Supporting information

For

On the Reactivity of Damaged Pyrimidines: DNA Cleavage via Hemiaminal Formation at the C4 Positions of the Saturated Thymine of Spore Photoproduct and Dihydrouridine

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Abbreviations used

SP TpT, 5-thyminyl-5,6-dihydrothymine, also called spore photoproduct; DMSO-d_6, dimethylsulfoxide-d_6; Abbreviations for NMR signal coupling are as follows: s, singlet; d, doublet; m, multiplet.

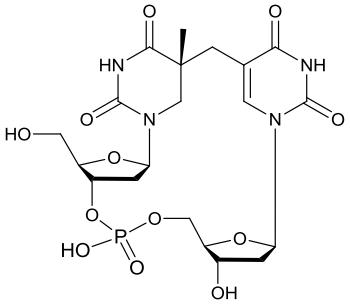
General Methods

All reagent grade chemicals were purchased from Sigma, Fisher, or VWR and used without further. The ^{1}H spectra were obtained on a Bruker 500 MHz NMR Fourier transform spectrometer. NMR spectra were recorded in sample solutions in deuterated DMSO (DMSO-d_6), with residual DMSO (δ 2.50 ppm for ^{1}H NMR) taken as the standard. The chemical shifts on NMR spectra were reported in parts per million (ppm).

HPLC analyses were performed at room temperature with a Waters (Milford, MA) breeze HPLC system coupled to a 2489 UV/Visible detector at 268 nm. An Agilent ZORBAX Bonus-RP column (5 μm particle size, 250 × 4.6 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 1 mL/min. Under this gradient, SP TpT was eluted at 11.4 min, and product **1** at 7.7 min.

The LC/MS assay of alkaline treatment of 5-mer oligonucleotide and dHdU was conducted using via an Agilent 6520 Accurate Mass Q-TOF LC/MS spectrometer using an Agilent Eclipse Plus C18 column (3.5 μm particle size, 100 × 4.6 mm i.d.). The column was equilibrated in solvent A (5 mM ammonium acetate in 99% water and 2% acetonitrile, pH 6.5) and compounds were eluted with an ascending gradient (2% ~ 10%) of acetonitrile (solvent B) in 20 minutes at a flow rate of 0.5 mL/min. Under this gradient, compound **6** was eluted at 6.8 min, compound **7** at 7.9 min, compound **8** at 8.4 min, 5-mer oligonucleotide TTSPT at 8.8 min, dHdU-H_2O (**9**) at 5.51 min and dHdU at 10.2 min. The mass signals were monitored under both positive and negative ion modes respectively.

Preparation of SP TpT



The synthesis of **SP TpT** was achieved using published procedures.[1] The structure of synthesized **(5*R*)-SP TpT** was confirmed by the NMR and the mass spectroscopy. ^{1}H NMR (DMSO-d_6): δ 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.

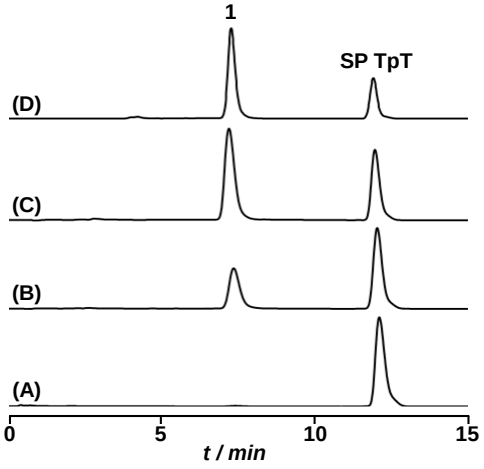


Figure S1. HPLC chromatograph (260 nm) of the SP hydration reaction in 50 mM K_2HPO_4 buffer at pH 11 (A), and in 50 mM (B), 100 mM (C), as well as 200 mM KOH (D) for 2-4 days at ambient temperature. The yield of **1** was only improved by 2 ~ 4% in 400 mM or 800 mM KOH; the chromatographs for these two reactions are not shown.

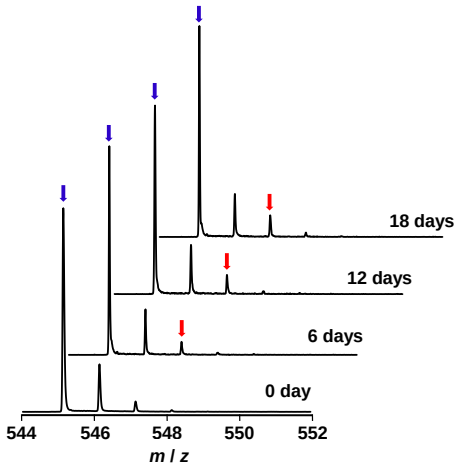


Figure S2. ESI-MS analyses of the ^{18}O incorporation into SP TpT at pH 7.4 and 37 $^{\circ}C$. The peaks pointed by blue arrows exhibit a mass of 545.1, corresponding to SP without any ^{18}O incorporation. The peaks pointed by red arrows exhibit a mass of 547.1, corresponding to the SP with one ^{18}O incorporated which overlaps with the SP n + 2 isotopic peak.

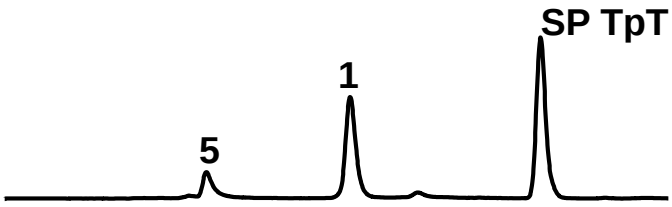


Figure S3. HPLC chromatograph (260 nm) of the SP hydration reaction in 200 mM KOH for 1 hour at 90 ºC. The formation of **5** from the decomposition of **1** is clearly observed.

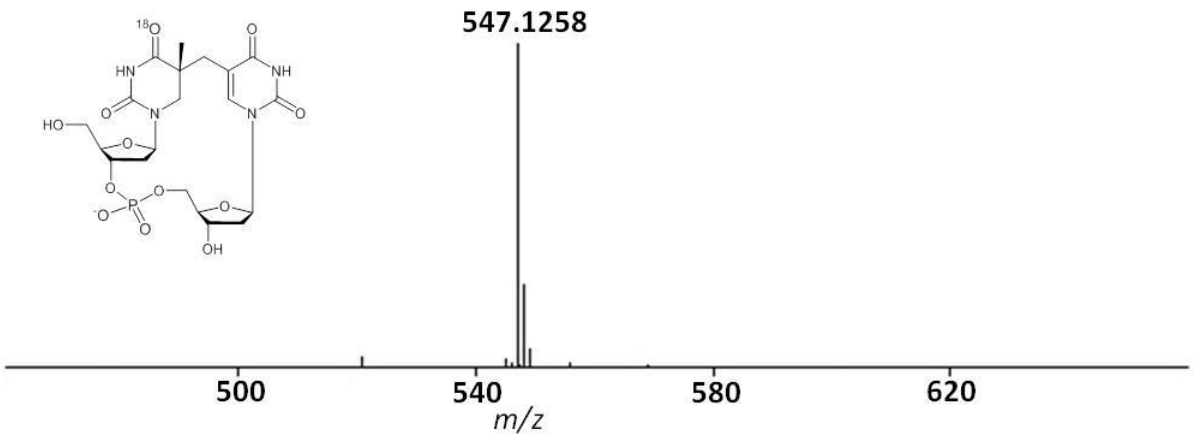


Figure S4. MS analysis of ^{18}O labeled SP.

