Supporting Information

New Metal-Free Route towards Imidazole-Substituted Uridine

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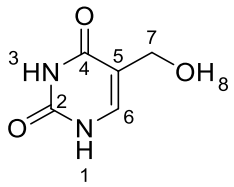
Supporting Information

EXPERIMENTAL SECTION

Materials and Methods

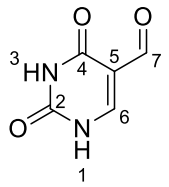
For all reactions, chemicals of analytical or synthetic grade were obtained from commercial sources and were used without further purification unless stated otherwise. The stationary phase in column chromatography was 70-230 mesh silica 60 (E.M. Merck). Analytical Thin Layer Chromatography (TLC) was performed on Alugram® silica gel UV254 mesh 60, 0.20mm (Macherey-Nagel). ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker Avance 300 (5mm BBO Probe). 2D NMR spectra on a Bruker Avance 500 a TXI Z gradient probe or Bruker Avance II 600 MHz with a 5 mm TCI HCN Z gradient cryoprobe. Chemical shifts are expressed as δ (part per million) down field from TMS (tetramethyl silane) for 1H and 13C-spectra. High Resolution Mass Spectrometry (HRMS) spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL/min and spectra were obtained in positive or negative ionization mode with a resolution of 15000 FWHM using leucine encephalin as lock mass. Melting points were collected by DSC (Mettler Toledo Star System) with Indium as calibration agent.

CAUTION: Pressure tube reactions were carried out in Ace pressure tubes (Sigma Aldrich) with front seal. The glassware was checked for any cracks before the reaction. The reaction itself was carried out behind blast screens and was cooled down before opening. Opening must be done carefully to release any overpressure resulting from gas build-up.



S1

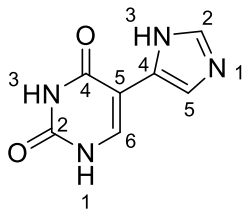
5-Hydroxymethyluracil procedure according to literature[1]: To a solution of uracil (8.8 g, 78.5 mmol, 1 eq) and paraformaldehyde (90%, 7.85 g, 0.236 mol, 3 eq) in water (200 mL), triethylamine (16.4 mL, 0.118 mol, 1.5 eq) was added. Heating to 65 °C turned the solution clear, continued by further stirring at 65 °C for 12 h. The water was removed by rotary evaporation until approximately 1/10 of volume remained. The residue was diluted with an equal amount of 95% ethanol, which was allowed to stand for 30 min at RT and further cooled at 4 °C. The white precipitate was filtered off and washed with 95% ethanol. The filtrate was used to repeat the process of concentration and precipitation three times, resulting in a combined 88% (9.82 g) yield. ^{1}H NMR (300 MHz, DMSO-d_6, δ): 11.05 (s, 1H, NH_3), 10.72 (s, 1H, NH^1), 7.23 (s, 1H, H^6), 4.85 (s, 1H, OH), 4.10 (s, 2H, H^7); ^{13}C NMR (75 MHz, DMSO- d_6, δ): 163.80 (C^4), 151.35 (C^2), 138.20 (C^6), 112.72 (C^5), 55.81 (C^7); m/z = 141.0 $[M-H]^-$; Mp = 192-6 °C (lit[1] = 195-200 °C)



1

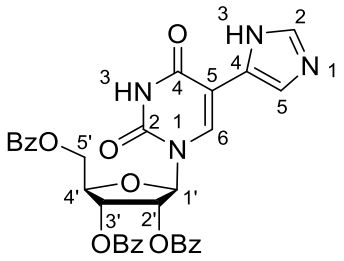
5-Formyluracil (1) *procedure according to literature*[1]*:* S1 (10.75 g, 75.6 mmol, 1 eq) was dissolved in H_2O (400 mL) by heating to 90 °C. The solution was then cooled to 45 °C followed by administration of $AgNO_3$ (0.4 g, 2.3 mmol, 3 mol%) and slow, partial addition of $K_2S_2O_8$ (37.5 g, 0.14 mol, 1.81 eq) which led to the gradual formation of a white precipitate. The reaction was left stirring for 20 min at 40 °C and 15 min at RT. The suspension was then placed at 4 °C, filtered and washed with cold water to yield 9.8 g 1 (93%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6, δ): 11.93 (bs, 1H, NH^3), 11.52 (s, 1H, NH^1), 9.73 (s, 1H, H^7),

8.14 (s, 1H, H^6); ^{13}C NMR (75 MHz, DMSO-d_6, δ): 191.64 (C^7), 167.68 (C^4), 155.69 (C^2), 154.54 (C^6), 115.30 (C^5); m/z = 139.1 $[M-H]^-$; Mp > 300 °C (lit[1] = 302-3 °C)



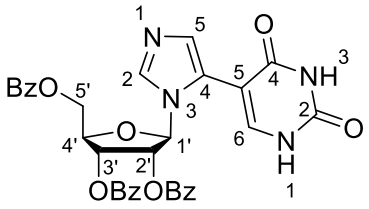
2a

5-(Imidazol-4-yl)uracil (2a) 7N NH_3 in MeOH (3 mL, 21.4 mmol, 3 eq) was added to 5-formyluracil **1** (1 g, 7.1 mmol, 1 eq) in MeOH (14 mL). The mixture was stirred and refluxed in a pressure tube (CAUTION cfr supra) for 1 hour before adding TosMIC (2.23 g, 11.4 mmol, 1.6 eq). After refluxing for another hour, the result was filtered, washed with Et_2O and 1.07 g (85% yield) of **1** as a light grey precipitate was obtained. ^1H NMR (300 MHz, DMSO-d_6, δ): 7.81 (s, 1H, U-H^6), 7.63 (s, 1H, Im-H^2), 7.50 (s, 1H, H^5); ^{13}C NMR (75 MHz, DMSO-d_6, δ): 162.59 (U-C^4), 150.75 (U-C^2), 135.52 (U-C^6), 135.10 (Im-C^2), 132.57 (Im-C^4), 113.77 (Im-C^5), 107.93 (U-C^5); HRMS (ESI-TOF) m/z : $[M-H]^-$ Calcd for $C_7H_5N_4O_2$ 177.04179;Found 177.0425; Mp > 300 °C (dec.) (lit[2] = 312-20 °C)



