SUPPORTING INFORMATION FOR

A toolbox for enzymatic modification of nucleic acids with photosensitizers for photodynamic therapy

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1. Materials and methods

Chemicals and agents used for the reactions were purchased from Sigma-Aldrich, Alfa Aesar, Fluorochem and Fisher scientific. All compounds were purified on a Büchi Pure C-850 FlashPrep with silica gel (230-400 mesh) from Büchi. Reaction progress was monitored by Thin layer chromatography which was performed using glass-backed plates with a thin silica gel layer (0.25 mm, UV254) from Macherey-Nagel. Purifications procedures employed an Äkta pure apparatus from GE Healthcare paired with a Kinetex 5 µm C18 100 A LC Column by Phenomenex or a DNA Pac PA 100 oligonucleotide column from Fisher Scientific.

NMR spectral data were obtained on a Bruker Avance 400 MHz (^{1}H NMR: 400 MHz, ^{13}C: 101 MHz, ^{31}P: 162 MHz, ^{11}B: 128 MHz) and 500 MHz Bruker UltraShield Avance II (^{1}H NMR: 500 MHz, ^{13}C: 126 MHz, ^{31}P: 202 MHz) spectrometers and, with reference points set to solvent signals.

The Thermofisher Q Exactive MS recorded high-definition electrospray ionization (ESI) mass spectra in the positive-ion mode. Solution compositions were 1:1 MeCN/H_2O with a 0.1% inclusion of formic acid. MALDI-TOF measurements were conducted on a Bruker ultrafleXtreme. For nucleoside triphosphates MALDI-TOF analysis: With 9-aminoacridine serving as the matrix for nucleotide detection using a linear negative mode.

All DNA primers and templates were purchased from Integrated DNA Technologies and Mycrosynth. A variety of DNA polymerases (Phusion, Hemo Klen Taq, Q5, Taq, *Bst*, Therminator, Vent (*exo*$^{-}$), Deep Vent (*exo*$^{-}$), Dpo4 (*Sulfolobus*), Deep Vent (*exo*$^{-}$), Phi29, Klenow fragment of DNA polymerase I of *E. coli* (Kf (*exo*$^{-}$) and TdT) as well as natural dNTPs and polymerases buffers, were obtained from New England Biolabs (NEB). UV-Vis analysis were conducted using the UV-VIS CARY 3500 COMPACT PELTIER from Agilent Technologies. All analyses were performed in either 100 µL or 1 mL cuvettes.

Acrylamide/bis(acrylamide) (29:1, 40%) was acquired from Fisher Scientific. PAGE gel analysis was carried out via fluorescence imaging on a GE Healthcare Typhoon Trio phosphorimager. Agaroses Gel (2% and 4%). E-GEL (Agarose 2% and 4%) sample loading buffer 1X was also from the same provider. The were stained with SYBER® GREEN with a loading buffer.

2. Chemical synthesis

Synthesis of compound **1**

Chlorin e_6 (41 mg, 0.069 mmol, 1 equiv.) was combined with HBTU (31.2 mg, 0.082 mmol, 1.2 equiv.) and DIPEA (35.9 µL, 26.6 mg, 0.206 mmol, 3 equiv.) in 10 mL of DMF. The mixture was stirred at 50°C for 1 hour and shielded from light using aluminum foil. Subsequently, Compound 5 (5.9 mg, 0.069 mmol, 1 equiv.) dissolved in 1 mL of DMF was added dropwise over a 30-minute period. The resulting dark green mixture was then stirred at room temperature for 12 hours. Volatiles were evaporated, and the residue was purified by flash column chromatography using a gradient of 1-20% 0.6 M KNO_3 and 1-20% H_2O in ACN. This yielded a dark green solid (23 mg, 50% yield).

^{1}H NMR (400 MHz, MeOD) δ 9.66 (s, 1H), 9.47 (s, 1H), 9.01 (s, 1H), 7.93 (dd, *J* = 17.8, 11.6 Hz, 1H), 6.18 (dd, *J* = 17.9, 1.4 Hz, 1H), 6.02 (dd, *J* = 11.6, 1.4 Hz, 1H), 5.58 (d, *J* = 17.8 Hz, 1H), 5.32 – 5.22 (m, 1H), 4.52 (dd, *J* = 16.9, 9.2 Hz, 4H), 3.58 (d, *J* = 7.8 Hz, 2H), 3.50 (s, 3H), 3.36 (s, 3H), 3.29 (s, 4H), 3.05 (d, *J* = 7.1 Hz, 4H), 2.76 – 2.72 (m, 1H), 2.65 – 2.57 (m, 2H), 2.30 – 2.17 (m, 5H).

HRMS (ESI) Calcd for $C_{36}H_{40}N_8O_5$, $[M]^+$=665.3184 , found $[M]^+$=665.3184.

Synthesis of compound **2**

To a solution of 2,4-dimethylpyrrole (1 mL, 9.7 mmol) in dry DCM (50 mL), methyl 8-chloro-8-oxooctanoate (0.96 mL, 4.9 mmol) was added dropwise over a period of 10 min at room temperature. The deep red solution was refluxed for 30 min. After cooling, the triethylamine was added (4.1 mL, 29.1 mmol), and the solution was stirred for 30 min at room temperature. BF_3-Et_2O (5.4 mL, 43.7 mmol) was added dropwise, and the mixture was refluxed for 1 h 30. After cooling, triethylamine was added (4.1 mL, 29.1 mmol) and BF_3-Et_2O (5.4mL, 43.7 mmol) were added. The reaction was refluxed for 1h. The deep red solution was washed with sat. $NaHCO_3$ solution, then 1M HCl solution. The organic phase was separated and dried over $MgSO_4$. After removal of the solvent under reduced pressure the crude was dissolved in Et_2O and washed with water. After column chromatography in PET/DCM (4:1 → 1:1 → 0:1), **2** was obtained as an orange solid. Yield: 0.792 g (41%). The NMR characterization is consistent with that reported in the literature.[1]

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.05 (s, 2H), 3.67 (s, 3H), 2.97 – 2.89 (m, 2H), 2.51 (s, 6H), 2.41 (s, 6H), 2.32 (t, J = 7.4 Hz, 2H), 1.70 – 1.59 (m, 4H), 1.51 (dd, J = 8.8, 7.0 Hz, 2H), 1.40 (td, J = 8.2, 5.9 Hz, 2H)

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.1 (Cq), 153.9 (Cq), 146.4 (Cq), 140.3 (Cq), 131.5 (Cq), 121.6 (CH), 51.4 (CH_3), 34.0 (CH_2), 31.8 (CH_2), 30.1 (CH_2), 29.0 (CH_2), 28.4 (CH_2), 24.89 (CH_2), 16.4 (CH_3), 14.5 (CH_3)

^{11}B NMR (128 MHz, $CDCl_3$) δ 0.58 (t, J = 32.9 Hz)

^{19}F NMR (377 MHz, $CDCl_3$) δ - 146.5 (q, J = 32.1 Hz)

HRMS (ESI-TOF) Calcd for $C_{21}H_{29}BF_2N_2O_2$: 413.2182, found [M]+: 413.2166.

Synthesis of compound **3**

To a solution of **2** (0.300 g, 0.77 mmol) in THF/H_2O (23/23 mL) LiOH.H_2O (0.065 g, 1.54 mmol) was added and the reaction mixture was stirred at 40°C for 4 h 30. After removal of the solvent under reduced pressure the crude was dissolved in 6M HCl and stirred at room temperature for 30 min. After extraction with EtOAc, **3** was obtained as a brown solid. Yield: 0.282 g (97%). The NMR characterization is consistent with that reported in the literature.[1].

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.05 (s, 2H), 2.99 – 2.89 (m, 2H), 2.51 (s, 6H), 2.41 (s, 6H), 2.37 (t, *J* = 7.3 Hz, 2H), 1.71 – 1.61 (m, 4H), 1.52 (t, *J* = 7.9 Hz, 2H), 1.46 – 1.38 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 179.7 (Cq), 154.2 (Cq), 146.7 (Cq), 140.6 (Cq), 131.8 (Cq), 122.0 (CH), 34.2 (CH_2), 32.1 (CH_2), 30.4 (CH_2), 29.2 (CH_2), 28.7 (CH_2), 25.0 (CH_2), 16.8 (CH_3), 14.8 (CH_3).

^{11}B NMR (128 MHz, $CDCl_3$) δ 0.57 (t, *J* = 33.1 Hz).

^{19}F NMR (377 MHz, $CDCl_3$) δ - 146.6 (q, J = 33.4 Hz).

HRMS (ESI-TOF) Calcd for $C_{20}H_{27}BF_2N_2O_2$: 375.2061, found [M]+: 375.2046.

Synthesis of compound **4**

To a solution of **3** (0.100 g, 0.27 mmol) in MeOH (35 mL), I_2 (0.178 g, 0.70 mmol) and HIO_3 (0.094 g, 0.54 mmol) were added, and the reaction mixture was stirred at room temperature for 30 min. **4** was obtained as red crystalline powder after filtration of the precipitate formed. Yield: 0.100 g (59%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.02 (s, 2H), 2.61 (s, 6H), 2.47 (s, 6H), 2.38 (m, 2H), 1.79 – 1.32 (m, 8H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 178.2 (Cq), 155.8 (Cq), 146.4 (Cq), 142.6 (Cq), 131.8 (Cq), 86.8 (Cq), 33.9 (CH_2), 31.9 (CH_2), 30.33 (CH_2), 29.6 (CH_2), 29.2 (CH_2), 24.9 (CH_2), 19.4 (CH_3), 16.5 (CH_3).

^{11}B NMR (128 MHz, $CDCl_3$) δ 0.36 (t, *J* = 32.1 Hz).

^{19}F NMR (377 MHz, $CDCl_3$) δ -145.90 (q, J = 32.0 Hz).

HRMS (ESI-TOF) Calcd for $C_{20}H_{25}BF_2I_2N_2O_2$: 628.0061, found [M]+: 628.0078.

Singlet oxygen quantum yield: Φ_Δ = 51%.

Synthesis of compound **5**

To a solution of 6-bromohexanoyl chloride (0.74 mL, 4.9 mmol) in dry DCM (50 mL), a solution of 2,4-dimethylpyrrole (1 mL, 9.7 mmol) in dry DCM (20 mL) was added at 0°C. After returning to room temperature the solution was stirred for 3 h 30. After cooling to 0°C, the triethylamine was added (4.1 mL, 29.1 mmol), and the solution was stirred for 30 min at room temperature. BF_3-Et_2O (5.4mL, 43.7 mmol) was added dropwise, and the mixture was stirred at room

temperature overnight. The deep red solution was washed with sat. $NaHCO_3$ solution, then 1M HCl solution. The organic phase was separated and dried over $MgSO_4$. After removal of the solvent under reduced pressure the crude was dissolved in ET_2O and washed with water. After column chromatography in PET/DCM (4:1 → 1:1 → 0:1), **5** was obtained as an orange solid. Yield: 0.914 g (47%) The NMR characterization is consistent with that reported in the literature.[2]

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.05 (s, 2H), 3.43 (t, *J* = 6.6 Hz, 2H), 2.96 (ddd, *J* = 8.3, 4.6, 2.4 Hz, 2H), 2.51 (t, *J* = 1.3 Hz, 6H), 2.41 (s, 6H), 1.97 – 1.88 (m, 2H), 1.66 (dt, *J* = 7.8, 3.6 Hz, 4H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 154.1 (Cq), 146.0 (Cq), 140.4 (Cq), 131.5 (Cq), 121.8 (CH), 33.5 (CH_2), 32.4 (CH_2), 31.0 (CH_2), 28.7 (CH_2), 28.4 (CH_2), 16.6 (CH_3), 14.6 (CH_3).

^{11}B NMR (160 MHz, $CDCl_3$) δ 0.59 (t, *J* = 33.0 Hz).

^{19}F NMR (377 MHz, $CDCl_3$) δ -146.68 (q, *J* = 31.1 Hz).

HRMS (ESI-TOF) Calcd for $C_{18}H_{24}BBrF_2N_2$: 418.1113, found [M]+: 418.1154.

Synthesis of compound **6**

To a solution of **5** (0.050 g, 0.13 mmol) in dry THF (1 mL) ether crown 18-C-6 (0.110 g, 0.42 mmol) and NaN_3 (0.027 g, 0.42 mmol) were adder under argon atmosphere and the reaction mixture was stirred at room temperature overnight. After column chromatography in PET/DCM (7:3 → 1:1), **6** was obtained as an orange solid. Yield: 0.041 g (87%).

The NMR characterization is consistent with that reported in the literature.[3]

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.05 (s, 2H), 3.31 (t, J = 6.4 Hz, 2H), 2.98 – 2.89 (m, 2H), 2.51 (s, 6H), 2.40 (s, 6H), 1.71 – 1.52 (m, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 154.1 (Cq), 146.0 (Cq), 140.4 (Cq), 131.5 (Cq), 121.8 (CH), 33.5 (CH_2), 32.4 (CH_2), 31.1 (CH_2), 28.7 (CH_2), 28.4 (CH_2), 16.6 (CH_3), 14.6 (CH_3).

^{11}B NMR (128 MHz, $CDCl_3$) δ 0.56 (t, *J* = 33.1 Hz).

^{19}F NMR (377 MHz, $CDCl_3$) δ -146.58 (q, *J* = 32.7 Hz).

HRMS (ESI-TOF) Calcd for $C_{18}H_{24}BF_2N_5K$: 398.172441, found [M]+: 398.173361.

Synthesis of compound **7**

To a solution of **6** (0.020 g, 0.06 mmol) in MeOH (7 mL), I_2 (0.041 g, 0.16 mmol) and HIO_3 (0.021 g, 0.12 mmol) were added, and the reaction mixture was stirred at room temperature for 30 min. After column chromatography in PET/DCM (9:1 → 1:1), **7** was obtained as a red crystalline powder. Yield: 0.023 g (62%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (s, 2H), 3.33 (t, *J* = 6.3 Hz, 2H), 3.08 – 2.94 (m, 2H), 2.62 (s, 6H), 2.47 (s, 6H), 1.71 – 1.55 (m, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 155.5 (Cq), 145.6 (Cq), 142.2 (Cq), 131.4 (Cq), 86.6 (Cq), 51.2 (CH_2), 31.2 (CH_2), 29.1 (CH2), 28.7 (CH2), 27.3 (CH_2), 19.0 (CH_3), 16.2 (CH_3).

^{11}B NMR (128 MHz, $CDCl_3$): δ 0.37 (t, *J* = 32.1 Hz).

^{19}F NMR (377 MHz, $CDCl_3$): δ -145.79 (q, *J* = 32.0 Hz).

HRMS (ESI-TOF) Calcd for $C_{18}H_{22}BF_2I_2N_5Na$: 633.991799, found [M]+: 633.989604.

Singlet oxygen quantum yield: Φ_Δ = 57%.

Synthesis of **dU$^{E\text{-}Ce6}$TP**

The general protocol for CuAAC was applied with 4 µmol of **dU^{E}TP** (2.03 mg), 4.9 µmol of azide **1** (3.29 mg), and the reaction was carried out in a H_2O/MeCN/DMF 3:2:7 mixture. After application of the general HPLC purification method, **dU$^{E\text{-}Ce6}$TP** was obtained as a dark green solid (0.92 mg; 19%).

^{31}P NMR (162 MHz, D_2O) δ -10.89 (d, *J* = 23.4 Hz, 1P), -11.58 (t, *J* = 19.6 Hz, 1P), -23.30 (m, 1P).

HRMS (ESI) Calcd for $C_{47}H_{53}CuN_{10}O_{19}P_3^-$, (*z* = 2) $[M\text{-}H]^{2-}$ = 607.5926, found $[M\text{-}H]^{2-}$ = 607.5927.

Synthesis of **dU$^{CO\text{-}Ce6}$TP**

The general protocol for SPAAC was applied with 3.2 µmol (3 mg) of **dU^{CO}TP**, 3.8 µmol (2.5 mg) of azide **1**, and the reaction was carried out in a H_2O/DMF 1:1 mixture. After application of the general HPLC purification method, **dU$^{CO\text{-}Ce6}$TP** was obtained as a dark green solid (0.88 mg; 17%).

^{31}P NMR (162 MHz, D_2O) δ -10.70 (d, *J* = 20.1 Hz, 1P), -11.72 (d, *J* = 21.4 Hz, 1P), -23.24 (t, *J* = 17.7 Hz, 1P).

HRMS (ESI) Calcd for $C_{75}H_{86}N_{13}O_{22}P_3^-$, (z = 2) $[M\text{-}H]^{2-}$ = 805.7547, found $[M\text{-}H]^{2-}$ = 805.7539

MALDI-TOF (linear negative): 1613.600.

Synthesis of **dU$^{Am\text{-}Ce6}$TP**

The general protocol for amide bond formation was applied with 3.2 µmol of **dU^{Am}TP**, 3.8 µmol of Ce_6, and the reaction was carried out in a H_2O/DMF 3:4 mixture. After application of the general HPLC purification method, **dU$^{Am\text{-}Ce6}$TP** was obtained as a dark green solid (1.2 mg; 17%).

^{1}H NMR (400 MHz, D_2O) δ 9.96 (s, 2H), 9.34 (s, 2H), 8.03 (m, 2H), 6.81 (t, J = 7.9 Hz, 1H), 6.37 (t, J = 7.8 Hz, 2H), 6.22 (t, J = 7.2 Hz, 2H), 5.69 (s, 2H), 5.17 (m, 5H), 4.22 (s, 1H), 3.83 (m, 4H), 3.52 (m, 6H), 3.36 (m, 4H), 2.41 (m, 10H), 1.58 (m, 9H).

^{31}P NMR (162 MHz, D_2O) δ -10.52 (d, *J* = 19.4 Hz, 1P), -11.88 (d, *J* = 20.8 Hz, 1P), -23.32 (t, *J* = 20.5 Hz, 1P).

HRMS (ESI) Calcd for $C_{52}H_{63}N_8O_{20}P_3^-$, (z = 2) $[M-2H]^{2-}$ = 605.1613, found $[M-2H]^{2-}$ = 605.1610.

MALDI-TOF (linear negative): 1210.734.

Synthesis of **dU$^{E\text{-}MB}$TP**

The general protocol for CuAAC was applied with 5.4 µmol (2.7 mg) of **dU^{E}TP**, 1.8 µmol (1 mg) of commercially available methylene blue azide, and the reaction was carried out in a H_2O/MeCN/DMF 2:1:1 mixture. After application of the general HPLC purification method, **dU$^{E\text{-}MB}$TP** was obtained as a blue solid (1.1 mg; 58%).

^{1}H NMR (500 MHz, D_2O) δ 8.00 (s, 1H), 7.90 (s, 1H), 7.61 – 7.35 (m, 2H), 7.14 (m, 2H), 6.96 (m, 2H), 6.01 (t, *J* = 6.8 Hz, 1H), 4.47 (m, 5H), 4.24 – 4.04 (m, 5H), 3.85 (t, *J* = 5.2 Hz, 3H), 3.56 (m, 16H), 3.39 – 3.25 (m, 6H), 2.35 (s, 2H), 2.27 (m, 3H).

^{31}P NMR (202 MHz, D_2O) δ -10.88 (d, *J* = 19.9 Hz, 1P), -11.47 (d, *J* = 20.3 Hz, 1P), -23.30 (t, *J* = 20.2 Hz, 1P).

HRMS (ESI) Calcd for $C_{38}H_{53}N_9O_{18}P_3S^-$, $[M-H]^-$= 1046.2291, found $[M-H]^-$= 1046.2298.

MALDI-TOF (linear negative): 1047.870.

Synthesis of **dU$^{CO\text{-}MB}$TP**

The general protocol for SPAAC was applied with 3 µmol (2.8 mg) of **dU^{CO}TP**, 1.5 µmol (1 mg) of commercially available methylene blue azide, and the reaction was carried out in a H_2O/DMF 2:1 mixture. After application of the general HPLC purification method, **dU$^{CO\text{-}MB}$TP** was obtained as a blue solid (1.7 mg; 67 %).

^{1}H NMR (500 MHz, D_2O) δ 7.79 (d, *J* = 3.0 Hz, 1H), 7.64 – 7.28 (m, 8H), 7.26 – 7.08 (m, 5H), 6.91 (m, 3H),5.84 (t, *J*= 8 Hz, 1H), 5.72 (d, *J* = 4.8 Hz, 1H), 4.53-4.29 (m, 4H), 4.20 – 3.84 (m, 7H), 3.77 – 3.26 (m, 17H), 3.01 (q, *J* = 7.42 Hz, 4H), 2.37 – 2.13 (m, 6H), 2.08 (s, 2H), 1.72 – 1.38 (m, 5H), 1.18 – 1.02 (m, 5H).

^{31}P NMR (202 MHz, D_2O) δ -10.58 (d, *J* = 15.6 Hz, 1P), -11.50 (d, *J* = 20.4 Hz, 1P), -22.87 (t, *J* = 18.0 Hz, 1P).

HRMS (ESI) Calcd for $C_{66}H_{84}N_{12}O_{21}P_3S^-$, $[M^+-2H]^-$ = 1503.4657, found $[M^+-2H]^-$ = 1503.4644.

MALDI-TOF (linear negative): 1506.44

Synthesis of **dU$^{Am\text{-}MB}$TP**

The general protocol for amide bond formation was applied with 5.1 µmol (3.2 mg) of **dU^{Am}TP**, 2.55 µmol (1 mg) of commercially available methylene blue carboxylic acid, and the reaction was carried out in a H_2O/DMF 1:1 mixture. After application of the general HPLC purification method, **dU$^{Am\text{-}MB}$TP** was obtained as a blue solid (1.8 mg; 73%).

^{1}H NMR (500 MHz. D_2O) δ 7.75 (s, 1H), 7.63 (m, 2H), 7.24 (d, *J* = 9.7 Hz, 2H), 7.06 (d, *J* = 10.9 Hz, 2H), 5.90 (t, *J* = 5.4 Hz, 1H), 4.49 (s, 1H), 4.07 (m, 4H), 3.80 (s, 2H), 3.57 (s, 2H), 3.24 (m, 8H), 2.36 – 2.01 (m, 7H), 1.60 – 1.39 (m, 4H).

^{31}P NMR (202 MHz, D_2O) δ -10.61 (d, *J* = 22.1 Hz, 1P), -11.60 (d, *J* = 20.4 Hz, 1P), -23.02 (t, *J* = 28.5 Hz, 1P).

HRMS (ESI) Calcd for $C_{36}H_{47}N_7O_{16}P_3S^-$, $[M^+-2H]^-$= 970.2018, found $[M^+-2H]^-$= 970.2033.

MALDI-TOF (linear negative): 971.826.

Synthesis of **dU$^{E\text{-}BDP}$TP**

The general protocol for CuAAC was applied with 4.1 µmol (2 mg) of **dU^{E}TP**, 5.3 µmol (3.2 mg) of azide **7**, and the reaction was carried out in a H_2O/MeCN/DMF/DMSO 4:1:3:10 mixture. After application of the general HPLC purification method, **dU$^{E\text{-}BDP}$TP** was obtained as a pink solid (1.1 mg; 25%).

^{1}H NMR (400 MHz, D_2O) δ 8.13 (s, 2H), 6.07 (m, 1H), 4.37 (m, 4H), 4.07 (m, 4H), 2.98 – 2.81 (m, 1H), 2.24 (m,10H), 2.10 (s, 3H), 1.93 (m, 7H), 1.31 (d, *J* = 7.3 Hz, 1H).

^{31}P NMR (202 MHz, D_2O) δ -11.00 (d, *J* = 20.4 Hz 1P), -11.58 (d, *J* = 20.8 Hz, 1P), -23.46 (d, *J* = 25.4 Hz, 1P).

HRMS (ESI) Calcd for $C_{29}H_{37}BF_2I_2N_7O_{14}P_3^-$, $[M-H]^-$= 1101.9693, found $[M-H]^-$= 1101.9700.

MALDI-TOF (linear negative): 1102.069.

Synthesis of **dU$^{CO\text{-}BDP}$TP**

The general protocol for SPAAC was applied with 3.2 µmol (3.0 mg) of **dU^{CO}TP**, 4.7 µmol (2.9 mg) of azide **7**, and the reaction was carried out in a H_2O/DMF/DMSO 3:3:10 mixture. After application of the general HPLC purification method, **dU$^{CO\text{-}BDP}$TP** was obtained as a pink solid (0.9 mg; 18%).

^{1}H NMR (400 MHz, D_2O) δ 7.90 (s, 1H), 7.27 (m, 10H), 5.96 (t, *J* = 8.7 Hz 1H), 4.45 (m, 4H), 4.03 (m, 8H), 2.18 (m, 10H), 2.10 (m, 5H), 2.02 – 1.80 (m, 9H), 1.78 (m, 5H).

^{31}P NMR (162 MHz, D_2O) δ -10.20 (d, *J* = 20.1 Hz, 1P), -11.47 (d, *J* = 20.4 Hz, 1P), -23.06 (t, *J* = 21.5 Hz, 1P).

HRMS (ESI) Calcd for $C_{57}H_{68}BF_2I_2N_{10}O_{17}P_3^-$, $[M-H]^-$= 1559.2062, found $[M-H]^-$= 1559.2039.

MALDI-TOF (linear negative): 1559.215.

Synthesis of **dU$^{Am\text{-}BDP}$TP**

The general protocol for amide bond formation was applied with 4.5 µmol of **dU^{Am}TP** (2.85 mg), 3 µmol of carboxylic acid **4** (1.88 mg), and the reaction was carried out in a H_2O/DMF 3:4

mixture. After application of the general HPLC purification method, **dU$^{Am\text{-}BDP}$TP** was obtained as a pink solid (1.6 mg; 43%).

^{1}H NMR (500 MHz, D_2O) δ 7.88 (s, 1H), 5.86 (t, *J* = 4.5 Hz, 1H), 4.41 (s, 1H), 3.98 (m, 4H), 2.10 (m, 16H), 1.56 – 1.16 (m, 7H).

^{31}P NMR (202 MHz, D_2O) δ -11.05 (d, *J* = 19.9 Hz, 1P), -11.78 (d, *J* = 20.1 Hz, 1P), -23.46 (t, *J* = 19.7 Hz, 1P).

HRMS (ESI) Calcd for $C_{38}H_{52}BF_2I_2N_6O_{16}P_3^-$, $[M\text{-}H]^-$= 1243.0736, found $[M\text{-}H]^-$= 1243.0746.

MALDI-TOF (linear negative): 1242.740.

Synthesis of **dU$^{E\text{-}Pery}$TP**

The general protocol for CuAAC was applied with 6.1 µmol (3 mg) of **dU^{E}TP**, 7.3 µmol (3.3 mg) of commercially available perylene azide, and the reaction was carried out in a H_2O/MeCN/DMF 8:3:18 mixture. After application of the general HPLC purification method, **dU$^{E\text{-}Pery}$TP** was obtained as a yellow solid (1.3 mg; 22%).

^{1}H NMR (400 MHz, D_2O) δ 7.84 (dd, J = 12.8, 7.5 Hz, 2H), 7.75 – 7.66 (m, 3H), 7.62 (t, J = 7.1 Hz, 2H), 7.50 (d, J = 8.4 Hz, 1H), 7.36 (q, J = 8.2 Hz, 2H), 7.31 (d, J = 1.5 Hz, 1H), 7.23 (dd, J = 7.7, 1.4 Hz, 1H), 7.04 (t, J = 7.7 Hz, 1H), 5.16 (t, J = 6.3 Hz, 1H), 4.45 – 4.36 (m, 2H), 4.20 (s, 1H), 3.90 (t, J = 4.9 Hz, 2H), 3.87 – 3.76 (m, 9H), 1.92 (d, J = 1.4 Hz, 8H).

^{31}P NMR (162 MHz, D_2O) δ -10.92 (d, *J* = 19.3 Hz, 1P), -11.52 (d, *J* = 20.2 Hz, 1P), -23.34 (t, *J* = 19.9 Hz, 1P).

HRMS (ESI) Calcd for $C_{38}H_{39}N_6O_{17}P_3^-$, $[M\text{-}H]^-$= 943.1512, found $[M\text{-}H]^-$= 943.1511.

MALDI-TOF (linear negative): 943.045.

Synthesis of **dU$^{CO\text{-}Pery}$TP**

The general protocol for SPAAC was applied with 3.2 µmol (3.0 mg) of **dU^{CO}TP**, 3.8 µmol (1.7 mg) of commercially available perylene azide, and the reaction was carried out in a H_2O/DMF 3:8 mixture. After application of the general HPLC purification method, **dU$^{CO\text{-}Pery}$TP** was obtained as a yellow solid (1.3 mg; 24%).

^{1}H NMR (400 MHz, D_2O) δ 7.92 – 7.77 (m, 2H), 7.70 – 7.40 (m, 5H), 7.41 – 7.03 (m, 5H), 7.00 – 6.80 (m, 3H), 6.79 – 6.51 (m, 3H), 6.40 (d, *J* = 7.3 Hz, 1H), 5.56 (d, *J* = 6.8 Hz, 1H), 5.22 (s, 2H), 4.36 (m, 2H), 4.17 – 3.88 (m, 7H), 3.69 (m, 7H), 3.37 (m, 4H), 3.00 (m, 2H), 2.28 (m, 2H), 1.89 (d, *J* = 1.2 Hz, 6H), 1.52 (m, 6H), 1.08 (t, *J* = 7.5 Hz, 4H).

^{31}P NMR (162 MHz, D_2O) δ -10.50 (d, *J* = 20.4 Hz, 1P), -11.56 (d, *J* = 20.4 Hz, 1P), -23.08 (t, *J* = 20.9 Hz, 1P).

HRMS (ESI) Calcd for $C_{67}H_{74}N_9O_{20}P_3^-$, $[M\text{-}H]^-$= 1400.3877, found $[M\text{-}H]^-$=1400.3880

MALDI-TOF (linear negative): 1401.512.

3. Oligonucleotides

Table S1. Sequence composition of templates and primers used in primer extension reactions

	Oligonucleotide sequence
P1	5'-FAM-TAC GAC TCA CTA TAG CCT C-3'
T1	3'-ATG CTG AGT GAT ATC GGA G**A**-5'
T2	3'-GTA CCC GCC GTA CCC TG**A** CTC G**A**G T**A**C G**A**T C-5'
P2	5'-FAM-CAT GGG CGG CAT GGG-3'
P3	5'-FAM-GGA TCC GAG CTC CAC GTG-3'
T3	3'-CCT AGG CTC GAG GTG CAC CG**A** TCC **A**CT C**A**C T**A**C **A**CT T**A**C C**A**C T**A**C TC**A** T**A**C CCC G**A**T CGC **A**GC TGG C**A**T GC-5'
P4	5'-FAM-GTG GTG CGA AAT TTC TGA C-3'
P5	3'-CGT ACA GTG ACT GCA CTC AC-5'
T4	3'-*CACCACGCTTTAAAGACTG* ATC GTG CAA TGC ACG TGA TCA CTG CTG ATC AGT AGC CGT A *CGTACAGTGACTGCACTCAC*-5'

[a] red bold As represent templating dA nucleotides and italicized regions represent primer binding regions.

4. Additional gel images

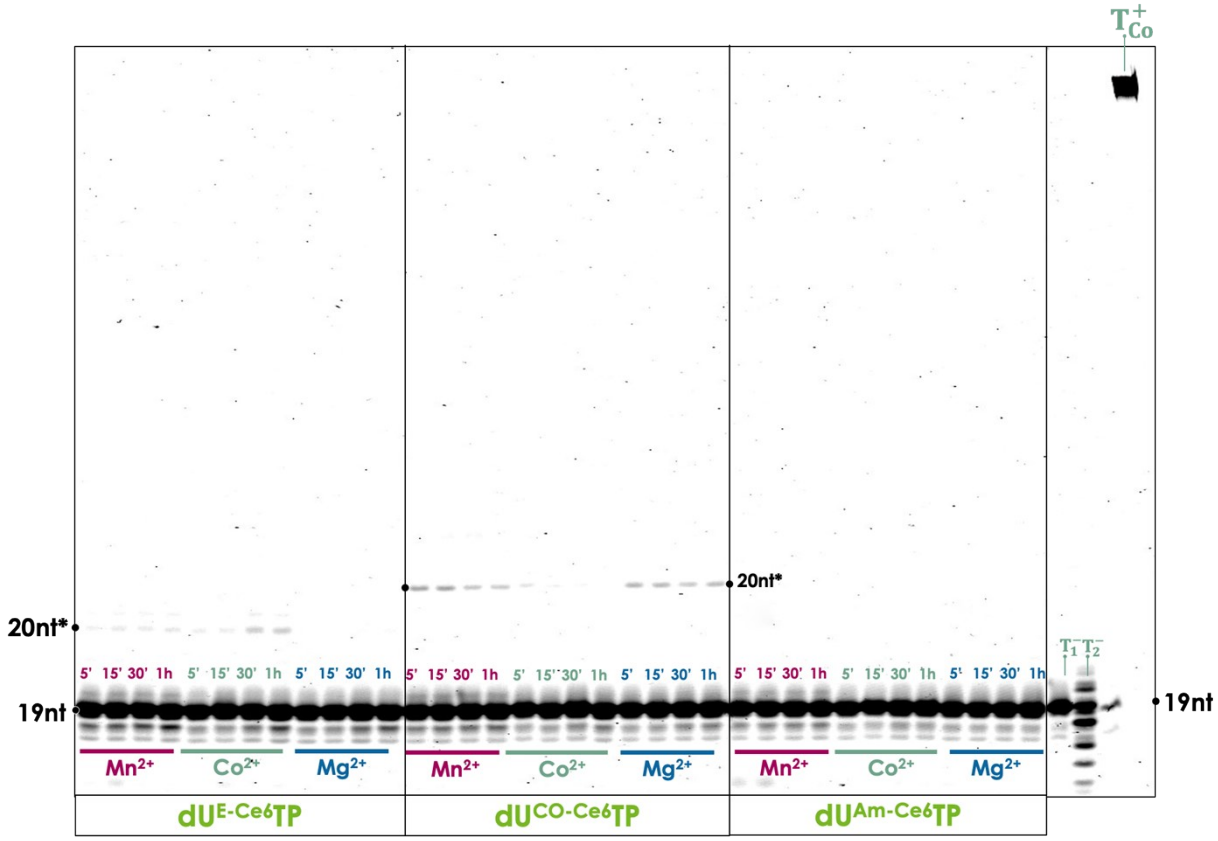


Fig. S1. PAGE gel (20%) analysis of tailing reactions with methylene blue containing nucleotides. Reactions were conducted with TdT (20 U) using primer **P1** (2 pmol) with 1 µL TdT 10X buffer, with 200 µM of either **dU$^{E\text{-}Ce6}$TP**, **dU$^{CO\text{-}Ce6}$TP**, or **dU$^{Am\text{-}Ce6}$TP** in the presence of either 1 mM Mn^{2+}, 0.25 mM Co^{2+}, or 1 mM Mg^{2+}. The reactions with modified nucleotides were incubated for 5 min, 15 min, 30 min and 1 h at 37°C. Controls: (T^{-}_{1}) negative control without TdT; (T^{-}_{2}) negative control without dTTP; (T^{+}_{Mn}; T^{+}_{Co};T^{+}_{Mg}) Positive control with a final concentration of 200 µM dTTP and 1 mM Mn^{2+}, 0.25 mM Co^{2+}, or 1 mM Mg^{2+} and 1 h incubation.

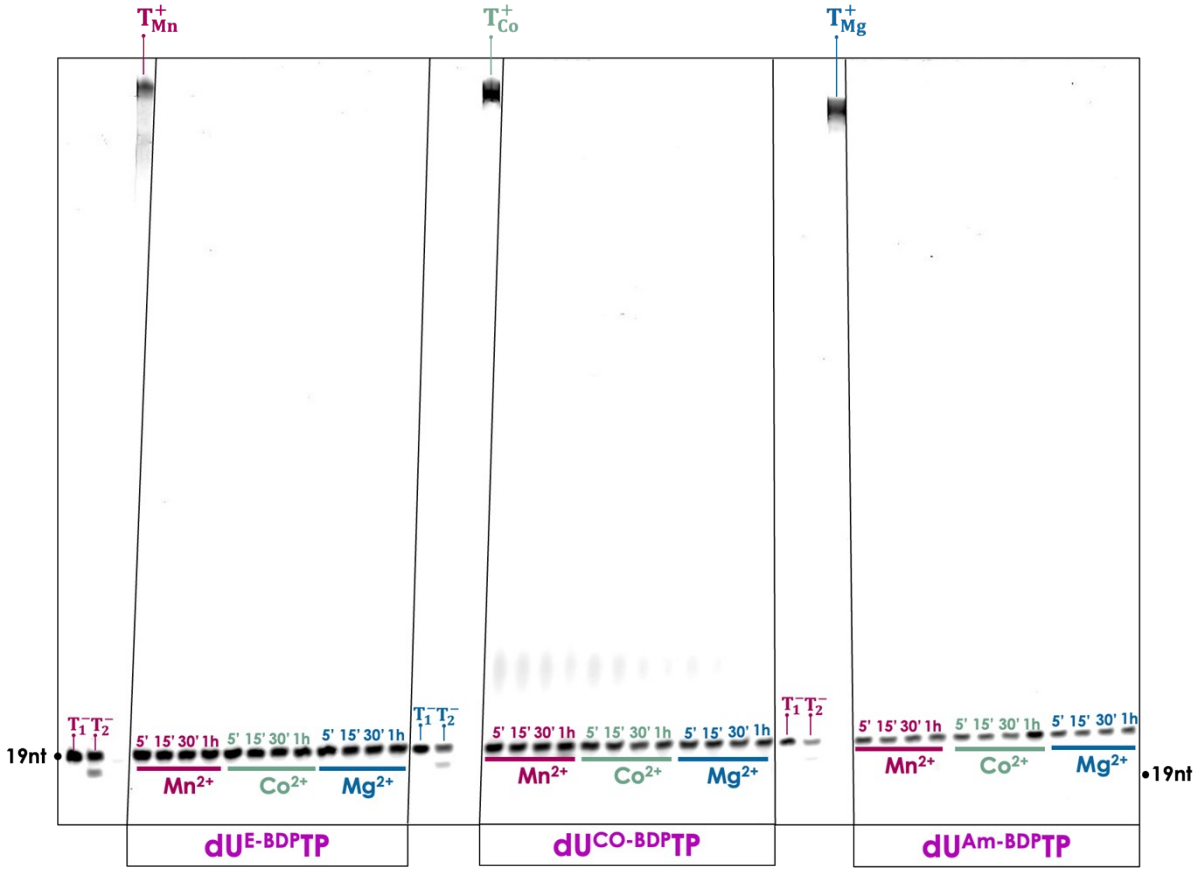


Fig. S2. PAGE gel (20%) analysis of tailing reactions with methylene blue containing nucleotides. Reactions were conducted with TdT (20 U) using primer **P1** (2 pmol) with 1 µL TdT 10X buffer, with 200 µM of either **dU$^{E\text{-}BDP}$TP**, **dU$^{CO\text{-}BDP}$TP**, or **dU$^{Am\text{-}BDP}$TP** in the presence of either 1 mM Mn^{2+}, 0.25 mM Co^{2+}, or 1 mM Mg^{2+}. The reactions with modified nucleotides were incubated for 5 min, 15 min, 30 min and 1 h at 37°C. Controls: (T^-_1) negative control without TdT; (T^-_2) negative control without dTTP; (T^+_{Mn}; T^+_{Co};T^+_{Mg}) Positive control with a final concentration of 200 µM dTTP and 1 mM Mn^{2+}, 0.25 mM Co^{2+}, or 1 mM Mg^{2+} and 1 h incubation.

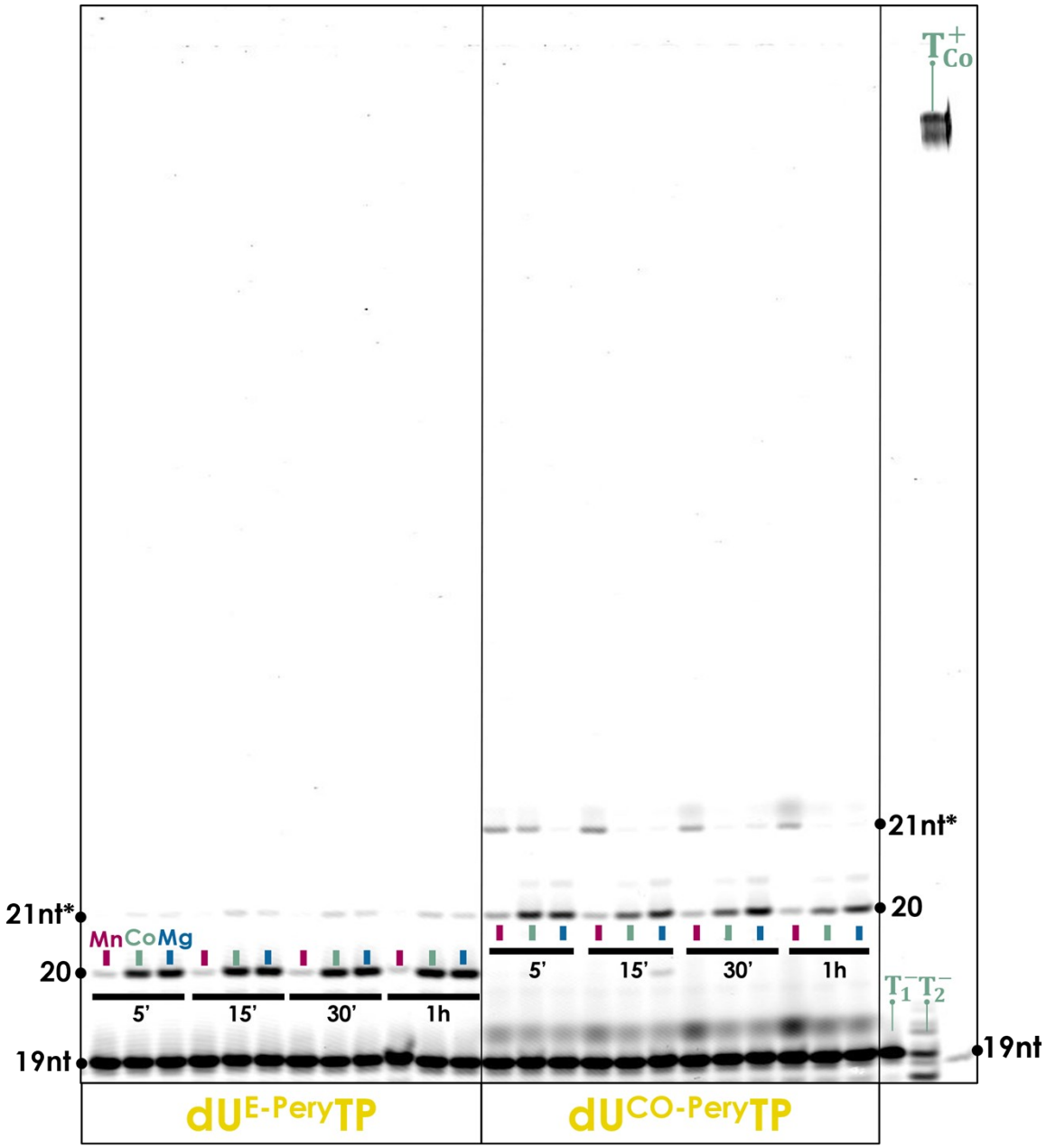


Fig. S2. PAGE gel (20%) analysis of tailing reactions with methylene blue containing nucleotides. Reactions were conducted with TdT (20 U) using primer **P1** (2 pmol) with 1 µL TdT 10X buffer, with 200 µM of either **dU$^{E\text{-}Pery}$TP** or **dU$^{CO\text{-}Pery}$TP** in the presence of either 1 mM Mn^{2+}, 0.25 mM Co^{2+}, or 1 mM Mg^{2+}. The reactions with modified nucleotides were incubated for 5 min, 15 min, 30 min and 1 h at 37°C. Controls: (T^{-}_{1}) negative control without TdT; (T^{-}_{2}) negative control without dTTP; (T^{+}_{Mn}; T^{+}_{Co};T^{+}_{Mg}) Positive control with a final concentration of 200 µM dTTP and 1 mM Mn^{2+}, 0.25 mM Co^{2+}, or 1 mM Mg^{2+} and 1 h incubation.

