Tightly linked morpholino-nucleoside chimeras: new, compact cationic oligonucleotide analogues

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Supporting Information

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General Information

TLC was performed on Kieselgel 60 F254 (Merck) with detection by UV-light (254 nm) and immersing into sulfuric acidic ammonium-molibdenate solution followed by heating. Flash column chromatography was performed on Silica gel 60 (Merck 0.040-0.063 mm). Organic solutions were dried over anhydrous Na_2SO_4, and concentrated in vacuum. Optical rotations were measured at room temperature with a Perkin-Elmer 241 automatic polarimeter. The ^1H NMR (400 and 500 MHz) and ^{13}C NMR (100 and 125 MHz) spectra were recorded with DRX-400, and Bruker Avance II 500 spectrometers at 25 ^oC. Chemical shifts are referenced to Me_4Si (0.00 ppm for ^1H), to the residual solvent signals (D_2O: 4.79, DMSO-d6: 2.50 ppm for ^1H) and to the residual solvent signals ($CDCl_3$: 77.16, DMSO-d6: 39.52, CD_3OD: 49.00 ppm for ^{13}C).

NMR spectra were recorded in D_2O at 300 K using a Bruker Avance II 500 MHz spectrometer (Bruker, Billerica, MA, USA) equipped with a txi triple-resonance probehead. DSS was used as internal reference for 0 ppm. Typical 90° pulses were 13 and 16 µs for ^1H and ^{13}C, and relaxation delays were generally in 2-3 s range. ^1H-^{13}C heteronuclear Single Quantum Correlation (HSQC) spectra were recorded in 4K using «hsqcetgpsi2» pulse program and four scans for each of the 256 increments in indirect dimension. The 2D spectra were processed with the aid of Topspin 3.1 software using Gaussian window function (Lb= -5, GB= 0.05) in F2 and cosine-square (QSINE, SSB = 2) in F1 dimension. To support the $^1H/^{13}C$ assignments heteronuclear multiple bond correlation experiments, HMBC (pulse program: "hmbcgplpndqf", 70 ms evolution time), and homonuclear correlated spectroscopy (COSY) with water presaturation (pulse program: "cosygpprqf"), The digital resolution of the processed spectra was typically 2–3 Hz.

The MALDI-TOF MS measurements were carried out with a Bruker Autoflex Speed mass spectrometer equipped with a time-of-flight (TOF) mass analyzer. In all cases 19 kV (ion source voltage 1) and 16.65 kV (ion source voltage 2) were used. For reflectron mode, 21 kV and 9.55 kV were applied as reflector voltage 1 and reflector voltage 2, respectively. A solid phase laser (355 nm, ≥100 µJ/pulse) operating at 500 Hz was applied to produce laser desorption and 3000 shots were summed. 2,5-Dihydroxybenzoic acid (DHB) was used as matrix and $F_3CCOONa$ as cationising agent in DMF.

ESI-QTOF MS measurements were carried out on a maXis II UHR ESI-QTOF MS instrument (Bruker), in positive ionization mode. The following parameters were applied for the electrospray ion source: capillary voltage: 3.6 kV; end plate offset: 500 V; nebulizer pressure: 0.5 bar; dry gas temperature: 200 °C and dry gas flow rate: 4.0 L/min. The MS method

was tuned according to the examined mass range, which was 200-1000 m/z. Constant background correction was applied for each spectrum, the background was recorded before each sample by injecting the blank sample matrix (solvent). Na-formate calibrant was injected after each sample, which enabled internal calibration during data evaluation. Mass spectra were recorded by otofControl version 4.1 (build: 3.5, Bruker) and processed by Compass DataAnalysis version 4.4 (build: 200.55.2969).

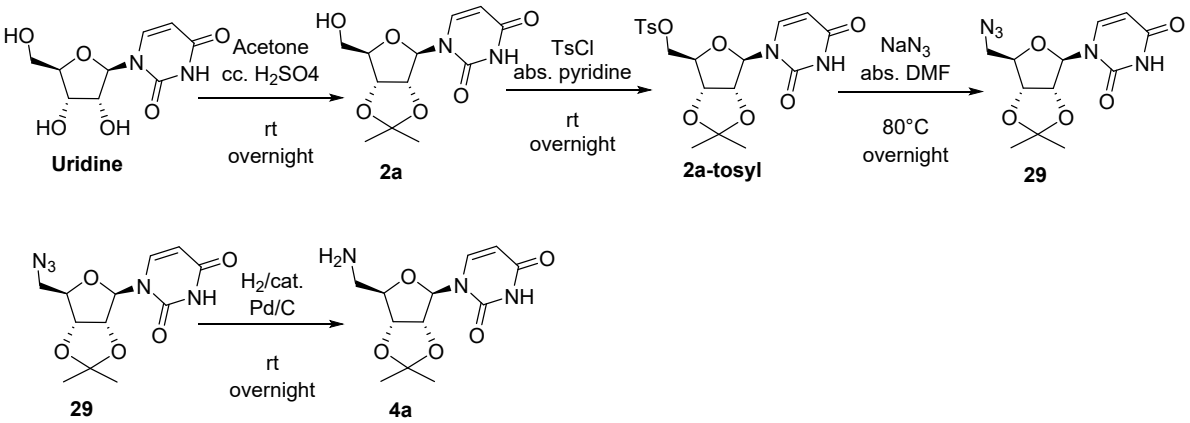
UV-VIS spectra of compound **15** in forms of TFA salt and free base were recorded by a Lambda 25 UV-VIS double-beam spectrophotometer in DMSO (*c* 1 µg/mL).

Dialdehydes **(3a-3f)** were prepared according to the literature procedure.[1]

1. Preparation of periodate resin[1]

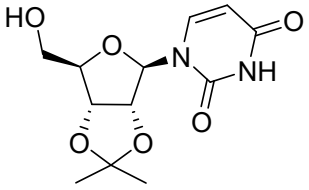
To a solution of NaOH (40 g) in water (3.0 l) Amberlite IRA-400 (200 g, 20-50 mesh Cl^- form) ion exchange resin was added and stirred for 2 hours. The resin was neutralized by washing with water and added to a suspension of $NaIO_4$ (233 g) in water (4-5 l). After stirring overnight the resin was filtered off, washed with water and dried in vacuum over P_2O_5 and KOH overnight. After drying, the resin was stored in the dark.

2. Synthesis of 5'-amino derivatives 4a-4c



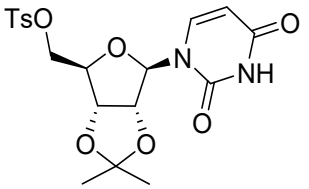
Scheme S1. Synthesis of **4a**

2',3'-*O*-Isopropylidene-uridine (2a)[2]



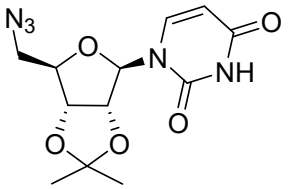
Uridine (10.0 g, 40.95 mmol) was dissolved in acetone (300 ml). and cc. H_2SO_4 (3 ml) was added to the reaction mixture and stirred overnight at room temperature. The solution was neutralized by Et_3N and then evaporated. The crude product was purified by flash column chromatography (CH_2Cl_2:acetone 6:4 →7:3) to yield **2a** (10.5 g, 91%) as a white foam. R*f*=0.42 (CH_2Cl_2:acetone 1:1).

2',3'-*O*-Isopropylidene-5'-*O*-(*p*-toluenesulfonyl)uridine (2a-tosyl)[3]



2a (10.5 g, 36.94 mmol) was dissolved in abs. pyridine (20 ml) and TsCl (10.6 g, 55.4 mmol, 1.5 equiv.) was added to the reaction mixture and stirred overnight at room temperature. The next day water (50 ml) was added to the reaction mixture and stirred for 30 minutes. Then the mixture was concentrated, the residue was dissolved in EtOAc (400 mL) and washed with H_2O and 10% solution of $NaHSO_4$ solution. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane:acetone 7:3 →6:4) to afford (**2a-tosyl**) (11.3 g, 70%) as a white foam. R*f*= 0.75 (CH_2Cl_2:acetone 6:4).

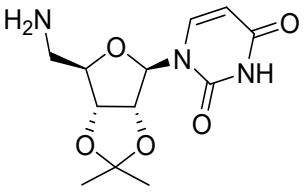
5'-Azido-5'-deoxy-2',3'-*O*-isopropylidene-uridine (29)[4]



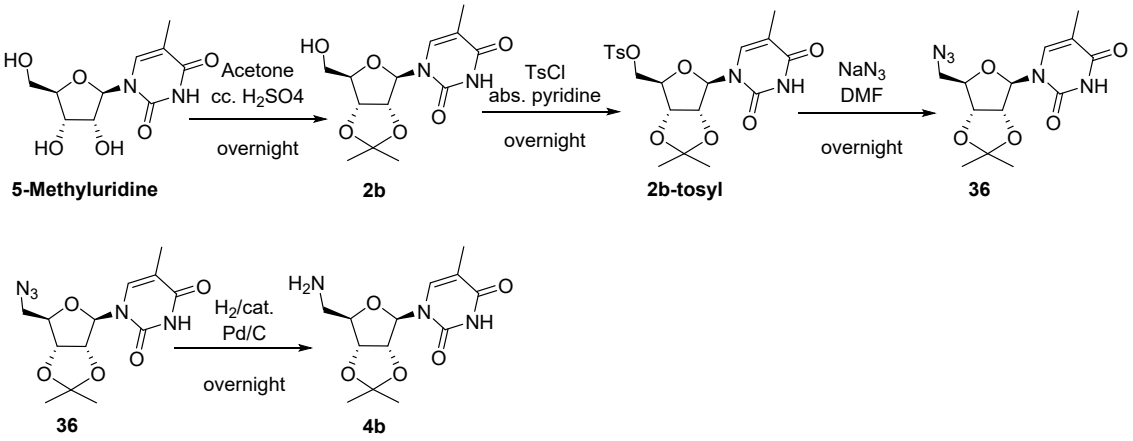
2a-tosyl (11.3 g, 23.37 mmol) was dissolved in abs. DMF (30 ml). and NaN_3 (8.4 g, 128.9 mmol, 5 equiv.) was added to the reaction mixture and stirred overnight at 80 °C. The reaction was quenched by adding 10 ml of MeOH and 10 mL of water and the mixture was stirred for 30 minutes. Then it was evaporated, the residue was dissolved in CH_2Cl_2 (500 ml) and washed with water. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (*n*-

hexane:acetone 7:3 → 6:4) to afford **29** (5.4 g, 68%) as a white foam. R*f*= 0.2 (*n*-hexane:acetone 7:3).

5'-Amino-5'-deoxy-2',3'-*O*-isopropylidene-uridine (4a)[5]

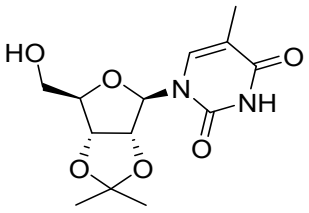


29 (5.4 g, 17.46 mmol) was dissolved in MeOH (30 ml) and Pd/C (1.1g, 20 m/m%) was added to the reaction mixture. The suspension was stirred under hydrogen atmosphere overnight. Then the solution was filtered through Celite and then was concentrated in vacuo. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 9:1) to afford **4a** (3.9 g, 80%) as a white foam. R*f*= 0.2 (CH_2Cl_2:MeOH 9:1).



Scheme S2. Synthesis of **4b**

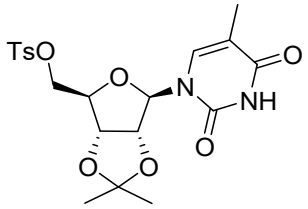
2',3'-*O*-Isopropylidene-5-methyluridine (2b)[3]



5-Methyluridine (2 g, 7.75 mmol) was dissolved in acetone (60 ml). and cc. H_2SO_4 (0.6 ml) was added to the reaction mixture and stirred overnight at room temperature. The solution was neutralized by Et_3N and then evaporated. The crude product was purified by flash column

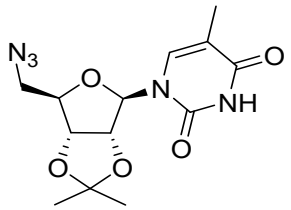
chromatography (CH_2Cl_2:acetone 7:3→1:1) to yield **2b** (2.3 g, 99%) as a white foam. R*f*=0.57 (CH_2Cl_2:acetone 1:1).

2',3'-*O*-Isopropylidene-5'-*O*-(*p*-toluenesulfonyl)-5-methyluridine (2b-tosyl)[3]



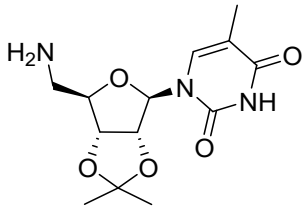
2b (2.3 g, 7.65 mmol) was dissolved in abs. pyridine (5 ml). and TsCl (2.19 g, 1.15 mmol, 1.5 equiv.) was added to the reaction mixture and stirred overnight at room temperature. After that, 50 ml water was added to the reaction mixture and stirred for 30 minutes. Then the mixture was concentrated, the residue was dissolved in EtOAc (200 ml) and washed with H_2O and 10% solution of $NaHSO_4$. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane:acetone 7:3 → 6:4) to afford **2b-tosyl** (2.7 g, 78%) as a white foam. R*f*= 0.75 (CH_2Cl_2:acetone 7:3).

5'-Azido-5'-deoxy-2',3'-*O*-isopropylidene-5-methyluridine (36)[6]

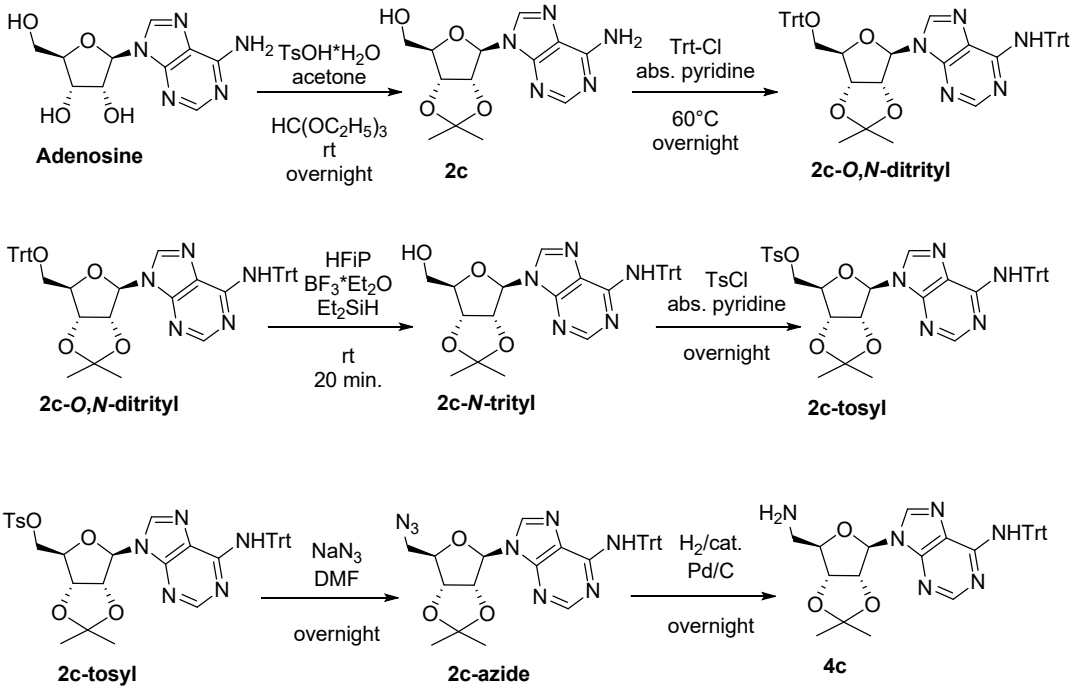


2b-tosyl (2.3 g, 5.1 mmol) was dissolved in abs. DMF (10 ml), NaN_3 (1.3g, 2.0 mmol, 4 equiv.) was added to the reaction mixture and stirred overnight at 80 °C. The reaction was quenched by adding 10 ml of MeOH and 10 ml of water and stirred for 30 minutes. Then it was evaporated, the residue was dissolved in CH_2Cl_2 (200 ml) and washed with water. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane:acetone 7:3) to afford **36** (1.34 g, 83%) as a white foam. R*f*= 0.2 (hexane:acetone 7:3).

5'-Amino-5'-deoxy-2',3'-*O*-isopropylidene-5-methyluridine (4b)

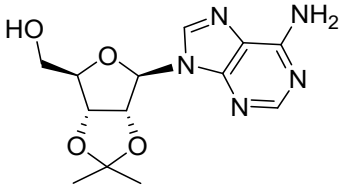


Compound **36** (1.2 g, 3.71 mmol) was dissolved in MeOH:DMF 10:1 (11 ml) and Pd-C (0.24 g, 20 m/m%) was added to the reaction mixture The suspension was stirred under hydrogen atmosphere overnight. Then the mixture was filtered through a pad of Celite and the filtrate was concentrated in vacuo. The crude product was purified by flash column chromatography (*n*-hexane:acetone 7:3 → 6:4) to afford **4b** (0.87 g, 79%) as a white foam. R*f*= 0.2 (CH_2Cl_2:MeOH 9:1). $[\alpha]_D$ −6.09 (*c*=0.23; $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (s, 1H, thymine H-6), 5.64 (s, 1H, H-1'), 5.01 (d, *J* = 6.3 Hz, 1H, H-2'), 4.85 – 4.79 (m, 1H, H-3'), 4.55 (br. s, 2H, NH_2), 4.08 (dd, *J* = 9.7, 4.8 Hz, 1H, H-4'), 3.07 (dd, *J* = 13.4, 4.2 Hz, 1H, H-5'a), 3.01 (dd, *J* = 13.4, 6.2 Hz, 1H, H-5'b), 1.90 (s, 3H, thymine CH_3), 1.56 (s, 3H, *i*-propylidene CH_3), 1.35 (s, 3H, *i*-propylidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5, 150.6 (2C, 2x *C*=O), 138.4 (1C,thymine C-6), 114.6 (1C, *i*-propylidene C_q), 111.2 (1C, thymine C-5), 93.9 (1C, C-1'), 87.6, 84.1, 81.3 (3C, C-2' & C-3' & C-4'), 43.4 (1C, C-5'), 27.2, 25.4 (2C, 2x *i*-propylidene *C*H_3), 12.4 (1C, thymine *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{13}H_{19}N_3NaO_5$ $[M+Na]^+$ 320.1222, found 320.1217.



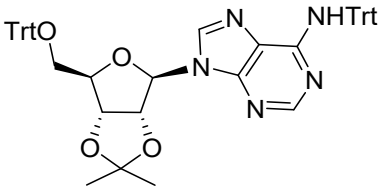
Scheme S3. Synthesis of **4c**

2',3'-*O*-Isopropylidene-adenosine (2c)[7]



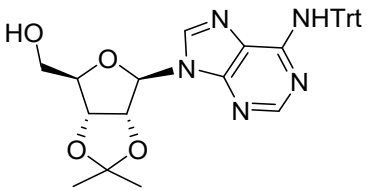
p-Toluenesulfonic acid monohydrate (10.5 g, 55 mmol) and triethyl orthoformate (39 ml, 234 mmol) were subsequently added into the solution of adenosine (13.35g, 48 mmol) in acetone (1.5 l). The resulted mixture was stirred at room temperature overnight. After neutralization with saturated aqueous solution of ammonium-hydroxide, the solution was evaporated until small volume and cooled to crystallize. After filtration, **2c** was obtained as white crystals (14.1 g, 92%). R*f*=0.49 (CH_2Cl_2:MeOH 9:1).

2',3'-*O*-Isopropylidene-5'-*O*-trityl-*N*-trityl-adenosine (2c-*O*,*N*-ditrityl)[8]



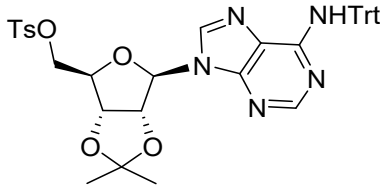
To a suspension of **2c** (1.34 g, 5.00 mmol) in abs. pyridine (30 ml) trityl chloride (4.18 g, 15.00 mmol, 3 equiv.) was added and the reaction mixture was heated to 60 ºC and stirred overnight. The reaction mixture was concentrated in *vacuo* and coevaporated with toluene. The residue was dissolved in CH_2Cl_2 (300 ml) and and successively washed with 10% aqueous solution of $NaHSO_4$, saturated aqueous solution of $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:acetone 9:1 →85:15) to yield compound **2c-*O*,*N*-ditrityl** (2.38 g, 63%) as a white solid. R*f*= 0.39 (CH_2Cl_2:acetone 85:15).

2',3'-*O*-Isopropylidene-*N*-trityl-adenosine (2c-*N*-trityl)[8]



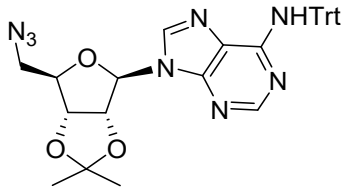
HFiP (12 ml), $BF_3 \cdot Et_2O$ (0.154 ml, 0.18 g, 0.13 equiv.), and Et_3SiH (11,65 ml, 8.5 g, 7.6 equiv.) were mixed and added to **2c-N-trityl** (7,6 g, 9.60 mmol). After 20 minutes, saturated aq. solution of $NaHCO_3$ was added to the reaction mixture and it was concentrated under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane:acetone 75:25) to afford **2c-*N*-trityl** (4,7 g, 89%) as a white foam. R*f*= 0.16 (*n*-hexane:acetone 75:25).

2',3'-*O*-isopropylidene-5'-*O*-(*p*-toluenesulfonyl)-*N*-trityl-adenosine (2c-tosyl)[8]



2c-*N*-trityl (4.7 g, 8.55 mmol) was dissolved in abs. pyridine (15 ml). and TsCl (3.26 g, 17.10 mmol, 2 equiv.) was added to the reaction mixture and stirred overnight at room temperature. After that, 50 ml of water was added to the reaction mixture and stirred for 30 minutes. Then the mixture was concentrated, the residue was dissolved in CH_2Cl_2 (300 mL) and washed with H_2O and 10% solution of $NaHSO_4$. The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane:acetone 85:15 → 8:2) to afford **2c-tosyl** (3.6 g, 60%) as a white foam. R*f*= 0.12 (CH_2Cl_2:acetone 8:2).

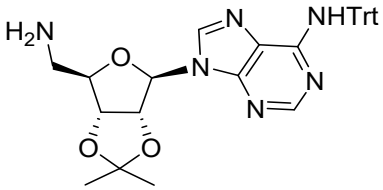
5'-Azido-5'-deoxy-2',3'-*O*-isopropylidene-*N*-trityl-adenosine (2c-azide)



2c-tosyl (3.6 g, 5.12 mmol) was dissolved in abs. DMF (15 ml). and NaN_3 (1.66 g, 25.58 mmol, 5 equiv.) was added to the reaction mixture and stirred overnight at 80 °C. The reaction was quenched by adding MeOH:H_2O 1:1 (10ml) and stirred for 30 minutes. Then it was evaporated, the residue was dissolved in CH_2Cl_2 (300 ml) and washed with water. The organic phase was dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane:acetone 8:2) to afford **2c-azide** (1.7 g, 57%) as a white foam. R*f*= 0.2 (hexane:acetone 7:3); $[\alpha]_D$ +9.44 (*c*=0.18; $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03, 7.87 (2 x s, 2H, H-2, H-8), 7.34 (m, *J* = 7.6 Hz, 6H), 7.26 (q, *J* = 9.3, 8.1 Hz, 9H), 6.97 (s, 1H, N*H*), 6.08 (s, 1H, H-1'), 5.43 – 5.40 (m, 1H, H-2'), 5.01 (dd, *J* = 6.0, 3.5 Hz, 1H, H-3'), 4.35 (q, *J* = 5.4 Hz, 1H, H-4'), 3.58 (h, *J* = 7.8, 7.1 Hz, 2H, H-5'a and H-5'b), 1.60 (s, 3H, *i*-propylidene CH_3), 1.36 (s, 3H, *i*-propylidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.3 (1C, C-6), 152.5 (1C, C-2), 148.2 (1C, C-4), 145.0 (3C, 3x Arom. C_q.), 132.0 (1C, C-8), 129.1, 128.6, 128.0, 127.9, 127.1 (15C, 15x Arom.), 121.6 (1C, adenine C_q), 114.8 (1C, *i*-propylidene C_q), 90.6 (1C, C-1'), 85.6, 84.1, 82.1 (3C, C-2' & C-3' & C-4'),

71.5 (1C, NHTrt C_q), 52.4 (1C, C-5'), 27.2, 25.4 (2C, 2x *i*-propylidene CH_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{32}H_{30}N_8NaO_3$ $[M+Na]^+$ 597.24, found 597.2328.

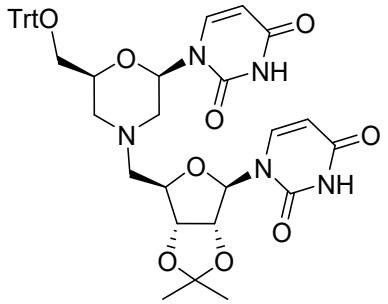
5'-Amino-5'-deoxy-2',3'-*O*-isopropylidene-*N*-trityl-adenosine (4c)



Compound **2c-tosyl** (1.7 g, 2.96 mmol) was dissolved in MeOH:DMF 10:3 (13 ml). and Pd-C (0.34 g, 20 m/m%) was added to the reaction mixture The suspension was stirred under hydrogen atmosphere overnight. Then the mixture was filtered through a pad of Celite and the filtrate was concentrated in vacuo. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 95:5) to afford **4c** (1.2 g, 73%) as a white foam. R*f*= 0.36 (CH_2Cl_2:MeOH 9:1). $[\alpha]_D$ −2.31 (*c*=0.13; $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H, adenine C*H*), 7.87 (s, 1H, adenine C*H*), 7.37 – 7.20 (m, 15H, 15x Arom. C*H*), 6.98 (s, 1H, N*H*-Trt), 5.99 (d, *J* = 3.1 Hz, 1H, H-1'), 5.44 (dd, *J* = 6.5, 3.2 Hz, 1H, H-2'), 4.99 (dd, *J* = 6.5, 3.5 Hz, 1H, H-3'), 4.23 (q, *J* = 4.2 Hz, 1H, H-4'), 3.03 (dd, *J* = 13.4, 4.3 Hz, 1H, H-5'a), 2.98 – 2.92 (m, 1H, H-5'b), 1.61 (s, 3H, *i*-propylidene CH_3), 1.36 (s, 3H, , *i*-propylidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.3 (1C, adenine C_q), 152.5 (1C, adenine *C*H), 145.0 (1C, adenine C_q), 140.1 (3C, 3x NHTrt Arom. C_q), 139.4 (1C, adenine *C*H), 129.1, 128.00, 127.0 (15C, 15x Arom. *C*H), 121.7 (1C, adenine C_q), 114.7 (1C, *i*-propylidene C_q), 90.7, 87.38, 83.5, 81.8 (4C, C-1' & C-2' & C-3' & C-4'), 71.5 (1C, NH-Trt C_q), 43.9 (1C, C-5'), 27.4 (1C, *i*-propilidene CH_3), 25.5 (1C, *i*-propilidene CH_3) ppm. ESI-ToF MS: *m/z* calcd for $C_{32}H_{33}N_6O_3$ $[M+H]^+$ 548.25, found 549.2606.

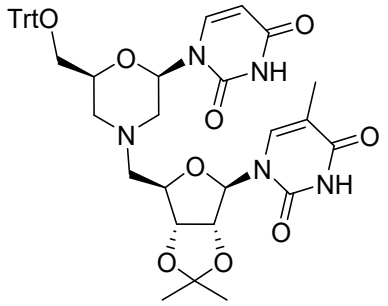
3. Sythesis of morpholino-ribonucleoside dimers 5-13 by double reductive amination-cyclization reactions

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-trityl-2-(uracil-1-yl)-morpholine-4-yl]-uridine (5)



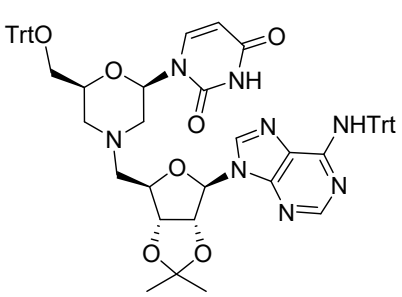
3a (0.2 g, 0.41 mmol) and **4a** (0.12 g, 0.41 mmol, 1.0 equiv.) were dissolved in EtOH (10 ml) and stirred at room temperature for 10 min. After that, AcOH (2 drops) and $NaCNBH_3$ (0.031 g, 0.49 mmol, 1.2 equiv.) were added and the mixture was stirred overnight. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane:acetone 65:35) to give **5** (0.17 g, 57%) as a white solid. R*f*= 0.30 hexane:acetone 1:1; $[\alpha]_D$ +31.7 (*c*=0.12; $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.39 (m, 7H, 2x uracil H-6 & 5x Arom. C*H*), 7.26 (dt, *J* = 15.6, 7.3 Hz, 10H, 10x Arom. C*H*), 5.77 (dd, *J* = 11.3, 7.7 Hz, 2H, uracil H-5 & morpholine H-2), 5.62 (d, *J* = 7.9 Hz, 1H, uracil H-5), 5.47 (d, 1H, H-1'), 5.11 (d, *J* = 6.4 Hz, 1H, H-2'), 4.73 (t, *J* = 5.5 Hz, 1H, H-3'), 4.26 (dt, *J* = 8.6, 4.1 Hz, 1H, H-4'), 4.15 – 4.05 (m, 1H, morpholine H-6), 3.23 (dt, *J* = 9.9, 4.3 Hz, 2H, morpholine H-3a & morpholine H-7a), 3.14 (dd, *J* = 10.0, 4.2 Hz, 1H, morpholine H-7b), 3.02 (d, *J* = 10.3 Hz, 1H, morpholine H-5a), 2.93 (d, *J* = 11.0 Hz, 1H, H-5'a), 2.64 (d, *J* = 12.3 Hz, 1H, H-5'b), 2.09 (d, *J* = 10.8 Hz, 1H, morpholine H-5b), 1.99 (t, *J* = 10.3 Hz, 1H, morpholine H-3b), 1.51 (s, 3H, *i*-propylidene C*H*$_3$), 1.30 (s, 3H, *i*-propylidene C*H*$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.1, 163.7, 150.2, 150.0 (4C, 4x uracil *C*=O), 143.7 (3C, 3x OTrt Arom. C_q), 139.9 (2C, 2x uracil C-6), 128.7, 128.6, 127.9, 127.2 (15 C, 15x Arom. *C*H), 114.3 (1C, *i*-propylidene C_q), 102.5, 102.2 (2C, 2x uracil C-5), 96.3 (1C, C-1'), 86.6 (1C, OTrt C_q), 85.1 (1C, C-4'), 84.4 (1C, C-2'), 82.9 (1C, C-3'), 79.5 (1C, morpholine C-2), 75.6 (1C, morpholine C-6), 64.7 (1C, morpholine C-7), 59.6 (1C, C-5'), 57.1 (1C, morpholine C-3), 53.1 (1C, morpholine C-5), 27.3 (1C, *i*-propylidene *C*H$_3$), 25.4 (1C, *i*-propylidene *C*H$_3$) ppm. MALDI-ToF MS: *m/z* calcd for $C_{40}H_{41}N_5NaO_9$ $[M+Na]^+$ 758.29, found 758.2797.

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-trityl-2-(uracil-1-yl)-morpholine-4-yl]-5-methyluridine (6)



3a (0.2 g, 0.41 mmol) was dissolved in EtOH (10 ml), **4b** (0.12 g, 0.41 mmol, 1.0 equiv.) was added and stirred at room temperature for 10 min. Then, AcOH (2 drops) and $NaCNBH_3$ (0.031 g, 0.49 mmol, 1.2 equiv.) were added and the reaction mixture was stirred overnight. Next day, the reaction mixture was diluted with water and extracted with CH_2Cl_2. The combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexane/acetone 65:35→6:4) to give **6** (0.141 g, 46%) as an amorphous solid. R*f*= 0.18 (*n*-hexane:acetone 6:4); $[\alpha]_D$ +42.0 (*c*=0.1; $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.38 (m, 6H, 5x Arom. C*H* & uracil H-6), 7.25 (dt, *J* = 22.2, 6.9 Hz, 10H, 10x Arom. C*H*), 7.02 (s, 1H, thymine H-6), 5.81 (d, *J* = 9.0 Hz, 1H, morpholine H-2), 5.75 (d, *J* = 8.1 Hz, 1H, uracil H-5), 5.46 (s, 1H, H-1'), 5.10 (d, *J* = 6.4 Hz, 1H, H-2'), 4.79 – 4.71 (m, 1H, H-3'), 4.23 (s, 1H, H-4'), 4.10 (d, *J* = 7.4 Hz, 1H, morpholine H-6), 3.21 (d, *J* = 5.2 Hz, 2H, morpholine H-7a & morpholine H-3a), 3.12 (d, *J* = 5.8 Hz, 1H, morpholine H-7b), 3.01 (d, *J* = 9.9 Hz, 1H, morpholine H-5a), 2.97 – 2.88 (m, 1H, H-5'a), 2.68 (d, *J* = 11.2 Hz, 1H, H-5'b), 2.14 – 2.06 (m, 1H, morpholine H-5b), 2.00 (dd, *J* = 12.6, 7.9 Hz, 1H, morpholine H-3b), 1.83 (s, 3H, thymine CH_3), 1.53 (s, 3H, *i*-propylidene CH_3), 1.31 (s, 3H, *i*-propylidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 163.8, 150.2, 150.1 (4C, 2x uracil *C*=O & 2x thymine *C*=O), 143.7 (3C, 3x OTrt Arom. C_q), 140.1 (1C, uracil C-6), 139.6 (1C, thymine C-6), 128.6, 127.9, 127.1 (15C, 15x Arom.), 114.3 (1C, *i*-propylidene C_q), 110.5 (1C, thymine C-5), 102.4 (1C, uracil C-5), 95.9 (1C, C-1'), 86.6 (1C, O-Trt C_q), 85.1 (1C, C-4'), 84.2 (1C, C-2'), 82.8 (1C, C-3'), 79.5 (1C, morpholine C-2), 75.5 (1C, morpholine C-6), 64.7 (1C, morpholine C-7), 59.8 (1C, C-5'), 57.0 (1C, morpholine C-3), 53.5 (1C, morpholine C-5), 27.2, 25.3 (2C, 2x *i*-propylidene *C*H_3), 12.3 (1C, thymine *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{41}H_{43}N_5NaO_9$ $[M+Na]^+$ 772.2958, found 772.2947.

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-trityl-2-(uracil-1)-yl-morpholine-4]-yl-*N*-trityl-adenosine (7), 5'-deoxy-2',3'-*O*-isopropylidene-5'-[3-etoxy 6-trityl-2-(uracil-1-yl)-morpholine-4-yl]-*N*-trityl-adenosine (7-B1) and **5'-deoxy-2',3'-*O*-isopropylidene-5'-[6-trityl-2-(uracil-1-yl)--3,4-dihydro-1,4-oxazine-4-yl]-*N*-trityl-adenosine (7-B2)**



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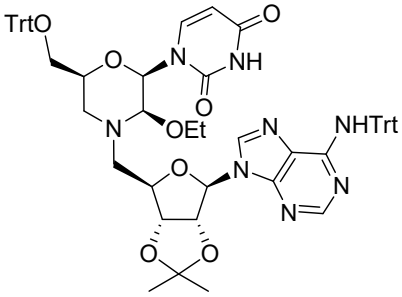
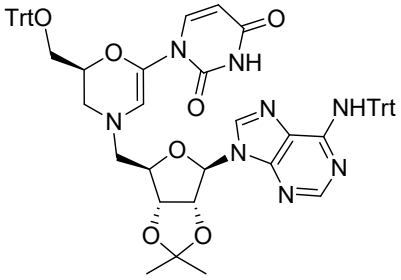


7-B1



7-B2

Method A

3a (0.2 g, 0.41 mmol) and **4c** (0.23 g, 0.41 mmol, 1.0 equiv.) were dissolved in EtOH (10 ml) and stirred at room temperature for 10 min. After that, AcOH (2 drops) and $NaCNBH_3$ (0.031 g, 0.49 mmol, 1.2 equiv.) were added and stirred overnight. Next day, The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane:acetone 65:35) to give **7** (0.17 g, 42%) as a white solid, **7-B1** (9% after double chromatographic purification) as a white foam and **7-B2** (5%, after double chromatographic purification) as a white foam.

Method B

3a (0.2 g, 0,41 mmol) and **4c** (0.36 g, 0,66 mmol, 1.6 equiv.) were dissolved in a 1: 1 mixture of EtOH and CH_2Cl_2 and stirred for 1 hour, then $NaCNBH_3$ (0.062 g, 0.99 mmol, 2.4 equiv.) and triethylamine (0.14 ml, 0.99 mmol, 2.4 equiv.) were added and stirred for another hour. After that the solution of TFA (0.165 ml) in EtOH (1 ml) was added dropwise over 10 minutes and the reaction mixture was stirred for additional 3 hours. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane:acetone 65:35) to give **7** (0.25 g, 62%) as a white solid.

Compound **7**: R*f*= 0.19 hexane:acetone 6:4); $[\alpha]_D$ +15.7 (*c*=0.28; $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H, adenine C*H*), 7.88 (s, 1H, adenine C*H*), 7.48 – 7.20 (m, 31H, 30 x Arom. C*H* & uracil H-6), 6.03 (d, *J* = 2.2 Hz, 1H, H-1'), 5.82 (dd, *J* = 9.7, 2.6 Hz, 1H, morpholine H-2), 5.72 (d, *J* = 8.1 Hz, 1H, uracil H-5), 5.40 (dd, *J* = 6.5, 2.2 Hz, 1H, H-2'), 4.97 (dd, *J* = 6.5, 4.5 Hz, 1H, H-3'), 4.33 (dt, *J* = 7.5, 4.5 Hz, 1H, H-4'), 4.07 (dtd, *J* = 10.5, 5.1, 2.2 Hz, 1H,

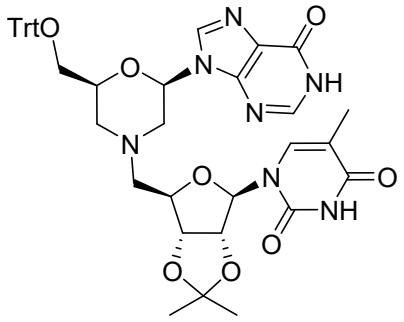
morpholine H-6), 3.26 (dd, *J* = 9.7, 5.0 Hz, 1H, morpholine H-7a), 3.14 – 3.03 (m, 2H, morpholine H-3a & morpholine H-7b), 2.92 (d, *J* = 9.8 Hz, 1H, morpholine H-5a), 2.84 (dd, *J* = 13.4, 4.5 Hz, 1H, H-5'a), 2.66 (dd, *J* = 13.3, 7.7 Hz, 1H, H-5'b), 2.09 (t, *J* = 11.0 Hz, 1H, morpholine H-5b), 2.03 – 1.90 (m, 1H, morpholine H-3b), 1.60 (s, 3H, *i*-propylidene CH_3), 1.35 (s, 3H, *i*-propylidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2, 154.3 (2C, 2x uracil *C*=O),152.3 (1C, adenine *C*H)* 149.9, 148.27 (2C, 2x adenine C_q), 145.0, 143.72 (6C, 3x OTrt Arom C_q & 3x NHTrt Arom. C_q), 140.0 (1C, uracil C-6), 139.2 (1C, adenine *C*H)*, 129.1, 128.71, 128.0, 127.3, 127.0 (30C, 30x Arom. *C*H), 121.6 (1C, adenine Cq), 114.9 (*i*-propylidene Cq), 102.5 (1C, uracil C-5), 90.3 (1C, C-1'), 86.8 (1C, OTrt C_q), 84.8 (1C, C-4'), 83.6 (1C-C-2'), 82.9 (1C, C-3'), 79.7 (1C, morpholine C-2), 75.7 (1C, morpholine C-6), 71.5 (1C, NHTrt C_q), 64.6 (1C, morpholine C-7), 60.0 (1C, C-5'), 56.8 (1C, morpholine C-3), 55.0 (1C, morpholine C-5), 27.3 (1C, *i*-propylidene *C*H_3), 25.6 (1C, *i*-propylidene *C*H_3) ppm.
*Note: Peak can only be seen in HSQC. MALDI-ToF MS: *m/z* calcd for $C_{60}H_{56}N_8NaO_7$ $[M+Na]^+$ 1023.43, found 1023.4165.

Compound **7-B1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H, adenine C*H*), 7.95 (s, 1H), 7.84 (s, 1H, adenine C*H*), 7.62 (d, *J* = 8.2 Hz, 1H, uracil H-6), 7.42 (dt, *J* = 6.2, 1.4 Hz, 4H, 4x Arom. C*H*), 7.33 (dt, *J* = 5.9, 1.6 Hz, 4H, 4x Arom. C*H*), 7.30 – 7.18 (m, 23H, 23x Arom. C*H*), 6.00 (d, *J* = 2.0 Hz, 1H, H-1'), 5.72 (d, *J* = 1.6 Hz, 1H, morpholine H-2), 5.65 (d, *J* = 8.2 Hz, 1H, uracil H-5), 5.40 (dd, *J* = 6.5, 2.0 Hz, 1H, H-2'), 4.97 (dd, *J* = 6.5, 4.4 Hz, 1H, H-3'), 4.31 (dt, *J* = 8.2, 4.1 Hz, 1H, H-4'), 4.10 (ddd, *J* = 10.2, 7.6, 4.6 Hz, 1H, morpholine H-6), 4.00 (d, *J* = 1.7 Hz, 1H, morpholine H-3), 3.25 (dd, *J* = 9.8, 5.3 Hz, 1H, morpholine H-7a), 3.15 (dq, *J* = 9.8, 7.3 Hz, 1H, OEt CH_2a), 3.02 (qd, *J* = 8.6, 5.5 Hz, 3H, OEt CH_2b & morpholine H-7b & H-5'a), 2.81 – 2.71 (m, 2H, H-5'b & morpholine H-5a), 2.54 (dd, *J* = 11.4, 2.8 Hz, 1H, morpholine H-5b), 1.60 (s, 3H, *i*-propylidene CH_3), 1.36 (s, 3H, *i*-propylidene CH_3), 0.80 (t, *J* = 6.9 Hz, 3H, OEt CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 154.2 (2C, 2x uracil *C*=O), 150.1, 148.1 (2C, 2x adenine C_q), 145.0, 143.8 (6C, 3x OTrt Arom. C_q & 3x NHTrt Arom. C_q), 142.3 (1C, uracil C-6), 129.0, 128.7, 127.9, 127.2, 126.9 (30 C, 30x Arom. *C*H), 121.5 (1C, adenine C_q), 114.7 (1C, *i*-propylidene C_q), 101.0 (1C, uracil C-5), 90.3 (1C, C-1'), 86.9 (1C, morpholine C-3), 86.6 (1C, OTrt C_q), 86.0, 83.6, 82.9 (3C, C-2' & C-3' & C-4'), 81.2 (1C, morpholine C-2), 76.0 (1C, morpholine C-6), 71.4 (1C, NHTrt C_q), 67.8 (1C, OEt *C*H_2), 64.6 (1C, morpholine C-7), 55.7 (1C, C-5'), 47.1 ('C, morpholine C-5), 27.3, 25.5 (2C, 2x *i*-propylidene *C*H_3), 15.4 (1C, OEt *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{62}H_{60}N_8NaO_8$ $[M+Na]^+$ 1067.4534,

found 1021.480 $[M\text{-}EtOH+Na]^+$. In the MALDI-spectrum, the mass of an ethanol-eliminated product was detected instead of the expected product.

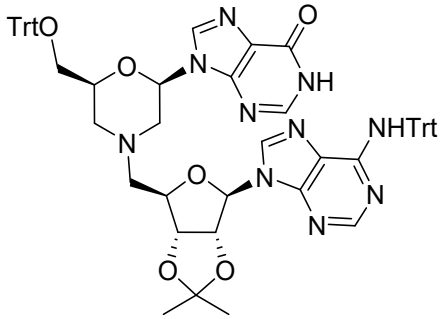
Compound **7-B2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H, adenine C*H*), 7.84 (s, 1H, adenine C*H*), 7.44 – 7.36 (m, 6H, 6x Arom. C*H*), 7.33 (d, J = 7.1 Hz, 7H, 7x Arom. C*H*), 7.23 (tt, J = 14.1, 6.8 Hz, 17H, 17x Arom. C*H*), 7.07 (d, J = 7.9 Hz, 1H, uracil H-6), 6.01 (dd, J = 10.3, 2.3 Hz, 1H, H-1'), 5.58 (d, J = 8.0 Hz, 1H, uracil H-5), 5.53 (s, 1H, morpholine H-3), 5.43 – 5.34 (m, 1H, H-2'), 4.95 (dd, J = 6.5, 4.2 Hz, 1H, H-3'), 4.30 (dt, J = 8.8, 4.6 Hz, 1H, H-4'), 4.17 (d, J = 6.3 Hz, 1H, morpholine H-6), 3.37 (dd, J = 9.9, 4.9 Hz, 1H, morpholine H-7a), 3.27 – 3.14 (m, 3H, morpholine H-7b & morpholine H-5a & H-5'a), 3.04 (ddd, J = 24.0, 13.1, 7.5 Hz, 2H, morpholine H-5b & H-5'b), 1.59 (s, 3H, *i*-propylidene CH_3), 1.34 (s, 3H, *i*-propylidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 154.3 (2C, 2x uracil *C*=O), 150.2, 148.2 (2C, 2x adenine C_q), 145.8 (1C, uracil C-6), 145.0, 143.7 (6X, 3x OTrt Arom. C_q & 3x NHTrt Arom. C_q) , 129.1, 128.7, 128.0, 127.26, 127.0 (30C, 30x Arom. *C*H), 121.5 (1C, adenine C_q), 115.2 (1C, morpholine C-3), 114.8 (1C, *i*-propylidene C_q), 102.0 (1C, uracil C-5), 90.4 (1C, C-1'), 87.0 (1C, OTrt C_q) 85.5 (1C, C-4'), 83.9 (1C, C-2'), 82.5 (1C, C-3'), 73.5 (1C, morpholine C-6), 71.5 (1C, NHTrt C_q), 63.4 (1C, morpholine C-7), 56.5 (1C, morpholine C-5), 49.1 (1C, C-5'), 27.3, 25.5 (2C, 2x *i*-propylidene *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{60}H_{54}N_8NaO_7$ $[M+Na]^+$ 1021.4115, found 1021.472.

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-triphenylmethoxymethyl-2-(hypoxanthine-9-yl)-morpholine-4-yl]-5-methyluridine (8)



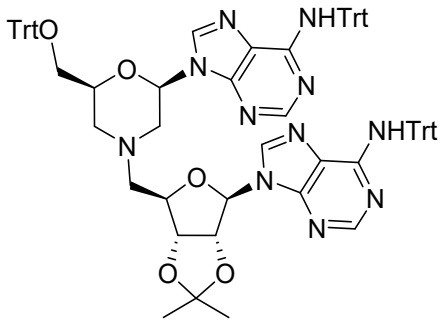
3b (0.23 g, 0.8 mmol) and **4b** (0.398 g, .0783 mmol) were dissolved in EtOH (10 ml) and stirred for 10 min. AcOH (2 drops) and $NaCNBH_3$ (0.059 g, 0.936 mmol, 1.2 equiv.) were added and the stirring was continued overnight. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (gradient elution CH_2Cl_2:acetone 6:4→1:1) to give **8** (0.31 g, 51%) as white foam. R*f*= 0.25 hexane:acetone 1:1); $[\alpha]_D$ +24.1 (*c*=0.22; $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.01 (s, 1H, thymine H-6), 7.43 (d, *J* = 7.6 Hz, 7H, arom.), 7.23 (dt, *J* = 25.6, 7.0 Hz, 11H, 11x Arom. C*H*), 7.08 (s, 1H), 5.95 (s, 1H, morpholine H-2), 5.53 (s, 1H, H-1'), 5.06 (s, 1H, H-2'), 4.75 (s, 1H, H-3'), 4.27 (s, 1H, H-4'), 4.17 (s, 1H, morpholine H-6), 3.30 (s, 2H, morpholine H-3a & morpholine H-7a), 3.11 (d, *J* = 17.2 Hz, 2H, morpholine H-7b & morpholine H-5a), 2.92 (s, 1H, H-5'a), 2.76 (d, *J* = 4.3 Hz, 1H, H-5'b), 2.42 (s, 1H, morpholine H-3b), 2.19 (s, 1H, morpholine H-5b), 1.81 (s, 3H, *i*-propylidene CH_3), 1.53 (s, 3H, *i*-propylidene CH_3), 1.30 (s, 3H, thymine CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 158.6 (2C,2x thymine *C*=O), 150.2, 148.1 (2C, 2x hypoxanthine C_q), 143.6 (3C, 3x OTrt Arom. C_q), 145.8, 139.3 (1C, thymine C-6), 129.3, 128.6, 127.8, 127.1 (15C, 15x Arom. *C*H), 124.1 (1C, hypoxanthine C_q), 114.4 (1C, *i*-propylidene C_q), 110.6 (1C, thymine C-5), 95.4 (1C, C-1'), 86.6 (1C, OTrt-C_q), 85.1 (1C, C-4'), 83.9 (1C, C-2'), 82.7 (1C, C-3'), 79.5 (1C, morpholine C-2), 75.4 (1C, morpholine C-6), 64.5 (1C, morpholine C-7), 60.2 (1C, C-5'), 58.1 (1C, morpholine C-2), 53.5 (1C, morpholine C-5), 27.1, 25.3 (2C, 2x *i*-propylidene *C*H_3), 12.2 (1C, thymine *C*H_3) ppm. MALDI-ToF MS: calcd for $C_{42}H_{43}N_7NaO_8$ $[M+Na]^+$ 796.3071, found 796.3083.

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-trityl-2-(hypoxanthine-9-yl)-morpholine-4-yl]-*N*-trityl-adenosine (9)



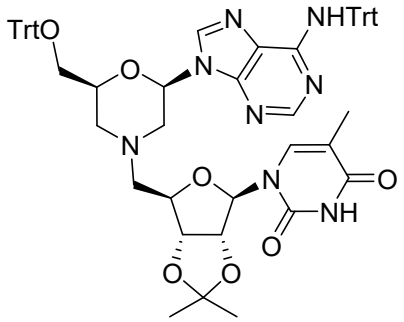
3b (0.27 g, 0.5 mmol) and **4c** (0.25 g, 0.5 mmol) were dissolved in EtOH (10 ml) and stirred for 10 min. AcOH (2 drops) and $NaCNBH_3$ (0.037 g, 0.6 mmol, 1.2 equiv.) were added and stirred overnight. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was diluted dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (gradient elution CH_2Cl_2:acetone 8:2→7:3→6:4) to give **9** (0.24 g, 48%) as white solid. R*f*= 0.46 (CH_2Cl_2:acetone 1:1); $[\alpha]_D$ +14.3 (*c*=0.3; $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22, 7.99, 7.98, 7.84 (4x s, 4x 1H, adenine H-2, H-8 & hypoxanthine H-2, H-8), 7.42 (d, *J* = 7.4 Hz, 6H, 6x Arom. C*H*), 7.32 (d, *J* = 7.2 Hz, 7H, 7x Arom. C*H*), 7.29 – 7.17 (m, 21H, 21x Arom. CH), 7.01 (s, 1H, N*H*), 6.01 (s, 1H, H-1'), 5.92 (d, *J* = 9.0 Hz, 1H, morpholine H-2), 5.40 (d, *J* = 6.4 Hz, 1H, H-2'), 5.00 – 4.96 (m, 1H, H-3'), 4.36 (dd, *J* = 10.6, 5.1 Hz, 1H, H-4'), 4.17 – 4.08 (m, 1H, morpholine H-6), 3.29 (dd, *J* = 9.4, 4.7 Hz, 1H, morpholine H-7a), 3.18 (d, *J* = 9.7 Hz, 1H, morpholine H-3a), 3.12 (dd, *J* = 8.4, 4.3 Hz, 1H, morpholine H-7b), 3.00 (d, *J* = 10.5 Hz, 1H, morpholine H-5a), 2.83 (dd, *J* = 13.2, 4.6 Hz, 1H, H-5'a), 2.72 (dd, *J* = 13.0, 7.4 Hz, 1H, H-5'b), 2.42 (t, *J* = 10.3 Hz, 1H, morpholine H-3b), 2.21 (dd, *J* = 21.5, 10.4 Hz, 1H, morpholine H-5b), 1.60 (s, 3H, *i*-propylidene CH_3), 1.36 (s, 3H, *i*-propylidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.1, 154.2 (2C, hypoxanthine C-6 & adenine C-6), 152.4, 148.4, 148.2 (2C, adenine & hypoxanthine C_q), 144.9, 143.7 (6C, 6x Trt Arom C_q), 140.3, 139.5 (2C, 2x hypoxanthine and adenine *C*H), 129.0, 128.7, 127.9, 127.2, 127.0 (30C, Arom. *C*H), 124.4, 121.6, 114.8 (1C, *i*-propylidene C_q), 90.4 (1C, C-1'), 86.8 (1C, OTrt C_q), 84.6 (1C, C-4'), 83.7 (1C, C-2'), 83.0 (1C, C-3'), 79.8 (1C, morpholine C-2), 75.6 (1C, morpholine C-6), 71.4 (1C, NHTrt C_q), 64.5 (1C, morpholine C-7), 59.9 (1C, C-5'), 57.7 (1C, morpholine C-3), 55.0 (1C, morpholine C-5), 27.3, 25.5 (2C, *i*-propylidene *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{61}H_{56}N_{10}NaO_6$ $[M+Na]^+$ 1047.4282, found 1047.4391.

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-trityl-2-(*N*-trityl-adenine-9-yl)-morpholine-4-yl]-*N*-trityl-adenosine (10)



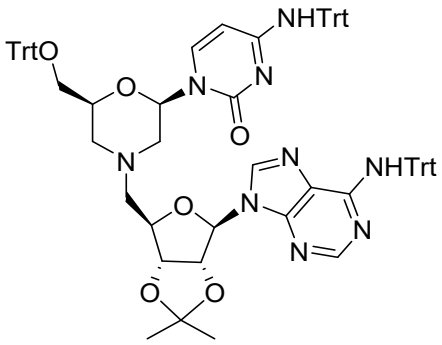
3c (0.749 g, 1.0 mmol) was dissolved in EtOH (20 ml) and **4c** (0.549 g, 1.0 mmol, 1.0 equiv) was added. The reaction mixture was stirred for 10 min at room temperature. Than, AcOH (2 drops) and $NaCNBH_3$ (0.075 g, 1.2 mmol, 1.2 equiv) were added and the reaction mixture was stirred overnight. Next day, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexane/acetone 8:2→7:3) because of the imperfect purification, the compound was re-purified by flash chromatography (gradient elution CH_2Cl_2:acetone 99:1→98:2→97:3→96:4→95:5) to give **10** (0.64 g, 50%) as a white solid. R*f*= 0.27 (hexane:acetone 7:3); $[\alpha]_D$ +6.00 (*c*=0.15; $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04, 8.00, 7.89, 7.79 (4xs, 4H, 2x adenine H-2, 4x adenine H-8), 7.42 (d, J = 7.5 Hz, 6H, 6x Arom. C*H*), 7.38 – 7.29 (m, 14H, 14x Arom.), 7.04 (s, 2H, 2x N*H*), 5.98 (d, J = 1.3 Hz, 1H, H-1'), 5.93 (dd, J = 9.8, 1.1 Hz, 1H, morpholine H-2), 5.40 (d, J = 6.4 Hz, 1H, H-2'), 4.98 (dd, J = 5.9, 4.3 Hz, 1H, H-3'), 4.35 (dd, J = 9.9, 5.2 Hz, 1H, H-4'), 4.18 – 4.08 (m, 1H, morpholine H-6), 3.30 (dd, J = 9.3, 4.8 Hz, 1H, morpholine H-7a), 3.14 (d, J = 10.4 Hz, 1H, morpholine H-3a), 3.10 – 3.05 (m, 1H, morpholine H-7b), 2.99 (d, J = 10.4 Hz, 1H, morpholine H-5a), 2.81 (dd, J = 13.1, 4.6 Hz, 1H, H-5'a), 2.66 (dd, J = 13.0, 7.2 Hz, 1H, H-5'b), 2.43 (t, J = 10.4 Hz, 1H, morpholine H-3b), 2.17 (t, J = 10.8 Hz, 1H, morpholine H-5b), 1.57 (s, 3H, *i*-propylidene CH_3), 1.33 (s, 3H, *i*-propylidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.2, 154.1, 152.4, 152.2, 148.2, 148.1 (6C, 2x adenine *C*H, 4x adenine C_q), 144.9, 144.9, 143.6 (3C, 3x OTrt Arom. C_q), 128.9, 128.6, 127.8, 127.1, 126.9 (45C, 45x Arom. *C*H), 121.6, 120.6 (2C, 2x adenine C_q), 114.6 (1C, *i*-propylidene C_q), 90.4 (1C, C-1'), 86.7 (1C, OTrt C_q), 84.7 (1C, C-4'), 83.6 (1C, C-2'), 82.9 (1C, C-3'), 79.5 (1C, morpholine C-2), 75.4 (1C, morpholine C-6), 71.4 (2C, 2x NHTrt C_q), 64.5 (1C, morpholine C-7), 59.7 (1C, C-5'), 57.5 (1C, morpholine C-3), 55.2 (1C, morpholine C-5), 27.2, 25.4 (2C, *i*-propylidene *C*H_3) ppm. MALDI-ToF MS *m/z* calcd for $C_{80}H_{71}N_{11}NaO_5$ $[M+Na]^+$: 1288.5537, found 1288.5447.

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-trityl-2-(6-*N*-trityl-adenine-1-yl)-morpholine-4-yl]-5-methyluridine (11)



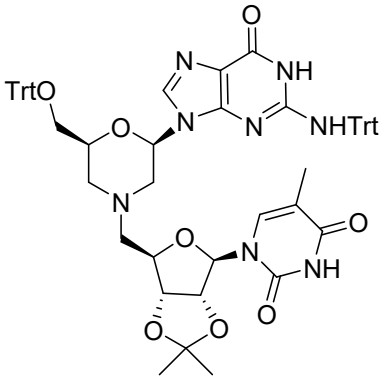
3c (0.3 g, 0.40 mmol) and **4b** (0.12 g, 0.40 mmol, 1.0 equiv.) were dissolved in EtOH (10 ml) and stirred at room temperature for 10 min. After 10 min, AcOH (2 drops) and $NaCNBH_3$ (0.030 g, 0.48 mmol, 1.2 equiv.) were added and stirred overnight. Next day, The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (gradient elution *n*-hexane:acetone 7:3→6:4) to give **11** (0.19 g, 46%) as a white solid. R*f*= 0.16 (*n*-hexane:acetone 7:3); $[\alpha]_D$ +17.5 (*c*=0.12; $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04, 7.98 (2x s, 2H, adenine H-2, H-8), 7.42 (d, *J* = 6.9 Hz, 9H, 9x Arom. C*H*), 7.35 (d, *J* = 7.2 Hz, 12H, 12x Arom. C*H*), 7.30 – 7.15 (m, 32H, 32x Arom. C*H*), 7.08 (s, 1H, N*H*), 6.95 (s, 1H, thymine H-6), 5.95 (d, *J* = 8.9 Hz, 1H, morpholine H-2), 5.46 (s, 1H, H-1'), 4.99 (d, *J* = 5.1 Hz, 1H, H-2'), 4.71 (dd, *J* = 12.0, 6.6 Hz, 1H, H-3'), 4.19 (d, *J* = 18.4 Hz, 2H, H-4' & morpholine H-6), 3.28 (s, 2H, morpholine H3a & morpholine H-7a), 3.10 (dd, *J* = 23.4, 10.3 Hz, 2H, morpholine H-7b & morpholine H-5a), 2.78 (dd, *J* = 16.7, 9.8 Hz, 2H, H-5'ab), 2.47 (t, *J* = 10.3 Hz, 1H, morpholine H-3b), 2.19 (t, *J* = 10.7 Hz, 1H, morpholine H-5b), 1.81 (s, 3H, thymine CH_3), 1.53 (s, 3H, *i*-propilidene CH_3), 1.26 (s, 3H, *i*-propilidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2 (1C, thymine *C*=O), 154.1 (1C, adenine C_q), 152.4 (2C, adenine C-2 & C-8), 150.2 (1C, thymine *C*=O), 148.3 (1C, adenine C_q), 145.0, 143.7 (6C, 3x OTrt Arom C_q & 3x NHTrt Arom C_q), 138.9 (1C, thymine C-6), 129.0, 128.7, 127.9, 127.1, 126.9 (30C, 30x Arom *C*H), 120.5 (1C, adenine C_q), 114.6 (1C, *i*-propylidene C_q), 110.9 (1C, thymine C-5), 95.2 (1C, C-1'), 86.7 (1C, OTrt-C_q), 85.1 (1C, C-4'), 84.0 (1C, C-2'), 82.6 (1C, C-3'), 79.4 (1C, morpholine C-2), 75.5 (1C, morpholine C-6), 71.4 (1C, NHTrt-C_q), 64.6 (1C, morpholine C-7), 59.9 (1C, C-5'), 58.0 (1C, morpholine C-3), 54.6 (1C, morpholine C-5), 27.3, 25.4 (2C, 2x *i*-propilidene *C*H_3), 12.3 (1C, thymine *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{61}H_{58}N_8NaO_7$ $[M+Na]^+$ 1037.4326, found 1037.4322.

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-trityl-2-(*N*-trityl-cytosine-1-yl)-morpholine-4-yl]-*N*-trityl-adenosine (12)



4c (1.42 g, 2.6 mmol) was dissolved in EtOH (30 ml) **3d** (1.95 mg, 2.6 mmol, 1.0 equiv.) were added. The reaction mixture was stirred for 10 min at room temperature. Than, AcOH (4 drops) and $NaCNBH_3$ (196 mg, 3.1 mmol, 1.2 equiv.) were added and the reaction mixture was stirred overnight. Next day, the reaction mixture was diluted with H_2O (200 ml) and extracted with CH_2Cl_2 (5x100 ml). The combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexane:acetone 1:1) to give to give **12** (1.85 g, 58%) as a yellowish foam. R*f*= 0.2 (*n*-hexane:acetone 1:1); $[\alpha]_D$ +5.0 (*c*=0.2; $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94, 7.83 (2x s, 2x1H, adenine H-2 & H-8), 7.40 – 7.31 (m, 15H, 15x Arom. C*H*), 7.31 – 7.17 (m, 41H, Arom. C*H* & cytidine H-6), 6.96 (s, 1H, adenine N*H*), 5.96 (d, *J* = 2.4 Hz, 1H, H-1'), 5.89 (dd, *J* = 9.4, 1.9 Hz, 1H, morpholine H-2), 5.34 (dd, *J* = 6.5, 2.4 Hz, 1H, H-2'), 5.04 (d, *J* = 7.7 Hz, 1H, cytidine H-5), 4.93 (dd, *J* = 6.4, 4.4 Hz, 1H, H-3'), 4.28 (dd, *J* = 11.1, 4.7 Hz, 1H, H-4'), 4.05 – 3.99 (m, 1H, morpholine H-6), 3.21 (d, J = 10.2 Hz, 1H, morpholine H-3a), 3.15 (dd, *J* = 9.8, 5.1 Hz, 1H, morpholine H-7a), 2.98 (dd, *J* = 9.8, 4.5 Hz, 1H, morpholine H-7b), 2.80 (d, *J* = 12.7 Hz, 1H, morpholine H-5a), 2.78 – 2.72 (m, 1H, H-5'a), 2.57 (dd, J = 13.4, 6.9 Hz, 1H, H-5'b), 2.04 – 1.96 (m, 1H, morpholine H-5b), 1.80 – 1.73 (m, 1H, morpholine H-3b), 1.59 (s, 3H, *i*-propylidene CH_3), 1.34 (s, 3H, *i*-propylidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4 (1C, cytosine C-2), 154.7, 154.2, 148.3 (3C, cytosine C-4 & 2x adenine C_q), 152.4, 139.2* (2C, adenine C-2 & C-8) 145.0, 144.0, 143.8 (9C, 3x OTrt Arom. C_q & 6x NHTrt Arom. C_q), 140.8 (1C, cytosine C-6), 129.1, 128.8, 128.7, 128.5, 127.9, 127.9, 127.7, 127.1, 127.0 (45C, 45x Arom. *C*H), 121.7 (1C, adenine C_q), 114.9 (1C, *i*-propylidene C_q), 94.6 (1C, cytosine C-5), 90.2 (1C, morpholine C-2), 86.6 (1C, OTrt C_q), 84.7 (1C, C-4'), 83.5 (1C, C-2'), 82.7 (1C, C-3'), 80.7 (1C, C-1'), 75.5 (1C, morpholine C-6), 71.4, 71.0 (2C, 2x NHTrt C_q), 64.9 (1C, morpholine C-7), 59.8 (1C, C-5'), 57.3 (1C, morpholine C-3), 55.0 (1C, morpholine C-5), 27.3, 25.5 (2C, 2x *i*-propyliene *C*H_3) ppm. *Signal can be seen only in HSQC. MALDI-ToF MS: *m/z* calcd for $C_{79}H_{71}N_9NaO_6$ $[M+Na]^+$ 1264.5425, found 1264.5487.

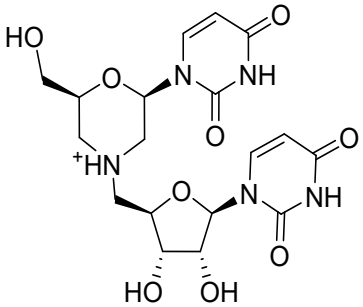
5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-(4,4'-trityl)-2-(2-*N*-trityl-guanine-9-yl) -morpholine-4-yl]-5-methyluridine (13)



3e (0.4 g, 0.5 mmol) and **4b** (0.19 g, 0.6 mmol, 1.2 equiv.) were dissolved in EtOH (10 ml) and stirred at room temperature for an hour. After that, glacial AcOH (0,03 ml, 0,5 mmol, 1.0 equiv.) and $NaCNBH_3$ (0.049 g, 0.8 mmol, 1.5 equiv.) were added and stirred overnight. Next day, The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexane:acetone 1:1) to crude **13** (0.25 g, 0.24 mmol, 50%) as a yellowish foam which was used for the deprotection without NMR characterization. (R*f*= 0.30 *n*-hexane:acetone 1:1). MALDI-ToF MS: *m/z* calcd for $C_{61}H_{58}N_8NaO_8$ $[M+Na]^+$ 1053.4378, found 1053.419.

4. Synthesis of deprotected ribonucleosyl-morpholino dinucleotides 14-22

5'-Deoxy-5'-[6-hydroxymethyl-2-(uracil-1-yl)-morpholine-4-yl]-uridine (14)

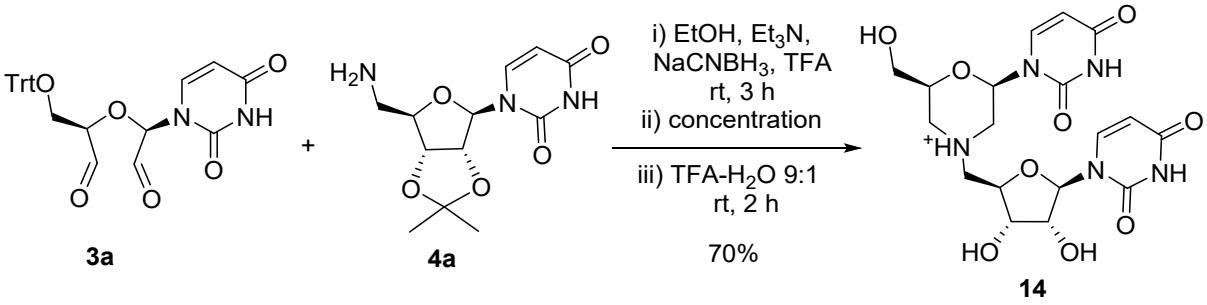


Method A

Compound **5** (0.1 g, 0.14 mmol) was dissolved in 90% aqueous TFA (5 ml) and Et_3SiH was added (0.65 ml, 1.011 mmol, 3.0 equiv.) and stirred for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene 3 times. Then the residue was purified by flash column chromatography ($CHCl_3$:MeOH 7:3) to give **14** (0.052

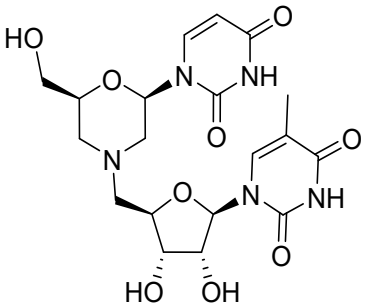
g, 87%) as an amorphous solid. R*f*= 0.26 (CH_2Cl_2:MeOH 7:3); $[\alpha]_D$ +23.00 (*c*=0.001; MeOH) ^{1}H NMR (400 MHz, D_2O) δ 7.73 (dd, *J* = 8.2, 1.1 Hz, 1H, uracil H-6), 7.57 (dd, *J* = 8.1, 1.1 Hz, 1H, uracil H-6), 5.77 (dt, *J* = 8.0, 1.5 Hz, 2H, 2x uracil H-5), 5.70 (dt, *J* = 4.2, 2.9 Hz, 2H, morpholine H-2 & H-1'), 4.24 (ddd, *J* = 5.8, 3.4, 1.1 Hz, 1H, H-2'), 4.18 – 4.08 (m, 1H, H-3'), 3.96 (ddd, *J* = 6.9, 5.6, 1.1 Hz, 1H, H-4'), 3.89 (ddd, *J* = 8.6, 5.1, 2.6 Hz, 1H, morpholine H-6), 3.65 (ddd, *J* = 12.3, 3.6, 1.1 Hz, 1H, morpholine H-7a), 3.64 – 3.55 (m, 1H, morpholine H-7b), 3.09 (d, *J* = 10.5 Hz, 1H, morpholine H-3a), 2.91 (d, *J* = 12.2 Hz, 1H, morpholine H-5a), 2.89 – 2.84 (m, 1H, H-5'a), 2.76 (dd, *J* = 13.9, 8.4 Hz, 1H, H-5'b), 2.29 (t, *J* = 10.8 Hz, 1H, morpholine H-3b), 2.16 (t, *J* = 11.4 Hz, 1H, morpholine H-5b) ppm. ^{13}C NMR (100 MHz, D_2O) δ 166.1, 165.9, 151.3, 151.0 (4C, 4x uracil *C*=O), 142.1, 142.0 (2C, 2x uracil C-6), 102.2, 102.1 (2C, 2x uracil C-5), 91.0 (1C, C-1'), 80.0 79.4, 76.3, 72.9, 71.3 (5C, C-2' & C-3' & C-4' & morpholine C-2 & morpholine C-6), 62.0 (1C, morpholine C-7), 59.3, 55.3 (2C, morpholine C-3 & morpholine C-5), 52.2 (1C, C-5') ppm. MALDI-ToF MS: *m/z* calcd for $C_{18}H_{23}N_5NaO_9$ $[M+Na]^+$ 476.1496, found 476.1388.

Method B (One-pot reaction)



3a (0.2 g, 0,41 mmol) and **4a** (0.19 g, 0,66 mmol, 1.6 equiv.) were dissolved in a 1: 1 mixture of EtOH and CH_2Cl_2 and stirred for 1 hour, then $NaCNBH_3$ (0.062 g, 0.99 mmol, 2.4 equiv.) and triethylamine (0.14 ml, 0.99 mmol, 2.4 equiv.) were added and stirred for another hour. After that the solution of TFA (0.165 ml) in EtOH (1 ml) was added dropwise over 10 minutes and the reaction mixture was stirred for additional 3 hours. The reaction mixture was concentrated under reduced pressure, then 90% aqueousTFA (8 ml) and Et_3SiH (0.198 ml, 1.2 mmol, 3.0 equiv.) were added to the mixture and it was stirred for 2 hours. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene 3 times. Then the residue was purified by flash column chromatography ($CHCl_3$:MeOH 8:2→7:3) to give **14** (0.13 g, 70% for 2 steps) as a white foam.

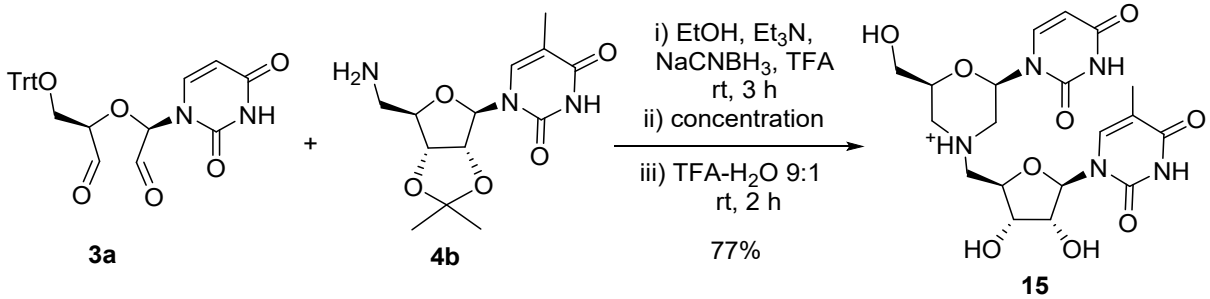
5'-Deoxy-5'-[6-hydroxymethyl-2-(uracil-1-yl)-morpholine-4-yl]-5-methyluridine (15)



Method A

90 % aqueous TFA (5.0 ml) and Et_3SiH (0.086 mL, 0.54 mmol, 3.0 equiv.) were added to **6** (0.13 g, 0.18 mmol). The reaction mixture was stirred for 2 h at room temperature. After that, the solution was concentrated under reduced pressure and co-evaporated with toluene 3 times. Then the crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 8:2) to give **15** (0.075 g, 89%) as a white foam. R*f*= 0.21 (CH_2Cl_2:MeOH 8:2); $[\alpha]_D$ +11.8 (*c*=0.11; DMSO) ^{1}H NMR (400 MHz, MeOD) δ 7.78 (d, *J* = 8.1 Hz, 1H, uracil H-6), 7.44 (s, 1H, thymine H-6), 5.89 (d, *J* = 8.9 Hz, 1H, morpholine H-2), 5.80 (d, *J* = 3.9 Hz, 1H, H-1'), 5.75 (d, *J* = 8.1 Hz, 1H, uracil H-5), 4.33 – 4.26 (m, 1H, H-2'), 4.17 (s, 1H, H-4'), 4.10 (t, *J* = 5.7 Hz, 1H, H-3'), 4.03 – 3.94 (m, 1H, morpoline H-6), 3.68 (d, *J* = 3.9 Hz, 2H, morpholine H-7ab), 3.29 (s, 1H, morpholine H-3a), 3.15 (d, *J* = 10.9 Hz, 1H, morpholine H-5a), 3.06 (d, *J* = 12.1 Hz, 1H, H-5'a), 3.02 – 2.94 (m, 1H, H-5'b), 2.50 (t, *J* = 10.5 Hz, 1H, morpholine H-3b), 2.41 (t, *J* = 11.1 Hz, 1H, morpholine H-5b), 1.89 (s, 3H, thymine CH_3) ppm. ^{13}C NMR (100 MHz, MeOD) δ 166.2, 165.9, 152.4, 151.7 (4C, 2x uracil *C*=O & 2x thymine *C*=O), 142.4 (1C, uracil C-6), 138.9 (1C, thymine C-6), 112.0 (1C, thymine C-5), 103.1 (1C, uracil C-5), 92.7 (1C, C-1'), 81.7 (1C, C-4'), 80.4 (1C, morpholine C-2), 77.7 (1C, morpholine C-6), 73.9 (1C, C-2'), 72.8 (1C, C-3'), 63.5 (1C, morpholine C-7), 60.7 (1C, C-5'), 56.7 (1C, morpholine C-3), 54.4 (1C, morpholine C-5), 12.4 (1C, thymine *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{19}H_{25}N_5NaO_9$ $[M+Na]^+$ 490.1550, found 490.1548.

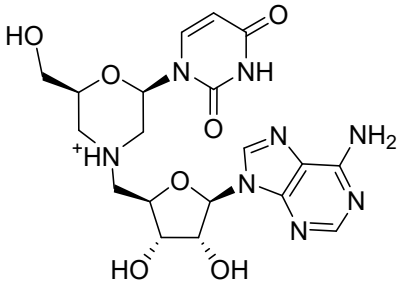
Method B (One-pot reaction)



3a **4b** **15**

3a (0.57 g, 1.2 mmol) and **4b** (0.56 g, 1.9 mmol, 1.6 equiv.) were dissolved in a 1: 1 mixture of EtOH and CH_2Cl_2 (20 ml) and stirred for 1 hour, then $NaCNBH_3$ (0.177 g, 2.8 mmol, 2.4 equiv.) and triethylamine (0.39 ml, 2.8 mmol, 2.4 equiv.) were added and stirred for another hour. After that the solution of TFA (0.19 ml) in EtOH (2 ml) was added dropwise over 10 minutes and the reaction mixture was stirred for additional 3 hours. The reaction mixture was concentrated under reduced pressure, then 90% aqueousTFA (10 ml) and Et_3SiH (0.564 ml, 3.5 mmol, 3.0 equiv.) were added to the mixture and it was stirred for 2 hours. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene 3 times. Then the residue was purified by flash column chromatography ($CHCl_3$:MeOH 85:15) to give **15** (0.42 g, 77% for 2 steps) as a white foam.

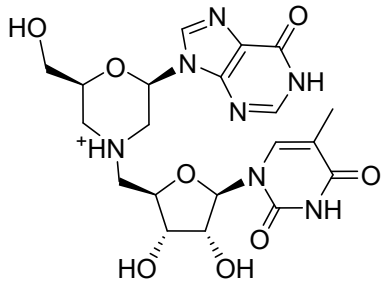
5'-Deoxy-5'-[6-hydroxymethyl-2-(uracil-1-yl)-morpholine-4-yl]- adenosine (16)



Compound **7** (0.13 g, 0.14 mmol) was dissolved in 90% aqueous TFA (5 ml) and Et_3SiH was added (0.13 ml, 0.76 mmol , 3.0 equiv.) and stirred for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene 3 times. Then the residue was purified by flash column chromatography ($CHCl_3$:MeOH 8:2) to give **16** (0.047 g, 52%) as an amorph solid. R*f*= 0.11 (CH_2Cl_2:MeOH 8:2); $[\alpha]_D$ +26.15 (*c*=0.003; H_2O) ^1H NMR (400 MHz, MeOD) δ 7.09 (d, *J* = 2.7 Hz, 1H, adenine C*H*), 7.00 (d, *J* = 3.8 Hz, 1H, adenine C*H*), 6.56 (dd, *J* = 8.1, 2.6 Hz, 1H, uracil H-6), 4.82 (dd, *J* = 4.3, 2.0 Hz, 1H, H-1'), 4.60 (dd, *J* = 8.0, 3.2 Hz, 1H, uracil H-5), 4.54 (dt, *J* = 10.0, 2.4 Hz, 1H, morpholine H-2), 3.54 (td, *J* = 4.6, 2.3 Hz, 1H, H-2'), 3.17 – 3.05 (m, 2Hm H-3' & H-4'), 2.68 (dt, *J* = 8.3, 4.1 Hz, 1H, morpholine H-6), 2.48 – 2.38 (m, 2H, morpholine H-7a,b), 1.98 – 1.87 (m, 1H, morpholine H-3a), 1.81 – 1.69 (m, 2H, H-5'a,b), 1.70 – 1.59 (m, 1H, morpholine H-5a), 1.08 – 0.98 (m, 2H, morpholine H-3b & morpholine H-5b) ppm. ^{13}C NMR (100 MHz, MeOD) δ 156.6, 151.7 (2C, 2x uracil *C*=O), 149.9 (1C, adenine C_q), 142.8 (1C, uracil C-6), 1030 (1C, uracil C-5), 89.6 (1C, C-1'), 82.7 (1C, C-4'), 80.6 (1C, morpholine C-2), 77.5 (1C, morpholine C-6), 74.2 (1C,

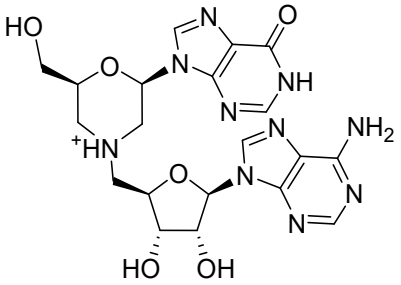
C-2'), 72.9 (1C, C-3'), 63.2 (1C, morpholine C-7), 60.5 (1C, C-5'), 56.8 (1C, morpholine C-3), 54.0 (1C, morpholine C-5) ppm. MALDI-ToF MS: *m/z* calcd for $C_{19}H_{24}N_8O_7$ $[M+Na]^+$ 499.1768, found 499.1660.

