Supporting Information

Design, Synthesis and Spectroscopic Properties of Extended and Fused Pyrrolo-dC and Pyrrolo-C Analogs

Mary S. Noé, Andro C. Ríos, and Yitzhak Tor*

Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093

*to whom correspondence should be addressed: ytor@ucsd.edu

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S.1 Materials and synthesis

General Procedures

Reagents were purchased from Sigma-Aldrich, Rasayan Inc. (Encinitas, CA), Acros and VWR, and were used without further purification unless otherwise specified. Solvents were purchased from Sigma-Aldrich and Fisher Scientific. Anhydrous *N*,*N*-dimethylformamide (DMF) and tetrahydrofuran (THF) were obtained using a two-column purification system (Glasscontour System, Irvine, CA). NMR solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). Reactions were monitored with analytical thin-layer chromatography (TLC) performed on pre-coated silica gel aluminum-backed plates (Merck Kieselgel 60 F254). All experiments involving air and/or moisture sensitive compounds were carried out under an argon atmosphere. Column chromatography was performed with silica gel particle size 40–63 μm. NMR spectra were obtained on Varian Mercury 400 MHz and Jeol ECA 500 MHz spectrometers. Mass spectra were obtained on an Agilent 6230 HR-ESI-TOF MS at the Molecular Mass Spectrometry Facility at the UCSD Chemistry and Biochemistry Department.

Abbreviations

DCM – dichlormethane
Dioxane – 1,4-dioxane
DMF – *N*,*N*-dimethylformamide
DMSO – dimethyl sulfoxide
dppf – 1,1′-bis(diphenylphosphino)ferrocene
MeCN – acetonitrile
MeOH – methanol
THF – tetrahydrofuran
TLC – thin layer chromatography

Synthesis

Synthesis of 2-ethynylthiophene via desilylation of 2-((Trimethylsilyl)-ethynyl)thiophene was reported elsewhere.[1] Syntheses of compounds **7**,[2] **13**,[3] **14**,[4] **17**,[5] and **19**[6] were previously reported.

5-(2-Ethynylthiophene)-2′-deoxycytidine (8). A solid mixture of 5-iodo-2′-deoxycytidine **(5)** (5.00 g, 14.1 mmol), $Pd(dppf)Cl_2$ (413 mg, 0.565 mmol), and copper(I) iodide (134 mg, 0.704 mmol) was placed in a flask. To the degassed flask containing the solids, was added previously purged (30 mins each) anhydrous DMF (47mL) and anhydrous triethylamine (7mL) via syringe. The flask was heated to 40°C and the orange suspension was allowed to stir for a few minutes. To the warmed suspension was added a previously purged (30 mins purging under argon) 2-ethynylthiophene (3.00 g, 27.8 mmol) in triethylamine (7mL) via syringe, as the reaction mixture turned yellow. The heat was increased to 50°C and reaction was allowed to stir for 3 hours with monitoring by TLC (9/1 DCM/MeOH). Upon completion, the orange-tan colored reaction mixture was removed from heat and allowed to cool to room temperature. Solvent volume was reduced to *ca*.1/3 of original amount under vacuum followed by dilution with DCM. The flask was placed on an ice bath to induce voluminous precipitation. The white solid was filtered off, and the supernatant was subjected to further rounds of precipitaion. The crude was combined and recrystallized in methanol to afford light-tan colored crystals of **8** (4.03 g, 12.1 mmol, 85%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.28 (s, 1H), 7.74 (s br, 1H(NH)), 7.62–7.60 (d, *J*= 5.0 Hz, 1H), 7.41–7.40 (d, *J*= 3.5 Hz, 1H), 7.09–7.07 (m, 1H), 7.02 (s br, 1H(NH)), 6.12–6.09 (t, *J*=6.5 Hz, 1H), 5.22–5.21 (d, *J*=4.2 Hz, 1H, OH), 5.10–5.08 (t, *J*=5.0 Hz, 1H, OH), 4.22–4.18 (m, 1H), 3.79–3.78 (d, 1H), 3.64–3.52 (m, 2H), 2.18–2.12 (m, 1H), 2.04–1.97 (m, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 164.2, 154.0, 145.5, 133.5, 129.0, 128.0, 122.6, 90.0, 88.0, 87.0, 86.0, 85.8, 70.8, 61.6, 41.5; HRMS: $[M + Na]^+$ calculated for $C_{15}H_{15}N_3O_4SNa^+$, 356.0675; found, 356.0677.

Extended (thien-2-yl)-pyrrolo-2′-deoxycytidine (1). A solution of **8** (3.00 g, 9.00 mmol) in ethanol (55mL) was heated to 45 °C. To the warmed solution was added a solution of $NaAu(Cl_4)•2H_2O$ (71 mg, 0.18 mmol) in ethanol (5mL). Reaction temperature was raised to 55–60 °C and allowed to reflux overnight. The reaction was monitored by TLC (97.5/2.5 MeCN/NH_4OH (aq)) and was stopped prematurely to minimize deglycosylation of cyclized product. After allowing the reaction mixture to cool, the solvent was removed under reduced pressure. The crude mixture was purified by silica column chromatography (98/2 MeCN/NH_4OH (aq)) to afford product **1** as a yellow solid (1.44 g, 4.32 mmol, 48%, not based on recovered starting material). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.85 (s, 1H), 8.61 (s, 1H), 7.56–7.54 (d, *J*=5.5 Hz, 1H), 7.53–7.52 (d, *J*=3.5 Hz, 1H), 7.11–7.09 (m, 1H), 6.43 (s, 1H), 6.22–6.20 (t, *J*=6.5 Hz, 1H), 5.26–5.25 (d, *J*=4.5, 1H, OH), 5.11–5.09 (t, *J*=5.3 Hz, 1H, OH), 4.23–4.20 (m, 1H), 3.88–3.86 (m, 1H), 3.67–3.57 (m, 2H), 2.35–2.31 (m, 1H), 2.02–1.97 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 160.4, 154.5, 136.8, 134.7, 134.5, 128.8, 127.1, 125.7, 109.7, 97.1, 88.6, 87.7, 70.6, 61.6, 42.1; HRMS: $[M + Na]^+$ calculated for $C_{15}H_{15}N_3O_4SNa^+$, 356.0675; found, 356.0677.

5-(2-Ethynylthiophene)-2′,3′,5′-tri-*O*-acetyl-cytidine (9). A solid mixture of **7** (2.20 g, 4.43 mmol), $Pd(dppf)Cl_2$ (163 mg, 0.223 mmol), and copper(I) iodide (48 mg, 0.252 mmol) was placed in a flask. To the degassed flask was added anhydrous DMF (15.mL) and anhydrous triethylamine (4.4 mL) via syringe. To the suspension was added 2-ethynylthiophene (1.35g, 12.5 mmol) in triethylamine (1mL) and anhydrous DMF (3 mL) via syringe as the reaction mixture turned yellow. The flask was allowed to stir overnight at room temperature with monitoring by TLC (95/5 DCM/MeOH). Upon completion, the orange-tan colored reaction mixture was diluted with cold methanol (20–30 mL) and allowed to stir to induce precipitation. The suspension was filtered and the supernatant was reduced in volume under vacuum. Further amounts of cold methanol were added to induce additional precipitation followed by filtration. The crude precipitate was combined and recrystallized in methanol to afford white crystals of **9** (1.65g, 3.47 mmol, 78%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.16 (s, 1H), 7.99 (s br, 1H, NH), 7.63–7.62 (m, 1H), 7.43–7.42 (m, 1H), 7.31 (s br, 1H, NH), 7.10–7.08 (m, 1H), 5.87–5.86 (d, *J*=4.2 Hz, 1H), 5.46–5.44 (m, 1H), 5.35–5.32 (t, *J*=6.2 Hz, 1H), 4.37–4.32 (m, 1H), 4.26–4.20 (m, 2H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 170.7, 170.1, 170.0, 164.5, 153.7, 146.9, 133.7, 129.6, 128.2, 122.5, 91.0, 90.3, 88.1, 85.0, 79.6, 73.3, 70.2, 63.5, 21.3, 20.99, 20.97; HRMS: $[M + Na]^+$ calculated for $C_{21}H_{21}N_3O_8SNa^+$, 498.0942; found, 498.0943.

Extended (thien-2-yl)-pyrrolocytidine (2). A solution of **9** (1.65 g, 3.46 mmol) in ethanol (21mL) was heated to 45 °C. To the warmed solution was added a solution of $NaAu(Cl_4)•2H_2O$ (27 mg, 0.068 mmol) in ethanol (2mL). Reaction temperature was raised to 70 °C, to encourage dissolution, and refluxed for > 24 hours. Reaction was monitored by TLC (99/1 MeCN/NH_4OH (aq)). During the course of the reaction, the gold catalyst also promoted minimal deacetylation, so small amounts of gold catalyst (5mg) were added to encourage progression of cyclization. Reaction was eventually stopped prematurely to prevent any metal catalyzed deglycosylation of cyclized product. Mixture was allowed to cool to room temperature, then solvent volume was reduced by ½ and diluted with dichloromethane (10mL) to encourage partial gravity filtration of gold-catalyst. To the mixture was added aqueous NH_4OH (37%, 2 – 3 mL) and let stir for 30-40 mins at room temperature. After complete deacetylation, residual ammonia gas was bubbled out before the remaining solvents were removed under vacuum. The yellow crude was purified by silica column chromatography (98/2 DCM/MeOH) to afford **2** as a yellow solid (414 mg, 1.18 mmol, 34%, not based on recovered starting material). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.87 (s, 1H, NH), 8.76 (s, 1H), 7.57–7.55 (m, 2H), 7.13–7.11 (m, 1H), 6.42 (s, 1H), 5.92 (d, *J*=1.8 Hz, 1H), 5.51–5.50 (d, *J*=4.1 Hz, 1H, OH), 5.25–5.22 (t, *J*=5.0, 1H, OH), 5.03–5.02 (d, *J*=5.2 Hz, 1H, OH), 4.00–3.99 (m, 2H), 3.95–3.93 (m, 1H), 3.82–3.77 (m, 1H), 3.67–3.62 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 160.4, 154.8, 137.2, 134.9, 134.4, 128.9, 127.2, 125.8, 109.8, 97.0, 91.8, 84.8, 75.7, 69.2, 60.6; HRMS: $[M + Na]^+$ calculated for $C_{16}H_{15}N_3O_5SNa^+$, 372.0625; found, 372.0625.

5-(Thien-2-yl)-2′,3′,5′-*O*-acetyl-uridine (16). A flask containing 5-iodo-2′,3′,5′-*O*-acetyl-uridine (**13**) (2.13 g, 4.29 mmol) and $PdCl_2(PPh_3)_2$ (151 mg, 0.214 mmol) dissolved in anhydrous DMF (43 mL) was heated while stirring to 80 °C. To this mixture, 2-tributylstannyl thiophene (1.57 mL, 4.93 mmol) was added via syringe and allowed to stir at 80 °C overnight until the flask contained an even black coating around a clear brown solution. The reaction mixture was removed from heated and reduced to a thick brown oil under reduced pressure. This oil was dissolved in MeCN (~40 mL) and rinsed five times with ample quantities of hexanes to help remove toxic tin compounds. The acetonitrile was removed under reduced pressure to produce a brown foam. The product was purified by silica column chromatography (9/1 DCM/acetone) and recrystallied from hot methanol to produce lovely white crystals (1.63 g, 3.60 mmol, 84% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.11 (s br, 1H), 7.75 (s, 1H), 7.43–7.42 (d, *J*=3.6 Hz, 1H), 7.31-7.30 (d, *J*=5.1 Hz, 1H), 7.06–7.03 (t, *J*=4.3 Hz, 1H), 6.17–6.16 (d, *J*=5.5 Hz, 1H), 5.41–5.35 (m, 2H), 4.46–4.35 (m, 3H), 2.14 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.4, 169.9, 160.9, 149.6, 134.3, 133.1, 127.4, 126.1, 125.5, 111.4, 94.5, 87.3, 80.6, 73.1, 70.6, 63.5, 21.0, 20.8, 20.7; HRMS: $[M + Na]^+$ calculated for $C_{19}H_{20}N_2O_9SNa^+$, 475.0782; found, 475.0780.

5-(3,5-Dibromothien-2-yl)-2′,3′,5′-*O*-acetyl-uridine (18). Bromine (0.37 mL, 7.1 mmol) in carbon tetrachloride (8.9 mL) was added dropwise over 30 minutes to solution of **16** (1.40g, 3.09 mmol) in 1,2-dichloroethane (44 mL) stirring at 0 °C. The solution was allowed to stir for an additional hour at 0 °C before quenching with DCM. The organic layer was washed with saturated $NaHCO_3$, brine, 5% $NaHSO_3$, and water, repeating as necessary until it has no remaining yellow color. The organic layer was dried over Na_2SO_4, filtered, and dried to an off-white foam under reduced pressure. The product was purified by silica column chromatography (9/1 DCM/acetone) to produce a white foam (1.74 g, 2.85 mmol, 92% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.00 (s br, 1H), 8.13 (s, 1H), 7.00 (s, 1H), 6.20–6.18 (m, 1H), 5.39–5.35 (m, 2H), 4.41–4.38 (m, 1H), 4.35–4.34 (m, 2H), 2.14 (s, 3H), 2.12 (s, 3H), 2.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.4, 169.9, 169.8, 160.9, 149.3, 138.8, 132.4, 129.3, 115.1, 108.5, 108.4, 87.3, 80.3, 73.3, 70.3, 63.4, 20.9, 20.8, 20.7; HRMS: $[M + Na]^+$ calculated for $C_{19}H_{18}Br_2N_2O_9SNa^+$, 630.8892; found, 630.8890.

5-(3-Bromothien-2-yl)-2′,3′,5′-*O*-acetyl-uridine (20). A mixture of solid **18** (1.70 g, 2.80 mmol) and zinc dust (454 mg, 6.95 mmol) was suspended in anhydrous DMF (35 mL). To this suspension acetic acid (0.30 mL, 5.00 mmol) and acetic anhydride (0.13 mL, 1.4 mmol) were added via syringe. The reaction was heated to 80 °C and stirred for 4 hrs, until small, round beads formed at the bottom of the clear yellow solution and no starting material remained (monitored by TLC). The reaction mixture was filtered through a cotton plug and the solvent was removed under reduced pressure to produce a yellow oil. The product was purified by silica column chromatography (92/8 DCM/acetone) and recrystallized from methanol and ethyl acetate to produce white crystals (1.22 g, 2.30 mmol, 82% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.77 (s br, 1H), 7.42–

7.40 (d, *J*=5.6 Hz, 1H), 7.04–7.02 (d, *J*=5.6 Hz, 1H), 6.23–6.20 (m, 1H), 5.41–5.37 (m, 2H), 4.41–4.38 (m, 1H), 4.34–4.33 (m, 2H), 2.14 (s, 3H), 2.13 (s, 3H), 1.94 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ170.4, 169.9, 169.8, 160.9, 149.4, 139.1, 130.4, 127.8, 127.7, 109.9, 108.9, 87.1, 80.3, 73.3, 70.3, 63.4, 20.77, 20.74, 20.68; HRMS: $[M + Na]^+$ calculated for $C_{19}H_{19}BrN_2O_9SNa^+$, 552.9887; found, 552.9886.

