Chromophoric Nucleoside Analogs: Synthesis and Characterization of 6-Aminouracil-Based Nucleodyes

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Supporting Information

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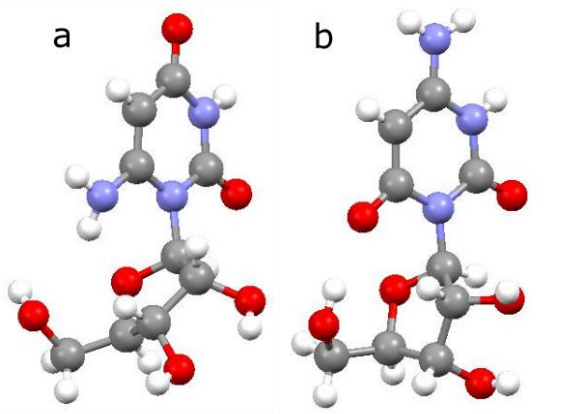


Figure S1. Crystal structures of (a) 6-aminouridine (**4**) and (b) 6-oxocytidine (**7**).

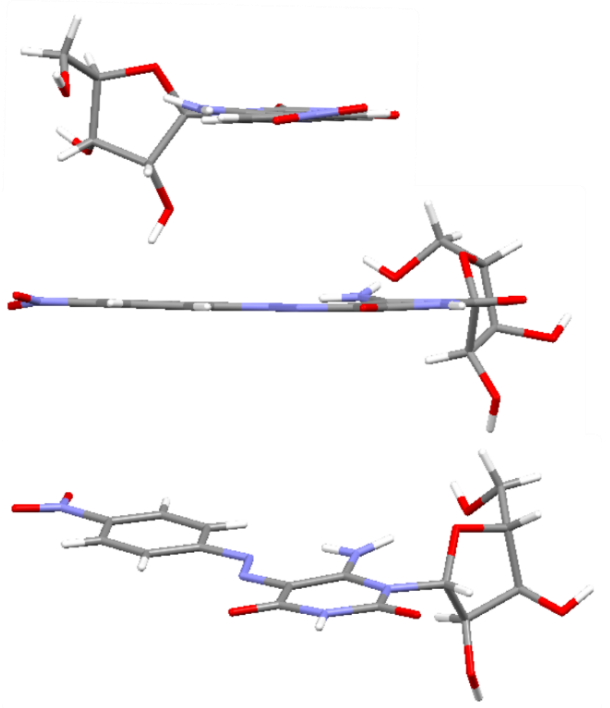


Figure S2. Side view images of the crystal structure of 6-amino-5-(4-nitrophenylazo) uridine (**5**).

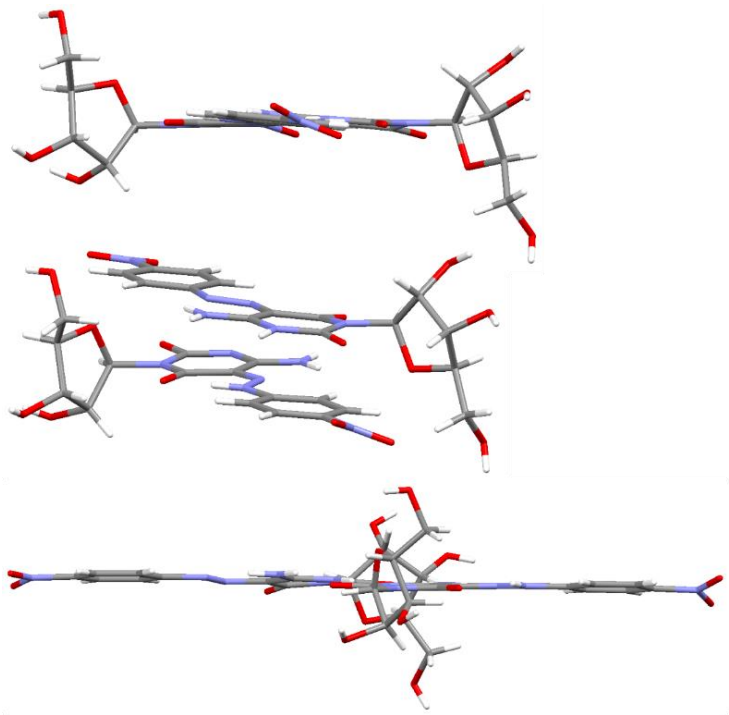


Figure S3. Side view images of the crystal structure of 5-(4-nitrophenylazo)-6-oxocytidine (**8**).

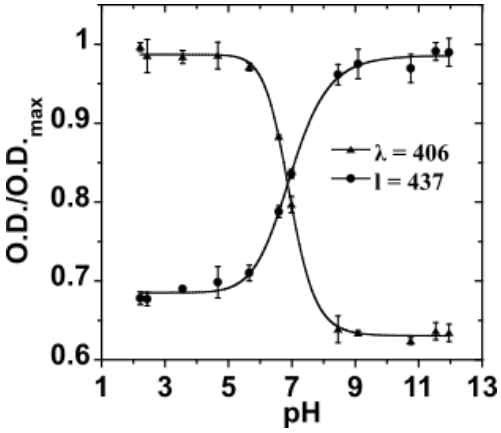


Figure S4. Correlation of optical density values to pH at Gaussian maxima λ = 406 nm (red) and λ = 437 nm (blue) for 5-(4-nitrophenylazo)-6-oxocytidine (**8**) give a slightly lower value than the wavelength maxima correlation indicating the transition at neutral pH (pK_{a1} = 7.0 ± 0.1).

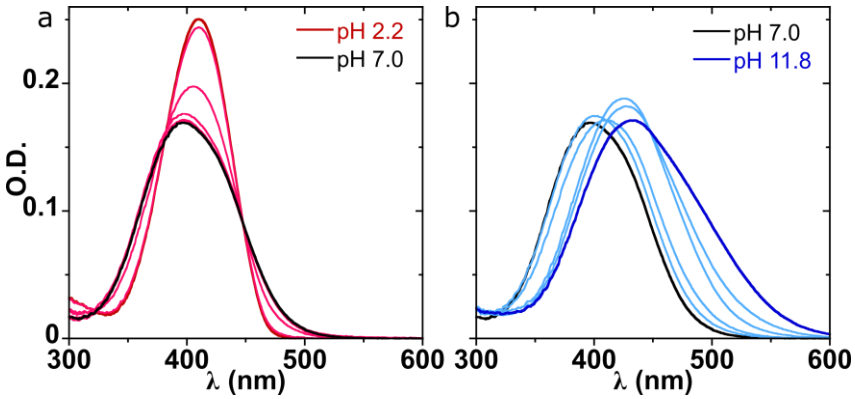


Figure S5. Split Figure 3a. Absorption spectra as function of pH for 6-amino-5-(4-nitrophenylazo) uridine **5** (5.9×10^{-6} M). (a) pH 2.2 – 7.0 ($pK_{a1} = 3.9 \pm 0.2$). (b) pH 7.0 – 11.8 ($pK_{a2} = 8.8 \pm 0.1$).

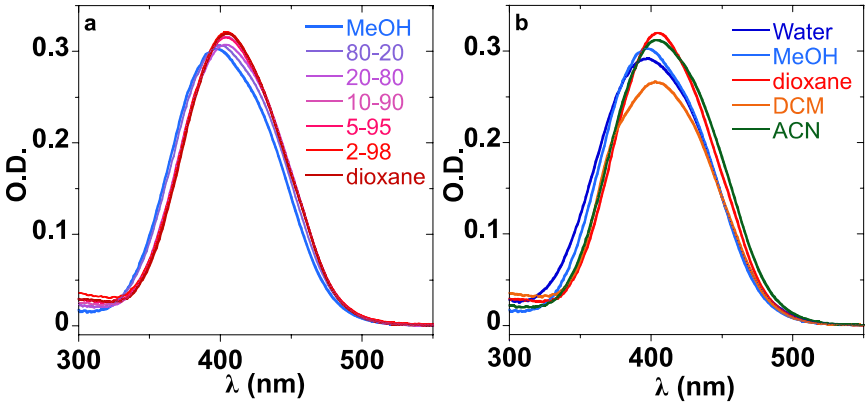


Figure S6. Absorption spectra as function of polarity for 6-amino-5-(4-nitrophenylazo) uridine (**5**, 1×10^{-5} M). (a) MeOH–dioxane solutions; (b) pure solvents.

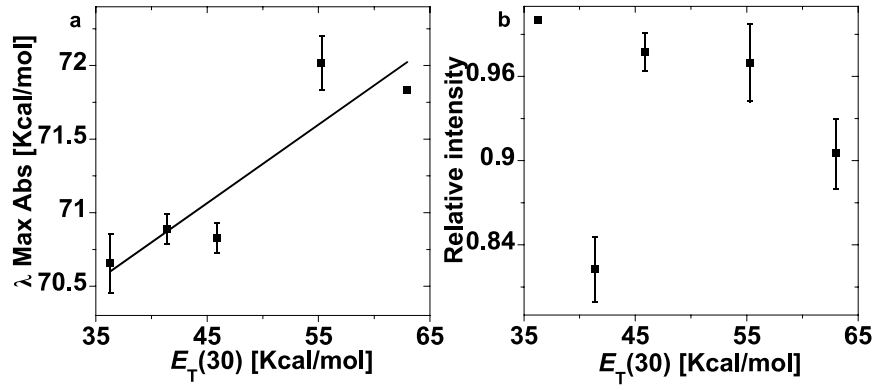


Figure S7. Assessing the effect of solvent polarity on absorption of **5** in pure solvents. (a) Correlation of absorption wavelength maxima and (b) absorption intensity (at the wavelength maxima) with ET30 values. The dramatic decrease in intensity (-18%) measured in pure DCM ($E_T(30)$ = 41.4 Kcal/mol) is likely due to solvent specific effects.

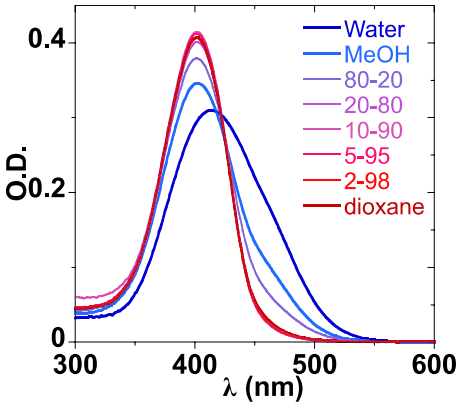


Figure S8. Absorption spectra as function of polarity for 5-(4-nitrophenylazo)-6-oxocytidine (**8**, 9×10^{-6} M) measured in MeOH–dioxane solutions. Absorption spectra in water (dark blue) displays a notable 10 nm bathochromic shift.

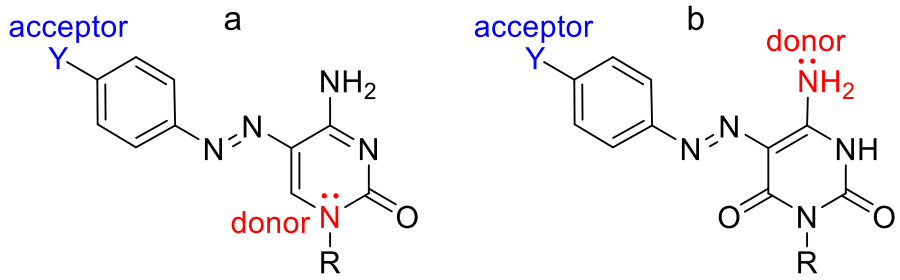


Figure S9. Design strategy for (a) cytidine and (b) 6-oxocytidine azo nucleodyes. R = ribofuranose or 2'-dexoyribofuranose.

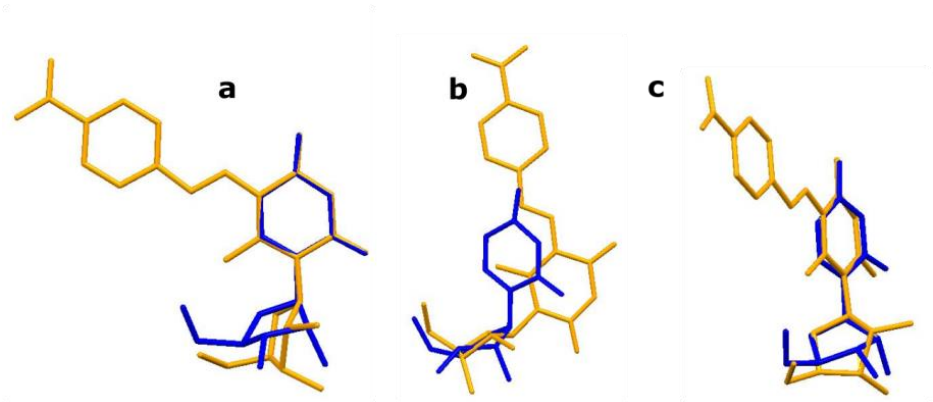


Figure S10. Overlap of uridine and 6-amino-5-(4-nitrophenylazo) uridine (**5**). a. pyrimidine core; b. sugar pucker; c. sugar and nucleobase.

