Supporting Information

*For*

# Probing the reaction mechanism of spore photoproduct lyase (SPL) via diastereoselectively labeled dinucleotide SP TpT substrates

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**Abbreviations used**

SP, 5-thyminyl-5,6-dihydrothymine, also called spore photoproduct; PivCl, trimethylacetyl chloride; DMTr, 4,4′-dimethoxytrityl; Py, pyridine; Ac, acetyl; TFA, trifluoroacetic acid; TLC, thin layer chromatography; $CDCl_3$, choloroform-$d_3$; $CD_3OD$, methanol-$d_4$; DMSO, dimethylsulfoxide-$d_6$; Abbreviations for NMR signal coupling are as follows: s, singlet; d, doublet; m, multiplet. EPR: electron paramagnetic resonance; MS: mass spectroscopy; ESI: electrospray ionization; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis.

**General Methods**

All DNA-modifying enzymes and reagents were purchased from Fermentas Life Sciences (Glen Burnie, MD). Oligonucleotide primers were obtained from Integrated DNA Technologies (Coralville, IA). *E. Coli* BL21(DE3) and expression vector pET-28a were purchased from Novagen (Madison, WI). The construct containing the SPL gene was co-expressed with plasmid pDB1282, which was a generous gift from Prof. Squire Booker at the Pennsylvania State University. 5′-deoxyadenosine (5′-dA) and *S*-adenosylmethionine (SAM) was purchased from Aldrich and used directly without further purification. All other buffers and chemicals were of the highest grade available.

All reagent grade chemicals were purchased from Sigma, Fisher, or VWR and used without further purification. All reactions were carried out using oven or flame-dried glassware under a nitrogen atmosphere in distilled solvents. Dichloromethane and pyridine were distilled over calcium hydride. Purification of reaction products was carried out by flash chromatography using silica gel (Dynamic Adsorbents Inc, 32-63 μm). For TLC analysis, precoated plates (w/h F254, Dynamic Adsorbents Inc, 0.25 mm thick) were used. The $^1H$ and $^{13}C$ NMR spectra were obtained on a Bruker 500 MHz NMR Fourier transform spectrometer. NMR spectra were recorded in sample solutions in deuterated chloroform ($CDCl_3$), with residual chloroform ($\delta$ 77.2 ppm for $^{13}C$ NMR) and TMS ($\delta$ 0 ppm for $^1H$ NMR), deuterated methanol ($\delta$ 3.31 ppm for $^1H$ NMR and $\delta$ 49.1 ppm for $^{13}C$ NMR) or deuterated methyl sulfoxide (DMSO-$d_6$), with residual methyl sulfoxide ($\delta$ 2.50 ppm for $^1H$ NMR and $\delta$ 39.5 ppm for $^{13}C$ NMR) taken as the standard. $H_3PO_4$ ($\delta$ 0 ppm for $^{31}P$ NMR) was used as the external standard for $^{31}P$ NMR spectrum. The chemical shifts in NMR spectra were reported in parts per million (ppm). MS analysis was obtained using Agilent 1100 series LC/MSD system with electrospray ionization. The TpT photoreaction was carried out using a Spectroline germicidal UV sterilizing lamp (Dual-tube, 15 w, intensity: 1550 uw/cm$^2$) with the samples ~5 cm to the lamp.

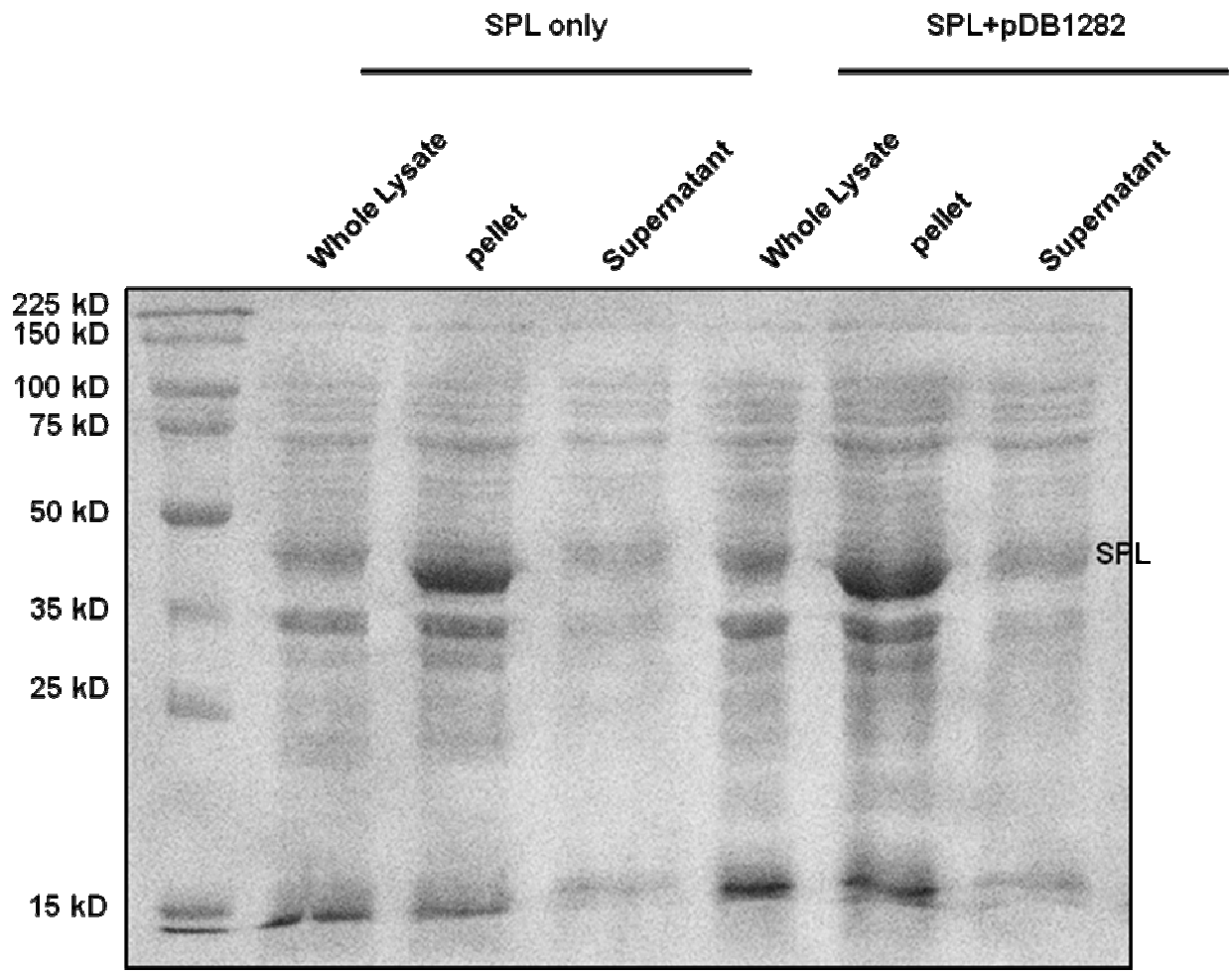



**Figure S1**. SDS-PAGE shows that co-expression of SPL gene with the pDB 1282 plasmid in *E.coli* at least doubles the amount of SPL in supernatant.

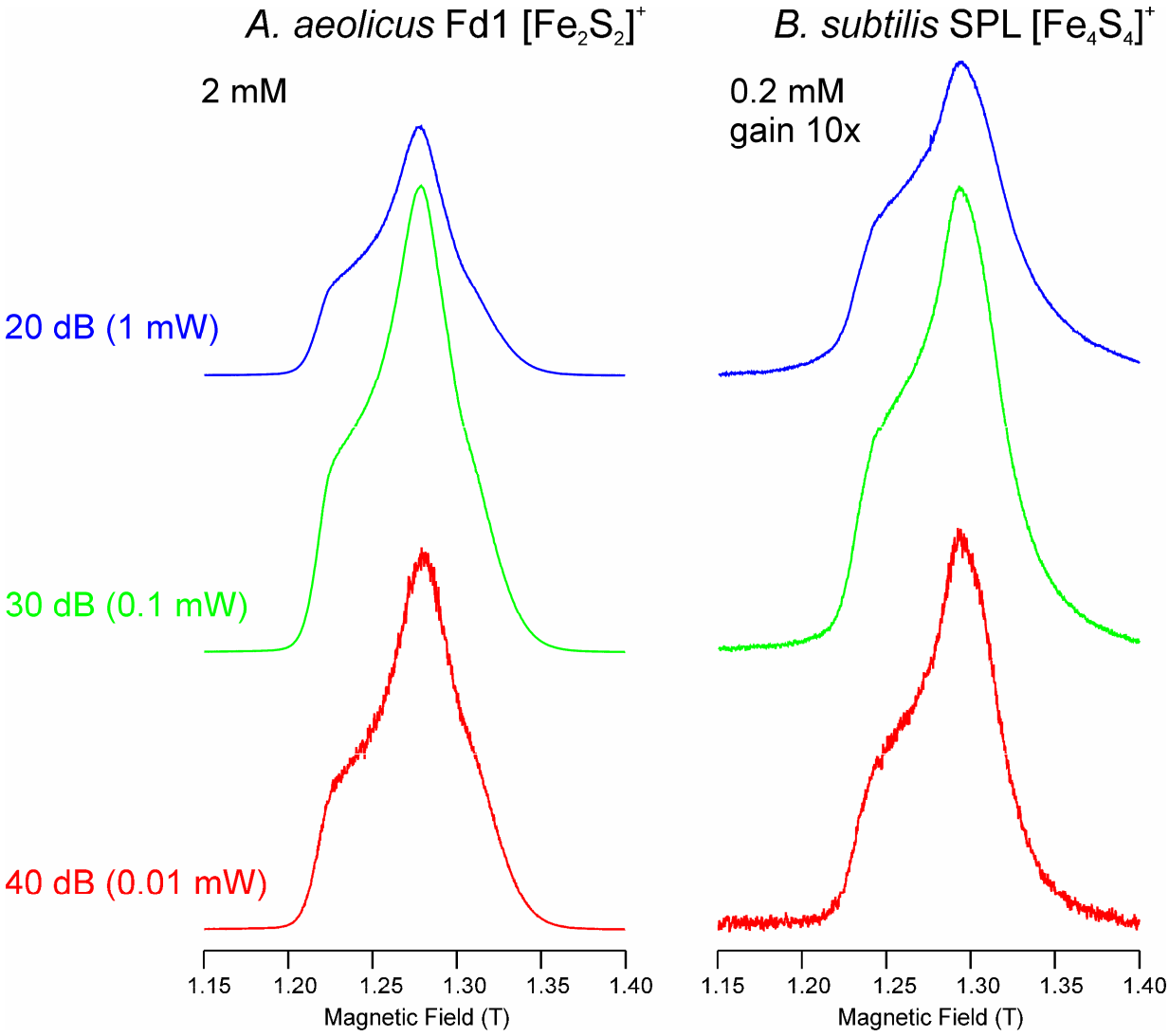
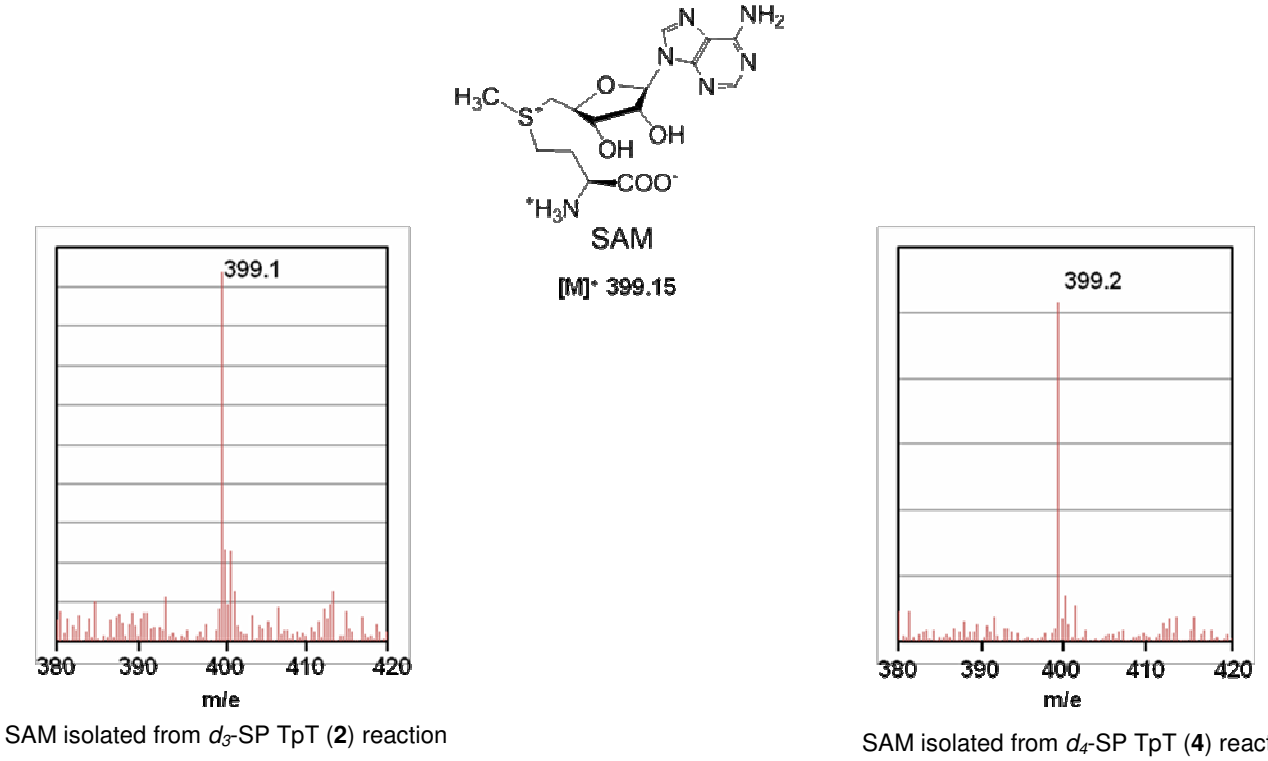



**Figure S2.** 35 GHz EPR spectra of dithionite-reduced *B. Subtilis* SPL and *A. Aeolicus* Fd1 obtained at microwave power of 10, 100 and 1000 μW respectively. At 10 μW, where saturation effects were minimized, the integrated intensity of a 2 mM solution of *A. Aeolicus* Fd1 was found to be ~ ten times that of SPL, suggesting a 200 μM concentration for the $[4Fe\text{-}4S]^{+}$ cluster in the SPL sample.




