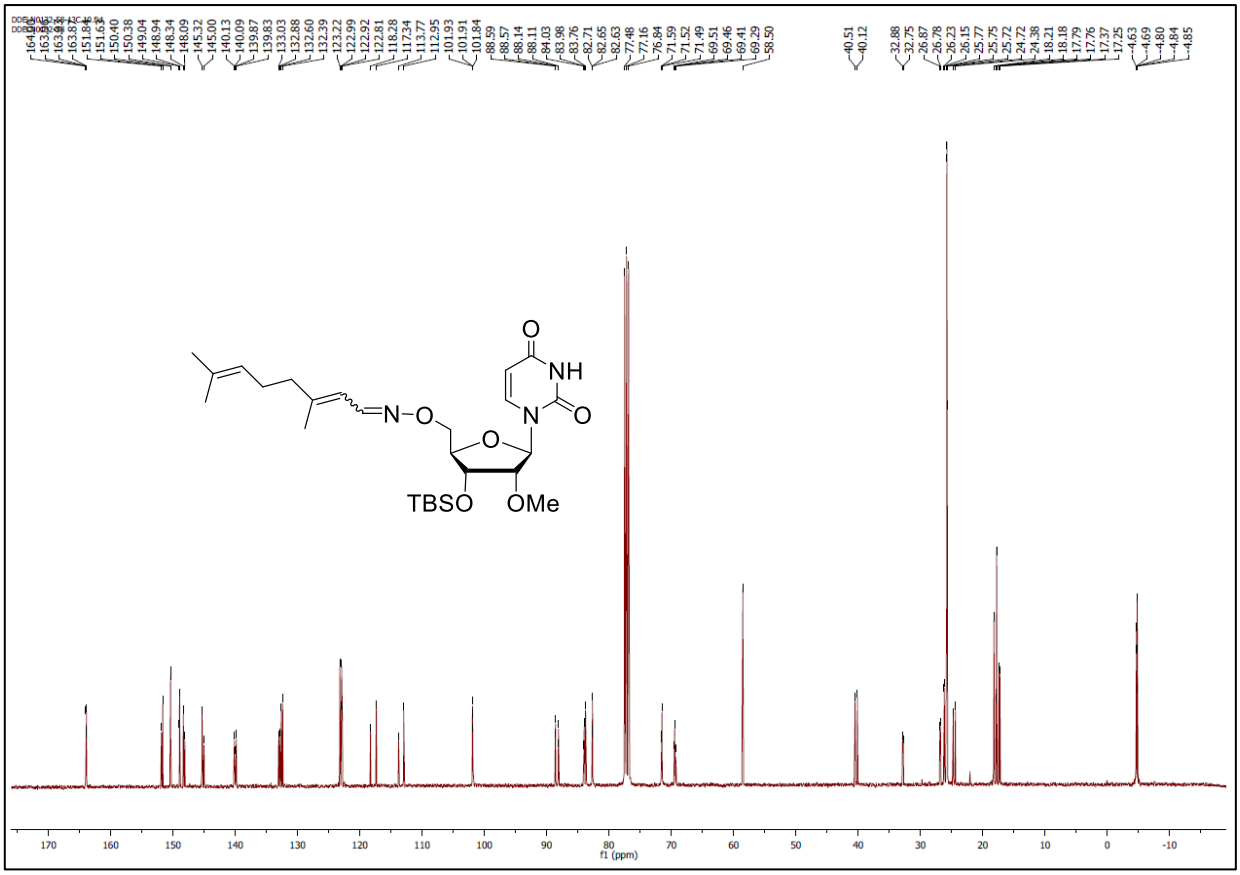
^{1}H NMR of compound **3** (400 MHz, DMSO-d_6)



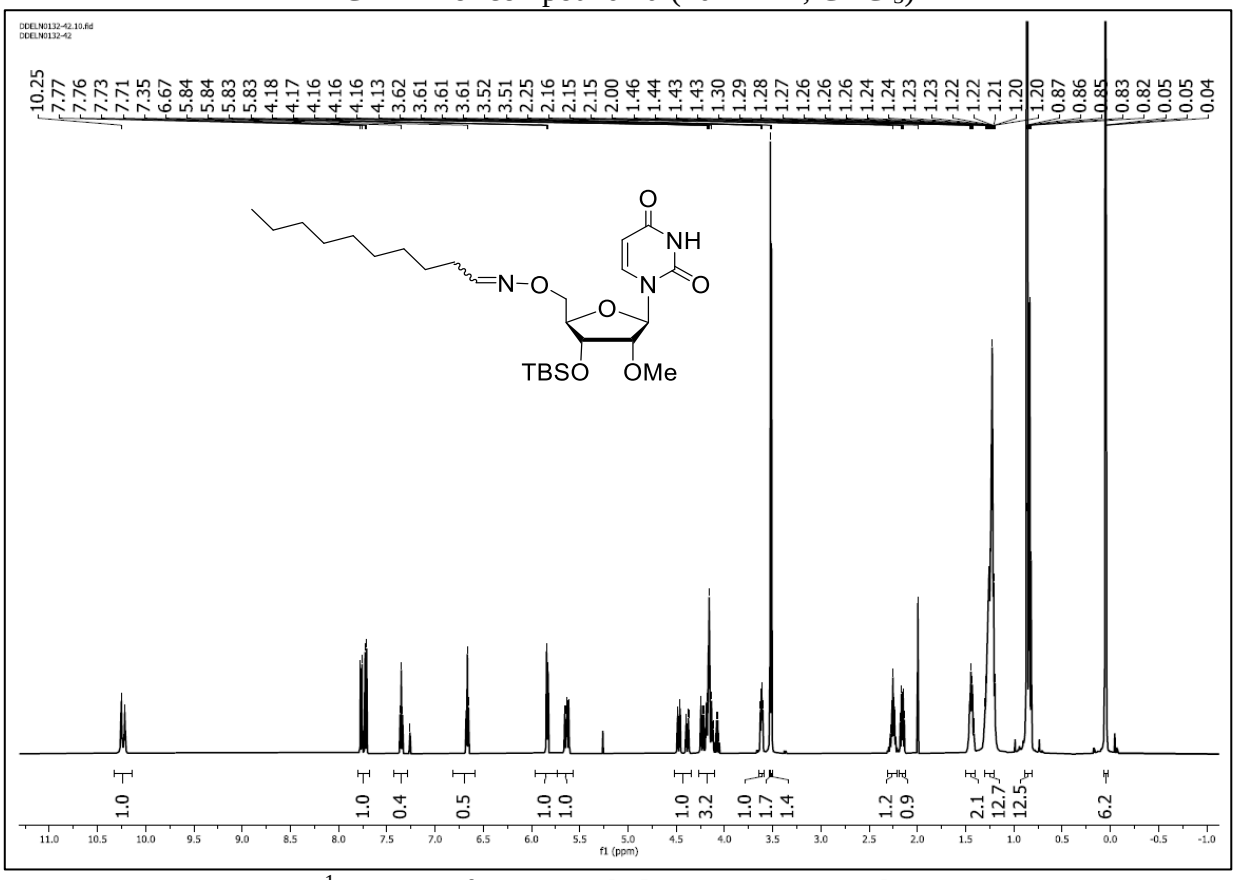
^{13}C NMR of compound **3** (101 MHz, DMSO-d_6)



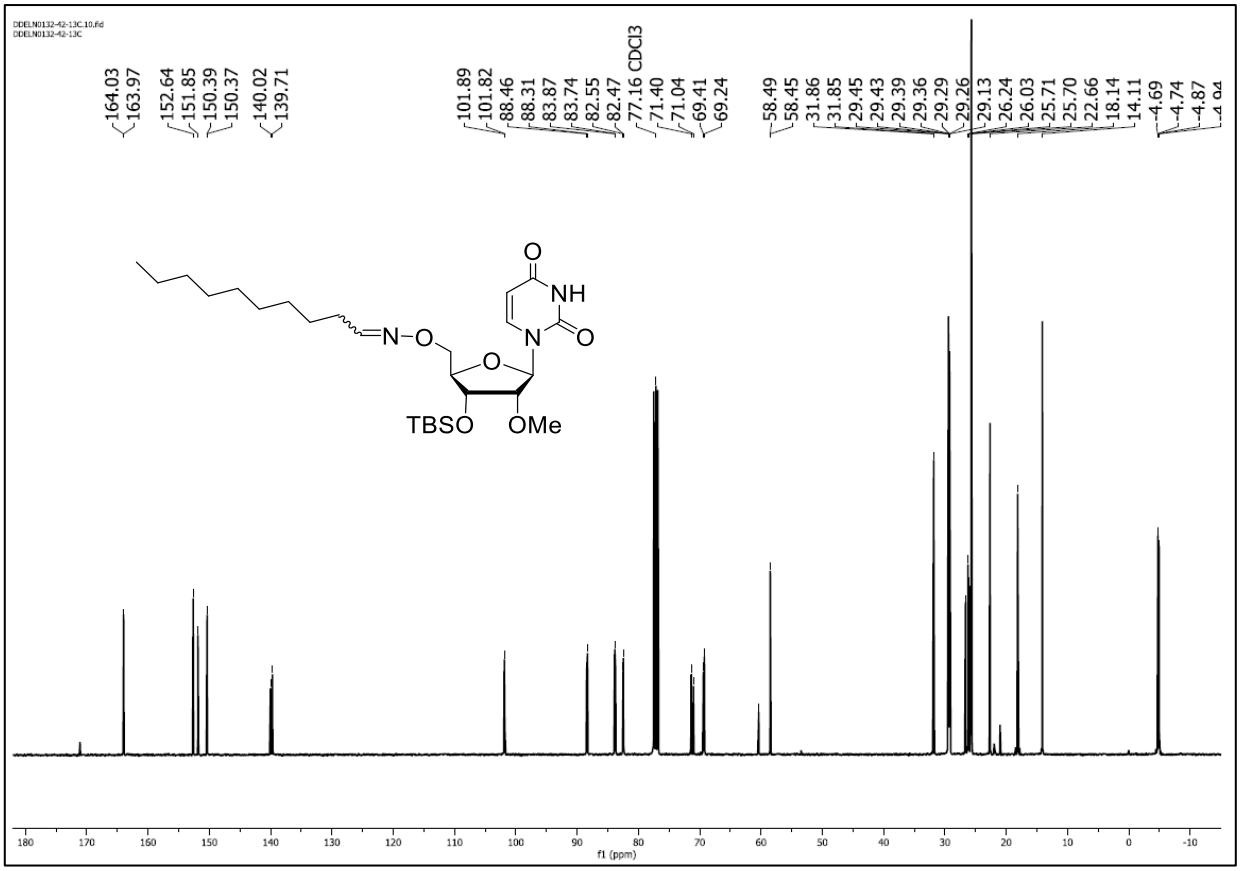
^{1}H NMR of compound **4a** (400 MHz, $CDCl_3$)



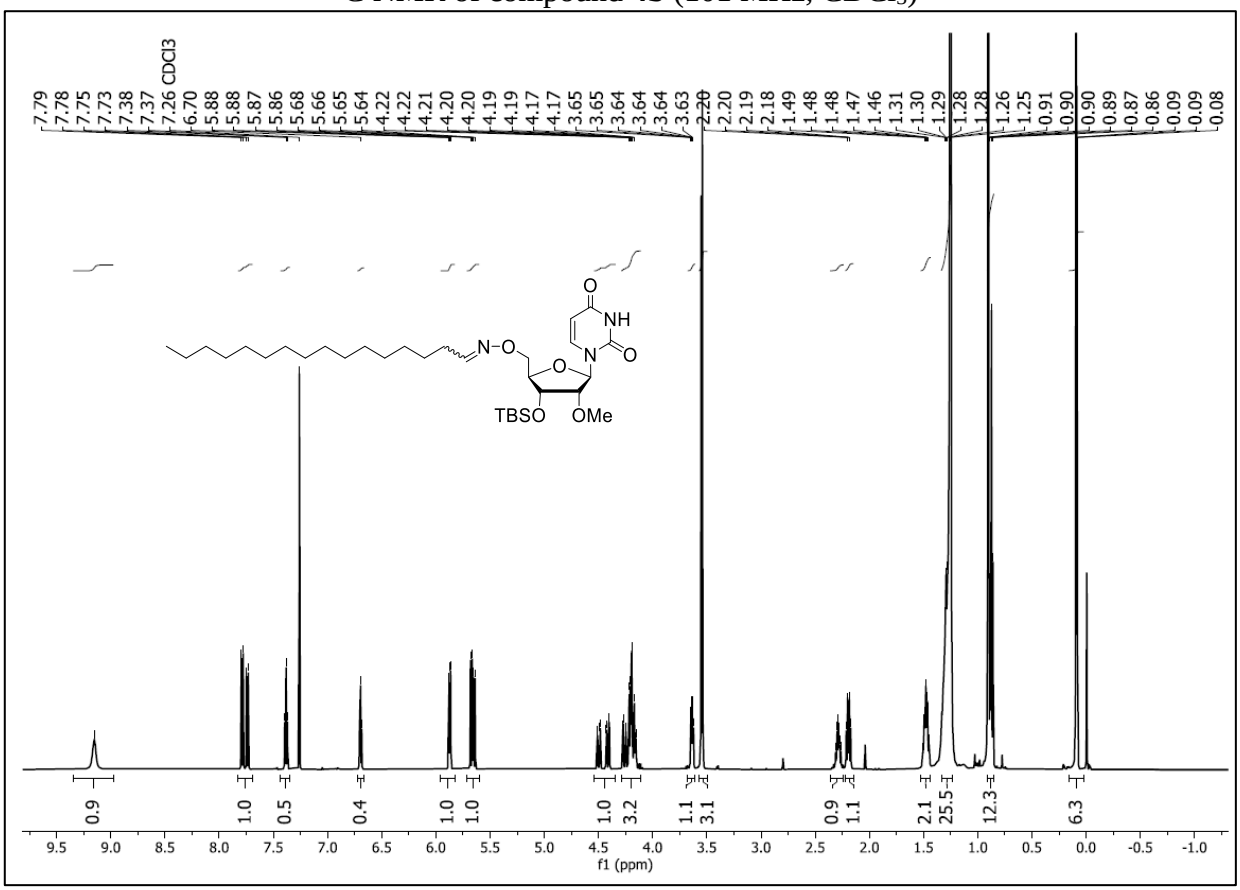
^{13}C NMR of compound **4a** (101 MHz, $CDCl_3$)



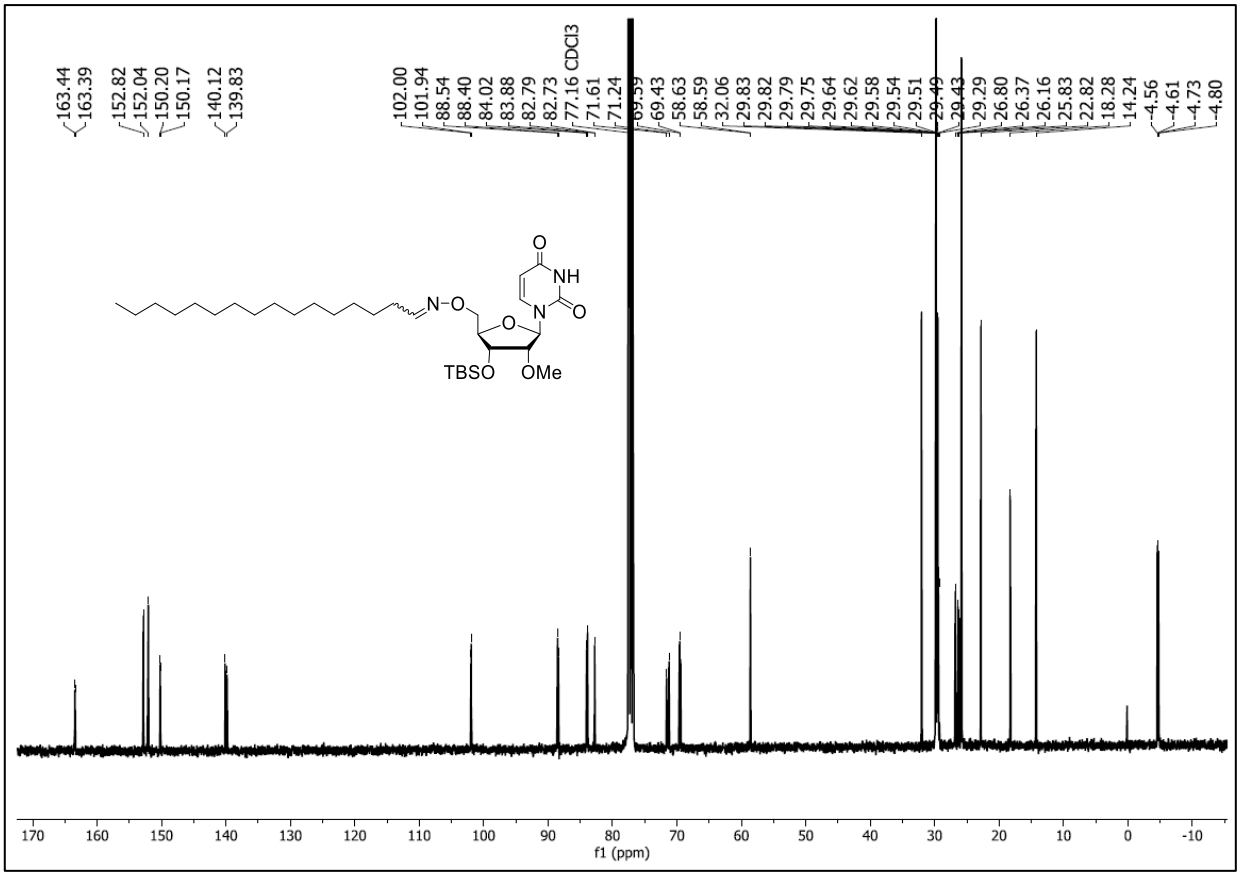
^{1}H NMR of compound **4b** (500 MHz, $CDCl_3$)



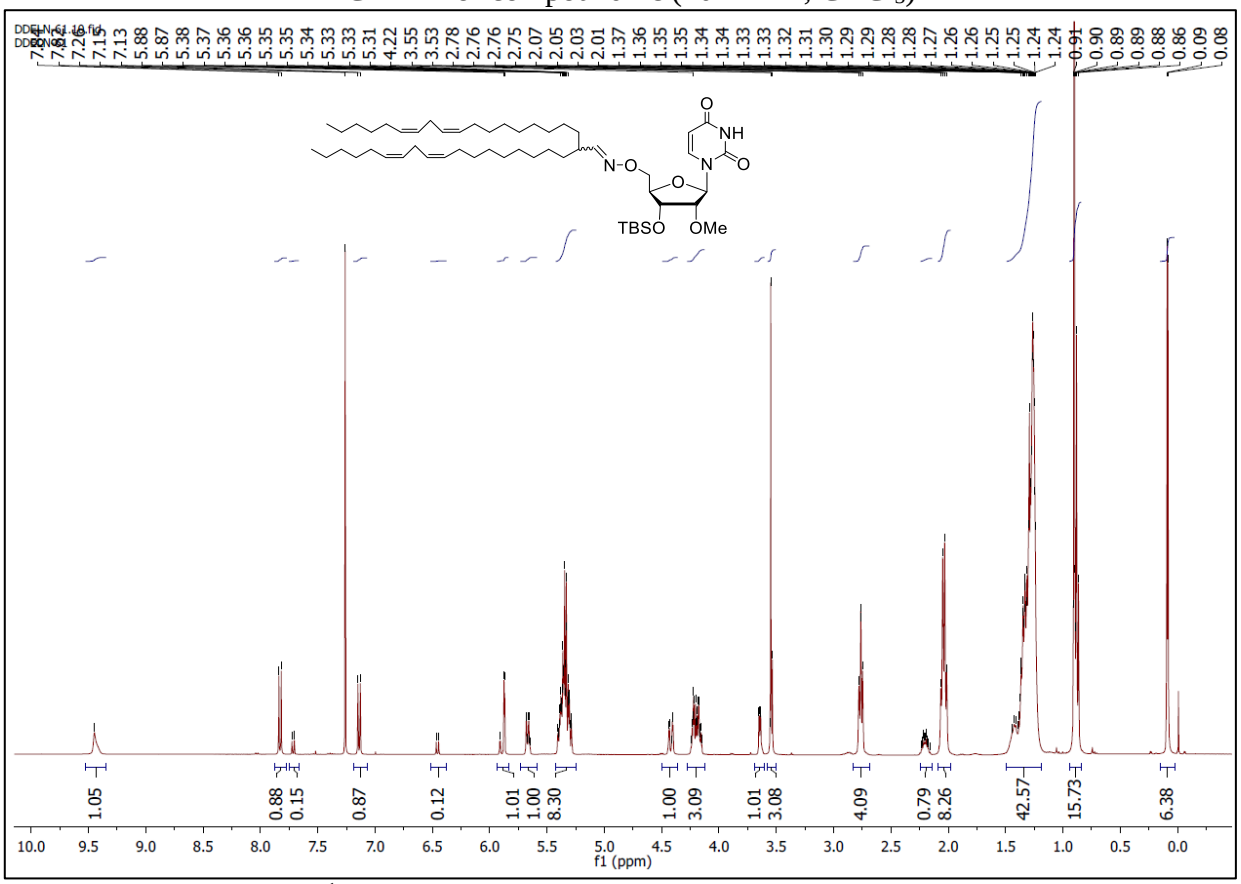
^{13}C NMR of compound **4b** (101 MHz, $CDCl_3$)



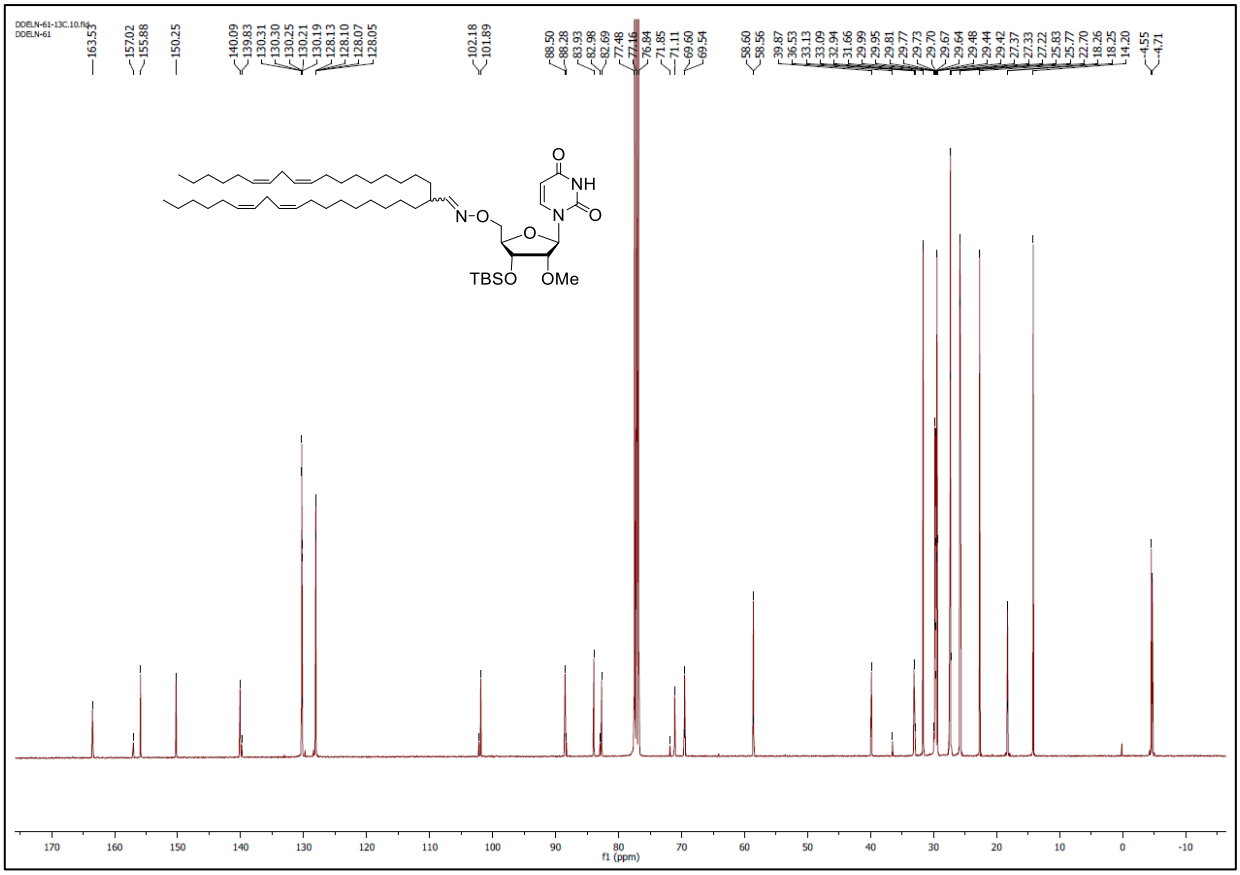
^{1}H NMR of compound **4c** (500 MHz, $CDCl_3$) ELN0132-52



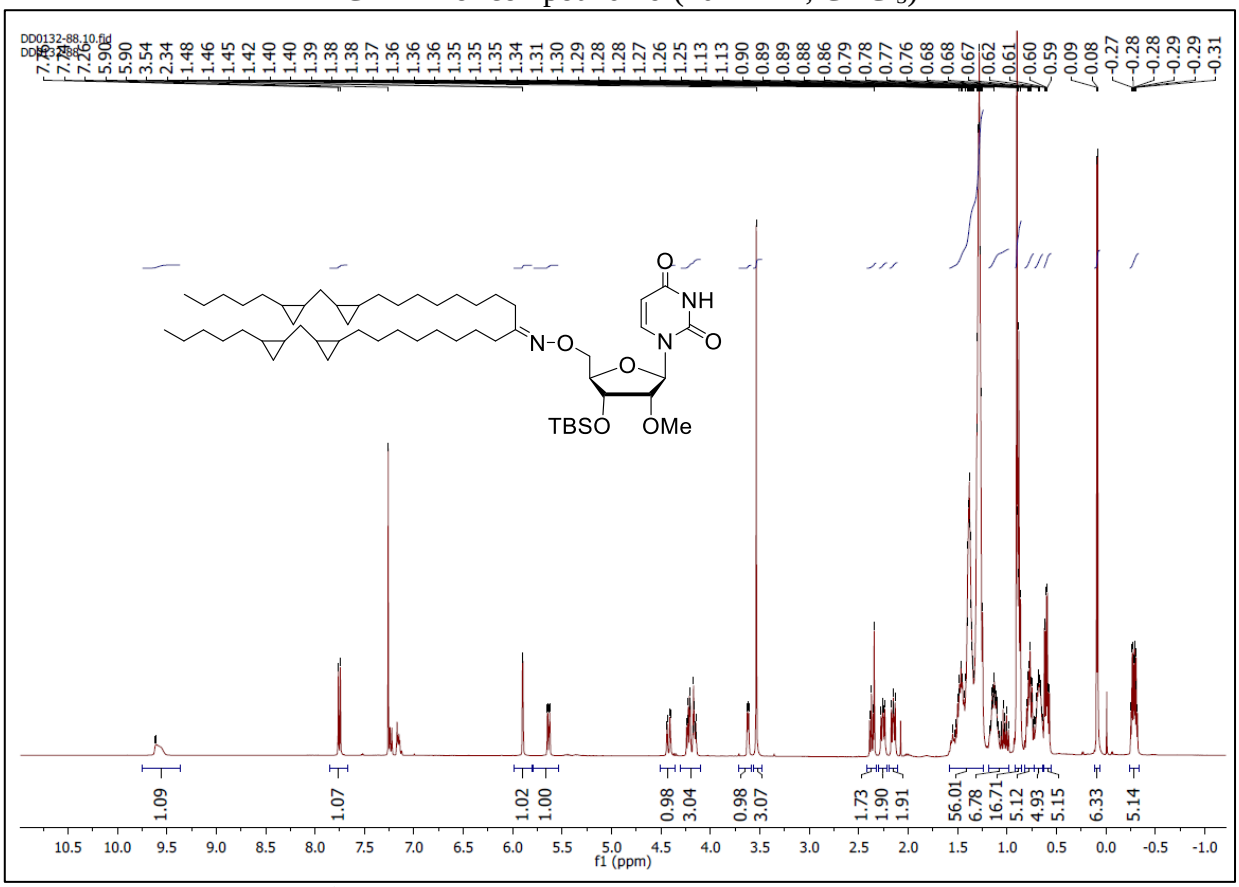
^{13}C NMR of compound **4c** (101 MHz, $CDCl_3$)



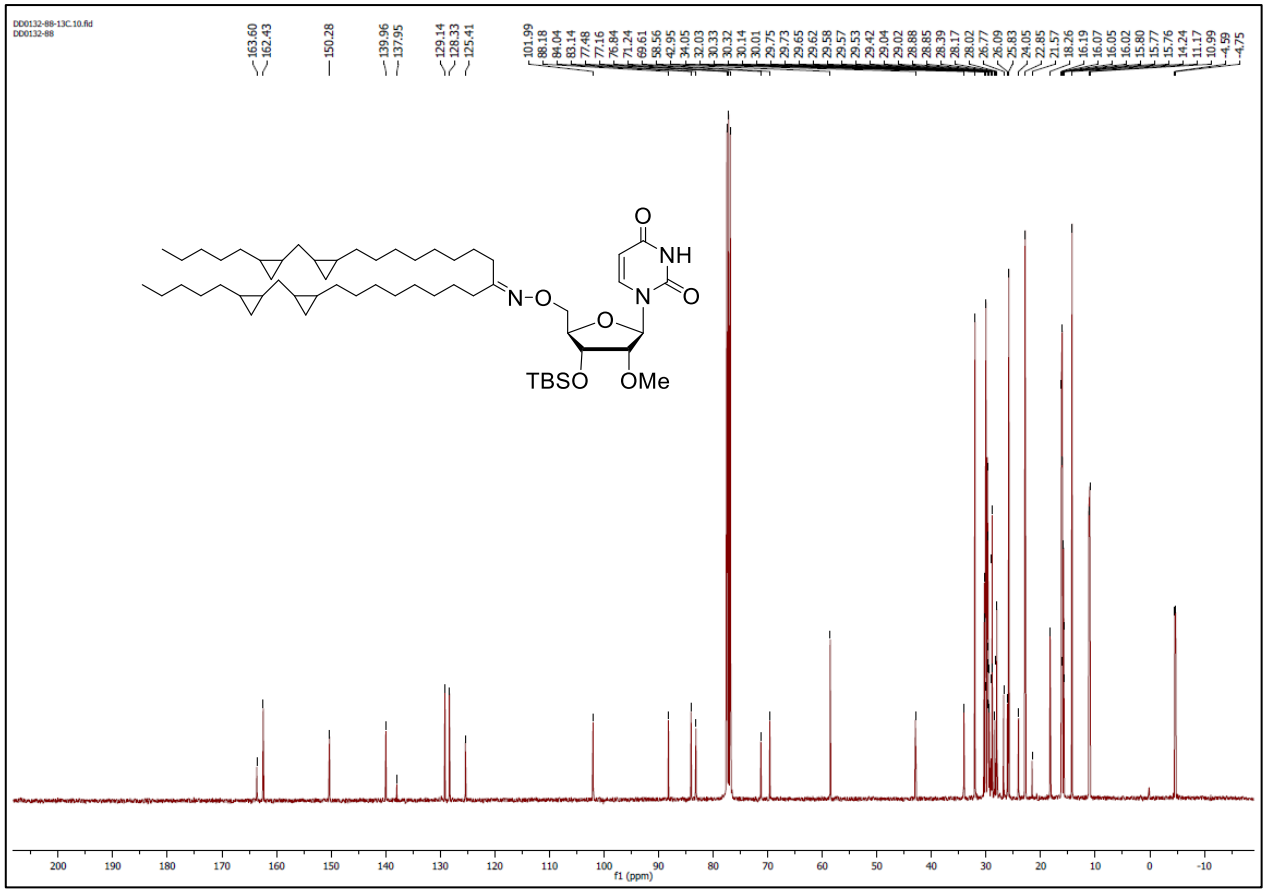
^{1}H NMR of compound **4d** (400 MHz, $CDCl_3$)



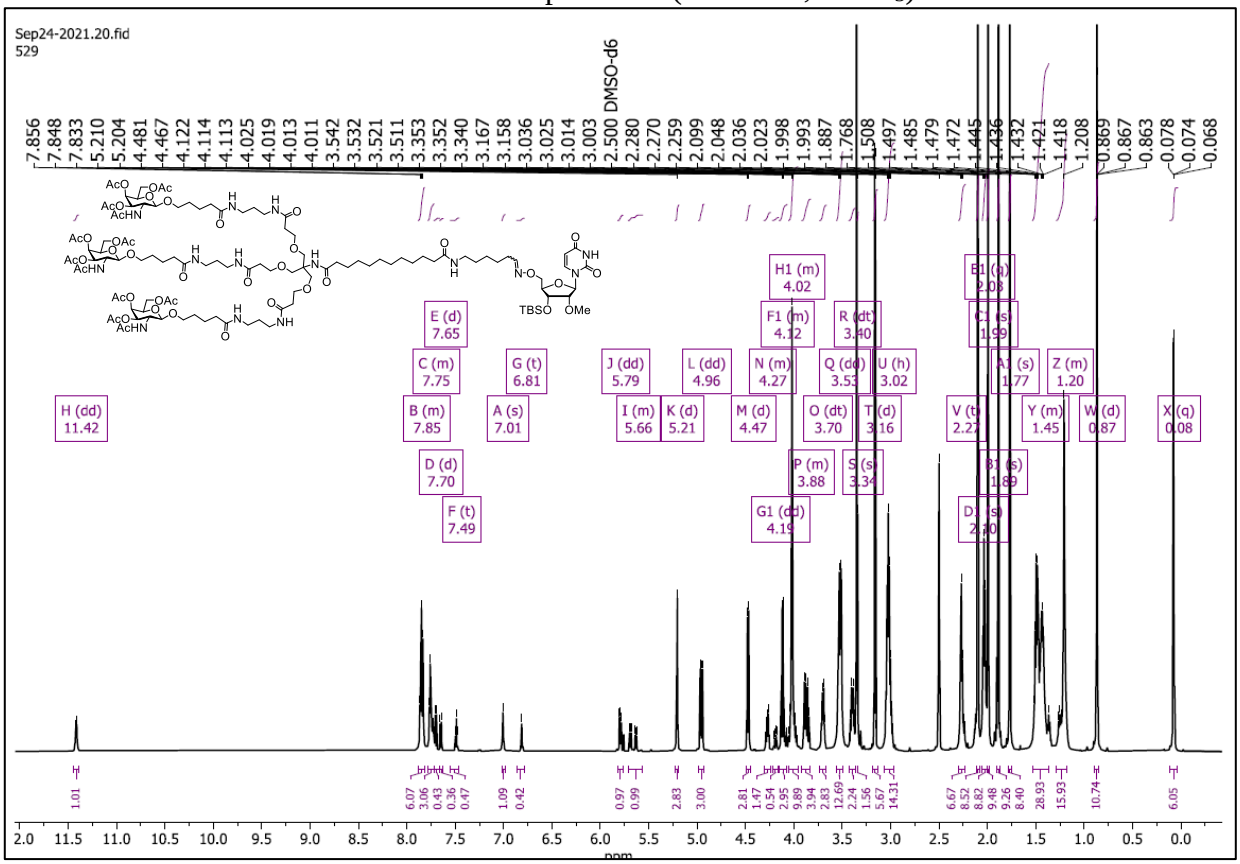
^{13}C NMR of compound **4d** (101 MHz, $CDCl_3$)



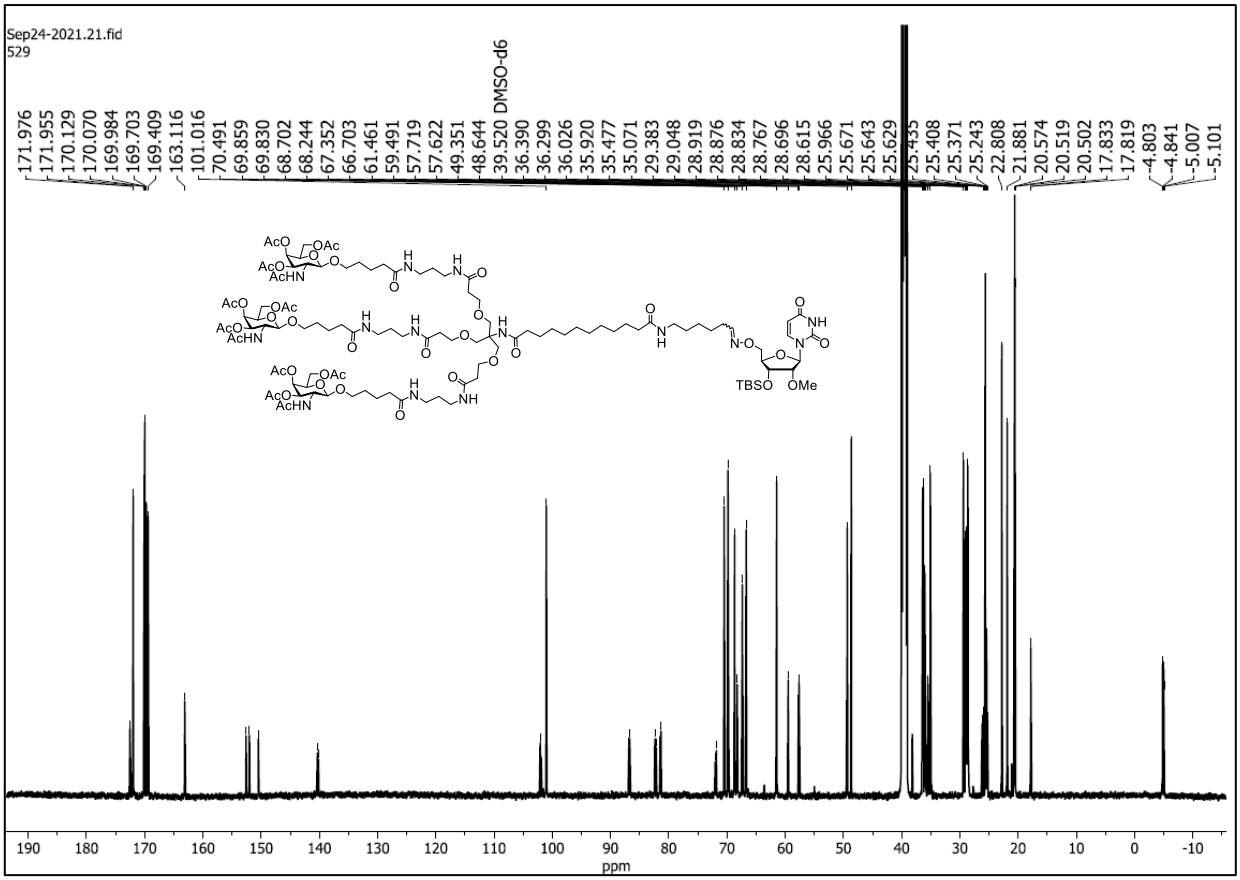
^{1}H NMR of compound **4e** (400 MHz, $CDCl_3$)



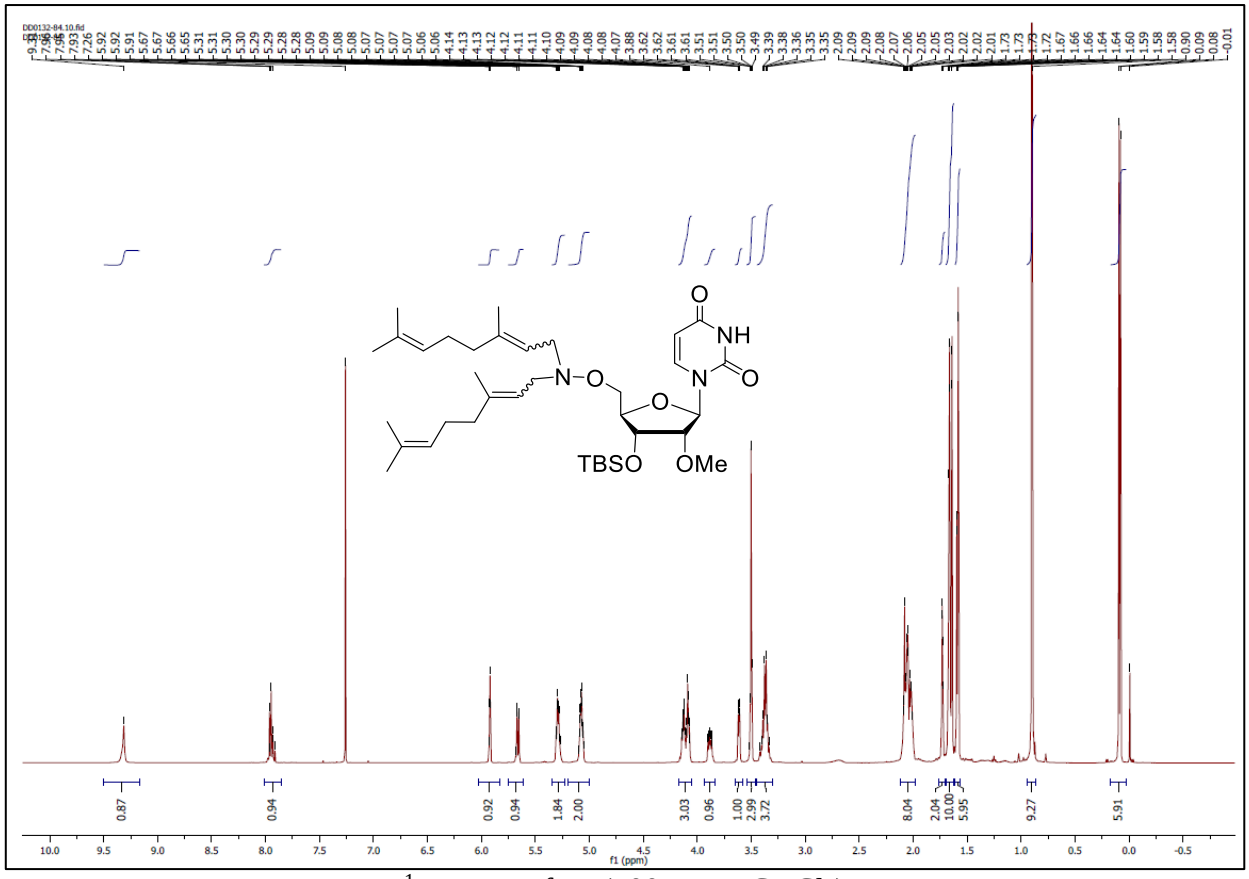
^{13}C NMR of compound **4e** (101 MHz, $CDCl_3$)



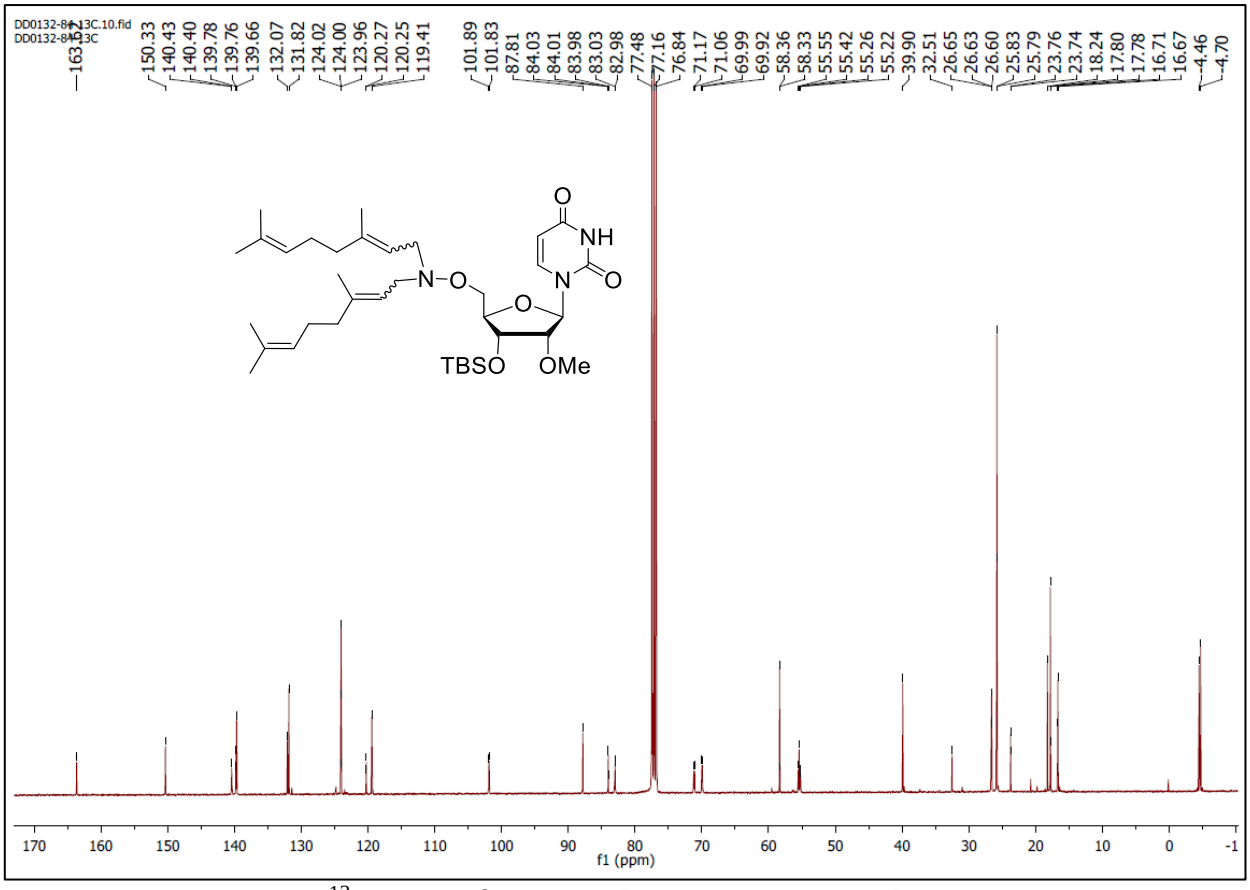
^{1}H NMR of compound **4f** (600 MHz, DMSO-d_6)



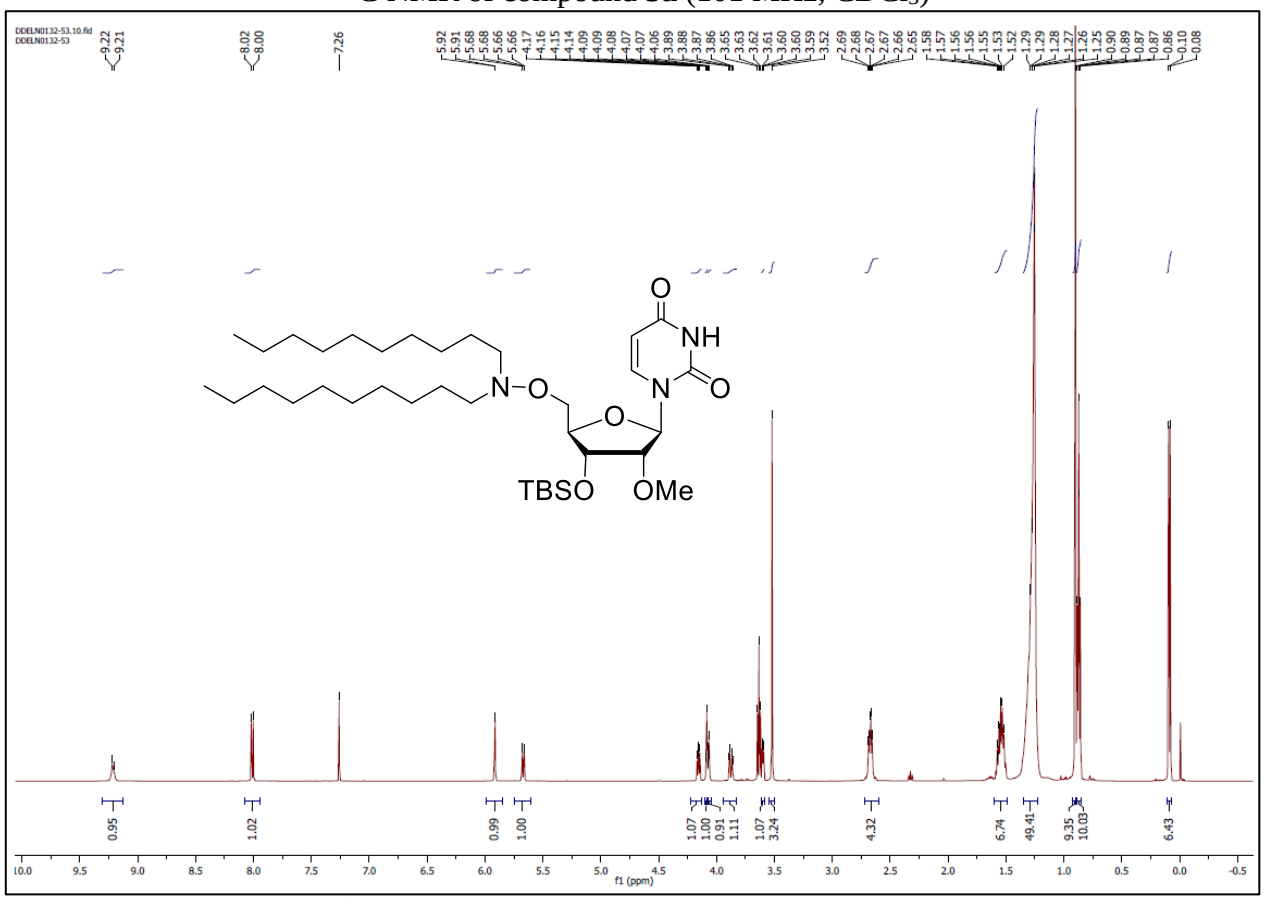
^{13}C NMR of compound **4f** (151 MHz, DMSO-d_6)



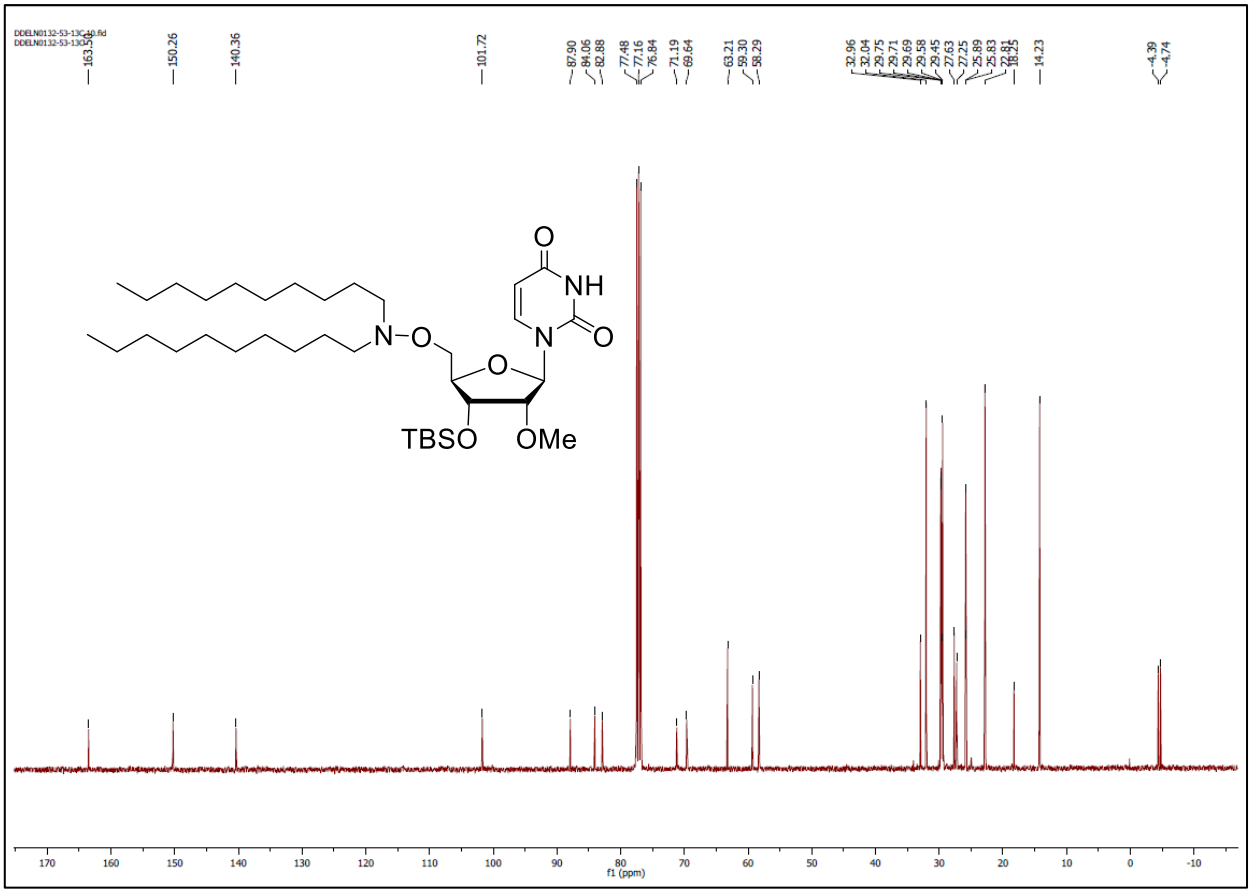
^{1}H NMR of **5a** (500 MHz, $CDCl_3$)



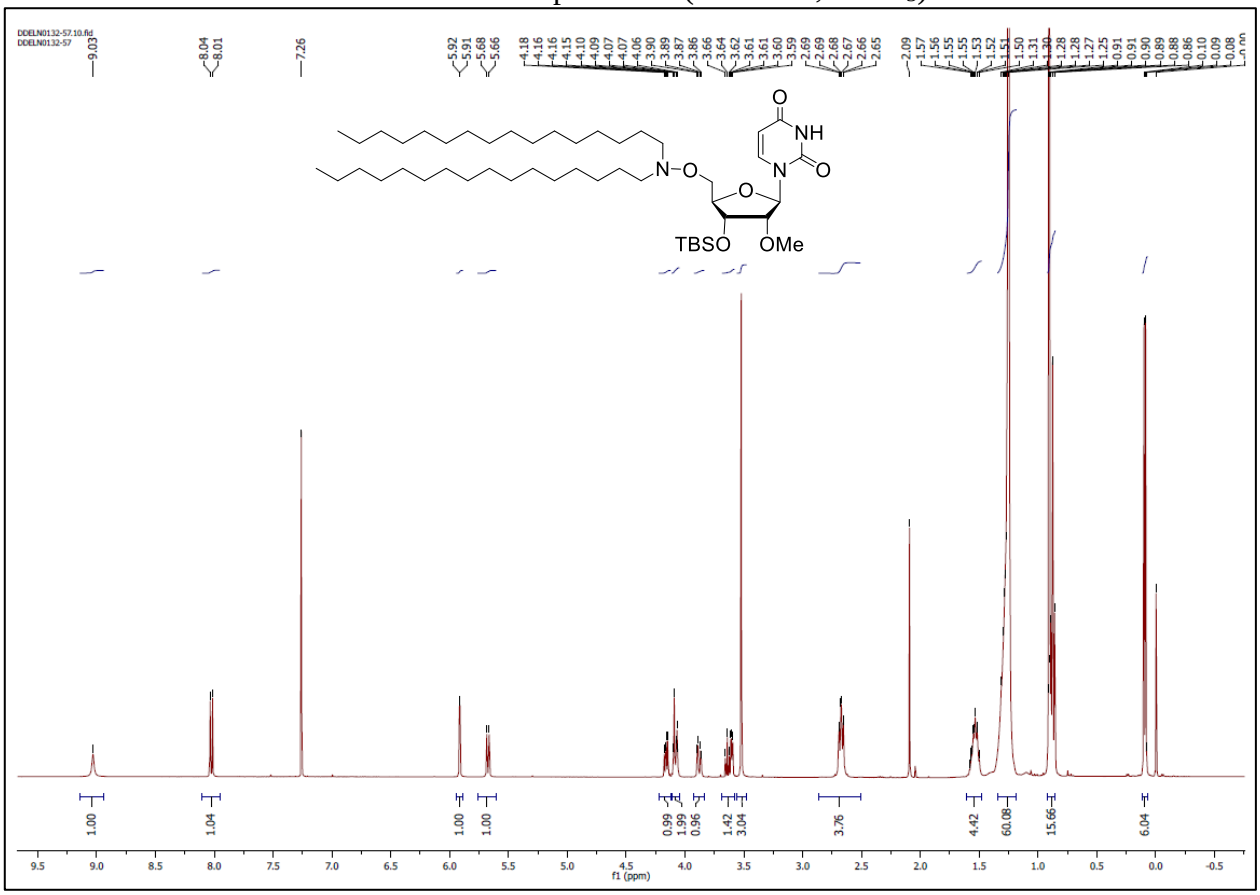
^{13}C NMR of compound **5a** (101 MHz, $CDCl_3$)