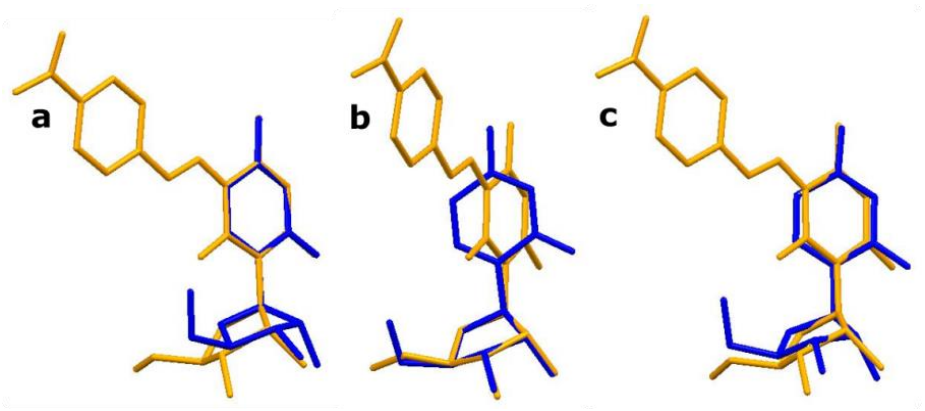


Figure S11. Overlap of cytidine and 5-(4-nitrophenylhydrazono)-6-oxocytidine (**8b**). a. pyrimidine core; b. sugar pucker; c. sugar and nucleobase.

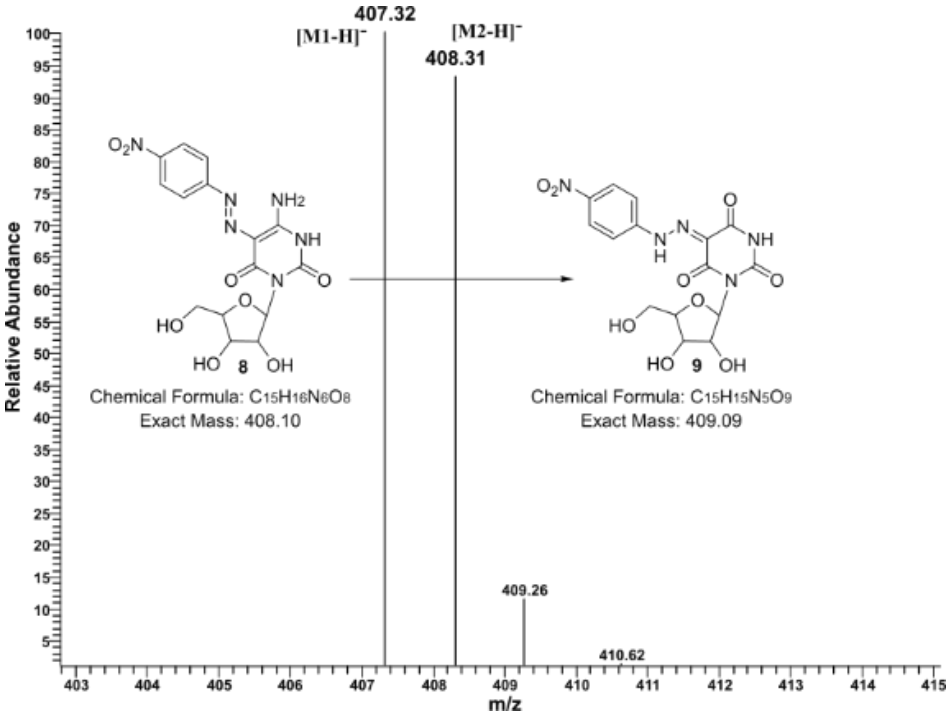
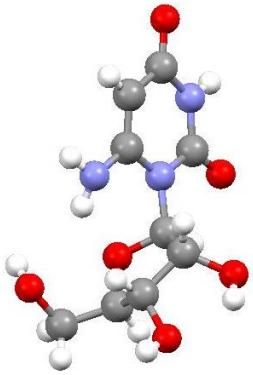


Figure S12. LR-MS analysis of crystallization solution gave the masses of both 5-(4-nitrophenylazo)-6-oxocytidine (**8**) and 5-(4-nitrophenylhydrazono)-6-oxouridine (**9**).

X-ray crystal structures

The single crystal X-ray diffraction studies were carried out on a single crystal diffractometer equipped with Cu K_α radiation (λ = 1.5478) and a Charge-Coupled-Device (CCD) Detector. Crystals were mounted on a Cryoloop with Paratone oil. Data were collected in a nitrogen gas stream at 100(2) K using ϕ and ϖ scans. The data were integrated using the Bruker SAINT software program and scaled using the SADABS software program. Solution by direct methods (SHELXT) produced a complete phasing model consistent with the proposed structure. All nonhydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-2014). All hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-2014. All other hydrogen atoms (H-bonding) were located in the difference map. There relative positions were restrained using DFIX commands and their thermals freely refined. Crystallographic data are summarized in Tables S1–S5.

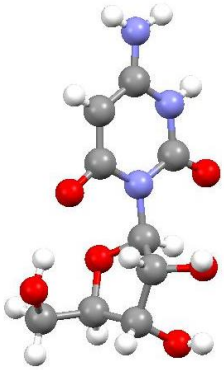
Table S1. Crystal data and structure refinement for Tor100, 6-aminouridine (**4**), CCDC 1437719.



Identification code	NF-218	
Empirical formula	C9 H13 N3 O6	
Formula weight	259.22	
Temperature	100.0 K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P 1 21 1	
Unit cell dimensions	a = 7.0881(3) Å	α= 90°.
	b = 6.6887(3) Å	β= 96.219(2)°.
	c = 11.6839(6) Å	γ = 90°.
Volume	550.68(4) $Å^3$	
Z	2	
Density (calculated)	1.563 Mg/m^3	
Absorption coefficient	1.148 mm^{-1}	
F(000)	272	
Crystal size	0.167 x 0.113 x 0.095 mm^3	
Theta range for data collection	3.805 to 68.031°.	
Index ranges	-8<=h<=8, -8<=k<=8, -14<=l<=13	
Reflections collected	17032	
Independent reflections	1962 [R(int) = 0.0344]	
Completeness to theta = 68.000°	97.6 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.1665 and 0.0631	
Refinement method	Full-matrix least-squares on F^2	

Data / restraints / parameters	1962 / 7 / 187
Goodness-of-fit on F^2	1.080
Final R indices [I>2sigma(I)]	R1 = 0.0232, wR2 = 0.0612
R indices (all data)	R1 = 0.0232, wR2 = 0.0613
Absolute structure parameter	0.04(3)
Extinction coefficient	n/a
Largest diff. peak and hole	0.181 and -0.180 e.$Å^{-3}$

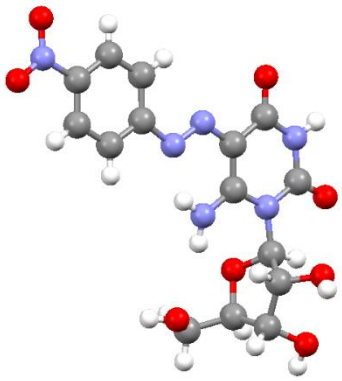
Table S2. Crystal data and structure refinement for Tor87, 6-oxocytidine (**7**), CCDC 1437716.



Report date	2014-07-02	
Identification code	NF141b	
Empirical formula	C9 H15 N3 O7	
Molecular formula	C9 H13 N3 O6, H2 O	
Formula weight	277.24	
Temperature	100.0 K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 7.11510(10) Å	α= 90°.
	b = 8.9631(2) Å	β= 90°.
	c = 18.7210(4) Å	γ = 90°.
Volume	1193.90(4) $Å^3$	
Z	4	
Density (calculated)	1.542 Mg/m^3	
Absorption coefficient	1.161 mm^{-1}	
F(000)	584	
Crystal size	0.251 x 0.153 x 0.142 mm^3	
Crystal color, habit	Colorless Block	
Theta range for data collection	4.724 to 68.193°.	
Index ranges	-8<=h<=7, -10<=k<=9, -21<=l<=22	
Reflections collected	7838	
Independent reflections	2168 [R(int) = 0.0216]	
Completeness to theta = 68.000°	99.7 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7530 and 0.6907	
Refinement method	Full-matrix least-squares on F^2	

Data / restraints / parameters	2168 / 8 / 204
Goodness-of-fit on F^2	1.097
Final R indices [I>2sigma(I)]	R1 = 0.0237, wR2 = 0.0618
R indices (all data)	R1 = 0.0241, wR2 = 0.0620
Absolute structure parameter	0.00(6)
Extinction coefficient	n/a
Largest diff. peak and hole	0.176 and -0.165 e.$Å^{-3}$

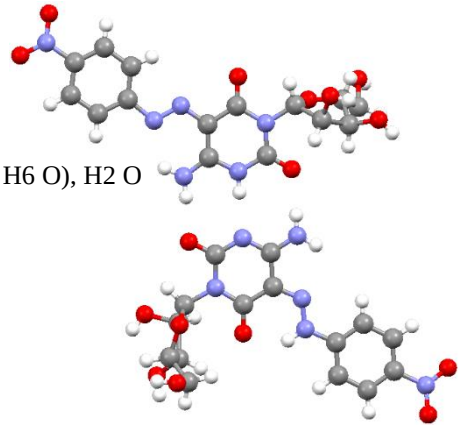
Table S3. Crystal data and structure refinement for Tor82, 6-amino-5-(4-nitrophenylazo) uridine (**5**), CCDC 1437715.



Report date	2014-05-28	
Identification code	NF221	
Empirical formula	C15 H20 N6 O10	
Molecular formula	C15 H16 N6 O8, 2(H2 O)	
Formula weight	444.37	
Temperature	100.0 K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P 1 21 1	
Unit cell dimensions	a = 4.9790(2) Å	α= 90°.
	b = 10.7305(4) Å	β= 96.594(2)°.
	c = 16.8422(7) Å	γ = 90°.
Volume	893.88(6) $Å^3$	
Z	2	
Density (calculated)	1.651 Mg/m^3	
Absorption coefficient	1.217 mm^{-1}	
F(000)	464	
Crystal size	0.157 x 0.035 x 0.021 mm^3	
Crystal color, habit	Yellow Needle	
Theta range for data collection	2.641 to 68.853°.	
Index ranges	-5<=h<=5, -12<=k<=12, -20<=l<=20	
Reflections collected	21896	
Independent reflections	3239 [R(int) = 0.0331]	
Completeness to theta = 68.000°	99.8 %	
Absorption correction	Semi-empirical from equivalents	

Max. and min. transmission	0.7531 and 0.6752
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3239 / 11 / 320
Goodness-of-fit on F^2	1.047
Final R indices [I>2sigma(I)]	R1 = 0.0242, wR2 = 0.0605
R indices (all data)	R1 = 0.0259, wR2 = 0.0614
Absolute structure parameter	0.06(7)
Extinction coefficient	n/a
Largest diff. peak and hole	0.209 and -0.168 e.Å^{-3}

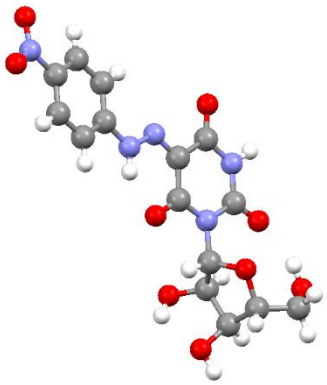
Table S4. Crystal data and structure refinement for Tor90, 5-(4-nitrophenylazo)-6-oxocytidine (**8**), CCDC 1437718.



Report date	2014-07-31	
Identification code	NF244	
Empirical formula	C31 H37 N12 O17.50	
Molecular formula	2(C15 H16 N6 O8), 0.5(C2 H6 O), H2 O	
Formula weight	857.72	
Temperature	100.0 K	
Wavelength	1.54178 Å	
Crystal system	Triclinic	
Space group	P 1	
Unit cell dimensions	a = 7.7143(2) Å	α= 94.0269(16)°.
	b = 10.5890(3) Å	β= 96.5699(17)°.
	c = 11.4023(3) Å	γ = 100.9764(16)°.
Volume	904.32(4) Å^3	
Z	1	
Density (calculated)	1.575 Mg/m^3	
Absorption coefficient	1.129 mm^{-1}	
F(000)	447	
Crystal size	0.113 x 0.032 x 0.021 mm^3	
Crystal color, habit	Orange Needle	
Theta range for data collection	3.920 to 68.399°.	
Index ranges	-9<=h<=9, -11<=k<=12, -13<=l<=13	
Reflections collected	21045	
Independent reflections	6016 [R(int) = 0.0439]	
Completeness to theta = 68.000°	97.8 %	

Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7531 and 0.6937
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6016 / 91 / 590
Goodness-of-fit on F^2	1.035
Final R indices [I>2sigma(I)]	R1 = 0.0506, wR2 = 0.1301
R indices (all data)	R1 = 0.0648, wR2 = 0.1402
Absolute structure parameter	0.08(13)
Extinction coefficient	n/a
Largest diff. peak and hole	0.462 and -0.260 e.$Å^{-3}$

Table S5. Crystal data and structure refinement for Tor89Cu, 5-(4-nitrophenylhydrazono)-6-oxouridine (**9**), CCDC 1437717.



