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Protocol

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Deoxyfluorination of phenols for chemoselective ^{18}F-labeling of peptides

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PART A

Supplementary Tutorial 1

Synthesis and characterization of phenol S19

NOTE: The synthesis of intermediate compounds **S16**, **S17** and **S18** are adapted from ref 1. Spectra for the intermediates were previously reported.[1]

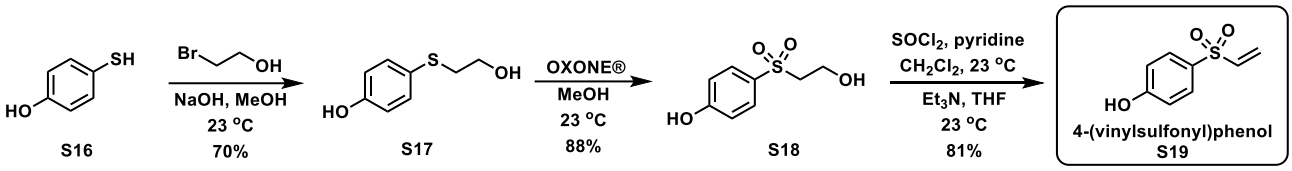


Fig. S1 | Synthesis of 4-(vinylsulfonyl)phenol.

Reagents

- 4-Mercaptophenol (CAS no. 637-89-8, Alfa Aesar, 97%, cat. No. AAL0442906)
- Methanol (CAS no. 67-56-1, ACS reagent, ≥ 99.8%, Fisher Scientific, cat. No. A412-4)
- 2-Bromoethanol (CAS no. 540-51-2, >95.0%, TCI America, cat. No. B0590)
- NaOH (CAS no. 1310-73-2, ACS reagent, Fisher Scientific, cat. No. S318-500)
- Diethyl ether (CAS no. 60-29-7, ≥99%, Fisher Scientific, cat. No. E138-20)
- Sodium bicarbonate (CAS no. 144-55-8, $NaHCO_3$, Fisher Scientific, cat. No. S233-10)
- Sodium sulfate (CAS no. 7757-82-6, Na_2SO_4, Sigma-Aldrich, cat. No. SX0760-10)
- Ethyl acetate (EtOAc, CAS no. 141-78-6, ≥99.5%, Fisher Scientific, cat. No. E145-20)
- Hexane (CAS no. 110-54-3, ≥98.5%, Fisher Scientific, cat. No. H292-20)
- OXONE® (CAS no. 70693-62-8, Alfa Aesar, cat. No. 89892-22)
- Sodium hydrogen sulfite (CAS no. 7631-90-5, Certified ACS, Fisher Scientific, cat. No. S654-500)
- Pyridine (CAS no. 110-86-1, 99%, Oakwood, cat. No. 005154)
- Dichloromethane (CAS no. 75-09-2, Fisher Scientific, cat. No. D151-4)
- Thionyl chloride (CAS no. 7719-09-7, ≥99%, Sigma-Aldrich, cat. No. 230464)
- Tetrahydrofuran (THF, CAS no. 109-99-9, ≥99%, Fisher Scientific, cat. No. 397-4)
- Triethylamine (Et_3N, CAS no. 121-44-8, Fisher Scientific, cat. No. O4885)
- Silica-gel (230-400 Mesh, Fisher, cat. No. S825-25)
- TLC plates (TLC Silica gel 60 F_{254} Aluminium sheets 20x20 cm, Supelco. cat. No. HX91185454)
- $CDCl_3$ (99.8%, Cambridge Isotope Laboratories, cat. No. DLM-7-100)
- DMSO-d_6 (99.9%, Cambridge Isotope Laboratories, cat. No. DLM-10-10)

Procedure

1| Weigh 2.0 g (15.8 mmol) 4-mercaptophenol **S16** into a 100-mL round-bottom flask containing a Teflon-coated magnetic stir bar. With a polypropylene syringe fitted with a 20-guage disposable needle, add 10 mL of methanol and cool the reaction mixture to –5 °C. With a polypropylene syringe fitted with a 20-guage disposable needle, add an aqueous solution of NaOH (1.0 N, 17.3 mL) dropwise over 30 mins at –5 °C. Prepare a solution of 2-bromoethanol (2.3 mL) in methanol (7 mL) and add the resulting solution dropwise, over 15 min at –5 °C, to the round-bottom flask. Stir the reaction mixture at 23 °C for 21 h. Concentrate the reaction mixture in a rotary evaporator and treat the crude residue with 5 mL of water and 20 mL of diethyl ether. After extraction and phase separation, wash the organic phase with 10 mL of saturated aqueous $NaHCO_3$ and 10 mL of brine. Dry the combined organic phases over Na_2SO_4 and remove the solvent under

reduced pressure to yield crude product. Purify the crude residue by flash column chromatography on silica-gel (10–30% ethyl acetate in *n*-hexane) to afford **S17** (1.87 g, 11 mmol, 70% yield) as a white solid.

2| Weigh 1.0 g (5.87 mmol) 4-((2-hydroxyethyl)thio)phenol **S17** into a 15-mL round bottom flask containing a Teflon-coated magnetic stir bar. With a polypropylene syringe fitted with a disposable needle, slowly add 5 mL of methanol and cool the reaction mixture to 10 °C. Add 5.43 g (8.82 mmol) of OXONE® at 10 °C, over 20 min. Stir the suspension at 23 °C (exothermic reaction) for 2 h. Using a Buchner filter funnel equipped with a medium porosity frit, filter the precipitate. Wash the filtrate with a 38- 40% (v/v) aqueous sodium hydrogen sulfite solution (0.5 mL) and adjust the pH of the reaction mixture to ~7.0 by adding 1.0 M aqueous NaOH. Filter the suspension and concentrate the filtrate to dryness under reduced pressure using a rotary evaporator at approximately 23 °C. Purify the crude residue by flash column chromatography on silica-gel (20–60% ethyl acetate in *n*-hexane) to afford 4-((2-hydroxyethyl)sulfonyl)phenol **S18** (1.0 g, 5.20 mmol, 88% yield) as a white solid.

3| Weigh 1.00 g (5.19 mmol) 4-((2-hydroxyethyl)sulfonyl)phenol **S18** into a 25-mL round bottom flask containing a Teflon-coated magnetic stir bar. With a polypropylene syringe fitted with a disposable needle, add 6 mL of dichloromethane followed by 0.8 mL (10 mmol) of pyridine at 23 °C. Cool the reaction mixture to 0 °C. With a polypropylene syringe fitted with a disposable needle, draw up 0.64 mL (8.8 mmol) thionyl chloride and add it to a vial containing 5 mL of dichloromethane. With a polypropylene syringe fitted with a disposable needle, draw up the thionyl chloride/dichloromethane solution and add it dropwise, over 15 min, to the reaction mixture in the 25-mL round bottom flask. Stir the reaction at 23 °C for 20 h. Dilute the reaction mixture with 1 mL brine and extract the suspension/contents of the flask. Combine the organic layers and wash them with 2 mL of brine and extract. Repeat and wash the organic layers with brine a second time. Combine the organic layers, add dry Na_2SO_4, and filter using a filter funnel. Concentrate the filtrate to dryness under reduced pressure using a rotary evaporator to afford 4-((2-chloroethyl)sulfonyl)phenol which was used directly without further purification.

4| Weigh 0.8 g (3.6 mmol) 4-((2-chloroethyl)sulfonyl)phenol into a 25-mL round bottom flask containing a Teflon-coated magnetic stir bar. With a polypropylene syringe fitted with a disposable needle, add 8 mL of THF. With a polypropylene syringe fitted with a disposable needle, draw up 0.76 mL (5.40 mmol) triethylamine and add it to a vial containing 5 mL of THF. With a polypropylene syringe fitted with a disposable needle, draw up the triethylamine/THF solution and add it to the reaction mixture in the 25-mL round bottom flask at 23 °C. Allow the reaction mixture to stir at 23 °C for 24 h. Using a filter funnel, filter the triethylamine hydrochloride salt that precipitates from of the reaction mixture. Concentrate the colorless filtrate to dryness under reduced pressure using a rotary evaporator at approximately 23 °C. Purify the crude solid by flash column chromatography on silica-gel (5–30% ethyl acetate in *n*-hexane) to give 4-(vinylsulfonyl)phenol **S19** (0.53 g, 2.90 mmol, 81% yield) as a white solid.

NMR Spectroscopy:

^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.62 (s, 1H), 7.63 (d, *J* = 8.8 Hz, 2H), 6.97 (dd, *J* = 16.4, 9.6 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 1H), 6.17 (d, *J* = 16.8 Hz, 1H), 6.04 (d, *J* = 9.6 Hz, 1H).
^{13}C NMR (100 MHz, DMSO-d_6, δ): 162.7, 139.9, 130.4, 129.5, 127.2, 116.5.
HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calc'd for $C_8H_8O_3SNa$ 207.0092; Found 207.0084.
Melting point: 64 – 66 °C

Supplementary Tutorial 2

Synthesis and characterization of fluorine-19 reference standard ^{19}F-9.[2,3]

NOTE: The synthesis of intermediate compounds **S20**, **S21** and **S22** are adapted from ref 1. Spectra for the intermediates were previously reported.[1]

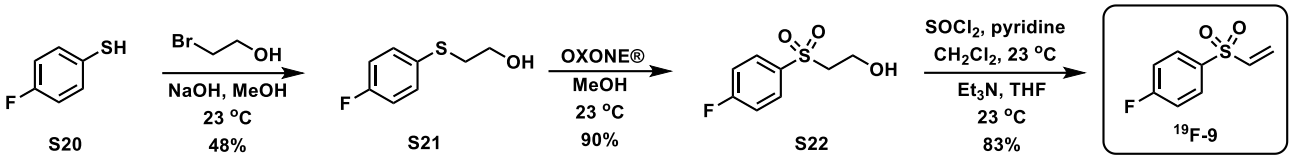


Fig. S2 | Synthesis of fluorine-19 reference standard.

Reagents

- Chemical reagents used in these steps are identical to those used for the preparation of 4-(vinylsulfonyl)phenol **S19** (Fig. S1) and reagent details are listed above, in Supplemental tutorial 1
- 4-fluorobenzenethiol (CAS no. 371-42-6, TCI America, >98.0%, cat. No. 50-014-34924)

Procedure

1| Weigh 280 mg (2.19 mmol) 4-fluorobenzenethiol **S20** (280 mg, 2.19 mmol) into a 4-dram borosilicate vial. With a polypropylene syringe fitted with a 20-guage disposable needle, add 3 mL of methanol and cool the vial to –5 °C. With a polypropylene syringe fitted with a 20-guage disposable needle, add aqueous NaOH (1N, 2.4 mL) in a dropwise fashion, over a period of 15 min and stir the reaction at –5 °C for 1 h. Prepare a solution of 2-bromoethanol (0.3 mL, 2.4 mmol) in methanol (1 mL) and add the resulting solution dropwise, over 10 min at –5 °C, to the 4-dram vial. Allow the vial to warm to room temperature and stir for 21 h at 23 °C. Remove the methanol under reduced pressure using a rotary evaporator at approximately 23 °C. Add 20 mL of diethyl ether and 5 mL of water to the crude residue and extract the organic layer from the aqueous layer. Separate the phases and extract the aqueous layer with diethyl ether again. Combine the organic layers and wash them with saturated aqueous $NaHCO_3$ and brine. Combine the organic layers, add dry Na_2SO_4, and filter using a filter funnel. Concentrate the filtrate to dryness under reduced pressure using a rotary evaporator and purify the crude residue by flash column chromatography on silica-gel (5–20% EtOAc in *n*-hexane) to obtain 2-((4-fluorophenyl)thio)ethanol **S21** (0.18 g, 1.05 mmol, 48% yield) as a light yellow oil.

2| Weigh 181 mg (1.05 mmol) 2-((4-fluorophenyl)thio)ethanol **S21** into a 15-mL round bottom flask containing a Teflon-coated magnetic stir bar. With a polypropylene syringe fitted with a disposable needle, slowly add 4 mL of methanol and cool the reaction mixture to 10 °C. Add 972 mg (1.58 mmol) of OXONE® at 10 °C, over

20 min. Stir the suspension at 23 °C (exothermic reaction) for 2 h. Using a Buchner filter funnel equipped with a medium porosity frit, filter the precipitate. Wash the filtrate with a 38–40% (v/v) aqueous sodium hydrogen sulfite solution (0.5 mL), dry the filtrate over Na_2SO_4 and concentrate to dryness under reduced pressure using a rotary evaporator to afford crude 2-((4-fluorophenyl)sulfonyl)ethanol **S22** (0.19 g, 0.95 mmol, 90% yield) as a light yellow oil which was used without further purification.

3| Weigh 190 mg (0.95 mmol) 2-((4-fluorophenyl)sulfonyl)ethanol **S22** into a 15-mL round bottom flask containing a Teflon-coated magnetic stir bar. With a polypropylene syringe fitted with a disposable needle, add 3 mL of dichloromethane followed by 0.15 mL (1.86 mmol) of pyridine at 23 °C. Cool the reaction mixture to 0 °C. With a microsyringe, draw up 119 µL (1.61 mmol) thionyl chloride and add it to a vial containing 1 mL of dichloromethane. With a polypropylene syringe fitted with a disposable needle, draw up the thionyl chloride/dichloromethane solution and add it dropwise, over 15 min, to the reaction mixture in the 15-mL round bottom flask. Stir the reaction at 23 °C for 20 h. Dilute the reaction mixture with 1 mL brine and extract the suspension/contents of the flask. Combine the organic layers and wash them with 2 mL of brine and extract. Repeat and wash the organic layers with brine a second time. Combine the organic layers, add dry Na_2SO_4, and filter using a filter funnel. Concentrate the filtrate to dryness under reduced pressure using a rotary evaporator to afford 1-((2-chloroethyl)sulfonyl)-4-fluorobenzene (0.20 g, 0.90 mmol, 94% yield) as a yellow solid. *Note:* 1-((2-chloroethyl)sulfonyl)-4-fluorobenzene is slightly unstable and undergoes spontaneous elimination to afford vinyl sulfone **^{19}F-9**, which can be seen in the NMR of this intermediate. For this reason, 1-((2-chloroethyl)sulfonyl)-4-fluorobenzene was taken on to the next step without further purification.

4| Weigh 202 mg (0.9 mmol) 1-((2-chloroethyl)sulfonyl)-4-fluorobenzene into a 15-mL round bottom flask containing a Teflon-coated magnetic stir bar. With a polypropylene syringe fitted with a disposable needle, add 3 mL of THF. With a microsyringe, draw up 187 µL (1.35 mmol) triethylamine and add it to a vial containing 1 mL of THF at 23 °C. With a polypropylene syringe fitted with a disposable needle, draw up the triethylamine/THF solution and add it to the reaction mixture in the 15-mL round bottom flask at 23 °C. Allow the reaction mixture to stir at 23 °C for 1 h. With a polypropylene syringe fitted with a disposable needle, add 1.0 mL of aqueous HCl (1.0 M) to quench the reaction. Add 4 mL of ethyl acetate to the flask and extract the aqueous phase twice using 3 mL of ethyl acetate. Combine the organic layers and wash them with 2 mL of brine and extract. Repeat and wash the organic layers with brine a second time. Combine the organic layers, add dry Na_2SO_4, and filter using a filter funnel. Concentrate the filtrate to dryness under reduced pressure using a rotary evaporator and purify by flash column chromatography on silica-gel (10–30% EtOAc in *n*-hexane) to afford fluoro-4-(vinylsulfonyl)benzene **^{19}F-9** (0.15 g, 0.79 mmol, 88% yield) as a light yellow oil.

Characterization data for fluoro-4-(vinylsulfonyl)benzene ^{19}F-9:

^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.91 (dd, *J* = 8.8, 4.8 Hz, 2H), 7.26-7.2 (m, 2H), 6.6 (dd, *J* = 16.4, 10 Hz, 2H), 6.46 (d, *J* = 16.4 Hz, 1H), 6.05 (d, *J* = 10 Hz, 1H).
^{13}C NMR (100 MHz, $CDCl_3$, δ): 165.8 (C-F, $^1J_{C\text{-}F}$ = 254.8 Hz), 138.4, 135.6 (C-F, $^4J_{C\text{-}F}$ = 3.2 Hz), 130.8 (C-F, $^3J_{C\text{-}F}$ = 9.7 Hz), 127.9, 116.8 (C-F, $^2J_{C\text{-}F}$ = 22.6 Hz).