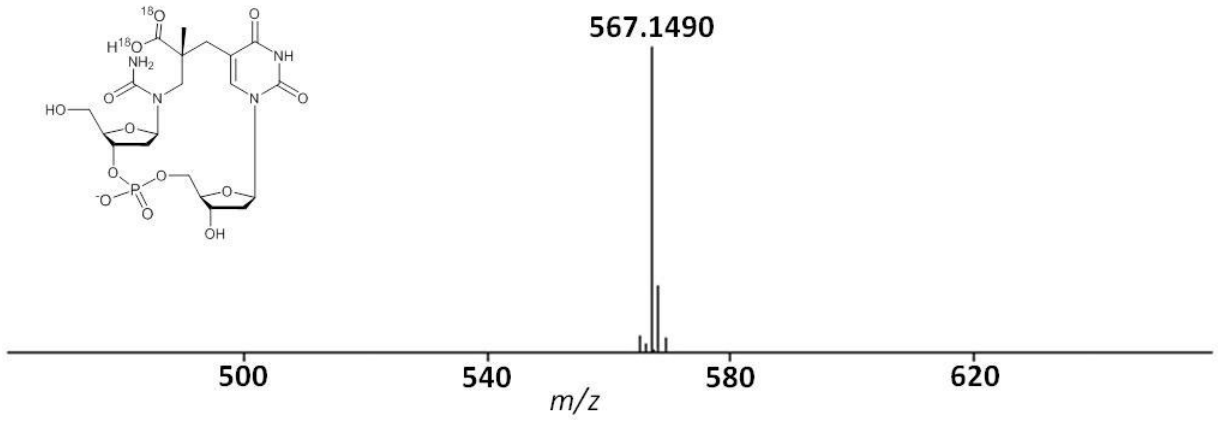
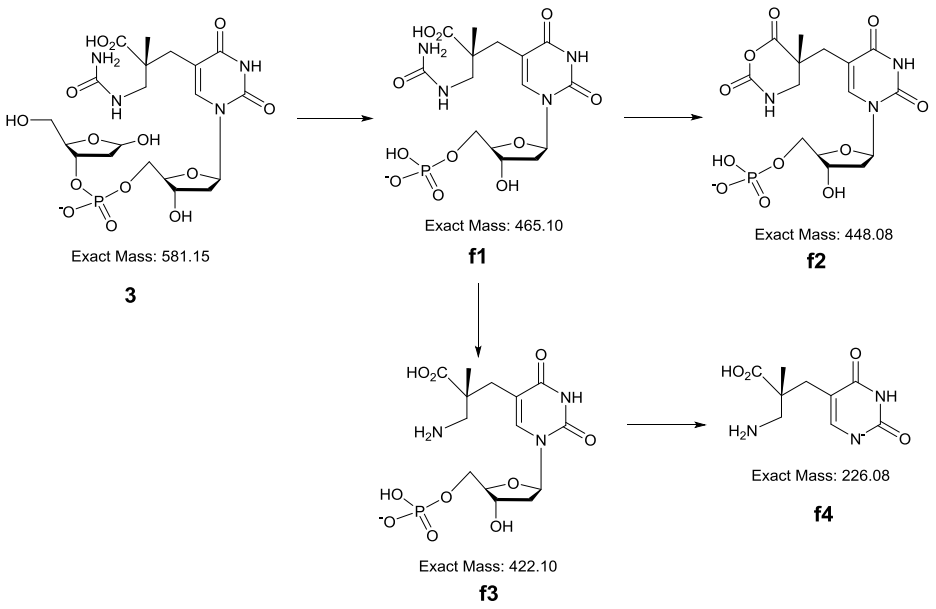
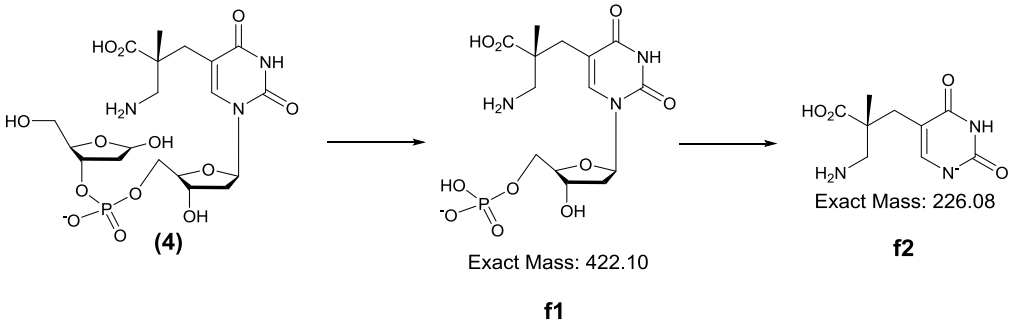


Figure S5. MS analysis of double-^{18}O labeled compound **1**.

(A)



(B)



(C)

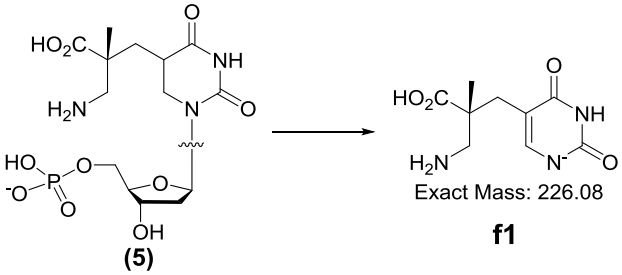


Figure S6. Possible structures of the fragments generated from the MS/MS analyses of the decomposition products **3** (A), **4** (B), and **5** (C) respectively.

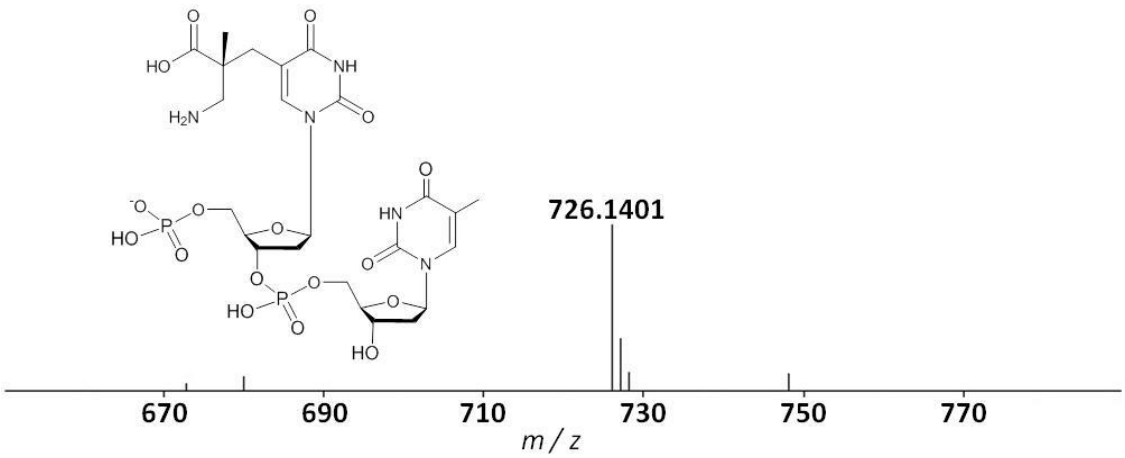


Figure S7. MS analysis of the TTSPT decomposition product **6**.