Report date	2014-07-09	
Identification code	NF244-CM	
Empirical formula	C15 H15 N5 O9	
Molecular formula	C15 H15 N5 O9	
Formula weight	409.32	
Temperature	100.0 K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 6.7215(4) Å	α= 90°.
	b = 10.5793(6) Å	β= 90°.
	c = 22.9108(14) Å	γ = 90°.
Volume	1629.16(17) $Å^3$	
Z	4	
Density (calculated)	1.669 Mg/m^3	
Absorption coefficient	1.218 mm^{-1}	
F(000)	848	
Crystal size	0.083 x 0.025 x 0.025 mm^3	
Crystal color, habit	Yellow Needle	
Theta range for data collection	3.859 to 69.421°.	
Index ranges	-8<=h<=8, -12<=k<=11, -27<=l<=27	
Reflections collected	19571	

Independent reflections	2981 [R(int) = 0.0556]
Completeness to theta = 68.000°	99.8 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7001 and 0.6086
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2981 / 5 / 282
Goodness-of-fit on F^2	1.048
Final R indices [I>2sigma(I)]	R1 = 0.0419, wR2 = 0.1033
R indices (all data)	R1 = 0.0475, wR2 = 0.1068
Absolute structure parameter	-0.01(13)
Extinction coefficient	n/a
Largest diff. peak and hole	0.443 and -0.260 e.$Å^{-3}$

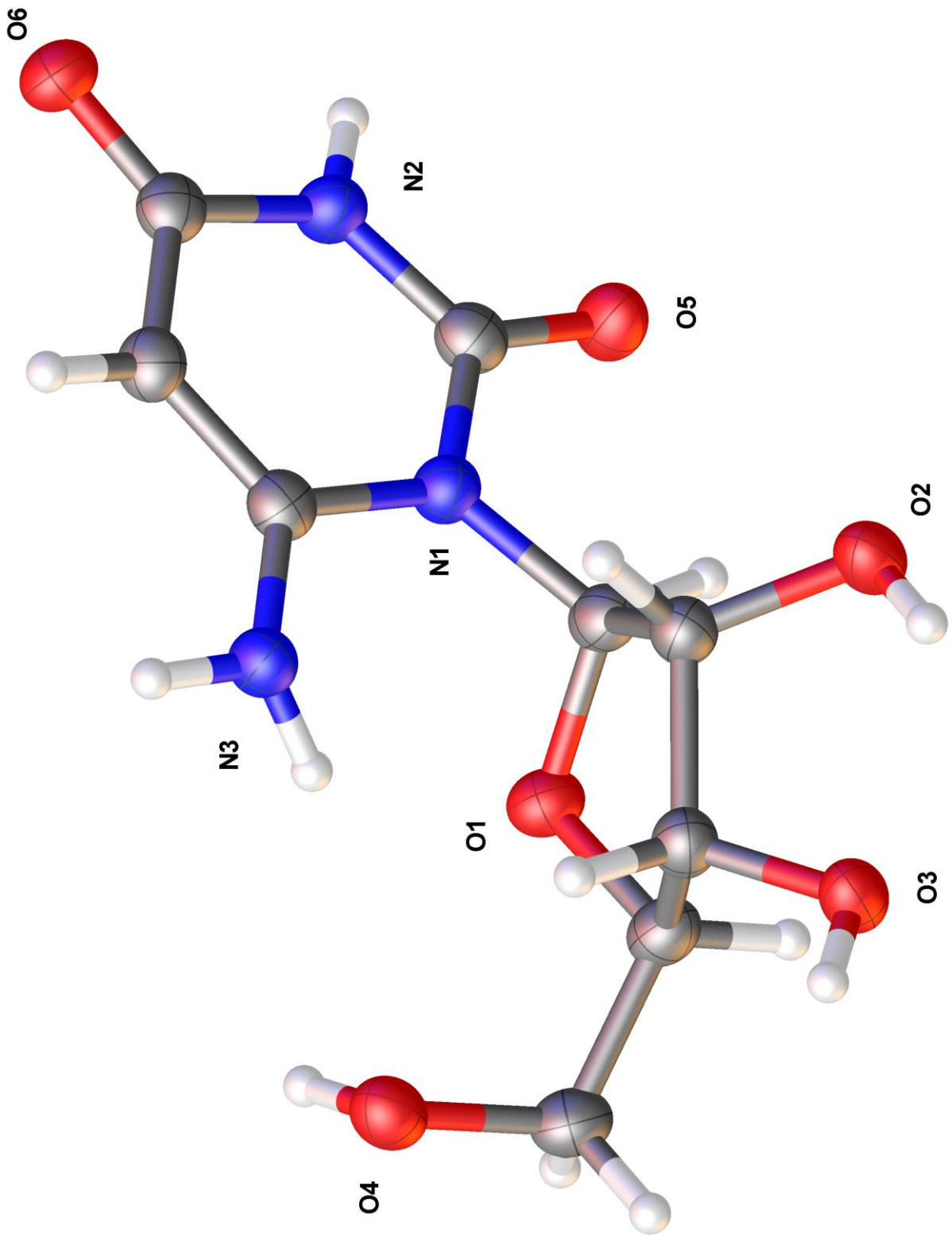


Figure S13. Crystal structure of 6-aminouridine (**4**) (Tor100, 50% contour percent probability).

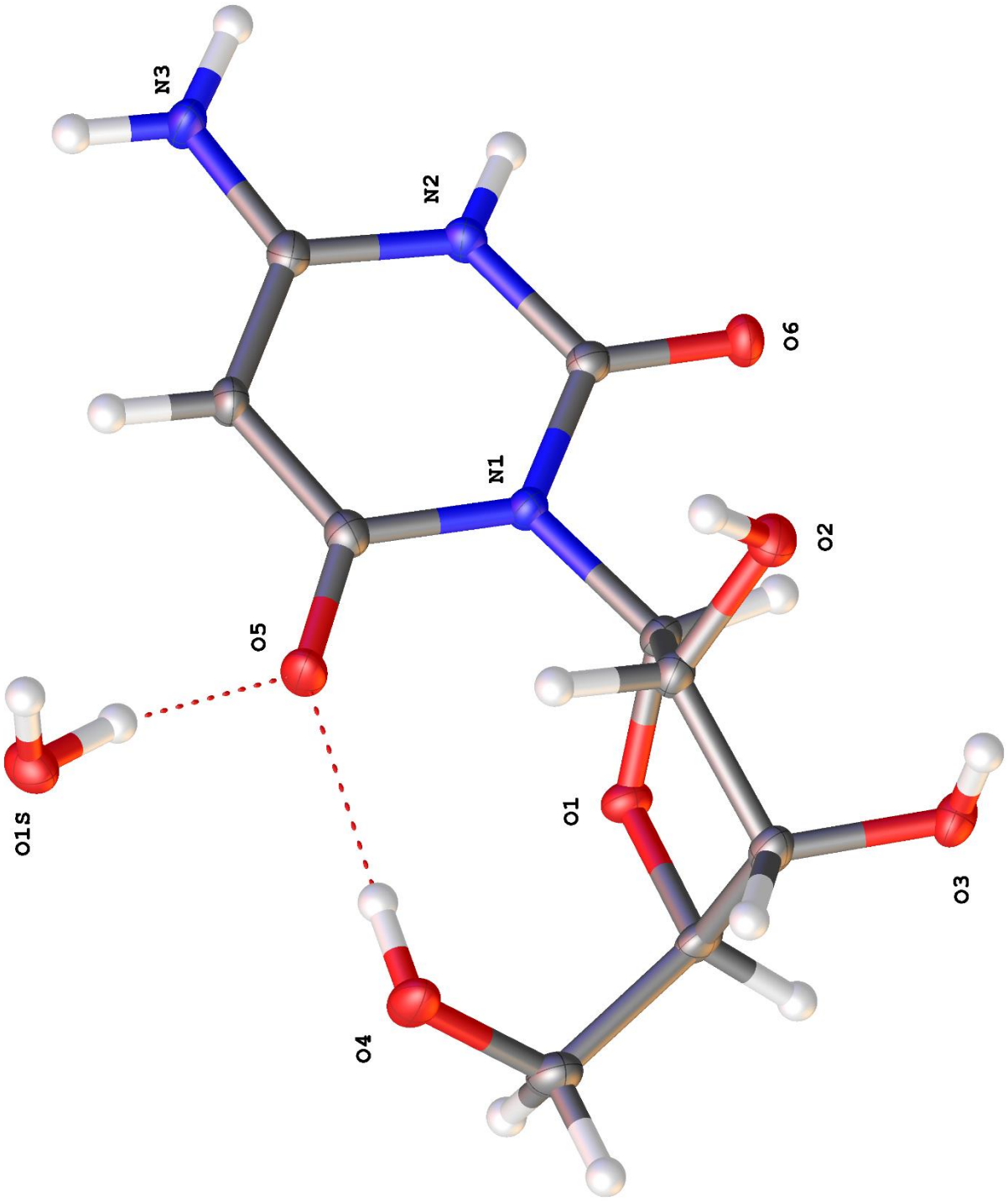


Figure S14. Crystal structure of 6-oxocytidine (**7**) (Tor87, 50% contour percent probability).

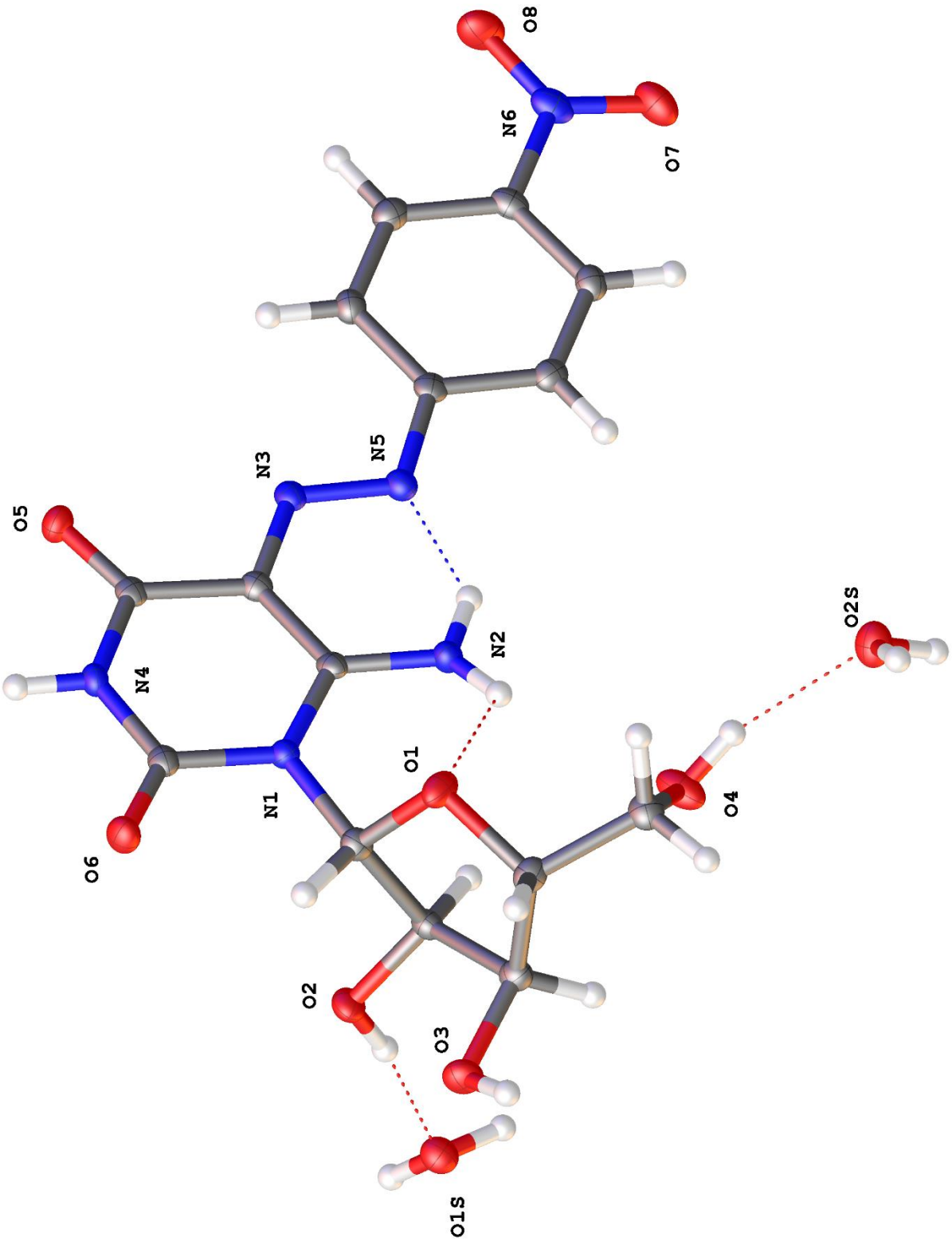


Figure S15. Crystal structure of 6-amino-5-(4-nitrophenylazo) uridine (**5**) (Tor82, 50% contour percent probability).

