Supporting Information

Synthesis of Perfluoro-*tert*-butyl Tyrosine, for Application in ^{19}F NMR, via a Diazonium Coupling Reaction

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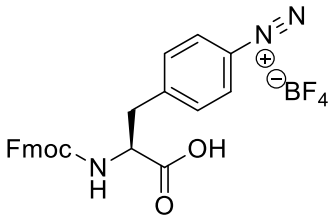
Contents

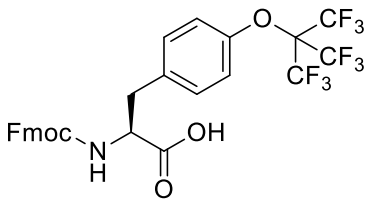
Materials

Fmoc-L-4-NH_2-Phe-OH, Fmoc-L-4-NHBoc-Phe-OH, Fmoc amino acids, HBTU, and HATU were purchased from Chem-Impex (Wood Dale, IL). Novagel Rink amide resin was purchased from EMD Millipore. All solvents, tetrafluoroboric acid, and sodium nitrate were purchased from Fisher Scientific. Perfluoro-*tert*-butanol and Fmoc-D-4-NH_2-Phe-OH were purchased from Matrix Scientific (Columbia, SC). All other reagents were purchased from Acros. Thin layer chromatography was conducted using silica gel glass-backed plates (Silicycle, 250 μm, 60 Å, F254). Flash chromatography was performed using 230-400 mesh (32-63 μm, 60 Å) silica gel from Silicycle. NMR spectra were recorded on a Brüker 400 MHz NMR spectrometer equipped with a cryogenic QNP probe or a Brüker 600 MHz NMR spectrometer equipped with a 5-mm Brüker SMART probe. High resolution mass spectrometry was performed on a Brüker 7 Tesla FT-MS. Peptides were characterized by ESI-MS (positive ion mode) on an LCQ Advantage (Finnigan) or an Acquity UPLC H-class SQD2 (Waters) mass spectrometer.



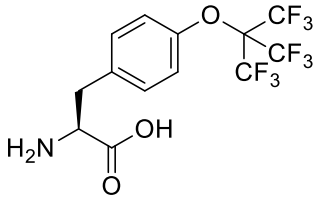
2

Fmoc-L-4-diazonium-phenylalanine tetrafluoroborate (2) Fmoc-L-4-amino-phenylalanine (0.306 g, 0.761 mmol) was dissolved in a 1:1 solution of tetrafluoroboric acid in water (0.2 mL, 48% to 50% w/w) and methanol (0.2 mL) in an open glass vial. The solution was placed in an ice bath before the dropwise addition of aqueous sodium nitrite (0.053 g, 76 mmol in 0.05 mL H_2O, 1.5 M) over two minutes. The reaction was allowed to warm to room temperature over the course of two hours. The crude product was diluted with water (1 mL) and extracted with ethyl acetate (2 × 1 mL). The crude product was dried under vacuum on a rotary evaporator to produce an orange-red solid (0.483 g, 0.761 mmol) which was used without further purification in the next step.[1,2] ***Warning***: diazonium salts are inherently prone to rapid generation of nitrogen gas. It is critical to use an open reaction vessel and low temperature to prevent accidental explosion. It is critical to immediately use the diazonium tetrafluoroborate to prevent complete drying and possible detonation.



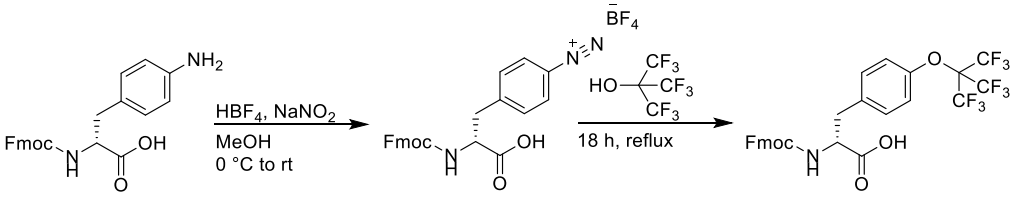
3

Fmoc-L-perfluoro-*tert*-butyl-tyrosine (3) Crude compound **2** (0.483 g, 0.761 mmol) was dissolved in perfluoro-*tert*-butanol (5 mL) in a round-bottom flask with a reflux condenser open to the atmosphere, and the resultant solution was stirred at reflux (bp of perfluoro-*tert*-butanol: 45 °C) for 18 hours. The excess solvent was removed under vacuum. The crude product was dissolved in ethyl acetate (15 mL) and washed with 1 M HCl (15 mL) and water (15 mL). The ethyl acetate was removed under vacuum. The crude product was purified via column chromatography (0 to 2% methanol in CH_2Cl_2) to yield a yellowish solid (0.289 g, 0.465 mmol) in 61% yield.[2] ^{1}H NMR (400 MHz, CD_3OD) δ 7.81-7.79 (d, *J* = 7.6 Hz, 2H), 7.64-7.60 (dd, *J* = 7.1, 7.1 Hz, 2H), 7.41-7.38 (dd, *J* = 7.5 Hz, 7.4 Hz, 2H), 7.32-7.28 (m, 4H), 7.15-7.13 (d, *J* = 8.4, 2H), 4.45-4.41 (m, 1H), 4.30-4.27 (m, 2H), 4.18-4.16 (m, 2H), 3.03-2.97 (m, 1H), 2.99-2.93 (m, 1H) ^{13}C NMR (150.8 MHz, CD_3OD) δ 173.4, 156.9, 151.4, 143.9, 143.7, 141.2, 136.8, 130.4, 127.3, 126.7, 124.8, 122.1, 121.4, 119.5, 118.5, 66.5, 55.2, 36.4. ^{19}F NMR (376.3 MHz, CD_3OD) δ -70.41. HRMS (LIFDI-TOF) m/z: $[M]^+$ calcd for $C_{28}H_{20}F_9NO_5$ 621.1198, found 621.1211.



4

L-Perfluoro-*tert*-butyl-tyrosine (4) Fmoc-perfluoro-*tert*-butyl-tyrosine (0.100 g, 0.16 mmol) was dissolved in 20% piperidine in acetonitrile (10 mL) and stirred at room temperature for 30 min. The solvent was removed before redissolving in ethyl acetate. The crude product was extracted from the solvent with water (1 × 5 mL) and 1 M HCl (1 × 5 mL). The solvent was removed under vacuum and dried to produce the product as off-white solid (0.042 g, 0.11 mmol) in 63% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.35-7.32 (dd, *J* = 5.5 Hz, 5.2 Hz, 2H), 7.11-7.06 (dd, *J* = 8.7 Hz, 8.7 Hz, 2H), 3.78-3.75 (dd, *J* = 6.4 Hz, 6.1 Hz, 1H), 3.28-3.27 (d, *J* = 4.4 Hz, 1H), 3.06-3.00 (m, 1H). ^{13}C NMR (150.8 MHz, CD_3OD) δ 169.7, 163.7, 161.2, 131.1, 131.0, 130.8, 130.2, 123.5, 115.5, 115.3, 53.7, 35.0. ^{19}F NMR (376.3 MHz, CD_3OD) δ -70.27. HRMS (HESI) m/z: $[M]^+$ calcd for $C_{13}H_{11}F_9NO_3$ 400.05998, found 400.05897.



5 **6** **7**

Scheme S1. **Synthesis of Fmoc-D-perfluoro-*tert*-butyl-tyrosine** (**7**). Compound **7** was synthesized from Fmoc-D-4-NH_2-Phe via the approach described above. The ^{1}H, ^{13}C, and ^{19}F NMR spectra of **7** were identical to those of compound **3**.

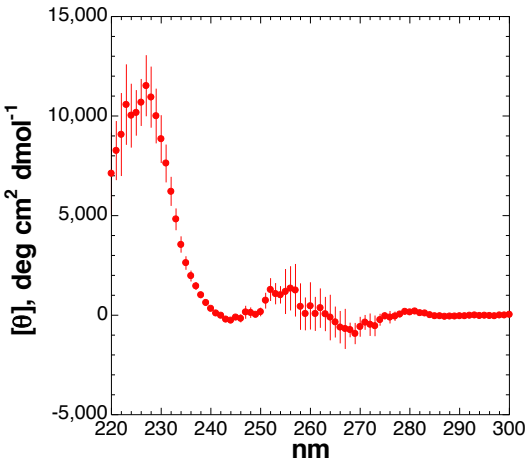


Figure S1. CD spectrum of Fmoc-perfluoro-*tert*-butyl tyrosine (**3**). Data were collected in methanol at 1.61 mM concentration. Error bars indicate standard error.

Circular Dichroism (CD)

Circular dichroism (CD) experiments were conducted on a Jasco model J-810 spectropolarimeter. All data were collected with a 1 mm cell (Starna Cells, Atascadero, CA). Data for compound **3** Fmoc-perfluoro-*tert*-butyl tyrosine were collected in methanol using 1.61 mM compound at 25 °C. Data were background-corrected but were not smoothed.

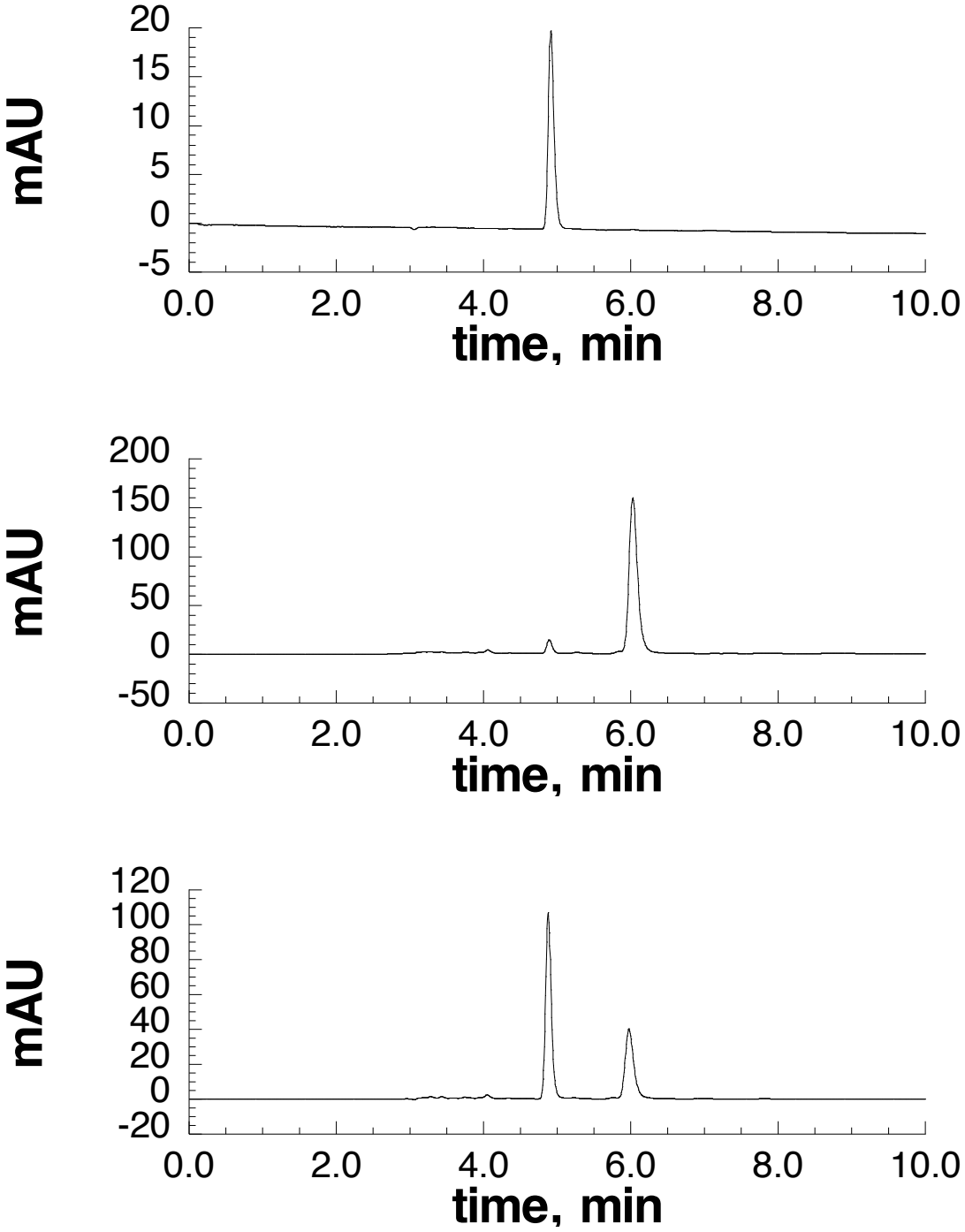


Figure S2. Analytical chiral HPLC chromatograms of purified **3** Fmoc-L-Tyr(C_4F_9)-OH (top) and **7** Fmoc-D-Tyr(C_4F_9)-OH (middle), and a co-injection of **3** and **7** (bottom) (UV detection at 254 nm) using isocratic 20% isopropanol in hexanes over 30 minutes on a Daicel ChiralPak 1A column (250 × 4.6 mm, 5 μm particle, 1.0 mL/min). Compound **3** exhibited greater than 98% ee by integration.

