Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


# Supporting Information

## Studies on sugar puckering and glycosidic stabilities of 3'-amino-5'-carboxymethyl-3',5'-dideoxy nucleoside mimics

Chandan Pal, Arventh Velusamy, Yashoda Krishna Sunkari and Tushar Kanti Chakraborty*

Department of Organic Chemistry, Indian Institute of Science, Bengaluru 560012, India

*tushar@iisc.ac.in*

## Table of Contents:

## 1. General experimental details:

All the reactions were carried out under an inert atmosphere in oven-dried glassware using dry solvents, unless otherwise stated. All the chemicals purchased from commercial suppliers were used as received unless otherwise stated. Reactions and chromatography fractions were monitored by Merck silica gel 60 F-254 glass TLC plates and visualized using UV light, 2.5% ethanolic anisaldehyde (with 1% AcOH and 3.3% conc. $H_2SO_4$) and ninhydrin or chlorine/o-tolidine-heat as developing agents. Flash column chromatography was performed with 60–120 mesh silica gel and the yields reported herein refer to chromatographically and spectroscopically pure compounds.

**Instrumentation:**

The NMR spectra were recorded in $D_2O$, $CDCl_3$ or DMSO-$d_6$ on 400, 700 or 800 MHz instruments at 300 K(unless otherwise mentioned) and were calibrated to the residual solvent peaks [$CHCl_3$ 7.26 ppm and 77.0 ppm, DMSO 2.50 ppm and 40.0 ppm, $D_2O$ 4.79 ppm or (5.00 ppm at 278K)]. Herein, multiplicities are abbreviated as: brs = broad singlet; s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet. All IR data were recorded as a neat liquid or as KBr pellets using a PerkinElmer RXIFTIR spectrophotometer. Mass spectra were obtained under electron spray ionisation (ESI), while HRMS spectra were taken with a 3000 mass spectrometer using a Waters Agilent 6520-Q-TOFMS/ MS system and JEOL-AccuTOF JMS-T100 LC. RP-HPLC was performed on a Shimadzu ISO 9001 HPLC system (model no. LC-20AD) equipped with a 5 µ Shimadzu's C18 column (4.6 × 250 mm) and 5 µ Shimadzu's C18 column (20 × 250 mm) in combination with eluants A ($H_2O$, 0.1% TFA) and B (MeCN, 0.1% TFA) with a flow rate of 1 mL/min and 10 mL/min respectively and a photodiode array detector setting of λ = 210–270 nm.

## 2. Experimental procedures and data for the compounds:

**General procedure for acylation:** Triphenylphosphine (2 equiv.) was added to the 3'-azido-3'-deoxy nucleoside derivatives[10c] (1 equiv.) in MeOH at room temperature and stirred at the same temperature for 20 mins. To the same reaction mixture $Ac_2O$ (1.5 equiv.) and $Et_3N$ (1.5 equiv.) were added and stirred overnight at room temperature. Solvent was removed under reduced pressure and the residue was purified by silica gel chromatography.

**Data for compound 1:** Scale of reaction 24 mg, 0.06 mmol; chromatographic purification (4-8 % MeOH in EtOAc) afforded **1** (20 mg, 89%) as a solid foam. $\mathbf{R_f}$ = 0.33 ($SiO_2$, 16% MeOH in $CHCl_3$); **RP-HPLC,** $\mathbf{t_R}$ = 21.8 mins (initial 5 mins 5% B then linear gradient 5->100% B in 20 mins); $\mathbf{[\alpha]_D}$ = 15.78 (c 0.58, MeOH); **IR (neat):** $\mathbf{\nu_{max}}$ 3655, 2927, 2857, 2365, 1743, 1546, 1454, 1169 $cm^{-1}$; **$^1$H NMR ( DMSO-$d_6$, 400 MHz)**: $\delta$ 8.28 (s, 1H), 8.15 (s, 1H), 7.85 (d, $J$ = 8.40 Hz, 1H), 7.28 (s, 2H), 5.97 (d, $J$ = 4.7 Hz, 1H), 5.88 (d, $J$ = 2.6 Hz, 1H), 4.55 (t, $J$ = 6.4 Hz, 1H), 4.45 (dd, $J$ = 8.3, 6.4 Hz, 1H), 4.03 - 3.96 (m, 2H), 3.86 (dt, $J$ = 8.1, 4.3 Hz, 1H), 2.43 - 2.30 (m, 2H), 1.99 - 1.80 (m, 5H), 1.12 (t, $J$ = 7.1 Hz, 3H); **$^{13}$C NMR (DMSO-$d_6$, 100 MHz)**: $\delta$ 172.4, 169.5, 156.0, 152.7, 149.0, 139.4, 119.1, 89.5, 80.5, 79.1, 72.8, 59.8, 53.9, 29.9, 28.1, 22.6, 14.0; **HRMS (ESI-Q-TOF)**: calcd for $C_{16}H_{22}N_6O_5Na$ ($[M+Na]^+$) 401.1549, found 401.1546.

**Data for compound 2:** Scale of reaction 108.3 mg, 0.2842 mmol; HPLC purification employing a linear gradient 10 - 100% B in 25 mins at 10 mL/min afforded **2** (40.5 mg, 40%) as a solid foam. **RP-HPLC,** $\mathbf{t_R}$ = 16.21 mins (initial 5 mins, 5% B and then linear gradient 5 - 100% B in 20 mins); $\mathbf{[\alpha]_D}$ = 67.61 (c 0.45, MeOH); **IR (neat):** $\mathbf{\nu_{max}}$ 3642, 2928, 2583, 2364, 2077, 1665, 1457, 1016 $cm^{-1}$; **$^1$H NMR ( DMSO-$d_6$, 400 MHz)**: $\delta$ 11.36 ( brs, 1H), 7.81 (d, $J$ = 7.8 Hz, 1H), 7.62 (d, $J$ = 8.1 Hz, 1H), 5.86 (d, $J$ = 2.8 Hz, 1H), 5.65 - 5.63 (m, 2H), 4.15 - 4.06 (m, 2H), 4.06 - 4.0 (m, 2H), 3.8 - 3.72 (m, 1H), 2.45 - 2.30 (m, 2H), 1.95 - 1.78 (m, 5H), 1.16 (t, $J$ = 7.1 Hz,

3H); **$^{13}$C NMR (DMSO-$d_6$, 100 MHz)**: 172.5, 169.6, 163.2, 150.4, 141.4, 101.9, 91.2, 80.2, 79.2, 72.6, 59.9, 53.6, 30.0, 27.9, 22.7, 14.1; **HRMS (ESI-Q-TOF):** calcd for $C_{15}H_{21}N_3O_7Na$ ($[M+Na]^+$) 378.1277, found 378.1275.

**Data for compound 3:** Scale of reaction 108.2 mg, 0.2847 mmol; chromatographic purification (3-10 % MeOH in EtOAc) afforded **3** (42.3 mg, 42%) as a solid foam. **$R_f$** = 0.21 ($SiO_2$, 14% MeOH in $CHCl_3$); **RP-HPLC, $t_R$** = 19.23 mins (initial 5 mins, 5% B and then linear gradient 5 - 100% B in 20 mins); **$[\alpha]_D$** = 81.43 (c 1.73, MeOH); **IR (neat): $\nu_{max}$** 3387, 2510, 2131, 1650, 1454, 1282, 1200, 1017 $cm^{-1}$; **$^1$H NMR (800 MHz, DMSO-$d_6$):** $\delta$ 7.78 (d, $J$ = 8.2 Hz, 1H), 7.54 (d, $J$ = 7.4 Hz, 1H), 7.24 (brs, 1H), 7.12 (brs, 1H), 5.86 (brs, 1H), 5.76 (d, $J$ = 7.0 Hz, 1H), 5.64 (d, $J$ = 1.7 Hz, 1H), 4.06 - 4.0 (m, 3H), 4.0 - 3.98 (m, 1H), 3.78 (dt, $J$ = 8.7, 4.0 Hz, 1H), 2.45 - 2.40 (m, 1H), 2.39 - 2.34 (m, 1H), 1.92 - 1.80 (m, 5H), 1.16 (t, $J$ = 7.1 Hz, 3H); **$^{13}$C NMR (DMSO-$d_6$, 100 MHz):** $\delta$ 172.5, 169.5, 165.7, 155.0, 141.5, 94.2, 92.1, 79.7, 73.3, 59.8, 53.8, 30.2, 27.9, 22.6, 14.1; **HRMS (ESI-Q-TOF):** calcd for $C_{15}H_{22}N_4O_6Na$ ($[M+Na]^+$) 377.1437, found 377.1435.

**Data for compound 4:** Scale of reaction 48.8 mg, 0.074 mmol; solvent was removed under reduced pressure after acylation and the residue was treated with a 2-3 mL of methanol saturated with ammonia and left to stir for 1.5 h. The final residue after solvent removal was purified by HPLC employing a linear gradient 5 - 30% B in 30 mins at 10 mL/min to afford **4** (17.17 mg, 49%) as a solid foam. **RP-HPLC, $t_R$** = 15.57 mins (initial 5 mins, 5% B and then linear gradient 5 - 100% B in 20 mins); **$[\alpha]_D$** = 17.43 (c 0.44, MeOH); **IR (neat): $\nu_{max}$** 3393, 2928, 2860, 2364, 1859, 1650, 1457,1375, 1199, 1016 $cm^{-1}$; **$^1$H NMR (DMSO-$d_6$, 400 MHz):** $\delta$ 10.83 (brs, 1H), 7.89 (d, $J$ = 8.0 Hz, 1H), 7.83 (s, 1H), 6.53 (brs, 2H), 5.93 (brs, 1H), 5.66 (d, $J$ = 3.2 Hz, 1H), 4.43 (dd, $J$ = 6.2, 3.2 Hz, 1H), 4.25 (dd, $J$ = 14.7, 6.6 Hz, 1H), 4.01 (dq, J = 7.1, 2.4 Hz, 2H),

3.81 (dt, *J* = 8.0, 4.3 Hz, 1H), 2.46 - 2.28 (m, 2H), 1.99 – 1.78 (m, 5H), 1.14 (t, *J* = 7.1 Hz, 3H); **$^{13}$C NMR (DMSO-$d_6$, 100 MHz):** 172.5, 169.6, 156.7, 153.7, 150.9, 135.3, 116.7, 88.4, 80.3, 72.7, 59.8, 54.0, 29.9, 28.2, 22.7, 14.0; **HRMS (ESI-Q-TOF):** calcd for $C_{16}H_{22}N_6O_6Na$ ($[M+Na]^+$) 417.1499, found 417.1496.

**Data for compound 9:** Scale of reaction using $N^6$-Bz-3'-azido-3'-deoxy-adenine nucleoside derivative of **7** - 33.2 mg, 0.0578 mmol; chromatographic purification (2-3% MeOH in EtOAc) afforded **9** (26.8 mg, 85.2%) as a solid foam. **$R_f$** = 0.36 ($SiO_2$, 8% MeOH in $CHCl_3$); **$[\alpha]_D$** = -5.2 (c 1.0, MeOH); **IR (neat): $\nu_{max}$** 3581, 3401 2920, 2854, 2554, 2362, 2066, 1994, 1603, 1410, 1219, 1021 $cm^{-1}$; **$^1$H NMR ($CDCl_3$, 400 MHz):** $\delta$ 9.41 (brs, 1H), 8.56 (s, 1H), 8.13 (s, 1H), 7.94 (d, *J* = 7.6 Hz, 2H), 7.54 (t, *J* = 7.2 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 2H), 7.31 - 7.25 (m, 5H), 6.67 (d, *J* = 7.2 Hz, 1H), 6.12 (brs, 1H), 5.99 (d, *J* = 2.1 Hz, 1H), 5.04 (dd, *J* = 12.3, 9.0 Hz, 2H), 4.66 (d, *J* = 4.2 Hz, 1H), 4.42 (dd, *J* = 14.1, 6.2 Hz, 1H), 4.07 (dt, *J* = 8.5, 3.5 Hz, 1H), 2.61 - 2.44 (m, 2H), 2.24 - 2.14 (m, 1H), 2.10 - 1.99 (m, 1H), 1.92 (s, 3H); **$^{13}$C NMR ($CDCl_3$, 100 MHz):** $\delta$ 173.1, 171.1, 165.3, 152.4, 151.0, 149.4, 141.7, 135.9, 133.3, 133.1, 129.0, 128.6, 128.3, 128.0, 123.3, 91.0, 82.4, 74.4, 66.5, 54.3, 30.6, 28.5, 23.3; **HRMS (ESI-Q-TOF):** calcd for $C_{28}H_{28}N_6O_6Na$ ($[M+Na]^+$) 567.1968, found 567.1973.

**Data for compound 10:** Scale of reaction using Bn-ester of 3'-azido-3'-deoxy-uracil nucleoside derivative of **7** - 80.7 mg, 0.1821 mmol; chromatographic purification (1-3 % MeOH in EtOAc) afforded **10** (26.8 mg, 53%) as a solid foam. **$R_f$** = 0.32 ($SiO_2$, 8% MeOH in $CHCl_3$); **$[\alpha]_D$** = 49.75 (c 1.9, MeOH); **IR (neat):** $\nu_{max}$ 3309, 2926, 2389, 2291, 1684, 1544, 1380, 1264, 1165, 1098 $cm^{-1}$; **$^1$H NMR ($CDCl_3$, 400 MHz):** $\delta$ 10.52 (brs, 1H), 7.58 (d, *J* = 8.2 Hz, 1H), 7.36 - 7.29 (m, 5H), 6.86 (d, *J* = 6.9 Hz, 1H), 5.74 (d, *J* = 8.1 Hz, 1H), 5.67 (s, 1H), 5.11 (dd, *J* = 25.7, 13.5 Hz, 2H), 4.28 (d, *J* = 4.0 Hz, 1H), 4.15 - 4.05 (m, 2H), 2.62 - 2.55 (m, 2H), 2.21 - 2.01 (m, 3H), 1.99 (s,

3H); **$^{13}$C NMR ($CDCl_3$, 100 MHz):** $\delta$ 172.9, 171.0, 164.3, 150.8, 139.6, 135.9, 128.6, 128.4, 102.4, 92.8, 82.0, 75.1, 66.6, 53.6, 31.2, 28.3, 23.2; **HRMS (ESI-Q-TOF):** calcd for $C_{20}H_{23}N_3O_7Na$ ($[M+Na]^+$) 440.1434, found 440.1434.

**Data for compound 17a:** Scale of reaction using 2-*N*-acetyl-6-*O*-diphenylcarbamoyl-3'-azido-3'-deoxy-guanine nucleoside derivative of **7 -** 95.2 mg, 0.1324 mmol; chromatographic purification (2-3% MeOH in EtOAc) afforded **17a** (78 mg, 85%) as a solid foam. $\mathbf{R_f}$ = 0.56 ($SiO_2$, 8% MeOH in $CHCl_3$); $\mathbf{[\alpha]_D}$ = 32.27 (c 1.0, MeOH); **IR (neat):** $\mathbf{\nu_{max}}$ 3578, 3411, 2925, 2853, 2718, 2631,2361, 1806, 1737, 1620, 1593, 1451, 1291, 1177, 1024 $cm^{-1}$; **$^1$H NMR (DMSO-$d_6$, 400 MHz**): $\delta$ 10.72 (s, 1H), 8.63 (s, 1H), 7.95 (d, $J$ = 8.2 Hz, 1H), 7.53 - 7.41 (m, 8H), 7.36 - 7.27 (m, 7H), 6.01 (d, $J$ = 4.8 Hz, 1H), 5.90 (d, $J$ = 3.3 Hz, 1H), 5.03 (dd, $J$ = 15.4, 2.8 Hz, 2H), 4.71 - 4.63 (m, 1H), 4.38 (dd, $J$ = 14.7, 6.8 Hz, 1H), 3.96 - 3.87 (m, 1H), 2.48 - 2.38 (m, 2H), 2.20 (s, 3H), 2.01 - 1.92 (m, 2H), 1.89 (s, 3H); **$^{13}$C NMR (DMSO-$d_6$, 100 MHz):** $\delta$ 172.4, 169.6, 168.9, 155.2, 154.3, 152.3, 150.1, 144.0, 141.6, 136.1, 129.4, 128.4, 127.9, 127.8, 120.2, 89.4, 79.2, 72.4, 65.4, 54.0, 29.9, 28.1, 24.5, 22.7; **HRMS (ESI-Q-TOF):** calcd for $C_{36}H_{35}N_7O_8Na$ ($[M+Na]^+$) 716.2445, found 716.2440.

**General procedure for dimer synthesis:**

The acid fragment produced from by benzyl ester deprotection from the acetamide derivative is coupled to the amine component obtained by converting the azide in the intermediate **7** (1.5 equiv.) to an amine using triphenylphosphine (3 equiv.) in MeOH. To the acid fragments were then added HATU (1.5 equiv.), HOBt (1.5 equiv.) and DIPEA (5 equiv.) in a mixture of DMF and DCM (1:4) at 0 °C followed by the addition of amine dissolved in minimum amount of the same solvent mixture. The reaction mixture was stirred overnight under inert atmosphere to produce the dimer which after coupling was treated with saturated methanolic ammonia solution

to remove the nucleobase protecting groups with concomitant trans-esterification of benzyl ester to the methyl ester in the C-terminus to furnish the desired dimer. Solvent was removed under reduced pressure and the residue was purified by HPLC.

**Synthesis of compound 5:** The acylated compound **9** (30 mg, 0.055 mmol) was taken in dry MeOH along with Pd/C (50% wt) at 0 °C temperature. To the same reaction mixture $Et_3SiH$ (160 µL, 1.023 mmol) was added and stirred overnight at room temperature. The reaction mixture was filtered through Celite and the solvent was removed under reduced pressure and the residue was stirred with HOBt (12.6 mg, 0.083 mmol), HBTU (31.3 mg, 0.083 mmol) and DIPEA (50 µl, 0.276 mmol) in a mixture of DMF and DCM (1:4 ratio) at 0 °C under inert atmosphere. Then compound **11** which was synthesized by treating uracil nucleoside azide derivative of **7** (36.6 mg, 0.0827 mmol) with triphenylphosphine (43.3 mg, 0.165 mmol) in MeOH at r.t. for 20 mins, was concentrated, dried under vacuum and transferred under inert atmosphere using the same solvent mixture to the activated acid solution. The coupling reaction mixture was allowed to stir overnight under room temperature. Solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (2-12% MeOH in EtOAc) to afford the coupled dimer as a solid foam. This was treated with a 2-3 mL of methanol saturated with ammonia and left to stir for 12 h at room temperature. After solvent evaporation under reduced pressure, the residue was purified by HPLC (0-25% in 42 mins in $CH_3CN$/water as mobile phase solvent) to afford the compound **5** (12.6 mg, 35%). **RP-HPLC, $t_R$** = 34.49 mins (initial 3 mins, 0% B and then linear gradient 0 - 25% B in 42 mins); **$[\alpha]_D$** = -8.0 (c 0.05, MeOH); **IR (neat): $\nu_{max}$** 3662, 3337, 2892, 2260, 2126, 1986, 1673, 1047 $cm^{-1}$; **$^1$H NMR (DMSO-$d_6$, 400 MHz**): $\delta$ 11.34 (brs, 1H), 8.30 (s, 1H), 8.16 (s, 1H), 7.90 (d, $J$ = 8.4 Hz, 1H), 7.78 (d, $J$ = 7.6 Hz, 1H), 7.59 (d, $J$ = 8.2 Hz, 1H), 7.28 (s, 2H), 6.00 (brs, 1H), 5.88 (d, $J$ = 2.7 Hz, 1H), 5.83 (brs, 1H), 5.65 - 5.60 (m, 2H), 4.57 -

4.52 (m, 1H), 4.46 - 4.38 (m, 1H), 4.13 - 4.05 (m, 2H), 3.90 - 3.83 (m, 1H), 3.77 - 3.68 (m, 1H), 3.56 (s, 3H), 2.46 - 2.28 (m, 4H), 2.25 - 2.16 (m, 1H), 1.90 (s,3H), 1.88-1.76 (m, 3H); **$^1$H NMR (10% $D_2O$ in $H_2O$, 700 MHz, 298 K):** $\delta$ 8.36 (s, 1H), 8.34 (s, 1H), 8.26 (d, $J$ = 8.4 Hz, 1H), 8.03 (d, $J$ = 8.6 Hz, 1H), 7.57 (d, $J$ = 8.4 Hz, 1H), 6.05 (d, $J$ = 1.60 Hz, 1H), 5.83 (d, $J$ = 8.51 Hz, 1H), 5.59 (s, 1H), 4.18 - 4.12 (m, 2H), 4.10 - 4.06 (m, 1H), 3.88 - 3.83 (m, 1H), 3.60 (s, 3H), 2.53 - 2.39 (m, 5H), 2.15 - 2.09 (m, 1H), 2.04 (s, 3H), 1.94-1.82 (m, 2H); **$^{13}$C NMR** (**DMSO-$d_6$, 100 MHz**): $\delta$ 172.8, 171.9, 169.5, 156.0, 152.7, 150.4, 149.0, 141.2, 139.3, 120.6, 119.1, 101.9, 91.0, 89.4, 80.9, 80.0, 72.8, 72.5, 54.0, 53.6, 51.3, 31.6, 29.7, 28.9, 27.8, 22.7; **HRMS (ESI-Q-TOF):** calcd for $C_{26}H_{33}N_9O_{10}$ ([M+Na]$^+$) 654.2246, found 654.2297.

**Synthesis of compound 6:** The acylated compound **10** (34.6 mg, 0.083 mmol) was taken in dry MeOH along with Pd/C (50% wt) at 0 °C temperature. To the same reaction mixture $Et_3SiH$ (190 µL, 1.663 mmol) was added and stirred overnight at room temperature. The reaction mixture was filtered through Celite and the solvent was removed under reduced pressure and the residue was stirred with HOBt (19 mg, 0.125 mmol), HBTU (47.2 mg, 0.0125 mmol) and DIPEA (72 µL, 0.42 mmol) in a mixture of DMF and DCM (1:4 ratio) at 0 °C under inert atmosphere. Compound **12** which was synthesized by treating 6-*N*-Bz-adenine nucleoside azide derivative of **7** (71.1 mg, 0.125 mmol) with triphenylphosphine (65.3 mg, 0.25 mmol) in MeOH at r.t. for 20 mins, was concentrated, dried under vacuum and transferred under inert atmosphere using the same solvent mixture to the activated acid solution. The coupling reaction mixture was allowed to stir overnight under room temperature. Solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (2-12% MeOH in EtOAc) to afford the coupled dimer as a solid foam. This was treated with a 2-3 mL of methanol saturated with ammonia and left to stir for 12 h at room temperature. After evaporation of the solvent under

reduced pressure, the residue was purified by hplc (3-30% in 27 mins in $CH_3CN$/water as mobile phase solvent) to afford the compound **6** (17.4 mg, 32%). **RP-HPLC, $t_R$** = 26.42 mins (initial 3 mins, 3% B and then linear gradient 3 - 30% B in 27 mins); **$[\alpha]_D$** = 16. 85 (c 1.0, MeOH); **IR (neat): $\nu_{max}$** 3662, 3337, 2892, 2260, 2126, 1986, 1673, 1047 $cm^{-1}$; **$^1$H NMR (DMSO-$d_6$, 400 MHz)**: $\delta$ 11.35 (brs, 1H), 8.29 (s, 1H), 8.15 (s, 1H), 7.85 (dd, $J$ = 8.2, 14.4 Hz, 2H), 7.62 (d, $J$ = 8.0 Hz, 1H), 7.30 (s, 2H), 5.97 (brs, 1H), 5.89 - 5.84 (m, 2H), 5.67 - 5.63 (m, 2H), 4.59 - 4.54 (m, 1H), 4.49 - 4.42 (m, 1H), 4.13 - 4.09 (m, 2H), 3.90 - 3.83 (m, 1H), 3.80 - 3.73 (m, 1H), 3.53 (s, 3H), 2.46 - 2.31 (m, 3H), 2.29 - 2.20 (m, 1H), 1.97 - 1.90 (m, 2H), 1.88 (s, 3H), 1.86 - 1.73 (m, 2H); **$^1$H NMR (10% $D_2O$ in $H_2O$ at 278K, 800 MHz):** $\delta$ 8.06 (s, 1H), 8.05 (s, 1H), 7.94 (d, $J$ = 8.4 Hz, 1H), 7.83 (d, $J$= 3.84 Hz, 1H), 7.34 (d, $J$ = 9.0 Hz, 1H), 5.76 (s, 1H), 5.50 (d, $J$ = 9.1 Hz, 1H), 5.40 (s, 1H), 4.09 (d, $J$ = 6.0 Hz, 1H), 3.89 - 3.83 (m, 2H), 3.77 - 3.72 (m, 1H), 3.26 (s, 3H), 2.23 - 2.16 (m, 5H), 1.80 - 1.76 (m, 2H), 1.73 (s, 3H), 1.72 - 1.67 (m, 2H); **$^{13}$C NMR (DMSO-$d_6$, 100 MHz):** $\delta$ 173.0, 172.0, 169.6, 163.1, 155.3, 150.4, 141.2, 139.8, 131.2, 128.2, 127.4, 102.0, 91.0, 89.5, 80.6, 80.5, 72.9, 72.5, 53.9, 53.8, 51.3, 31.7, 29.7, 28.7, 28.1, 22.7; **HRMS (ESI-Q-TOF):** calcd for $C_{26}H_{33}N_9O_{10}$ ($[M+Na]^+$) 654.2246, found 654.2254.