5-(3-Bromothien-2-yl)-2′-deoxycytidine (21). A mixture of solid **19** (221 mg, 0.467 mmol) and *p*-toluenesulfonyl chloride (178 mg, 0.933 mmol) was dissolved in anhydrous acetonitrile (15.6 mL). Anhydrous triethylamine (0.2 mL, 1.4 mmol) was added and the reaction was allowed to stir at room temperature overnight. The reaction was monitored by TLC (9/1 DCM/acetone) until no starting material was visible. Reaction was placed directly into a pressure tube to which 37% aqueous NH_4OH (~15 mL) was added. The reaction was heated to 40 °C, stirring for roughly 72 hours, then concentrated to dryness under reduced pressure. Off-white solid was purified by silica column chromatography (9/1 DCM/MeOH) to produce a white, powdery solid (153 mg, 0.394 mmol, 84% yield). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.20 (s, 1H), 7.60–7.59 (d, *J*=5.2 Hz, 1H), 7.13–7.12 (d, *J*=5.2 Hz, 1H), 6.27–6.24 (t, *J*=6.0 Hz, 1H), 4.38–4.35 (m, 1H), 3.95–3.92 (m, 1H), 3.79–3.66 (m, 2H), 2.44–2.39 (m, 1H), 2.22–2.16 (m, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ 164.2, 156.4, 143.5, 130.9, 128.9, 128.2, 113.1, 99.9, 87.8, 86.7, 70.5, 61.2, 41.3; HRMS: $[M + Na]^+$ calculated for $C_{13}H_{14}BrN_3O_4SNa^+$, 409.9781; found, 409.9783.

5-(3-Bromothien-2-yl)-cytidine (22). A mixture of solid **20** (495 mg, 0.931 mmol) and *p*-toluenesulfonyl chloride (355 mg, 1.86 mmol) was dissolved in anhydrous acetonitrile (31 mL). Anhydrous triethylamine was added and the reaction was allowed to stir at room temperature overnight. The reaction was monitored by TLC (9/1 DCM/acetone) until no starting material was visible. Reaction was placed directly into a pressure tube to which 37% aqueous NH_4OH (~30 mL) was added. The reaction was heated to 40 °C, stirring for roughly 72 hours, then concentrated to dryness under reduced pressure. Off-white solid was purified by silica column chromatography (9/1 DCM/MeOH) to produce a white, powdery solid (294 mg, 0.726 mmol, 78% yield). ^{1}H NMR (CD_3OD, 400 MHz): δ 8.30 (s, 1H), 7.61–7.60 (d, *J*=5.4 Hz, 1H), 7.13–7.12 (d, *J*=5.4 Hz, 1H), 5.90–5.89 (d, *J*=2.9 Hz, 1H), 4.20–4.15 (m, 2H), 4.05–4.02 (m, 1H), 3.88–3.84 (m, 1H), 3.73–3.69 (m, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ 164.2, 156.6, 143.8, 130.8, 128.8, 128.2, 122.3, 113.1, 91.2, 84.6, 75.3, 69.2, 60.2; HRMS: $[M + Na]^+$ calculated for $C_{13}H_{14}BrN_3O_5SNa^+$, 425.9730; found, 425.9728.

Fused-thieno-[2,3-*d*]-pyrrolo-2′-deoxycytidine (3). A mixture of solid **21** (150 mg, 0.386 mmol), cesium carbonate (252 mg, 0.772 mmol), and copper(I) iodide (7 mg, 0.0386 mmol) was dissolved in anhydrous DMF (13 mL). *N*,*N*′-dimethylethylenediamine (8uL, 0.08 mmol) was added and the reaction was allowed to stir at 60 °C overnight as it turned from blue to green to light brown. The reaction was monitored by TLC (98/2 MeCN/NH_4OH (aq)) as a fluorescent green spot appeared along with the disappearance of the starting material. The reaction was cooled to room temperature and several drops of saturated EDTA solution were added. The reaction was evaporated to dryness under reduced pressure, resulting in a brown solid. This mixture was purified by silica column chromatography (98/2 MeCN/NH_4OH (aq)) to produce a light yellow solid (45 mg, 0.147 mmol, 37% yield). ^{1}H NMR (CD_3OD, 400 MHz): δ 9.02 (s, 1H), 7.50–7.49 (d, *J*=5.2 Hz, 1H), 7.06–7.05 (d, *J*=5.2 Hz, 1H), 6.41–6.38 (t, *J*=6.1 Hz, 1H), 4.44–4.40 (m, 1H), 4.05–4.02 (m, 1H), 3.95–3.91 (m, 1H), 3.84–3.80 (m, 1H), 2.60–2.54 (m, 1H), 2.24–2.18 (m, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 162.7, 155.3, 144.3, 134.1, 128.1, 114.2, 112.0, 105.2, 88.0, 87.9, 70.0, 61.0, 41.7; HRMS: $[M + Na]^+$ calculated for $C_{13}H_{13}N_3O_4SNa$, 330.0519; found, 330.0523.

Fused-thieno-[2,3-*d*]-pyrrolo-cytidine (4). A mixture of solid **22** (100 mg, 0.247 mmol), cesium carbonate (161 mg, 0.494 mmol), and copper(I) iodide (355 mg, 1.86 mmol) was dissolved in anhydrous DMF (13 mL). *N*,*N*′-dimethylethylenediamine (8uL, 0.14 mmol) was added and the reaction was allowed to stir at 60 °C overnight as it turned from blue to green to light brown. The reaction was monitored by TLC (97/3 MeCN/NH_4OH (aq)) as a fluorescent green spot appeared along with the disappearance of the starting material. The reaction was cooled to room temperature and several drops of saturated EDTA solution were added. The reaction was evaporated to dryness under reduced pressure, resulting in a brown solid. This mixtre was purified by silica column chromatography (97/3 MeCN/NH_4OH (aq)) to produce a light yellow solid (42 mg, 0.13 mmol, 52% yield). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.74 (s br, 1H), 8.99 (s, 1H), 7.59–7.58 (d, *J*=5.1 Hz, 1H), 7.08–7.07 (d, *J*=5.1 Hz, 1H), 5.94 (s, 1H), 5.51 (s br, 1H), 5.31–5.30 (m, 1H), 5.05–5.04 (m, 1H), 4.12–3.94 (m, 3H), 3.84–3.81 (m, 1H), 3.67–3.64 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.7, 154.5, 144.5, 135.5, 128.9, 113.9, 113.4, 104.4, 91.6, 84.7, 75.5, 69.1, 60.6; HRMS: $[M - H]^-$ calculated for $C_{12}H_{12}N_3O_5S^-$, 322.0503; found, 322.0502.

S.2 Photophysical studies

S2.1 Experimental details and calculations

PydC and PyC were obtained from Berry & Associates (Dexter, MI). Stock solutions of nucleosides were prepared by dissolving a known mass of the compound in spectrophotometric grade DMSO (Sigma Alrich). DMSO stock solutions were

prepared in concentrations of 3.77×10^{-3} M, 3.55×10^{-3} M, 3.00×10^{-3} M, 2.86×10^{-3} M, 5.42×10^{-3} M, and 5.38×10^{-3} M for PydC, PyC, **1**, **2**, **3**, and **4**, respectively. Water-dioxane mixtures were prepared with spectrophotometric grade 1,4-dioxane and de-ionized water. A series of solutions containing 0, 10, 30, 70 and 100 v/v % water in dioxane were prepared. $E_T(30)$ values for each solvent mixture was determined by dissolving a small amount of Reichardt's dye in the solution and measuring the most red-shifted absorption maximum. This value was converted into $E_T(30)$ using the formula: $E_T(30) = 28592/\lambda_{abs}$.[7] All absorption measurements were obtained at 21 °C on a Shimadzu UV 2450 absorption spectrometer using a quartz cuvette with a 1.0 cm path length (Hellma GmbH & Co KG, Müllheim, Germany). Steady state fluorescence measurements were obtained at 21 °C on a Jobin Yvon Horiba FLuoroMax-3 luminescence spectrometer using a 500 µL quartz fluorescence cell with a 1.0 cm path length (Hellma GmbH & Co KG, Müllheim, Germany). A variety of slit widths were used to obtain the ful range of spectra for each nucleoside. Each spectroscopy sample was prepared by diluting the DMSO stock solutions so that each solution contained only 0.4 v/v % DMSO. Three replicates of each spectroscopy measurements were taken and demonstrated negligible error.

Exctinction coefficients were determined by making Beer's Law plots of concentration versus absorbance for each nucleoside at four or more different concentrations. Each Beer's Law plot was determined three times for each nucleoside in each solvent, with negligible error. The relative quantum yields of each compound were determined using the formula: $\Phi_u = \Phi_s \times (A_s/A_u) \times (I_u/I_s) \times (n_u^2/n_s^2)$,[8] where each absorbance (A) and integrated area of the emission spectrum (I) was measured in triplicate. For PydC and PyC, anthracene in ethanol (λ_{abs} = 367, Φ = 0.27)[9] was used as a standard. Additionally, the quantum yields of PydC and PyC in ethanol, 0.22 and 0.18, respectively, were determined. For compounds **1**–**4**, coumarin 1 dissolved in acetonitrile (λ_{abs} = 367, Φ = 1.03)[10] was used as a standard. The refractive index of each solvent was obtained from *The CRC Handbook of Chemistry and Physics (92nd ed.)* **2011**– **2012**.

S2.2 Spectra and graphs

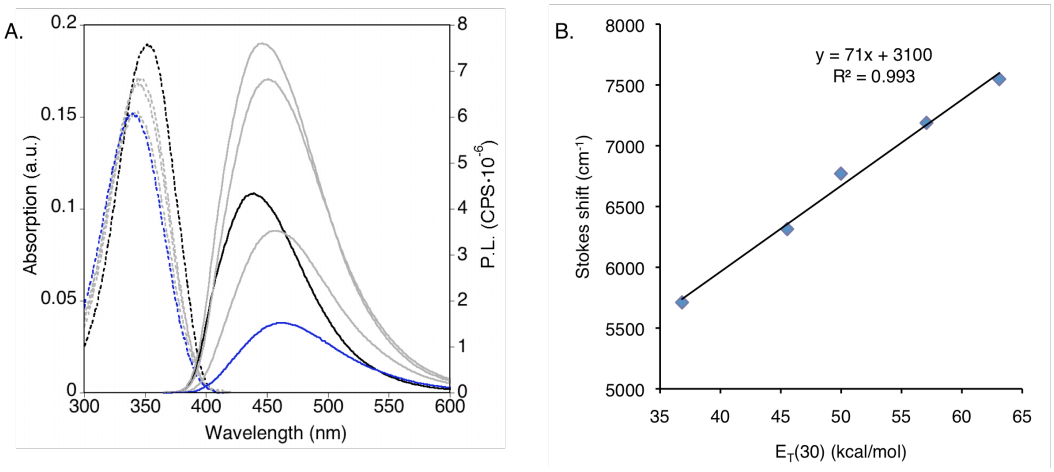


Figure S2.1A. Absorption (dashed) and emission (solid) spectra of PydC in water (blue) and dioxane (black) and mixtures (grey). **1B.** Linear relationship between stokes shift and solvent polarity ($E_T(30)$) of PydC in dioxane, water and mixtures.

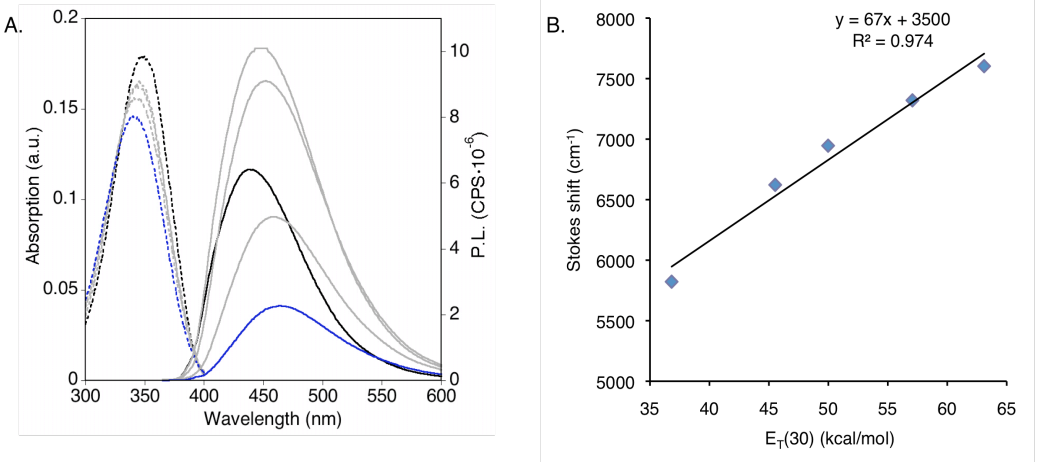


Figure S2.2A. Absorption (dashed) and emission (solid) spectra of PyC in water (blue) and dioxane (black) and mixtures (grey). **2B.** Linear relationship between stokes shift and solvent polarity ($E_T(30)$) of PyC in dioxane, water and mixtures.

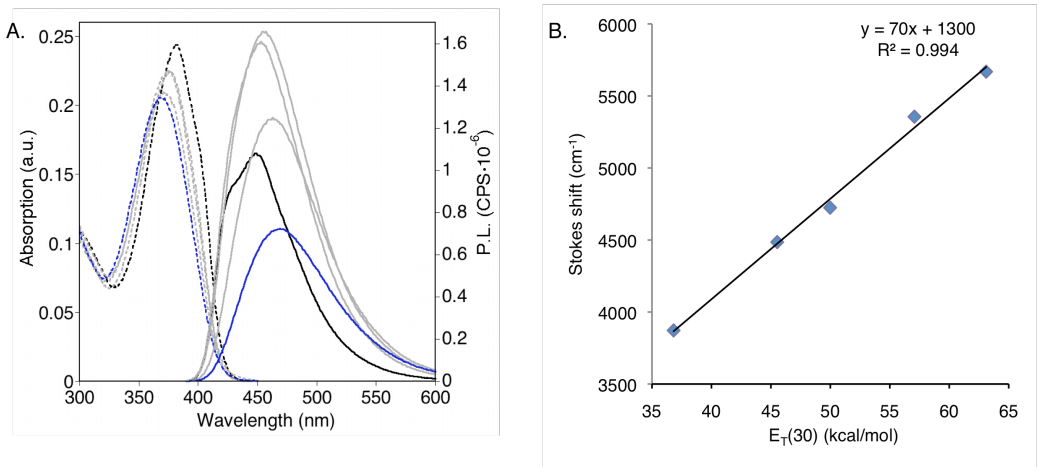


Figure S2.3A. Absorption (dashed) and emission (solid) spectra of **1** in water (blue) and dioxane (black) and mixtures thereof (grey). **3B.** Linear relationship between stokes shift and solvent polarity ($E_T(30)$) of **1** in dioxane, water and their mixtures.

