Supporting Information

A semi-rigid isoindoline-derived nitroxide spin label for RNA

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MALDI-TOF MS analysis of oligoribonucleotides

The incorporation of ^{Im}**Um** into oligoribonucleotides was confirmed by MALDI-TOF MS analysis. The instrument was calibrated with an external standard prior to measurements. The calculated and observed monoisotopic masses of the oligonucleotides are listed in **Table S1**.

Table S1. Monoisotopic masses of oligoribonucleotides

Sr. No.	Sequence	Monoisotopic mass (M+H) (calculated)	Monoisotopic mass (M+H) (observed)
1	GACCUCGCAUCGUG	4420.625	4420.796
2	CACGAUGCGAGGUC	4483.658	4484.344
6	CACGA^{Im}**Um**GCGAGGUC	4725.788	4725.827
1	GUCGACGGAAGUCGACAGUA	6466.935	6468.196
2	UACUGUCGACUUCCGUCGAC	6277.834	6278.168
5	UAC^{Im}**Um**GUCGACUUCCGUCGAC	6519.964	6520.514
8	G ^{Im}**Um**CGACGGAAGUCGACAGUA	6709.065	6709.359
7	G ^{Im}**Um**CGACGGAAG ^{Im}**Um**CGACAGUA	6951.195	6951.807

Preparation of molecular models

Helix structures were generated using the program Hyperchem. An A form RNA helix was generated with standard parameters and the spin-labeled nucleoside built into the structure. The modified base pair, along with the flanking base pairs, were subsequently minimized by using the Amber force-field, while keeping the rest of the helix frozen.

T_M measurements

To determine if the **^{Im}Um** affected the stability of RNA duplexes, the thermal denaturation curves of unmodified and spin-labeled oligomers were determined. RNA 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 or pH 5.0), annealed and diluted to 1.0 mL with the phosphate buffer (pH 7.0 or pH 5.0 accordingly) and degassed with argon. The samples were heated up from 20 °C to 90 °C (1.0 °C/min) and absorbance at 260 nm was recorded at 1.0 °C intervals (**Figure S1A-E**).

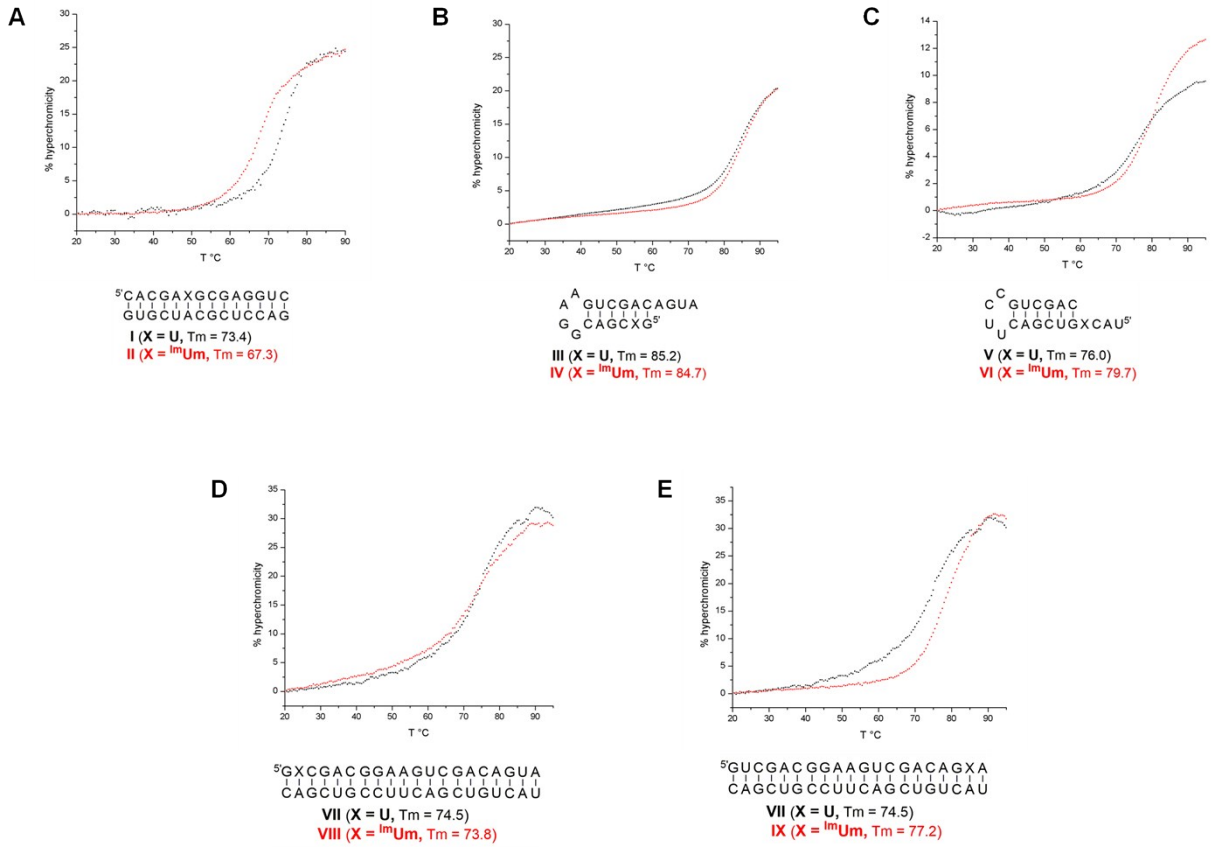


Figure S1. Thermal denaturation curves for **^{Im}Um**-containing RNA oligonucleotides.

CD measurements

To determine if the **^{Im}Um** spin labels had any effect on the RNA duplex conformation, circular dichroism (CD) spectra of a 14-mer unmodified and a spin-labeled RNA hairpin and duplex were recorded. The RNA samples (2.5 nmol of duplexes) were dissolved in 100 µL of phosphate buffer (10 mM phosphate, 100 mM NaCl, 0.1 mM Na_2EDTA, pH 7.0) 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. The annealed samples were diluted to 200 µL with the same buffer. CD spectra of the **^{Im}Um**-modified and unmodified oligoribonucleotides were almost identical, confirming that **^{Im}Um** is well tolerated in an A-form helix (**Figure 2**).