Peptide Synthesis and Purification

Ac-T(**Tyr(C_4F_9)**)PN-NH_2 was synthesized on NovaGel PEG-polystyrene graft Rink amide resin (EMD Millipore, 0.67 meq) by standard solid-phase peptide synthesis. Amide coupling reactions with Fmoc-perfluoro-*tert*-butyl tyrosine were conducted using 2 equivalents each of amino acid and HATU. The reaction was allowed to proceed for 24 hours at room temperature. The amide coupling reaction time was not optimized for yield; extended amide coupling reaction times were necessary for previous perfluoro-*tert*-butyl amino acids and were applied to this system. Subsequent amide coupling reactions were conducted as double coupling reactions (2 hours for the first coupling, 1 hour for the second coupling) using HATU as a coupling reagent. Ac-T(4-NH_2-Phe)PN-NH_2 was synthesized on Rink amide resin with 2 equivalents each of Fmoc-(Boc-4-NH_2)-Phe and coupling reagent. All other coupling reactions were performed with 4 equivalents each of amino acid and coupling reagents for 1 hour. All peptides were acetylated on the N terminus (5% acetic anhydride in pyridine, 3 mL, 3 × 5 min) and contained a C-terminal amide. Ac-T(4-NH_2-Phe)PN-NH_2 was subjected to cleavage from the resin and deprotection for 3 hours in 1 mL reaction volume of 90% TFA with 5% H_2O and 5% triisopropylsilane. Ac-T(**Tyr(C_4F_9)**)PN-NH_2 was subjected to cleavage and deprotection using 95% TFA, 2.5% H_2O, and 2.5% thioanisole for 60 minutes. TFA was removed by evaporation under nitrogen over 30 minutes, after which the peptides were dissolved in 1 mL 500 mM phosphate buffer (pH 7.2) without the use of ether precipitation. Crude peptide solutions were filtered using a 0.45 μm syringe filter before injection on the HPLC. Peptides were purified by reverse phase HPLC (Vydac semipreparative C18, 10 × 250 mm, 5 μm particle size, 300 Å pore) using a 60 minute linear gradient of 0 to 60% buffer B (20% H_2O, 80% acetonitrile, 0.05% TFA) in buffer A (98% H_2O, 2% acetonitrile, 0.06% TFA) for Ac-T(**Tyr(C_4F_9)**)PN-NH_2 or 0 to 20% buffer B in buffer A for Ac-T(4-NH_2-Phe)PN-NH_2. Analytical data for peptides: Ac-T(**Tyr(C_4F_9)**)PN-NH_2 [t_R, 46.7 min; expected mass, 752.5, observed mass, 753.5 $(M + H)^+$], Ac-T(4-NH_2-Phe)PN-NH_2 [t_R, 8.2 min; expected mass, 533.2, observed mass, 534.3 $(M + H^+)$].

Solution Phase Synthesis of Ac-T(Tyr(C_4F_9))PN-NH_2 from Ac-T(4-NH_2-Phe)PN-NH_2

Purified and lyophilized Ac-T(4-NH_2-Phe)PN-NH_2 was dissolved in a 1:1 solution of tetrafluoroboric acid in water (0.2 mL, 48% to 50% w/w) and methanol (0.2 mL) in an open glass vial. The solution was placed in an ice bath before the dropwise addition of aqueous sodium nitrite (0.053 g, 76 mmol in 0.05 mL H_2O, 1.5 M) over two minutes. The reaction was allowed to warm to room temperature over the course of two hours. The solvent was removed by lyophilization and used without further purification. The peptide was redissolved in perfluoro-*tert*-butanol (500 μL) and the solution warmed in an oil bath at 45 °C for 18 hours in an open glass vial fitted with an open tube (the top of a 15 mL conical vial) which functioned as a modified condenser; some loss of solvent was observed over the course of the reaction. The perfluoro-*tert*-butanol was removed by evaporation and the crude peptide was dissolved in methanol (500 μL) before injection on the HPLC. ***Warning***: diazonium salts are inherently prone to rapid generation of nitrogen gas. It is critical to use an open reaction vessel and low temperature to prevent accidental explosion. It is critical to immediately use the diazonium tetrafluoroborate to prevent drying and accidental detonation.

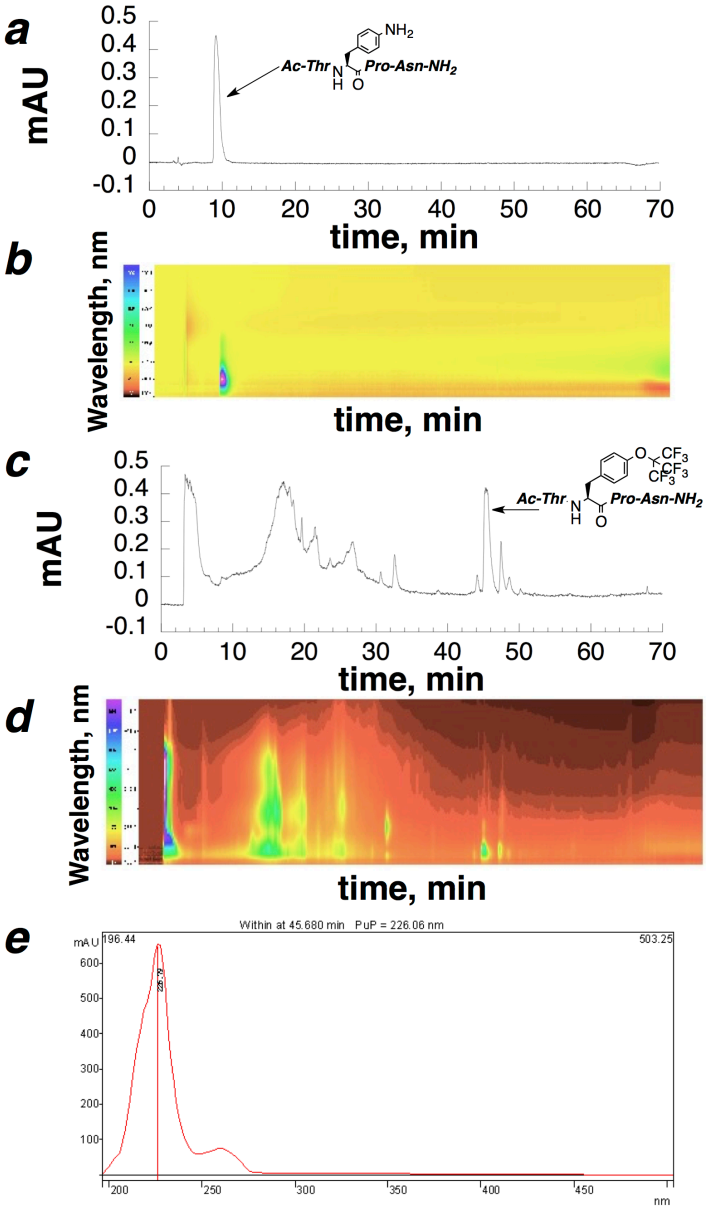


Figure S3. Purified Ac-T(4-NH_2-Phe)PN-NH_2 (a, b) was converted to Ac-T(Tyr(C_4F_9))PN-NH_2 via diazonium formation followed by heating at 45 °C with perfluoro-*tert*-butyl alcohol (c, d). (a, c): HPLC chromatograms with absorbance at 215 nm; (b, d): diode array analysis; (e) UV-Vis spectrum via the diode array of the major peak at 45.8 nm, exhibiting a spectrum in TFA-acetonitrile-water above 230 nm that is similar to that of the purified peptide (Figure S9). Diode array analysis (d) of the product chromatogram indicates that most other peaks visible in the HPLC chromatogram do not ideally match the UV signature of peptides (maximum absorbance below 230 nm), and specifically match neither the starting material nor product, and thus are likely not the result of side reactions leading to other peptide products. As observed in Figure S9, Ac-T(Tyr(C_4F_9))PN-NH_2 has a λ_{max} below 230 nm, associated with absorbance of the amide bonds. Thus, any compounds with a global maximum absorbance at greater than 230 nm are not associated with this peptide or potential decomposition products, and are likely due to small molecule impurities. Peptides in the vicinity of the product peptide peak do exhibit peptide-like UV signatures and likely represent side reaction products. In addition, this peptide product (major peak at t_R = 46 minutes) was isolated and characterized by ^{1}H NMR, and exhibits an identical NMR spectrum to the peptide synthesized using **3**.

NMR Spectroscopy of Peptides

Peptide NMR experiments were performed on a Brüker 600 MHz (^{19}F 564.5 MHz) NMR spectrometer equipped with a 5-mm Brüker SMART probe in 90% H_2O with 5 mM phosphate buffer (pH 4), 25 mM NaCl, and 10% D_2O. TSP was used for referencing in ^{1}H NMR. Residual TFA was used for referencing in ^{19}F NMR. All 1-D ^{1}H NMR spectra were collected with a Brüker w5 watergate pulse sequence with a 2 to 3 second relaxation delay. TOCSY spectra were collected using a watergate TOCSY pulse sequence. ^{19}F NMR data were collected on a Brüker 600 MHz (^{19}F 564.5 MHz) NMR spectrometer equipped with a 5-mm Brüker SMART probe without the use of proton decoupling. A 0.8 second acquisition time was used with a 200 ppm or an 8 ppm sweep width unless otherwise noted.

Stability of Perfluoro-*tert*-butyl Tyrosine Under Standard Solid-Phase Peptide Synthesis and TFA Deprotection and Cleavage Conditions

Substituted aromatic amino acids have the potential to decompose under TFA cleavage and deprotection conditions. Upon subjection to cleavage and deprotection in 95% TFA with 2.5% thioanisole and 2.5% water for 90 min, the product Ac-T(**Tyr(C_4F_9)**)PN-NH_2 was afforded in good yield (Figure S4). Cleavage and deprotection in 90% TFA with 5% TIS and 5% water, however, led to elimination of the perfluoro-*tert*-butyl ether (Figure S4). This product decomposition is most likely due to hydride delivery via the TIS, allowing reaction with the perfluoro-*tert*-butyl ether as a leaving group. Ac-TFPN-NH_2 was isolated from the cleavage and deprotection reactions utilizing TIS. The elimination product was purified and characterized by HPLC, mass spectrometry, and ^{1}H and TOCSY NMR spectroscopy, with the observed product identical to the previously synthesized peptide Ac-TFPN-NH_2.[4] These results indicate that TIS should be avoided during TFA cleavage and deprotection reactions with perfluoro-*tert*-butyl tyrosine.