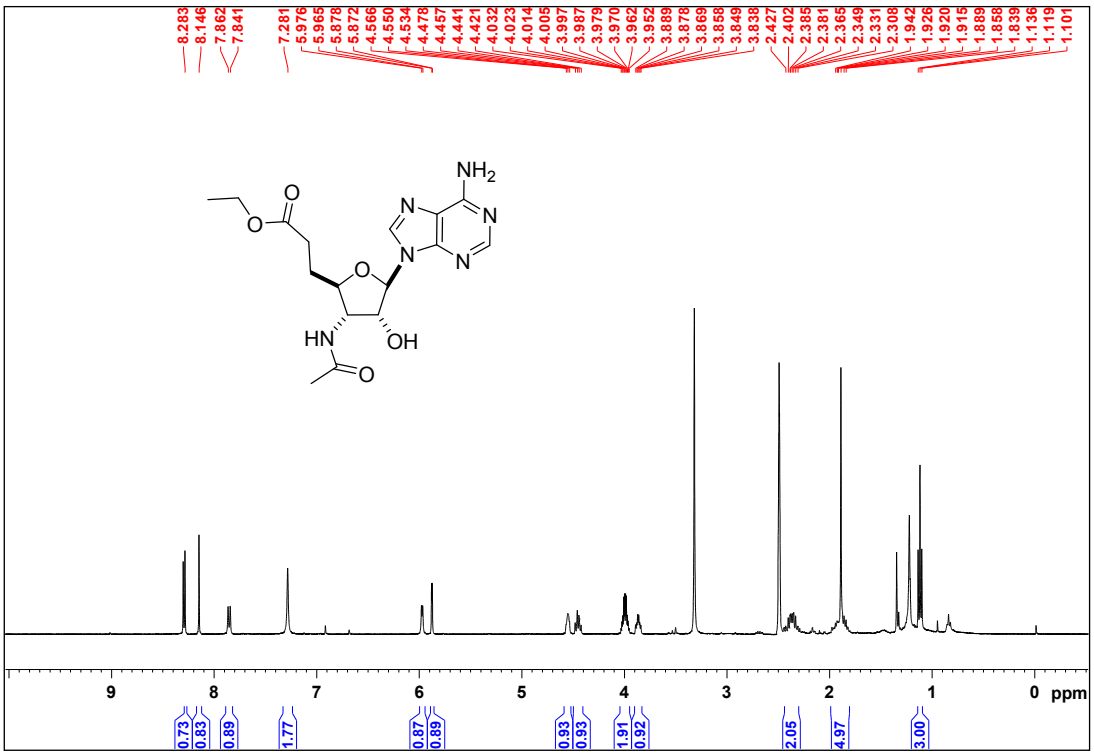
**Synthesis of compound 16:** The acylated compound **9** (23.5 mg, 0.04 mmol) was taken in dry MeOH along with Pd/C (50% wt) at 0 °C temperature. To the same reaction mixture $Et_3SiH$ (130 µL, 0.802 mmol) was added and stirred overnight at room temperature. The reaction mixture was filtered through Celite and the solvent was removed under reduced pressure and to the residue was added 2-3 mL of methanol saturated with ammonia and left to stir for 12 h at room temperature. After solvent evaporation under reduced pressure, HPLC purification of the residue (3-13% in 10 mins in $CH_3CN$/water as mobile phase solvent) afforded **16** (10 mg, 71%) as a white solid. **RP-HPLC, $t_R$** = 11.88 mins (Initial 3 mins, 2.5% B and then linear gradient 2.5 -

100% B in 20 mins); **[α]$_D$** = 5.7 (c 0.1, MeOH); **IR (neat)**: $\nu_{max}$ 3887, 3572, 2982, 1984, 1692, 1217 cm$^{-1}$; **$^1$H NMR ($D_2O$, 400 MHz):** $\delta$ 8.44 (brs, 1H), 8.43 (brs, 1H), 6.11 (d, $J$ = 1.6 Hz, 1H), 4.65 - 4.58 (m, 1H), 4.25 - 4.06 (m, 2H), 2.55 - 2.45 (m, 2H), 2.18 - 2.07 (m, 1H), 2.05 (s, 3H), 2.03 - 2.0 (m, 1H); **$^{13}$C NMR ($D_2O$, 100 MHz):** $\delta$ 182.0, 174.5, 162.6, 152.8, 139.8, 118.9, 89.4, 81.1, 73.5, 54.7, 48.9, 33.5, 29.5, 21.9; **HRMS (ESI-Q-TOF**): calcd for $C_{14}H_{18}N_6O_5$ ($[M+Na]^+$) 373.1236, found 373.1251.

**Synthesis of compound 17:** The acylated compound **17a** (44 mg, 0.06347 mmol) was taken in dry MeOH along with Pd/C (50% wt) at 0 °C temperature. To the same reaction mixture $Et_3SiH$ (200 μL, 1.269 mmol) was added and stirred overnight at room temperature. Solvent was removed under reduced pressure and to the residue was added 2-3 mL of methanol saturated with ammonia and left to stir for 12 h at room temperature. After solvent evaporation under reduced pressure, hplc purification (3-20% in 19 mins in $CH_3CN$/water as mobile phase solvent) afforded **17** (14 mg, 60%) as a white solid**. RP-HPLC, $t_R$** = 12.05 mins (Initial 3 mins, 1.5% B and then linear gradient 1.5 - 100% B in 20 mins); **[α]$_D$** = 16.6 (c 0.44, MeOH); **IR (neat):** $\nu_{max}$ 3410, 2921, 2851, 2358, 1645, 1202, 1022 cm$^{-1}$; **$^1$H NMR ($D_2O$, 400 MHz):** $\delta$ 7.90 (s, 1H), 5.83 (brs, 1H), 4.70 - 4.64 (m, 1H), 4.52 - 4.46 (m, 1H), 4.13 - 4.06 (m, 1H), 2.39 - 2.18 (m, 2H), 2.06 (s, 3H), 2.04 - 1.91 (m, 2H); **$^{13}$C NMR ($D_2O$, 100 MHz):** $\delta$ 182.0, 174.4, 154.4, 151.1, 137.4, 116.6, 89.4, 80.9, 73.2, 54.6, 48.8, 33.3, 29.3, 21.8; **HRMS (ESI-Q-TOF):** calcd for $C_{14}H_{18}N_6O_6$ ($[M+Na]^+$) 389.1186, found 389.1194.

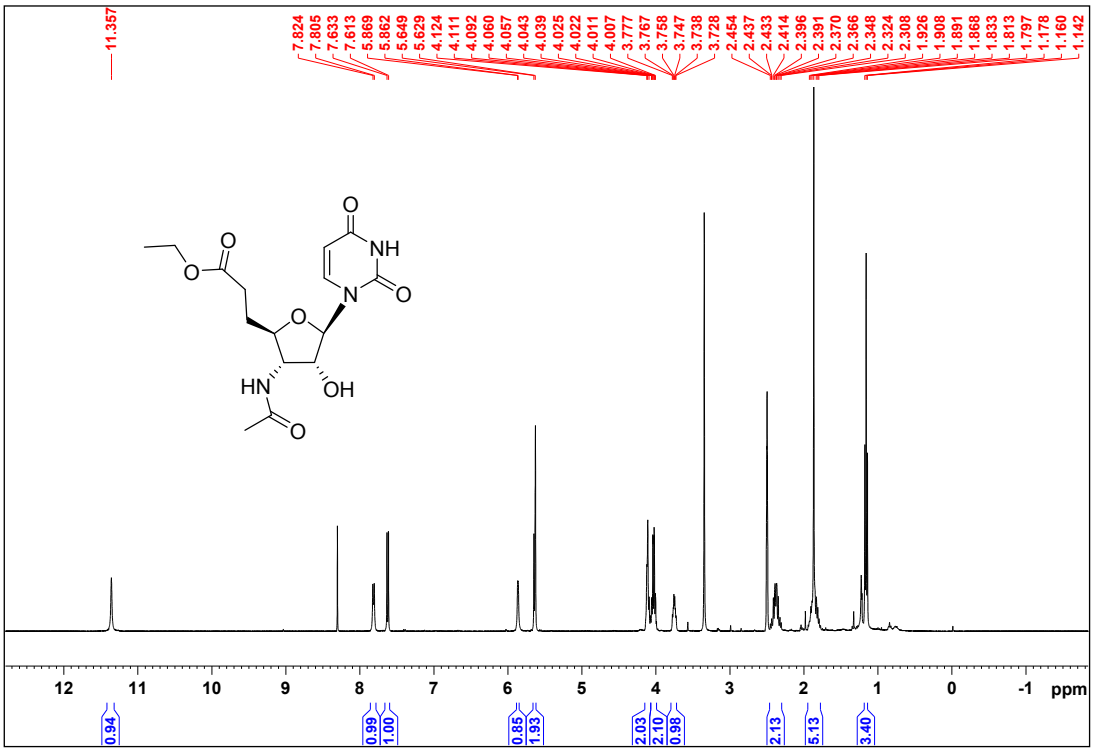
## 2. Spectra ($^{1}$H and $^{13}$C NMR) of Compounds:

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

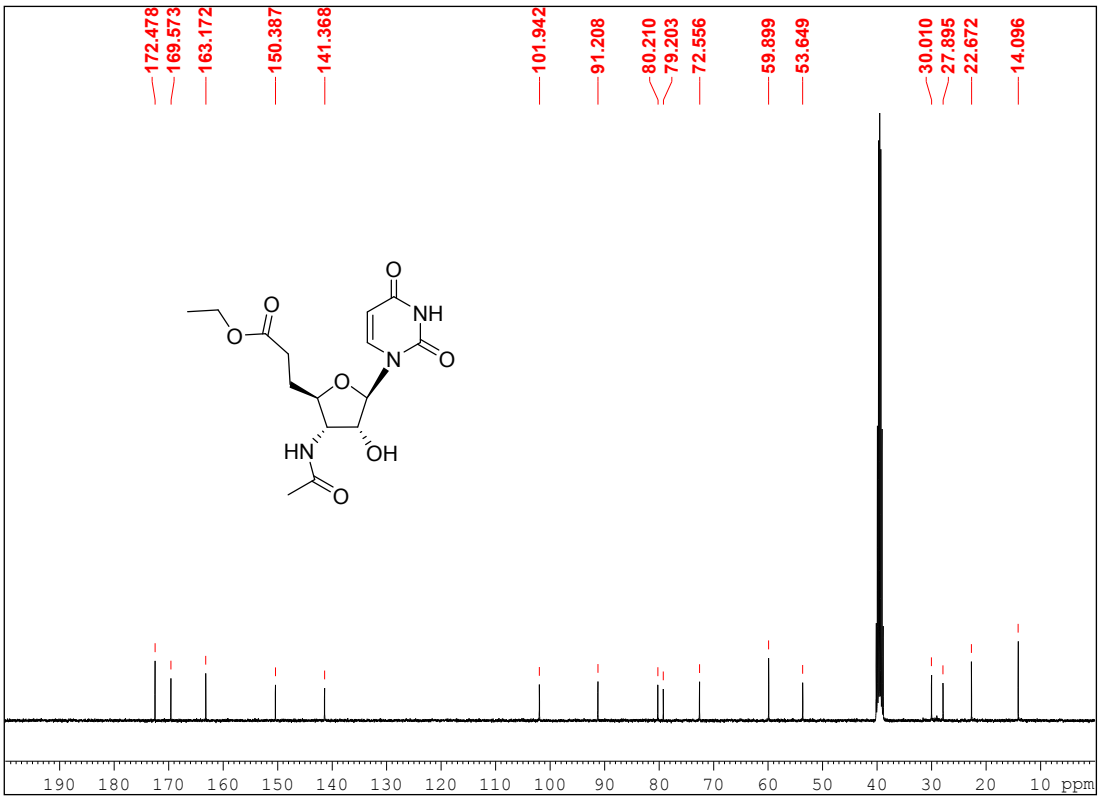



$^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of compound **1**

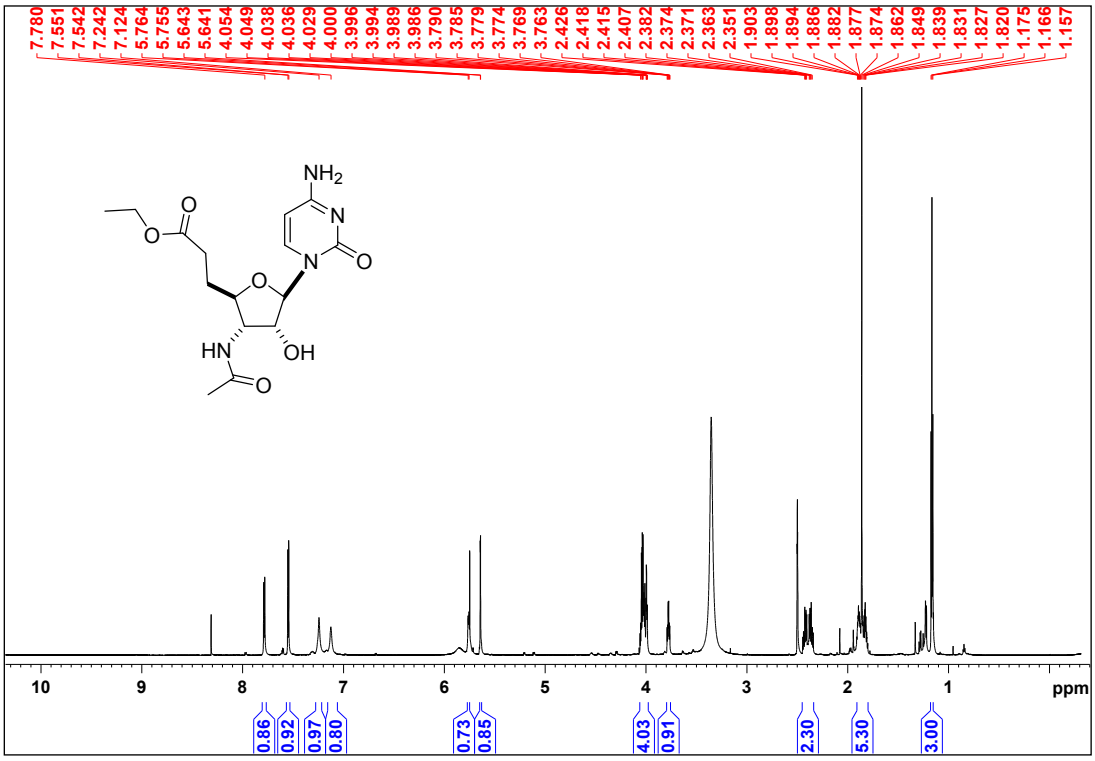
$^{1}$H NMR spectrum (800 MHz, DMSO-$d_6$) of compound **2**



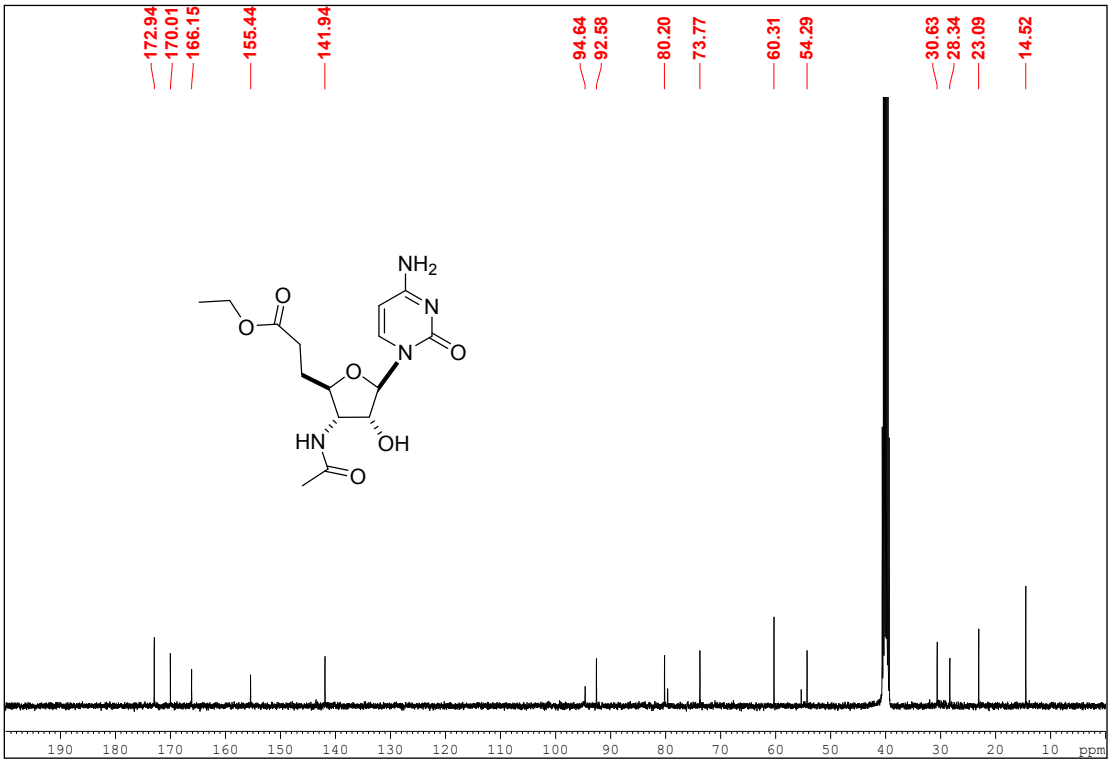

$^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of compound **2**



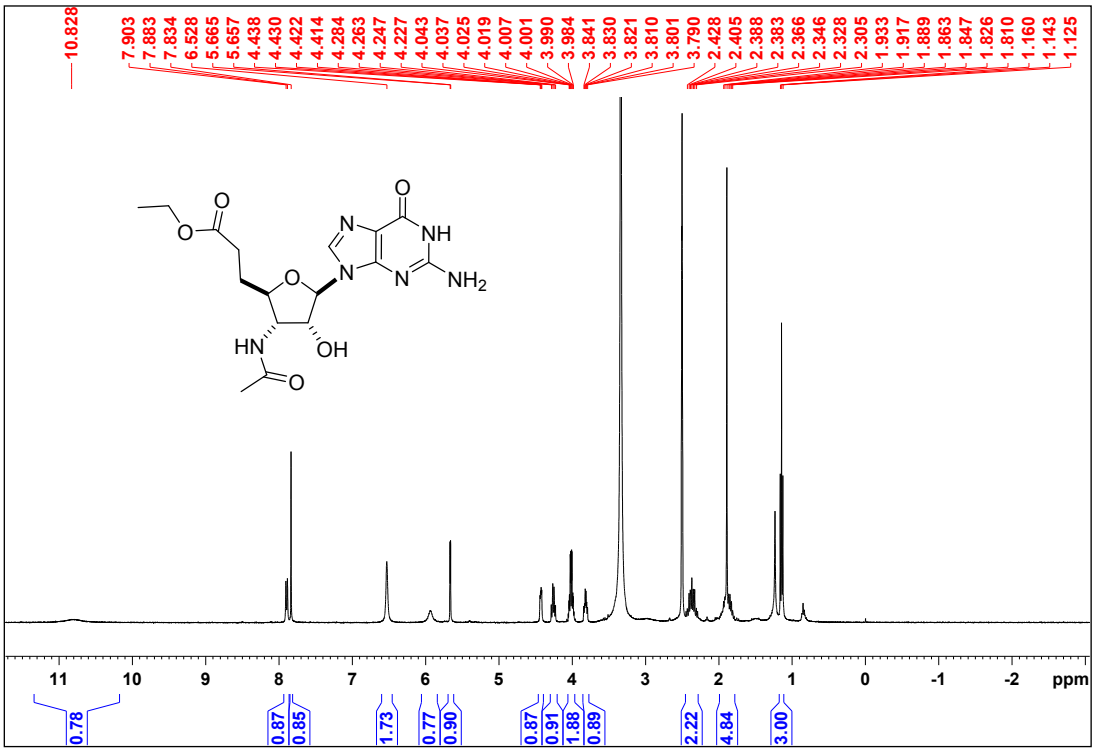
$^{1}$H NMR spectrum (800 MHz, DMSO-$d_6$) of compound **3**