SAM isolated from $d_3$-SP TpT (**2**) reaction

SAM isolated from $d_4$-SP TpT (**4**) reaction

**Figure S3**. ESI-MS spectra of SAM isolated from SPL reaction when $d_3$-SP TpT (**2**) or $d_4$-SP TpT (**4**) was used as the enzyme substrate.

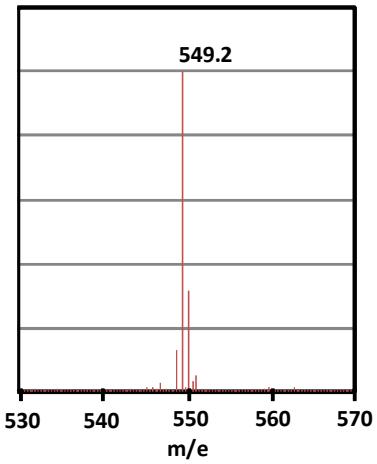



**Figure S4**. ESI-MS spectra of TpT isolated from SPL reaction in $D_2O$ when $d_4$-SP TpT (**4**) was used as the enzyme substrate. The vast majority of the TpT product contained four deuteriums as indicated by the $[M - H]^-$ signal of 549.2.

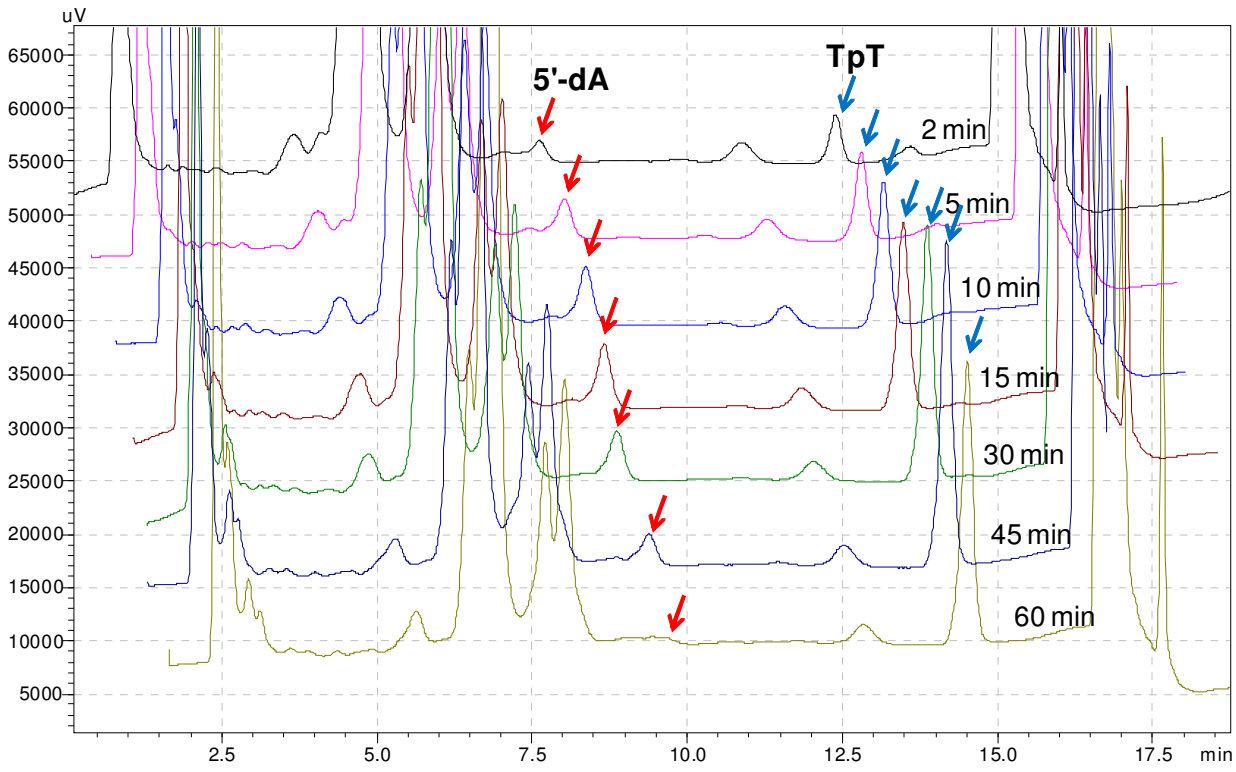
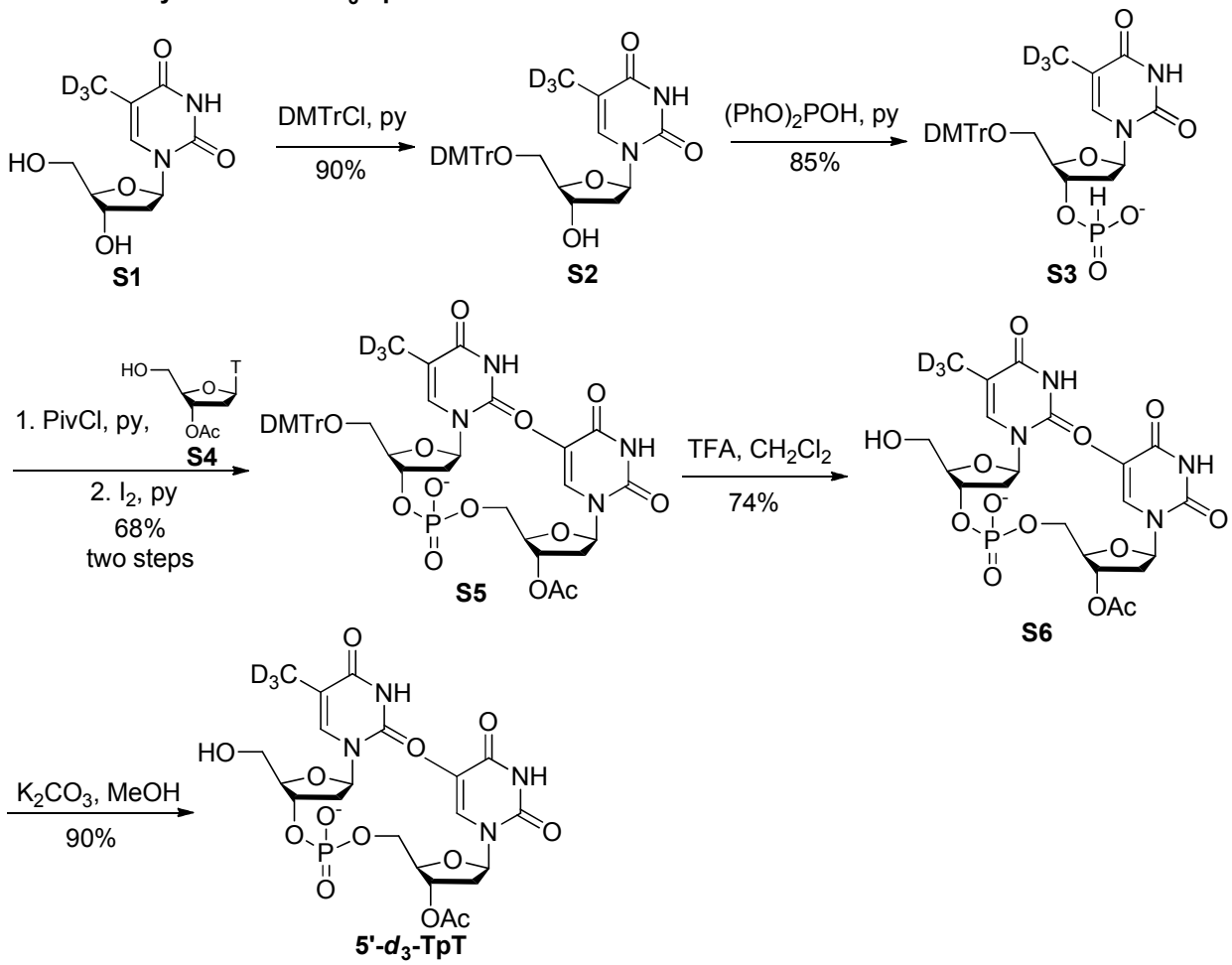



**Figure S5**. HPLC chromatograph of 5′-dA and TpT formation in a 1-hr period of SP repair. The reaction solution contained 30 μM SPL, 3 mM correspondent SP TpT substrate, 300 μM SAM, and 2 mM DTT in a final volume of 400 μL of buffer containing 25 mM Tris-HCl, 300 mM NaCl and 10% glycerol at pH 7.0. Sodium dithionite (final concentration 1 mM) was added as a reductant to initiate the enzyme reaction. The reactions were carried out under anaerobic conditions at ambient temperature for various periods of time. At each time point, 50 μL of the solution was taken out to an Eppendorf tube, quenched by 5 μL of 3 M HCl and analyzed by HPLC.

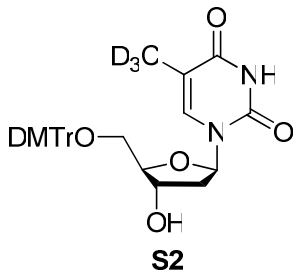
**Preparation of 5′-$d_3$-TpT via chemical synthesis and 5′-$d_3$-SP TpT (3) via photolysis**

The labeled dinucleotide 5′-$d_3$-TpT was synthesized under a similar procedure as described in our previous publication.[1] Briefly, the C3 hydroxyl group of labeled $d_3$-thymidine **S1**, which was prepared according to the literature method,[2] was protected with a DMTr moiety to yield **S2**. Compound **S2** was then coupled to a non-labeled thymidine via the traditional H-phosphate chemistry.[3] Subsequent de-protection afforded the selectively labeled 5′-$d_3$-TpT. The synthetic details are described as the follows.

**SHEME S1: Synthesis of 5'-$d_3$-TpT**

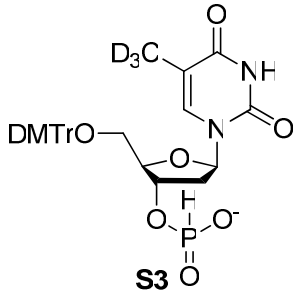



**Protection of the 5′-OH moiety in S1 to yields DMTr-$d_3$-thymidine (S2).**



A mixture of **S1** (2.05 g, 8.35 mmol) and 4,4′-dimethoxytrityl chloride (3.50 g, 10.3 mmol) was stirred in pyridine (40 mL) at 0 °C. After 18 h, the reaction was quenched with MeOH (3 mL), transferred into a saturated $NaHCO_3$ solution (25 mL), and extracted with EtOAc (3 × 100 mL). The organic layers were combined, washed with brine (20 mL), dried over $Na_2SO_4$, and concentrated by rotary evaporation. Purification of the resulting residue via flash chromatography (eluent: hexane/EtOAc/MeOH = 1/1/0.1) yielded **S2** as a white solid (4.12 g, 90%). $^1$H NMR ($CDCl_3$): $\delta$ 2.27-2.35 (m, 1H), 2.44 (ddd, $J$ = 2.5, 5.8, 13.6 Hz, 1H), 3.35 (dd, $J$ = 2.9, 10.5 Hz, 1H), 3.45 (dd, $J$ = 3.0, 10.5 Hz, 1H), 3.76 (s, 6H), 4.05-4.11 (m, 1H), 4.54-4.60 (m, 1H), 6.44 (dd, $J$ = 5.9, 7.9 Hz, 1H), 6.80-6.84 (m, 4H), 7.19-7.24 (m, 1H), 7.25-7.32 (m, 6H), 7.37-7.42 (m, 2H), 7.61 (s, 1H), 9.69 (s, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$ 41.1, 55.3, 63.8, 72.6, 84.9, 86.5, 87.0, 111.3, 113.4, 127.2, 128.1, 128.2, 130.2, 135.5, 135.9, 144.5, 150.8, 158.8, 164.3; ESI-MS (positive mode) calcd for $C_{31}H_{29}D_3N_2NaO_7$: 570.2 ($M + Na^+$), found 570.2.