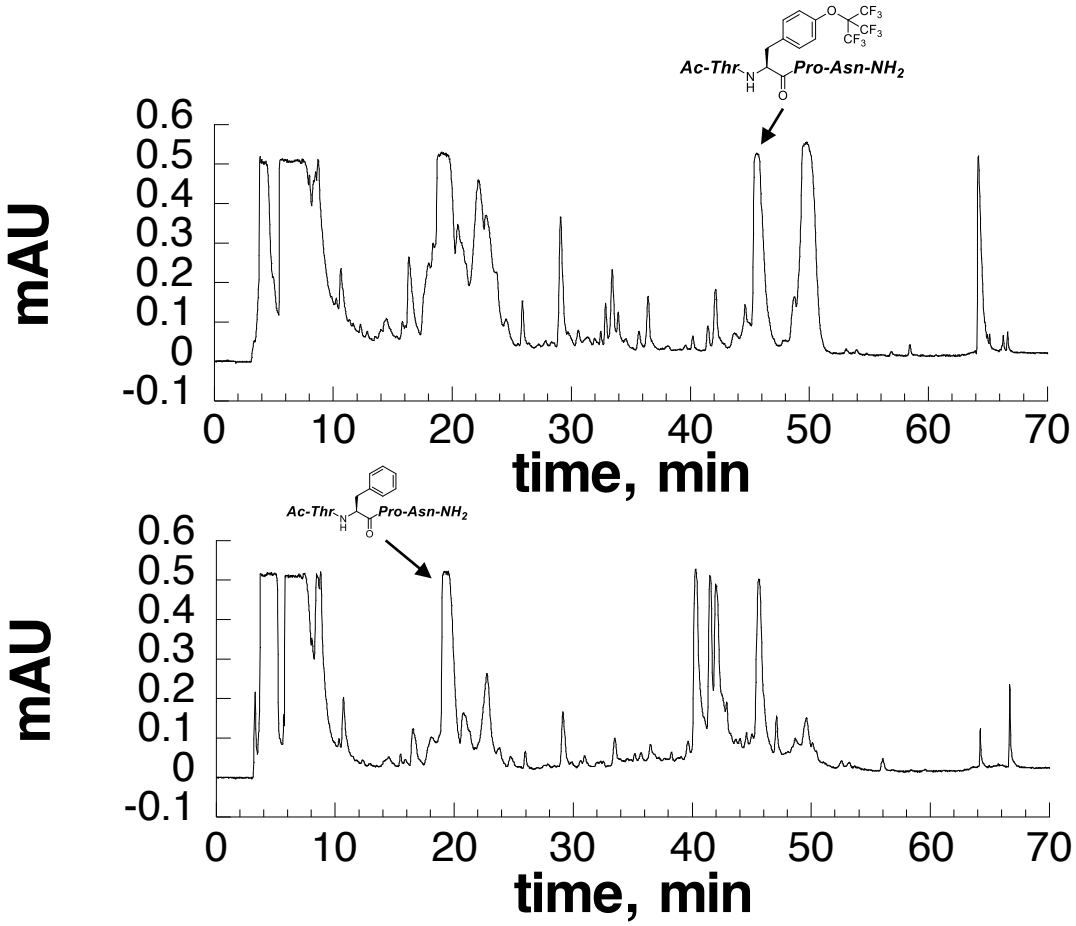


Figure S4. HPLC chromatograms of cleavage and deprotection reactions of Ac-T(Tyr(C_4F_9))PN-NH_2 in 95% TFA with 2.5% thioanisole and 2.5% water for 90 min (top) and in 90% TFA with 5% TIS and 5% water for 3 hours (bottom). *No ether precipitation was used after TFA cleavage and deprotection*, which results in additional non-peptide products derived from the cleavage/deprotection reagent cocktail being observed by HPLC.

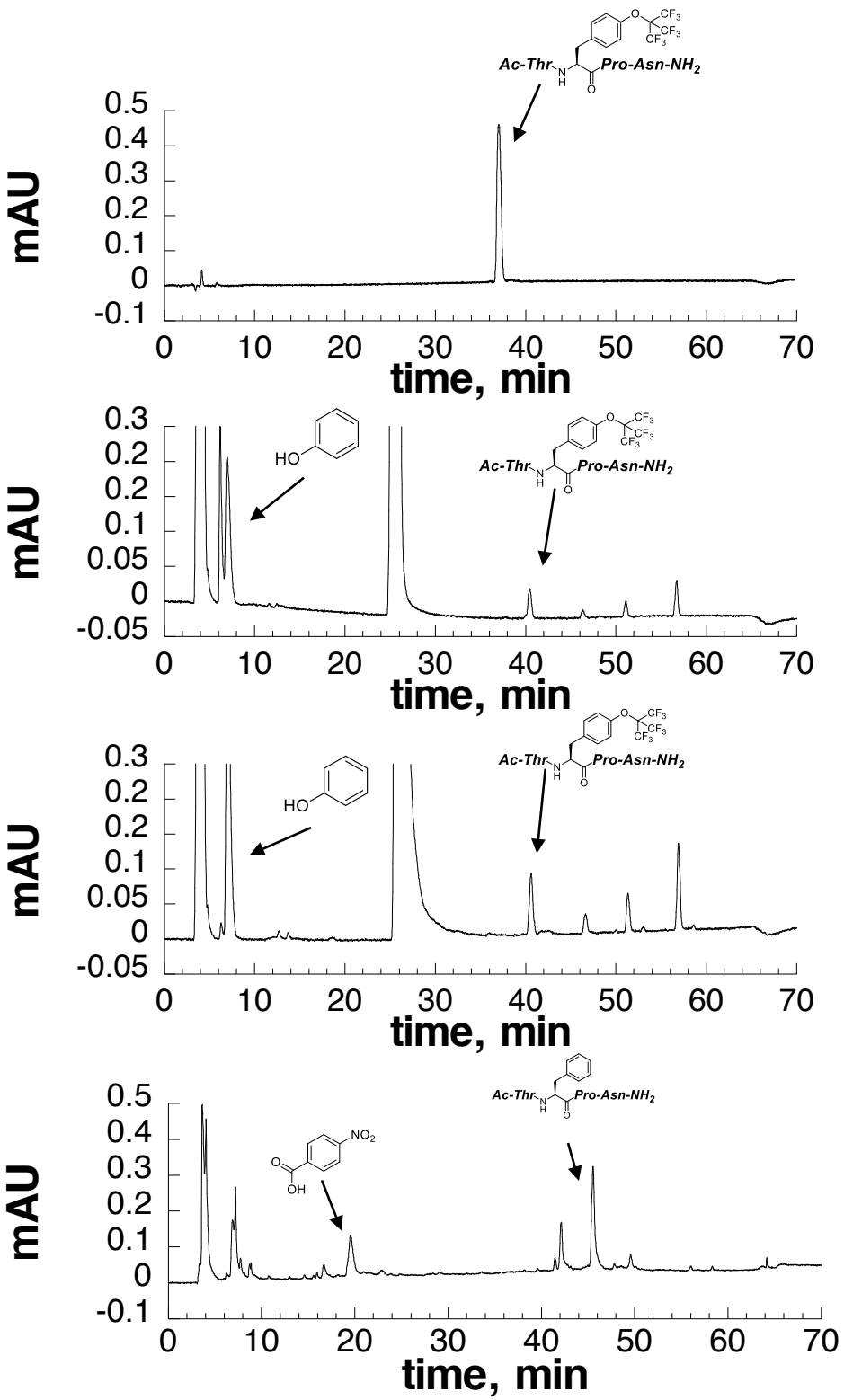


Figure S5. HPLC chromatograms of cleavage and deprotection reactions on the purified peptide Ac-T(Tyr(C_4F_9))PN-NH_2 (top) and peptide in 95% TFA with 2.5% thioanisole and 2.5% water at 0 hours (t_0) (second) and 6 hours (third) (HPLC conditions: 0-60% buffer B in buffer A over 60 min at 1 mL/min). Peptide and *p*-nitrobenzoic acid (internal standard) peaks were integrated and indicated that minimal side reaction had occurred over 6 hours. (bottom) Peptide in 90% TFA with 5% TIS and 5% water for 3 hours (HPLC conditions: 0-60% buffer B in buffer A over 60 min at 4 mL/min).

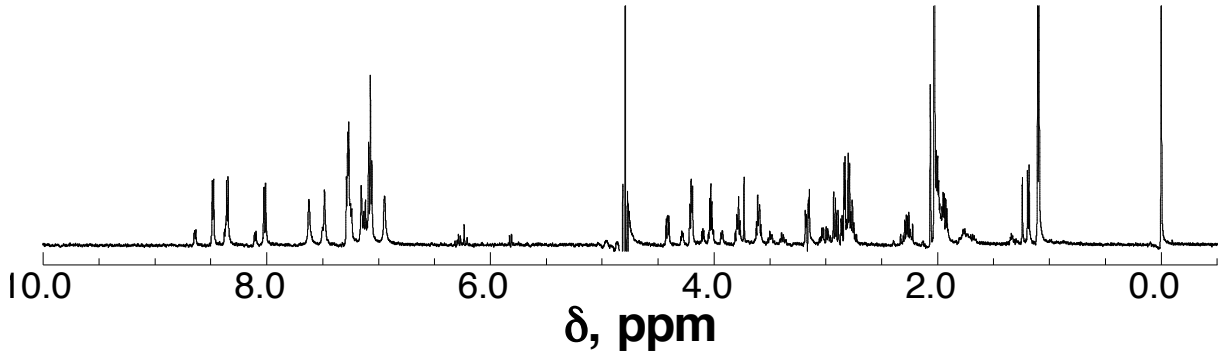


Figure S6. ^{1}H NMR spectrum of the peptide Ac-TFPN-NH_2 in 90% H_2O/10%D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl that was isolated by HPLC at t_R = 19.2 min from the cleavage and deprotection reaction of Ac-T(**Tyr(C_4F_9)**)PN-NH_2 with 90% TFA, 5% TIS, and 5% H_2O for three hours.

Due to the instability of the perfluoro-*tert*-butyl tyrosine under some cleavage and deprotection conditions, we verified that the peptide was stable under optimized cleavage and deprotection conditions as well as standard peptide synthesis conditions. Purified Ac-T(**Tyr(C_4F_9)**)PN-NH_2 was subjected to the optimized cleavage and deprotection conditions as well as the cleavage conditions that promote elimination of the perfluoro-*tert*-butyl group. After three hours in 95% TFA with 5% TIS and 5% water, Ac-TFPN-NH_2 was observed by HPLC (Figure S5). When subjected to optimized TFA cleavage/deprotection conditions (95% TFA with 2.5% thioanisole and 2.5% water), no elimination was observed after six hours. The peptide was also stable for 24 hours under standard amide coupling (8% DIPEA in DMF) and standard Fmoc deprotection (20% piperidine in DMF), with no identifiable side products observed by HPLC (Figure S7 and S8).