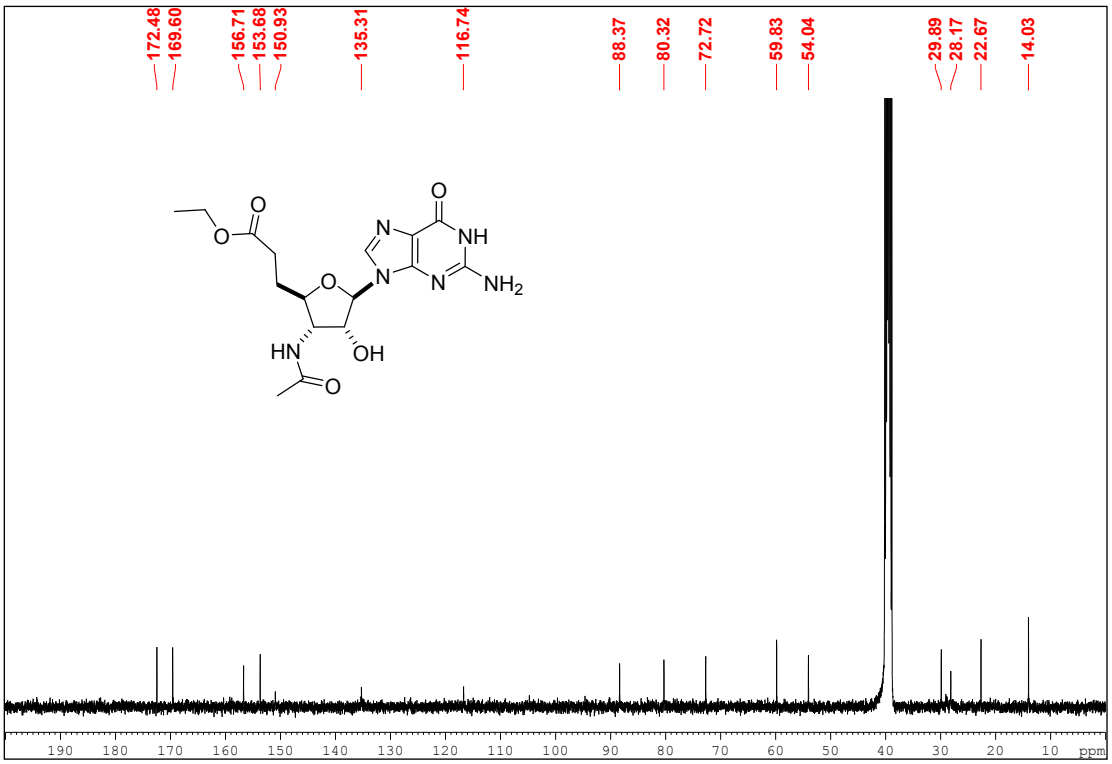

$^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of compound **3**



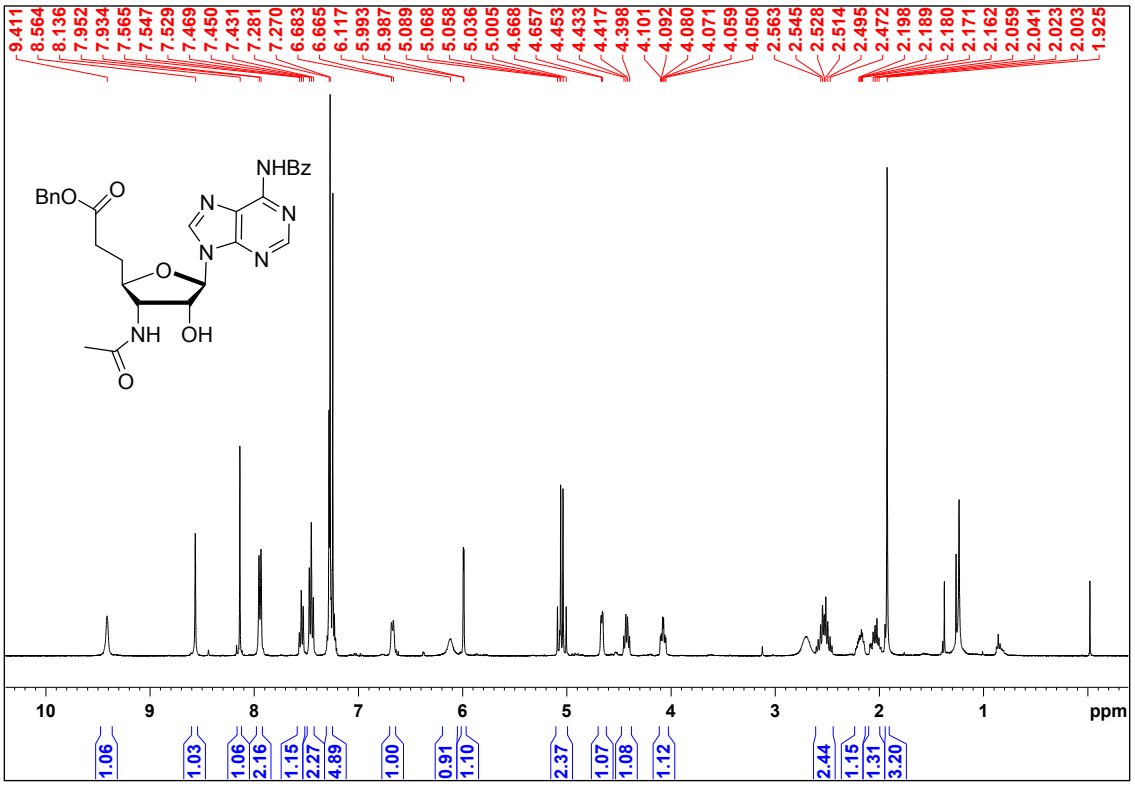
$^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **4**



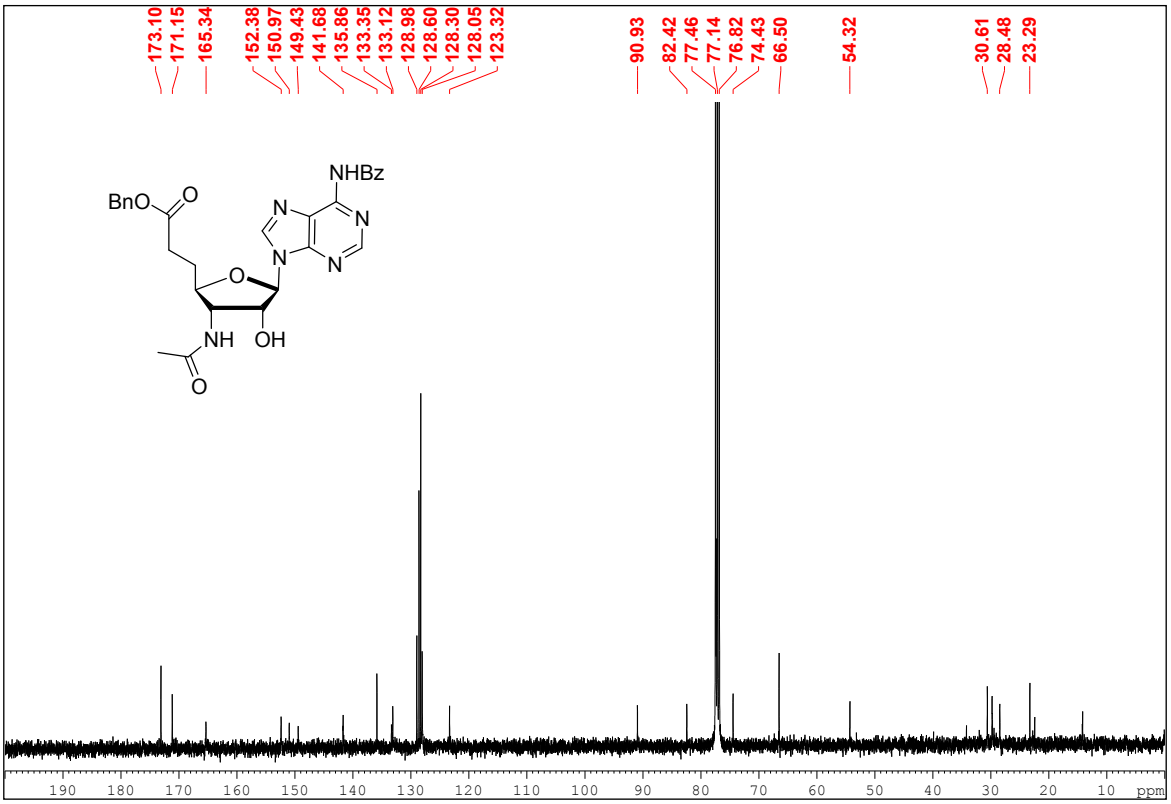

$^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of compound **4**



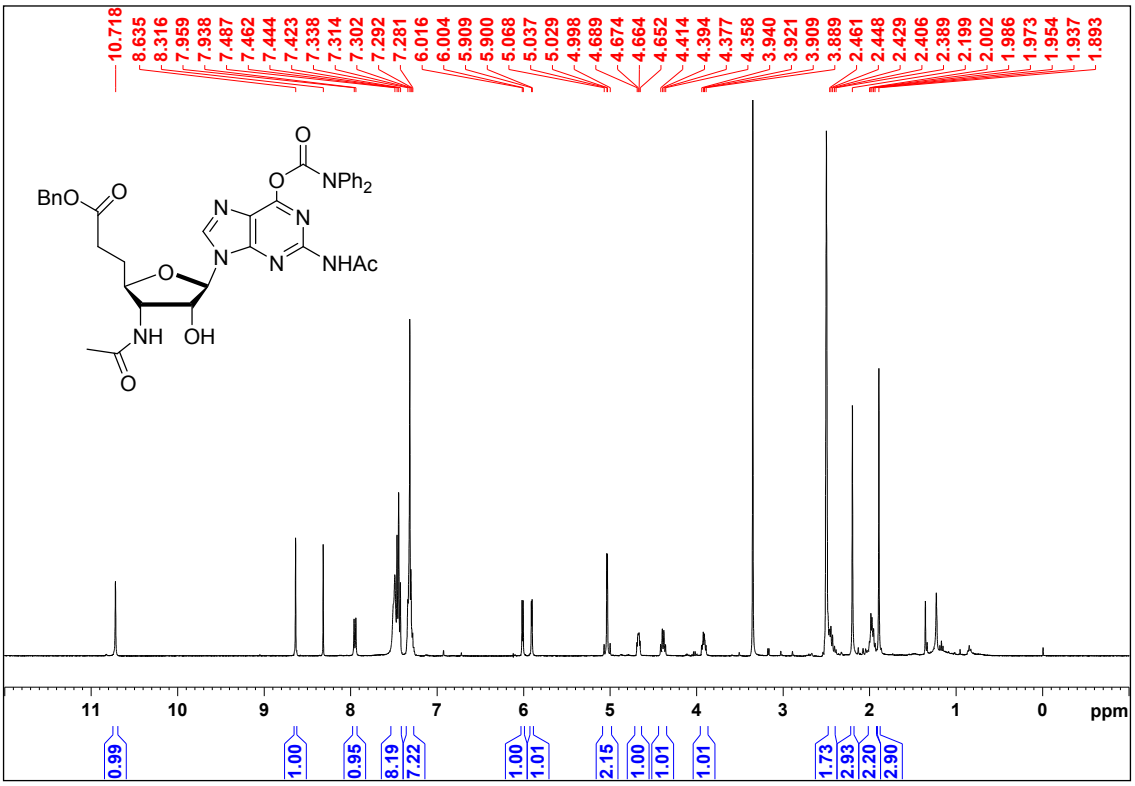
$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **9**



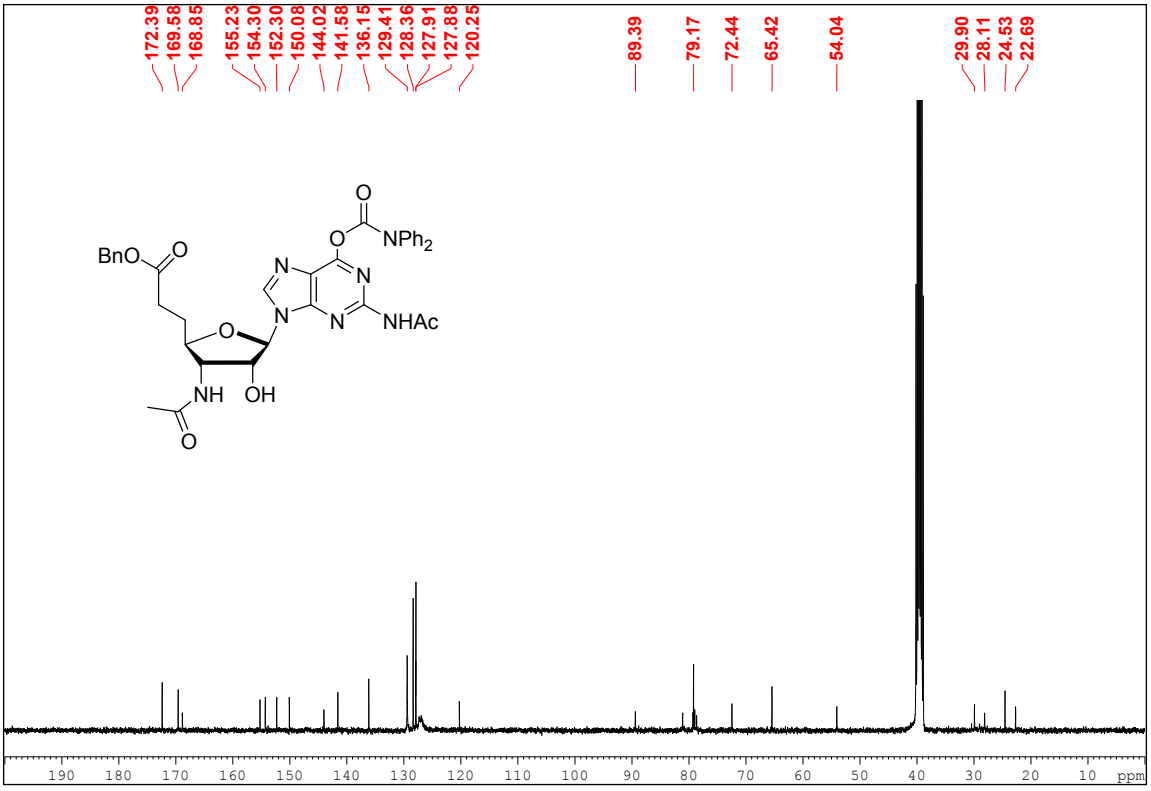

$^{13}$C NMR spectrum (100 MHz, $CDCl_3$) of compound **9**



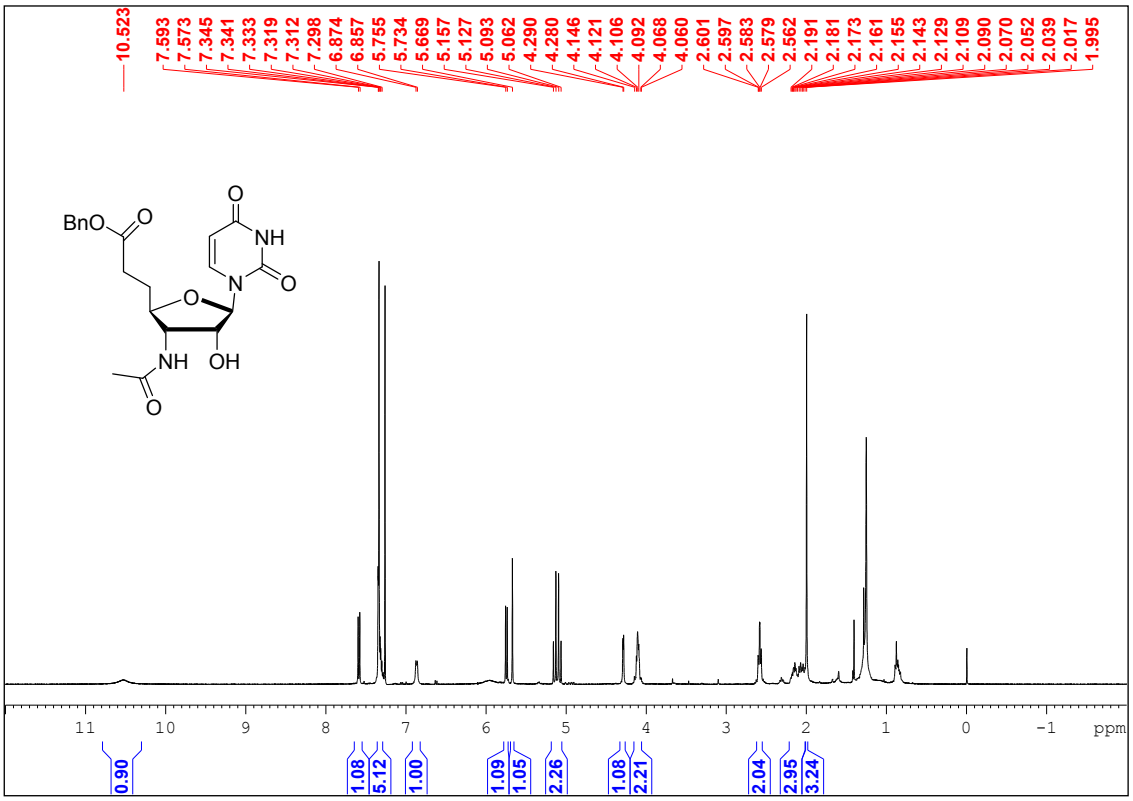
$^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **17a**



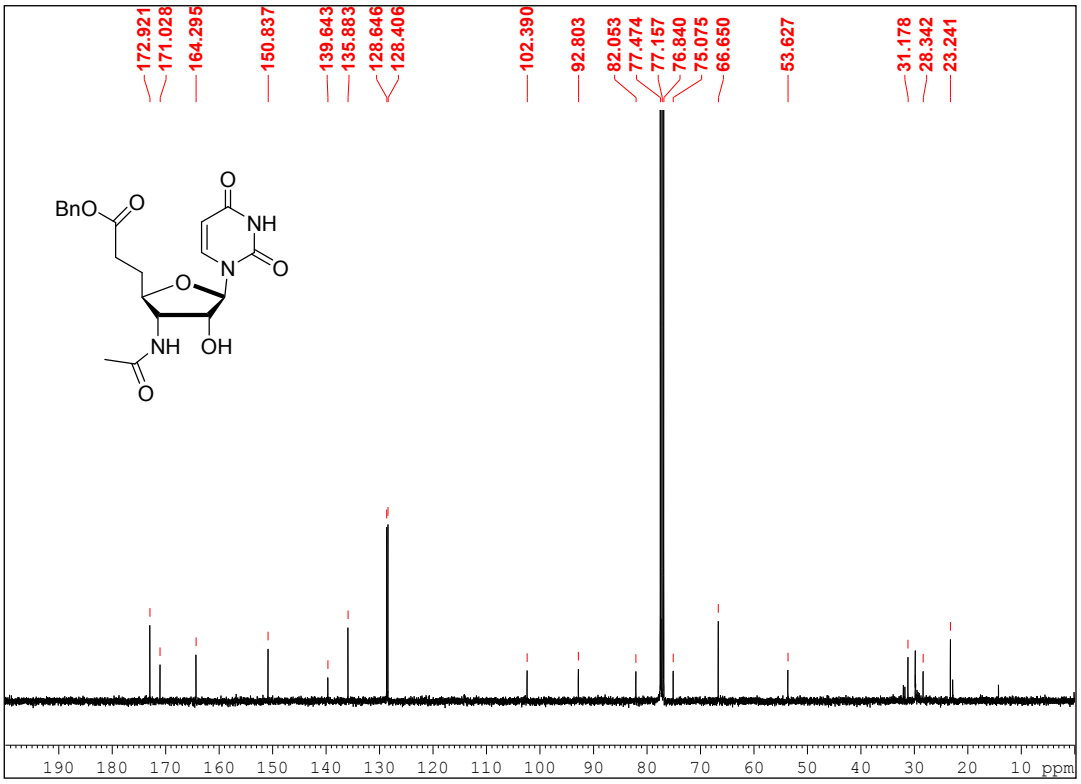

$^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of compound **17a**



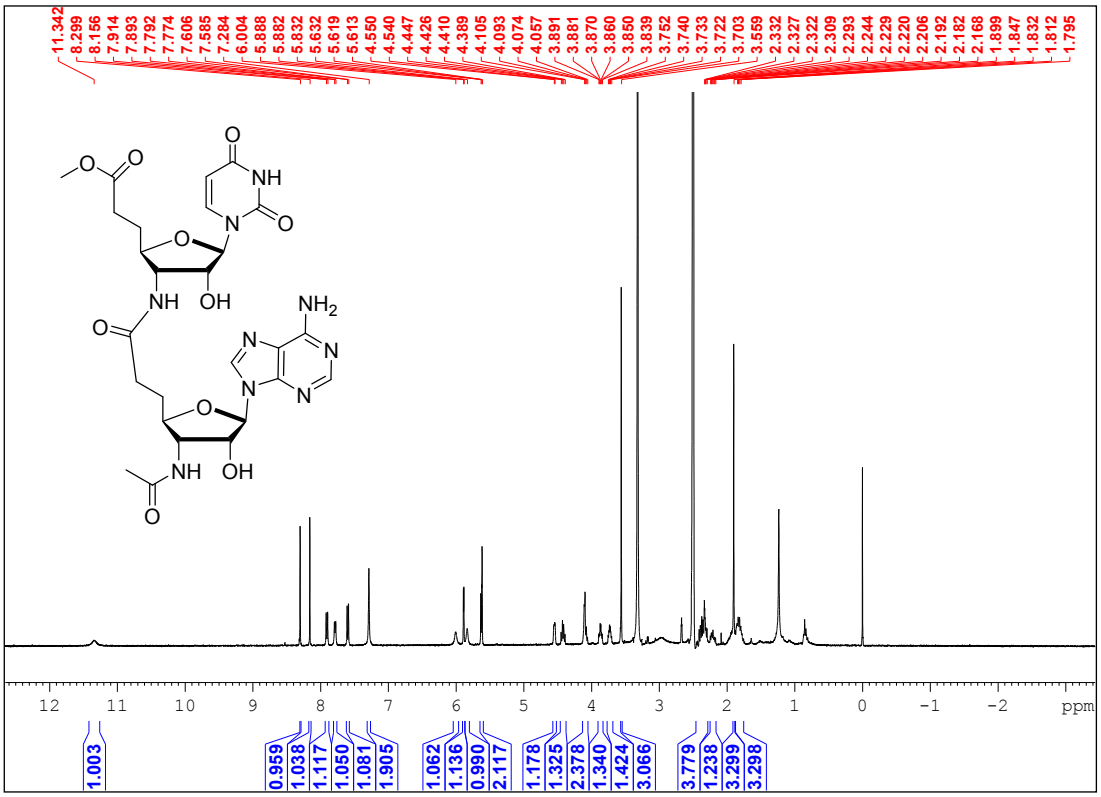
$^{1}$H NMR spectrum (400 MHz, $CDCl_3$) of compound **10**