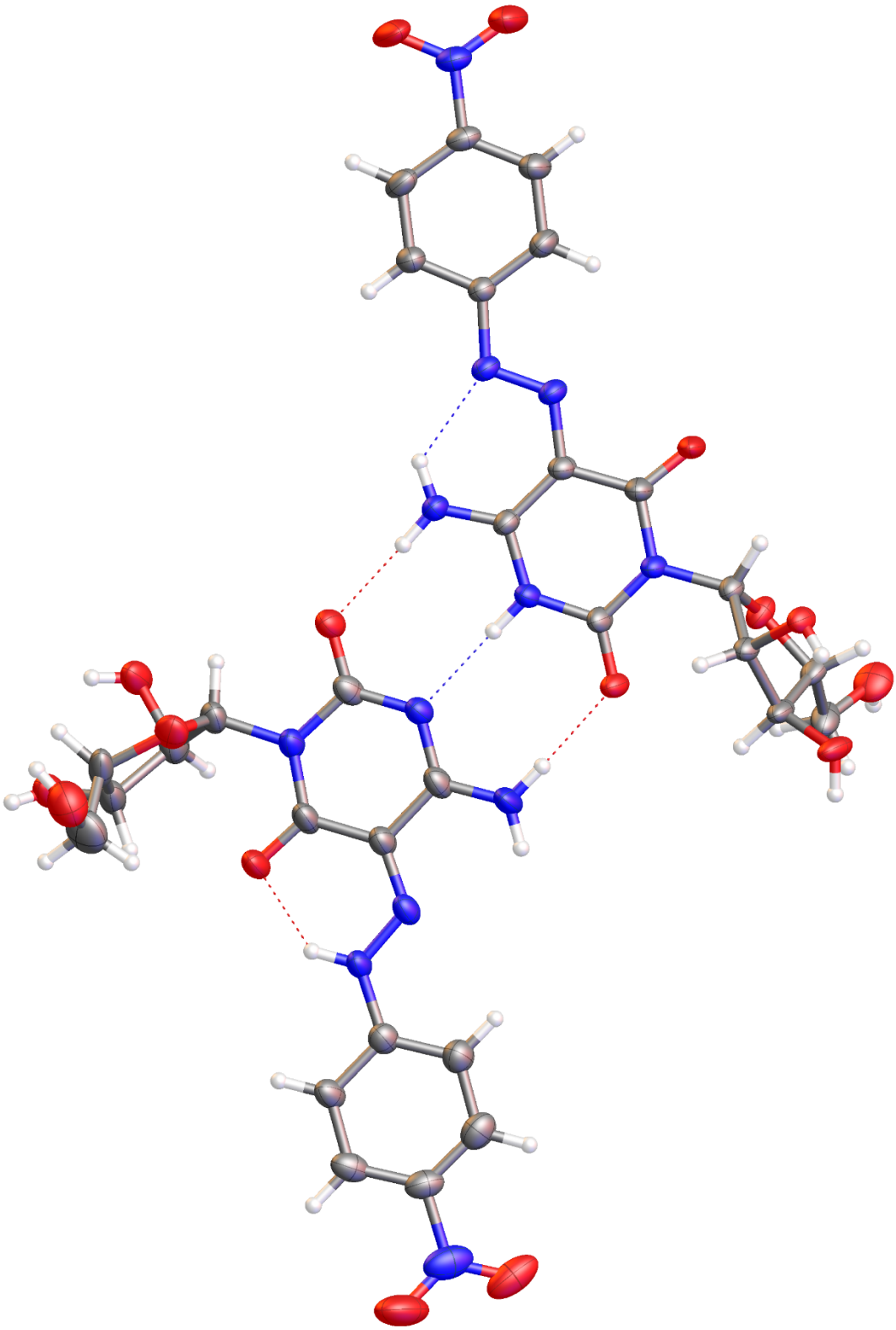


Figure S16. Crystal structure of 5-(4-nitrophenylazo)-6-oxocytidine (**8**) (Tor90, 50% contour percent probability).

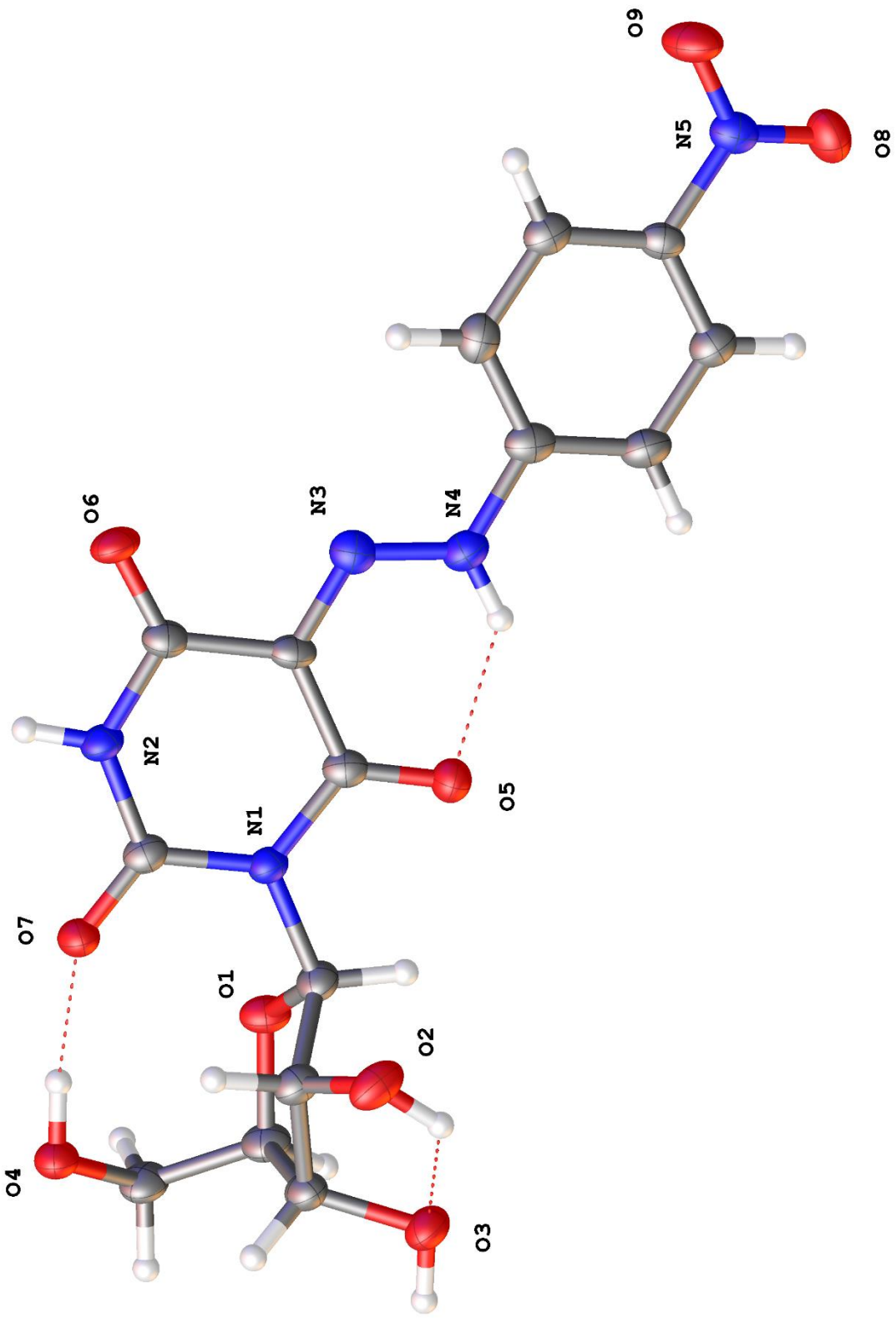


Figure S17. Crystal structure of 5-(4-nitrophenylhydrazono)-6-oxouridine (**9**) (Tor89Cu, 50% contour percent probability).

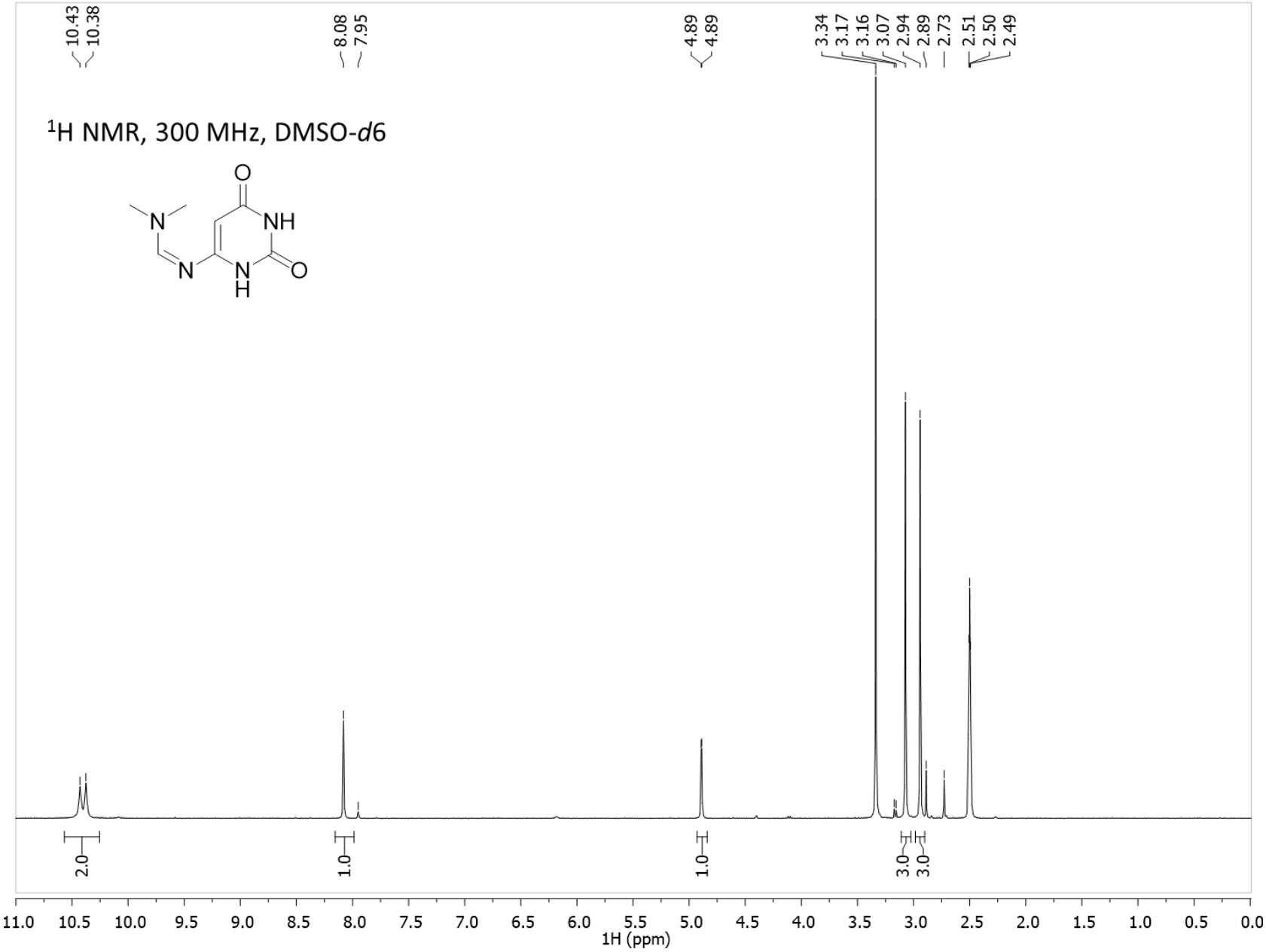


Figure S18. ^{1}H NMR of N^{6}-DMF 6-amino uracil (**2**) (traces of DMF and MeOH)

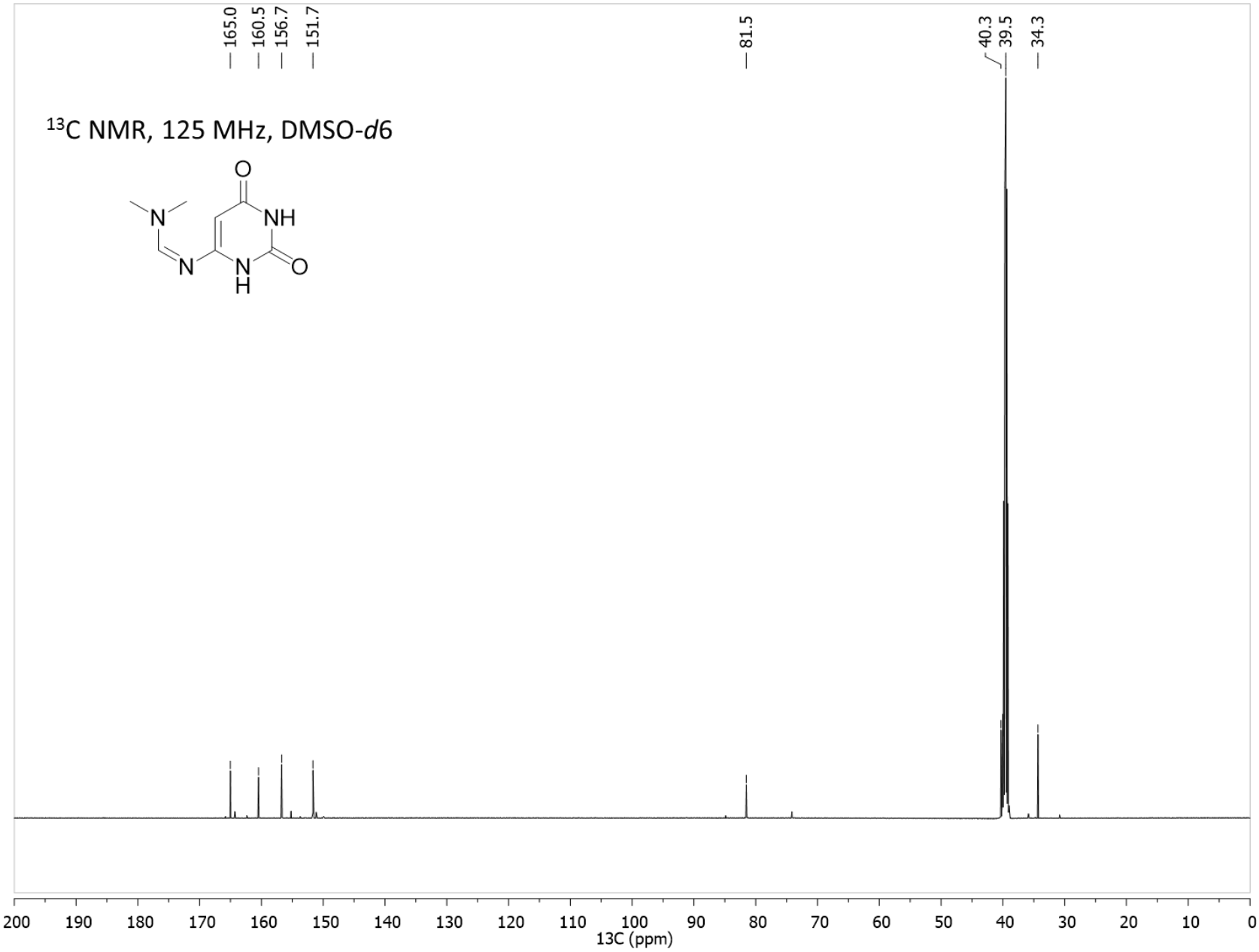


Figure S19. ^{13}C NMR of N^6-DMF 6-amino uracil (**2**)

