Hydrogen-bonding controlled rigidity of an isoindoline-derived nitroxide spin label for nucleic acids

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Table of Contents

General materials and methods

Thymidine was purchased from Rasayan Inc. USA. Other chemicals were purchased from Sigma Aldrich and used without further purification. Water was purified on MILLI-Q water purification system. Thin layer chromatography (TLC) was carried out using glass plates pre-coated with silica gel (0.25 mm, F-254) from Silicycle. Compounds were visualized by UV light and staining with *p*-anisaldehyde. Flash column chromatography was performed using ultra pure flash silica gel (Silicycle, 230-400 mesh size, 60 Å). Dichloromethane and pyridine were freshly distilled over calcium hydride prior to use. Anhydrous triethylamine, n-hexane and ethyl acetate were used directly as received. All moisture and air sensitive reactions were carried out in oven dried glassware under an inert argon atmosphere. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 400 MHz spectrometer. ^{1}H NMR chemical shifts are reported in parts per million (ppm) relative to the residual proton signal of solvents $CDCl_3$ (7.26 ppm), d_6-DMSO (2.50 ppm), d_4-MeOH (3.31 and 4.84 ppm) for ^{1}H NMR and $CDCl_3$ (77.0 ppm), d_6-DMSO (39.43 ppm), d_4-MeOH (49.05) for ^{13}C NMR. ^{31}P NMR chemical shifts are reported relative to 85% H_3PO_4 as an external standard. Commercial grade $CDCl_3$ was passed over basic alumina shortly before use with tritylated compounds. NMR spectra of compounds containing a nitroxide radical show significant broadening, sometimes to the extent that some nuclei are not seen in the spectra.[1,2] Therefore, integration of ^{1}H NMR spectra of nitroxides is not reported. Mass spectrometric analyses of all organic compounds were performed on an HR-ESI-MS (Bruker, MicroTof-Q) in positive ion mode. UV-VIS spectra were recorded on a PerkinElmer Lambda 25 UV/VIS spectrometer. CD spectra were recorded on a JASCO J-810 spectropolarimeter at 25 °C with path length of 1 mm (Hellma), 10 scans, scanned from 500 nm to 200 nm with response of 1s, data pitch of 0.1 nm and band width of 1.0 nm. Molecular weight (MW) of oligonucleotides was determined by MALDI-TOF analysis (Bruker, Autoflex III).

List of abbreviations

*m*CPBA	*meta*Chloroperbenzoic acid
DMTrCl	4,4'-Dimethoxytrityl chloride
EPR	Electron paramagnetic resonance

Synthesis and purification of oligonucleotides

All commercial phosphoramidites, columns and solutions were purchased from ChemGenes Corporation. Unmodified and spin-labelled oligonucleotides were synthesized by a trityl-off synthesis on a 1.0 μmol (1000 Å CPG columns) scale using phosphoramidites with standard base protection on an automated DNA synthesizer ASM800 (Biossset, Russia). The spin-labelled DNA was prepared by using previously reported protocols;[3] the spin-labelled phosphoramidite was incorporated into the oligonucleotides by manual coupling by pausing the synthesizer program after completion of the prior cycle, removing the column from the synthesizer and running 200 μL of standard activator solution and 200 μL of a 0.05 M solution of spin-labelled phosphoramidite in 1,2 dichloroethane back and forth through the column for 10 min. Then the column was re-mounted on the synthesizer to complete the cycle. The oligos were deprotected from solid support using 33% aq. NH_3 at 55 °C for 8 h and purified by 20% denaturing polyacryamide gel electrophoresis (DPAGE). The DNA bands were visualized by UV and excised from the gel, crushed and soaked in 10 mL buffer (250 mM NaCl, 10 mM Tris, 1 mM Na_2EDTA, pH 7.5) for 12 h. A polyethersulfone membrane (0.25 μm, disposable filter device from Whatman) was used for filtration of the DNA elution solutions, which were desalted using Sep-Pak cartridges (Waters Corporation) according to manufacturer's instructions. Concentrations of oligonucleotides were calculated by Beer's law based on measurements of UV absorbance at 260 nm using extinction coefficients (ε) which were determined by using UV WinLab oligonucleotide calculator (V2.85.04, PerkinElmer).

Figure S1 shows an analytical denaturing polyacrylamide gel of spin-labelled and unmodified (T instead of ^{X}**U**) oligonucleotides. Mobility of spin-labelled oligomers is slower, as seen from the lane 2 and 4 where spin-labelled and unmodified oligomers were co-spotted.

Lane	Sample
1	GAC CTC G^{Im}**U**A TCG TG
2	GAC CTC GTA TCG TG + GAC CTC G^{Im}**U**A TCG TG
3	GAC CTC GTA TCG TG
4	GAC CTC GTA TCG TG + GAC CTC G^{Ox}**U**A TCG TG
5	GAC CTC G^{Ox}**U**A TCG TG

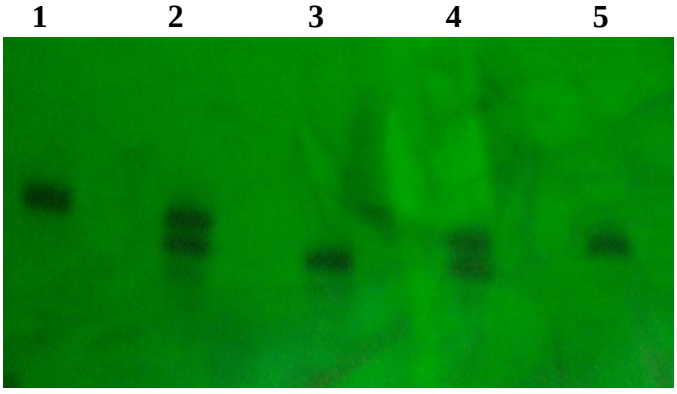


Fig. S1 Analytical gel of spin-labelled and unmodified oligonucleotides.

EPR measurements

2.0 nmol of spin-labelled, single-stranded DNA and 2.4 nmol of its complementary strand was dissolved in phosphate buffer (10 mM phosphate, 100 mM NaCl, 0.1 mM Na_2EDTA, pH 7.0) (10 μL, oligo final conc. 200 μM) and annealed using the following annealing protocol: 90 °C for 2 min, 60 °C for 5 min, 50 °C for 5 min, 40 °C for 5 min, 22 °C for 15 min. Samples (10 μL) were placed in a quartz capillary for EPR measurements. Continuous wave (CW) EPR spectra were recorded on a MiniScope MS200 spectrometer using 100 kHz modulation frequency, 1.0 G modulation amplitude and 2.0 mW microwave power. Each spectrum was scanned 100-120 times. The temperature was regulated by Magnettech temperature controller M01 with error ± 0.5 °C.

MALDI-Tof MS analysis of oligonucleotides

The incorporation of ^{Im}**U** spin label was confirmed by MALDI-Tof MS analysis. The instrument was calibrated with external standard prior to measuring the mass of spin-labelled oligonucleotide. The calculated monoisotopic mass of spin-labelled single strand GAC CTC G^{Im}**U**A TCG TG is 4467.84 and observed mass is 4468.07.

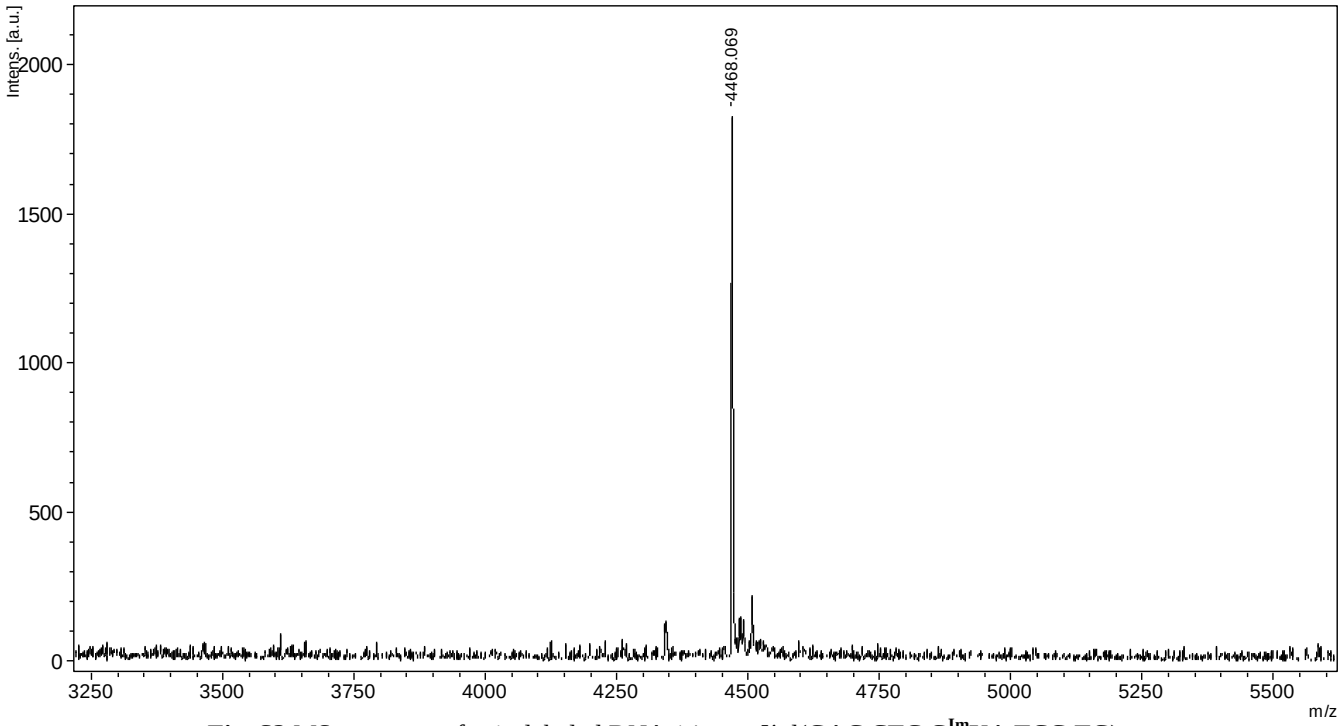


Fig. S2 MS spectrum of spin-labeled DNA 14-mer 5′-d(GAC CTC G^{Im}**U**A TCG TG).

CD measurements

To determine if the spin labels had any effect on the DNA duplex conformation, circular dichroism (CD) spectra of 14-mer unmodified and ^{Im}**U** and ^{Ox}**U** spin-labelled duplex were recorded. The DNA samples (2.5 nmol of duplex) were dissolved in phosphate buffer (10 mM phosphate, 100 mM NaCl, 0.1 mM Na_2EDTA, pH 7.0), annealed and diluted to 200 µL with the same buffer. Both spectra possessed negative and positive molar ellipticities at ca. 250 and 280 nm respectively, which is characteristic of a right-handed B-DNA indicating that the spin-labelled nucleosides ^{Im}**U (Figure 3A)** and ^{Ox}**U (Figure 3B)** do not alter the conformation of duplex DNA.

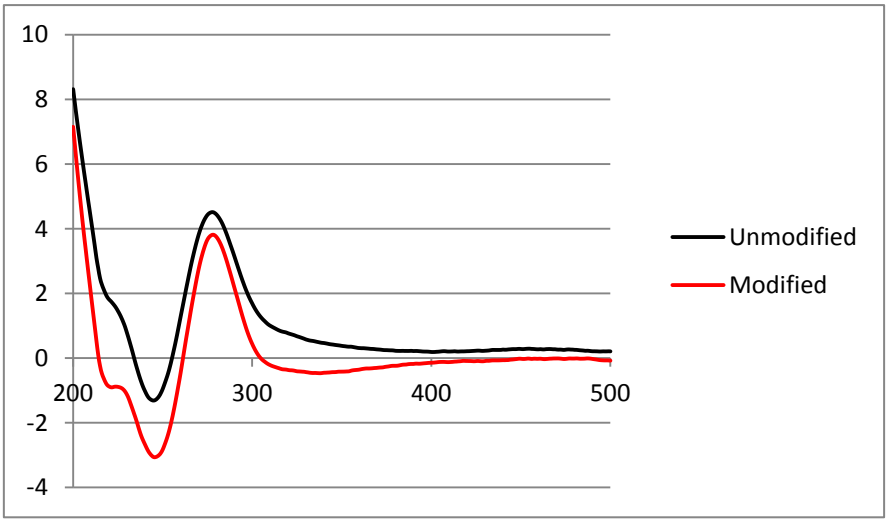


Fig. S3A CD spectra of an unmodified 14-mer DNA duplex (black) and spin-labelled ^{Im}**U**-containing DNA duplex (red).

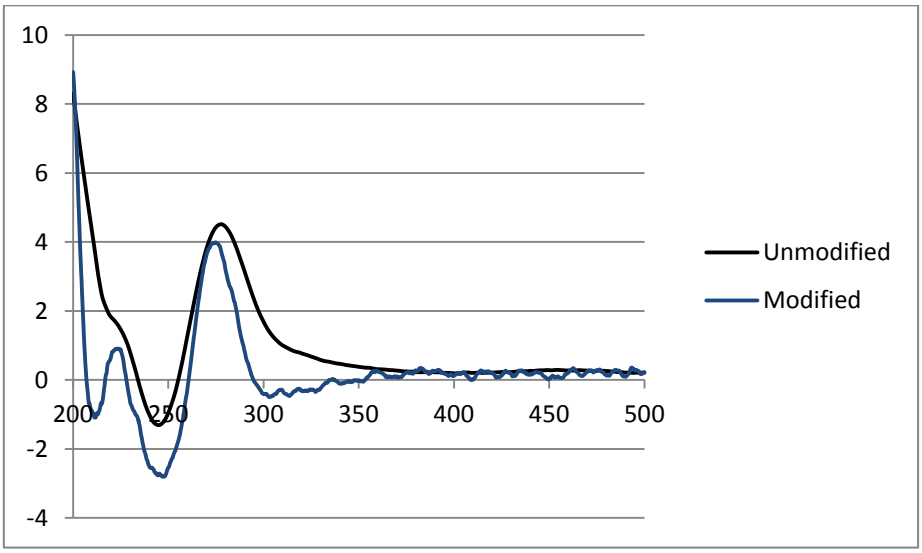


Fig. S3B CD spectra of an unmodified 14-mer DNA duplex (black) and spin-labelled ^{Ox}**U**-containing DNA duplex (blue).

Tm measurements

To determine if ^{Im}**U** and ^{Ox}**U** affect the stability of DNA duplexes, the thermal denaturation curves of unmodified and spin-labelled oligomers were determined. DNA samples (4.0 nmol of each strand) were dissolved in phosphate buffer (100 µL) (10 mM phosphate, 100 mM NaCl, 0.1 mM Na_2EDTA, pH 7.0), annealed and diluted to 1.0 mL with same phosphate buffer and degassed using argon. The samples were heated up from 20 °C to 90 °C (1.0 °C/min) and absorbance at 260 nm recorded. **Table S1** shows melting temperatures (T_Ms) of spin-labelled DNAs **2** and **3** and the unmodified 14 mer.

Table S1. Melting temperatures of DNA duplexes

Duplex	**Sequence**	**T_M(°C)**	**ΔT_M(°C)**
1	5'-GAC CTC GTA TCG TG 3'-CTG GAG CAT AGC AC	59.5 ± 1.0	
2	5'-GAC CTC G^{Im}**U**A TCG TG 3'-CTG GAG C AT AGC AC	55.5 ± 0.5	-4.0
3	5'-GAC CTC G^{Ox}**U**A TCG TG 3'-CTG GAG C AT AGC AC	53.5 ± 0.5	-6.0

T_M is melting temperature of duplex and ΔT_M is difference between unmodified and modified oligos.

