Electronic Supplementary Material (ESI) for ChemComm.
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Hetero-Diels-Alder Click Reaction of Dithioesters for a Catalyst-Free Indirect ^{18}F-Radiolabelling of Peptides

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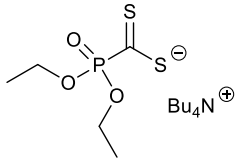
1 General information

Reagents were obtained from commercial sources and used without any further purification. Thin-layer chromatography was performed on Merck silica gel 60F254 plates. VWR silica gel (40-63 µm) was used for chromatography columns. Semi-preparative reverse-phase HPLC purifications were performed on a Waters SunFire C18 OBD Prep column (5 µm, 19 × 150 mm) on a Gilson PLC2020 system. Reverse-phase flash purifications were performed on prepacked Puriflash C18 columns from Interchim on a Gilson PLC2020 system. Analytical reverse-phase HPLC were performed on a Kinetex EVO C18 column (5 µm, 4.6 mm × 150 mm) on an Agilent Technologies 1200 series HPLC system using a linear gradient (5% to 100% v/v in 7.3 min, flow rate of 1.6 mL.min^{-1}) of solvent B (0.1% v/v TFA in MeCN) in solvent A (0.1% v/v TFA in H_2O). ^{1}H NMR spectra were recorded at 400 MHz and 500 MHz, ^{13}C NMR spectra were recorded at 101 MHz and 126 MHz, ^{19}F NMR spectra were recorded at 376 MHz and 471 MHz and ^{31}P NMR spectra were recorded at 162 MHz and 202 MHz on a Bruker Advance spectrometer. Chemical shifts are reported in parts per million (ppm), coupling constants (J) are reported in hertz (Hz). Signals are described as s (singlet), d (doublet), t (triplet), q (quadruplet), p (pentuplet) and m (multiplet). Low resolution mass spectra (LRMS) and high resolution mass spectra (HRMS) were obtained on an Agilent Technologie 6520 Accurare-Mass Q.Tof LC/MS apparatus equipped with a Zorbax SB C18 column (1.8 µm, 2.1 × 50 mm) using electrospray ionization (ESI) and a time-of-flight analyzer (TOF).

2 Synthetic procedures

2.1 Synthesis of phosphonodithioformate salt S1

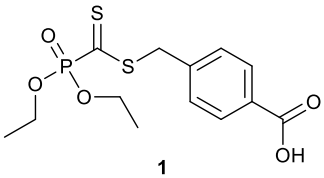


S1

Modified procedure:[1] Diethyl phosphite (1 equiv, 0.83 g, 0.77 mL, 6 mmol) was placed in DMF (35 mL). Then cesium carbonate (3 equiv, 5.86 g, 18 mmol) and TBAI (3 equiv, 6.65 g, 18 mmol) were added under inert atmosphere. This solution was stirred for 10 min at room temperature then CS_2 (3 equiv, 1.37 g, 1.09 mL, 18 mmol) was added and the reaction mixture was stirred for an additional hour at the same temperature. The salts were filtered and washed with EtOAc before evaporating the filtrate under reduced pressure. The residue was taken in EtOAc/*n*-heptane (10 mL/5 mL), filtered and washed with *n*-heptane. Finally, the filtrate was evaporated under reduced pressure to give the corresponding dithioformate salt **S1** as a brown oil (1.87 g, 68%). Product **S1** was used directly in the next step.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 (p, *J* = 7.1 Hz, 4H, P-O-C***H₂***-CH_3), 3.29 - 3.17 (m, 8H, N(C***H₂***-CH_2-CH_2-CH_3)$_4$), 1.71 - 1.55 (m, 8H, N(CH_2-C***H₂***-CH_2-CH_3)$_4$), 1.43 (h, *J* = 7.4 Hz, 8H, N(CH_2-CH_2-C***H₂***-CH_3)$_4$), 1.31 (td, *J* = 7.0, 0.7 Hz, 6H, P-O-CH_2-C***H₃***), 0.98 (t, *J* = 7.3 Hz, 12H, N(CH_2-CH_2-CH_2-C***H₃***)$_4$). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 63.4 (d, $^{2}J_{C-P}$ = 7.2 Hz, P-O-***C***H_2-CH_3), 58.9 (N(***C***H_2-CH_2-CH_2-CH_3)$_4$), 24.1 (N(CH_2-***C***H_2-CH_2-CH_3)$_4$), 19.8 (N(CH_2-CH_2-***C***H_2-CH_3)$_4$), 16.5 (d, $^{3}J_{C-P}$ = 6.3 Hz, P-O-***C***H_2-CH_3), 13.7 (N(CH_2-CH_2-CH_2-***C***H_3)$_4$). **^{31}P NMR** (162 MHz, $CDCl_3$) δ -2.5.

2.2 Synthesis of phosphonodithioester 1



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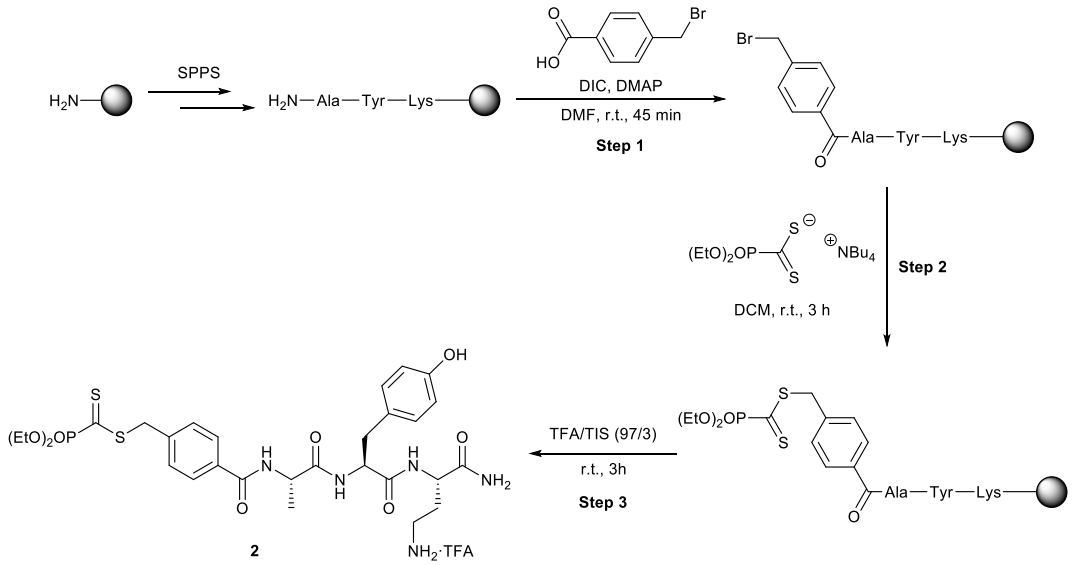
CAS RN: 1415386-07-0

To a solution of dithioester salt **S1** (1 equiv, 640 mg, 1.4 mmol) in MeCN (6 mL) was added 4-(bromomethyl)benzoic acid (1.5 equiv, 450 mg, 2.1 mmol). The reaction mixture was stirred overnight and then diluted in EtOAc and washed with brine. The organic phase was dried over Na_2SO_4 and concentrated in vacuo. Purification on gel silica column chromatography with EtOAc/*n*-heptane/AcOH (59/40/1) gave the corresponding dithioester as a pink oil. The product was precipitated in pentane with the help of ultrasounds to give **1** as a pink solid (310 mg, 63%).[2]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 - 7.80 (m, 2H, C***H^{Ar}***), 7.28 - 7.17 (m, 2H, C***H^{Ar}***), 4.38 (s, 2H, S-C***H$_2$***), 4.23 - 4.01 (m, 4H, P-O-C***H$_2$***-CH_3), 1.21 (td, *J* = 7.1, 0.8 Hz, 6H, P-O-CH_2-C***H$_3$***). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 227.6 (d, $^1J_{C\text{-}P}$ = 176.1 Hz, ***C***=S), 170.6 (***C***=O), 139.9 (***C$_q^{Ar}$***), 130.6 (***C^{Ar}***), 129.4 (***C^{Ar}***), 129.3 (***C$_q^{Ar}$***), 65.0 (d, $^2J_{C\text{-}P}$ = 7.0 Hz, P-O-***C***H_2-CH_3), 39.8 (d, $^3J_{C\text{-}P}$ = 2.9 Hz, S-***C***H_2), 16.3 (d, $^3J_{C\text{-}P}$ = 6.2 Hz, P-O-CH_2-***C***H_3). **^{31}P NMR** (162 MHz, $CDCl_3$) δ -3.0; **R_f** = 0.30 (70% EtOAc in *n*-heptane + 1% AcOH).

2.3 Synthesis of phosphonodithioester-tripeptide 2



SPPS: The first three amino acids were introduced on a rink resin (0.69 mmol.g^{-1}, 0.11 mmol scale) following a classical procedure for manual solid phase peptide synthesis with Fmoc-Lys(Boc)-OH, Fmoc-Tyr(O*t*Bu)-OH and Fmoc-Ala-OH. Manual SPPS was performed in polypropylene tubes equipped with polyethylene frits and polypropylene caps using an orbital agitator shaking device. The Fmoc-protected resin was swollen for 1 h in DCM and the excess of solvent was removed by filtration. N-terminal-Fmoc-group was removed by using a 20% (v/v) solution of piperidine in DMF (2 times for 15 min). All Fmoc-deprotection steps were performed in the same way. The piperidine solution was drained off and the resin was washed three times with successively DMF, CH_2Cl_2 and MeOH. All Fmoc-protected amino acids (4 equiv) were coupled in DMF (2.5 mL per 0.1 mmol of resin) for 45 min using HATU (3.8 equiv) and DIEA (12 equiv) as activating agents. The excess of solvent was removed by filtration and the resin was washed three times with successively DMF, CH_2Cl_2 and MeOH. With the three amino acids coupled on the resin, N-terminal-Fmoc-group was removed by using a 20% (v/v) solution of piperidine in DMF (2 times for 15 min).

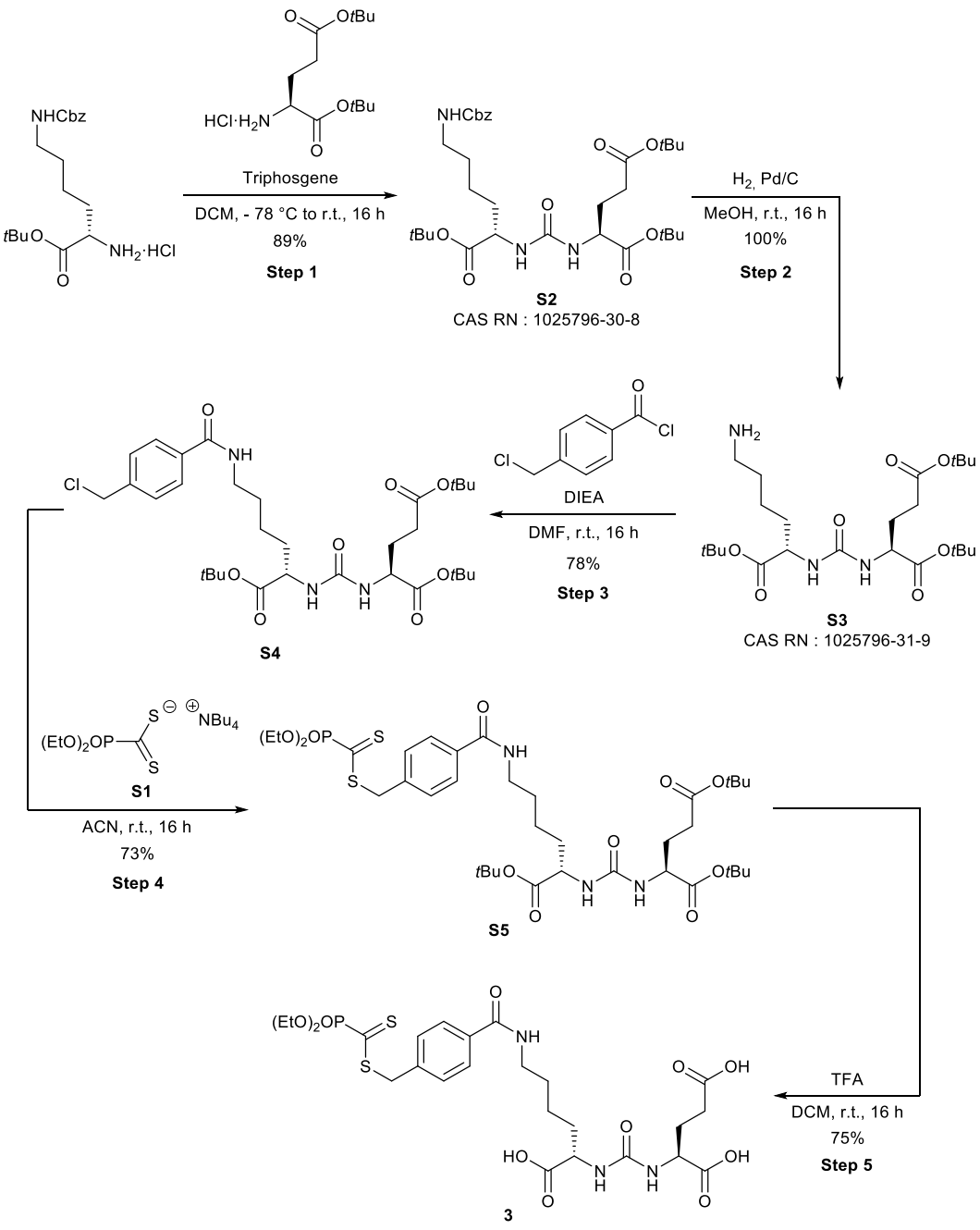
Step 1: Then 4-(bromomethyl)benzoic acid (230 mg, 10 equiv) was dissolved in CH_2Cl_2 at 0 °C in a round-bottom flask and a solution of DIC (85 µL, 5 equiv) in CH_2Cl_2 was added. The reaction mixture was stirred 20 min at 0 °C and evaporated under reduced pressure. The resulting slurry was suspended in DMF (3 mL) and transferred in the tube containing the resin previously swollen in DMF for 20 min and filtered. DMAP (1 mg, 0.1 equiv) was added and the resin was shaked for 1 h. The excess of solvent was removed by filtration and the resin was washed three times with successively DMF, CH_2Cl_2 and MeOH and a final CH_2Cl_2 wash was performed.

Step 2: Phosphonodithioformate salt **S1** (150 mg, 0.33 mmol, 3 equiv) was then dissolved in CH_2Cl_2 (14 mL) and added on the resin which was shaked for 3 h before being filtered and washed as previously.

Step 3: Peptide cleavage and deprotection was performed with a solution of TFA/TIS (6 mL, 97/3 v/v) and this solution was filtered and poured in CH_2Cl_2. The resin was washed with CH_2Cl_2, solvents were evaporated and the desired dithioester-peptide was precipitated by trituration in cold Et_2O and washed by trituration three times with Et_2O (the supernatant was removed with a syringe each time). The resulting dithioester-tripeptide **2** was isolated as a pink solid (73 mg, 81%) used without further purification.

^{1}H NMR (400 MHz, DMSO) δ 9.16 (s, 1H), 8.55 (d, *J* = 7.1 Hz, 1H), 7.89 (dd, *J* = 8.1, 3.7 Hz, 2H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.65 (s, 3H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.20 (s, 1H), 7.09 (s, 1H), 7.00 (d, *J* = 8.3 Hz, 2H), 6.58 (d, *J* = 8.3 Hz, 2H), 4.66 (s, 2H), 4.39 (q, *J* = 7.3 Hz, 2H), 4.16 (dtd, *J* = 8.8, 4.3, 2.0 Hz, 4H), 2.93 (dd, *J* = 14.0, 4.6 Hz, 1H), 2.75 (q, *J* = 8.2 Hz, 4H), 1.73 - 1.62 (m, 1H), 1.50 (s, 4H), 1.35 - 1.20 (m, 11H); **^{31}P NMR** (202 MHz, DMSO) δ -2.64; **HRMS** (ESI-TOF): Calculated for $C_{31}H_{45}N_5O_8PS_2$ $[M+H]^+$: 710.2447, Found: 710.2450 (Δ_{HRMS} = 0.42 ppm).

2.4 Synthesis of phosphonodithioester-PSMA 3



Step 1: *L*-Glutamic di-*tert*-butyl ester hydrochloride (1.7 equiv, 674.27 mg, 2.28 mmol) was suspended in CH_2Cl_2 (20 mL) and Et_3N (5.5 equiv, 740 mg, 1.02 mL, 7.3 mmol) was added and the solution was cooled down to - 78 °C. Then a solution of triphosgene (0.57 equiv, 227 mg, 0.13 mL, 0.76 mmol) in CH_2Cl_2 (6.6 mL) was added dropwise and the reaction mixture was stirred 30 min at r.t. Then H-Lys(Z)-O*t*Bu hydrochloride (1 equiv, 500 mg, 1.34 mmol) was added, followed by Et_3N (1 equiv, 136 mg, 0.19 mL, 1.34 mmol). The mixture was stirred overnight at r.t. The solution was diluted in CH_2Cl_2 (30 mL) and washed twice with 60 mL of water. The organic phase was dried on Na_2SO_4 and evaporated. The crude oil was then purified by gel silica chromatography (50/50 EtOAc/*n*-heptane) to afford the pure product **S2** as a clear oil (740 mg, 89%).[3]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 - 7.27 (m, 5H, C***H^{Ar}***), 5.18 - 4.99 (m, 5H), 4.32 (s, 2H), 3.18 (d, *J* = 7.4 Hz, 2H), 2.40 - 2.20 (m, 2H), 2.16 - 2.00 (m, 1H), 1.91 - 1.71 (m, 2H), 1.71 - 1.56 (m, 3H), 1.52 (t, *J* = 6.8 Hz, 2H), 1.48 - 1.39 (m, 27H, C(C***H$_3$***)$_3$).

Step 2: **S2** (1 equiv, 740 mg, 1.19 mmol) was dissolved in MeOH (25 mL) and the flask was purged with Argon. Then Pd/C (24 mg) was added and H_2 was bubbled in the reaction mixture for 5 min. The solution was then stirred overnight under an H_2 balloon. The next day the reaction mixture was filtered on celite with MeOH and the solvent was carefully evaporated to afford **S3** as a white foam (600 mg, 100%). Analyses were in agreement with literature data.[2]

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 3H), 4.51 - 4.18 (m, 2H), 3.11 (s, 2H), 2.43 - 2.24 (m, 2H), 2.12 - 2.01 (m, 1H), 1.94 - 1.69 (m, 5H), 1.66 - 1.51 (m, 2H), 1.49 - 1.37 (m, 27H, C(C***H$_3$***)$_3$).

Step 3: **S3** (1 equiv, 160 mg, 0.33 mmol) was dissolved in DMF (4.6 mL) then DIEA (2 equiv, 87 mg, 0.11 mL, 0.67 mmol) and 4-(chloromethyl)benzoyl chloride (2 equiv, 127 mg, 0.67 mmol) were added sequentially. The solution was stirred at room temperature for 20 h and the solvent was evaporated. The crude was directly purified by silica gel chromatography (EtOAc/*n*-heptane, 40/60) to afford the product **S4** as a white foam after careful evaporation of the solvent (166 mg, 78%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 - 7.81 (m, 2H, C***H^{Ar}***), 7.46 - 7.33 (m, 2H, C***H^{Ar}***), 7.18 (t, J = 5.6 Hz, 1H), 5.55 (s, 1H), 5.41 (s, 1H), 4.57 (s, 2H), 4.21 (dt, *J* = 11.5, 5.6 Hz, 2H), 3.56 - 3.28 (m, 2H), 2.37 - 2.15 (m, 2H), 1.99 (m, 1H), 1.74 (m, 2H), 1.67 - 1.47 (m, 3H), 1.51 - 1.33 (m, 28H, C(C***H$_3$***)$_3$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.8, 172.5, 172.4, 167.4, 157.5, 140.6, 134.8, 128.6, 127.9, 82.6, 82.0, 80.9, 53.7, 53.3, 45.6, 39.9, 32.6, 31.7, 29.0, 28.2, 28.1, 28.1, 23.1.; **HRMS** (ESI-TOF): Calculated for $C_{32}H_{50}ClN_3NaO_8$ $[M+Na]^+$: 662.3184, Found: 662.3207 (Δ_{HRMS} = 3.47 ppm).

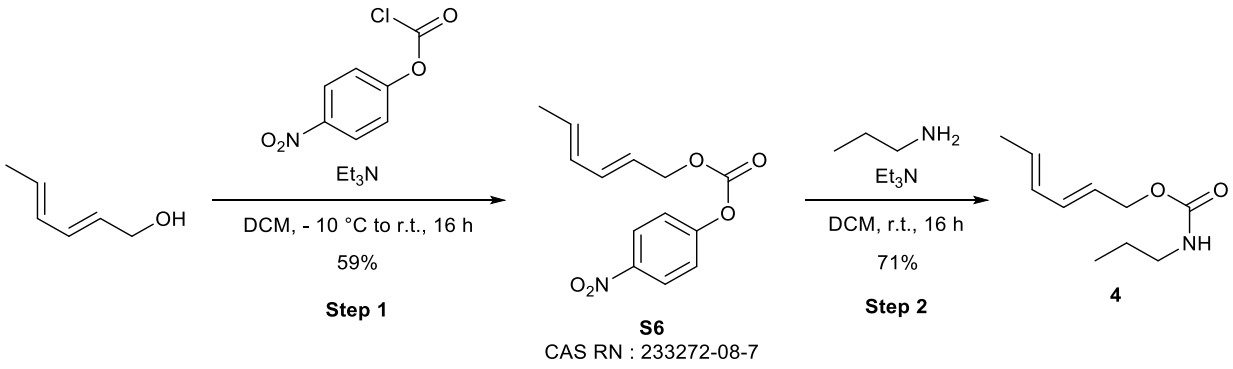
Step 4: Phosphonodithioformate salt **S1** (1 equiv, 119 mg, 0.26 mmol) was dissolved in acetonitrile (1 mL) and the benzyl chloride derivative **S4** (167 mg, 0.28 mmol) dissolved in acetonitrile (1 mL) was added. The mixture was stirred at room temperature for 20 h as it became pinker. The solvent is evaporated, and the product was directly purified by silica gel chromatography (EtOAc/*n*-heptane, 80/20) to afford **S5** as a pink oil (156 mg, 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 - 7.73 (m, 2H, C***H^{Ar}***), 7.25 (m, 3H), 5.64 (s, 1H), 5.48 (s, 1H), 4.39 (s, 2H), 4.24 - 4.08 (m, 5H), 3.43 - 3.19 (m, 2H), 2.30 - 2.11 (m, 2H), 1.94 (m, 2H), 1.79 - 1.60 (m, 2H), 1.49 (m, 3H), 1.32 (s, 9H, C(C***H$_3$***)$_3$), 1.32 (s, 9H, C(C***H$_3$***)$_3$), 1.30 (s, 9H, C(C***H$_3$***)$_3$), 1.26 (td, *J* = 7.1, 0.8 Hz, 6H, P-O-CH_2-C***H$_3$***); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 228.01 (d, *J* = 175.3 Hz), 173.1, 172.5, 172.3, 157.5, 137.0, 134.5, 129.3, 128.0, 82.3, 81.7, 80.7, 64.9 (d, *J* = 6.9 Hz), 53.6, 53.5, 53.1, 40.2 (d, *J* = 2.9 Hz), 39.9, 32.5, 31.7, 29.0, 28.2, 28.13, 28.07, 28.03, 23.1, 16.4 (d, *J* = 6.1 Hz); **HRMS** (ESI-TOF): Calculated for $C_{37}H_{61}N_3O_{11}PS_2$ $[M+H]^+$: 818.3485, Found: 818.3505 (Δ_{HRMS} = 2.44 ppm).

Step 5: Tri-*tert*-butyl ester **S5** (1 equiv, 156 mg, 0.19 mmol) was dissolved in CH_2Cl_2 (2 mL) and TFA (0.5 mL) was added. The mixture is stirred overnight at room temperature for the full deprotection. The volatiles were removed in vacuo and the crude mixture was purified in reversed phase chromatography with a gradient from 5% to 100% of solvent A (MeCN + 0.1% TFA) in solvent B (H_2O + 0.1% TFA) in 40 min. The fractions containing the product were freeze-dried to afford the desired product **3** as a pink solid (93 mg, 75%).

^{1}H NMR (400 MHz, MeOD) δ 7.83 - 7.71 (m, 2H, C***H^{Ar}***), 7.54 - 7.39 (m, 2H, C***H^{Ar}***), 4.65 (s, 2H), 4.35 - 4.18 (m, 6H), 3.38 (td, *J* = 7.0, 2.2 Hz, 2H), 2.49 - 2.32 (m, 2H), 2.20 - 2.08 (m, 1H), 1.94 - 1.81 (m, 2H), 1.75 - 1.59 (m, 3H), 1.54 - 1.43 (m, 2H), 1.35 (td, *J* = 7.0, 0.8 Hz, 6H, P-O-CH_2-C***H$_3$***); **^{13}C NMR** (126 MHz, MeOD) δ 173.7, 173.4, 173.2, 168.2, 158.6, 158.6, 137.9, 134.1, 129.1, 127.4, 65.1, 65.1, 52.8, 52.2, 39.3, 39.1, 39.0, 31.6, 29.5, 28.5, 27.1, 22.7, 15.2, 15.2; **^{31}P NMR** (202 MHz, DMSO) δ -3.39; **HRMS** (ESI-TOF): Calculated for $C_{25}H_{37}N_3O_{11}PS_2$ $[M+H]^+$: 650.1607, Found: 650.1627 (Δ_{HRMS} = 3.08 ppm).

2.5 Synthesis of acyclic diene 4



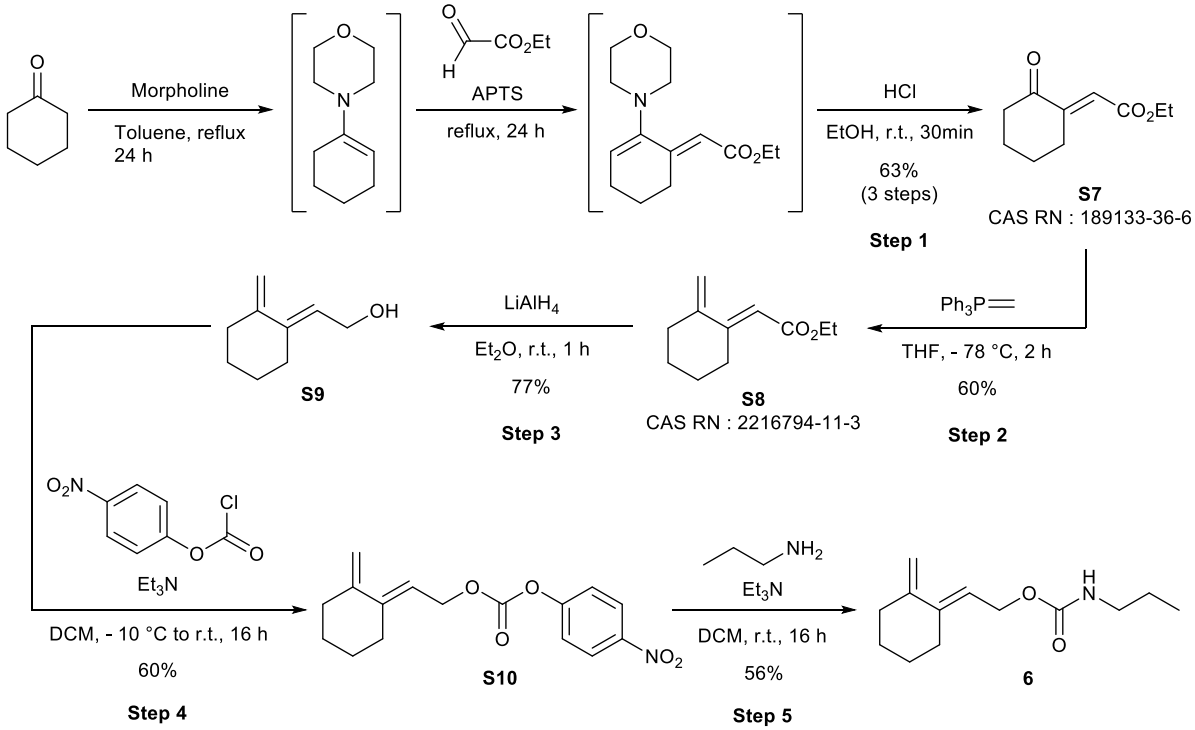
Step 1: (2E,4E)-hexadien-1-ol (1 equiv, 1 g, 1.15 mL, 10.2 mmol) was dissolved in CH_2Cl_2 (50 mL) and 4-nitrophenyl chloroformate (1.1 equiv, 2.3 g, 11.2 mmol) was added. The mixture was cooled to - 10 °C in an ice-salt bath before TEA (2 equiv, 2.06 g, 2.83 mL, 20.4 mmol) was added dropwise. The reaction mixture turned yellow and was stirred letting it return to room temperature overnight. The reaction mixture was partitioned between EtOAc (15 ml $mmol^{-1}$) and saturated $NH_4Cl_{(aq)}$ (7.5 ml $mmol^{-1}$). The organic phase was washed with saturated $NH_4Cl_{(aq)}$ (2 × 7.5 ml $mmol^{-1}$), dried (Na_2SO_4) and concentrated under reduced pressure. The crude product is purified by silica gel chromatography (EtOAc/*n*-heptane 5/95) affording the *p*-nitrophenyl allyl carbonate **S6** (1.59 g, 59%).[4]

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 - 8.10 (m, 2H, C***H*^{Ar}**), 7.34 - 7.17 (m, 2H, C***H*^{Ar}**), 6.28 - 6.19 (m, 1H, C***H*^{diene}**), 6.02 - 5.89 (m, 1H, C***H*^{diene}**), 5.75 - 5.64 (m, 1H, C***H*^{diene}**), 5.61 - 5.51 (m, 1H, C***H*^{diene}**), 4.64 (d, *J* = 6.9 Hz, 2H, CH=CH-C***H*$_2$**-O), 1.65 (dd, *J* = 6.7, 1.6 Hz, 3H, CH=CH-C***H*$_3$**).

Step 2: To a solution of a propylamine (1 equiv, 180 mg, 0.25 mL, 3.0 mmol) in CH_2Cl_2 (7.1 mL) was added TEA (2 equiv, 615 mg, 0.84 mL, 6.1 mmol) and a solution of **S6** (1 equiv, 800 mg, 3.0 mmol) in CH_2Cl_2 (3.55 mL). The reaction mixture was left overnight at room temperature. The solvent was removed in vacuo and the resulting yellow oil was redissolved in CH_2Cl_2 and washed several times with brine, saturated $NaHCO_3$, and 2 M NaOH until the organic phase was colourless. The organic phase was dried (Na_2SO_4) and concentrated in vacuo to give **4** as a yellowish oil (400 mg, 71%). Product **4** was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 - 6.15 (m, 1H, C***H*^{diene}**), 6.08 - 6.00 (m, 1H, C***H*^{diene}**), 5.80 - 5.67 (m, 1H, C***H*^{diene}**), 5.67 - 5.56 (m, 1H, C***H*^{diene}**), 4.73 (s, 1H, N***H***), 4.54 (d, *J* = 6.6 Hz, 2H, CH=CH-C***H*$_2$**-O), 3.12 (q, *J* = 6.7 Hz, 2H, NH-C***H*$_2$**), 1.74 (d, *J* = 6.8 Hz, 3H, CH=CH-C***H*$_3$**), 1.50 (h, *J* = 7.4 Hz, 2H, CH_2-C***H*$_2$**-CH_3), 0.90 (t, *J* = 7.4 Hz, 3H, CH_2-CH_2-C***H*$_3$**); **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 154.4, 134.3, 130.9, 130.6, 124.6, 65.2, 42.8, 23.2, 18.1, 11.2. **HRMS** (ESI-TOF): Calculated for $C_{10}H_{17}NNaO_2$ $[M+Na]^+$: 206.1157, Found : 206.1157 (Δ_{HRMS} = 0 ppm).

2.6 Synthesis of exocyclic diene 6



Step 1: Following a procedure described in the literature,[5] to a mixture of cyclohexanone (1.15 equiv, 6.63 g, 7 mL, 67.6 mmol) in toluene (90 mL) was added morpholine (1.73 equiv, 8.86 g, 8.95 mL, 101.7 mmol) and the reaction vessel was fitted with a Dean Stark apparatus and heated to 130 °C for 16 h. The reaction mixture was cooled to room temperature and Ethyl glyoxalate (50% w/w in toluene, 1 equiv, 12 g, 11.63 mL, 58.8 mmol) and *p*-toluenesulfonic acid monohydrate (0.04 equiv, 0.45 g, 2.4 mmol) were added and the mixture was refluxed with a Dean Stark for a further 24 h. The reaction mixture was cooled to room temperature and solvent removed in vacuo. The crude residue was dissolved in EtOH (10 mL) and treated with HCl (2M, 10 mL) and stirred at room temperate for 30 min. Water (30 mL) was added and the mixture was extracted with EtOAc (3 x 40 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo before being purified via column chromatography (5% EtOAc in *n*-heptane) to afford **S7** as an oil (6.71 g, 63%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (t, *J* = 2.3 Hz, 1H, C=C***H***-CH_2), 4.20 (q, *J* = 7.1 Hz, 2H, O-C***H₂***-CH_3), 3.09 (td, *J* = 6.5, 2.3 Hz, 2H, C***H₂***-C=CH), 2.53 (t, *J* = 6.8 Hz, 2H, C***H₂***-CO), 1.98 - 1.87 (m, 2H, CH_2-C***H₂***-CH_2-CH_2), 1.87 - 1.74 (m, 2H, CH_2-C***H₂***-CH_2-CH_2), 1.29 (t, *J* = 7.1 Hz, 3H, O-CH_2-C***H₃***).

Step 2: Following a procedure described in the literature,[5] to a suspension of methyl triphenylphosphonium bromide (1 equiv, 11.5 g, 32 mmol) in dry THF (96 mL) was added *n*-buthyllithium (1 equiv, 12.9 mL, 32 mmol, 2.5 M in hexanes) at -78 °C and the mixture was stirred and allowed to warm to room temperature. After 45 min the mixture was cooled back down to -78 °C and diene **S7** (1 equiv, 5.9 g, 32 mmol) in THF (32 mL) was added dropwise over 10 min. The mixture was stirred at this temperature for a further 30 min before being warmed to 0 °C, quenched with water (100 mL), extracted with CH_2Cl_2 (3 x 100 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The crude product was purified by gel column chromatography (2 % EtOAc in *n*-heptane) to afford the pure product as a clear to yellowish oil (3.47 g, 60%). (*Note:* the dienyl esters are isolable however, they are known to decompose within two days in the fridge).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (t, *J* = 1.5 Hz, 1H, C=C***H***-CH_2), 4.99 (dt, *J* = 2.0, 1.0 Hz, 1H, C=C***H₂***), 4.76 (q, *J* = 1.4 Hz, 1H, C=C***H₂***), 4.16 (q, *J* = 7.1 Hz, 2H, O-C***H₂***-CH_3), 2.94 - 2.88 (m, 2H, C***H₂***-CH_2-CH_2-CH_2), 2.35 - 2.29 (m, 2H, C***H₂***-CH_2-CH_2-CH_2), 1.73 - 1.64 (m, 4H, CH_2-C***H₂***-C***H₂***-CH_2), 1.28 (t, *J* = 7.1 Hz, 3H, O-CH_2-C***H₃***).

Step 3: A mixture of diene-ester **S8** (1 equiv, 3.47 g, 19.3 mmol), Et_2O (100 mL), and LAH (1.15 equiv, 833 mg, 22 mmol) was stirred at r.t. for 1 h, then cooled to 0 °C, and treated with aq 2 M H_2SO_4 (150 mL). The aqueous phase was extracted with Et_2O (3 × 100 mL), the combined organic layers were washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The crude product was purified on silica gel chromatography (30 % EtOAc in *n*-heptane) to afford the pure product **S9** as a clear oil (2.06 g, 77 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.66 (tt, *J* = 7.0, 1.5 Hz, 1H, C=C***H***-CH_2), 4.86 (dd, *J* = 2.4, 1.2 Hz, 1H, C=C***H₂***), 4.62 (dt, *J* = 2.6, 1.4 Hz, 1H, C=C***H₂***), 4.19 (d, *J* = 7.0 Hz, 2H, C=CH-C***H₂***-O), 2.29 - 2.23 (m, 4H, C***H₂***-CH_2-CH_2-C***H₂***), 1.75 - 1.53 (m, 4H, CH_2-C***H₂***-C***H₂***-CH_2), 1.36 (bs, 1H, O***H***). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 150.5, 143.8, 121.6, 108.3, 58.9, 35.3, 28.8, 26.9, 26.3.

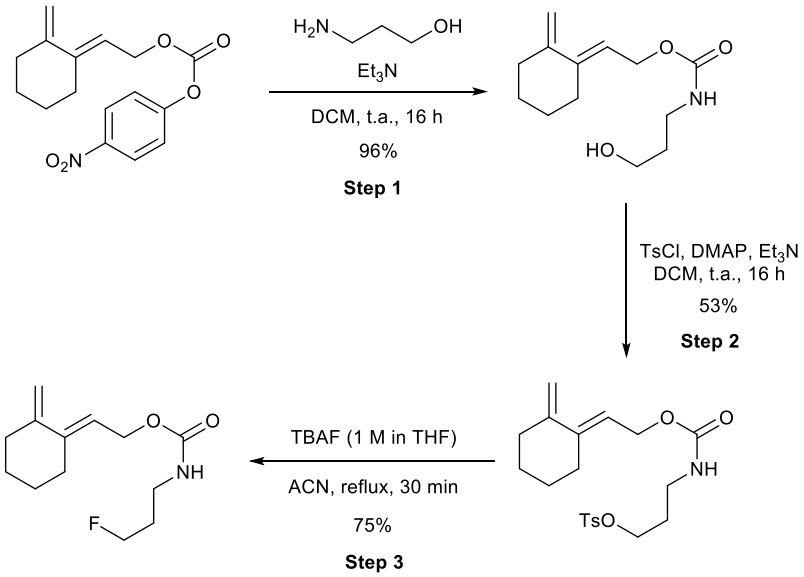
Step 4: **S9** (1 equiv, 2.06 g, 14.9 mmol) was dissolved in CH_2Cl_2 (75 mL) and 4-nitrophenyl chloroformate (1.1 equiv, 3.3 g, 16.4 mmol) was added. The mixture was cooled to - 10 °C in an ice-salt bath before TEA (2 equiv, 4.1 mL, 29.8 mmol) was added dropwise. The reaction mixture turned yellow and was stirred overnight letting it slowly return to room temperature. The reaction mixture was partioned between EtOAc (250 mL) and saturated $NH_4Cl_{(aq)}$ (100 mL). The organic phase was washed with saturated $NH_4Cl_{(aq)}$ (2 × 100 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (5% EtOAc in *n*-heptane) afforded the corresponding product **S10** as a white to yellowish solid (2.72 g, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 - 7.95 (m, 2H, C***H^{Ar}***), 7.34 - 7.17 (m, 2H, C***H^{Ar}***), 5.53 (tt, *J* = 7.4, 1.5 Hz, 1H, C=C***H***-CH_2), 4.78 (dt, *J* = 2.2, 1.1 Hz, 1H, C=C***H₂***), 4.70 (d, *J* = 7.4 Hz, 2H, C=CH-C***H₂***-O), 4.56 (dt, *J* = 2.6, 1.4 Hz, 1H, C=C***H₂***), 2.41 - 1.90 (m, 4H, C***H₂***-CH_2-CH_2-C***H₂***), 1.70 - 1.34 (m, 4H, CH_2-C***H₂***-C***H₂***-CH_2). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 155.7, 152.5, 149.9, 148.2, 145.4, 125.3, 121.8, 114.7, 109.3, 65.5, 35.3, 29.1, 26.8, 26.2.

Step 5a: To a solution of propylamine (1 equiv, 43 mg, 0.06 mL, 0.72 mmol) in CH_2Cl_2 (1.7 mL) was added TEA (2 equiv, 145.46 mg, 0.2 mL, 1.44 mmol) and a solution of **S10** (1 equiv, 220 mg, 0.72 mmol) in CH_2Cl_2 (0.8 mL). The reaction mixture was left overnight at room temperature. The solvent was removed in vacuo and the resulting yellow oil was redissolved in CH_2Cl_2 and washed several times with brine, saturated $NaHCO_3$, and 2 M NaOH until the organic phase was colorless. The organic phase was dried (Na_2SO_4) and concentrated in vacuo to give **6** as a yellowish oil (90 mg, 56%). Product **6** was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.54 (t, *J* = 7.1 Hz, 1H, C=C***H***-CH_2), 4.83 (dd, *J* = 2.4, 1.2 Hz, 1H, C=C***H₂***), 4.80 (s, 1H, N***H***), 4.59 (bs, C=CH-C***H₂***-O), 4.57 (s, 1H, C=C***H₂***), 3.11 (q, *J* = 6.7 Hz, 2H, NH-C***H₂***), 2.29 - 2.18 (m, 4H, C***H₂***-CH_2-CH_2-C***H₂***), 1.66 - 1.55 (m, 4H, CH_2-C***H₂***-C***H₂***-CH_2), 1.48 (h, *J* = 7.3 Hz, 2H, CH_2-C***H₂***-CH_3), 0.88 (t, *J* = 7.4 Hz, 3H, CH_2-CH_2-C***H₃***); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.6, 150.2, 145.2, 117.2, 108.5, 61.0, 42.7, 35.3, 28.9, 26.9, 26.1, 23.2, 11.2. **HRMS** (ESI-TOF): Calculated for $C_{13}H_{21}NNaO_2$ $[M+Na]^+$: 246.1470 ; 246.1467 (Δ_{HRMS} = 1.22 ppm).

2.7 Synthesis of exocyclic fluorinated diene F-8



Step 5b: To a solution of 3-aminopropanol (1.5 equiv, 1.04 mL, 13.6 mmol) in CH_2Cl_2 (21 mL) was added TEA (3 equiv, 3.8 mL, 27.2 mmol) and a solution of **S10** (1 equiv, 2.72 g, 9 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was left overnight at room temperature. The solvent was removed in vacuo and the resulting yellow oil was redissolved in CH_2Cl_2 and washed several times with brine, saturated $NaHCO_3$, and 2 M NaOH until the organic phase was colorless. The organic phase was dried (Na_2SO_4) and concentrated in vacuo to give **S11** as a yellow oil (2.06 g, 96%). The product was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.55 (t, *J* = 7.1 Hz, 1H, C=C***H***-CH_2), 5.11 (bs, 1H, N***H***), 4.90 - 4.79 (m, 1H, C=C***H₂***), 4.66 - 4.56 (m, 3H, C=C***H₂*** + C=CH-C***H₂***-O), 3.65 (t, *J* = 5.7 Hz, 2H, C***H₂***-OH), 3.31 (q, *J* = 6.2 Hz, 2H, NH-C***H₂***), 2.32 - 2.19 (m, 4H, C***H₂***-CH_2-CH_2-C***H₂***), 1.75 - 1.55 (m, 6H, C***H₂***-CH_2-OH + CH_2-C***H₂***-C***H₂***-CH_2). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 157.6, 150.2, 145.7, 116.9, 108.6, 61.4, 59.5, 37.6, 35.3, 32.7, 28.9, 26.9, 26.1. **HRMS** (ESI-TOF): Calculated for $C_{13}H_{21}NNaO_3$ $[M+Na]^+$: 262.1419, Found: 262.1414 (Δ_{HRMS} = 1.91 ppm).

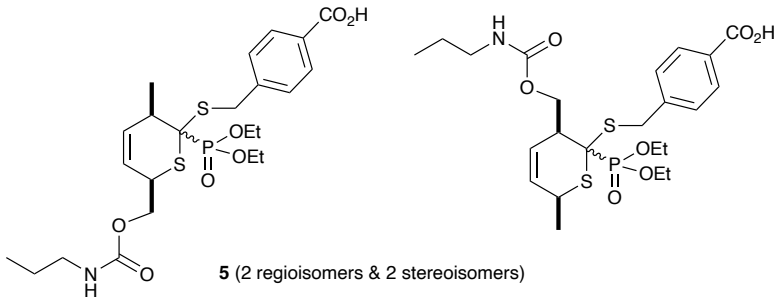
Step 6: To a solution of **S11** (1 equiv, 2.06 g, 8.6 mmol) in CH_2Cl_2 (50 mL) was added TEA (1 equiv, 1.2 mL, 8.6 mmol) and DMAP (0.1 equiv, 100 mg, 0.86 mmol) and stirred 5 min at r.t. Then para-toluenesulfonyl chloride (1.05 equiv, 1.72 g, 9 mmol) was added and the solution was stirred overnight at room temperature. Evaporation of the solvent gave the crude product which was purified by gel column chromatography (40% EtOAc in *n*-heptane) giving product TsO-**8** as a clear oil (1.81 g, 53%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 - 7.62 (m, 2H, C***H^{ArTs}***), 7.25 (d, *J* = 8.1 Hz, 2H, C***H^{ArTs}***), 5.45 (tt, *J* = 7.1, 1.5 Hz, 1H, C=C***H***-CH_2), 4.76 (dd, *J* = 2.4, 1.1 Hz, 1H, C=C***H₂***), 4.74 (s, 1H, N***H***), 4.53 (dd, *J* = 2.6, 1.3 Hz, 1H, C=C***H₂***), 4.48 (d, *J* = 7.1 Hz, 2H, C=CH-C***H₂***-O), 3.99 (t, *J* = 6.0 Hz, 2H, C***H₂***-OTs), 3.13 (q, *J* = 6.4 Hz, 2H, NH-C***H₂***), 2.35 (s, 3H, C***H$_3^{Ts}$***), 2.16 (td, *J* = 7.3, 3.8 Hz, 4H, C***H₂***-CH_2-CH_2-C***H₂***), 1.76 (p, *J* = 6.3 Hz, 2H, C***H₂***-CH_2-OTs), 1.63 - 1.44 (m, 4H, CH_2-C***H₂***-C***H₂***-CH_2). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 156.5, 150.2, 145.5, 145.0, 132.9, 129.9, 127.9, 117.0, 108.6, 67.8, 61.2, 37.2, 35.3, 29.1, 28.9, 26.9, 26.1, 21.7. **HRMS** (ESI-TOF): Calculated for $C_{20}H_{27}NNaO_5S$ $[M+Na]^+$: 416.1508, Found: 416.1518 (Δ_{HRMS} = 2.40 ppm).

Step 7: To a solution of TsO-**8** (1 equiv, 600 mg, 1.52 mmol) in acetonitrile (24 mL) was added TBAF (1M in THF, 1.5 equiv, 2.3 mL, 2.3 mmol) and the mixture was stirred 30 min at reflux. The solvent was carefully evaporated, and the crude was purified on gel silica column chromatography (10% to 30% EtOAc in *n*-heptane) to give the corresponding product **F-8** as a clear oil (264 mg, 72%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.57 (t, *J* = 6.8 Hz, 1H, C=C***H***-CH_2), 4.89 (bs, 1H), 4.86 (m, 1H), 4.64 - 4.59 (m, 3H), 4.52 (dt, $^2J_{H\text{-}F}$ = 47.2, *J* = 6.1 Hz, 2H, C***H₂***-F), 3.33 (q, *J* = 6.4 Hz, 2H, NH-C***H₂***), 2.26 (m, 4H, C***H₂***-CH_2-CH_2-C***H₂***), 1.92 (dp, $^3J_{H\text{-}F}$ = 27.6, *J* = 6.0 Hz, 2H, C***H₂***-CH_2-F), 1.63 (m, 4H, CH_2-C***H₂***-C***H₂***-CH_2). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 156.6, 150.2, 145.5, 117.0, 108.6, 82.1 (d, $^1J_{C\text{-}F}$ = 164.3 Hz), 61.1, 37.7, 35.3, 30.7 (d, $^2J_{C\text{-}F}$ = 19.5 Hz), 28.9, 26.9, 26.1. **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) δ -221.0. **HRMS** (ESI-TOF): Calculated for $C_{13}H_{20}FNNaO_2$ $[M+Na]^+$: 264.1376, Found: 264.1381 (Δ_{HRMS} = 1.89 ppm); **Rf** = 0.40 (30% EtOAc in *n*-heptane).

2.8 Cycloaddition between 1 and 4: Synthesis of 5



5 (2 regioisomers & 2 stereoisomers)

Dithioester **1** (1 equiv, 35 mg, 0.1 mmol) was dissolved in *i*PrOH (1.5 mL) and diene **4** (1 equiv, 18 mg, 0.1 mmol) was dissolved in *i*PrOH (1.5 mL) and H_2O (7 mL). The diene solution was heated to 60 °C and the solution got clearer before the dithioester solution was added. The reaction was complete after 24 h (checked by HPLC). Isopropanol and water were evaporated, then the residue was dissolved in DMSO and directly purified by semi-preparative reversed-phase HPLC chromatography (Gilson PLC2020 system) on a SunFire C18 column (5 µm, 19 × 150 mm) using a linear gradient (10% to 95% in 40 min, flow-rate of 18 mL.min^{-1}) of solvent B (0.1% TFA in MeCN, v/v) in solvent A (0.1% TFA in H_2O, v/v). Detection was set at 220 and 254 nm. Two fractions containing the desired products were freeze-dried to give the purified product **5** as a white foam (25 mg, 47%) as a mixture of four regio/stereoisomers observed by ^{31}P NMR.

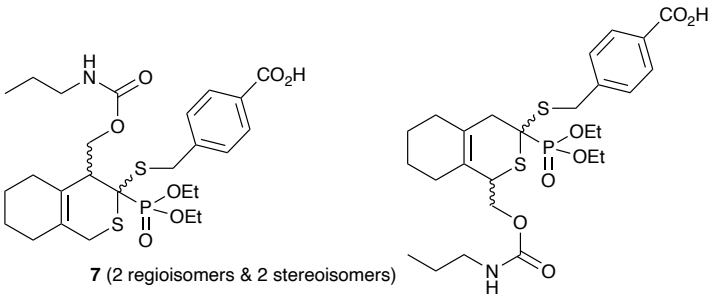
^{31}P{^{1}H} NMR (162 MHz, $CDCl_3$) δ 20.1, 19.6, 19.5, 18.3 (15:24:29:32 ratio); **HRMS** (ESI-TOF): Calculated for $C_{23}H_{35}NO_7PS_2$ $[M+H]^+$: 532.1593; Found: 532.1596.

Two fractions containing each one pair of isomers were isolated for NMR analysis.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 8.5, 2.3 Hz, 2H), 7.45 - 7.36 (m, 2H), 6.00 - 5.87 (m, 1H), 5.86 - 5.80 (m, 1H), 4.71 (d, *J* = 10.3 Hz, 2H), 4.43 - 4.19 (m, 5H), 4.16 (d, *J* = 11.9 Hz, 1H), 3.69 (s, 3H), 3.12 (s, 4H), 1.49 (q, *J* = 7.2 Hz, 2H), 1.43 (dd, *J* = 7.2, 2.4 Hz, 3H), 1.41 - 1.34 (m, 6H), 0.93 - 0.84 (m, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.0, 156.2, 143.1, 132.1, 130.4, 129.6, 128.2, 65.4 (d, *J* = 7.4 Hz), 65.2, 64.0 (d, *J* = 7.9 Hz), 42.8, 40.4, 35.5, 35.1, 23.2, 20.9, 16.5 (d, *J* = 5.5 Hz), 16.4 (d, *J* = 5.9 Hz), 11.2. **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$) δ 19.6.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 - 7.92 (m, 2H), 7.42 - 7.34 (m, 2H), 5.71 (d, *J* = 11.0 Hz, 1H), 5.62 (m, 1H), 4.56 (s, 1H), 4.34 - 4.17 (m, 5H), 4.10 (d, *J* = 12.1 Hz, 2H), 3.52 (d, *J* = 7.6 Hz, 1H), 3.07 (t, *J* = 7.1 Hz, 2H), 2.63 (d, *J* = 8.2 Hz, 1H), 1.45 (h, *J* = 7.4 Hz, 2H), 1.32 (t, *J* = 7.1 Hz, 6H), 1.25 (d, *J* = 7.2 Hz, 3H), 0.85 (t, *J* = 7.4 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.11, 143.32, 130.84, 130.44, 129.51, 128.11, 125.83, 66.4, 64.9, 55.7, 42.9 39.54, 36.46, 32.48, 23.18, 18.99, 16.45 (d, *J* = 5.7 Hz), 16.37 (d, *J* = 5.8 Hz), 11.19. **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$) δ 18.3.

2.9 Cycloaddition between 1 and 6: Synthesis of 7



7 (2 regioisomers & 2 stereoisomers)

Dithioester **1** (1 equiv, 33 mg, 0.1 mmol) was dissolved in *i*PrOH (1.4 mL) and diene **6** (1 equiv, 21 mg, 0.1 mmol) was dissolved in *i*PrOH (1.4 mL) and H_2O (6.5 mL). The diene solution was heated to 60 °C and the solution got clearer before the dithioester solution was added. The reaction was complete after 10 min (checked by HPLC). Isopropanol and water were evaporated, then the residue was dissolved in DMSO and directly purified by semi-preparative reversed-phase HPLC chromatography (Gilson PLC2020 system) on a SunFire C18 column (5 µm, 19 × 150 mm) using a linear gradient (10% to 95% in 40 min, flow-rate of 18 mL.min^{-1}) of solvent B (0.1% TFA in MeCN, v/v) in solvent A (0.1% TFA in H_2O, v/v). Detection was set at 220 and 254 nm. Fractions containing the desired products were freeze-dried to give the purified product **7** as a white foam (35 mg, 65%), as a mixture of four regio/stereoisomers observed by ^{31}P NMR.

^{31}P{^{1}H} NMR (162 MHz, $CDCl_3$) δ 20.7, 19.8, 19.4, 18.3 (24:11:17:48 ratio); **HRMS** (ESI-TOF): Calculated for $C_{26}H_{39}NO_7PS_2$ $[M+H]^+$: 572.1906; Found: 572.1912.

Two fractions containing each one pair of isomers were isolated for NMR analysis.

