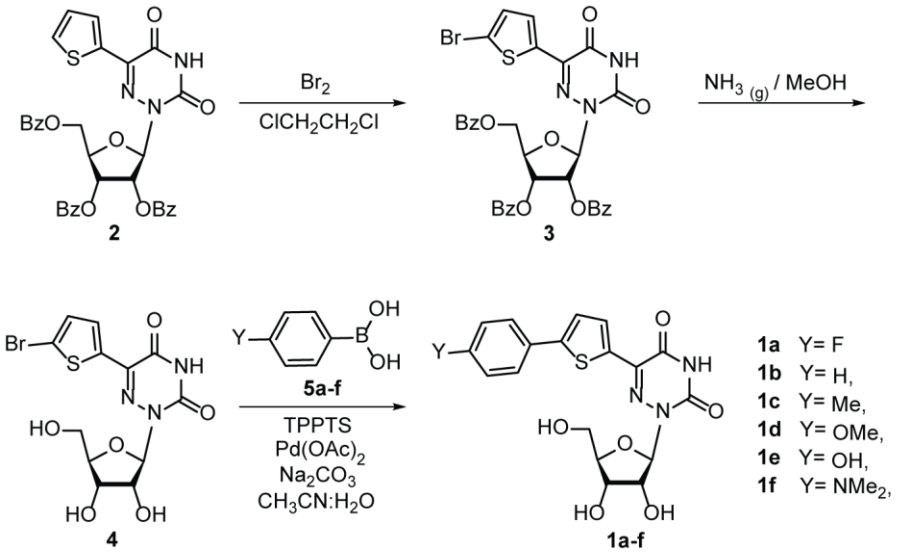
Visibly Emissive and Responsive Extended 6-Aza-Uridines

Patrycja A. Hopkins, Renatus W. Sinkeldam, Yitzhak Tor*

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, USA. * Correspondence to: ytor@ucsd.edu

Table of Contents

S1. Synthesis

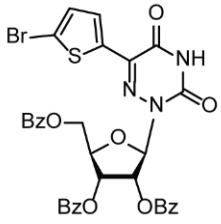


Scheme S1. Synthesis of nucleosides **1a-f.**

S1.1. Synthesis: General

Palladium acetate, (4-(dimethylamino)phenyl)boronic acid, 1,2-dichloroethane and dry acetonitrile were purchased from Sigma-Aldrich. Sodium thiosulphate was purchased from Amresco. Ammonium chloride was purchased from Mallinckrodt. Sodium sulfate, sodium carbonate and all solvents were purchased from Fisher. Tris(3-sulfophenyl)phosphine trisodium salt was purchased from Alfa-Aesar. (4-Methoxyphenyl)boronic acid was purchased from Frontier Scientific. (4-hydroxyphenyl)boronic, (4-methylphenyl)boronic, phenylboronic and (4-fluorophenyl)boronic were purchased from Combi-Blocks. All reagents and solvents were used without further purification. Moisture and oxygen sensitive reactions were performed in an inert argon atmosphere. 5-(thiophene-2-yl)-6-aza-2',3',5'-tribenzoyl-uridine **2** synthesized by previously published method in our laboratory.[1]

S1.2. Synthesis

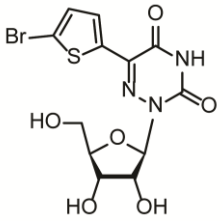


Synthesis of 5-(5-bromothiophene-2-yl)-6-aza-2',3',5'-tribenzoyl-uridine (3). 5-(thiophene-2-yl)-6-aza-2',3',5'-tribenzoyl-uridine **2** (1.1 g, 1.72 mmol) was dissolved in 1,2-dichloroethane (22 mL) at rt. Bromine (0.18 mL, 3.51 mmol) was then added and the reaction mixture left to stir at rt. After 1h, saturated aqueous solution of sodium thiosulphate (0.5 mL) was added and mixed until the red color changed to yellow. The reaction mixture was washed with water (3 × 100 mL). The combined organic layers were dried over Na_2SO_4 filtered over a glass frit and concentrated to dryness. Purification of the crude residue by column chromatography (silica, 7 *v*% DCM in EtOAc) yielded **3** as an almost white foam (1.18 g, 1.64 mmol, 95%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.65 (br s, 1H), 8.05 – 7.73 (m, 7H), 7.72 – 7.20 (m, 10H) 6.49 (s, 1H), 6.10 – 5.95 (m, 2H), 4.90 – 4.75 (m, 1H), 4.68 (dd, *J* = 12.2, 3.4 Hz, 1H), 4.56 (dd, *J* = 12.2, 4.6 Hz, 1H);

^{13}C NMR (125 MHz, DMSO-d_6) δ 165.85, 165.14, 165.01, 155.85, 148.21, 137.93, 135.56, 134.44, 134.29, 133.93, 131.46, 129.92, 129.82, 129.76, 129.60, 129.46, 129.30, 129.15, 128.97, 128.92, 117.13, 88.11, 78.79, 74.36, 71.05, 63.67.

HR-MS-ESI: m/z calcd. for $C_{33}H_{24}BrN_3O_9S$ $(M+Na)^+$: 740.0309, found 740.0307.

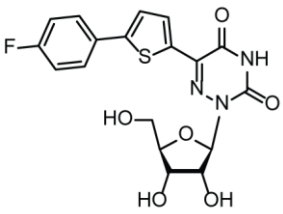


5-(5-bromothiophen-2-yl)-6-aza-uridine (4). A solution of **3** (1.14 g, 1.58 mmol) in saturated methanolic ammonia (10 mL) was heated at 60 °C in a pressure vessel for 24h. The mixture was cooled to rt, concentrated and the residue was triturated with DCM (3 × 60 mL). Purification of the crude residue by recrystallization from water/methanol (60 mL/20 mL) yielded **4** as a white solid (495.5 mg, 1.22 mmol, 77%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.50 (br s, 1H), 7.75-7.67 (m, 1H), 7.35-7.25 (m, 1H), 5.98 (d, *J* = 2.0 Hz, 1H), 5.32 (d, *J* = 4.7 Hz, 1H), 5.09 (d, *J* = 6.1 Hz, 1H), 4.65 (t, *J* = 5.6 Hz, 1H), 4.28 – 4.12 (m, 2H), 3.88 – 3.78 (m, 1H), 3.64 – 3.54 (m, 1H), 3.49 – 3.38 (m, 1H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 155.69, 148.53, 137.10, 136.47, 131.56, 129.71, 116.23, 89.99, 85.14, 73.72, 70.80, 62.36.

HR-MS-ESI: m/z calcd. for $C_{12}H_{12}BrN_3O_6S$ $(M-H)^-$: 403.9557, found: 403.9558.



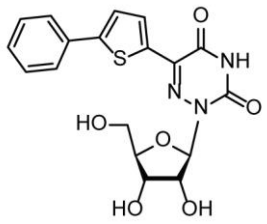
Synthesis of 5-(5-(4-fluorophenyl)thiophen-2-yl)-6-aza-uridine (1a). All solvents were purged with argon. Under argon, sodium carbonate (400 mg, 3.77 mmol) was dissolved in water (6 mL). Then **4** (500 mg, 1.23 mmol), (4-fluorophenyl)boronic acid **5a** (258.3 mg, 1.85 mmol) and dry acetonitrile were added to the solution and additionally purged with argon for 15 minutes. Tris(3-sulfophenyl)phosphine trisodium salt (175 mg, 0.31 mmol) and palladium acetate (13.8 mg, 0.06 mmol) were added and the reaction mixture was heated and kept at 60 °C for 18h. The mixture was cooled to rt, neutralized with saturated aqueous solution of ammonium chloride and diluted with methanol (100 mL). A black precipitate was filtrate off, and remaining filtrate was concentrated to dryness. The residue was triturated with water (3 × 60 mL). Purification of the crude solid by recrystallization from methanol (50 mL) yielded **1a** as a bright yellow solid (337.0 mg, 0.80 mmol, 65%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.48 (br s, 1H), 7.96 (d, *J* = 4.0 Hz, 1H), 7.80 – 7.72 (m, 2H), 7.54 (d, *J* = 4.0 Hz, 1H), 7.34 – 7.26 (m, 2H), 6.00 (d, *J* = 3.0 Hz, 1H), 5.36 (d, *J* = 4.5 Hz, 1H), 5.13 (d, *J* = 6.0 Hz, 1H), 4.70 (t, *J* = 6.0 Hz, 1H), 4.32 – 4.19 (m, 2H), 3.87 –.3.81 (m, 1H), 3.66 – 3.59 (m, 1H), 3.51 – 3.43 (m, 1H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 163.47, 161.51, 155.72, 148.61, 145.29, 137.72, 134.32, 130.79, 130.20, 130.18, 128.18, 128.12, 124.97, 116.77, 116.60, 90.03, 85.24, 73.63, 70.95, 62.56.

^{19}F NMR (280 MHz, DMSO-d_6) δ 116.78

HR-MS-ESI: m/z calcd. for $C_{18}H_{16}FN_3O_6S$ $(M-H)^-$: 420.0671, found: 420.0673

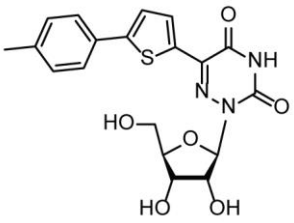


Synthesis of 5-(5-phenylthiophen-2-yl)-6-aza-uridine (1b).
Using the procedure described for **1a**, but starting from **5b, 1b** was obtained as a bright yellow solid (335.2 mg, 0.83 mmol, 68%).

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 12.46 (br s, 1H), 7.97 (d, *J* = 4.0 Hz, 1H), 7.80 – 7.64 (m, 2H), 7.57 (d, *J* = 4.0 Hz, 1H), 7.51 – 7.40 (m, 2H), 7.40 – 7.27 (m, 1H), 6.00 (d, *J* = 3.2 Hz, 1H), 5.33 (d, *J* = 5.2 Hz, 1H), 5.11 (d, *J* = 6.0 Hz, 1H), 4.68 (t, *J* = 6.0 Hz, 1H), 4.36 – 4.16 (m, 2H), 3.90 –.3.78 (m, 1H), 3.70 – 3.56 (m, 1H), 3.54 – 3.42 (m, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$) δ 155.73, 148.61, 146.45, 137.77, 134.32, 133.51, 130.81, 129.73, 128.90, 126.01, 124.85, 90.06, 85.24, 73.65, 70.97, 62.58.

HR-MS-ESI: m/z calcd. for $C_{18}H_{17}N_3O_6S$ $(M-H)^-$: 402.0765, found: 402.0767.

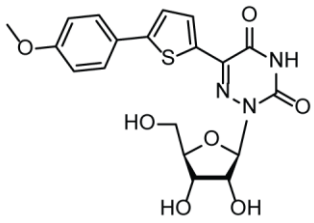


Synthesis of 5-(5-(4-methyl)phenyl)thiophen-2-yl)-6-aza-uridine (1c).
Using the procedure described for **1a**, but starting from **5c, 1c** was obtained as a a bright yellow solid (354.5 mg, 0.85 mmol, 69%).

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 12.46 (br s, 1H), 7.95 (d, *J* = 4.0 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 4.0 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 6.00 (d, *J* = 3.0 Hz, 1H), 5.36 (d, *J* = 4.5 Hz, 1H), 5.14 (d, *J* = 6.0 Hz, 1H), 4.70 (t, *J* = 6.0 Hz, 1H), 4.34 – 4.18 (m, 2H), 3.90 –.3.80 (m, 1H), 3.68 – 3.57 (m, 1H), 3.53 – 3.42 (m, 1H) 2.33 (s, 3H).

^{13}C NMR (125 MHz, DMSO-d$_6$) δ 155.73, 148.61, 146.67, 138.48, 137.80, 133.74, 130.85, 130.78, 130.24, 125.90, 124.25, 90.01, 85.24, 73.62, 70.97, 62.59, 21.27.

HR-MS-ESI: m/z calcd. for $C_{19}H_{19}N_3O_6S$ $(M-H)^-$ 416.0922, found: 416.0923.

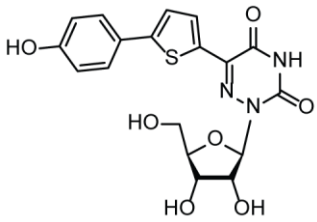


Synthesis of 5-(5-(4-methoxyphenyl)thiophen-2-yl)-6-aza-uridine (1d).
Using the procedure described for **1a**, but starting from **5d, 1d** was obtained as a yellow solid (351.9 mg, 0.81 mmol, 66%).

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 12.45 (br s, 1H), 7.95 (d, *J* = 4.0 Hz, 1H), 7.70 – 7.60 (m, 2H), 7.44 (d, *J* = 4.0 Hz, 1H), 7.05 – 6.97 (m, 2H), 6.01 (d, *J* = 3.0 Hz, 1H), 5.37 (d, *J* = 5.0 Hz, 1H), 5.14 (d, *J* = 6.0 Hz, 1H), 4.71 (t, *J* = 5.5 Hz, 1H), 4.32 – 4.18 (m, 2H), 3.87 –.3.82 (m, 1H), 3.79 (s, 3H), 3.69 – 3.59 (m, 1H), 3.53 – 3.43 (m, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$) δ 159.94, 155.74, 148.62, 146.66, 137.85, 133.22, 130.96, 127.44, 126.19, 123.61, 115.10, 90.02, 85.26, 73.63, 71.01, 62.64, 55.74.

HR-MS-ESI: m/z calcd. for $C_{19}H_{19}N_3O_7S$ $(M-H)^-$ 432.0874, found 432.0874



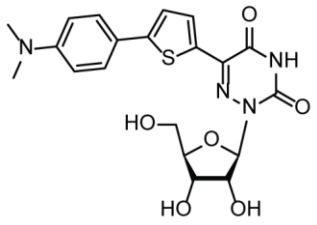
Synthesis of 5-(5-(4-hydroxyphenyl)thiophen-2-yl)-6-aza-uridine (1e).

Using the procedure described for **1a**, but starting from **5e, 1e** was obtained as a off-yellow solid (382.5 mg, 0.91 mmol, 74%).

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 12.42 (br s, 1H), 9.80 (br s, 1H), 7.92 (d, *J* = 4.0 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 4.0 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 2H), 5.99 (d, *J* = 2.8 Hz, 1H), 5.32 (d, *J* = 4.8 Hz, 1H), 5.09 (d, *J* = 6.0 Hz, 1H), 4.68 (t, *J* = 5.6 Hz, 1H), 4.34 – 4.15 (m, 2H), 3.90 –.3.77 (m, 1H), 3.68 – 3.56 (m, 1H), 3.53 – 3.40 (m, 1H).

^{13}C NMR (125 MHz, DMSO-d$_6$) δ 158.41, 155.87, 148.73, 147.23, 137.89, 132.78, 130.99, 127.53, 124.66, 122.98, 116.42, 90.01, 85.23, 73.62, 71.03, 62.68.

HR-MS-ESI: m/z calcd. for $C_{18}H_{17}N_3O_7S$ $(M-H)^-$ 418.0714, found 418.0715.



Synthesis of 5-(5-(4-(dimethylamino)phenyl)thiophen-2-yl)-6-aza-uridine (1f).