5'-Deoxy-5'-[6-hydroxymethyl-2-(hypoxanthine-9-yl)-morpholine-4-yl]-5-methyluridine (17)



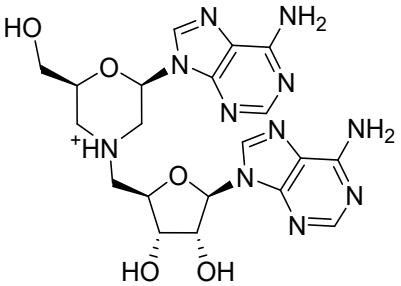
Compound **8** (0.26 g, 0.337 mmol) was dissolved in 90% aqueous TFA (5 ml) and Et_3SiH was added (0.16 ml, 1.011 mmol, 3.0 equiv.) and stirred for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene 3 times. Then the residue was purified by flash column chromatography ($CHCl_3$:MeOH 7:3) to give **17** (0.14 g, 86%) as an amorphous solid. R*f*= 0.26 (CH_2Cl_2:MeOH 7:3); $[\alpha]_D$ −1.875 (*c*=0.16; DMSO) ^{1}H NMR (400 MHz, DMSO) δ 8.28, 8.08 (2xs, 2x 1H, hypoxanthine H-2 & H-8), 7.44 (s, 1H, thymine H-6), 5.76 (dd, *J* = 10.0, 2.2 Hz, 1H, morpholine H-2), 5.72 (d, *J* = 4.9 Hz, 1H, H-1'), 5.37 (s, 1H, 2'-O*H*), 5.16 (s, 1H, 3'O*H*), 4.82 (t, *J* = 5.5 Hz, 1H, morpholine 7 O*H*), 4.08 (s, 1H, H-2'), 3.92 (s, 2H, H-3' & H-4'), 3.84 – 3.76 (m, 1H, morpholine H-6), 3.44 (qd, *J* = 11.5, 5.7 Hz, 2H, morpholine H-7ab), 3.11 (d, *J* = 10.1 Hz, 1H, morpholine H-3a), 2.96 (d, *J* = 10.8 Hz, 1H, morpholine H-5a), 2.78 (t, *J* = 10.7 Hz, 2H, morpholine H-3b & H-5'a), 2.69 (dd, *J* = 13.4, 6.4 Hz, 1H, H-5'b), 2.11 (t, *J* = 11.0 Hz, 1H, morpholine H-5b), 1.80 (s, 3H, thymine C*H*$_3$) ppm. ^{13}C NMR (100 MHz, DMSO) δ 163.7, 156.6, 150.7, 147.8, 123.9 (5C, hypoxanthine C-4, C-5 & C-6 and thymine C-2 & C-4), 146.4*, 138.8* (2C, hypoxanthine C-2 & C-8) 136.6 (1C, thymine C-6), 109.8 (1C, thymine C-5), 88.7 (1C, C-1'), 80.9 (1C, C-4'), 79.1 (1C, morpholine C-2), 76.9 (1C, morpholine C-6), 72.1 (1C, C-2'), 71.2 (1C, C-3'), 61.9 (1C, morpholine C-7), 59.4 (1C, C-5'), 56.0 (1C, morpholine C-3), 54.3 (1C, morpholine C-5), 12.1 (1C, thymine *C*H$_3$) ppm. *Note: Peak can only be seen in HSQC spectrum. MALDI-ToF MS: *m/z* calcd for $C_{20}H_{25}N_7NaO_8$ $[M+Na]^+$ 514.1765, found 514.1643.

5'-Deoxy-5'-[6-hydroxymethyl-2-(hypoxanthine-9-yl)-morpholine-4-yl]-adenosine (18)



Compound **9** (0.21 g, 0.206 mmol) was dissolved in 90% aqueous TFA (5 ml) and Et_3SiH (0.197 ml, 1.24 mmol, 6.0 equiv.) was added and stirred for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene 3 times. The crude product was purified by flash column chromatography (gradient elution CH_2Cl_2:MeOH 7:3→6:4) to give **18** (0.090 g, 84%) as white solid. R*f*= 0.25 (CH_2Cl_2:MeOH 6:4); $[\alpha]_D$ −6.47 (*c*=0.17; DMSO) ^{1}H NMR (400 MHz, DMSO) δ 8.34, 8.25, 8.15, 8.07 (4xs, 4x1H, 2x H-2 & 2xH-8), 7.28 (s, 2H, NH_2), 5.87 (d, J = 4.4 Hz, 1H, H-1'), 5.74 (d, J = 9.5 Hz, 1H, morpholine H-2), 4.60 (t, J = 4.7 Hz, 1H, H-2'), 4.22 (t, J = 5.2 Hz, 1H, H-3'), 4.08 (dd, J = 9.5, 5.6 Hz, 1H, H-4'), 3.82 – 3.74 (m, 1H, morpholine H-6), 3.42 – 3.38 (m, 2H, morpholine H-7ab), 3.10 (d, J = 10.3 Hz, 1H, morpholine H-3a), 2.95 (d, J = 10.9 Hz, 1H, morpholine H-5a), 2.85 (dd, J = 13.5, 3.3 Hz, 1H, H-5'a), 2.77 (d, J = 10.5 Hz, 1H, morpholine H-3b), 2.75 – 2.70 (m, 1H, H-5'b), 2.09 (t, J = 10.9 Hz, 1H, morpholine H-5b) ppm. ^{13}C NMR (100 MHz, DMSO) δ 156.8, 156.1, 149.3, 147.8, 123.9, 119.2 (6C, 6x hypoxanthine and adenine C_q), 88.0 (1C, C-1'), 81.6 (1C, C-4'), 79.1 (1C, morpholine C-2), 76.9 (1C, morpholine C-6), 72.8 (1C, C-2'), 71.6 (1C, C-3'), 61.9 (1C, morpholine C-7), 59.6 (1C, C-5'), 56.0 (1C, morpholine C-3), 54.3 (1C, morpholine C-5) ppm. MALDI-ToF MS: *m/z* calcd for $C_{20}H_{24}N_{10}NaO_6$ $[M+Na]^+$ 523.1778, found 523.1791.

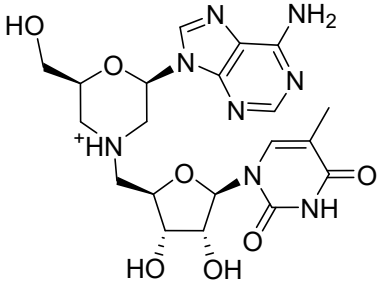
5'-Deoxy-5'-[(2-adenine-9-yl)-6-hydroxymethyl-morpholine-4-yl]-adenosine (19)



Compound **10** (0.27 g, 0.213 mmol) was dissolved in 90% aqueous TFA (5 ml), then Et_3SiH (0.31 ml, 1.92 mmol, 9.0 equiv.) was added and stirred for 2 h at room temperature. The reaction mixture was diluted with toluene, the solvent was evaporated under reduced pressure and the

residue was purified by flash column chromatography (CH_2Cl_2:MeOH 7:3→6:4) to give **19** (0.076 g, 72%) as a white solid. R*f*= 0.25 (CH_2Cl_2:MeOH 7:3); $[\alpha]_D$ −5.0 (*c*=0.1; DMSO) ^{1}H NMR (400 MHz, DMSO) δ 8.35 (s, 1H, adenine C*H*), 8.31 (s, 1H, adenine C*H*), 8.16 (s, 1H, adenine C*H*), 7.30 (d, *J* = 4.1 Hz, 4H, 2x NH_2), 5.88 (d, *J* = 4.5 Hz, 1H, H-1'), 5.78 (dd, *J* = 10.0, 1.9 Hz, 1H, morpholine H-2), 5.64 (s, 1H, 3'O*H*), 5.40 (s, 1H, 2'O*H*), 4.81 (s, 1H, morpholine 7-O*H*), 4.60 (t, *J* = 4.6 Hz, 1H, H-2'), 4.23 (t, *J* = 5.0 Hz, 1H, H-3'), 4.09 (dd, *J* = 9.7, 5.7 Hz, 1H, H-4'), 3.85 – 3.74 (m, 1H, morpholine H-6), 3.40 (s, 2H, morpholine H-7a,b), 3.11 (d, *J* = 10.4 Hz, 1H, morpholine H-3a), 2.96 (d, *J* = 10.5 Hz, 1H, morpholine H-5a), 2.90 – 2.84 (m, 1H, H-5'a), 2.82 (d, *J* = 11.2 Hz, 1H, morpholine H-3b), 2.74 (dd, *J* = 13.5, 7.0 Hz, 1H, H-5'b), 2.09 (t, *J*= 10.9 Hz, 1H, morpholine H-5b) ppm. ^{13}C NMR (100 MHz, DMSO) δ 156.1, 152.8, 152.7, 149.3, 149.0, 119.2, 118.5 (8C, adenine carbons), 88.0 (1C, C-1'), 81.6 (1C, C-4'), 78.9 (1C, morpholine C-1), 76.8 (1C, morpholine C-6), 72.8 (1C, C-2'), 71.6 (1C, C-3'), 62.0 (1C, morpholine C-7), 59.6 (1C, C-5'), 56.0 (1C, morpholine C-3), 54.4 (1C, morpholine C-5) ppm. MALDI-ToF MS: *m/z* calcd for $C_{20}H_{25}N_{11}NaO_5$ $[M+Na]^+$ 522.1938, found 522.1922.

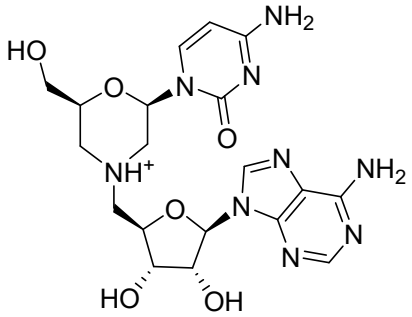
5'-Deoxy-5'-[2-(adenine-9-yl)-6-hydroxymethyl-morpholine-4-yl]-5-methyluridine (20)



90 % aqueous TFA (5.0 ml) and Et_3SiH (0.13 ml, 0.845 mmol, 6.0 equiv.) were added to **11** (0.14 g, 0.141 mmol). The reaction mixture was stirred for 2 h at room temperature. After 2 h, the solution was concentrated under reduced pressure, and the crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 7:3) to give **20** (0.053 g, 77%) as a white solid. R*f*= 0.15 (CH_2Cl_2:MeOH 8:2); $[\alpha]_D$ +0.01 (*c*=0.3; DMSO) ^{1}H NMR (400 MHz, DMSO) δ 8.34, 8.17 (2xs, 2x1H, adenine H-2 & H-8), 7.46 (s, 1H, thymine H-6), 7.32 (s, 2H, NH_2), 5.84-5.77 (1H, m, morpholine H-2), 5.76-5.71 (m, 1H, H-1'), 5.45 (s, 1H, 2'O*H*), 5.27 (s, 1H, 3'O*H*), 4.88 (s, 1H, morpholine 7-O*H*), 4.10 (s, 1H, H-2'), 3.94 (s, 2H, H-4', H-3'), 3.81 (dd, *J* = 4.9, 3.0 Hz, 1H, morpholine H-6), 3.12 (d, *J* = 10.5 Hz, 1H, morpholine H-3a), 2.97 (d, *J* = 10.3 Hz, 1H, morpholine H-5a), 2.81 (d, *J* = 11.7 Hz, 2H, morpholine H-3b & H-5'a), 2.74 – 2.65 (m, 2H, H-5'b & morpholine H-3b), 2.11 (t, *J* = 10.9 Hz, 1H, morpholine H-5b), 1.80 (s, 3H,

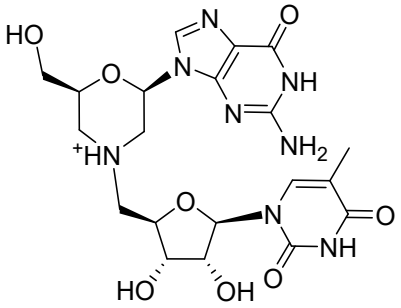
thymineCH_3) ppm. ^{13}C NMR (100 MHz, DMSO) δ 163.8, 156.1, 152.8, 150.7, 149.0 (5C, 2x thymine*C*=O & 2x adenine C_q, adenine *C*H), 136.7 (1C, thymine C-6), 118.5 (1C, adenine C_q), 109.9 (1C, thymine C-5), 88.8 (1C, C-1'), 78.9 (1C, morpholine C-2), 76.8 (1C, morpholine C-6), 72.1 (1C, C-2'), 81.0, 71.2 (2C, C-4' & C-3'), 62.1 (1C, morpholine C-7), 59.4 (1C, C-5'), 56.1 (1C, morpholine C-3), 54.4 (1C, morpholine C-5), 12.1 (1C, thymine *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{20}H_{26}N_8NaO_7$ $[M+Na]^+$ 513.1822, found 513.1816.

5'-Deoxy-5'-[2-(cytosine-1-yl)-6-hydroxymethyl-morpholine-4-yl]-adenosine (21)



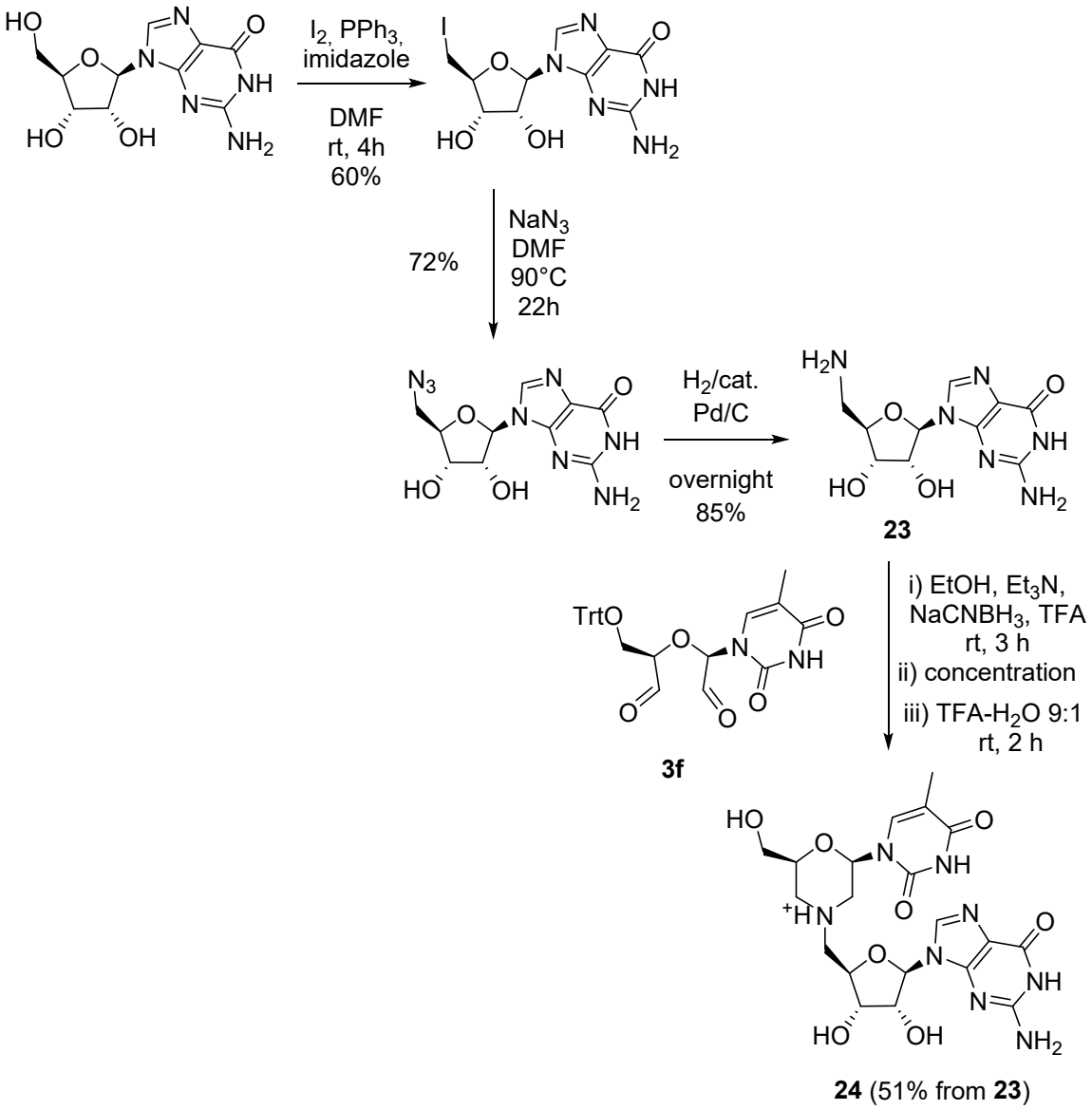
Compound **12** (1.6 g, 1.29 mmol) was dissolved in 90% TFA (20 ml) and Et_3SiH (1.85 ml, 11.6 mmol, 9.0 equiv.) was added and stirred at room temperature for 2 h. The reaction mixture was diluted with toluene an concentrated under reduced pressure. The crude product was purified by flash column chromatography ($CHCl_2$:MeOH 6:4) to give **21** (0.3g, 50%) as a white solid. R*f* = 0.22 ($CHCl_2$:MeOH 6:4); $[\alpha]_D$ +16.0 (*c*=0.15; DMSO). ^{1}H NMR (400 MHz, DMSO) δ 8.33, 8.15 (2x s, 2x 1H, adenine H-2, H-8), 7.58 (d, *J* = 7.4 Hz, 1H, cystosine H-6), 7.28 (s, 2H, NH_2), 7.19 (s, 1H), 5.87 (d, *J* = 4.4 Hz, 1H, H-1'), 5.73 (d, *J* = 7.4 Hz, 1H, cytosine H-5), 5.65 (d, *J* = 7.9 Hz, 1H, morpholine H-2), 5.50 (s, 1H, 2'O*H*), 5.25 (s, 1H, 3'O*H*), 4.75 (s, 1H, morpholine 7-O*H*), 4.57 (s, 1H, H-2'), 4.19 (s, 1H, H-3'), 4.03 (dd, *J* = 9.3, 6.2 Hz, 1H, H-4'), 3.72 – 3.64 (m, 1H, morpholine H-6), 3.39 (HDO + morpholine H-7a,b), 2.89 (t, *J* = 12.6 Hz, 2H, morpholine H-3a & H-5a), 2.79 (dd, *J* = 13.4, 3.1 Hz, 1H, H-5'a), 2.65 (dd, *J* = 13.4, 7.0 Hz, 1H, H-5'b), 2.06 – 1.92 (m, 2H, morpholine H-3b & H-5b) ppm. ^{13}C NMR (100 MHz, DMSO) δ 165.4 (1C, cytosine C-2), 156.1, 154.2, 149.3 (3C, 2x adenine C_q & cytosine C-4), 152.7 (1C, adenine *C*H), 141.5 (1C, cytosine C-6), 119.2 (1C, adenosine C_q), 94.1 (1C, cytosine C-5), 88.1 (1C, C-1'), 81.6 (1C, C-4'), 79.6 (1C, C-1'), 76.7 (1C, morpholine C-6), 72.8 (1C, C-2'), 71.6 (1C, C-3'), 62.1 (1C, morpholine C-7), 59.6 (1C, C-5'), 56.6 (morpholine C-3), 54.3 (1C, morpholine C-5) ppm. MALDI-ToF MS: *m/z* calcd for $C_{19}H_{25}N_9NaO_6$ $[M+Na]^+$ 498.1825, found 498.1813.

5'-Deoxy-5'-[2-guanosine-9-yl-6-hydroxymethyl-morpholine-4-yl]-5-methyluridine (22)



90 % aqueous TFA (15 ml) and Et_3SiH (0.116 ml, 0.73 mmol, 3.0 equiv.) were added to **13** (0.25 g, 0.24 mmol). The reaction mixture was stirred for 2 h at room temperature. After that, the solution was concentrated under reduced pressure, and the crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 7:3→6:4) to give **22** (0.08 g, 67%) as a white foam. R*f*= 0.15 (CH_2Cl_2:MeOH 7:3); $[\alpha]_D$ +30.0 (*c*=0.1; MeOH). ^{1}H NMR (400 MHz, DMSO) δ 11.06 (d, *J* = 193.6 Hz, 1H, base N*H*), 8.35 (s, 1H, base N*H*), 7.86 (s, 1H, guanine H-8), 7.45 (d, *J* = 3.9 Hz, 1H, thymine H-6), 6.65 (s, 2H, guanine NH_2), 5.72 (d, *J* = 4.9 Hz, 1H, H-1'), 5.56 (dd, *J* = 10.1, 2.4 Hz, 1H, morpholine H-2), 5.39 (d, *J* = 5.4 Hz, 1H, H-2' O*H*), 5.21 (d, *J* = 16.2 Hz, 1H, H-3' O*H*), 4.84 (t, *J* = 5.8 Hz, 1H, morpholine H-7 O*H*), 4.11 (d, *J* = 17.5 Hz, 1H, H-2'), 3.92 (dd, *J* = 6.7, 4.0 Hz, 2H, H-3' & H-4'), 3.73 (dtd, *J* = 10.7, 5.3, 2.2 Hz, 1H, morpholine H-6), 3.46 – 3.41 (m, 2H, morpholine H-7a,b), 3.04 (d, *J* = 8.9 Hz, 1H, morpholine H-3a), 2.94 (d, *J* = 11.2 Hz, 1H, morpholine H-5a), 2.77 (dd, *J* = 13.6, 3.6 Hz, 1H, H-5'a), 2.70 – 2.60 (m, 2H, H-5'b & morpholine H-3b), 2.07 (t, *J* = 10.9 Hz, 1H, morpholine H-3b), 1.80 (s, 1H, morpholine H-5b) ppm. ^{13}C NMR (100 MHz, DMSO) δ 166.0, 158.7, 154.8 (3C, 2x thymine *C*=O & guanine *C*=O), 152.0, 151.8 (2C, 2x guanine C_q), 138.3 (1C, thymine C-6), 116.7 (1C, guanine C_q), 111.8 (1C, thymine C-5), 90.5 (1C, C-1'), 82.0 (1C, C-3'), 79.5 (1C, morpholine C-6), 77.4 (1C, morpholine C-2), 73.3 (1C, C-2'), 72.3 (1C, C-4'), 63.0 (1C, morpholine C-7), 60.4 (1C, C-5'), 57.2 (1C, morpholine C-3), 54.3 (1C, morpholine C-5), 13.0 ('C, thymine *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{20}H_{26}N_8NaO_8$ $[M+Na]^+$ 529.1874, found 529.1843.

5'-Deoxy-5'-[6-hydroxymethyl-2-(thymine-1-yl)-morpholine-4-yl]-5-guanosine (24)



3f (0.9 g, 1.8 mmol) and 5'-amino-5'-deoxy-guanosine (**23**)[9] (0.82 g, 2.9 mmol, 1.6 equiv.) were dissolved in a 1: 1: 1 mixture of EtOH, CH_2Cl_2 and DMF (30 ml) and stirred for 1 hour, then $NaCNBH_3$ (0.27 g, 4.3 mmol, 2.4 equiv.) and triethylamine (0.61 ml, 4.3 mmol, 2.4 equiv.) were added and stirred for another hour. After that the solution of TFA (0.25 ml) in EtOH (3 ml) was added dropwise over 10 minutes and the reaction mixture was stirred for additional 3 hours. The reaction mixture was concentrated under reduced pressure, then 90% aqueousTFA (10 ml) and Et_3SiH (0.867 ml, 5.4 mmol, 3.0 equiv.) were added to the mixture and it was stirred for 2 hours. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene 3 times. Then the residue was purified by flash column chromatography ($CHCl_3$:MeOH 1:1→4:6) to give **24** (0.46 g, 51% for 2 steps) as a white foam.

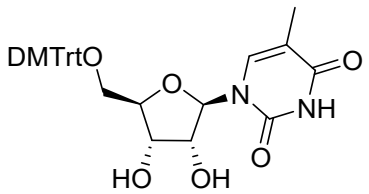
R*f*=0.15 (CH_2Cl_2:MeOH 1:1); $[\alpha]_D$ -7.86 (*c*=0.14; DMSO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90, 7.56 (2x s, 2H, guanine H-8 & thymine H-6), 5.81 – 5.67 (m, 1H, H-1'), 5.62 (d, *J* = 9.7

Hz, 1H, morpholine H-2), 4.43 (t, J = 5.1 Hz, 1H, H-2'), 4.10 (t, J = 5.4 Hz, 1H, H-3'), 4.02 (t, J = 5.5 Hz, 1H, H-4'), 3.71 (s, 1H, D_2O & morpholine H-6), 3.44 (d, J = 5.0 Hz, 2H, morpholine H-7a,b), 2.96 – 2.74 (m, 2H, morpholine H-3a & morpholine H-5a), 2.66 (dd, J = 13.7, 6.9 Hz, 1H, H-5'a), 2.54 (s, 1H, H-5'b), 2.25 (t, J = 10.5 Hz, 1H, morpholine H-3b), 2.05 (t, J = 11.0 Hz, 1H, morpholine H-5b), 1.79 (s, 3H thymine CH_3)ppm. ^{13}C NMR (101 MHz, DMSO) δ 164.03, 157.19, 153.83, 151.58, 150.33 (5C, 2x thymine *C*=O & 3x guanine C_q), 136.86, 136.83 (2C, guanine C-8 & thymine C-6) 116.93 (1C, guanine C_q), 109.90 (1C, thymine C-5), 87.39 (1C, C.-1'), 81.85, (1C, C-4'), 79.08 (1C, morpholine C-2), 77.06 (1C, morpholine C-6), 73.04 (1C, C-2'), 71.78 (1C, C-3'), 62.19 (1C, morpholine C-7), 59.92 (1C, C-5'), 55.84 (1C, morpholine C-3), 54.21 (1C, morpholine C-5), 12.25 (1C, thymine CH_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{20}H_{26}N_8NaO_8$ $[M+Na]^+$ 529.1874, found 529.1781.

5. Synthesis of a 2'-deoxyribonucleosyl morpholino dinucleotide 29

5.1. Synthesis of 5'-O-DMTr-protecetd secodialdehyde from 5-methyluridine

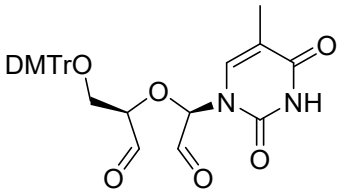
5'-*O*-(4,4'-Dimethoxytrityl)-5-methyluridine (25)[10]



5-Methyluridine (1.0 g, 3.87 mmol) and DMTrCl (1.57 g, 4.64 mmol, 1.2 equiv.) were dissolved in abs. pyridine (5 ml) and stirred at room temperature overnight. Next day, the reaction mixture was diluted with EtOAc and extracted with 10% aqueous solution of $NaHSO_4$. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2:acetone 7:3→6:4) to give **25** (1.91 g, 88%) as a white foam. R*f*=0.3 (CH_2Cl_2:acetone 6:4); $[\alpha]_D$ +3.4(*c*=0.29; $CHCl_3$) ^{1}H NMR (400 MHz, DMSO) δ 7.52 (s, 1H, H-6), 7.40 (d, J = 7.3 Hz, 2H, 2x Arom. C*H*), 7.29 (dt, J = 16.8, 7.6 Hz, 8H, 8x Arom. C*H*), 6.91 (d, J = 8.8 Hz, 4H, 4x Arom. C*H*), 5.82 (d, J = 5.2 Hz, 1H, H-1'), 5.48 (d, J = 5.7 Hz, 1H, 2'O*H*), 5.18 (d, J = 5.5 Hz, 1H, 3'O*H*), 4.20 (dd, J = 10.8, 5.4 Hz, 1H, H-2), 4.12 (dd, J = 10.1, 5.1 Hz, 1H, H-3'), 3.97 (dd, J = 6.9, 3.9 Hz, 1H, H-4'), 3.74 (s, 6H, 2x DMTrO CH_3), 3.25 (dd, J = 10.6, 4.1 Hz, 1H, H-5'a), 3.19 (dd, J = 10.5, 2.4 Hz, 1H, H-5'b), 2.51 (dt, J = 3.5, 1.7 Hz, 1H), 1.42 (s, 3H, thymine CH_3) ppm. ^{13}C NMR (100 MHz, DMSO) δ 163.7, 158.2, 150.7 (4C, 2x *C*=O & 2x Arom. C_q), 144.8 (1C, Arom. C_q),

135.9 (1C, C-6), 135.4, 135.2 (2C, 2x Arom. C_q), 129.8, 128.0, 127.7, 126.9, 113.3 (13C, 13x Arom. *C*H), 109.6 (1C, C-5), 88.0, 82.9, 73.3, 70.2 (4C, C-1', C-2', C-3', C-4'), 85.9 (1C, DMTrC_q), 63.5 (1C, C-5'), 55.1 (2C, 2x DMTrO CH_3), 11.7 (1C, thymine CH_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{31}H_{32}N_2NaO_8S$ $[M+Na]^+$ 583.206, found 583.182.

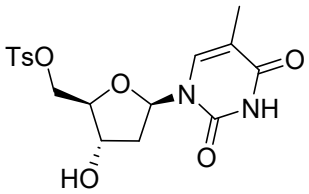
5'-*O*-(4,4'-Dimethoxytrityl)-5-methyluridine-secodialdehyde (26)



1.0 g (1.8 mmol) **25** was dissolved in MeOH (40 ml), then 4.0 g IO_4^- resin was added to the solution and was stirred overnight in dark. Next day, the reaction mixture was filtered through a pad of Celite, and concentrated *in vacuo*. The crude product **26** was used for the reductive amination reaction without purification.(R*f*= 0.57 CH_2Cl_2:MeOH 1:1).

5.2. Synthesis of 5'-amino 5'-deoxy-thymidine

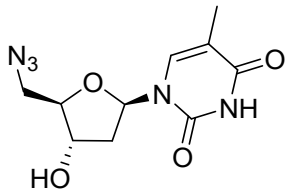
5'-*O*-(*p*-Toluenesulfonyl)-thymidine (27)[11]



Thymidine (2.0 g, 8.2 mmol) was dissolved in dry pyridine and cooled to 0 °C. TsCl (1.96 g, 10.3 mmol, 1.25 equiv.) was added and the reaction mixture was stirred overnight at 0 °C. Next day, H_2O (10 ml) was added and stirred for 10 min. The reaction mixture was diluted with EtOAc, extracted with H_2O and 10% aqueous solution of $NaHSO_4$. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2:acetone 96:4→95:5) to give **27** (2.0 g, 62%) as a white solid. R*f*= 0.30 (CH_2Cl_2:acetone 9:1); $[\alpha]_D$ +22.9 (0.44g/100mL; DMSO) ^{1}H NMR (400 MHz, DMSO) δ 7.80 (d, J = 8.3 Hz, 2H, 2x Arom.C*H*), 7.48 (d, J = 8.1 Hz, 2H, 2x Arom. C*H*), 7.39 (s, 1H, H-6), 6.16 (t, J = 6.9 Hz, 1H, H-1'), 5.45 (d, J = 4.4 Hz, 1H), 4.27 (dd, J = 10.9, 3.3 Hz, 1H, H-5'a), 4.18 (dd, J = 10.8, 5.9 Hz, 2H), 2.51 (s, 1H), 2.42 (s, 3H, tosyl CH_3), 2.16 (dt, J = 13.7, 6.9 Hz, 1H, H-2'a), 2.07 (ddd, J = 13.5, 6.5, 4.0 Hz, 1H, H-2'b), 1.77 (s, 3H, thymine

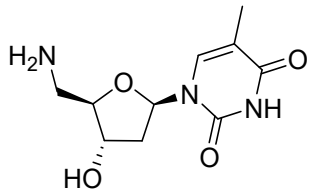
CH_3) ppm. ^{13}C NMR (100 MHz, DMSO) δ 163.7, 150.4 (2C, 2x *C*=O), 145.2 (1C, tosyl C_q), 135.9 (1C, thymine C-6), 132.1 (1C, tosyl C_q), 130.2 127.7 (4C, 4x Arom. *C*H), 109.8 (1C, C-5), 84.0, 83.2, 70.0 (3C, C-1', C-3', C-4'), 70.2 (1C, C-5'), 38.4 (1C, C-2'), 21.1 (1C, tosyl *C*H$_3$), 12.1 (1C, thymine *C*H$_3$) ppm. MALDI-ToF MS: *m/z* calcd for $C_{17}H_{20}N_2NaO_7S$ $[M+Na]^+$ 419.0889, found 419.0826.

5'-Azido-5'-deoxy-thymidine (28)[12]



Compound **27** (1.92 g, 4.87 mmol) was dissolved in dry DMF (10 ml). NaN_3 (1.26 g, 19.5 mmol, 4.0 equiv.) was added and stirred overnight at 80°C. Next day, the reaction mixture was diluted with EtOAc and extracted with H_2O. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2:MeOH 95:5) to give **28** (0.74 g, 57%) as a white solid. R*f*= 0.43 (CH_2Cl_2:MeOH 95:5); $[\alpha]_D$=+79.14 (*c*=0.35; DMSO) ^{1}H NMR (400 MHz, DMSO) δ 7.50 (d, *J* = 1.1 Hz, 1H, H-6), 6.21 (t, *J* = 7.0 Hz, 1H, H-1'), 5.42 (s, 1H), 4.25 – 4.15 (m, 1H, H-3'), 3.85 (dd, *J* = 8.9, 5.2 Hz, 1H, H-4'), 3.56 (d, *J* = 5.3 Hz, 2H, H-5'ab), 2.51 (dt, *J* = 3.5, 1.7 Hz, 1H, O*H*), 2.26 (dt, *J* = 13.9, 7.0 Hz, 1H, H-2'a), 2.09 (ddd, *J* = 13.5, 6.5, 3.7 Hz, 1H, H-2'b), 1.80 (s, 3H, thymine C*H*$_3$) ppm. ^{13}C NMR (100 MHz, DMSO) δ 163.7, 150.5 (2C, 2x *C*=O), 136.1 (1C, thymine C-6), 109.9 (1C, thymine C-5), 84.6, 83.9, 70.8 (3C, C-1', C-3', C-4'), 51.7 (1C, C-5'), 38.1 (1C, C-2), 12.1 (1C, thymine *C*H$_3$) ppm. MALDI-ToF MS: : *m/z* calcd for $C_{10}H_{13}N_5NaO_4$ $[M+Na]^+$ 290.0865, found 290.0869.

5'-Amino-5'-deoxy-thymidine (29)[13]

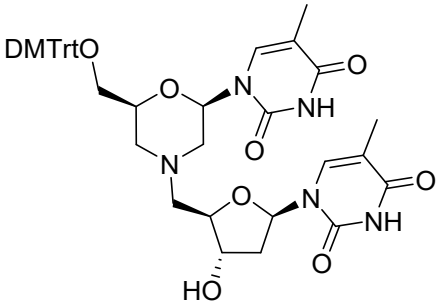


Compound **28** (0.7 g, 2.61 mmol) was dissolved in a mixture of MeOH (10 ml) and DMF (2 ml) and Pd/C (0.070 g) was added. The reaction mixture was stirred under H_2 athmosphere overnight. The reaction mixture was filtered through a pad of Celite, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 6:4) to give **29** (0.38 g, 60%) as white solid. R*f*= 0.17 (CH_2Cl_2:MeOH 6:4); $[\alpha]_D$=+22.7 (*c*=0.15; DMSO)

^{1}H NMR (400 MHz, DMSO) δ 7.65 (s, 1H, H-6), 6.14 (t, J = 6.9 Hz, 1H, H-1'), 4.20 (dt, J = 6.3, 3.3 Hz, 1H, H-3'), 3.65 (dd, J = 8.5, 5.1 Hz, 1H, H-4'), 2.73 (d, J = 5.4 Hz, 2H, H-5'a,b), 2.51 (d, J = 1.6 Hz, 1H), 2.14 (dd, J = 13.5, 7.3 Hz, 1H, H-2'a), 2.04 (ddd, J = 13.3, 6.2, 3.4 Hz, 1H, H-2'b), 1.79 (s, 3H, thymine CH_3) ppm. ^{13}C NMR (100 MHz, DMSO) δ 163.8, 150.5 (2C, 2xCO), 136.3 (1C, C-6), 109.6 (1C, C-5), 87.8, 83.4, 70.8 (3C, C-1', C-3', C-4'), 43.7 (1C, C-5'), 39.0 (1C, C-2'), 12.2 (1C, thymine CH$_3$) ppm. MALDI-ToF MS: *m/z* calcd for $C_{10}H_{15}N_3NaO_4$ $[M+Na]^+$ 264.0960, found 264.1013.

5.3. Double reductive amination cyclization reaction

5'-Deoxy-5'-[6-(4,4'-triphenylmethyloxymethyl)-2-(thymine-1-yl)-morpholine-4-yl]-thymidine (30)

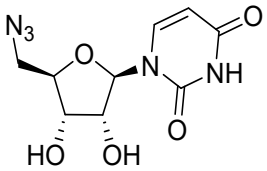


Compound **26** (0.77 g, 1.37 mmol), and **29** (0.33 g, 1.37 mmol) were dissolved in EtOH (20 ml) and stirred for 10 min at room temperature. AcOH (3 drops) and $NaCNBH_3$ (0.103 g, 1.64 mmol, 1.2 equiv.) were added and the stirring was continued overnight. The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2, the organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 98:2→95:5) to give **30** (0.47 g, 45%) as a white solid. R*f*= 0.28 (CH_2Cl_2:MeOH 95:5); $[\alpha]_D$=+7.6(*c*=0.25; DMSO) ^{1}H NMR (400 MHz, DMSO) δ 7.54 (s, 1H, thymine H-6), 7.48 (s, 1H, thymine H-6), 7.38 (d, J = 7.6 Hz, 2H, 2x Arom. CH), 7.33 – 7.19 (m, 7H, 7x Arom. CH), 6.87 (d, J = 8.6 Hz, 4H, 4x Arom. CH), 6.18 (t, J = 6.7 Hz, 1H, thymidine H-1'), 5.70 (dd, J = 9.8, 1.6 Hz, 1H, morpholine H-2), 5.35 (s, 1H, OH), 4.19 (s, 1H, H-3'), 4.02 – 3.93 (m, 1H, morpholine H-6), 3.90 – 3.82 (m, 1H, H-4'), 3.74 (s, 6H, 2x OCH_3), 3.11 (dd, J = 9.1, 5.0 Hz, 1H, morpholine H-7a), 2.99 (d, J = 10.6 Hz, 1H, morpholine H-5a), 2.93 (d, J = 9.3 Hz, 2H, morpholine H-7b & morpholine H-3a), 2.76 (dd, J = 13.2, 2.9 Hz, 1H, H-5'a), 2.63 (dd, J = 13.4, 7.0 Hz, 1H, H-5'b), 2.38 – 2.26 (m, 1H, morpholine H-3b), 2.20 (dt, J = 13.2, 6.6 Hz, 1H, H-2'a), 2.14 – 2.04 (m, 2H, H-2'b & morpholine H-5b), 1.78 (s, 6H, 2x thymine CH_3) ppm. ^{13}C NMR (100 MHz, DMSO) δ 163.7, 163.6, 150.5, 150.1 (4C, 4x C=O),

136.3, 136.1 (2C, 2x thymine C-6), 158.1, 144.8, 135.5, 135.4 (5C, 5x Arom C_q), 129.7, 127.9, 127.7, 126.7, 113.2 (13C, 13x Arom. *C*H), 109.9, 109.6 (2C, 2x thymine C-5), 85.5 (1C, DMTrO C_q), 83.6, 83.6 (2C, C-1' & C-4'), 78.7 (1C, morpholine C-2), 74.8 (1C, morpholine C-6), 71.4 (1C, C-3'), 64.3 (1C, morpholine C-7), 59.0 (1C, C-5'), 55.0 (2C, 2x O*C*H$_3$), 54.9 (1C, morpholine C-3), 54.7 (1C, morpholine C-5), 38.3 (1C, C-2'), 12.2, 12.1 (2C, 2x thymine *C*H$_3$) ppm. MALDI-ToF MS: *m/z* calcd for $C_{20}H_{27}N_5NaO_8$ $[M\text{-}DMTr+Na]^+$ 488.176, found 488.1761.

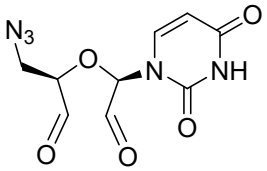
6. Synthesis of tri- and tetranucleotide derivatives 37 and 45

5'-Azido-5'-deoxyuridine (32)[4]



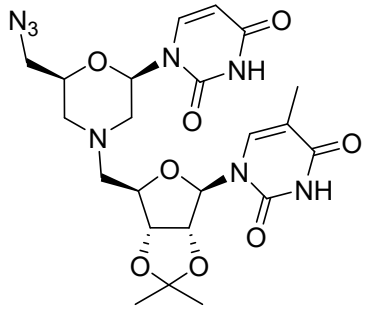
90% aqueous TFA (20 ml) was added to the 2',3'-O-isopropylidene derivative **31** (1,7 g, 5.5 mmol) and stirred at room temperature for 4 hours. After that the mixture was evaporated and co-evaporated with toluene. The crude product was purified by flash column chromatography (CH_2Cl_2:acetone 7:3→4:6) to give **32** (1 g, 69%) as a white solid. R*f*= 0.29 (CH_2Cl_2:acetone 1:1).

5'-Azido-uridine-secodialdehyde (33)



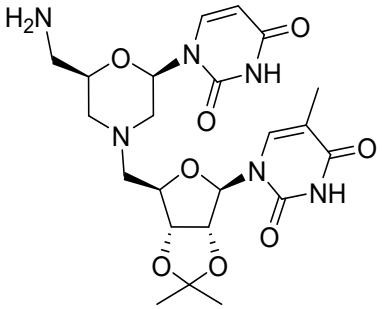
0.66 g (1.2 mmol) **32** was dissolved in MeOH (15 ml), then 2.6 g IO_4^- resin was added to the solution and was stirred overnight in dark. Next day, the reaction mixture was filtered through a pad of Celite, and concentrated *in vacuo*. The crude product **33** was used for the reductive amination reaction without purification.(R*f*= 0.62 CH_2Cl_2:MeOH 8:2).

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-azidomethyl-2-(uracil-1-yl)-morpholine-4-yl]-5-methyluridine (34)



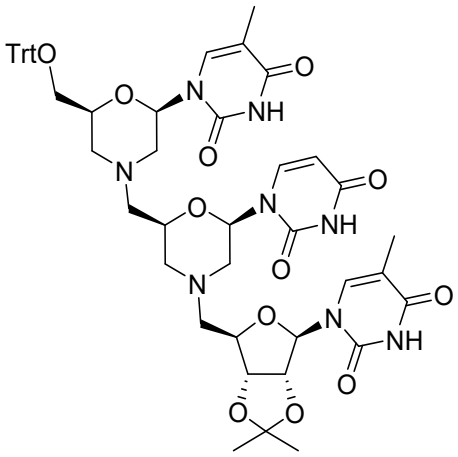
Compound **33** (1.75 g, 6.55 mmol) and **4b** (1.95 g, 6.55 mmol, 1.0 equiv.) were dissolved in EtOH (30 ml) and stirred at room temperature for 10 min. After that, AcOH (2 drops) and $NaCNBH_3$ (0.49 g, 6.86 mmol, 1.2 equiv.) were added and stirred overnight. Next day, The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 97:3→95:5) to give **34** (1.67 g, 47%) as a white solid. R*f*= 0.17 (CH_2Cl_2:MeOH 97:3); $[\alpha]_D$=+89.41 (*c*=0,34; $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, *J* = 8.1, 1.4 Hz, 1H, uracil H-6), 7.08 (d, *J* = 1.5 Hz, 1H, thymine H-6), 5.81 (dd, *J* = 9.8, 2.5 Hz, 1H, morpholine H-2), 5.76 (dd, *J* = 8.2, 1.6 Hz, 1H, uracil H-5), 5.45 (d, *J* = 1.6 Hz, 1H, H-1'), 5.11 (dd, *J* = 6.5, 1.6 Hz, 1H, H-2'), 4.75 (dd, *J* = 6.6, 4.8 Hz, 1H, H-3'), 4.22 (dt, *J* = 8.8, 4.0 Hz, 1H, H-4'), 4.07 (qd, *J* = 6.8, 4.4, 3.8 Hz, 1H, morpholine H-6), 3.50 (dd, *J* = 13.2, 3.7 Hz, 1H, morpholine H-7a), 3.29 (dd, *J* = 13.4, 5.0 Hz, 1H, morpholine H-7b), 3.20 (d, *J* = 10.6 Hz, 1H, morpholine H-3a), 3.04 – 2.93 (m, 1H, morpholine H-5a), 2.93 (d, *J* = 9.2 Hz, 1H, H-5'a), 2.70 (dd, *J* = 13.1, 3.3 Hz, 1H, H-5'b), 2.17 (t, *J* = 11.0 Hz, 1H, morpholine H-5b), 2.08 (t, *J* = 10.5 Hz, 1H, morpholine H-3b), 1.87 (s, 3H, thymine C*H*$_3$), 1.54 (s, 3H, *i*-propylidene C*H*$_3$), 1.33 (s, 3H, *i*-propylidene C*H*$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 163.8, 150.2 150.1 (4C, 2x uracil *C*=O & 2x thymine *C*=O), 139.8 (1C, uracil C-6), 139.7 (1C, thymine C-6), 114.4 (1C, *i*-propylidene C_q), 110.5 (1C, thymine C-5), 102.7 (1C, uracil C-5), 95.9 (1C, C-1'), 84.8 (1C, C-4'), 84.0 (1C, C-2'), 82.7 (1C, C-3'), 79.5 (1C, morpholine C-2), 75.2 (1C, morpholine C-6), 59.4 (1C, morpholine C-7), 56.4 (1C, C-5'), 53.0 (1C, morpholine C-3), 52.4 (1C, morpholine C-5), 27.1 (1C, *i*-propylidene *C*H$_3$), 25.2 (1C, *i*-propylidene *C*H$_3$), 12.2 (1C, thymine *C*H$_3$) ppm. MALDI-ToF MS: *m/z* calcd for $C_{22}H_{28}N_8NaO_8$ $[M+Na]^+$ 555.2030, found 555.1936.

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-aminomethyl-2-(uracil-1-yl)-morpholine-4-yl]-5-methyluridine (35)



Compound **34** (1 g, 1.88 mmol) was dissolved in MeOH:DMF 1:1 (20 ml). and Pd-C (0.2 g, 20 m/m%) was added to the reaction mixture The mixture was stirred overnight at room temperature under H_2 atmosphere. Then the solution was filtered through a pad of Celite, and concentrated in vacuo. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 8:2) to afford **35** (0.73 g, 76%) as a white foam. R*f*= 0.20 (CH_2Cl_2:MeOH 8:2); $[\alpha]_D$=+37.5 (*c*=0.28; MeOH) ^{1}H NMR (400 MHz, DMSO) δ 7.71 (d, *J* = 8.1 Hz, 1H, uracil H-6), 7.59 (s, 1H, thymine H-6), 5.80 – 5.71 (m, 1H, H-1'), 5.61 (t, *J* = 7.7 Hz, 2H, uracil H-5 & morpholine H-2), 4.98 (dd, *J* = 6.5, 2.4 Hz, 1H, H-2'), 4.70 (dd, *J* = 6.6, 4.5 Hz, 1H, H-3'), 4.13 (dd, *J* = 7.3, 4.3 Hz, 1H, H-4'), 3.80 – 3.57 (m, 1H, morpholine H-6), 3.00 – 2.83 (m, 2H, morpholine H-3a & morholine H-5a), 2.74 – 2.60 (m, 4H, H-5'a,b & morpholine H-7a,b), 2.17 (t, *J* = 10.5 Hz, 1H, morpholine H-3b), 1.96 (t, *J* = 11.0 Hz, 1H, morpholine H-5b), 1.78 (s, 3H, thymine C*H*$_3$), 1.49 (s, 3H, i-propylidene C*H*$_3$), 1.29 (s, 3H, i-propylidene C*H*$_3$) ppm. ^{13}C NMR (100 MHz, DMSO) *δ* 163.9, 163.0, 150.3, 150.0 (4C 2x uracil *C*=O) & 2x thymine *C*=O), 141.0 (1C, uracil C-6), 138.1 (1C, thymine C-6), 113.5 (*i*-propylidene C_q), 109.6 (1C, thymine C-5), 101.8 (1C, uracil C-5), 91.3 (1C, C-1'), 83.1 (2C, C-2' & C-4'), 82.0 (1C, C-3'), 78.9 (1C, morpholine C-2), 76.7 (1C, morpholine C-6), 59.3 (1C, morpholine C-7), 55.7 (1C, morpholine C-3), 54.2 (1C, morpholine C-5), 43.3 (1C, C-5'), 27.0 (1C, *i*-propyledene *C*H$_3$), 25.3 (1C, *i*-propyledene *C*H$_3$), 12.1 (1C, thymine *C*H$_3$) ppm. MALDI-ToF MS: *m/z* calcd for $C_{22}H_{30}N_6NaO_8$ $[M+Na]^+$ 529.2125, found 529.2232.

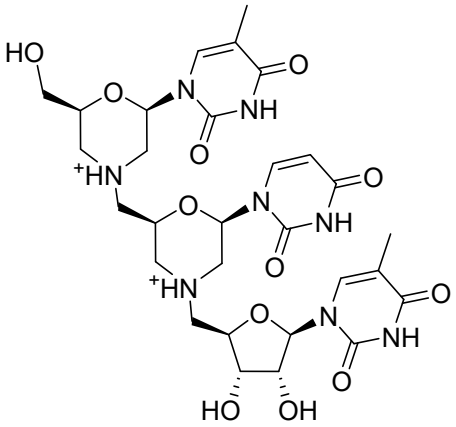
5'-Deoxy-2',3'-*O*-isopropylidene-5'-{2-(uracil-1-yl)-6-[2-(thymine-1-yl)-6-trityl-morpholine-4-yl-methyl]-morpholine-4-yl}5-methyluridine (36)



Compound **3f** (0.58 g, 1.16 mmol) and **35** (0.59 g, 1.16 mmol, 1.0 equiv.) were dissolved in EtOH (15 ml) and stirred at room temperature for 10 min. After that, AcOH (2 drops) and $NaCNBH_3$ (0.087 g, 1.39 mmol, 1.2 equiv.) were added and stirred overnight. Next day, The reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 97:3) to give **36** (0.13 g, 41%) as a white solid. R*f*= 0.29 (CH_2Cl_2:acetone 1:1); $[\alpha]_D$=+1.82 (*c*=0.11; $CHCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.94, 9.59, 9.34 (3x s, 3H, 3x N*H*), 7.42 (d, *J* = 7.6 Hz, 9H), 7.30 – 7.18 (m, 17H, 15x Arom. C*H* & thymine H-6' & uracil H-6), 7.01 (s, 1H, thymine H-6), 5.79 (d, *J* = 9.2 Hz, 1H, morpholine H-2'), 5.75 (d, *J* = 9.1 Hz, 1H, morpholine H-2), 5.54 (s, 1H, uracil H-5), 5.49 (s, 1H, H-1'), 5.07 (s, 1H, H-2'), 4.74 (s, 1H, H-3'), 4.23 (s, 1H, H-4'), 4.05 (s, 2H, morpholine H-6 & morpholine H-6'), 3.27 (dd, *J* = 9.0, 4.3 Hz, 1H, morpholine H-7a), 3.16 – 3.05 (m, 3H, morpholine H-3a & morpholine H-3'a & morpholine H-7'b), 2.95 (d, *J* = 11.3 Hz, 2H, morpholine H-5a & morpholine H-5'a), 2.90 – 2.82 (m, 1H, H-5'a), 2.74 (d, *J* = 11.4 Hz, 1H, H-5'b), 2.60 (s, 1H, morpholine H-7a), 2.54 (d, *J* = 9.5 Hz, 1H, morpholine H-7b), 2.09 (t, *J* = 11.1 Hz, 1H, morpholine H-5'b), 2.02 (t, *J* = 10.1 Hz, 3H, morpholine H-5b & morpholine H-3b & morpholine H-3'b), 1.89 (s, 3H, thymine CH_3), 1.85 (s, 3H, thymine CH_3), 1.55 (s, 3H, *i*-propylidene CH_3), 1.33 (s, 3H, *i*-propylidene CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.2, 163.85, 150.4, 150.2, 150.0 (6C, 6x *C*=O), 143.9 (3C, 3x OTrt Arom. C_q), 139.8, 139.4, 135.6 (3C, 2x thymine C-6 & uracil C-6), 128.8, 128.0, 127.3 (15C, 15x Arom. *C*H), 114.6 (1C, i-propylidene C_q), 111.1, 111.0 (2C, 2x thymine C-5), 102.6 (1C, uracil C-5), 95.8 (1C, C-1'), 86.9 (1C, OTrt C_q), 85.1 (1C, C-4'), 84.1 (1C, C-2'), 83.0 (1C, C-3'), 79.7 (2C, morpholine C-2 & morpholine C-2'), 75.6 (2C, morpholine C-6 & morpholine C-6'), 64.8 (1C, morpholine C-7'), 60.3 (1C, morpholine C-7), 60.0 (1C, C-5'), 56.9, 56.7 (2C, morpholine C-3 & morpholine

C-3'), 55.5, 55.2 (2C, morpholine C-5 & morpholine C-5'), 27.4, 25.5 (2C, 2x *i*-propylidene CH_3), 12.6, 12.4 (2C, 2x thymine CH_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{51}H_{56}N_8NaO_{12}$ $[M+Na]^+$ 995.4015, found 995.3074.

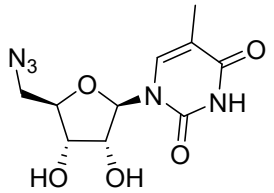
5'-Deoxy-5'-{2-8uracil-1-yl)-6-[2-(thymine-1-yl)-6-trityl-morpholine-4-yl-methyl]-morpholine-4-yl}5-methyluridine (37)



90% aqueous TFA (5 ml) and Et_3SiH (0.064 mmol, 0.047 g, 3 equiv.) were added to **36** (0.13 g, 0.13 mmol) and stirred at room temperature for 2 hours. After that, the mixture was evaporated and co-evaporated with toluene. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 8:2) to give **37** (0,08 g, 85%) as a white solid. R*f*= 0.21 (CH_2Cl_2:MeOH 8:2); $[\alpha]_D$=+19.1 (*c*=0.22; DMSO) ^{1}H NMR (400 MHz, DMSO) δ 11.35 (d, *J* = 21.7 Hz, 3H, 3x N*H*), 7.64 (d, *J* = 8.1 Hz, 1H, uracil H-6), 7.55 (s, 1H, thymine H-6), 7.45 (s, 1H, thymine H-6'), 5.73 (d, *J* = 4.8 Hz, 1H, H-1'), 5.61 (dd, *J* = 14.0, 8.9 Hz, 3H, uracil H-5 & 1.morpholine H-2 & 2. morpholine H-2), 5.42 (d, *J* = 4.7 Hz, 1H, 2'-O*H*), 5.21 (s, 1H, 3'-O*H*), 4.83 (t, *J* = 5.4 Hz, 1H, morpholine 7'-O*H*), 4.09 (d, *J* = 4.7 Hz, 1H, H-2'), 4.00 – 3.85 (m, 3H, H-3' & H-4', morpholine H-6), 3.78 – 3.62 (m, 1H, morpholine H-6'), 3.41 (s, 2H + H_2O, morpholine H-7' a,b). 2.96 (d, *J* = 9.9 Hz, 1H, morpholine H-5'a), 2.88 (t, *J* = 10.7 Hz, 1H, 3H, morpholine H-5a & morpholine H-3a & morpholine H-3'a), 2.77 (d, *J* = 11.0 Hz, 1H, H-5'a), 2.67 – 2.42 (m, , morpholine H-5'b & morpholine H-7a,b + solvent), 2.20 (t, *J* = 10.4 Hz, 2H, morpholine H-3b & morpholine H-3'b), 2.01 (dt, *J* = 20.8, 10.9 Hz, 2H, morpholine H-5b & morpholine H-5'b), 1.79 (d, *J* = 7.9 Hz, 6H, 2x thymine C*H*$_3$) ppm. ^{13}C NMR (100 MHz, DMSO) δ 163.8, 163.7, 163.0, 150.7, 150.1, 150.1 (6C, 6x *C*=O), 140.8* (1C, uracil C-6) 136.7, 136.5 (2C, thymine C-5 & thymine C-5'), 109.9, 109.5 (2C, 2x thymine C_q), 102.0 (1C, uracil C-5), 88.8 (1C, C-1'), 80.9 (1C, C-4'), 79.0 (1C, morpholine C-2), 78.8 (1C, morpholine C-2'), 76.9 (1C, morpholine C-6), 73.4 (1C, morpholine C-6'), 72.1 (1C, C-2'), 71.4 (1C, C-3'), 62.0

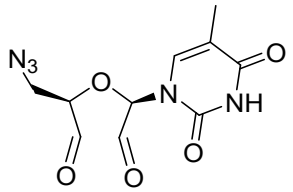
(1C, morpholine C-7'), 59.9 (1C, morpholine C-7), 59.5 (1C, C-5'), 55.7 (2C, morpholine C-3 & morpholine C-3') 55.4* (1C, morpholine C-5') 12.1, 12.0 (2C, 2x thymine CH_3) ppm. *Note that peaks can only be seen in HSQC. MALDI-ToF MS: *m/z* calcd for $C_{29}H_{38}N_8NaO_{12}$ $[M+Na]^+$ 713.2609, found 713.2506.

5'-Azido-5'-deoxy-5-methyluridine (39)[6]



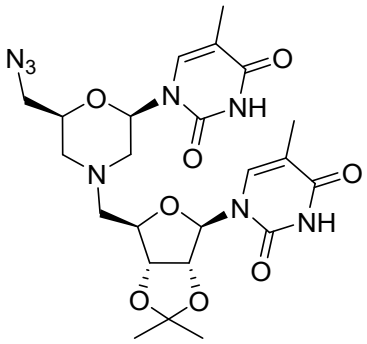
90% aqueous TFA (40 ml) was added to the 2',3'-*O*-isopropylidene derivative **38** (3.4 g, 10.52 mmol) and stirred at room temperature for 4 hours. After that the mixture was evaporated and co-evaporated with toluene. The crude product was purified by flash column chromatography (CH_2Cl_2:acetone 1:1) to give **39** (2.65 g, 89%) as a white solid. R*f*= 0.28 (CH_2Cl_2:acetone 1:1).

5'-Azido-5'-deoxy-5-methyluridine-secodialdehyde (40)



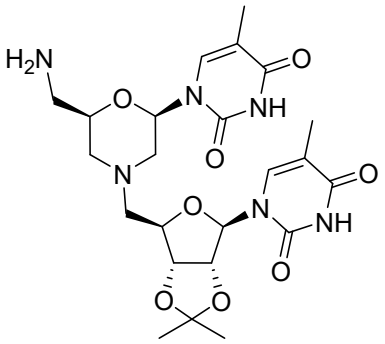
2.2 g (7.7 mmol) **39** was dissolved in MeOH (80 ml), then 8.8 g IO_4^- resin was added to the solution and was stirred overnight in dark. Next day, the reaction mixture was filtered through a pad of Celite, and concentrated *in vacuo*. The crude product **40** was used for the reductive amination reaction without purification. (R*f*= 0.70 CH_2Cl_2:MeOH 1:1).

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-azidomethyl-2-(thymine-1-yl)-morpholine-4-yl]-5-methyluridine (41)



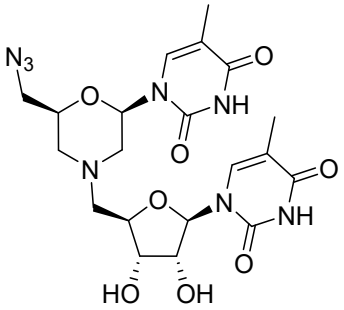
40 (1.8 g, 6.6 mmol) and **4b** (2.54 g, 8.53 mmol, 1.0 equiv.) were dissolved in EtOH (30 ml) and stirred at room temperature for 10 min. After that, AcOH (2 drops) and $NaCNBH_3$ (0.64 g, 10.2 mmol, 1.2 equiv.) were added and stirred overnight. Next day, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 97:3) to give **41** (1.5 g, 42%) as a white solid. R*f*= 0.63 (CH_2Cl_2:MeOH 9:1); $[\alpha]_D$=+90.31 (*c*=0,32; $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (s, 1H, thymine H-6), 7.08 (s, 1H, thymine H-6), 5.80 (dd, *J* = 9.8, 2.6 Hz, 1H, morpholine H-2), 5.45 – 5.42 (m, 1H, H-1'), 5.14 – 5.10 (m, 1H, H-2'), 4.75 (dd, *J* = 6.5, 4.6 Hz, 1H, H-3'), 4.24 (dt, *J* = 8.9, 4.2 Hz, 1H, H-4'), 3.53 (dd, *J* = 13.3, 3.6 Hz, 1H, morpholine H-6), 3.27 (d, *J* = 4.6 Hz, 1H, morpholine H-7a), 3.24 – 3.16 (m, 2H, morpholine H-7b & morpholine H-3a), 3.01 (q, *J* = 6.7 Hz, 2H, morpholine H-5a & H-5'a), 2.73 – 2.63 (m, 1H, H-5'b), 2.20 (t, *J* = 11.1 Hz, 1H, morpholine H-5b), 2.10 (t, *J* = 10.5 Hz, 1H, morpholine H-3b), 1.90 (s, 3H, thymine CH_3), 1.86 (s, 3H, thymine CH_3), 1.52 (s, 3H, *i*-propylidene CH_3), 1.30 (s, 3H, *i*-propylidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5, 164.1, 150.3, 150.2 (4C, 4x thymine *C*=O), 139.9, 135.37 (2C, 2x thymine C-6), 114.2 (1C, *i*-propylidene C_q), 111.2, 110.4 (2C, 2x thymine C-5), 96.1 (1C, C-1'), 84.8(1C, C-4'), 84.2 (1C, C-2'), 82.8 (1C, C-3'), 79.3 (1C, morpholine C-2), 75.1 (1C, morpholine C-6), 59.4 (1C, morpholine C-7), 56.3 (1C, C-5'), 52.7 (1C, morpholine C-3), 52.5 (1C, morpholine C-5), 27.2, 25.3 (2C, 2x i-propylidene *C*H_3), 12.5, 12.3 (2C, 2x thymine *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{23}H_{30}N_8NaO_8$ $[M+Na]^+$:569.2187, found 569.2107.

5'-Deoxy-2',3'-*O*-isopropylidene-5'-[6-aminomethyl-2-(thymine-1-yl)-morpholine-4-yl]-5-methyluridine (42)



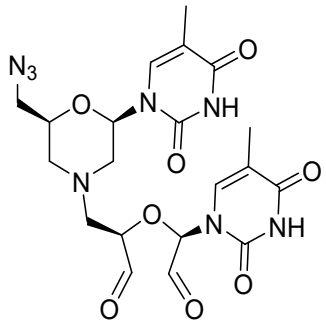
Compound **41** (1.5 g, 2.74 mmol) was dissolved in MeOH:DMF 1:1 (10 ml:10 ml) and Pd-C (0.3g, 20 m/m%) was added to the reaction mixture The mixture was stirred overnight at room temperature under H_2 atmosphere gas was bubbled through the solvent. Then the solution was filtered through a pad of Celite, and concentrated in vacuo. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 8:2) to afford **42** (1.1 g, 77%) as a white foam. R*f*= 0.22 (CH_2Cl_2:MeOH 8:2); $[\alpha]_D$=+81.88 (*c*=0.16; $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 1H, thymine H-6), 7.18 (s, 1H, thymine H-6), 5.82 (dd, *J* = 9.9, 2.6 Hz, 1H, morpholine H-2), 5.54 (d, *J* = 1.9 Hz, 1H, H-1'), 5.05 (dd, *J* = 6.6, 2.0 Hz, 1H, H-2'), 4.75 (dd, *J* = 6.6, 5.0 Hz, 1H, H-3'), 4.18 (dt, *J* = 6.3, 5.1 Hz, 1H, H-4'), 3.85 (dddd, *J* = 10.4, 6.9, 4.7, 2.2 Hz, 1H, morpholine H-6), 3.05 (dt, *J* = 10.8, 2.1 Hz, 1H, morpholine H-3a), 2.95 – 2.89 (m, 1H, morpholine H-5a), 2.83 – 2.80 (m, 2H, morpholine H-7a,b), 2.79 – 2.77 (m, 2H, H-5'a,b), 2.16 (t, 1H, morpholine H-3b), 2.02 (t, *J* = 10.9 Hz, 1H, morpholine H-5b), 1.92 (s, 3H, thymine C*H*$_3$), 1.91 (s, 3H, thymine C*H*$_3$), 1.56 (s, 3H, *i*-propylidene C*H*$_3$), 1.35 (s, 3H, *i*-propylidene C*H*$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7, 164.4, 150.2, 150.2 (4C, 4x thymine *C*=O), 139.1, 135.9 (2C, 2x thymine C-6), 114.5 (1C, *i*-propylidene C_q), 110.7, 110.6 (2C, 2x thymine C-5), 94.7 (1C, C-1'), 84.5, 83.6, 82.2 (3C, 3x skeleton C), 79.4, 77.0 (2C, morpholine C-2 & morpholine C-6), 59.4, 56.3, 54.3, 43.3 (4C, morpholine C-3 & morpholine C-5 & morpholine C-7 & C-5'), 26.9, 25.0 (2C, 2x i-propylidene *C*H$_3$), 12.0, 11.9 (2C, 2x thymine *C*H$_3$) ppm. MALDI-ToF MS: *m/z* calcd for $C_{22}H_{32}N_6NaO_8$ $[M+Na]^+$ 543.2282, found 543.2190.

5'-Deoxy-5'-[6-azidomethyl-2-(thymine-1-yl)-morpholine-4-yl]-5-methyluridine (43)



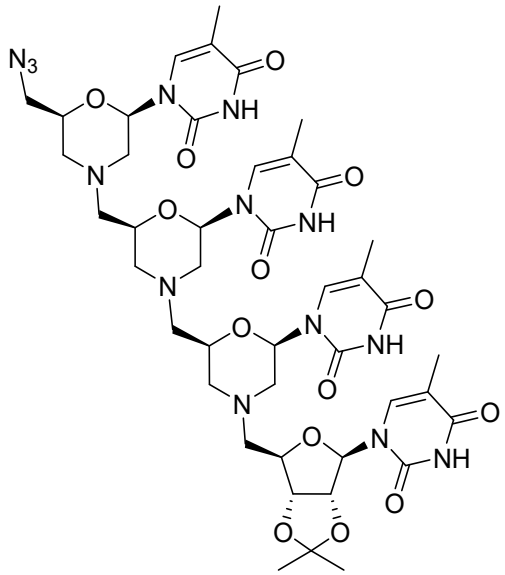
90% TFA (20 ml) was added to **41** (1.4 g, 2.56 mmol) and stirred at room temperature for 4 hours. After that the mixture was evaporated and coevaporated with toluene. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 1:1) to give **43** (1.1 g, 85%) as a white solid. R*f*= 0.25 (CH_2Cl_2:MeOH 9:1); $[\alpha]_D$=+60.0 (*c*=0.37; MeOH) ^{1}H NMR (400 MHz, MeOD) δ 7.52 (s, 1H, thymine H-6), 7.40 (s, 1H, thymine H-6), 5.91 (dd, *J* = 10.0, 2.6 Hz, 1H, morpholine H-2), 5.75 (d, *J* = 4.0 Hz, 1H, H-1'), 4.30 (dd, *J* = 5.7, 4.0 Hz, 1H, H-2'), 4.17 – 4.07 (m, 3H, H-3' & H-4' & morpholine H-6), 3.55 (dd, *J* = 13.4, 3.8 Hz, 1H, H-5'a), 3.40 – 3.33 (m, 1H, H-5'b), 3.33 – 3.26 (m, 1H, morpholine H-5a), 3.13 (d, *J* = 11.1 Hz, 1H, morpholine H-3a), 3.09 – 2.95 (m, 2H, morpholine H-7a,b), 2.56 (t, *J* = 10.8 Hz, 1H, morpholine H-5b), 2.48 (t, *J* = 11.3 Hz, 1H, morpholine H-3b), 1.87 (s, 3H, thymine C*H*$_3$), 1.86 (s, 3H, thymine C*H*$_3$) ppm. ^{13}C NMR (100 MHz, MeOD) δ 166.2, 166.0, 152.3, 151.8 (4C, 4x thymine *C*=O), 139.1, 137.4 (2C, 2x thymine C-6), 112.0, 111.9 (2C, 2x thymine C-5), 93.2 (1C, C-1'), 81.7, 80.29, 76.1, 73.9, 72.9 (5C, 3x skeleton C & morpholine C-2 & morpholine C-6), 60.6 (1C, morpholine C-7), 56.5 (1C, morpholine C-5), 54.6 (1C, morpholine C-3), 53.2 (1C, C-5'), 12.4, 12.4 (2C, 2x thymine *C*H$_3$) ppm. MALDI-ToF MS: *m/z* calcd for $C_{20}H_{26}N_8NaO_8$ $[M+Na]^+$ 529.1874, found 529.1768.

5'-Deoxy-5'-[6-azidomethyl-2-(thymine-1-yl)-morpholine-4-yl]-5-methyluridine-secodialdehyde (44)



1.0 g (1.8 mmol) **43** was dissolved in MeOH (40 ml), then 4.0 g IO_4^- resin was added to the solution and was stirred overnight in dark. Next day, the reaction mixture was filtered through a pad of Celite, and concentrated *in vacuo*. The crude product **44** was used for the reductive amination reaction without purification.(R*f*= 0.75 CH_2Cl_2:MeOH 1:1).

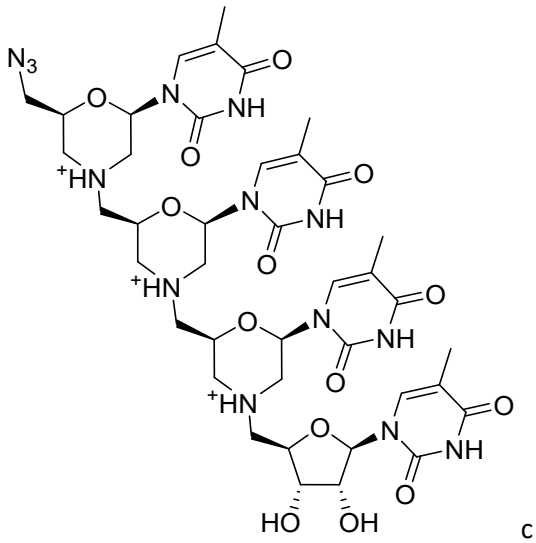
5'-Deoxy-2',3'-*O*-isopropylidene-{2(thymine 1-yl)-6-[2-(thymine-1-yl)-6-azidomethyl-morpholine-4-ylmethyl-(2-(thymine-1-yl))-morpholine-4-ylmethyl]-morpholine-4-ylmethyl}-5-methyluridine (45)



Compound **44** (0.75 g, 1.49 mmol) was dissolved in EtOH (30 ml) and **42** (0.77 g, 1.49 mmol, 1.0 equiv.) was added and stirred at room temperature for 10 min. Then, AcOH (2 drops) and $NaCNBH_3$ (0.110 g, 1.75 mmol, 1.2 equiv.) were added and the reaction mixture was stirred for overnight. Next day, the reaction mixture was diluted with water and extracted with CH_2Cl_2. The combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2:acetone 1:1→CH_2Cl_2:MeOH 9:1) to give **45** (0.64 g, 47%) as an amorph solid. R*f*= 0.41 (CH_2Cl_2:acetone 1:1); $[\alpha]_D$=+76.36 (*c*=0.33; $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$+ MeOD) δ 7.33, 7.24, 7.21 (4x s, 4H, rybothymidine H-6 & thymine H-6 & thymine H-6' & thymine H-6"), 5.86 – 5.71 (m, 3H, morpholine H-2 & morpholine H-2' & morpholine H-2"), 5.60 (s, 1H, H-1'), 5.07 – 5.02 (m, 1H, H-2'), 4.79 – 4.73 (m, 1H, H-3'), 4.26 – 4.17 (m, 1H, H-4'), 4.14 (s, 1H, morpholine H-6), 4.10 – 4.01 (m, 2H, morpholine H-6' & morpholine H-6"), 3.51 (dd, *J* = 13.2, 3.6 Hz, 1H, morpholine H-7"a), 3.37 – 3.29 (m, 1H, morpholine H-7"b), 3.09 (dt, *J* = 21.5, 10.2 Hz, 3H, morpholine H-5a & morpholine H-3a & morpholine H-3'a & morpholine H-3"a), 3.00 – 2.90 (m, 2H, morpholine H-5a & morpholine H-5"a), 2.83 – 2.67 (m, 4H, H-5'a,b & morpholine H-7a & morpholine H-7'a), 2.57 (dd, *J* = 12.6, 4.1 Hz, 1H, morpholine H-7'b), 2.44 (d, *J* = 11.5 Hz, 1H, morpholine H-7b), 2.18 (dq, *J* = 19.3, 10.6, 9.6 Hz, 5H, morpholine H-3b & morpholine H-3'b & morpholine H-3"b & morpholine H-5'a & morpholine H5"b), 2.02 (dd, *J* = 25.1, 12.5 Hz, 2H,morpholine H-5b & morpholine H-5'b), 1.92 – 1.85 (m,

12H, 4x thymine CH_3), 1.57 (s, 3H, *i*-propylidene CH_3), 1.36 (s, 3H, *i*-propylidene CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$+ MeOD) δ 164.6, 164.4, 164.3, 150.1, 149.9 (8C, 8x thymine *C*=O), 139.0, 135.7 (4C, 4x thymine C-6), 114.4 (1C, *i*-propylidene C_q), 110.5 (4C, 4x thymine C-5), 94.2 (1C, C-1), 84.4 (1C, C-4'), 83.6 (1C, C-2'), 82.1 (1C, C-3'), 79.8 (1C, morpholine C-2"), 78.9 (2C, morpholine C-2 & morpholine C-2'), 74.9 (1C, morpholine C-6"), 72.8 (1C, morpholine C-6'), 72.3 (1C, morpholine C-6), 59.9 (1C, morpholine C-7), 59.1 (2C, morpholine C-7' & C-5'), 56.1 (2C, morpholine C-3 & morpholine C-3'), 55.5 (1C, morpholine C-3"), 54.9, 54.6 (2C, morpholine C-5 & morpholine C-5') 53.8 (1C, morpholine C-5"), 52.1 (1C, morpholine C-7"), 26.8, 24.9 (2C, 2x *i*-propylidene *C*H_3), 11.9, 11.8 (4C, 4x thymine *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{43}H_{56}N_{14}NaO_{14}$ $[M+Na]^+$ 1015.4100, found 1015.4007.

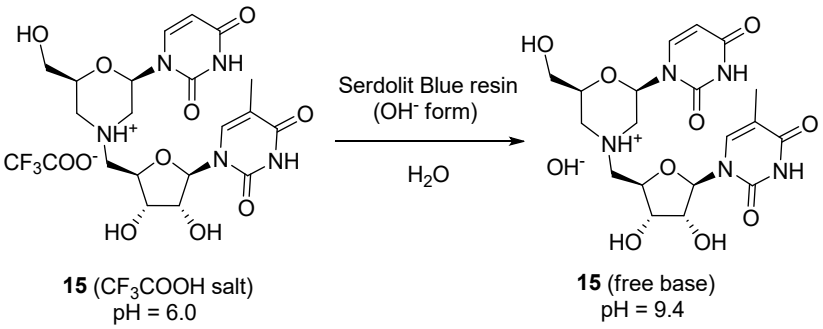
5'-Deoxy-{2-(thymine 1-yl)-6-[2-(thymine 1-yl)-6-azidomethyl-morpholine-4-ylmethyl-(2-(thymine-1-yl))-morpholine-4-ylmethyl]-morpholine-4-ylmethyl}-5-methyluridine (46)



90% aqueous TFA (5 ml) was added to **45** (0.44 g, 0.45 mmol) and stirred at room temperature for 2 hours. After that the mixture was evaporated and co-evaporated with toluene. The crude product was purified by flash column chromatography (CH_2Cl_2:MeOH 9:1) to give **46** (2.65 g, 89%) as a white solid. R*f*= 0.18 (CH_2Cl_2:MeOH 9:1); $[\alpha]_D$=+214.4 (*c*=0.18; MeOH) ^{1}H NMR (400 MHz, DMSO) δ 11.42 (d, *J* = 11.8 Hz, 2H, 2x N*H*), 11.35 (s, 1H, N*H*), 7.54 (s, 4H, 4x thymine H-6), 7.46 (s, 2H), 5.74 (s, 2H), 5.67 (s, 3H), 4.09 (s, 3H), 3.93 (d, *J* = 18.5 Hz, 8H), 3.53 (d, *J* = 11.2 Hz, 1H), 3.48 – 3.41 (m, 1H), 3.40 – 3.29 (m, 2H), 3.17 (s, 3H), 2.94 (s, 8H), 2.69 (d, *J* = 41.2 Hz, 5H), 2.51 (s, 3H), 2.34 (s, 3H), 2.12 (s, 2H), 1.79 (d, *J* = 9.8 Hz, 18H, 4x thymine CH_3) ppm. ^{13}C NMR (100 MHz, DMSO) δ 163.8, 163.6, 163.5, 158.0, 150.7, 150.1, 15.0 (8C, 8x *C*=O), 136.8, 136.3, 136.2, 136.1 (4C, 4x thymine C-6), 110.0, 109.7, 109.7, 109.6

(4C, 4x thymine C-5), 89.0, 78.6, 74.8, 72.0, 71.3 (9C, 4x skeleton *C*H 5x morpholine *C*H), 59.5, 56.0, 55.1, 51.7 (10C, 3x morpholine C-7 & 3x morpholine C-5 & 3x morpholine C-3 & C-5'), 48.6 (1C, morpholine *C*H), 18.6, 12.1, 12.1 (4C, 4x thymine *C*H_3) ppm. MALDI-ToF MS: *m/z* calcd for $C_{40}H_{52}N_{14}NaO_{14}$ $[M+Na]^+$ 975.3787, found 975.3600.

NMR study of compound **15** in forms of TFA salt and free base



Scheme S4. Conversion of TFA salt of **15** into free base

Treatment of 15 with anion exchange resin

Compound **15** (CF_3COOH salt) (0.14 g, 0.11 mmol) was dissolved in H_2O and Serdolit Blue OH^- anion exchange resin (200 mg) was added and stirred for 10 minutes. After that the resin was filtered and compound **15** (free base) was concentrated under reduced pressure. The acidity of H_2O solution of **15** was measured prior and after the treatment of anion exchange resin, pH(TFA salt) = 6.0; pH(free base) = 9.4.

NMR measurments

Highest field NMR spectra were recorded at 300 K using a BrukerNEO/Avance III 700 MHz spectrometer (Bruker, Billerica, MA, USA) equipped with prodigy triple-resonance probehead. Typical 90° pulses were 8.3, and 12 us for 1H and 13C excitations and relaxation delays were in the 2.5-3 s range. Direct chemical shift referencing was performed for 1H using TMS or solvent chemical shifts (DMSO-d6, 2.51 and 40 ppm for ^1H and ^{13}C). Improved off-resonance ROESY spectra[14] were measured at 500 MHz (Bruker Avance II. spectrometer) with 300 ms mixing time and 55° tilt angle of the spin-lock field of 8.3 kHz strength. The spectra were processed using Topspin 3.1 or 4.1 softwares.