Characteristic NMR signals of fraction 1:

Ratio Maj/min = 55/45

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.3 Hz, 2H, C***H*$_{Ar}$**, Maj), 8.00 (d, *J* = 8.3 Hz, 2H, C***H*$_{Ar}$**, min), 7.46 (d, *J* = 8.3 Hz, 2H, C***H*$_{Ar}$**, Maj), 7.42 (d, *J* = 8.4 Hz, 2H, C***H*$_{Ar}$**, min), 4.80 - 4.50 (m, 3H), 4.39 - 3.72 (m, 15H), 3.55 (m, 1H), 3.10 - 2.98 (m, 6H), 2.86 - 2.76 (m, 2H), 2.27 - 1.36 (m, 16H), 1.51 - 1.35 (m, 6H), 1.32 - 1.21 (m, 12H, OCH_2C***H*$_3$**), 0.91 (t, *J* = 7.4 Hffz, 3H, CH_2CH_2C***H*$_3$**, Maj), 0.86 (t, *J* = 7.4 Hz, 3H, CH_2CH_2C***H*$_3$**, min).**^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.5 (***C***=O), 170.4 (***C***=O), 143.8, 143.1, 130.3, 130.3, 129.6, 129.4, 128.4, 128.1, 65.8, 64.8 (d, *J* = 7.6 Hz, O***C***H_2CH_3), 64.6 (d, *J* = 7.7 Hz, O***C***H_2CH_3), 64.1 (d, *J* = 7.9 Hz, O***C***H_2CH_3), 53.60 (d, *J* = 161.6 Hz, ***C*$_q$**P), 44.3, 42.7, 42.4, 36.7, 36.3, 35.0, 32.2, 30.9, 30.6, 30.3, 29.1, 23.2, 22.9, 22.9, 22.8, 22.5, 16.58 (d, *J* = 5.6 Hz, OCH_2***C***H_3, Maj), 16.52 (d, *J* = 5.8 Hz, OCH_2***C***H_3, min), 11.2 (***C***H_3, Maj), 11.1 (***C***H_3, min). **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$) δ 20.7 (Maj), 19.44 (min).

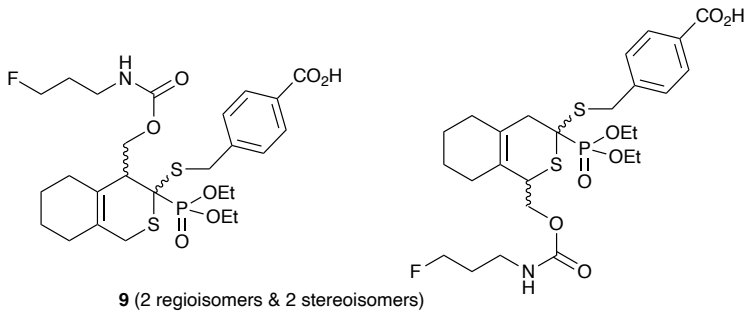
Characteristic NMR signals of fraction 2:

Ratio Maj/min = 75/25

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.4 Hz, C***H*$_{Ar}$**, 2H), 8.01 (d, *J* = 8.4 Hz, C***H*$_{Ar}$**, 2H), 7.45 (d, *J* = 8.1 Hz, C***H*$_{Ar}$**, 2H), 7.41 (d, *J* = 7.9 Hz, C***H*$_{Ar}$**, 2H), 4.79 - 3.95 (m, $PO(OC$***H*$_2$**$CH_3)_2$, 17H), 3.48 - 2.97 (m, 6H), 2.78 - 2.48 (m, 3H), 2.42 - 1.72 (m, 9H), 2.00 - 1.38 (m, 22H), 1.40 - 1.32 (m, 12H, $PO(OCH_2C$***H*$_3$**$)_2$), 0.91 (t, *J* = 7.4 Hz, 6H,

$NHCH_2CH_2C\boldsymbol{H_3}$). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.2 (***C***=O), 156.6, 143.4, 130.4 (***C*** *Ar*, Maj), 129.5 (***C*** *Ar*, Maj), 129.4 (***C*** *Ar*, Maj), 128.4 (***C*** *Ar*, min), 125.8, 65.5, 65.0, 64.8, 64.6, 64.1, 45.4, 42.8, 37.6, 36.5, 36.2, 31.6, 31.1, 30.4, 27.7, 23.2, 23.02, 22.98, 22.6, 16.5 ($PO(OCH_2\boldsymbol{C}H_3)_2$, Maj), 16.2 (d, *J* = 6.1 Hz, $PO(OCH_2\boldsymbol{C}H_3)_2$, min), 11.22 (***C***$H_3$, Maj), 11.19 (***C***$H_3$, min). **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$) δ 19.8 (min), 18.3 (Maj).

2.10 Cycloaddition between 1 and F-8: Synthesis of F-9



9 (2 regioisomers & 2 stereoisomers)

Dithioester **1** (1 equiv, 49 mg, 0.14 mmol) was dissolved in *i*PrOH (2.2 mL) and diene **8** (1 equiv, 34 mg, 0.14 mmol) was dissolved in *i*PrOH (2.2 mL) and H_2O (10 mL). The diene solution was heated to 60 °C and the solution got clearer before the dithioester solution was added. The reaction was complete after 10 min (checked by HPLC). Isopropanol and water were evaporated, then the residue was dissolved in DMSO and directly purified by semi-preparative reversed-phase HPLC chromatography (Gilson PLC2020 system) on a SunFire C18 column (5 µm, 19 × 150 mm) using a linear gradient (10% to 95% in 40 min, flow-rate of 18 mL.min^{-1}) of solvent B (0.1% TFA in MeCN, v/v) in solvent A (0.1% TFA in H_2O, v/v). Detection was set at 220 and 254 nm. Fractions containing the desired products were freeze-dried to give the purified product **F-9** as a white foam (54 mg, 66%) as a mixture of four regio/stereoisomers observed by ^{31}P and ^{19}F NMR.

^{19}F{^{1}H} NMR (471 MHz, DMSO) δ -76.55 (F-TFA), -221.49, -221.51, -221.55, -221.57 (F-cycloaducts); **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$) δ 20.8, 19.8, 19.4, 18.2 (27:9:16:48 ratio); **HRMS** (ESI-TOF): Calculated for $C_{26}H_{38}FNO_7PS_2$ $[M+H]^+$: 590.1811; Found: 590.1811, 590.1809.

Two fractions containing each one pair of isomers were isolated for NMR analysis.

Characteristic NMR signals of fraction 1:

Ratio Maj/min = 55/45

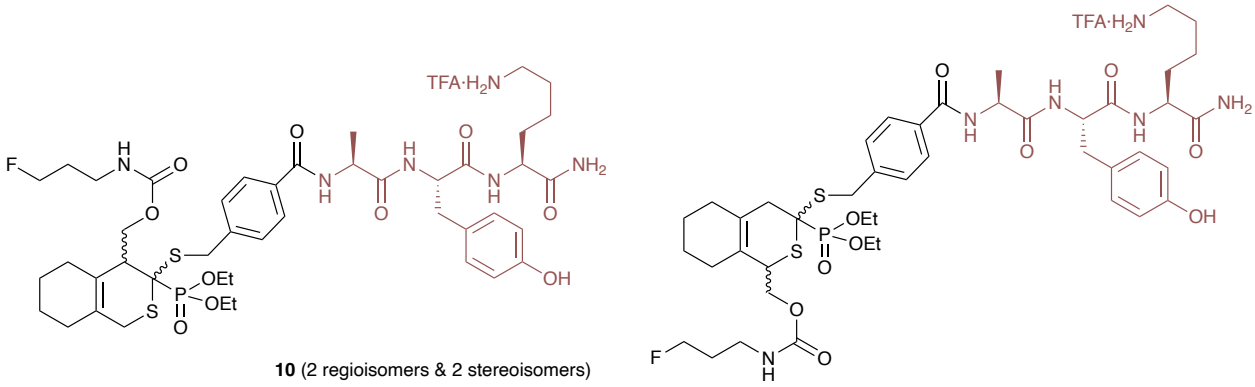
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 3.1 Hz, C***H***$_{Ar}$, 2H), 8.00 (d, *J* = 3.3 Hz, C***H***$_{Ar}$, 2H), 7.49 - 7.44 (m, C***H***$_{Ar}$, 2H), 7.44 - 7.39 (m, C***H***$_{Ar}$, 2H), 5.78 - 3.53 (m, 23H), 3.41 - 2.80 (m, 8H), 2.32 - 1.42 (m, 21H), 1.36 (dt, *J* = 11.9, 7.1 Hz, $OCH_2C\boldsymbol{H_3}$, 12H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.5 (***C***=O), 170.4 (***C***=O), 156.3, 156.1, 143.8, 143.1, 130.34, 130.29, 129.6, 129.4, 129.3, 128.6, 128.3, 127.6, 126.3, 82.6, 81.3, 66.0, 65.0 (d, *J* = 9.2 Hz, $O\boldsymbol{C}H_2CH_3$), 64.7 (d, *J* = 7.4 Hz, $O\boldsymbol{C}H_2CH_3$), 64.6 (d, *J* = 7.7 Hz, $O\boldsymbol{C}H_2CH_3$), 64.0 (d, *J* = 7.8 Hz, $O\boldsymbol{C}H_2CH_3$), 54.7 (d, *J* = 161.2 Hz, ***C***$_q$P), 53.6 (d, *J* = 161.4 Hz, ***C***$_q$P), 44.3, 42.3, 37.7, 36.8, 36.4, 35.1, 32.2, 30.9, 30.7, 30.5, 30.4, 29.1, 22.9, 22.9, 22.8, 22.5, 16.59 (d, *J* = 5.5 Hz, $(PO(OCH_2\boldsymbol{C}H_3)_2)$, 16.52 (d, *J* = 5.7 Hz, $(PO(OCH_2\boldsymbol{C}H_3)_2)$; **^{19}F{^{1}H} NMR** (471 MHz, DMSO) δ -76.55 (F-TFA), -221.54 (Maj), -221.56 (min); **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$) δ 20.8 (Maj), 19.4 (min).

Characteristic NMR signals of fraction 2:

Ratio Maj/min = 80/20

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (m, C***H***$_{Ar}$, 2H), 7.48 - 7.43 (m, C***H***$_{Ar}$, 2H), 7.40 (d, *J* = 8.0 Hz, C***H***$_{Ar}$, 2H), 5.88 - 3.93 (m, 22H), 3.55 - 2.15 (m, 12H), 2.07 - 1.46 (m, 18H), 1.46 - 1.27 (m, $OCH_2C\boldsymbol{H_3}$, 12H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.1 (***C***=O), 156.6, 156.2, 143.2, 143.0, 130.3, 129.5, 129.4, 128.6, 127.6, 127.6, 126.0, 82.6, 81.3, 66.4, 65.6 (d, *J* = 7.9 Hz, $O\boldsymbol{C}H_2CH_3$), 65.0, 64.7 (d, *J* = 7.8 Hz, $O\boldsymbol{C}H_2CH_3$), 64.6 (d, *J* = 7.4 Hz, $O\boldsymbol{C}H_2CH_3$), 64.1 (d, *J* = 8.3 Hz, $O\boldsymbol{C}H_2CH_3$), 55.2 (d, *J* = 157.8 Hz, ***C***$_q$P, Maj), 52.5 (d, *J* = 159.6 Hz, ***C***$_q$P, min), 45.3, 37.8, 37.5, 37.5, 36.5, 36.2, 31.6, 31.0, 30.7, 30.6, 30.4, 29.5, 27.7, 27.6, 23.0, 23.0, 23.0, 22.6, 16.52 (d, *J* = 5.6 Hz, $(PO(OCH_2\boldsymbol{C}H_3)_2$, Maj), 16.20 (d, *J* = 6.6 Hz, $(PO(OCH_2\boldsymbol{C}H_3)_2$, min); **^{19}F{^{1}H} NMR** (471 MHz, DMSO) δ -76.55 (F-TFA), -221.50 (min), -221.56 (Maj) **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$) δ 19.8 (min), 18.3 (Maj).

2.11 Cycloaddition between 2 and F-8: Synthesis of F-10

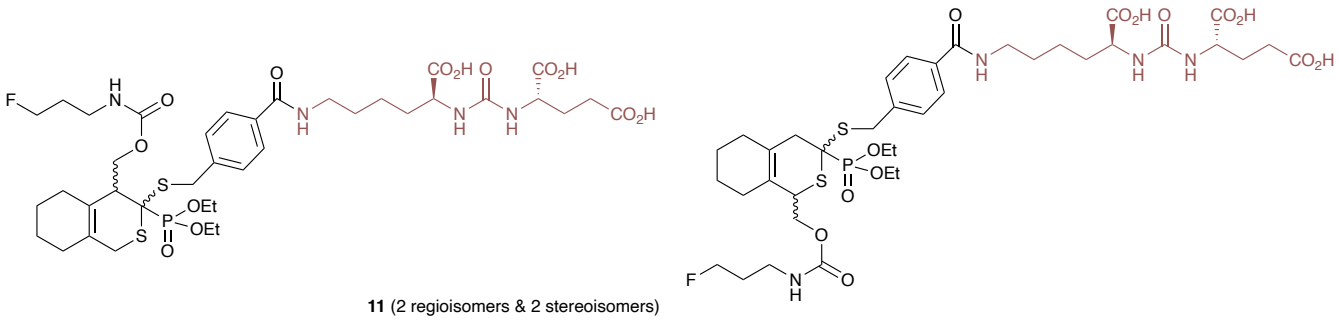


10 (2 regioisomers & 2 stereoisomers)

First, diene **F-8** (2.93 mg, 12 µmol) was dissolved in isopropanol (60 µL) in a small vial and was heated to 60 °C under stirring. Then dithioester-tripeptide **2** (10 mg, 12 µmol) was dissolved in water (540 µL, milli-Q grade) and this solution was added on the diene solution. After 2 min, the pink color of dithioester-tripeptide **2** vanished and completion was confirmed by HPLC analysis. Isopropanol was evaporated and the aqueous crude solution was directly purified by semi-preparative reversed-phase HPLC chromatography (Gilson PLC2020 system) on a SunFire C18 column (5 µm, 19 × 150 mm) using a linear gradient (10% to 95% in 40 min, flow-rate of 18 mL.min^{-1}) of solvent B (0.1% TFA in MeCN, v/v) in solvent A (0.1% TFA in H_2O, v/v). Detection was set at 220 and 254 nm. Fractions containing the desired products were freeze-dried to give the purified products **F-10** as a white foam (8.1 mg, 63%) as a mixture of four regio/stereoisomers observed by ^{31}P and ^{19}F NMR.

$^{19}F\{^1H\}$ NMR (471 MHz, DMSO) δ -76.55 (F-TFA), -222.33, -222.34, -222.35, -222.36 (F-cycloaducts); **$^{31}P\{^1H\}$ NMR** (202 MHz, DMSO) δ 20.33, 19.79, 19.43, 18.94 (22/9/20/49); **HRMS** (ESI-TOF): Calculated for $C_{44}H_{65}FN_6O_{10}PS_2$ $[M+H]^+$: 951.3925; Found: 951.3918.

2.12 Cycloaddition between 3 and F-8: Synthesis of F-11



11 (2 regioisomers & 2 stereoisomers)

Phosphonodithioester **3** (1 equiv, 31 mg, 0.048 mmol) was dissolved in *i*PrOH (0.715 mL) and diene **F-8** (1 equiv, 13 mg, 0.048 mmol) was dissolved in *i*PrOH (0.715 mL) and H_2O (3.4 mL). The diene solution was heated to 60 °C and the solution got clearer before the dithioester solution was added. The reaction was complete after 10 min (checked by HPLC). Isopropanol was evaporated and the aqueous crude solution was directly purified by semi-preparative reversed-phase HPLC chromatography (Gilson PLC2020 system) on a SunFire C18 column (5 µm, 19 × 150 mm) using a linear gradient (10% to 95% in 40 min, flow-rate of 18 mL.min^{-1}) of solvent B (0.1% TFA in MeCN, v/v) in solvent A (0.1% TFA in H_2O, v/v). Detection was set at 220 and 254 nm. Fractions containing the desired peptide were freeze-dried to give the purified products **F-11** as a white foam (27 mg, 64%) as a mixture of four regio/stereoisomers observed by ^{31}P and ^{19}F NMR.

^1H NMR (400 MHz, DMSO) δ 8.43 (q, *J* = 5.4 Hz, 1H), 7.78 (dd, *J* = 8.4, 2.1 Hz, 2H), 7.44 - 7.04 (m, 3H), 6.32 (t, *J* = 9.1 Hz, 2H), 4.72 - 2.96 (m, 19H), 2.37 - 1.41 (m, 18H), 1.39 - 1.22 (m, 8H); **$^{19}F\{^1H\}$ NMR** (376 MHz, DMSO) δ -221.00, -221.01, -221.03; **$^{31}P\{^1H\}$ NMR** (162 MHz, DMSO) δ 20.4, 19.8, 19.5, 18.9 (27:9:16:48 ratio); **HRMS** (ESI-TOF): Calculated for $C_{38}H_{57}FN_4O_{13}PS_2$ $[M+H]^+$: 891.3085; Found (2 fractions isolated): 891.3087, 891.3067.

Two fractions containing each one a pair of isomers were isolated for NMR analysis. Samples were too small for ^{13}C analysis.

Characteristic NMR signals of fraction 1:

Ratio Maj/min = 83/17

^{1}H NMR (400 MHz, DMSO) δ 8.43 (q, *J* = 5.4 Hz, 1H), 7.78 (dd, *J* = 8.4, 2.1 Hz, 2H), 7.39 (dd, *J* = 14.3, 8.3 Hz, 3H), 6.32 (t, *J* = 9.2 Hz, 2H), 4.73 - 2.85 (m, 19H), 2.30 - 1.47 (m, 18H), 1.28 (qd, *J* = 7.0, 1.2 Hz, 8H). **^{19}F{^{1}H} NMR** (376 MHz, DMSO) δ -221.00 (Maj), -221.03 (min); **^{31}P{^{1}H} NMR** (162 MHz, DMSO) δ 19.5 (min), 18.9 (Maj).

Characteristic NMR signals of fraction 2:

Ratio Maj/min = 70/30

^{1}H NMR (400 MHz, DMSO) δ 8.42 (t, *J* = 5.6 Hz, 1H), 7.82 - 7.71 (m, 2H), 7.44 - 7.05 (m, 3H), 6.32 (t, *J* = 9.1 Hz, 2H), 4.63 - 2.94 (m, 19H), 2.29 - 1.46 (m, 18H), 1.40 - 1.21 (m, 8H); **^{19}F{^{1}H} NMR** (376 MHz, DMSO) δ -221.00 (min), -221.03 (Maj); **^{31}P{^{1}H} NMR** (162 MHz, DMSO) δ 20.4 (Maj), 18.9 (min).

3 Theoretical kinetic study

This theoretical kinetic study was performed in classical conditions for radiochemistry with ^{18}F with a concentration of the fluorinated compound "**F**" of 5 µM with an excess of the dithioester partner "**P**" with 10 equivalents. In these conditions, equation (1) can become (2) by considering **[P]** as a constant leading after integration to equation (3).

$$\frac{d[F]}{dt} = k * [F] * [P] \quad (1)$$

$$\frac{d[F]}{dt} = k_{app} * [F] \; with \; k_{app} = k * [P]_0 \quad (2)$$

$$[F] = [F]_0 * \; e^{-k_{app} * t} \quad (3)$$

Theoretical conversions "C" were then calculated with equation (4) and plotted with different values for the second order rate constant:

$$C = \frac{[F]_0 - [F]}{[F]_0} = \; 1 - e^{-k_{app} * t} \quad (4)$$

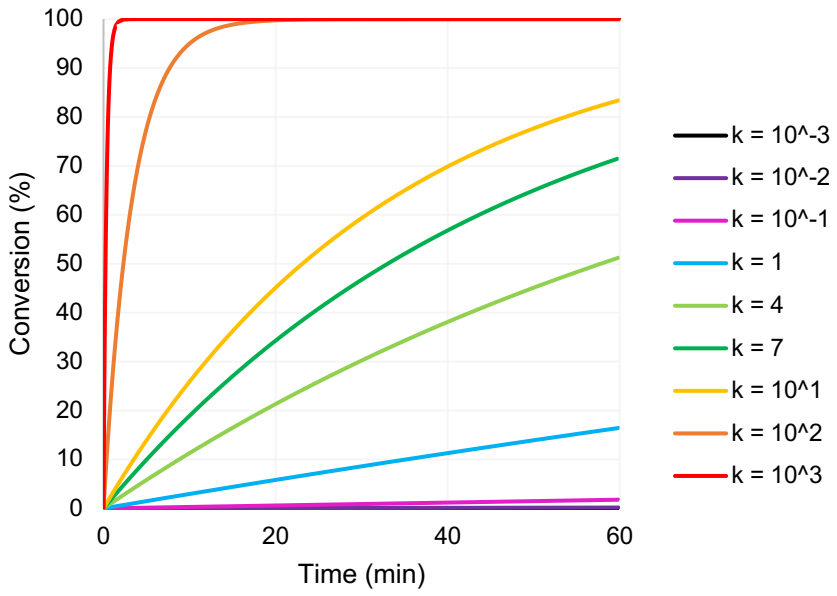


Figure S1. Evolution of the conversion in pseudo-first order reactions with classical conditions in radiochemistry (Concentration of 5 µM of ^{18}F-substrates and 10 equivalents of the partner).

4 Kinetic studies

For the kinetics studies, we chose to use stoichiometric ratios between starting materials. In these conditions, the kinetics follows the subsequent law with [R] being the concentration of one starting material and k being the second order rate constant:

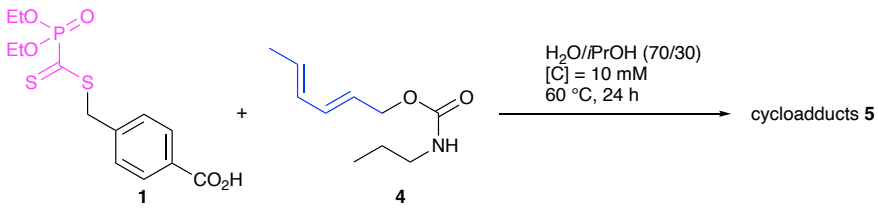
$$-\frac{d[R]}{dt} = k * [R]^2$$

Through integration of the previous expression, we can obtain the following equation:

$$\frac{1}{[R]} = \frac{1}{[R]_0} + kt$$

The second order reaction rate can thus be obtained with a plot of the inverse concentration (1/[R]) against time as the slope of the line of best fit. The kinetics of each reaction was followed by UV-vis absorption at the corresponding wavelength.

For the acyclic diene **4**, a typical reaction was set up in a round-bottom flask. Acyclic diene **4** (0.1 mmol) was dissolved in *i*PrOH (1.5 mL) and H_2O (7 mL). This solution was heated up to 60 °C. A solution of dithioester **1** (0.1 mmol) in *i*PrOH (1.5 mL) was then added. The absorption at 530 nm corresponding to the n to π* transition of the dithioester was measured over time with ε = 24 $M^{-1}.cm^{-1}$. Samples were collected regularly from the solution and were kept warm to avoid precipitation of the adducts.



Scheme S1. HDA reaction for kinetic study using acyclic diene **4**.

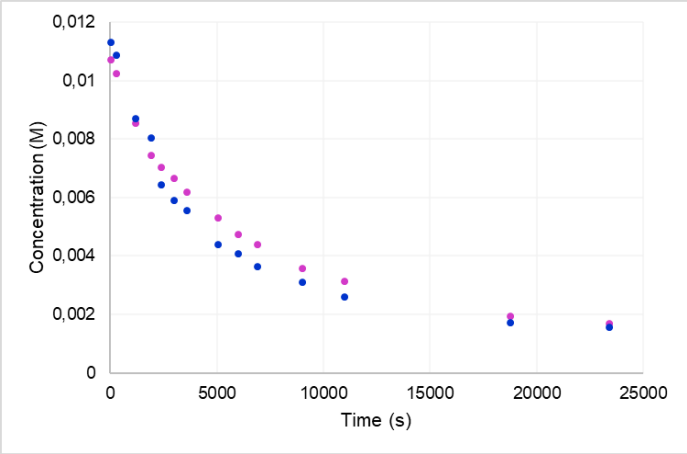


Figure S2. Evolution of the concentration of dithioester **1** over time with the acyclic diene **4** (n = 2).

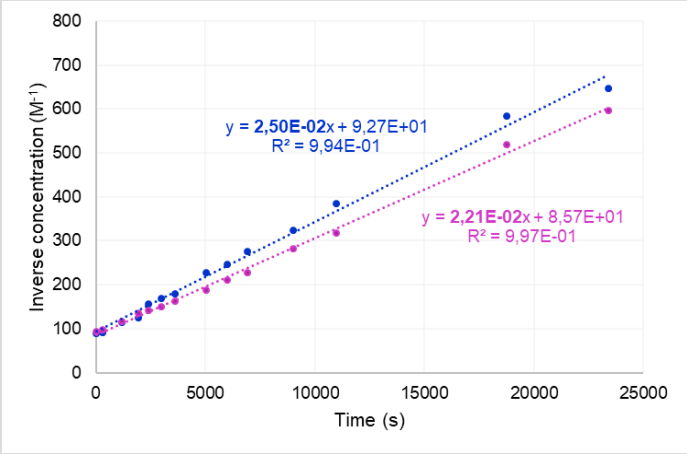
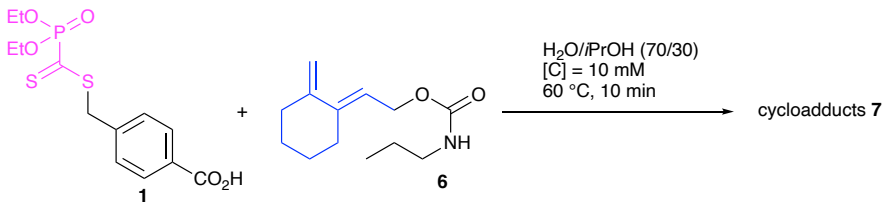


Figure S3. Determination of the second order rate constant for the acyclic diene **4** in H_2O/*i*PrOH (7/3) (n = 2).

For the cyclic diene **6**, a typical reaction was set up in a round-bottom flask. 100 µL of a 22 mM solution of cyclic diene **6** (22 µmol in 1 mL of *i*PrOH) was added in a 7/3 solution of H_2O/*i*PrOH (22 mL). This solution was heated up to 60 °C. Then 100 µL of a 22 mM solution of dithioester **1** (22 µmol in 1 mL of *i*PrOH) was then added. The absorption at 330 nm corresponding to the π to π* transition of the dithioester was measured over time with ε = 10500 $M^{-1}.cm^{-1}$. Samples were collected regularly from the solution and were kept warm to avoid precipitation of the adducts.



Scheme S2. HDA reaction for kinetic study using cyclic diene **6**.

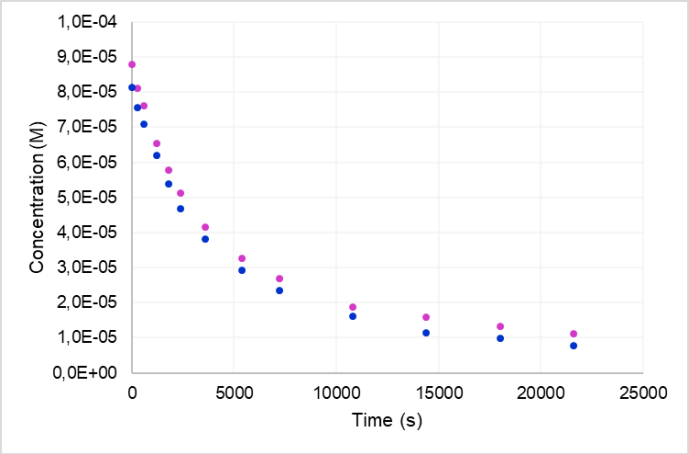


Figure S4. Evolution of the concentration of dithioester **1** over time with cyclic diene **6** (n = 2).

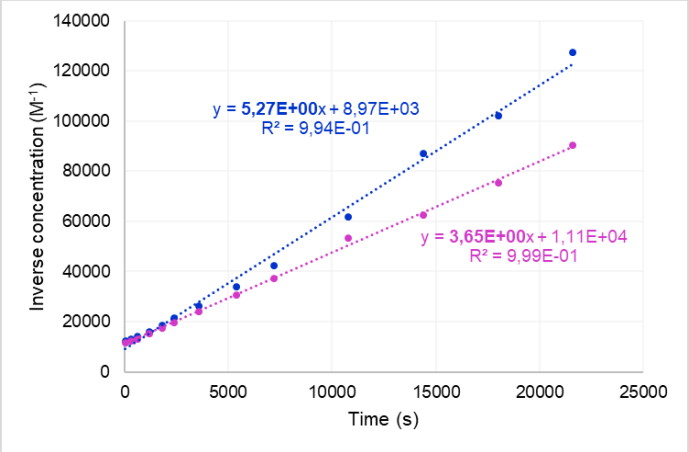


Figure S5. Determination of the second order rate constant for the cyclic diene **6** in H_2O/*i*PrOH (7/3) (n = 2).

5 Radiochemistry

5.1 General materials and methods

All reagents for radiochemistry were used without further purification.

K_2CO_3 99.99% and Kryptofix $K_{2.2.2}$ (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo-[8.8.8]-hexacosane), anhydrous acetonitrile 99.8%, ethanol (absolute, HPLC grade), propan-2-ol (HPLC grade), hydrochloric acid, trifluoroacetic acid (HPLC grade), sodium acetate (analytical grade) and acetic acid (HPLC grade) were purchased from Merck. Accell plus QMA carbonate light cartridges were obtained from Waters (130 mg sorbent, Part No 186004051) and used as received, C18 SepPak (tC18 environmental WAT 036800 and C18 WAT 020515) were purchased from Waters and pre-conditioned with 5 mL of MeCN or 5 mL of ethanol followed by 10 mL of pure H_2O before use.

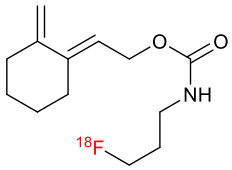
[^{18}O]H_2O ([^{18}O] 98%) for [^{18}F]fluoride production was purchased from Nukem isotopes GmbH-Germany.
Pure H_20 (18.2 MΩ) was produced with a Purelab option Q purification system (Veolia®).
Sodium chloride 0.9% sterile solution was purchased from B BRAUN medical.

HPLC Dionex® U3000 equipped with a UV-Vis DAD detector, a radioactivity detector (NaI) and a 20 µL injection loop was used for radio-chemical conversion determination, quality control and molar activity determination (columns, eluents and gradients are reported in each experimental section). The radioactive detector is placed after the UV-Vis DAD detector generating a delay (function of tubing lengh and flow) between the two signals. The difference observed between the shape of radioactive signals versus UV-Vis detection is due to the difference in size of the detection cells and the rate of acquisition of both detector (1 Hz for the radioactive channel versus 5Hz for UV-Vis channel)

[^{18}F]fluoride production was performed with an ACSI® 24 MeV cyclotron by proton irradiation of a 1mL volume niobium target at an energy of 16.5 MeV and an intensity of 35 µA. After 5 min of cooling the radioactivity was transferred to the hot cell under He pressure then the transfer lines and the target were rinsed twice (2x1 mL) with pure water. Total transferred activity was measured (well counter in the hot cell) and further transferred to the Raytest module reception vial under He pressure. Residual activity in the intermediate vial after transfer was counted to determine the activity used in each radiosynthesis (a typical activity of 23-24 GBq for a 11 µA.h irradiation was transferred in the hot cell).

Automated synthesis
A Raytest R&D synchrom dual reactor was used for the automated radiosynthesis with some modifications. Reactor 1 is used for the synthesis of the prosthetic group ([^{18}**F]-8**) and the second reactor is used for the Thia-Diels-Alder reaction. Outlet of the pump and vents are connected to gas bags to avoid any radioactive releases in the hot cell ventilation system, 3 additional valves (G3-G5) and a 12 mL vial were added to measure the ^{18}F activity transferred from the target. The system is equipped with a semi preparative HPLC (Knauer) including an isocratic pump, a 254 nm fixed wavelength UV detector, a radioactivity detector and a 5 mL stainless steel injection loop. Purification of **[18F]-8** was done on a syncronis 250x10 mm (5 µm) semi-preparative column.
For the synthesis of **[^{18}F]-11** a second HPLC purification on a semi-preparative Kinetex column was necessary. An USB remotely controlled 6 ways-2 positions Titan MX II valve (Rheodyne) was inserted as a column selector enabling to shift between the two different columns. Injection loop and tubing common for the two purifications processes are washed automatically with MeCN after use. For the purification of **[^{18}F]-11** a gradient was obtained by adding an automated injection pump (KdScientific) performing a direct injection of MeCN at a flow rate of 3.5 mL/min in the solvent bottle (50 mL, MeCN/H_2O+0.1%TFA 20/80 V/V) with constant stirring. A second dilution flask (50 mL falcon tube) was added for the dilution of the final product in water before C18 seppak formulation.

5.2 Synthesis of diene [^{18}F]-8

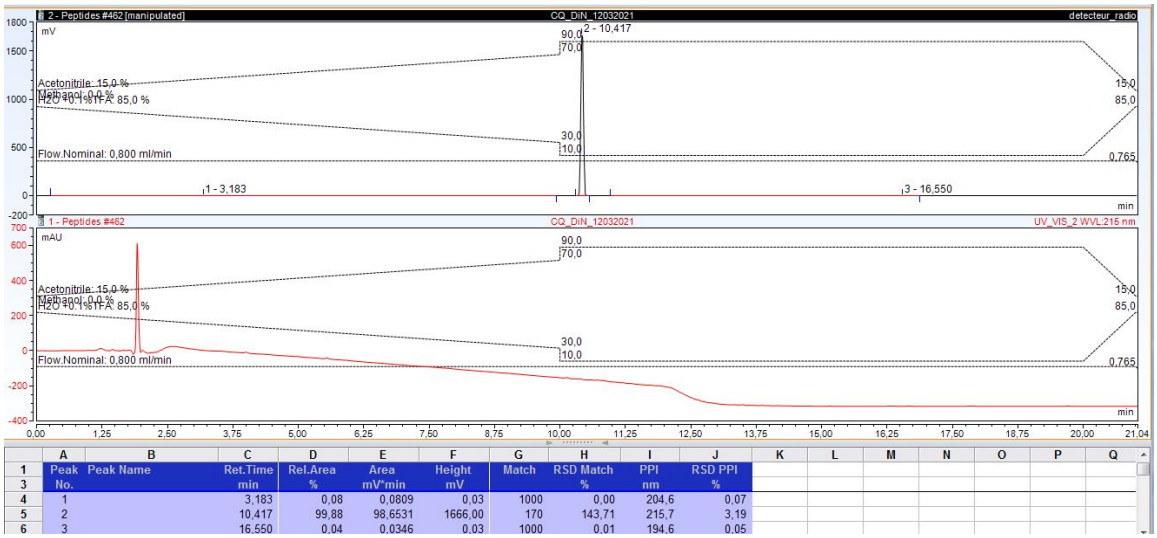


[^{18}F]-8

Automated synthesis was done on a Raytest R&D Synchrom EVO III module. [^{18}F]fluoride production was performed with an ACSI® 24 MeV cyclotron by proton irradiation of a 1mL volume ([^{18}O]H_2O >97%) niobium target at an energy of 16.5 MeV and a beam intensity of 35 µA. The radioactivity was transferred to the hot cell under He pressure then the transfer lines and the target were rinsed twice (2x1 mL) with pure water. Total transferred activity was measured (well counter in the hot cell) and further transferred to the Raytest module reception vial under He pressure. Residual activity in the intermediate vial after transfer was counted to determine the activity used in each radiosynthesis (a typical activity of 23 GBq for a 11 µA.h irradiation was transferred in the hot cell).

The activity (22.4-23.0 GBq) received in the intermediate vial was transferred onto the automated module and was then trapped on a QMA cartridge (Waters Sep-Pak Light Accell plus QMA). After drying, the [^{18}F]fluoride was eluted into reactor 1 with a solution of $K_{2.2.2}$ (Aldrich, 14 mg in 800 µl of MeCN) and K_2CO_3 (Aldrich 0,59 mg in 360 µl of water). The solution was evaporated under reduced pressure and an argon flow at 90 °C with iterative additions of MeCN (3 times, total volume used 1.7 mL). After evaporation, tosylate TsO-**8** (5.0 mg, 12-13 µmol) in 1 mL of MeCN was added and the sealed reactor was heated at 95 °C for 10 min. After cooling to 45 °C, the crude solution was diluted with H_2O (2.2 mL) and MeCN (2 mL). The whole solution was injected in HPLC (5 mL injection loop) and purified on a Syncronis C18 column (Thermo scientific, 250x10 mm, 5 µm) using MeCN/H_2O (60/40 V/V) at a flow rate of 4 mL/min (conversion according to semi-preparative HPLC on radioactive channel = 90%, R_t = 14'50'').

The collected solution (3 min, 12 mL) was diluted in 40 mL of water and passed through two stacked C18 cartridges (tC18 environmental WAT 036800 and C18 WAT 020515, WATERS). The cartridges were then washed with 3 mL of water to remove the solvents and dried under Argon flow. The purified product [^{18}F]-**8** was eluted with 4 mL of alcohol (*i*PrOH or EtOH HPLC grade) for further use in manual synthesis.
The mean molar activity (at the end of synthesis) was determined to be 150 GBq/µmol (determination was done by analytical HPLC with a calibration curve at 215 nm). [^{18}F]-**8** was obtained with a radiochemical yield of 70% (decay corrected) and a total synthesis time of 52 min.
Analytical HPLC was performed on a C18 Kinetex (Phenomenex, 5 µm EVO 4.6x150 mm) using a gradient of MeCN/H_2O+0.1%TFA from 15/85 V/V to 70/30 in 10 min then 90/10 V/V during 15 min at a flow rate of 0.8 mL/min. Rt = 10.47 min.



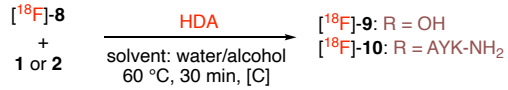
Peak No.	Peak Name	Ret.Time min	Rel.Area %	Area mV*min	Height mV	Match	RSD Match %	PPI nm	RSD PPI %
1		3,183	0,08	0,0809	0,03	1000	0,00	204,6	0,07
2		10,417	99,88	98,6531	1666,00	170	143,71	215,7	3,19
3		16,550	0,04	0,0346	0,03	1000	0,01	194,6	0,05

Figure S6. Analytical HPLC of **[^{18}F]-8** at the end of synthesis. Top radioactive channel and bottom UV channel at 215 nm. Integration table refers to the radioactive channel

5.3 Manual syntheses of [^{18}F]-9 and [^{18}F]-10 (Table S1)

For all manual synthesis a batch of diene [^{18}F]-**8** was produced on large scale (starting from 23 GBq of [^{18}F]fluoride) using the automated described method. A solution of [^{18}F]-**8** (9-10 GBq) in H_2O/*i*PrOH (70/30 V/V, 5.15-5.25 mL, 1.7-1.9 GBq/mL at the end of synthesis) was obtained after purification and formulation. From this solution samples were used for molar activity (A_m, GBq/□mol) determination and quality control. For each manual reaction 20 µL of the solution were used ensuring a constant amount of diene [^{18}F]-**8** in each reaction. The amount of diene (0.2-0.3 nmol in each aliquot) was calculated using the pre-determined molar activity and the activity (measured in a calibrated well counter) of a 20 µL solution of [^{18}F]-**8**.
Manual syntheses were performed in 2 mL Eppendorf tubes. Reactions were carried out at 60-63 °C, samples were heated using a stirring heating bath during 30 min. For entries 1-2 ([^{18}F]-**9**) the total reaction volume was ajusted to 50 µL, 200 µL for entries 3-4 ([^{18}F]-**10**) and 70 µL for entry 5 ([^{18}F]-**10**). Dithioester **1** was dissolved in *i*PrOH and dithioester **2** was dissolved in water containing 0.1% AcOH. [^{18}F]-**8** was dissolved in H_2O/*i*PrOH (for entries 1-2 and H_2O/EtOH (for entries 3-5). The total reaction volume was adjusted by addition of water containing 0.1% AcOH and the appropriate alcohol (*i*PrOH or EtOH) to reach the total volume and the correct ratio of solvents. Monitoring of reaction was performed by C18 analytical HPLC using the following conditions.
[^{18}F]-**9**: C18 column Acclaim 120, 3 µm 4.6x150 mm (Thermo scientific) using a gradient of MeCN in AcONa 50 mM from 5/95 V/V to 65/35 V/V in 20 min then 65/35 V/V during 10 min at a flow rate of 0.8 mL/min. Rt = 8.35 min.
[^{18}F]-**10**: C18 column Kinetex, 5□µm 4.6x150 mm (Phenomenex) using a gradient of MeCN in H_2O+0.1%TFA from 15/85 V/V to 70/30 in 10 min then 90/10 V/V during 15 min at a flow rate of 0.8 mL/min. Rt = 7.40 min.

Table S1. Radiofluorination via thia-Diels-Alder reactions with diene [^{18}F]-**8**.



Entry	Substrate (nmol); [C] (µM)[d]	water/ alcohol	Product	product/ [^{18}F]-**8**[e]
1[a]	**1** (30); 600	70/30[b]	[^{18}F]-**9**	100/0
2[a]	**1** (3); 60	70/30[b]	[^{18}F]-**9**	83/17
3[a]	**2** (3); 15	80/20[c]	[^{18}F]-**10**	10/90
4[a]	**2** (3); 15	60/40[c]	[^{18}F]-**10**	0/100
5[a]	**2** (3); 45	70/30[c]	[^{18}F]-**10**	77/23

[a] Manual synthesis with a constant amount of diene, analyzed after 30 min. [b] Ratio water/isopropanol. [c] Ratio water/ethanol. [d] The volume of the solvent was adjusted to the amount of dithioester. [e] Ratio measured by HPLC on the radioactive channel.

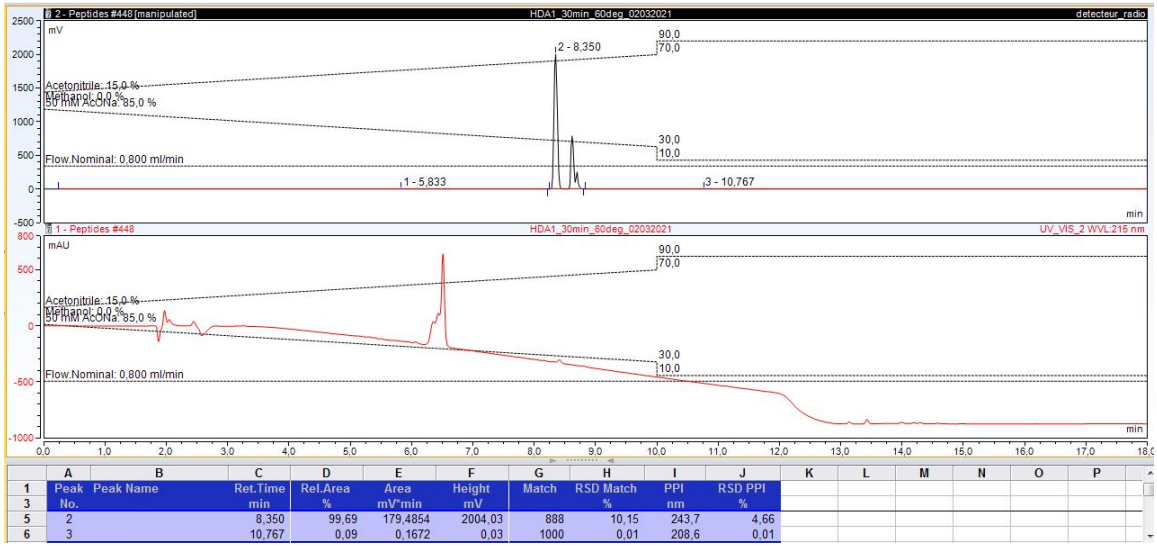


Figure S7. HPLC analysis (Table 1, entry 1) with a radioactive detector (top) and a 215 nm UV detector (bottom). Adducts [^{18}F]-**9** can be seen at 8.35 min.

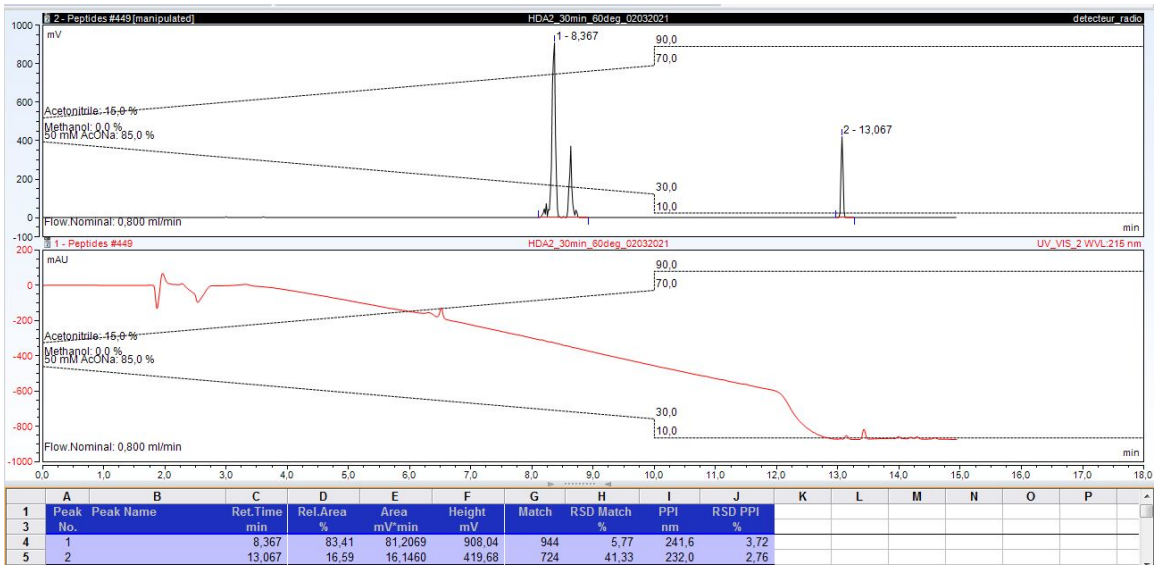
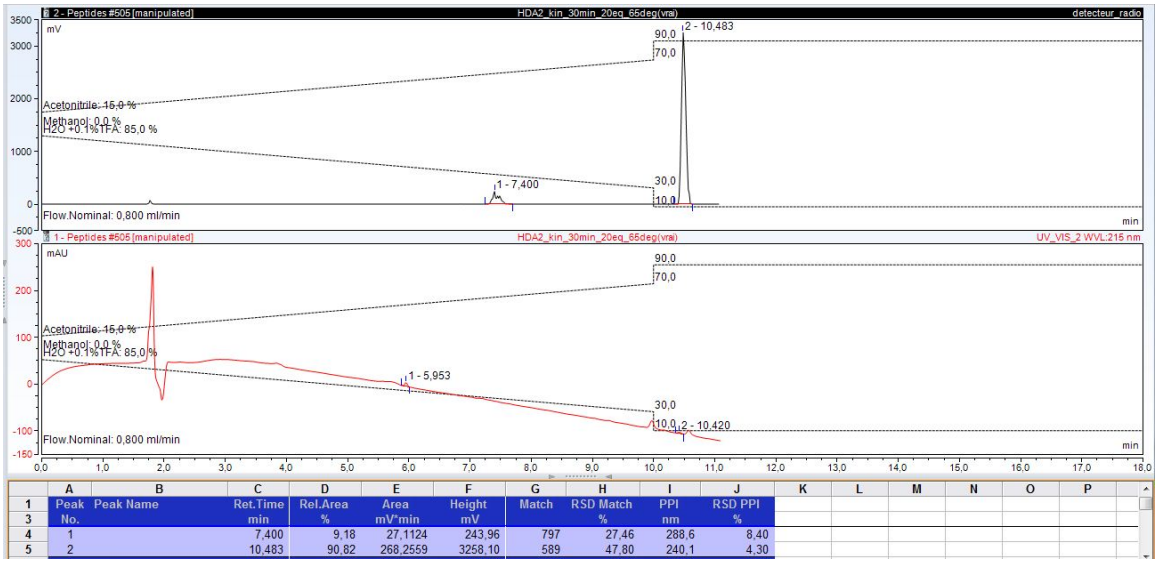
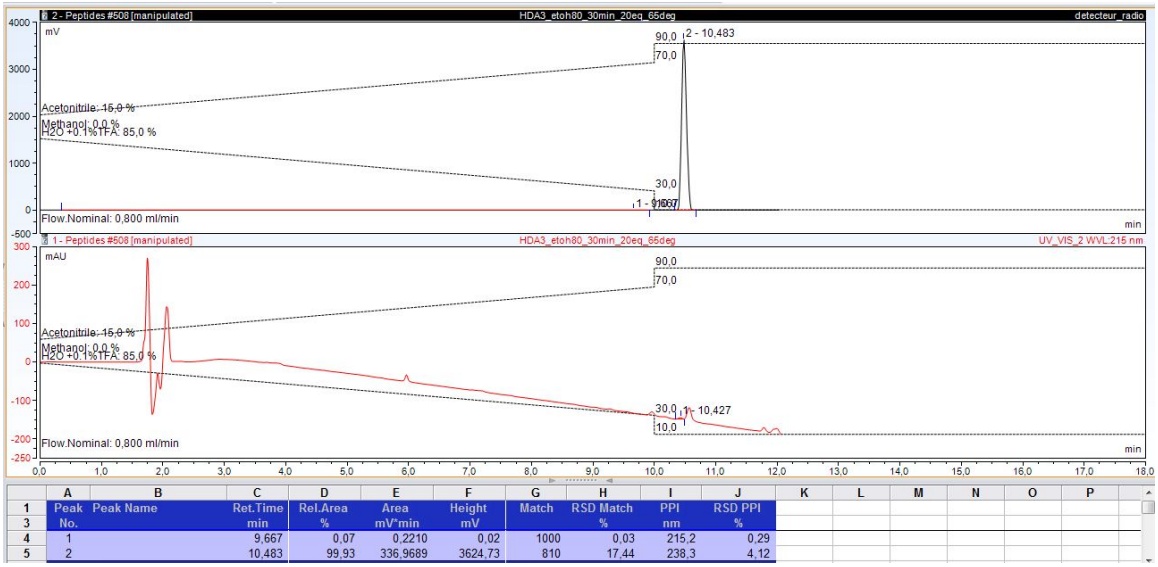


Figure S8. HPLC analysis (Table 1, entry 2) with a radioactive detector (top) and a 215 nm UV detector (bottom). Adducts [^{18}F]-**9** can be seen at 8.37 min while diene [^{18}F]-**8** can be seen at 13.07 min.



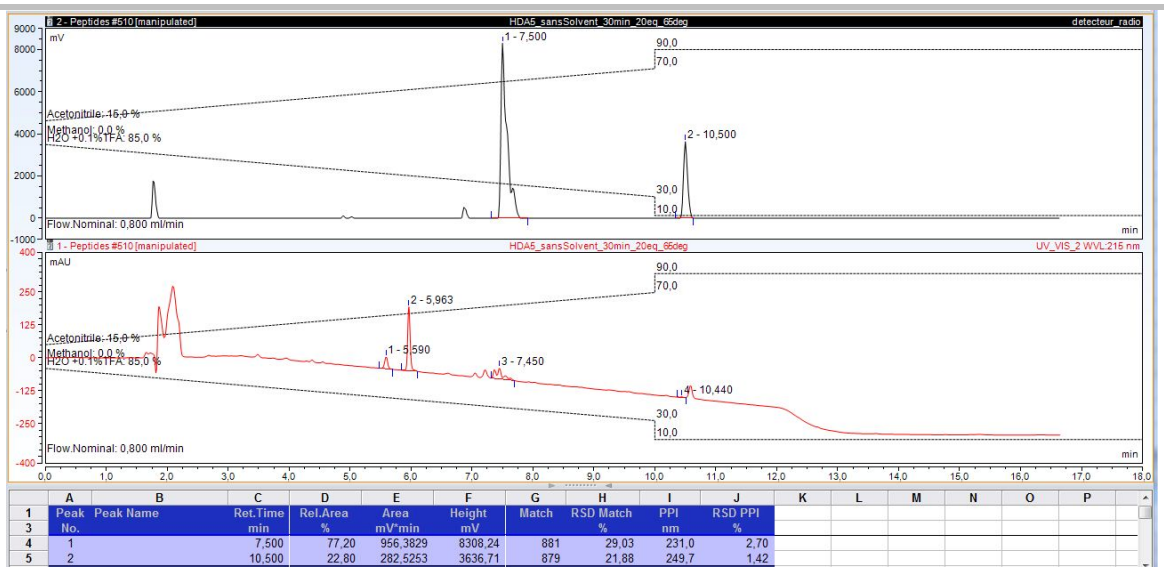
Peak No.	Peak Name	Ret.Time min	Rel.Area %	Area mV*min	Height mV	Match	RSD Match %	PPI nm	RSD PPI %
1		7,400	9,18	27,1124	243,96	797	27,46	288,6	8,40
2		10,483	90,82	268,2559	3258,10	589	47,80	240,1	4,30

Figure S9. HPLC analysis (Table 1, entry 3) with a radioactive detector (top) and a 215 nm UV detector (bottom). Adducts [^{18}F]-**10** can be seen at 7.40 min while diene [^{18}F]-**8** can be seen at 10.48 min.



Peak No.	Peak Name	Ret.Time min	Rel.Area %	Area mV*min	Height mV	Match	RSD Match %	PPI nm	RSD PPI %
1		9,667	0,07	0,2210	0,02	1000	0,03	215,2	0,29
2		10,483	99,93	336,9689	3624,73	810	17,44	238,3	4,12

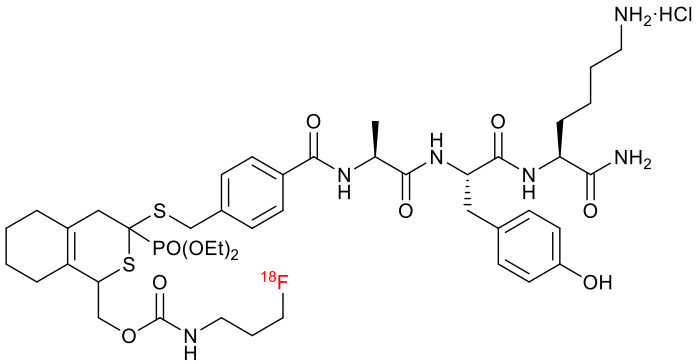
Figure S10. HPLC analysis (Table 1, entry 4) with a radioactive detector (top) and a 215 nm UV detector (bottom). Diene [^{18}F]-**8** can be seen at 10.48 min.



Peak No.	Peak Name	Ret.Time min	Rel.Area %	Area mV*min	Height mV	Match	RSD Match %	PPI nm	RSD PPI %
1		7,500	77,20	956,3829	8308,24	881	29,03	231,0	2,70
2		10,500	22,80	282,5253	3636,71	879	21,88	249,7	1,42

Figure S11. HPLC analysis (Table 1, entry 5) with a radioactive detector (top) and a 215 nm UV detector (bottom). Adducts [^{18}F]-**10** can be seen at 7.50 min while diene [^{18}F]-**8** can be seen at 10.50 min.