**Preparation of the $d_3$-thymidine H-phosphonate (S3).**

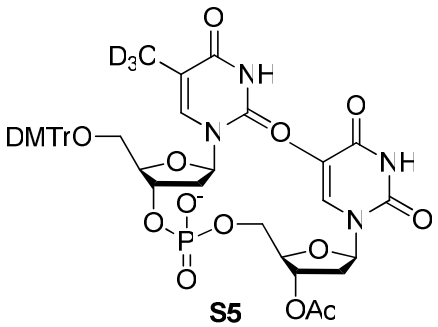



**S3**

To the solution of nucleoside **S2** (3.50 g, 6.39 mmol) in pyridine (30 mL), diphenylphosphite (8.50 mL, 44.2 mmol) was added. After 15 min (TLC analysis) the reaction mixture was quenched by addition of a mixture of water-triethylamine (1:1 v/v, 11.5 mL). The solvent was subsequently evaporated and the residue partitioned between methylene dichloride (250 mL) and 5% *aq.* $NaHCO_3$ (50 mL). The organic layer was washed twice with 5% *aq.* $NaHCO_3$ (34 mL), dried over $Na_2SO_4$ and finally evaporated to yield an oil. Purification of the oil via flash chromatography (eluent: $CH_2Cl_2$/MeOH /$Et_3N$ = 20/1/1) yielded **S3** (3.85 g, 85%) as a white solid. $^1$H NMR ($CDCl_3$): $\delta$ 2.40 (ddd, $J$ = 6.0, 8.3, 13.5 Hz, 1H), 2.60 (ddd, $J$ = 2.2, 5.6, 13.5 Hz, 1H), 3.39 (dd, $J$ = 2.7, 10.6 Hz, 1H), 3.48 (dd, $J$ = 2.7, 10.6 Hz, 1H), 3.78 (s, 6H), 4.25-4.30 (m, 1H), 5.00-5.05 (m, 1H), 6.47 (dd, $J$ = 5.6, 8.3 Hz, 1H), 6.80-6.85 (m, 4H), 7.20-7.24 (m, 1H), 7.25-7.33 (m, 6H), 7.38-7.43 (m, 2H), 7.59 (s, 1H), 9.93 (brs, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$ 39.7, 55.2, 63.5, 74.1, 84.4, 85.2, 86.9, 111.0, 113.2, 127.0, 127.9, 128.2, 130.1, 130.2,

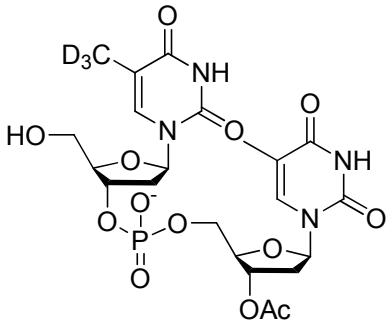
135.3, 135.4, 135.7, 144.3, 150.7, 158.6, 164.2; ESI-MS (positive mode) calcd for $C_{31}H_{31}D_3N_2O_9P$: 612.2 ($M + H^+$), found 612.3.

**Preparation of the protected 5′-$d_3$-TpT dinucleotide (S5).**




To a mixture of **S3** (3.00 g, 4.20 mmol) and **S4** (1.44 g, 5.04 mmol) in pyridine (35 mL), PivCl (1.50 mL, 12.6 mmol) was added. After 15 min, the reaction mixture was diluted with EtOAc and quenched by addition of saturated $NaHCO_3$ (60 mL). The organic phase was dried over $Na_2SO_4$ and concentrated by rotary evaporation. The resulting residue was dissolved in a 0.2 M $I_2$ solution (90 mL) in Py/$H_2O$/THF (95/5/5). After stirring for 10 min, the reaction was quenched with *aq.* $NaHCO_3$/$Na_2S_2O_3$ solution (60 mL). The solvent was then removed via rotary evaporation and the resulting residue partitioned between dichloromethane (300 mL) and water (60 mL). The aqueous phase was extracted with dichloromethane (2 × 60 mL). The combined organic layers were dried over $Na_2SO_4$ and concentrated under vacuum. Purification of the resulting residue via flash chromatography (eluent: $CH_2Cl_2$/MeOH/$Et_3N$ = 20/1/1) yielded **S5** (2.83 g, 68%) as a white solid. $^1$H NMR ($CDCl_3$): $\delta$ 1.91 (s, 3H), 2.06 (s, 3H), 2.22-2.30 (m, 2H), 2.37 (ddd, $J$ = 5.6, 9.1, 13.5 Hz, 1H), 2.64 (dd, $J$ = 5.5, 13.5 Hz, 1H), 3.37 (dd, $J$ = 2.1, 10.4 Hz, 1H), 3.50 (dd, $J$ = 2.5, 10.4 Hz, 1H), 3.77 (s, 6H), 4.02-4.13 (m, 3H), 4.30-4.33 (m, 1H), 5.00-5.05 (m, 1H), 5.25-5.29 (m, 1H), 6.42 (t-like, $J$ = 7.4 Hz, 1H), 6.46 (dd, $J$ = 5.3, 8.9 Hz, 1H), 6.79-6.84 (m, 4H), 7.19-7.23 (m, 1H), 7.23-7.30 (m, 6H), 7.35-7.40 (m, 2H), 7.62 (s, 1H), 7.78 (s, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$ 12.5, 21.0, 37.2, 39.6, 55.3, 64.0, 65.6, 75.5, 76.6, 83.8, 84.4, 84.6, 85.4, 87.0, 111.2, 111.6, 113.3, 127.1, 127.8, 128.3, 130.2, 130.3, 135.3 (2C), 135.8, 136.0, 144.3, 150.8, 150.9, 158.7 (2C), 164.0, 164.1, 170.4; ESI-MS (positive mode) calcd for $C_{43}H_{45}D_3N_4O_{15}P$: 894.3 ($M + H^+$), found 894.3.

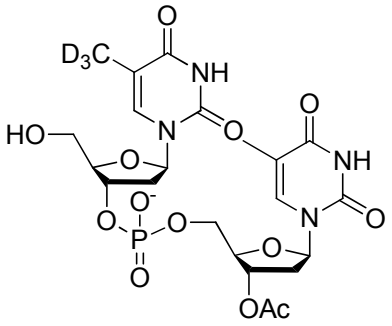
**De-protection of S5 to yield S6.**



**S6**

To a solution of **S5** (1.25 g, 1.25 mmol) in $CH_2Cl_2$ (8 mL), TFA (1.5 mL) was added dropwise. The reaction was allowed to proceed for 5 min, and the solvent removed under vacuum. Purification of the resulting residue via flash chromatography (eluent: $CH_2Cl_2$/MeOH/$Et_3N$ = 10/1/1) yielded **S6** (0.64 g, 74%) as a white solid. $^1$H NMR ($CDCl_3$): $\delta$ 1.92 (s, 3H), 2.09 (s, 3H), 2.24-2.40 (m, 3H), 2.44-2.52 (m, 1H), 3.80 (dd, $J$ = 3.3, 10.7 Hz, 1H), 3.88 (d, $J$ = 10.7 Hz, 1H), 4.05-4.28 (m, 4H), 4.95-5.05 (m, 1H), 5.35-5.45 (m, 1H), 6.22 (t-like, $J$ = 6.4 Hz, 1H), 6.39 (t-like, $J$ = 7.3 Hz, 1H), 7.57 (s, 1H), 7.74 (s, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$ 12.6, 21.2, 37.4, 39.2, 62.1, 65.7, 75.0, 75.4, 83.8, 84.6, 84.8, 86.2, 110.8, 111.5, 136.0, 136.2, 150.8, 151.0, 164.3, 164.4, 170.6; ESI-MS (positive mode) calcd for $C_{22}H_{27}D_3N_4O_{13}P$: 592.2 ($M + H^+$), found 592.2.

**Deacetylation of S6 to yield 5′-$d_3$-TpT.**

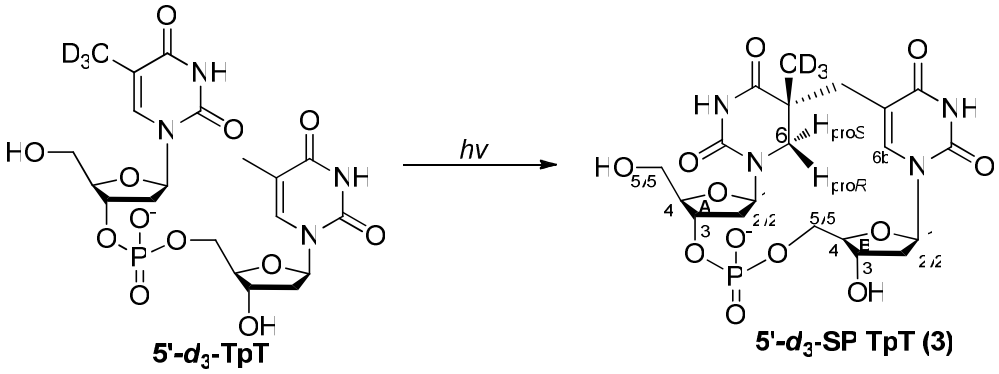


**5'-$d_3$-TpT**

A mixture of **S6** (600 g, 0.87 mmol) and $K_2CO_3$ (600 mg, 4.38 mmol) were stirred in MeOH (8 mL) at room temperature for overnight. The reaction mixture was subsequently neutralized with HOAc. After removal of the MeOH under vacuum, the crude product was purified by reverse phase HPLC affording 5′-$d_3$-TpT (425 mg, 90%) as a white solid. $^1$H NMR ($CD_3OD$): $\delta$ 1.90 (s, 3H), 2.20-2.32 (m, 2H), 2.32-2.39 (m, 1H), 2.51 (ddd, $J$ = 2.1, 5.8, 14.1 Hz, 1H), 3.77 (dd, $J$ = 3.2, 12.1 Hz, 1H), 3.80 (dd, $J$ = 3.2, 12.1 Hz, 1H), 4.03-4.08 (m, 1H), 4.18-4.21 (m, 1H), 4.23 (ddd, $J$ = 4.1, 5.8, 11.3 Hz, 1H), 4.29 (ddd, $J$

= 3.1, 5.6, 11.3 Hz, 1H), 4.42-4.47 (m, 1H), 5.01-5.06 (m, 1H), 6.27-6.32 (m, 2H), 7.60 (s, 1H), 7.79 (s, 1H); $^{13}$C NMR ($CD_3OD$): $\delta$ 12.5, 39.6, 40.7, 62.6, 67.9, 71.9, 79.4, 86.1, 86.3, 86.4, 87.3, 111.7, 111.9, 137.7, 137.9, 152.3 (2C), 166.3; ESI-MS (positive mode) calcd for $C_{20}H_{25}D_3N_4O_{12}P$: 550.2 ($M + H^+$), found 550.2.

**Photolysis of 5′-$d_3$-TpT to generate 5′-$d_3$-SP TpT (3).**[1]

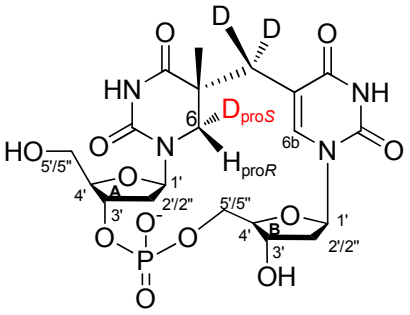



A 100 mL 10 mM aqueous solution of TpT was prepared by titration with 0.3 M NaOH to pH = 7.0. After removal of water under vacuum, the resulting TpT sodium salt was dissolved in 60 mL MeOH. The solution was divided into equal fractions that were poured in two 15 × 20 cm plates. Methanol evaporation afforded nice thin films. The films were then exposed for 20 min to the UVC (254 nm) light. The products were subsequently dissolved in water and purified by reverse phase HPLC in the gradient mode using triethyl ammonium acetate, pH 6.5, and acetonitrile as solvents. The 5′-$d_3$-SP TpT (**3**) was collected with a yield of *ca.* 0.8%. $^1$H NMR (DMSO): $\delta$ 2.10-2.21 (m, 4H), 2.53 (d, $J$ = 14.0 Hz, 1H), 2.61 (d, $J$ = 14.0 Hz, 1H), 3.01 (d, $J$ = 12.7 Hz, 1H), 3.15 (d, $J$ = 12.7 Hz, 1H), 3.37-3.45 (m, 2H), 3.45-2.50 (m, 1H), 3.75-3.82 (m, 1H), 3.86-3.93 (m, 1H), 4.24-4.29 (m, 1H), 4.41-4.49 (m, 1H), 5.29 (d, $J$ = 4.3 Hz, 1H), 5.47 (brs, 1H), 5.89 (t, $J$ = 7.0 Hz, 1H), 6.00 (t, $J$ = 6.1 Hz, 1H), 7.50 (s, 1H), 10.1 (s, 1H), 11.3 (s, 1H); ESI-MS (positive mode) calcd for $C_{20}H_{25}D_3N_4O_{12}P$: 550.2 ($M + H^+$), found 550.2.

**Preparation of $d_3$-SP TpT (3) and $d_4$-SP TpT (4) via photolysis and SP TpT (1) by organic synthesis**

The detailed preparation and characterization of $d_3$-SP TpT (**2**), $d_4$-SP TpT (**4**) and SP TpT (**1**) can be found in our previous publication. [1]

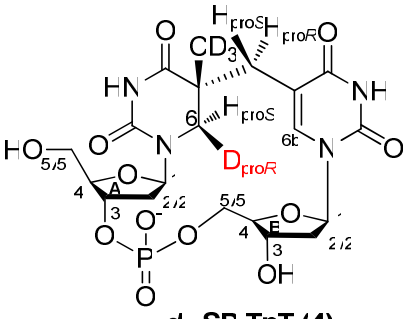
***d*$_3$-SP TpT (2) generated by UV photolysis.**




***d*$_3$-SP TpT (2)**

$^1$H NMR (DMSO): $\delta$ 2.10-2.23 (m, 4H), 3.01 (s, 1H), 3.39-3.43 (m, 1H), 3.41-3.47 (m, 1H), 3.50 (dd, $J$ = 4.7, 12.6 Hz, 1H), 3.77-3.83 (m, 2H), 3.88-3.93 (m, 1H), 4.25-4.30 (m, 1H), 4.43-4.50 (m, 1H), 5.30 (brs, 1H), 5.48 (brs, 1H), 5.91 (dd, $J_1$ = $J_2$ = 7.0 Hz, 1H), 6.00 (t, $J$ = 6.0 Hz, 1H), 7.52 (s, 1H), 10.1 (s, 1H), 10.9 (brs, 1H); ESI-MS (positive mode) calcd for $C_{20}H_{25}D_3N_4O_{12}P$: 550.2 ($M + H^+$), found 550.2.