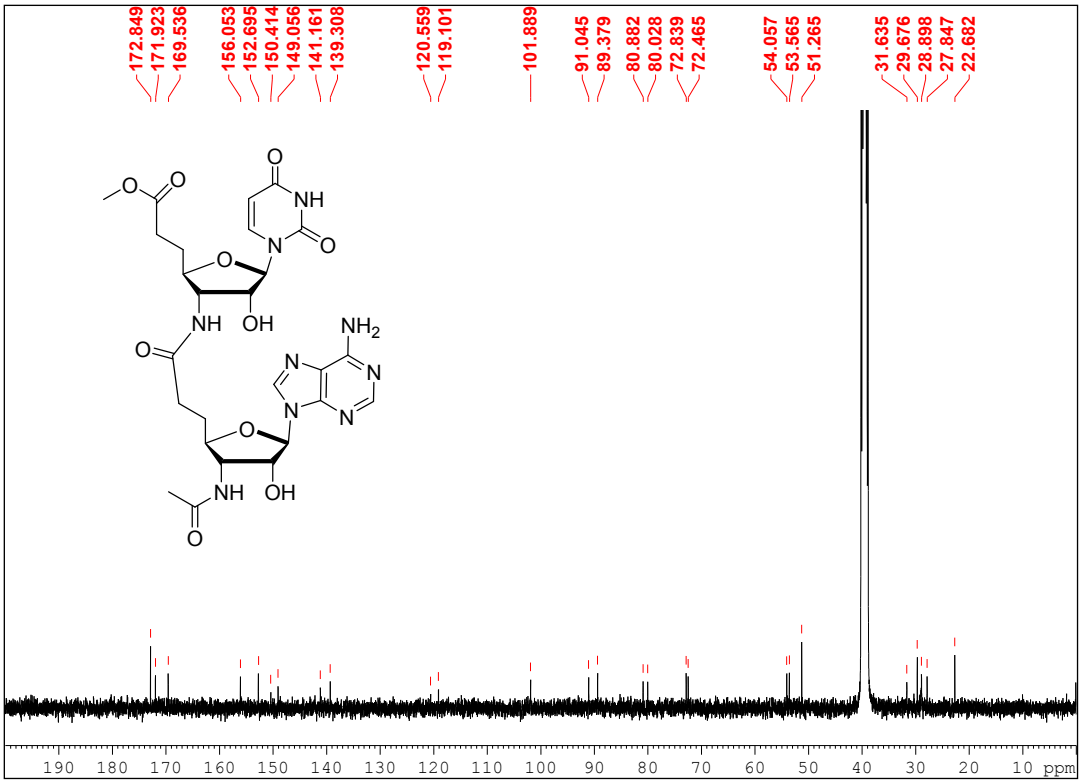

$^{13}$C NMR spectrum (100 MHz, $CDCl_3$) of compound **10**



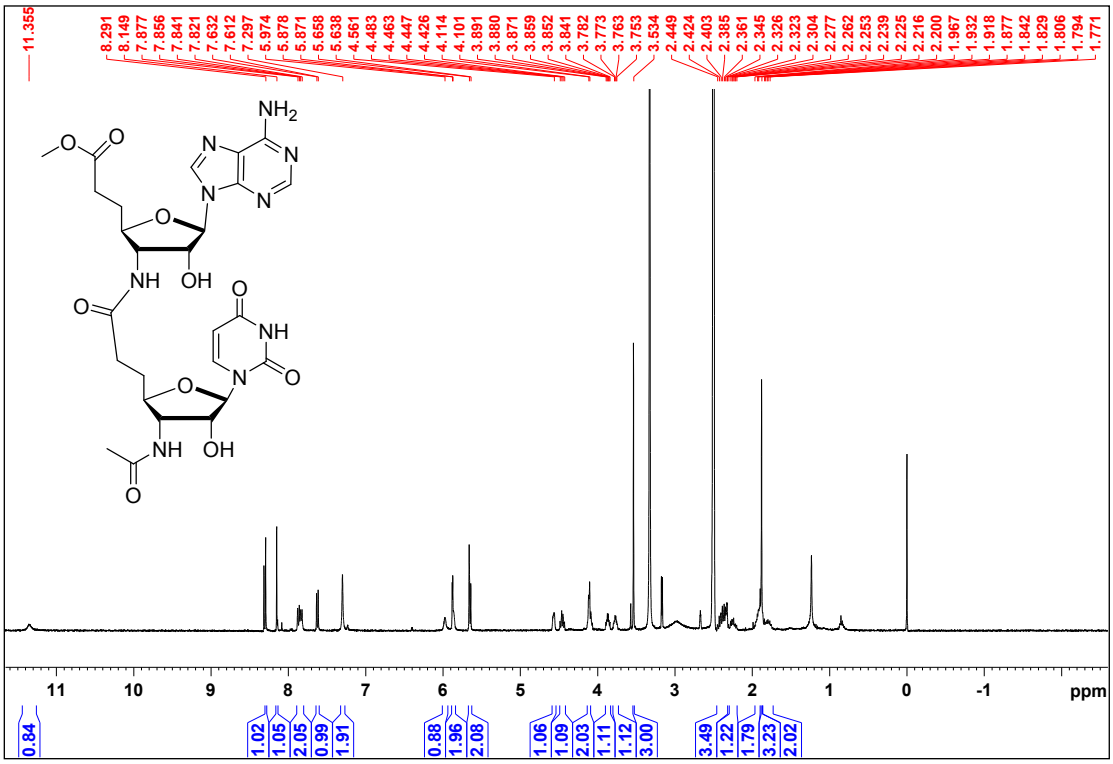
$^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **5**



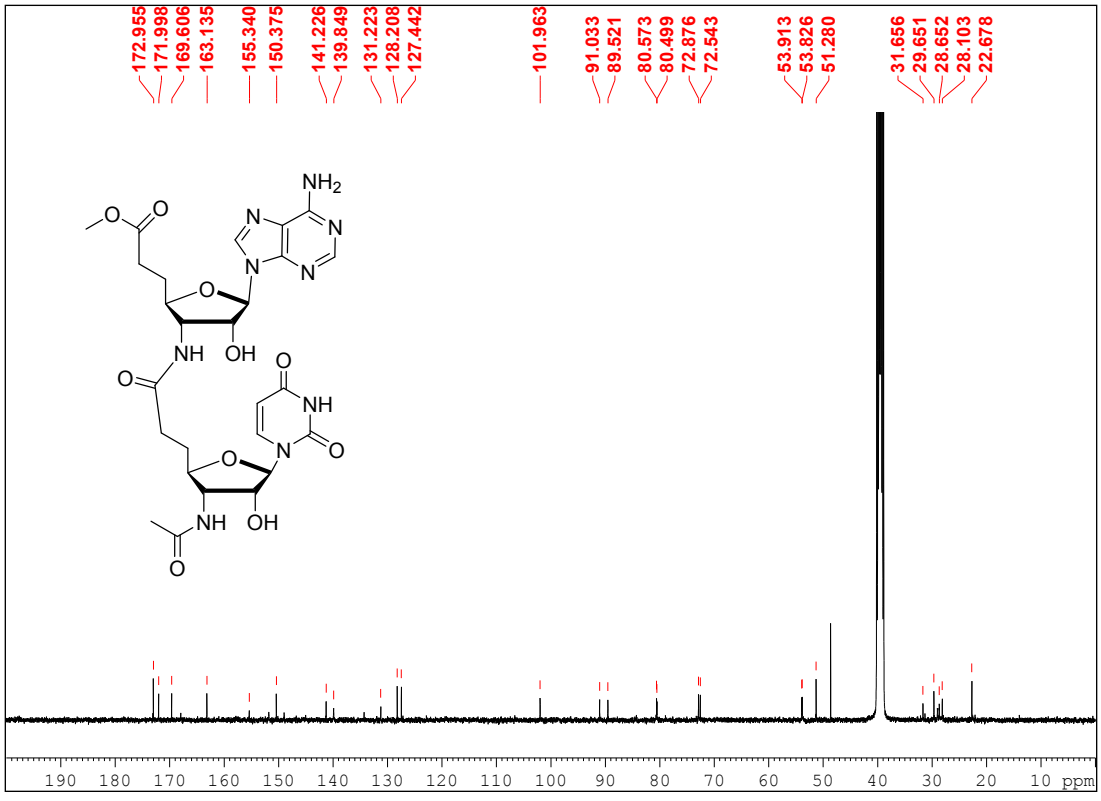

$^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of compound **5**



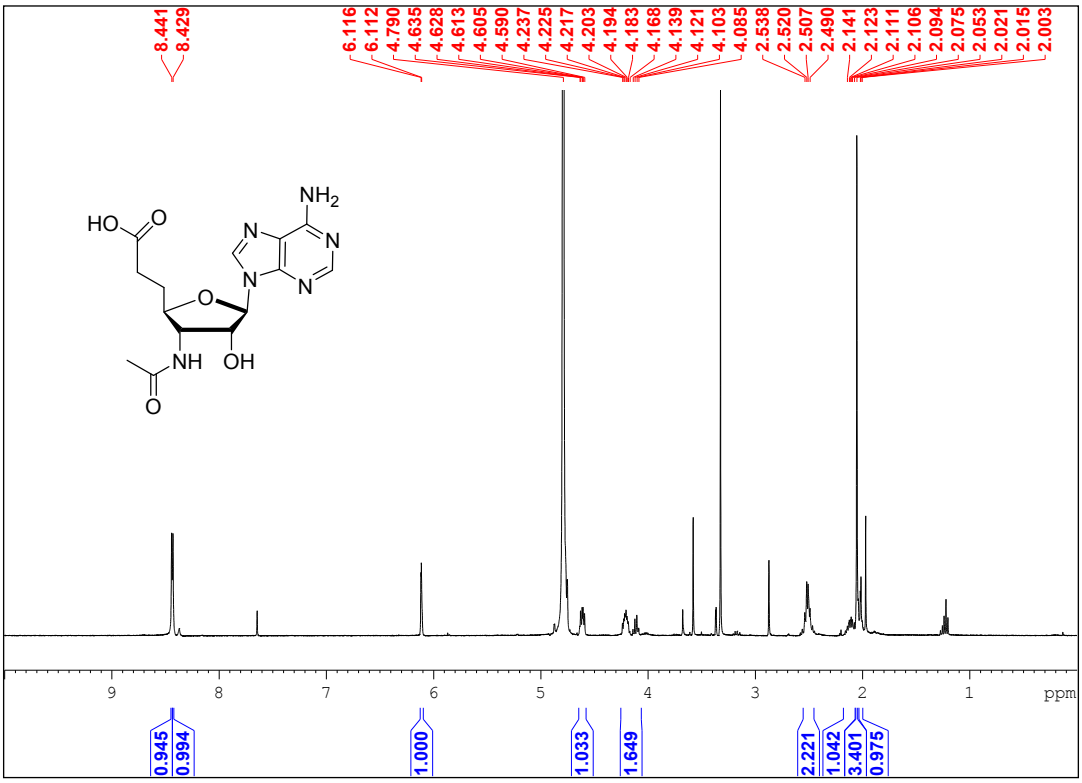
$^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$) of compound **6**



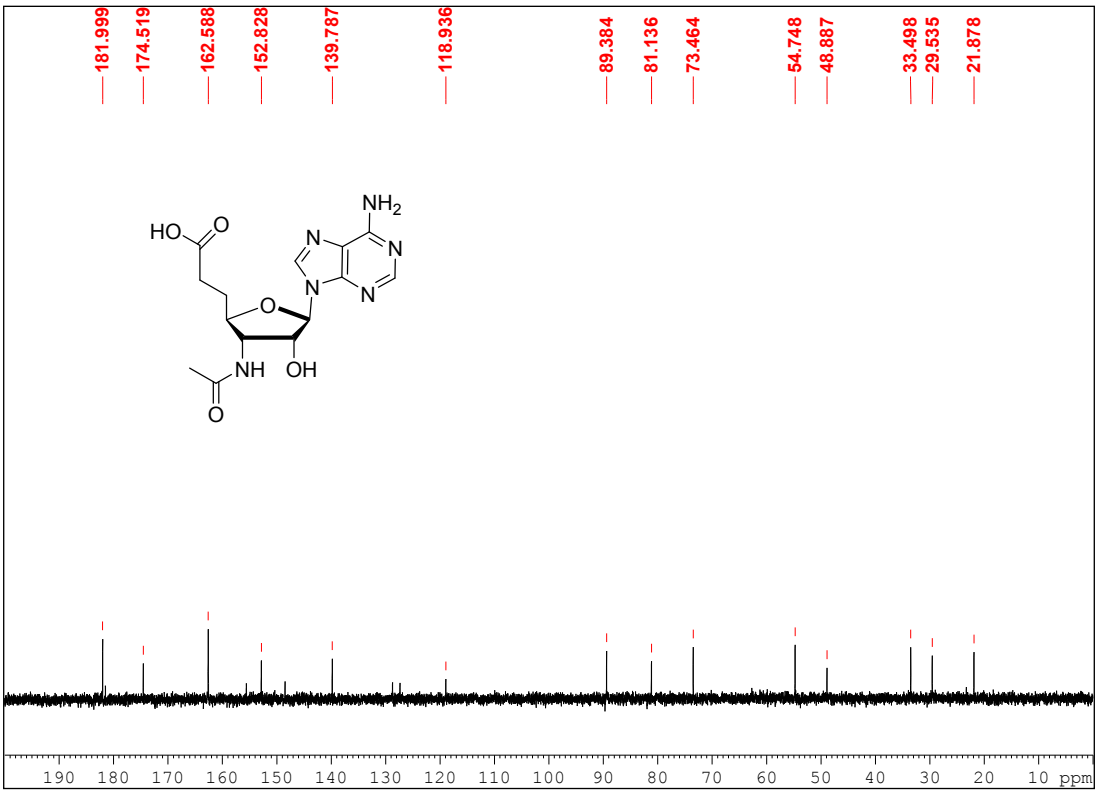
$^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$) of compound **6**



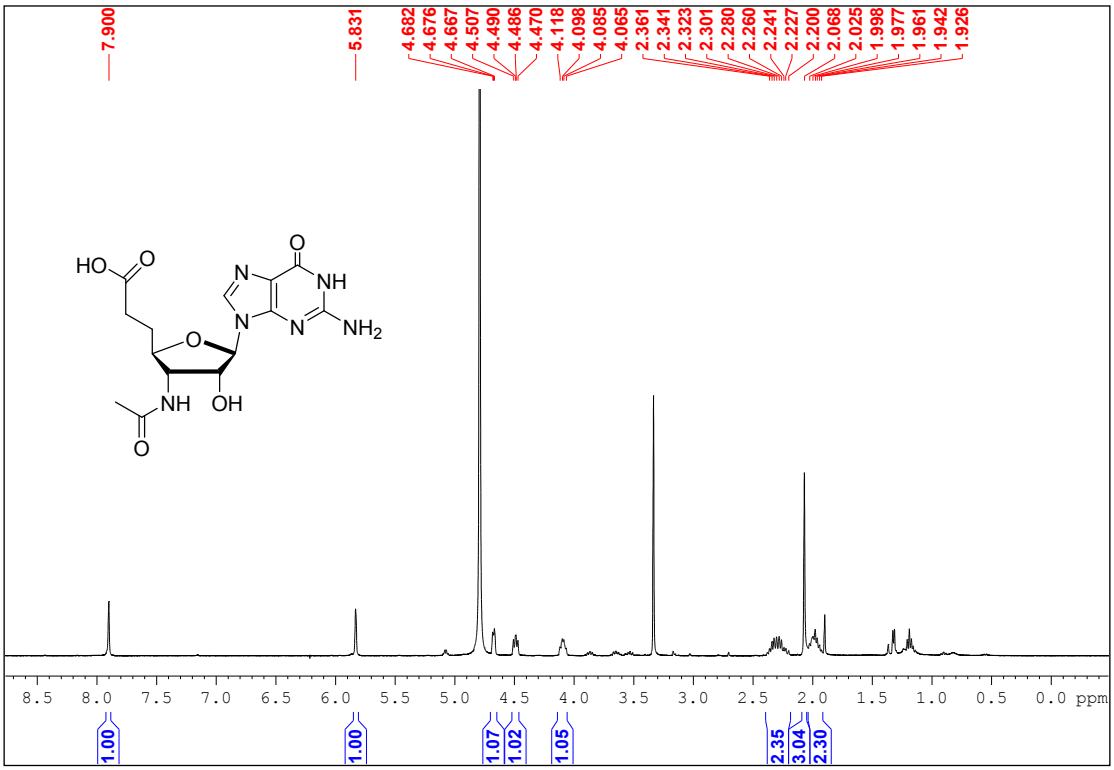
$^{1}$H NMR spectrum (400 MHz, $D_2O$) of compound **16**



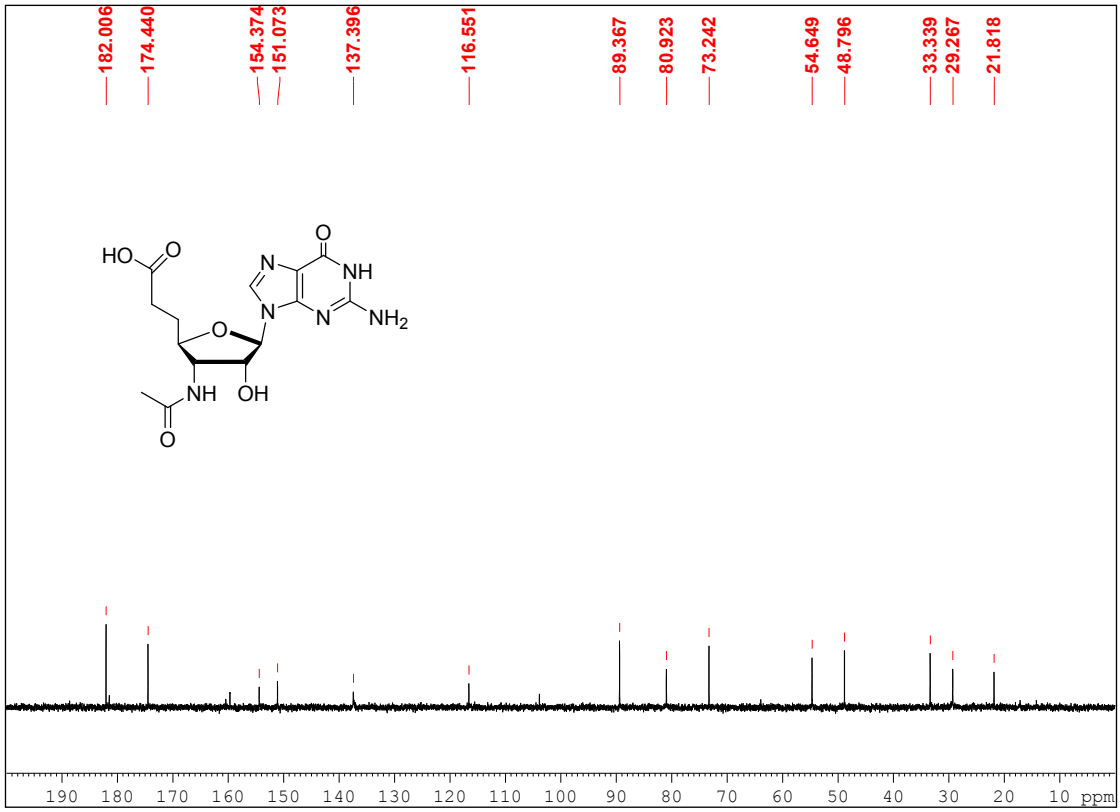

$^{13}$C NMR spectrum (100 MHz, $D_2O$) of compound **16**



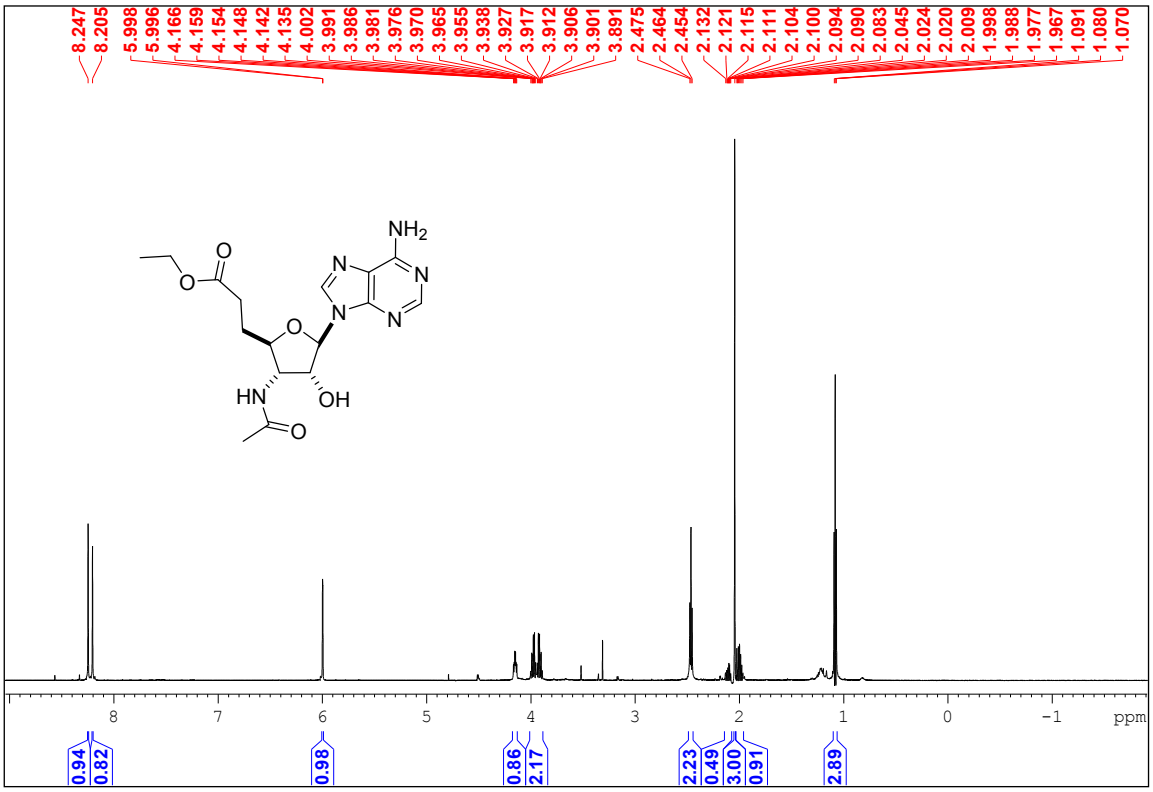
$^{1}$H NMR spectrum (400 MHz, $D_2O$) of compound **17**