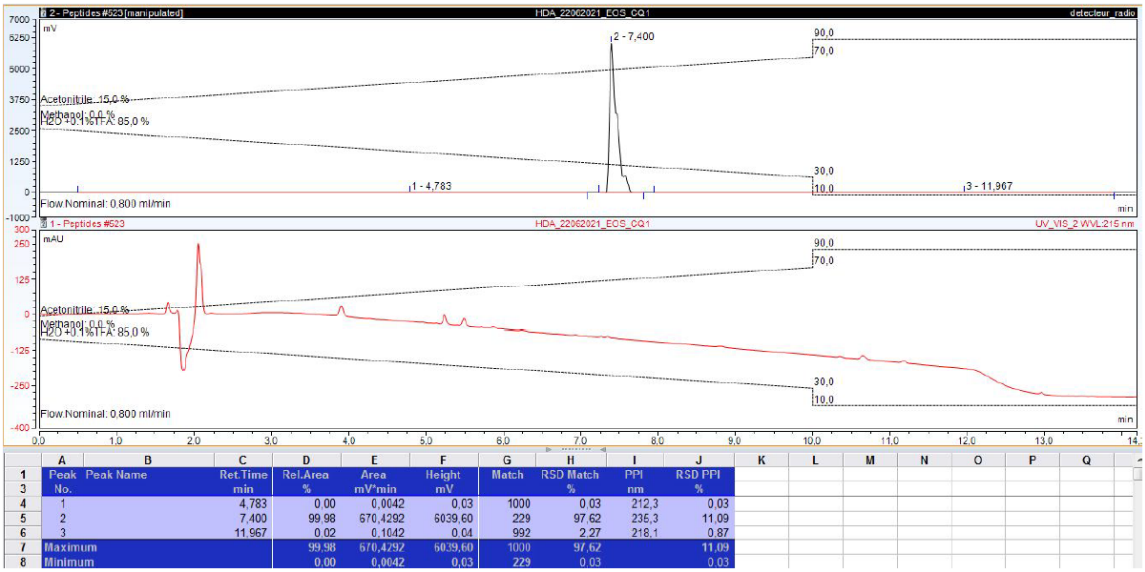
5.4 Automated synthesis of radiolabeled tripeptide [^{18}F]-10



[^{18}F]-10

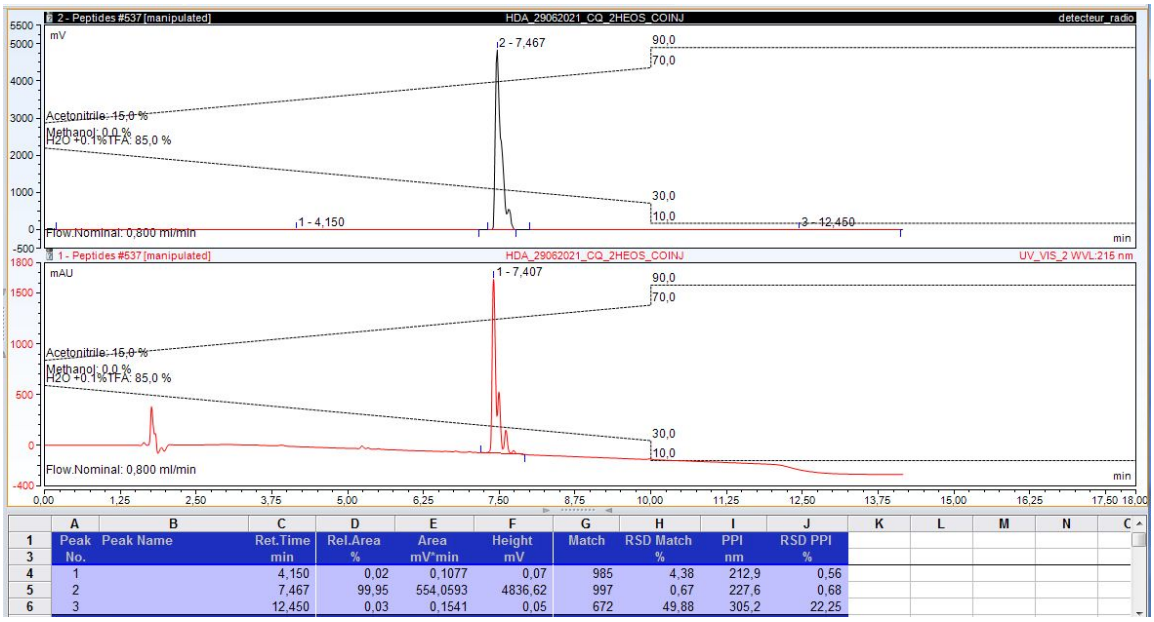
The automated synthesis was done on a Raytest R&D Synchrom EVO III module. [^{18}F]fluoride was produced by an ACSI® 24 MeV cyclotron by proton irradiation of a 1mL volume niobium target ([^{18}O]H_2O >97%) at an energy of 16.5 MeV and a beam intensity of 35 µA. The radioactivity was transferred to the hot cell under He pressure then the transfer lines and the target were rinsed twice (2x1 mL) with pure water. Total transferred activity was measured (well counter in the hot cell) and further transferred to the Raytest module reception vial under He pressure. Residual activity in the intermediate vial after transfer was counted to determine the activity used in each radiosynthesis (a typical activity of 23 GBq for a 11 µA.h irradiation was transferred in the hot cell).
The total activity (23 GBq) received in the intermediate vial was transferred onto the automated module and was trapped on a QMA cartridge (Waters Sep-Pak Light Accell plus QMA). After drying, the [^{18}F]fluoride was eluted into reactor 1 with a solution of $K_{2.2.2}$ (Aldrich, 14 mg in 800 µl of MeCN) and K_2CO_3 (Aldrich 0,59 mg in 360 µl of water). The solution was evaporated under reduced pressure and an argon flow at 90 °C with iterative additions of MeCN (3 times, total volume used 1.7 mL). After evaporation, tosylate TsO-**8** (5.0 mg, 12.5 µmol) in 1 mL of MeCN was added and the sealed reactor was heated at 95 °C for 10 min. After cooling to 45 °C, the crude solution was diluted with H_2O (2.1 mL) and MeCN (2 mL). The whole solution was injected in HPLC (5 mL injection loop) and purified on a Syncronis C18 column (Thermo scientific, 250x10 mm, 5 µm) using MeCN/H_2O (60/40 V/V) at a flow rate of 4 mL/min (conversion according to semi-preparative HPLC on radioactive channel = 90%, R_t = 15'05''). The collected solution (3 min, 12 mL) was diluted in 38 mL of water and passed through two stacked C18 cartridges (tC18 environmental WAT 036800 and C18 WAT 020515, WATERS). The cartridges were then washed with 3 mL of water to remove the solvents and dried under Argon flow. The purified product [^{18}F]-**8** was eluted with 1.85 mL of EtOH (HPLC grade) in the pre-heated (65 °C) second reactor.
After addition of tripeptide-dithioester **2** (0.6 mg, 0.7 µmol) in 4.2 mL of water (containing 0.15% AcOH) the reactor was set under pressure (Argon, 1800 mBar) and close. The solution was stirred and heated 45 min at 65 °C. The

solution was diluted with 12 mL of water, passed over a C18 cartridge (C18 WAT 020515, Waters) and after short drying the cartridge was washed with 2.5 mL of diethyl ether, dried under Argon pressure and washed again with 7.5 mL of H_2O/EtOH 95/5. The final product was eluted with a solution of ethanol (1.15 mL) and HCl 0.2M (250 µl) in a vial containing 4 mL of water. A radiochemical yield of 26.5% (54% decay corrected yield from start of synthesis SOS) was obtained after 112 min of synthesis (including the synthesis of [^{18}F]-**8**, purification and formulation) and with a radioactive purity above 99% (analytical HPLC). The molar activity (end of synthesis) was determined to be 78 GBq/µmol (±12 GBq/µmol, n = 2) using a calibration curve at 215 nm. Purified product was analyzed by HPLC with a C18 Kinetex, 5 µm 4.6x150 mm (Phenomenex) using a gradient of MeCN in H_2O+0.1%TFA from 15/85 V/V to 70/30 in 10 min then 90/10 V/V during 15 min at a flow rate of 0.8 mL/min. Rt = 7.40 min.



Peak No.	Peak Name	Ret.Time min	Rel.Area %	Area mV*min	Height mV	Match	RSD Match %	PPI nm	RSD PPI %
1		4,783	0,00	0,0042	0,03	1000	0,03	212,3	0,03
2		7,400	99,98	670,4292	6039,60	229	97,62	236,3	11,09
3		11,967	0,02	0,1042	0,04	992	2,27	218,1	0,87
Maximum			99,98	670,4292	6039,60	1000	97,62		11,09
Minimum			0,00	0,0042	0,03	229	0,03		0,03

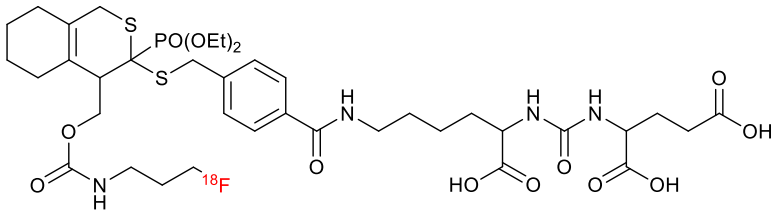
Figure S12. HPLC analysis of the adducts [^{18}F]-**10** with a radioactive detector (top) and a 215 nm UV detector (bottom). The radioactive purity was obtained by integration of the product compared with the rest of the chromatogram.



Peak No.	Peak Name	Ret.Time min	Rel.Area %	Area mV*min	Height mV	Match	RSD Match %	PPI nm	RSD PPI %
1		4,150	0,02	0,1077	0,07	985	4,38	212,9	0,56
2		7,467	99,95	554,0593	4836,62	997	0,67	227,6	0,68
3		12,450	0,03	0,1541	0,05	672	49,88	305,2	22,25

Figure S13. HPLC analysis of the co-injection of authentic non-radioactive reference **F-10** and adducts [^{18}F]-**10** with a radioactive detector (top) and a 215 nm UV detector (bottom).

5.5 Automated Synthesis of radiolabeled PSMA [^{18}F]-11



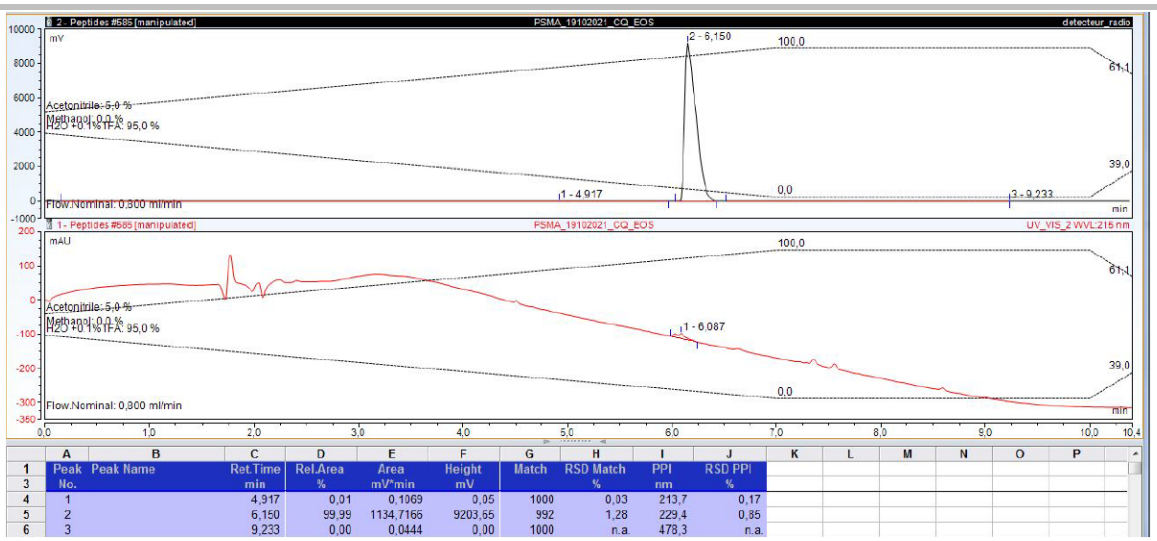
[^{18}F]-11

The fully automated synthesis of PSMA [^{18}F]-**11** was done on a Raytest R&D Synchrom EVO III module. [^{18}F]fluoride was produced by an ACSI® 24 MeV cyclotron by proton irradiation of enriched water ([^{18}O]H_2O >97%) in a 1mL volume niobium target at an energy of 16.5 MeV and a beam intensity of 35 µA. The radioactivity was transferred to the hot cell under He pressure then the transfer lines and the target were rinsed twice (2x1 mL) with pure water. Total transferred activity was measured (well counter in the hot cell) and further transferred to the Raytest module reception vial under He pressure. Residual activity in the intermediate vial after transfer was counted to determine the activity used in each radiosynthesis (a typical activity of 23 GBq for a 11 µA.h irradiation was transferred in the hot cell).

The total activity (23 GBq) received in the intermediate vial was transferred onto the automated module and was trapped on a QMA cartridge (Waters Sep-Pak Light Accell plus QMA). After drying, the [^{18}F]fluoride was eluted into reactor 1 with a solution of $K_{2.2.2}$ (Aldrich, 12 mg in 800 µl of MeCN) and K_2CO_3 (Aldrich 0,59 mg in 360 µl of water). The solution was evaporated under reduced pressure and an argon flow at 90 °C with iterative additions of MeCN (3 times, total volume used 1.7 mL). After evaporation, tosylate TsO-**8** (5.0 mg, 12.5 µmol) in 1 mL of MeCN was added and the sealed reactor was heated at 95 °C for 10 min. After cooling to 45 °C, the crude solution was diluted with H_2O (2.15 mL) and MeCN (1.9 mL). The whole solution was injected in HPLC (5 mL injection loop) and purified on a Syncronis C18 column (Thermo scientific, 250x10 mm, 5 µm) using MeCN/H_2O (60/40 V/V) at a flow rate of 4 mL/min (conversion according to semi-preparative HPLC on radioactive channel = 91%, R_t = 15'24''). The collected solution (4 min, 16 mL) was diluted in 38 mL of water and passed through two stacked C18 cartridges (tC18 environmental WAT 036800 and C18 WAT 020515, WATERS). The cartridges were then washed with 3 mL of water to remove the solvents and dried under Argon flow. The purified product [^{18}F]-**8** was eluted with 1.70 mL of EtOH (HPLC grade) in the pre-heated (65 °C) second reactor.

PSMA-dithioester **3** (1+/- 0.1 mg, 1.5 µmol) in 3.275 mL of water (containing 0.15% AcOH) and 75 µL of EtOH, was added and the reactor was set under pressure (Argon, 1800 mBar) and closed. The solution was stirred and heated 45 min at 62-65 °C. After cooling at 50 °C the solution was injected in HPLC (5 mL injection loop) and purified on a Kinetex EVO C18 column (Thermo scientific, 150x10 mm, 5 µm) using H_2O/MeCN + 0.1% TFA (80/20 v/v) at a flow rate of 4 mL/min with an increasing gradient of MeCN (addition 3.75 mL/min over 10 min with mixing). The purified product (Rt = 11 min, 4 mL) was collected and diluted in 30 mL of water and passed over a C18 cartridge (C18 WAT 020515, WATERS). The cartridge was washed with 5 mL of water to remove solvents and TFA and then dried before elution with 1 mL of ethanol. The collected fraction was further diluted with NaCl 0.9% (9 mL passed over the C18 seppak cartridge). A radiochemical yield of 20.5% (47% decay corrected yield from start of synthesis SOS) was obtained after 125 min of synthesis (including the synthesis of [^{18}F]-**8**, purification and formulation) and with a radioactive purity above 99% (analytical HPLC). The mean molar activity (end of synthesis, n = 3) was determined to be 80 ± 20 GBq/µmol using a calibration curve at 254 nm.

Analytical HPLC of **[^{18}F]-11** was performed on C18 Kinetex column (5 µm 4.6x150 mm, Phenomenex) using a gradient of MeCN in H_2O+0.1%TFA from 5/95 V/V to 100/0 in 7 min then 100/0 V/V during 5 min at a flow rate of 0.8 mL/min. Rt = 6.15 min.



	Peak No.	Peak Name	Ret.Time min	Rel.Area %	Area mV*min	Height mV	Match	RSD Match %	PPI nm	RSD PPI %
4	1		4,917	0,01	0,1069	0,05	1000	0,03	213,7	0,17
5	2		6,150	99,99	1134,7166	9203,55	992	1,28	229,4	0,85
6	3		9,233	0,00	0,0444	0,00	1000	n.a.	478,3	n.a.

Figure S14. HPLC analysis of the adducts [^{18}F]-**11** with a radioactive detector (top) and a 215 nm UV detector (bottom). The radioactive purity (> 99%) was obtained by integration of the product compared with the rest of the chromatogram.

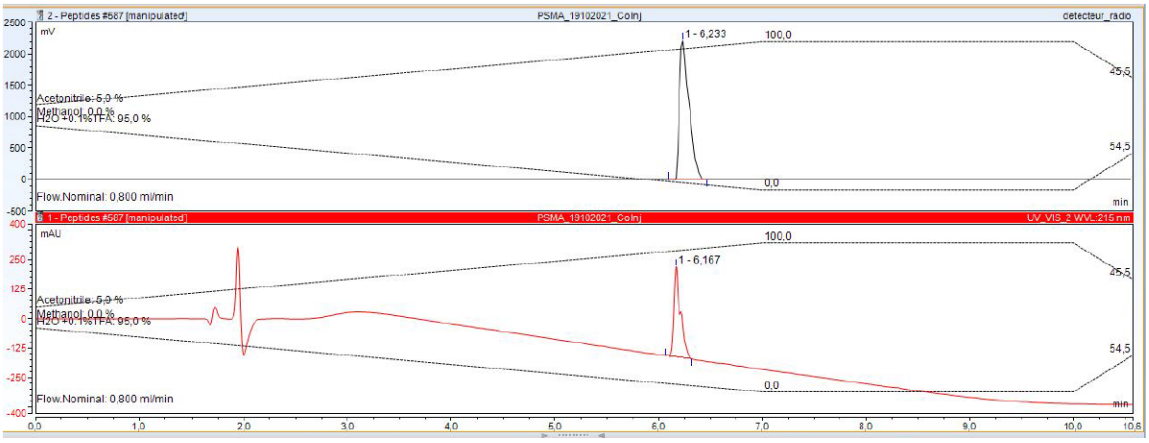
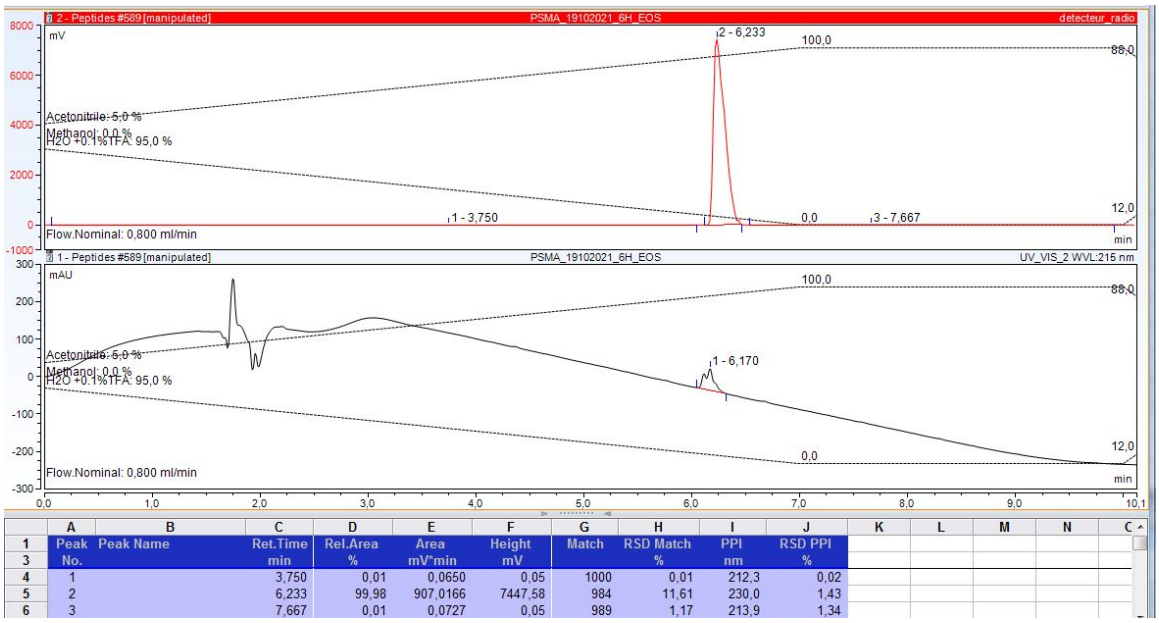


Figure S15. HPLC analysis of the co-injection of the authentic non radioactive reference **F-11** and adducts [^{18}F]-**11** with a radioactive detector (top) and a 215 nm UV detector (bottom).



	Peak No.	Peak Name	Ret.Time min	Rel.Area %	Area mV*min	Height mV	Match	RSD Match %	PPI nm	RSD PPI %
4	1		3,750	0,01	0,0650	0,05	1000	0,01	212,3	0,02
5	2		6,233	99,98	907,0166	7447,58	984	11,61	230,0	1,43
6	3		7,667	0,01	0,0727	0,05	989	1,17	213,9	1,34

Figure S16. HPLC analysis of the adducts [^{18}F]-**11** 6h after the end of the synthesis with a radioactive detector (top) and a 215 nm UV detector (bottom). The radioactive purity (> 99%) was obtained by integration of the product compared with the rest of the chromatogram.

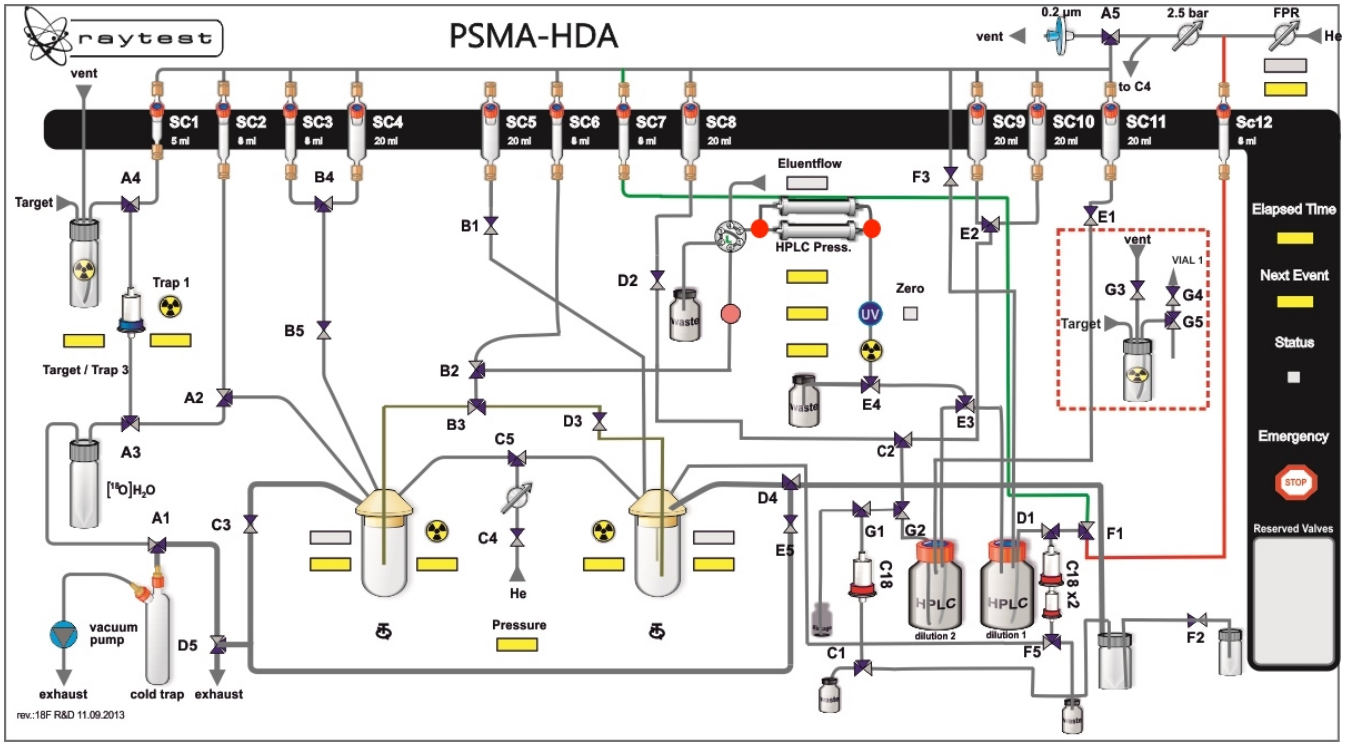


Figure S17. Set-up of the Raytest R&D Synchrom EVO III module for the radiosynthesis of [^{18}F]-**11**. The red dots figure the additional remotely controlled valve enabling use of 2 HPLC columns on the same set up. The red square figures the set up for the collect and counting of the [^{18}F]fluoride from the target.

5.6 Cellular study and PET imaging

All *in vivo* procedures were carried out following approved experimental protocols and guidelines for the well-being and use of animals and were approved by the Strasbourg ethics committee for animal experimentation and the French ministry of research (CREMEAS, Strasbourg, France, Apafis #9919).
Animals were housed with constant temperature (22 °C) and humidity (40%) and a 12-h light-dark cycles. They were allowed free access to food and water until the beginning of the imaging procedure.
4-week-old, athymic nude male mice (NMRI-Foxn1 nu/nu) were purchased from Janvier Laboratories (Saint Berthevin, France) and housed under pathogen-free conditions.
LNCap cells were purchased from DSMZ (German collection of microorganisms and cell culture GmbH, Germany).
PET imaging was performed with a preclinical IRIS PET-CT system (Inviscan, France). True coincidences were defined as two photons detected within a window of 5 ns coincidence time and with an energy between 250-750 keV. For static PET exam the coincidences were acquired for 10 min and for dynamic PET scans 18 frames of 5 min were acquired. Data were then reconstructed into a 201x201x120 volume by iterative 3D ordered-subset expectation-maximization algorithm with full correction for normalization, random coincidences, radioactive decay and dead time (attenuation and scattering were not corrected). For all images, the voxel sizes were 0.42 mm in the transverse plane with a 0.855 mm thickness.
PET data were treated using the AMIDE software package (http://amide.sourceforge.net/). An elliptic volume of interest (VOI) was manually drawn around the tumor or the organs on the image. For dynamic acquisition, VOI were propagated over the 18 frames and values were expressed as the percentage of dose injected per gram (%ID/g).

5.6.1 Biological stability

Stability in mice serum using **[^{18}F]-11**

Serum from mice was obtained from ThermoFischer scientific and used as such (100 µl per test). To Eppendorf tubes containing serum was added 50 µl of formulated ^{18}F-PSMA **[^{18}F]-11** (10% ethanol in 0.9% NaCl). The content was mixed (vortex 15 seconds) and incubated in a water bath at 37 °C with gentle stirring. After the incubation time (5 min, 30 min, 60 min) tubes were set on ice and treated by addition of 50 µl of MeCN (containing 0.1% TFA). After mixing (vortex) tubes were centrifuged (8000 RPM during 3 min) and the liquid phase was collected and filtered (0.2 µm PTFE filter, whatman, 13 mm). After filtration, samples were analyzed by reverse phase HPLC (column Kinetex EVO C18 150x4.6 mm, Phenomenex) with a mobile phase MeCN/H_2O (containing 0.1% TFA) using a fast gradient (5/95 to 100/0 within 10 min). Percentages of recovered activity (decay corrected to addition time) after incubation and treatment were higher than 90%.

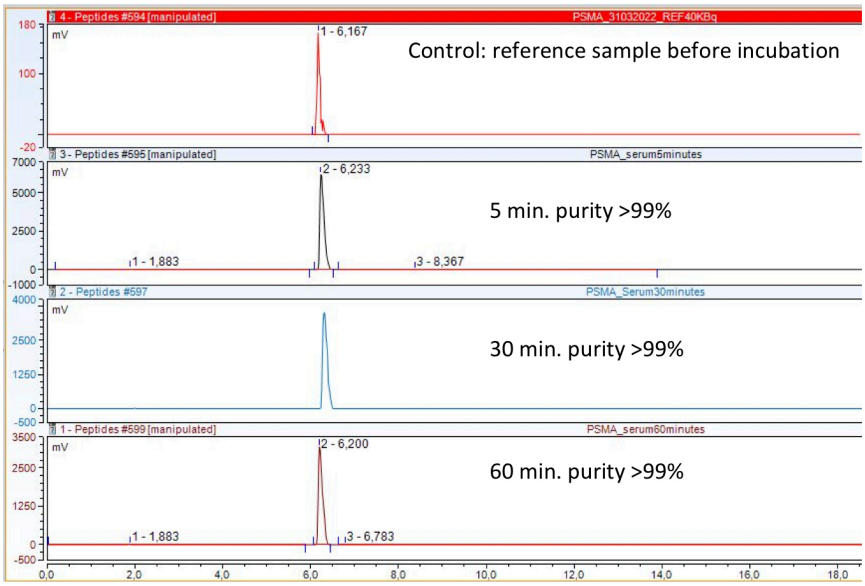


Figure S18. HPLC analysis (radioactive channel) of reference **[^{18}F]-11** (top) and extracted products after serum incubation at 5, 30 and 60 min respectively.

Stability in whole mice blood using **F-11** (non-radioactive form)

Stability in whole blood of mice was performed using the non-radioactive compound **F-11**. Analytical method was developed and a calibration curve was realized using a Shimadzu LC-MS/MS 8030 (UPLC coupled to a triple quadrupole mass detector using a phenomenex kinetex column 2.6 µm C18 100A°, 50 x 2.1 mm and a gradient H_2O/MeCN containing 0.5% HCO_2H at 0.5 mL.min^{-1}). A stock solution of **F-11** in DMSO (10 mM) was prepared and further diluted to 0.1 mM with DMSO. The solution was added to whole mice blood (total volume 400 µL) to reach a final concentration of 1 µM. Samples were incubated at 37 °C during 5, 15, 30, 60 and 120 min under agitation. For each assay, 20 µL were withdrawn and mixed with 100 µL of H_2O/MeCN (1/1) containing 0.5% of $ZnSO_4$. Samples were mixed 5 minutes (vortex), then centrifuged 5 minutes at 15 000 G (at 16 °C). Each sample was analyzed by LC-MS/MS (3 µL injected volume) and the concentration of **F-11** was determined using the calibration curve.
The compound was found to be stable over 120 min under these conditions with no apparent variations of the concentration of **F-11** during the incubation time.

5.6.2 LNCap cells

Cells were grown in 75 cm2 flasks containing 12 mL RPMI-1640 media (Gibco 21875034) supplemented with 20% inactivated fetal bovine serum, 1% Penicillin-Streptomycin (Gibco 15140122), 1% Glutamax (Gibco 35050038). Cells were maintained and cultured at 37 °C in a 5% CO_2 humidified atmosphere twice per week. At 70-80% confluency cells were harvested (with 2 mL of trypsin-EDTA) and seeded again.

5.6.3 *Cellular uptake*

Adherent LNCap cells (200 000) per well were maintained at 37 °C under 5% CO_2 in humidified atmosphere (incubator Incusafe, phcbi). After 4 h, the culture medium was removed by aspiration (Vacusip, INTEGRA) and replace by HBSS (Hank's Balanced Salt Solution, GIBCO) preheated at 37 °C (see below for added volume).
The purified and formulated radiotracer (15 µl, 4.51 MBq at time of addition) was dissolved in preheated (37 °C) HBSS. To 3 wells of the 6-wells plates were added 900 µL of HBSS and 100 µl of ^{18}F-radiotracer (top row, n = 3). In bottom row (n = 3 per plate) 800 µL of HBSS was added with 200 µl of a solution of ^{18}F-tracer pre-mixed with 2-PMPA (Aldrich, 1mg/mL in HBSS) 1/1 (V/V) to reach a final volume of 1mL per well. Cells were then incubated 15 min or 1 hour in humidified atmosphere at 37 °C under 5% CO_2. After incubation, the radioactive solution was removed by gentle aspiration and cells were washed 3 times with cold (5 °C) NaCl solution (1mL each time, 0.9% NaCl, Bbraun). After washing, cells were covered with 1mL of 1N NaOH and let to dissolve 30 min at room temperature. The solutions were set in tubes and counted on a HIDEX gamma counter for 3 min each. Results (mean values n = 3 and standard deviation) are expressed as the percentage of the added dose per 200 000 cells (per well) and all values are decay corrected to the time of addition of the radiotracer onto the cells.

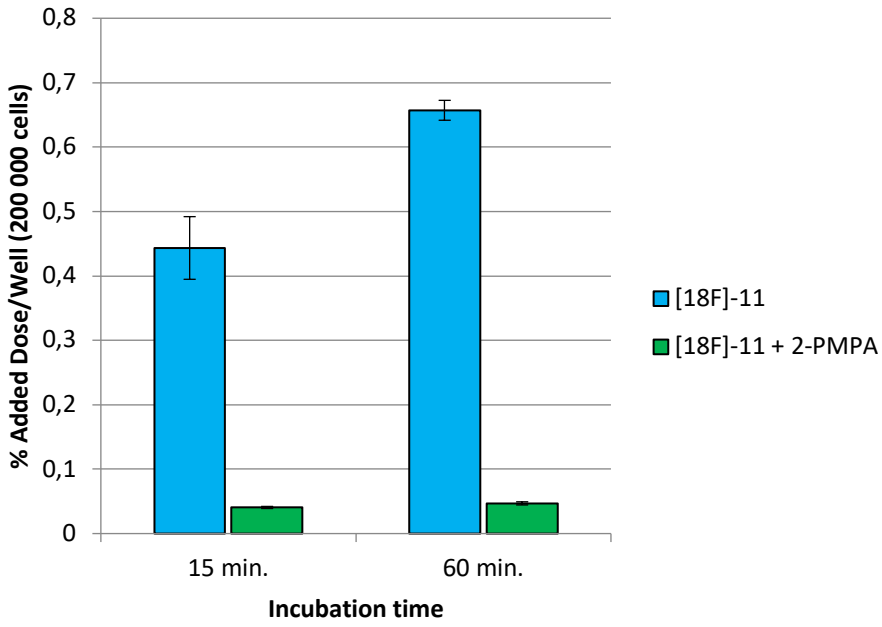


Figure S19. Histogram of cellular incubation of **[18F]-11** with and without 2-PMPA (competitor ligand) at 15 and 60 min of incubation time.

5.6.4 *Injection of cells on athymic nude mice*

10x10^6 LNCap cells (DSMZ, German collection of microorganisms and cell culture GmbH, Germany) in 70 µl of PBS/Matrigel (Corning) 1/1 (v/v, in a syringe cooled on ice bed to avoid solidification of matrigel) were injected on back of the right shoulder to 7 weeks old athymic nude male mice (n = 14, weight 22.3-25.2 g) under isoflurane (2%) anesthesia.
Growth of tumors was monitored by caliper measurements (long and small axis). Due to the low tumorogenicity of LNCap cells a minimum of 35 days of growth was necessary to obtain vascularized tumors with an anatomical volume inferior to 100 mm^3.
At the time of imaging only 3 mice on 14 developed tumors sufficiently vascularized for PET imaging. Remaining mice with no visible tumors or not vascularized tumors (translucent aspect) were scanned too. All mice (with or without tumors) were killed when the last day of the approved protocol was reached.

5.6.5 *Injection of [^{18}F]-11, PET imaging and quantification*

9.2 ± 2 MBq of **[^{18}F]-11** were diluted in 0.9% NaCl for injection to reach a total volume of 200 µl. The mice were maintained under 2.5% isoflurane anaesthesia and injected *via* the tail vein. After injection the mice were quickly placed under the PET imaging system and a 90 min dynamic acquisition was performed (start time 4 min after the

end of injection). For static acquisition, mice were kept awake for 60 min before a 10 min acquisition scan. During acquisition, mice were kept under an atmosphere of isoflurane (2-3% in air) and heated. Mice were placed tail first and supine.

VOI (volume of interest) were manually drawn on coronal, sagittal and axial views for the tumor and the organs. For blood activity measurement from PET images a VOI was located inside the heart on the first frame of the dynamic acquisition and propagated over the 18 frames.
A VOI (identical to the one used for the tumor) was set on the opposite shoulder (left shoulder) to be used as a reference tissue for quantification.
For Liver quantification a VOI was located on the left and/or right lobe of liver (with exclusion of gall bladder).
For Kidneys, a VOI was set on one of the kidneys (right or left indifferently).
All values (mean values and standard deviations) obtained are expressed as percentage of injected dose per gram (% ID/g, assuming 1mL = 1g) and corrected for decay (to the time of injection).

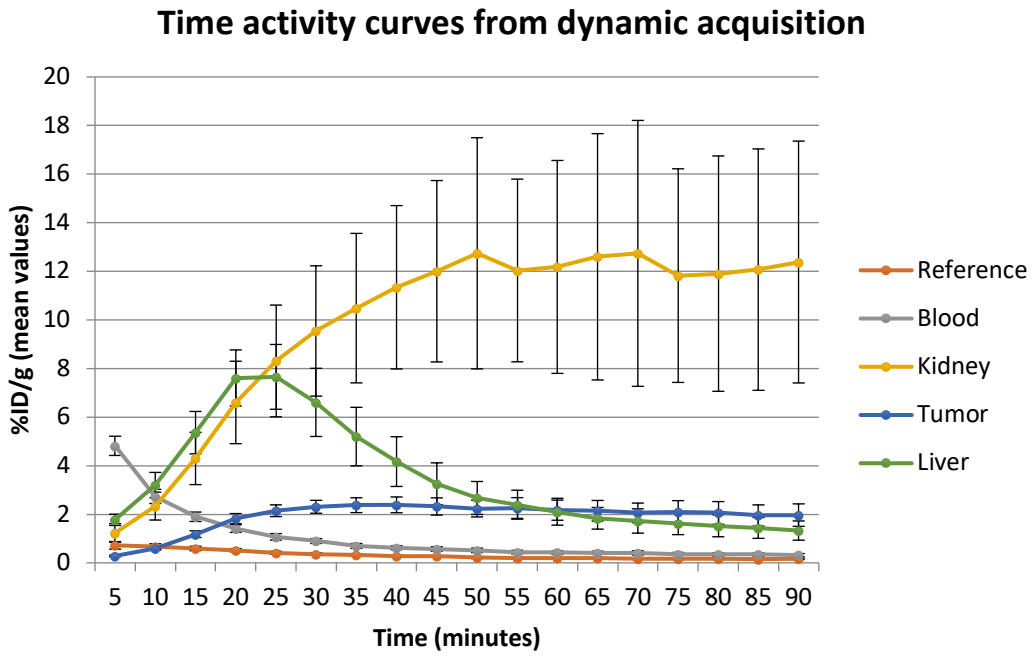


Figure S20. Time activity curve, over 90 min, obtained from ROI (region of interest) set on different organs during a dynamic acquisition.

5.6.6 Additional PET Images

MIP (Maximum Intensity Projection) images were obtained using OSIRIX software. The white arrow pointing at the tumor. Axial, Coronal and Sagittal views of mice were obtained using Amide software. The white arrow pointing at the tumor. All images are scaled from 0 to 10% of the injected dose per gram (%ID/G, assessing 1 g = 1 mL).

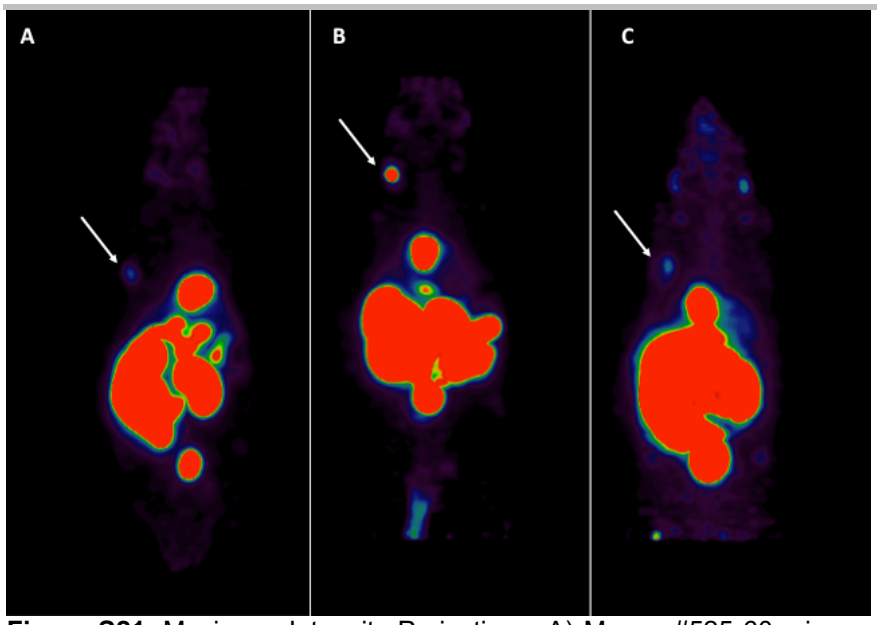


Figure S21. Maximum Intensity Projections. A) Mouse #525 60 min post-injection of 10.85 MBq of **[^{18}F]-11**. B) Mouse 529 60 min post-injection of 8.36 MBq of **[^{18}F]-11**. C) Mouse #530 60 min post-injection of 11.56 MBq of **[^{18}F]-11**. White arrow pointing at the tumor.

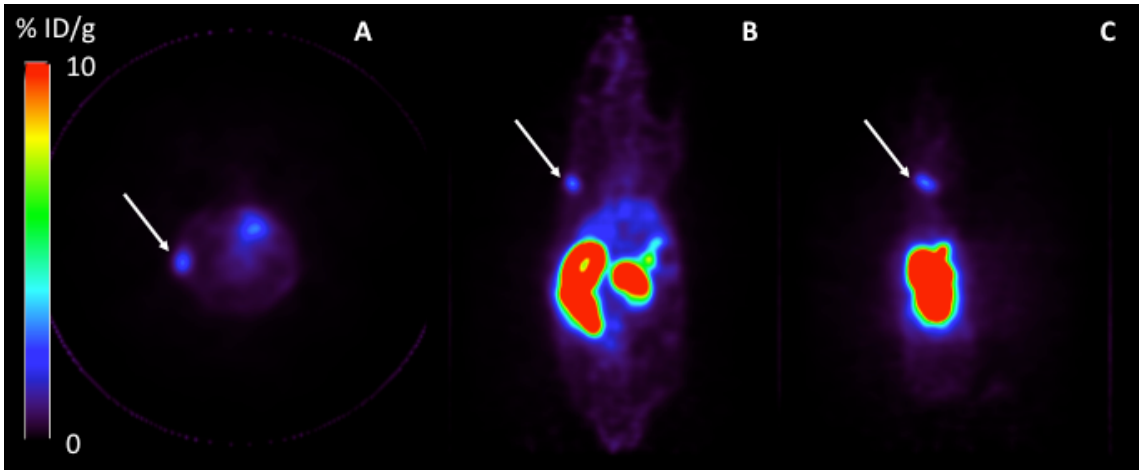


Figure S22. Mouse # 525. 60 minutes post injection of 10.85 MBq of **[^{18}F]-11**. A) Axial view. B) Coronal view. C) Sagittal view. White arrow pointing at the tumor.

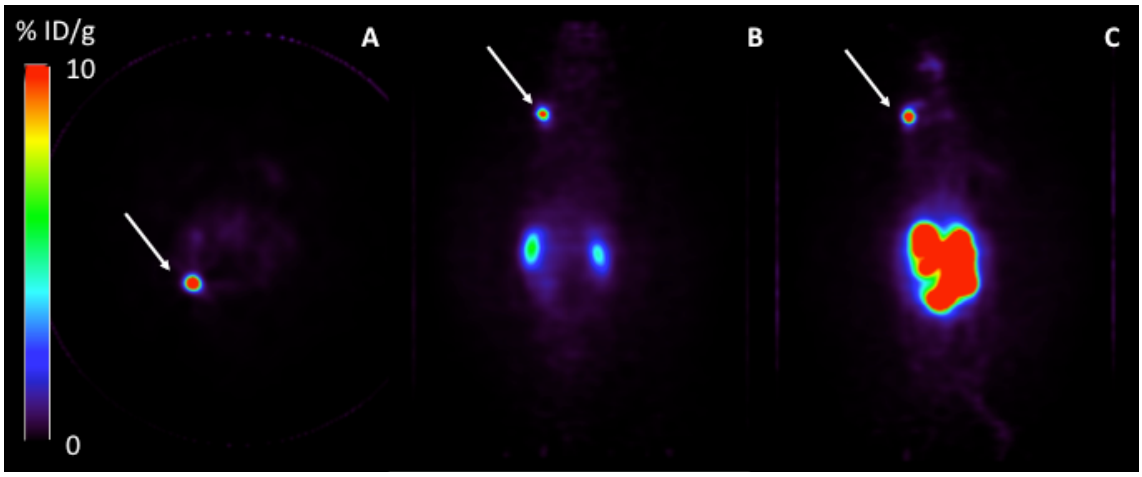


Figure S23. Mouse # 529. 60 minutes post injection of 8.36 MBq of **[^{18}F]-11**. A) Axial view. B) Coronal view. C) Sagittal view. White arrow pointing at the tumor.

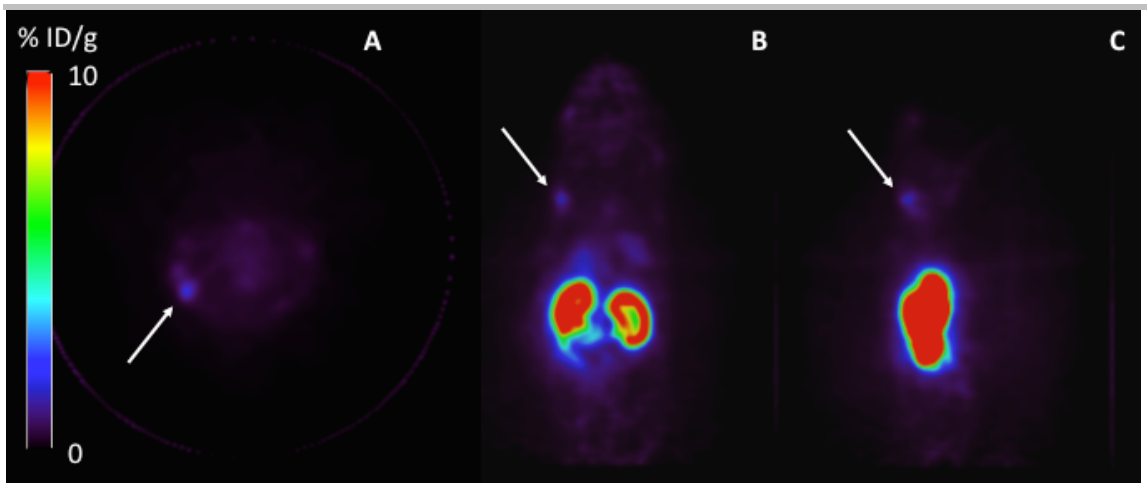
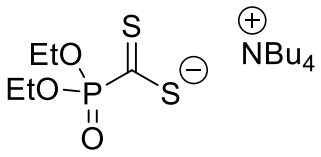


Figure S24. Mouse # 530. 60 minutes post injection of 11.56 MBq of **[^{18}F]-11**. A) Axial view. B) Coronal view. C) Sagittal view. White arrow pointing at the tumor.

6 References

[1] Modified procedure from: D. L. Fox, N. R. Whitely, R. J. Cohen, R. N. Salvatore, Synlett 2003, **13**, 2037-2041.
[2] N. Motzner, I. Lamparth, K. Rist, C. Barner-Kowollik, A. Schenzel, M. Langer, Polymerizable compositions based on thermally splittable thermolabile compounds for use in adhesives, composites, stereolithog. materials etc. in dental materials. WO2017064042, **2020**.
[3] V. I. Böhmer, W. Szymanski, K.-O. van den Berg, C. Mulder, P. Kobauri, H. Helbert, D. van der Born, F. Reebing, A. Huizing, M. Klopstra, D. F. Samplonius, I. F. Antunes, J. W. A. Sijbesma, G. Luurtsema, W. Helfrich, T. J. Visser, B. L. Feringa, P. H. Elsinga, *Chem. Eur. J.* 2020, **26**, 10871-10881.
[4] D. Craig, M. I. Lansdell, S. E. Lewis, *Tetrahedron Lett.* 2007, **44**, 7861-7864.
[5] R. M. Gillard, J. E. M. Fernando, D. W. Lupton, *Angew. Chem. Int. Ed.* 2018, **57**, 4712-4716.