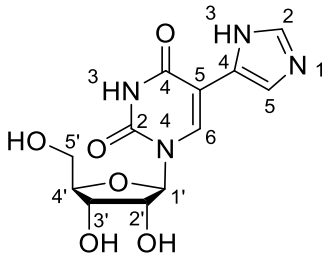
3a

2',3',5'-Tribenzoyl 5-(imidazol-4-yl)uridine (3a) To a two-necked, flame dried, roundbottom flask under atmosphere was added imidazole **2a** (0.2 g, 1.1 mmol, 1.2 eq), followed by the addition of DCE (20 mL) and bis(trimethylsilyl)acetamide (1.14 mL, 6 mmol, 5 eq). The resulting suspension was refluxed until it became transparent, followed by cooling to RT. 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (0.47 g, 0.94 mmol, 1 eq) and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.26 mL, 1.4 mmol, 1.5 eq) were subsequently added under continuous stirring. The resulting mixture was heated for 2d at 60°C. The reaction was quenched by the addition of Et_3N (2 mL), followed by immediate dilution with DCM and washing by sat. $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, followed by purification of the crude residue by column chromatography (R_f=0.42 DCM/MeOH (19:1 v/v)). The product was isolated as an off-white foam (0.50 g, 73% yield) and identified by ^1H-NMR and MS, awaiting further identification by 2D-NMR after deprotection of the benzyl-groups. ^1H NMR (600 MHz, $CDCl_3$-*d*, δ): 10.78 (bs, 1H), 9.87 (bs, 1H), 8.01 (d, $^3J_{HH}$ = 7.2 Hz, 3H), 7.89 – 7.81 (m, 4H), 7.52 (t, $^3J_{HH}$ = 7.4 Hz, 1H), 7.49 – 7.43 (m, 2H), 7.42 – 7.37 (m, 3H), 7.30 – 7.27 (m, 3H), 7.26 – 7.24 (m, 1H), 7.02 (s, 1H), 6.24 (d, $3J_{HH}$ = 3.2 Hz, 1H), 5.98 – 5.92 (m, 1H), 5.80 (t, $^3J_{HH}$ = 5.7 Hz, 1H), 4.83 – 4.72 (m, 2H), 4.67 – 4.60 (m, 1H). ^{13}C NMR (151 MHz, $CDCl_3$-d_6, δ): 166.13, 165.22, 164.82, 162.86, 151.85, 142.19, 136.26, 133.79, 133.69, 133.51, 130.31, 129.71, 129.65, 129.12, 129.02, 128.64, 128.55, 128.45, 128.40, 128.31, 128.21, 125.28, 123.69, 103.75, 88.30, 79.76, 75.12, 70.69, 63.24, 29.68. HRMS (ESI-TOF) m/z : $[M+H]^+$ Calcd for $C_{33}H_{27}N_4O_9$ 623.17723; Found 623.1768



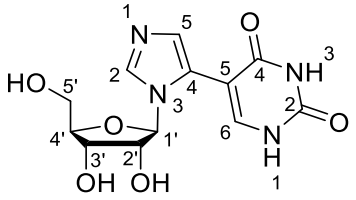
3b

5-(8-(2',3',5'-Tribenzoyl)ribofuranos-1'-yl)-imidazol-4-yl)uracil (3b) This side-product was isolated by column chromatography (R_f=0.35 DCM/MeOH (19:1 v/v))as an off-white foam (61.6 mg, 9% yield) and identified to be an isomer of **2a** by ^{1}H-NMR and MS, awaiting further identification by 2D-NMR after deprotection of the benzyl-groups.^{1}H NMR (300 MHz, $CDCl_3$-*d*, *δ*) 10.81 (bs, 1H), 9.90 (bs, 1H), 8.00 (d, $^3J_{HH}$ = 6.8 Hz, 3H), 7.84 (t, $^3J_{HH}$ = 8.5 Hz, 4H), 7.56 – 7.35 (m, 7H), 7.29 (s, 2H), 7.24 (s, 1H), 7.02 (s, 1H), 6.23 (d, $^3J_{HH}$ = 3.1 Hz, 1H), 5.94 (s, 1H), 5.79 (t, $^3J_{HH}$ = 5.7 Hz, 1H), 4.82 – 4.69 (m, 2H), 4.62 (d, $^3J_{HH}$ = 8.7 Hz, 1H). HRMS (ESI-TOF) m/z : $[M+H]^+$ Calcd for $C_{33}H_{27}N_4O_9$ 623.17723;Found 623.1769; m/z : $[M+Na]^+$ Calcd for $C_{33}H_{26}N_4O_9Na$ 645.15921;Found 645.1578



4a

5-(Imidazol-4-yl)uridine (4a) 7N NH_3 in MeOH (20 mL) was added to **3a** (320 mg, 0.51 mmol) in a pressure tube (CAUTION cfr supra) overnight at RT. The reaction mixture was evaporated *in vacuo*, followed by recrystallization of the residue in MeOH/iPrOH. The obtained precipitate was filtered off, washed with small amounts of cold iPrOH and Et_2O, followed by drying *in vacuo*, yielding 160 mg (quant. yield) of a white foam identified to be the product by 2D-NMR. ^{1}H NMR (600 MHz, DMSO-d_6, *δ*) 12.07 (s, 1H), 11.49 (s, 1H), 8.26 (s, 1H), 7.65 (s, 1H), 7.54 (s, 1H), 5.90 (d, $^3J_{HH}$ = 6.1 Hz, 1H), 5.40 (d, $^3J_{HH}$ = 5.5 Hz, 1H), 5.14 (d, $^3J_{HH}$ = 4.3 Hz, 1H), 5.02 (t, $^3J_{HH}$ = 4.8 Hz, 1H), 4.10 (dd, $^3J_{HH}$ = 7.0, 3.6 Hz, 1H), 3.97 (d, $^3J_{HH}$ = 3.6 Hz, 1H), 3.87 (q, $^3J_{HH}$ = 3.7 Hz, 1H), 3.62 – 3.51 (m, 2H), 3.17 (d, $^3J_{HH}$ = 5.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6, *δ*) 161.52, 150.25, 135.20, 134.06, 132.20, 114.44, 109.42, 87.49, 85.11, 73.34, 70.47, 61.58. HRMS (ESI-TOF) m/z : $[M+H]^+$ Calcd for $C_{12}H_{15}N_4O_6$ 311.09860; Found 311.0995.