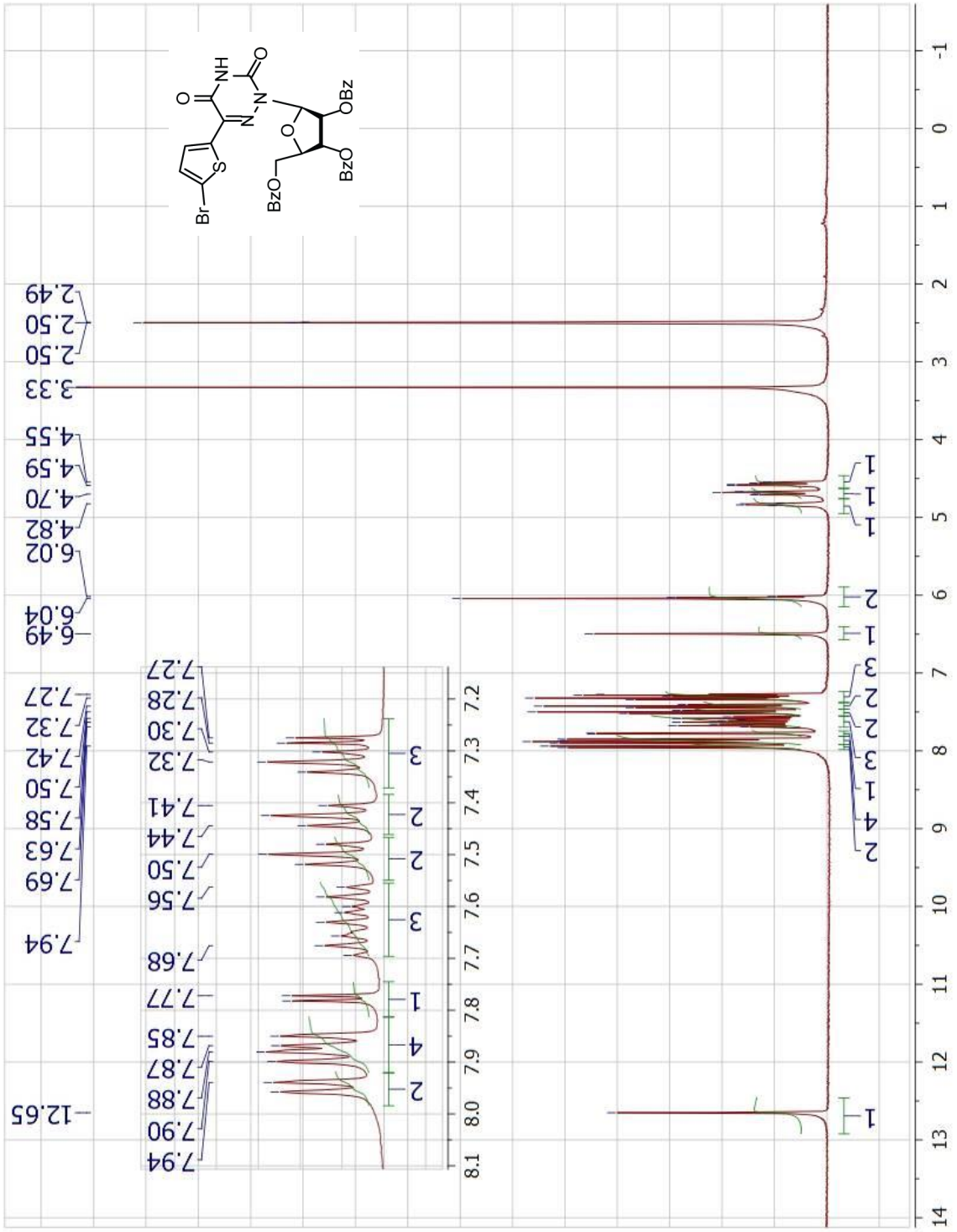
Using the procedure described for **1a**, but starting from **5f, 1f** was obtained as a red solid (269.3 mg, 0.60 mmol, 49%).

^{1}H NMR (500 MHz, DMSO-d$_6$) δ 12.41 (br s, 1H), 7.93 (d, *J* = 4.0 Hz, 1H), 7.57 – 7.49 (m, 2H), 7.33 (d, *J* = 4.0 Hz, 1H), 6.80 – 6.73 (m, 2H), 6.00 (d, *J* = 3.0 Hz, 1H), 5.32 (d, *J* = 5.0 Hz, 1H), 5.10 (d, *J* = 6.5 Hz, 1H), 4.68 (t, *J* = 5.5 Hz, 1H), 4.32 – 4.16 (m, 2H), 3.88 –.3.80 (m, 1H), 3.67 – 3.57 (m, 1H), 3.52 – 3.43 (m, 1H), 2.95 (s, 6H).

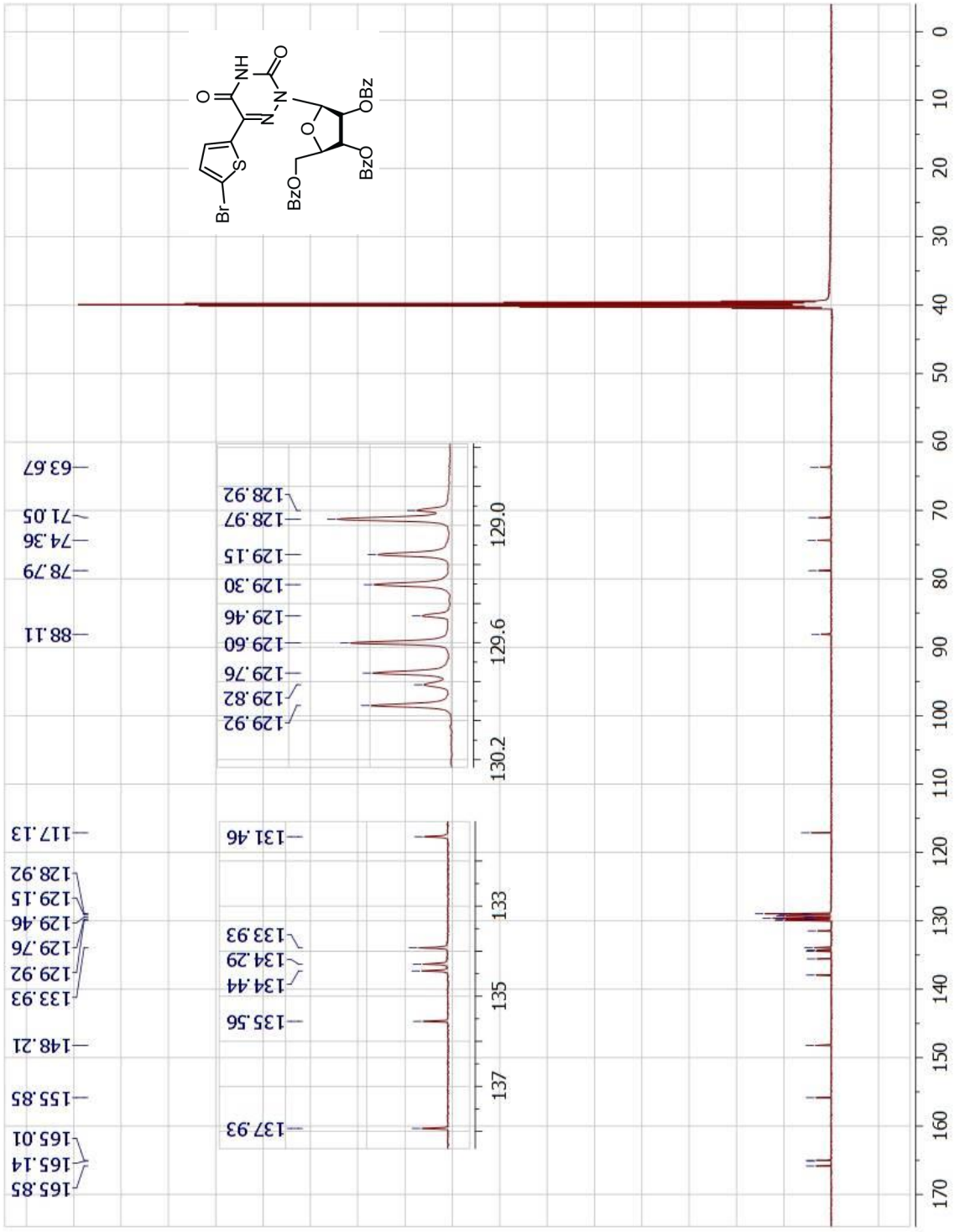
^{13}C NMR (125 MHz, DMSO-d$_6$) δ 156.07, 150.76, 148.93, 147.86, 137.93, 131.91, 131.08, 126.93, 121.92, 121.24, 112.73, 90.01, 85.23, 73.62, 71.07, 62.76, 40.33.

HR-MS-ESI: m/z calcd. for $C_{20}H_{22}N_4O_6S$ $(M-H)^-$ 445.1187, found 445.1190

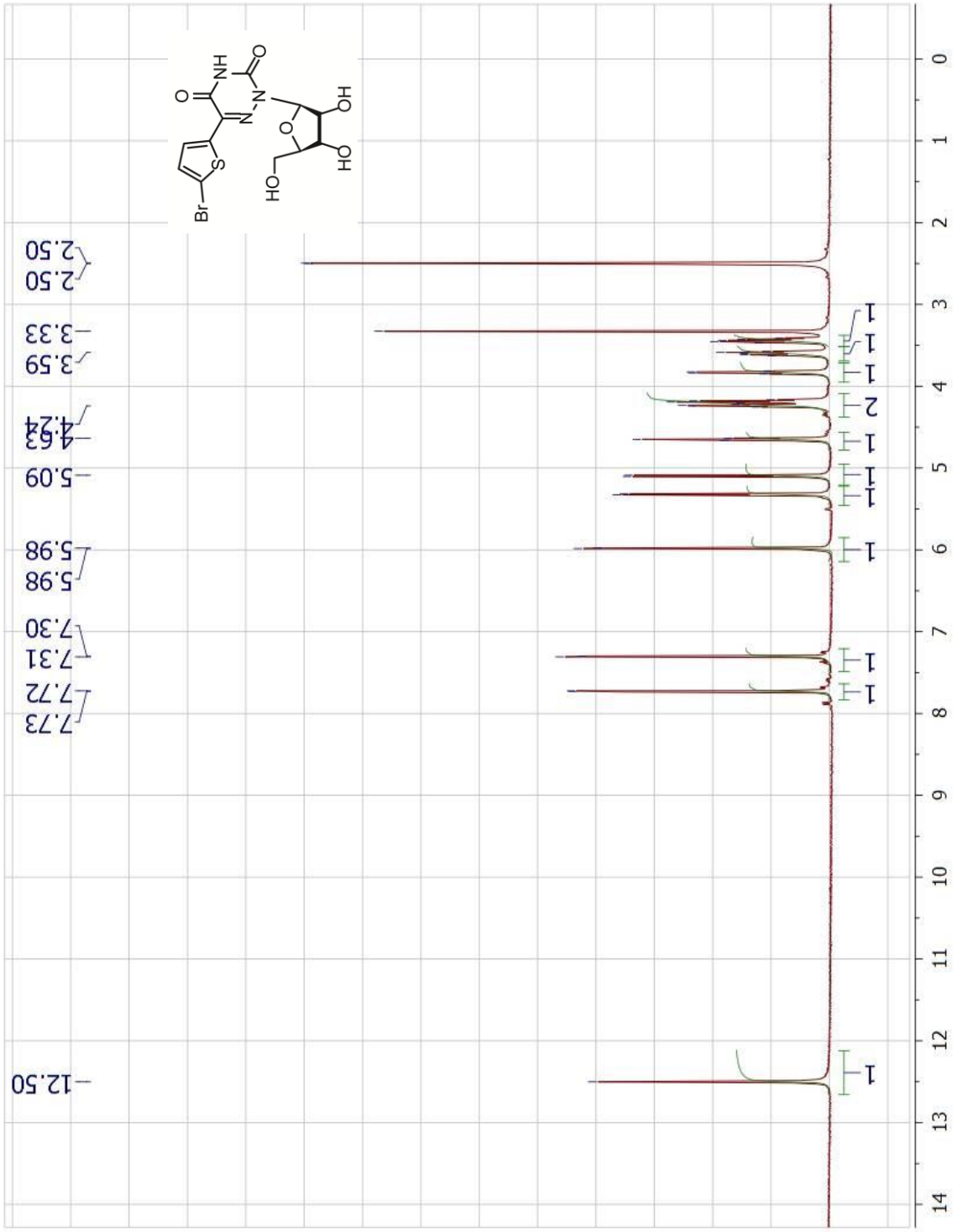
S1.3 ^{1}H-NMR and ^{13}C-NMR spectra of 1a-f



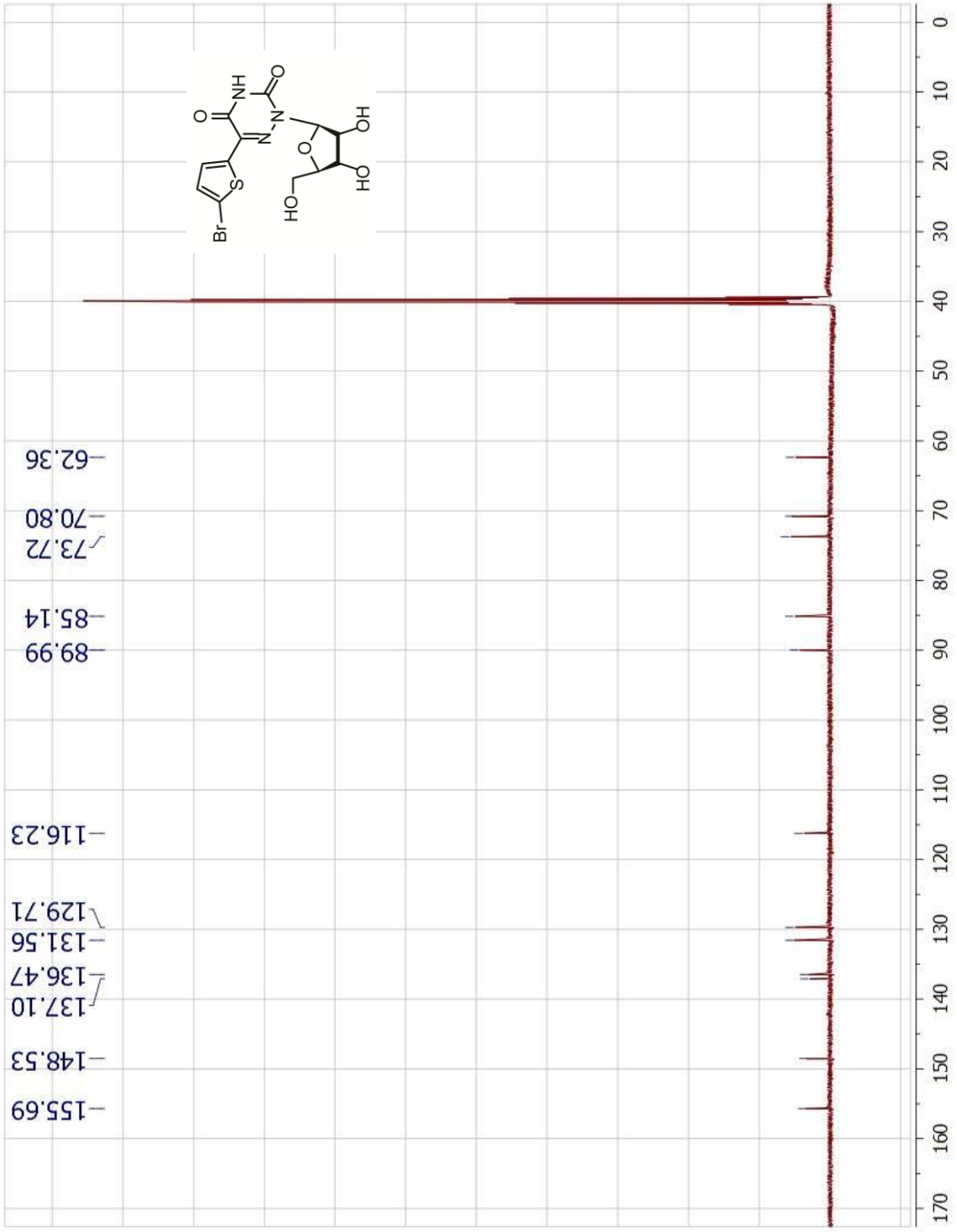
^{1}H-NMR of 5-(5-bromothiophene-2-yl)-6-aza-2,3,5-tribenzoyl-uridine (3).