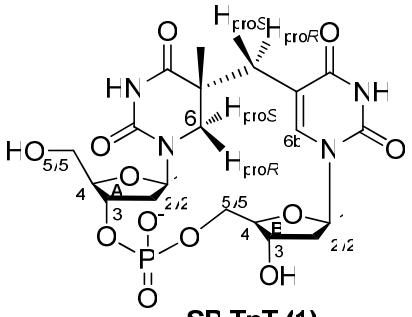
***d*$_4$-SP TpT (4) generated via UV photolysis.**




***d*$_4$-SP TpT (4)**

$^1$H NMR (DMSO): $\delta$ 2.10-2.16 (m, 3H), 2.16-2.23 (m, 1H), 2.53 (d, $J$ = 14.0 Hz, 1H), 2.59 (d, $J$ = 14.0 Hz, 1H), 3.14 (s, 1H), 3.39 (dd, $J$ = 4.4, 11.6 Hz, 1H), 3.45 (ddd, $J$ = 4.0, 4.4, 6.9 Hz, 1H), 3.51 (dd, $J$ = 4.0, 11.6 Hz, 1H), 3.75-3.80 (m, 1H), 3.80-3.85 (m, 1H), 3.87-3.93 (m, 1H), 4.22-4.28 (m, 1H), 4.43-4.52 (m, 1H), 5.93 (dd, $J_1$ = $J_2$ = 7.0 Hz, 1H), 5.99 (dd, $J$ = 4.3, 8.4 Hz, 1H), 7.52 (s, 1H), 10.1 (s, 1H); ESI-MS (positive mode) calcd for $C_{20}H_{24}D_4N_4O_{12}P$: 551.2 ($M + H^+$), found 551.2.

**Chemically synthesized SP TpT (1).**



SP TpT (1)

The synthesis of SP TpT (**1**) was achieved using published procedures.[4,5] The structure of synthesized (5*R*)-SP TpT (**1**) was confirmed by the NMR and the mass spectroscopy. $^1$H NMR (DMSO): $\delta$ 1.02 (s, 3H), 2.04-2.14 (m, 3H), 2.14-2.23 (m, 1H), 2.49 (d, $J$ = 14.0 Hz, 1H), 2.58 (d, $J$ = 14.0 Hz, 1H), 3.02 (d, $J$ = 12.7 Hz, 1H), 3.15 (d, $J$ = 12.7 Hz, 1H), 3.35 (dd, $J$ = 4.2, 11.7 Hz, 1H), 3.42 (ddd, $J$ = 4.0, 4.2, 6.9 Hz, 1H), 3.48 (dd, $J$ = 4.0, 11.7 Hz, 1H), 3.73-3.78 (m, 1H), 3.78-3.86 (m, 1H), 3.87-3.94 (m, 1H), 4.21-4.26 (m, 1H), 4.41-4.49 (m, 1H), 5.89 (dd, $J_1$ = $J_2$ = 7.1 Hz, 1H), 5.95 (dd, $J$ = 4.0, 8.1 Hz, 1H), 7.51 (s, 1H), 10.0 (s, 1H), 11.2 (s, 1H); ESI-MS (positive mode) calcd for $C_{20}H_{28}N_4O_{12}P$: 547.1 (M + $H^+$), found 547.2.

**Table S1.** $^1$H NMR Data of the synthesized SP TpT (**1**), $d_3$-SPTpT (**2**), 5′-$d_3$-SP TpT (**3**), $d_4$-SPTpT (**4**) and the literature values for the chemical shift.

| identification of proton | $^1$H chemical shift (*ppm*) | | | | |
|---|---|---|---|---|---|
| | literature value [6] | Synthesized [4,5] | UV generated | | |
| | | SP TpT (**1**) | $d_3$-SP TpT (**2**) | $d_3$-SP TpT (**3**) | $d_4$-SP TpT (**4**) |
| $H_{proS}$ /$H_{proR}$ | 2.64 / 2.50 | 2.58 / 2.49 | none | 2.61 / 2.53 | 2.59 / 2.53 |
| $CH_3$ | 1.03 | 1.02 | - | none | none |
| 6-$H_{proS}$ /6-$H_{proR}$ | 3.15 / 2.98 | 3.15 / 3.02 | none /3.01 | 3.15 / 3.01 | 3.14 / none |
| $H_{1'A}$ | 5.99 | 5.95 | 6.00 | 6.00 | 5.99 |
| $H_{2'A}$ / $H_{2''A}$ | 2.11 | 2.09 | 2.16 | 2.16 | 2.14 |
| $H_{3'A}$ | 4.43 | 4.45 | 4.45 | 4.45 | 4.48 |
| $H_{4'A}$ | 3.41 | 3.42 | 3.44 | 3.42 | 3.45 |
| $H_{5'A}$ / $H_{5''A}$ | 3.44 | 3.42 | 3.45 | 3.47 | 3.45 |
| $H_{6b}$ | 7.50 | 7.51 | 7.52 | 7.50 | 7.52 |
| $H_{1'B}$ | 5.86 | 5.89 | 5.91 | 5.89 | 5.93 |
| $H_{2'B}$ / $H_{2''B}$ | 2.16 | 2.14 | 2.16 | 2.16 | 2.20 |
| $H_{3'B}$ | 4.27 | 4.24 | 4.27 | 4.27 | 4.25 |
| $H_{4'B}$ | 3.76 | 3.75 | 3.79 | 3.78 | 3.78 |
| $H_{5'B}$ / $H_{5''B}$ | 3.87 | 3.87 | 3.86 | 3.89 | 3.86 |
| NH | 10.1 / 7.1 | 10.1 / 11.2 | 10.1 / 10.9 | 10.1 / 11.3 | 10.1 / - |

***N*, *N'*-Dimethyl-4,4'-bipyridinium iodide (methyl viologen or MV).** A mixture of 4, 4′-bipyridine (0.25 g, 1.60 mmol) and methyl iodide (0.568 g, 4.0 mmol) was refluxed in $CH_3CN$ (15 mL) for 12 h. A red solid was precipitated. The mixture was filtered and washed with $CH_3CN$ and dried to afford the product as a red solid (0.646 g, 92%). $^1$H NMR ($CDCl_3$): $\delta$ 3.39 (s, 6H), 7.68 (d, $J$ = 6.5 Hz, 4H), 8.19 (d, $J$ = 6.5 Hz, 4H); $^{13}$C NMR ($CDCl_3$): $\delta$ 48.3, 126.2, 126.4, 146.7, 148.4; ESI-MS (positive mode) calcd for $C_{12}H_{14}N_2^+$: 186.1 (M + e), found 186.1.