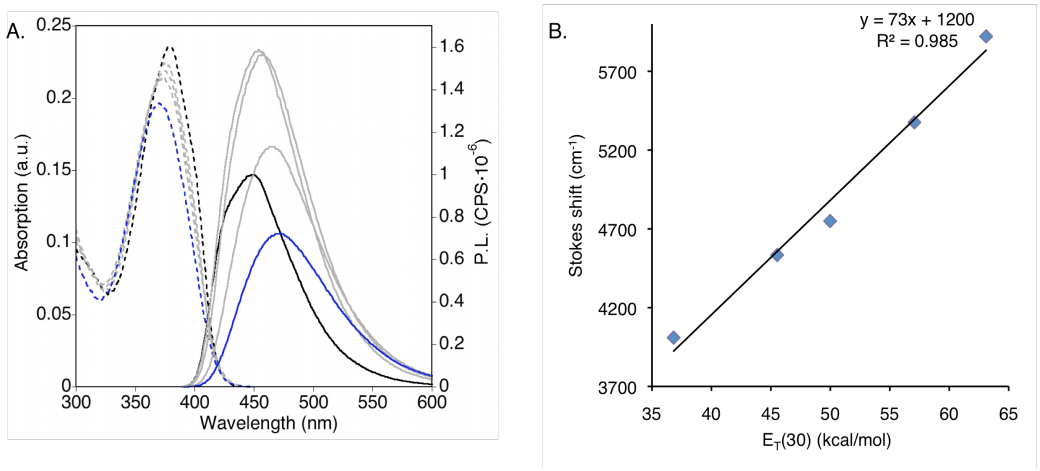


Figure S2.4A. Absorption (dashed) and emission (solid) spectra of **2** in water (blue) and dioxane (black) and mixtures thereof (grey). **4B.** Linear relationship between stokes shift and solvent polarity ($E_T(30)$) of **2** in dioxane, water and their mixtures.

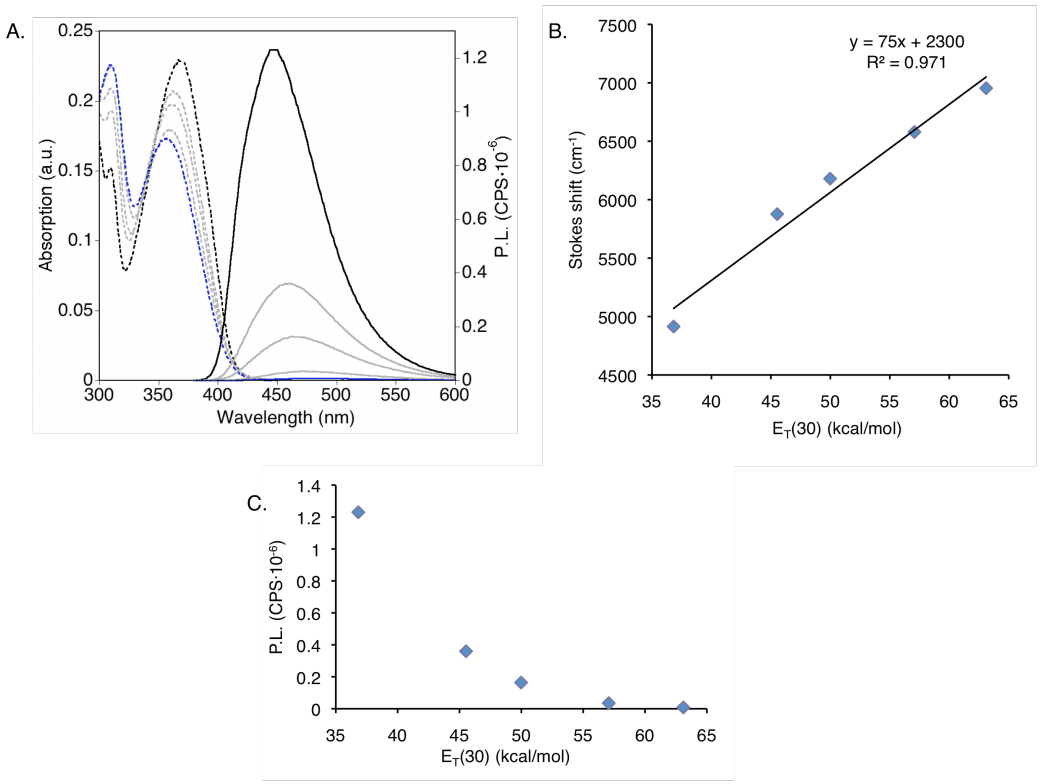


Figure S2.5A. Absorption (dashed) and emission (solid) spectra of **3** in water (blue) and dioxane (black) and mixtures thereof (grey). **5B.** Linear relationship between stokes shift and solvent polarity ($E_T(30)$) of **3** in dioxane, water and their mixtures. **5C**. Relationship between emission intensity and solvent polarity of **3** in dioxane, water and their mixtures.

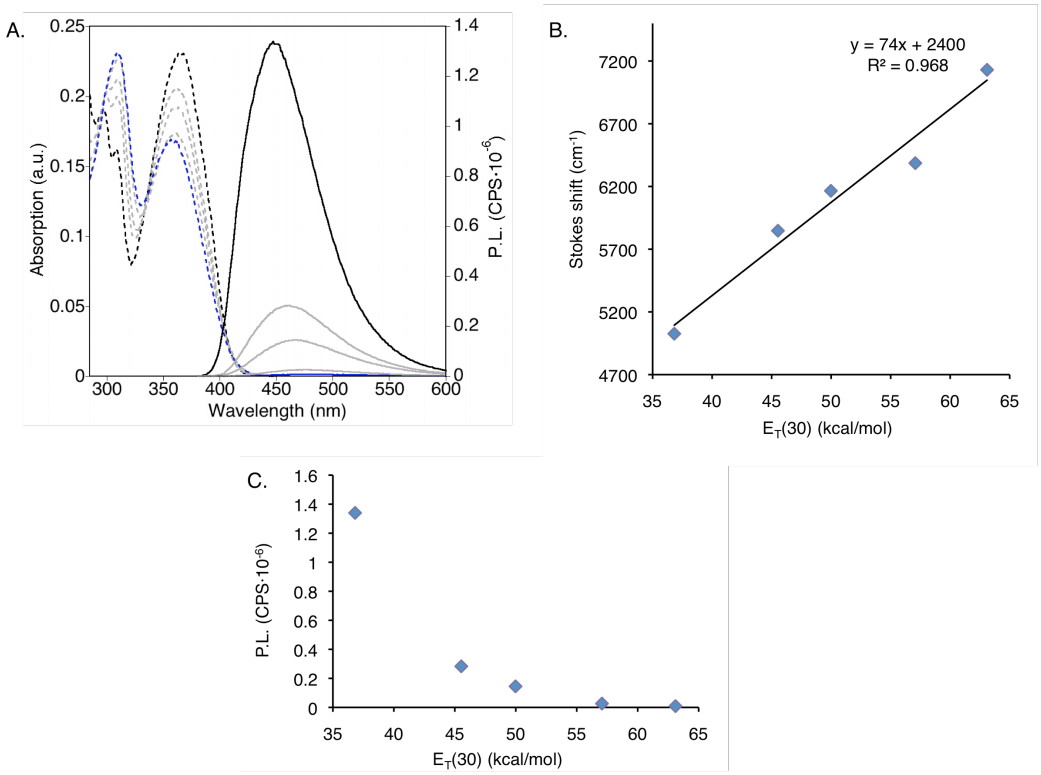
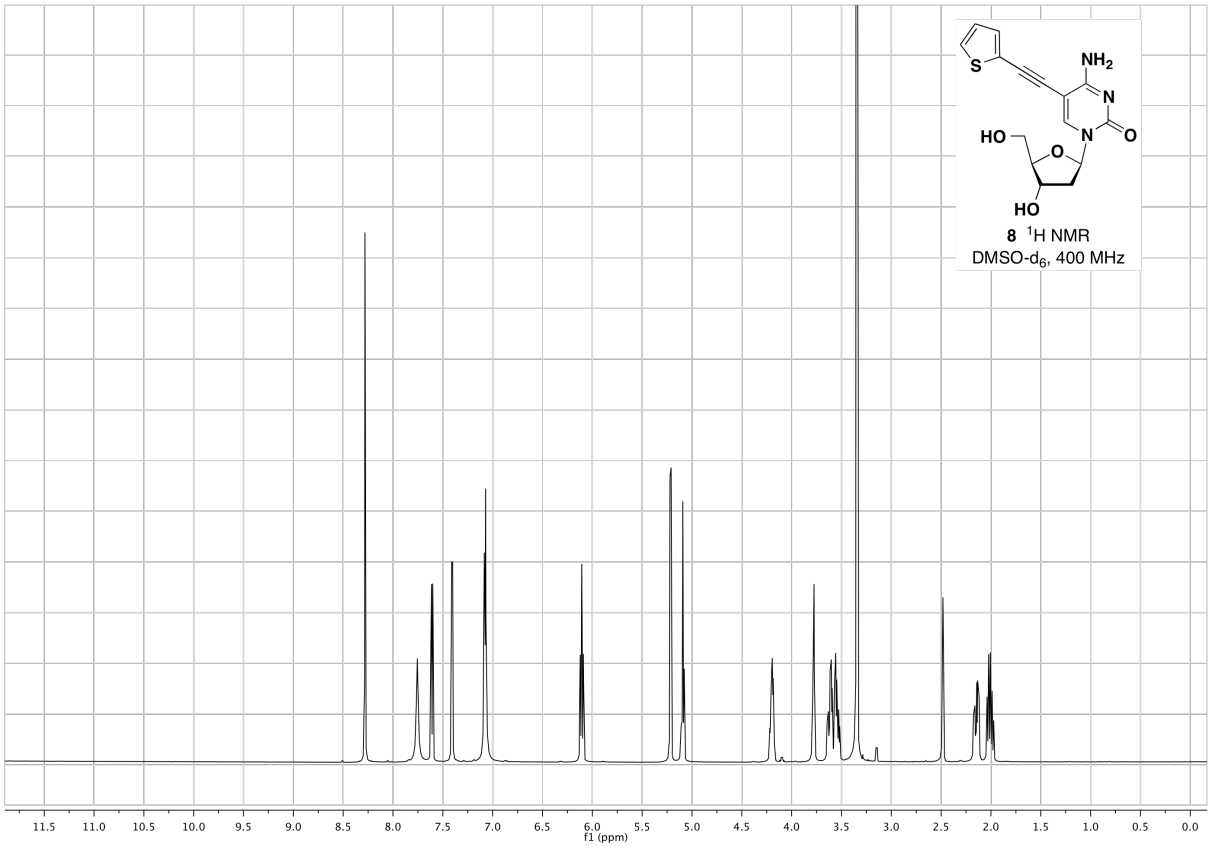
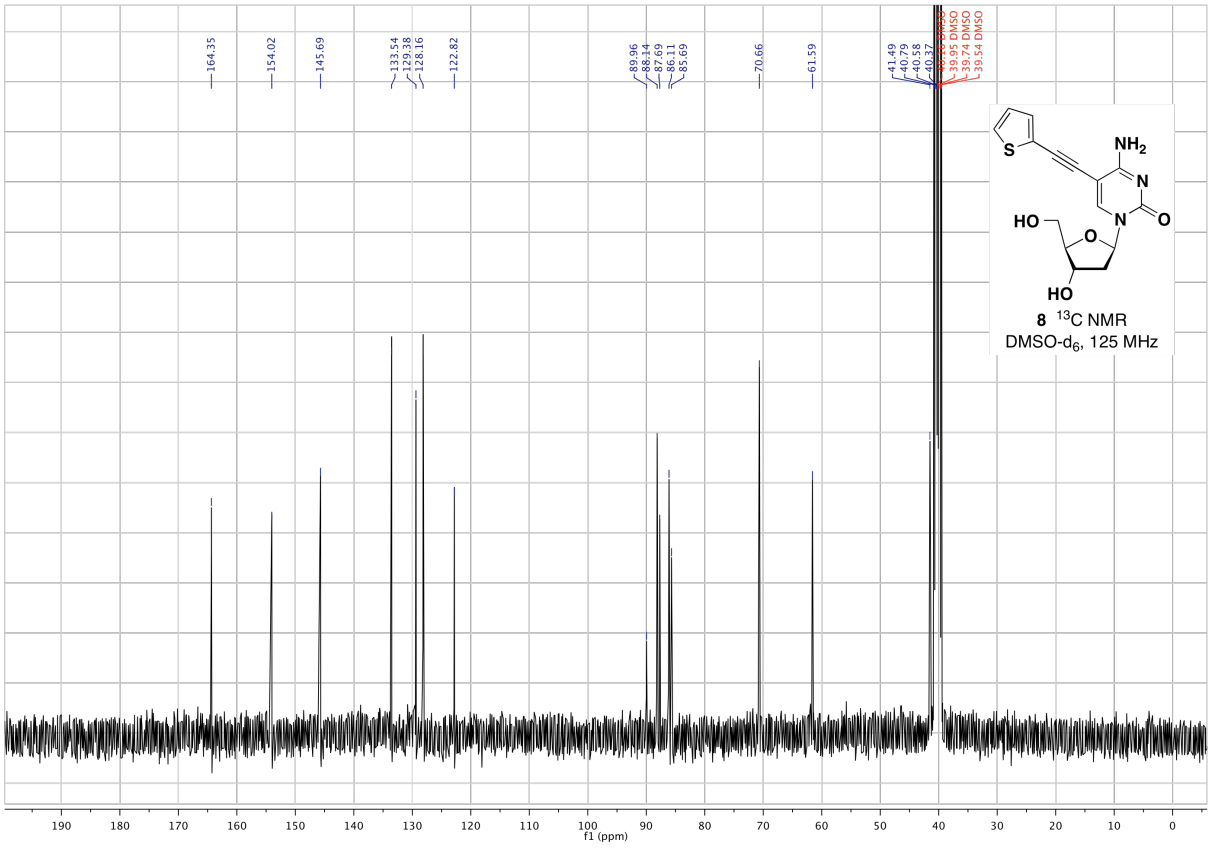
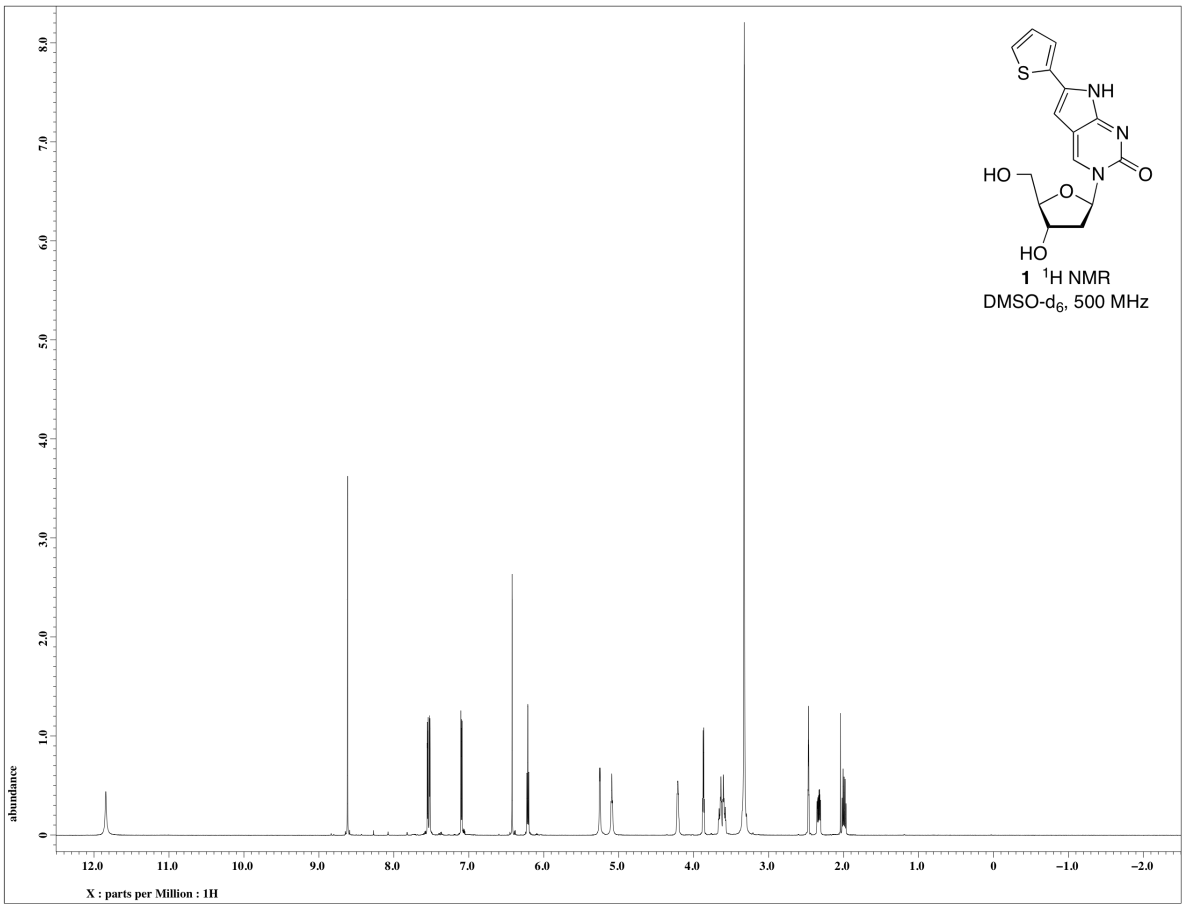