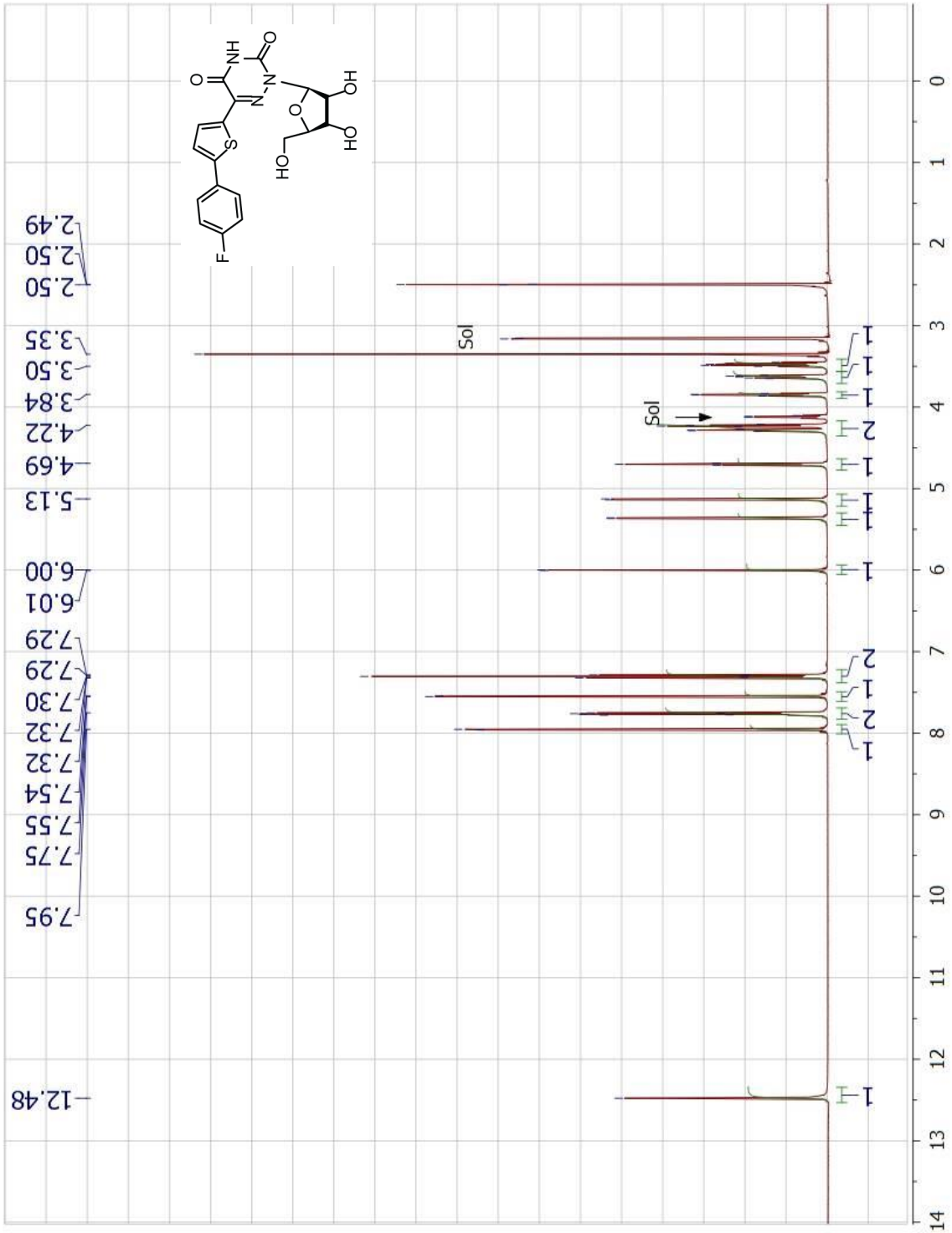
^{13}C-NMR of 5-(5-bromothiophene-2-yl)-6-aza-2,3,5-tribenzoyl-uridine (3).



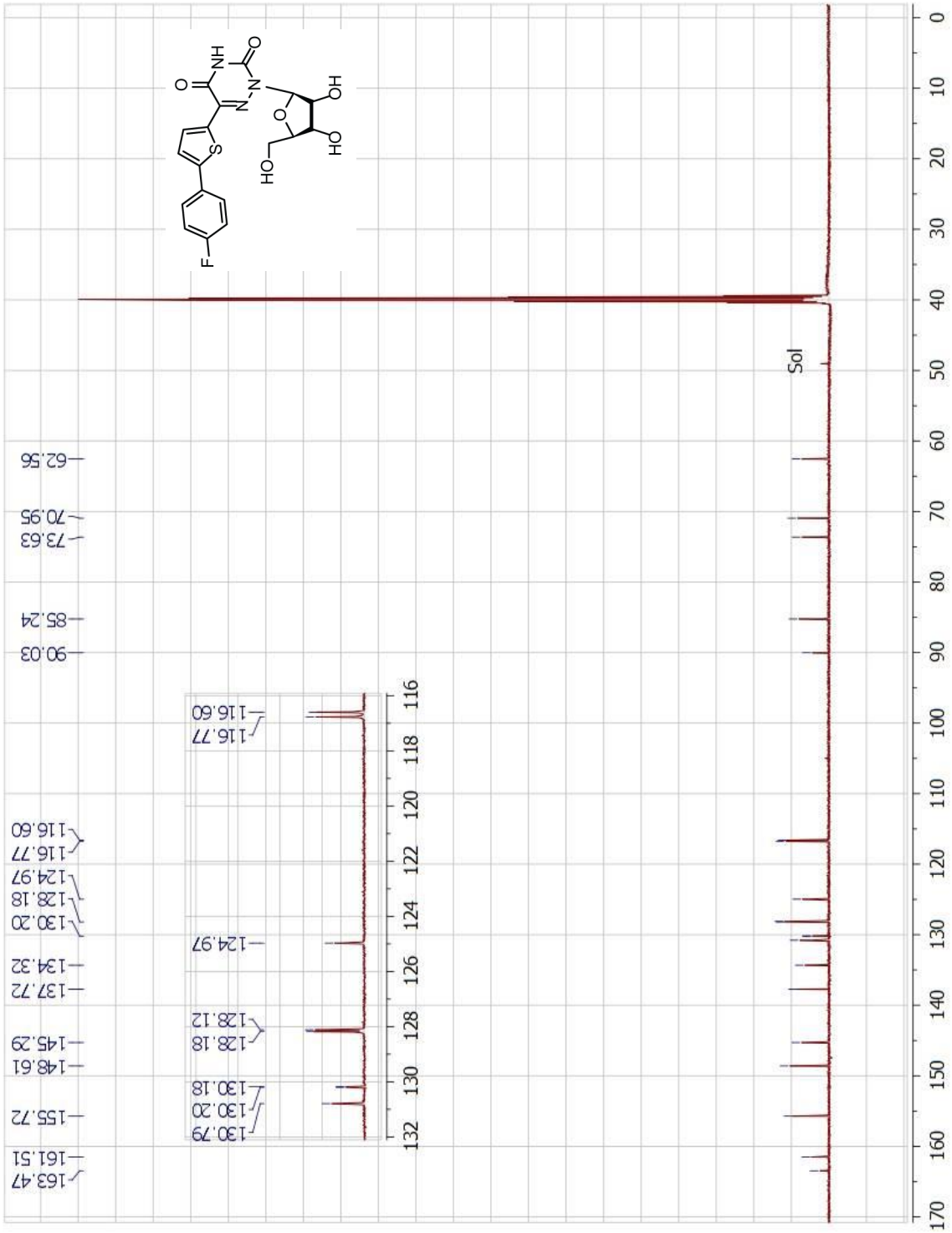
^{1}H-NMR of 5-(5-bromothiophen-2-yl)-6-aza-uridine (4).



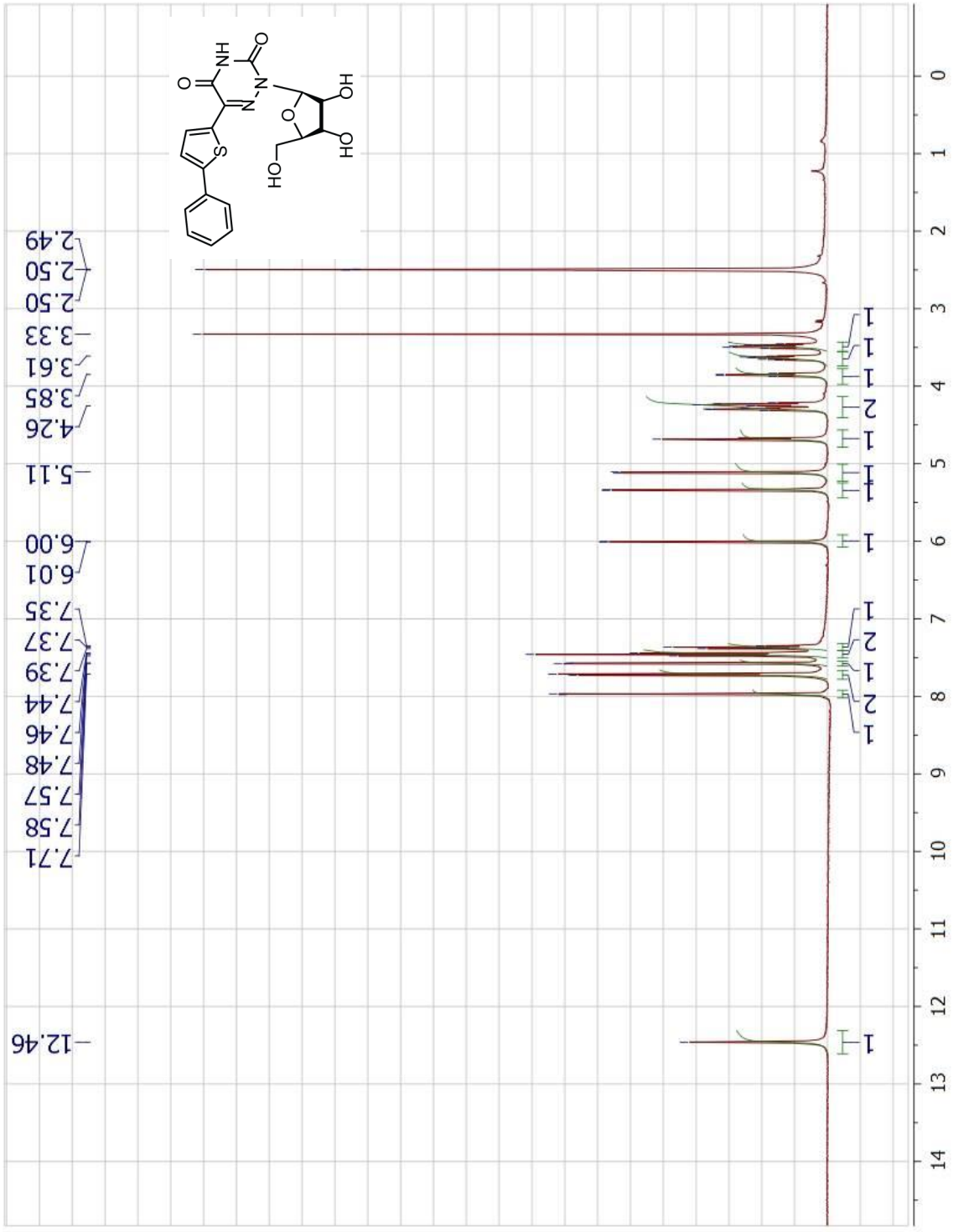
^{13}C-NMR of 5-(5-bromothiophen-2-yl)-6-aza-uridine (4).



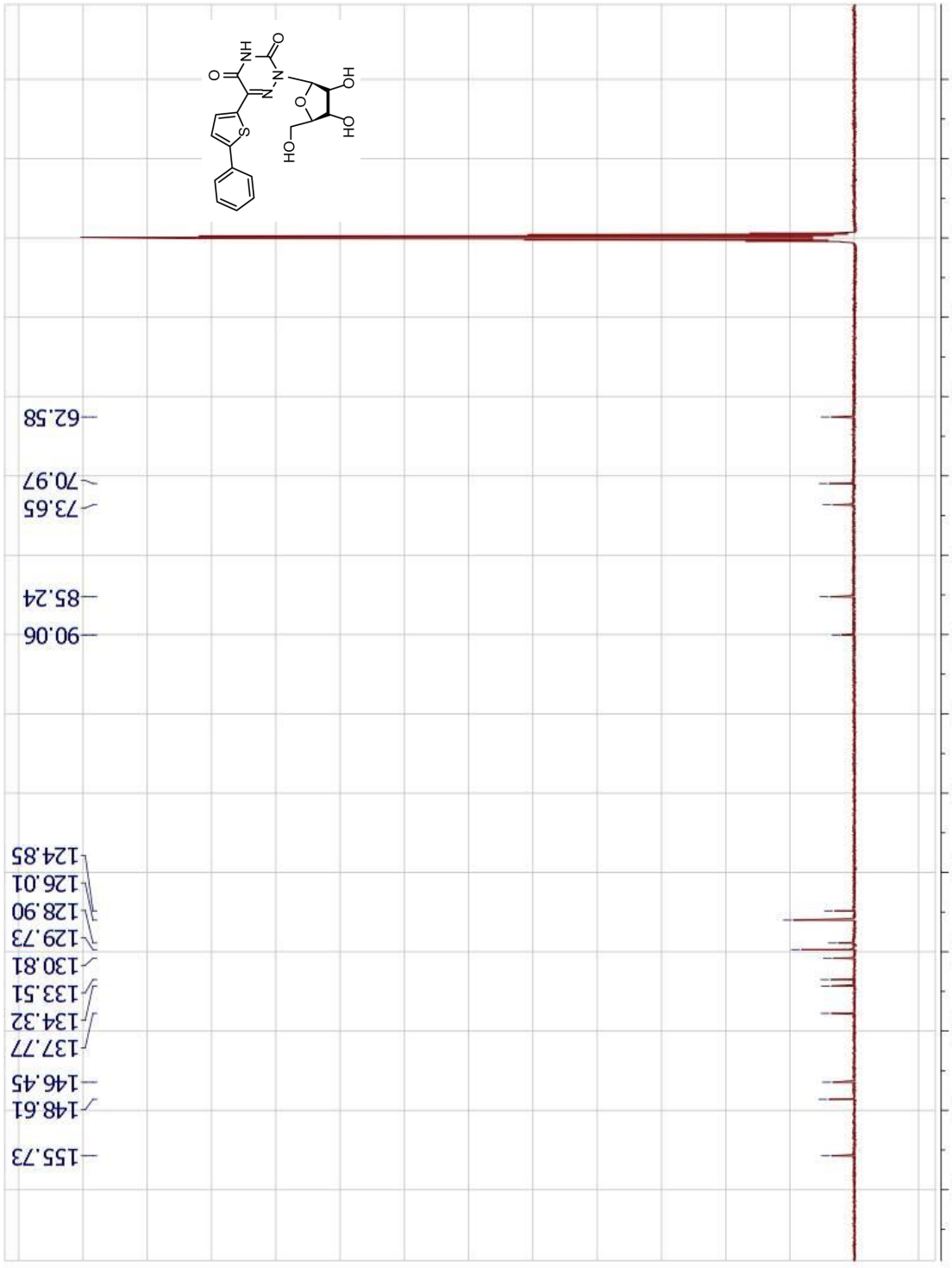
^{1}H-NMR of 5-(5-(4-fluorophenyl)thiophen-2-yl)-6-aza-uridine (1a).