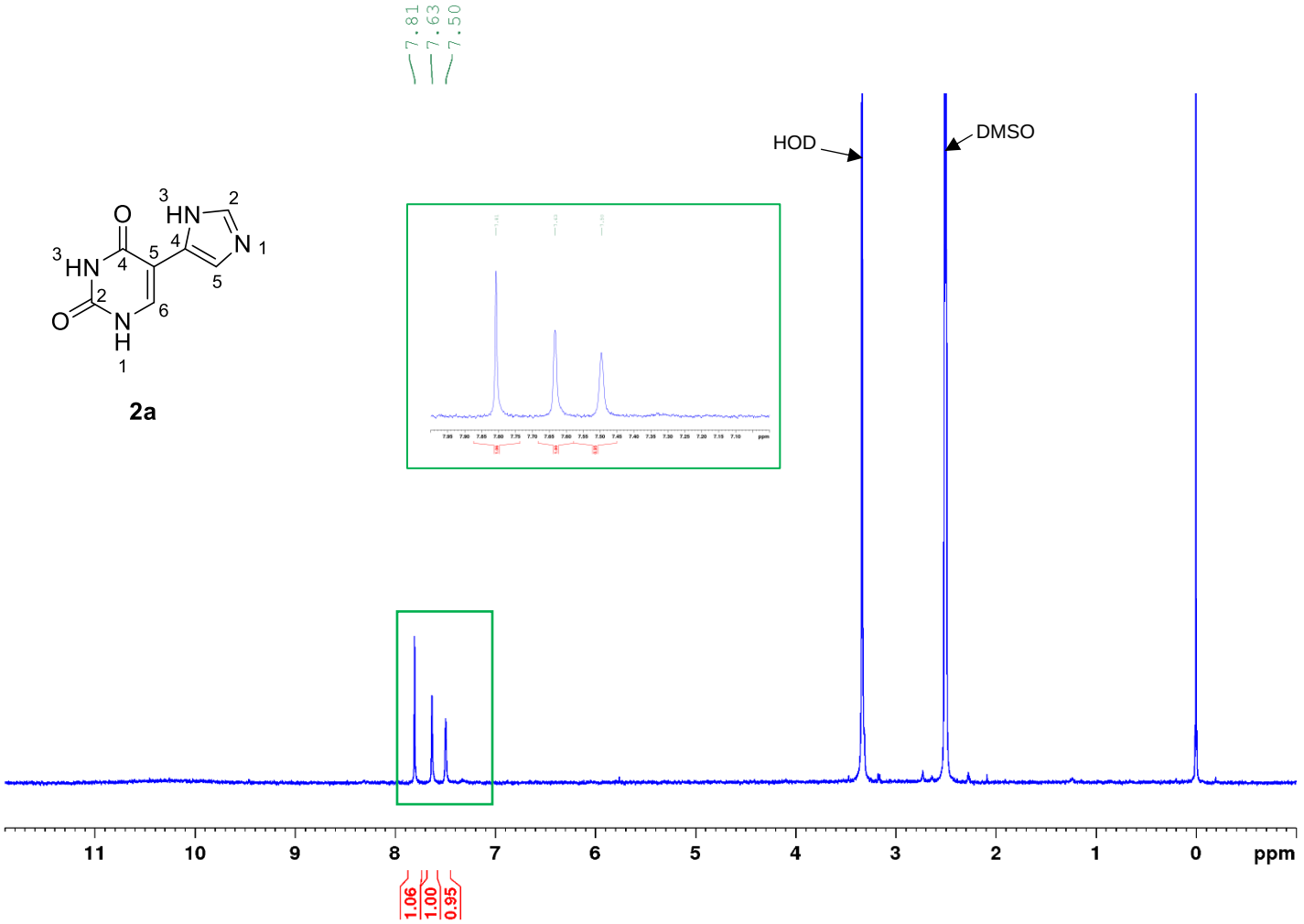
4b

5-(8-Ribofuranos-1'-yl)-imidazol-4-yl)uracil (4b) 7N NH_3 in MeOH (10 mL) was added to **3b** (120 mg, 0.19 mmol) in a pressure tube (CAUTION cfr supra) overnight at RT. The reaction mixture was evaporated *in vacuo*, followed by purification by column chromatography (R_f=0.15 DCM/MeOH/NH_4OH (aq) (17:7:1 v/v)) to obtain an off-white solid. An analytical sample was recrystallized in MeOH/iPrOH. The obtained precipitate was filtered off, washed with small amounts of cold iPrOH and Et_2O, followed by drying *in vacuo*, to obtain 60 mg (quant. yield) white foam identified to be the product by 2D-NMR. Single crystals of $C_{12}H_{14}N_4O_6$·HCl [**4b**·HCl] were obtained by dissolving an analytical sample in boiling MeOH, a drop of 0.1M HCl was added and the solution was slowly evaporated at ambient temperature and atmosphere. ^{1}H NMR (400 MHz, MeOD-d_4, *δ*): 8.16 (s, 1H), 7.57 (s, $^3J_{HH}$ = 4.6 Hz, 1H), 6.98 (s, $^3J_{HH}$ = 1.1 Hz, 1H), 5.56 (d, $^3J_{HH}$ = 4.7

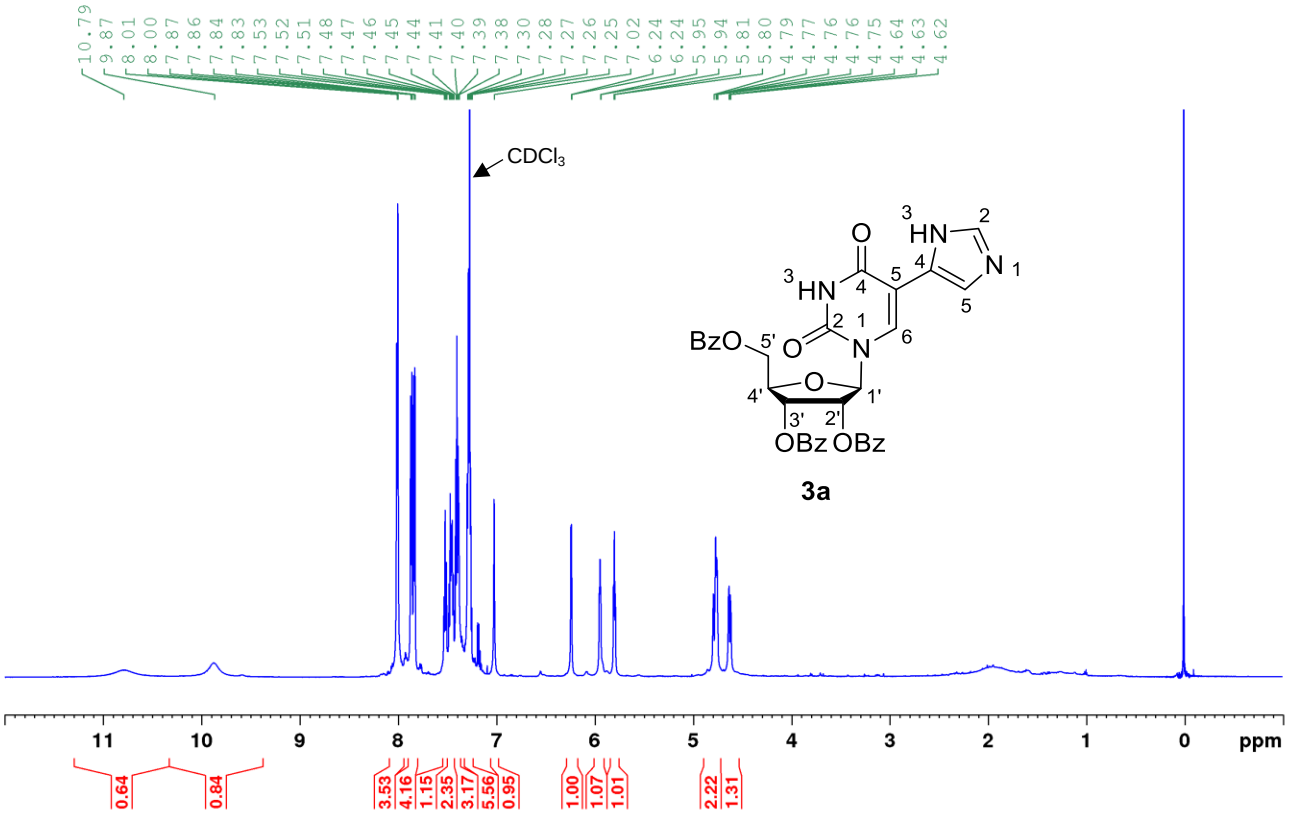
Hz, 1H), 4.28 (t, $^{3}J_{HH}$ = 5.0 Hz, 1H), 4.21 (t, $^{3}J_{HH}$ = 5.0 Hz, 1H), 4.00 (dd, $^{3}J_{HH}$ = 7.9, 3.4 Hz, 1H), 3.86 – 3.78 (m, 1H), 3.74 – 3.64 (m, 1H). ^{13}C NMR (151 MHz, MeOD-d_4, δ): 162.76, 151.11, 144.93, 134.31, 125.56, 122.48, 99.42, 90.68, 85.20, 75.83, 68.85, 59.96; HRMS (ESI-TOF) m/z : $[M+H]^+$ Calcd for $C_{12}H_{15}N_4O_6$ 311.09860;Found 311.0988