Comparision of the mobility of ^{Im}U, Ç and ^{Ox}U in DNA

The mobility of the spin label ^{Im}**U** was compared with the rigid spin label **Ç** and spin label ^{Ox}**U** in the same DNA sequence at various temperatures. The mobility of ^{Im}**U**-DNAs is identical with rigid spin label **Ç** at -10 °C (**Figure S4**). The mobility of ^{Im}**U**-modified DNA increased upon increasing the temperature and became nearly identical with that of ^{Ox}**U**-modified DNA at 20 °C.

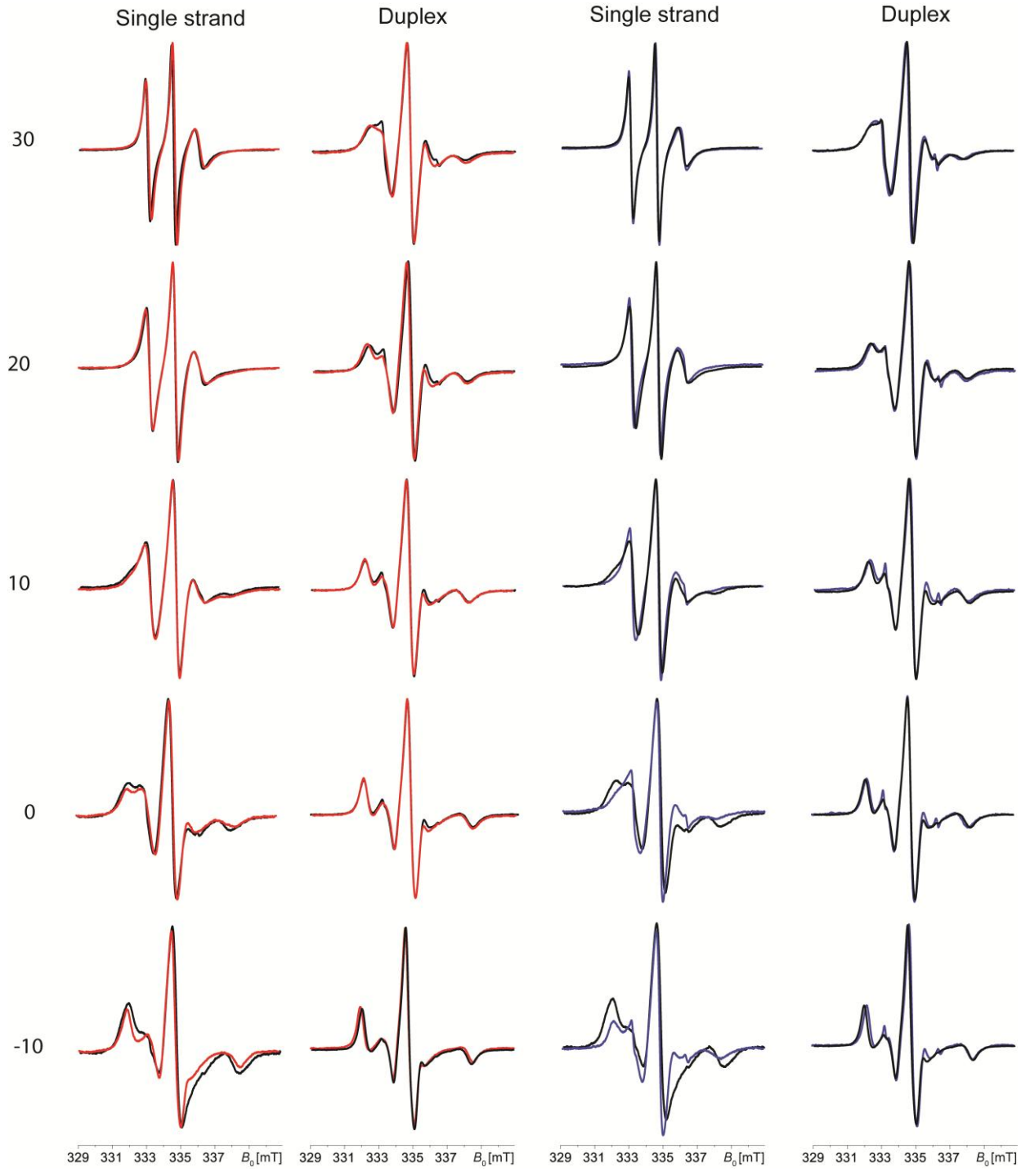


Fig. S4 Comparison of EPR spectra of ^{Im}**U** (black), **Ç** (red) and ^{Ox}**U** (blue) in duplex and single-stranded DNAs at various temperatures.

Base-pairing of ^{Im}U detected by EPR

Comparing the EPR spectra of four duplexes, in which ^{Im}**U** was paired with either A, T, G or C, yielded four distinguishable spectra (**Figure S5A**). The ^{Im}**U**•A pair had the lowest mobility, while the ^{Im}**U**•T pair had the highest mobility. Addition of Hg^{2+} ions, known to form metallo base-pairs in T•T mismatches,[4] yielded an EPR spectrum that was nearly identical to the ^{Im}**U**•A duplex (**Figure S5B**).

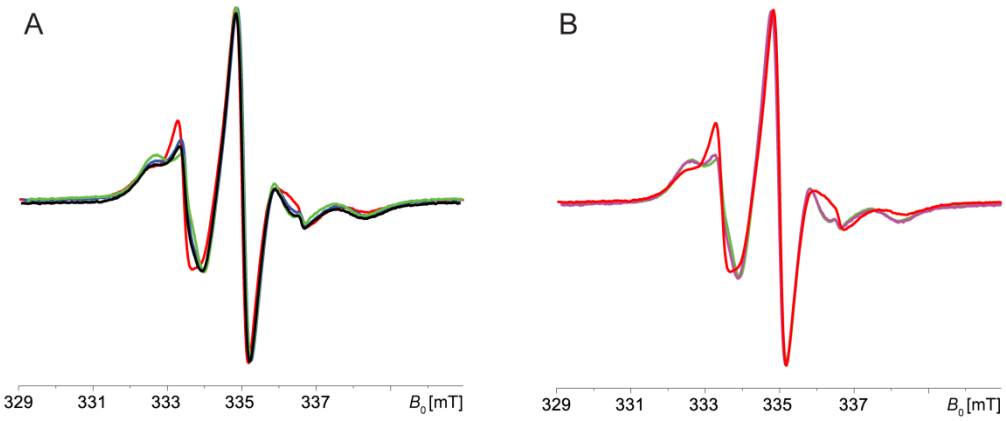
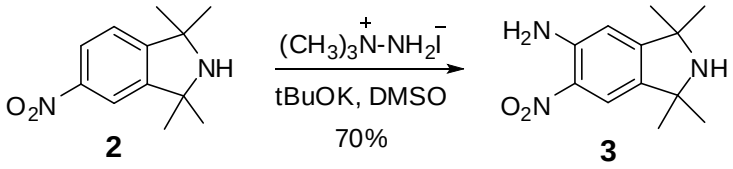


Fig. S5 EPR spectra of ^{Im}**U**-labelled DNA duplexs (14-mer) (**A**) with A (green), C (blue), G (black) and T (red). (**B**) ^{Im}**U** paired with A (green), T (red) and after addition of Hg^{2+} to the ^{Im}**U**•T mismatch (magenta) at 25 °C.



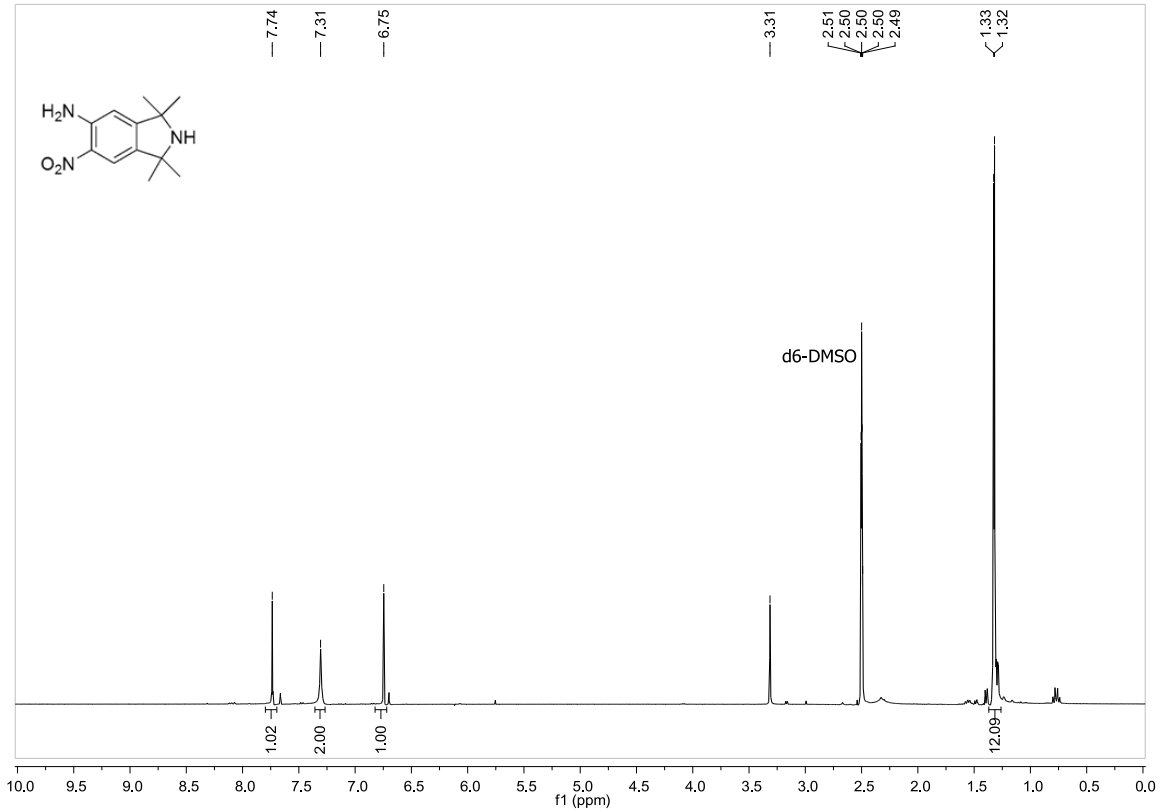
1,1,3,3-Tetramethyl-6-nitroisoindolin-5-amine (3). To a solution of N,N,N-trimethylhydrazine iodide (1.83 g, 99.85 mmol) in DMSO (15 mL) was added t-BuOK (1.12 g, 99.85 mmol) and stirred reaction for 30 min. To this solution, compound **2** (1.0 g, 45.39 mmol) was added and the reaction stirred for 14 h at 22 °C. The reaction mixture was poured into 50 mL of ice-cold water, followed by extraction with CH_2Cl_2 (3 x 50 mL), the combined organic phases dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by flash silica gel column chromatography using a gradient elution (CH_2Cl_2:MeOH; 100:0 to 90:10) to give compound **3** as a yellow solid (0.750 g, 70% yield).

^{1}H NMR (d_6-DMSO): δ 7.74 (s, 1H), 7.31 (s, 2H), 6.75 (s, 1H), 1.32 (d, J = 3.0 Hz, 12H).

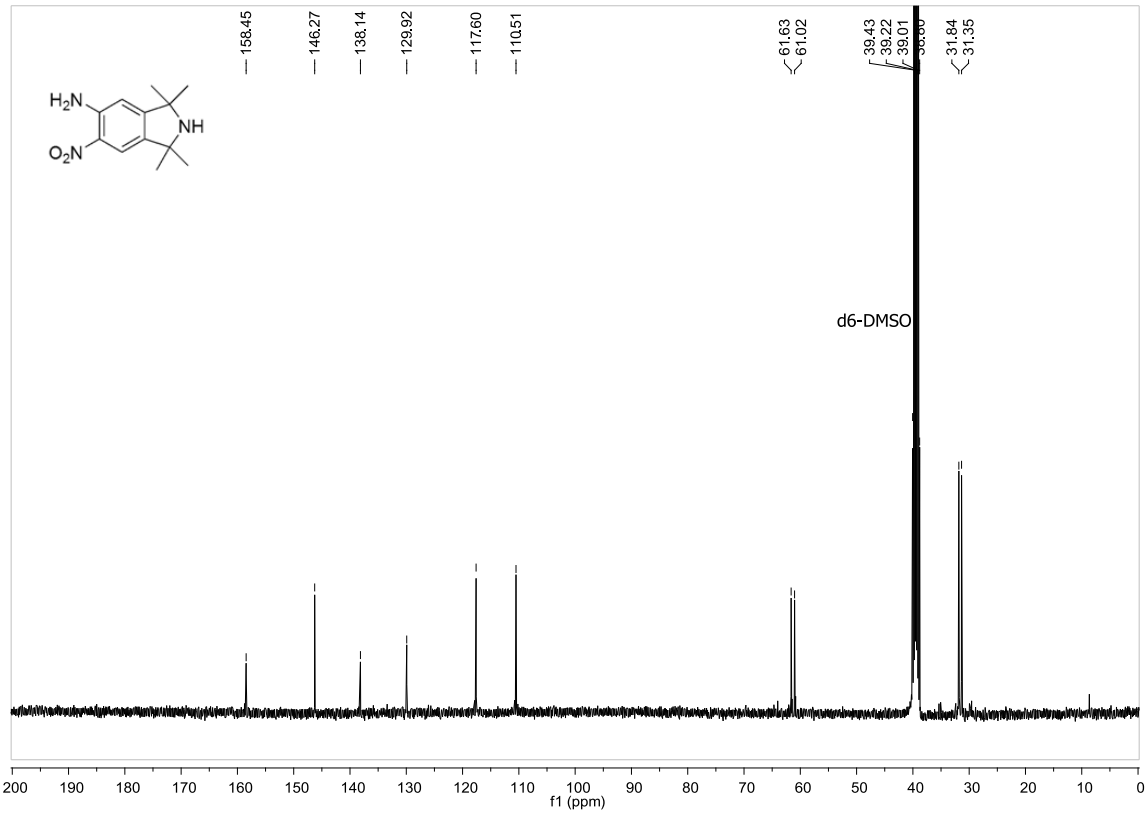
^{13}C NMR (d_6-DMSO): δ 158.45, 146.27, 138.14, 129.92, 117.60, 110.51, 61.63, 61.02, 31.84, 31.35.

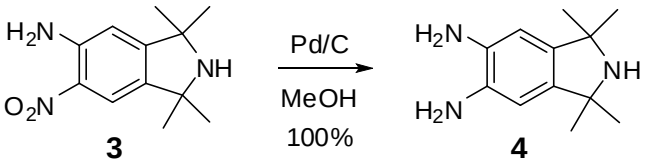
HR-ESI-MS $(M + H)^+$: calcd. for $C_{12}H_{18}N_2O_2$ 236.1394, found 236.1371.