^{19}F NMR (376 MHz, $CDCl_3$, δ): -103.4.
HRMS (ESI-TOF) m/z: [M+Na] + Calc'd for $C_8H_7FO_2SNa$ 209.0049; Found 209.0041.

ANTICIPATED RESULTS

Analytical data

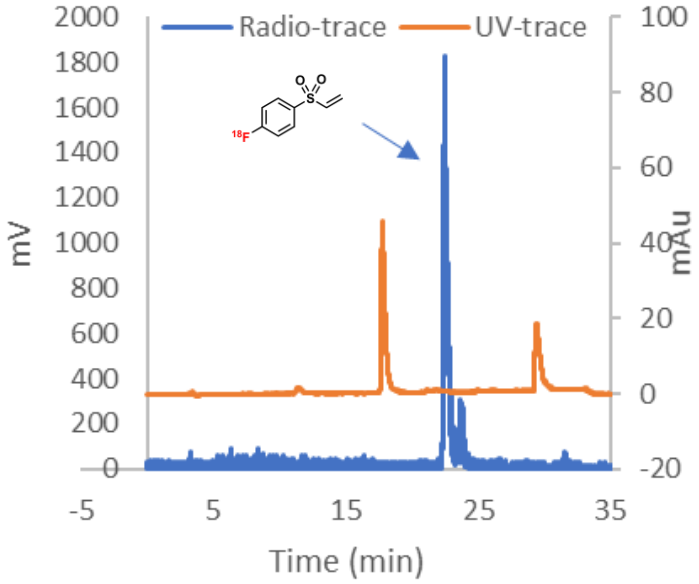


Fig. S3 | Analytical radio-HPLC with 254 nm UV trace (orange) and radioactive trace (blue) of cartridge purified **^{18}F-FVSB (^{18}F-9)**, obtained in 85% radiochemical purity (RCP).

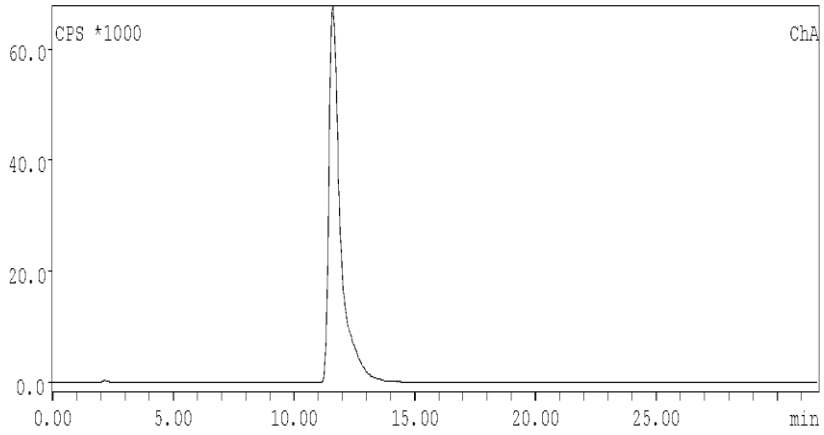


Fig. S4 | Analytical HPLC chromatogram obtained for HPLC purified **^{18}F-FVSB (^{18}F-9)**.

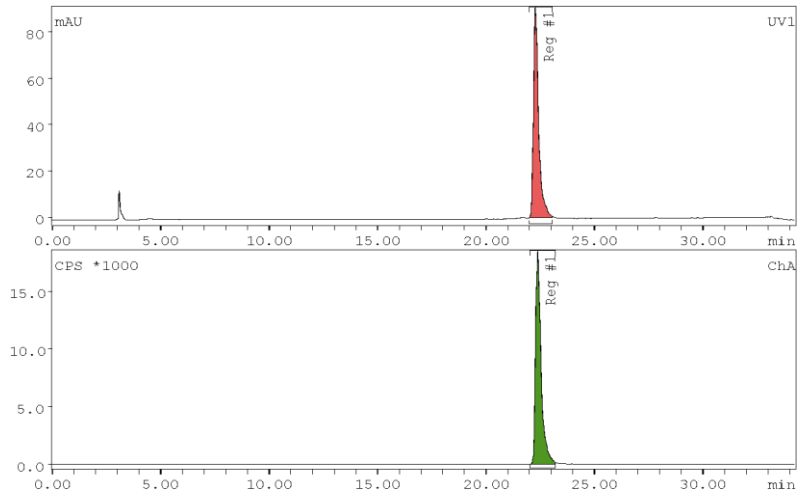


Fig. S5 | Coinjection of HPLC purified **^{18}F-FVSB (^{18}F-9)** spiked with an aliquot of **^{19}F-FVSB (^{19}F-9)** reference standard using an analytical HPLC column. γ-trace (lower) and 254 nm UV trace (upper).

Supplementary Tutorial 3

Synthesis and characterization of fluorine-19 reference standard for the radiolabeled L-glutathione conjugate

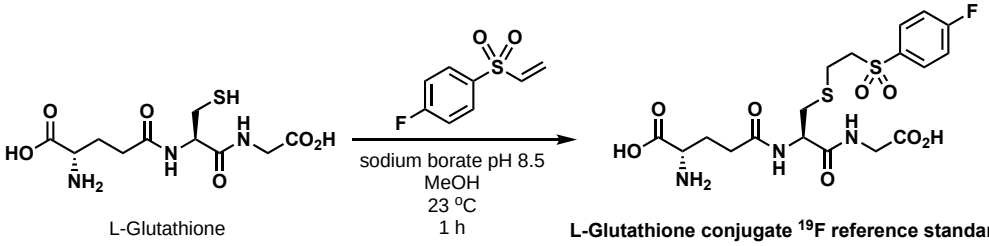


Fig. S6 | Synthesis of fluorine-19 reference standard.

Reagents

- Fluoro-4-(vinylsulfonyl)benzene (CAS no. 28122-14-7, Enamine Building Blocks, >95.0%, cat. No. EN300-115569; alternative: preparation from 4-fluorobenzenethiol as described in the Supplementary tutorial 2)
- Borate Buffer (0.5 M Borate buffer, pH 8.5, VWR, cat. No. AAJ60803-AK)
- Methanol (CAS no. 67-56-1, Sigma-Aldrich, cat. No. 322415-100mL, anhydrous 99.8%)
- L-Glutathione, reduced, free acid (CAS no. 70-18-8, Sigma-Aldrich, cat. No. 3541-5GM)

Procedure

1| Add fluoro-4-(vinylsulfonyl)benzene (10 mg, 53.7 µmol) and L-glutathione (16.5 mg, 53.7 µmol) to a 4-dram vial equipped with a 10 × 3 mm magnetic stir bar.

2| Add 500 µL methanol and 800 µL sodium borate buffer pH 8.5 and stir the reaction mixture at 23 °C for 1 h.

3| Purify the reaction mixture with semi-preparative HPLC. Load 150 µL of the vial contents onto the HPLC

loop each time and inject the reaction mixture onto the semi-preparative HPLC column (Phenomenex reverse-phase Luna, 10 × 250 mm, 5 µm) for purification (the reaction mixture was sequentially purified in 250 µL aliquot injections). Set the flow rate to 3 mL·min^{-1}, with an isocratic mixture of 5:95 (MeCN:water, 0.1% TFA, v:v) for 3 minutes, followed by a linear gradient to 80:20 (MeCN:water, 0.1% TFA, v:v) over 30 minutes. Combine the collected fractions containing the product and concentrate *in vacuo* to dryness to obtain the fluorine-19 reference standard (12 mg, 45% yield) as a white solid.

Characterization data for the L-glutathione conjugate fluorine-19 reference standard:
^{1}H NMR (400 MHz, DMSO-d_6, δ): 8.50 (t, *J* = 6.0 Hz, 1H), 8.25 (d, *J* = 8.4 Hz, 1H), 7.95 (dd, *J* = 9.2, 5.2 Hz, 2H), 7.47 (m, 2H), 4.38 (m, 1H), 3.69 (dd, *J* = 6.0, 3.2 Hz, 2H), 3.62-3.54 (m, 3H), 2.84 (dd, *J* = 14.0, 4.8 Hz, 1H), 2.68 (dd, *J* = 9.6, 6.4 Hz, 2H), 2.55 (dd, *J* = 14.0, 9.6 Hz, 1H), 2.29-2.25 (m, 2H), 1.94-1.88 (m, 2H).
^{13}C NMR (100 MHz, DMSO-d_6, δ): 171.8, 171.3, 171.2, 170.9, 165.6 (d, *J* = 251.2 Hz), 135.5 (d, *J* = 2.9 Hz), 131.6 (d, *J* = 9.7 Hz), 117.2 (d, *J* = 22.7 Hz), 55.4, 52.8, 52.4, 41.3, 34.2, 31.5, 26.9, 24.4.
^{19}F NMR (376 MHz, DMSO-d_6, δ): -104.56.
HRMS (ESI-TOF) m/z: Calc'd for $C_{18}H_{25}FN_3O_8S_2$[M+H] 494.1067; found 494.1077.

Molar activity determination for the ^{18}F-labeled L-glutathione conjugate

Using the authentic reference material for the L-glutathione conjugate (Fig. S6) a standard curve was generated by integration of the UV absorbance signal (at 254 nm) of five different known amounts (performed in triplicate):

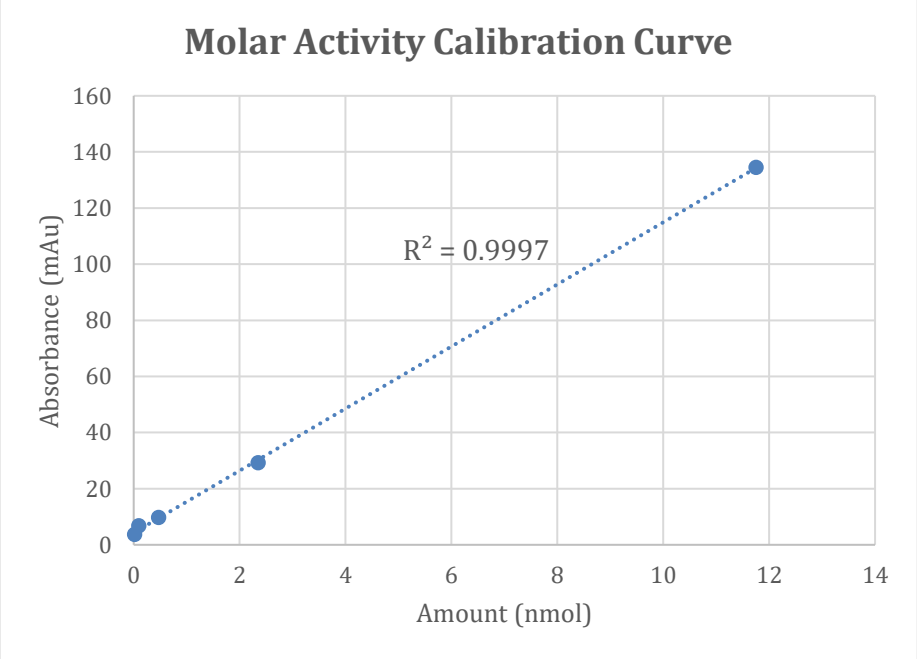


Fig. S7 | Standard curve of the UV absorbance vs amount of the authentic reference standard ^{19}F-L-glutathione conjugate.

^{18}F-Deoxyfluorination of uronium precursor **10** was performed and subsequent peptide labeling was conducted to furnish the ^{18}F-labeled L-glutathione conjugate, which was purified by semi-preparative HPLC. The reaction mixture was concentrated and dissolved in 100 µL methanol. An aliquot of purified peptide was injected into an analytical HPLC for analysis. From comparison with the standard curve, it was determined that the molar activity of the sample (from n = 3 trials) was 1.28 ± 0.10 Ci·µmol^{-1} (47.36 ± 3.7 GBq·µmol^{-1}).

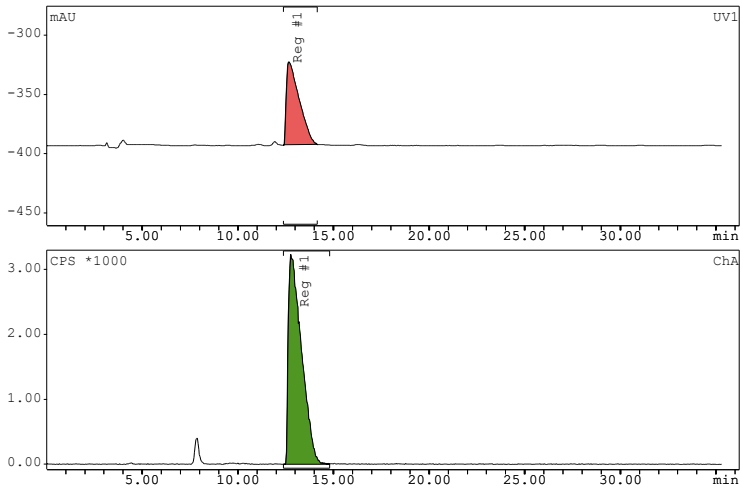
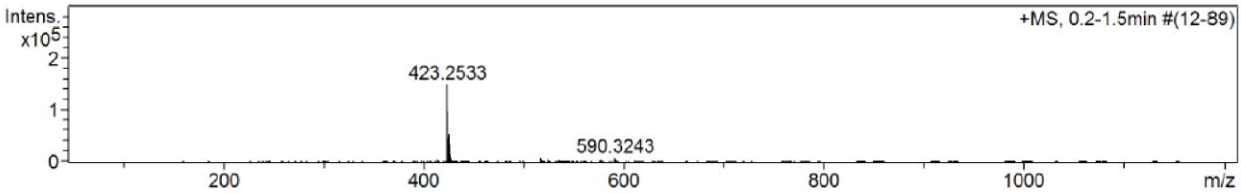


Fig. S8 | Coinjection of HPLC purified ^{18}F-labeled L-glutathione conjugate spiked with an aliquot of the authentic reference standard using an analytical HPLC column. (γ-trace = blue, 254 nm UV trace = orange).

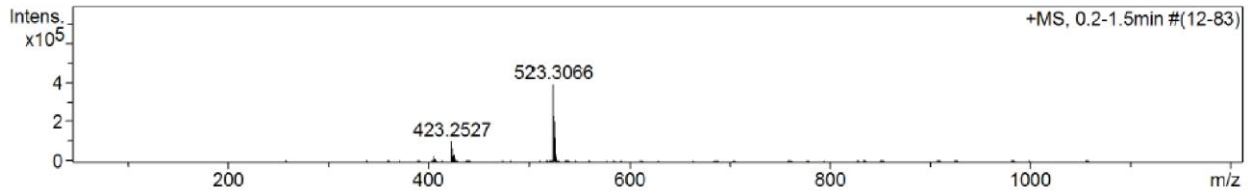
Monitoring the production of uronium labeling precursor via mass spectrometry

Formation of the uronium precursor from the reaction between a phenol and chloroimidazolium **1** can be followed by removing aliquots from the reaction mixture at regular intervals and analyzing them by mass spectrometry. The reaction is completed when the peak at m/z = 423.25, which corresponds to chloroimidazolium chloride **1** is no longer observed.

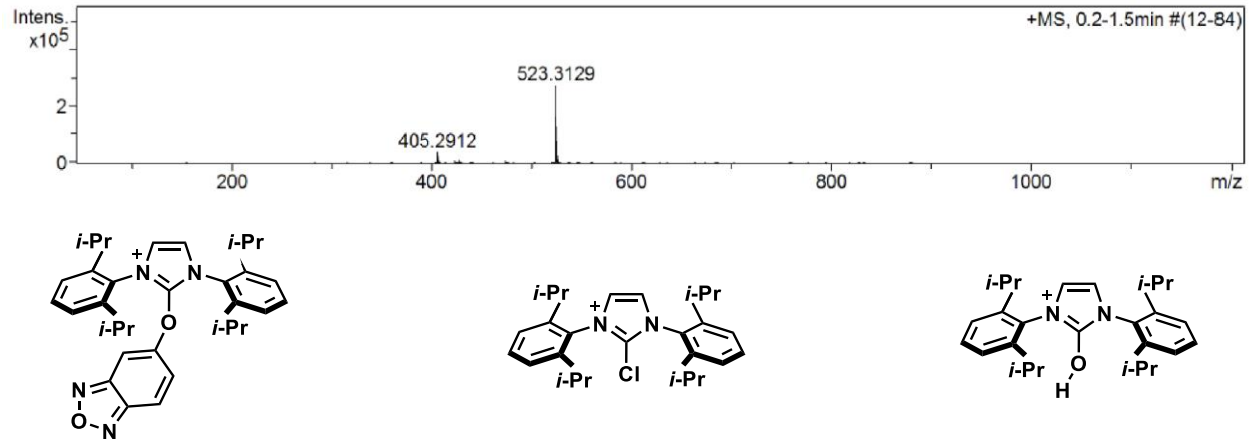
Reaction time: 1 min



Reaction time: 1.5 hours



Reaction time: 4 hours



Chemical Formula: $C_{33}H_{39}N_4O_2^+$
Exact Mass: 523.30675
m/z: 523.30675 (100.0%)
524.31011 (35.7%)

Chemical Formula: $C_{27}H_{36}ClN_2^+$
Exact Mass: 423.25615
m/z: 423.25615 (100.0%)
425.25320 (32.0%)
424.25951 (29.2%)

Chemical Formula: $C_{27}H_{37}N_2O^+$
Exact Mass: 405.29004
m/z: 405.29004 (100.0%)
406.29340 (29.2%)

Fig. S9 | The presence of the uronium product for a sample phenol, 5-hydroxybenzofurazan, and imidazolium chloride reagent **1** in the reaction mixture can be conveniently determined by mass spectrometry.