Table S1. ^{13}C and ^{1}H NMR chemical shift assignments of salt form and free base form of **15**

Chemical shifts in ppm							
15 (TFA salt)					**15** (base form)		
Assignment	Type	^{13}C	^{1}H	^{1}H	^{13}C	^{1}H	^{1}H
Base							
u2,t2	C=O	151.0, 150.5			151.9, 151.6		
u4,t4	C=O	164.1, 163.2			165.6, 164.9		
u5	CH	102.4	5.63		102.3	5.57	
u6	CH	141.1	7.66		140.8	7.60	
t5	C	110.3			110.2		
t6	CH	137.1	7.40		136.9	7.40	
Me-t5	CH_3	12.3	1.82		12.6	1.79	
Furanose ring							
1'	CH	90.1	5.74		89.4	5.73	
2'	CH	72.7	4.12		72.7	4.06	
3'	CH	71.8	3.94		71.7	3.88	
4'	CH	80.9	4.13		81.3	4.13	
5'	CH_2	59.7	2.87	2.82	59.9	2.73	2.62
Morpholine ring							
2	CH	79.2	5.71		79.5	5.62	
3	CH_2	55.8	3.06	2.43	56.5	2.91	2.17
5	CH_2	54.2	2.92	2.24	54.6	2.88	2.01
6	CH	76.9	3.81		77.31	3.7	
7	CH_2	62.4	3.49		62.5	3.43	
TFA							
	CF_3	117.6 (q)			-		
	C=O	158.2 (q)			-		

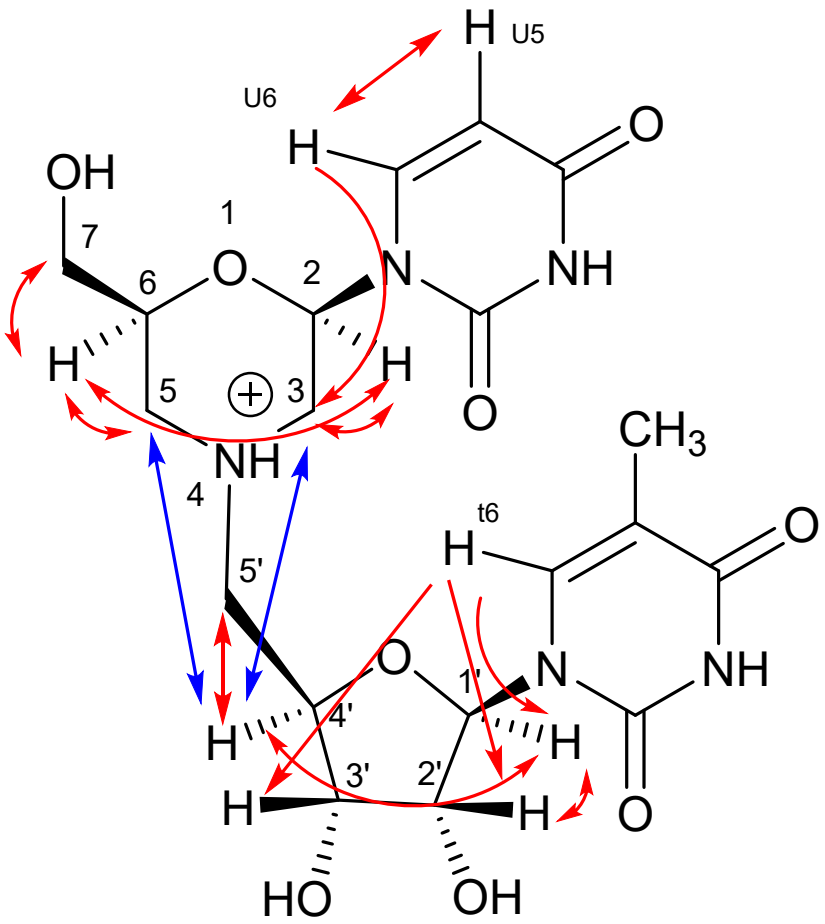


Figure S1. ROESY connectivities of compound **15**. (Blue arrows: internucleotid H-H connectivities, Red arrows: H-H connectivities within on nucleoside unit)

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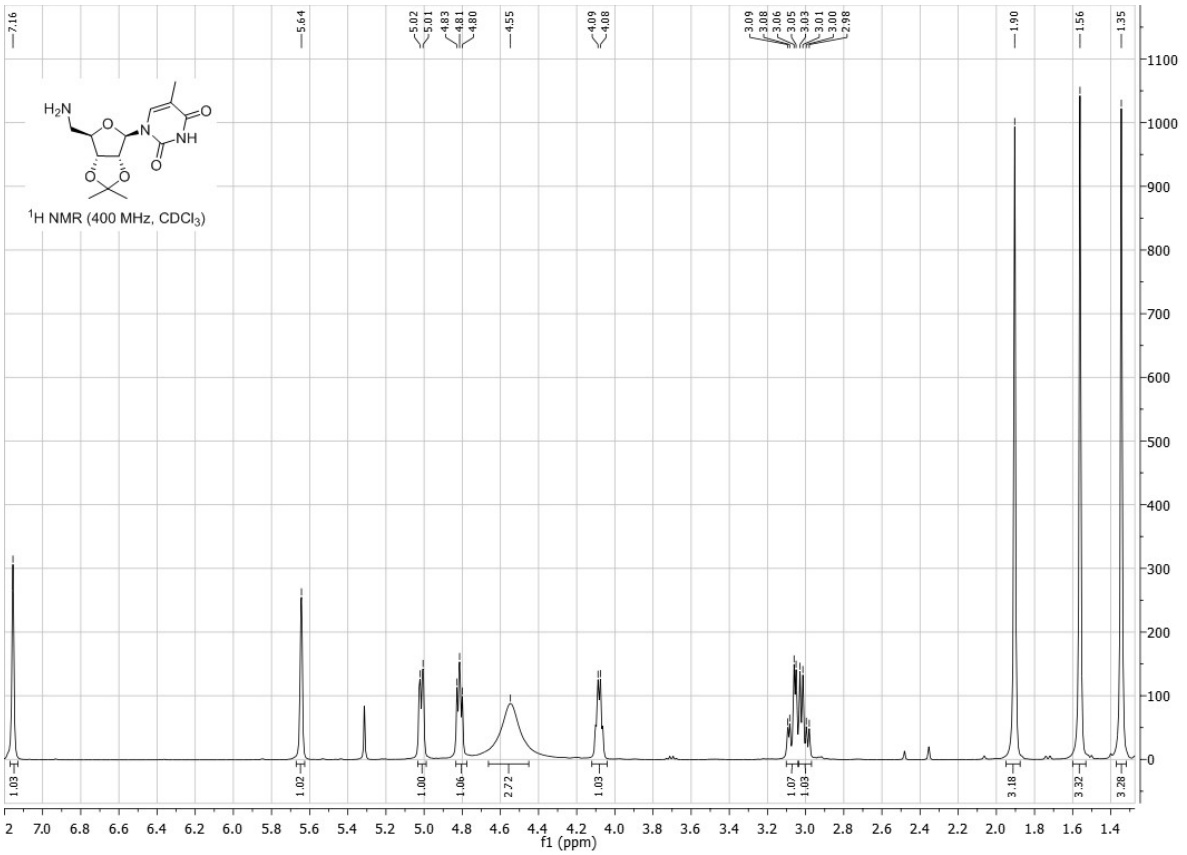
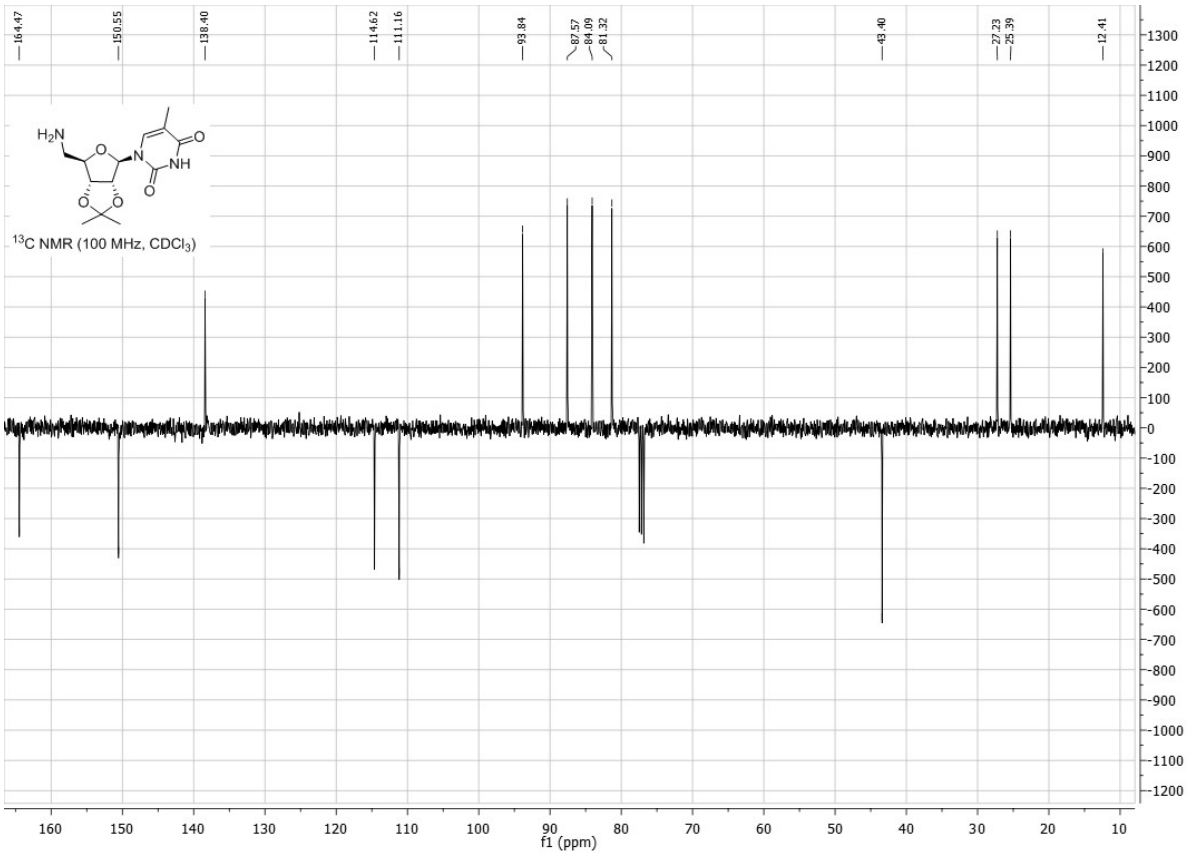
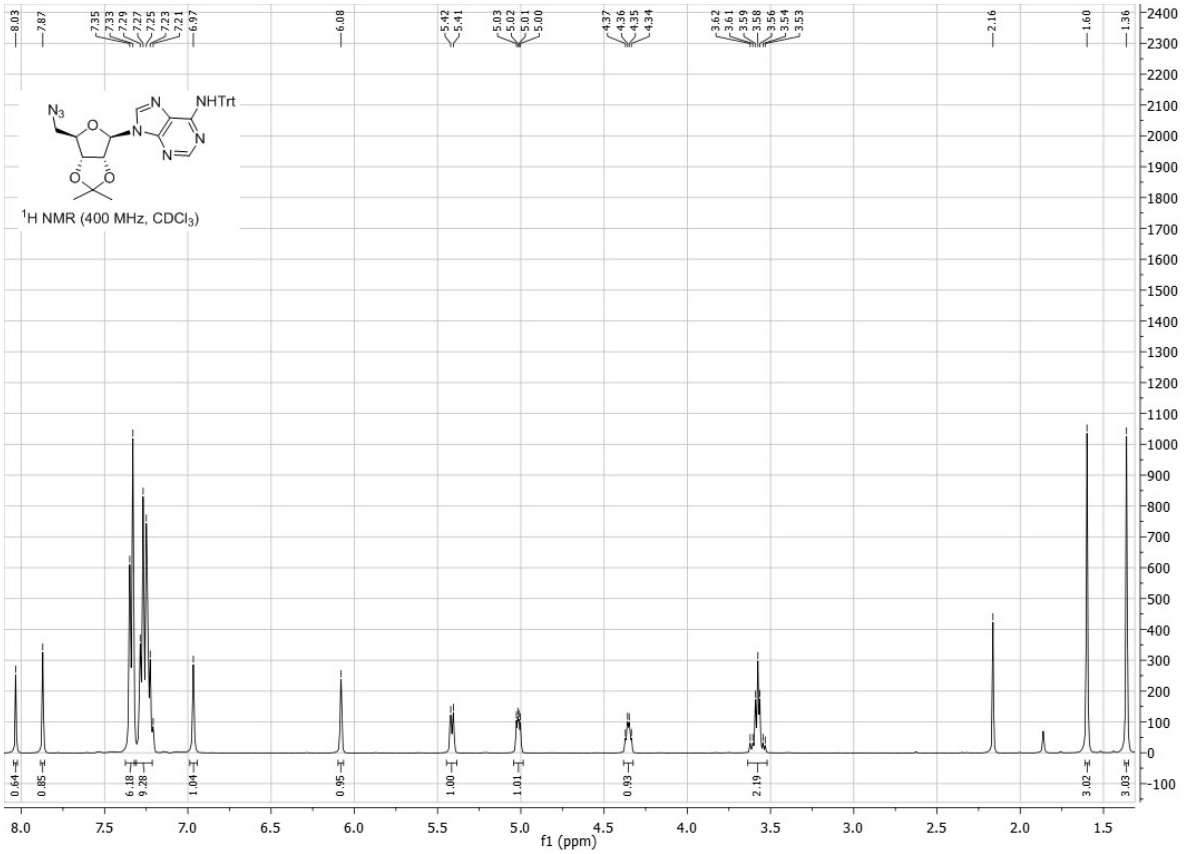
NMR spectra of the compounds

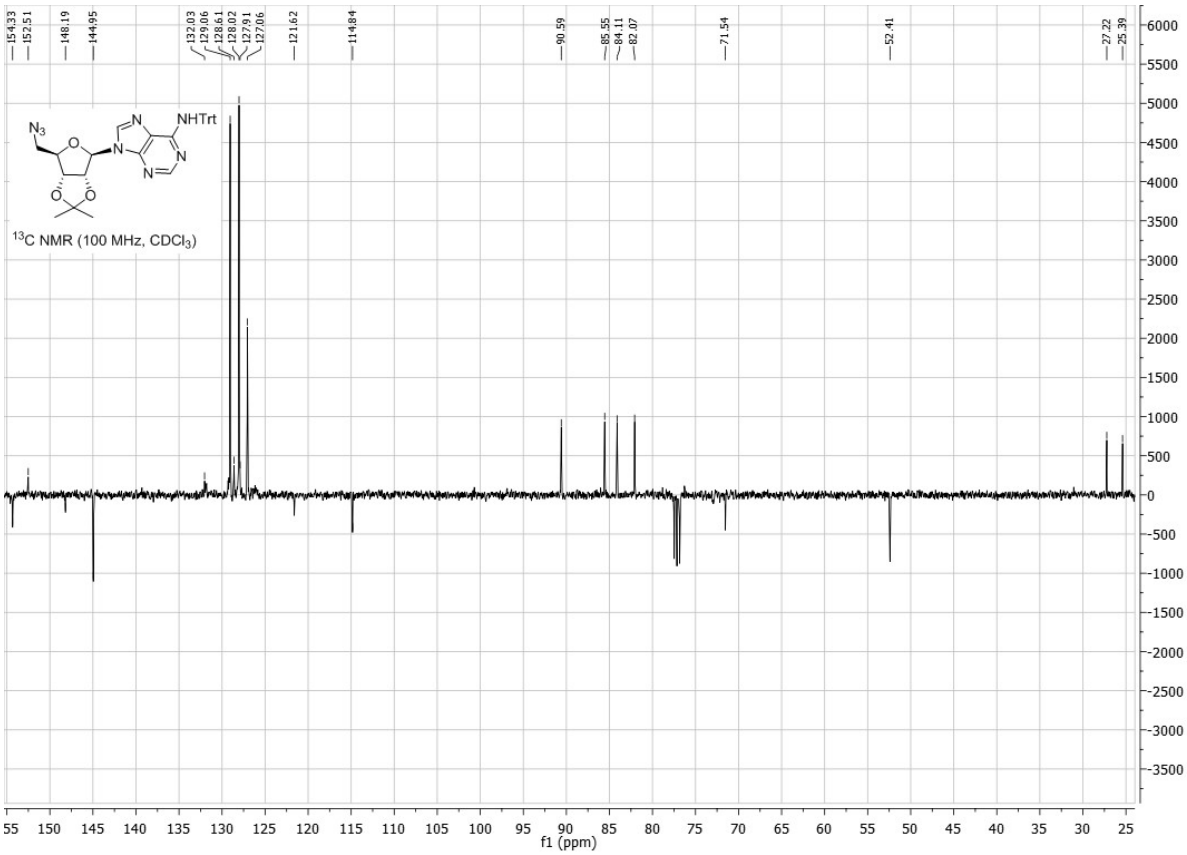
NMR spectra of compound 4b



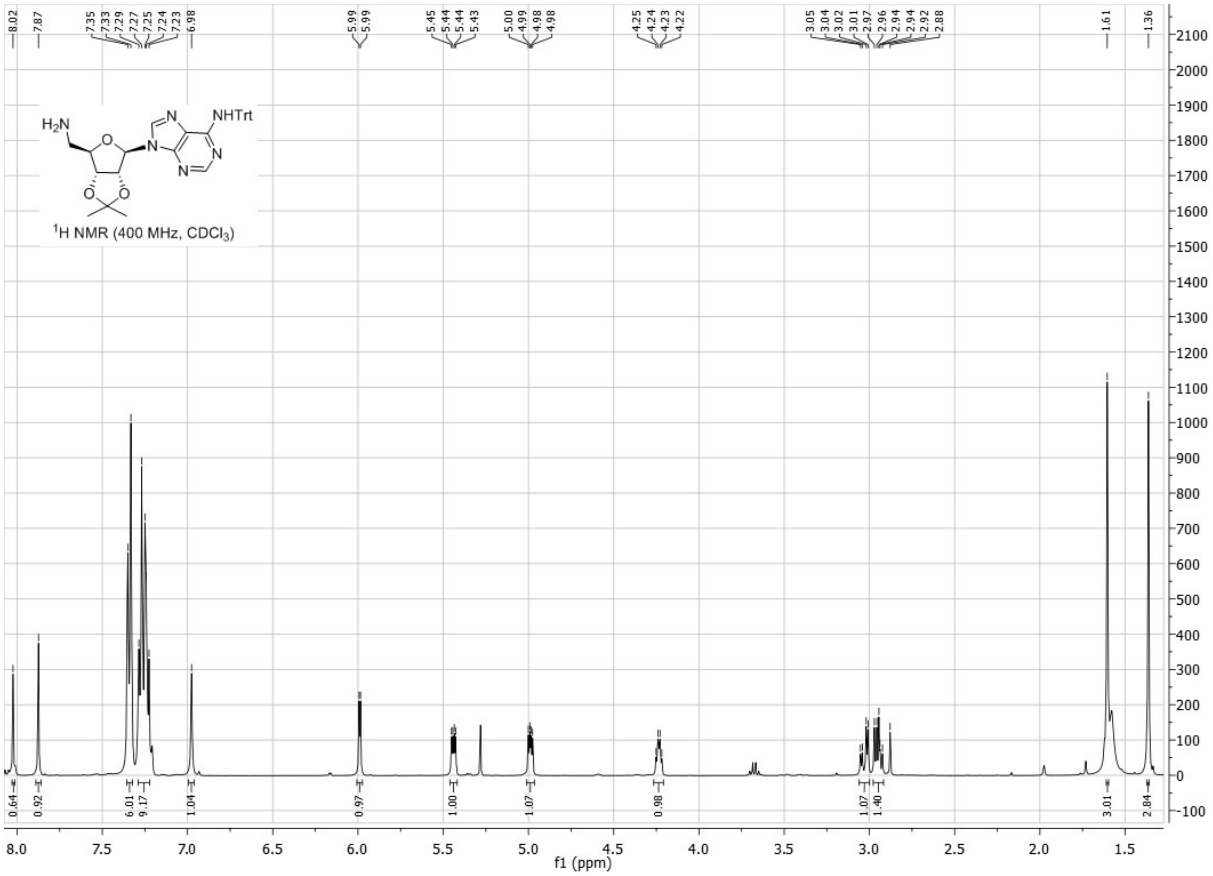


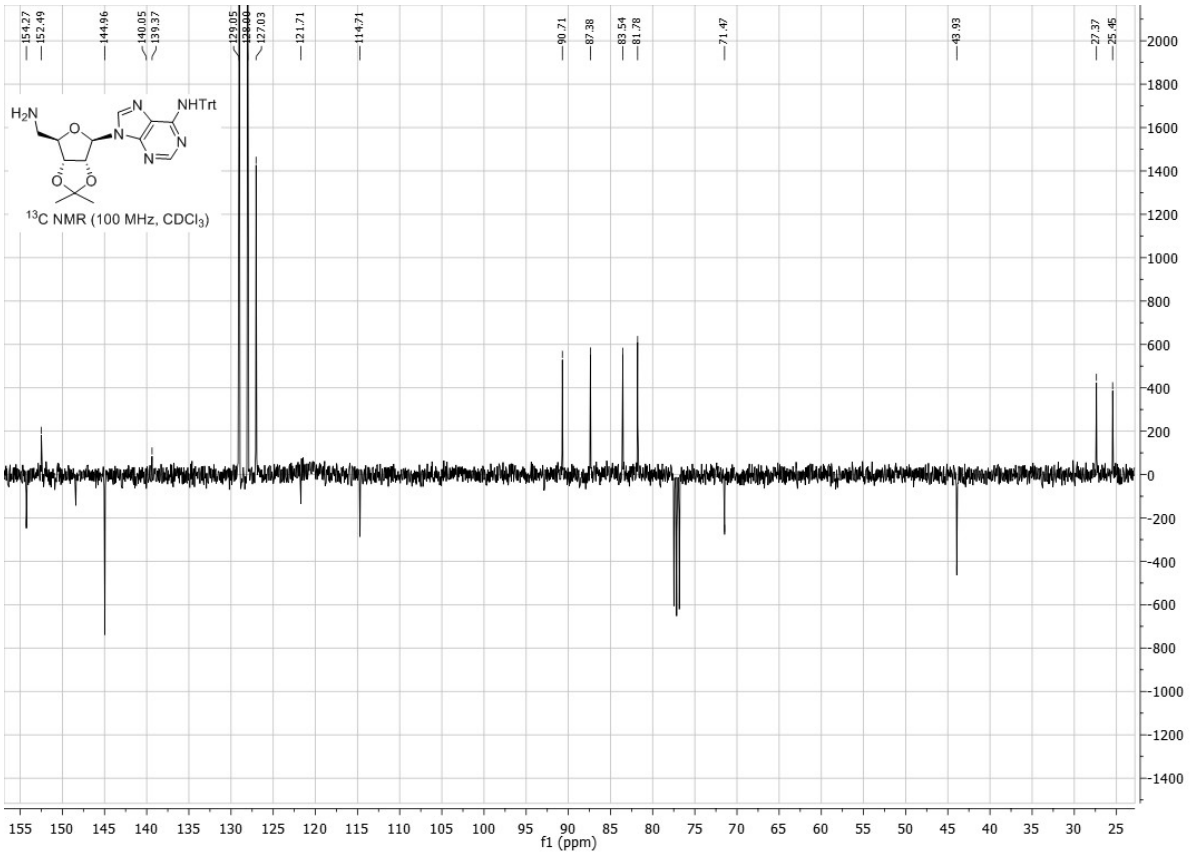
NMR spectra of compound 2c-azide



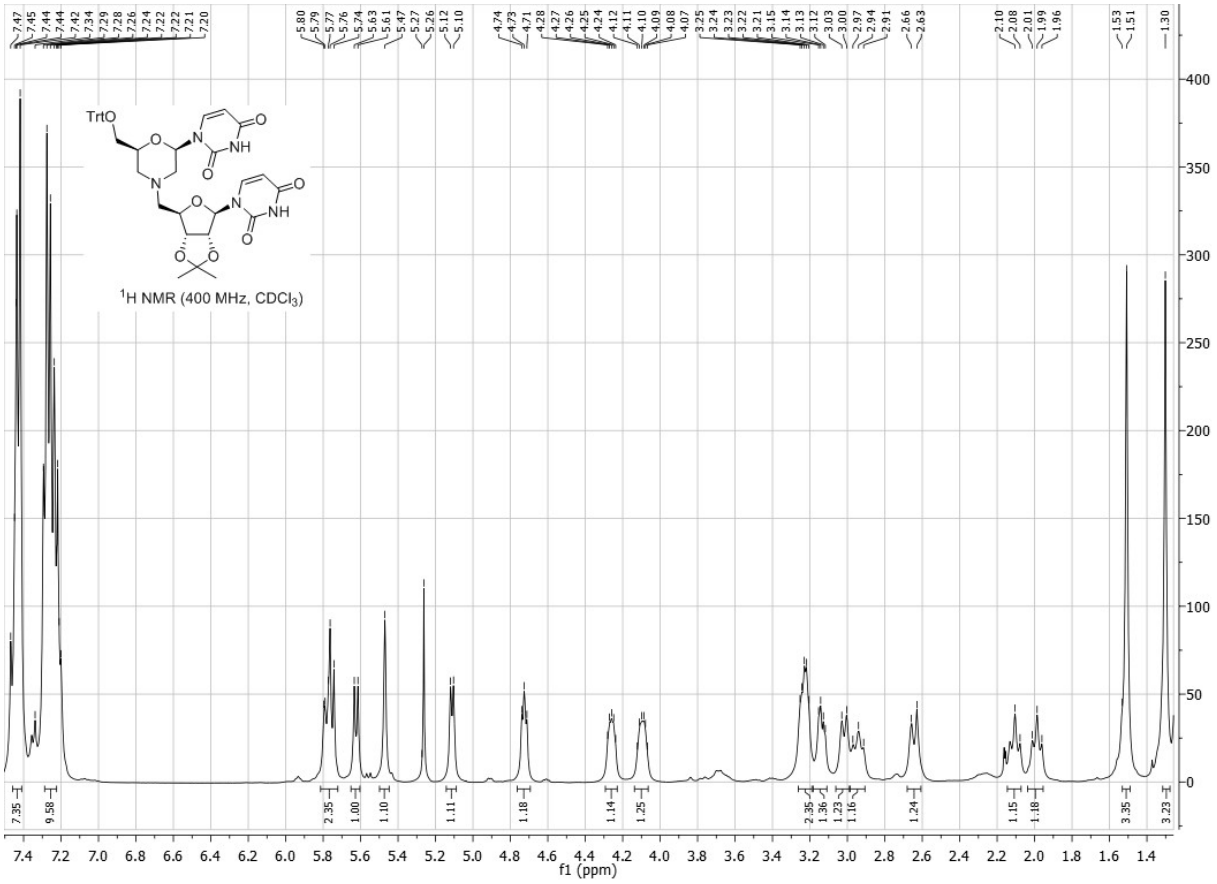


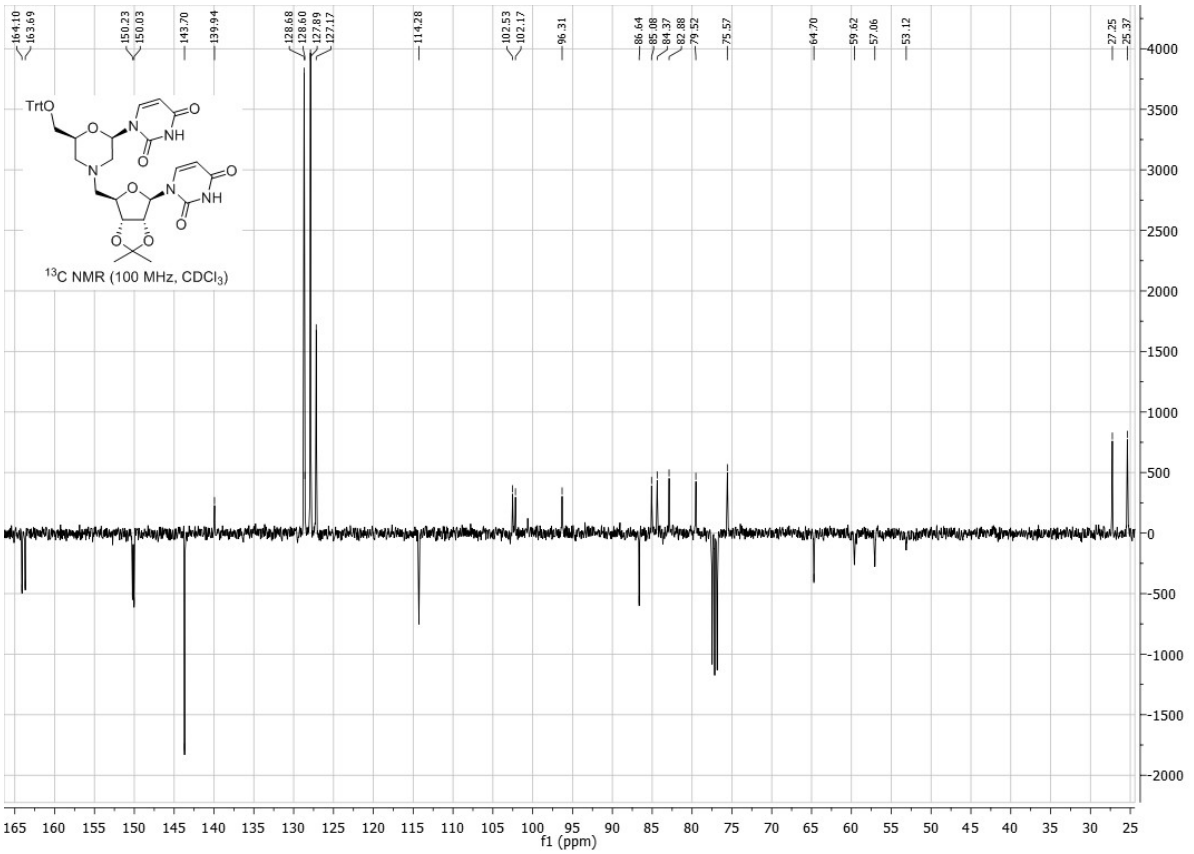
NMR spectra of compound 4c

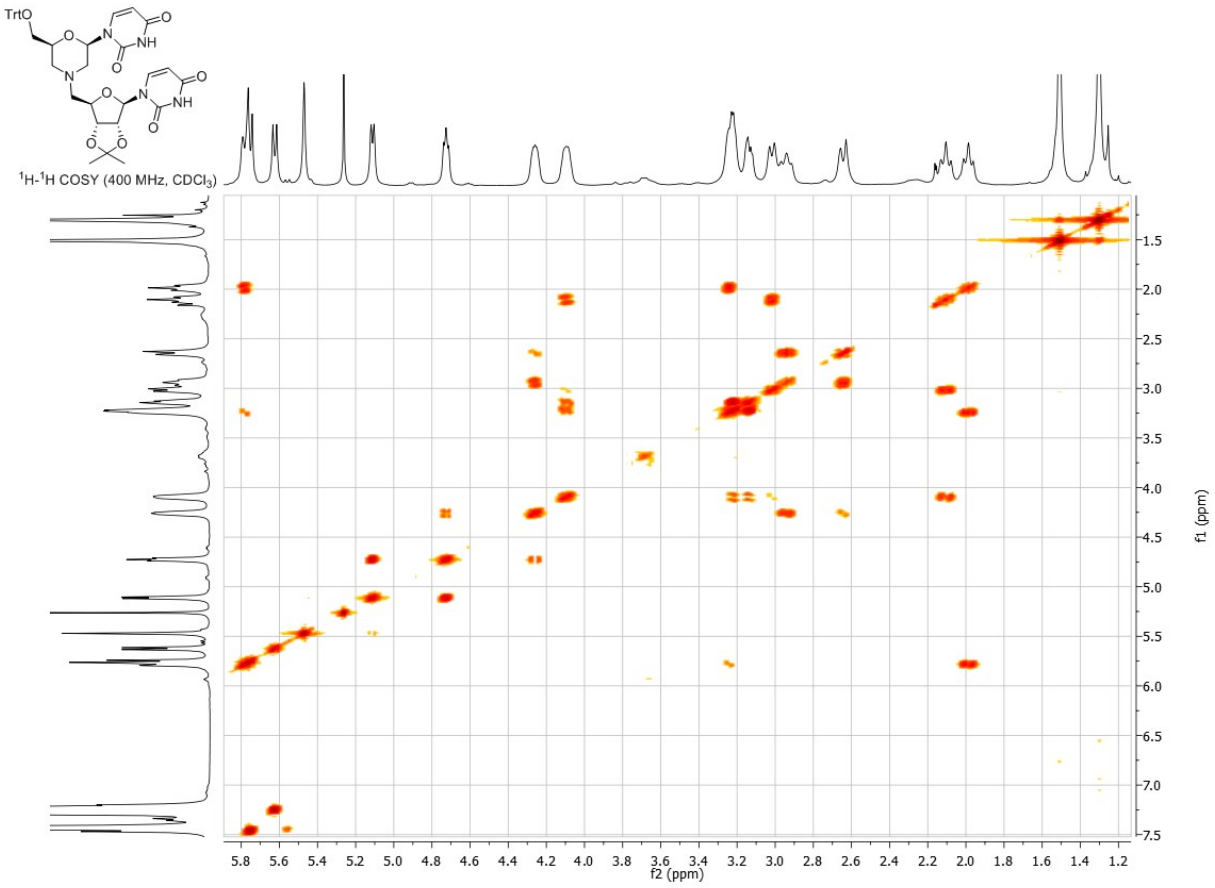


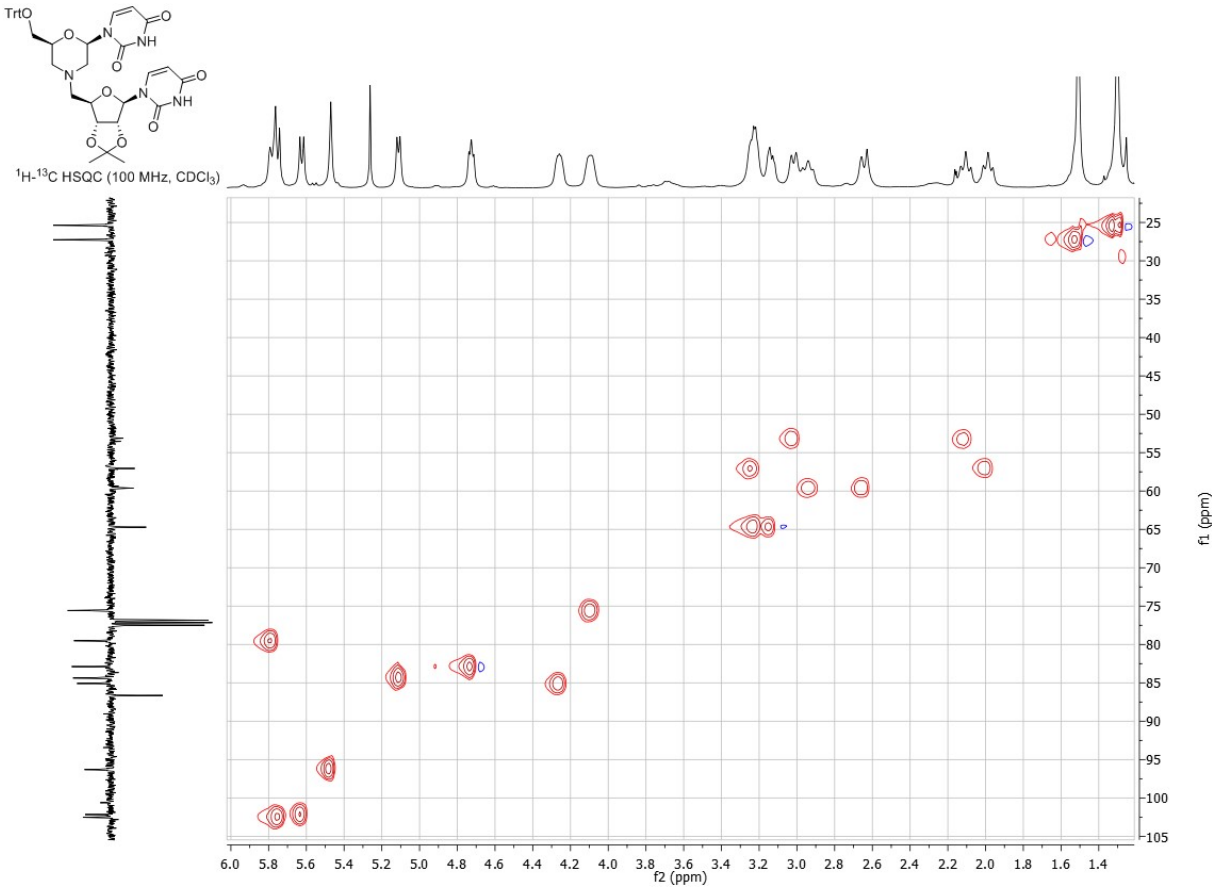


NMR spectra of compound 5

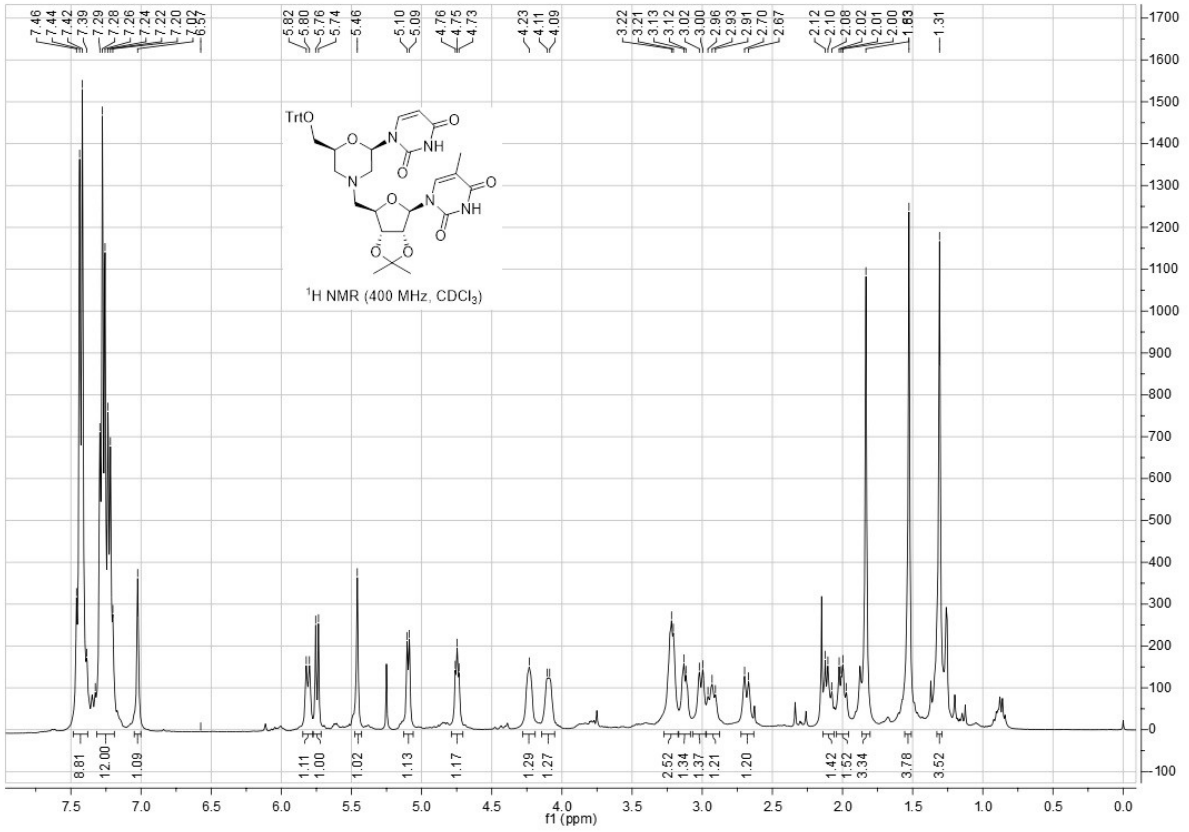


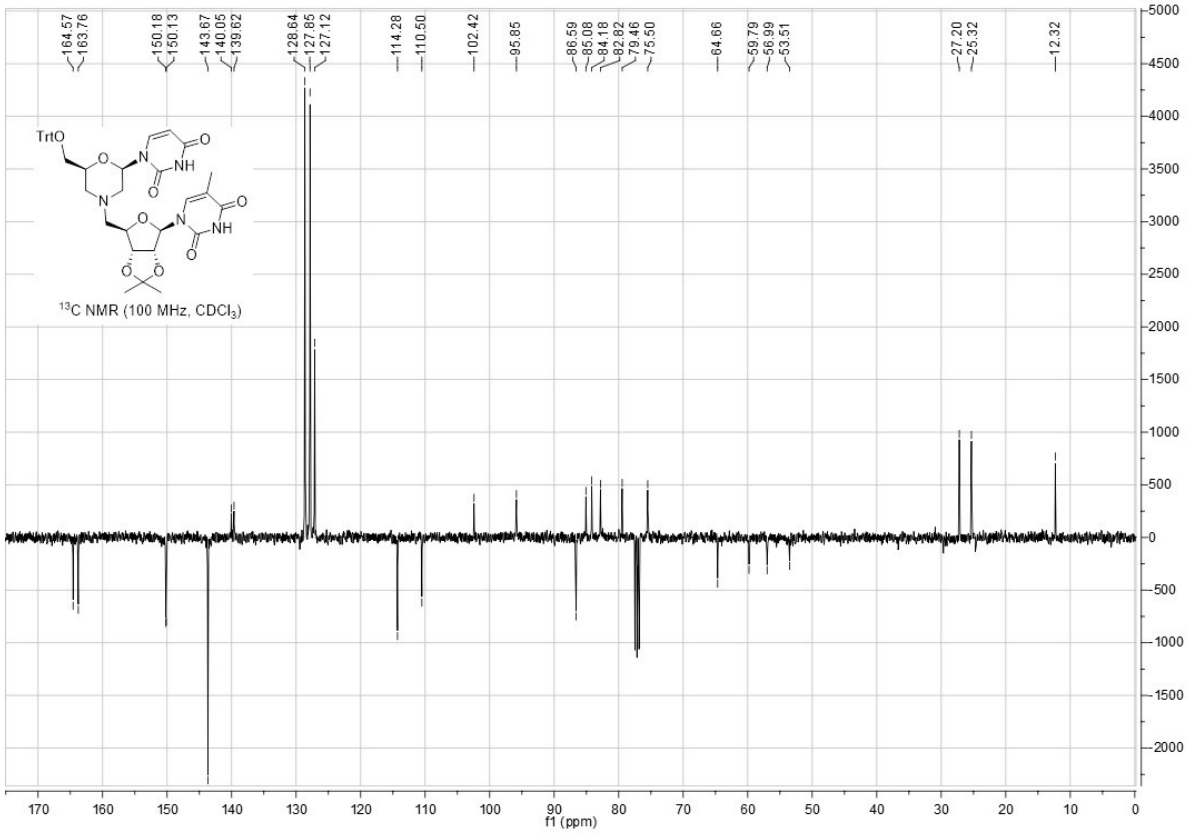


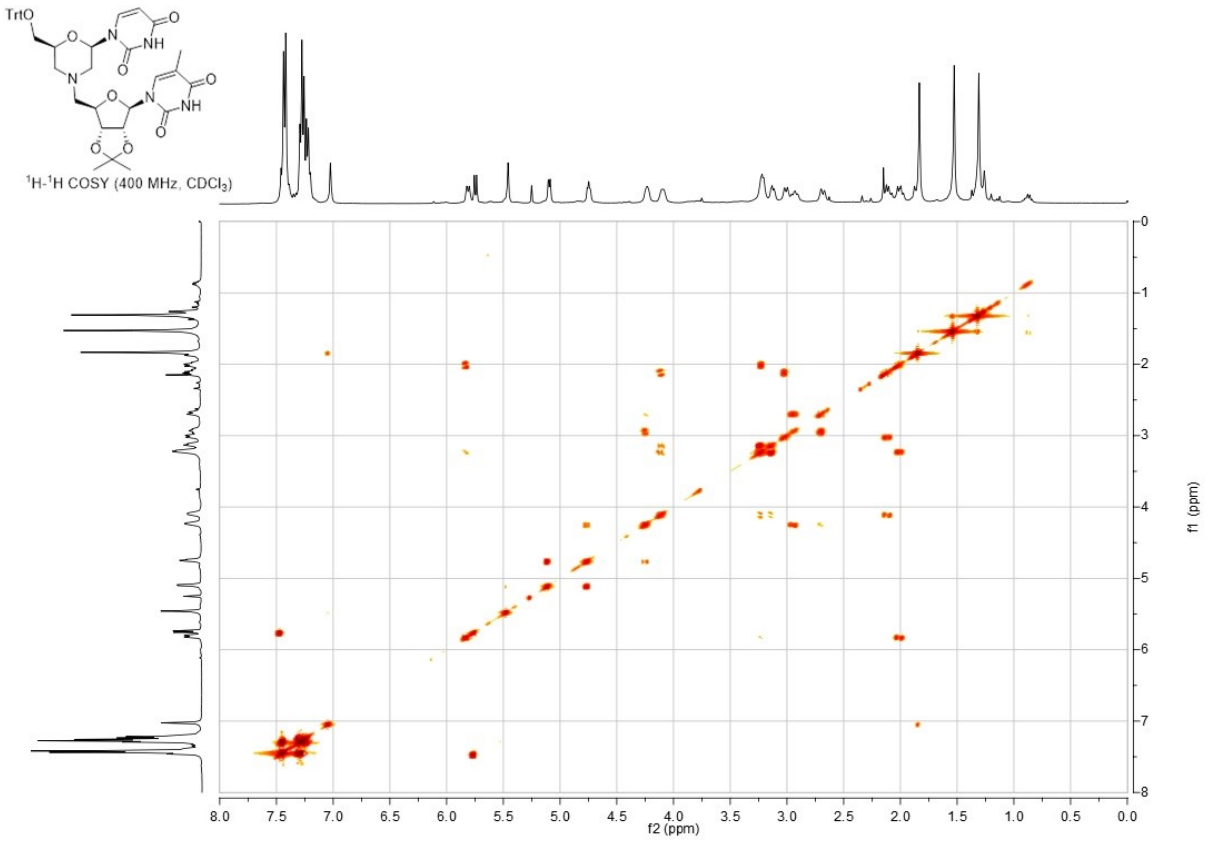

TrtO
NH
O
^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f1 (ppm)
f2 (ppm)


TrtO
NH
O
^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$)
f1 (ppm)
f2 (ppm)

NMR spectra of compound 6



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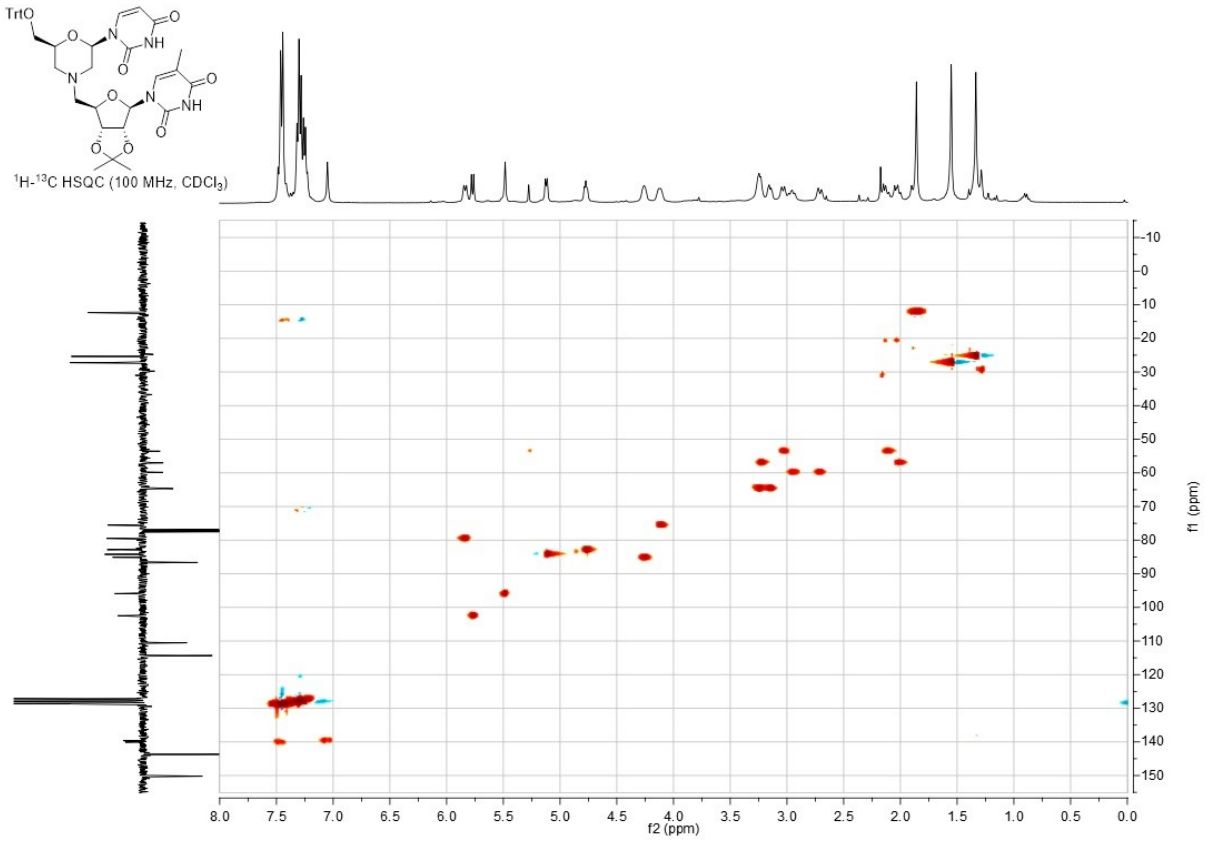
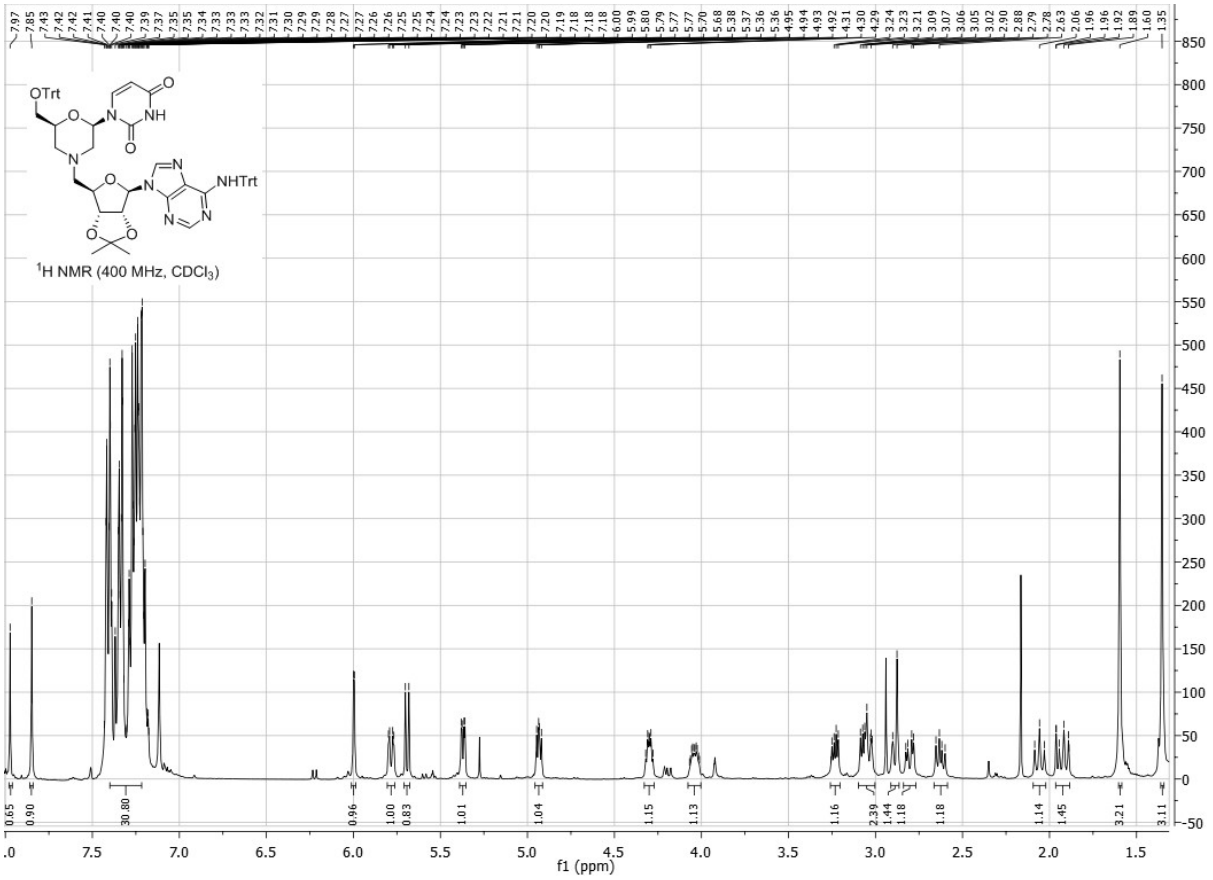
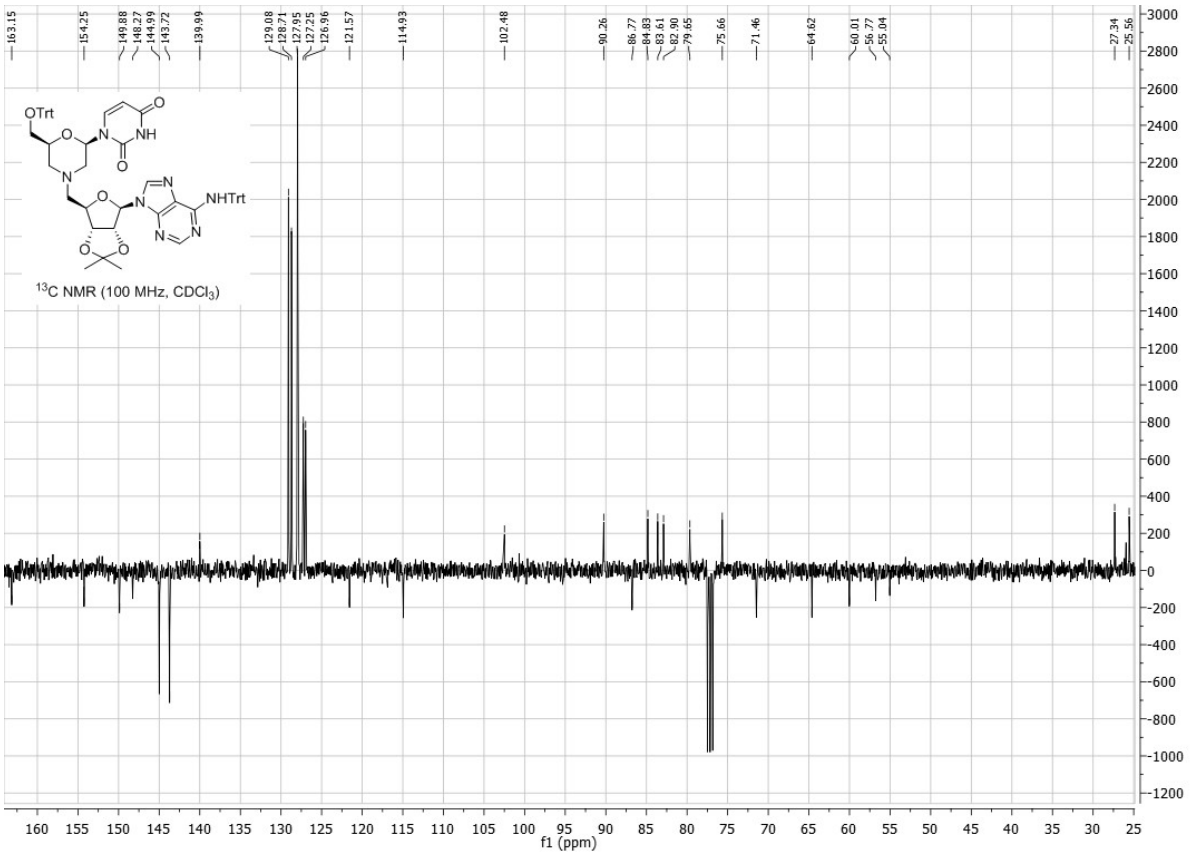
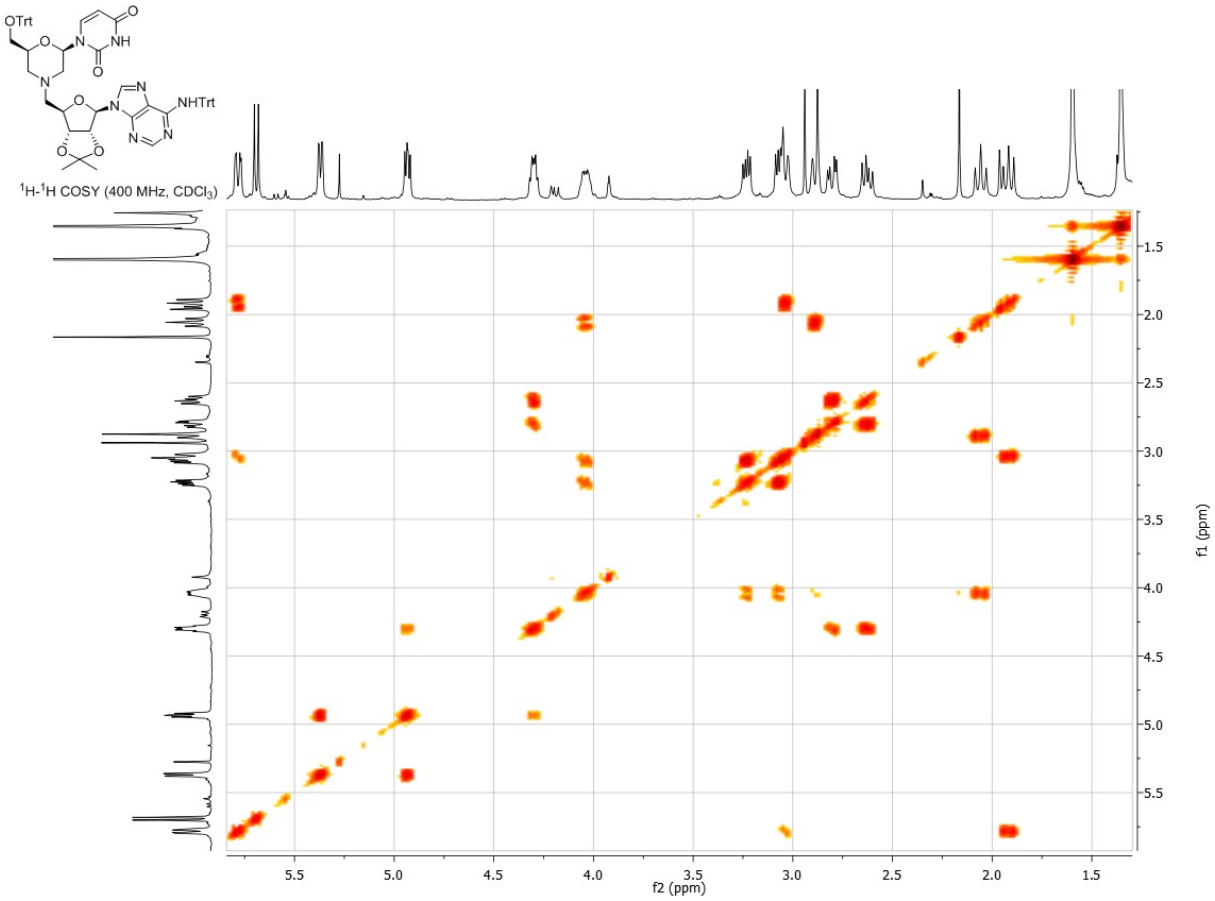
^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