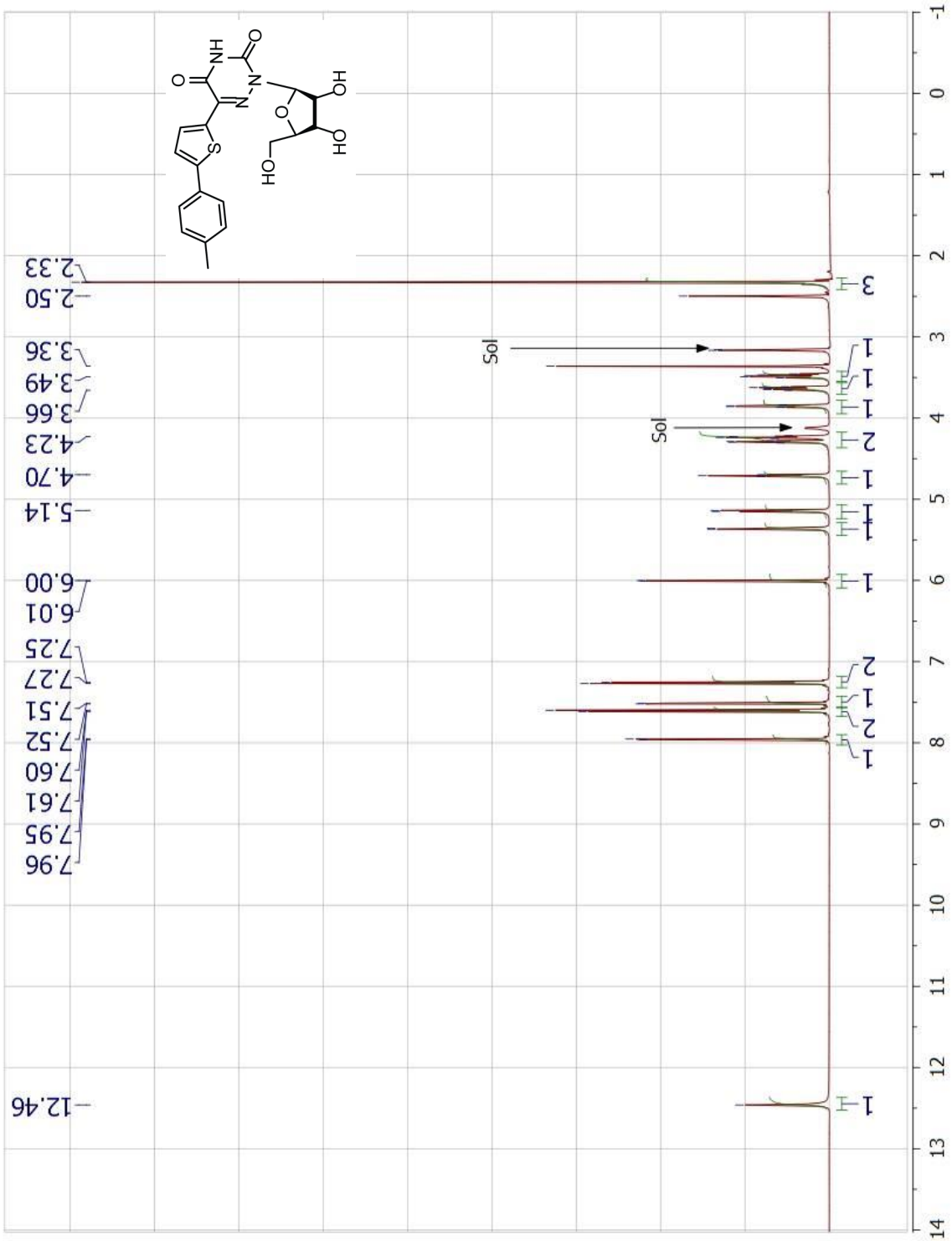
^{13}C-NMR of 5-(5-(4-fluorophenyl)thiophen-2-yl)-6-aza-uridine (1a).



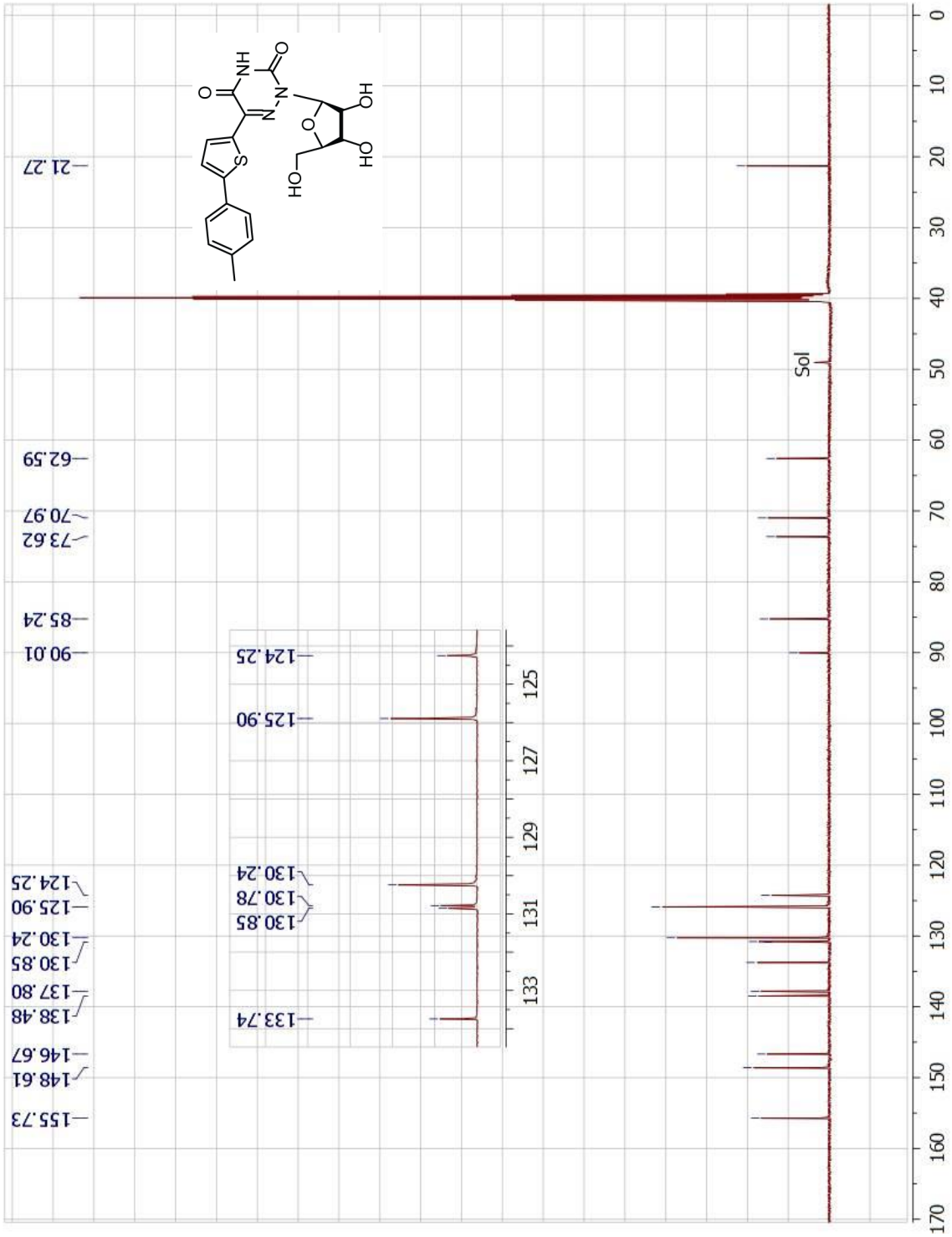
^{1}H-NMR of 5-(5-phenylthiophen-2-yl)-6-aza-uridine (1b).



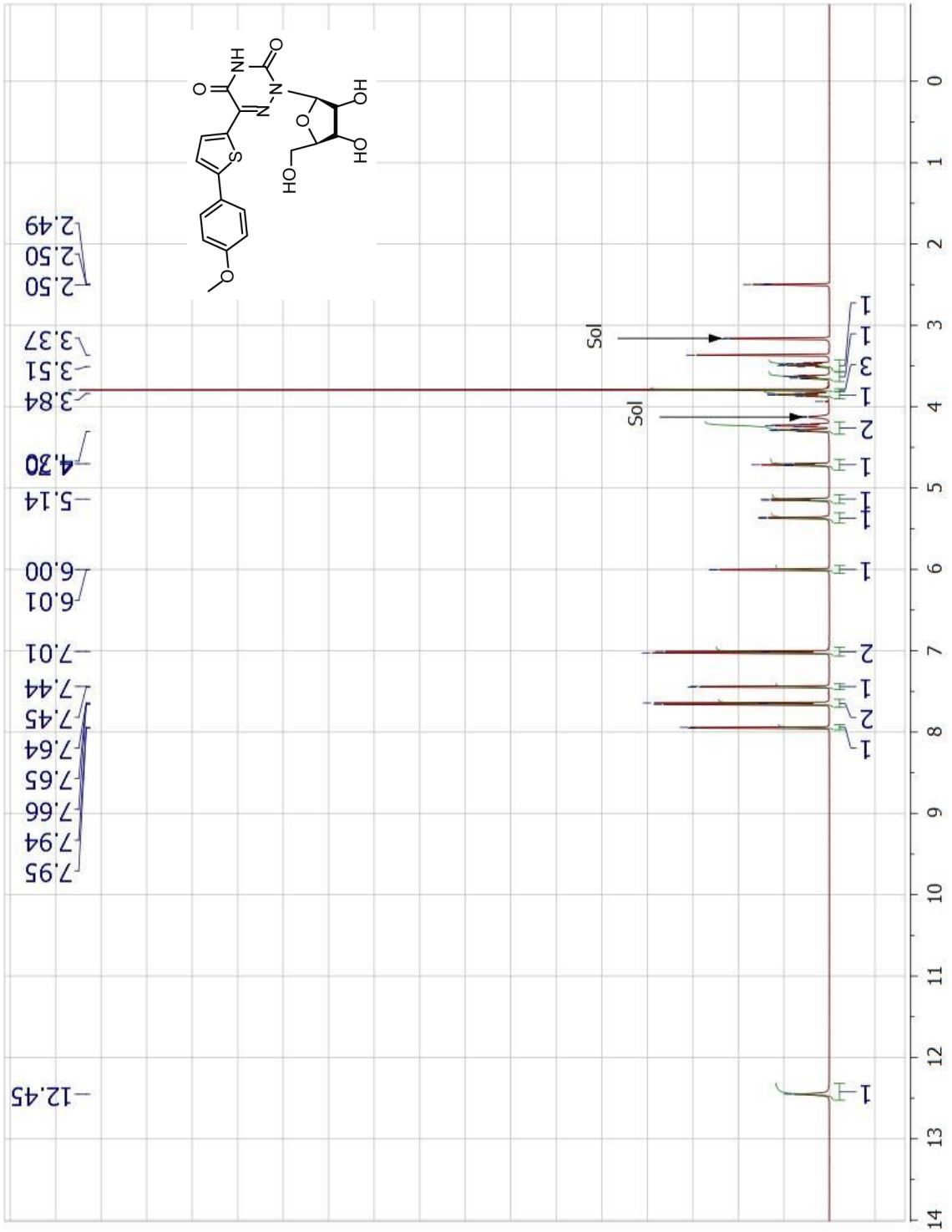
^{13}C-NMR of 5-(5-phenylthiophen-2-yl)-6-aza-uridine (1b).



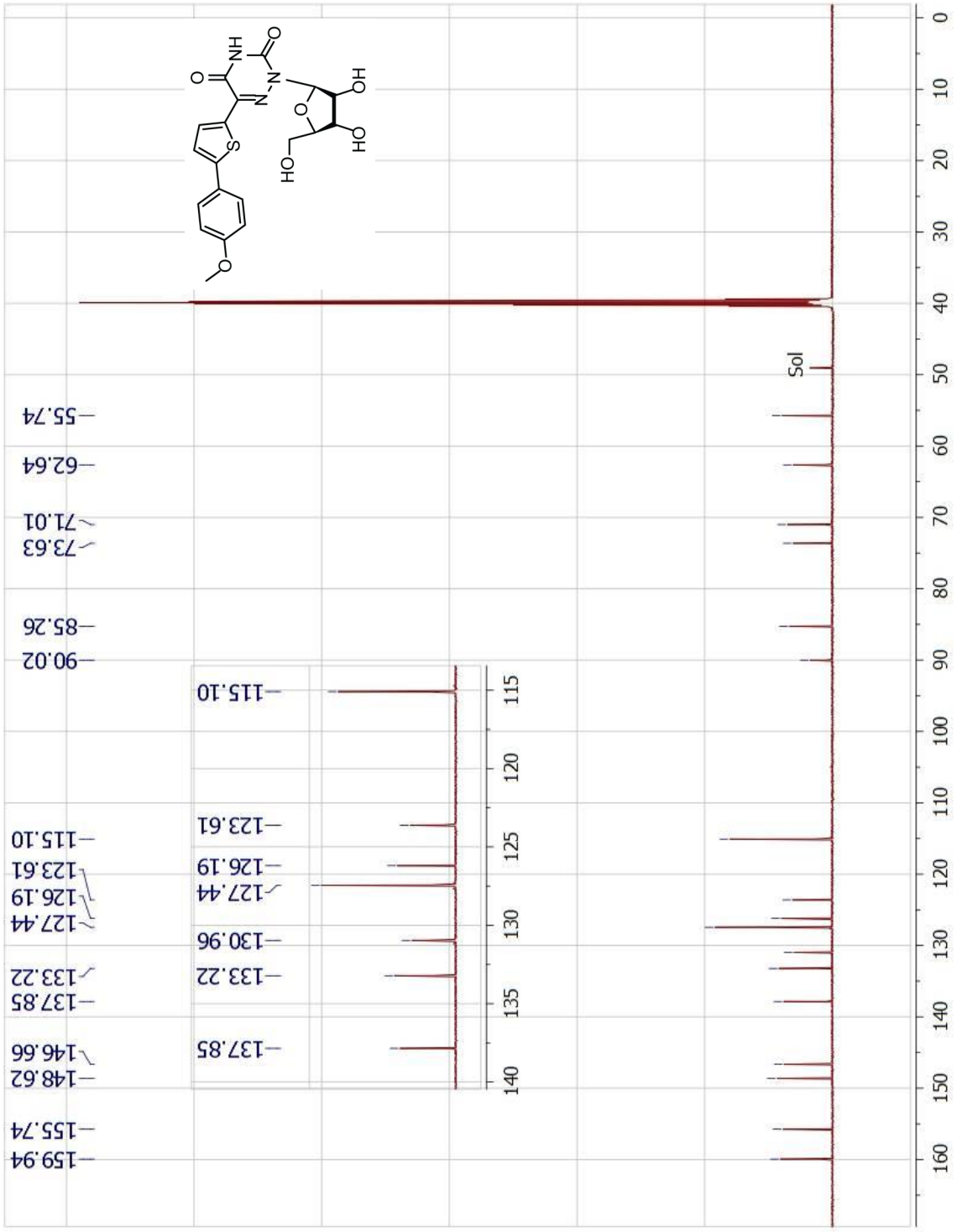
^{1}H-NMR of 5-(5-(4-methyl)phenyl)thiophen-2-yl)-6-aza-uridine (1c).