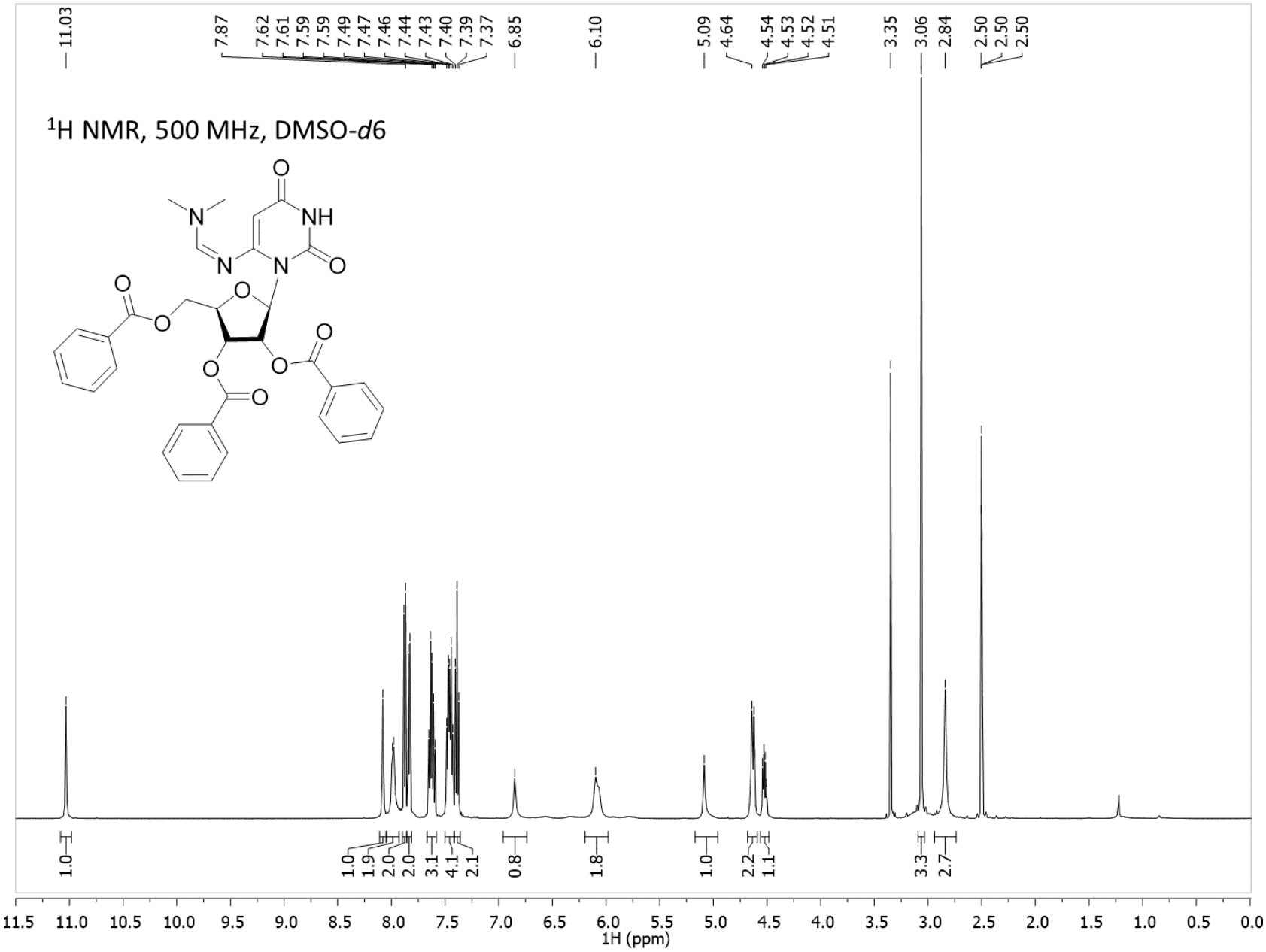


Figure S20. ^{1}H NMR of N^{6}-DMF 2',3',5'-tri-*O*-benzoyl 6-amino uridine (**3**)

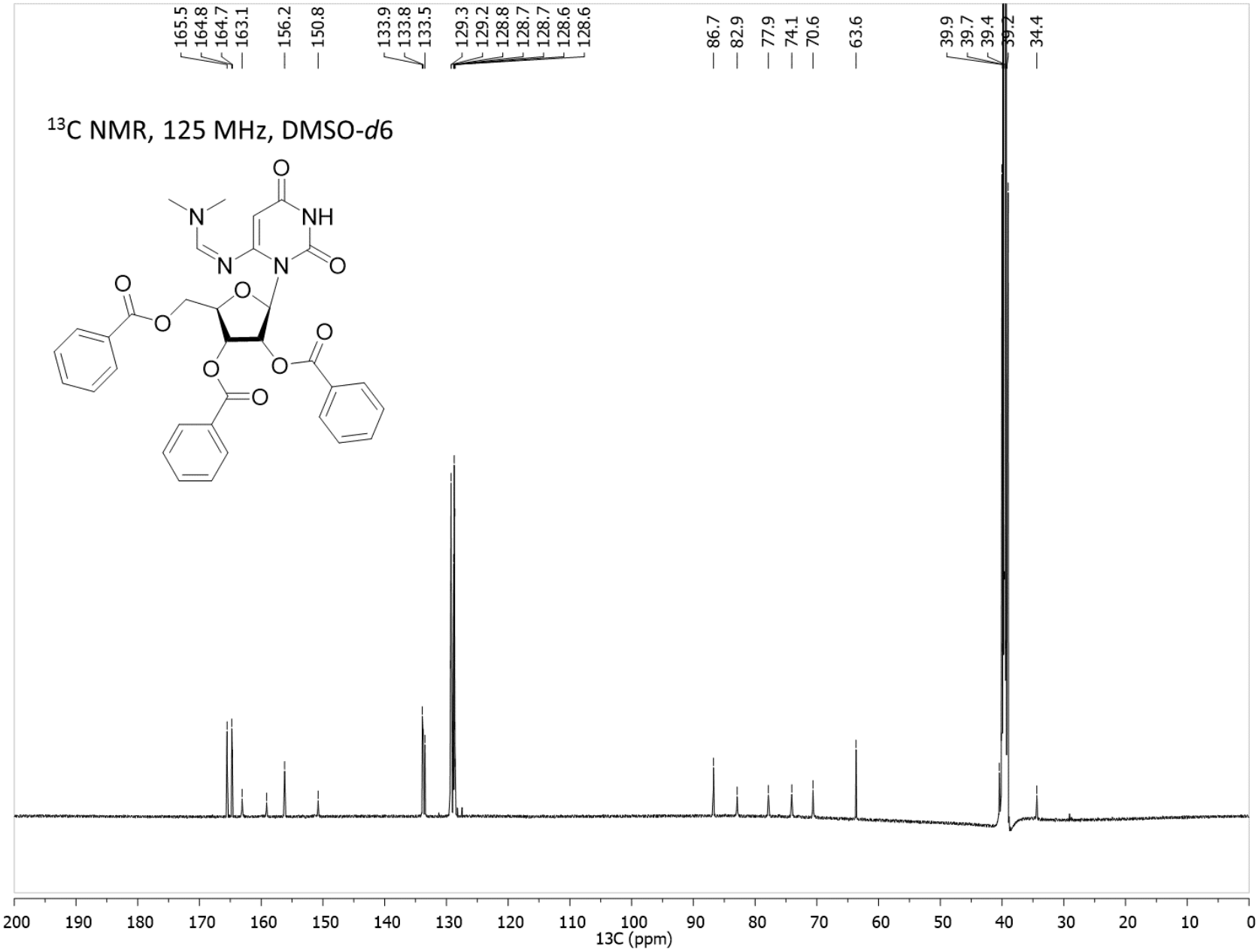


Figure S21. ^{13}C NMR of N^6-DMF 2',3',5'-tri-*O*-benzoyl 6-amino uridine (**3**)

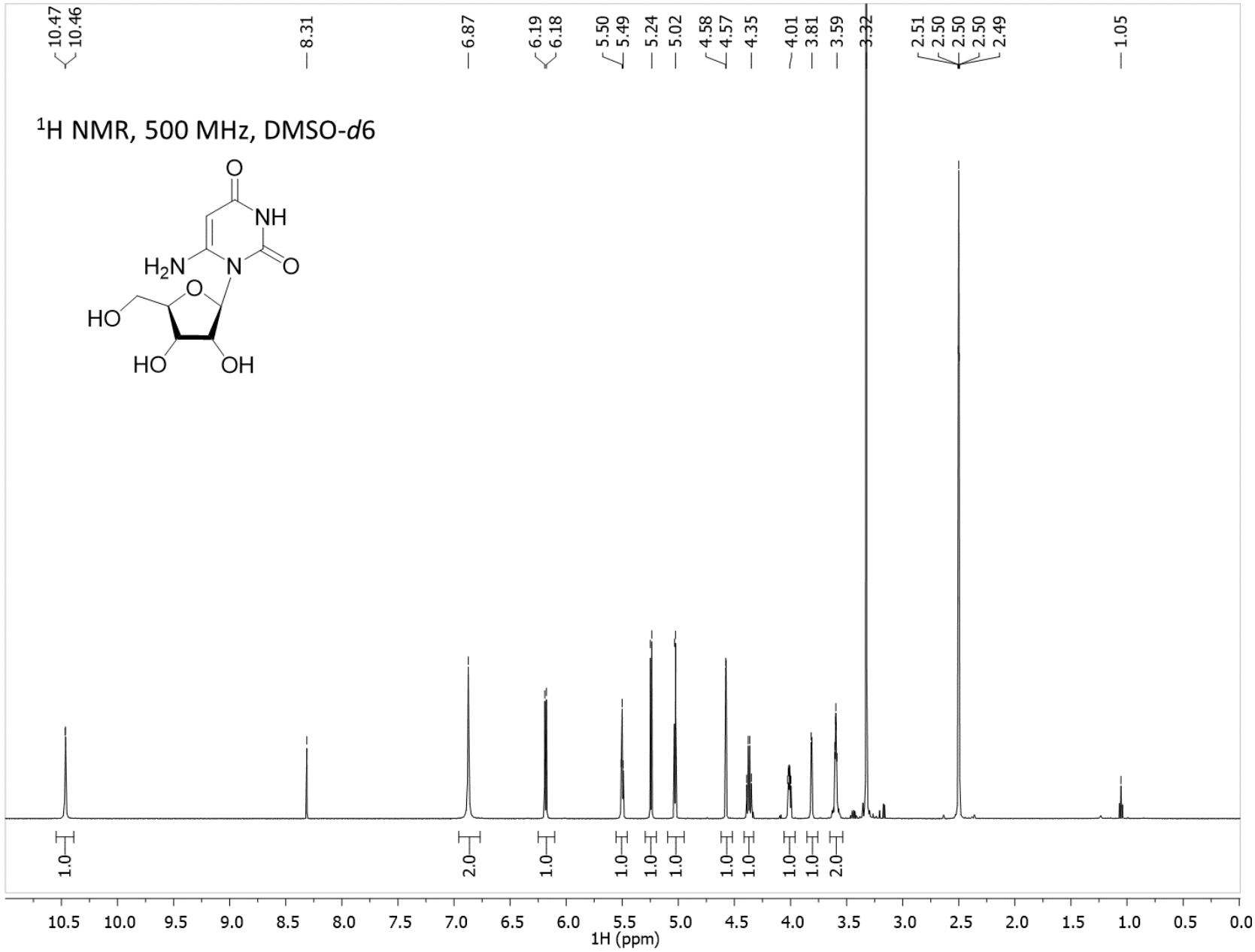


Figure S22. ^{1}H NMR of 6-amino uridine (**4**)