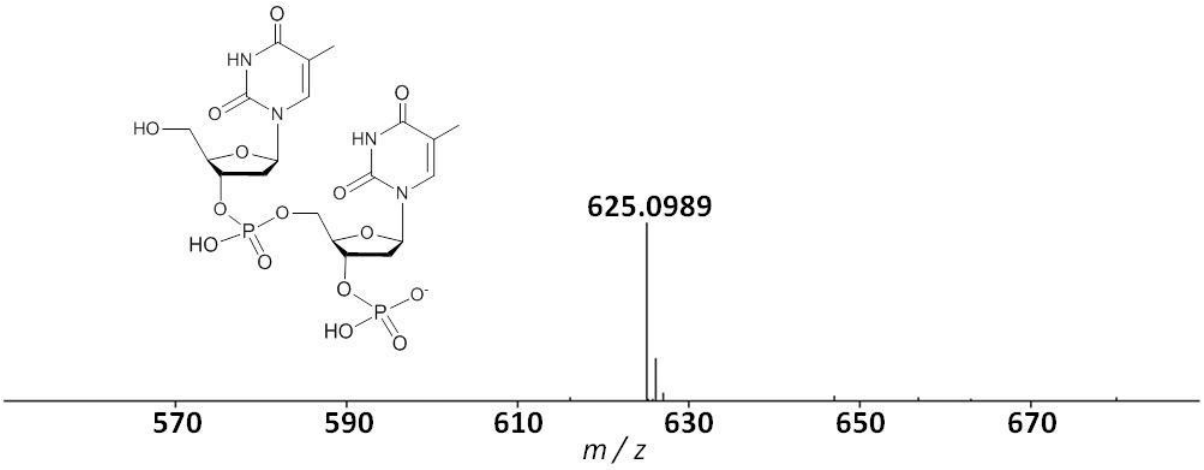


Figure S8. MS analysis of the TTSPT decomposition product **7**.

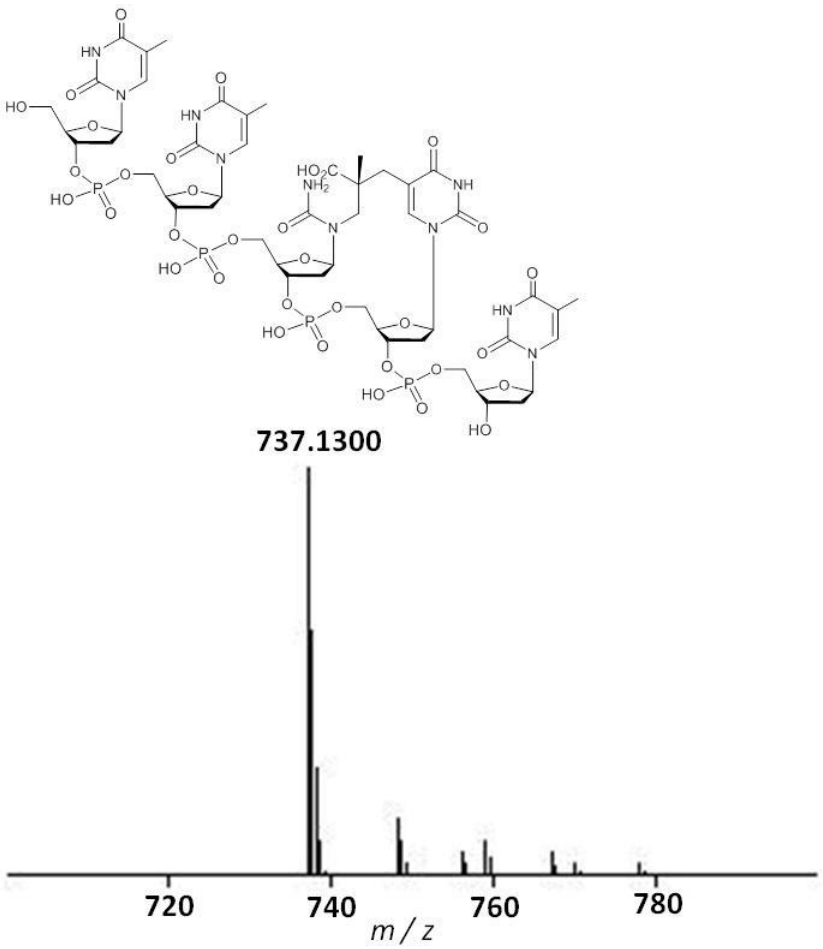
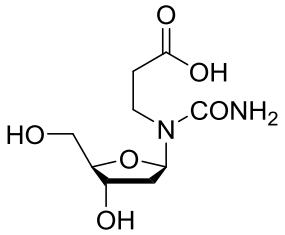


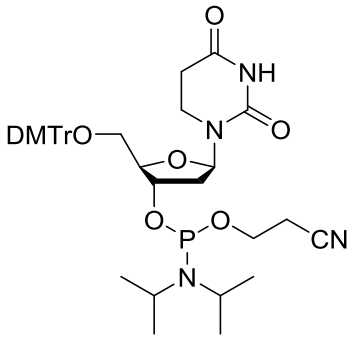
Figure S9. ESI-MS analysis of the TTSPT hydrate product **8**.

dHdU water addition product



20 mg dHdU was dissolved in 0.2 M KOD/D_2O solution (0.5 mL). The mixture was set at room temperature for 2 h to fully convert the dHdU to its water addition product and then directly used for NMR analysis. ^{1}H NMR (D_2O): δ 2.00 (ddd, J = 2.6, 5.8, 13.9 Hz, 1H), 2.22 (ddd, J = 6.8, 9.1, 13.9 Hz, 1H), 2.40-2.53 (m, 2H), 3.40-3.52 (m, 2H), 3.65 (dd, J = 5.5, 12.1 Hz, 1H), 3.71 (dd, J = 4.3, 12.1 Hz, 1H), 3.79-3.84 (m, 1H), 4.28-4.34 (m, 1H), 5.99 (dd, J = 5.8, 9.1 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 36.3, 37.7, 39.7, 61.9, 71.1, 84.8, 86.0, 160.7, 180.3; HRMS (M^-) calcd for $C_9H_{15}N_2O_6^-$: 247.0936 (M^-), found.