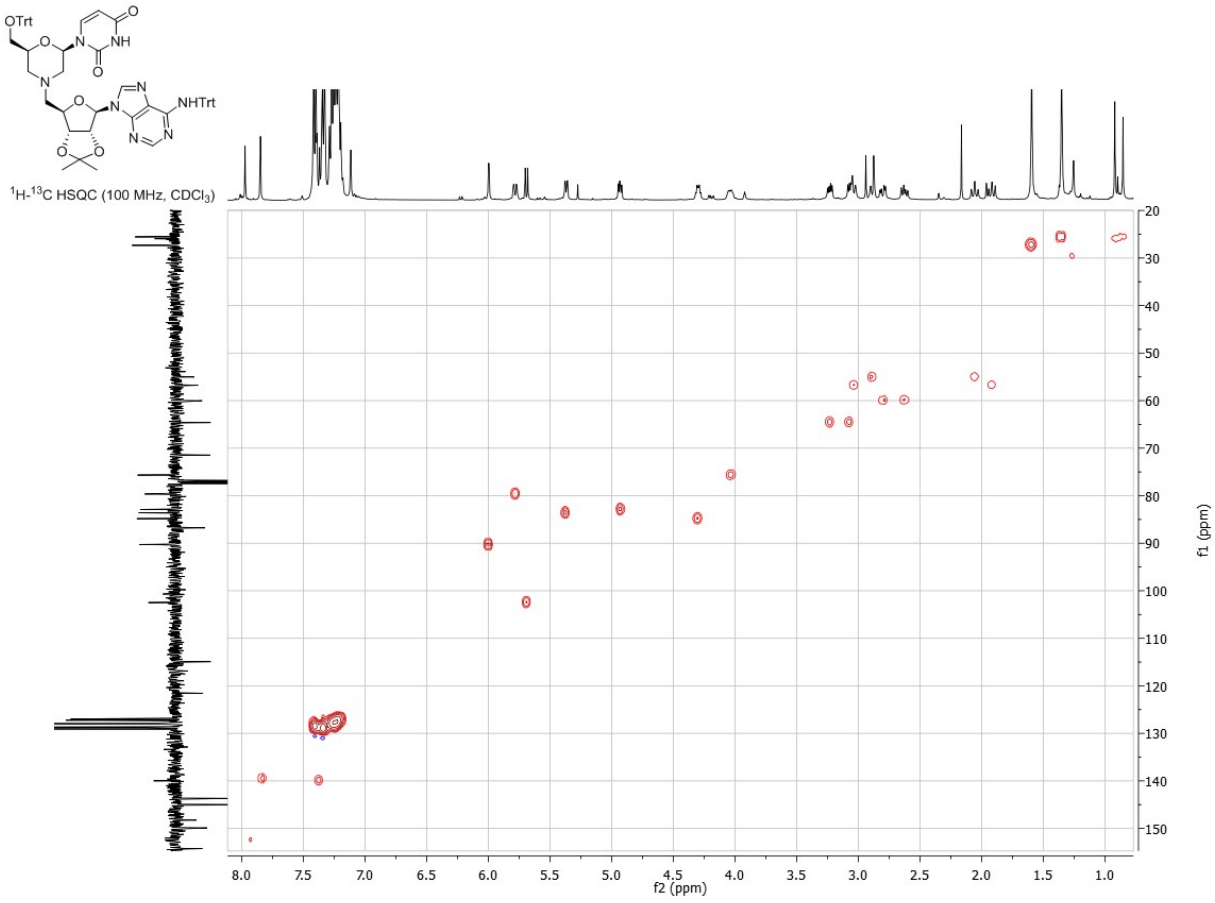

TrtO
^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

NMR spectra of compound 7

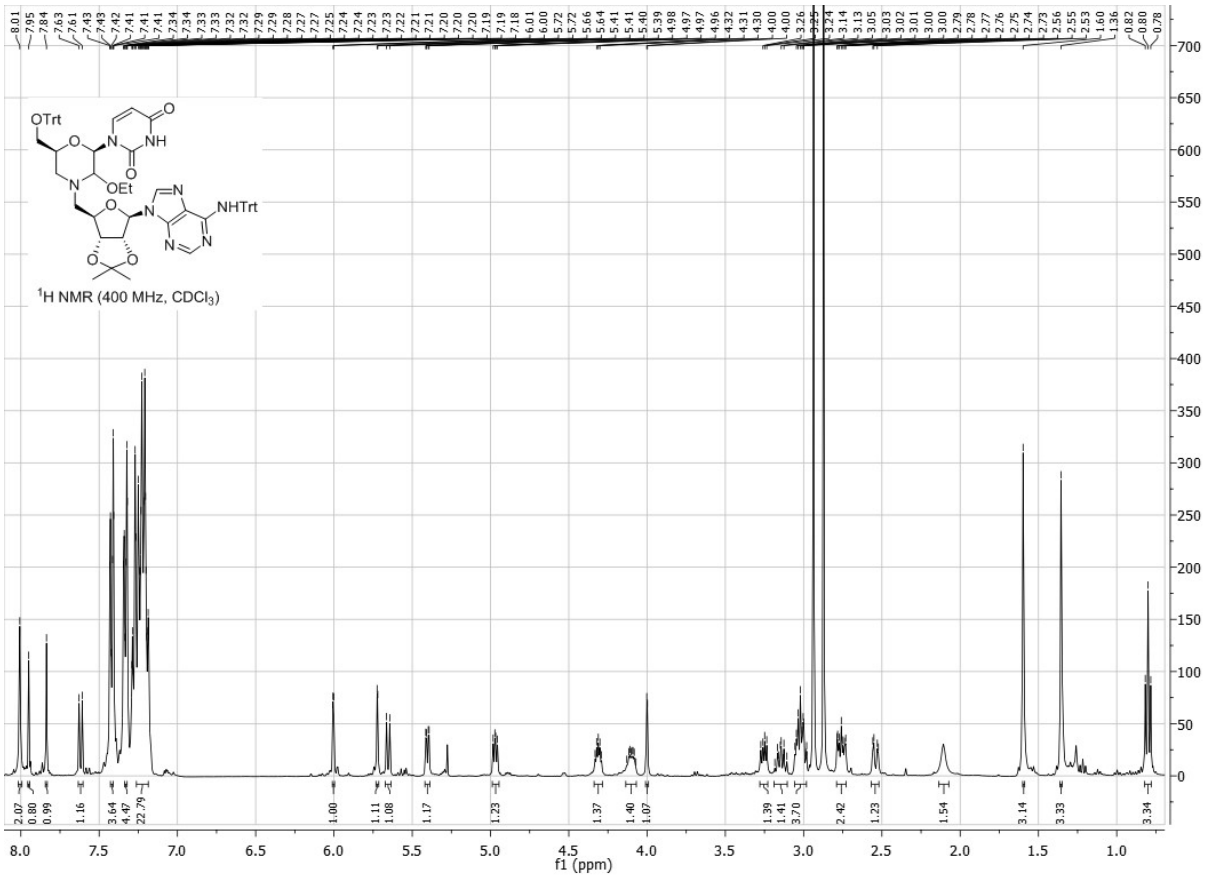


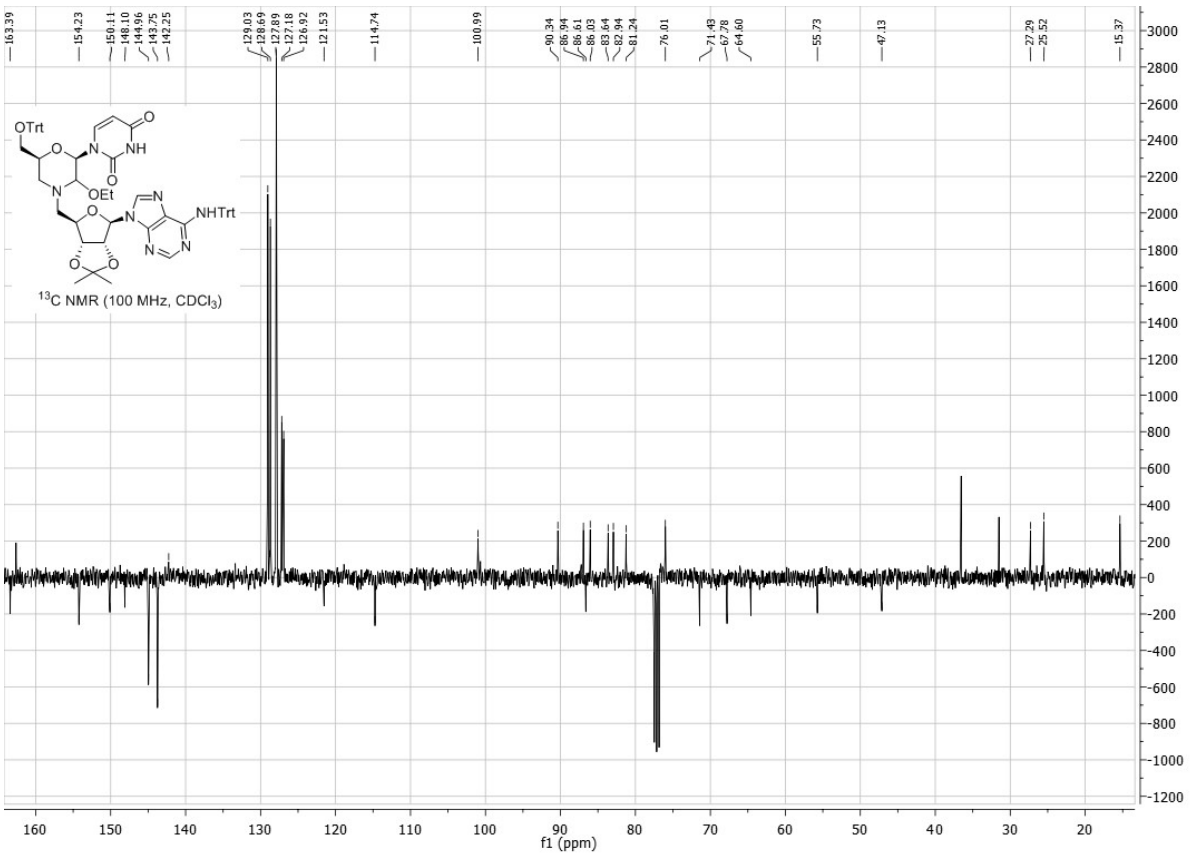


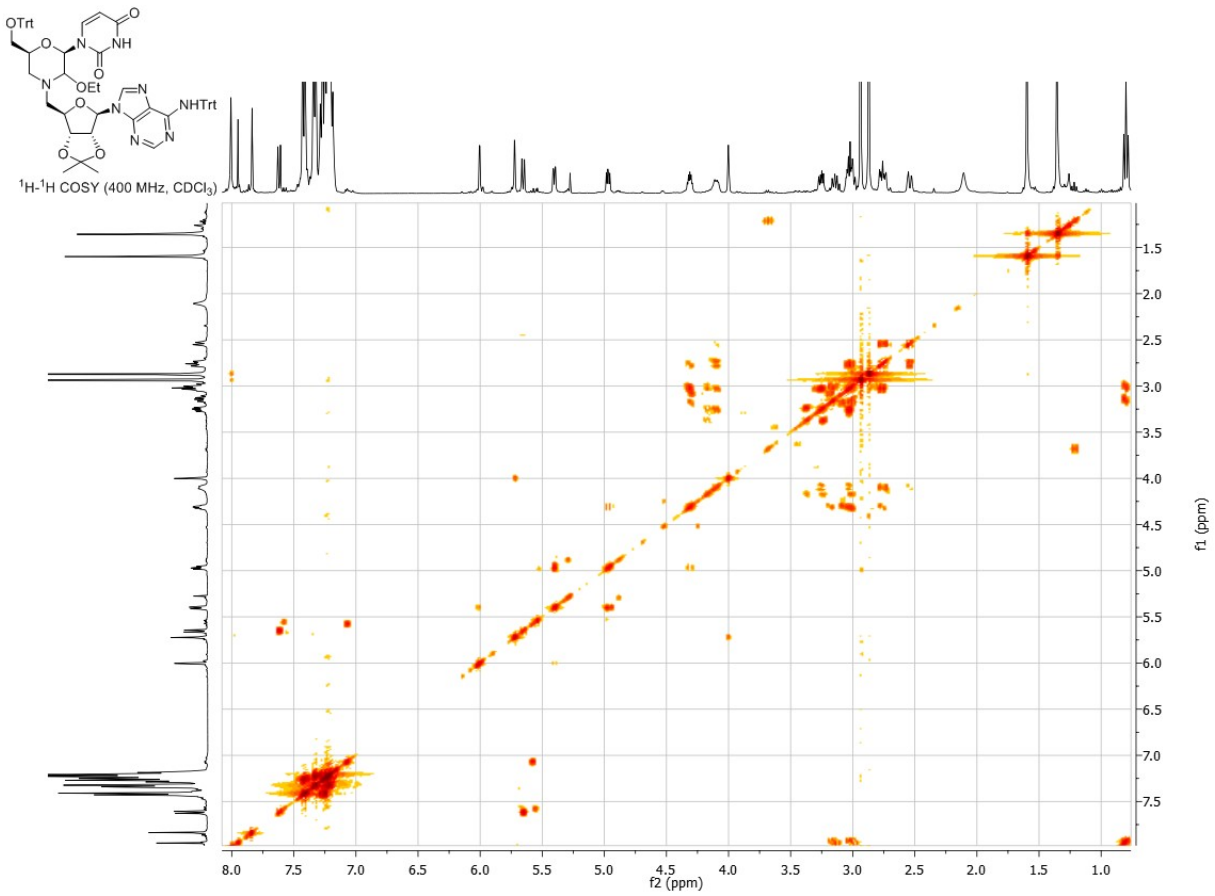

OTrt
NHTrt
^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

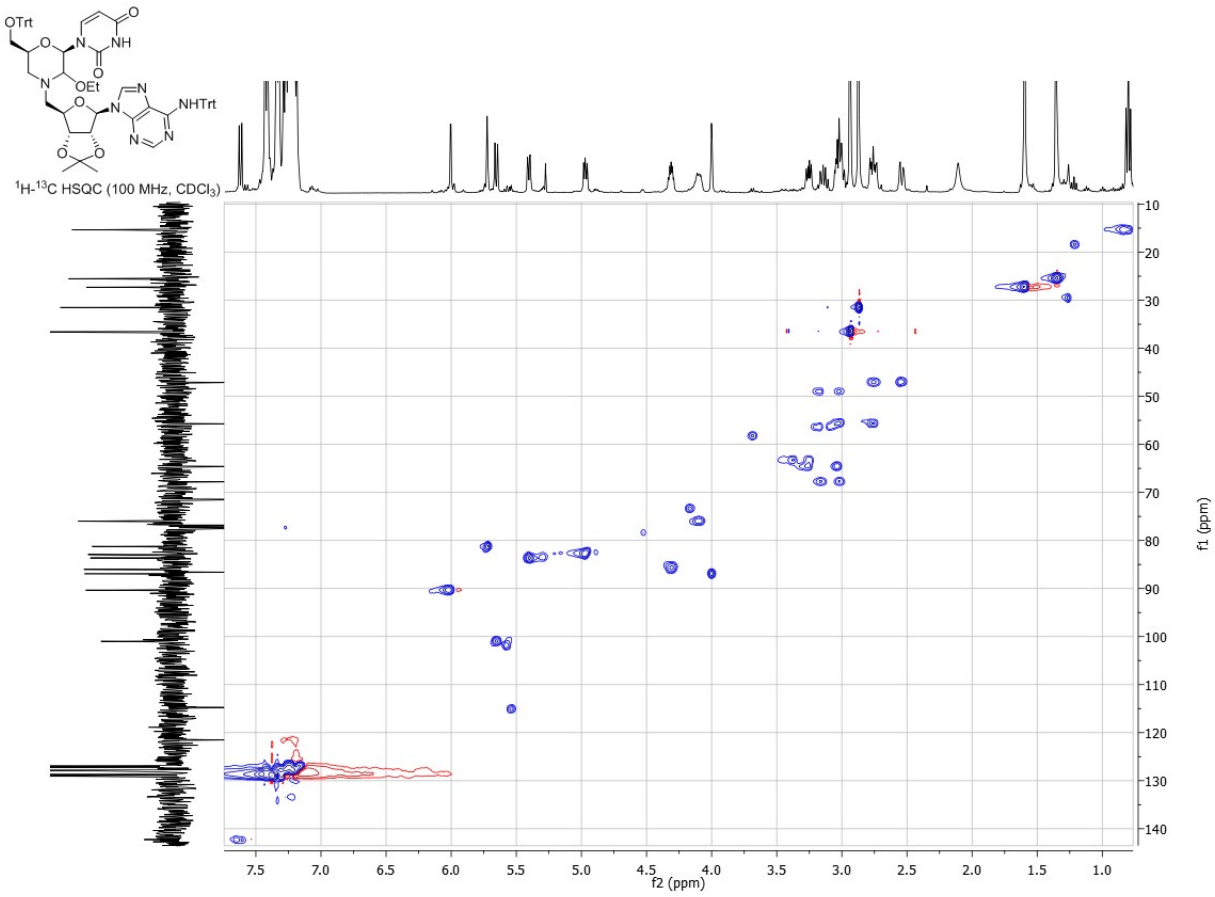

OTrt
NHTrt
^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

NMR spectra of compound 7-B1

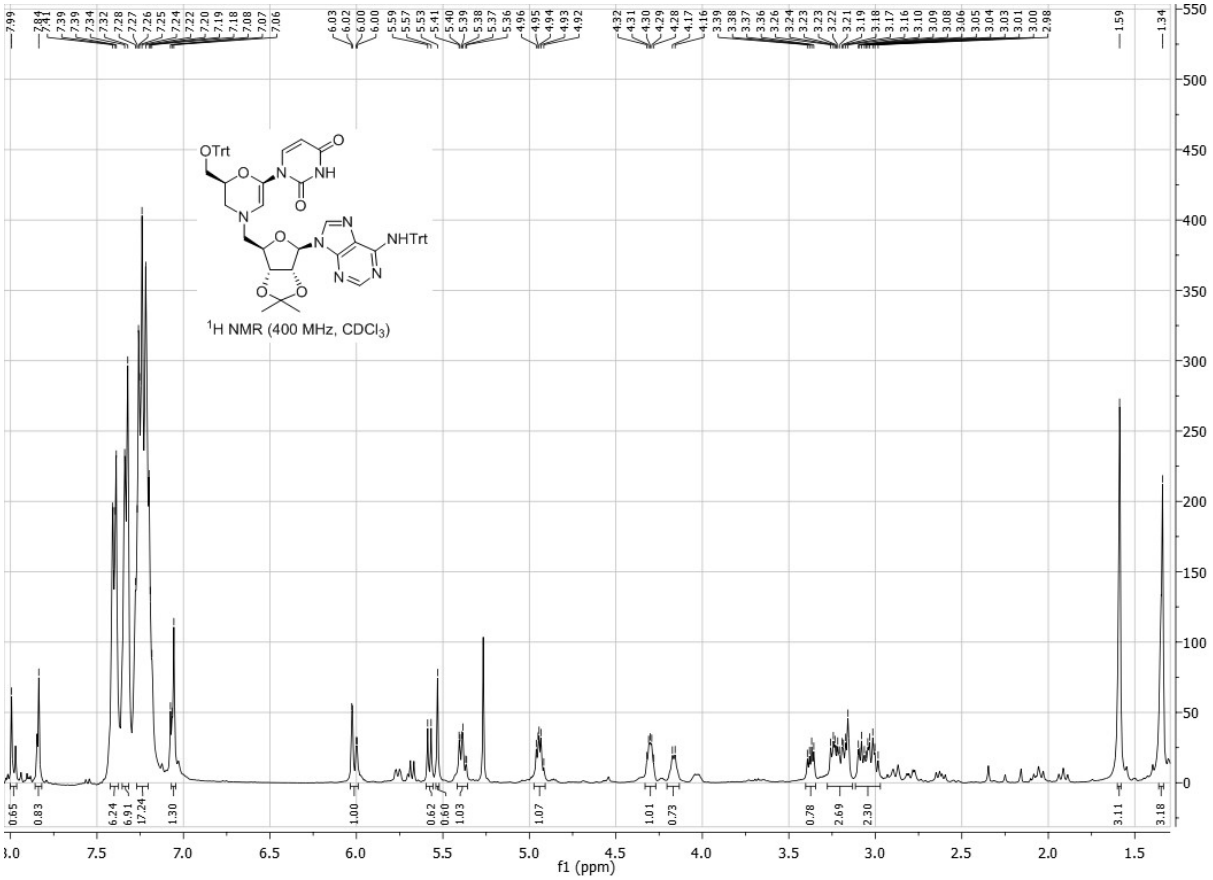


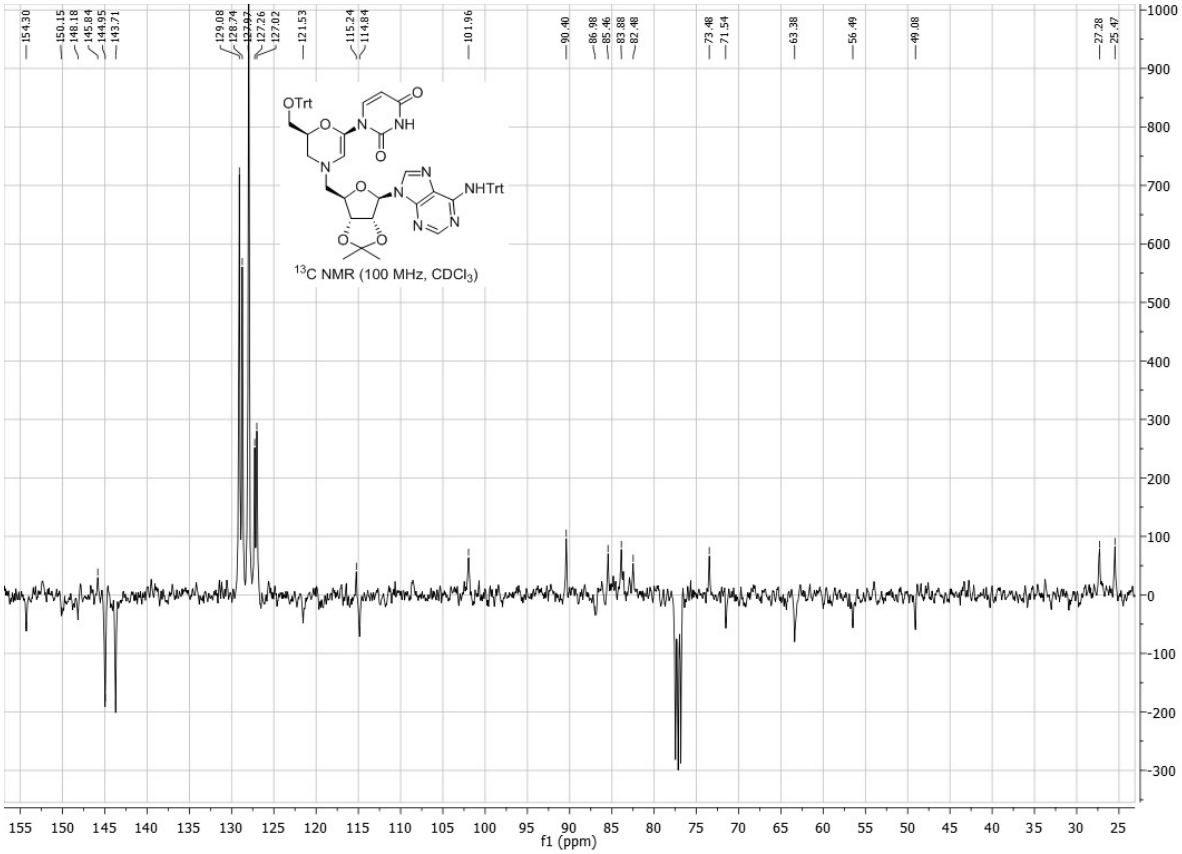


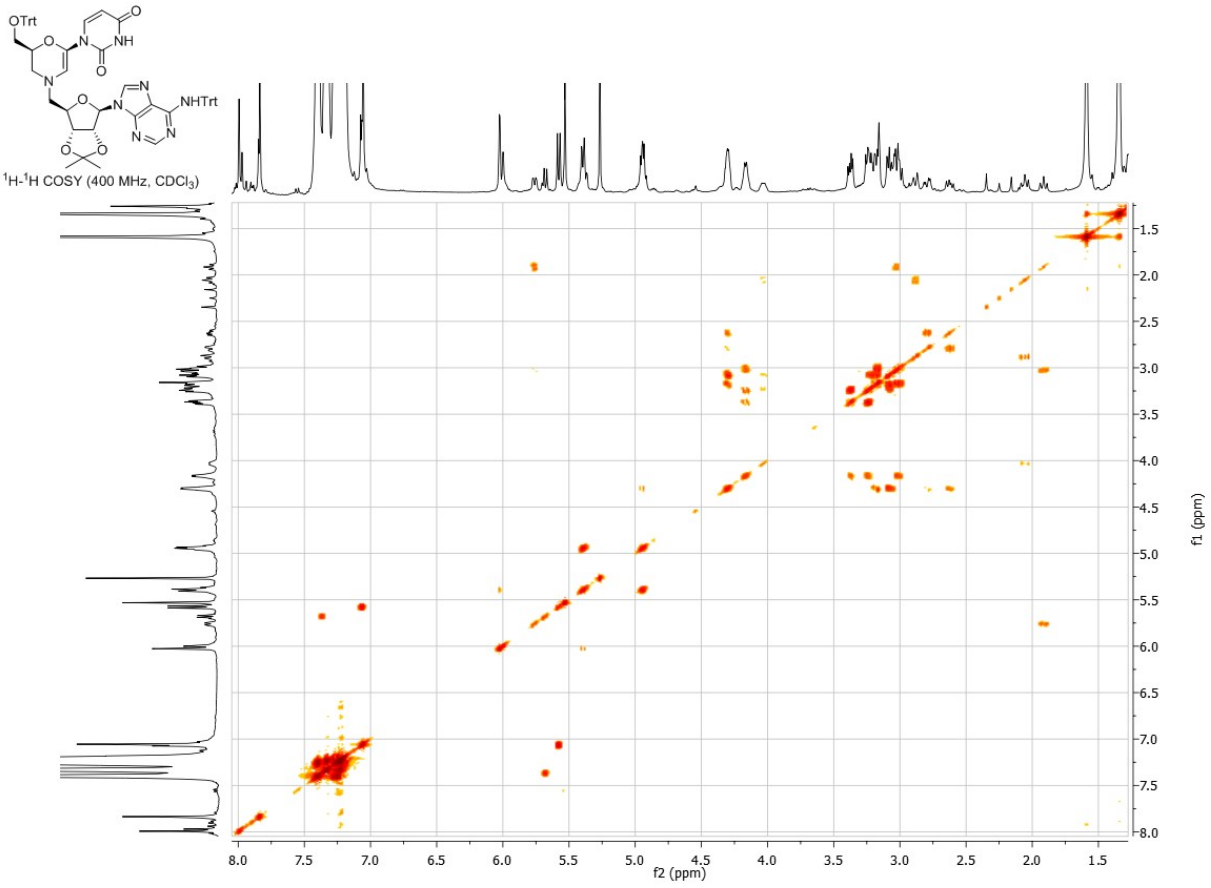

OTrt
OEt
NHTrt
^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

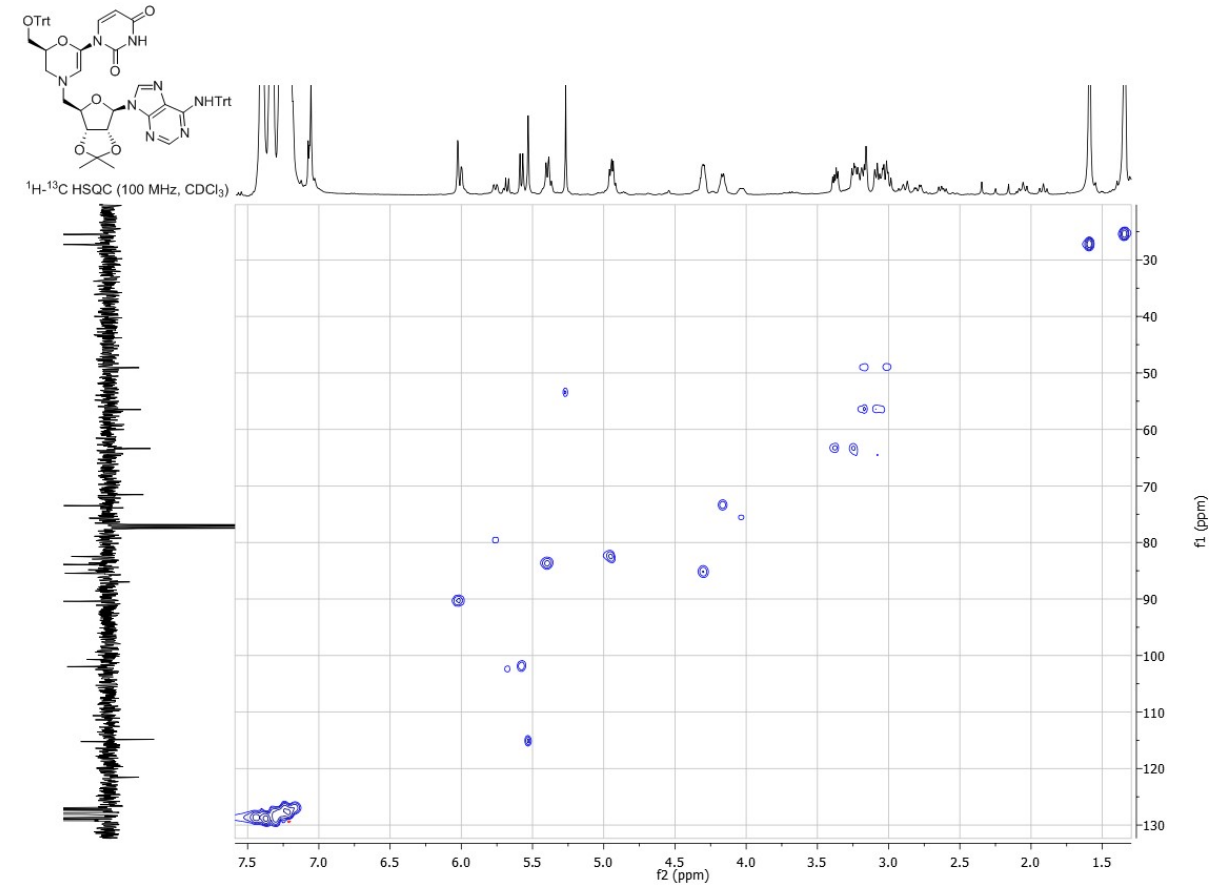

OTrt
OEt
NHTrt
^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

NMR spectra of compound 7-B2

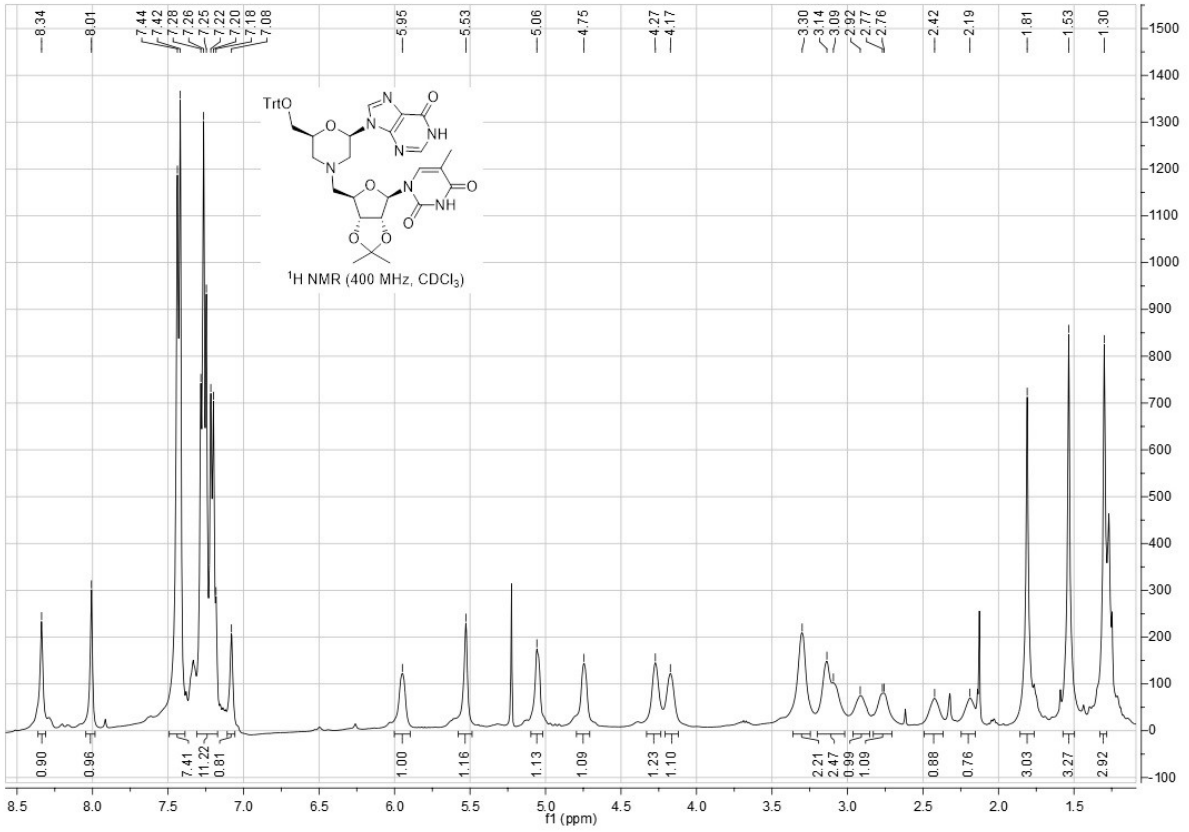


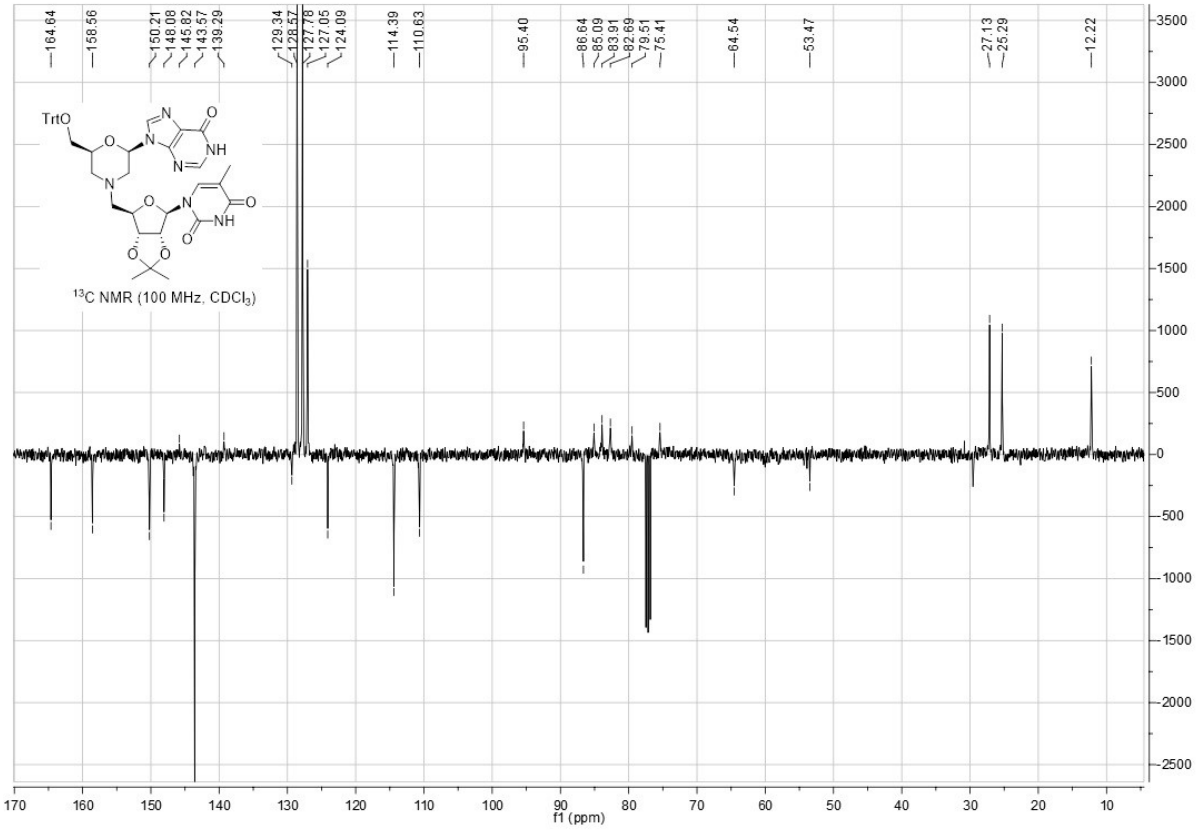


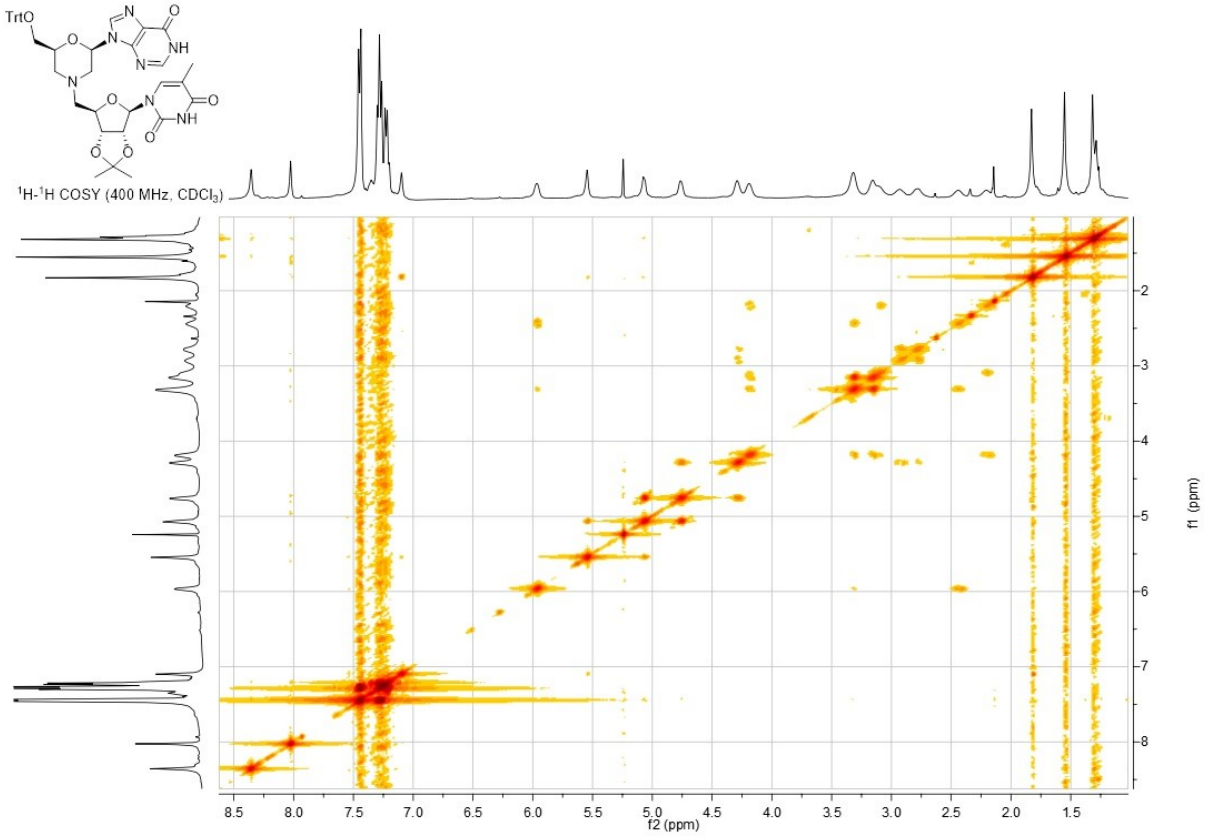

OTrt
NH
O
NHTrt
N
O
^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

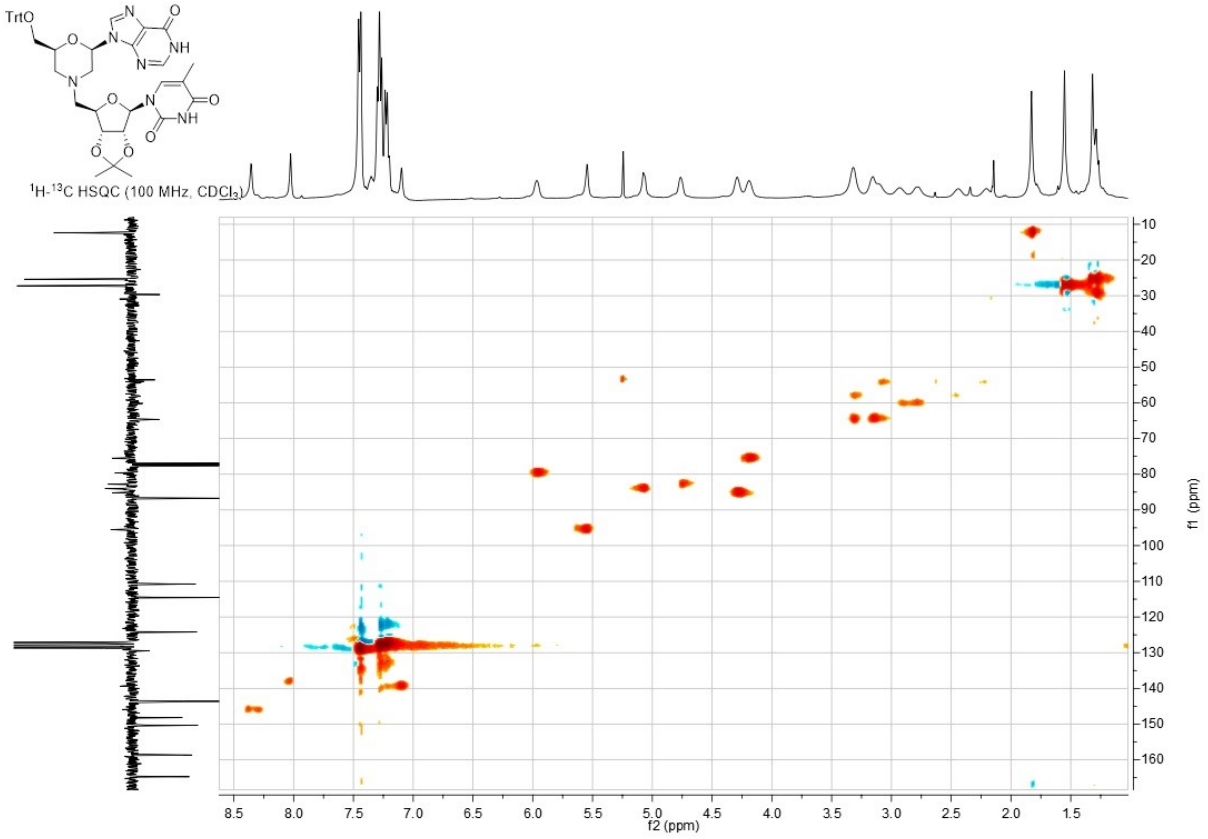

OTrt
NH
O
NHTrt
N
O
^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

NMR spectra of compound 8

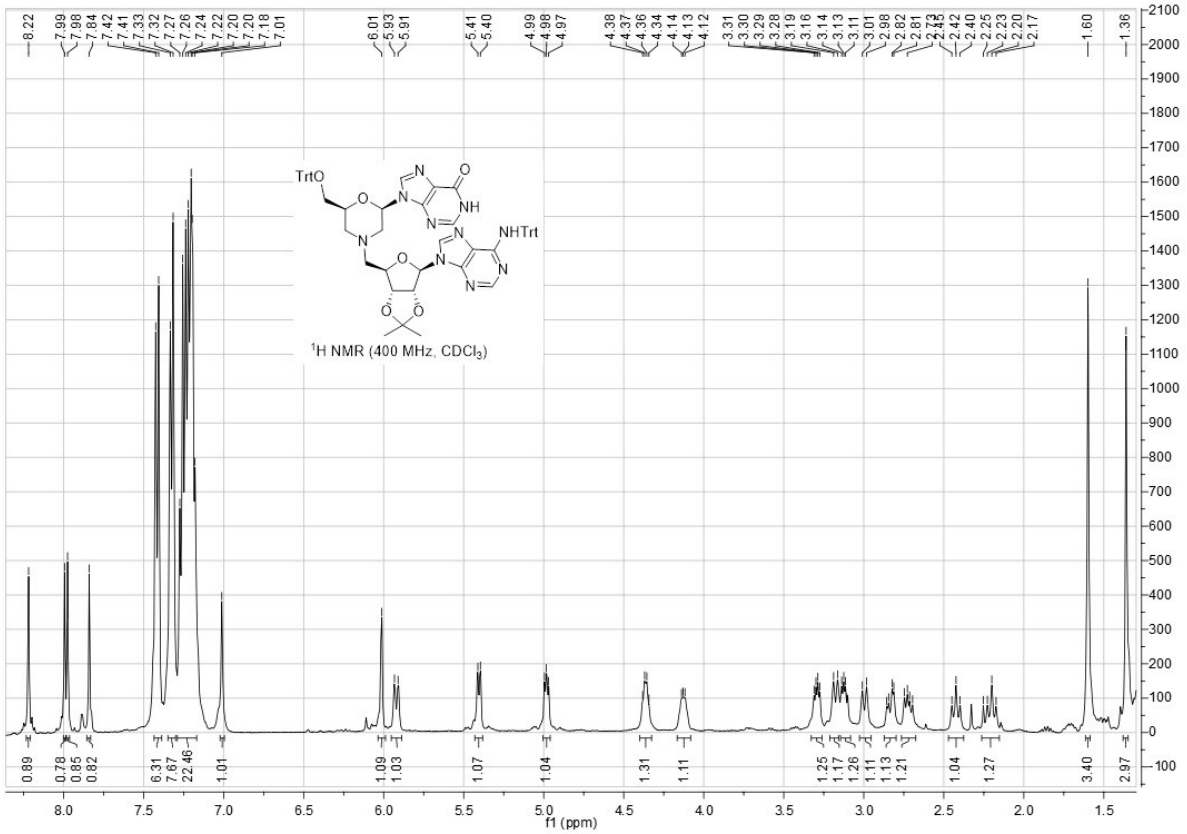


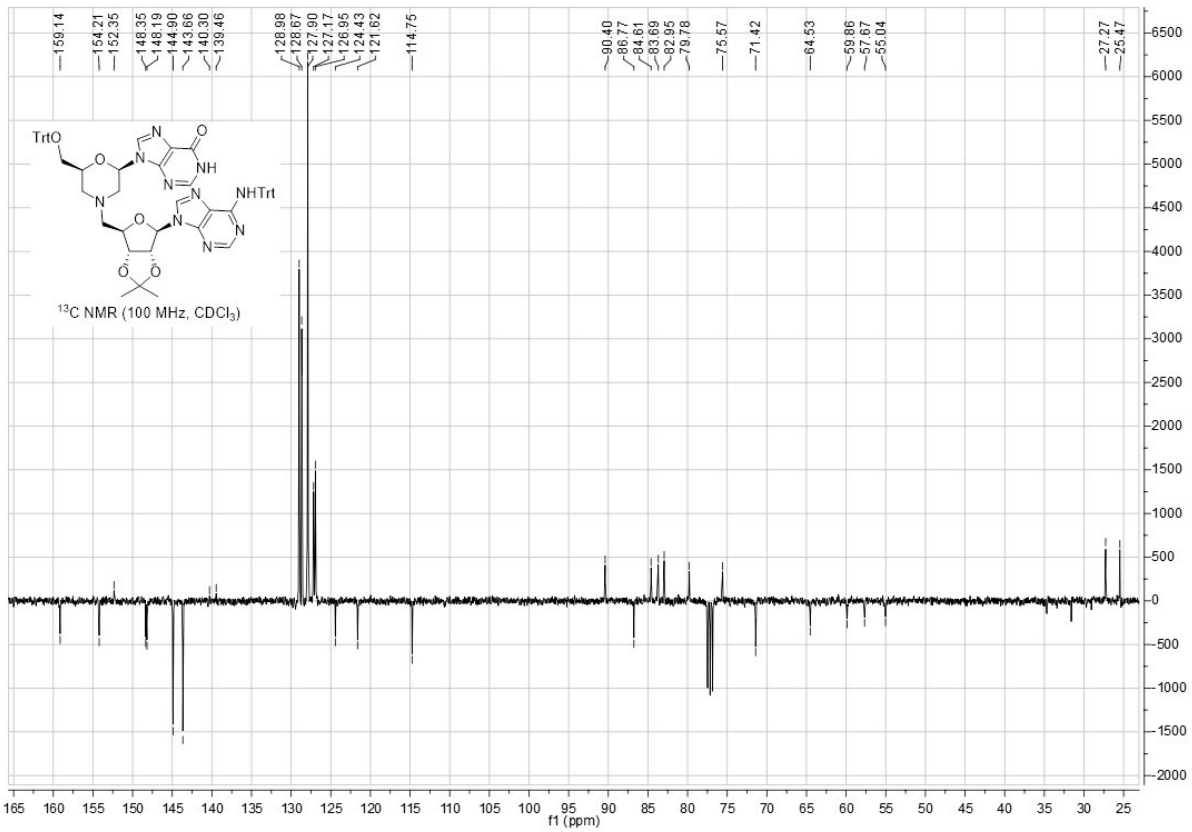


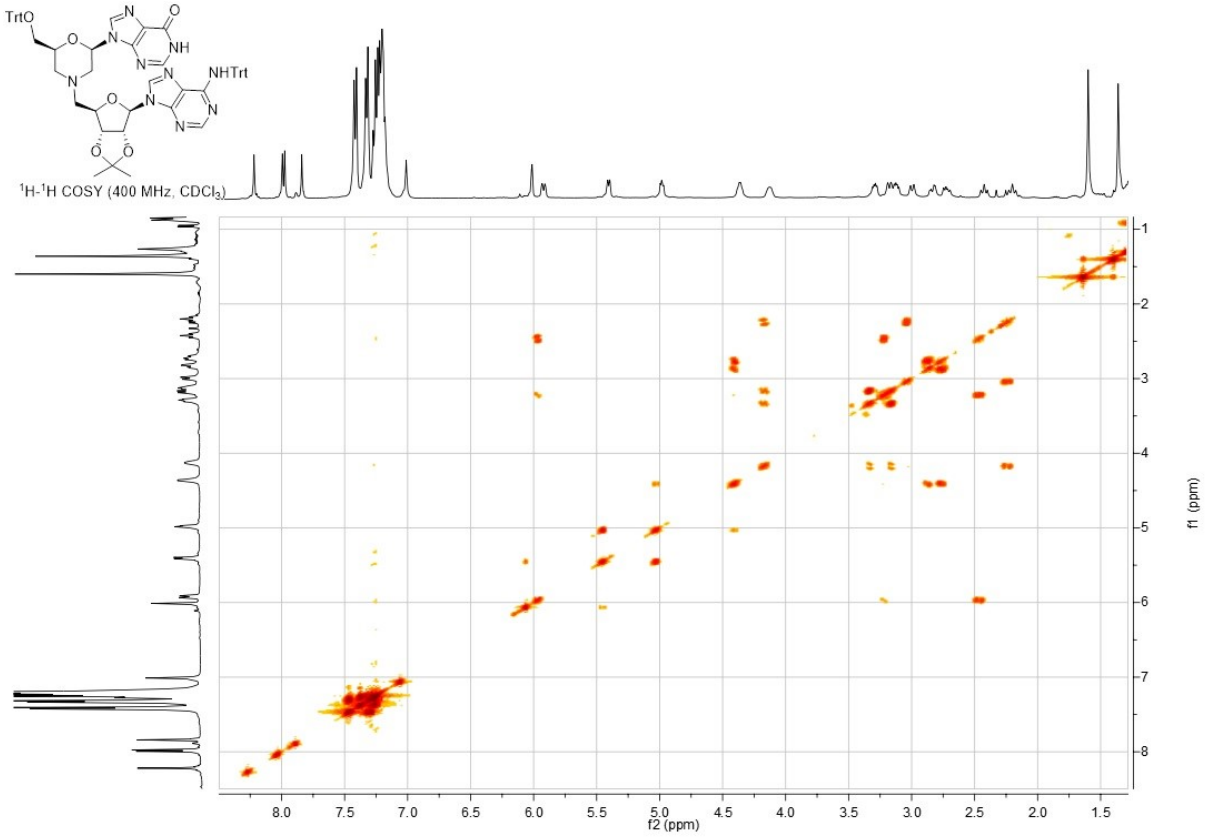

TrtO
NH
NH
O
O
^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

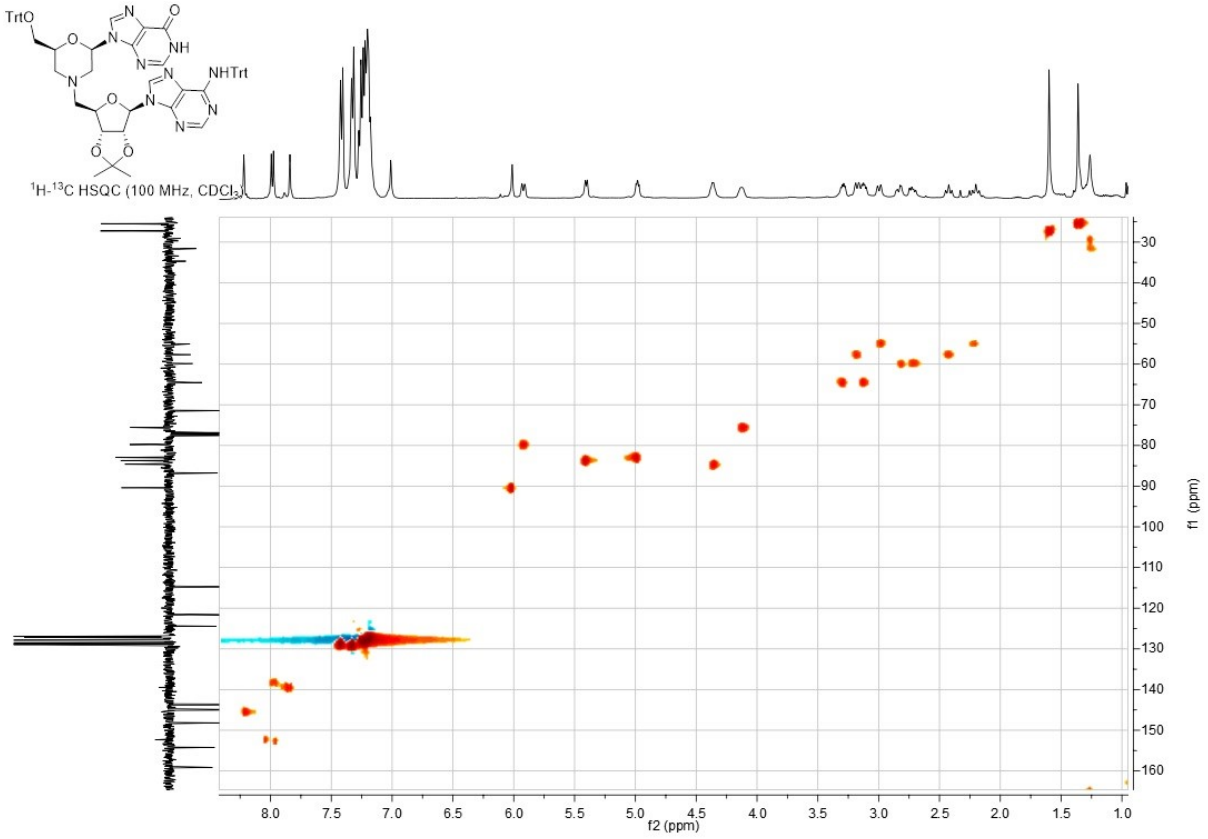

TrtO
NH
NH
O
O
^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

NMR spectra of compound 9

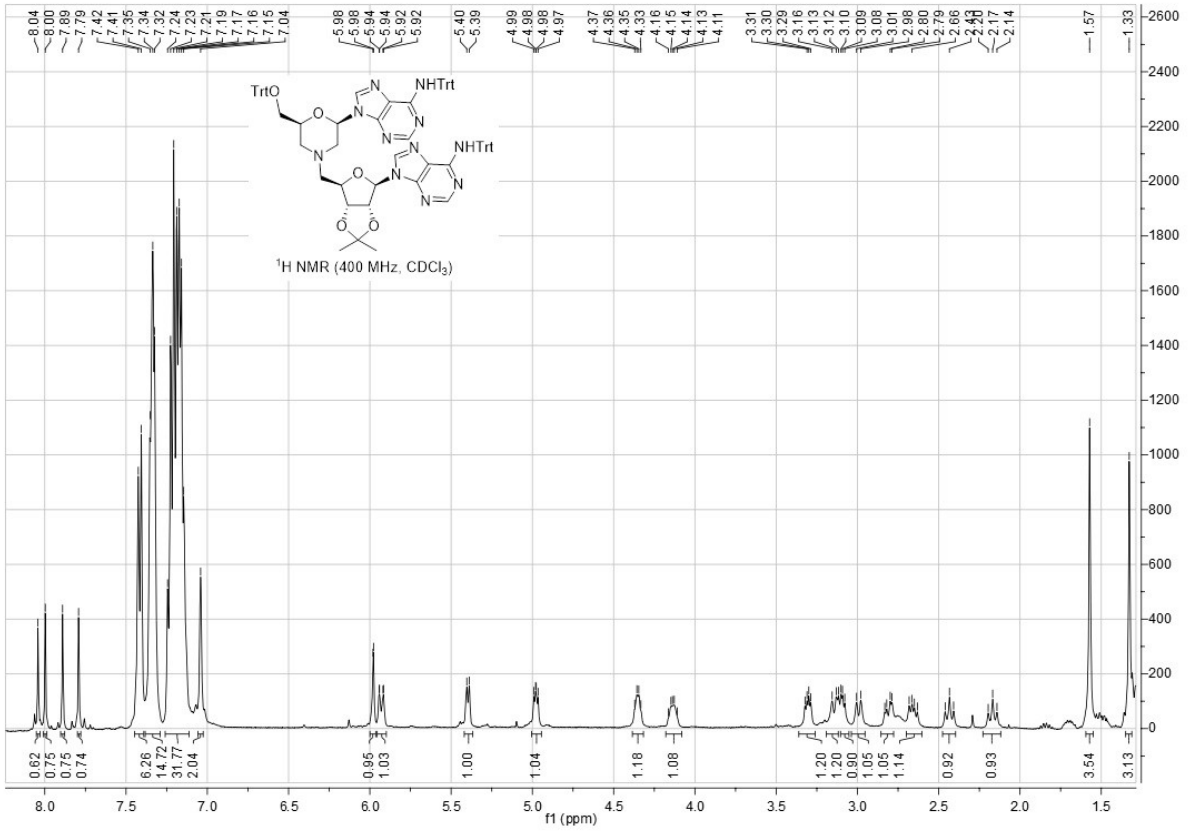


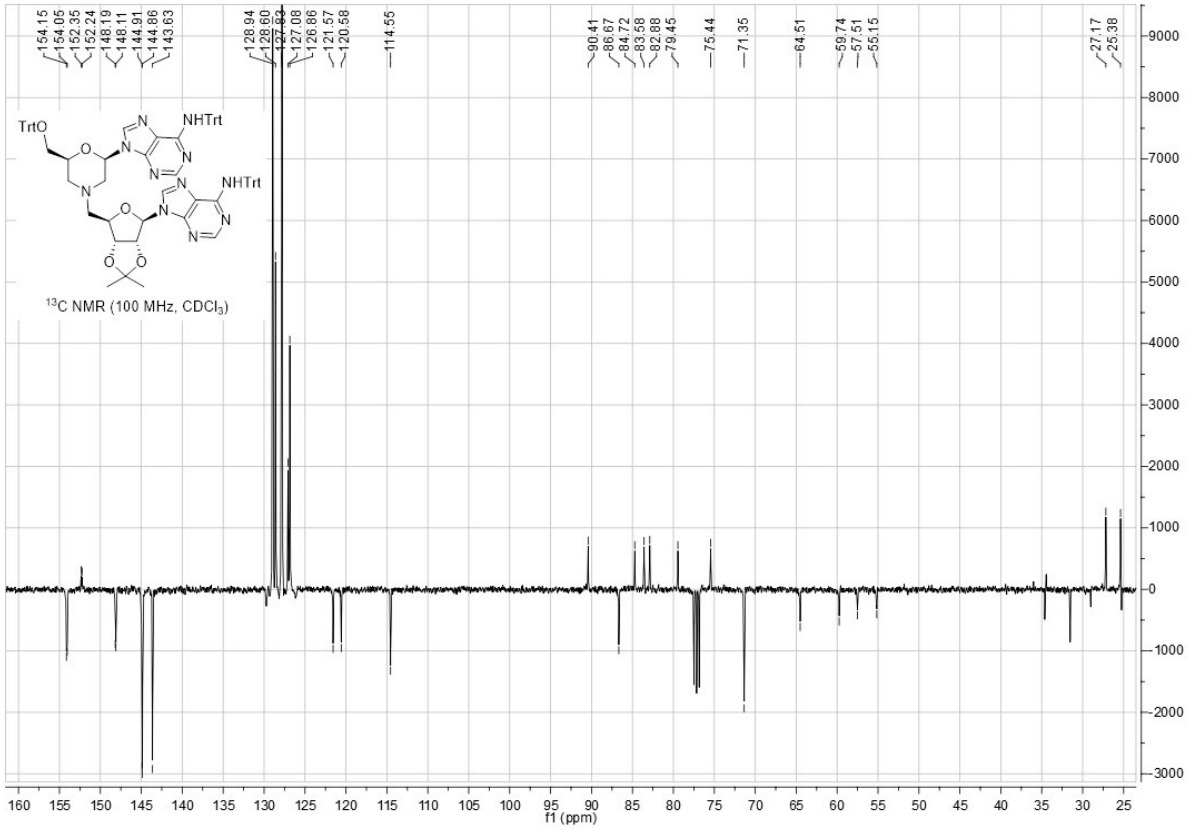


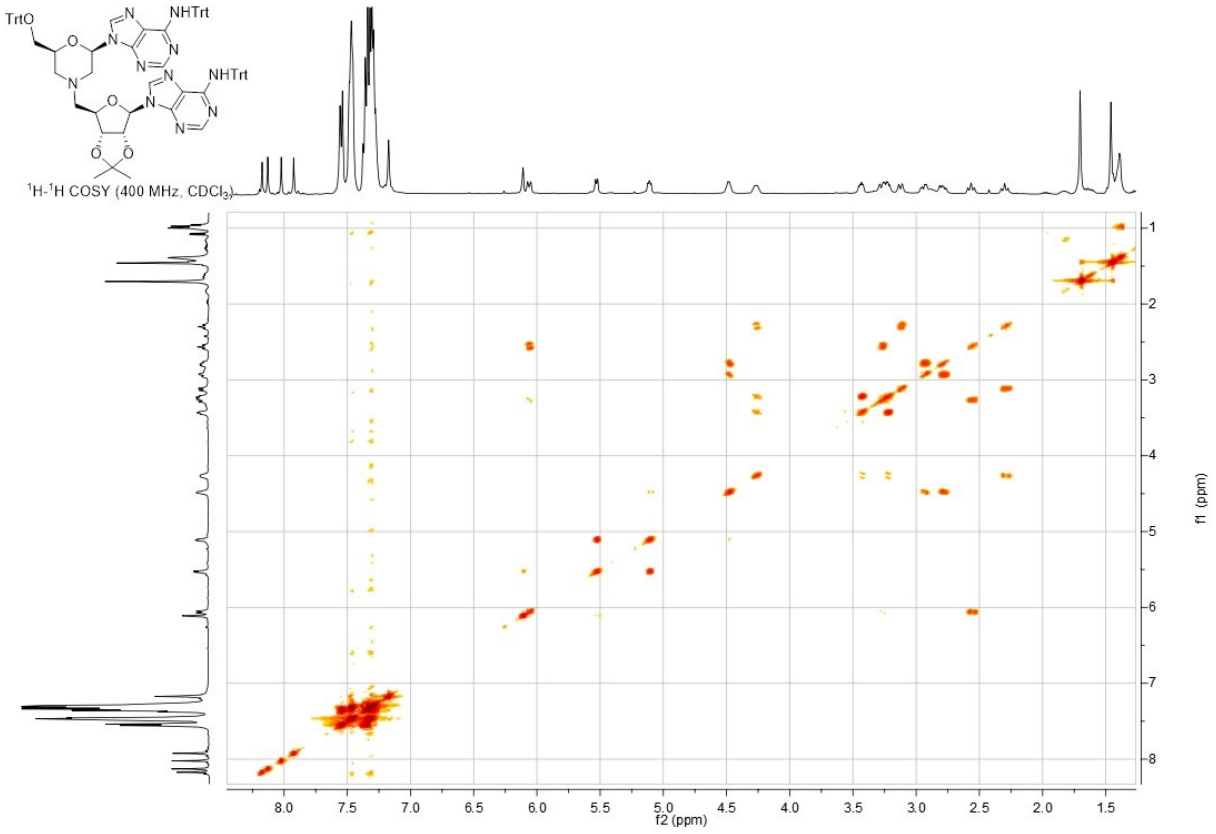

TrtO
NHTrt
^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

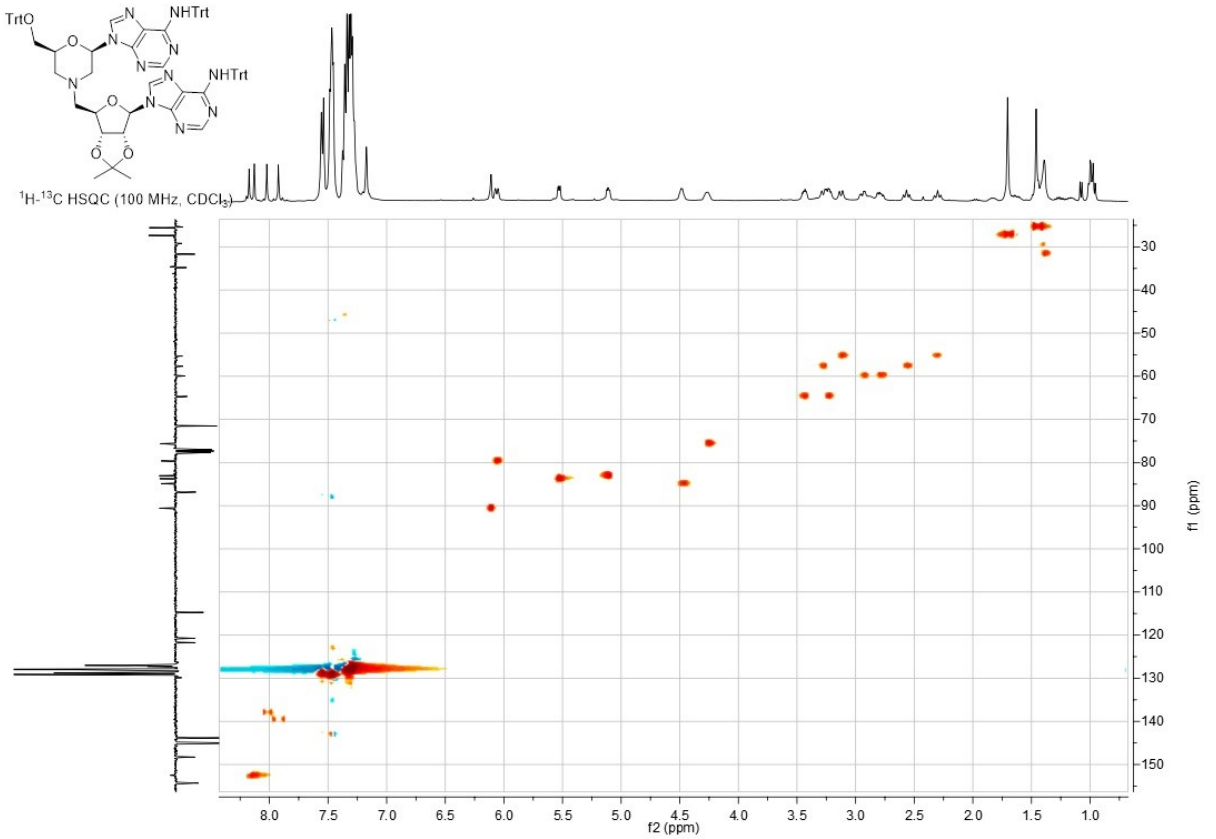

TrtO
NHTrt
^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

NMR spectra of compound 10

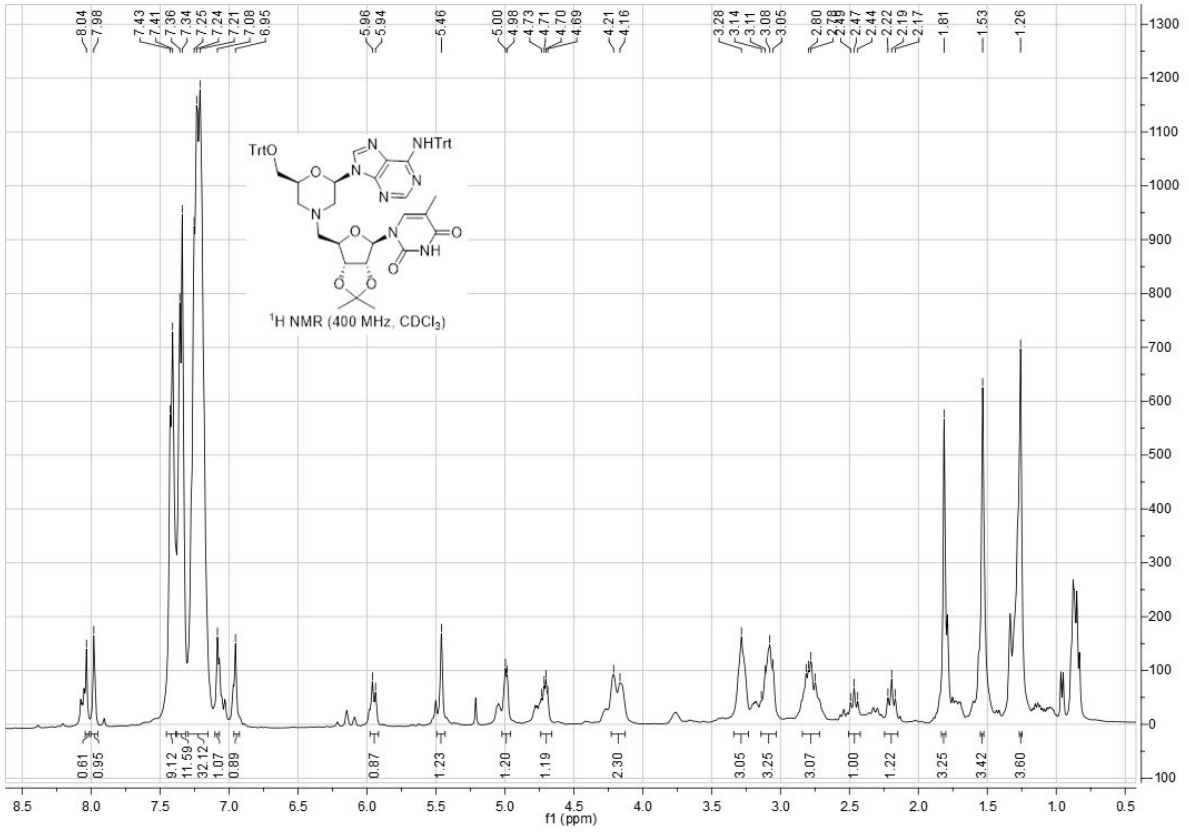


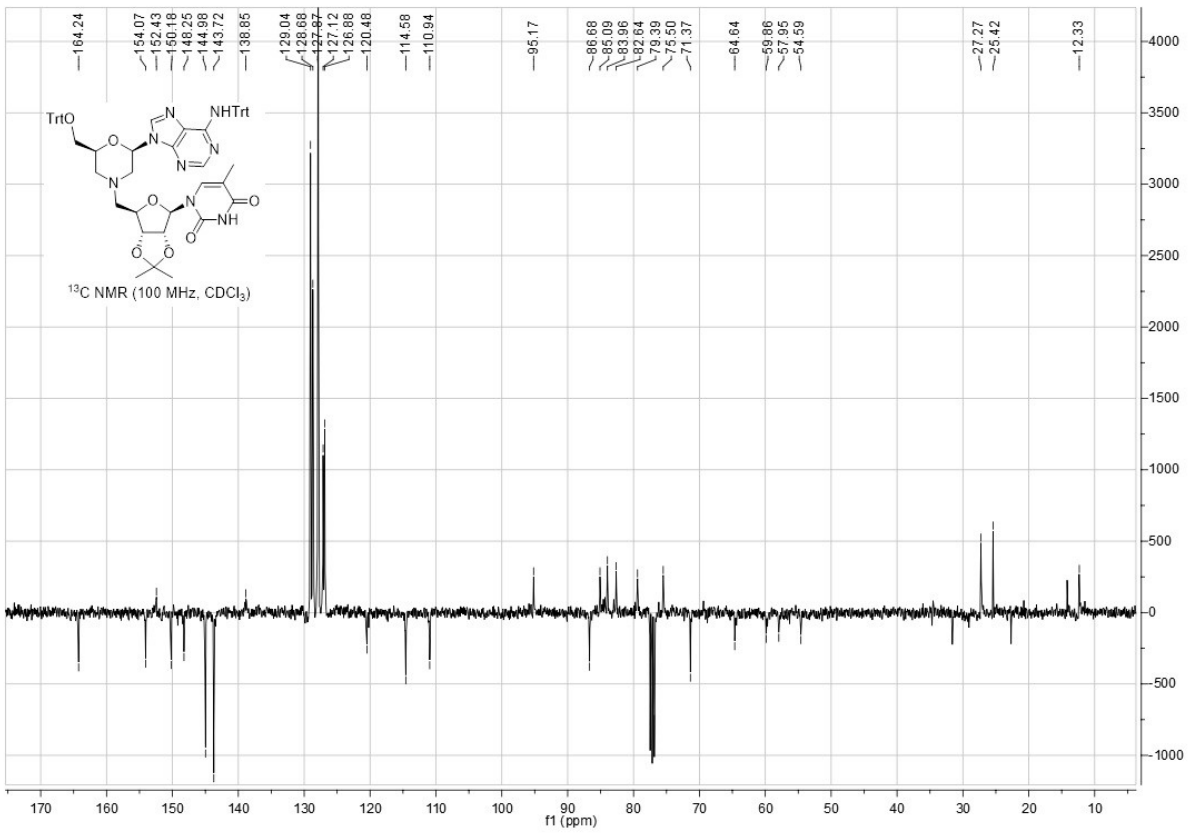


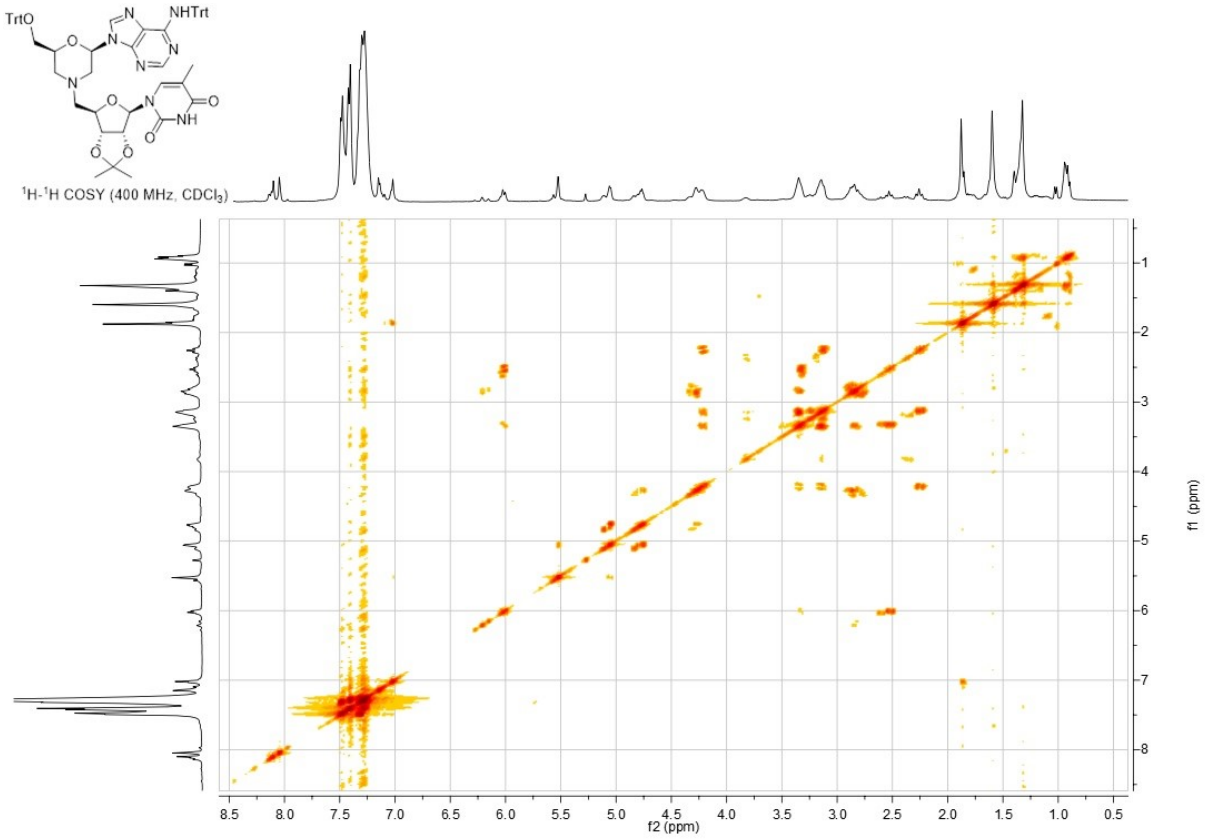

TrtO
NHTrt
NHTrt
^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

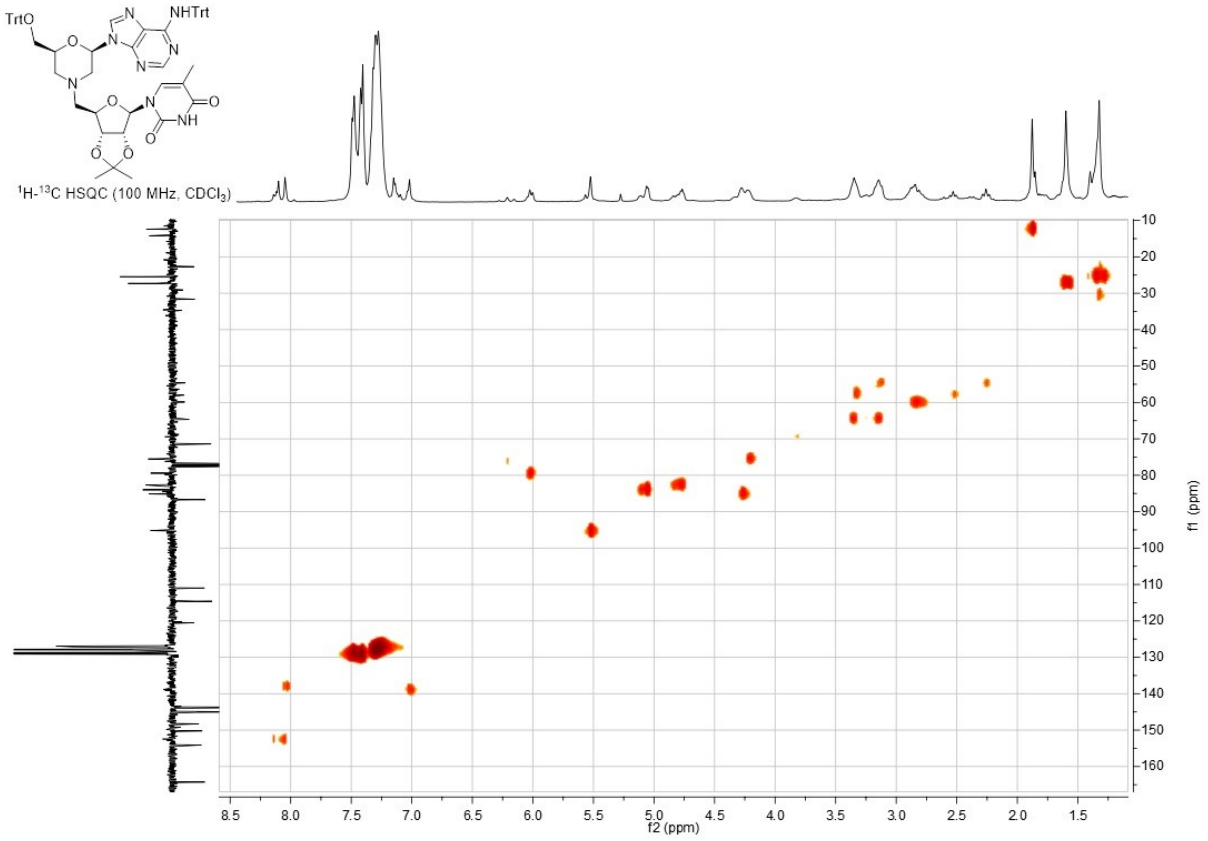

TrtO
NHTrt
NHTrt
^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

NMR spectra of compound 11

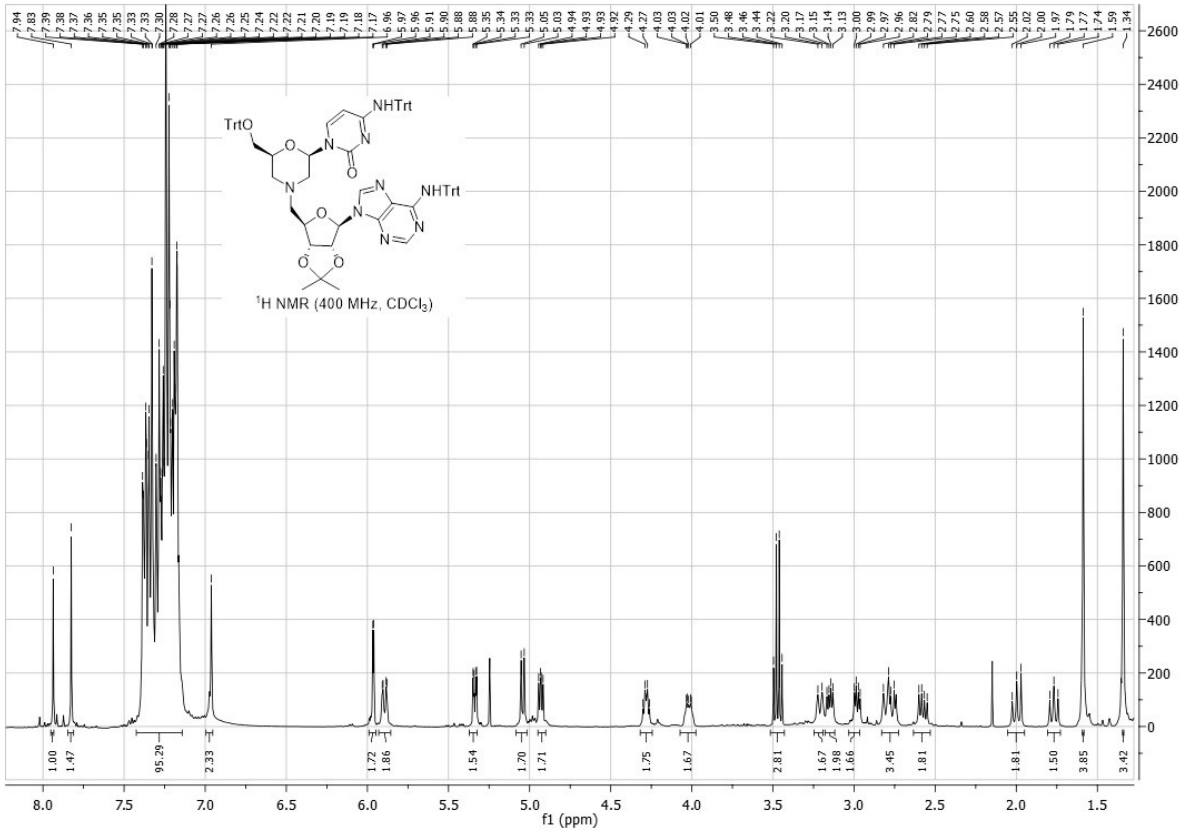


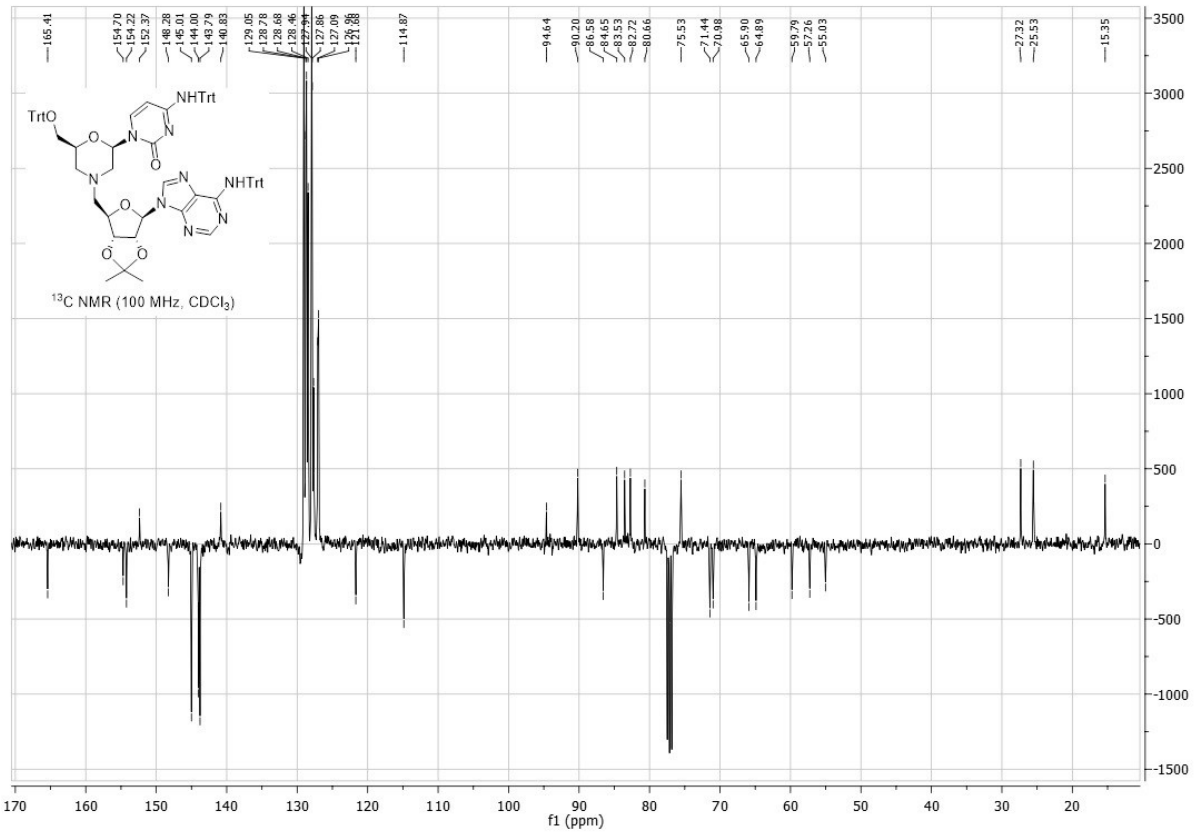


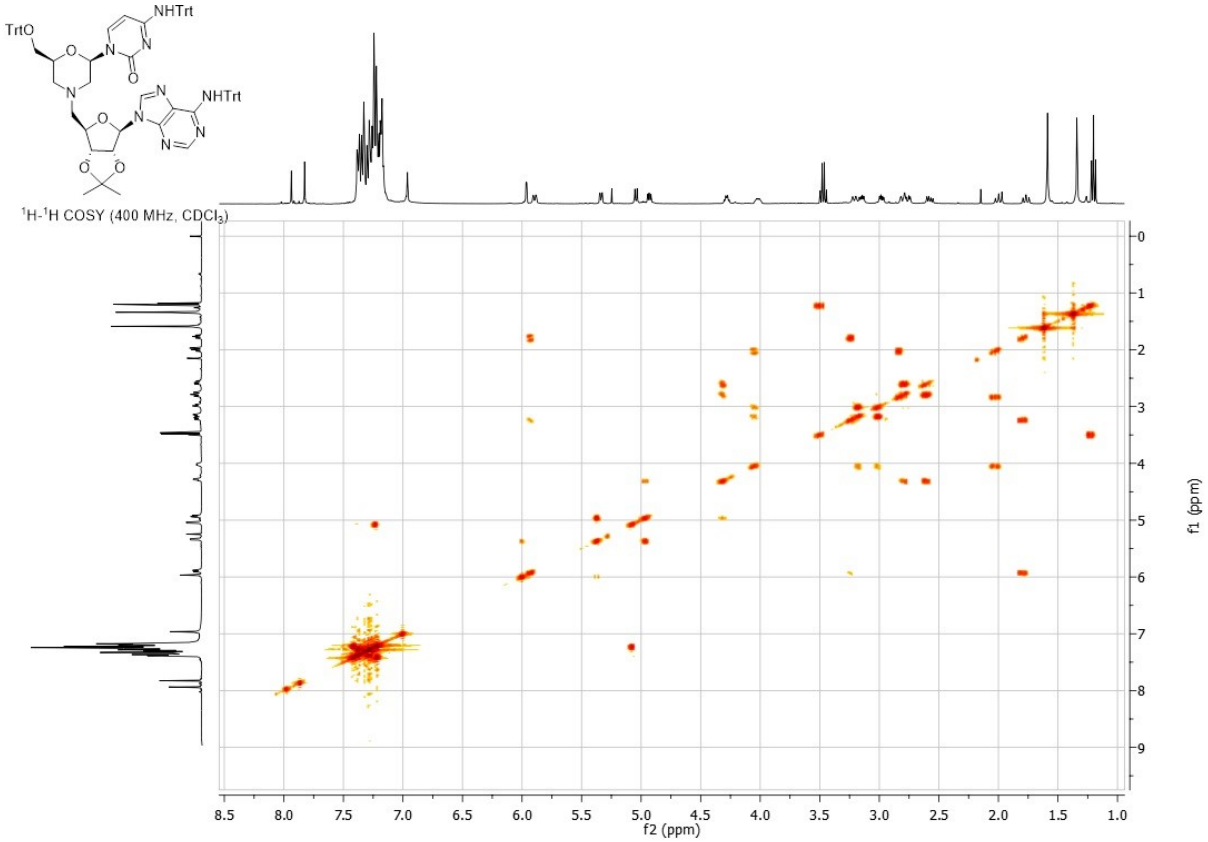

TrtO
NHTrt
NH
O
^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

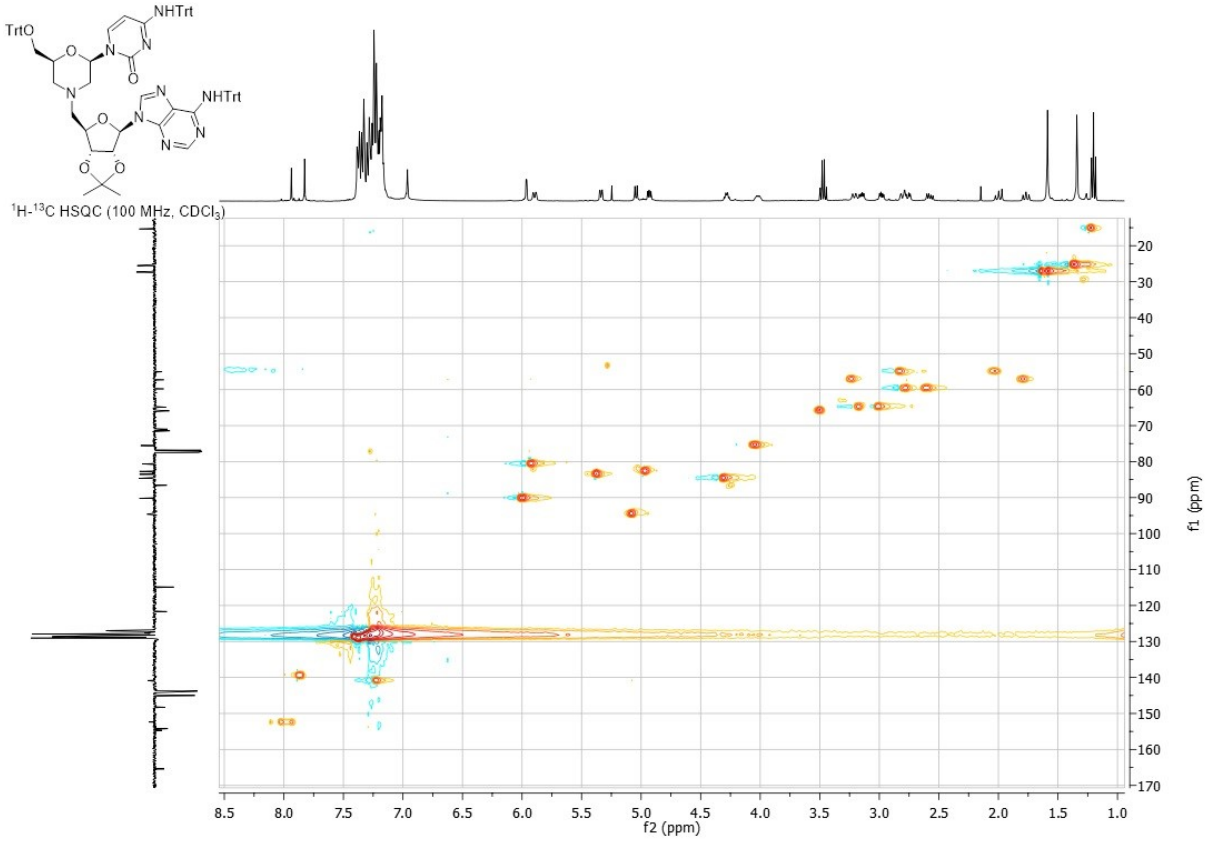

TrtO
NHTrt
NH
O
^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

NMR spectra of compound 12

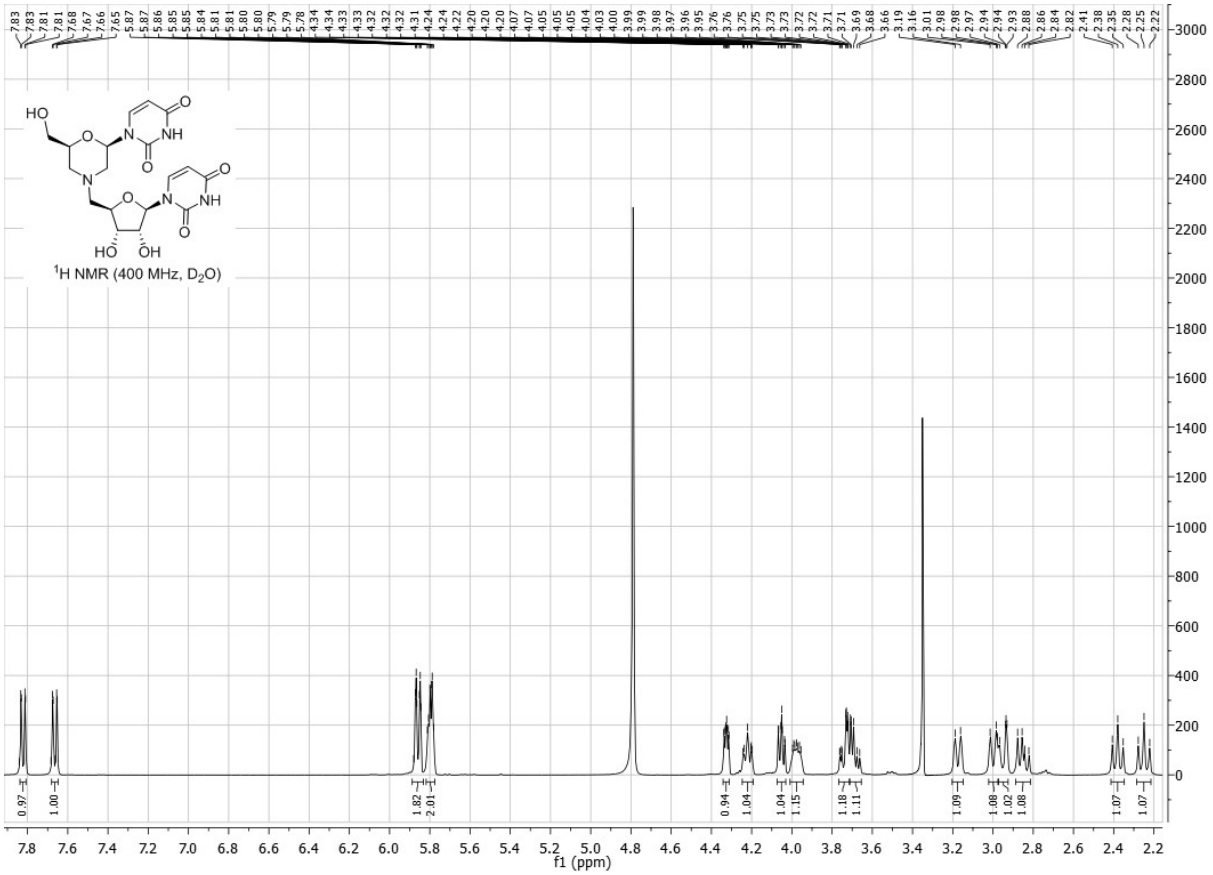


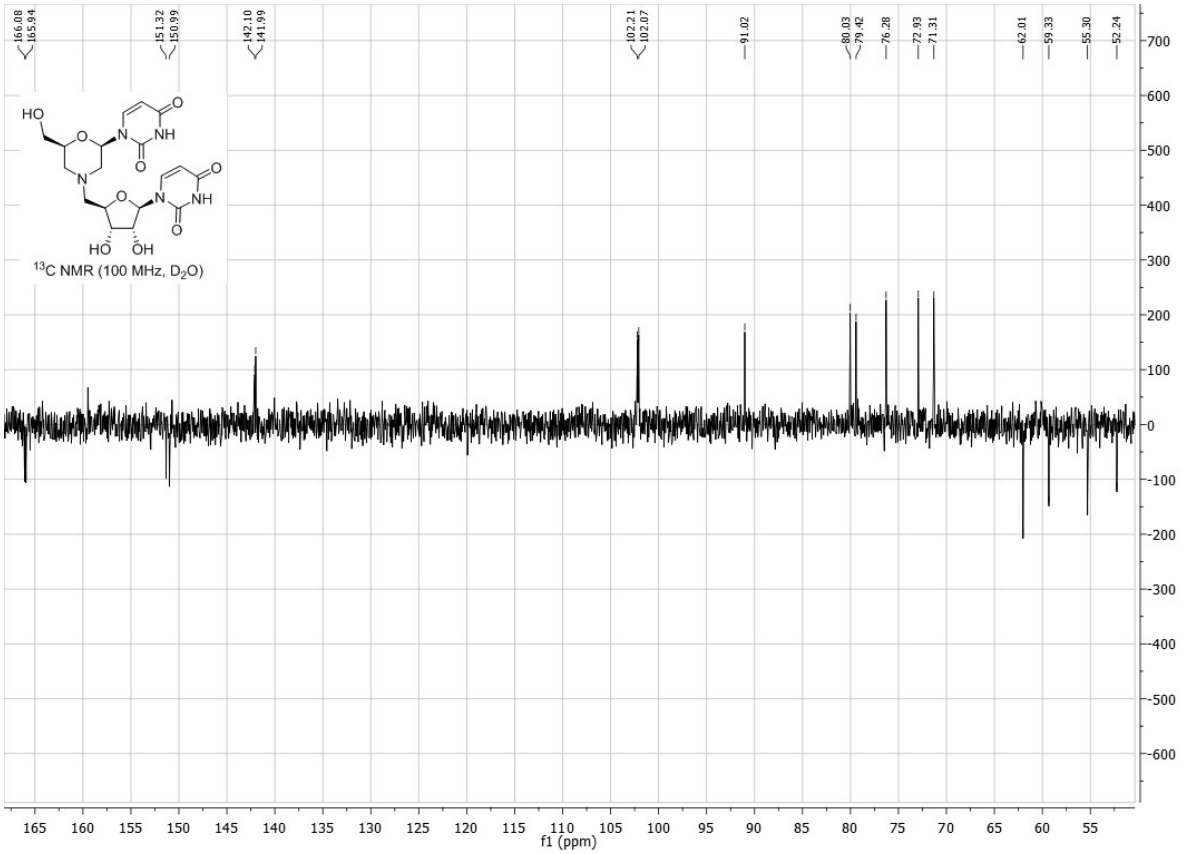


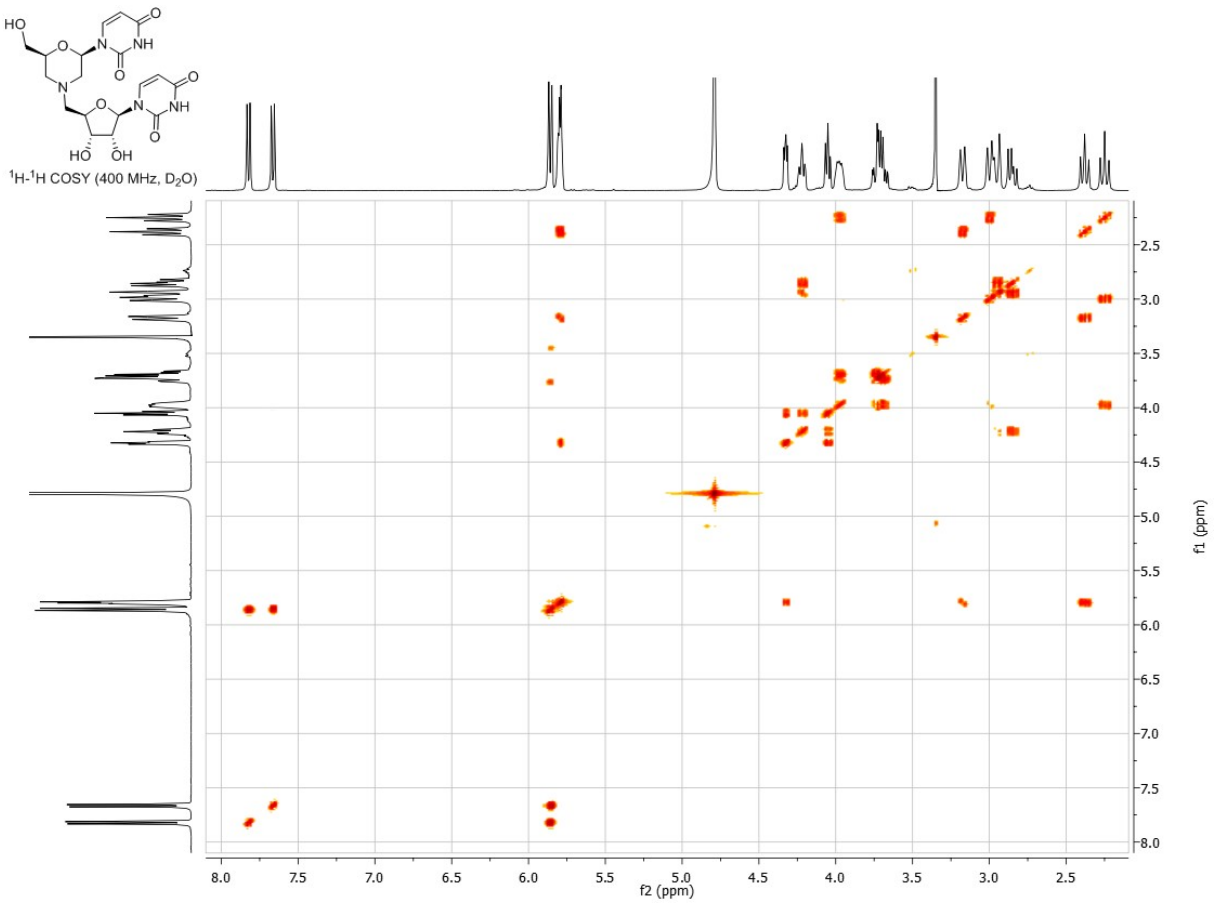

TrtO
NHTrt
NHTrt
^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)