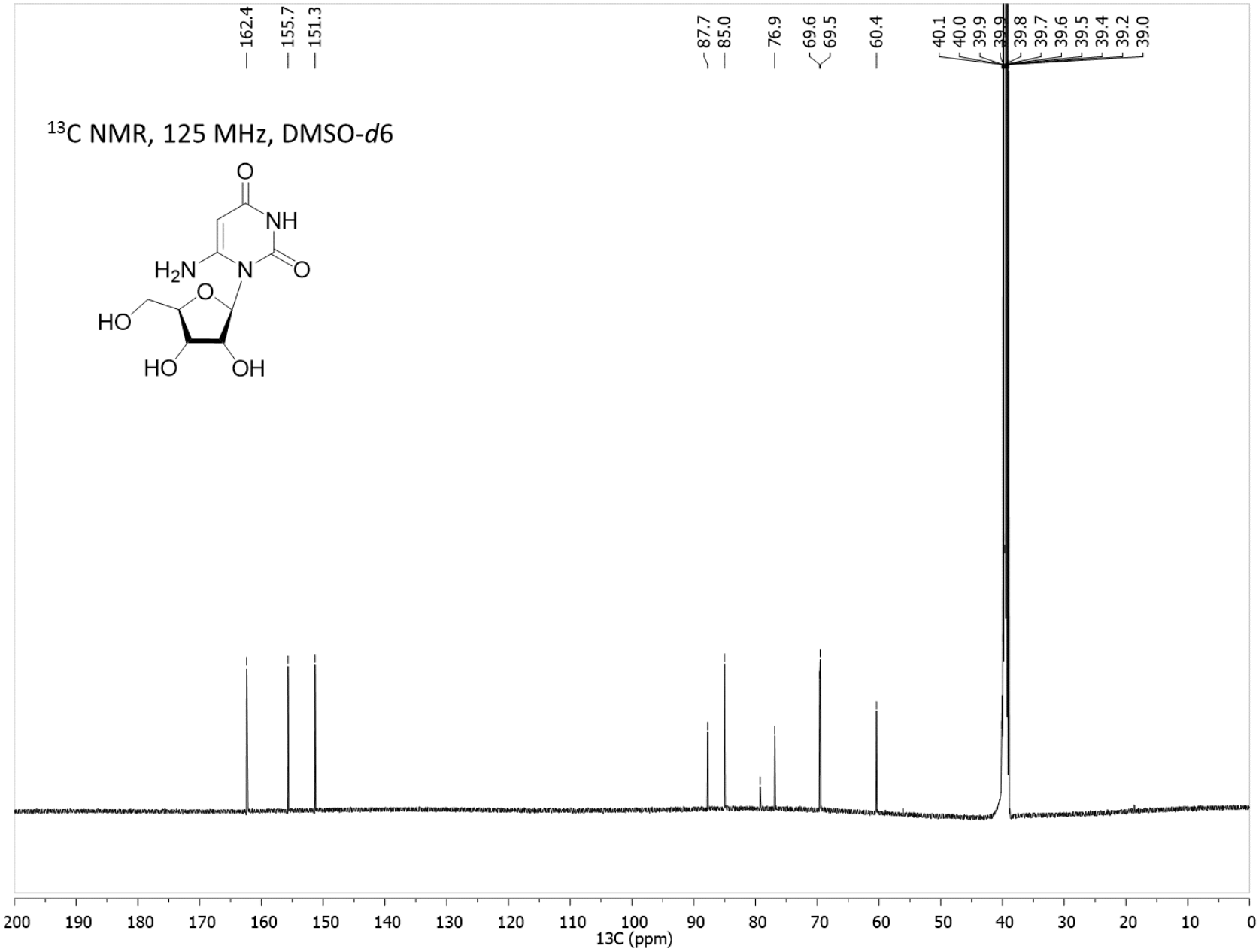


Figure S23. ^{13}C NMR of 6-amino uridine (**4**)

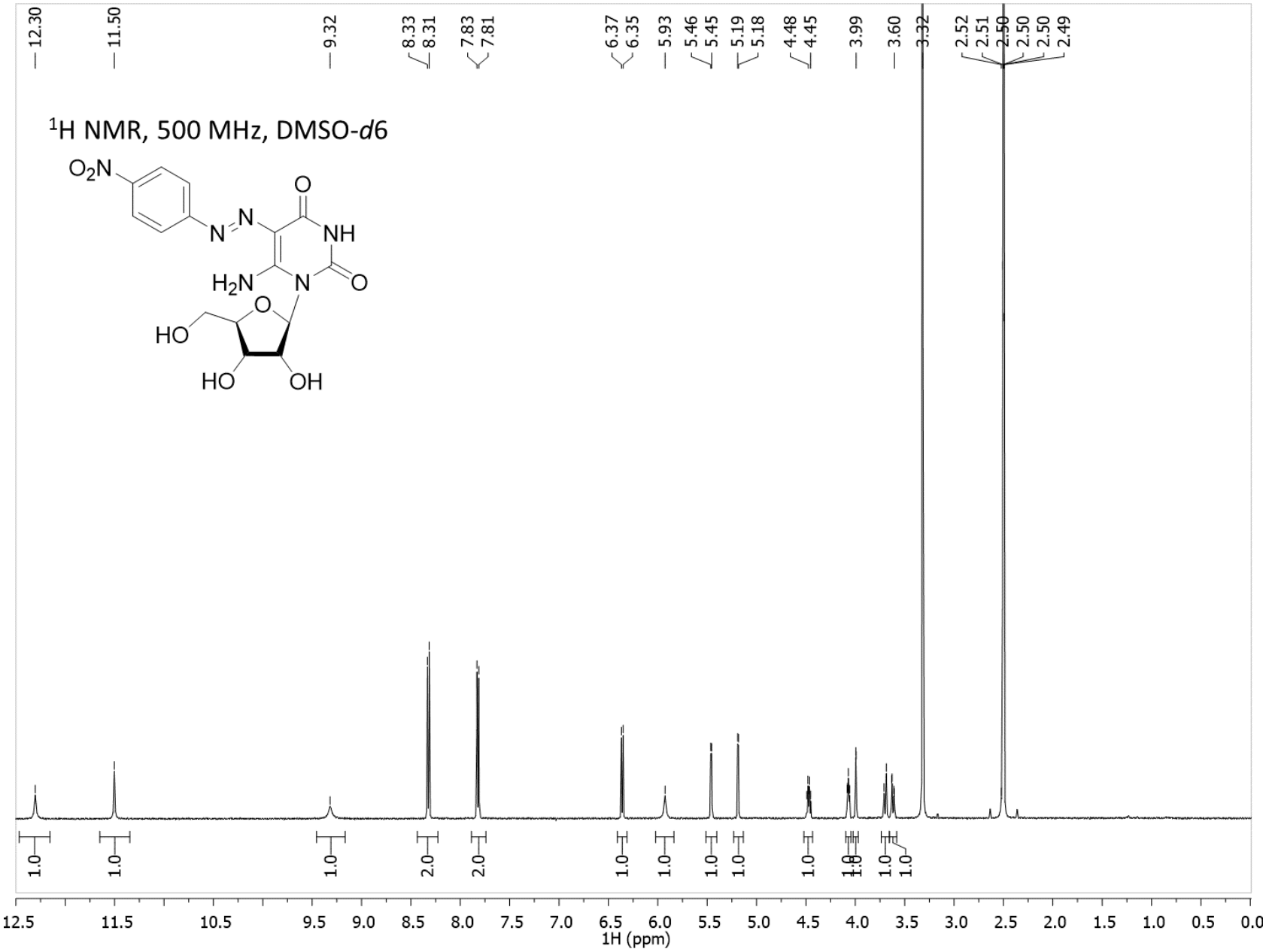


Figure S24. ^{1}H NMR of 6-amino-5-(4-nitrophenylazo) uridine (**5**)

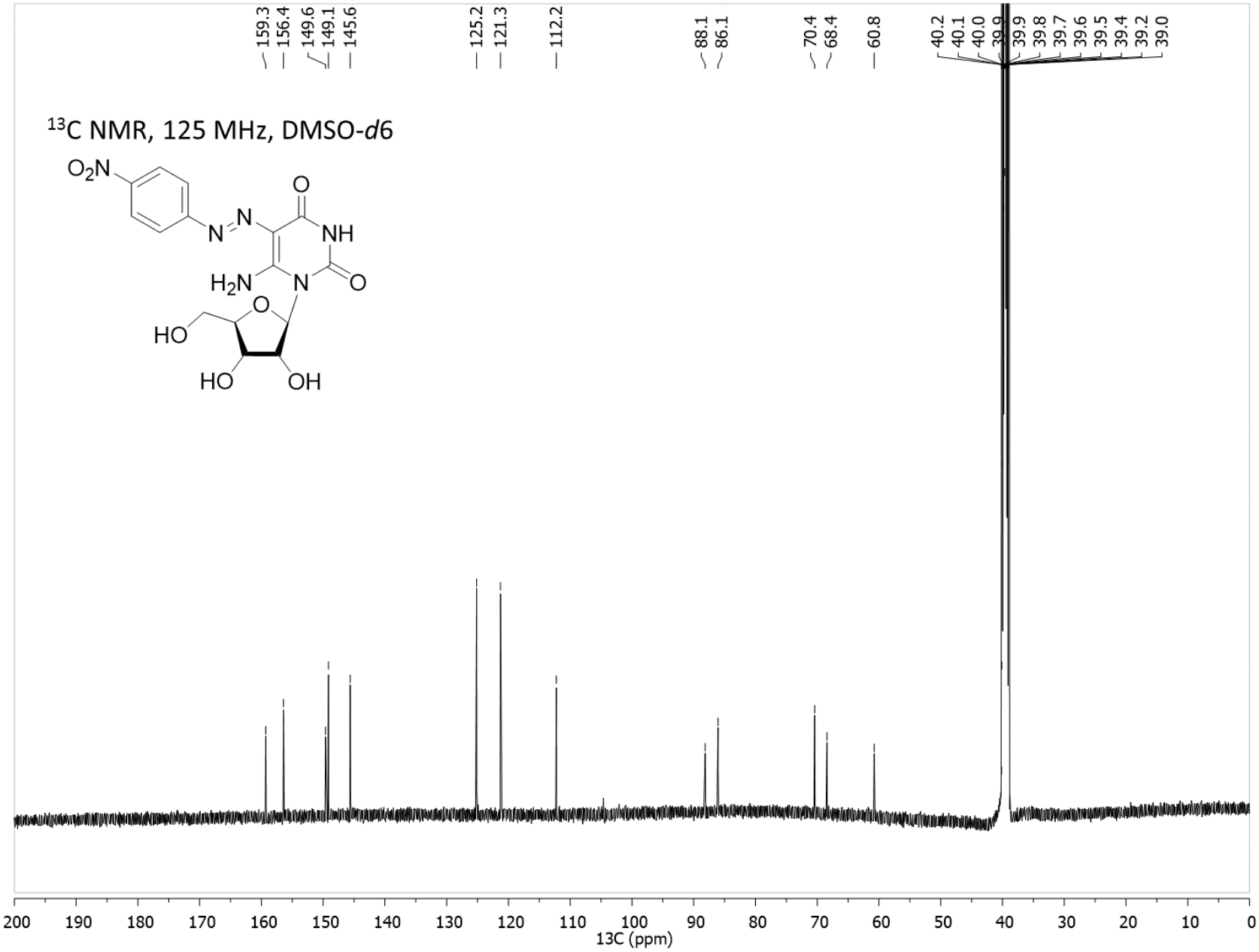


Figure S25. ^{13}C NMR of 6-amino-5-(4-nitrophenylazo) uridine (**5**)

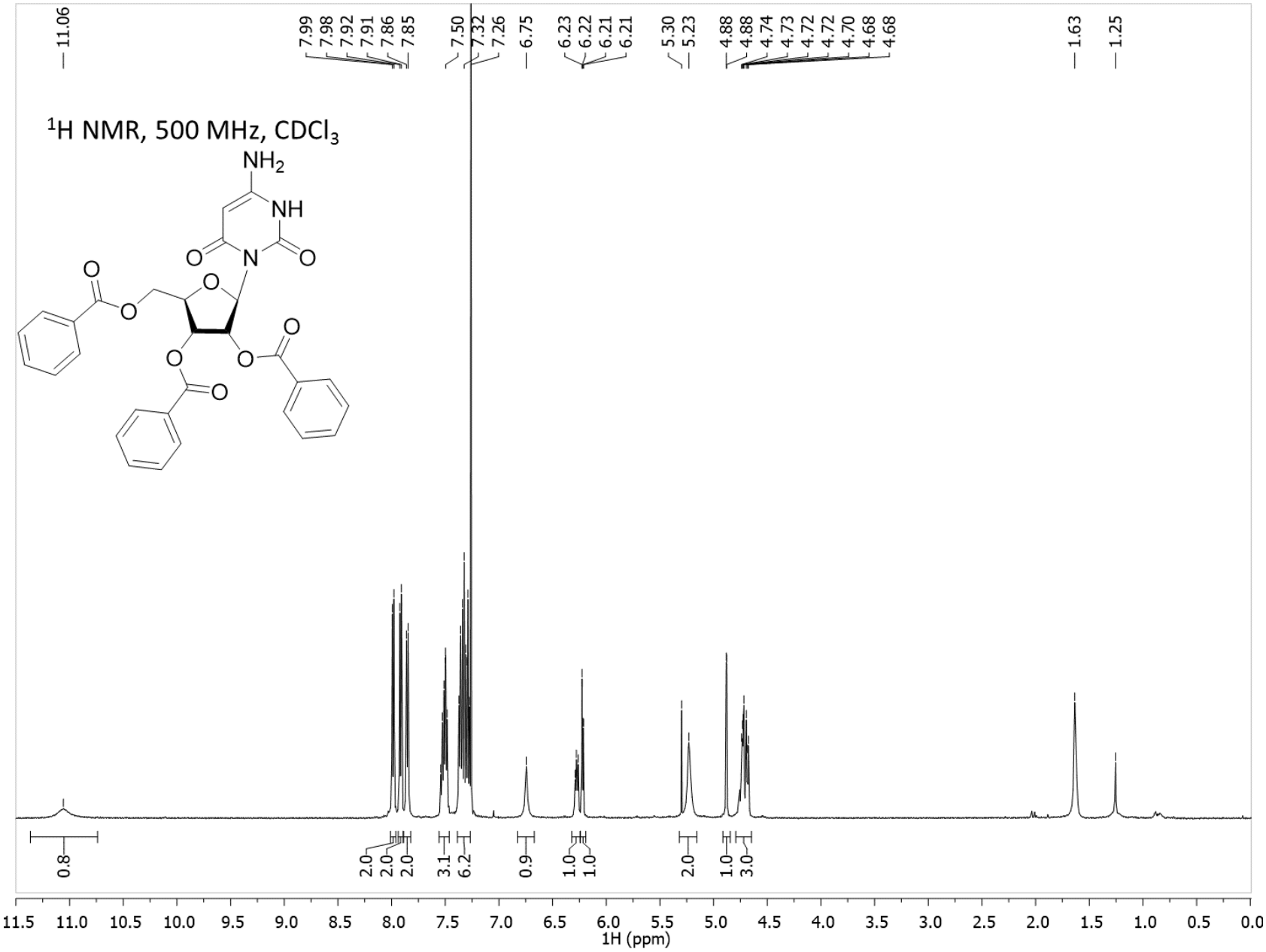


Figure S26. ^{1}H NMR of 2',3',5'-tri-O-benzoyl 6-oxocytidine (**6**).