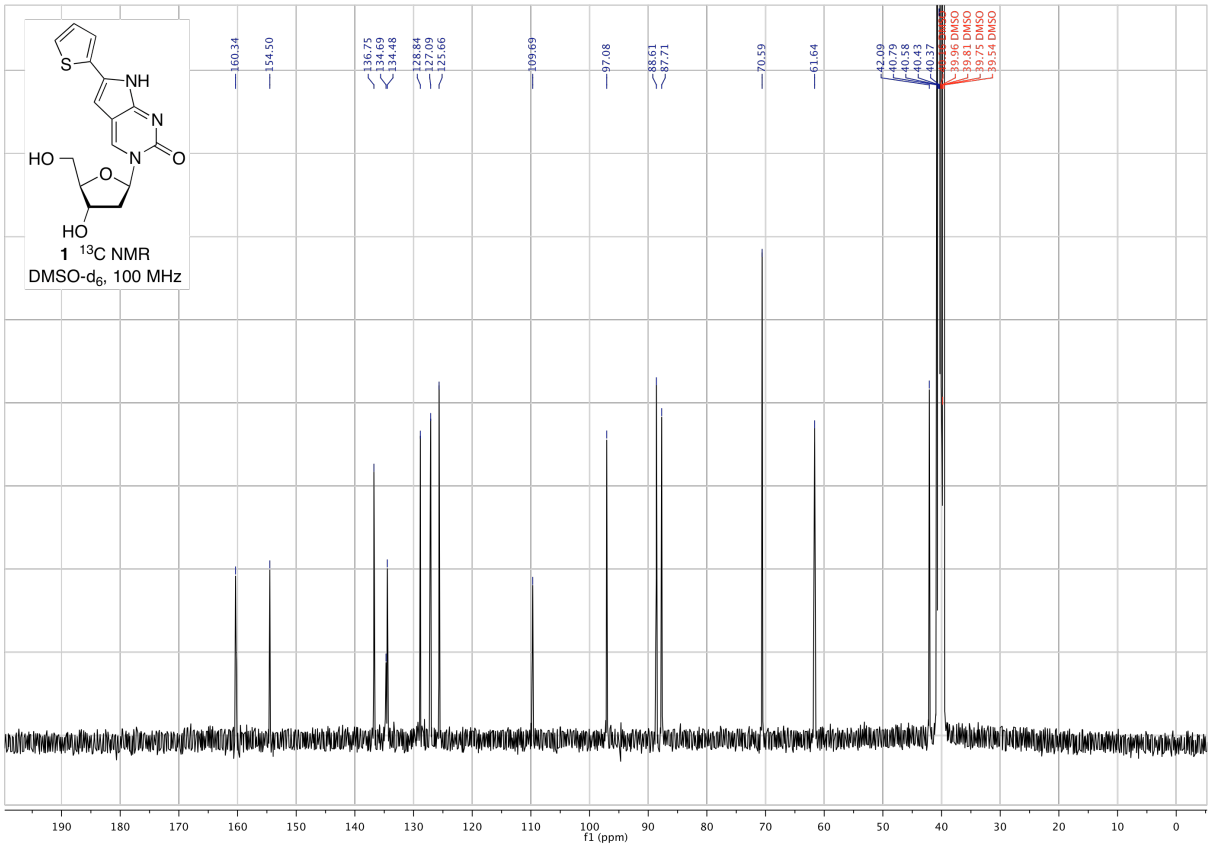
Figure S2.6A. Absorption (dashed) and emission (solid) spectra of **4** in water (blue) and dioxane (black) and mixtures thereof (grey). **6B.** Linear relationship between stokes shift and solvent polarity ($E_T(30)$) of **3** in dioxane, water and their mixtures. **6C**. Relationship between emission intensity and solvent polarity of **3** in dioxane, water and their mixtures.

