Supporting information

For

An unexpected deamination reaction after hydrolysis of the pyrimidine (6-4) pyrimidone photoproduct

Gengjie Lin[†,‖], *Yajun Jian*[†,‖] Hao Ouyang,[§] and Lei Li [†,‡]*

[†]Department of Chemistry and Chemical Biology, Indiana University-Purdue University Indianapolis (IUPUI), 402 North Blackford Street, Indianapolis, Indiana, 46202

[‡]Department of Biochemistry and Molecular Biology & Department of Dermatology, Indiana University School of Medicine, , Indianapolis, Indiana 46202

[§]Preclinical Pharmacology, Johnson& Johnson, 199 Grandview Road, Skillman, NJ, 08558.

lilei@iupui.edu

[‖]These authors contributed equally

Abbreviations used

6-4PP, pyrimidine (6-4) pyrimidone photoproduct; abbreviations for NMR signal coupling are as follows: s, singlet; d, doublet; m, multiplet.

General Methods

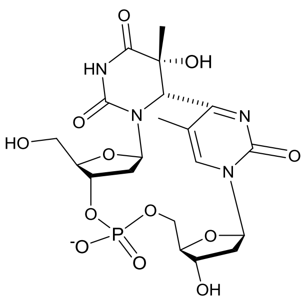
All reagent grade chemicals were purchased from Sigma, Fisher, or VWR and used without further purification. The ^{1}H NMR spectra were obtained on a Bruker 500 MHz NMR Fourier transform spectrometer. NMR spectra were recorded in sample solutions in d_4-methanol, with residual methanol (δ 3.31 ppm for ^{1}H NMR and δ 49.0 ppm for ^{13}C NMR), or in deuterated water (D_2O), with residual H_2O (δ 4.79 ppm for ^{1}H NMR) taken as the standard. The chemical shifts on NMR spectra were reported in parts per million (ppm). The photoreaction was carried out using a Spectroline germicidal UV sterilizing lamps (254 nm, Dual-tube, 15 w, intensity: 1.55mw/cm^2) with the samples ~10 cm to the lamp.

HPLC analysis was performed at room temperature with a Waters (Milford, MA) breeze HPLC system coupled to a 2489 UV/Visible detector at 302 nm. An Agilent ZORBAX Bonus-RP column (5 µm particle size, 250 × 4.6 mm i.d.) was equilibrated in solvent A (20 mM ammonium acetate in 99% water and 1% acetonitrile, pH 6.5) and compounds were eluted with an ascending gradient (1% ~ 10%) of acetonitrile in 20 minutes at a flow rate of 1 mL/min. Under this gradient, **1** was eluted at 4.5 min and 6-4PP at 7.6 min. Semi-preparative HPLC was performed at room temperature with the same Waters HPLC setup. An XBridge™ OST C18 column (2.5 µm particle size, 50 × 10 mm i.d.) was equilibrated in solvent A (10 mM ammonium acetate in 99% water and 1% acetonitrile, pH 6.5) and compounds were eluted with an ascending gradient (1% ~ 10%) of acetonitrile in 20 minutes at a flow rate of 4.73 mL/min. The LC/MS and MS/MS analyses were conducted via an Agilent 6520 Accurate Mass Q-TOF LC/MS spectrometer.

Preparation of 6-4PP and [^{15}N]-6-4PP

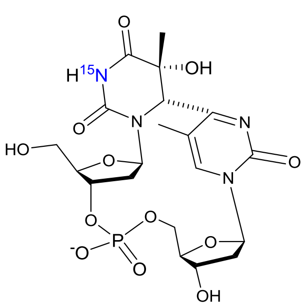
The preparation of 6-4PP and [^{15}N]-6-4PP was achieved using published procedures from TpT and [^{15}N]-TpT[1,2].

6-4PP



^{1}H NMR (d_4-methanol): δ1.49 (dd, J = 7.6, 13.8 Hz, 1H), 1.64 (s, 3H), 1.92-2.01 (m, 1H), 2.34 (s, 3H), 2.43-2.52 (m, 1H), 2.94 (ddd, J = 2.7, 7.2, 14.5 Hz, 1H), 3.51 (dt, J = 8.9, 3.0 Hz, 1H), 3.65 (d, J = 11.7 Hz, 1H), 3.82 (dd, J = 3.4, 12.9 Hz, 1H), 3.90 (dd, J = 2.6, 12.9 Hz, 1H), 3.94-4.03 (m, 3H), 4.80-4.87 (m, 1H), 5.07 (s, 1H), 6.13 (dd, J = 1.2, 8.7 Hz, 1H), 6.52 (dd, J = 2.7, 7.8 Hz, 1H), 7.84 (s, 1H); ^{13}C NMR (d_4-methanol): δ13.6, 25.7, 35.5, 35.8, 57.7, 59.2, 64.6, 69.7, 69.9, 71.9, 82.0, 82.8, 86.5, 87.5, 115.8, 142.8, 153.1, 156.9, 173.3, 175.4. ESI-MS (negative mode) calcd for $C_{20}H_{26}N_4O_{12}P^-$: 545.1290, found 545.1287.

[^{15}N]-6-4PP



^{1}H NMR (d_4-methanol): δ1.51 (dd, J = 7.6, 13.5 Hz, 1H), 1.65 (s, 3H), 1.93-2.01 (m, 1H), 2.34 (s, 3H), 2.43-2.52 (m, 1H), 2.94 (ddd, J = 2.9, 7.3, 14.6 Hz, 1H), 3.52 (dt, J = 8.9, 3.0 Hz, 1H), 3.66 (*d*, J = 11.8 Hz, 1H), 3.82 (dd, J = 3.5, 12.9 Hz, 1H), 3.90 (dd, J = 2.7, 12.9 Hz, 1H), 3.92-4.03 (m, 3H), 4.80-4.86 (m, 1H), 5.08 (s, 1H), 6.12 (dd, J = 1.4, 8.9 Hz, 1H), 6.52 (dd, J = 2.5, 7.6 Hz, 1H), 7.84 (s, 1H); ^{13}C NMR (d_4-methanol): δ13.5, 25.6, 35.7, 35.9, 57.8, 59.3, 64.5, 69.7, 70.0, 71.9, 82.1, 82.8, 86.4, 87.5, 115.8, 142.8, 153.1 (d, $J_{C\text{-}N}$ = 18.2 Hz), 156.9, 173.3 (d, $J_{C\text{-}N}$ = 11.0 Hz), 175.4. ESI-MS (negative mode) calcd for $C_{20}H_{26}N_3{}^{15}NO_{12}P^-$: 546.1261, found 546.1252.

Formation of 1 in 0.2 M KOH

6-4PP was dissolved in 0.2 M KOH to a final concentration of 0.75 mM. The resulting solution was allowed to sit at room temperature for various time periods. 1 µl of the reaction mixture was extracted and immediately analyzed by HPLC.

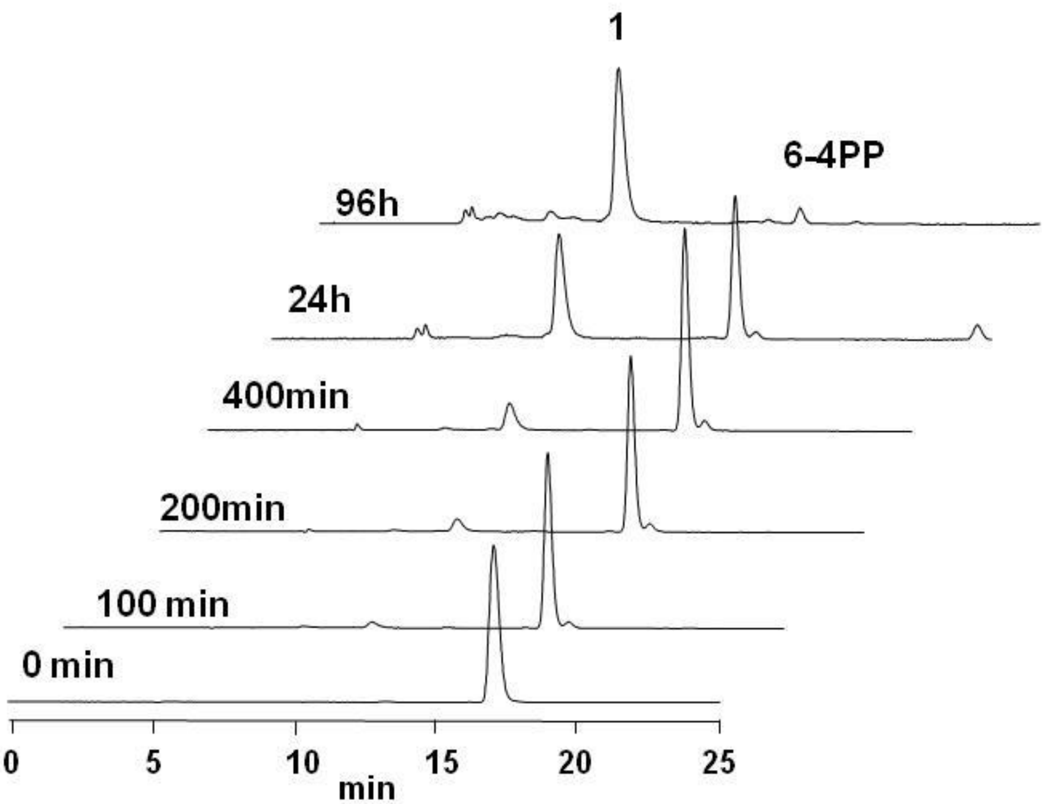
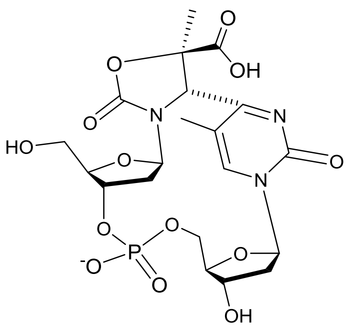


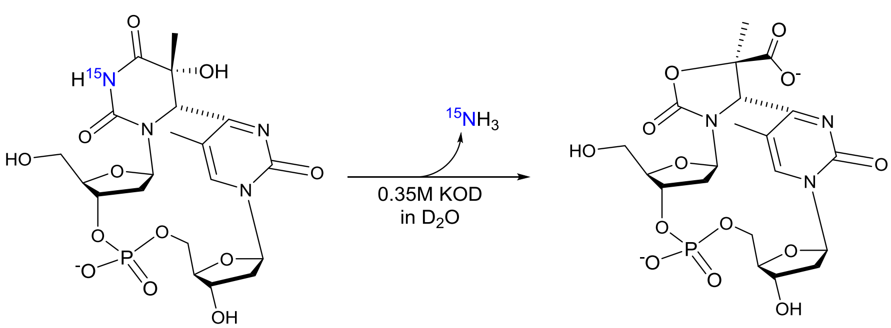
Figure S1. HPLC chromatograph of the 6-4PP hydrolysis reaction under 0.2 M KOH at ambient temperature monitored at the 302 nm wavelength of the UV detector. A new product **1** was generated at ~ 90% yield after the 4-day reaction. Additionally, the putative 6-4PP water adduct reported previously was not observed during the course of the reaction.

Compound 1



^{1}H NMR (d_4-methanol): δ1.36 (s, 3H), 1.74-1.80 (m, 1H), 1.94-2.04 (m, 1H), 2.40 (s, 3H), 2.46-2.55 (m, 1H), 3.02 (ddd, J = 2.0, 7.0, 14.6 Hz, 1H), 3.47 (dt, J = 8.5, 3.6 Hz, 1H), 3.68 (d, J = 11.7 Hz, 1H), 3.71 (dd, J = 4.1, 12.8 Hz, 1H), 3.83 (dd, J = 3.0, 12.8, Hz, 1H), 3.91-3.98 (m, 1H), 3.98-4.05 (m, 2H), 4.82-4.88 (m, 1H), 5.58 (s, 1H), 5.77 (d, J = 8.4 Hz, 1H), 6.50 (dd, J = 2.0, 7.6 Hz, 1H), 8.04 (s, 1H); ^{13}C NMR (d_4-methanol): δ12.8, 18.9, 34.9, 35.8, 59.0, 60.2, 64.6, 69.4, 70.4, 81.5, 82.6, 82.8, 86.6, 87.8, 114.0, 144.7, 156.9, 157.5, 173.4, 176.3. ESI-MS (negative mode) calcd for $C_{20}H_{25}N_3O_{13}P^-$: 546.1130, found 546.1132.

Loss of an ammonia after 6-4PP alkaline treatment: monitoring the reaction using ^{15}N-labeled 6-4PP in 0.30 M KOD in D_2O



^{15}N labeled dinucleotide 6-4PP was dissolved in 0.30 M KOD in D_2O (0.40 mL) to a final concentration of 55.0 mM. The resulting solution was allowed to sit at room temperature for various periods of time and ^{15}N NMR spectra were collected at different time points. (A sample solution containing $^{15}NH_4Cl$ (3 mg) in 0.30 M KOD in D_2O (0.5 mL) was used as an external standard for ^{15}N-NMR spectrum.)