^{1}H NMR spectrum of 3



^{13}C NMR spectrum of 3





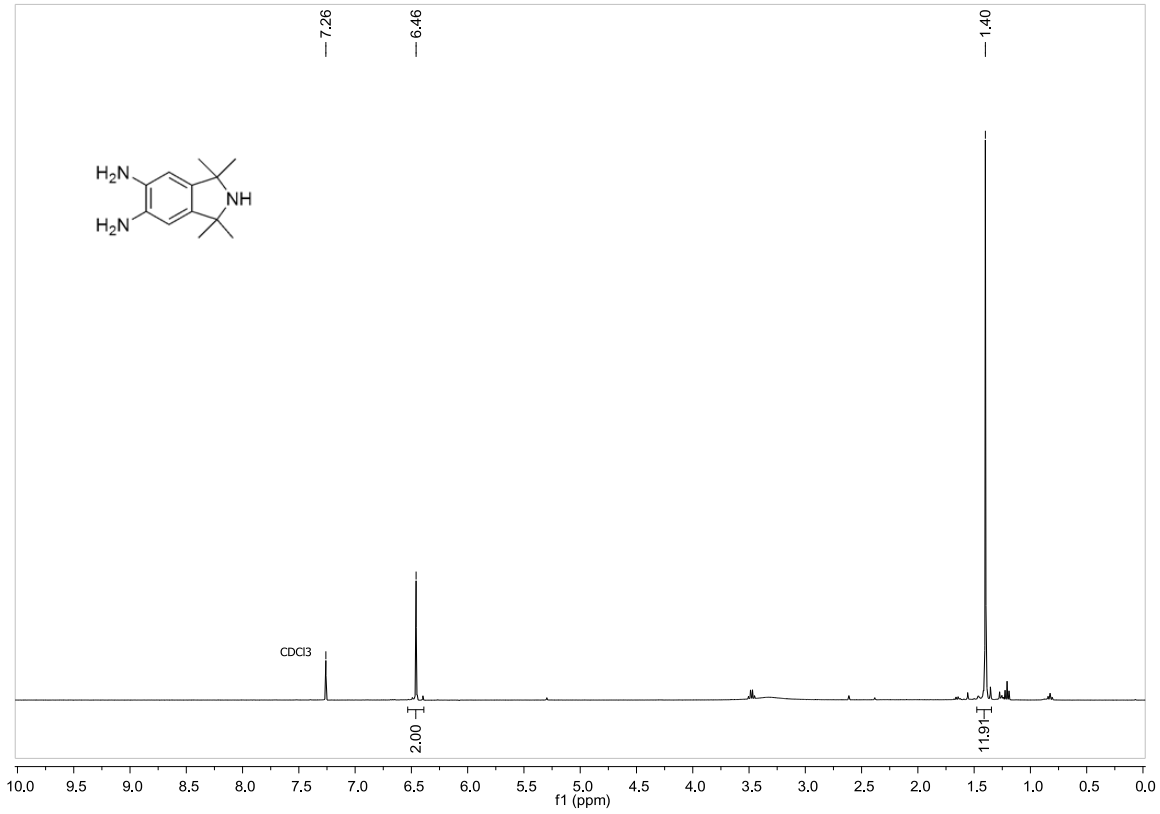
1,1,3,3-Tetramethylisoindoline-5,6-diamine (4). To a solution of compound **3** (200.0 mg, 0.85 mmol) in MeOH (10 mL) was added 10% Pd/C (20.0 mg) and the mixture hydrogenated at 55 psi and 22 °C for 2 h. The reaction mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo* to yield 1,1,3,3-tetramethylisoindoline-5,6-diamine (174.0 mg, 100%) which was used for the next reaction without further purification.

^{1}H NMR ($CDCl_3$): δ 6.46 (s, 2H), 1.40 (s, 12H).

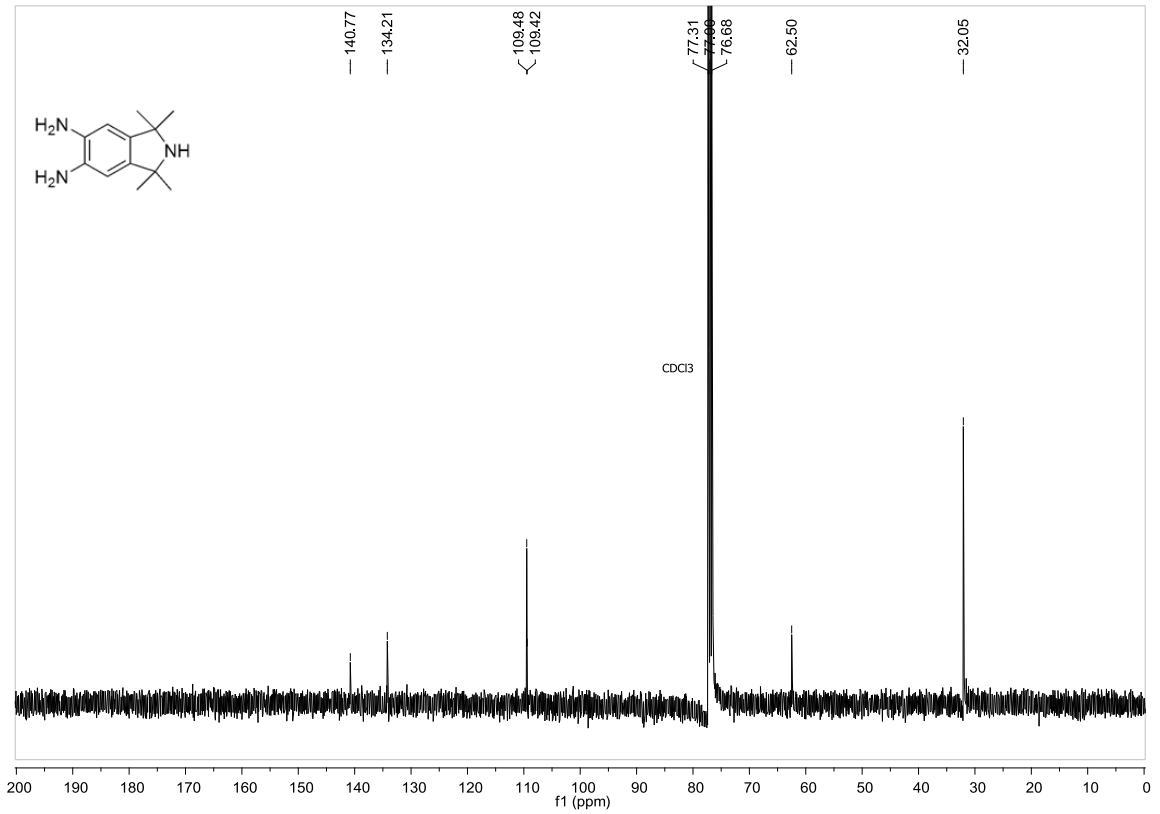
^{13}C NMR ($CDCl_3$): δ 140.77, 134.21, 109.48, 109.42, 62.50, 32.05.

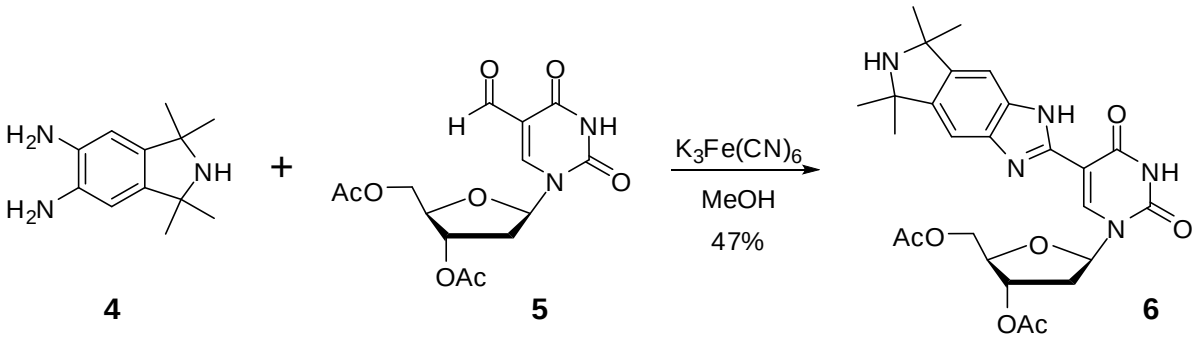
HR-ESI-MS $(M + H)^+$: calcd. for $C_{12}H_{18}N_3O_2$ 206.1652, found 206.1652.

^{1}H NMR spectrum of 4



^{13}C NMR spectrum of 4





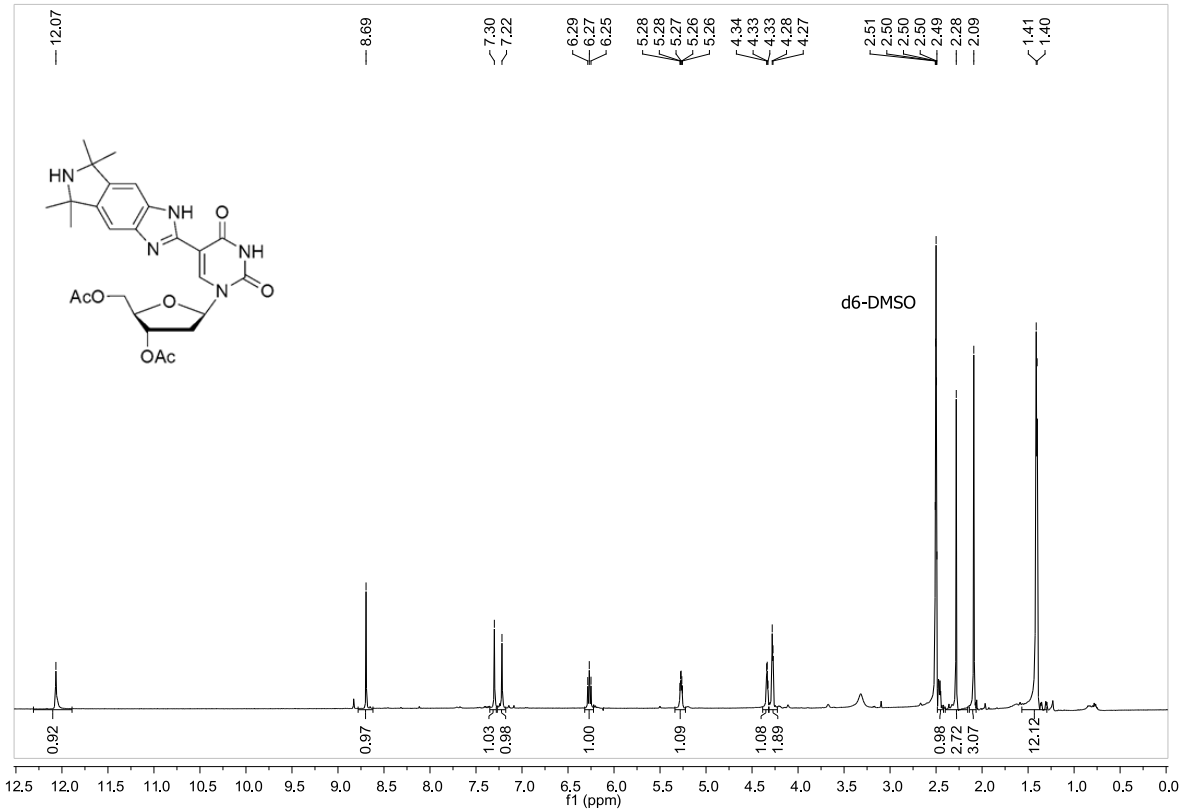
Compound 6. 3',5'-Di-*O*-acetyl-5-formyl-2'-deoxyuridine (**5**) (346.0 mg, 1.02 mmol) and 1,1,3,3-tetramethylisoindoline-5,6-diamine (**4**) (174.0 mg, 0.85 mmol) were dissolved in MeOH (9 mL), $K_3Fe(CN)_6$ (402.0 mg, 1.22 mmol) added and the reaction stirred for 14 h at 22 °C. The reaction mixture was concentrated *in vacuo* and the crude product was purified by flash silica gel column chromatography using a gradient elution (CH_2Cl_2:MeOH; 98:02 to 90:10) to give compound **6** as a dark yellow solid (210.0 mg, 47% yield).

^{1}H NMR (d_6-DMSO): δ 12.07 (s, 1H), 8.69 (s, 1H), 7.30 (s, 1H), 7.22 (s, 1H), 6.27 (t, *J* = 7.0 Hz, 1H), 5.27 (dt, *J* = 5.6, 2.7 Hz, 1H), 4.38 – 4.32 (m, 1H), 4.28 (d, *J* = 3.4 Hz, 2H), 2.49 – 2.42 (m, 1H), 2.28 (s, 3H), 2.09 (s, 3H), 1.41 (d, *J* = 4.0 Hz, 12H).

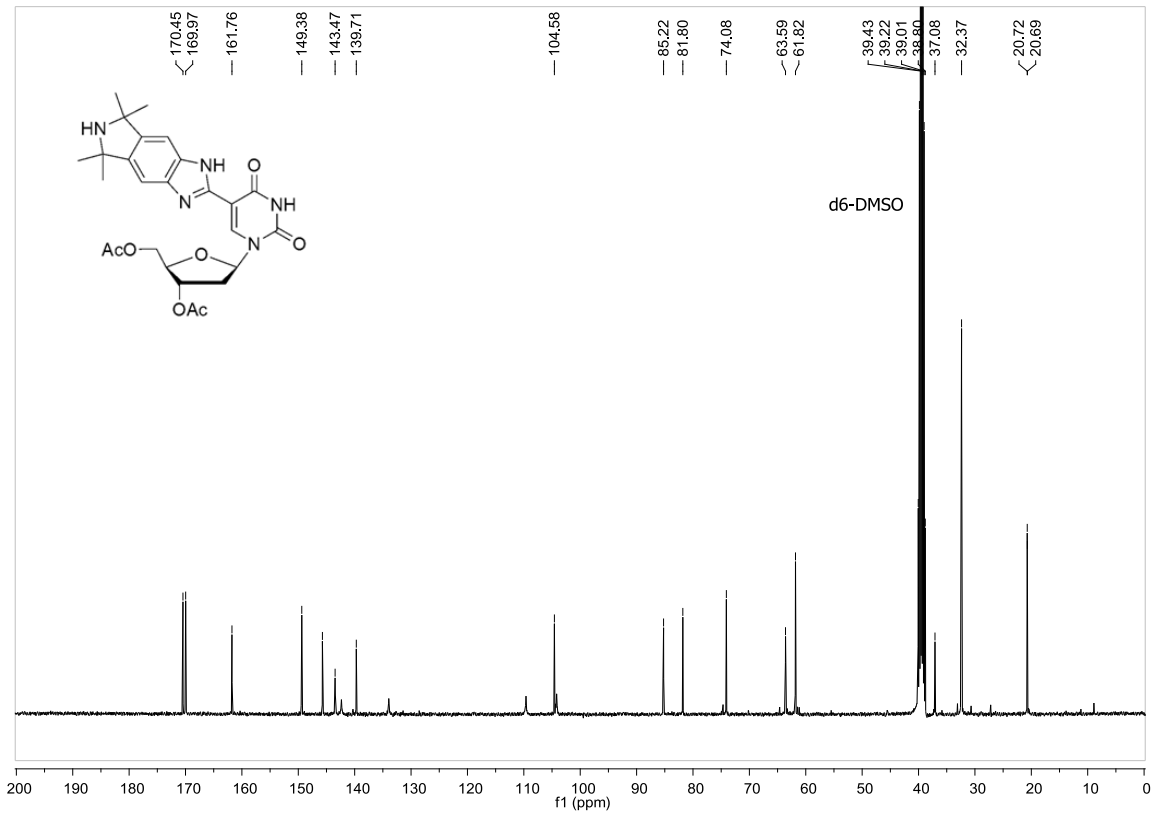
^{13}C NMR (d_6-DMSO): δ 170.45, 169.97, 161.76, 149.38, 145.72, 143.47, 139.71, 104.58, 85.22, 81.80, 74.08, 63.59, 61.82, 37.08, 32.37, 20.72, 20.69.

