Supporting information

for

Oxidation and reduction of 5-(2'-Deoxyuridinyl)methyl radical

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Abbreviations used

DMTr, 4,4′-dimethoxytrityl;TFA, trifluoroacetic acid; TLC, thin layer chromatography;$CDCl_3$, choloroform-d_3; CD_3OD, methanol-d_4;Abbreviations for NMR signal coupling are as follows: s, singlet; d, doublet; m, multiplet.MS: mass spectroscopy; MS/MS: tandem mass spectrometry, ESI: electrospray ionization.

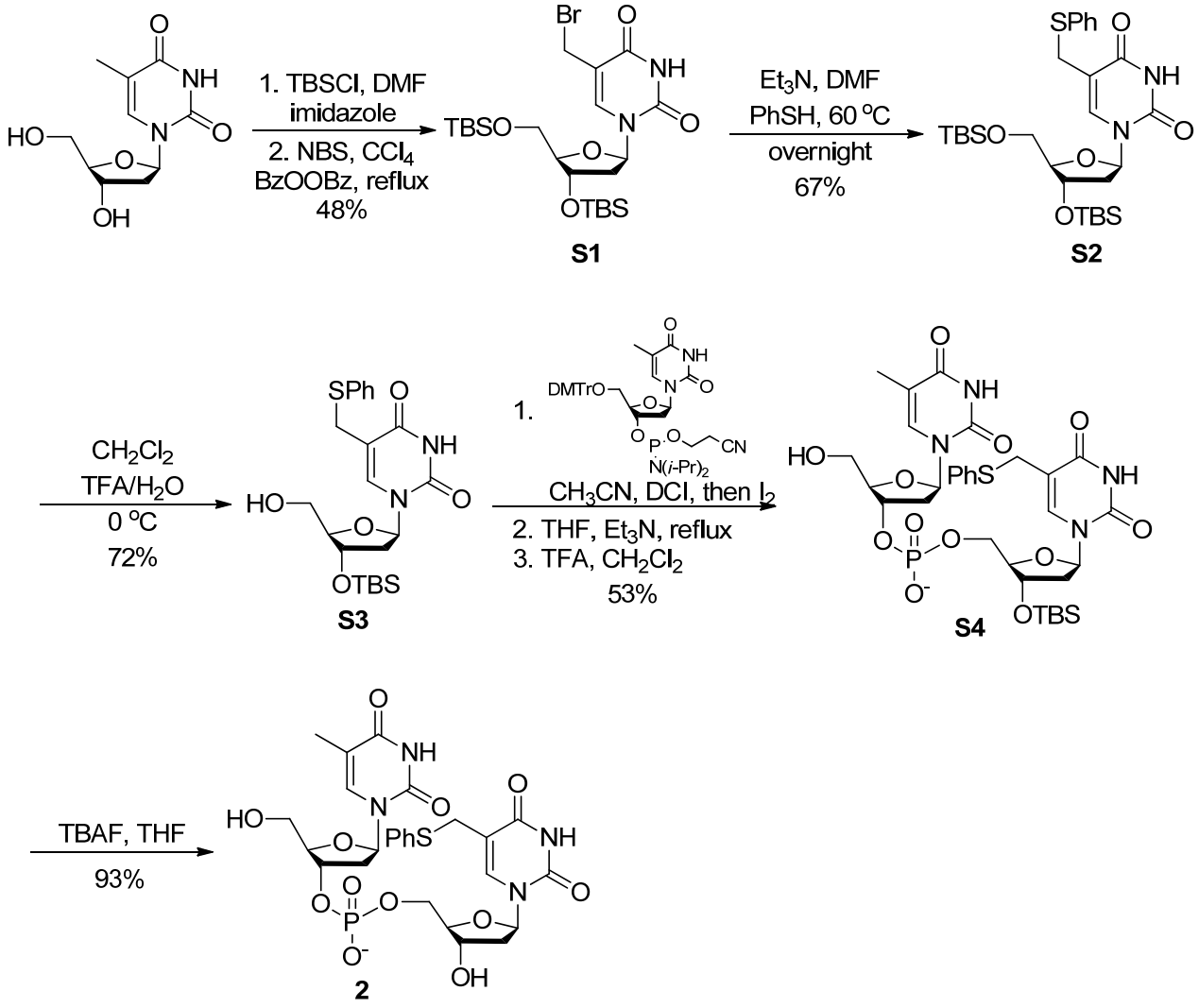
General Methods

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. 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 (δ 77.2 ppm for ^{13}C NMR) and TMS (δ 0 ppm for ^1H NMR) ordeuteratedmethanol (δ 3.31 ppm for ^1H NMR and δ 49.1 ppm for ^{13}C NMR) taken as the standard. The chemical shifts in NMR spectra were reported in parts per million (ppm).The TpTSPh photoreaction was carried out using a Spectroline germicidal UV sterilizing lamp (Dual-tube, 15w, intensity: 1550μwcm^{-2}) with the samples ~25 cm to the lamp.

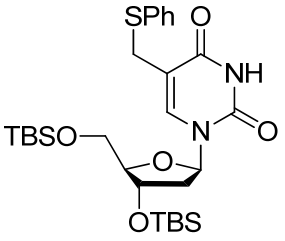
i. Synthesis of TpTSPh

The bromomethylthymidine**S1**[1] wasreacted with PhSH in DMF to afford compound **S2**. After selective deprotection, compound **S3** was then coupled to thymidinevia the traditional phosphoramidite chemistry. Subsequent de-protection afforded the TpTSPh (**2**).

Scheme S1



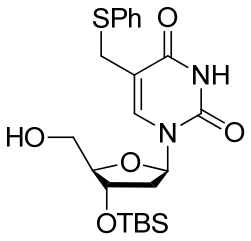
Synthesis of compound S2.



S2

A solution containing **S1**[1] (3.50 g, 6.36 mmol), TEA (1.5 mL) and PhSH (0.78 mL, 7.63 mmol) in anhydrous DMF (15 mL) was stirred at 70 °C for one day. The reaction was quenched by water (75 mL). The mixture was extracted with Et_2O (5 × 50 mL). The collected extracts were washed with brine (20 mL) and dried over anhydrous sodium sulfate. After filtration and concentration under vacuum, the residue was purified by flash chromatography (n-Hexane/EtOAc = 1:2) to afford compound **S2** as a yellow oil (2.50 g, 67%). ^{1}H NMR ($CDCl_3$): δ0.06 (s, 3H), 0.07 (s, 3H), 0.08 (s, 6H), 0.88 (s, 9H), 0.90 (s, 9H), 1.73-1.81 (m, 1H), 2.19 (ddd, J = 2.6, 5.9, 13.3 Hz, 1H), 3.63-3.69 (m, 2H), 3.78 (d, J = 14.2 Hz, 1H), 3.83 (d, J = 14.2 Hz, 1H), 3.83-3.88 (m, 1H), 4.26-4.34 (m, 1H), 6.25 (dd, J = 6.1, 7.6 Hz, 1H), 7.18-7.25 (m, 1H), 7.25-7.31 (m, 2H), 7.31 (s, 1H), 7.32-7.38 (m, 2H), 10.0 (s, 1H); ^{13}C NMR ($CDCl_3$): δ-5.4, -5.3, -4.8, -4.6, 18.0, 18.4, 25.8, 25.9, 30.9, 40.9, 62.9, 72.1, 84.9, 87.6, 110.9, 126.9, 129.0, 131.2, 135.3, 137.2, 150.3, 162.9; ESI-MS (positive mode) calcd for $C_{28}H_{47}N_2O_5SSi_2^+$: 579.3 ($M + H^+$), found 579.3.

Synthesis of compound S3.

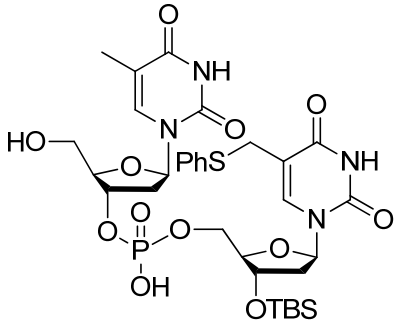


S3

The bis-TBS-protected thymidine **S2** (3.10 g, 5.35 mmol) was dissolved in CH_2Cl_2 (40 mL) and cooled to 0 °C. A TFA/water mixture (4 mL, 10:1) was added dropwise. The reaction mixture was stirred for 4 h at 0 °C. The solution was diluted with CH_2Cl_2 (100 mL), washed with ice-cold water (2 × 50 mL) and saturated aqueous sodium chloride (10 mL) and dried over anhydrous sodium sulfate. After filtration and concentration under vacuum, the residue was purified by flash chromatography (n-

Hexane/EtOAc/MeOH = 1:1:0.2) to afford compound **S3** as a colorless oil (1.79 g, 72%).^{1}H NMR ($CDCl_3$): δ0.05 (s, 6H), 0.87 (s, 9H), 1.94-2.01 (m, 1H), 2.12 (ddd, J = 3.8, 6.4, 13.5 Hz, 1H), 2.75 (t, J = 5.2 Hz, 1H), 3.62 (ddd, J = 3.4, 5.2, 11.9 Hz, 1H), 3.72-3.80 (m, 1H), 3.76 (d, J = 14.9 Hz, 1H), 3.83 (d, J = 14.9 Hz, 1H), 3.83-3.87 (m, 1H), 4.31-4.36 (m, 1H), 6.14 (t, J = 6.7 Hz, 1H), 7.16-7.23 (m, 1H), 7.24-7.29 (m, 2H), 7.29-7.34 (m, 2H), 7.42 (s, 1H), 9.96 (s, 1H); ^{13}C NMR ($CDCl_3$): δ-4.9, -4.8, 17.9, 25.7, 30.4, 40.7, 61.9, 71.5, 85.9, 87.5, 110.8, 126.8, 129.0, 130.7, 135.2, 138.2, 150.3, 163.0; ESI-MS (positive mode) calcd for $C_{22}H_{33}N_2O_5SSi^+$: 465.2 ($M + H^+$), found 465.2.

Synthesis of compound S4.

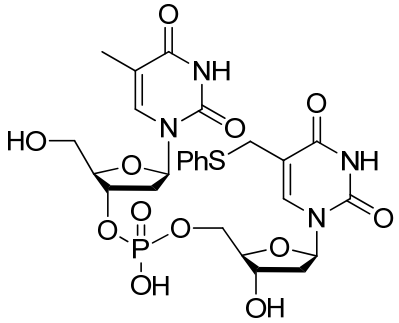


S4

Compound **S3** (561 mg, 1.21 mmol) and dT-phosphoramidite monomer (1.00 g, 1.34 mmol) were dissolved in ACN (8.5 mL) and put under an argon atmosphere. 4,5-Dicyanoimidazole (474 mg, 4.02 mmol) was added and the mixture was stirred for 30 min. Water (0.31 mL) was added, and the mixture was stirred for 2 min. A solution of I_2 (0.2 M, in THF/Py 4:1, 13.5 mL) was added, and the mixture was stirred for 30 min. The reaction mixture was diluted with EtOAc(50 mL), washed with aq. saturated $Na_2S_2O_3$/$NaHCO_3$ solution (5 mL). The organic layer was dried over anhydrous sodium sulfate. After filtration and concentration under vacuum, the residue was purified by flash chromatography to afford the coupling intermediate as anoff-white solid. The crude product was dissolved in THF/Et_3N (1:1, 15 mL) and refluxed for overnight. After evaporation of the solvent and dried in vacuum, the residue was dissolved in CH_2Cl_2 (10 mL), TFA (1 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 (CH_2Cl_2/MeOH/Et_3N = 10:1:0.3) yielded **S4** (490mg, 53%) as a white solid.^{1}H NMR ($CDCl_3$): δ0.01 (s, 3H), 0.02 (s, 3H), 0.81 (s, 9H), 1.79-1.87 (m, 1H), 1.80 (s, 3H), 2.02-2.08 (m,1H), 2.17-2.25 (m, 1H), 2.37-2.44 (m, 1H), 3.68-3.75 (m, 1H), 3.78-3.84 (m, 1H), 3.84-4.01 (m, 5H), 4.03-4.10 (m, 1H), 4.28-4.33 (m, 1H), 4.89-4.96 (m, 1H), 6.14 (t, J= 6.5 Hz, 1H), 6.17 (t, J = 6.7 Hz, 1H), 7.08-7.12 (m, 1H), 7.19-7.24 (m, 2H), 7.32-7.37 (m, 2H), 7.49 (s, 1H), 7.59 (s,

1H); ^{13}C NMR ($CDCl_3$): δ-4.8, -4.7, 12.6, 17.9, 25.7, 30.1, 39.1, 40.7, 62.1, 64.9, 72.2, 74.9, 84.9, 85.0, 86.1, 86.4, 110.6, 110.8, 126.4, 128.9, 130.3, 135.6, 136.2, 137.9, 150.5, 150.7, 162.9, 164.4; ESI-MS (positive mode) calcd for $C_{32}H_{46}N_4O_{12}PSSi^+$: 769.2 ($M + H^+$), found 769.2.

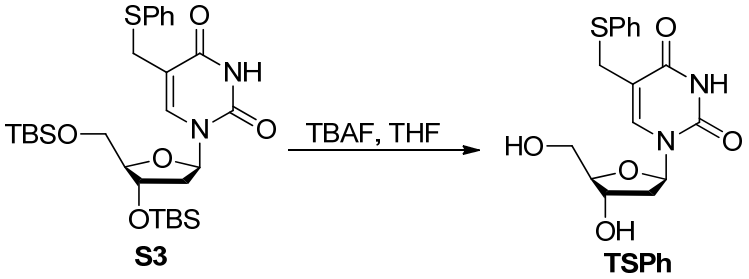
Synthesis of compound S5.



2

To a solution of compound **S4** (210 mg, 0.27 mmol) in THF (5 mL) was added TBAF (1.0 M in THF, 0.75 mL) and stirred for overnight. After the evaporation of the solvent under vacuum, the residue was purified by reverse phase HPLC in the gradient mode using CH_3CN/aqueous ammonium acetate (10 mM)as the solvent. The compound **2** was isolated as a colorless solid (164 mg, 93%).^{1}H NMR (d_4-methanol): δ1.86 (s, 3H), 1.86-1.95 (m, 1H), 2.14 (ddd, J = 4.1, 6.4, 13.6 Hz, 1H), 2.26 (ddd, J = 6.1,7.1, 13.8 Hz, 1H), 2.48 (ddd, J = 2.8, 5.9, 13.8 Hz, 1H), 3.77-3.81 (m, 2H), 3.86 (d, J = 13.6 Hz, 1H), 3.92-3.96 (m, 1H), 3.93 (d, J = 13.6 Hz, 1H), 3.96-4.03 (m, 2H), 4.14-4.17 (m, 1H), 4.27-4.32 (m, 1H), 4.83-4.89 (m, 1H), 6.17 (t, J = 6.6 Hz, 1H), 6.28 (dd, J = 6.0, 7.9 Hz, 1H), 7.22-7.27 (m, 1H), 7.31-7.36 (m, 2H), 7.40-7.44 (m, 2H), 7.49 (s, 1H), 7.82 (s, 1H); ^{13}C NMR (d_4-methanol): δ12.5, 31.6, 40.1, 40.7, 62.8, 66.1, 71.8, 76.8, 85.9, 86.3, 87.0, 87.7, 111.7, 111.9, 128.1, 130.2, 132.9, 136.4, 138.1, 139.2, 151.9, 152.3, 164.8, 166.4; ESI-MS (positive mode) calcd for $C_{26}H_{32}N_4O_{12}PS^+$: 655.1 ($M + H^+$), found 655.1.

ii. Synthesis of TSPh



A solution containing **S3**(800 mg, 1.38 mmol), TBAF (4.14 mmol) in THF (20 mL) was stirred at room temperature for overnight. The solvent was removed under vacuum and the residue was purified by flash chromatography (EtOAc/MeOH= 10:1) to afford **TSPh**as a white solid (440 mg, 92%).[1]H NMR (d_4-methanol): δ1.79 (ddd, $J_1 = J_2 = 6.4$ Hz, $J_3 = 13.6$ Hz, 1H), 2.11 (ddd, $J = 3.5$, 6.2, 13.6 Hz, 1H), 3.60 (dd, $J = 3.8$, 12.0 Hz, 1H), 3.65 (dd, $J = 3.4$, 12.0 Hz, 1H), 3.74 (d, $J = 14.1$ Hz, 1H), 3.82 (d, $J = 14.1$ Hz, 1H), 3.82-3.86 (m, 1H), 4.20-4.24 (m, 1H), 6.18 (t, $J = 6.8$ Hz, 1H), 7.22-7.27 (m, 1H), 7.29-7.33 (m, 2H), 7.34-7.38 (m, 2H), 7.56 (s, 1H);[13]C NMR (d_4-methanol): δ31.8, 41.2, 62.8, 72.1, 86.1, 88.7, 111.8, 128.2, 130.2, 132.9, 136.3, 139.2, 151.9, 164.8; ESI-MS (positive mode) calcd for $C_{16}H_{19}N_2O_5S^+$: 351.1($M + H^+$), found 351.1.