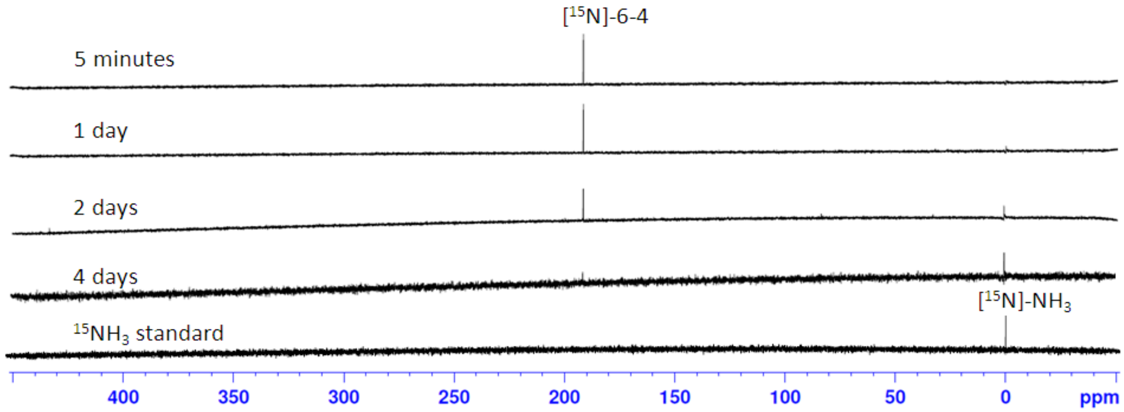
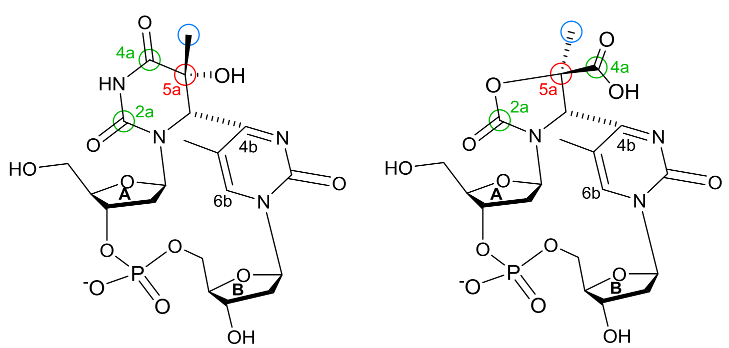


Figure S2. ^{15}N-NMR spectra describing the reaction of ^{15}N-labeled 6-4PP in 0.30 M KOD at ambient temperature in D_2O. The ^{15}N peak (191.7 ppm), corresponding to ^{15}N-labeled 6-4PP, decreased over time as the reaction proceeded. A new ^{15}N NMR signal (0 ppm), corresponding to $^{15}NH_3$, increased accordingly, indicating that a molecule of ammonia was generated during the reaction.

The product 1 generated from alkaline treatment of 6-4PP possesses a five-membered ring structure rather than an anhydride structure:

(a) Comparison of the ^{13}C NMR spectra of 6-4PP and compound 1

Table S1. ^{13}C NMR data of 6-4PP and **1**



identification of carbon	^{13}C chemical shift (ppm)		
	6-4PP	**1**	$\Delta\delta$(ppm)
1A	82.0	81.5	-0.5
2A	35.5	34.9	-0.6
3A	69.9	70.3	0.4
4A	82.8	82.6	-0.2

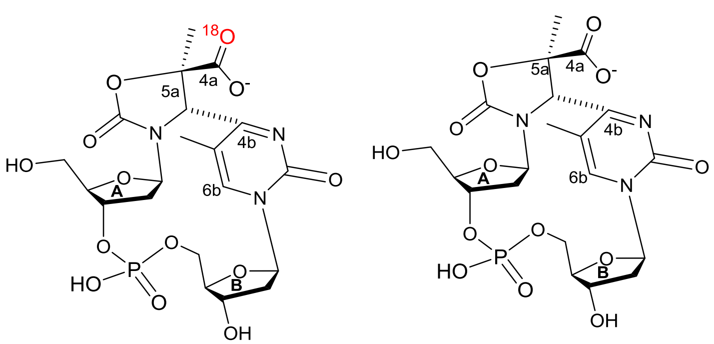
5A	59.2	60.2	1.0
1B	87.5	87.9	0.4
2B	35.8	35.8	0.0
3B	69.7	69.4	-0.3
4B	86.5	86.6	0.1
5B	64.6	64.7	0.1
Me_a	25.7	18.9	-6.8
2a	153.1	157.5	4.4
4a	173.3	176.3	3.0
5a	71.9	82.8	10.9
6a	57.7	59.0	1.3
Me_b	13.6	12.9	-0.7
2b	156.9	156.9	0.0
4b	175.4	173.4	-2.0
5b	115.8	114.0	-1.8
6b	142.8	144.7	1.9

(b) ^{18}O label results in ^{13}C NMR signal shift only at C_{4a}, but not at C_{2a}

Preparation of [^{18}O]-1

6-4PP (21 mg) was dissolved in 0.35 M KOH in $H_2{}^{18}O$ (95% ^{18}O) to a final concentration of 0.75 mM. The resulting solution was allowed to sit at room temperature for two days and the resulting **[^{18}O]-1** was purified by preparative HPLC.

1 and [^{18}O]-1 in a ~ 1:1 mixture



1 : 1 mixture

^{1}H NMR (D_2O): δ1.37 (s, 3H), 1.63 (dd, J = 7.0, 13.9 Hz, 1H), 2.07-2.17 (m, 1H), 2.33 (s, 3H), 2.55-2.65 (m, 1H), 3.07 (ddd, J = 1.9, 7.0, 14.9 Hz, 1H), 3.57-3.63 (m, 1H), 3.67 (dd, J = 4.1, 13.1 Hz, 1H), 3.71 (d, J = 12.3 Hz, 1H), 3.82-3.92 (m, 1H), 3.86 (d, J = 13.1 Hz, 1H), 3.98 (dd, J = 5.6, 12.3 Hz, 1H), 4.12-4.17 (m, 1H), 4.78-4.87 (m, 1H), 5.48 (s, 1H), 5.83 (d, J = 8.4 Hz, 1H), 6.48 (dd, J = 2.0, 7.5 Hz, 1H), 8.11 (s, 1H); ^{13}C NMR (D_2O): δ13.1, 18.8, 34.2, 35.1, 59.0, 59.2, 64.98, 65.03,, 69.6, 69.7, 81.6, 82.1, 83.6, 85.9, 88.4, 115.0, 145.8, 157.1, 158.0, 172.4, 177.1 and 177.0 (2C; due to the chemical shift effects of ^{18}O, C_{4a} exhibits as two peaks; 177.0617 and 177.0327). ESI-MS (negative mode) calcd. for $C_{20}H_{25}N_3O_{12}{}^{18}OP^-$: 548.1173, found 548.1175.

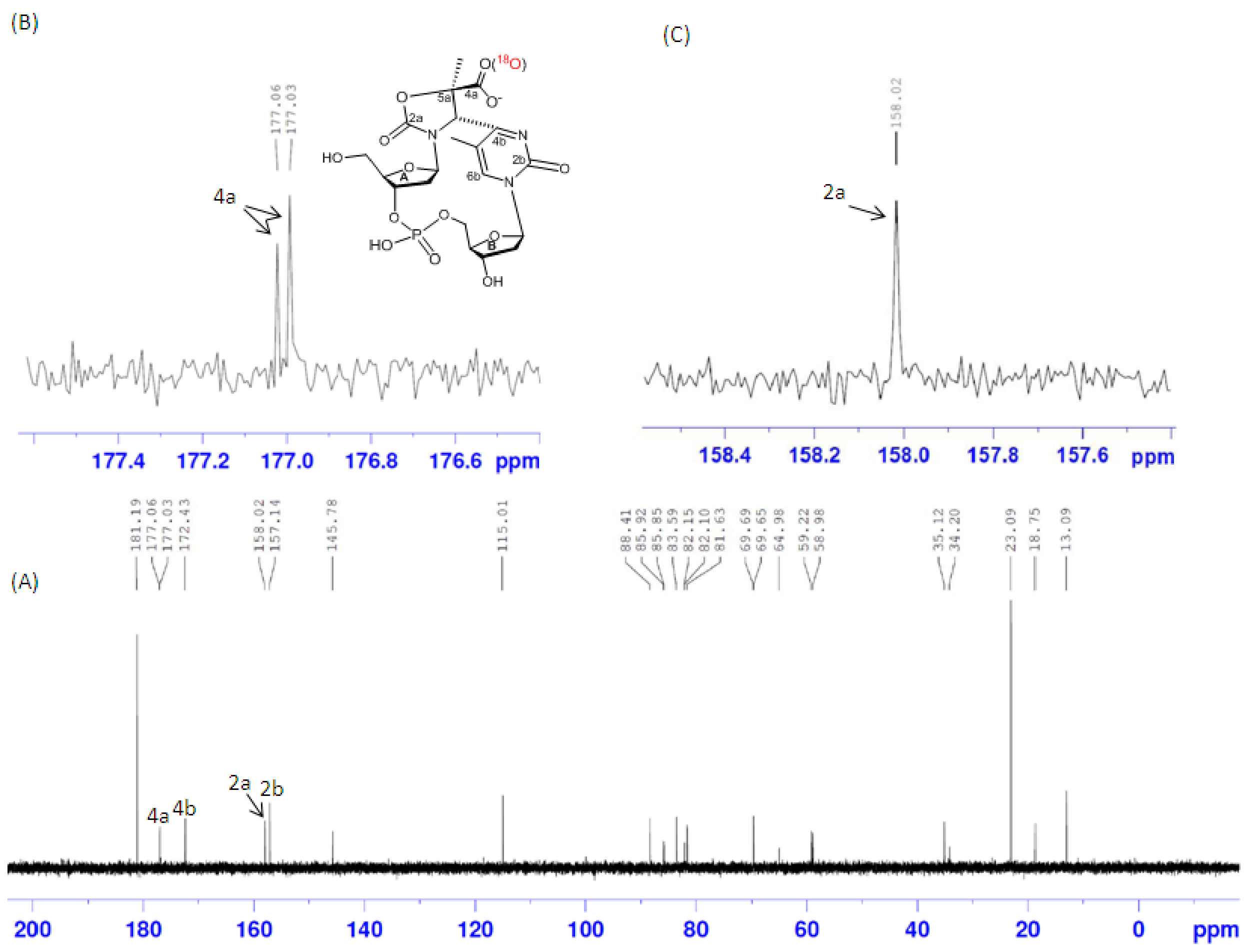


Figure S3. (B) ^{13}C NMR spectrum of unlabeled **1**/[^{18}O]-labeled **1** as a 1:1.8 mixture. (B) and (C) Zoom-in view of the region corresponding to ^{13}C NMR signals of C_{4a} and C_{2a} respectively.

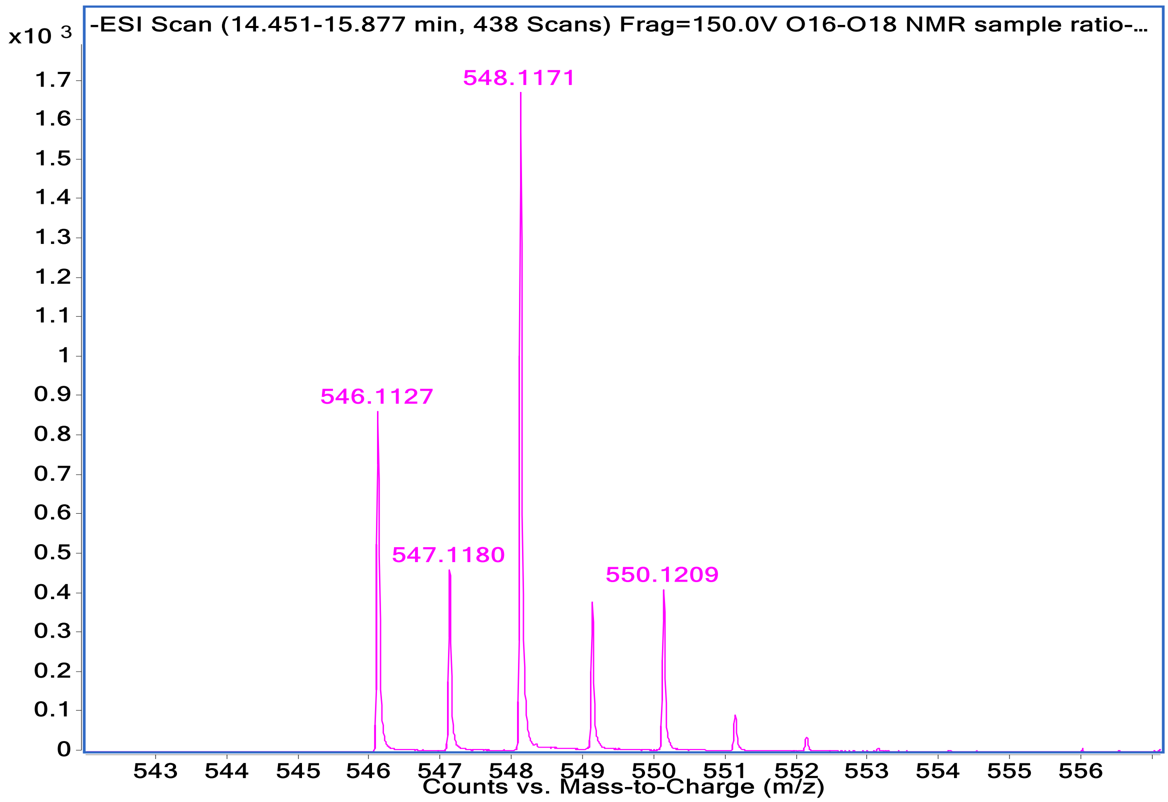


Figure S4. ESI-MS analysis of the unlabeled and ^{18}O-labeled **1** used for the ^{13}C NMR studies above. The ratio between the unlabeled ($[M - H]^-$ signal at 546.1 amu) and ^{18}O-labeled **1** ($[M - H]^-$ signal at 548.1 amu) was found to be 1 : 1.8.