"Reverse elution" workflow for troubleshooting low elution of fluorine-18 from anion exchange cartridge

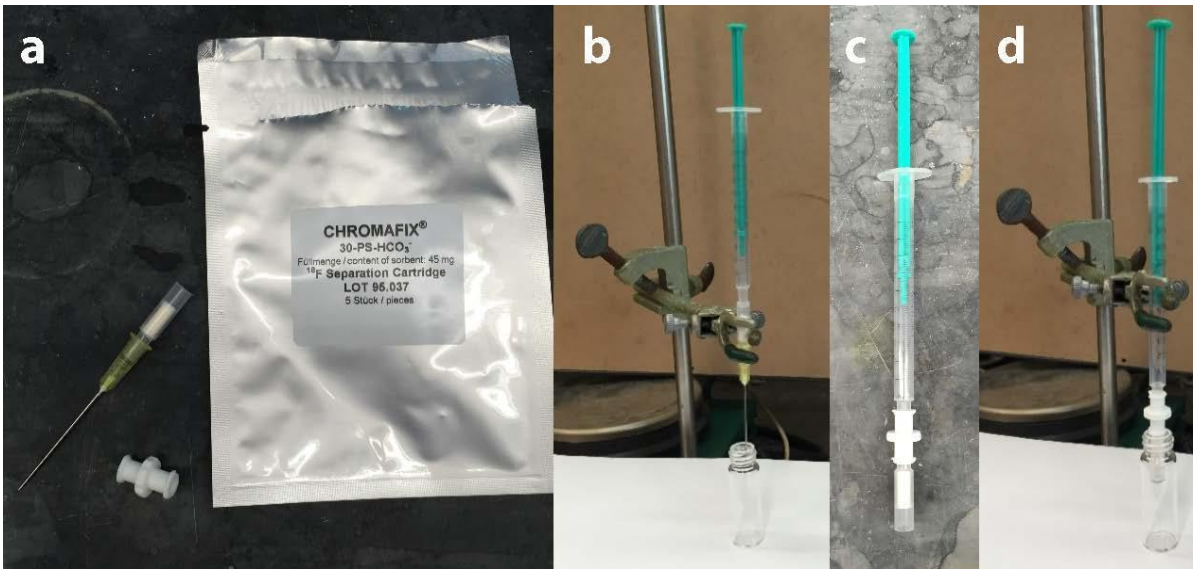


Fig. S10 | **Photographs of the reverse elution workflow. a,** A needle is affixed to a Chromafix cartridge; female-female luer adaptor **b,** Fluorine-18 loading set-up **c,** Cartridge fitted with a female-female luer adaptor **d,** Fluorine-18 elution set-up.

PART B

Supplementary Tutorial 4

Synthesis and characterization of Bis(cyclopentadienyl)ruthenium(II) (S23)

NOTE: The synthesis of intermediate compounds **S23** is adapted from previously reported method.[4]

Reagents

- Ruthenium trichloride hydrate (metal content: 38%–43% ruthenium; CAS no. 14898-67-0, Johnson Matthey, cat no. Ru-131)
- Absolute ethanol (CAS no. 64-17-5, Sigma Aldrich, cat no. 200-578-6)
- Cyclopentadiene dimer (CAS no. 77-73-6, Alfa Aesar, cat no. 32546). Cyclopentadiene monomer was obtained prior to the synthesis by cracking at ~160 °C and distilling to collect the product.[5]
- Zinc dust (~325 mesh, 99.9% (metals basis); CAS no. 7440-66-6, Alfa Aesar, cat no. 39694)
- Toluene (CAS no. 108-88-3, Oqema, cat no. 202011000)
- $CDCl_3$ (CAS no. 865-49-6, Deutero, cat no. 00405)

Spectroscopy and Instruments

- NMR spectra were recorded on a Varian Unity/Inova 600 spectrometer operating at 600 MHz and 151 MHz for ^{1}H and ^{13}C acquisitions, respectively. Chemical shifts are reported in ppm with the solvent residual peak as the internal standard. For ^{1}H NMR: $CDCl_3$, δ 7.26; CD_3CN, δ 1.96; DMSO-d_6, δ 2.50. For ^{13}C NMR: $CDCl_3$, δ 77.16; CD_3CN, δ 1.32; CD_2Cl_2, δ 53.84; DMSO-d_6, δ 39.52[6]. ^{19}F NMR spectra were referenced using a unified chemical shift scale based on the ^{1}H resonance of tetramethylsilane (1% v/v solution in the respective solvent).[7] Data is reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sext = sextet, sept = septet, m = multiplet, bs = broad singlet; coupling constants are reported in Hz.
- High-resolution mass spectra (HRMS) were obtained using Q Exactive Plus from Thermo.

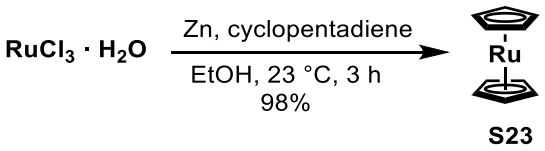


Fig. S11 | Synthesis of Bis(cyclopentadienyl)ruthenium(II) (**S23**)

1. Add 6.0 g ruthenium trichloride hydrate (29 mmol, 1.0 equiv) and 85 mL absolute ethanol (c = 0.34 M) to a 250 mL two-neck round-bottom flask equipped with a Teflon-coated magnetic stirring bar and a thermometer.
2. Place the reaction flask in an ice bath, cool the reaction mixture to 0 °C and, add 24 mL cyclopentadiene (0.29 mol, 10 equiv)[4] via syringe to the dark red solution.
3. Add 19 g zinc dust (~325 mesh, 99.9% (metals basis), 0.29 mol, 10 equiv) over 60 minutes in 10 equal portions to the stirred solution, maintaining the temperature between 0 °C and 10 °C during the addition.

! CAUTION Use of large zinc particles can lead to the accumulation of zinc and a delayed exothermic reaction.

? TROUBLESHOOTING

4. Stir the reaction mixture at 0 °C for 30 minutes, then remove the ice bath and continue stirring for 3 hours.
5. Filter the suspension over a 60 mL Büchner funnel with microporosity (code M) and wash the metallic grey solid with hot toluene (100 °C, 4 × 85 mL).

! CAUTION Toluene is a highly flammable solvent. Take toluene in a round bottom flask attached to a reflux condenser and heat it to 80-90 °C. After heating, pour the hot solvent carefully and keep it away from an open flame.

6. Concentrate the filtrate on rotatory evaporation to dryness, dissolve the brown residue in toluene (0.30 L) at 23 °C and pass through a plug of silica gel (15 g), and subsequently rinse with toluene (0.15 L).

? TROUBLESHOOTING

7. Concentrate the resulting yellowish solution *in vacuo* to dryness to afford Bis(cyclopentadienyl)ruthenium(II) (**S23)** (6.6 g, 28.4 mmol, 98% yield).

Table S1│Troubleshooting table for the synthesis of Bis(cyclopentadienyl)ruthenium(II) (**S23**).

Step	Problem	Possible reasons	Solution
3	Insufficient or no stirring	The stirring bar got stuck to the glass wall	Shake the flask until stirring is possible or use a long needle to move the stirring bar
3	Delayed exothermic reaction	Too large zinc particle size leading to zinc accumulation	Use zinc dust with a particle size of ~325 mesh
3	Temperature rises above 10 °C	Too quick addition of zinc	Divide zinc addition into smaller portions
6	The filtrate looks brownish	The impurities were not removed via the silica plug	Use more silica
6	Low yield of product obtained	The concentration in step 6 was not complete	Re-concentrate the solution again for longer at lower vacuum and higher temperature and filter through another silica plug
		Product was stuck on the filter	Rinse the plug with hot toluene

NMR Spectroscopy:

^{1}H NMR (600 MHz, $CDCl_3$, 25 °C, δ): 4.56 (s).

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$, °25 C, δ): 70.2.

HRMS-EI (m/z) calc'd for C10H10Ru $[M]^+$, 231.98205; found, 231.98208; deviation: –0.13 ppm.

Supplementary Tutorial 5

Synthesis and characterization of [(Cp)Ru(η^6-naphthalene)]·$CF_3SO_3^-$ (S24)

NOTE: The synthesis of intermediate compounds **S24** is adapted from previously reported method.[4]

Reagents

- Naphthalene (CAS no. 91-20-3, Alfa Aesar, cat no. A13188)
- Aluminum trichloride (CAS no. 7446-70-0, Alfa Aesar, cat no. 44470)
- Aluminum powder (CAS no. 7429-90-5, Strem Chemicals, cat no. MFCD00134029)
- Decalin (CAS no. 91-17-8, Alfa Aesar, cat no. A13883)
- Titanium tetrachloride (CAS no. 7550-45-0, ABCR GmbH, cat no. AB354527)
- HCl solution (38%, w/w; CAS no. 7647-01-0, JT Baker, cat no. MFCD00011324)
- Hydrogen peroxide solution (CAS no. 7722-84-1, JT Baker, cat no. 15558324)
- Sodium triflate (CAS no. 2926-30-9, TCI Deutschland GmbH, cat no. T1550)
- Dichloromethane (CAS no. 75-09-2, Sigma-Aldrich, cat no. DX0835)
- Sodium sulfate (CAS no. 7757-82-6, VWR International GmbH, cat no. 231-820-9)
- Diethyl ether (CAS no. 60-29-7, VWR, cat no. 1.00921.6025)

Spectroscopy and Instruments (same as in supplementary tutorial 3)

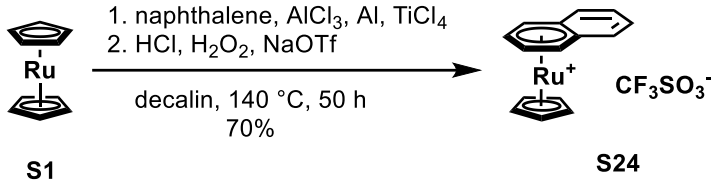


Fig. S12 | Synthesis of [(Cp)Ru(η^6-naphthalene)]·$CF_3SO_3^-$ (S24)

1. Under an inert atmosphere, add 6.0 g **S23** (26 mmol, 1.0 equiv), 33.3 g naphthalene (260 mmol, 10.0 equiv), 3.5 g $AlCl_3$ (26 mmol, 1.0 equiv), and 0.35 g aluminum powder (~325 mesh, 99.7%, 13 mmol, 0.50 equiv) to a 500 mL oven-dried two-neck round-bottom flask equipped with a reflux condenser and a Teflon-coated egg-shaped magnetic stirring bar.
2. Add 0.14 L dry decalin (c = 0.19 M) followed by dropwise addition of 1.4 mL $TiCl_4$ (13 mmol, 0.50 equiv) via a syringe.

! CAUTION Titanium tetrachloride reacts strongly exothermic with water and the humidity in the air. Furthermore, it can lead to corrosion of plastic syringes after a while.

▲ CRITICAL STEP The solids must not get stuck to the wall as this will reduce the reaction surface.

3. Heat the resulting red suspension to 140 °C and stir for 50 hours.

? TROUBLESHOOTING

4. Remove the oil bath, cool to room temperature, and pour the reaction mixture onto a mixture of 0.2 kg ice, 37 mL aqueous concentrated HCl-solution (38%, w/w), and 26 mL H_2O_2 (30% solution in H_2O).

5. Separate the aqueous layer from the organic layer with the aid of a separatory funnel, and wash the aqueous layer with pentane (2 × 50 mL).
6. Extract the combined organic layers with 30 mL water.
7. Add the combined aqueous layers back to the reaction flask, and add 8.9 g sodium triflate (52 mmol, 2.0 equiv) to the flask.
8. Stir the resulting orange solution for 15 minutes and extract the suspension with dichloromethane (5 × 0.13 L).
9. Dry the combined organic layers over sodium sulfate, filter, and concentrate *in vacuo* to dryness.
10. Dissolve the brown residue in 15 mL dichloromethane and then add it dropwise through a syringe-filter to 0.17 L vigorously stirred diethyl ether.

▲ **CRITICAL STEP** Slow addition is required or otherwise, impurities are trapped in the precipitate.

11. Filter the suspension through a 60 mL Büchner funnel with microporosity (code M), wash with diethyl ether (2 × 15 mL), and dry *in vacuo* to afford **S24** (5.4 g, 18.3 mmol, 70% yield).

Table S2│Troubleshooting table for the synthesis of [(Cp)Ru(η^6-naphthalene)]·$CF_3SO_3^-$ (**S24**).

Step	Problem	Possible reasons	Solution
3	Insufficient or no stirring	Stirring bar got stuck to the glass wall	Shake the flask until stirring is possible or use a long needle to move the stirring bar
			Use overhead stirrer

NMR Spectroscopy:

^{1}H NMR (600 MHz, $CDCl_3$, 25 ºC, δ): 7.76 (dd, *J* = 6.7, 3.2 Hz, 2H), 7.58 (dd, *J* = 6.8, 3.1 Hz, 2H), 7.10 (dd, *J* = 4.4, 2.4 Hz, 2H), 6.40 (dd, *J* = 4.4, 2.4 Hz, 2H), 5.05 (s, 5H).

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$, 25 ºC, δ): 131.6, 129.5, 97.2, 86.3, 84.1, 80.1.

^{19}F NMR (565 MHz, $CDCl_3$, 25 ºC, δ): −77.1.

HRMS-ESI (m/z) calc'd for C15H13Ru $[M-OTf]^+$, 295.00553; found, 295.00552; deviation: –0.02 ppm..

Supplementary Tutorial 6

Synthesis and characterization of [Fmoc-tyrosine(RuCp)-OH]·CF_3CO_2 (12)

NOTE: The synthesis of intermediate compounds **12** is adapted from previously reported method.[4]

Reagents

- L-tyrosine (CAS no. 60-18-4, ABCR GmbH, cat no. AB119378)
- Deionized water
- Trifluoroacetic acid (CAS no. 76-05-1, ABCR GmbH, cat no. AB212435)
- Sodium carbonate
- Fmoc-N-hydroxysuccinimide ester (Fmoc-OSu) (CAS no. 82911-69-1, ABCR GmbH, cat no. AB181827)
- Hexane (CAS no. 110-54-3, Oqema, cat no. 202011000)
- Dioxane (CAS no. 123-91-1, ABCR GmbH, cat no. AB208550)
- Ethyl acetate (CAS no. 141-78-6, Firma Oqema GmbH, cat no. 813379)
- Acetonitrile (CAS no. 75-05-8, Sigma-Aldrich, cat no. 34851, HPLC, gradient grade, ≥99.9%)
- Column chromatography was performed using silica gel (40–63 µm particle size) purchased from Geduran.
- CD_3OD (CAS no. 811-98-3, Euriso-top, cat no. D024FE)

Spectroscopy and Instruments

- NMR and HRMS (same as mentioned in supplementary tutorial 3)
- 40 W blue LED (Kessil A 160WE Tuna Blue).
- Thin layer chromatography (TLC) was performed using EMD TLC plates pre-coated with 250 µm thickness silica gel 60 F254 plates and visualized by fluorescence quenching under UV light.