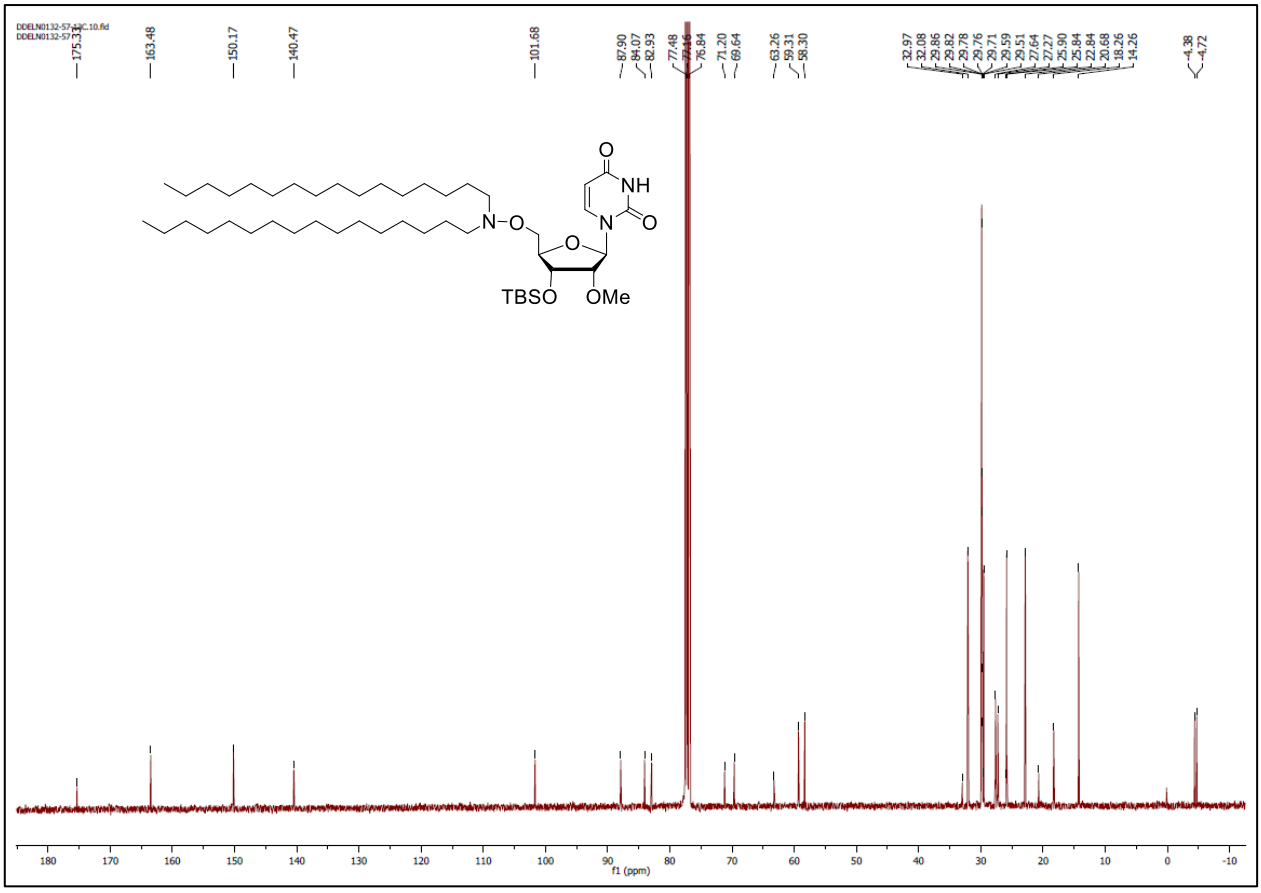
^{1}H NMR of compound **5b** (500 MHz, $CDCl_3$)



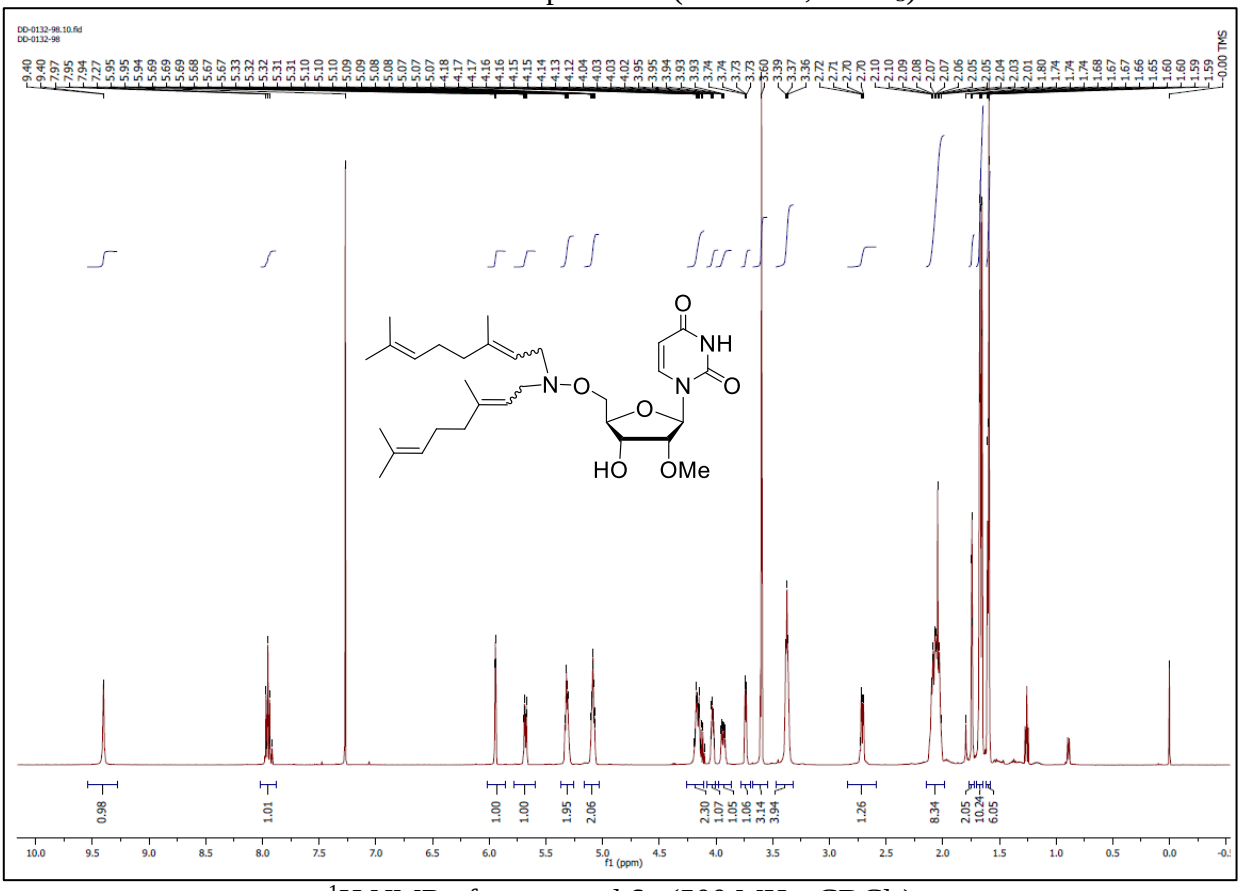
^{13}C NMR of compound **5b** (101 MHz, $CDCl_3$)



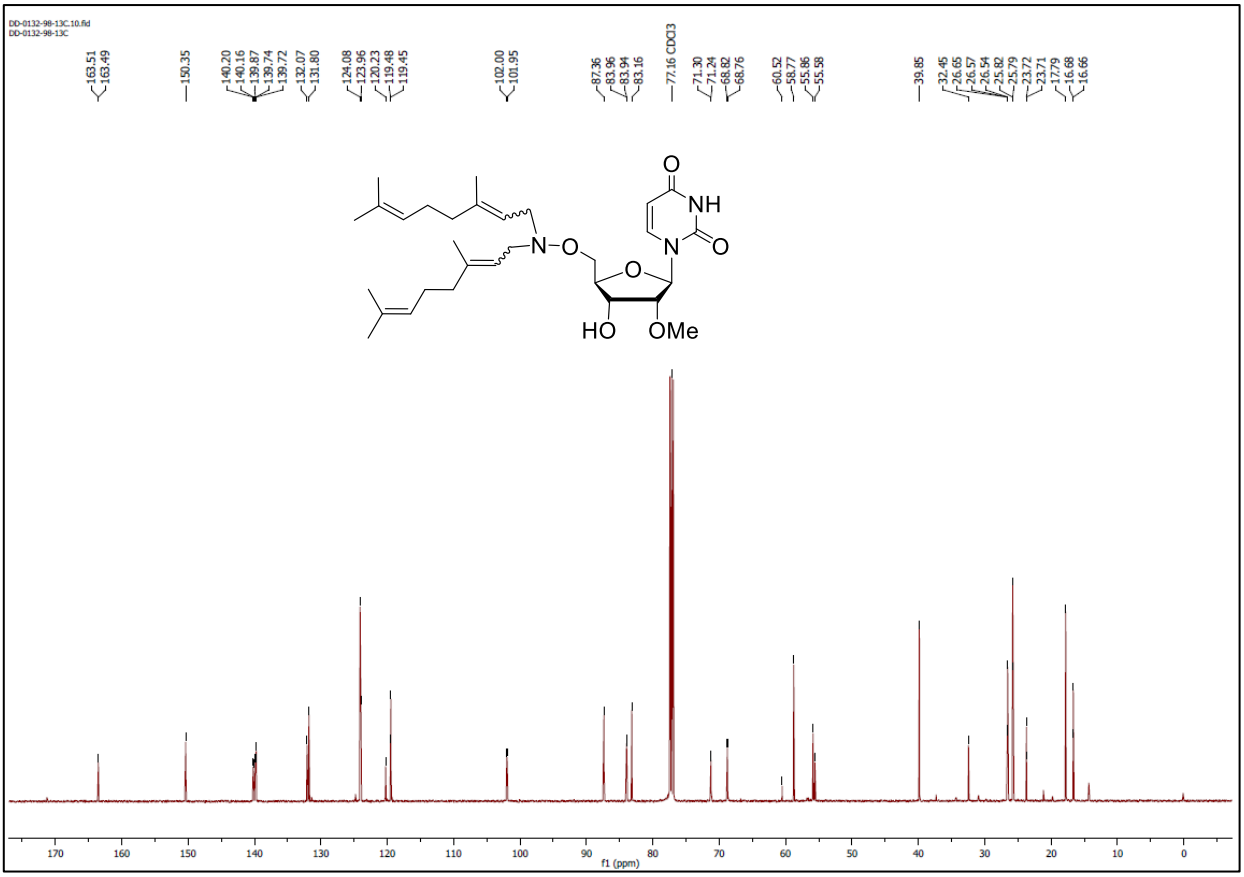
^{1}H NMR of compound **5c** (400 MHz, $CDCl_3$)



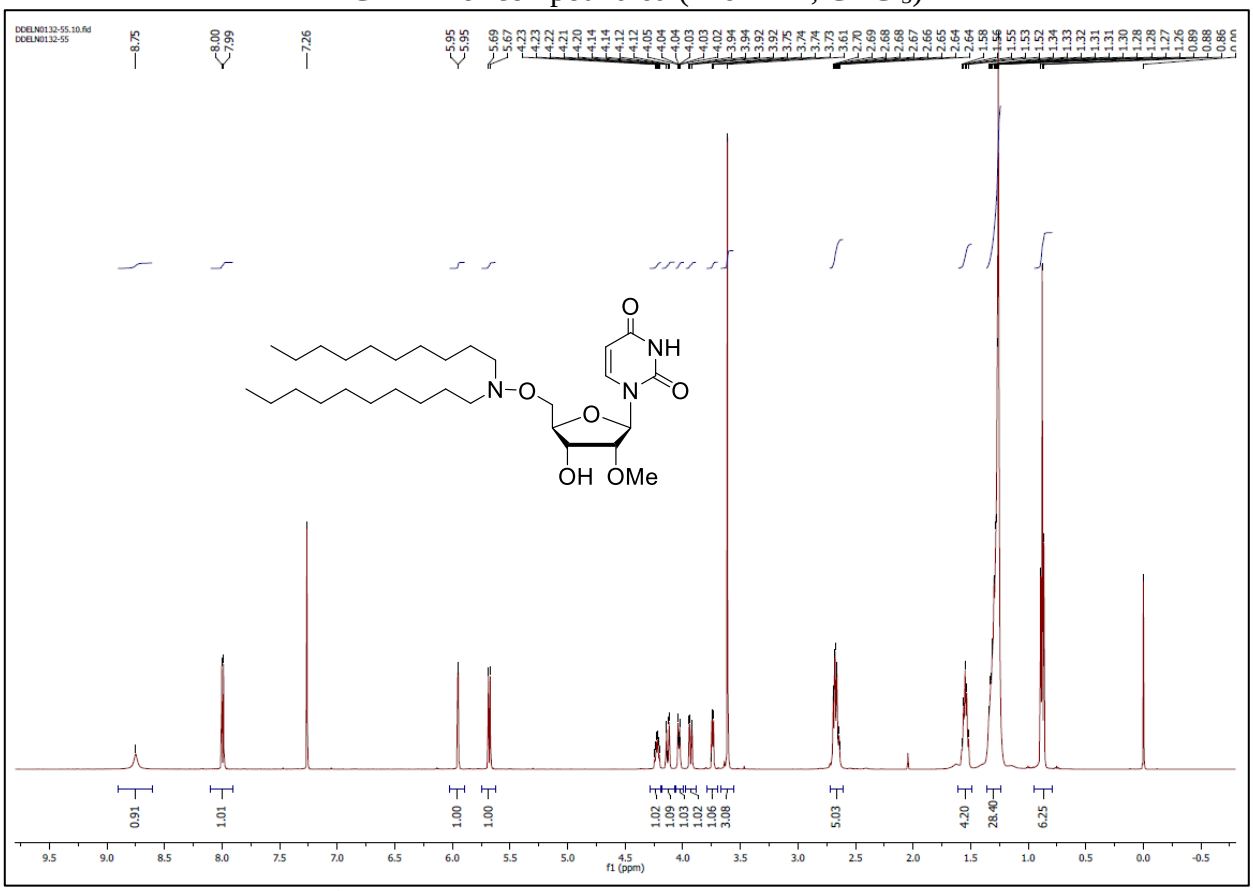
^{13}C NMR of compound **5c** (101 MHz, $CDCl_3$)



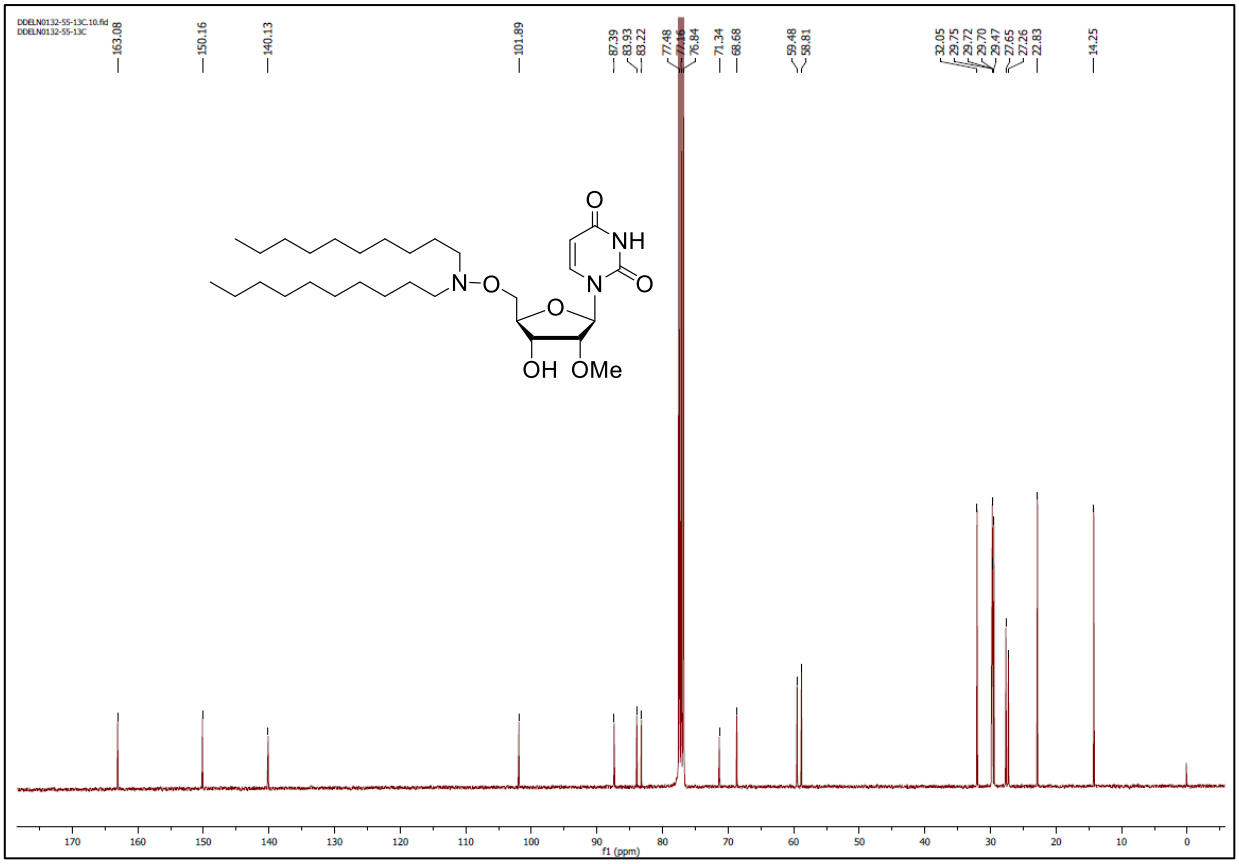
^{1}H NMR of compound **6a** (500 MHz, $CDCl_3$)



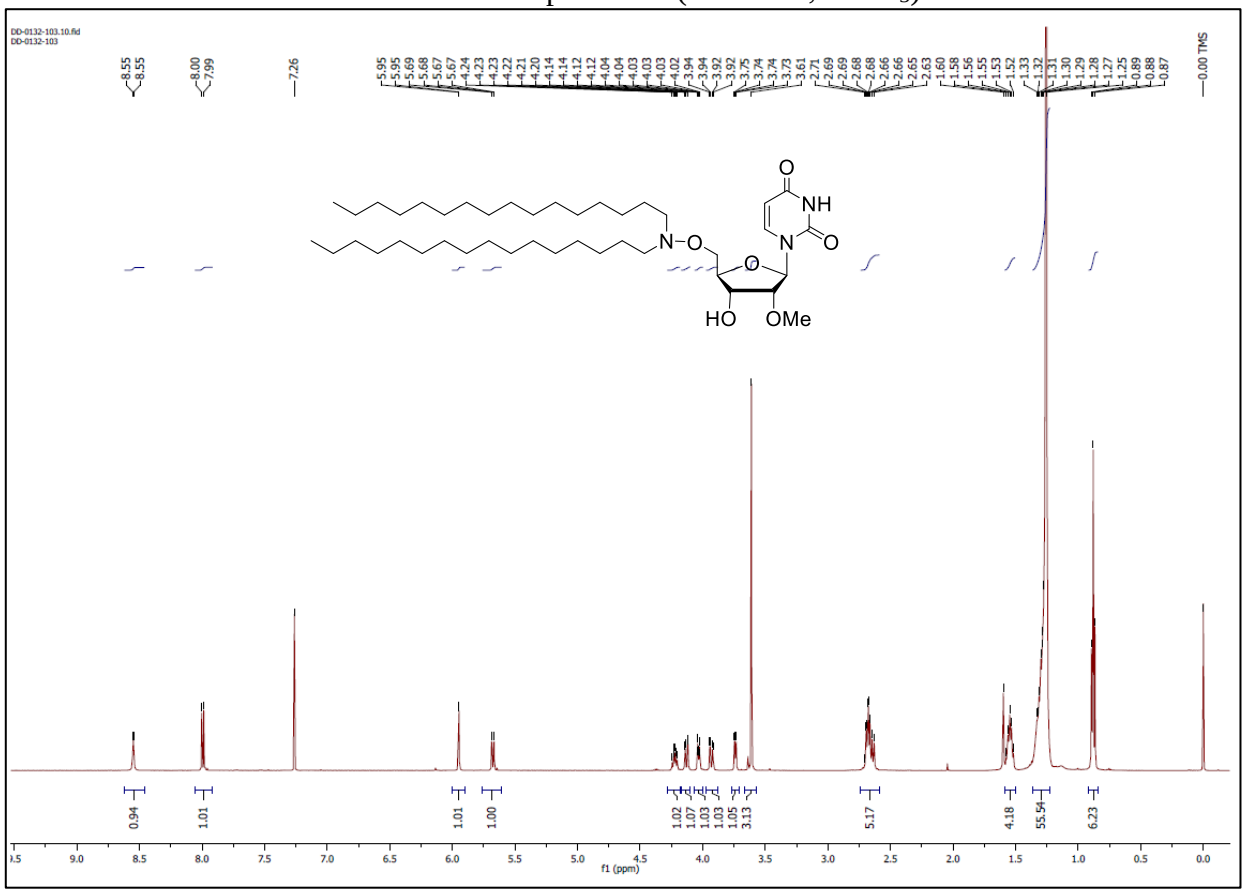
^{13}C NMR of compound **6a** (126 MHz, $CDCl_3$)



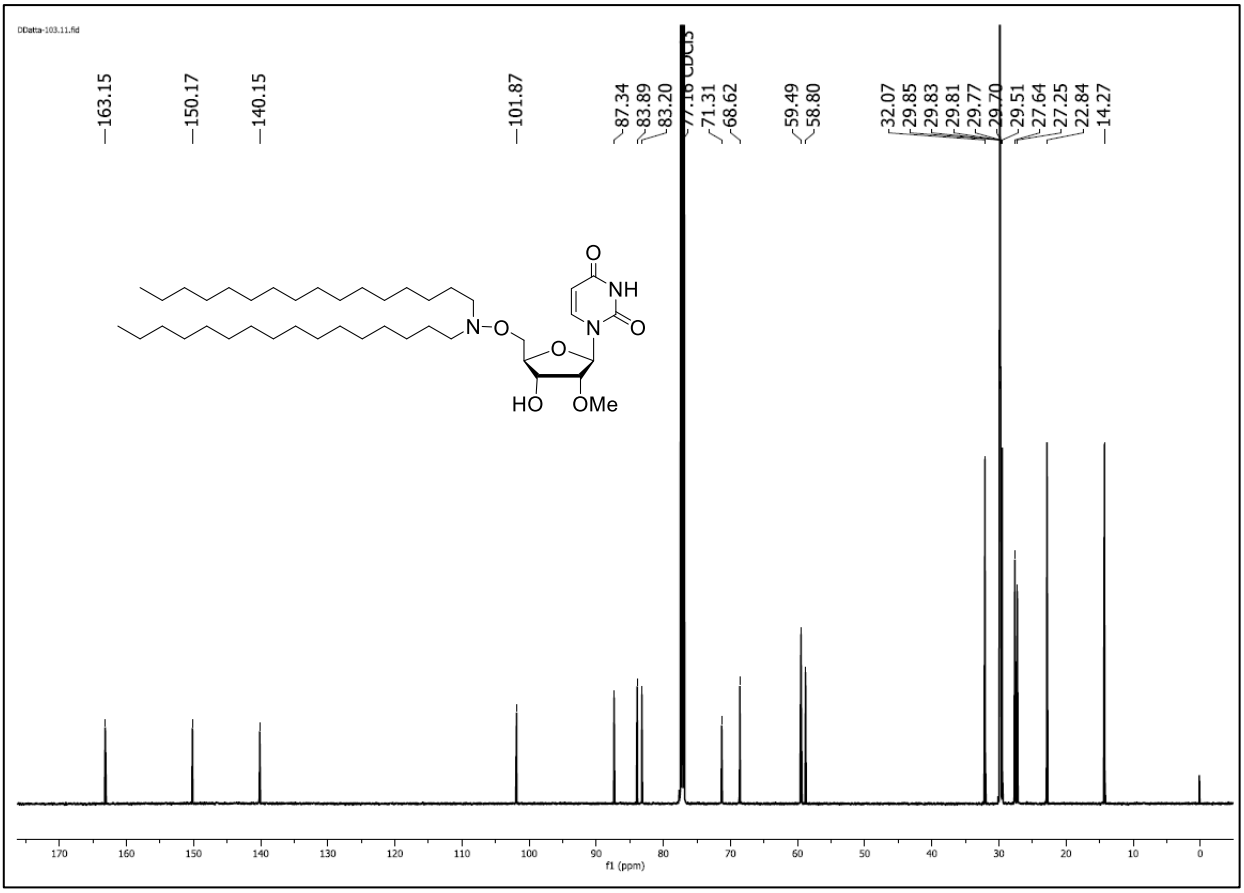
^{1}H NMR of compound **6b** (500 MHz, $CDCl_3$)



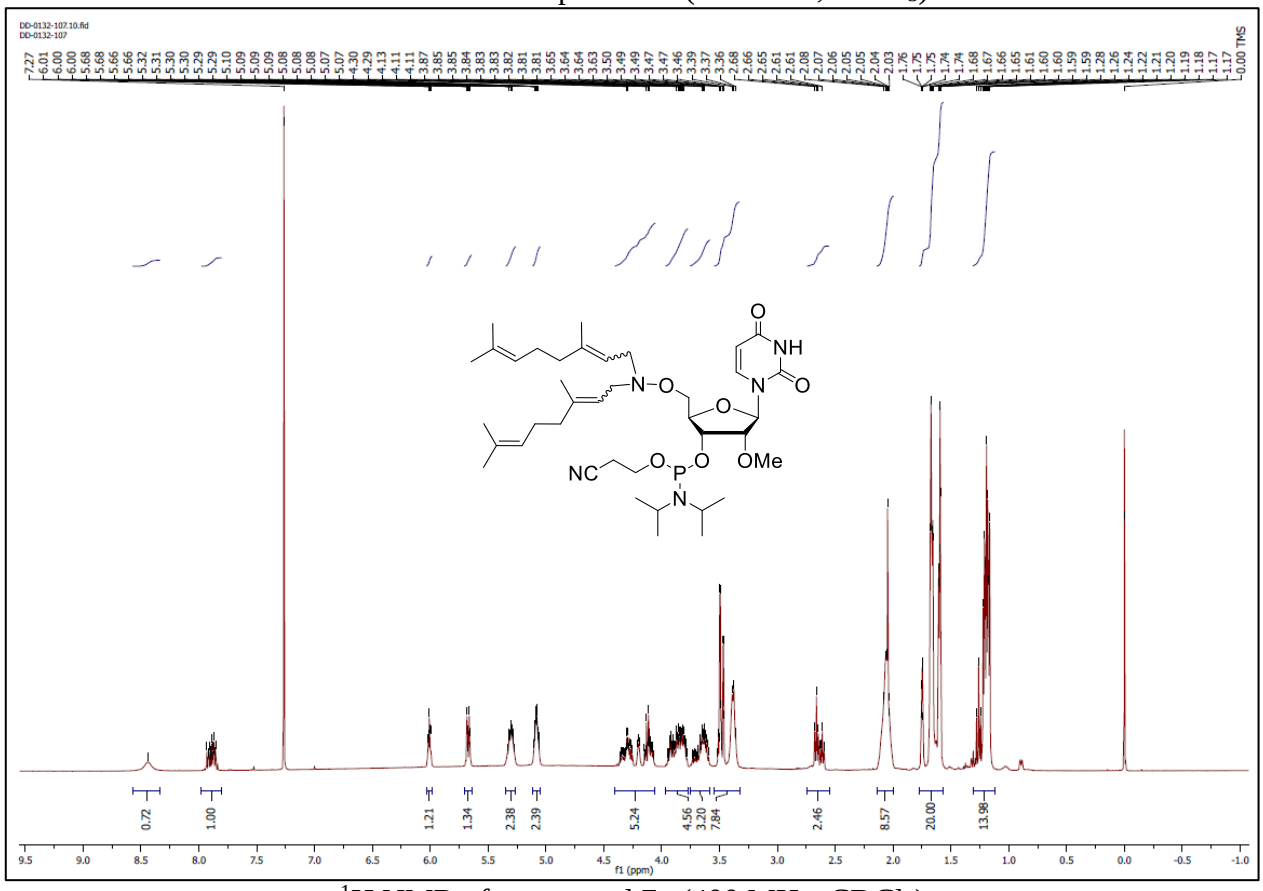
^{13}C NMR of compound **6b** (101 MHz, $CDCl_3$)



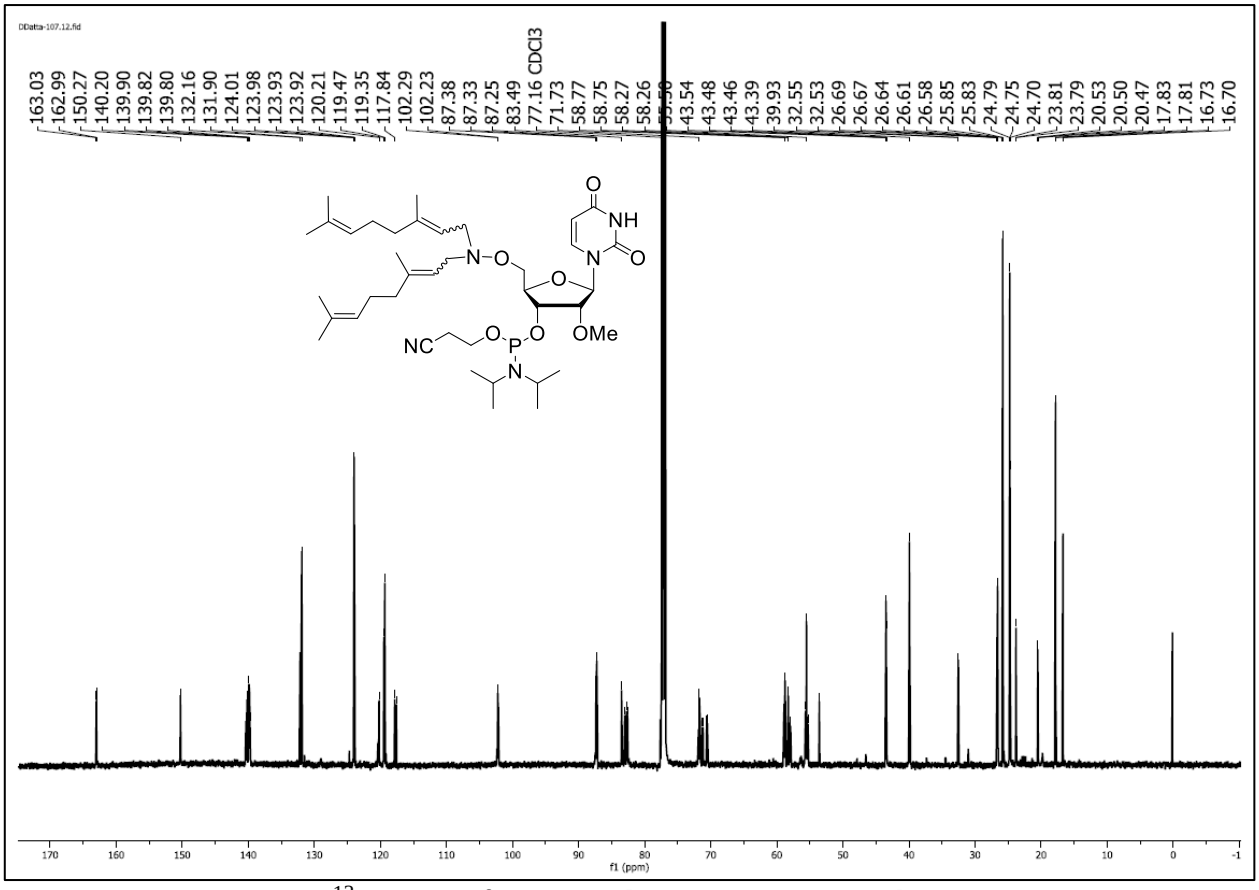
^{1}H NMR of compound **6c** (500 MHz, $CDCl_3$)



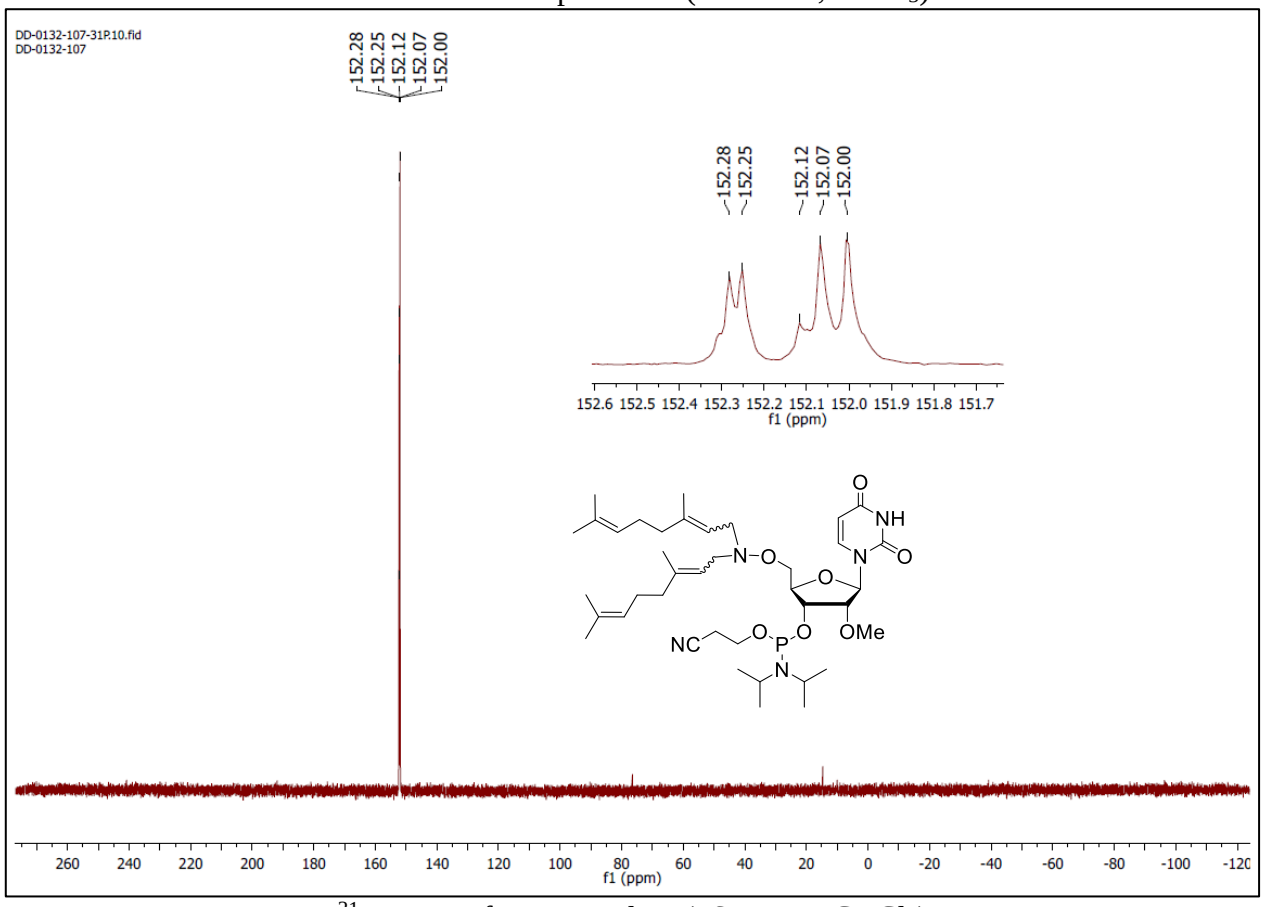
^{13}C NMR of compound **6c** (151 MHz, $CDCl_3$)



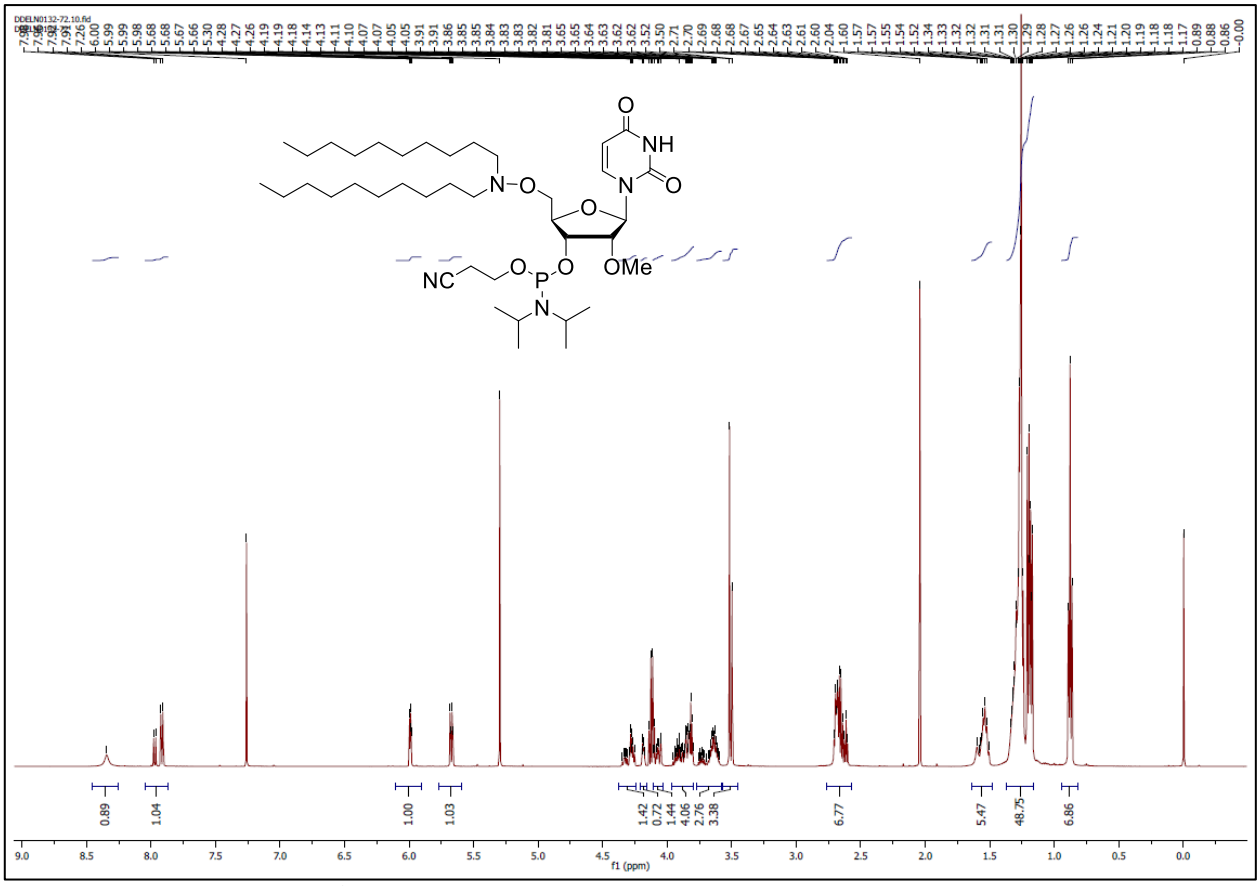
^{1}H NMR of compound **7a** (400 MHz, $CDCl_3$)



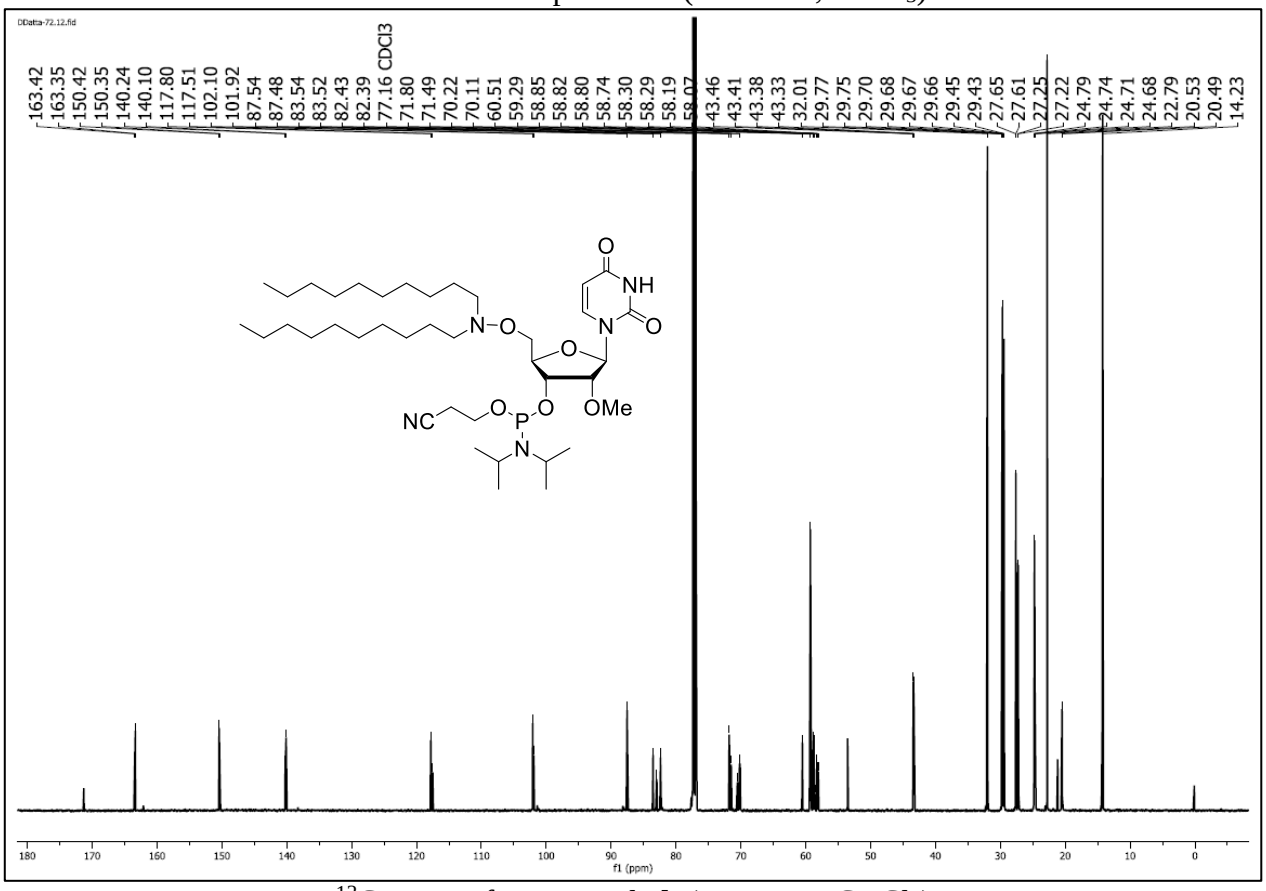
^{13}C NMR of compound **7a** (151 MHz, $CDCl_3$)



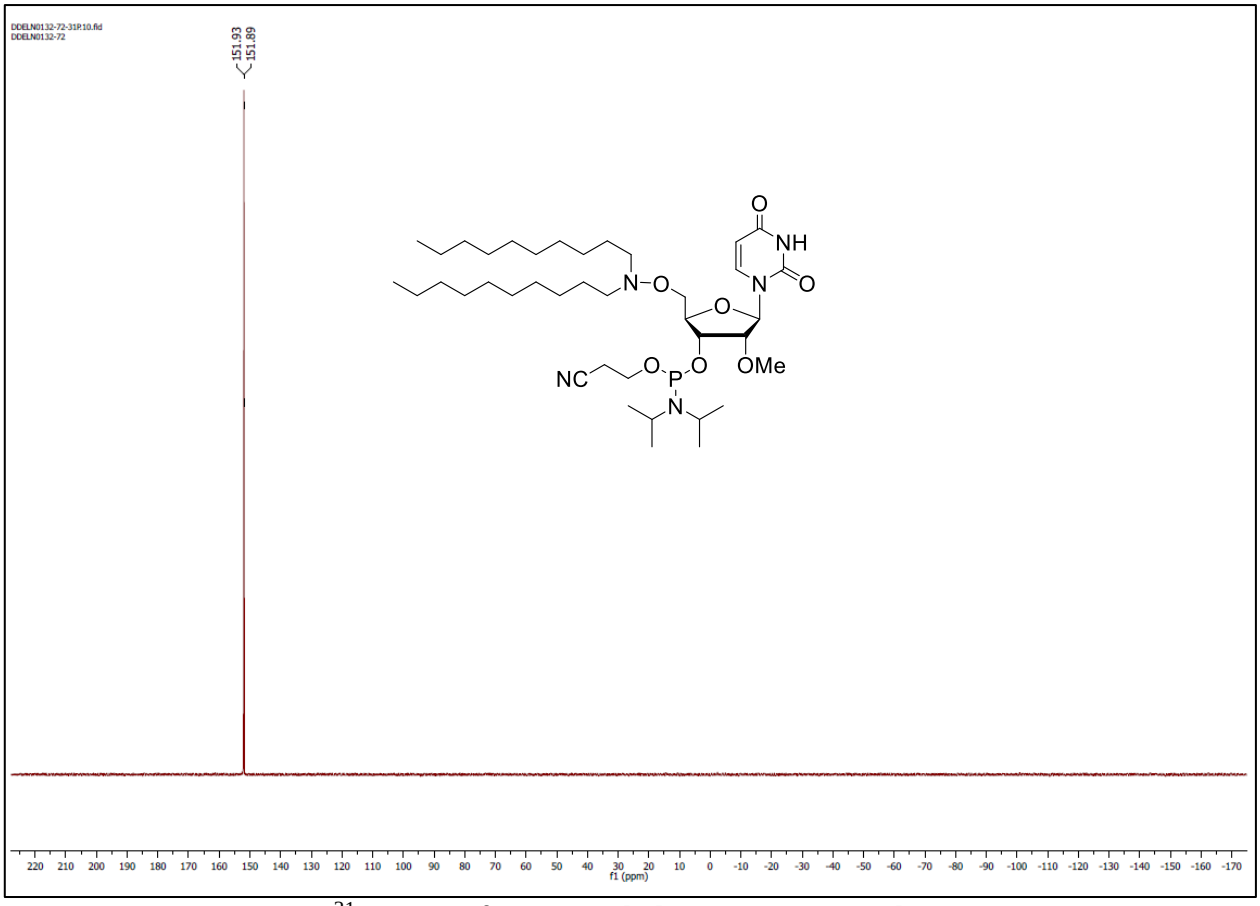
^{31}P NMR of compound **7a** (162 MHz, $CDCl_3$)



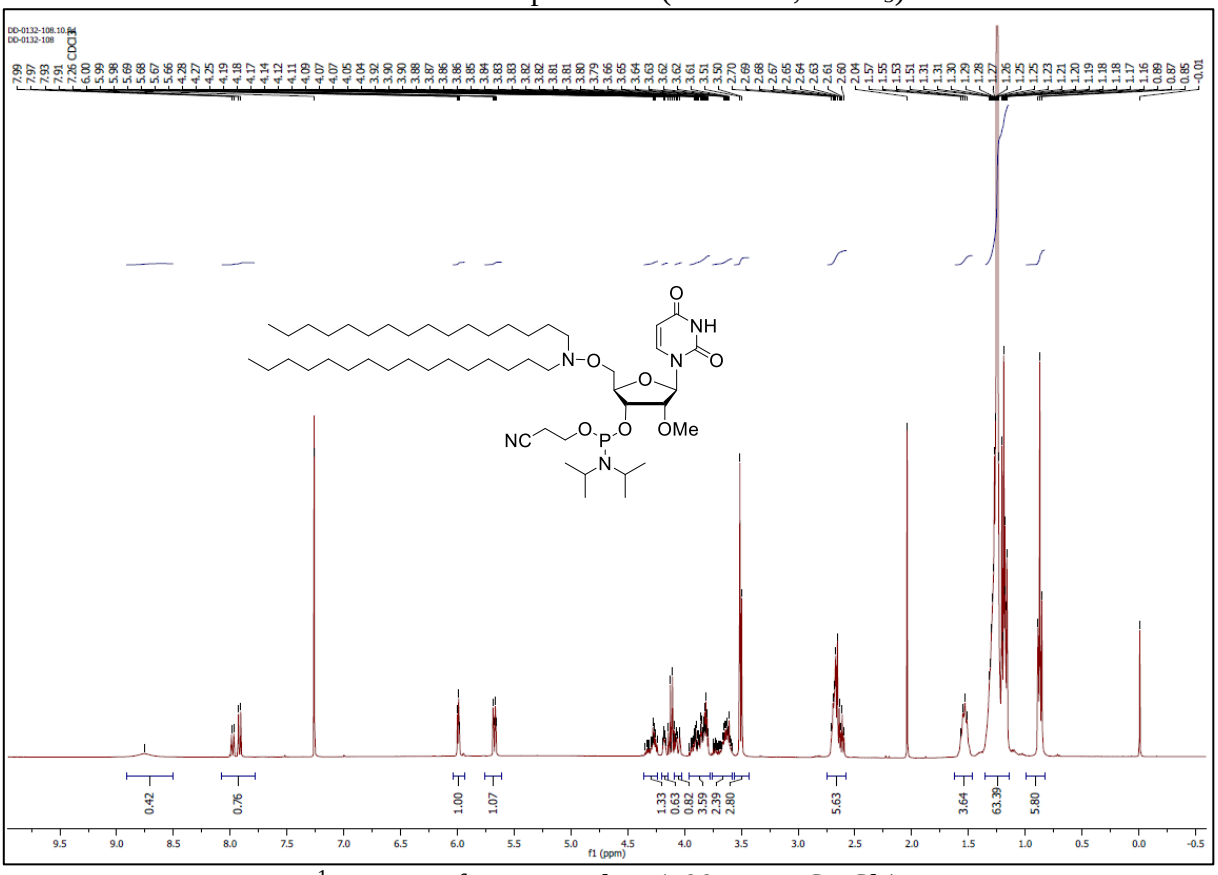
^{1}H NMR of compound **7b** (500 MHz, $CDCl_3$)



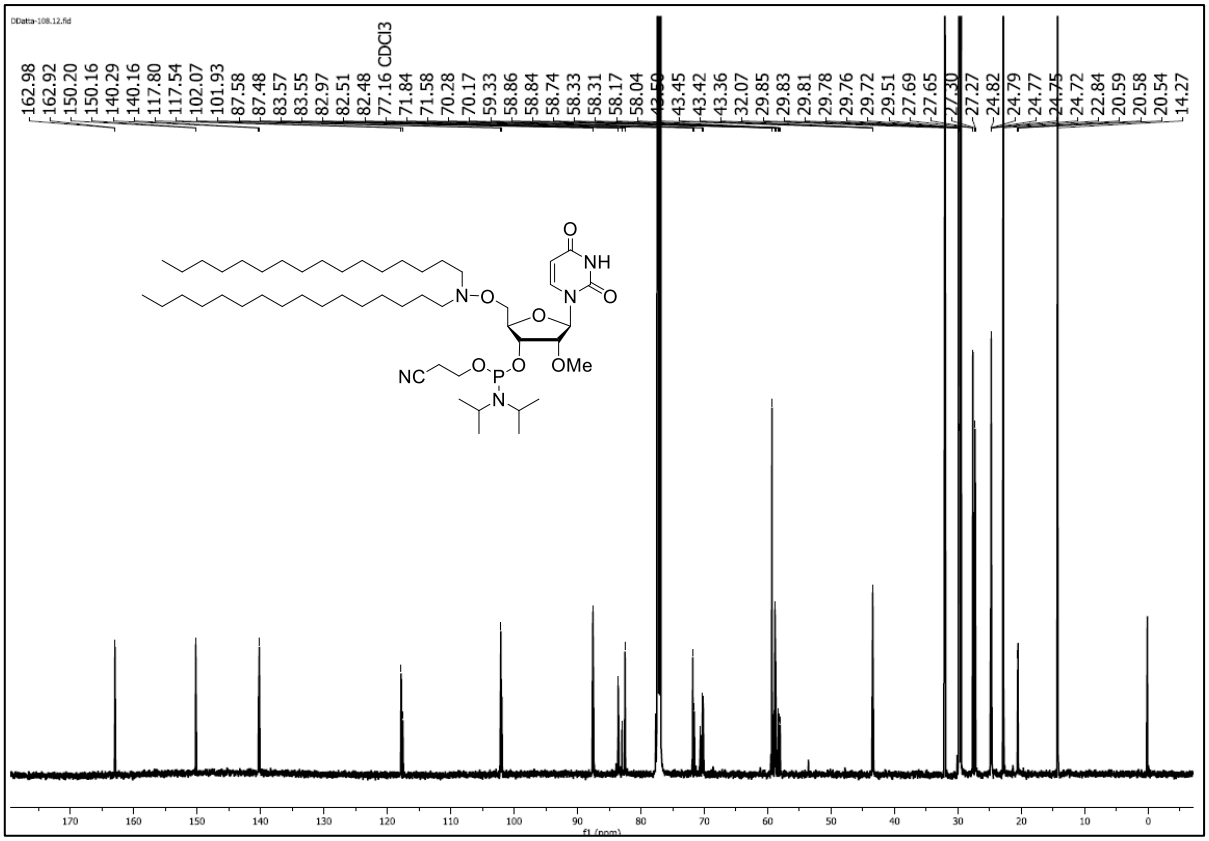
^{13}C NMR of compound **7b** (151 MHz, $CDCl_3$)



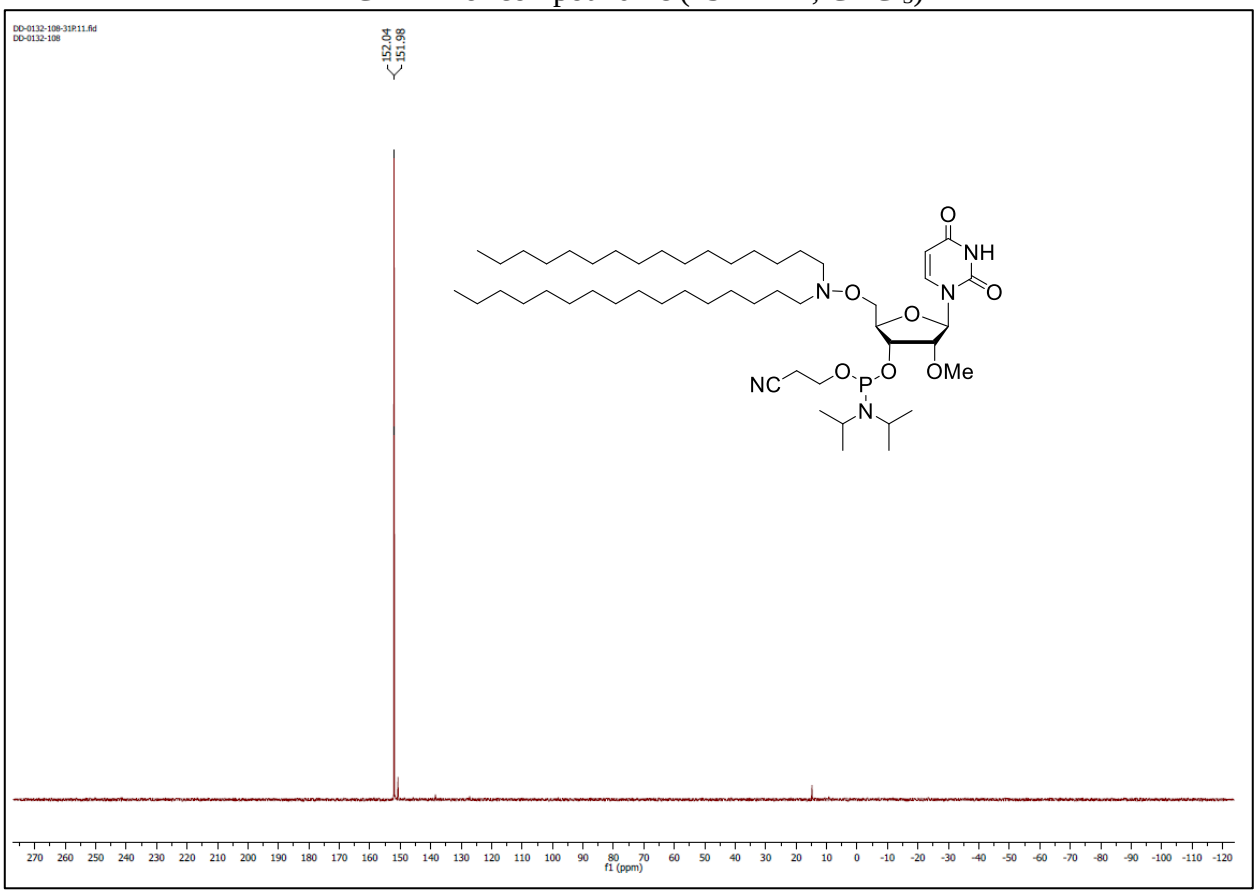
^{31}P NMR of compound **7b** (162 MHz, $CDCl_3$)