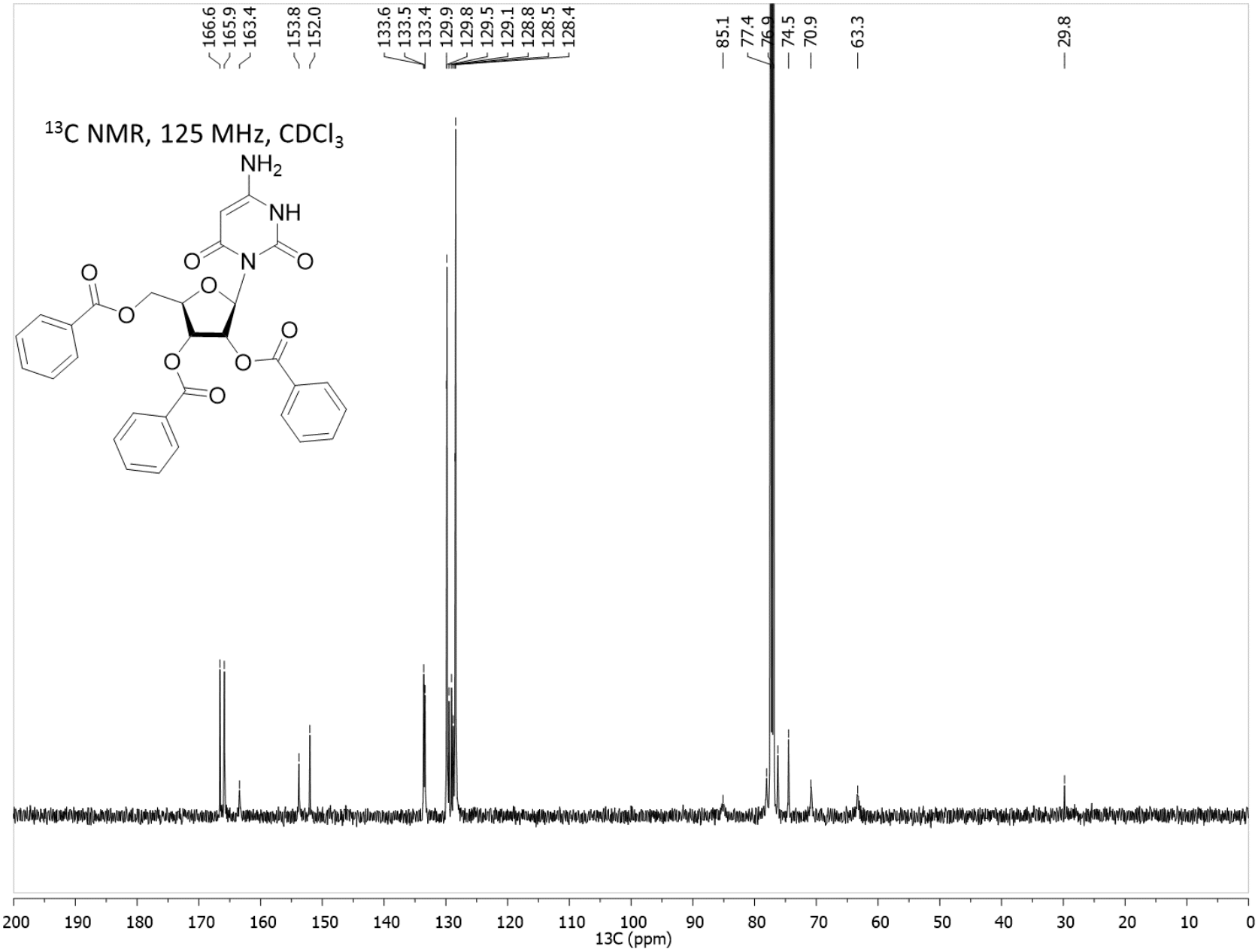


Figure S27. ^{13}C NMR of 2',3',5'-tri-O-benzoyl 6-oxocytidine (**6**).

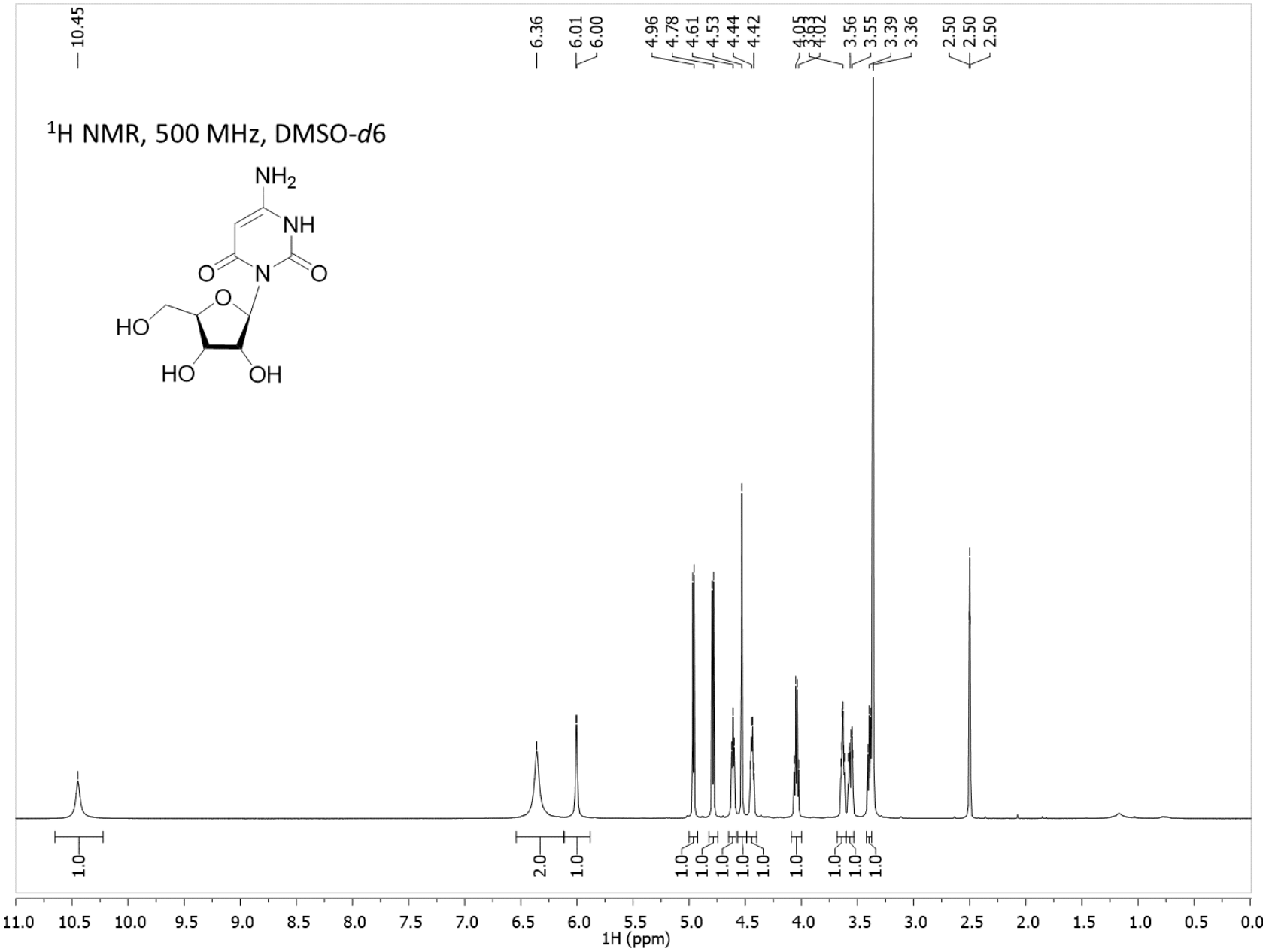


Figure S28. ^{1}H NMR of 6-oxocytidine (**7**)

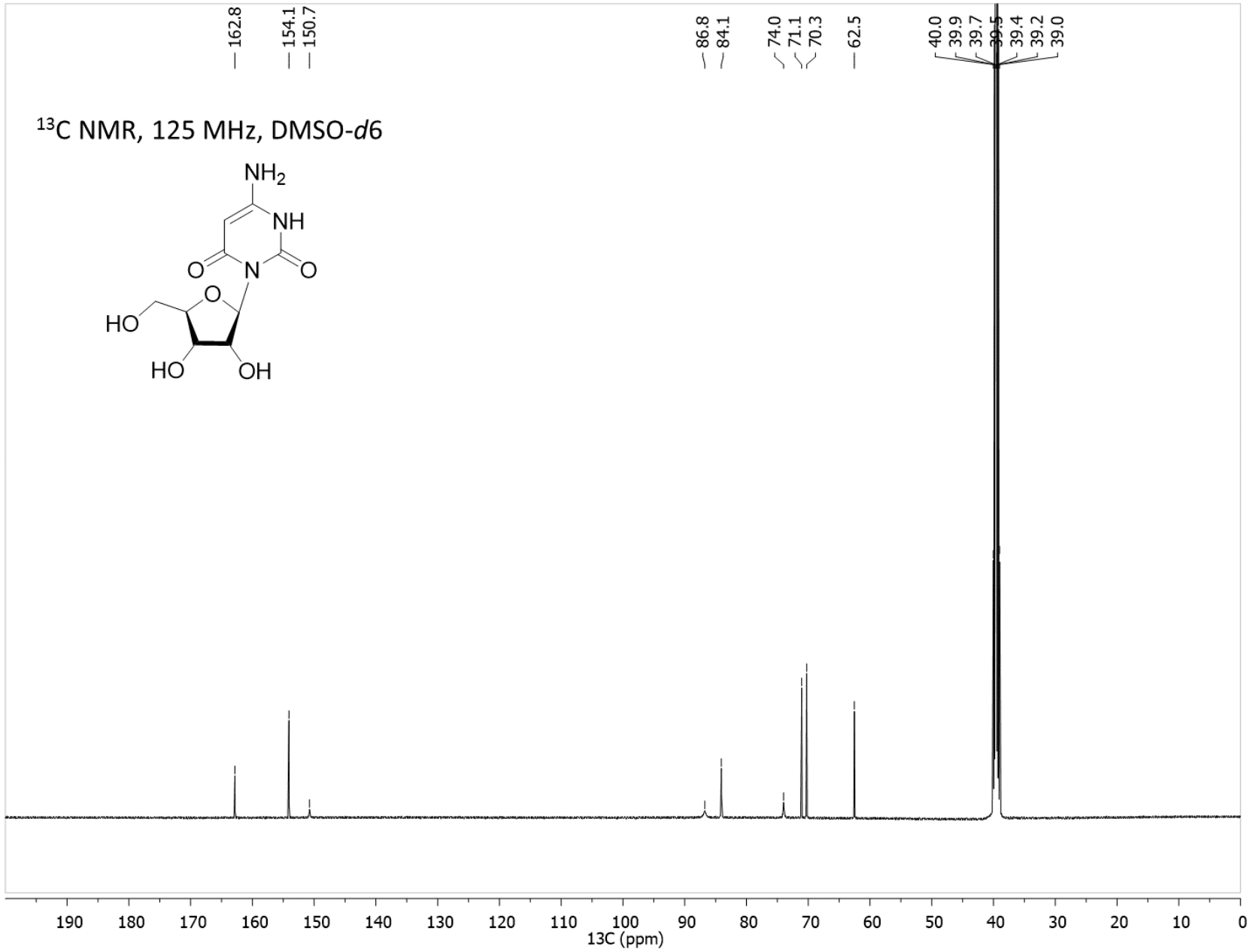


Figure S29. ^{13}C NMR of 6-oxocytidine (**7**)