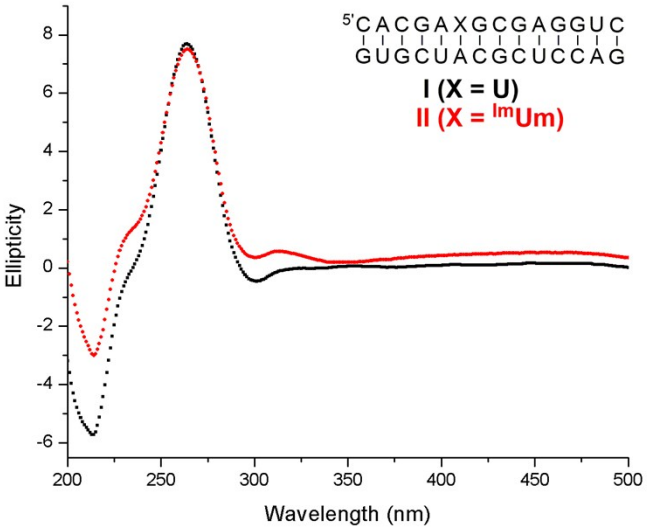
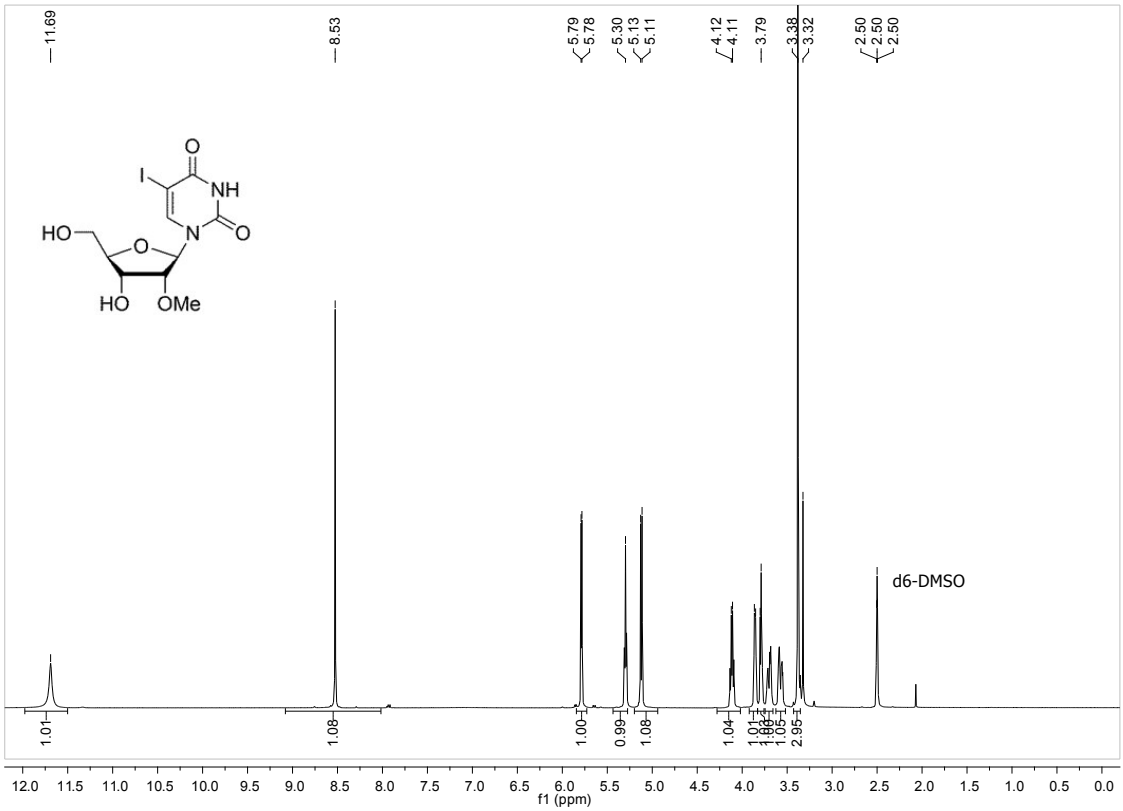
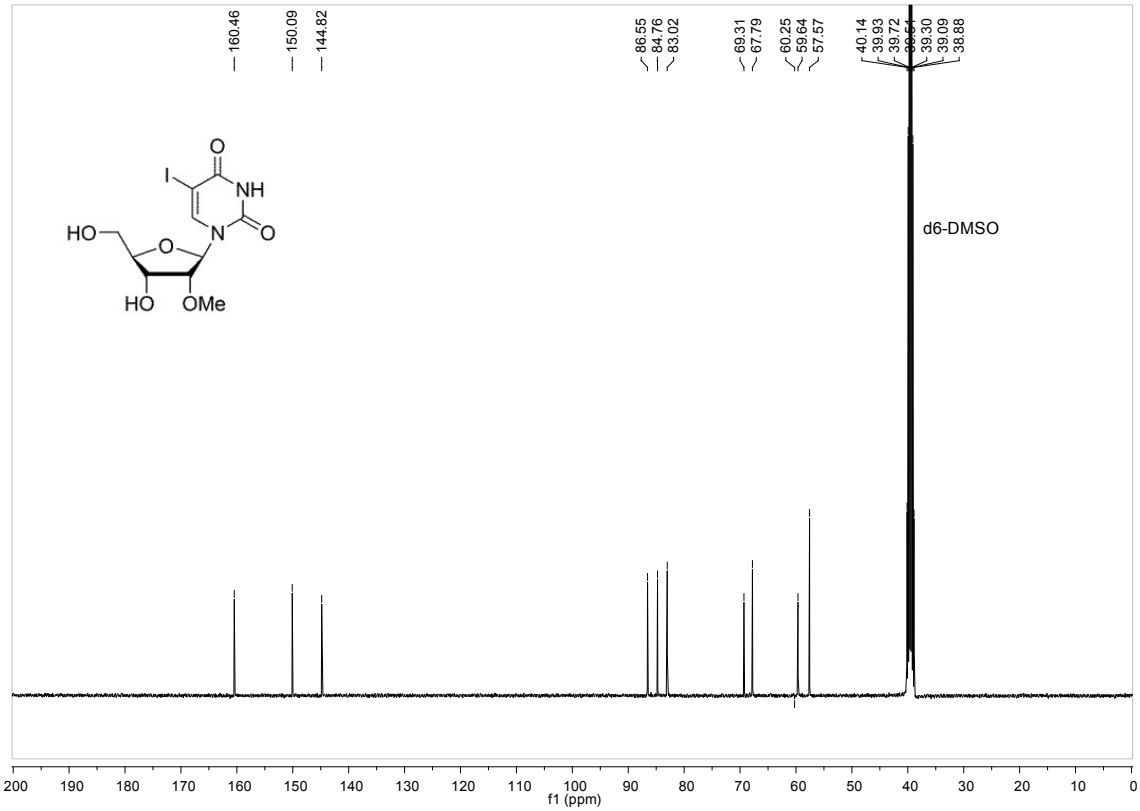


Figure S1. CD curves for **^{Im}Um**-containing RNA oligonucleotides.