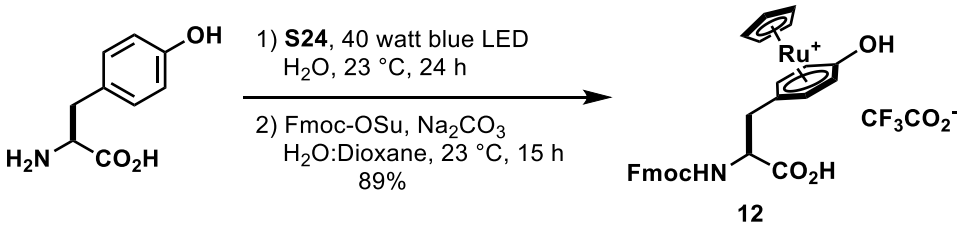


Fig. S13 | Synthesis of [Fmoc-tyrosine(RuCp)-OH]·CF_3CO_2 (**12**)

1. Add 5.0 g [(Cp)Ru(η^6-naphthalene)]·CF_3SO_3 (**S24)** (11 mmol, 1.0 equiv), 2.6 g *L*-tyrosine (14 mmol, 1.2 equiv), 0.56 L water (c = 0.02 M), and 2.2 mL trifluoroacetic acid acid (28 mmol, 2.5 mmol) to a 1.0 L round-bottom flask equipped with a Teflon-coated magnetic stirring bar.
2. Irradiate the yellow suspension for 24 h with blue LED light (Kessil A 160WE Tuna Blue, 40 W).

▲ CRITICAL STEP The tyrosine must be fully consumed at this point; failure will result in tyrosine contamination, which challenges the purification procedure, and column chromatography must be used instead. If required, the reaction time needs to be adjusted and more ruthenium precursor needs to be added. The conversion of the reaction can be monitored by HPLC analysis.

? TROUBLESHOOTING

3. The aqueous suspension was washed with hexane (2 x 0.15 L), and the combined organic layers were extracted with 35 mL water.
4. Basify the resulting beige suspension by slow addition of 6.0 g sodium carbonate (56 mmol, 5.0 equiv)
5. Cool the suspension to 0 °C with an ice bath and add a solution of 5.7 g Fmoc-OSu (17 mmol, 1.5 equiv) in 0.19 L dioxane.
6. Stir the reaction mixture at 0 °C for 1 hour, then at 23 °C for 14 hours.
7. Concentrate the solution by rotary evaporation to two-thirds of the original volume and wash the aqueous layer with dichloromethane (3 × 0.15 L).
8. Acidify the aqueous layer with TFA to pH 3 and then extract with DCM (4 × 0.23 L).
9. Dry the combined organic layers over sodium sulfate, filter, and concentrate *in vacuo* to dryness.
10. The residual brown solid was purified by column chromatography (EtOAc:MeCN:TFA 90:9:0.1, v:v:v) to afford **12** as a yellow powder (6.8 g, 10 mmol, 91% yield).

Table S3│Troubleshooting table for the synthesis of [Fmoc-tyrosine(RuCp)-OH]·CF_3CO_2 (**12**).

Step	Problem	Possible reasons	Solution
2	Low conversion of **S24** observed	Insufficient irradiation due to the formation of deposits on the wall	Remove solid with a spatula and then irradiate
		Slow reaction due to turbidity.	Increase the reaction time
2	Low conversion of L-tyrosine	Sufficient amount of **S24** not added	Add more **S24**

R_f = 0.55 (EtOAc:MeCN:TFA, 8:2:0.1, v:v:v).

NMR Spectroscopy:
^{1}H NMR (600 MHz, CD_3OD, 25 ºC, δ): 7.81 (d, *J* = 7.6, 2H), 7.64 (dd, *J* = 10.8, 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.33 – 7.30 (m, 2H), 6.03 (dd, *J* = 6.3, 1.6 Hz, 1H), 6.02 – 5.96 (m, 3H), 5.30 (s, 5H), 4.47 – 4.33 (m, 3H), 4.20 (t, *J* = 6.5 Hz, 1H), 2.99 (dd, *J* = 14.2, 4.6 Hz, 1H), 2.71 (dd, *J* = 14.2, 9.4 Hz, 1H).
^{13}C{^{1}H} NMR (151 MHz, CD_3OD, 25 ºC, δ): 173.7, 158.3, 145.3, 145.1, 142.7, 135.1, 128.8, 128.2, 126.1, 121.0, 99.2, 86.8, 86.6, 81.3, 75.9, 75.7, 67.7, 56.3, 36.9.
^{19}F NMR (471 MHz, CD_3OD, 25 ºC, δ): −76.9.
HRMS-ESI (m/z) calc'd for C29H26NO5Ru $[M-CF3CO2]^+$,570.08490; found, 570.08517; deviation: –0.47 ppm.

Supplementary Tutorial 7

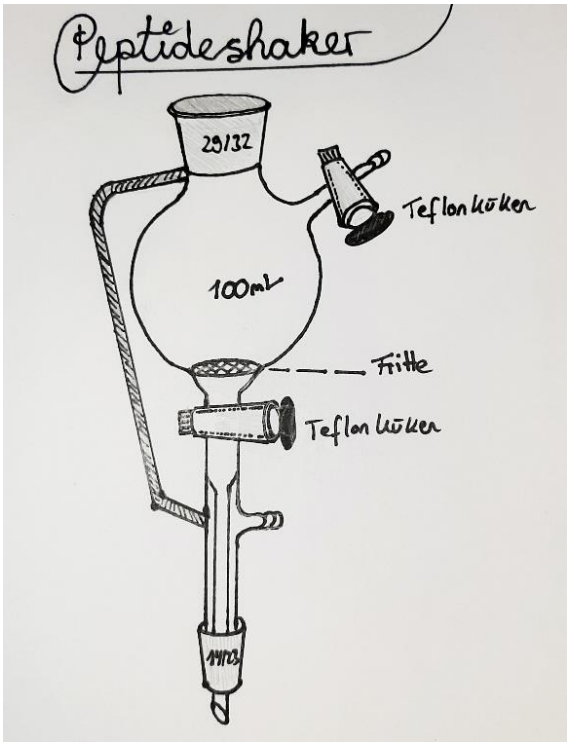


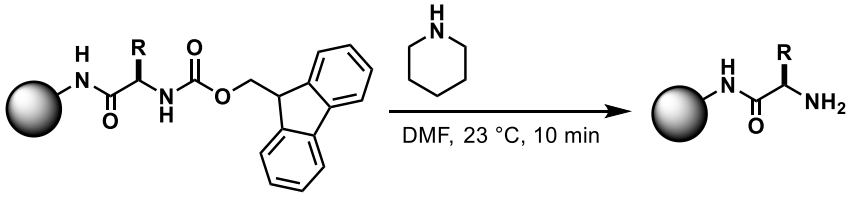
Fig. S14 | Schematic diagram of peptide synthesis vessel (self-made).

Peptides are synthesized by solid-phase peptide synthesis using the Fmoc/*t*Bu-orthogonal strategy on a Fmoc-Rink-Amid-2CT resin (200–400 mesh, 1% DVB, 0.68 mmol · g^{-1}).[8]

General procedures:

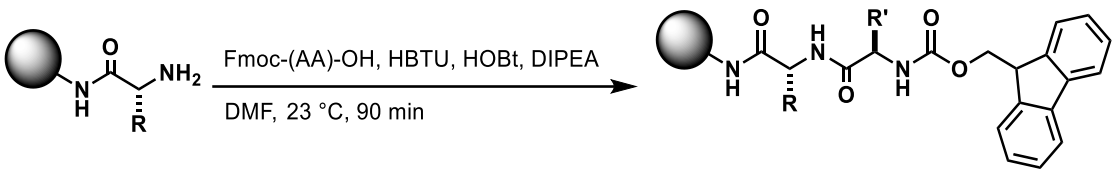
Washing: Add the stated washing-solvent (20 mL · g^{-1} resin) into the peptide synthesis vessel. Shake the suspension with the aid of a Heidolph Vibramax 100 (150 rpm) for 2 minutes at 23 °C, and then remove the liquid via vacuum filtration.

Deprotection:



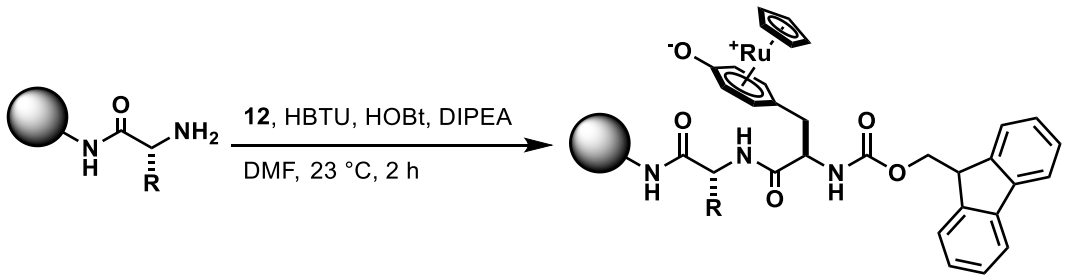
1. Add 20% piperidine in DMF (v/v, 20 mL·g^{-1} resin) into the peptide synthesis vessel containing resin-bound Fmoc-protected peptide, and shake the suspension for 5 minutes at 23 °C.
2. Remove the liquid via vacuum filtration. Repeat this deprotection sequence a second time, and then wash with DMF (3 × 20 mL·g^{-1} resin) for 2 minutes each time.

HBTU/HOBt coupling:



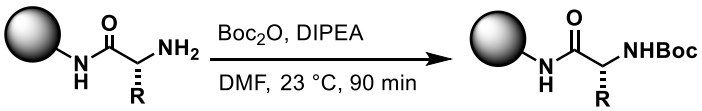
1. Add Fmoc-protected amino acid (Fmoc-(AA)-OH, 4.00 equiv), HBTU (3.90 equiv), HOBt hydrate (3.90 equiv), DIPEA (8.00 equiv), and DMF (10 mL·g^{-1} resin) to a round-bottom flask equipped with a Teflon-coated magnetic stirring bar.
2. Stir the solution for 15 minutes at 23 °C and then add it into the peptide synthesis vessel.
3. Shake the vessel for 90 minutes at 23 °C, and remove the liquid via vacuum filtration.
4. Wash the resin with DMF (3 × 10 mL·g^{-1} resin) for 2 minutes each time.

[Fmoc-Tyr(RuCp)-OH]·CF_3CO_2 coupling:



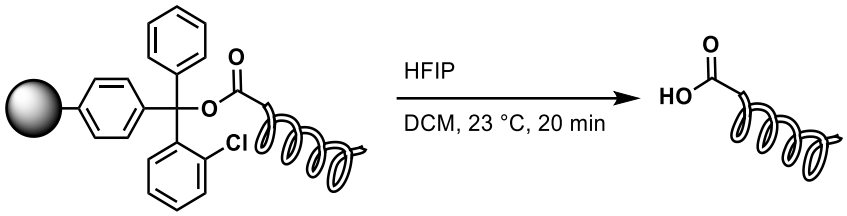
1. Add [Fmoc-Tyr(RuCp)-OH]·CF_3CO_2 (**12**) (2.00 equiv), HBTU (1.90 equiv), HOBt hydrate (1.90 equiv), DIPEA (16.0 equiv), and DMF (10 mL·g^{-1} resin) to a round-bottom flask equipped with a Teflon-coated magnetic stirring bar.
2. Stir the solution for 1 minute at 23 °C and then add it into the peptide synthesis vessel.
3. Shake the vessel for 2 h at 23 °C, and remove the liquid via vacuum filtration.
4. Wash the resin with DMF (3 × 10 mL · g^{-1} resin) for 2 minutes each time.

Boc protection:



1. Add a solution of di-*tert*-butyldicarbonate (Boc_2O) (4.00 equiv) and DIPEA (8.00 equiv) in DMF (20.0 mL·g^{-1} resin) to the resin bound peptide.
2. Shake the peptide synthesis vessel for 2 hours at 23 °C.
3. Remove the liquid via vacuum filtration, and wash the resin with DMF (3 × 20 mL·g^{-1} resin) for 2 minutes each time.

Cleavage conditions:



1. Wash the resin with DCM (3 × 20 mL·g^{-1} resin) for 2 minutes each time.
2. Then add a solution of 20% of hexafluoroisopropanol (HFIP) in DCM (v:v, 50 mL·g^{-1} resin) to the resin, and shake the suspension for 20 minutes at 23 °C.
3. Collect the liquid via vacuum filtration. Add a solution of 20% of HFIP in DCM (v:v, 50 mL·g^{-1} resin) to the resin, and shake the suspension for 50 minutes at 23 °C.
4. Collect the liquid via vacuum filtration, concentrate the combined organic layers *in vacuo* to dryness and analyze via LC-MS.

Synthesis and characterization of the ^{19}F-standard H-Leu-Phe(4-[^{18}F]F)-Glu-Met-Lys-NH$_2$ (^{19}F-14)

NOTE: The synthesis of intermediate compounds **^{19}F-14** is adapted from previously reported method.[4]

Reagents

- Fmoc-Rink-Amid-2CT resin (200–400 mesh, 1% DVB, 0.68 mmol·g−1, Iris-Biotech, cat no. BR-1315)
- Fmoc-L-Lys(Boc)-OH (CAS no. 71989-26-9, Iris-Biotech, cat no. FAA1125)
- Fmoc-*L*-Met-OH (CAS no 71989-28-1, Iris-Biotech, cat no. FAA1150)
- Fmoc-*L*-Glu(^{t}Bu)-OH·H$_2$O (CAS no 71989-18-9, Iris-Biotech, cat no. FSP1045)
- Fmoc-*L*-Leu-OH (CAS no 35661-60-0, Iris-Biotech, cat no. FAA1120)
- Fmoc-4-fluoro-L-phenylalanine (Fmoc-Phe(4-F)-OH) (CAS no. 169243-86-1, ABCR GmbH, cat no. AB169268)
- 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate (HBTU) (CAS no. 94790-37-1, Iris-Biotech, cat no. RL-1030)
- 1-hydroxybenzotriazol (HOBt) (CAS no 123333-53-9, Sigma Aldrich, cat no. 103542)
- Diisopropylethylamine (DIPEA) (CAS no 7087-68-6, Iris-Biotech, cat no. 117299)
- Dichloromethane (DCM) (CAS no. 75-09-2, Sigma-Aldrich, cat no. DX0835)
- Dimethylformamide (DMF) (CAS no. 68-12-2, Iris-Biotech, cat no. SOL-004, peptide grade)
- Piperidine (CAS no. 110-89-4, Iris-Biotech, cat no. SOL-010)

Spectroscopy and Instruments

- NMR spectra were recorded on a Bruker Ascend™ 500 spectrometer operating at 500 MHz, 471 MHz and 126 MHz, for ^{1}H, ^{19}F and ^{13}C acquisitions, respectively; or on a Varian Unity/Inova 600 spectrometer operating at 600 MHz and 151 MHz for ^{1}H and ^{13}C acquisitions, respectively.
- HRMS (same as mentioned in supplementary tutorial 3)
- Preparative high-performance liquid chromatographic (prep-HPLC) separation was executed on Shimadzu Prominence Preparative HPLC system
- Product was lyophilized using BÜCHI Lyovapor™ L-200

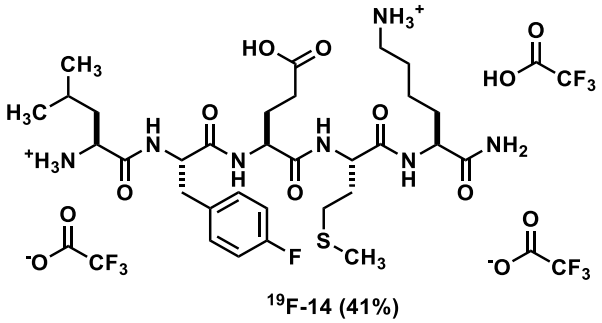


Fig. S15 | Synthesis of the fluorine-19 standard (**^{19}F-14**)

1. Charge a peptide synthesis vessel (100 mL) with Fmoc-Rink-Amid-2CT resin (200–400 mesh, 1% DVB, 0.68 mmol·g^{-1}, 0.74 g, 0.50 mmol, 1.0 equiv) and DCM (45 mL, 26 g·L^{-1}).
2. Shake the resulting suspension (150 rpm) for 30 minutes at 23 °C with the aid of a Heidolph Vibramax 100, and then remove the liquid via vacuum filtration.
3. Wash the resin with DMF (3 × 10 mL) for 2 minutes each time.
4. Add 20 mL mixture of piperidine in DMF (1:5, v:v) into the peptide synthesis vessel, and shake the suspension (150 rpm) for 5 minutes at 23 °C.
5. Remove the liquid via vacuum filtration.
6. Repeat this deprotection sequence (step 4–5) once, and then wash the resin with DMF (3 × 20 mL) for 2 minutes each time.
7. Add Fmoc-Lys-OH (0.93 g, 2.0 mmol, 4.0 equiv), HBTU (0.74 g, 1.9 mmol, 3.9 equiv), HOBt hydrate (0.26 g, 1.9 mmol, 3.9 equiv), DIPEA (0.74 mL, 4.0 mmol, 8.0 equiv), and DMF (10 mL) into a 25 mL round-bottom flask equipped with a Teflon-coated magnetic stirring bar.
8. Stir the yellow solution for 15 minutes at 23 °C and then add it into the peptide synthesis vessel.
9. Shake the vessel (150 rpm) for 90 minutes at 23 °C with the aid of a Heidolph Vibramax 100, and then remove the liquid via vacuum filtration.
10. Repeat steps 3–6.
11. Add Fmoc-Met-OH (0.74 g, 2.0 mmol, 4.0 equiv), HBTU (1.5 g, 3.9 mmol, 3.9 equiv), HOBt hydrate (0.53 g, 3.9 mmol, 3.9 equiv), DIPEA (1.4 mL, 1.0 g, 8.0 mmol, 8.0 equiv), and DMF (10 mL) into a 20 mL round-bottom flask equipped with a Teflon-coated magnetic stirring bar.