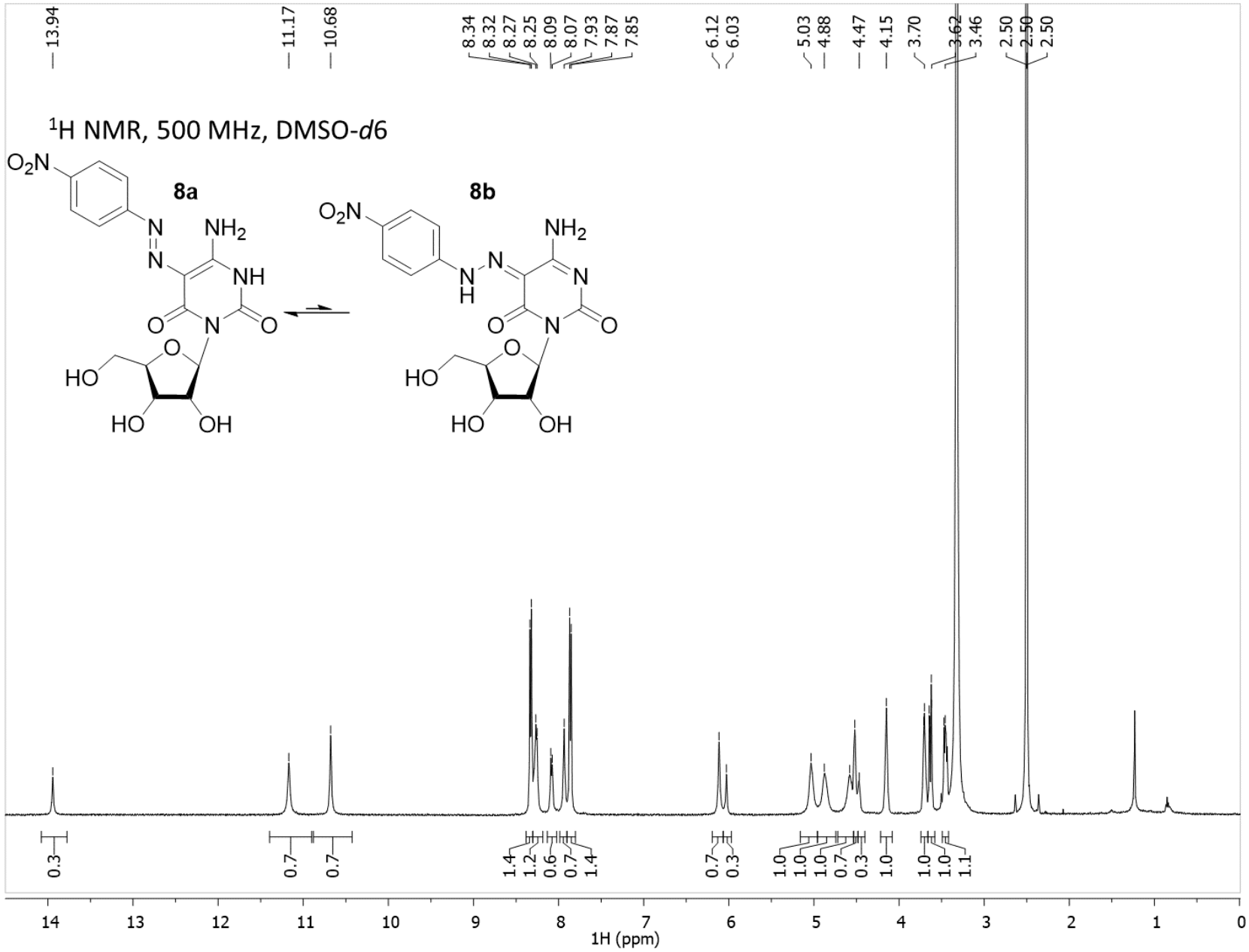


Figure S30. ^{1}H NMR of 5-(4-nitrophenylazo)-6-oxocytidine (**8**)

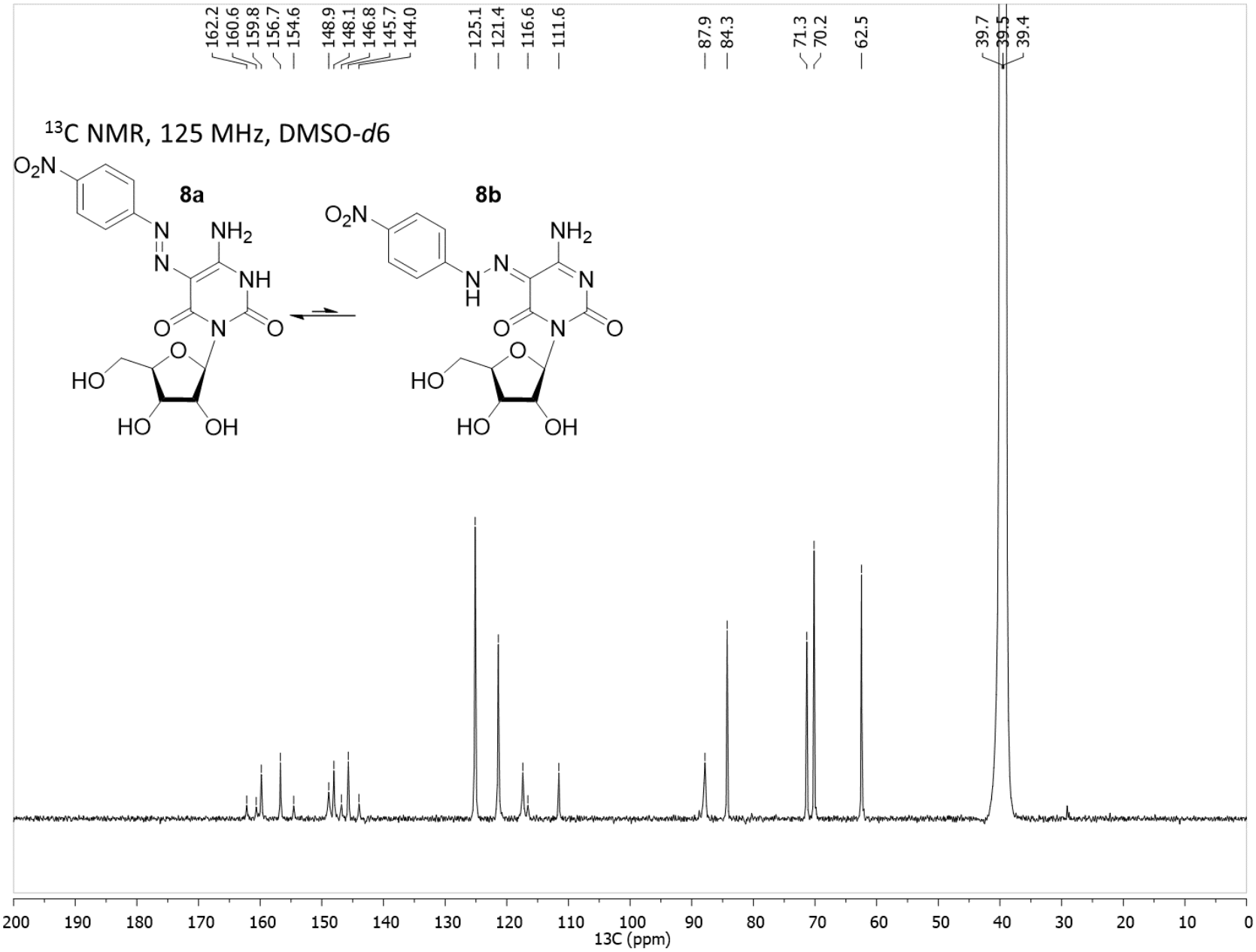


Figure S31. ^{13}C NMR of 5-(4-nitrophenylazo)-6-oxocytidine (**8**)

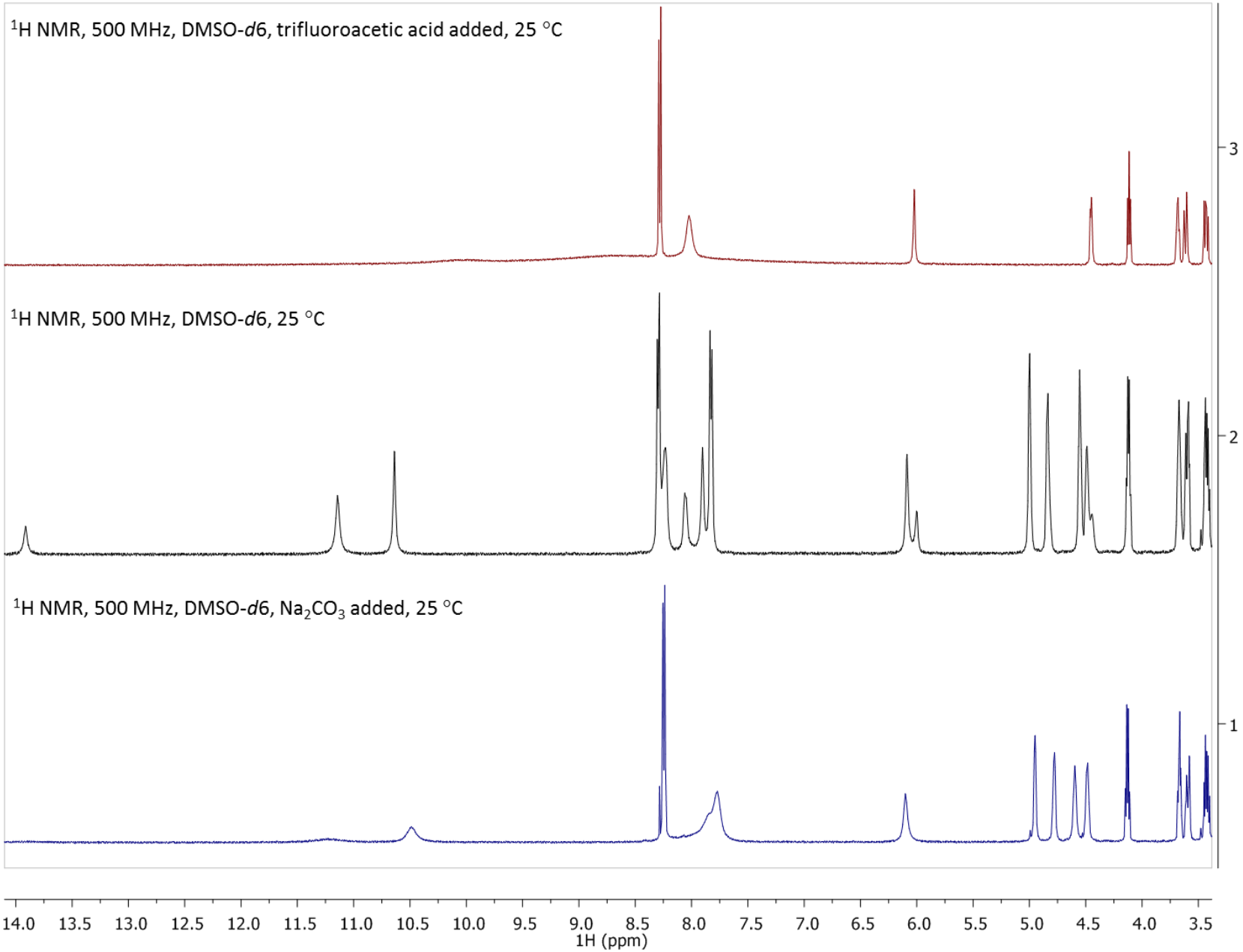


Figure S32. ^{1}H NMR study of 5-(4-nitrophenylazo)-6-oxocytidine (**8**), 25 °C

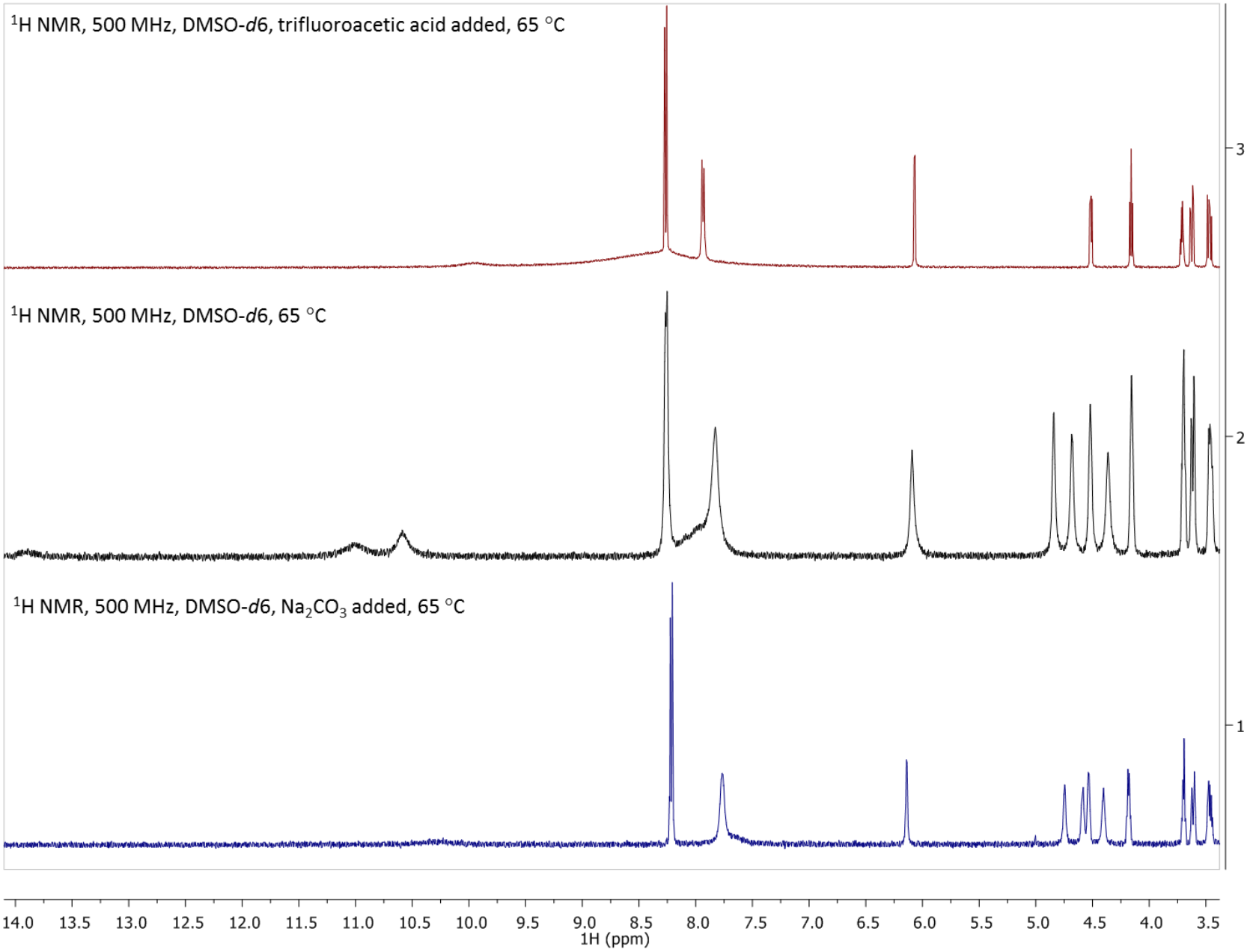


Figure S33. ^{1}H NMR study of 5-(4-nitrophenylazo)-6-oxocytidine (**8**), 65 °C