CHEMBIOCHEM

Supporting Information

An Expedient Synthesis of Flexible Nucleosides through Enzymatic Glycosylation of Proximal and Distal Fleximer Bases

Sophie Vichier-Guerre,[a] Therese C. Ku,[b] Sylvie Pochet,*[a] and Katherine L. Seley-Radtke*[b]

cbic_201900714_sm_miscellaneous_information.pdf

Author Contributions

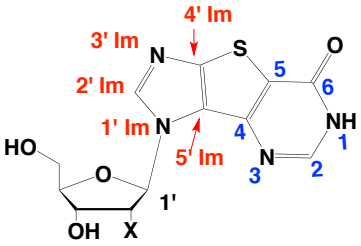
S.V.-G. Investigation: Lead; Methodology: Lead
T.C.K. Investigation: Supporting; Methodology: Supporting; Writing - Original Draft: Equal; Writing - Review & Editing: Equal
S.P. Funding acquisition: Equal; Project administration: Equal; Resources: Equal; Supervision: Equal; Writing - Original Draft: Equal; Writing - Review & Editing: Equal
K.L.S.-R. Conceptualization: Equal; Funding acquisition: Equal; Project administration: Equal; Resources: Equal; Supervision: Equal; Writing - Original Draft: Equal; Writing - Review & Editing: Equal.

Table of contents

Only the spectra (as recorded below) are shown.

Compound	Advance 400	Advance 600	Neo 800
2a	^{1}H	^{13}C	
2b	^{1}H	^{13}C	
3a	^{1}H, ^{13}C		
4a	^{1}H, ^{13}C		
5a			^{1}H, ^{13}C, HMBC
5b			^{1}H, ^{13}C, HMBC
5c			^{1}H, ^{13}C, HMBC
3c	^{1}H, ^{13}C		
5d		^{1}H, ^{13}C, HMBC	
5e		^{1}H, ^{13}C, HMBC	

Nomenclature used for NMR description



General procedures. Canonical nucleobases and nucleosides were purchased from Sigma-Aldrich or Carbosynth. Flex-bases **1**-**5** were synthesized according to published procedures.[1,2] Analytical HPLC was carried out on an Agilent system (1200 series) using a C18 reverse phase column (Kromasil, 5 μm, 100 Å, 4.6x150 mm) at a flow rate of 1mL/min and a linear gradient of acetonitrile in 10 mM triethylammonium acetate buffer over 20 min (Gradients: G1, 0 to 20 %; G2, 0 to 40%). Purification by preparative HPLC was carried out on an Agilent 1100 Series system on a C18 reverse phase column (Kromasil, 5 μm, 100Å, 10x250 mm) using a flow rate of 4.0 mL/min and a linear gradient of acetonitrile in 10 mM triethylammonium acetate buffer over 20 min. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance 400, Advance 600 or Avance Neo 800. Chemical shifts are given in ppm (δ) relative to residual solvent peak, coupling constants (J) are reported in Hertz and standard abbreviations are used. Assignment of ^{1}H and ^{13}C signals was performed by analysis of the correlated homonuclear ^{1}H,^{1}H-COSY and heteronuclear ^{1}H,^{13}C-HMBC, ^{1}H,^{13}C-HSQC spectra. High-resolution mass spectra were recorded on a Waters Q-TOF micro MS instrument under electrospray ionization in positive ionization mode using a mobile phase of acetonitrile/water with 0.1 % formic acid.

NDT-catalyzed transglycosylation reaction. Nucleoside 2'-deoxyribosyltransferases class II from *L. leichmannii* (*Ll*NDT) was produced and purified as described previously.[3] Typically, reaction mixtures (0.1 mL) contained a flex-base (1 μmol) as the acceptor and thymidine (4 μmol) as the donor. A 0.2 M stock solution of flex-base in DMSO was added slowly to 10 mM citrate buffer (pH 6.5) containing thymidine to reach a final volume of 0.1 mL. Reaction was started by adding NDT to the mixture (variable amounts) and incubation was carried out at 37°C. Conversion was monitored by analytical reverse phase HPLC. Optimal conditions leading to all possible glycosylated products were scaled-up to the preparative scale, and the corresponding nucleosides were isolated after purification by reverse phase HPLC and fully characterized.

NDT-catalyzed transglycosylation of flex-base 2. Compound **2** (4.0 mg, 0.02 mmol) and thymidine (20.0 mg, 0.08 mmol) in citrate buffer (4.0 mL, 5% v/v DMSO) were incubated in the presence of NDT (25 μL) at 37°C for 4 h (75% conversion). After purification by reverse phase HPLC, three compounds were isolated, the starting material **2**, and the C4- and C5-pyrimidinyl 2'-deoxyribosylimidazoles derivatives (**2a** and **2b**).

2-Amino-4-methoxy-5-[1-(2'-deoxy-β-D-ribofuranosyl)-1*H*-imidazol-4-yl]-pyrimidine (2a): 0.8 mg (13% yield); t_R = 14.88 min (G1); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.20-2.28 (ddd, J = 2.9, 5.9, 13.1 Hz, 1H, H-2'), 2.35-2.42 (ddd, J = 5.9, 7.3, 13.1 Hz, 1H, H-2"), 3.49-3.55 (m, 2H, H-5', H-5"), 3.78-3.84 (m, 1H, H-4'), 3.94 (s, 3H, OCH_3), 4.28-4.35 (m, 1H, H-3'), 4.89 (t, J = 5.2 Hz, 1H, OH-5'), 5.23 (d, J = 3.8 Hz, 1H, OH-3'), 6.03 (t, J = 6.5 Hz, 1H, H-1'), 6.50 (br s, 2H, NH_2), 7.47 (d, J = 1.0 Hz, 1H, H-5 Im), 7.87 (br s, 1H, H-2 Im), 8.59 (s, 1H, H-6 Pyr); ^{13}C NMR (200 MHz, DMSO-d_6): δ 41.2, 53.1, 62.2, 71.3, 86.4, 88.1, 104.9, 115.1, 134.6, 136.8, 155.8, 162.3, 165.8; HRMS (ESI-TOF) m/z calcd for [$C_{13}H_{17}N_5O_4$ + H]: 308.1359, found: 308.1355.

2-Amino-4-methoxy-5-[1-(2'-deoxy-*β*-D-ribofuranosyl)-1*H*-imidazol-5-yl]-pyrimidine (2b): 1.0 mg (16% yield); t_R = 12.77 min (G1); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.12-2.20 (ddd, J = 3.4, 6.0, 13.3 Hz, 1H, H-2'), 2.30-2.38 (m, 1H, H-2'), 3.47-3.53 (m, 2H, H-5', H-5"), 3.70-3.75 (m, 1H, H-4'), 3.82 (s, 3H, OCH_3), 4.20-4.28 (m, 1H, H-3'), 4.88 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.18 (d, *J* = 4.7 Hz, 1H, OH-3'), 5.57 (dd, *J* = 6.2, 7.9 Hz, 1H, H-1'), 6.79 (d, *J* = 0.9 Hz, 1H, H-4 Im), 6.84 (br s, 2H, NH_2), 7.90 (s, 1H, H-6 Pyr), 8.00 (d, *J* = 0.9 Hz, 1H, H-2 Im); ^{13}C NMR (200 MHz, DMSO-d_6): δ 41.7, 53.7, 62.0, 71.1, 84.6, 87.9, 98.9, 125.8, 128.2, 136.4, 159.9, 164.2, 167.5; HRMS (ESI-TOF) m/z calcd for [HRMS (ESI-TOF) m/z calcd for [$C_{13}H_{17}N_5O_4$ + H]: 308.1359, found: 308.1355.

2,6-Dimethoxy-4-[1-(2'-deoxy-*β*-D-ribofuranosyl)-1*H*-imidazol-4-yl]-pyrimidine (3a). Compound **3a** was obtained starting from **3** (8.0 mg, 0.039 mmol) and thymidine (40.0 mg, 0.17 mmol) in the presence of NDT (100 μL) in citrate buffer (4.0 mL, 5% v/v DMSO) at 37°C overnight (100% conversion). Purification by reverse phase HPLC gave **3a** (8.2 mg, 66% yield); t_R = 13.33 min (G2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.24-2.32 (ddd, *J* = 3.3, 6.2, 13.2 Hz, 1H, H-2'), 2.37-2.46 (ddd, *J* = 6.0, 7.3, 13.3 Hz, 1H, H-2"), 3.48-3.61 (m, 2H, H-5', H-5"), 3.62-3.87 (m, 1H, H-4'), 3.91 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.30-4.37 (m, 1H, H-3'), 4.95 (br s, 1H, OH-5'), 5.28 (br s, 1H, OH-3'), 6.11 (t, *J* = 6.1 Hz, 1H, H-1'), 6.83 (s, 1H, H-6 Pyr), 8.00 (d, *J* = 1.3 Hz, 1H, H-5 Im), 8.04 (d, J = 1.3 Hz, 1H, H-2 Im); ^{13}C NMR (100 MHz, DMSO- d_6): δ 41.5, 54.1, 54.7, 62.1, 71.1, 86.4, 88.3, 94.5, 119.1, 138.0, 139.8, 162.6, 165.5, 172.3; HRMS (ESI-TOF) m/z calcd for [$C_{14}H_{18}N_4O_5$ + H]: 323.1356, found: 323.1352.

2-Amino-6-methoxy-4-[1-(2'-deoxy-*β*-D-ribofuranosyl)-1*H*-imidazol-4-yl]-pyrimidine (4a). Compound **4a** was obtained starting from **4** (5.5 mg, 0.029 mmol) and thymidine (27.5 mg, 0.11 mmol) in the presence of NDT (35 μL) in citrate buffer (2.5 mL, 5% v/v DMSO) at 37°C overnight (100% conversion). Purification by reverse phase HPLC gave **4a** (3.2 mg, 36% yield); t_R = 15.42 min (G1); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.11-2.41 (ddd, *J* = 3.1, 6.1, 13.3 Hz, 1H, H-2'), 2.32-2.43 (ddd, *J* = 5.9, 7.4, 13.3 Hz, 1H, H-2'), 3.51 (br s, 2H, H-5', H-5"), 3.81-3.85 (m, 4H, H-4', OCH_3), 4.29-4.34 (m, 1H, H-3'), 4.92 (br s, 1H, OH-5'), 5.27 (br s, 1H, OH-3'), 6.08 (t, *J* = 6.4 Hz, 1H, H-1'), 6.40 (br s, 2H, NH_2), 6.45 (s, 1H, H-6 Pyr), 7.76 (d, *J* = 1.2 Hz, 1H, H-5 Im), 7.94 (d, *J* = 1.2 Hz, 1H, H-2 Im); ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.5, 53.3, 62.2, 71.2, 86.2, 88.2, 90.3, 117.5, 137.6, 140.8, 161.9, 163.9, 171.1; HRMS (ESI-TOF) m/z calcd for [$C_{13}H_{17}N_5O_4$ + H]: 308.1359, found: 308.1363.

NDT-catalyzed transglycosylation of imidazo[4',5':4,5]-thieno[3,2-*d*]pyrimidin-5(6*H*)-one (5). Compound **5** (3.25 mg, 0.016 mmol) and thymidine (16.3 mg, 0.07 mmol) in citrate buffer (2.0 mL, 5% v/v DMSO) were incubated in the presence of NDT (20 μL) at 37°C overnight (75% conversion). After purification by reverse phase HPLC, three compounds were isolated.