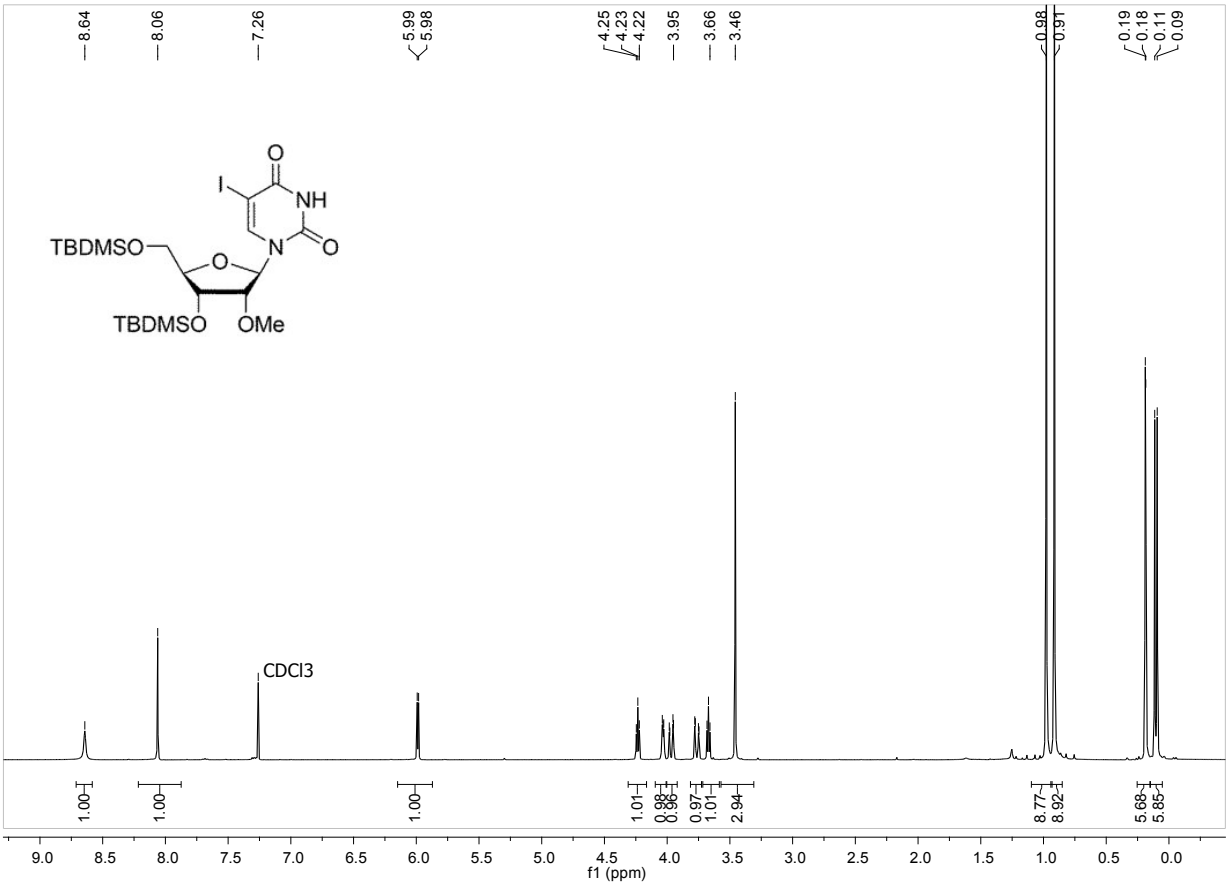
^{1}H NMR spectrum of **1**



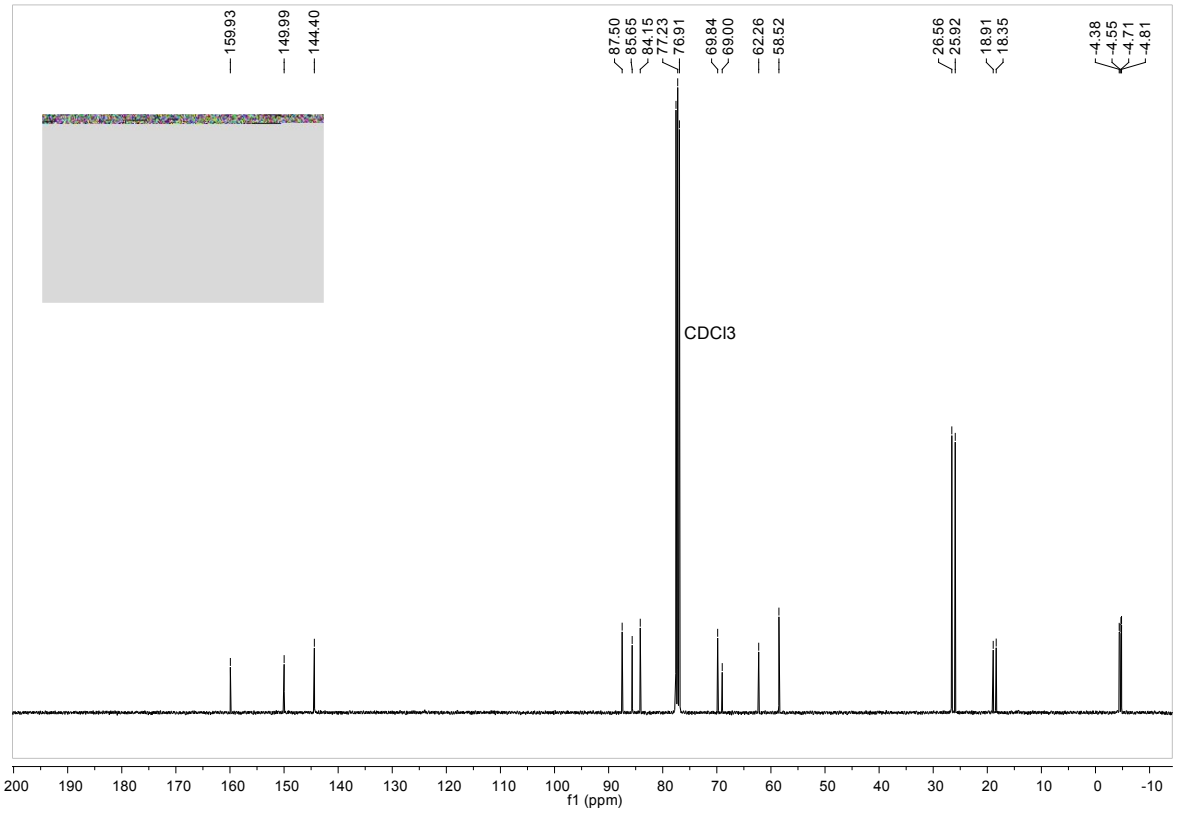
^{13}C NMR spectrum of **1**



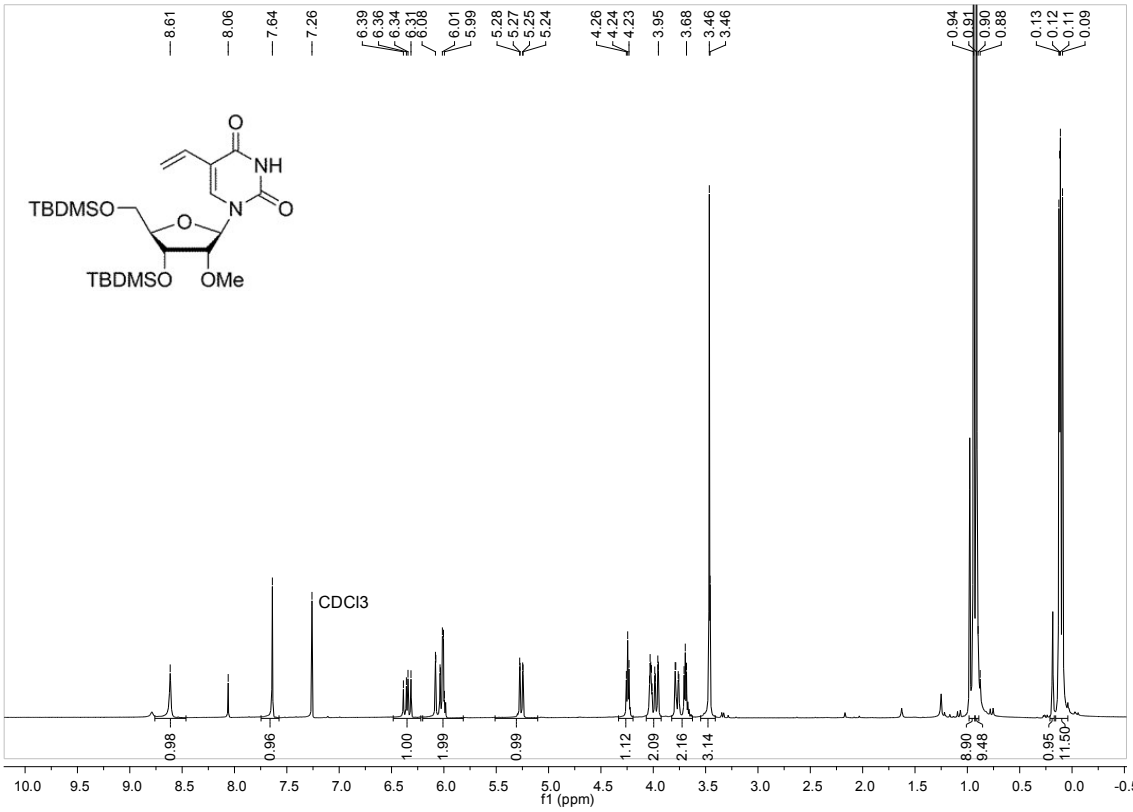
^{1}H NMR spectrum of **2**



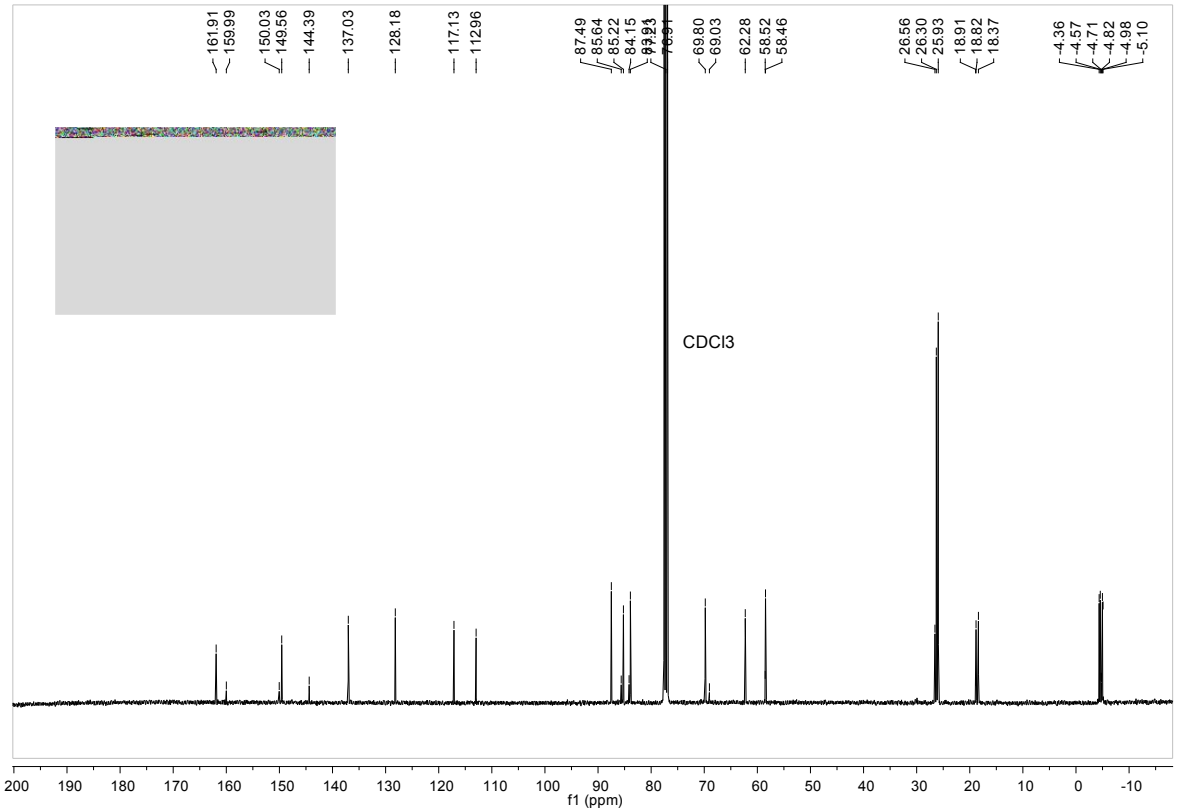
^{13}C NMR spectrum of **2**



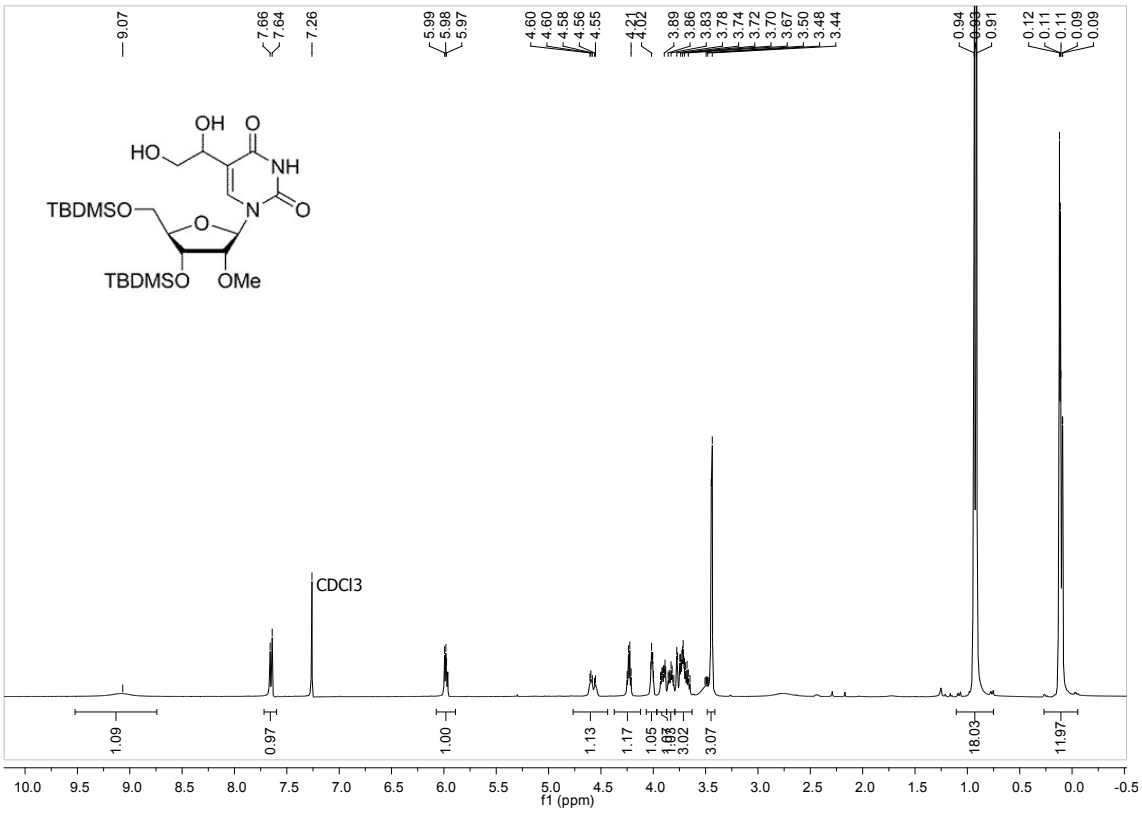