dHdU phosphoramidite



The product was isolated as a mixture of isomers. ^{1}H NMR (MeOD): δ 1.06 (s, 3H), 1.07 (s, 3 H), 1.15 (s, 6H), 1.16 (s, 6H), 1.17 (s, 3H), 1.18 (s, 3H), 2.10-2.18 (m, 1H), 2.19-2.32 (m, 3H), 2.37-2.52 (m, 6H), 2.64-2.68 (m, 2H), 3.23-3.30 (m, 2H), 3.32-3.40 (m, 4H), 3.46-3.74 (m, 9H), 3.73 (s, 12H), 3.75-3.85 (m, 1H), 3.93-3.98 (m, 1H), 3.99-4.03 (m, 1H), 4.54-4.64 (m, 2H), 6.24-6.30 (m, 2H), 6.79-6.83 (m, 8H), 7.15-7.20 (m, 2H), 7.22-7.34 (m, 12H), 7.39-7.45 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 20.8, 20.9, 25.0, 25.1, 31.8, 37.0, 37.3, 44.3, 44.4, 55.7, 59.6, 64.3, 74.3, 74.6, 85.0, 85.1, 85.3, 87.6, 114.2, 119.4, 119.6, 128.0, 128.8, 129.4, 131.3, 136.9, 137.0, 146.2, 154.6, 160.1, 172.5; HRMS (M^+) calcd for $C_{39}H_{50}N_4O_8P^+$: 733.3361 (M^-), found 733.3350.

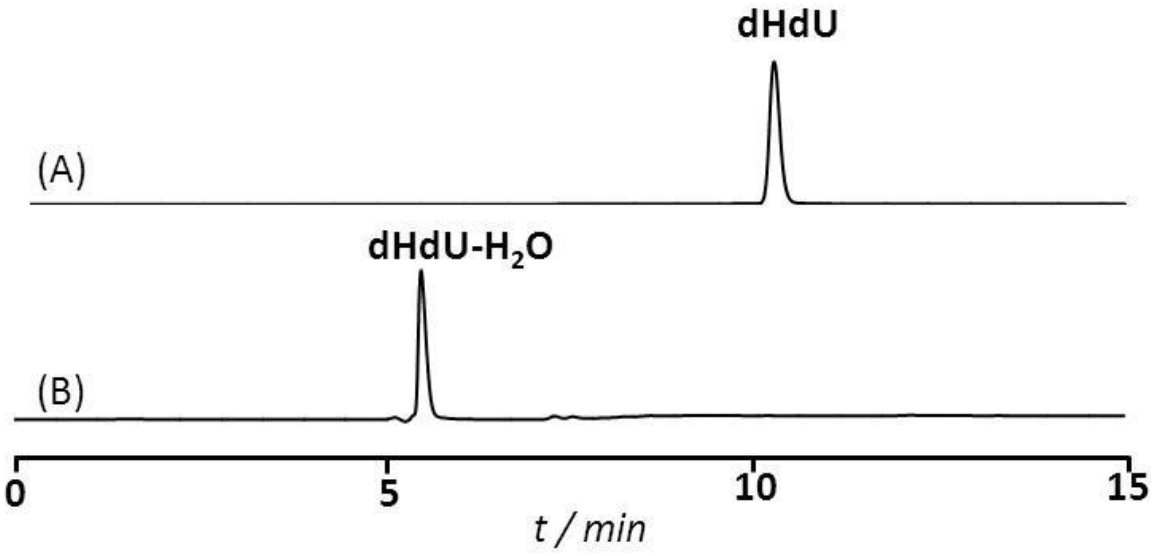
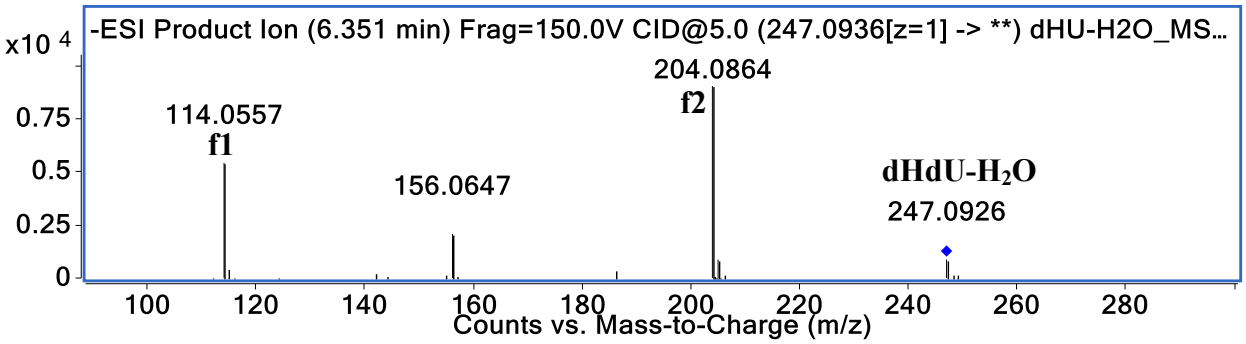
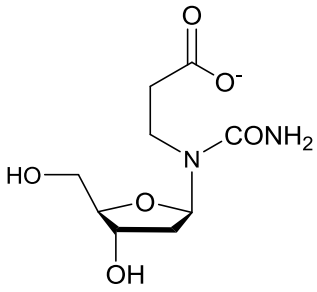


Figure S10. HPLC chromatograph (230 nm) of the dHdU hydration reaction in 0.2 M KOH at room temperature for 0 min (A), and for 30 min (B).



Structure of dHdU H_2O-addition product

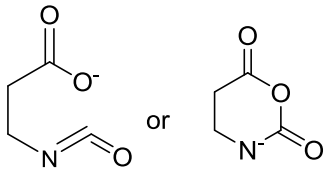


dHdU-H_2O

Chemical Formula: $C_9H_{15}N_2O_6^-$

Exact Mass: 247.0936

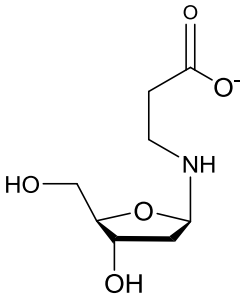
Fragment structure (negative mode)



f1

Chemical Formula: $C_4H_4NO_3^-$

Exact Mass: 114.0197

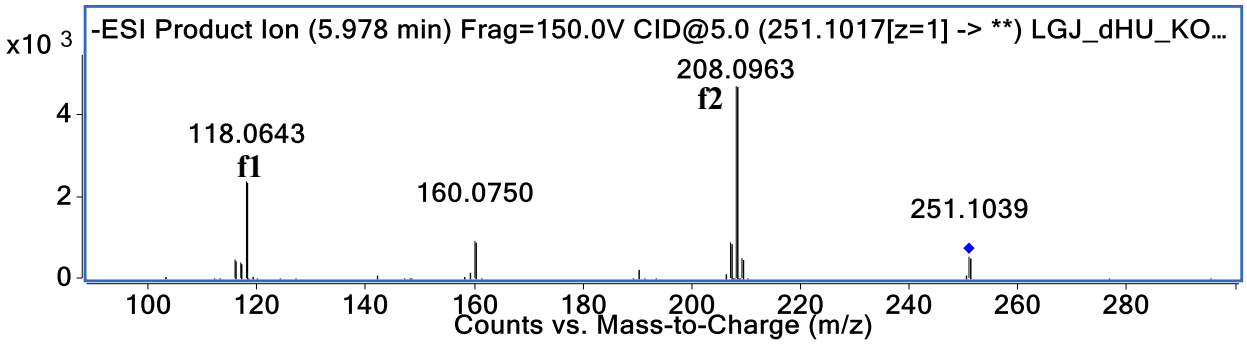


f2

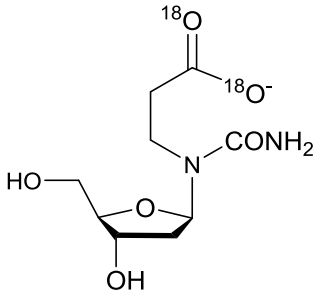
Chemical Formula: $C_8H_{14}NO_5^-$

Exact Mass: 204.0877

Figure S11. MS-MS analysis of the dHdU H_2O adduct **9**.