▲ **CRITICAL STEP** Full conversion after each coupling step needs to be ensured as otherwise purification becomes very difficult. To check conversion, remove a small portion (~1 mg) of the resin, wash, deprotect and cleave the peptide (step 27). Then, compare the retention time of starting material and coupled peptide with an analytical HPLC (YMC-Triart C18 column, 150 x 4.6 mm, flow rate = 1.0 mL·min^{-1}) with an isocratic elution using 35:65 0.1% TFA in H_2O:MeOH (v:v).

12. Stir the yellow solution for 15 minutes at 23 °C and then add it into the peptide synthesis vessel.
13. Shake the vessel for 90 minutes at 23 °C with the aid of a Heidolph Vibramax 100, and then remove the liquid via vacuum filtration.
14. Repeat steps 3–6.

15. Add Fmoc-Glu(^{t}Bu)-OH (0.85 g, 2.0 mmol, 4.0 equiv), HBTU (0.74 g, 1.9 mmol, 3.9 equiv), HOBt hydrate (0.26 g, 1.9 mmol, 3.9 equiv), DIPEA (0.74 mL, 4.0 mmol, 8.0 equiv), and DMF (10 mL) into a round-bottom flask (25 mL) equipped with a Teflon-coated magnetic stirring bar.
16. Stir the yellow solution for 15 minutes at 23 °C and then add it into the peptide synthesis vessel.
17. Shake the vessel for 90 minutes at 23 °C with the aid of a Heidolph Vibramax 100, and then remove the liquid via vacuum filtration.
18. Repeat steps 3–6.
19. Add Fmoc-*L*-Phe(4-F)-OH (0.81 g, 2.0 mmol, 4.0 equiv), HBTU (0.74 g, 1.9 mmol, 3.9 equiv), HOBt hydrate (0.26 g, 1.9 mmol, 3.9 equiv), DIPEA (0.74 mL, 4.0 mmol, 8.0 equiv), and DMF (10 mL) into a round-bottom flask (25 mL) equipped with a Teflon-coated magnetic stirring bar.
20. Stir the yellow solution for 15 minutes at 23 °C and then add it into the peptide synthesis vessel.
21. Shake the vessel for 90 minutes at 23 °C with the aid of a Heidolph Vibramax 100, and then remove the liquid via vacuum filtration.
22. Repeat steps 3–6.
23. Add Fmoc-L-Leu-OH (0.71 g, 2.0 mmol, 4.0 equiv), HBTU (0.74 g, 1.9 mmol, 3.9 equiv), HOBt hydrate (0.26 g, 1.9 mmol, 3.9 equiv), DIPEA (0.74 mL, 4.0 mmol, 8.0 equiv), and DMF (10 mL) into a round-bottom flask (25 mL) equipped with a Teflon-coated magnetic stirring bar.
24. Stir the yellow solution for 15 minutes at 23 °C and then add it into the peptide synthesis vessel.
25. Shake the vessel for 90 minutes at 23 °C with the aid of a Heidolph Vibramax 100, and then remove the liquid via vacuum filtration.
26. Wash the resin with DMF (3 × 10 mL) for 2 minutes each time.
27. Add a mixture of 8.8 mL TFA, 0.50 g DTT, 0.50 mL water, and 0.25 mL triisopropylsilane to the suspension was shake at 23 °C for 2 hours.
28. Filter the reaction mixture into a 100 mL round-bottom flask containing 80 mL diethyl ether.
29. Filter the resulting suspension and the filter cake is purified by HPLC on an YMC Pack Pro C18 column ((30 × 150 mm, 5 µm + 30 × 50 mm, 5 µm), flow rate = 42.5 mL·min^{-1}, 35 °C) with an isocratic eluent 35:65 (0.1% TFA in H2O:MeOH, v:v).
30. Collect and combine the fraction containing product (t ≈ 12.2 min) and lyophilize to dryness to afford **^{19}F-14** as a colorless solid (0.14 g, 0.2 mmol, 41% yield).

NMR Spectroscopy:

^{1}H NMR (500 MHz, $(CD_3)_2SO$, 25 ºC, δ): 12.15 (s, 1H), 8.70 (d, *J* = 7.4 Hz, 1H), 8.34 (d, *J* = 7.7 Hz, 1H), 8.15 (dd, *J* = 7.7, 3.1 Hz, 1H), 8.11 – 8.02 (m, 4H), 7.72 (s, 3H), 7.42 – 7.37 (m, 1H), 7.35 – 7.28 (m, 2H), 7.15 – 7.08 (m, 2H), 7.05 (d, *J* = 3.1 Hz, 1H), 4.61 (td, *J* = 8.3, 4.4 Hz, 1H), 4.42 (dtd, *J* = 13.7, 7.8, 5.8 Hz, 1H), 4.29 (p, *J* = 7.4 Hz, 1H), 4.21 – 4.12 (m, 1H), 3.74 (dd, *J* = 8.7, 5.1 Hz, 1H), 3.02 (dd, *J* = 14.5, 4.9 Hz, 1H), 2.86 – 2.65 (m, 4H), 2.53 (d, *J* = 1.6 Hz, 3H), 2.31 – 2.17 (m, 2H), 2.09 – 1.86 (m, 2H), 1.80 – 1.60 (m, 3H), 1.54 (dtt, *J* = 13.6, 10.0, 5.2 Hz, 5H), 1.38 – 1.22 (m, 2H), 0.88 (dd, *J* = 6.4, 4.0 Hz, 6H).

^{13}C{^{1}H} NMR (151 MHz, $(CD_3)_2SO$, 25 ºC, δ): 174.3, 173.5, 173.5, 171.1, 170.6, 170.5, 160.3 (d, $^1J_{C\text{-}F}$ = 241.3 Hz), 133.8, 131.1 (d, $^3J_{C\text{-}F}$ = 7.9 Hz), 115.0 (d, $^2J_{C\text{-}F}$ = 20.9 Hz), 54.2, 52.4, 51.6, 51.4, 50.9, 49.3, 49.1, 40.5, 38.8, 38.0, 36.3, 31.3, 30.5, 27.3, 26.8, 25.3, 23.5, 22.9, 22.4, 21.7.

^{19}F NMR (471 MHz, $(CD_3)_2SO$, 25 ºC, δ): −77, –118.1.

HRMS-ESI (m/z) calc'd for $C_{31}H_{51}FN_7O_7S$ $[M-CF_3CO_2-2CF_3CO_2H]^+$, 684.35492; found, 684.35469; deviation: −0.34 ppm.

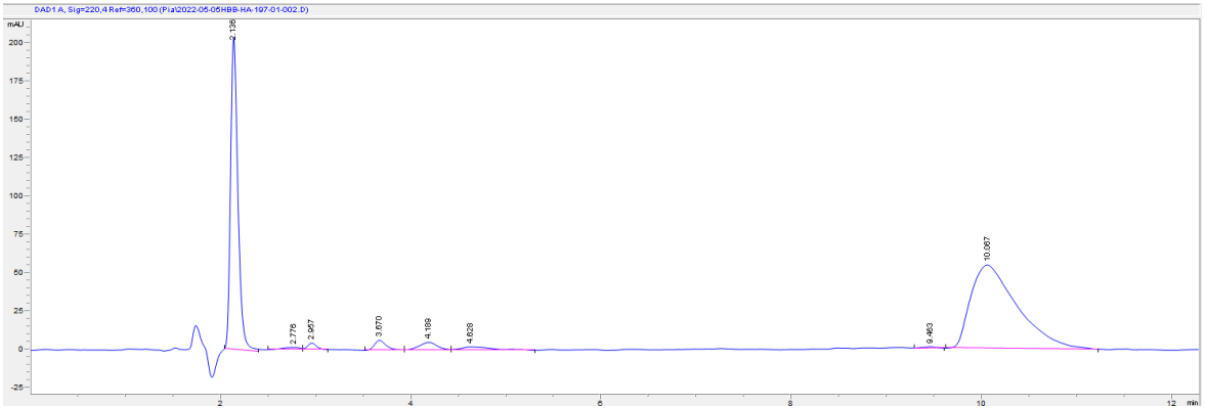
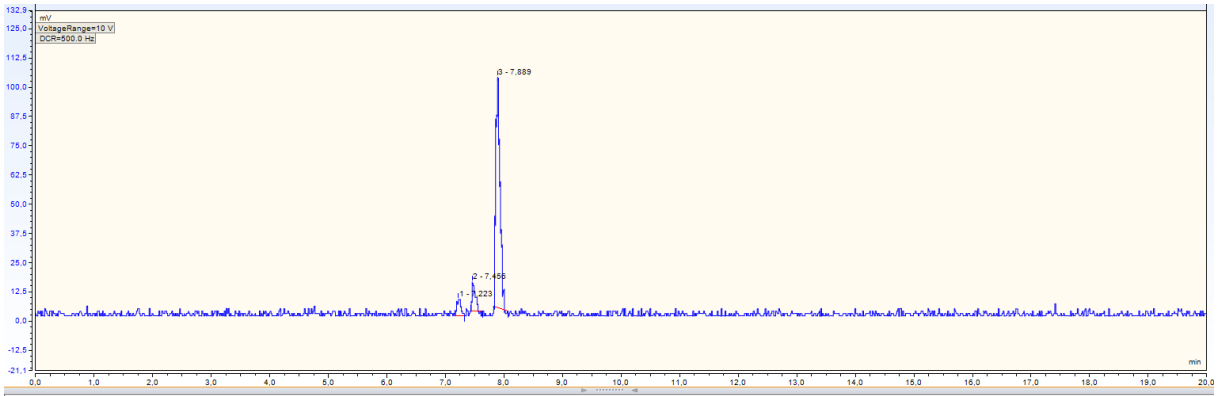


Fig. S16 | Analytical HPLC trace of S6 (YMC-Triart C18 column, 150 x 4.6 mm, flow rate = 1.0 mL·min^{-1}) with an isocratic elution using 35:65 0.1% TFA in H_2O:MeOH (v:v).

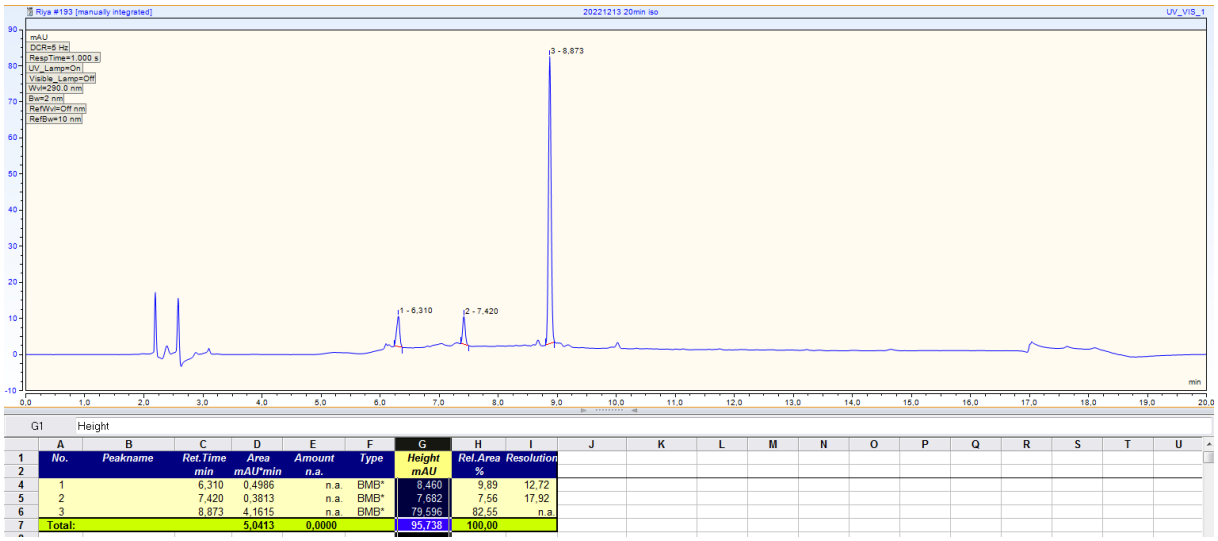
Molar activity determination of H-Leu-Phe(4-[^{18}F]F)-Glu-Met-Lys-NH_2 (^{18}F-14)



G1 Height

	A	B	C	D	E	F	G	H	I
1	No.	Peakname	Ret.Time	Area	Amount	Type	Height	Rel.Area	Resolution
2			min	mV*min	n.a.		mV	%	
4	1		7,223	0,5774	n.a.	BMB*	7,096	6,05	2,07
5	2		7,456	0,7802	n.a.	BMB*	12,589	8,17	3,84
6	3		7,889	8,1938	n.a.	BMB*	98,551	85,79	n.a.
7	Total:			9,5515	0,0000		118,236	100,00	

Fig. S17 |. Radio-HPLC trace of the isolated compound H-Leu-Phe(4-[^{18}F]F)-Glu-Met-Lys-NH_2 (**^{18}F-14**) (15 kBq) (column 2).



G1 Height

No.	Peakname	Ret.Time min	Area mAU*min	Amount n.a.	Type	Height mAU	Rel.Area %	Resolution
1		6,310	0,4986	n.a.	BMB*	8,460	9,89	12,72
2		7,420	0,3813	n.a.	BMB*	7,682	7,56	17,92
3		8,873	4,1615	n.a.	BMB*	79,596	82,55	n.a.
Total:			5,0413	0,0000		95,738	100,00	

Fig. S18 | UV-HPLC trace of the isolated compound H-Leu-Phe(4-[^{18}F]F)-Glu-Met-Lys-NH_2 (**^{18}F-14**) (15 kBq) at 290 nm (column 2).

Using the authentic reference material **^{19}F-14** a standard curve was generated by integration of the UV absorbance signal (at 290 nm) of five different known amounts

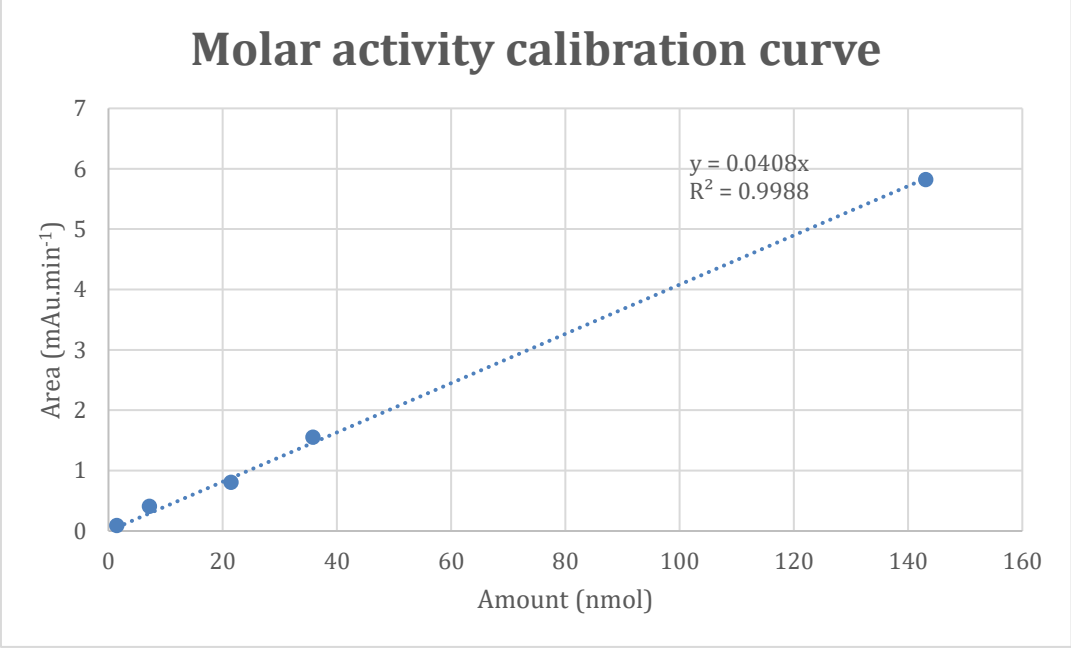


Fig. S19 | Calibration curve acquired with **^{19}F-14** at 290 nm for determination of molar activity.