NMR

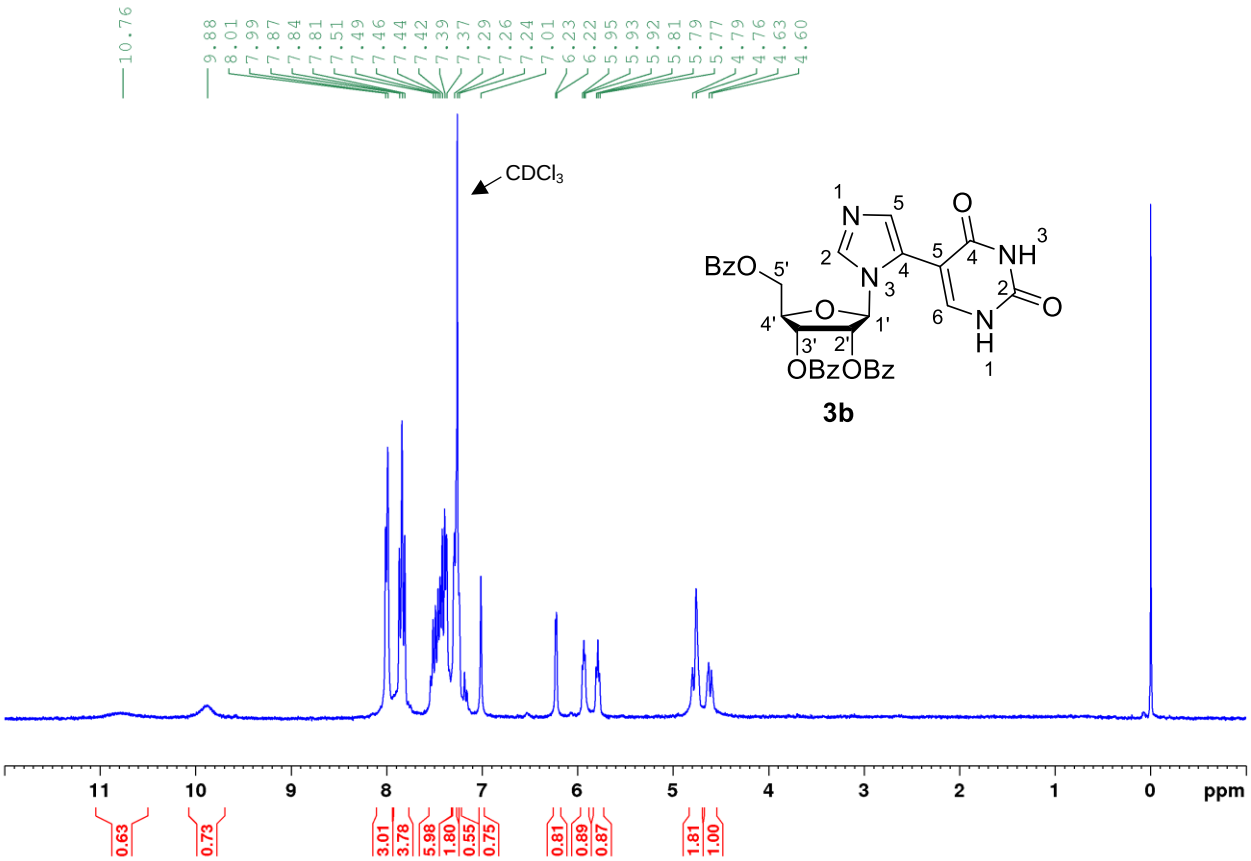
5-(Imidazol-4-yl)uracil (2a) ^{1}H NMR (300 MHz, DMSO-d_6)



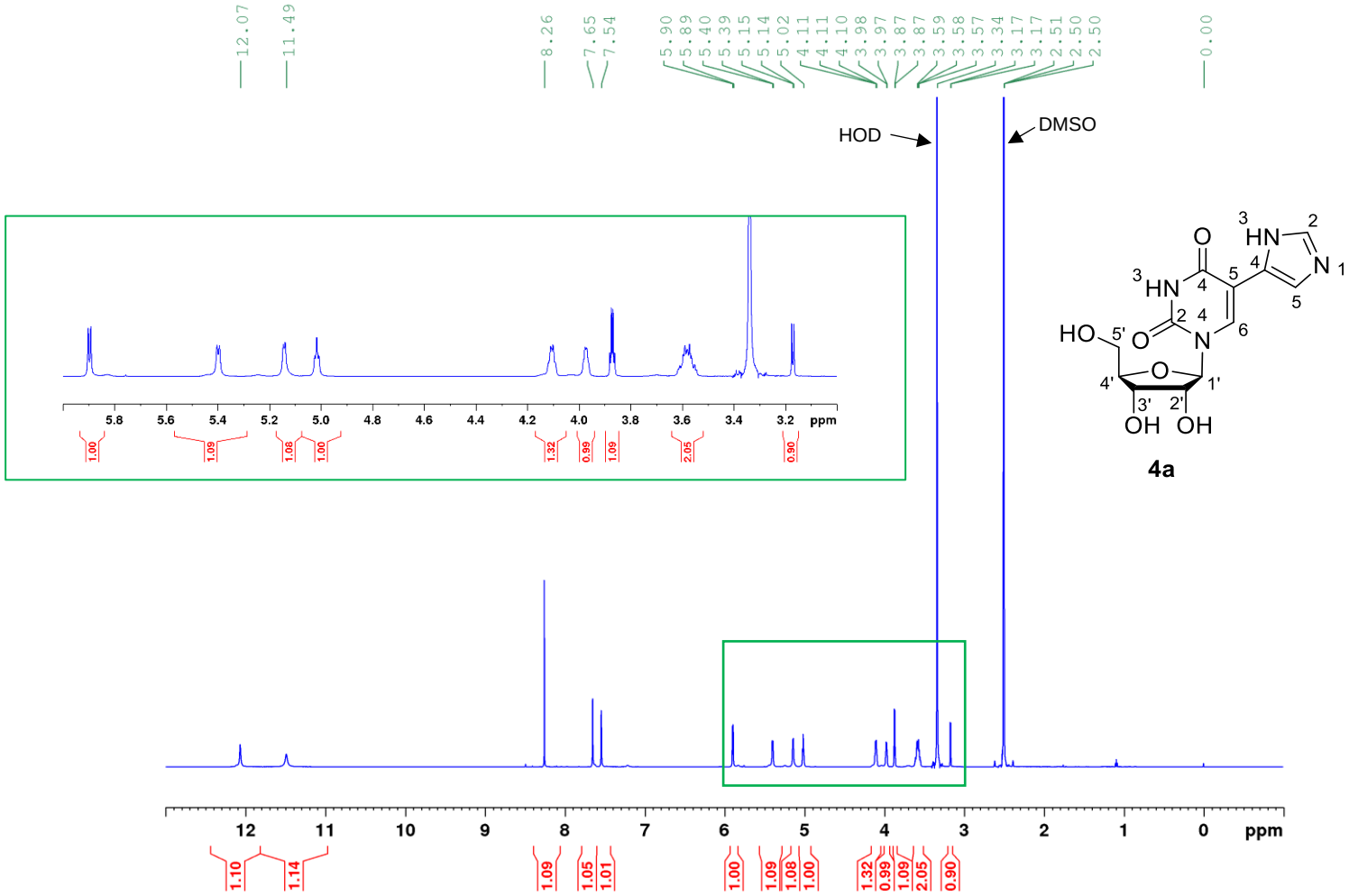
2',3',5'-Tribenzoyl 5-(imidazol-4-yl)uridine (3a) ^{1}H NMR (600 MHz, $CDCl_3$-*d*, *δ*)