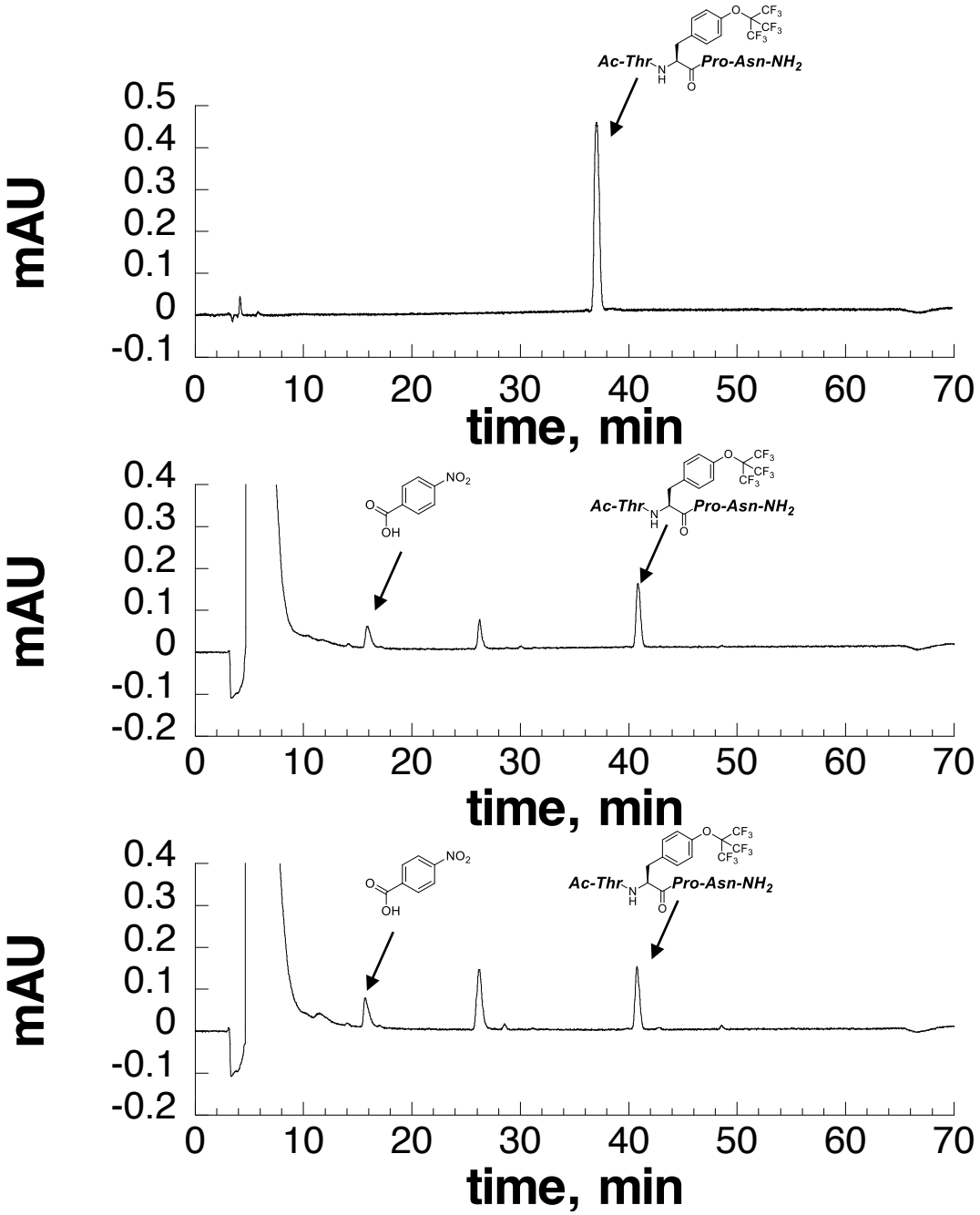


Figure S7. Ac-T(**Tyr(C_4F_9)**)PN-NH_2 is stable in DMF with 8% DIPEA for a minimum of 24 hours at room tempearture. Purified Ac-T(**Tyr(C_4F_9)**)PN-NH_2 (top) was subjected to a solution of 8% DIPEA in DMF with *p*-nitrobenzoic acid as a standard. HPLC chromatograms were recorded at time = 0 (center) (1:2.54 ratio of standard to peptide) and 24 hours (bottom) (1:2.55 ratio of standard to peptide) using a 60 minute linear gradient of 0 to 70% buffer B (20% H_2O, 80% acetonitrile, 0.05% TFA) in buffer A (98% H_2O, 2% acetonitrile, 0.06% TFA). Integration of the standard and the peptide peaks, as well as the absence of new peak formation, demonstrate that the peptide Ac-T(**Tyr(C_4F_9)**)PN-NH_2 is stable under these conditions.

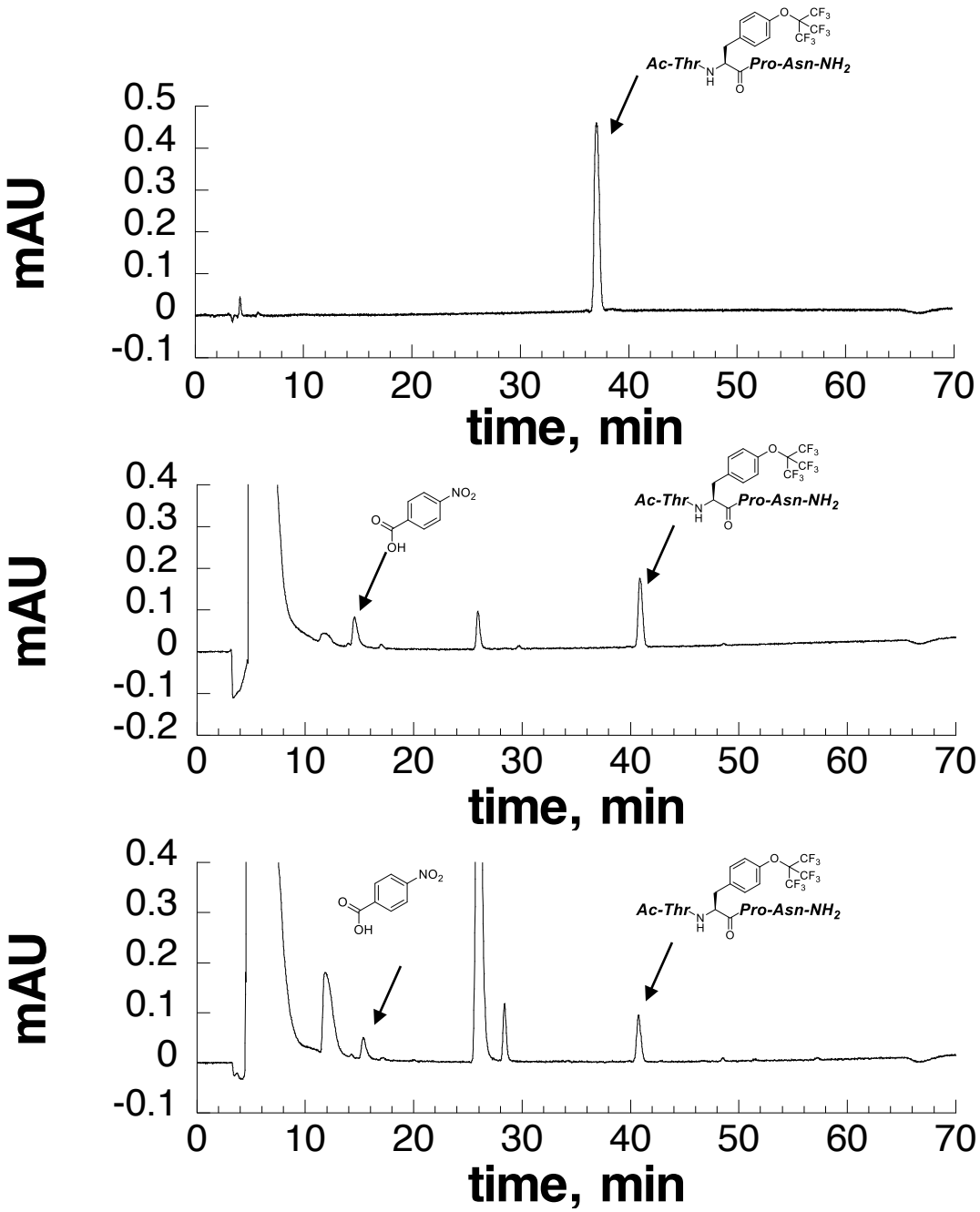


Figure S8. Ac-T(**Tyr(C$_4$F$_9$)**)PN-NH$_2$ is stable in DMF with 20% piperidine for a minimum of 24 hours at room temperature. Purified Ac-T(**Tyr(C$_4$F$_9$)**)PN-NH$_2$ (top) was subjected to a solution of 20% piperidine in DMF with *p*-nitrobenzoic acid as a standard. HPLC chromatograms were recorded at time = 0 (center) (1:1.85 ratio of standard to peptide) and 24 hours (bottom) (1:2.06 ratio of standard to peptide) using a 60 minute linear gradient of 0 to 70% buffer B (20% H_2O, 80% acetonitrile, 0.05% TFA) in buffer A (98% H_2O, 2% acetonitrile, 0.06% TFA). Integration of the standard and the peptide peaks, as well as the absence of new peak formation, demonstrates that the peptide Ac-T(**Tyr(C$_4$F$_9$)**)PN-NH$_2$ is stable under these conditions.

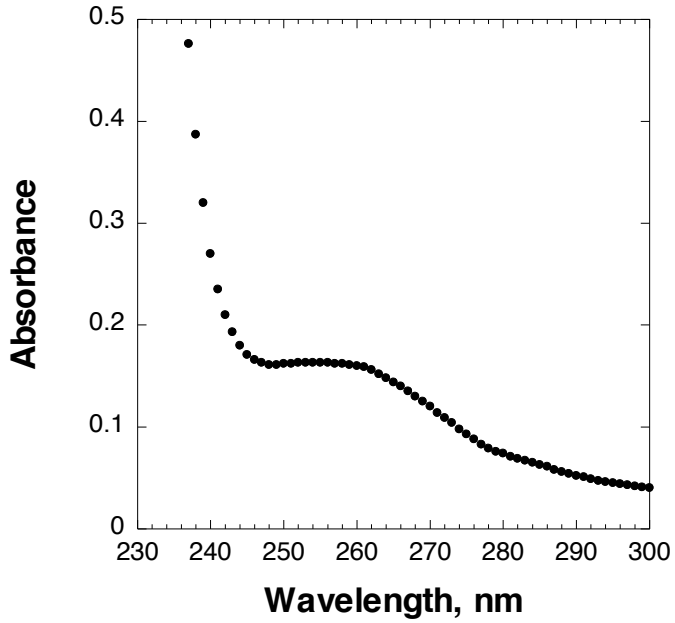


Figure S9. UV spectrum of the peptide Ac-T(**Tyr(C_4F_9)**)PN-NH_2 in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl. The extinction coefficient was calculated to be 1620 M^{-1} cm^{-1} at 254 nm using a 1 cm pathlength and 101 μM concentration. Concentration was determined by NMR using 1 mM maleic acid as a standard.

UV Spectroscopy of Perfluoro-*tert*-butyl Tyrosine

The purified peptide Ac-T(**Tyr(C_4F_9)**)PN-NH_2 was dissolved in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl. Absorbance spectra were collected on a Perkin-Elmer Lambda 25 UV-Vis spectrometer in a 1 cm cell. Absorbance was measured from 300 nm to 220 nm with a slit width of 1 nm. The λ_{max} was determined to be 254nm.

Determination of Detection Limit of Perfluoro-*tert*-butyl Tyrosine in a Peptide by ^{19}F NMR

Ac-T(**Tyr(C_4F_9)**)PN-NH_2 was dissolved in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl and the concentration quantified using 1 mM maleic acid as a standard. The peptide was diluted under the same conditions to 500 nM or 200 nM peptide concentration. ^{19}F peptide NMR spectra were recorded using a 8 ppm sweep width without decoupling and a 3 second relaxation delay.