(c) ROE correlation of 6-4PPand compound 1

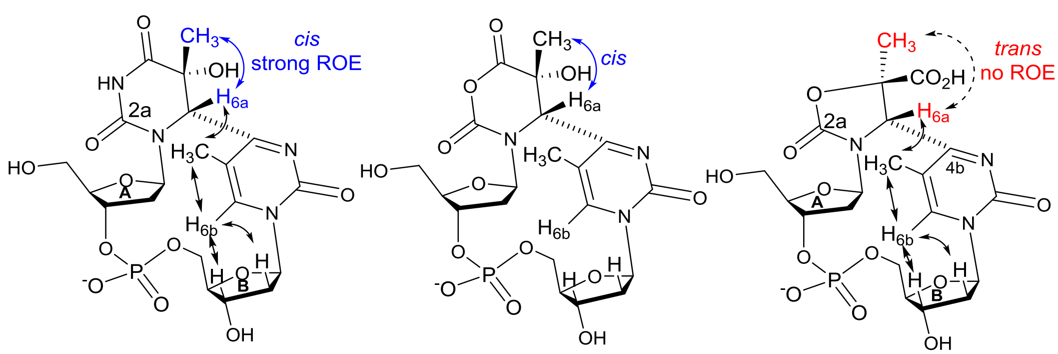


Figure S5. Comparison of the ROESY spectra of 6-4PP and **1**. In 6-4PP, the H_{6a} atom interacts with both methyl groups. In compound **1**, the corresponding ROESY signal disappears due to the *trans* conformation of H_{6a} and Me_a.

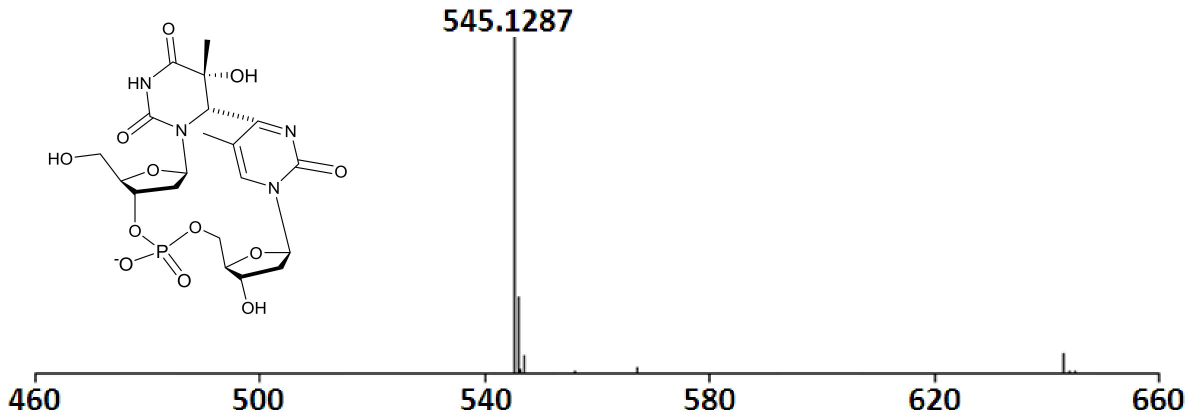


Figure S6. ESI-MS spectrum of the 6-4PP in negative ion mode ([M – H]$^{-}$ species).

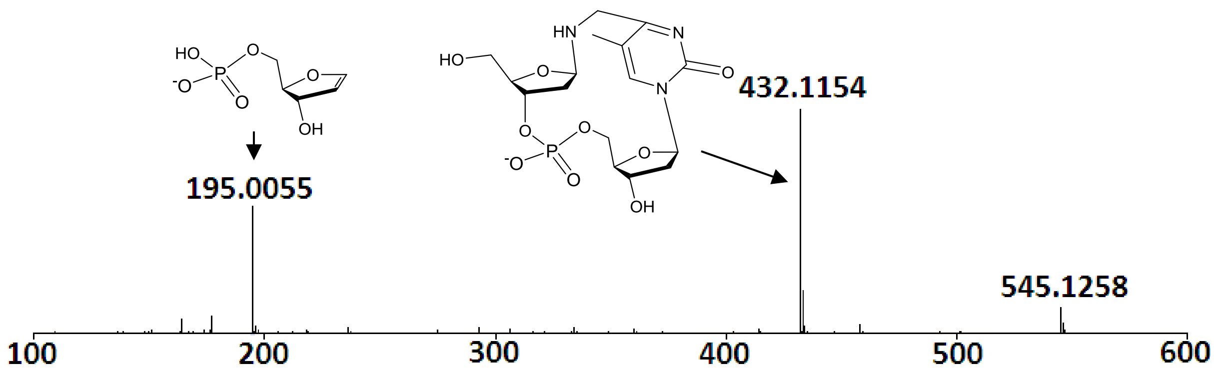


Figure S7. MS-MS spectrum and the fragment structures of the 6-4PP under negative ion mode.

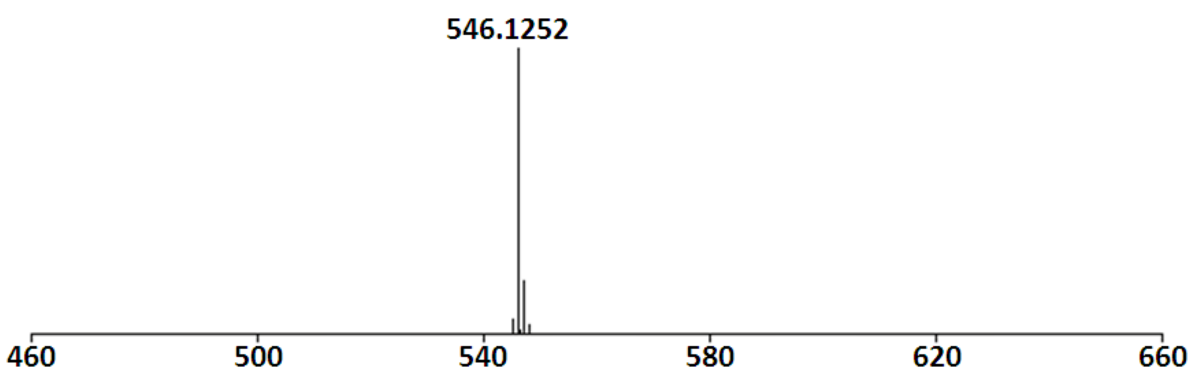


Figure S8. ESI-MS spectrum of [^{15}N]-6-4PP under negative ion mode ([M – H]$^{-}$ species).

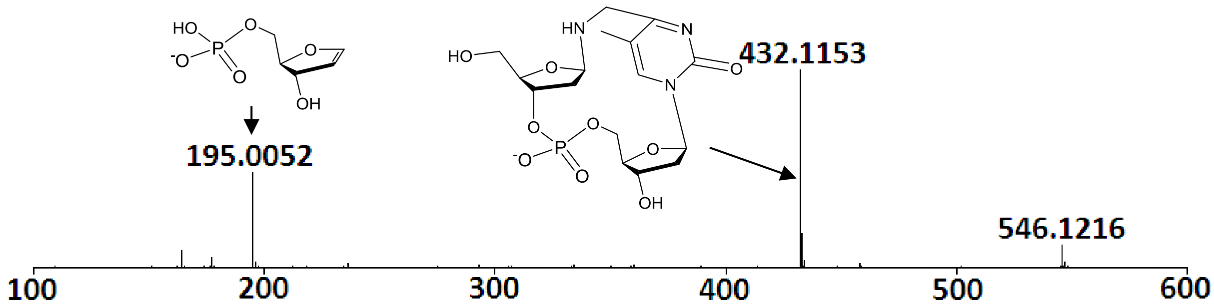


Figure S9. MS-MS spectrum and fragment structures of the [^{15}N]-6-4PP under negative ion mode.

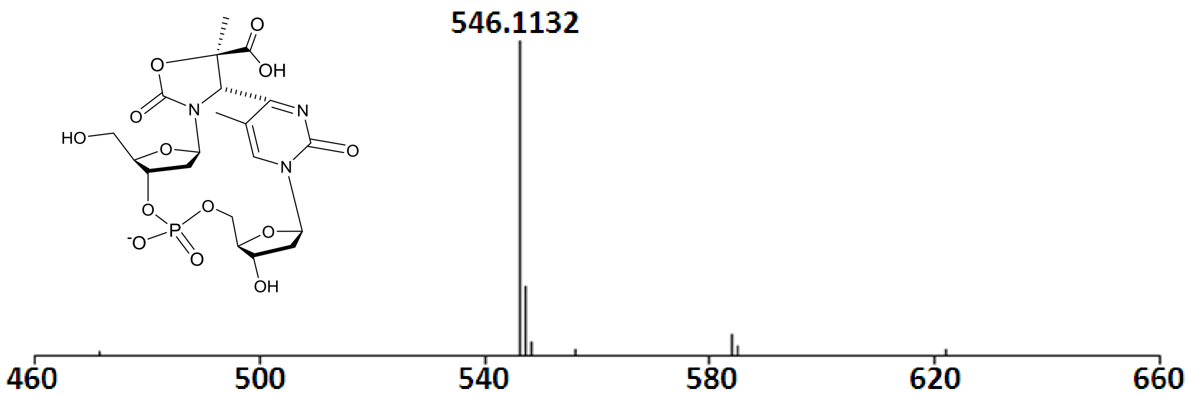


Figure S10. ESI-MS spectrum of product **1** under negative ion mode ([M – H]$^{-}$ species).

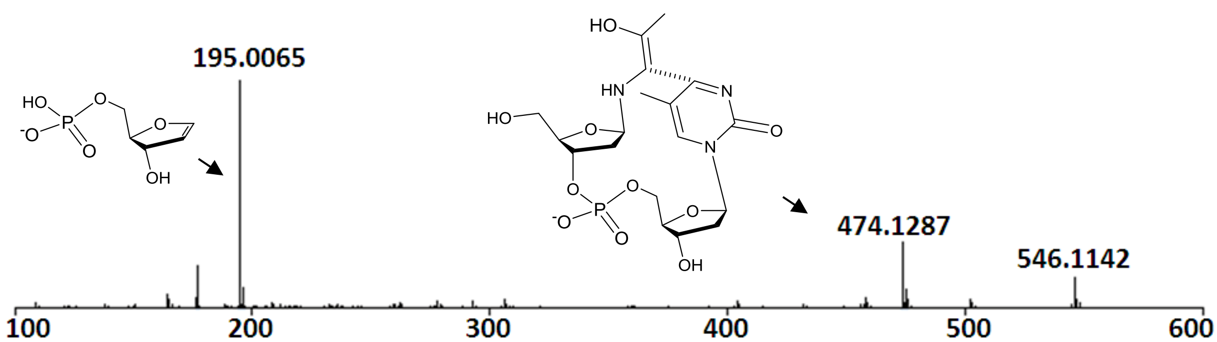


Figure S11. MS-MS spectrum and fragment structures of **1** under negative ion mode.

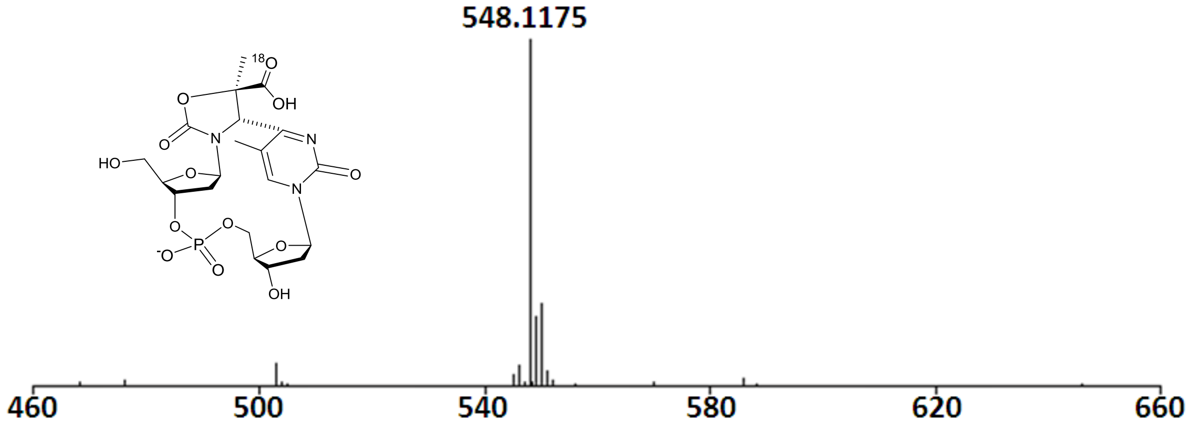


Figure S12. ESI-MS spectrum of [^{18}O]-**1** under negative ion mode ([M – H]$^{-}$ species).