1'-(2'-deoxy-*β*-D-ribofuranosyl)-1H-imidazo[4',5':4,5]-thieno[3,2-*d*]pyrimidin-5(6*H*)-one (5a): 0.8 mg (15% yield); t_R = 13.33 min (G1); ^{1}H NMR (800 MHz, DMSO-d_6): δ 2.29-2.37 (m, 1H, H-2'), 2.74-2.83 (m, 1H, H-2"), 3.50-3.69 (m, 2H, H-5', H-5"), 3.88-3.95 (m, 1H, H-4'), 4.37-4.45 (m, 1H, H-3'), 4.25 (br s, 1H, OH-5'), 5.31 (d, *J* = 3.4 Hz, 1H, OH-3'), 6.52 (dd,

J = 5.8, 8.0 Hz, 1H, H-1'), 8.22 (s, 1H, H-2 Pyr), 8.49 (s, 1H, H-2' Im), 12.62 (br s, 1H, NH); ^{13}C NMR (200 MHz, DMSO-d_6): δ 41.1, 62.2, 71.1, 86.3, 88.7, 121.4, 121.3, 128.3, 143.4, 144.7, 148.7, 160.1; HRMS (ESI-TOF) m/z calcd for [$C_{12}H_{12}N_4O_4S$ + H]: 309.0658, found: 309.0646.

1-(2'-deoxy-β-D-ribofuranosyl)-6-(2'-deoxy-β-D-ribofuranosyl)-1*H*-imidazo[4',5':4,5]thieno[3,2-d]pyrimidin-5(6*H*)-one (5b): 1.2 mg (17% yield); t_R = 16.75 min (G1); ^{1}H NMR (800 MHz, DMSO-d_6: δ 2.23-2.43 (m, 3H, H-2'a, H-2'b, H-2"b), 2.72-2.79 (m, 1H, H-2"a), 3.53-3.72 (m, 4H, H-5', H-5"), 3.89-3.97 (m, 2H, H-4'), 4.31-4.37 (m, 1H, H-3'b), 4.38-4.43 (m, 1H, H-3'a), 4.96 (br s, 1H, OH-5'), 5.13 (br s, 1H, OH-5', 5.30-5.36 (m, 2H, OH-3'), 6.48 (t, *J* = 6.1 Hz, 1H, H-1'b), 6.55 (t, *J* = 7.2 Hz, 1H, H-1'a), 8.56 (s, 1H, H-2' Im), 8.83 (s, 1H, H-2 Pyr); ^{13}C NMR (200 MHz, DMSO-d_6): δ 40.5, 42.1, 61.7, 62.1, 70.4, 71.5, 85.2, 86.3, 88.6, 88.7, 120.3, 128.2, 142.3, 145.0, 146.6, 149.5, 157.0; HRMS (ESI-TOF) m/z calcd for [$C_{17}H_{20}N_4O_7S$ + Na]: 447.0950, found: 447.0958.

3-(2'-deoxy-β-D-ribofuranosyl)-3*H*-imidazo[4',5':4,5]-thieno[3,2-*d*]pyrimidin-5(6*H*)-one (5c): 0.6 mg (11% yield); t_R = 11.25 min (G1); ^{1}H NMR (800 MHz, DMSO-d_6): δ 2.32-2.37 (ddd, *J* = 3.5, 5.8, 13.3 Hz, 1H, H-2'), 2.74-2.83 (ddd, *J* = 5.8, 7.2, 13.3 Hz 1H, H-2"), 3.56 (br s, 2H, H-5', H-5"), 3.89-3.92 (m, 1H, H-4'), 4.36 (br s, 1H, H-3'), 4.92 (br s, 1H, OH-5'), 5.41 (br s, 1H, OH-3'), 6.30 (dd, *J* = 6.2, 7.1 Hz, 1H, H-1'), 8.21 (s, 1H, H-2 Pyr), 8.32 (s, 1H, H-2' Im), 12.80 (br s, 1H, NH); ^{13}C NMR (200 MHz, DMSO-d_6): δ 39.3, 62.3, 71.2, 86.5, 88.4, 112.3, 120.3, 133.9, 142.3, 143.8, 148.2, 159.6; HRMS (ESI-TOF) m/z calcd for [$C_{12}H_{12}N_4O_4S$ + H]: 309.0658, found: 309.0660.

PNP-catalyzed transglycosylation reaction.

Bacterial purine nucleoside phosphorylase (N2415, 10 U/mg) was purchased from Sigma-Aldrich. Enzymatic reaction mixtures (0.1 mL) contained a flex-base (1 μmol) and adenosine as donor (2 μmol). A 0.2 M stock solution of flex-base in DMSO was added slowly to 10 mM phosphate buffer (pH 7.4) containing adenosine to reach a final volume of 0.1 mL. The reaction was started by adding PNP (2 μL at 0.1 U/μL) and run at 37°C or 50°C. Conversion was monitored by analytical reverse phase HPLC. Products were purified by reverse phase HPLC and characterized by NMR. Due to the limited quantities available, some ^{13}C NMR and heteronuclear correlation spectra are missing.

2,6-Dimethoxy-4-(1-β-D-ribofuranosyl-1H-imidazol-4-yl)-pyrimidine (3c). Compound **3c** was obtained starting from **3** (3.0 mg, 0.015 mmol) and adenine (0.03 mmol) in the presence of PNP (30 μL, 3 U) for 16 h at 50°C (80% conversion). Purification by reverse phase HPLC gave 3.45 mg (68% yield) of **3c**. t_R = 12.6 min (G2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.53-3.60 (m, 1H, H-5'), 3.60-3.67 (m, 1H, H-5"), 3.91 (s, 3H, OCH_3), 3.91 (br s, 1H, H-4'), 3.94 (s, 3H, OCH_3), 4.05-4.09 (m, 1H, H-3'), 4.21 (t, J = 5.4 Hz, 1H, H-2'), 5.06 (br s, 1H, OH-5'), 5.20 (br s, 1H, OH-3'), 5.46 (br s, OH-2'), 5.64(d, J = 6.02 Hz, 1H, H-1'), 6.84 (s, 1H, H-6 Pyr), 8.01 (d, J = 1.3 Hz, 1H, H-5 Im), 8.06 (d, *J* = 1.3 Hz, 1H, H-2 Im); ^{13}C NMR (100 MHz, DMSO-d_6): 54.1, 54.7, 61.8, 71.0, 76.1, 86.2, 90.2, 94.5, 119.2, 138.3, 139.9, 162.5, 165.5, 172.3; HRMS (ESI-TOF) m/z calcd for [$C_{14}H_{18}N_4O_6$+H]: 339.1305, found: 339.1303

PNP-catalyzed transglycosylation of imidazo[4',5':4,5]-thieno[3,2-*d*]pyrimidin-5(6H)-one (5). Compound **5** (1.0 mg, 4.8 μmol) and adenine (2 equiv) were incubated in the presence of PNP (10 μL, 1 U) for 16 h at 37°C (80% conversion). After purification by reverse phase HPLC, two compounds were isolated.

1-(*β*-D-ribofuranosyl)-1*H*-imidazo[4',5':4,5]thieno[3,2-*d*]pyrimidin-5(6*H*)-one (5d): 0.4 mg (26% yield). t_R = 16.5 min (G1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.53-3.65 (m, 1H, H-5'), 3.64-3.76 (m, 1H, H-5"), 4.00 (br s, 1H, H-4'), 4.16 (br s, 1H, H-3'), 4.59 (br s, 1H, H-2'), 5.20 (br s, 1H, OH-3'), 5.48 (br s, 1H, OH-2'), 5.90 (br s, 1H, OH-5'), 5.95 (d, J = 7.2 Hz, 1H, H-1'), 8.15 (s, 1H, H-2), 8.44 (s, 1H, H-2' Im); ^{13}C NMR (150 MHz, DMSO-d_6): 62.2, 70.8, 74.7, 86.9, 89.7, 121.6, 128.4, 143.4, 145.8, 150.6, 162.6 (^{13}C missing); HRMS (ESI-TOF) m/z calcd for [$C_{12}H_{11}N_4O_5S$ + H]: 325.0607, found: 325.0609.

3-(*β*-D-ribofuranosyl)-3*H*-imidazo[4',5':4,5]thieno[3,2-*d*]pyrimidin-5(6*H*)-one (5e): 0.2 mg (13% yield). t_R = 13.8 min (G1). ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.55-3.71 (m, 2H, H-5', H-5"), 3.96 (br s, 1H, H-4'), 4.08 (br s, 1H, H-3'), 4.31 (br s, 1H, H-2'), 5.04 (br s, 1H, OH-5'), 5.32 (br s, 1H, OH-3'), 5.61 (br s, OH-2'), 5.74 (t, J = 6.9 Hz, 1H, H-1'), 8.06 (br s, 1H, H-2 Pyr), 8.12 (s, 1H, H-2' Im); ^{13}C NMR (150 MHz, DMSO-d_6): 62.5, 71.2, 73.6, 86.2, 89.8, 121.6, 128.4, 143.4, 145.8, 150.6, 162.6 (^{13}C missing); HRMS (ESI-TOF) m/z calcd for [$C_{12}H_{11}N_4O_5S$ + H]: 325.0607, found: 325.0607.

References

[1] Seley, K. L.; Januszczyk, P.; Hagos, A.; Zhang, L.; Dransfield, D. T. *J. Med. Chem.* **2000**, *43*, 4877-83.

[2] Ku, T.; Lopresti, N.; Shirley, M.; Mori, M.; Marchant, J.; Heng, X.; Botta, M.; Summers, M. F.; Seley-Radtke, K. L. *Bioorg. Med. Chem.* **2019**, *27*, 2883-2892.

[3] Kaminski, P. A. *J. Biol. Chem.* **2002**, 277, 14400-14407

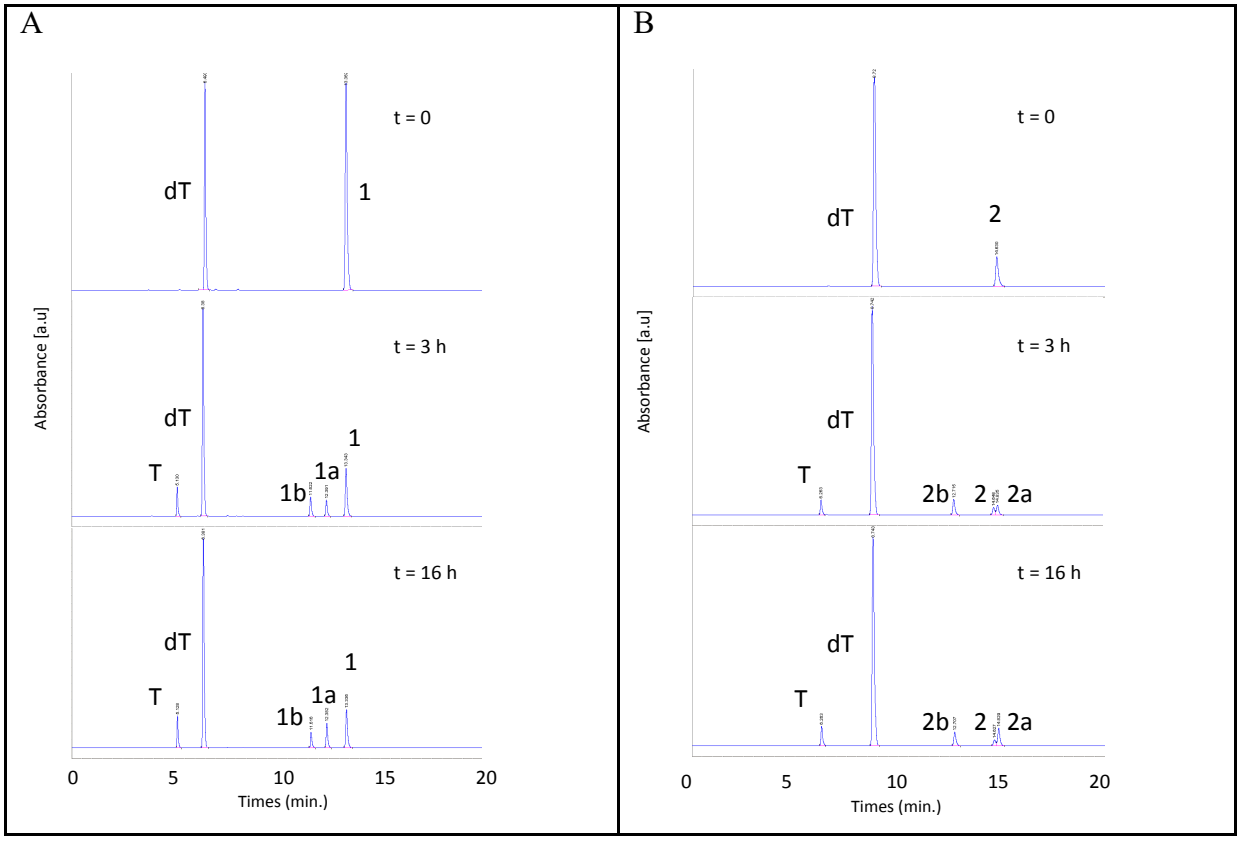


Figure S1. HPLC monitoring of the transglycosylation reaction of **1** (panel A) or **2** (panel B) in the presence of thymidine (4 equiv) and NDT (1.25 μL/μmol acceptor) at t = 0, 3 h and 16 h at 37°C. HPLC conditions: 0–40% (panel A) or 0–20% (panel B) linear gradient of acetonitrile in 10 mM TEAA buffer pH 6.0 over 20 min at a flow rate of 1mL min^{-1}. Detection at 254 nm.