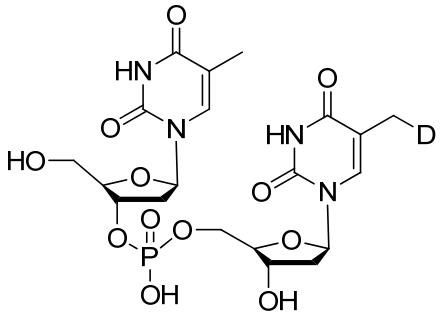
iii. Photoreactions of TpTSPh

Photoreactions of TpTSPh under anaerobic condition. TpTSPhwas dissolved in degassed H_2O to a final concentration of 1.0mM.40μL of the resulting solution was transferred to a small tube in a Coy anaerobic chamber. After addition of the trapping or reducing reagent (PhSH, DTT, 4-hydroxythiophenol, or $Na_2S_2O_4$), the resulting solution was diluted with water to a total volume of 200 μL. The solution wasthen irradiated for 5 minutes under the 254 nm UV light using a Spectroline germicidal UV sterilizing lamp (Dual-tube, 15w, intensity: 1550μw cm^{-2}) with the samples ~25 cm to the lamp. The resulting reaction mixture wasfurther diluted with 400μL H_2O and used directly for LC-MS analysis (100 μL for each injection).

Photoreaction of TpTSPh in the air. TpTSPh was dissolved in H_2O to a final concentration of 0.2 mM.200μL of this solution was irradiated for 5 minutes in the air at the 254 nm using a Spectroline germicidal UV sterilizing lamp (Dual-tube, 15w, intensity: 1550μw cm^{-2}) with the samples ~25 cm to the lamp. The resulting reaction mixture was diluted with 400μL H_2O and used directly for LC-MS analysis.

Photoreaction of TpTSPh in CH_3OD. The reaction was conducted under the procedure described above except using CH_3OD as solvent. The solvent was removed after the reaction and resulting compounds were re-dissolvedin H_2O for HPLC analysis.

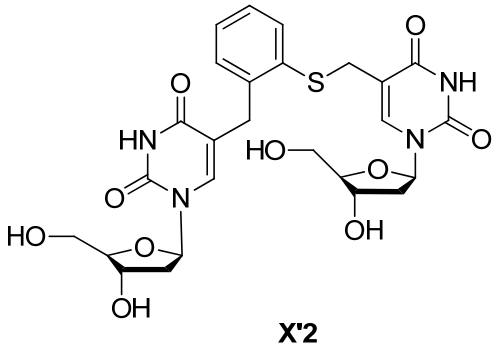
iv. Photoreaction of 2 in D_2O.



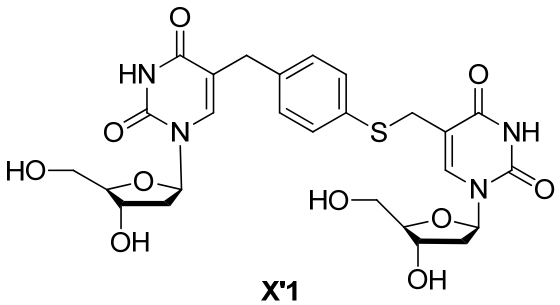
***d*$_1$-TpT**

A degassed D_2O solution of TpTSPh (10 mM, 50 mL) was irradiated under anaerobic condition for 10 minutes. The resulting reaction mixtures were filtered and then purified by reverse phase HPLC in the gradient mode using CH_3CN/aqueous ammonium acetate (10 mM) as solvent. The collected d_1-TpT was desalted by cation ion exchange resin and dried as a colorless solid (14 mg, 5.1%). ^{1}H NMR (d_4-methanol): δ1.90 (s, 3H), 1.91 (brs, 2H), 2.25-2.31 (m, 2H), 2.31-2.39 (m, 1H), 2.52 (dd, J = 5.7, 14.0 Hz, 1H), 3.77-3.84 (m, 2H), 4.03-4.09 (m, 1H), 4.17-4.29 (m, 3H), 4.43-4.51 (m, 1H), 4.98-5.06 (m, 1H), 6.27-6.34 (m, 2H), 7.65 (s, 1H), 7.83 (s, 1H). ESI-MS (negative mode) calcd for $C_{20}H_{26}DN_4O_{12}P^-$: 546.2, found 546.2.

v. Preparation of addition-elimination products X'1 and X'2.



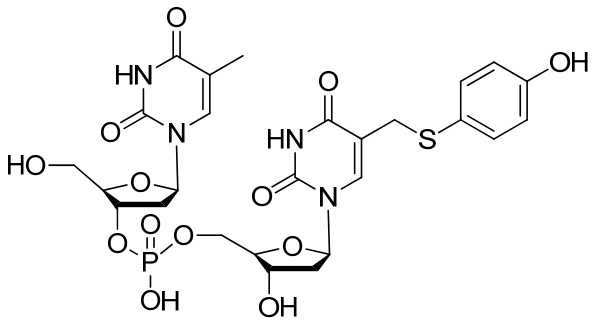
X'2



X'1

A degassed H_2O solution of TSPh (~2mM, 200 mL) was irradiated under anaerobic condition for 10 minutes. The resulting reaction mixtures were filtered and then purified by reverse phase HPLC in the gradient mode using CH_3CN/H_2O as solvent.Product **X'2** was collected and dried as a white solid (19 mg, 8%).^{1}H NMR (D_2O): δ1.72 (ddd, $J_1 = J_2 = 6.5$ Hz, $J_3 = 14.2$ Hz, 1H), 2.16 (ddd, $J_1 = J_2 = 6.5$ Hz, $J_3 = 14.2$ Hz, 1H), 2.22 (ddd, $J = 4.5, 6.5, 14.2$ Hz, 1H), 2.36 (ddd, $J = 4.2, 6.5, 14.2$ Hz, 1H), 3.46 (dd, $J = 4.9, 12.2$ Hz, 1H), 3.53-3.59 (m, 2H), 3.61 (d, $J = 15.9$ Hz, 1H), 3.65 (dd, $J = 3.7, 12.2$ Hz, 1H), 3.68 (d, $J = 15.9$ Hz, 1H), 3.74 (d, $J = 14.2$ Hz, 1H), 3.80 (d, $J = 14.2$ Hz, 1H), 3.88-3.92 (m, 1H), 3.93-3.97 (m, 1H), 4.12-4.17 (m, 1H), 4.27-4.32 (m, 1H), 6.13 (t, $J = 6.6$ Hz, 1H), 6.20 (t, $J = 6.6$ Hz, 1H), 7.16 (s, 1H), 7.19 (s, 1H), 7.31-7.37 (m, 3H), 7.45-7.50 (m, 1H);^{13}C NMR (D_2O): δ30.5, 30.8, 38.7, 38.8, 60.8, 61.1, 69.9, 70.5, 84.4, 85.1, 86.3, 86.4, 110.5, 114.0, 127.9, 128.4, 130.7, 132.6, 134.0, 137.7, 138.0, 140.5, 150.9, 151.1, 164.1, 164.9; ESI-MS (positive mode) calcd for $C_{26}H_{31}N_4O_{10}S^+$: 591.2 ($M + H^+$), found 591.2;Product **X'1** was collected and dried as a white solid (13 mg, 5.4%).^{1}H NMR (d_4-methanol): δ1.76 (ddd, $J_1 = J_2 = 6.7$ Hz, $J_3 = 13.6$ Hz, 1H), 2.10 (ddd, $J = 3.6, 6.2, 13.6$ Hz, 1H), 2.20 (ddd, $J_1 = J_2 = 6.7$ Hz, $J_3 = 13.6$ Hz, 1H), 2.28 (ddd, $J = 3.7, 6.3, 13.6$ Hz, 1H), 3.60 (dd, $J = 3.6, 12.0$ Hz, 1H), 3.62 (s, 2H), 3.64 (dd, $J = 3.3, 12.0$ Hz, 1H), 3.67 (dd, $J = 3.6, 12.0$ Hz, 1H), 3.69 (d, $J = 13.8$ Hz, 1H), 3.74 (dd, $J = 3.3, 12.0$ Hz, 1H), 3.78 (d, $J = 13.8$ Hz, 1H), 3.81-3.85 (m, 1H), 3.89-3.93 (m, 1H), 4.21-4.25 (m, 1H), 4.34-4.38 (m,1H), 6.17 (t, $J = 6.8$ Hz, 1H), 6.27 (t, $J = 6.7$ Hz, 1H), 7.22 (d, $J = 8.3$ Hz, 2H), 7.29 (d, $J = 8.3$ Hz, 2H), 7.52 (s, 1H), 7.88 (s, 1H);^{13}C NMR (d_4-methanol): δ32.2, 33.2, 41.2, 41.3, 62.8 (2C), 72.1 (2C), 86.0, 86.6, 88.8, 88.9, 111.8, 114.8, 130.4, 133.6 (2C), 133.7 (2C), 139.2, 139.5, 140.2, 151.9, 152.2, 164.8, 165.7; ESI-MS (positive mode) calcd for $C_{26}H_{31}N_4O_{10}S^+$: 591.2 ($M + H^+$), found 591.2.

vi. Preparation of TpTS(4-OH)C_6H_4.



TpTS(4-OH)C_6H_4

A degassed H_2O solution of TpTSPh (~2 mM, 50 mL) and 4-hydroxybenzenethiol (0.2 mmol, 25.2 mg) was irradiated under anaerobic condition for 15 minutes. The resulting reaction mixture was filtered and then purified by reverse phase HPLC in the gradient mode using CH_3CN/aqueous ammonium acetate (10 mM) as solvent. Product **TpTS(4-OH)C_6H_4** was collected and dried as a white solid (4.2 mg, 6.2%).^{1}H NMR (D_2O): δ1.75-1.88 (m, 1H), 1.77 (s, 3H), 2.12-2.21 (m, 1H), 2.23-2.32 (m, 1H), 2.45 (ddd, J = 3.6, 5.9, 14.1 Hz, 1H), 3.57 (d, J = 13.5 Hz, 1H), 3.64 (d, J = 13.5 Hz, 1H), 3.69 (dd, J = 4.5, 12.6 Hz, 1H), 3.74 (dd, J = 3.3, 12.6 Hz, 1H), 3.85-4.00 (m, 4H), 4.06-4.13 (m, 1H), 4.19-4.25 (m, 1H), 6.09 (t, J = 6.5 Hz, 1H), 6.12 (t, J = 6.9 Hz, 1H), 6.80 (d, J = 8.6 Hz, 1H), 7.05 (s, 1H), 7.20 (d, J = 8.6 Hz, 1H), 7.55 (s, 1H); ESI-MS (positive mode) calcd for $C_{26}H_{32}N_4O_{13}PS^+$: 671.2($M + H^+$), found 671.2.

vii. HPLC and LC-MS Assays.

HPLC assay for product preparation Preparative HPLC was performed at room temperature with a Waters (Milford, MA) breeze HPLC system with a 2489 UV/Visible detector at 268 nm. An Agilent SB-phenyl column (5 μm particle size, 250 × 9.4 mm i.d.) was equilibrated in solvent A (10mM ammonium acetate in water, pH 6.5) and compounds were eluted with an ascending gradient (2%~30%) of acetonitrilein 25 minutes at a flow rate of 6 mL/min. The identity of the products was confirmed by LC-MS spectrometry and NMR spectroscopy.

LC-MS assay for product analysis LC-MS analyses were conducted via an Agilent 6130 Quadrupole LC/MS spectrometer coupled to an Agilent 1100 series chromatography system using anAgilent Eclipse XDB-C18 column (5 μm particle size, 150 × 4.6 mm i.d.). The column was equilibrated in solvent A (5 mM ammonium acetate in 95% water and 5% acetonitrile, pH 6.5) and compounds were eluted with an ascending gradient (2%~15% in the first 20 minutes followed by 15~95% in the next 10 minutes) of solvent B (5 mM ammonium acetate in a 1:1 acetonitrile/methanol mixture) at a flow rate of 1 mL/min. Under this gradient, $TpTSO_2H$ was eluted at 10.3 min, TpHmU at 17.2 min, TpHpmU at 17.4 min, TpT at 18.7 min, TpfU at 18.9 min, TpT-DTT at 22.9/23.1 min, compound X at 23.8 min, $TpTS(4\text{-}OH)C_6H_4$ at 24.6 min, TpTSPh at 25.3 min.The mass signals were monitored under positive and negative ion mode respectively.

MS□MS assay The MS-MS assay was conducted using via an Agilent 6520 Accurate Mass Q-TOF LC/MS spectrometer. The compounds X, X1′, X2′ and $TpTS(4\text{-}OH)C_6H_4$ isolated from the preparative HPLC as described above were injected directly into spectrometer with solvent C (0.1% TFA in a 1:1 acetonitrile/methanol mixture) as the carrier solvent.

Determination of response factors. The response factors of photoproduct P and TpTSPh at 268 nm were calculated using TpT as an internal standard and the formula: $R_f = ([P]/[S])\times(A_S/A_P)$. Here, [P] is the concentration of the compound of interest and [S] is the concentration of the internal standard. A_P and A_s are the integration areas of the peaks corresponding to the compound of interest and standard respectively. The response factors of TpTOH and $TpTSO_2H$ are treated as TpT due to similar molar extinction coefficients of these three compounds.[2] The response factor of TpT-DTT adduct is treated as TpT and TpT-4-SC_6H_4OH adduct is treated as TpTSPh.

Compound	Response Factor
TpT	1.00
TpTSPh	0.94
X	0.54

Table S1. Response factors using TpT as internal standard.

Calculation of the yield of the photoreaction

The yield of the photoproduct was calculated using the literature method.[3] The relative molarity of each photoproduct is calculated using the formula: $M_P = A_P \times R_{f\text{-}P}$. To determine of the amount of the consumed TpTSPh, the TpTSPh was subjected for HPLC analyses before and after the UV irradiation. The decreased integration area A_c of TpTSPh was then converted to the relative molarity of TpTSPh which was consumed during the photoreaction using the following formula: $M_c = A_c \times R_{f\text{-}TpTSPh}$. The yield of each photoproducts was derived from the formula $\eta_P = M_P / M_c$. (Note: in the case where compound X is generated by a bimolecular coupling, formula $\eta_P = M_P / M_c'$ is used instead where $M_c' = M_c - M_x$)

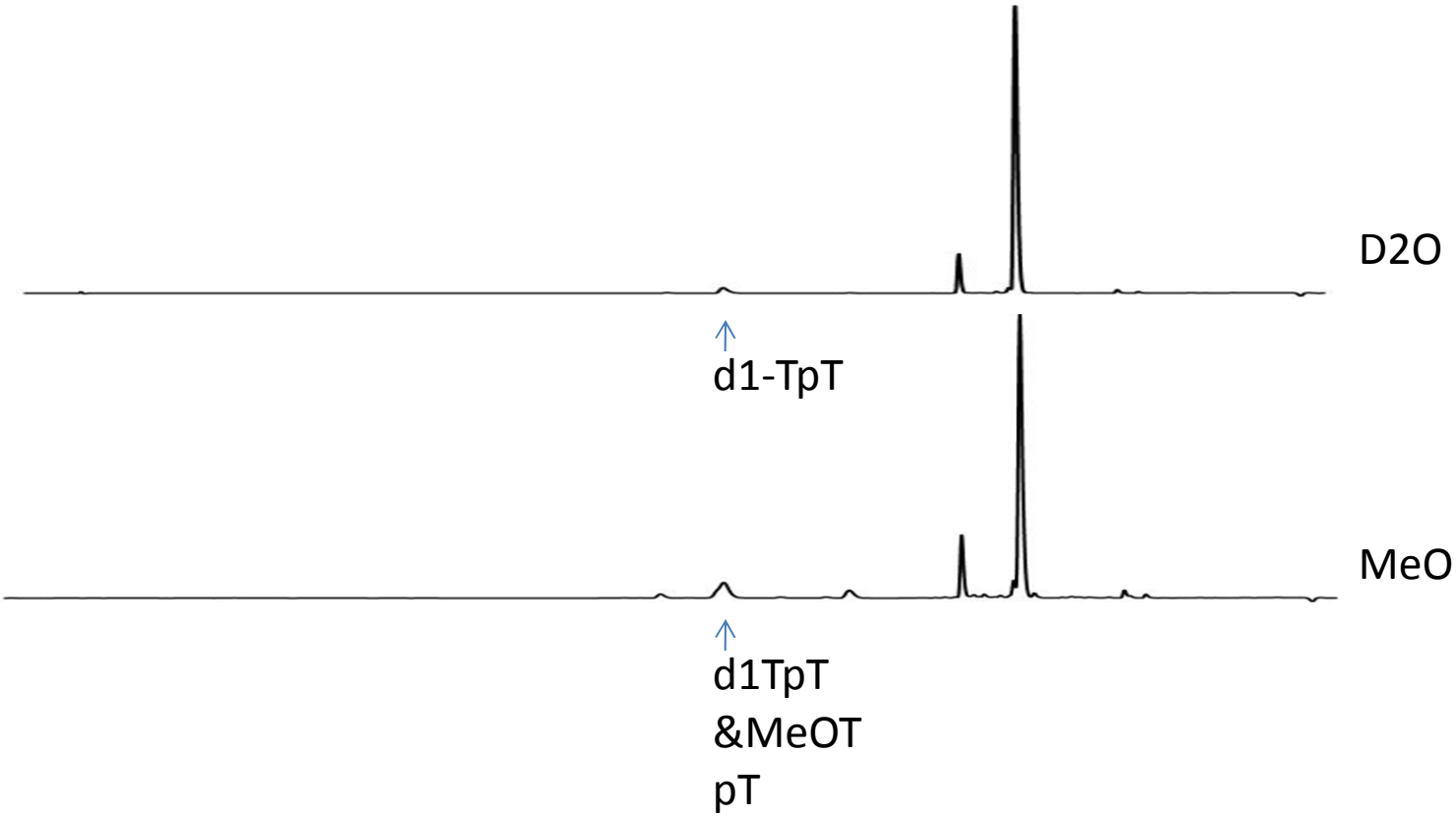


Figure S1.HPLC chromatograph of the TpTSPh photoreaction under 254 nm UV light for 10 minutes in D_2O or CH_3OD under an inert atmosphere.