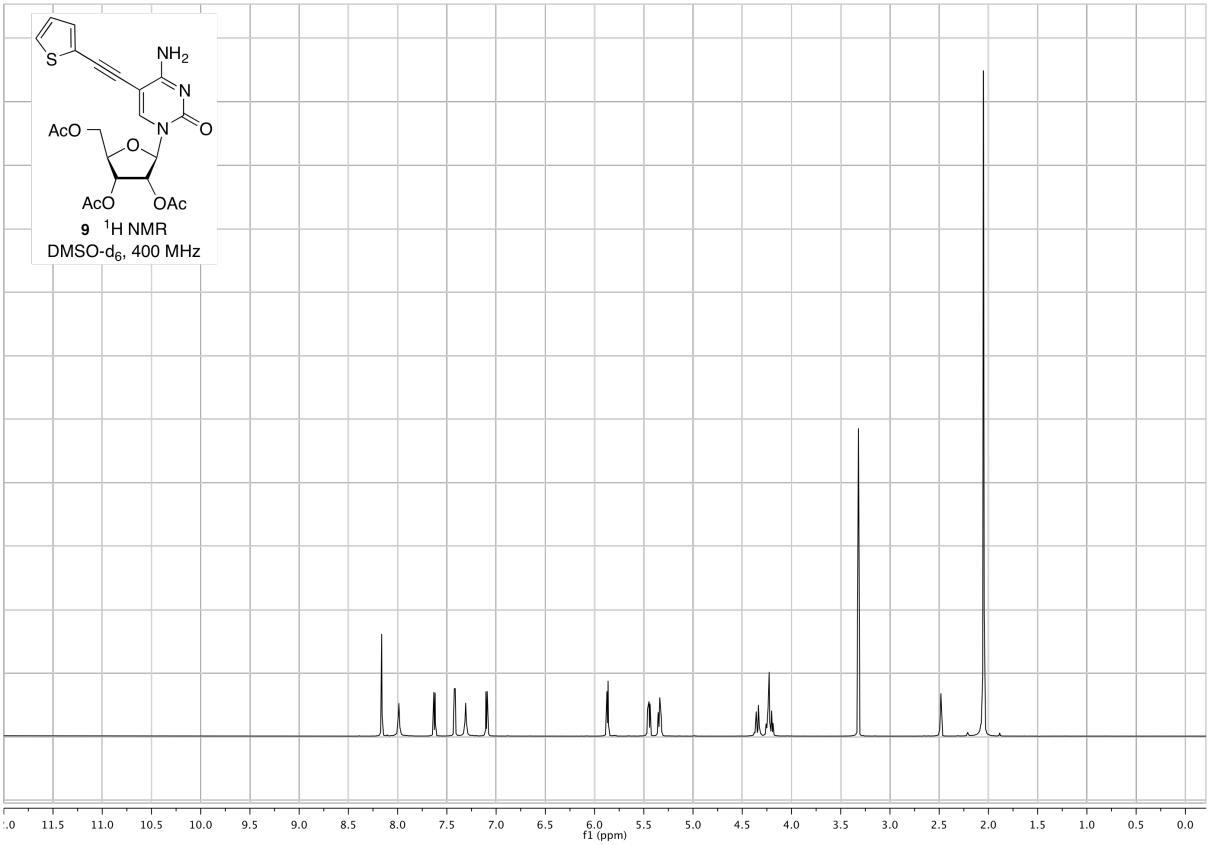
S.3 NMR Spectra

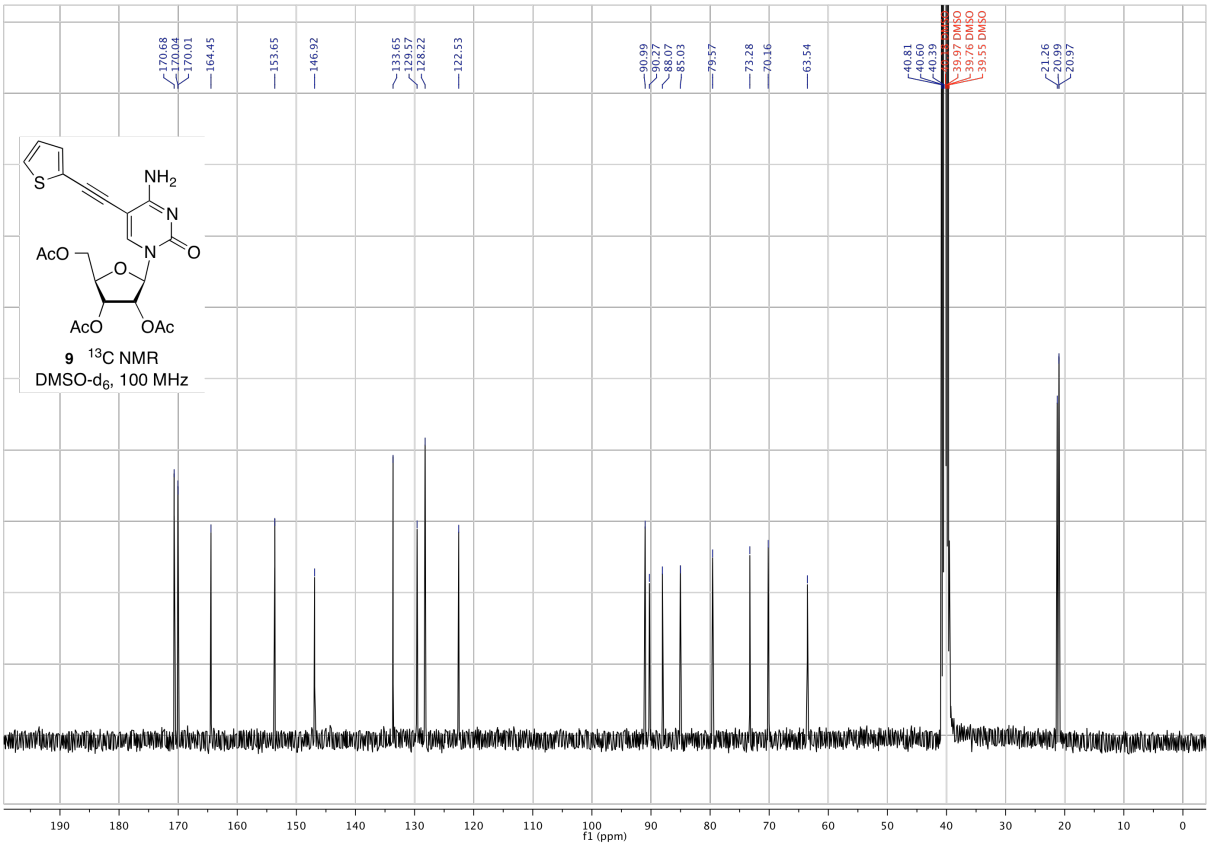


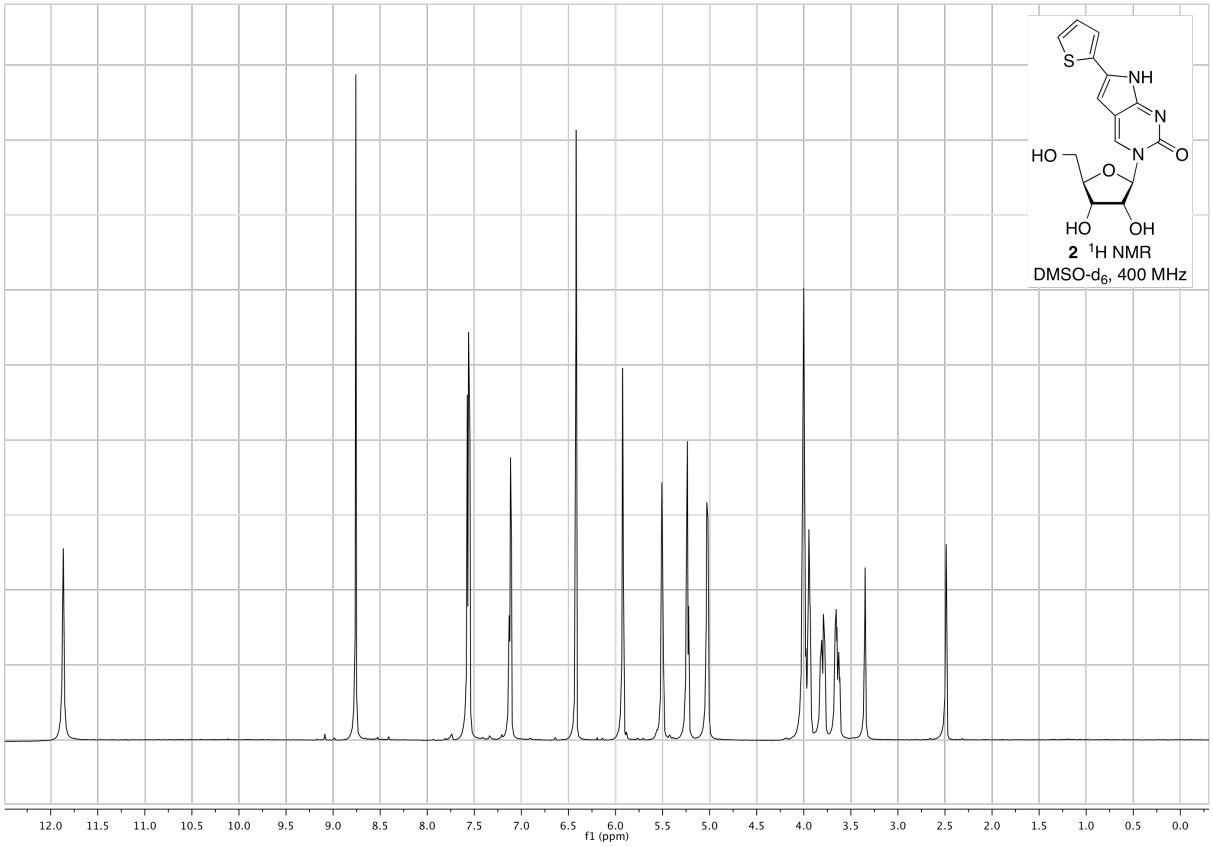


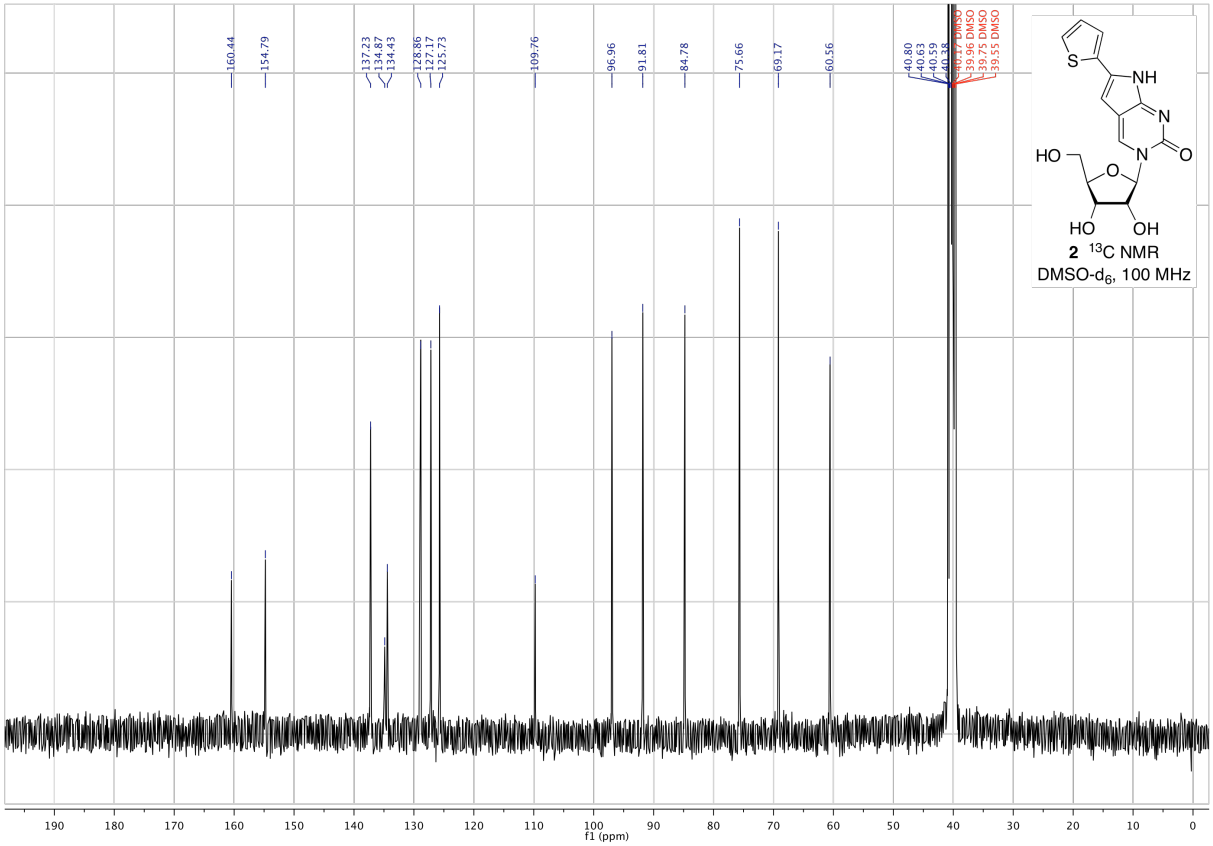

abundance
X : parts per Million : 1H
1 ^{1}H NMR
DMSO-d$_6$, 500 MHz

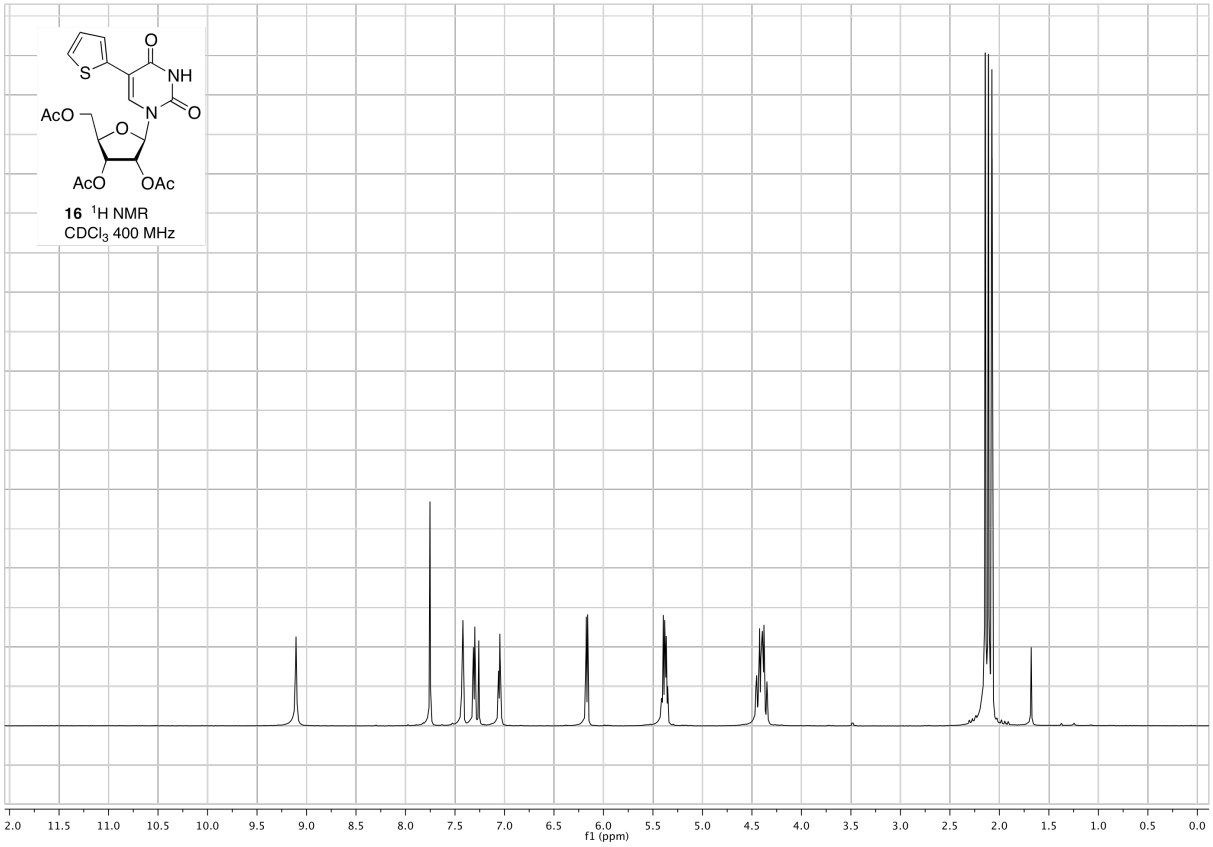

160.34
154.50
136.75
134.69
134.48
128.84
127.09
125.66
109.69
97.08
88.61
87.71
70.59
61.64
42.09
40.79
40.58
40.43
40.37
39.96 DMSO
39.81 DMSO
39.75 DMSO
39.54 DMSO
f1 (ppm)
1 ^{13}C NMR
DMSO-d$_6$, 100 MHz

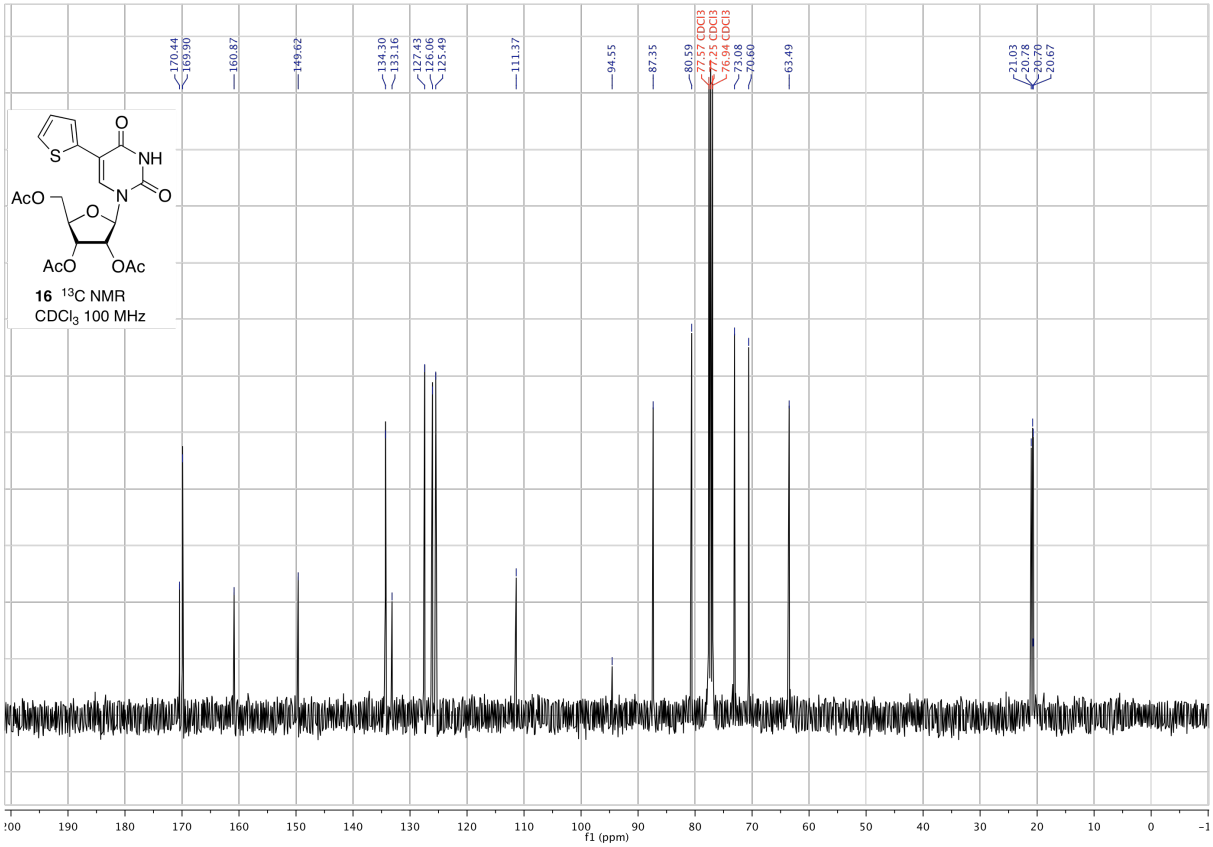

NH2
S
N
N
O
AcO
O
AcO
OAc
9 1H NMR
DMSO-d6, 400 MHz
f1 (ppm)

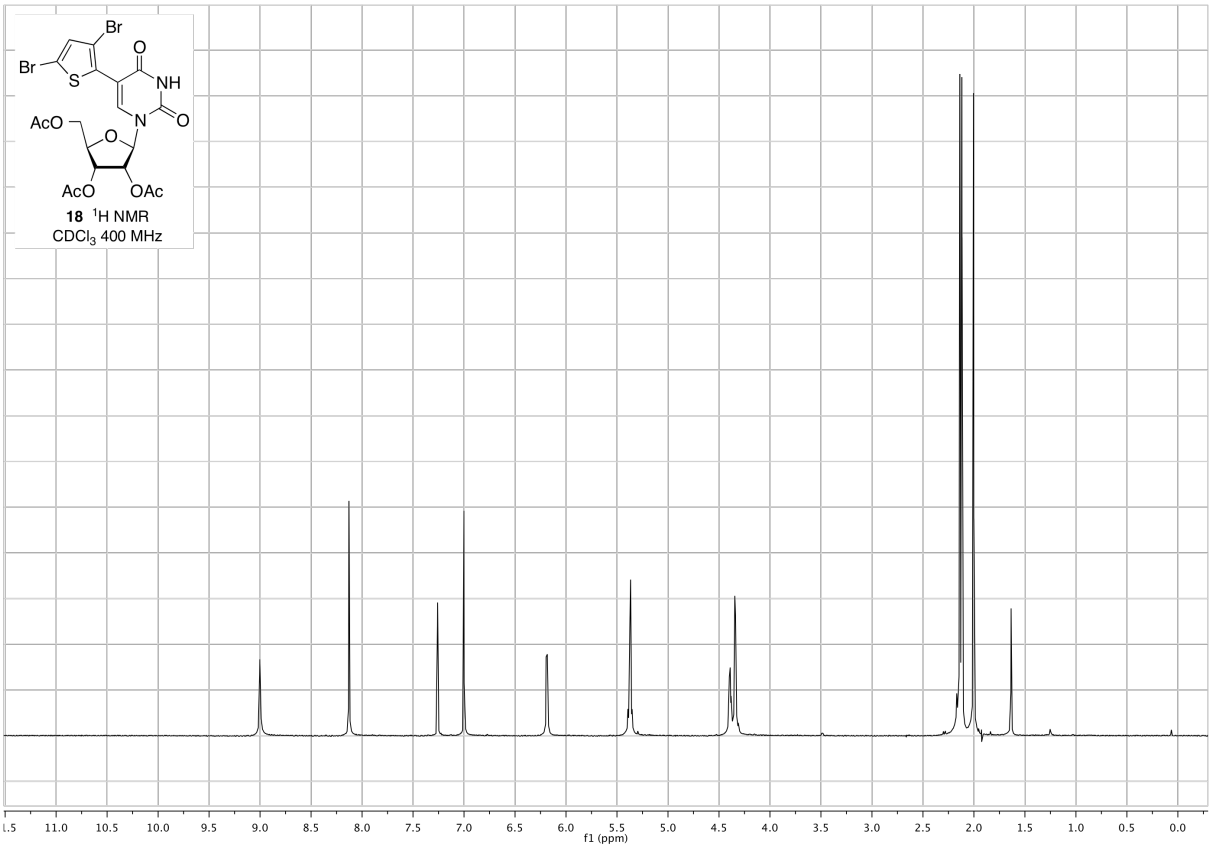

170.68
170.04
170.01
164.45
153.65
146.92
133.65
129.57
128.22
122.53
90.99
90.27
88.07
85.03
79.57
73.28
70.16
63.54
40.81
40.60
40.39
39.97 DMSO
39.76 DMSO
39.55 DMSO
21.26
20.99
20.97
NH2
S
N
N
O
AcO
O
AcO
OAc
9 13C NMR
DMSO-d6, 100 MHz
f1 (ppm)