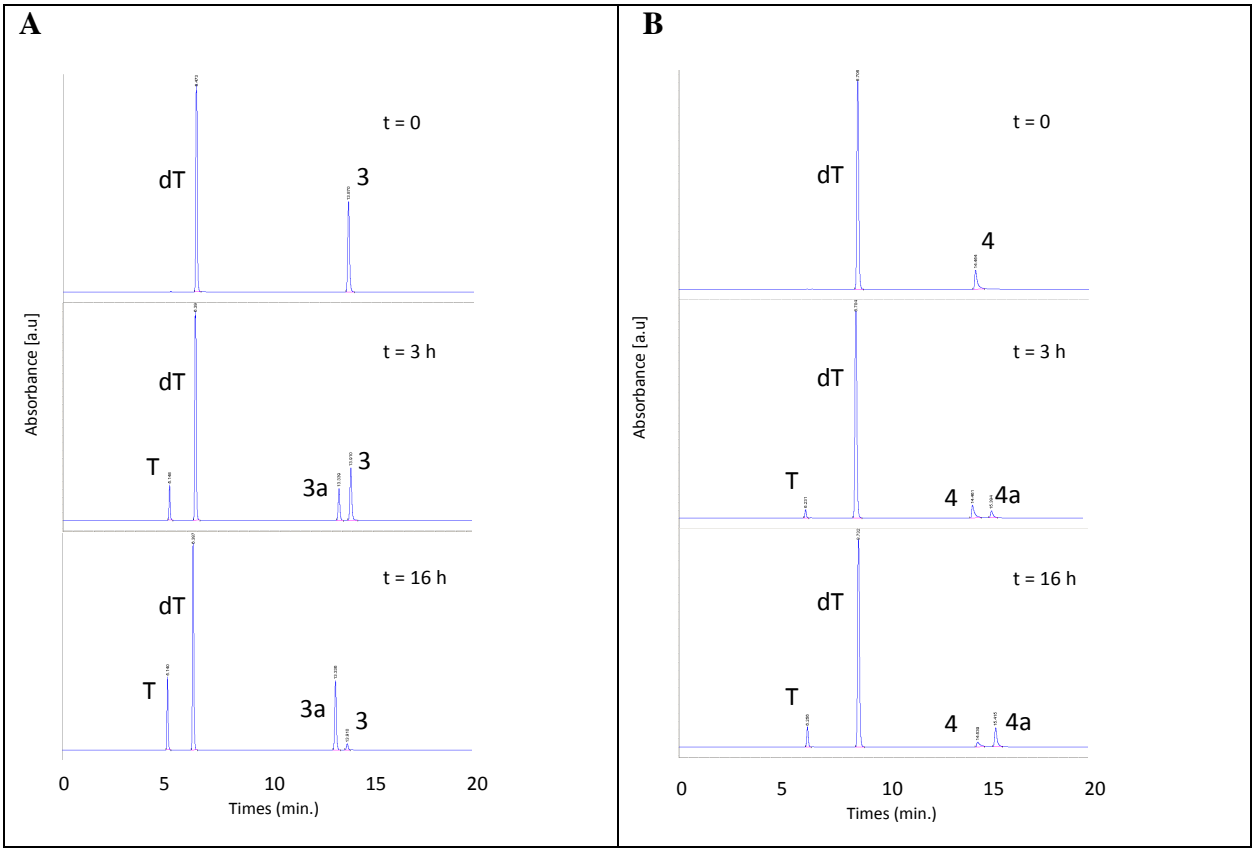


Figure S2. HPLC monitoring of the transglycosylation reaction of **3** (panel A) or **4** (panel B) in the presence of thymidine (4 equiv) and NDT (1.25 μL/μmol acceptor) at t = 0, 3 h and 16 h at 37°C. HPLC conditions: 0–40% (panel A) or 0–20% (panel B) linear gradient of acetonitrile in 10 mM TEAA buffer pH 6.0 over 20 min at a flow rate of 1mL min^{-1}. Detection at 254 nm.

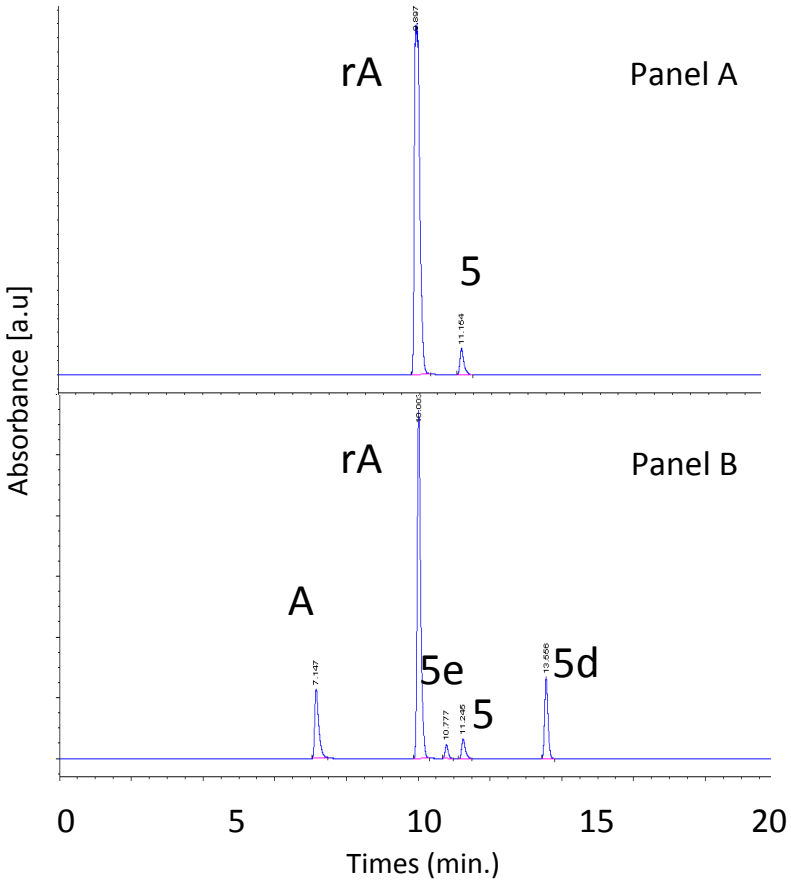
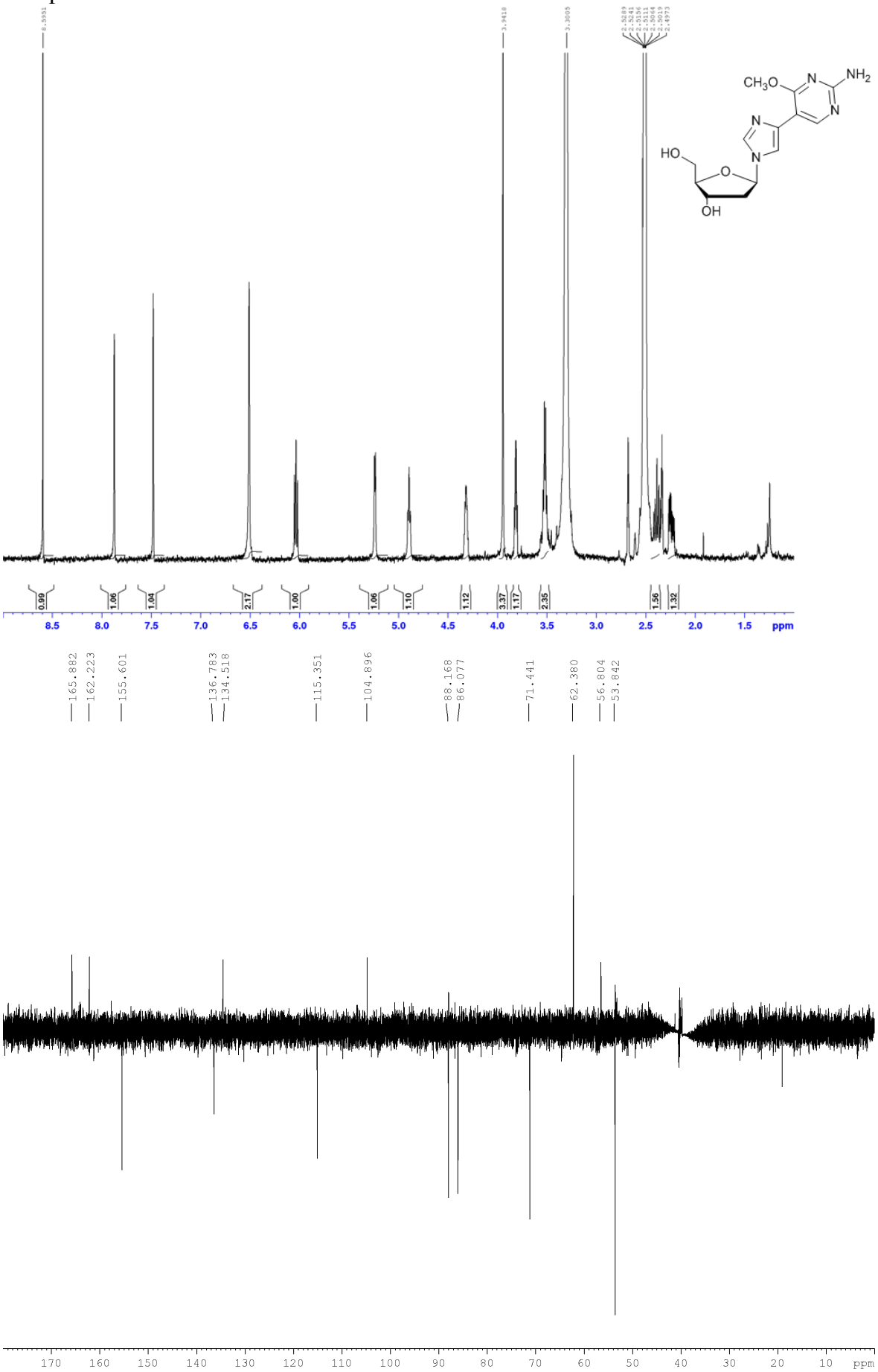
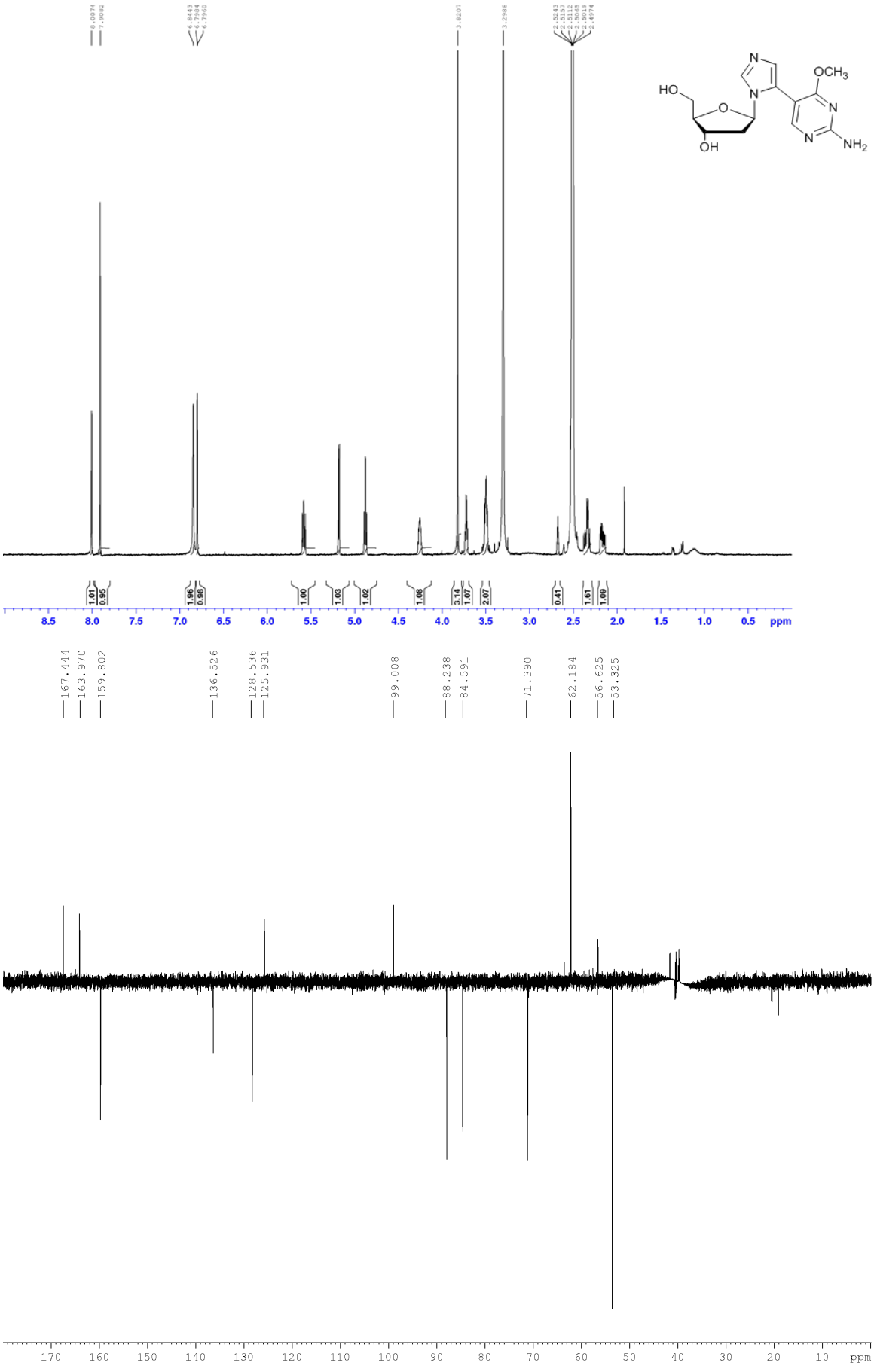