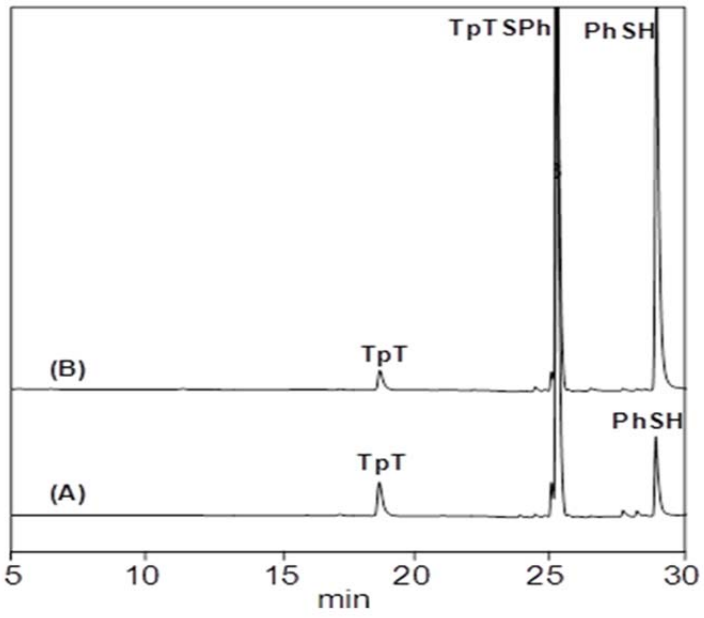


Figure S2.HPLC chromatograph of the TpTSPh photoreaction in the presence of (A) 1 mMPhSH and (B) 5 mMPhSH.

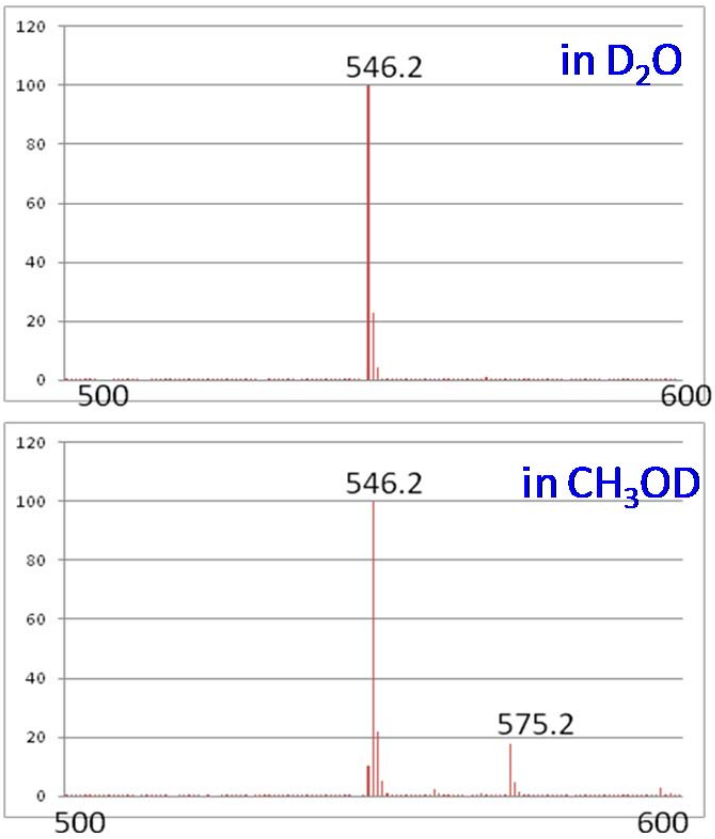


Figure S3.ESI-MS of TpTformed in the TpTSPh photoreaction in D_2O and CH_3OD respectively under negative ion mode. d_1-TpT is suggested to be produced in both cases.

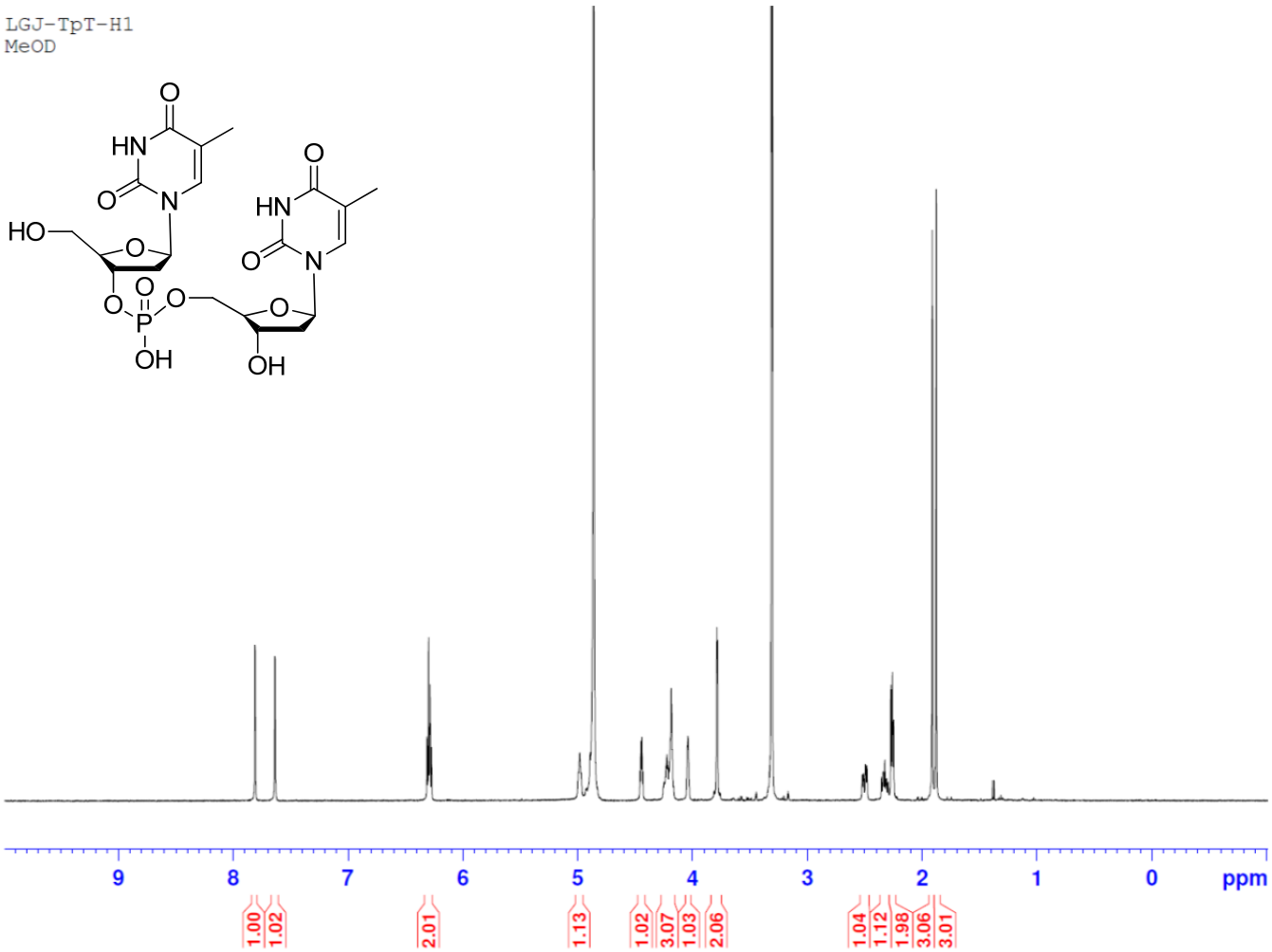


Figure S4.[1]H NMR spectrum of the regular dinucleotide TpT. CD_3OD was used as the NMR solvent. The signal integration confirms the presence of both methyl groups.

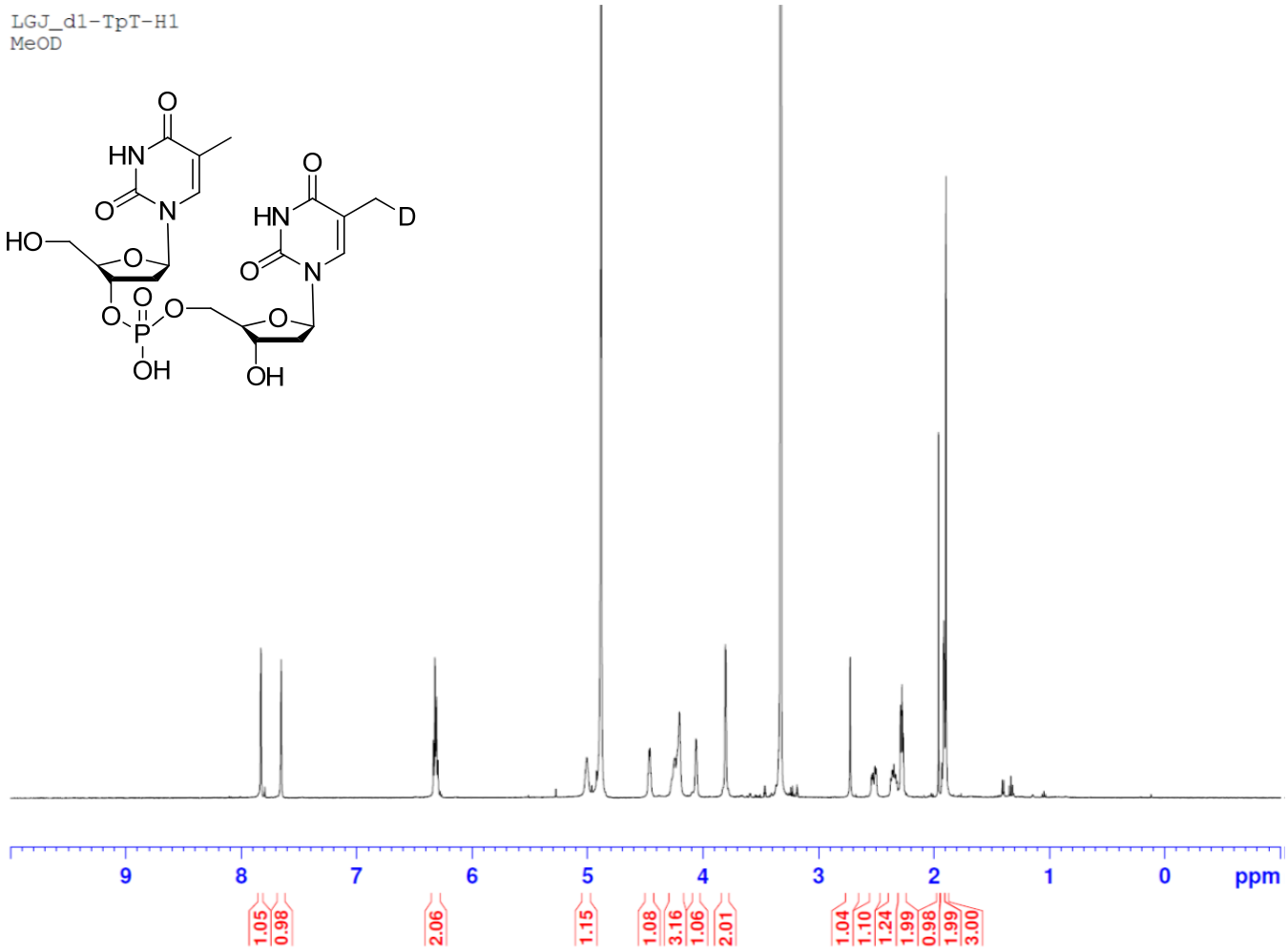


Figure S5.[1]H-NMR spectrum of the dinucleotide d_1-TpT obtained from the TpTSPh photoreaction in D_2O. The spectrum was recorded in CD_3OD. The signal integration indicates that one protium of a methyl group has been substituted by a deuterium.

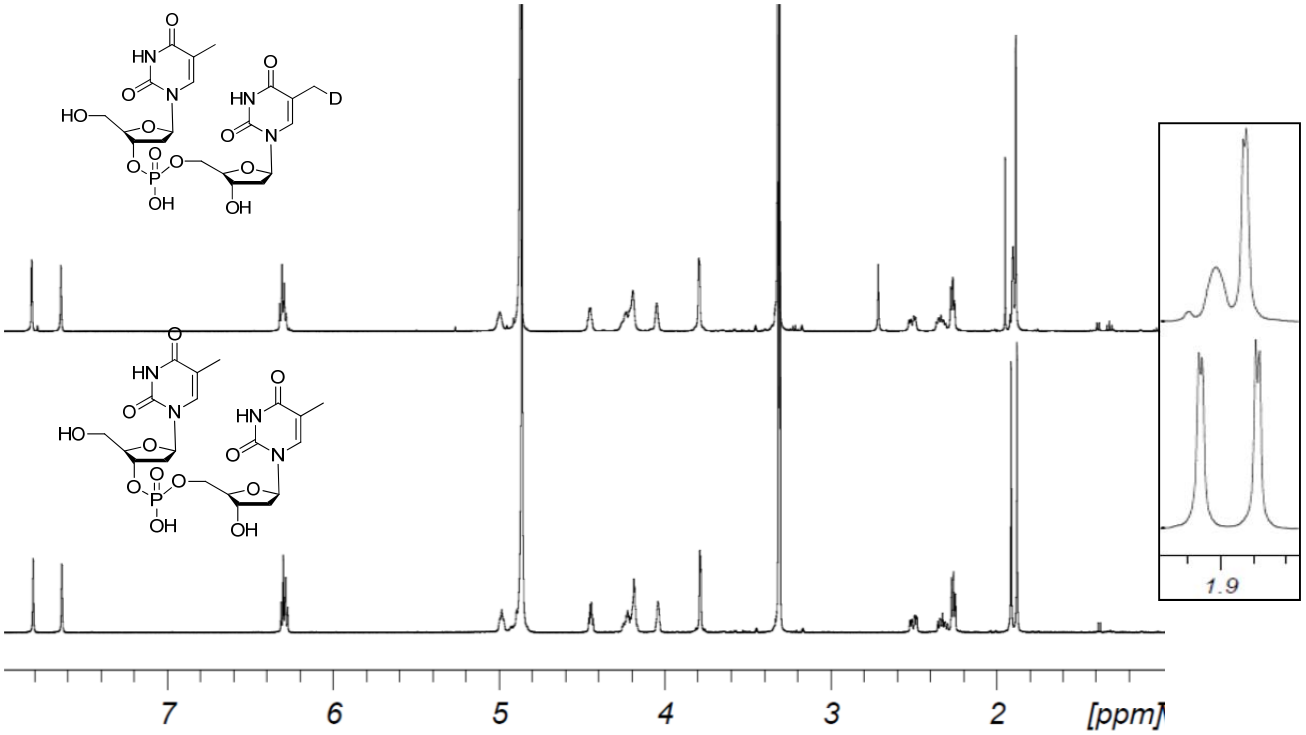
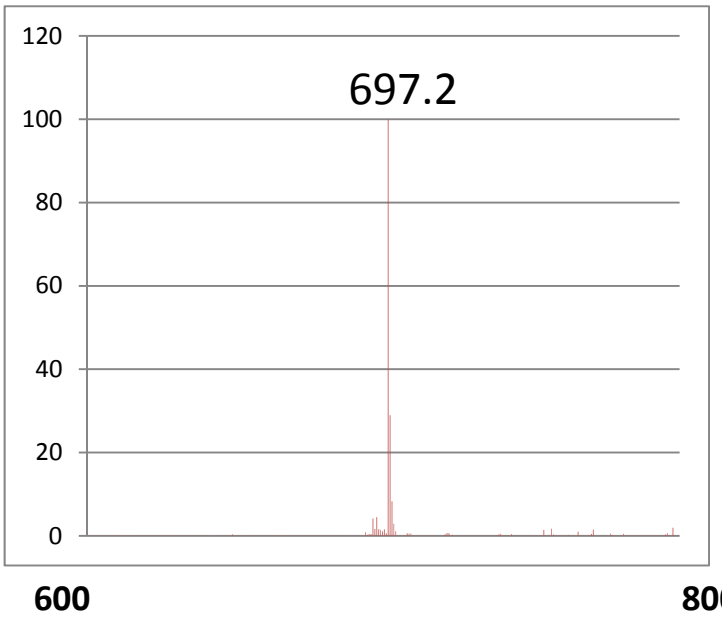
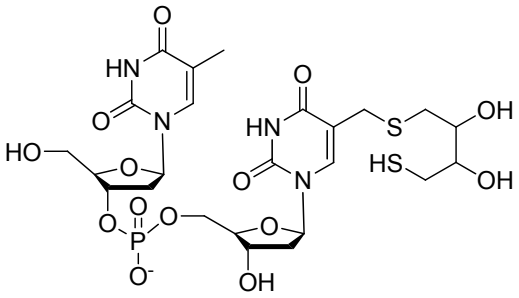


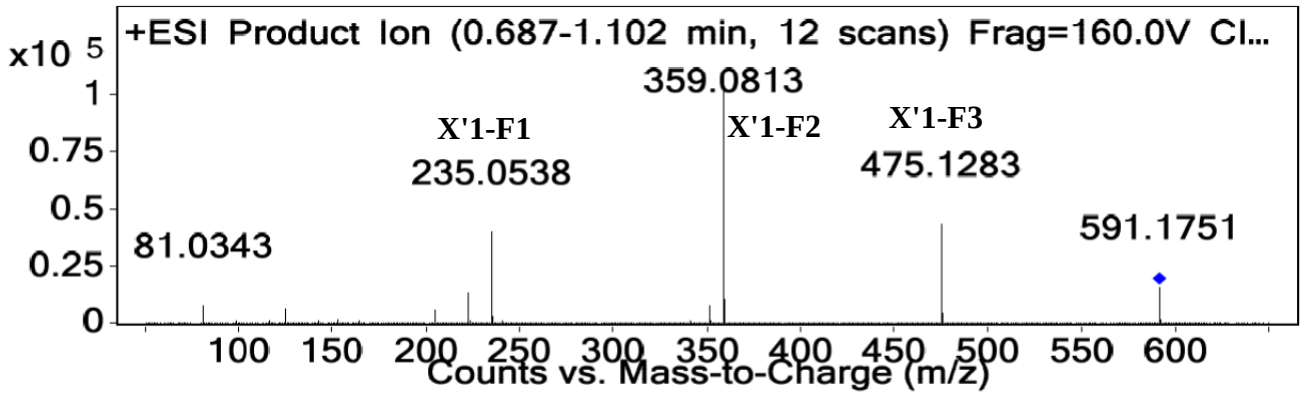
Figure S6.Side-by-side comparison of the ^{1}H NMR spectra for the dinucleotide TpT and d_1-TpT obtained from the TpTSPh photoreaction. The NMR signals corresponding to the methyl groups of the dinucleotides have been zoomed in as shown in the spectra on the right. The d_1-TpT possesses a broadened NMR signal as the result of the deuterium substitution in the CH_2D group.