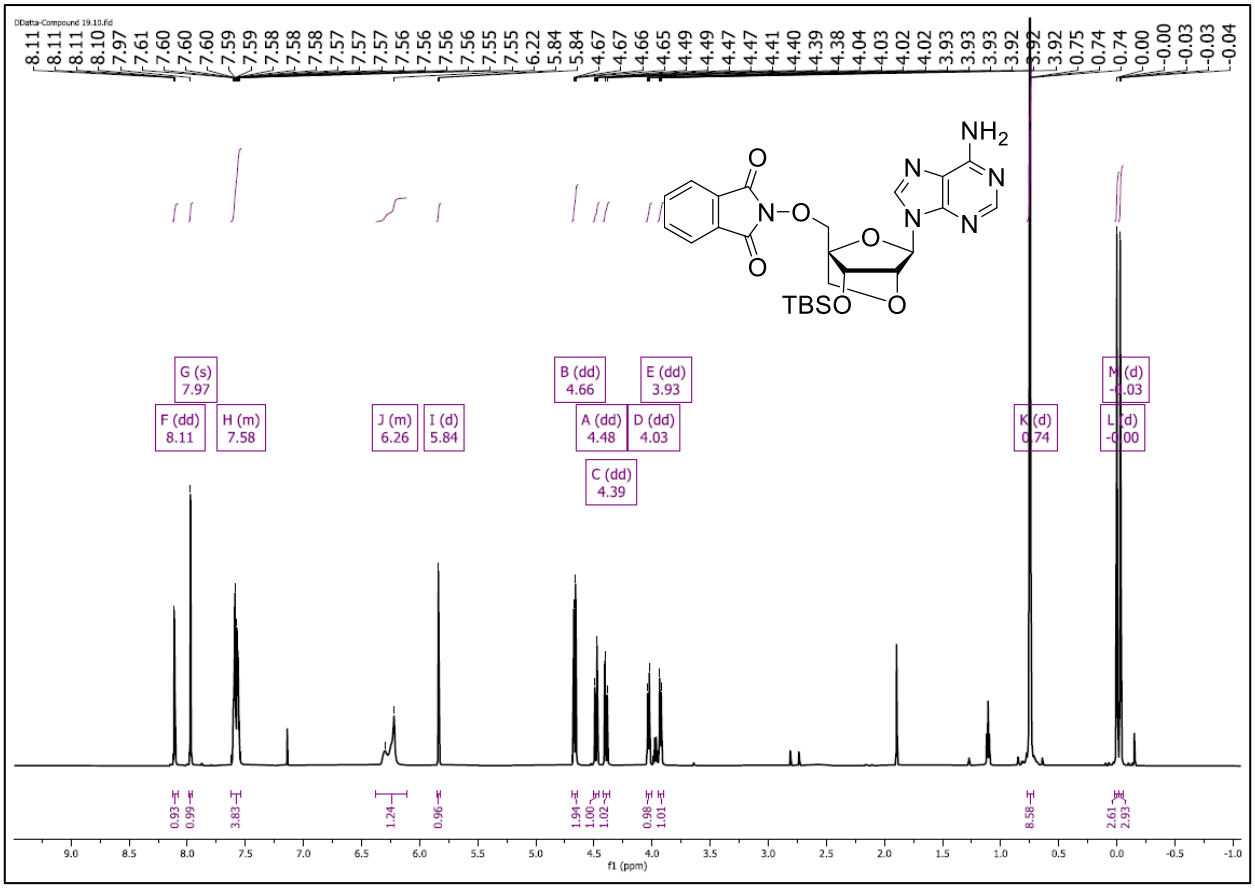
^{1}H NMR of compound **7c** (400 MHz, $CDCl_3$)



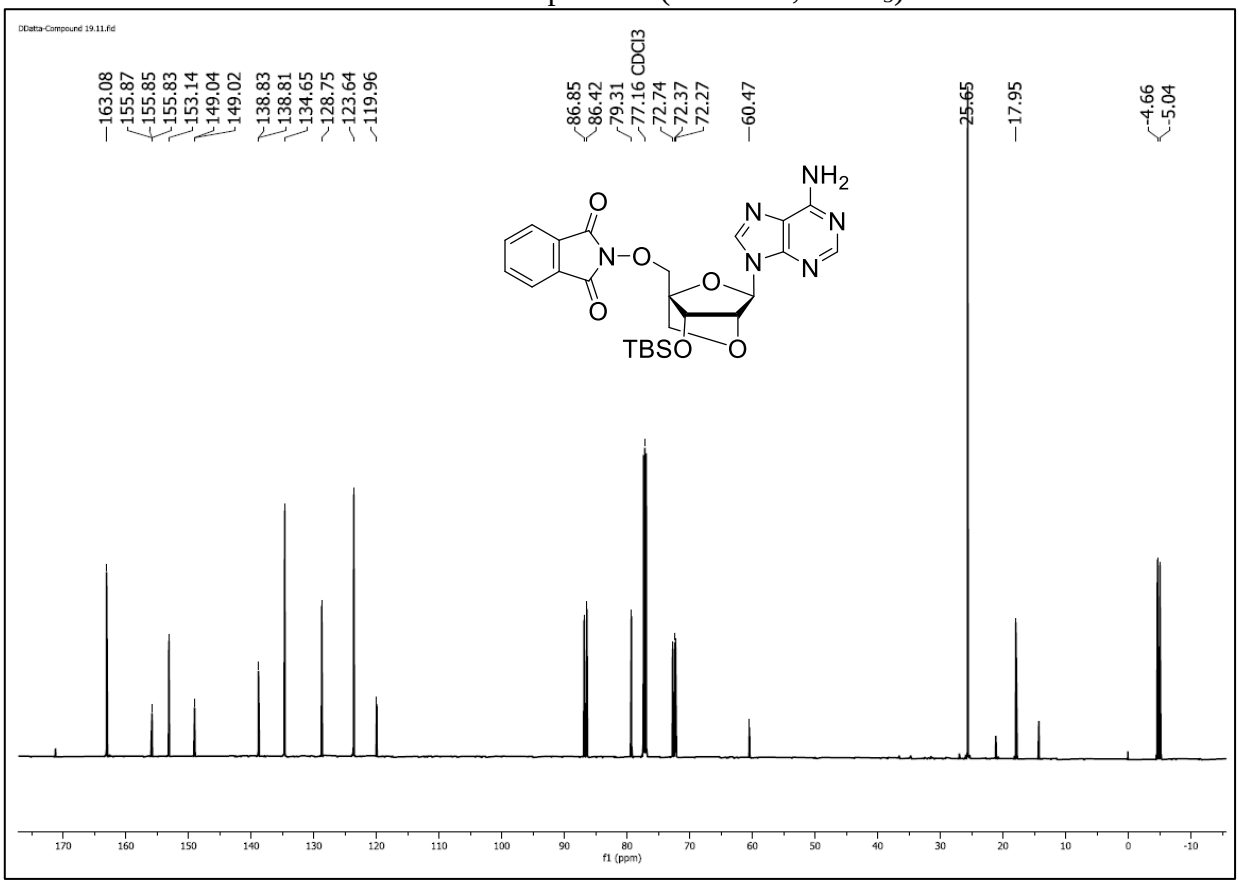
^{13}C NMR of compound **7c** (151 MHz, $CDCl_3$)



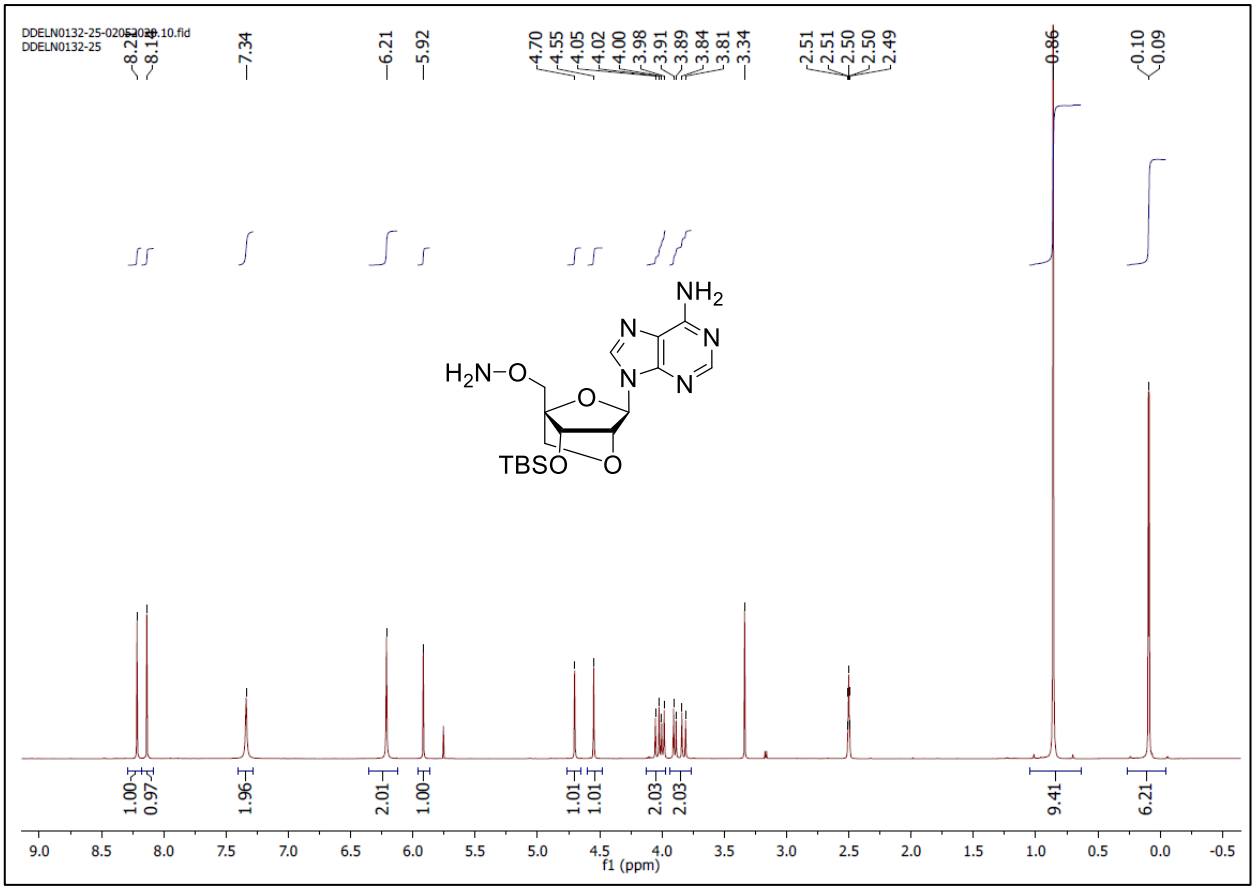
^{31}P NMR of compound **7c** (162 MHz, $CDCl_3$)



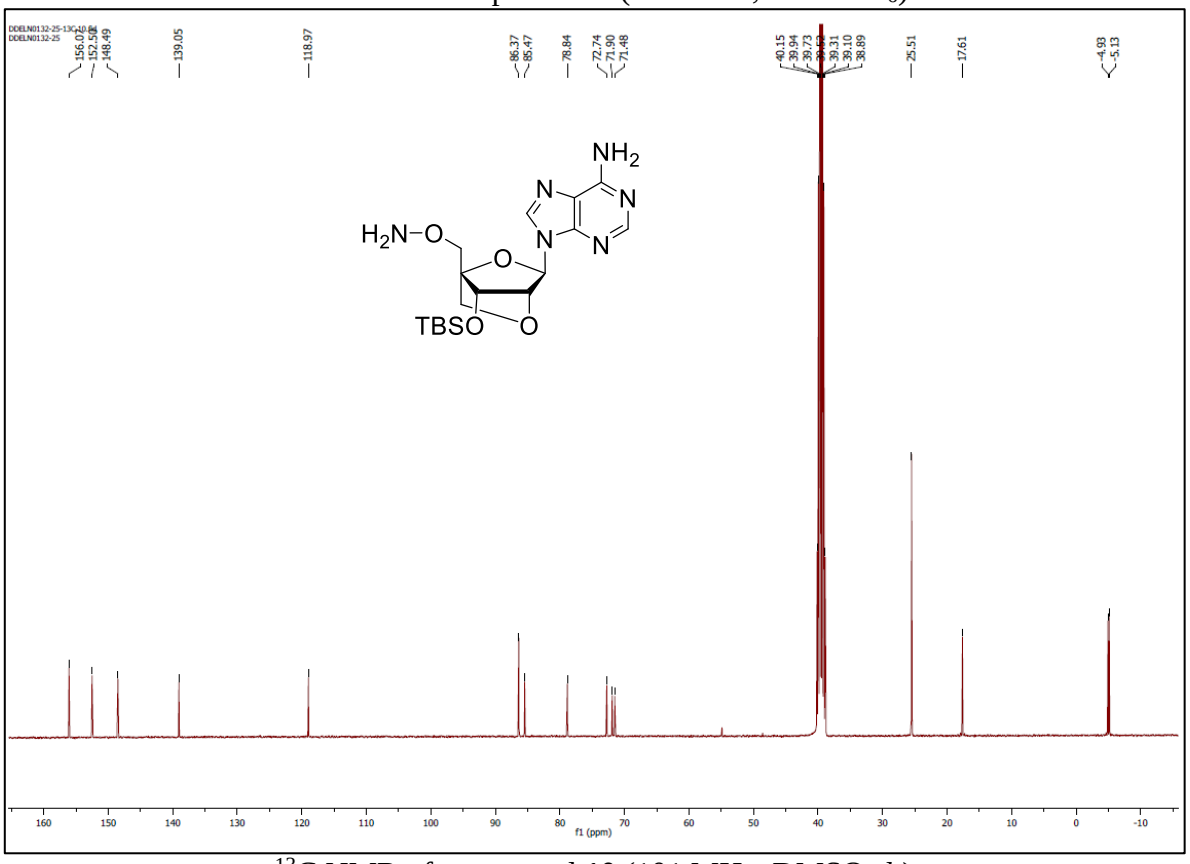
^{1}H NMR of compound **9** (600 MHz, $CDCl_3$)



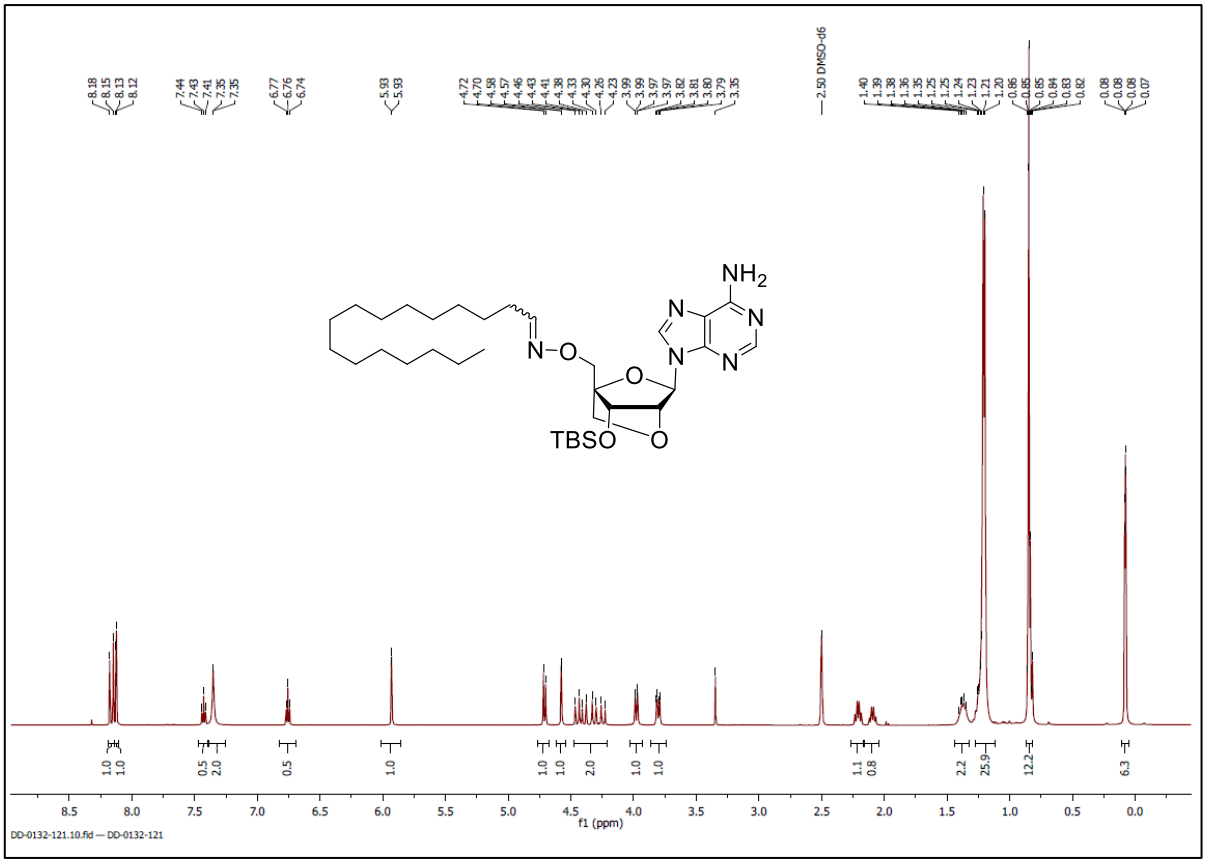
^{13}C NMR of compound **9** (151 MHz, $CDCl_3$)



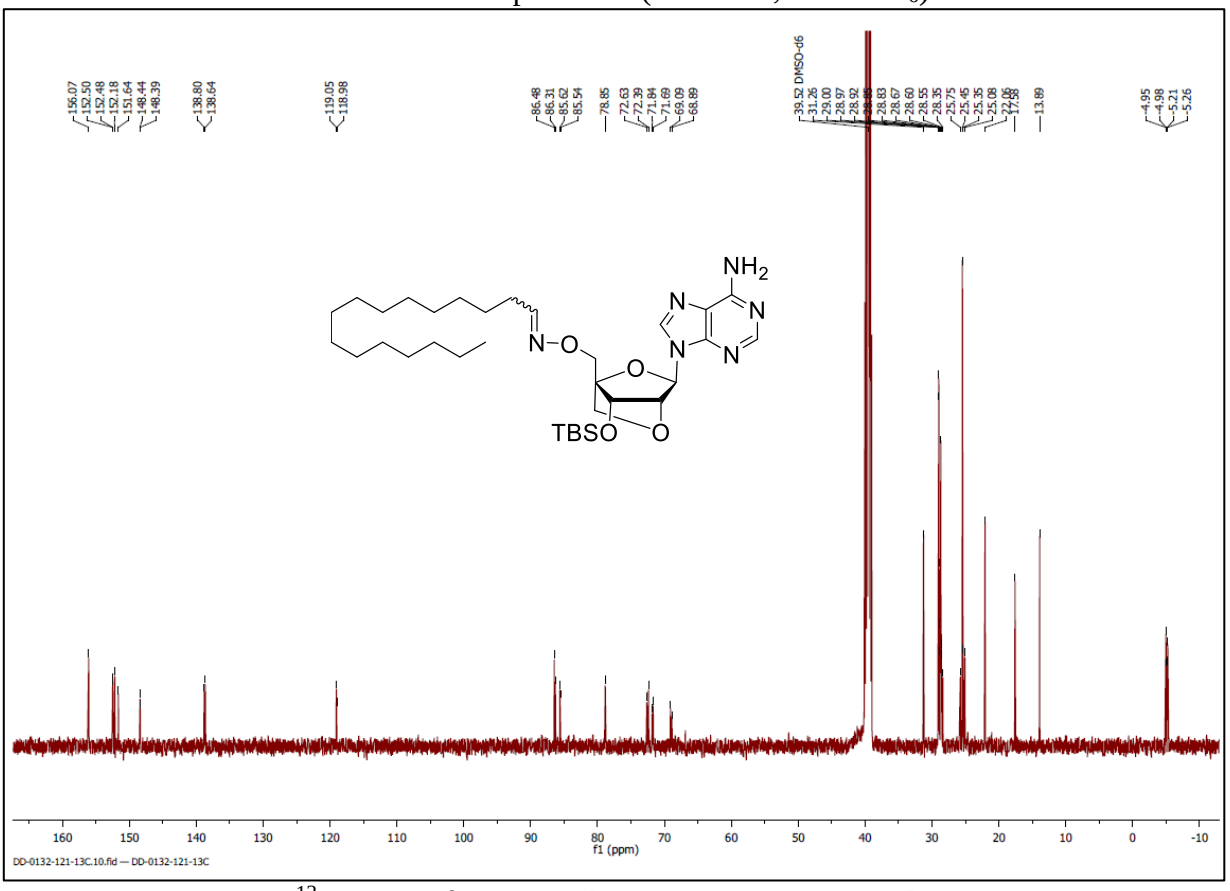
^{1}H NMR of compound **10** (400 MHz, DMSO-d_6)



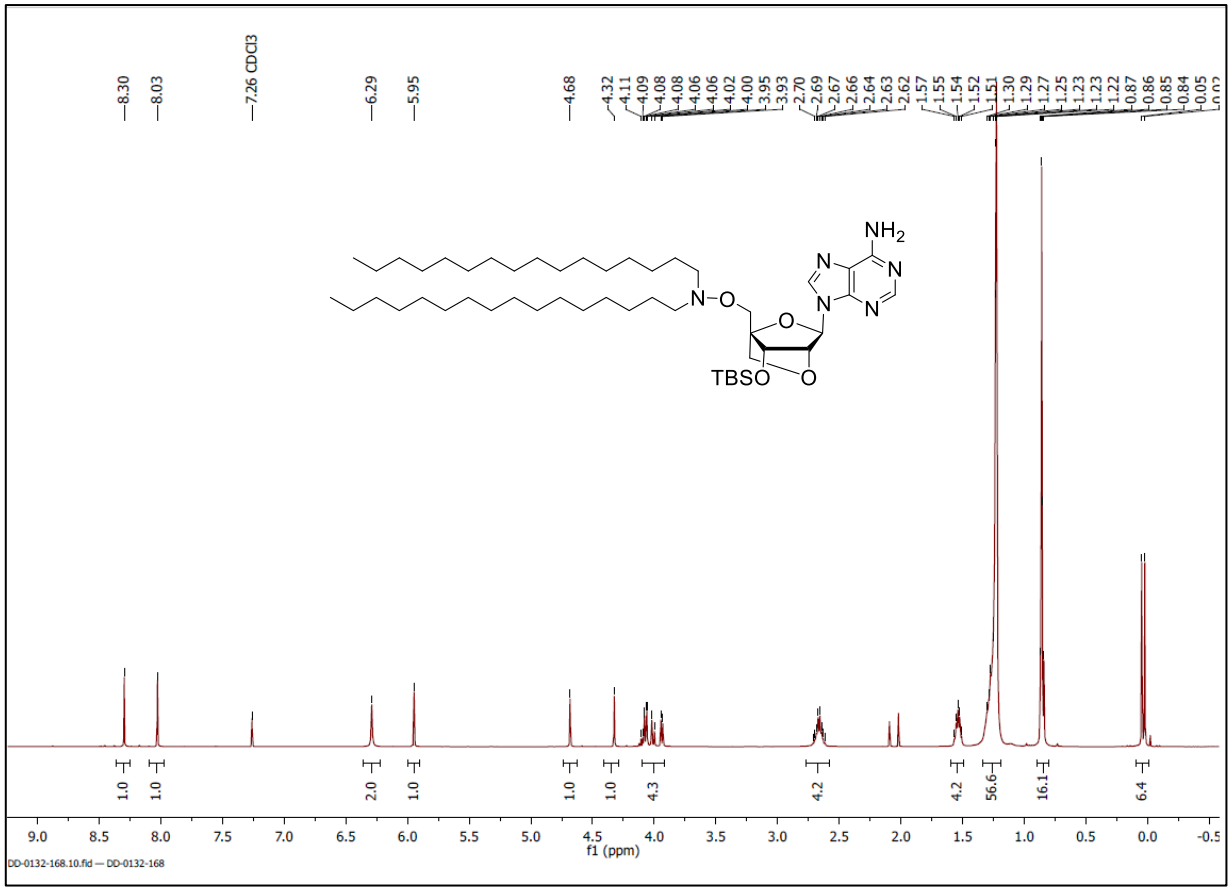
^{13}C NMR of compound **10** (101 MHz, DMSO-d_6)



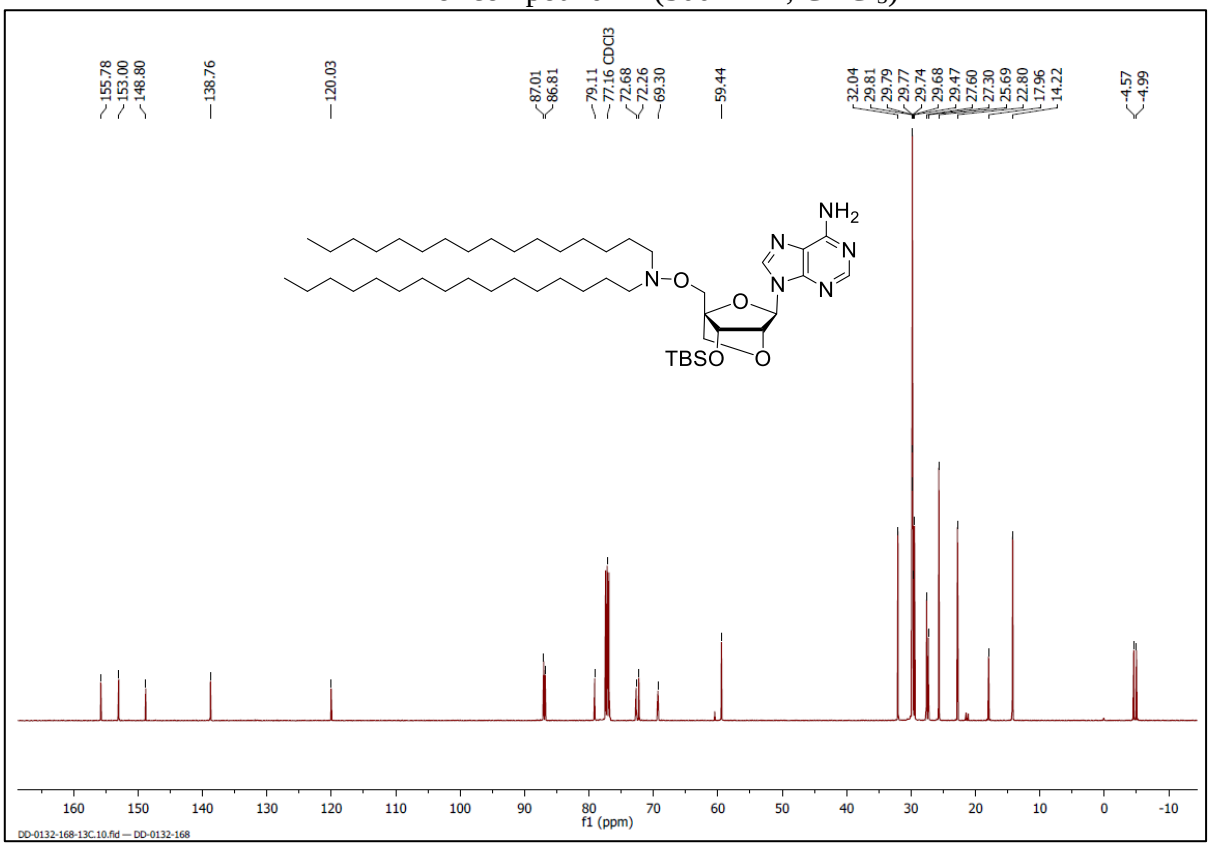
^{1}H NMR of compound **11** (400 MHz, DMSO-d_6)



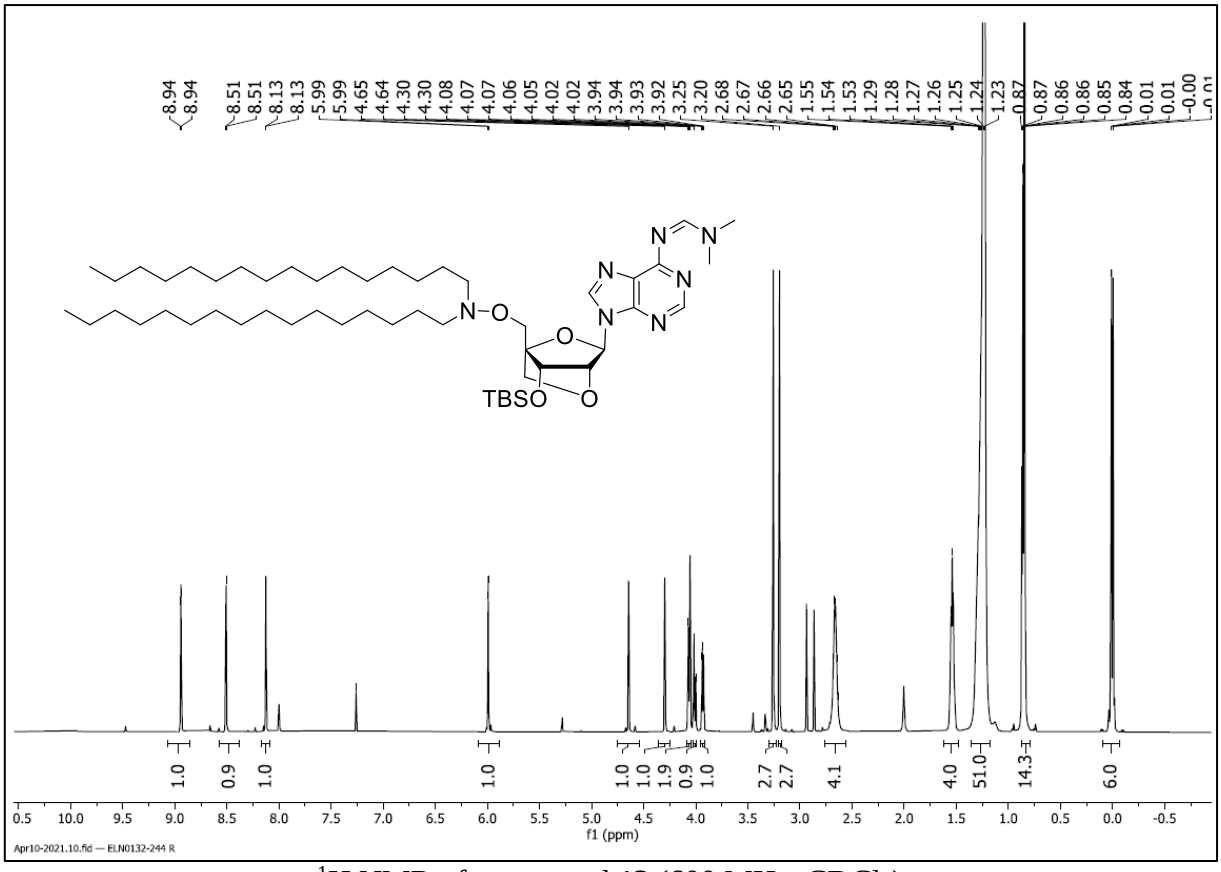
^{13}C NMR of compound **11** (126 MHz, DMSO-d_6)



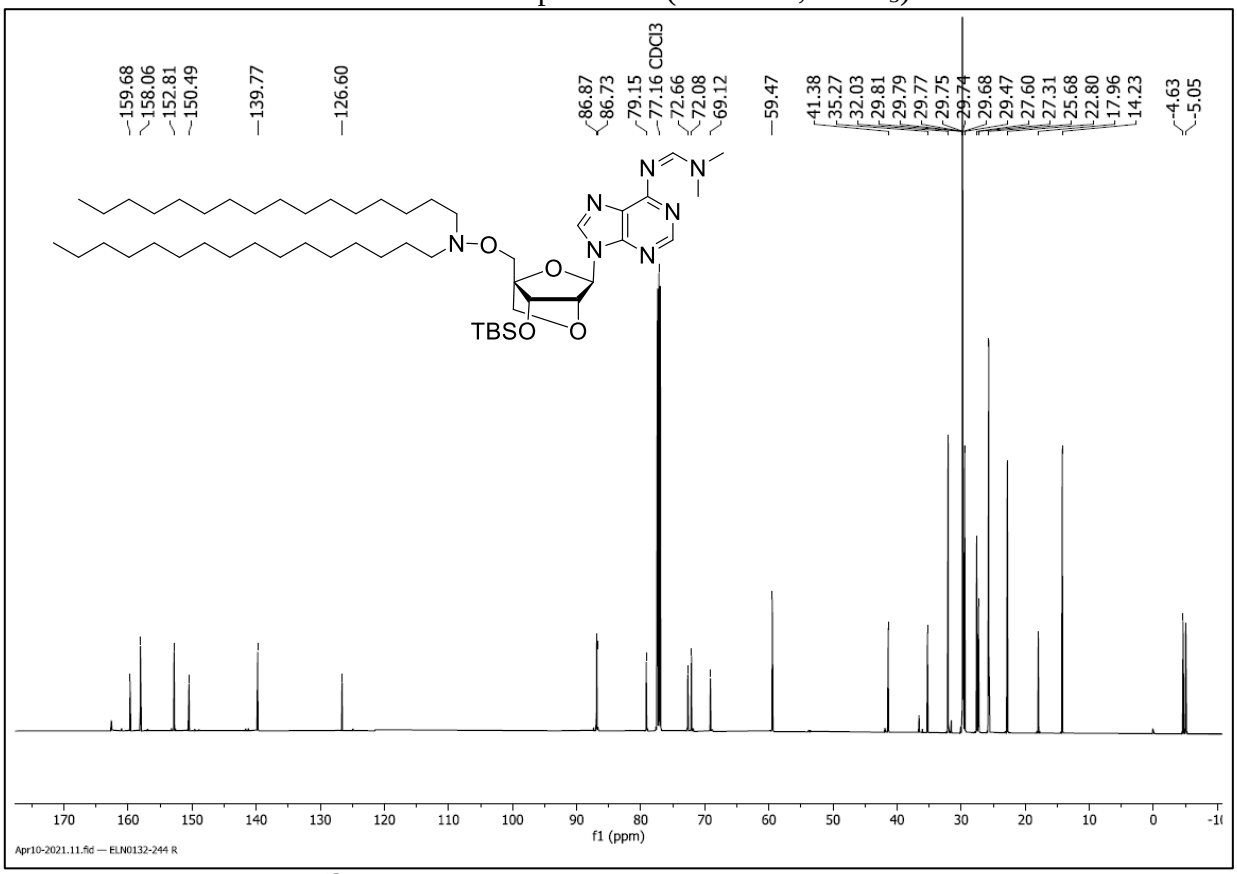
^{1}H NMR of compound **12** (500 MHz, $CDCl_3$)



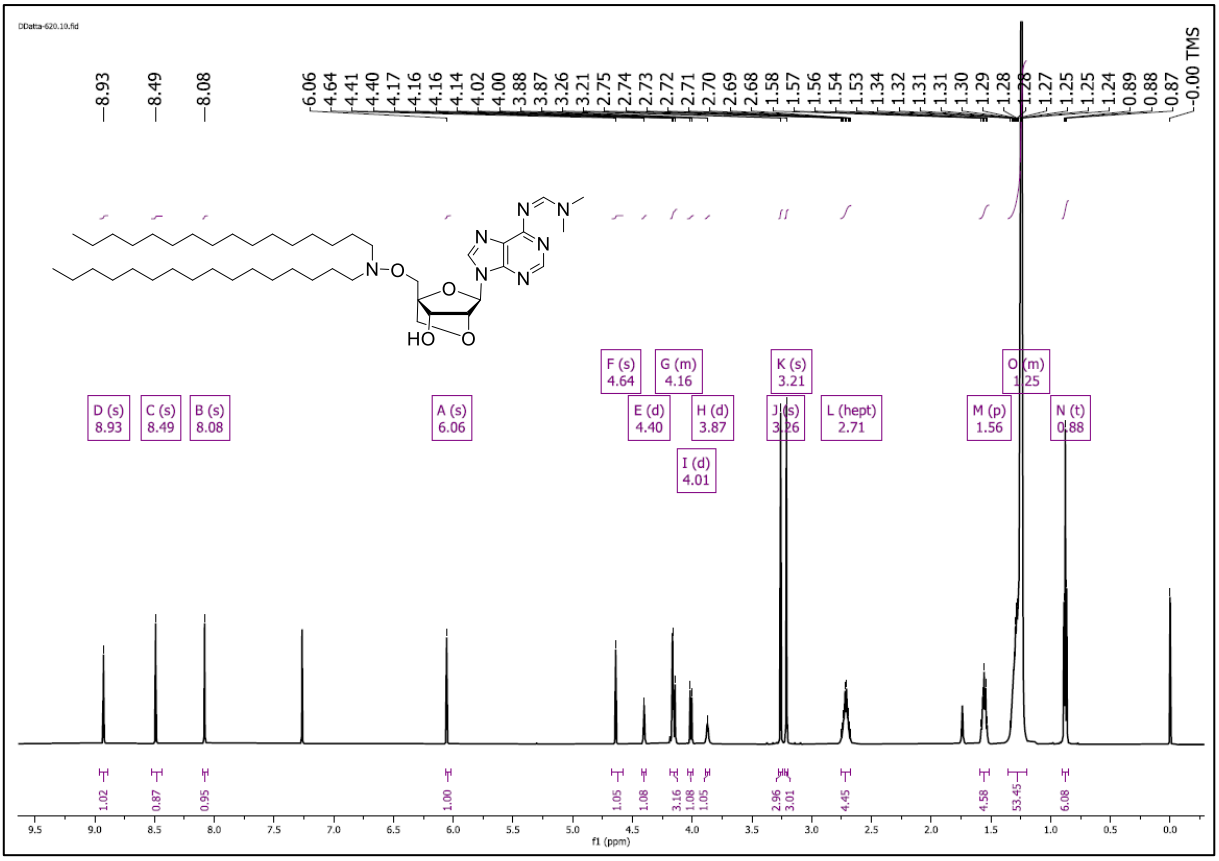
^{13}C NMR of compound **12** (126 MHz, $CDCl_3$)



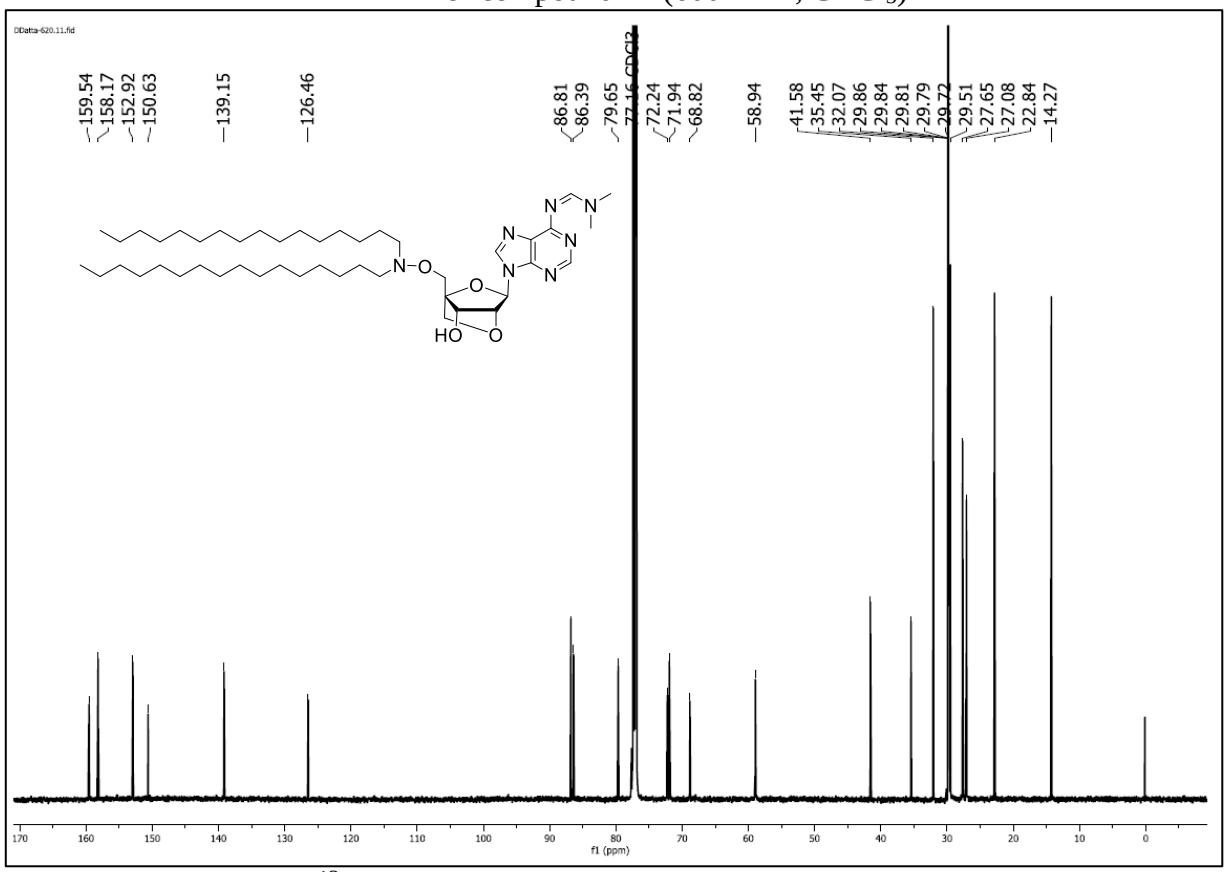
^{1}H NMR of compound **13** (600 MHz, $CDCl_3$)



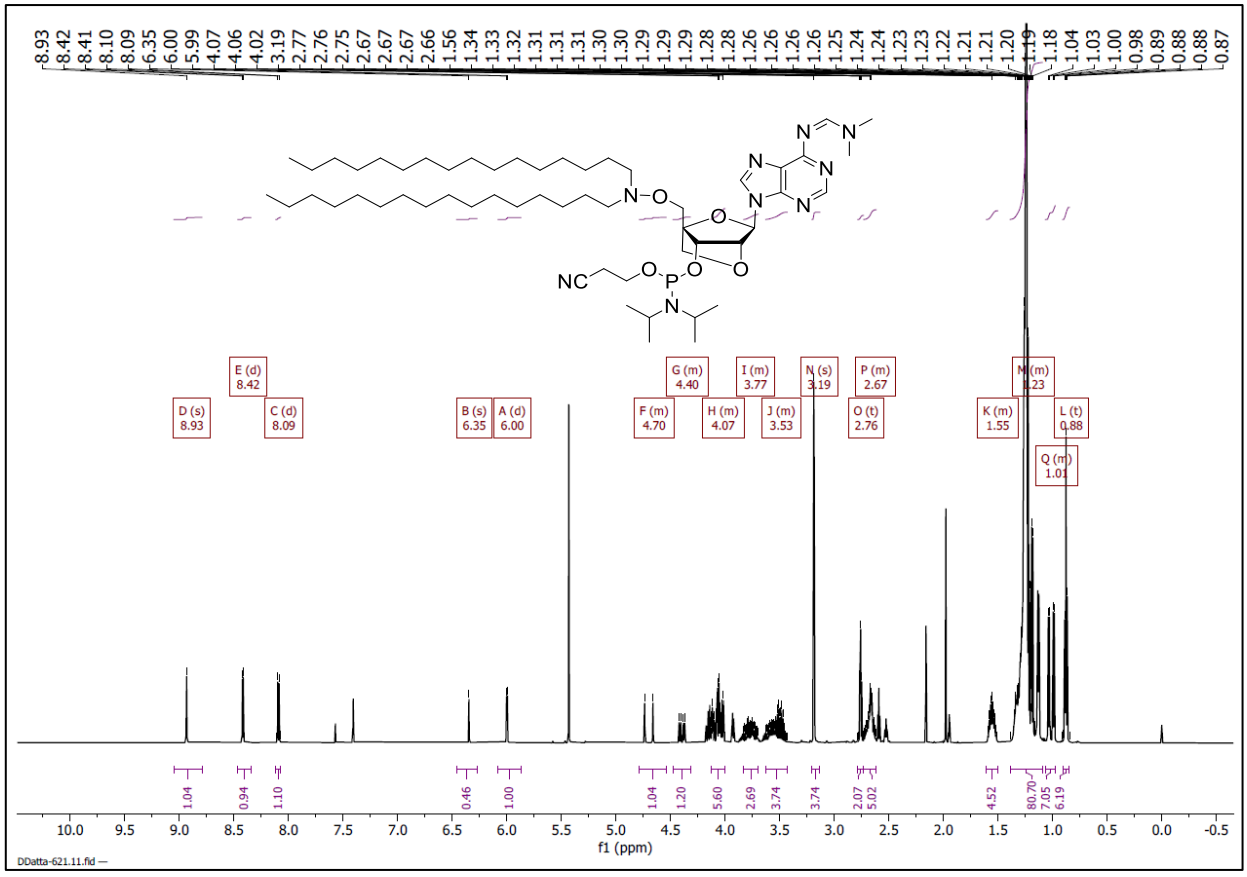
^{13}C NMR of compound **13** (151 MHz, $CDCl_3$)



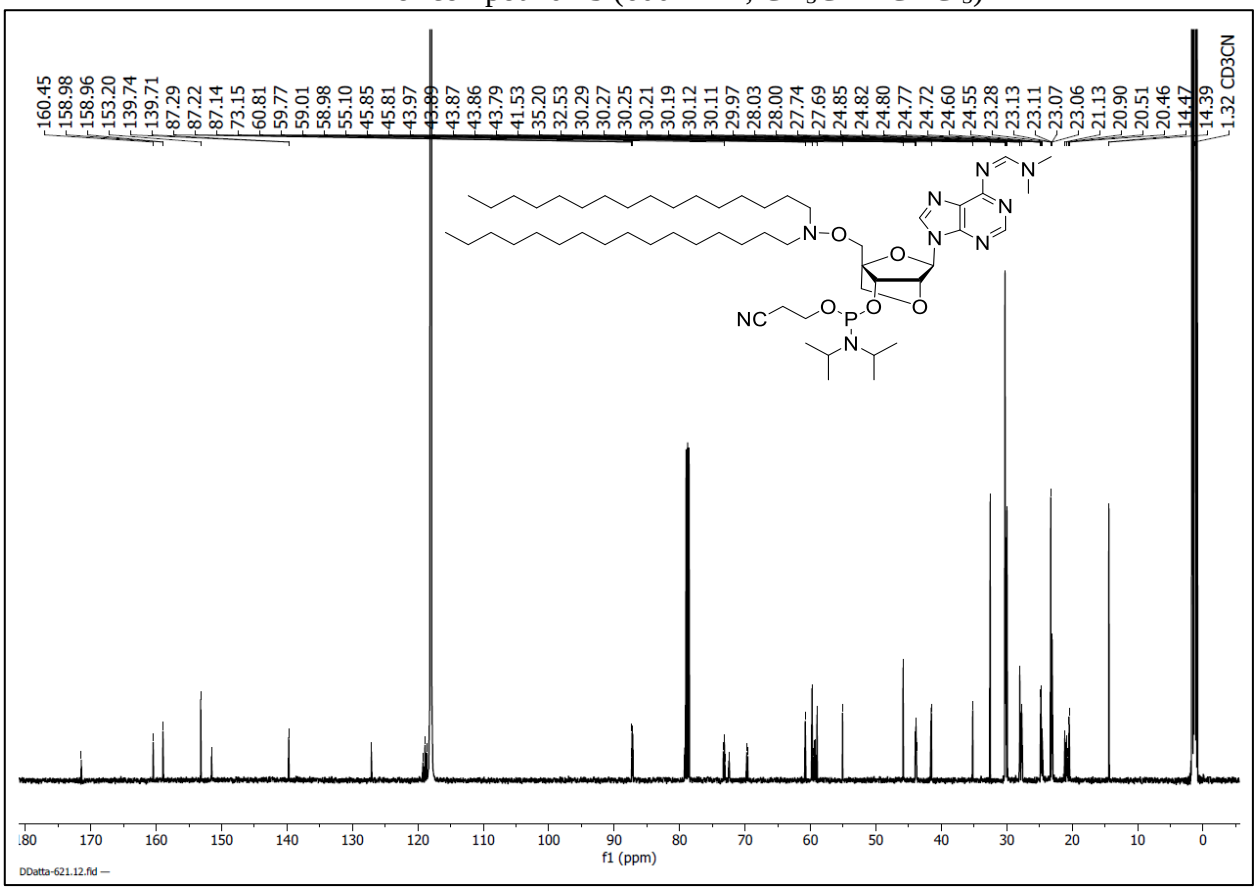
^{1}H NMR of compound **14** (600 MHz, $CDCl_3$)



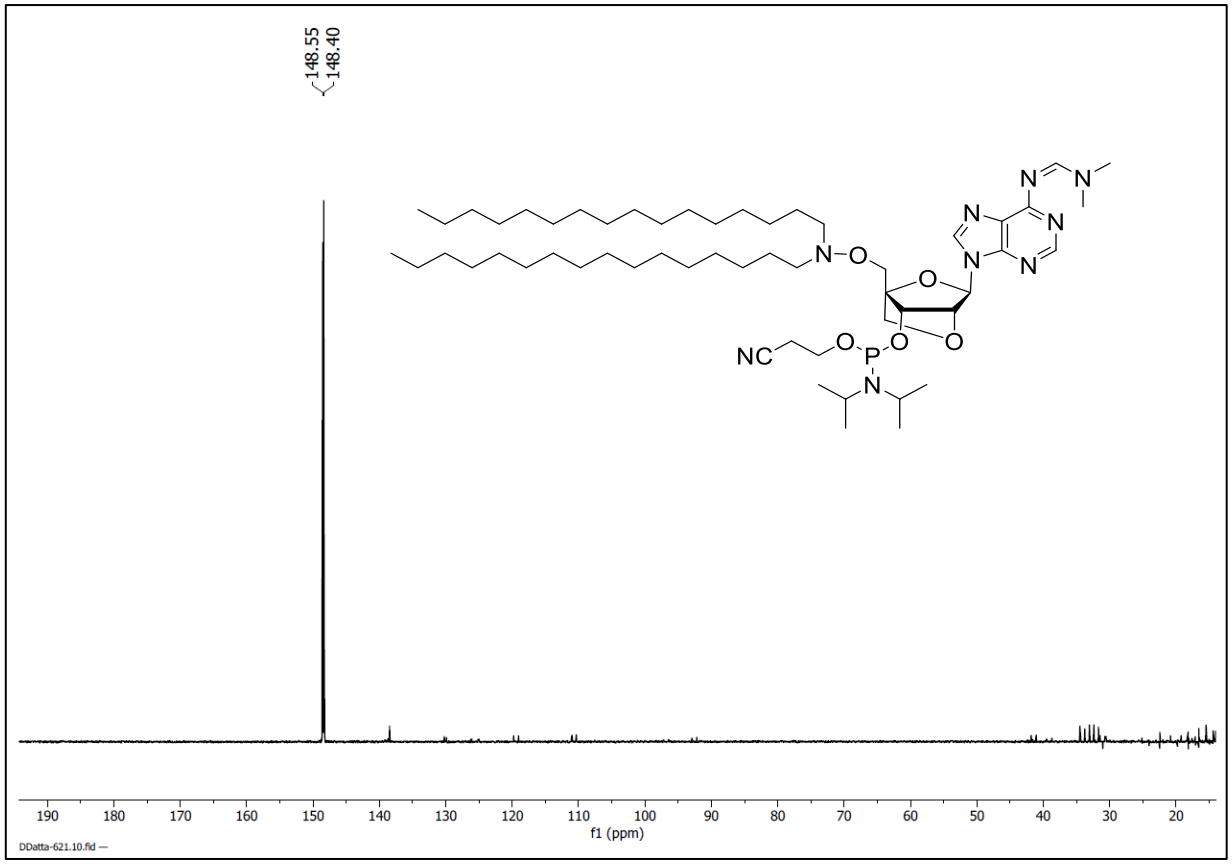
^{13}C NMR of compound **14** (151 MHz, $CDCl_3$)



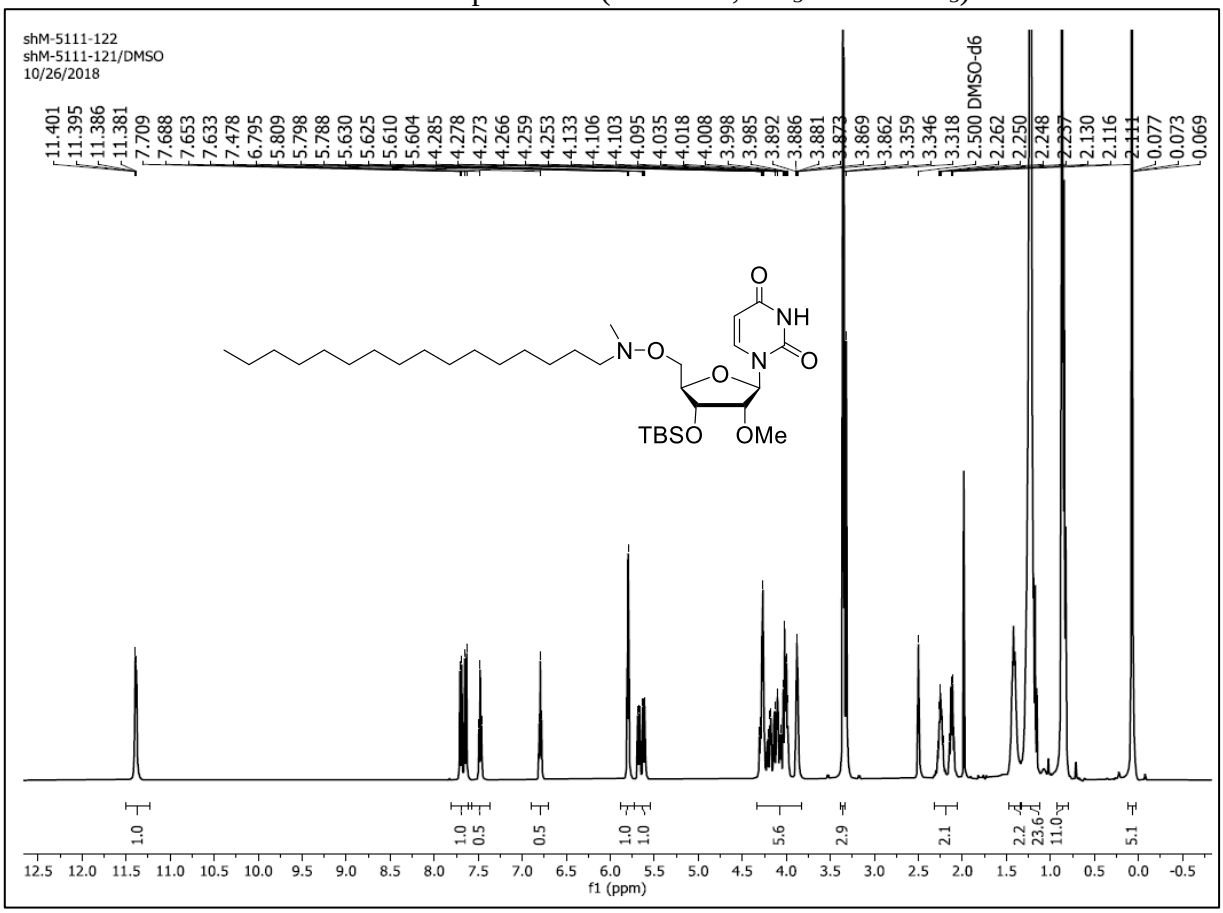
^{1}H NMR of compound **15** (600 MHz, CD_3CN+ $CDCl_3$)



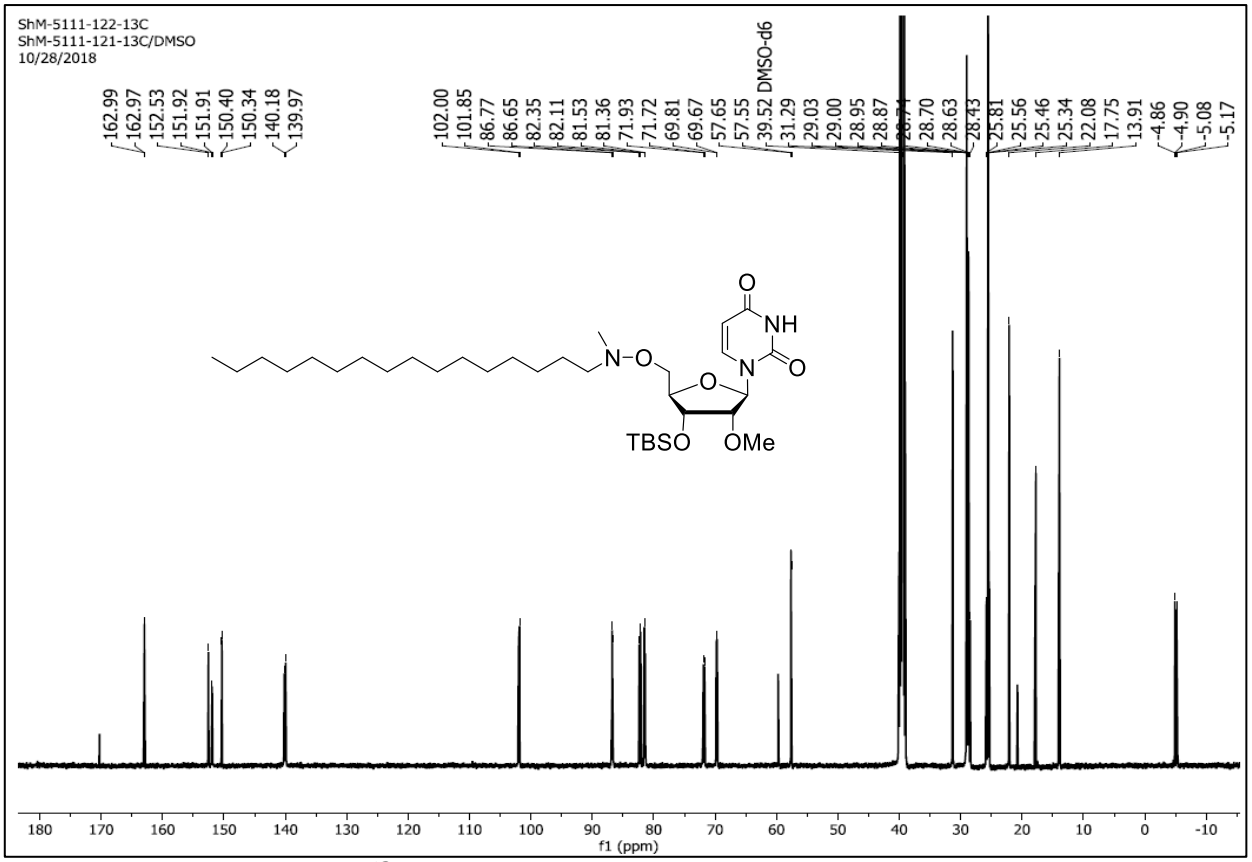
^{13}C NMR of compound **15** (151 MHz, CD_3CN+ $CDCl_3$)