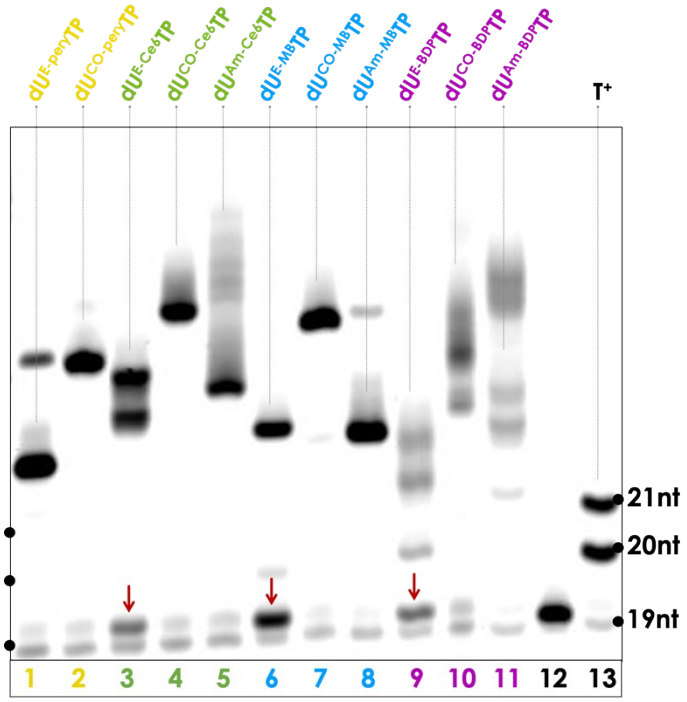


Fig. S3. Gel (PAGE 20%) analysis of PEX reactions carried out with primer **P1** (10 pmol), template **T1** (15 pmol), with all PS-modified nucleotides (200 µM), Vent (*exo*$^-$) (5U) at 60°C for 2 h. Lane 1: **dU$^{E\text{-}Pery}$TP**; lane 2: **dU$^{CO\text{-}Pery}$TP**; lane 3: **dU$^{E\text{-}Ce6}$TP**; lane 4: **dU$^{CO\text{-}Ce6}$TP**; lane 5: **dU$^{Am\text{-}Ce6}$TP**; lane 6: **dU$^{E\text{-}MB}$TP**; lane 7: **dU$^{CO\text{-}MB}$TP**; lane 8: **dU$^{Am\text{-}MB}$TP**; lane 9: **dU$^{E\text{-}BDP}$TP**; lane 10: **dU$^{CO\text{-}BDP}$TP**; lane 11: **dU$^{Am\text{-}BDP}$TP**; lane 12: Negative control reaction without polymerase; lane 13: positive control reaction with natural dTTP. 19 nt corresponds to the length of **P1**, 20 and 21 nt corresponds to the n+1 and n+2 products obtained with dTTP. Red arrows indicate incomplete reactions.

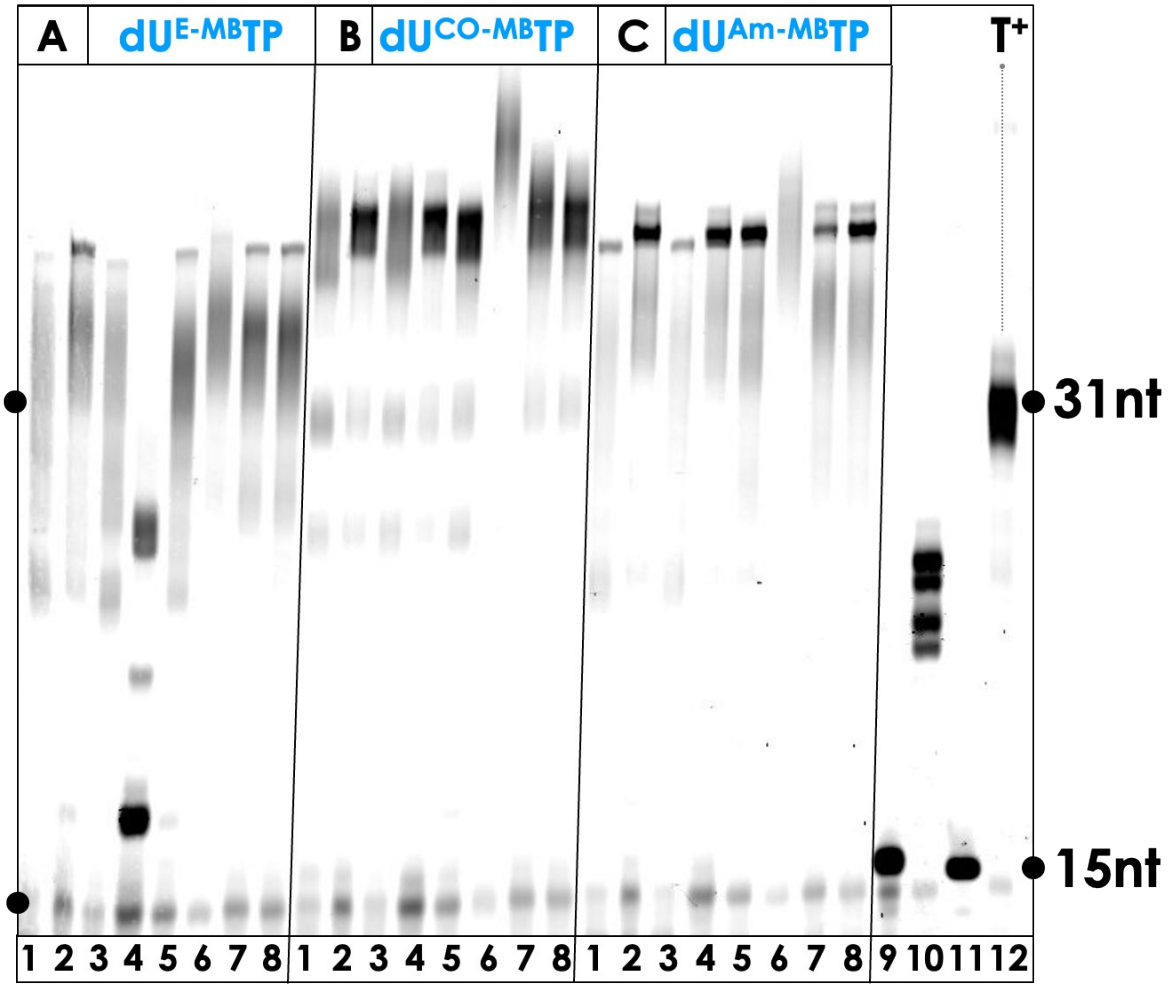


Fig. S4. PAGE gel (20%) analysis of PEX reactions (3 hour) using primer **P2** (10 pmol) and template **T2** (15 pmol) with natural dNTPs (200 μM) and the modified triphosphates (200 μM) **dU$^{E\text{-}MB}$TP**, **dU$^{CO\text{-}MB}$TP**, and **dU$^{Am\text{-}MB}$TP**. Polymerases used: lane 1) Phusion (2 U); lane 2) Hemo KlenTaq (8 U); lane 3) Q5 (2 U); lane 4) Taq (5 U); lane 5) *Bst* (8 U); lane 6) Therminator (2 U); lane 7) Vent (*exo*$^-$) (2 U); lane 8) Deep Vent (*exo*$^-$) (2 U); lane 9) Negative control reaction without polymerase; 10) Negative control reaction with only dATP, dGTP, and dCTP; lane 11) Primer **P2** 12) positive control reaction with natural dNTPs and Vent (*exo*$^-$) (2 U). 15 nucleotides (nt) correspond to **P2** and 31nt to full-length product.

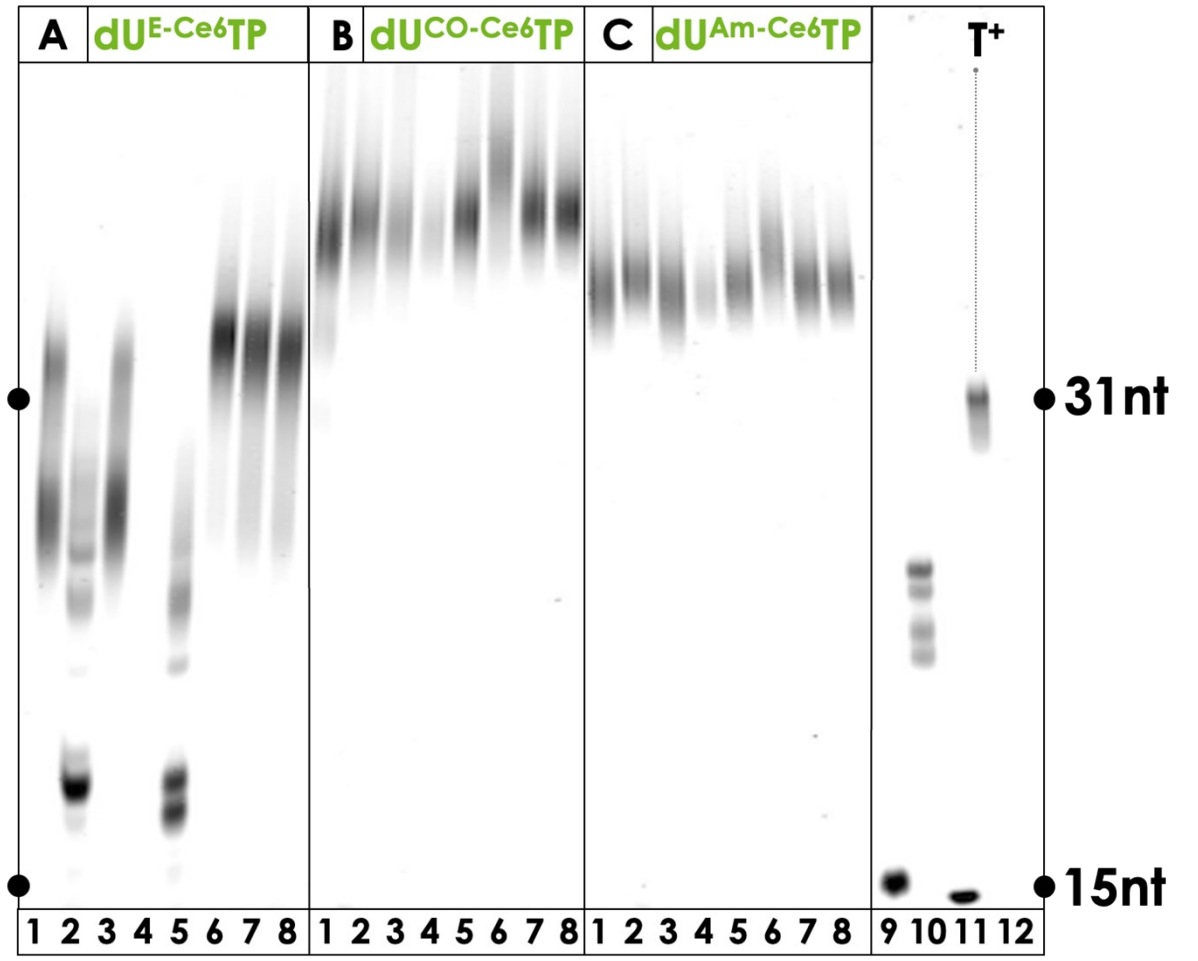


Fig. S5. PAGE gel (20%) analysis of PEX reactions (3 hour) using primer **P2** (10 pmol) and template **T2** (15 pmol) with natural dNTPs (200 μM) and the modified triphosphates (200 μM) **dU$^{E\text{-}Ce6}$TP**, **dU$^{CO\text{-}Ce6}$TP**, and **dU$^{Am\text{-}Ce6}$TP**. Polymerases used: lane 1) Phusion (2 U); lane 2) Hemo KlenTaq (8 U); lane 3) Q5 (2 U); lane 4) Taq (5 U); lane 5) *Bst* (8 U); lane 6) Therminator (2 U); lane 7) Vent (*exo*$^-$) (2 U); lane 8) Deep Vent (*exo*$^-$) (2 U); lane 9) Negative control reaction without polymerase; 10) Negative control reaction with only dATP, dGTP, and dCTP; lane 11) Primer **P2** 12) positive control reaction with natural dNTPs and Vent (*exo*$^-$) (2 U). 15 nucleotides (nt) correspond to **P2** and 31nt to full-length product.

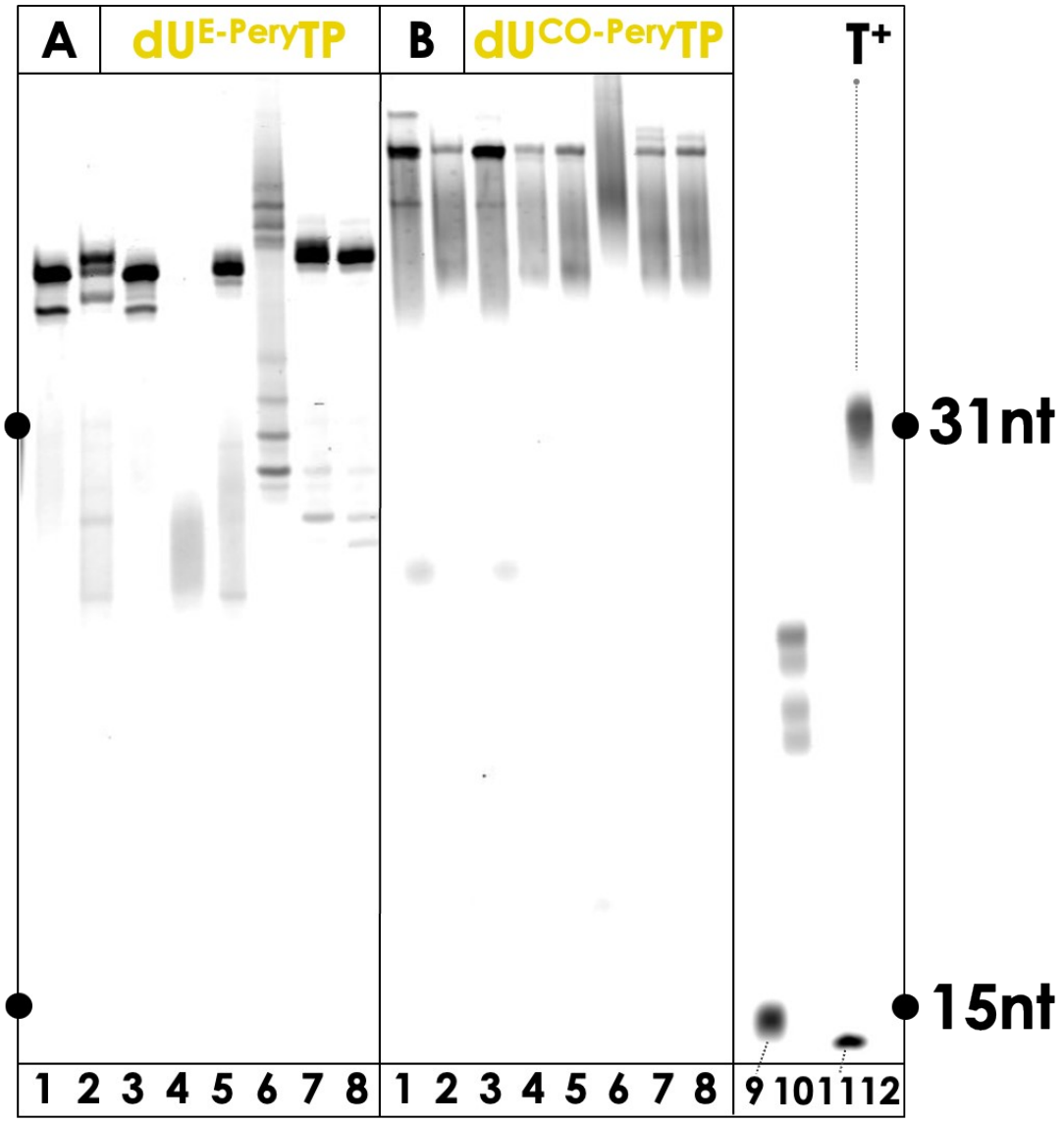


Fig. S6. PAGE gel (20%) analysis of PEX reactions (3 hour) using primer **P2** (10 pmol) and template **T2** (15 pmol) with natural dNTPs (200 μM) and the modified triphosphates (200 μM) **dU$^{E\text{-}Pery}$TP** and **dU$^{CO\text{-}Pery}$TP**. Polymerases used: lane 1) Phusion (2 U); lane 2) Hemo KlenTaq (8 U); lane 3) Q5 (2 U); lane 4) Taq (5 U); lane 5) *Bst* (8 U); lane 6) Therminator (2 U); lane 7) Vent (*exo*$^-$) (2 U); lane 8) Deep Vent (*exo*$^-$) (2 U); lane 9) Negative control reaction without polymerase; 10) Negative control reaction with only dATP, dGTP, and dCTP; lane 11) Primer **P2** 12) positive control reaction with natural dNTPs and Vent (*exo*$^-$) (2 U). 15 nucleotides (nt) correspond to **P2** and 31nt to full-length product.

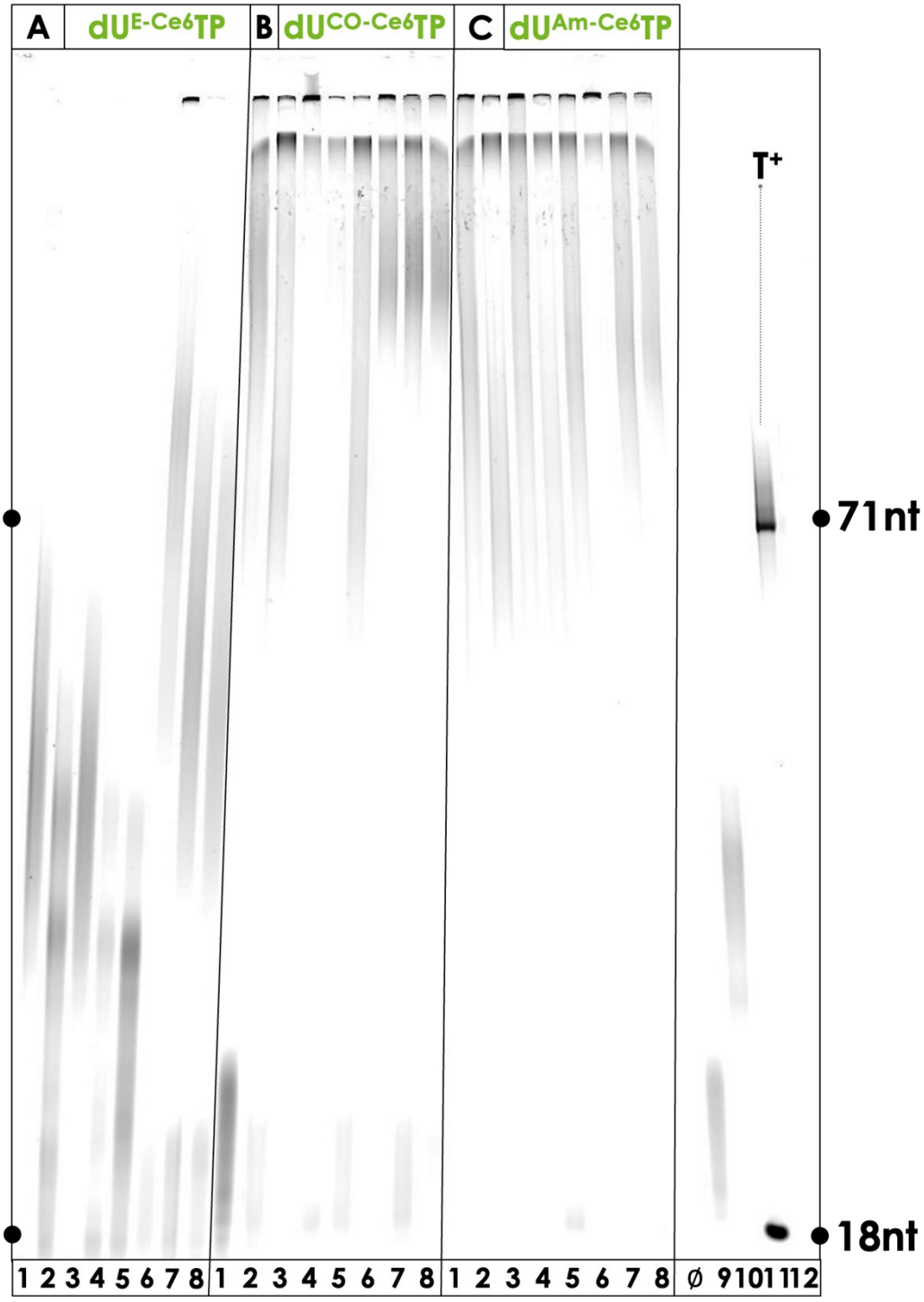


Fig. S7. PAGE gel (20%) analysis of PEX reactions (3 hour) using primer **P3** (10 pmol) and template **T3** (15 pmol) with natural dNTPs (200 μM) and the modified triphosphates (200 μM) **dU$^{E\text{-}Ce6}$TP**, **dU$^{CO\text{-}Ce6}$TP**, and **dU$^{Am\text{-}Ce6}$TP**. Polymerases used: lane 1) Phusion (2 U); lane 2) Hemo KlenTaq (8 U); lane 3) Q5 (2 U); lane 4) Taq (5 U); lane 5) *Bst* (8 U); lane 6) Therminator (2 U); lane 7) Vent (*exo*$^-$) (2 U); lane 8) Deep Vent (*exo*$^-$) (2 U); lane 9) Negative control reaction without polymerase; 10) Negative control reaction with only dATP, dGTP, and dCTP; lane 11) Primer **P3** 12) positive control reaction with natural dNTPs and Vent (*exo*$^-$) (2 U). 18 nucleotides (nt) correspond to **P3** and 71nt to full-length product.

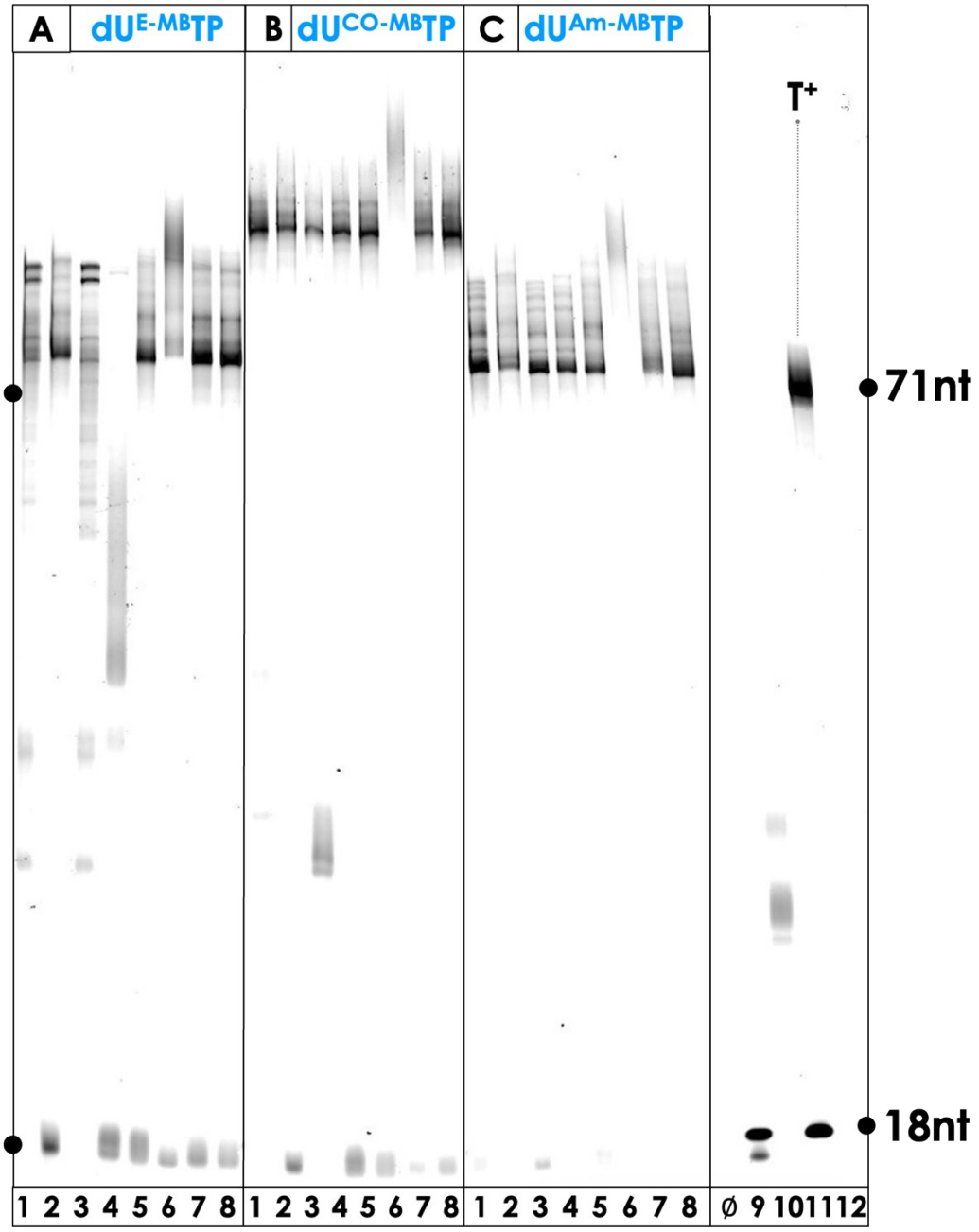


Fig. S8. PAGE gel (20%) analysis of PEX reactions (3 hour) using primer **P3** (10 pmol) and template **T3** (15 pmol) with natural dNTPs (200 μM) and the modified triphosphates (200 μM) **dU$^{E\text{-}MB}$TP**, **dU$^{CO\text{-}MB}$TP**, and **dU$^{Am\text{-}MB}$TP**. Polymerases used: lane 1) Phusion (2 U); lane 2) Hemo KlenTaq (8 U); lane 3) Q5 (2 U); lane 4) Taq (5 U); lane 5) *Bst* (8 U); lane 6) Therminator (2 U); lane 7) Vent (*exo*$^-$) (2 U); lane 8) Deep Vent (*exo*$^-$) (2 U); lane 9) Negative control reaction without polymerase; 10) Negative control reaction with only dATP, dGTP, and dCTP; lane 11) Primer **P3** 12) positive control reaction with natural dNTPs and Vent (*exo*$^-$) (2 U). 18 nucleotides (nt) correspond to **P3** and 71nt to full-length product.

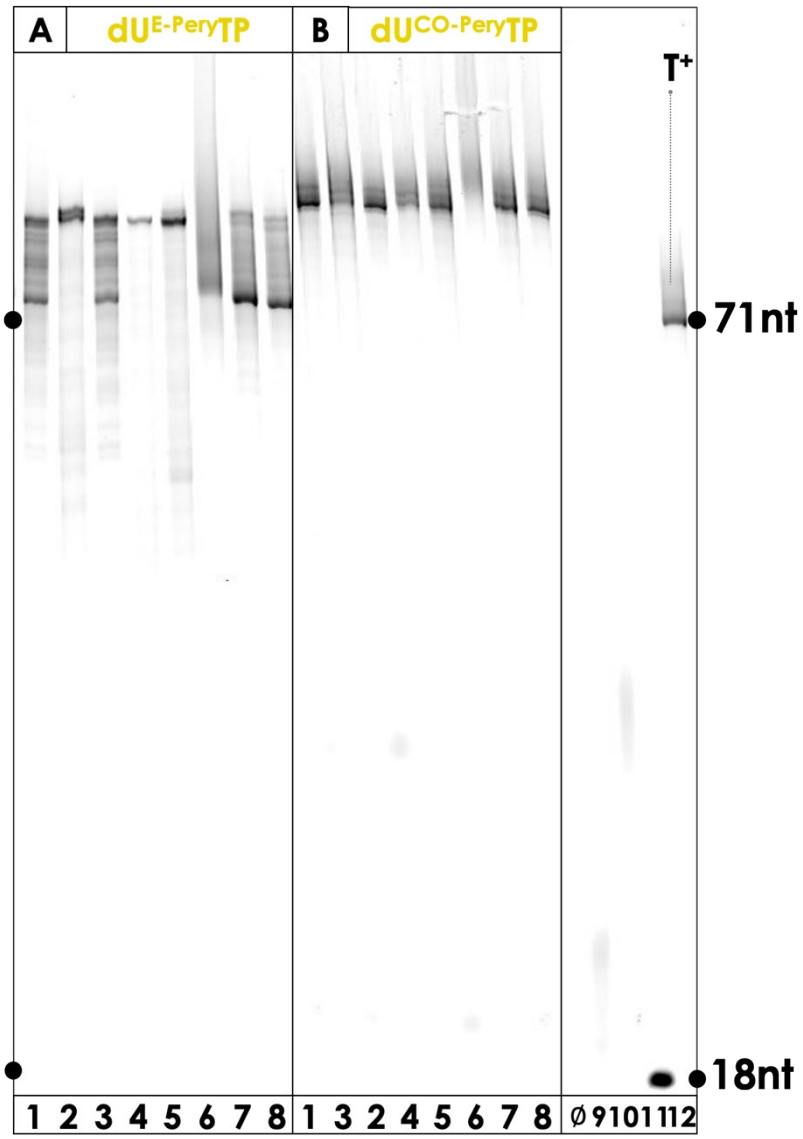


Fig. S9. PAGE gel (20%) analysis of PEX reactions (3 hour) using primer **P3** (10 pmol) and template **T3** (15 pmol) with natural dNTPs (200 μM) and the modified triphosphates (200 μM) **dU$^{E\text{-}Pery}$TP** and **dU$^{CO\text{-}Pery}$TP**. Polymerases used: lane 1) Phusion (2 U); lane 2) Hemo KlenTaq (8 U); lane 3) Q5 (2 U); lane 4) Taq (5 U); lane 5) *Bst* (8 U); lane 6) Therminator (2 U); lane 7) Vent (*exo*$^-$) (2 U); lane 8) Deep Vent (*exo*$^-$) (2 U); lane 9) Negative control reaction without polymerase; 10) Negative control reaction with only dATP, dGTP, and dCTP; lane 11) Primer **P3** 12) positive control reaction with natural dNTPs and Vent (*exo*$^-$) (2 U). 18 nucleotides (nt) correspond to **P3** and 71nt to full-length product.

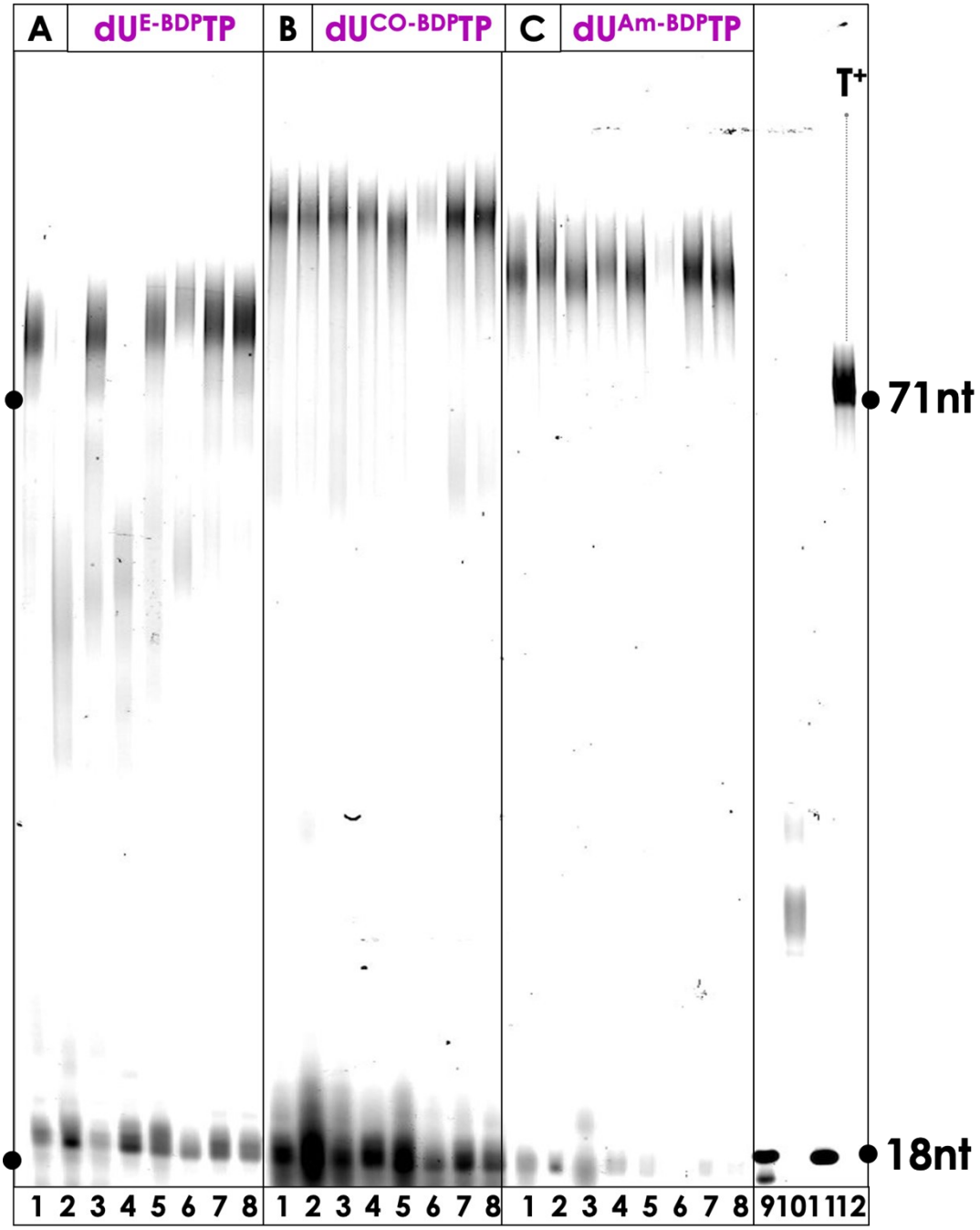


Fig. S10. PAGE gel (20%) analysis of PEX reactions (3 hour) using primer **P3** (10 pmol) and template **T3** (15 pmol) with natural dNTPs (200 μM) and the modified triphosphates (200 μM) **dU$^{E\text{-}BDP}$TP**, **dU$^{CO\text{-}BDP}$TP**, and **dU$^{Am\text{-}BDP}$TP**. Polymerases used: lane 1) Phusion (2 U); lane 2) Hemo KlenTaq (8 U); lane 3) Q5 (2 U); lane 4) Taq (5 U); lane 5) *Bst* (8 U); lane 6) Therminator (2 U); lane 7) Vent (*exo*$^-$) (2 U); lane 8) Deep Vent (*exo*$^-$) (2 U); lane 9) Negative control reaction without polymerase; 10) Negative control reaction with only dATP, dGTP, and dCTP; lane 11) Primer **P2** 12) positive control reaction with natural dNTPs and Vent (*exo*$^-$) (2 U). 15 nucleotides (nt) correspond to **P2** and 31nt to full-length product.

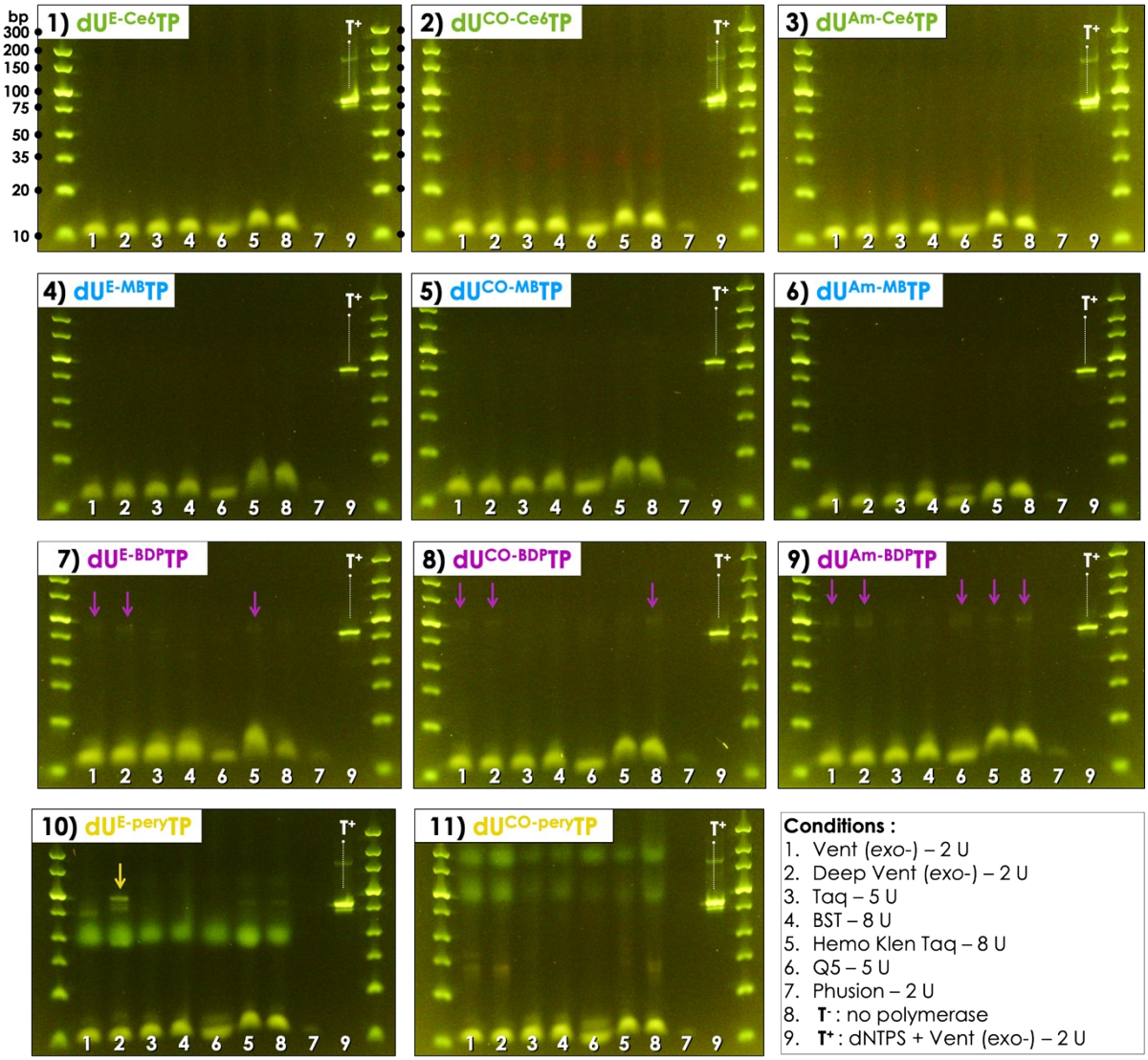


Fig. S11. Gel image (agarose 4% stained with SYBER®□ GREEN) analysis of PCR reactions with primers **P4** and **P5** (6 μM), template **T4** (0.1 μM), natural dNTPs (200 μM), **dU^{PS}TPs** (200 μM), Mg^{2+} (2 mM) and polymerases for a total reaction volume of 25 μL. The gels appear as follows: **1) dU$^{E\text{-}Ce6}$TP; 2) dU$^{CO\text{-}Ce6}$TP; 3) dU$^{Am\text{-}Ce6}$TP; 4) dU$^{E\text{-}MB}$TP; 5) dU$^{CO\text{-}MB}$TP; 6) dU$^{Am\text{-}MB}$TP; 7) dU$^{E\text{-}BDP}$TP; 8) dU$^{CO\text{-}BDP}$TP; 9) dU$^{Am\text{-}BDP}$TP; 10) dU$^{E\text{-}Pery}$TP; 11) dU$^{CO\text{-}Pery}$TP**. Lanes of the gels: 1. Vent (*exo*$^-$) (2 U); 2. Deep Vent (2 U); 3. Taq (5 U); 4. *Bst* (8 U); 5. Hemo KlenTaq (8 U); 6. Q5 (2 U); 7. Phusion (2 U); 8. negative control reaction without polymerase; 9. positive control reactions with natural dNTPs with Vent (*exo*$^-$) (2 U) polymerase. The PCR program was: [95 °C 5 minutes, (95 °C 30 sec, 57 °C 30 sec, 72 °C 2 minutes) X 25 cycles] or [72 °C 5 minutes, 95 °C 30 sec, (98 °C 30 sec, 72 °C 30 sec, 72 °C 30 sec) X 25 cycles] depending on the polymerase used.

5. LC-MS analysis of modified DNA

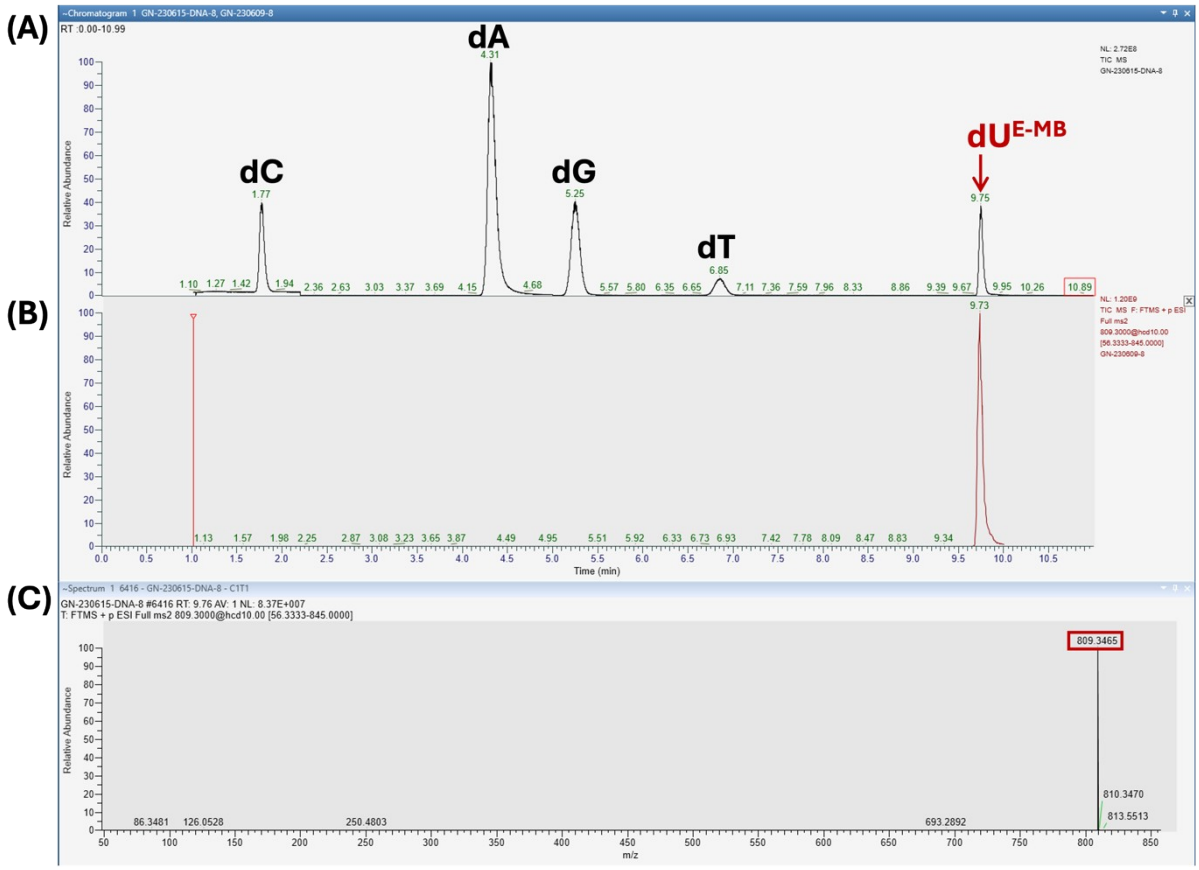


Fig. S12. LC-MS Digestion chromatograms for **dU$^{E\text{-}MB}$**. (A) LC-MS chromatogram showing the nucleoside digestion profile of ds DNA after PEX reaction with template **T1** and primer **P1**. The chromatogram reveals five distinct peaks, four of which correspond to the canonical nucleosides of the template and primer: deoxycytidine (dC), deoxyadenine (dA), deoxyguanine (dG) and deoxythymidine (dT). The additional peak represents the modified deoxyuridine (**dU$^{E\text{-}MB}$**). (B) LC chromatogram of phosphatase treated nucleotide serves as the (**dU$^{E\text{-}MB}$**) standard. (C) MS spectrum **dU$^{E\text{-}MB}$** after phosphatase treatment.