7 NMR and HRMS analyses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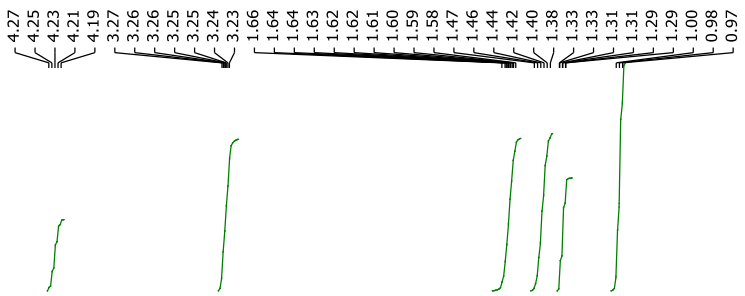
EtO
P
S
O
NBu4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3.27
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3.26
3.25
3.25
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1.66
1.64
1.64
1.63
1.62
1.62
1.61
1.60
1.59
1.58
1.47
1.46
1.44
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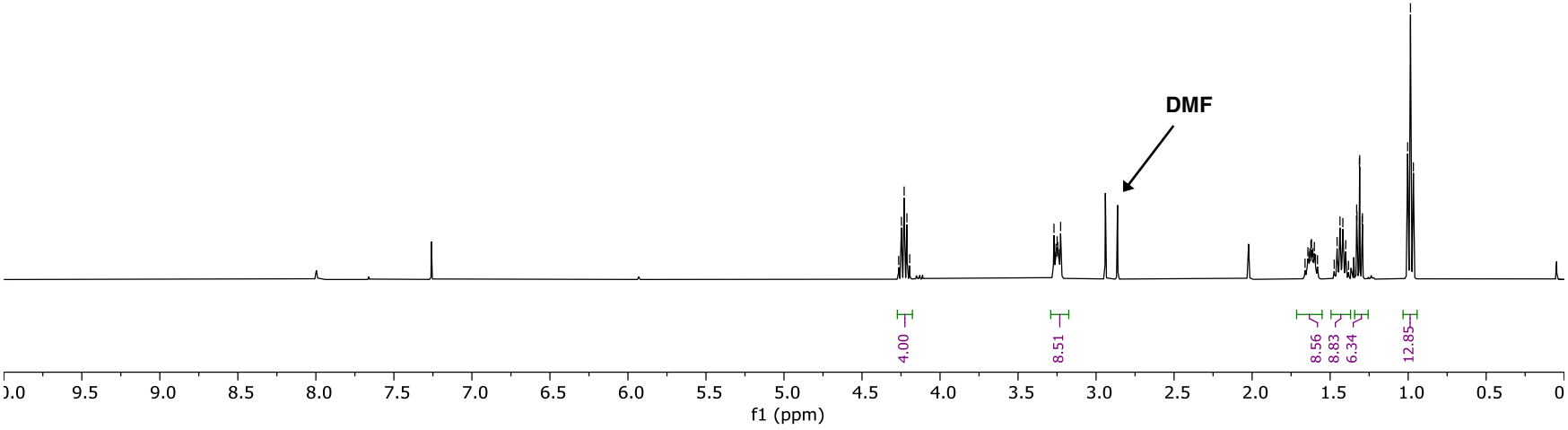
0.97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6.5
6.0
5.5
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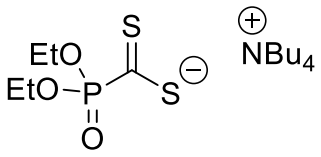
0
f1 (ppm)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EtO
P
O
S
S
NBu4

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13.73
00
190
180
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100
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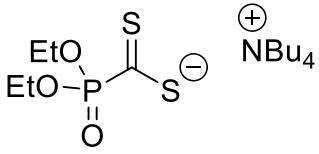
10
f1 (ppm)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EtO
P
S
O
NBu4

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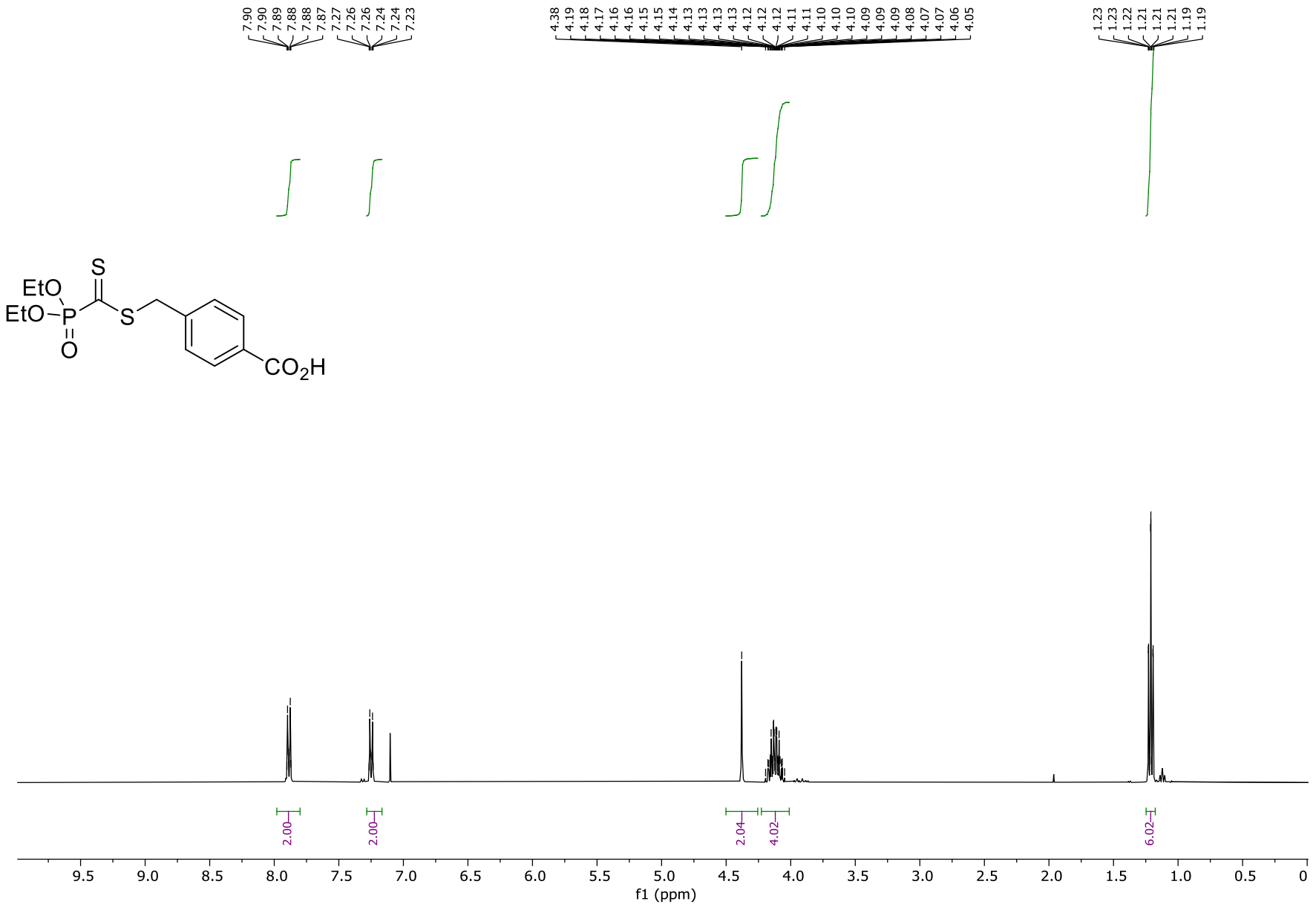
f1 (ppm)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4.07
4.07
4.06
4.05
1.23
1.23
1.22
1.21
1.21
1.21
1.19
1.19
EtO
EtO
P
O
S
S
CO2H
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2.00
2.04
4.02
6.02
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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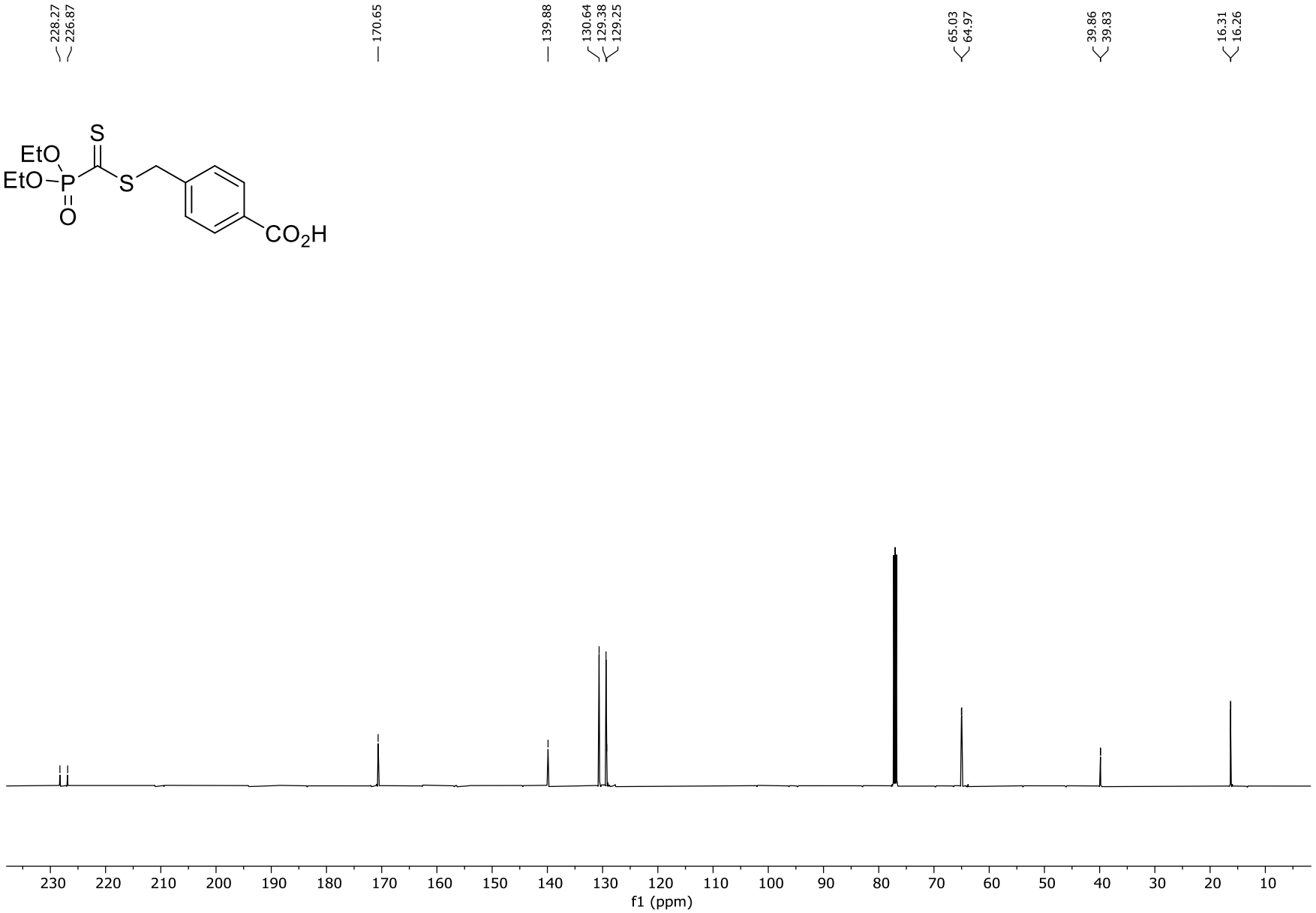
f1 (ppm)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226.87
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129.25
65.03
64.97
39.86
39.83
16.31
16.26
EtO
EtO
P
O
S
S
CO2H
230
220
210
200
190
180
170
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140
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120
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100
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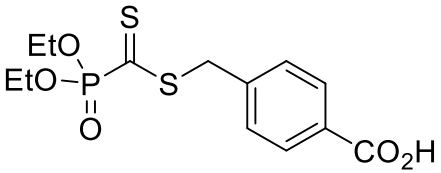
60
50
40
30
20
10
f1 (ppm)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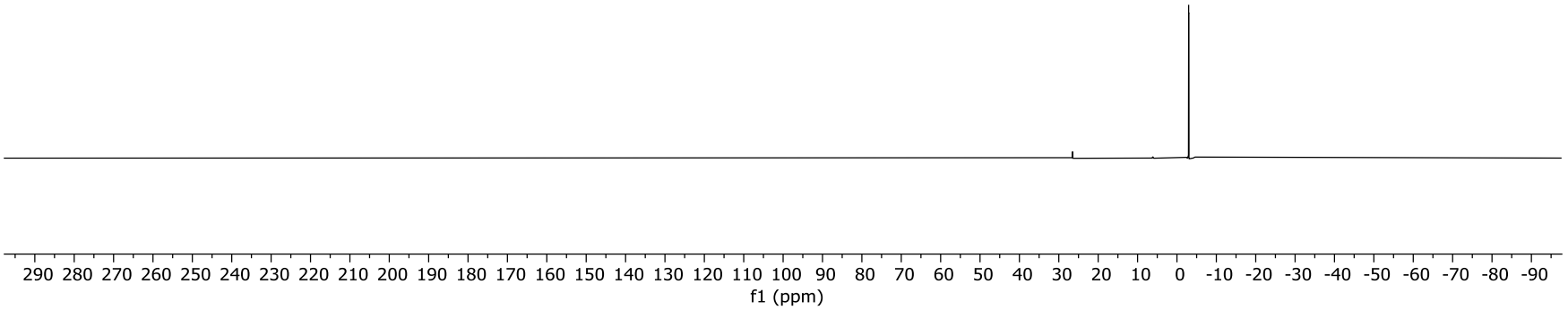
EtO
EtO
P
O
S
CO2H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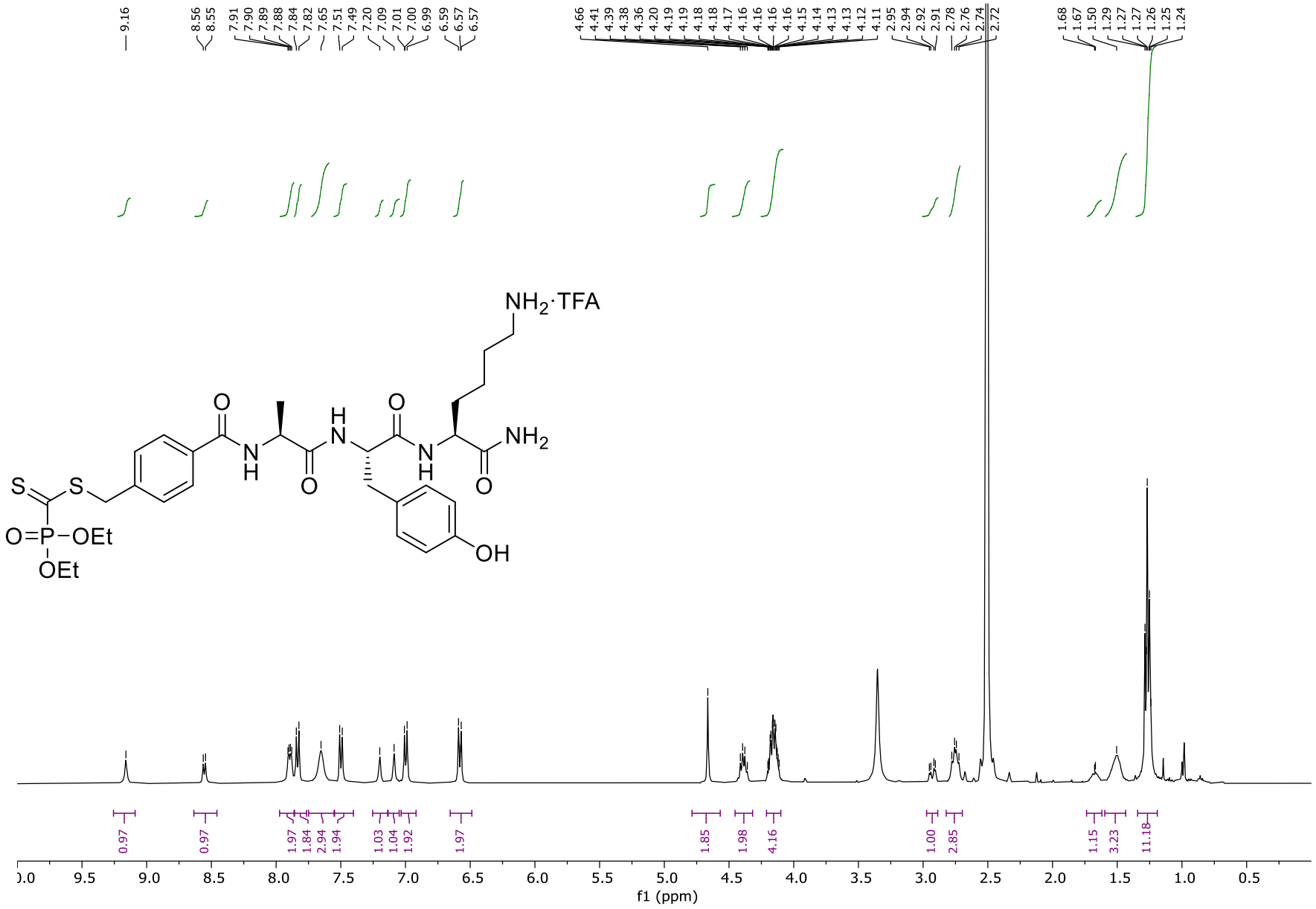
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7.00
6.99
6.59
6.57
6.57
4.66
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4.39
4.38
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4.20
4.19
4.19
4.18
4.18
4.17
4.16
4.16
4.16
4.16
4.15
4.14
4.13
4.13
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4.11
2.95
2.94
2.92
2.91
2.78
2.76
2.74
2.72
1.68
1.67
1.50
1.29
1.27
1.27
1.26
1.25
1.24
NH2·TFA
S
S
O=P−OEt
OEt
O
N
H
O
H
N
O
N
H
NH2
O
O
OH
0.97
0.97
1.97
1.84
2.94
1.94
1.03
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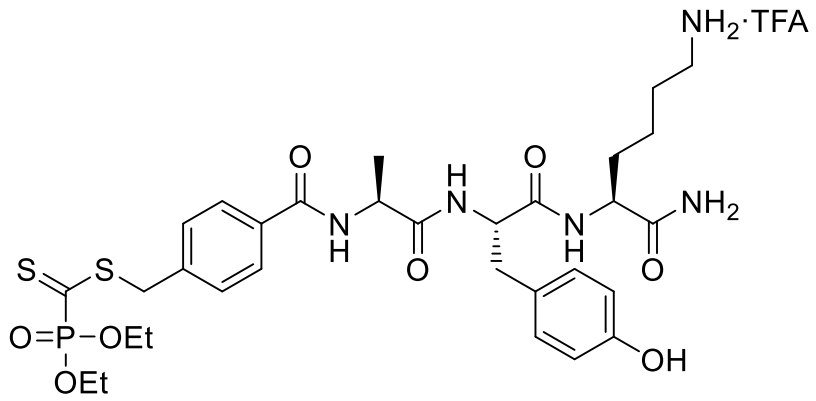
3.23
11.18
f1 (ppm)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H
O
N
H
O
NH2
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S
S
O=P−OEt
OEt
OH

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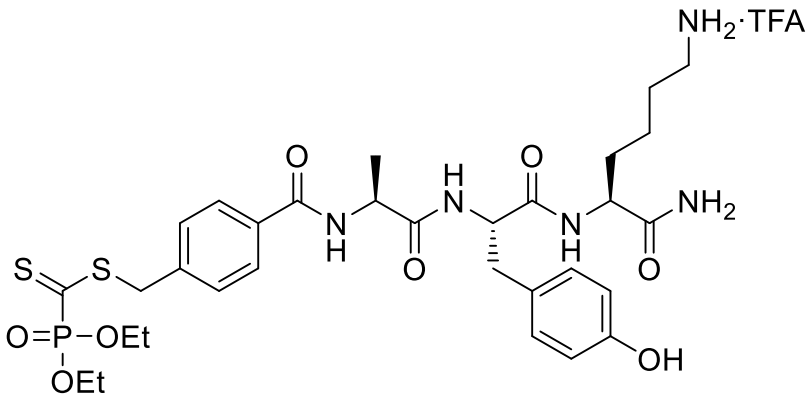
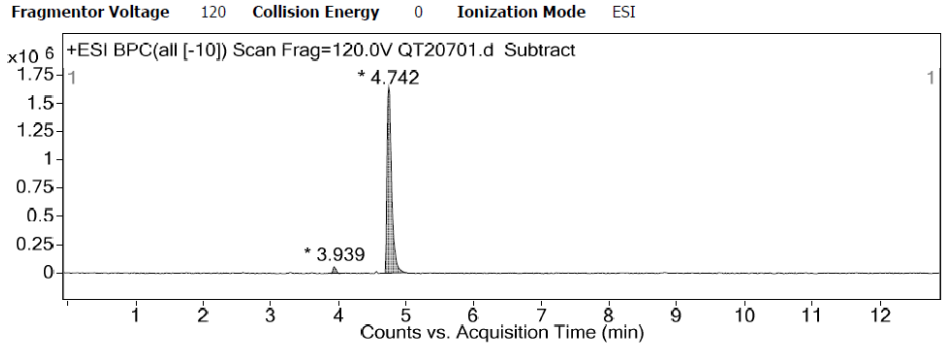
f1 (ppm)





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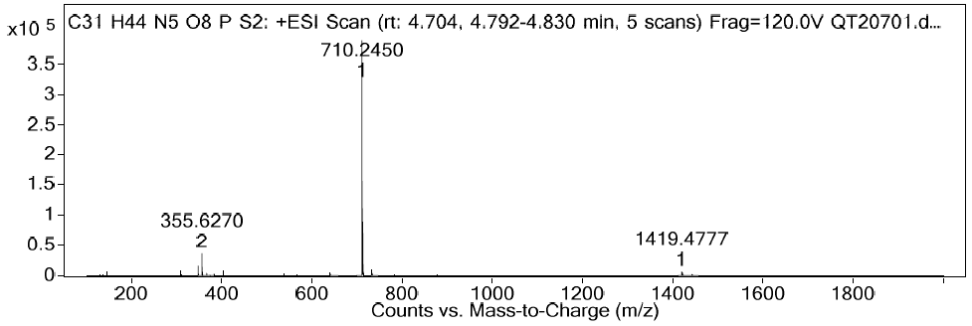

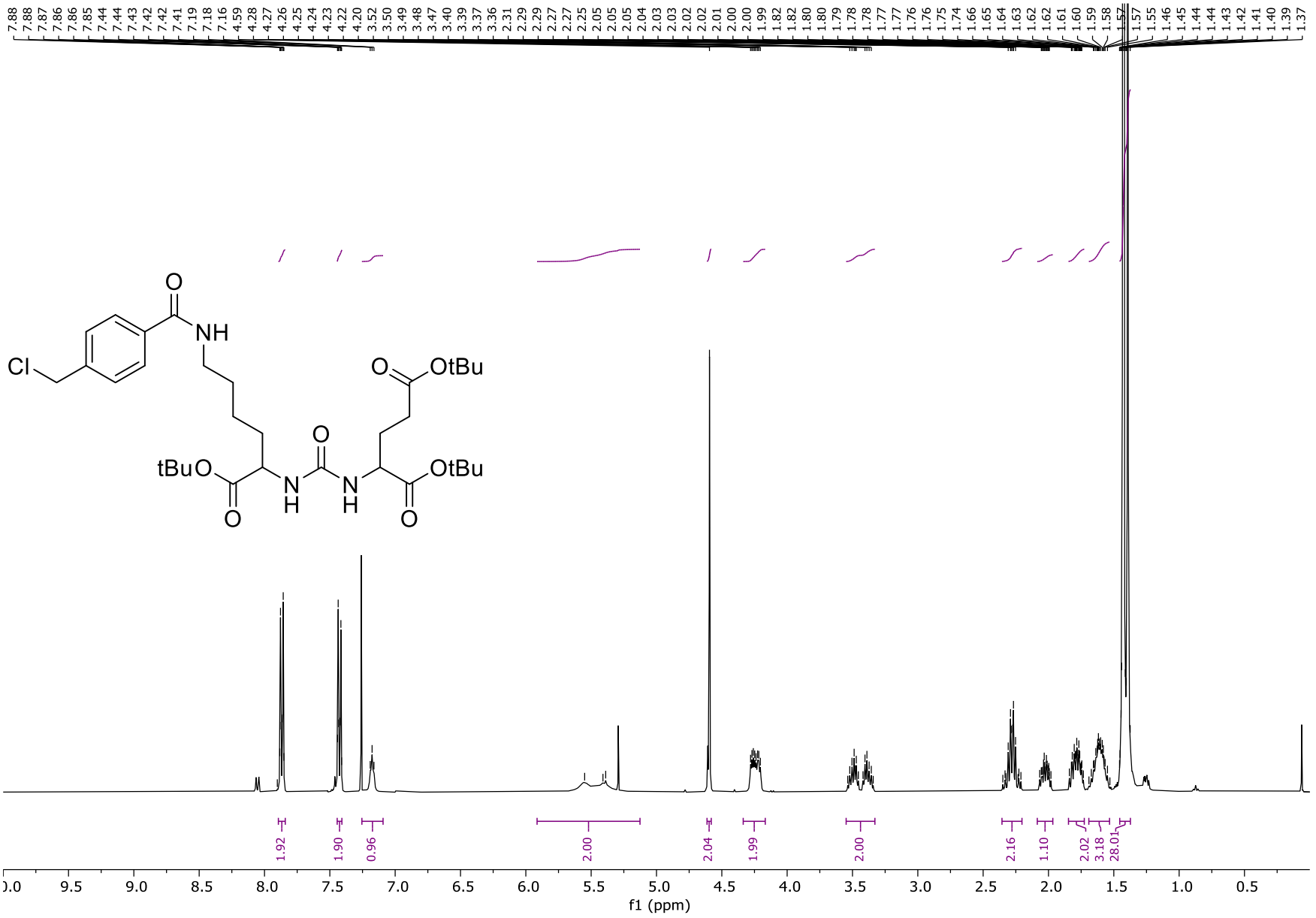
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OtBu
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1.10
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3.18
28.01
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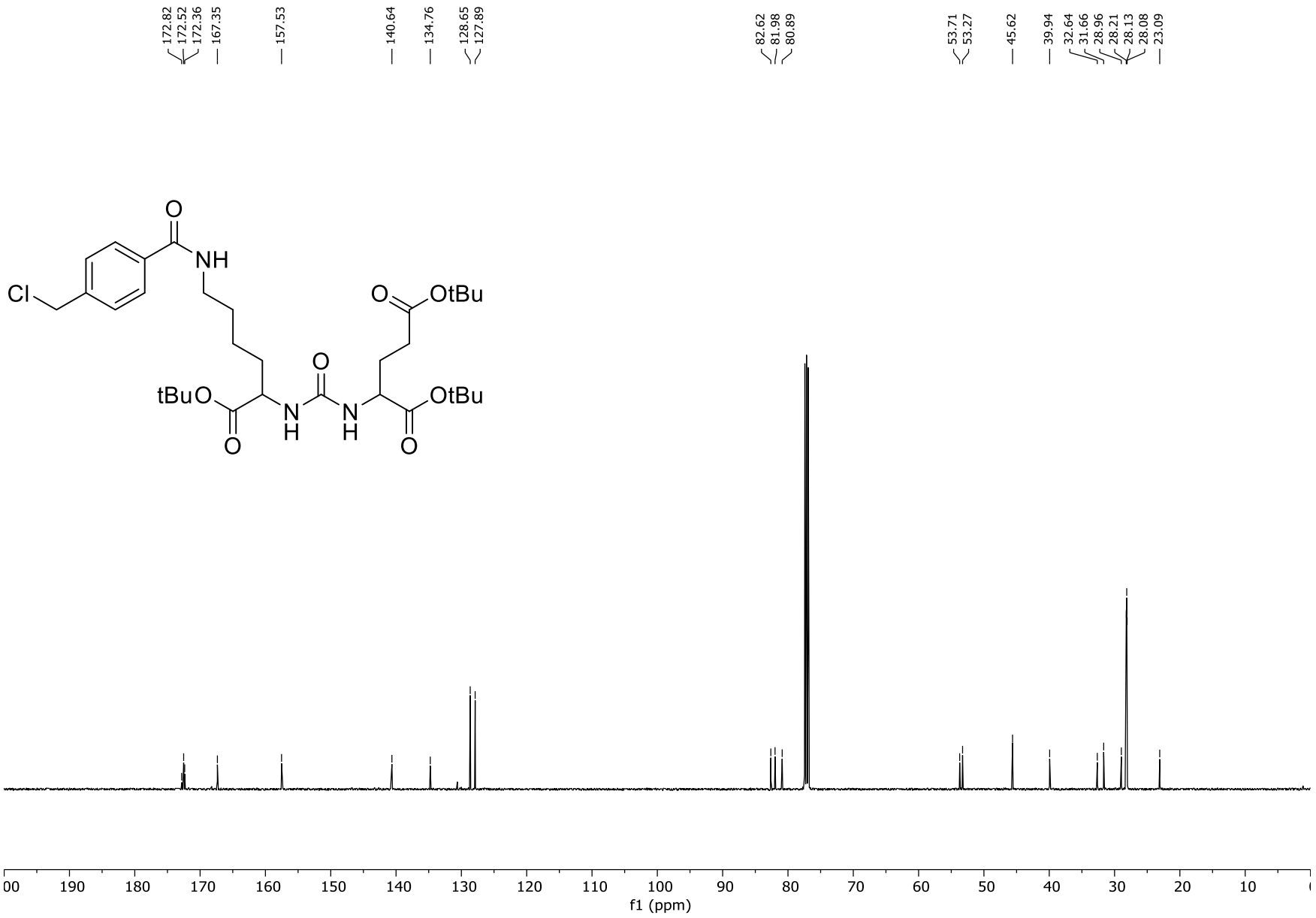
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28.08
23.09
O
NH
Cl
O
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tBuO
O
N
H
N
H
O
OtBu
O
O
00
190
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60
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20
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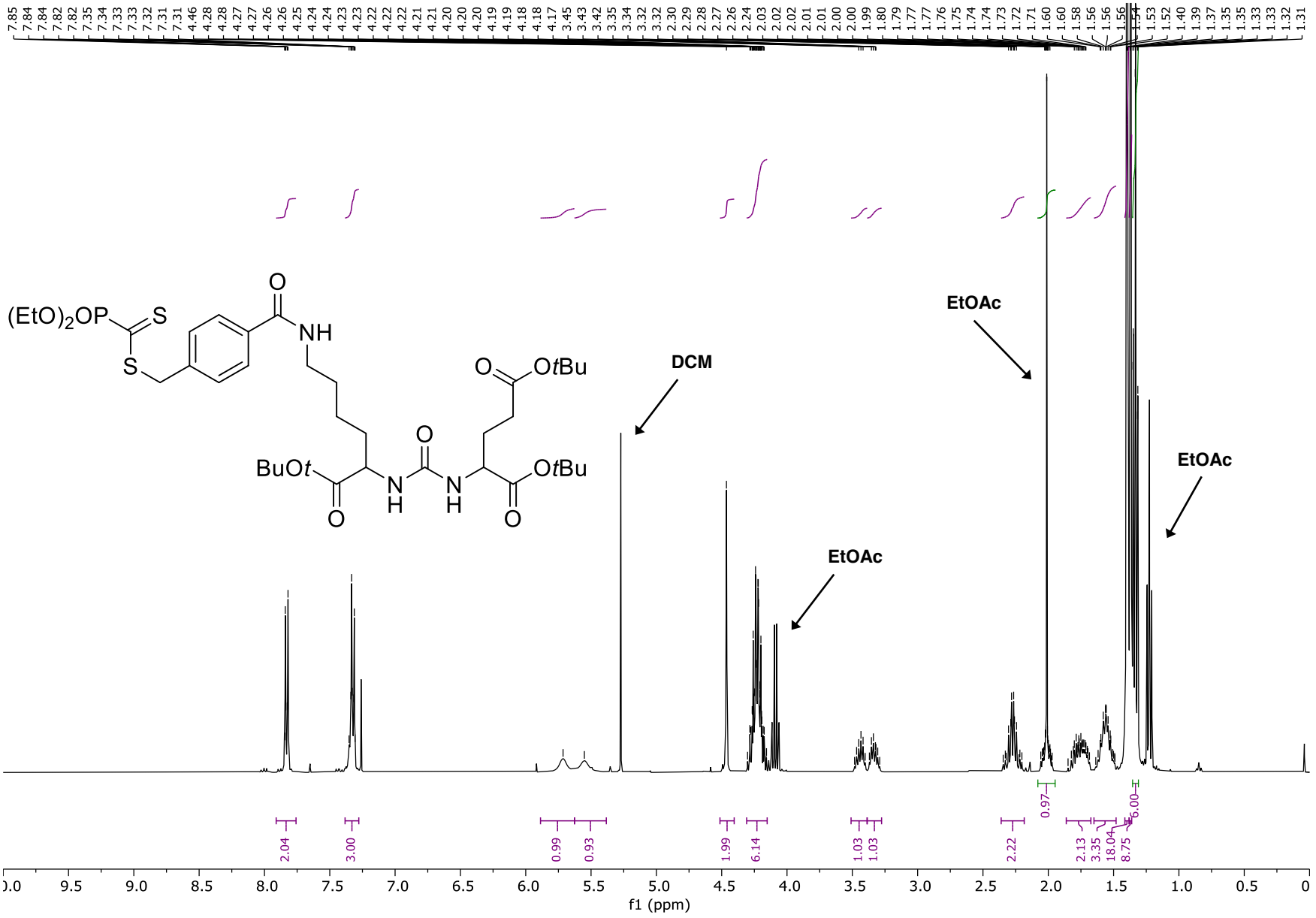
f1 (ppm)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BuOt
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EtOAc
EtOAc
EtOAc
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3.00
0.99
0.93
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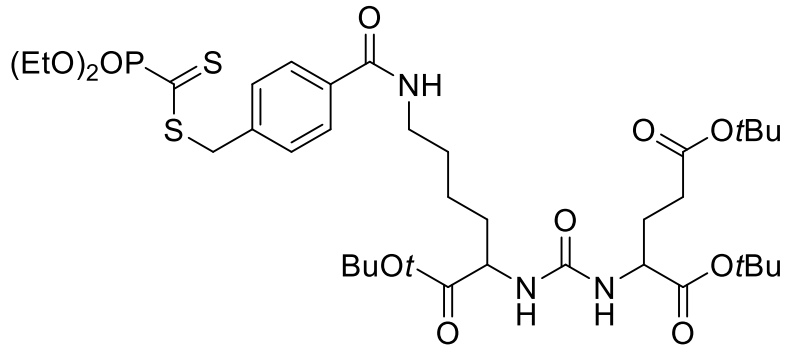
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O
OtBu
BuOt
O
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H
N
H
O
OtBu
O
O

-2.86
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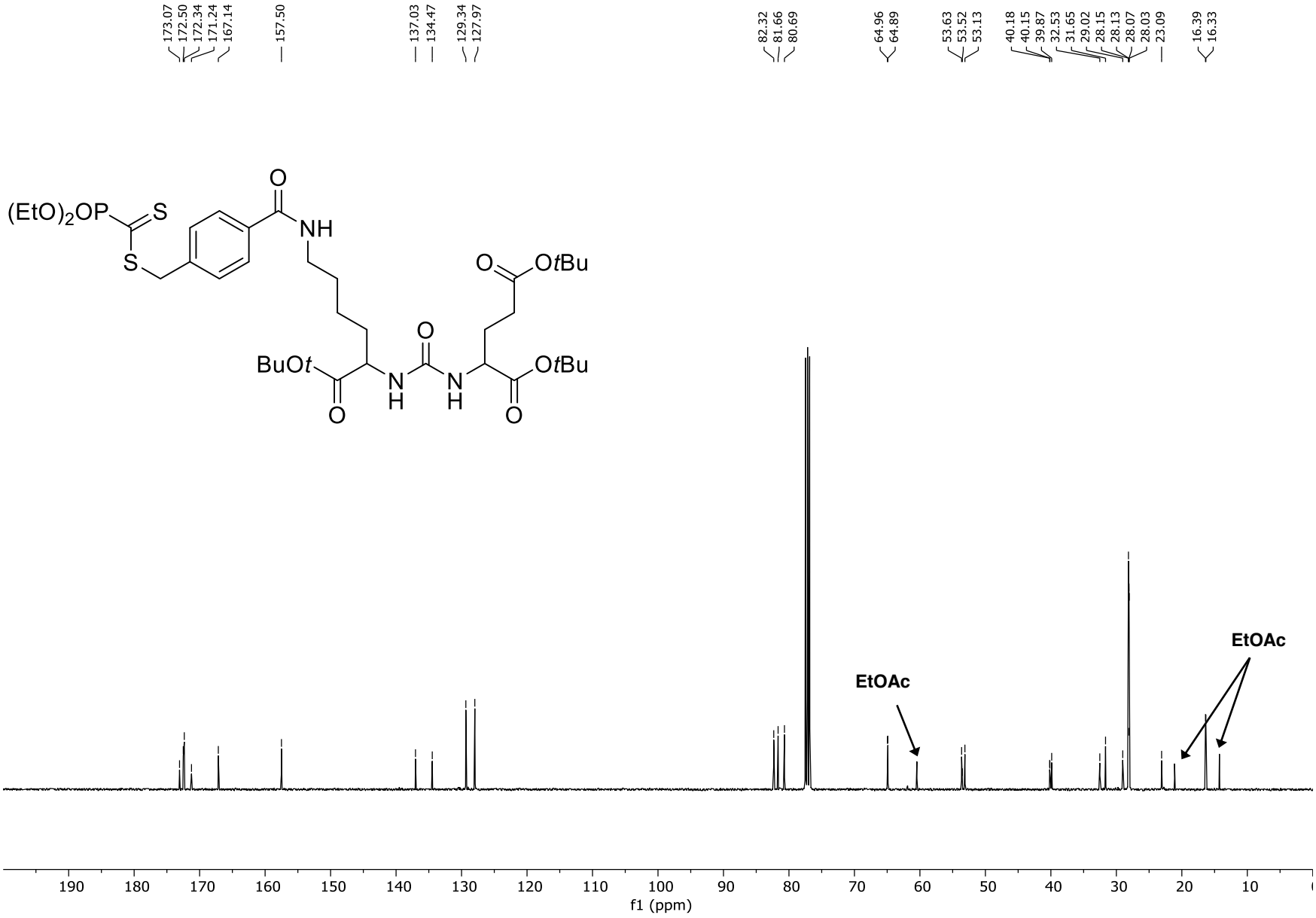
f1 (ppm)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29.02
28.15
28.13
28.07
28.03
23.09
16.39
16.33
(EtO)2OP
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S
O
NH
O
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BuOt
O
N
H
O
N
H
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OtBu
EtOAc
EtOAc
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60
50
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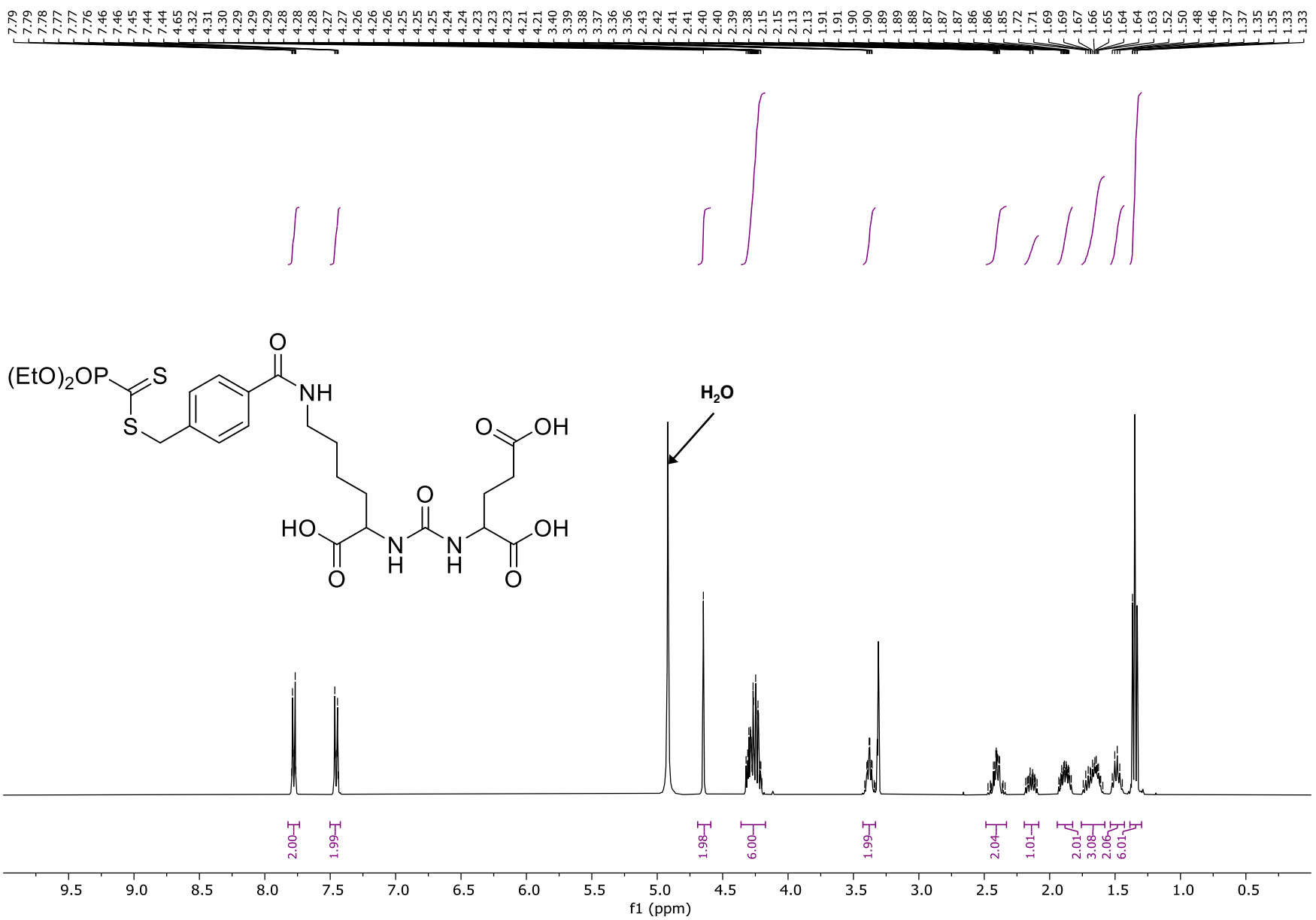
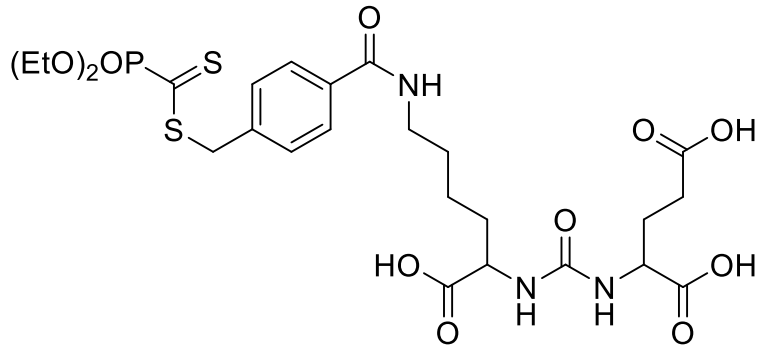
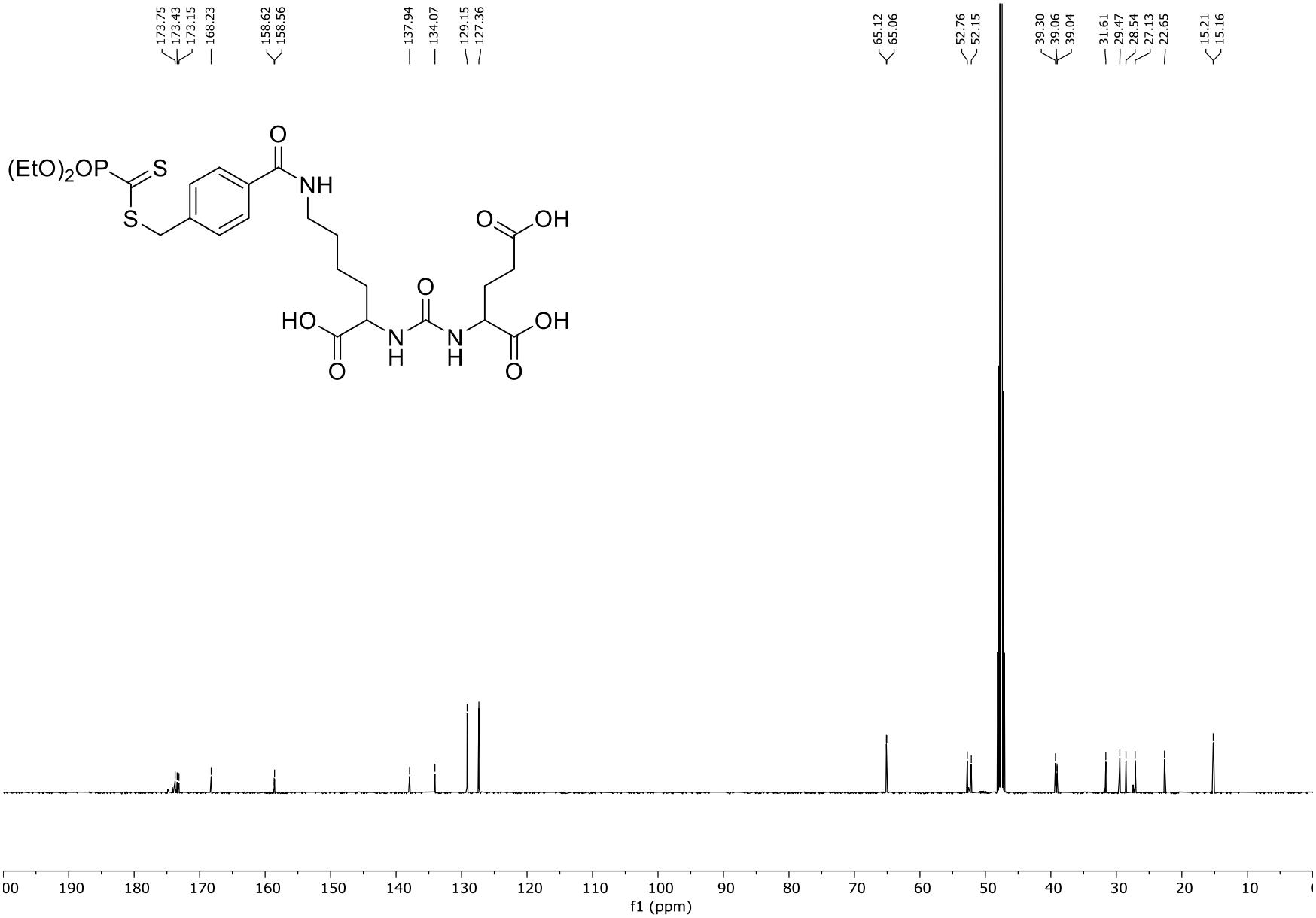
30
20
10
f1 (ppm)


7.79 7.79 7.78 7.77 7.77 7.76 7.46 7.46 7.45 7.44 7.44 4.65 4.32 4.31 4.30 4.29 4.29 4.29 4.28 4.28 4.28 4.27 4.27 4.26 4.26 4.26 4.25 4.25 4.25 4.24 4.24 4.23 4.23 4.23 4.21 4.21 3.40 3.39 3.38 3.37 3.36 3.36 2.43 2.42 2.41 2.41 2.40 2.40 2.39 2.38 2.15 2.15 2.13 2.13 1.91 1.91 1.90 1.90 1.89 1.89 1.88 1.87 1.87 1.87 1.86 1.86 1.85 1.72 1.71 1.69 1.69 1.67 1.66 1.65 1.64 1.64 1.63 1.52 1.50 1.48 1.46 1.37 1.37 1.35 1.35 1.33 1.33
(EtO)2OP
S
S
O
NH
HO
O
N
H
O
N
H
O
OH
O
OH
H2O
2.00
1.99
1.98
6.00
1.99
2.04
1.01
2.01
3.08
2.06
6.01
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


(EtO)2OP
S
S
O
NH
HO
O
N
H
O
N
H
O
OH
OH
O

-3.39
290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)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173.15
168.23
158.62
158.56
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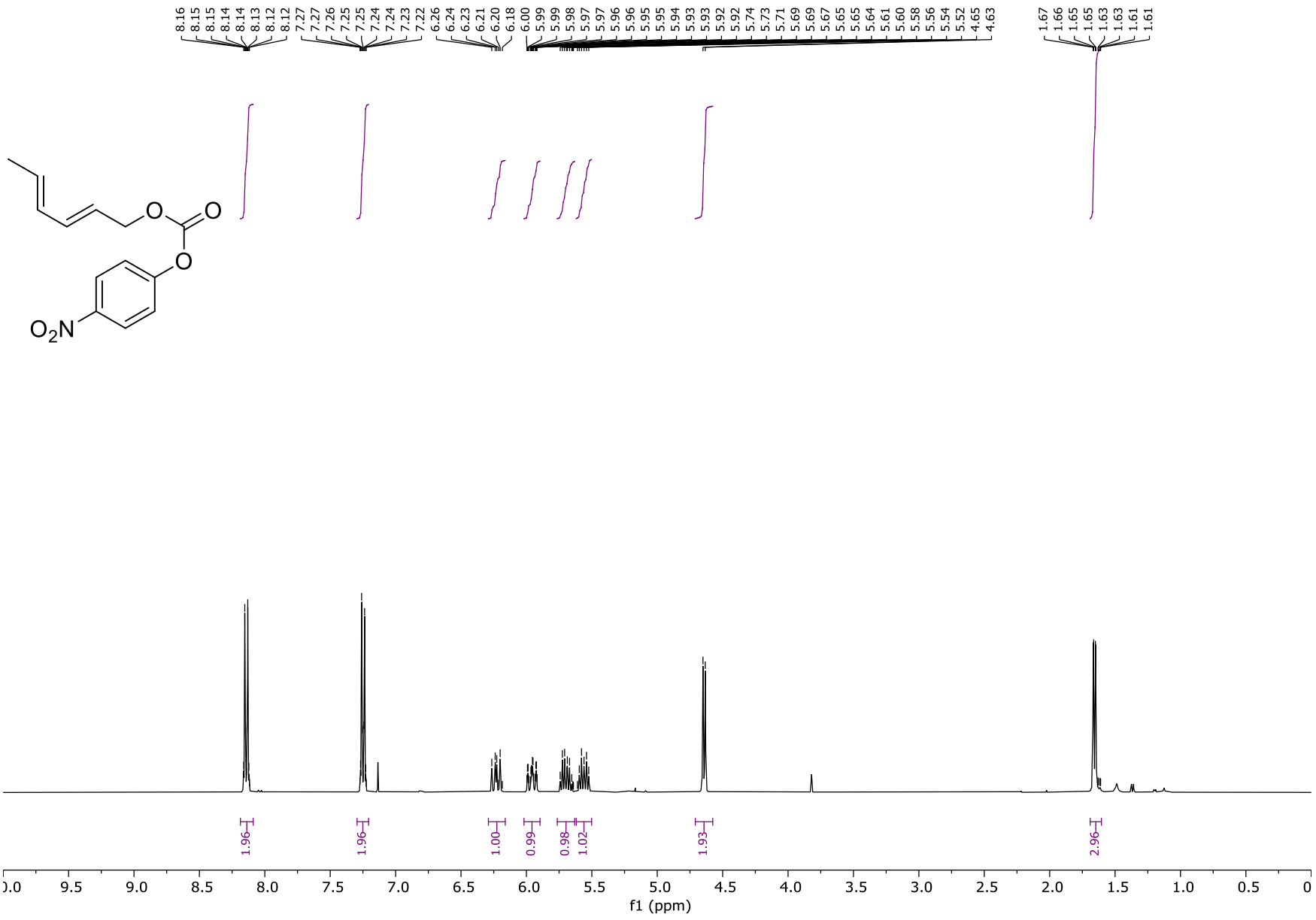
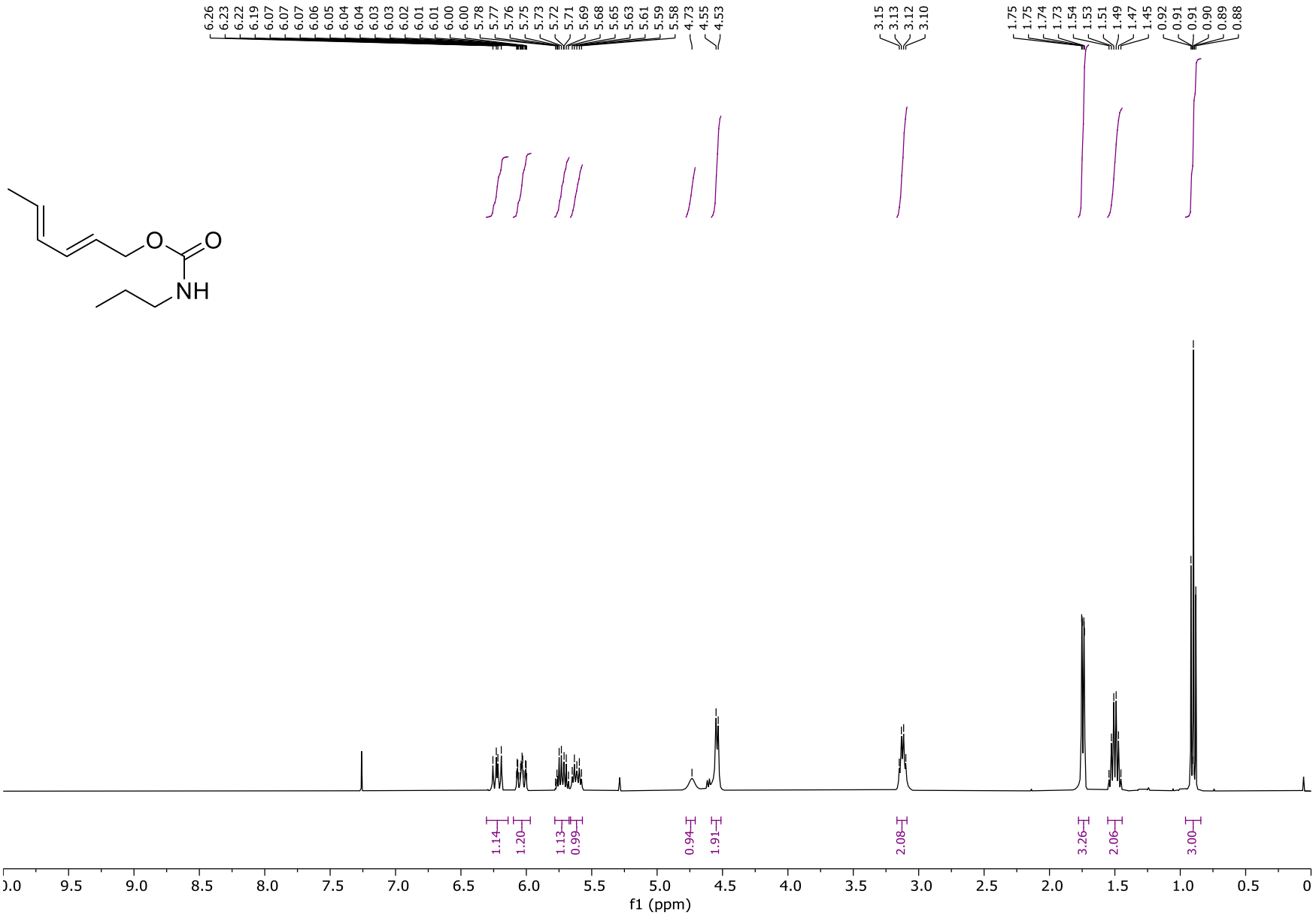
134.07
129.15
127.36
65.12
65.06
52.76
52.15
39.30
39.06
39.04
31.61
29.47
28.54
27.13
22.65
15.21
15.16
(EtO)2OP
S
S
O
NH
O
OH
HO
O
N
H
O
N
H
OH
O
f1 (ppm)


8.16 8.15 8.15 8.14 8.14 8.13 8.12 8.12
7.27 7.27 7.26 7.25 7.25 7.24 7.24 7.23 7.22
6.26 6.24 6.23 6.21 6.20 6.18
6.00 5.99 5.99 5.98 5.97 5.97 5.96 5.96 5.95 5.95 5.94 5.93 5.93 5.92 5.92 5.74 5.73 5.71 5.69 5.69 5.67 5.65 5.65 5.64 5.61 5.60 5.58 5.56 5.54 5.52 4.65 4.63
1.67 1.66 1.65 1.65 1.63 1.63 1.61 1.61
O
O
O
O2N
1.96
1.96
1.00
0.99
0.98
1.02
1.93
2.96
0.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)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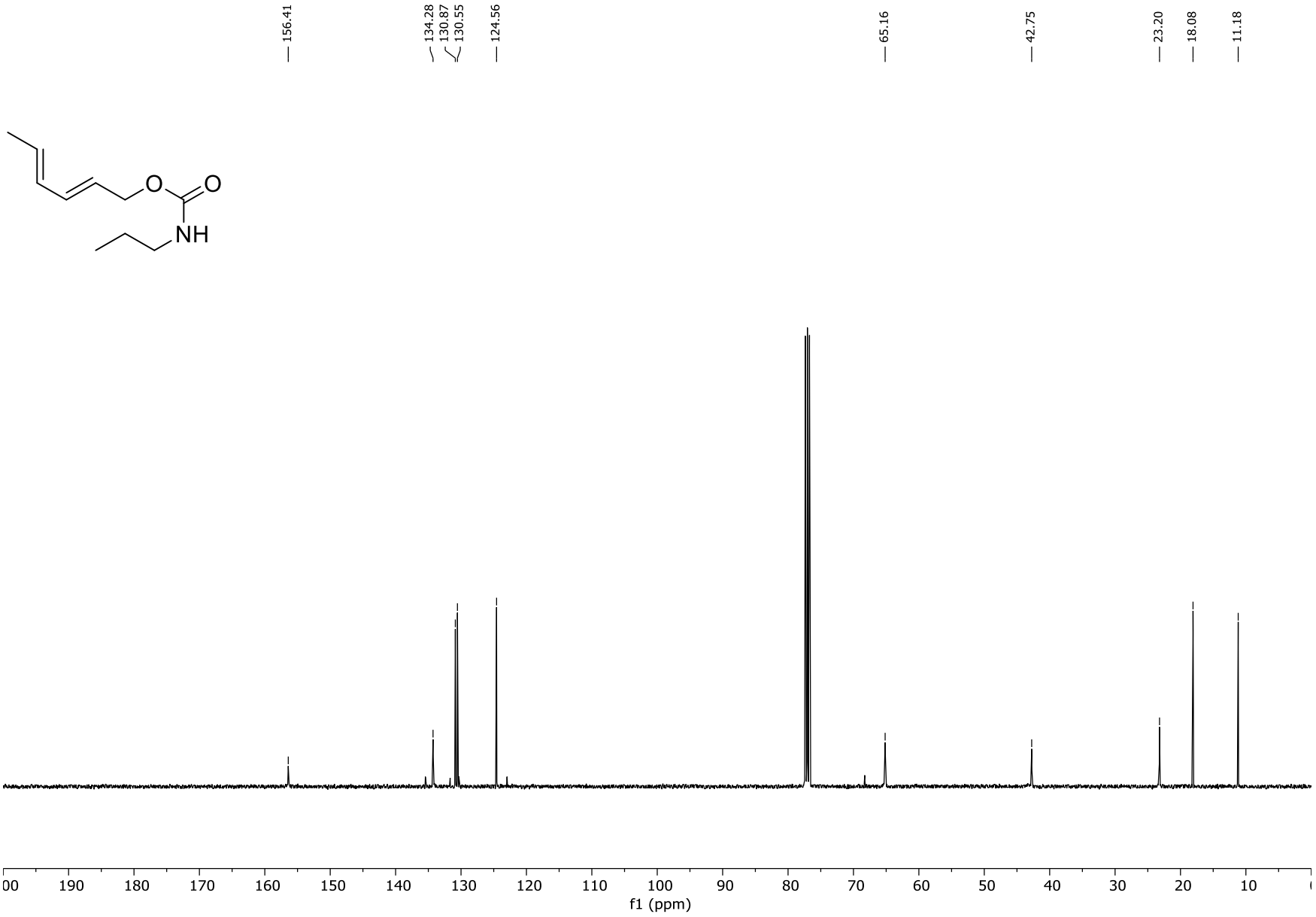
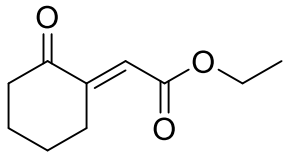
6.23
6.22
6.19
6.07
6.07
6.07
6.06
6.05
6.04
6.04
6.03
6.03
6.02
6.01
6.01
6.00
6.00
5.78
5.77
5.76
5.75
5.73
5.72
5.71
5.69
5.68
5.65
5.63
5.61
5.59
5.58
4.73
4.55
4.53
3.15
3.13
3.12
3.10
1.75
1.75
1.74
1.73
1.54
1.53
1.51
1.49
1.47
1.45
0.92
0.91
0.91
0.90
0.89
0.88
O
O
NH
1.14
1.20
1.13
0.99
0.94
1.91
2.08
3.26
2.06
3.00
f1 (ppm)