^{1}H NMR spectrum of **3**



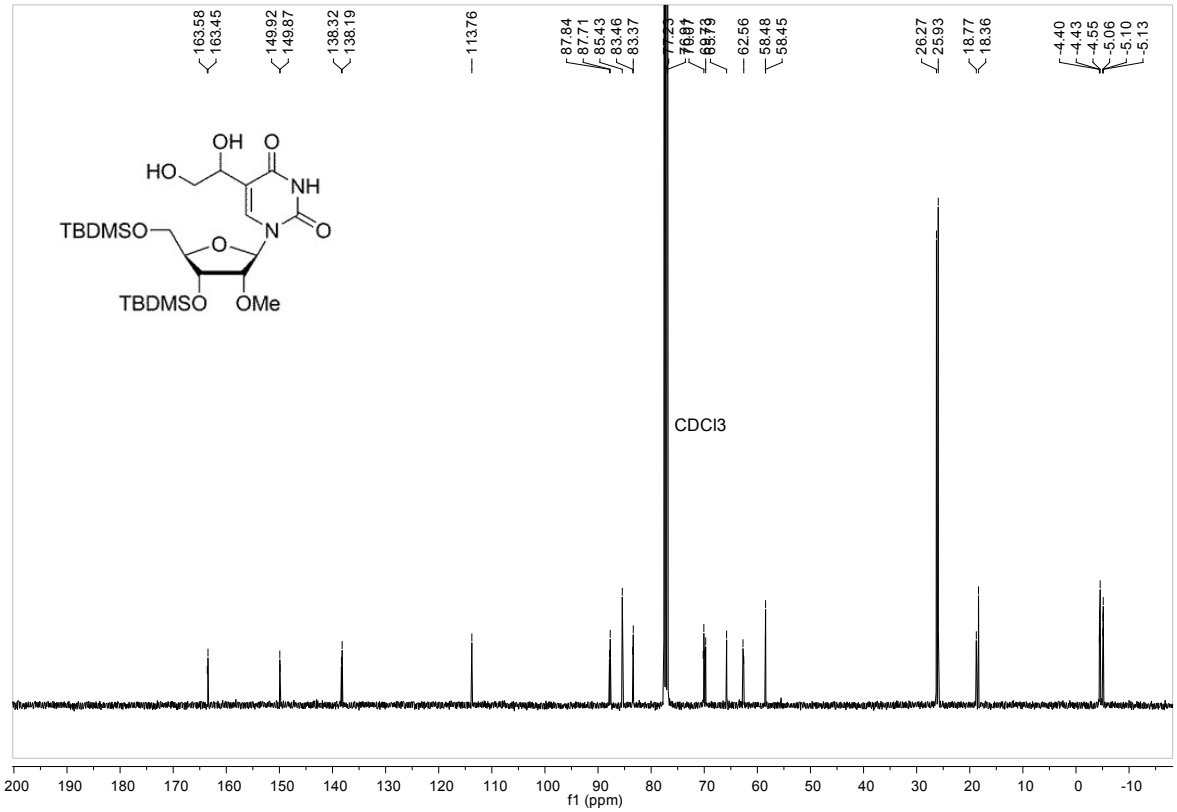
^{13}C NMR spectrum of **3**


161.91
159.99
150.03
149.56
144.39
137.03
128.18
117.13
112.96
87.49
85.64
85.22
84.15
69.80
69.03
62.28
58.52
58.46
26.56
26.30
25.93
18.91
18.82
18.37
-4.36
-4.57
-4.71
-4.82
-4.98
-5.10
CDCl3
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