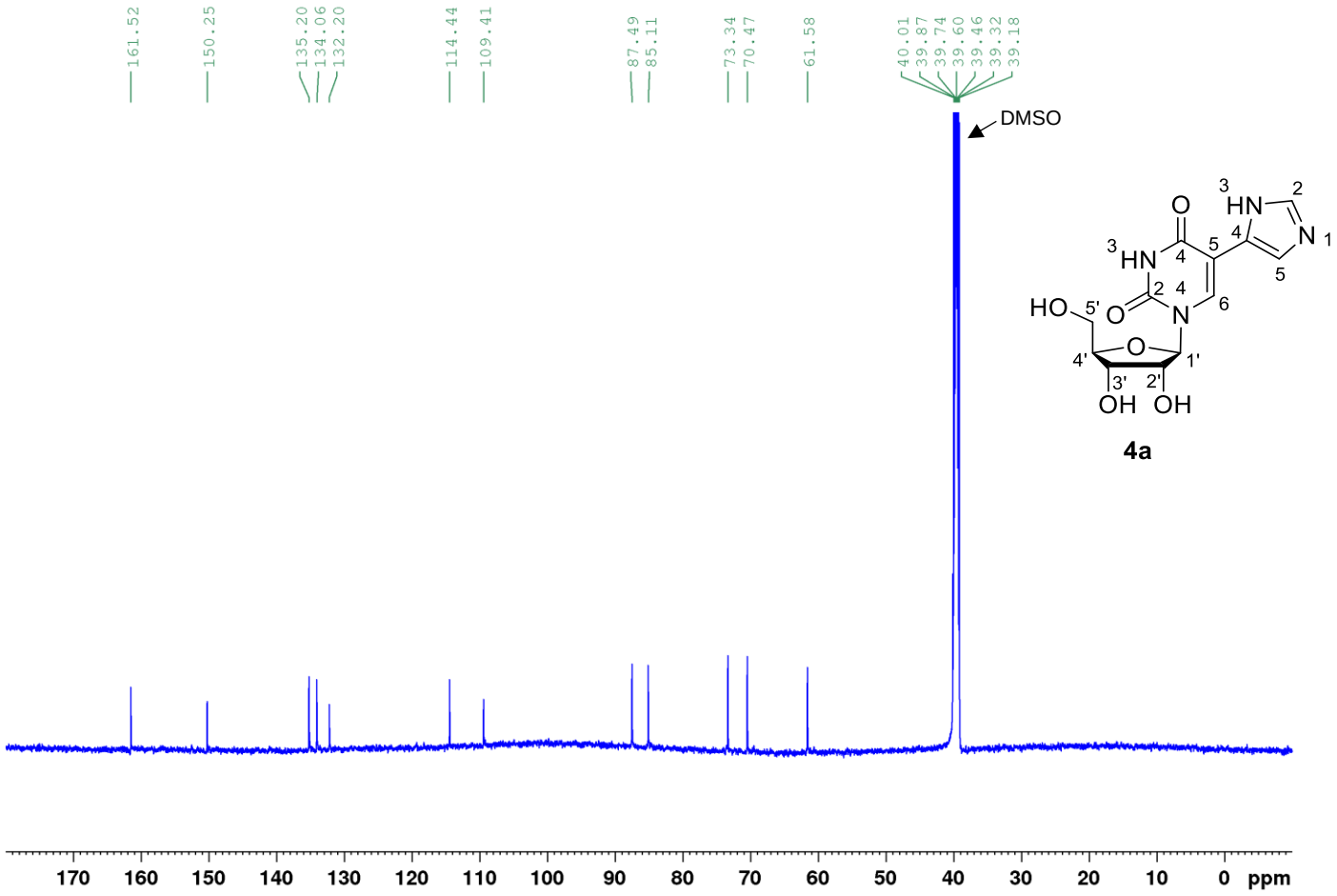
5-(8-(2',3',5'-Tribenzoyl)ribofuranos-1'-yl)-imidazol-4-yl)uracil (3b): ^{1}H NMR (300 MHz, $CDCl_3$)



5-(Imidazol-4-yl)uridine (4a): ^{1}H NMR (600 MHz, DMSO-d_6)

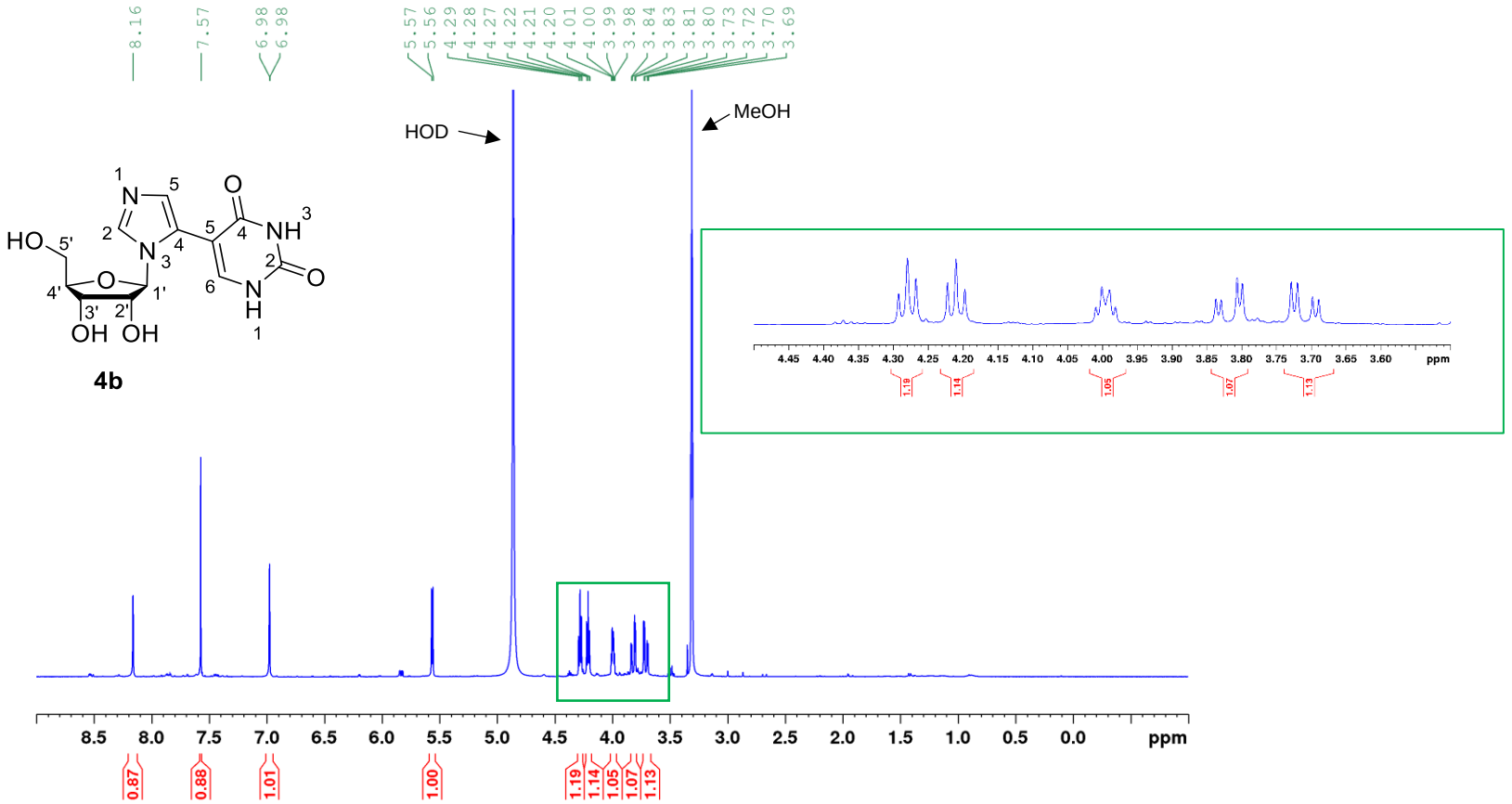


5-(Imidazol-4-yl)uridine (4a): ^{13}C NMR (151 MHz, DMSO-d_6)