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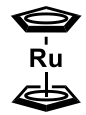
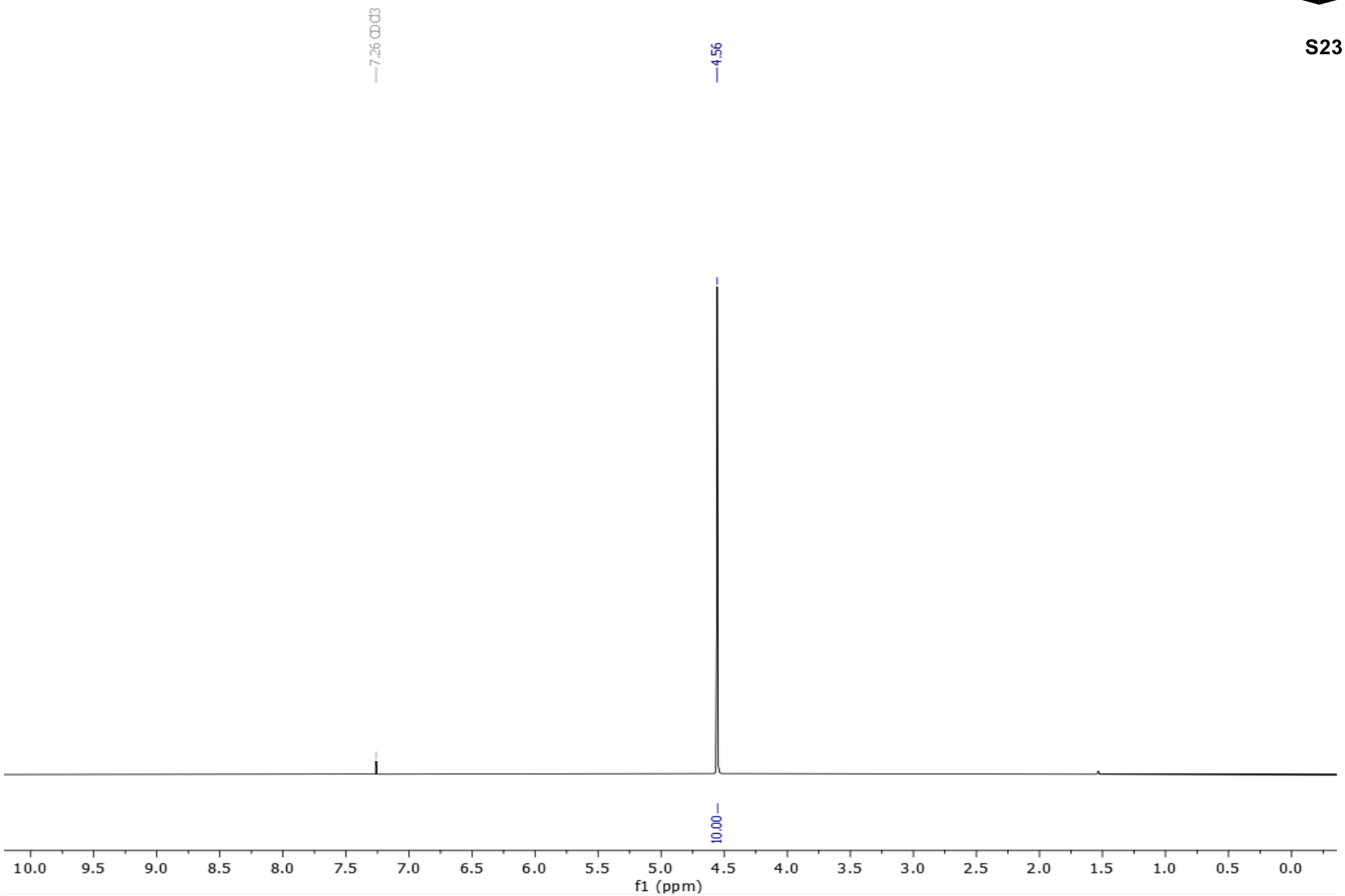
SPECTROSCOPIC DATA

^{1}H NMR of bis(cyclopentadienyl)ruthenium(II) (S23):

600 MHz, $CDCl_3$, 25 ºC

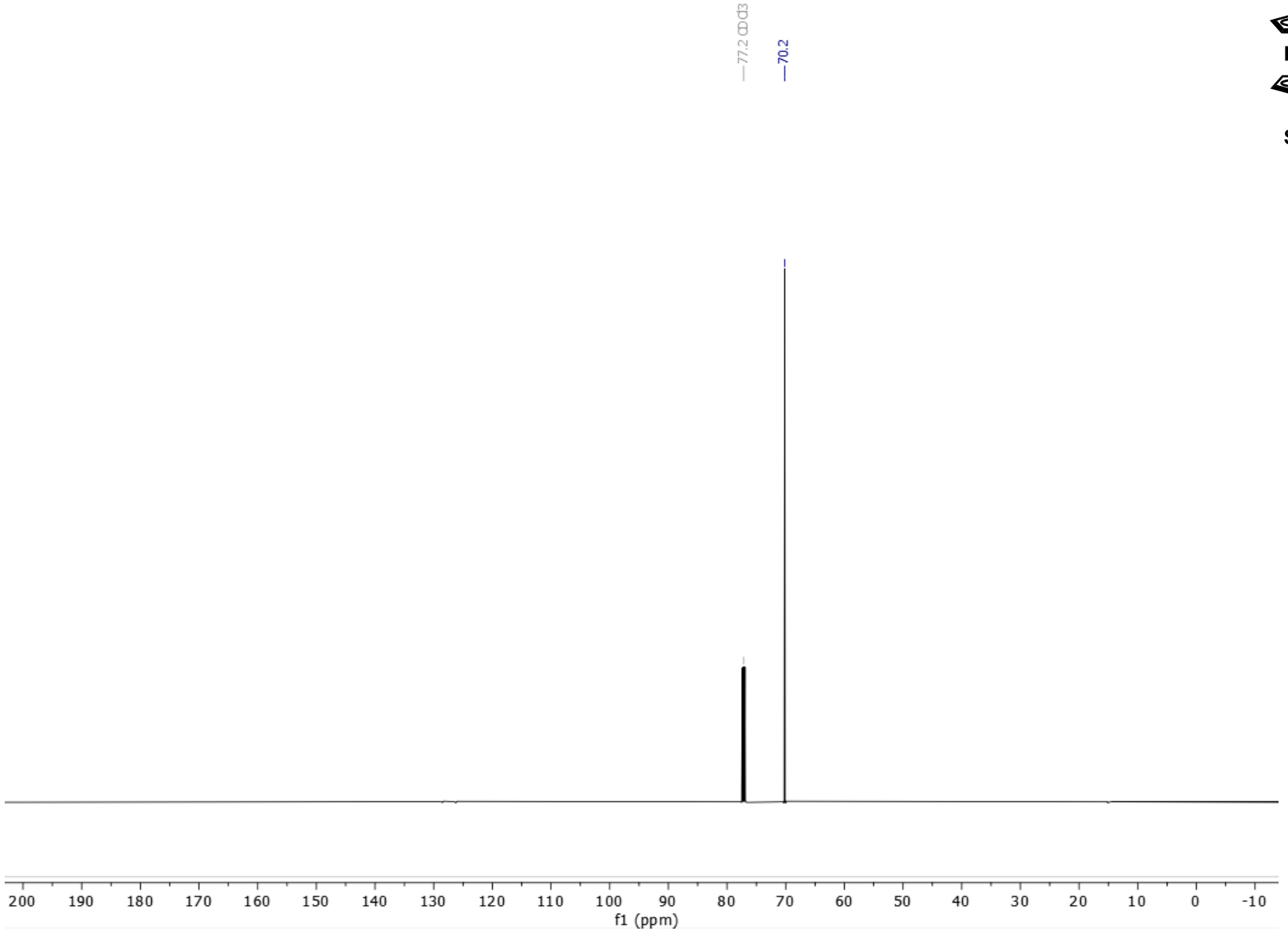


S23



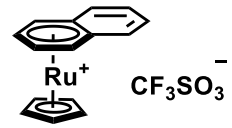
^{13}C NMR of bis(cyclopentadienyl)ruthenium(II) (S23):

151 MHz, $CDCl_3$, 25 ºC

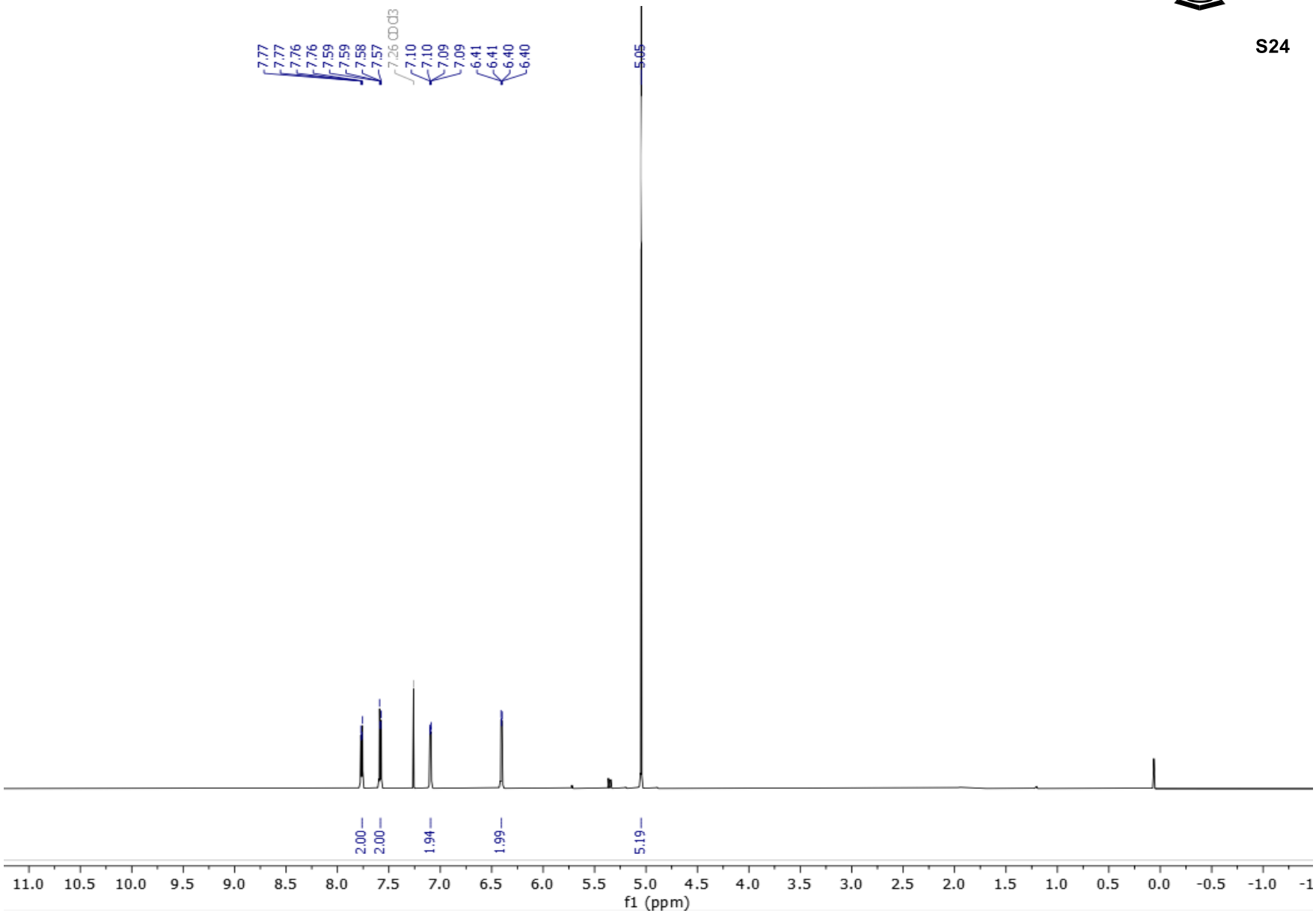


^{1}H NMR of [(Cp)Ru(η^{6}-naphthalene)]·CF_3SO_3 (S24):

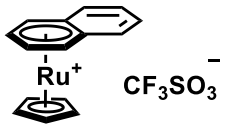
600 MHz, $CDCl_3$, 25 ºC



S24

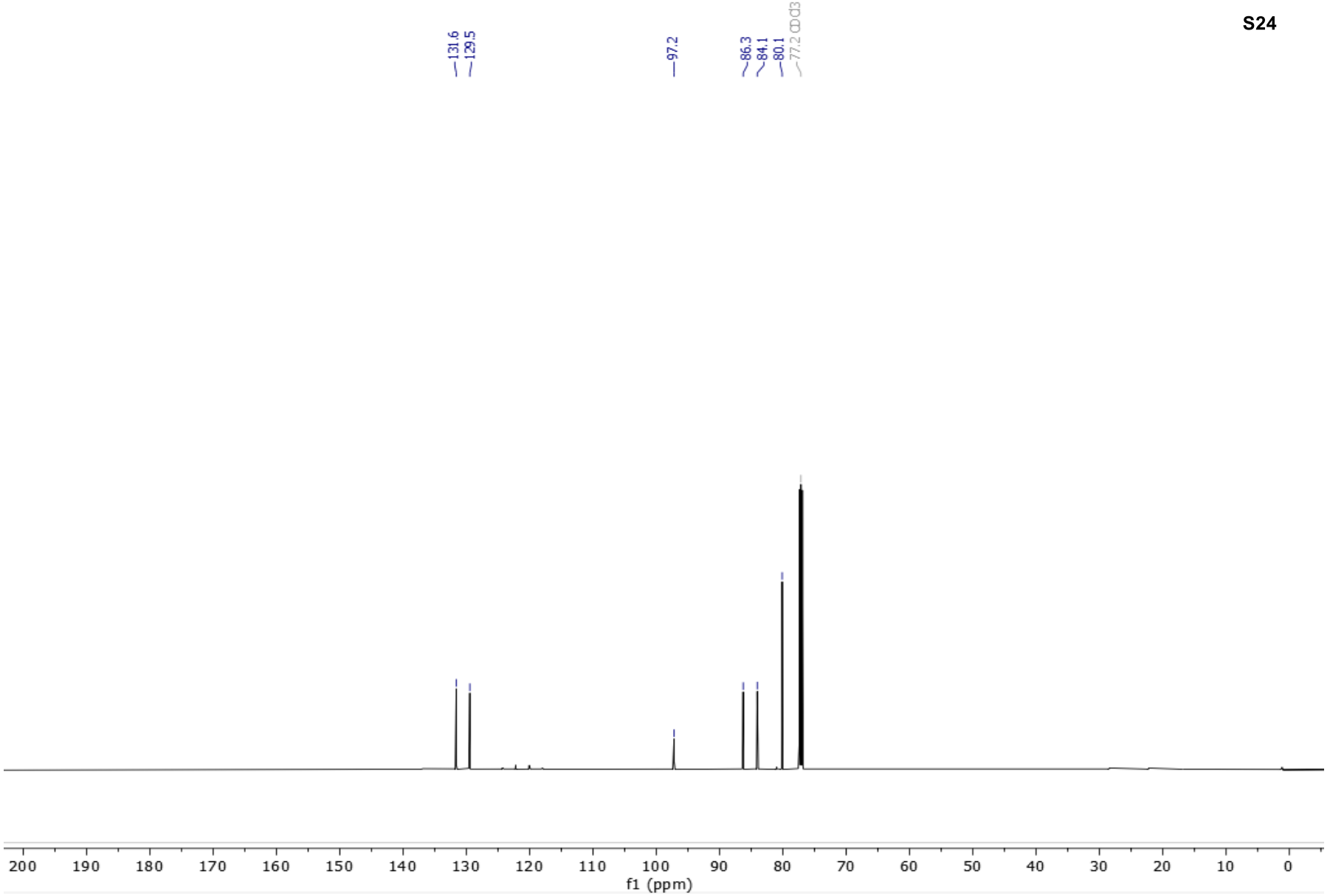


^{13}C NMR of [(Cp)Ru(η^6-naphthalene)]·CF_3SO_3 (S24):