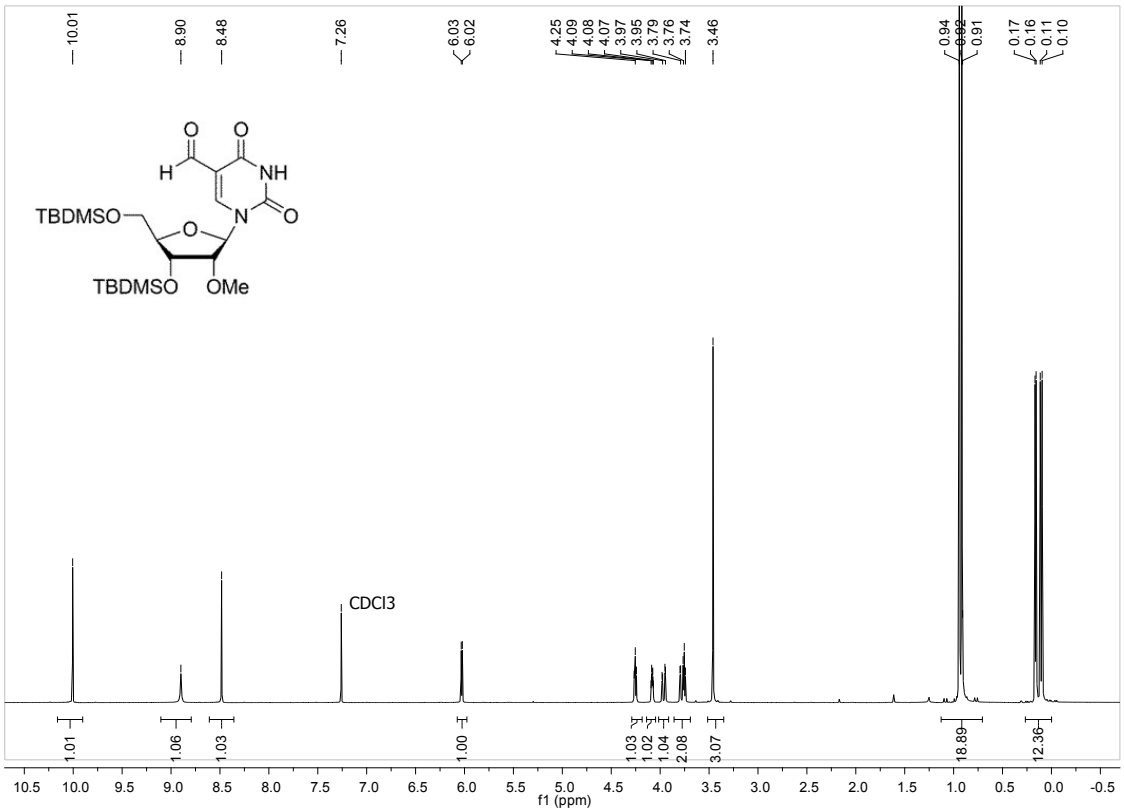
^{1}H NMR spectrum of the intermediate diol **4A**



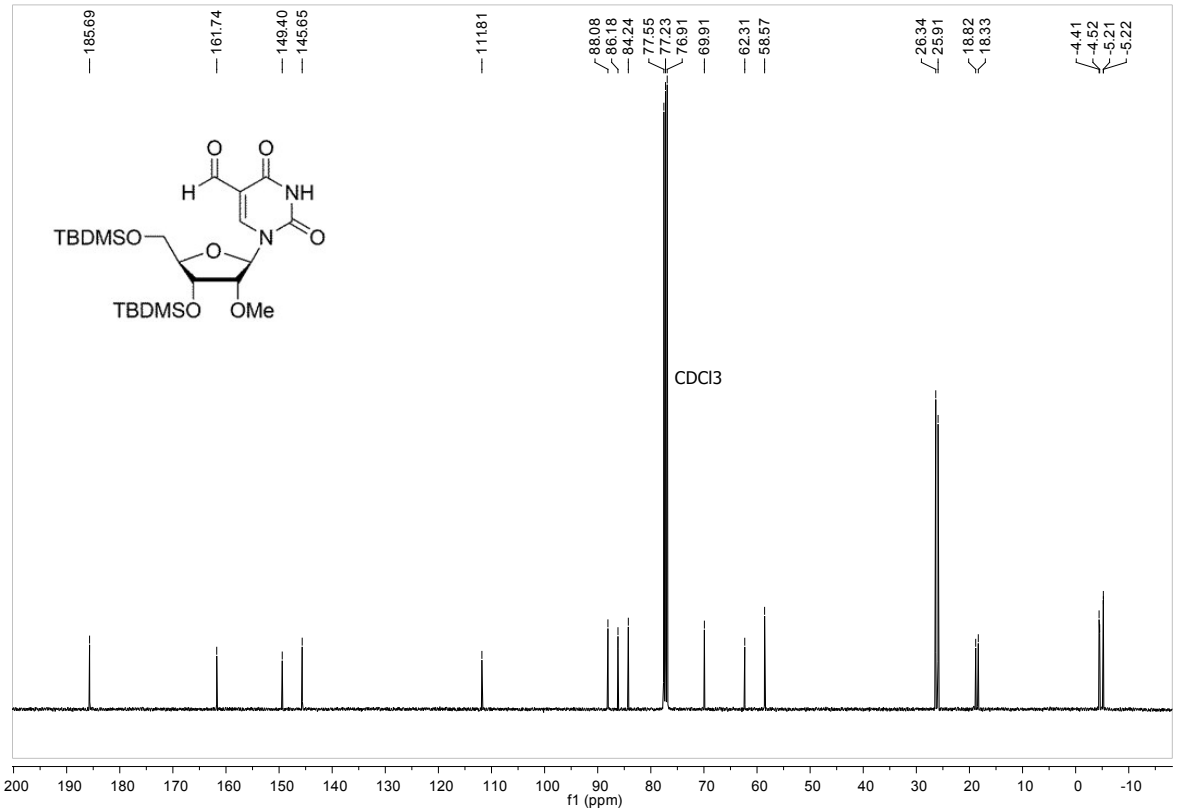
^{13}C NMR spectrum of the intermediate diol **4A**