TrtO
NHTrt
NHTrt
^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

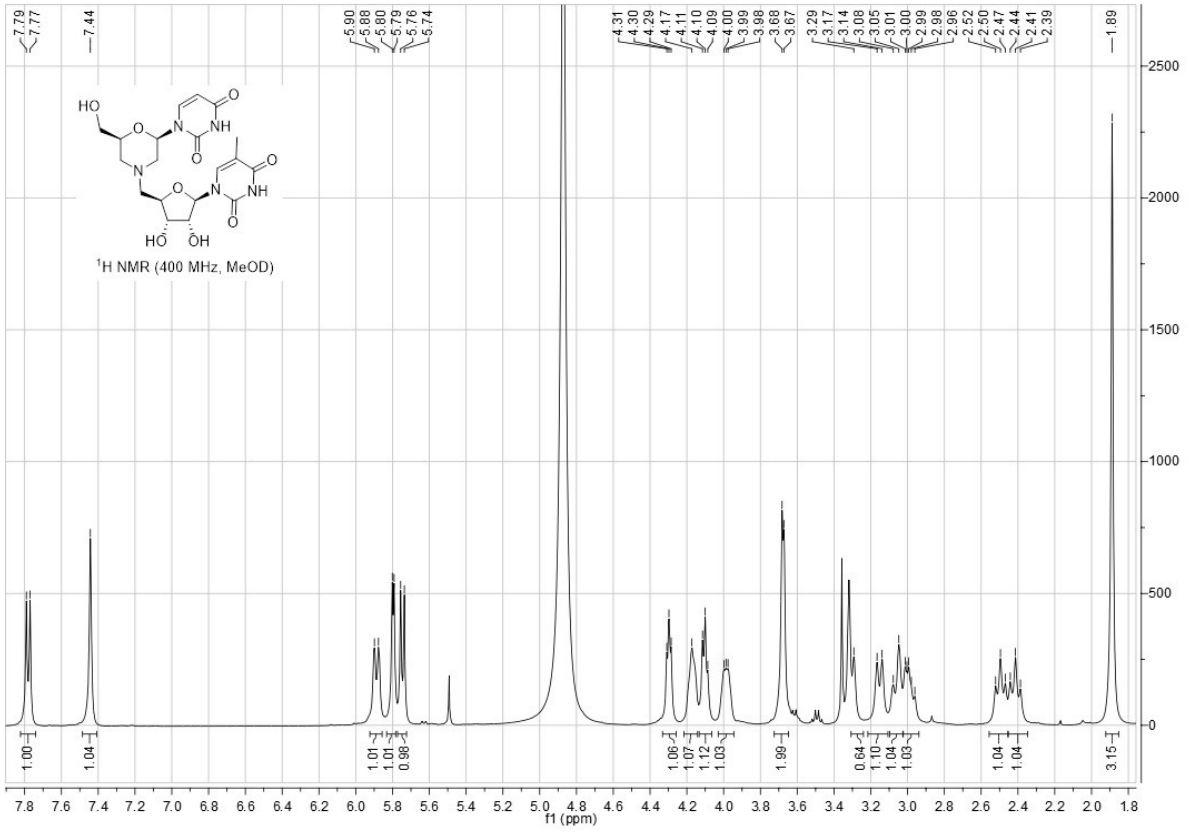
NMR spectra of compound 14

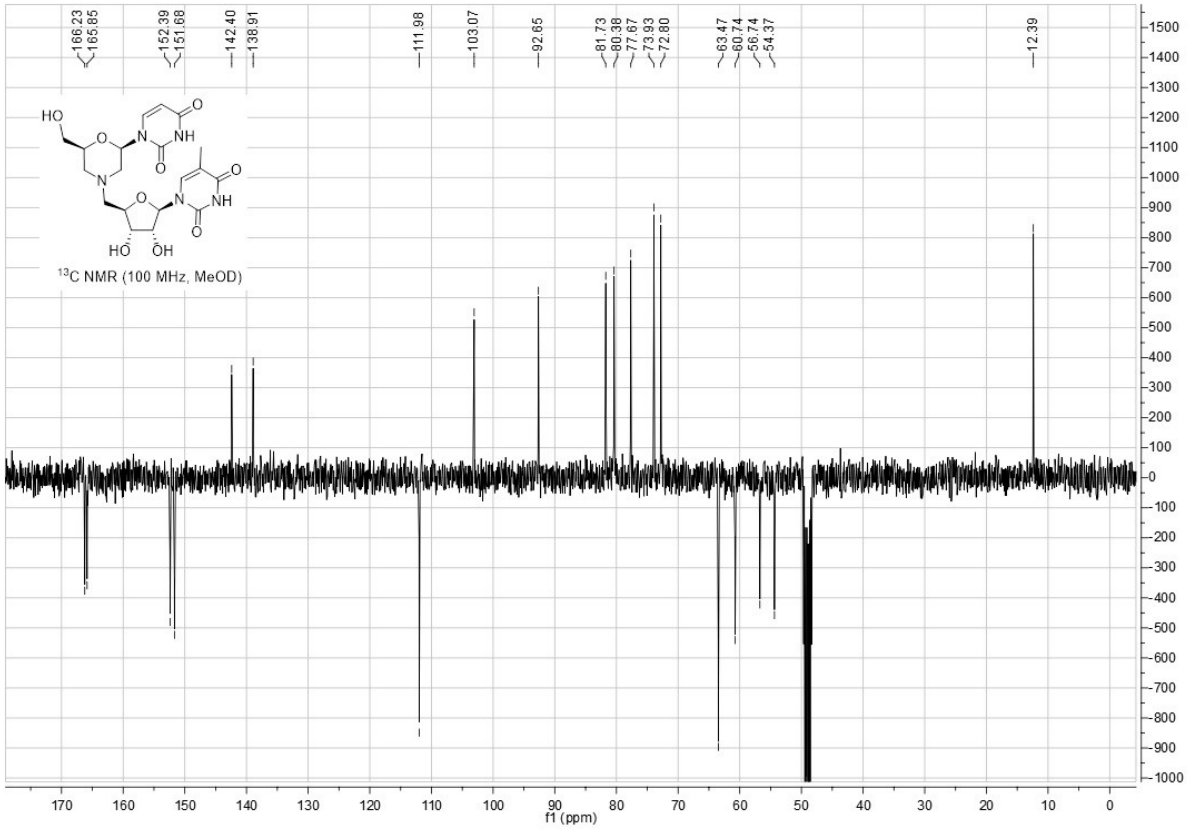


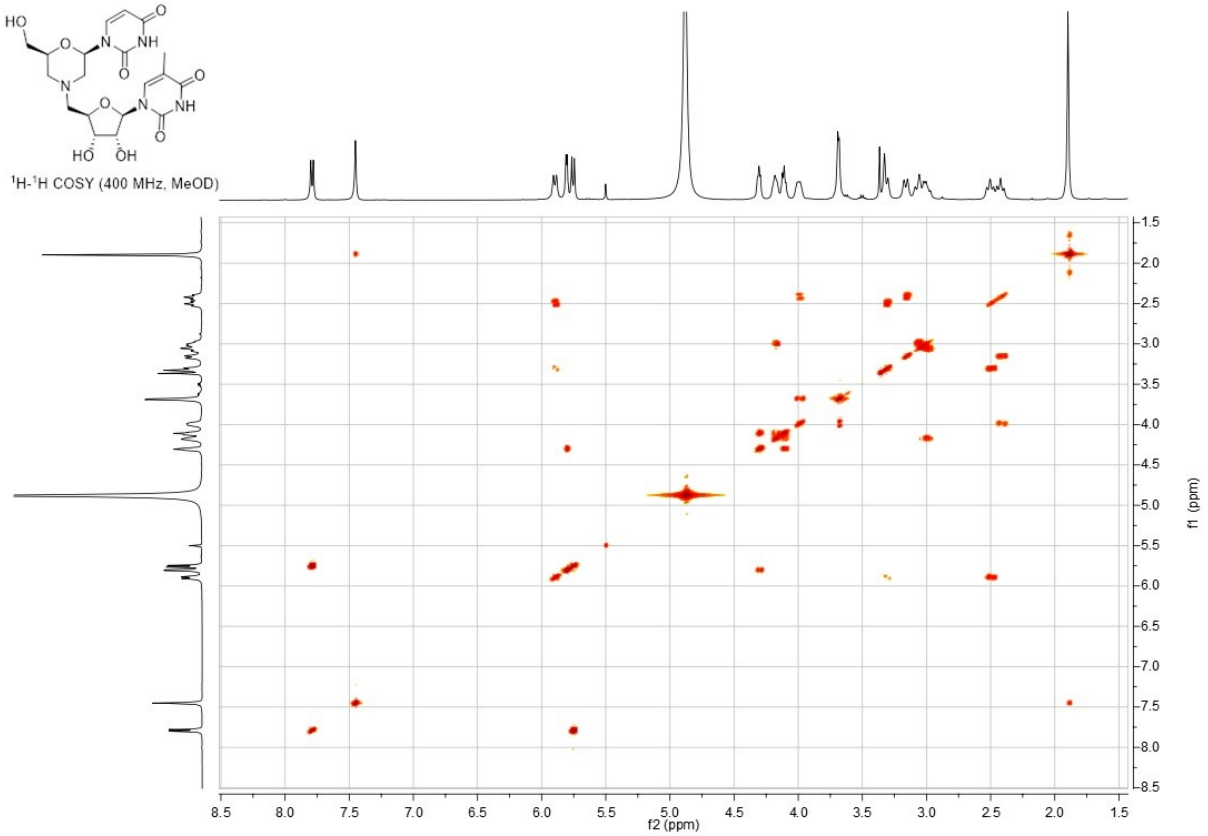


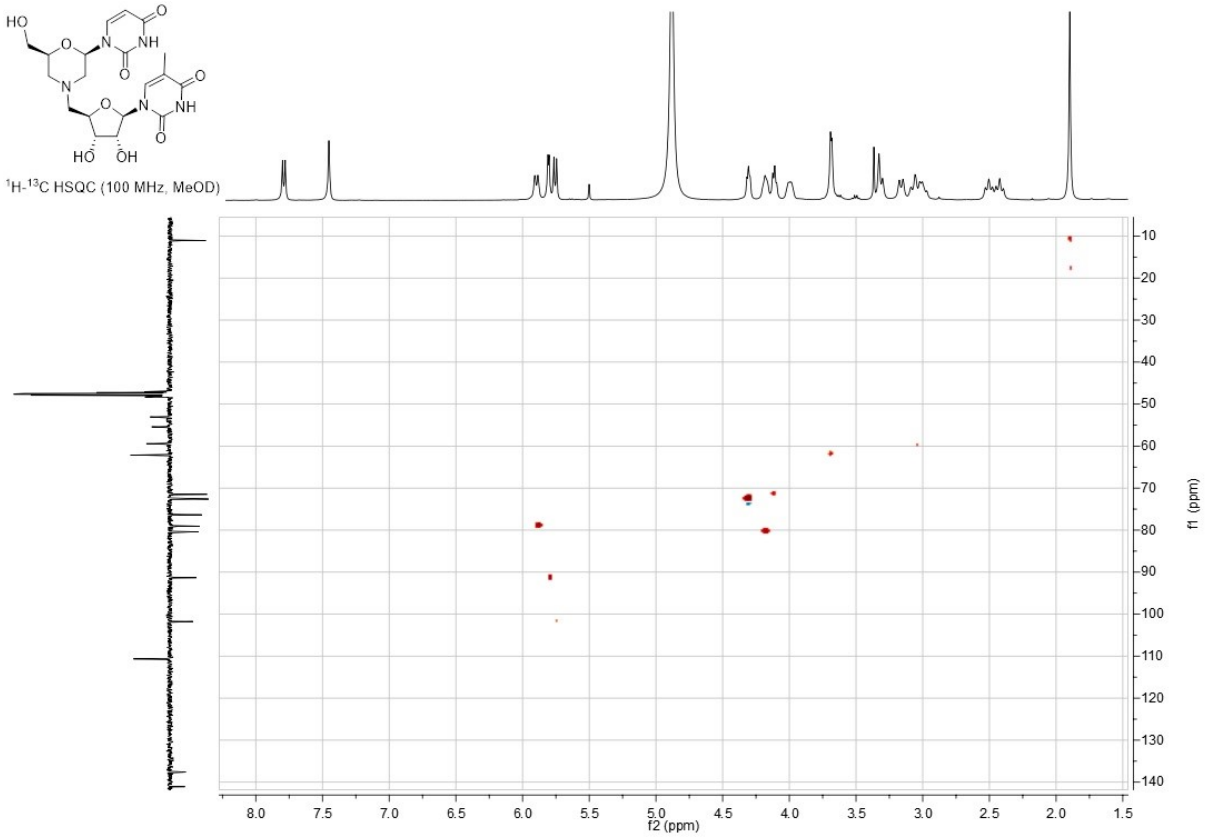

HO
O
NH
O
N
O
N
O
NH
O
HO
OH
^{1}H-^{1}H COSY (400 MHz, D_2O)
f1 (ppm)
f2 (ppm)

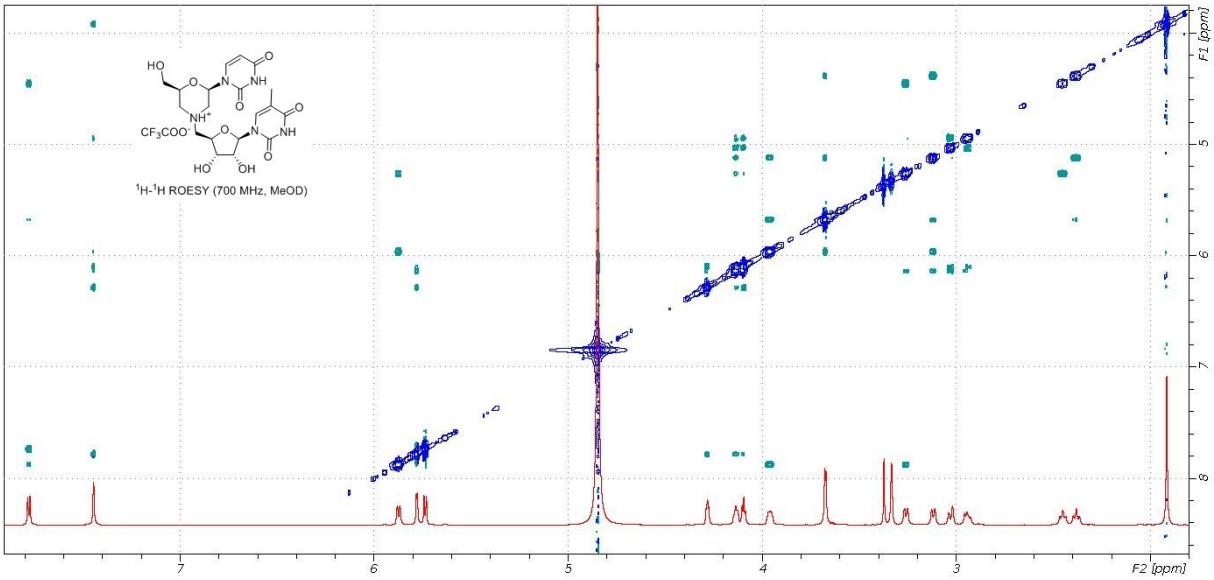
NMR spectra of compound 15 in MeOD



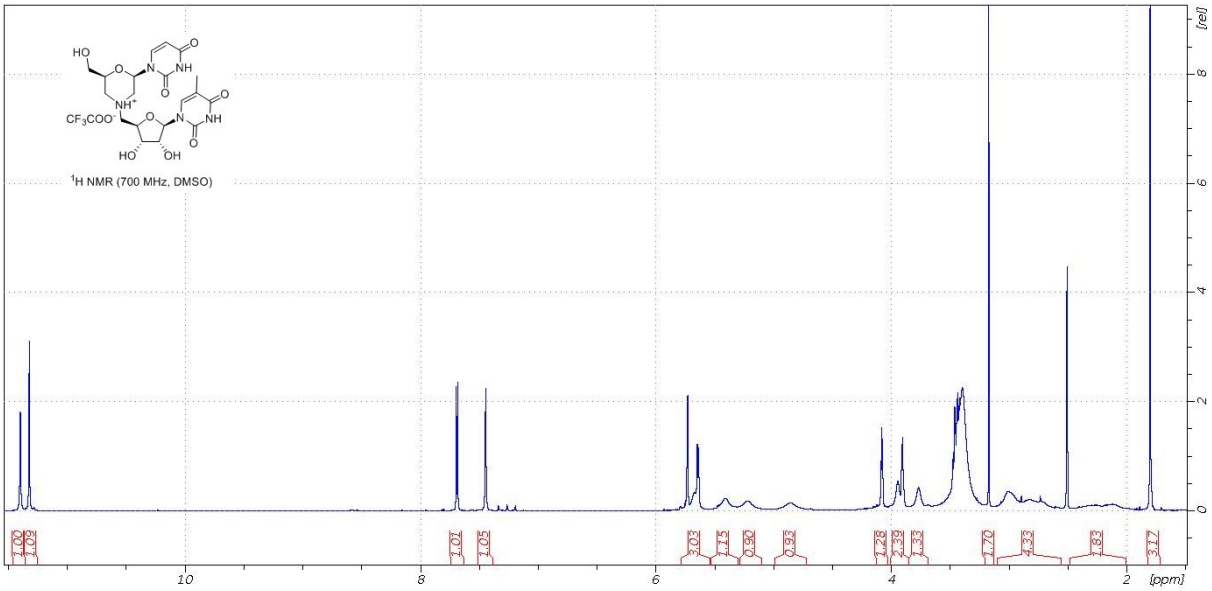


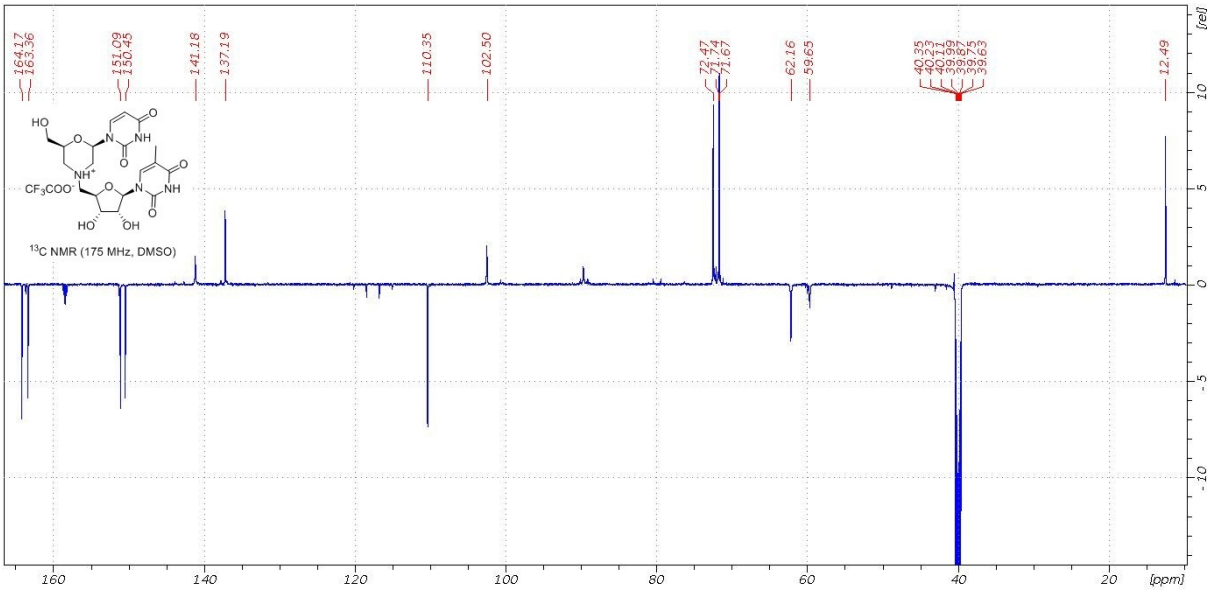

HO
O
NH
N
O
O
NH
O
HO
OH
^{1}H-^{1}H COSY (400 MHz, MeOD)
f2 (ppm)
f1 (ppm)


HO
O
NH
N
O
O
NH
O
HO
OH
^{1}H-^{13}C HSQC (100 MHz, MeOD)
f2 (ppm)
f1 (ppm)

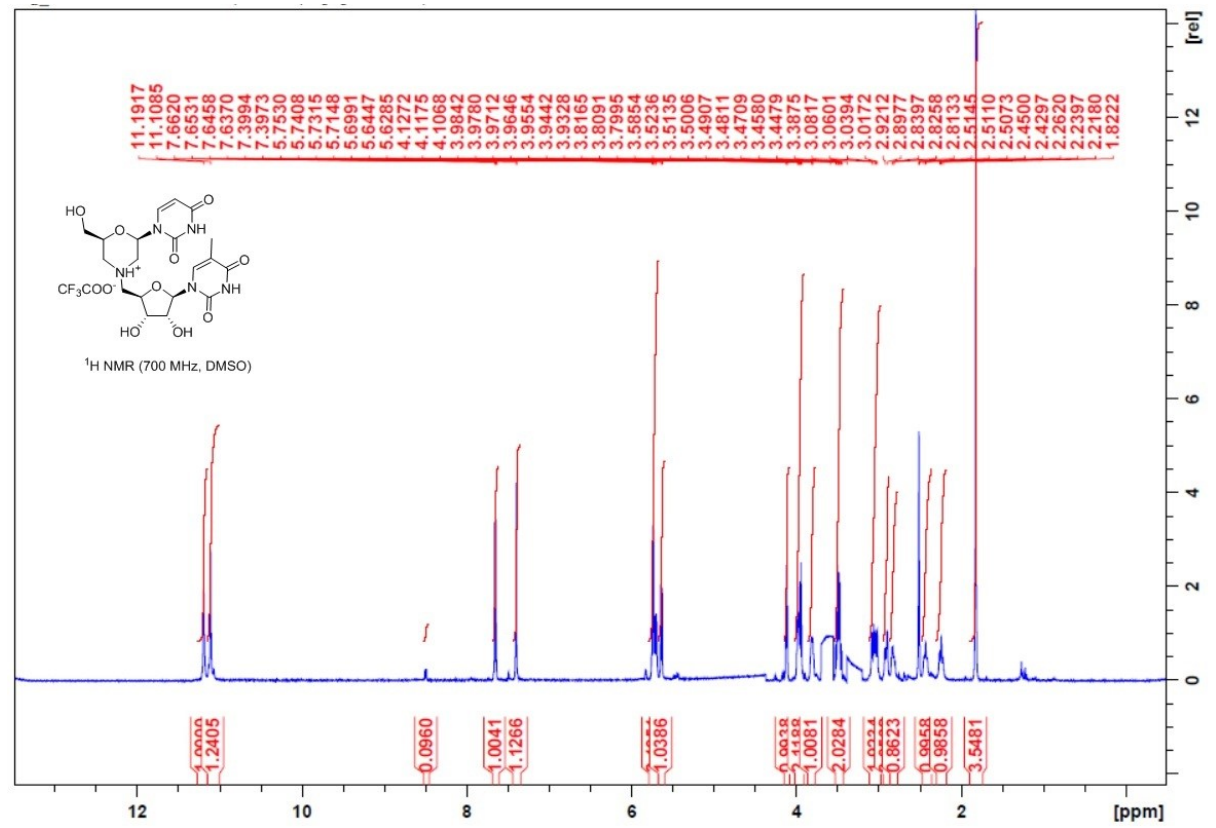


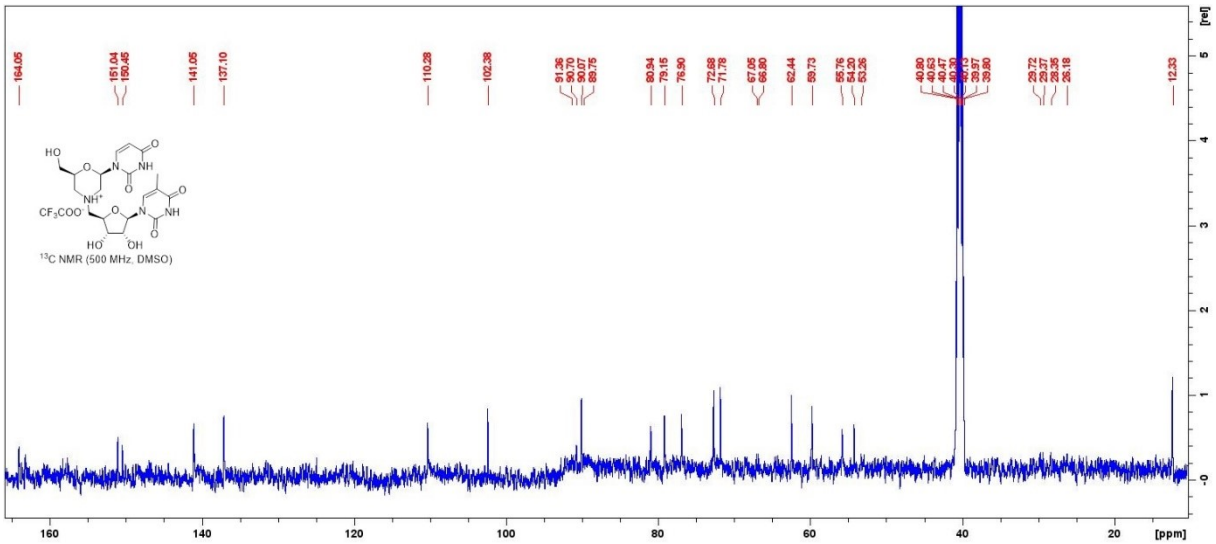
NMR spectra of compound 15 (TFA salt) in DMSO at 300 K

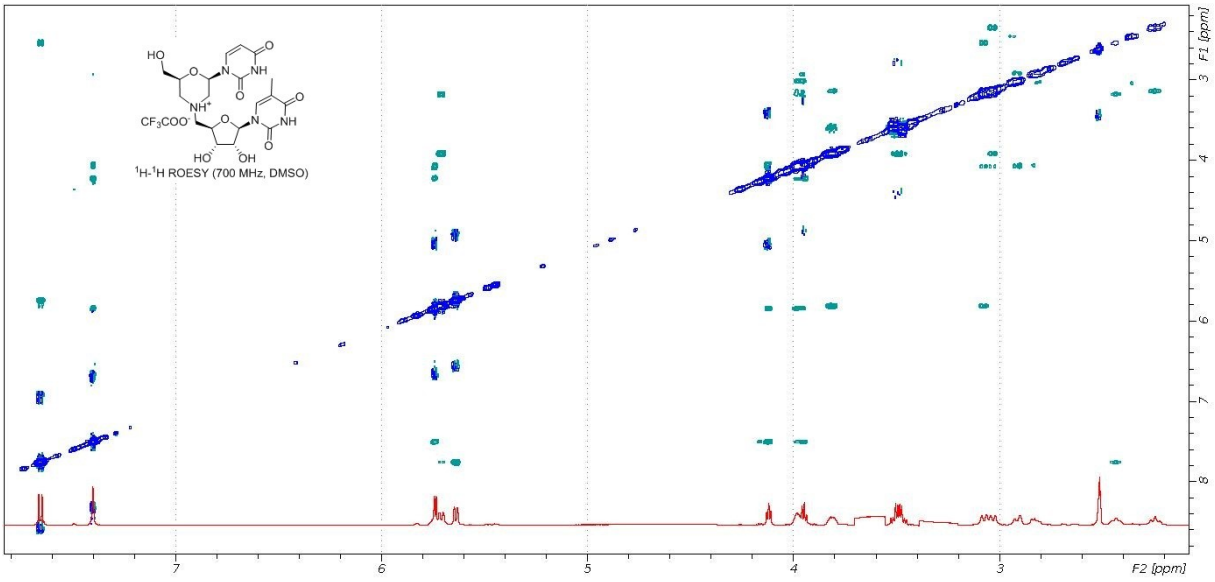




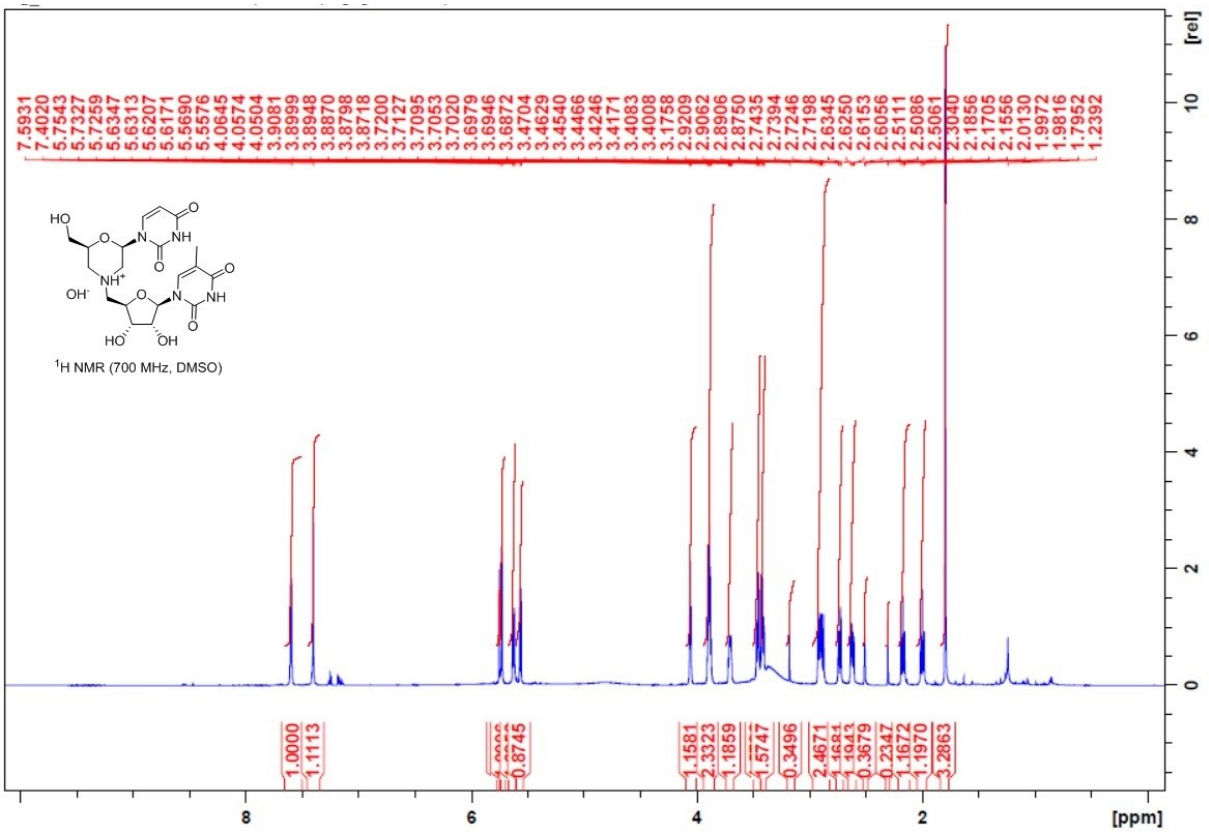
NMR spectra of compound 15 (TFA salt) in DMSO at 340 K

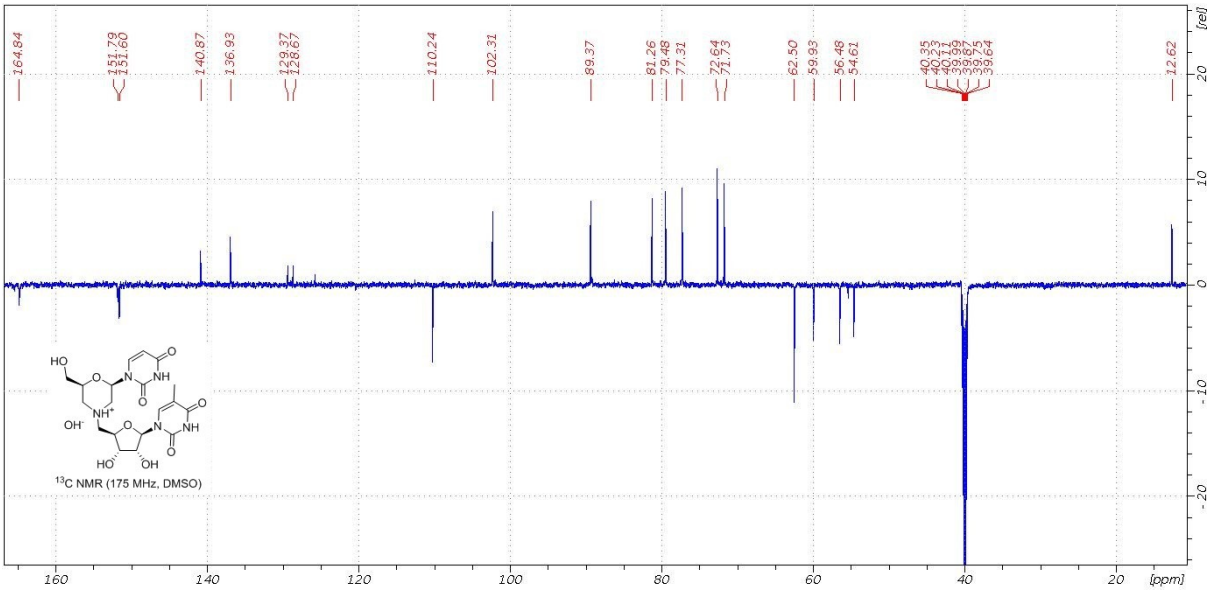


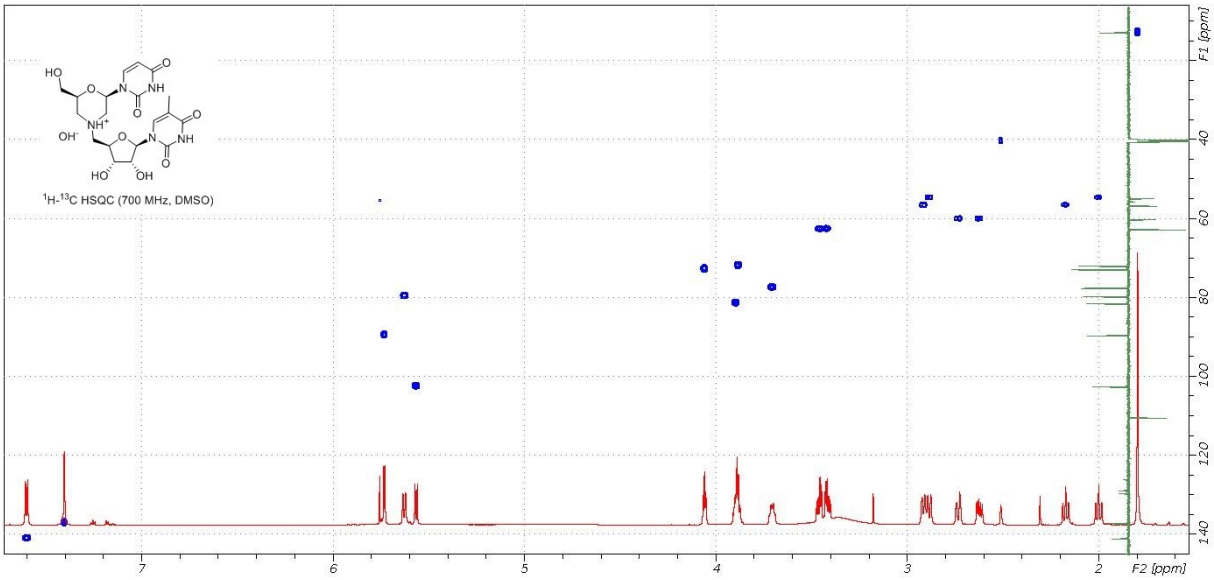


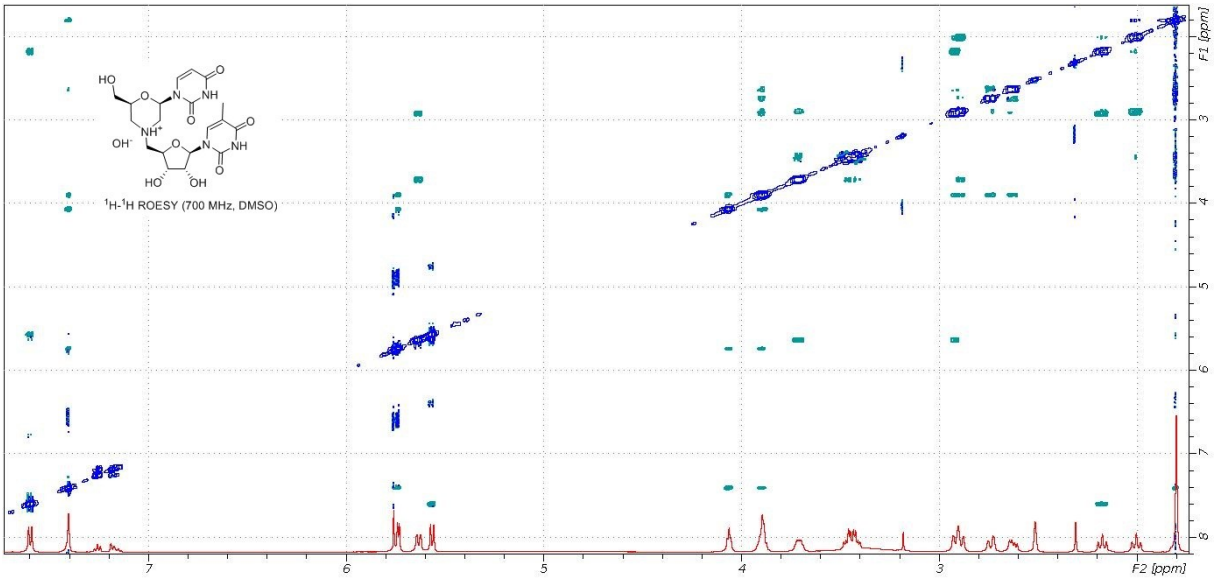


NMR spectra of compound 15 (base form) in DMSO at 300 K

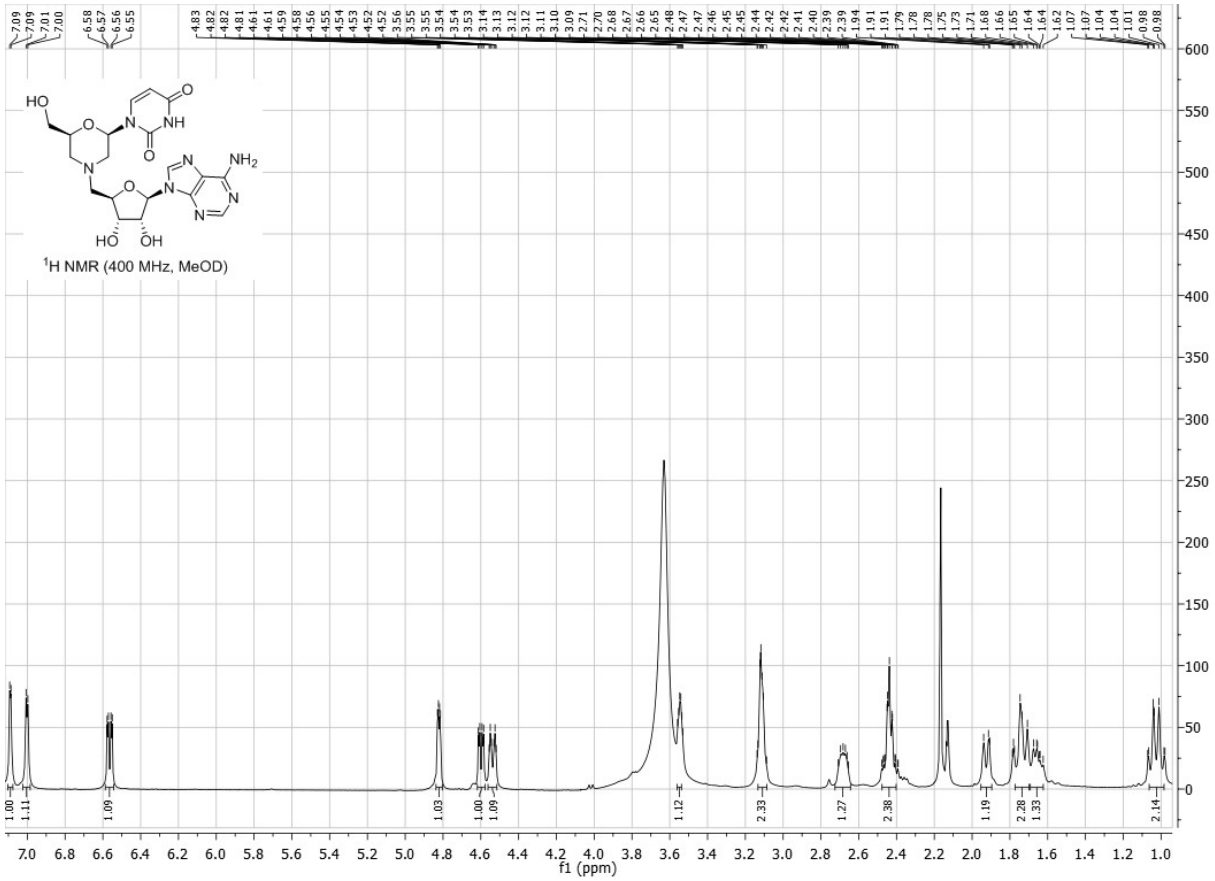


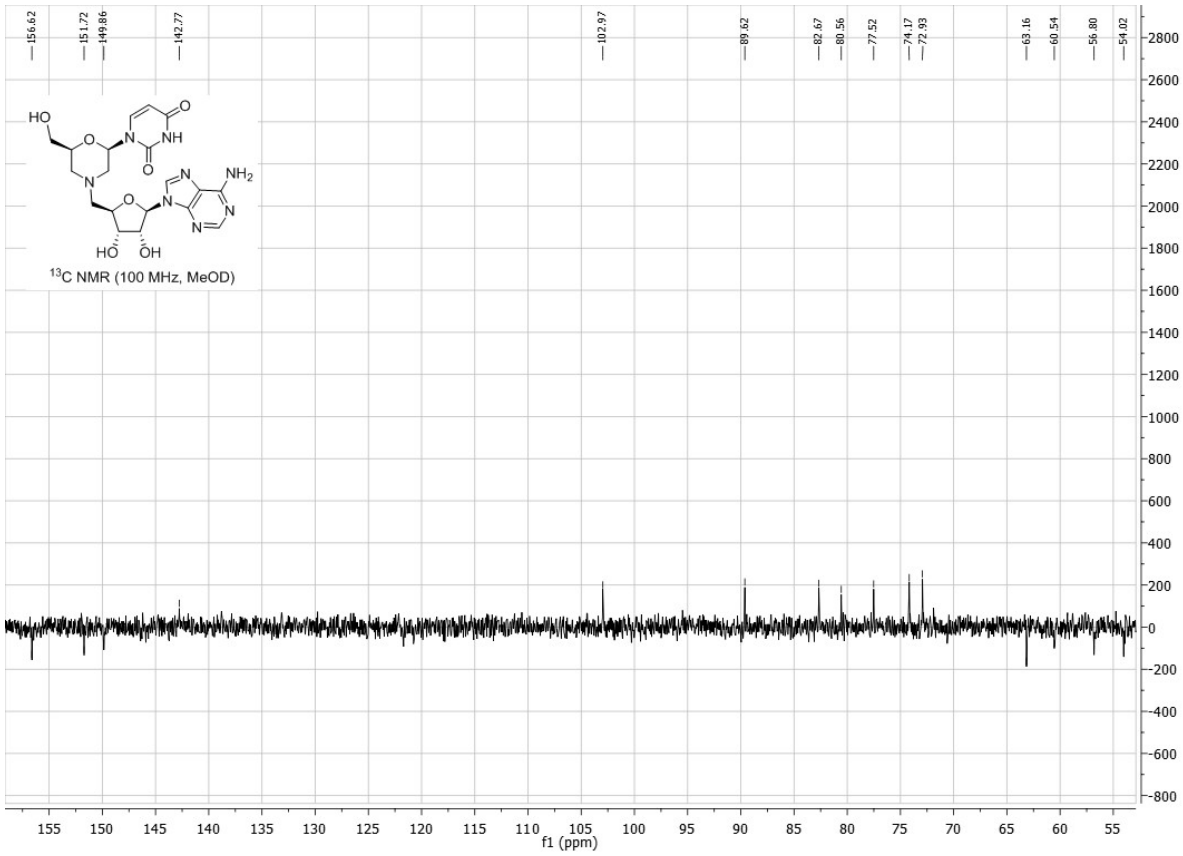

164.84
151.79
151.60
140.87
136.93
129.37
128.67
110.24
102.31
89.37
81.26
79.48
77.31
72.64
71.73
62.50
59.93
56.48
54.61
40.35
40.23
40.11
39.99
39.87
39.75
39.64
12.62
[rel]
20
10
0
-10
-20
160
140
120
100
80
60
40
20
[ppm]
HO
O
N
NH
O
NH+
OH-
HO
OH
13C NMR (175 MHz, DMSO)

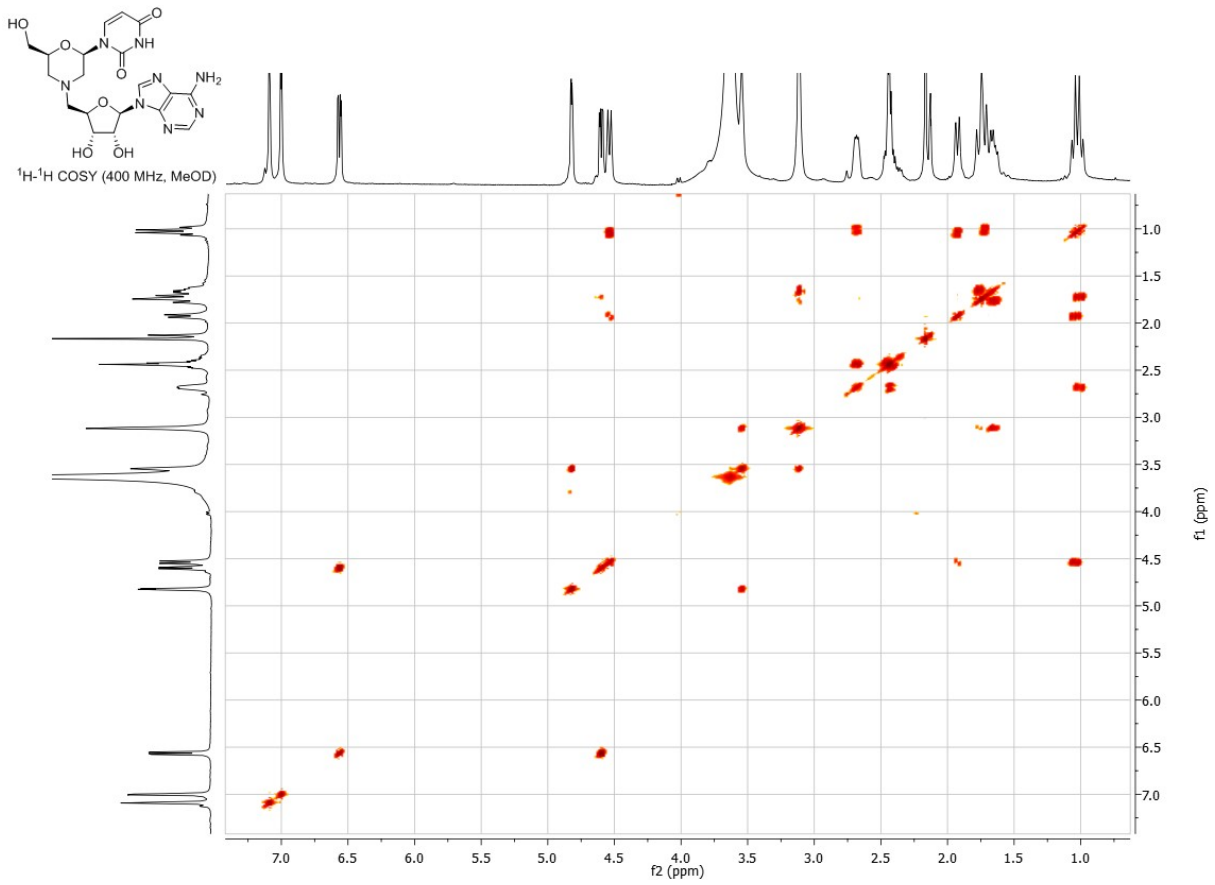

HO
O
N
NH
O
NH+
OH-
HO
OH
1H-13C HSQC (700 MHz, DMSO)
F1 [ppm]
40
60
80
100
120
140
7
6
5
4
3
2
F2 [ppm]

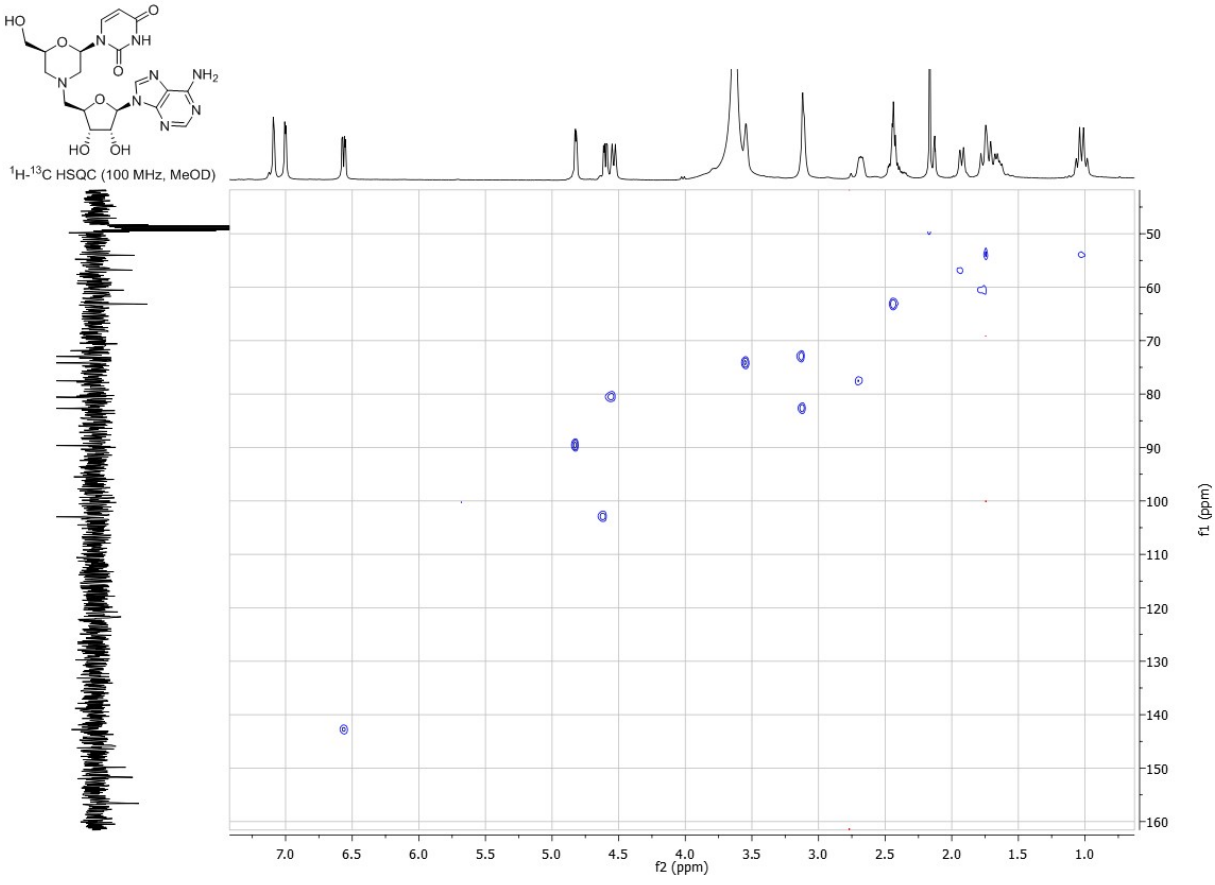

HO
O
N
NH
O
NH+
OH-
HO
OH
1H-1H ROESY (700 MHz, DMSO)
F1 [ppm]
3
4
5
6
7
8
7
6
5
4
3
F2 [ppm]

NMR spectra of compound 16

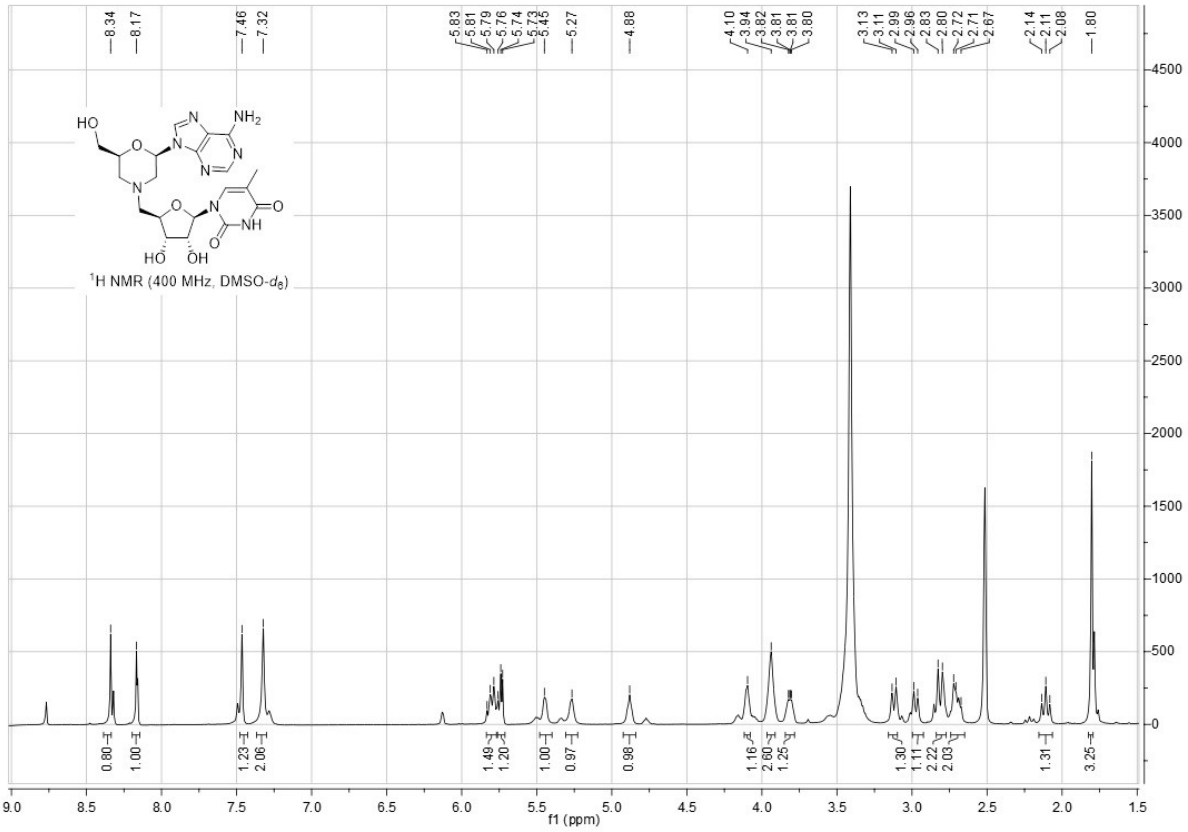


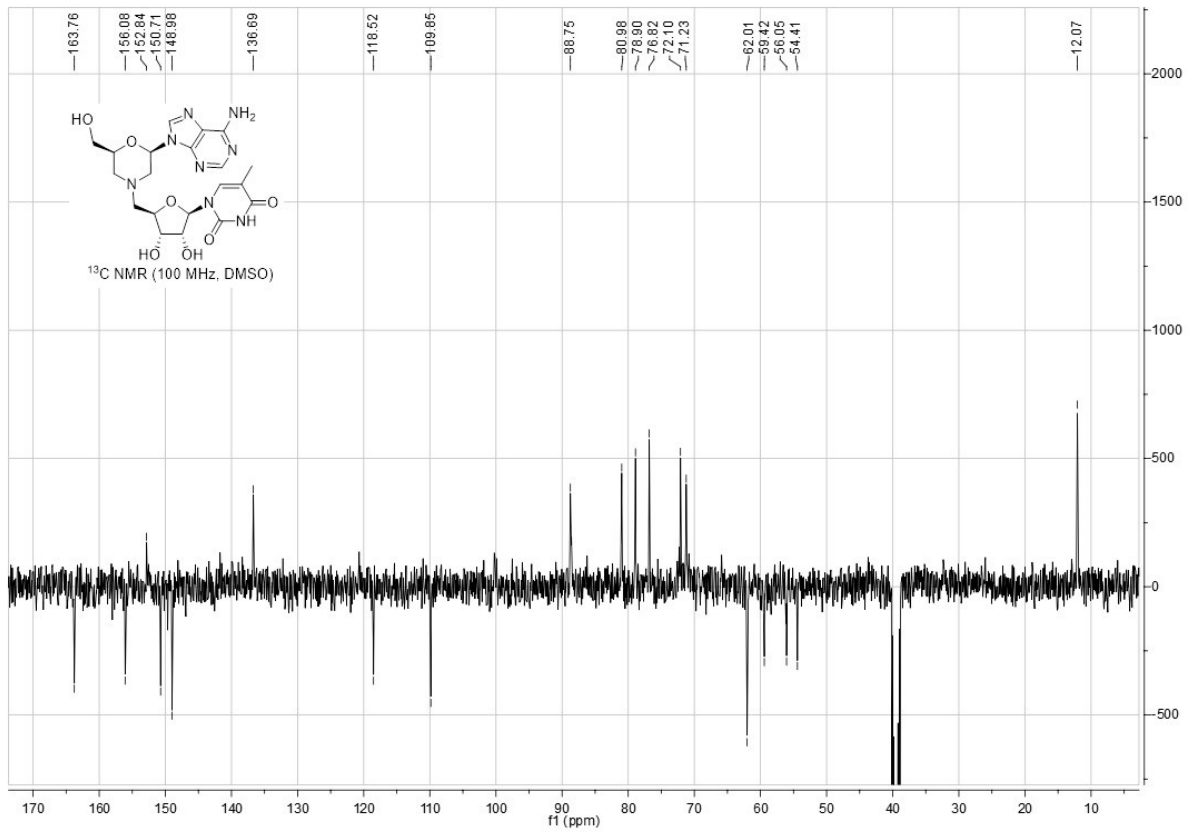


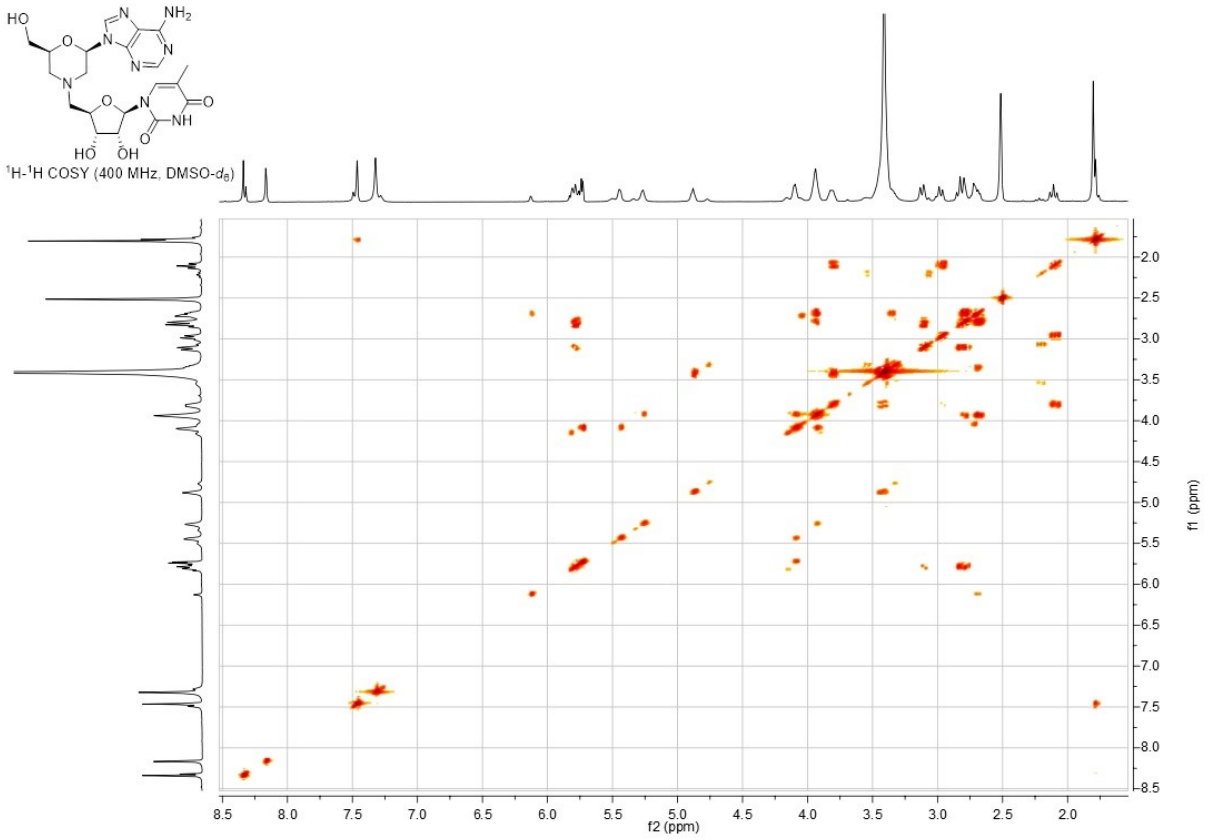

HO
O
NH
O
N
N
NH2
N
N
HO
OH
1H-1H COSY (400 MHz, MeOD)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
f1 (ppm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f2 (ppm)

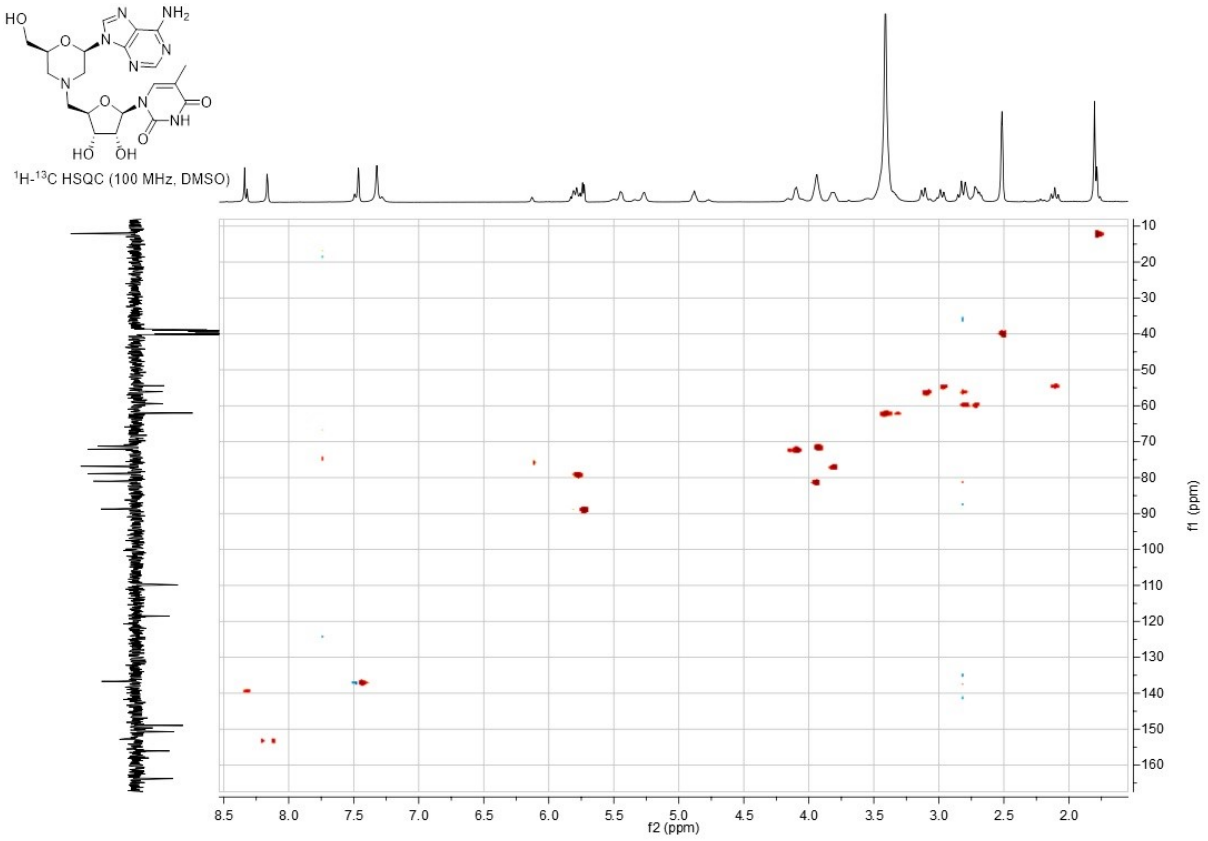

HO
O
NH
O
N
N
NH2
N
N
HO
OH
1H-13C HSQC (100 MHz, MeOD)
50
60
70
80
90
100
110
120
130
140
150
160
f1 (ppm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f2 (ppm)

NMR spectra of compound 17

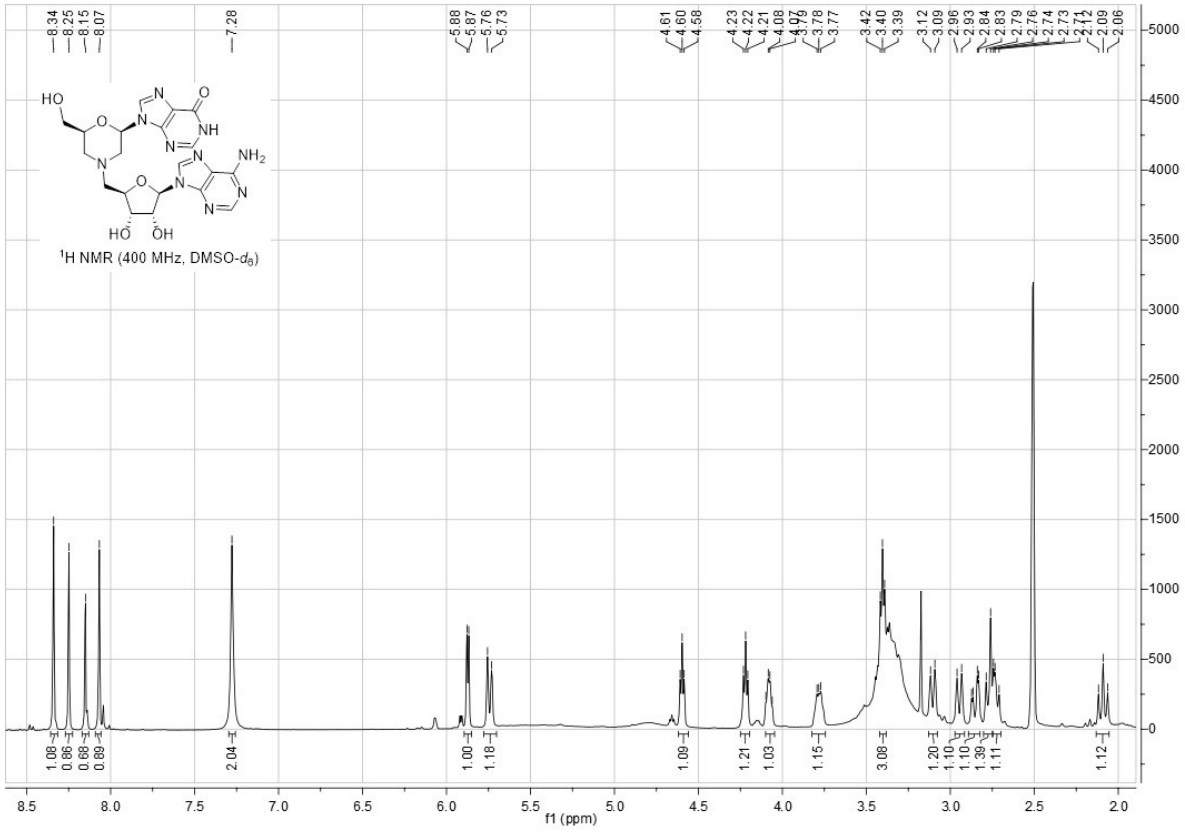


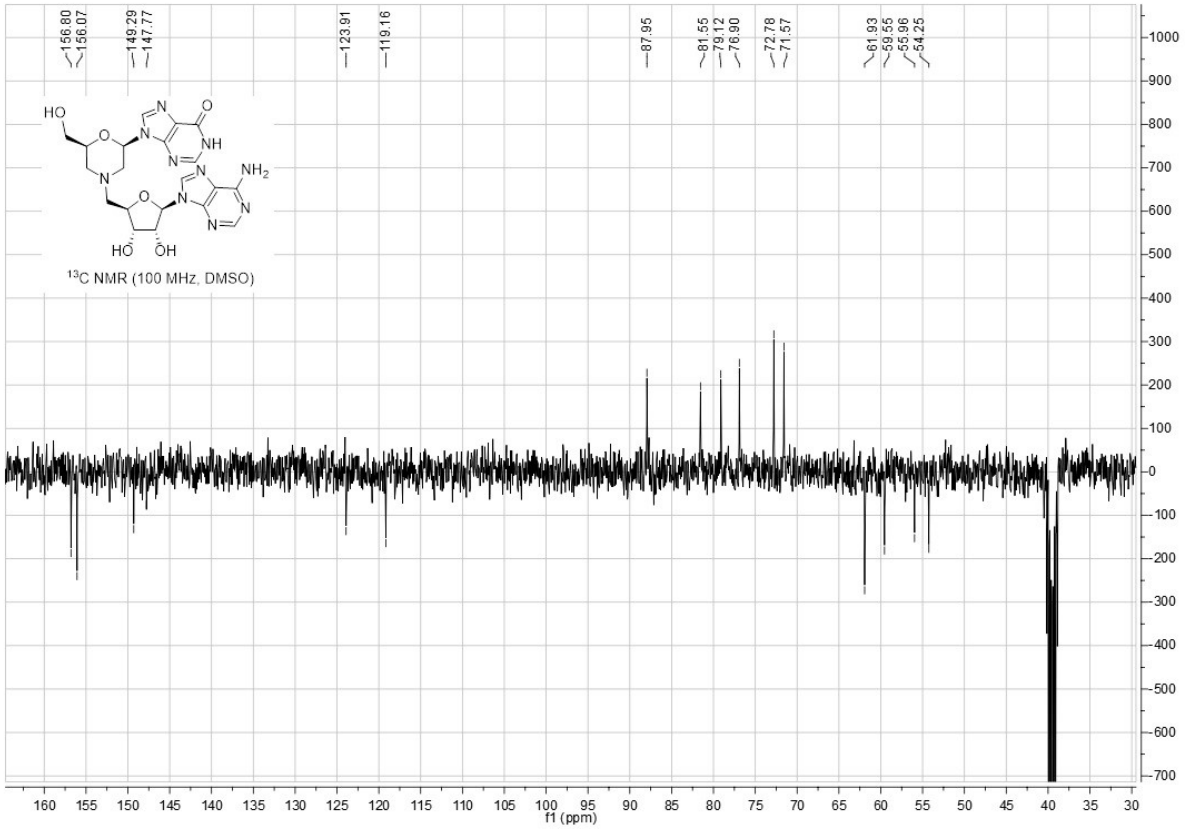


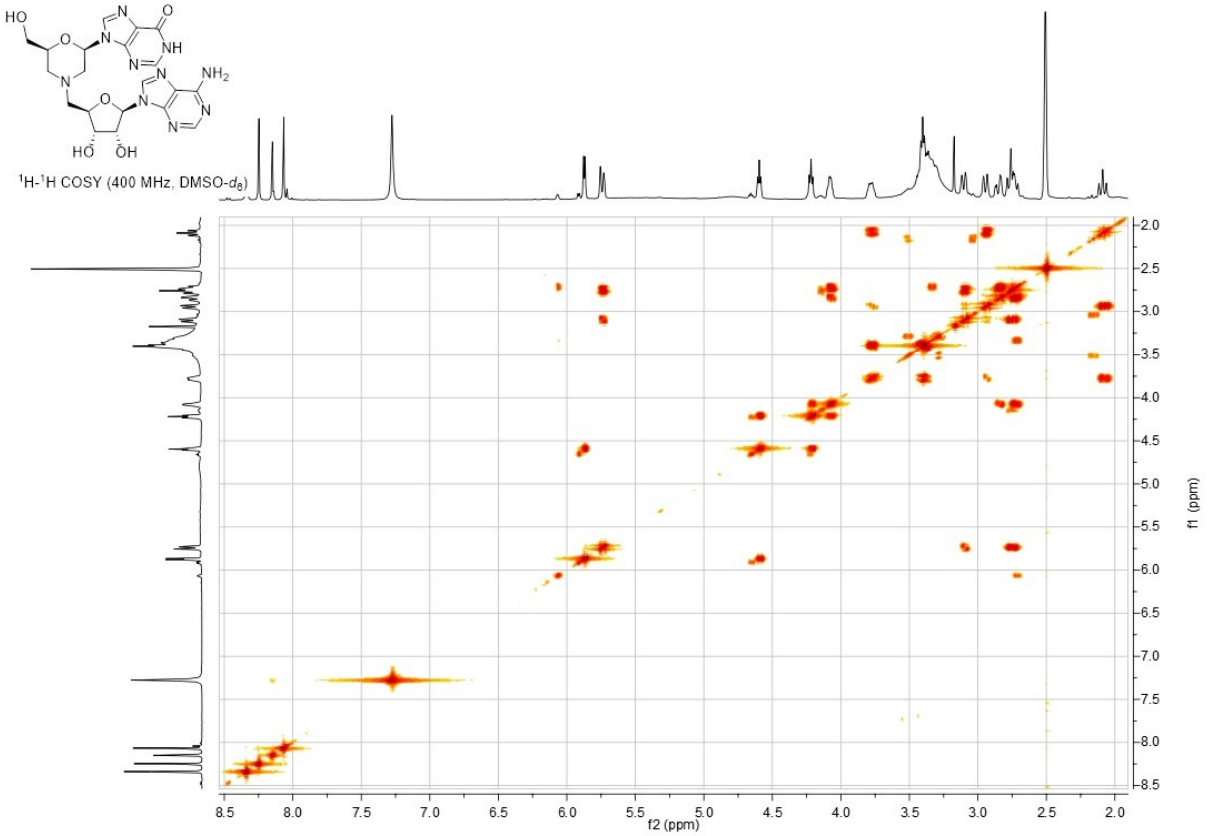

HO
NH2
N
O
NH
HO
OH
1H-1H COSY (400 MHz, DMSO-d6)
f1 (ppm)
f2 (ppm)

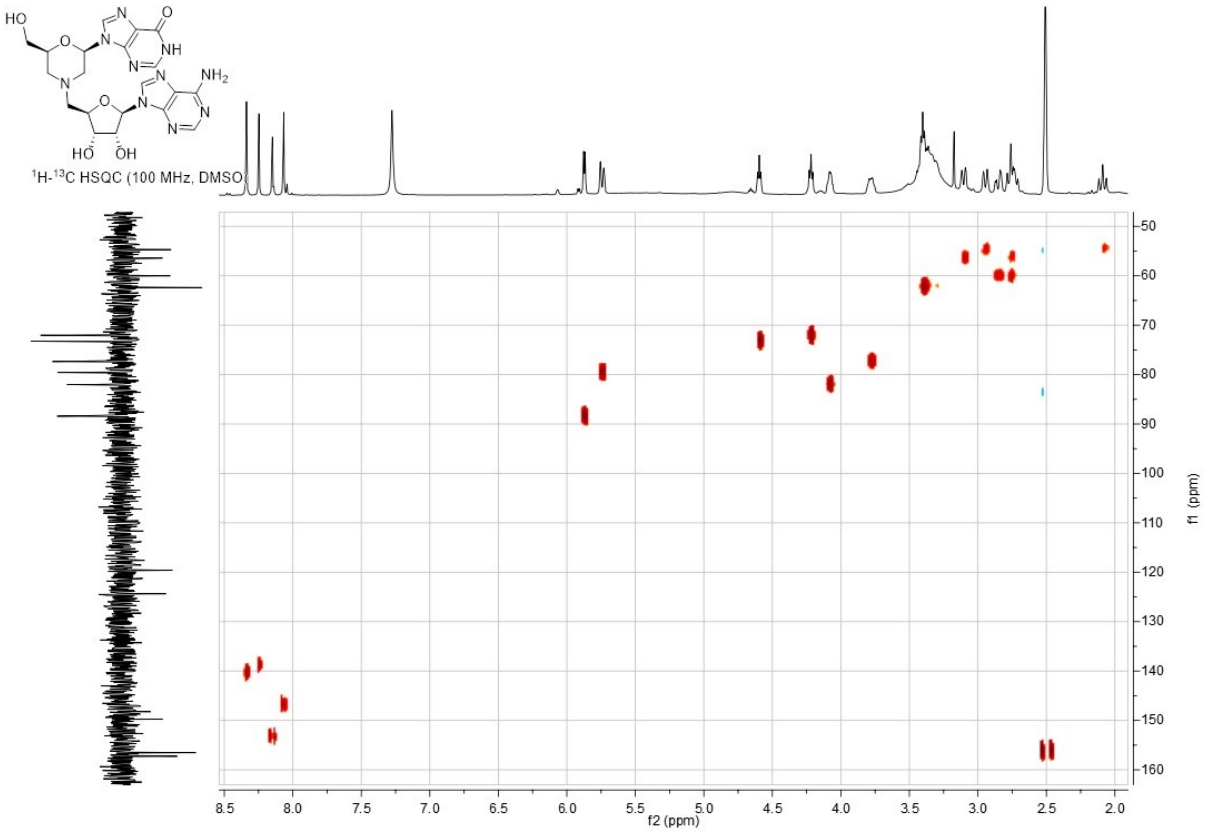

HO
NH2
N
O
NH
HO
OH
1H-13C HSQC (100 MHz, DMSO)
f1 (ppm)
f2 (ppm)

NMR spectra of compound 18

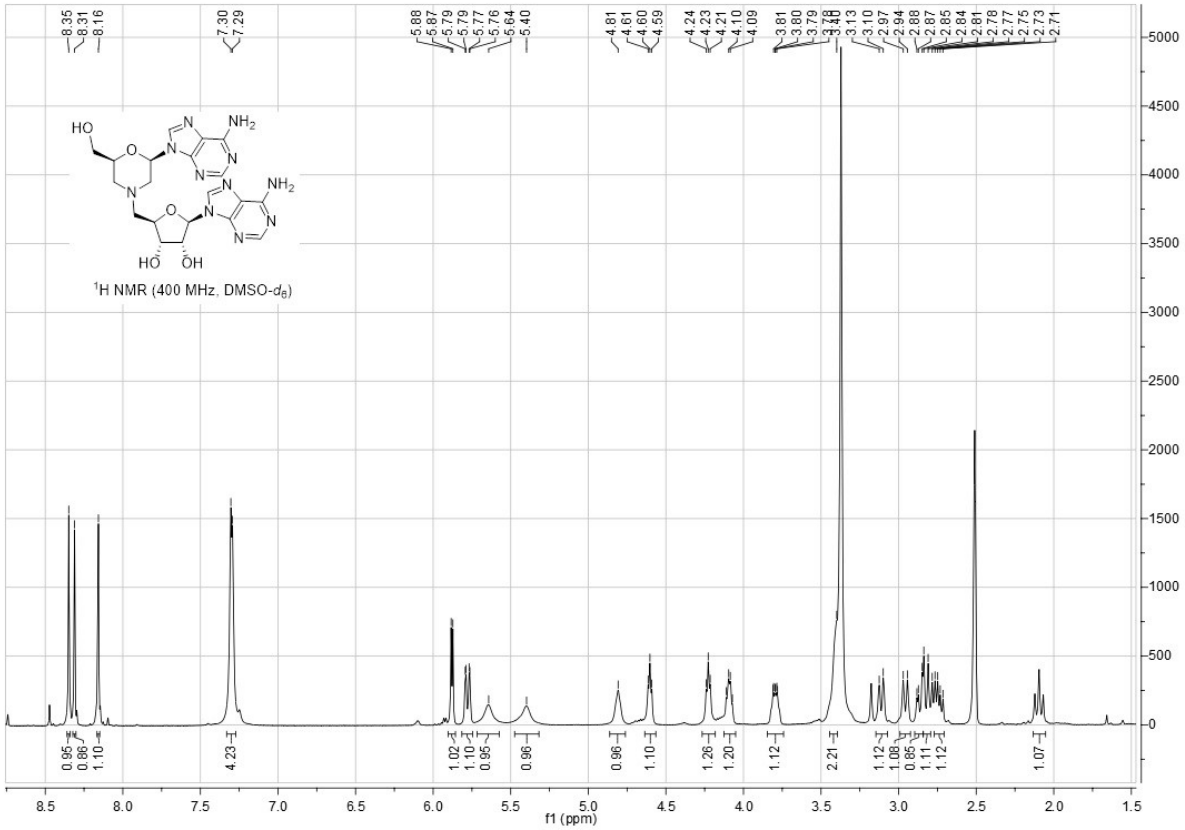


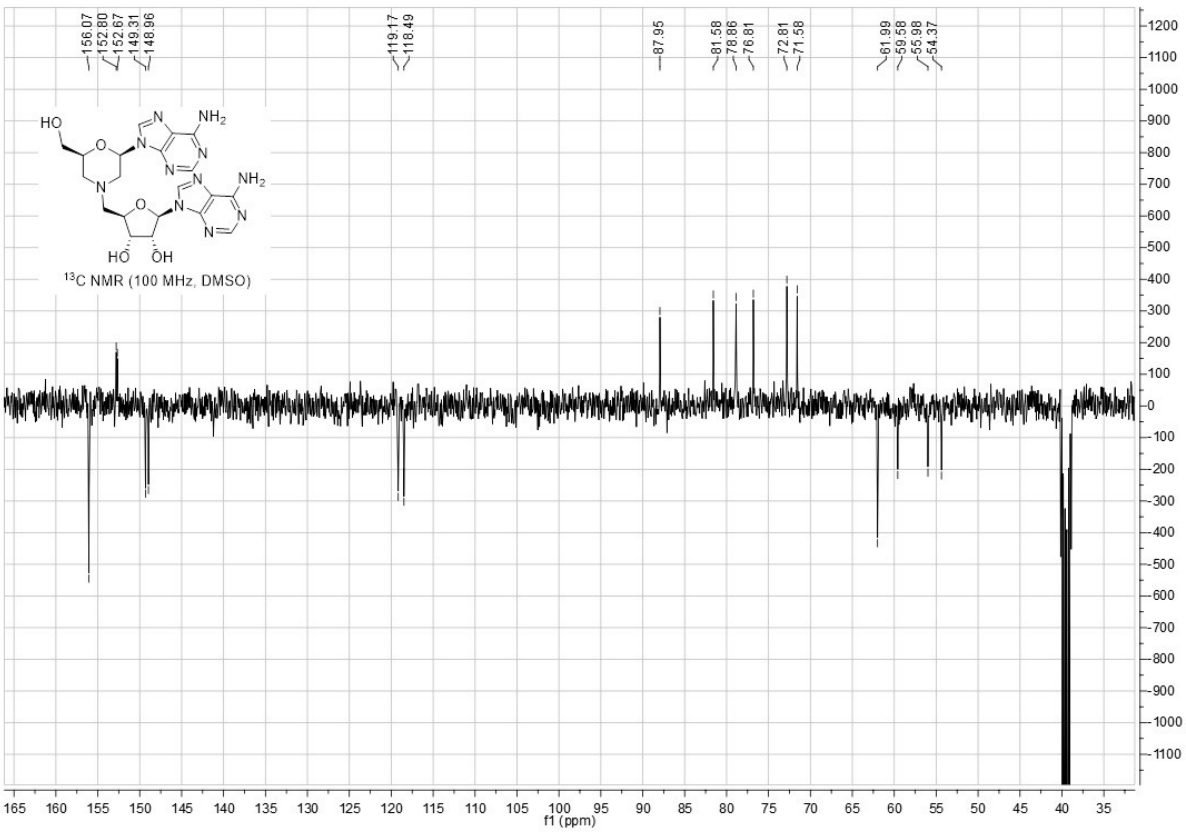


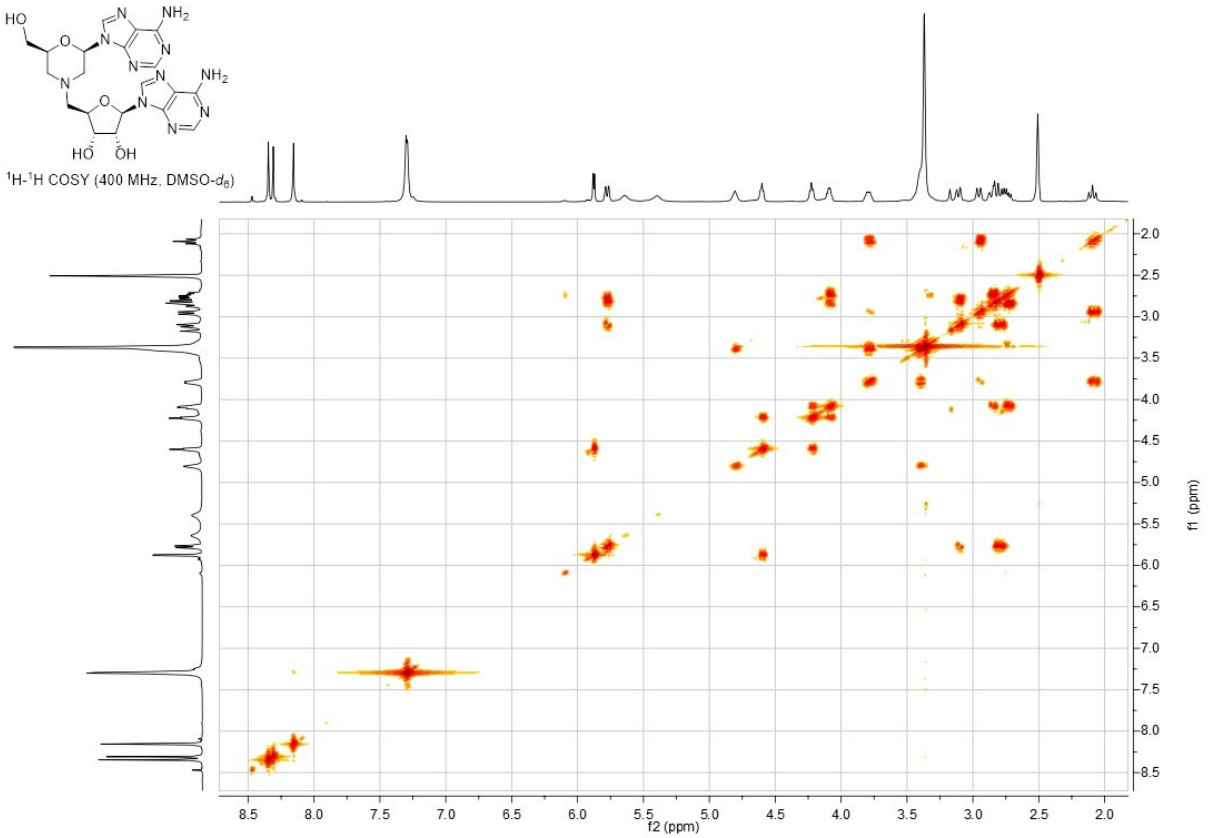

HO
O
NH
N
NH2
HO
OH
1H-1H COSY (400 MHz, DMSO-d6)
f2 (ppm)
f1 (ppm)