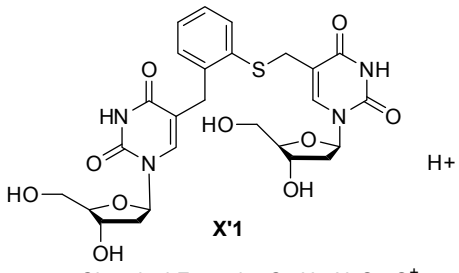


ESI-MS (negative mode)
calcd for $C_{24}H_{34}N_4O_{14}PS_2^-$: 697.1

Figure S7.ESI-MS spectrum of the TpT-DTT adduct under the negative ion mode.



Structure of compound X'1

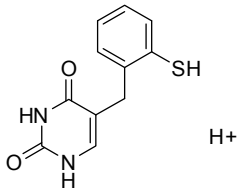


X'1

Chemical Formula: $C_{26}H_{31}N_4O_{10}S^+$

Exact Mass: 591.1755

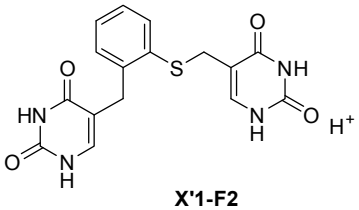
Fragment Structure (Positive mode)



X'1-F1

Chemical Formula: $C_{11}H_{11}N_2O_2S^+$

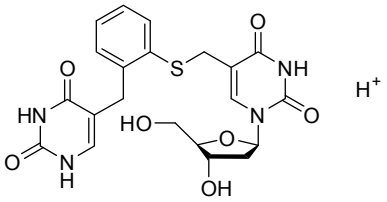
Exact Mass: 235.0536



X'1-F2

Chemical Formula: $C_{16}H_{15}N_4O_4S^+$

Exact Mass: 359.0809

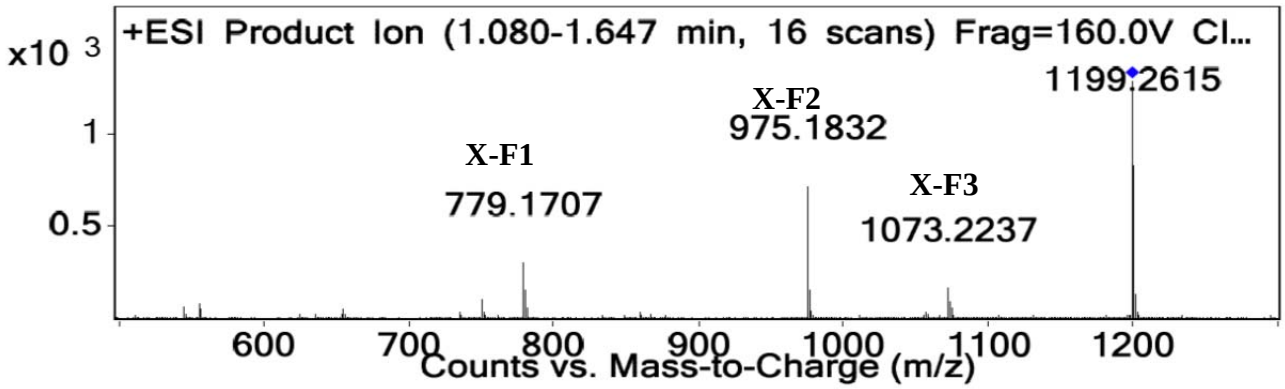


X'1-F3

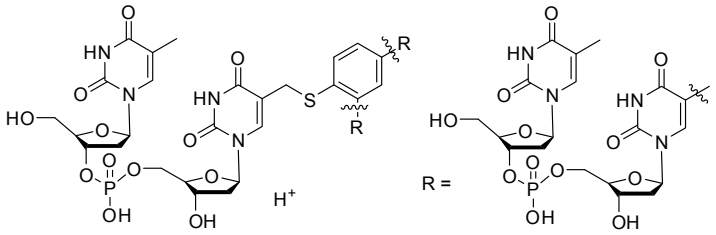
Chemical Formula: $C_{21}H_{23}N_4O_7S^+$

Exact Mass: 475.1282

Figure S8. MS/MS analysis of the addition-elimination productsX'1.The product X'2exhibits an identical MS spectrum to that of the X'1.



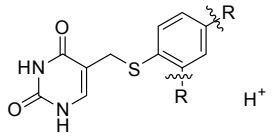
Structure of compound X'1



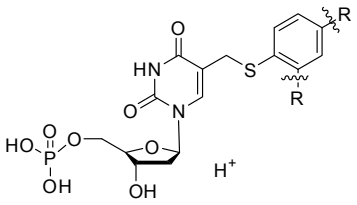
Chemical Formula: $C_{46}H_{57}N_8O_{24}P_2S^+$
Exact Mass: 1199.2676

X

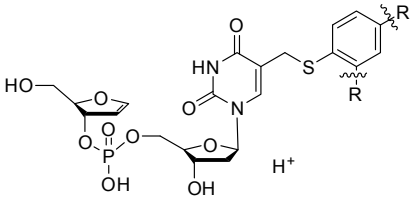
Fragment Structure (Positive mode)



X-F1
Chemical Formula: $C_{31}H_{36}N_6O_{14}PS^+$
Exact Mass: 779.1742

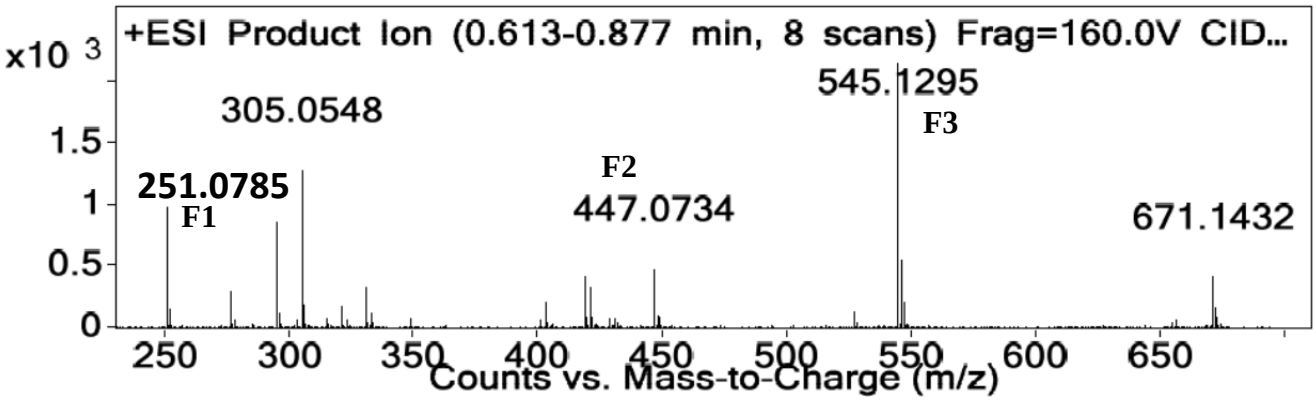


X-F2
Chemical Formula: $C_{36}H_{45}N_6O_{20}P_2S^+$
Exact Mass: 975.1879

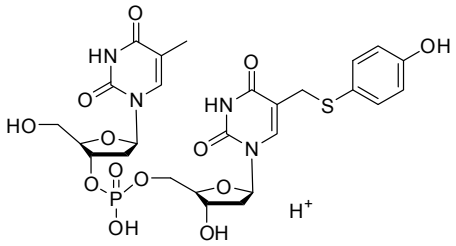


X-F2
Chemical Formula: $C_{41}H_{51}N_6O_{22}P_2S^+$
Exact Mass: 1073.2247

Figure S9. MS/MS spectrum of addition-elimination product X formed in the TpTSPh photoreaction under the positive ion mode.



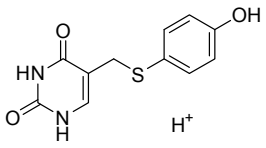
Structure of compound TpT(4-OH)SC$_6$H$_4$



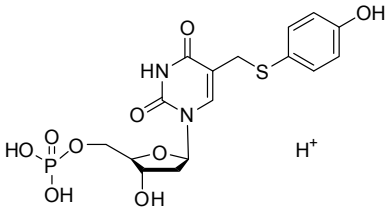
Chemical Formula: $C_{26}H_{32}N_4O_{13}PS^+$
Exact Mass: 671.1419

TpTS(4-OH)C$_6$H$_4$

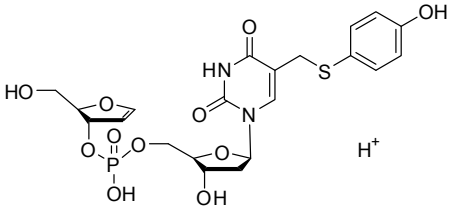
Fragment Structure (Positive mode)



F1
Chemical Formula: $C_{11}H_{11}N_2O_3S^+$
Exact Mass: 251.0485



F2
Chemical Formula: $C_{16}H_{20}N_2O_9PS^+$
Exact Mass: 447.0622



F3
Chemical Formula: $C_{21}H_{26}N_2O_{11}PS^+$
Exact Mass: 545.0989

Figure S10. MS/MS of TpTS(4-OH)C$_6$H$_4$ formed under the TpTSPh photoreaction in the presence of 1 mM 4-hydroxythiophenol (positive ion mode).

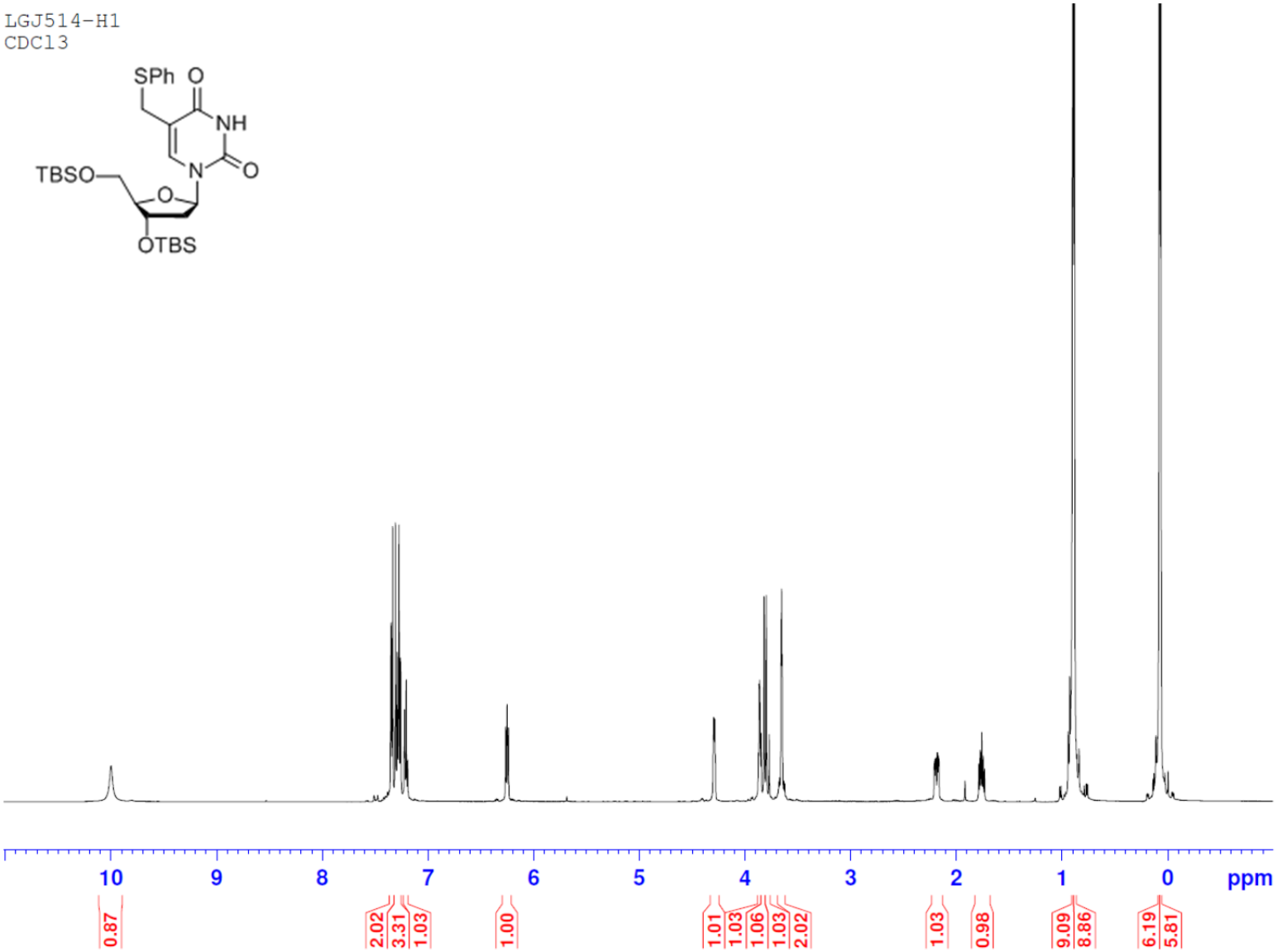


Figure S11. ^{1}H NMR spectrum of compound **S2**.