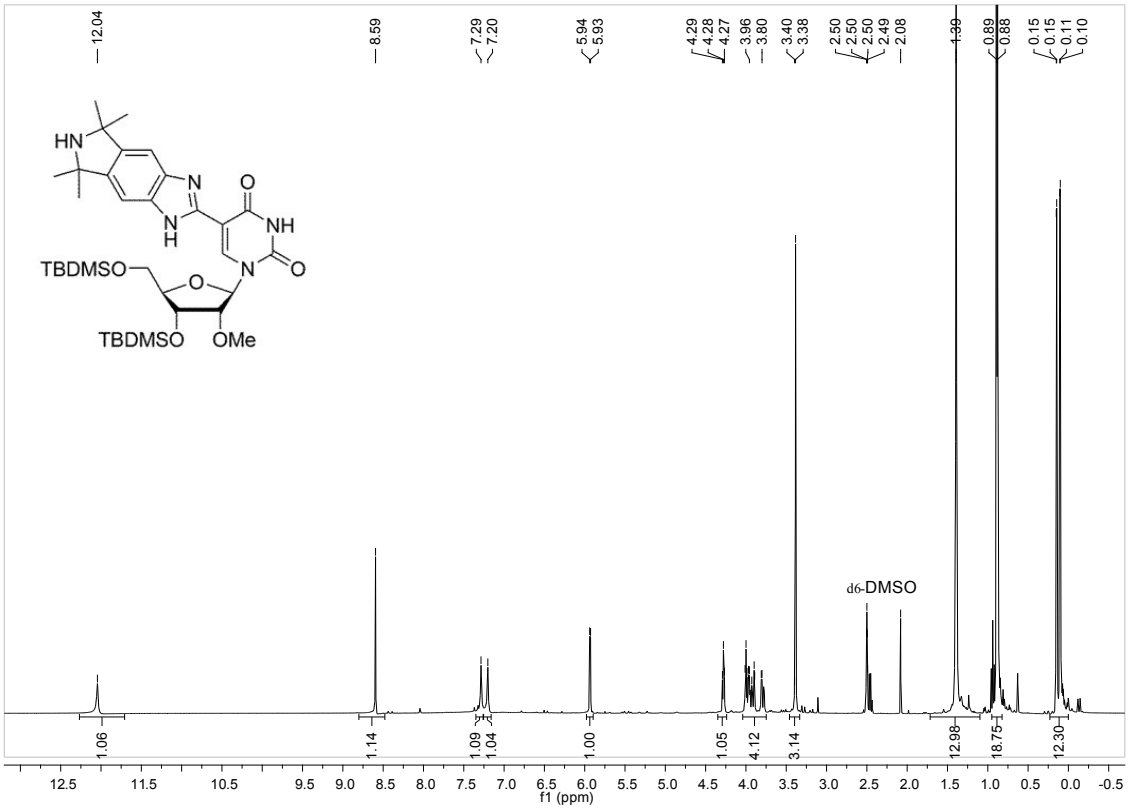
^{1}H NMR spectrum of **4**



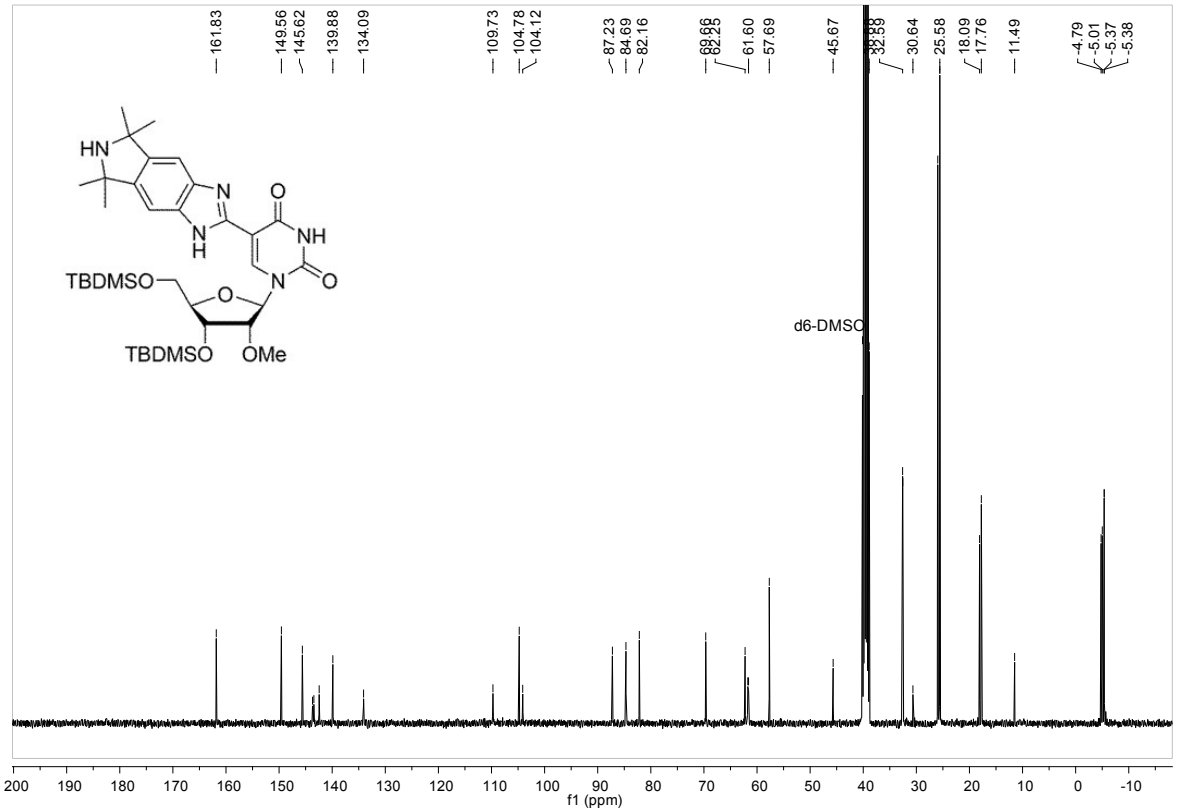
^{13}C NMR spectrum of **4**



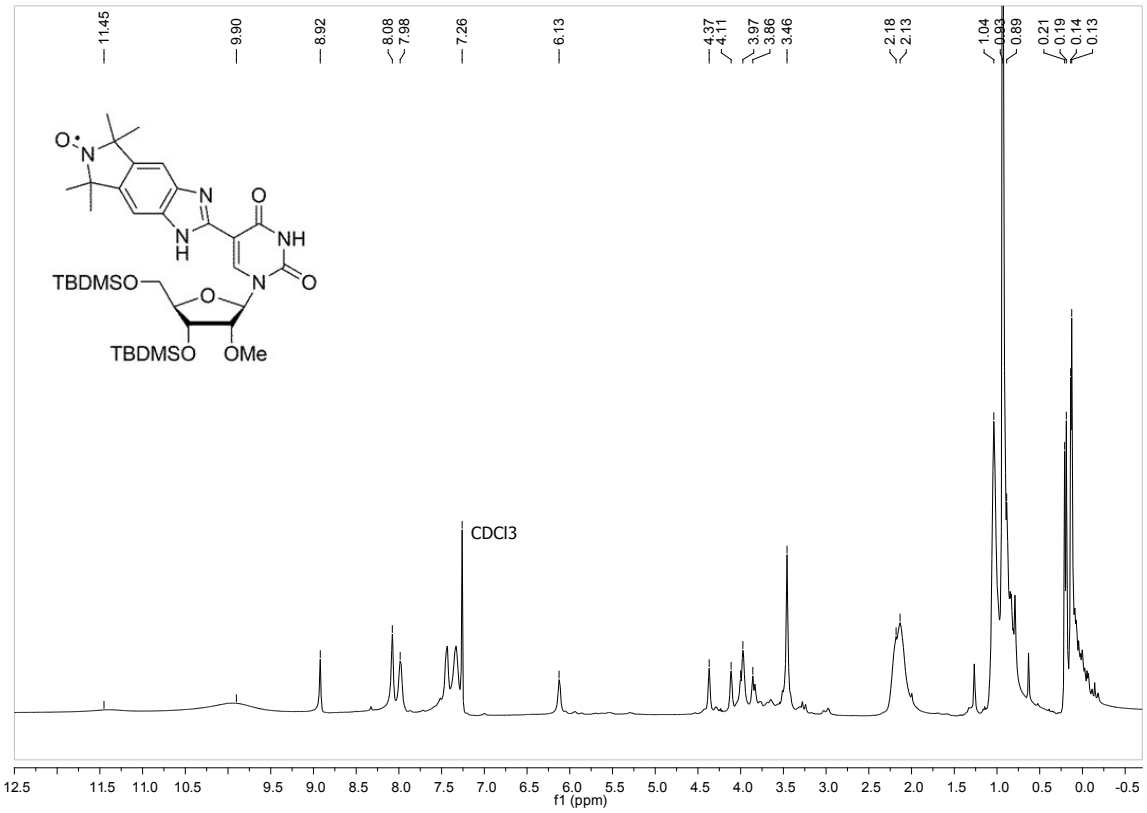
^{1}H NMR spectrum of **6**



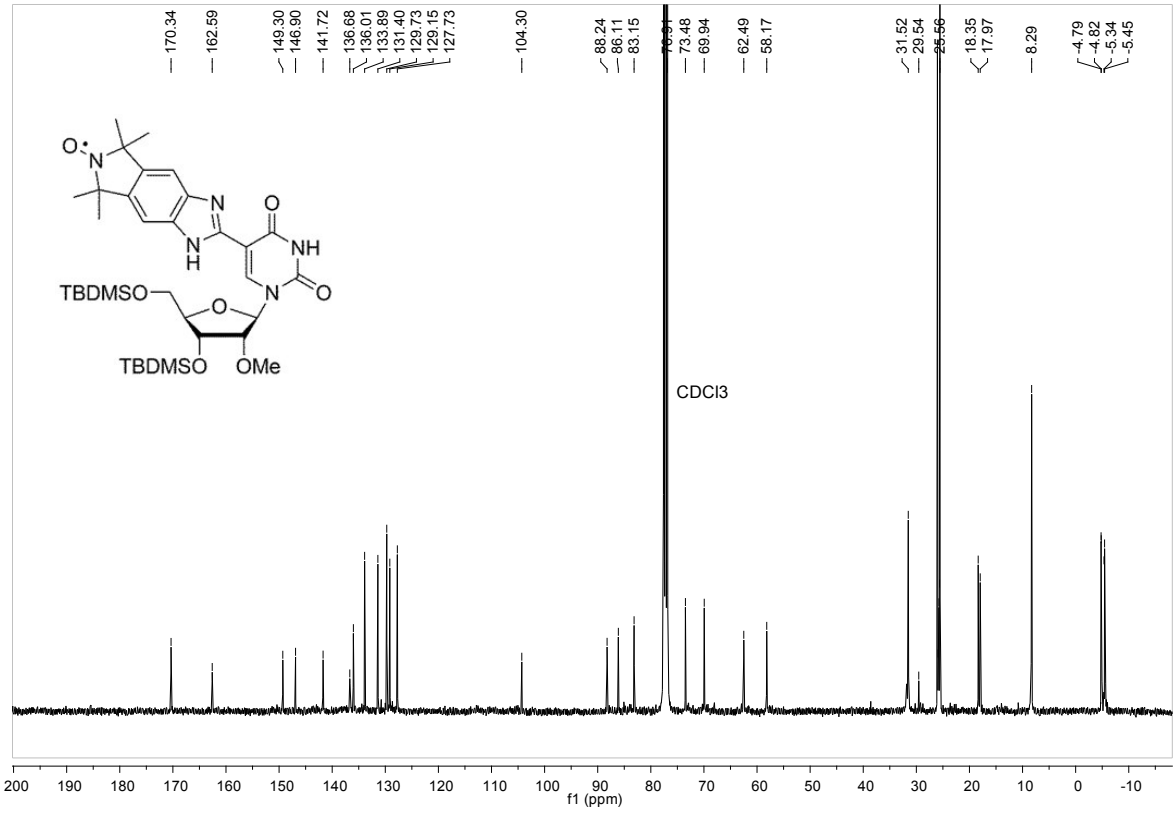