156.41
134.28
130.87
130.55
124.56
65.16
42.75
23.20
18.08
11.18
O
O
NH
f1 (ppm)


O
O
O

6.47
6.47
6.46
4.22
4.20
4.19
4.17
3.11
3.10
3.09
3.08
3.07
3.07
3.07
3.07
2.54
2.53
2.52
2.51
1.94
1.94
1.93
1.93
1.92
1.92
1.92
1.91
1.91
1.91
1.90
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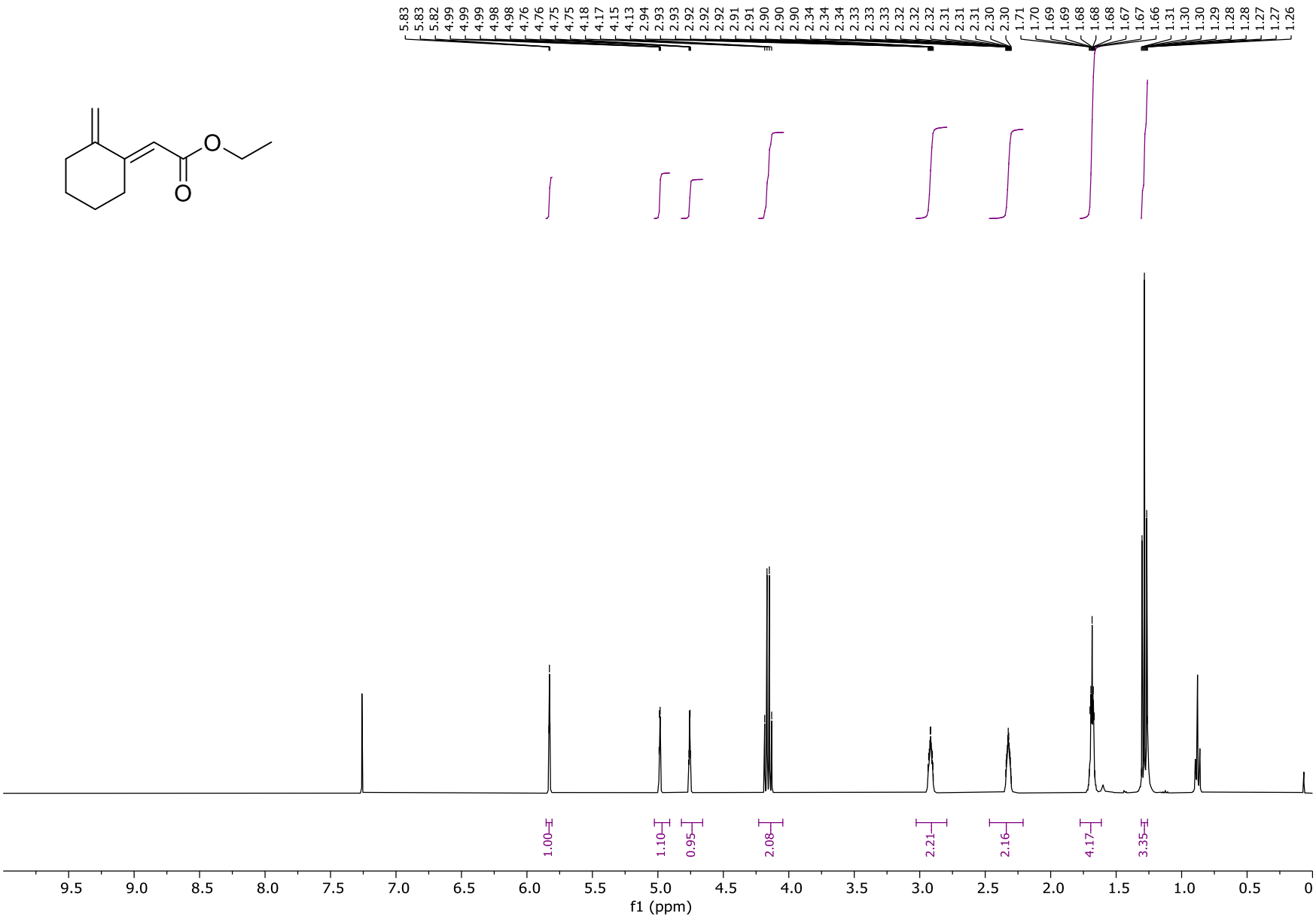
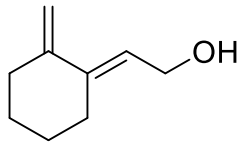
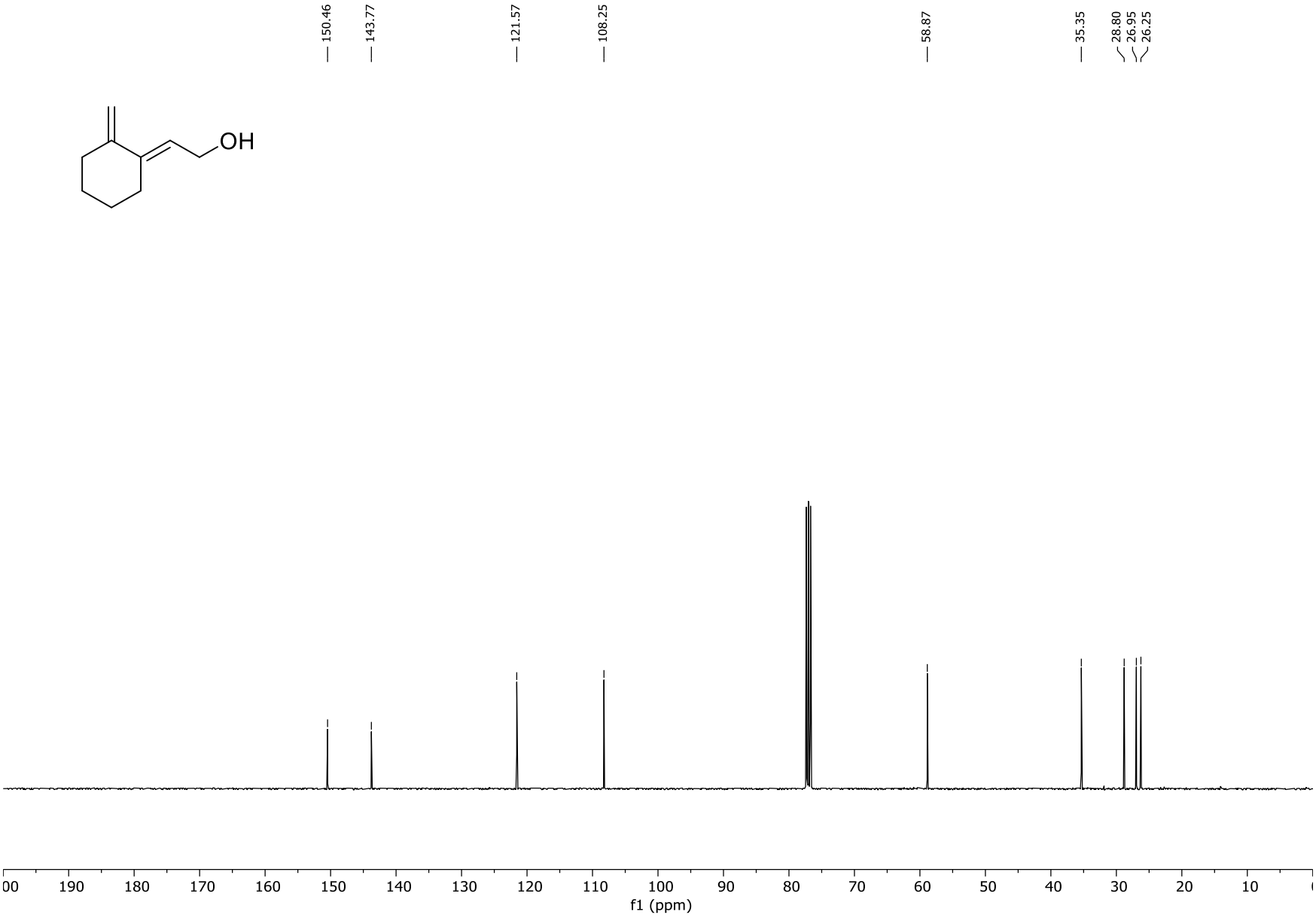
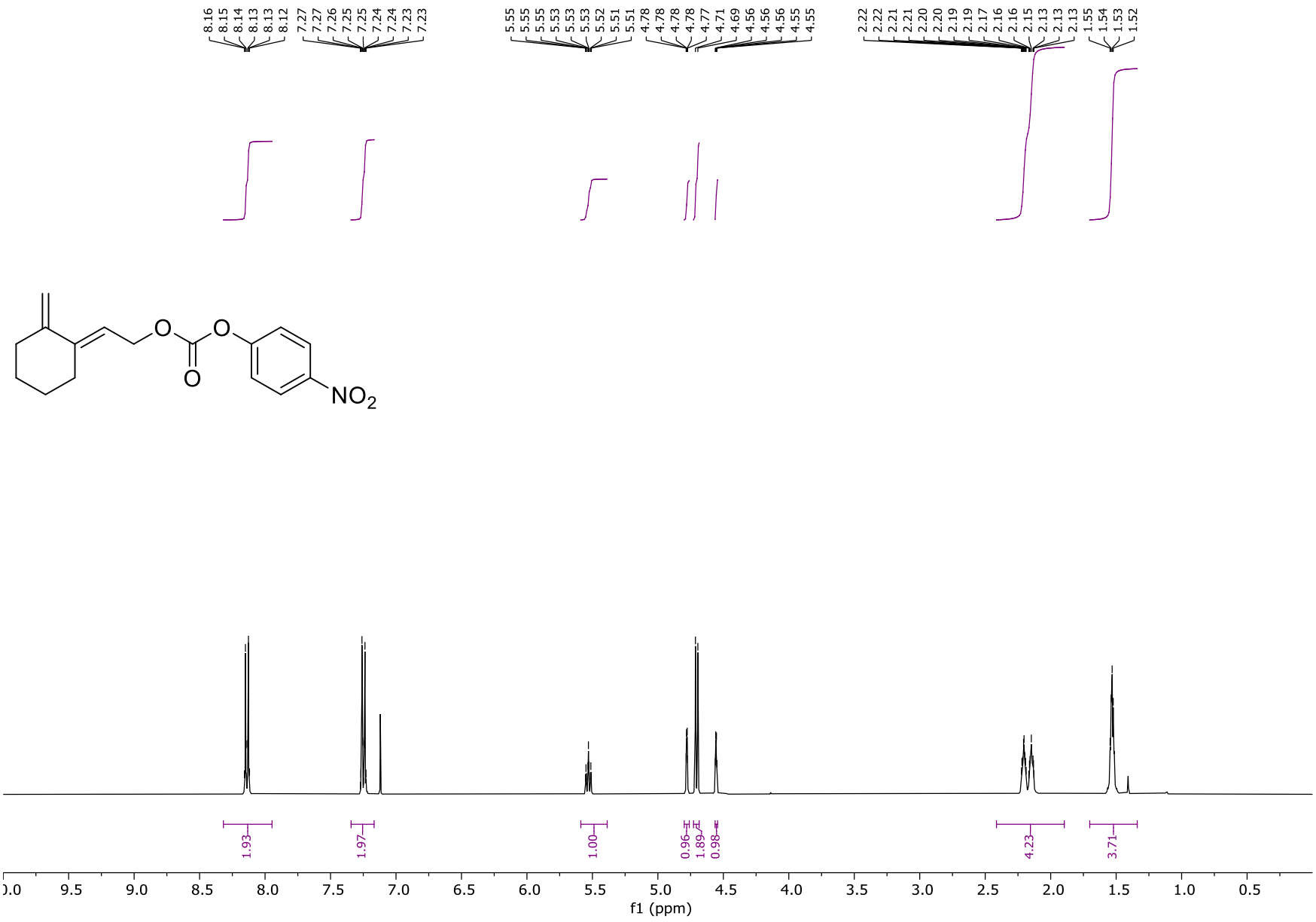
1.82
1.81
1.81
1.80
1.80
1.79
1.78
1.78
1.78
1.77
1.76
1.30
1.29
1.29
1.27
1.00
2.22
2.36
1.97
2.27
2.20
3.31
f1 (ppm)

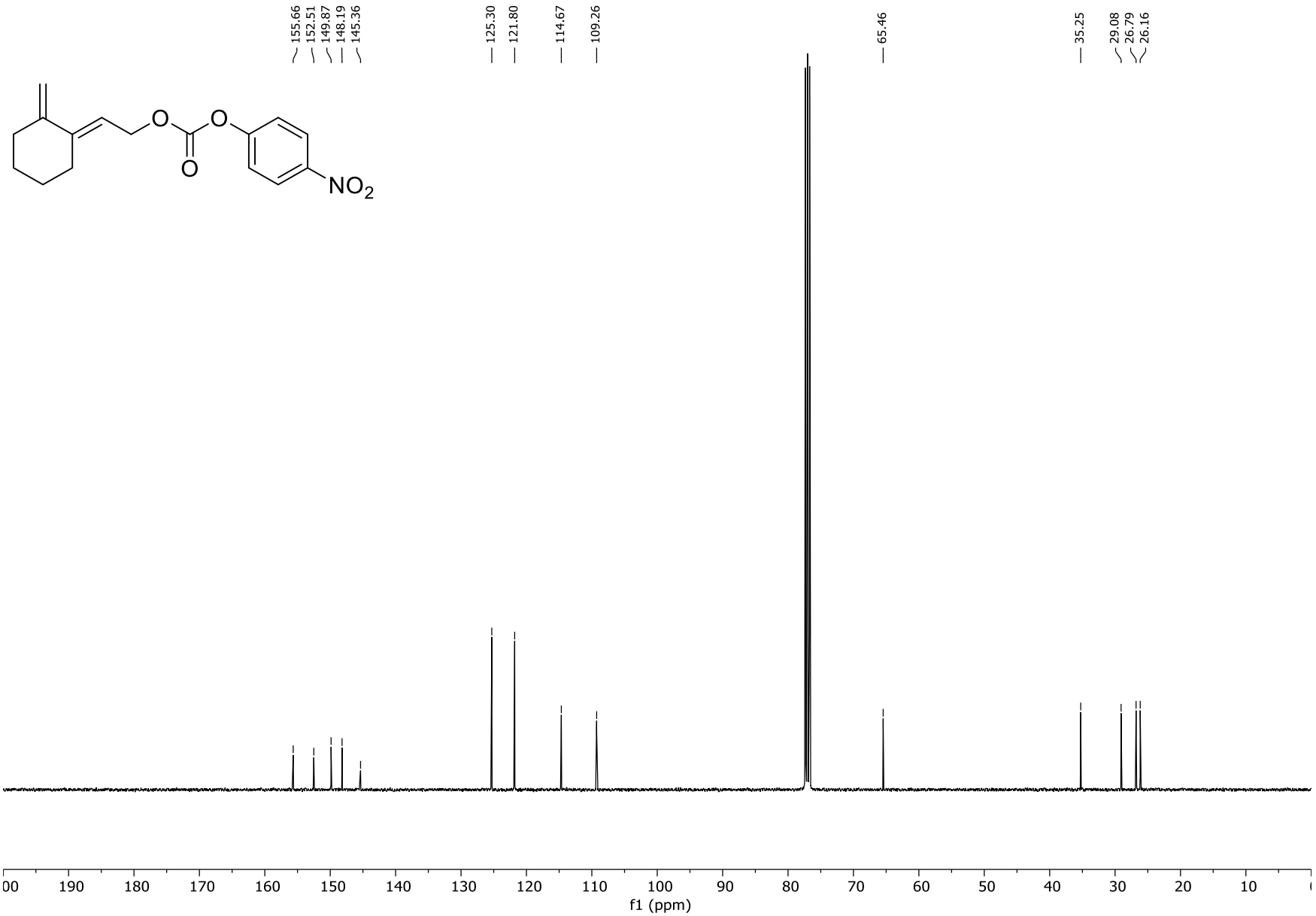

5.83 5.83 5.82 4.99 4.99 4.99 4.98 4.98 4.76 4.76 4.75 4.75 4.18 4.17 4.15 4.13 2.94 2.93 2.93 2.92 2.92 2.92 2.91 2.91 2.90 2.90 2.90 2.34 2.34 2.34 2.33 2.33 2.33 2.32 2.32 2.32 2.31 2.31 2.31 2.30 2.30 1.71 1.70 1.69 1.69 1.68 1.68 1.68 1.67 1.67 1.66 1.31 1.30 1.30 1.29 1.28 1.28 1.27 1.27 1.26
O
O
1.00
1.10
0.95
2.08
2.21
2.16
4.17
3.35
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)

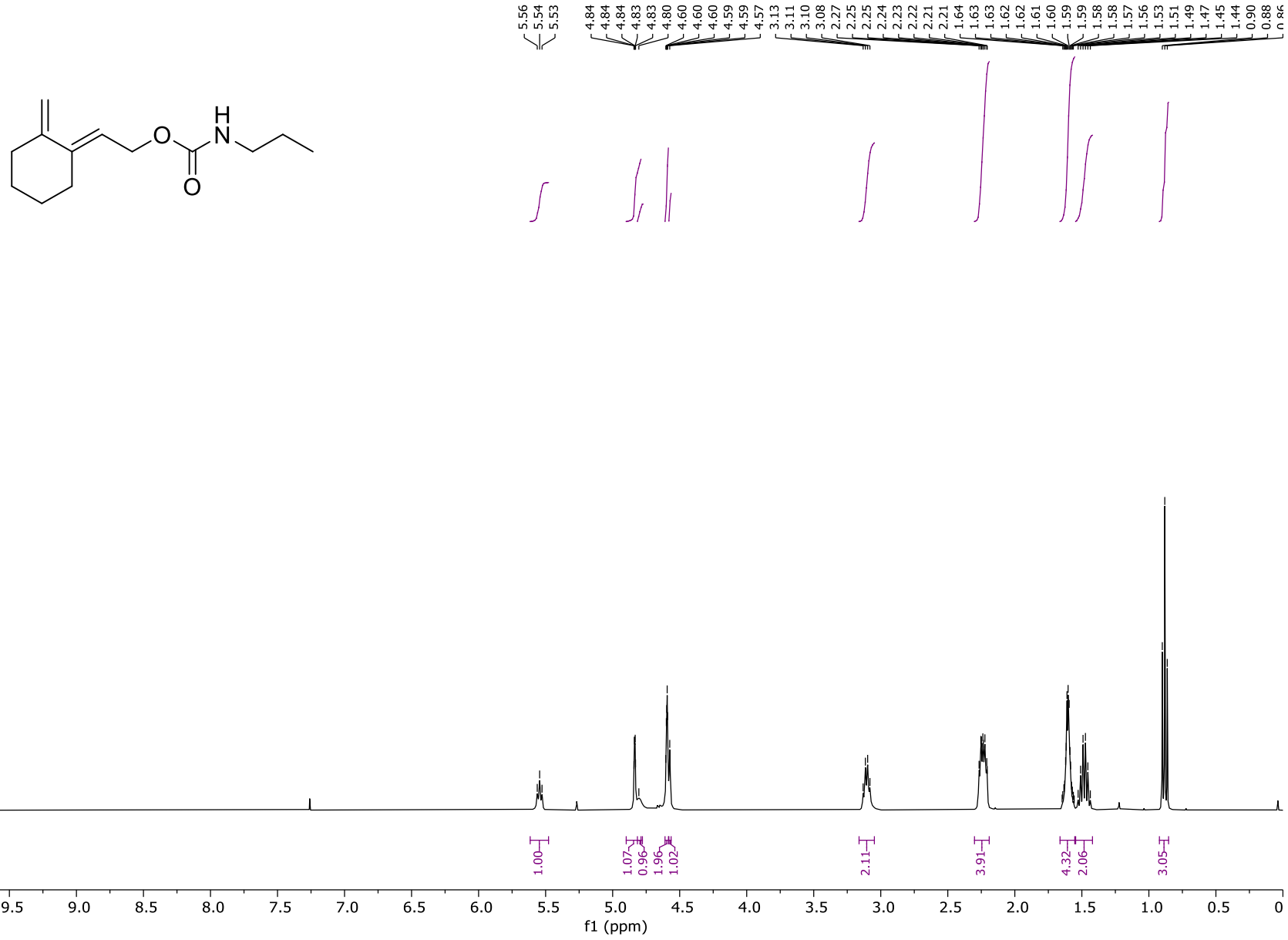

OH

5.68
5.67
5.67
5.66
5.66
5.65
5.64
5.64
5.63
4.87
4.87
4.87
4.86
4.86
4.63
4.63
4.62
4.62
4.62
4.20
4.18
2.28
2.27
2.27
2.27
2.26
2.26
2.26
2.25
2.25
2.24
2.24
2.23
1.67
1.66
1.66
1.66
1.65
1.65
1.65
1.64
1.64
1.64
1.63
1.63
1.62
1.62
1.61
1.61
1.00
1.00
1.03
1.93
4.05
4.18
1.02
f1 (ppm)


150.46
143.77
121.57
108.25
58.87
35.35
28.80
26.95
26.25
OH
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


8.16 8.15 8.14 8.13 8.13 8.12
7.27 7.27 7.26 7.25 7.25 7.24 7.24 7.23 7.23
5.55 5.55 5.55 5.53 5.53 5.52 5.51 5.51
4.78 4.78 4.78 4.77 4.71 4.69 4.56 4.56 4.56 4.55 4.55
2.22 2.22 2.21 2.21 2.20 2.20 2.19 2.19 2.17 2.16 2.16 2.15 2.13 2.13
1.55 1.54 1.53 1.52
O
O
O
NO2
1.93
1.97
1.00
0.96
1.89
0.98
4.23
3.71
f1 (ppm)


155.66
152.51
149.87
148.19
145.36
125.30
121.80
114.67
109.26
65.46
35.25
29.08
26.79
26.16
O
O
O
NO2
f1 (ppm)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5.53
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4.84
4.84
4.83
4.83
4.80
4.60
4.60
4.60
4.59
4.59
4.57
3.13
3.11
3.10
3.08
2.27
2.25
2.24
2.23
2.22
2.21
2.21
1.64
1.63
1.63
1.62
1.62
1.61
1.60
1.59
1.59
1.58
1.58
1.57
1.56
1.53
1.51
1.49
1.47
1.45
1.44
0.90
0.88
O
H
N
O
1.00
1.07
0.96
1.96
1.02
2.11
3.91
4.32
2.06
3.05
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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3.5
3.0
2.5
2.0
1.5
1.0
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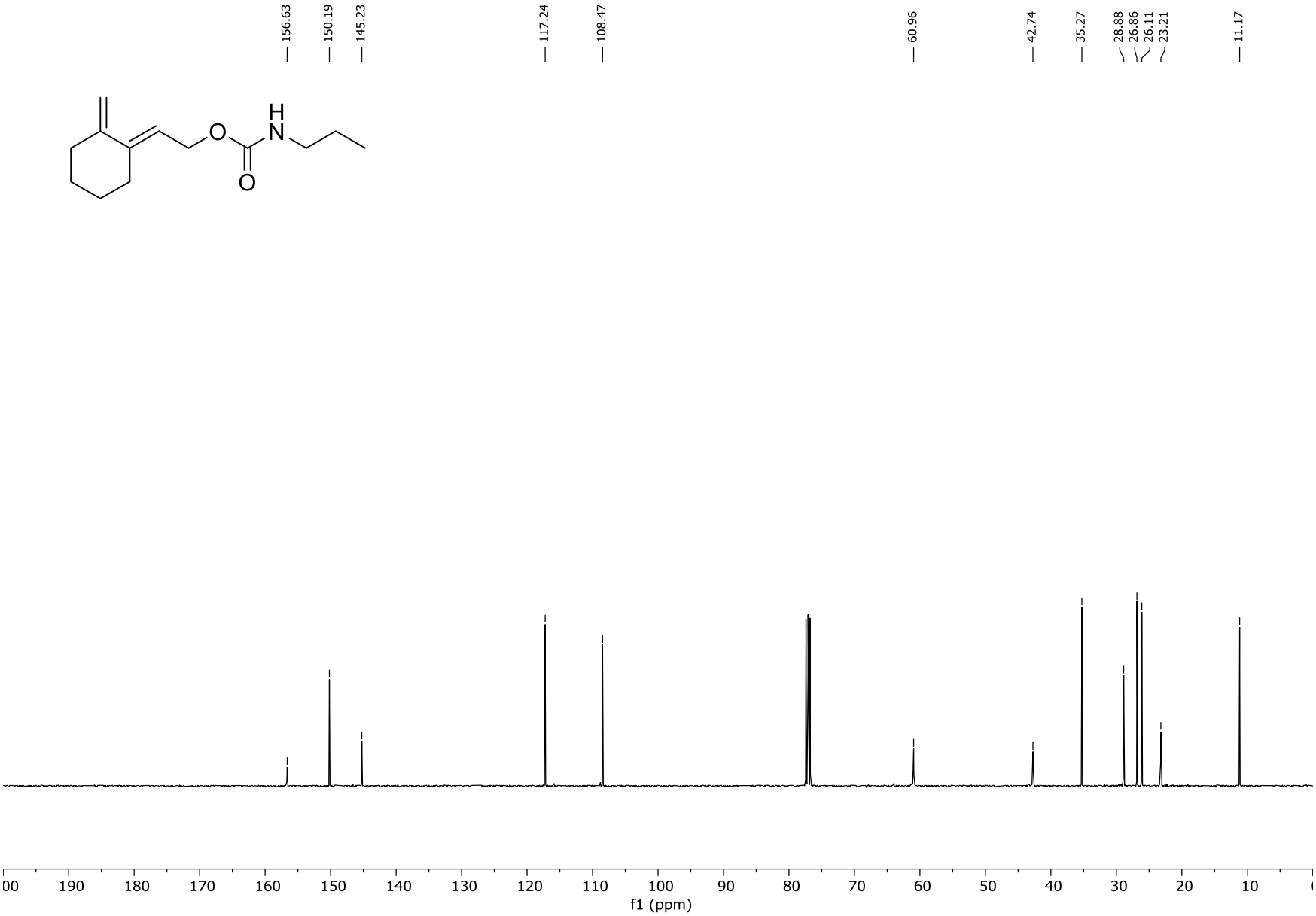
0
f1 (ppm)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60.96
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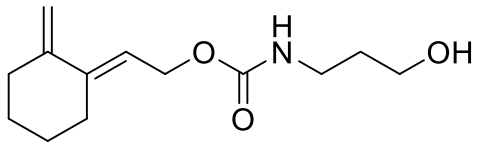
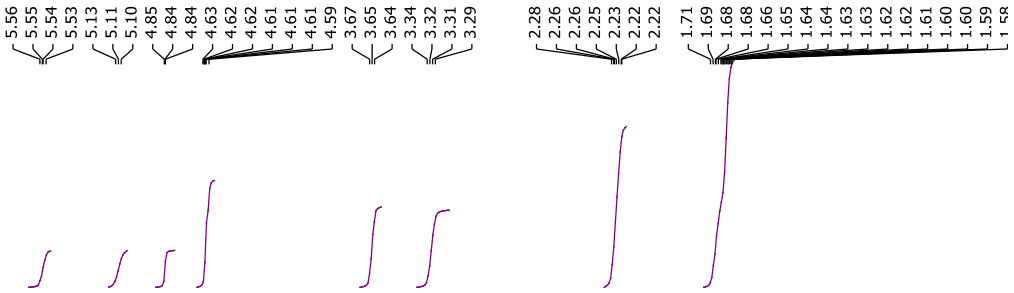
35.27
28.88
26.86
26.11
23.21
11.17
O
H
N
O
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


H
N
O
O
OH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4.84
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4.61
4.61
4.59
3.67
3.65
3.64
3.34
3.32
3.31
3.29
2.28
2.26
2.26
2.25
2.23
2.22
2.22
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1.69
1.68
1.68
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1.63
1.62
1.62
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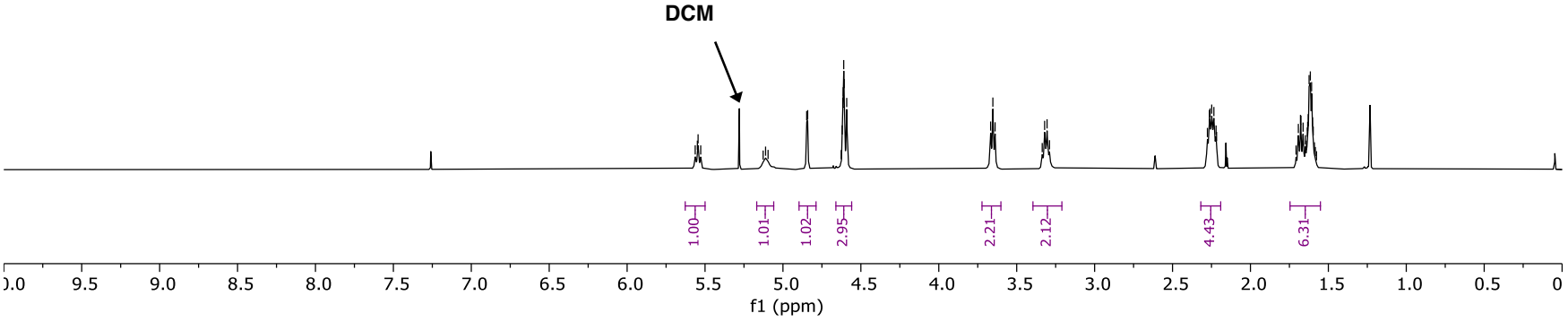
1.60
1.59


DCM
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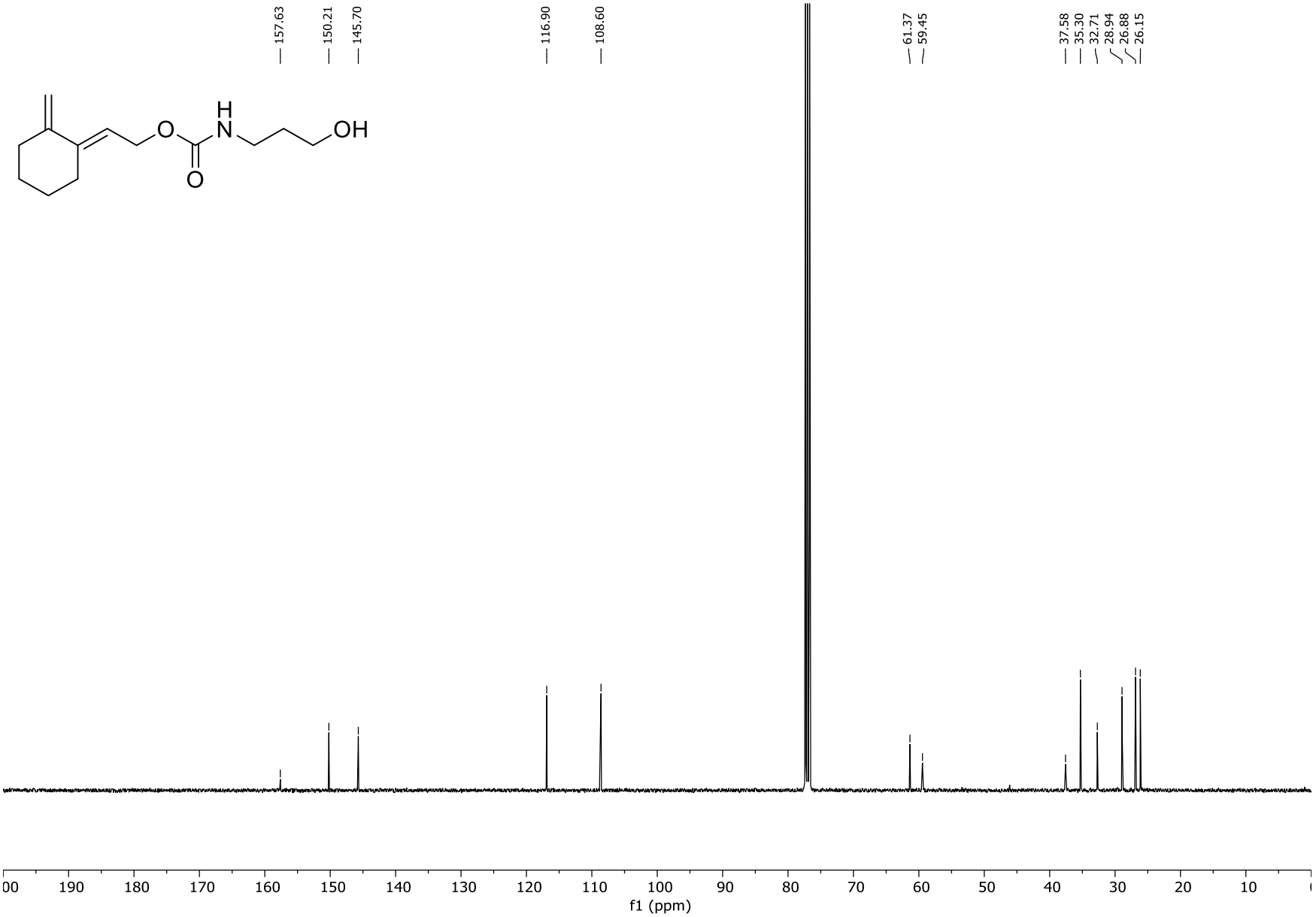
1.01
1.02
2.95
2.21
2.12
4.43
6.31
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
f1 (ppm)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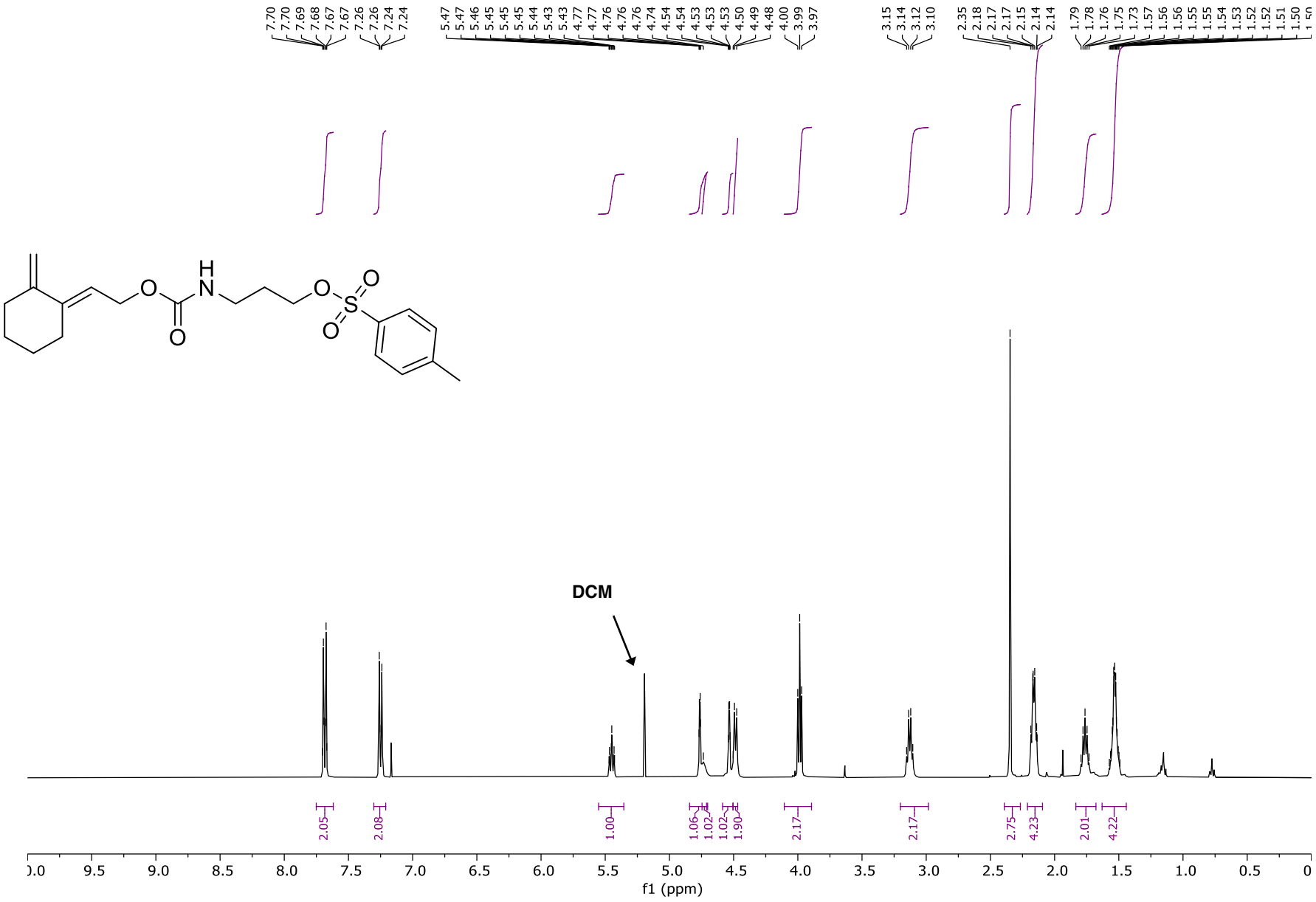
150.21
145.70
116.90
108.60
61.37
59.45
37.58
35.30
32.71
28.94
26.88
26.15
H
N
O
O
OH
f1 (ppm)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7.70
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7.68
7.67
7.67
7.26
7.26
7.24
7.24
5.47
5.47
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5.45
5.45
5.44
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5.43
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4.77
4.76
4.76
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4.74
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4.54
4.53
4.53
4.50
4.49
4.48
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2.18
2.17
2.17
2.15
2.14
2.14
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1.75
1.73
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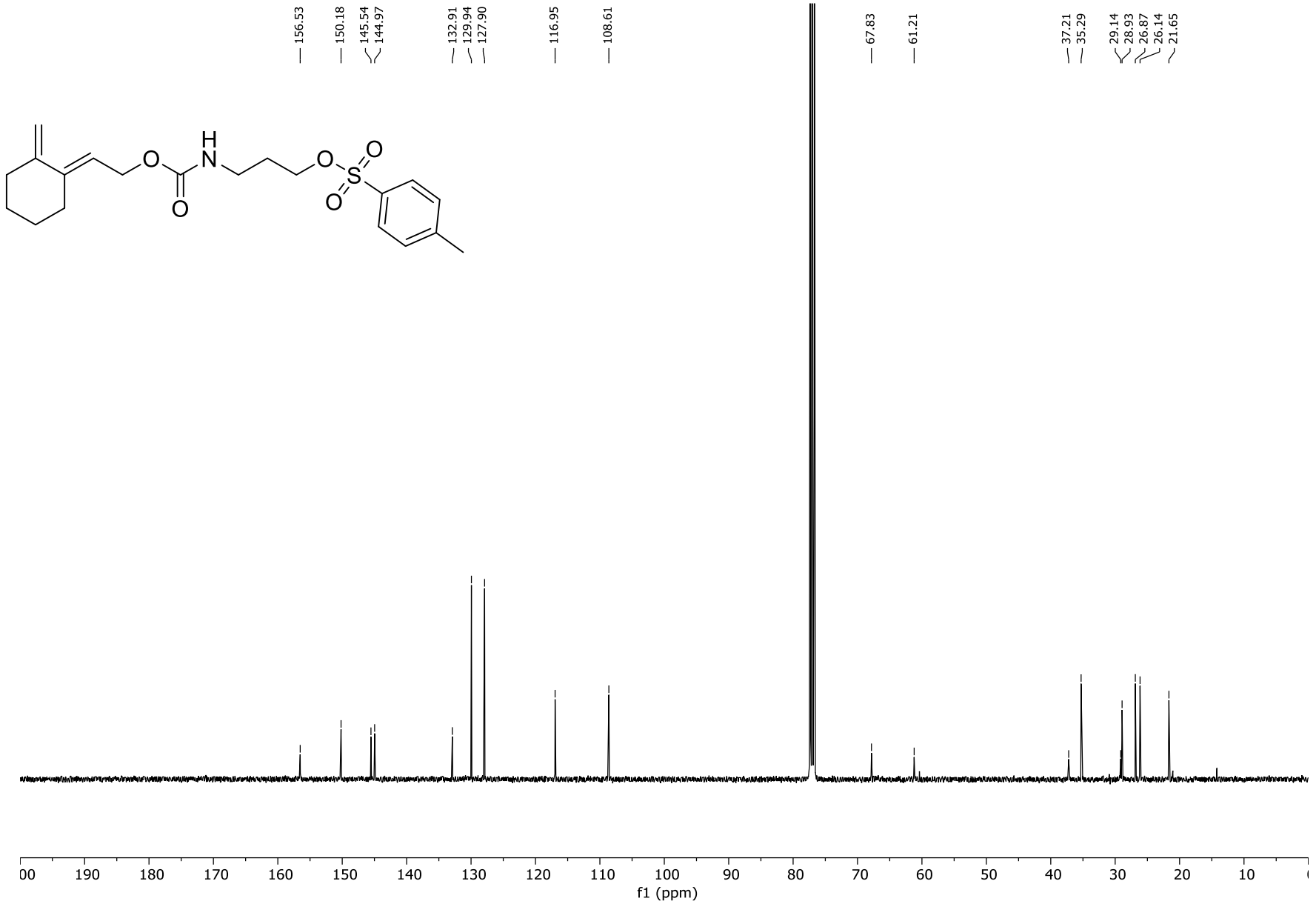
1.56
1.56
1.55
1.55
1.54
1.53
1.52
1.52
1.51
1.50
O
H
N
O
O
S
O
O
DCM
2.05
2.08
1.00
1.06
1.02
1.02
1.90
2.17
2.17
2.75
4.23
2.01
4.22
f1 (ppm)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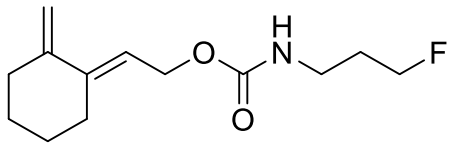
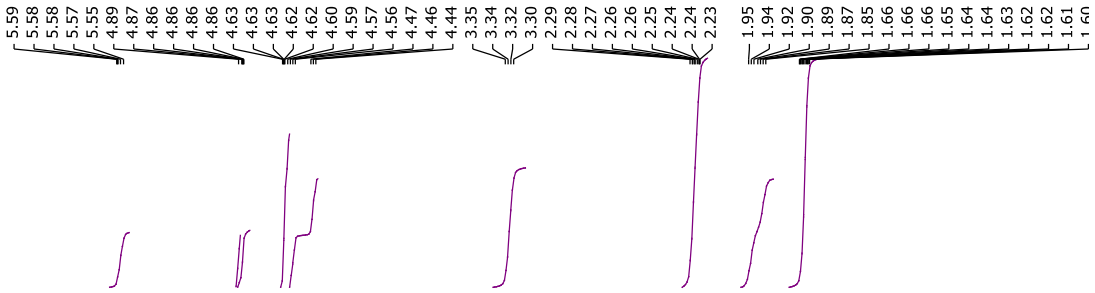
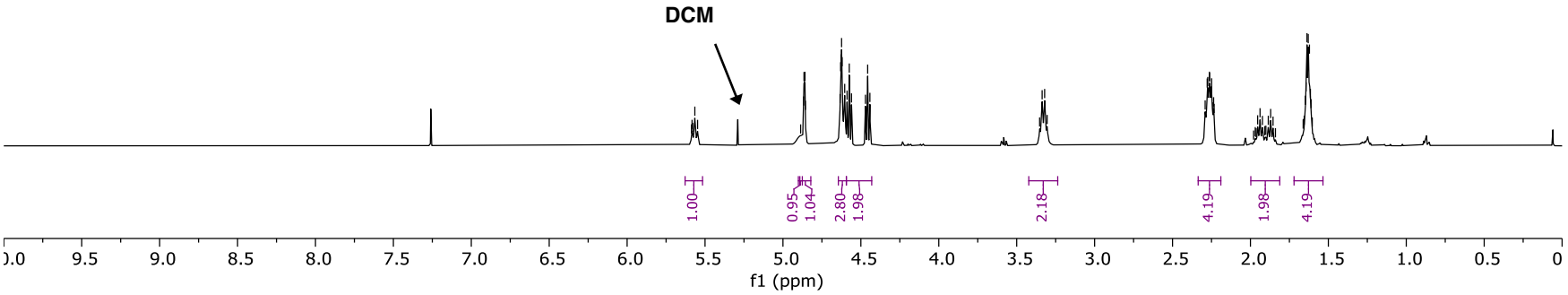
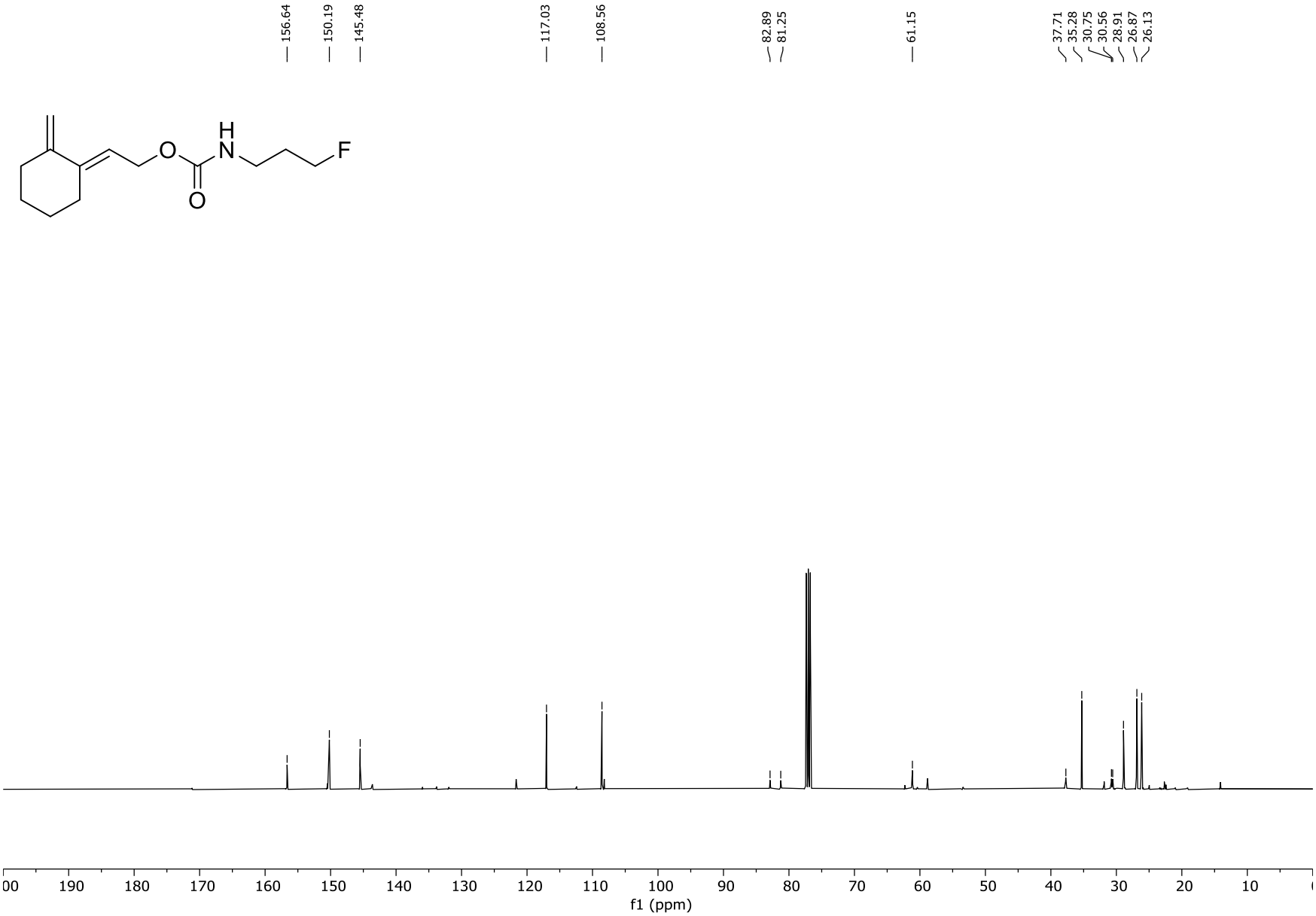
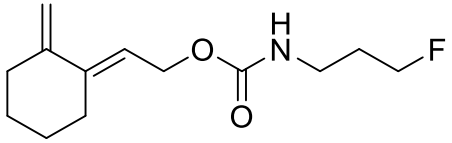
129.94
127.90
116.95
108.61
67.83
61.21
37.21
35.29
29.14
28.93
26.87
26.14
21.65
H
N
O
O
O
O
S
O
O
f1 (ppm)


H
N
O
O
F

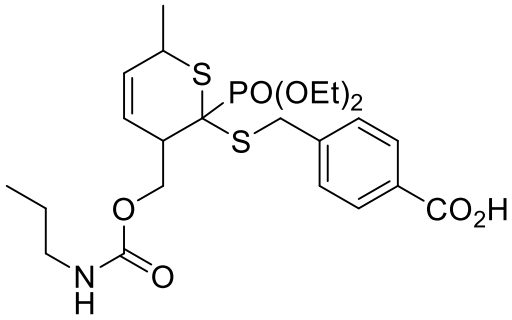

5.59 5.58 5.58 5.57 5.55 4.89 4.87 4.86 4.86 4.86 4.86 4.63 4.63 4.63 4.62 4.62 4.60 4.59 4.57 4.56 4.47 4.46 4.44 3.35 3.34 3.32 3.30 2.29 2.28 2.27 2.26 2.26 2.25 2.24 2.24 2.23 1.95 1.94 1.92 1.90 1.89 1.87 1.85 1.66 1.66 1.66 1.65 1.64 1.64 1.63 1.62 1.62 1.61


DCM
1.00
0.95
1.04
2.80
1.98
2.18
4.19
1.98
4.19
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)

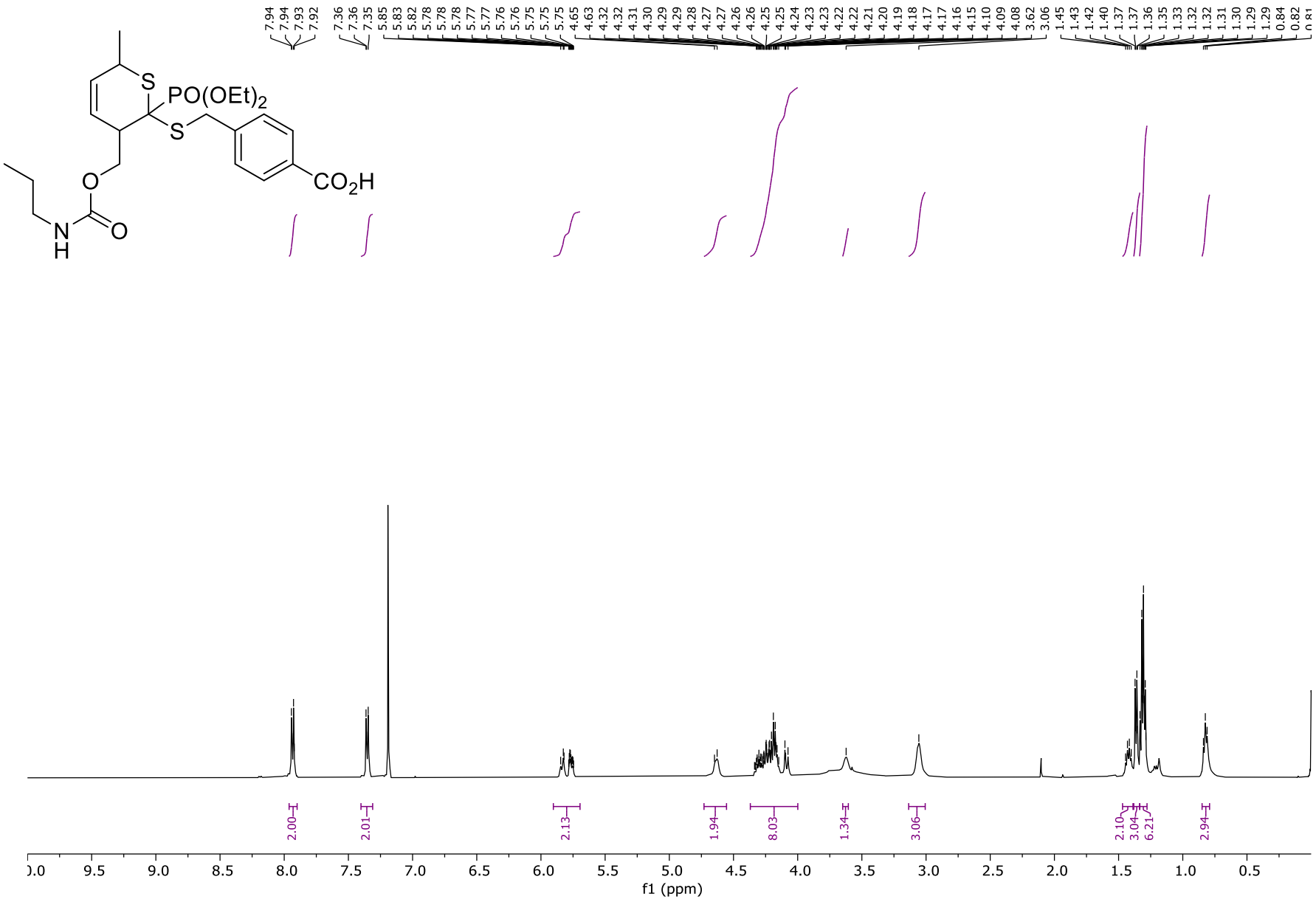

156.64
150.19
145.48
117.03
108.56
82.89
81.25
61.15
37.71
35.28
30.75
30.56
28.91
26.87
26.13
H
N
O
O
F
f1 (ppm)