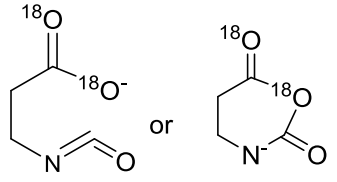


Structure of doubly- O^{18} labeled dHdU water adduct (9)



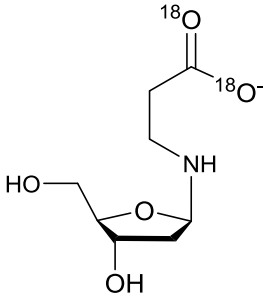
Chemical Formula: $C_9H_{15}N_2O_4{}^{18}O_2{}^-$
Exact Mass: 251.10

Fragment structure (negative mode)



f1

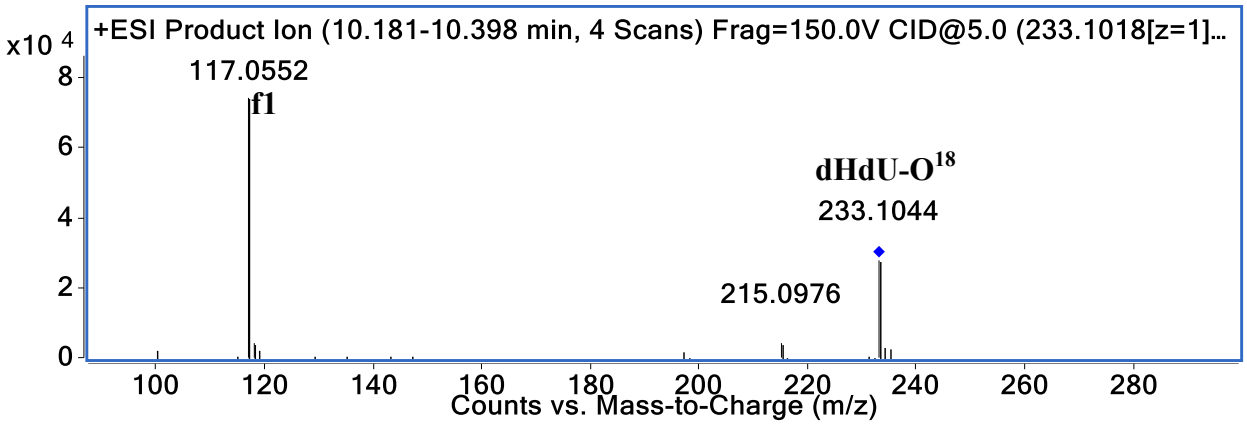
Chemical Formula: $C_4H_4NO{}^{18}O_2{}^-$
Exact Mass: 118.03



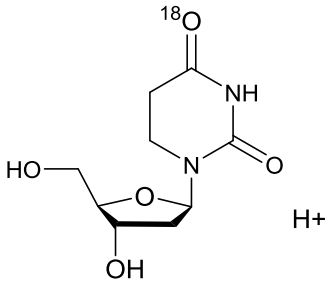
f2

Chemical Formula: $C_8H_{14}NO_3{}^{18}O_2{}^-$
Exact Mass: 208.10

Figure S12. MS-MS analysis of doubly-O^{18}-labeled dHdU water adduct **9**.



Structure of O^{18} exchanged dHdU addition product

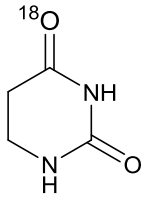


dHdU-O^{18}

Chemical Formula: $C_9H_{15}N_2O_4{}^{18}O^+$

Exact Mass: 233.1018

Fragment structure (positive mode)



f1

Chemical Formula: $C_4H_7N_2O^{18}O^+$

Exact Mass: 117.0544

Figure S13. MS-MS analysis of O^{18} exchanged dHdU.

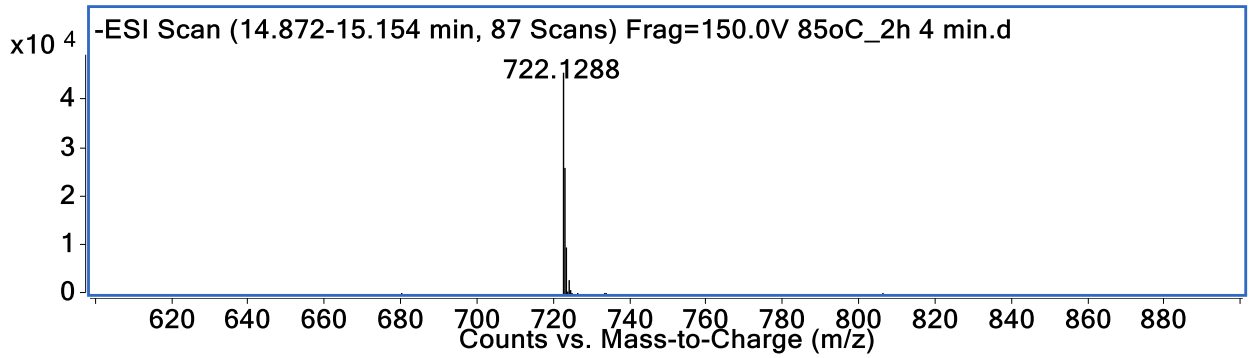


Figure S14. MS analysis of the **TTdHdUTT**.

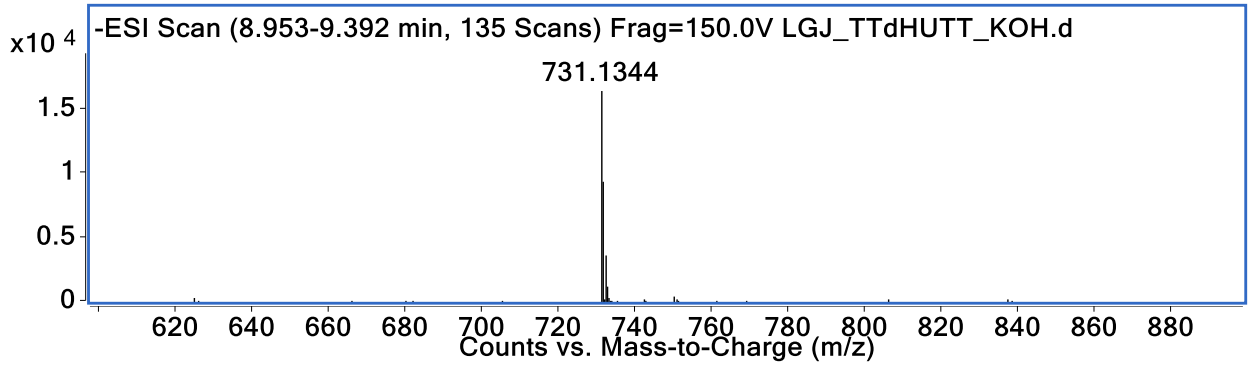


Figure S15. MS analysis of the **TT(9)TT**.