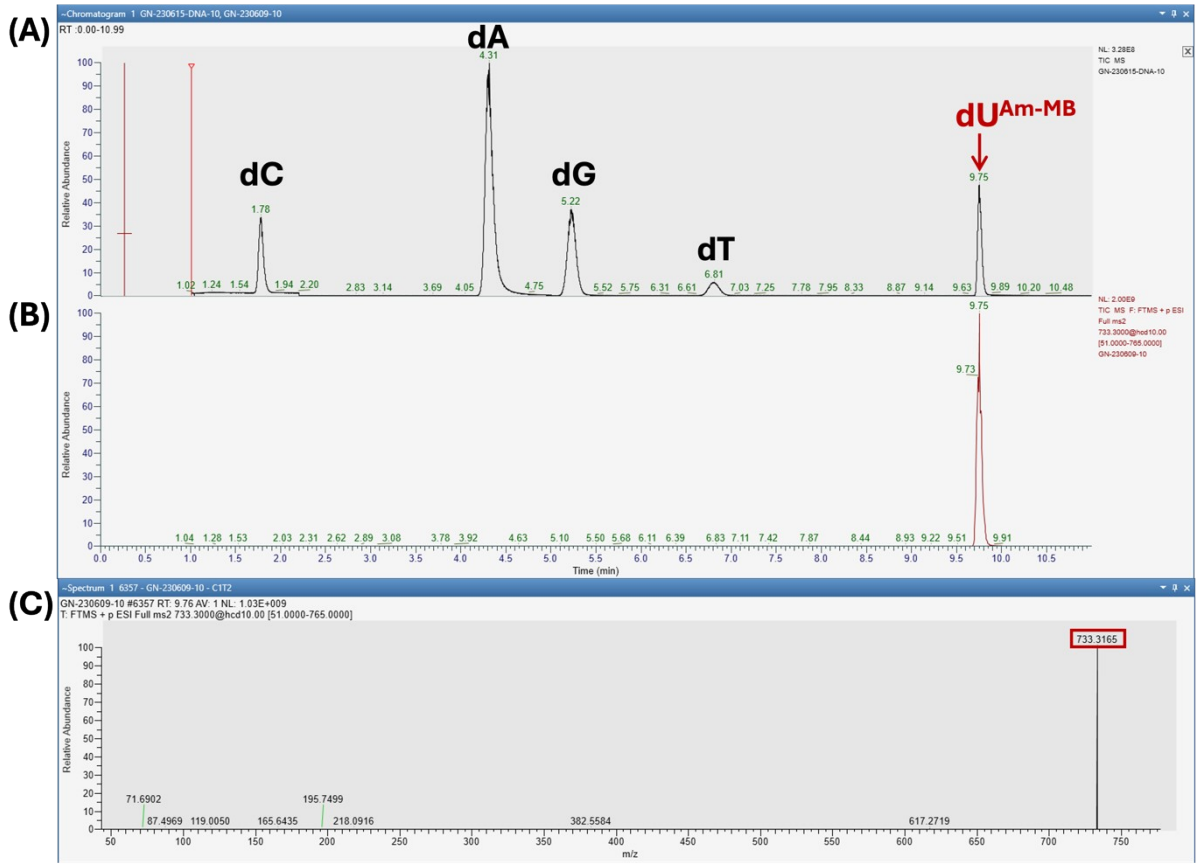


Fig. S13. LC-MS Digestion chromatograms for **dU^{Am-MB}**. (A) LC-MS chromatogram showing the nucleoside digestion profile of ds DNA after PEX reaction with template **T1** and primer **P1**. The chromatogram reveals five distinct peaks, four of which correspond to the canonical nucleosides of the template and primer: deoxycytidine (dC), deoxyadenine (dA), deoxyguanine (dG) and deoxythymidine (dT). The additional peak represents the modified deoxyuridine (**dU^{Am-MB}**). (B) LC chromatogram of phosphatase treated nucleotide serves as the (**dU^{Am-MB}**) standard. (C) MS spectrum **dU^{Am-MB}** after phosphatase treatment.

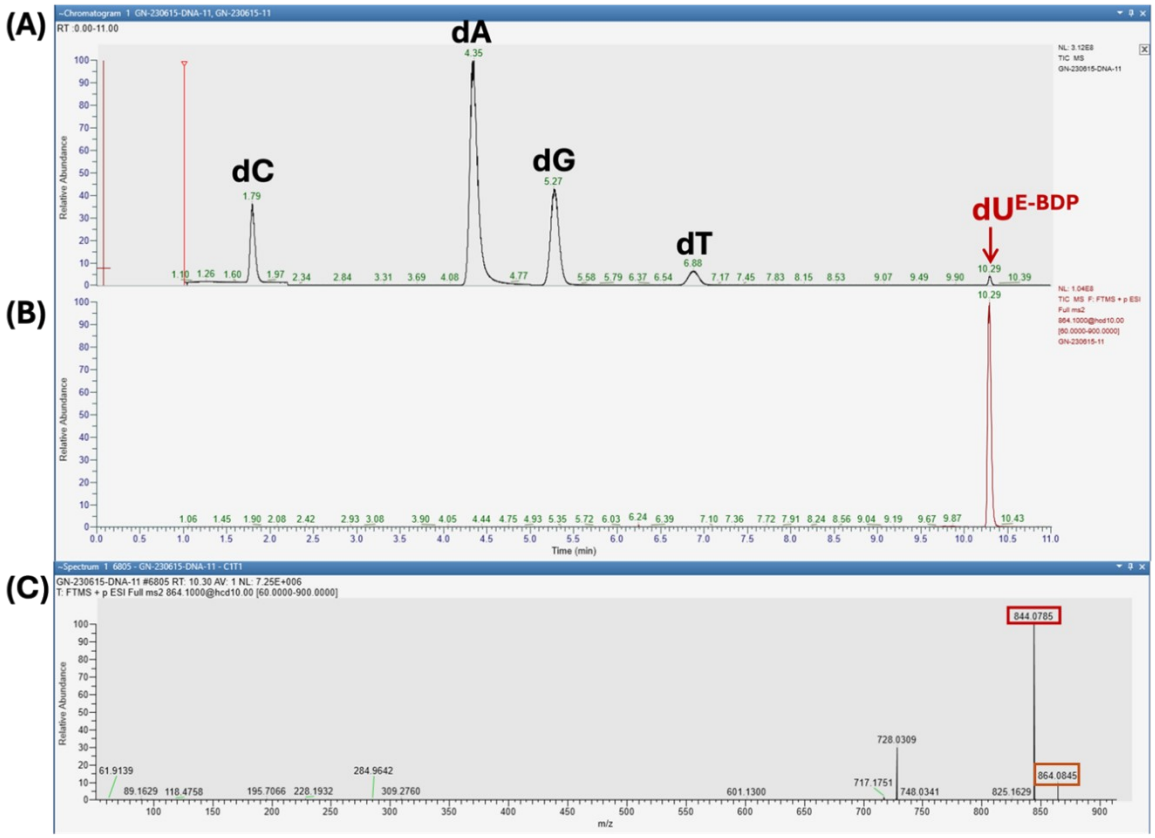


Fig. S14. LC-MS Digestion chromatograms for **dU$^{E\text{-}BDP}$**. (A) LC-MS chromatogram showing the nucleoside digestion profile of ds DNA after PEX reaction with template **T1** and primer **P1**. The chromatogram reveals five distinct peaks, four of which correspond to the canonical nucleosides of the template and primer: deoxycytidine (dC), deoxyadenine (dA), deoxyguanine (dG) and deoxythymidine (dT). The additional peak represents the modified deoxyuridine (**dU$^{E\text{-}BDP}$**). (B) LC chromatogram of phosphatase treated nucleotide serves as the (**dU$^{E\text{-}BDP}$**) standard. (C) MS spectrum **dU$^{E\text{-}BDP}$** after phosphatase treatment.

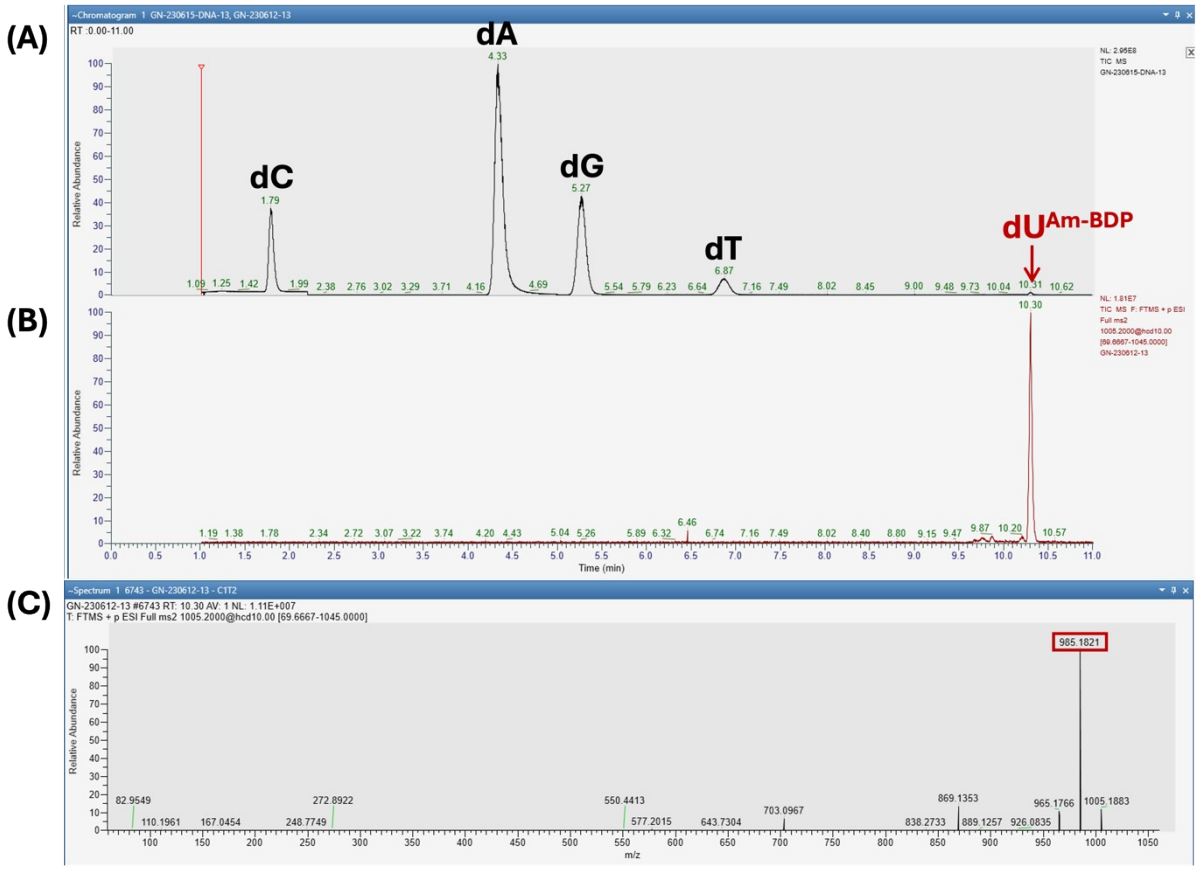


Fig. S15. LC-MS Digestion chromatograms for **dU$^{Am\text{-}BDP}$**. (A) LC-MS chromatogram showing the nucleoside digestion profile of ds DNA after PEX reaction with template **T1** and primer **P1**. The chromatogram reveals five distinct peaks, four of which correspond to the canonical nucleosides of the template and primer: deoxycytidine (dC), deoxyadenine (dA), deoxyguanine (dG) and deoxythymidine (dT). The additional peak represents the modified deoxyuridine (**dU$^{Am\text{-}BDP}$**). (B) LC chromatogram of phosphatase treated nucleotide serves as the (**dU$^{Am\text{-}BDP}$**) standard. (C) MS spectrum **dU$^{Am\text{-}BDP}$** after phosphatase treatment.

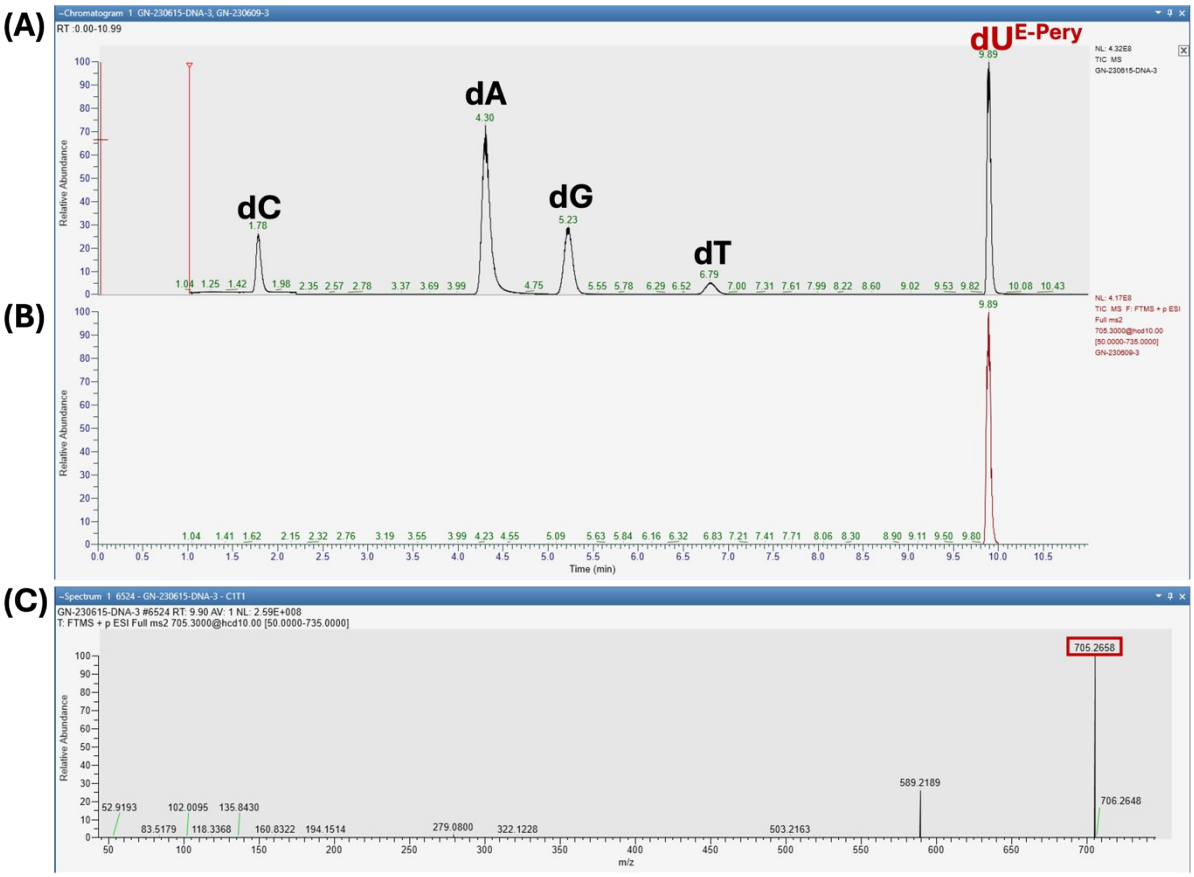


Fig. S16. LC-MS Digestion chromatograms for **dU$^{E\text{-}Pery}$**. (A) LC-MS chromatogram showing the nucleoside digestion profile of ds DNA after PEX reaction with template **T1** and primer **P1**. The chromatogram reveals five distinct peaks, four of which correspond to the canonical nucleosides of the template and primer: deoxycytidine (dC), deoxyadenine (dA), deoxyguanine (dG) and deoxythymidine (dT). The additional peak represents the modified deoxyuridine (**dU$^{E\text{-}Pery}$**). (B) LC chromatogram of phosphatase treated nucleotide serves as the (**dU$^{E\text{-}Pery}$**) standard. (C) MS spectrum **dU$^{E\text{-}Pery}$** after phosphatase treatment.

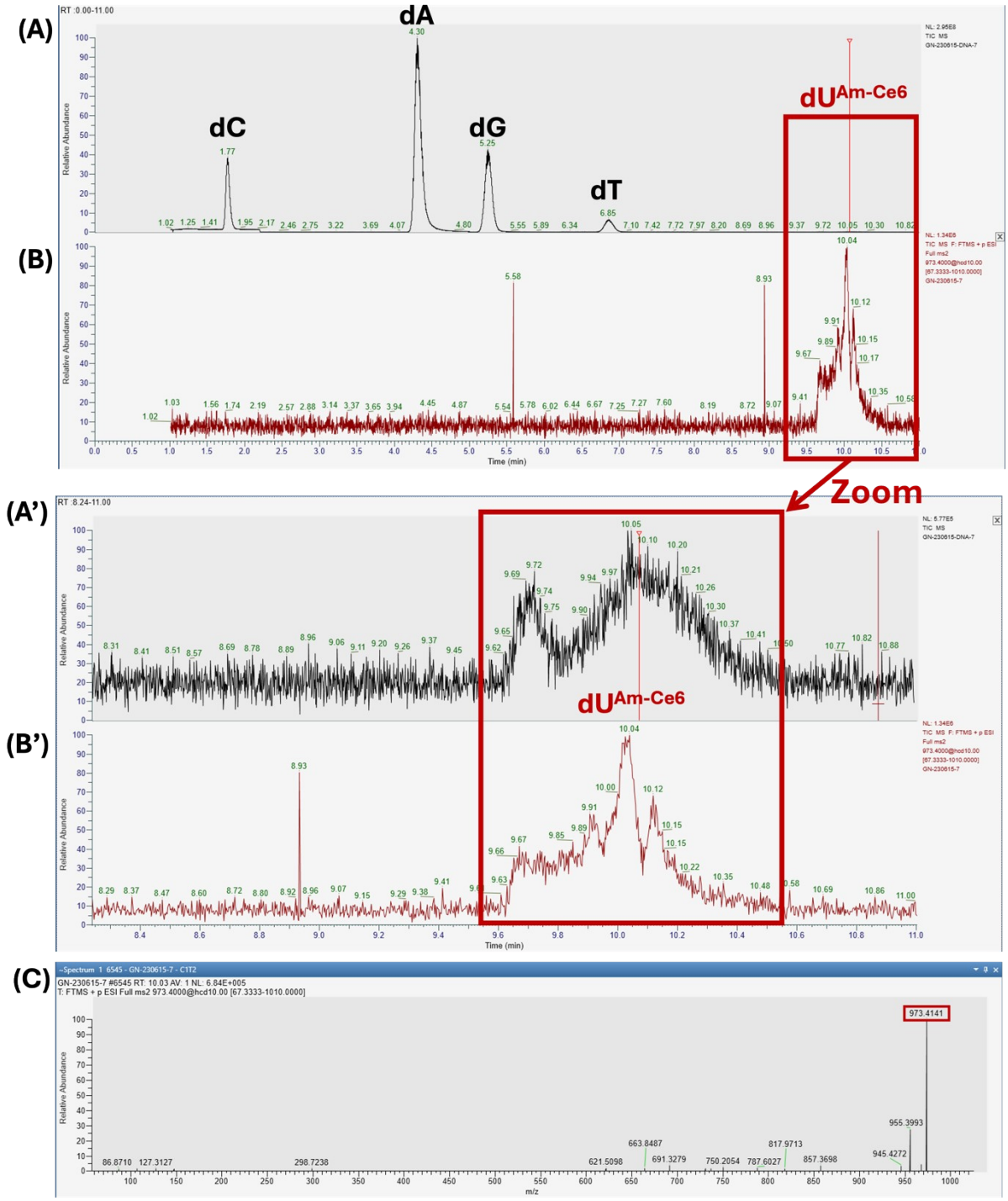
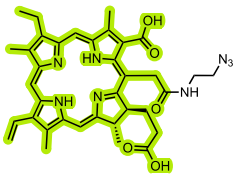


Fig. S17. LC-MS Digestion chromatograms for **dU$^{Am\text{-}Ce6}$**. (A) LC-MS chromatogram showing the nucleoside digestion profile of ds DNA after PEX reaction with template **T1** and primer **P1**. The chromatogram reveals five distinct peaks, four of which correspond to the canonical nucleosides of the template and primer: deoxycytidine (dC), deoxyadenine (dA), deoxyguanine (dG) and deoxythymidine (dT). The additional peak represents the modified deoxyuridine (**dU$^{Am\text{-}Ce6}$**). (B) LC chromatogram of phosphatase treated nucleotide serves as the (**dU$^{Am\text{-}Ce6}$**) standard. (C) MS spectrum **dU$^{Am\text{-}Ce6}$** after phosphatase treatment. A') and B') represent inset zooms on A) and B), respectively.

6. References

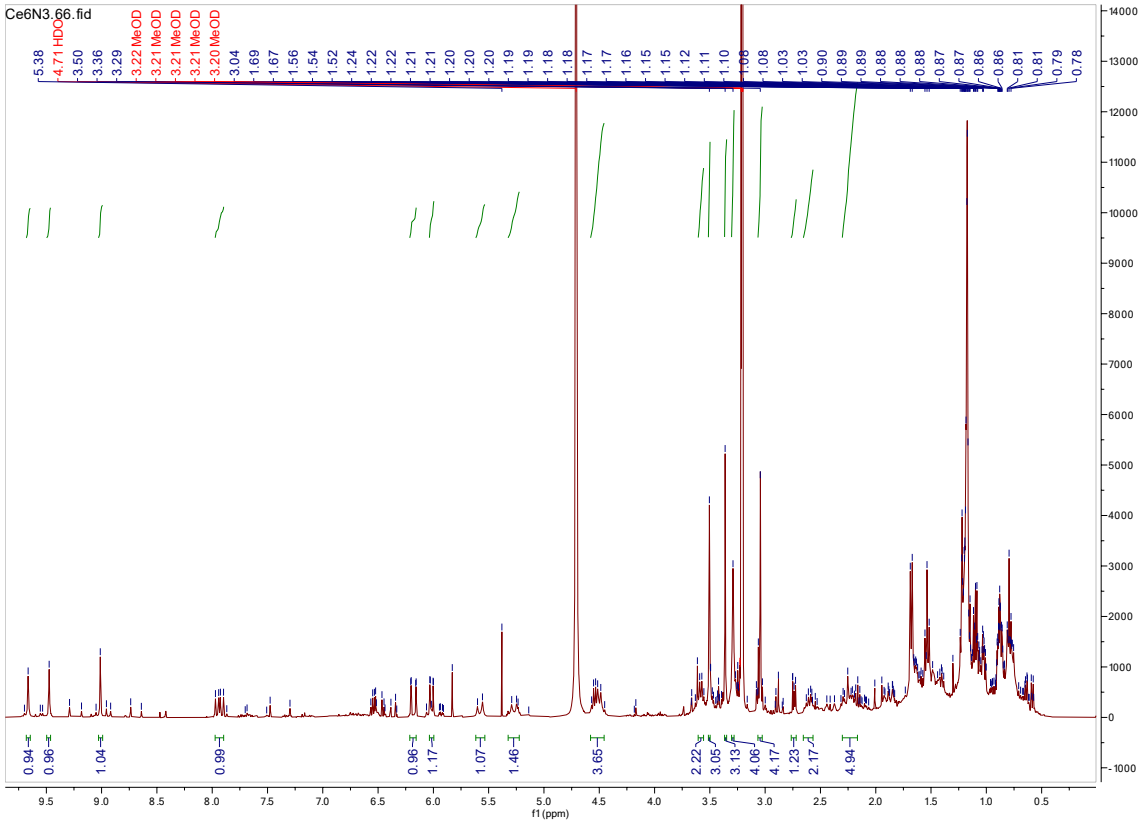
1. N. Elumalai, A. Berg, K. Natarajan, A. Scharow and T. Berg, *Angew. Chem. Int. Ed.*, 2015, **54**, 4758-4763.
2. Q. Guan, L.-L. Zhou, Y.-A. Li and Y.-B. Dong, *Chem. Commun.*, 2019, **55**, 14898-14901.
3. M. Macias-Contreras, K. L. Daykin, J. T. Simmons, J. R. Allen, Z. S. Hooper, M. W. Davidson and L. Zhu, *Org. Biomol. Chem.*, 2017, **15**, 9139-9148.

7. Characterization of compounds

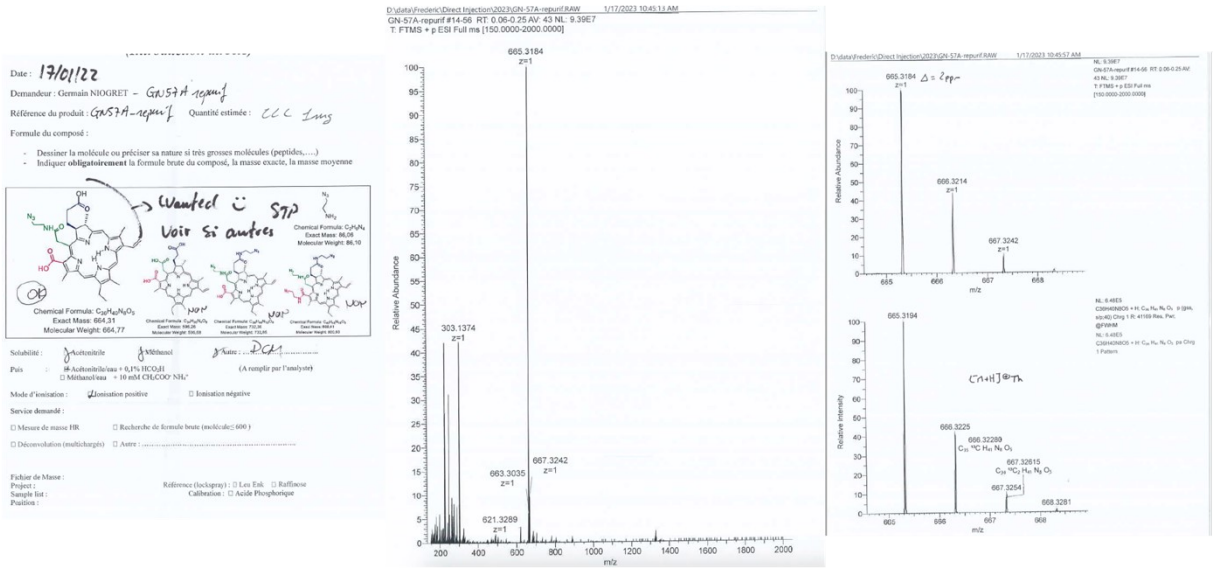


Chemical Formula: $C_{36}H_{40}N_8O_5$
Exact Mass: 664,31
Molecular Weight: 664,77

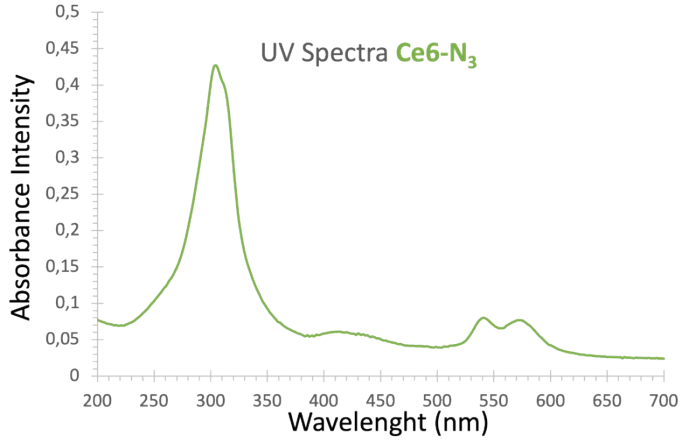
Compound **1**



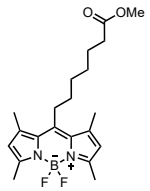
*^{1}H NMR spectrum of **Ce6-N_3** (400 MHz in CD_3OD)*



HR-MS ESI spectrum (negative mode) of ***Ce6-N$_3$***

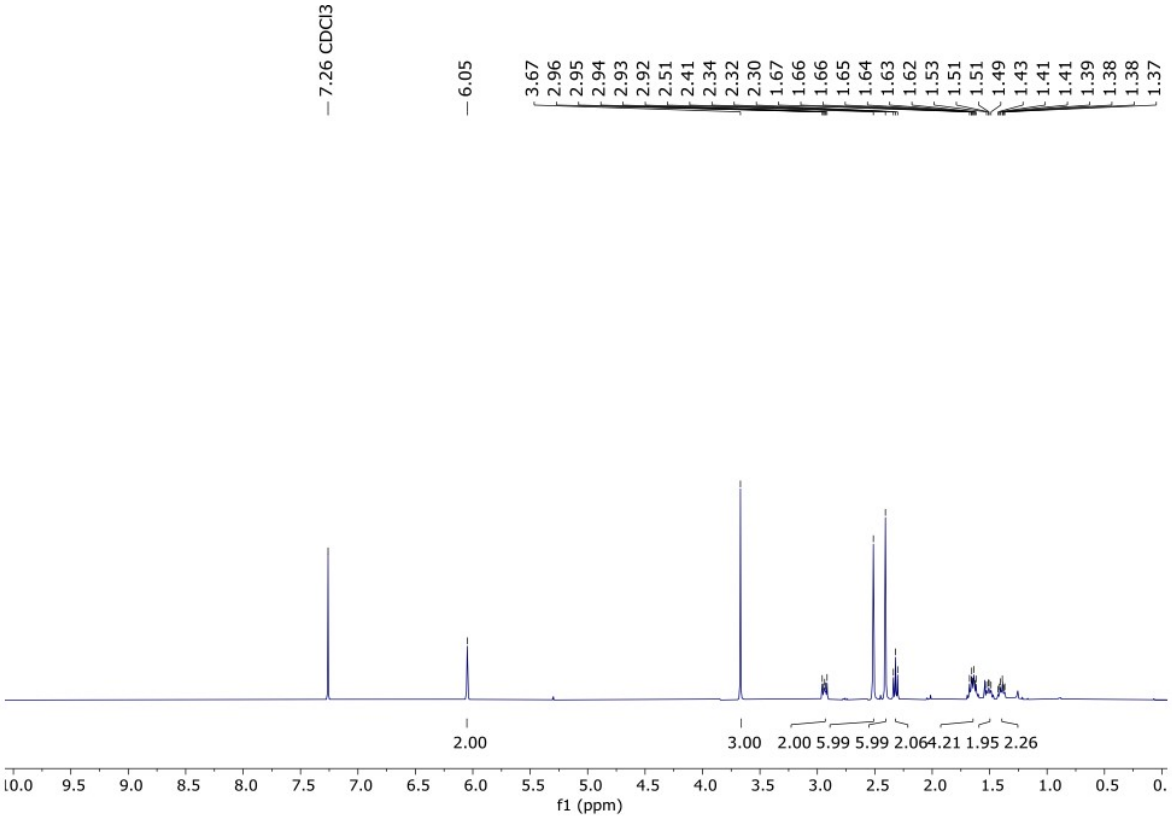


UV spectrum in ultra-pure water of ***Ce6-N$_3$***

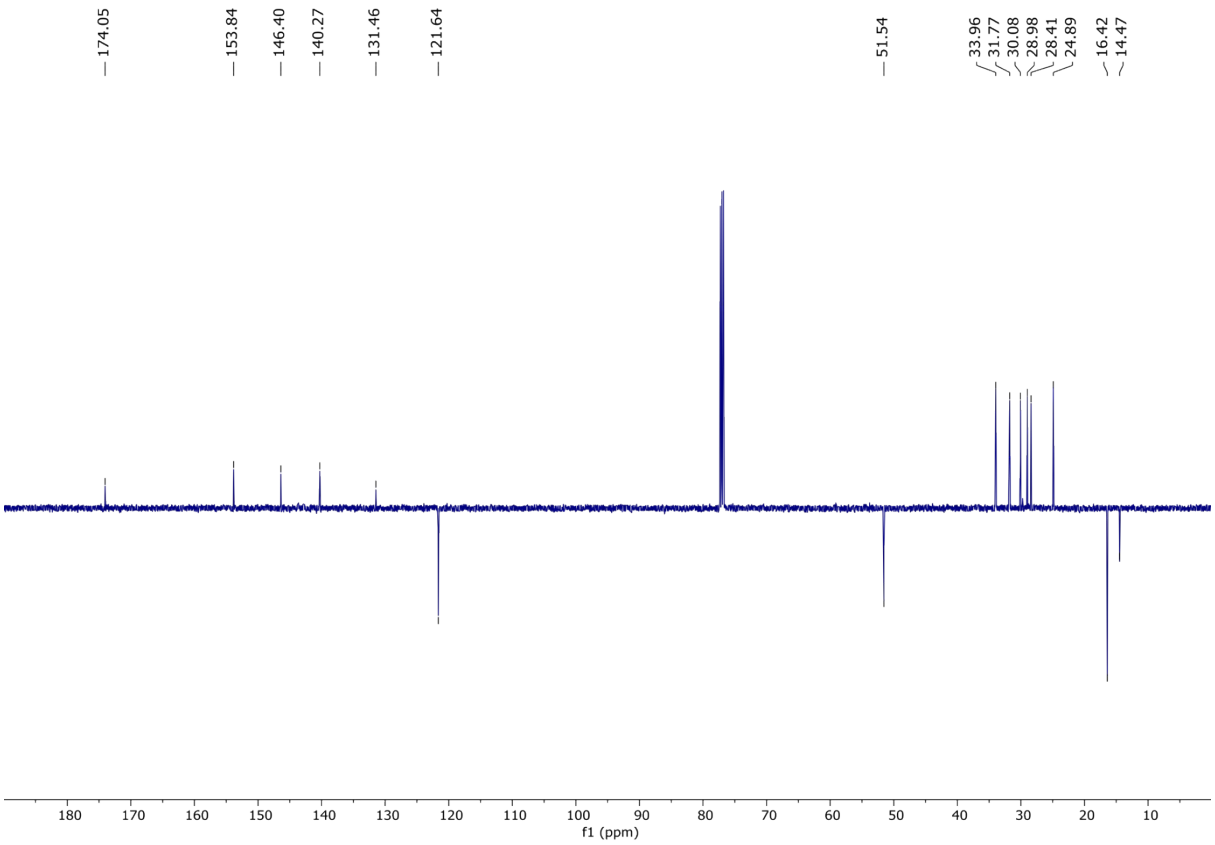


Chemical Formula: $C_{21}H_{29}BF_2N_2O_2$
Exact Mass: 390,23
Molecular Weight: 390,28

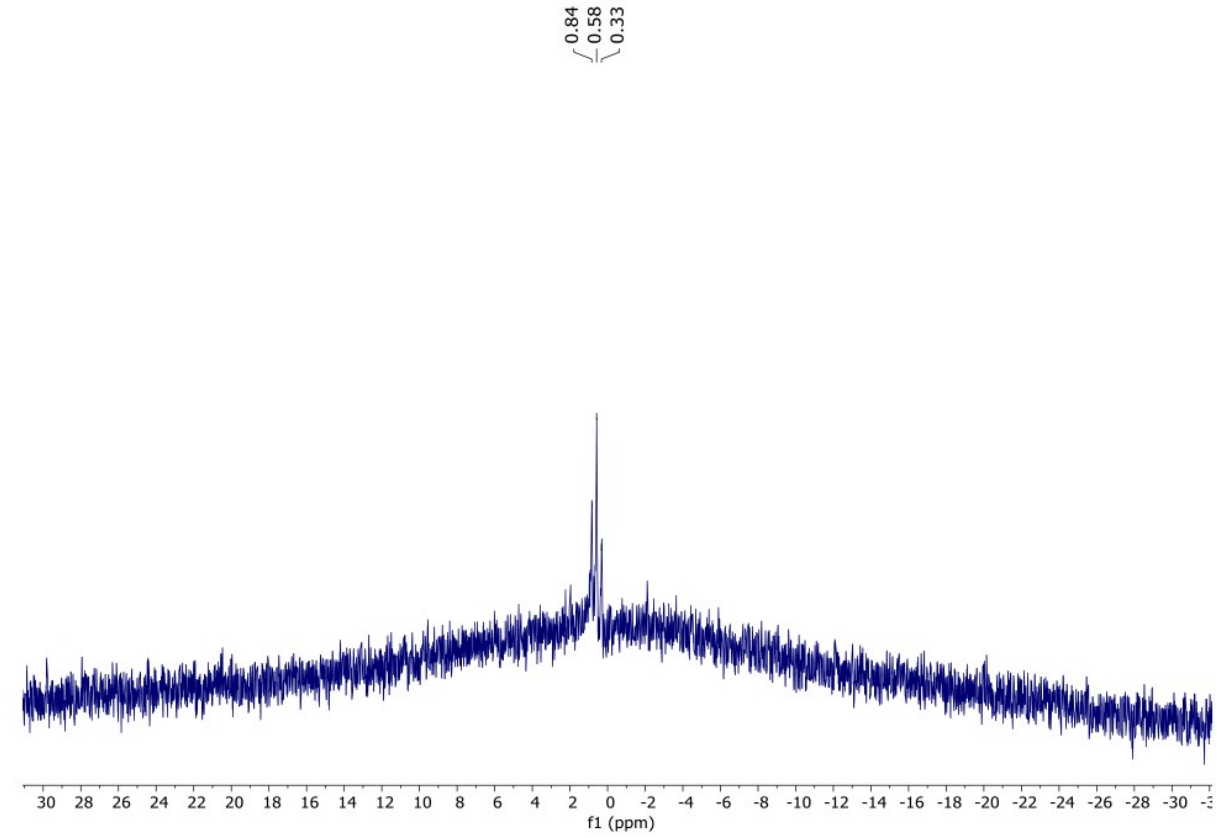
Compound **2**



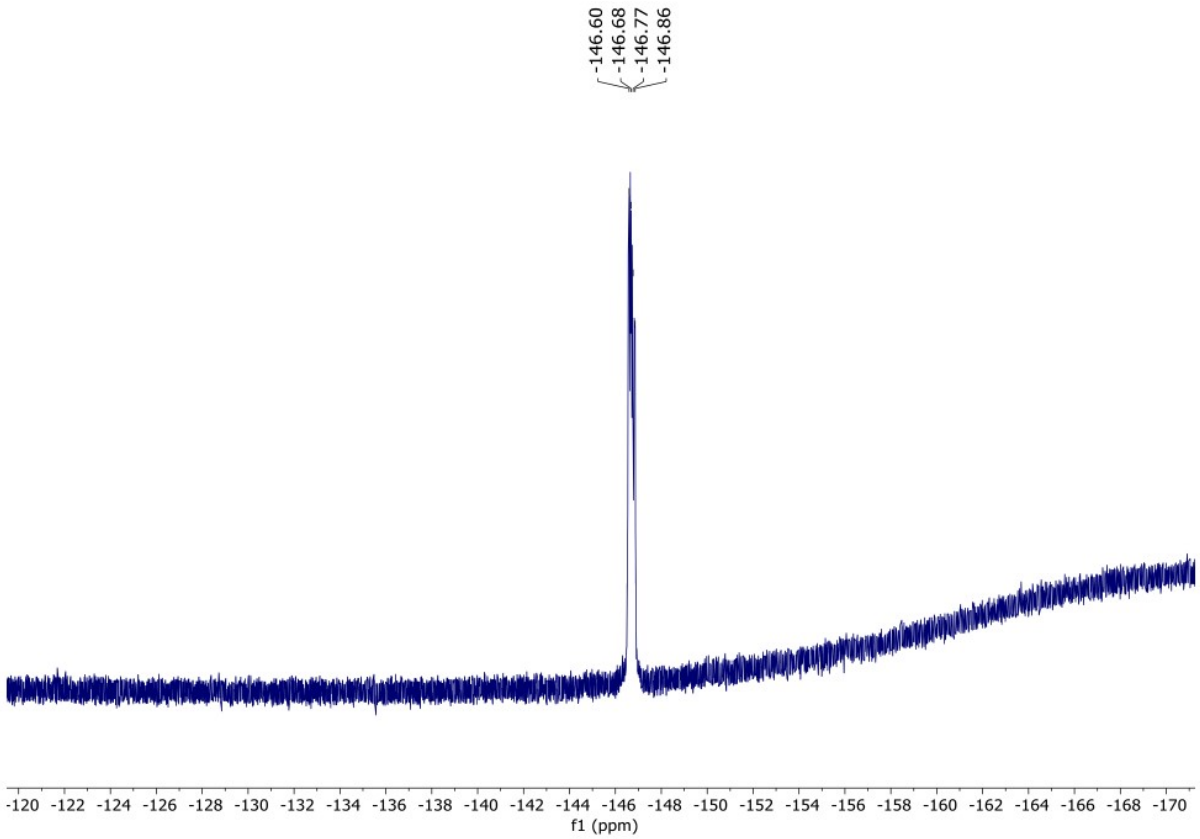
*^{1}H NMR spectrum of compound **2** (400 MHz in $CDCl_3$)*



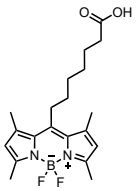
*^{13}C NMR spectrum of compound **2** (126 MHz in $CDCl_3$)*



*^{11}B NMR spectrum of compound **2** (128 MHz in $CDCl_3$)*

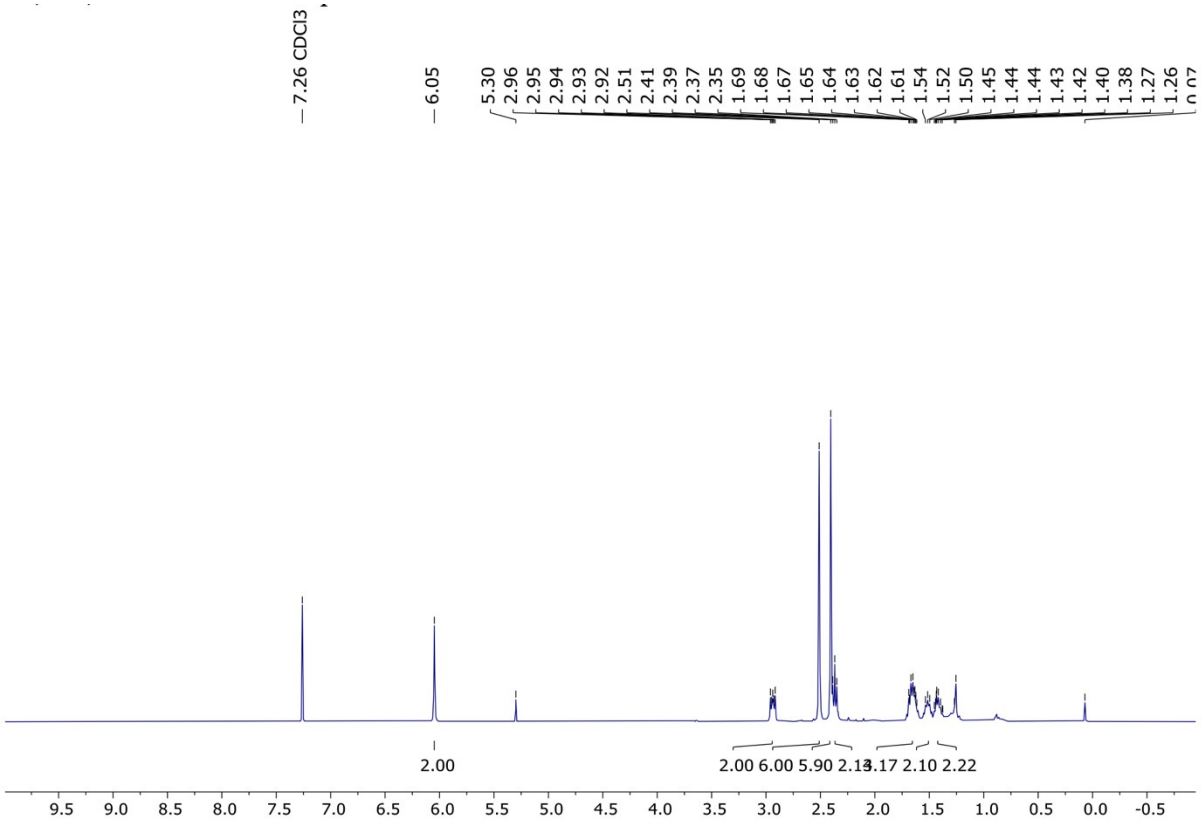


*^{19}F NMR spectrum of compound **2** (377 MHz in $CDCl_3$)*

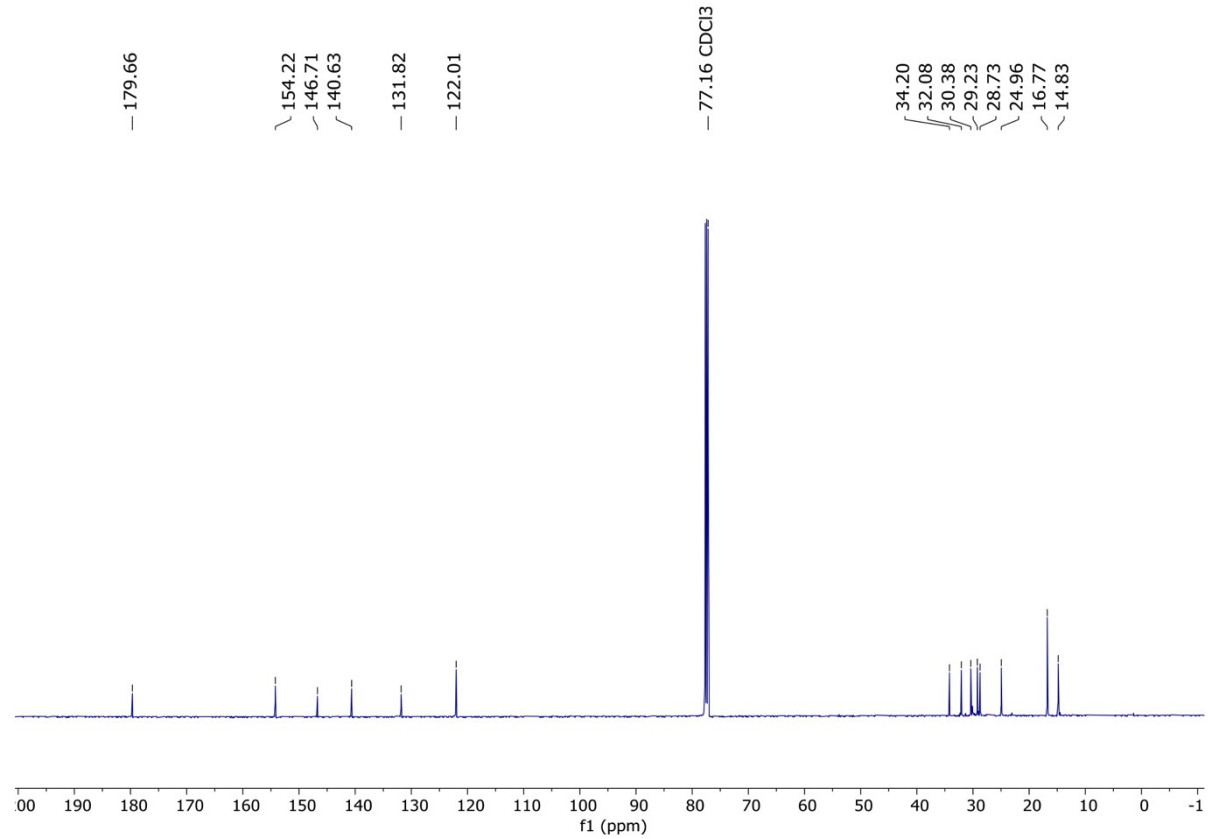


Chemical Formula: $C_{20}H_{27}BF_2N_2O_2$
Exact Mass: 376,21
Molecular Weight: 376,25