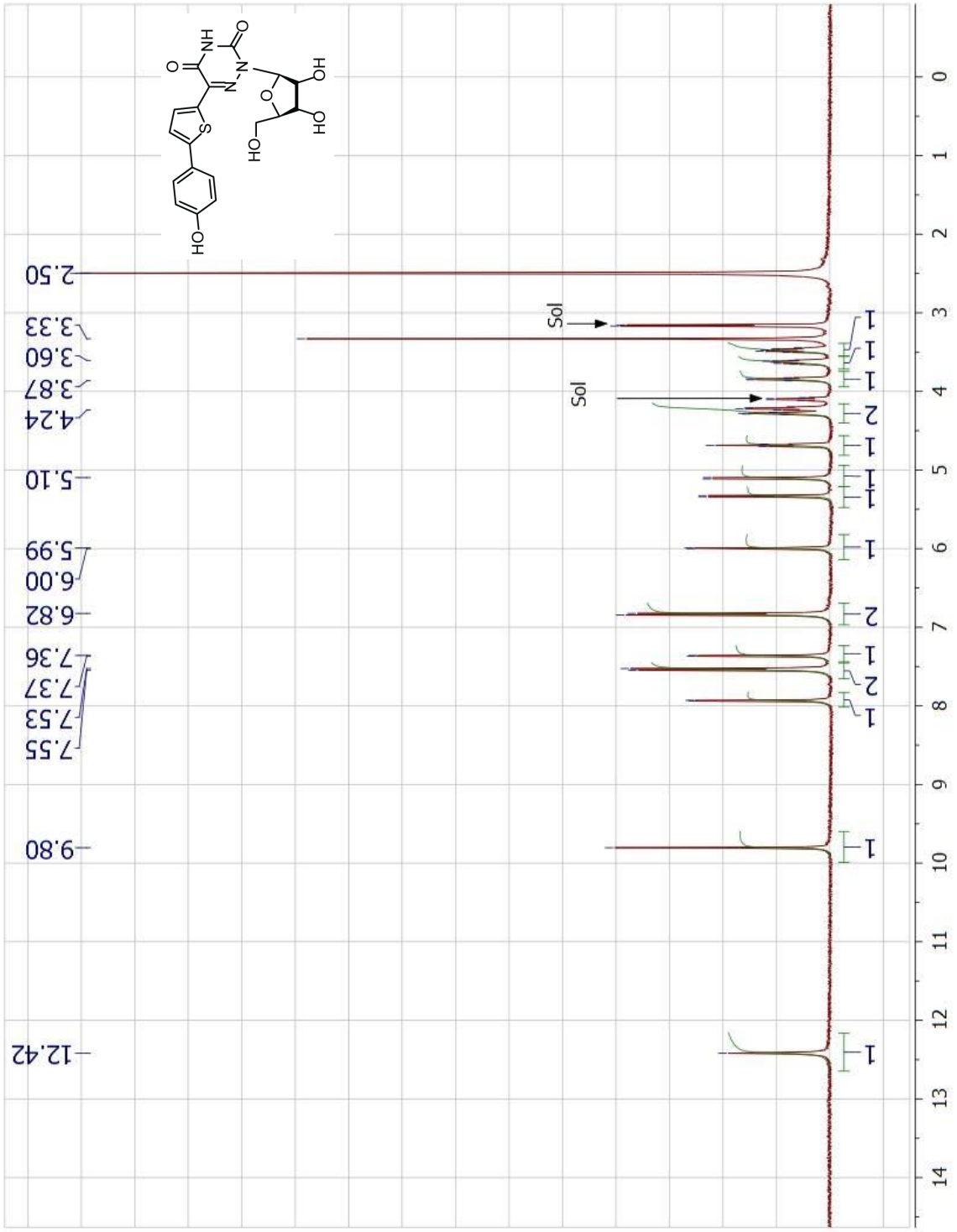
^{13}C-NMR of 5-(5-(4-methyl)phenyl)thiophen-2-yl)-6-aza-uridine (1c).



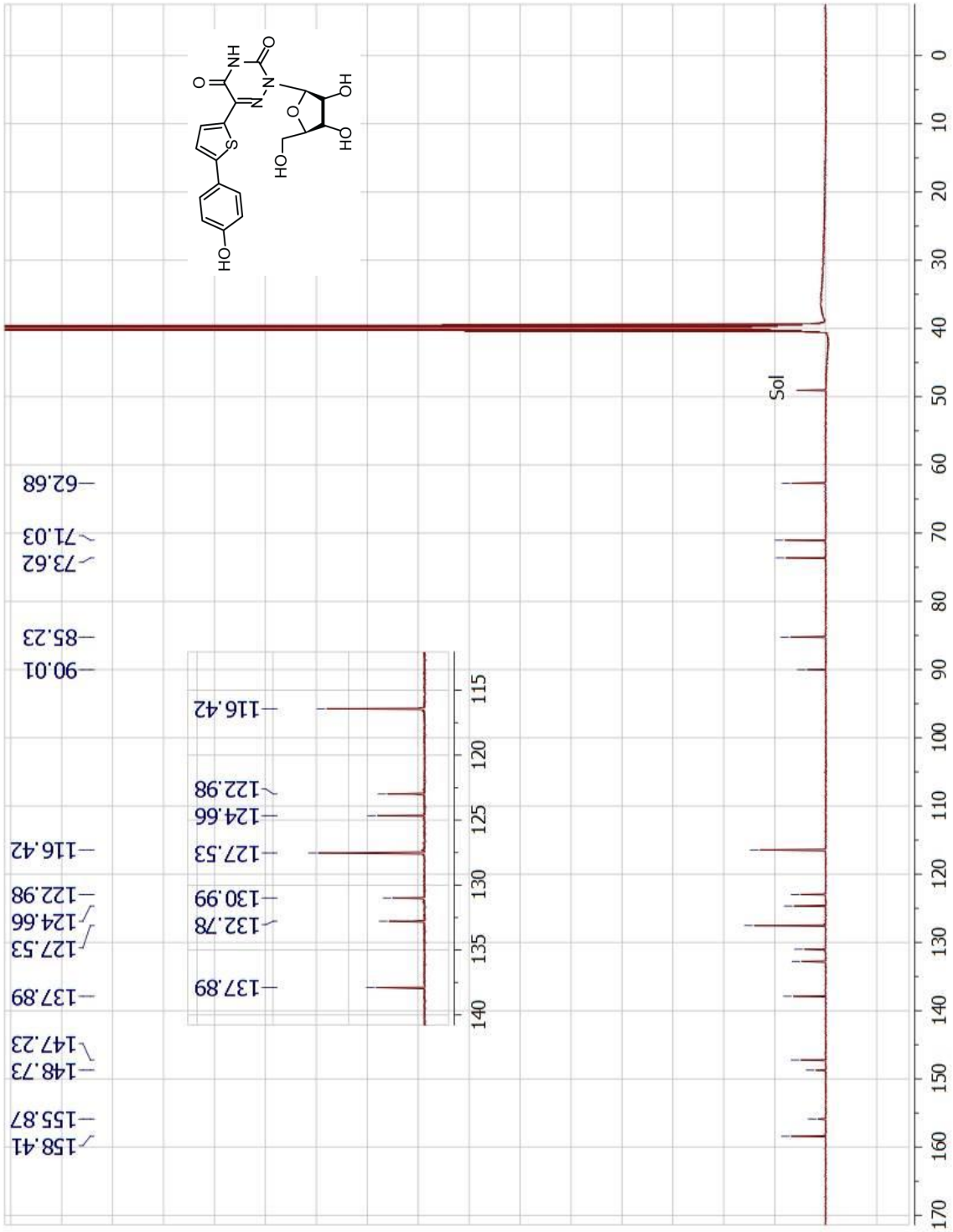
^{1}H-NMR of 5-(5-(4-methoxyphenyl)thiophen-2-yl)-6-aza-uridine (1d).



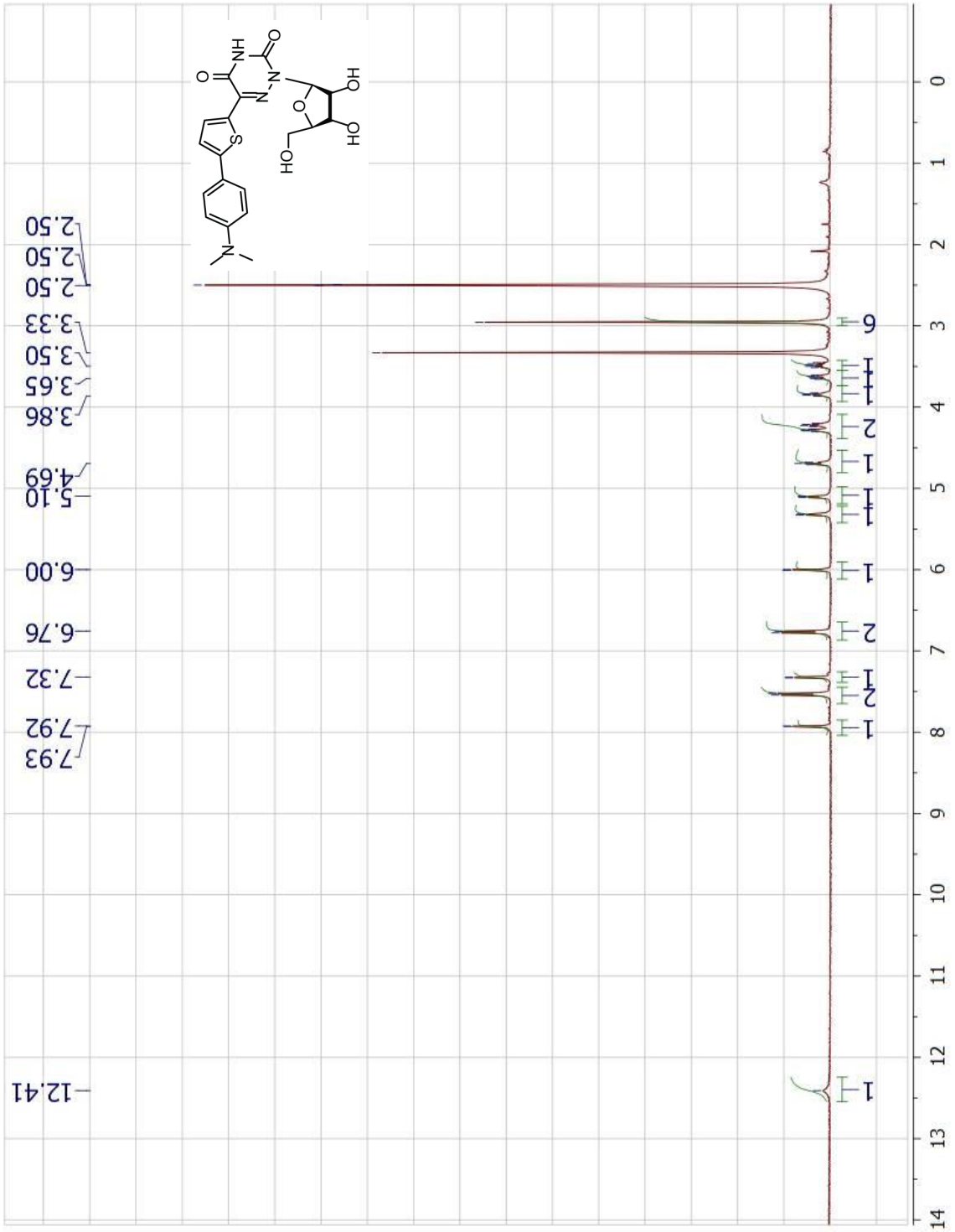
^{13}C-NMR of 5-(5-(4-methoxyphenyl)thiophen-2-yl)-6-aza-uridine (1d).