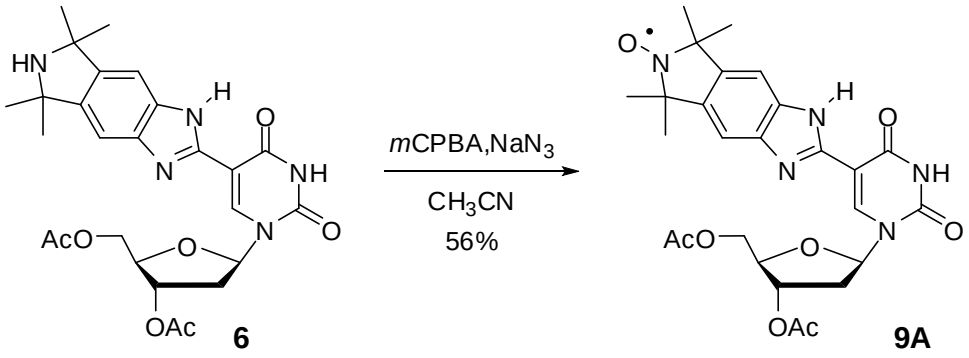
HR-ESI-MS $(M + H)^+$: calcd. for $C_{26}H_{32}N_5O_7$ 526.2223, found 526.2353.

^{1}H NMR spectrum of 6



^{13}C NMR spectrum of 6





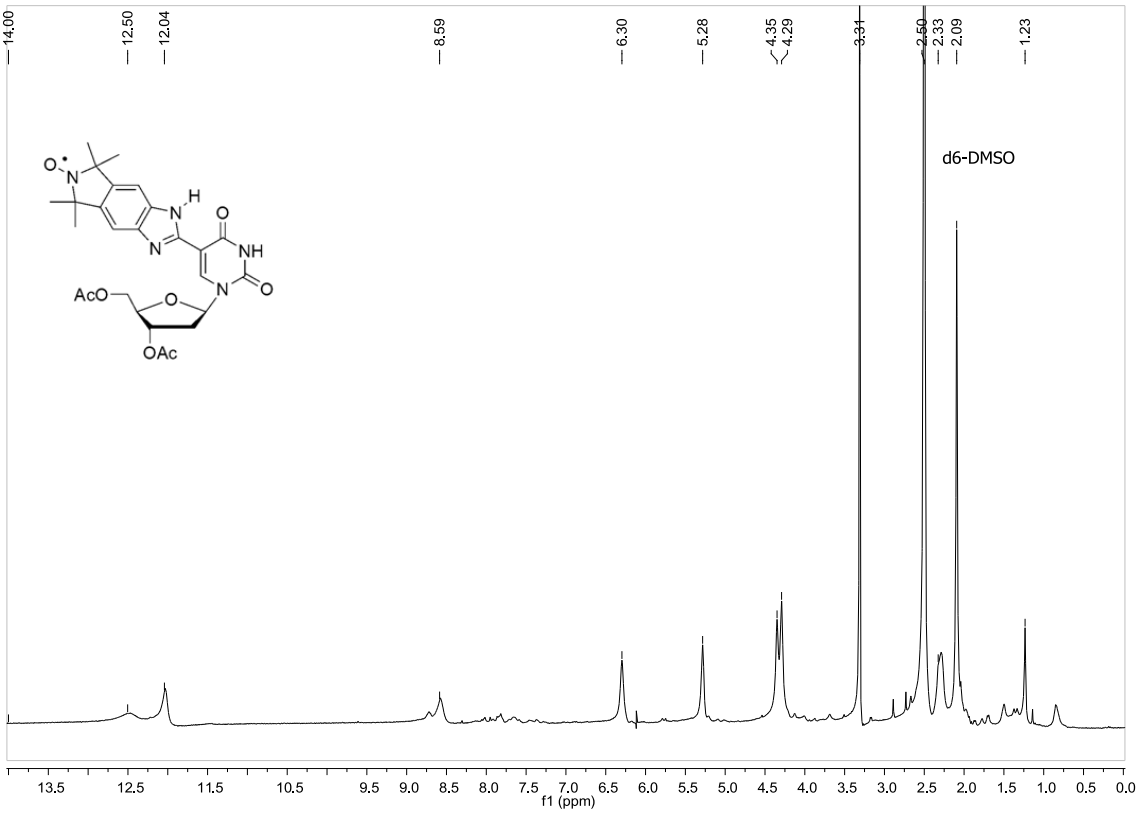
Compound 9A. To a suspension of **6** (200.0 mg, 0.38 mmol) in CH_3CN (20 mL) was added NaN_3 (99.0 mg, 1.52 mmol) and the reaction stirred at 22 °C. After 30 min, *m*CPBA (131.0 mg, 0.76 mmol) was added. After 4 h the reaction mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using a gradient elution (CH_2Cl_2:MeOH; 100:0 to 95:5) to give compound **9A** as a yellow solid (150.0 mg, 56.0% yield).

^{1}H NMR (d_6-DMSO): δ 12.50 (br s), 12.04 (br s), 8.59 (br s), 6.30 (br s), 5.28 (br s), 4.32 (br s), 2.33 (br s), 2.09 (br s), 1.23 (br s).

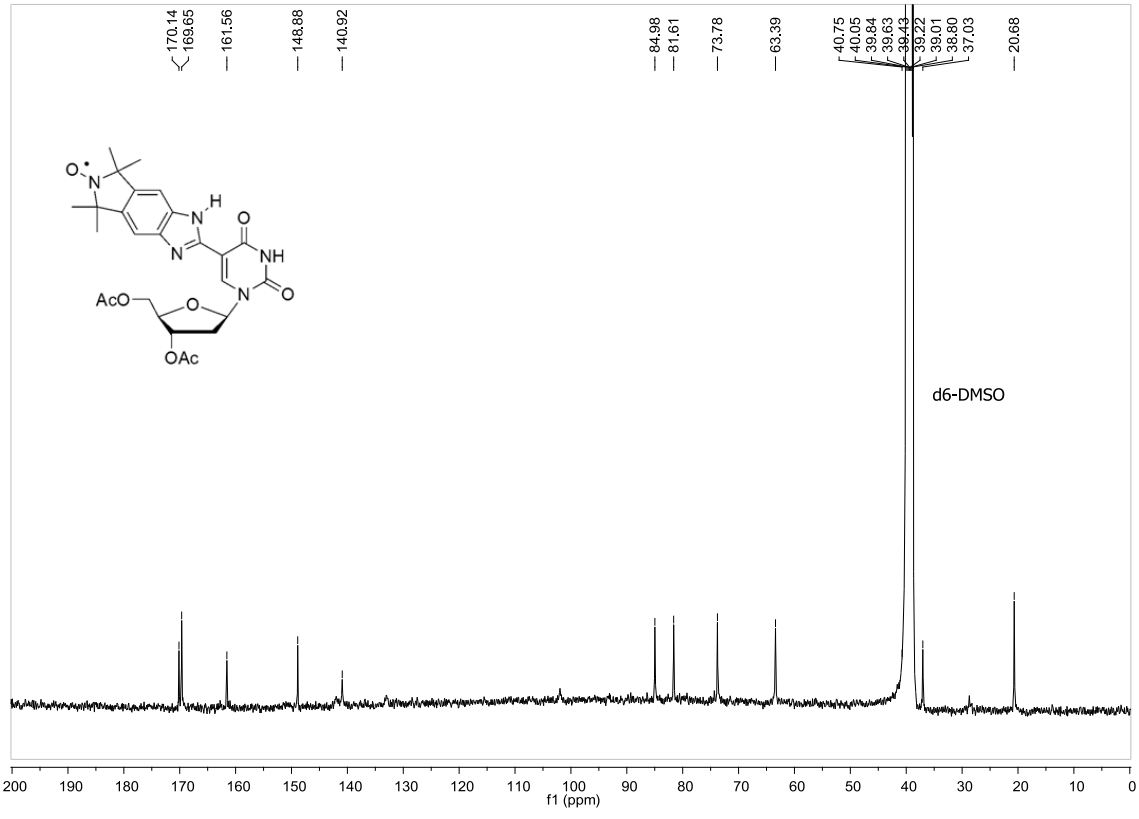
^{13}C NMR (d_6-DMSO): δ 170.14, 169.65, 161.56, 148.88, 140.92, 84.98, 81.61, 73.78, 63.39, 37.03, 20.68.

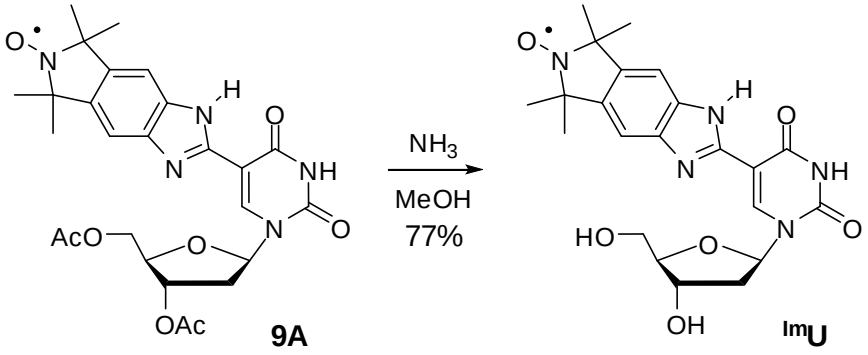
HR-ESI-MS $(M + H)^+$: calcd. for $C_{26}H_{30}N_5O_8$ 541.1267, found 541.2194.

^{1}H NMR spectrum of 9A



^{13}C NMR spectrum of 9A





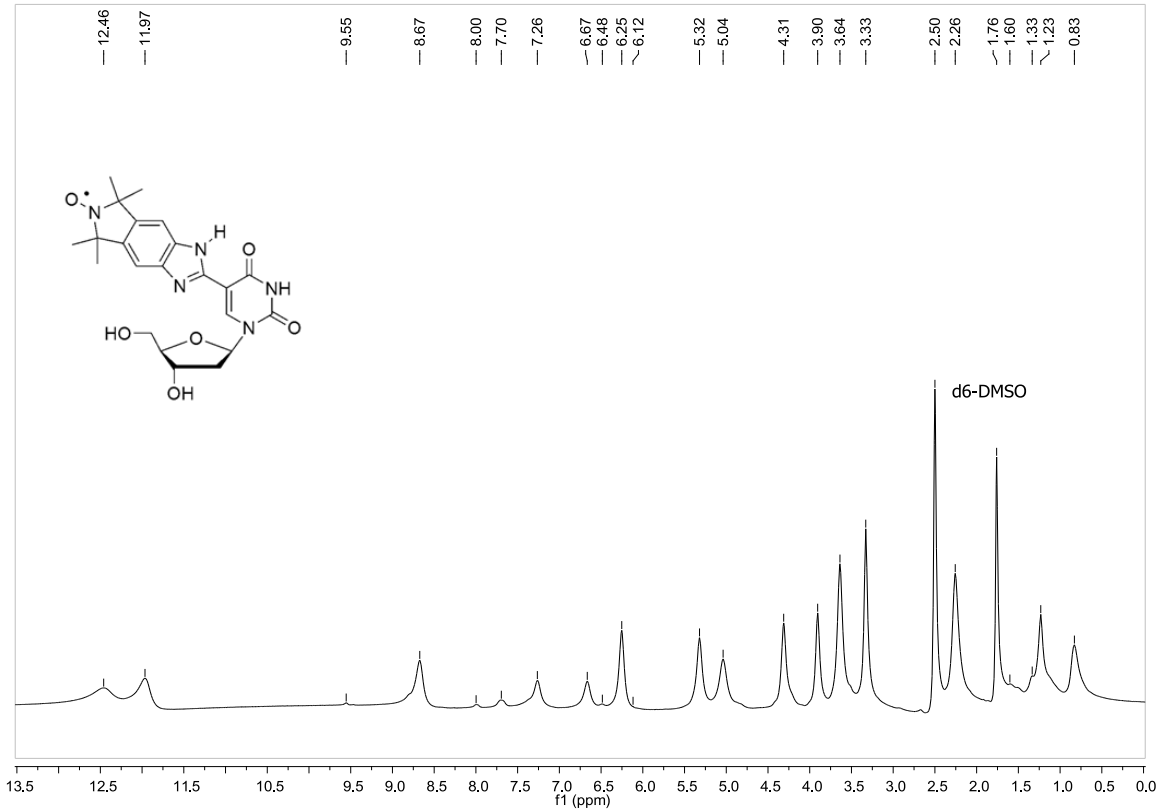
Spin-labelled nucleoside ^{Im}U. A solution of **9A (**150.0 mg, 0.28 mmol) in MeOH saturated with NH_3 (5.00 mL) was stirred at 22 °C for 16 h. The reaction mixture was concentrated *in vacuo* and the crude product purified by flash column chromatography, eluting with (CH_2Cl_2:MeOH; 98:2 to 92:8) to give ^{Im}**U** as a yellowish solid (98.0 mg, 77.0 %).

^{1}H NMR (*d*$_6$-DMSO): δ 12.46 (br s), 11.97 (br s), 9.55 (br s), 8.67 (br s), 8.00 (br s), 7.70 (br s), 7.26 (br s), 6.67 (br s), 6.48 (br s), 6.25 (br s), 6.12 (br s), 5.32 (br s), 5.04 (br s), 4.31 (br s), 3.90 (br s), 3.64 (br s), 3.33 (br s), 2.26 (br s), 1.76 (br s), 1.60 (br s), 1.33 (br s), 1.23 (br s), 0.83 (br s).

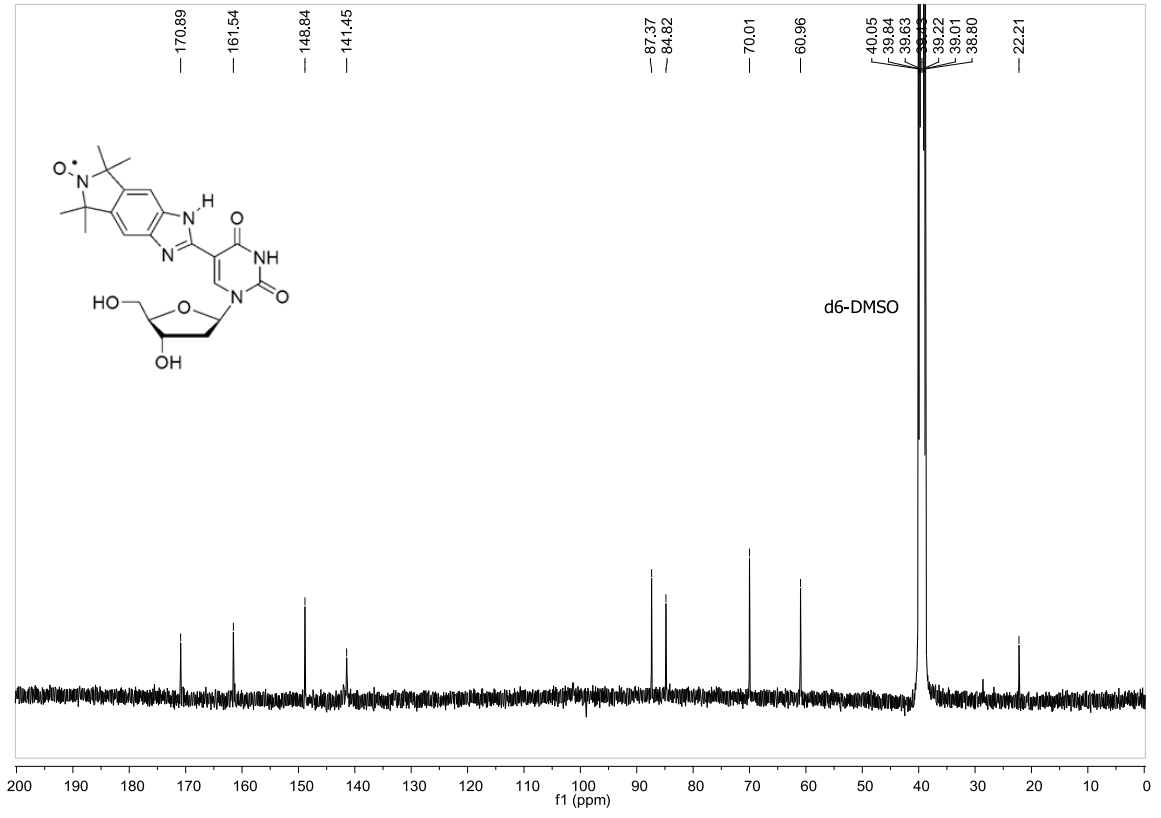
^{13}C NMR (*d*$_6$-DMSO): δ 170.89, 161.54, 148.84, 141.45, 87.37, 84.82, 70.01, 60.96, 22.21.