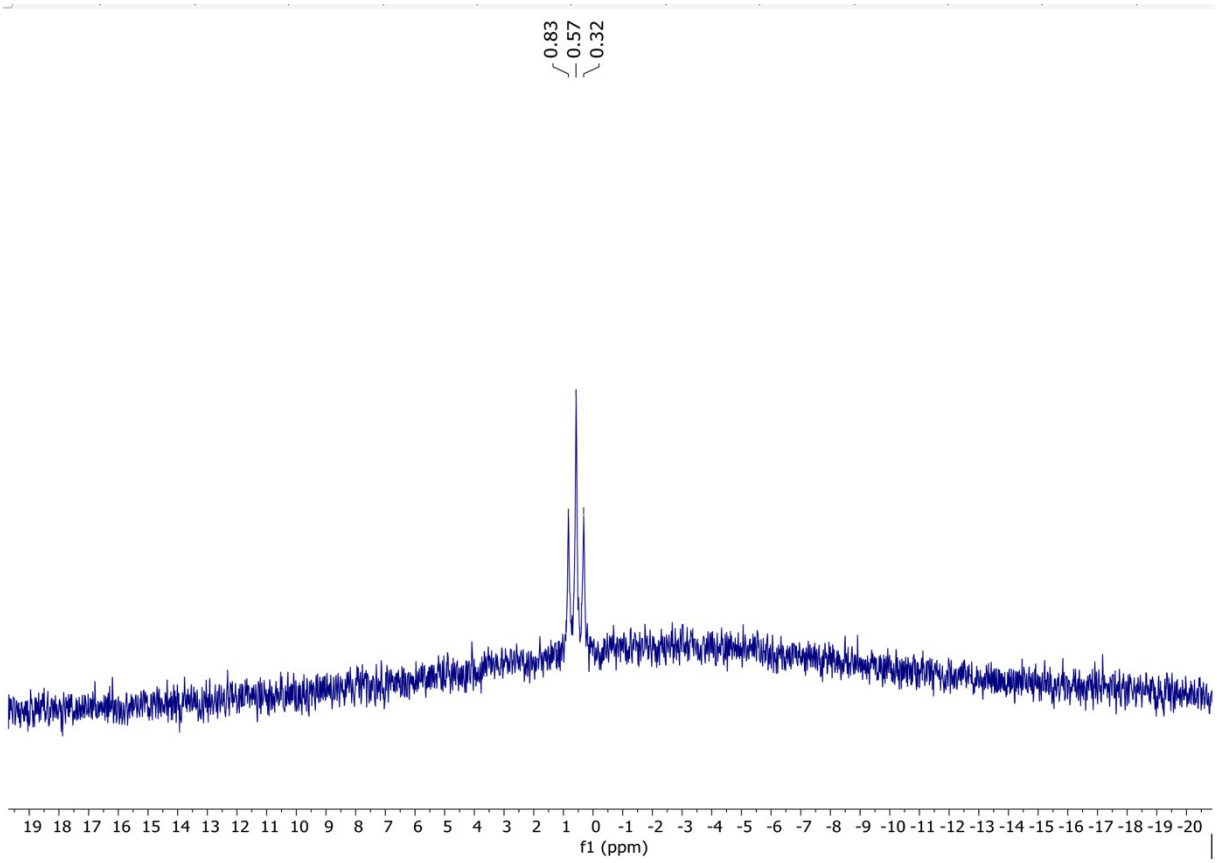
Compound **3**



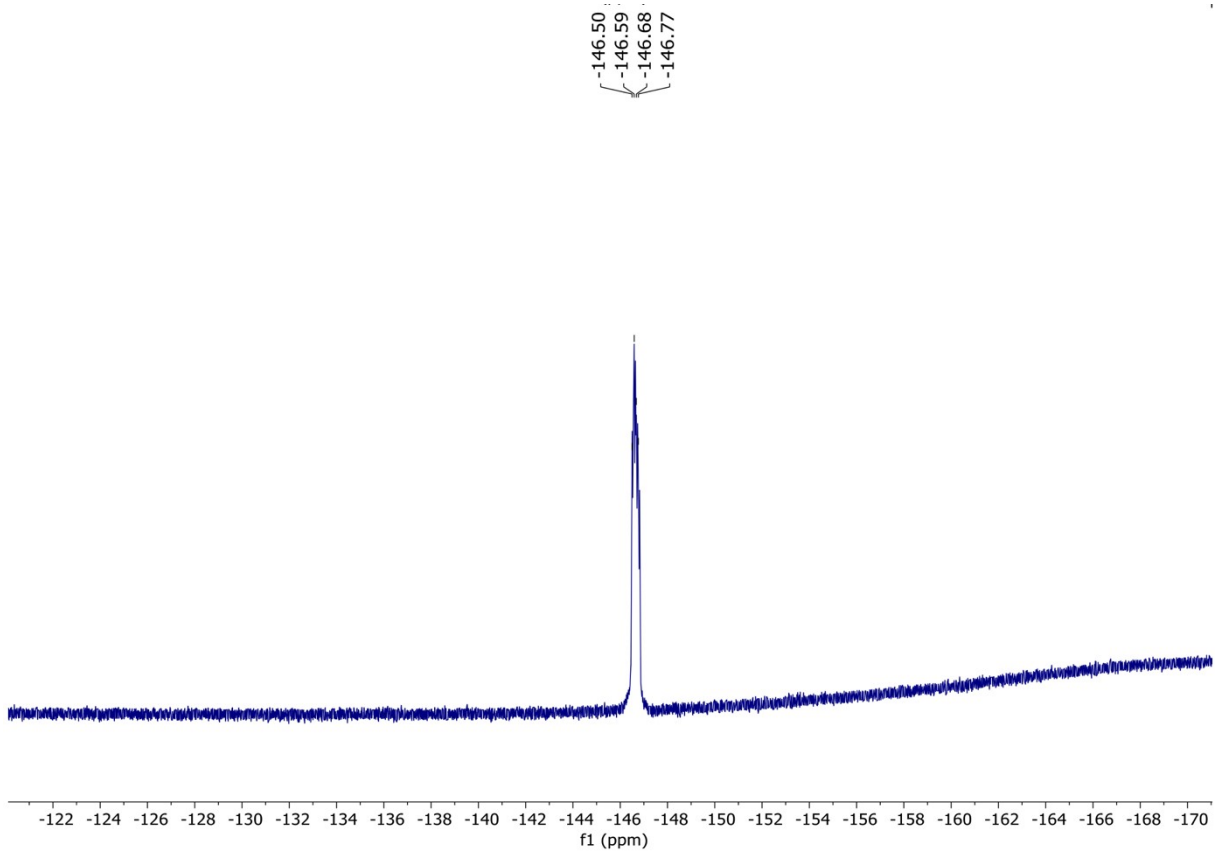
*[1]H NMR spectrum of compound **3** (400 MHz in $CDCl_3$)*



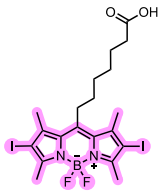
*[13]C NMR spectrum of compound **3** (126 MHz in $CDCl_3$)*



*^{11}B NMR spectrum of compound **3** (128 MHz in $CDCl_3$)*

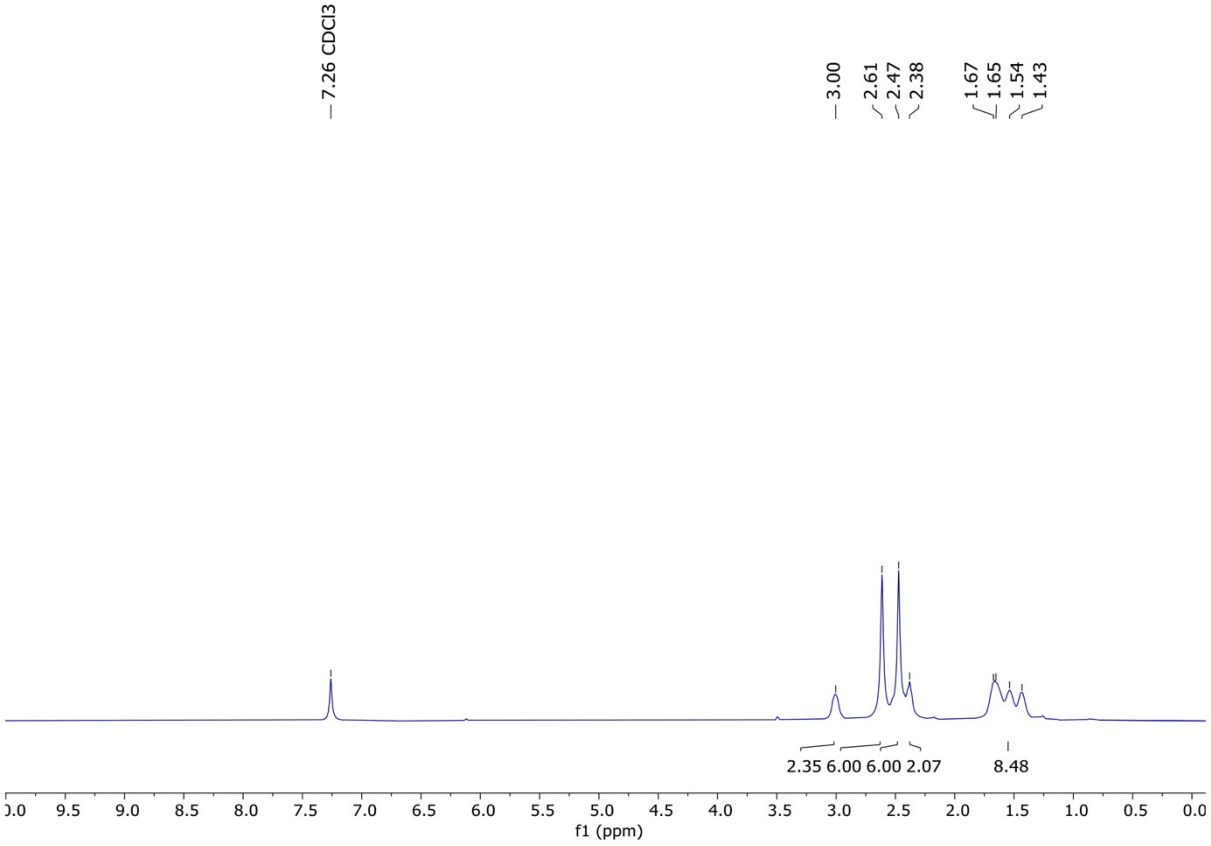


*^{19}F NMR spectrum of compound **3** (377 MHz in $CDCl_3$)*

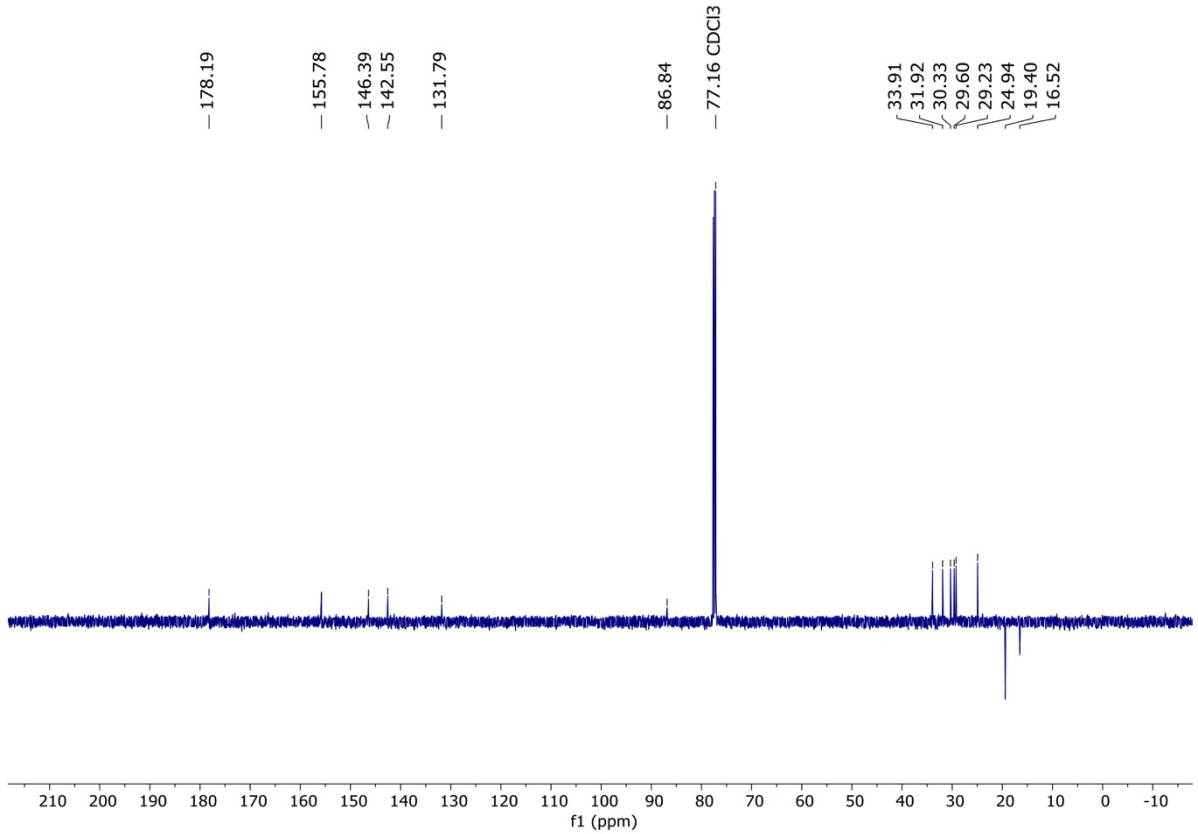


Chemical Formula: $C_{20}H_{25}BF_2I_2N_2O_2$
Exact Mass: 628,01
Molecular Weight: 628,05

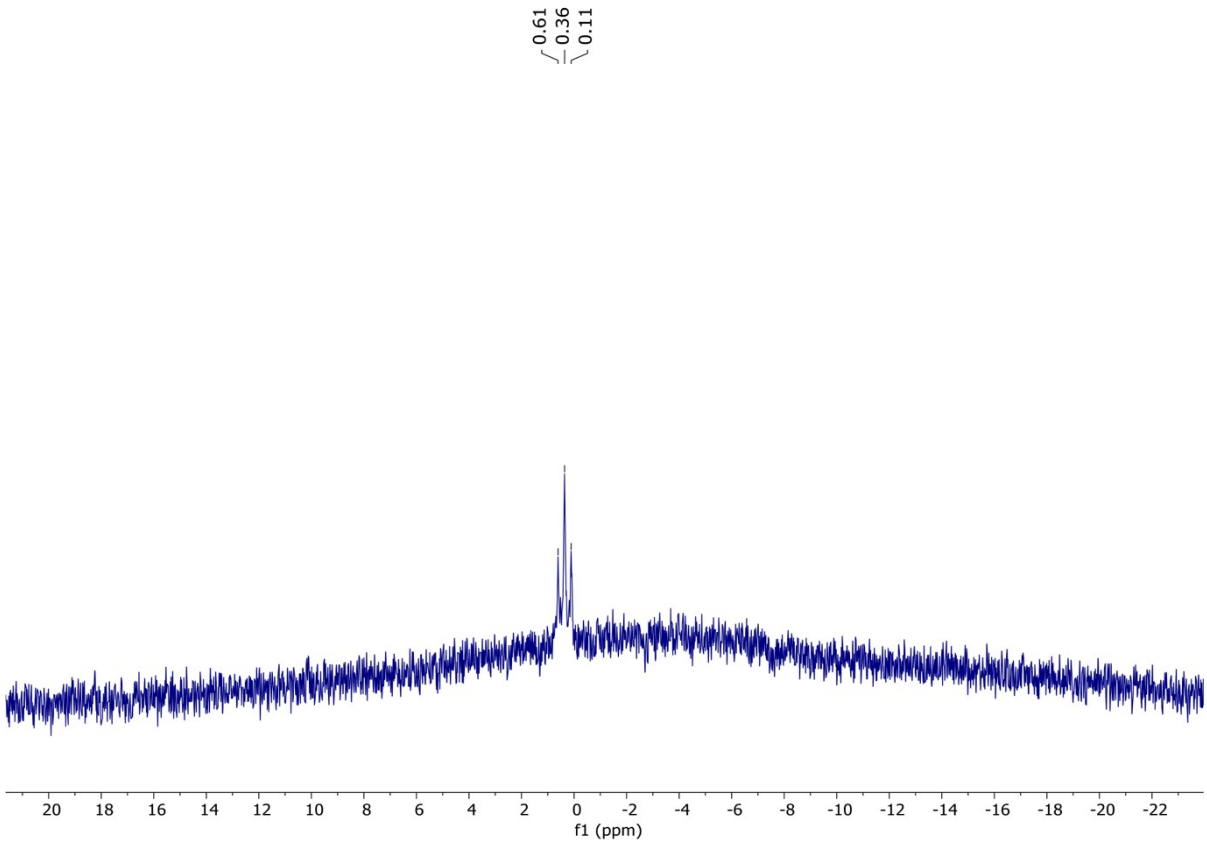
Compound **4**



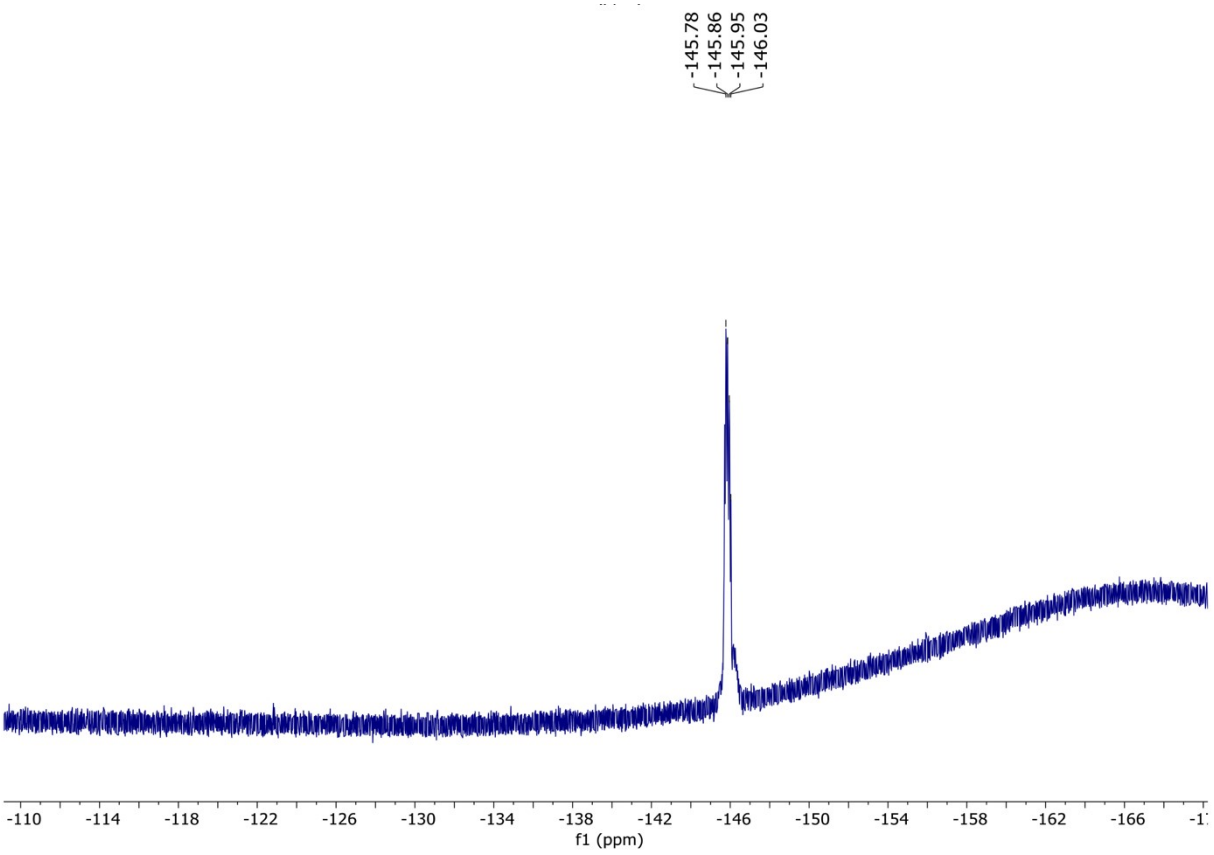
*^{1}H NMR spectrum of compound **4** (400 MHz in $CDCl_3$)*



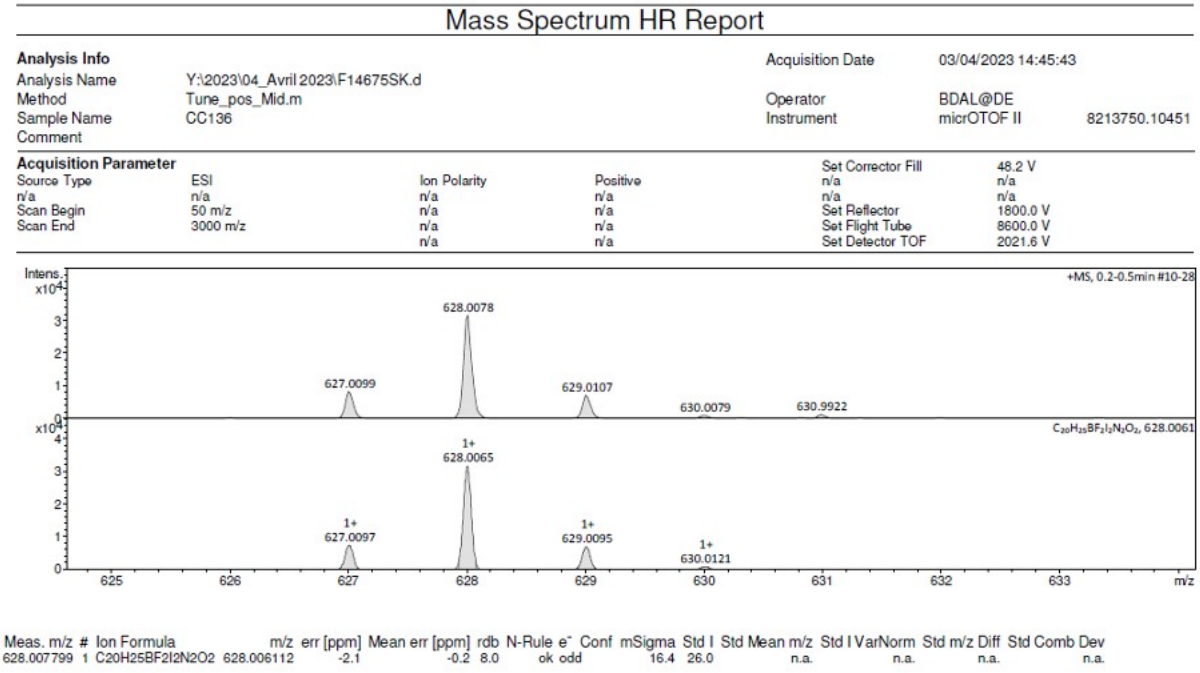
*^{13}C NMR spectrum of compound **4** (126 MHz in $CDCl_3$)*



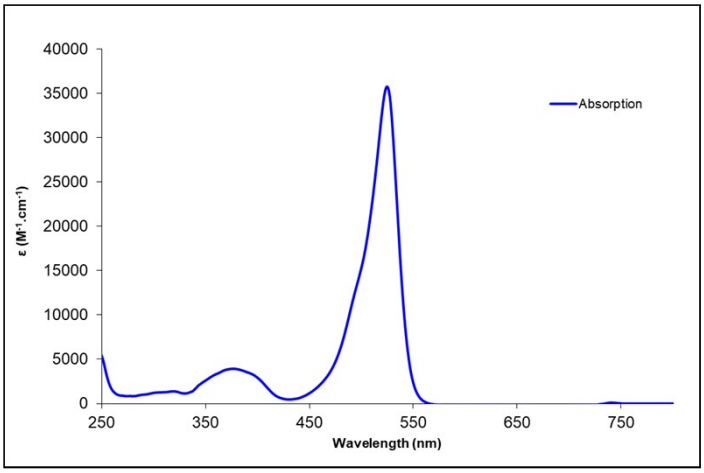
*^{11}B NMR spectrum of compound **4** (128 MHz in $CDCl_3$)*



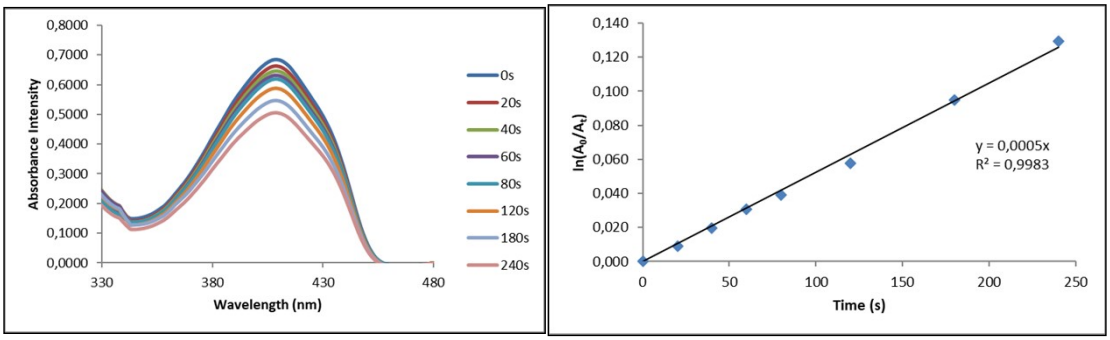
*^{19}F NMR spectrum of compound **4** (377 MHz in $CDCl_3$)*



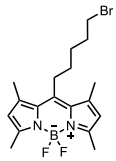
*HR-MS ESI spectrum of compound **4.***



*UV spectrum of compound **4** in EtOH. Emission and excitation curves are not shown, as emission is too weak to be recorded accurately. λ_{max}= 525 nm; ε_{525} = 89360 $M^{-1}.cm^{-1}$*

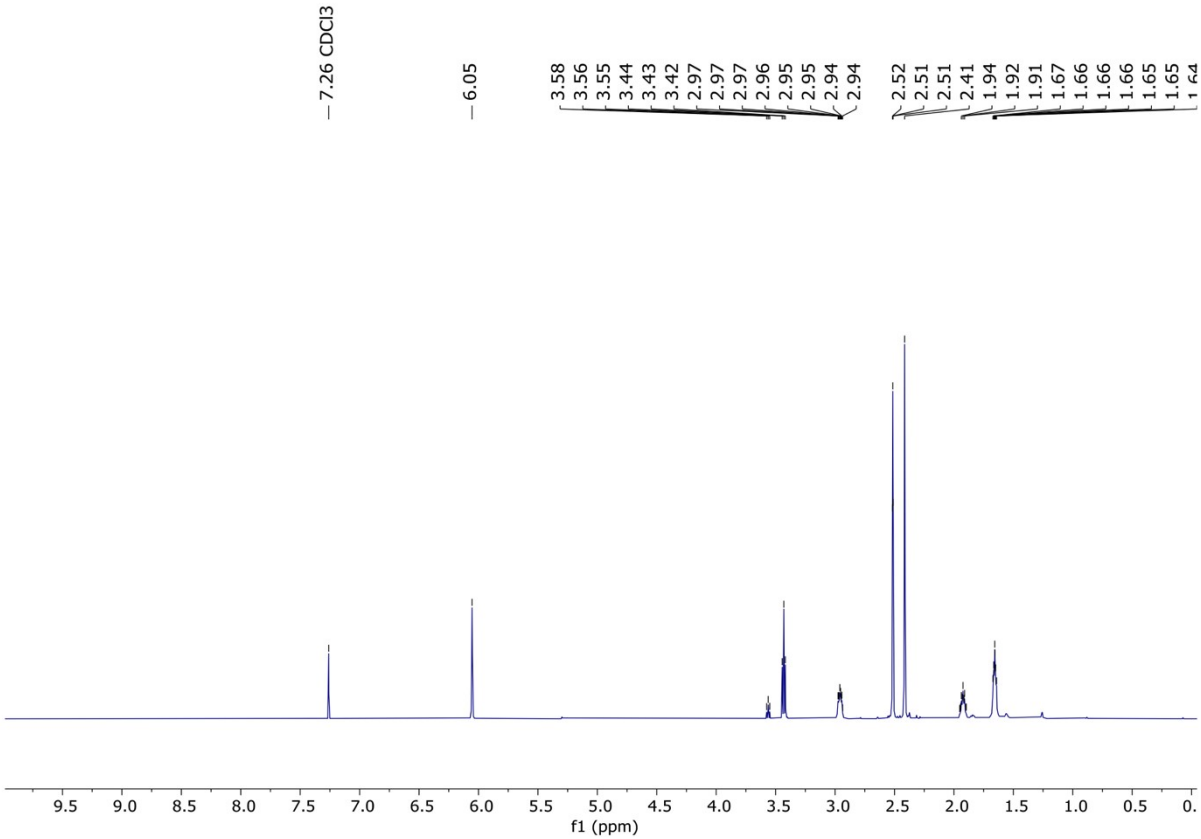


*Singlet oxygen measurement of compound **4***

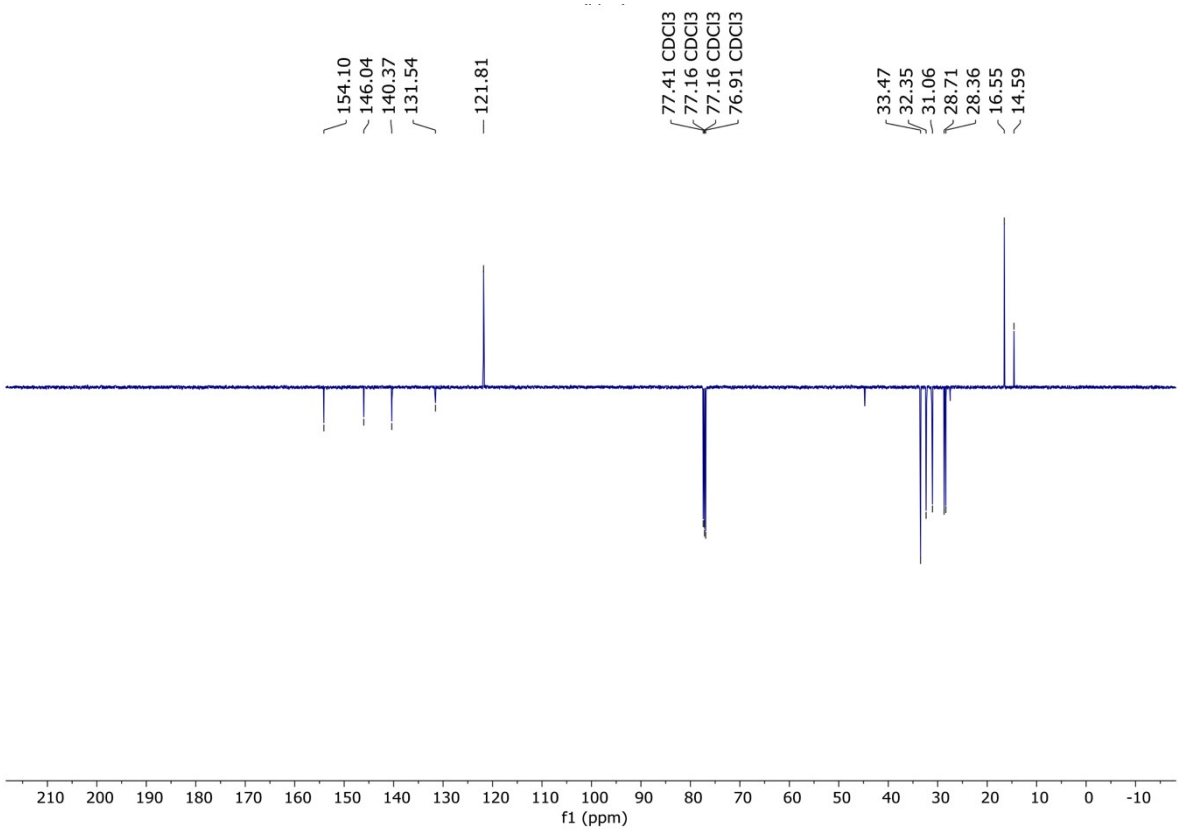


Chemical Formula: $C_{18}H_{24}BBrF_2N_2$
Exact Mass: 396,12
Molecular Weight: 397,11

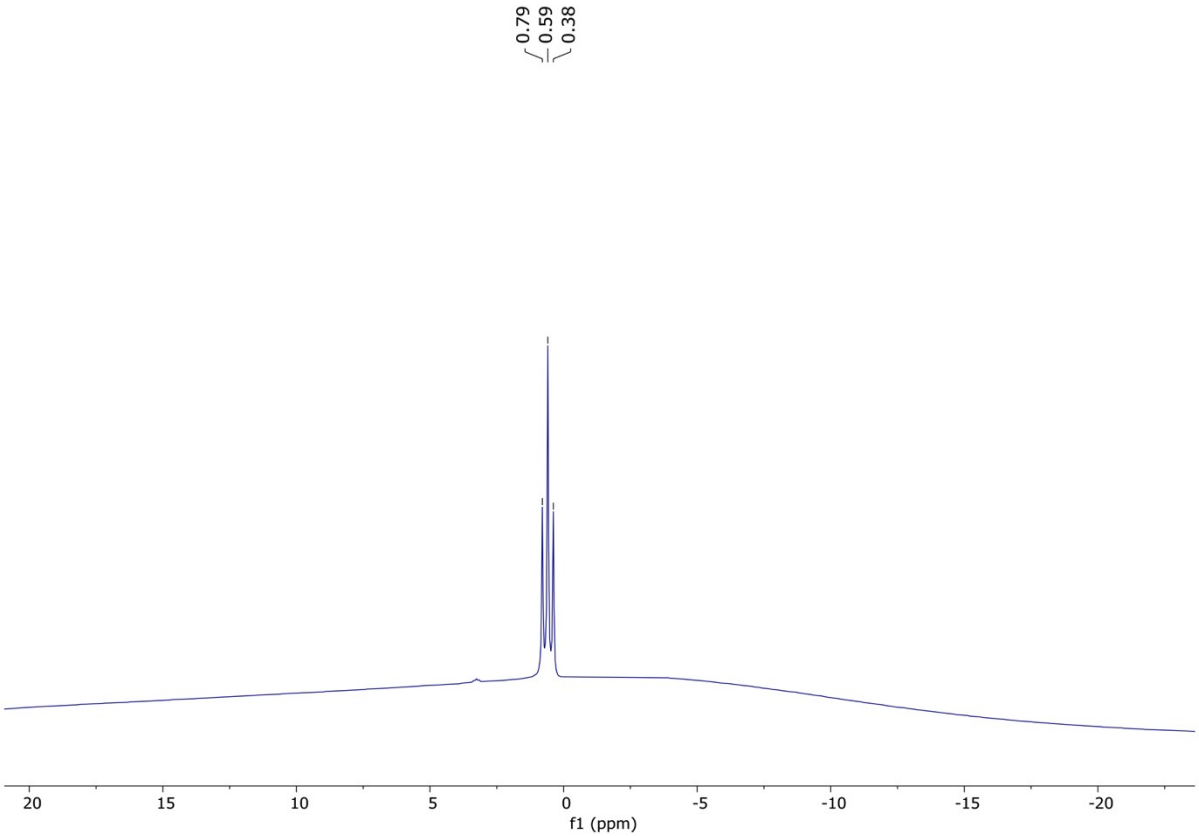
Compound **5**



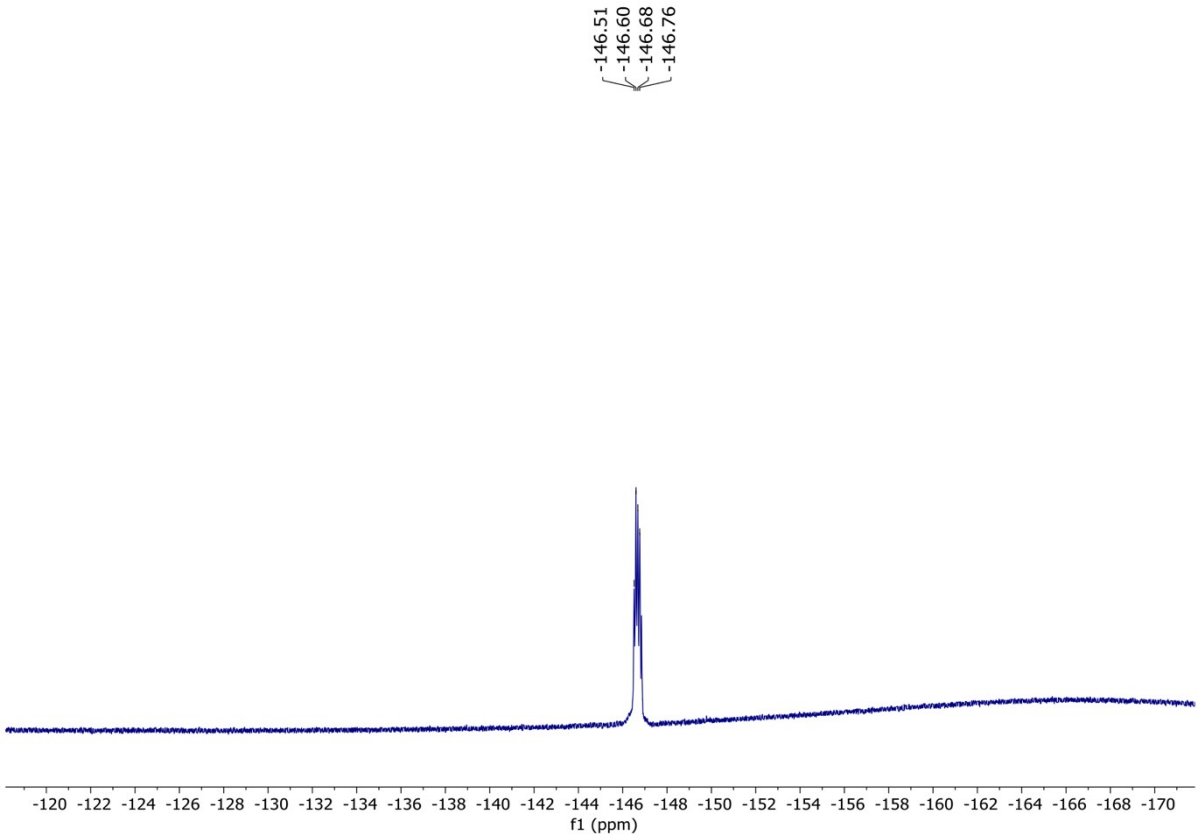
*^{1}H NMR spectrum of compound **5** (400 MHz in $CDCl_3$)*



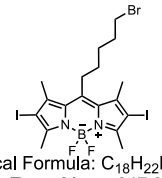
*^{13}C NMR spectrum of compound **5** (126 MHz in $CDCl_3$)*