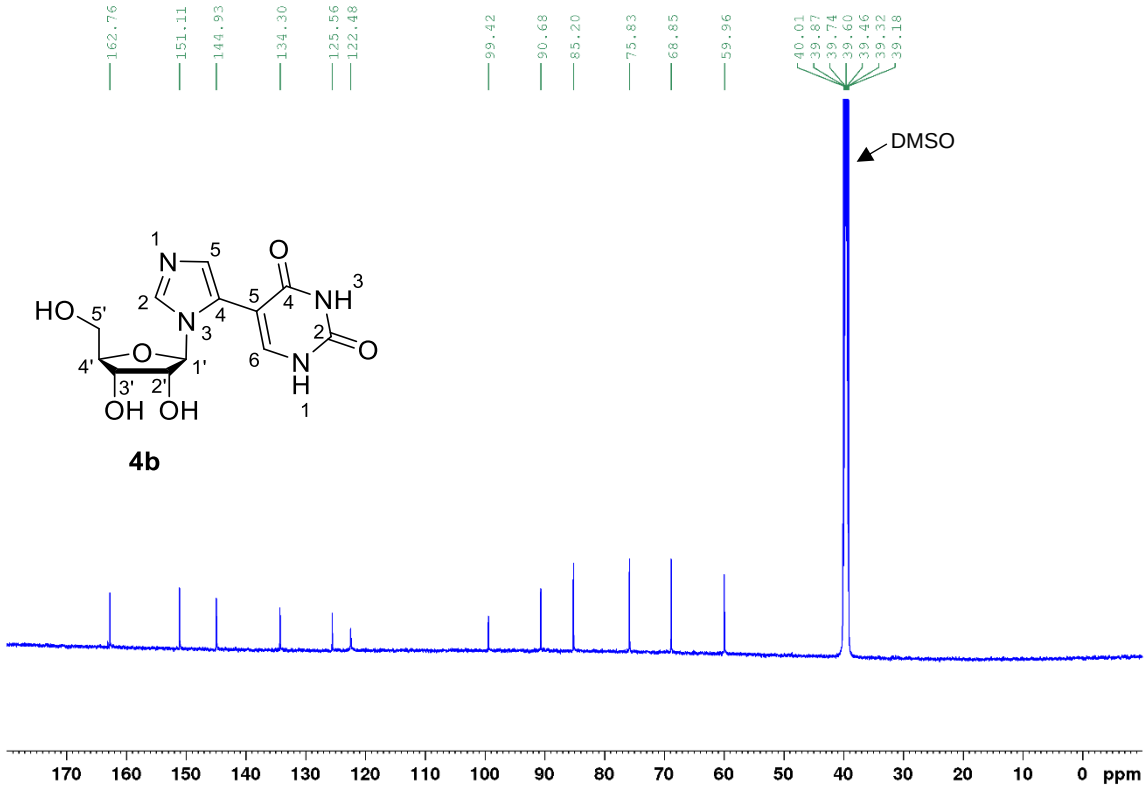


4a

5-(8-Ribofuranos-1'-yl)-imidazol-4-yl)uracil (4b) ^{1}H NMR (400 MHz, MeOD-d_4)



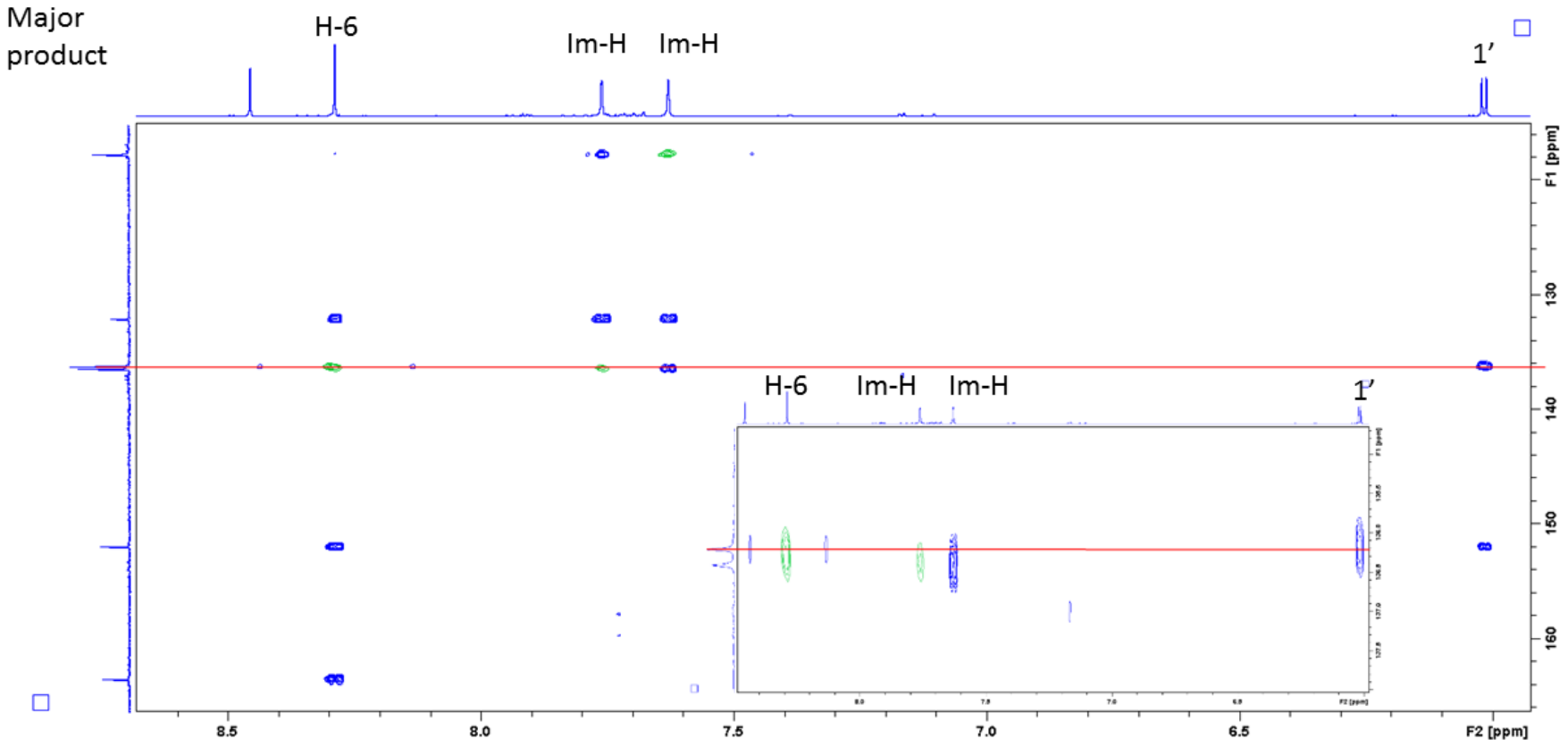
5-(8-Ribofuranos-1'-yl)-imidazol-4-yl)uracil (4b) ^{13}C NMR (151 MHz, DMSO-d_6)


162.76
151.11
144.93
134.30
125.56
122.48
99.42
90.68
85.20
75.83
68.85
59.96
40.01
39.87
39.74
39.60
39.46
39.32
39.18
DMSO
HO
5'
4'
3'
2'
1'
OH
OH
O
N
NH
O
H
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