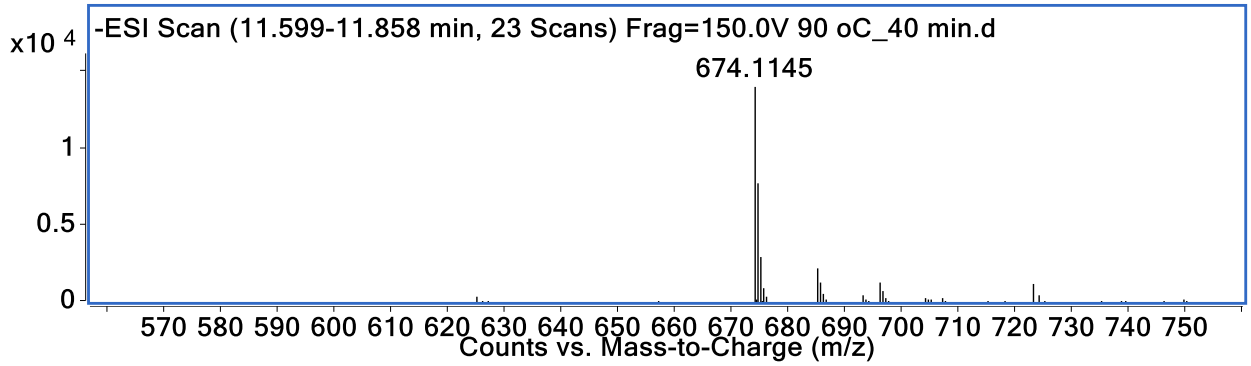


Figure S16. MS analysis of the **TT(9)TT** decomposition product **10**.

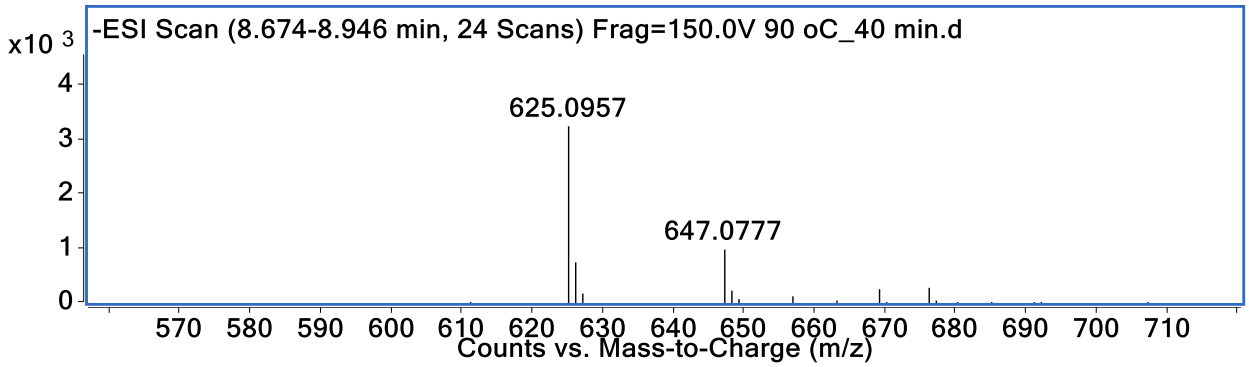


Figure S17. MS analysis of the **TT(9)TT** decomposition product **11**.

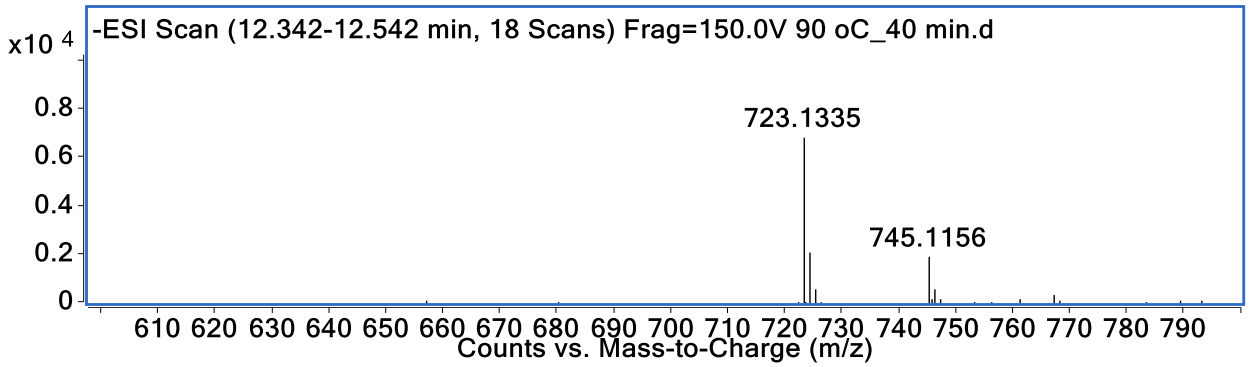


Figure S18. MS analysis of the **TT(9)TT** decomposition product **12**.