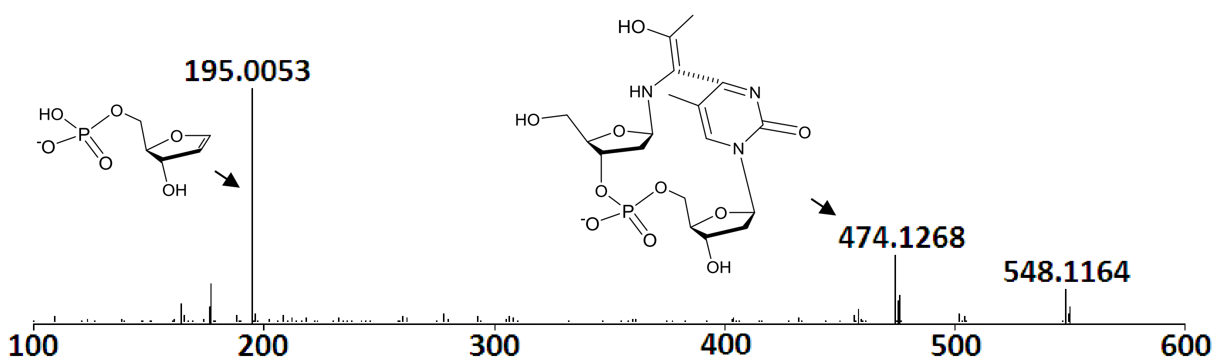


Figure S13. MS-MS spectrum and fragment structures of [^{18}O]-**1** under negative ion mode.

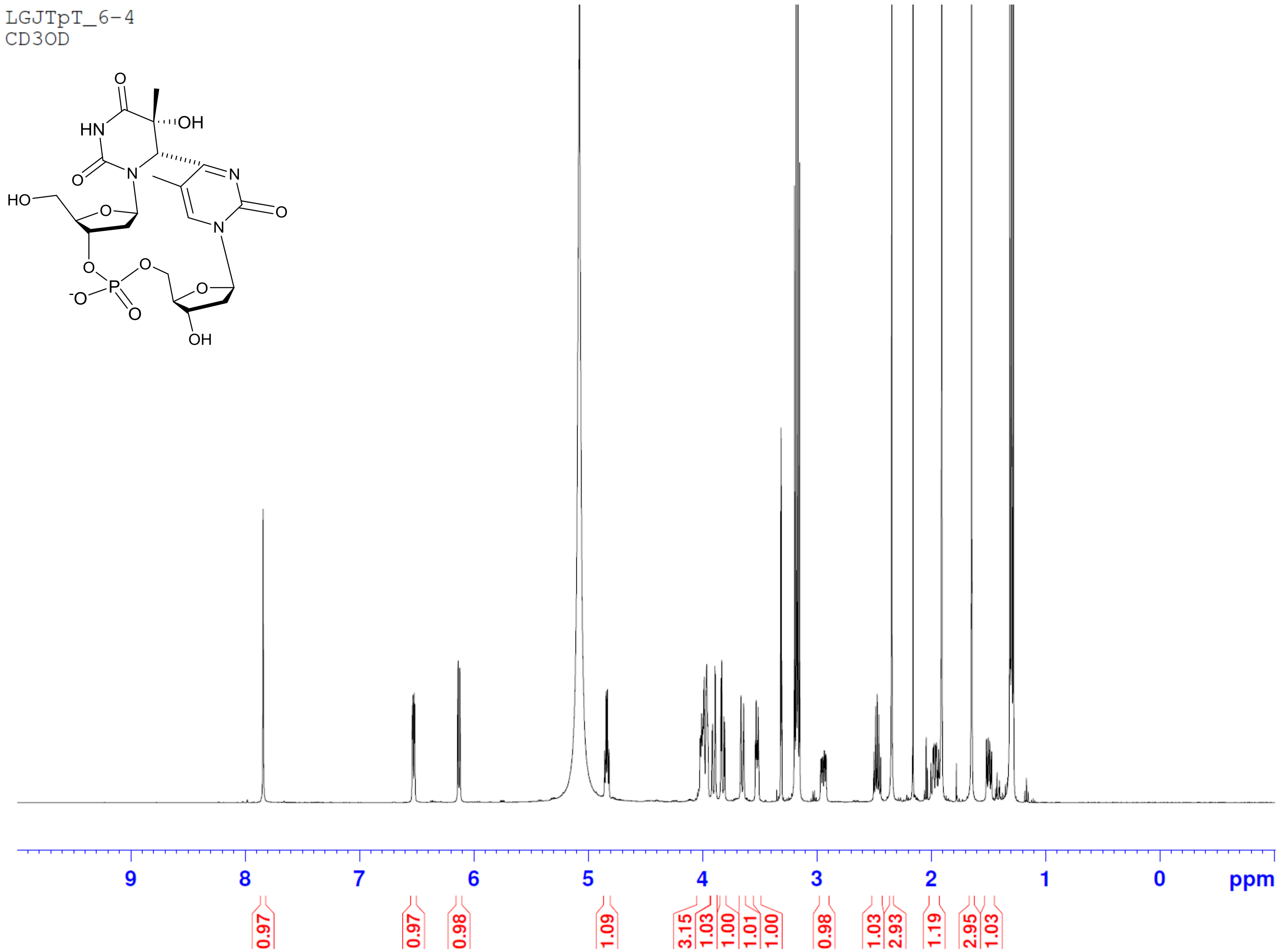


Figure S14. ^{1}H-NMR spectrum of 6-4PP in d_4-methanol.

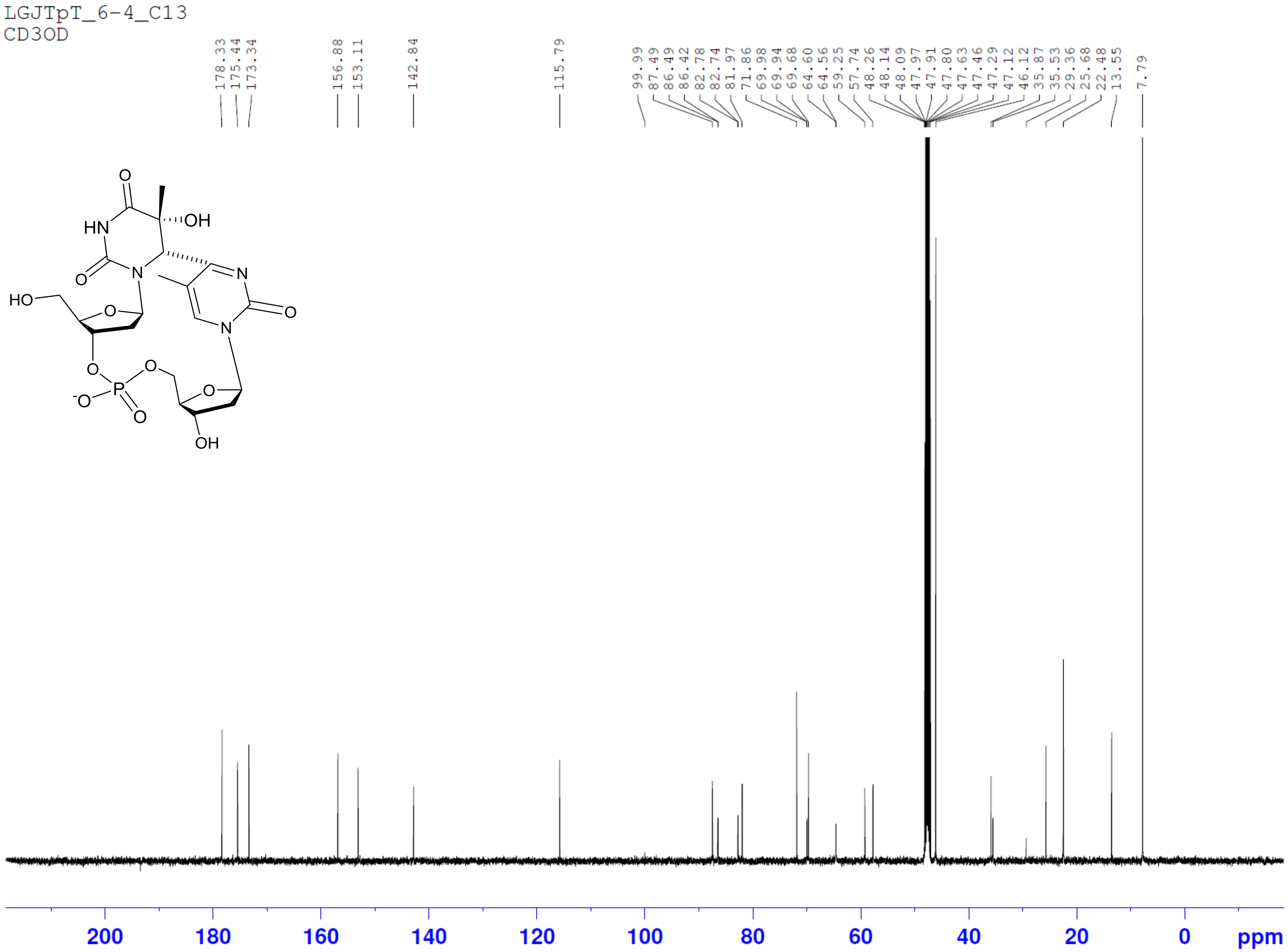


Figure S15. ^{13}C-NMR spectrum of 6-4PP in d_4-methanol.

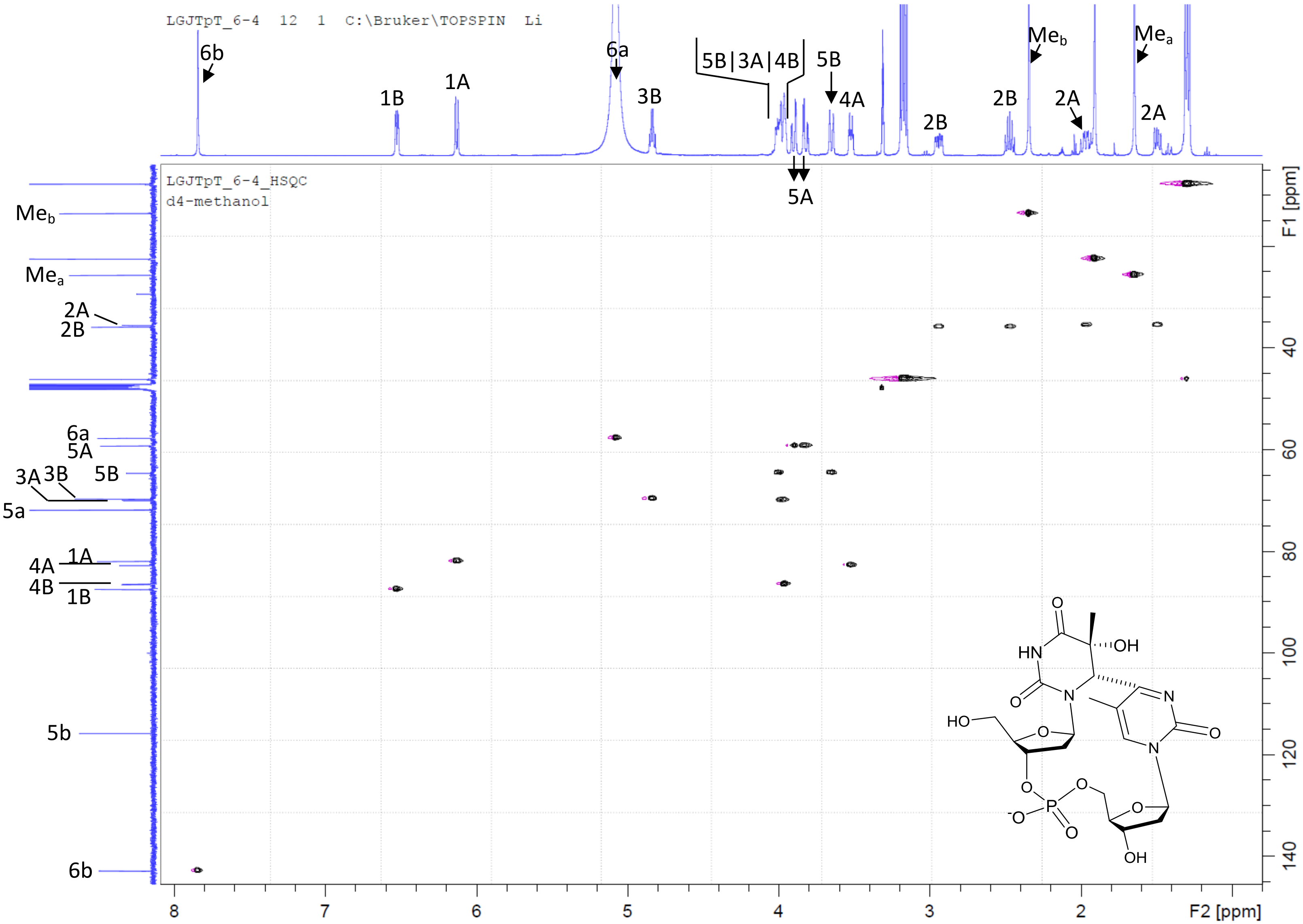


Figure S16. HSQC spectrum of 6-4PP in d_4-methanol.