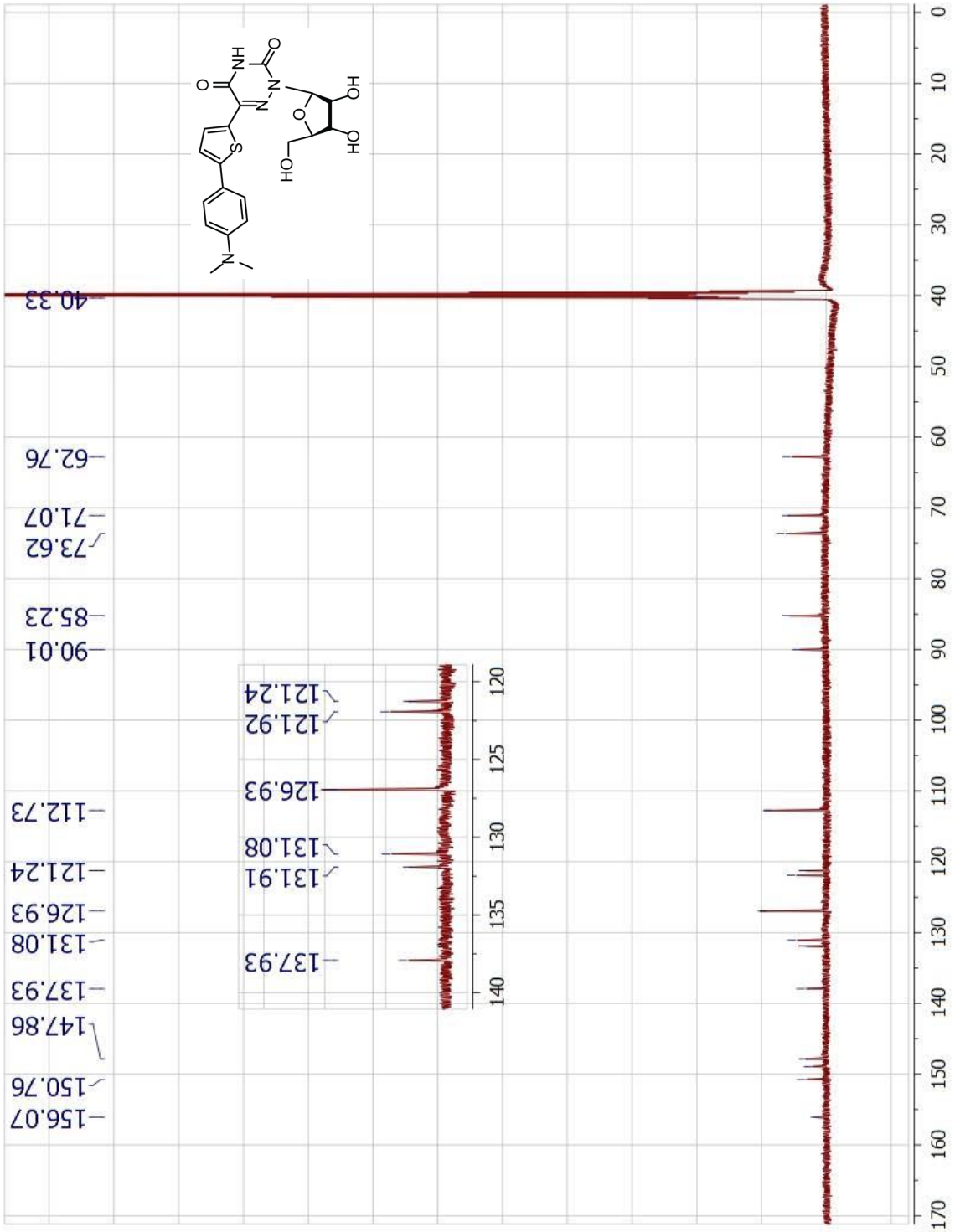
^{1}H-NMR of 5-(5-(4-hydroxyphenyl)thiophen-2-yl)-6-aza-uridine (1e).



^{13}C-NMR of 5-(5-(4-hydroxyphenyl)thiophen-2-yl)-6-aza-uridine (1e).



^{1}H-NMR of 5-(5-(4-(dimethylamino)phenyl)thiophen-2-yl)-6-aza-uridine (1f).



^{13}C-NMR of 5-(5-(4-(dimethylamino)phenyl)thiophen-2-yl)-6-aza-uridine (1f).

S2. Crystal structures

Experimental Summary

The single crystal X-ray diffraction studies were carried out on a Bruker Kappa APEX-II CCD diffractometer equipped with Mo K_α radiation (λ = 0.71073 Å). A 0.103 x 0.011 x 0.005 mm colorless needle was mounted on a Cryoloop with Paratone oil. Data were collected in a nitrogen gas stream at 100(2) K using ϕ and ϖ scans. Crystal-to-detector distance was 30 mm and exposure time was 40 seconds per frame using a scan width of 1.0°. Data collection was 99.6% complete to 25.00° in θ. A total of 27161 reflections were collected covering the indices, -26<=h<=26, -7<=k<=7, -15<=l<=15. 3051 reflections were found to be symmetry independent, with a R_{int} of 0.0621. Indexing and unit cell refinement indicated a *C*-centered, monoclinic lattice. The space group was found to be *C*2. 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-2013). 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-2013. Crystallographic data are summarized in Table 1-4.

Crystal structure(s) were deposited at the Cambridge Crystallographic Data Centre.
The data have been assigned to the following deposition numbers.

Summary of Data CCDC 1018947
Compound Name: **5-(5-bromothiophen-2-yl)-6-aza-uridine (4)**
Formula: C12 H12 Br1 N3 O6 S1
Unit Cell Parameters: a 21.582(3) b 5.6109(7) c 12.5574(14) C2

Summary of Data CCDC 1018950
Compound Name: **5-(5-(4-fluorophenyl)thiophen-2-yl)-6-aza-uridine (1a)**
Formula: 2(C18 H16 F1 N3 O6 S1),C1 H4 O1
Unit Cell Parameters: a 9.4297(5) b 10.0067(4) c 10.3843(4) P1

Summary of Data CCDC 1025557
Compound Name: **5-(5-phenylthiophen-2-yl)-6-aza-uridine (1b)**
Formula: C18 H17 N3 O6 S1,0.5(C1 H4 O1)
Unit Cell Parameters: a 20.4544(12) b 5.5733(3) c 16.1707(10) C2

Summary of Data CCDC 1018949
Compound Name: **5-(5-(4-methyl)phenyl)thiophen-2-yl)-6-aza-uridine (1c)**
Formula: C19 H19 N3 O6 S1,C1 H4 O1
Unit Cell Parameters: a 9.4923(7) b 19.9718(15) c 10.7103(8) P21

Summary of Data CCDC 1018948
Compound Name: **5-(5-(4-methoxyphenyl)thiophen-2-yl)-6-aza-uridine (1d)**
Formula: C19 H19 N3 O7 S1,C1 H4 O1
Unit Cell Parameters: a 9.0722(10) b 10.3704(11) c 12.3104(14) P1

Summary of Data CCDC 1025556
Compound Name: **5-(5-(4-(dimethylamino)phenyl)thiophen-2-yl)-6-aza-uridine (1f)**
Formula: 2(C20 H22 N4 O6 S1),C2 H6 O1 S1
Unit Cell Parameters: a 8.9567(7) b 19.8870(16) c 24.7770(19) P212121

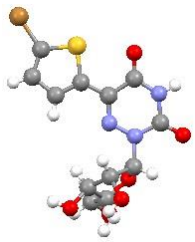


Figure S2.1. X-ray crystal structure of **5-(5-bromothiophen-2-yl)-6-aza-uridine (4)**.

Table S2.1. Crystal data and structure refinement for Tor75 **5-(5-bromothiophen-2-yl)-6-aza-uridine (4)**.

Identification code	PH140	
Empirical formula	C12 H12 Br N3 O6 S	
Molecular formula	C12 H12 Br N3 O6 S	
Formula weight	406.22	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C 1 2 1	
Unit cell dimensions	a = 21.582(3) Å	α= 90°.
	b = 5.6109(7) Å	β= 98.665(4)°.
	c = 12.5574(14) Å	γ = 90°.
Volume	1503.3(3) $Å^3$	
Z	4	
Density (calculated)	1.795 Mg/m^3	
Absorption coefficient	2.909 mm^{-1}	
F(000)	816	
Crystal size	0.103 x 0.011 x 0.005 mm^3	
Crystal color, habit	Colorless Needle	
Theta range for data collection	1.640 to 26.440°.	
Index ranges	-26<=h<=26, -7<=k<=7, -15<=l<=15	
Reflections collected	27161	
Independent reflections	3051 [R(int) = 0.0621]	
Completeness to theta = 25.000°	99.6 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.0926 and 0.0654	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3051 / 5 / 224	
Goodness-of-fit on F^2	1.032	
Final R indices [I>2sigma(I)]	R1 = 0.0251, wR2 = 0.0457	
R indices (all data)	R1 = 0.0321, wR2 = 0.0469	
Absolute structure parameter	0.039(4)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.417 and -0.408 $e.Å^{-3}$	

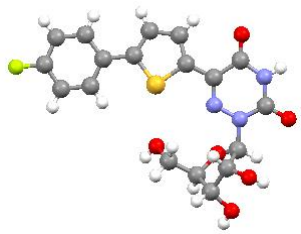


Figure S2.2. X-ray crystal structure of **5-(5-(4-fluorophenyl)thiophen-2-yl)-6-aza-uridine (1a).**

Table S2.2. Crystal data and structure refinement for tor85 **5-(5-(4-fluorophenyl)thiophen-2-yl)-6-aza-uridine (1a)**.

Identification code	PH188	
Empirical formula	C37 H36 F2 N6 O13 S2	
Formula weight	874.84	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P 1	
Unit cell dimensions	a = 9.4297(5) Å	α= 74.895(2)°.
	b = 10.0067(4) Å	β= 77.875(2)°.
	c = 10.3843(4) Å	γ= 76.982(2)°.
Volume	909.69(7) $Å^3$	
Z	1	
Density (calculated)	1.597 Mg/m^3	
Absorption coefficient	0.237 mm^{-1}	
F(000)	454	
Crystal size	0.290 x 0.080 x 0.040 mm^3	
Theta range for data collection	2.058 to 26.443°.	
Index ranges	-11<=h<=11, -12<=k<=12, -12<=l<=13	
Reflections collected	18448	
Independent reflections	7444 [R(int) = 0.0511]	
Completeness to theta = 25.000°	100.0 %	
Absorption correction	Multi-scan	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	7444 / 4 / 535	
Goodness-of-fit on F^2	1.019	
Final R indices [I>2sigma(I)]	R1 = 0.0401, wR2 = 0.0910	
R indices (all data)	R1 = 0.0453, wR2 = 0.0944	
Absolute structure parameter	0.03(4) [stereochem. confirmed]	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.501 and -0.459 e.$Å^{-3}$	

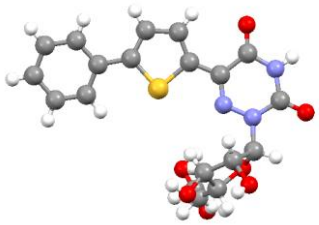


Figure S2.3. X-ray crystal structure of **5-(5-phenylthiophen-2-yl)-6-aza-uridine (1b).**

Table S2.3. Crystal data and structure refinement for tor93 **5-(5-phenylthiophen-2-yl)-6-aza-uridine (1b)**.

Identification code	PH189	
Empirical formula	C18.50 H19 N3 O6.50 S	
Molecular formula	C18 H17 N3 O6 S, 0.5(C H4 O)	
Formula weight	419.43	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C 1 2 1	
Unit cell dimensions	a = 20.4544(12) Å	α= 90°.
	b = 5.5733(3) Å	β= 93.062(4)°.
	c = 16.1707(10) Å	γ = 90°.
Volume	1840.80(19) Å^3	
Z	4	
Density (calculated)	1.513 Mg/m^3	
Absorption coefficient	0.223 mm^{-1}	
F(000)	876	
Crystal size	0.053 x 0.005 x 0.005 mm^3	
Crystal color, habit	Orange Needle	
Theta range for data collection	1.994 to 25.732°.	
Index ranges	-24<=h<=24, -6<=k<=6, -19<=l<=19	
Reflections collected	18065	
Independent reflections	3409 [R(int) = 0.1124]	
Completeness to theta = 25.000°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3409 / 23 / 284	
Goodness-of-fit on F^2	1.052	
Final R indices [I>2sigma(I)]	R1 = 0.0708, wR2 = 0.1881	
R indices (all data)	R1 = 0.0915, wR2 = 0.2038	
Absolute structure parameter	0.03(19)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.550 and -0.493 e.Å^{-3}	

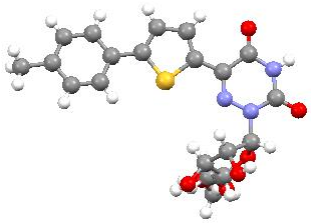


Figure S2.4. X-ray crystal structure of **5-(5-(4-methyl)phenyl)thiophen-2-yl)-6-aza-uridine (1c).**

Table S2.4. Crystal data and structure refinement for tor81 **5-(5-(4-methyl)phenyl)thiophen-2-yl)-6-aza-uridine (1c)**.

Identification code	PH187	
Empirical formula	C20 H23 N3 O7 S	
Formula weight	449.47	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21	
Unit cell dimensions	a = 9.4923(7) Å	α= 90°.
	b = 19.9718(15) Å	β= 95.314(3)°.
	c = 10.7103(8) Å	γ = 90°.
Volume	2021.7(3) $Å^3$	
Z	4	
Density (calculated)	1.477 Mg/m^3	
Absorption coefficient	0.210 mm^{-1}	
F(000)	944	
Crystal size	0.300 x 0.100 x 0.050 mm^3	
Theta range for data collection	2.039 to 28.349°.	
Index ranges	-12<=h<=12, -26<=k<=26, -14<=l<=12	
Reflections collected	31888	
Independent reflections	10067 [R(int) = 0.0496]	
Completeness to theta = 25.000°	99.9 %	
Absorption correction	Multi-scan	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	10067 / 1 / 571	
Goodness-of-fit on F^2	1.012	
Final R indices [I>2sigma(I)]	R1 = 0.0444, wR2 = 0.1009	
R indices (all data)	R1 = 0.0562, wR2 = 0.1077	
Absolute structure parameter	0.00(3)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.771 and -0.401 $e.Å^{-3}$	

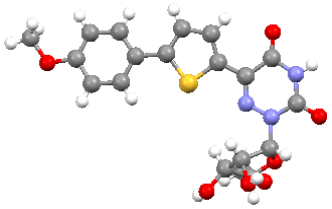


Figure S2.5. X-ray crystal structure of **5-(5-(4-methoxyphenyl)thiophen-2-yl)-6-aza-uridine (1d).**

Table S2.5. Crystal data and structure refinement for tor76 **5-(5-(4-methoxyphenyl)thiophen-2-yl)-6-aza-uridine (1d)**.

Identification code	PH176	
Empirical formula	C20 H23 N3 O8 S	
Formula weight	465.47	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Triclinic	
Space group	P 1	
Unit cell dimensions	a = 9.0722(10) Å	α= 100.154(4)°.
	b = 10.3704(11) Å	β= 103.516(4)°.
	c = 12.3104(14) Å	γ = 105.629(4)°.
Volume	1048.5(2) $Å^3$	
Z	2	
Density (calculated)	1.474 Mg/m^3	
Absorption coefficient	1.857 mm^{-1}	
F(000)	488	
Crystal size	0.340 x 0.080 x 0.060 mm^3	
Theta range for data collection	3.820 to 68.341°.	
Index ranges	-10<=h<=10, -10<=k<=12, -14<=l<=14	
Reflections collected	8418	
Independent reflections	5428 [R(int) = 0.0362]	
Completeness to theta = 66.000°	95.7 %	
Absorption correction	Multi-scan	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5428 / 3 / 582	
Goodness-of-fit on F^2	1.042	
Final R indices [I>2sigma(I)]	R1 = 0.0466, wR2 = 0.1257	
R indices (all data)	R1 = 0.0475, wR2 = 0.1270	
Absolute structure parameter	0.056(12)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.503 and -0.443 $e.Å^{-3}$	

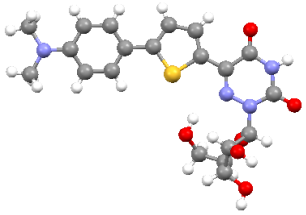


Figure S2.6. X-ray crystal structure of **5-(5-(4-(dimethylamino)phenyl)thiophen-2-yl)-6-aza-uridine (1f).**

Table S2.6. Crystal data and structure refinement for Tor91 **5-(5-(4-(dimethylamino)phenyl)thiophen-2-yl)-6-aza-uridine (1f).**

Report date	2014-08-14	
Identification code	Tor91	
Empirical formula	C21 H25 N4 O6.50 S1.50	
Molecular formula	C20 H22 N4 O6 S, 0.5(C2 H6 O S)	
Formula weight	485.54	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 8.9567(7) Å	α= 90°.
	b = 19.8870(16) Å	β= 90°.
	c = 24.7770(19) Å	γ = 90°.
Volume	4413.3(6) $Å^3$	
Z	8	
Density (calculated)	1.461 Mg/m^3	
Absorption coefficient	0.244 mm^{-1}	
F(000)	2040	
Crystal size	0.377 x 0.035 x 0.031 mm^3	
Crystal color, habit	Orange Needle	
Theta range for data collection	2.048 to 26.413°.	
Index ranges	-10<=h<=11, -24<=k<=24, -30<=l<=30	
Reflections collected	29602	
Independent reflections	9013 [R(int) = 0.0799]	
Completeness to theta = 25.000°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.0932 and 0.0655	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	9013 / 8 / 633	
Goodness-of-fit on F^2	1.013	
Final R indices [I>2sigma(I)]	R1 = 0.0530, wR2 = 0.0978	
R indices (all data)	R1 = 0.0948, wR2 = 0.1119	
Absolute structure parameter	-0.04(5)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.745 and -0.320 $e.Å^{-3}$	

S3. X-ray crystal structure: packing.