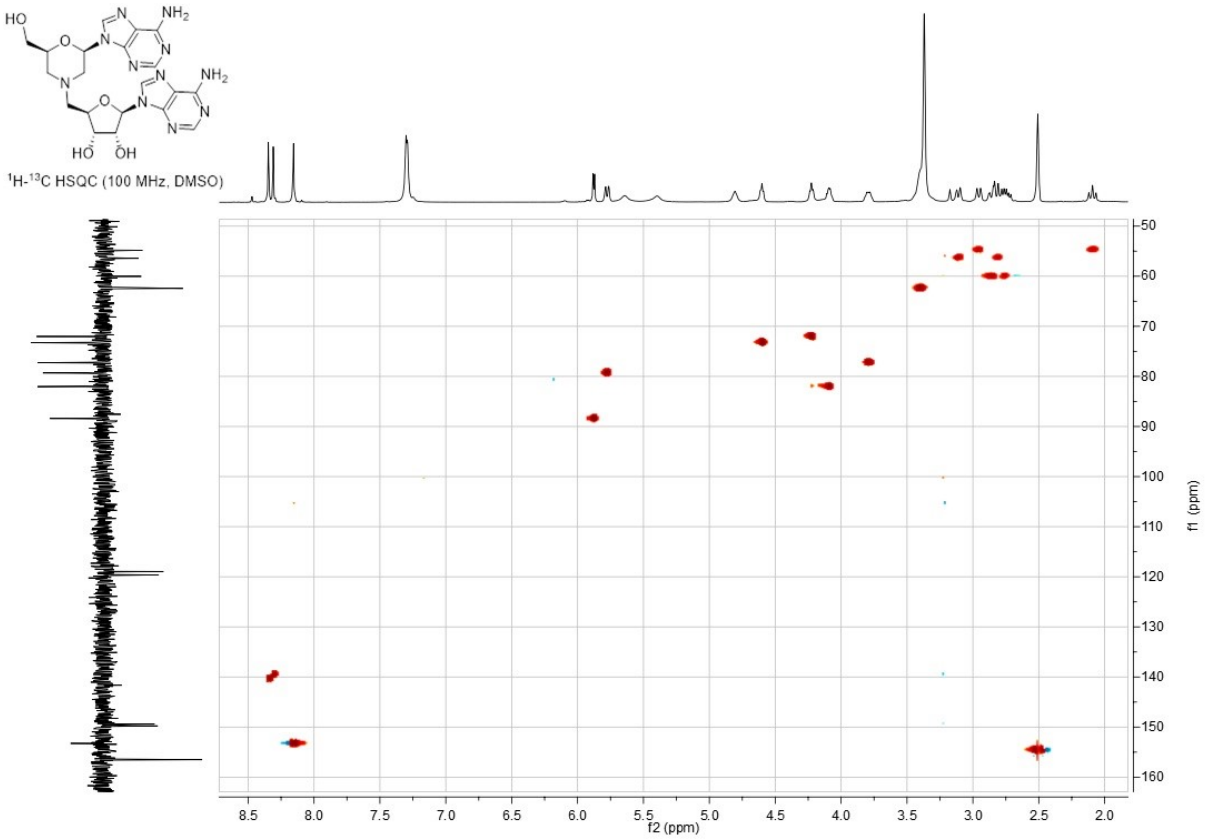

HO
O
NH
N
NH2
HO
OH
1H-13C HSQC (100 MHz, DMSO
f2 (ppm)
f1 (ppm)

NMR spectra of compound 19

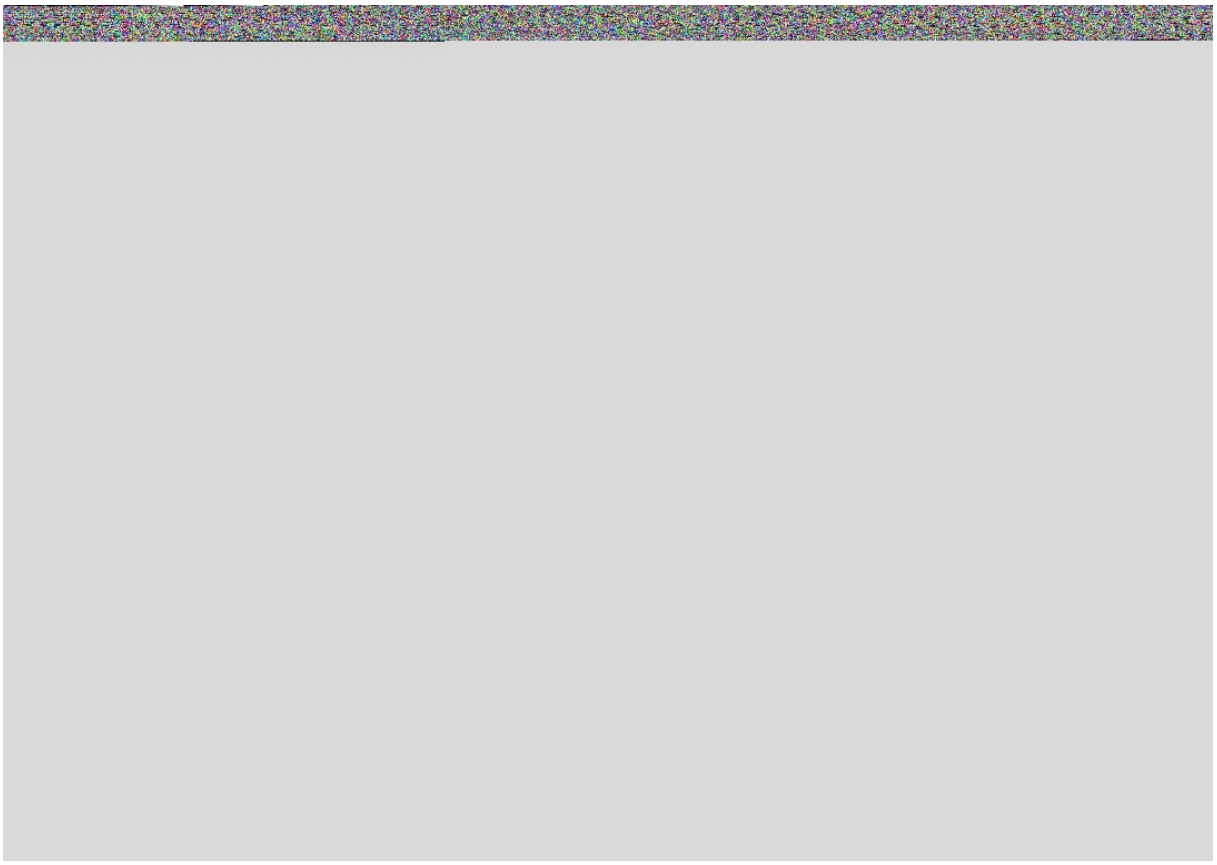


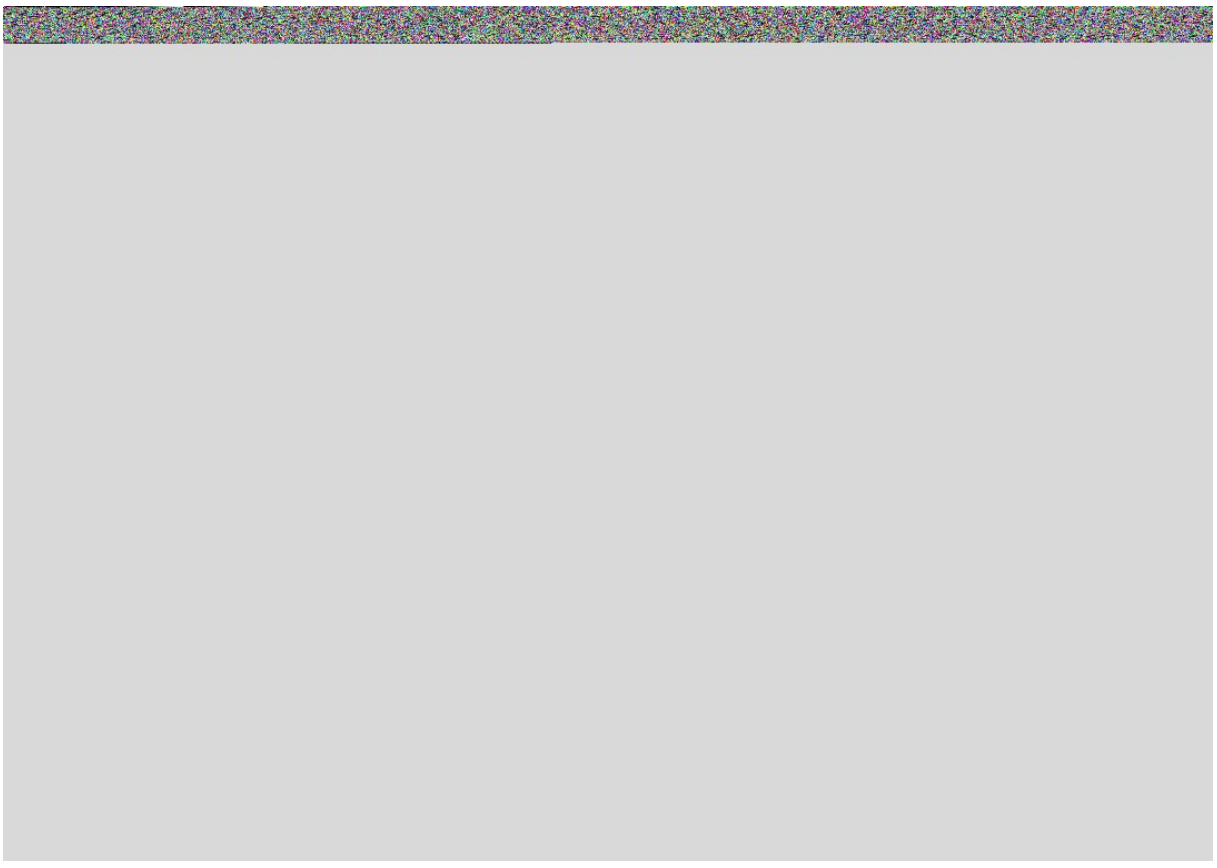



HO
NH2
NH2
HO
OH
1H-1H COSY (400 MHz, DMSO-d6)
f1 (ppm)
f2 (ppm)

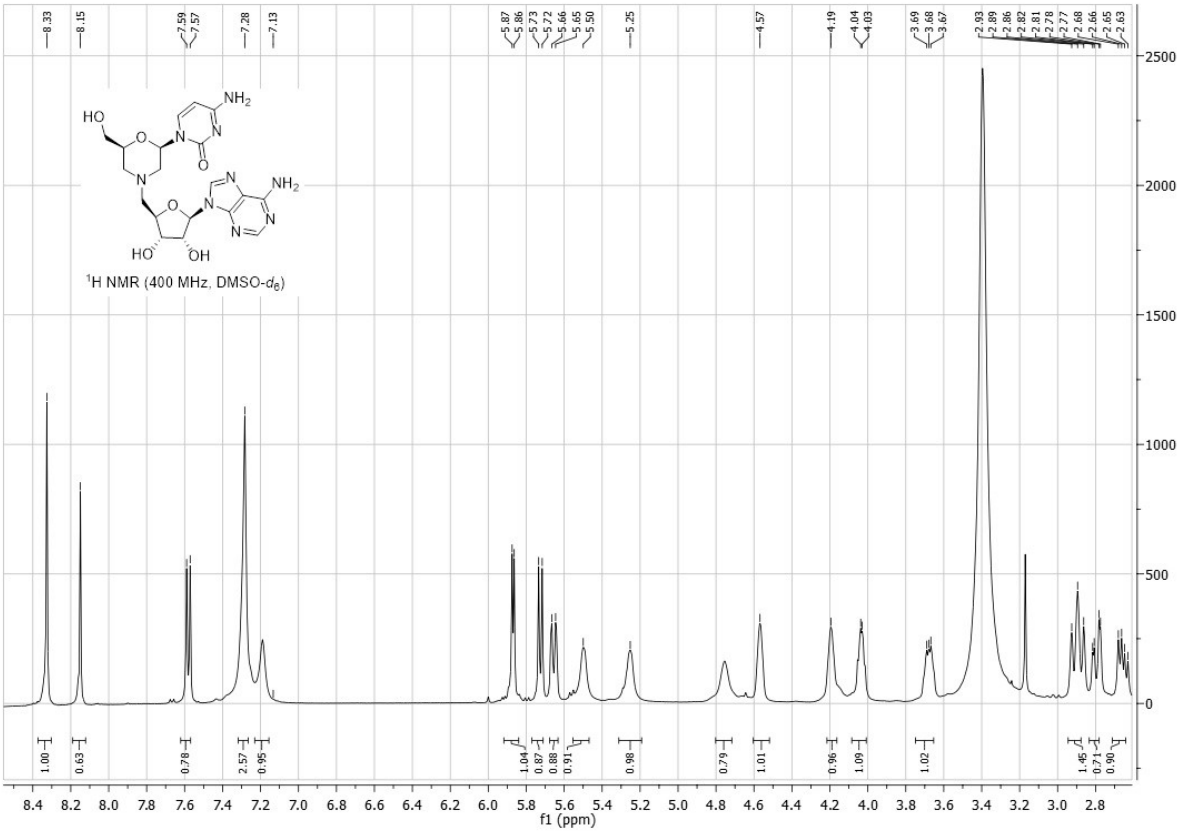

HO
NH2
NH2
HO
OH
1H-13C HSQC (100 MHz, DMSO)
f1 (ppm)
f2 (ppm)

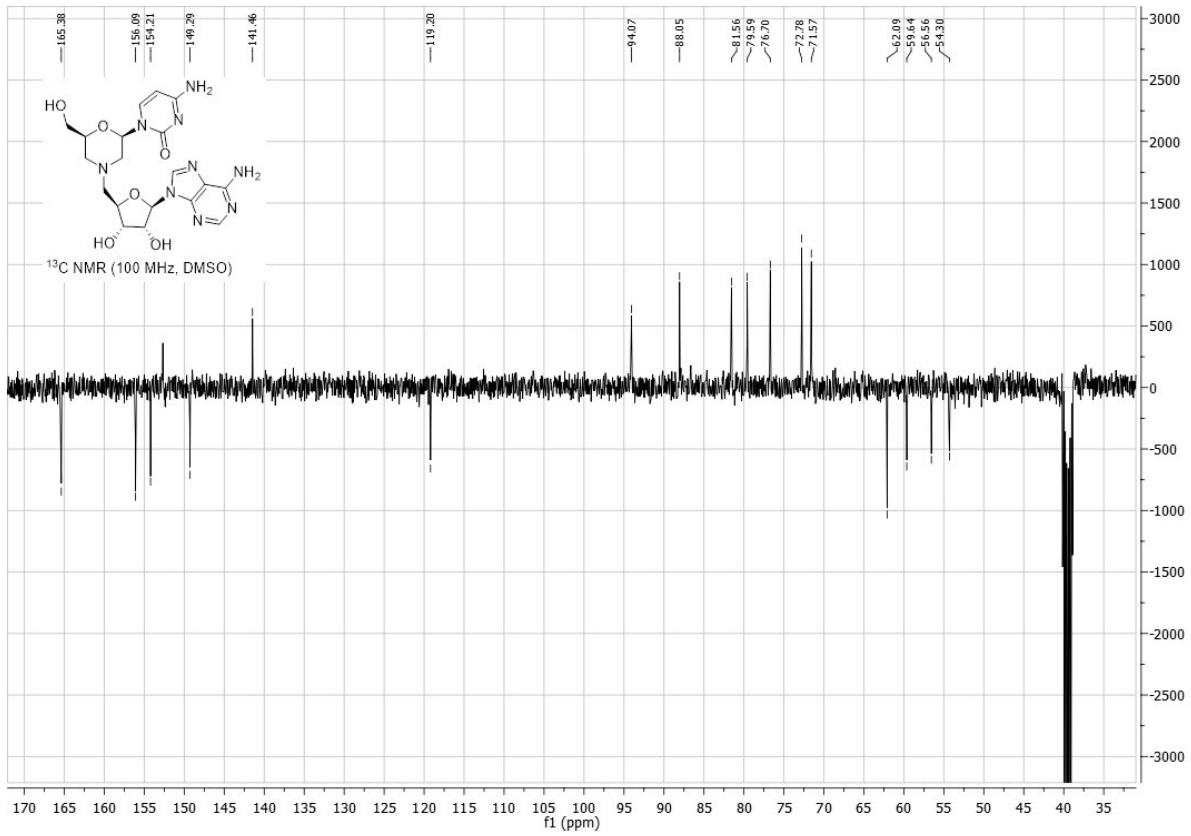
NMR spectra of compound 20

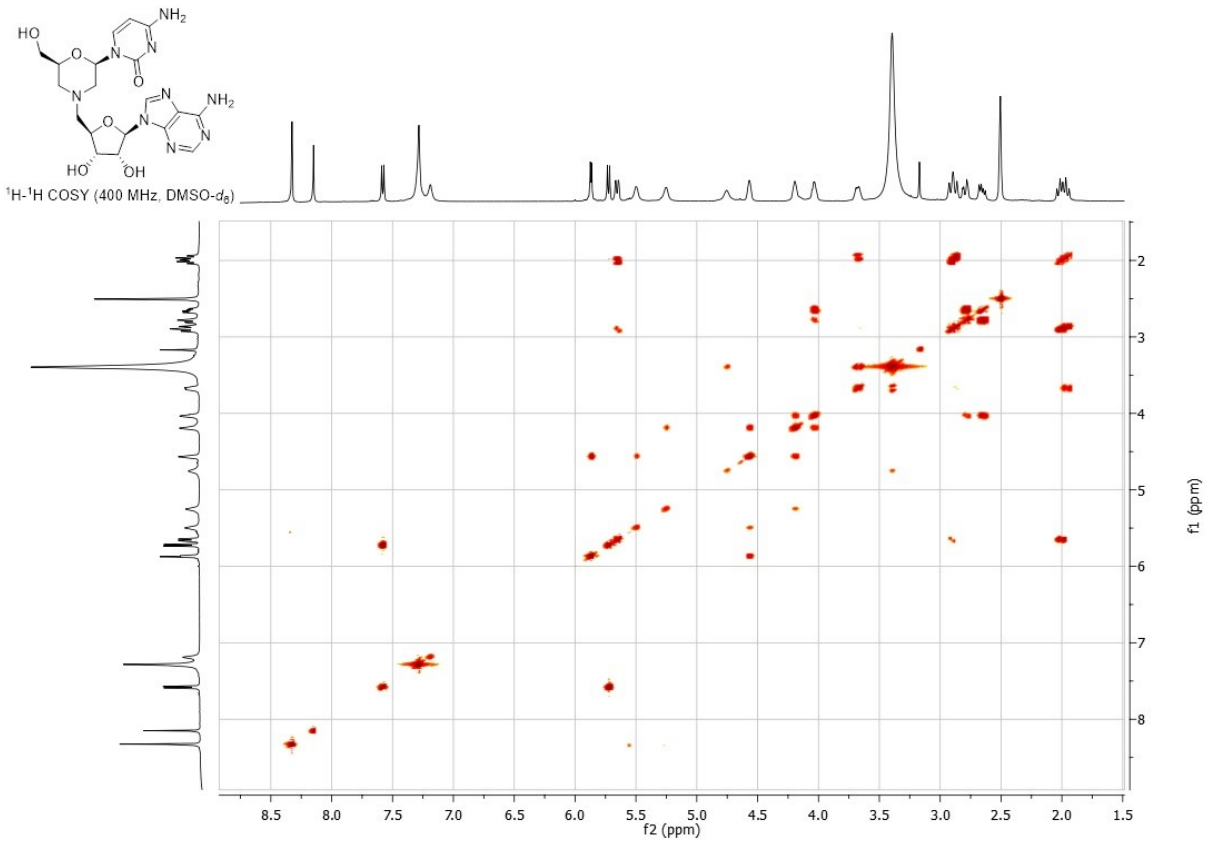


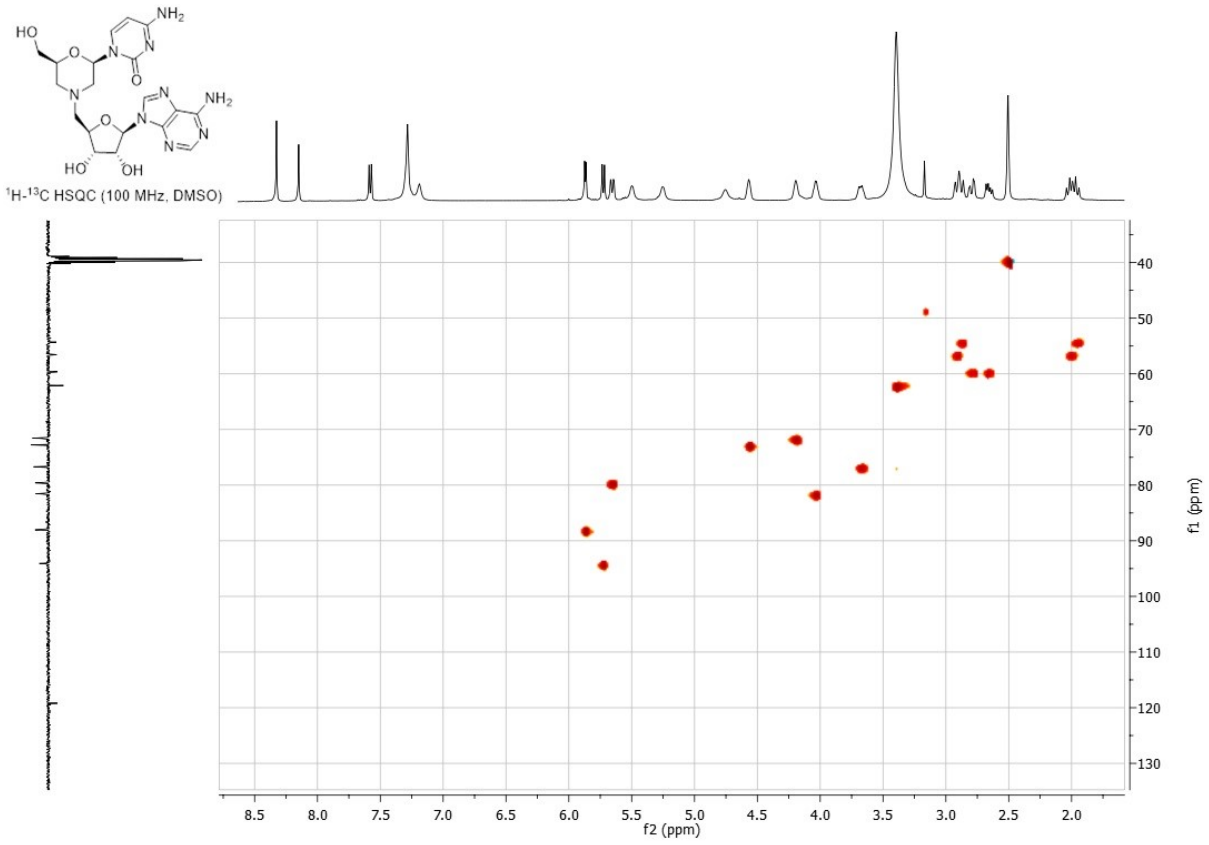


NMR spectra of compound 21

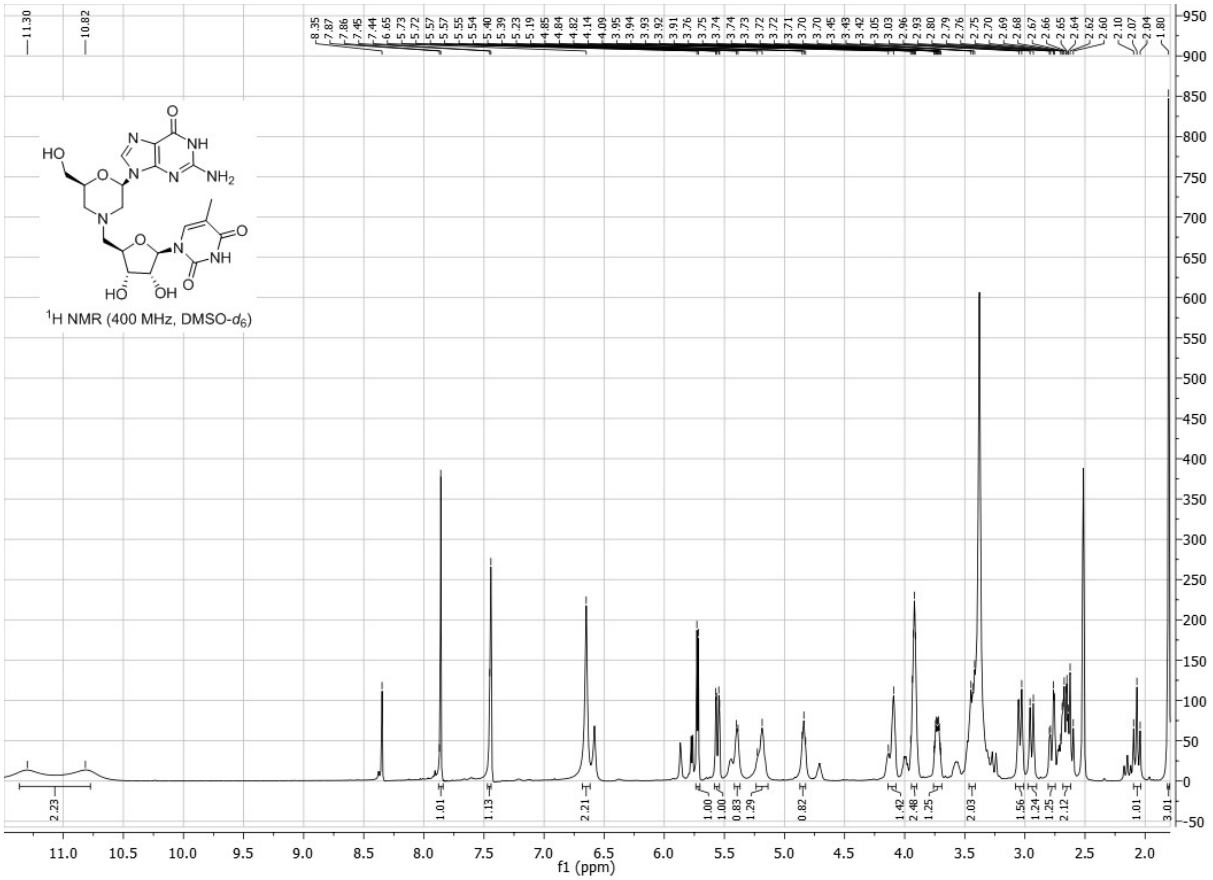


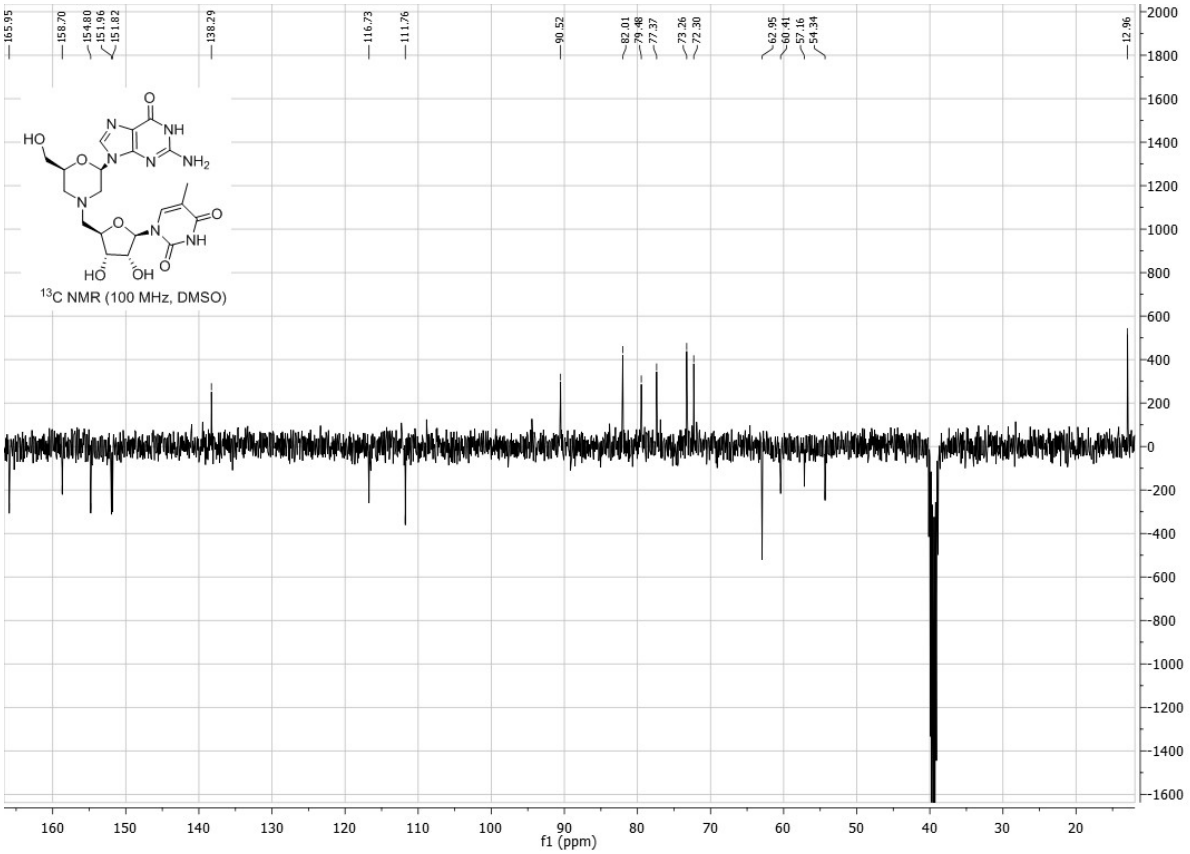


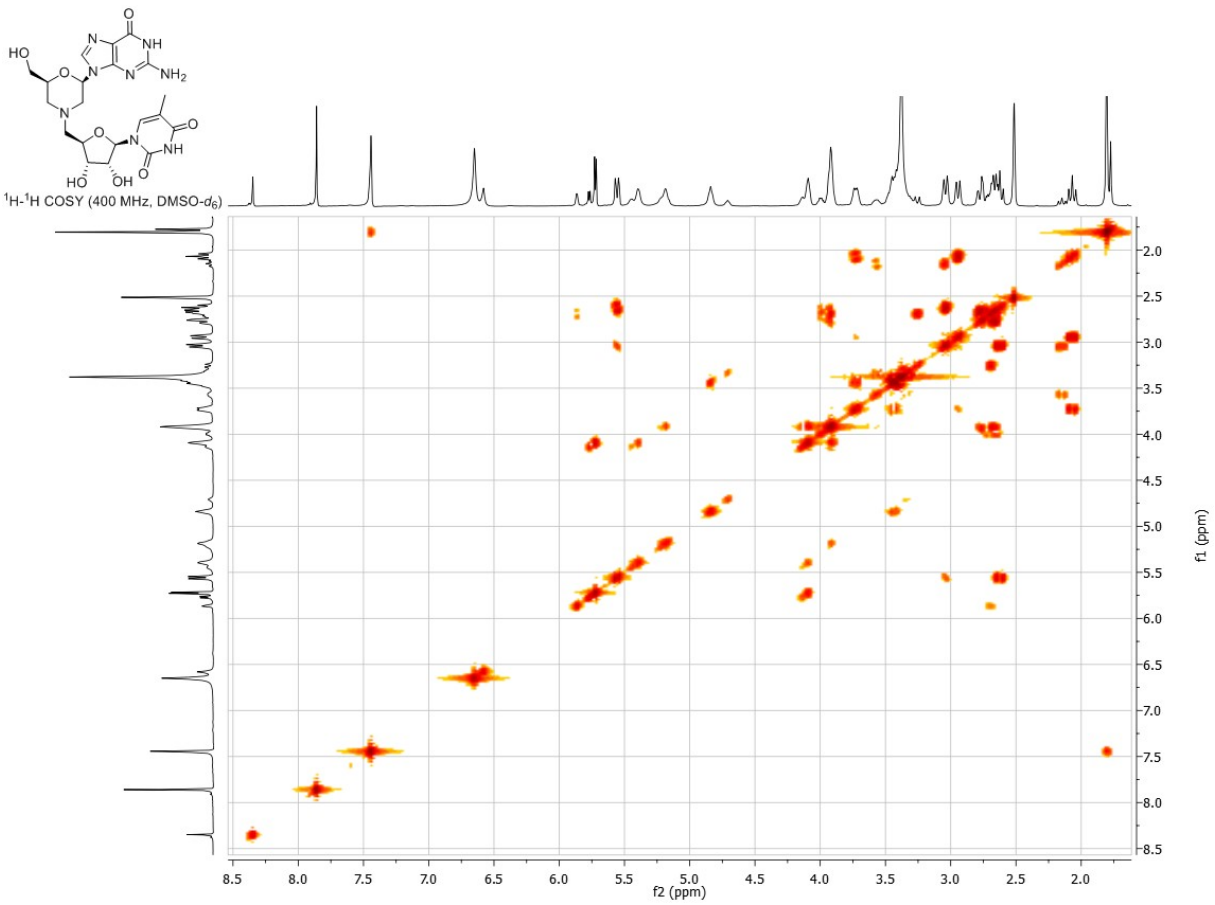

HO
NH2
NH2
HO
OH
1H-1H COSY (400 MHz, DMSO-d6)
f2 (ppm)
f1 (ppm)

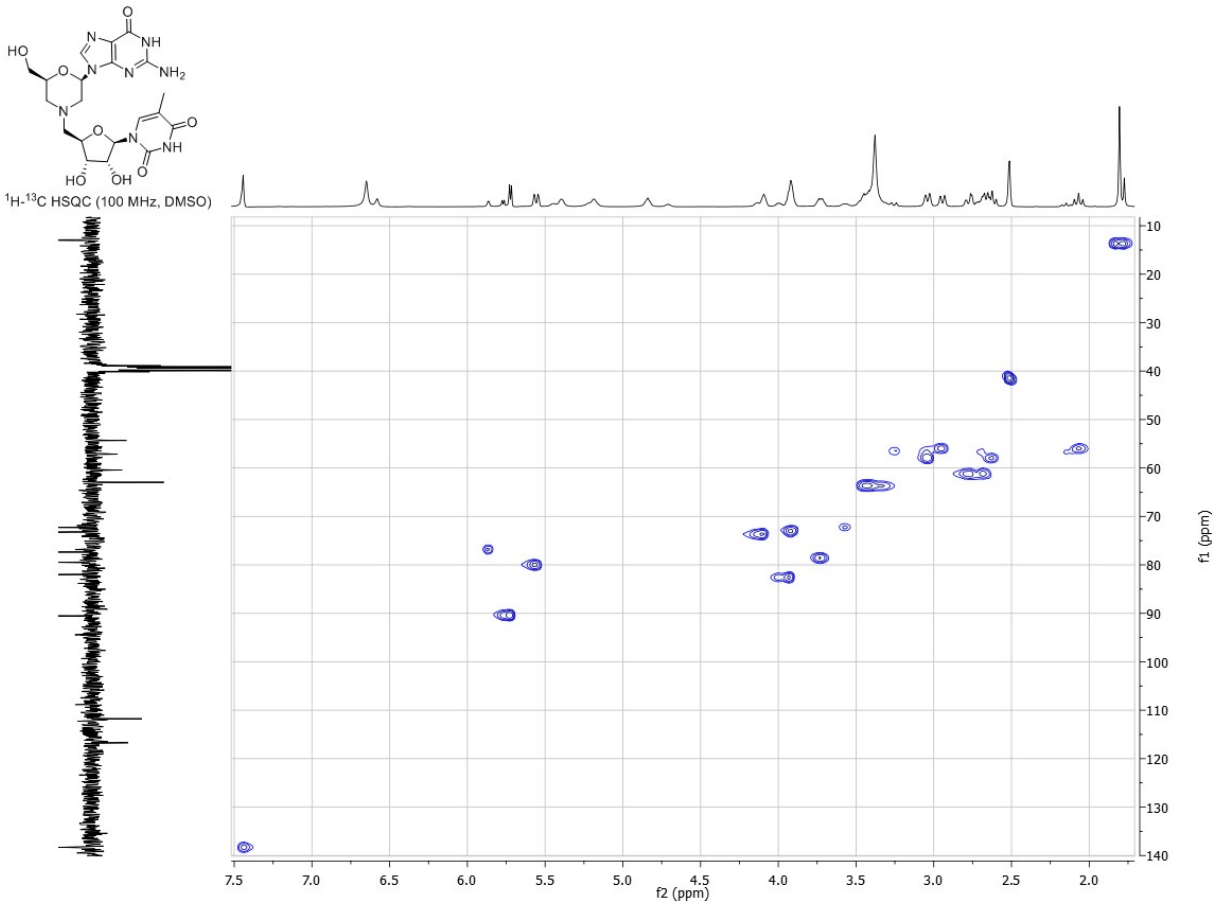

HO
NH2
NH2
HO
OH
1H-13C HSQC (100 MHz, DMSO)
f2 (ppm)
f1 (ppm)

NMR spectra of compound 22

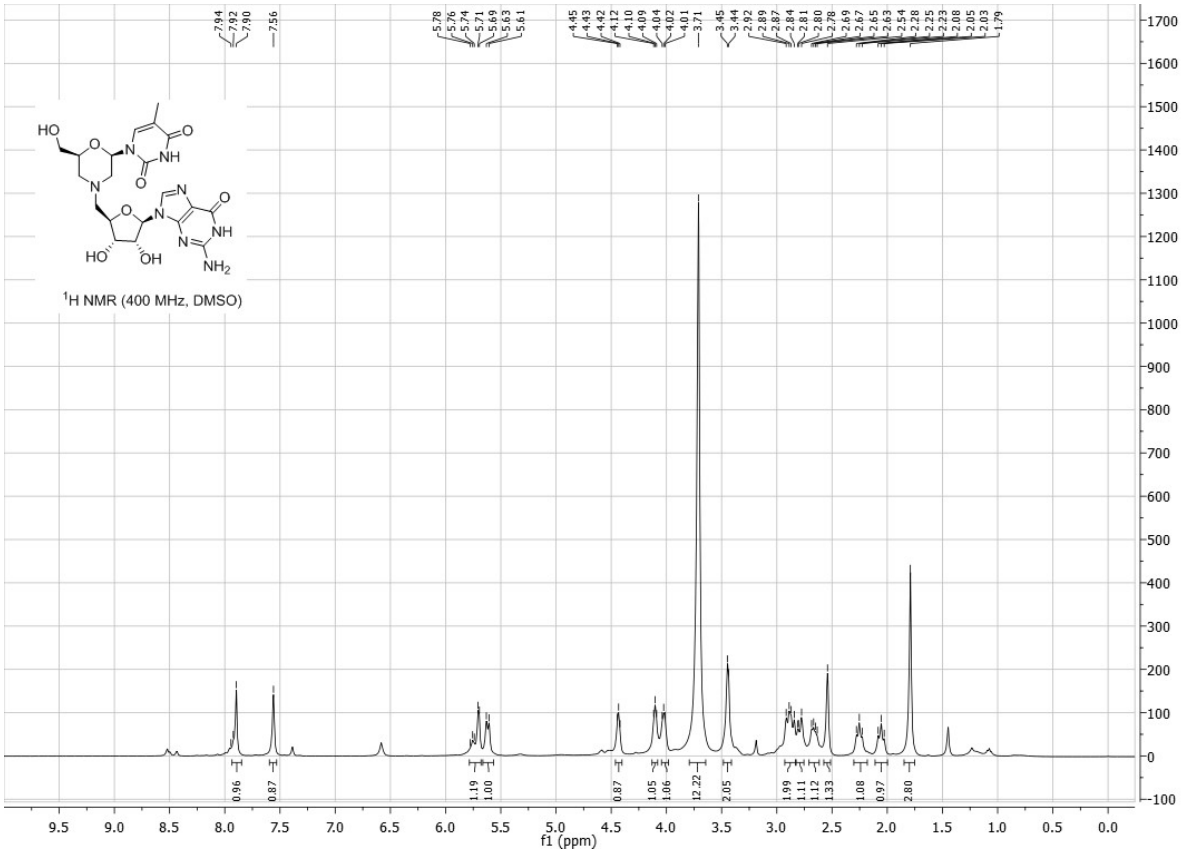


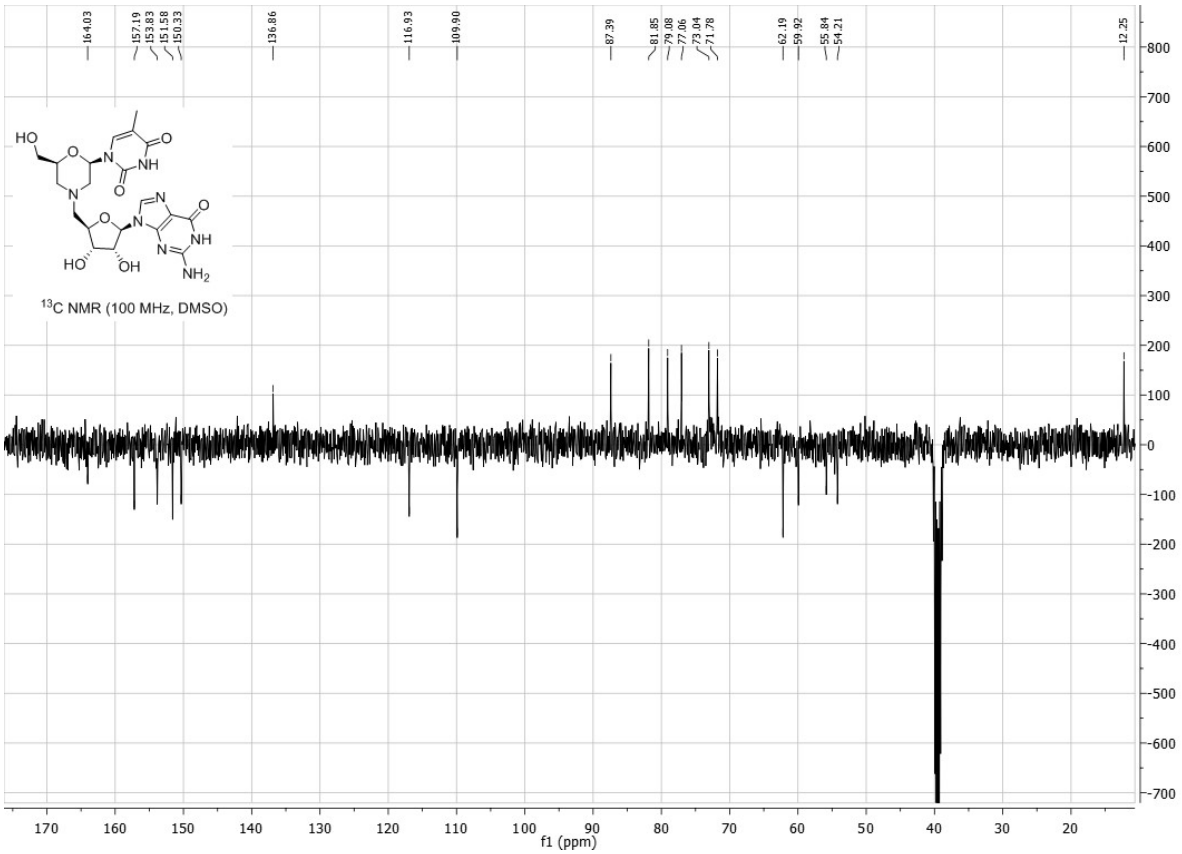


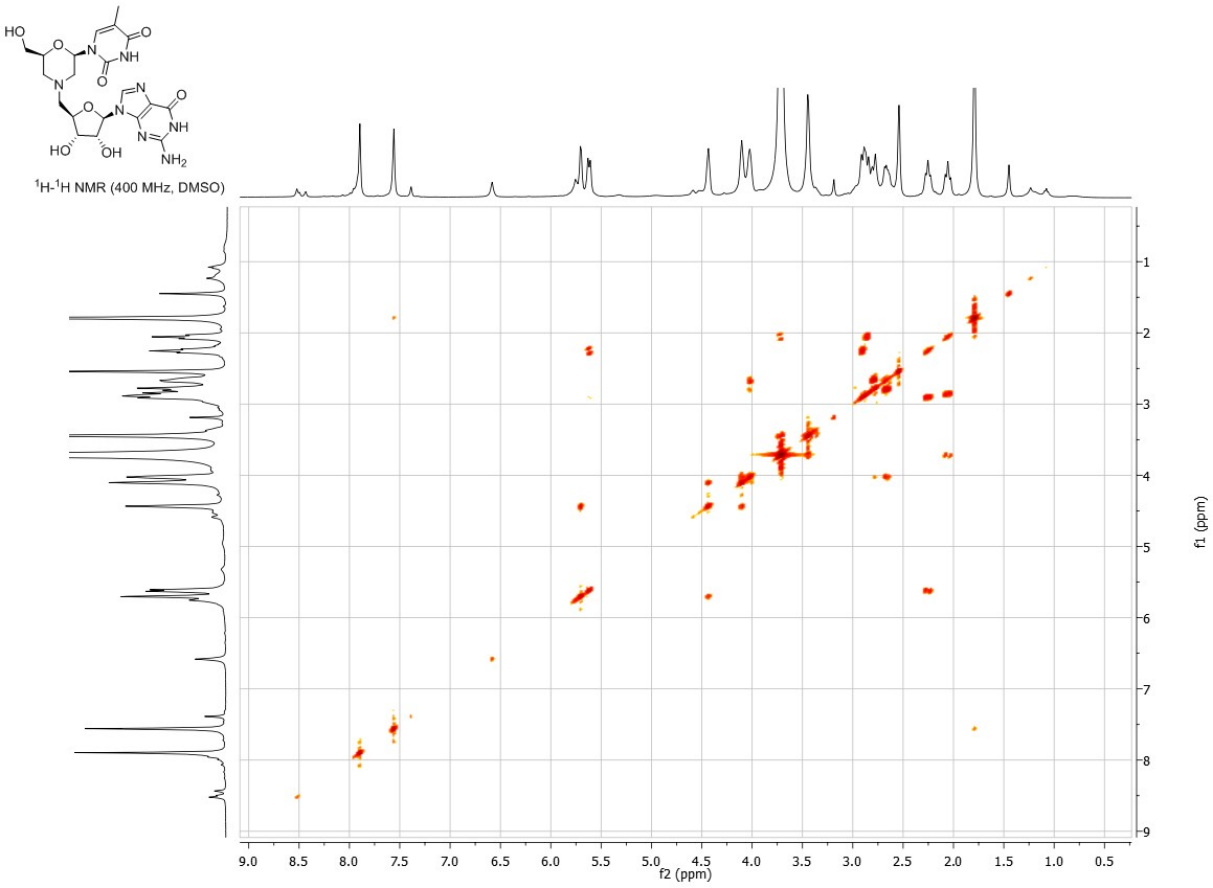

HO
O
NH
N
N
NH2
O
N
O
NH
O
HO
OH
1H-1H COSY (400 MHz, DMSO-d6)
f1 (ppm)
f2 (ppm)

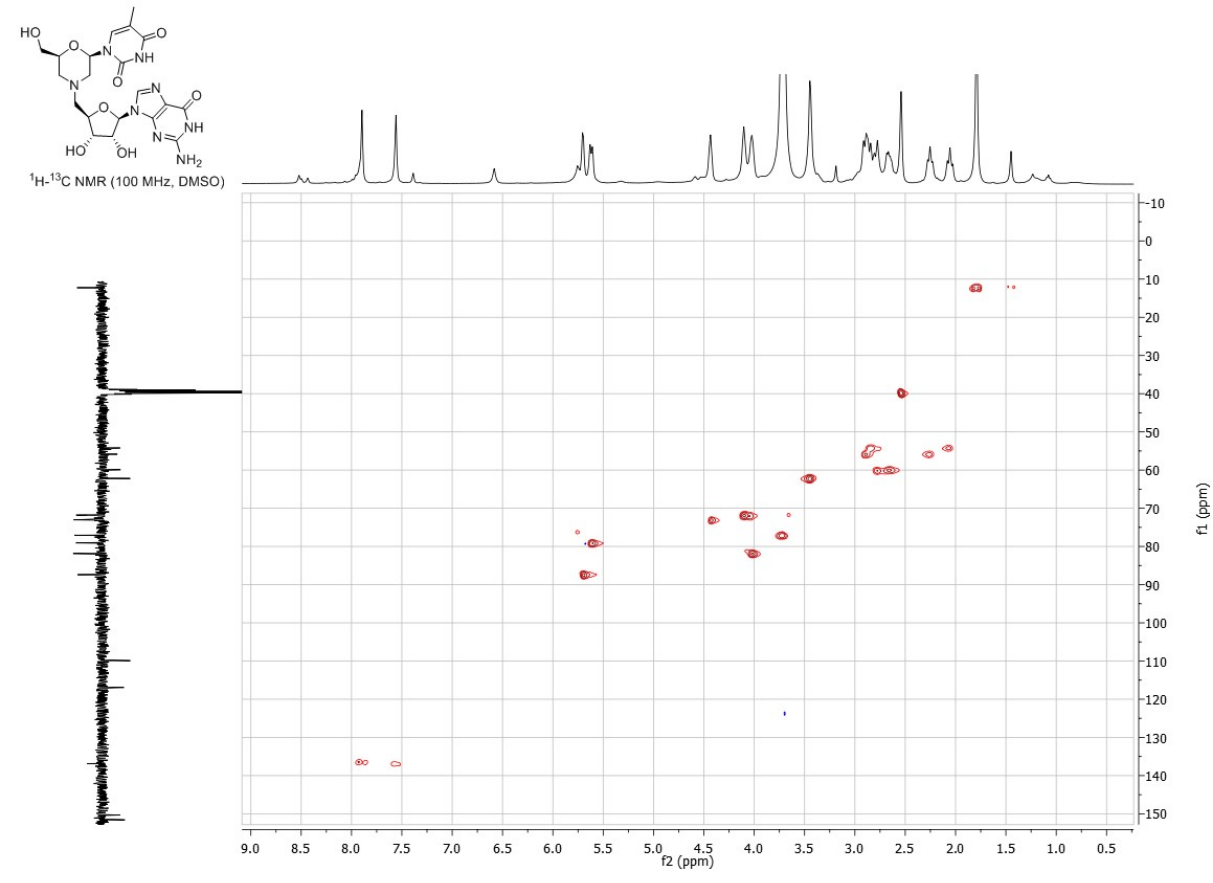

HO
O
NH
N
N
NH2
O
N
O
NH
O
HO
OH
1H-13C HSQC (100 MHz, DMSO)
f1 (ppm)
f2 (ppm)

NMR spectra of compound 24

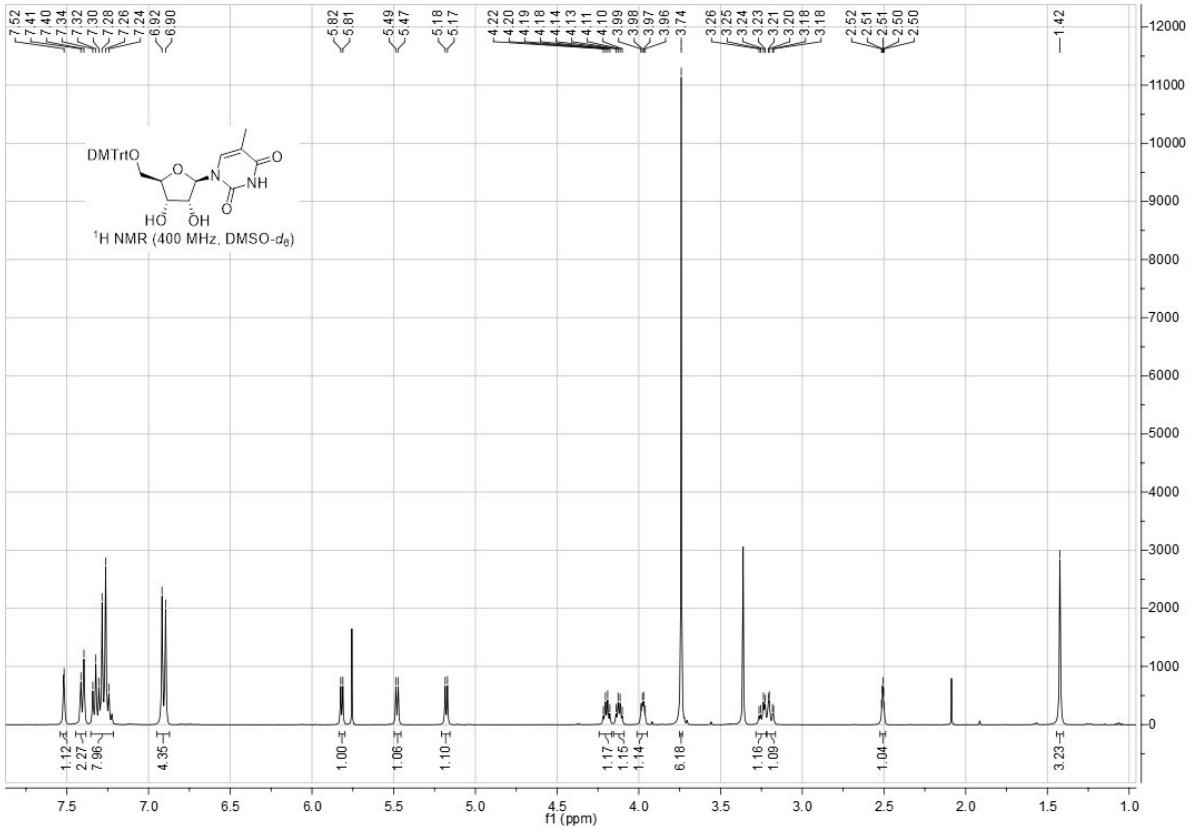


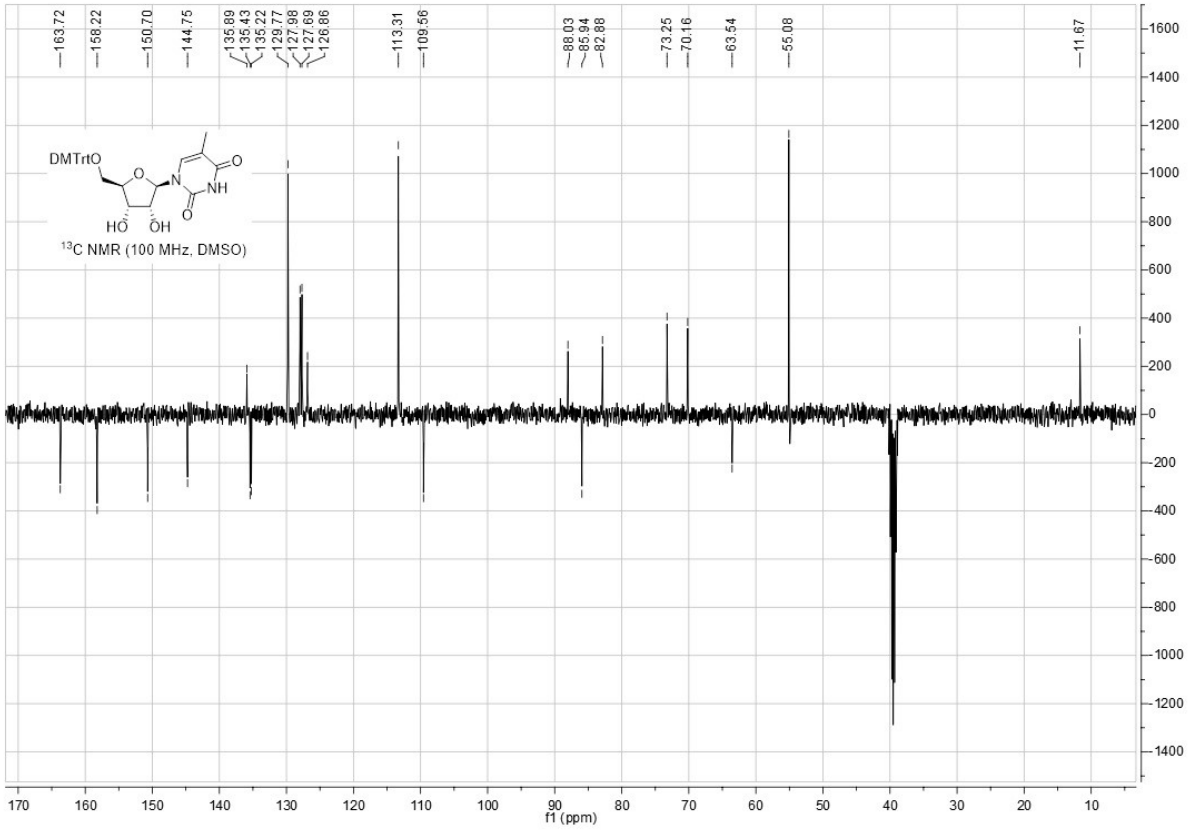


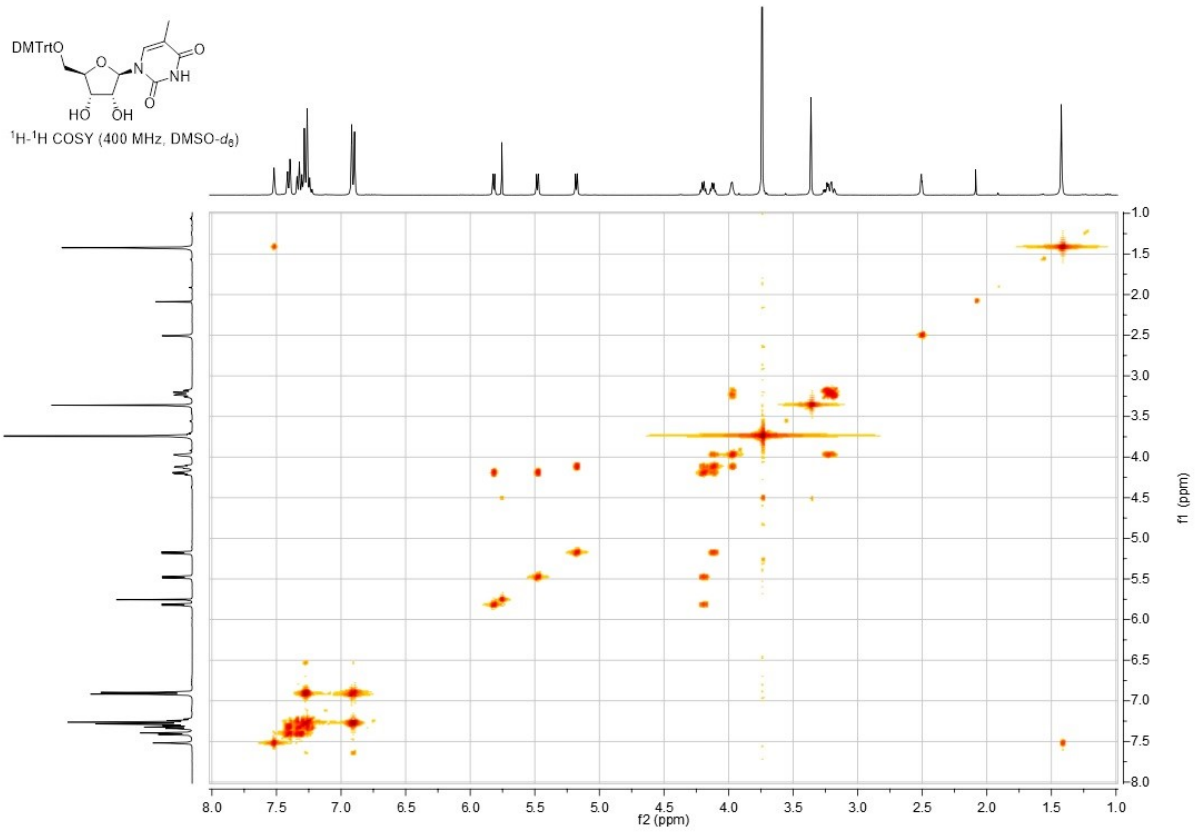

HO
O
NH
N
O
HO
OH
NH
NH2
1H-1H NMR (400 MHz, DMSO)
f2 (ppm)
f1 (ppm)


HO
O
NH
N
O
HO
OH
NH
NH2
1H-13C NMR (100 MHz, DMSO)
f2 (ppm)
f1 (ppm)

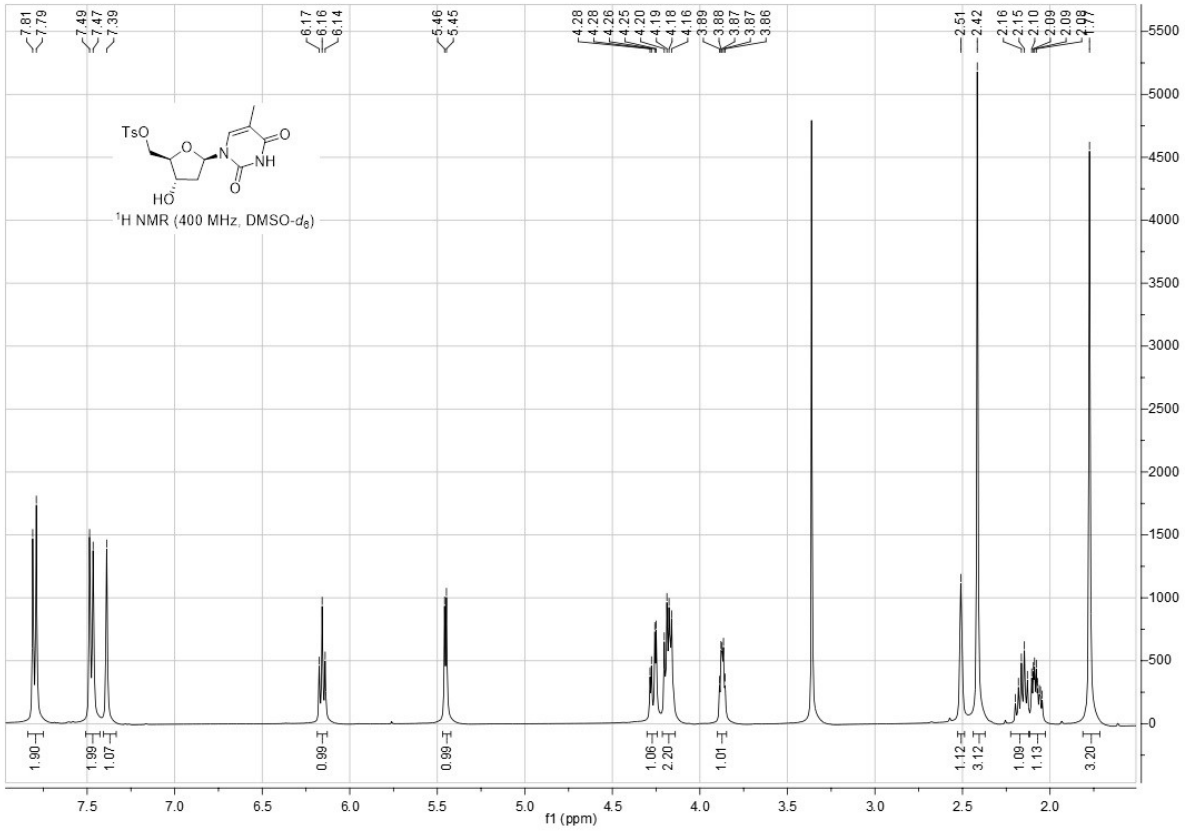
NMR spectra of compound 25

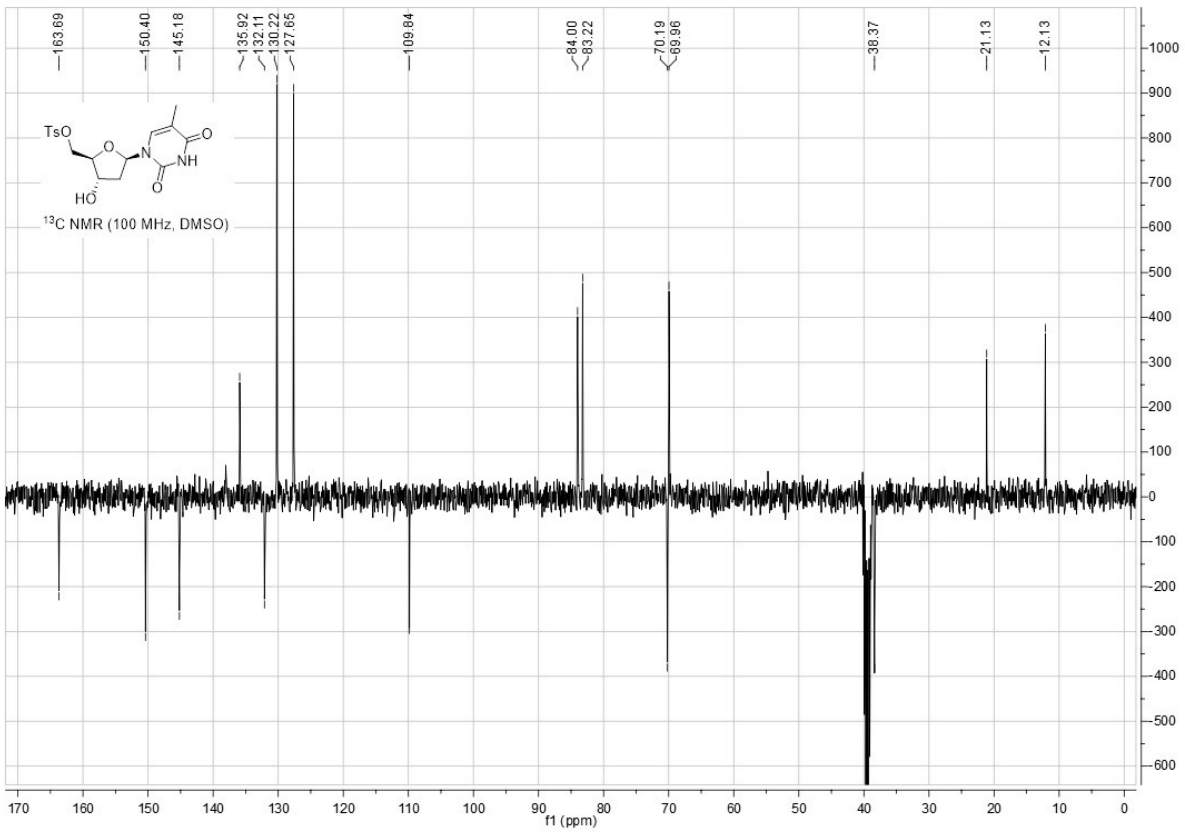




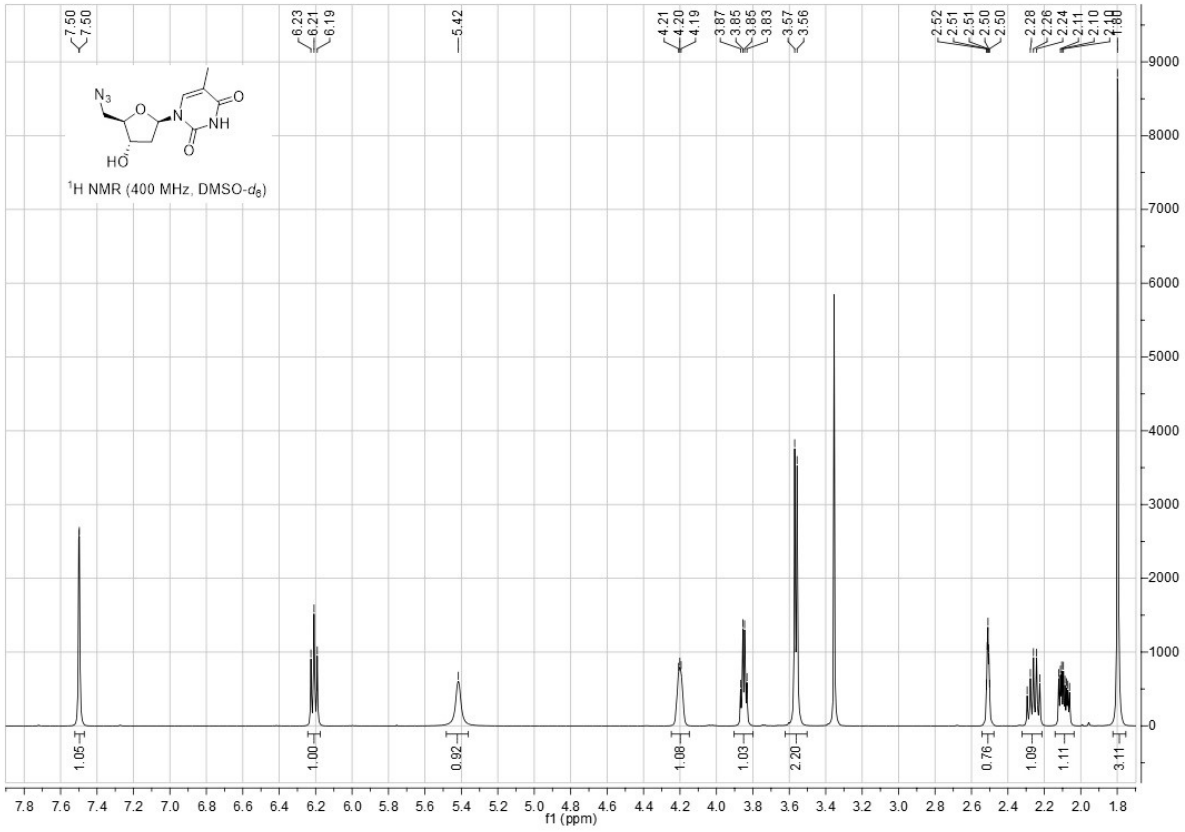

DMTrtO
O
NH
O
HO
OH
^{1}H-^{1}H COSY (400 MHz, DMSO-d_6)
f2 (ppm)
f1 (ppm)

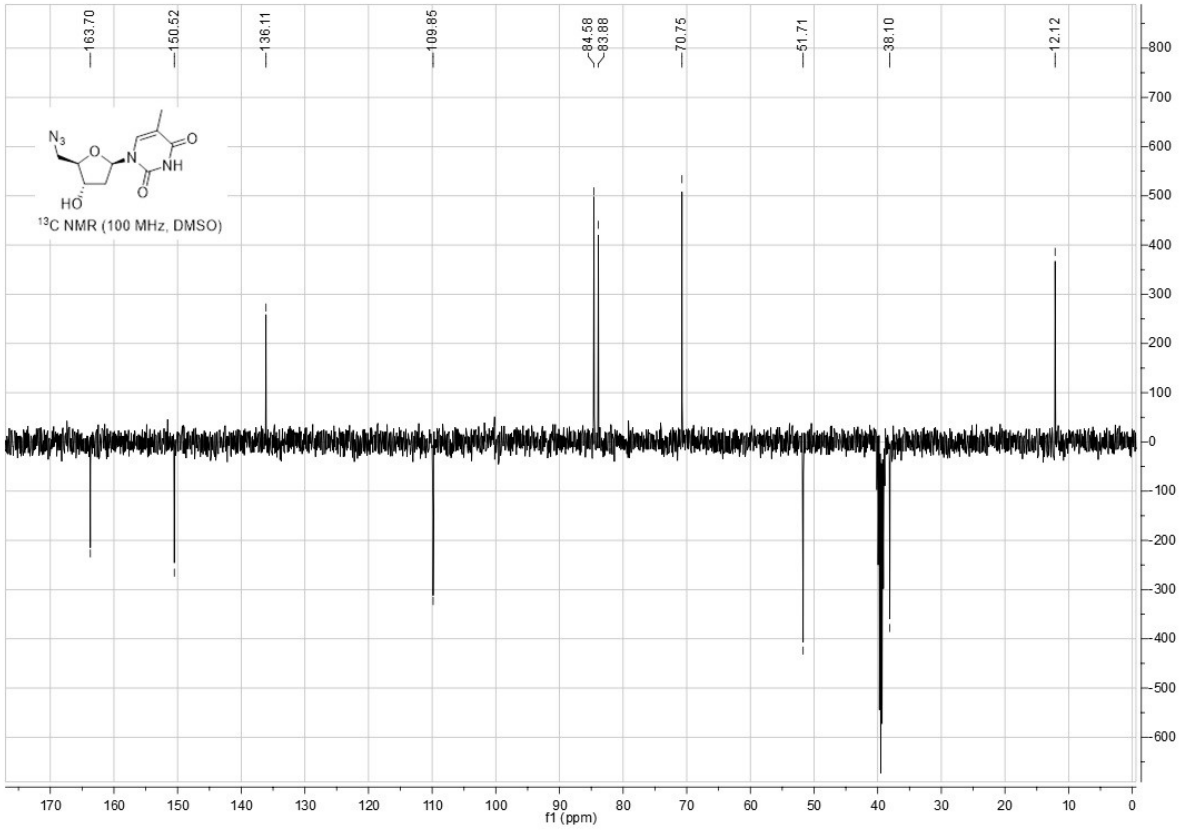
NMR spectra of compound 27



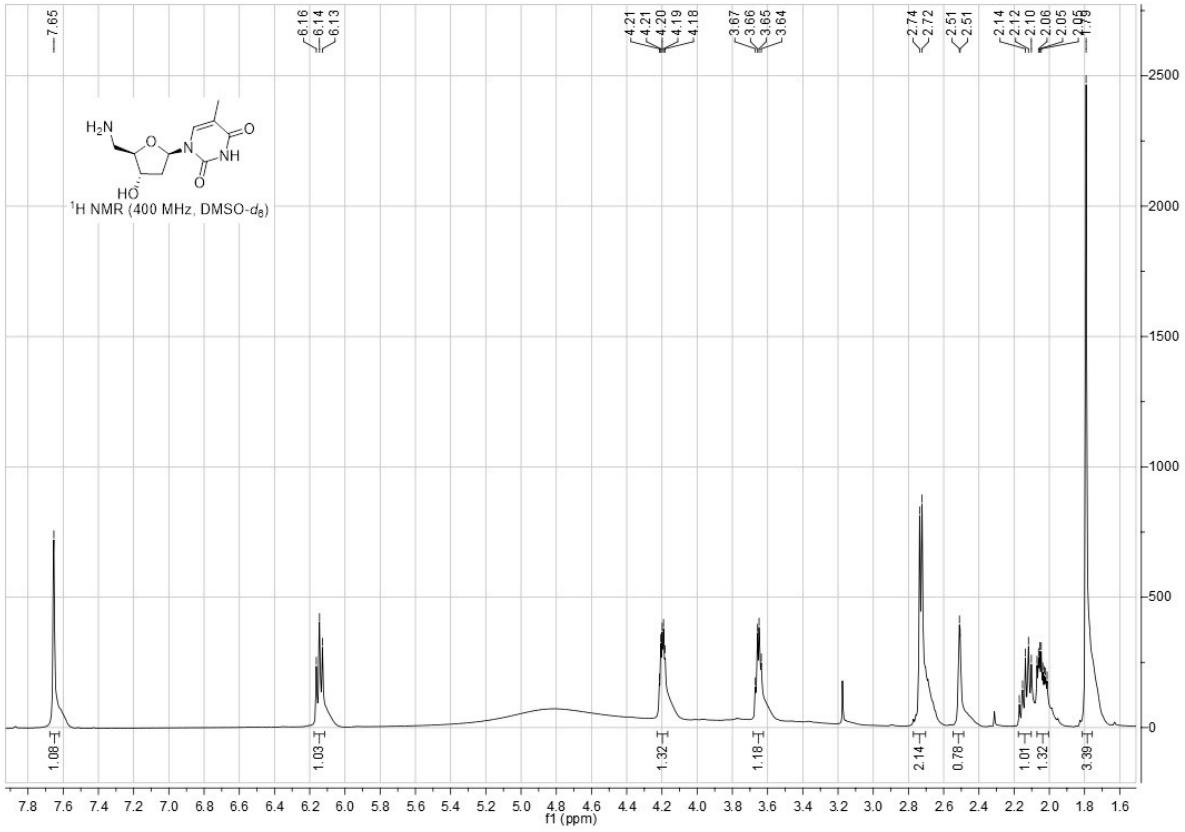


NMR spectra of compound 28

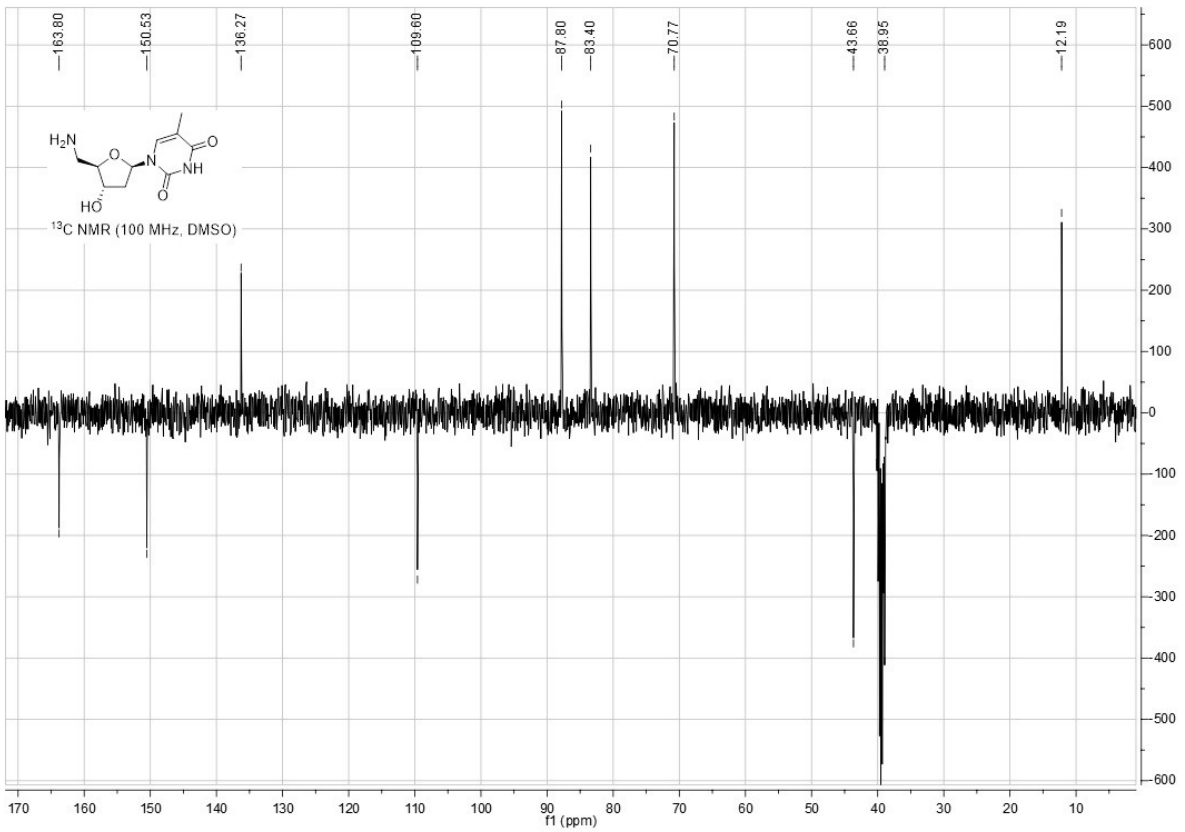




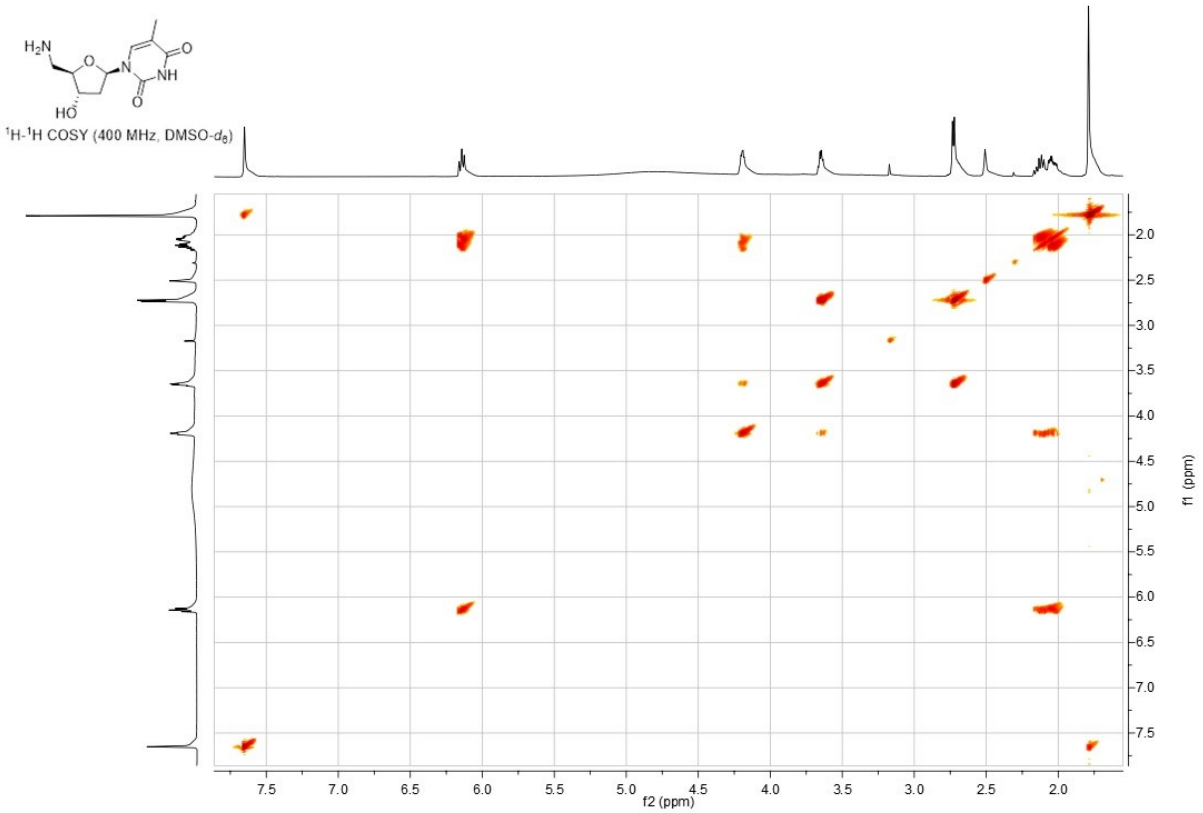
NMR spectra of compound 29



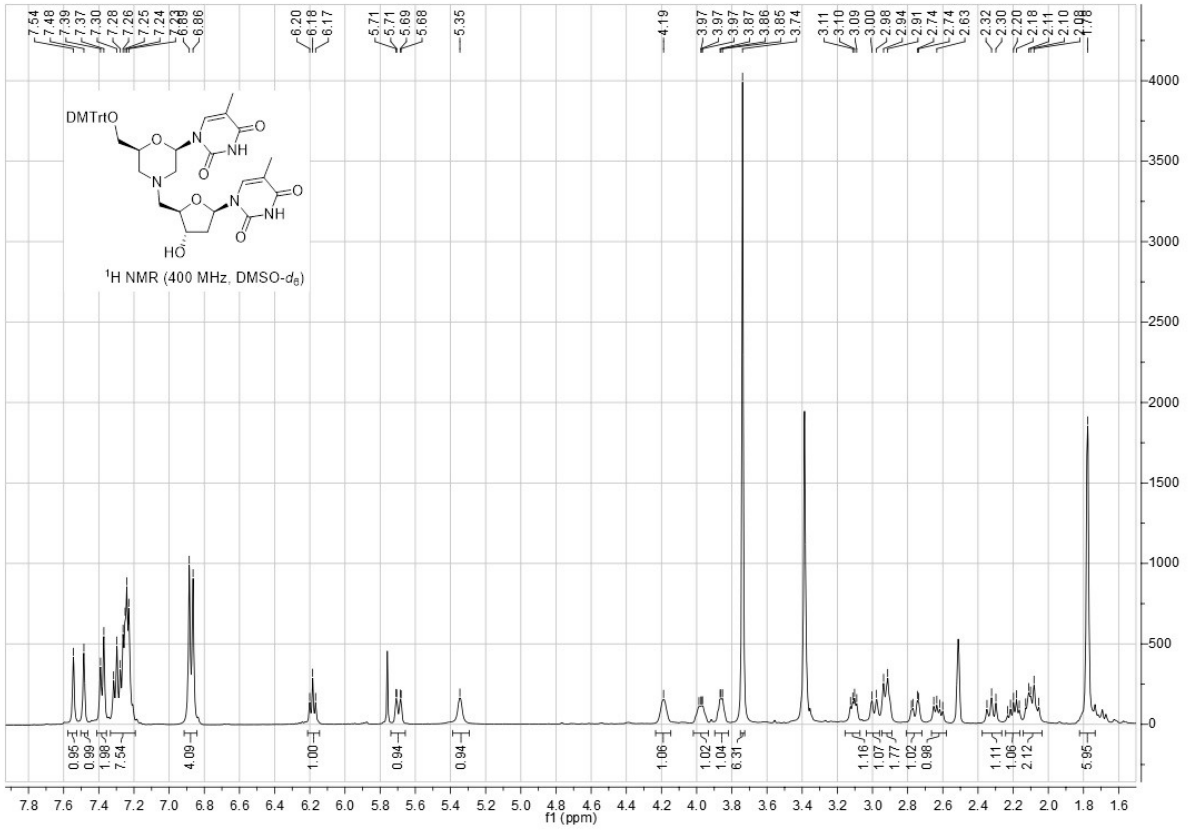
^{1}H NMR (400 MHz, DMSO-d_6)