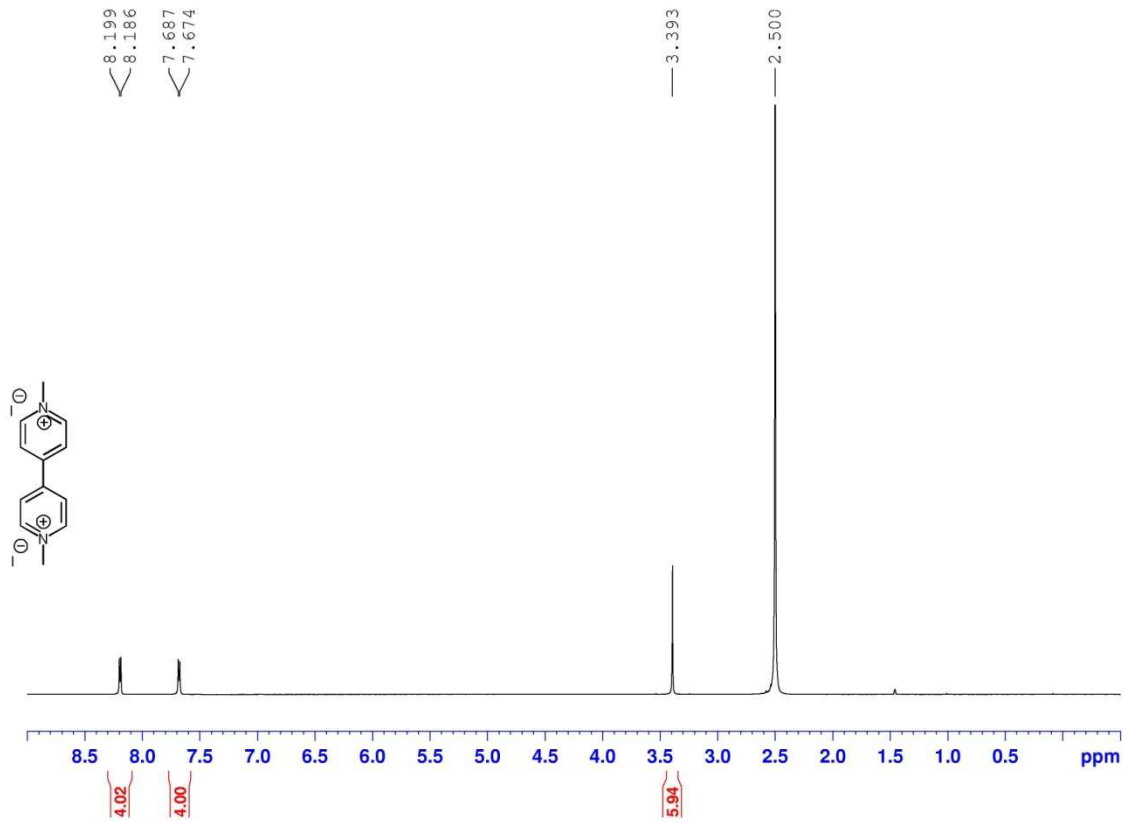



**Figure S6.** $^1$H NMR spectrum of MV.

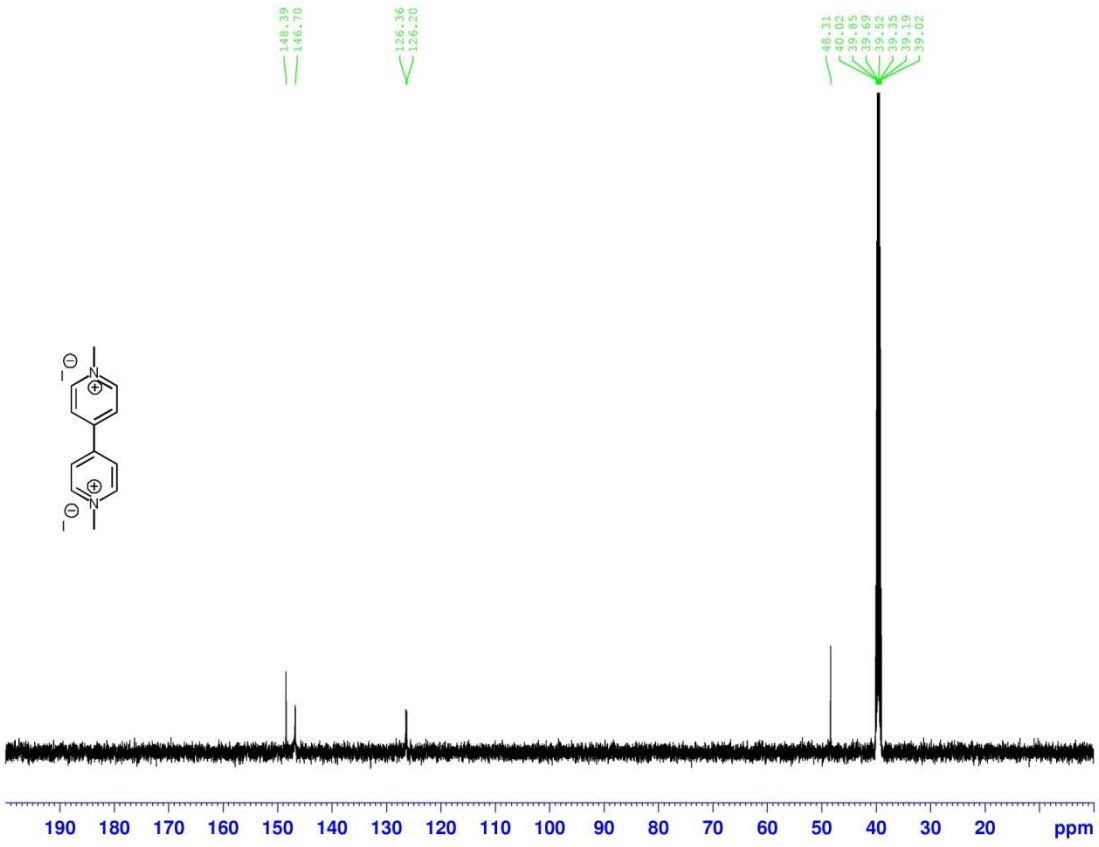



**Figure S7.** $^{13}$C NMR spectrum of MV.

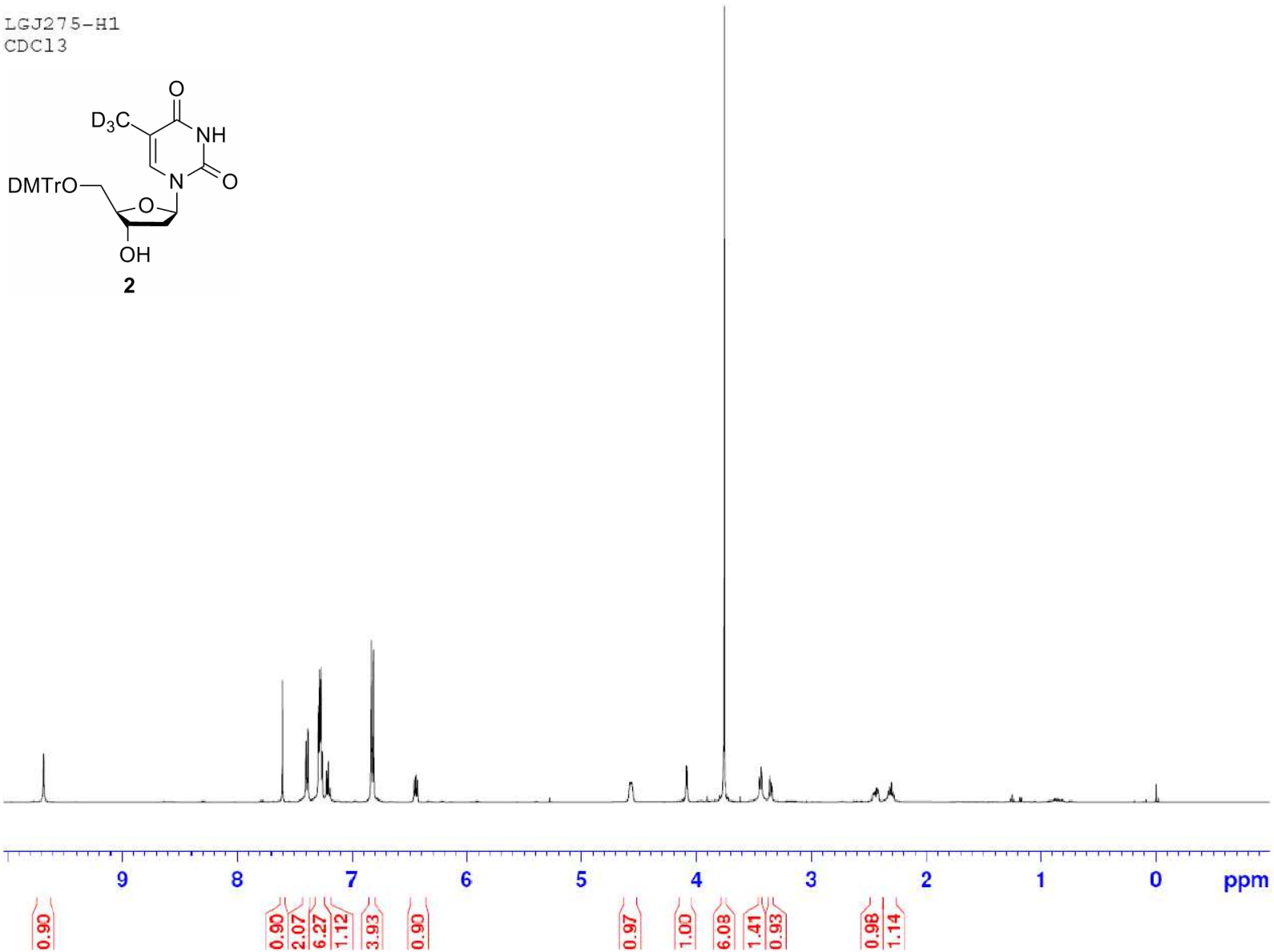



**Figure S8.** $^{1}$H NMR spectrum of compound **S2**.

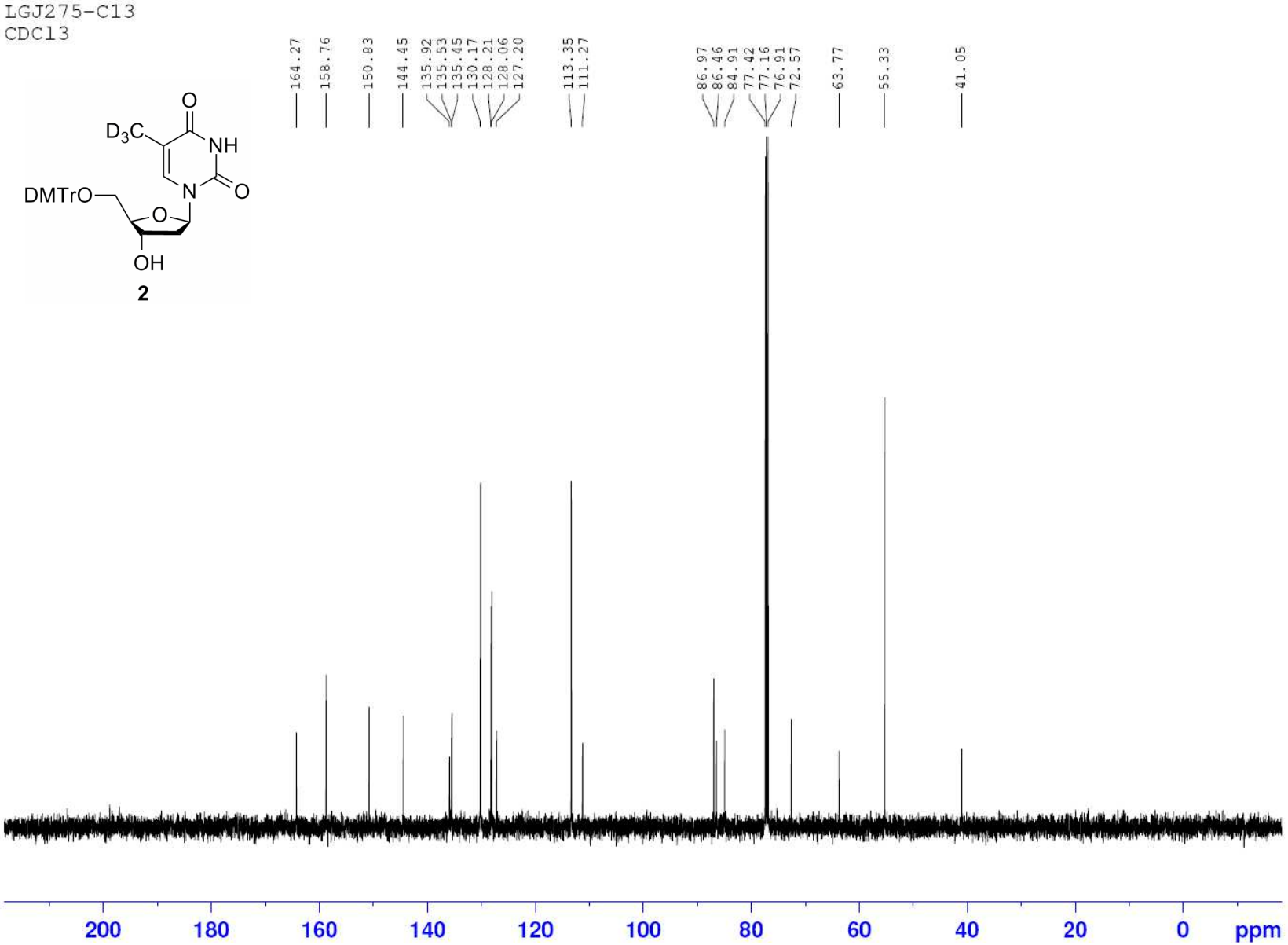



**Figure S9.** $^{13}$C NMR spectrum of compound **S2**.

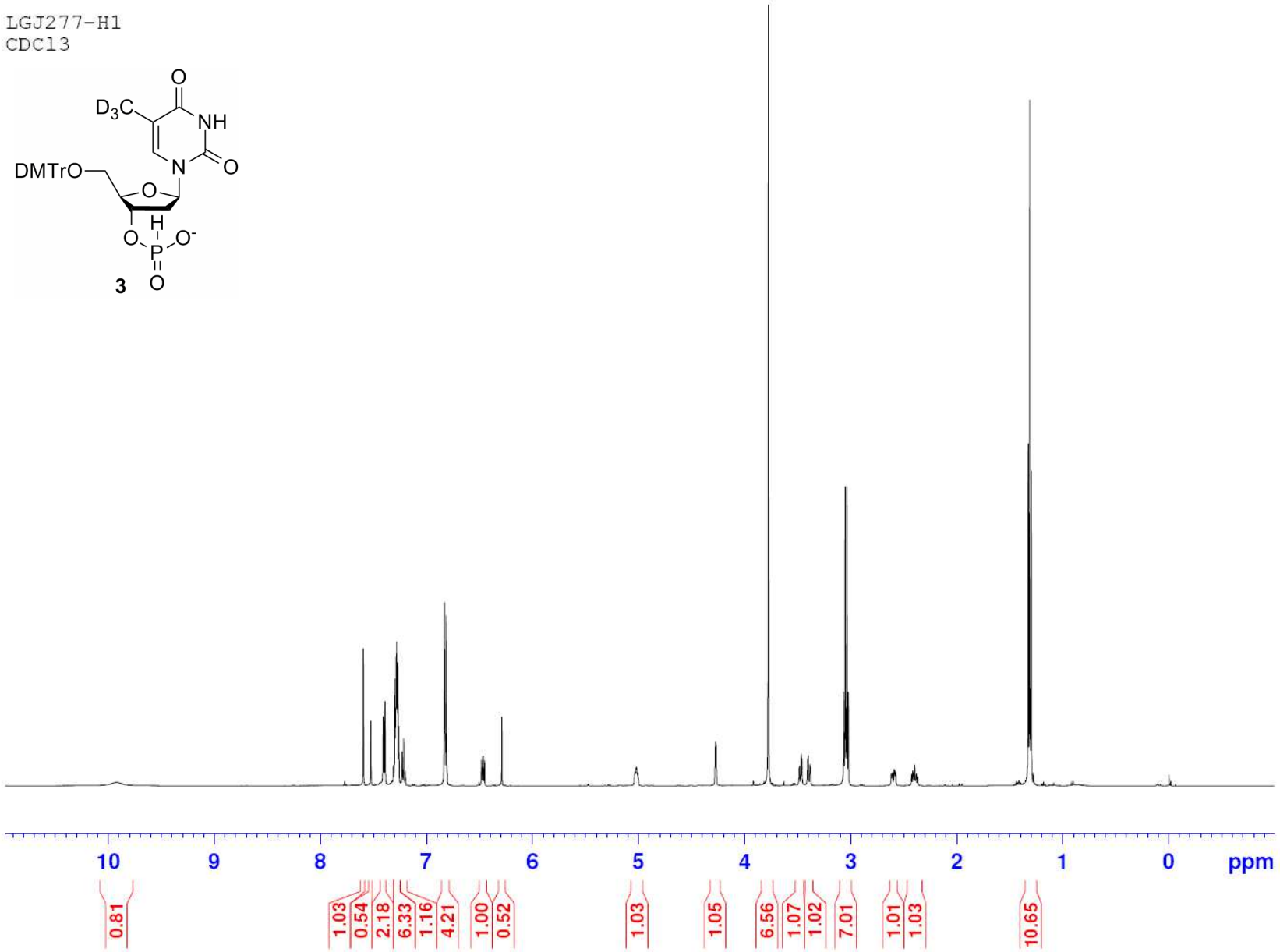