Figure S3. HPLC monitoring of the transglycosylation reaction of **5** in the presence of adenosine (2 equiv) and PNP (6.25 μL/μmol acceptor) at t = 0 (panel A) and 12 h (panel B) at 37°C. HPLC conditions: 0–20% linear gradient of acetonitrile in 10 mM TEAA buffer pH 6.0 over 20 min at a flow rate of 1mL min^{-1}. Detection at 254.

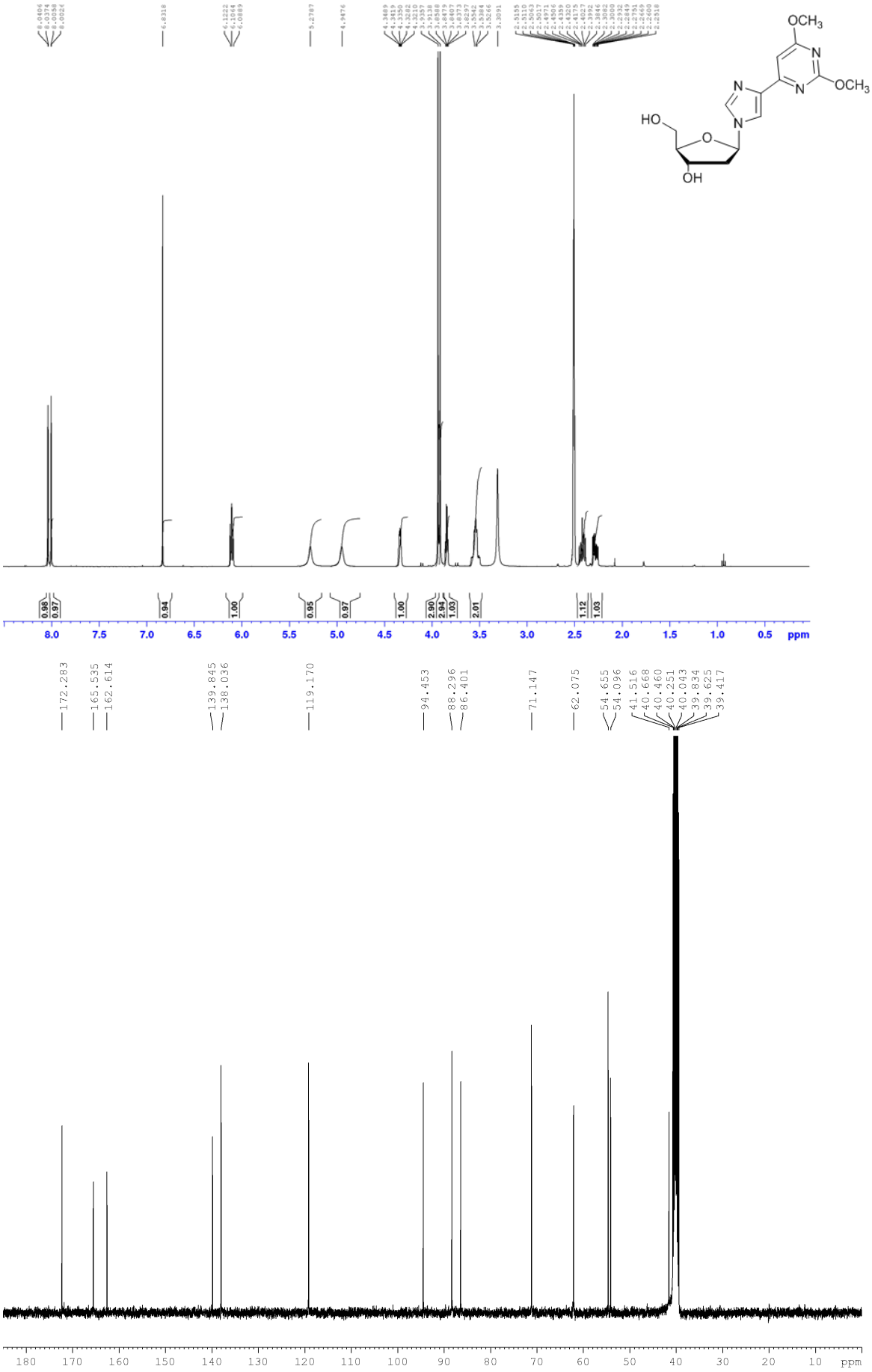
Compound **2a**


8.5991
3.9418
3.3005
2.5089
2.5243
2.5398
2.5553
2.5708
2.5864
2.6019
2.4973
CH3O
N
NH2
N
N
HO
O
N
OH
0.99
1.06
1.04
2.17
1.00
1.06
1.10
1.12
3.37
1.17
2.35
1.56
1.32
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
165.882
162.223
155.601
136.783
134.518
115.351
104.896
88.168
86.077
71.441
62.380
56.804
53.842
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