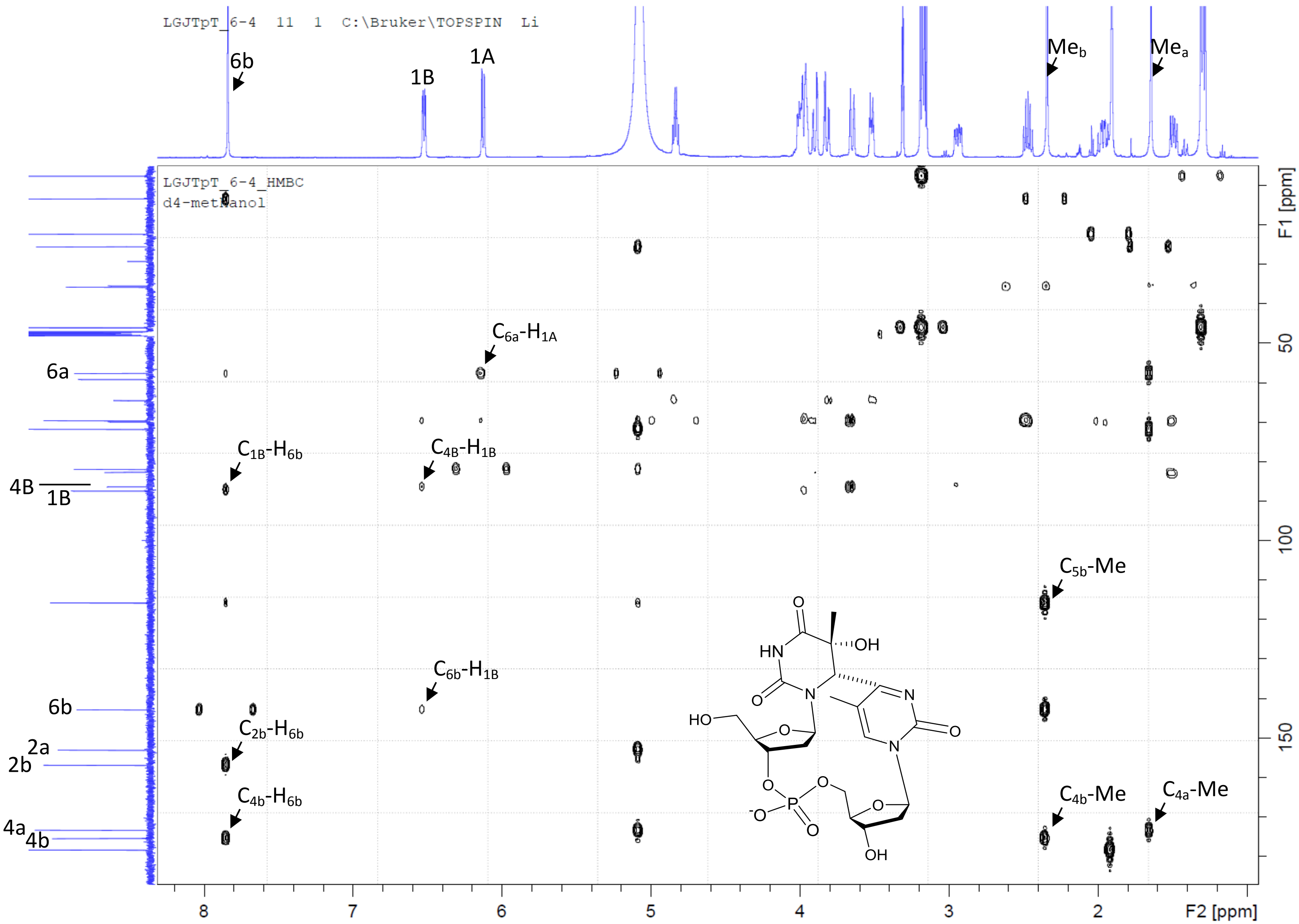


Figure S17. HMBC spectrum of 6-4PP in d_4-methanol.

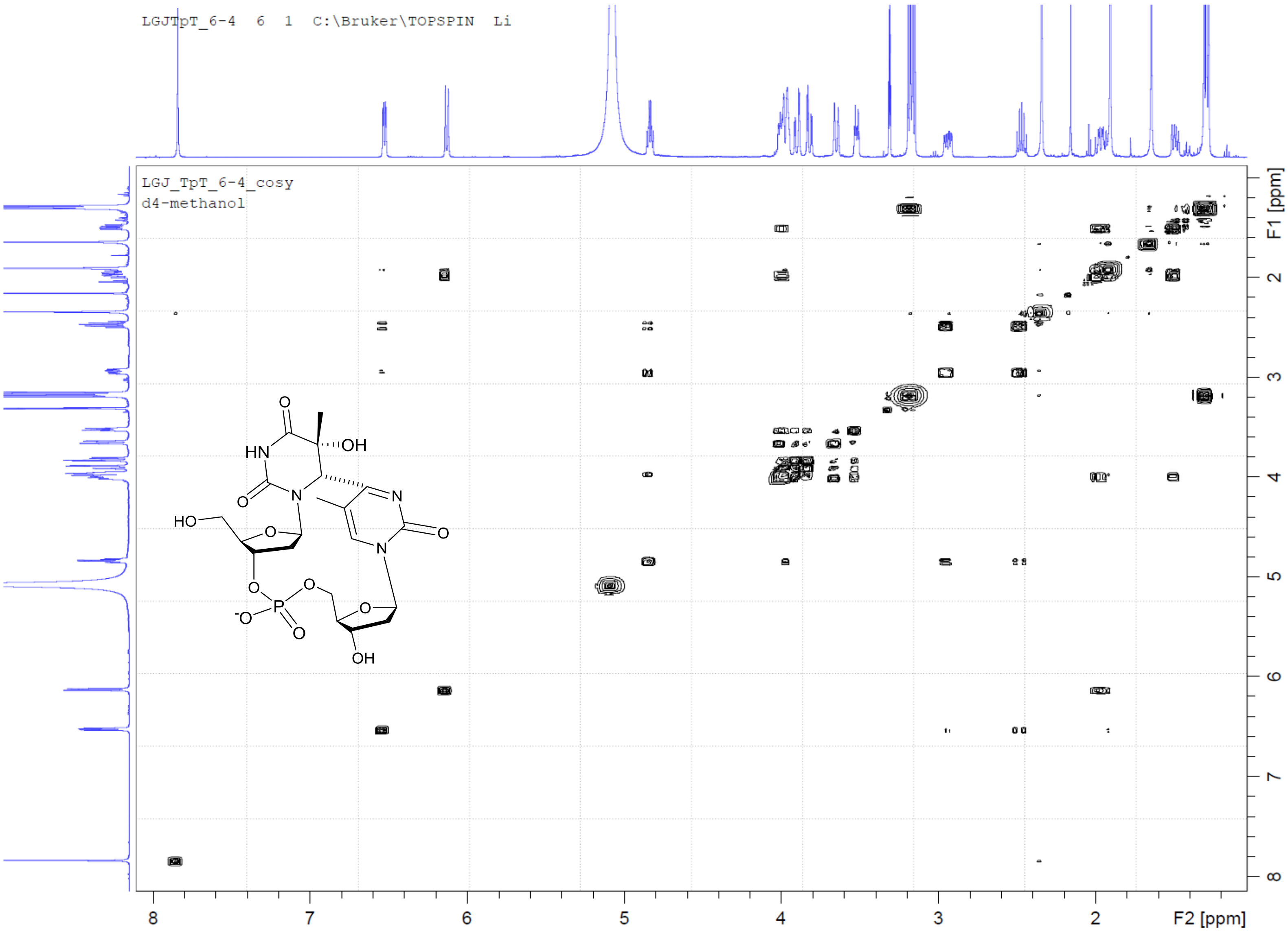


Figure S18. COSY spectrum of 6-4PP in d_4-methanol.

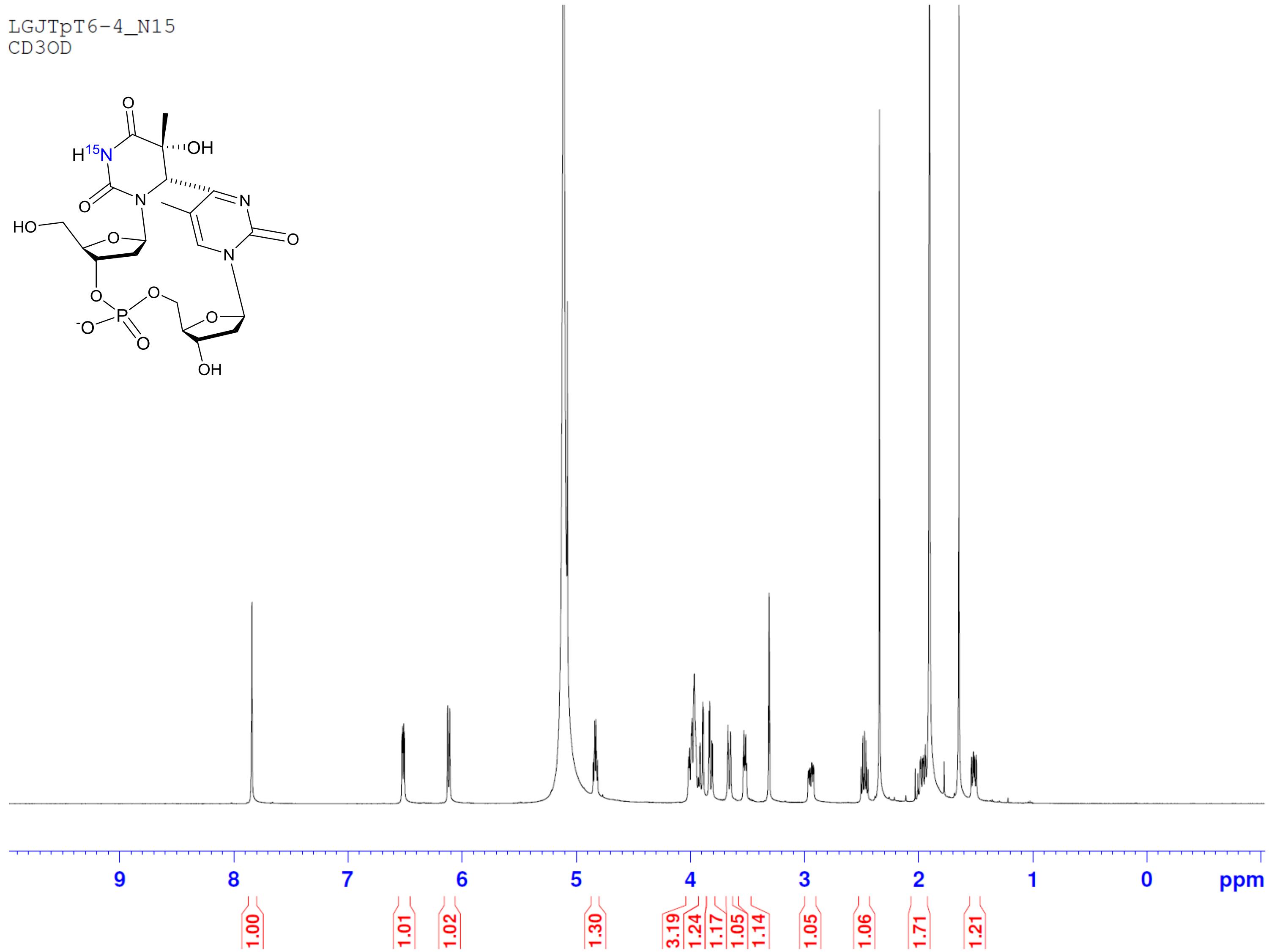


Figure S19. ^{1}H NMR spectrum of [^{15}N]-6-4PP in d_4-methanol.

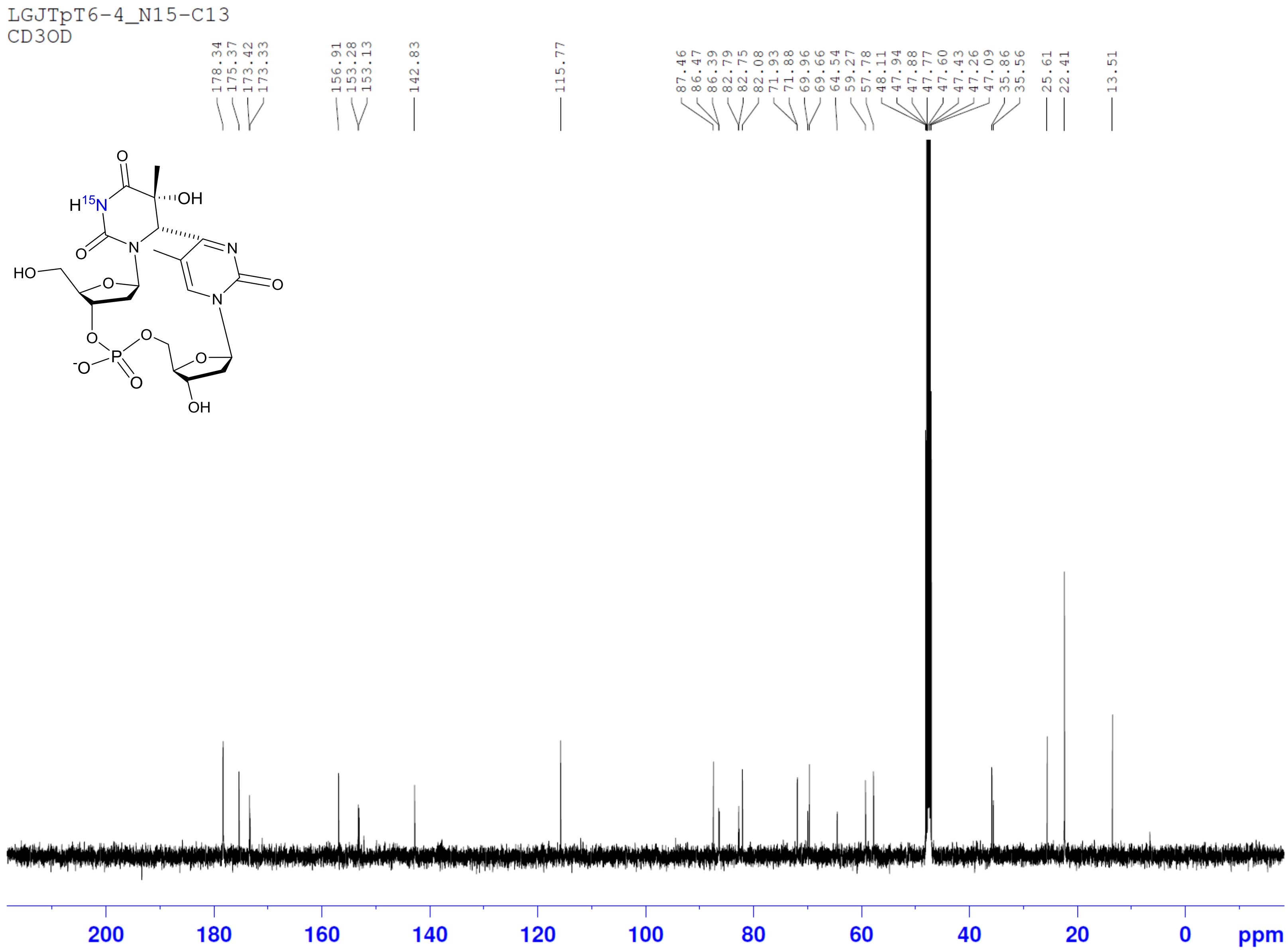


Figure S20. ^{1}H NMR spectrum of [^{15}N]-6-4PP in d_4-methanol.