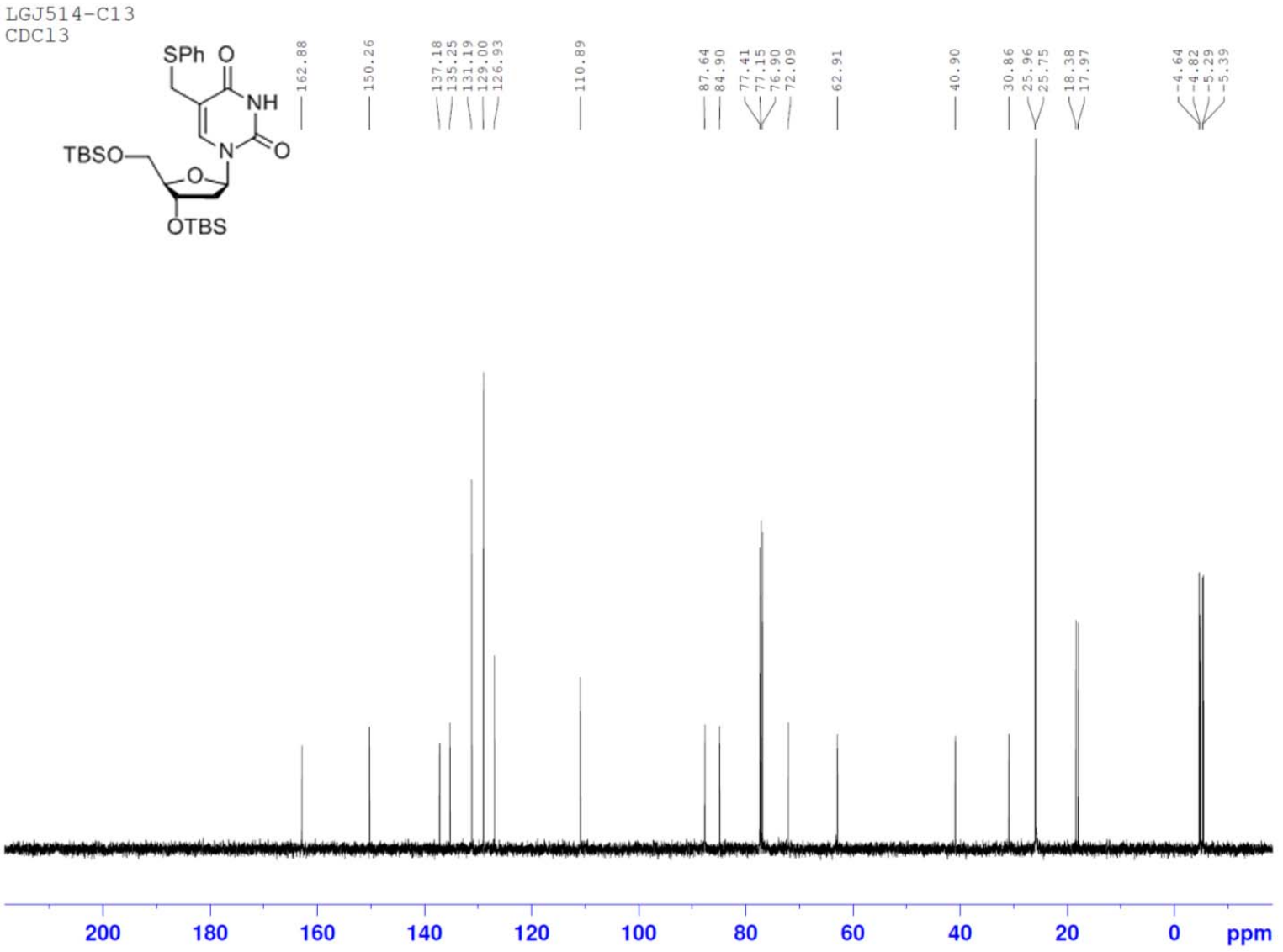


Figure S12. ^{13}C NMR spectrum of compound **S2**.

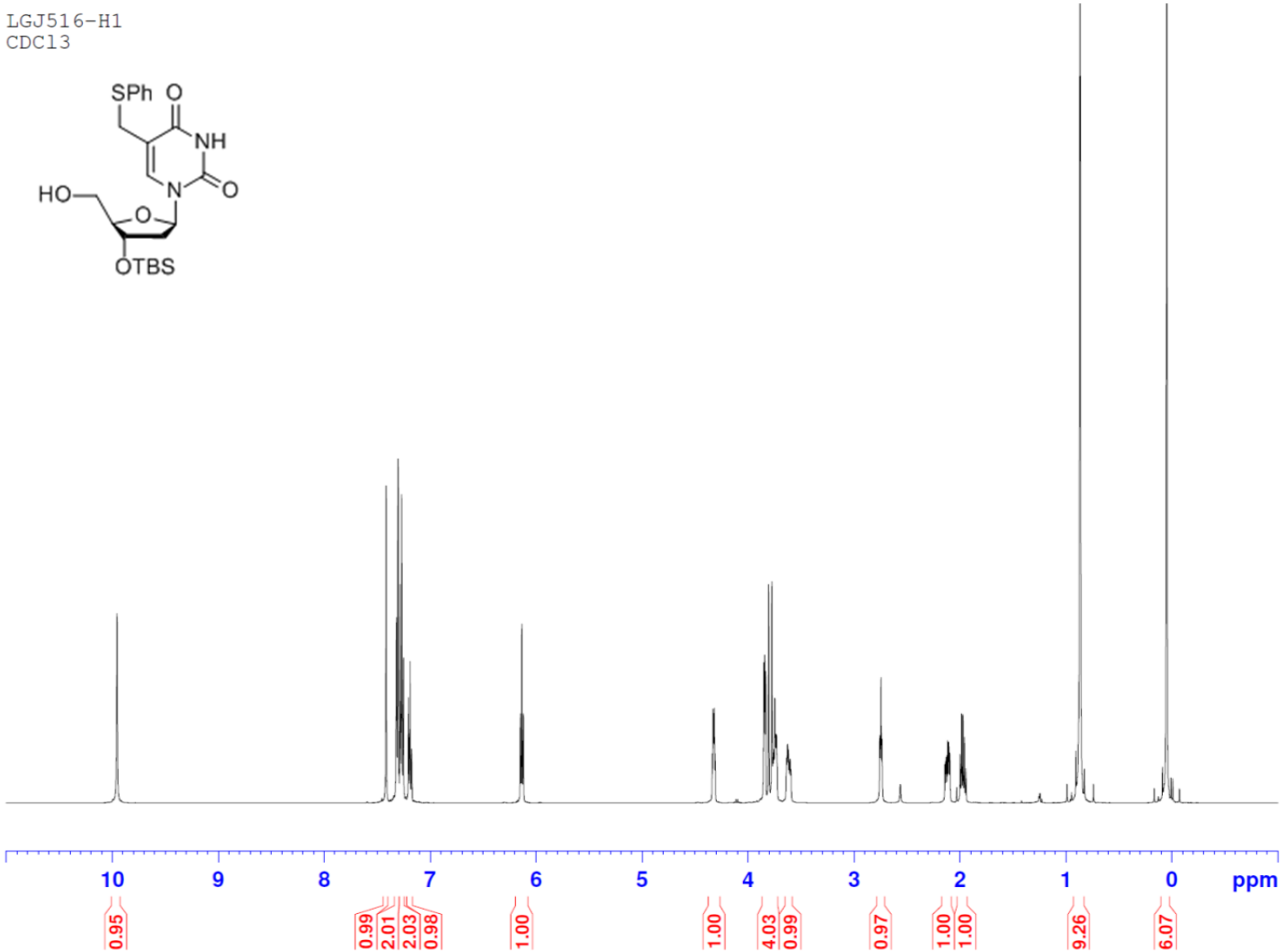


Figure S13. ^{1}H NMR spectrum of compound **S3**.

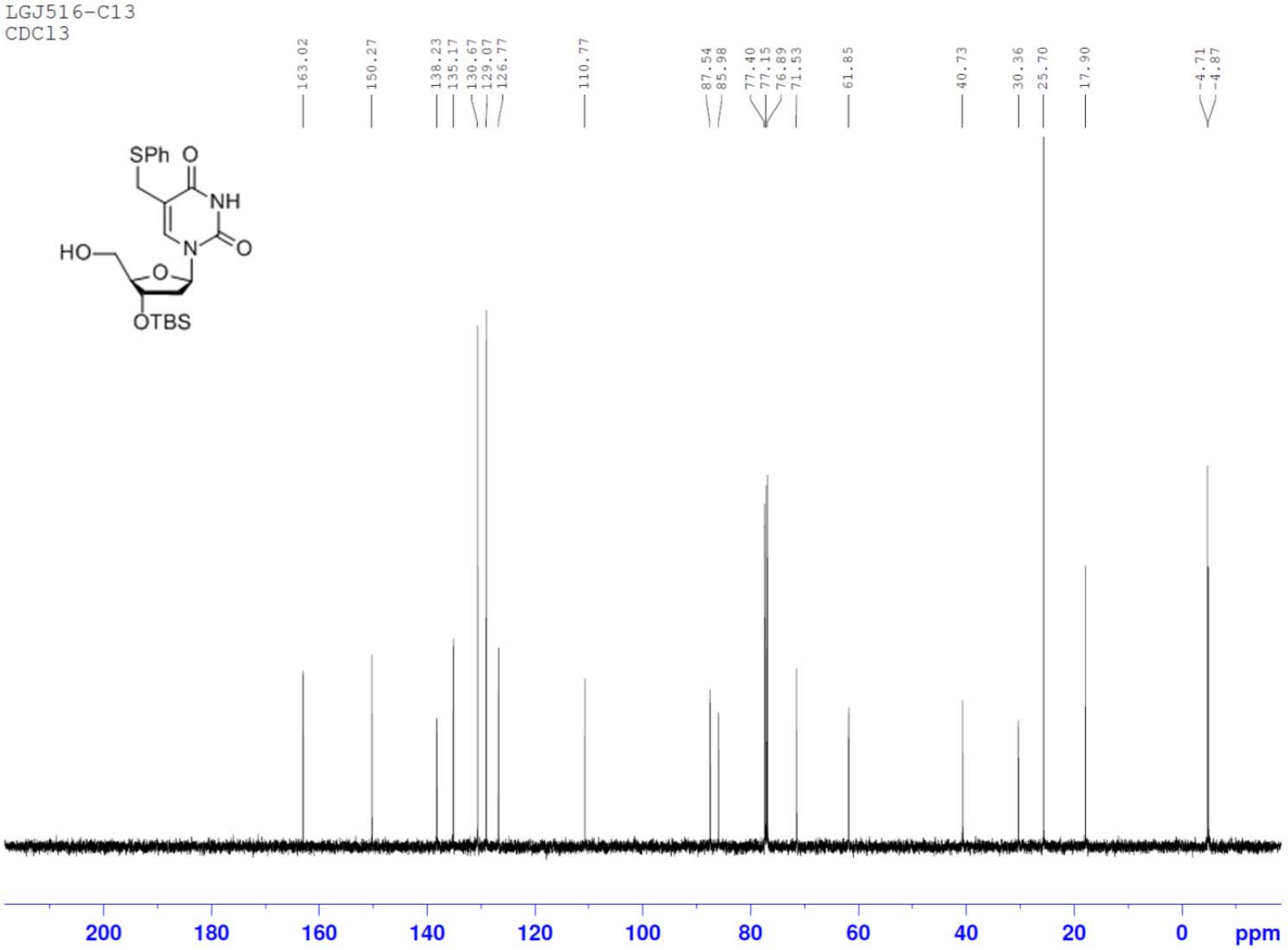


Figure S14. ^{13}C NMR spectrum of compound **S3**.

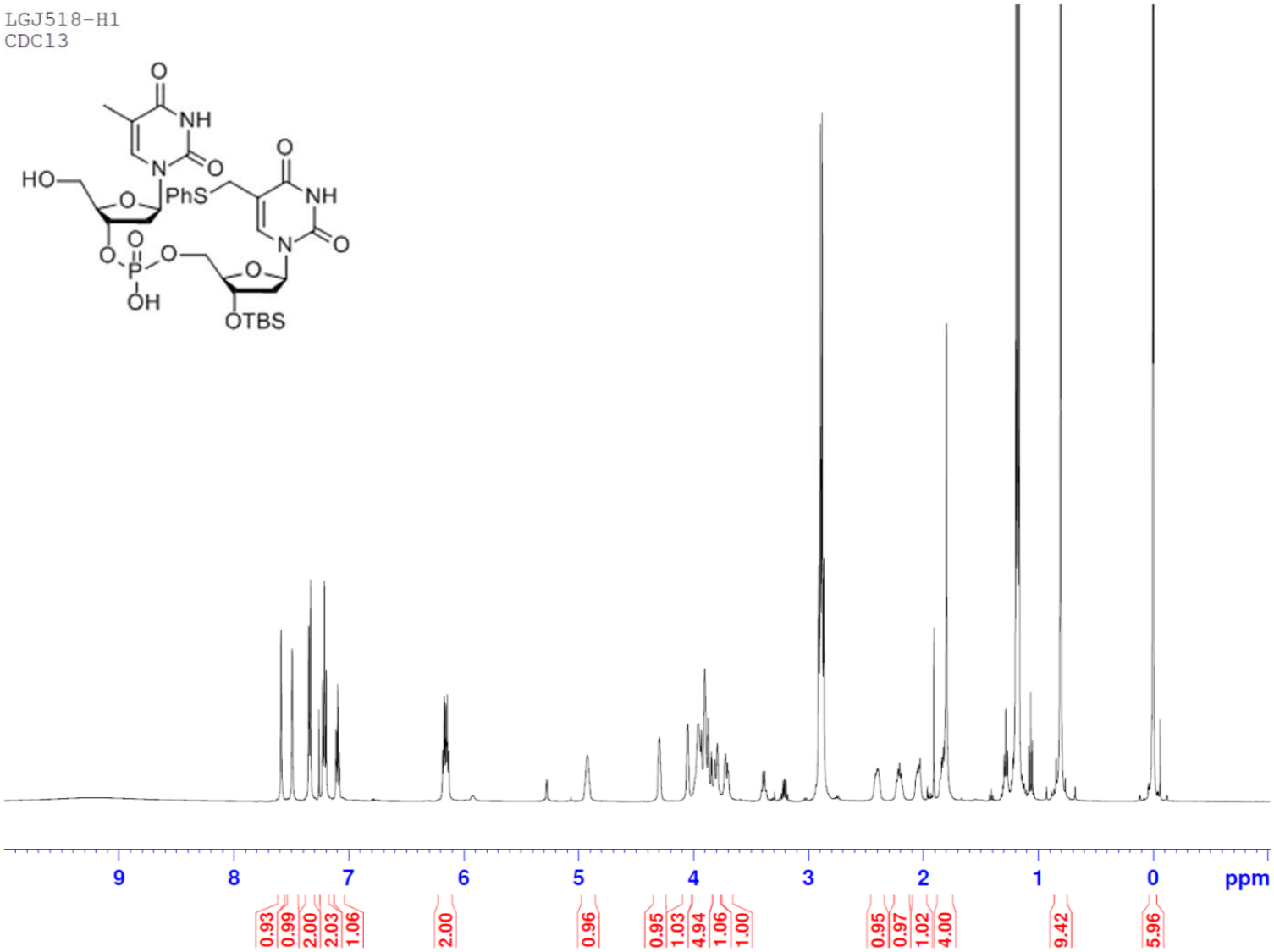


Figure S15. ^{1}H NMR spectrum of compound **S4**.

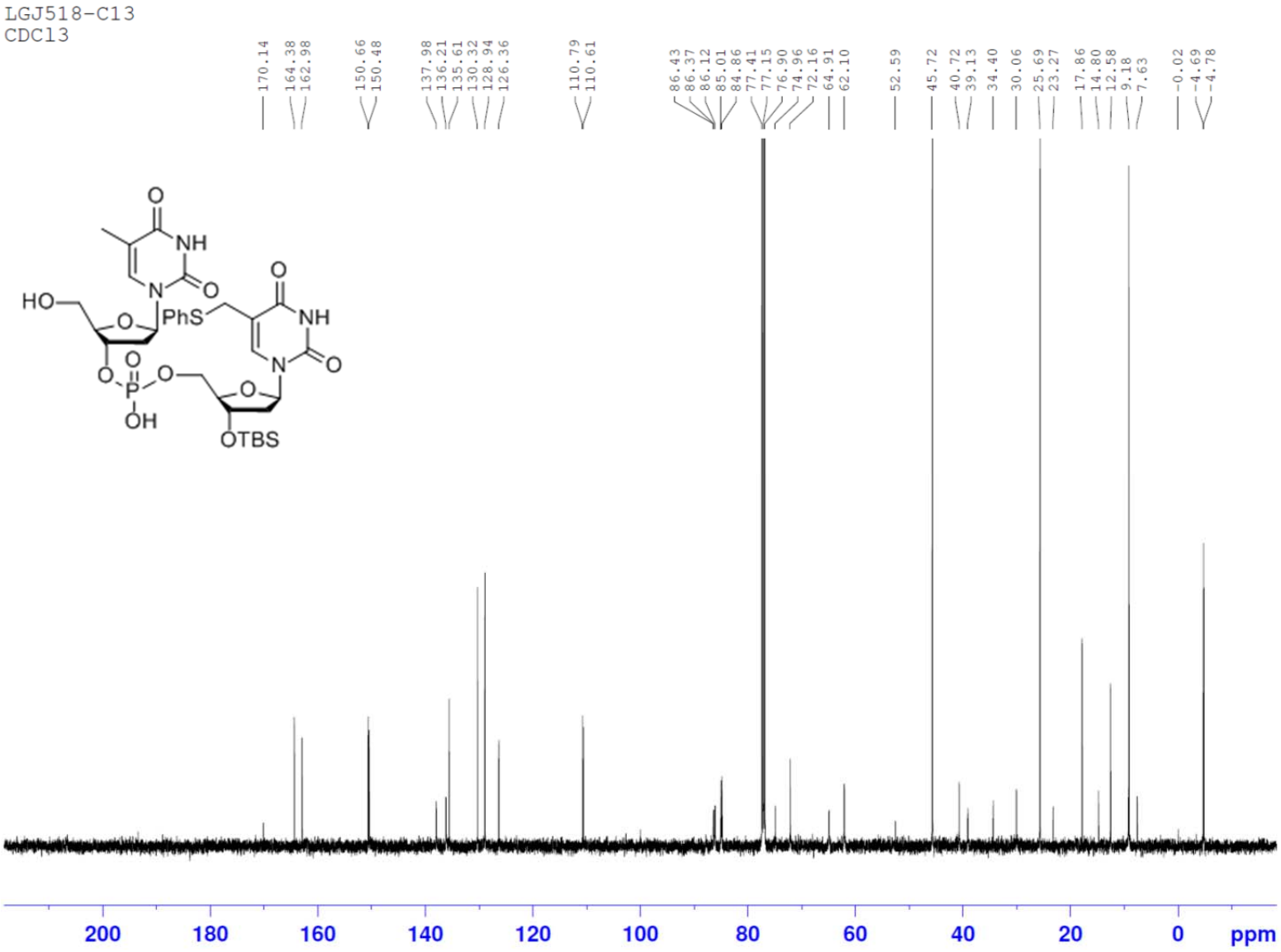


Figure S16. ^{13}C NMR spectrum of compound **S4**.