*^{11}B NMR spectrum of compound **5** (128 MHz in $CDCl_3$)*

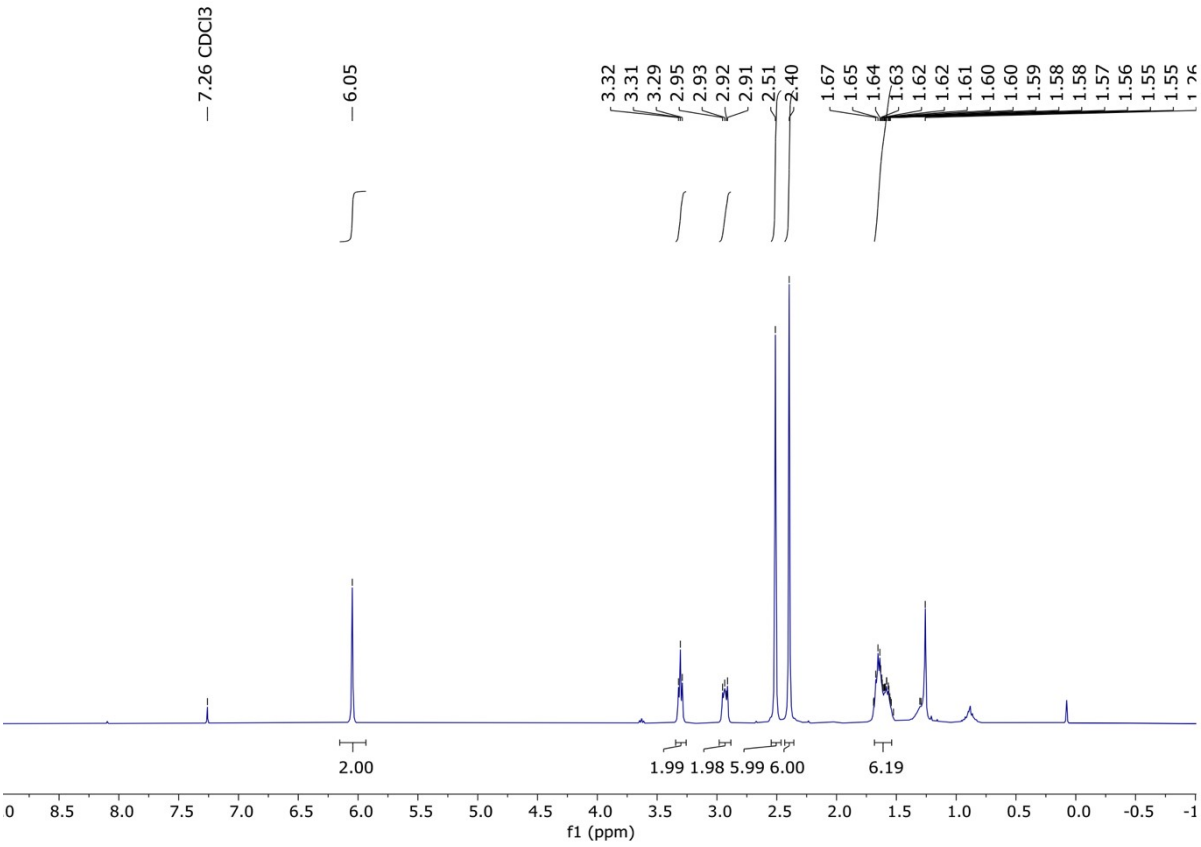


*^{19}F NMR spectrum of compound **5** (377 MHz in $CDCl_3$)*

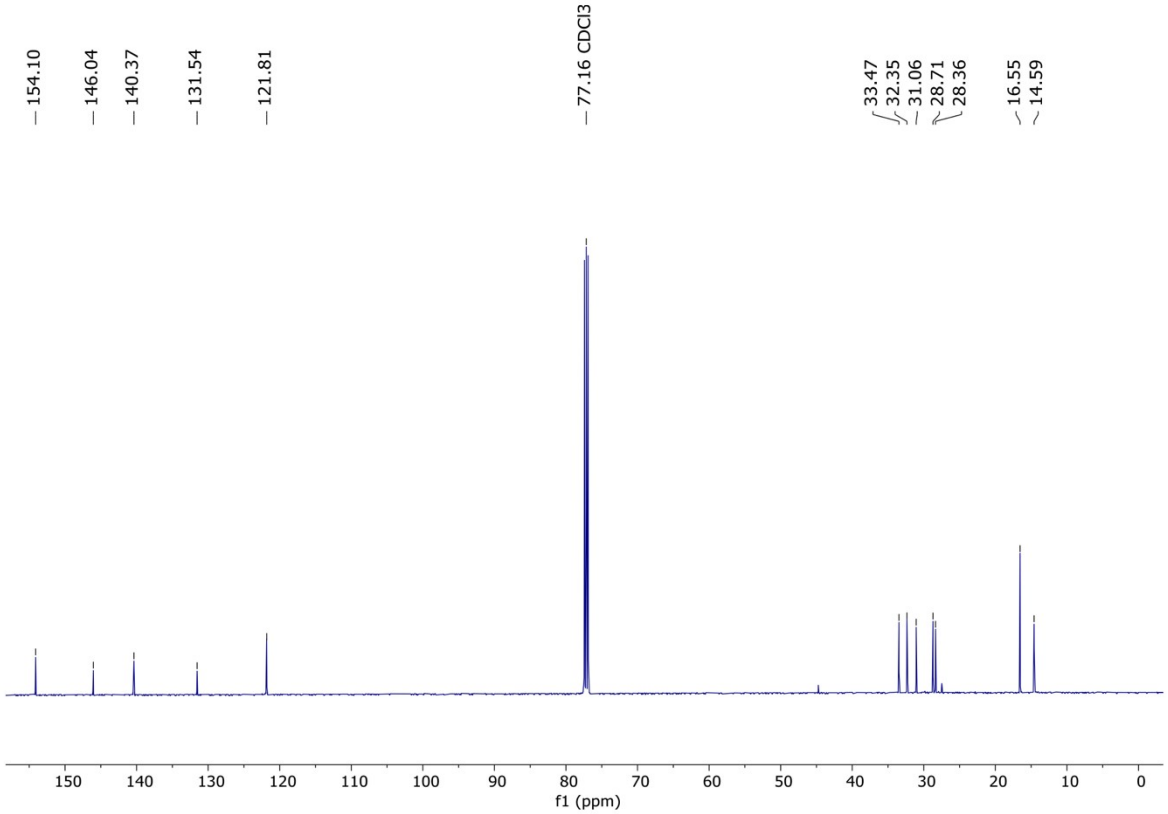


Chemical Formula: $C_{18}H_{22}BBrF_2I_2N_2$
Exact Mass: 647,91
Molecular Weight: 648,91

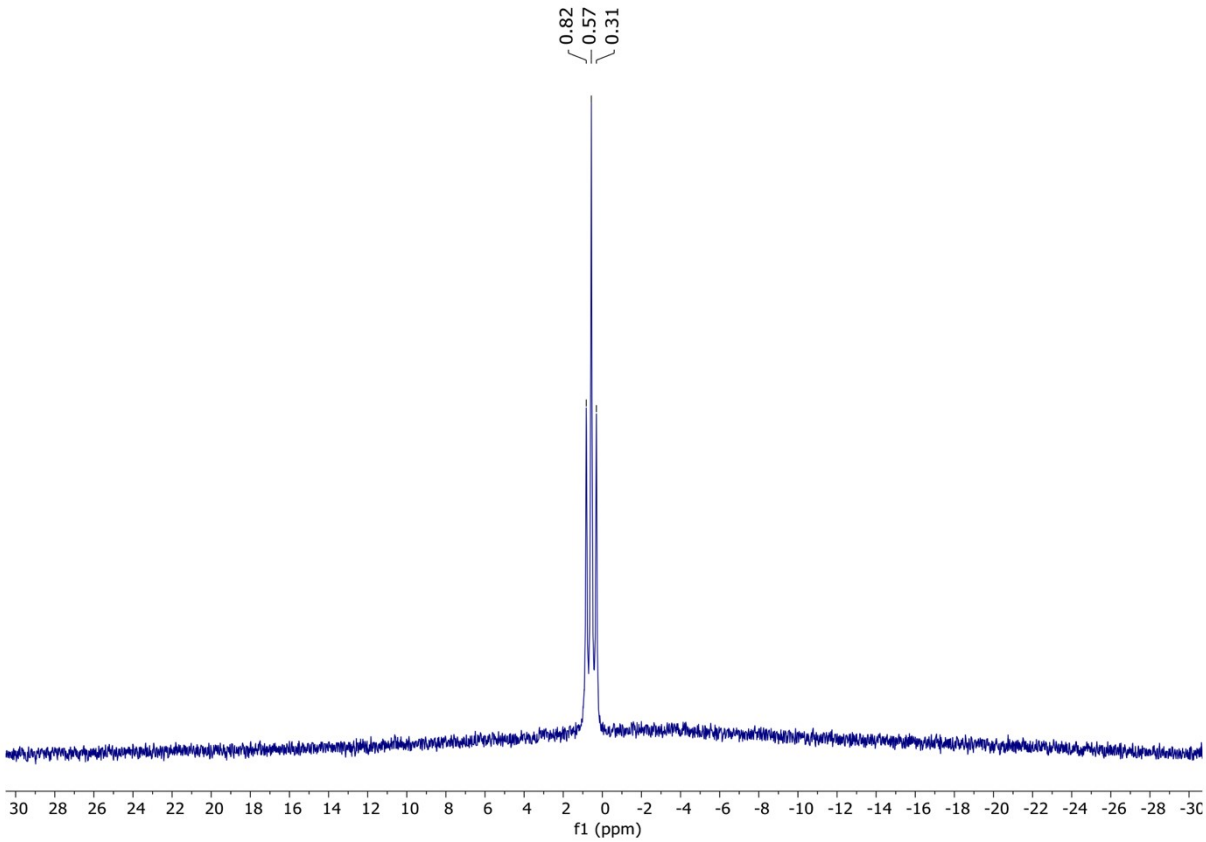
Compound **6**



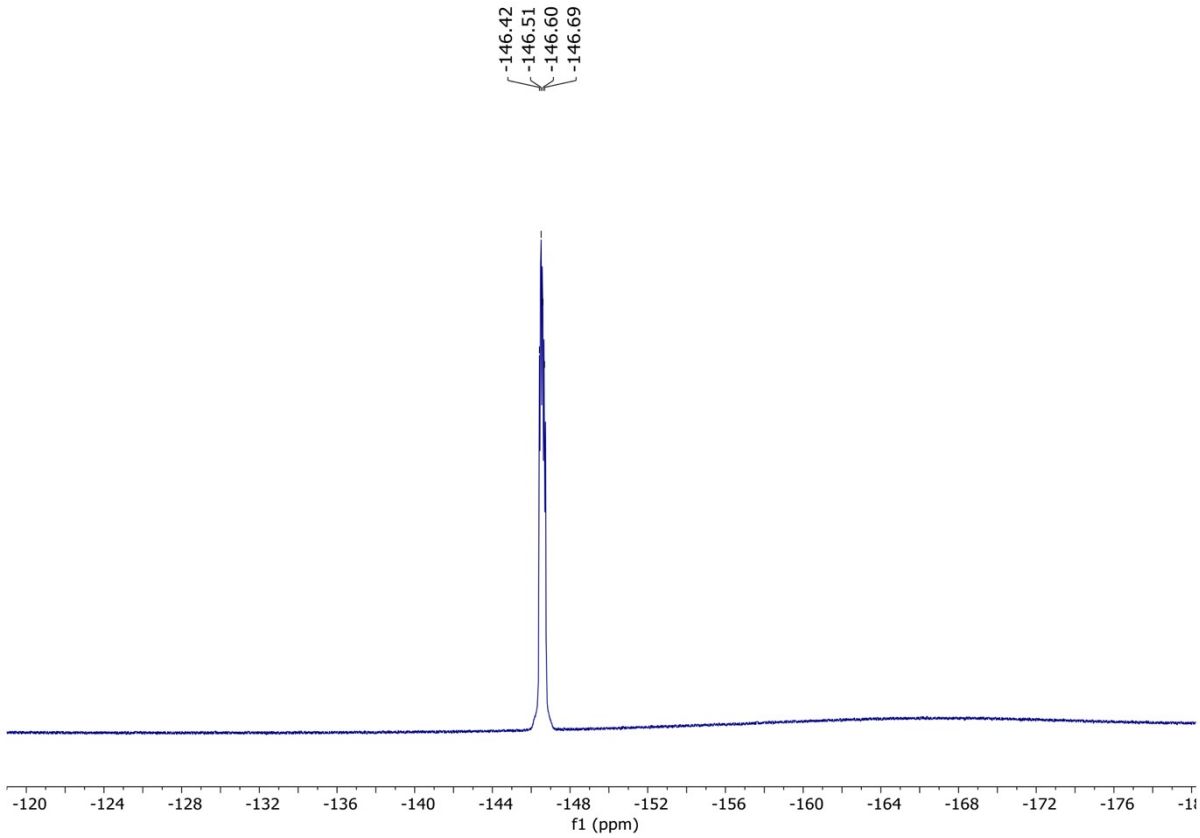
*^{1}H NMR spectrum of compound **6** (400 MHz in $CDCl_3$)*



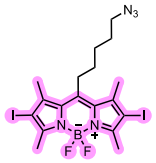
*^{13}C NMR spectrum of compound **6** (126 MHz in $CDCl_3$)*



*^{11}B NMR spectrum of compound **6** (128 MHz in $CDCl_3$)*

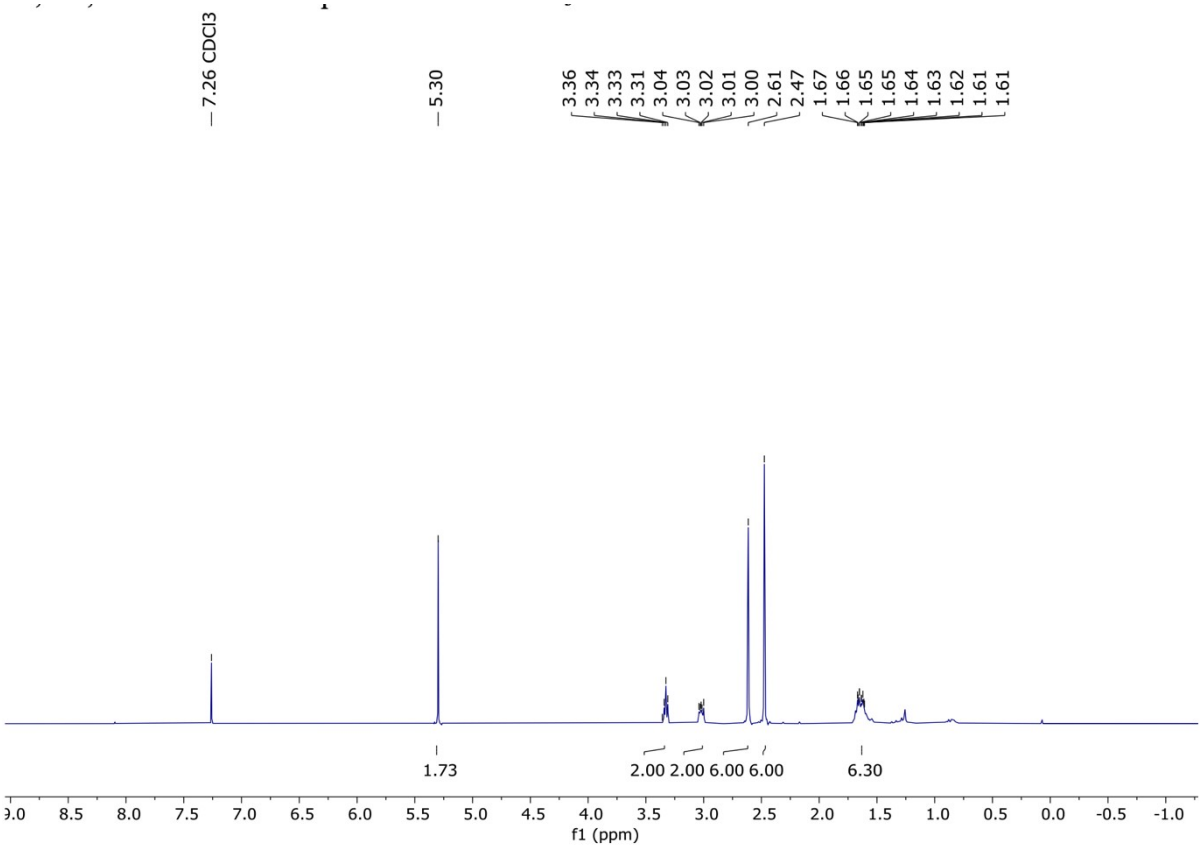


*^{19}F NMR spectrum of compound **6** (377 MHz in $CDCl_3$)*

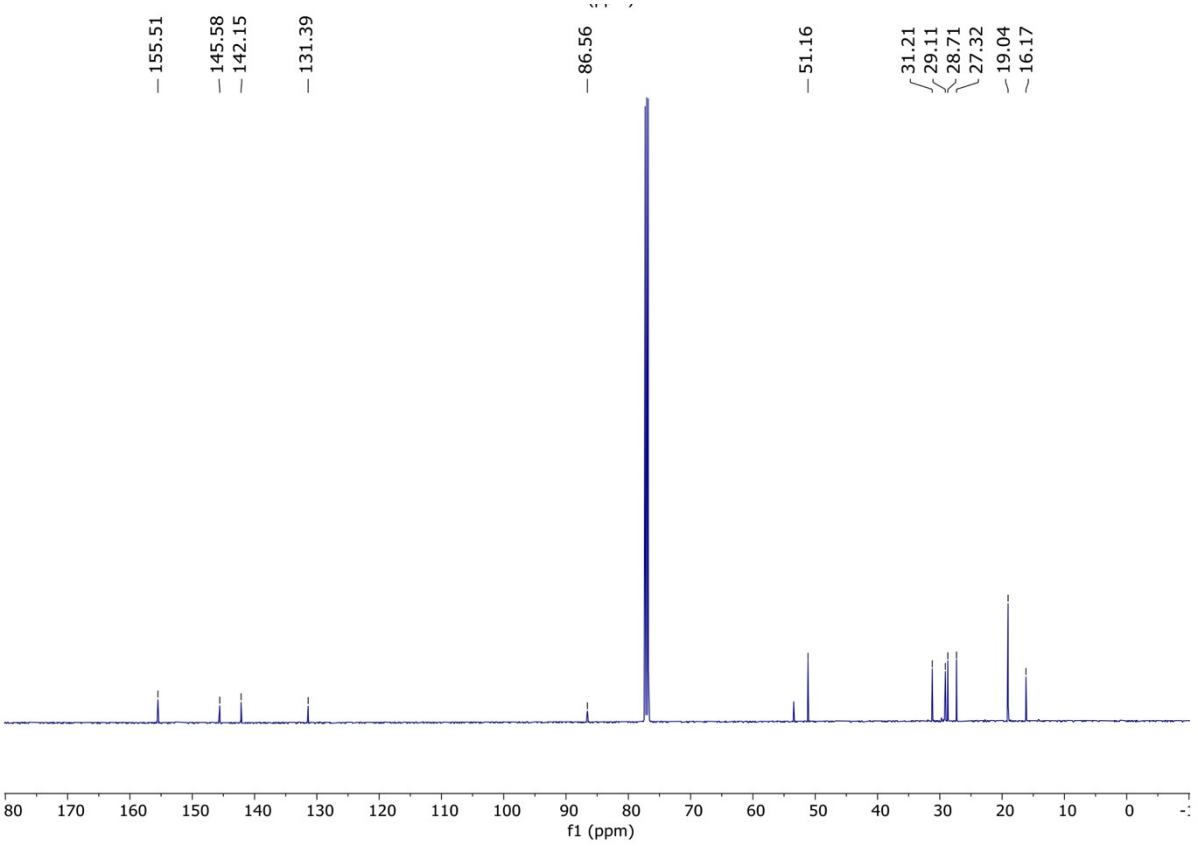


Chemical Formula: $C_{18}H_{22}BF_2I_2N_5$
Exact Mass: 611,00
Molecular Weight: 611,02

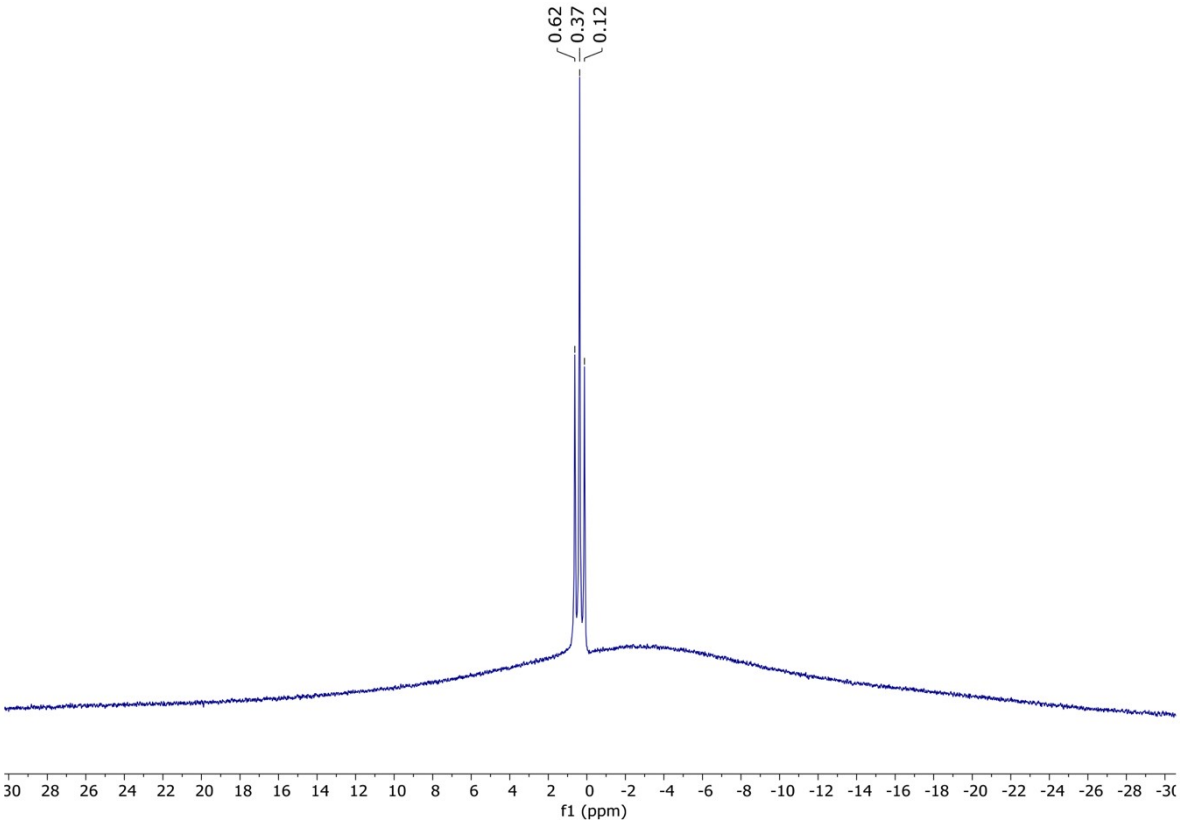
Compound **7**



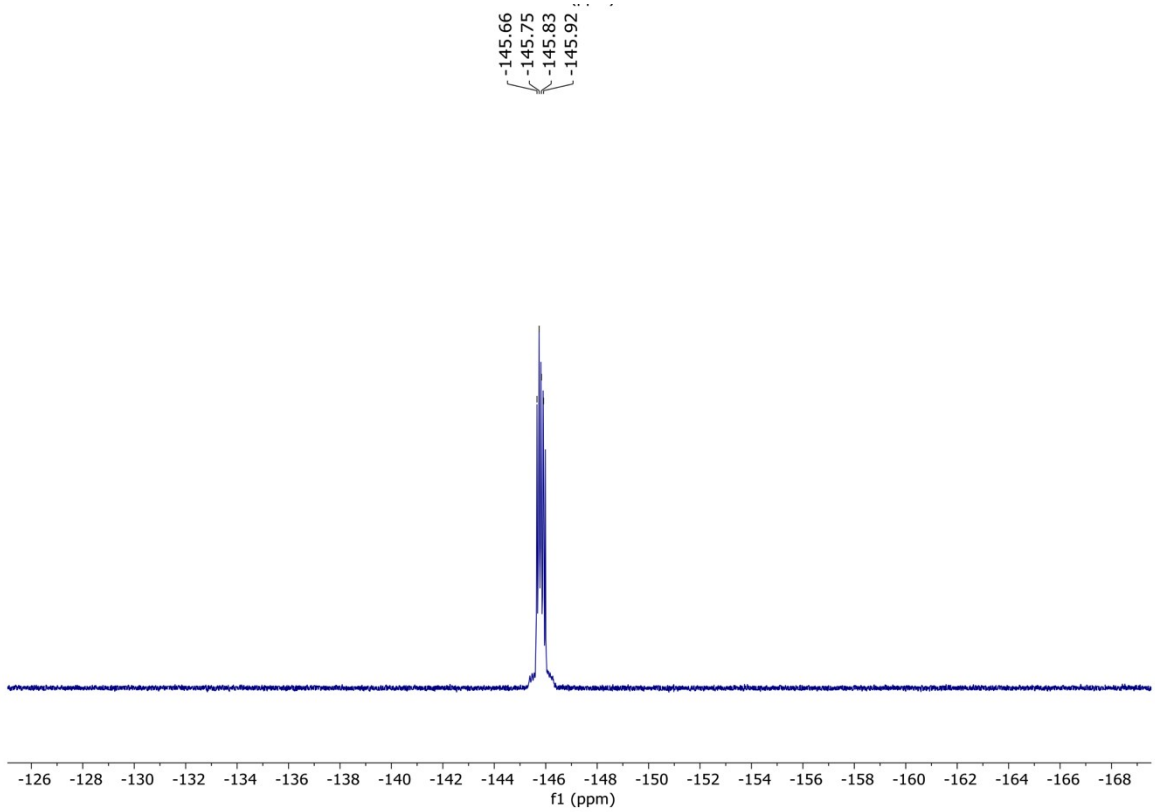
*^{1}H NMR spectrum of compound **7** (400 MHz in $CDCl_3$)*



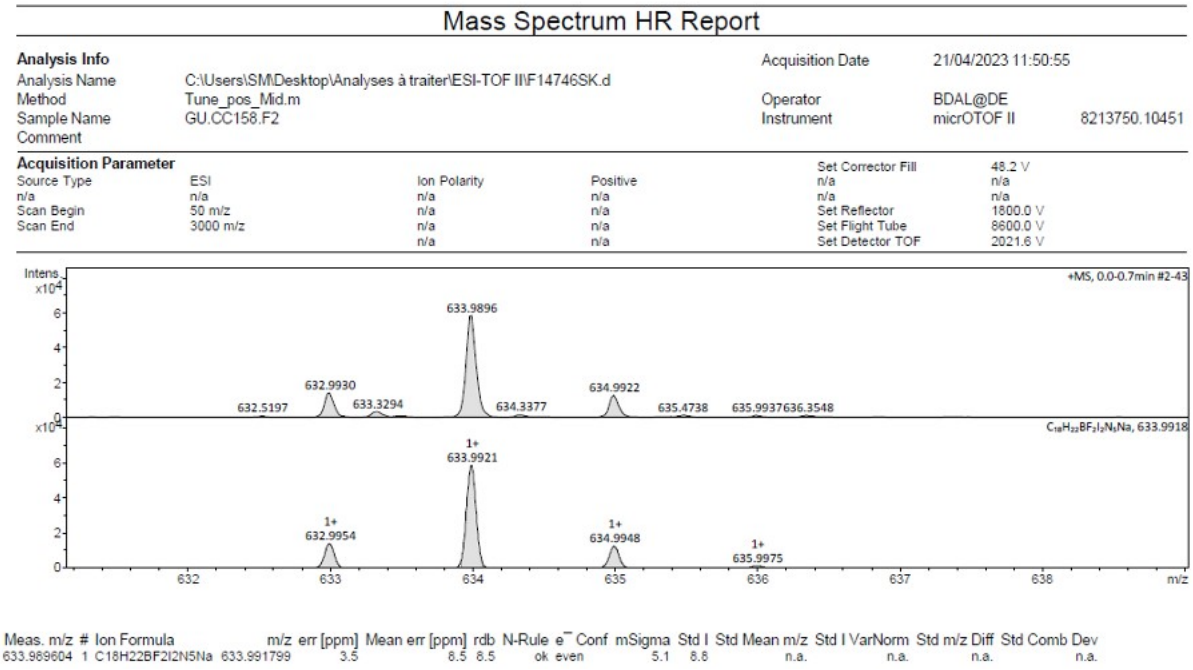
*^{13}C NMR spectrum of compound **7** (126 MHz in $CDCl_3$)*



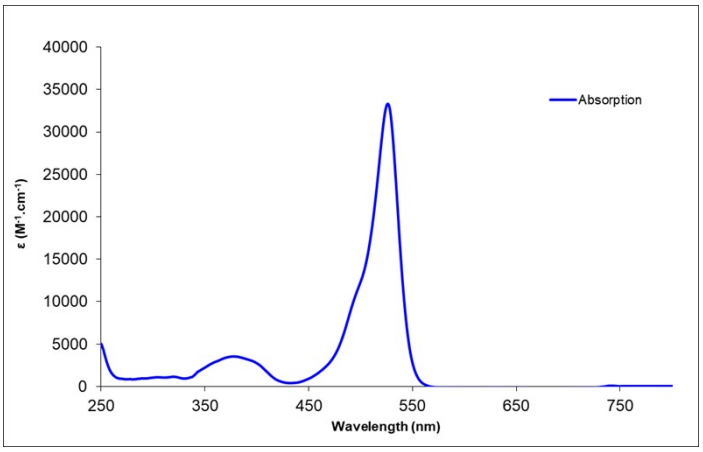
*^{11}B NMR spectrum of compound **7** (128 MHz in $CDCl_3$)*



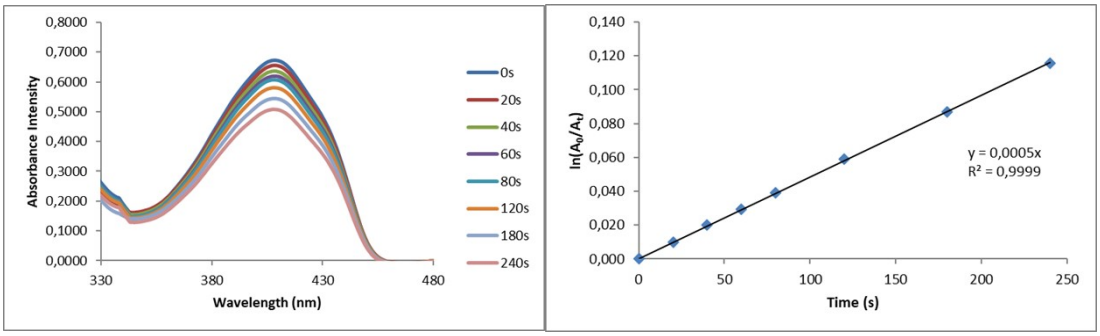
*^{19}F NMR spectrum of compound **7** (377 MHz in $CDCl_3$)*



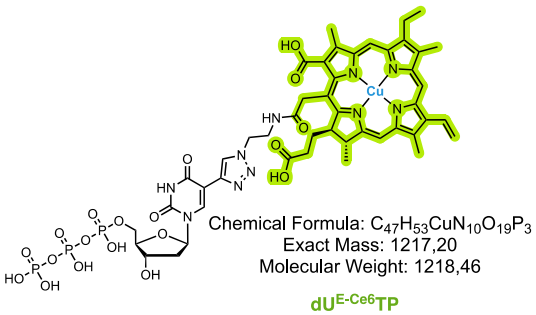
*HR-MS ESI spectrum of compound **7.***



*UV spectrum of compound **7**. Emission and excitation curves are not shown, as emission is too weak to be recorded accurately. λ_{max}= 526 nm; ε_{526} = 83230 $M^{-1}.cm^{-1}$*

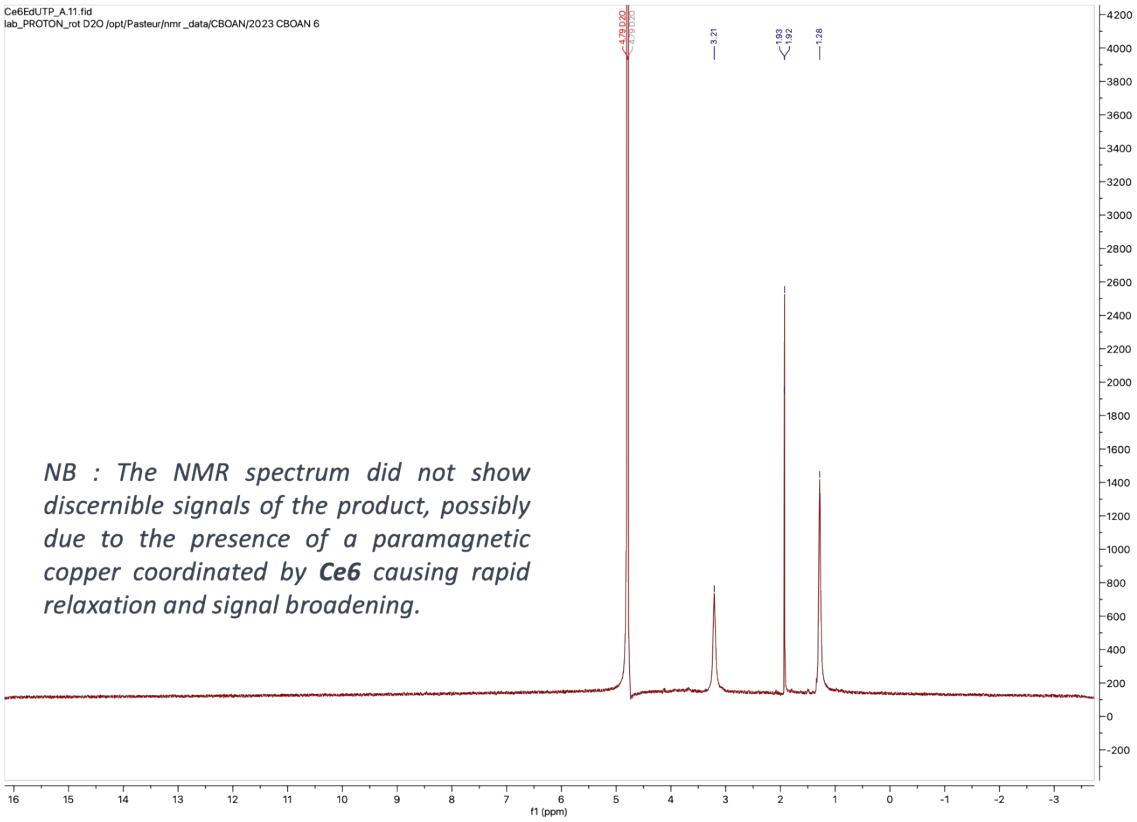


*Singlet oxygen measurement of compound **7**.*

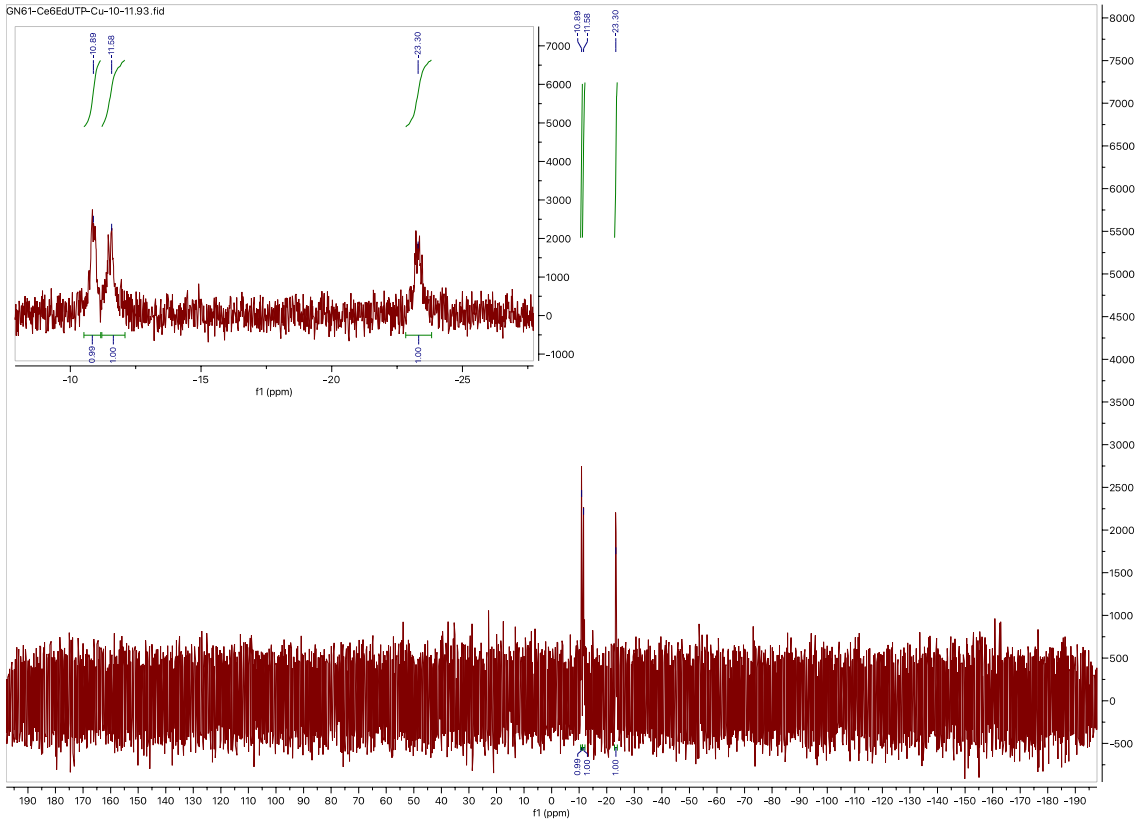


Chemical Formula: $C_{47}H_{53}CuN_{10}O_{19}P_3$
Exact Mass: 1217,20
Molecular Weight: 1218,46

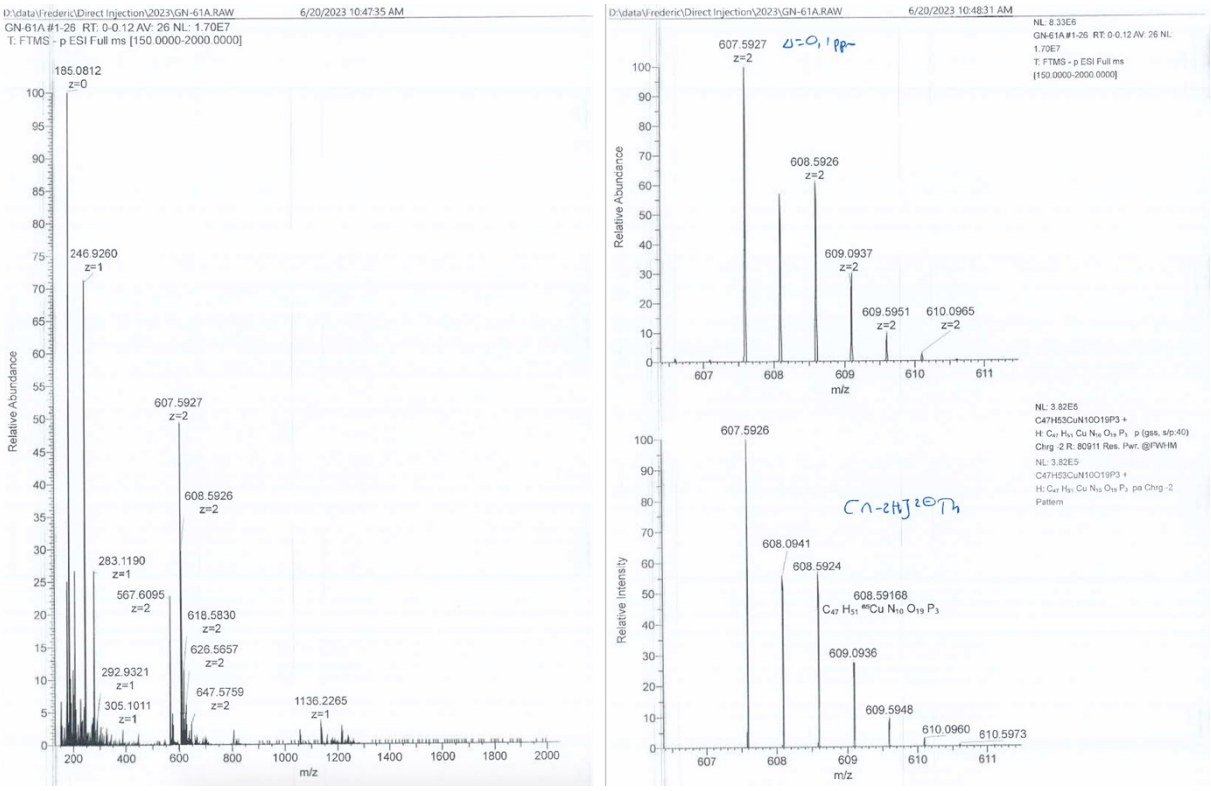
dU$^{E\text{-}Ce6}$TP



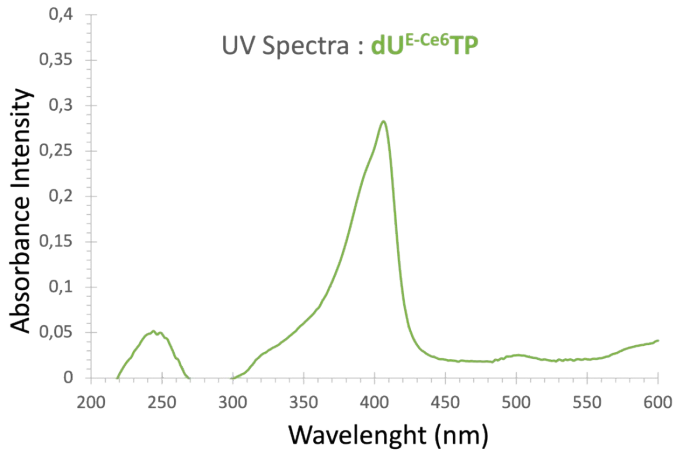
*^{1}H NMR spectrum of **dU$^{E\text{-}Ce6}$TP** (400 MHz in D_2O).*



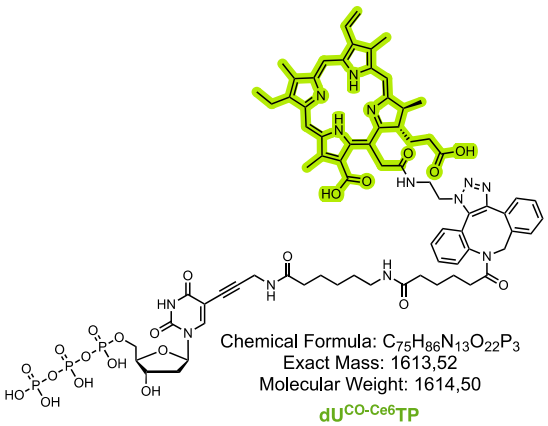
*^{31}P NMR spectrum of **dU$^{E\text{-}Ce6}$TP** (162 MHz in D_2O)*



*HR-MS ESI spectrum (negative mode) of **dU$^{E\text{-}Ce6}$TP**.*

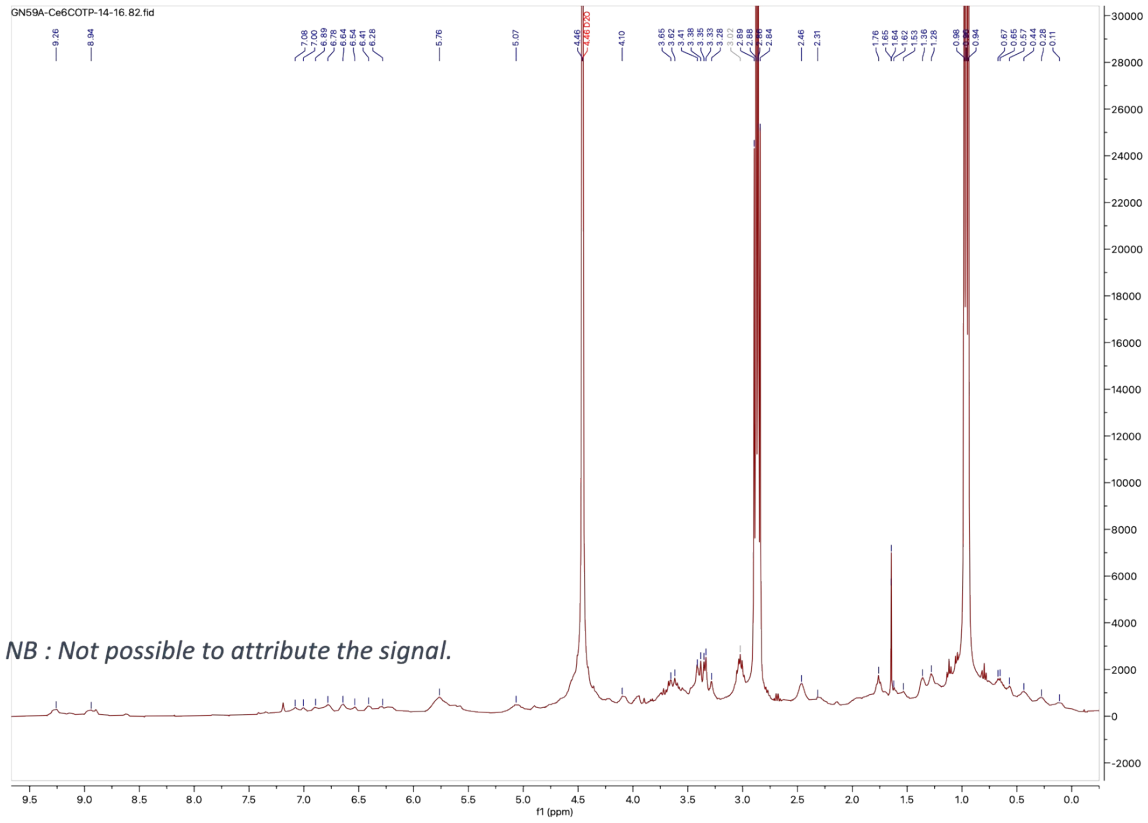


UV spectrum in ultra-pure water of ***dU$^{E\text{-}Ce6}$TP***

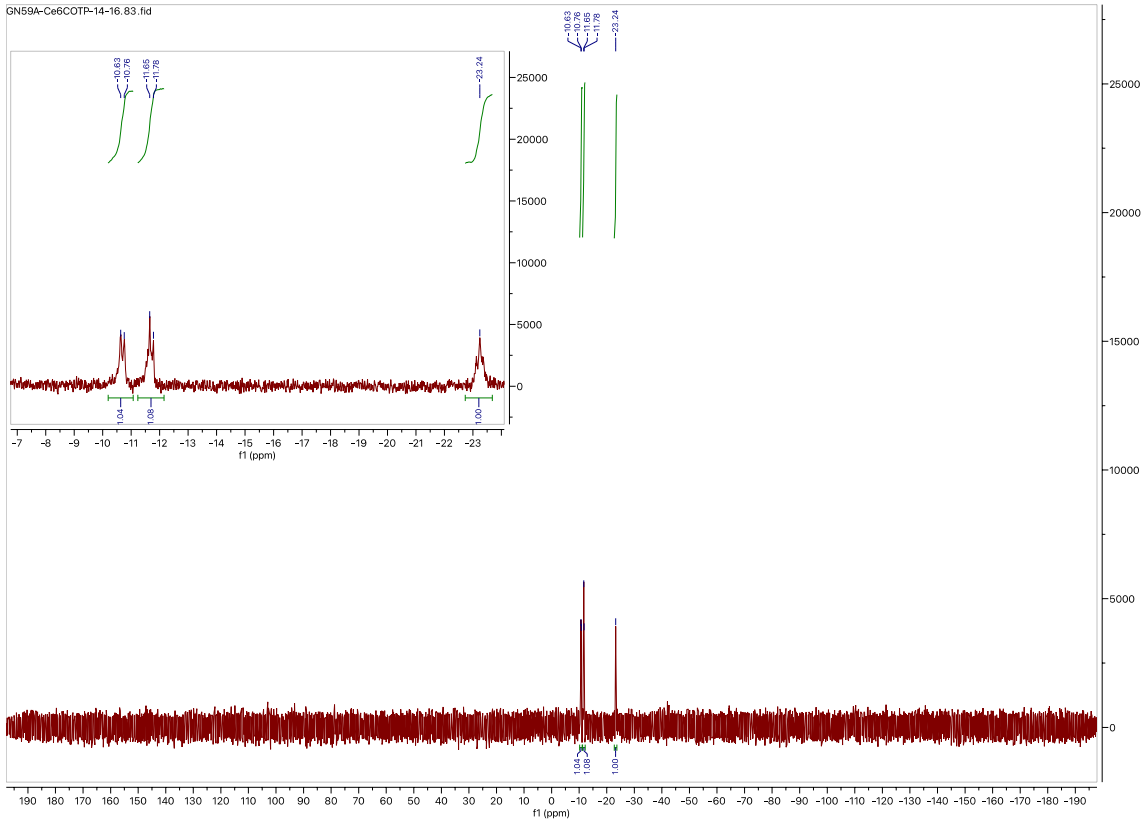


Chemical Formula: $C_{75}H_{86}N_{13}O_{22}P_3$
Exact Mass: 1613,52
Molecular Weight: 1614,50