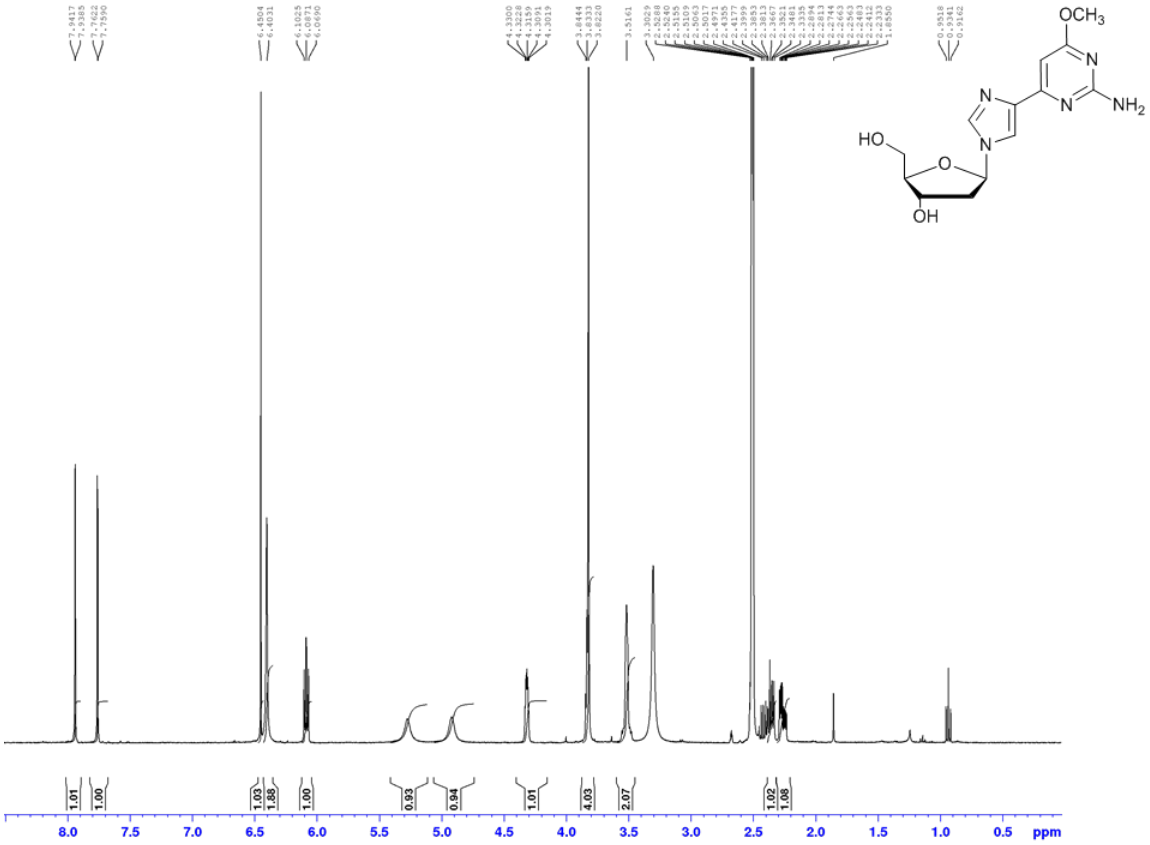
Compound **2b**

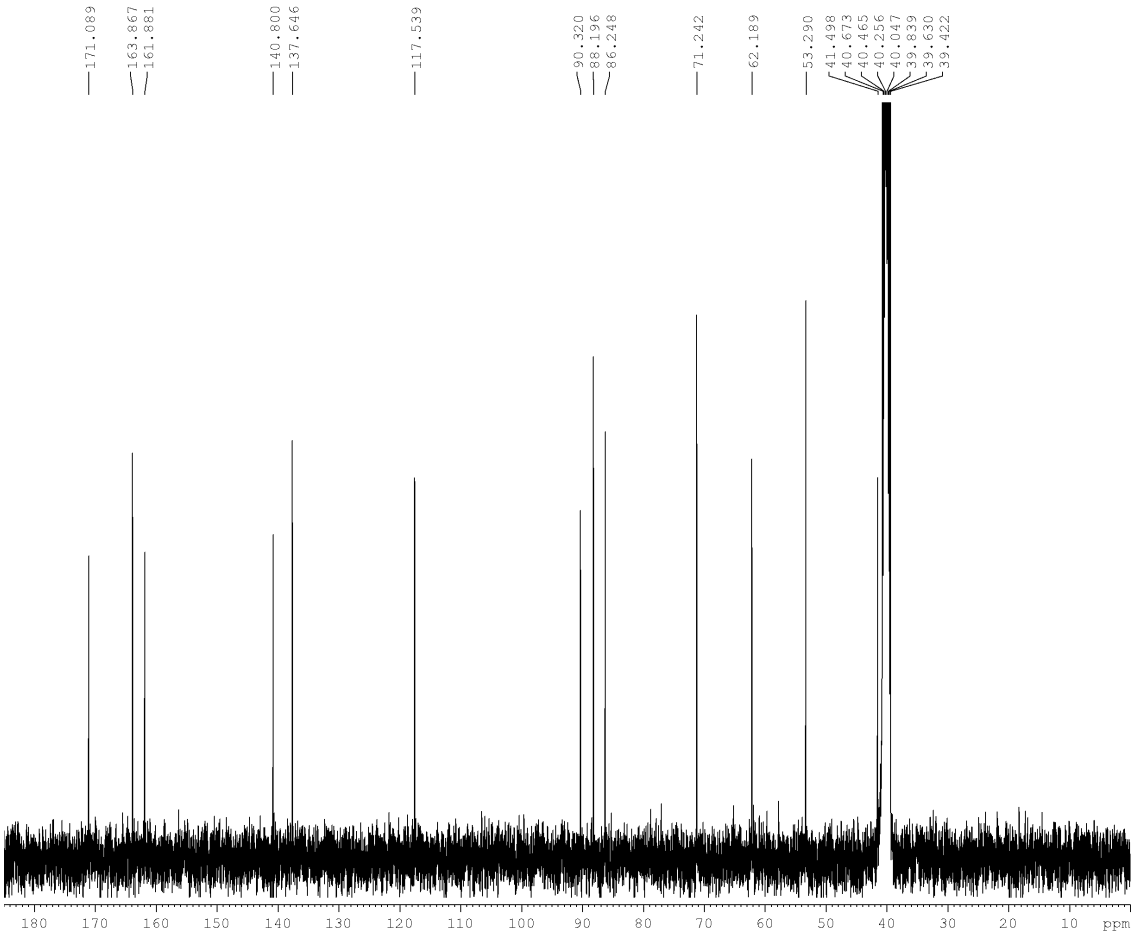

HO
O
OH
N
N
OCH3
N
N
NH2
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
167.444
163.970
159.802
136.526
128.536
125.931
99.008
88.238
84.591
71.390
62.184
56.625
53.325
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

Compound **3a**

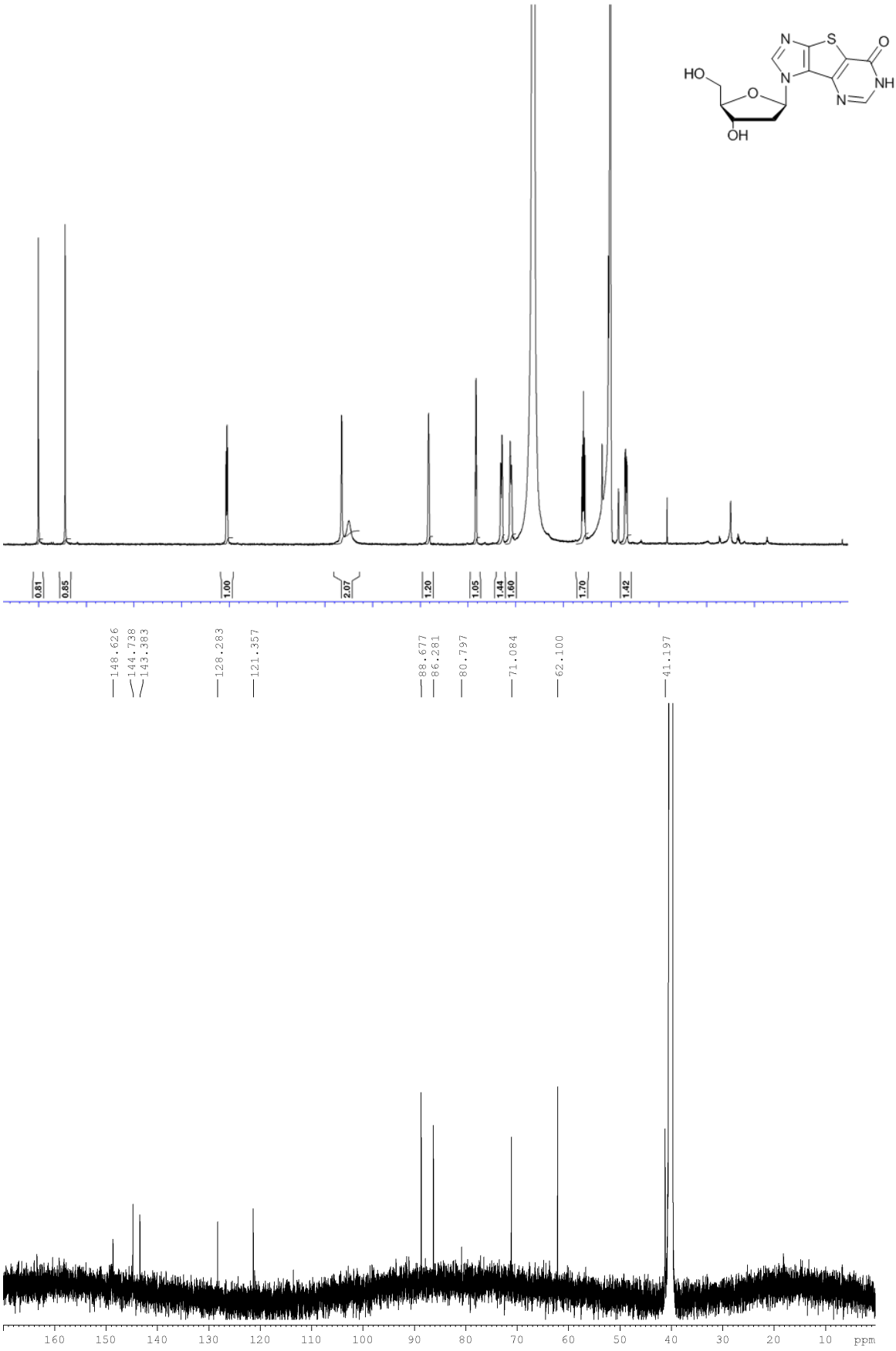

OCH3
N
N
N
N
OCH3
HO
O
OH
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
172.283
165.535
162.614
139.845
138.036
119.170
94.453
88.296
86.401
71.147
62.075
54.655
54.096
41.516
40.668
40.460
40.251
40.043
39.834
39.625
39.417
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

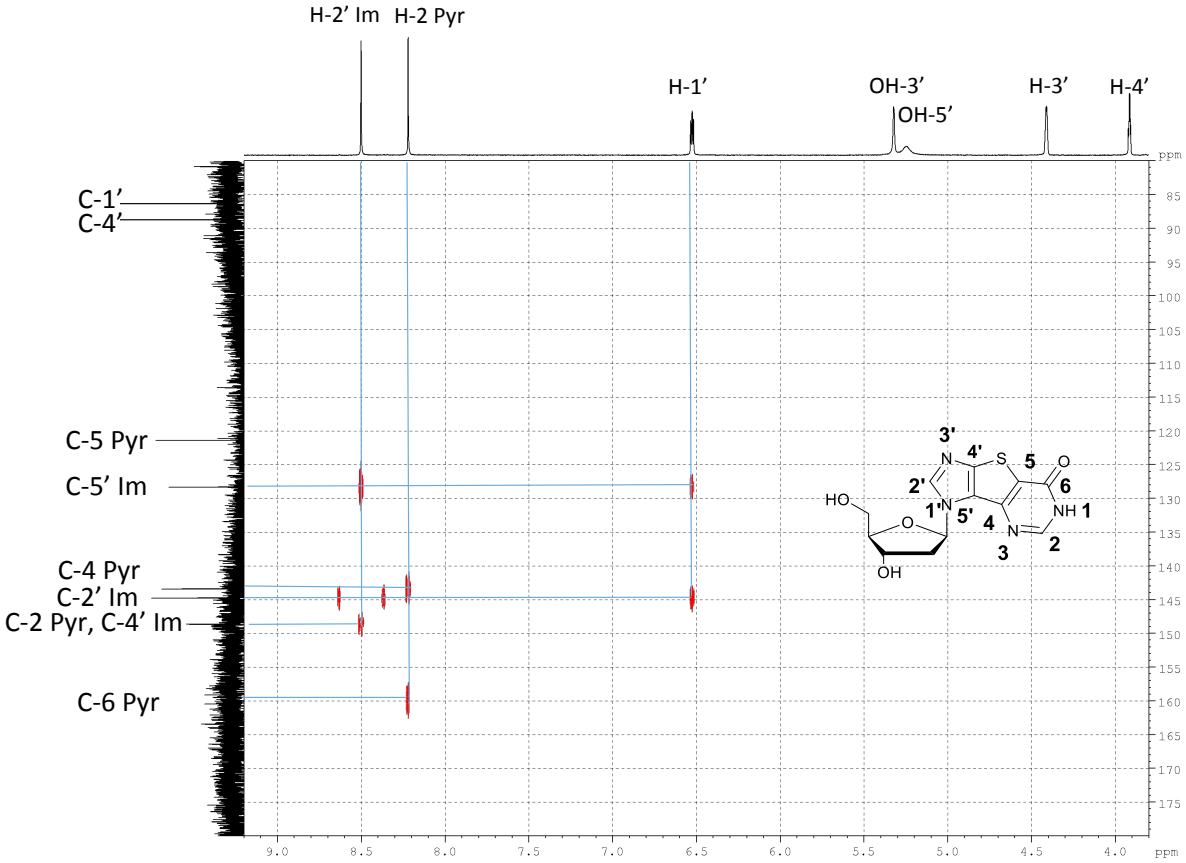
Compound **4a**


OCH3
N
N
N
N
NH2
HO
O
OH
1.01
1.00
1.03
1.88
1.00
0.93
0.94
1.01
4.03
2.07
1.02
1.08
8.0
7.5
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
0.5
ppm