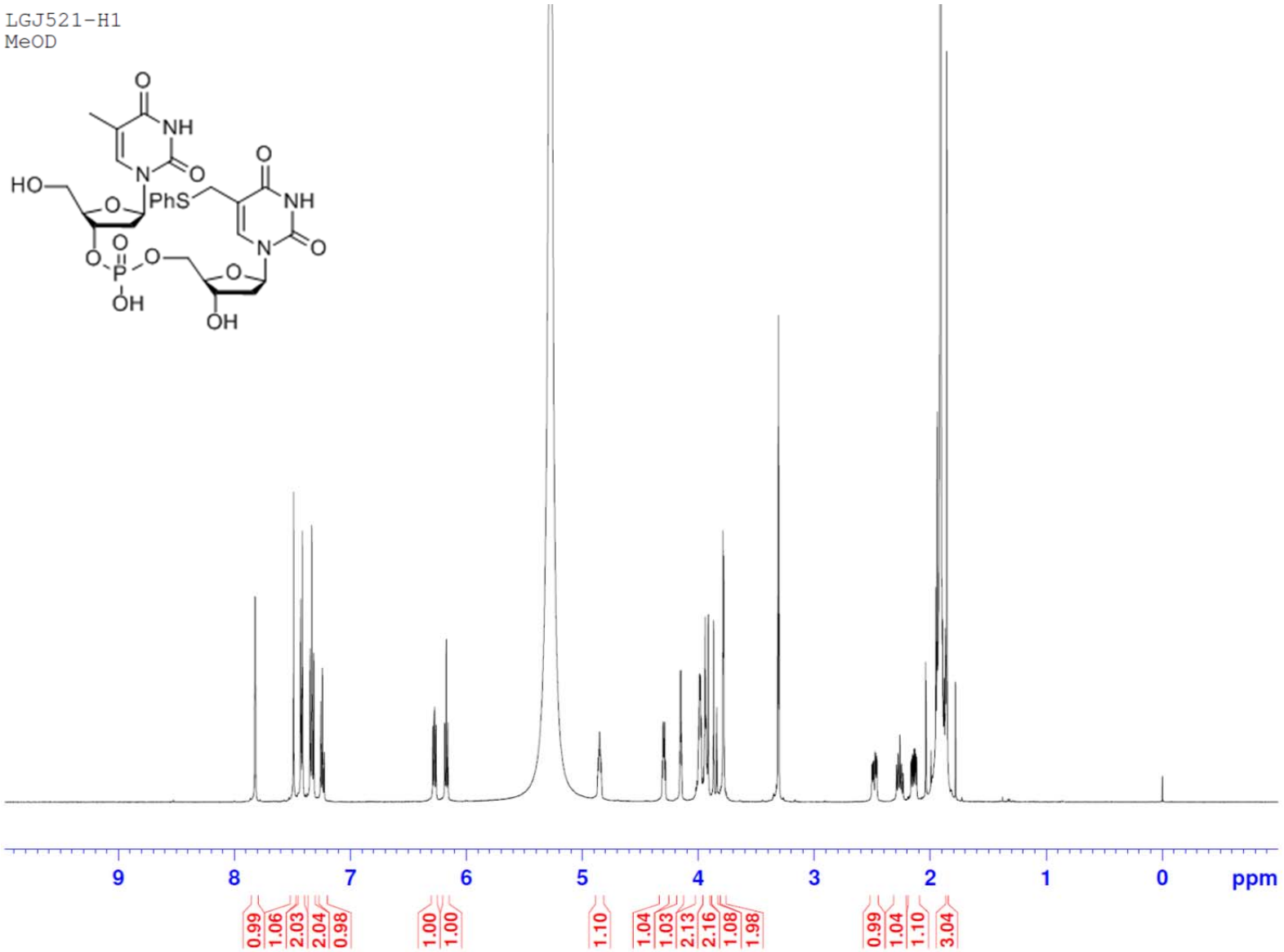


Figure S17. ^{1}H NMR spectrum of compound **2**.

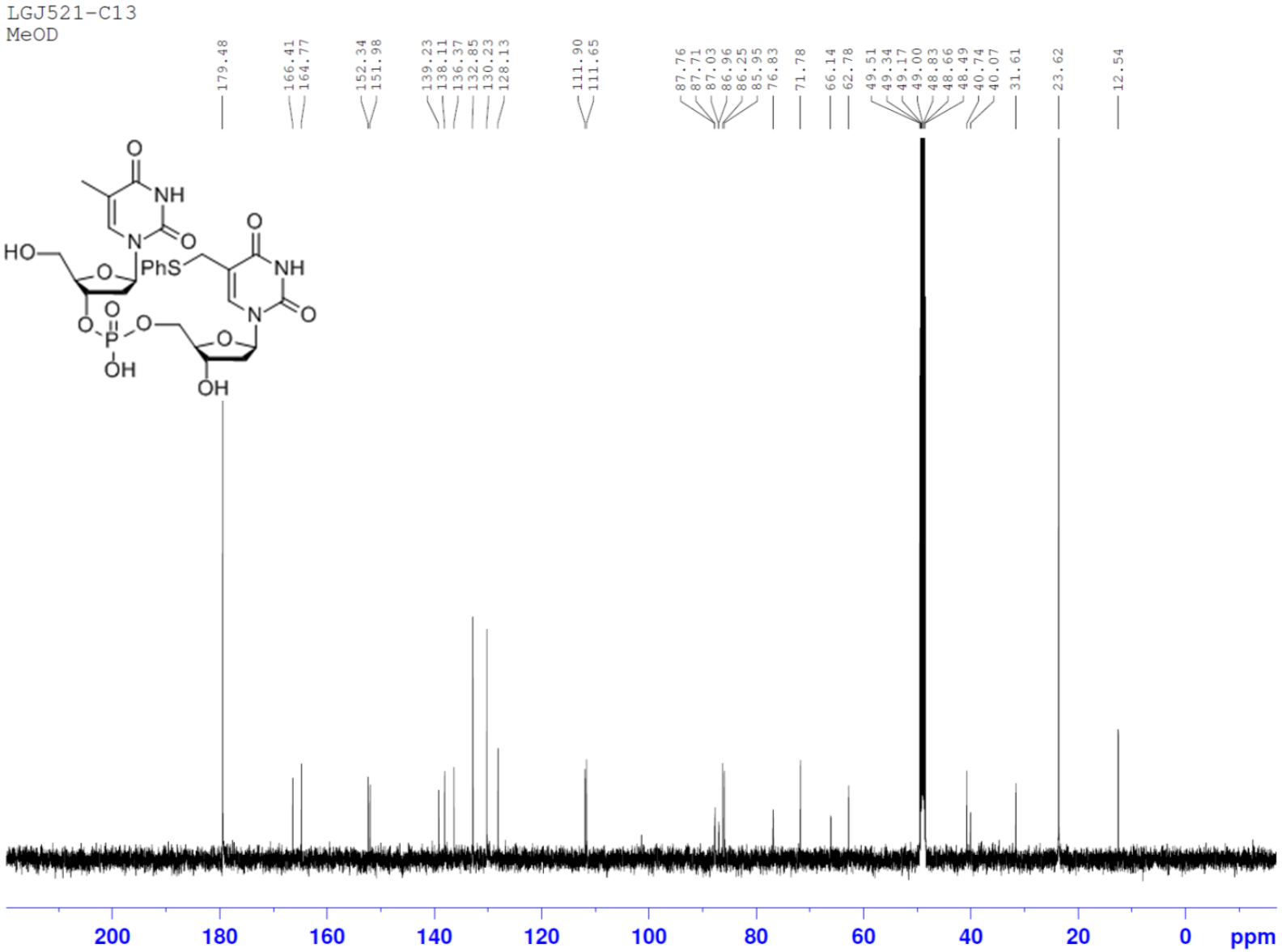


Figure S18. ^{13}C NMR spectrum of compound **2**.

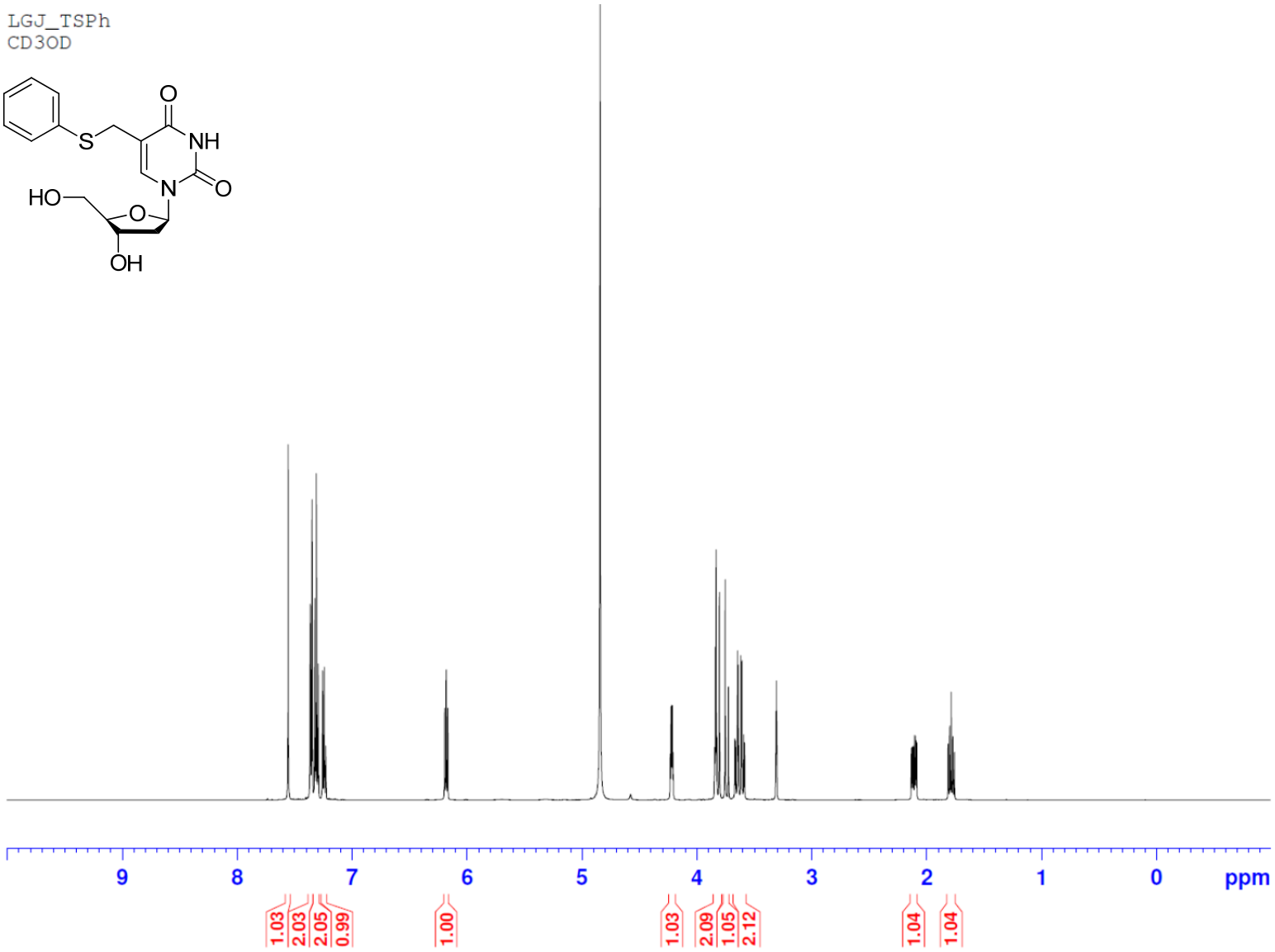


Figure S19. ^{1}H NMR spectrum of compound **TSPh**.

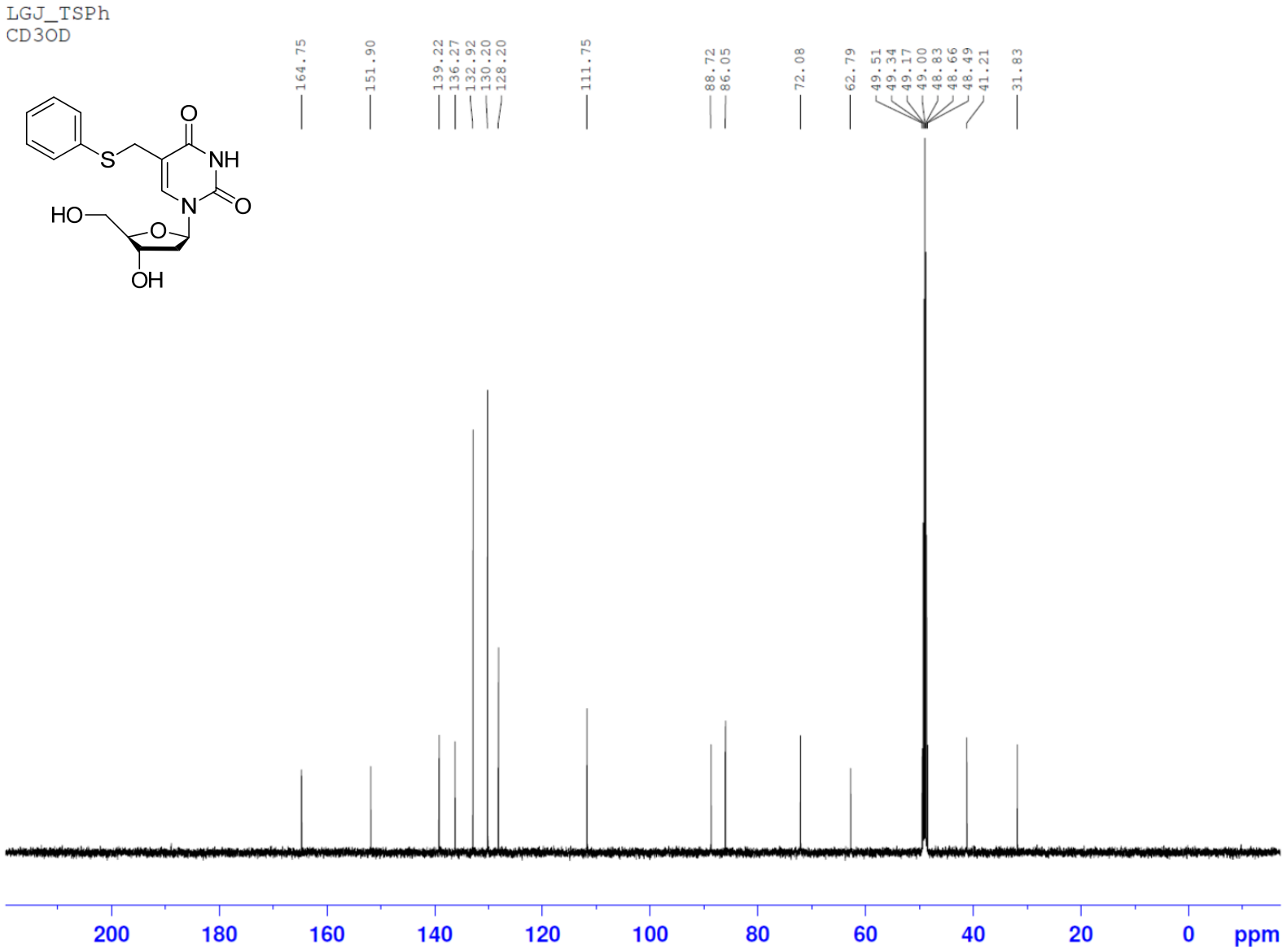


Figure S20. ^{13}C NMR spectrum of compound **TSPh**.