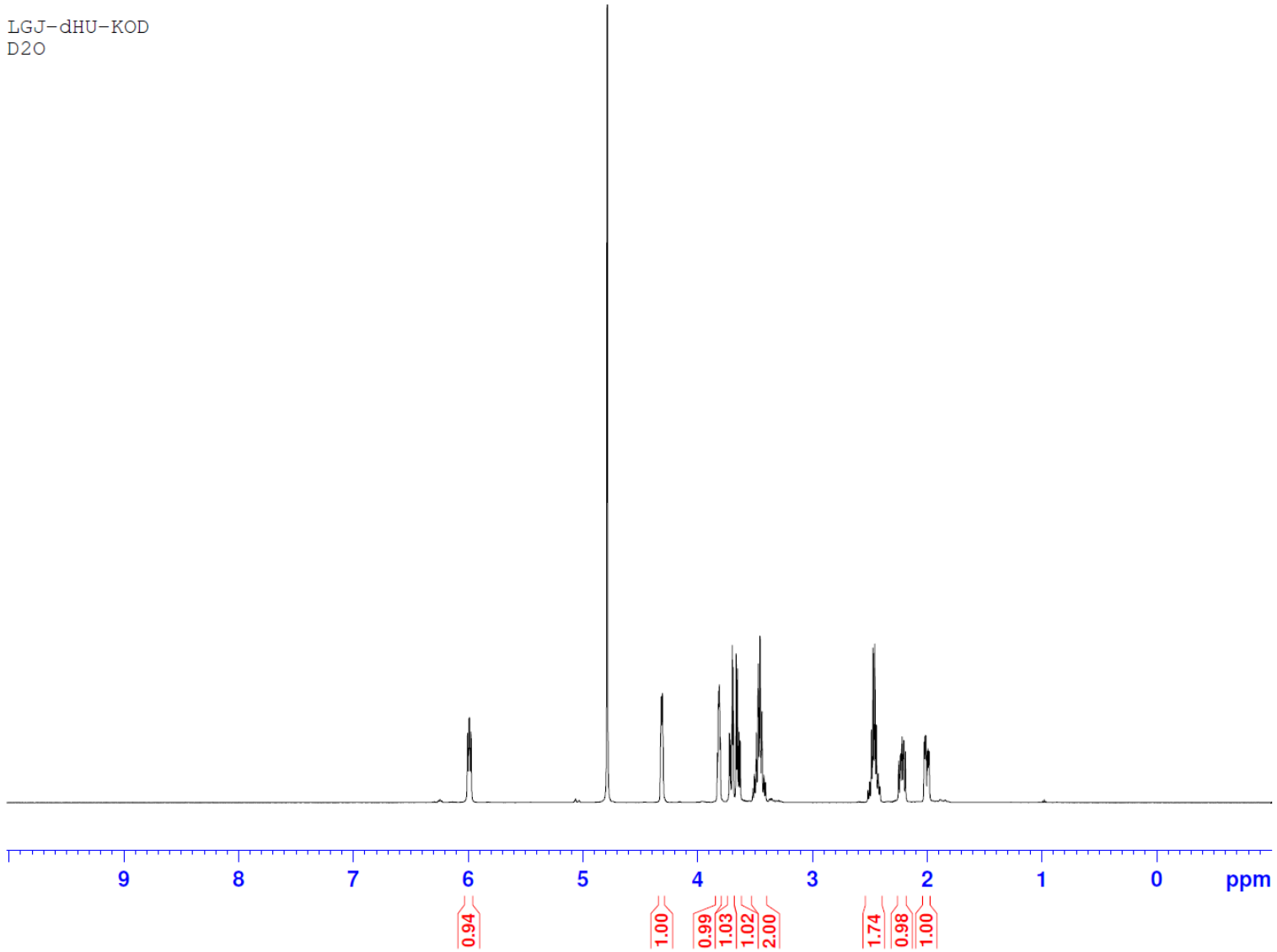


Figure S19. ^{1}H NMR spectrum of dHdU water adduct **9** in D_2O.

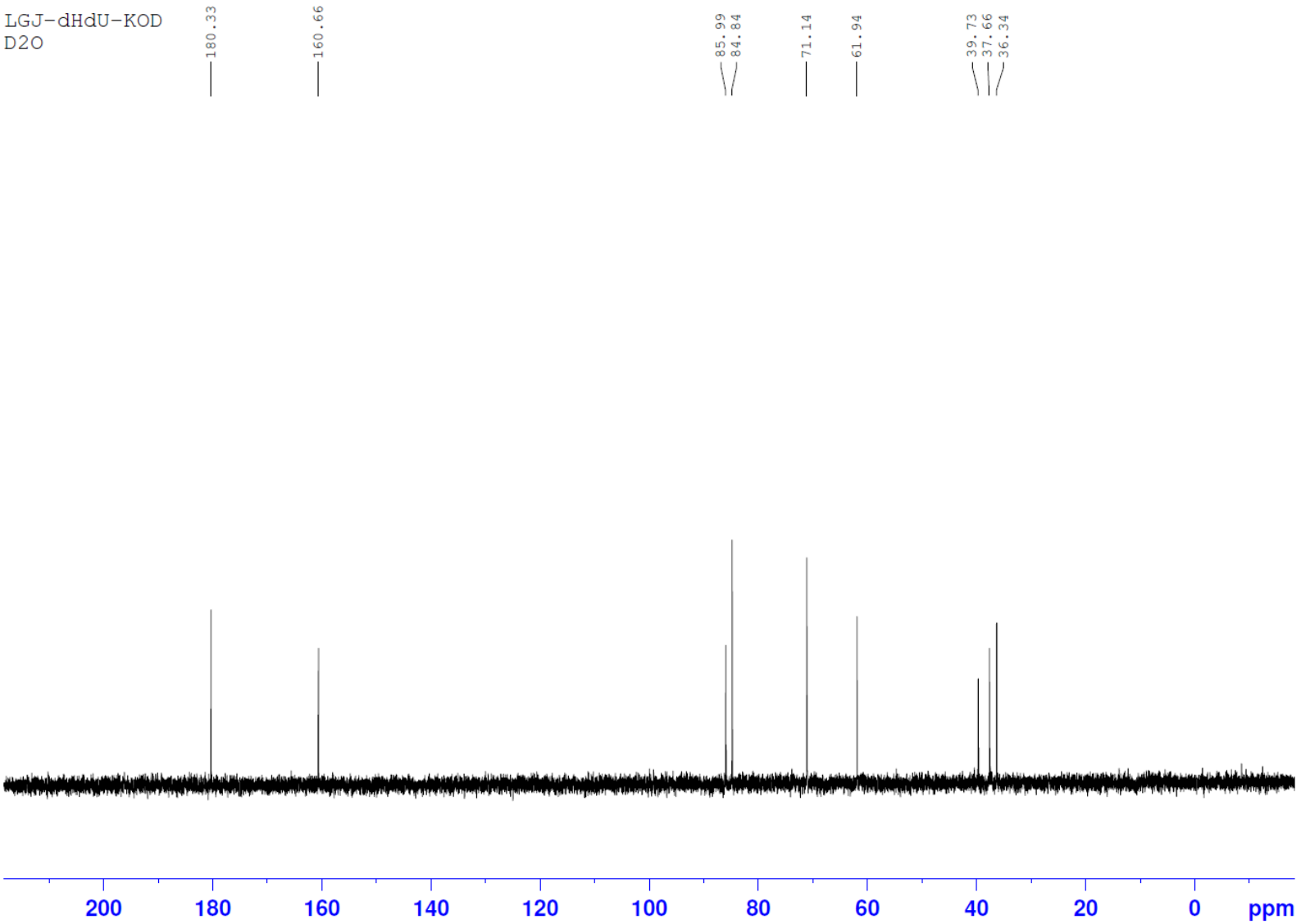


Figure S20. ^{13}C NMR spectrum of dHdU water adduct **9** in D_2O.