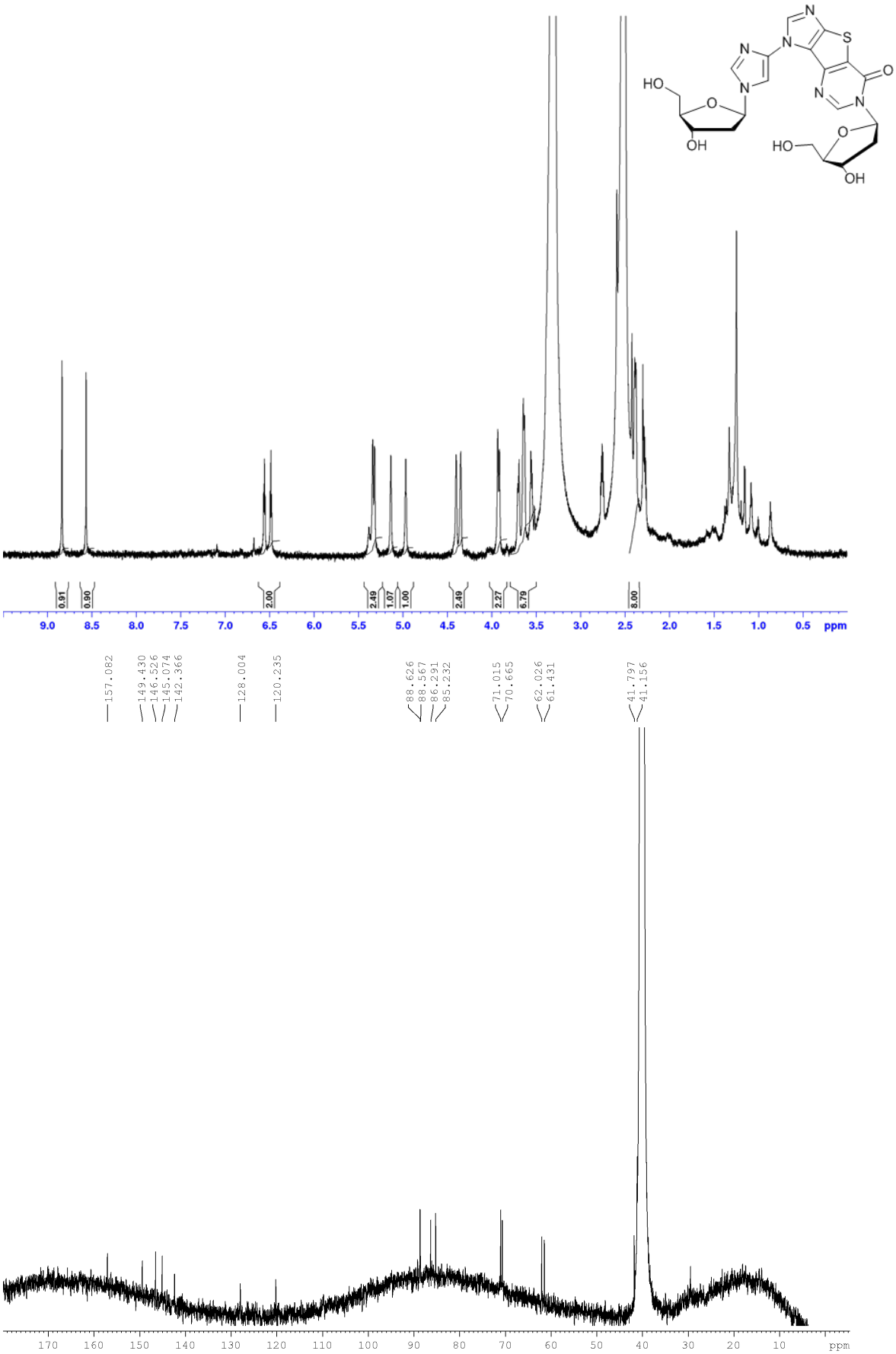

171.089
163.867
161.881
140.800
137.646
117.539
90.320
88.196
86.248
71.242
62.189
53.290
41.498
40.673
40.465
40.256
40.047
39.839
39.630
39.422
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

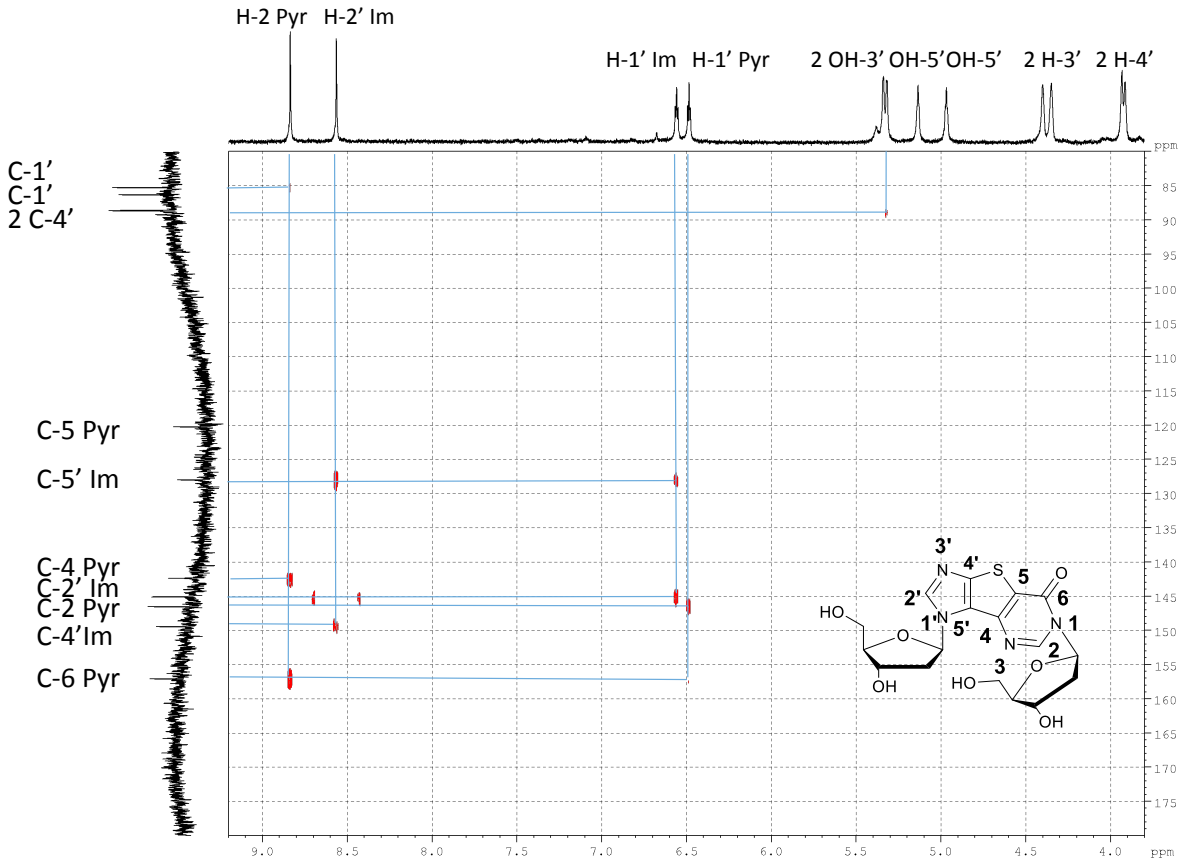
Compound **5a**


HO
O
OH
N
S
O
N
NH
N
0.81
0.85
1.00
2.07
1.20
1.05
1.44
1.60
1.70
1.42
148.626
144.738
143.383
128.283
121.357
88.677
86.281
80.797
71.084
62.100
41.197
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

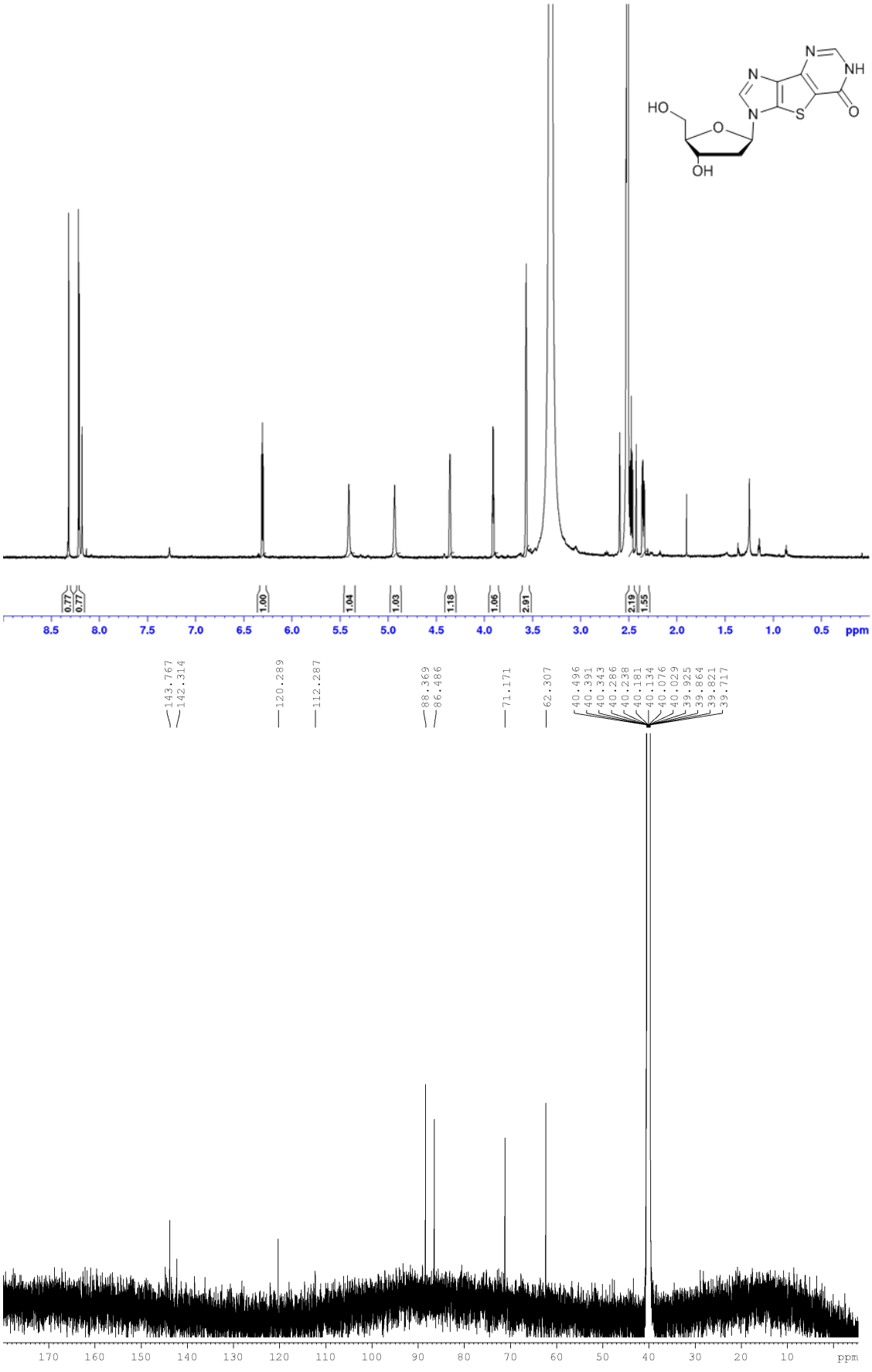

H-2’ Im
H-2 Pyr
H-1’
OH-3’
OH-5’
H-3’
H-4’
C-1’
C-4’
C-5 Pyr
C-5’ Im
C-4 Pyr
C-2’ Im
C-2 Pyr, C-4’ Im
C-6 Pyr
HO
OH
O
N
S
NH
3'
4'
5
6
2'
1'
5'
4
3
2
1
ppm