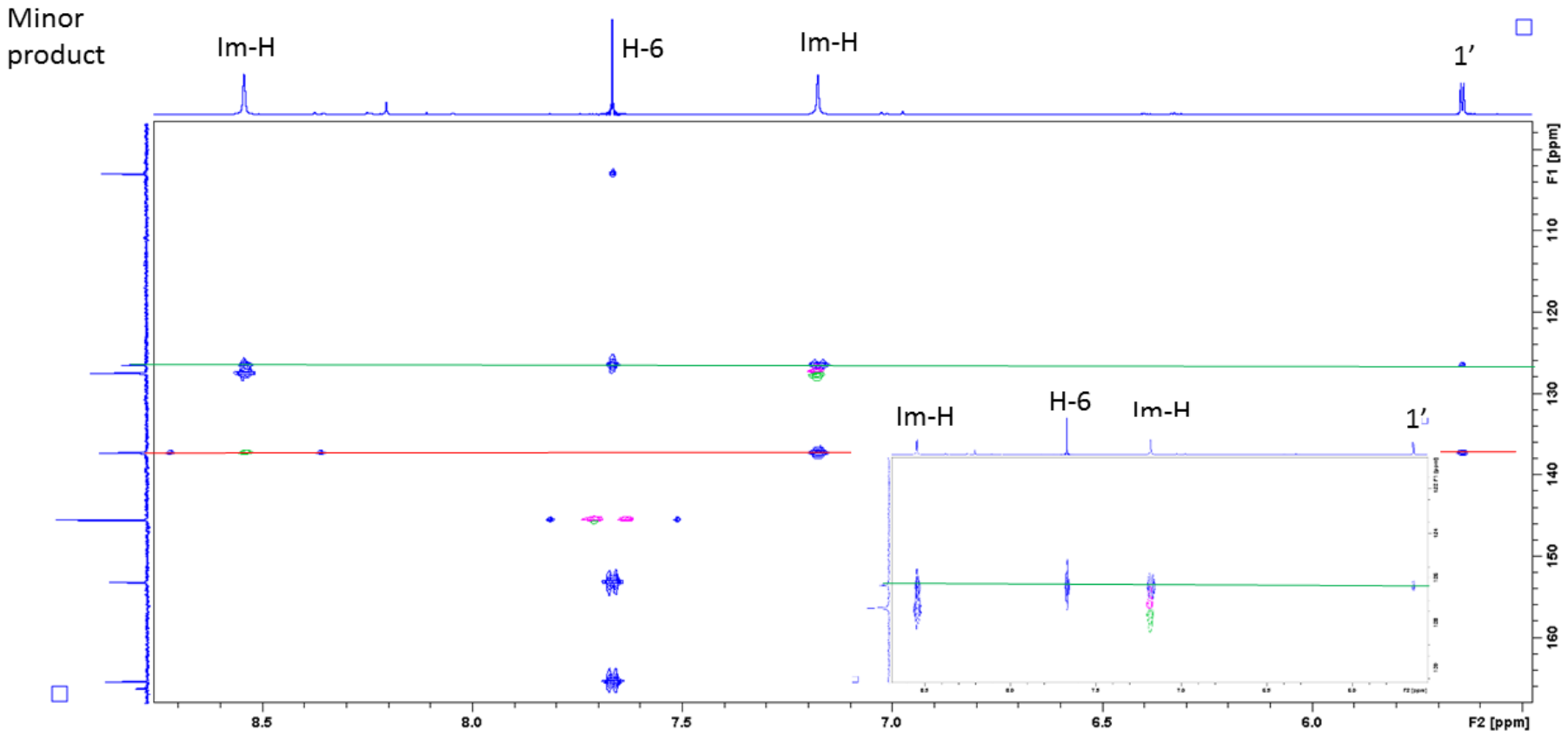
4b

NMR analysis of products formed:

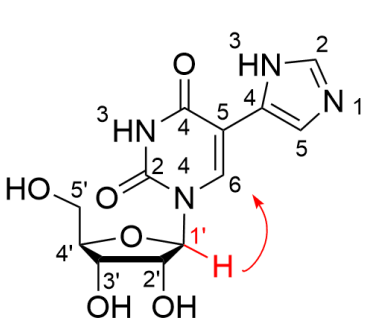
2D ^{13}C-^{1}H HMBC (blue)-HSQC (green-pink) in MeOD-d_4



2D ^{13}C-^{1}H HMBC (blue)-HSQC (green-pink) in MeOD-d_4

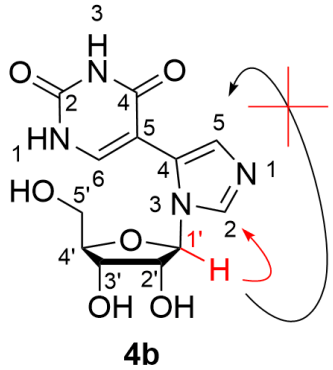


Hypothesized structures and rationale for assignment of structures



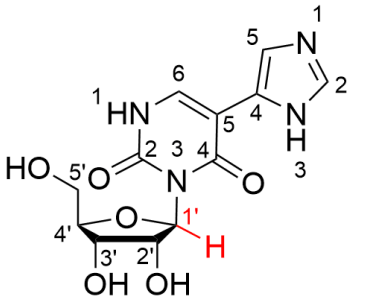
4a

vs.



4b

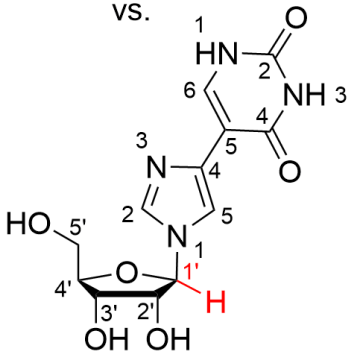
vs.



4c

vs.

vs.



4d

The **major product** shows the anomeric ($H^{1'}$) proton of the sugar interacting with the C^6 of uracil indicating **4a** as the only possible structure from all hypothesized structures (red line). The insert illustrates further that this interaction is attributed to the C^6 of uracil and not a carbon from one of the imidazole CH positions.

Similarly, the **minor product** shows interaction of only one imidazole carbon to the sugar $H^{1'}$ (red line) indicating **4b** as the only possible structure. The insert (including the green line) illustrates that these crosspeaks do not come from the interaction of the 1' position with the other imidazole proton.

On the other hand, **4d** would have shown interactions between the two imidazole carbons and $H^{1'}$ and **4c** would not show any interactions between aromatic protons and the anomeric position.

X-ray crystallographic data for 4b·HCl

Single crystals of $C_{12}H_{14}N_4O_6$·HCl [**4b·HCl**] were obtained by dissolving an analytical sample in boiling MeOH, a drop of 0.1M HCl was added and the solution was slowly evaporated at ambient temperature and atmosphere. A suitable crystal was selected and X-ray intensity data were collected on an Agilent SuperNova diffractometer with Eos CCD detector using MoK_α radiation (λ = 0.71073 Å). The crystal was kept at 293(2) K during data collection. Data frames were processed (unit cell determination, intensity data integration, correction for Lorentz and polarization effects, and empirical absorption correction) using CrysAlis PRO[3]. Using Olex2[4], the structure was solved with the ShelXT[5] structure solution program using Intrinsic Phasing and refined with the ShelXL[6] refinement package using full-matrix least-squares minimization on F^2. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms H2, H3, H4, H5, H2A and H3A were located from a difference electron density map and refined freely. The other hydrogen atoms were placed in idealized positions and included as riding contributions with isotropic temperature factors fixed at 1.2 times U_{eq} of the parent atoms. CCDC 1996102 contains the supplementary crystallographic data for **4b**·HCl and can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336033; or deposit@ccdc.cam.ac.uk).

Table S1. Crystal data and structure refinement for 4b·HCl

Empirical formula	$C_{12}H_{14}N_4O_6$·HCl
Formula weight	346.73
Temperature/K	293(±2)
Crystal system	monoclinic
Space group	$P2_1$
a/Å	4.8027(±0.0003)
b/Å	12.6451(±0.0006)
c/Å	11.8960(±0.0007)
α/°	90
β/°	90.448(±0.005)
γ/°	90
Volume/Å^3	722.43(±0.07)
Z	2
ρ_{calc}g/cm^3	1.594
μ/mm^{-1}	0.304
F(000)	360.0
Crystal size/mm^3	0.2 × 0.15 × 0.1
Radiation	MoKα (λ = 0.71073 Å)
2Θ range for data collection/°	6.444 to 52.744
Index ranges	-6 ≤ h ≤ 5, -15 ≤ k ≤ 15, -14 ≤ l ≤ 14
Reflections collected	14478
Independent reflections	2920 [R_{int} = 0.0920, R_{sigma} = 0.0542]
Data/restraints/parameters	2920/7/232
Goodness-of-fit on F^2	1.077

Final R indexes [I>=2σ (I)]	R_1 = 0.0460, wR_2 = 0.1098
Final R indexes [all data]	R_1 = 0.0522, wR_2 = 0.1166
Largest diff. peak/hole / e Å^{-3}	0.48/-0.27
Flack parameter	-0.12(±0.06)

Table S2. Hydrogen bond interactions for 4b·HCl

D	H	A	D-H	H…A	D…A	D-H…A
O2	H2	O5^{i}	0.82(±0.06)	1.94(±0.06)	2.734(±0.005)	164(±6)
N2	H2A	O2^{ii}	0.87(±0.06)	2.58(±0.08)	3.114(±0.006)	120(±6)
N2	H2A	O3^{ii}	0.87(±0.06)	1.93(±0.05)	2.756(±0.005)	157(±6)
O3	H3	Cl1^{iii}	0.80(±0.05)	2.25(±0.05)	3.030(±0.005)	168(±6)
N3	H3A	Cl1^{i}	0.84(±0.05)	2.39(±0.04)	3.216(±0.004)	172(±7)
N4	H4	Cl1^{iv}	0.87(±0.05)	2.43(±0.06)	3.154(±0.004)	141(±6)
O5	H5	O6^{ii}	0.82(±0.06)	1.90(±0.06)	2.684(±0.005)	161(±6)

Symmetry codes: (i) 1-x,1/2+y,1-z; (ii) 2-x,-1/2+y,1-z; (iii) 1+x,y,z; (iv) x,y,1+z.