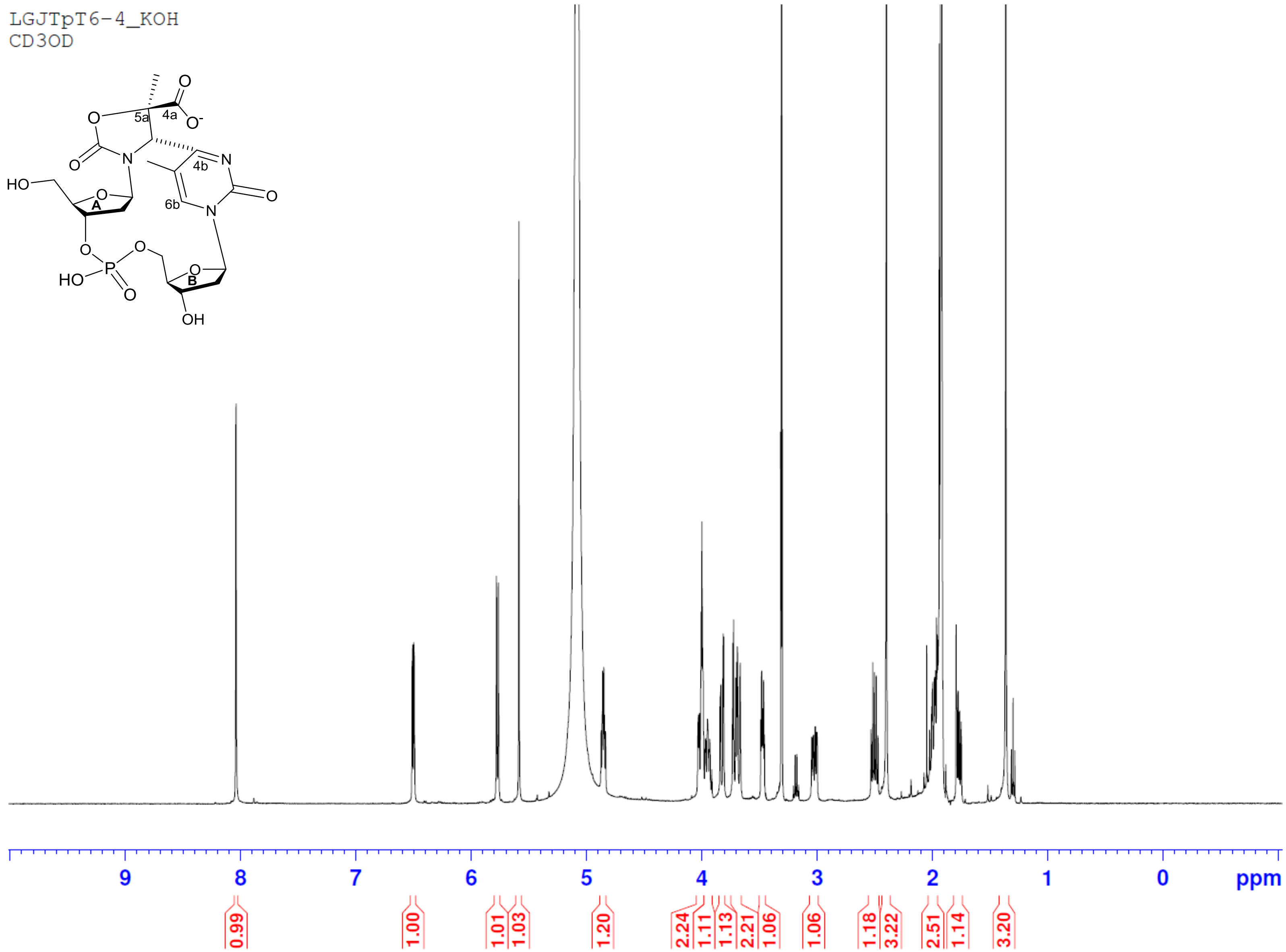


Figure S21. ^{1}H NMR spectrum of **1** in d_4-methanol.

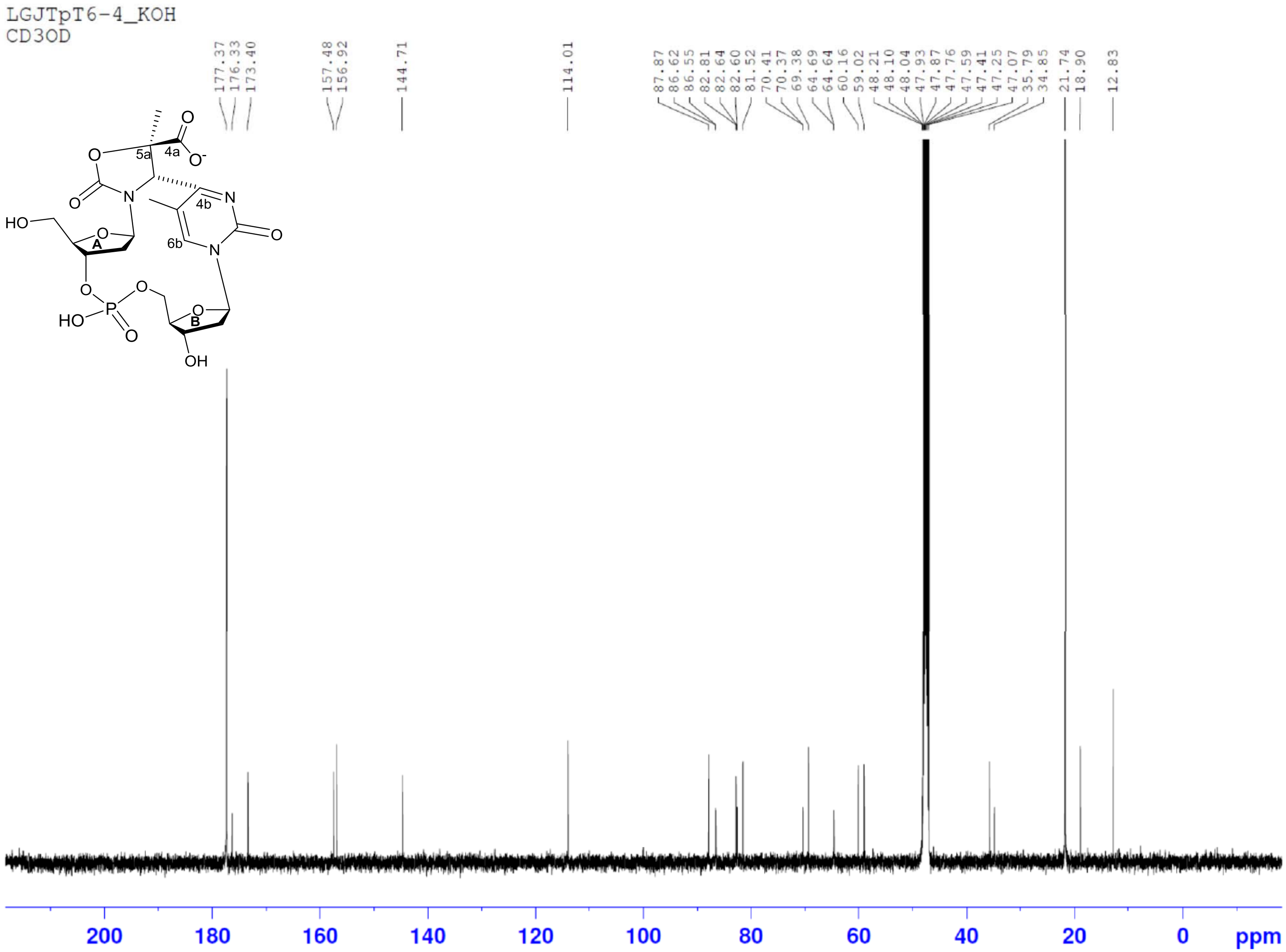


Figure S22. ^{13}C NMR spectrum of **1** in d_4-methanol.

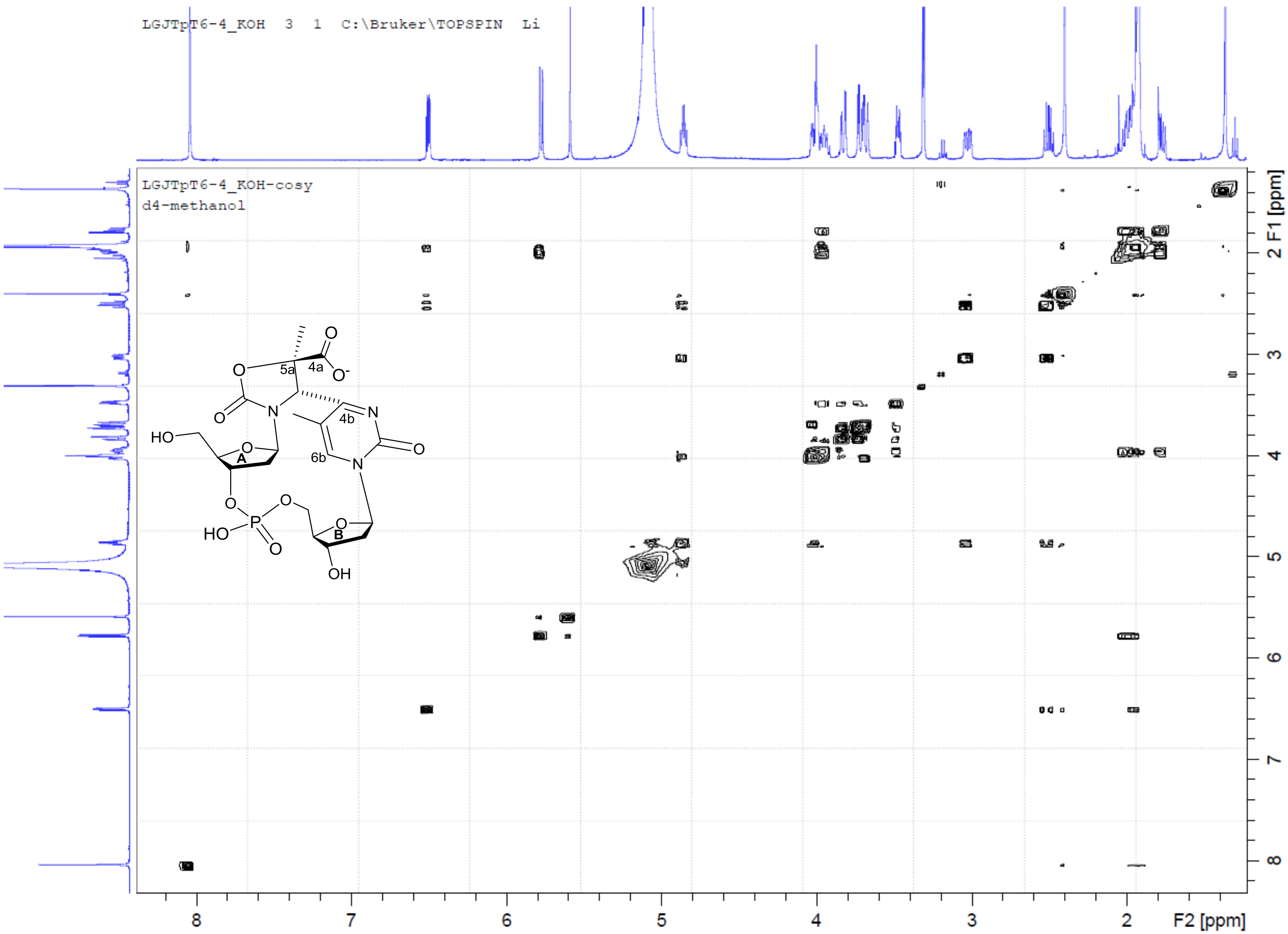


Figure S23. COSY spectrum of **1** in d_4-methanol.