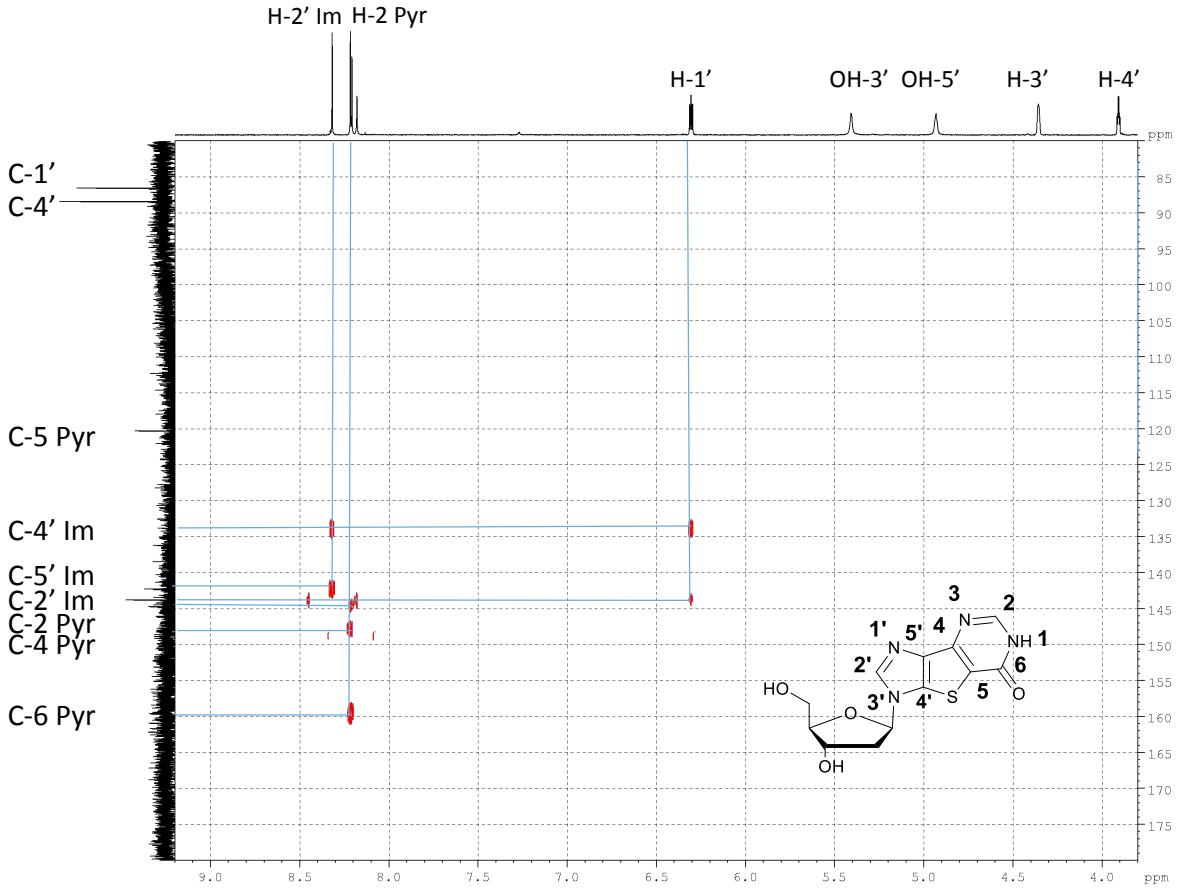
Compound **5b**


HO
O
OH
N
N
N
N
S
O
N
N
HO
O
OH
0.91
0.90
2.00
2.49
1.07
1.00
2.49
2.27
6.79
8.00
9.0
8.5
8.0
7.5
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
0.5
ppm
157.082
149.430
146.526
145.074
142.366
128.004
120.235
88.626
88.567
86.291
85.232
71.015
70.665
62.026
61.431
41.797
41.156
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

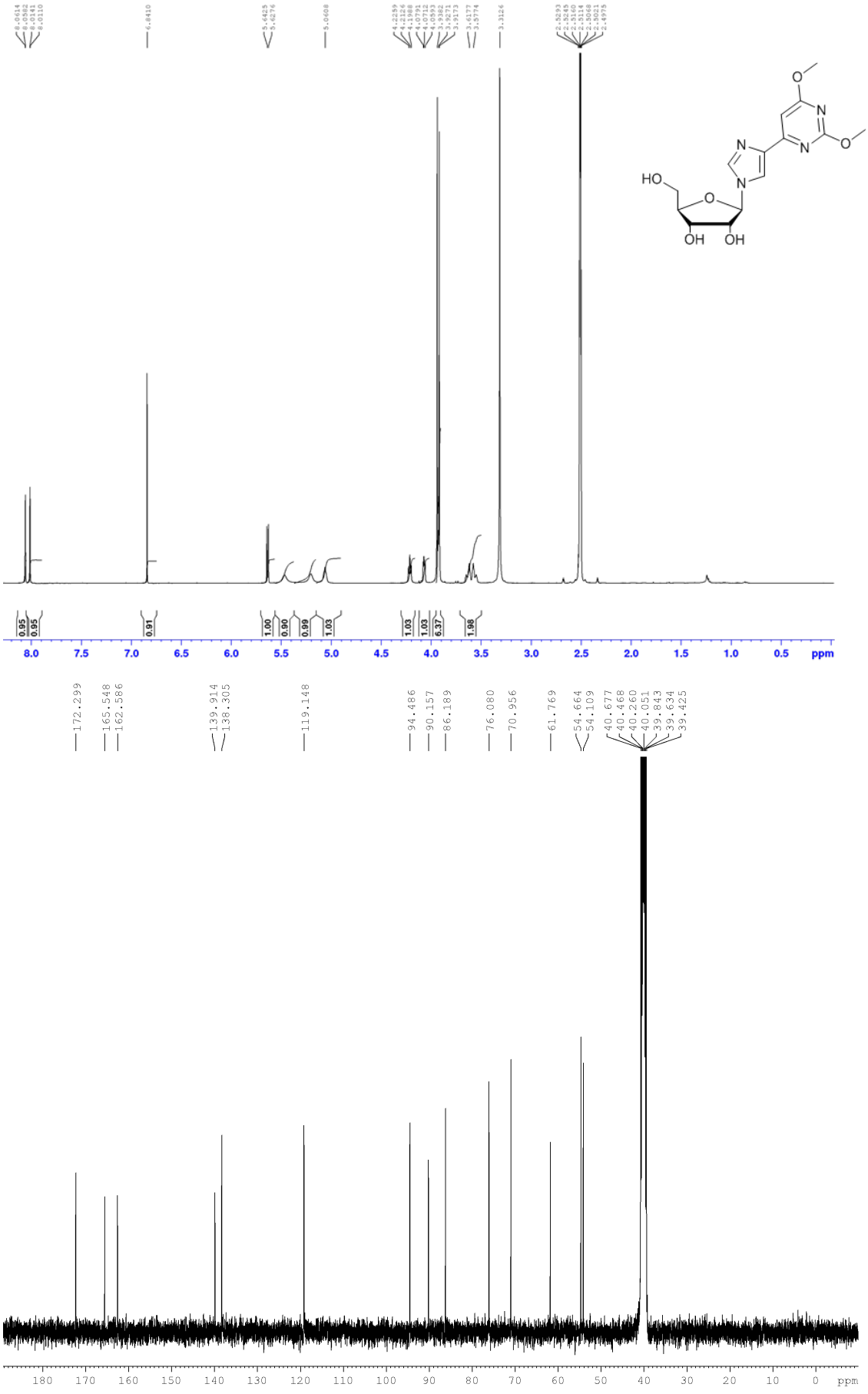

H-2 Pyr
H-2' Im
H-1' Im
H-1' Pyr
2 OH-3'
OH-5'
OH-5'
2 H-3'
2 H-4'
ppm
C-1'
C-1'
2 C-4'
C-5 Pyr
C-5' Im
C-4 Pyr
C-2' Im
C-2 Pyr
C-4'Im
C-6 Pyr
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm
HO
OH
HO
OH
N
S
O
O
N
N
O
3'
4'
5
6
2'
1'
5'
4
1
2
3

Compound **5c**

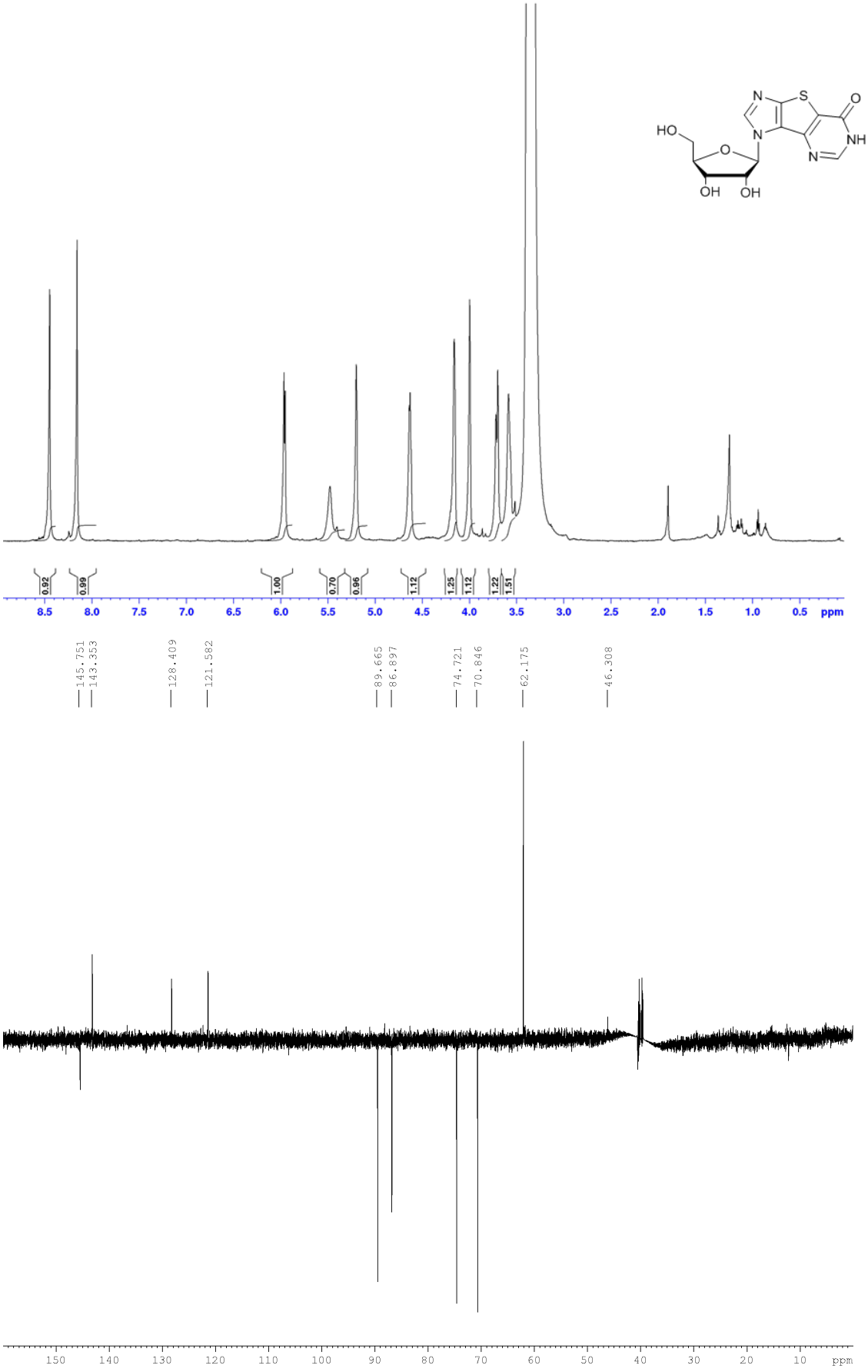

HO
O
OH
N
N
N
NH
S
O
0.77
0.77
1.00
1.04
1.03
1.18
1.06
2.91
2.19
1.55
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
143.767
142.314
120.289
112.287
88.369
86.486
71.171
62.307
40.496
40.391
40.343
40.286
40.238
40.181
40.134
40.076
40.029
39.925
39.864
39.821
39.717
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