^{13}C NMR spectrum of **6**



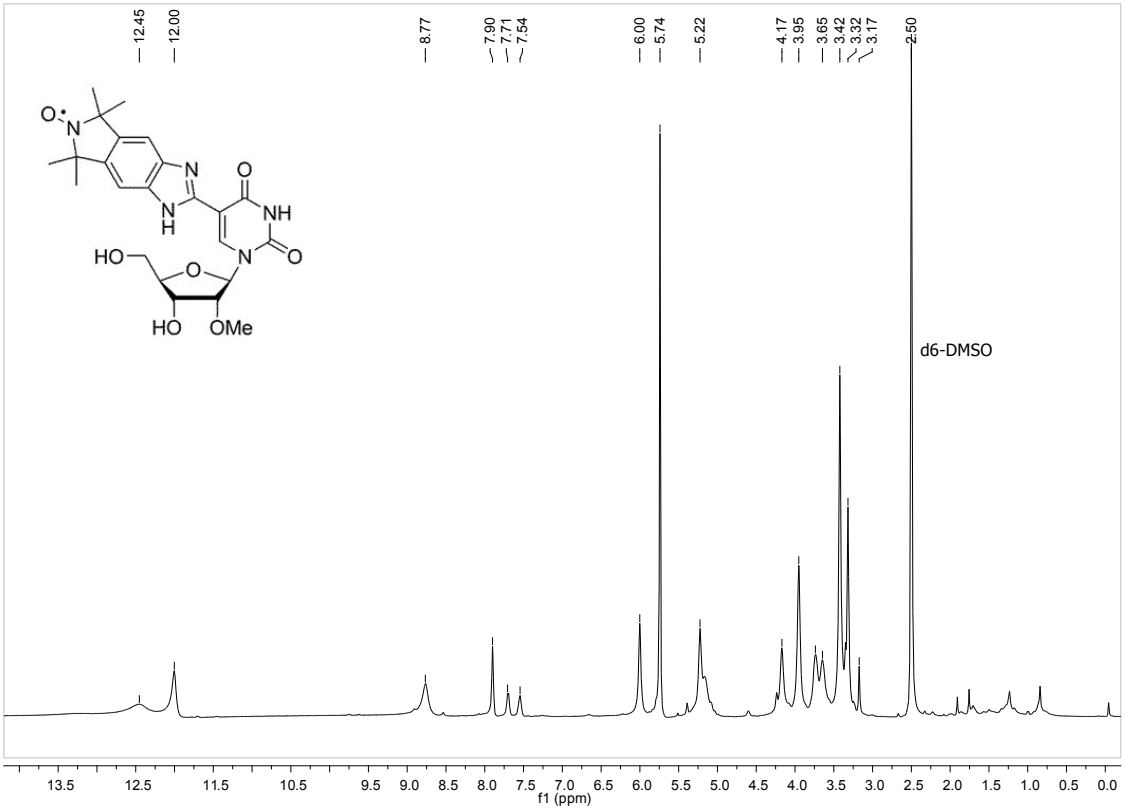
^{1}H NMR spectrum of **7**



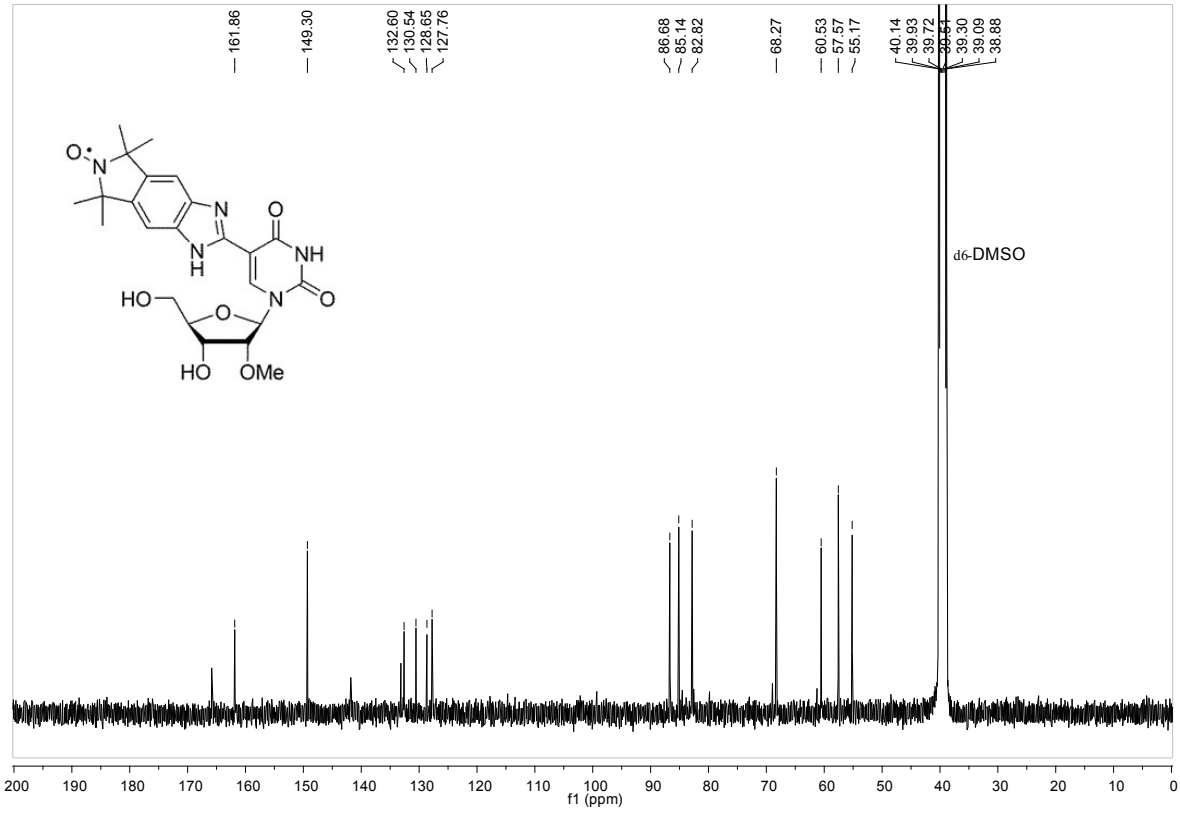
^{13}C NMR spectrum of **7**



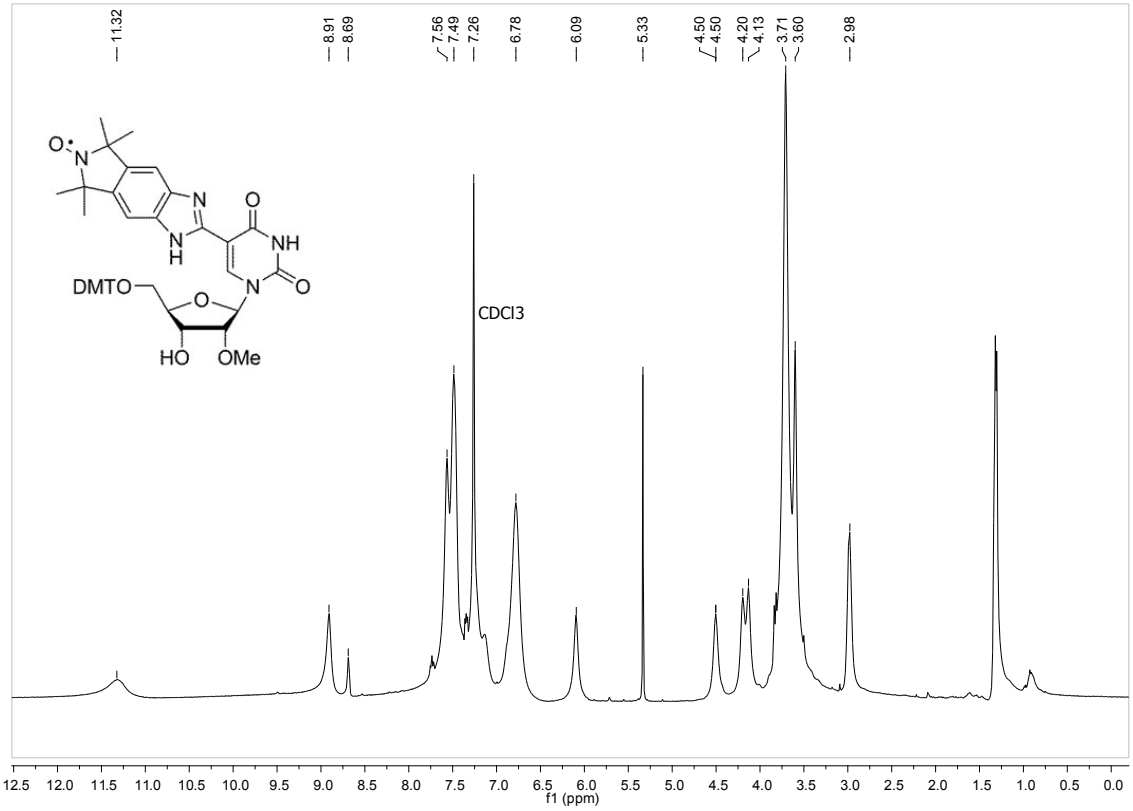
^{1}H NMR spectrum of $^{Im}U_m$