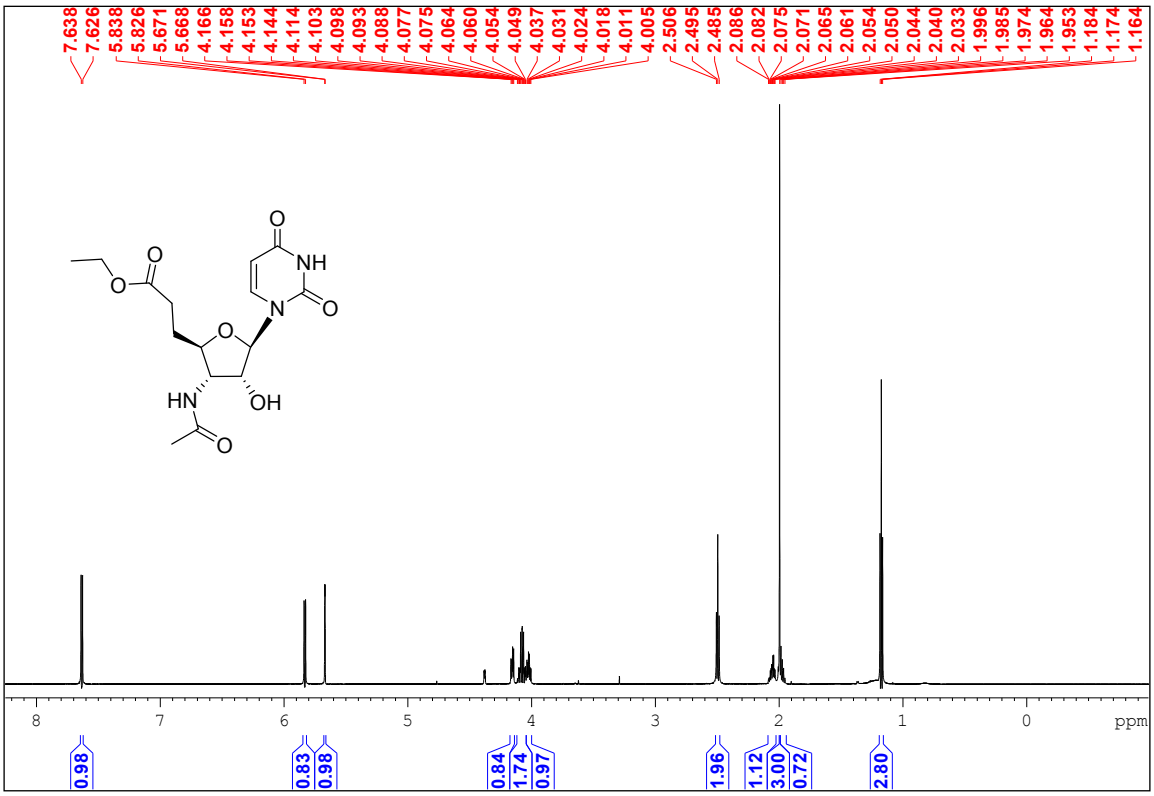

$^{13}$C NMR spectrum (100 MHz, $D_2O$) of compound **17**



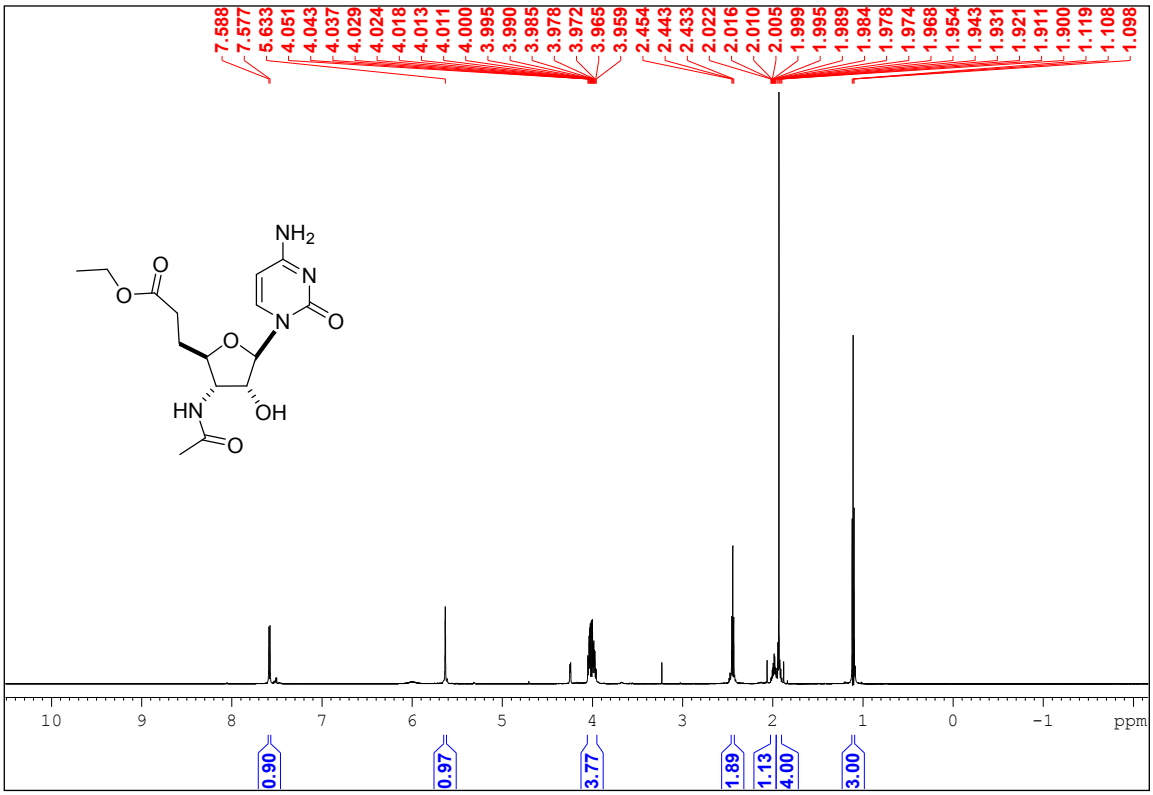
$^1$H NMR spectrum (700 MHz, $D_2O$) of compound **1**



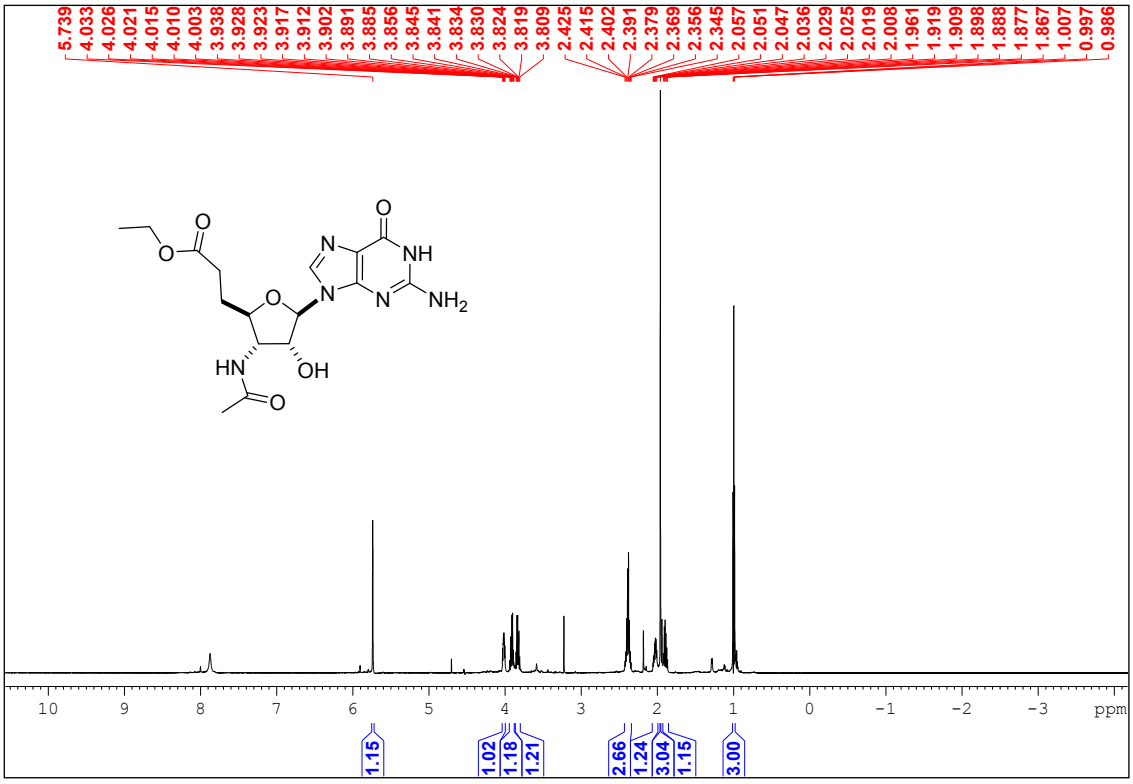

$^1$H NMR spectrum (700 MHz, $D_2O$) of compound **2**



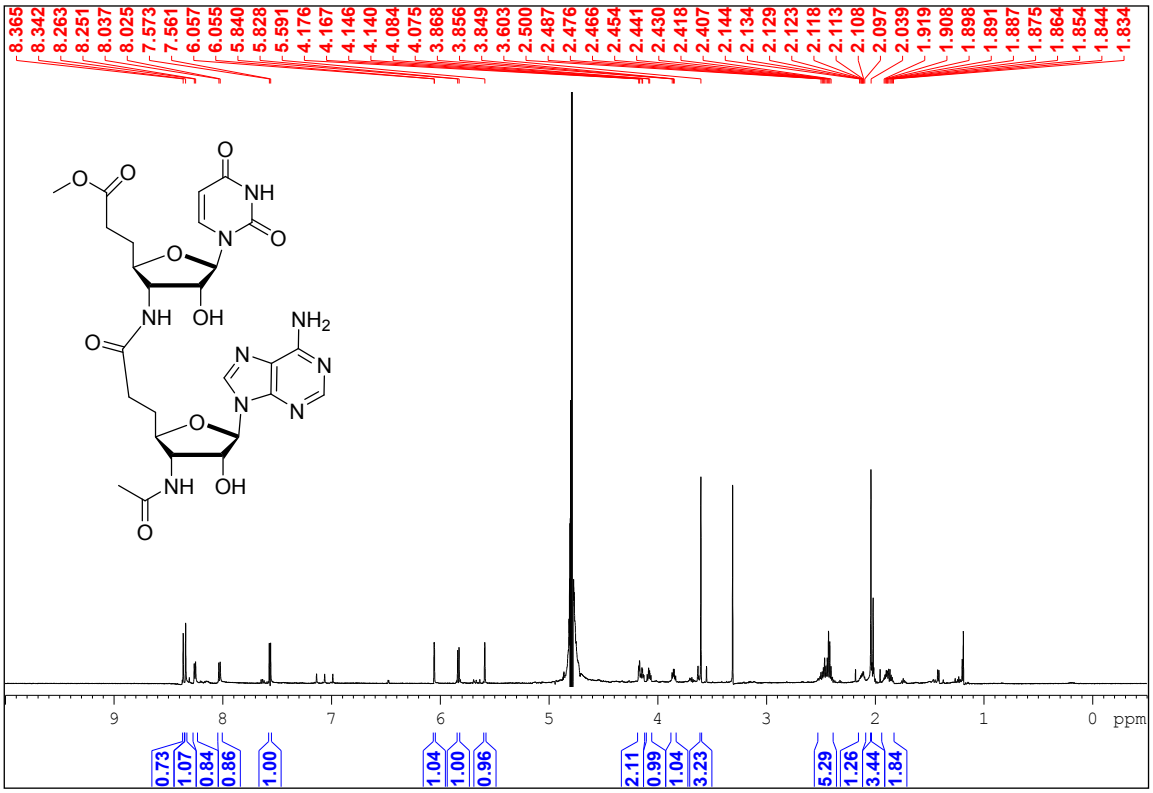
$^1$H NMR spectrum (700 MHz, $D_2O$) of compound **3**



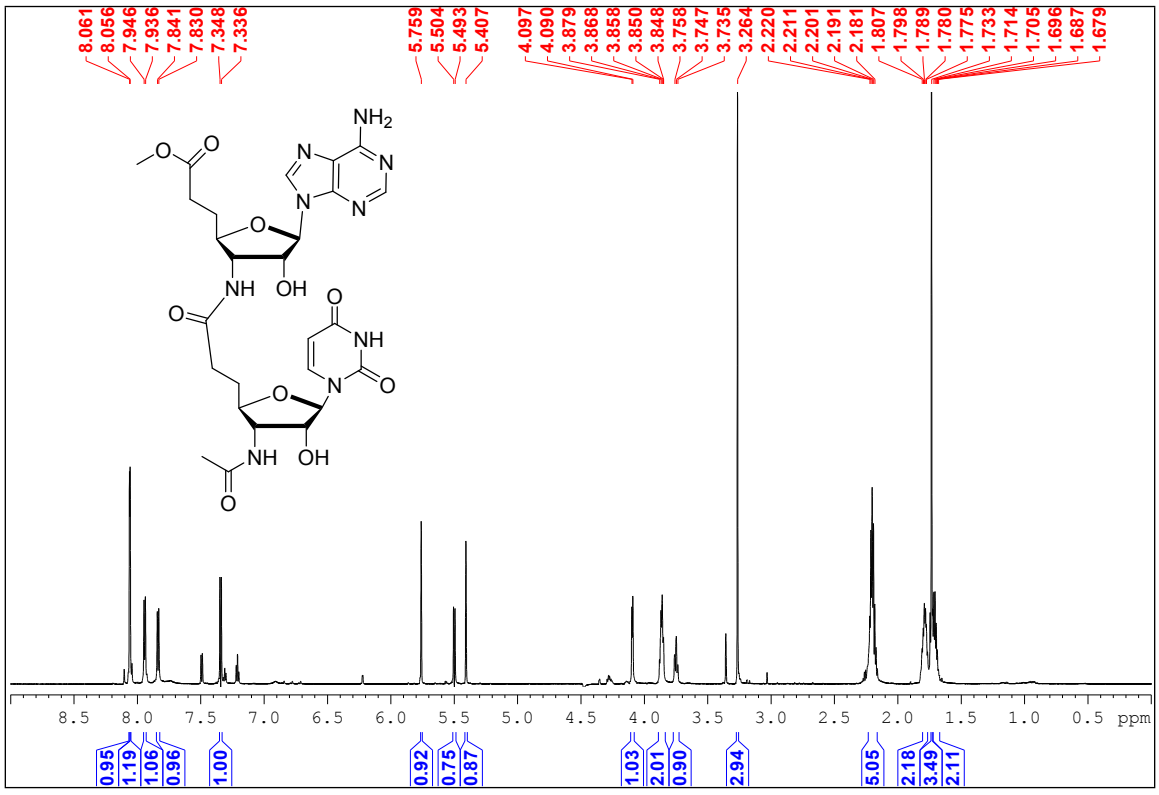

$^1$H NMR spectrum (700 MHz, $D_2O$) of compound **4**



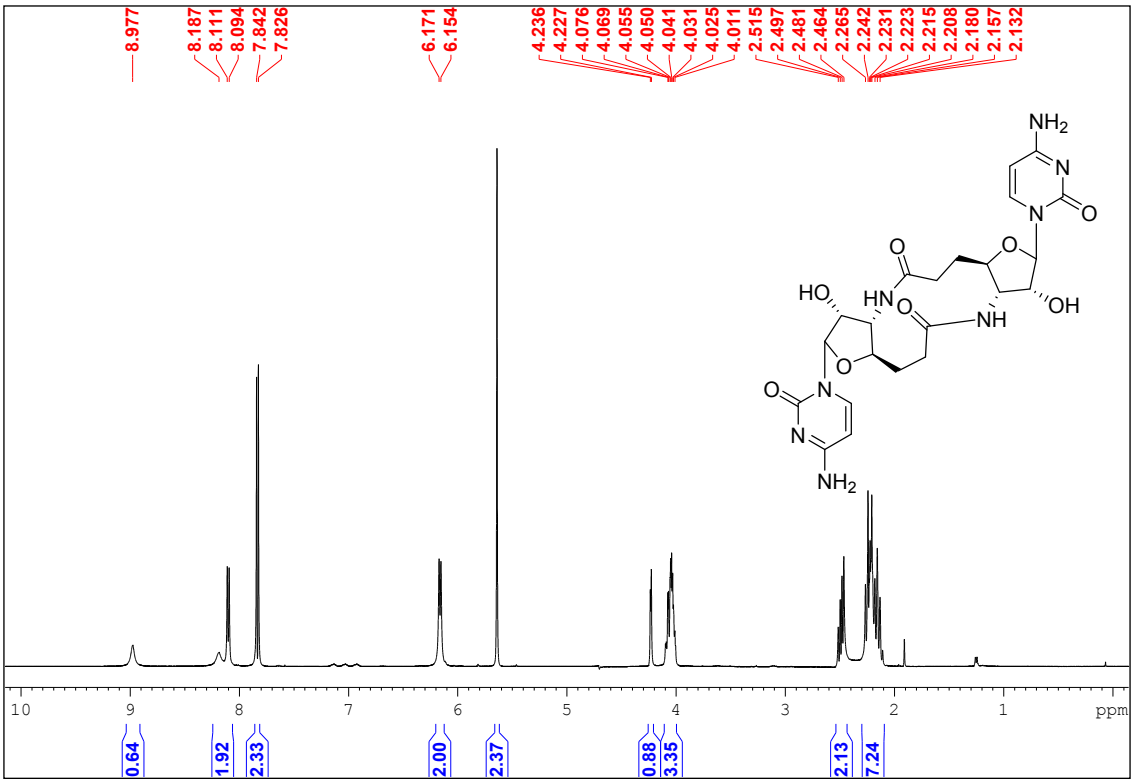
$^{1}$H NMR spectrum (700 MHz, 10% $D_2O$ in $H_2O$) of compound **5**



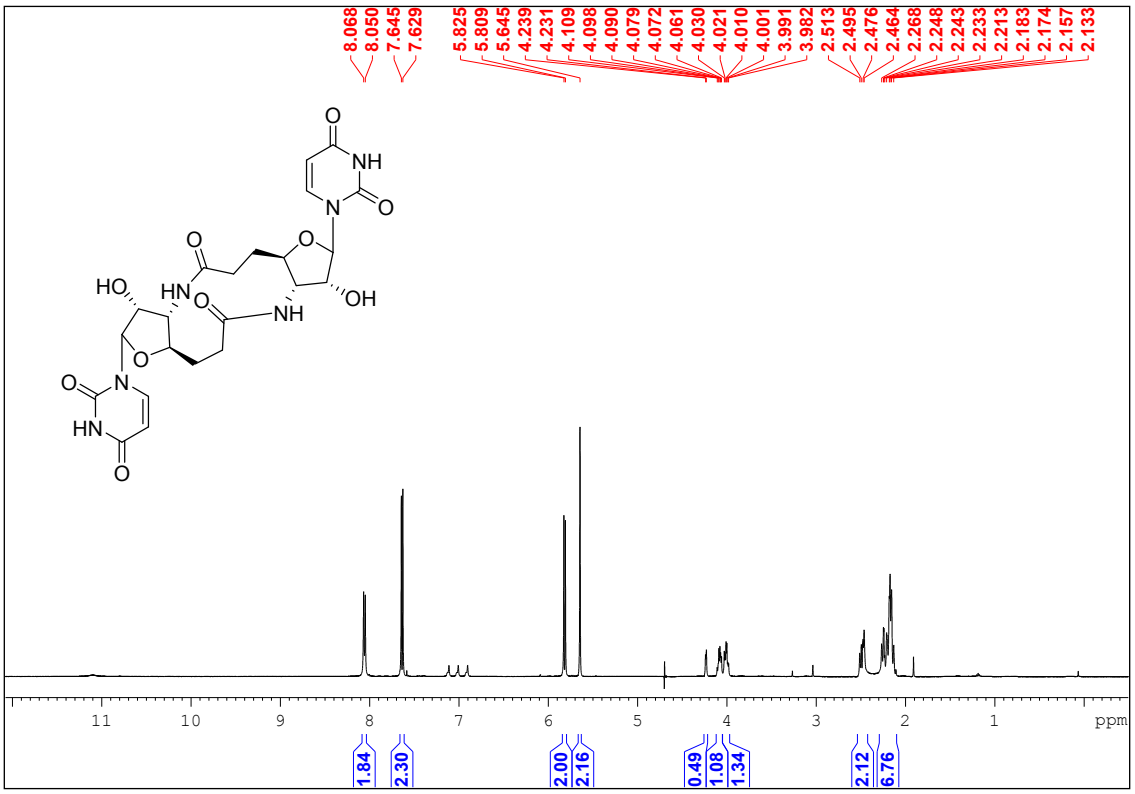
$^{1}$H NMR spectrum (800 MHz, 10% $D_2O$ in $H_2O$) of compound **6**