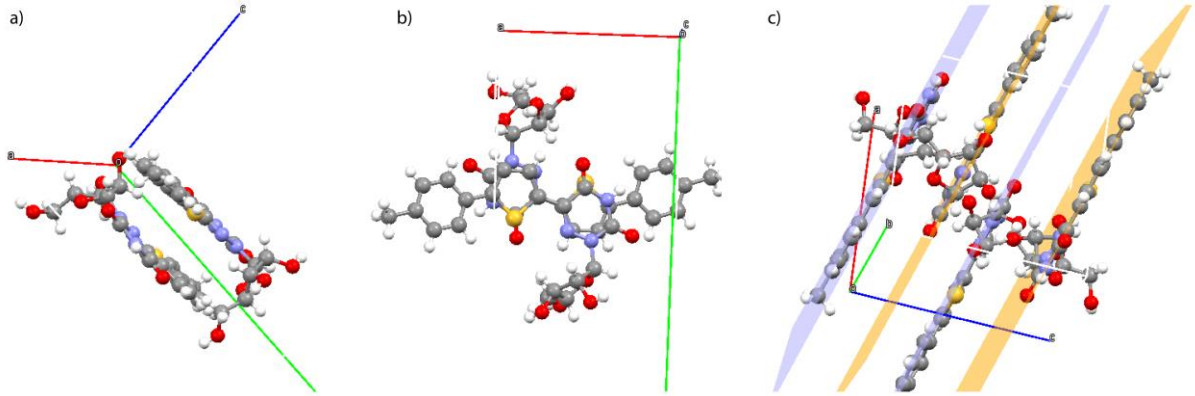


Figure S3.1. Crystal packing of derivative **5-(5-(4-methoxyphenyl)thiophen-2-yl)-6-aza-uridine (1d)** in three different views (a) side view (b) highlighting he overlap of the thiophene and the 6-aza-uridine rings (c) highlighting the aromatic layers.

S4. Photophysics

Spectroscopic grade methanol and ethanol were obtained from Sigma Aldrich. Spectroscopic grade dioxane was obtained from Acros. Aqueous samples were prepared with de-ionized water. For all spectroscopic measurements a 1 cm four-sided Helma quartz cuvette was used. All spectroscopy samples were prepared from concentrated DMSO stock solutions, hence, all samples contain 0.4 v% or 0.2 v% DMSO.

Absorption spectra were measured on a Shimadzu UV-2450 UV-Vis spectrophotometer with 1 nm resolution and corrected for the blank. The sample temperature was kept constant at 20 °C using a thermostat.

Steady state emission and excitation spectra were taken on a PTI luminescence spectrometer with a 1 nm resolution. The sample temperature was kept constant at 20 °C with a Quantum Northwest TLC50 fluorescence cuvette holder in conjunction with a software controllable TC 125 temperature controller.

The polarity dependent steady state fluorescence studies were performed using an excitation wavelength (λ_{ex}) of:

	Dioxane-methanol		**Water**	
Sample	**λ_{ex} (nm)**	**Concentration (μM)**	**λ_{ex} (nm)**	**Concentration (μM)**
1a	365	4.25	335	4.25
1b	370	3.75	365	3.75
1c	365	6.71	360	3.35
1d	375	2.00	370	2.00
1e	385	1.60	370	1.60
1f	410	2.13	390	2.13
slit-widths	0.50 mm = 2.0 nm		1.00 mm = 4.0 nm	

Stokes shifts were calculated in cm^{-1} then converted to kcal/mol by multiplication with 0.0028591 to plot the polarity sensitivity correlations.

Samples' $E_T(30)$ values (in kcal/mol) were determined by dissolving a small amount of Reichardt's dye in the solvent (mixture) used to prepare the sample.[2] The observed long wavelength absorption maximum in nm ($\lambda_{abs\ max}$) was converted to the sample $E_T(30)$ value according the following equation:

$$E_T(30) = \frac{28591}{\lambda_{\text{abs max}}}$$

Quantum yields were determined using Coumarin-102 in ethanol (Φ = 0.80) as a standard for **1a-e**, and Coumarin-153 in ethanol (Φ = 0.38) for **1f** using dilute sample solutions with an O.D. < 0.05 at the λ_{ex}, using the following equation:

$$\Phi_s = \frac{I_s}{I_{ref}} \cdot \frac{O.D._{ref}}{O.D._s} \cdot \left(\frac{n_s}{n_{ref}} \right)^2 \cdot \Phi_{ref}$$

Here Φ, I, O.D. and *n* stand for quantum yield, integrated emission intensity, optical density at λ_{ex} and refractive index (n_{water} = 1.333, $n_{dioxane}$ = 1.42, $n_{methanol}$ = 1.326, $n_{ethanol}$ = 1.361), respectively. Sample and reference are denoted by *s* and *ref*, respectively. The λ_{ex} is in a very close proximity for the sample and reference solutions to circumvent correction of the difference in excitation energy at different wavelengths.

For dioxane solutions:

Sample	λ_{ex} (nm)
1a	368
1b	371
1c	375
1d	383
1e	385
1f	421

The excitation wavelengths for methanol and water solutions were kept the same as reported above for polarity dependent steady state fluorescence studies.

S5. Sensitivity to polarity

Sensitivity to solvent polarity was studied in methanol, dioxane, and their mixtures (10, 20, 30, 40, 50, 60, 70, 80, and 90 v/v % of methanol in dioxane) at 20 °C. Emission spectra were recorded after excitation at the long wavelength emission maximum (O.D.'s < 0.1). The same samples were used to obtain excitation spectra probing at:

Sample	λ_{em} (nm)
1a	470
1b	480
1c	475
1d	480
1e	500
1f	560

All experiments were performed in triplicate with negligible differences; hence only one series is shown.

S5.1. Absorption and steady-state emission spectroscopy for 1a in methanol/dioxane

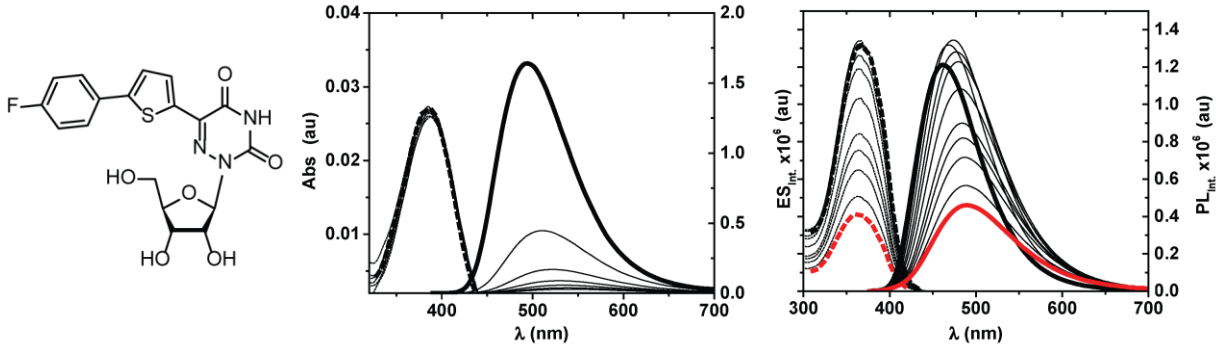


Figure 5.1.1. Assessing the effect of solvent polarity on absorption and excitation (dotted line), and emission (solid line), in dioxane (bold black line) and methanol (bold red line) and their mixtures (black lines).

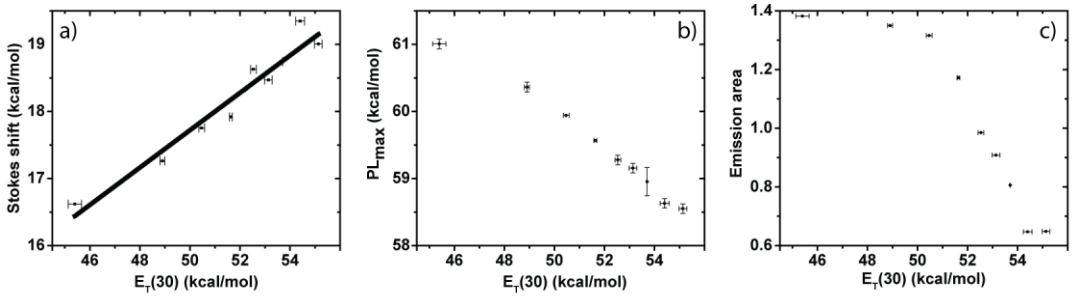


Figure 5.1.2. Correlating $E_T(30)$ vs. Stokes shift (slope:0.28 and R^2:0.97) (a), PL_{max} (b), emission area (c) values obtained from dioxane–methanol mixtures 90%:10% → 90%:10%.

S5.2. Absorption and steady-state emission spectroscopy for 1b in methanol/dioxane

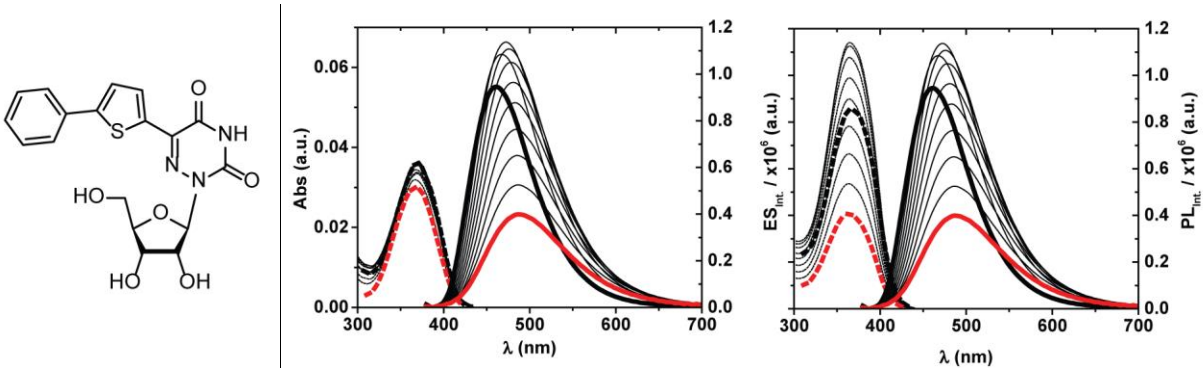


Figure 5.2.1. Assessing the effect of solvent polarity on absorption and excitation (dotted line), and emission (solid line), in dioxane (bold black line) and methanol (bold red line) and their mixtures (black lines).

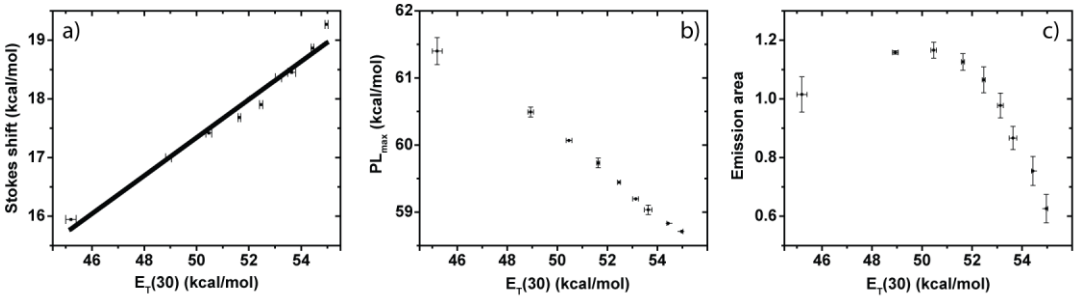


Figure 5.2.2. Correlating $E_T(30)$ vs. Stokes shift (slope:0.33 and R^2:0.99) (a), PL_{max} (b), emission area (c) values obtained from dioxane–methanol mixtures 90%:10% → 90%:10%.

S5.3. Absorption and steady-state emission spectroscopy for 1c in methanol/dioxane

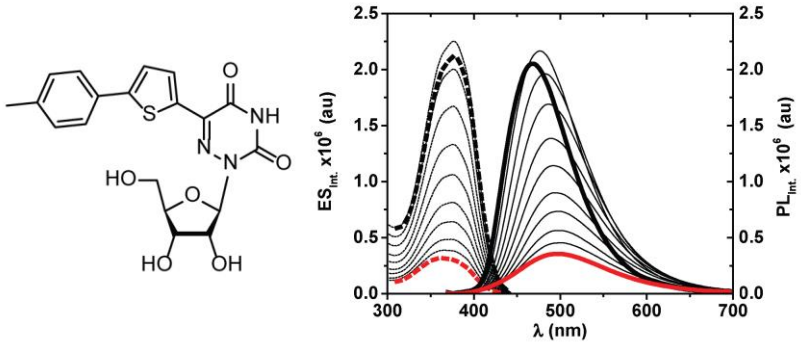


Figure 5.3.1. Assessing the effect of solvent polarity on excitation (dotted line), and emission (solid line), in dioxane (bold black line) and methanol (bold red line) and their mixtures (black lines).

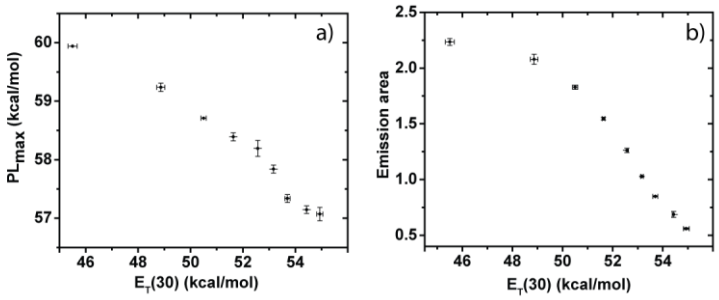


Figure 5.3.2. Correlating $E_T(30)$ vs. PL_{max} (a), emission area (b) values obtained from dioxane–methanol mixtures 90%:10% → 40%:60%.