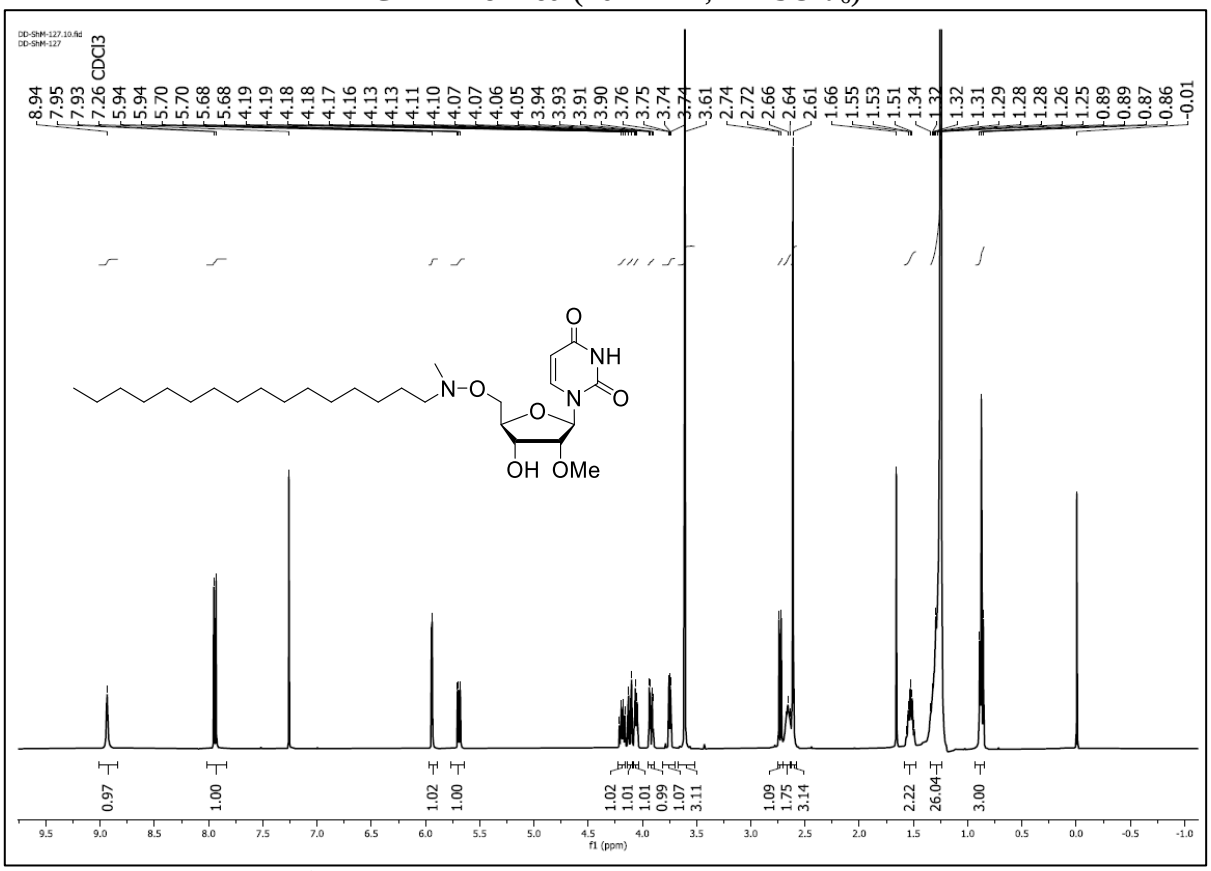
^{31}P NMR of compound **15** (243 MHz, CD_3CN+ $CDCl_3$)



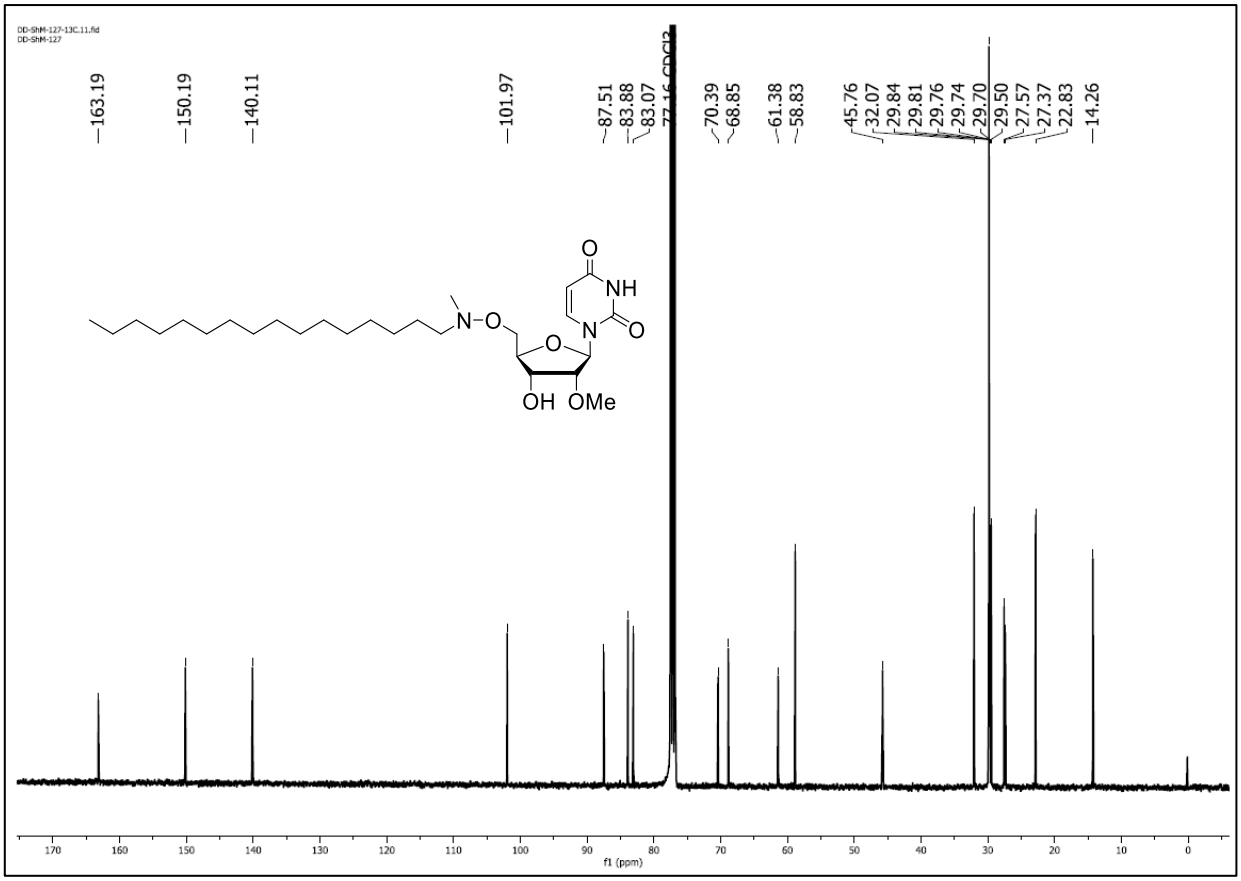
^{1}H NMR of **16a** (400 MHz, DMSO-d_6)



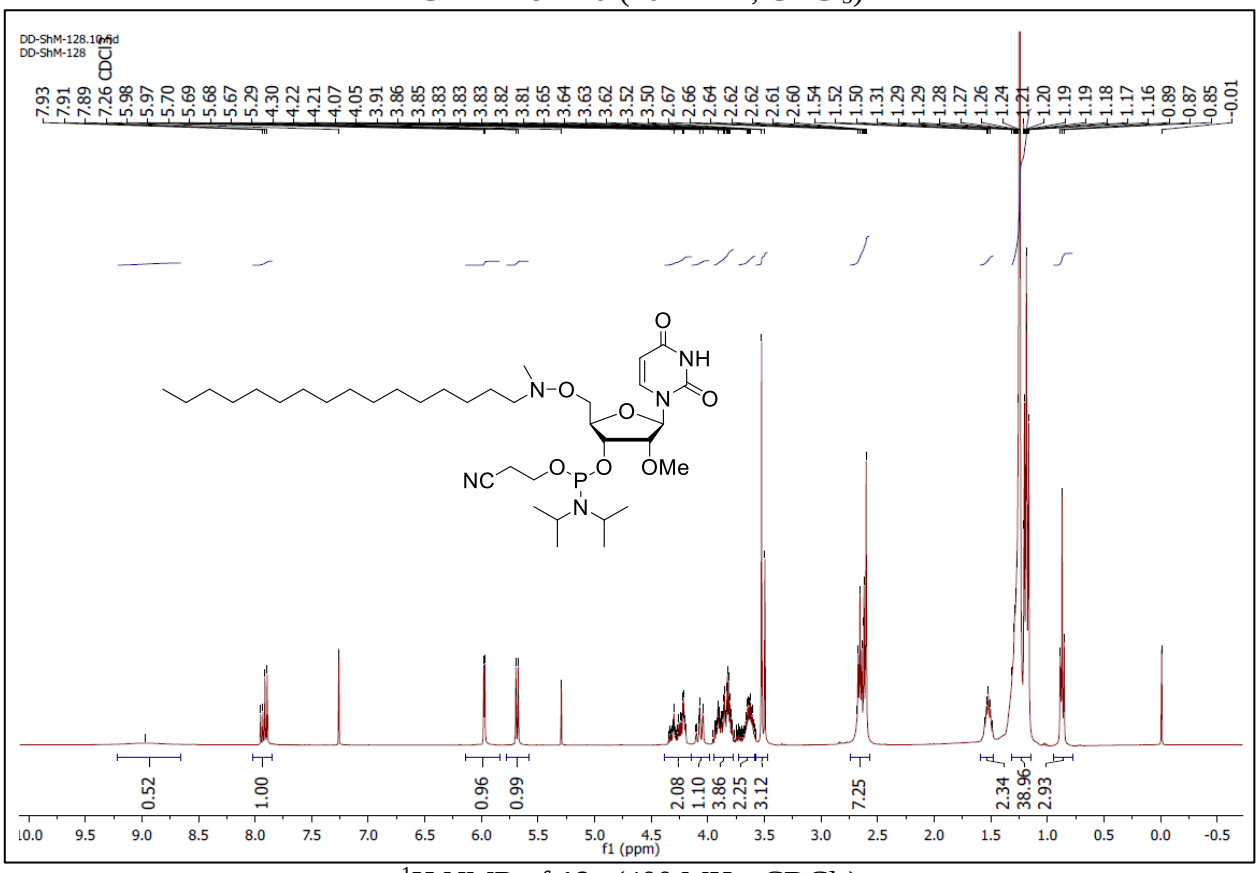
^{13}C NMR of **16a** (101 MHz, DMSO-d_6)



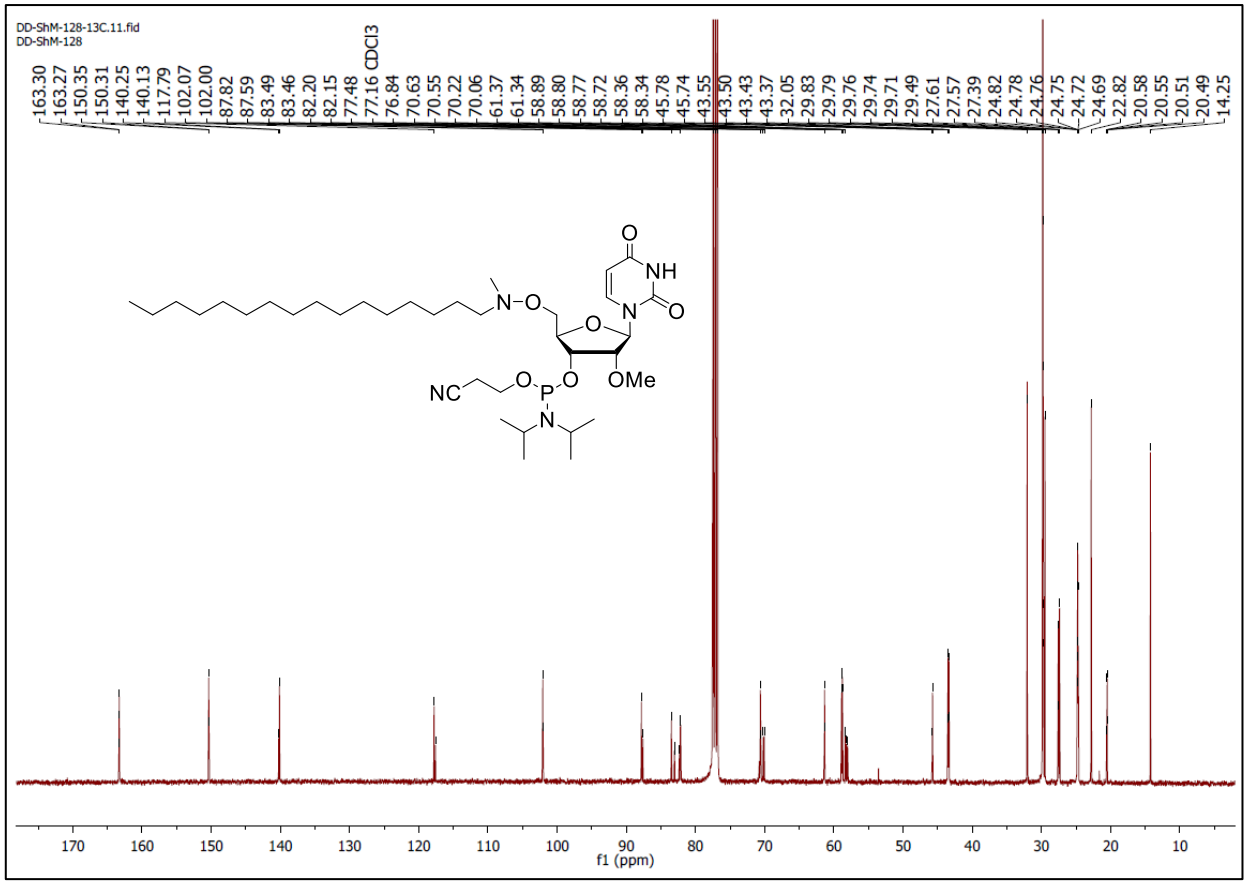
^{1}H NMR of compound **17a** (400 MHz, $CDCl_3$)



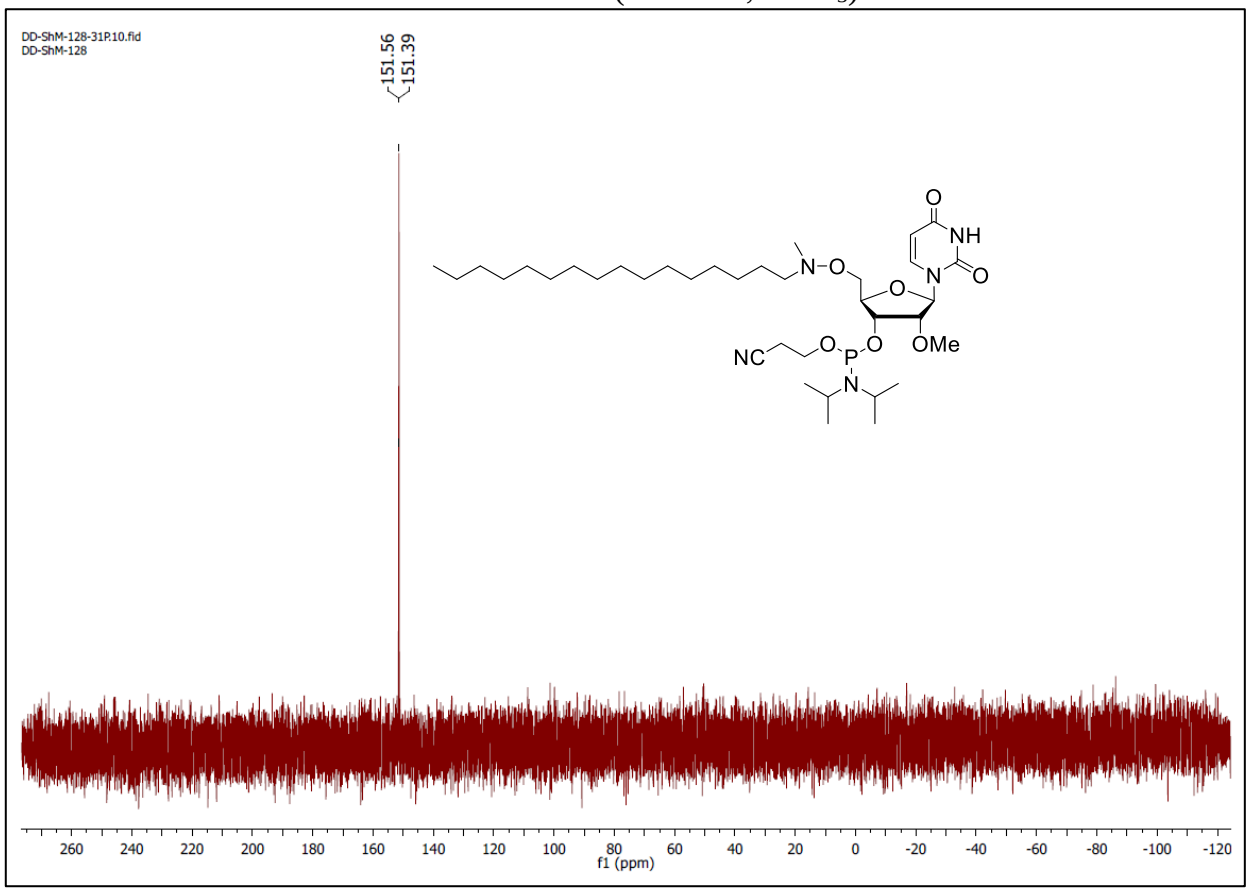
^{13}C NMR of **17a** (101 MHz, $CDCl_3$)



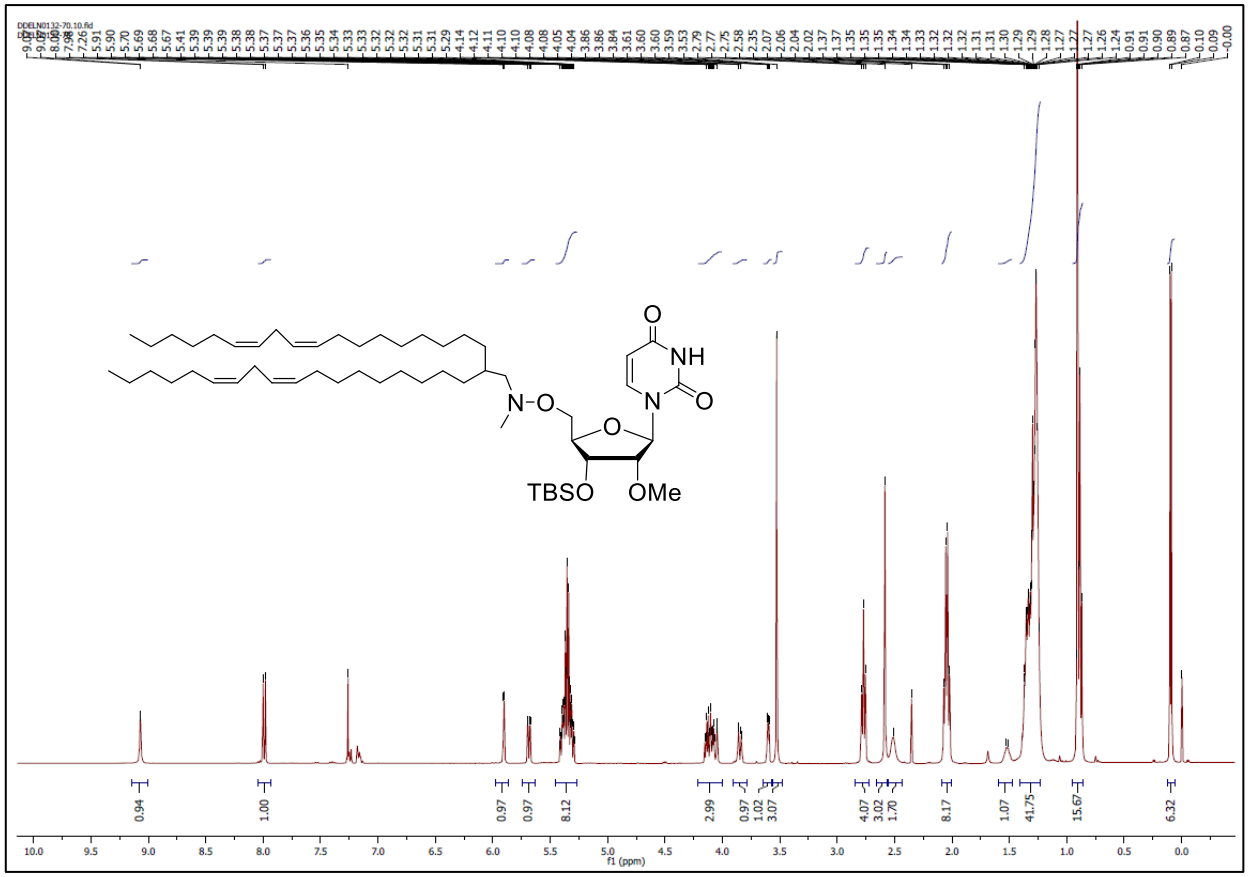
^{1}H NMR of **18a** (400 MHz, $CDCl_3$)



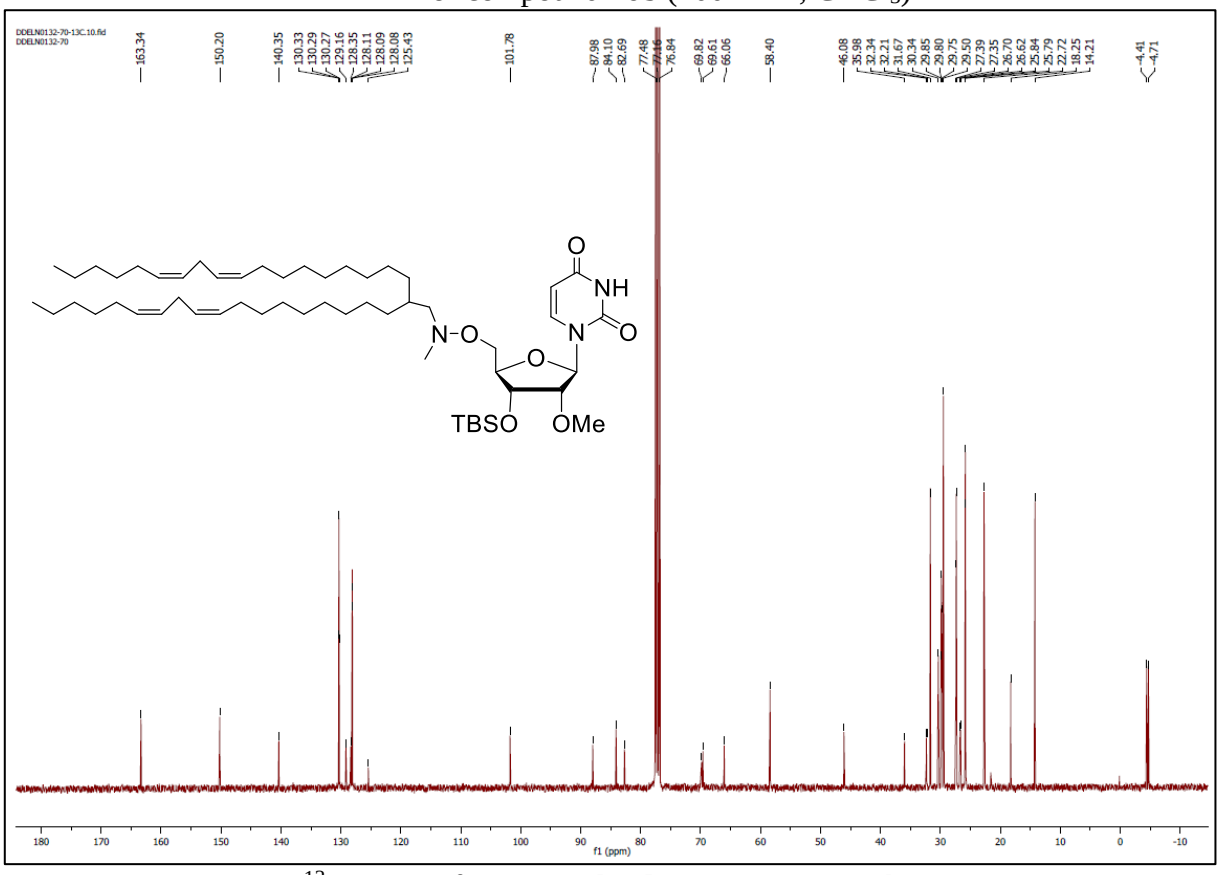
^{13}C NMR of **18a** (101 MHz, $CDCl_3$)



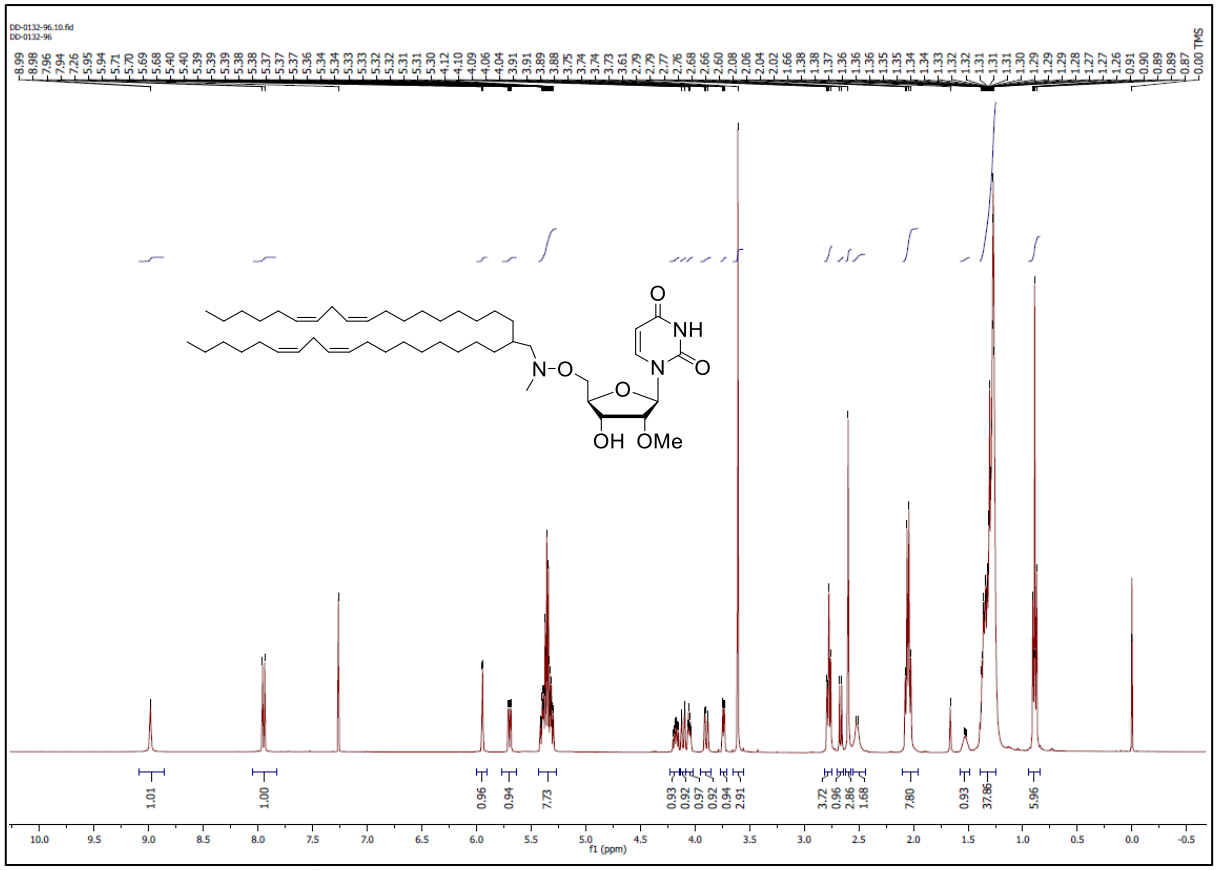
^{31}P NMR of **18a** (162 MHz, $CDCl_3$)



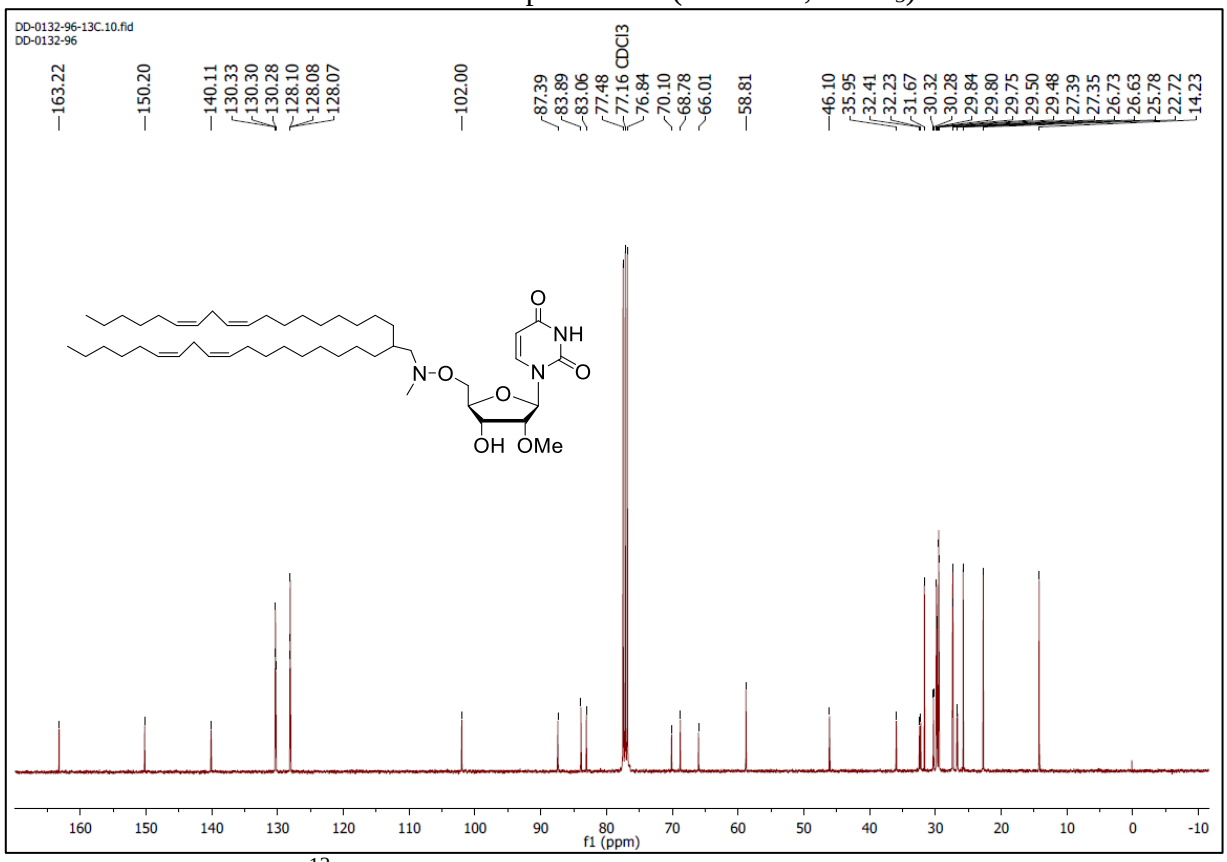
^{1}H NMR of compound **16b** (400 MHz, $CDCl_3$)



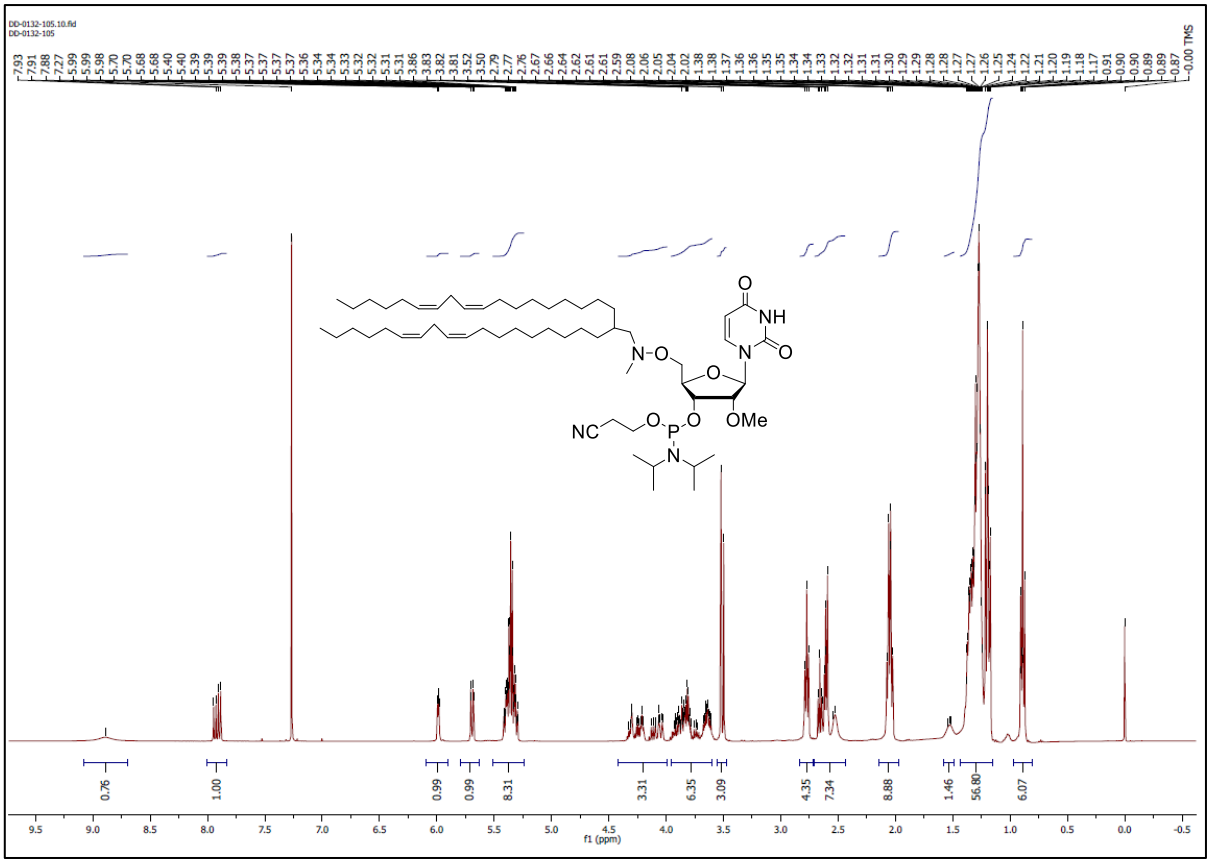
^{13}C NMR of compound **16b** (101 MHz, $CDCl_3$)



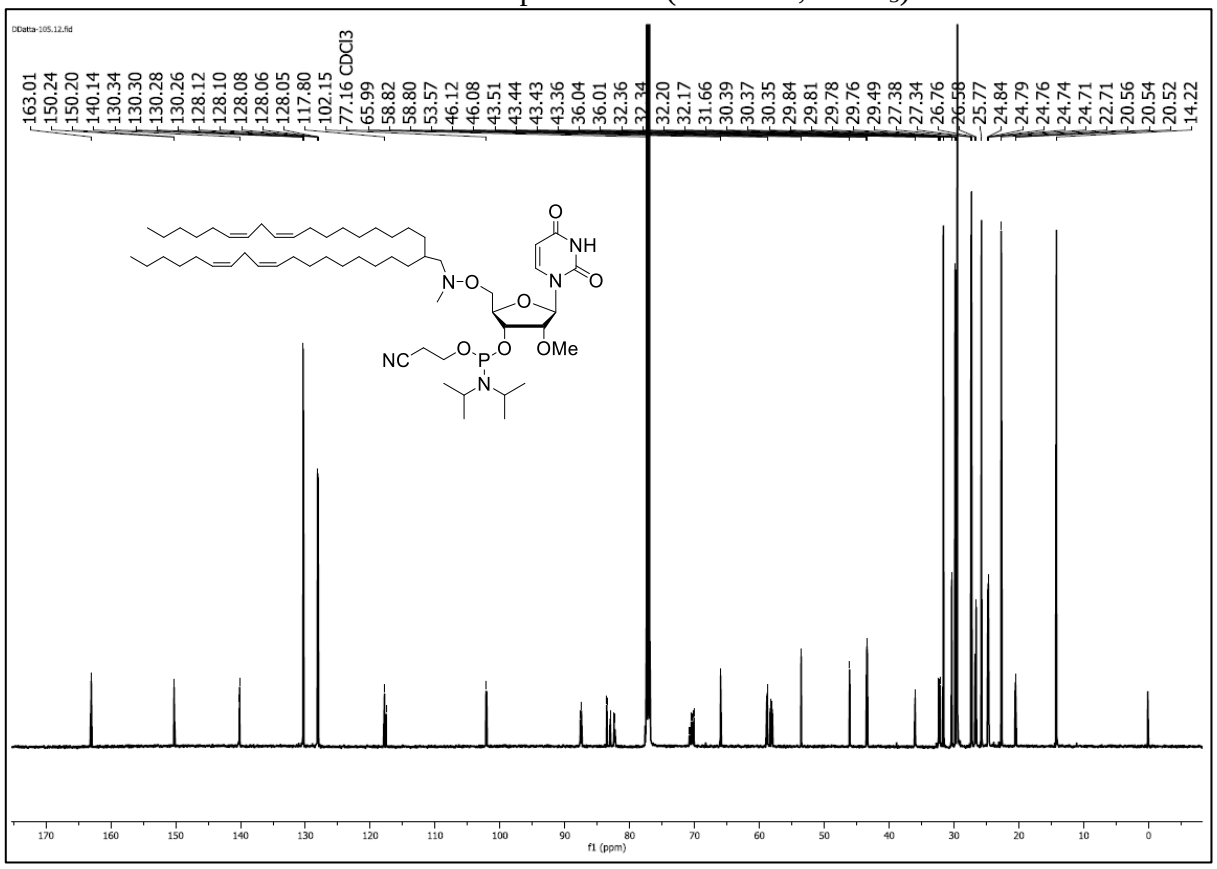
^{1}H NMR of compound **17b** (400 MHz, $CDCl_3$)



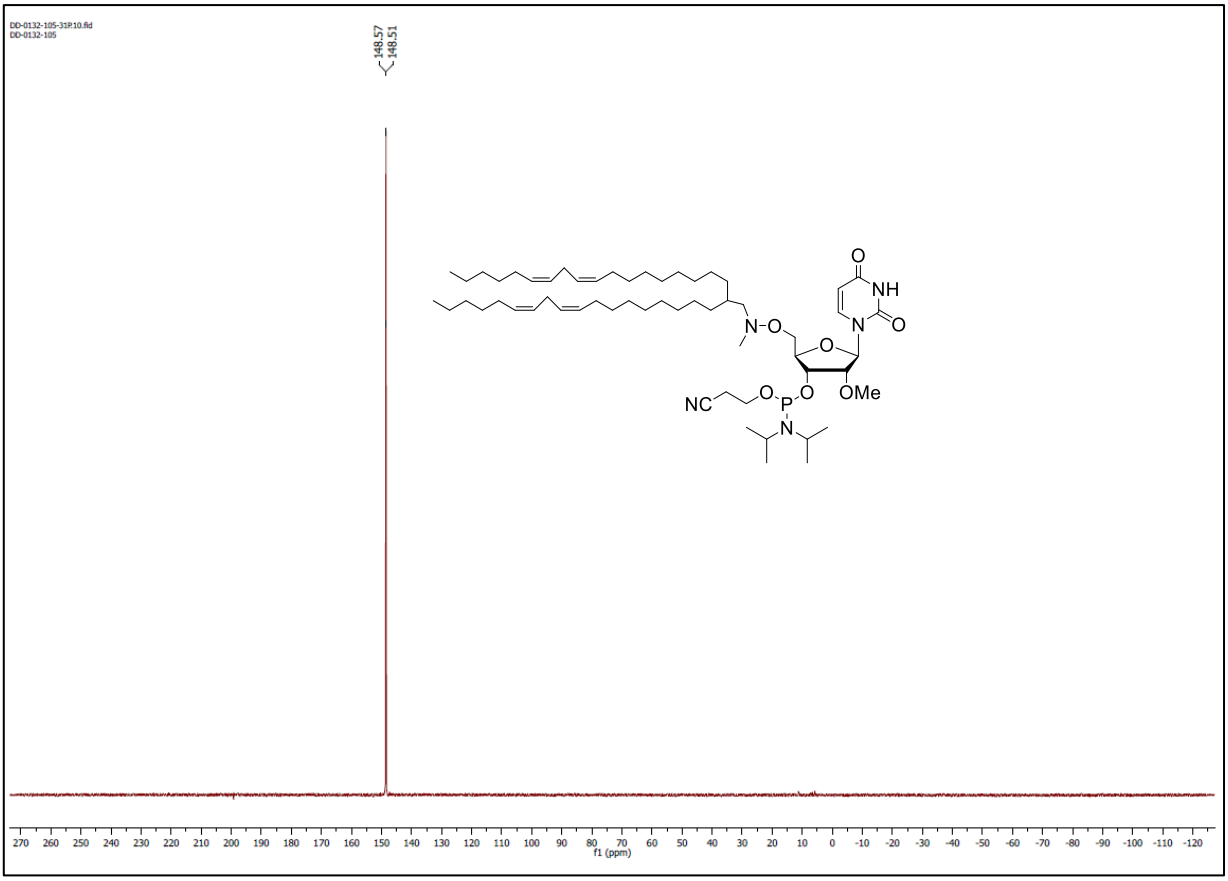
^{13}C NMR of compound **17b** (101 MHz, $CDCl_3$)



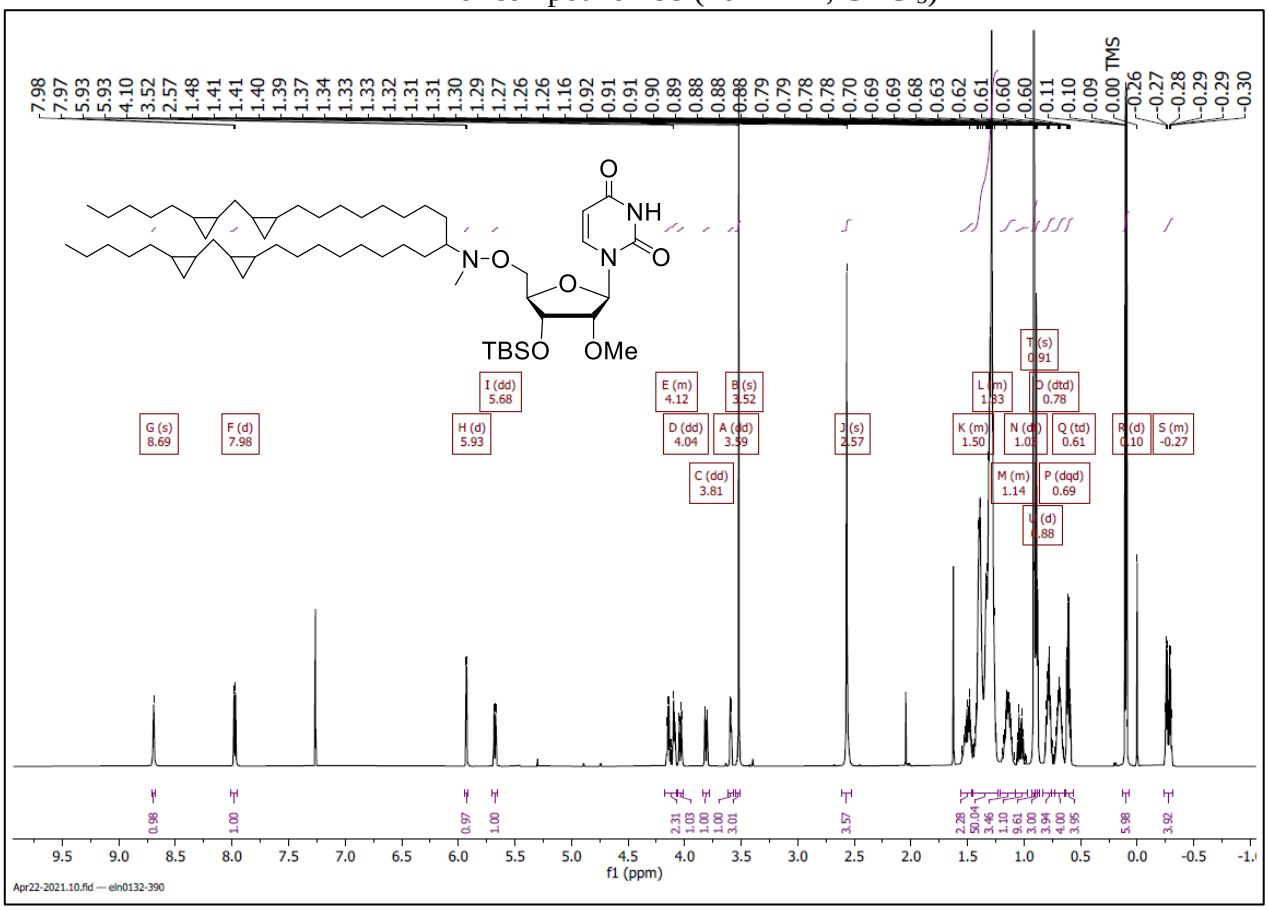
^{1}H NMR of compound **18b** (400 MHz, $CDCl_3$)



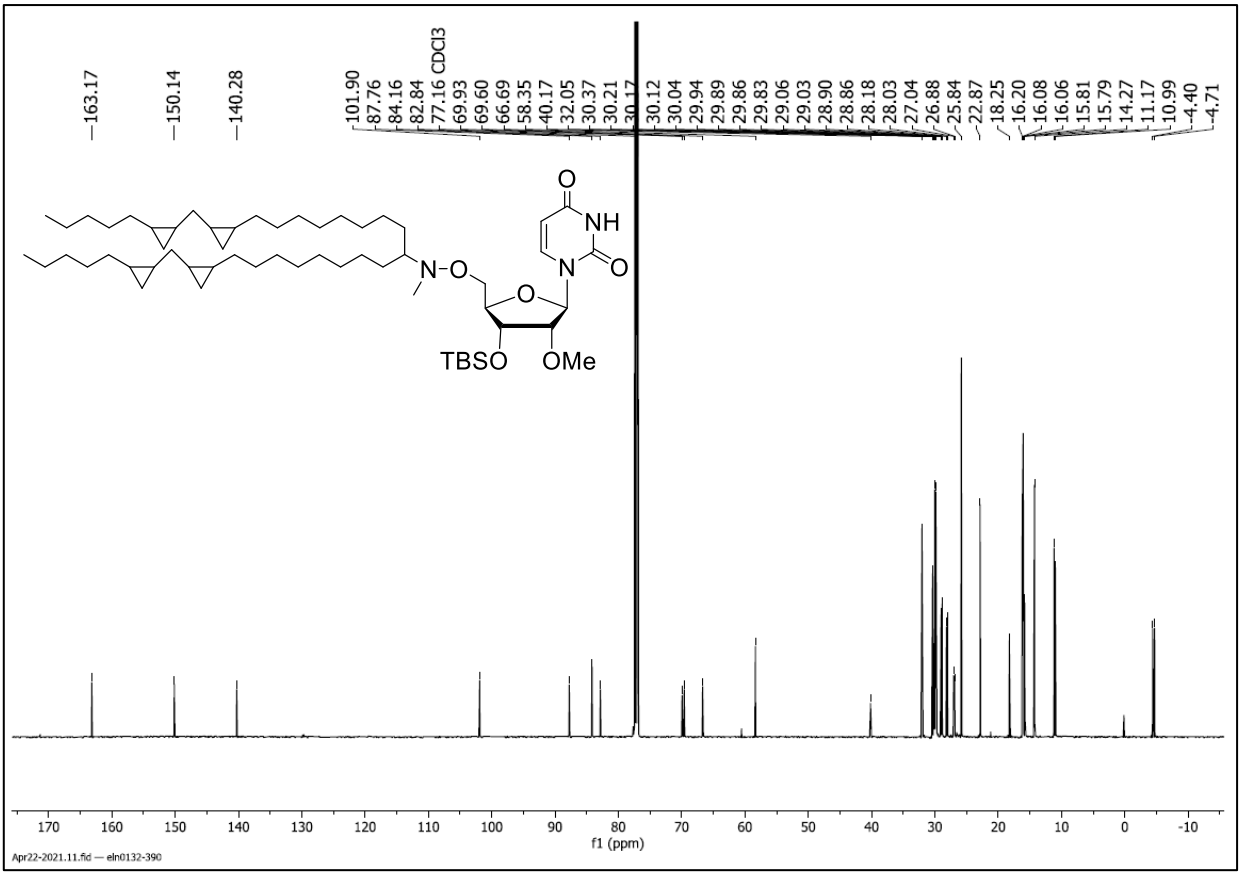
^{13}C NMR of compound **18b** (151 MHz, $CDCl_3$)



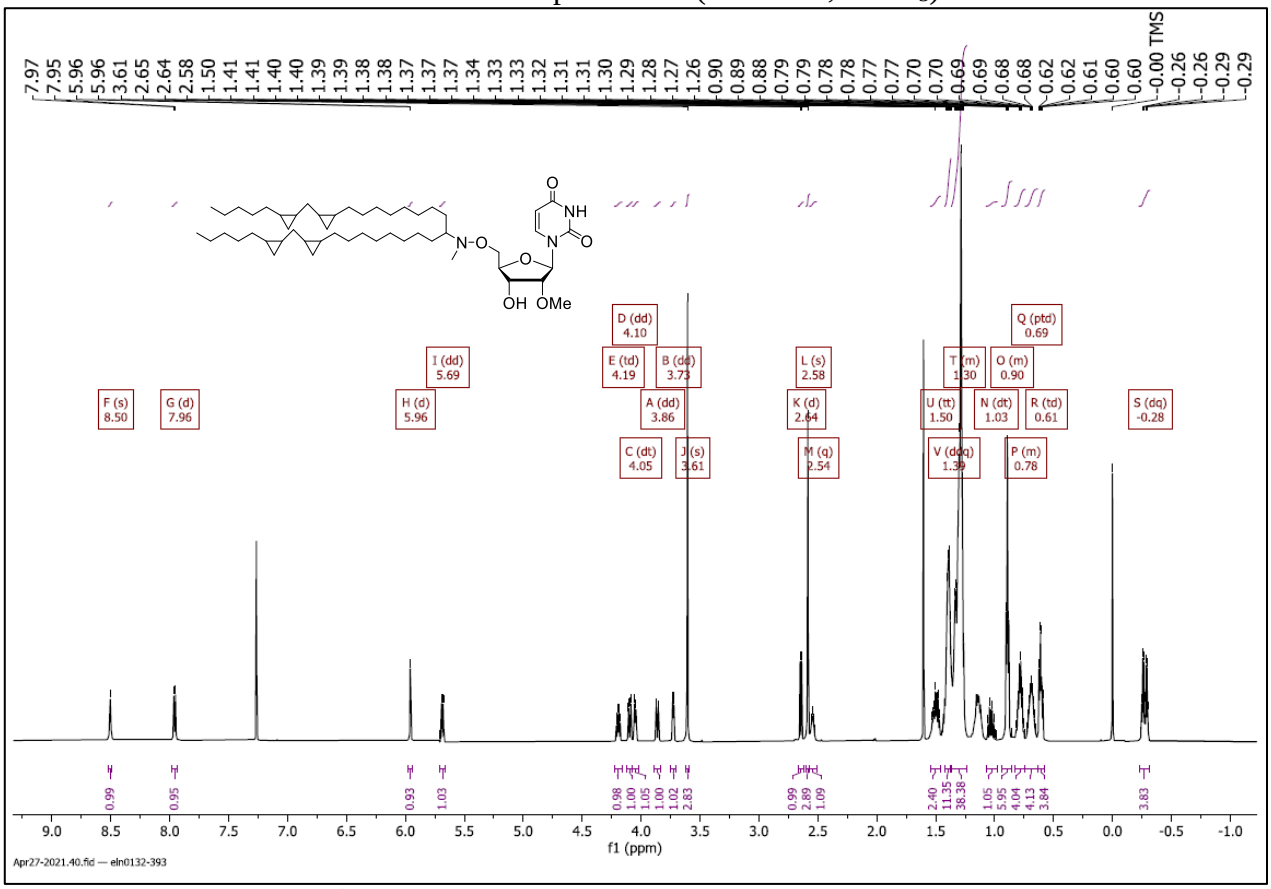
^{31}P NMR of compound **18b** (162 MHz, $CDCl_3$)



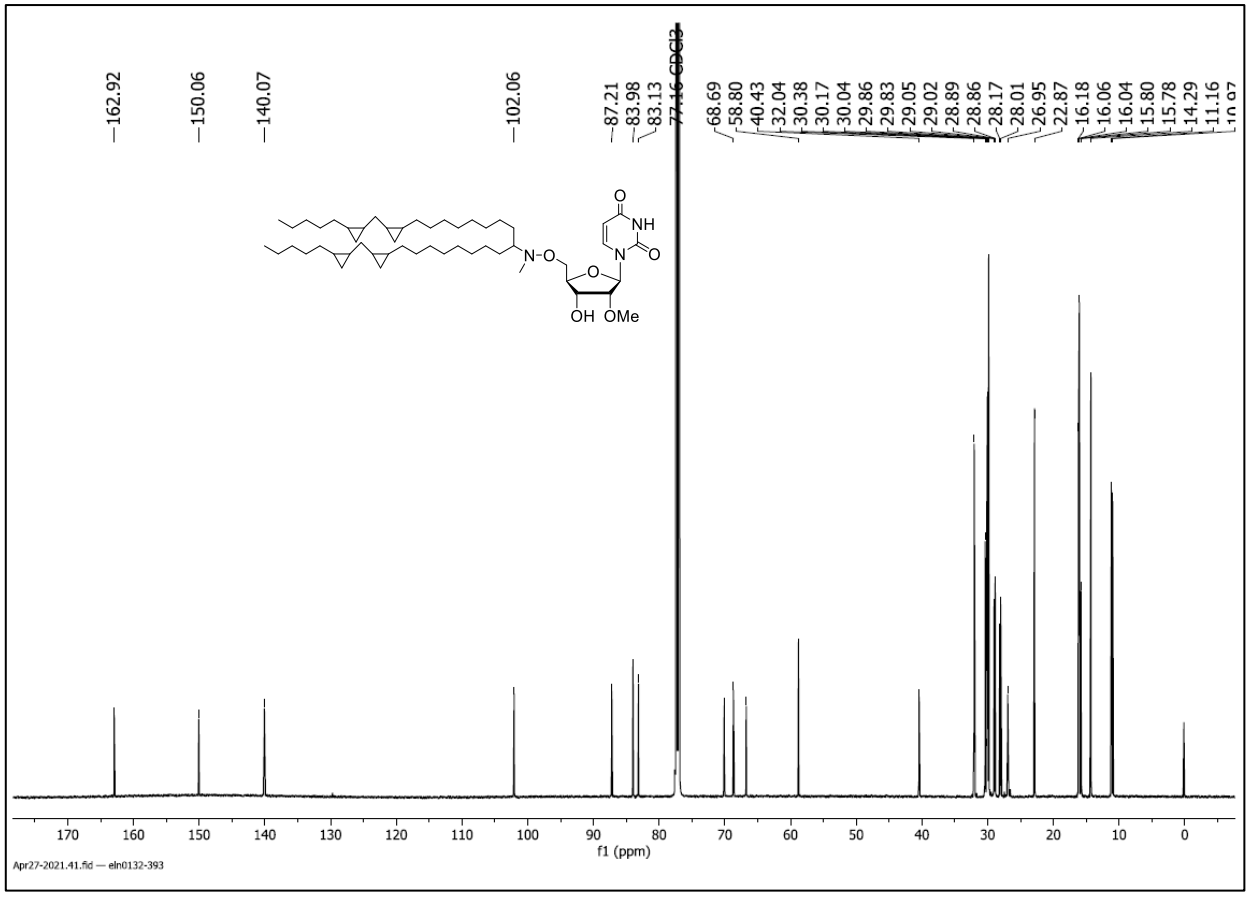
^{1}H NMR of compound **16c** (600 MHz, $CDCl_3$)