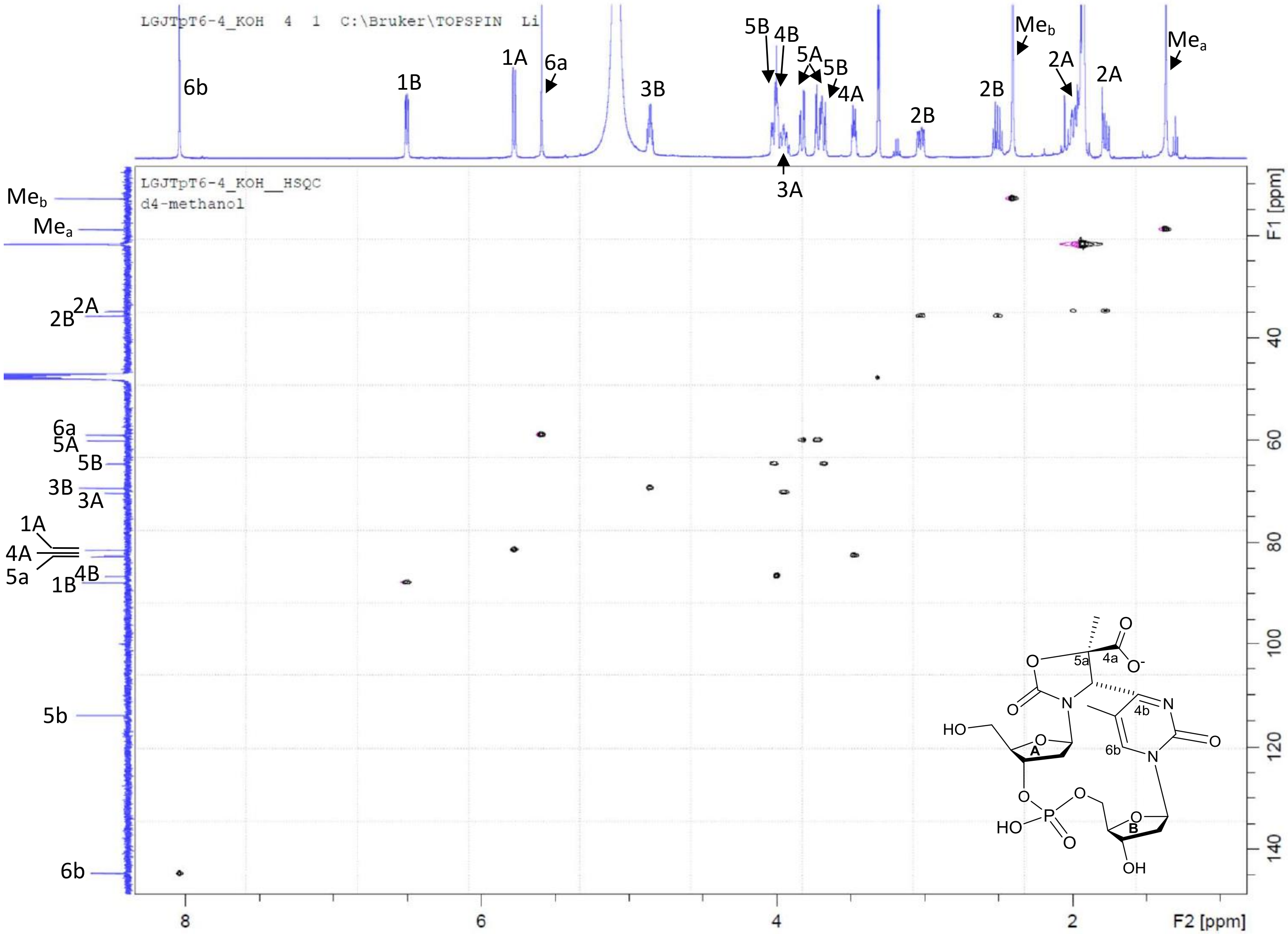


Figure S24. HSQC spectrum of **1** in d_4-methanol.

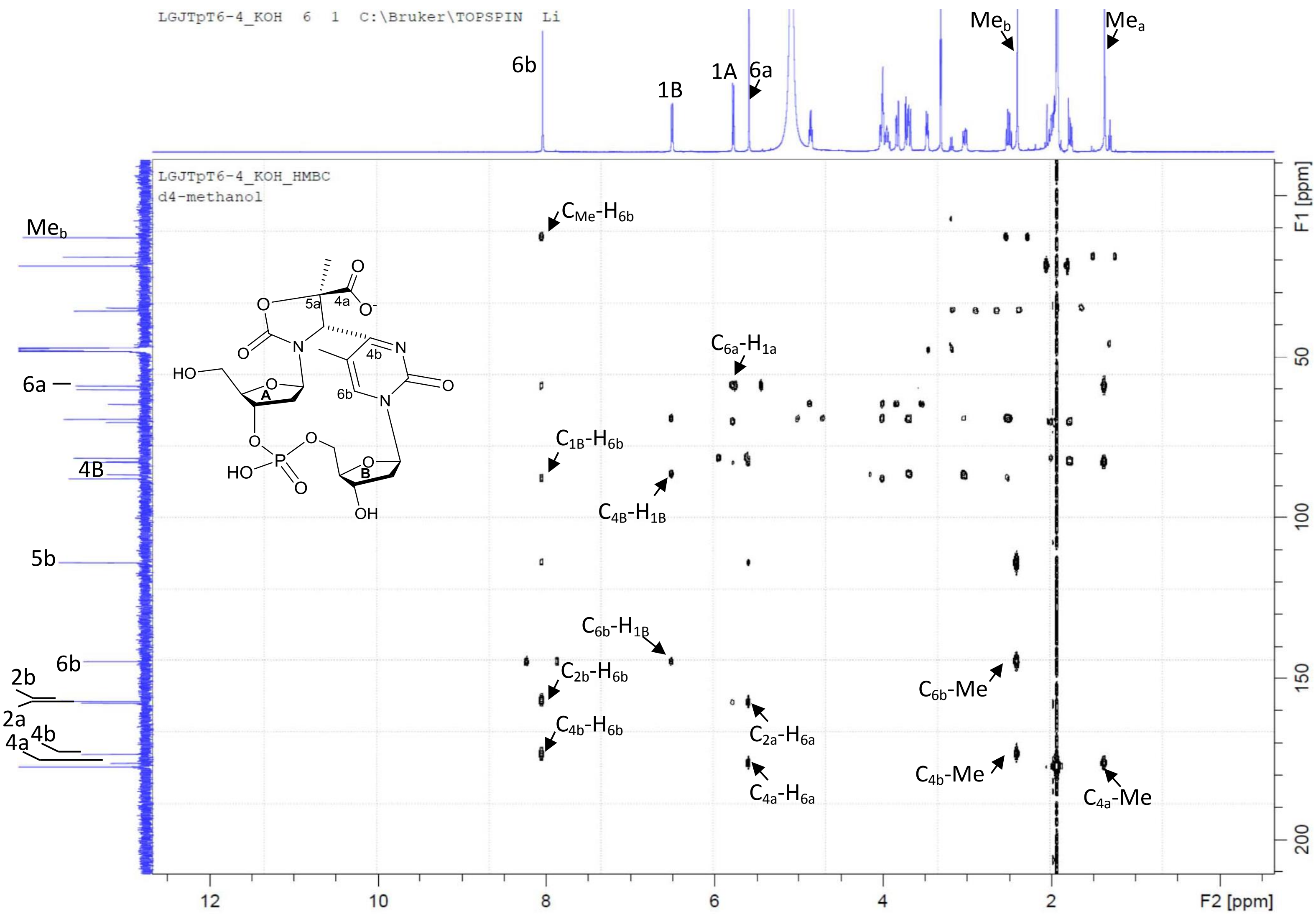


Figure S25. HMBC spectrum of **1** in d_4-methanol.

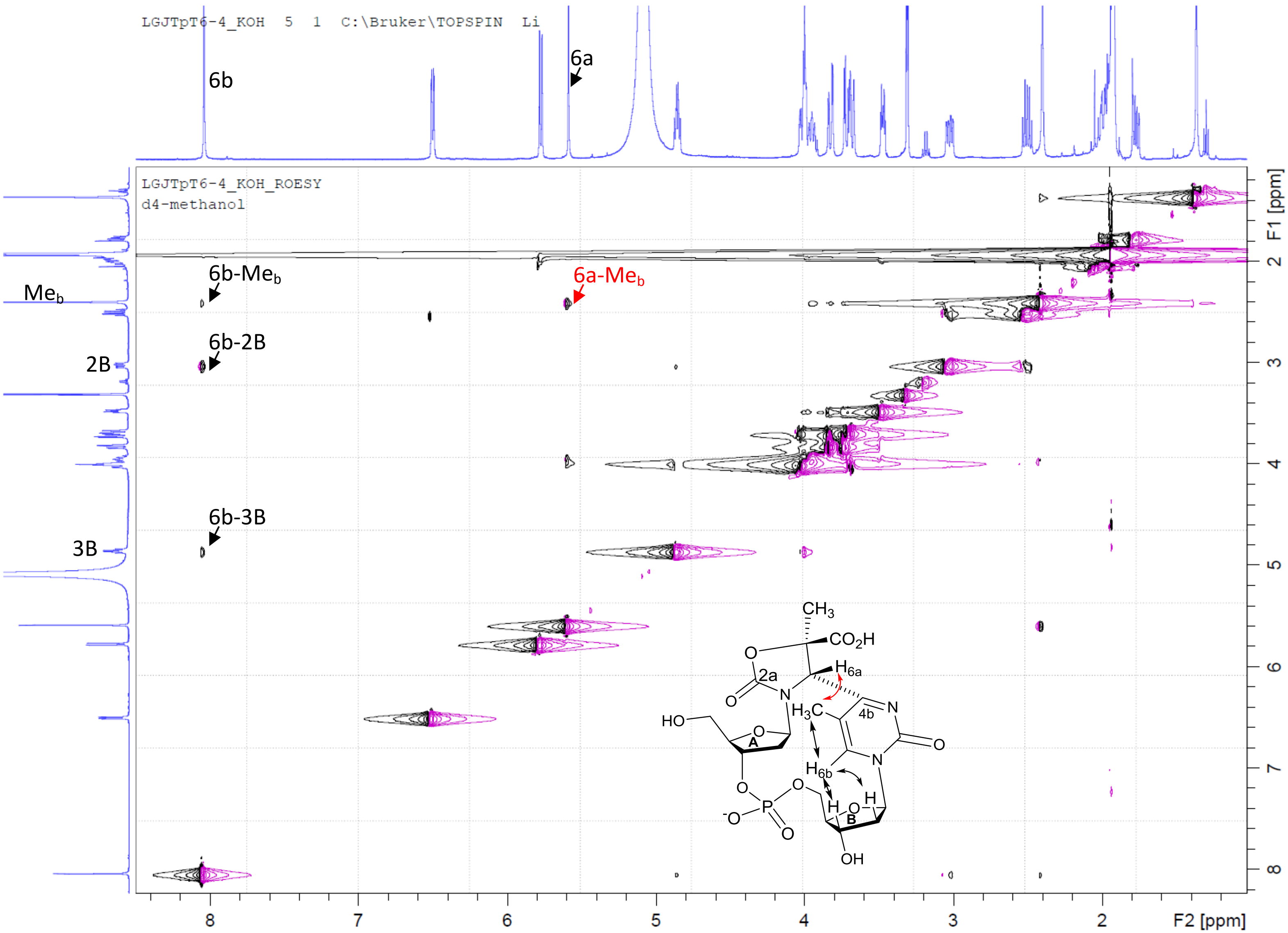


Figure S26. ROESY spectrum of **1** in d_4-methanol.

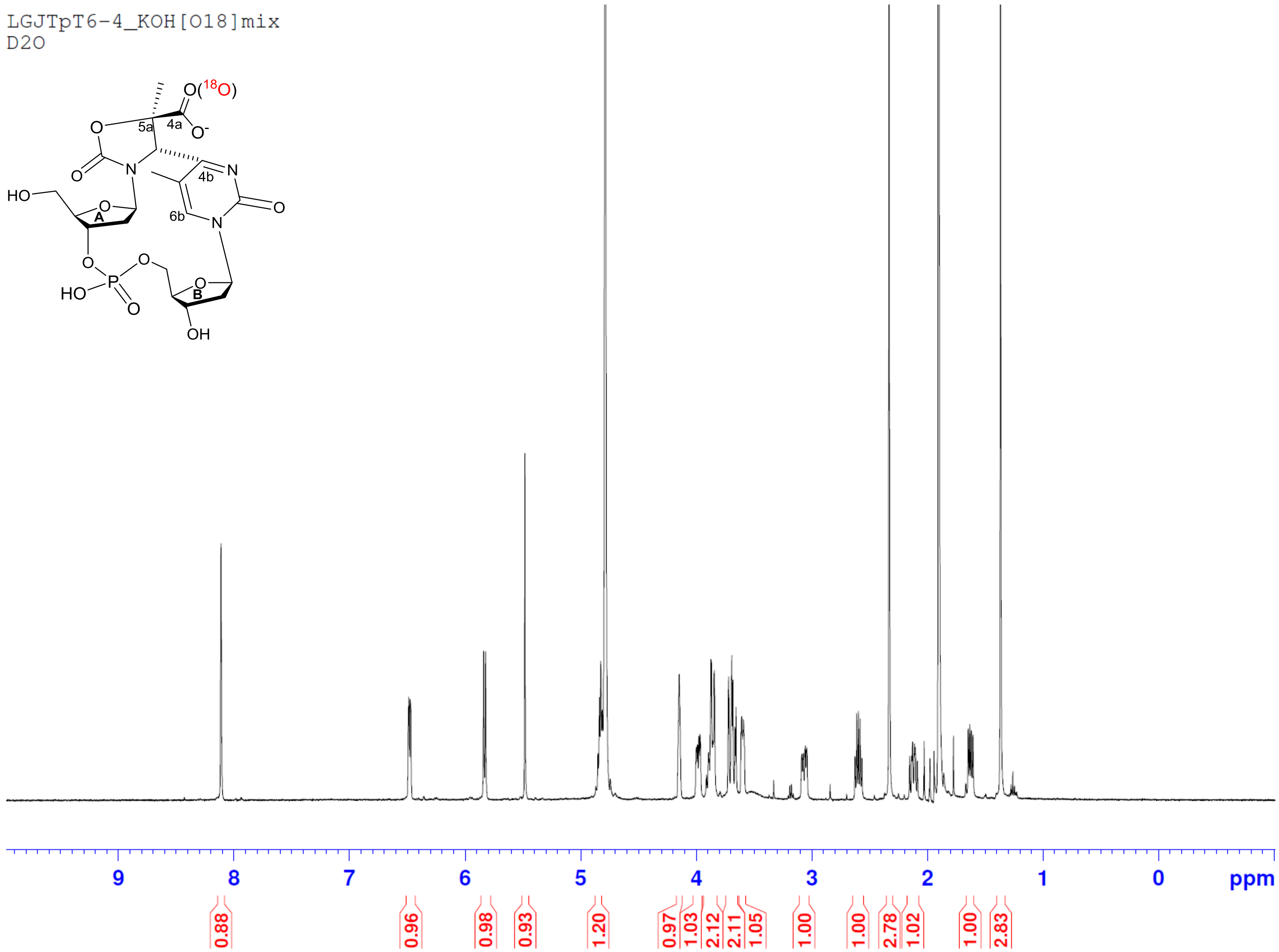


Figure S27. ^{1}H NMR spectrum of **1** and [^{18}O]-**1** as a~ 1 : 1 mixture in D_2O.