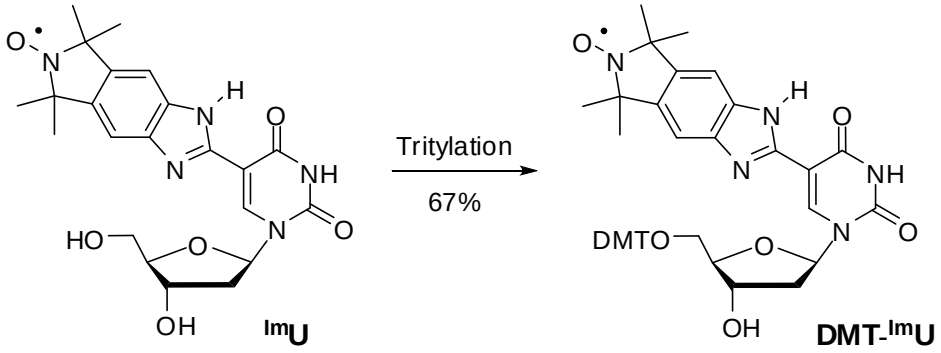
HR-ESI-MS $(M + H)^+$: calcd. for $C_{22}H_{27}N_5O_6$ 457.1959, found 457.1959.

^{1}H NMR spectrum of ^{Im}U



^{13}C NMR spectrum of ^{Im}U





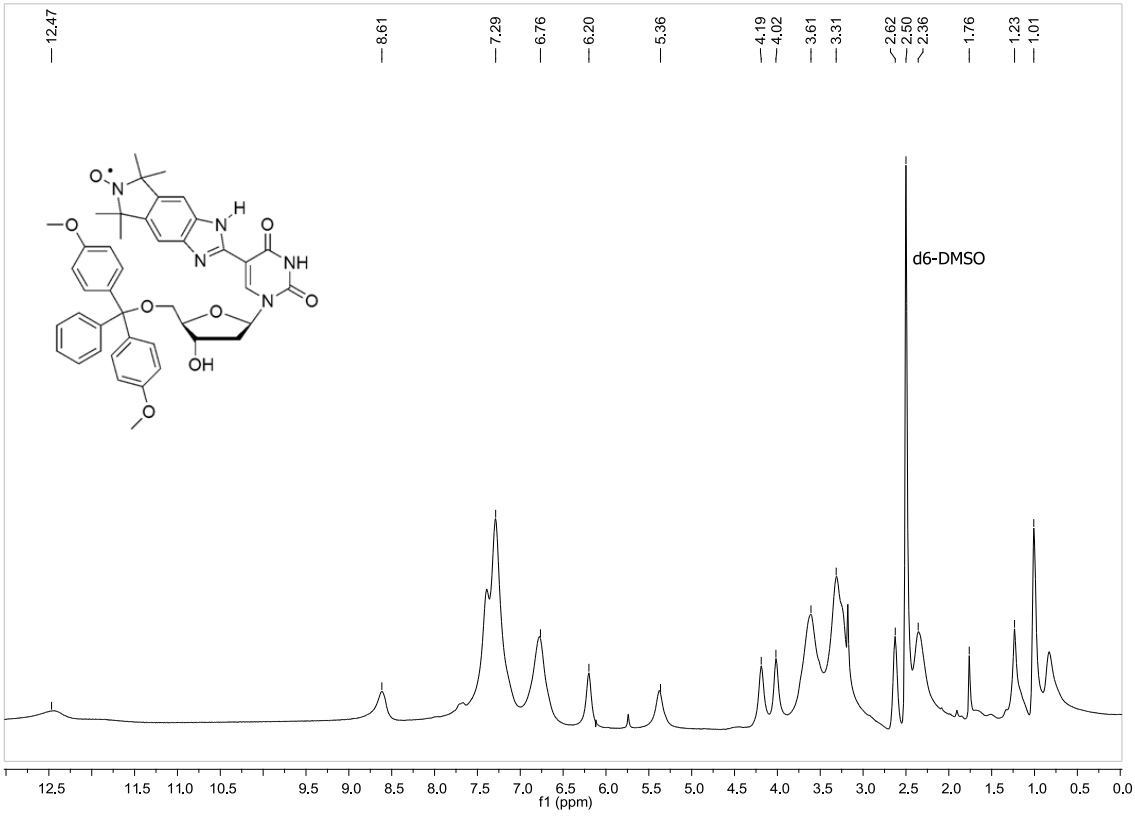
Compound DMT- ^{Im}***U***. ^{Im}**U** (90.0 mg, 0.20 mmol), DMTrCl (120.0 mg, 0.36 mmol) and DMAP (2.4 mg, 0.02 mmol) were weighed into a round bottom flask and kept under vacuum for 16 h. Pyridine (4.0 mL) was added and the solution was stirred at 22 °C for 2 h. MeOH (100 µL) was added and the solvent was removed *in vacuo* to give a crude orange solid. The solid was purified by column chromatography using a gradient of (CH_2Cl_2:MeOH; 100:0 to 97.5:2+0.5% Et_3N), using a column that was prepared in 99.5% CH_2Cl_2 + 0.5% Et_3N. Compound **DMT-** ^{Im}**U** was obtained as a yellow solid (100.0 mg, 67.0%).

^{1}H NMR (d_6-DMSO): δ 12.47 (br s), 8.61 (br s), 7.29 (br s), 6.76 (br s), 6.20 (br s), 5.36 (br s), 4.19 (br s), 4.02 (br s), 3.61 (br s), 3.31 (br s), 2.62 (br s), 2.36 (br s), 1.76 (br s), 1.23 (br s), 1.01 (br s).

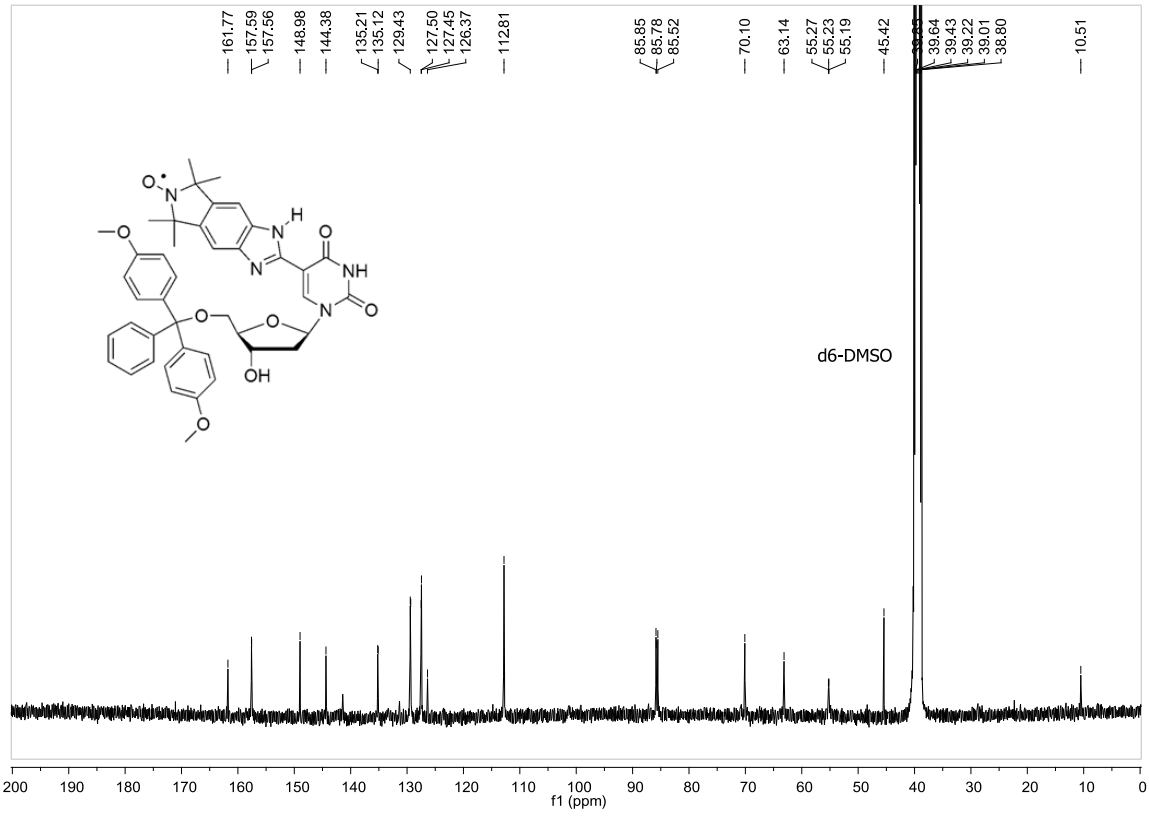
^{13}C NMR (d_6-DMSO): δ 161.77, 157.59, 157.56, 148.98, 144.38, 135.21, 135.12, 129.43, 129.35, 127.50, 127.45, 126.37, 112.81, 85.85, 85.78, 85.52, 70.10, 63.14, 55.27, 55.23, 55.19, 45.42, 10.51.

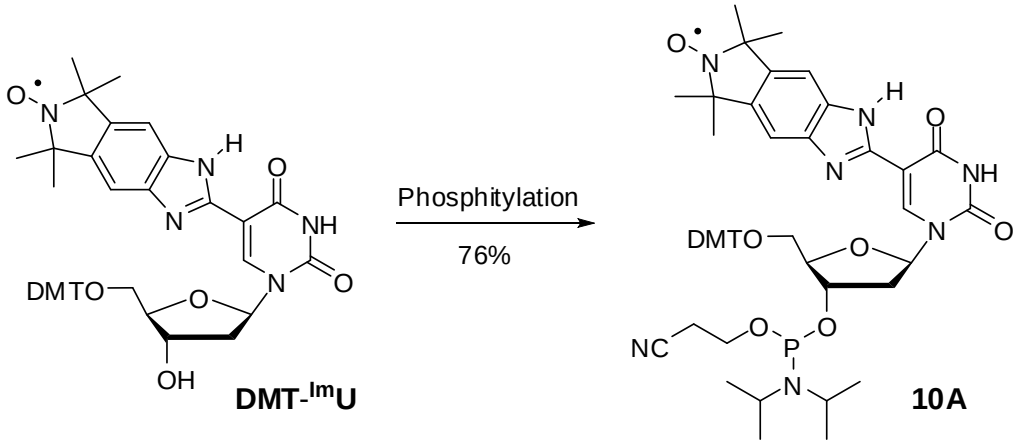
HR-ESI-MS $(M + H)^+$: calcd. for $C_{43}H_{45}N_5O_8$ 759.3263, found 759.3264.

^{1}H NMR spectrum of DMT-^{Im}U



^{13}C NMR spectrum of DMT-^{Im}U





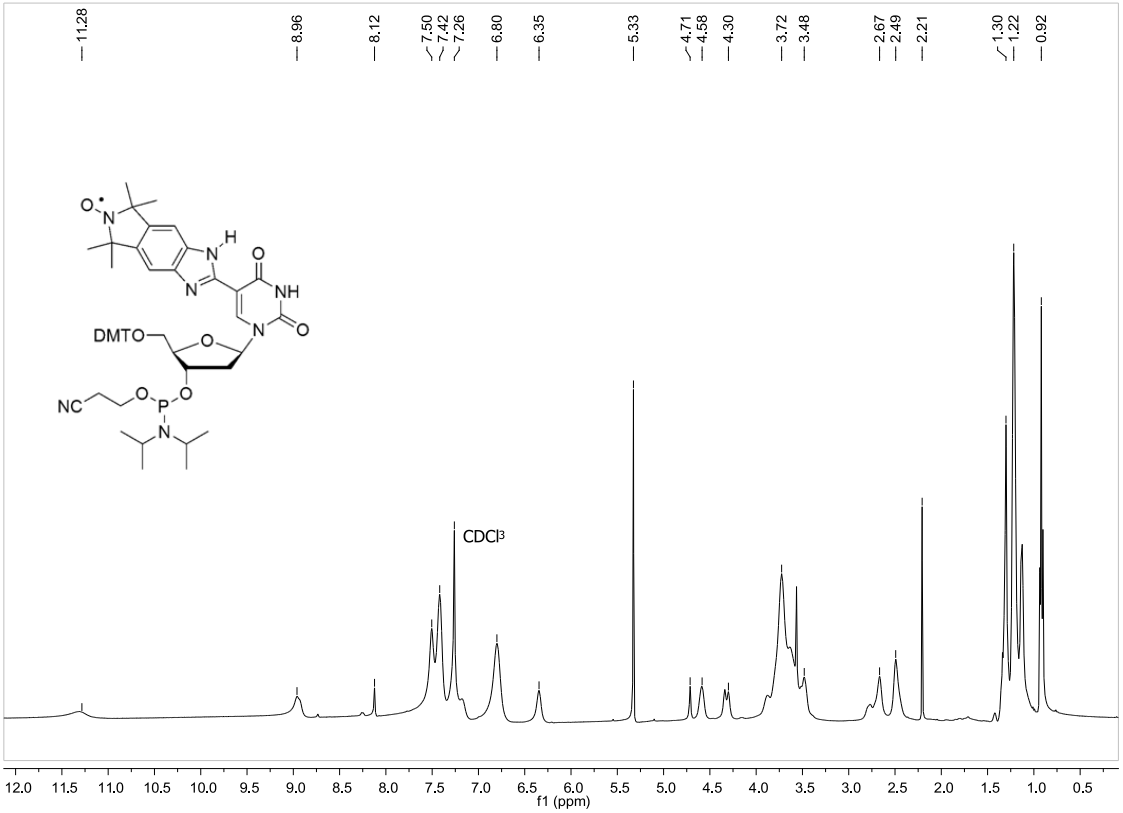
^{Im}U phosphoramidite (10A). **DMT-^{Im}U** (50.0 mg, 0.07 mmol) and diisopropyl ammonium tetrazolide (15.0 mg, 0.09 mmol) were dissolved in pyridine (2 mL) and the pyridine removed *in vacuo*. The residue was kept under vacuum for 19 h, followed by dissolution in CH_2Cl_2 (3.5 mL), CH_3CN (2.0 mL) and addition of 2-cyanoethyl N,N,N',N'-tetraisopropyl phosphoramidite (26.0 mg, 0.09 mmol). The reaction mixture was stirred at 22 °C for 2 h, diluted with CH_2Cl_2 (10 mL) and washed successively with saturated aq. $NaHCO_3$ (3x10 mL) and saturated aq. NaCl (2 x 10 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a crude solid. The solid was purified by precipitation by first dissolving it in CH_2Cl_2 (1 mL) followed by addition of n-hexane (80 mL). The liquid was decanted and the operation repeated twice to furnish phosphoramidite **10A** as a yellow solid (48.0 mg, 76%).

^{1}H NMR ($CDCl_3$): δ 11.28 (br s), 8.96 (br s), 8.12 (br s), 7.50 (br s), 7.42 (br s), 7.26 (br s), 6.80 (br s), 6.35 (br s), 5.33 (br s), 4.71 (br s), 4.58 (br s), 4.30 (br s), 3.72 (br s), 3.48 (br s), 2.67 (br s), 2.49 (br s), 2.21 (br s), 1.30 (br s), 1.22 (br s), 0.92 (br s).