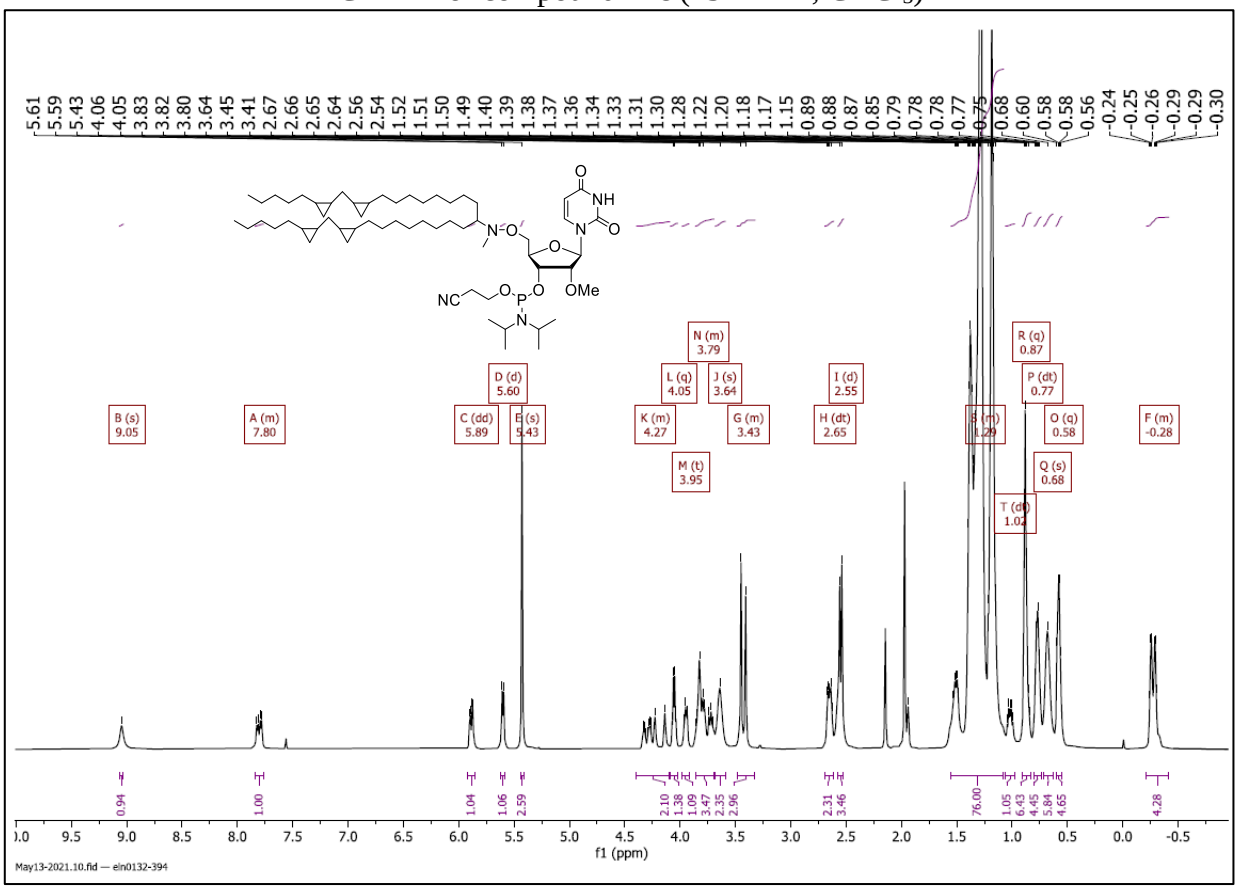
^{13}C NMR of compound **16c** (151 MHz, $CDCl_3$)



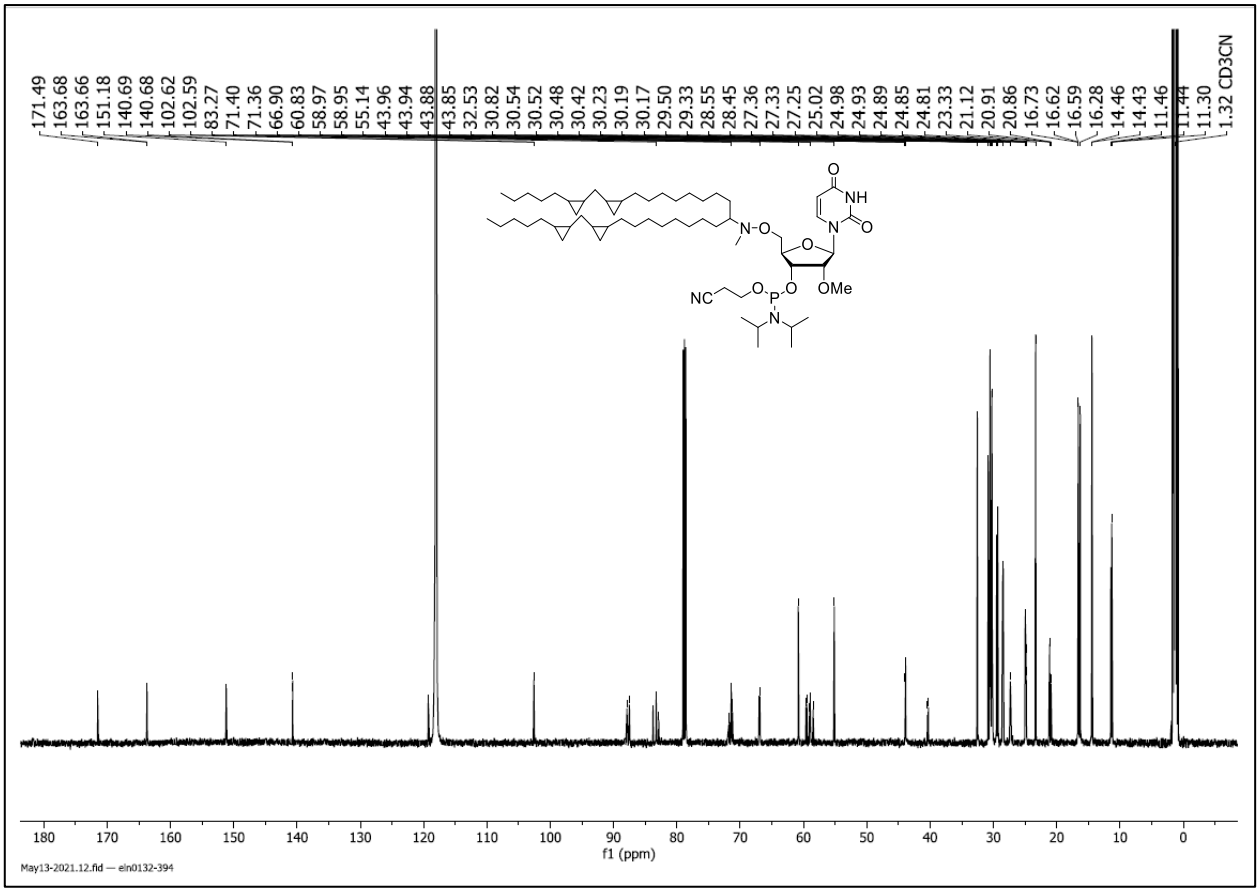
^{1}H NMR of compound **17c** (600 MHz, $CDCl_3$)



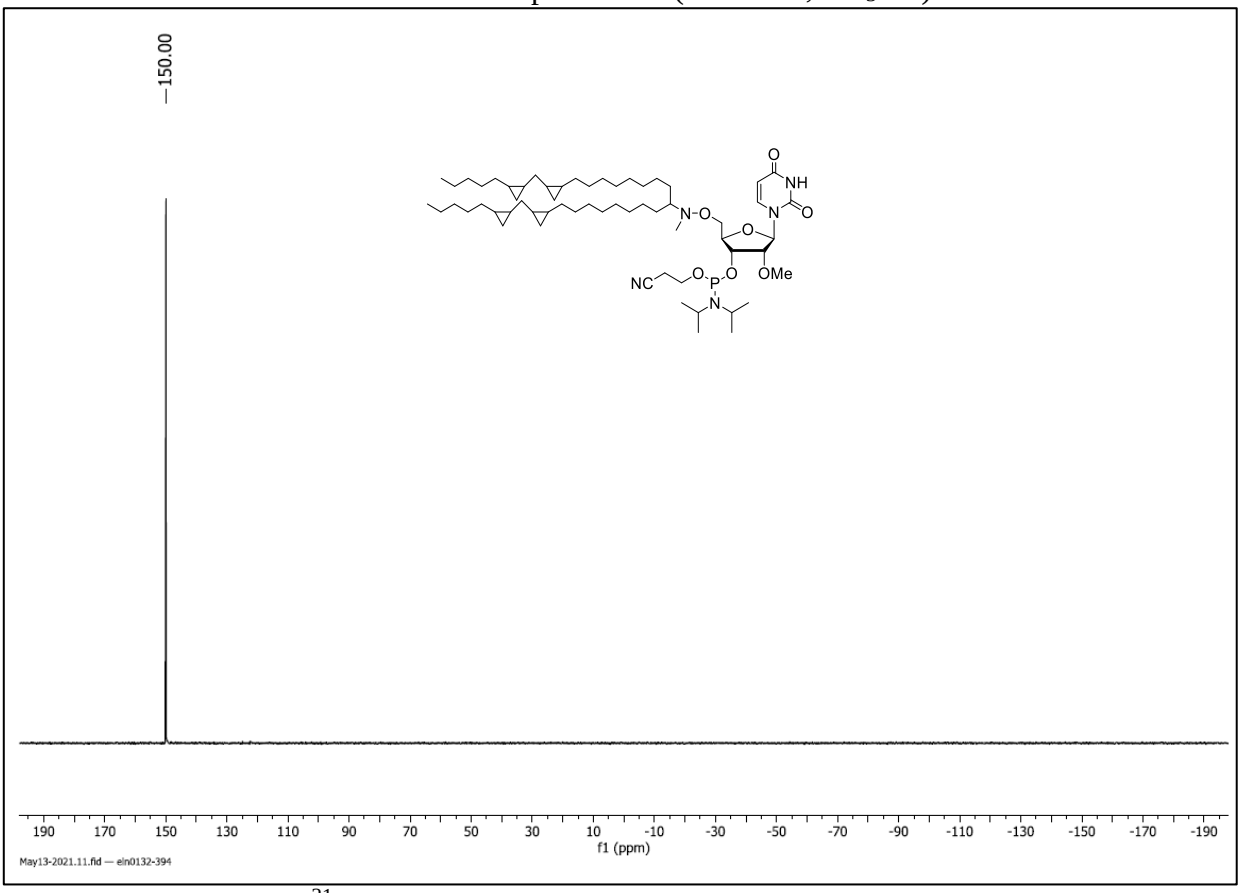
^{13}C NMR of compound **17c** (151 MHz, $CDCl_3$)



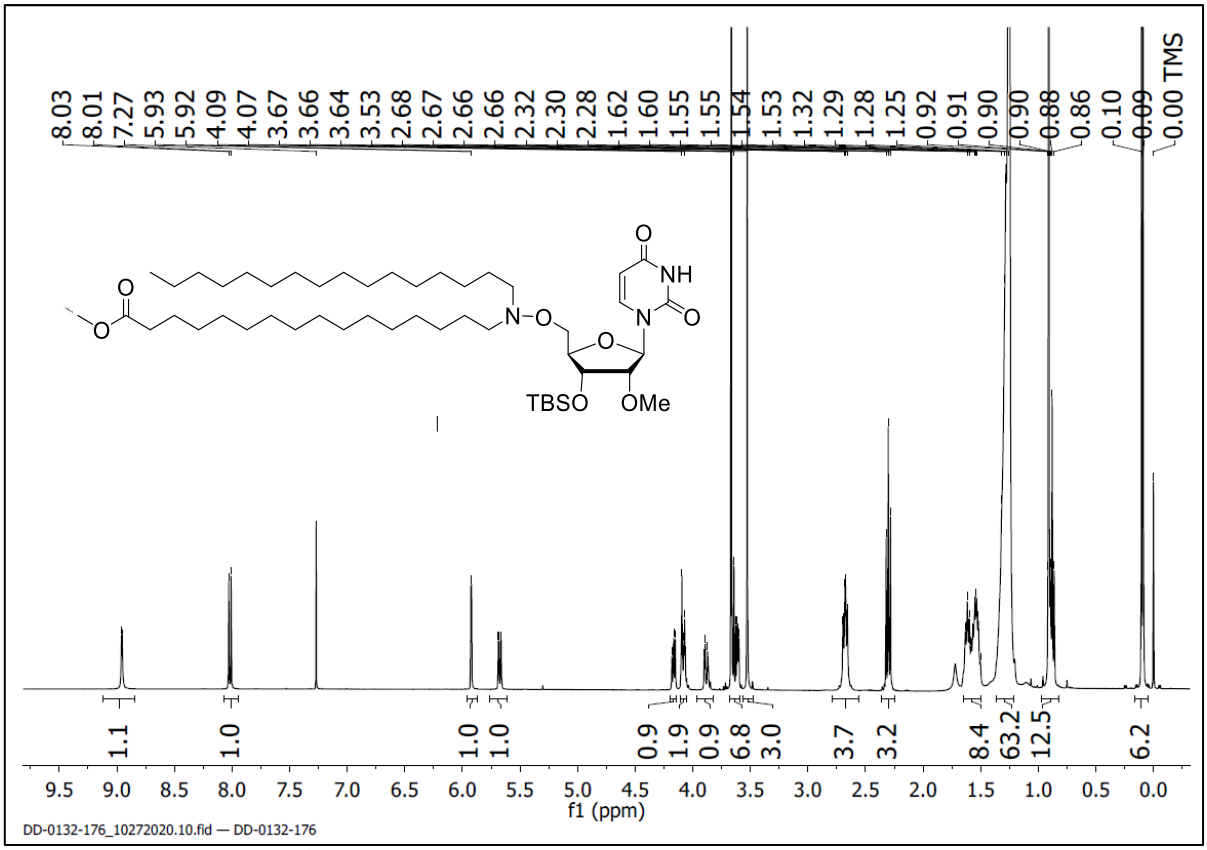
^{1}H NMR of compound **18c** (600 MHz, CD_3CN)



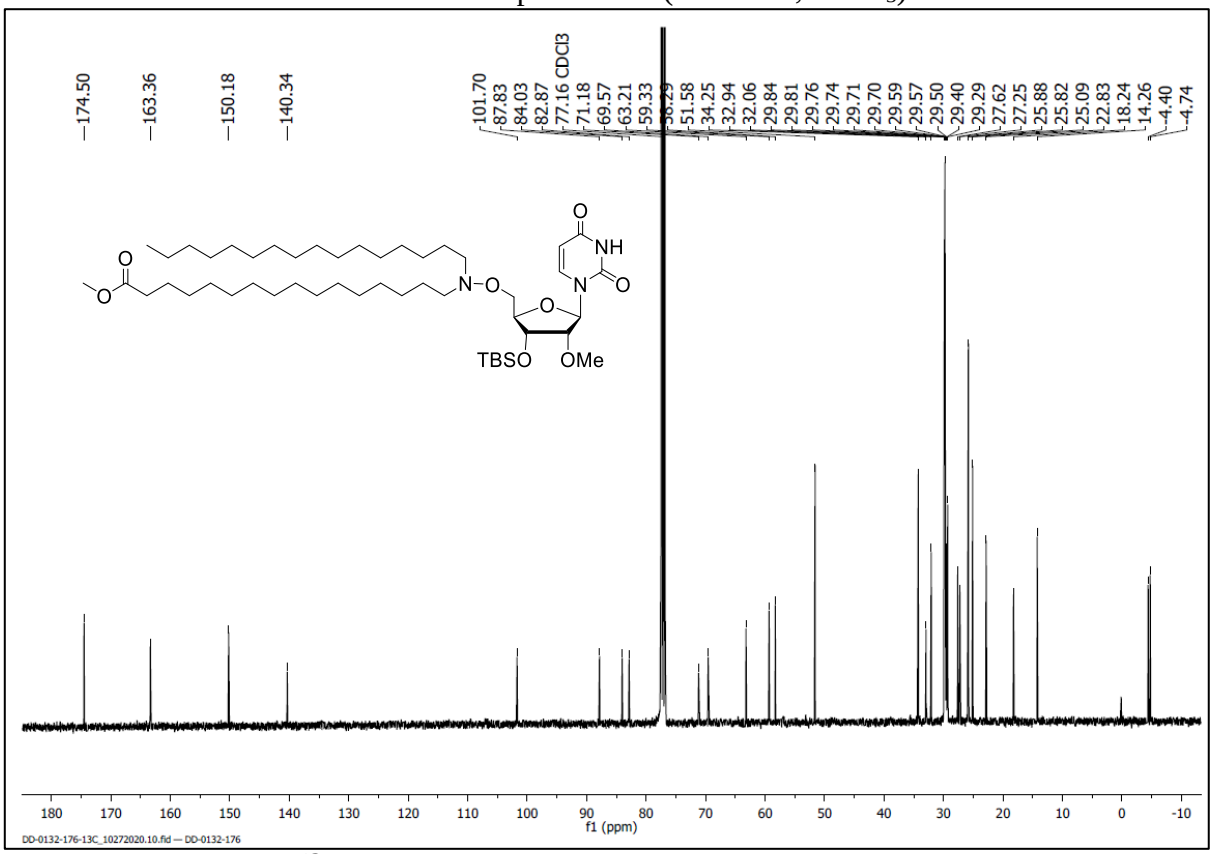
^{13}C NMR of compound **18c** (151 MHz, CD_3CN)



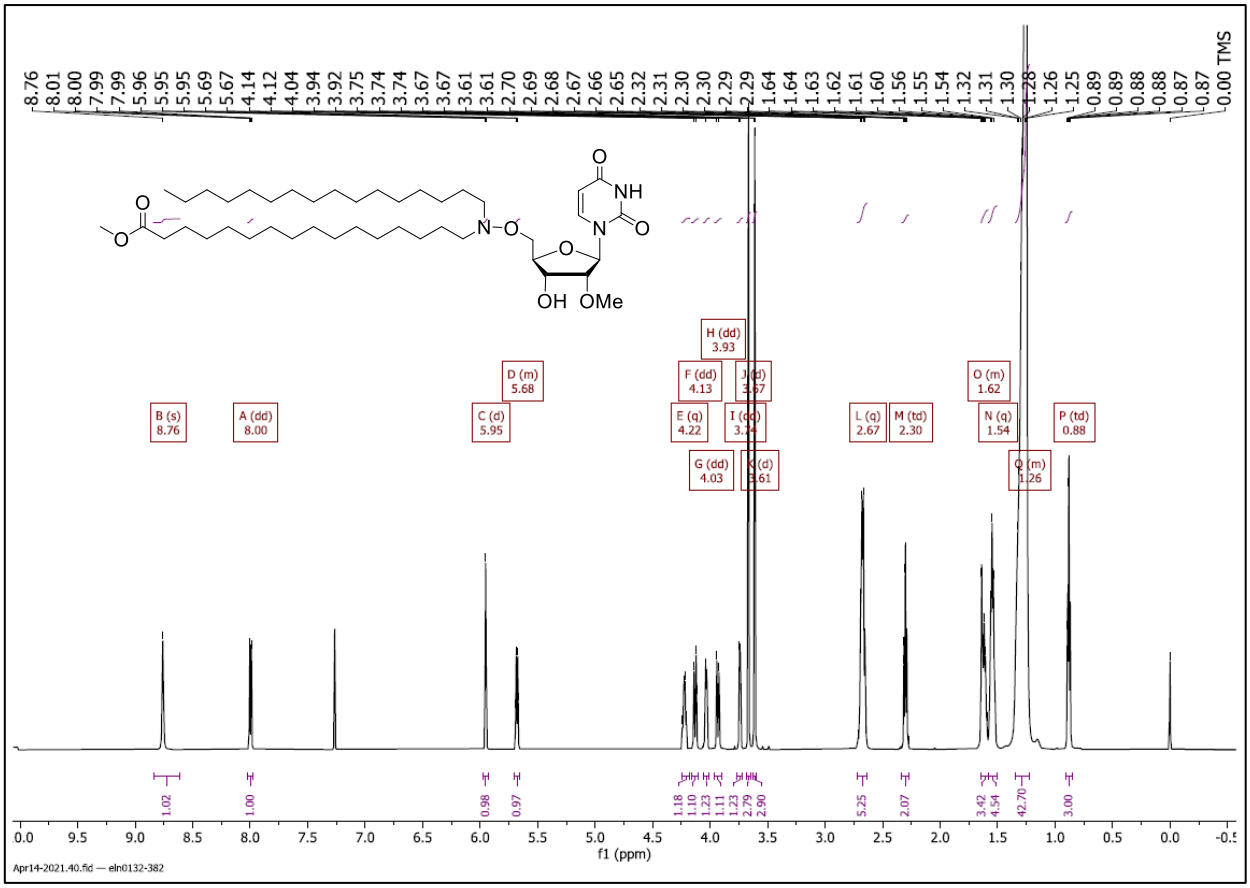
^{31}P NMR of compound **18c** (243 MHz, CD_3CN)



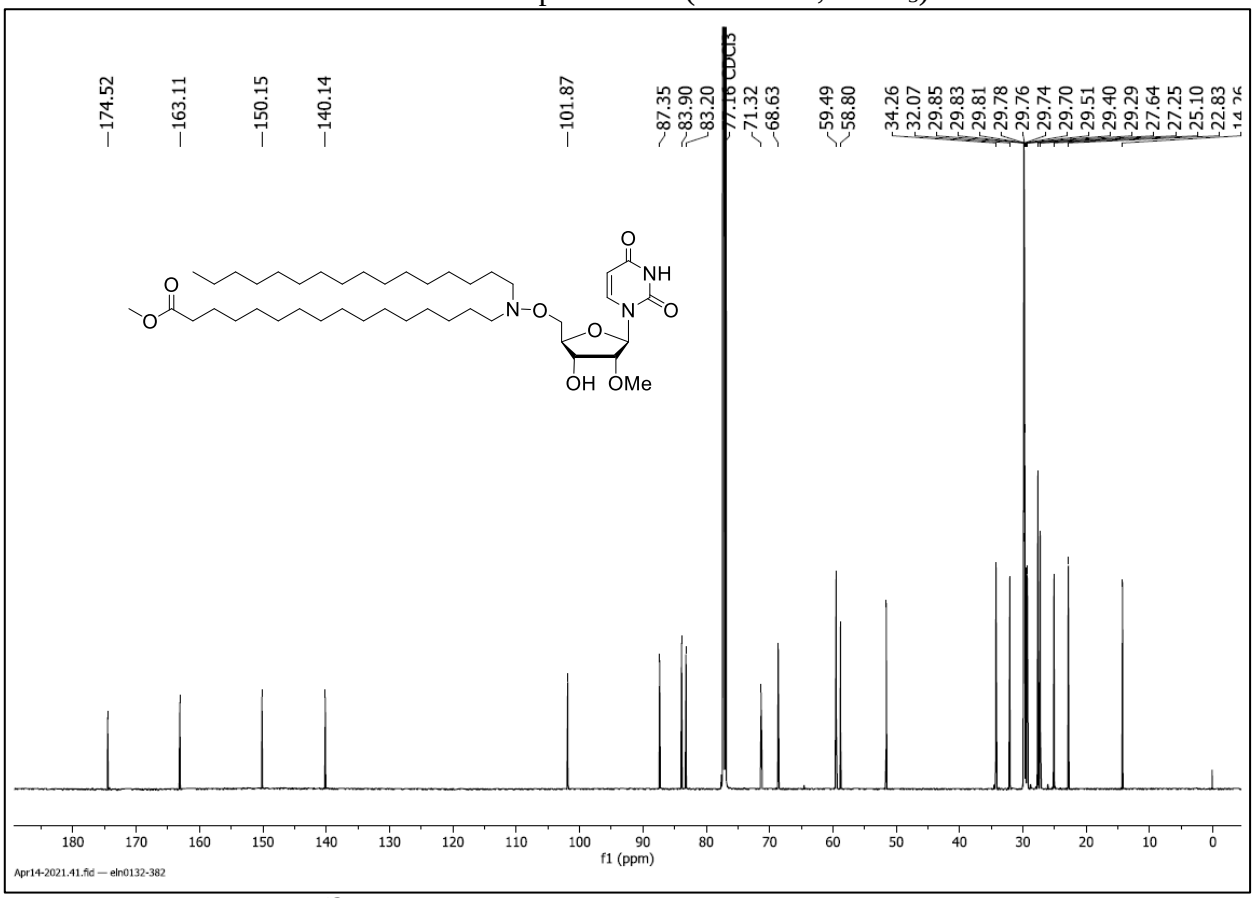
^{1}H NMR of compound **16d** (400 MHz, $CDCl_3$)



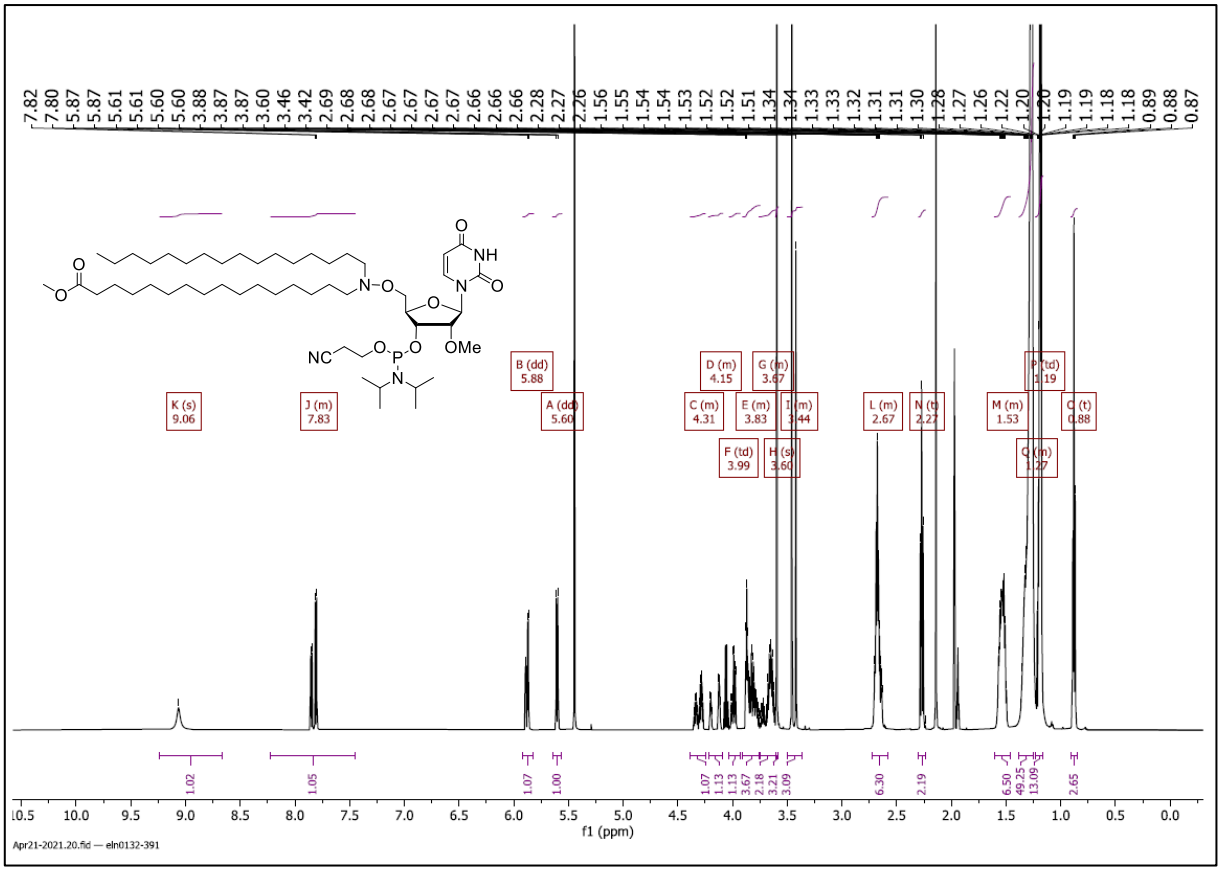
^{13}C NMR of compound **16d** (101 MHz, $CDCl_3$)



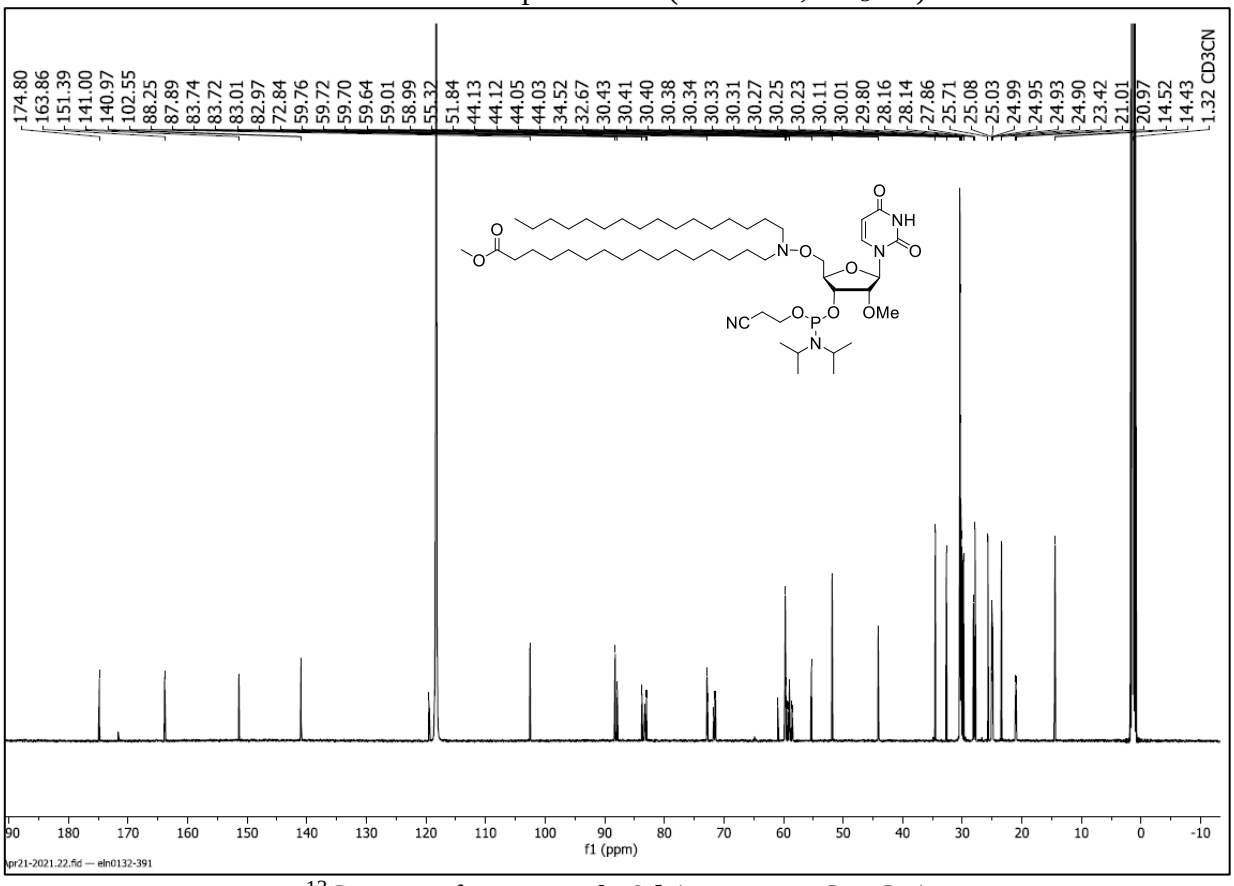
^{1}H NMR of compound **17d** (600 MHz, $CDCl_3$)



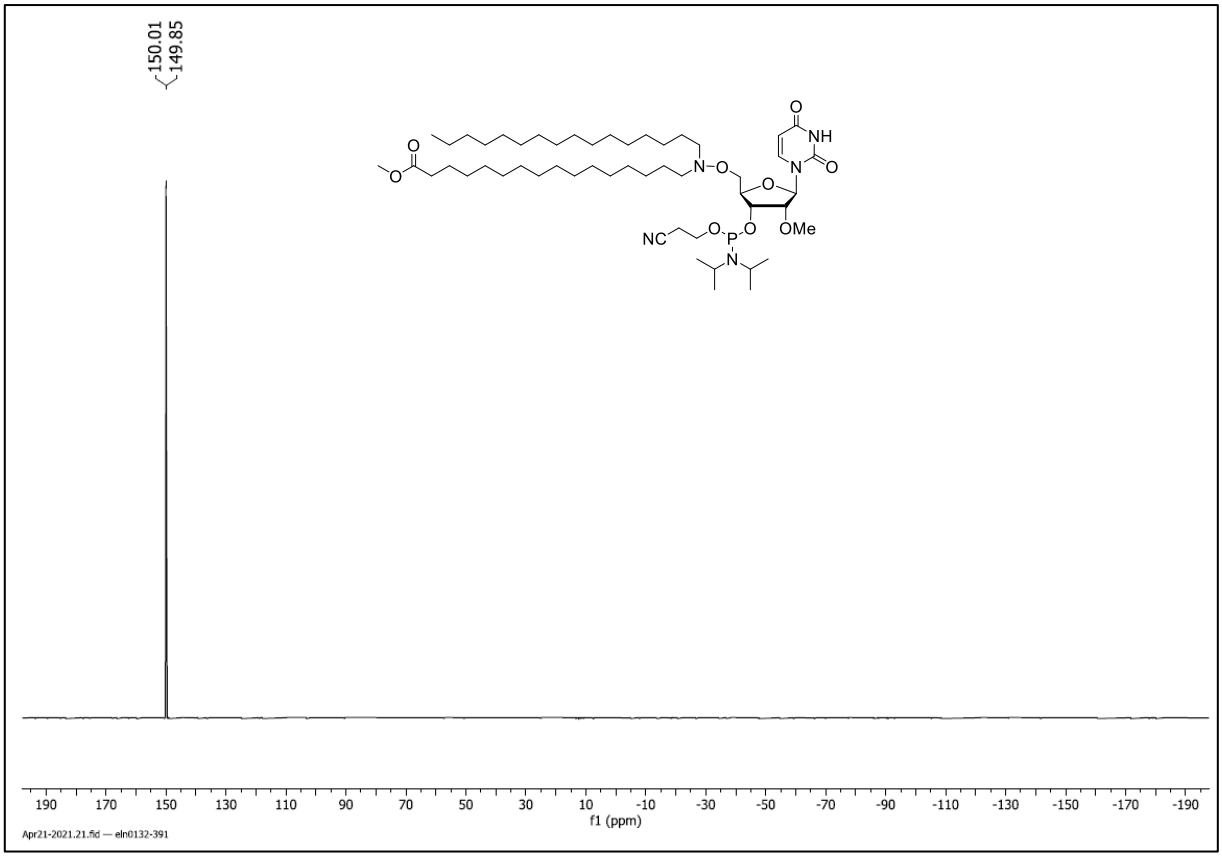
^{13}C NMR of compound **17d** (151 MHz, $CDCl_3$)



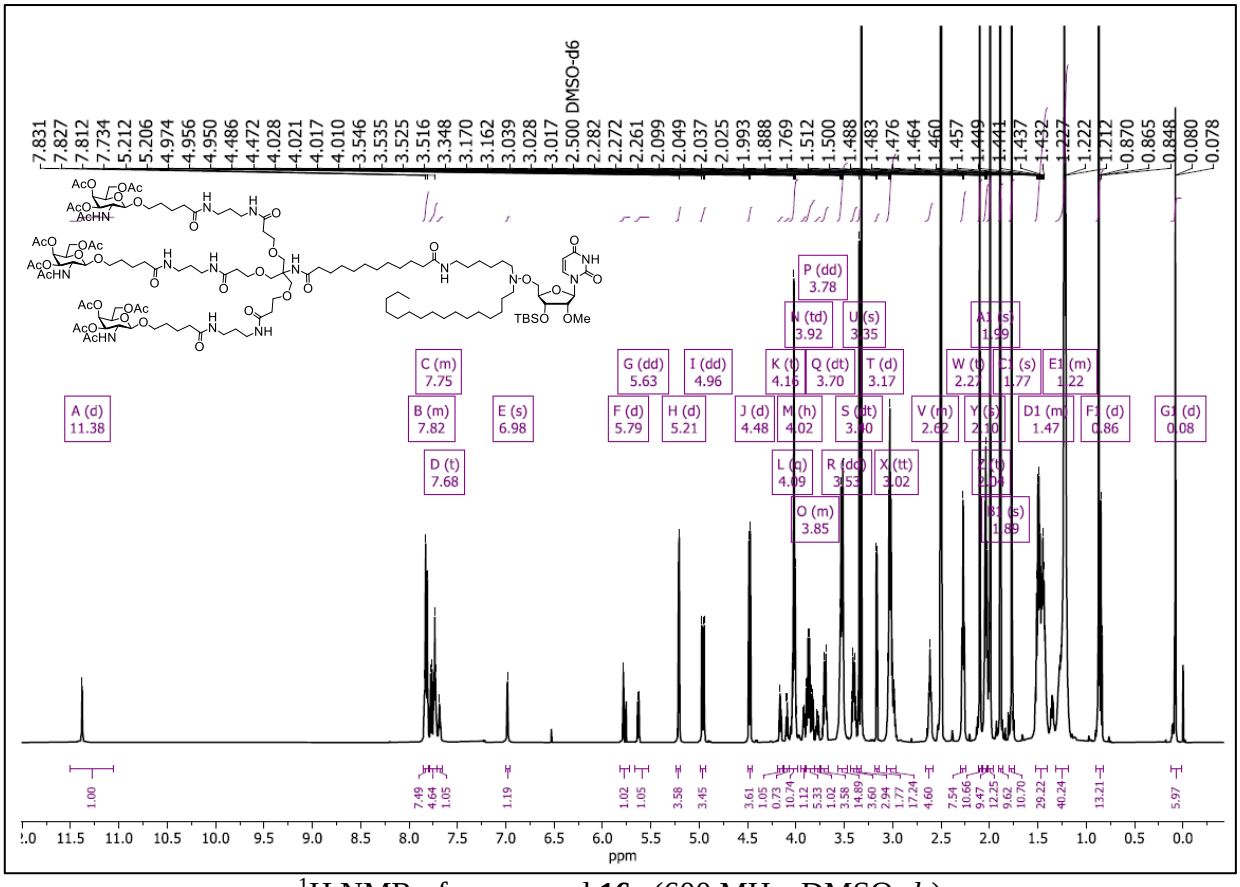
^{1}H NMR of compound **18d** (600 MHz, CD_3CN)



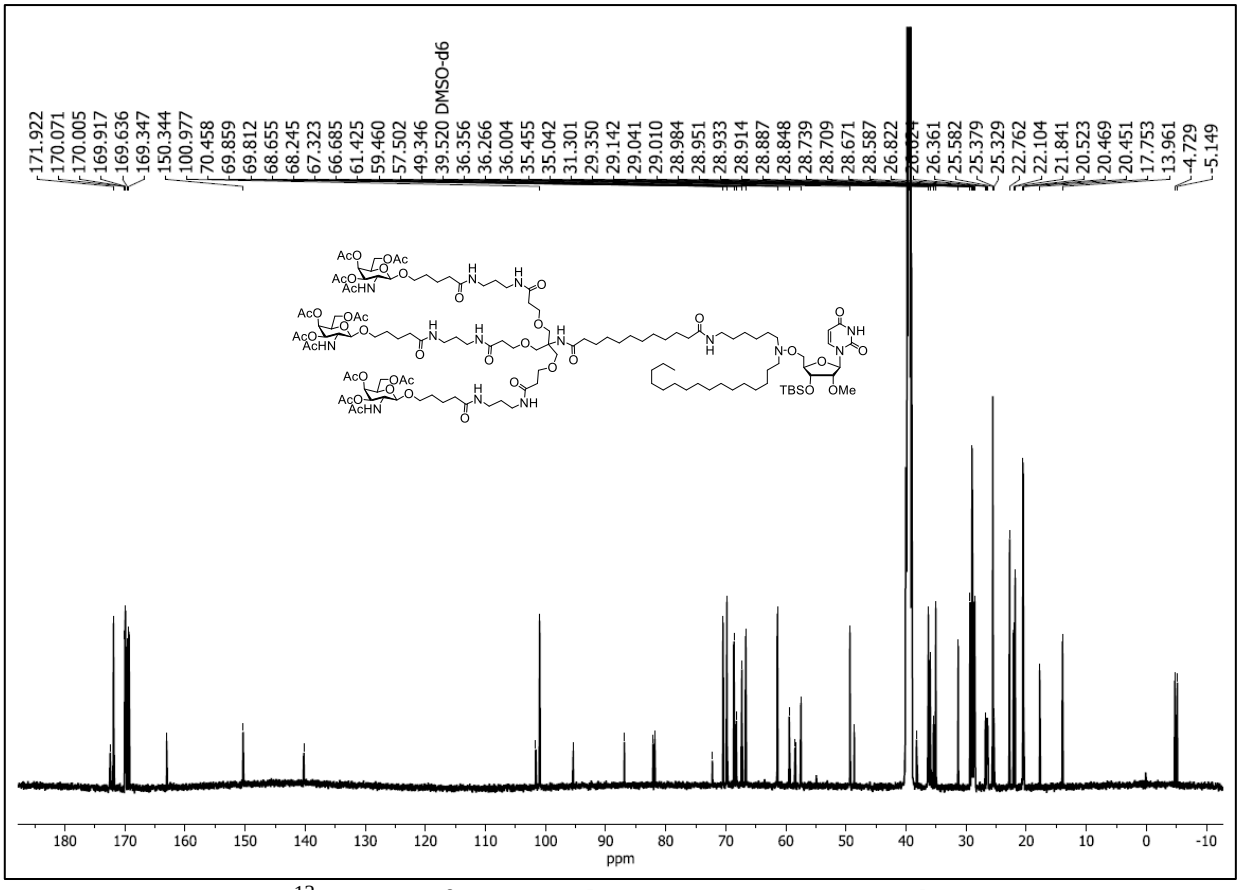
^{13}C NMR of compound **18d** (151 MHz, CD_3CN)



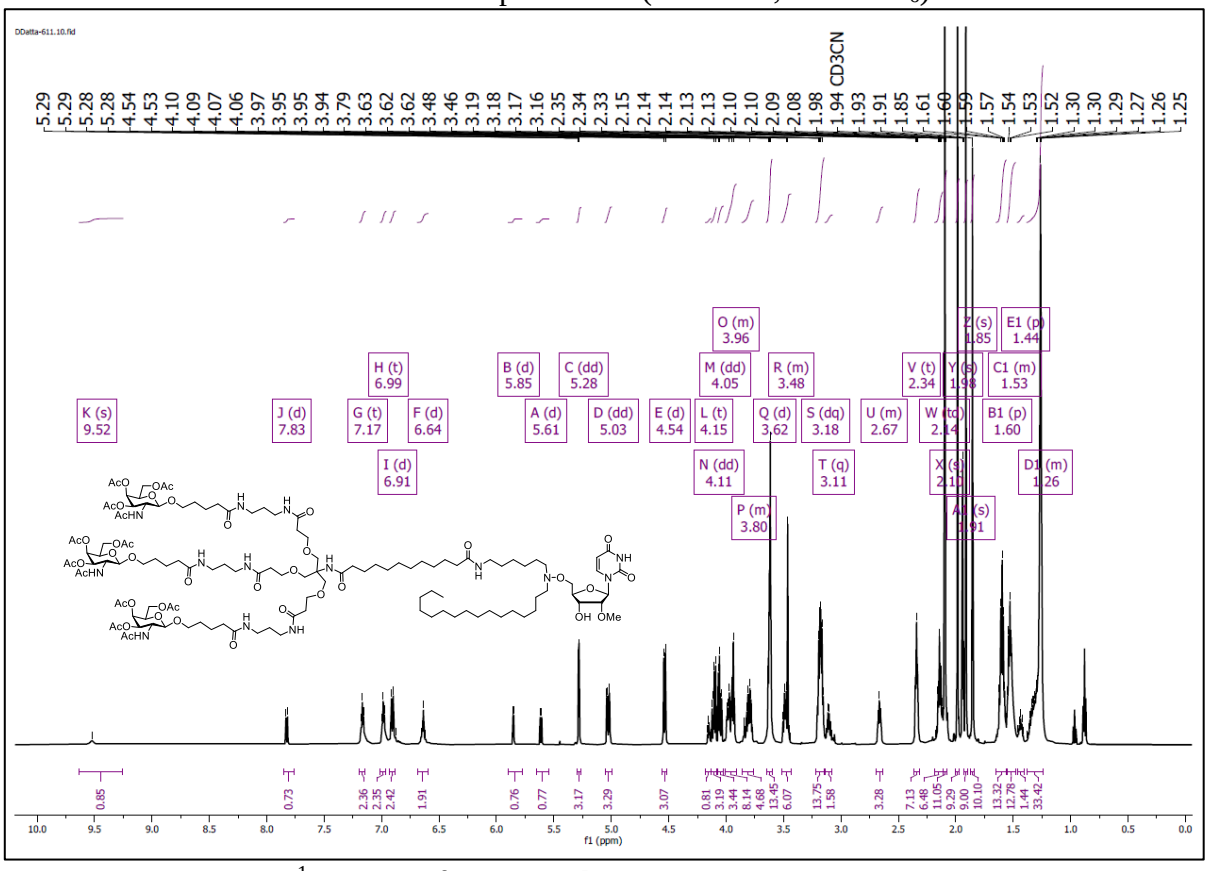
^{31}P NMR of compound **18d** (243 MHz, CD_3CN)



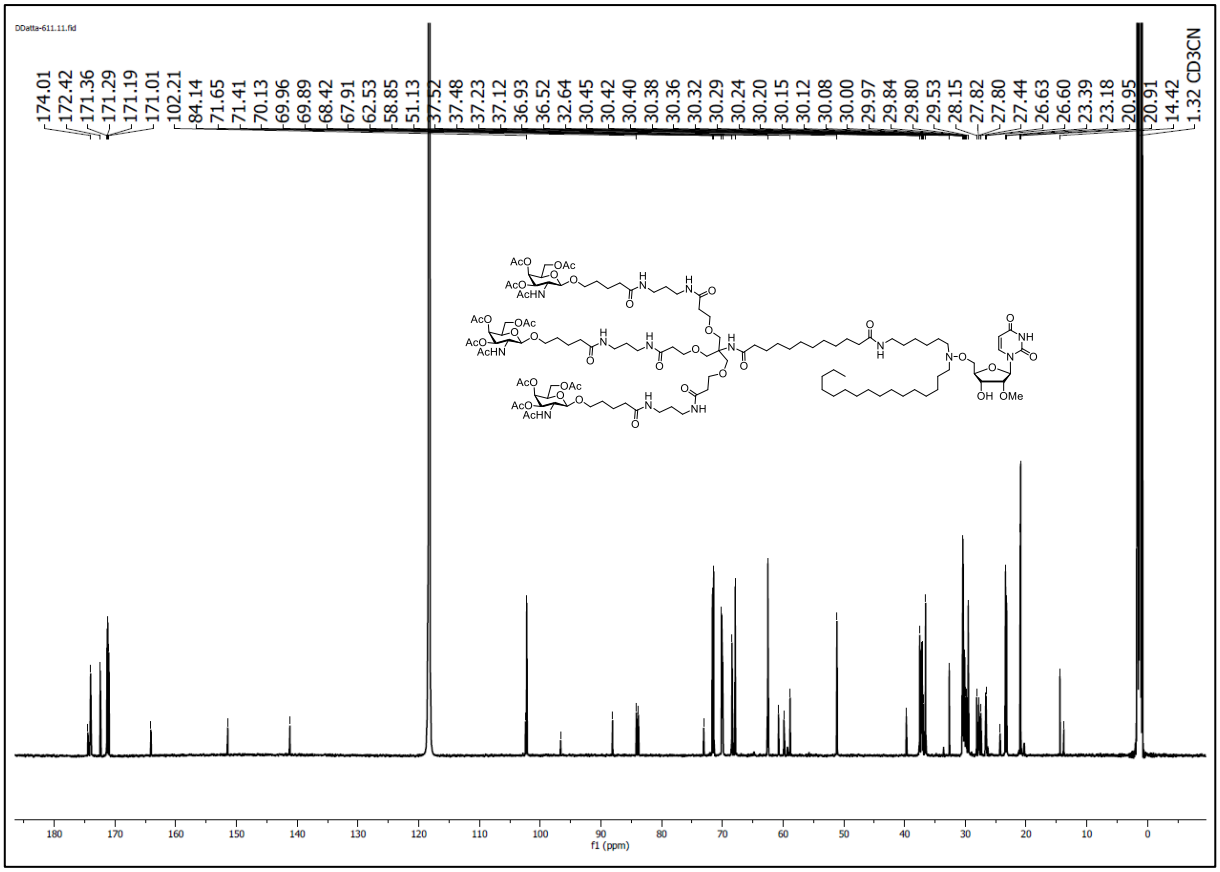
^{1}H NMR of compound **16e** (600 MHz, DMSO-d_6)



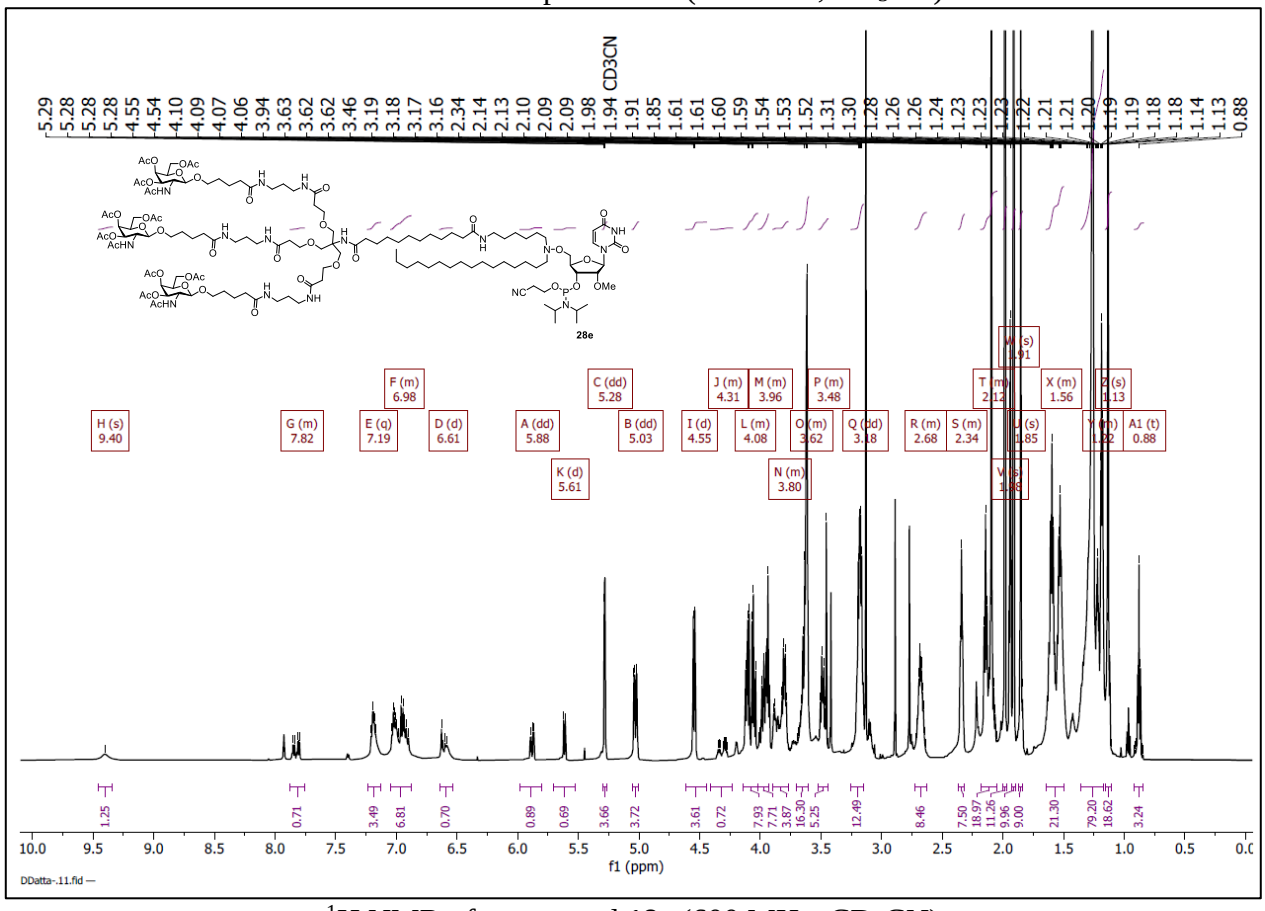
^{13}C NMR of compound **16e** (151 MHz, DMSO-d_6)



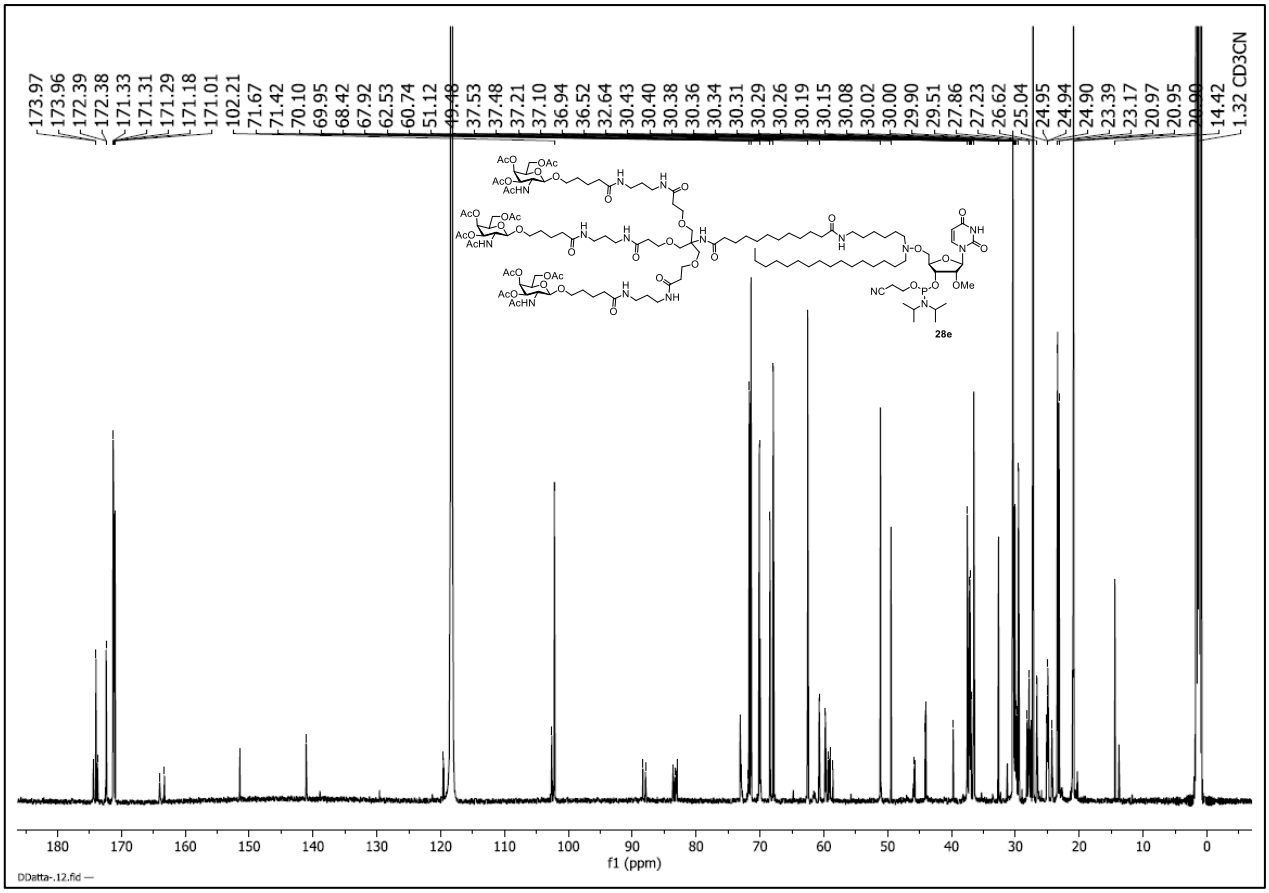
^{1}H NMR of compound **17e** (600 MHz, CD_3CN)