$^{1}$H NMR spectrum (500 MHz, 10% $D_2O$ in $H_2O$) of compound **13**



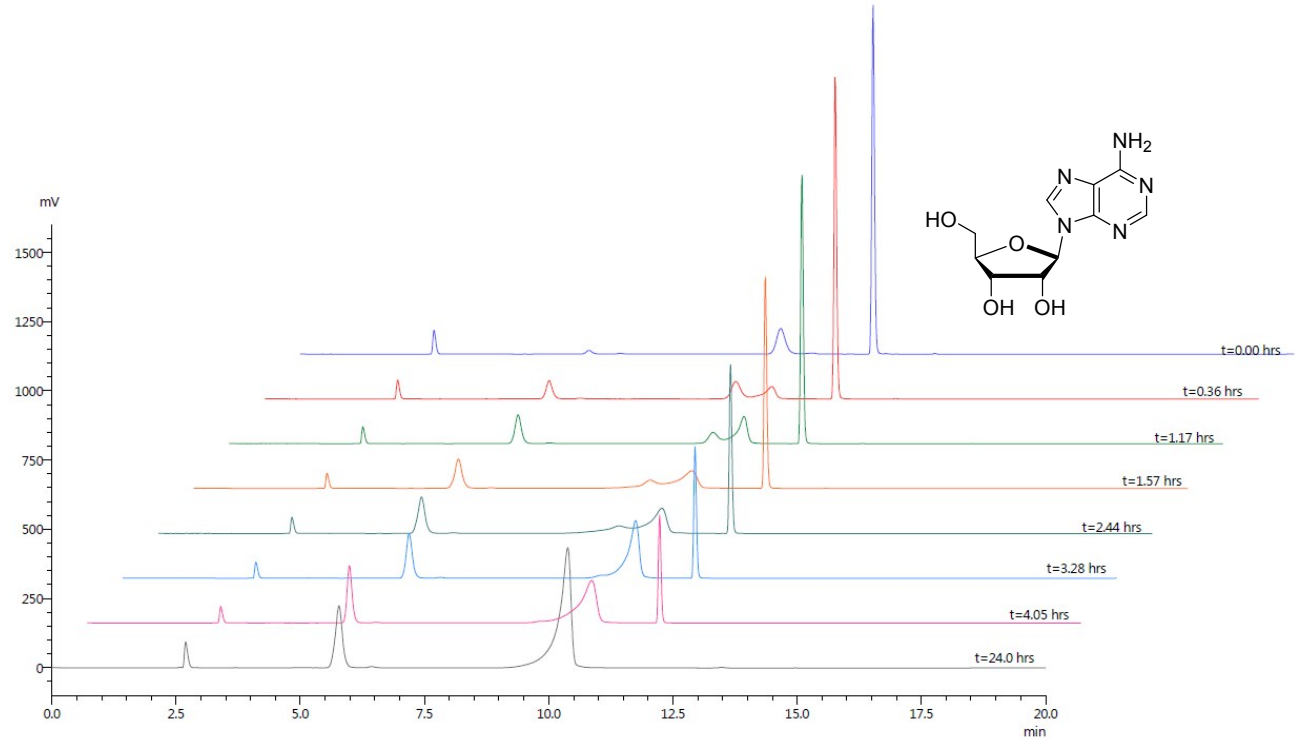

$^{1}$H NMR spectrum (500 MHz, 10% $D_2O$ in $H_2O$) of compound **14**

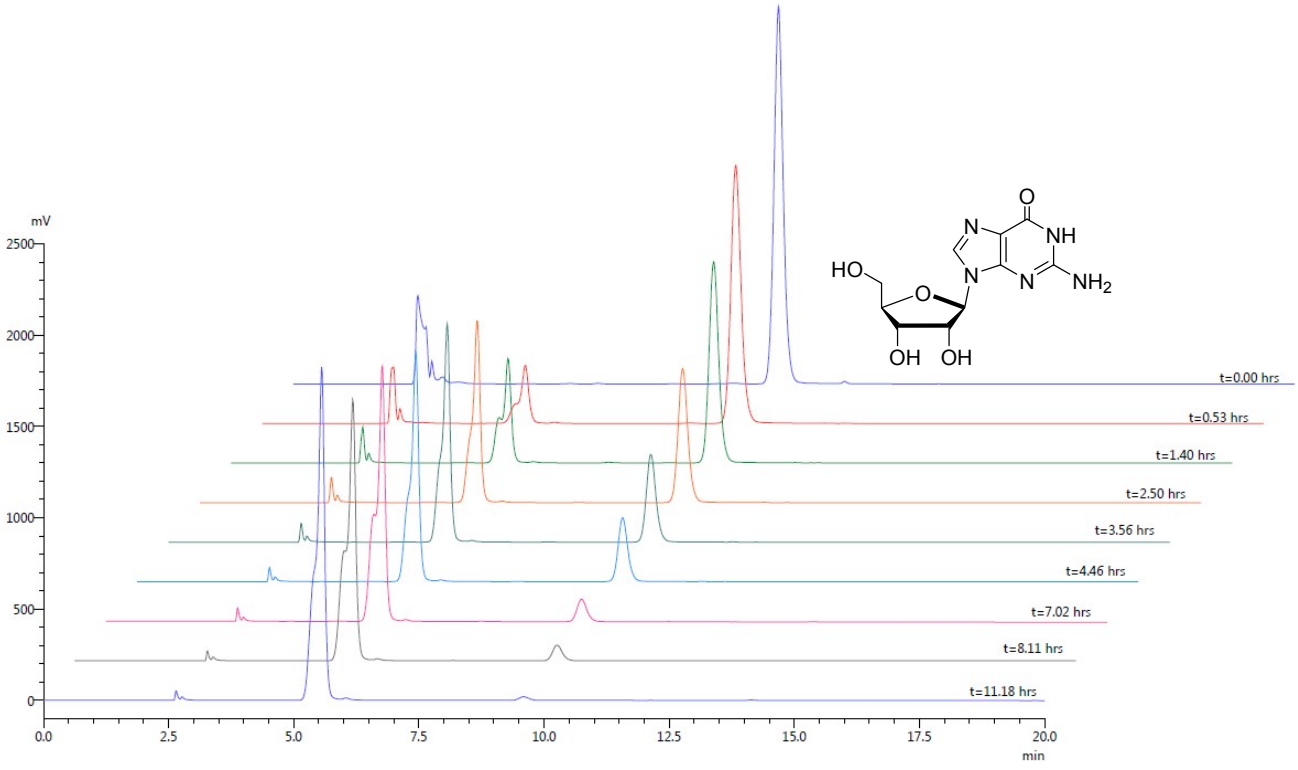


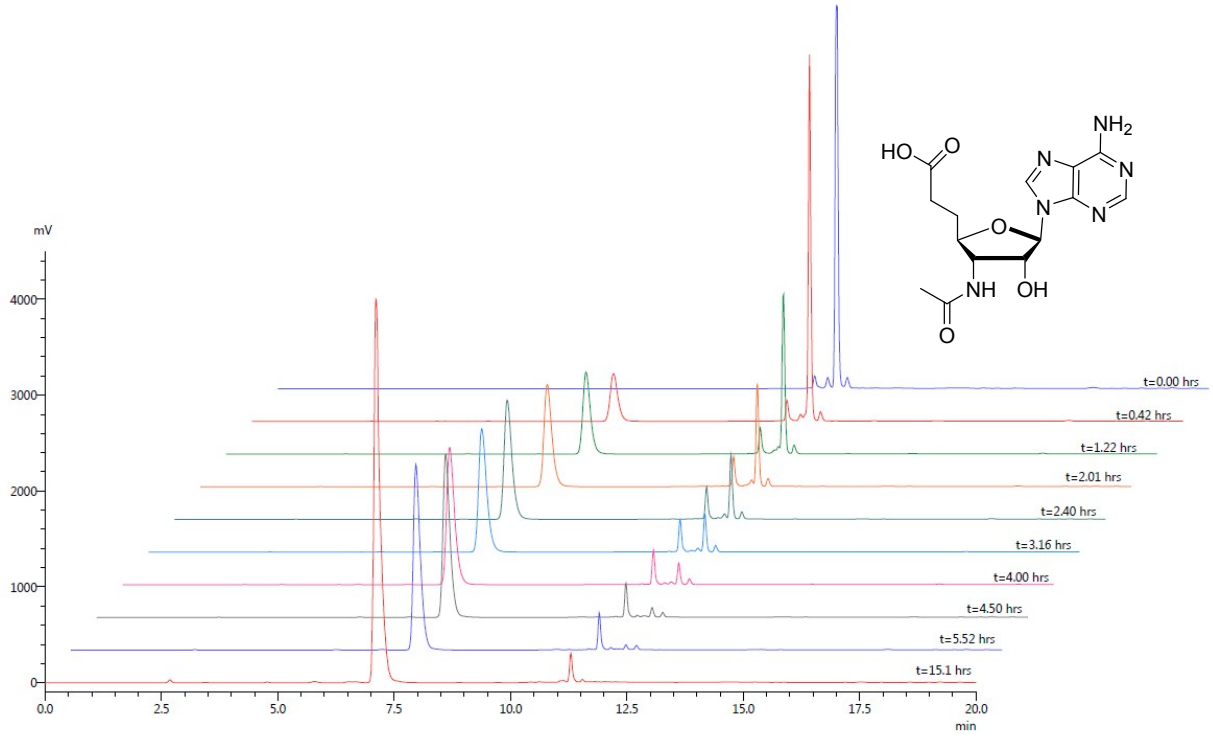
**Depurination experiments:**

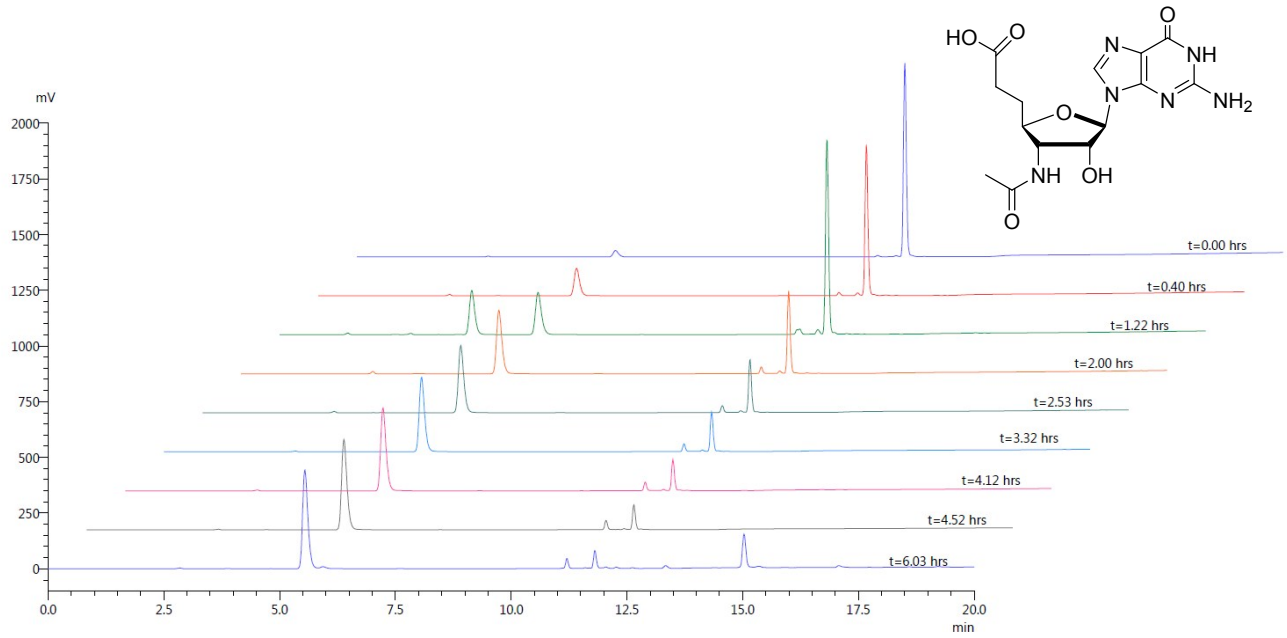
Experimentally, the deglycosylation rates were measured under standardized acidic conditions (0.1M HCl) for all nucleosides in order to obtain the apparent first order rates and half-lives. Initially, the monitoring of the hydrolysis was experimented with UV-Vis spectrophotometer. But limitations like building up of solvent bubbles in the cuvette over time, prevented real-time monitoring of the reactions. The well-known stability of riboglycosidic linkages makes it unpractical, in terms of time scale, to study deglycosylation kinetics at lower temperatures.

Reaction mixtures for deglycosylation kinetics of ribonucleosides at pH 1 (0.1M HCl) were prepared in 5 mL vials by rapidly mixing 0.5mL of 0.2M HCl and 0.5 mL of stock nucleoside solution and placing it in a temperature controlled (±1°C) oil bath. At designated time points, 20-μL aliquot of the reaction mixture was removed and was injected into a HPLC running a binary gradient. The decay of the starting materials was calculated as a percentage decrease in the peak area at a particular wavelength (254 nm). The percentages were then plotted as function of time and fitted to an exponential curve to get the apparent first-order rate contents. The half-lives at 50% of substrate were obtained from the fitted graph directly.


mV
1500
1250
1000
750
500
250
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
min
t=0.00 hrs
t=0.36 hrs
t=1.17 hrs
t=1.57 hrs
t=2.44 hrs
t=3.28 hrs
t=4.05 hrs
t=24.0 hrs
NH2
N
N
N
N
HO
O
OH
OH

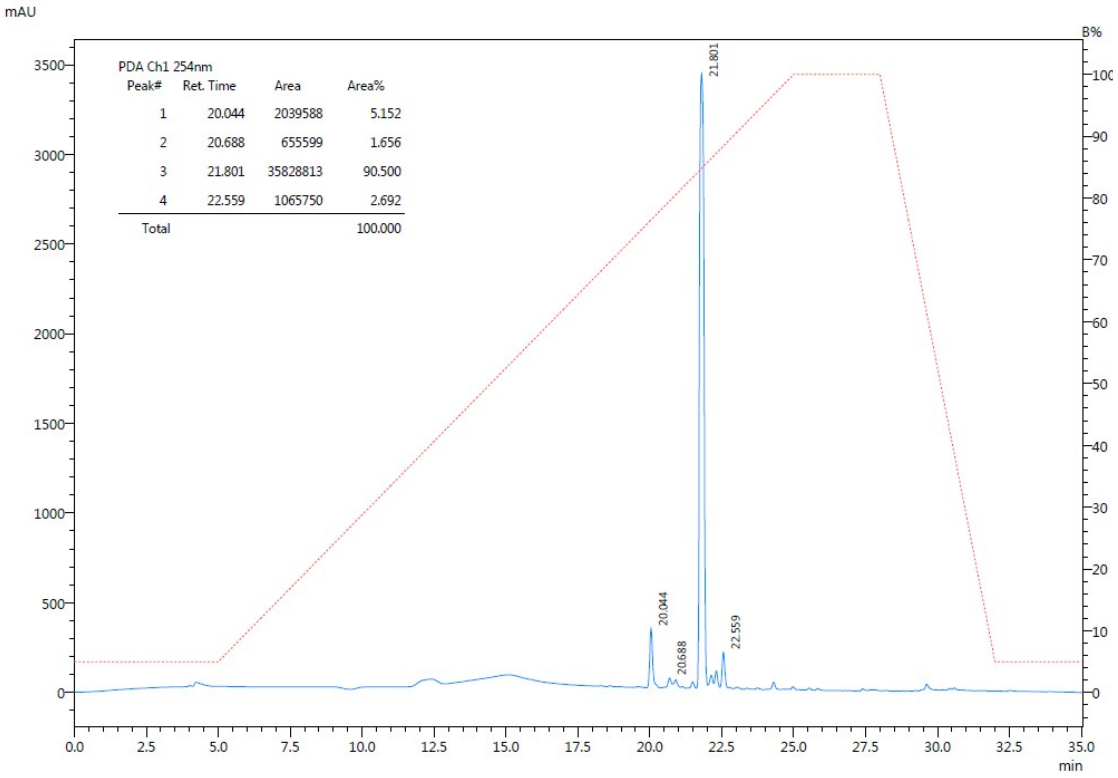

mV
2500
2000
1500
1000
500
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
min
t=0.00 hrs
t=0.53 hrs
t=1.40 hrs
t=2.50 hrs
t=3.56 hrs
t=4.46 hrs
t=7.02 hrs
t=8.11 hrs
t=11.18 hrs
O
N
NH
HO
N
N
NH2
O
OH
OH


mV
4000
3000
2000
1000
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
min
t=0.00 hrs
t=0.42 hrs
t=1.22 hrs
t=2.01 hrs
t=2.40 hrs
t=3.16 hrs
t=4.00 hrs
t=4.50 hrs
t=5.52 hrs
t=15.1 hrs
HO
O
NH2
N
N
N
N
O
NH
OH
O

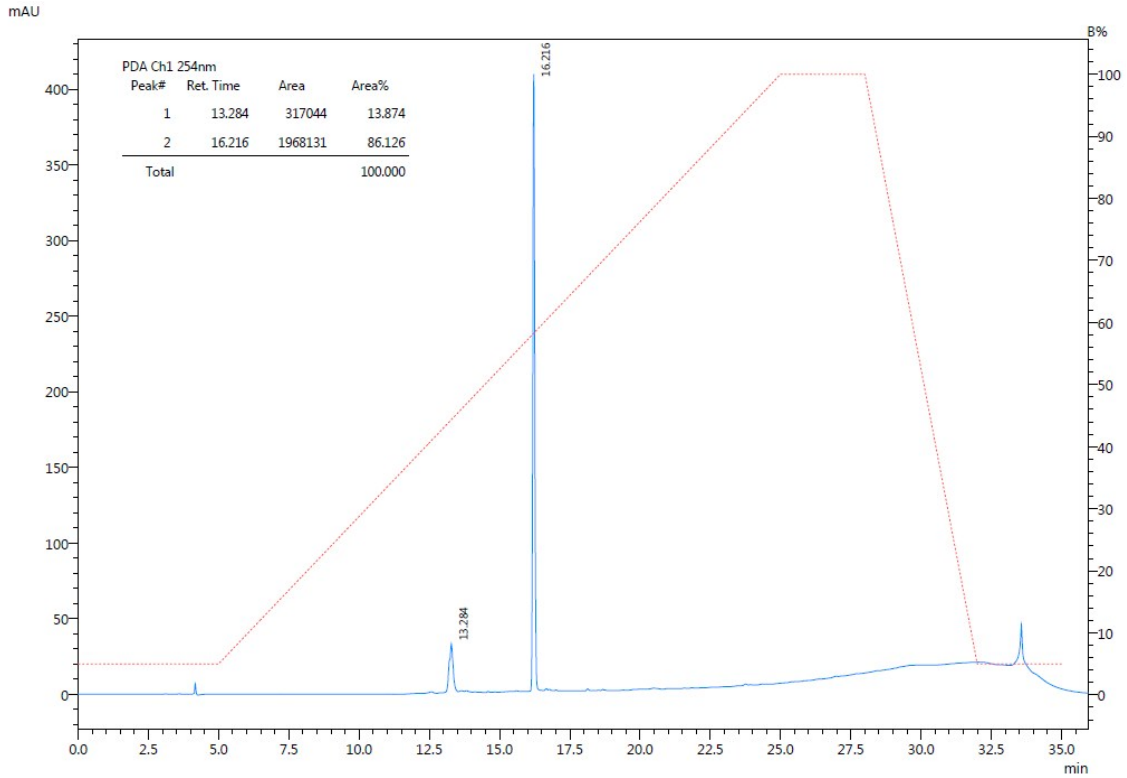

mV
2000
1750
1500
1250
1000
750
500
250
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
min
t=0.00 hrs
t=0.40 hrs
t=1.22 hrs
t=2.00 hrs
t=2.53 hrs
t=3.32 hrs
t=4.12 hrs
t=4.52 hrs
t=6.03 hrs
HO
O
O
N
NH
N
N
NH2
O
NH
OH
O