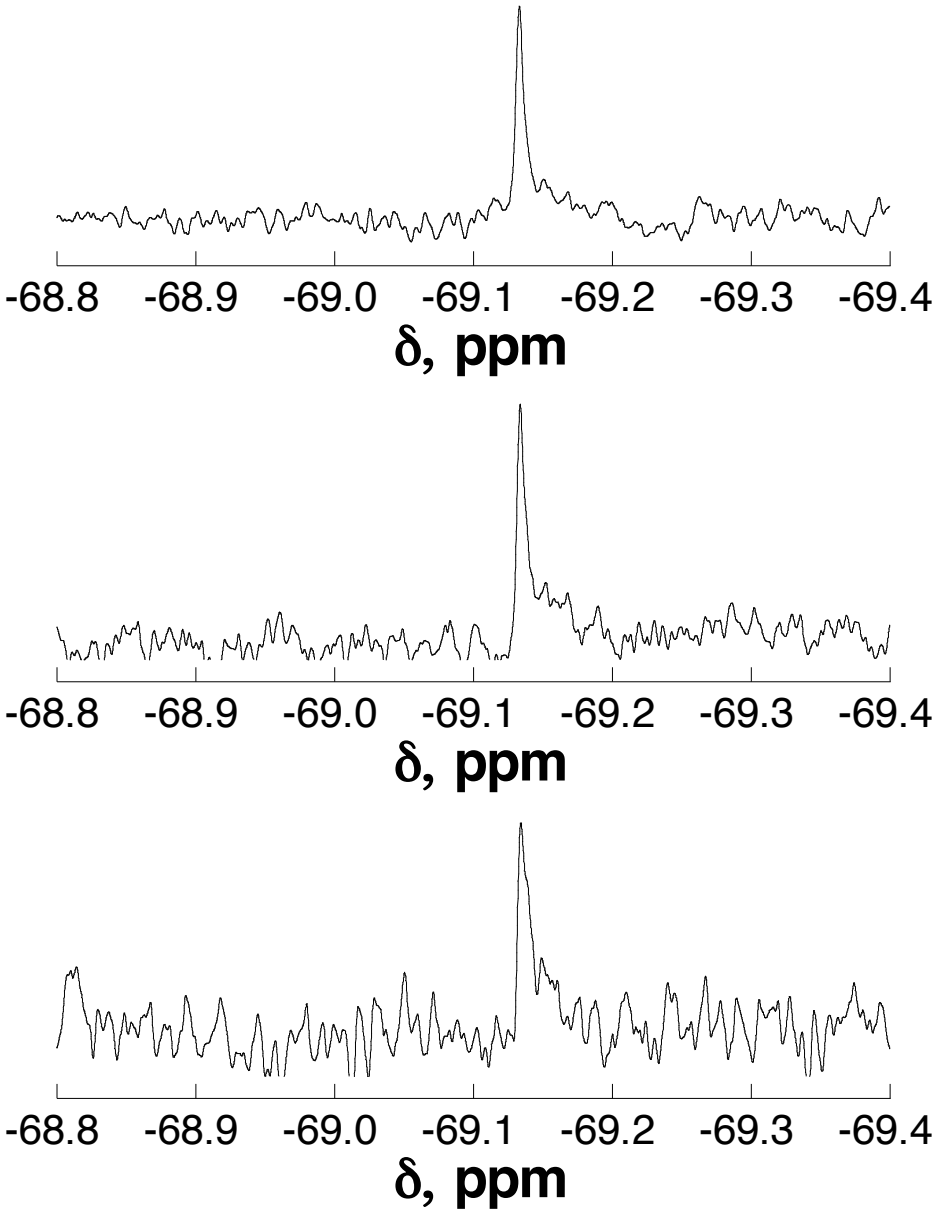


Figure S10. ^{19}F NMR spectrum of the peptide Ac-T(**Tyr(C_4F_9)**)PN-NH_2 in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl at 500 nM peptide concentration. Data were collected as above using 32 scans (2 min, 18 sec), signal-to-noise ratio (S/N) = 19.6 (top); 16 scans (1 min, 16 sec), S/N = 14.3 (middle); or 8 scans (46 sec), S/N = 8.9 (bottom). Experiments were conducted with 4 dummy scans, 0.8 sec acquisition time, and a 3.0 sec relaxation delay. Signal-to-noise ratio was calculated by the SNR peak calculator in MestReNova version 10.

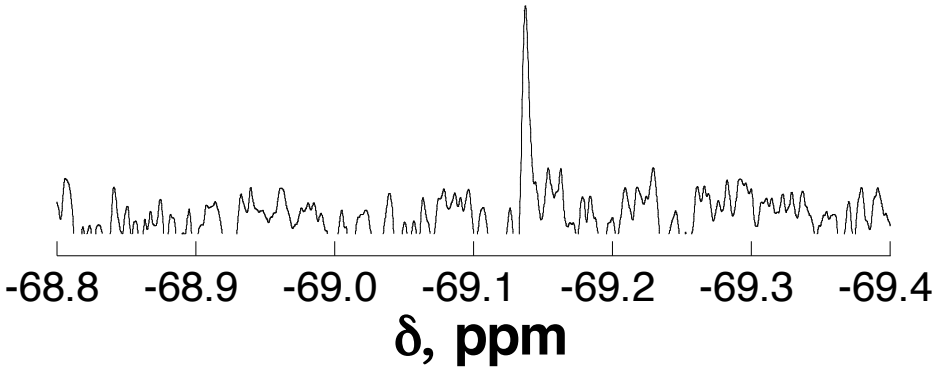


Figure S11. ^{19}F NMR spectrum of the peptide Ac-T(**Tyr(C_4F_9)**)PN-NH_2 in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl at 500 nM peptide concentration. Data were collected as above using 8 scans (28 sec), S/N = 10.1. Experiments were conducted with 2 dummy scans, 0.8 sec acquisition time, and a 2.0 sec relaxation delay. Signal-to-noise ratio was calculated by the SNR peak calculator in MestReNova version 10.

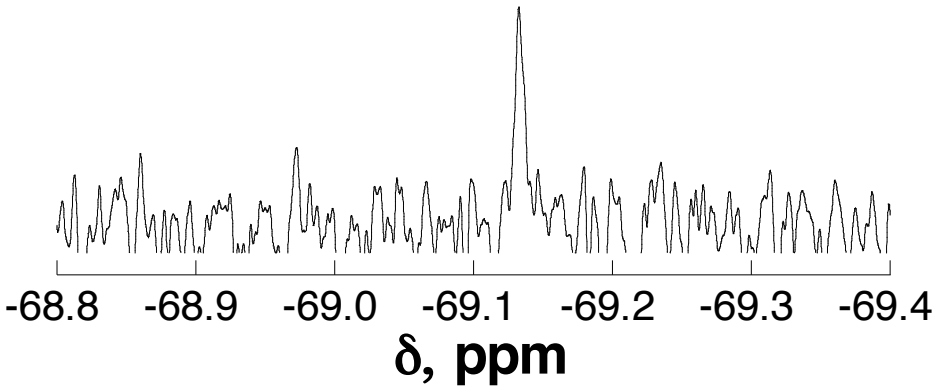


Figure S12. ^{19}F NMR spectrum of the peptide Ac-T(**Tyr(C_4F_9)**)PN-NH_2 in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl at 200 nM peptide concentration. Data were collected in 5 minutes and 23 seconds with 128 scans, S/N = 7.4. The data were collected with 0.91 seconds acquisition time and a 3.0 second relaxation delay. Data were processed with exponential multiplication and a line broadening of 2 Hz. Signal-to-noise ratio was calculated by the SNR peak calculator in MestReNova version 10.

Aromatic Electronic Effects of a Perfluoro-*tert*-butyl Ether

Aromatic-proline sequences account for a significantly higher frequency of *cis* amide bonds compared to other X-Pro amide bonds, as determined by examining structures within the PDB.[7] These sequences are often found in turn structures and can be an integral part of protein folding events. The Zondlo lab has previously developed the Ac-TYPN-NH_2 peptide as a model system to study *cis-trans* isomerization in aromatic-proline sequences and was optimized to maximize *cis* amide bond formation.[3] We have introduced unnatural amino acids at both the aromatic and proline sites in order to tune the interaction.[4] In the case of substituted aromatic

derivatives, more electron-donating substituents favor *cis* amide bond formation, while electron-withdrawing substituents favor *trans* amide bond formation. This electronic effect is due to a C–H/π interaction between the proline H_α proton and the aromatic ring when in the *cis* amide conformation. Electron-donating substituents increase electron density in the aromatic system, allowing for a stronger C–H/π interaction, while electron-withdrawing groups weaken the interaction by reducing electron density from the aromatic system.

In the case of the perfluoro-*tert*-butyl tyrosine, we set out to quantify the effect of the perfluoro-*tert*-butyl group on the aromatic system. Fluorinated amino acids are known to favor beta structure within a protein context, often disrupting other types of secondary structures. Fluorine is a highly electronegative atom which can affect the conformational preferences within an aromatic-proline sequence. Perfluoro-*tert*-butyl tyrosine was introduced into the Ac-TXPN-NH_2 peptide model system. All canonical aromatic amino acids as well as a large set of unnatural aromatic amino acids have previously been examined within this context.[6] ^{1}H NMR spectroscopy of the amide region was used to quantify the $K_{trans/cis}$ of 4.1 (ΔG = -0.83 kcal mol^{-1}) for perfluoro-*tert*-butyl tyrosine, compared to Ac-TYPN-NH_2 which has a $K_{trans/cis}$ of 2.7 (ΔG = -0.59 kcal mol^{-1}).

Comparison of the ^{1}H and TOCSY spectra of the peptides peptides Ac-TYPN-NH_2 and Ac-T(**Tyr(C_4F_9)**)PN-NH_2 also indicates modest changes as a result of the addition of the perfluoro-*tert*-butyl group. The protons on the aromatic ring are modestly shifted downfield, as a result of the perfluoro-*tert*-butyl modification. There are also changes in chemical shifts in the amide region, which are consistent with previous results in the presence of a peptide with modestly electron-withdrawing substitution on the aromatic ring (Table S1 and Figures S13-S14). Pro H_α in the *cis* conformation also directly correlates with aromatic electronic properties due to its interaction with the aromatic ring. In general, smaller chemical shift changes are observed in the *trans* conformation than the *cis* conformation. Analysis of the northeast quadrant (aliphatic-aliphatic correlations) of the TOCSY spectra (Figures S15-S20) reveals only small differences of chemical shifts in this region.

	δ, Tyr(C_4F_9), *trans*	δ,Tyr(C_4F_9), *cis*	δ, Tyr, *trans*	δ, Tyr, *cis*
H_N	8.40	8.39	8.31	8.39
H_α	4.92	4.62	4.90	4.90
$H_{\beta1}$	2.93	3.03	2.86	2.91
$H_{\beta2}$	3.20	3.20	3.12	3.03
Ring	7.33, 7.29	7.33, 7.28	7.17, 6.89	7.14, 6.88
Pro H_α	4.40	4.00	4.42	3.83

Table S1. Chemical shift comparisons for Tyr(C_4F_9) and Tyr protons within the peptides Ac-T(**Tyr(C_4F_9)**)PN-NH_2 and Ac-TYPN-NH_2 in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl.

	Tyr(C_4F_9) *trans*, ppm	$^3J_{\alpha N}$, Hz	Tyr *trans*, ppm	$^3J_{\alpha N}$, Hz	Tyr(C_4F_9) *cis*, ppm	$^3J_{\alpha N}$, Hz	Tyr *cis*, ppm	$^3J_{\alpha N}$, Hz
Thr	7.96	8.0	8.20	8.0	8.07	8.0	8.13	8.1
Tyr	8.40	8.4	8.29	8.3	8.39	n.d.	8.37	8.4
Asn	8.49	7.3	8.40	8.4	8.68	7.2	8.61	8.6

Table S2. Chemical shift and coupling constant comparisons for amide protons of the peptides Ac-T(**Tyr(C_4F_9)**)PN-NH_2 and Ac-TYPN-NH_2 in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl. The Tyr(C_4F_9) *cis* $^3J_{\alpha N}$ coupling constant was not determined due to spectral overlap with Tyr(C_4F_9) *trans* peak.