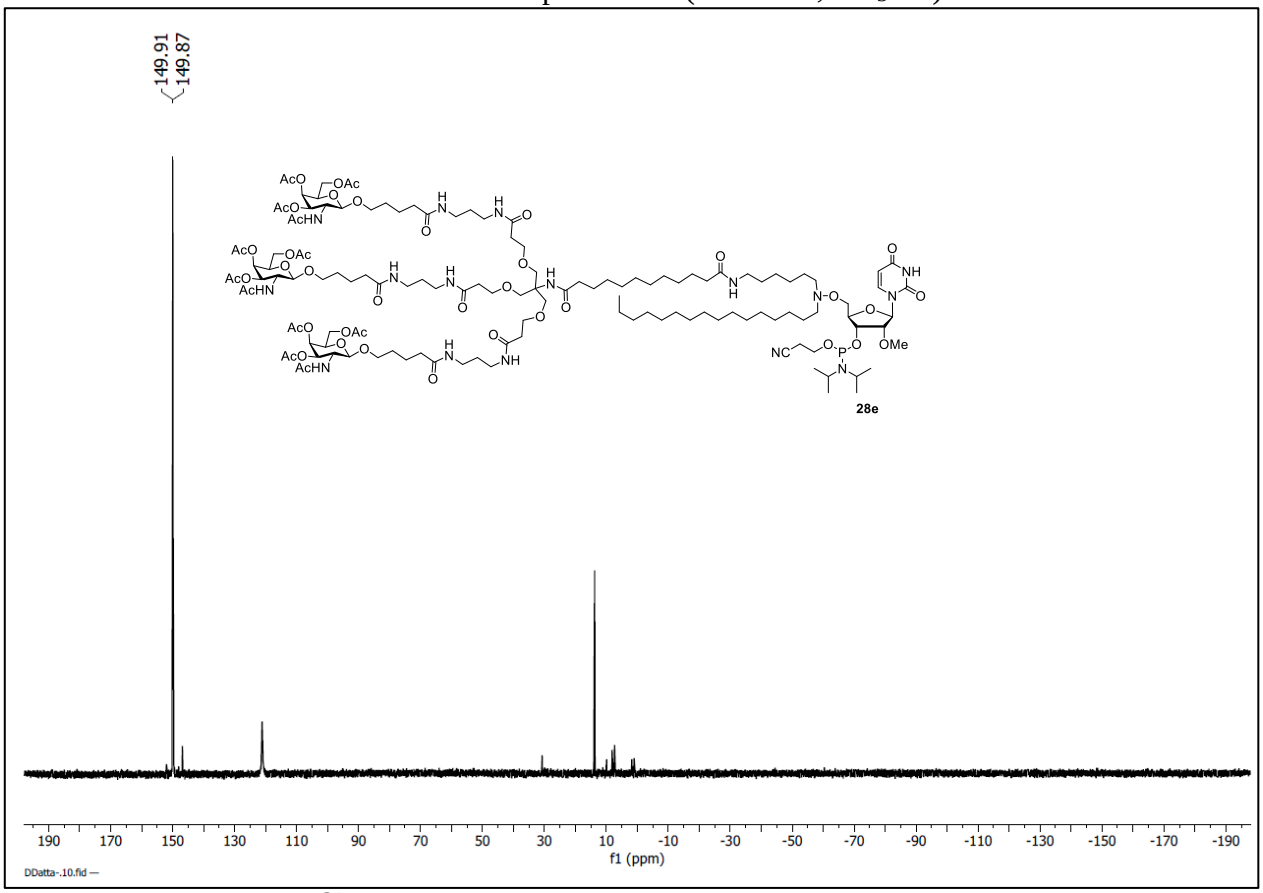
^{13}C NMR of compound **17e** (151 MHz, CD_3CN)



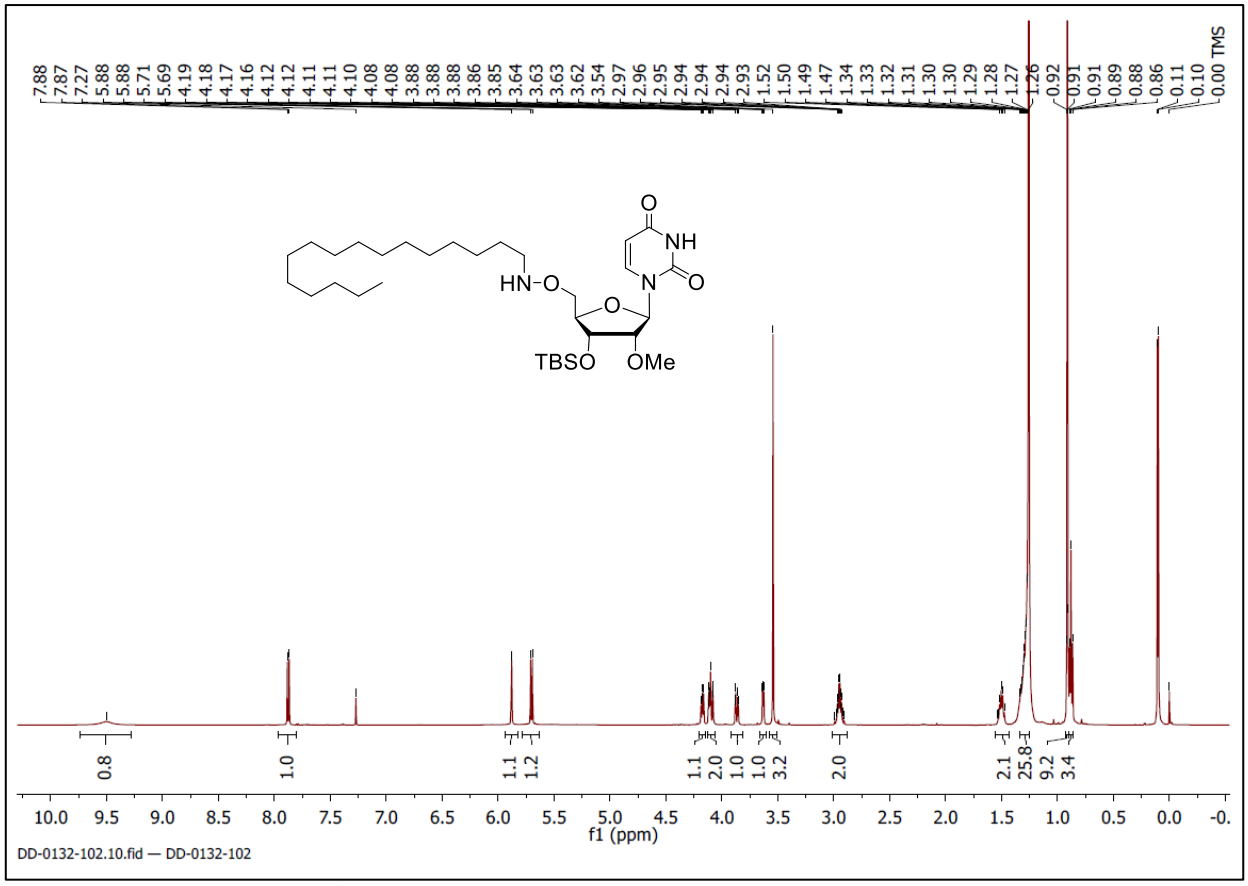
^{1}H NMR of compound **18e** (600 MHz, CD_3CN)



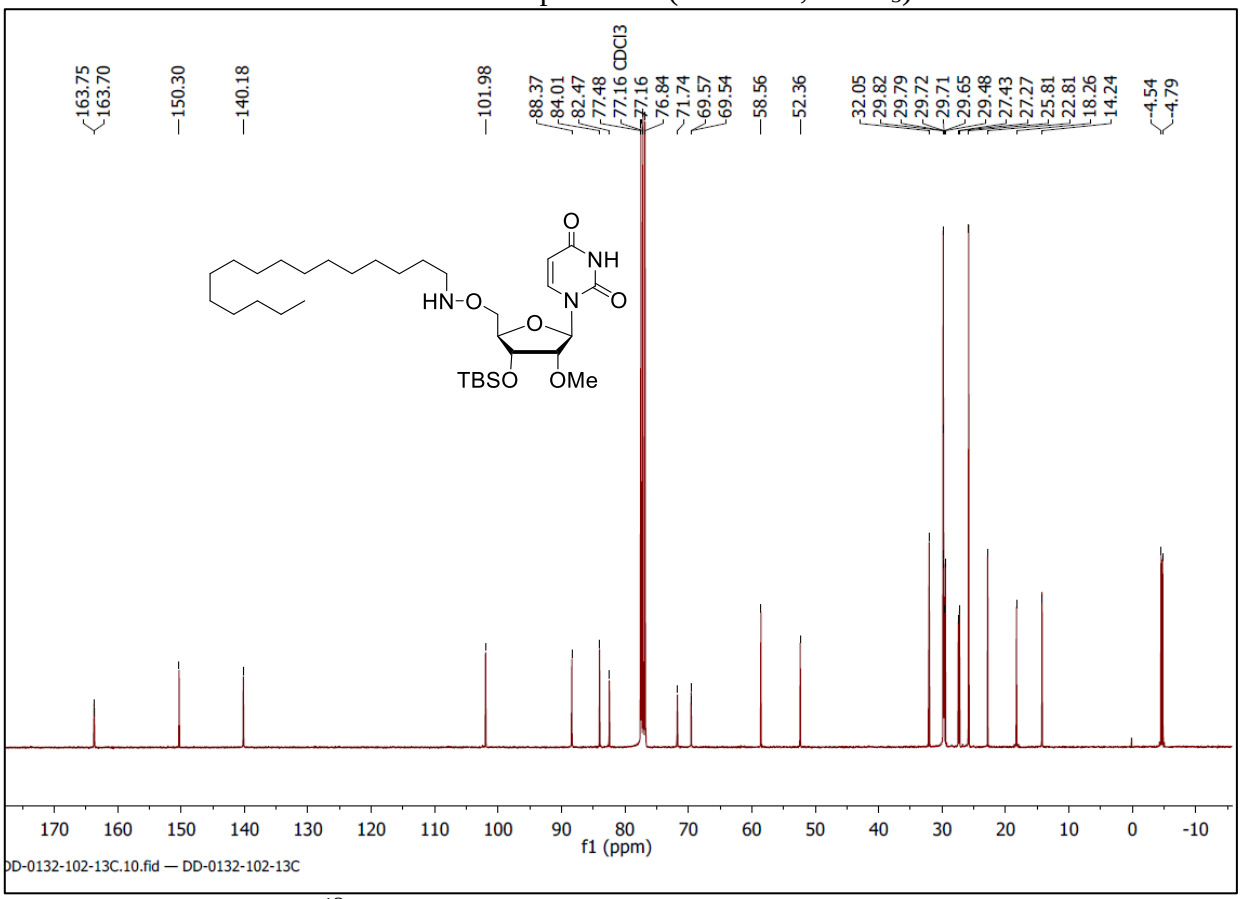
^{13}C NMR of compound **18e** (151 MHz, CD_3CN)



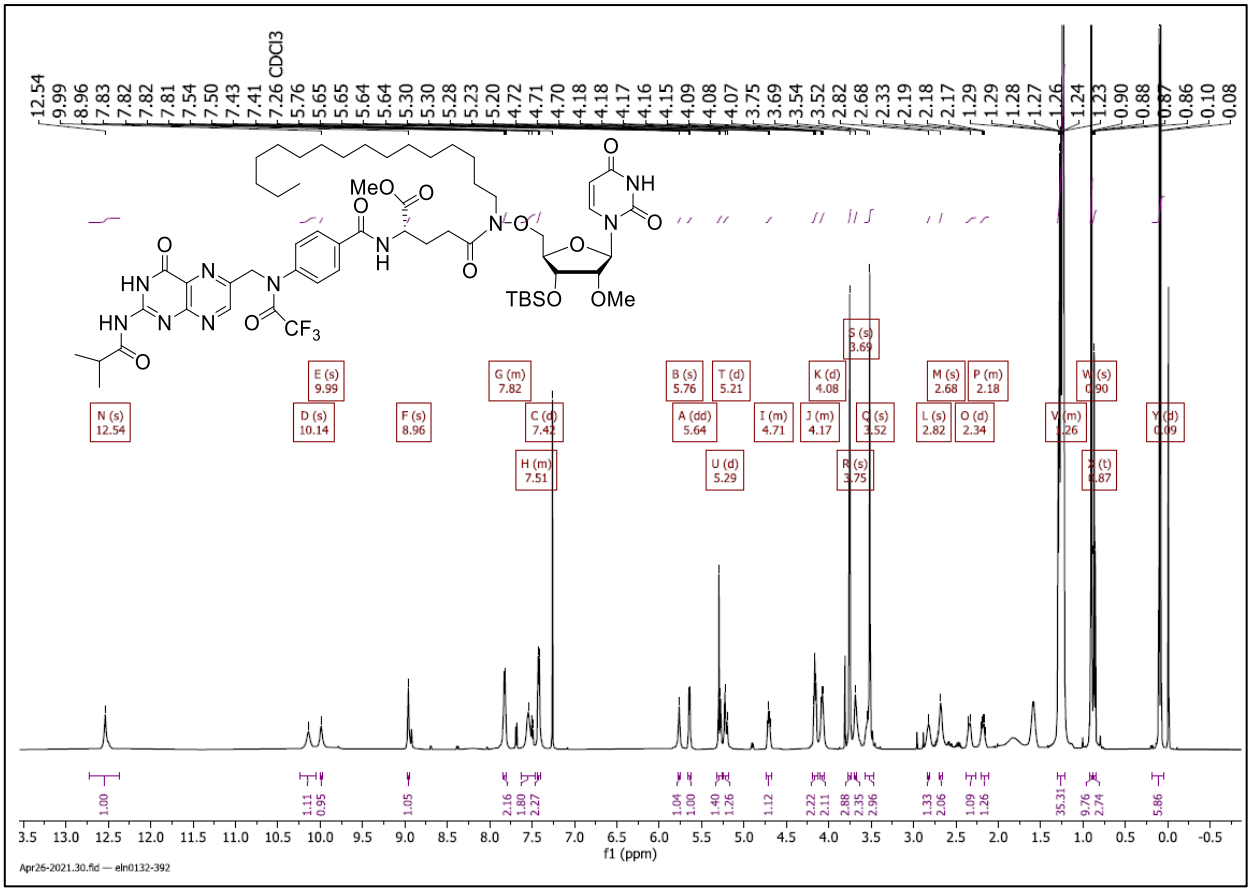
^{31}P NMR of compound **18e** (243 MHz, CD_3CN)



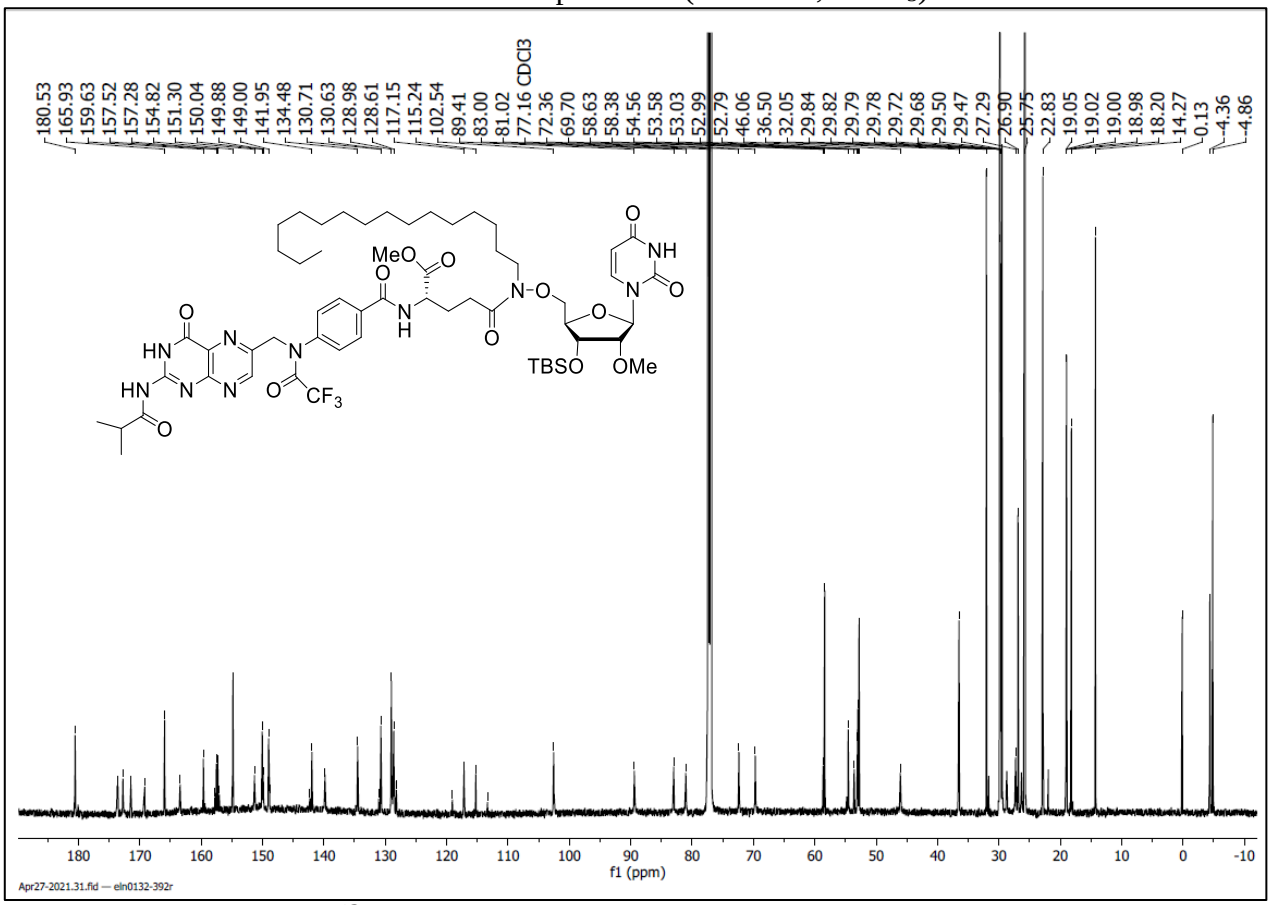
^{1}H NMR of compound **19** (500 MHz, $CDCl_3$)



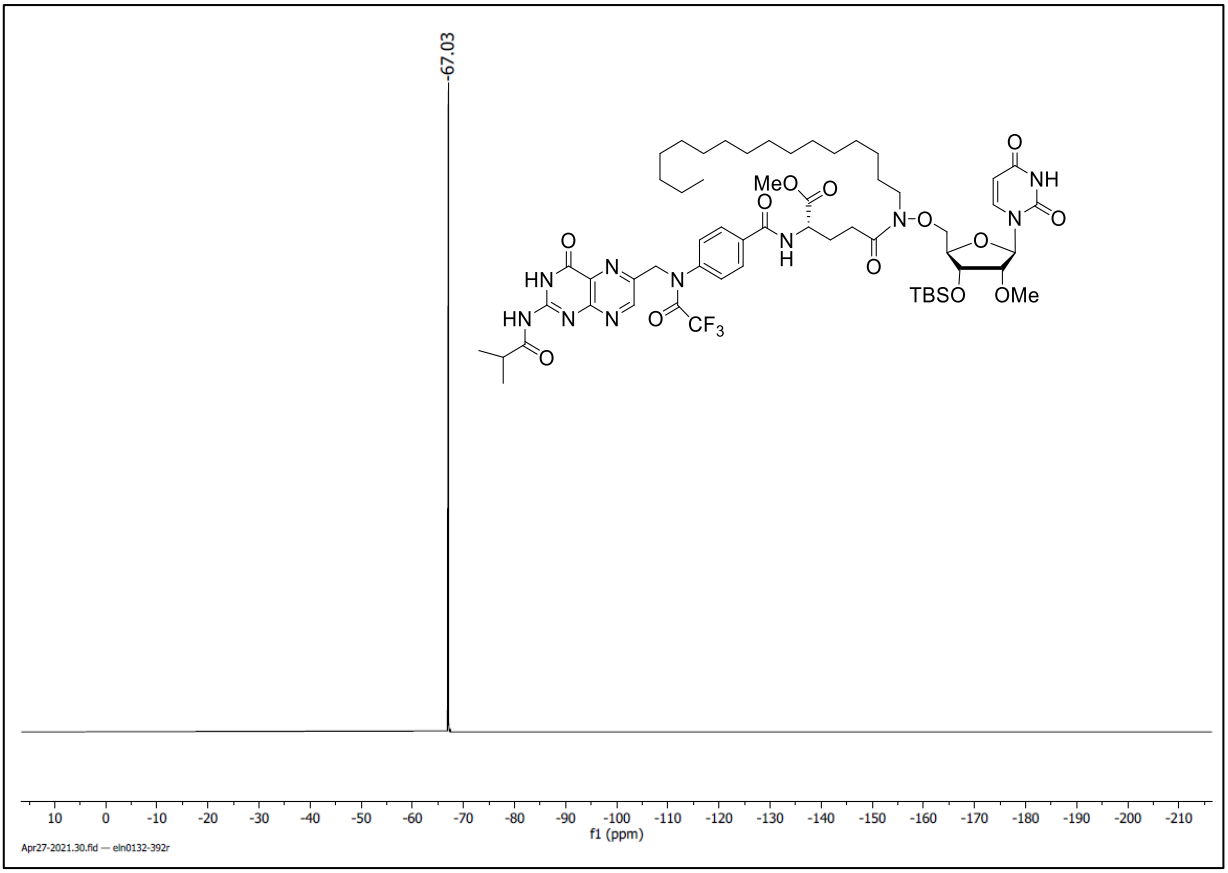
^{13}C NMR of compound **19** (101 MHz, $CDCl_3$)



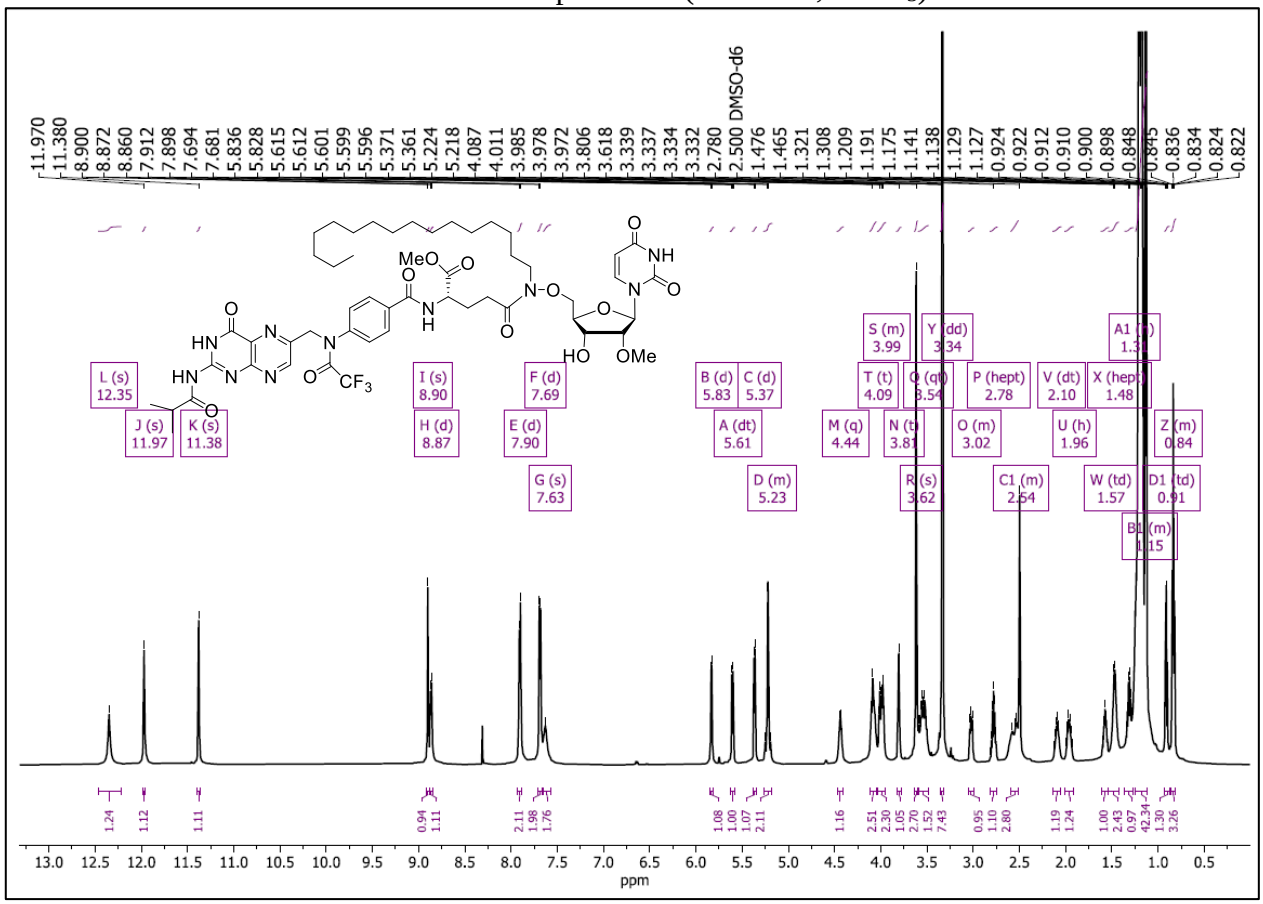
^{1}H NMR of compound **20** (600 MHz, $CDCl_3$)



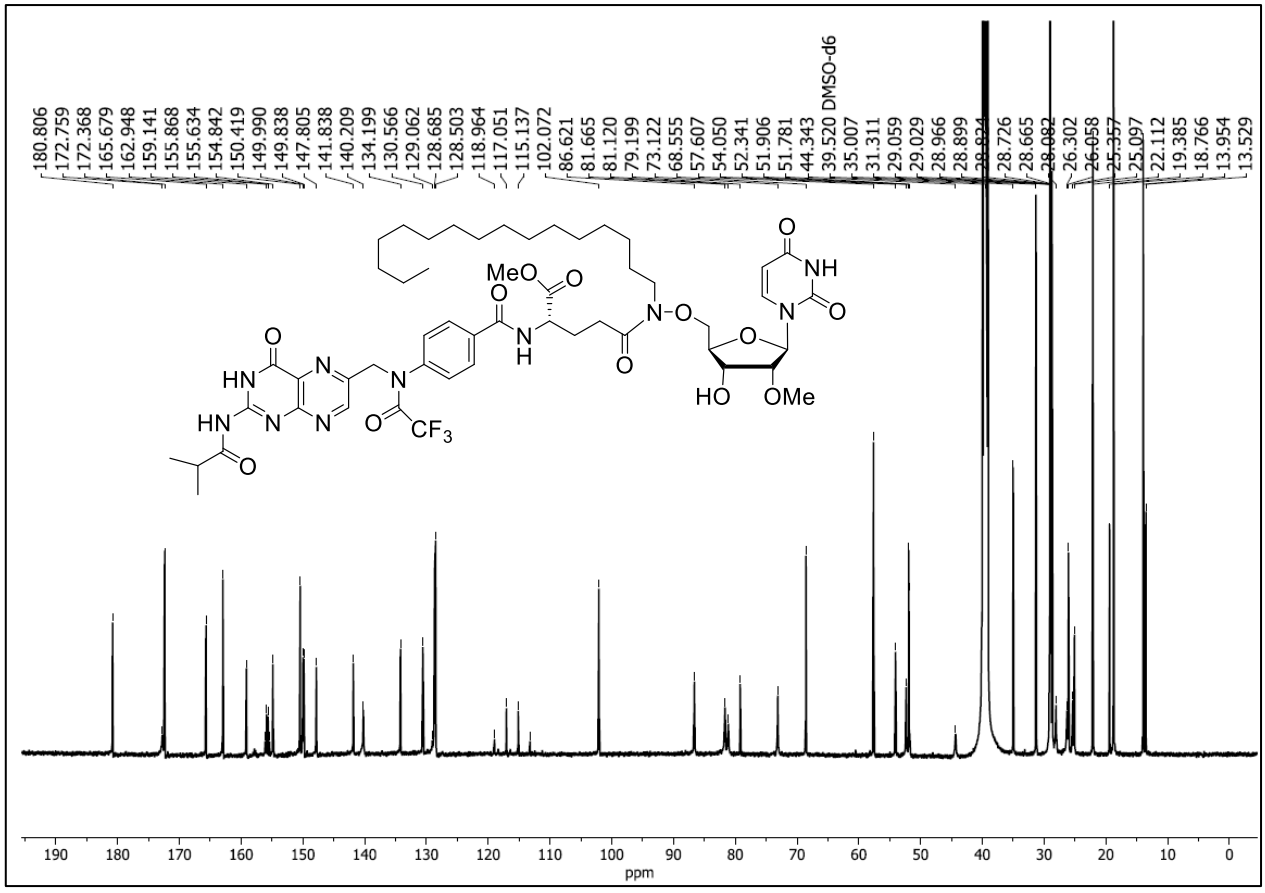
^{13}C NMR of compound **20** (151 MHz, $CDCl_3$)



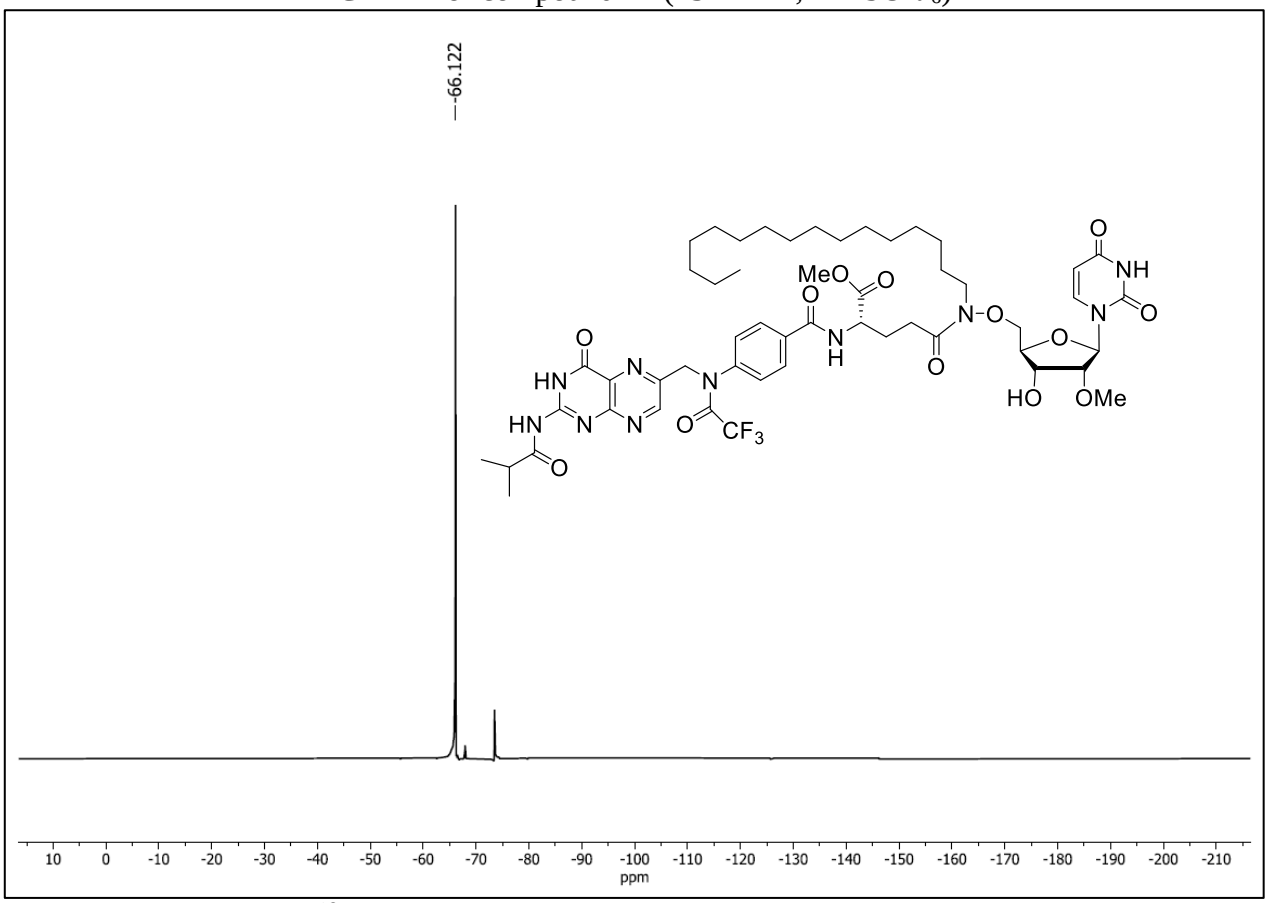
^{19}F NMR of compound **20** (565 MHz, $CDCl_3$)



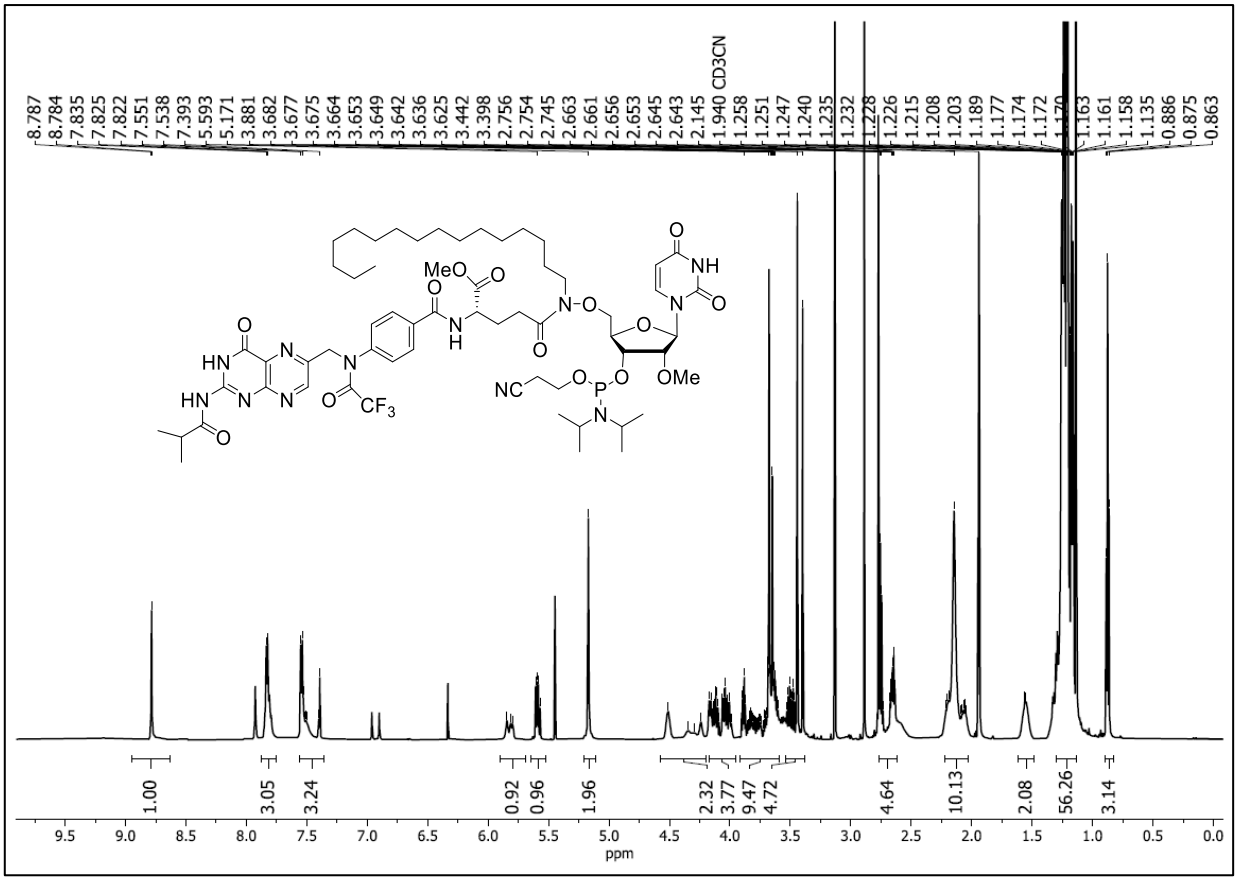
^{1}H NMR of compound **22** (600 MHz, DMSO-d_6)



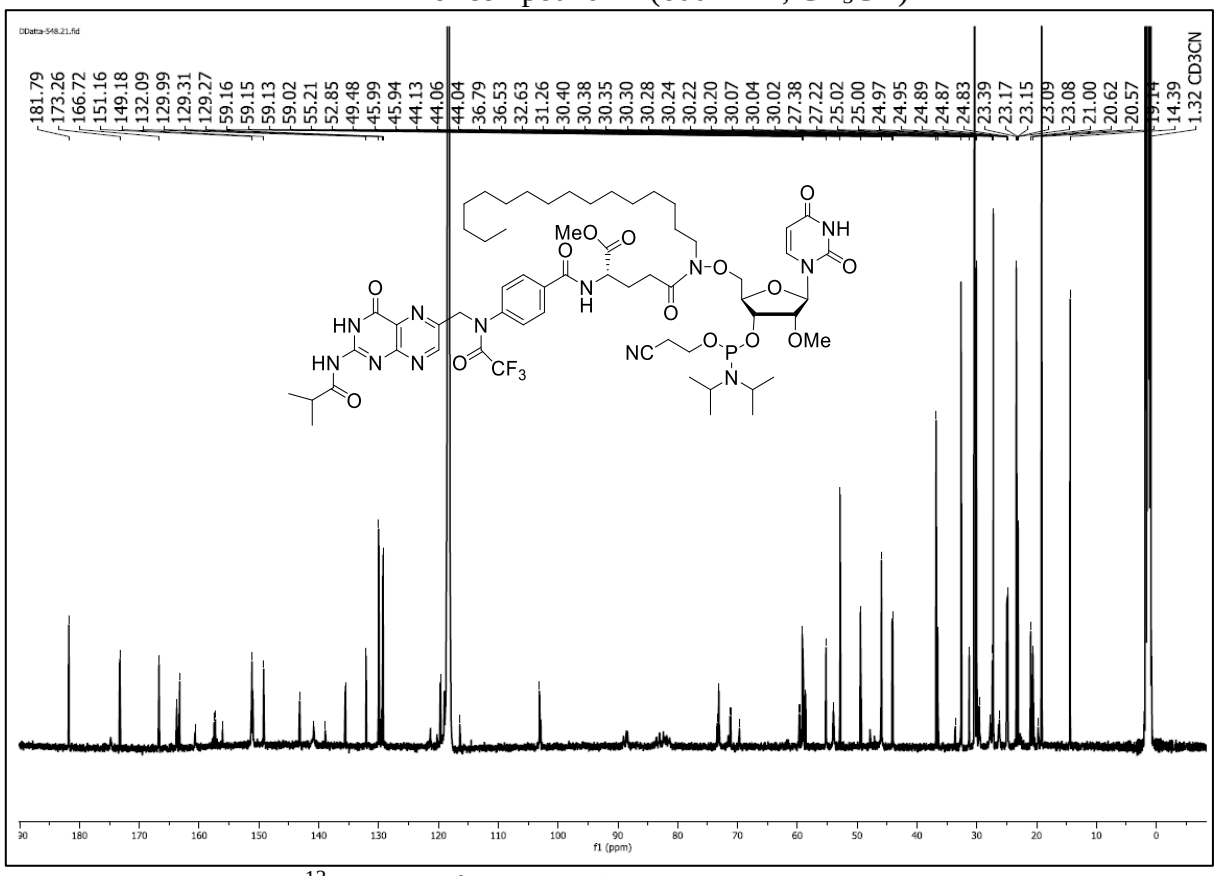
^{13}C NMR of compound **22** (151 MHz, DMSO-d_6)



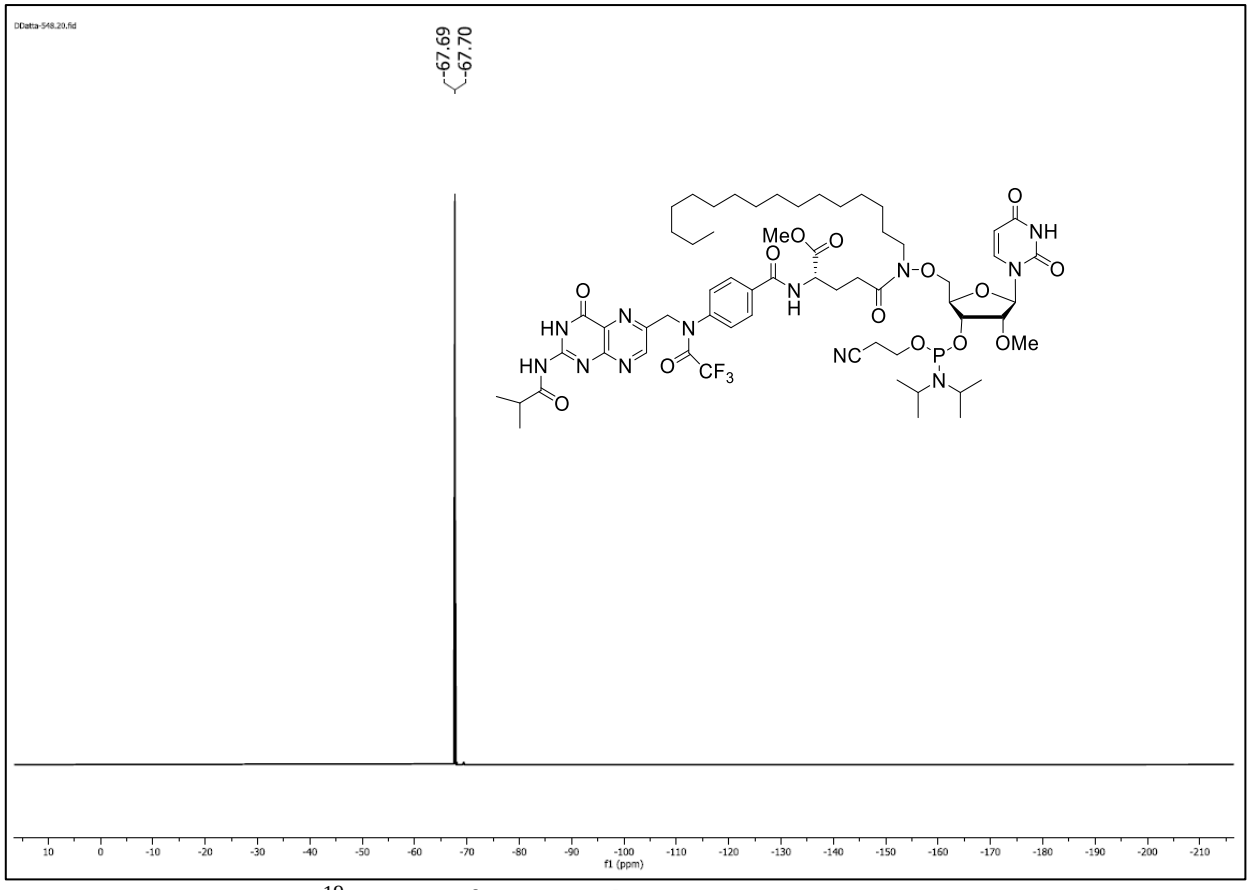
^{19}F NMR of compound **22** (565 MHz, DMSO-d_6)



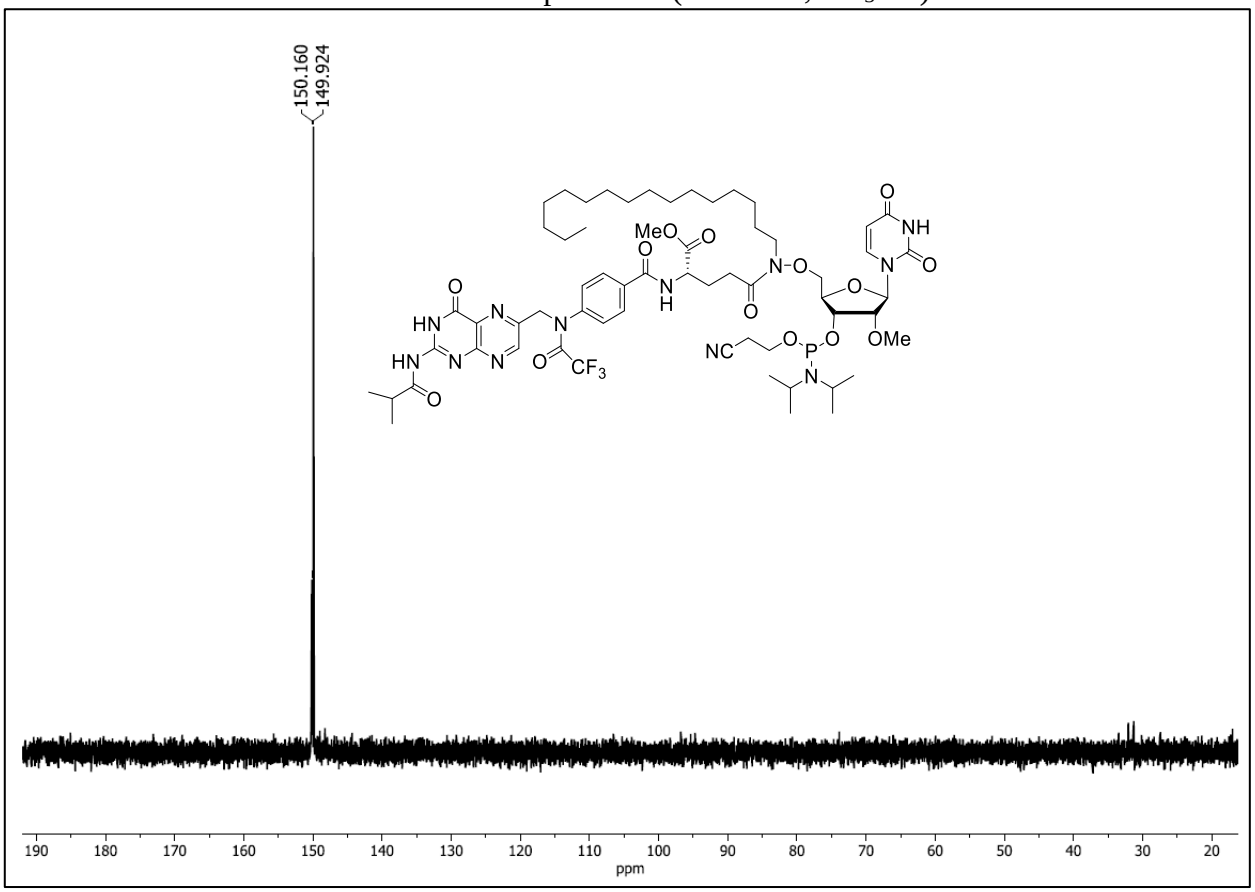
^{1}H NMR of compound **24** (600 MHz, CD_3CN)



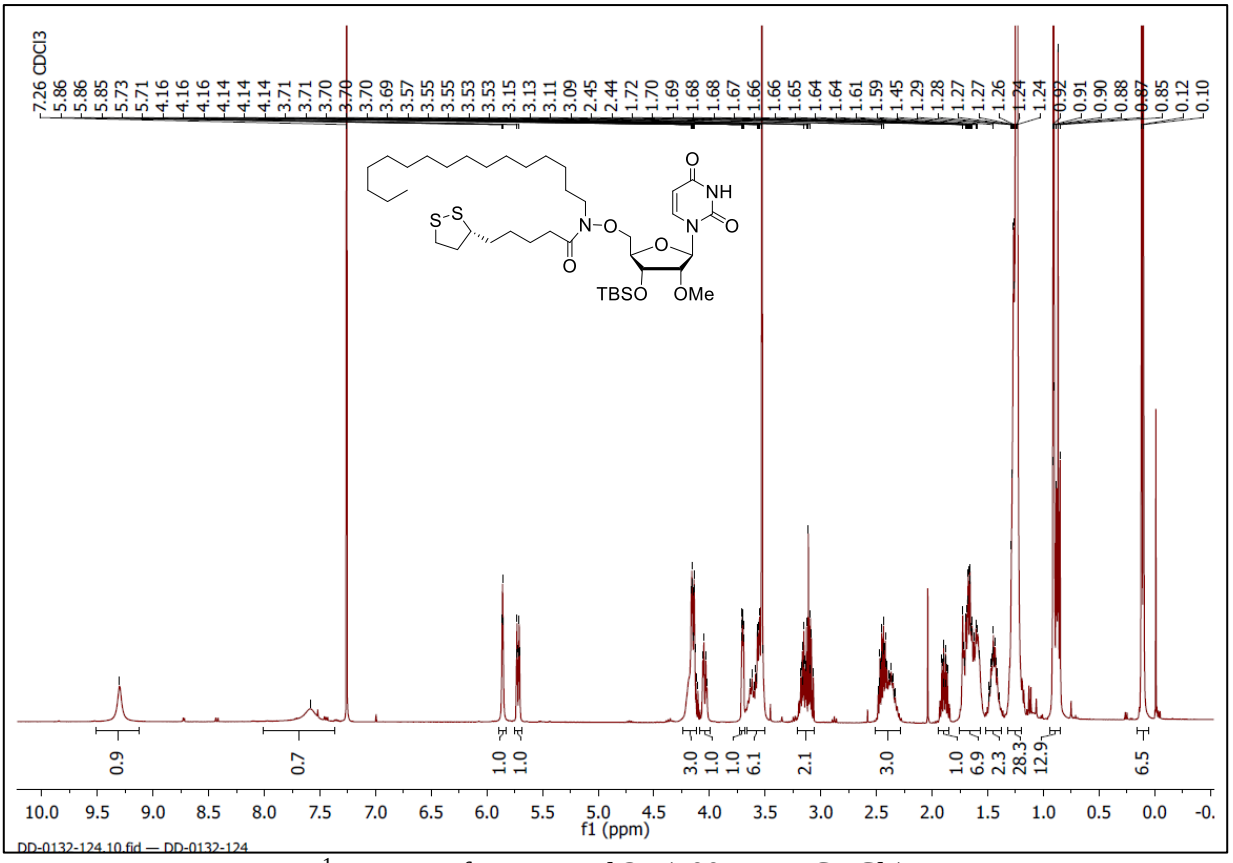
^{13}C NMR of compound **24** (151 MHz, CD_3CN)



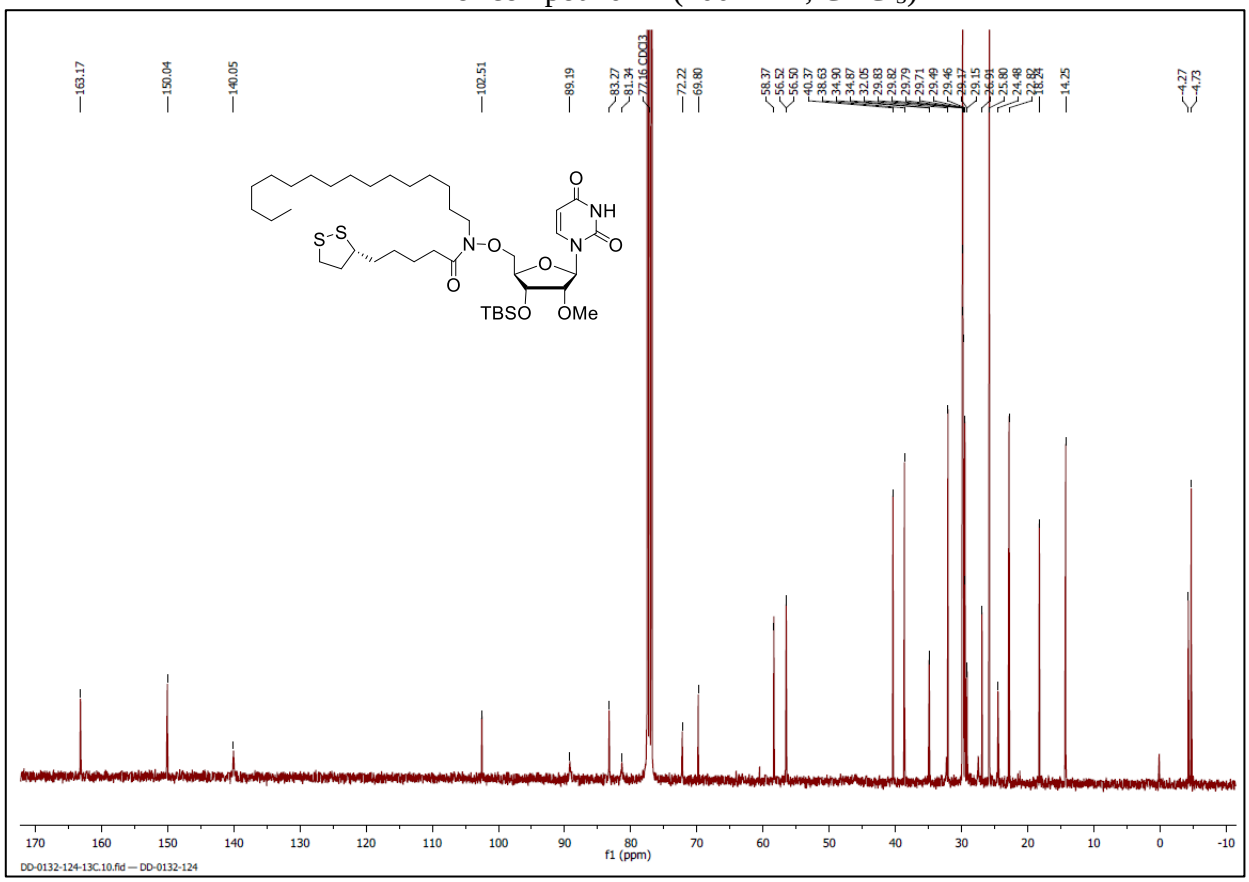
^{19}F NMR of compound **24** (565 MHz, CD_3CN)



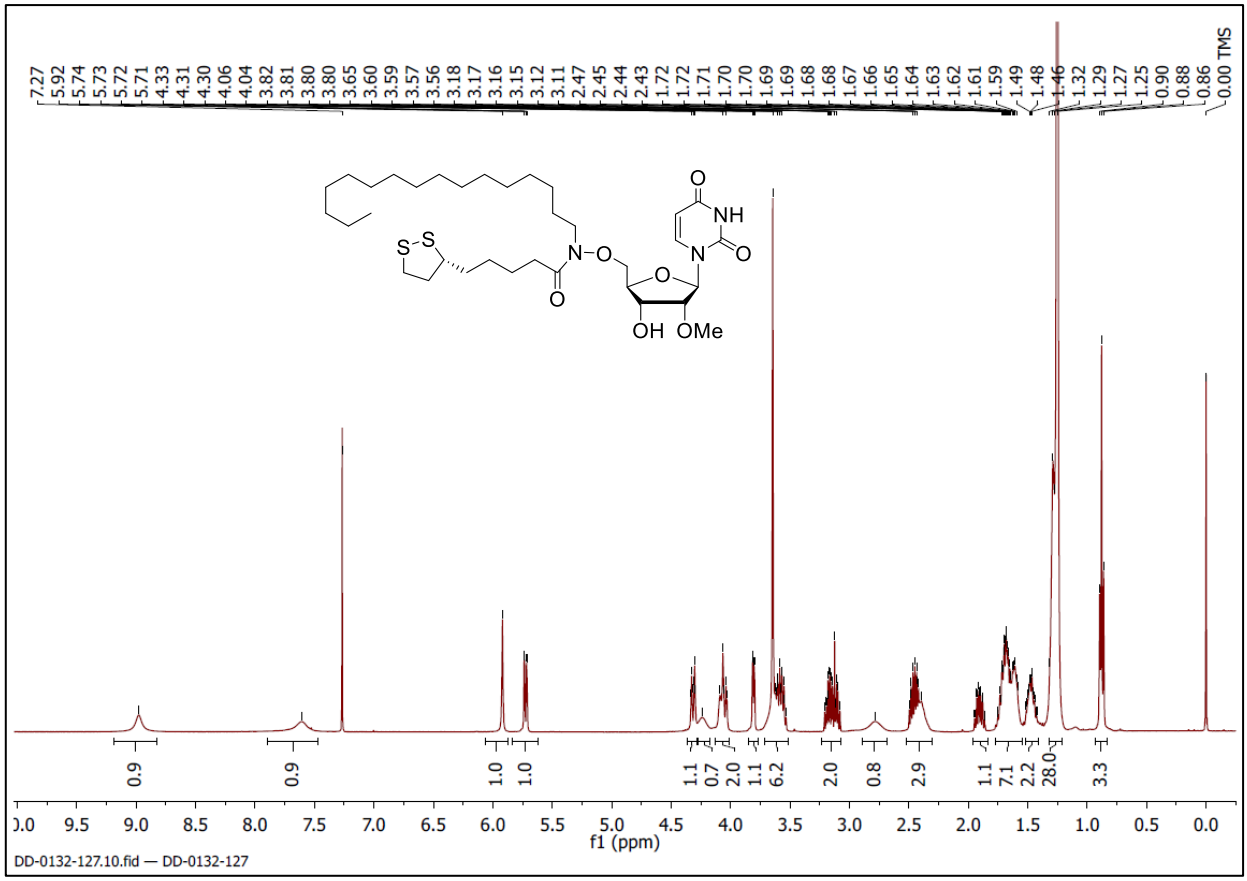
^{31}P NMR of compound **24** (243 MHz, CD_3CN)