**RP-HPLC Chromatograms:**

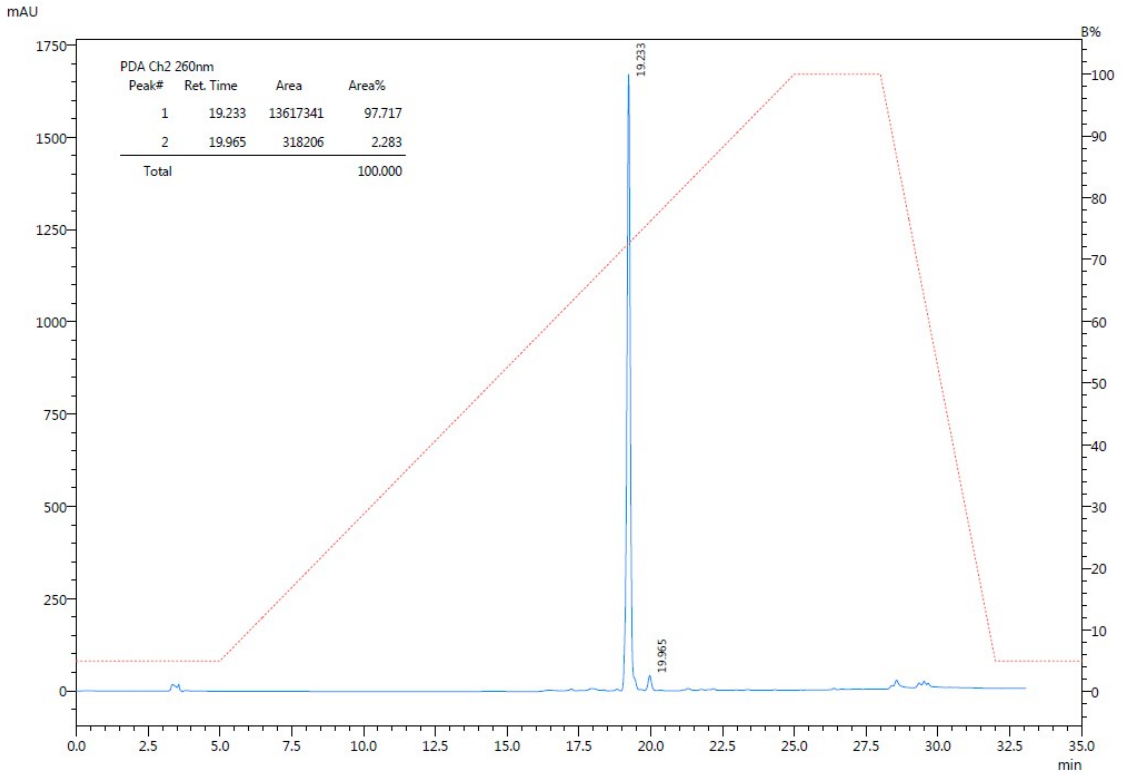
HPLC trace of compound **1**



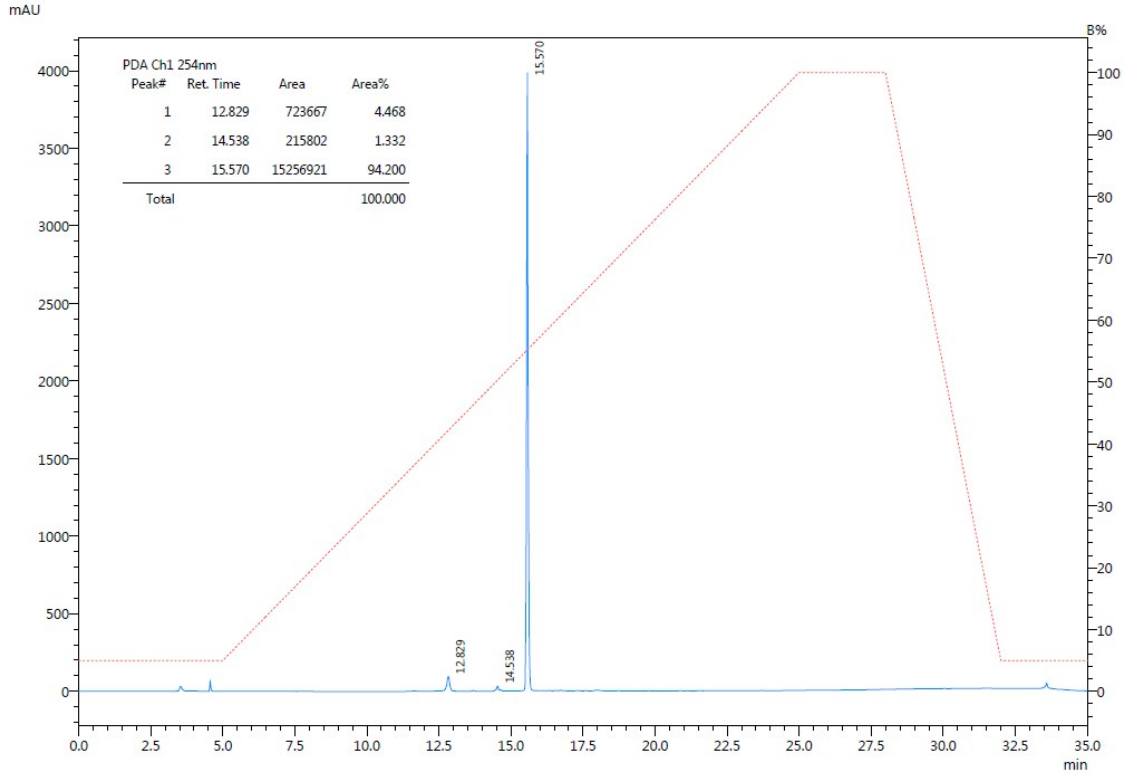

HPLC trace of compound **2**



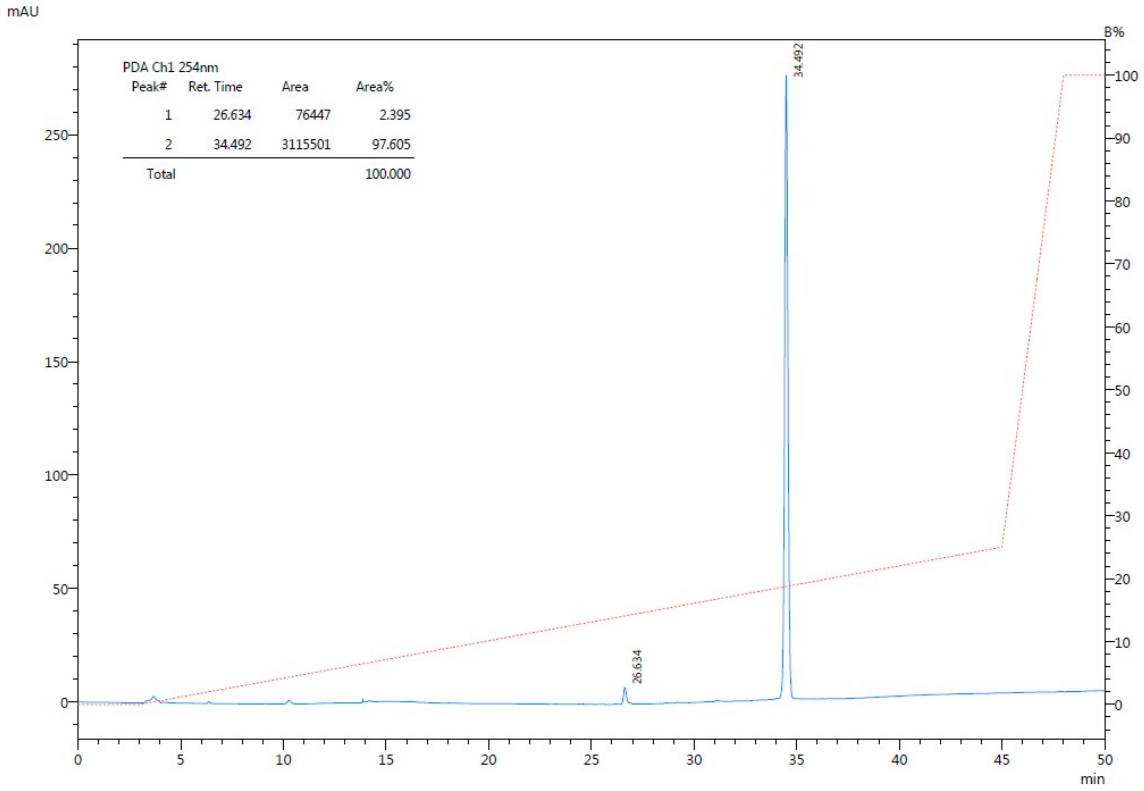
HPLC trace of compound **3**



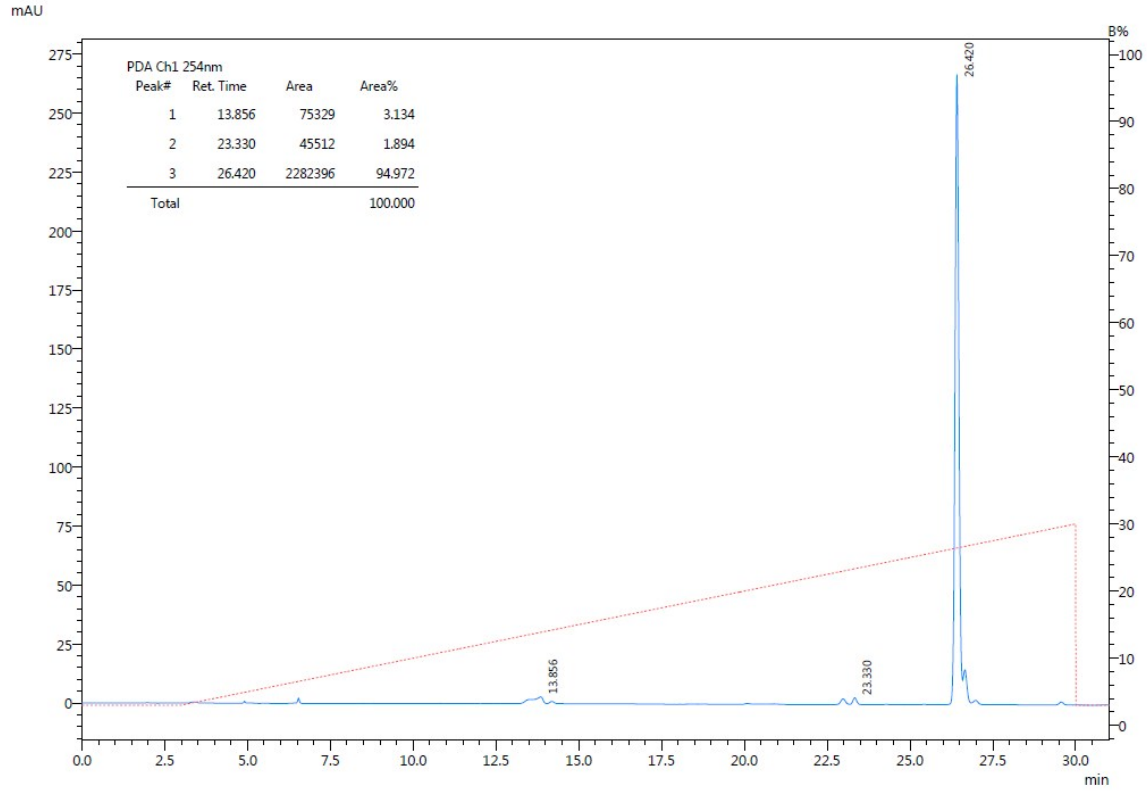

HPLC trace of compound **4**



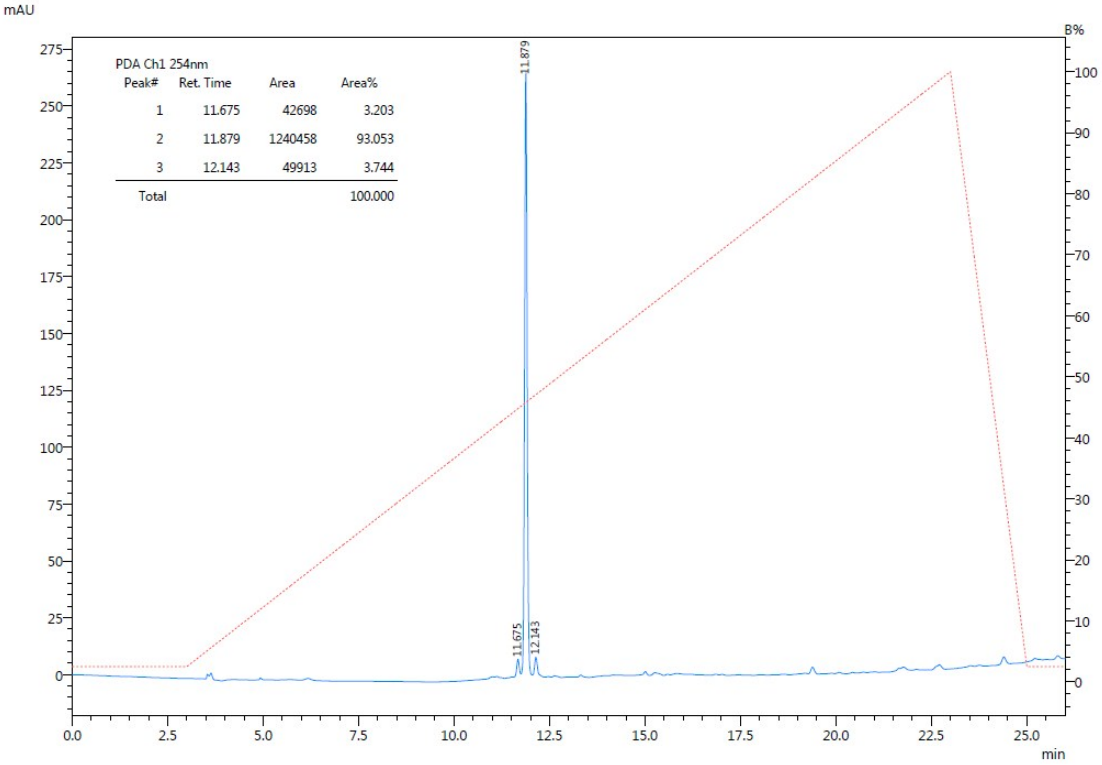
HPLC trace of compound **5**



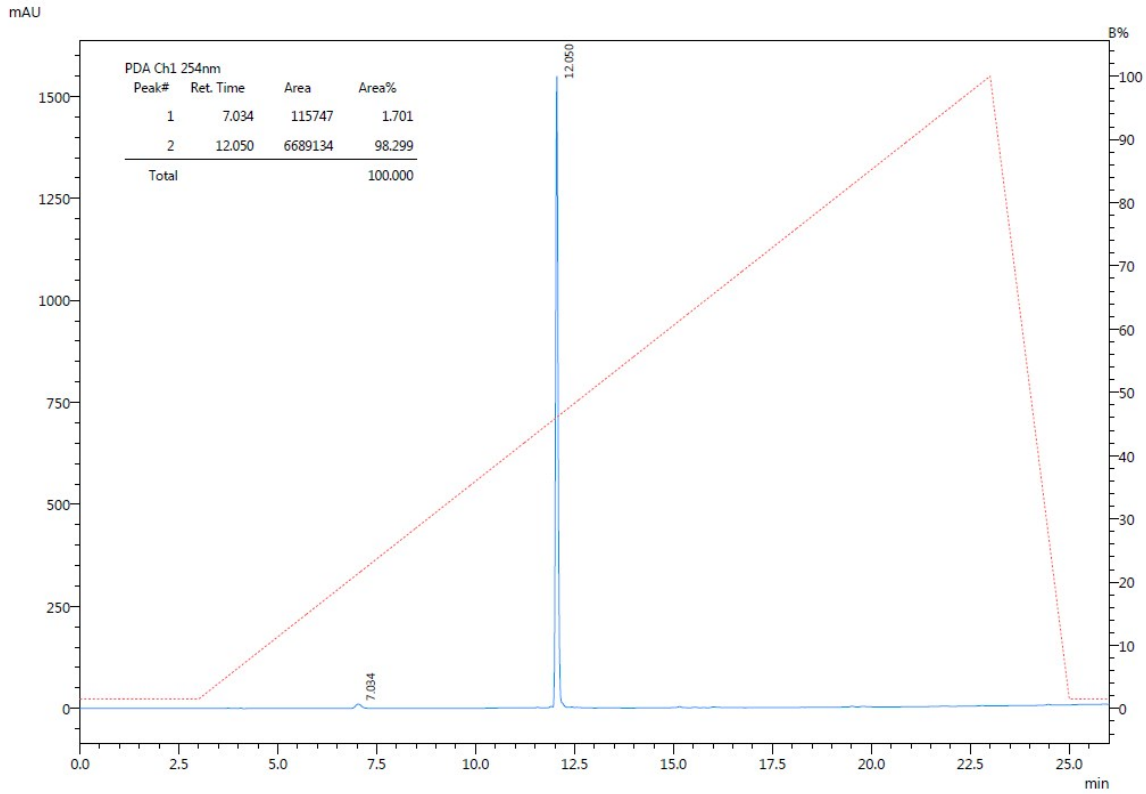

HPLC trace of compound **6**



HPLC trace of compound **16**



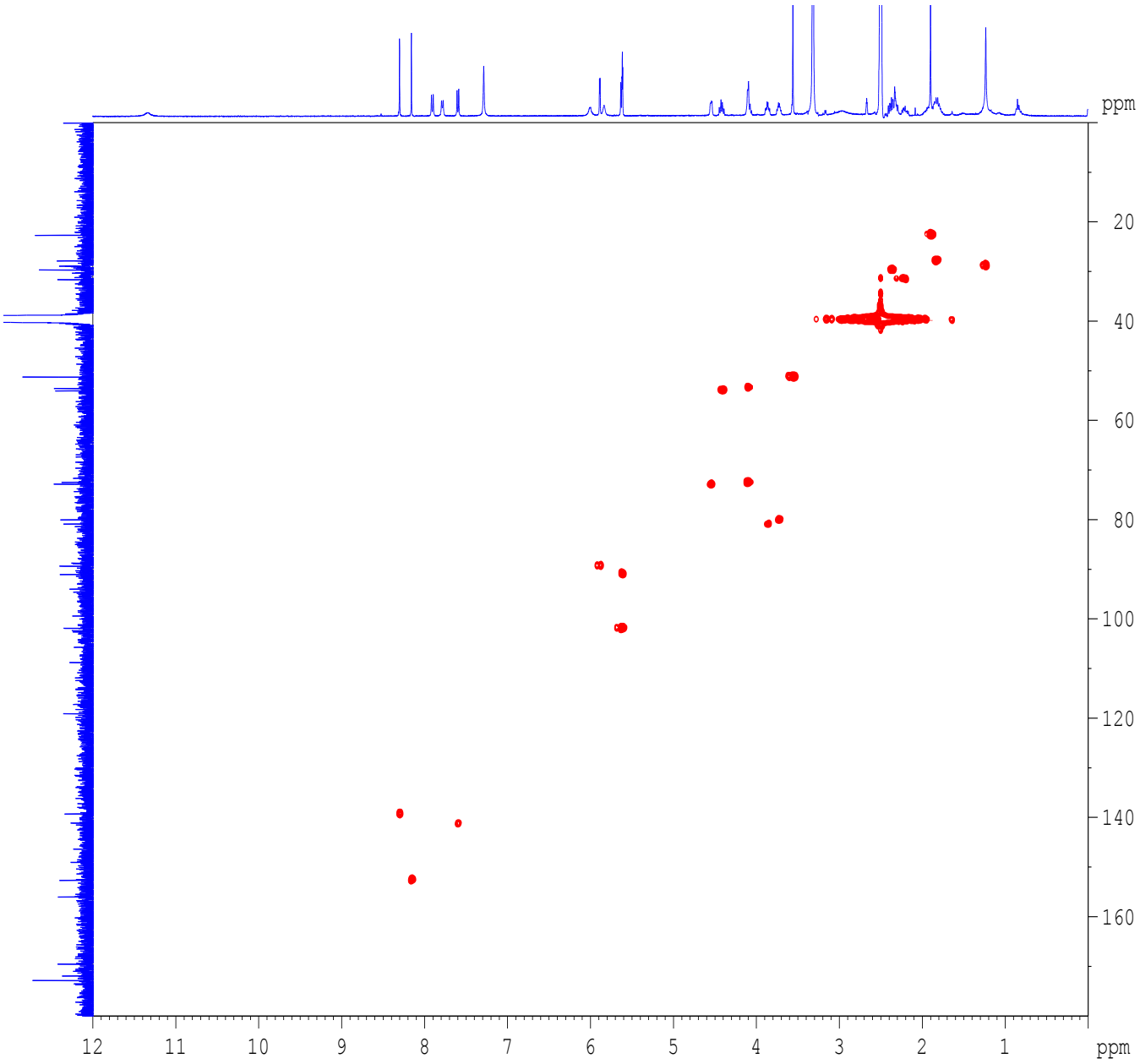

HPLC trace of compound **17**



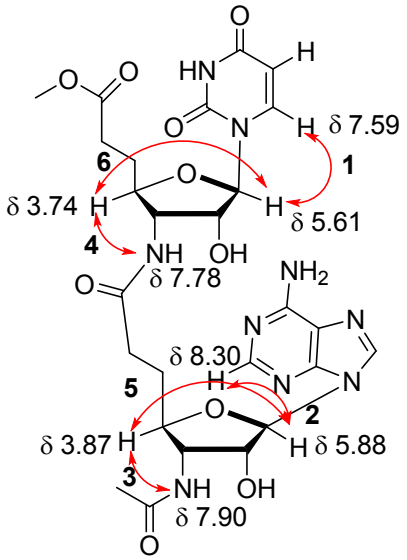
**NMR Studies:**

Solution 1D NMR spectra were recorded on 400 MHz spectrometers and 2D NMR spectra were recorded on 400, 700 or 800 MHz spectrometers (unless mentioned otherwise) at room temperature or else as mentioned using 2-10 mM concentration of compounds in appropriate solvents using TMS as internal standard or the solvent signals as secondary standards and the chemical shifts (δ) are shown in ppm scales. $^{13}C$ spectra were recorded at 100 MHz (unless mentioned otherwise) with complete proton decoupling. The proton resonance assignments for the compounds **5**, **6** were carried out by using $^{13}C$-$^{1}H$ Heteronuclear single quantum coherence spectroscopy (HSQY) and nuclear Overhauser effect spectroscopy (NOESY). All the experiments were carried out in the phase sensitive mode. The spectra were acquired with 2048x512, 2048x400 or 2048x256 free induction decays (FID) with relaxation delay 1.5 - 2s. The NOESY experiments were performed in DMSO-$d_6$ and 10% $D_2O$ in $H_2O$ with water suppression using excitation sculpting with gradients and with mixing time of 0.1 - 0.4s.

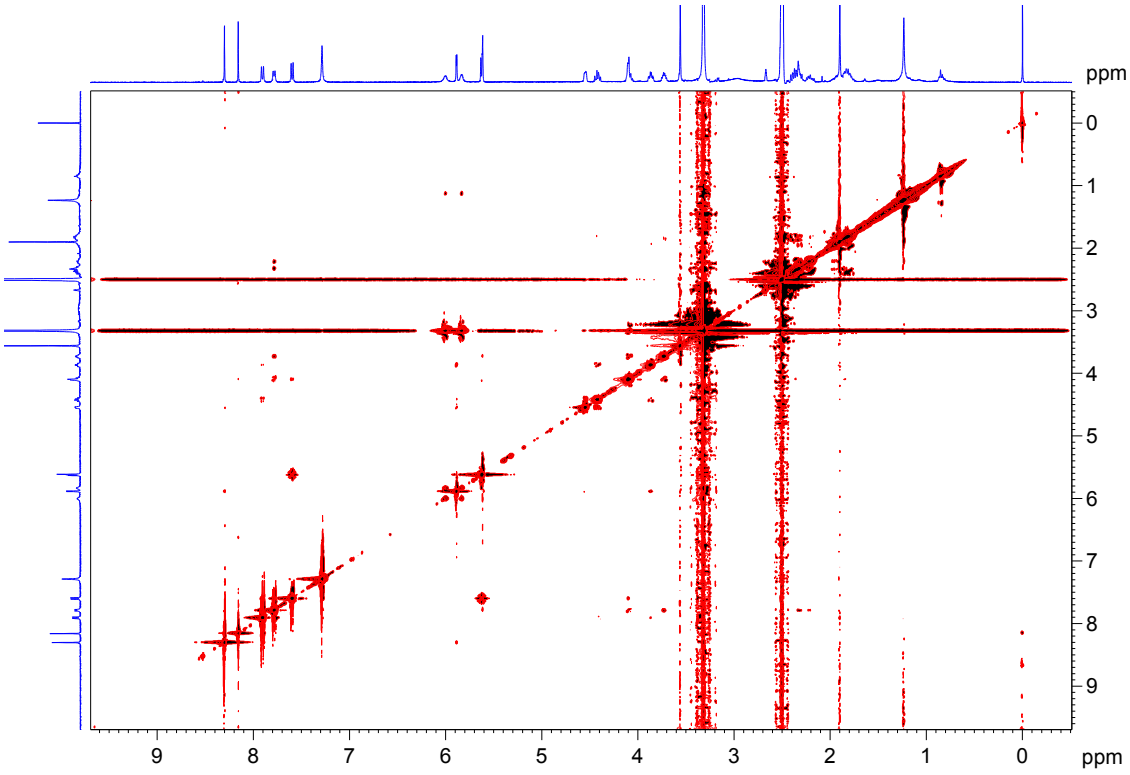
$^{13}C$ - $^{1}H$ HSQC of compound **5** in DMSO-$d_6$