Calculation of Hammett Sigma Constant of a Perfluoro-*tert*-butyl Ether

Hammett constants (σ) are a measure of electron-donating or electron-withdrawing effects of functional groups on an aromatic system. The σ value is determined by measuring the p*K*a of substituted benzoic acids. The location of the substituent on the aromatic ring gives rise to differing σ values, where σ_{para} is a measure of both resonance and inductive effects, while σ_{meta} is primarily a measure of the inductive effects Currently, there is no measured value for the perfluoro-*tert*-butyl ether group. In order to understand the electronic properties of the synthesized amino acid, the σ value for the perfluoro-*tert*-butyl ether was calculated. Previously in the Zondlo lab, a linear free energy relationship was observed between the log($K_{trans/cis}$) in Ac-T(4-Z-Phe)PN-NH_2 peptides and σ value of Z of 4-Z-Phe amino acids. A series of peptides was synthesized with known σ values of the 4-substituent (Z). The $K_{trans/cis}$ was calculated by

measuring the ratio of *trans* and *cis* amide bond populations by ^{1}H NMR. The series of peptides covered both a large range of $K_{trans/cis}$ values, and the data were fit to both σ_{para} and σ_{meta} values. The calculated σ_{para} value for the 4-perfluoro-*tert*-butyl ether is 0.30 using equation (1) with ρ = 0.295. The σ_{para} value is seemingly low for a derivative containing nine fluorine atoms; however, it has previously been demonstrated that both distance from the ring and ether linkages reduce the electron-withdrawing effect of the fluorines. In this case, all nine fluorines are four bonds separated from the aromatic ring. As seen in Table 1, the addition of an ether linkage significantly reduces the electron-withdrawing effect in all previously examined cases. This observation can be attributed to the electron-donating nature of oxygen. Furthermore, as the fluorines move further away from the ring system, they have less of an electron withdrawing-effect on the ring system.

(1) $\log(K_{trans/cis}) = 0.2954\sigma_{para} + 0.523$

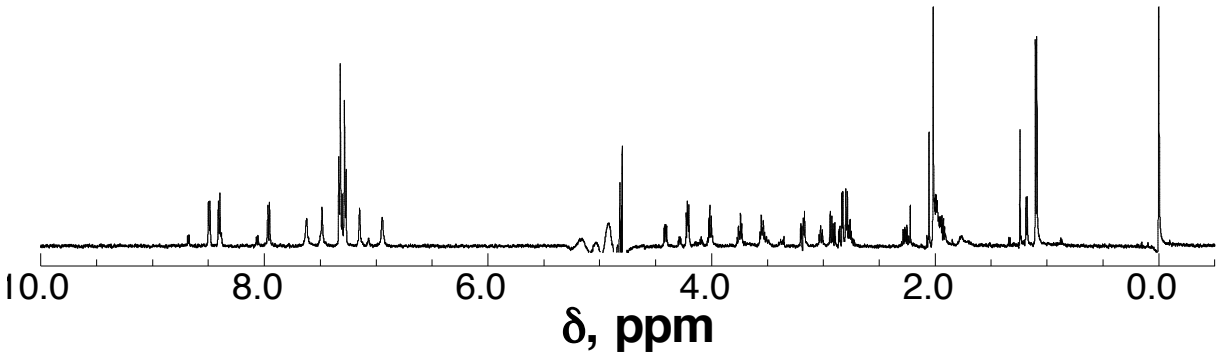


Figure S13. ^{1}H NMR spectrum of the peptide Ac-T(**Tyr(C_4F_9)**)PN-NH_2 in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl.

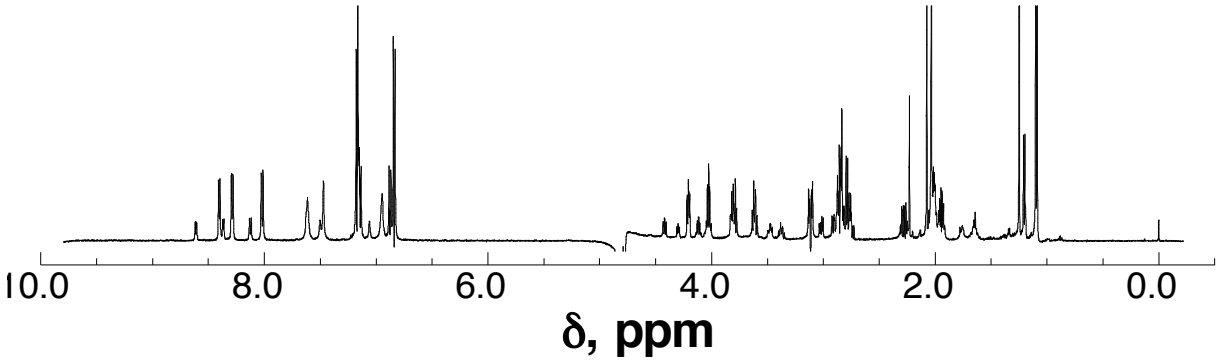


Figure S14. ^{1}H NMR spectrum of the peptide Ac-TYPN-NH_2 in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl.

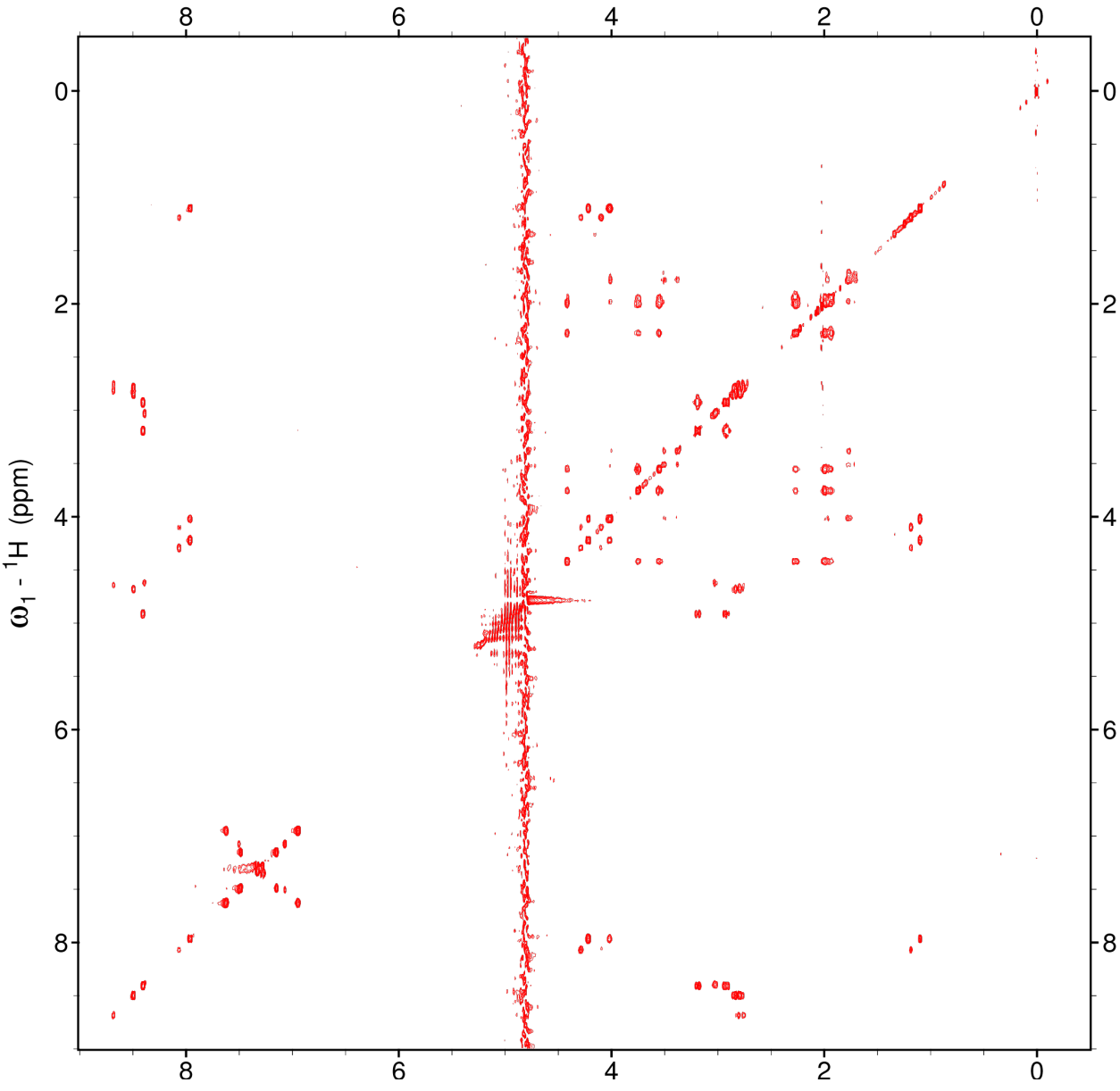


Figure S15. TOCSY spectrum of the peptide Ac-T(**Tyr(C_4F_9)**)PN-NH_2 in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl.

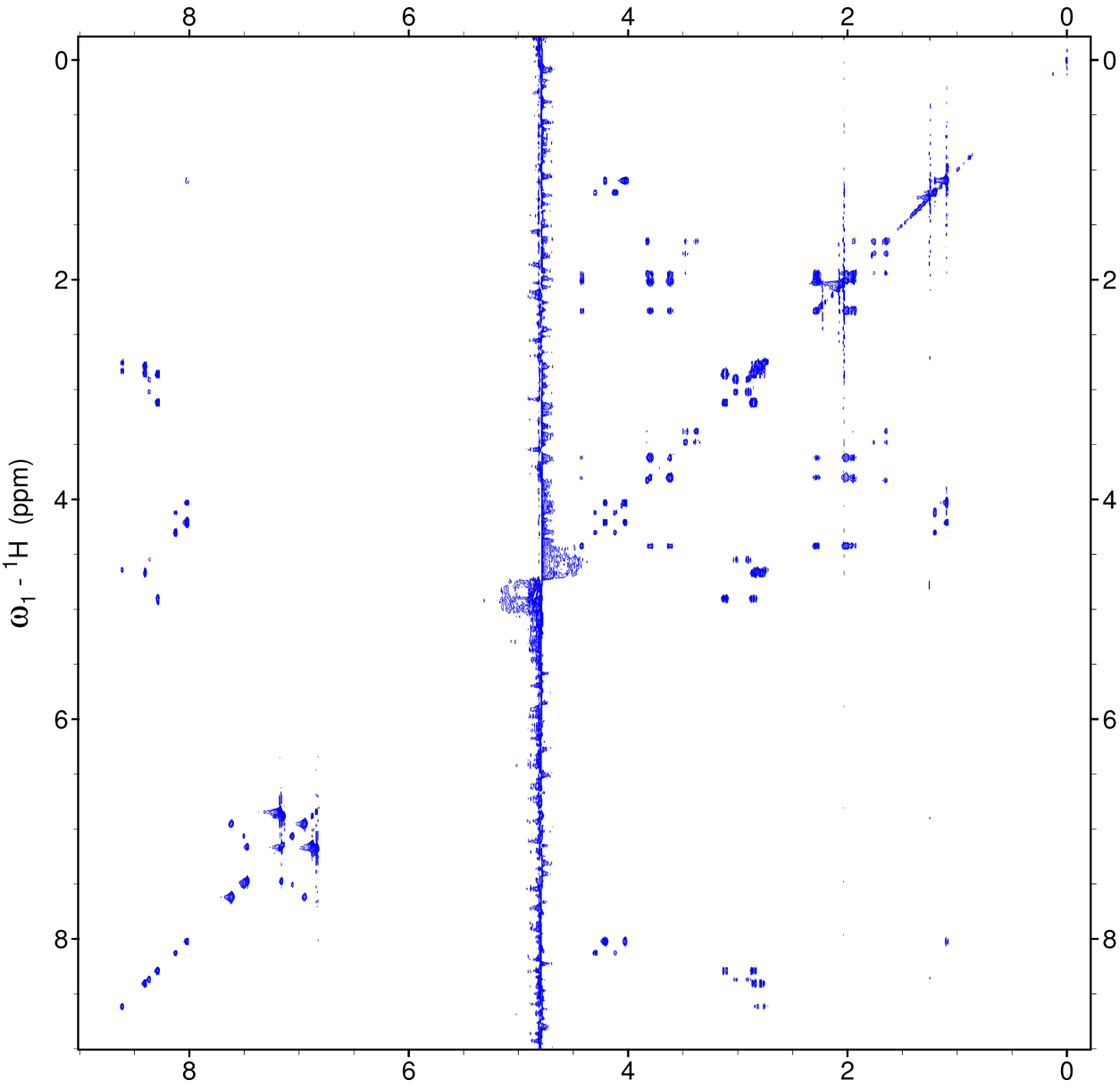


Figure S16. TOCSY spectrum of the peptide Ac-TYPN-NH_2 in 90% H_2O/10%D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl.