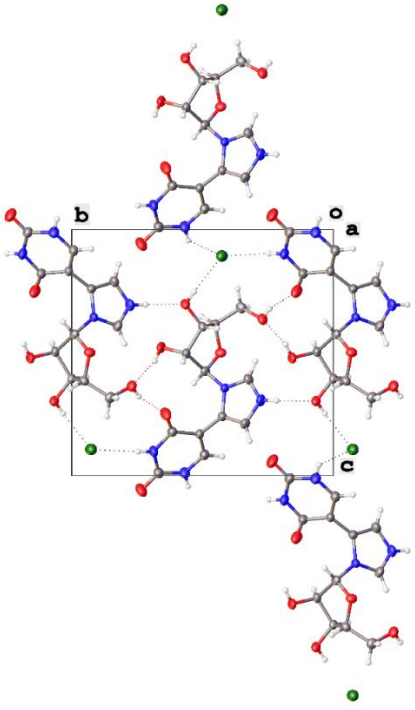


Figure S1. Crystal packing of **4b·HCl** showing O-H…O, N-H…O, O-H…Cl and N-H…Cl hydrogen bonds.

Fluorescence and absorption spectroscopy

Experimental

For solubility reasons, the stock solutions for the spectroscopic measurements were prepared in DMSO and further diluted to 1×10^{-5} M using commercially available solvents that were used as received. The final DMSO content was around 0.3 %. The UV-Visible absorption spectra were recorded on a Lambda 40 spectrophotometer with blank correction. For the fluorescence experiments a Horiba Jobin-Yvon Fluorolog FL3-22 fluorimeter was used with corrections for the excitation beam intensity, the wavelength dependent sensitivity of the detector and the optical path. Absolute fluorescence quantum yields were measured on the same machine using an integrating sphere and homemade cylindrical quartz cuvettes.[7] When recording the Rayleigh scatter, a neutral density filter of 0.3 % was used.

The details of the time-correlated single photon counting setup are published elsewhere.[8] The obtained fluorescence decay was fitted with a function of the form

$$I(t) = A \cdot \exp\left(\frac{-t}{\tau}\right)$$

using the homemade TRFA program.

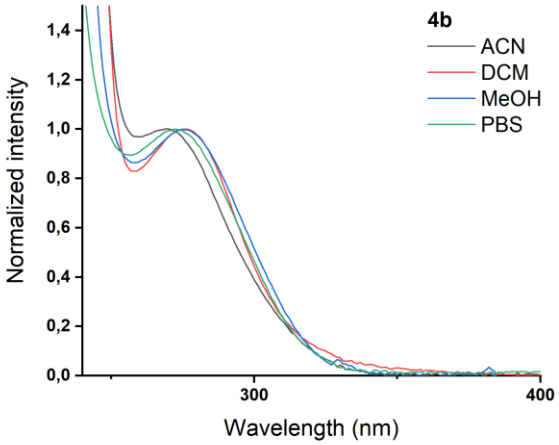


Figure S2. Normalised absorption spectra of **4b** in acetonitrile (ACN), dichloromethane (DCM), methanol (MeOH) and phosphate buffered saline.

Table S3. Maxima of the $S_0 \rightarrow S_1$ transition for **4b** in acetonitrile (ACN), dichloromethane (DCM), methanol (MeOH) and phosphate buffered saline (PBS).

Solvent	λ_{abs} (nm)
ACN	270
DCM	276
MeOH	275
PBS	272

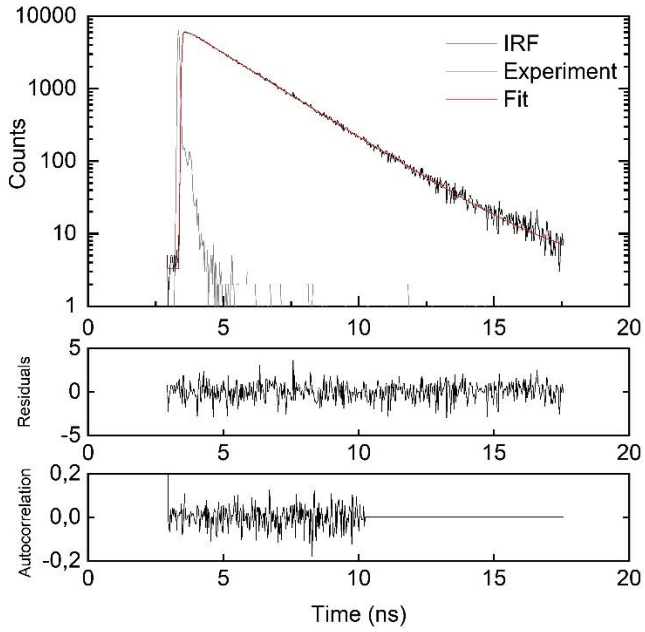


Figure S3. Time-correlated single photon counting fluorescence decay of **4a** in PBS. In the main panel, the instrument response function (IRF), the data (Experiment) and a mono-exponential fit (τ = 1.88 ns, χ^2 = 1.015) are displayed. The bottom two panels show residuals and autocorrelation associated with this fit. Excitation occurred at 330 nm, detection at 430 nm.

Table S4. Extinction coefficients of 4a and 4b in acetonitrile (ACN), dichloromethane (DCM), methanol (MeOH) and phosphate buffered saline (PBS) at maximum absorbance (Table 3 for **4a** and S3 for **4b**) and 260nm.

4a	ε_{max} ($M^{-1}cm^{-1}$)	ε_{260} ($M^{-1}cm^{-1}$)	**4b**	ε_{max} ($M^{-1}cm^{-1}$)	ε_{260} ($M^{-1}cm^{-1}$)
PBS	8400	5800	PBS	5500	5000
DCM	7600	4900	DCM	6900	5700
ACN	9300	4200	ACN	5900	5700
MeOH	9300	3800	MeOH	5300	4600

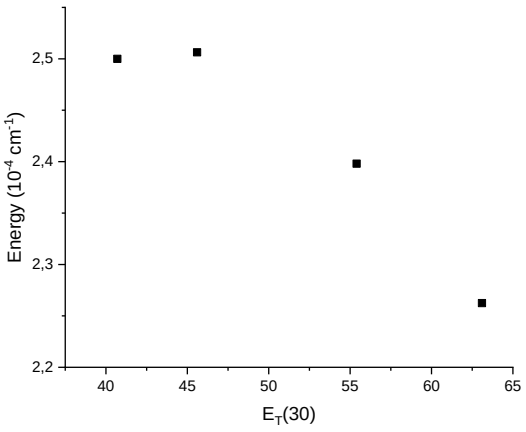


Fig. S4 Emission energy at the maximum in function of E_T(30)-values. E_T(30) DCM = 40.7; E_T(30) ACN = 45.6, E_T(30) MeOH = 55.6; E_T(30) H2O = 63.1[9]

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