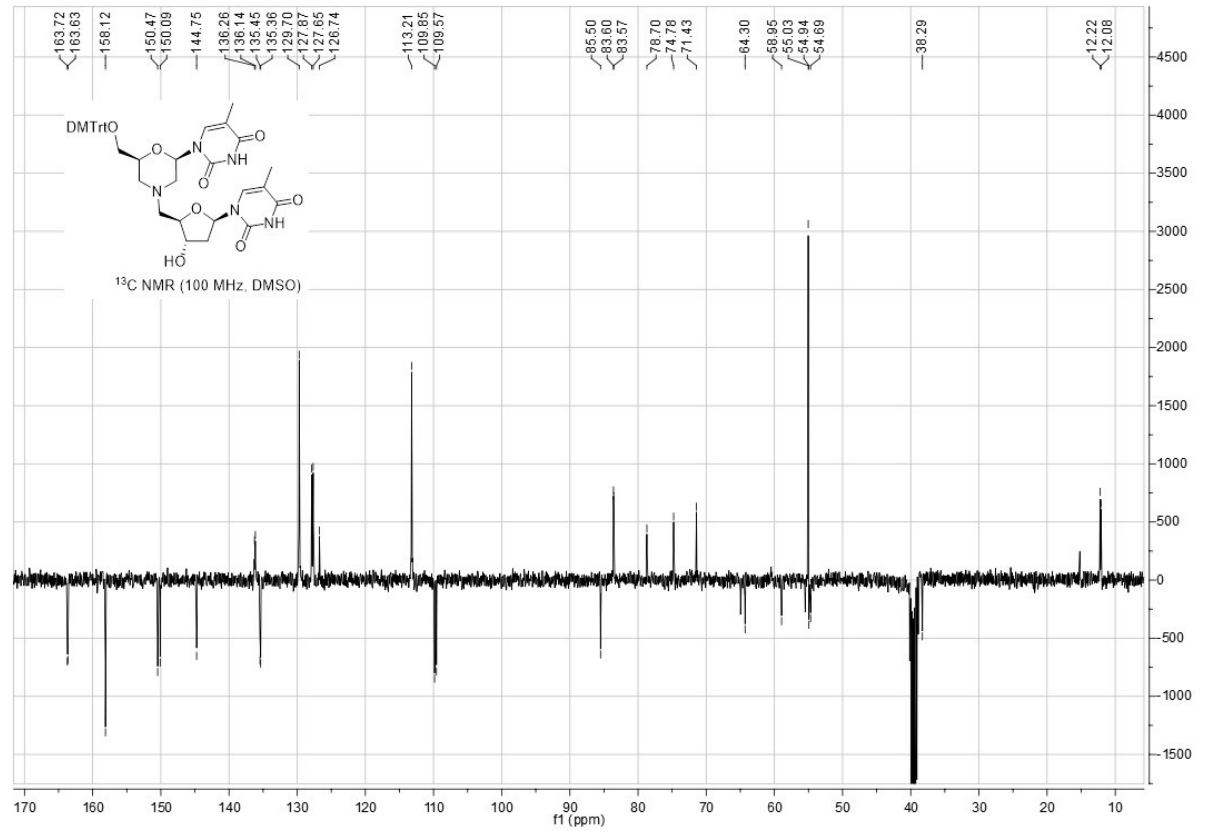


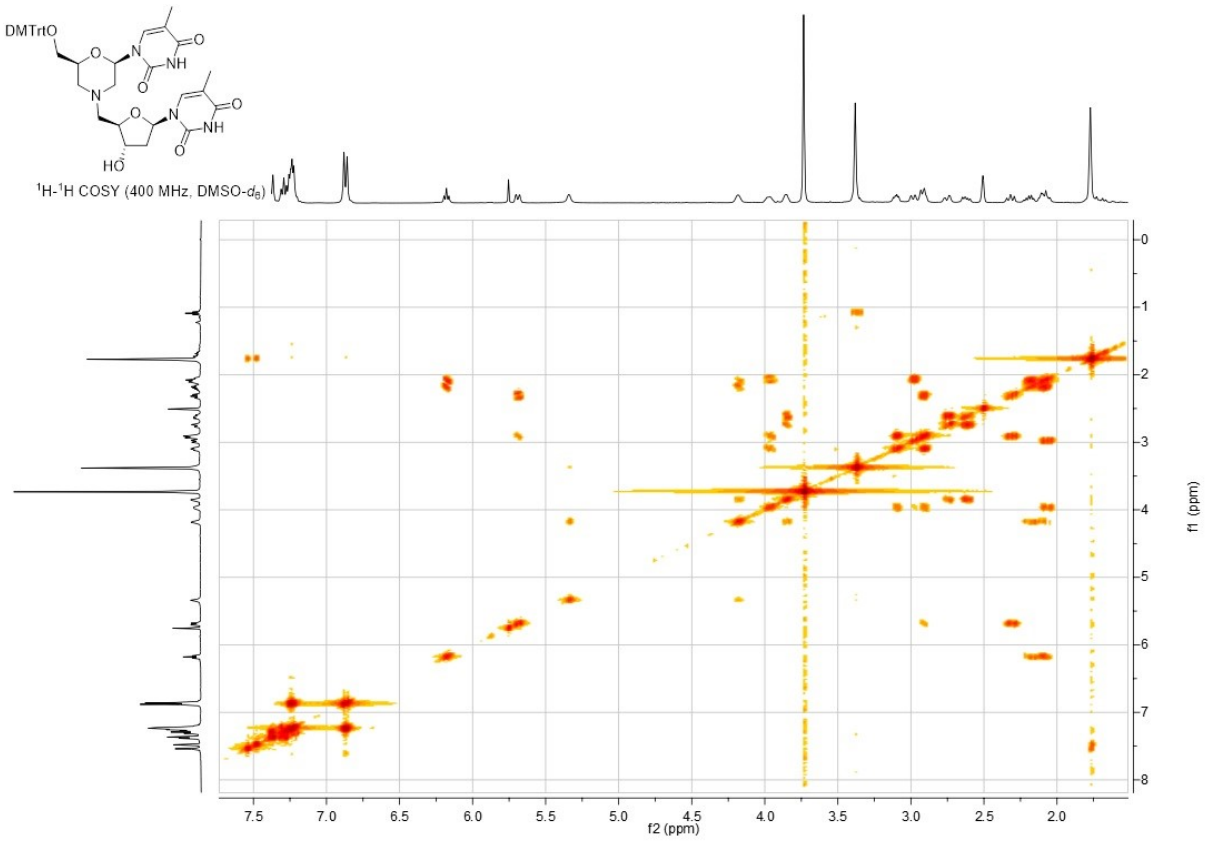
^{13}C NMR (100 MHz, DMSO)

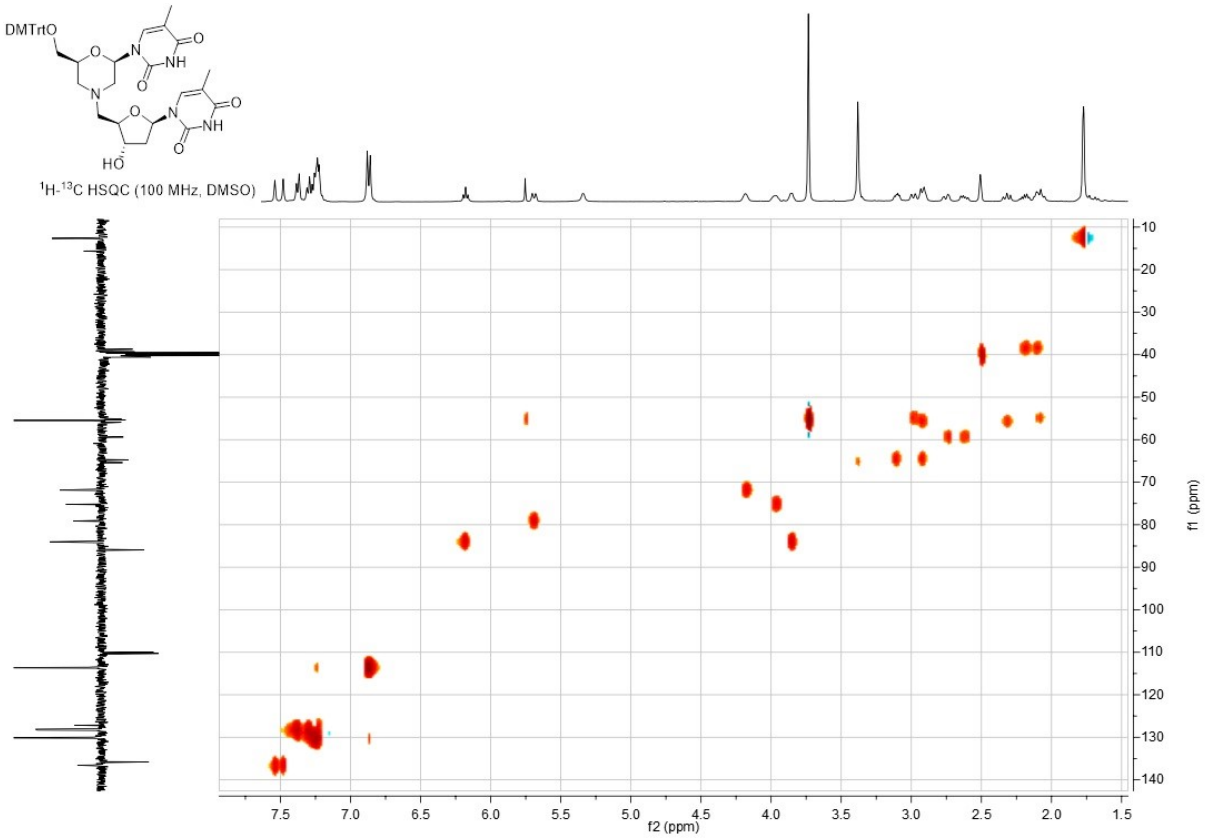


NMR spectra of compound 30

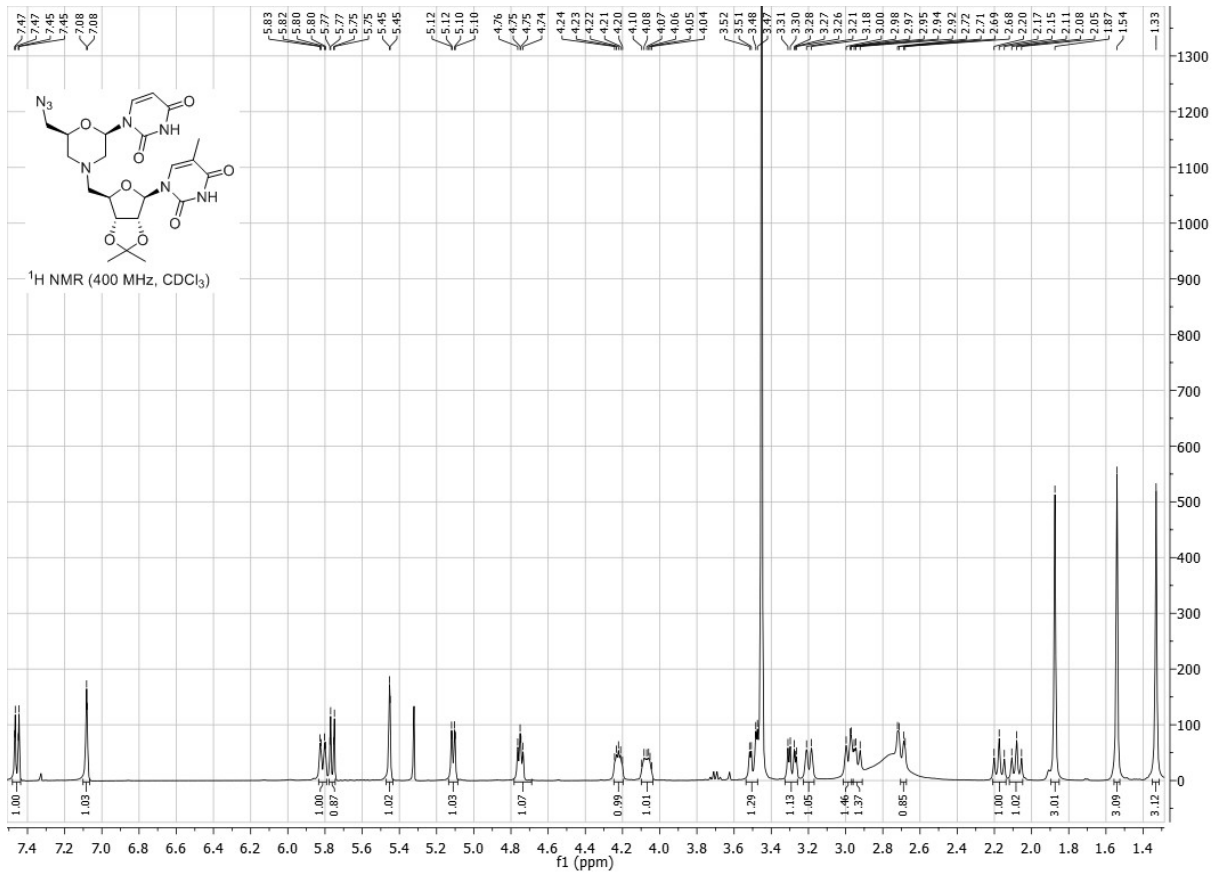


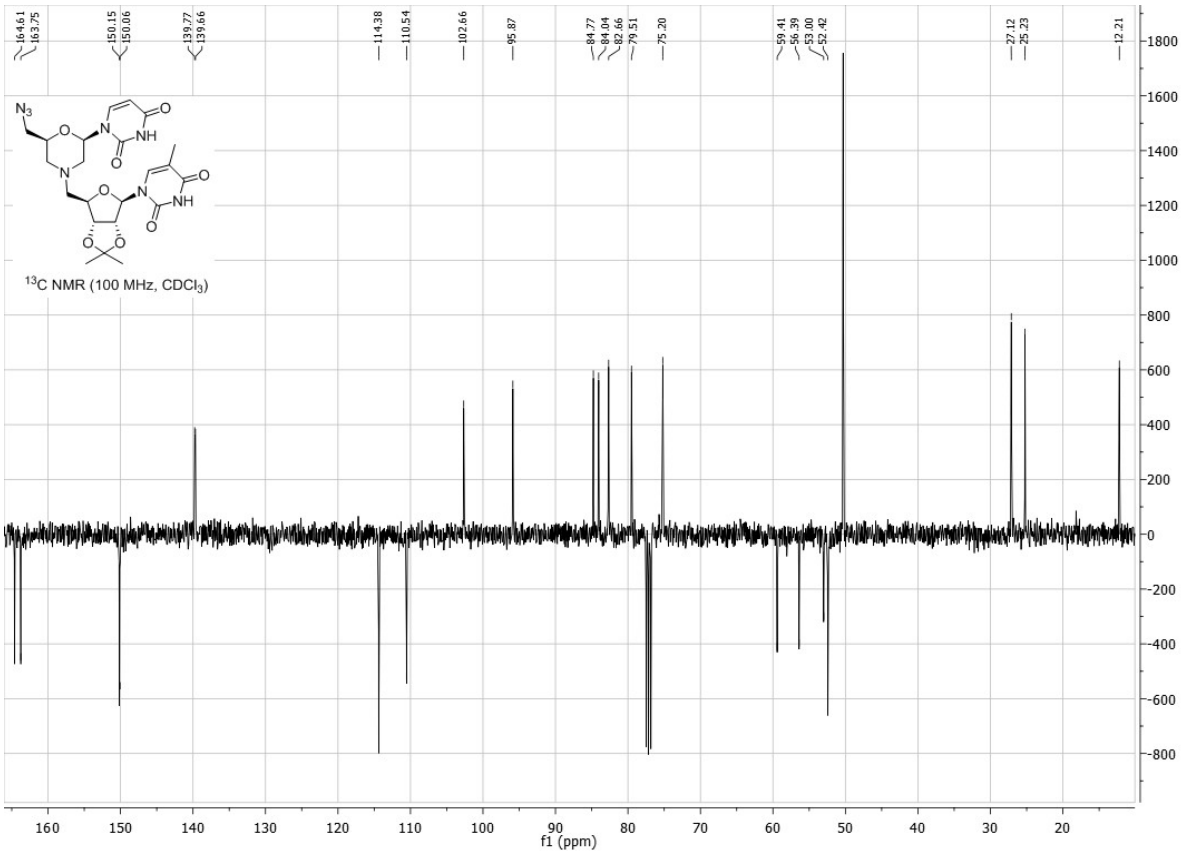

163.72
163.63
158.12
150.47
150.09
144.75
136.26
136.14
135.45
135.36
129.70
127.87
127.65
126.74
113.21
109.85
109.57
85.50
83.60
83.57
78.70
74.78
71.43
64.30
58.95
55.03
54.94
54.69
38.29
12.22
12.08
DMTrtO
NH
HO
13C NMR (100 MHz, DMSO)
f1 (ppm)

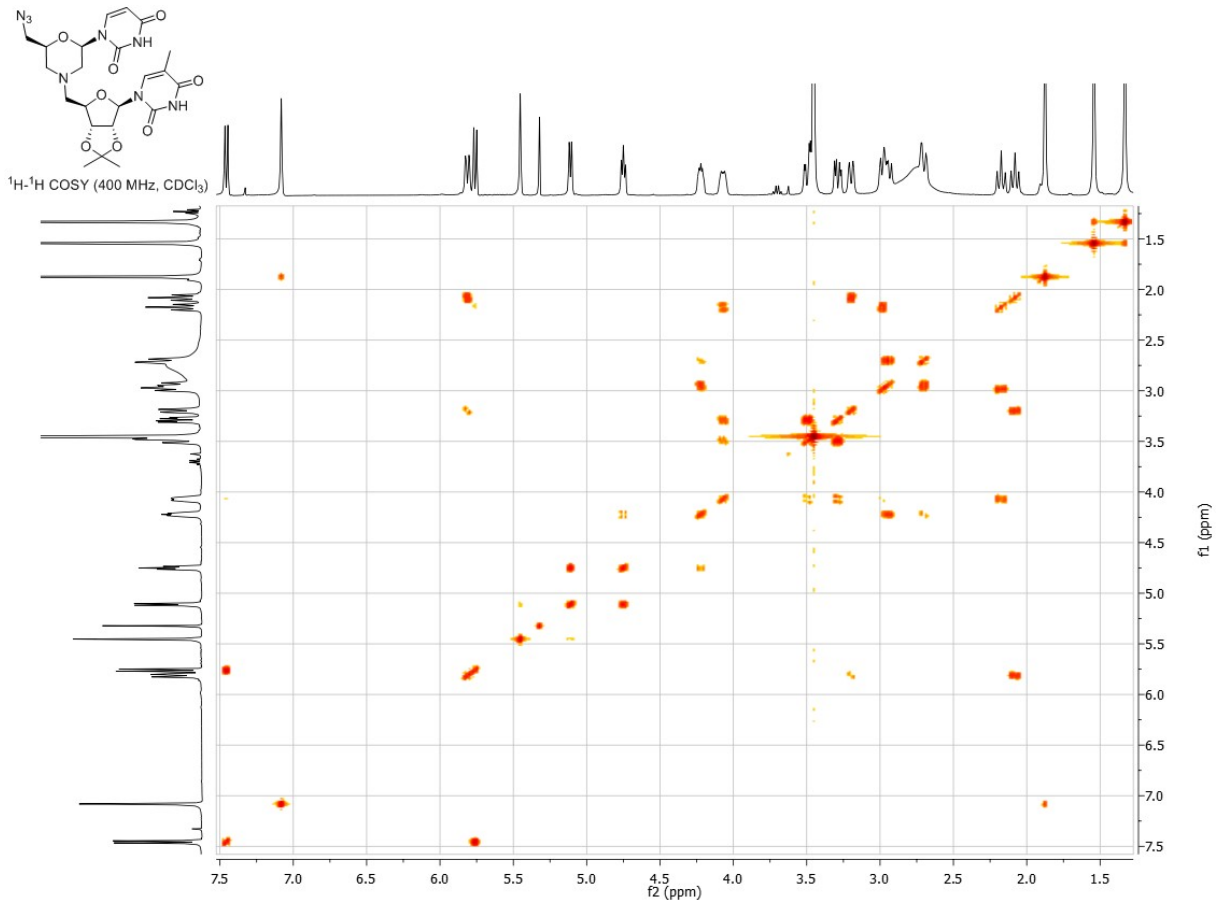

DMTrtO
NH
HO
1H-1H COSY (400 MHz, DMSO-d6)
f2 (ppm)
f1 (ppm)

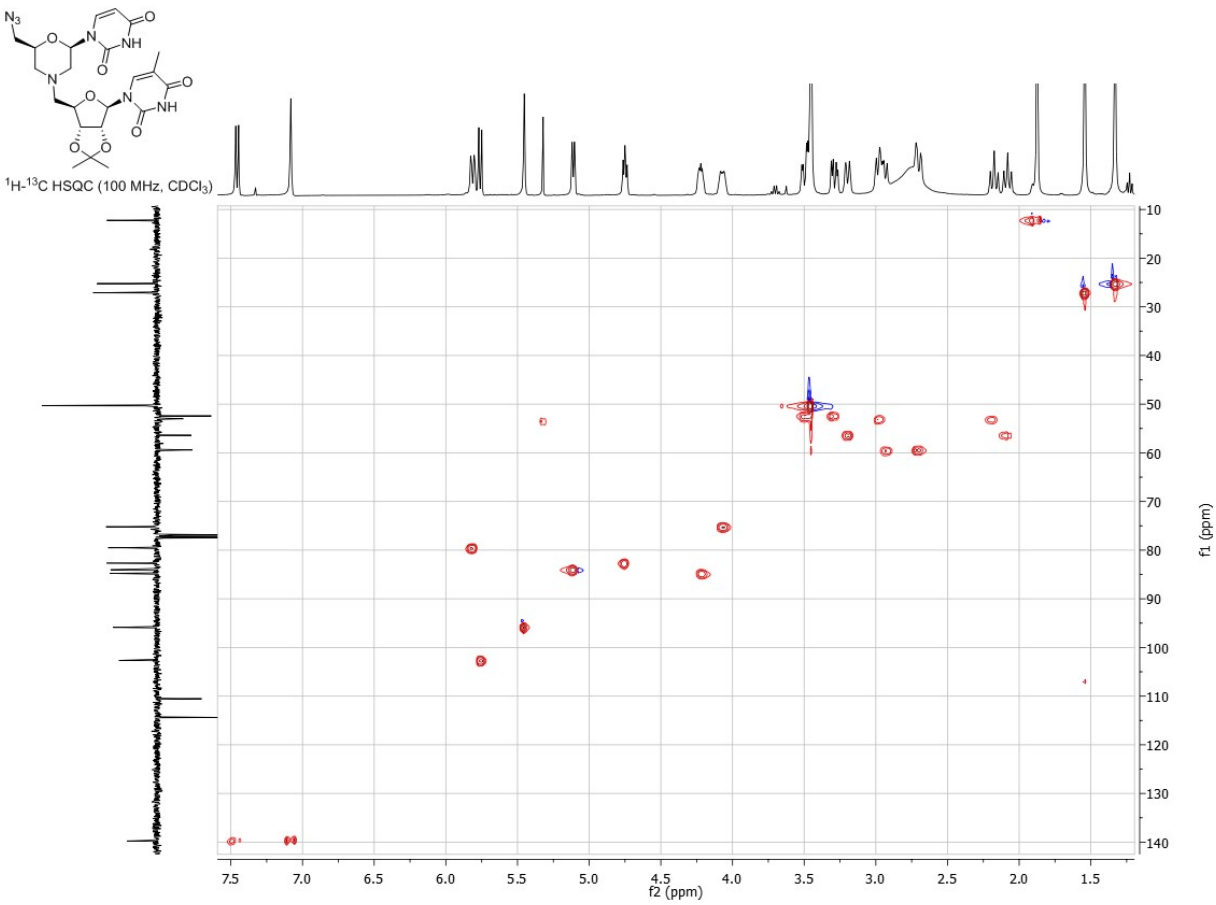


NMR spectra of compound 34

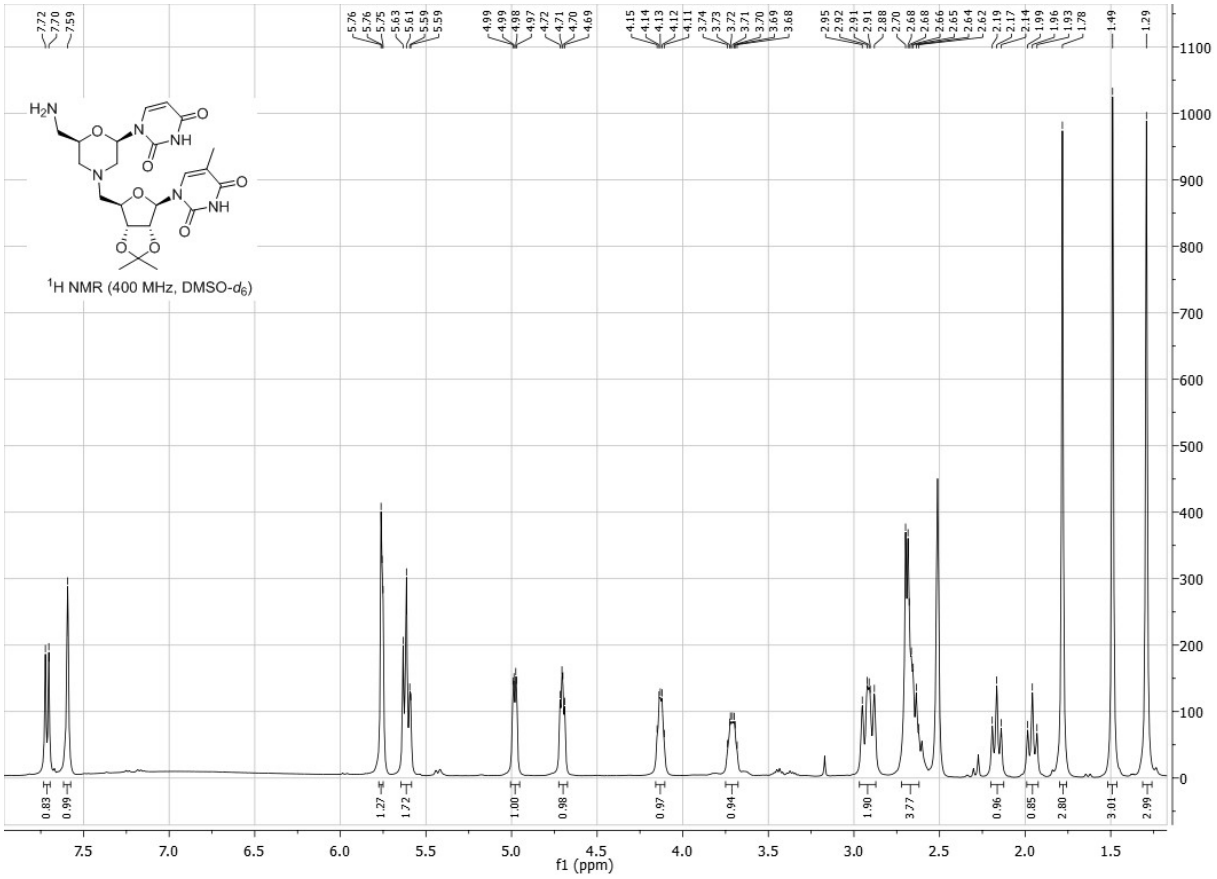


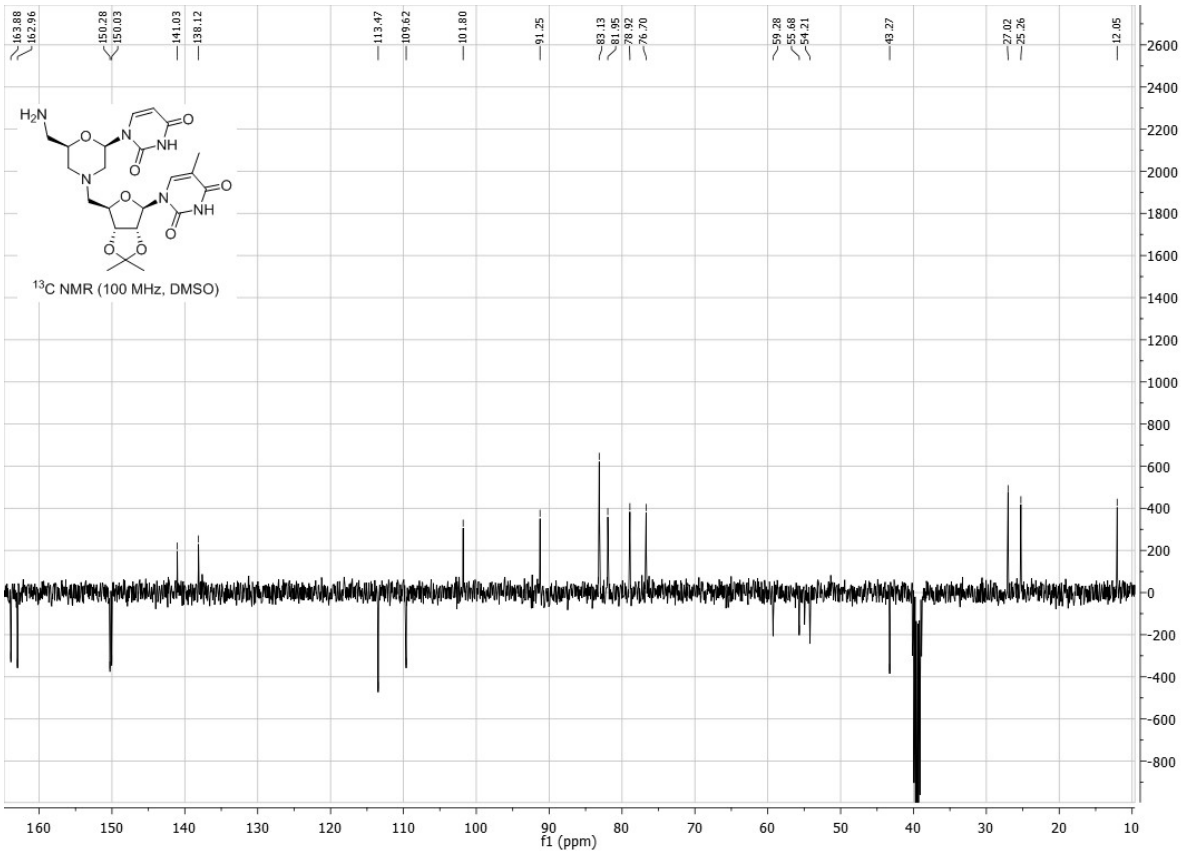

164.61
163.75
150.15
150.06
139.77
139.66
114.38
110.54
102.66
95.87
84.77
84.04
82.66
79.51
75.20
59.41
56.39
53.00
52.42
27.12
25.23
12.21
^{13}C NMR (100 MHz, CDCl$_3$)
f1 (ppm)

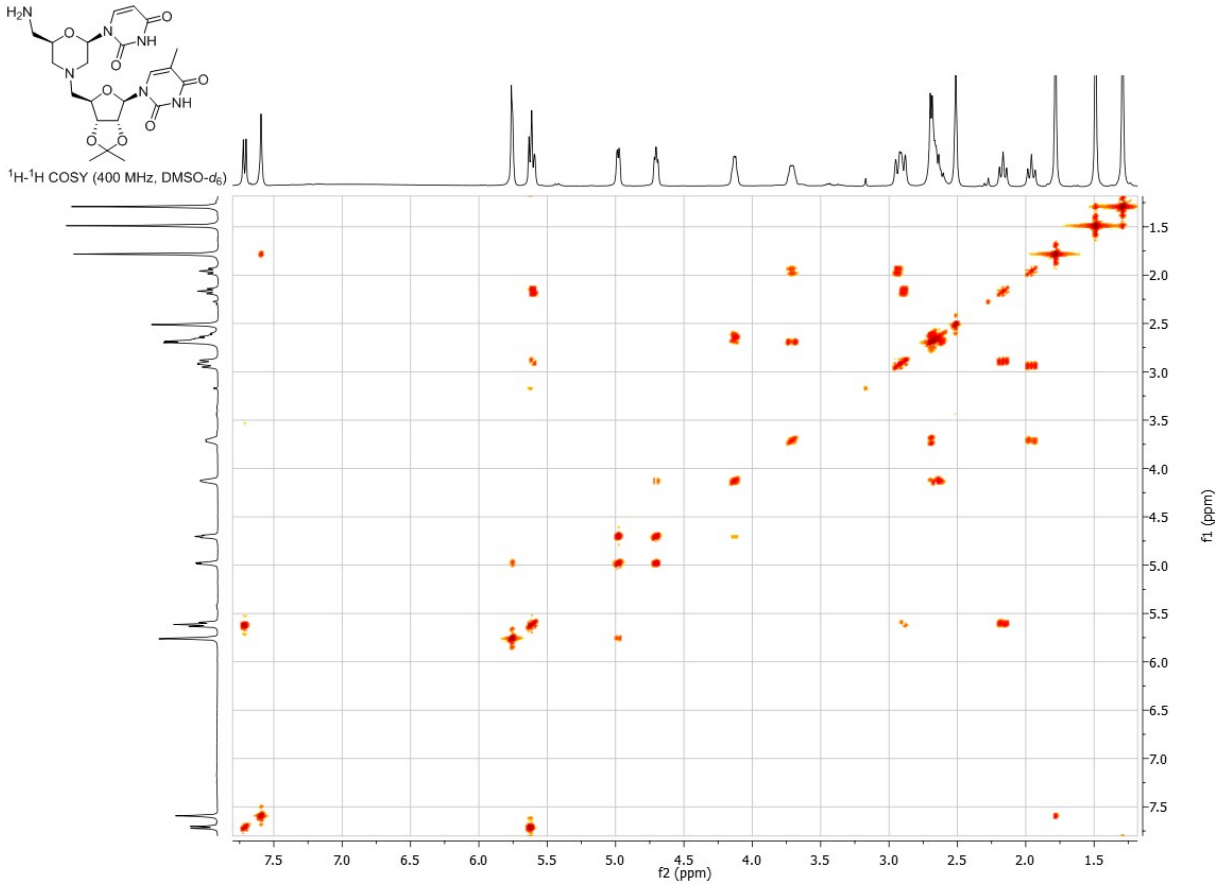

^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

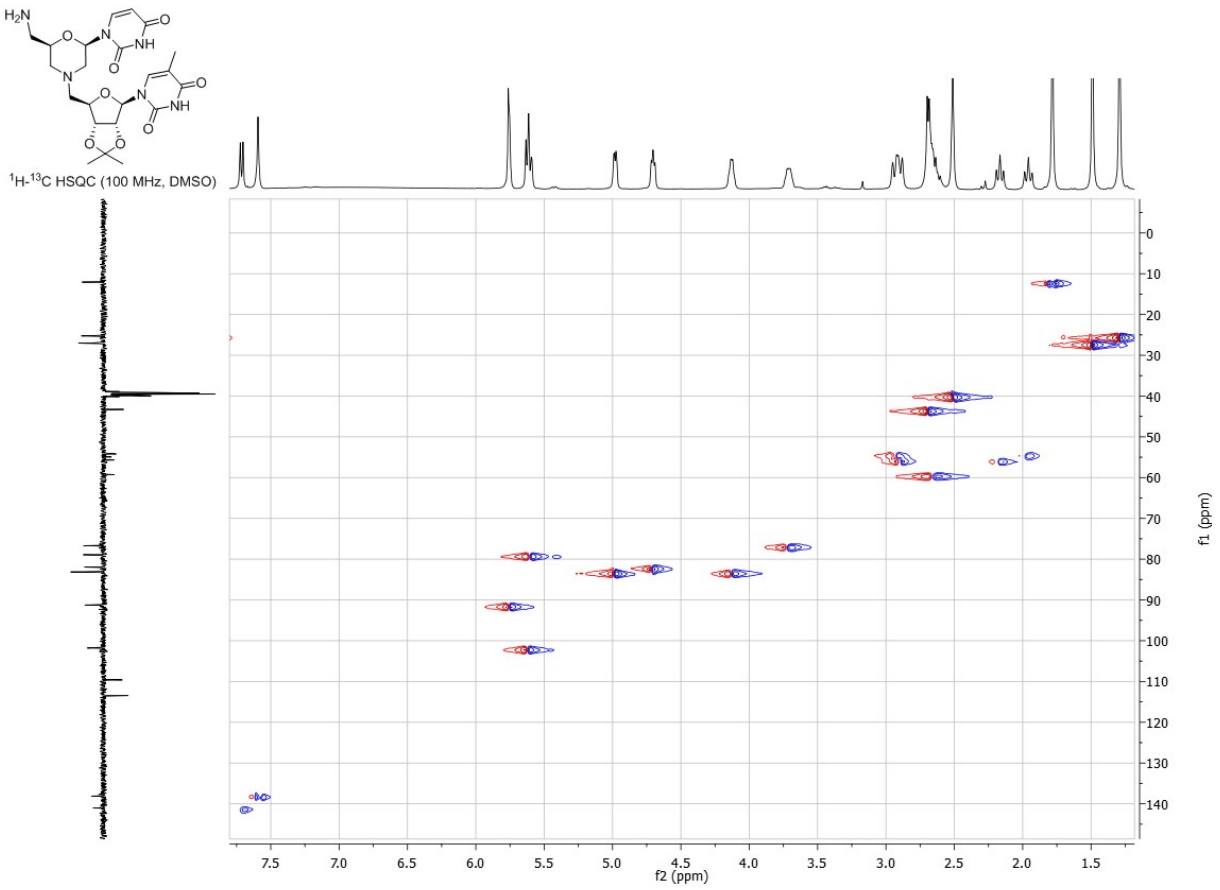


NMR spectra of compound 35

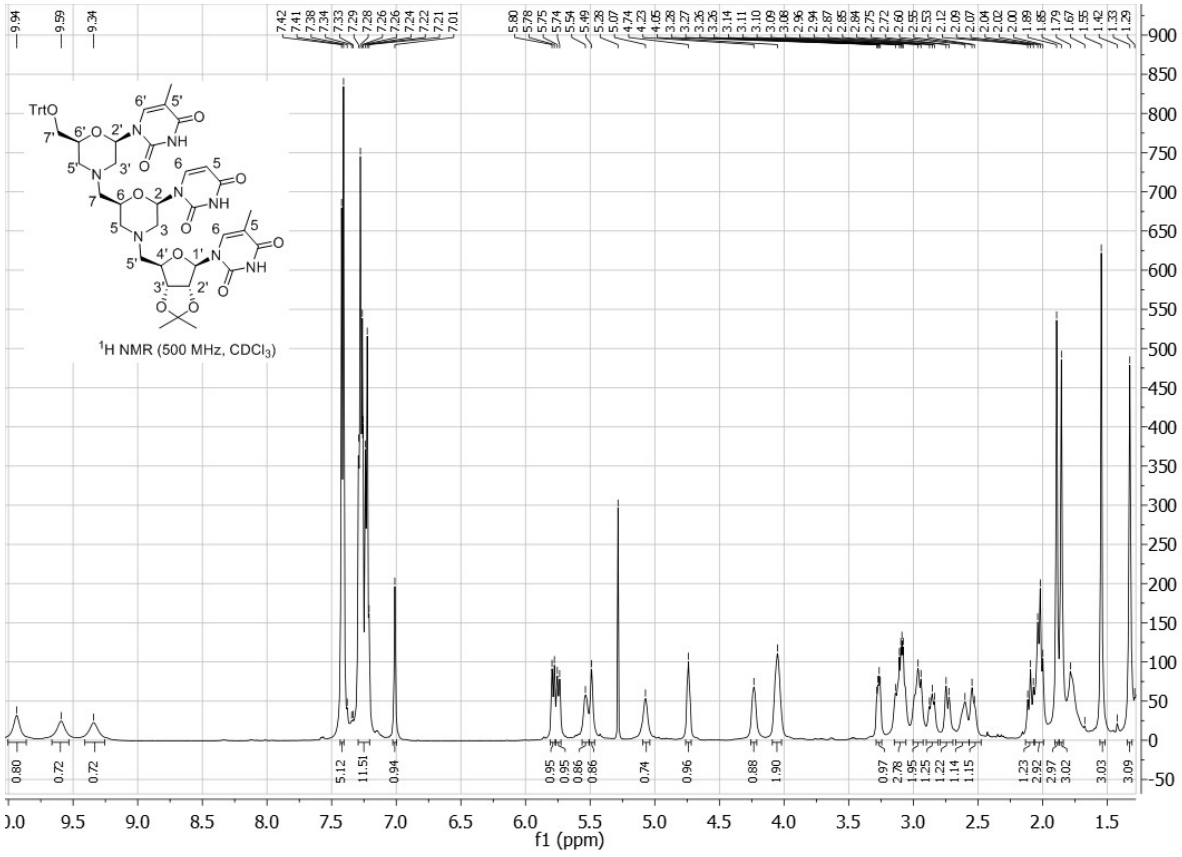


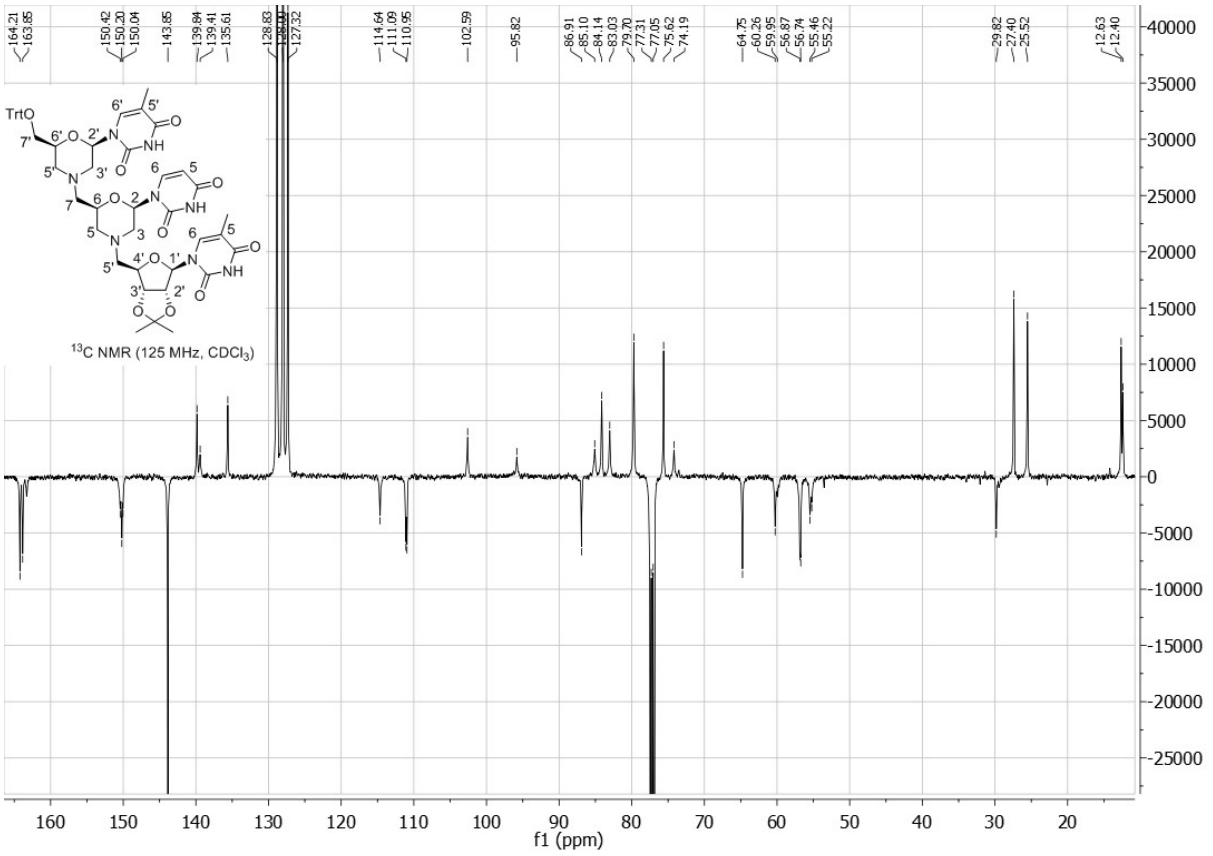

163.88
162.96
150.28
150.03
141.03
138.12
113.47
109.62
101.80
91.25
83.13
81.95
78.92
76.70
59.28
55.68
54.21
49.27
27.02
25.26
12.05
^{13}C NMR (100 MHz, DMSO)
f1 (ppm)

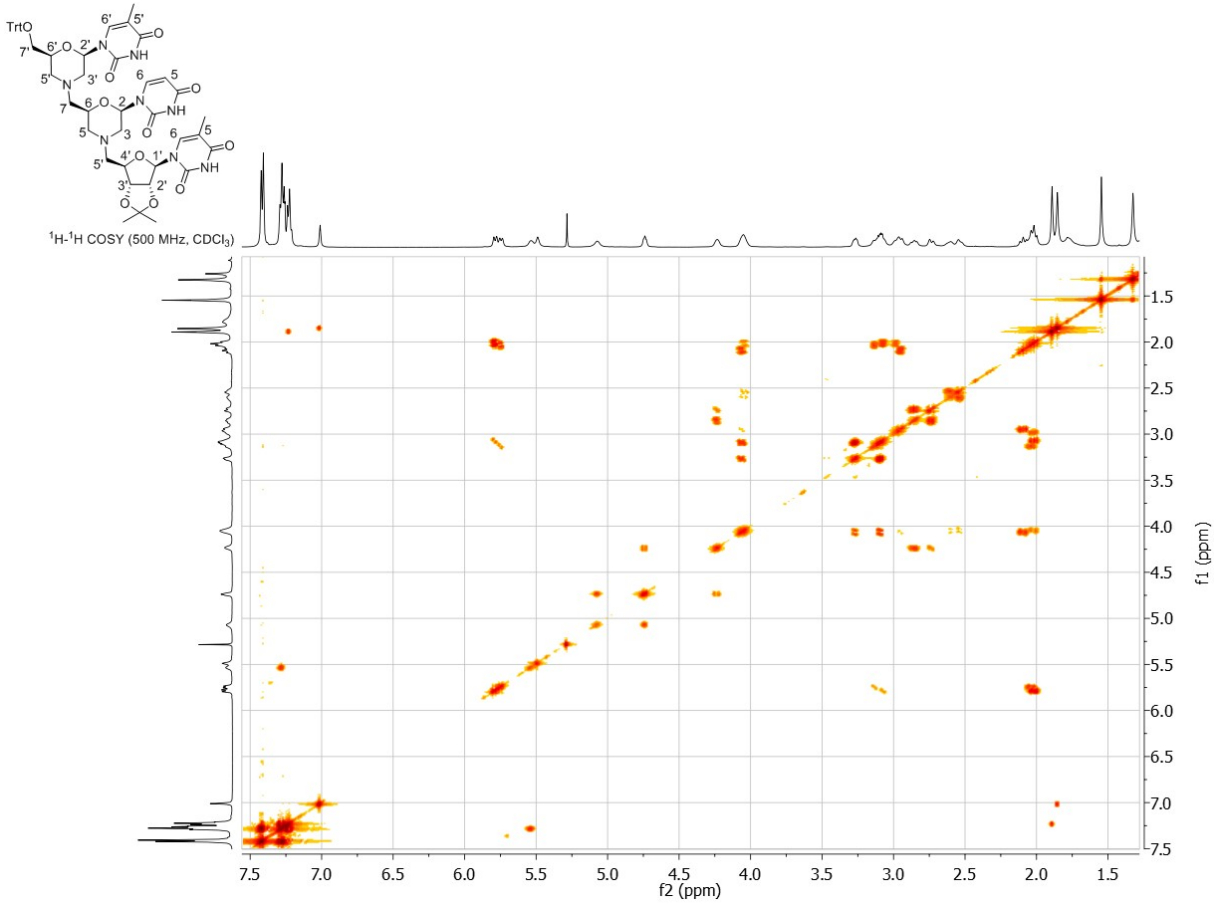

^{1}H-^{1}H COSY (400 MHz, DMSO-d_6)
f2 (ppm)
f1 (ppm)

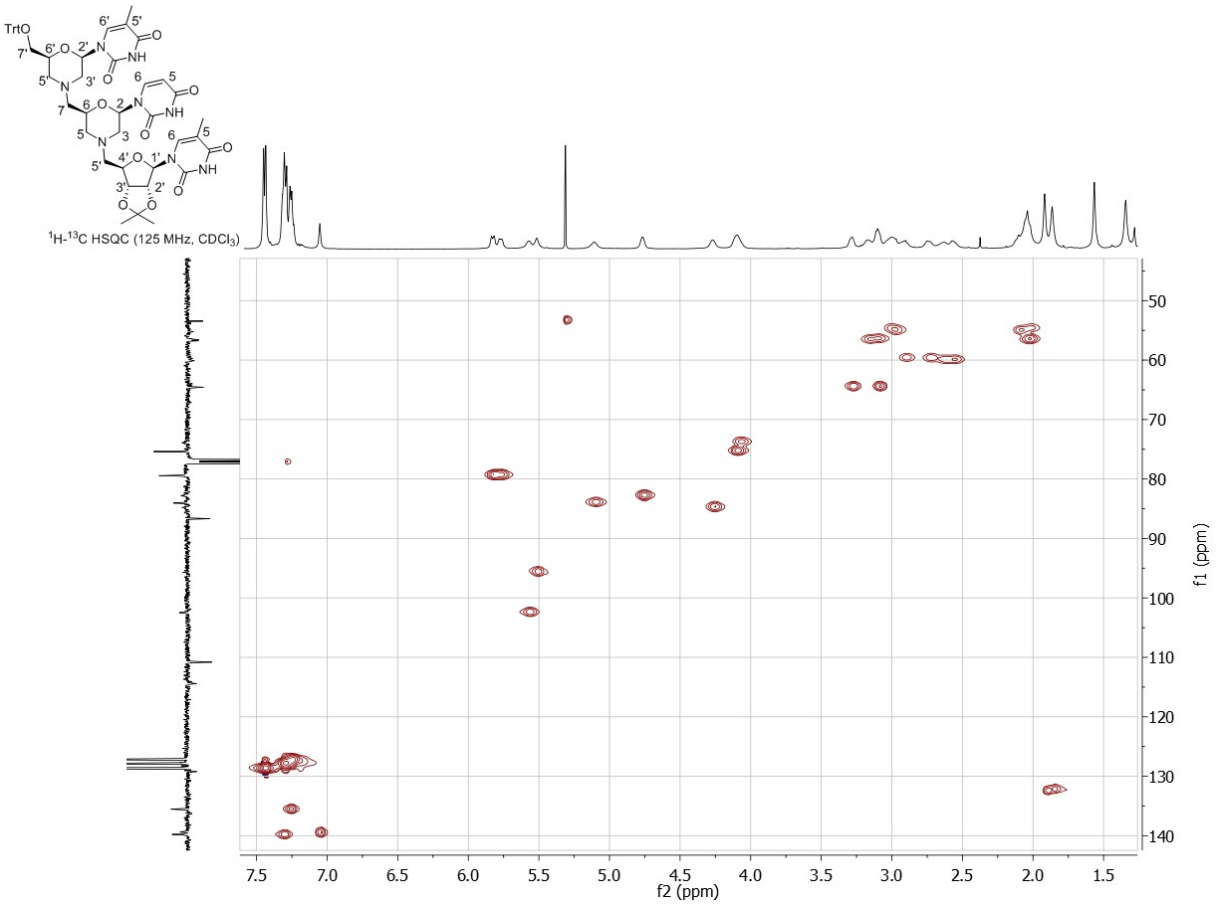


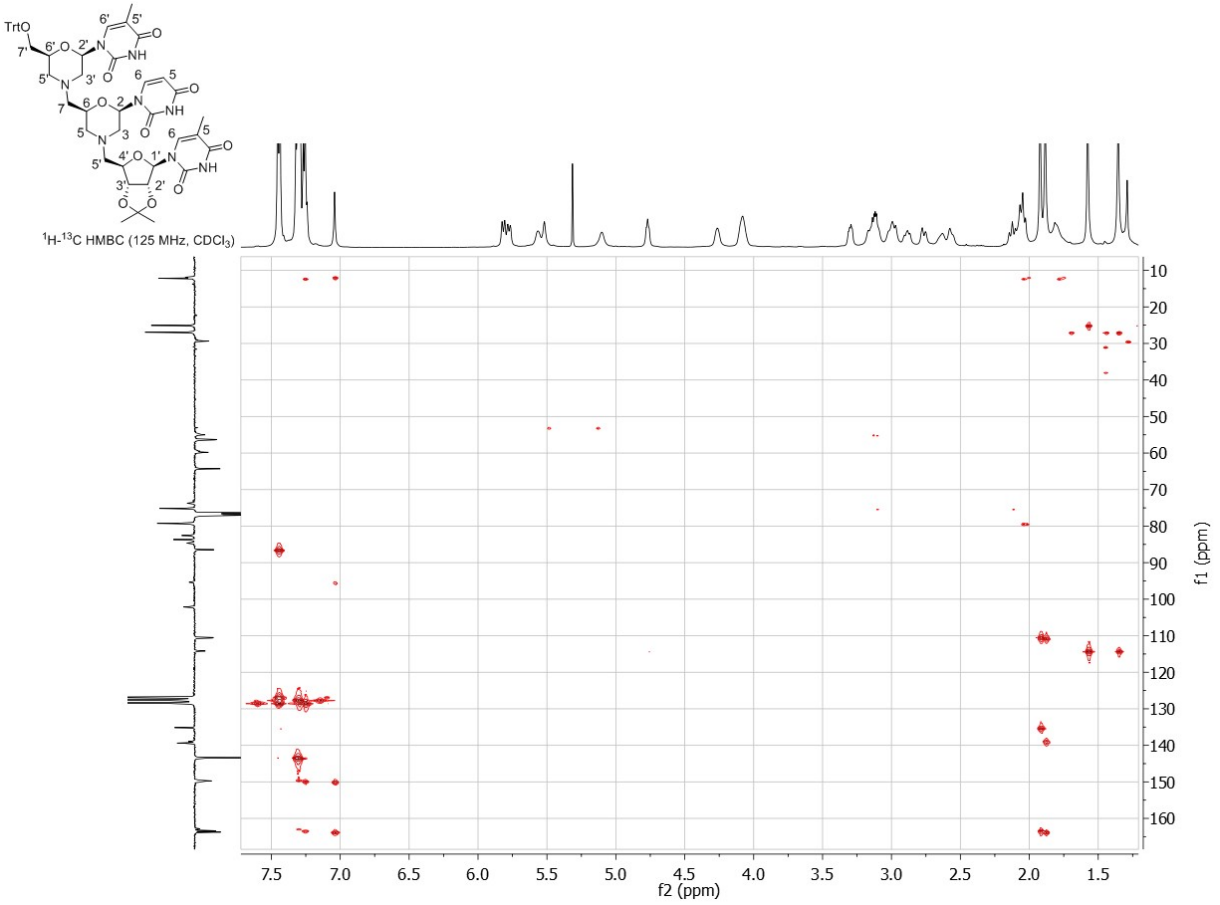
NMR spectra of compound 36



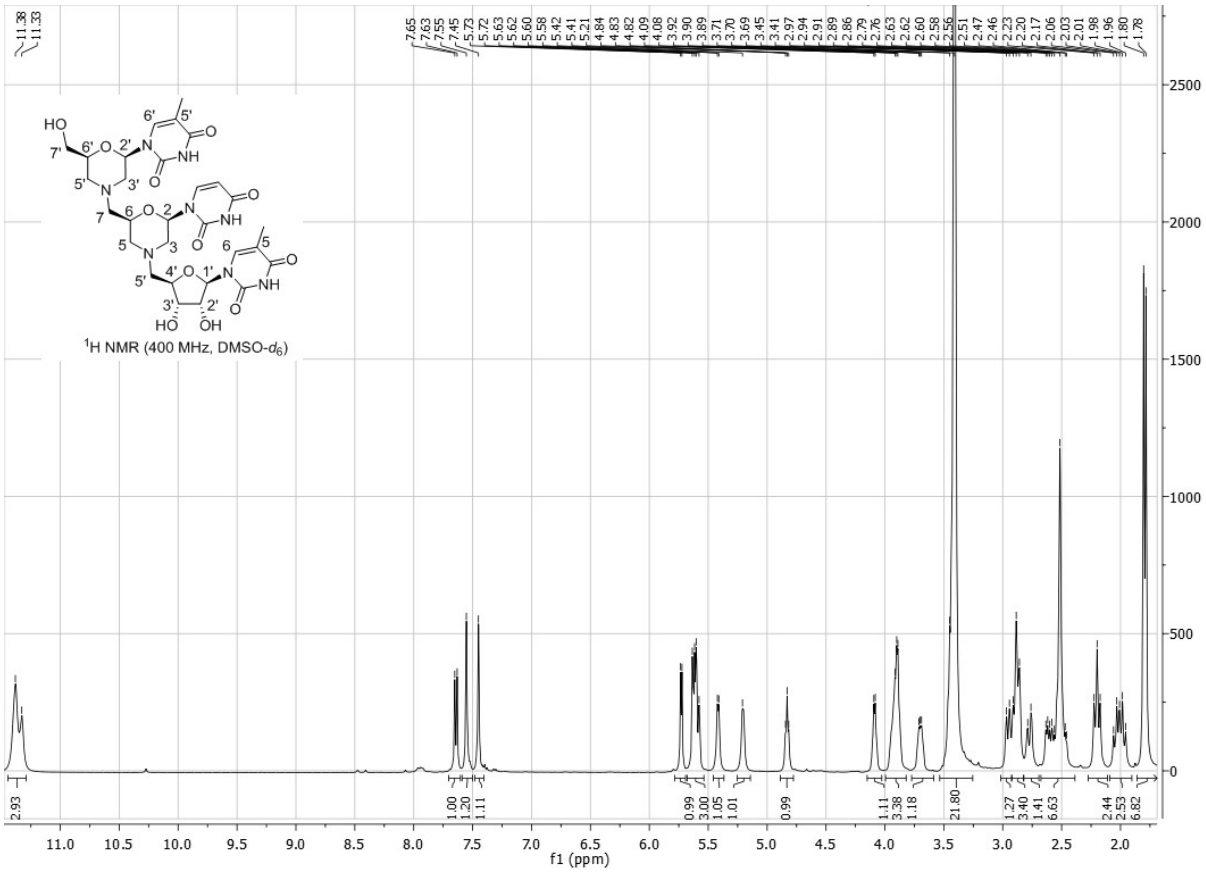

164.21
163.85
150.42
150.20
150.04
143.85
139.84
139.41
135.61
128.83
128.01
127.32
114.64
111.09
110.95
102.59
95.82
86.91
85.10
84.14
83.03
79.70
77.31
77.05
75.62
74.19
64.75
60.26
59.95
56.87
56.74
55.46
55.22
29.82
27.40
25.52
12.63
12.40
TrtO
^{13}C NMR (125 MHz, CDCl$_3$)
f1 (ppm)

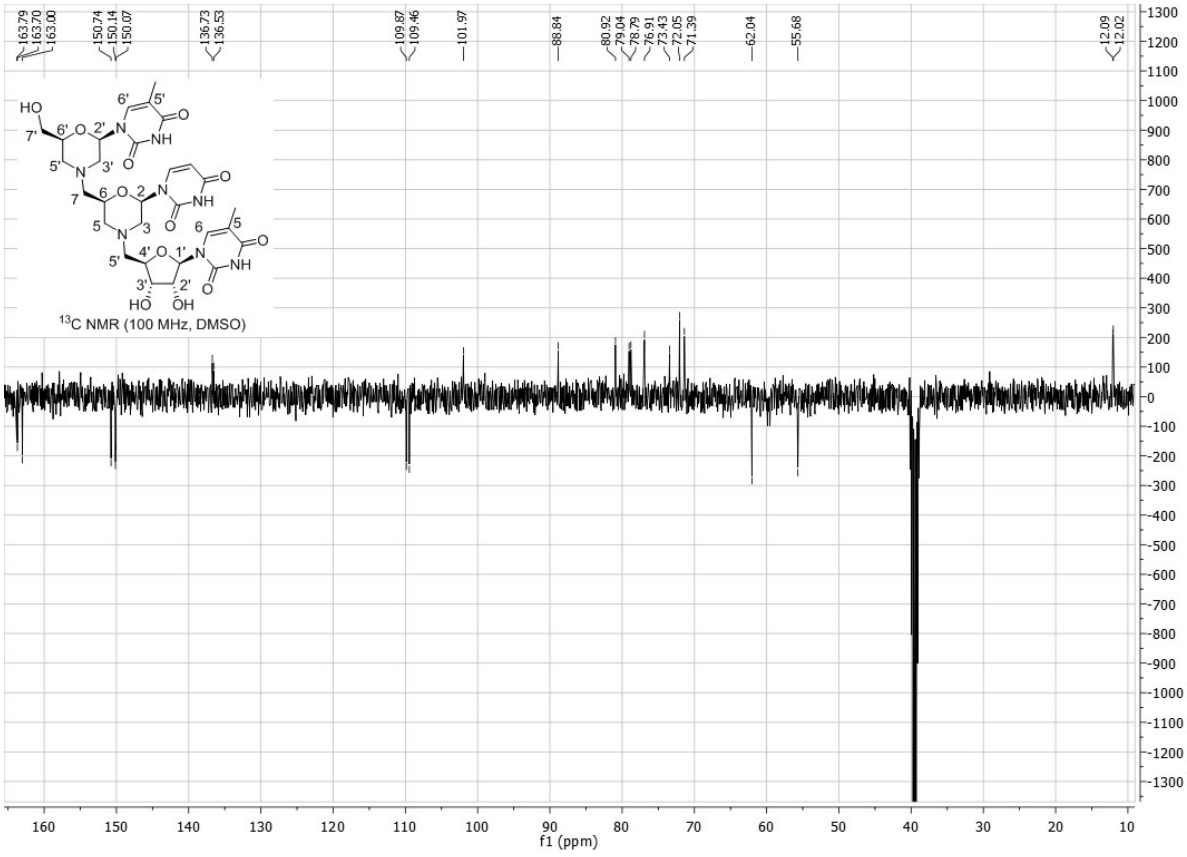

TrtO
^{1}H-^{1}H COSY (500 MHz, CDCl$_3$)
f1 (ppm)
f2 (ppm)

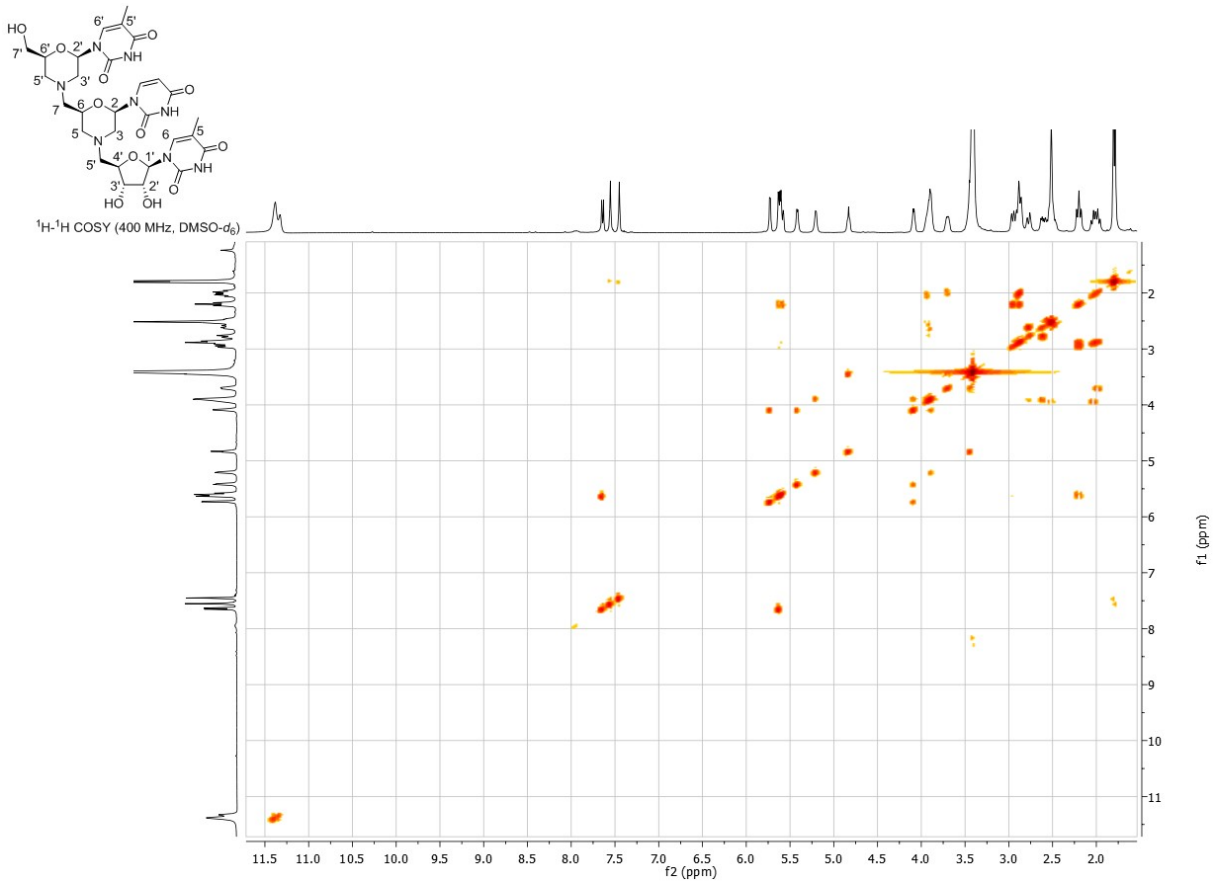

TrtO
^{1}H-^{13}C HSQC (125 MHz, CDCl$_3$)
f1 (ppm)
f2 (ppm)

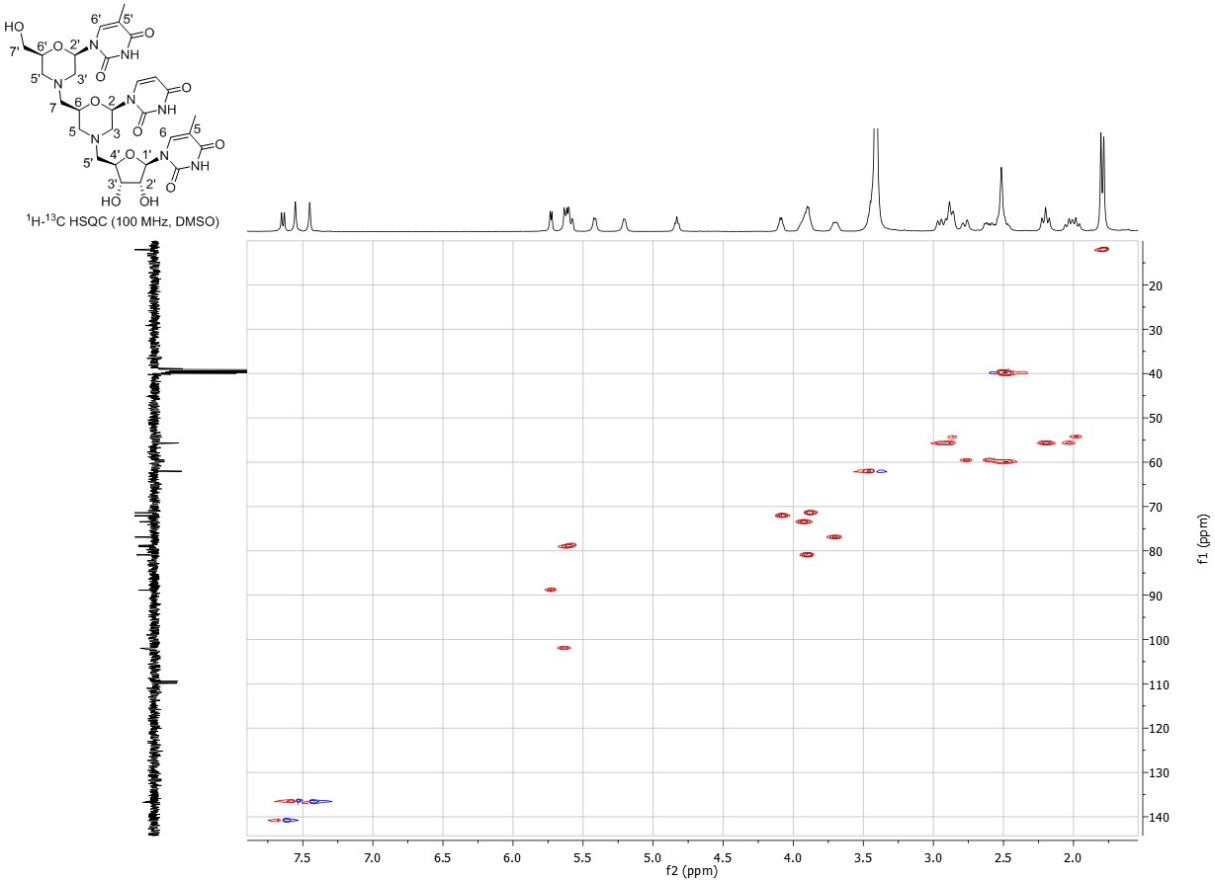

TrtO
^{1}H-^{13}C HMBC (125 MHz, CDCl$_3$)
f1 (ppm)
f2 (ppm)

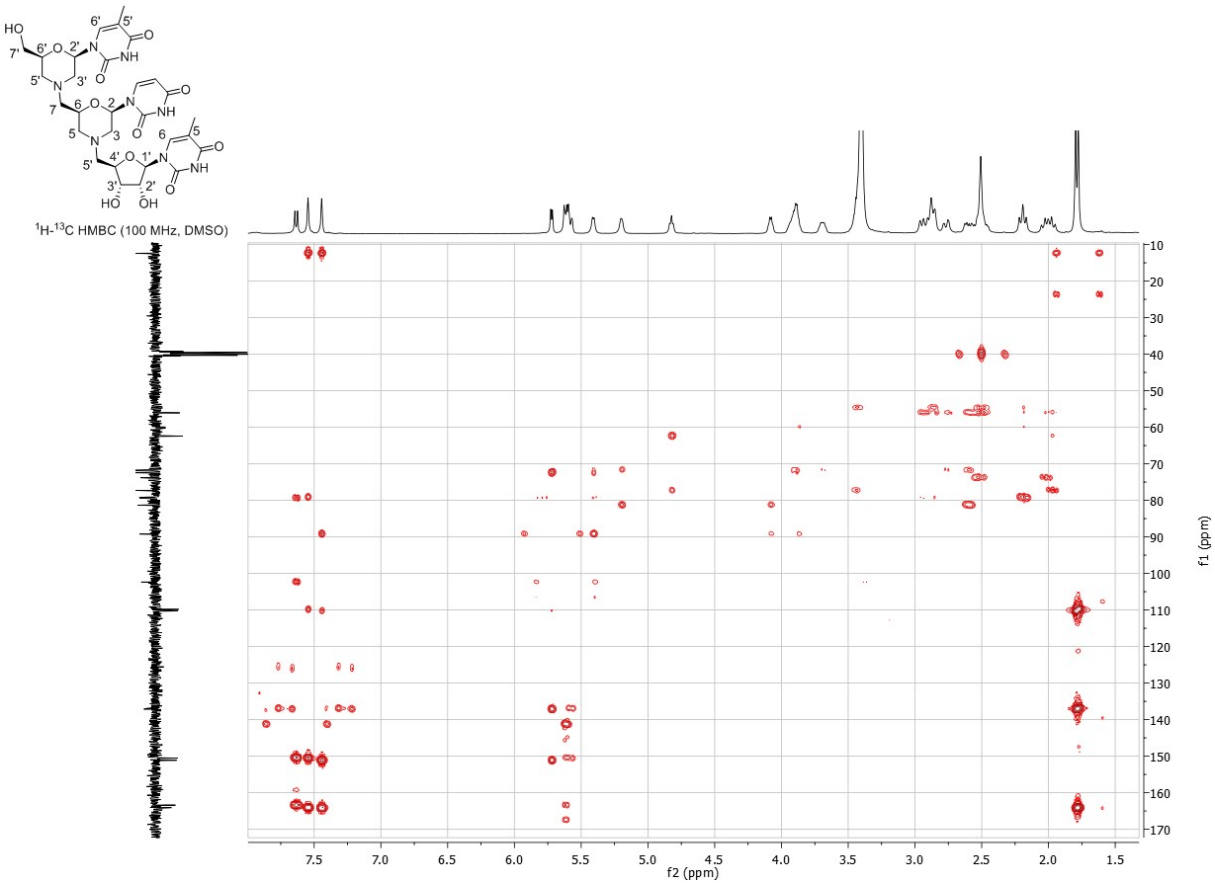
NMR spectra of compound 37

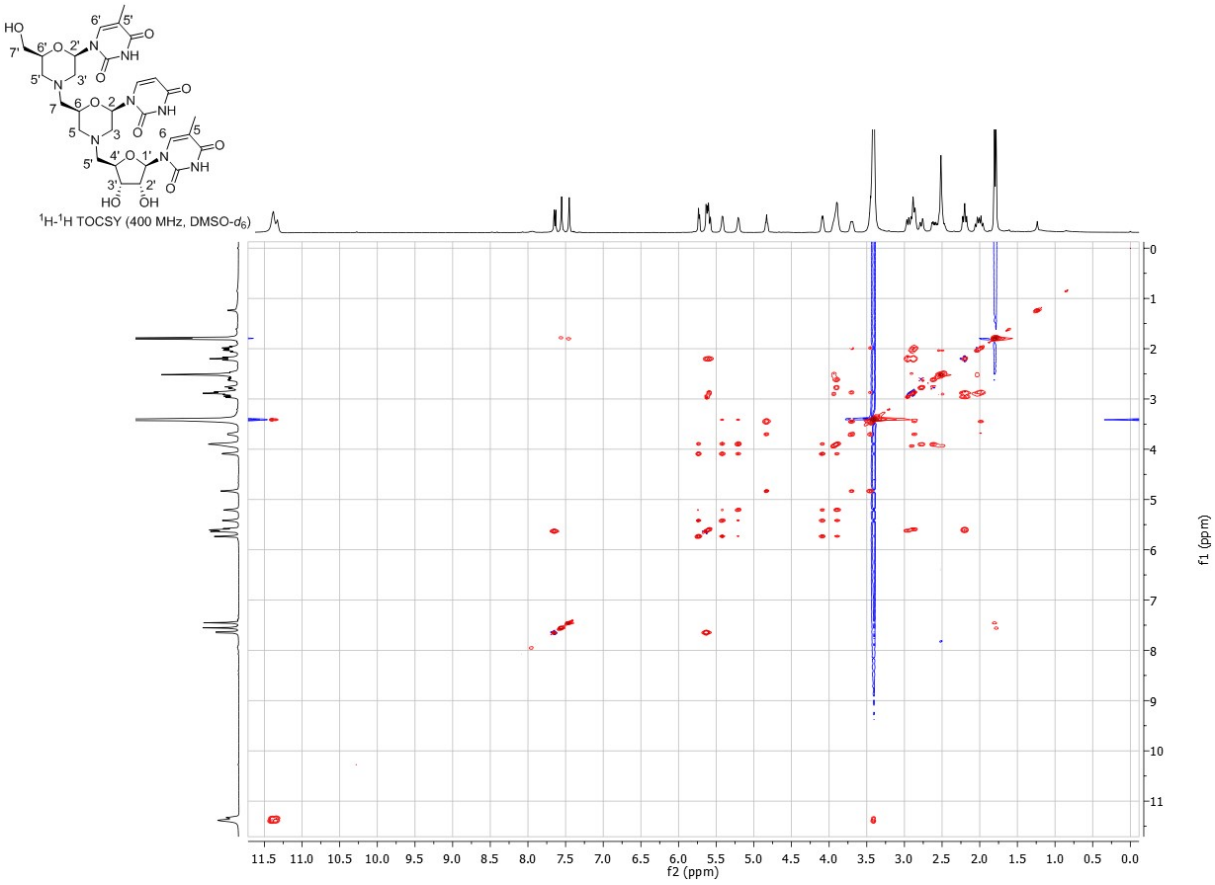




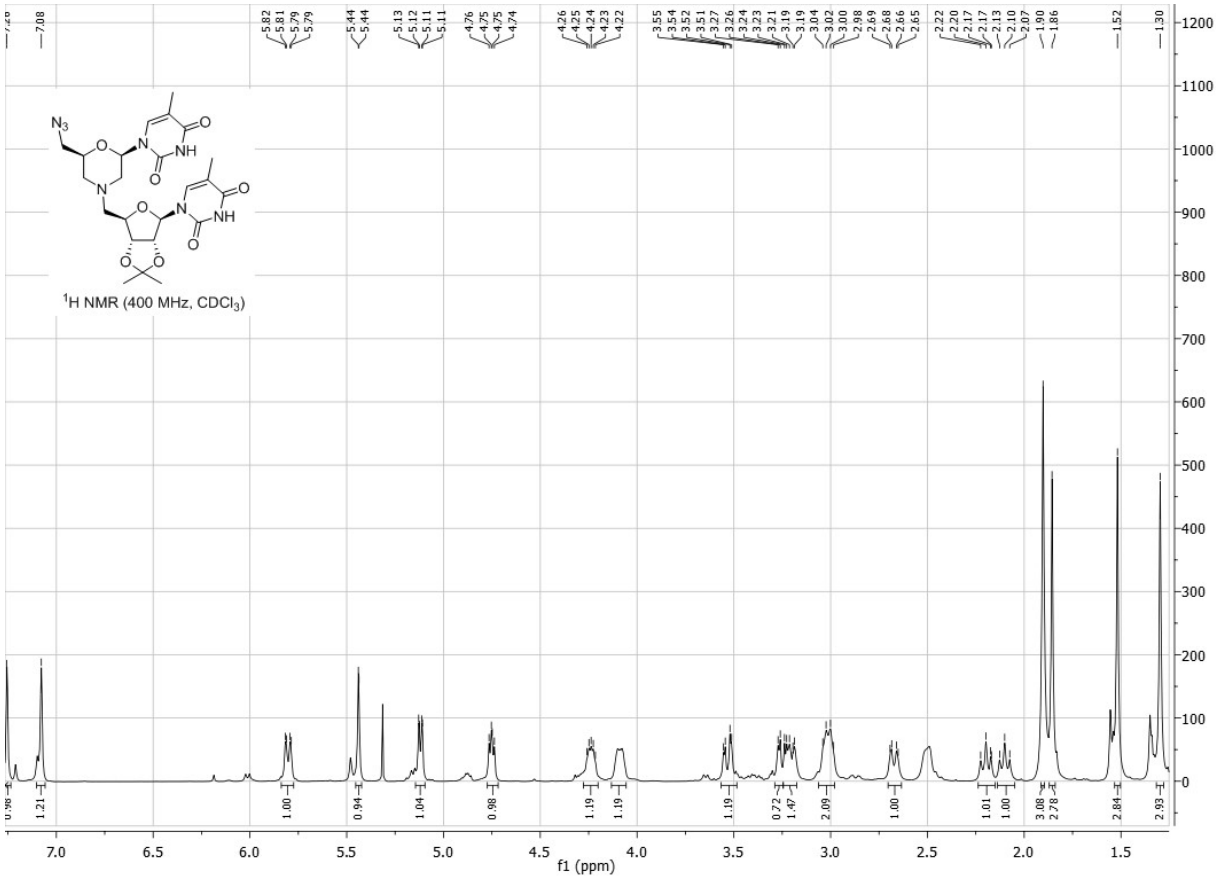

HO
6'
5'
7'
6'
O
2'
N
NH
O
5'
N
3'
O
7
6
O
2
N
NH
O
O
5
N
3
6
5
O
5'
4'
O
1'
N
NH
O
3'
2'
HO
OH
^{1}H-^{1}H COSY (400 MHz, DMSO-d_6)
f1 (ppm)
f2 (ppm)