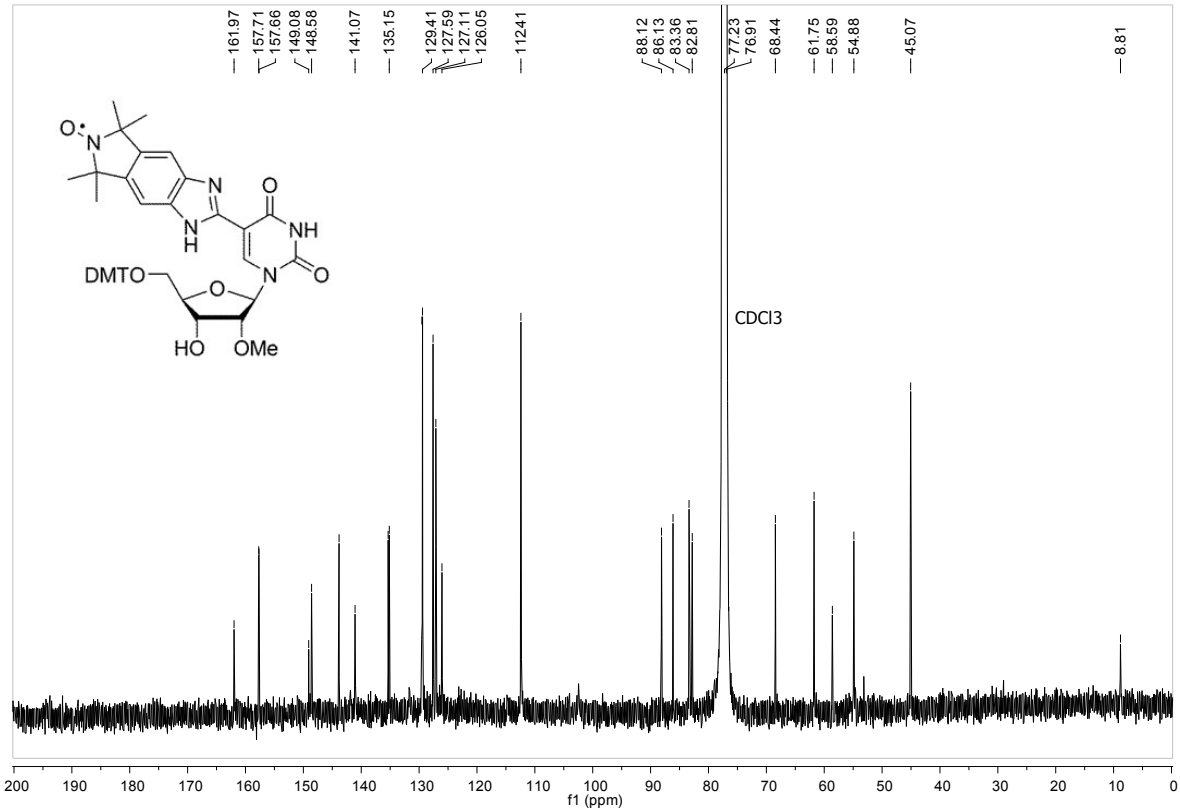
^{13}C NMR spectrum of $^{Im}U_m$



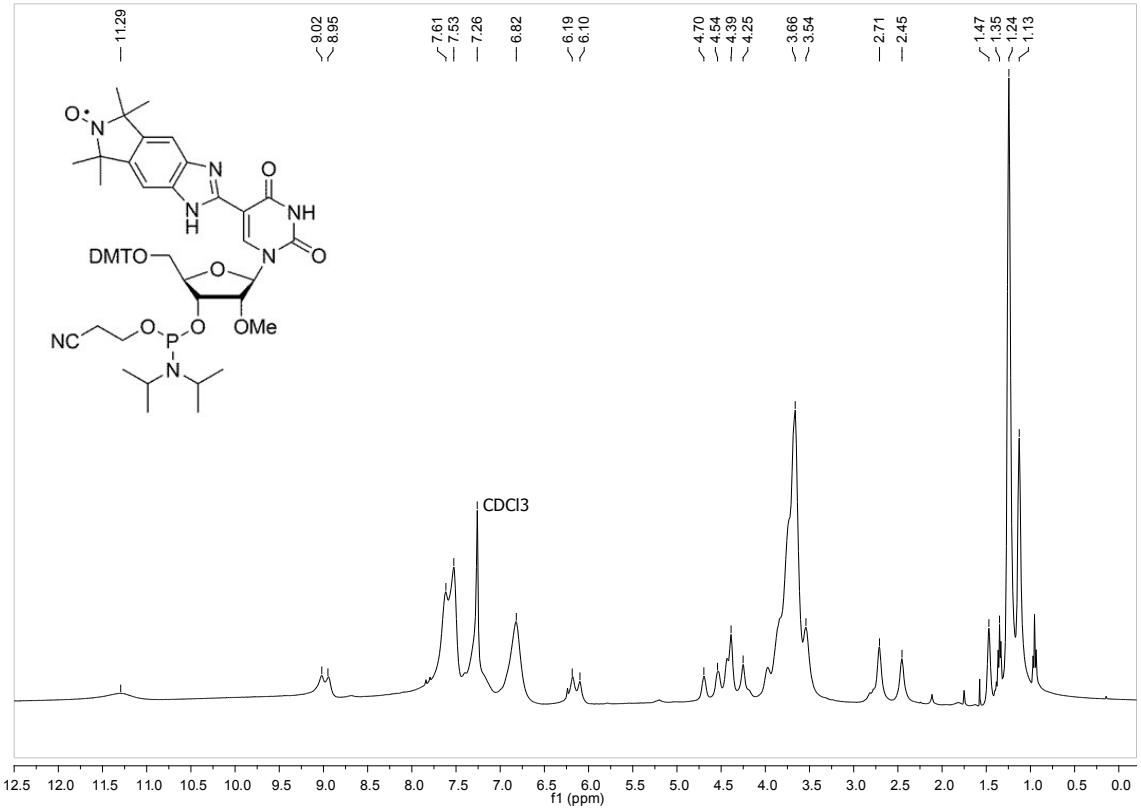
^{1}H NMR spectrum of **8**



^{13}C NMR spectrum of **8**



^{1}H NMR spectrum of **9**



^{31}P NMR spectrum of **9**