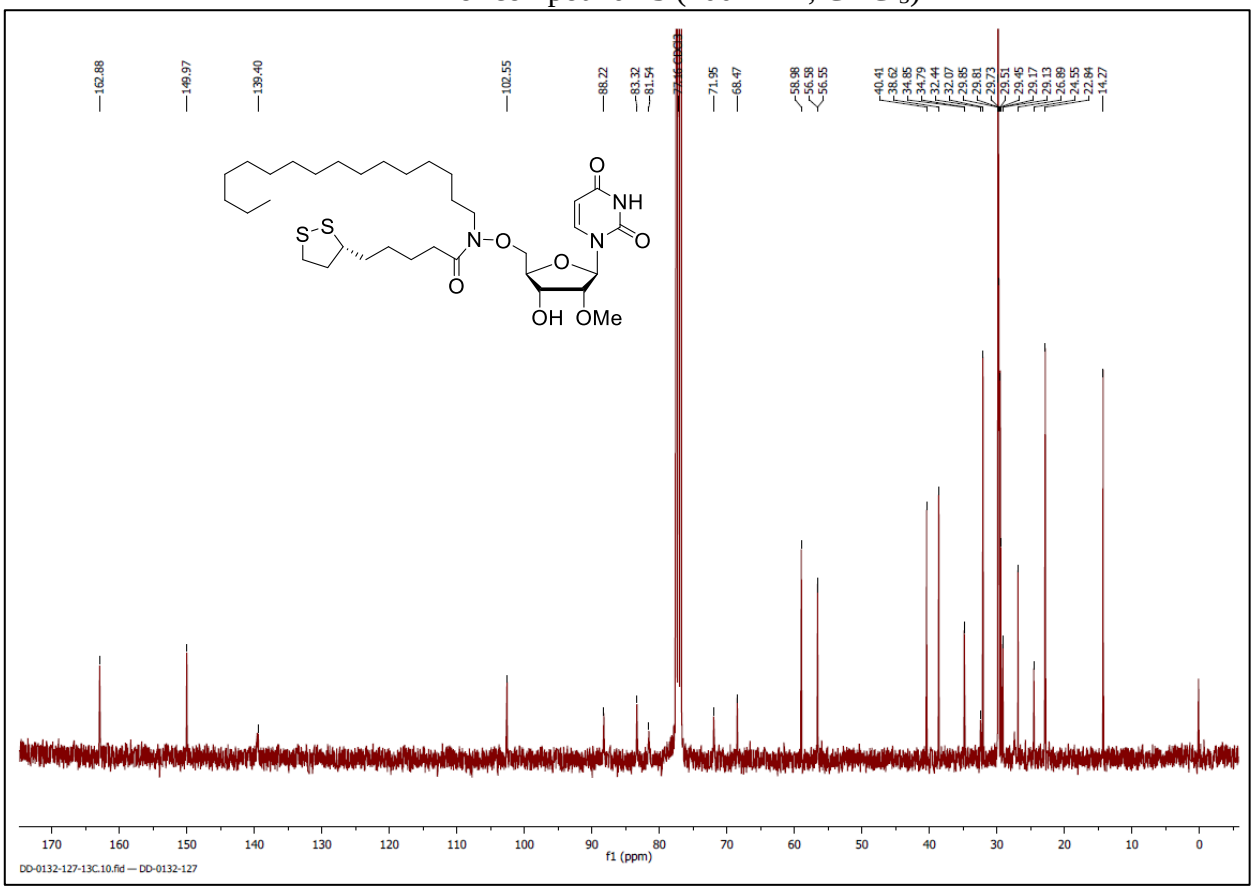
^{1}H NMR of compound **21** (400 MHz, $CDCl_3$)



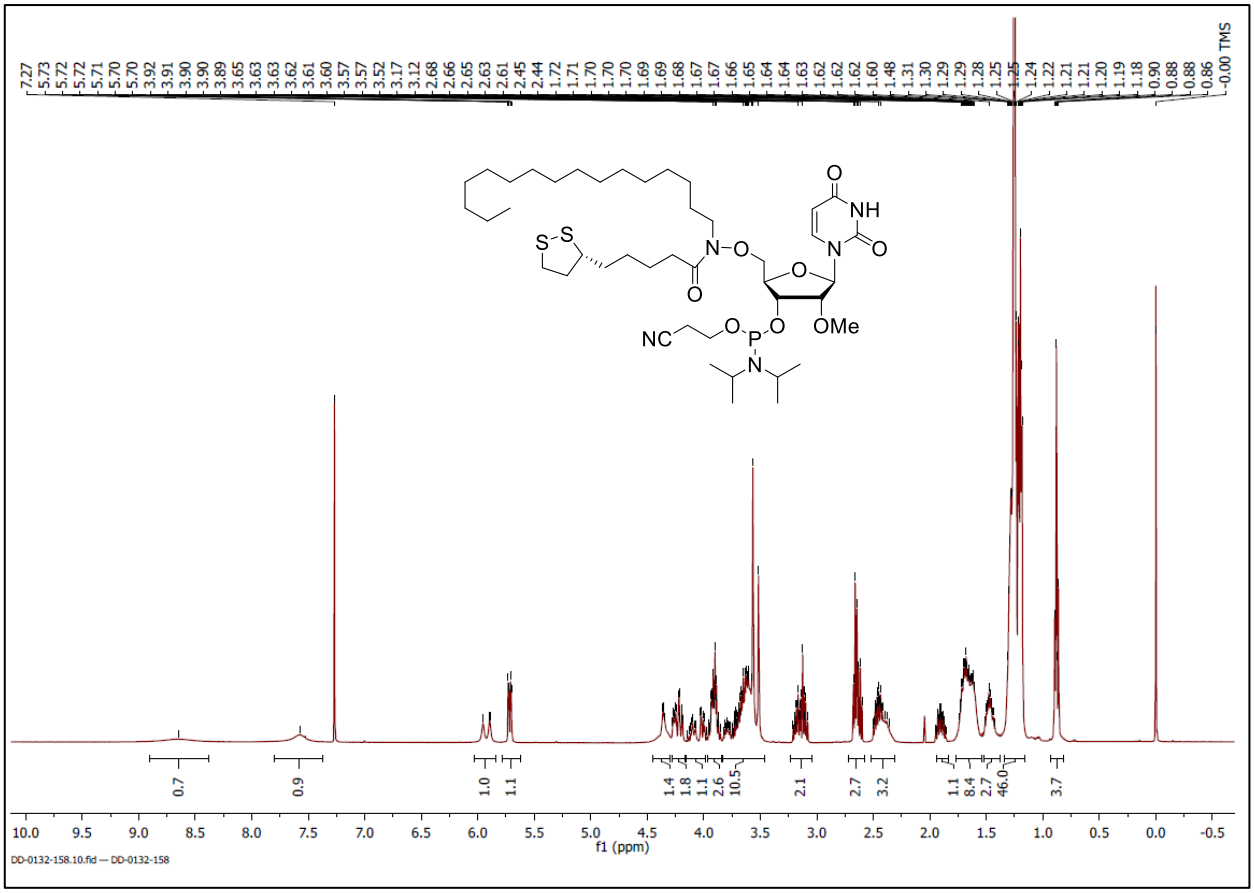
^{13}C NMR of compound **21** (101 MHz, $CDCl_3$)



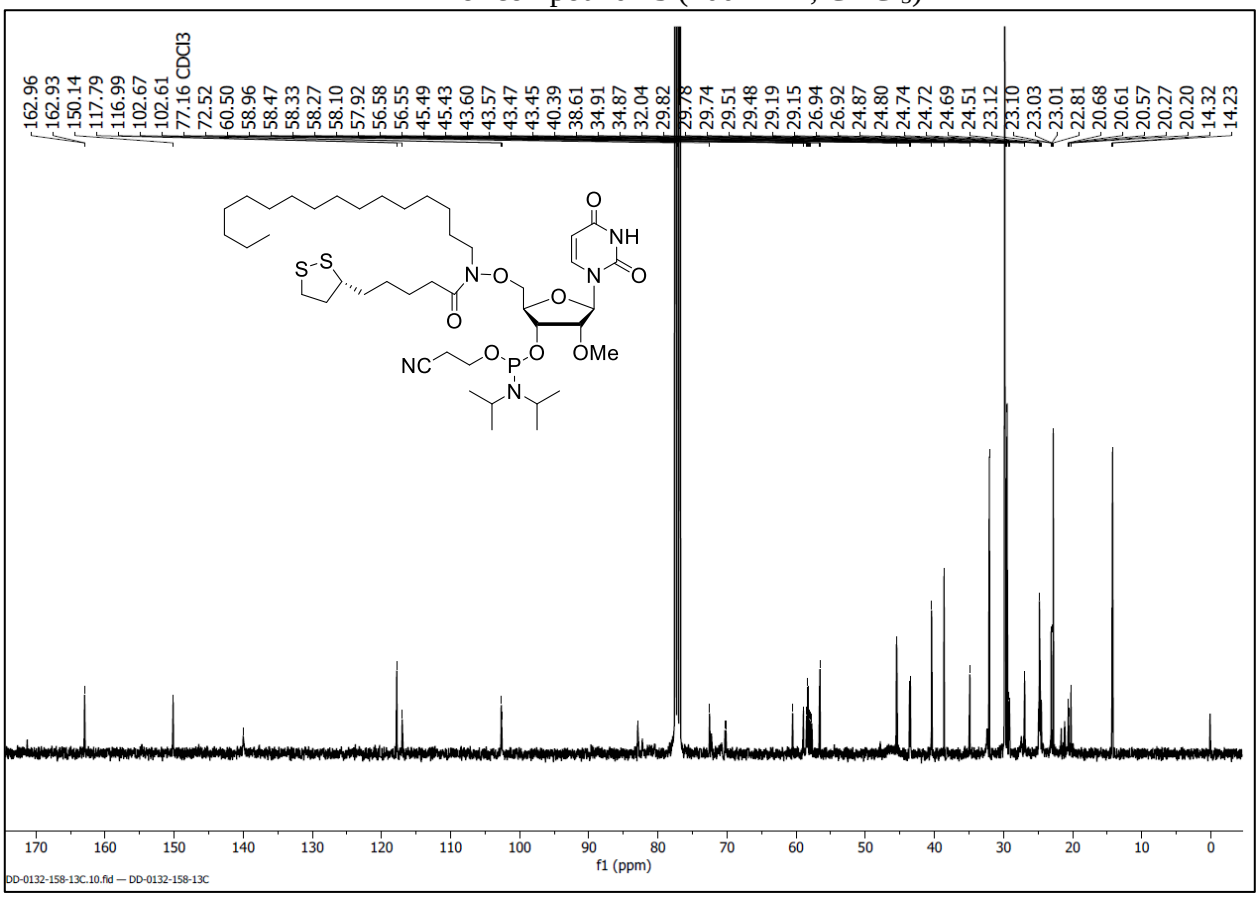
^{1}H NMR of compound **23** (400 MHz, $CDCl_3$)



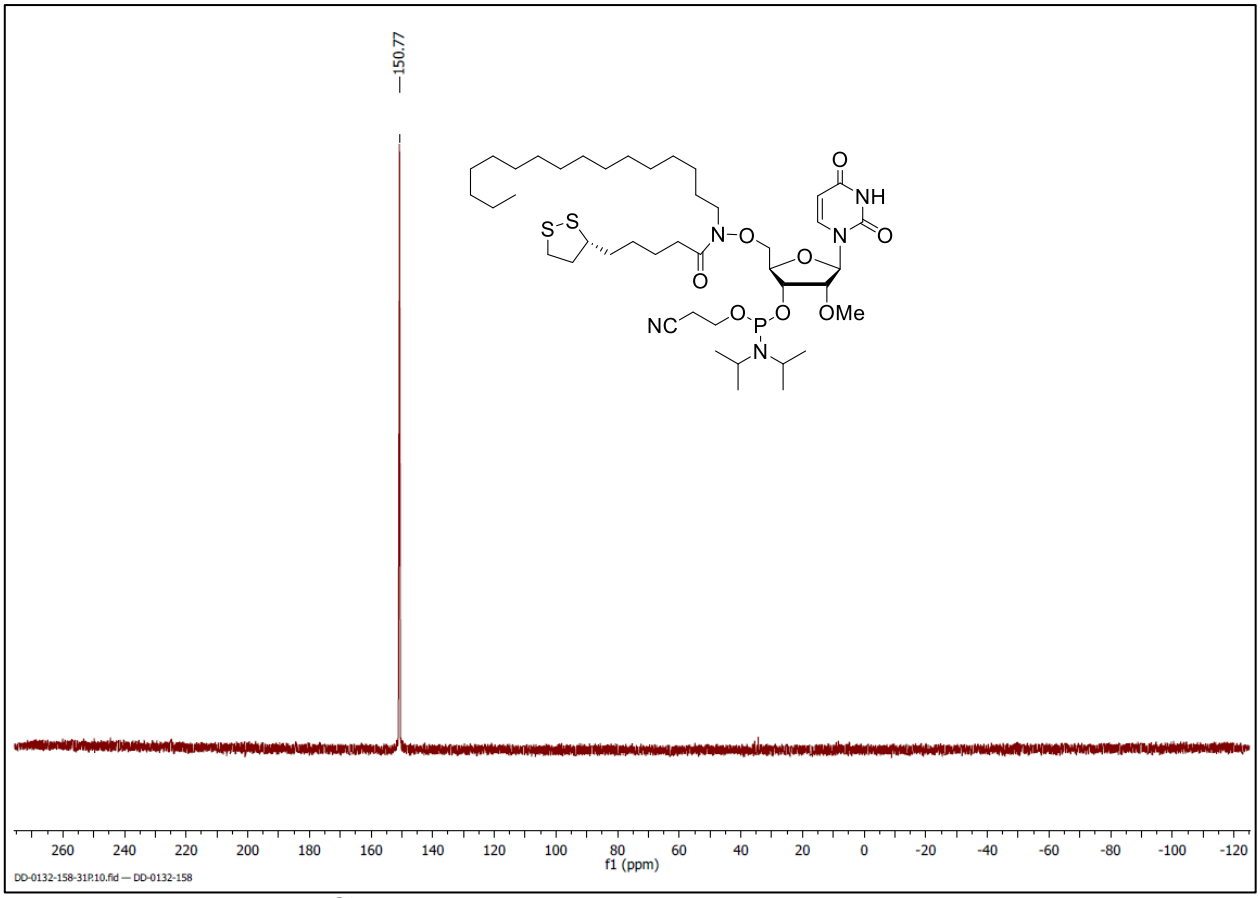
^{13}C NMR of compound **23** (101 MHz, $CDCl_3$)



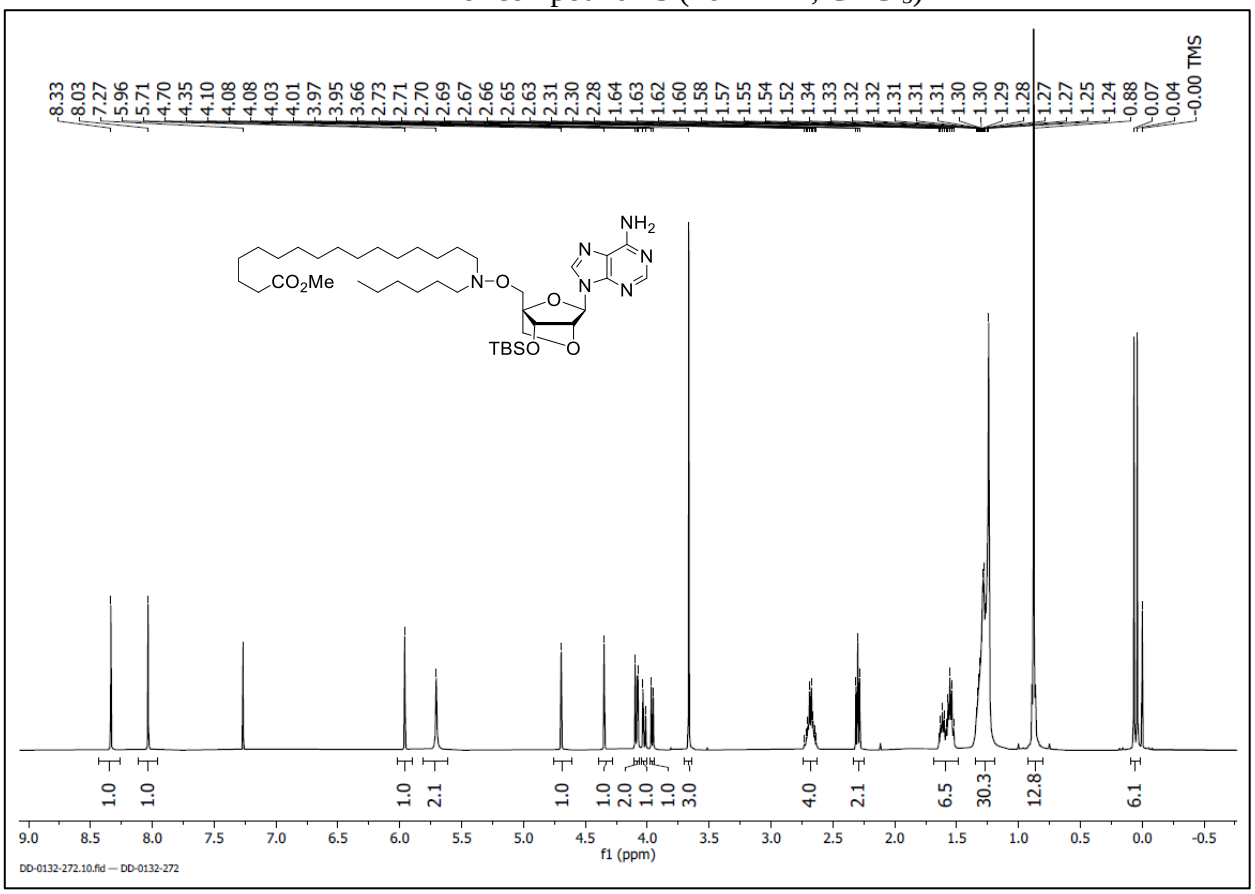
^{1}H NMR of compound **25** (400 MHz, $CDCl_3$)



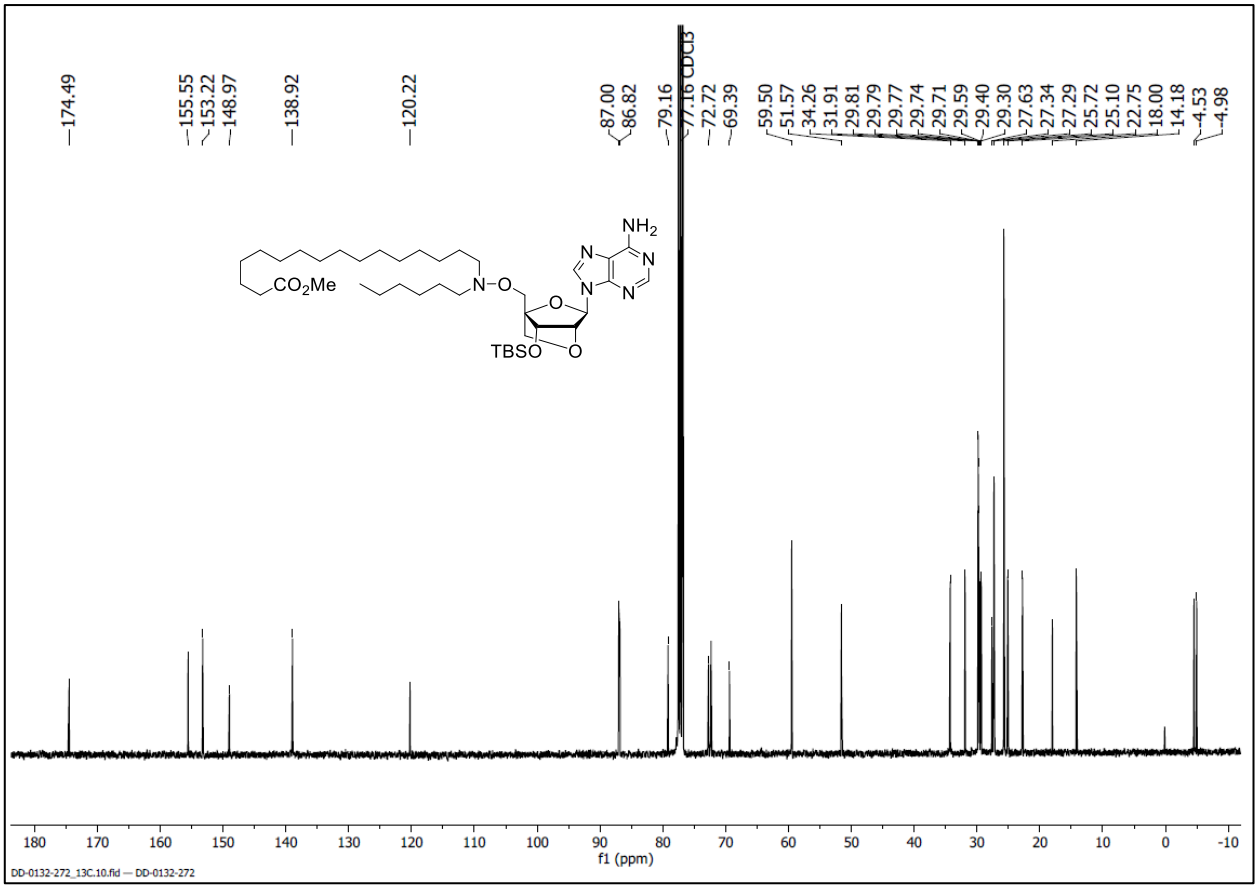
^{13}C NMR of compound **25** (101 MHz, $CDCl_3$)



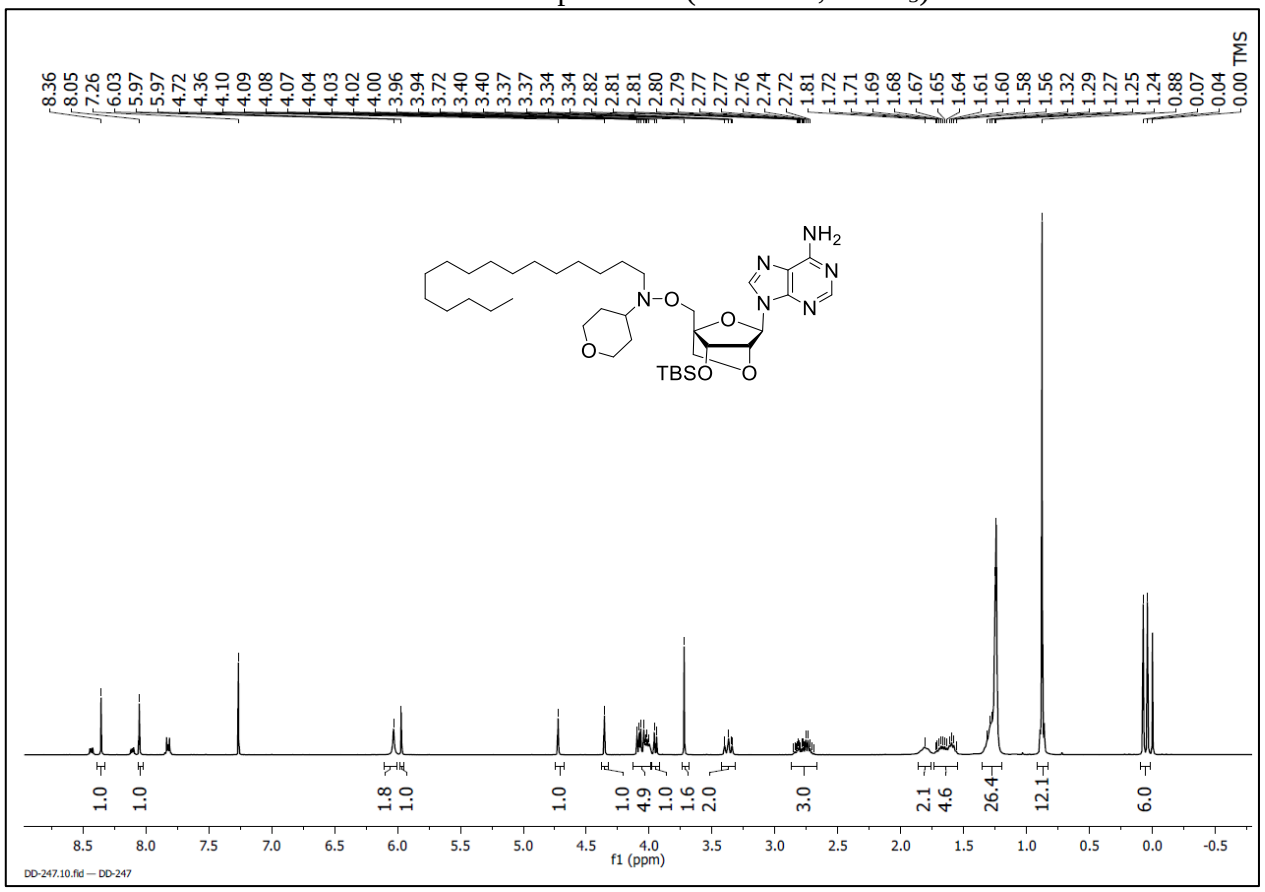
^{31}P NMR of compound **25** (162 MHz, $CDCl_3$)



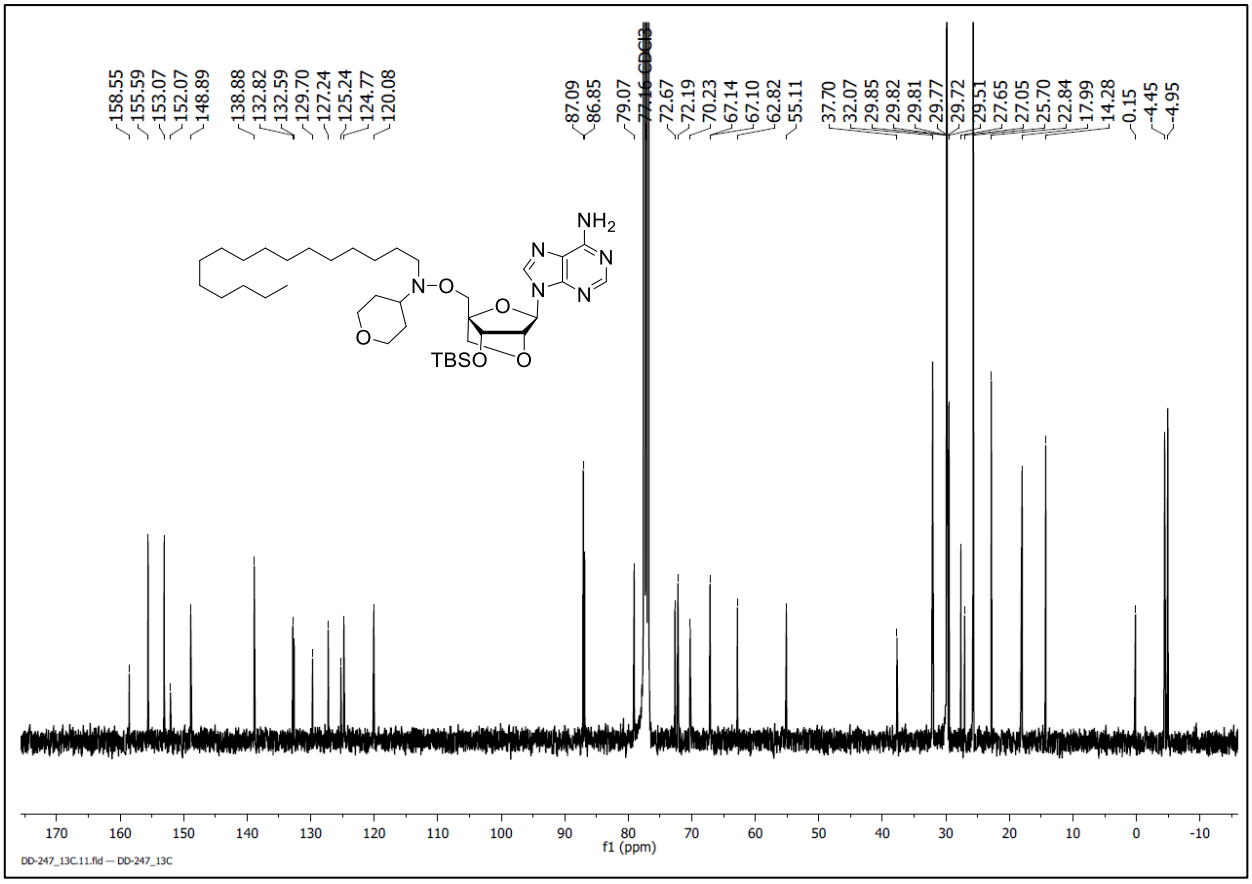
^{1}H NMR of compound **26** (500 MHz, $CDCl_3$)



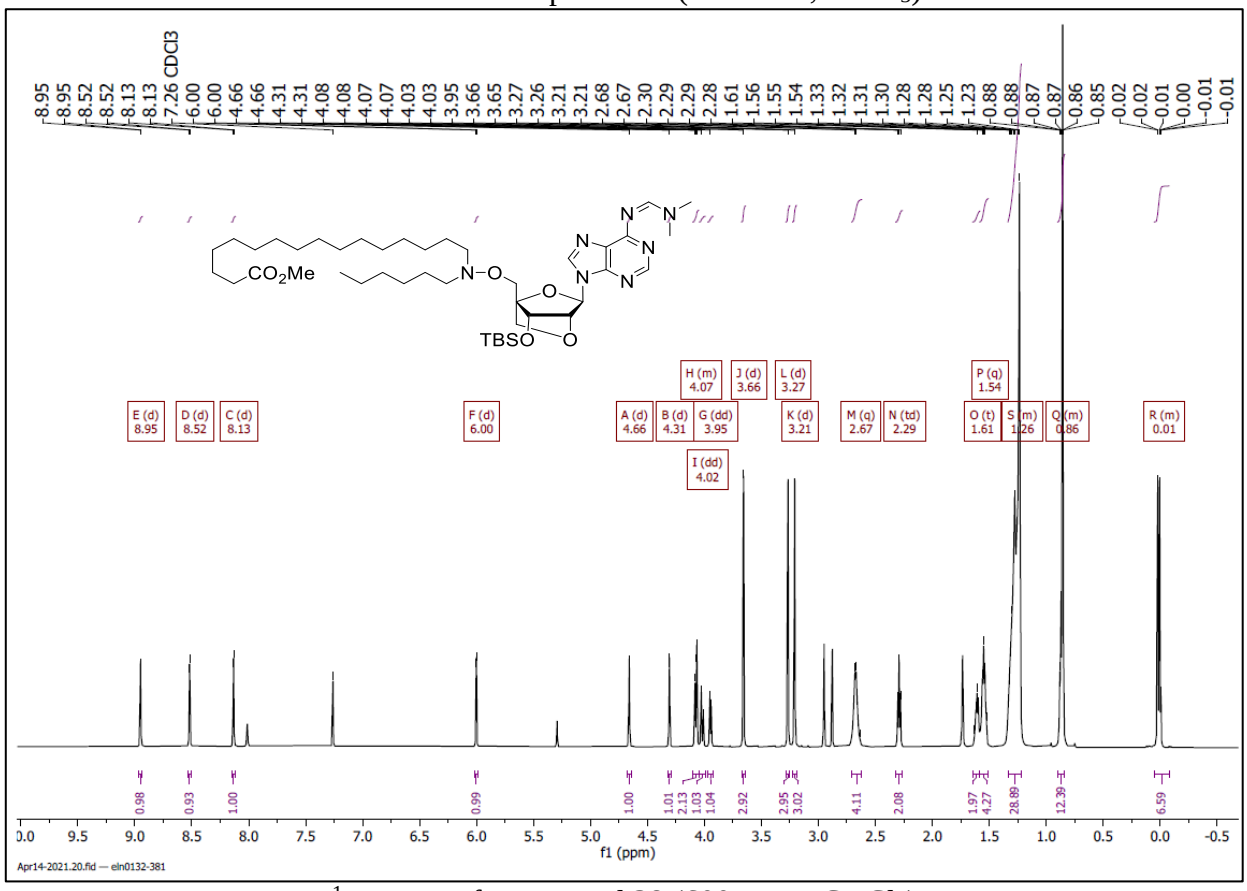
^{13}C NMR of compound **26** (101 MHz, $CDCl_3$)



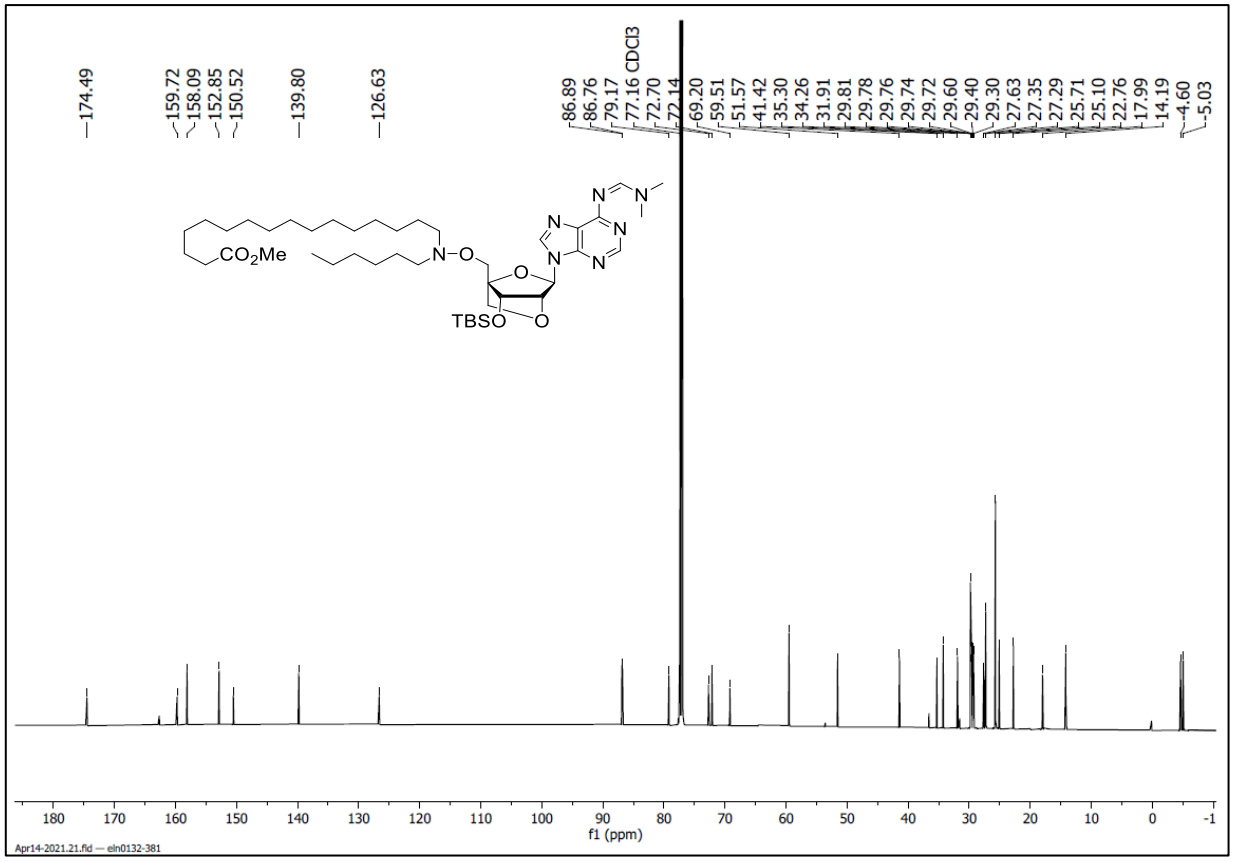
^{1}H NMR of compound **27** (400 MHz, $CDCl_3$)



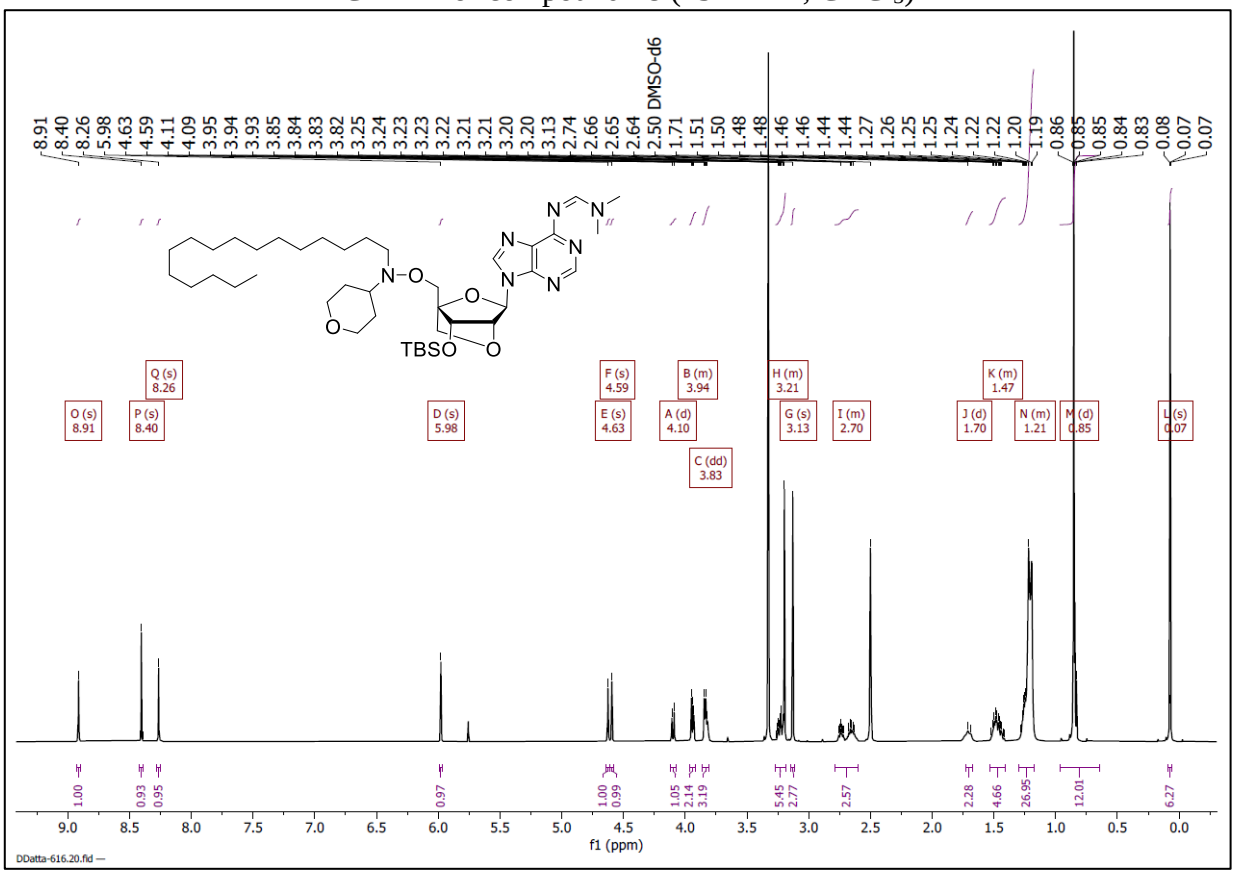
^{13}C NMR of compound **27** (101 MHz, $CDCl_3$)



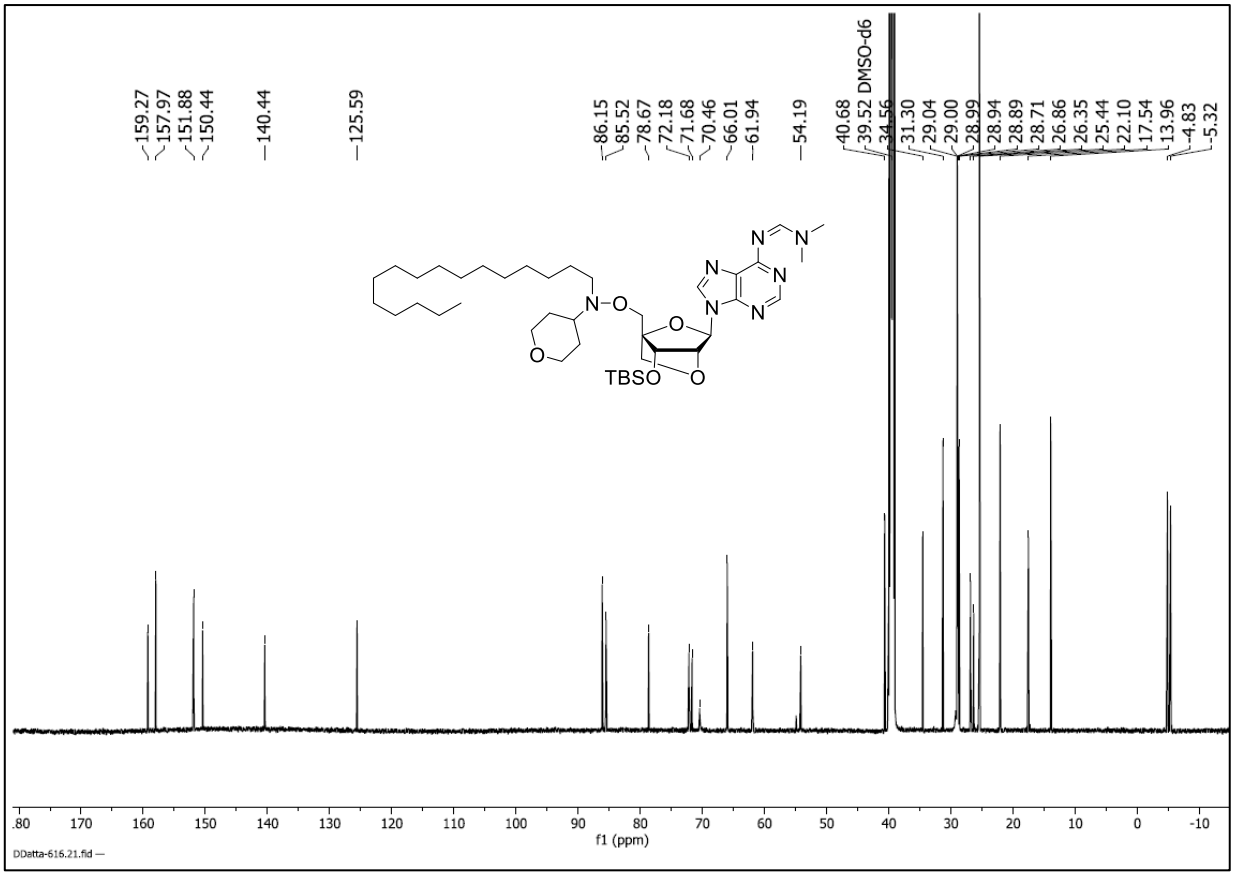
^{1}H NMR of compound **28** (600 MHz, $CDCl_3$)



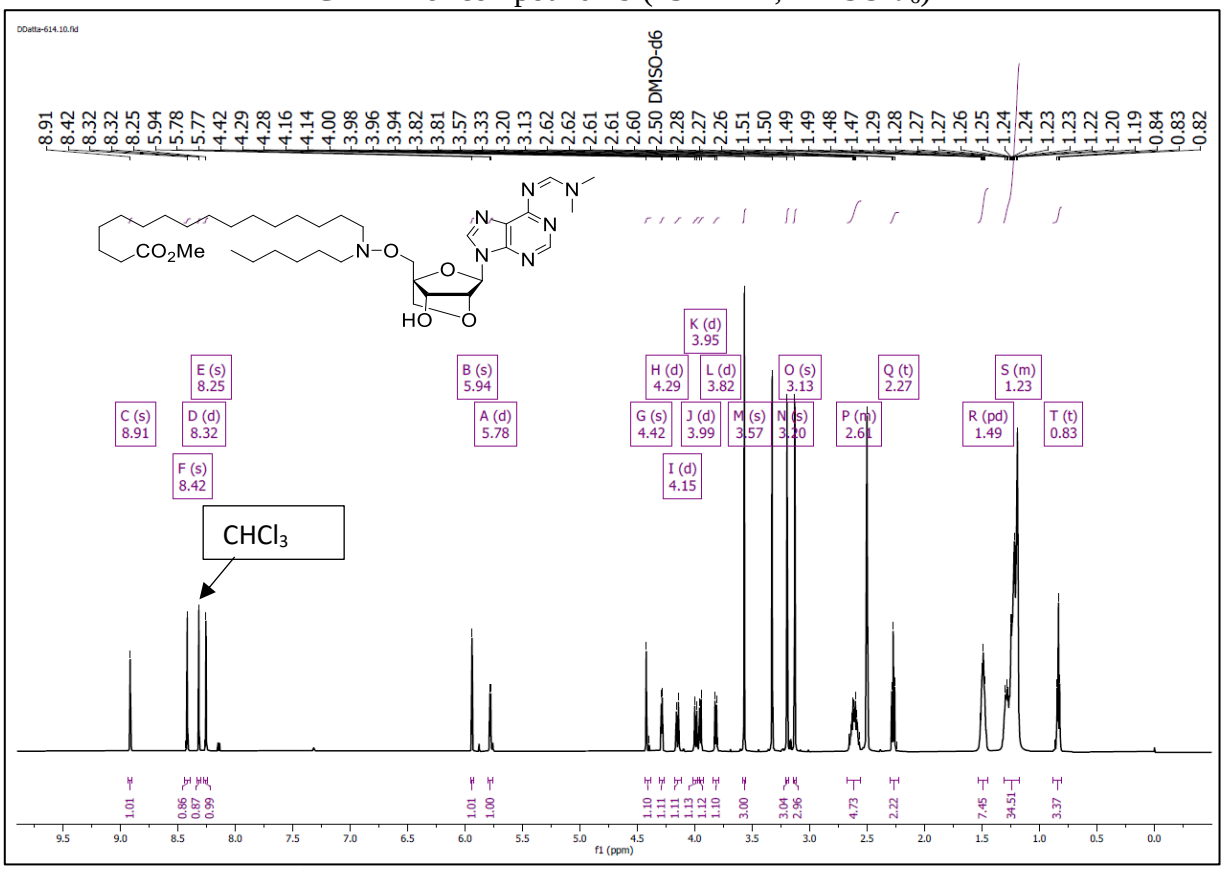
^{13}C NMR of compound **28** (151 MHz, $CDCl_3$)



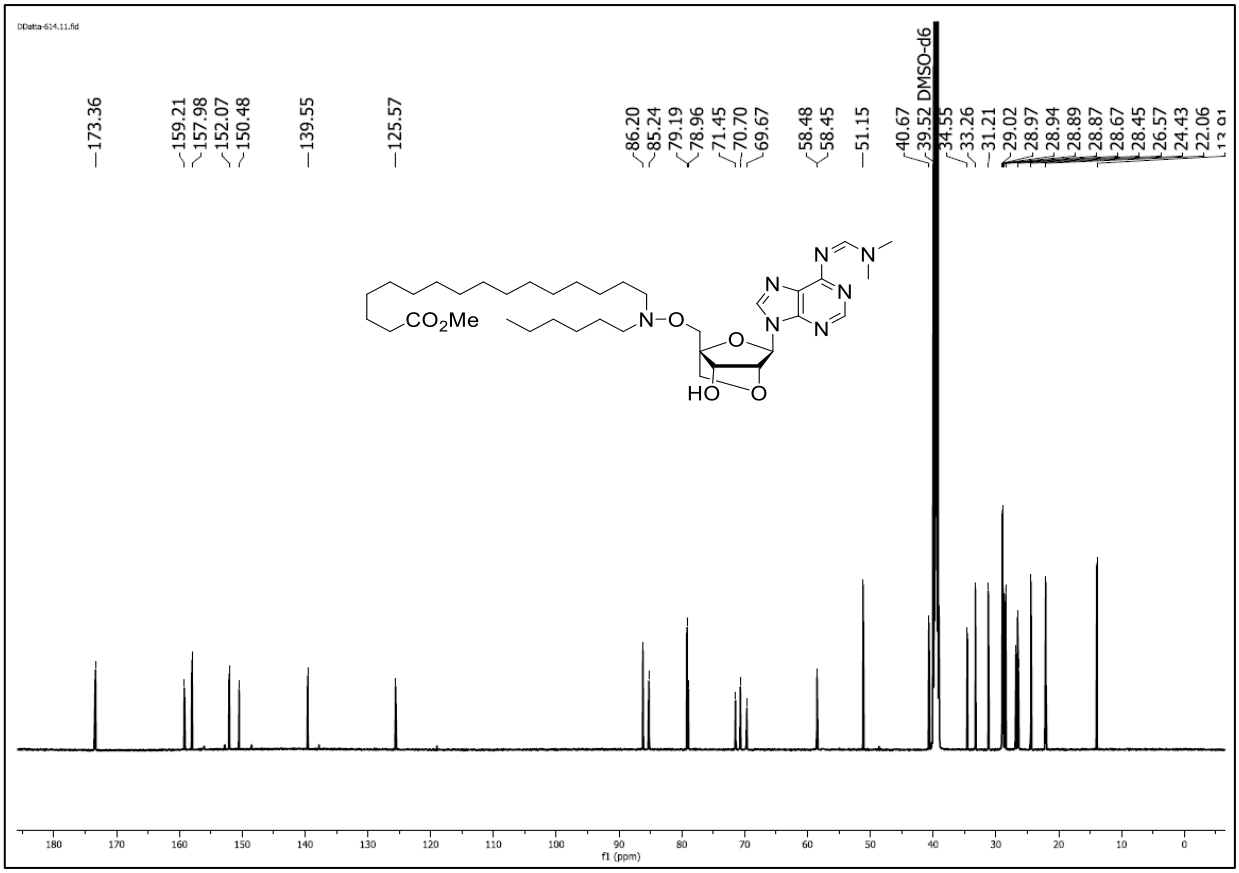
^{1}H NMR of compound **29** (600 MHz, DMSO-d_6)



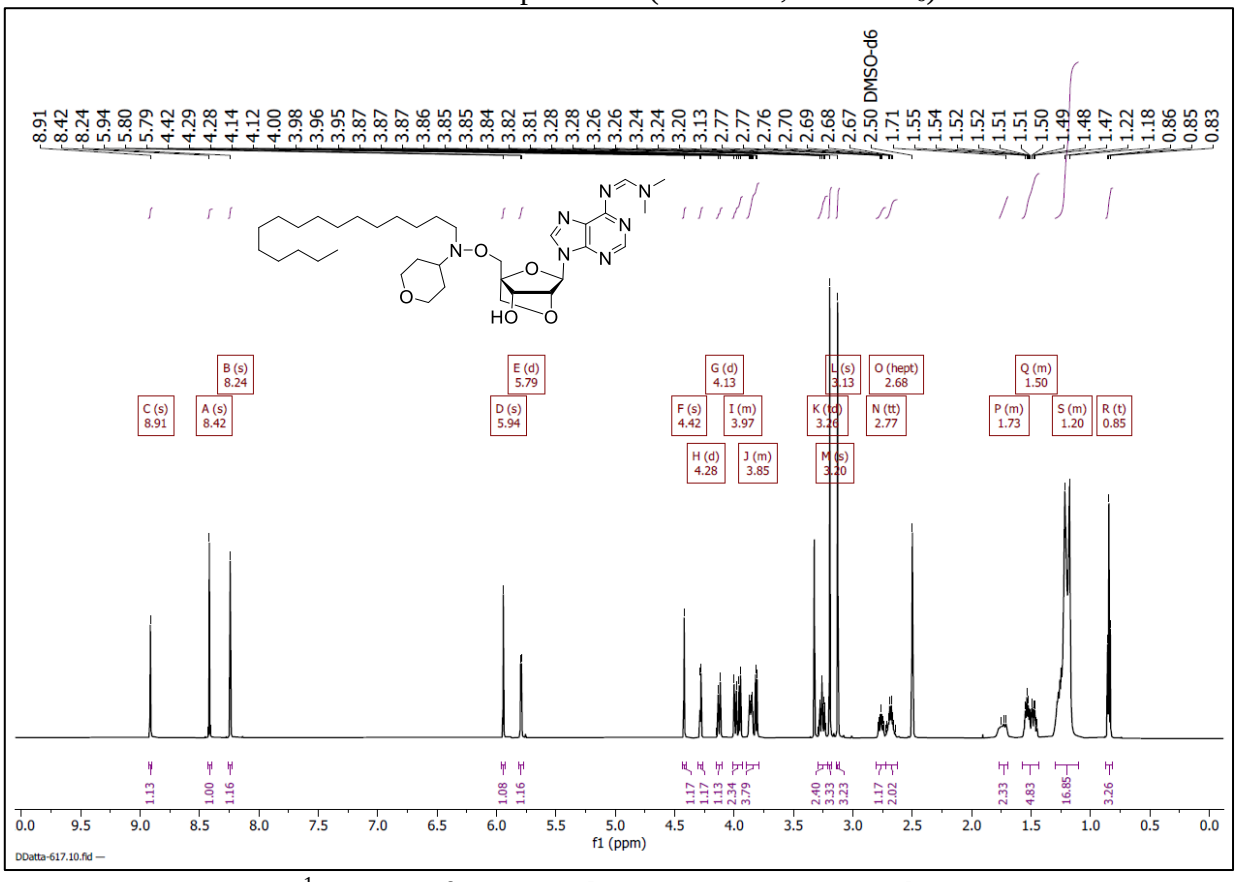
^{13}C NMR of compound **29** (151 MHz, DMSO-d_6)



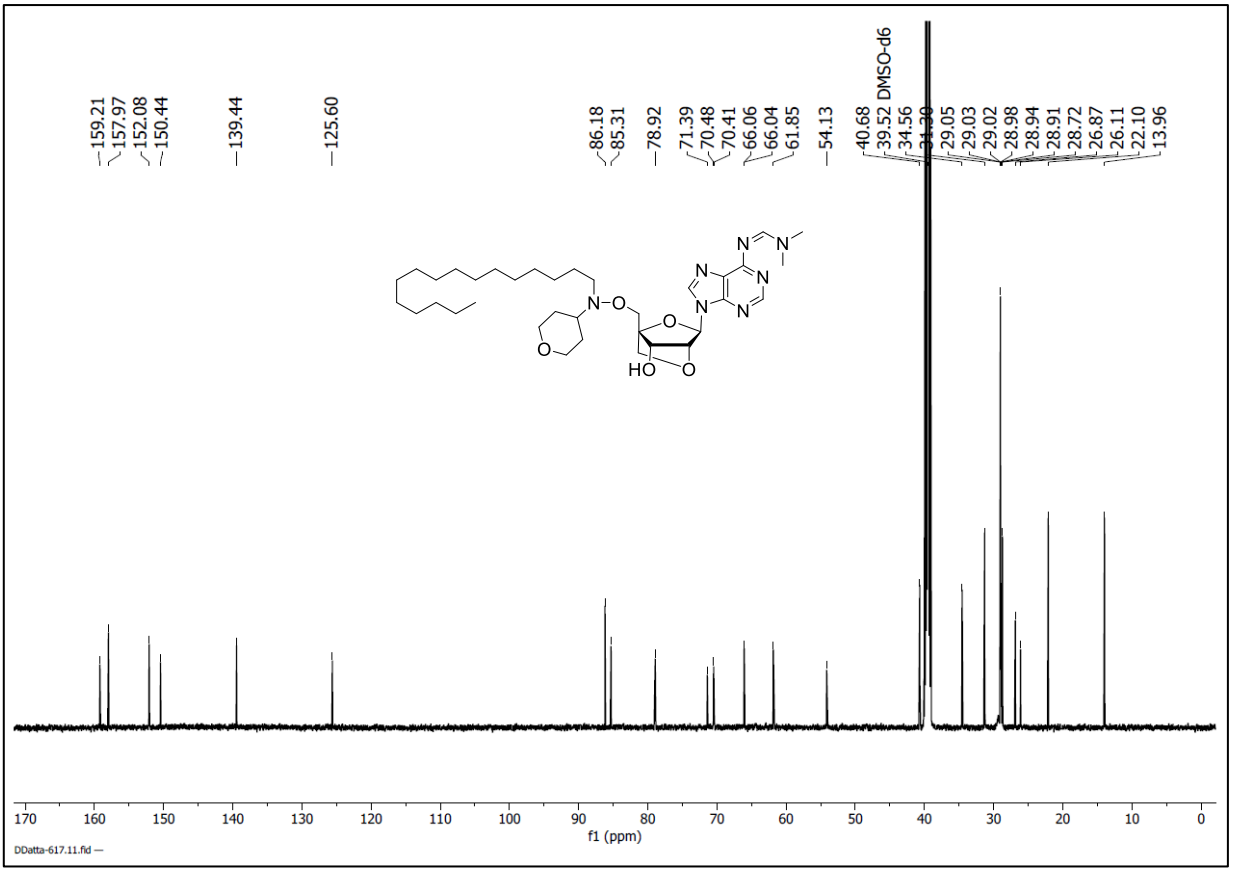
^{1}H NMR of compound **30** (600 MHz, DMSO-d_6)