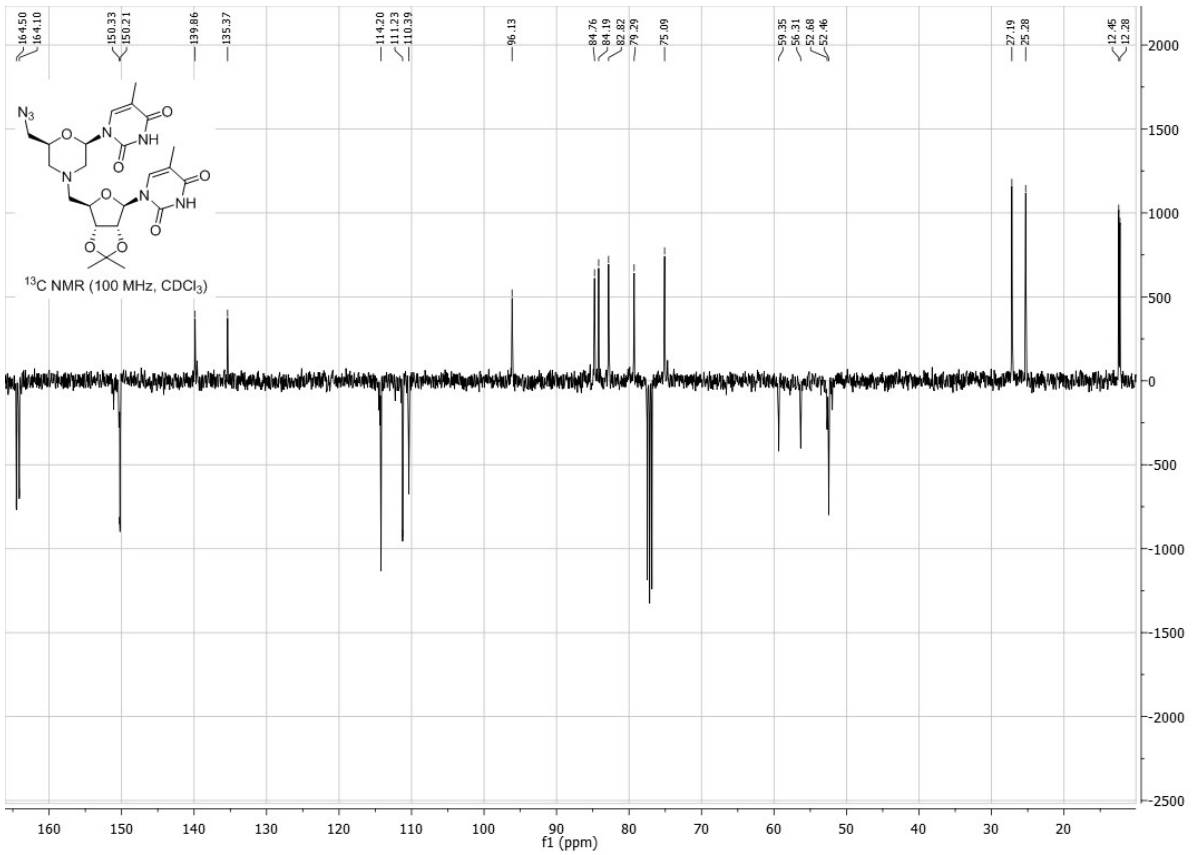

HO
6'
5'
7'
6'
O
2'
N
NH
O
5'
N
3'
O
7
6
O
2
N
NH
O
O
5
N
3
6
5
O
5'
4'
O
1'
N
NH
O
3'
2'
HO
OH
^{1}H-^{13}C HSQC (100 MHz, DMSO)
f1 (ppm)
f2 (ppm)

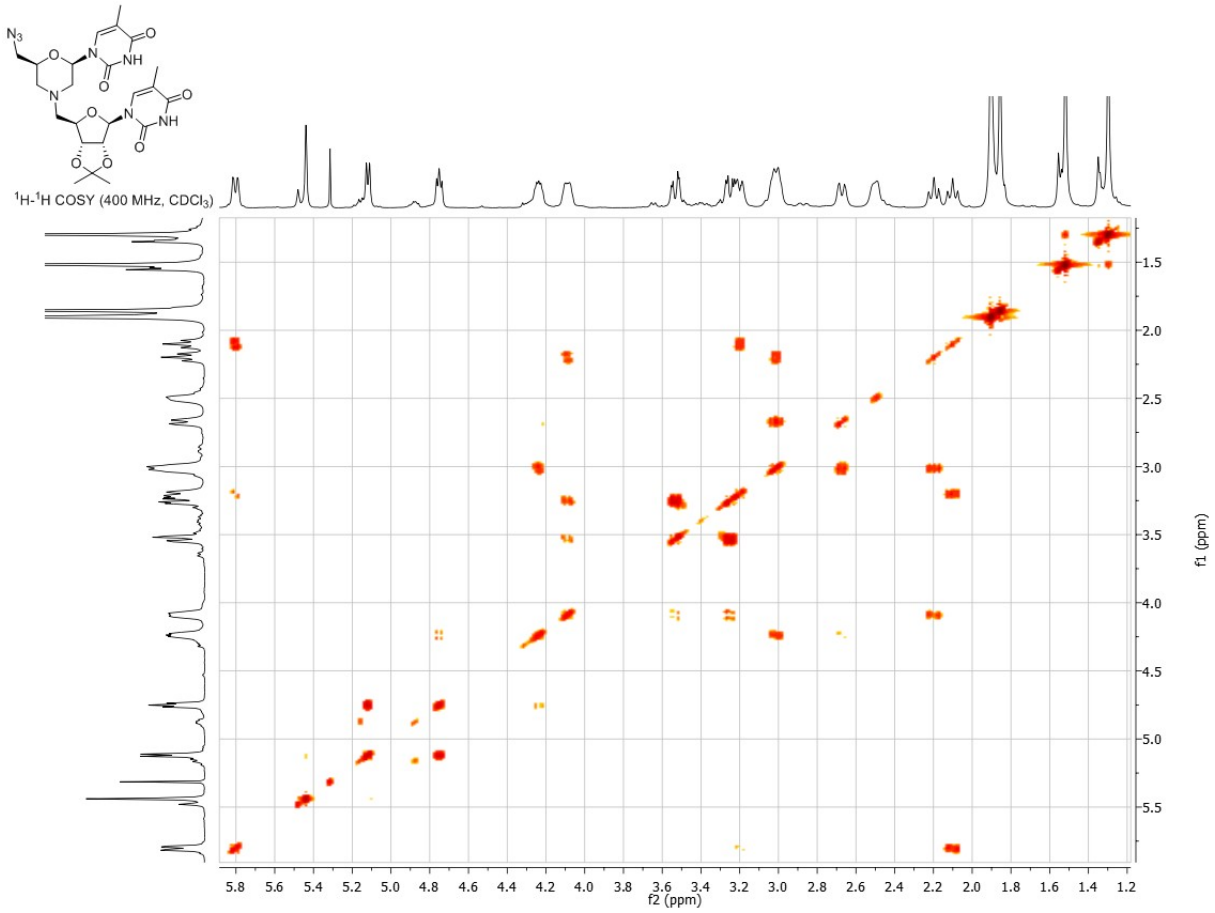

^{1}H-^{13}C HMBC (100 MHz, DMSO)
f2 (ppm)
f1 (ppm)

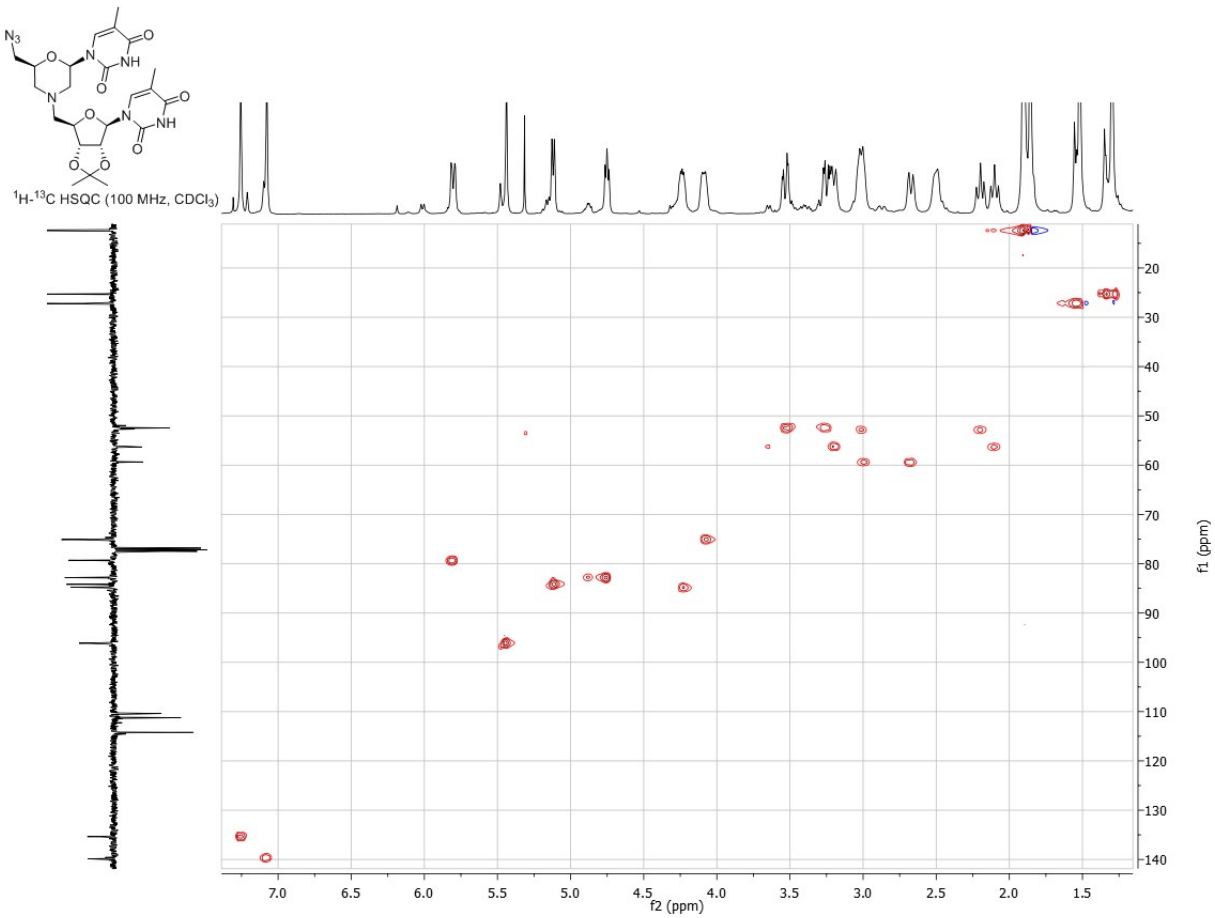

^{1}H-^{1}H TOCSY (400 MHz, DMSO-d_6)
f2 (ppm)
f1 (ppm)

NMR spectra of compound 41

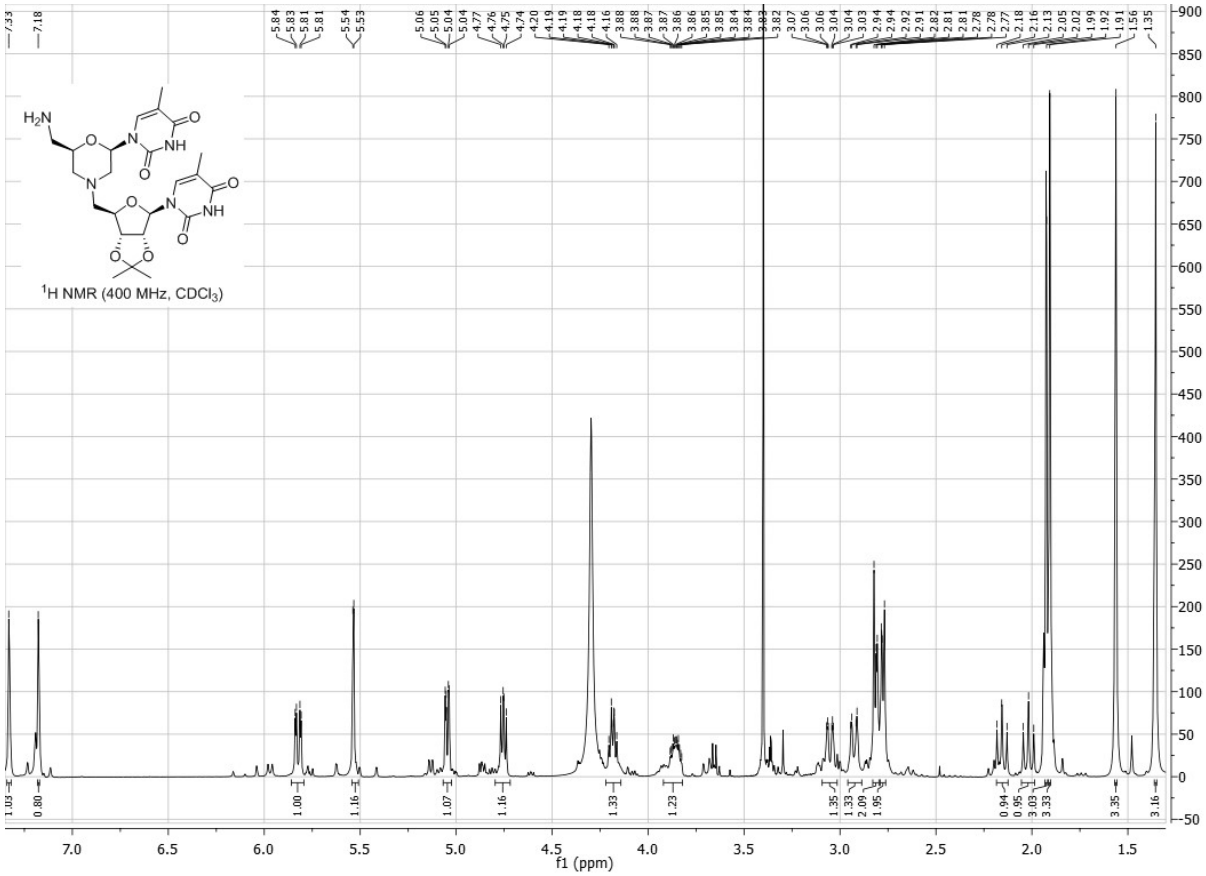


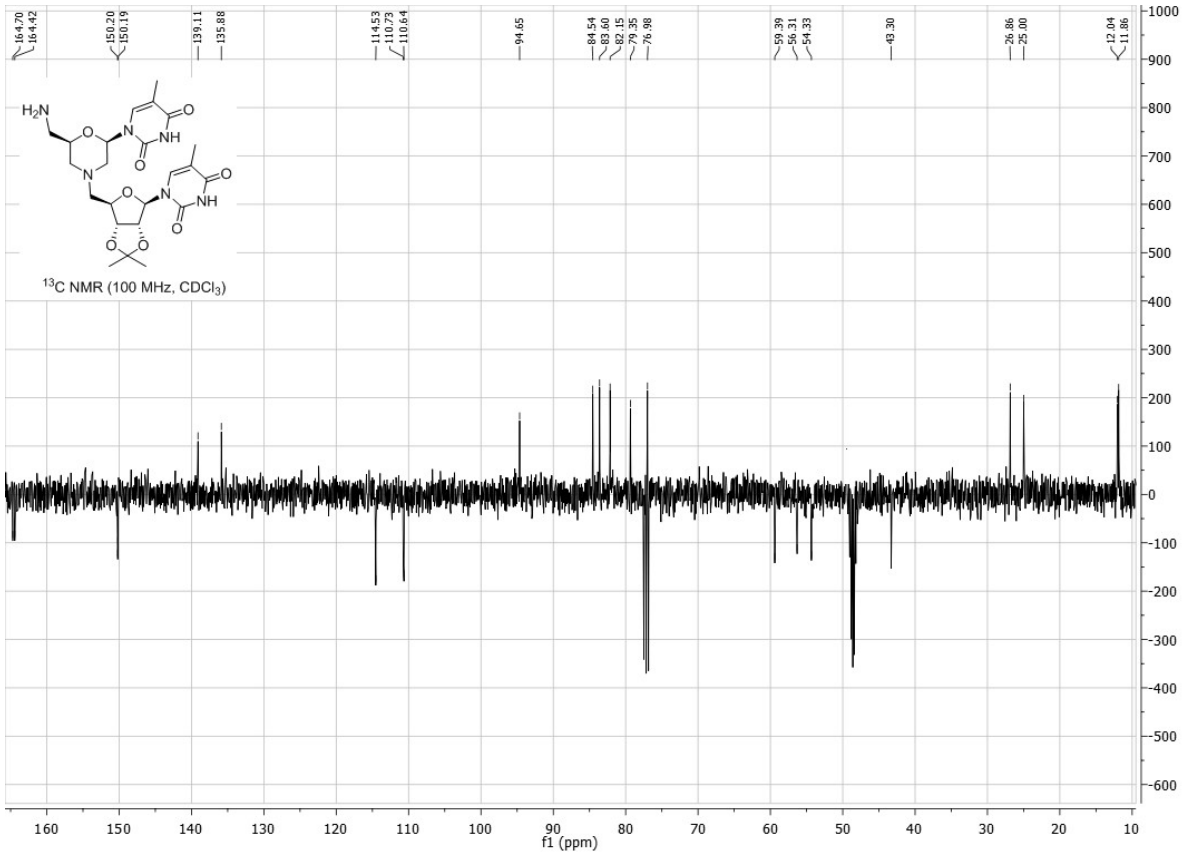


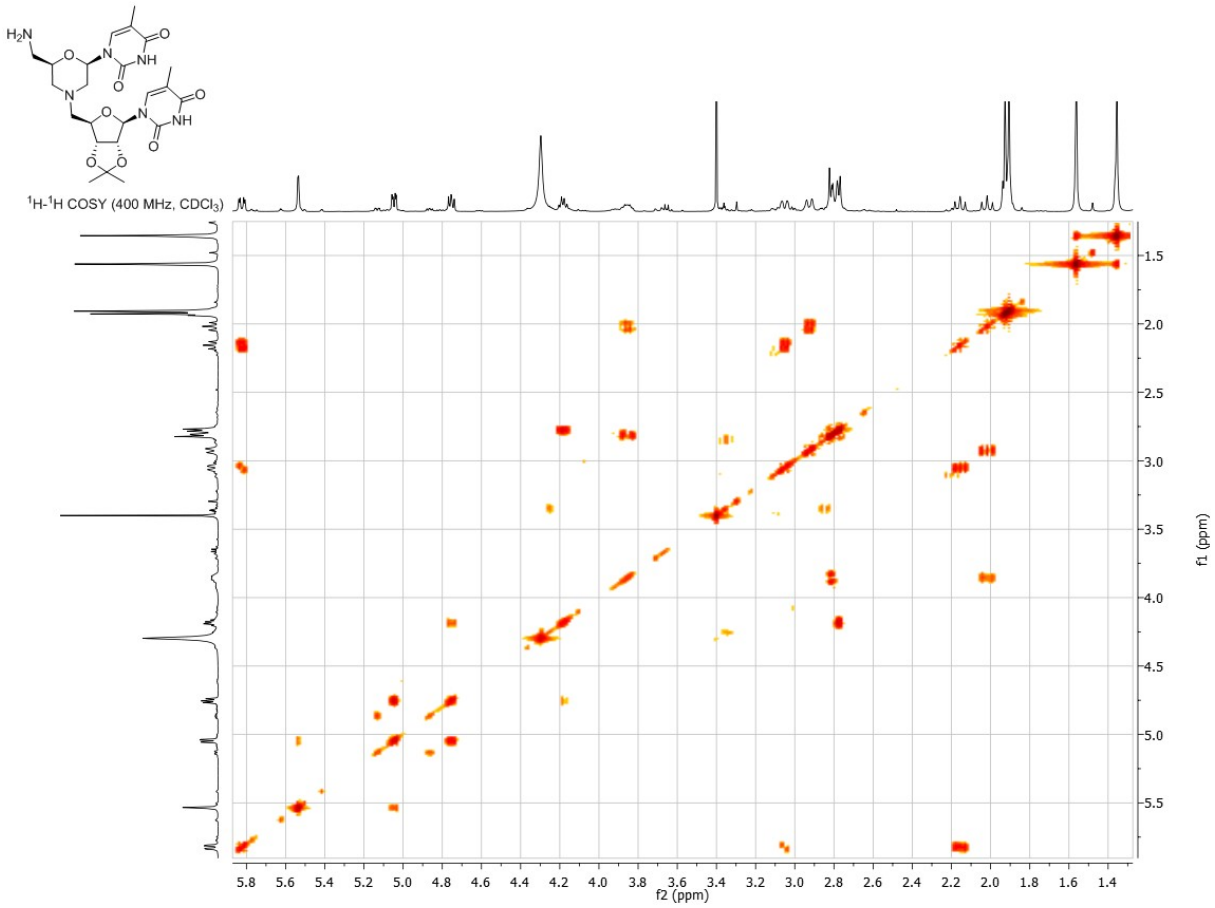

^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)


^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

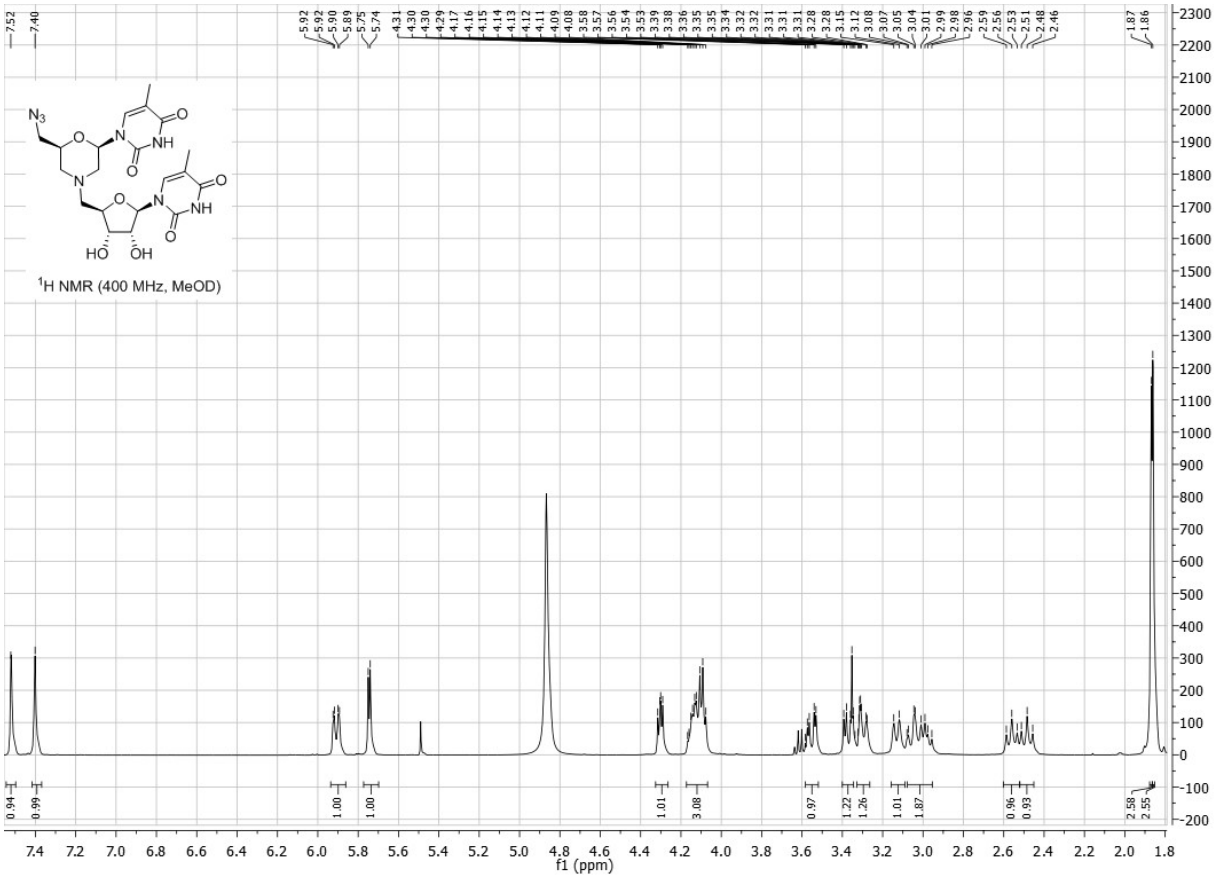
NMR spectra of compound 42

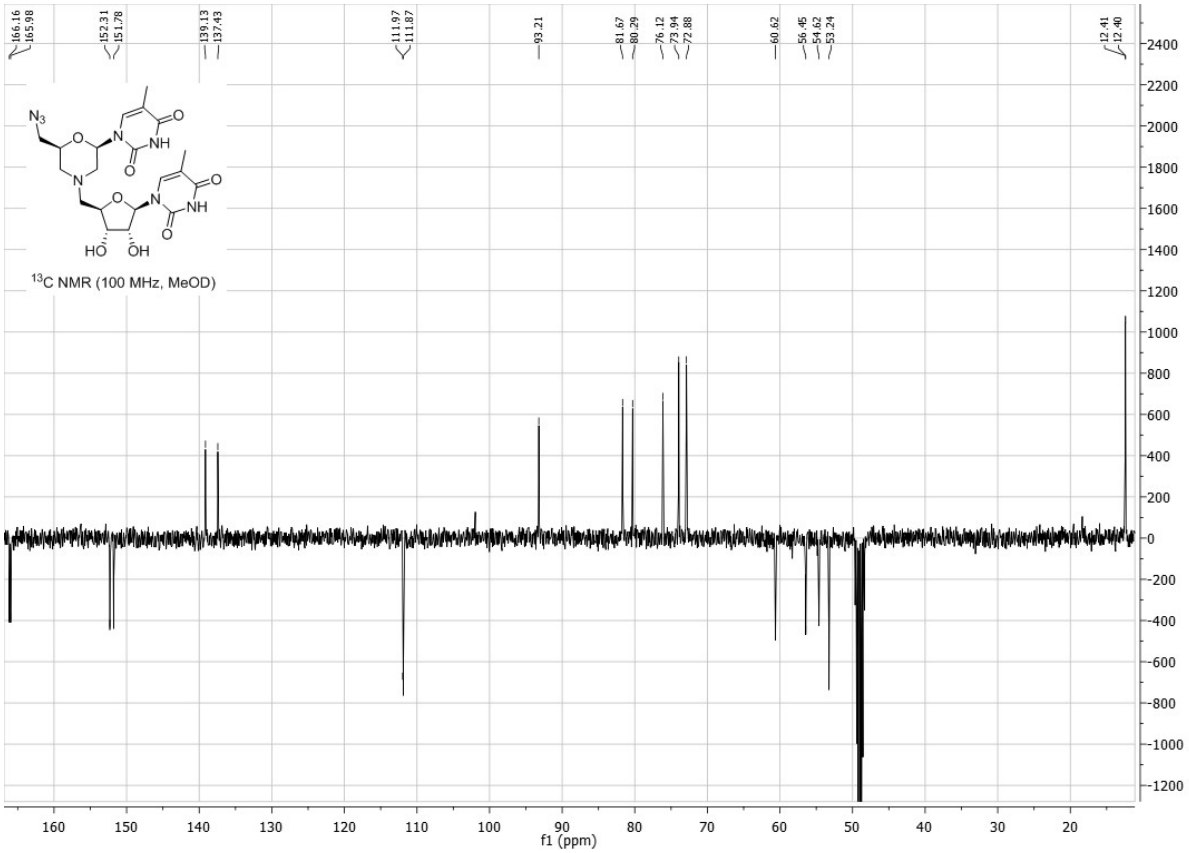


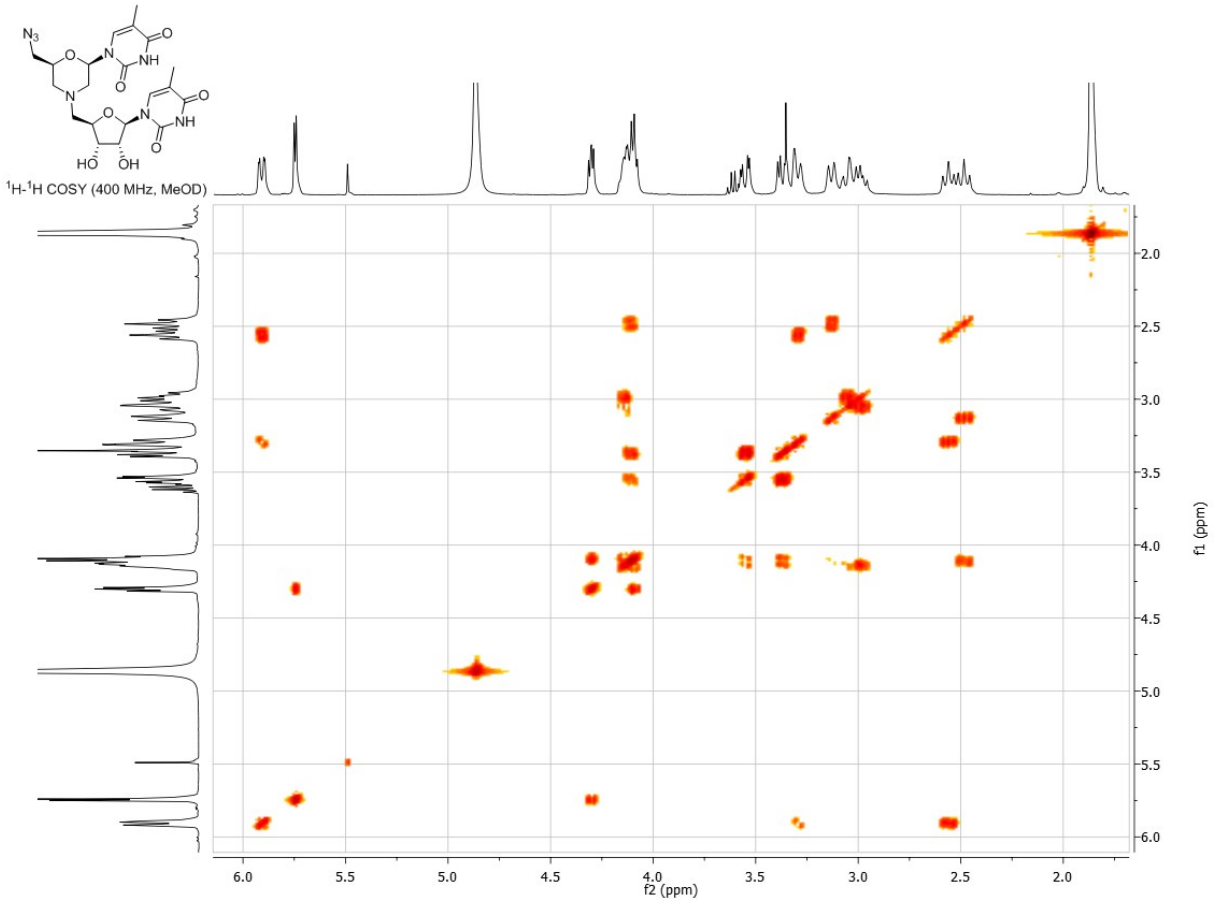


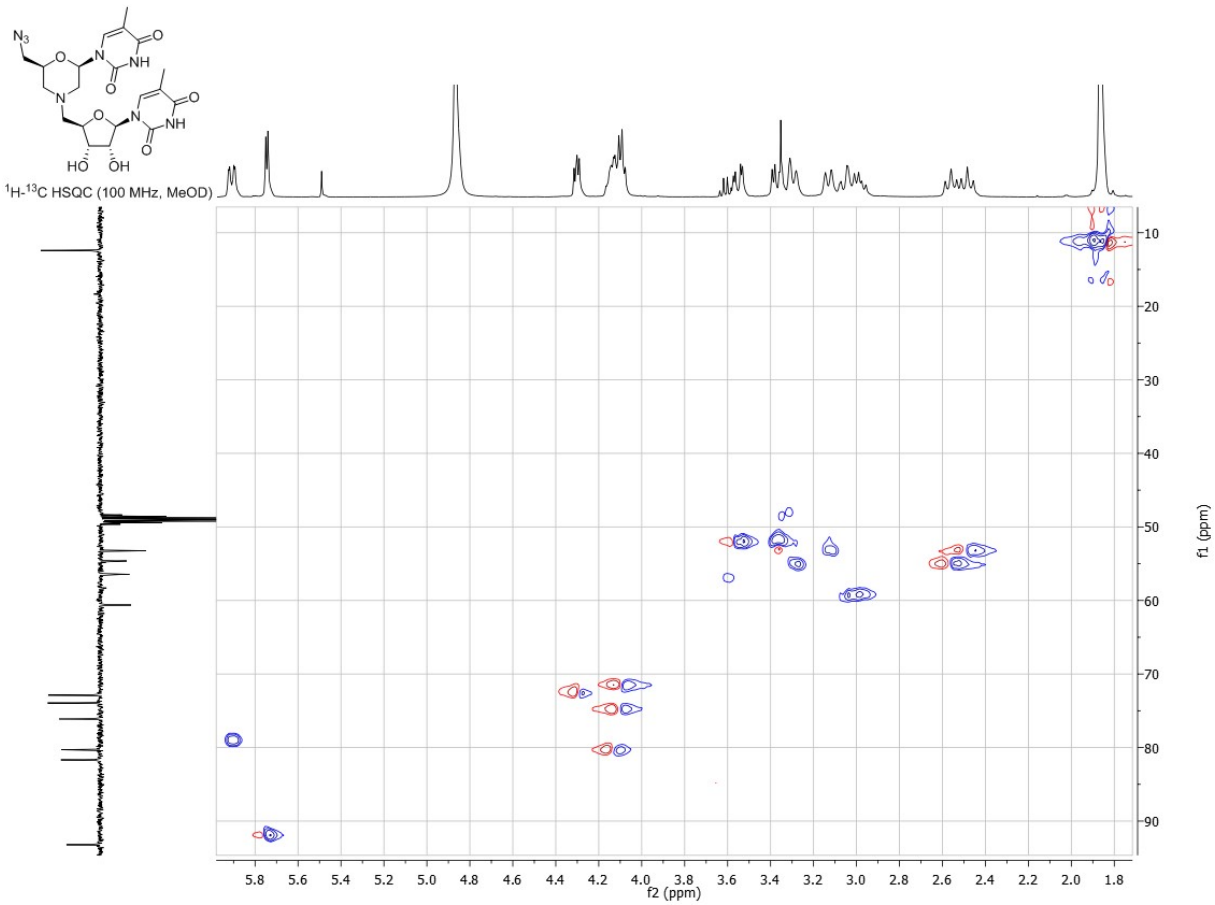

^{1}H-^{1}H COSY (400 MHz, CDCl$_3$)
f2 (ppm)
f1 (ppm)

NMR spectra of compound 43

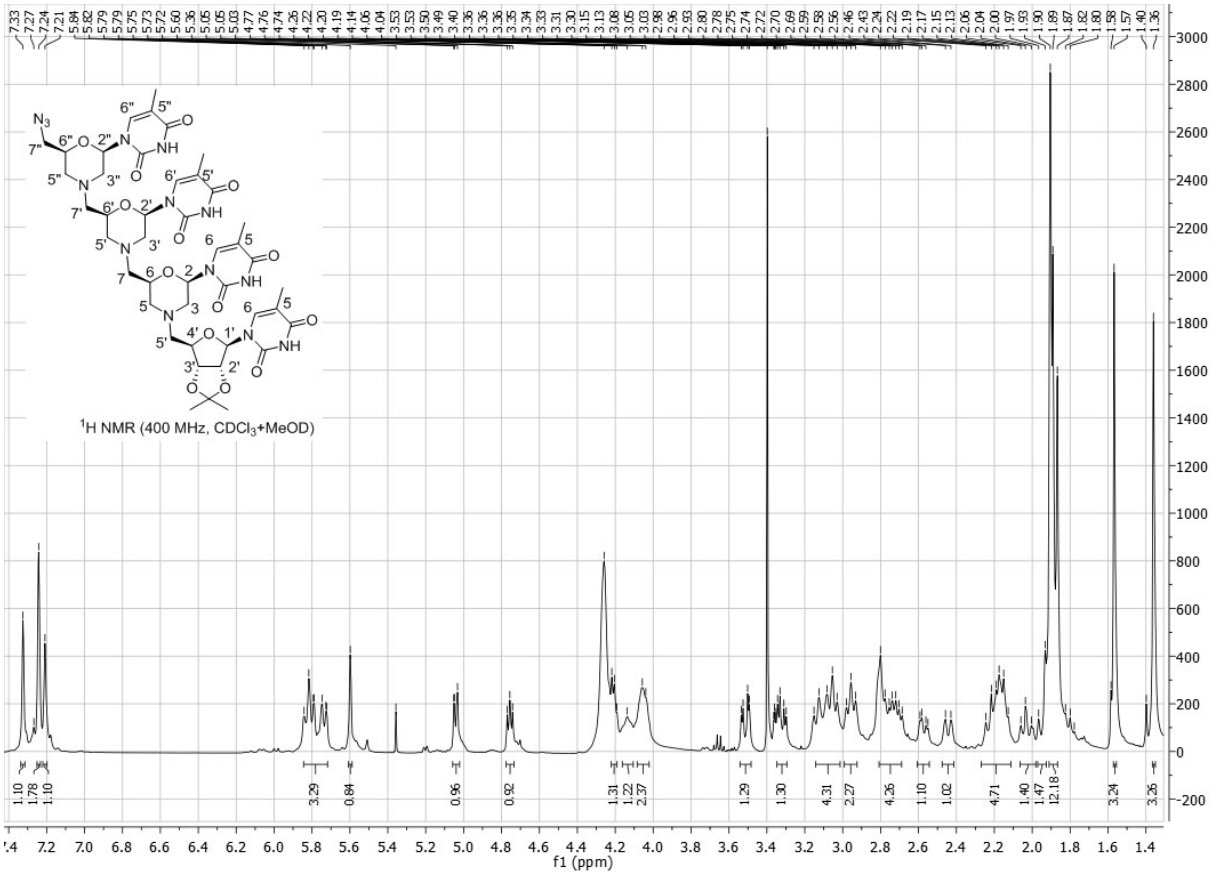


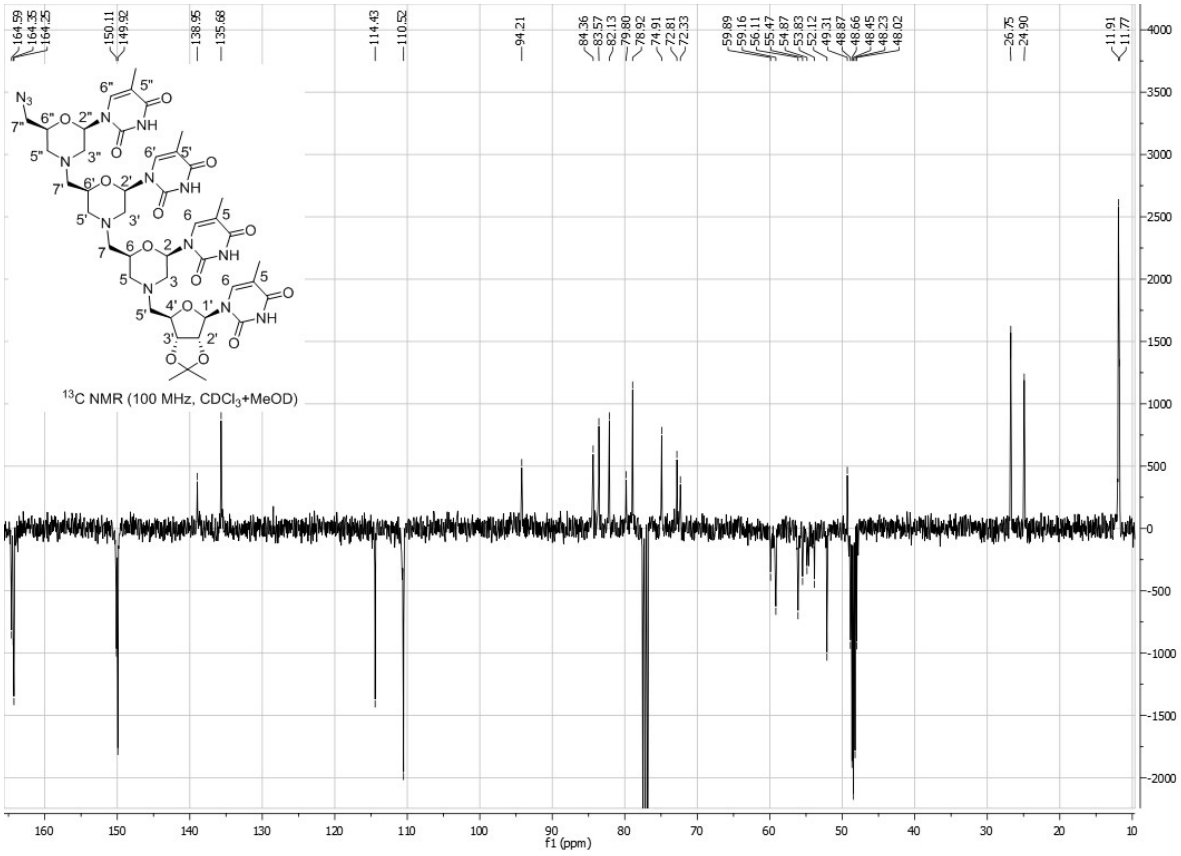


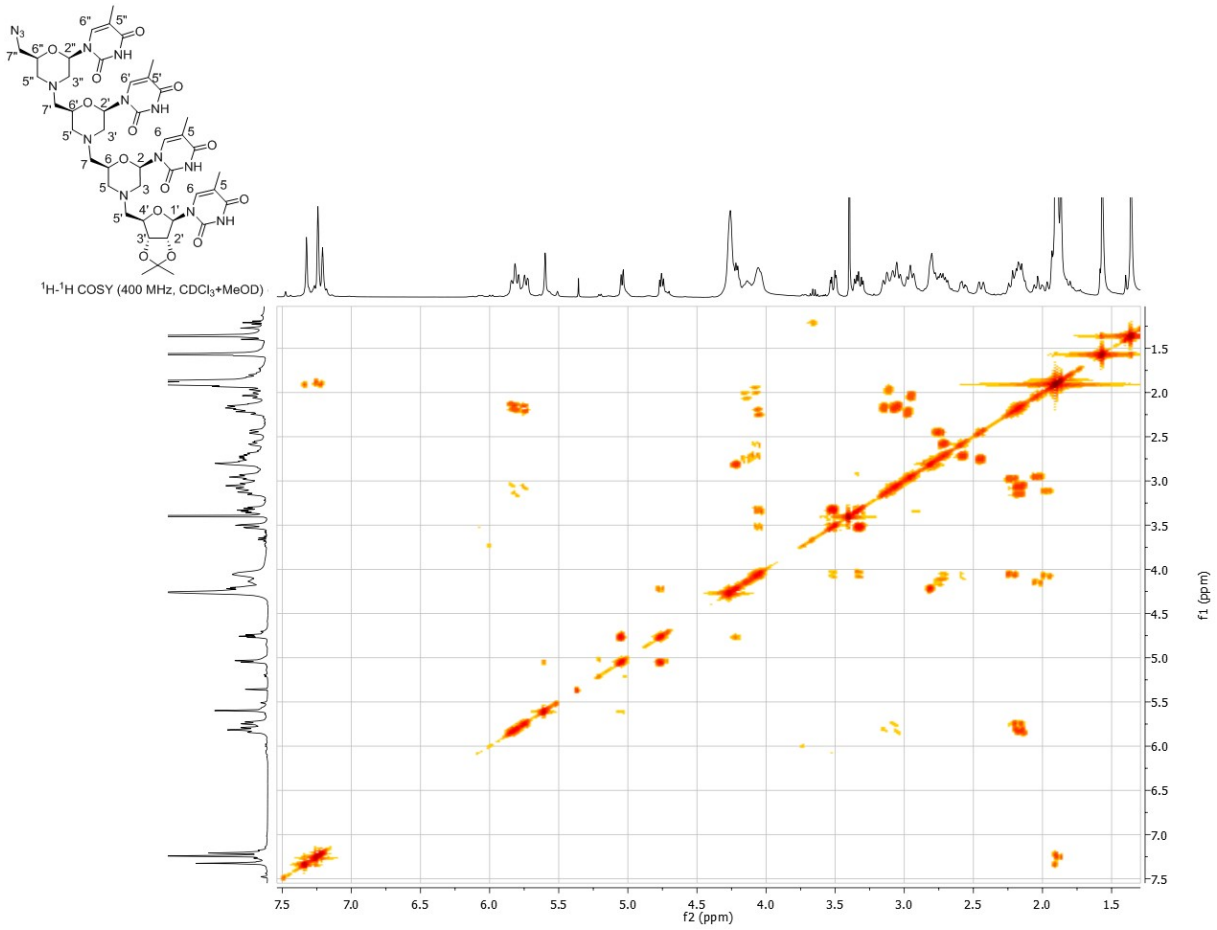

^{1}H-^{1}H COSY (400 MHz, MeOD)
f2 (ppm)
f1 (ppm)

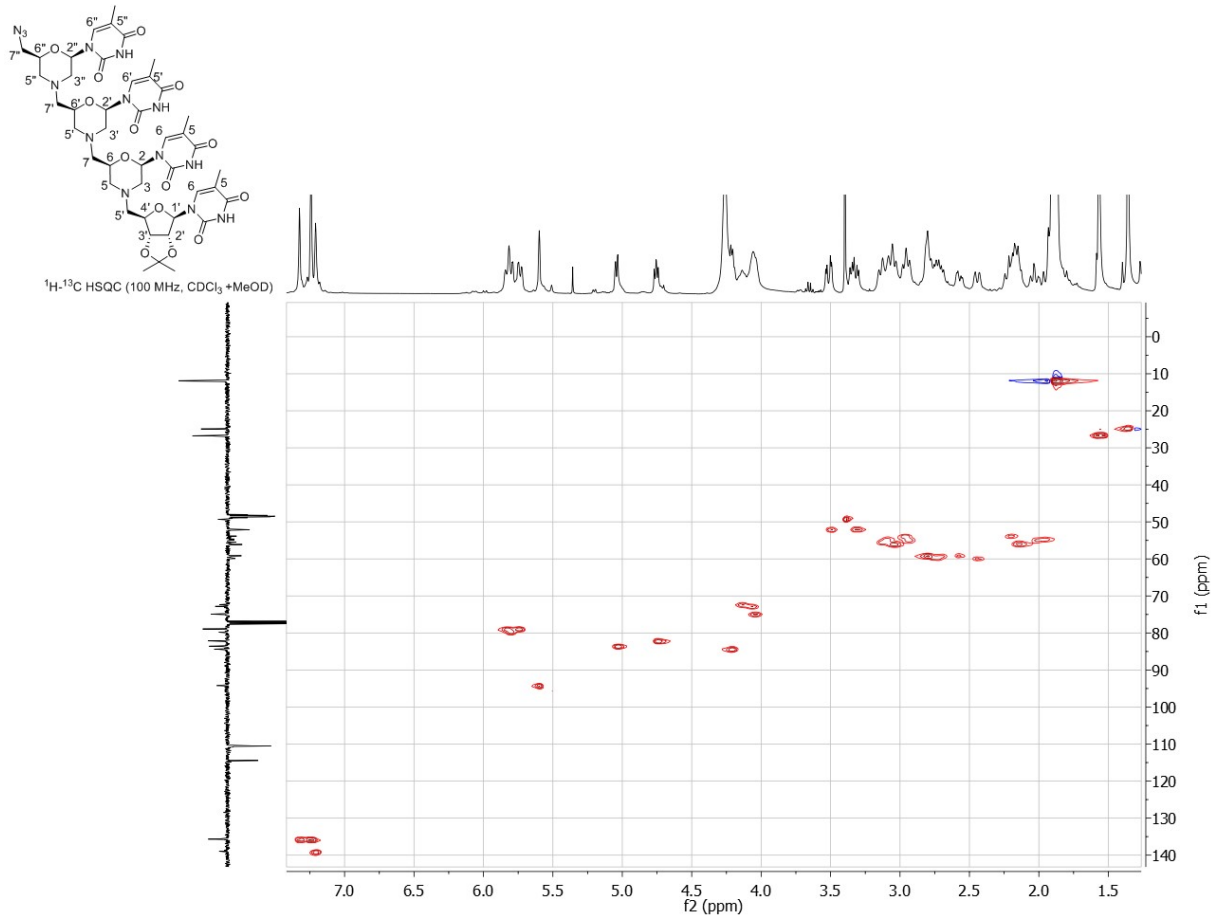

^{1}H-^{13}C HSQC (100 MHz, MeOD)
f2 (ppm)
f1 (ppm)

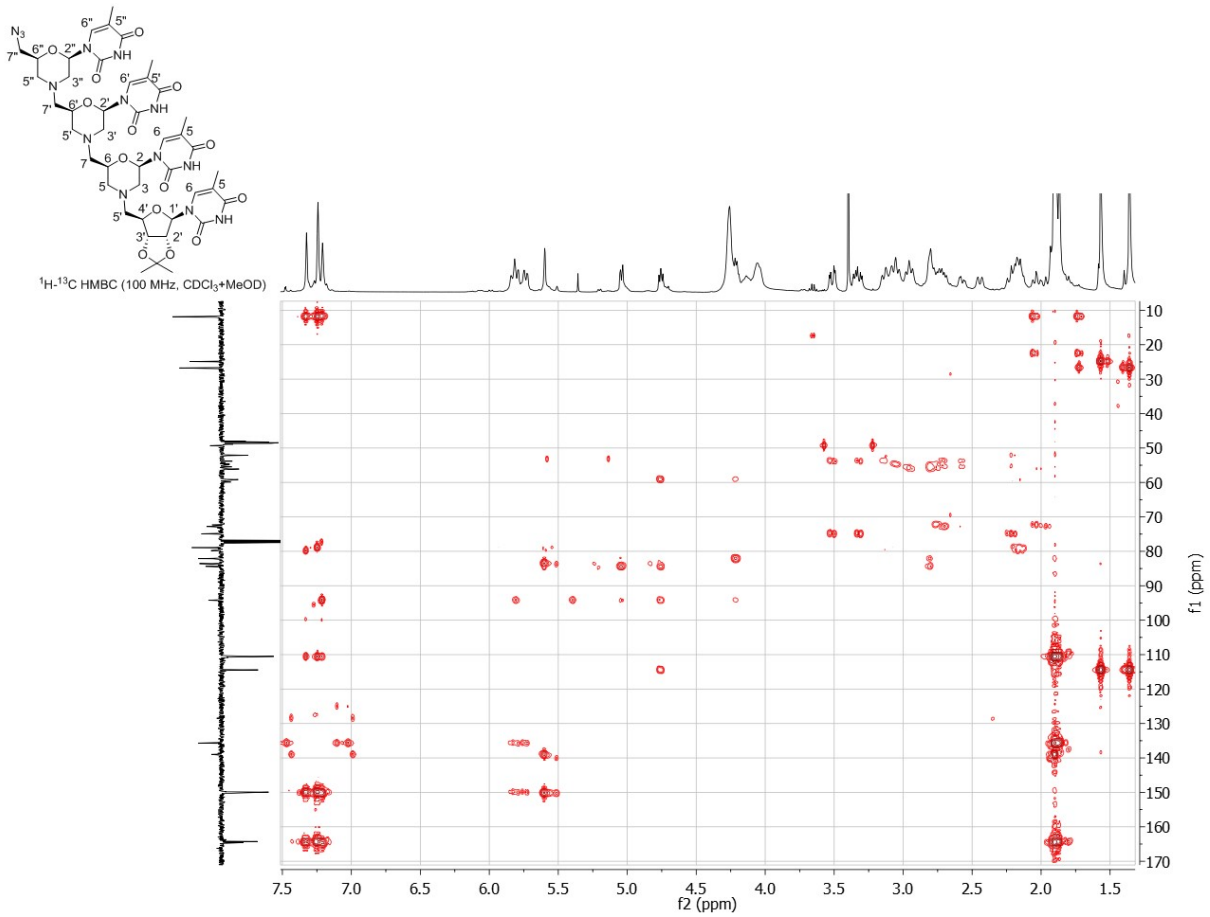
NMR spectra of compound 45

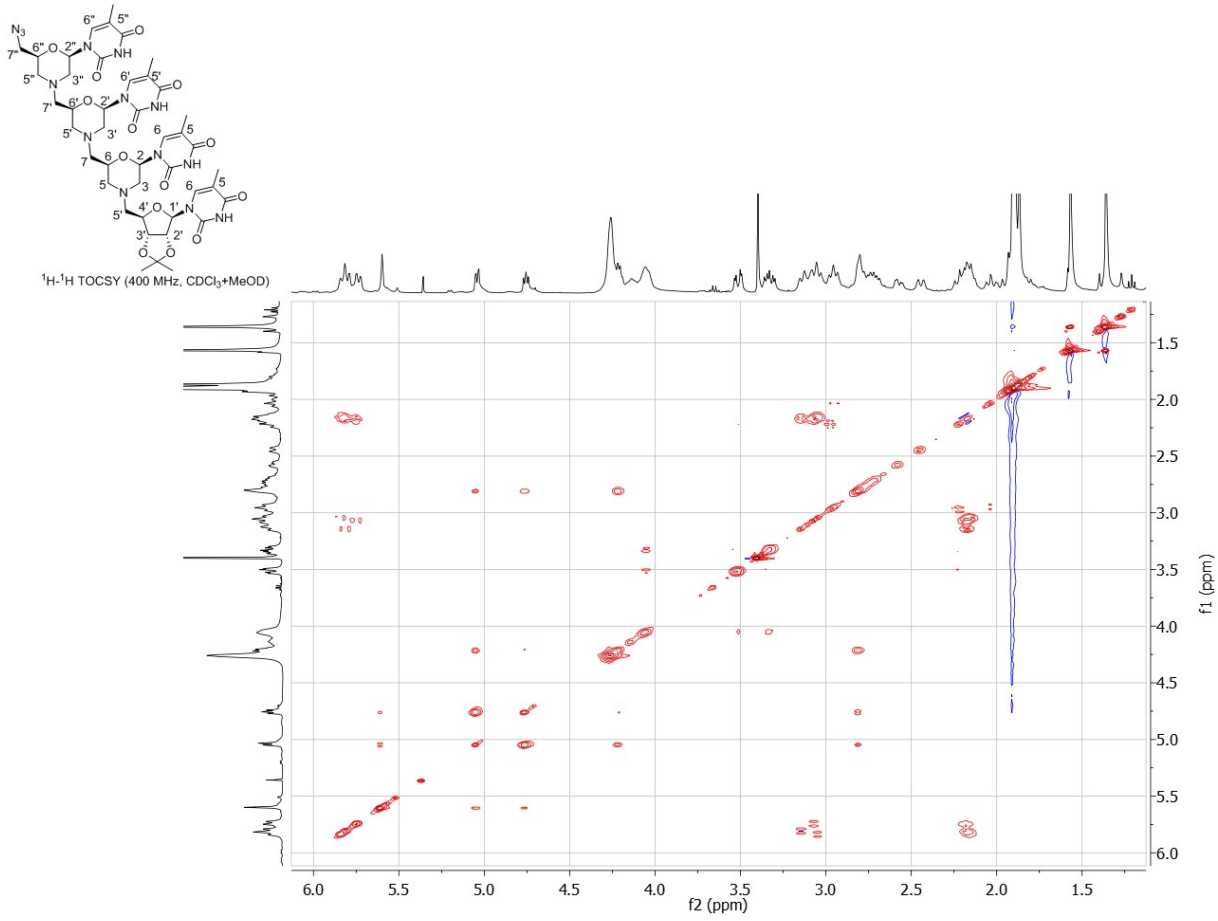




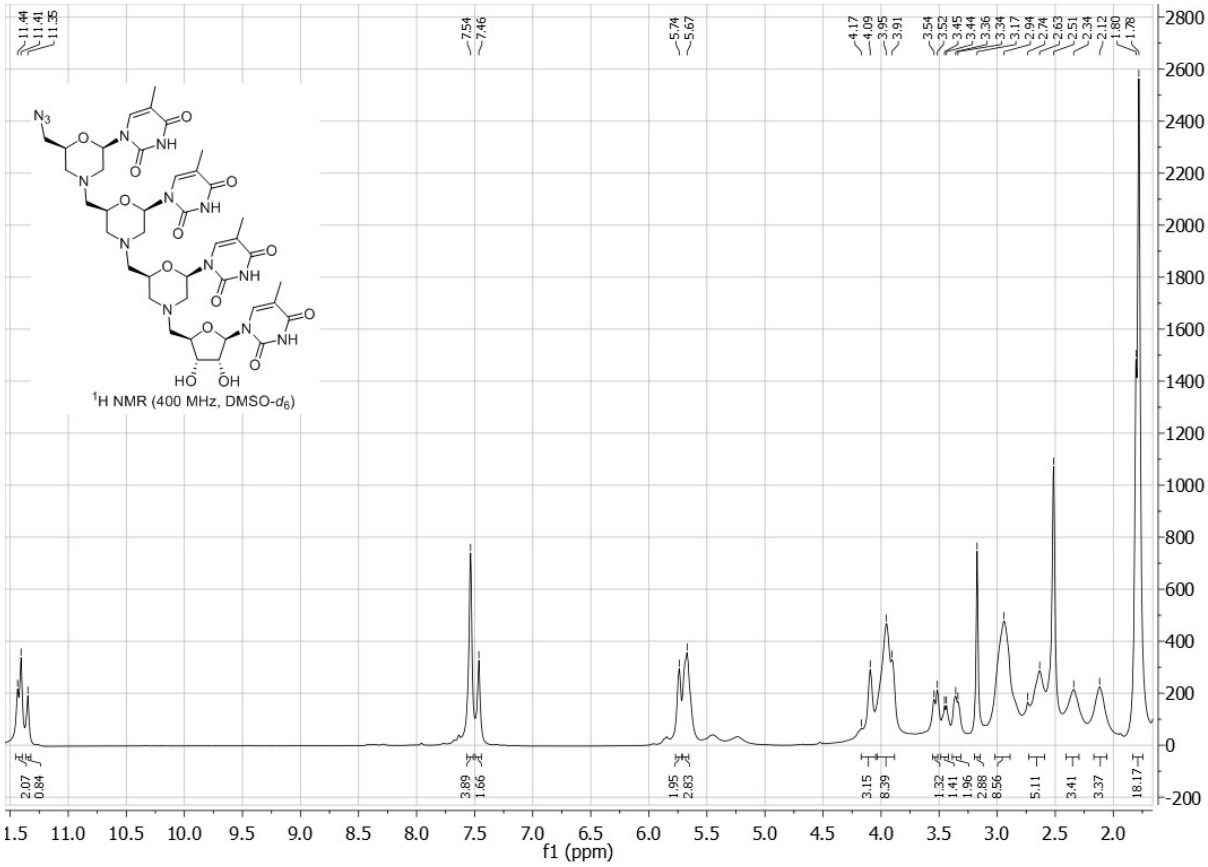

^{1}H-^{1}H COSY (400 MHz, CDCl$_3$+MeOD)
f2 (ppm)
f1 (ppm)

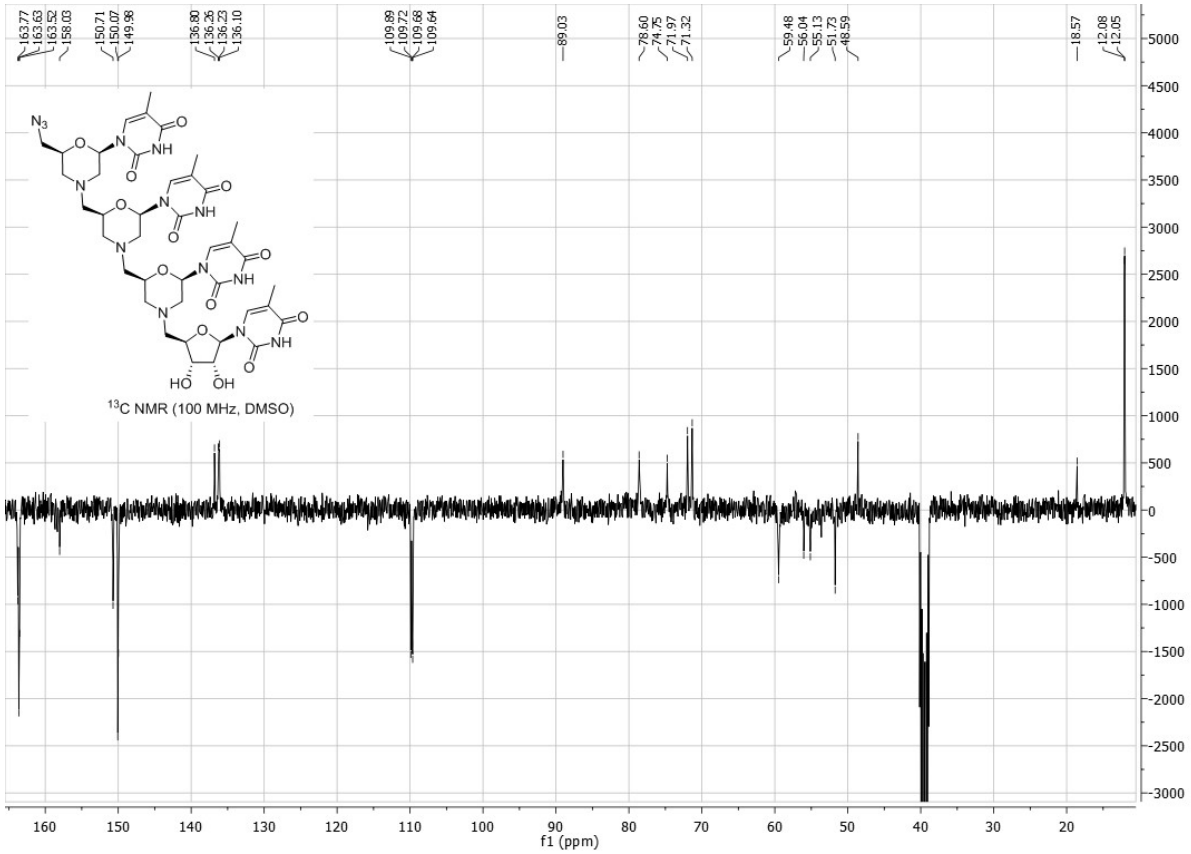

^{1}H-^{13}C HSQC (100 MHz, CDCl$_3$ +MeOD)
f2 (ppm)
f1 (ppm)

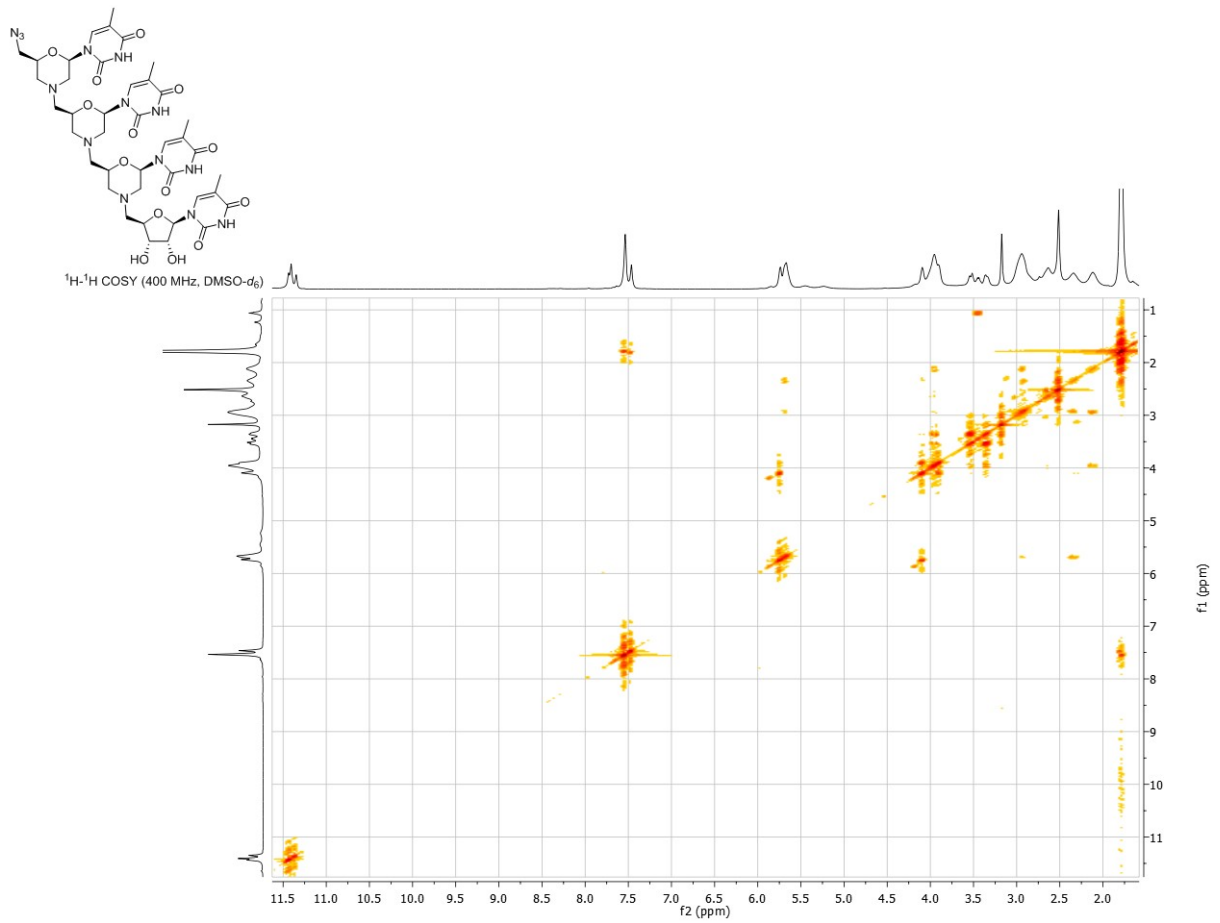

^{1}H-^{13}C HMBC (100 MHz, CDCl$_3$+MeOD)
f2 (ppm)
f1 (ppm)

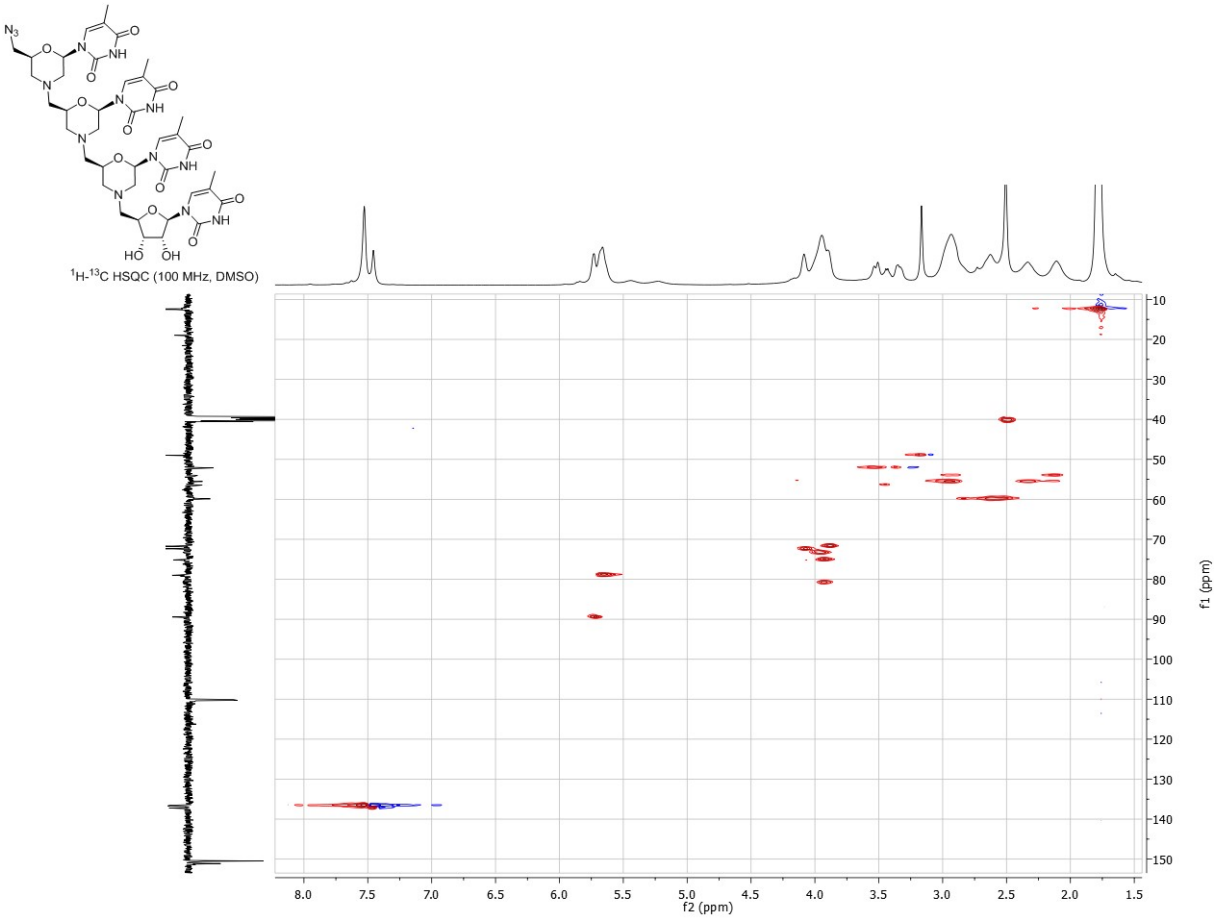

^{1}H-^{1}H TOCSY (400 MHz, CDCl$_3$+MeOD)
f2 (ppm)
f1 (ppm)

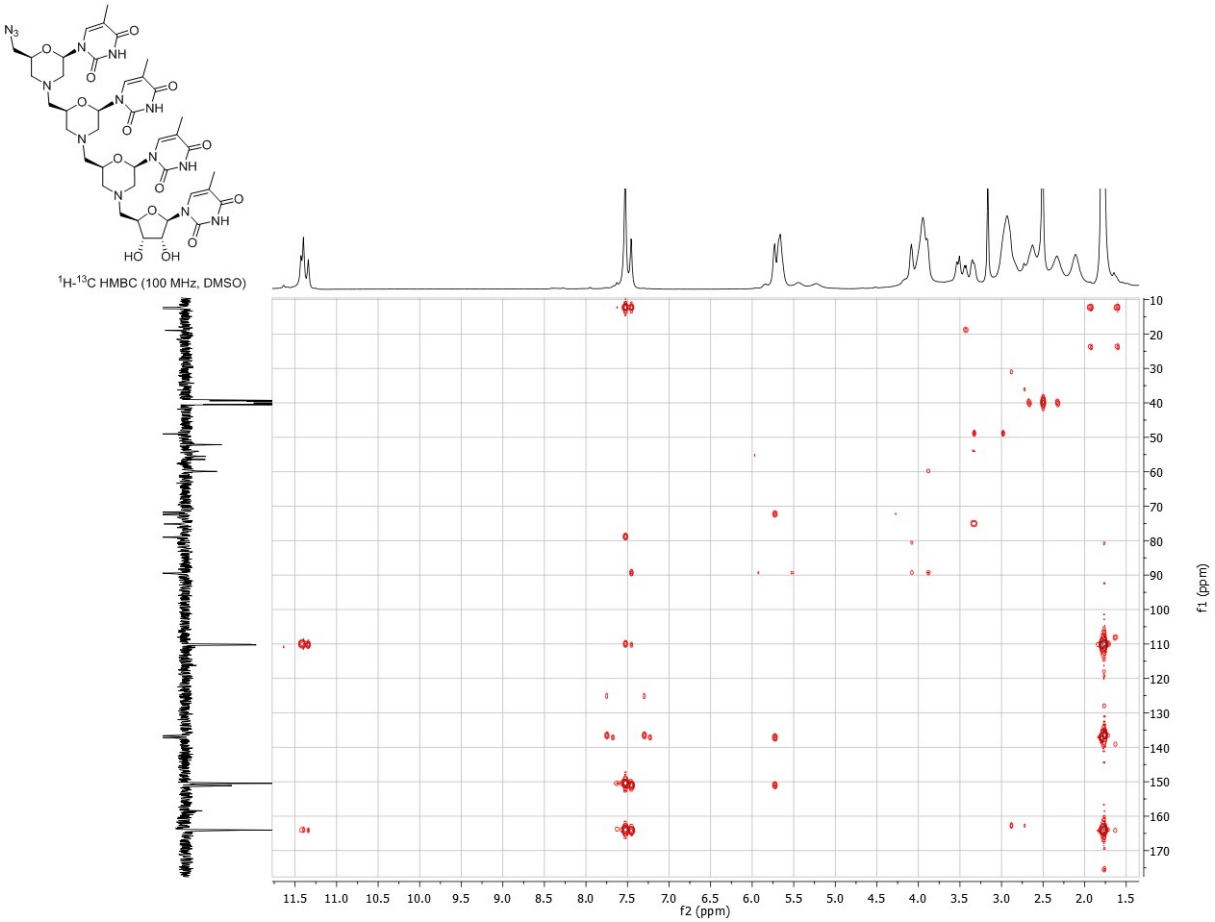
NMR spectra of compound 46

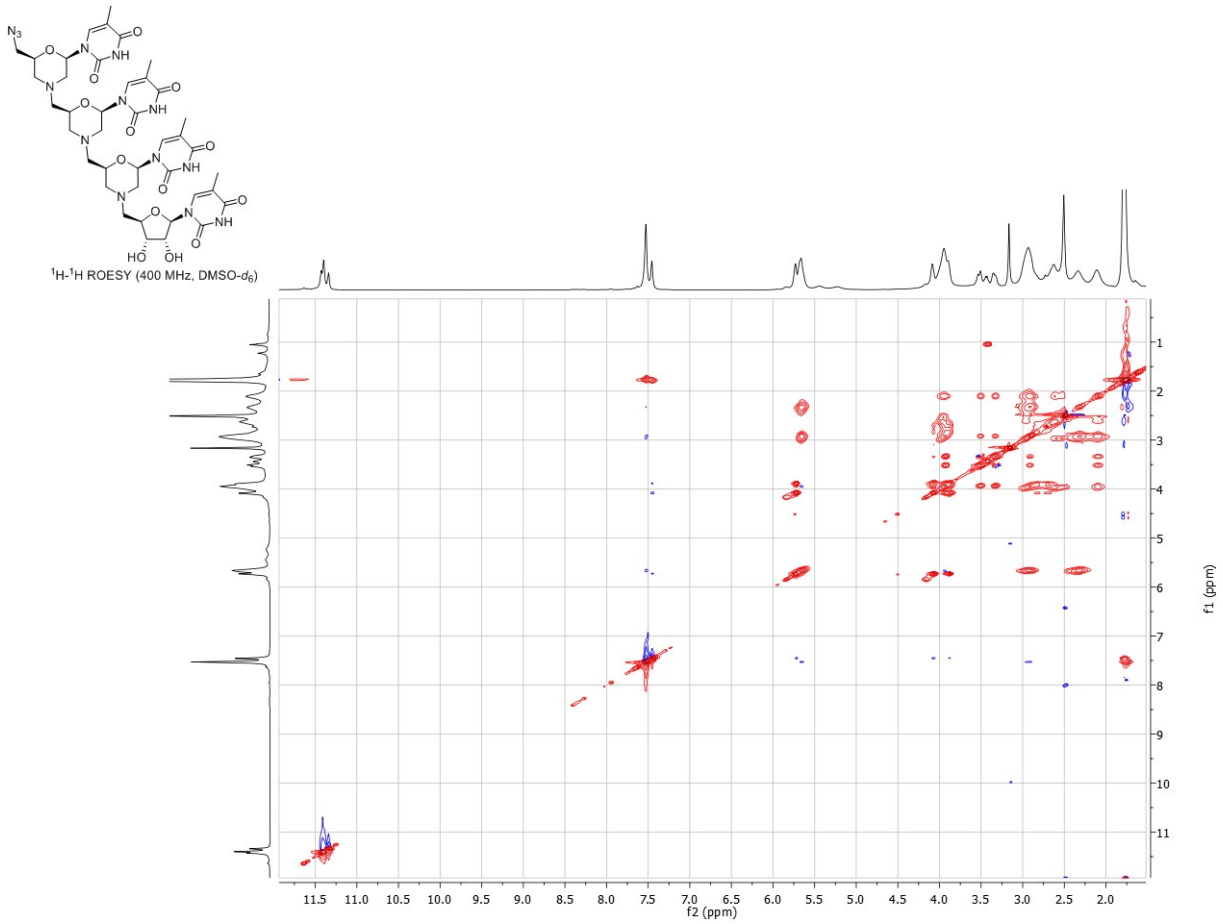




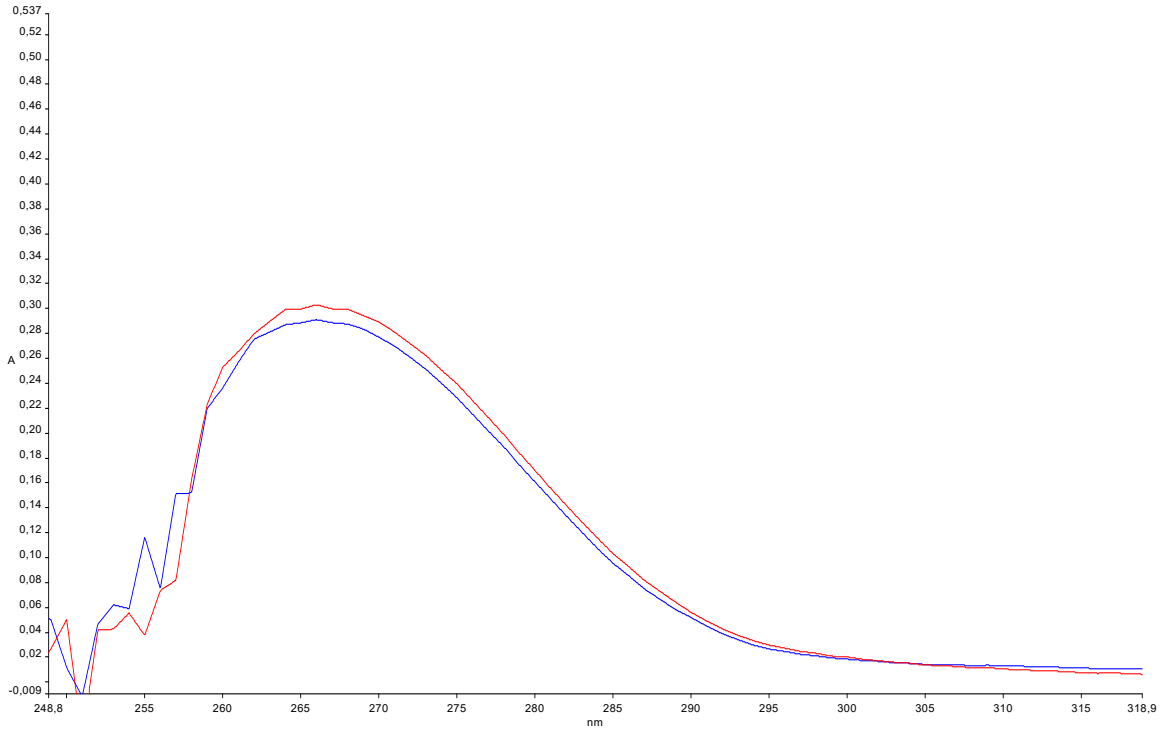

^{1}H-^{1}H COSY (400 MHz, DMSO-d_6)
f2 (ppm)
f1 (ppm)


^{1}H-^{13}C HSQC (100 MHz, DMSO)
f2 (ppm)
f1 (ppm)


^{1}H-^{13}C HMBC (100 MHz, DMSO)
f2 (ppm)
f1 (ppm)


N3
O
NH
HO
OH
1H-1H ROESY (400 MHz, DMSO-d6)
f2 (ppm)
f1 (ppm)

UV spectra of compound **15** in forms of TFA salt and free base



15 TFA salt; **15 free base**

Figure S2. Superimposed UV-VIS spectra of **15-TFA salt** and **15-free base**