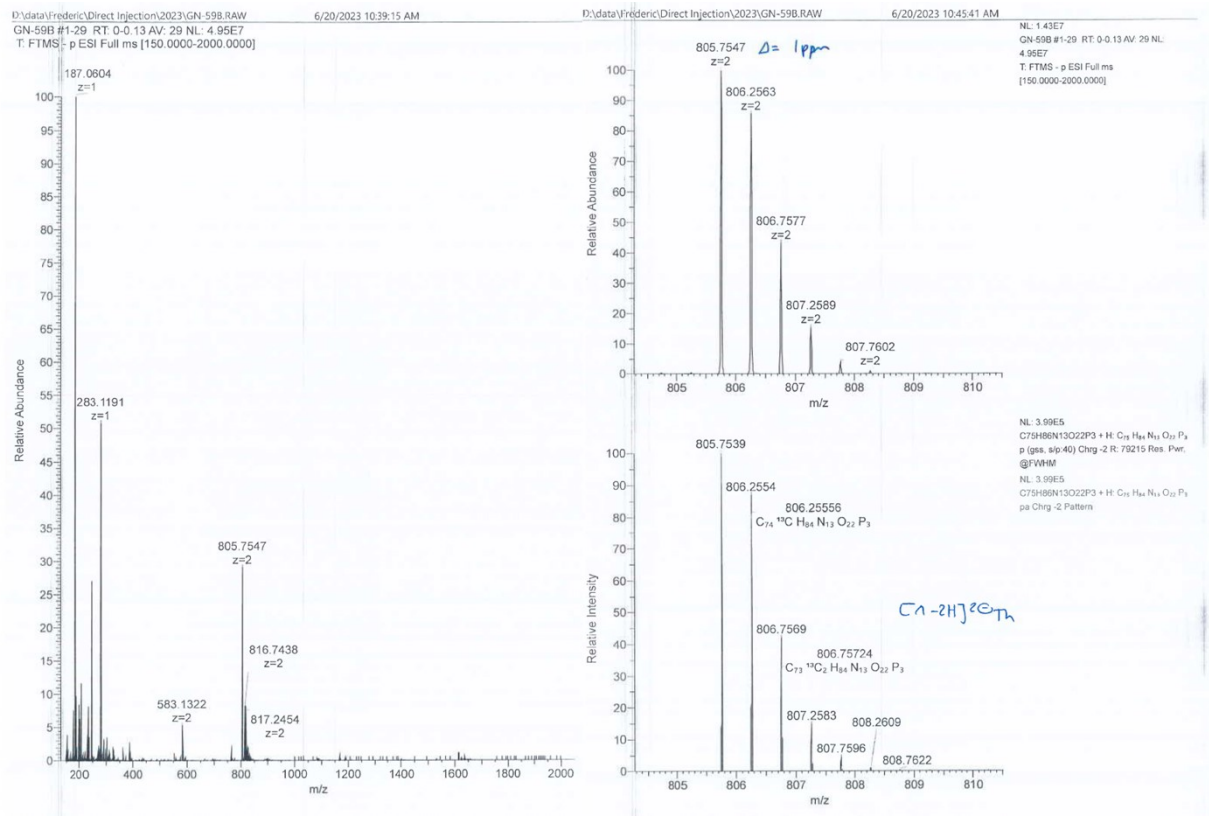
dU$^{CO\text{-}Ce6}$TP



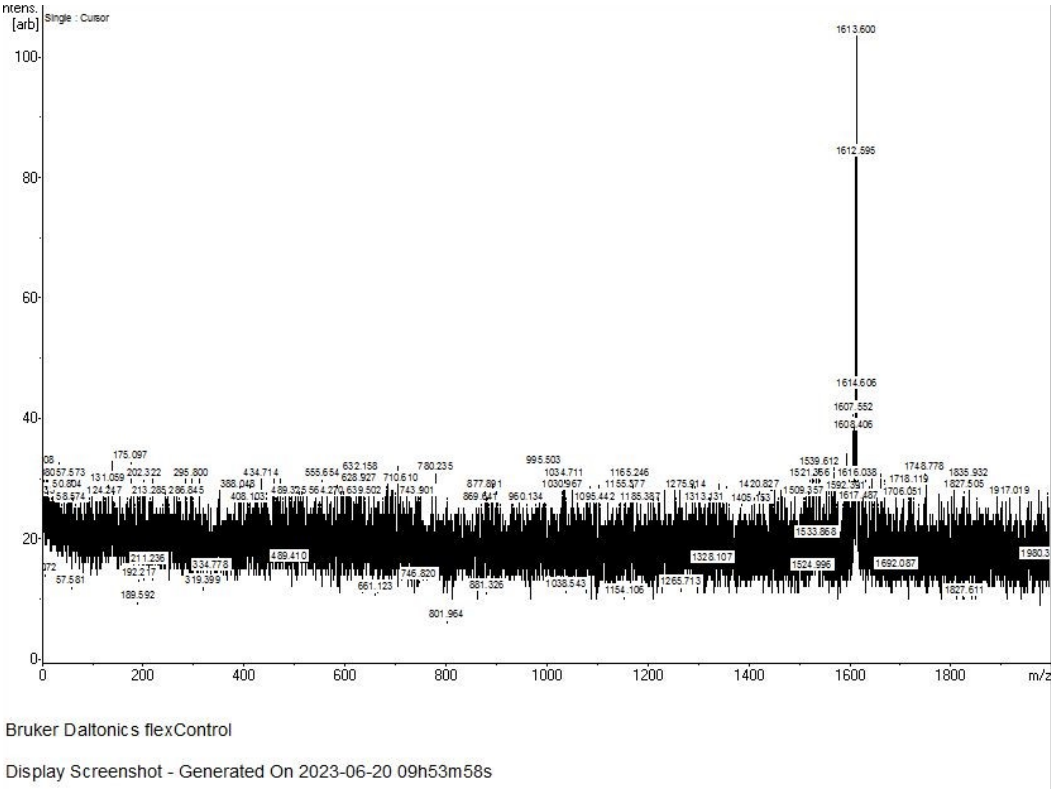
*^{1}H NMR spectrum of **dU$^{CO\text{-}Ce6}$TP** (400 MHz in D_2O)*



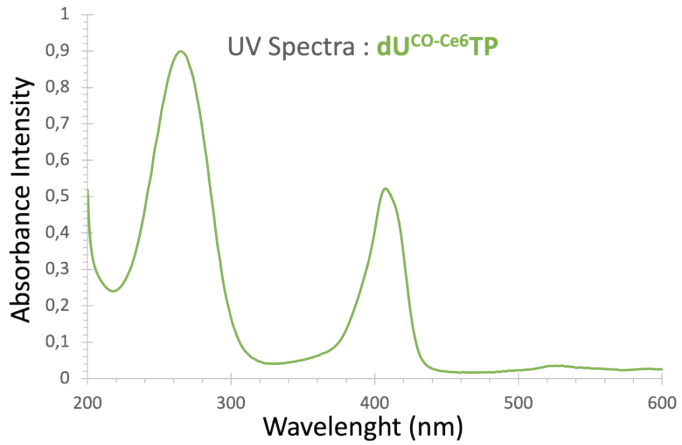
*^{31}P NMR spectrum of **dU$^{CO\text{-}Ce6}$TP** (162 MHz in D_2O)*



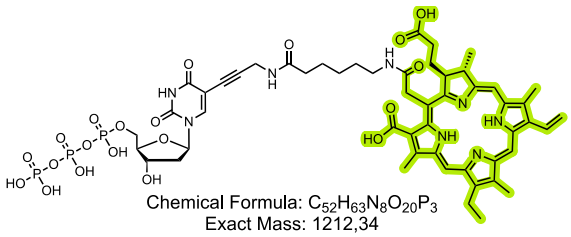
*HR-MS ESI spectrum (negative mode) of **dU$^{CO\text{-}Ce6}$TP***



MALDI-TOF spectrum (linear negative mode) of ***dU$^{CO\text{-}Ce6}$TP***

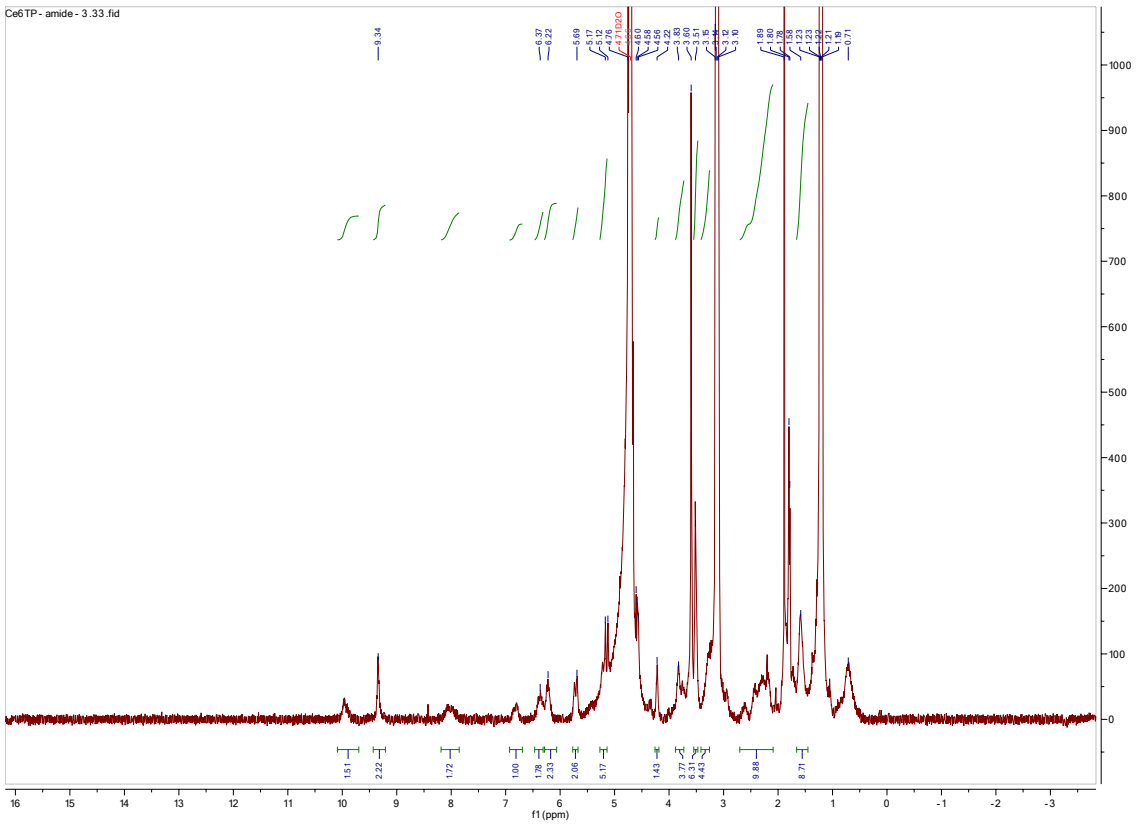


UV spectrum in ultra-pure water of ***dU$^{CO\text{-}Ce6}$TP***

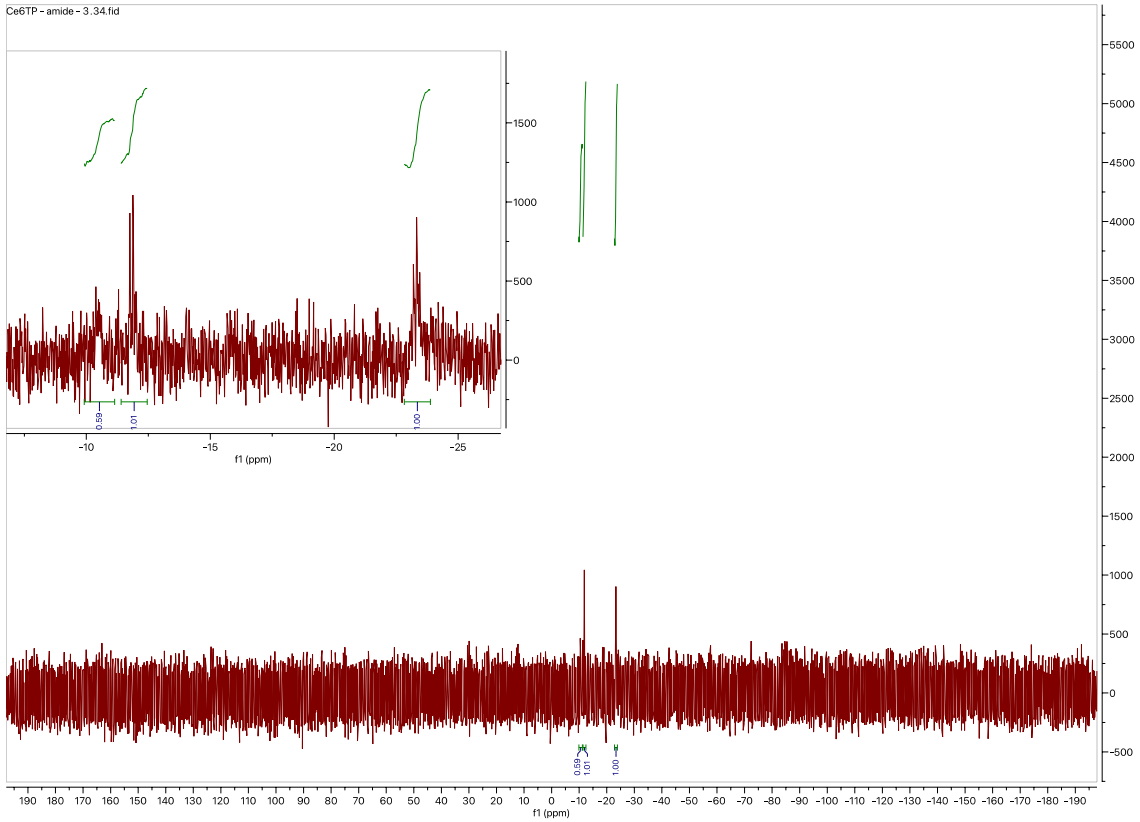


Chemical Formula: $C_{52}H_{63}N_8O_{20}P_3$
Exact Mass: 1212,34
Molecular Weight: 1213,03

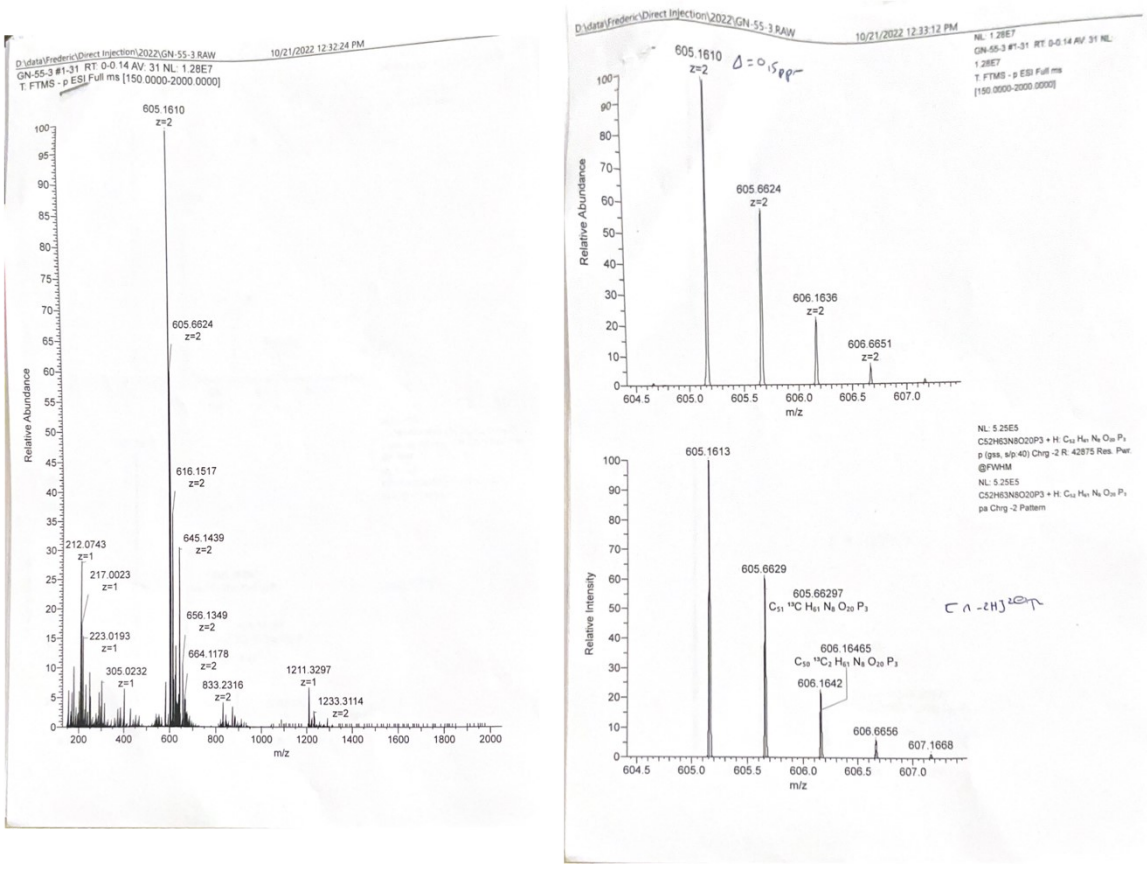
dU$^{Am\text{-}Ce6}$TP



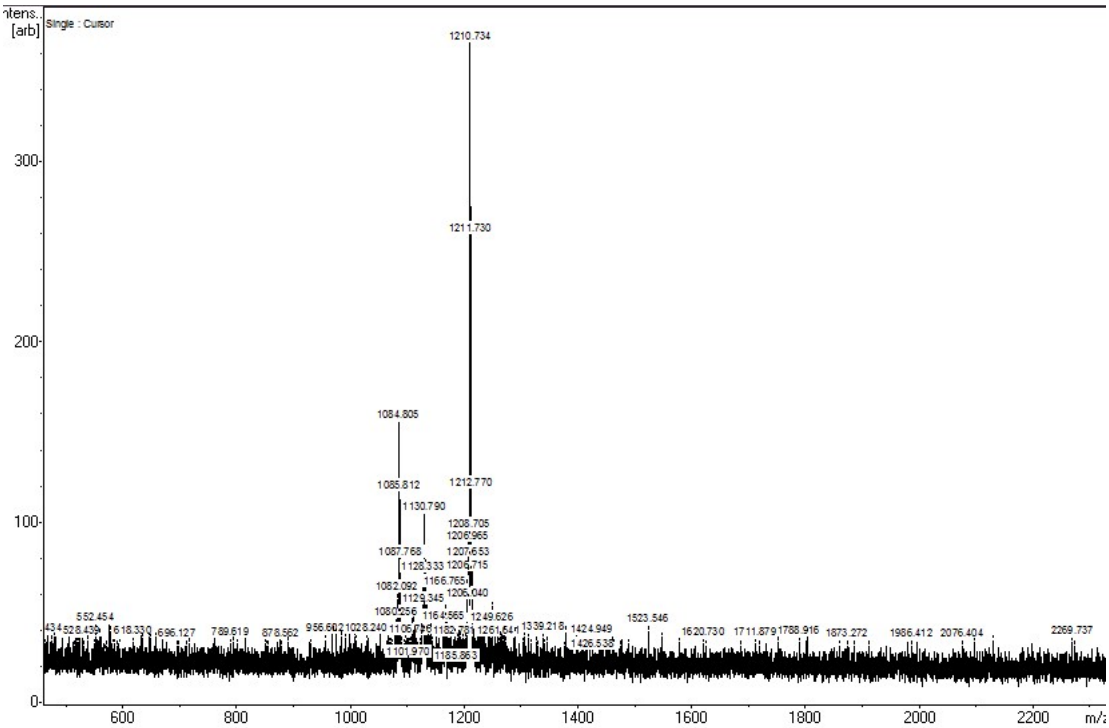
*^{1}H NMR spectrum of **dU$^{Am\text{-}Ce6}$TP** (400 MHz in D_2O)*



*^{31}P NMR spectrum of **dU$^{Am\text{-}Ce6}$TP** (162 MHz in D_2O)*



*HR-MS ESI spectrum (negative mode) of **dU$^{Am\text{-}Ce6}$TP***



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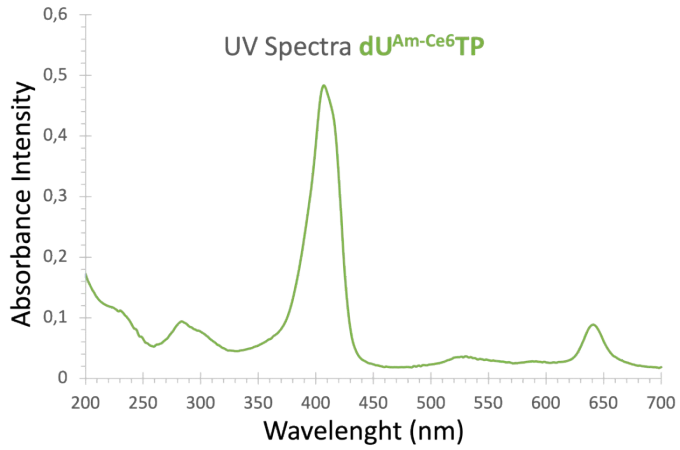
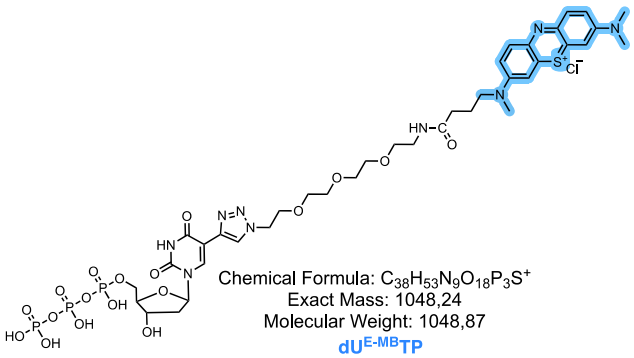
MALDI-TOF spectrum (linear negative mode) of ***dU$^{Am\text{-}Ce6}$TP***

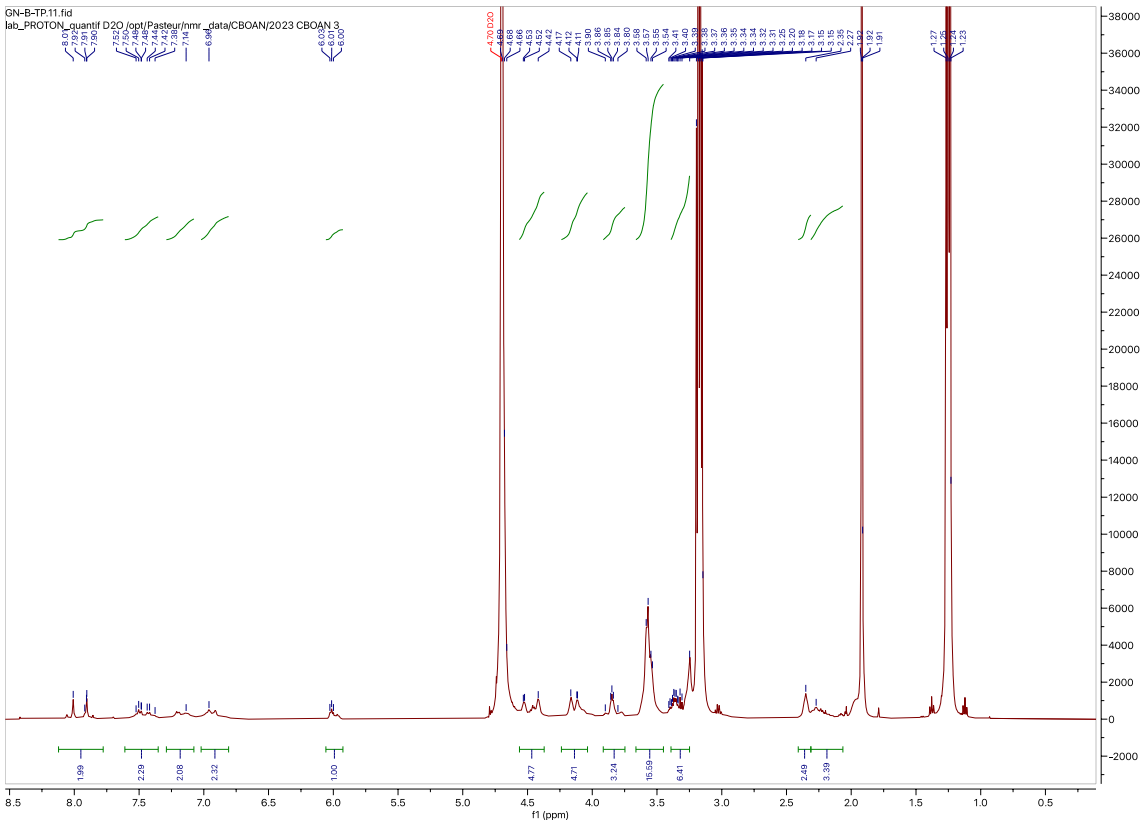


UV spectrum in ultra-pure water of ***dU$^{Am\text{-}Ce6}$TP***

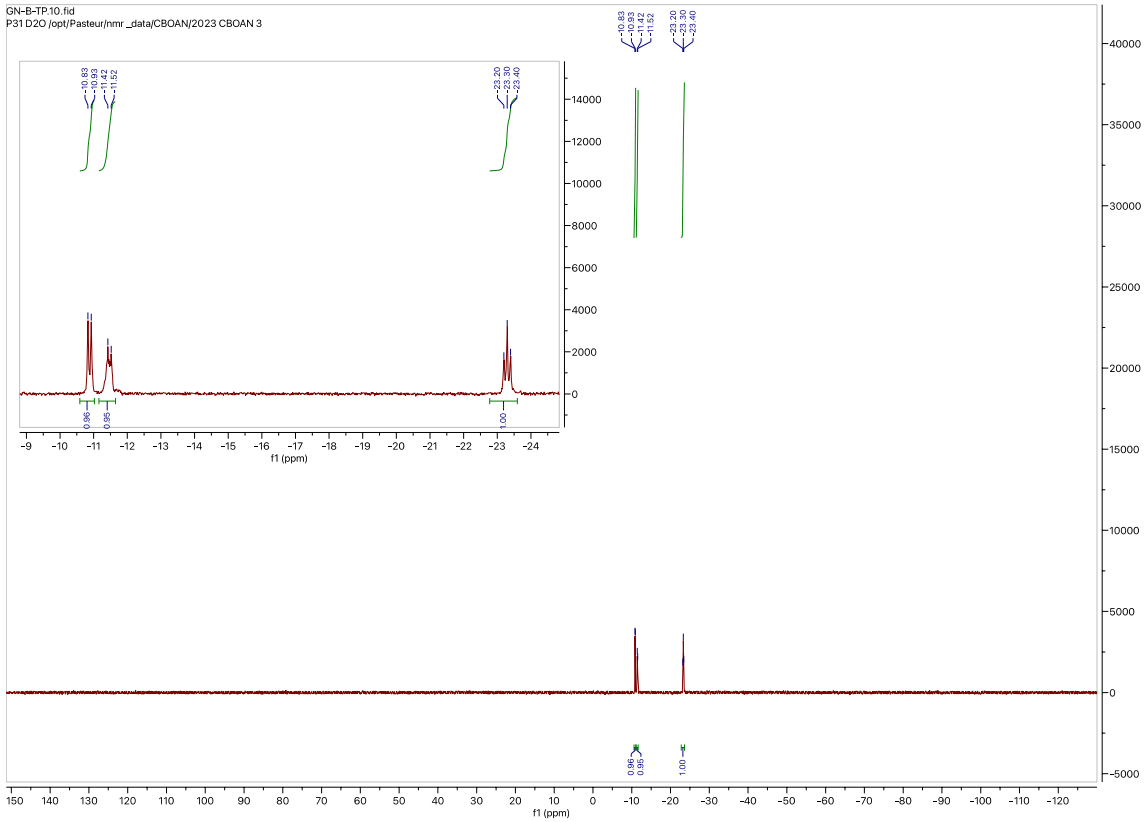


Chemical Formula: $C_{38}H_{53}N_9O_{18}P_3S^+$
Exact Mass: 1048,24
Molecular Weight: 1048,87

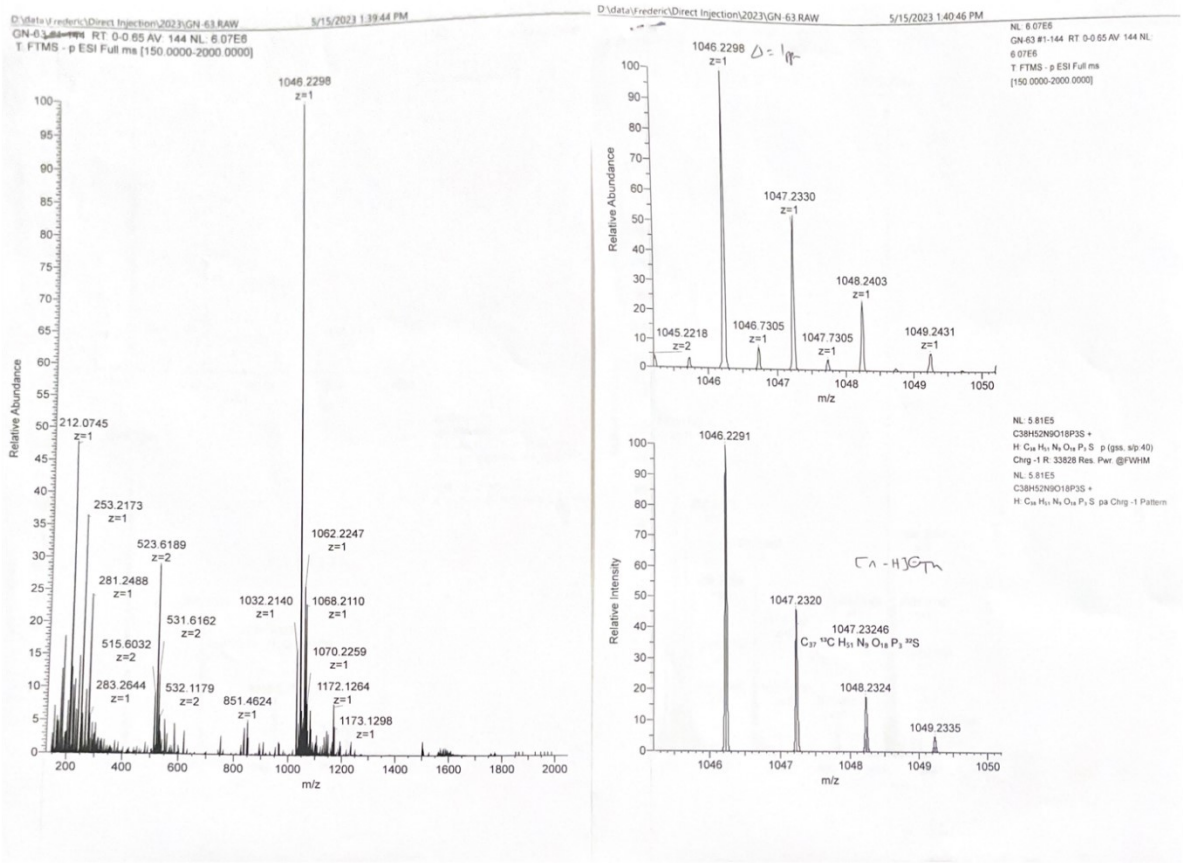
dU$^{E\text{-}MB}$TP



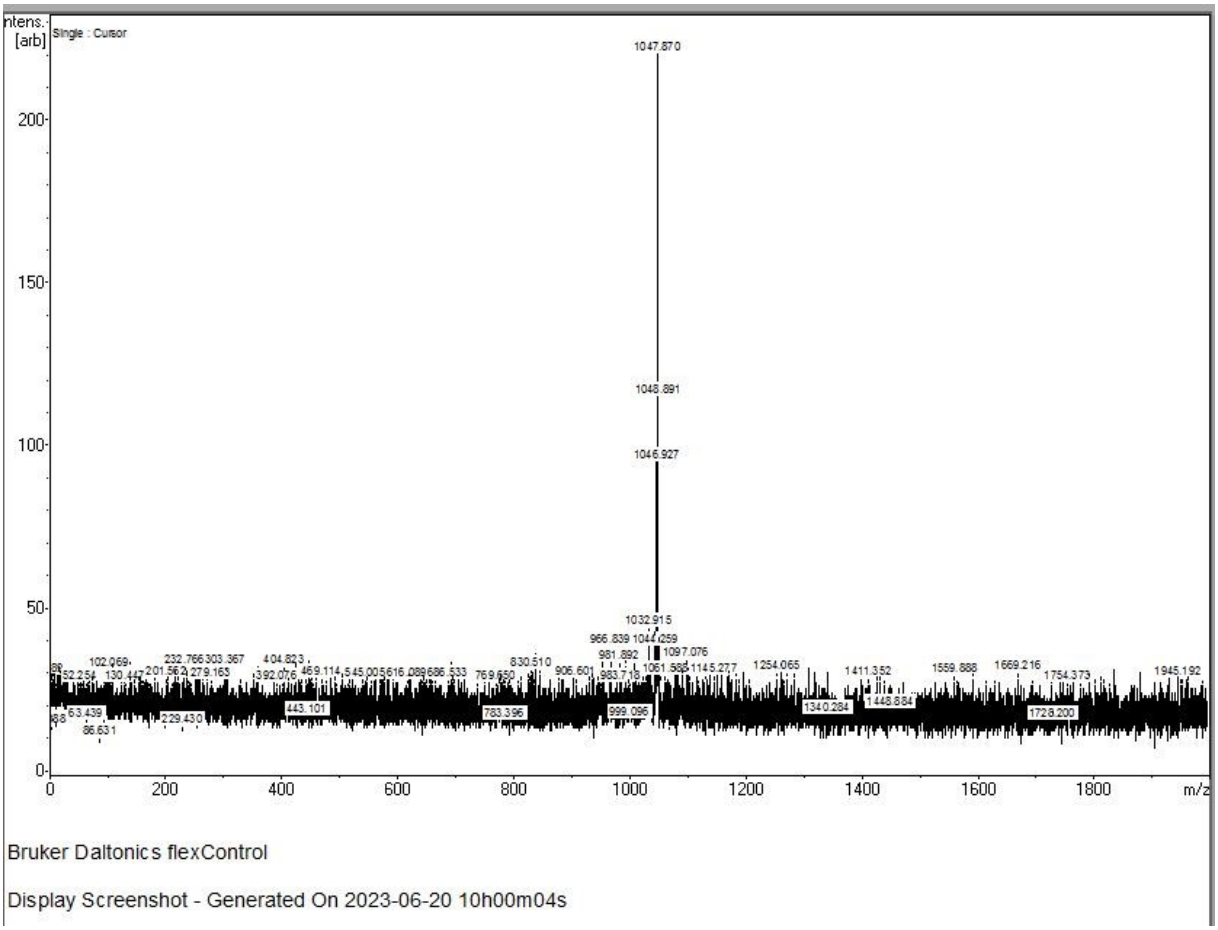
*^{1}H NMR spectrum of **dU$^{E\text{-}MB}$TP** (500 MHz in D_2O)*



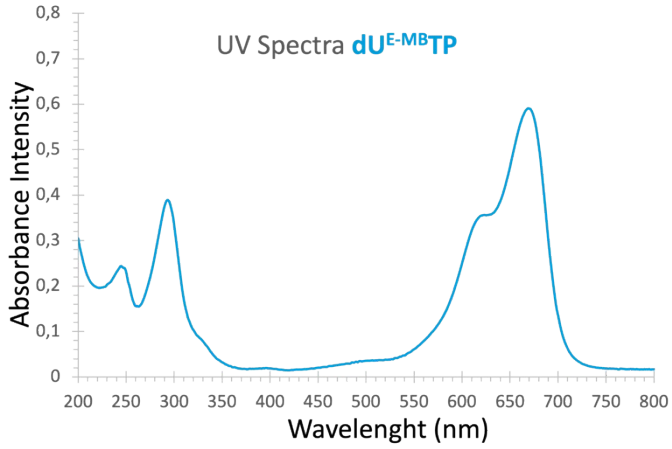
*^{31}P NMR spectrum of **dU$^{E\text{-}MB}$TP** (202 MHz in D_2O)*



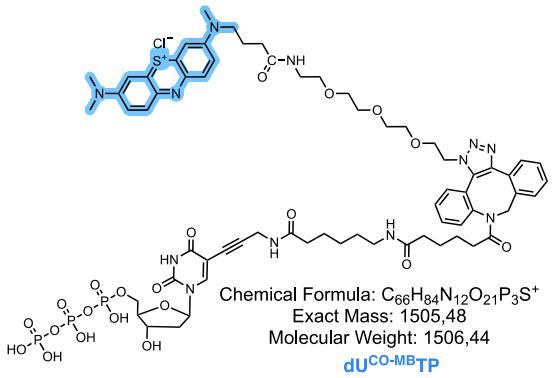
*HR-MS ESI spectrum (negative mode) of **dU$^{E\text{-}MB}$TP.***