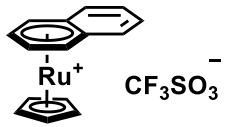
S24

151 MHz, $CDCl_3$, 25 ºC

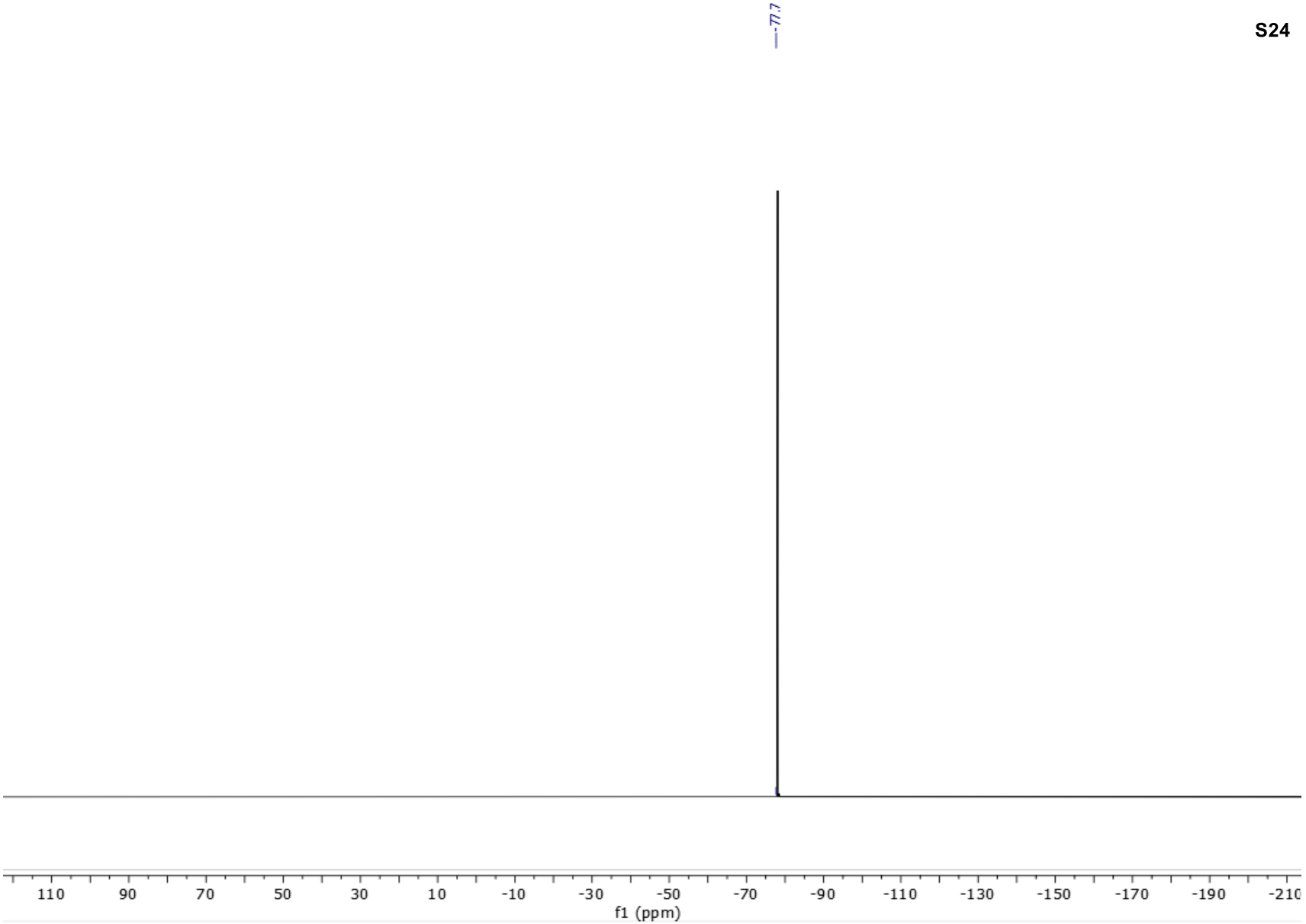


^{19}F NMR of [(Cp)Ru(η^6-naphthalene)]·CF_3SO_3 (S24):

565 MHz, $CDCl_3$, 25 ºC

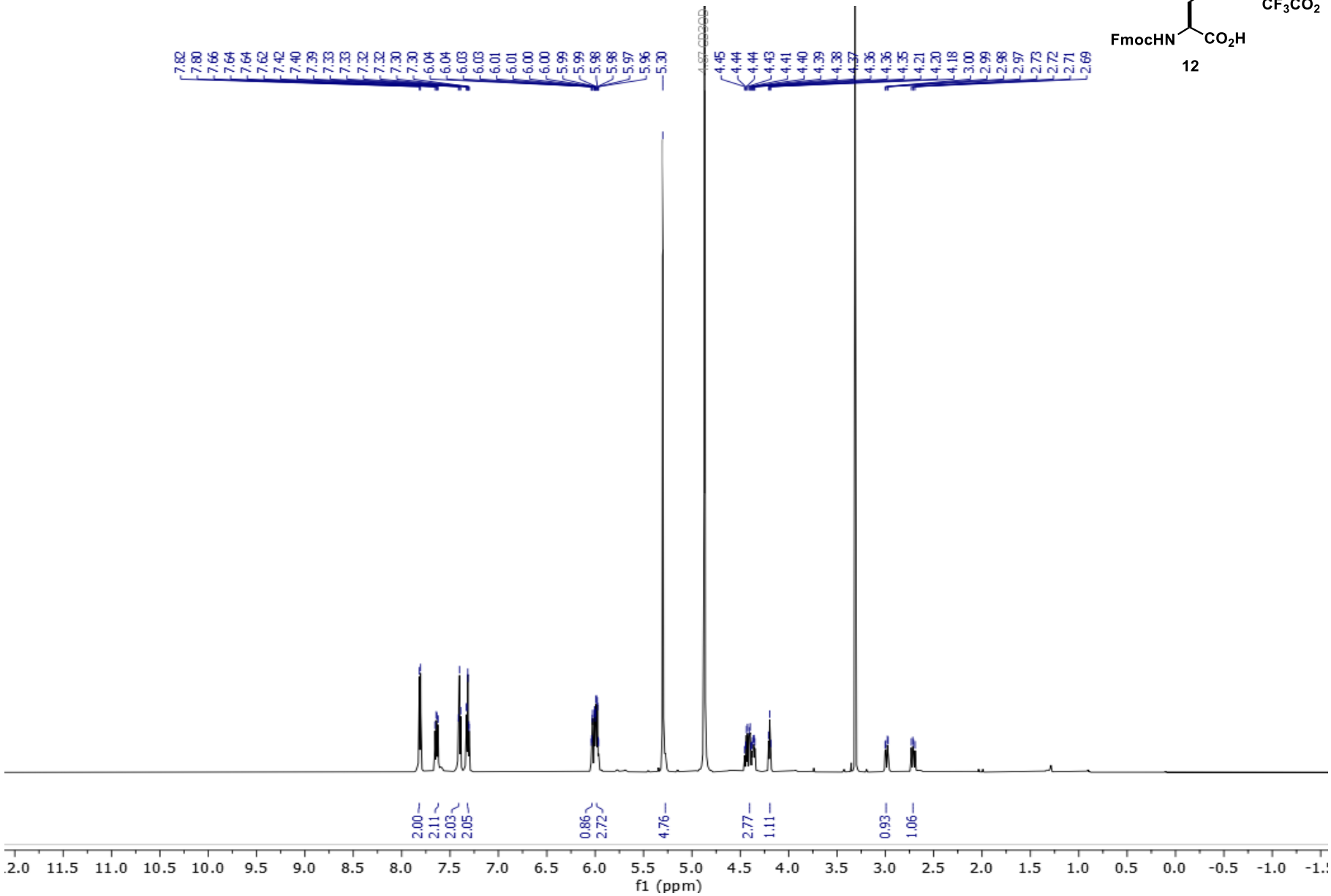


S24



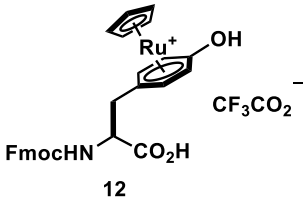
^{1}H NMR of [Fmoc-tyrosine(RuCp)-OH]·CF_3CO_2 (12):

600 MHz, CD_3OD, 25 ºC



^{13}C NMR of [Fmoc-tyrosine(RuCp)-OH]·CF_3CO_2 (12):

151 MHz, CD_3OD, 25 ºC



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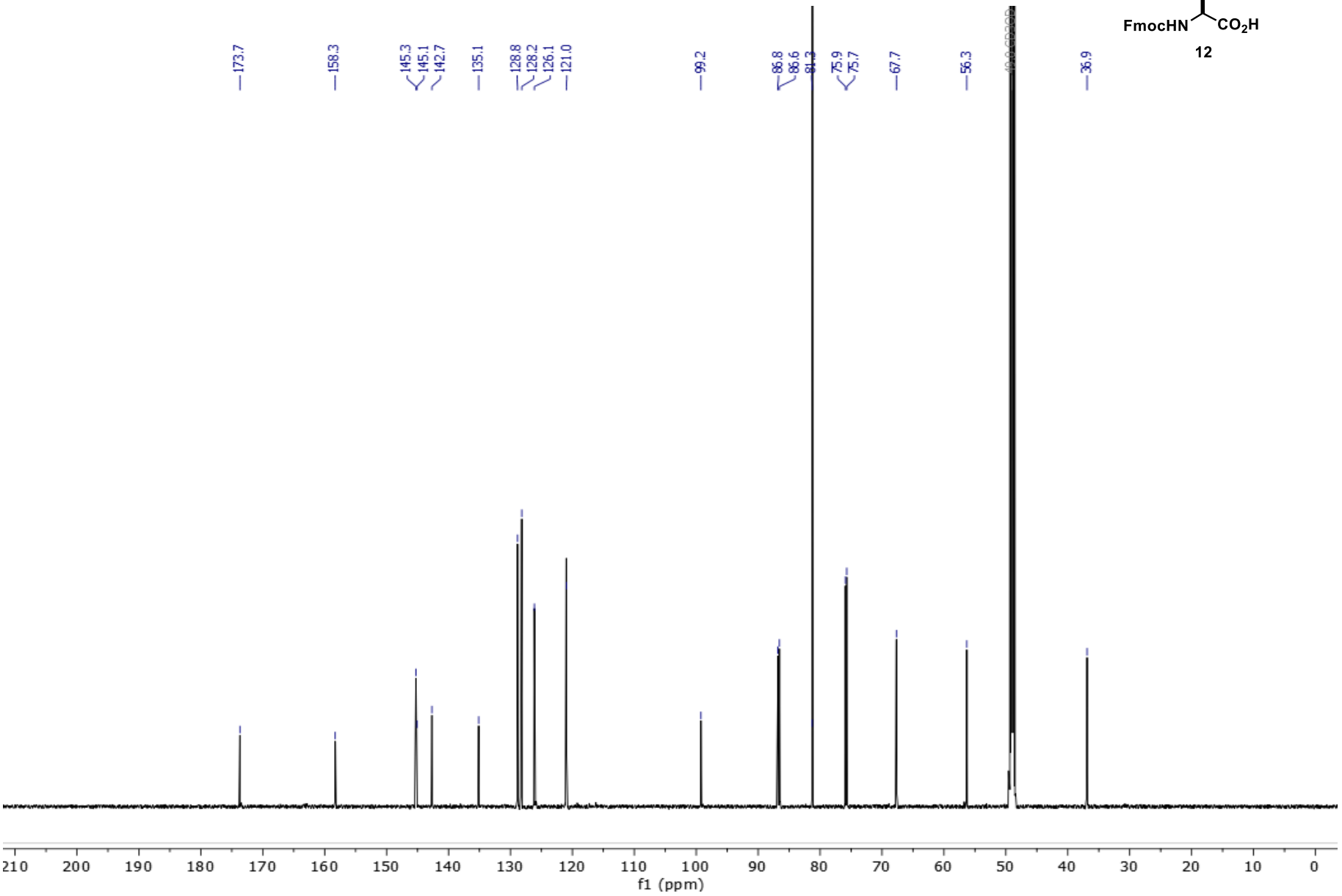
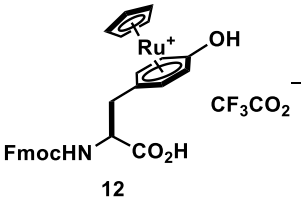


^{19}F NMR of [Fmoc-tyrosine(RuCp)-OH]·CF_3CO_2 (12):