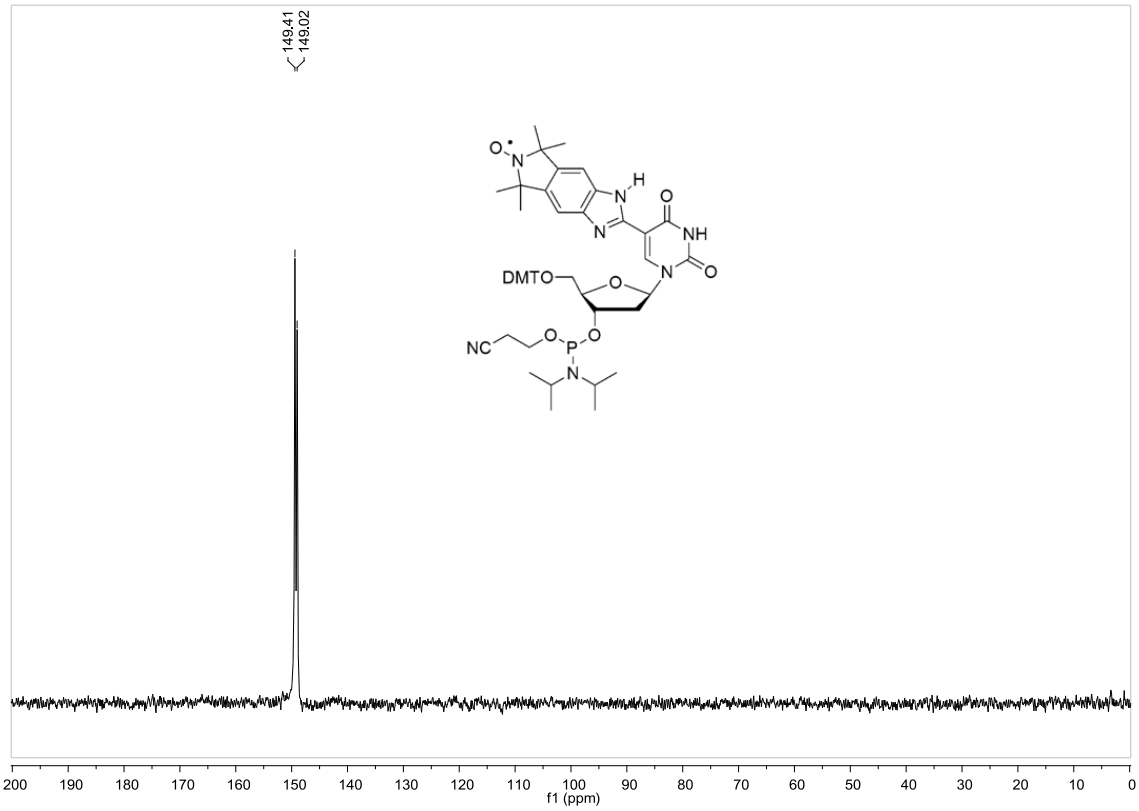
^{31}P NMR ($CDCl_3$): δ 149.41, 149.02.

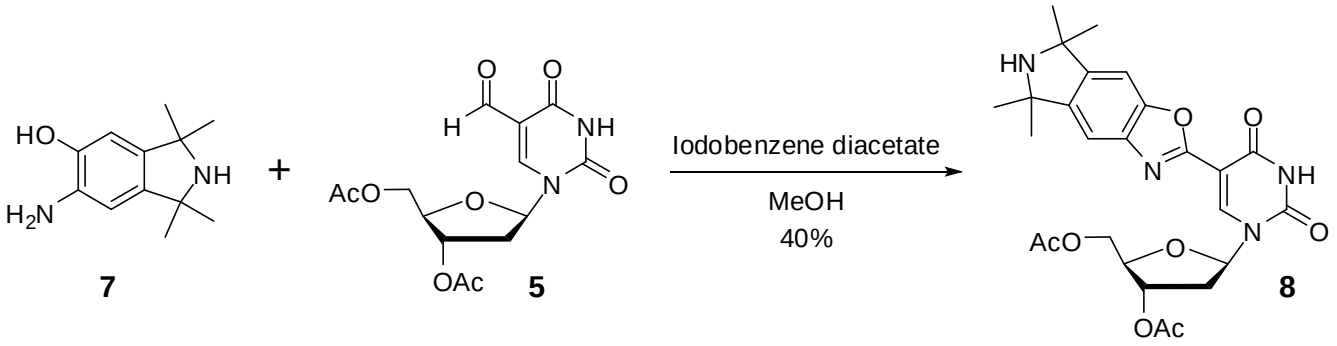
HR-ESI-MS $(M + H)^+$: calcd. for $C_{52}H_{62}N_7O_9P$ 959.4341, found 959.4368

^{1}H NMR spectrum of 10A



^{31}P NMR spectrum of 10A





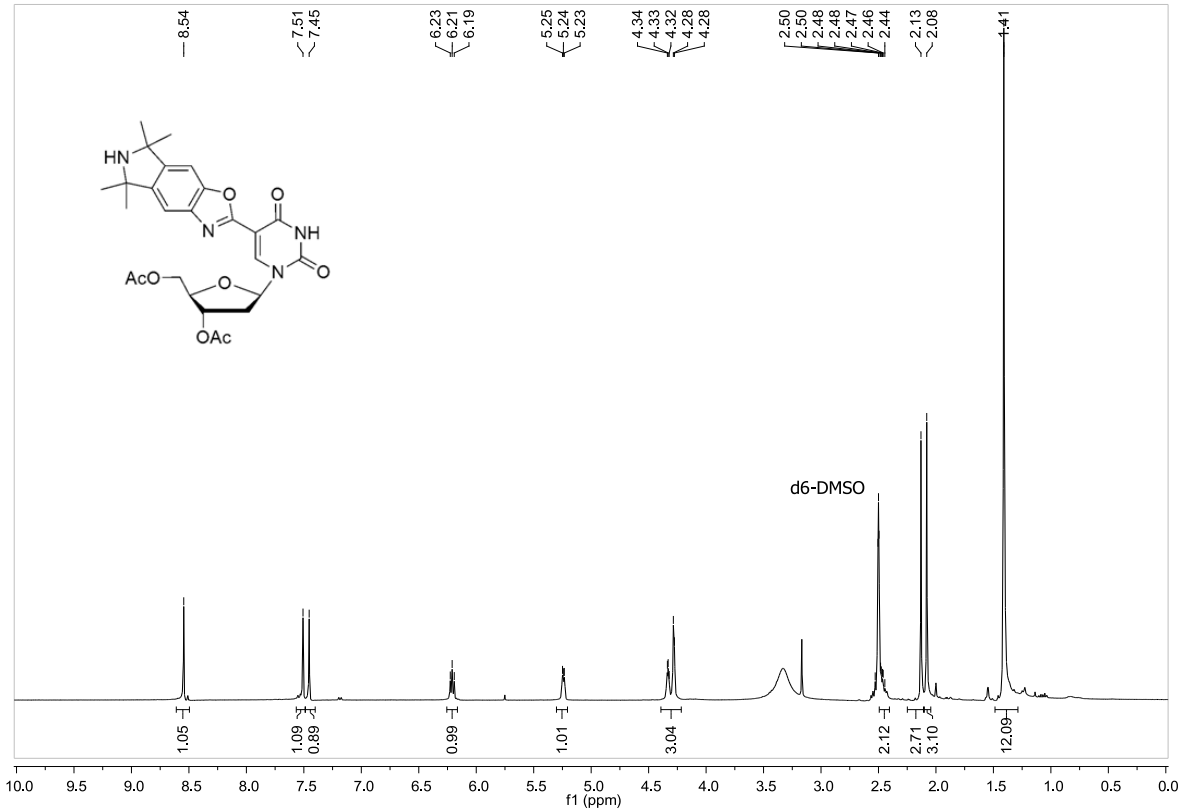
Compound 8. To a solution of compound **7** (349.0 mg, 1.69 mmol) in MeOH (18 mL) was added 3',5'-di-*O*-acetyl-5-formyl-2'-deoxyuridine (**5**) (633.0 mg, 1.86 mmol) and the reaction heated at 40 °C for 2 h, followed by cooling to 0 °C and adding the iodobenzene diacetate (599.0 mg, 1.86 mmol) all at once. Reaction was allow warm slowly to 22 °C and then stirred for 16 h. The solvent was removed *in vacuo* and the crude mixture purified by silica gel column chromatography using a gradient elution (CH_2Cl_2:MeOH; 100:0 to 80:20) to give compound **8** as a grey coloured solid (350.0 mg, 40% yield).

^{1}H NMR (d_6-DMSO): δ 8.54 (s, 1H), 7.51 (s, 1H), 7.45 (s, 1H), 6.21 (t, J = 6.9 Hz, 1H), 5.30 – 5.21 (m, 1H), 4.39 – 4.22 (m, 3H), 2.49 – 2.40 (m, 2H), 2.13 (s, 3H), 2.08 (s, 3H), 1.41 (s, 12H).

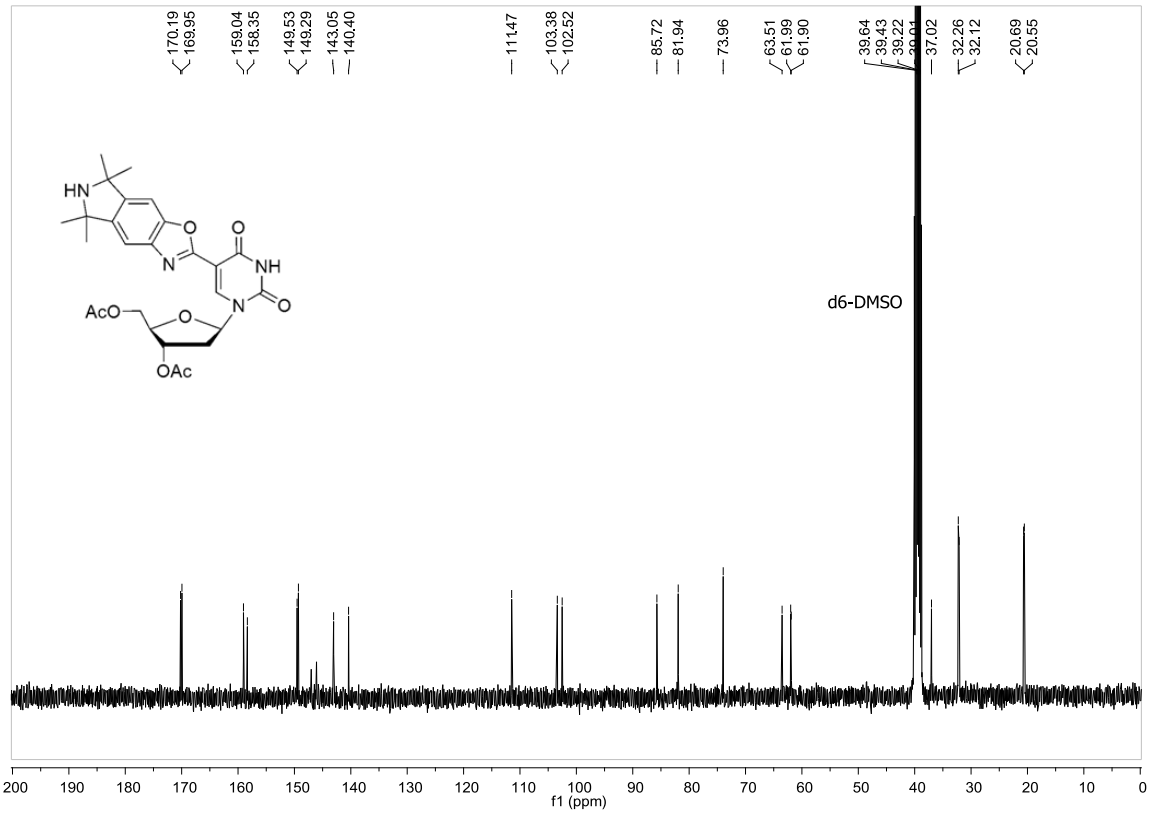
^{13}C NMR (d_6-DMSO): δ 170.19, 169.95, 159.04, 158.35, 149.53, 149.29, 143.05, 140.40, 111.47, 103.38, 102.52, 85.72, 81.94, 73.96, 63.51, 61.99, 61.90, 37.02, 32.26, 32.12, 20.69, 20.55.

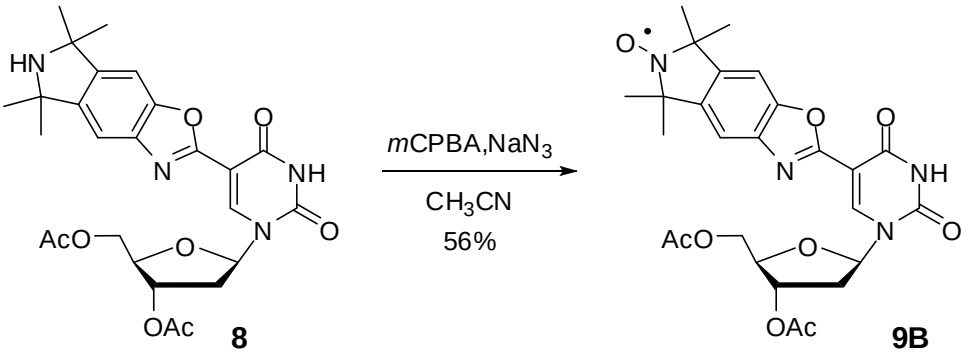
HR-ESI-MS $(M + H)^+$: calcd. for $C_{26}H_{31}N_4O_8$ 527.2136, found 527.2146.

^{1}H NMR spectrum of 8



^{13}C NMR spectrum of 8





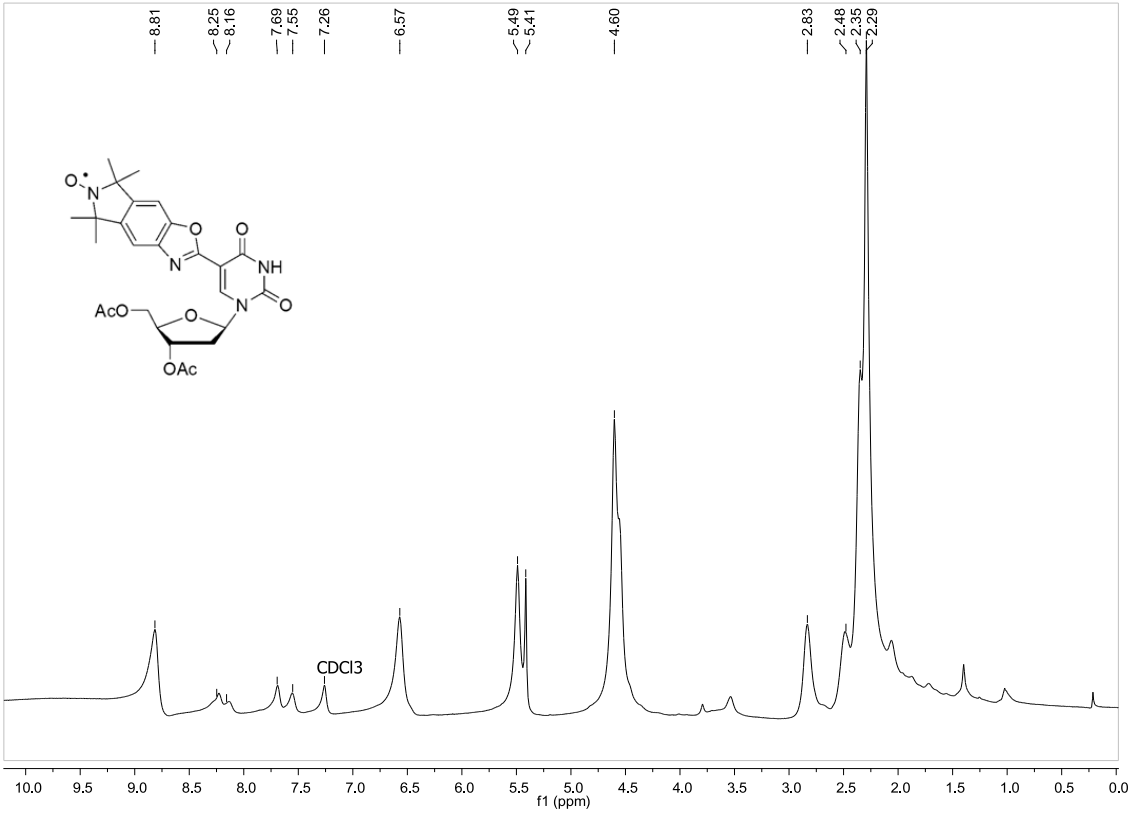
Compound 9B. To a suspension of **8** (175.0 mg, 0.33 mmol) in CH_3CN (18 mL) was added NaN_3 (87.0 mg, 1.33 mmol) and the reaction stirred at 22 ^oC. After 30 min *m*CPBA (115.0 mg, 0.66 mmol) was added. After 14 h the reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient elution (CH_2Cl_2:MeOH; 100:0 to 96:4) to give compound **9B** as a yellow solid (90.0 mg, 50% yield).