**Figure S10.** $^{1}$H NMR spectrum of compound **S3**.

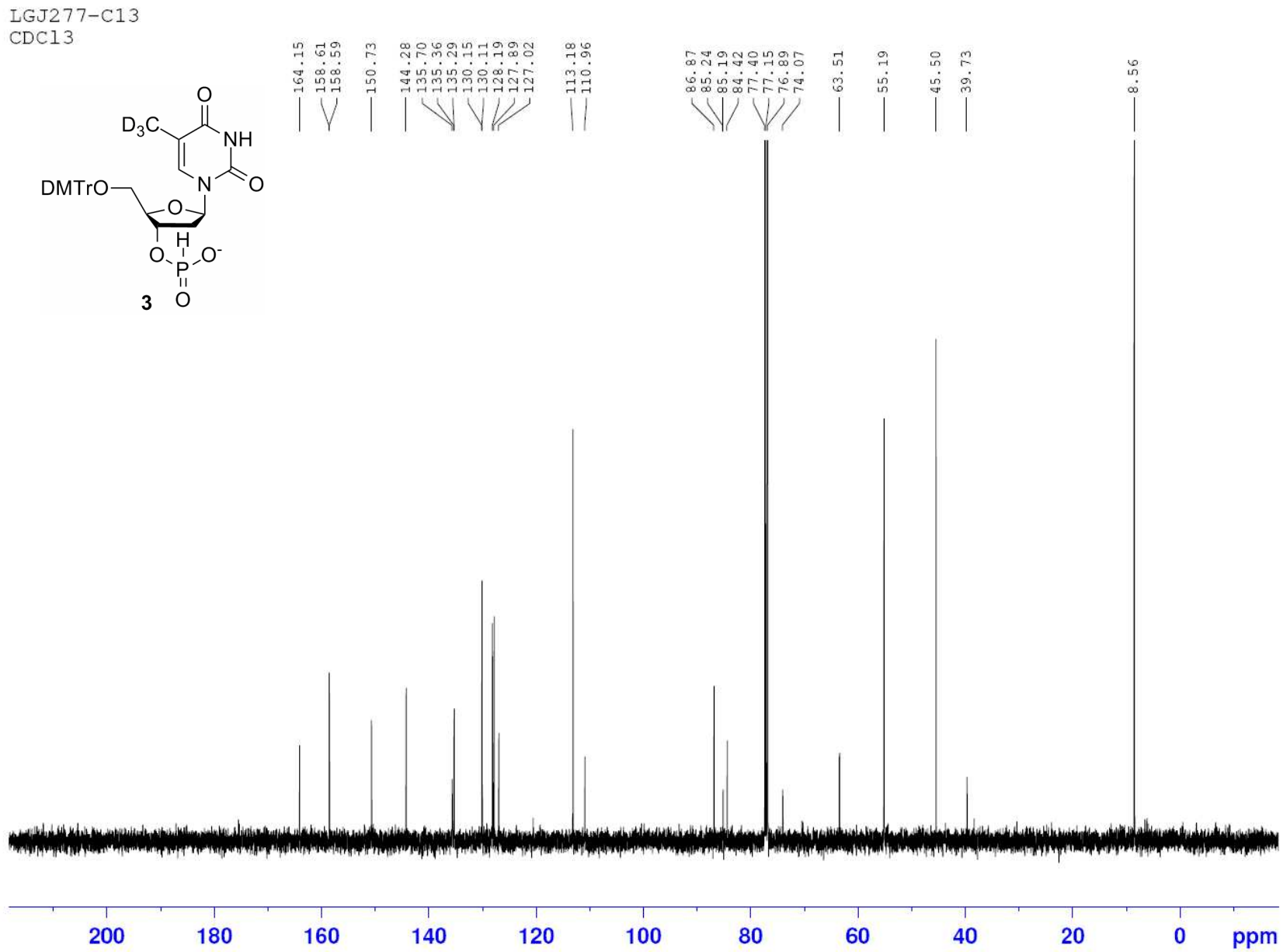



**Figure S11.** $^{13}$C NMR spectrum of compound **S3**.

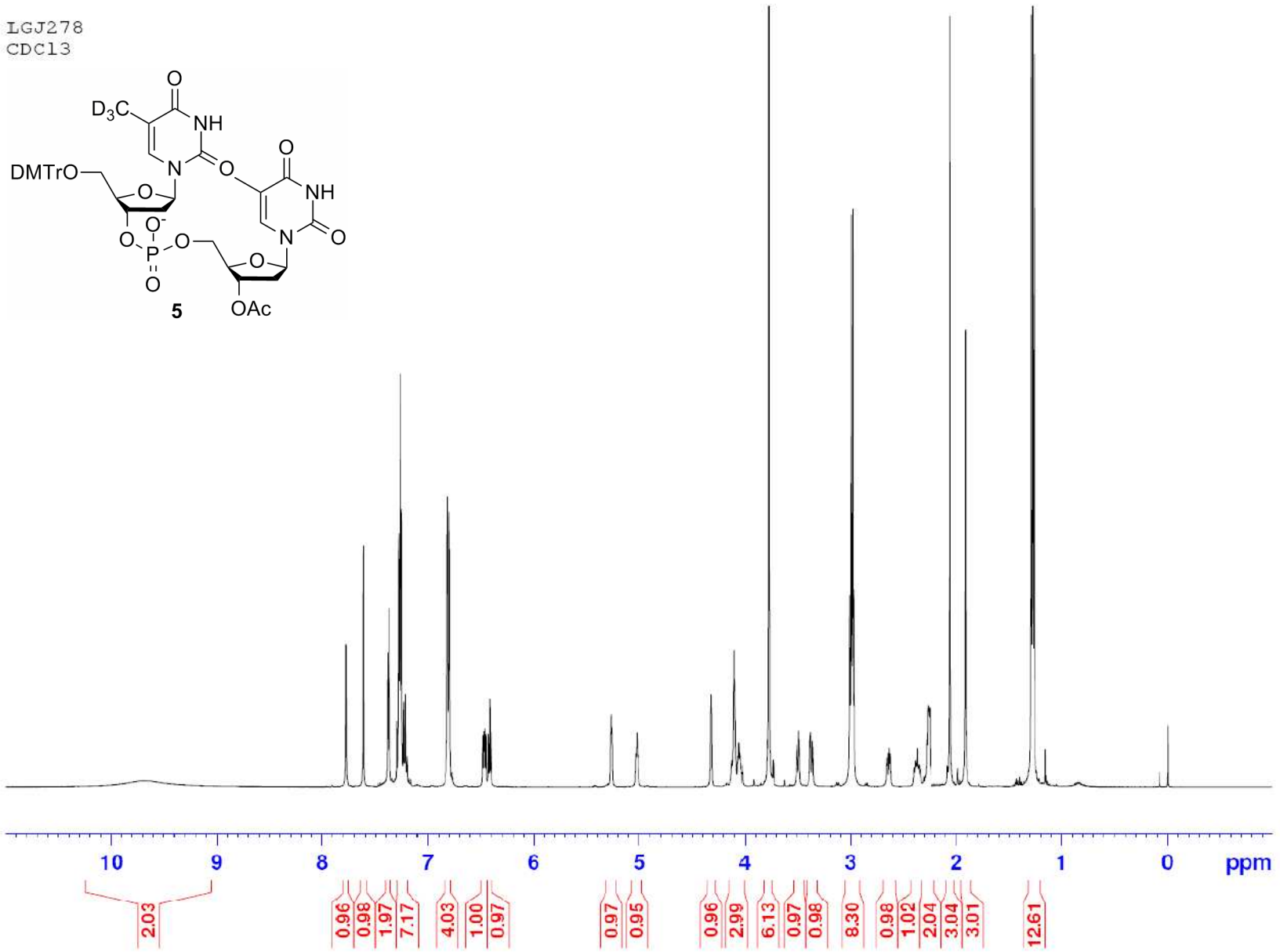



**Figure S12.** $^{1}$H NMR spectrum of compound **S5**.

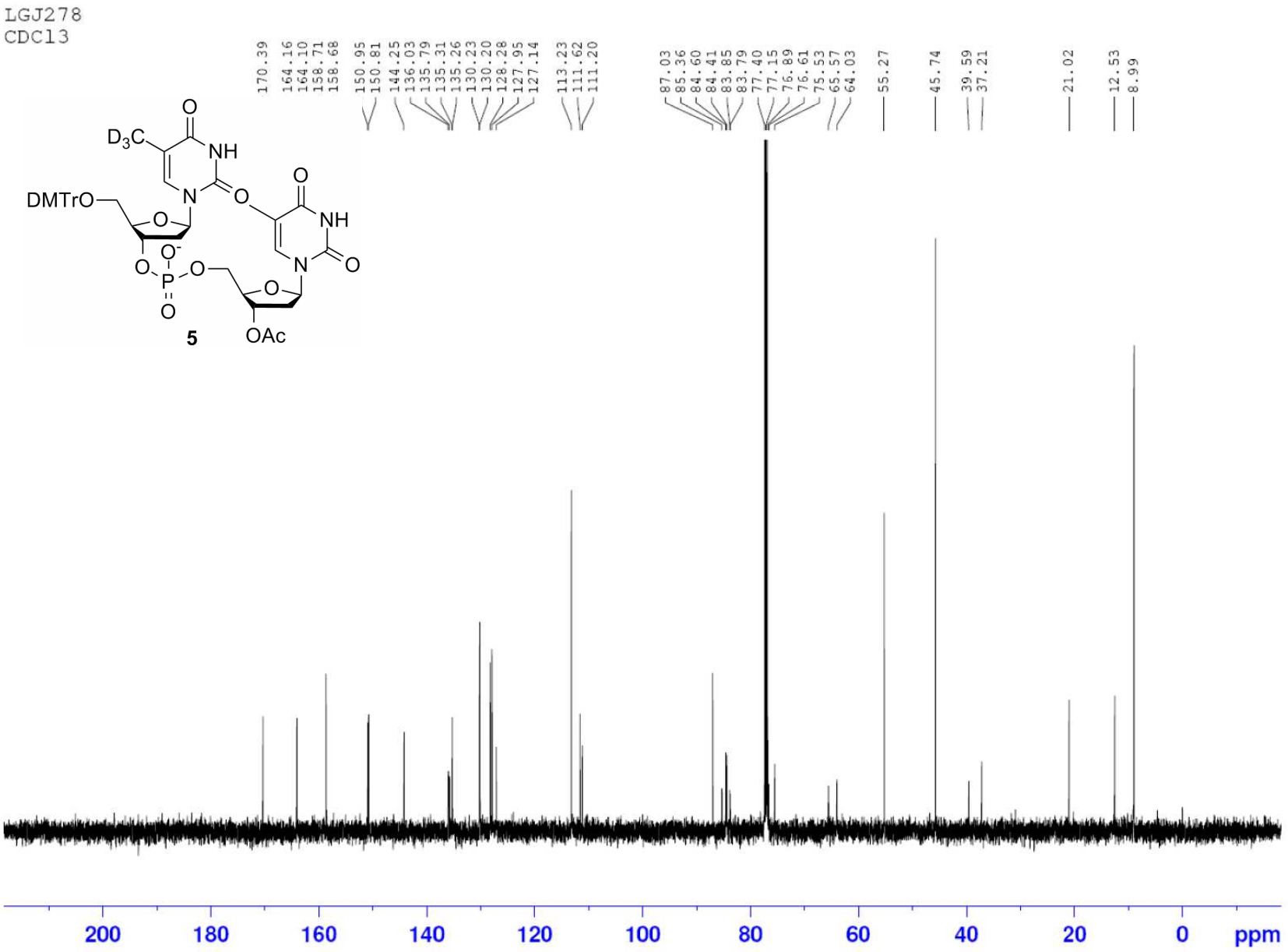



**Figure S13.** $^{13}C$ NMR spectrum of compound **S5**.

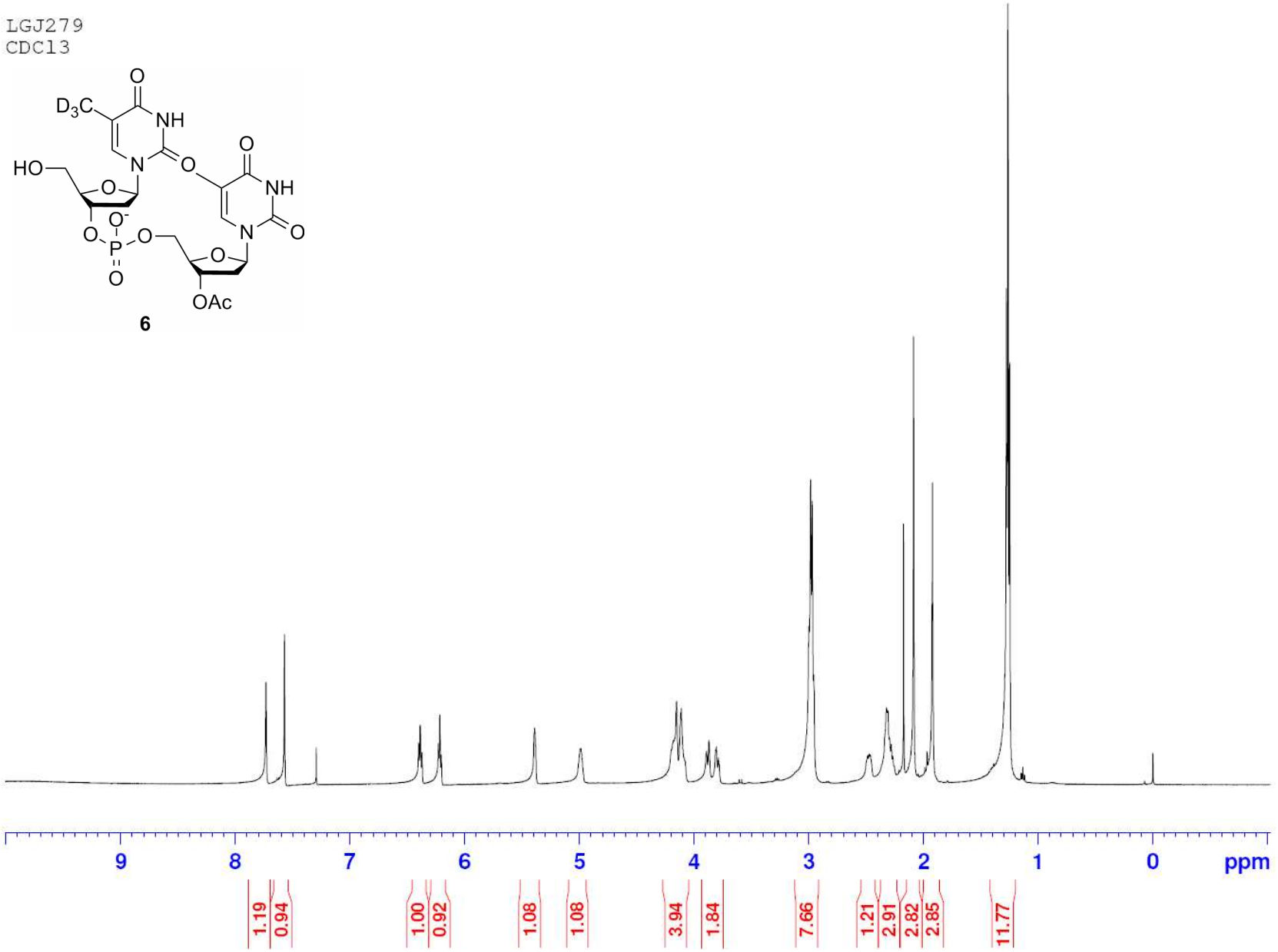