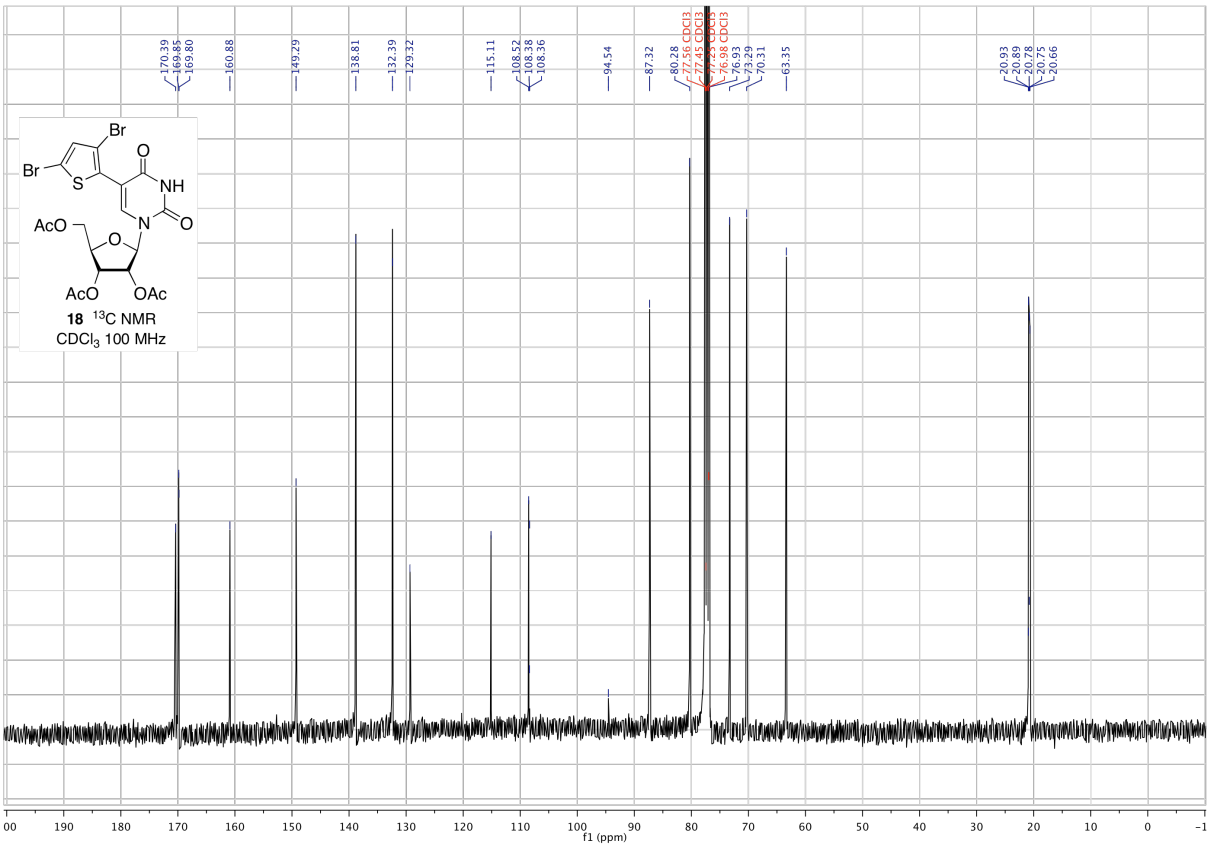

S
NH
N
N
O
HO
O
HO
OH
2 1H NMR
DMSO-d6, 400 MHz
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

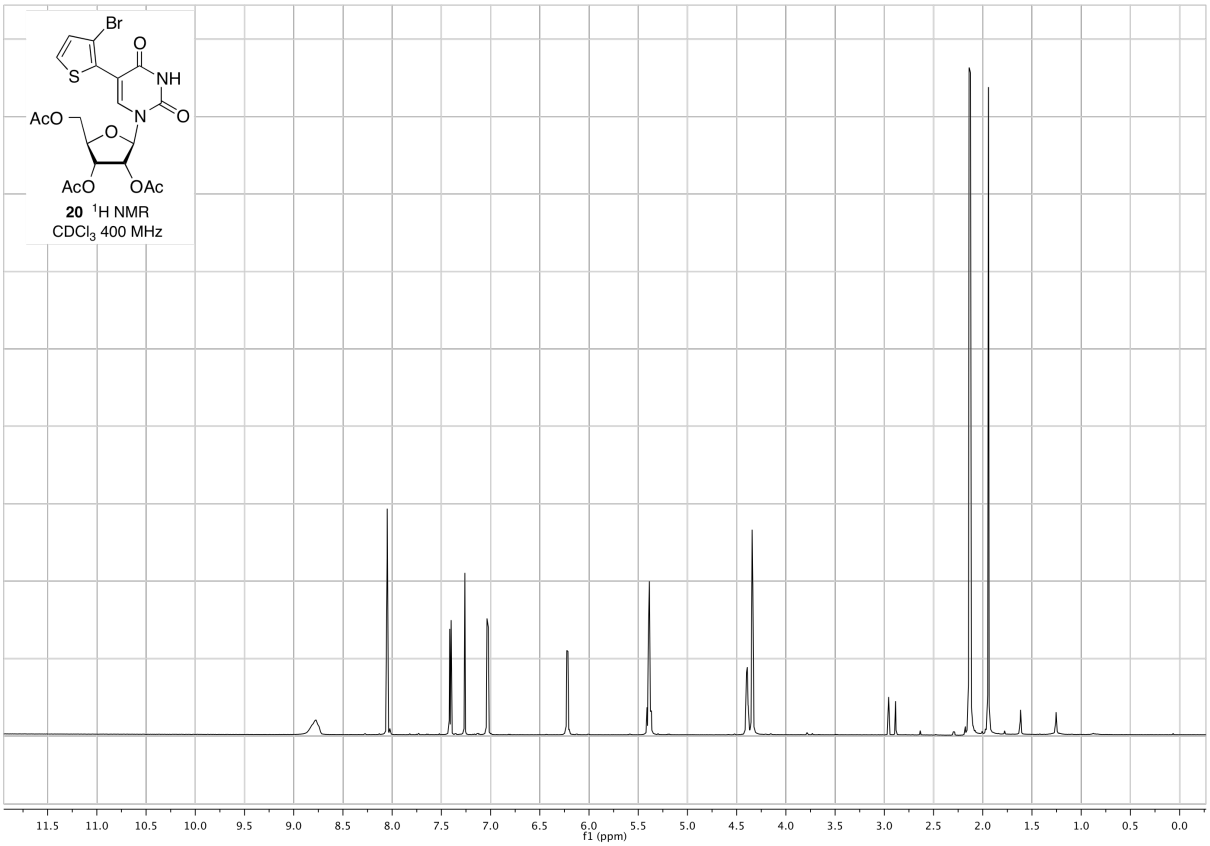

160.44
154.79
137.23
134.87
134.43
128.86
127.17
125.73
109.76
96.96
91.81
84.78
75.66
69.17
60.56
40.80
40.63
40.59
40.38
39.96 DMSO
39.75 DMSO
39.55 DMSO
S
NH
N
N
O
HO
O
HO
OH
2 13C NMR
DMSO-d6, 100 MHz
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

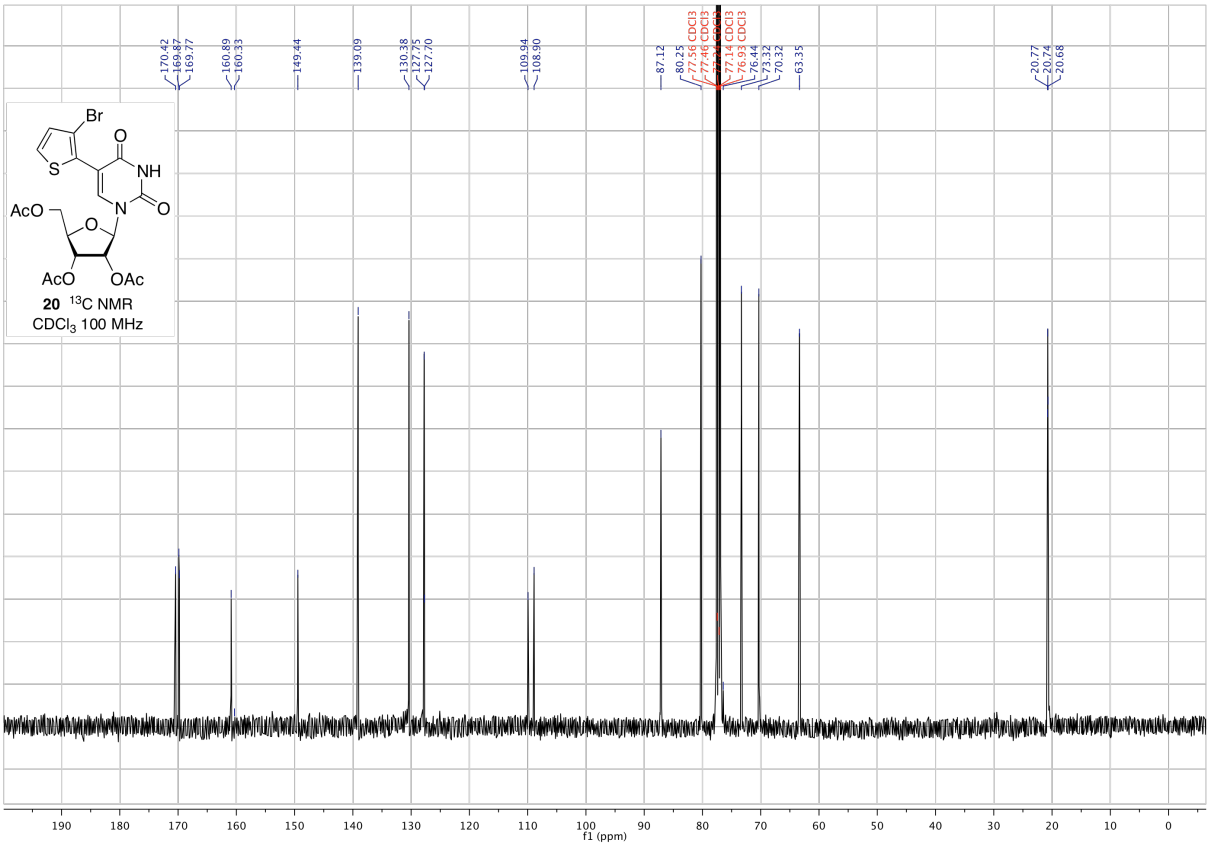

AcO
AcO
OAc
NH
O
O
O
S
N
16 1H NMR
CDCl3 400 MHz
f1 (ppm)

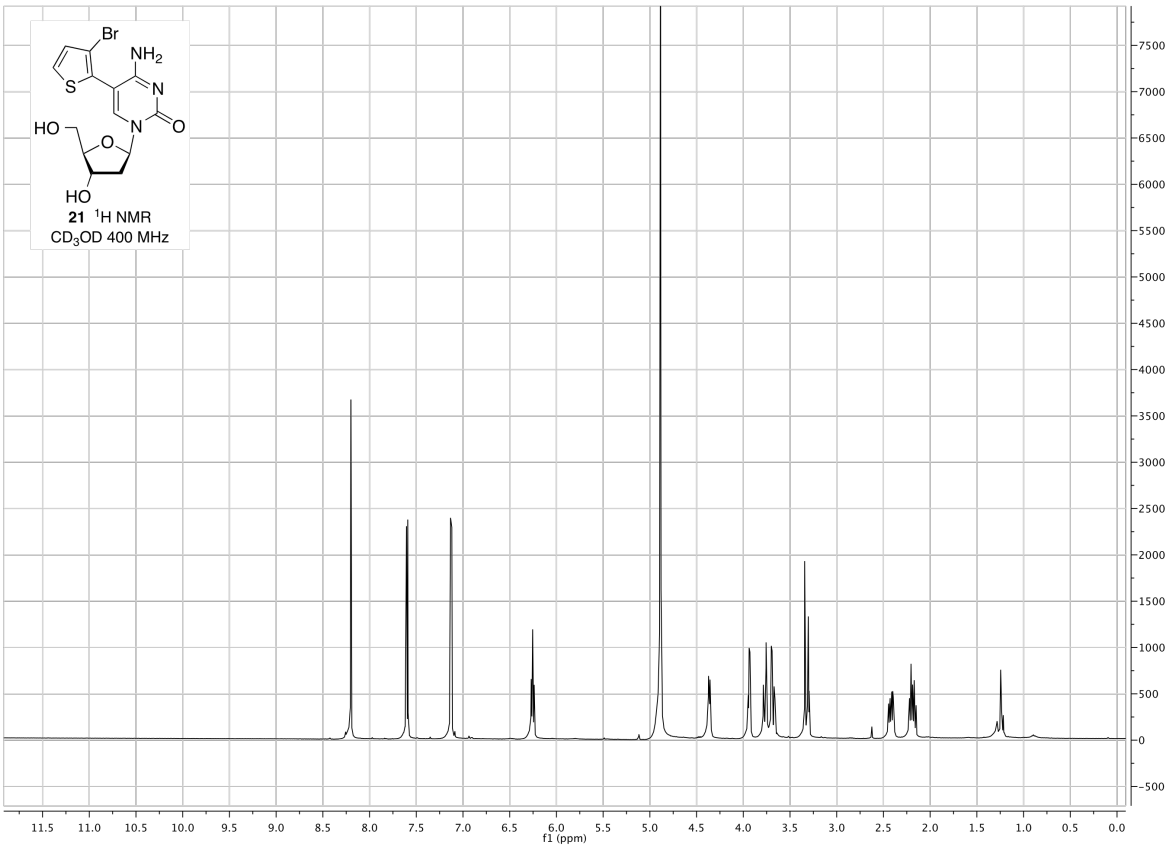

170.44
169.90
160.87
149.62
134.30
133.16
127.43
126.06
125.49
111.37
94.55
87.35
80.59
77.57 CDCl3
77.25 CDCl3
76.94 CDCl3
73.08
70.60
63.49
21.03
20.78
20.70
20.67
AcO
AcO
OAc
NH
O
O
O
S
N
16 13C NMR
CDCl3 100 MHz
f1 (ppm)