^{1}H NMR ($CDCl_3$): δ 8.81 (br s), 8.25 (br s), 8.16 (br s), 7.69 (br s), 7.55 (br s), 7.26 (br s), 6.57 (br s), 5.49 (br s), 5.41 (br s), 4.60 (br s), 2.83 (br s), 2.48 (br s), 2.35 (br s), 2.29 (br s).

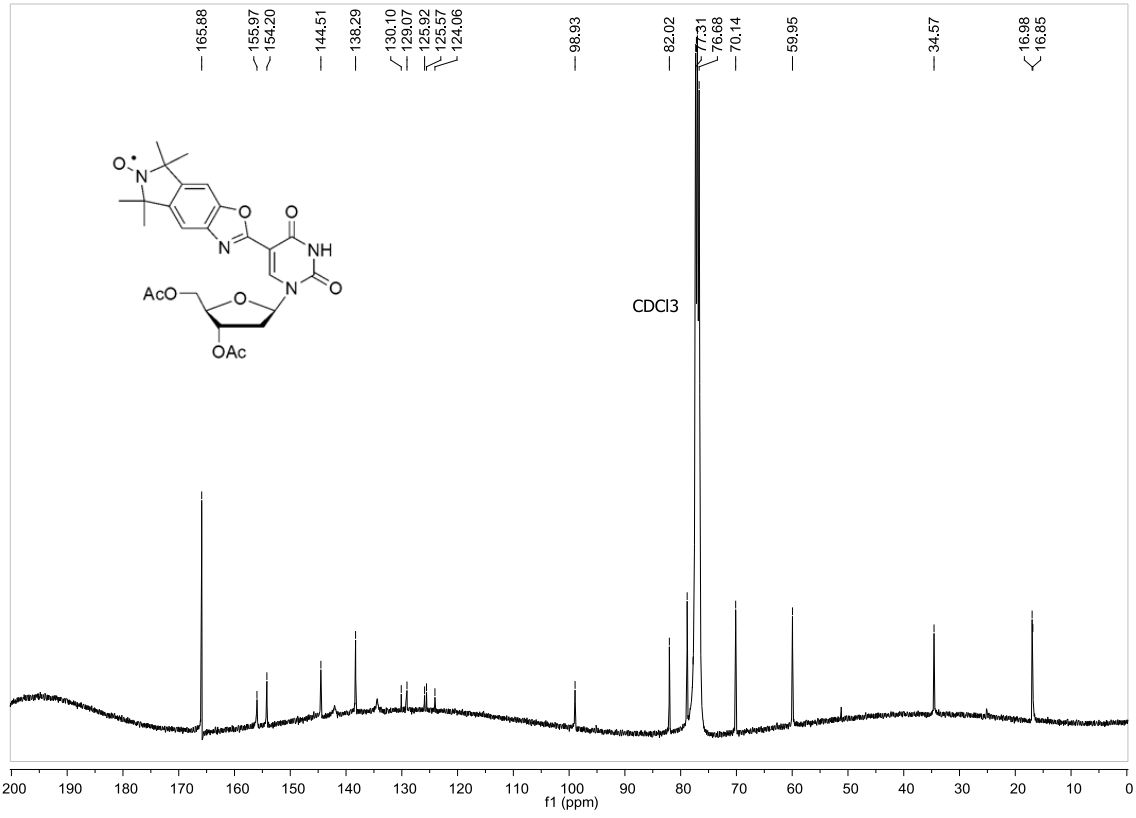
^{13}C NMR ($CDCl_3$): δ 165.88, 155.97, 154.20, 144.51, 138.29, 130.10, 129.07, 125.92, 125.57, 124.06, 98.93, 82.02, 78.84, 76.68, 70.14, 59.95, 34.57, 16.98, 16.85.

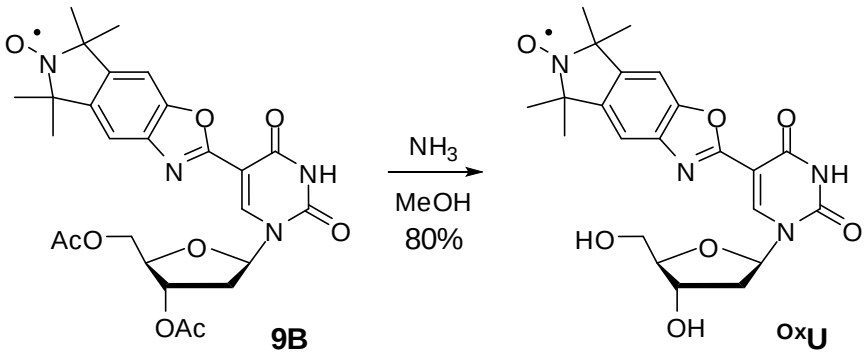
HR-ESI-MS $(M + Na)^+$: calcd. for $C_{26}H_{29}N_4O_9Na$ 564.1827, found 564.1779.

^{1}H NMR spectrum of 9B



^{13}C NMR spectrum of 9B





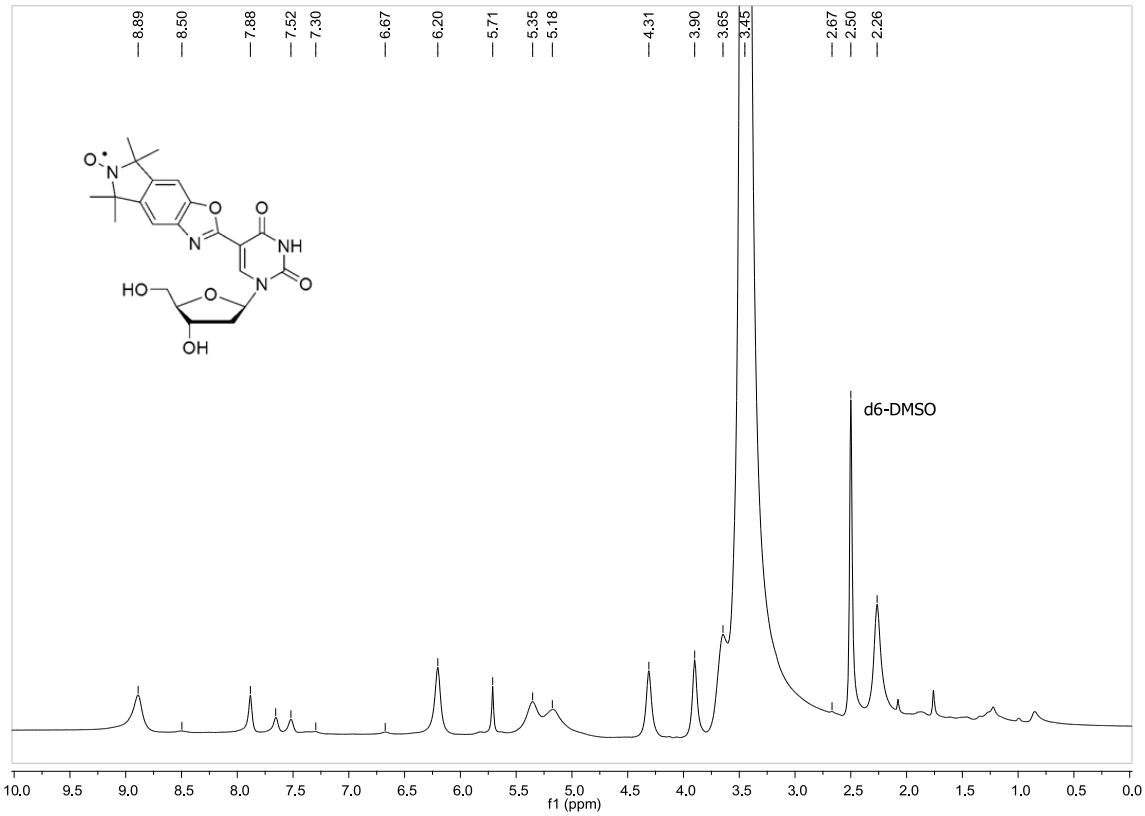
Spin-labelled nucleoside ^{Ox}***U***. A solution of **9B (**174.0 mg, 0.32 mmol) in MeOH saturated with NH_3 (4.00 mL) was stirred at 22 °C for 14 h. The reaction mixture was concentrated *in vacuo* and the residue purified by flash column chromatography eluting with (CH_2Cl_2:MeOH; 98:2 to 92:8) to give ^{Ox}**U** as yellow solid (118.0 mg, 80.0%).

^{1}H NMR (d_6-DMSO): δ 8.89 (br s), 8.50 (br s), 7.88 (br s), 7.52 (br s), 7.30 (br s), 6.67 (br s), 6.20 (br s), 5.71 (br s), 5.35 (br s), 5.18 (br s), 4.31 (br s), 3.90 (br s), 3.65 (br s), 2.67 (br s), 2.26 (br s).

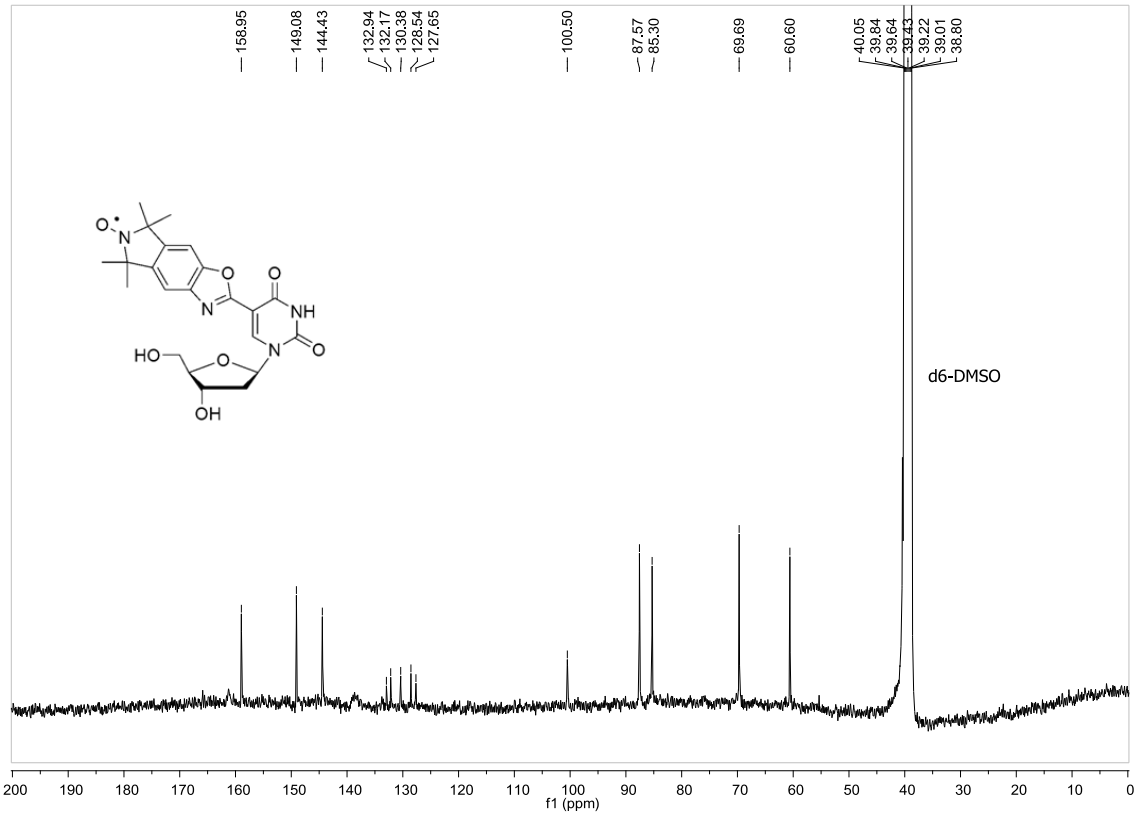
^{13}C NMR (d_6-DMSO): δ 158.95, 149.08, 144.43, 132.94, 132.17, 130.38, 128.54, 127.65, 100.50, 87.57, 85.30, 69.69, 60.60.

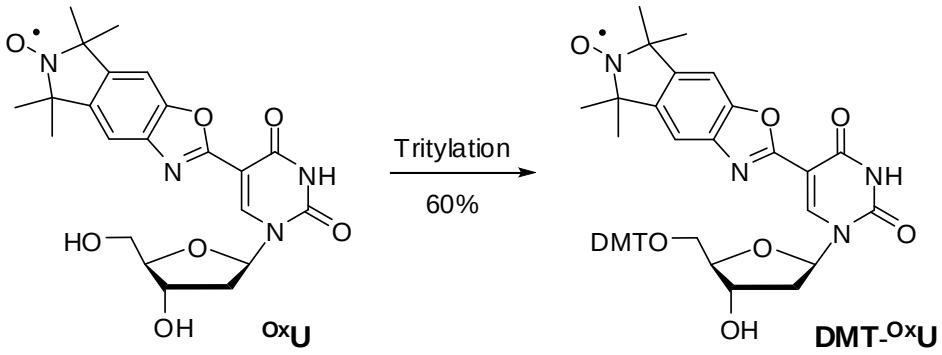
HR-ESI-MS $(M + Na)^+$: calcd. for $C_{22}H_{25}N_4O_7Na$ 480.1615, found 480.1603.