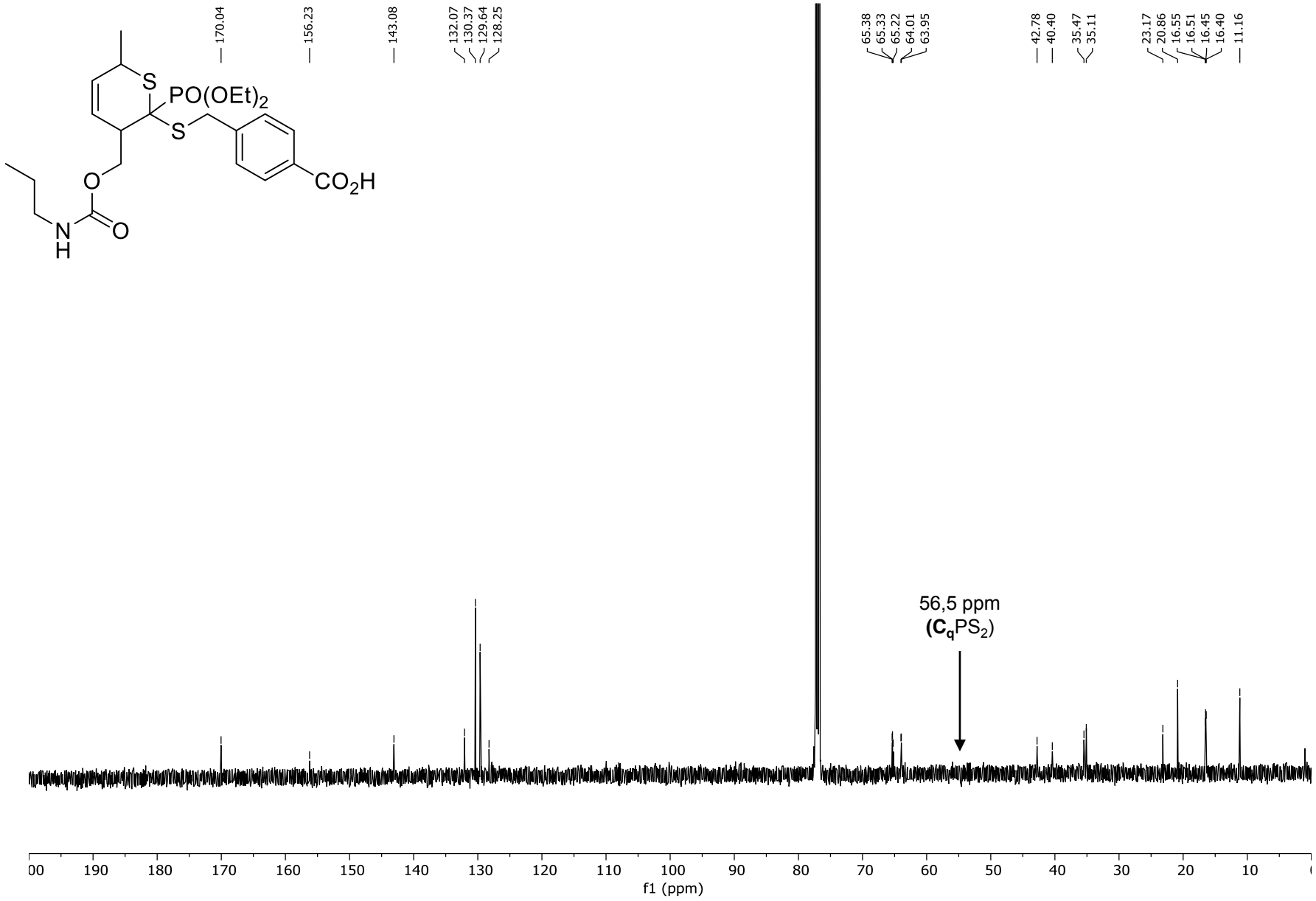

H
N
O
O
F

-221.00
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
-240
f1 (ppm)

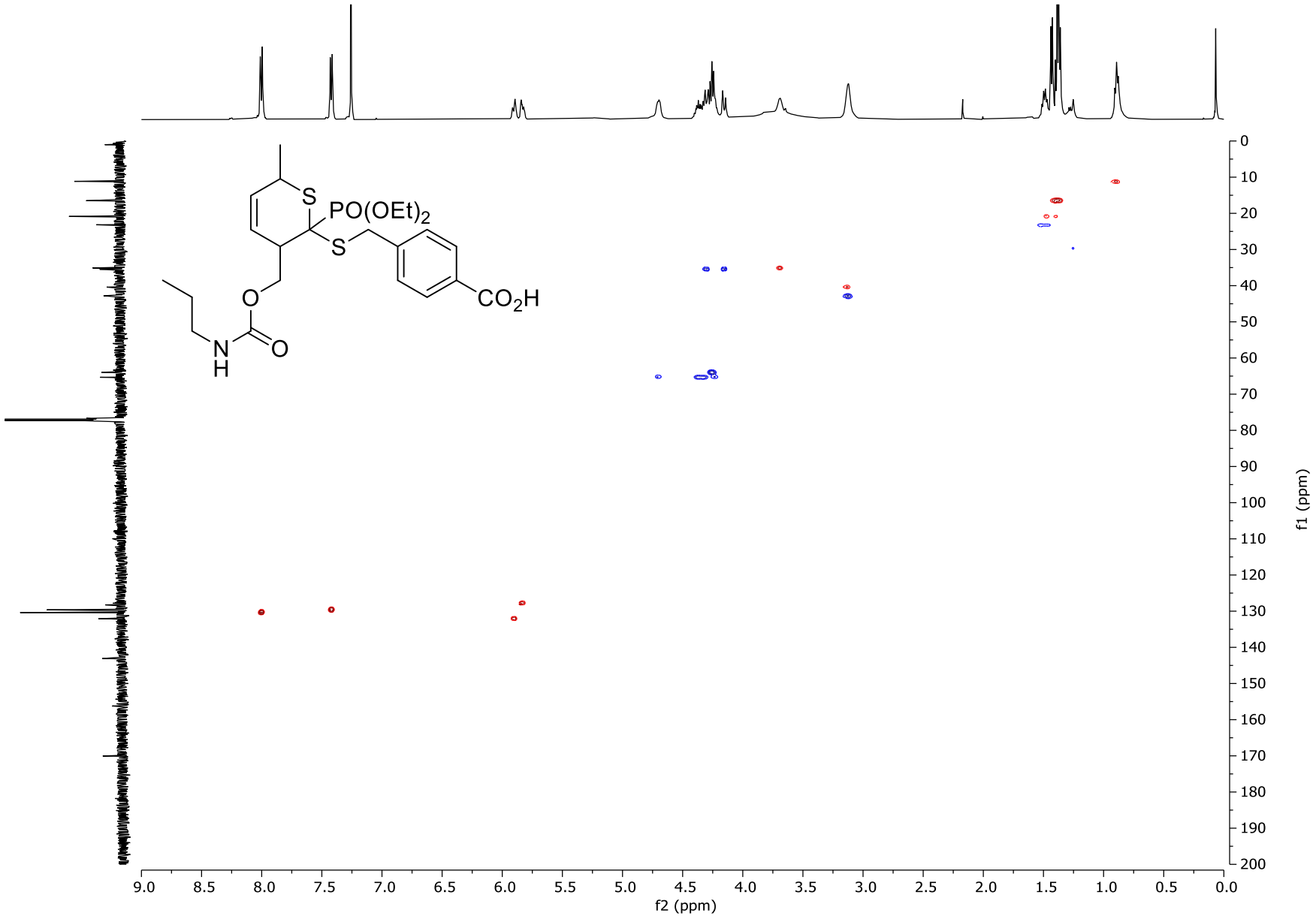

S
PO(OEt)2
S
O
CO2H
N
H
O

20.07
19.63
19.49
18.28
14.70
24.15
29.00
32.08
290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)

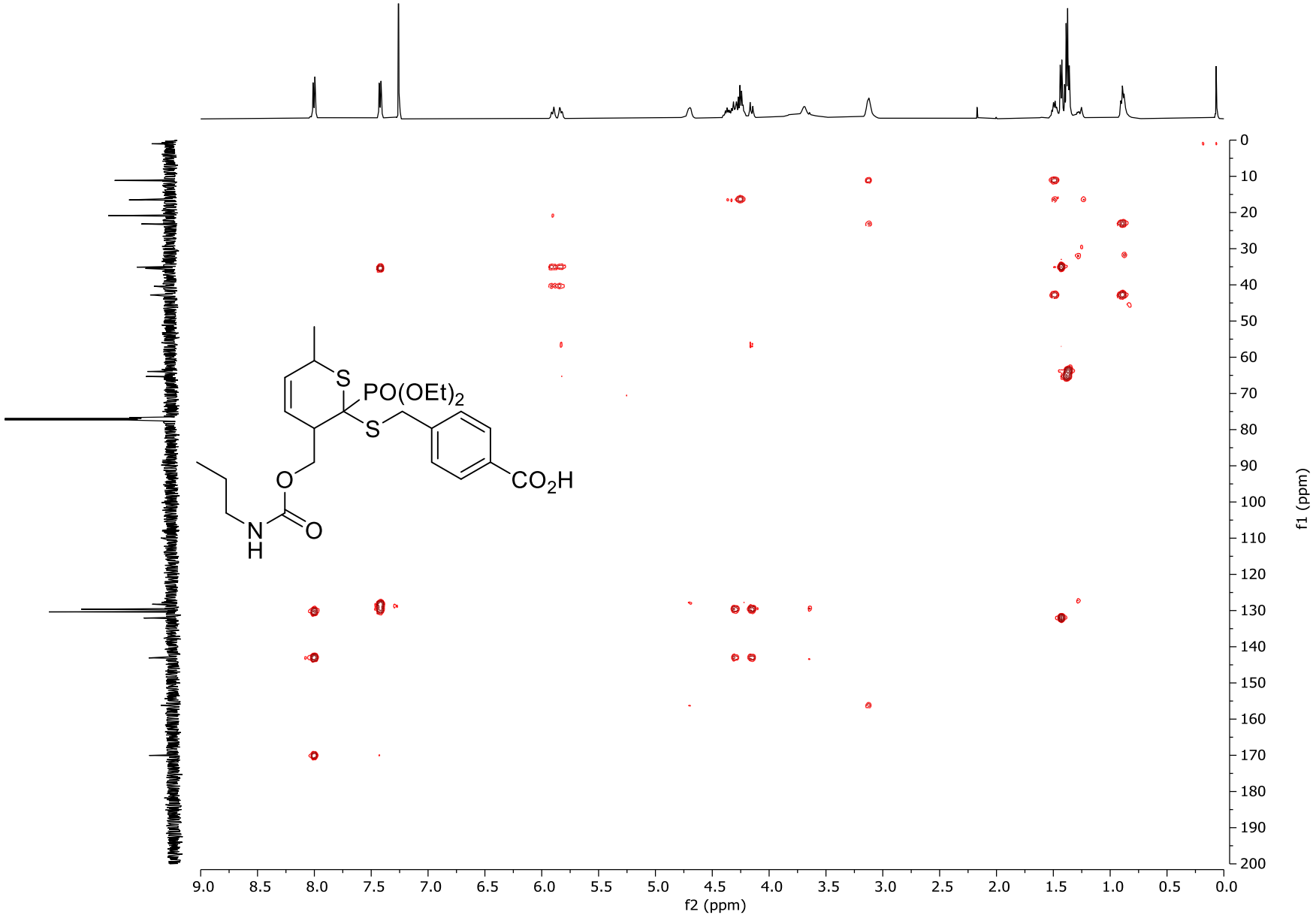

PO(OEt)2
S
S
O
N
H
O
CO2H
f1 (ppm)

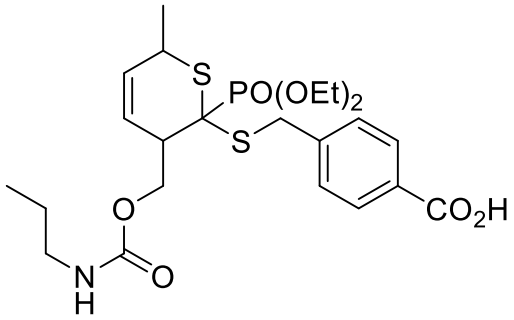

170.04
156.23
143.08
132.07
130.37
129.64
128.25
65.38
65.33
65.22
64.01
63.95
42.78
40.40
35.47
35.11
23.17
20.86
16.55
16.51
16.45
16.40
11.16
S
PO(OEt)2
S
O
CO2H
N
H
O
56,5 ppm
(CqPS2)
f1 (ppm)

Fraction 1

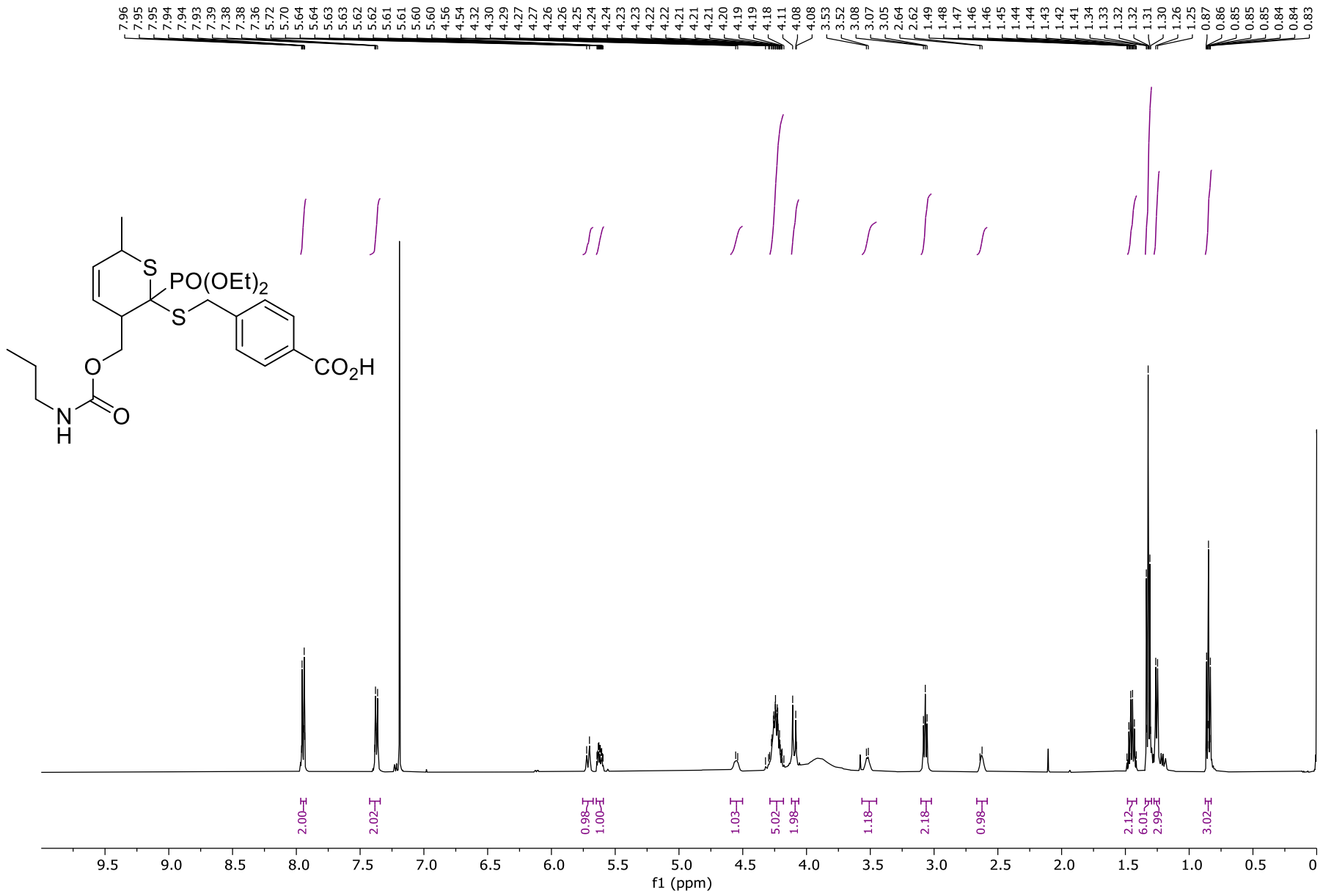


Fraction 1

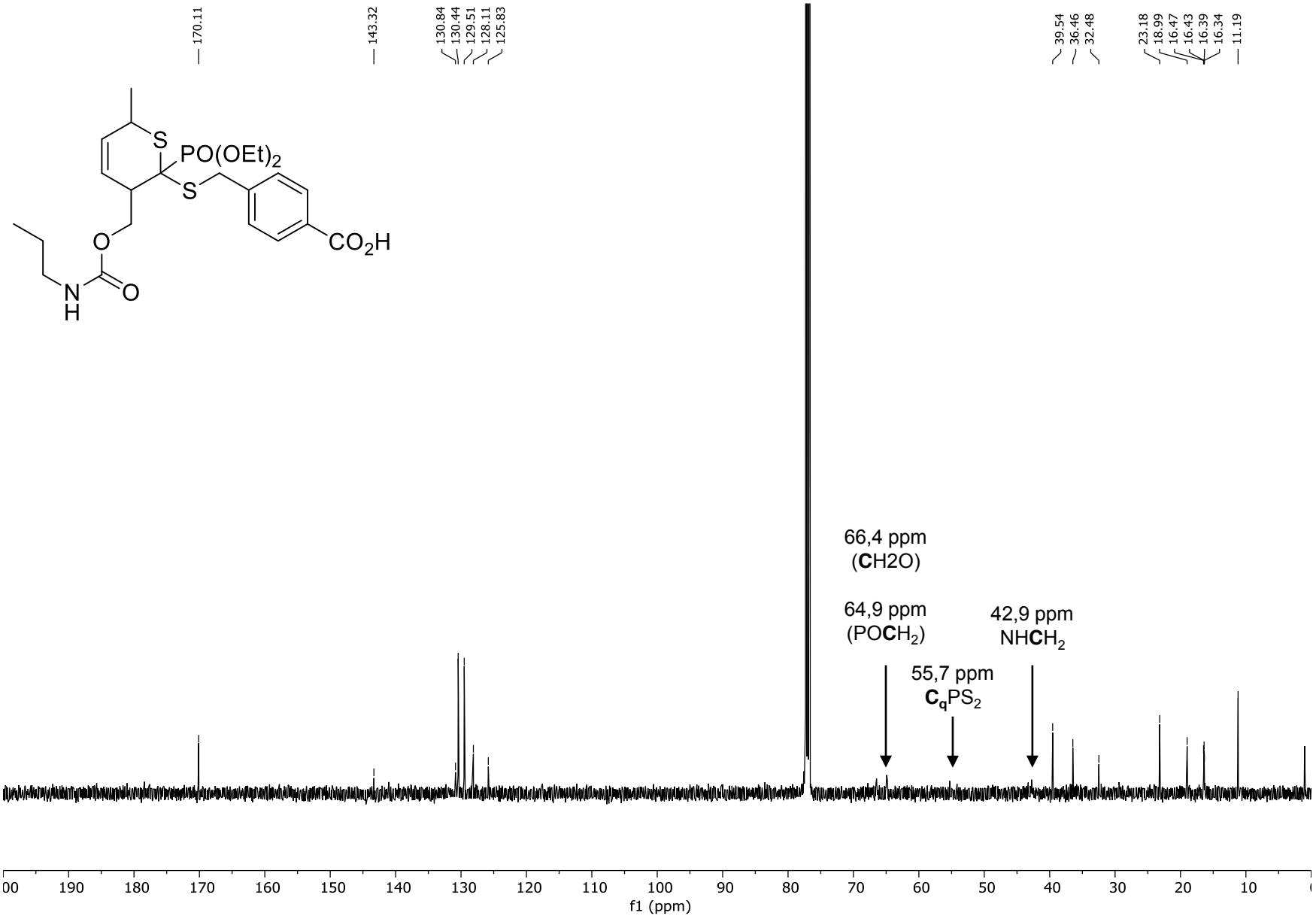



S
PO(OEt)2
S
O
CO2H
N
H
O

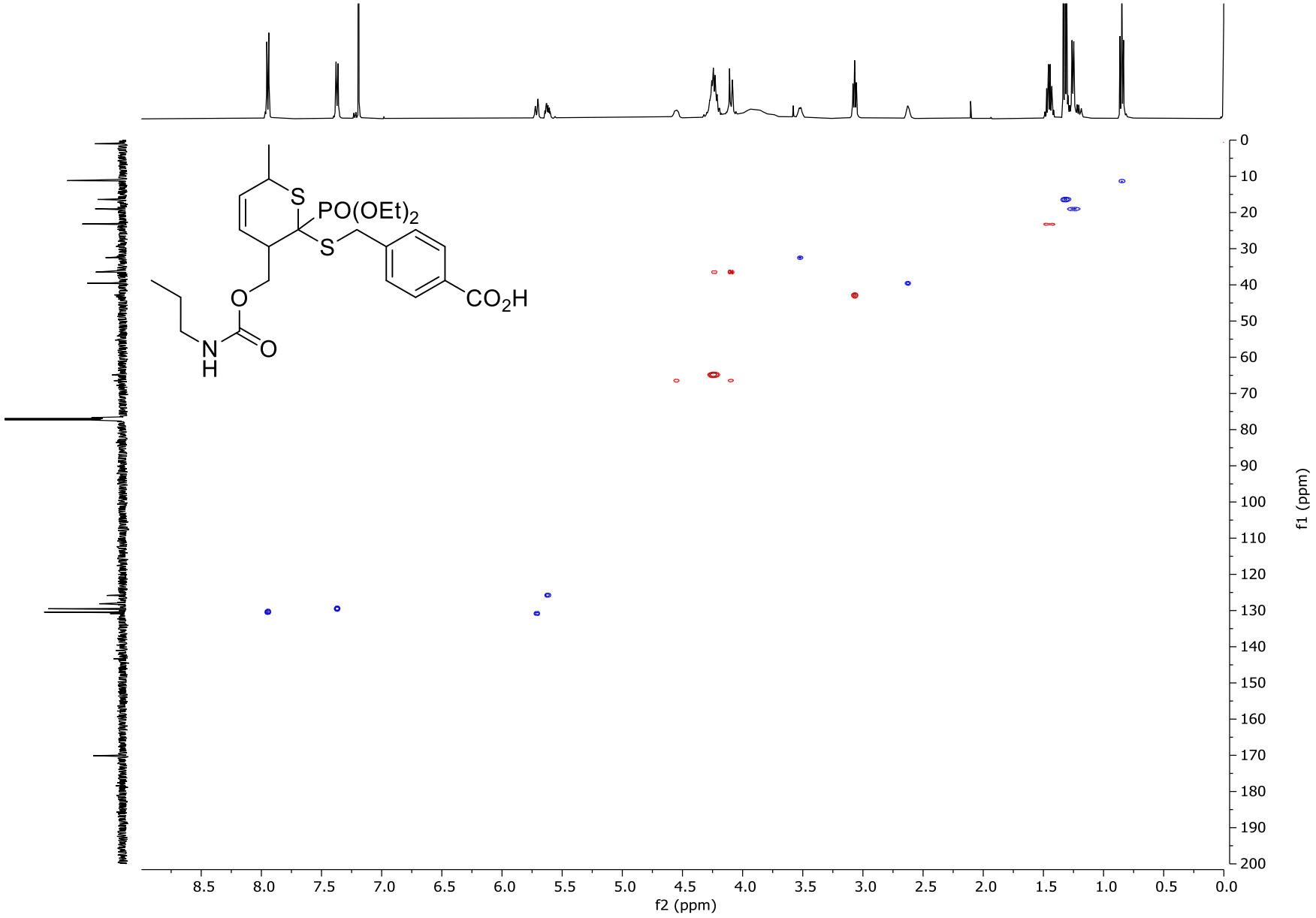
19.63
290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)


7.96 7.95 7.95 7.94 7.94 7.93 7.39 7.38 7.38 7.36 5.72 5.70 5.64 5.64 5.63 5.63 5.62 5.62 5.61 5.61 5.60 5.60 4.56 4.54 4.32 4.30 4.29 4.27 4.27 4.26 4.26 4.25 4.24 4.24 4.23 4.23 4.22 4.22 4.21 4.21 4.21 4.20 4.19 4.19 4.18 4.11 4.08 4.08 3.53 3.52 3.08 3.07 3.05 2.64 2.62 1.49 1.48 1.47 1.46 1.46 1.45 1.44 1.44 1.43 1.42 1.41 1.34 1.33 1.32 1.32 1.31 1.30 1.26 1.25 0.87 0.86 0.85 0.85 0.85 0.84 0.84 0.83
S
PO(OEt)2
S
O
CO2H
N
H
O
2.00
2.02
0.98
1.00
1.03
5.02
1.98
1.18
2.18
0.98
2.12
6.01
2.99
3.02
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)

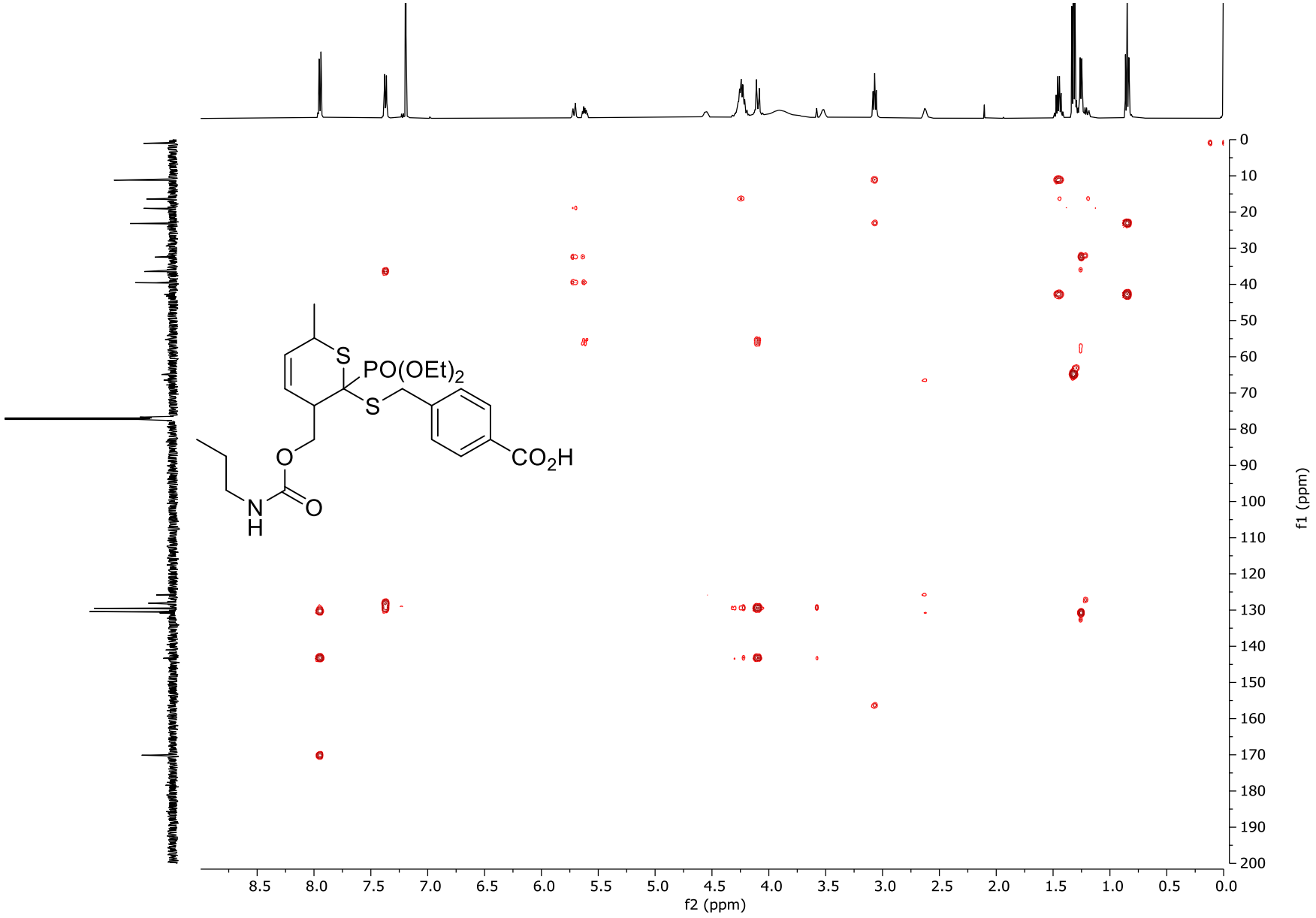
Fraction 2

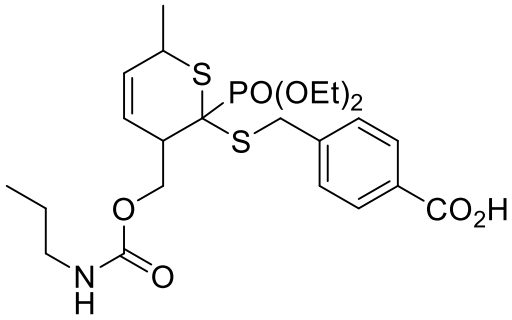

170.11
143.32
130.84
130.44
129.51
128.11
125.83
39.54
36.46
32.48
23.18
18.99
16.47
16.43
16.39
16.34
11.19
S
PO(OEt)2
S
O
CO2H
N
H
O
66,4 ppm
(CH2O)
64,9 ppm
(POCH2)
55,7 ppm
CqPS2
42,9 ppm
NHCH2
f1 (ppm)

Fraction 2

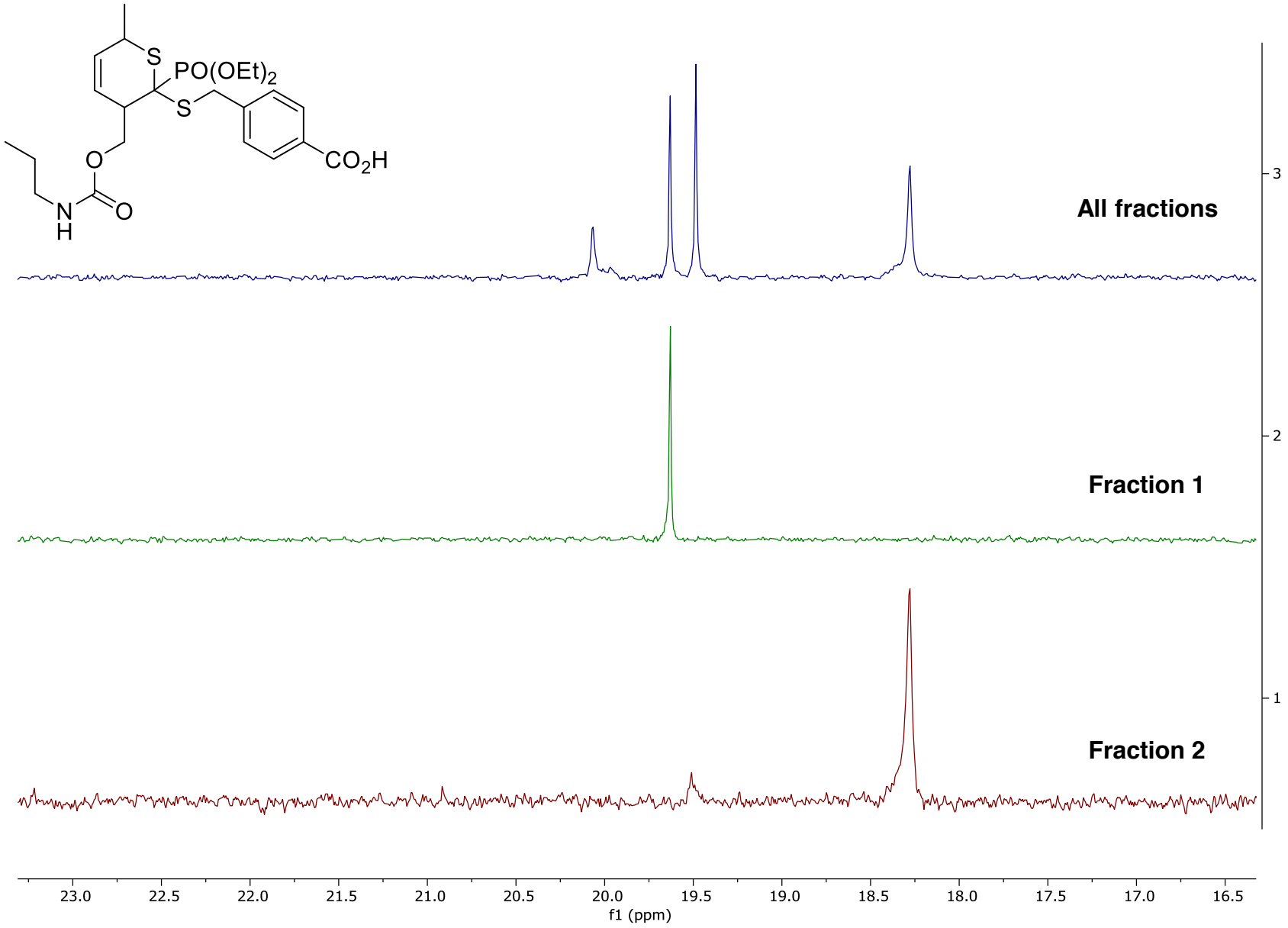


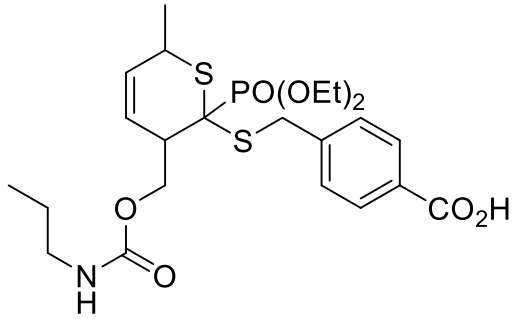
Fraction 2

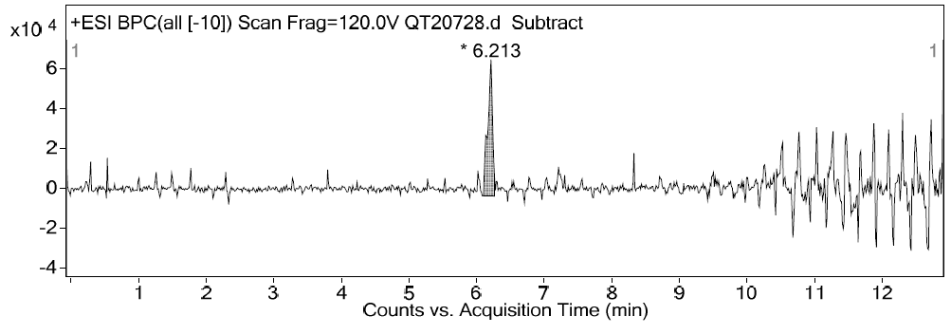



S
PO(OEt)2
S
O
CO2H
N
H
O

18.28
290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)

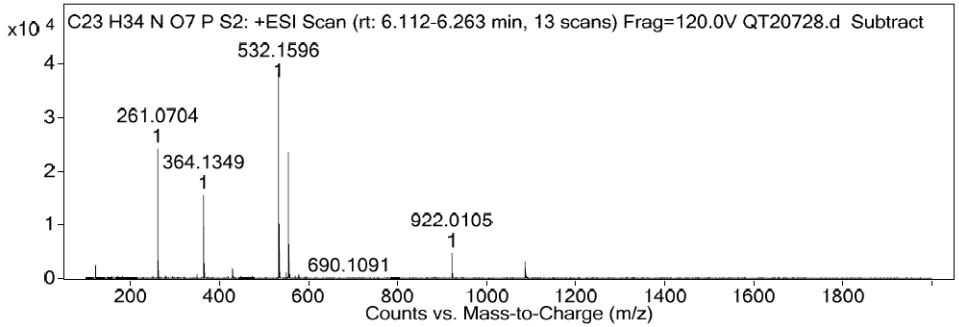

PO(OEt)2
S
S
O
O
N
H
CO2H
All fractions
Fraction 1
Fraction 2
3
2
1
23.0
22.5
22.0
21.5
21.0
20.5
20.0
19.5
19.0
18.5
18.0
17.5
17.0
16.5
f1 (ppm)

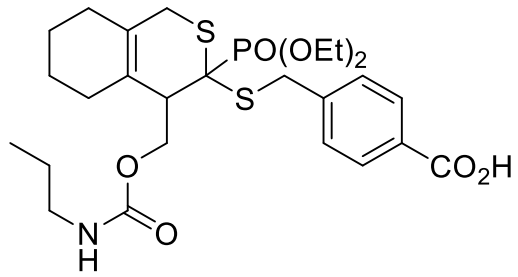




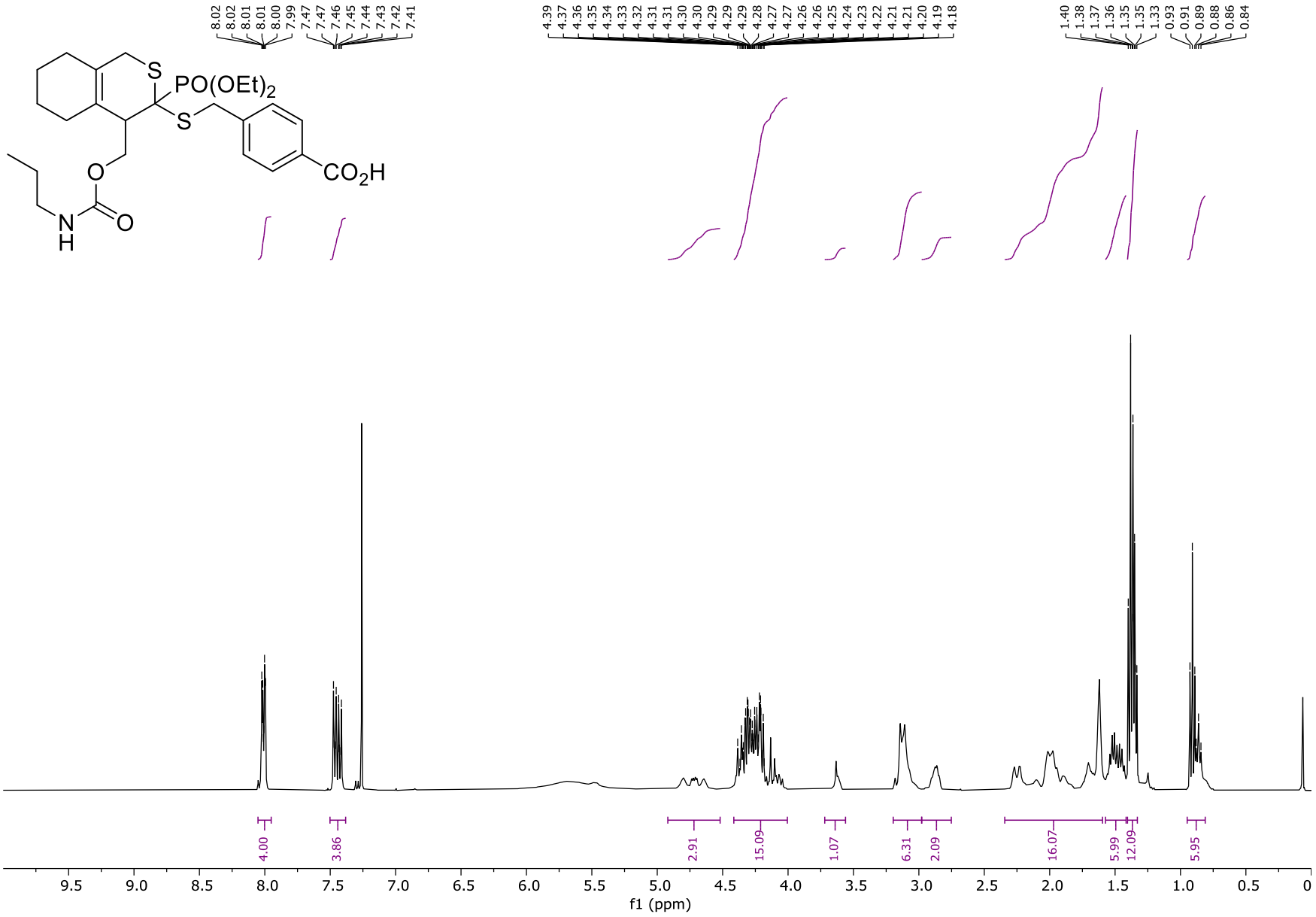
Integration Peak List

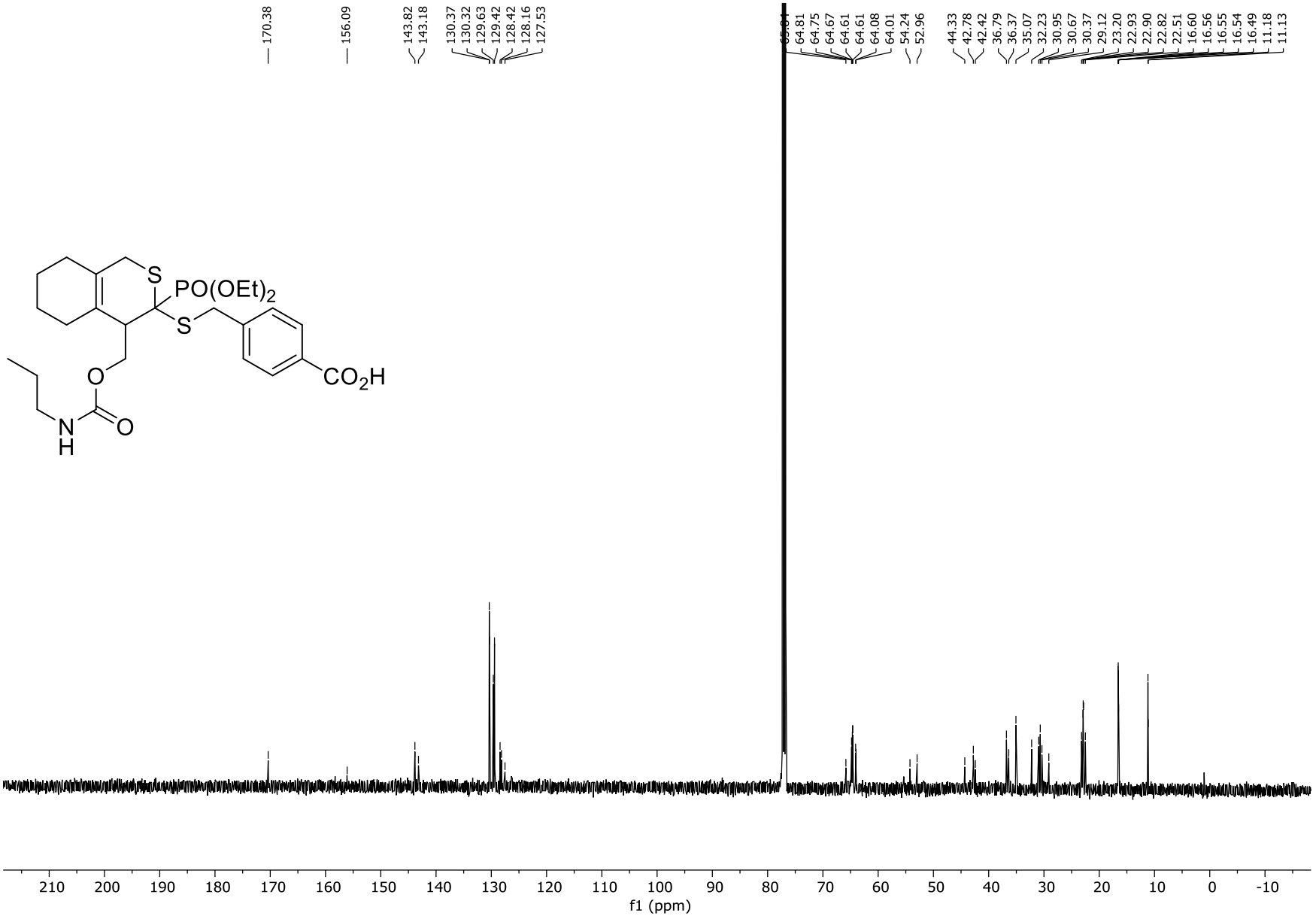
Peak	Start	RT	End	Height	Area	Area %	AreaSum%
1	6.085	6.213	6.263	67996.8	335085.19	100	100

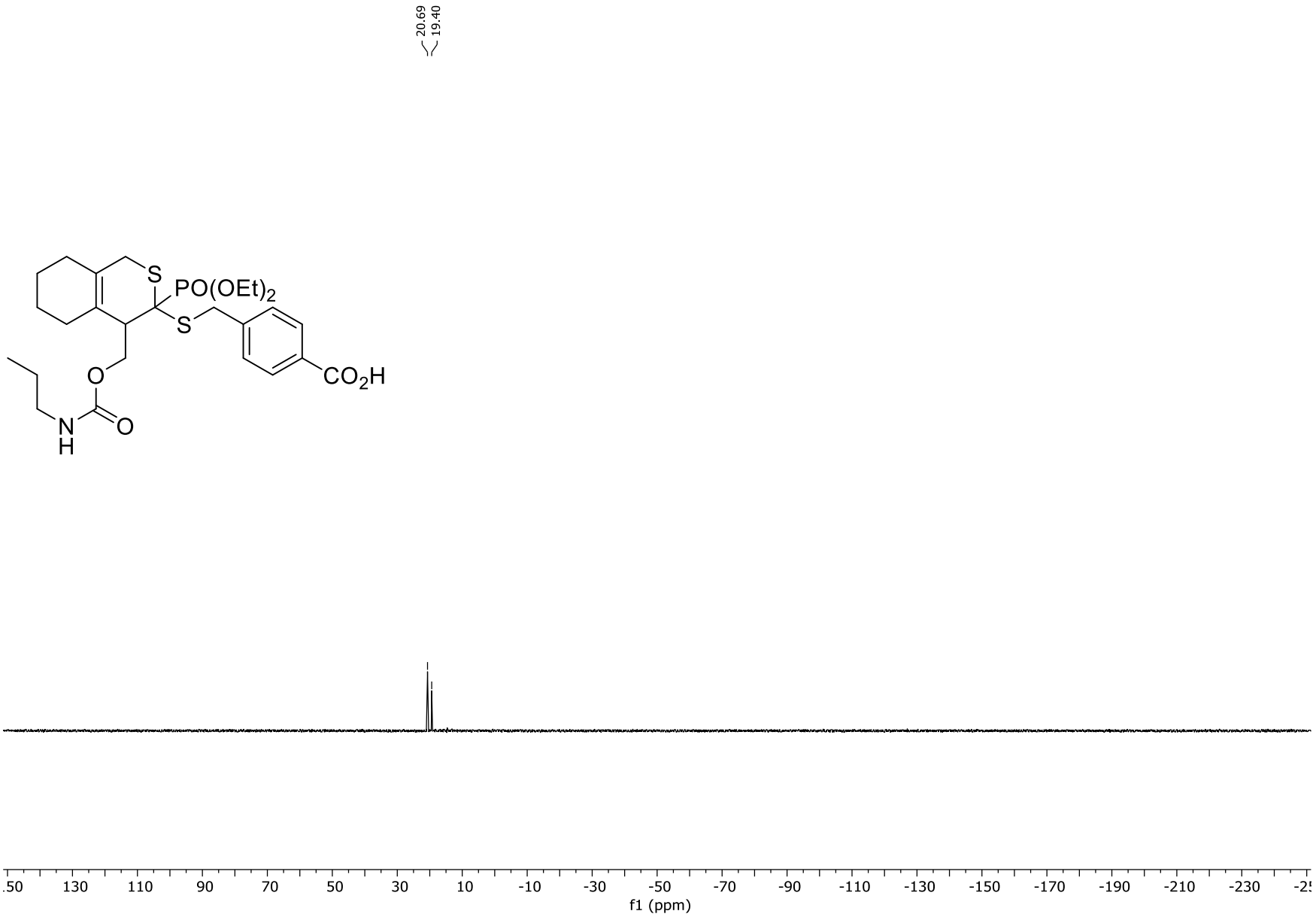


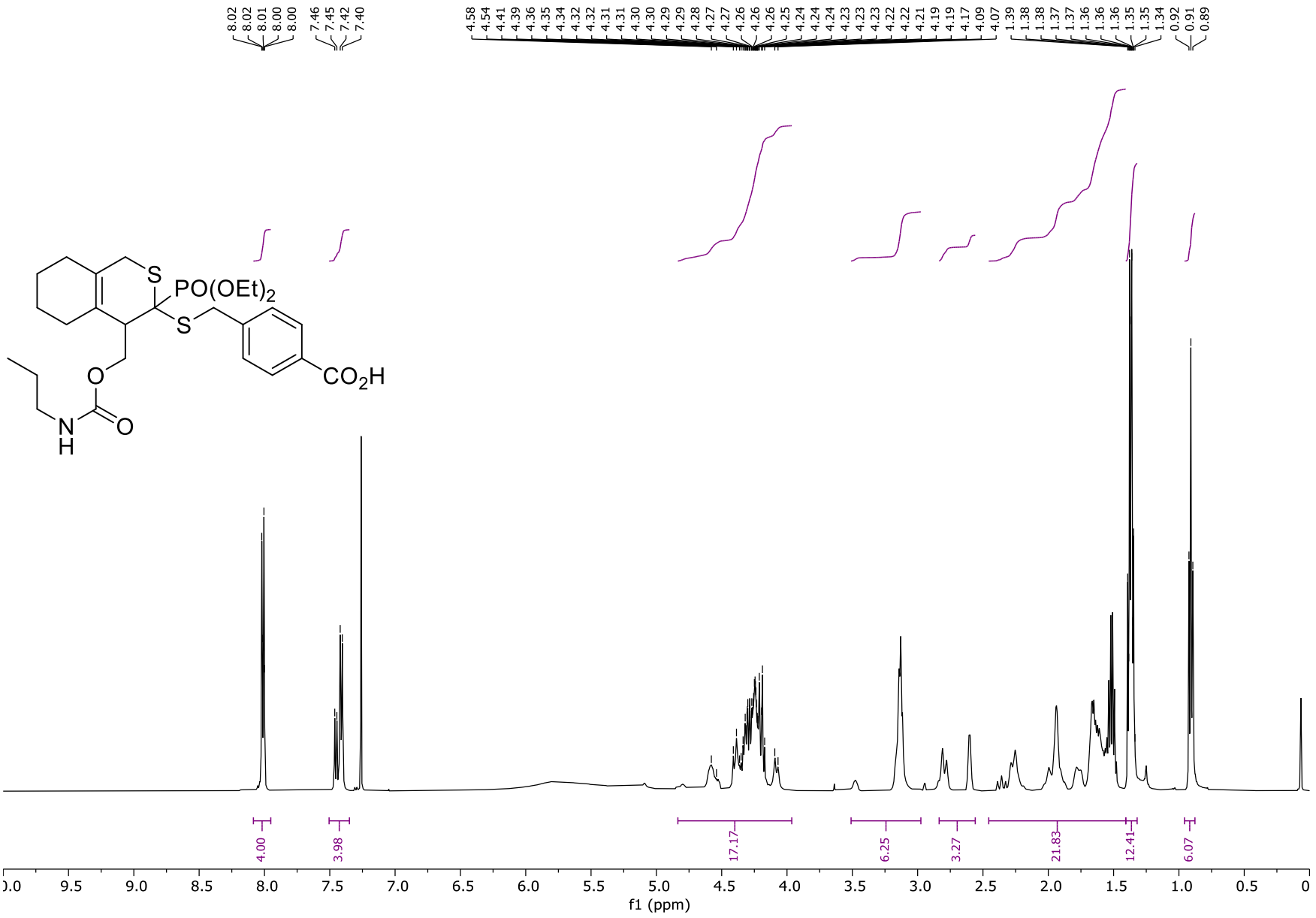

S
PO(OEt)2
S
O
CO2H
N
H
O

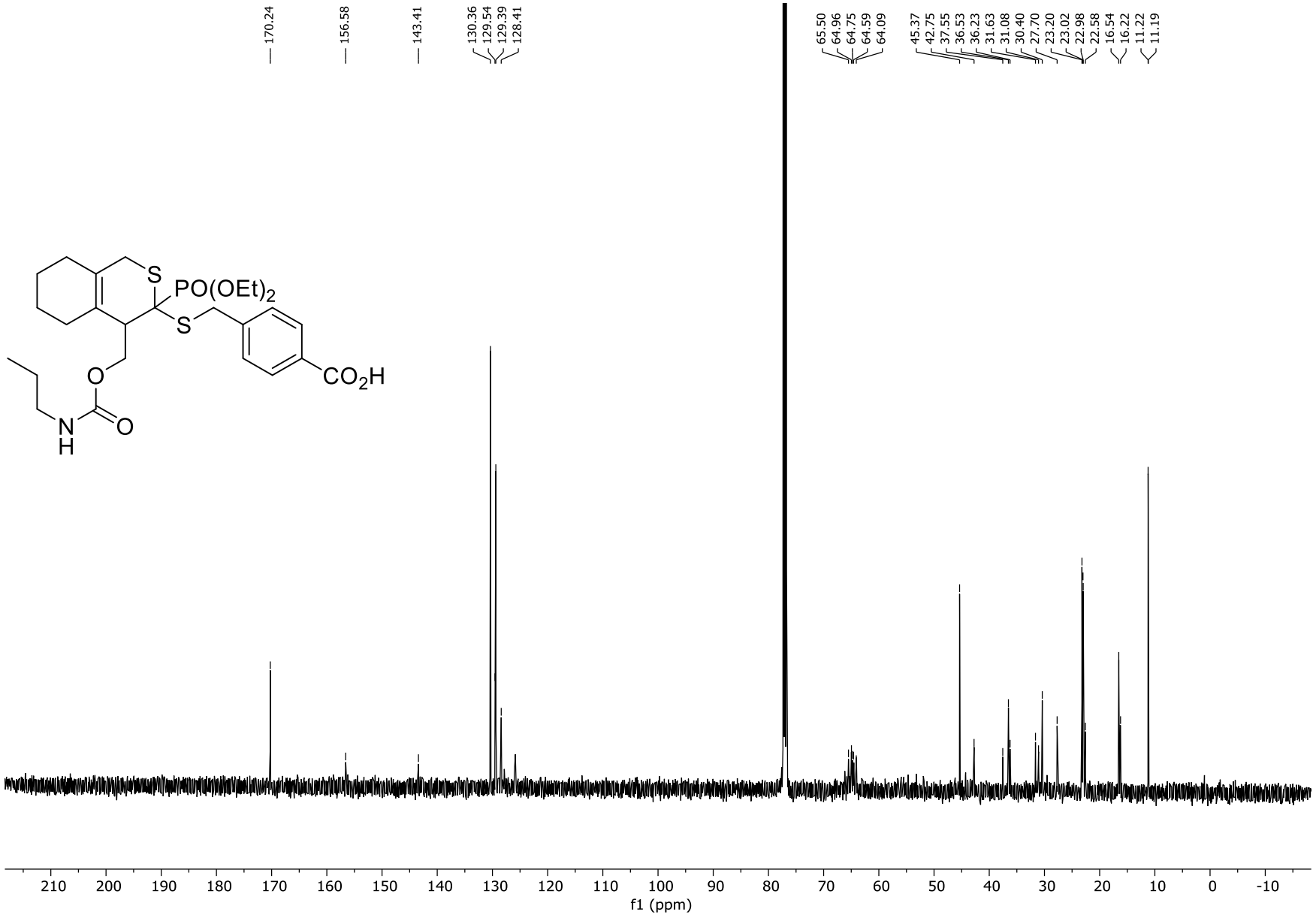
20.73
19.75
19.42
18.29
24.30
10.83
17.31
47.60
290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)