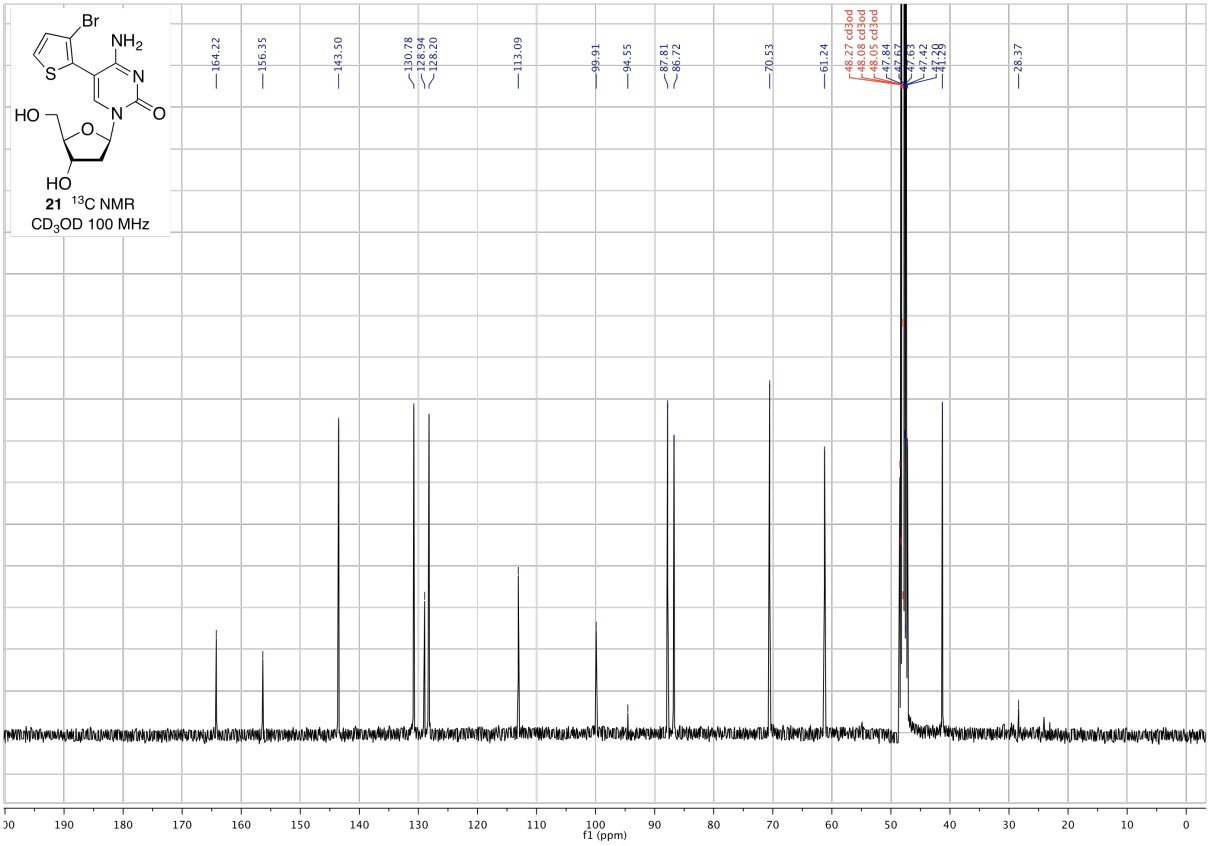

Br
Br
S
O
NH
N
O
AcO
O
AcO
OAc
18 ^{1}H NMR
$CDCl_3$ 400 MHz
f1 (ppm)

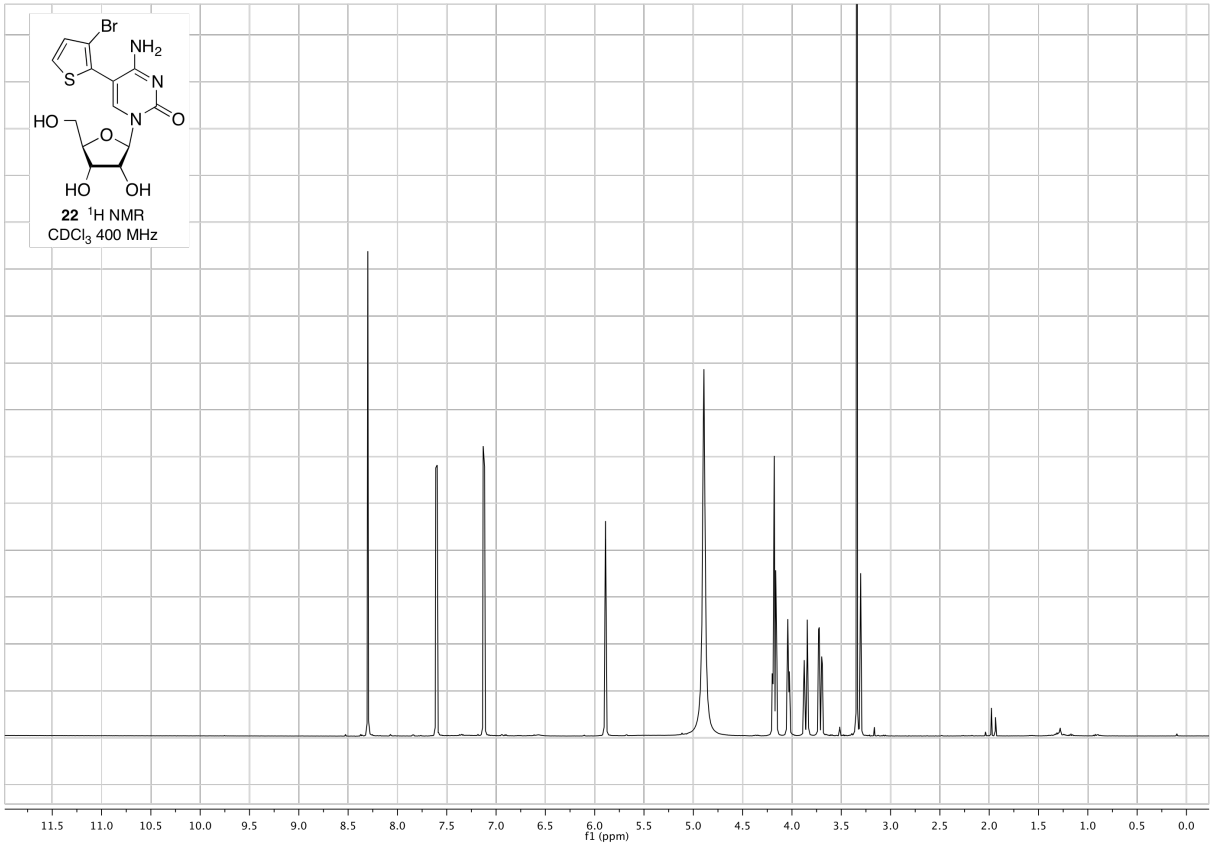

170.39
169.85
169.80
160.88
149.29
138.81
132.39
129.32
115.11
108.52
108.38
108.36
94.54
87.32
80.28
77.56 CDCl3
77.45 CDCl3
77.23 CDCl3
76.98 CDCl3
76.93
73.29
70.31
63.35
20.93
20.89
20.78
20.75
20.66
Br
Br
S
O
NH
N
O
AcO
O
AcO
OAc
18 ^{13}C NMR
$CDCl_3$ 100 MHz
f1 (ppm)

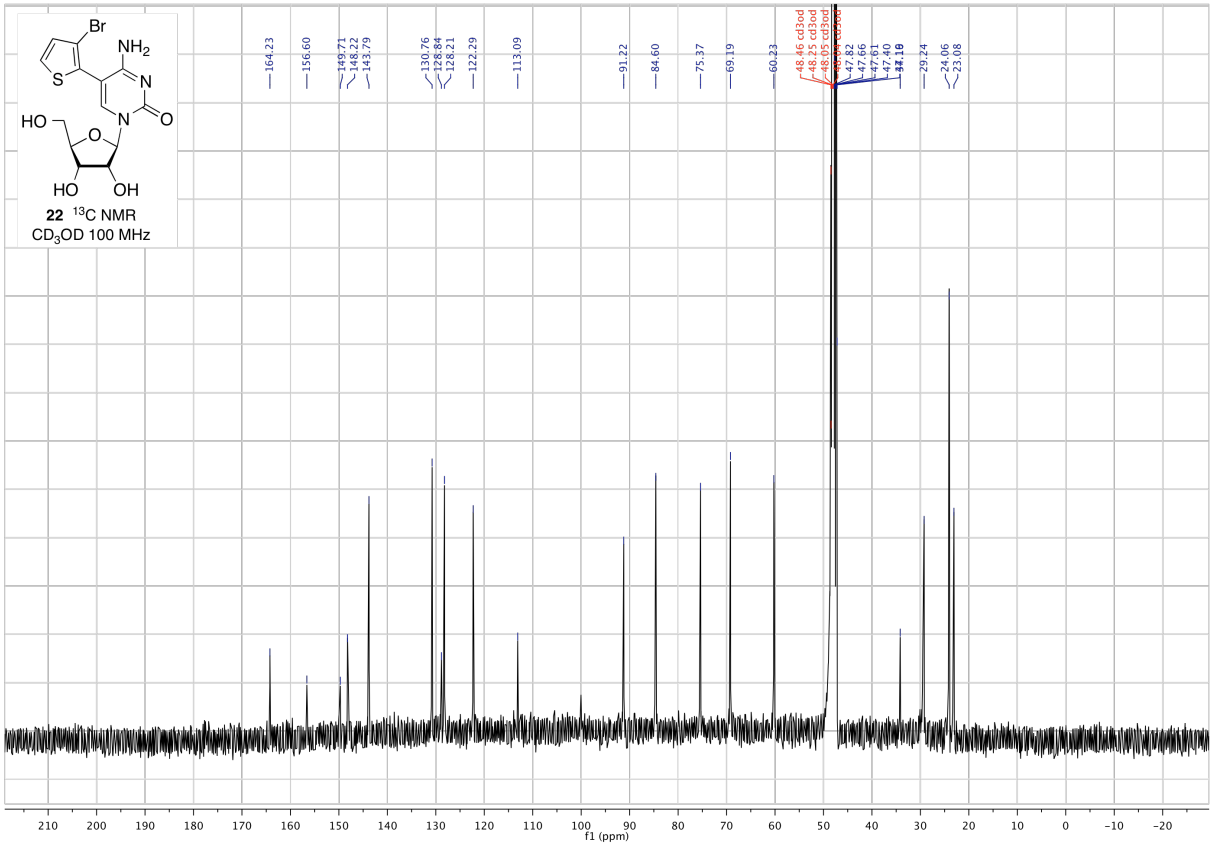

Br
O
S
NH
N
O
AcO
O
AcO
OAc
20 1H NMR
CDCl3 400 MHz
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

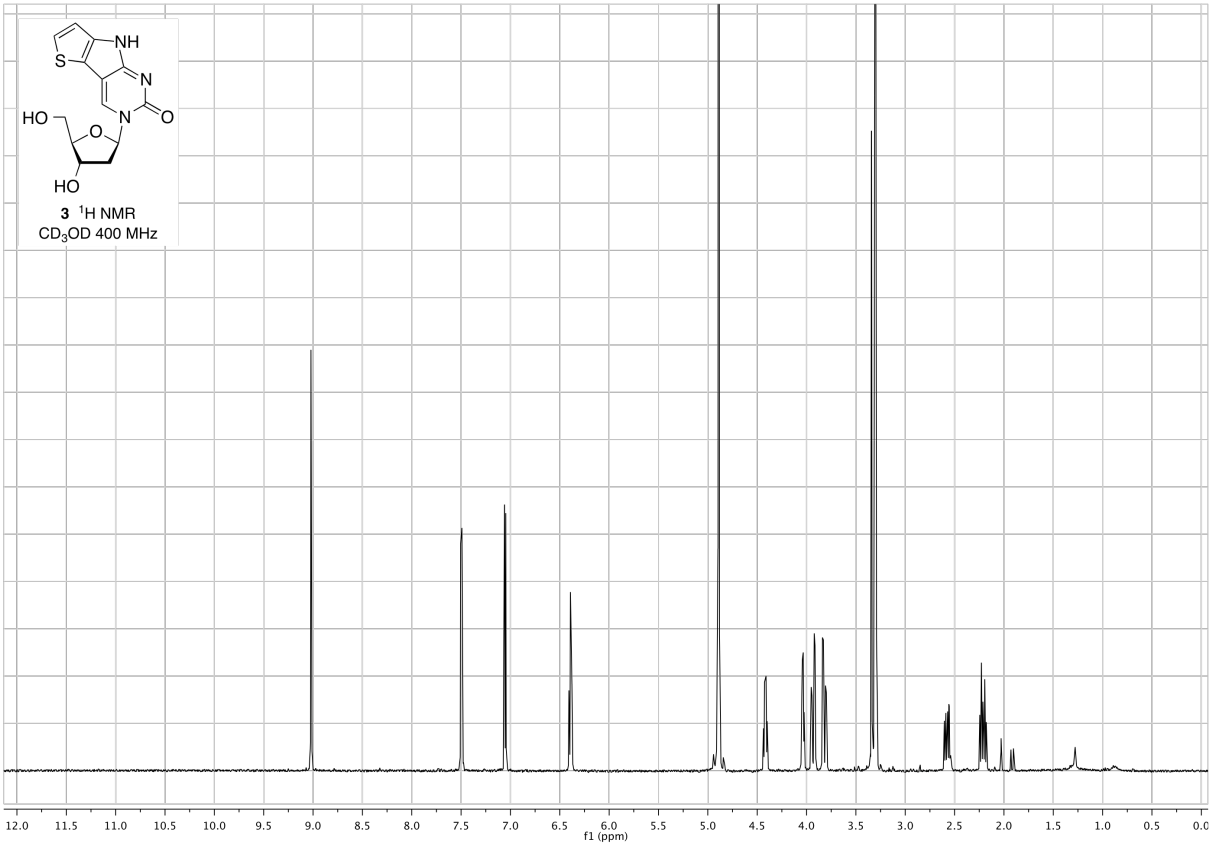

170.42
169.87
169.77
160.89
160.33
149.44
139.09
130.38
127.75
127.70
109.94
108.90
87.12
80.25
77.56 CDCl3
77.40 CDCl3
77.14 CDCl3
76.93 CDCl3
76.44
73.32
70.32
63.35
20.77
20.74
20.68
Br
O
S
NH
N
O
AcO
O
AcO
OAc
20 13C NMR
CDCl3 100 MHz
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

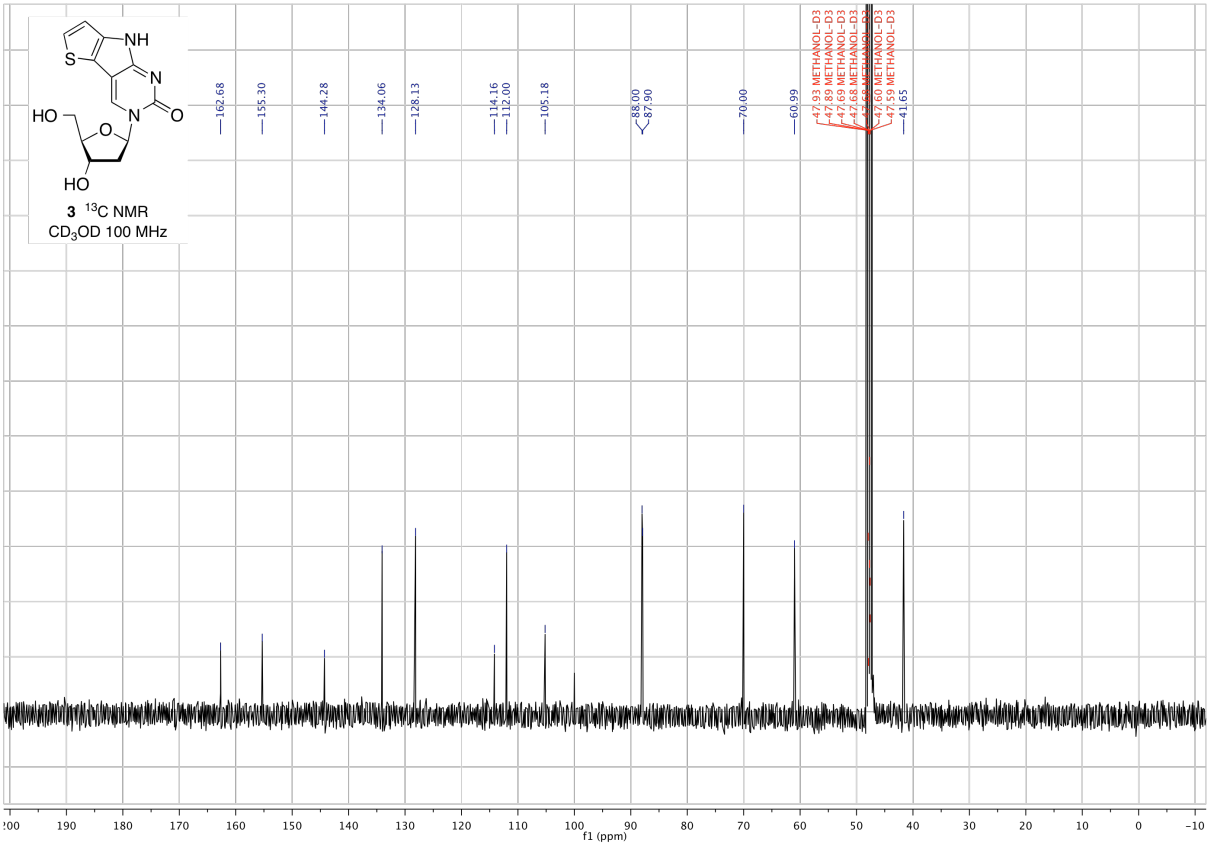

Br
NH2
S
N
N
O
HO
O
HO
21 1H NMR
CD3OD 400 MHz
f1 (ppm)