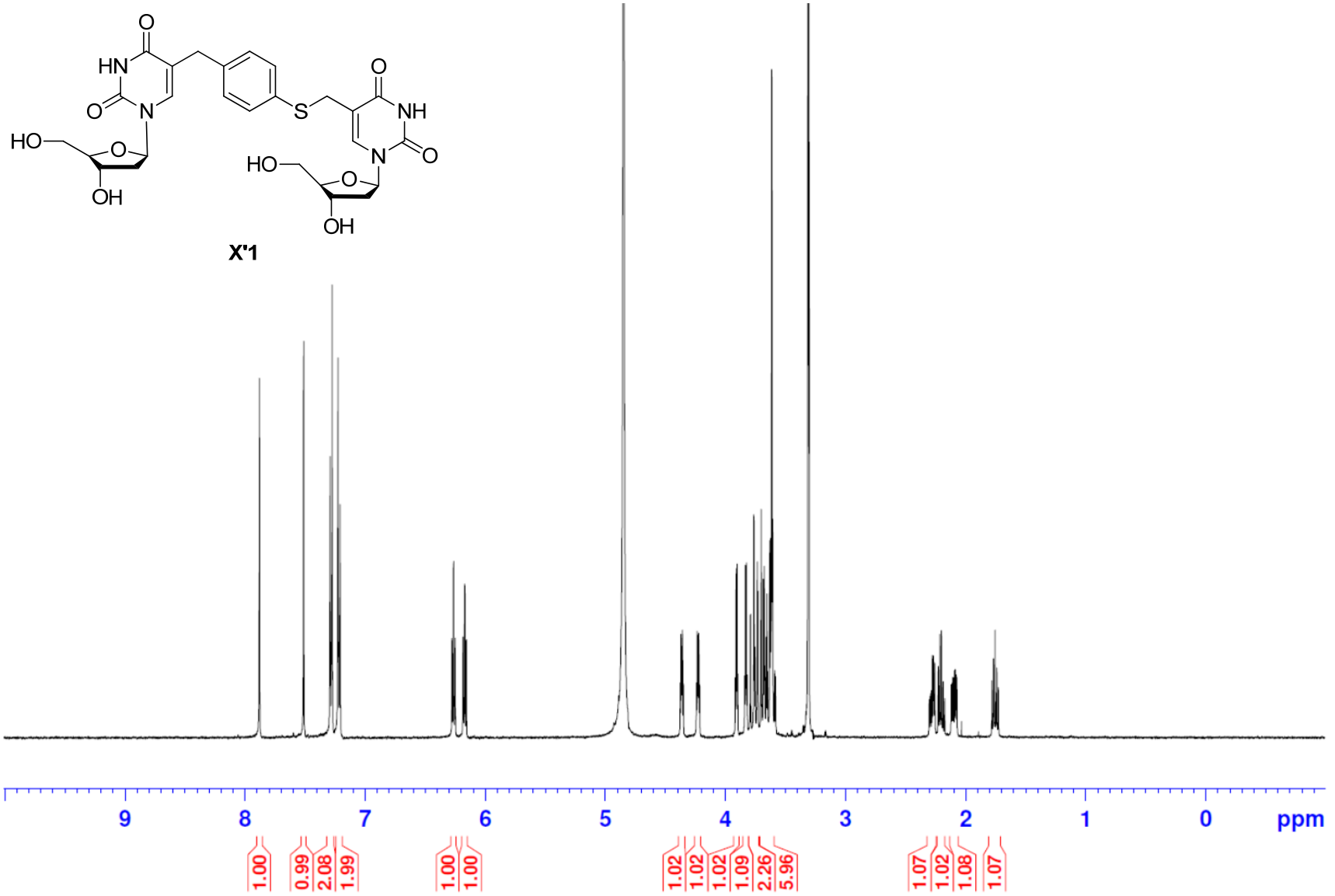


Figure S21. ^{1}H NMR spectrum of addition-elimination product **X'1**.

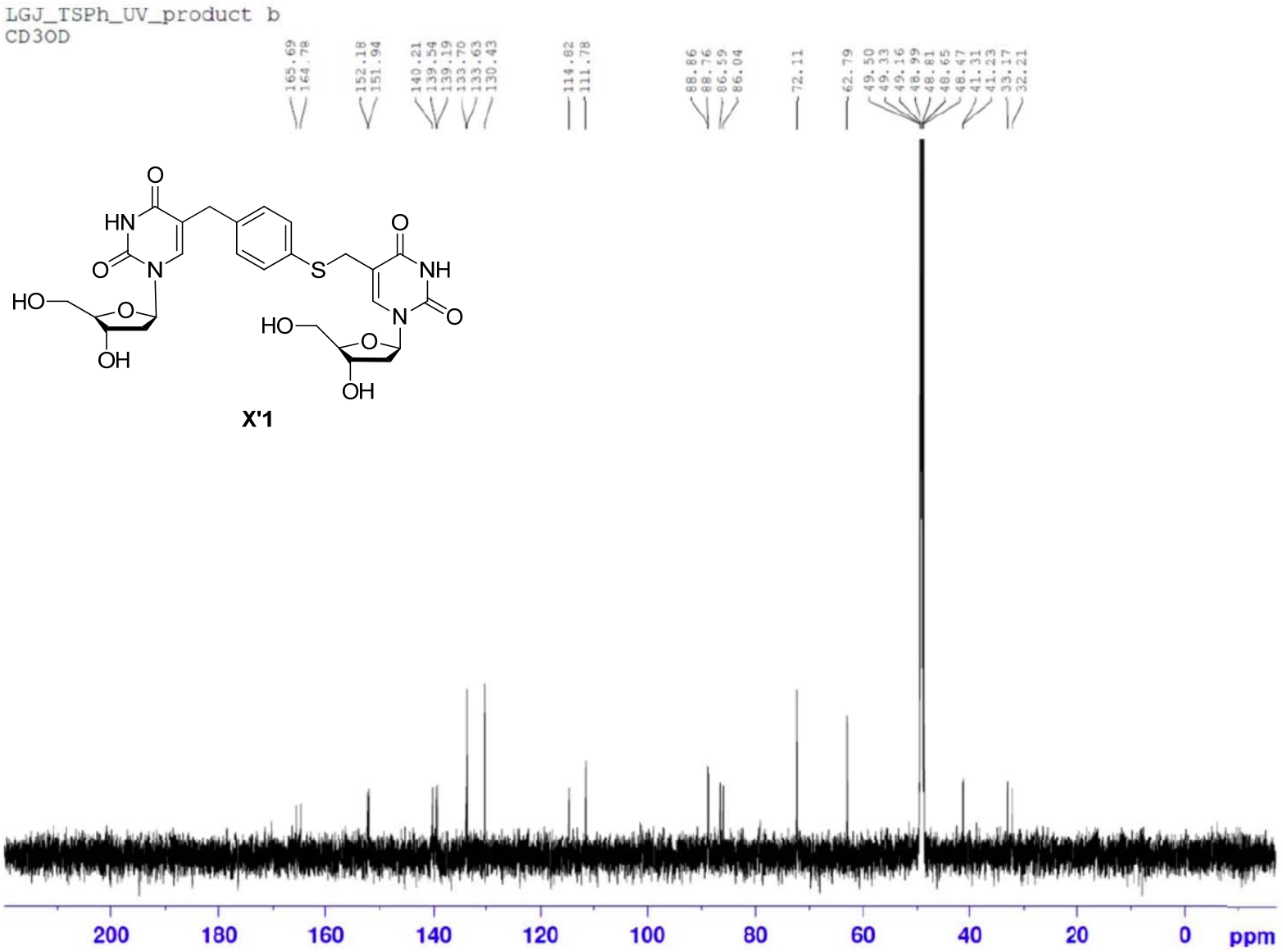


Figure S22. ^{13}C NMR spectrum of addition-elimination product **X'1**.

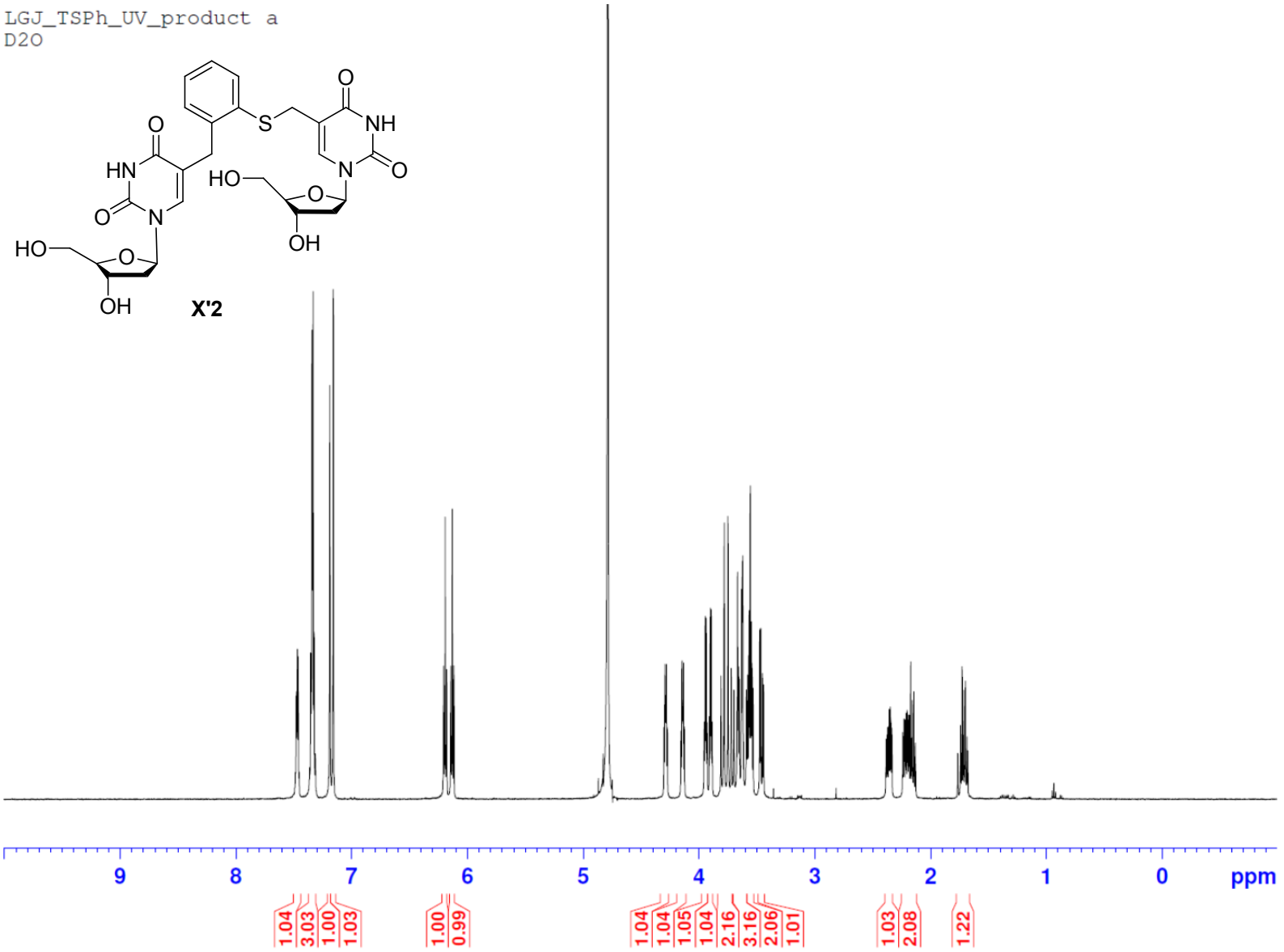


Figure S23. ^{1}H NMR spectrum of addition-elimination product **X'2**.

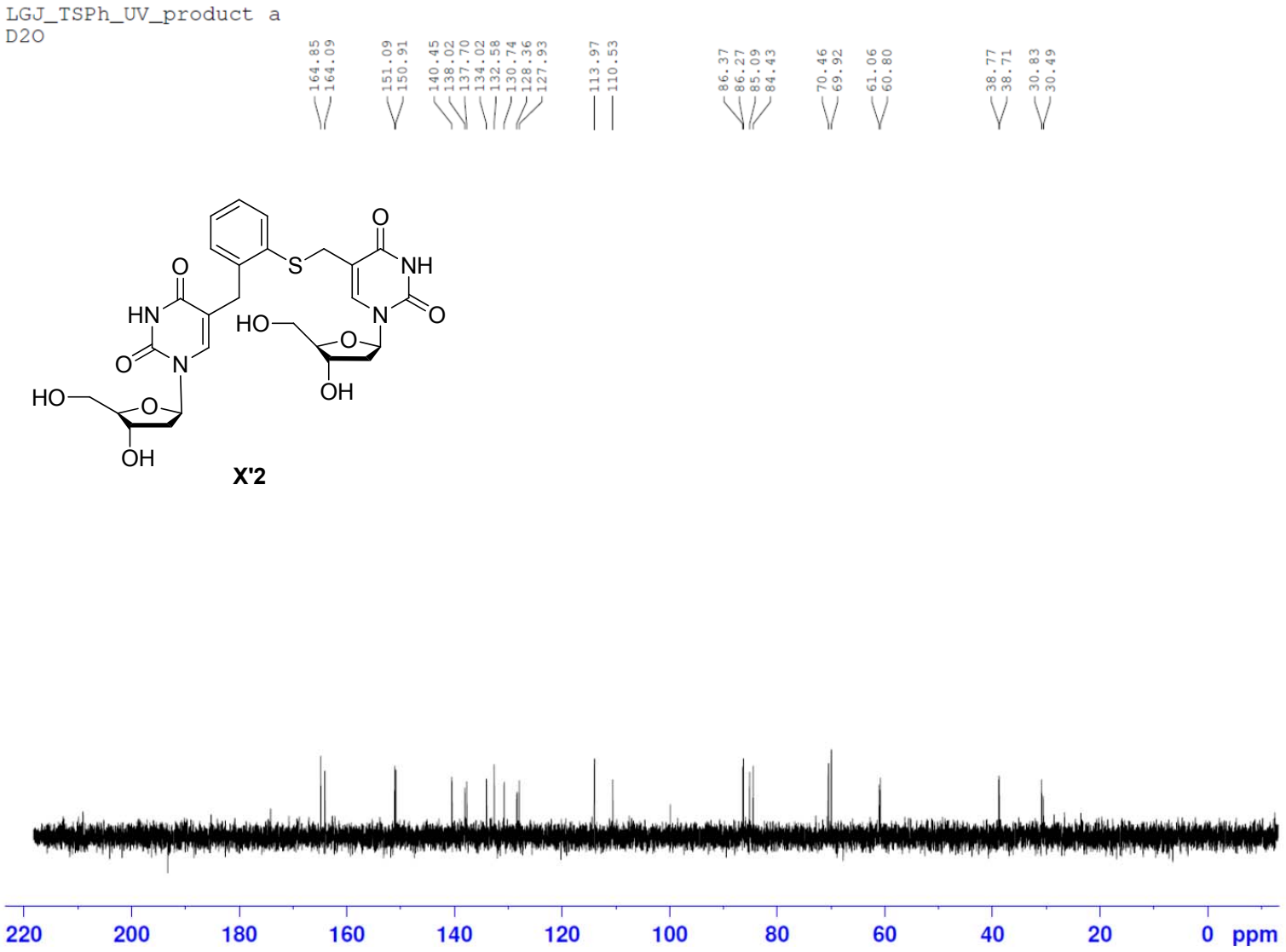


Figure S24. ^{13}C NMR spectrum of addition-elimination product **X'2**.

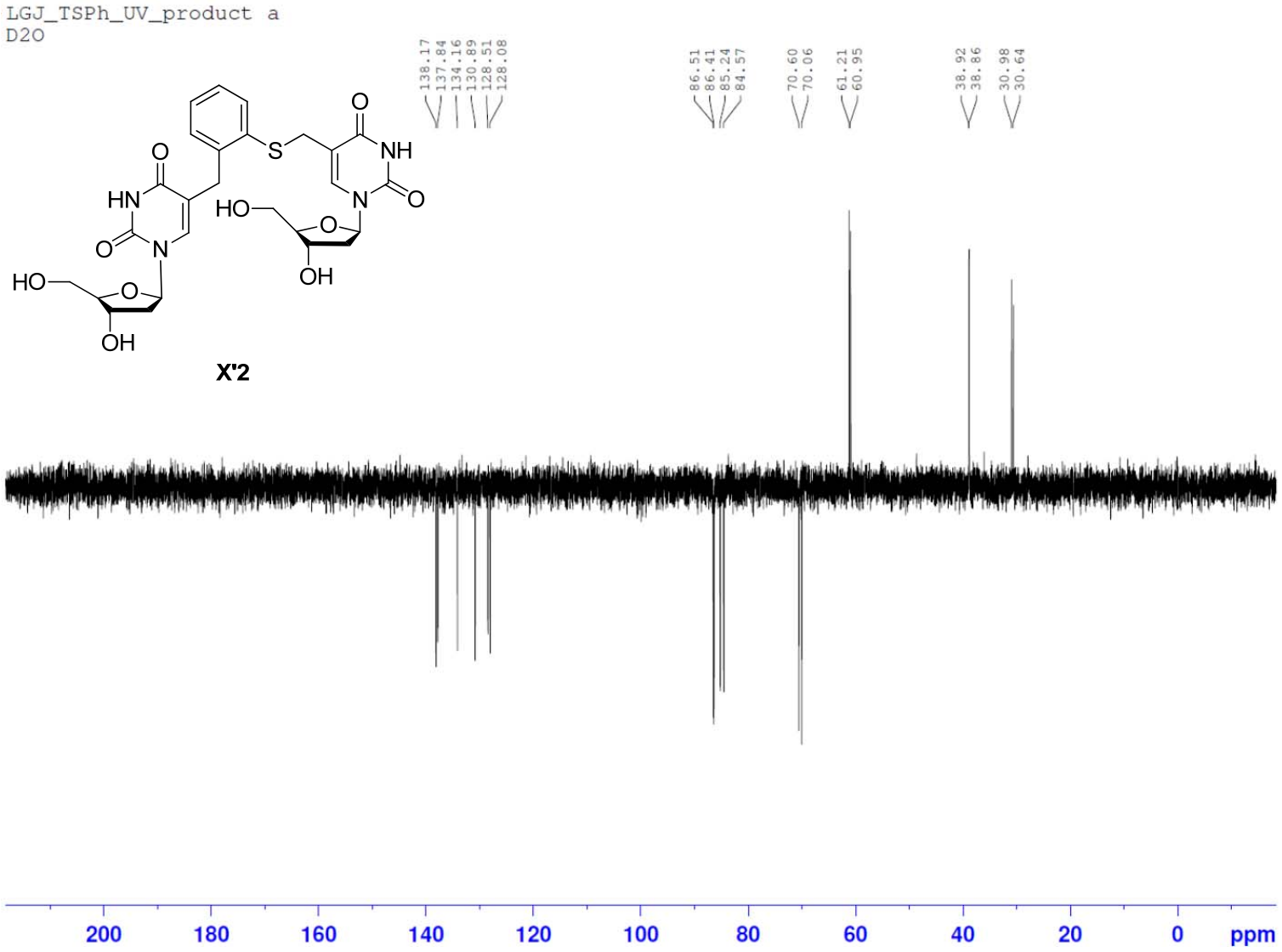


Figure S25. DEPT135 spectrum of addition-elimination product **X'2**.