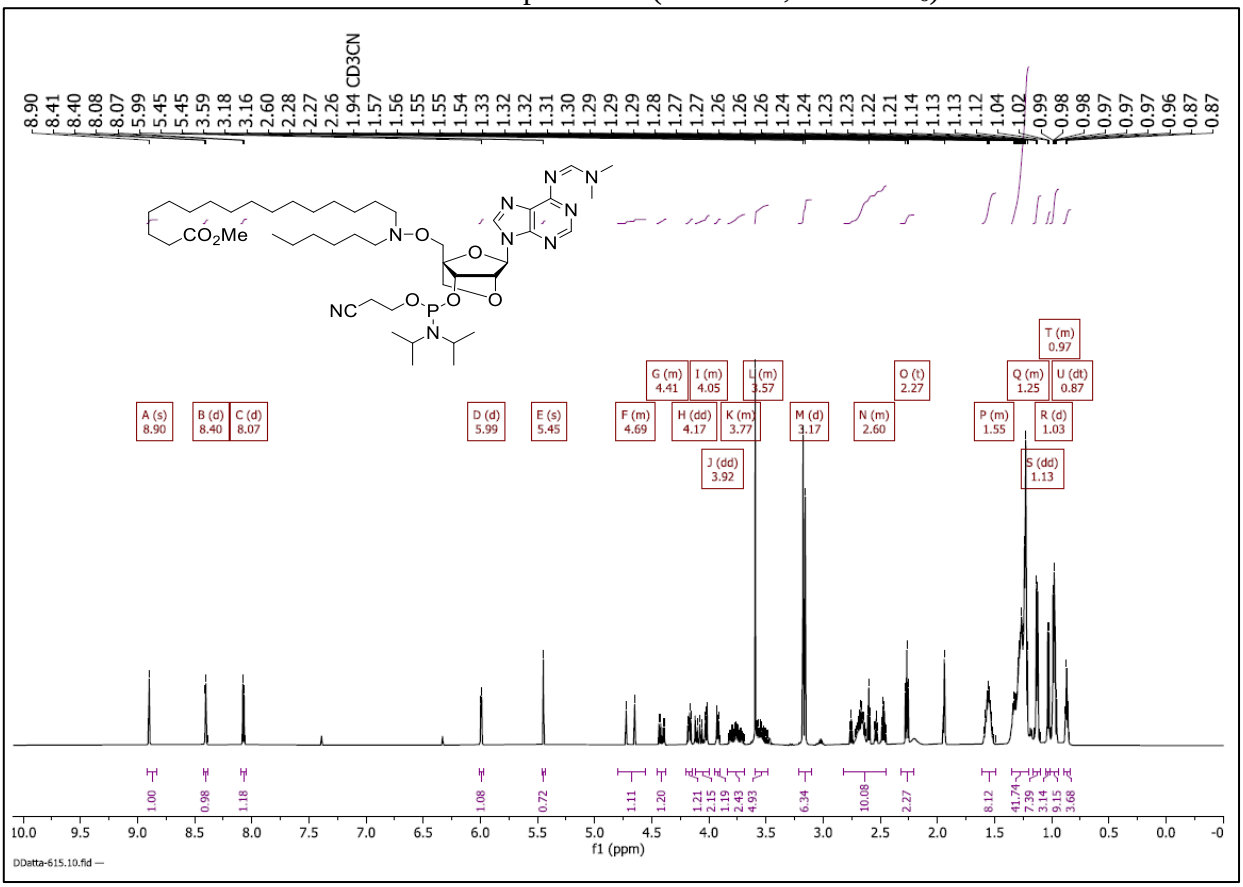
^{13}C NMR of compound **30** (151 MHz, DMSO-d_6)



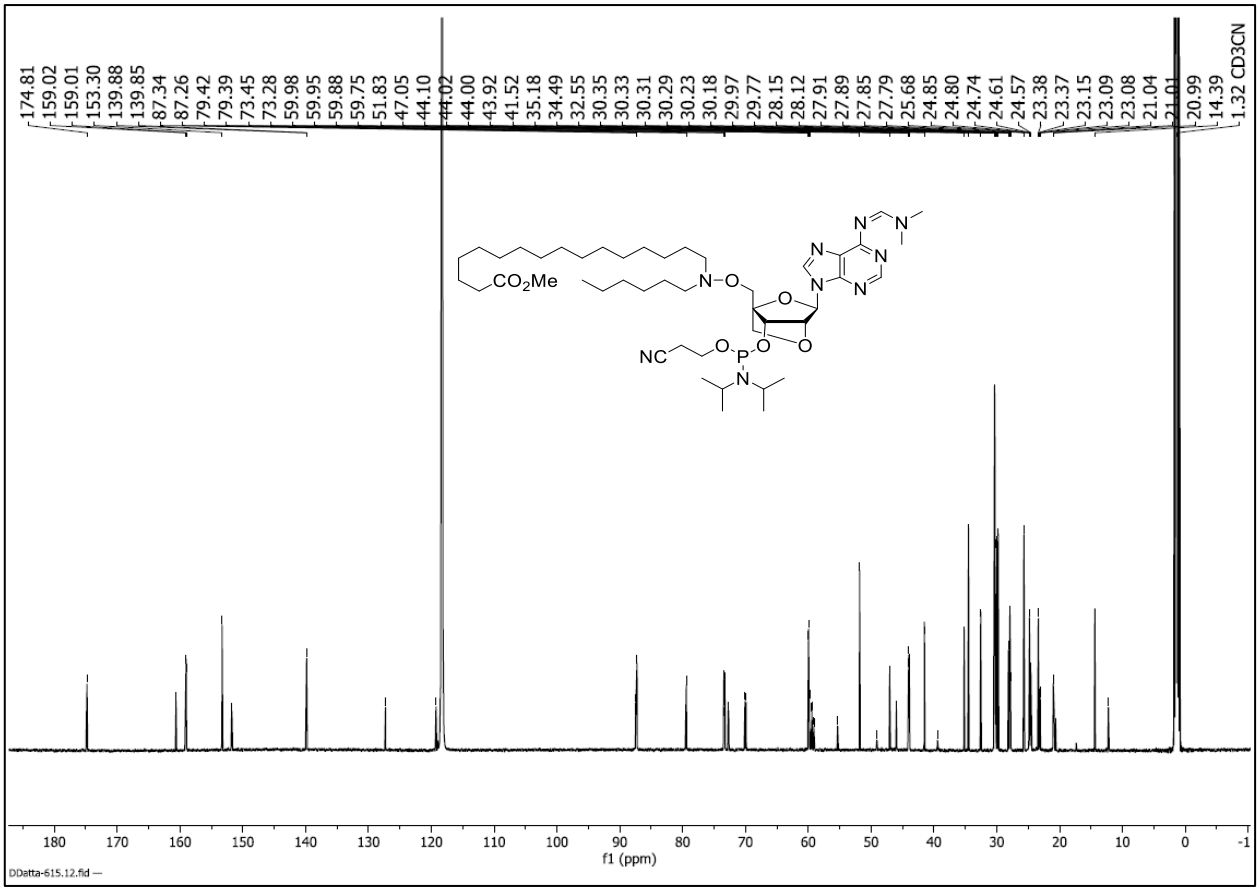
^{1}H NMR of compound **31** (600 MHz, DMSO-d_6)



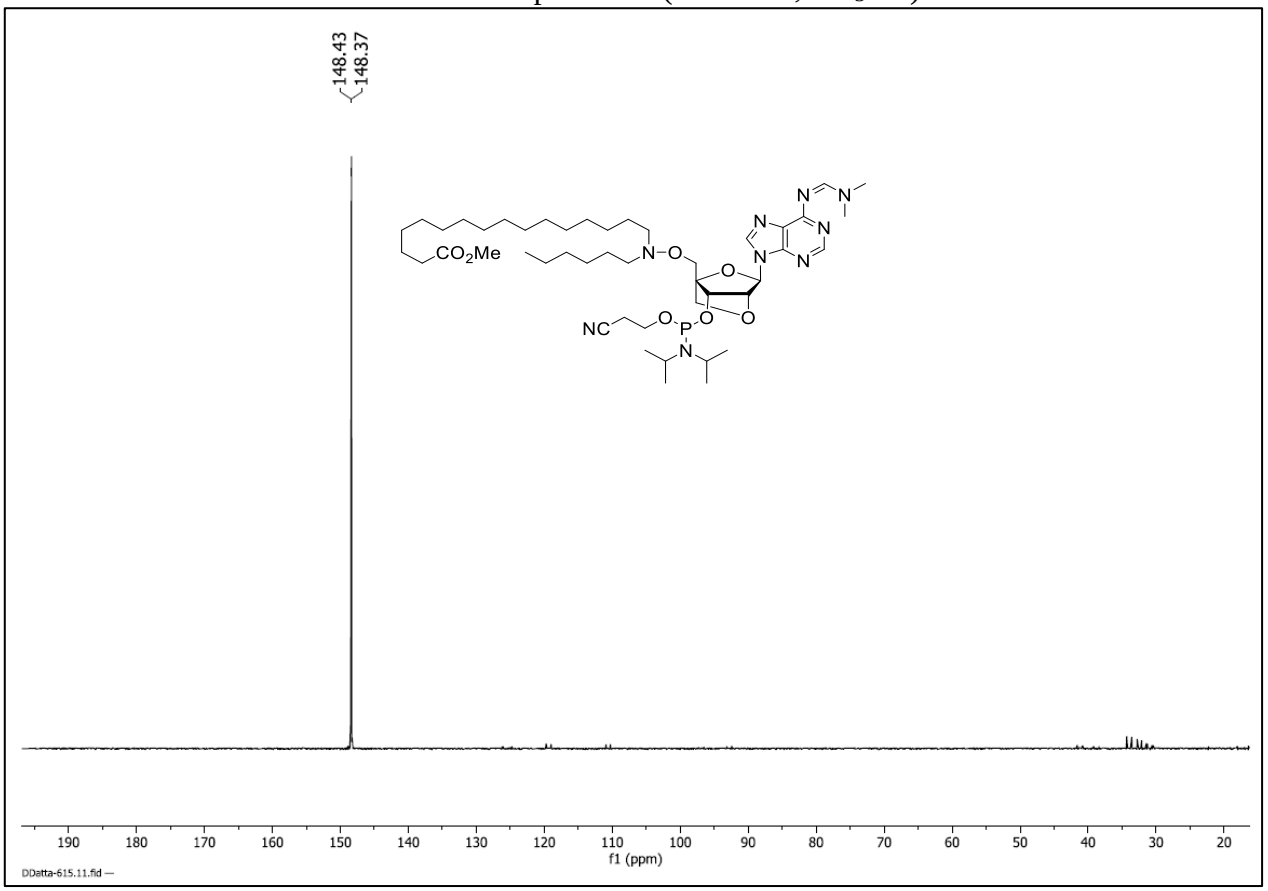
^{13}C NMR of compound **31** (151 MHz, DMSO-d_6)



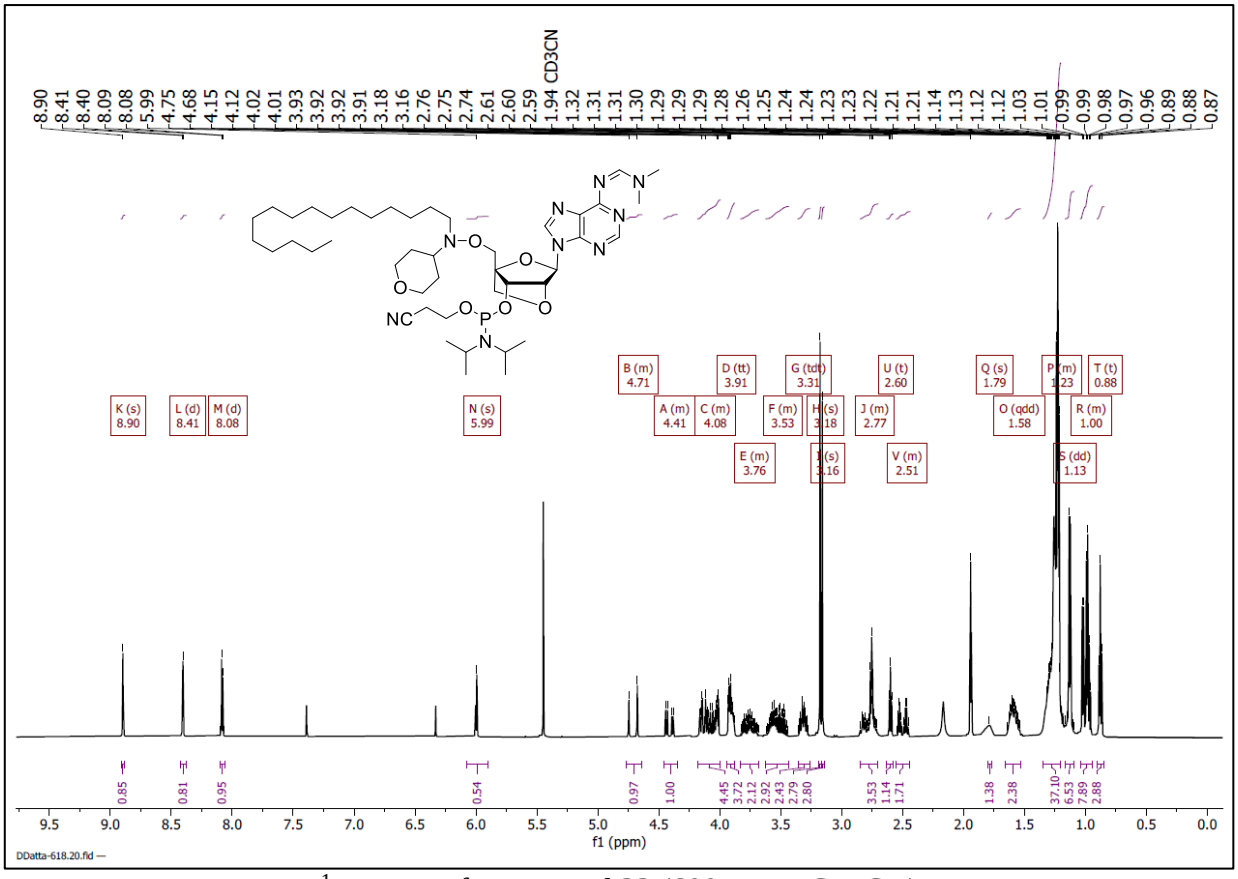
^{1}H NMR of compound **32** (600 MHz, CD_3CN)



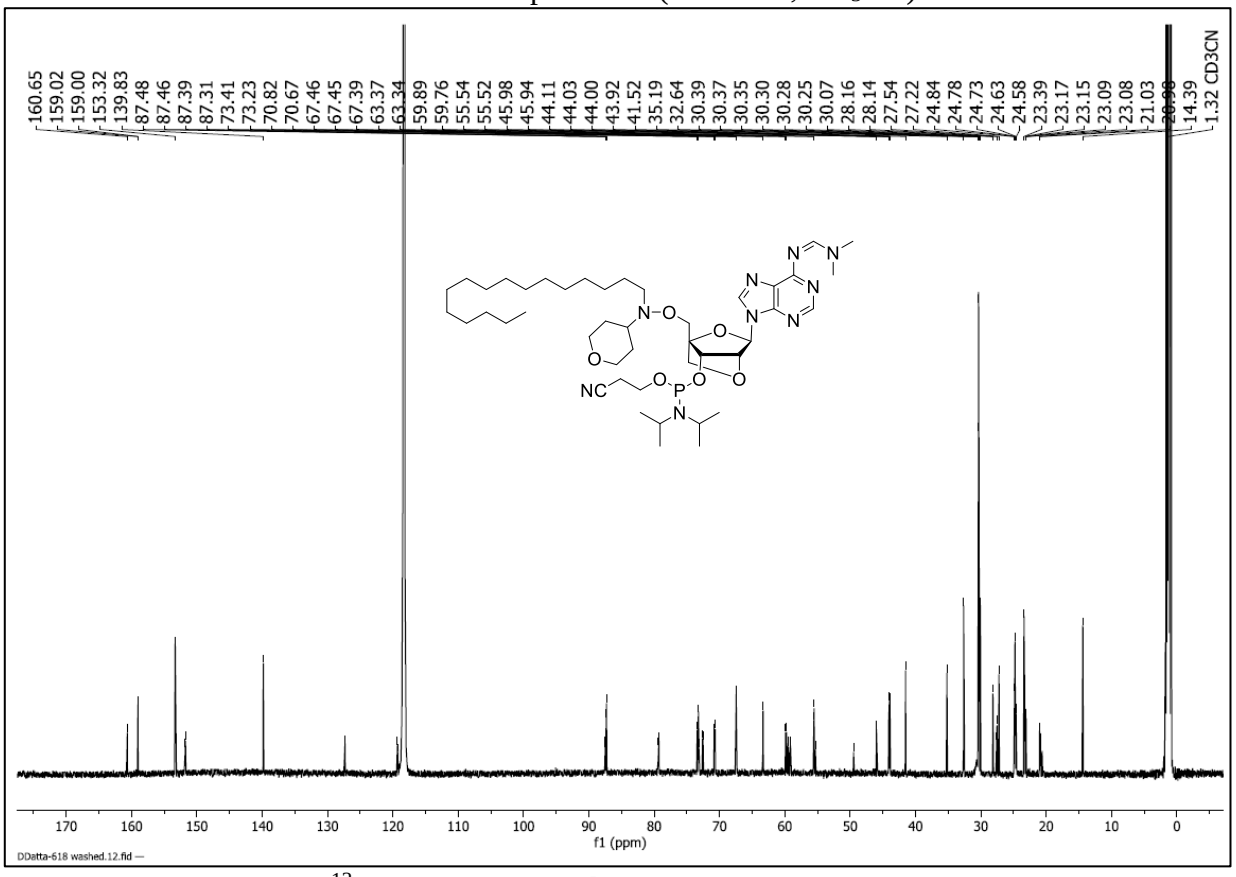
^{13}C NMR compound **32** (151 MHz, CD_3CN)



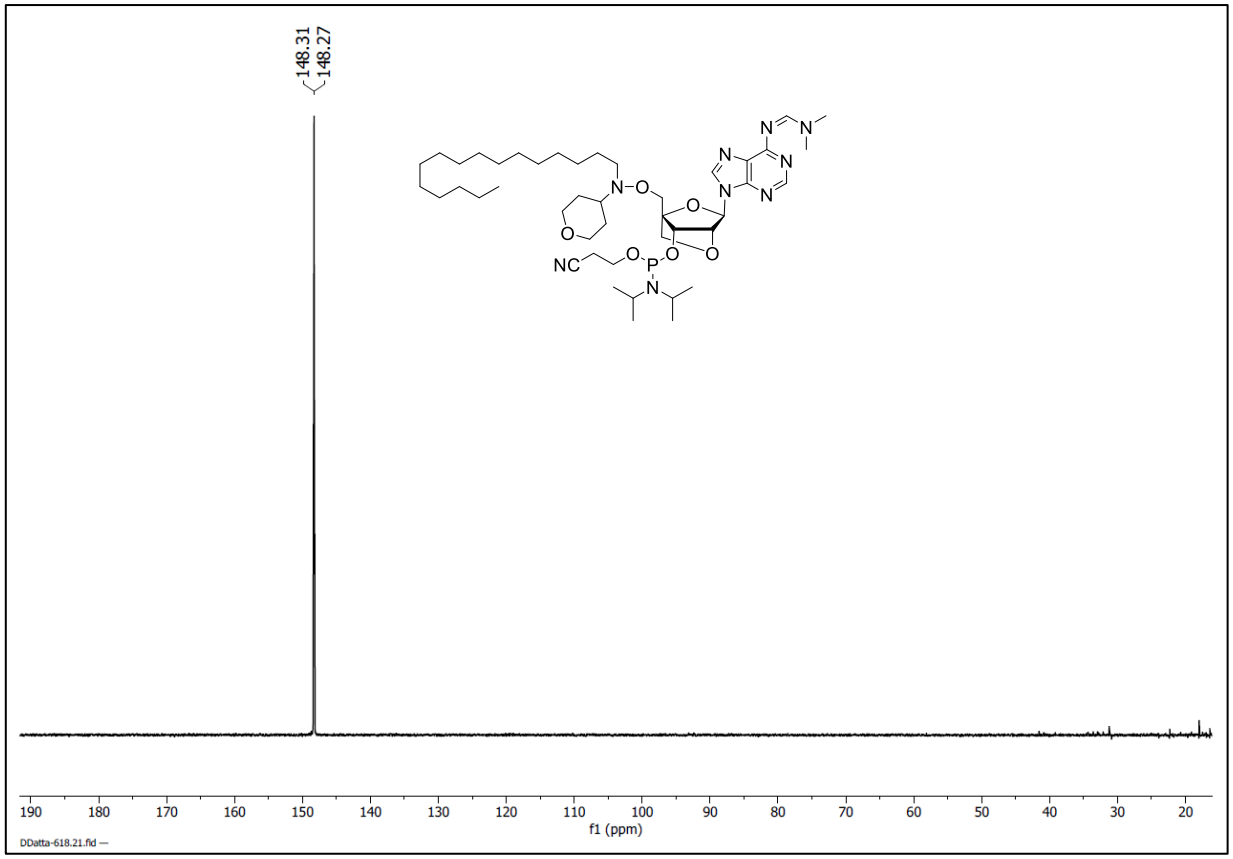
^{31}P NMR of compound **32** (243 MHz, CD_3CN)



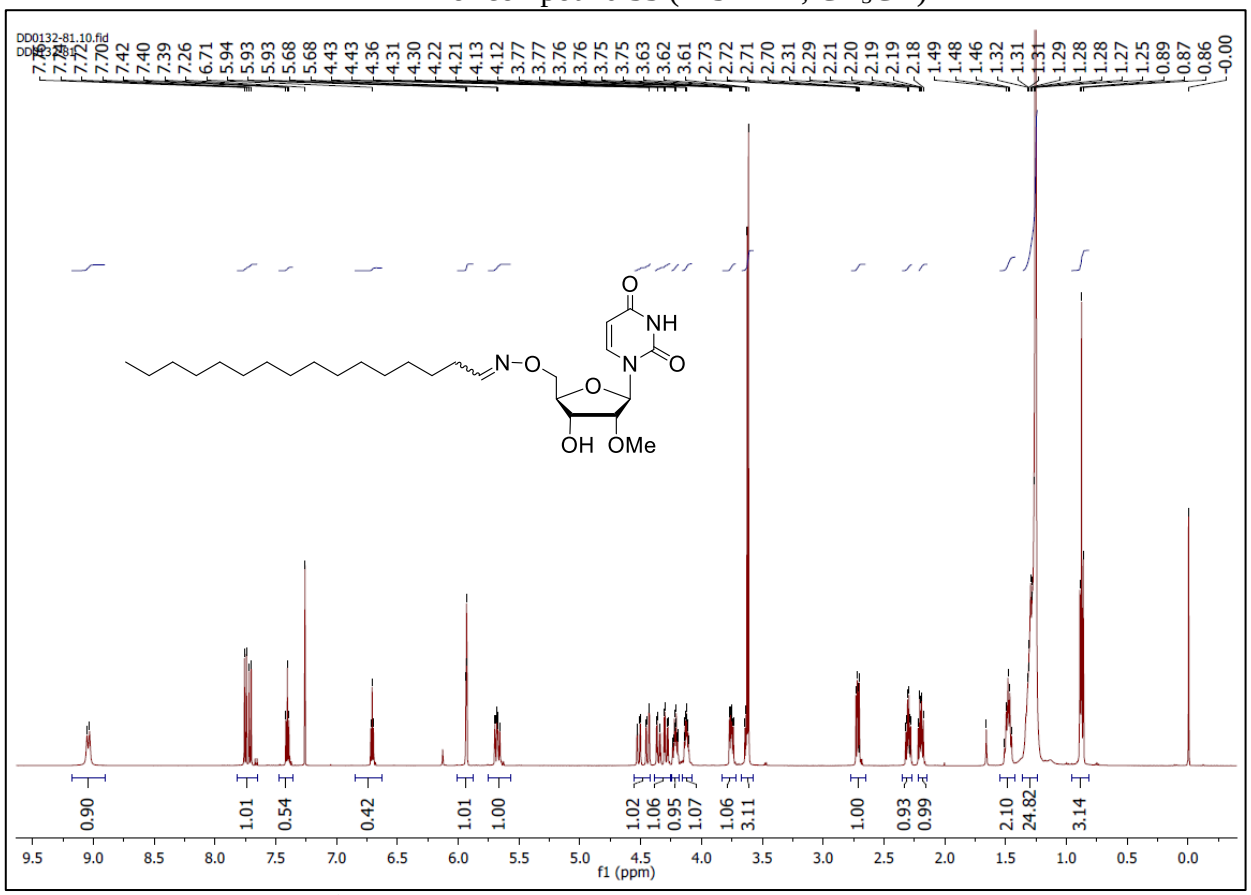
^{1}H NMR of compound **33** (600 MHz, CD_3CN)



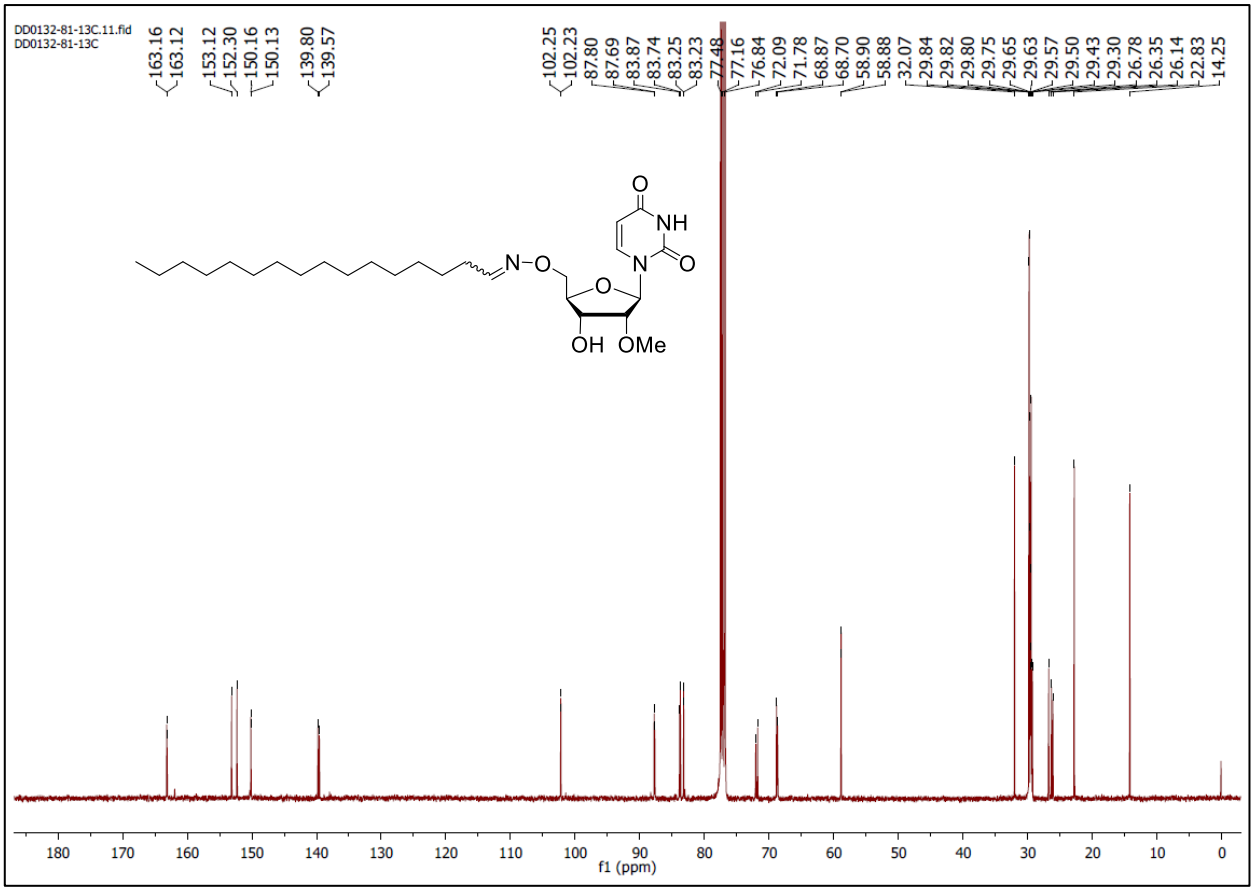
^{13}C NMR compound **33** (151 MHz, CD_3CN)



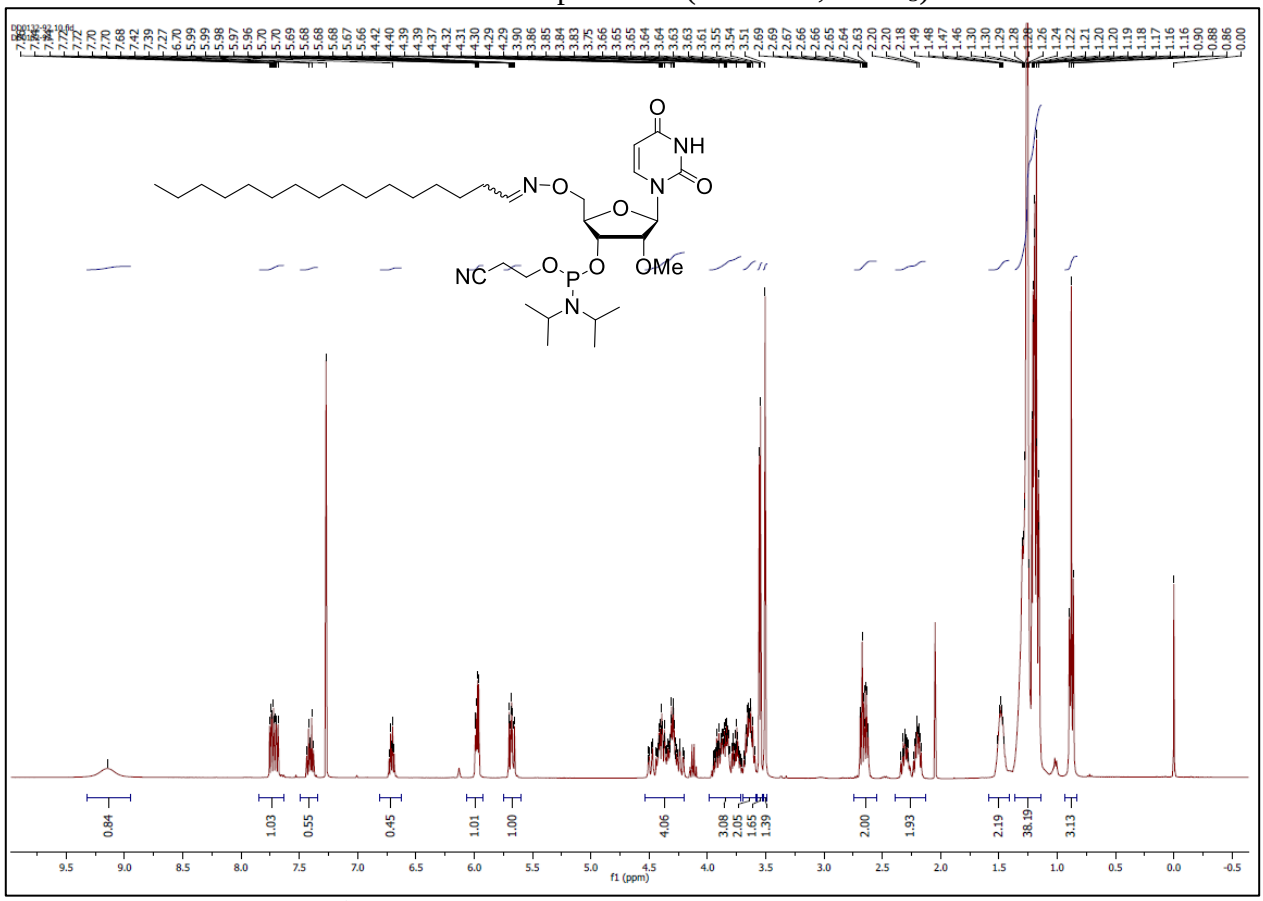
^{31}P NMR of compound **33** (243 MHz, CD_3CN)



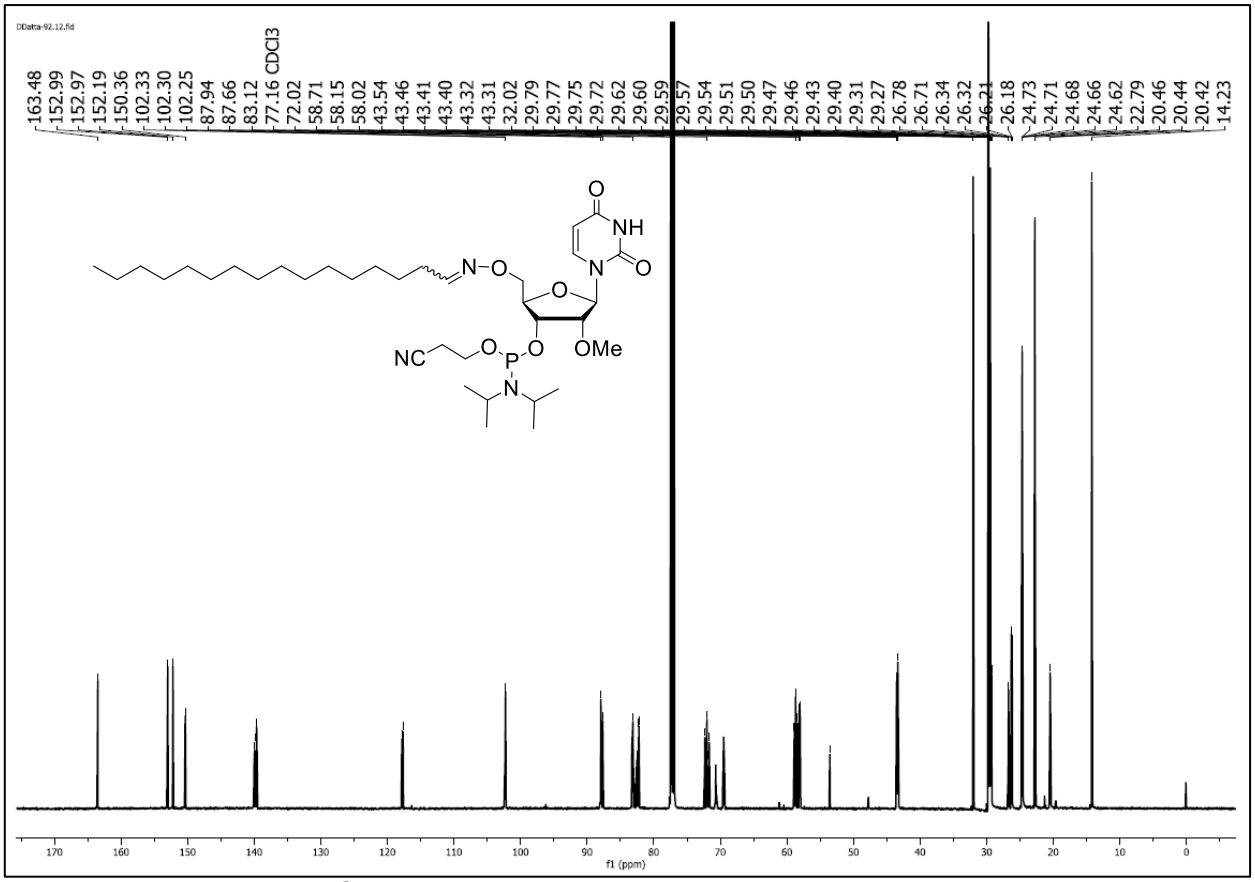
^{1}H NMR of compound **34** (500 MHz, $CDCl_3$)



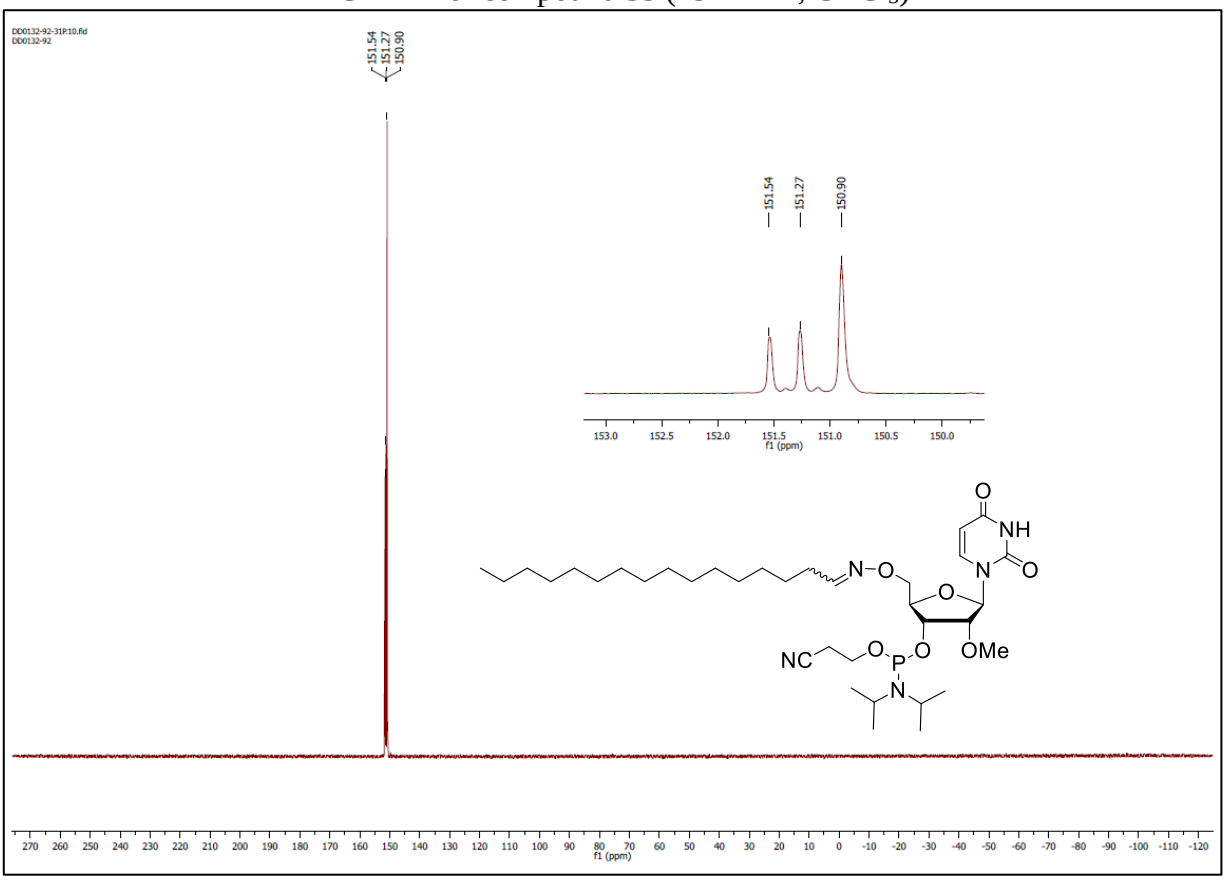
^{13}C NMR of compound **34** (101 MHz, $CDCl_3$)



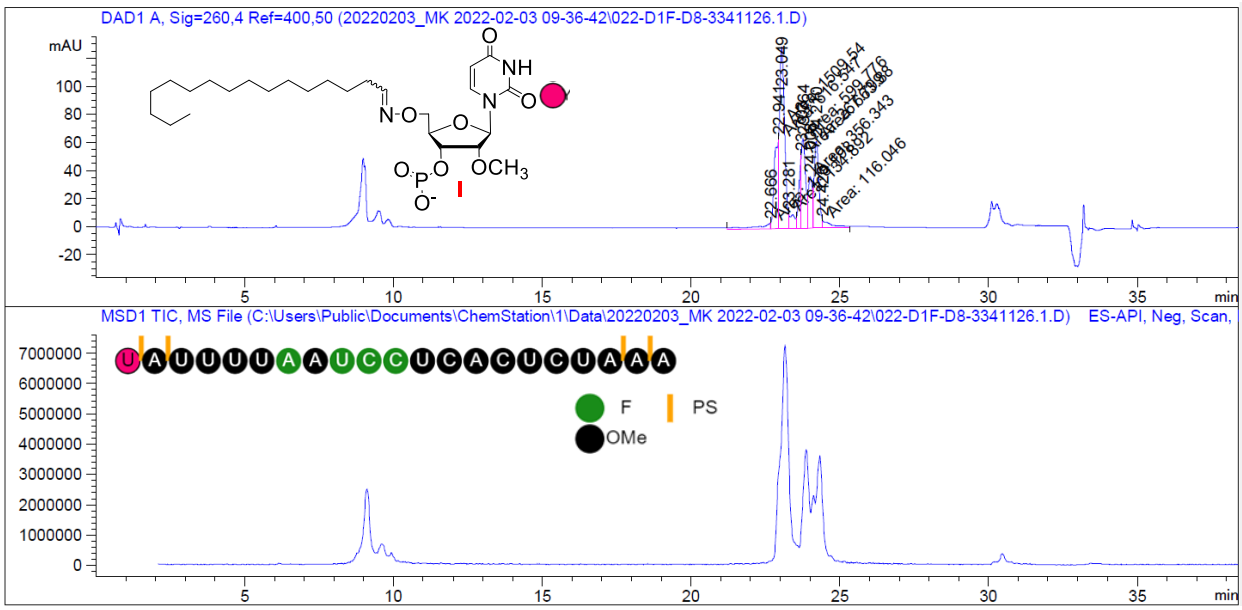
^{1}H NMR of compound **35** (400 MHz, $CDCl_3$)



^{13}C NMR of compound **35** (151 MHz, $CDCl_3$)

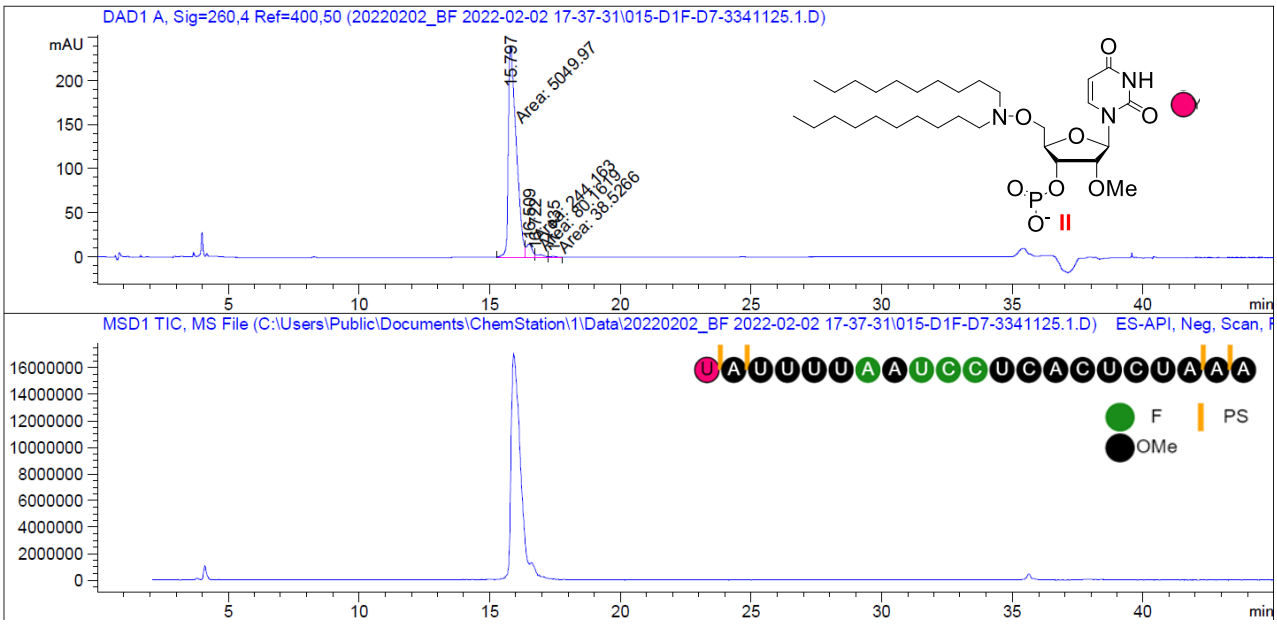


^{31}P NMR of compound **35** (162 MHz, $CDCl_3$)



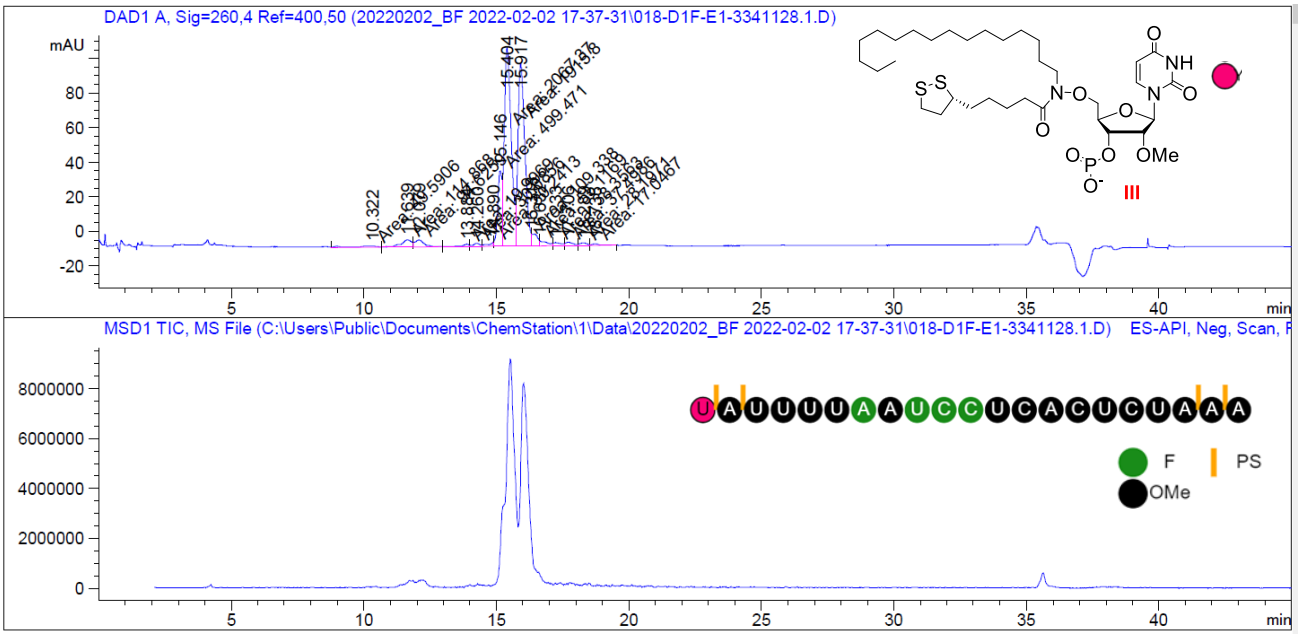
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	22.666	MF	0.4995	118.19809	3.94391	2.6967
2	22.941	FM	0.1617	616.54657	63.56105	14.0667
3	23.049	FM	0.1947	1509.54004	129.20488	34.4406
4	23.281	FM	0.2179	134.89249	10.31874	3.0776
5	23.703	MF	0.0861	267.79868	51.85812	6.1099
6	23.764	FM	0.1566	599.77600	63.83652	13.6841
7	24.008	FM	0.1631	356.34317	36.40572	8.1301
8	24.210	FM	0.1793	663.87970	61.72602	15.1466
9	24.420	FM	0.3491	116.04649	5.53993	2.6476

FigureS4: Reverse-phase HPLC profile for ON1



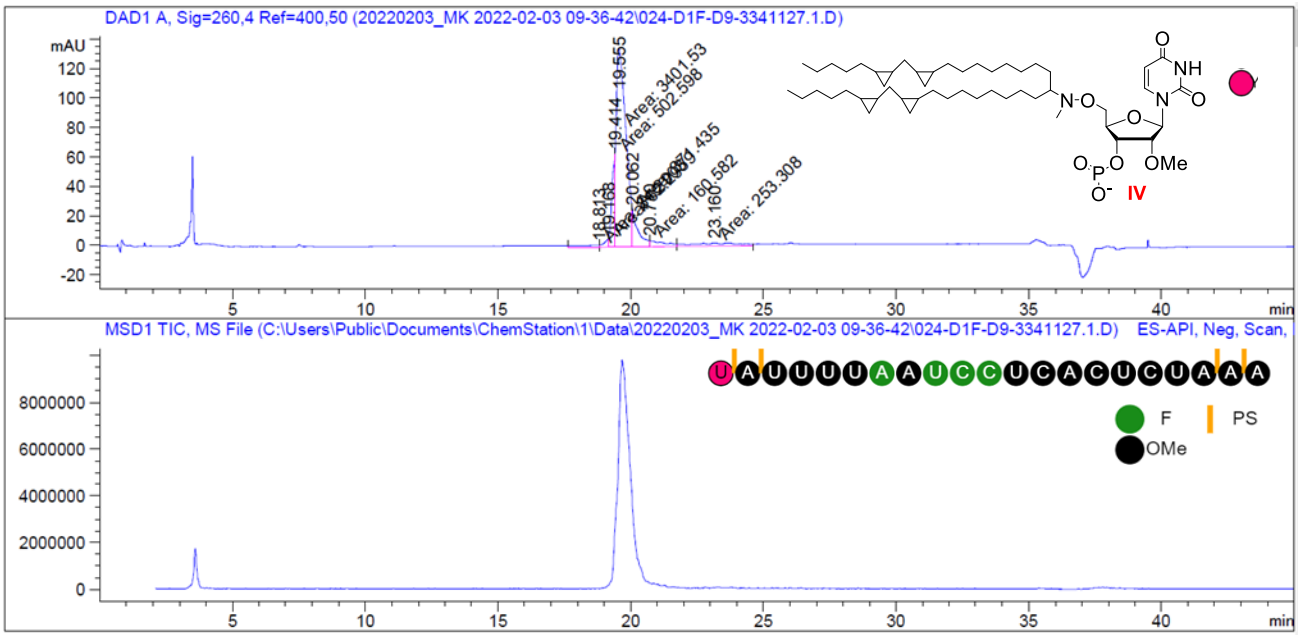
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.797	MF	0.3509	5049.97412	239.88747	93.2964
2	16.509	MF	0.2623	244.16330	15.51295	4.5108
3	16.722	MF	0.2240	80.16187	4.19234	1.4810
4	17.435	FM	0.3822	38.52655	1.67991	0.7118

Figure S5: Reverse-phase HPLC profile for ON2



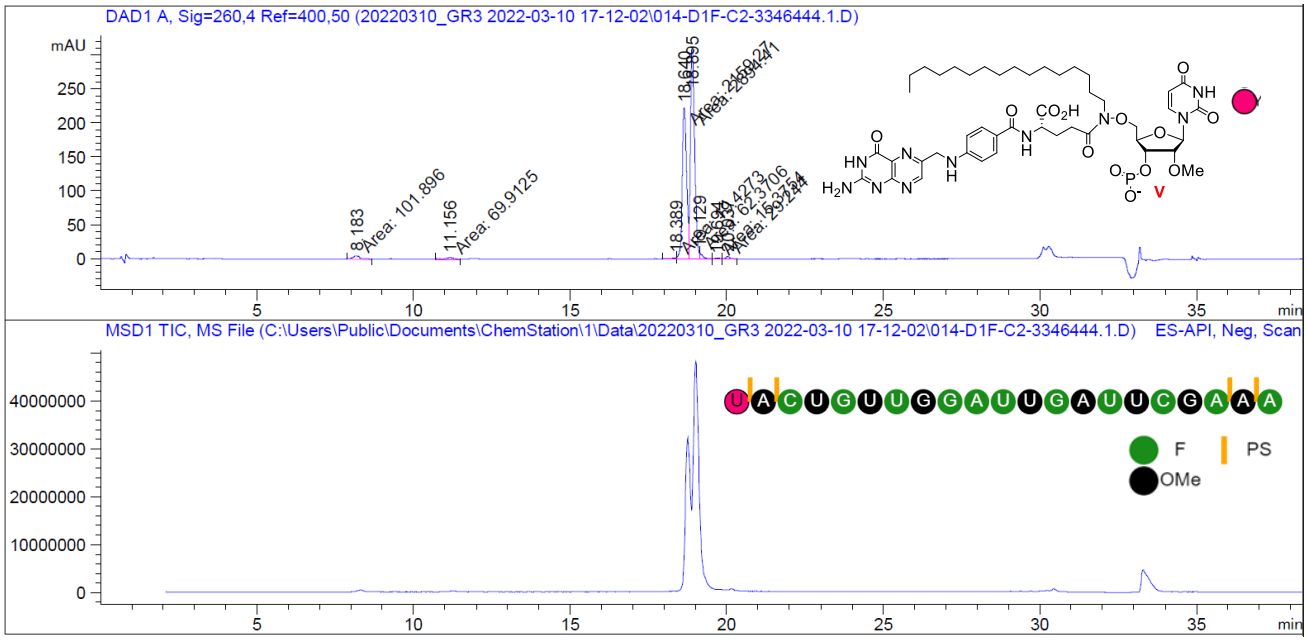
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
6	14.890	MF	0.2061	30.24129	2.44589	0.5921
7	15.146	MF	0.1908	499.47083	43.62690	9.7790
8	15.404	MF	0.3001	2067.36792	114.82887	40.4763
9	15.917	FM	0.3017	1915.79810	105.84090	37.5088
10	16.318	MF	0.2526	109.33765	7.21351	2.1407
11	16.632	MF	0.2081	55.11687	3.09261	1.0791
12	17.233	MF	0.3562	38.35630	1.79476	0.7510
13	17.706	MF	0.3316	37.49855	1.88493	0.7342
14	18.289	MF	0.3091	28.19712	1.52049	0.5521
15	18.735	FM	0.2886	17.04675	9.84512e-1	0.3338

Figure S7: Reverse-phase HPLC profile for ON3



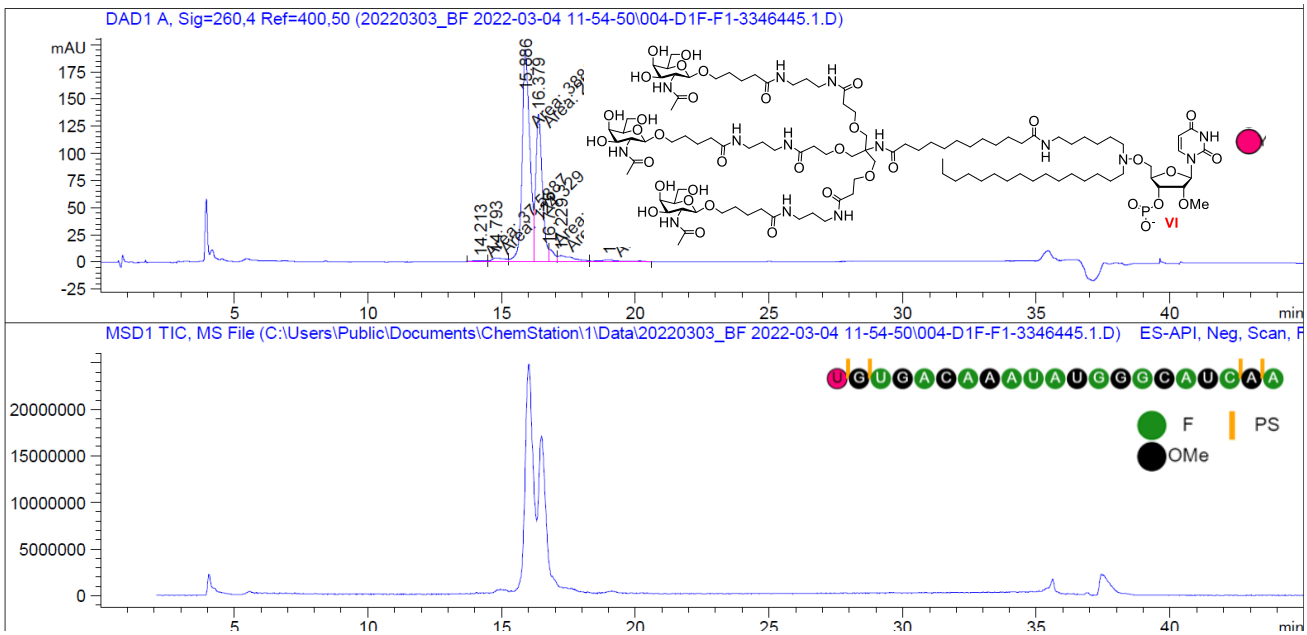
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.813	MF	0.9448	81.29025	1.43404	1.6820
2	19.168	FM	0.1832	62.29589	5.66804	1.2890
3	19.414	MF	0.1330	502.59784	62.98156	10.3992
4	19.555	FM	0.4205	3401.52979	134.83406	70.3808
5	20.062	FM	0.2434	371.43549	25.42860	7.6853

Figure S6: Reverse-phase HPLC profile for ON4



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.183	MM	0.3273	101.89648	5.18895	1.9039
2	11.156	MM	0.4317	69.91247	2.69913	1.3063
3	18.389	MF	0.1720	19.42729	1.88215	0.3630
4	18.640	MF	0.1611	2159.26953	223.37918	40.3458
5	18.895	FM	0.1545	2894.41040	312.23337	54.0819
6	19.129	MF	0.0856	62.37064	8.90647	1.1654
7	19.694	MF	0.2033	15.37537	1.26069	0.2873
8	20.037	FM	0.1472	29.24400	3.31092	0.5464

Figure S8: Reverse-phase HPLC profile for ON5



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.213	MF	0.5790	37.58873	1.08202	0.5469
2	14.793	MF	0.5653	124.32880	3.66564	1.8089
3	15.886	MF	0.3319	3889.23584	195.30348	56.5848
4	16.379	FM	0.2844	2316.53760	135.75987	33.7035
5	16.775	MF	0.1604	155.97365	11.39736	2.2693
6	17.229	MF	0.6953	235.96304	5.65607	3.4330
7	19.039	FM	0.8887	113.66112	2.13148	1.6537

Figure S9: Reverse-phase HPLC profile for ON6

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