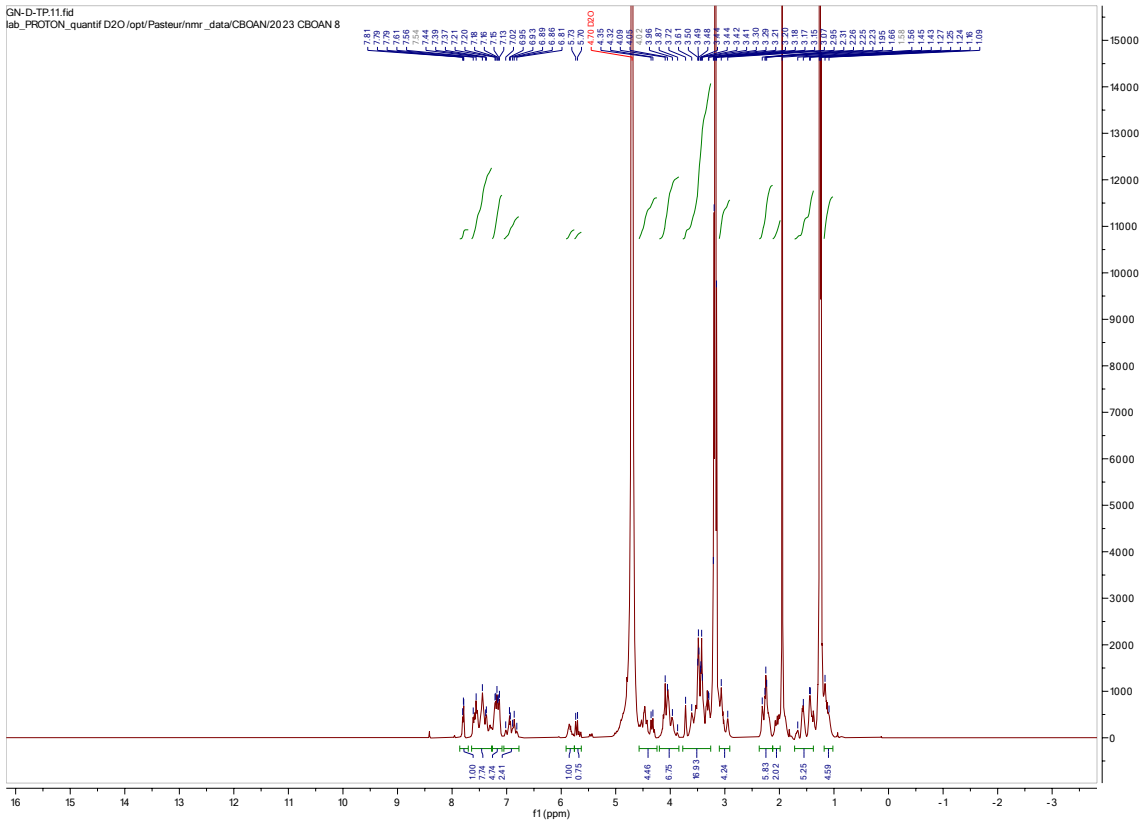


MALDI-TOF spectrum (linear negative mode) of ***dU$^{E\text{-}MB}$TP***

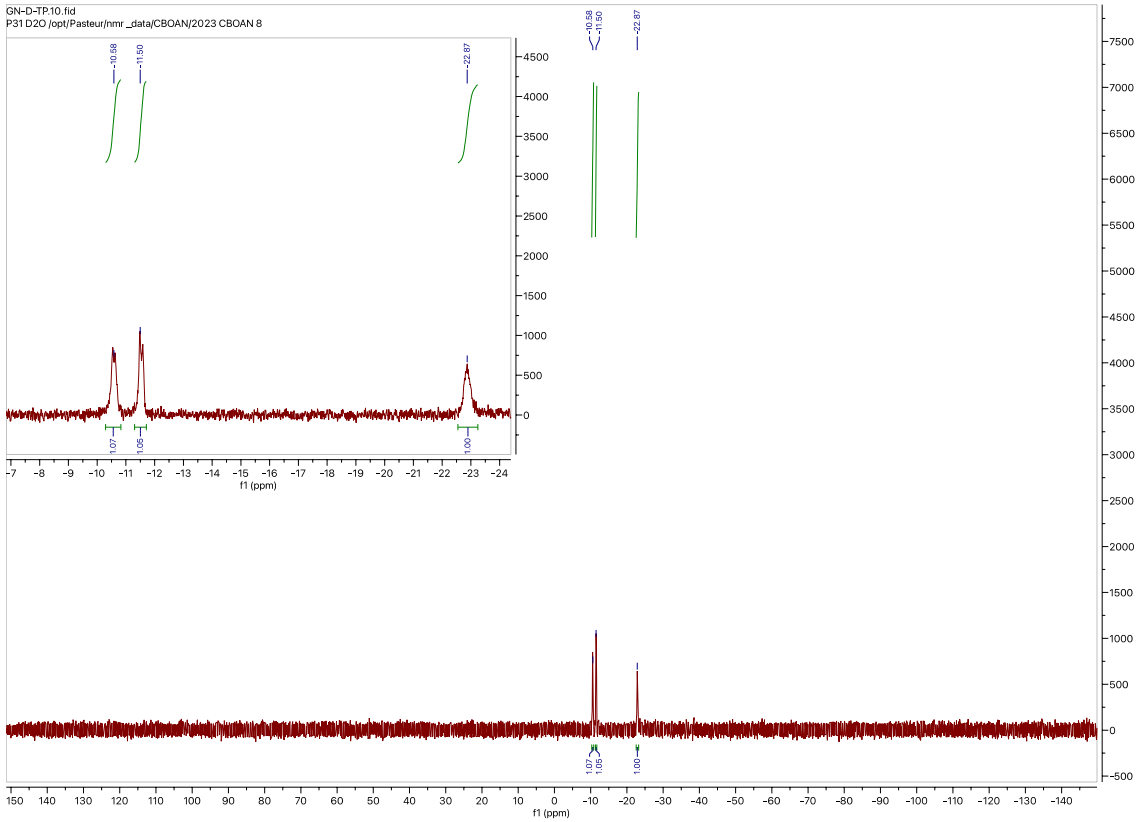


UV spectrum in ultra-pure water of ***dU$^{E\text{-}MB}$TP***

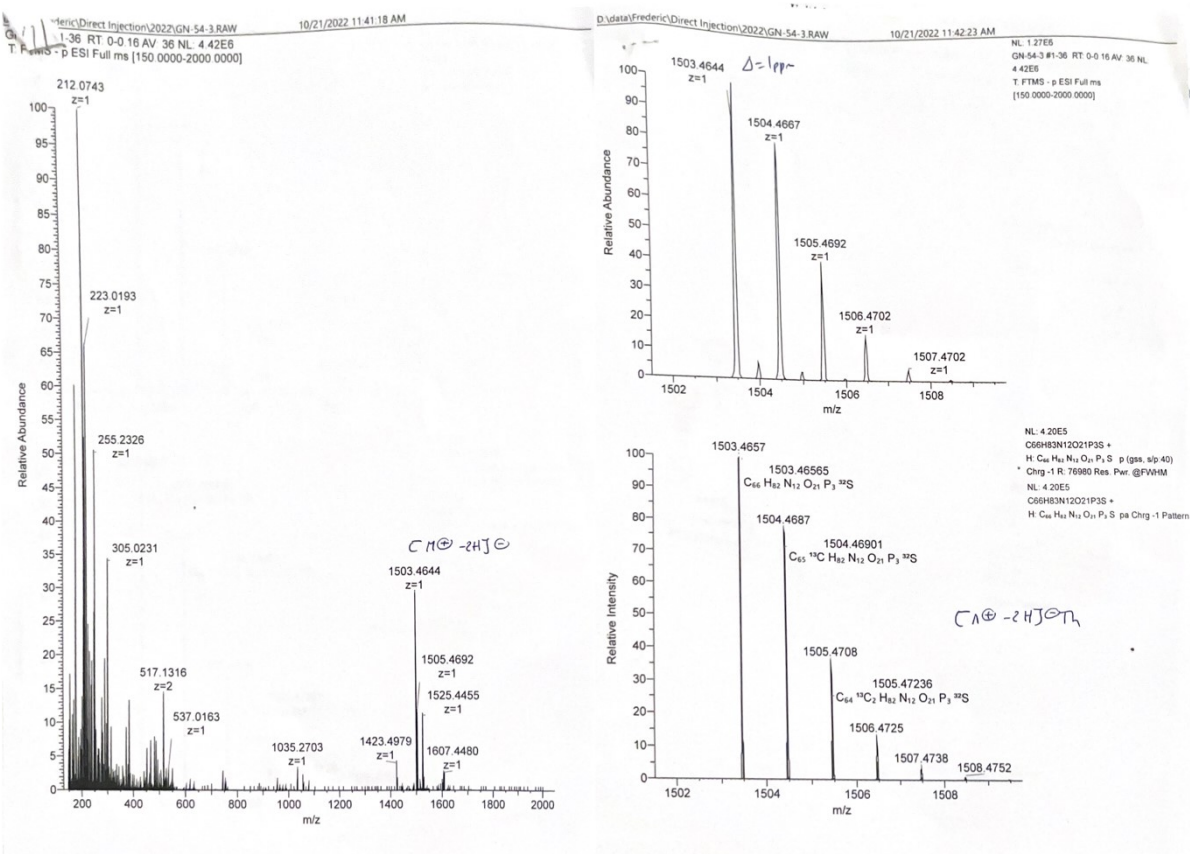




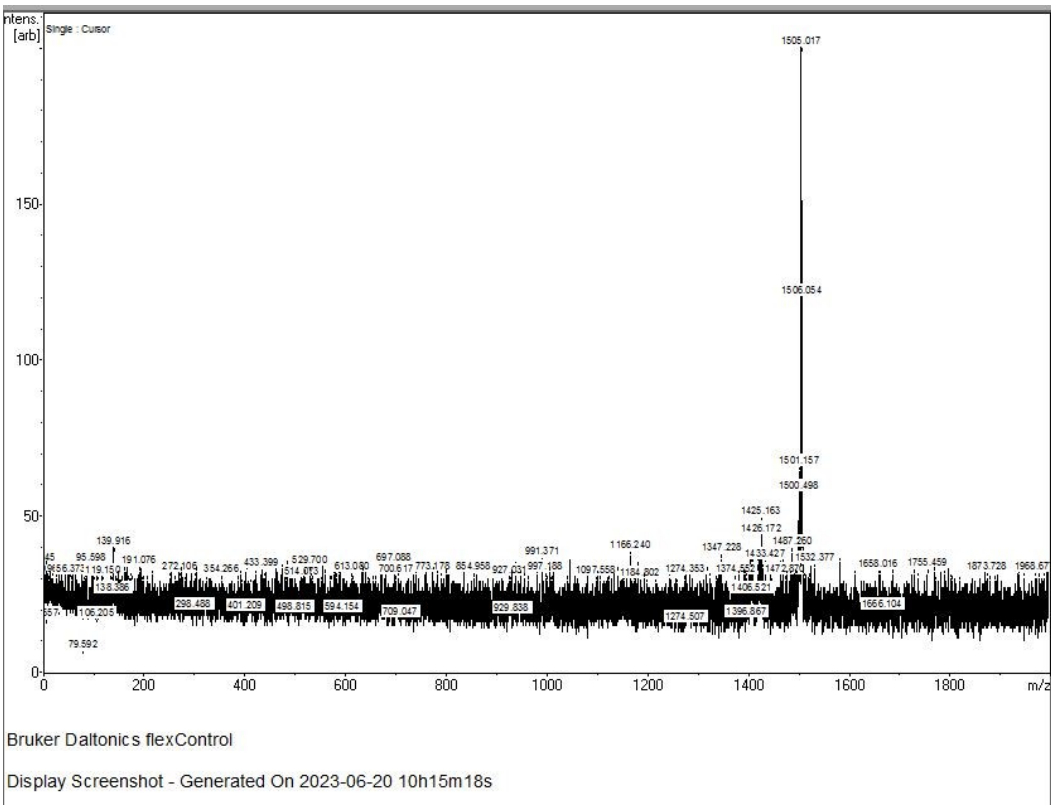
*^{1}H NMR spectrum of **dU$^{CO\text{-}MB}$TP** (500 MHz in D_2O)*



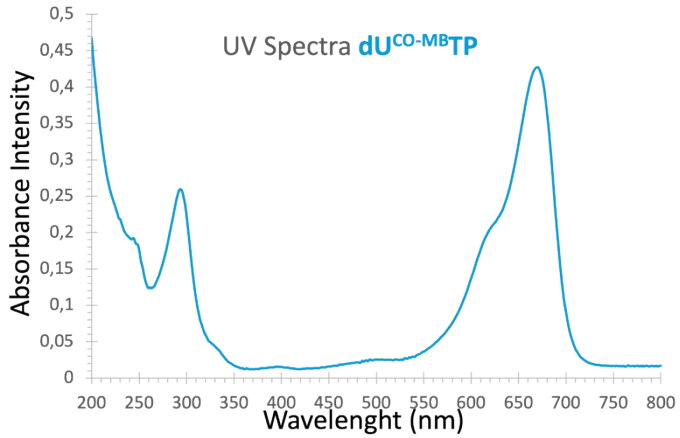
*^{31}P NMR spectrum of **dU$^{CO\text{-}MB}$TP** (202 MHz in D_2O)*



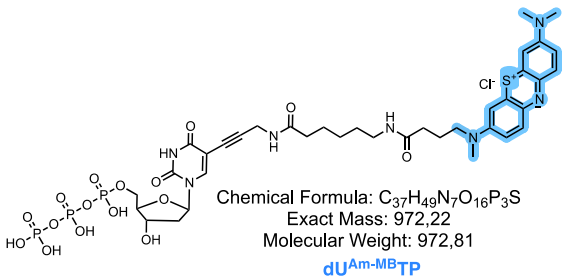
*HR-MS ESI spectrum (negative mode) of **dU$^{CO\text{-}MB}$TP.***



MALDI-TOF spectrum (linear negative mode) of ***dU$^{CO\text{-}MB}$TP***

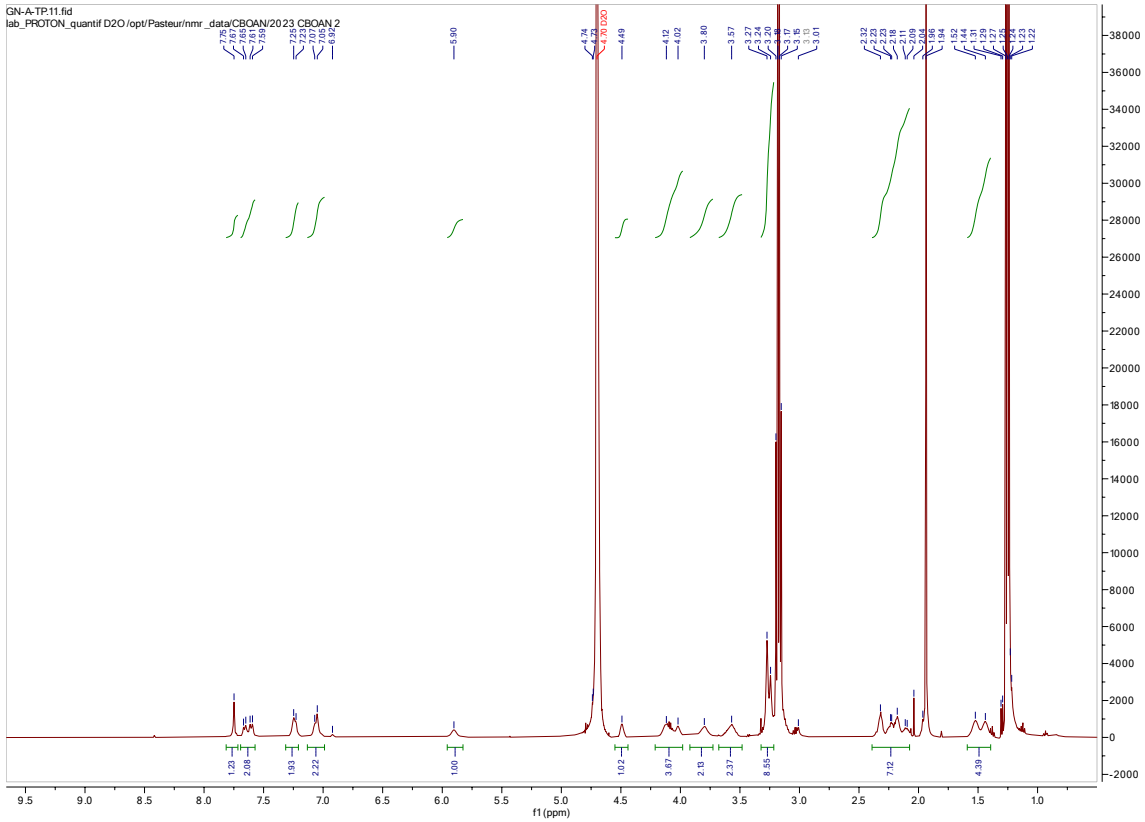


UV spectrum in ultra-pure water of ***dU$^{CO\text{-}MB}$TP***

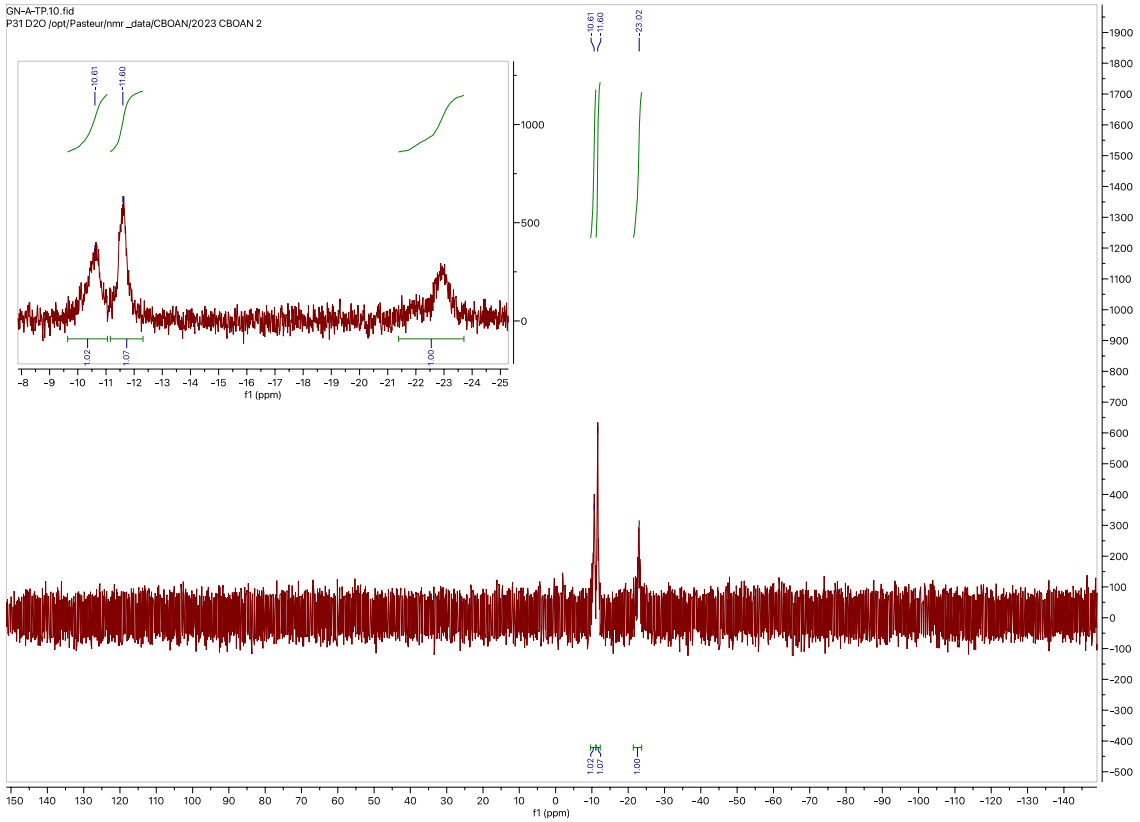


Chemical Formula: $C_{37}H_{49}N_7O_{16}P_3S$
Exact Mass: 972,22
Molecular Weight: 972,81

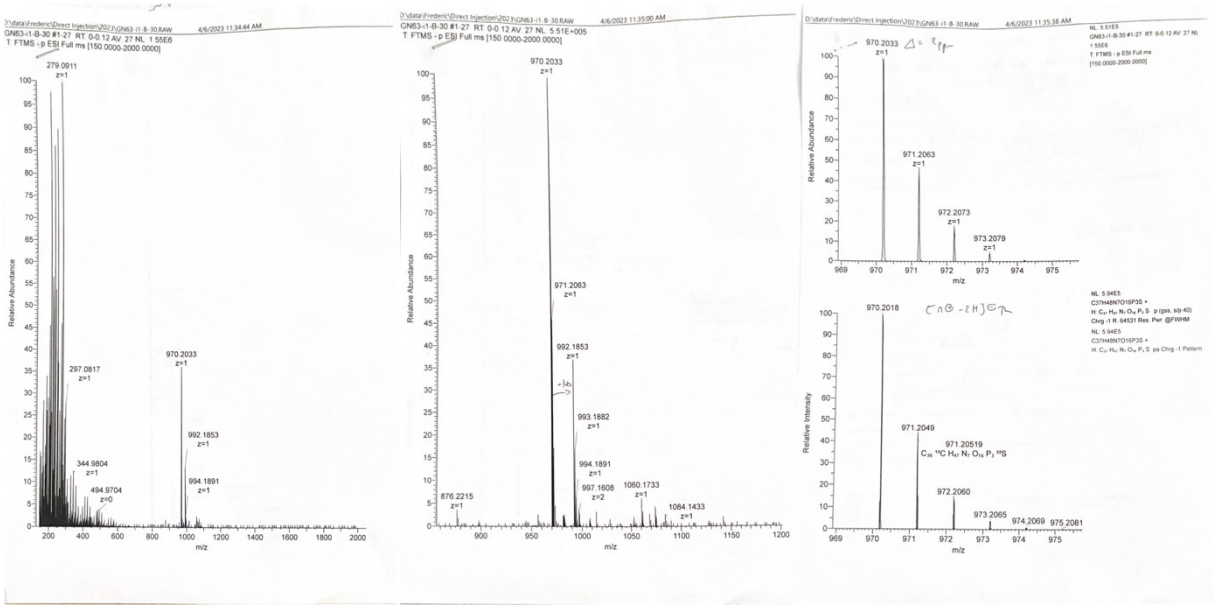
dU$^{Am\text{-}MB}$TP



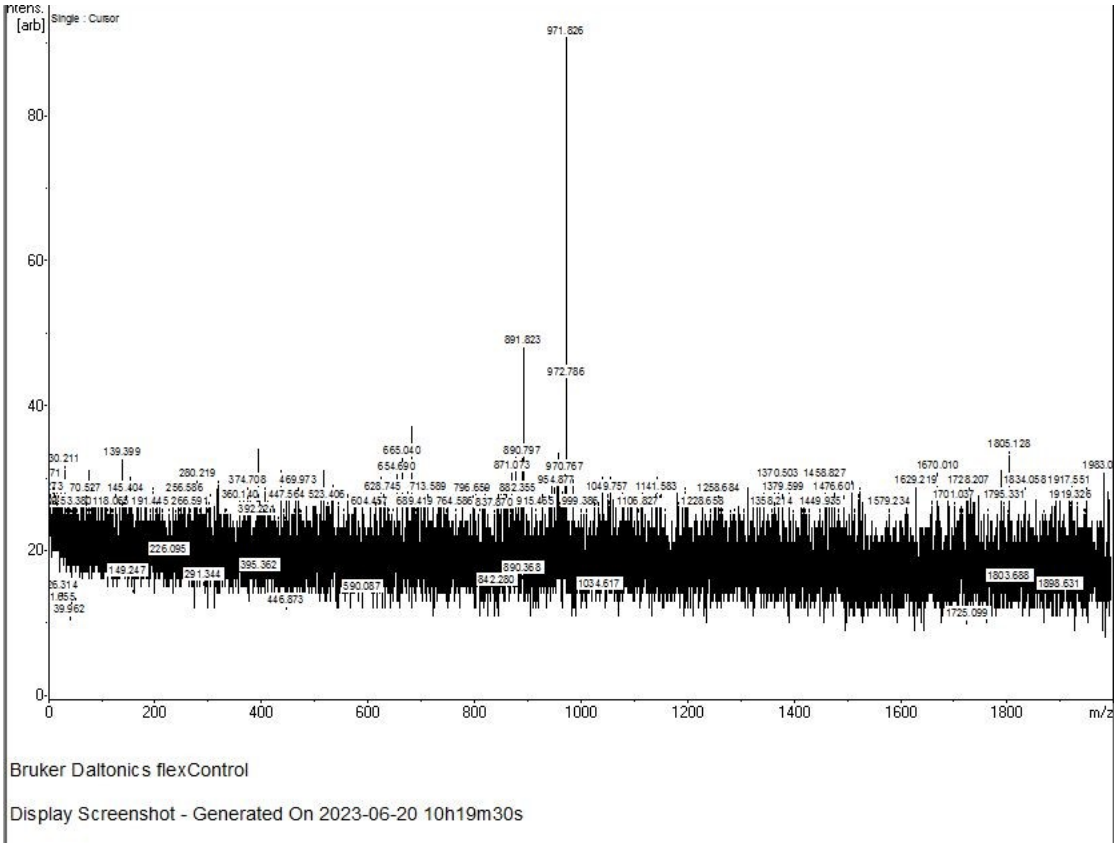
*^{1}H NMR spectrum of **dU$^{Am\text{-}MB}$TP** (500 MHz in D_2O)*



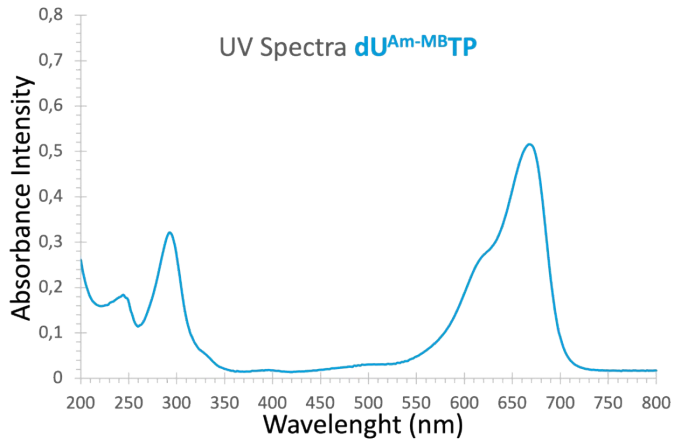
*^{31}P NMR spectrum of **dU$^{Am\text{-}MB}$TP** (202 MHz in D_2O)*



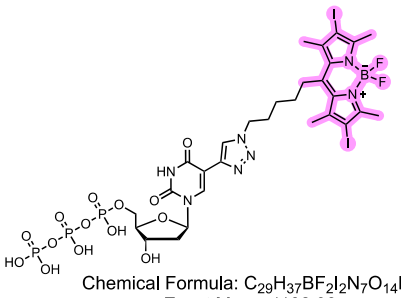
*HR-MS ESI spectrum (negative mode) of **dU$^{AM\text{-}MB}$TP.***



MALDI-TOF spectrum (linear negative mode) of ***dU$^{Am\text{-}MB}$TP***

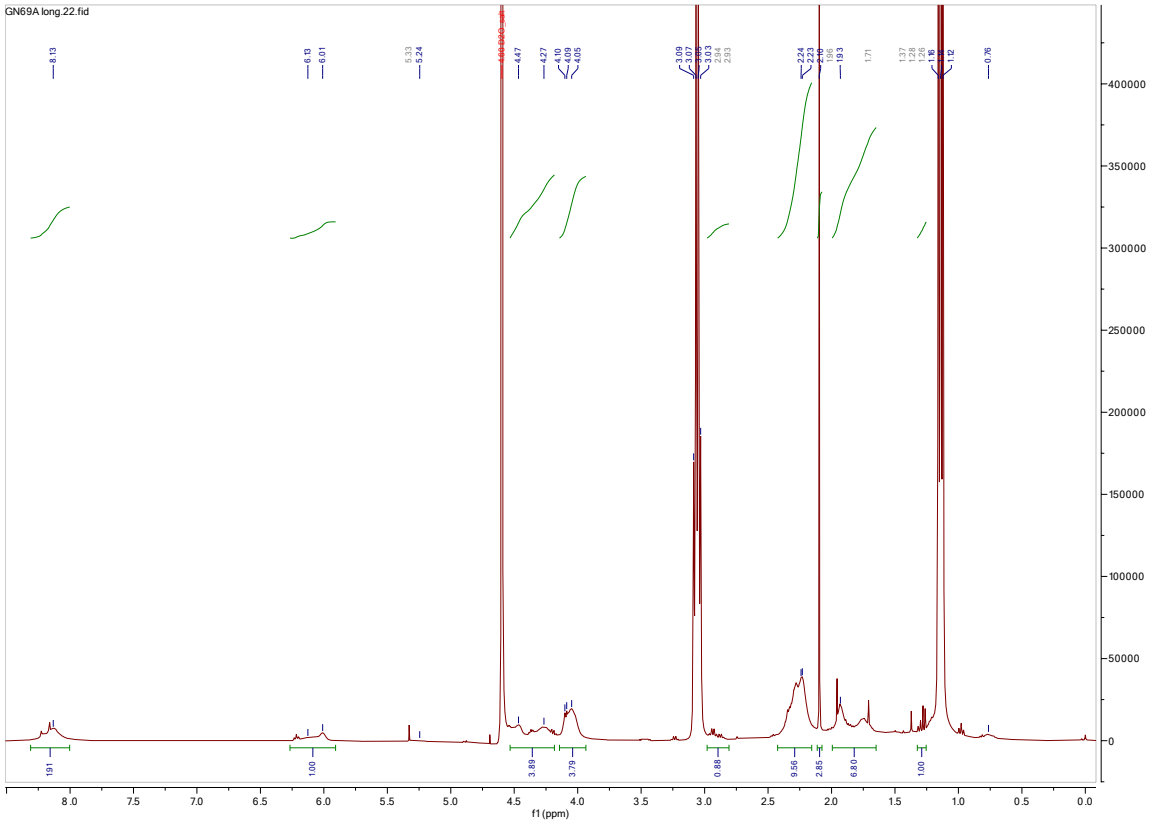


UV spectrum in ultra-pure water of ***dU$^{Am\text{-}MB}$TP***

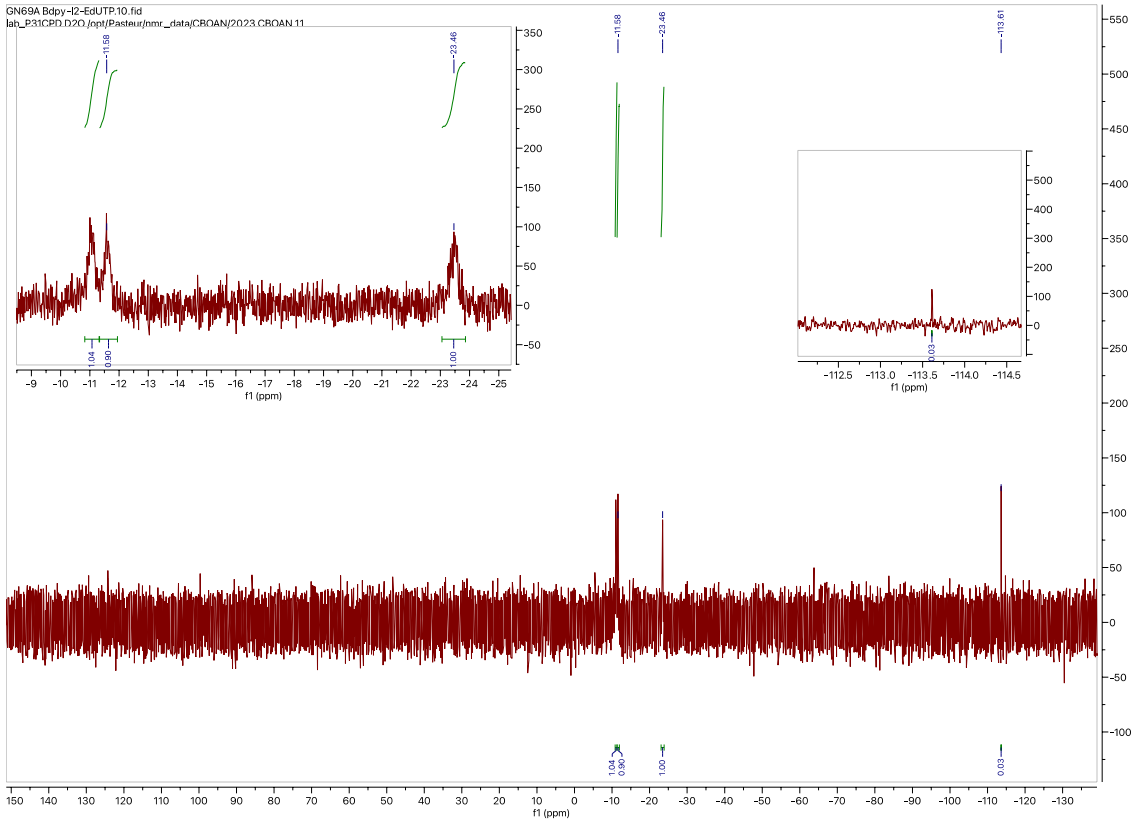


Chemical Formula: $C_{29}H_{37}BF_2I_2N_7O_{14}P_3$
Exact Mass: 1102,98
Molecular Weight: 1103,19

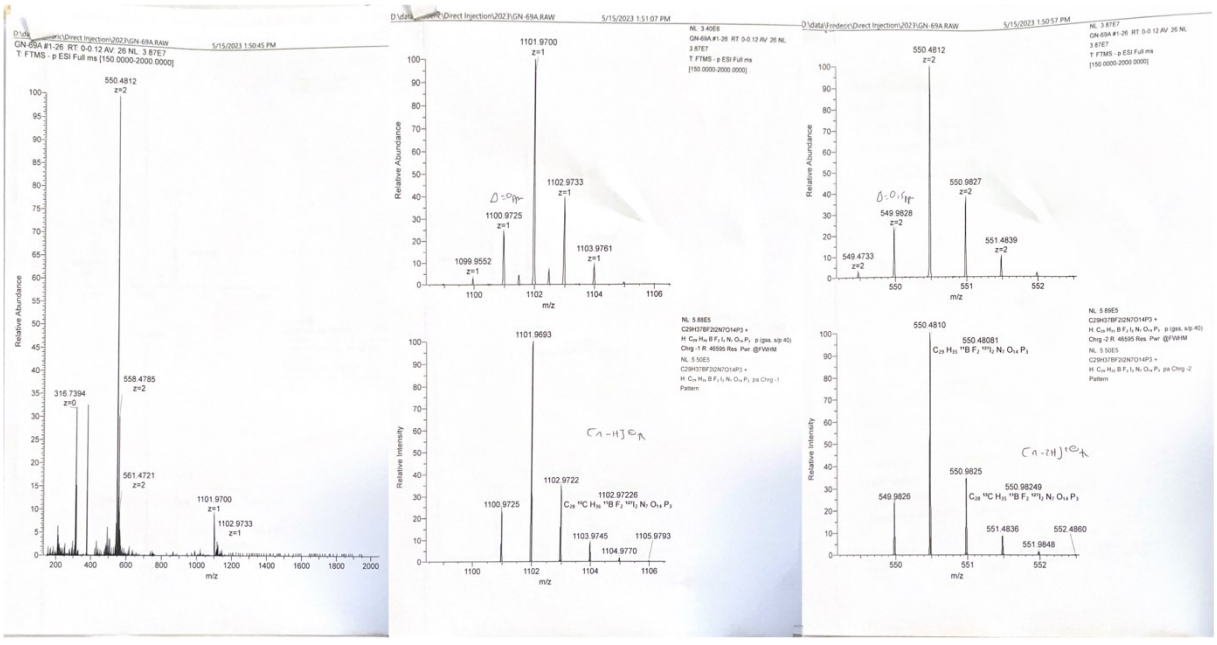
dU$^{E\text{-}BDP}$TP



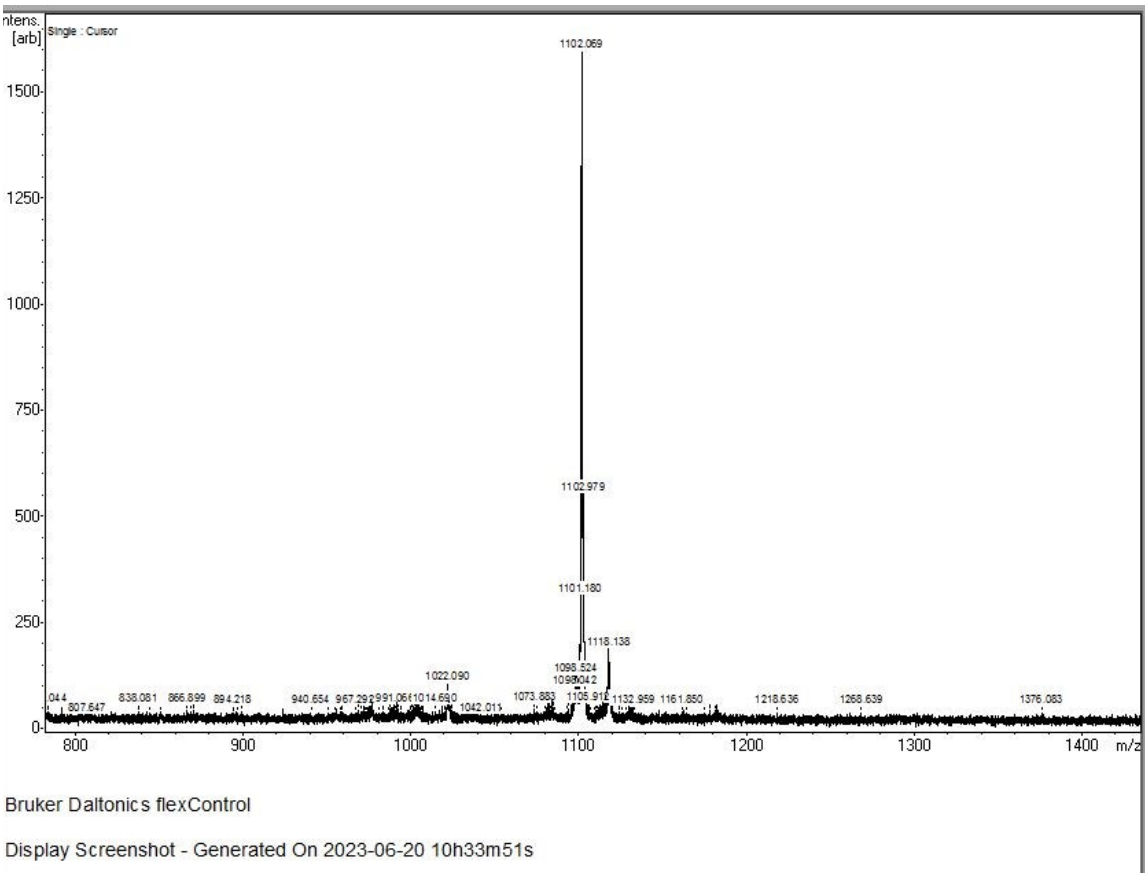
*^{1}H NMR spectrum of **dU$^{E\text{-}BDP}$TP** (400 MHz in D_2O)*



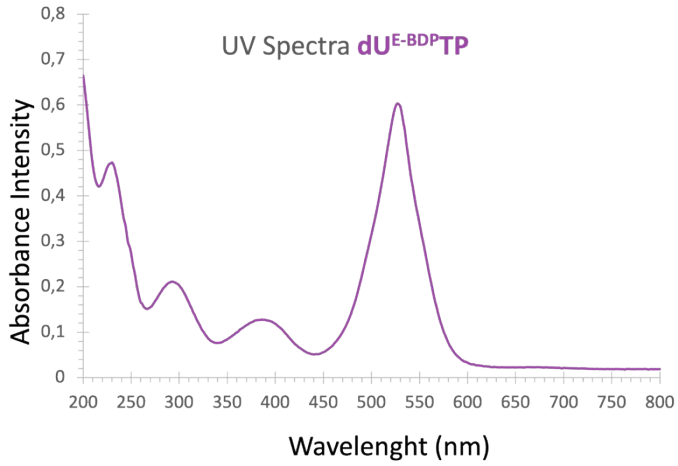
*^{31}P NMR spectrum of **dU$^{E\text{-}BDP}$TP** (202 MHz in D_2O)*



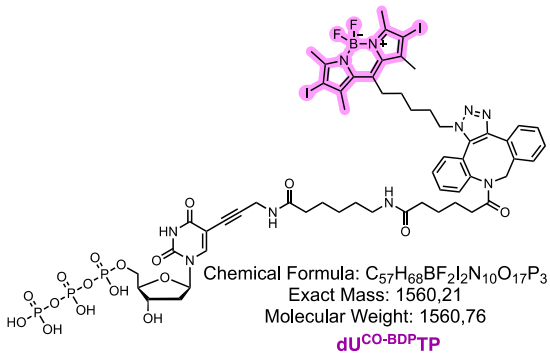
*HR-MS ESI spectrum (negative mode) of **dU$^{E\text{-}BDP}$TP.***



MALDI-TOF spectrum (linear negative mode) of ***dU$^{E\text{-}BDP}$TP.***

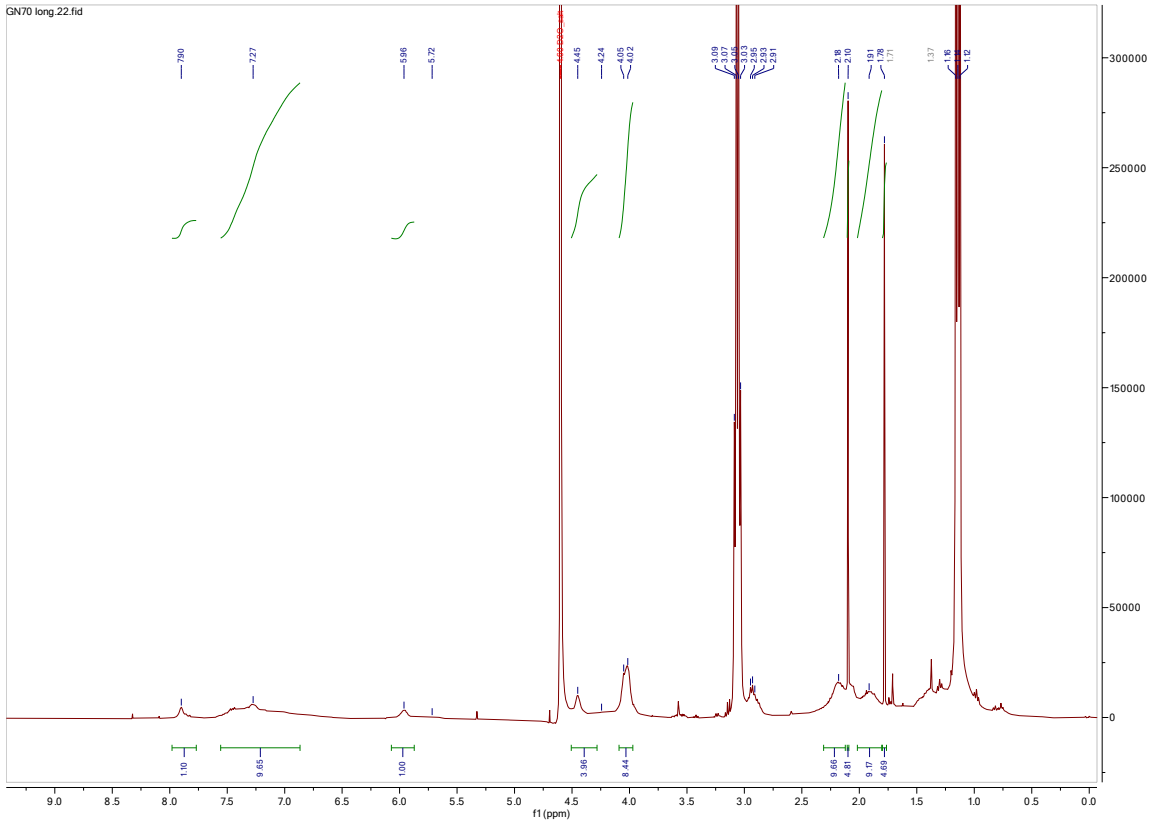


UV spectrum in ultra-pure water of ***dU$^{E\text{-}BDP}$TP***

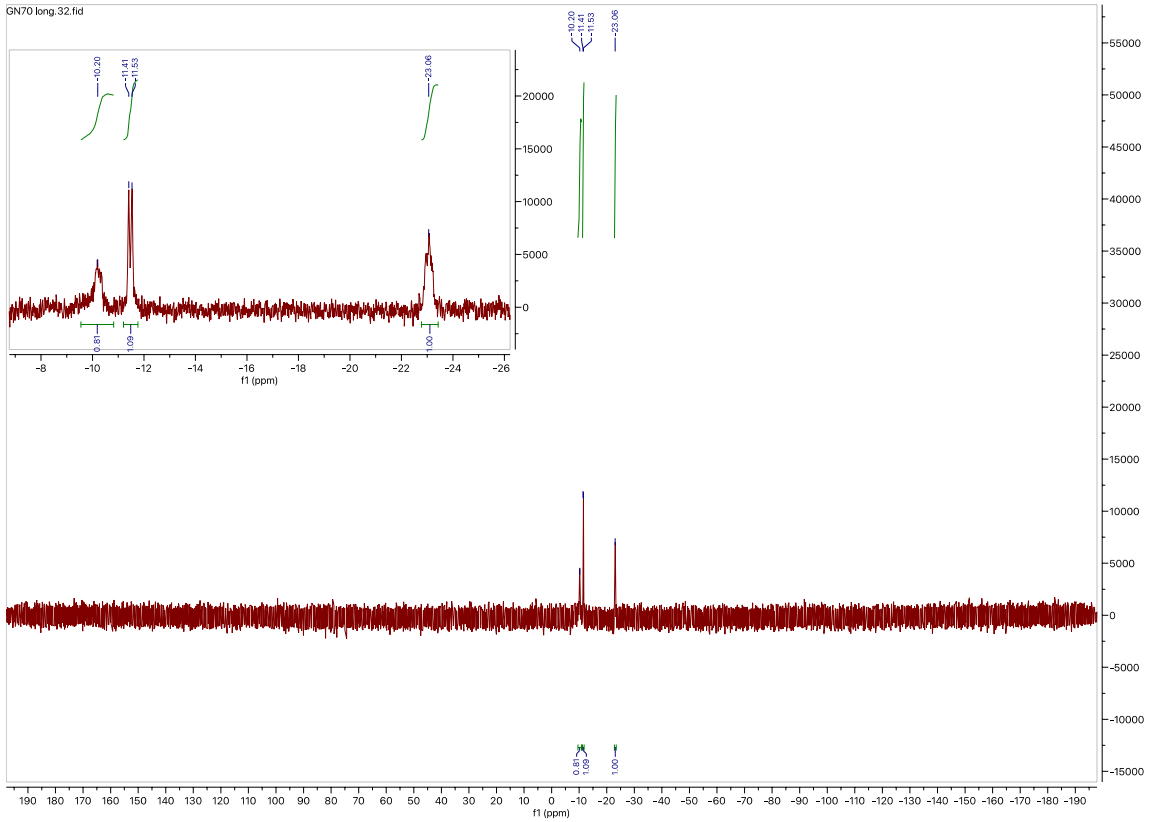


Chemical Formula: $C_{57}H_{68}BF_2I_2N_{10}O_{17}P_3$
Exact Mass: 1560,21
Molecular Weight: 1560,76