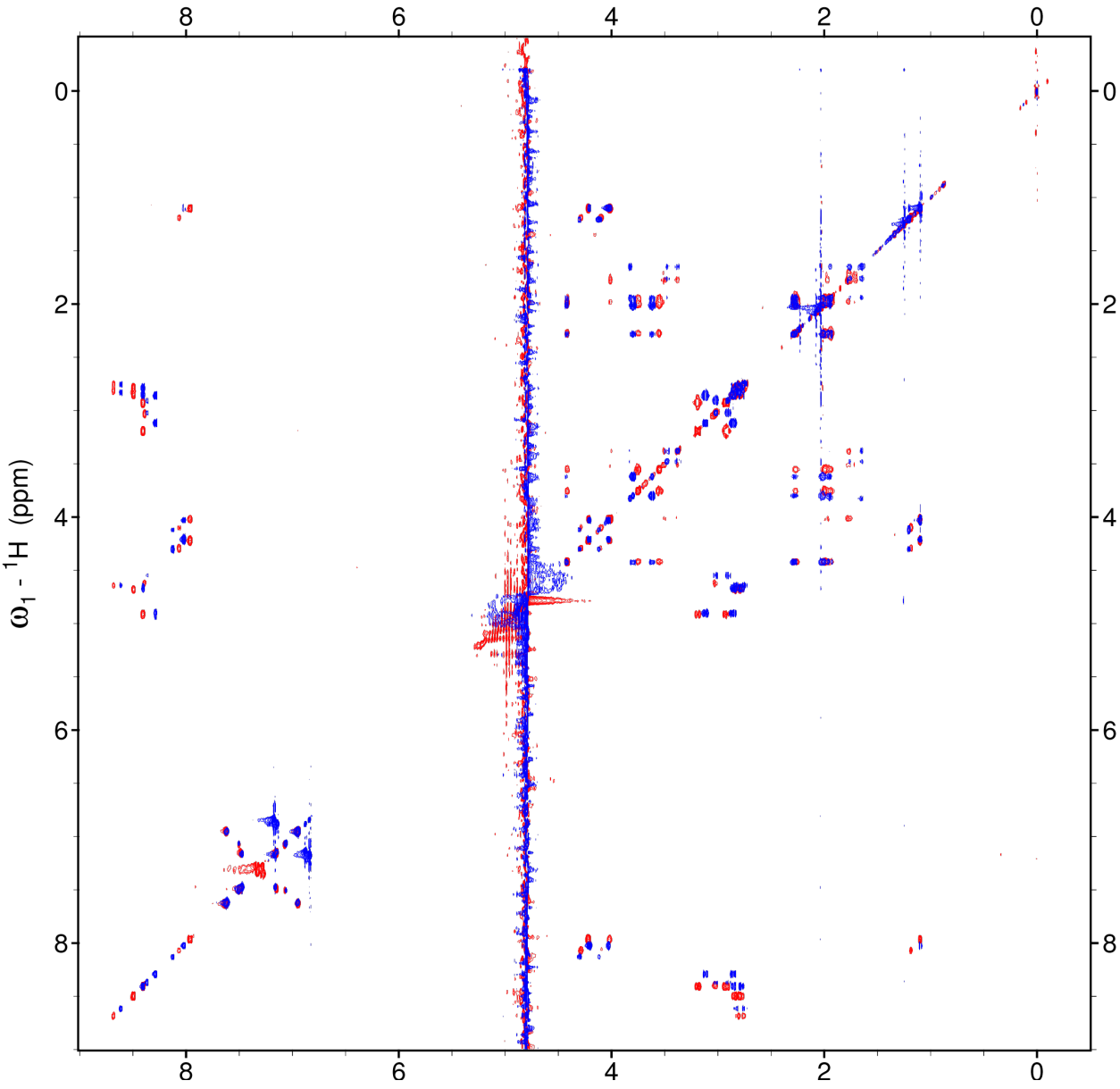


Figure S17. Superposition of the TOCSY spectra of the peptides Ac-T(**Tyr(C_4F_9)**)PN-NH_2 (red) and Ac-TYPN-NH_2 (blue) in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl.

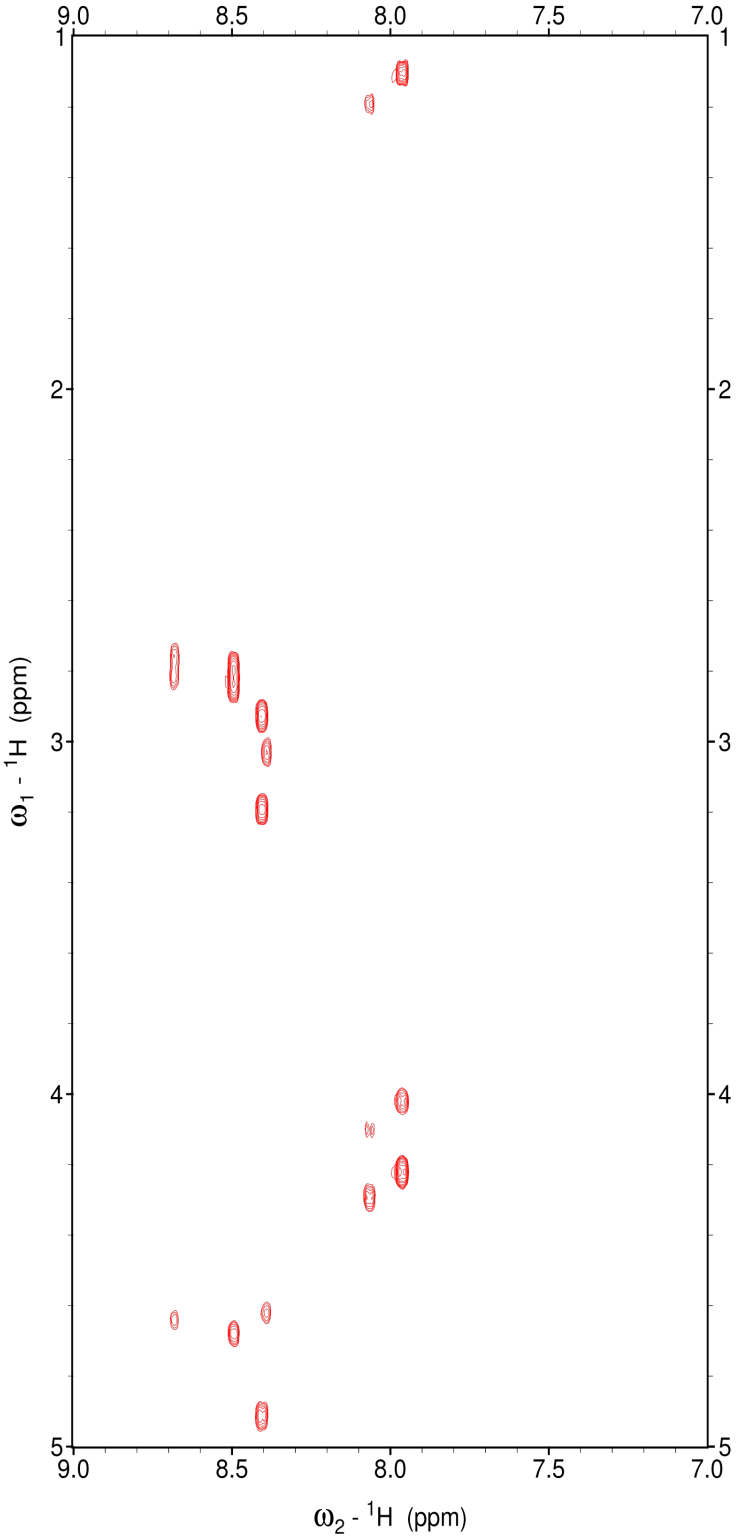


Figure S18. Fingerprint region of the TOCSY spectrum of the peptide Ac-T(**Tyr(C_4F_9)**)PN-NH_2 in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl.

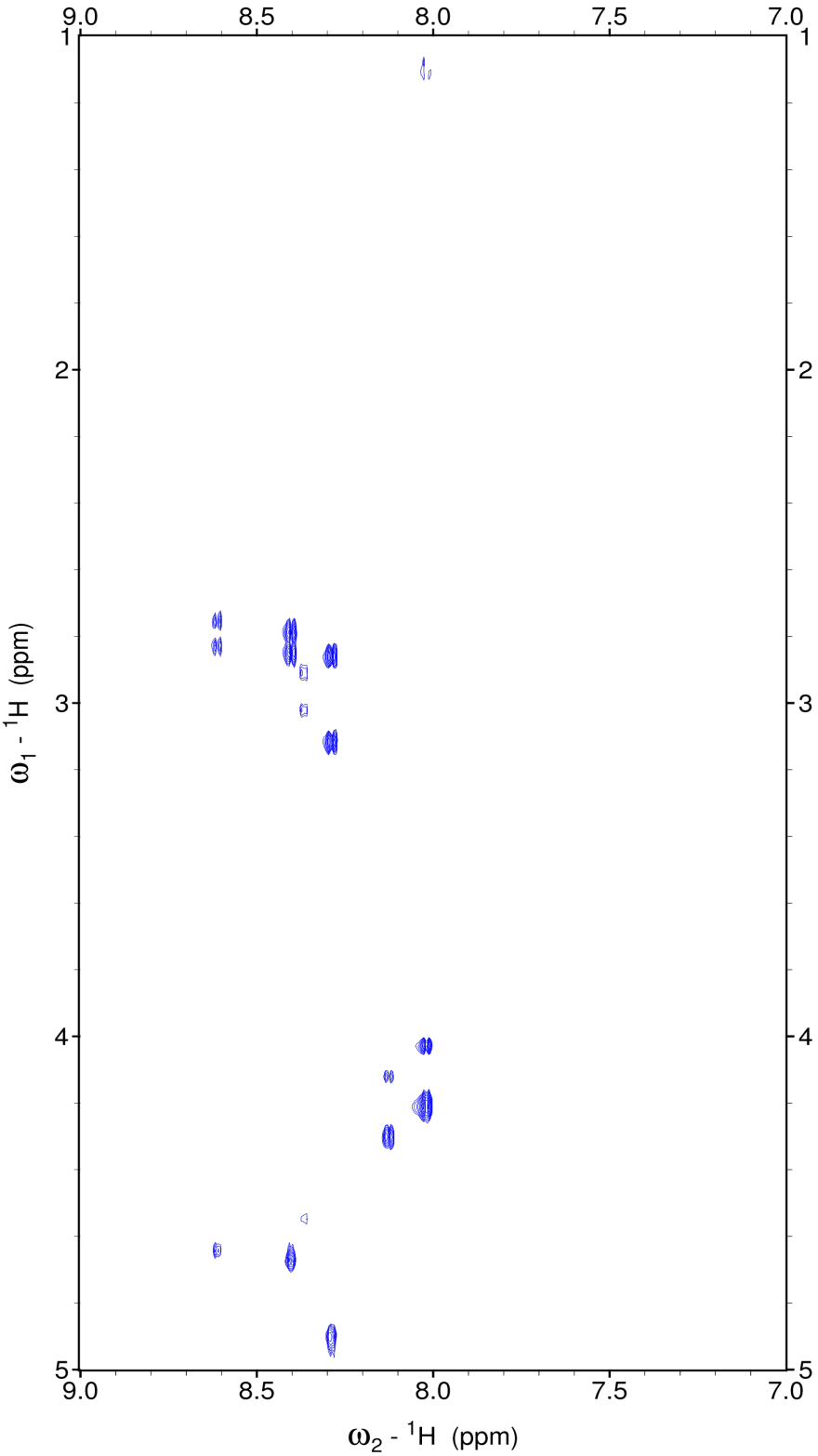


Figure S19. Fingerprint region of the TOCSY spectrum of the peptide Ac-TYPN-NH_2 in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl.