^{1}H NMR spectrum of ^{Ox}U



^{13}C NMR spectrum of ^{Ox}U





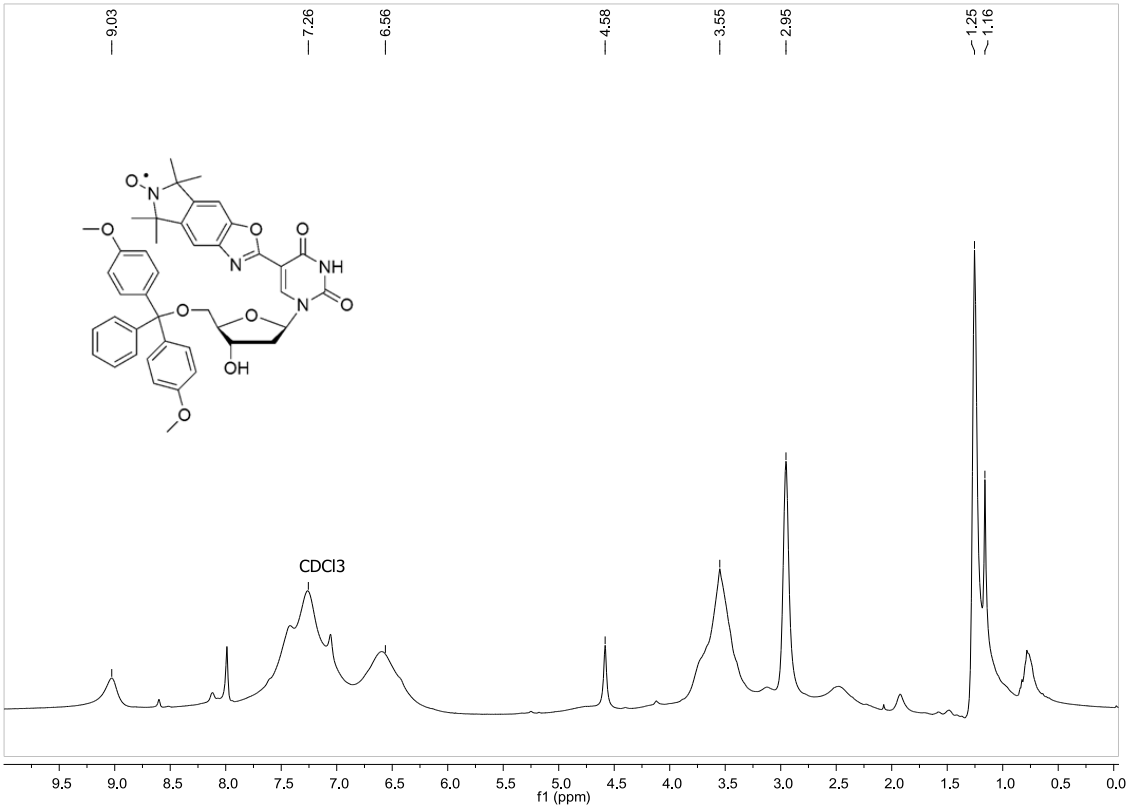
Compound DMT-^{Ox}U. **^{Ox}U** (45.0 mg, 0.10 mmol), DMTrCl (67.0 mg, 0.20 mmol) and DMAP (1.2 mg, 0.01 mmol) were weighed into a round bottom flask and kept *in vacuo* for 12 h. Pyridine (1.5 mL) was added and the solution was stirred at 22 °C for 3 h. MeOH (100 μL) was added and the solvent was removed *in vacuo* to obtain residue. The residue was purified by silica gel column chromatography using a gradient of (CH_2Cl_2:MeOH; 100:0 to 97.5:2 + 0.5% Et_3N). The column was prepared in 99.5% CH_2Cl_2 + 0.5% Et_3N. Compound **DMT-^{Ox}U** was obtained as a yellow solid (45.0 mg, 60.0%).

^{1}H NMR ($CDCl_3$): δ 9.03 (br s), 7.26 (br s), 6.56 (br s), 4.58 (br s), 3.55 (br s), 2.95 (br s), 1.25 (br s), 1.16 (br s).

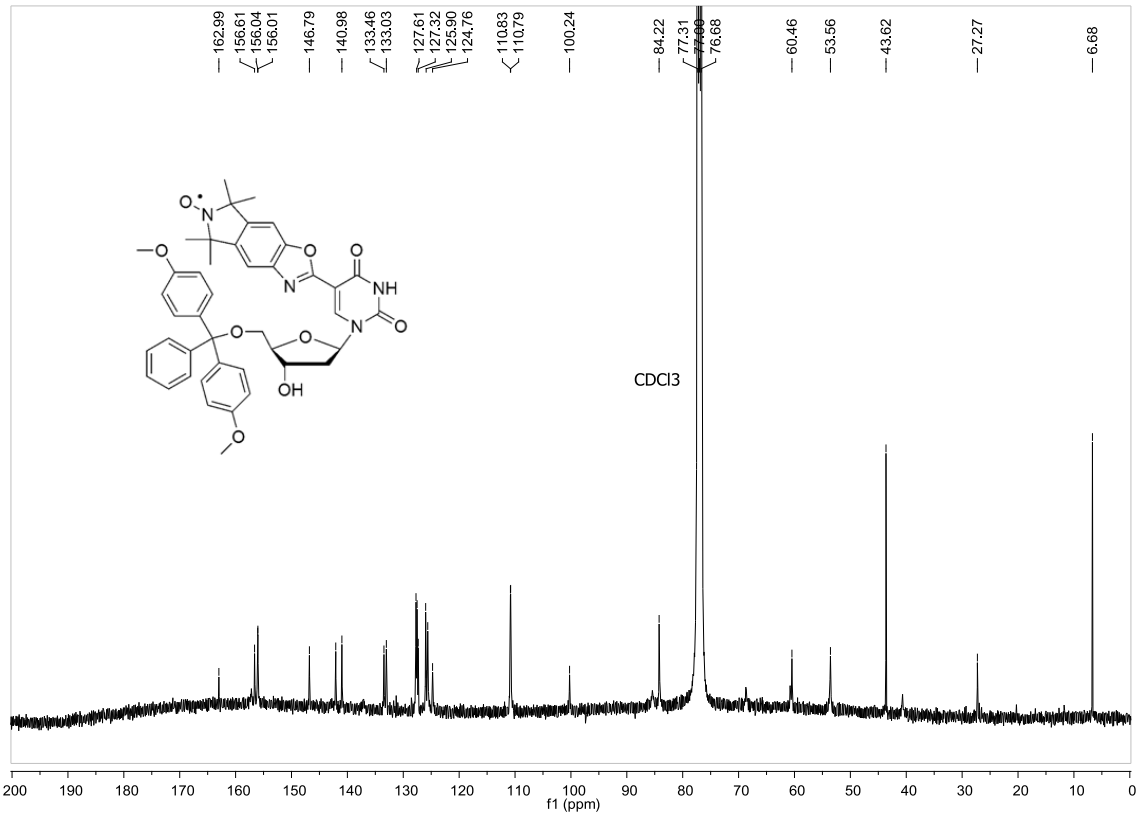
^{13}C NMR ($CDCl_3$): δ 162.99, 156.61, 156.04, 156.01, 146.79, 142.08, 140.98, 133.46, 133.03, 127.72, 127.61, 127.53, 127.32, 125.99, 125.90, 125.62, 124.76, 110.83, 110.79, 100.24, 84.22, 60.46, 53.56, 43.62, 27.27, 6.68.

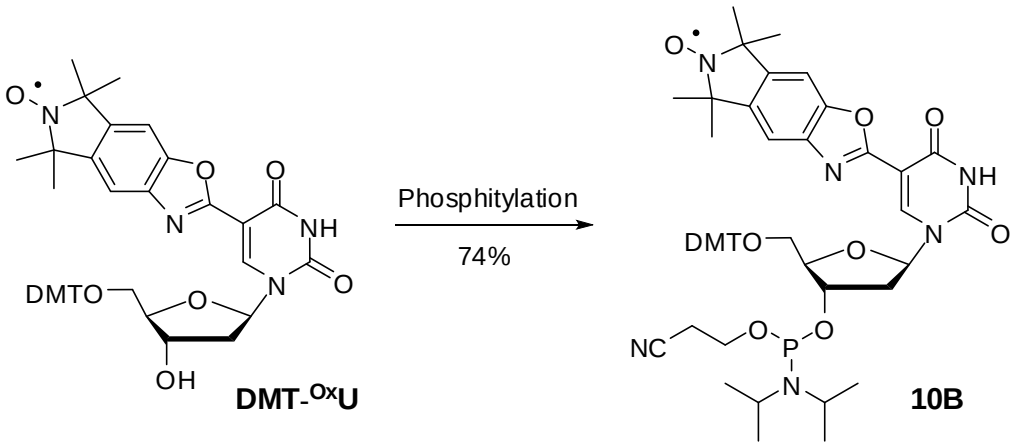
HR-ESI-MS $(M + Na)^+$: calcd. for $C_{43}H_{43}N_4O_9Na$ 782.2922, found 782.2912.

^{1}H NMR spectrum of DMT-^{Ox}U



^{13}C NMR spectrum of DMT-^{Ox}U





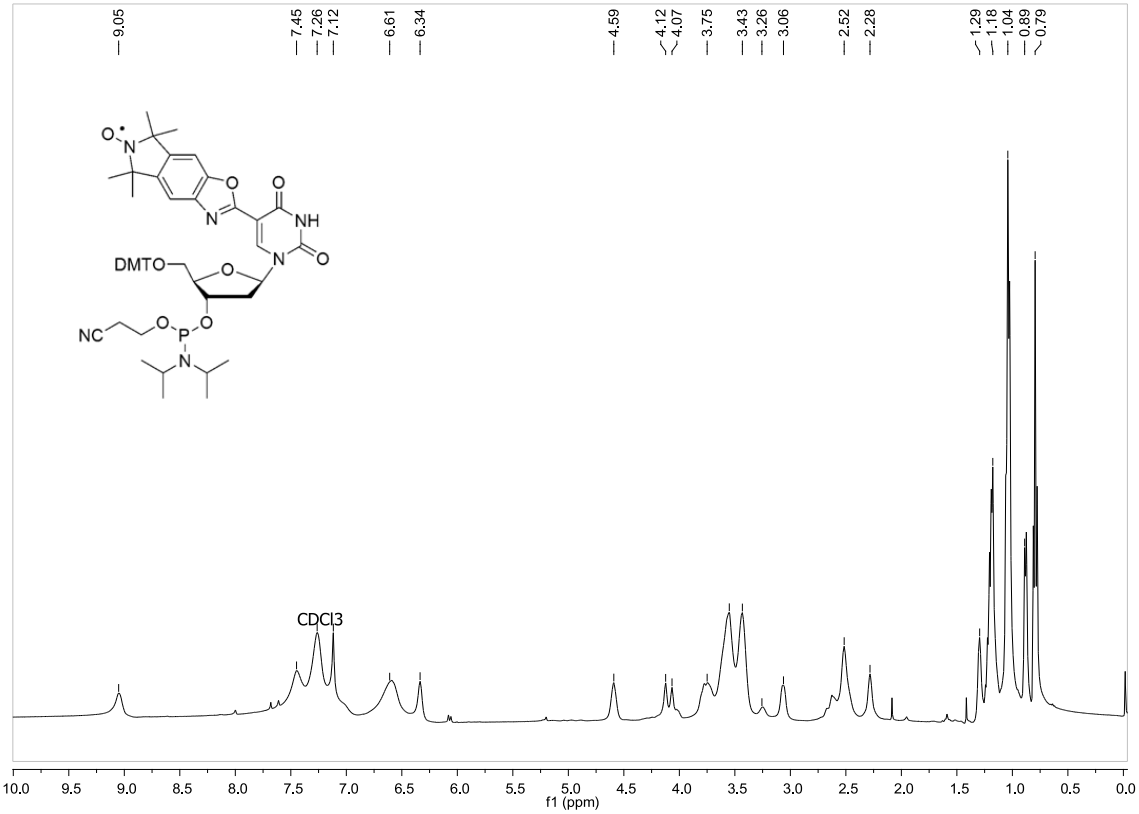
^{Ox}U Phosphoramidite (10B). A mixture of diisopropyl ammonium tetrazolide (9.4 mg, 0.05 mmol) and **DMT-^{Ox}U** (28.0 mg, 0.04 mmol) was dissolved in pyridine (2 mL), evaporated *in vacuo* and residue was kept under *vacuo* for 17 h. CH_2Cl_2 (2.0 mL) and 2-cyanoethyl N,N,N',N'-tetraisopropyl phosphoramidite (33.30 mg, 0.11 mmol) was added. The reaction mixture was stirred at 22 °C for 16 h, diluted with CH_2Cl_2 (10 mL) and washed successively with saturated aq. $NaHCO_3$ (3 x 10 mL) and saturated aq. NaCl (2 x 10 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a solid that was purified by precipitation by first dissolving it in CH_2Cl_2 (0.5 mL), followed by addition of petroleum ether (50 mL). The liquid was decanted and the operation repeated three times to furnish phosphoramidite **10B** as a yellowish solid (26 mg, 74%).

^{1}H NMR ($CDCl_3$): δ 9.05 (br s), 7.45 (br s), 7.26 (br s), 7.12 (br s), 6.61 (br s), 6.34 (br s), 4.59 (br s), 4.12 (br s), 4.07 (br s), 3.75 (br s), 3.55 (br s), 3.43 (br s), 3.26 (br s), 3.06 (br s), 2.52 (br s), 2.28 (br s), 1.29 (br s), 1.18 (br s), 1.04 (br s), 0.89 (br s), 0.79 (br s).

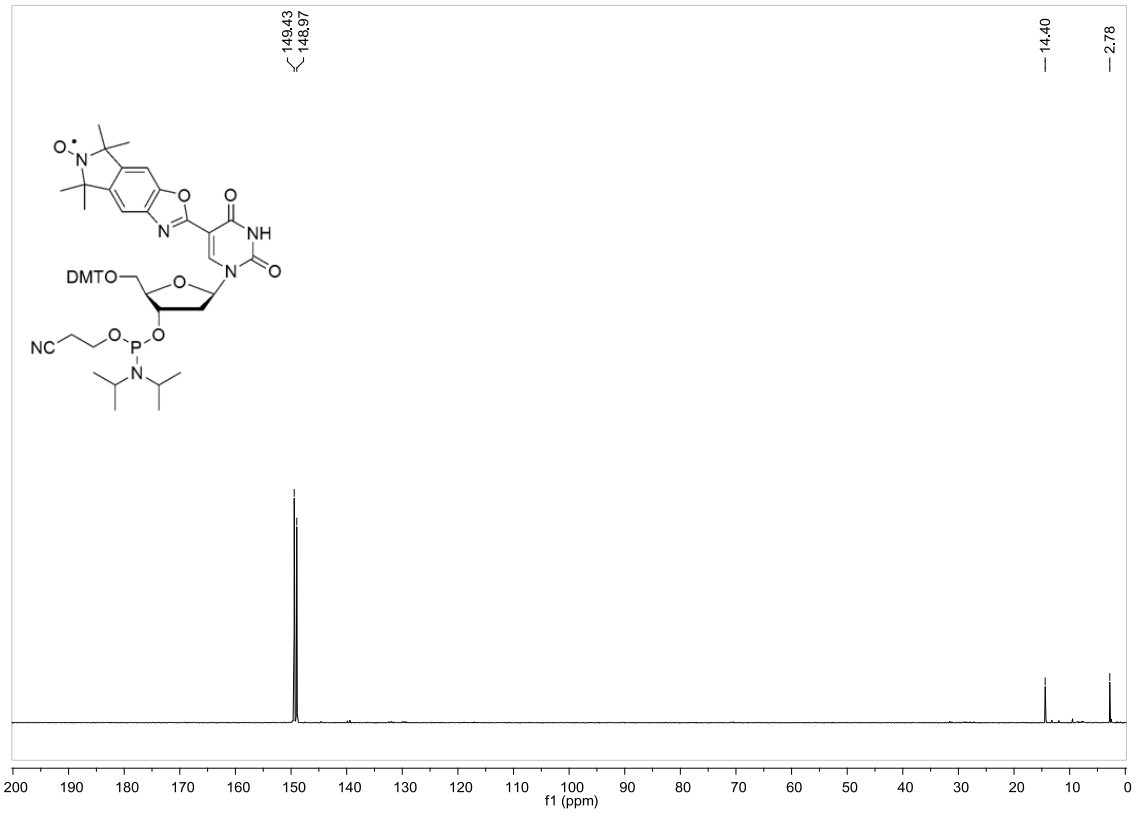
^{13}P NMR ($CDCl_3$): δ 149.43, 148.97, 14.40, 2.78.

HR-ESI-MS $(M + Na)^+$: calcd. for $C_{52}H_{60}N_6O_{10}PNa$ 982.4001, found 982.4007.

^{1}H NMR spectrum of 10B



^{31}P NMR spectrum of 10B



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