565 MHz, CD_3OD, 25 ºC



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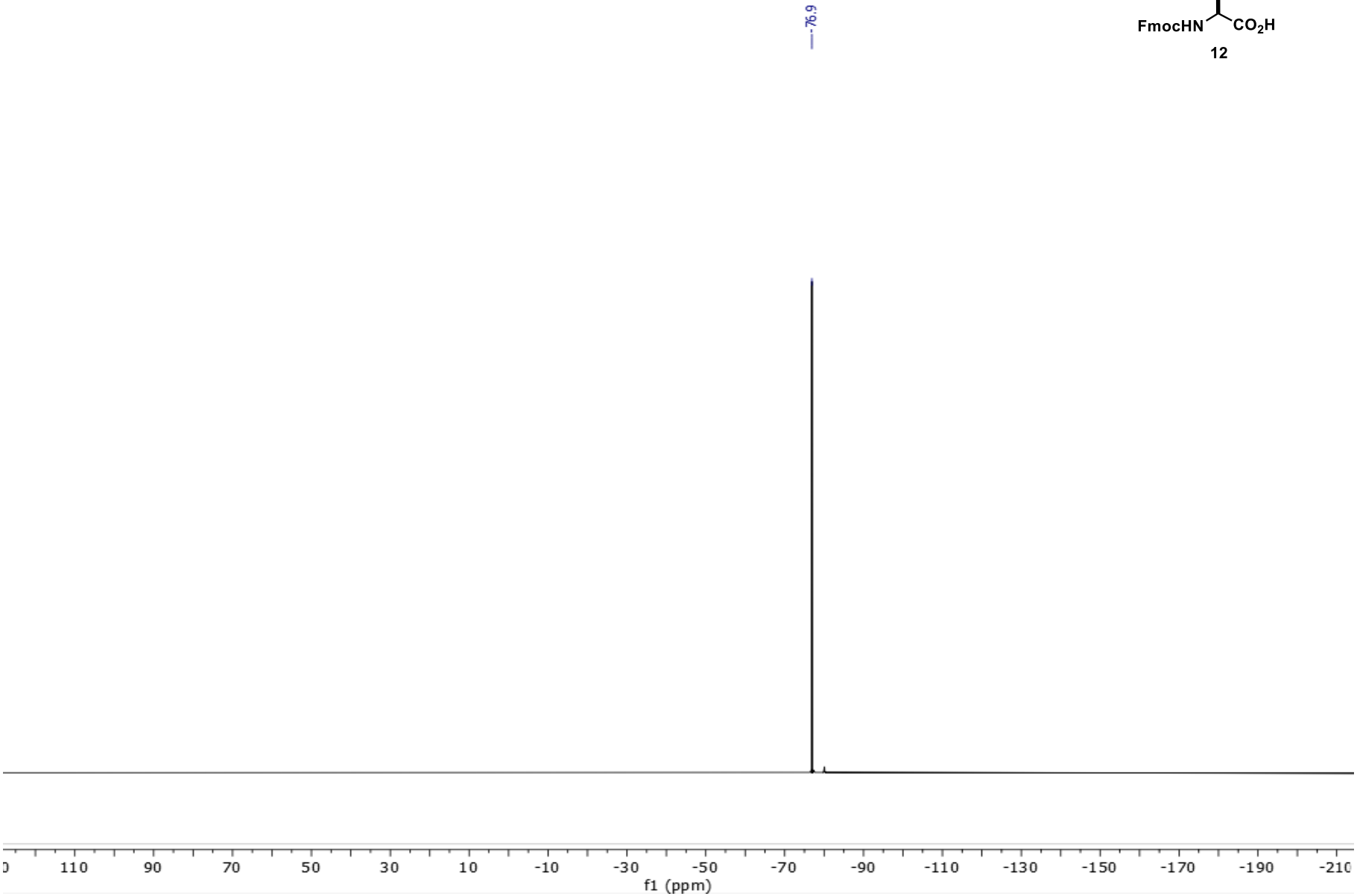
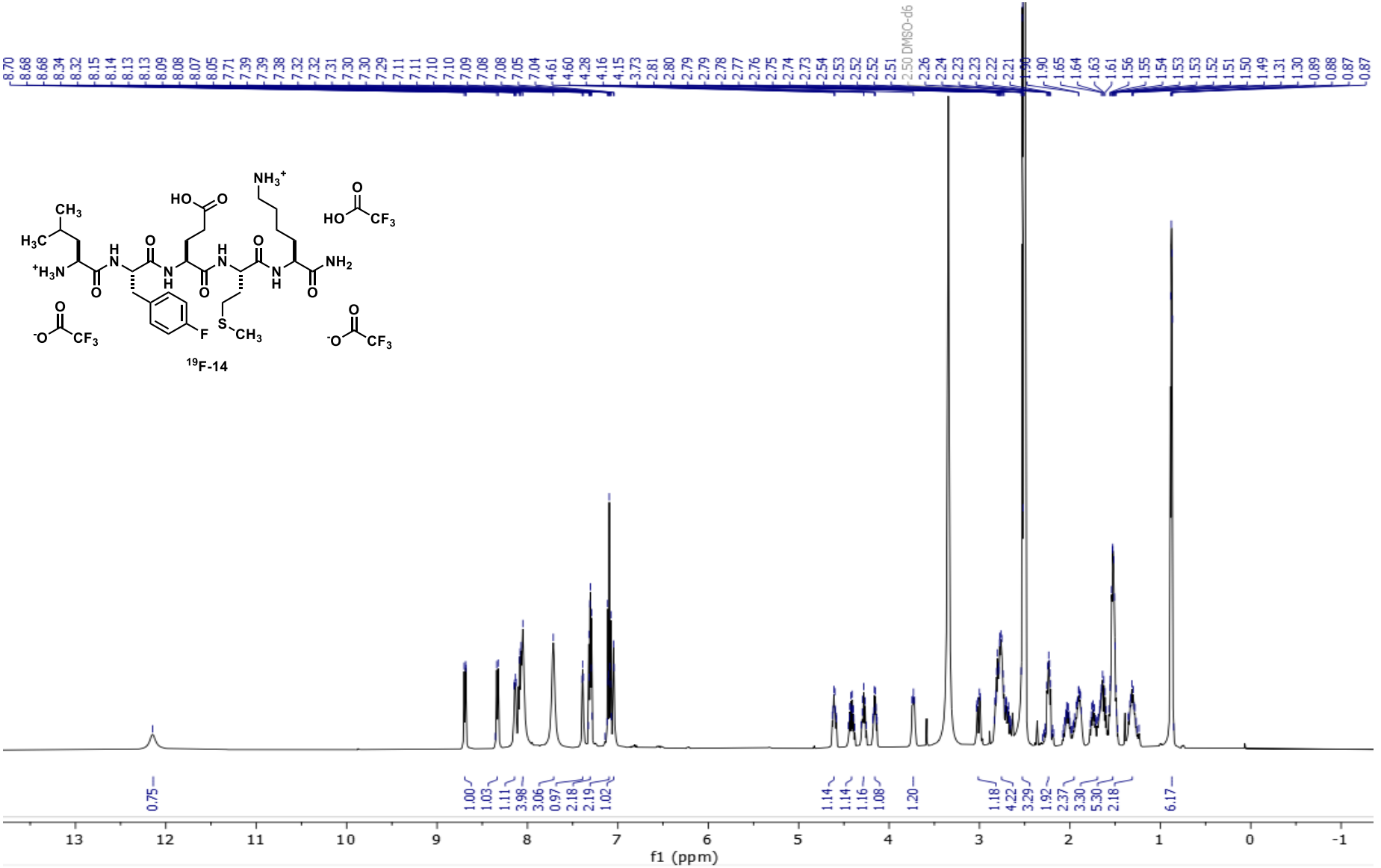


^{1}H NMR of [H-Leu-Phe(4-F)-Glu-Met-Lys-NH$_2$]·3CF$_3$CO$_2$H (^{19}F-14):

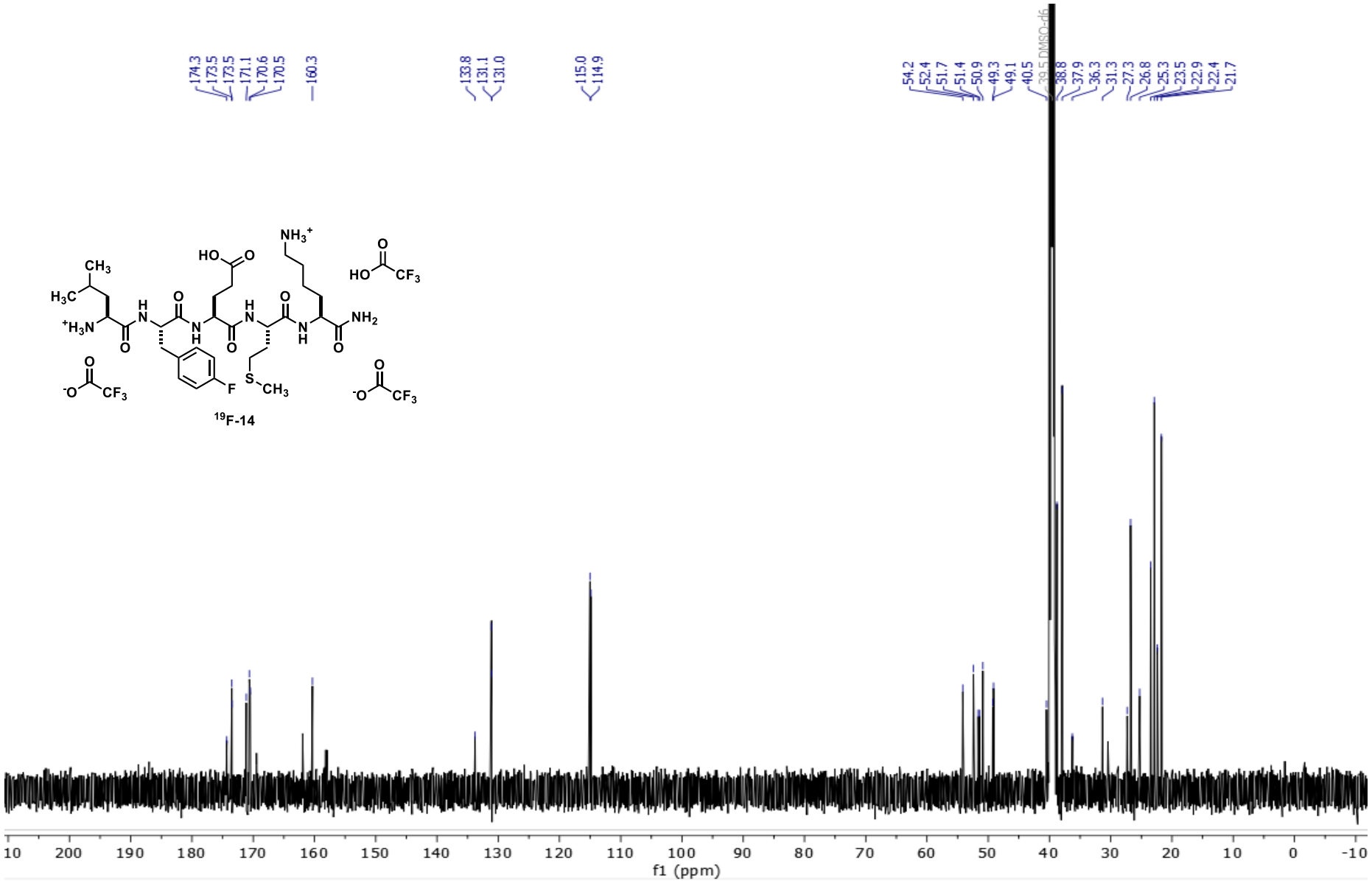
500 MHz, $(CD_3)_2SO$, 25 ºC



^{19}F-14

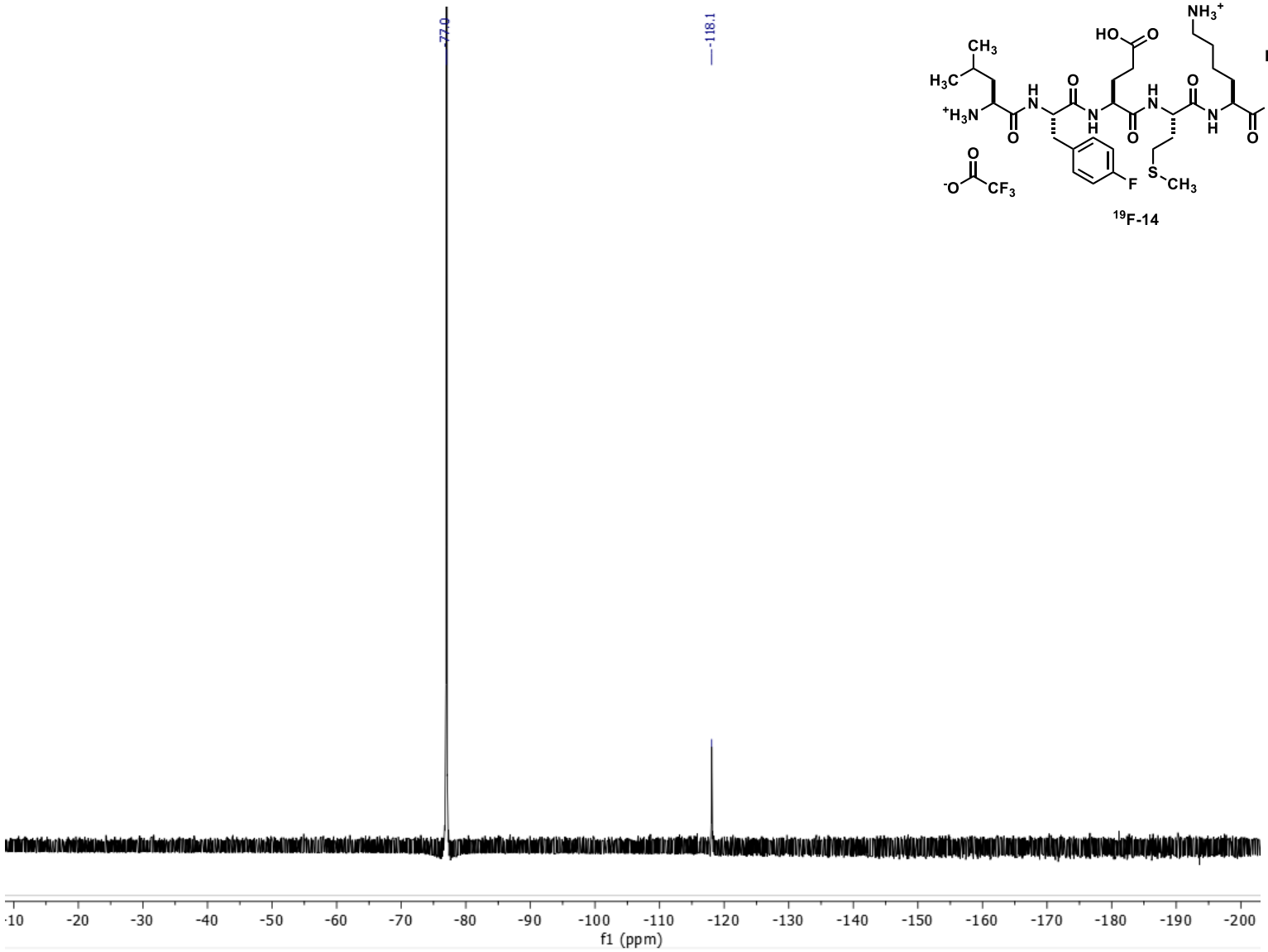
^{13}C NMR of [H-Leu-Phe(4-F)-Glu-Met-Lys-NH$_2$]·3CF$_3$CO$_2$H (^{19}F-14):

151 MHz, $(CD_3)_2SO$, 25 ºC



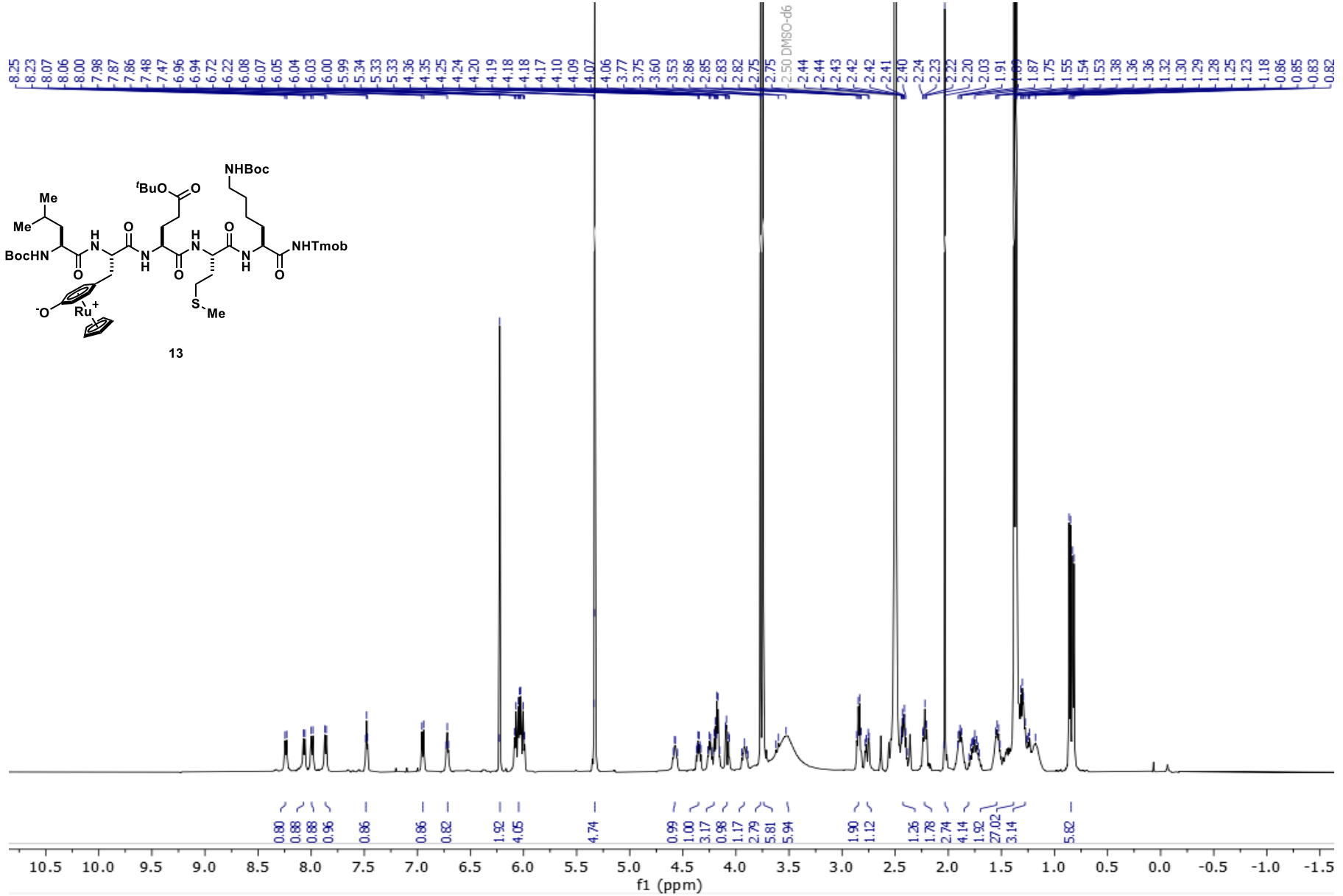
^{19}F NMR of [H-Leu-Phe(4-F)-Glu-Met-Lys-NH_2]·3CF_3CO_2H (^{19}F-14):

471 MHz, $(CD_3)_2SO$, 25 ºC



^{1}H NMR of Boc-Leu-Tyr(RuCp)-Glu(^{t}Bu)-Met-Lys(Boc)-NHTmob (13):

500 MHz, $(CD_3)_2SO$, 25 ºC



^{13}C NMR of Boc-Leu-Tyr(RuCp)-Glu(^{t}Bu)-Met-Lys(Boc)-NHTmob (13):

151 MHz,CD_3OD, 25 ºC