**Figure S14.** $^{1}$H NMR spectrum of compound **S6**.

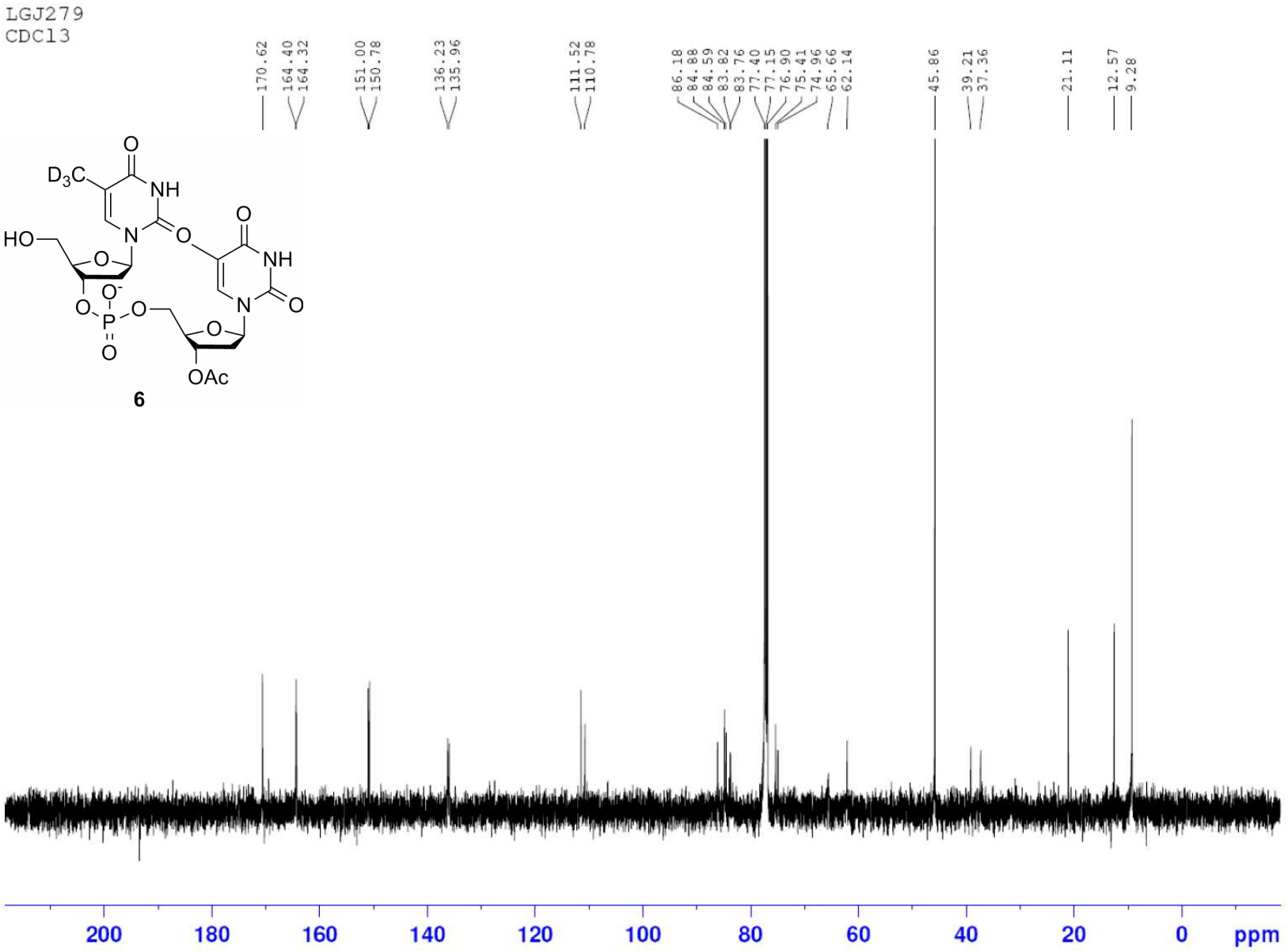



**Figure S15.** $^{13}$C NMR spectrum of compound **S6**.

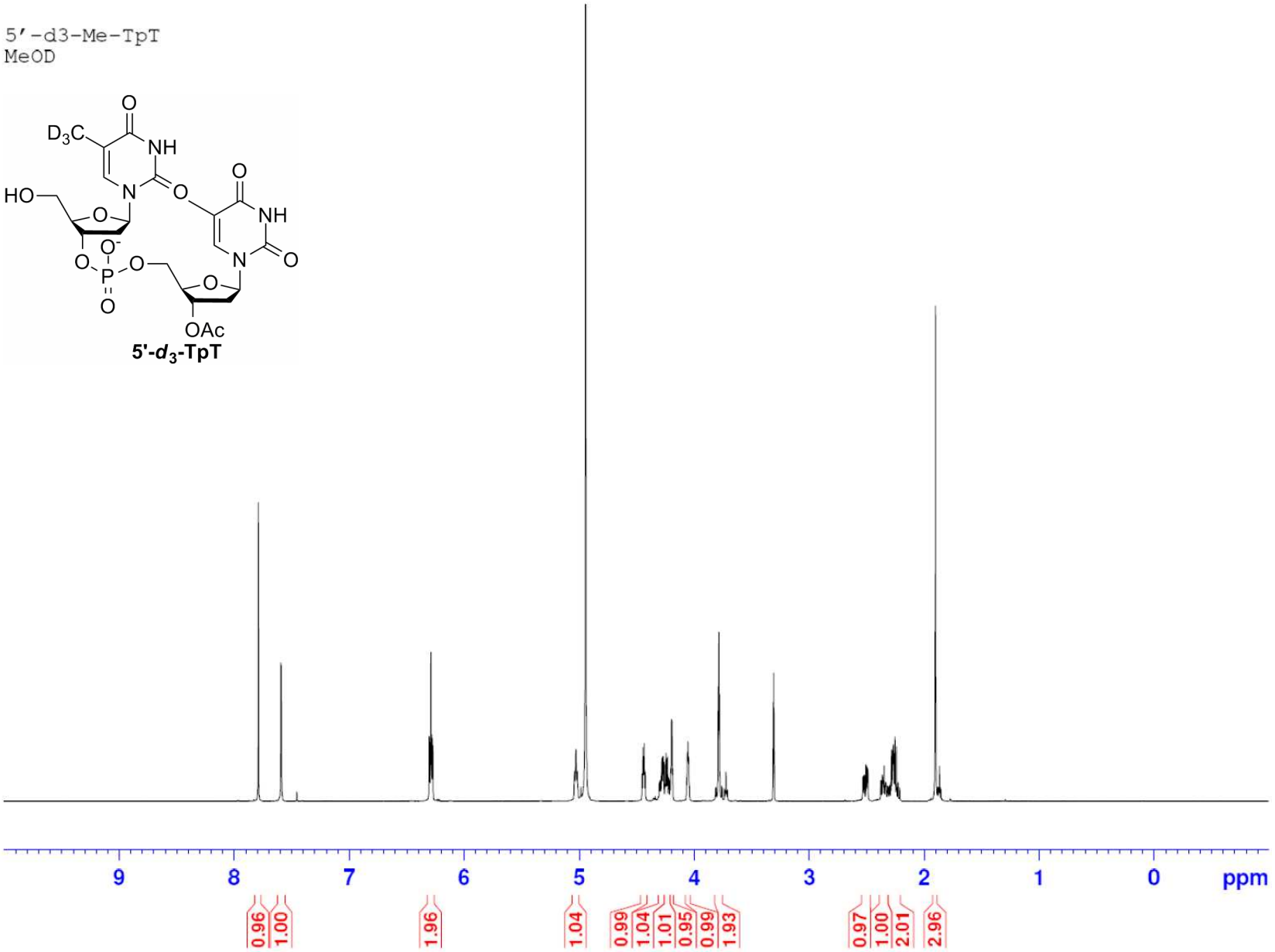



**Figure S16.** $^{1}$H NMR spectrum of **5′-$d_3$-TpT**.

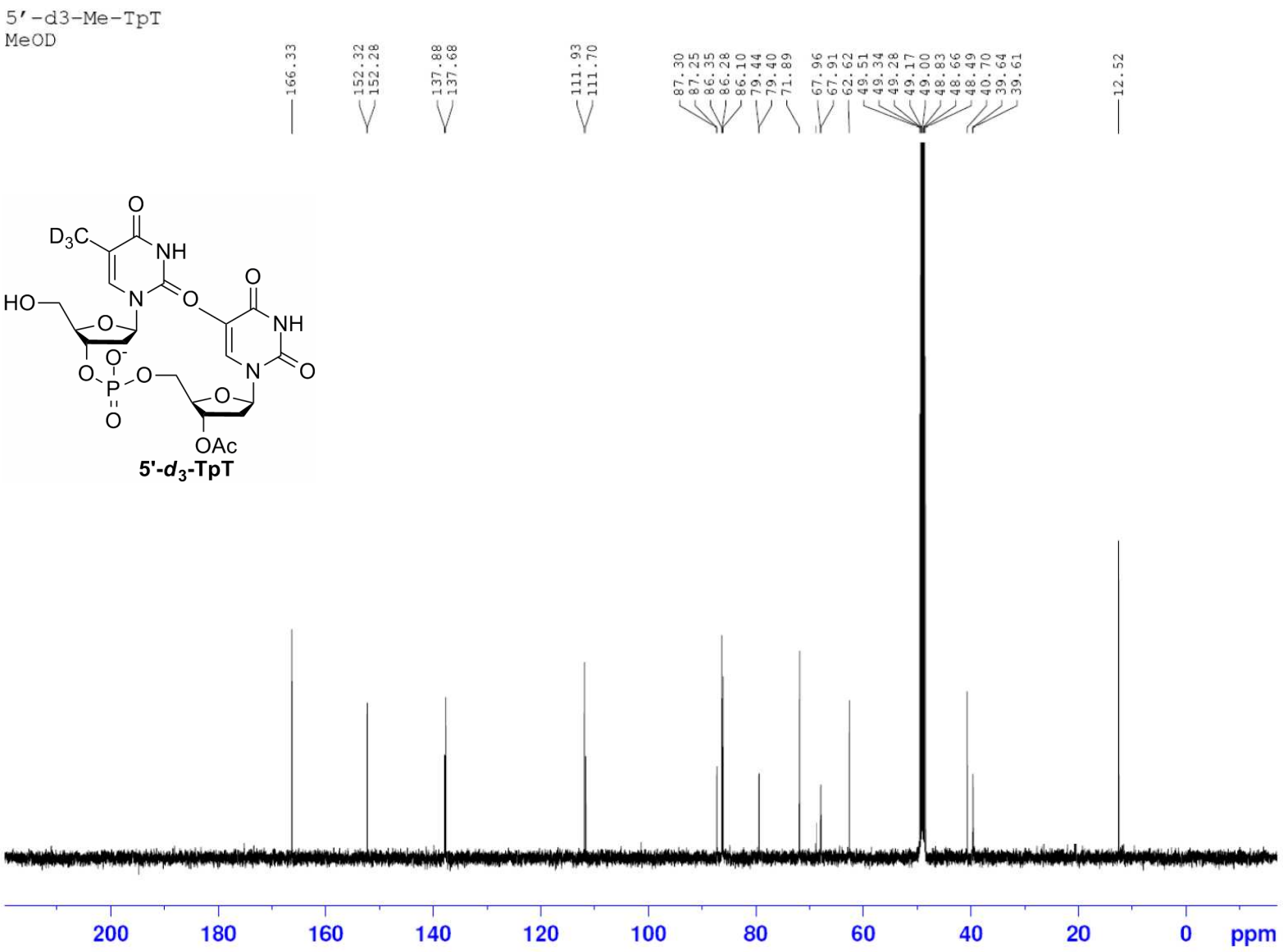



**Figure S17.** $^{13}$C NMR spectrum of **5′-*d*$_3$-TpT**.

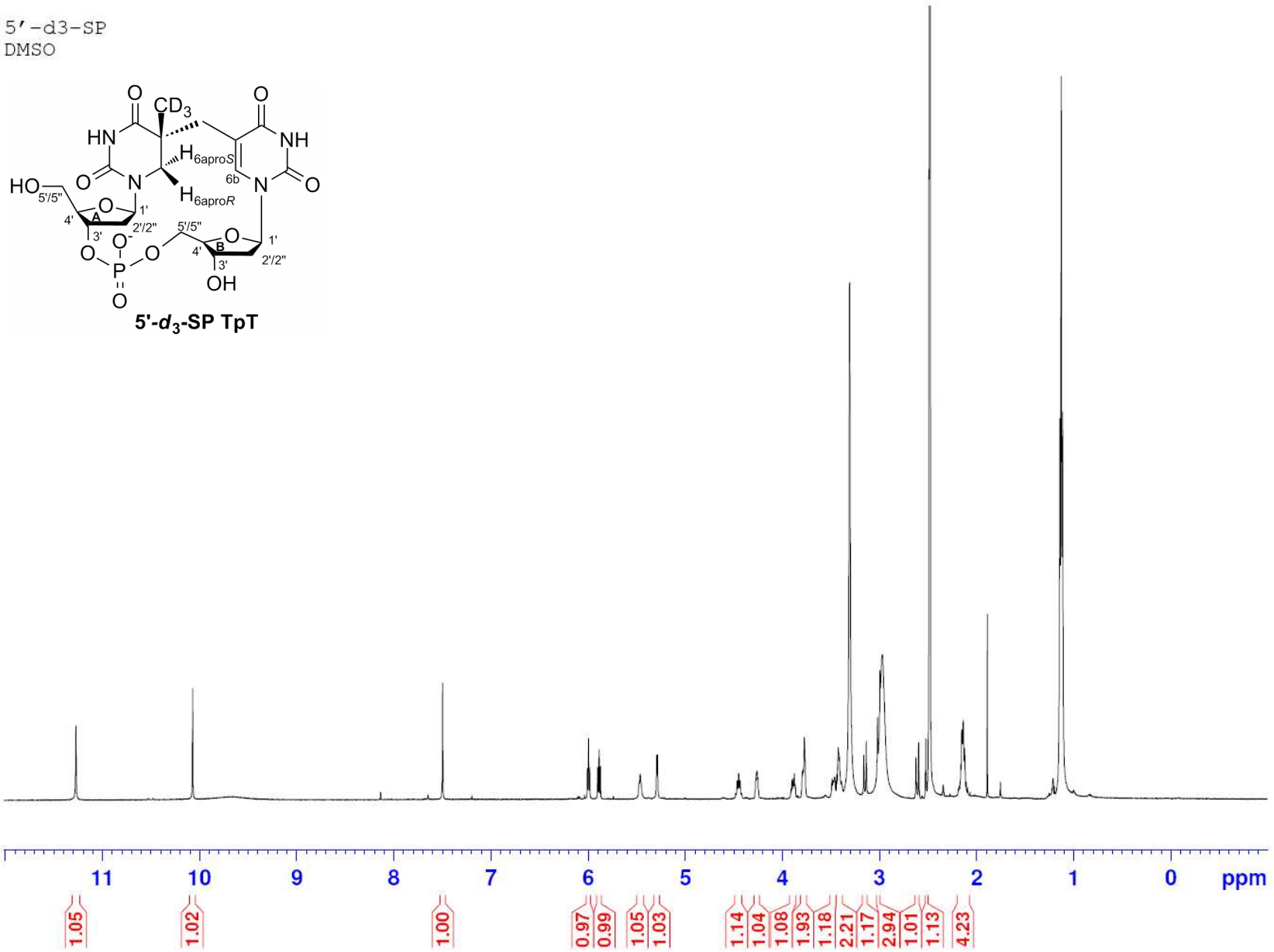