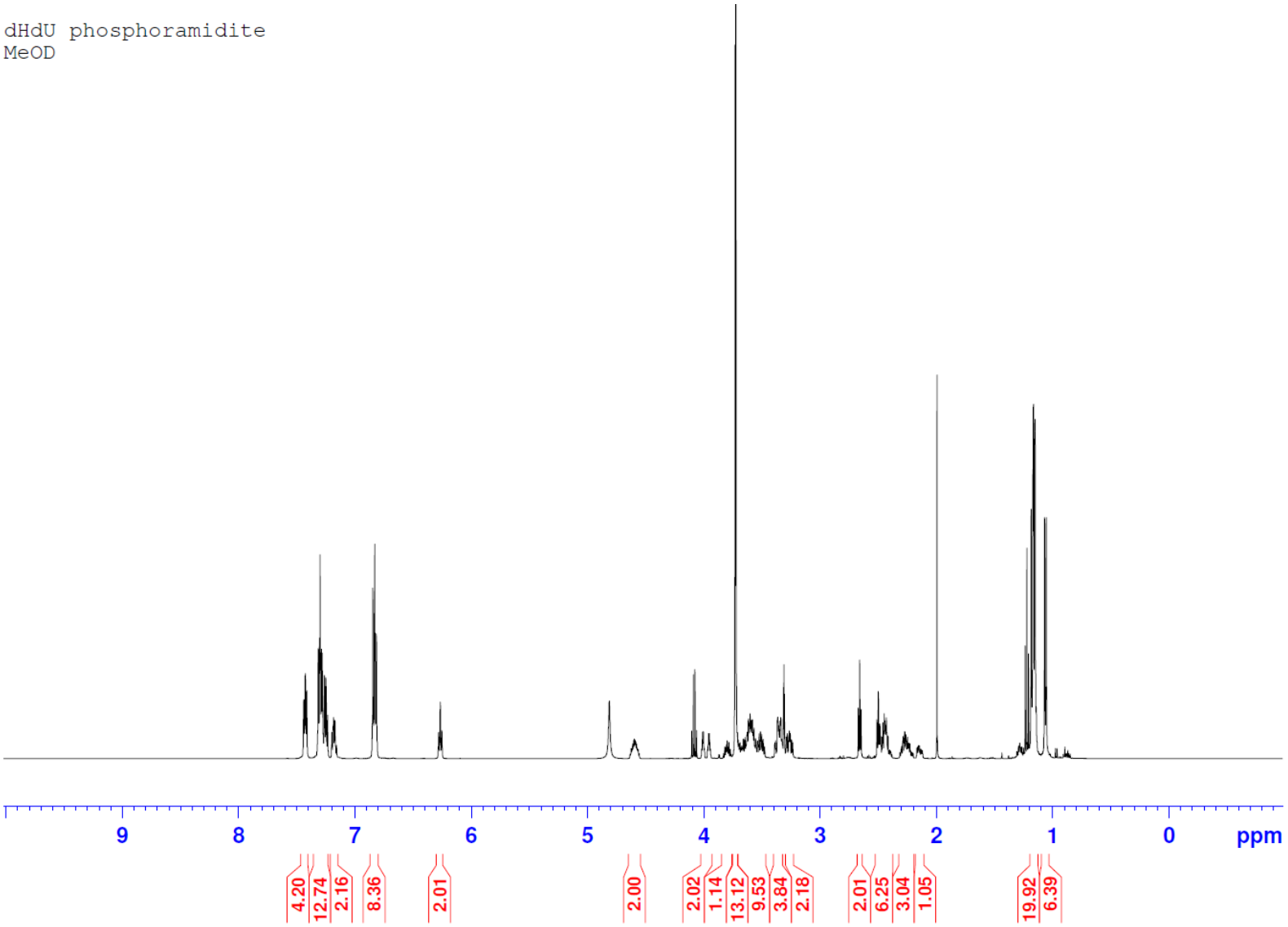


Figure S21. ^{1}H NMR spectrum of dHdU phosphoramidite in CD_3OD.

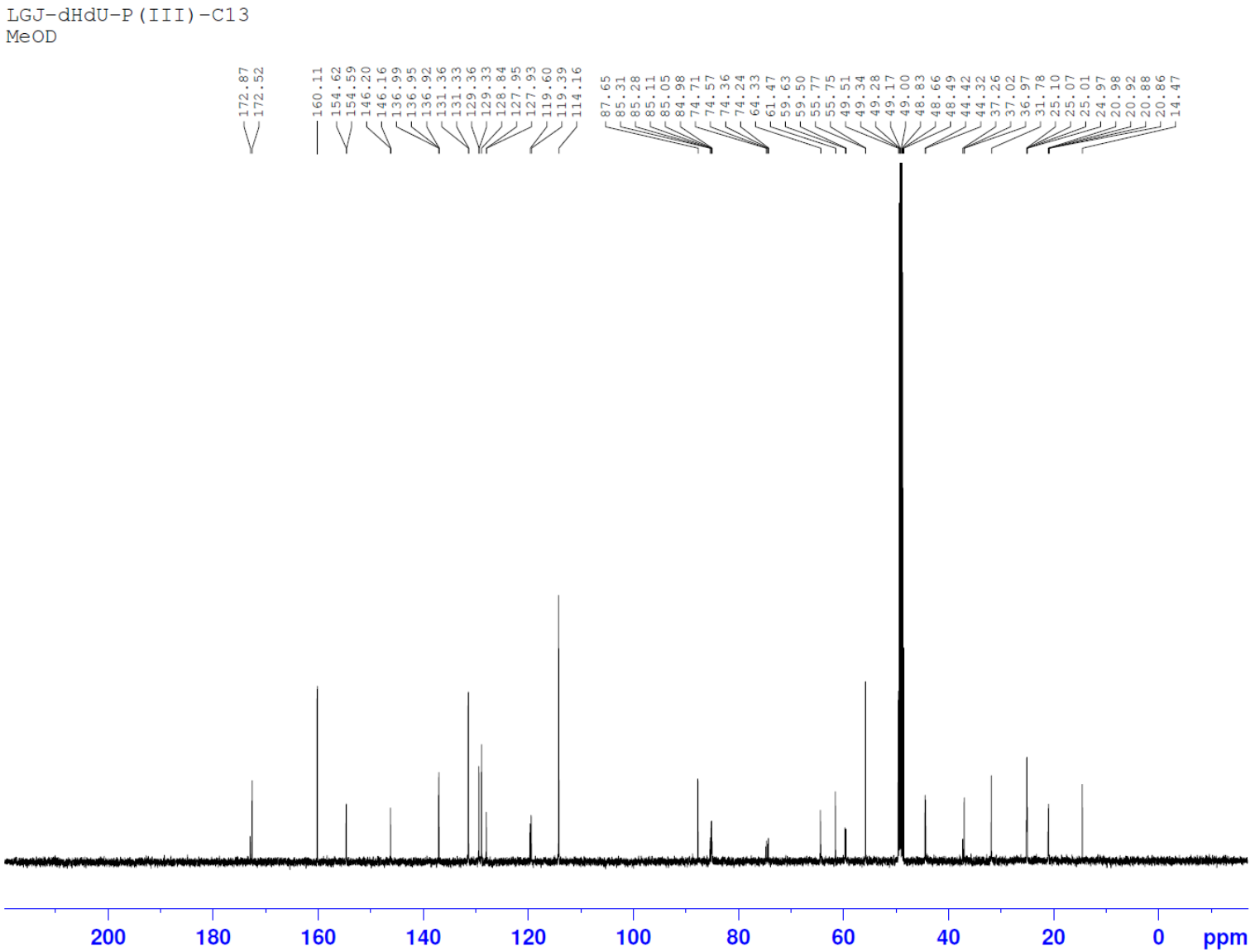


Figure S22. ^{13}C NMR spectrum of dHdU phosphoramidite in CD_3OD.

References

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