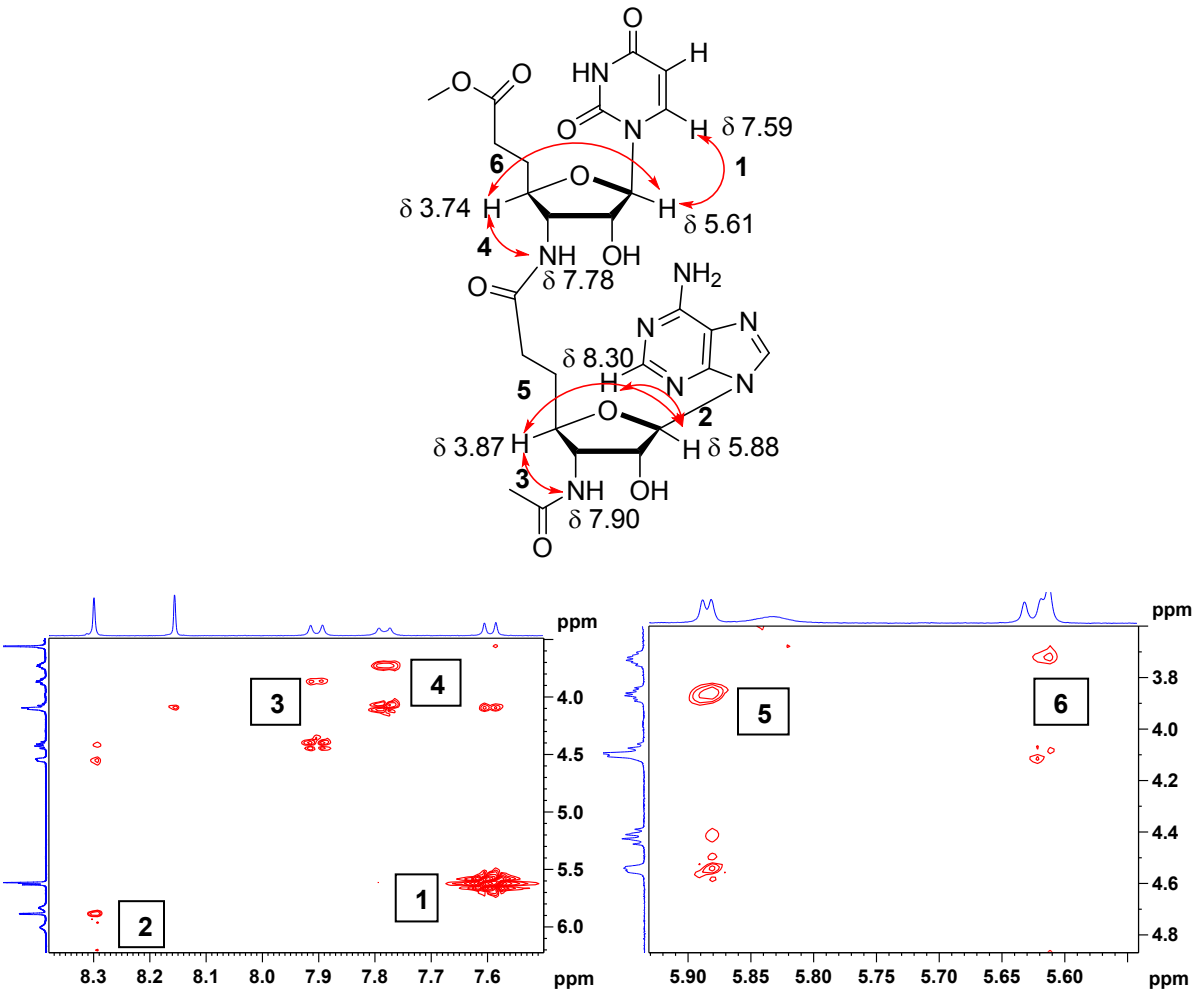


$^{1}$H - $^{1}$H NOESY of compound **5** in DMSO-$d_6$

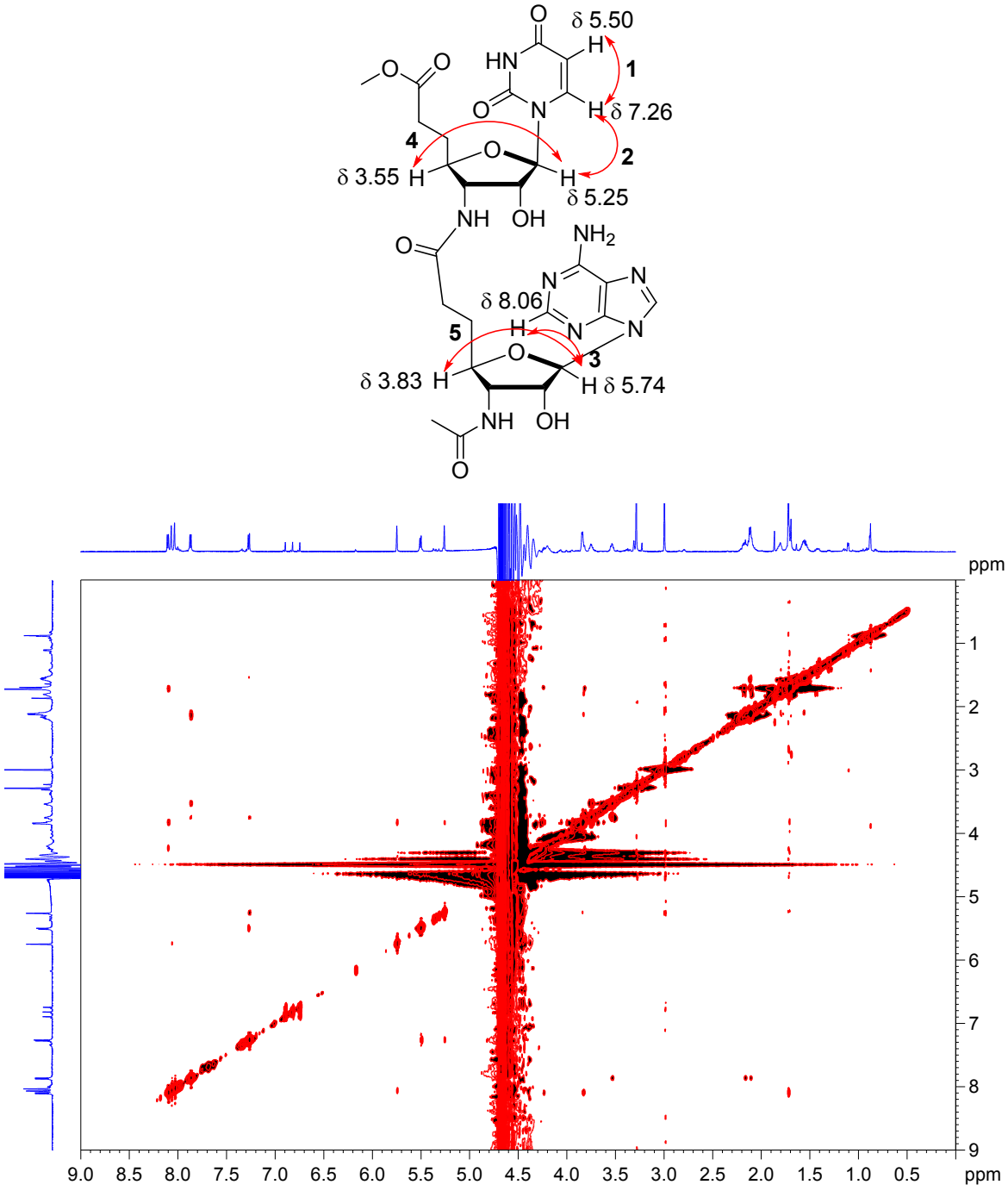


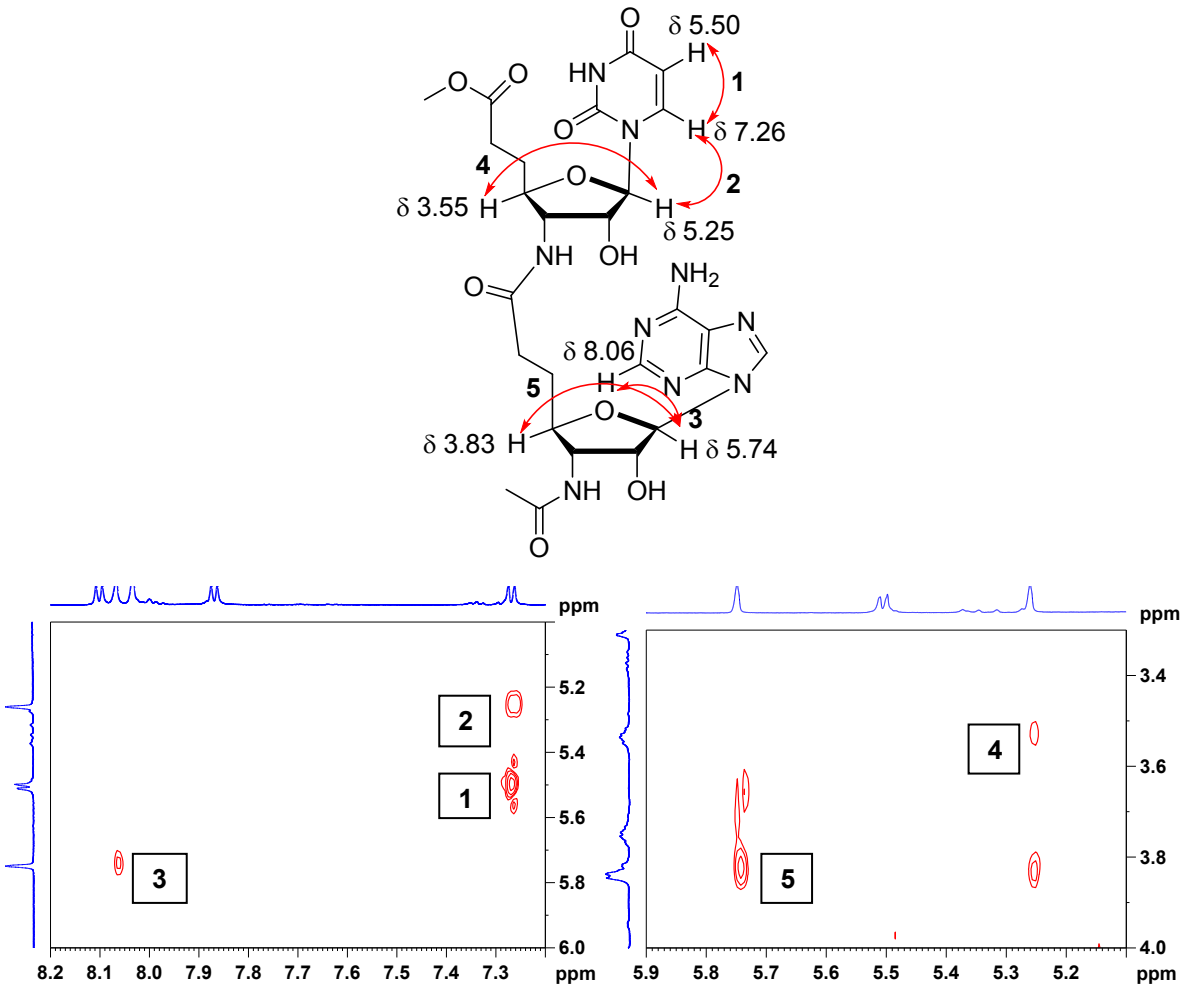

Characteristic NOE cross-peaks of compound **5**



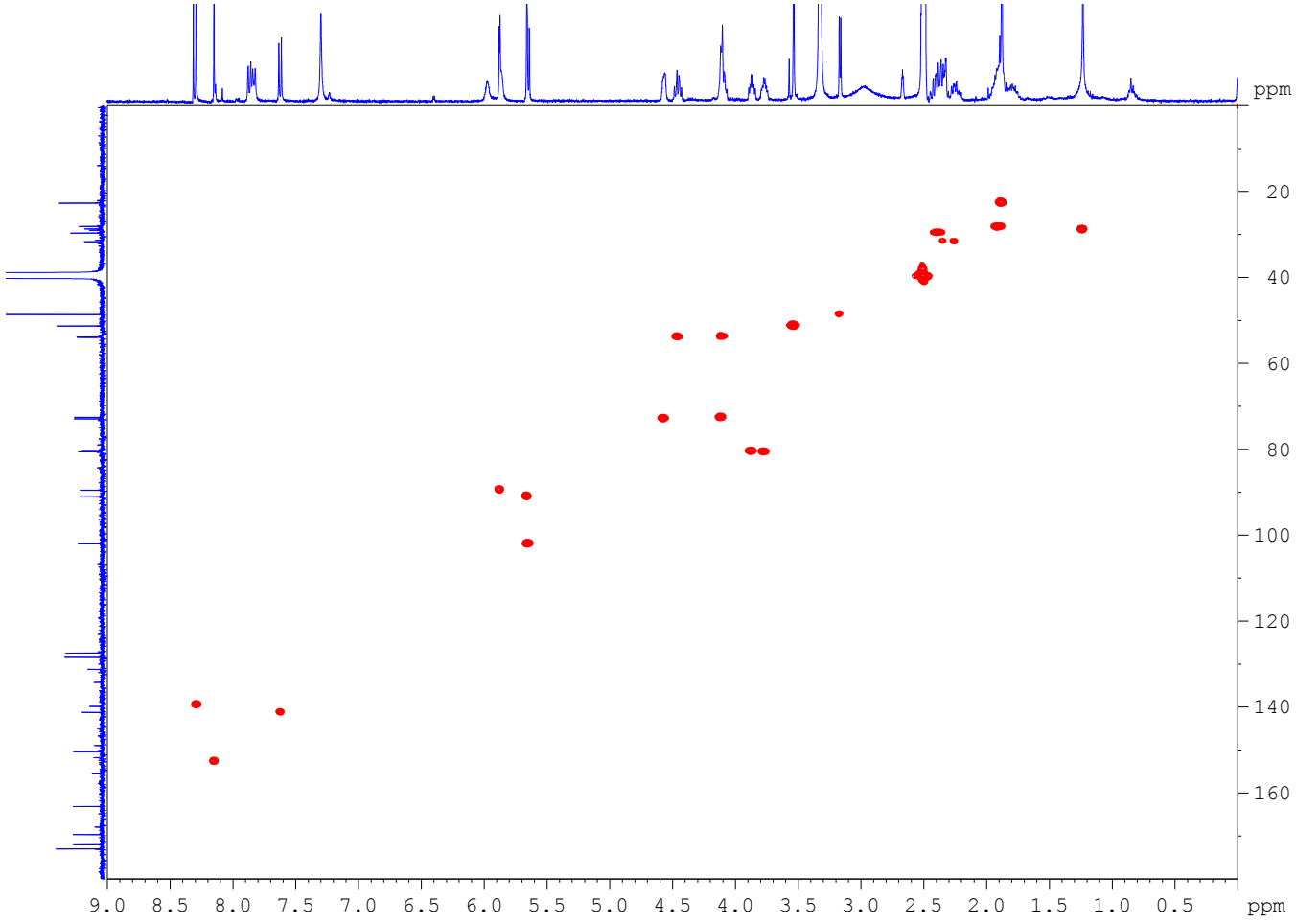

δ 7.59
δ 3.74
δ 5.61
δ 7.78
δ 8.30
δ 3.87
δ 5.88
δ 7.90
ppm
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
4.0
4.5
5.0
5.5
6.0
5.90
5.85
5.80
5.75
5.70
5.65
5.60
3.8
4.0
4.2
4.4
4.6
4.8

$^{1}$H - $^{1}$H NOESY of compound **5** in 10% $D_2O$ in $H_2O$-278K, 700 MHz

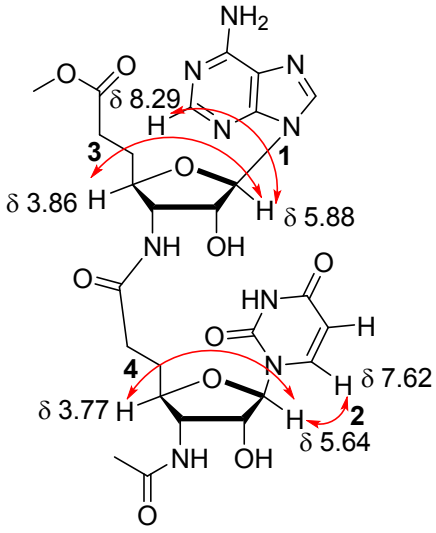


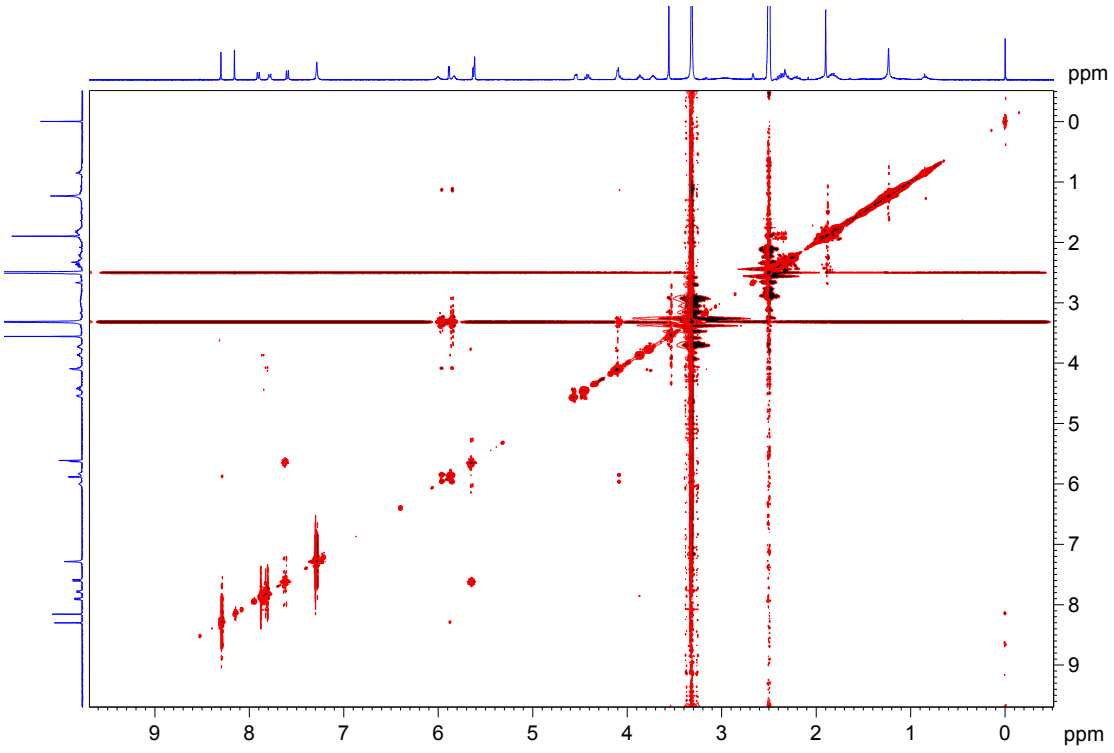

δ 5.50
δ 7.26
δ 3.55
δ 5.25
δ 8.06
δ 3.83
δ 5.74
1
2
3
4
5
ppm
8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3
5.2 5.4 5.6 5.8 6.0
5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2
3.4 3.6 3.8 4.0

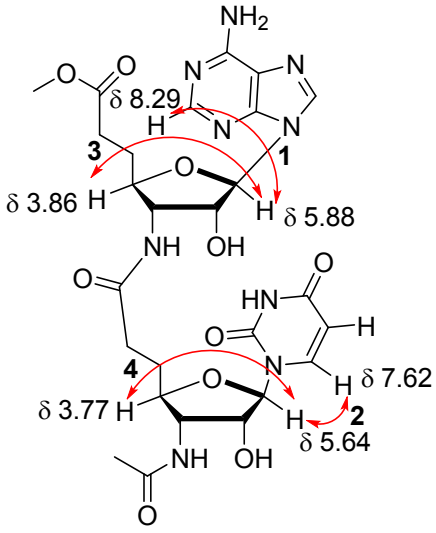
$^{13}C$ - $^{1}H$ HSQC of compound **6** in DMSO-$d_6$

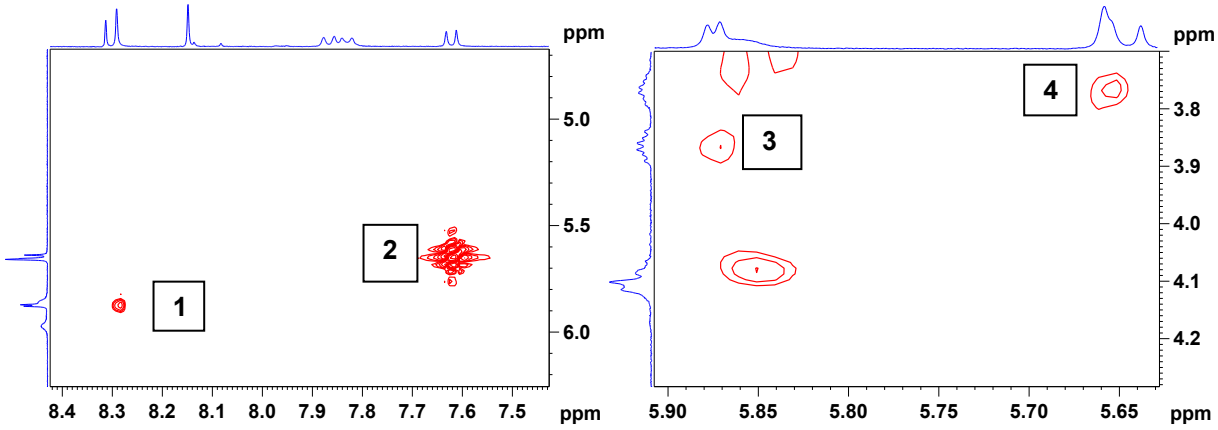


$^{1}$H - $^{1}$H NOESY of compound **6** in DMSO-$d_6$

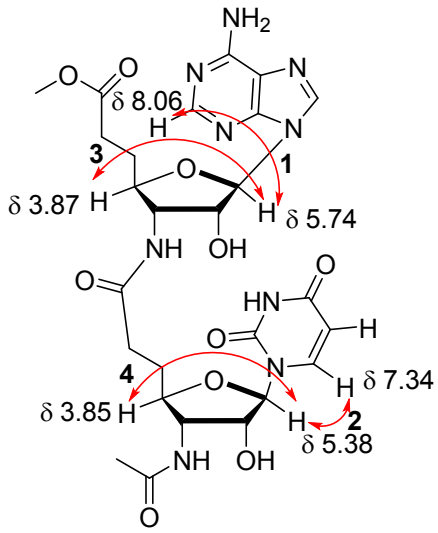


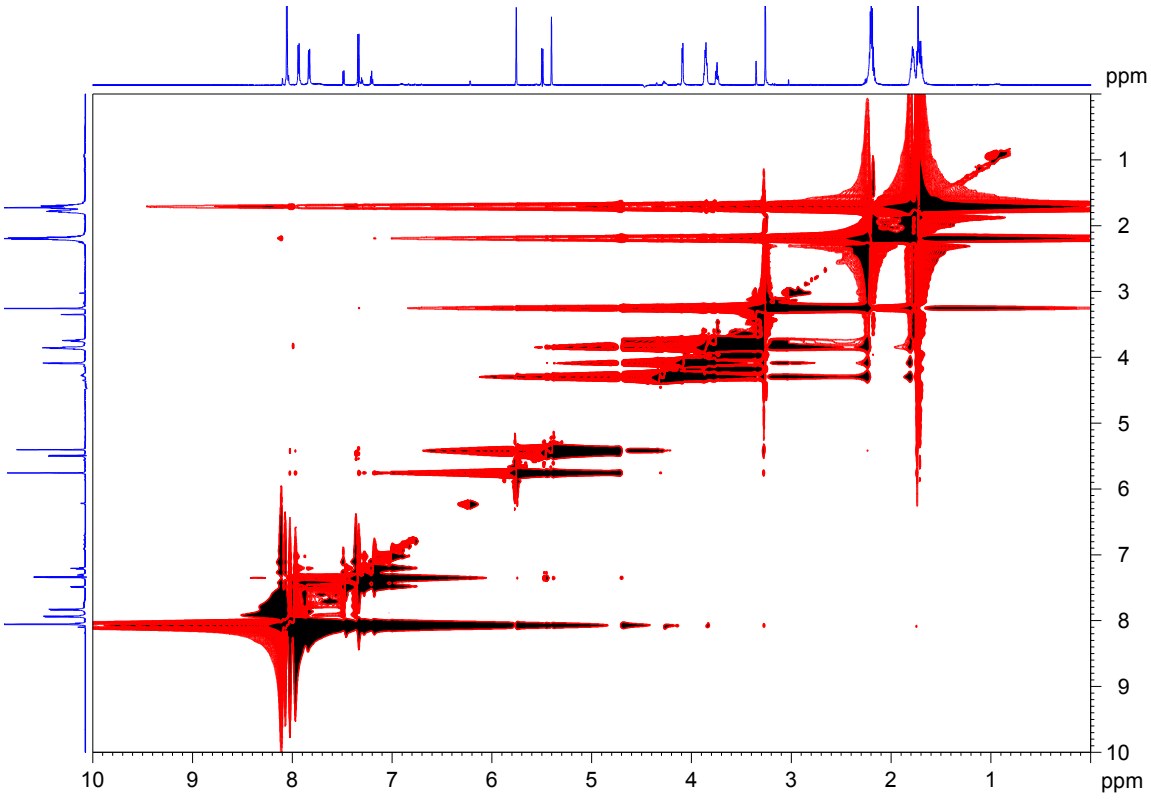



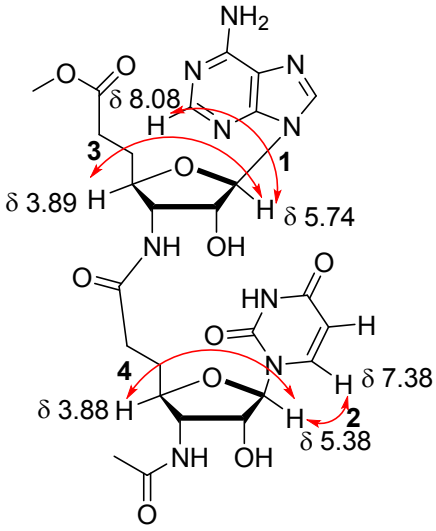

NH2
O
O
N
N
δ 8.29
H
N
N
3
1
O
δ 3.86 H
H δ 5.88
NH
OH
O
HN
O
H
O
N
4
H δ 7.62
O
δ 3.77 H
H
2
δ 5.64
NH
OH
O

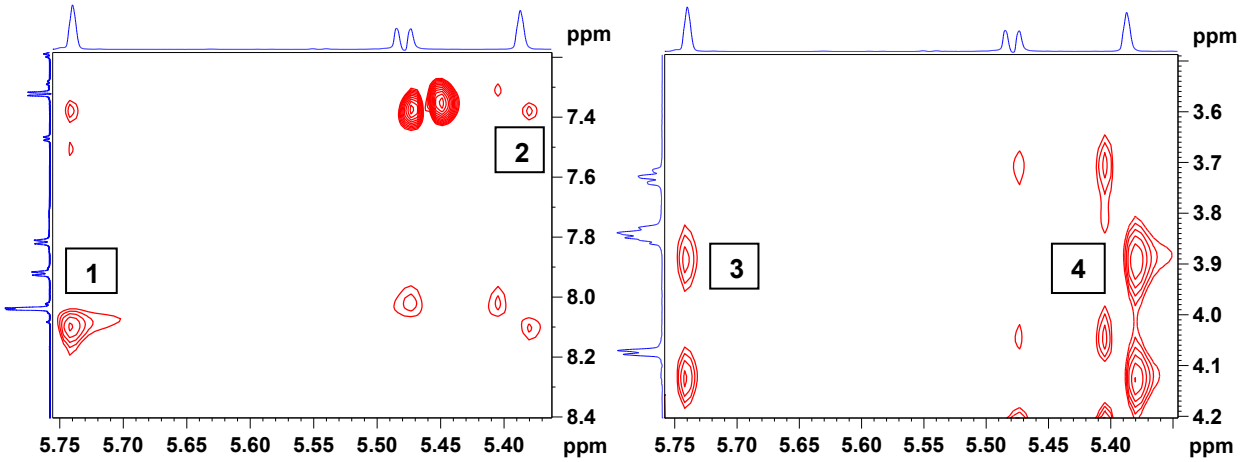

ppm
5.0
5.5
6.0
1
2
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
ppm
ppm
3.8
3.9
4.0
4.1
4.2
3
4
5.90
5.85
5.80
5.75
5.70
5.65
ppm

$^{1}$H - $^{1}$H NOESY of compound **6** in 10% $D_2O$ in $H_2O$-278K, 800 MHz




NH2
O
O
N
N
δ 8.08
H
N
N
3
1
O
δ 3.89 H
H δ 5.74
NH
OH
O
HN
O
H
N
4
O
H δ 7.38
δ 3.88 H
H
2
δ 5.38
NH
OH
O


ppm
7.4
7.6
7.8
8.0
8.2
8.4
1
2
5.75 5.70 5.65 5.60 5.55 5.50 5.45 5.40
ppm
ppm
3.6
3.7
3.8
3.9
4.0
4.1
4.2
3
4
5.75 5.70 5.65 5.60 5.55 5.50 5.45 5.40
ppm