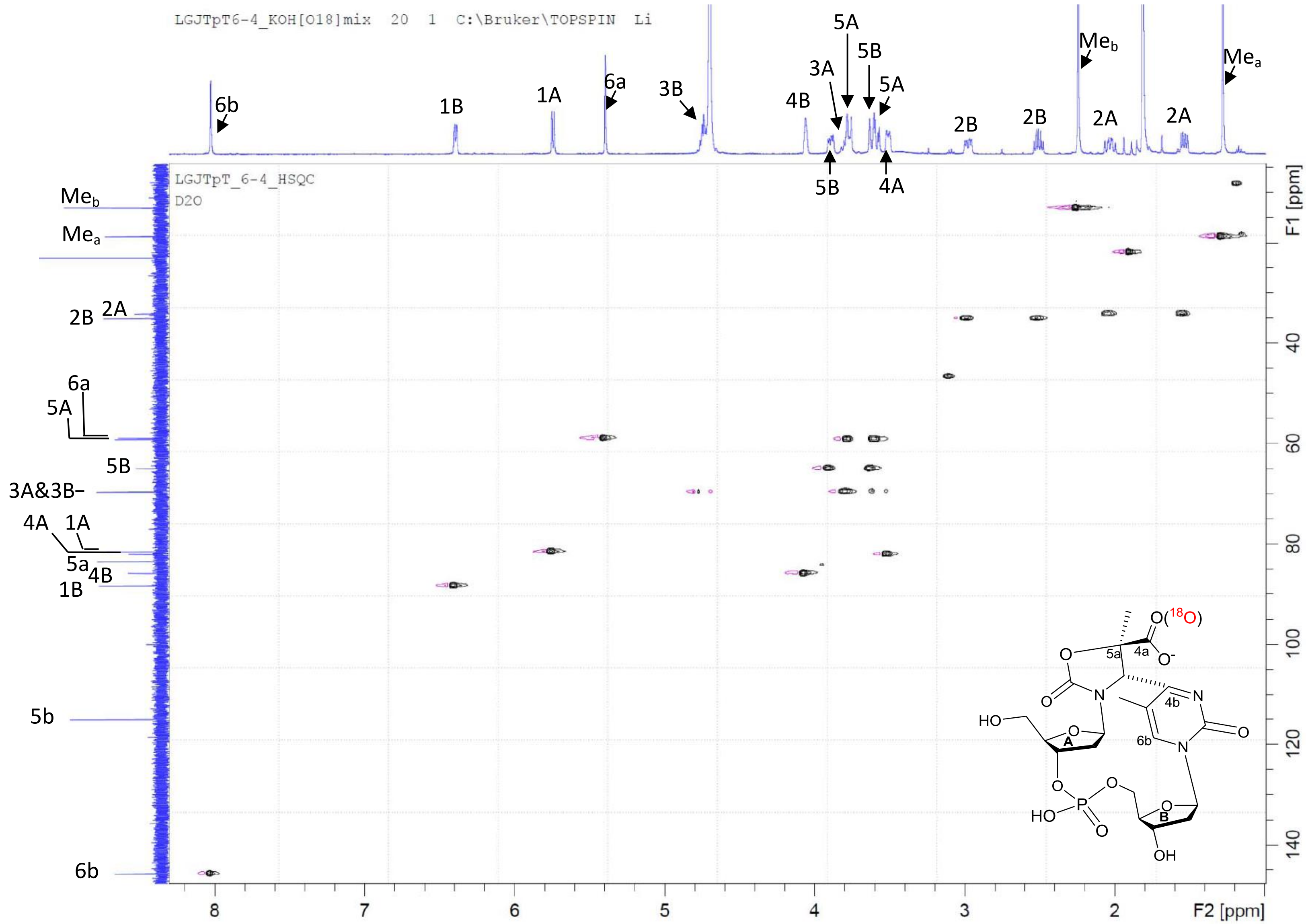


Figure S28. HSQC spectrum of **1** and [^{18}O]-**1** as a ~1 : 1 mixture in D_2O.

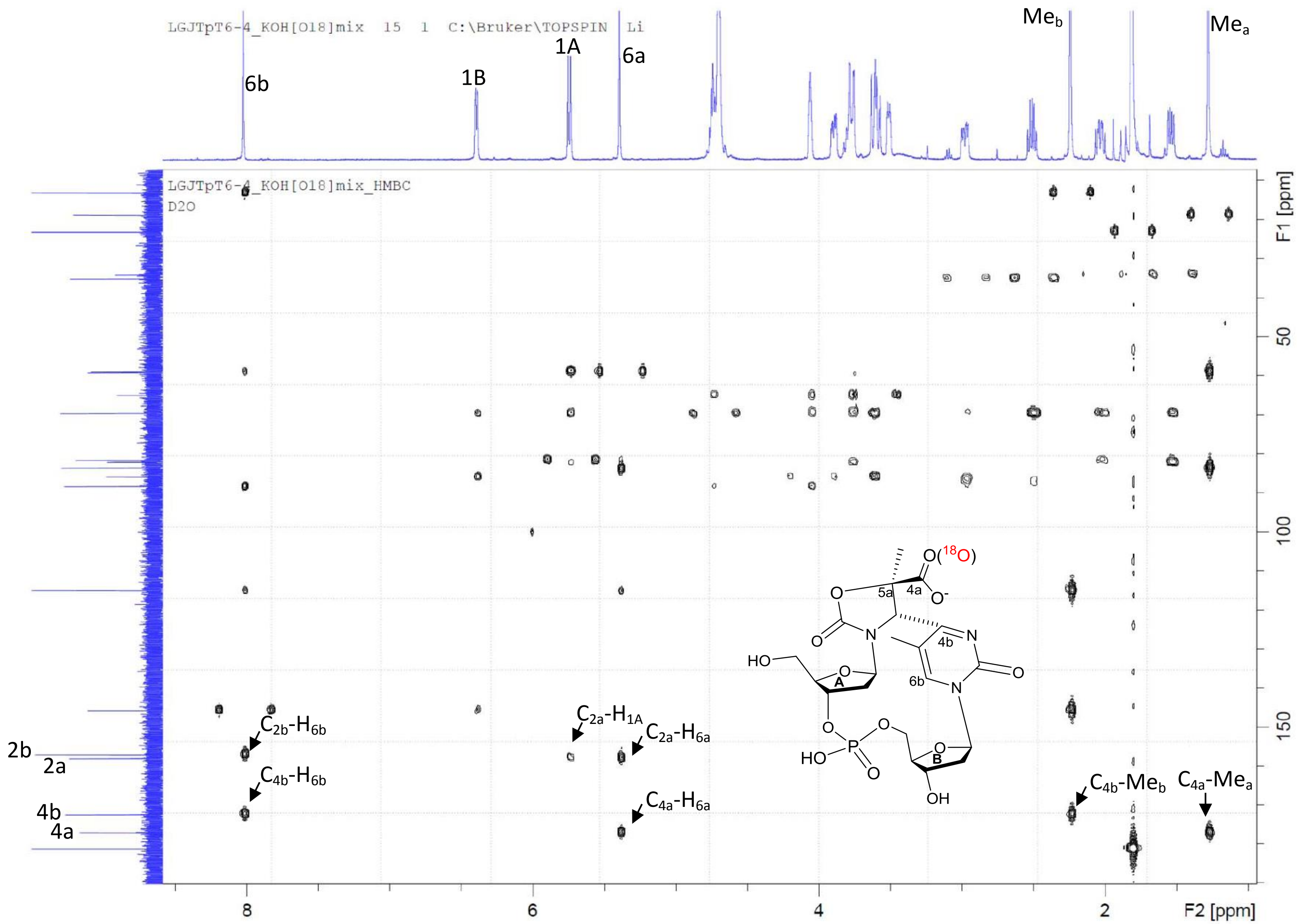


Figure S29. HMBC spectrum of **1** and [^{18}O]-**1** as a ~1 : 1 mixture in D_2O.

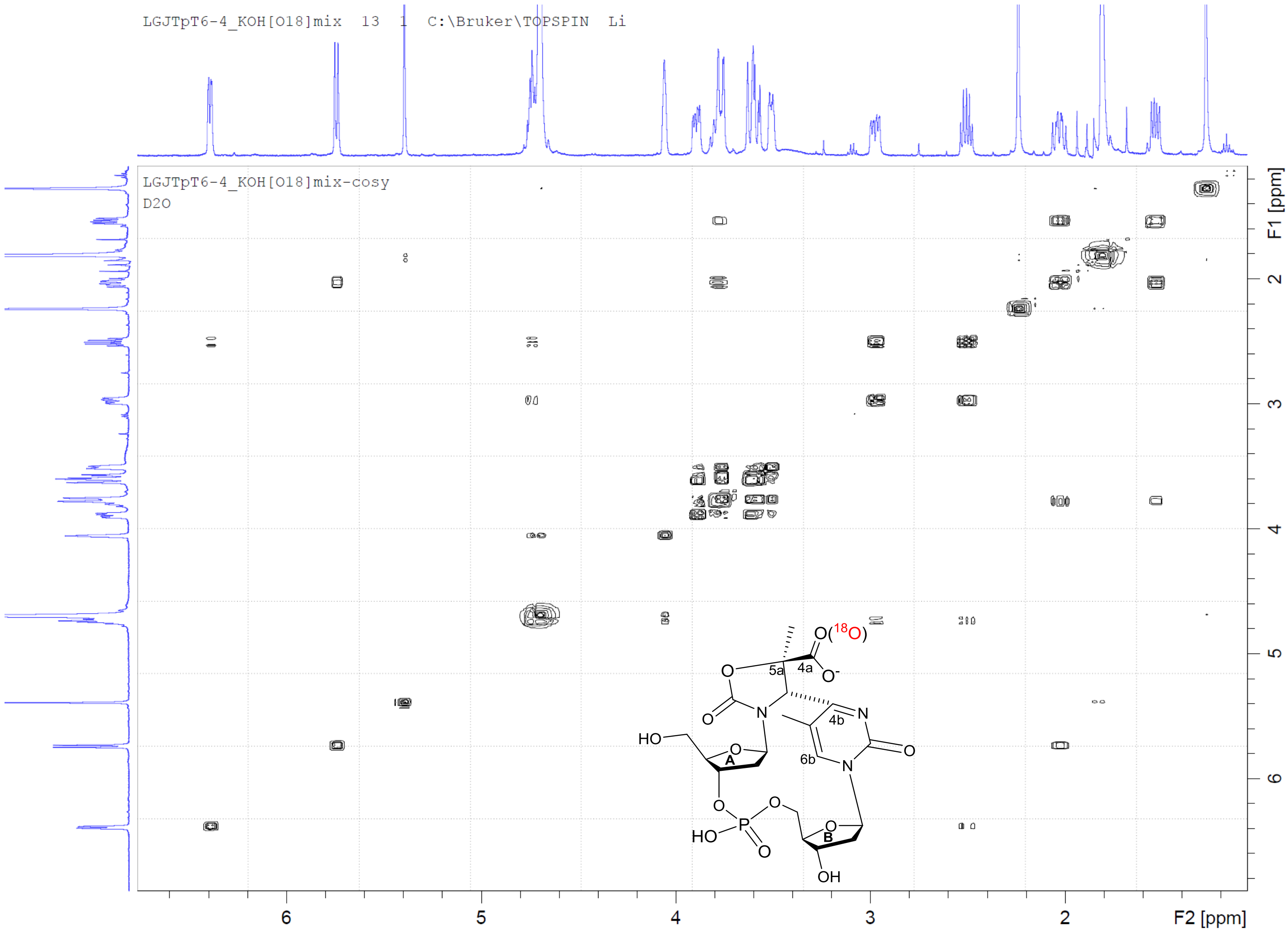


Figure S30. COSY spectrum of **1** and [^{18}O]-**1** as a~1 : 1 mixture in D_2O.

Reference

1. Ariza, X.; Bou, Valenti.;Vilarrasa J. *J. Am. Chem. Soc.* **1995**, *117*, 3665-3673.

2. Iwai, S.; Shimizu, M.; Kamiya, H.; Ohtsuka, E. *J. Am. Chem. Soc.* **1996**, *118*, 7642-7643.