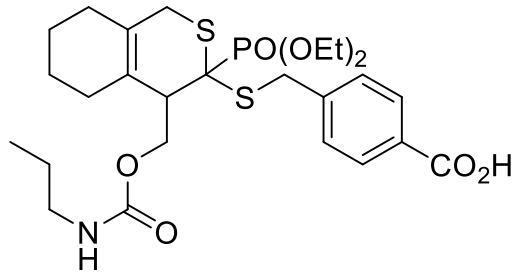

8.02
8.02
8.01
8.01
8.00
7.99
7.47
7.47
7.46
7.45
7.44
7.43
7.42
7.41
4.39
4.37
4.36
4.35
4.34
4.33
4.32
4.31
4.31
4.30
4.30
4.29
4.29
4.28
4.27
4.27
4.26
4.26
4.25
4.24
4.23
4.22
4.21
4.21
4.20
4.19
4.18
1.40
1.38
1.37
1.36
1.35
1.35
1.33
0.93
0.91
0.89
0.88
0.86
0.84
S
PO(OEt)2
S
O
CO2H
N
H
O
4.00
3.86
2.91
15.09
1.07
6.31
2.09
16.07
5.99
12.09
5.95
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
f1 (ppm)

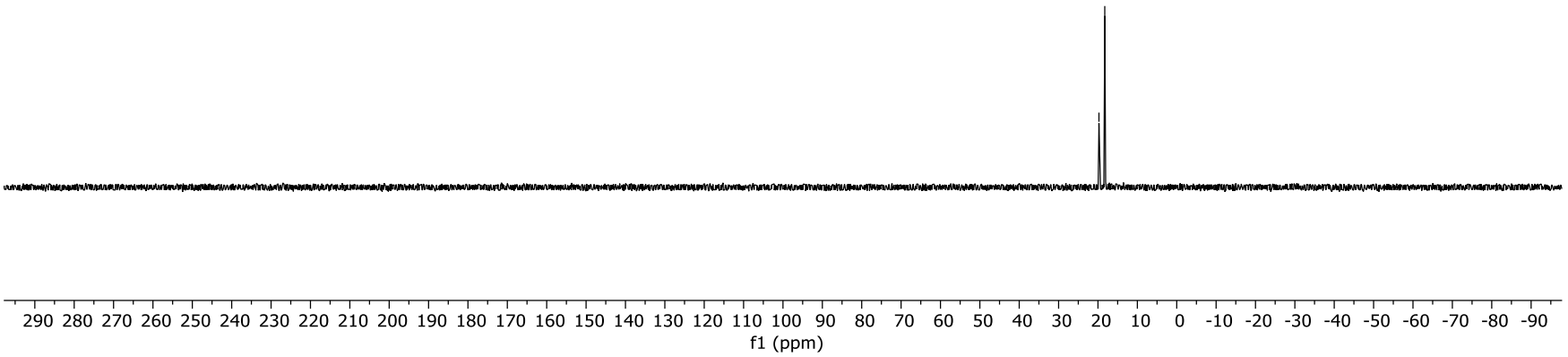

170.38
156.09
143.82
143.18
130.37
130.32
129.63
129.42
128.42
128.16
127.53
64.81
64.75
64.67
64.61
64.61
64.08
64.01
54.24
52.96
44.33
42.78
42.42
36.79
36.37
35.07
32.23
30.95
30.67
30.37
29.12
23.20
22.93
22.90
22.82
22.51
16.60
16.56
16.55
16.54
16.49
11.18
11.13
S
PO(OEt)2
S
O
CO2H
N
H
O
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

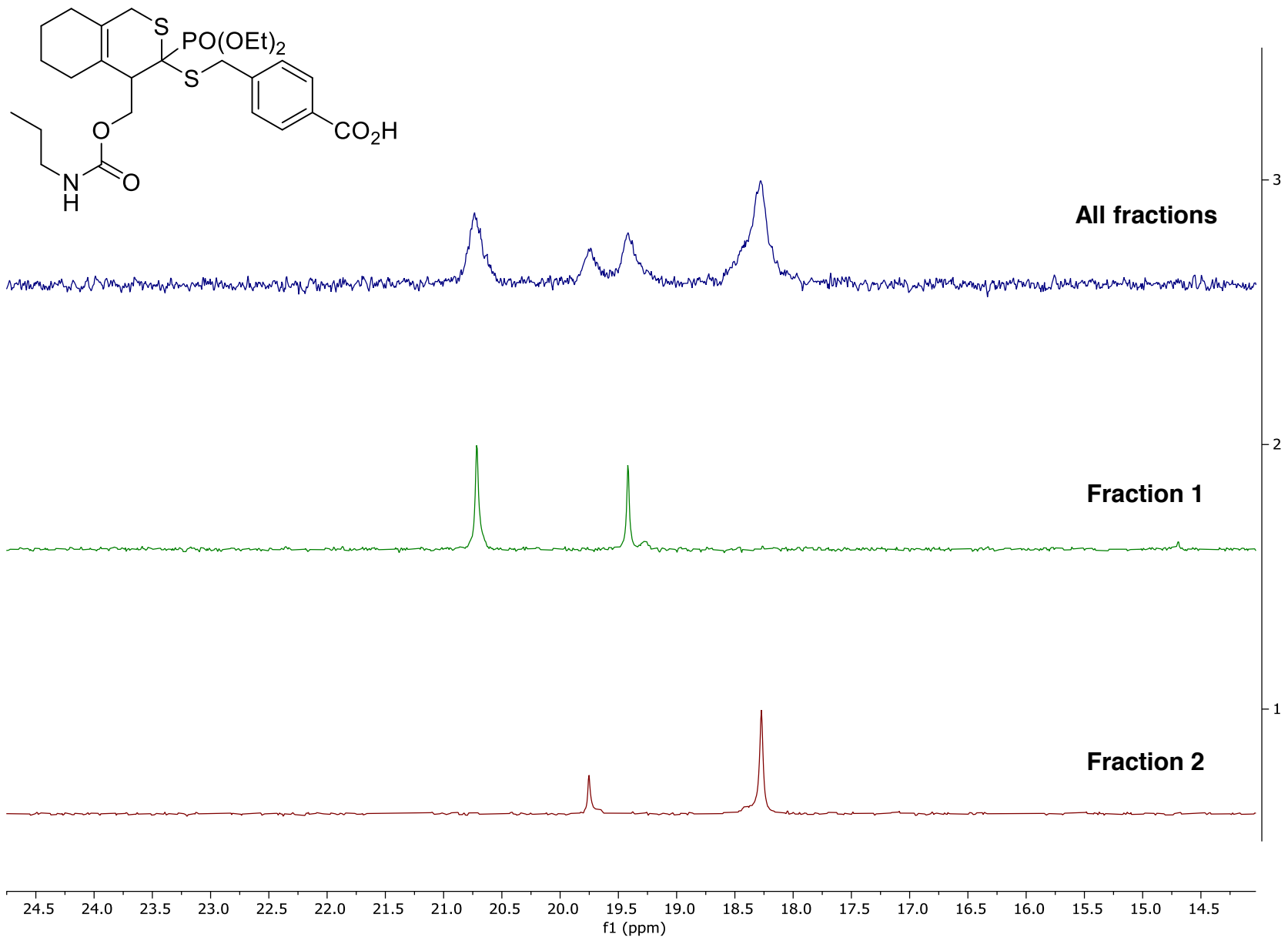

20.69
19.40
S
PO(OEt)2
S
O
CO2H
N
H
O
.50
130
110
90
70
50
30
10
-10
-30
-50
-70
-90
-110
-130
-150
-170
-190
-210
-230
-25
f1 (ppm)

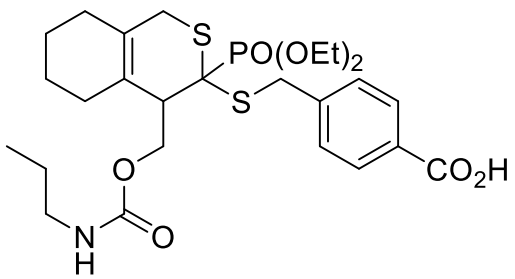

8.02
8.02
8.01
8.00
8.00
7.46
7.45
7.42
7.40
4.58
4.54
4.41
4.39
4.36
4.35
4.34
4.32
4.32
4.31
4.31
4.30
4.30
4.29
4.29
4.28
4.27
4.27
4.26
4.26
4.26
4.25
4.24
4.24
4.24
4.23
4.23
4.23
4.22
4.22
4.21
4.19
4.19
4.17
4.09
4.07
1.39
1.38
1.38
1.37
1.37
1.36
1.36
1.36
1.35
1.35
1.34
0.92
0.91
0.89
S
PO(OEt)2
S
O
CO2H
N
H
O
4.00
3.98
17.17
6.25
3.27
21.83
12.41
6.07
f1 (ppm)

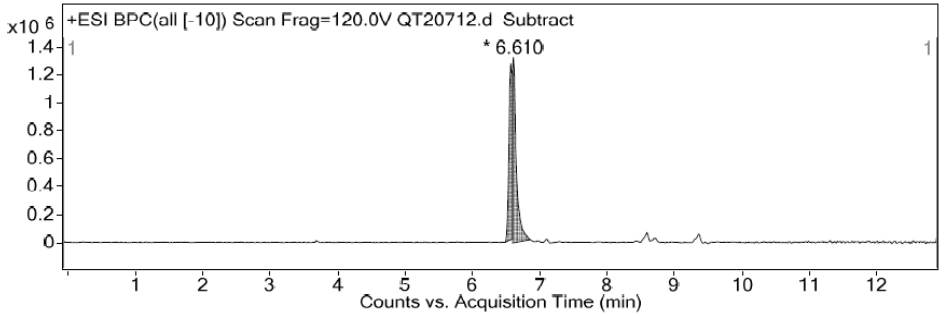

170.24
156.58
143.41
130.36
129.54
129.39
128.41
65.50
64.96
64.75
64.59
64.09
45.37
42.75
37.55
36.53
36.23
31.63
31.08
30.40
27.70
23.20
23.02
22.98
22.58
16.54
16.22
11.22
11.19
S
PO(OEt)2
S
O
CO2H
N
H
O
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


S
PO(OEt)2
S
O
CO2H
N
H
O


19.75
18.27
290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)

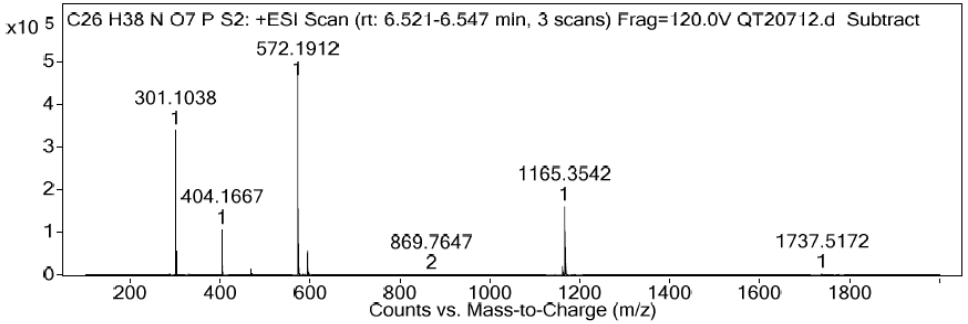

S
PO(OEt)2
S
O
CO2H
N
H
O
All fractions
Fraction 1
Fraction 2
3
2
1
24.5
24.0
23.5
23.0
22.5
22.0
21.5
21.0
20.5
20.0
19.5
19.0
18.5
18.0
17.5
17.0
16.5
16.0
15.5
15.0
14.5
f1 (ppm)

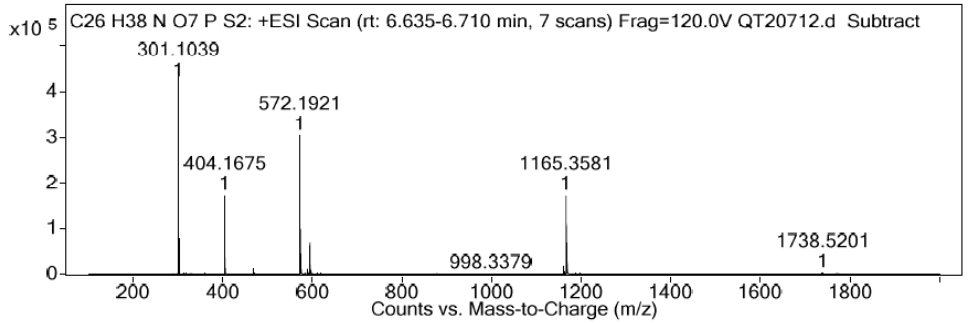


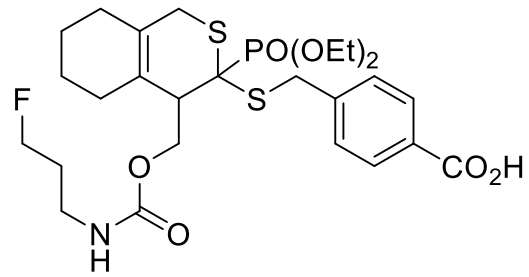


Integration Peak List

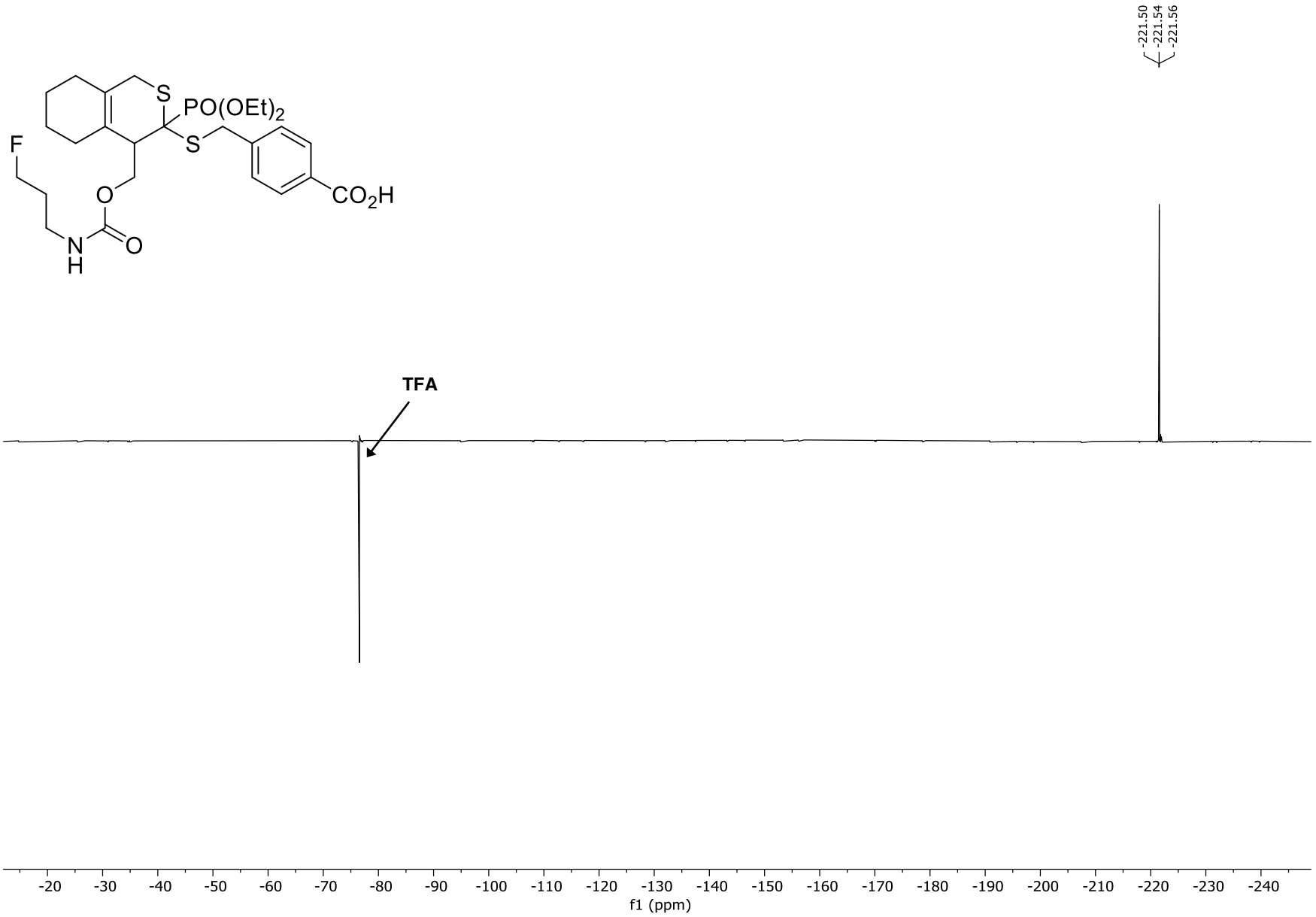
Peak	Start	RT	End	Height	Area	Area %	AreaSum%
1	6.483	6.572	6.597	1268516.58	4247224.39	90.23	47.43
2	6.597	6.61	6.862	1336055.52	4706914.58	100	52.57

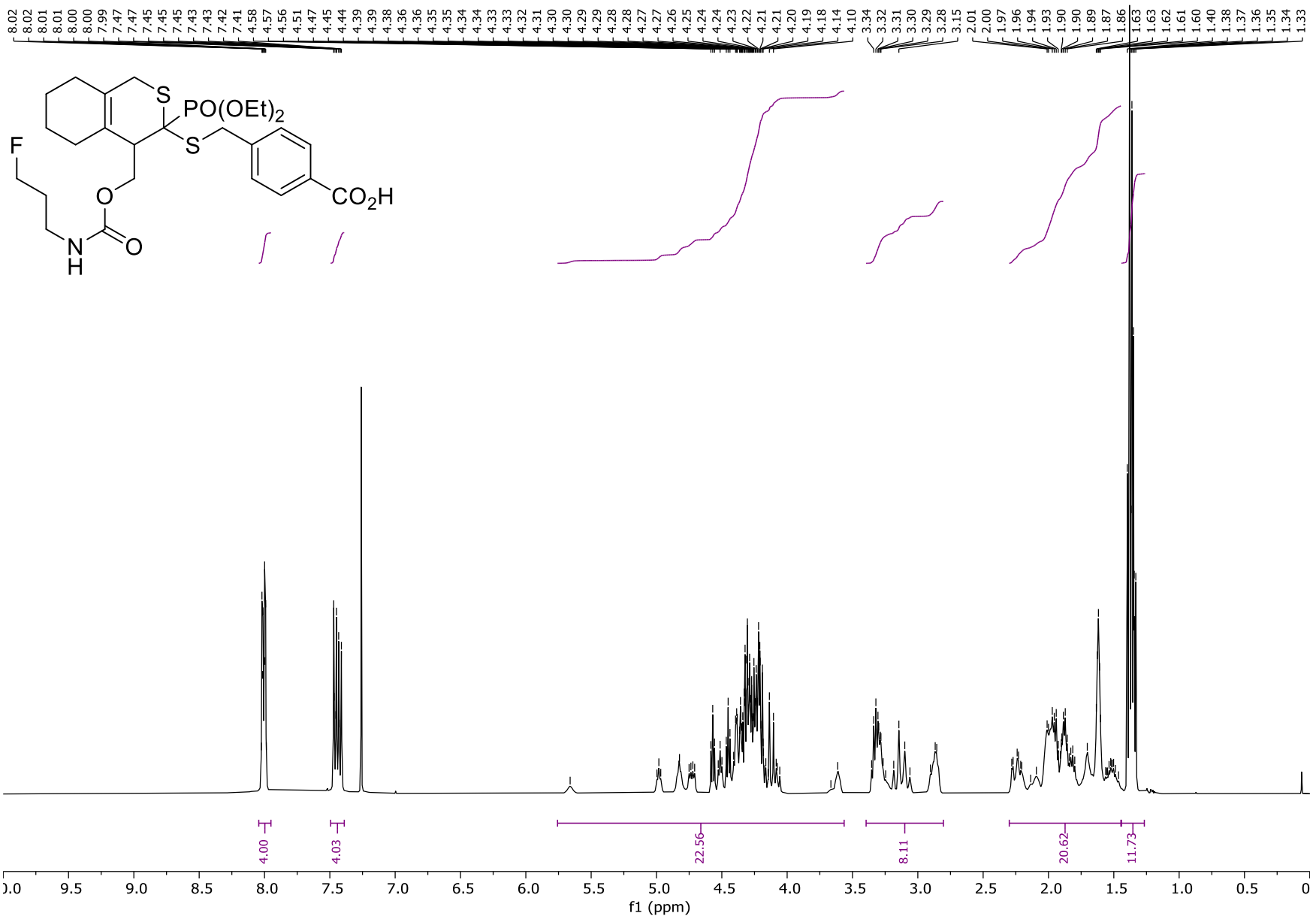


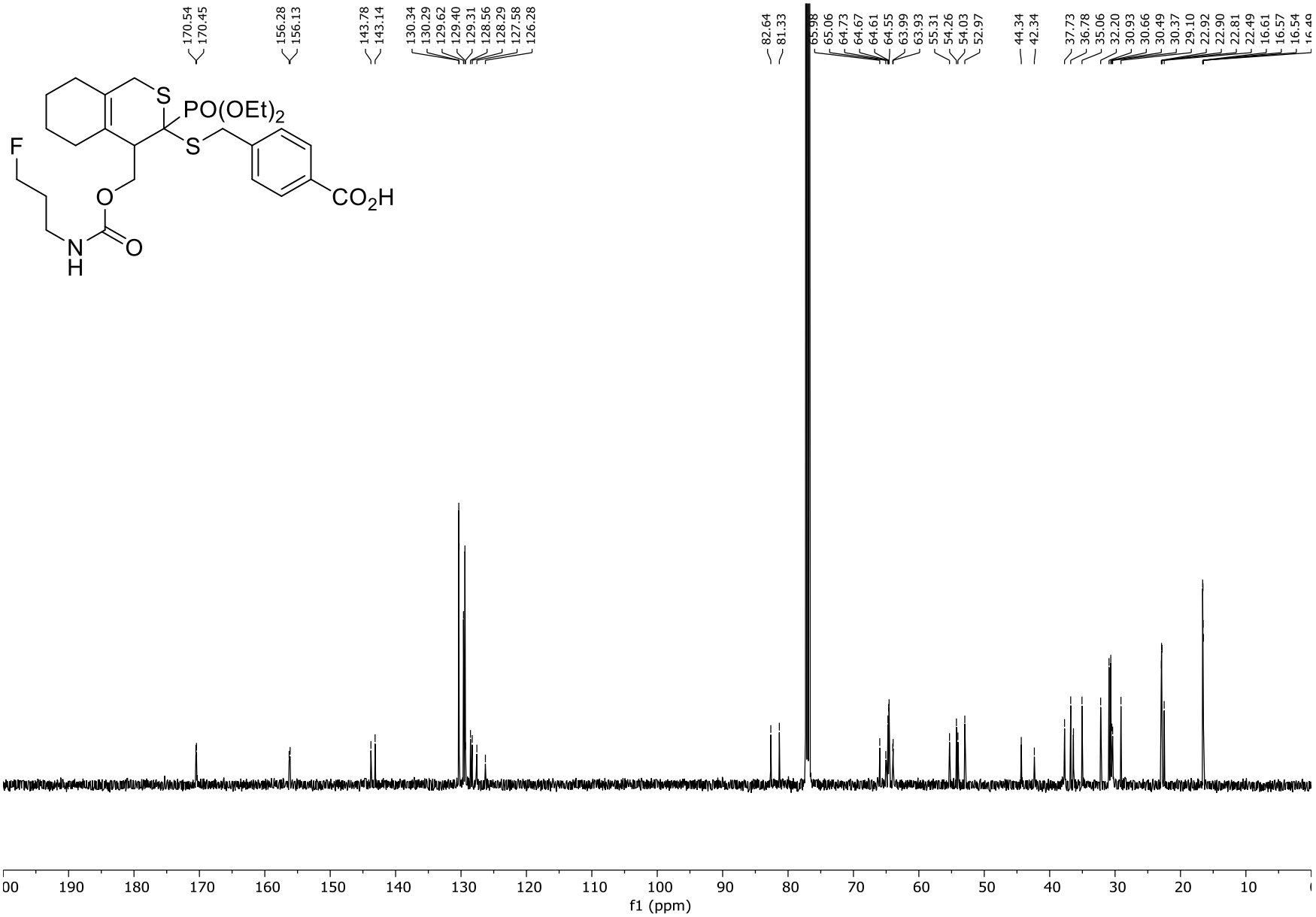


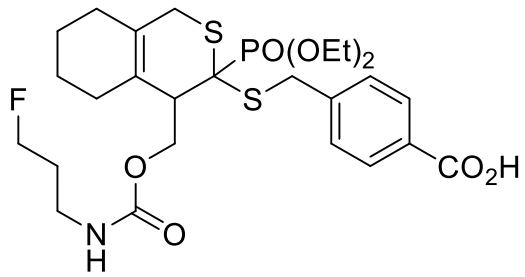

S
PO(OEt)2
S
F
O
CO2H
N
H
O

20.75
19.76
19.44
18.22
26.94
8.65
15.48
48.00
290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)

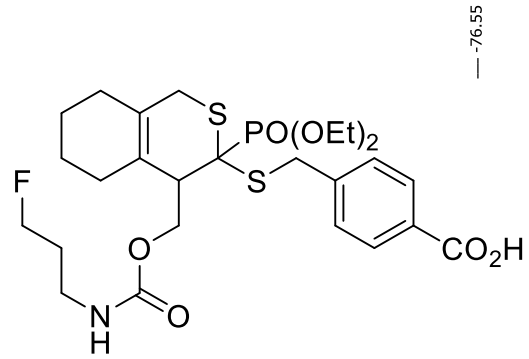

-221.50
-221.54
-221.56
S
PO(OEt)2
S
F
O
CO2H
N
H
O
TFA
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
-240
f1 (ppm)


8.02 8.02 8.01 8.01 8.00 8.00 7.99 7.47 7.47 7.45 7.45 7.45 7.43 7.43 7.42 7.41 4.58 4.57 4.56 4.51 4.47 4.45 4.44 4.39 4.39 4.38 4.36 4.36 4.35 4.35 4.34 4.34 4.33 4.33 4.32 4.31 4.30 4.30 4.29 4.29 4.28 4.28 4.27 4.27 4.26 4.25 4.24 4.24 4.23 4.22 4.21 4.21 4.20 4.19 4.18 4.14 4.10 3.34 3.32 3.31 3.30 3.29 3.28 3.15 2.01 2.00 1.97 1.96 1.94 1.93 1.90 1.90 1.89 1.87 1.86 1.63 1.63 1.62 1.61 1.60 1.40 1.38 1.37 1.36 1.35 1.34 1.33
S
PO(OEt)2
S
F
O
CO2H
N
H
O
4.00
4.03
22.56
8.11
20.62
11.73
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)


170.54
170.45
156.28
156.13
143.78
143.14
130.34
130.29
129.62
129.40
129.31
128.56
128.29
127.58
126.28
82.64
81.33
65.98
65.06
64.73
64.67
64.61
64.55
63.99
63.93
55.31
54.26
54.03
52.97
44.34
42.34
37.73
36.78
35.06
32.20
30.93
30.66
30.49
30.37
29.10
22.92
22.90
22.81
22.49
16.61
16.57
16.54
S
PO(OEt)2
S
F
O
CO2H
N
H
O
f1 (ppm)

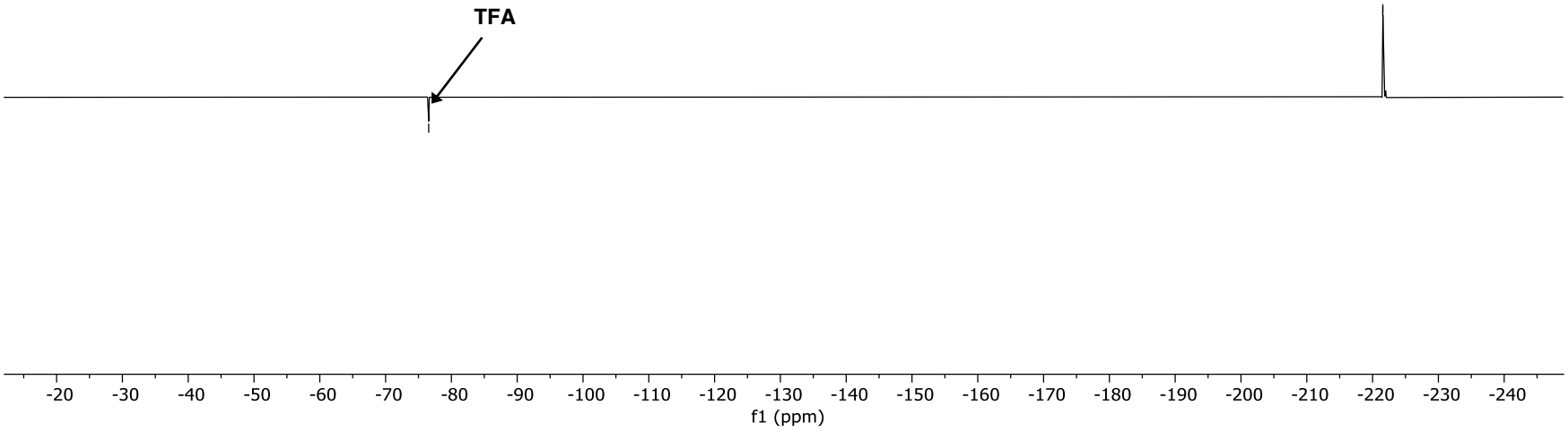

S
PO(OEt)2
S
F
O
CO2H
N
H
O

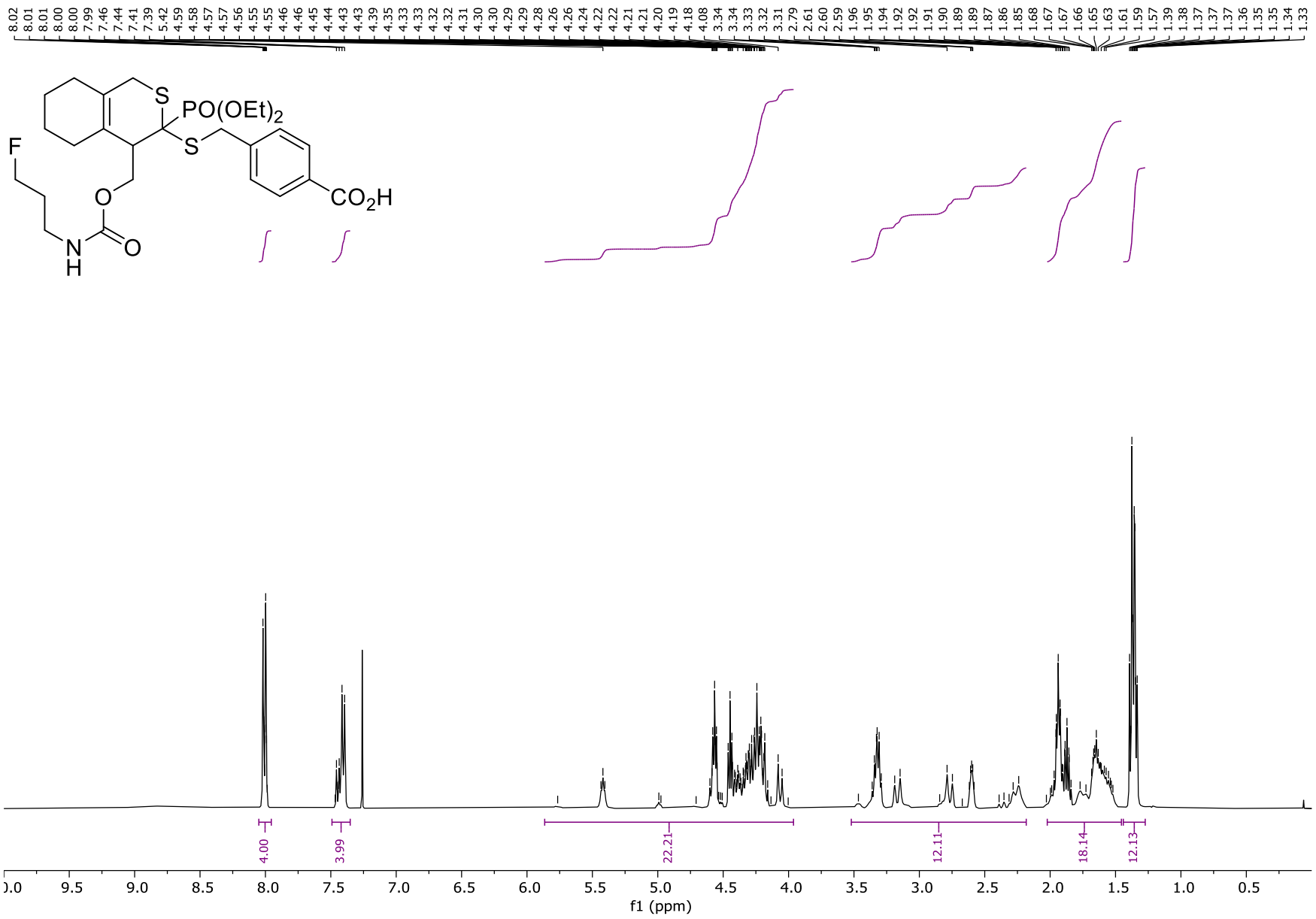
20.76
19.44
290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)

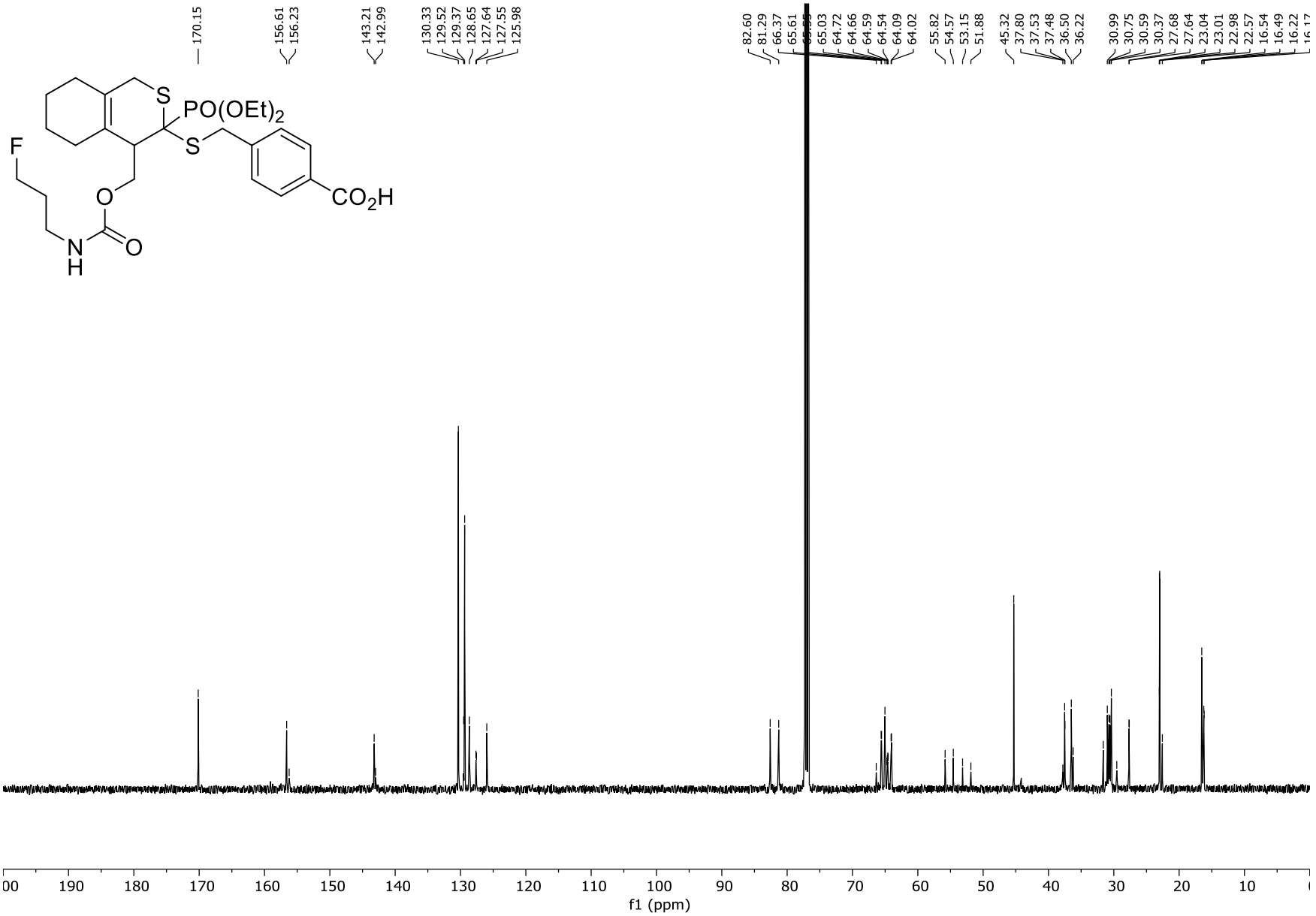


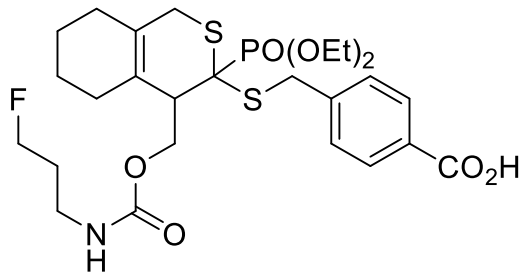
-76.55

-221.54
-221.56

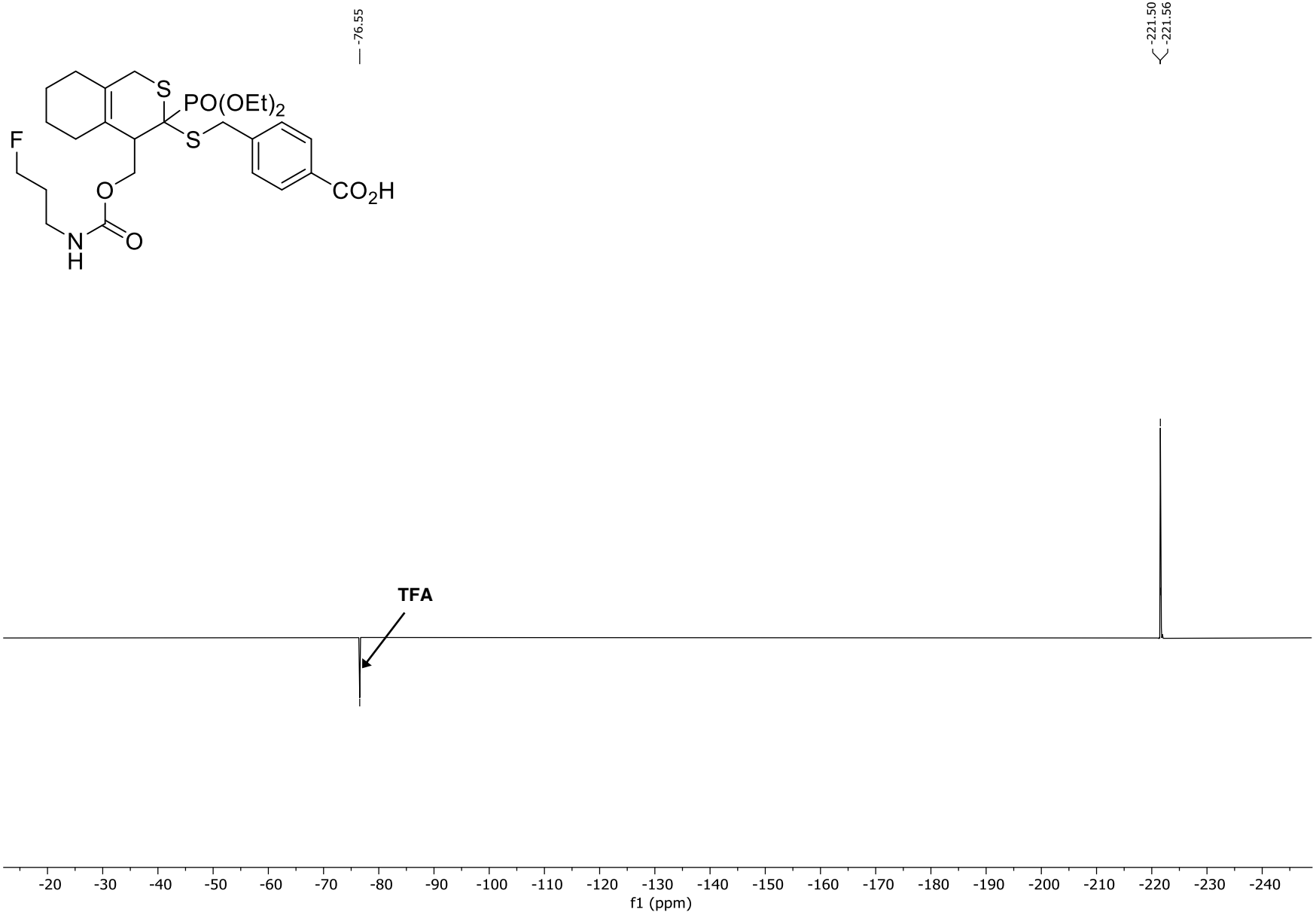


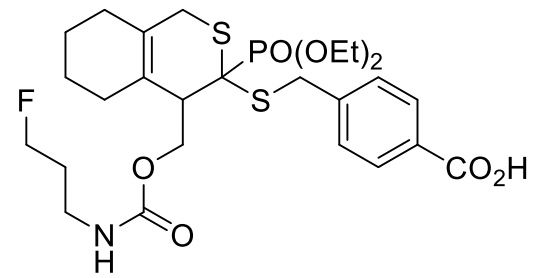

8.02 8.01 8.01 8.00 8.00 7.99 7.46 7.44 7.41 7.39 5.42 4.59 4.58 4.57 4.57 4.56 4.55 4.55 4.46 4.46 4.45 4.44 4.43 4.43 4.39 4.35 4.33 4.33 4.32 4.32 4.31 4.30 4.30 4.29 4.29 4.28 4.26 4.26 4.24 4.22 4.22 4.21 4.21 4.20 4.19 4.18 4.08 3.34 3.34 3.33 3.32 3.31 2.79 2.61 2.60 2.59 1.96 1.95 1.94 1.92 1.92 1.91 1.90 1.89 1.89 1.87 1.86 1.85 1.68 1.67 1.67 1.66 1.65 1.63 1.61 1.59 1.57 1.39 1.38 1.37 1.37 1.36 1.35 1.35 1.34 1.33
S
PO(OEt)2
S
F
O
CO2H
N
H
O
O
4.00
3.99
22.21
12.11
18.14
12.13
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


170.15
156.61
156.23
143.21
142.99
130.33
129.52
129.37
128.65
127.64
127.55
125.98
82.60
81.29
66.37
65.61
65.03
64.72
64.66
64.59
64.54
64.09
64.02
55.82
54.57
53.15
51.88
45.32
37.80
37.53
37.48
36.50
36.22
30.99
30.75
30.59
30.37
27.68
27.64
23.04
23.01
22.98
22.57
16.54
16.49
16.22
PO(OEt)2
S
S
F
O
N
H
O
CO2H
f1 (ppm)


S
PO(OEt)2
S
F
O
CO2H
N
H
O

19.80
18.25
290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)


-76.55
-221.50
-221.56
S
PO(OEt)2
S
F
O
CO2H
N
H
O
TFA
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
-240
f1 (ppm)



All fractions

Fraction 1

Fraction 2

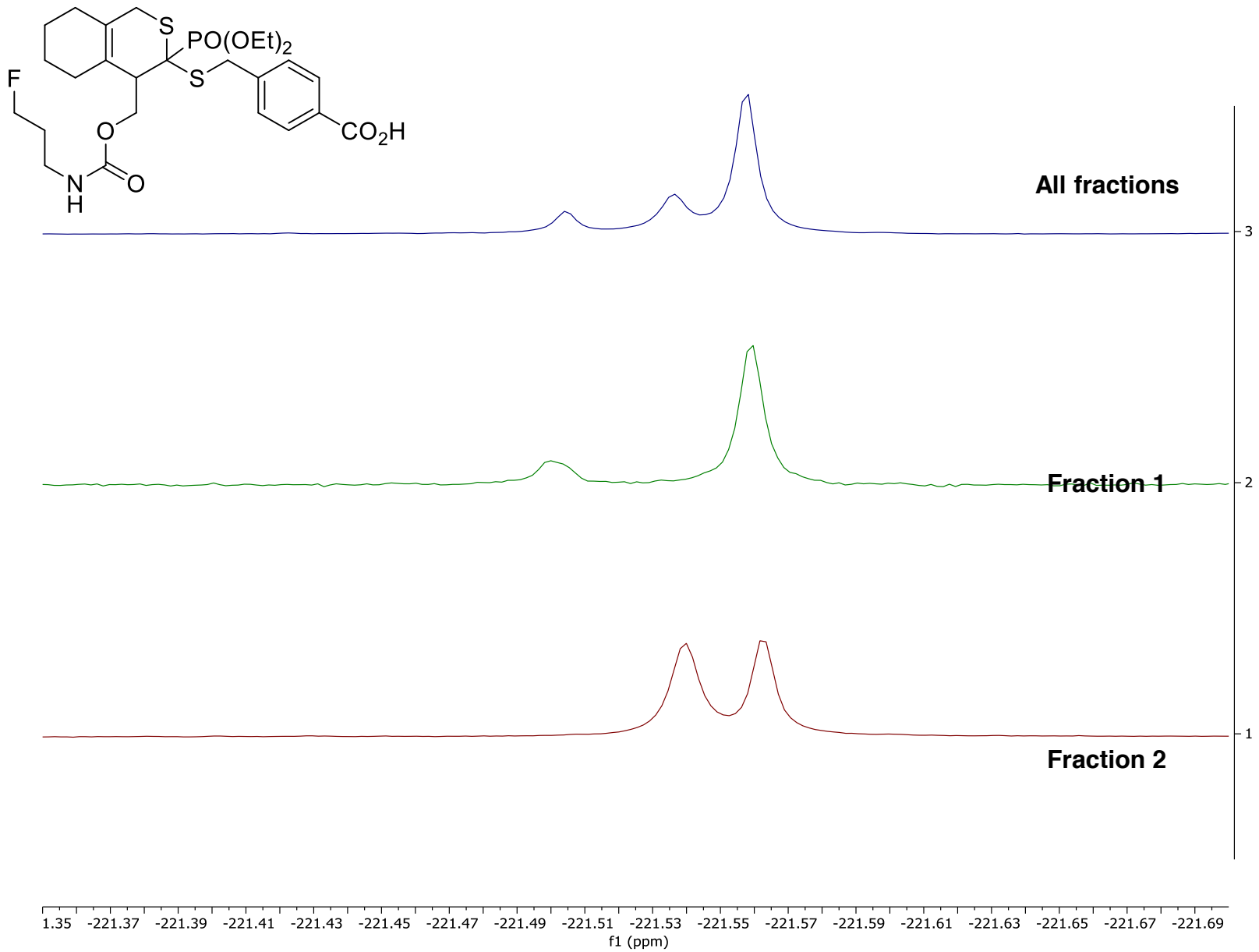
3

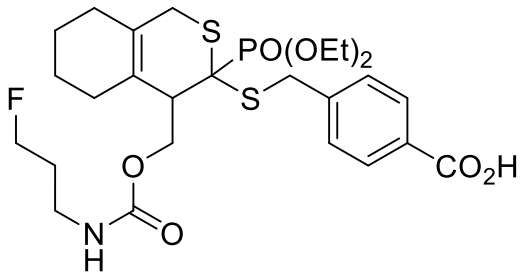
2

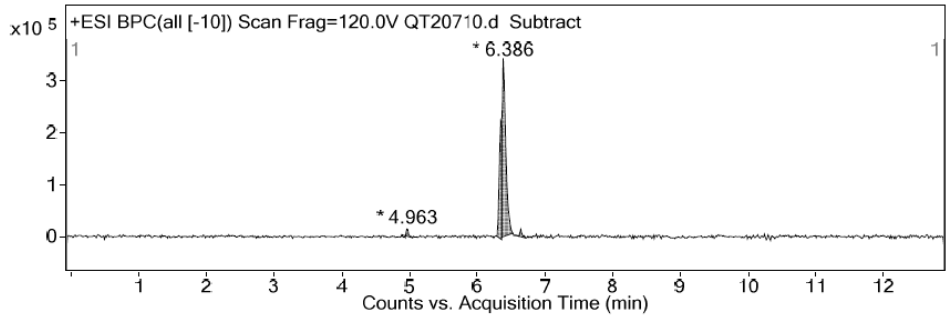
1

24.0 23.5 23.0 22.5 22.0 21.5 21.0 20.5 20.0 19.5 19.0 18.5 18.0 17.5 17.0 16.5 16.0 15.5 15.0

f1 (ppm)

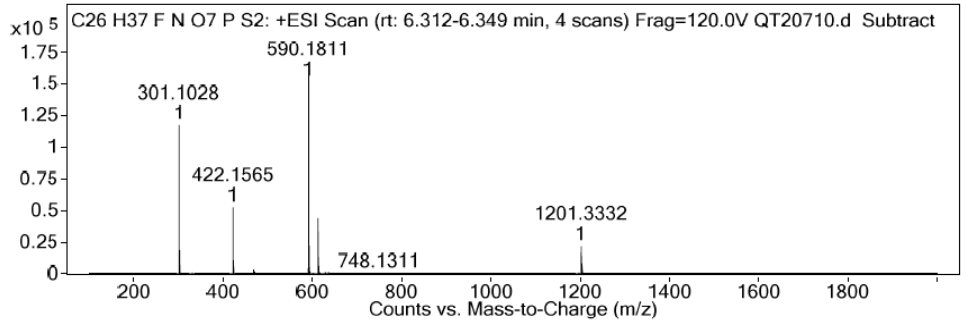

S
PO(OEt)2
S
F
O
CO2H
N
H
O
All fractions
3
Fraction 1
2
Fraction 2
1
1.35 -221.37 -221.39 -221.41 -221.43 -221.45 -221.47 -221.49 -221.51 -221.53 -221.55 -221.57 -221.59 -221.61 -221.63 -221.65 -221.67 -221.69
f1 (ppm)