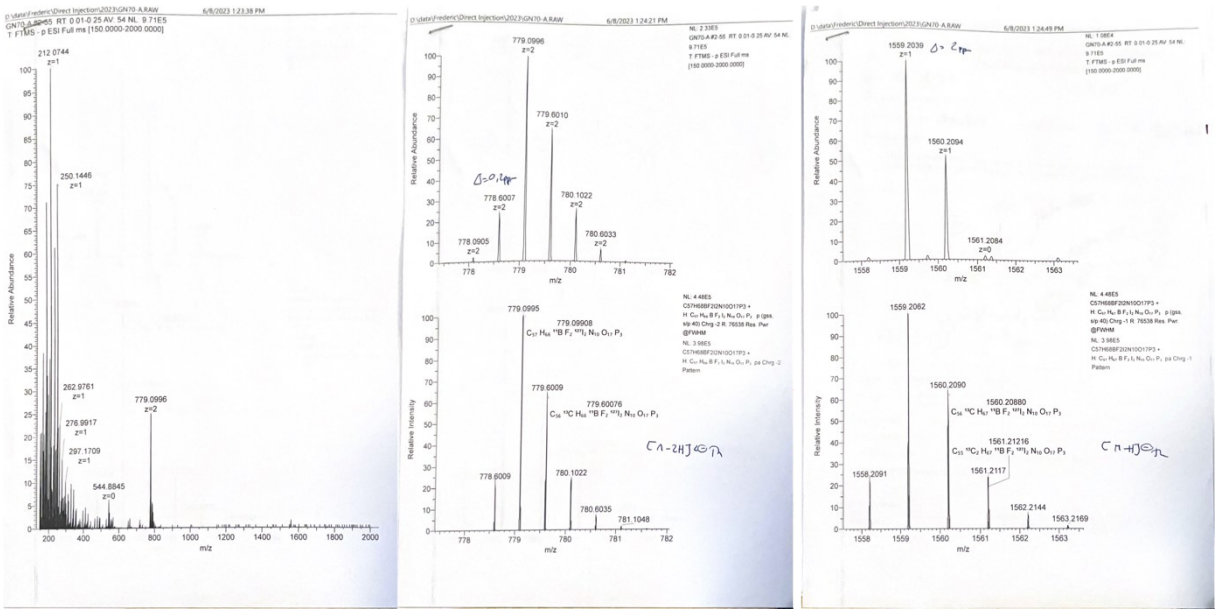
dU$^{CO\text{-}BDP}$TP



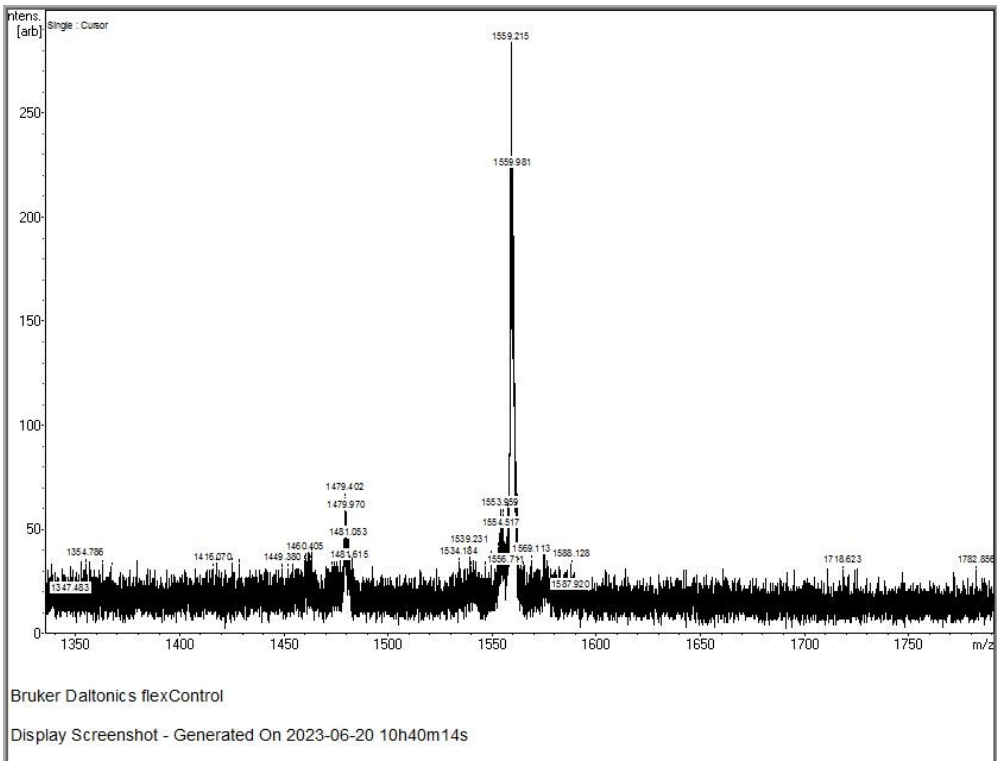
*^{1}H NMR spectrum of **dU$^{CO\text{-}BDP}$TP** (400 MHz in D_2O)*



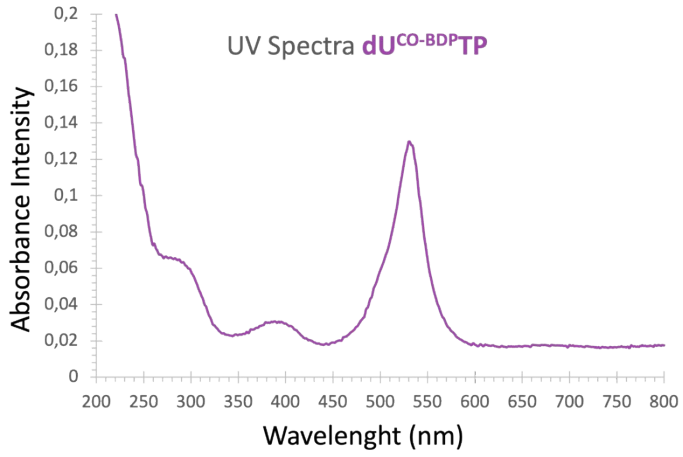
*^{31}P NMR spectrum of **dU$^{CO\text{-}BDP}$TP** (162 MHz in D_2O)*



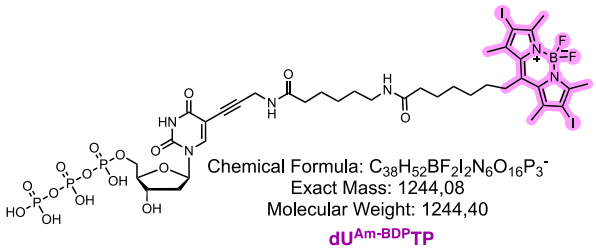
*HR-MS ESI spectrum (negative mode) of **dU$^{CO\text{-}BDP}$TP.***



38 MALDI-TOF spectrum (linear negative mode) of ***dU$^{CO\text{-}BDP}$TP***

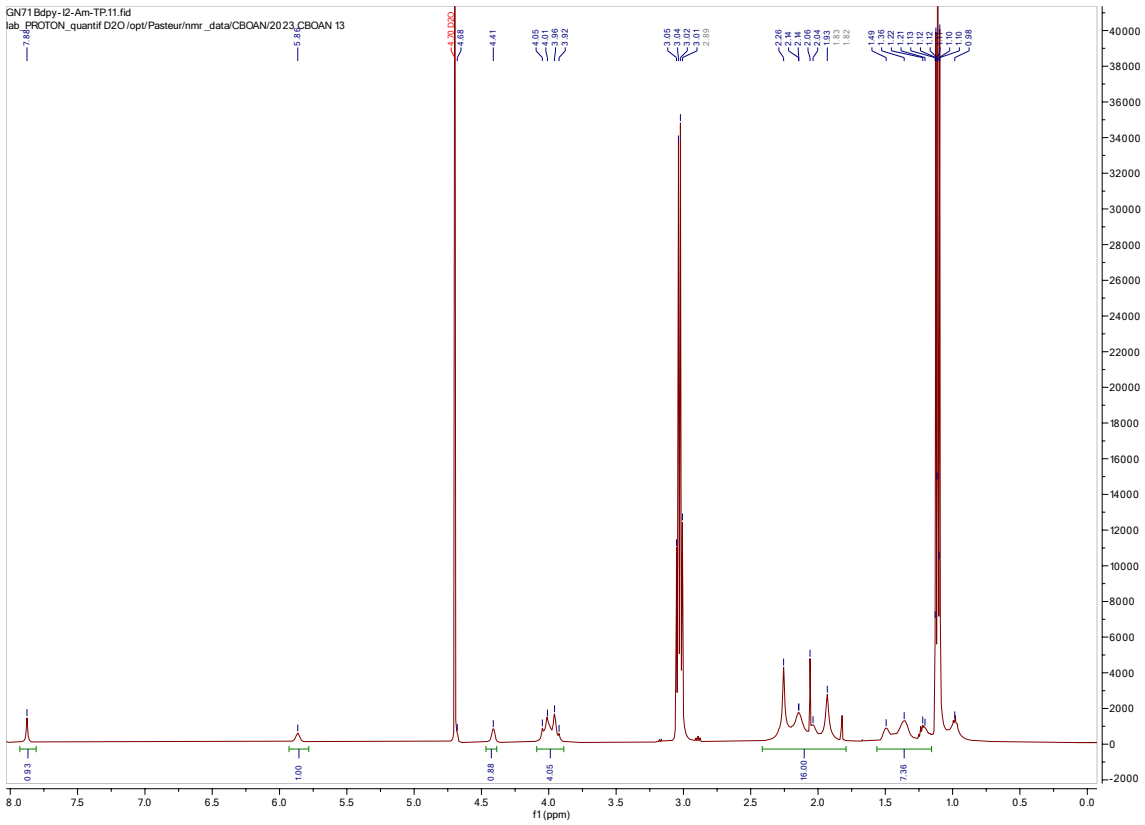


UV spectrum in ultra-pure water of ***dU$^{CO\text{-}BDP}$TP***

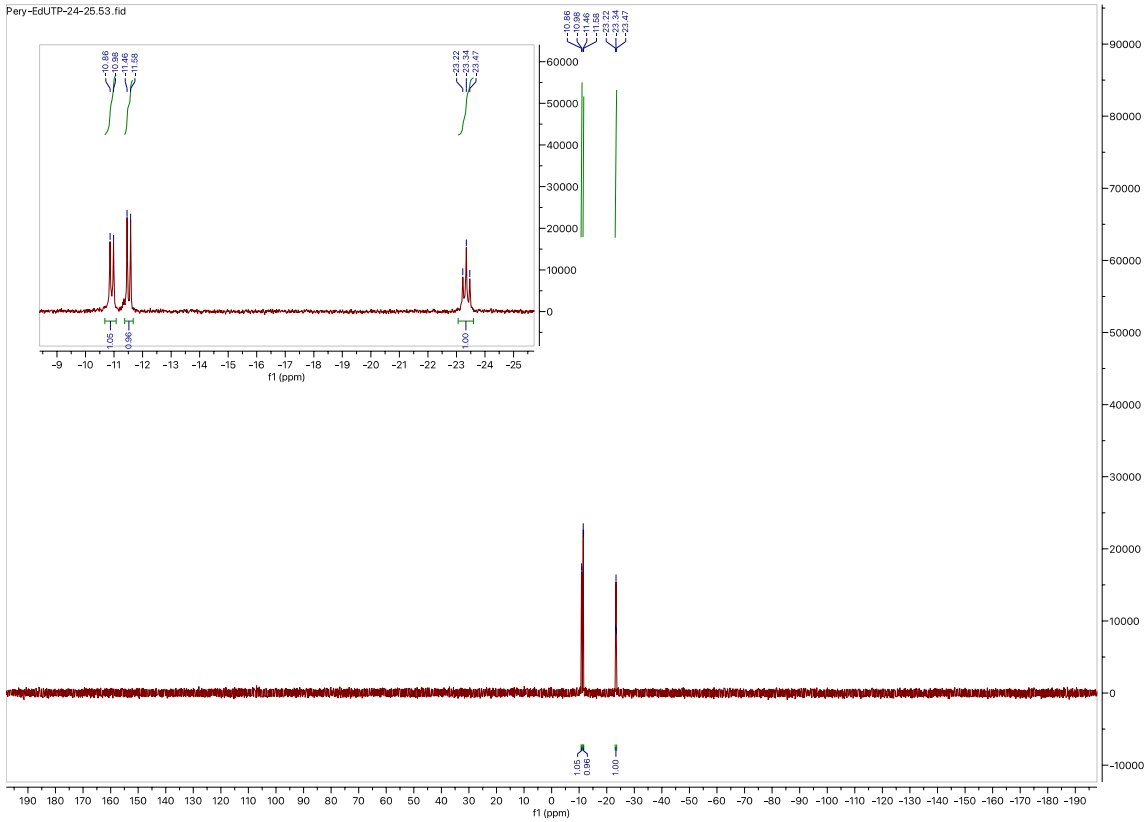


Chemical Formula: $C_{38}H_{52}BF_2I_2N_6O_{16}P_3^-$
Exact Mass: 1244,08
Molecular Weight: 1244,40

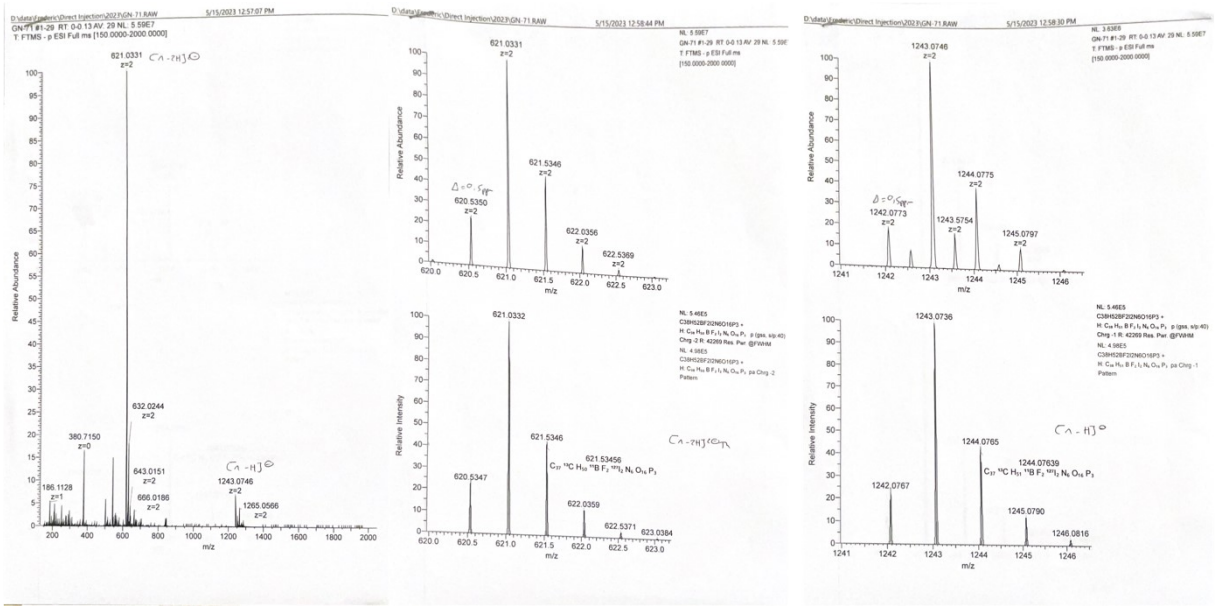
dU$^{Am\text{-}BDP}$TP



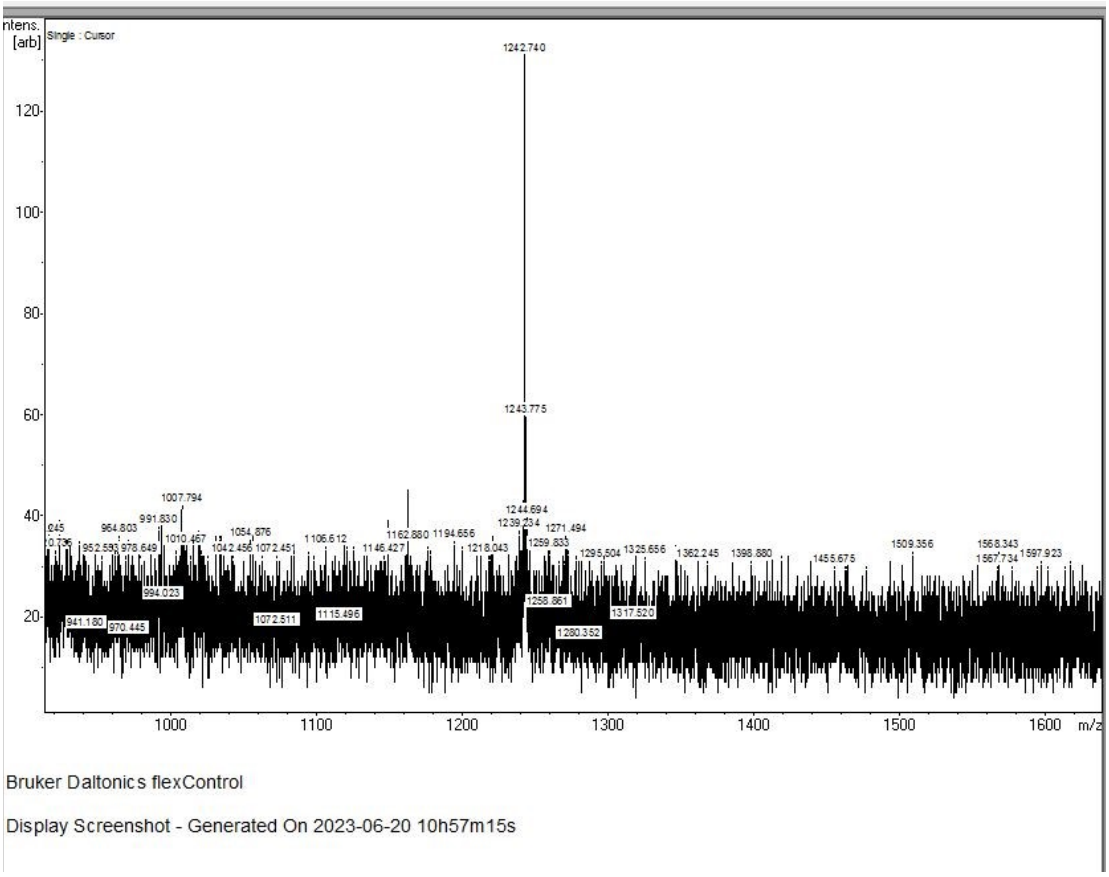
*^{1}H NMR spectrum of **dU$^{Am\text{-}BDP}$TP** (500 MHz in D_2O)*



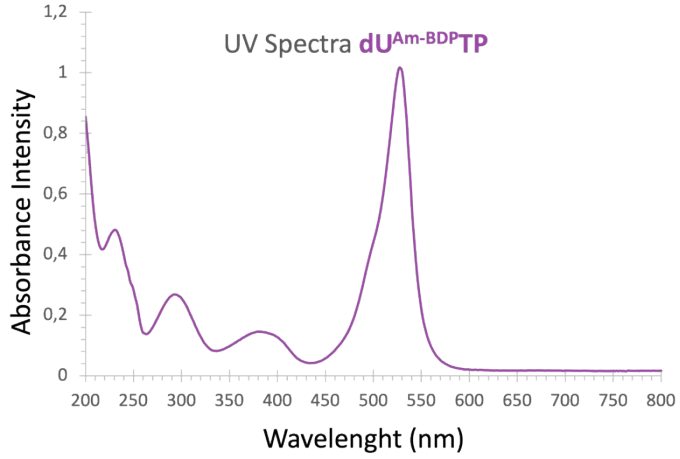
*^{31}P NMR spectrum of **dU$^{Am\text{-}BDP}$TP** (202 MHz in D_2O)*



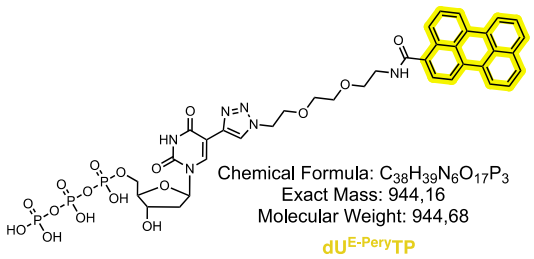
*HR-MS ESI spectrum (negative mode) of **dU$^{AM\text{-}BDP}$TP.***

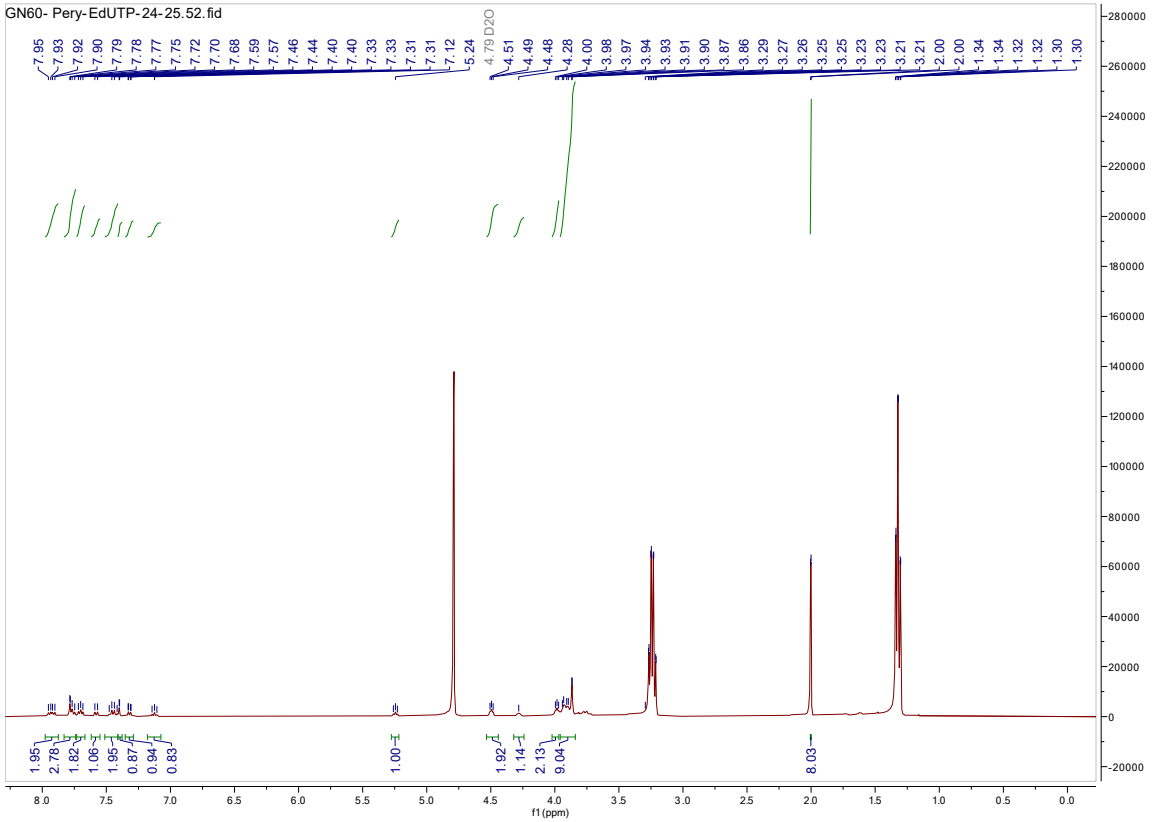


MALDI-TOF spectrum (linear negative mode) of ***dU$^{Am\text{-}BDP}$TP***

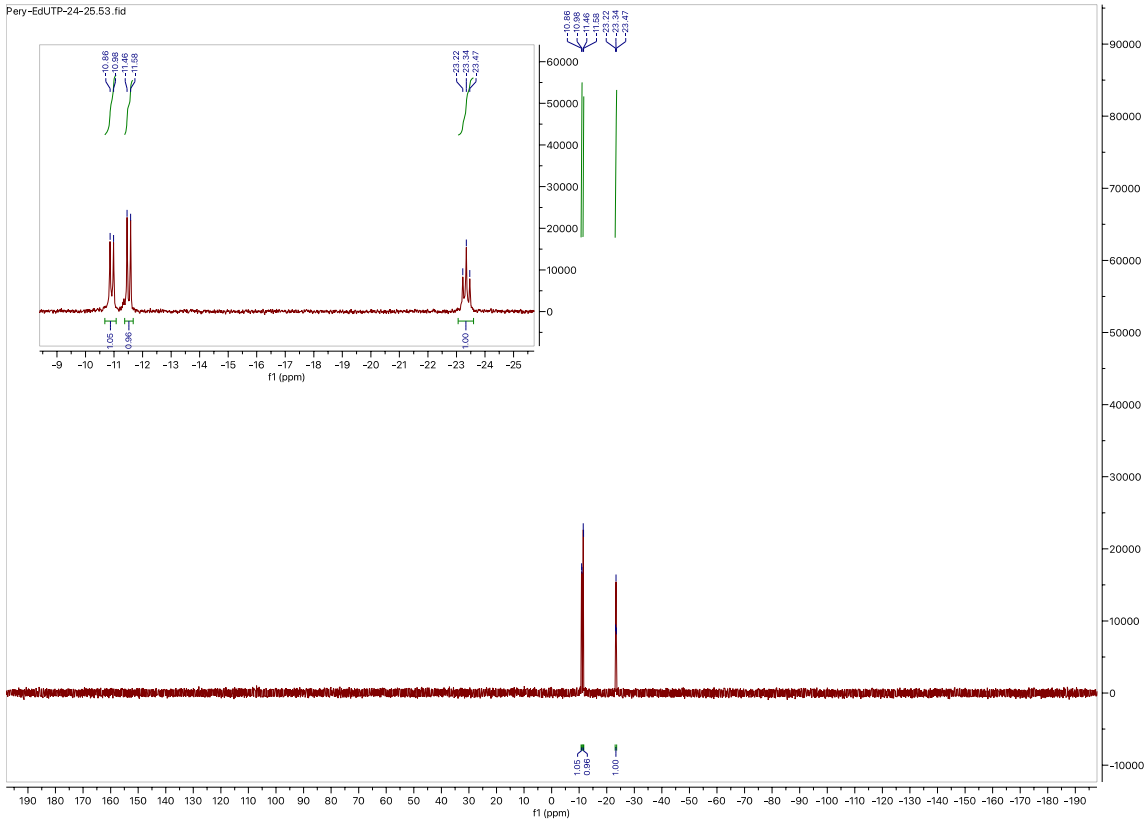


UV spectrum in ultra-pure water of ***dU$^{Am\text{-}BDP}$TP***

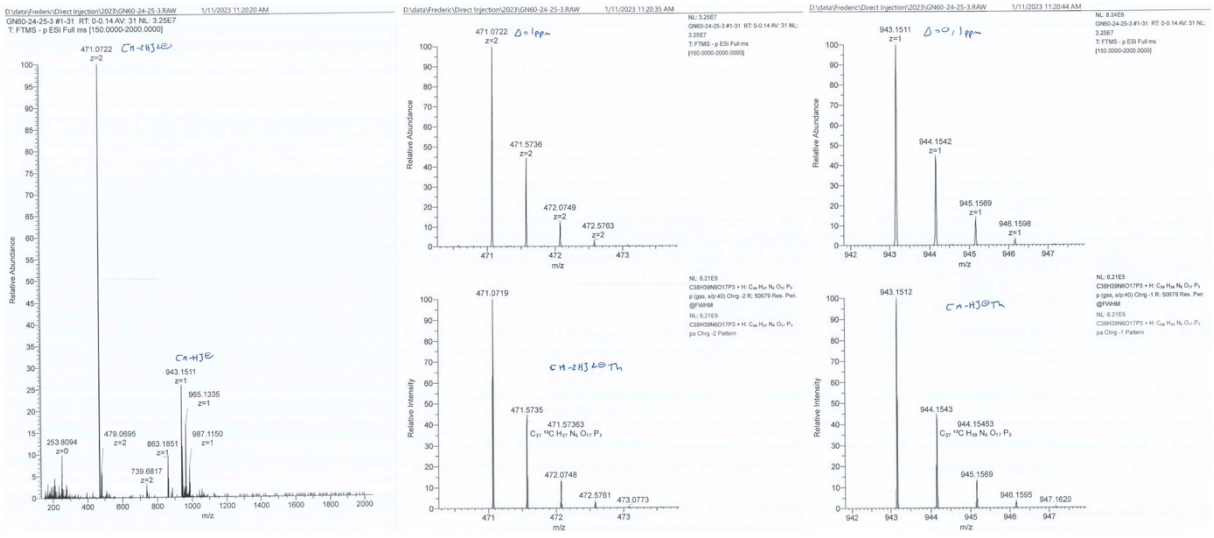




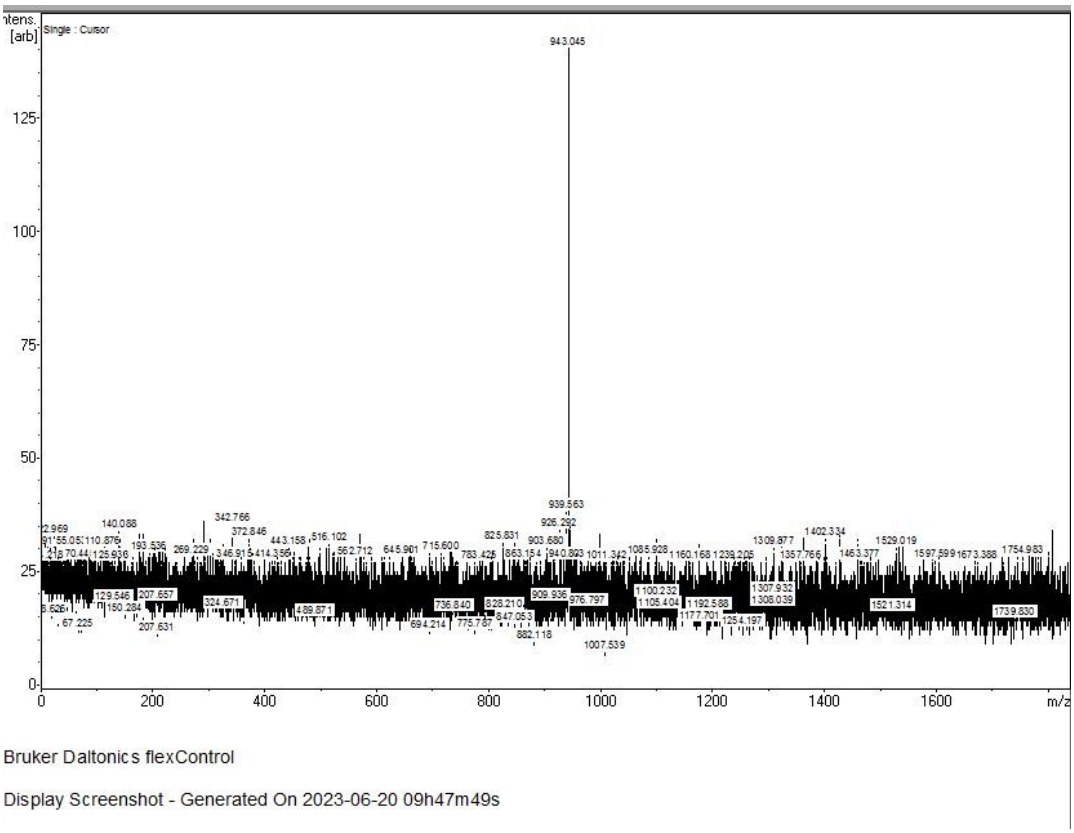
*^{1}H NMR spectrum of **dU$^{E\text{-}Pery}$TP** (400 MHz in D_2O)*



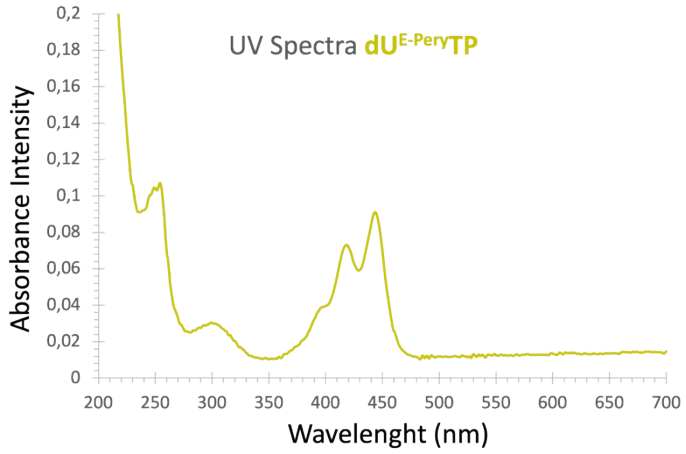
*^{31}P NMR spectrum of **dU$^{E\text{-}Pery}$TP** (162 MHz in D_2O)*



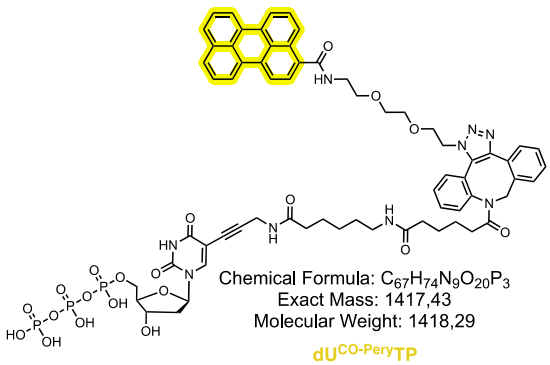
*HR-MS ESI spectrum (negative mode) of **dU$^{E\text{-}Pery}$TP.***

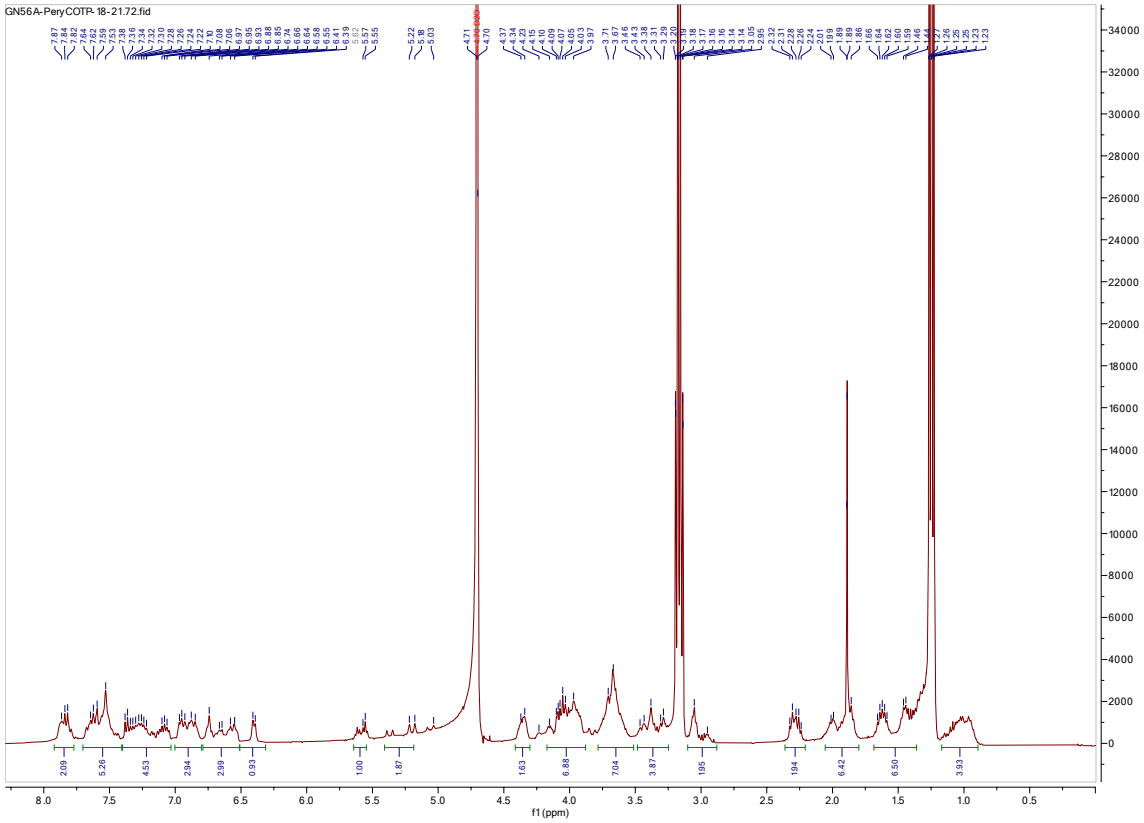


MALDI-TOF spectrum (linear negative mode) of ***dU$^{E\text{-}Pey}$TP***

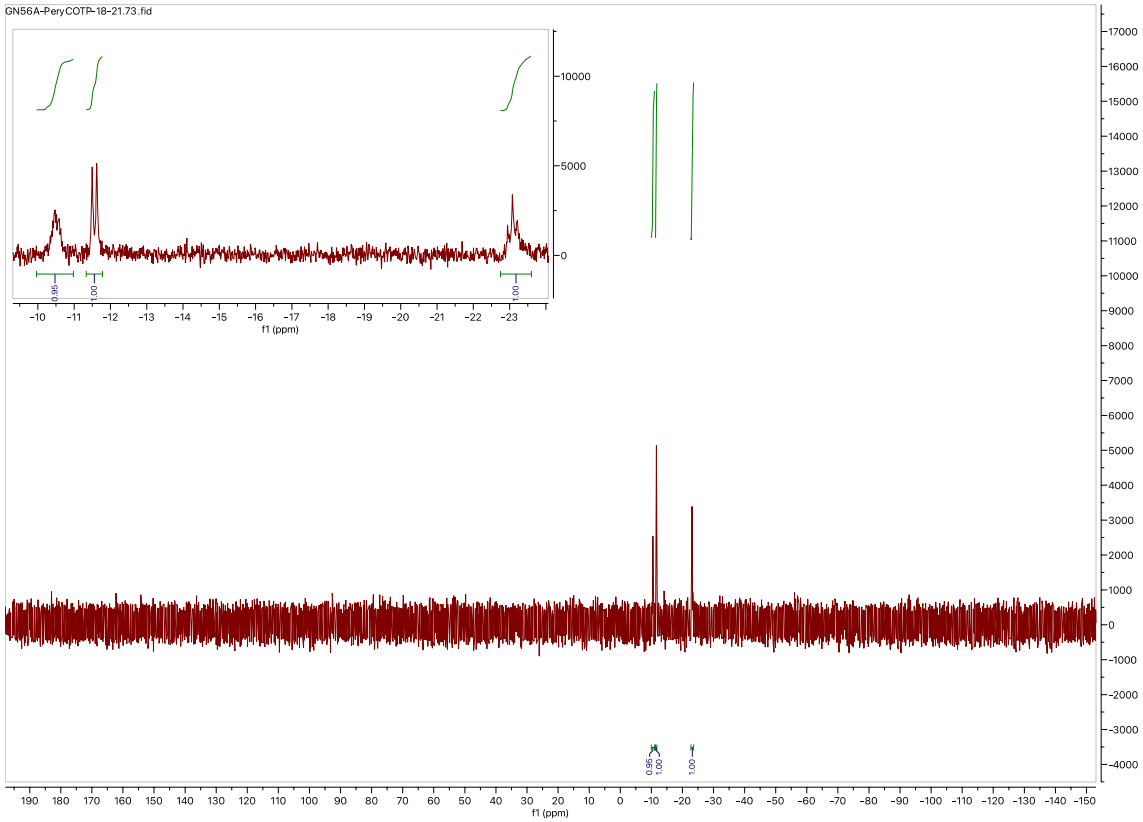


UV spectrum in ultra-pure water of ***dU$^{E\text{-}Pery}$TP***

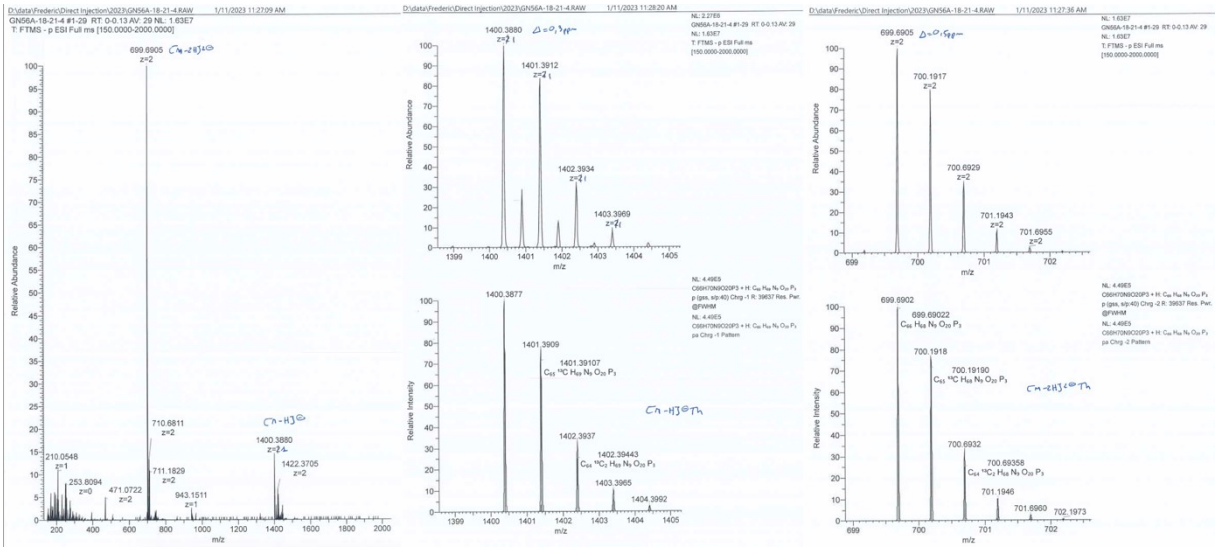




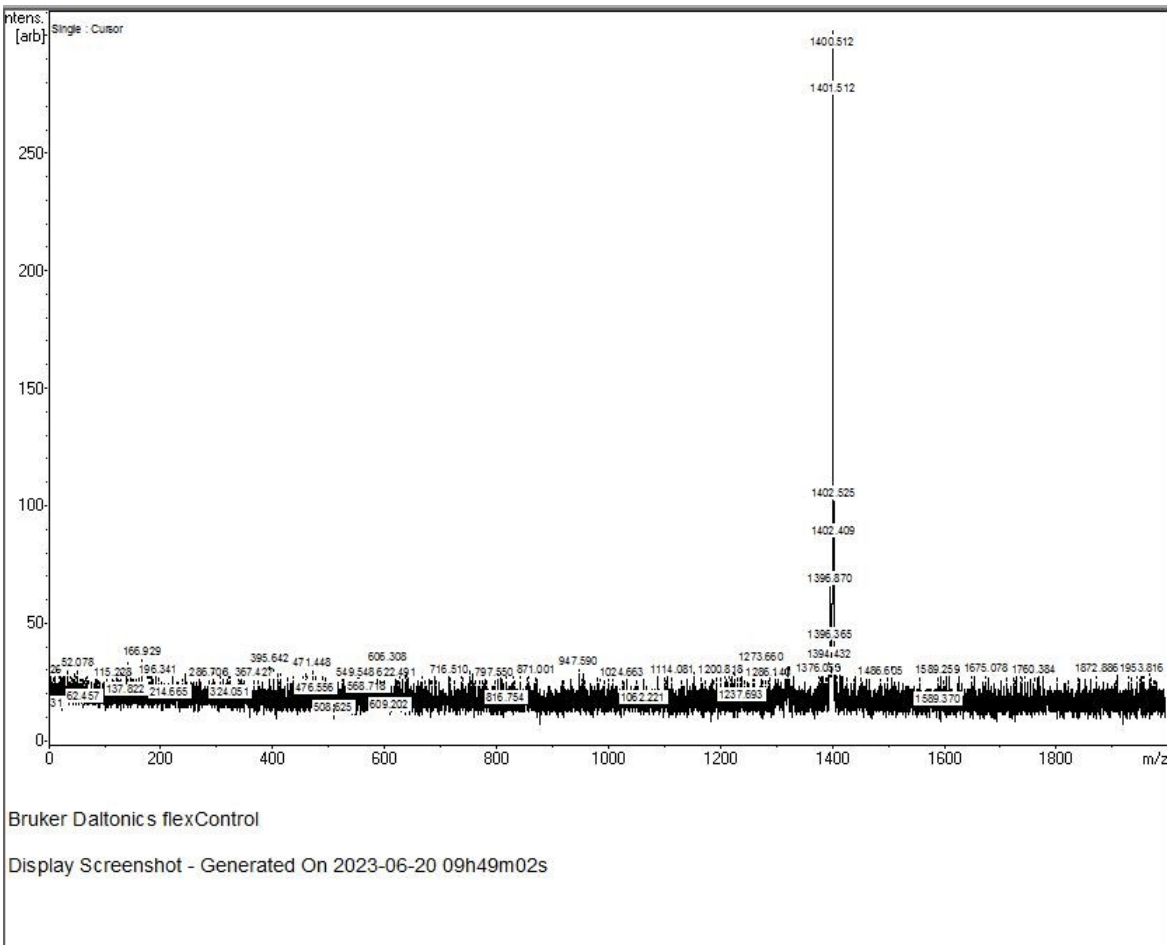
*^{1}H NMR spectrum of **dU$^{CO\text{-}Pery}$TP** (400 MHz in D_2O)*



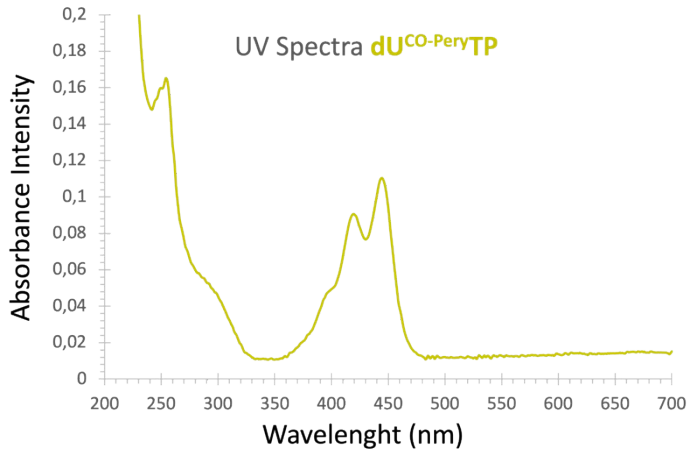
*^{31}P NMR spectrum of **dU$^{CO\text{-}Pery}$TP** (162 MHz in D_2O)*



*HR-MS ESI spectrum (negative mode) of **dU$^{CO\text{-}Pery}$TP.***



MALDI-TOF spectrum (linear negative mode) of ***dU$^{CO\text{-}Pery}$TP***



UV spectrum in ultra-pure water of ***dU$^{CO\text{-}Pery}$TP***