S5.4. Absorption and steady-state emission spectroscopy for **1d** in methanol/dioxane

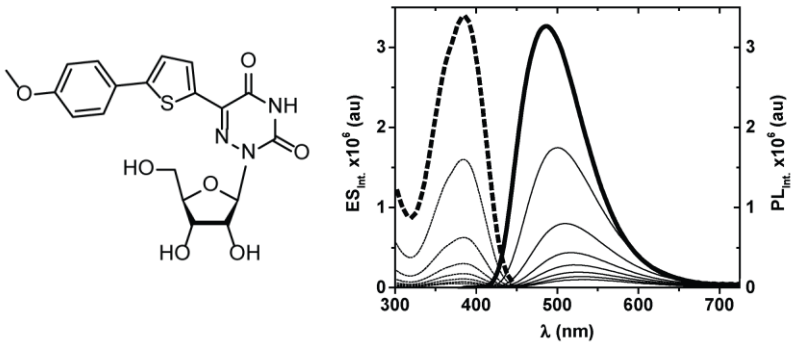


Figure 5.4.1. Assessing the effect of solvent polarity on excitation (dotted line), and emission (solid line), in dioxane (bold black line) and dioxane-methanol mixtures (black lines).

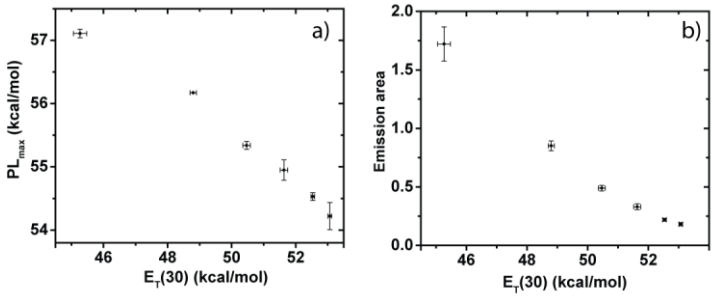


Figure 5.4.2. Correlating $E_T(30)$ vs. PL_{max} (a), emission area (b) values obtained from dioxane–methanol mixtures 90%:10% → 40%:60%.

S5.5. Absorption and steady-state emission spectroscopy for 1e in methanol/dioxane

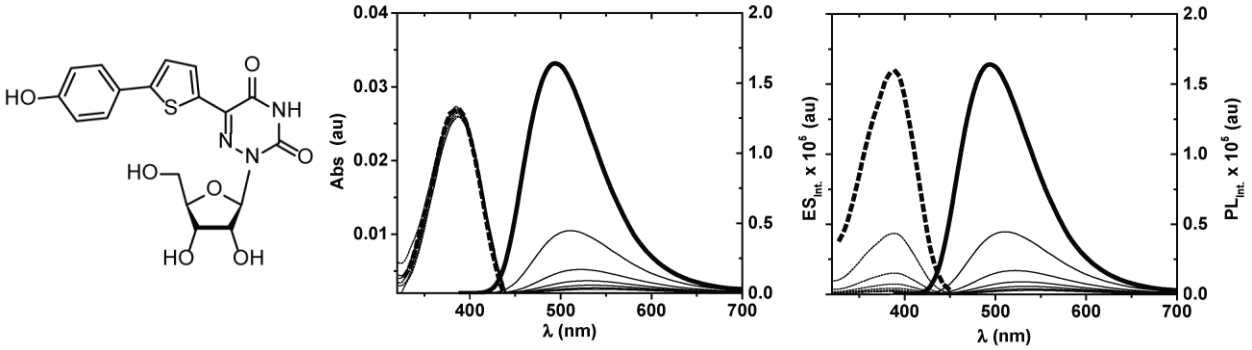


Figure 5.5.1. Assessing the effect of solvent polarity on absorption and excitation (dotted line), and emission (solid line), in dioxane (bold black line) and dioxane-methanol mixtures (black lines).

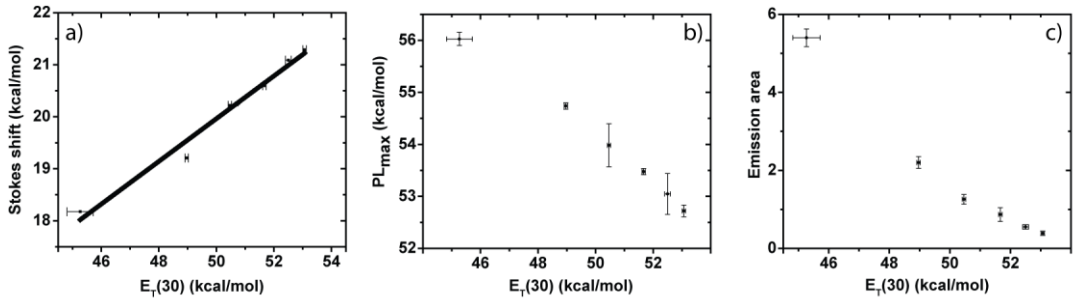


Figure 5.5.2. Correlating $E_T(30)$ vs. Stokes shift (slope:0.41 and R^2:0.99) (a), PL_{max} (b), emission area (c) values obtained from dioxane–methanol mixtures 90%:10% → 40%:60%.

S5.6. Absorption and steady-state emission spectroscopy for 1f in methanol/dioxane

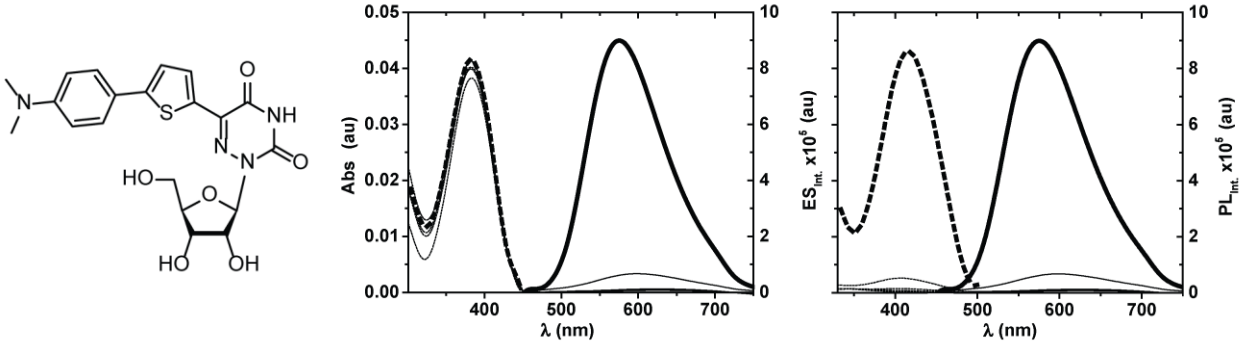


Figure 5.6.1. Assessing the effect of solvent polarity on absorption and excitation (dotted line), and emission (solid line), in dioxane (bold black line) and dioxane-methanol mixtures (black lines).

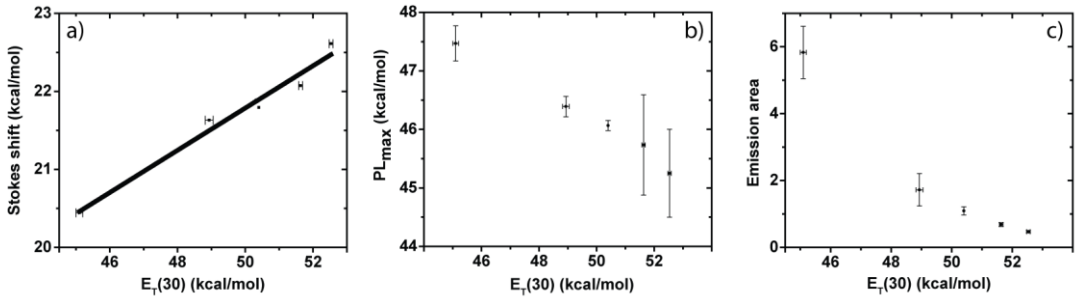


Figure 5.6.2. Correlating $E_T(30)$ vs. Stokes shift (slope:0.27 and R^2:0.99) (a), PL_{max} (b), emission area (c) values obtained from dioxane–methanol mixtures 90%:10% → 50%:50%.

S6. Absorption and steady-state emission spectroscopy for 1a-f in water

The water steady state fluorescence studies were performed using an excitation wavelength (λ_{ex}) of:

Sample	λ_{ex} (nm)
1a	355
1b	365
1c	360
1d	370
1e	370
1f	390

The same samples were used to obtain excitation spectra probing at:

Sample	λ_{em} (nm)
1a	450
1b	450
1c	475
1d	490
1e	475
1f	475

All experiments were performed in triplicate with negligible differences; hence only one series is shown.

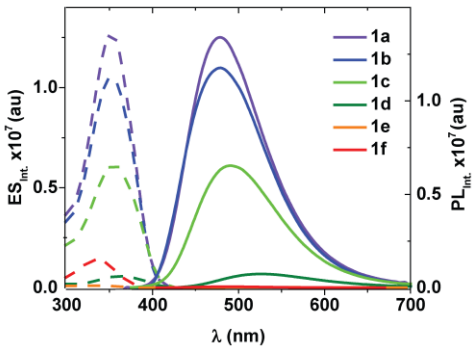


Figure S6.1. Excitation (dotted line) and emission (solid line) spectra for **1a** (purple), **1b** (blue), **1c** (green), **1d** (dark green), **1e** (orange), **1f** (red) in dioxane (a) and water (b).. Emission was recorded after excitation at $\lambda_{abs\ max}$ for each derivatives (values in Table above). Slits width 1.00mm.

S7. Sensitivity to pH

S7.1. Absorption and steady-state emission spectroscopy for 1c

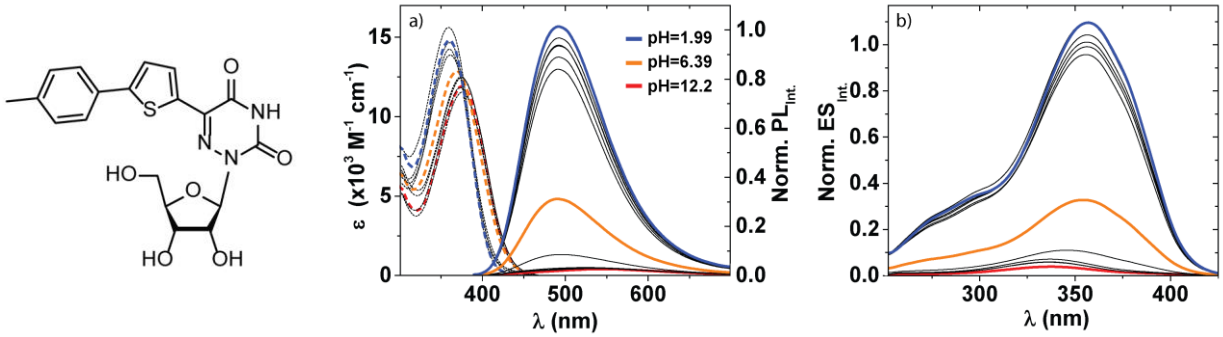


Figure S7.1. Assessing the effect of pH in aqueous buffers of pH 1.99 (red line), pH 6.39 (orange line) and pH 12.2 (blue line) and intermediate pH values (black lines) on (a) absorption (dotted line) and emission (solid line) (emission spectra were recorded after excitation at 370nm) and on (b) excitation (solid line) (spectra were recorded probing at 500nm).

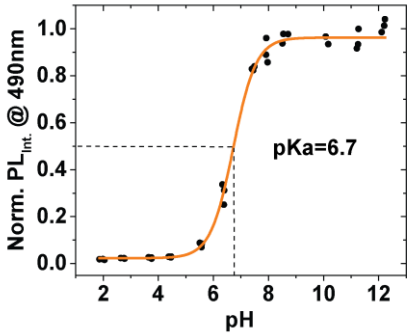


Figure S7.2. A plot of the normalized emission intensity as function of sample pH values (solid circles), with a sigmoidal fit (orange line) using OriginPro. The dashed lines illustrate a graphical determination of the pKa value (R^2: 0.99).

S8. **Enlarged Figure 3c and Figure 3d.**

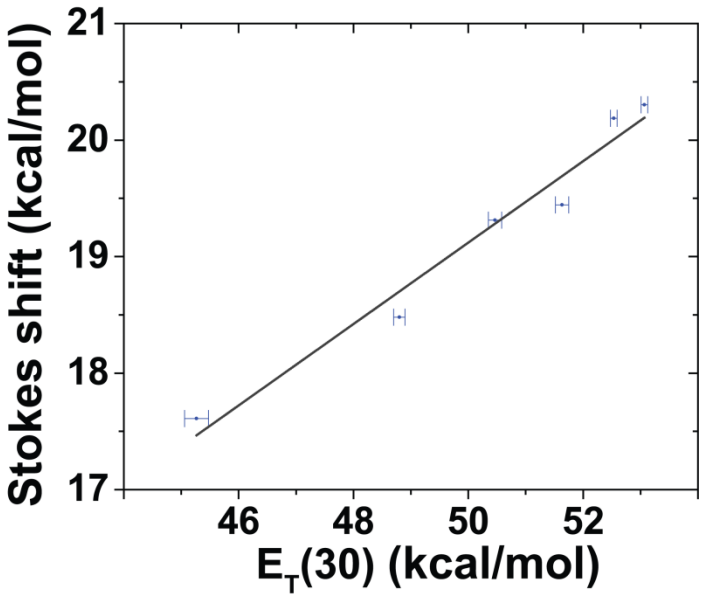


Figure 8.1. Correlating $E_T(30)$ vs. Stokes shift (slope:0.36 and R^2=0.95) values obtained from dioxane–methanol mixtures 90%:10% → 90%:10%.

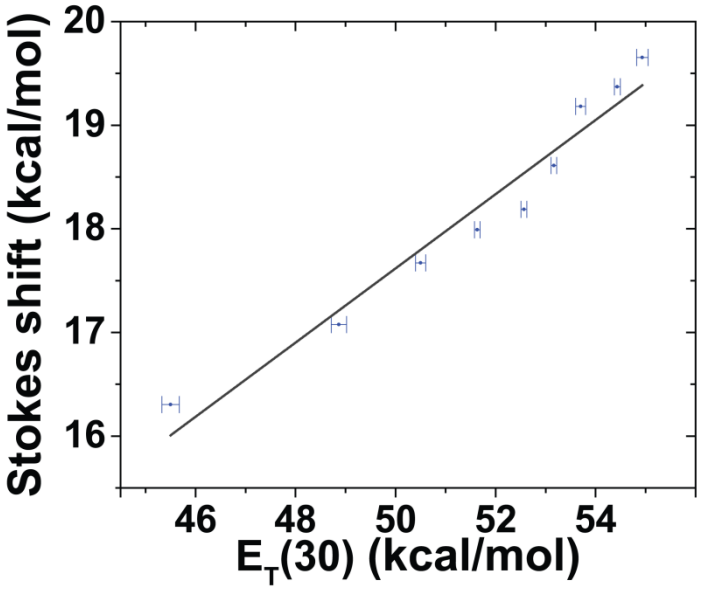


Figure 8.2. Correlating $E_T(30)$ vs. Stokes shift (slope:0.35 and R^2=0.98) values obtained from dioxane–methanol mixtures 90%:10% → 40%:60%.

S9. References

(1) Sinkeldam, R. W.; Hopkins, P. A.; Tor, Y. *ChemPhysChem* **2012**, *13*, 3350.
(2) Lakowicz, J. R. *Principles of fluorescence spectroscopy*; 3rd ed.; Springer: New York, 2006.