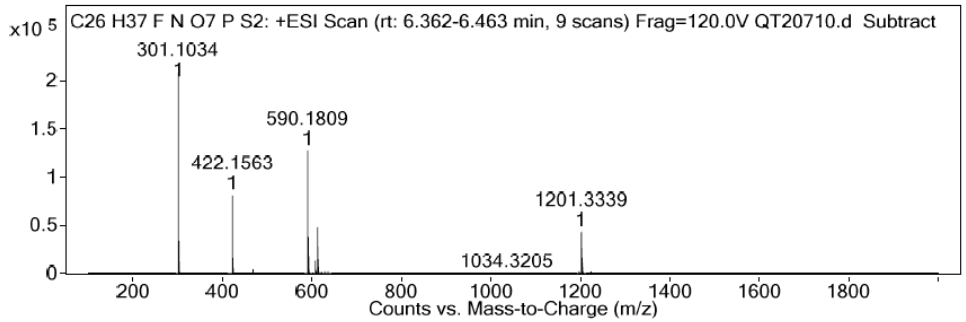


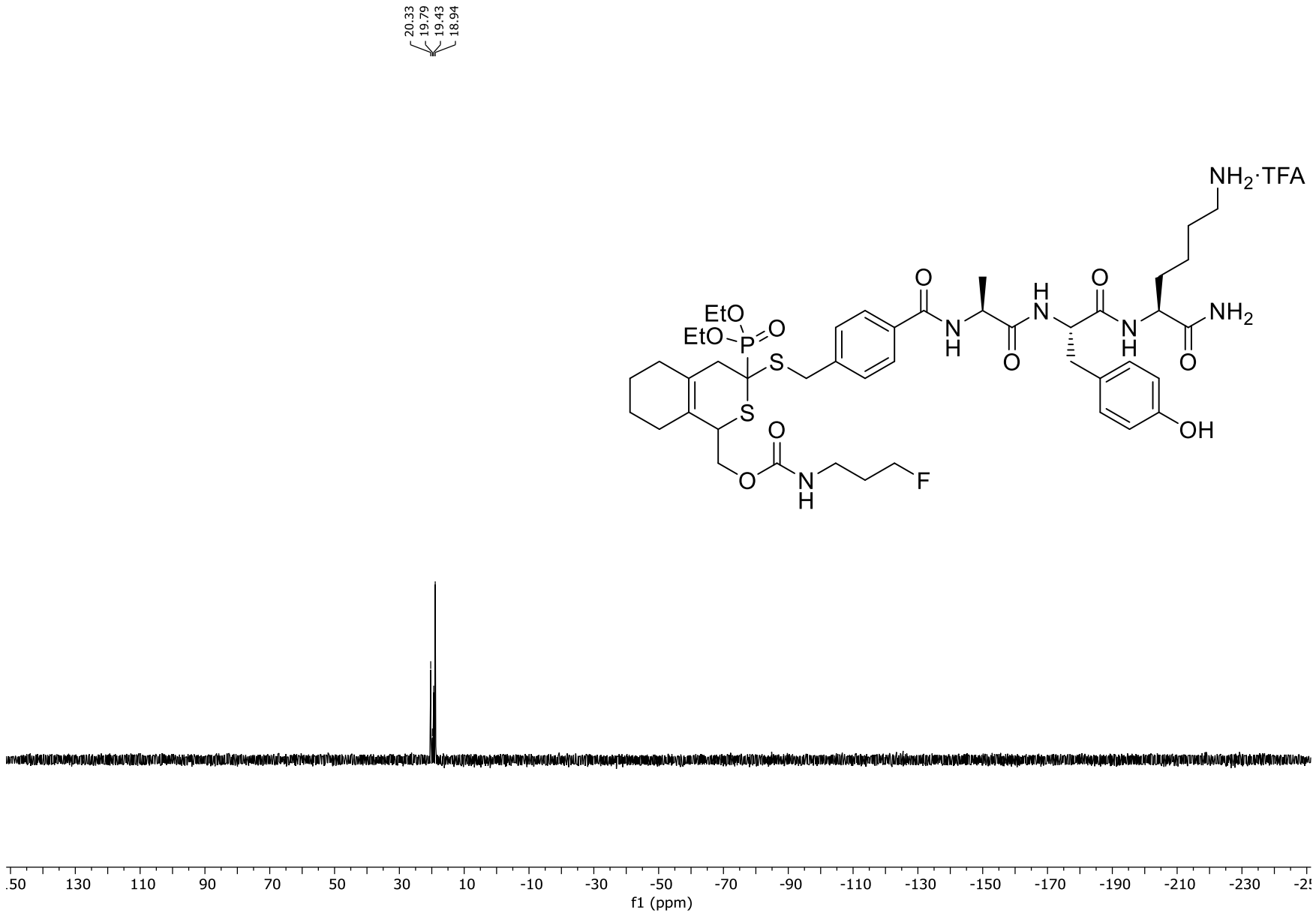


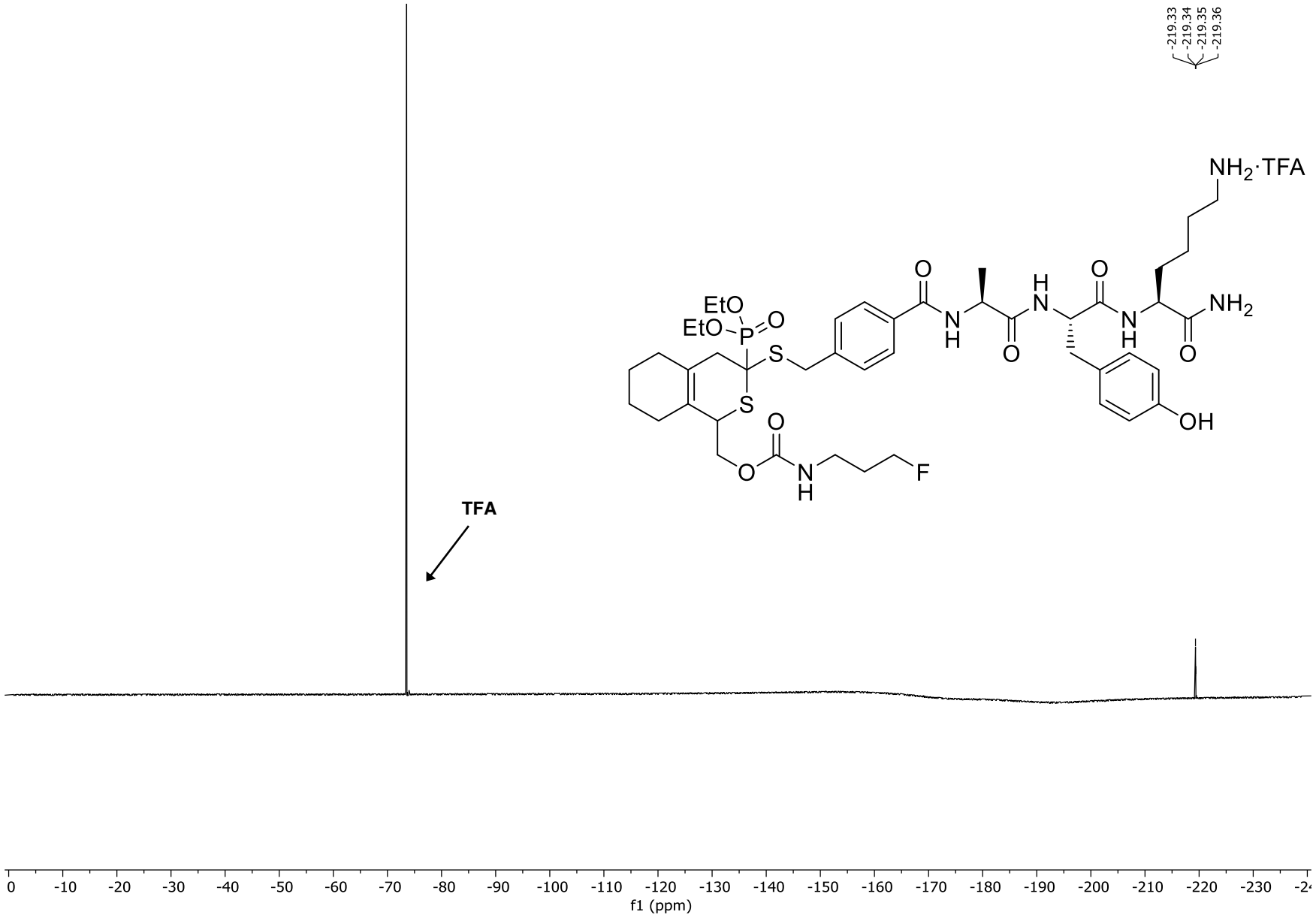
Integration Peak List

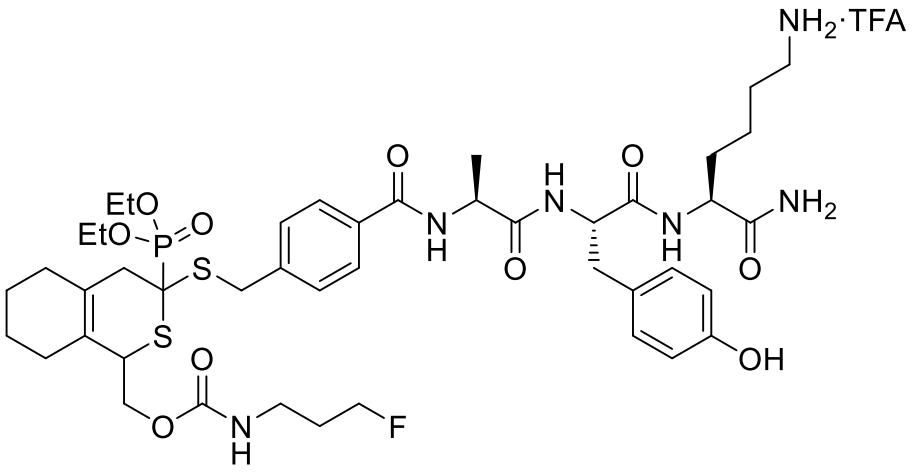
Peak	Start	RT	End	Height	Area	Area %	AreaSum%
1	4.886	4.963	5.048	13608.02	23997.55	1.93	1.28
2	6.295	6.346	6.359	226871.06	575841	46.41	30.75
3	6.359	6.386	6.528	344342.68	1240832.25	100	66.25
4	6.554	6.643	6.721	14268.32	32195.06	2.59	1.72

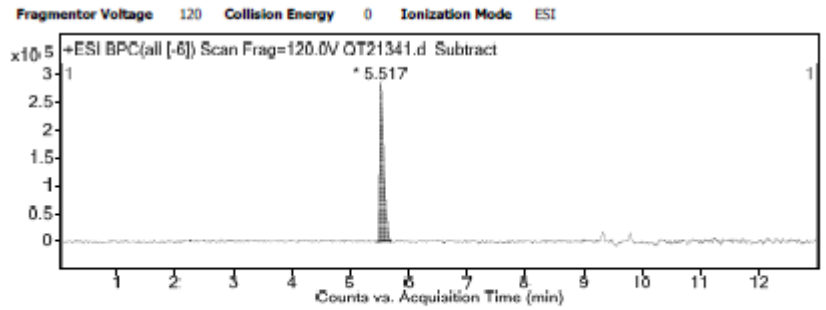





20.33
19.79
19.43
18.94
NH2·TFA
EtO
EtO
P
O
S
S
O
O
N
H
O
H
N
O
N
H
O
NH2
OH
O
O
N
H
F
f1 (ppm)

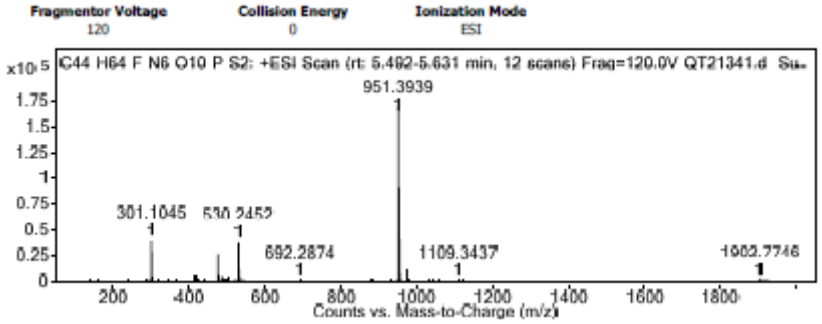

-219.33
-219.34
-219.35
-219.36
NH2·TFA
EtO
EtO
P
O
S
S
O
O
N
H
O
N
H
O
NH2
O
OH
O
N
H
F
TFA
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
f1 (ppm)

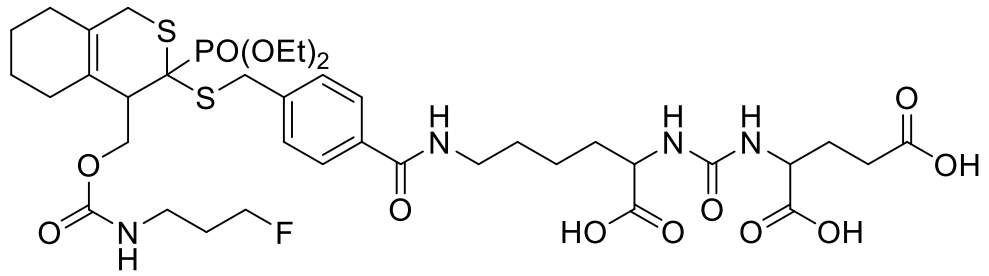


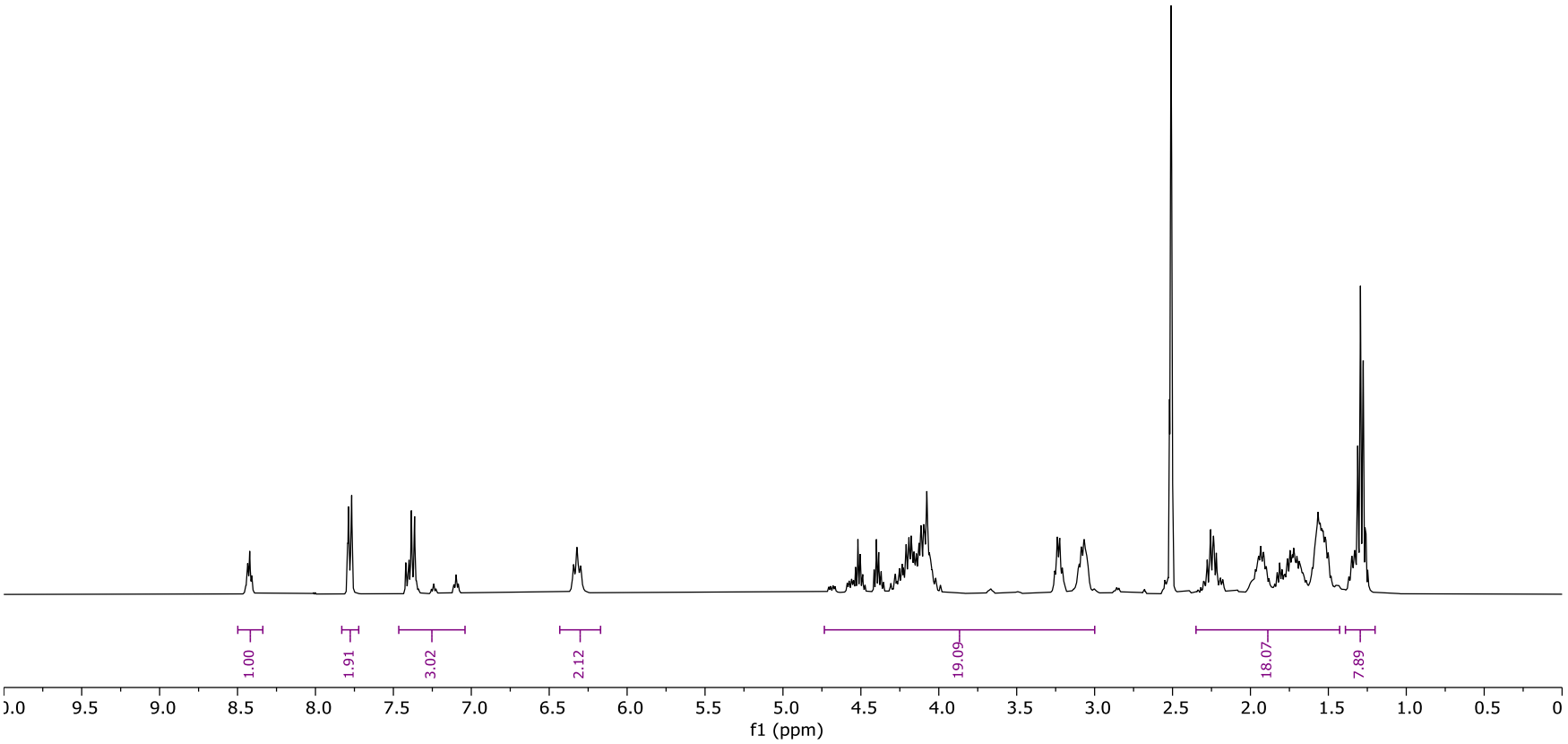


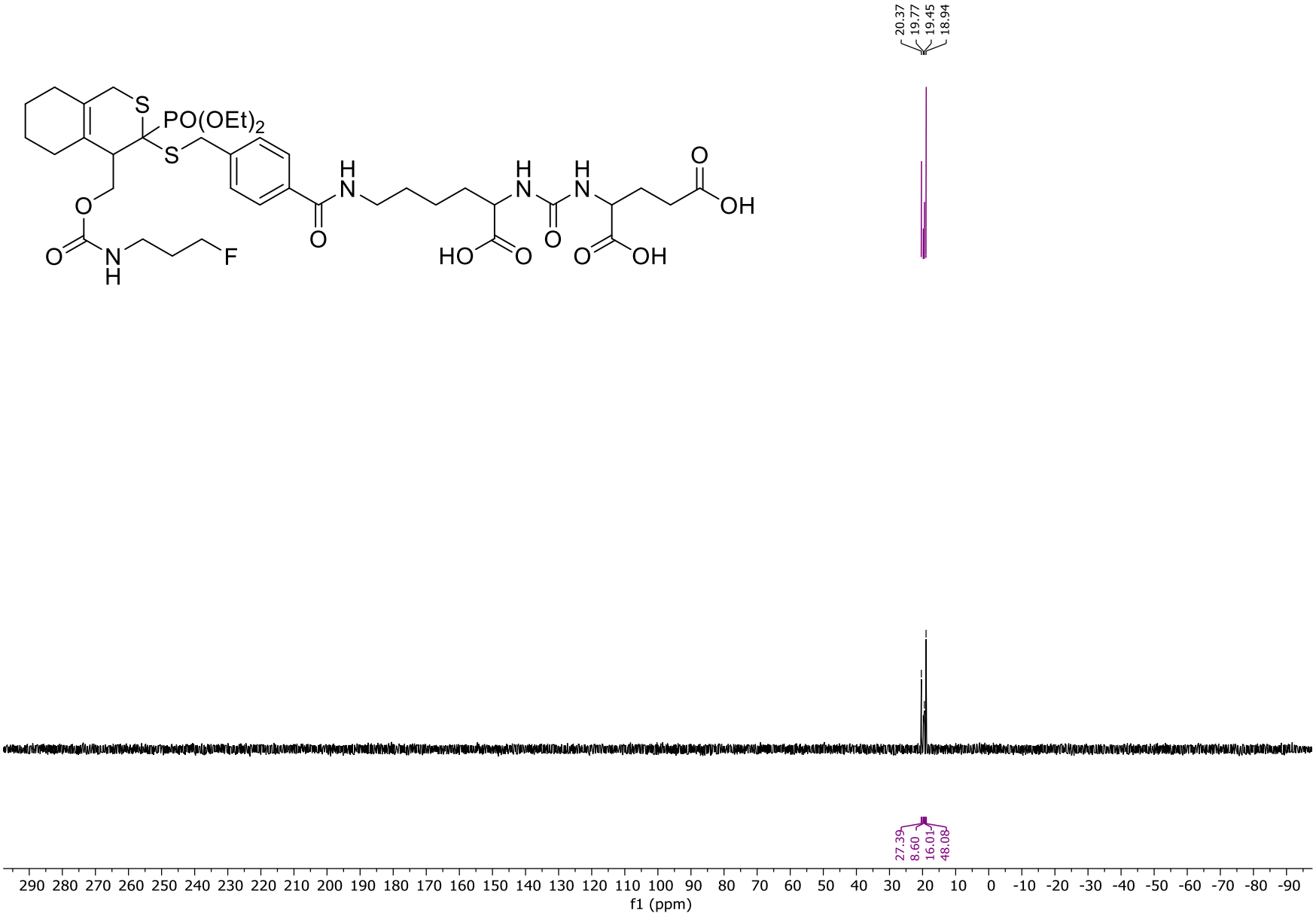
Integration Peak List

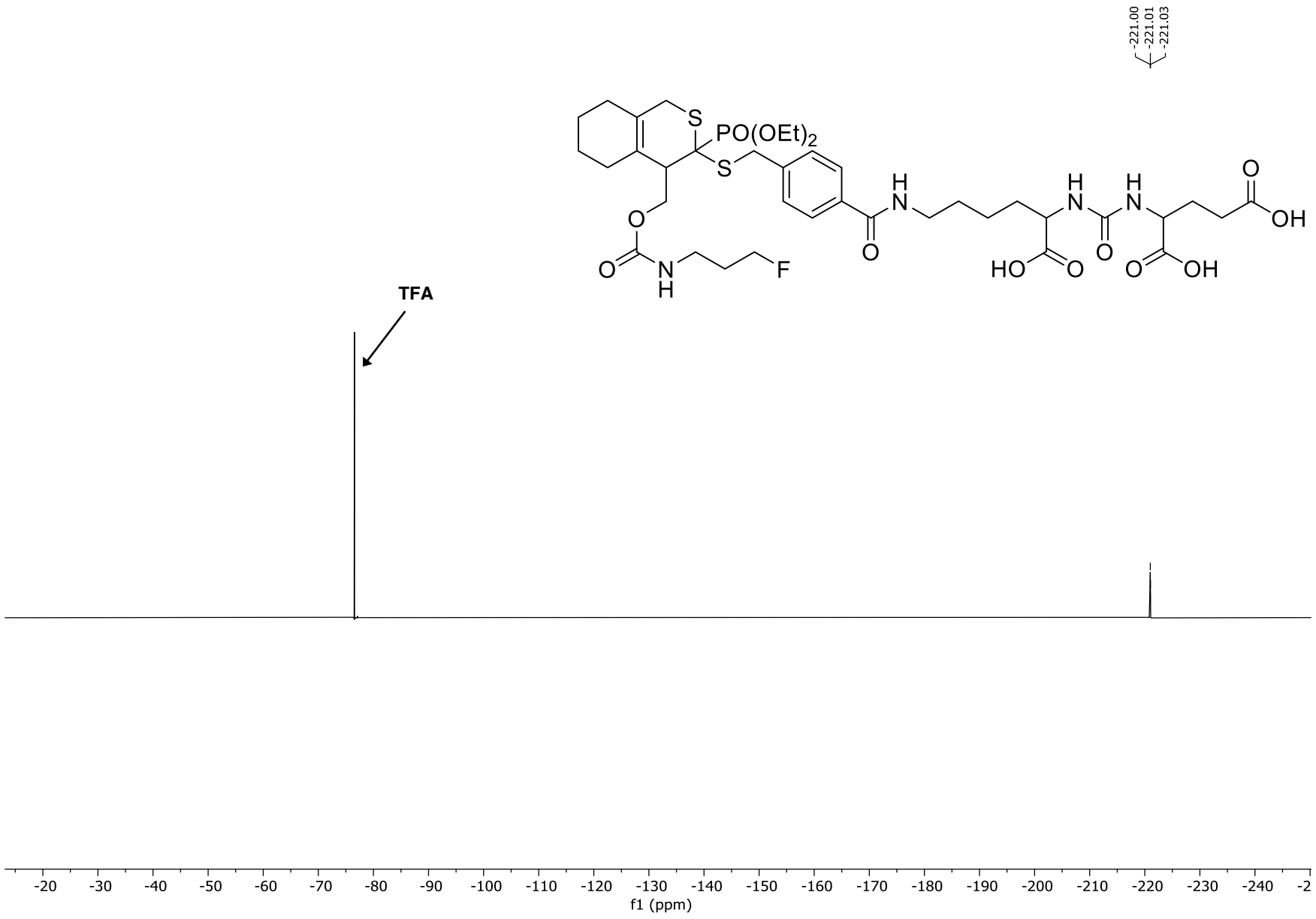
Peak	Start	RT	End	Height	Area	Area %	AreaSum%
1	5.452	5.517	5.695	287163.84	1417831.09	100	100

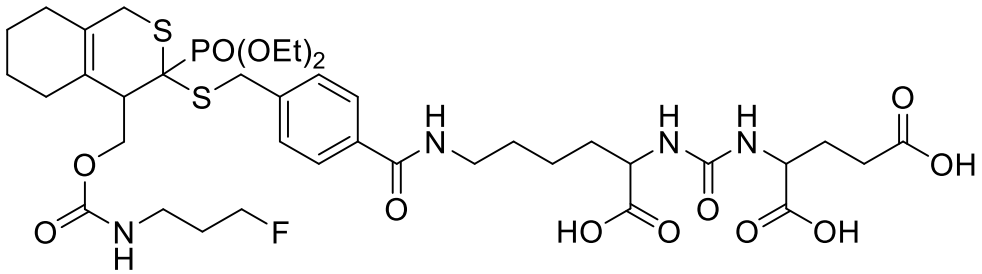


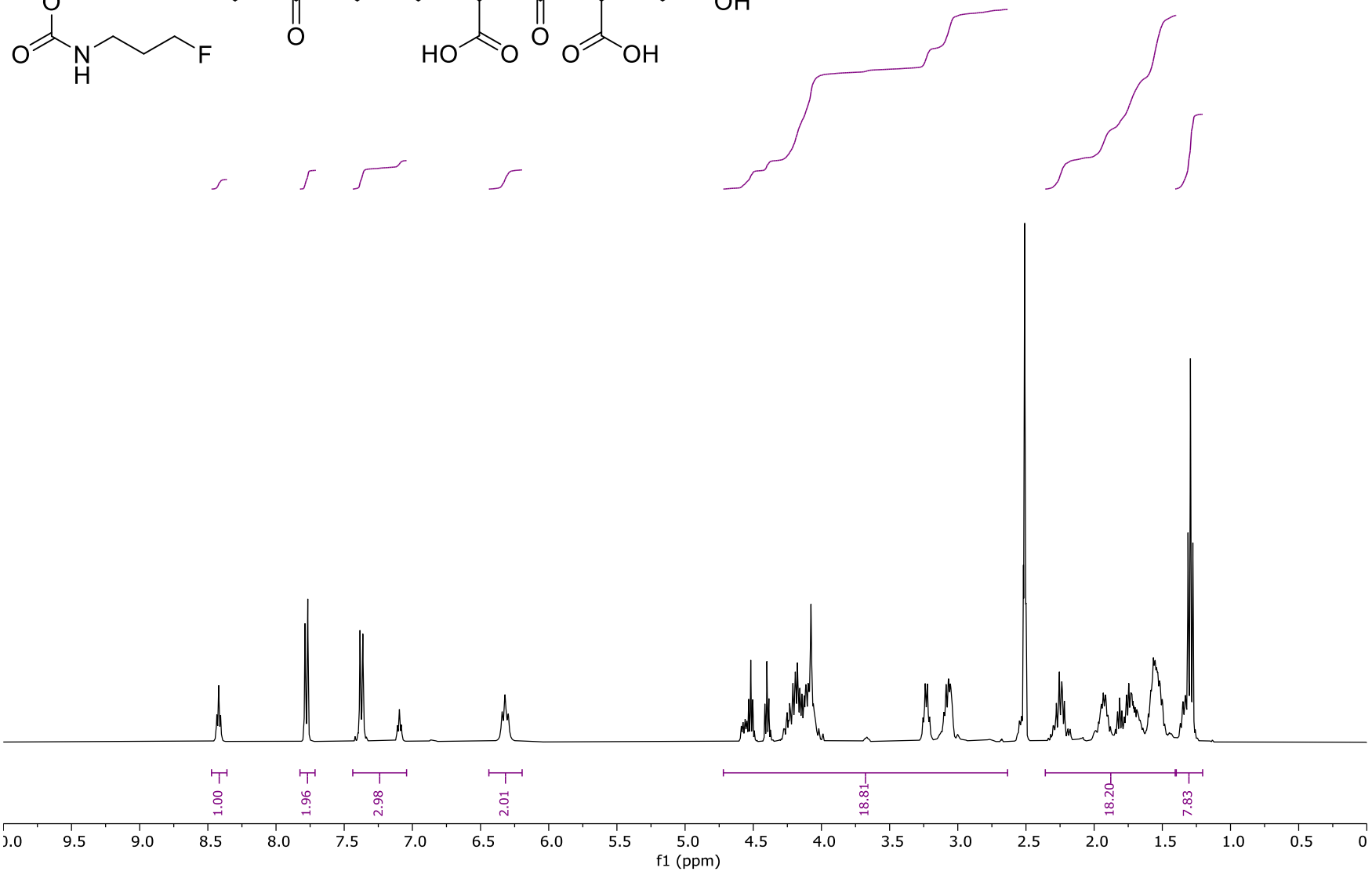

S
PO(OEt)2
S
O
O
N
H
F
H
N
O
H
N
HO
O
H
N
O
O
OH
O
OH

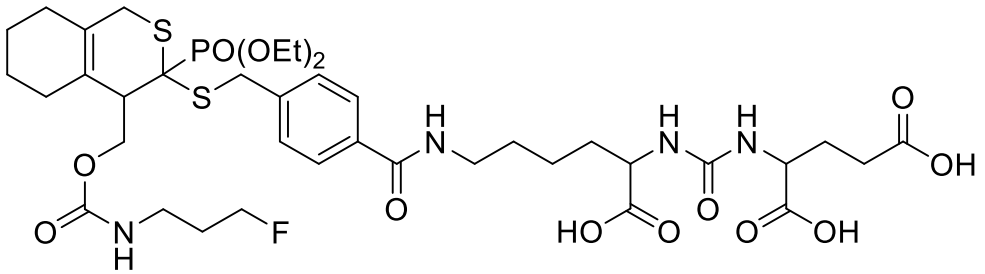

1.00
1.91
3.02
2.12
19.09
18.07
7.89
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
f1 (ppm)


20.37
19.77
19.45
18.94
27.39
8.60
16.01
48.08
290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)

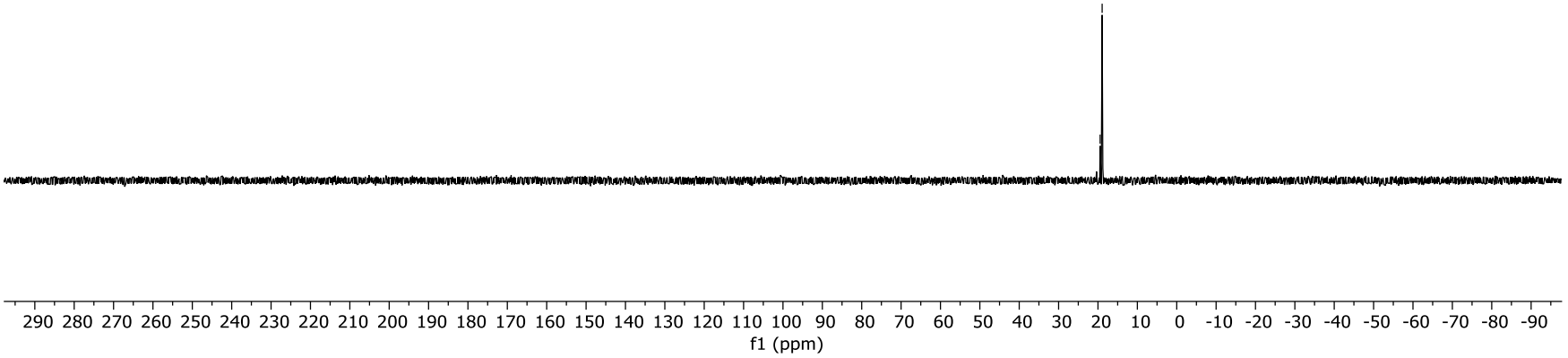

-221.00
-221.01
-221.03
S
PO(OEt)2
S
O
O
N
H
F
H
N
O
H
N
HO
O
H
N
O
O
H
N
O
OH
O
OH
TFA
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
-240
-2
f1 (ppm)

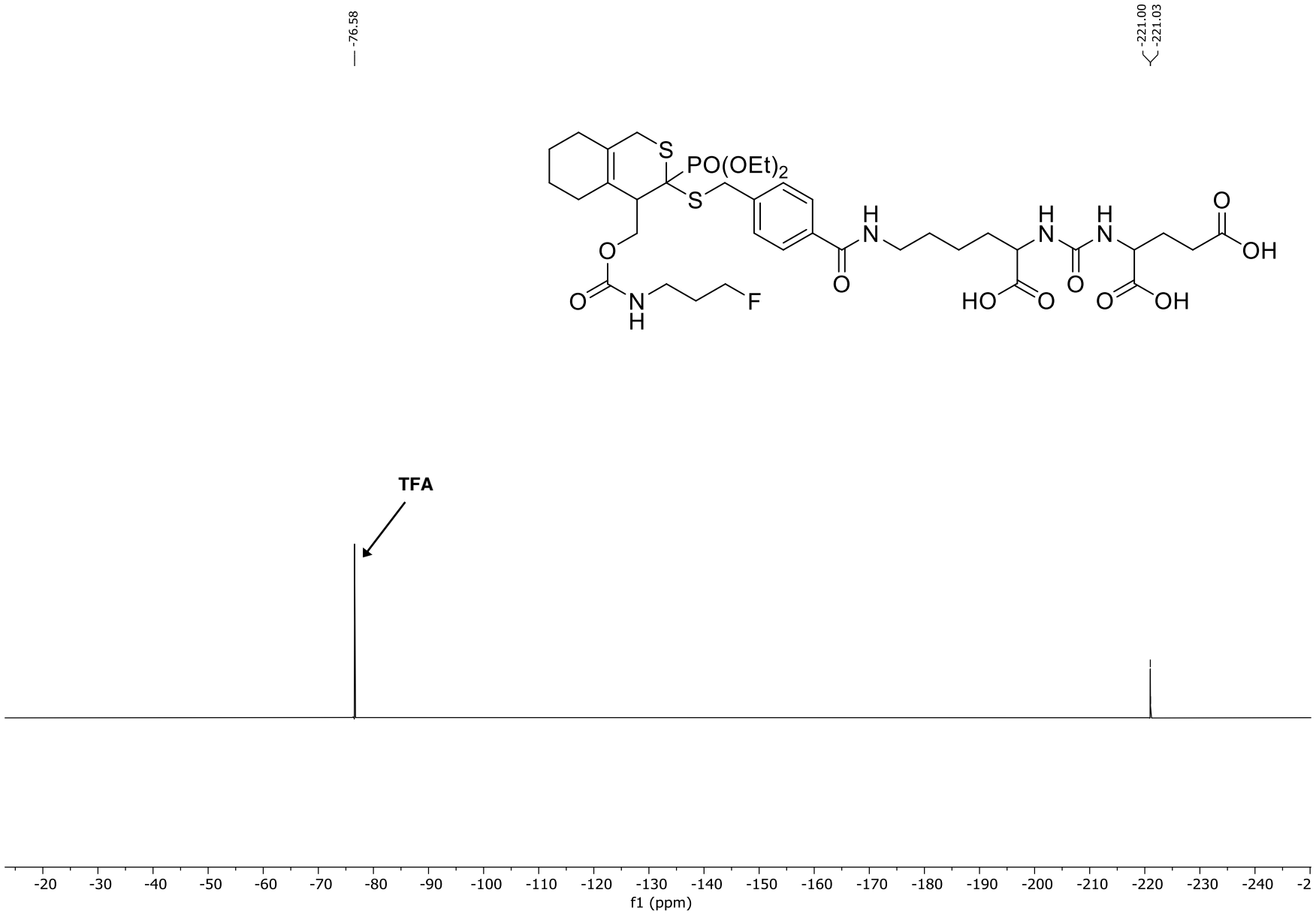

S
PO(OEt)2
S
O
O
N
H
F
H
N
O
H
N
HO
O
H
N
O
O
O
OH
O
OH

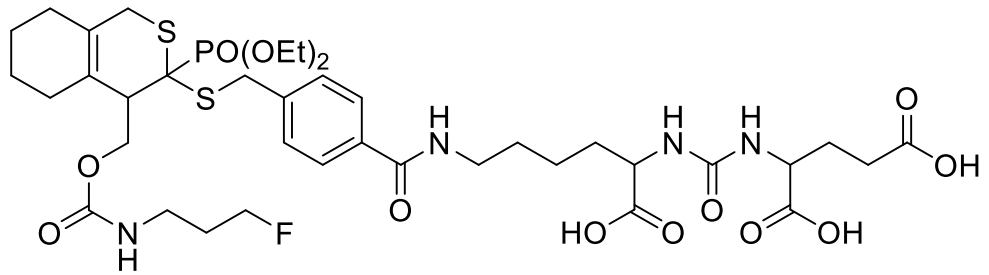

1.00
1.96
2.98
2.01
18.81
18.20
7.83
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
f1 (ppm)


S
PO(OEt)2
S
O
O
N
H
F
H
N
O
H
N
HO
O
H
N
O
O
H
N
O
OH
O
OH

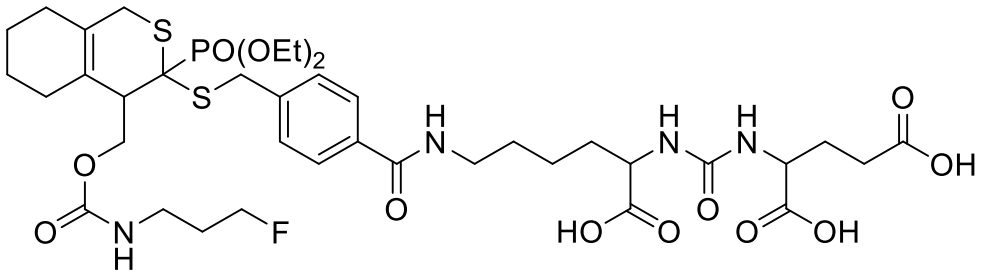
19.45
18.94


290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)

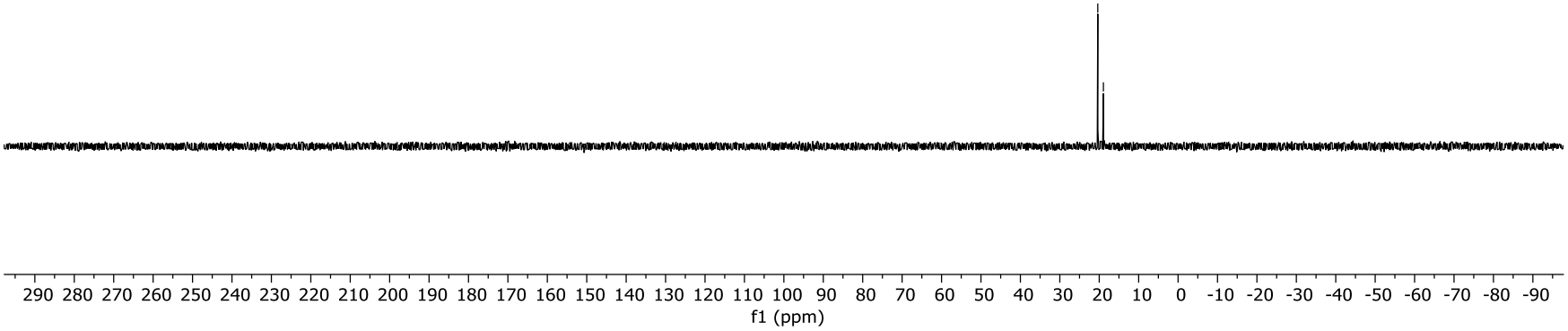

-76.58
-221.00
-221.03
S
PO(OEt)2
S
O
O
N
H
F
H
N
O
H
N
O
HO
O
H
N
O
O
OH
O
OH
TFA
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
-240
-2
f1 (ppm)

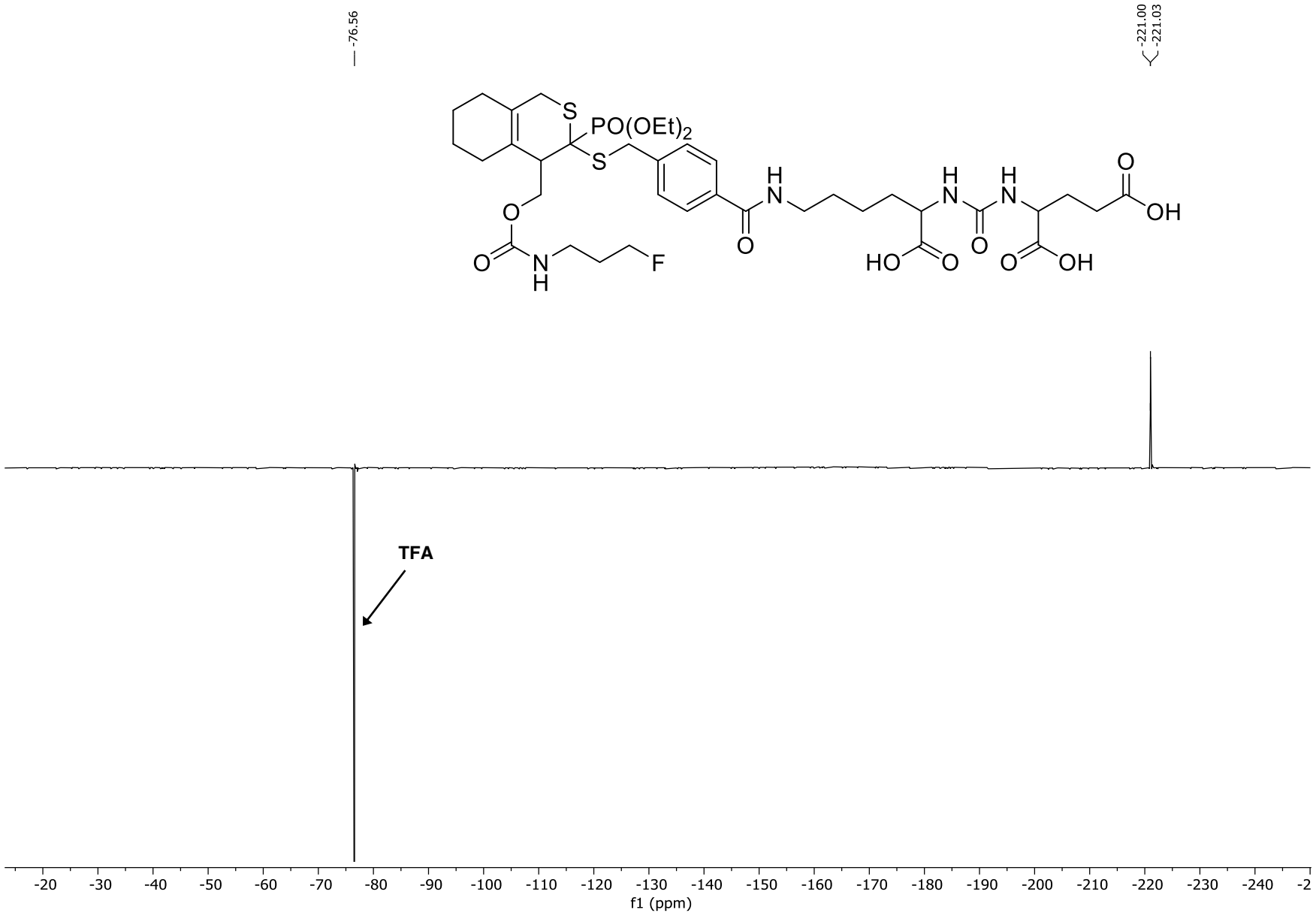

S
PO(OEt)2
S
O
O
N
H
F
H
N
O
H
N
HO
O
H
N
O
O
OH
O
OH

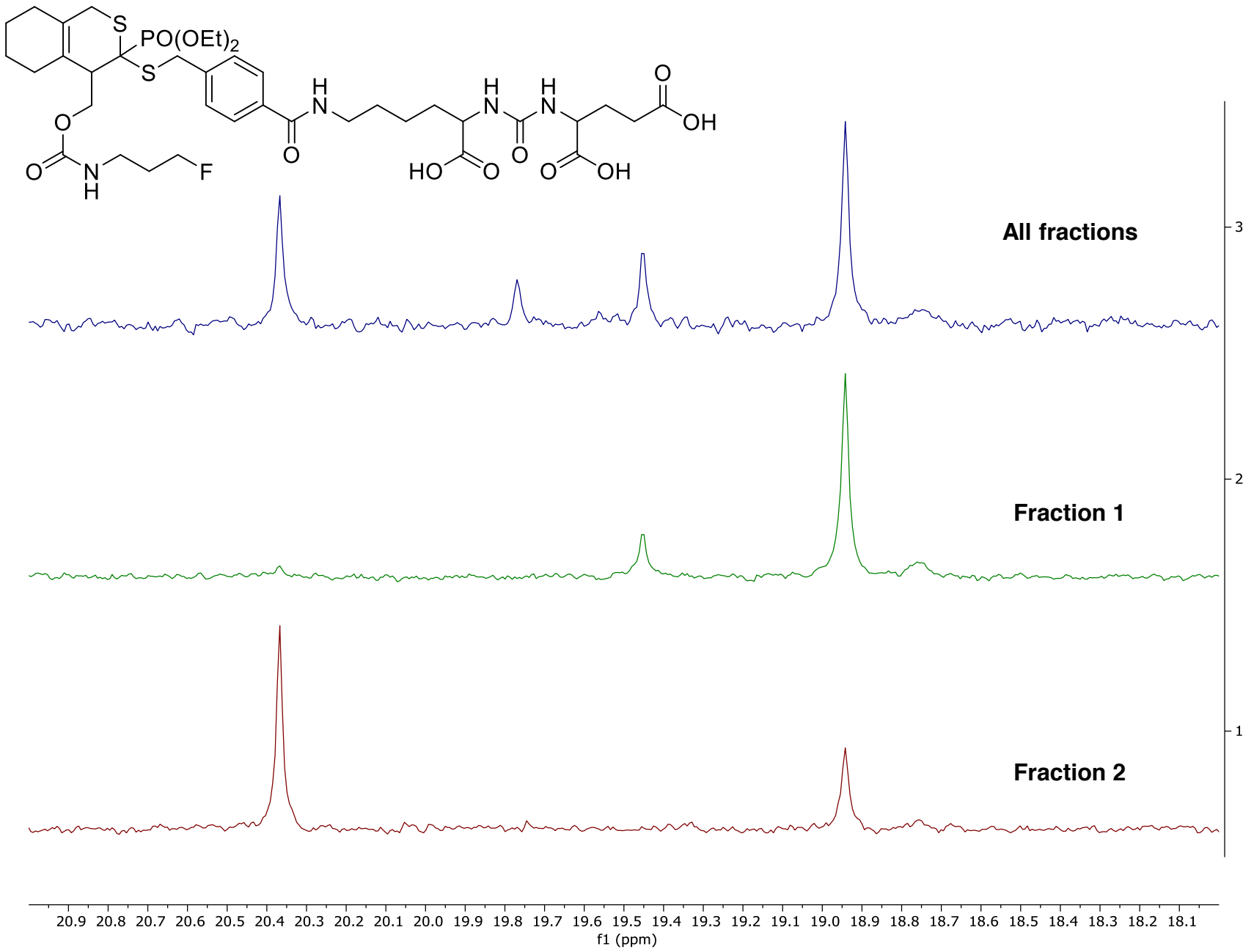
1.00
1.98
3.06
1.94
11.94
7.15
3.02
14.88
7.95
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
f1 (ppm)

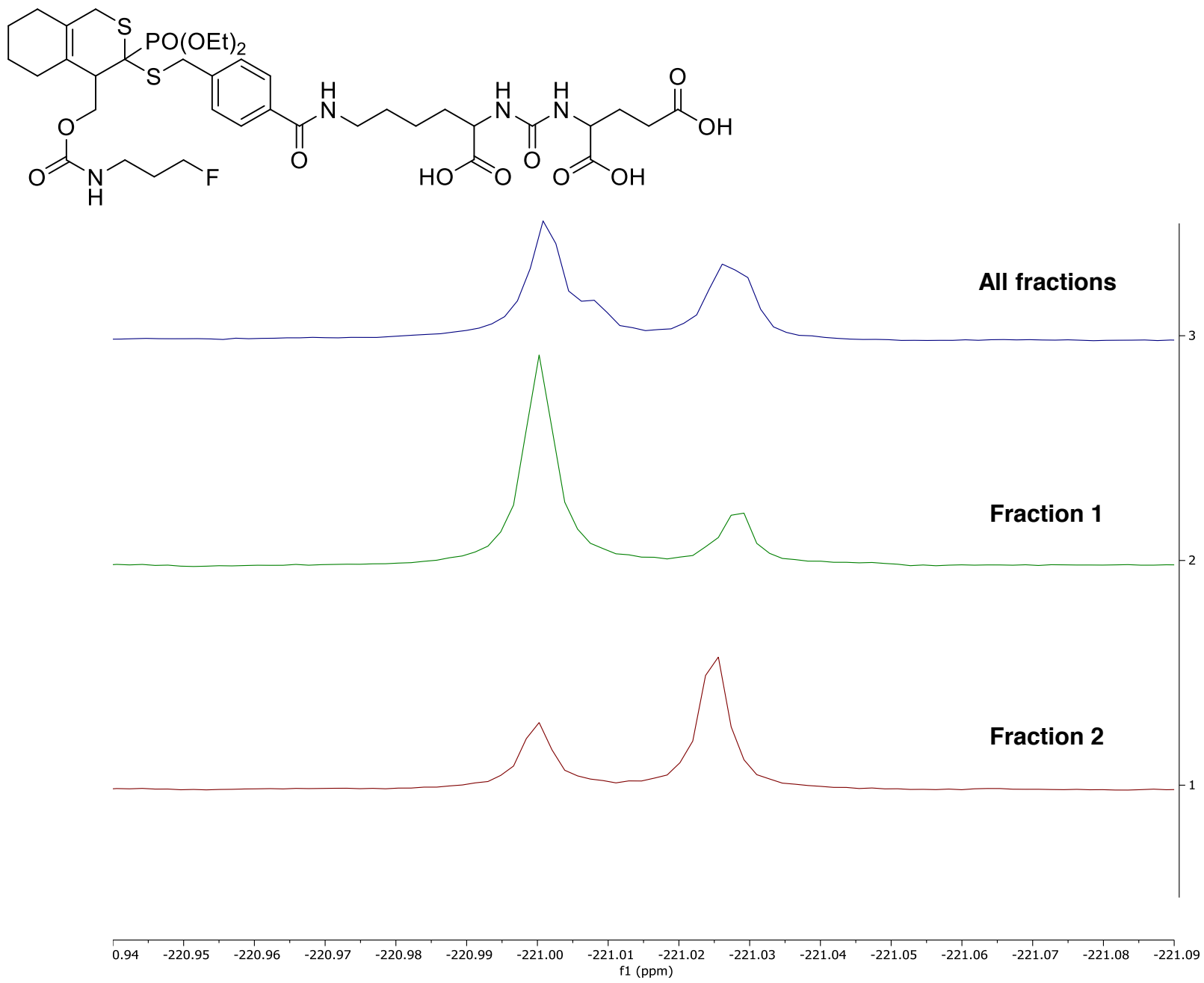

S
PO(OEt)2
S
O
O
N
H
F
H
N
O
H
N
HO
O
H
N
O
O
H
N
O
OH
O
OH

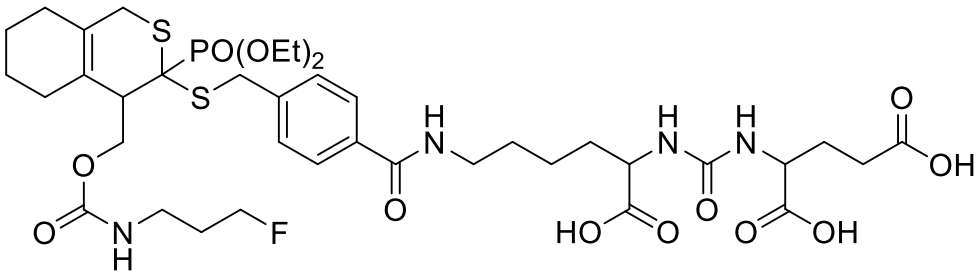
20.37
18.94

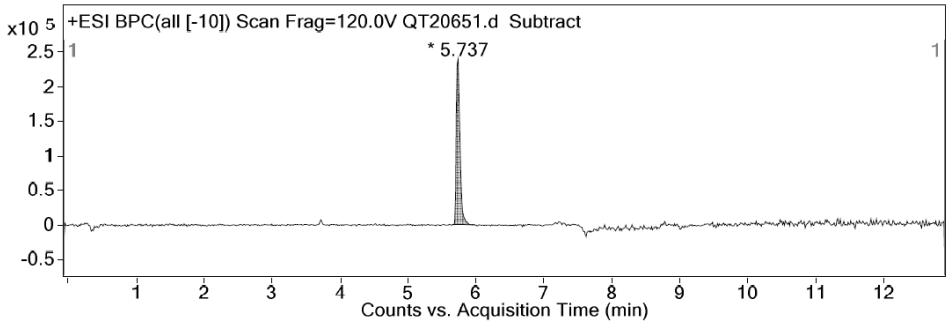

290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90
f1 (ppm)


-76.56
-221.00
-221.03
S
PO(OEt)2
S
O
O
N
H
F
H
N
O
H
N
HO
O
H
N
O
H
N
O
O
OH
O
OH
TFA
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
-240
-2
f1 (ppm)


PO(OEt)2
S
S
O
O
N
H
F
H
N
O
H
N
HO
O
H
N
O
O
OH
O
OH
All fractions
Fraction 1
Fraction 2
3
2
1
20.9 20.8 20.7 20.6 20.5 20.4 20.3 20.2 20.1 20.0 19.9 19.8 19.7 19.6 19.5 19.4 19.3 19.2 19.1 19.0 18.9 18.8 18.7 18.6 18.5 18.4 18.3 18.2 18.1
f1 (ppm)

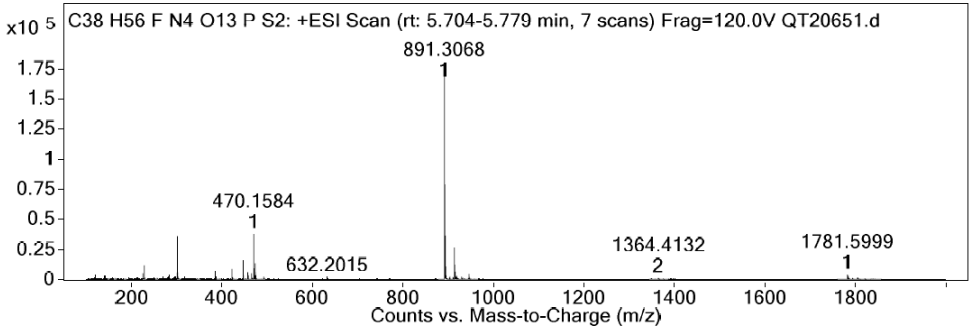

PO(OEt)2
S
S
O
O
N
H
F
H
N
O
H
N
HO
O
H
N
O
O
OH
O
OH
All fractions
Fraction 1
Fraction 2
3
2
1
0.94
-220.95
-220.96
-220.97
-220.98
-220.99
-221.00
-221.01
-221.02
-221.03
-221.04
-221.05
-221.06
-221.07
-221.08
-221.09
f1 (ppm)

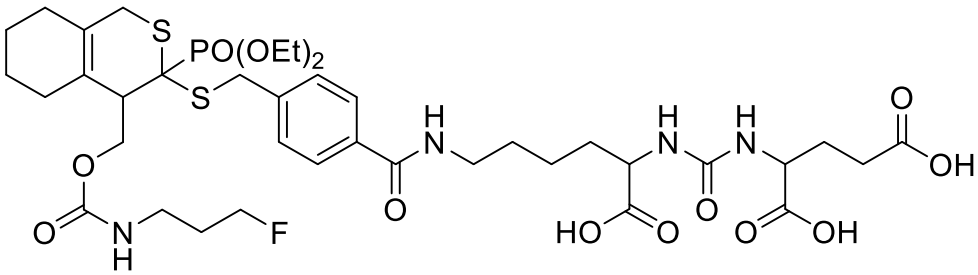


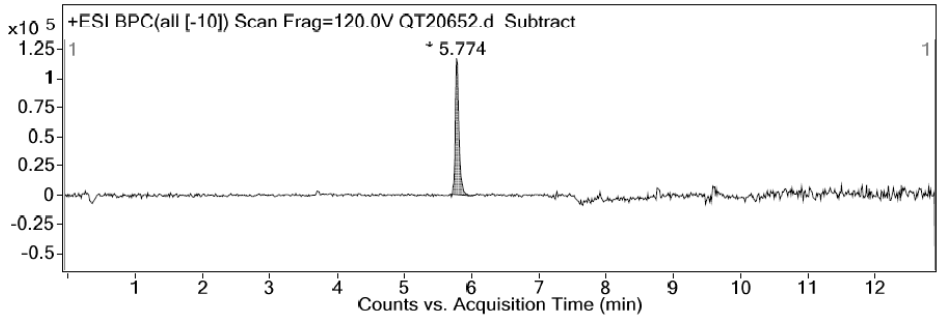


Integration Peak List

Peak	Start	RT	End	Height	Area	Area %	AreaSum%
1	5.659	5.737	5.963	240109.87	907406.7	100	100







Integration Peak List

Peak	Start	RT	End	Height	Area	Area %	AreaSum%
1	5.684	5.774	6.019	116765.24	437453.51	100	100