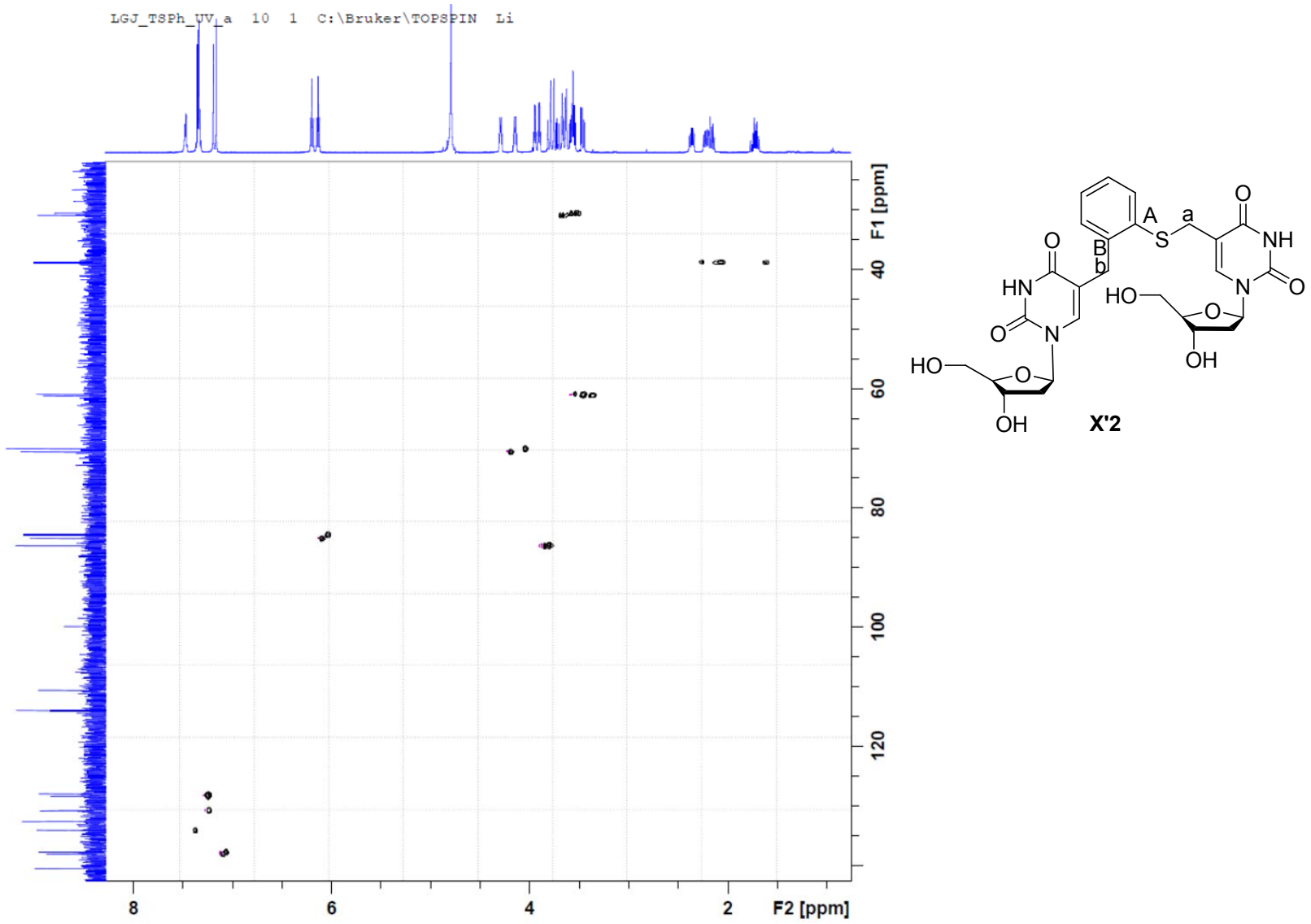


Figure S26. HSQC spectrum of addition-elimination product **X'2**.

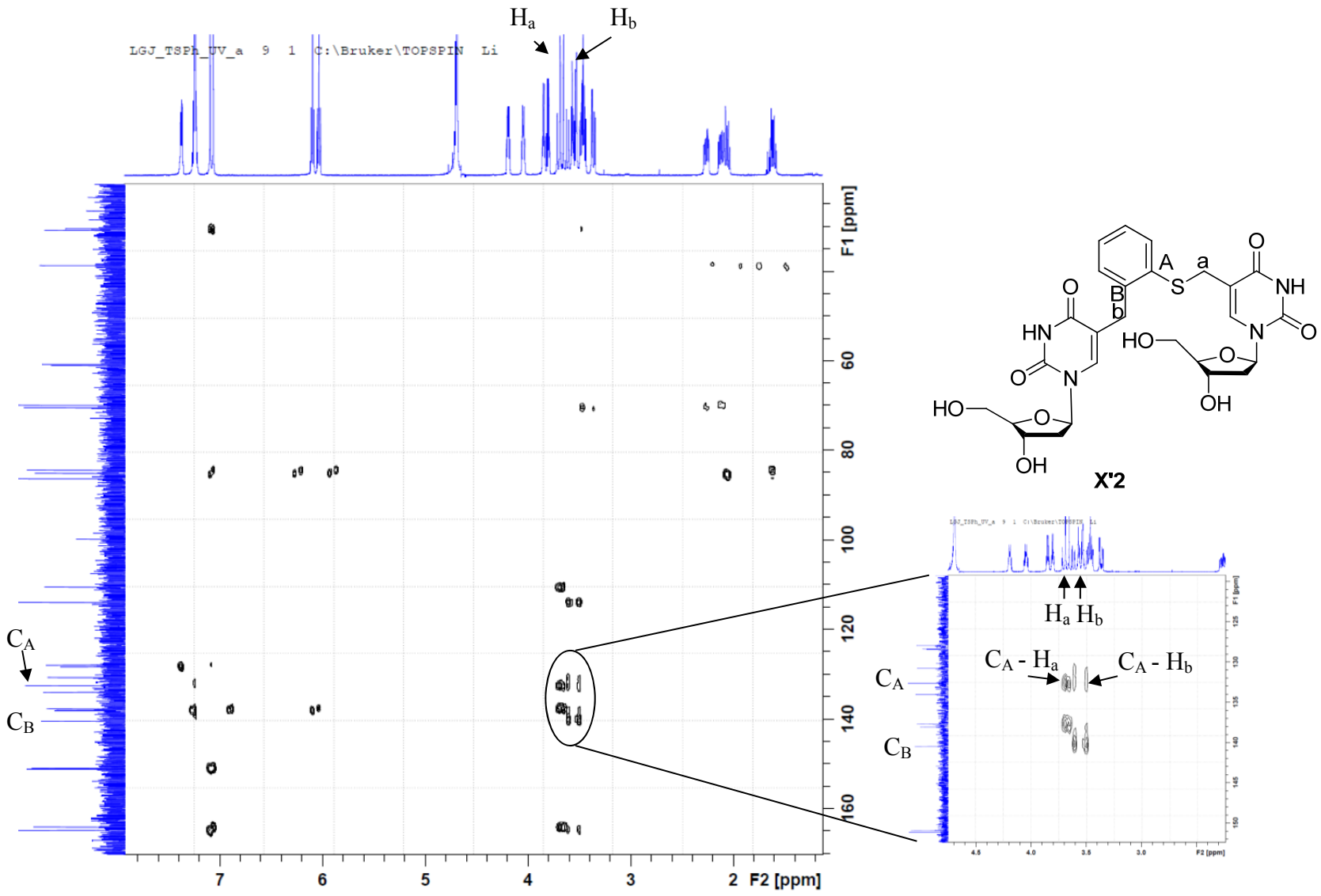


Figure S27. HMBC spectrum of addition-elimination product **X'2**.

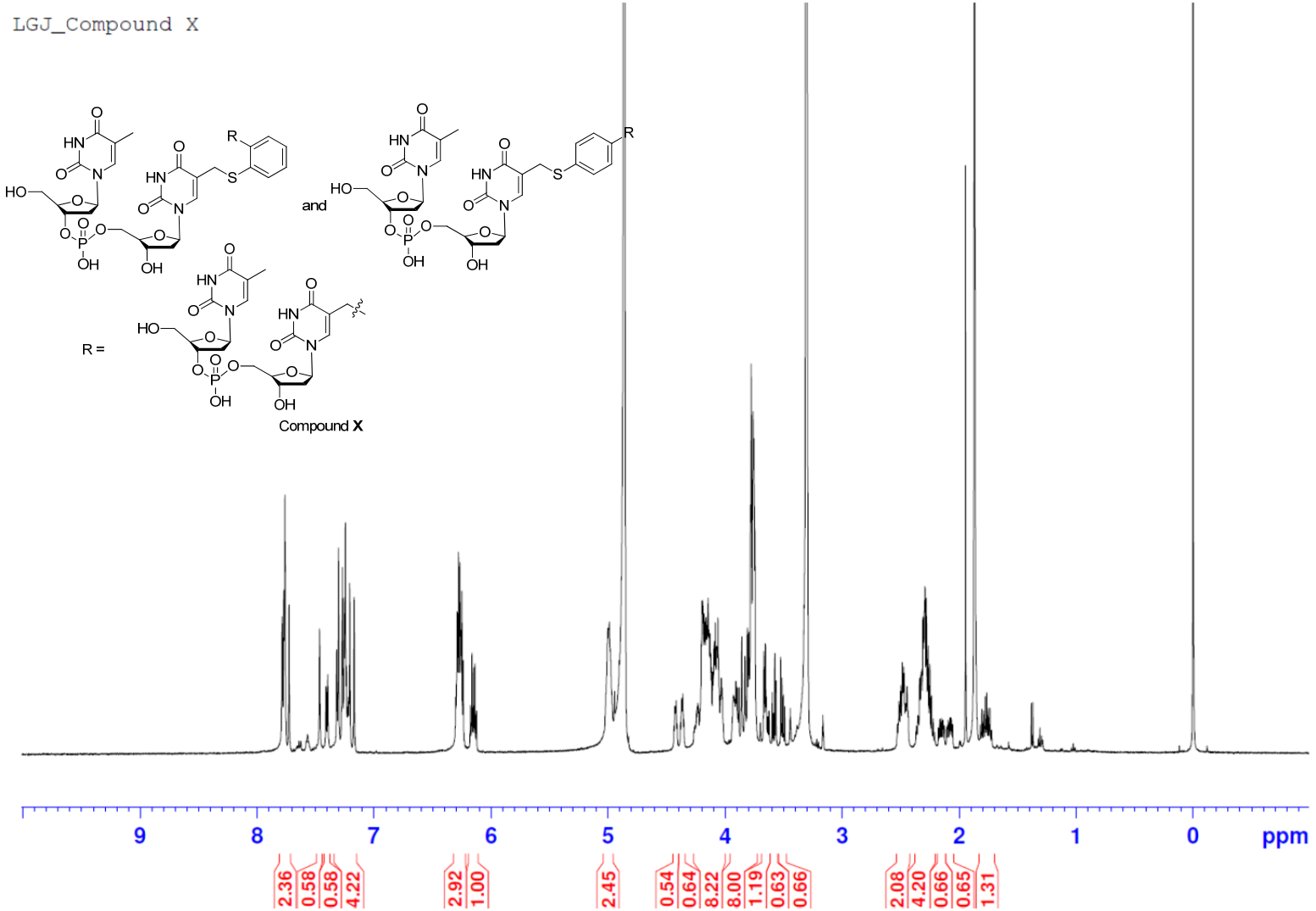


Figure S28. ^{1}H spectrum of addition-elimination product **X**. **X** is a mixture of the para and ortho addition products; therefore, no structural assignment can be obtained from the ^{1}H NMR spectrum.

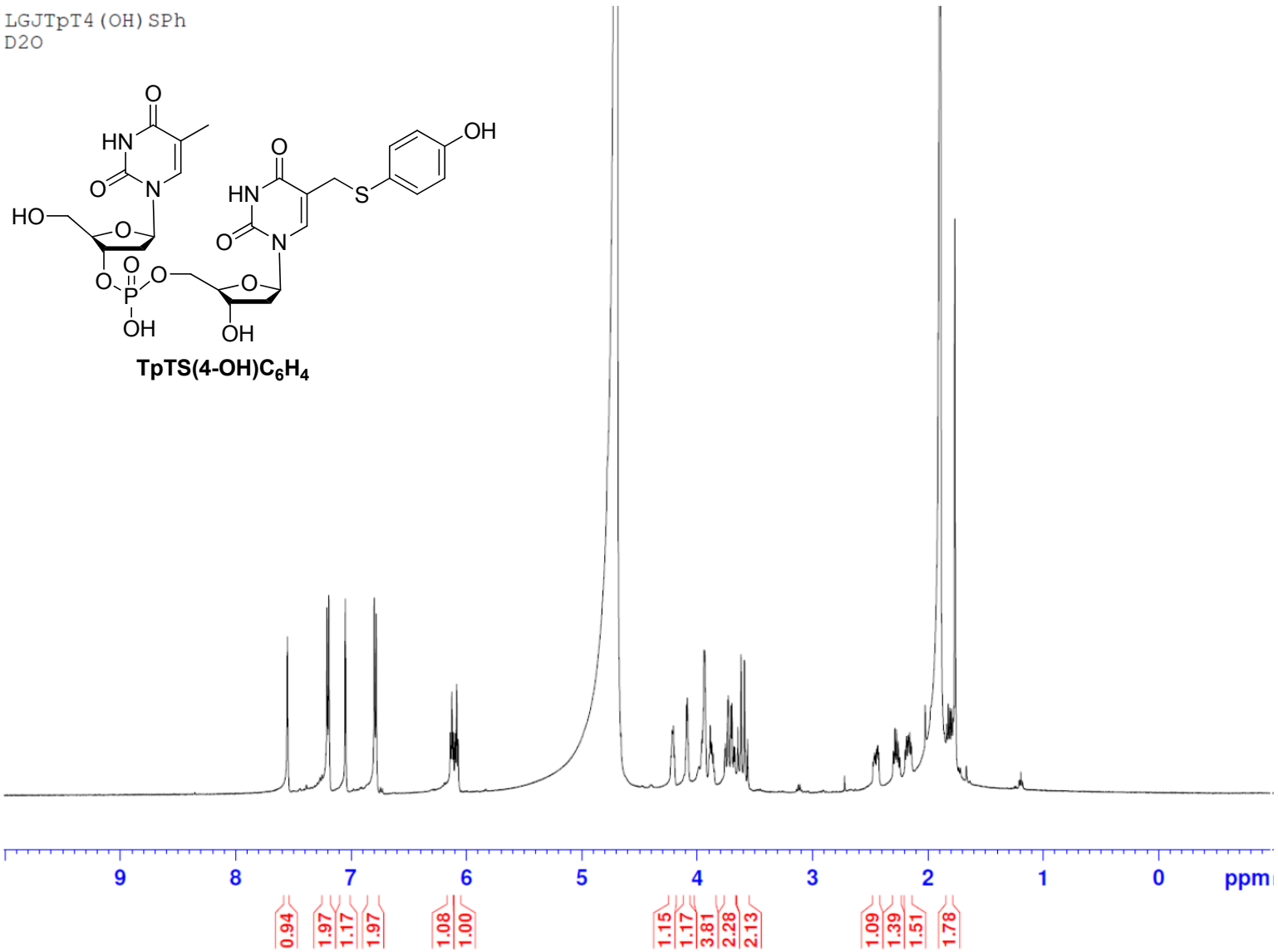


Figure S29. ^{1}H spectrum of **TpTS(4-OH)C_6H_4**.

References

(1) B. Bornemann, A. Marx, *Bioorg. Med. Chem.* **2006**, *14*, 6235-6238.

(2) L. Yang, G. Lin, R. S. Nelson, Y. Jian, J. Telser, L. Li, *Biochemistry* **2012**, *51*, 7173-7188.

(3) J. M. N. San Pedro, M. M. Greenberg, *Org. Lett.* **2012**, *14*, 2866-3869.