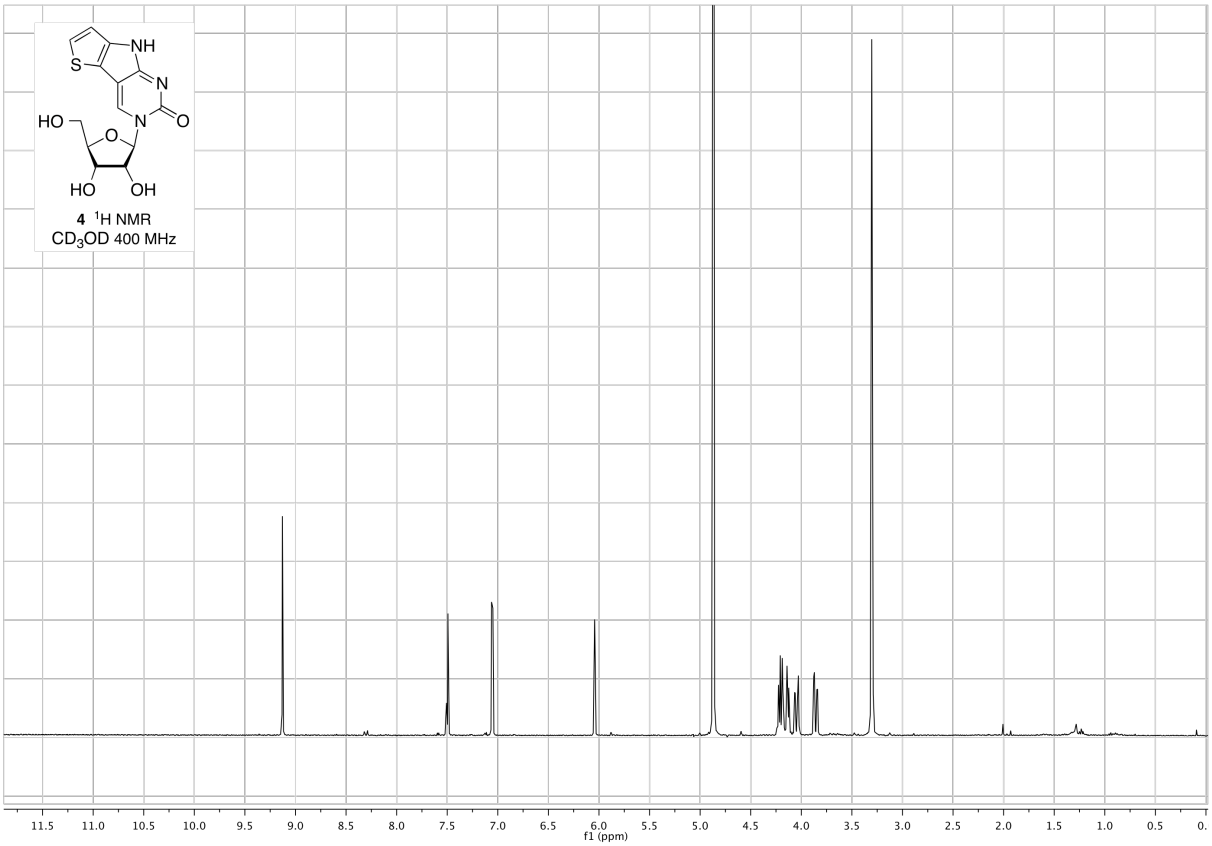

Br
NH2
S
N
N
O
HO
O
HO
21 13C NMR
CD3OD 100 MHz
164.22
156.35
143.50
130.78
128.94
128.20
113.09
99.91
94.55
87.81
86.72
70.53
61.24
48.27 cd3od
48.08 cd3od
48.05 cd3od
47.84
47.63
47.42
47.28
47.09
28.37
f1 (ppm)

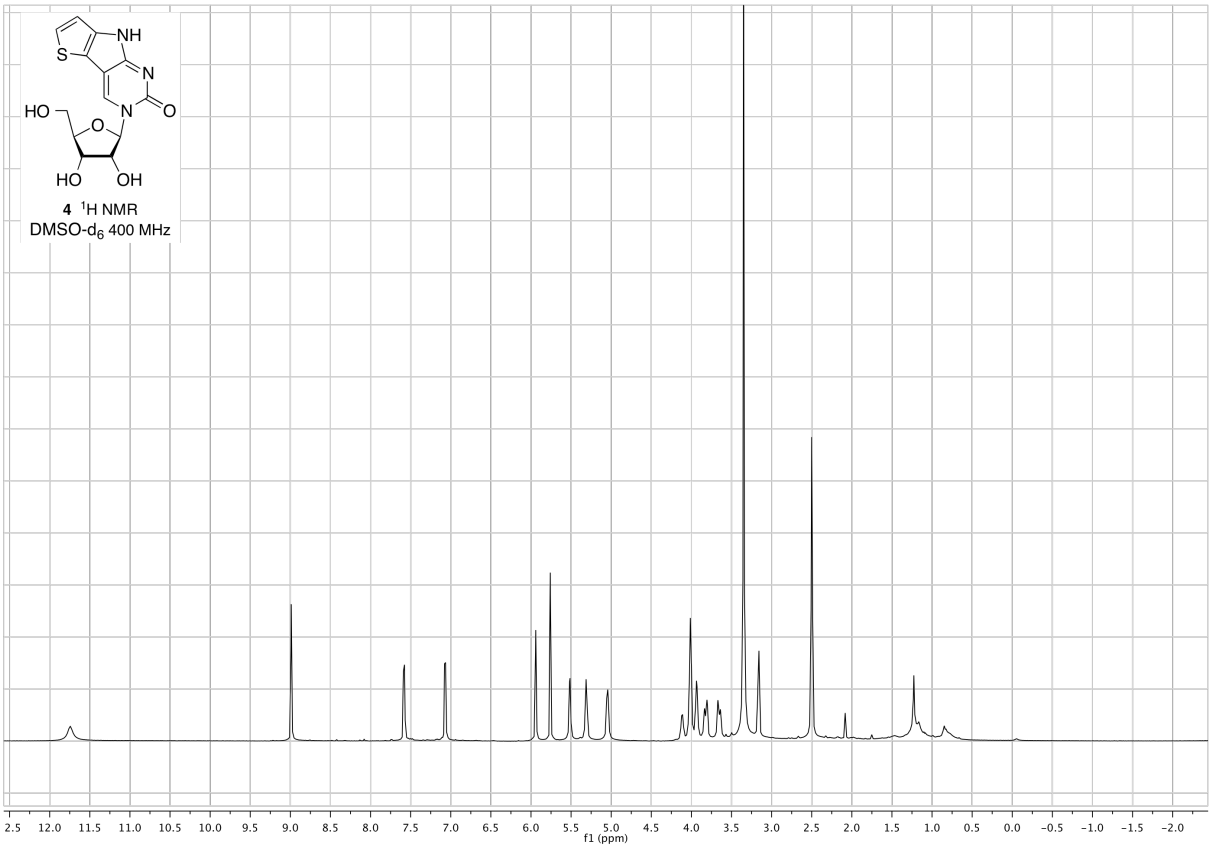

Br
NH2
S
N
N
O
HO
O
HO
OH
22 1H NMR
CDCl3 400 MHz
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

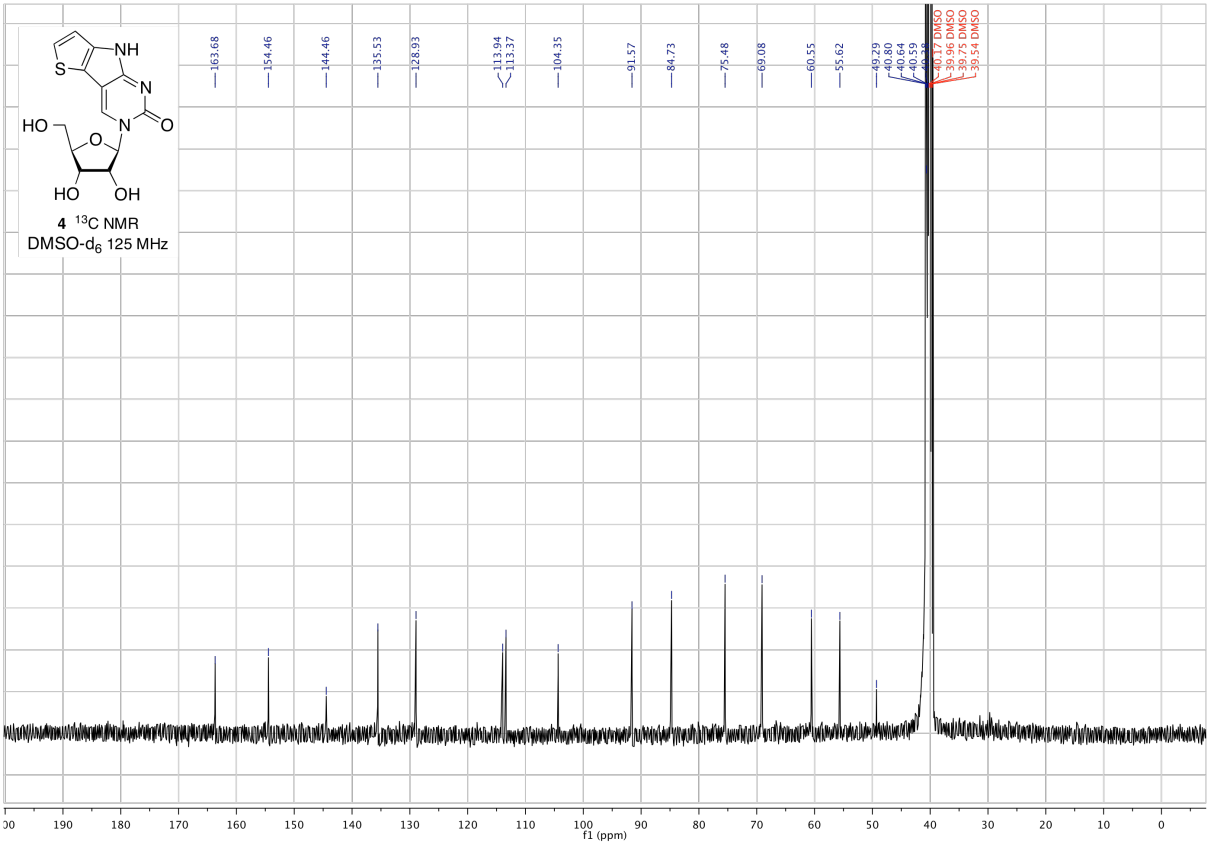

Br
NH2
S
N
N
O
HO
O
HO
OH
22 13C NMR
CD3OD 100 MHz
164.23
156.60
149.71
148.22
143.79
130.76
128.84
128.21
122.29
113.09
91.22
84.60
75.37
69.19
60.23
48.46 cd3od
48.25 cd3od
48.05 cd3od
47.82
47.66
47.61
47.40
47.19
29.24
24.06
23.08
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


NH
S
N
HO
N
O
O
HO
3 ^{1}H NMR
CD_3OD 400 MHz
12.0 11.5 11.0 10.5 10.0 9.5 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 0.0
f1 (ppm)


NH
S
N
HO
N
O
O
HO
3 ^{13}C NMR
CD_3OD 100 MHz
162.68
155.30
144.28
134.06
128.13
114.16
112.00
105.18
88.00
87.90
70.00
60.99
47.93 METHANOL-D3
47.89 METHANOL-D3
47.69 METHANOL-D3
47.68 METHANOL-D3
47.60 METHANOL-D3
47.59 METHANOL-D3
41.65
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


NH
S
N
HO
N
O
O
HO
OH
4 1H NMR
CD3OD 400 MHz
11.5 11.0 10.5 10.0 9.5 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 0.
f1 (ppm)


NH
S
N
HO
N
O
O
HO
OH
4 1H NMR
DMSO-d6 400 MHz
2.5 12.0 11.5 11.0 10.5 10.0 9.5 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 0.0 −0.5 −1.0 −1.5 −2.0
f1 (ppm)



S4. References

(1) Carpita, A.; Rossi, R.; Veracini, C. A. *Tetrahedron* **1985**, *41*, 1919.
(2) Piton, N.; Mu, Y.; Stock, G.; Prisner, T. F.; Schiemann, O.; Engels, J. W. *Nucleic Acids Re.s* **2007**, *35*, 3128.
(3) Asakura, J.; Robins, M. J. *J. Org. Chem.* **1990**, *55*, 4928.
(4) Wigerinck, P.; Pannecouque, C.; Snoeck, R.; Claes, P.; De Clercq, E.; Herdewijn, P. *J. Med. Chem.* **1991**, *34*, 2383.
(5) Liu, J.; Aerschot, A. V.; Luyten, I.; Wigerinck, P.; Pannecouque, C.; Balzarini, J.; Clercq, E. D.; Herdewijn, P. *Nucleosides Nucleotides* **1995**, *14*, 525.
(6) Luyten, I.; Jie, L.; Vanaerschot, A.; Pannecouque, C.; Wigerinck, P.; Rozenski, J.; Hendrix, C.; Wang, C.; Wiebe, L.; Balzarini, J.; Declercq, E.; Herdewijn, P. *Antivir. Chem. Chemoth.* **1995**, *6*, 262.
(7) Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319.
(8) Lakowicz, J. R. *Principles of fluorescence spectroscopy,* 3rd. ed.; Springer: New York, 2006.
(9) Eaton, D. F. *Pure Appl. Chem.* **1988**, *60*, 1107.
(10) Jones, G.; Jackson, W. R.; Choi, C. Y.; Bergmark, W. R. *J. Phys. Chem.* **1985**, *89*, 294.