**Figure S18.** $^{1}$H NMR spectrum of **5′-$d_3$-SP TpT (3)**.

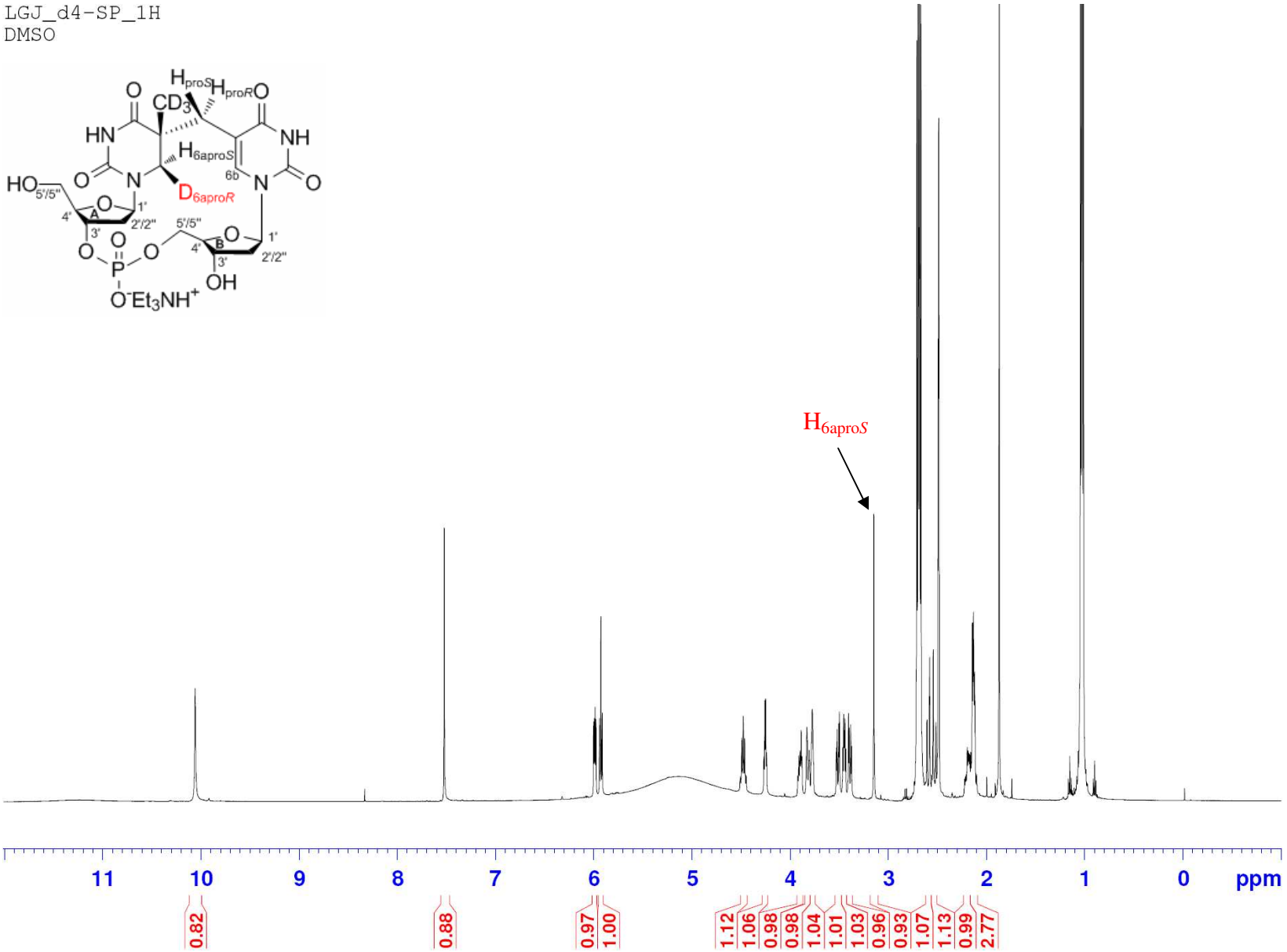



**Figure S19.** $^{1}$H NMR spectrum of ***d*$_4$-SP TpT (4)**.

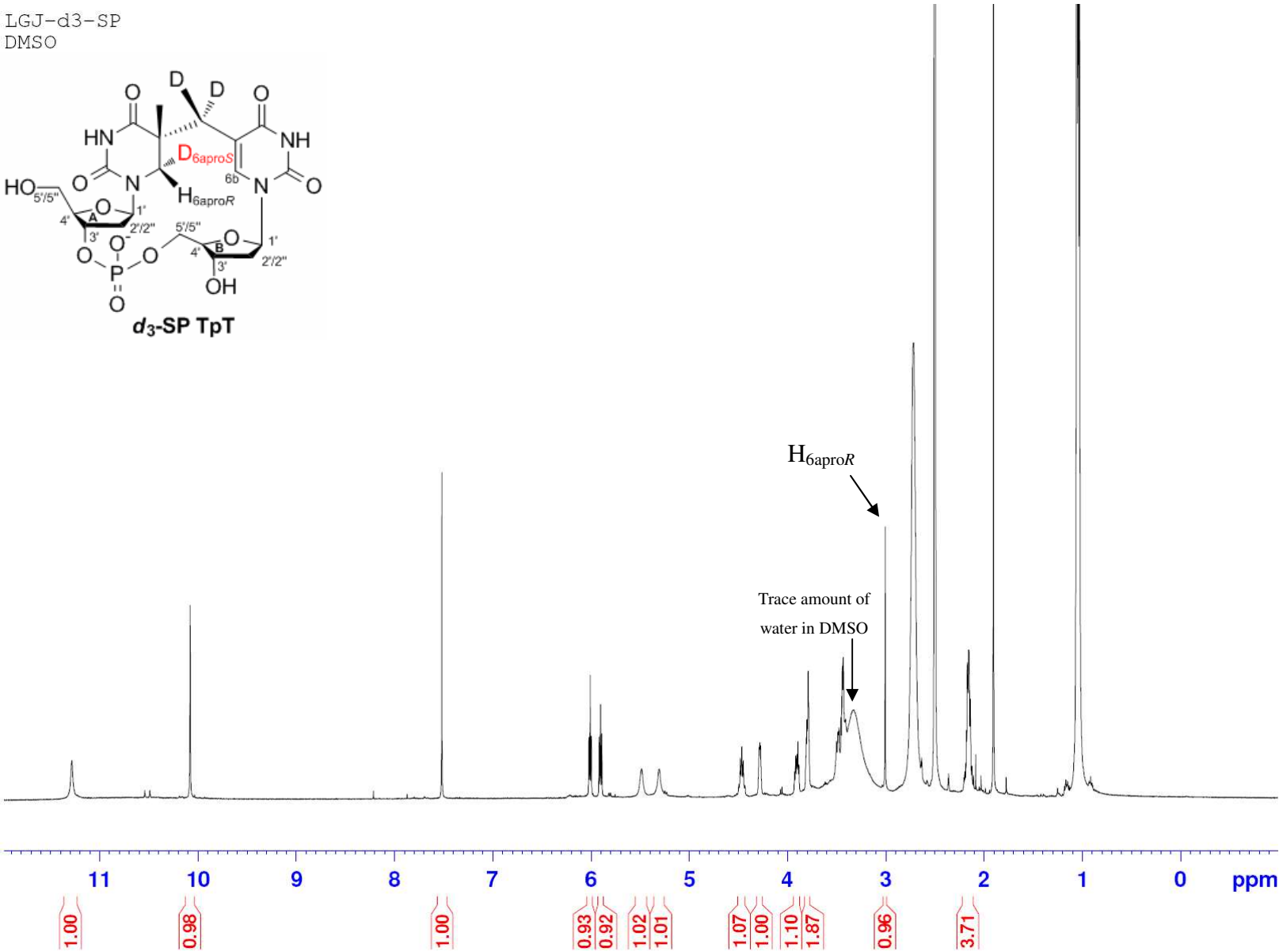



**Figure S20.** $^{1}$H NMR spectrum of ***d*$_3$-SP TpT (2)**.

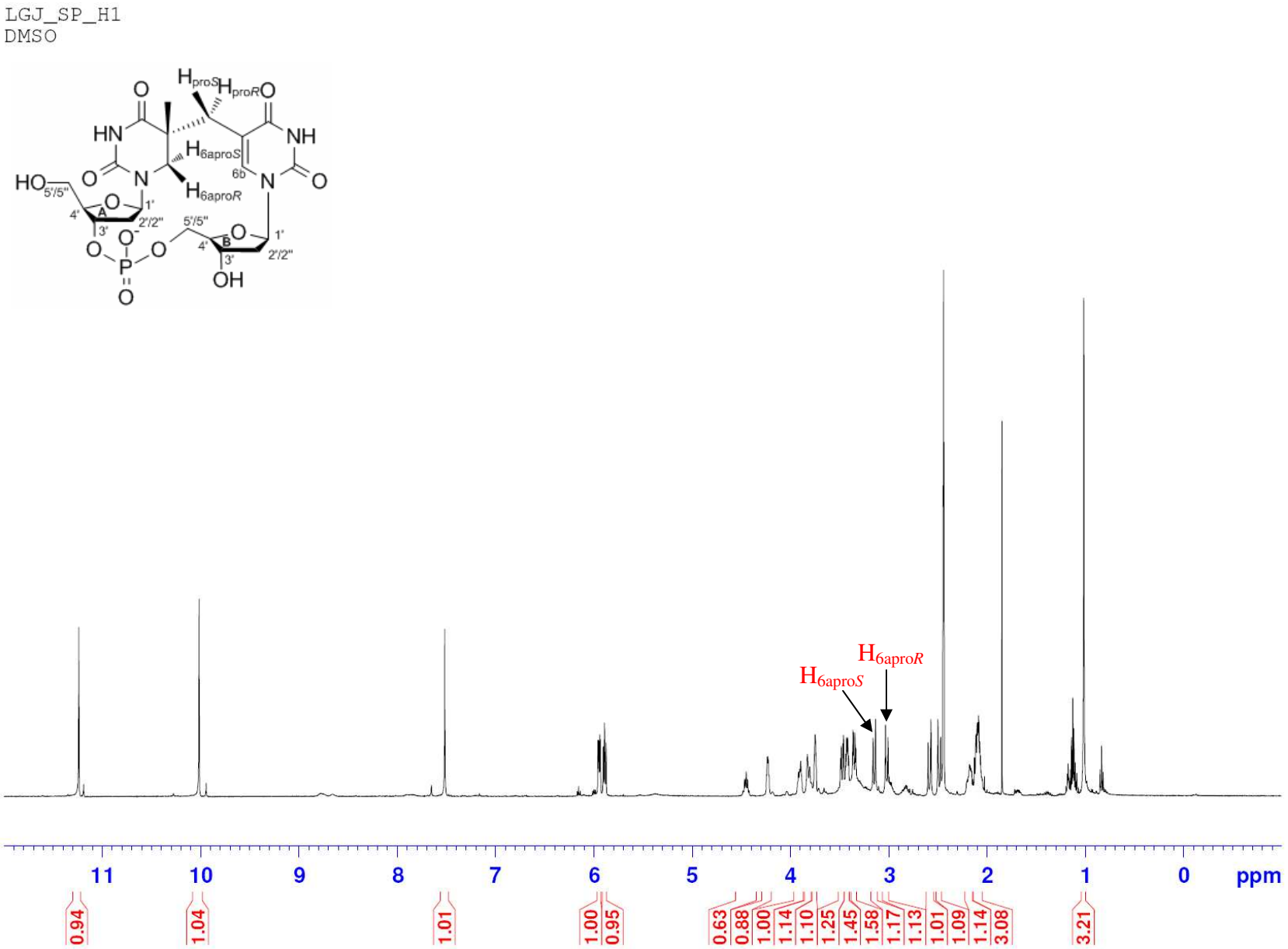



**Figure S21.** $^{1}$H NMR spectrum of **SP TpT (1)**.

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