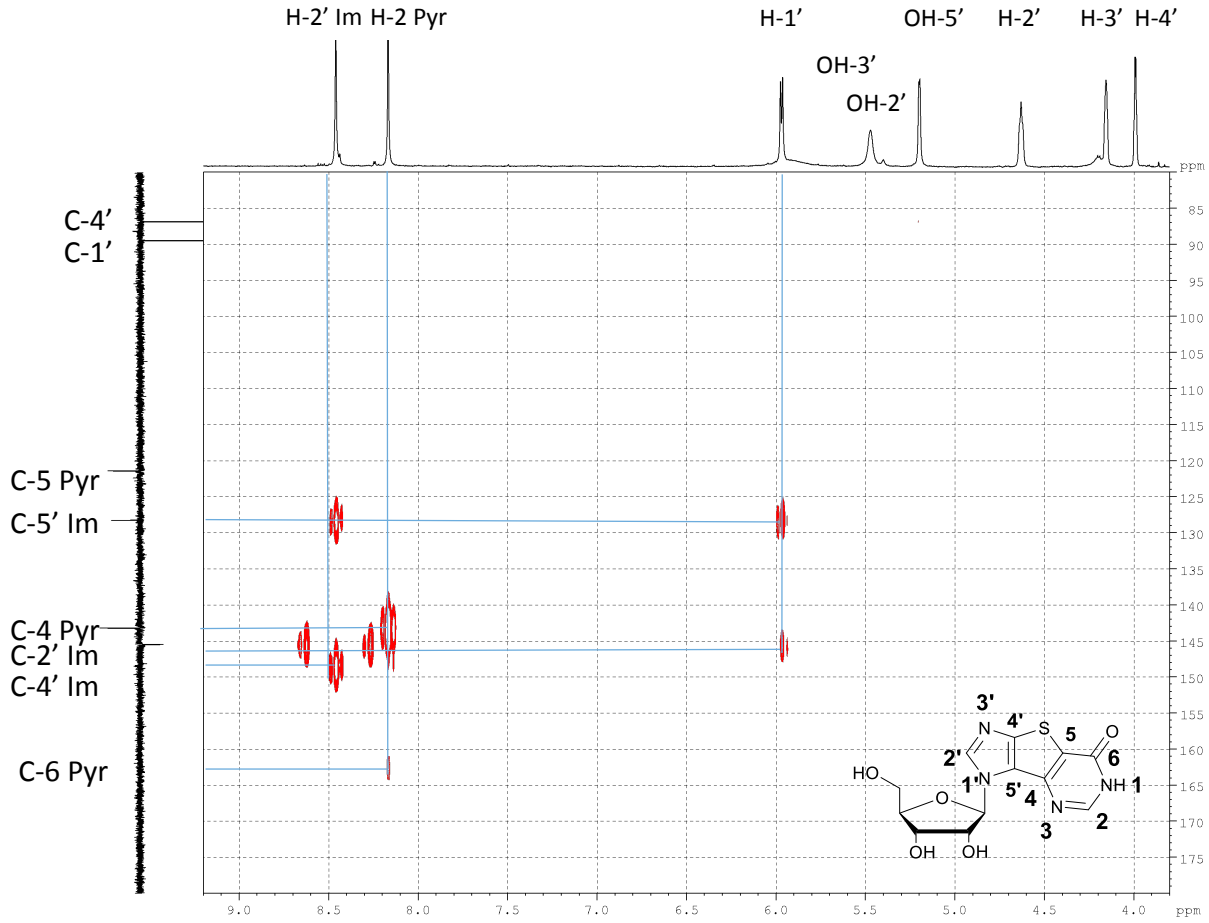

H-2' Im
H-2 Pyr
H-1'
OH-3'
OH-5'
H-3'
H-4'
C-1'
C-4'
C-5 Pyr
C-4' Im
C-5' Im
C-2' Im
C-2 Pyr
C-4 Pyr
C-6 Pyr
ppm
HO
OH
O
N
NH
S

Compound **3c**

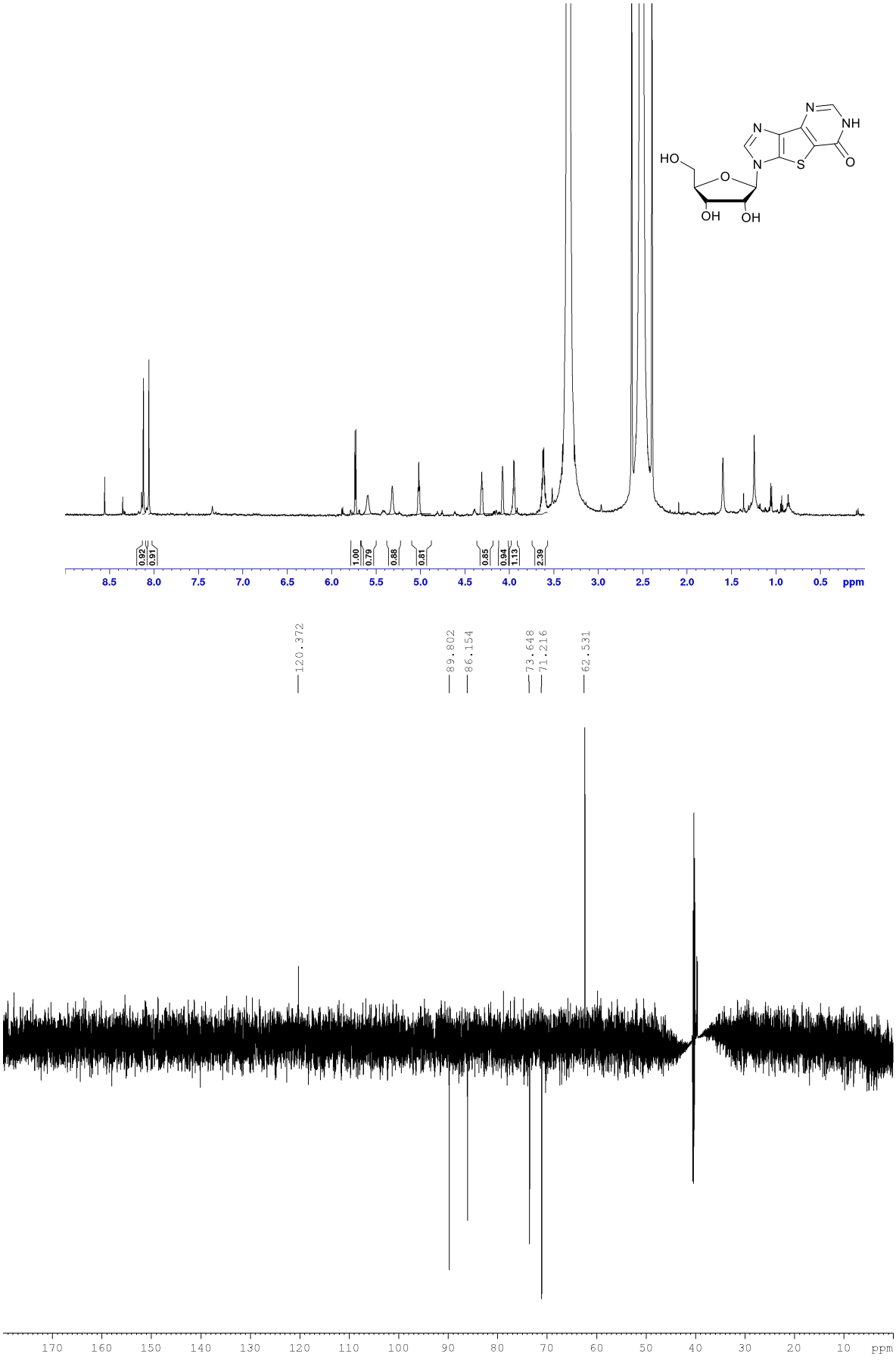

HO
OH
OH
O
N
N
N
N
O
O
8.0614
8.0582
8.0141
8.0110
6.8410
5.6625
5.6274
5.0608
4.2259
4.2126
4.1908
4.1721
4.0712
4.0593
3.9382
3.9271
3.9173
3.6177
3.5774
3.3126
2.5293
2.5160
2.5014
2.4868
2.5021
2.4975
0.95
0.95
0.91
1.00
0.90
0.99
1.03
1.03
1.03
6.37
1.98
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
172.299
165.548
162.586
139.914
138.305
119.148
94.486
90.157
86.189
76.080
70.956
61.769
54.664
54.109
40.677
40.468
40.260
40.051
39.843
39.634
39.425
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

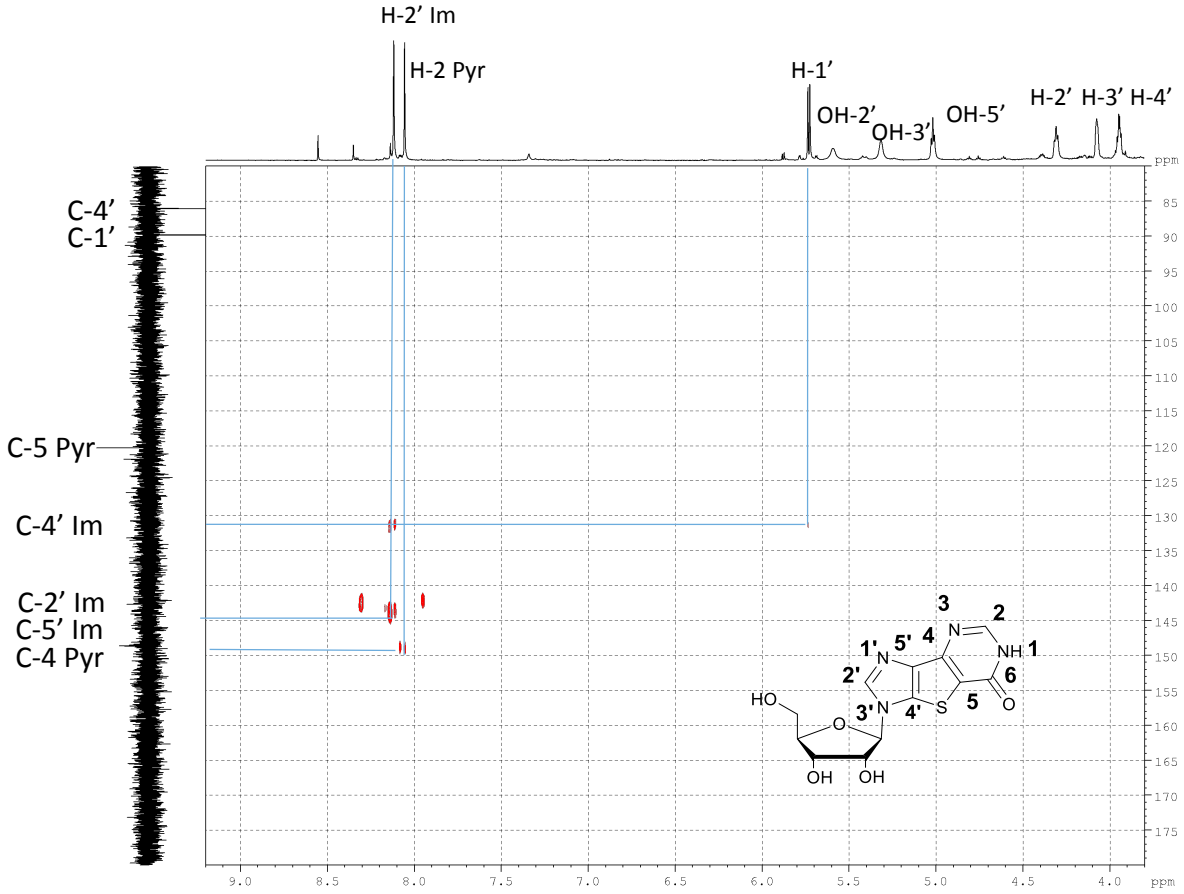
Compound **5d**


HO
O
N
S
O
NH
N
OH
OH
0.92
0.99
1.00
0.70
0.96
1.12
1.25
1.12
1.22
1.51
8.5
8.0
7.5
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
0.5
ppm
145.751
143.353
128.409
121.582
89.665
86.897
74.721
70.846
62.175
46.308
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


H-2' Im
H-2 Pyr
H-1'
OH-5'
H-2'
H-3'
H-4'
OH-3'
OH-2'
ppm
C-4'
C-1'
C-5 Pyr
C-5' Im
C-4 Pyr
C-2' Im
C-4' Im
C-6 Pyr
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm
HO
OH
OH
O
N
S
O
NH
1
2
3
4
5
6
1'
2'
3'
4'
5'

Compound **5e**


HO
O
OH
OH
N
NH
N
N
S
O
0.92
0.91
1.00
0.79
0.88
0.81
0.85
0.94
1.13
2.39
8.5
8.0
7.5
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
0.5
ppm
120.372
89.802
86.154
73.648
71.216
62.531
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


H-2’ Im
H-2 Pyr
H-1’
OH-2’
OH-3’
OH-5’
H-2’ H-3’ H-4’
C-4’
C-1’
C-5 Pyr
C-4’ Im
C-2’ Im
C-5’ Im
C-4 Pyr
ppm
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0