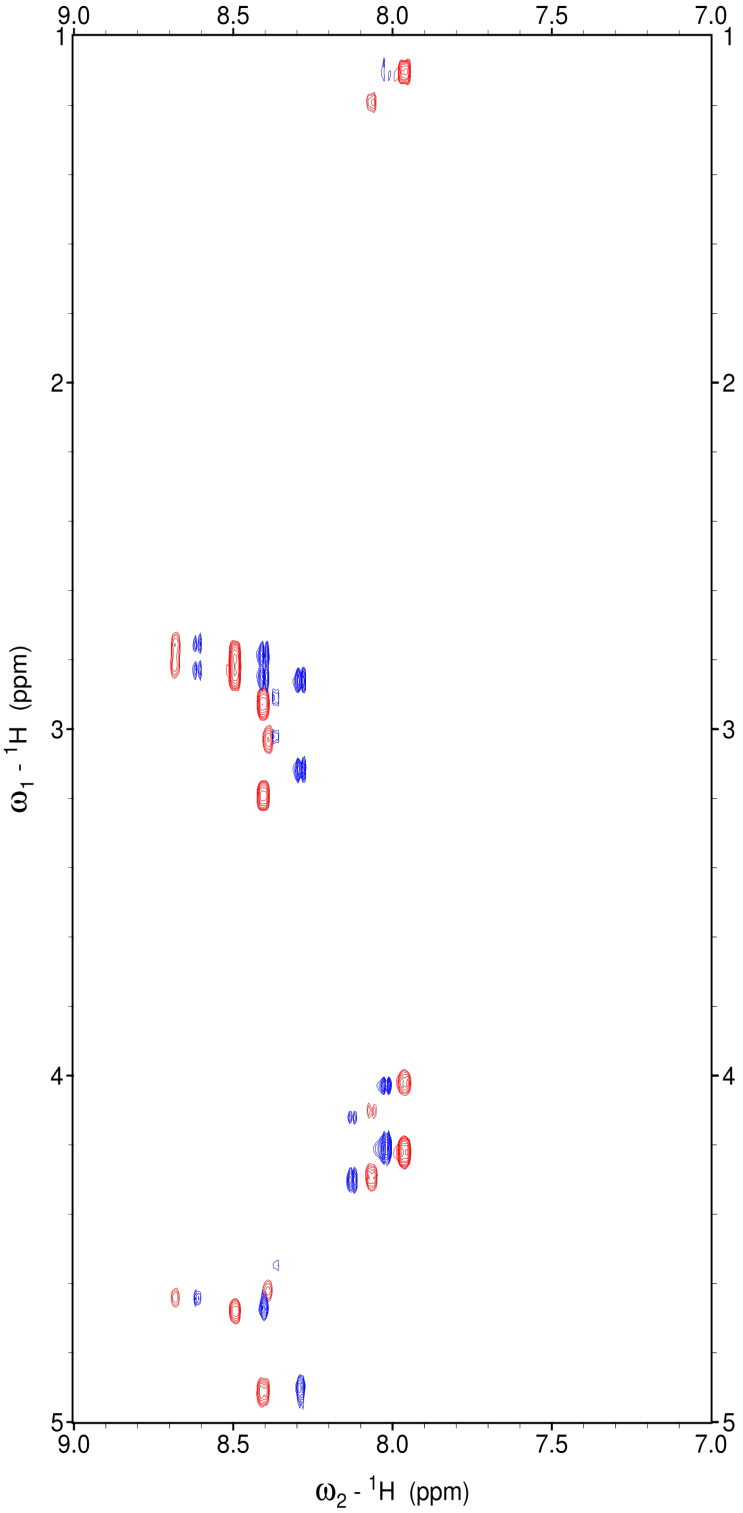


Figure S20. Superposition of the fingerprint region of the TOCSY spectra of the peptides Ac-T(**Tyr(C_4F_9)**)PN-NH_2 (red) and Ac-TYPN-NH_2 (blue) in 90% H_2O/10% D_2O with 5 mM phosphate buffer (pH 4) and 25 mM NaCl.

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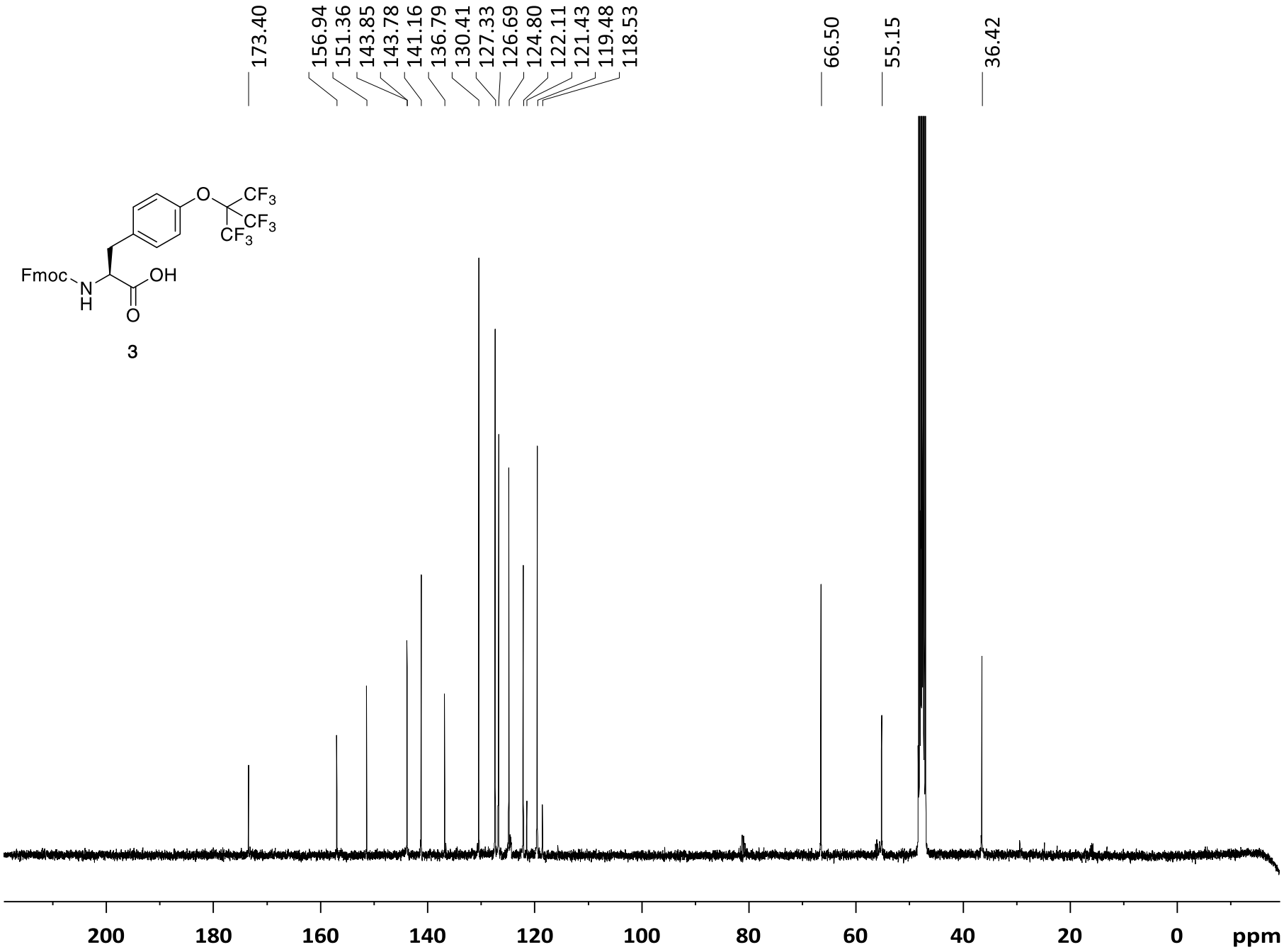
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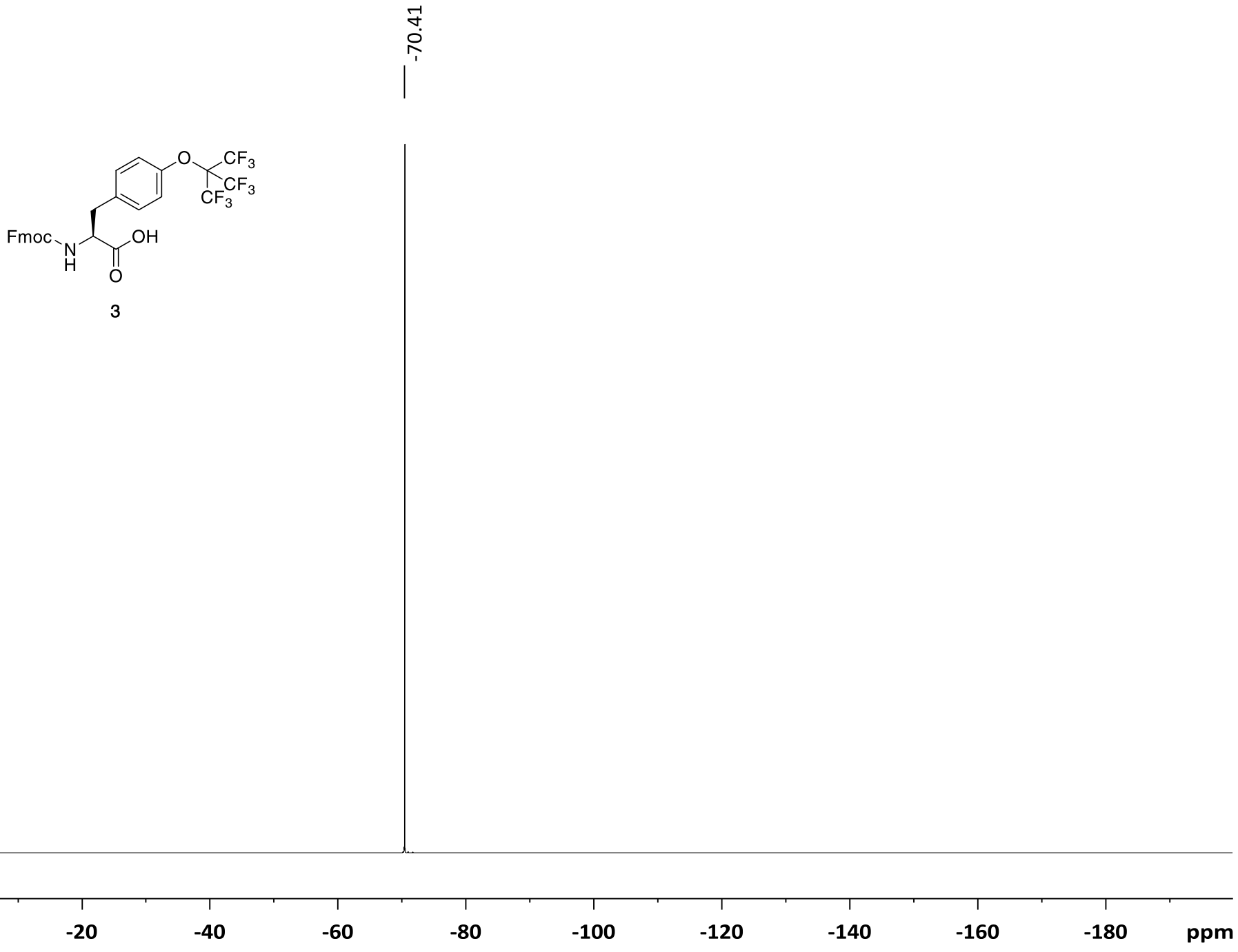
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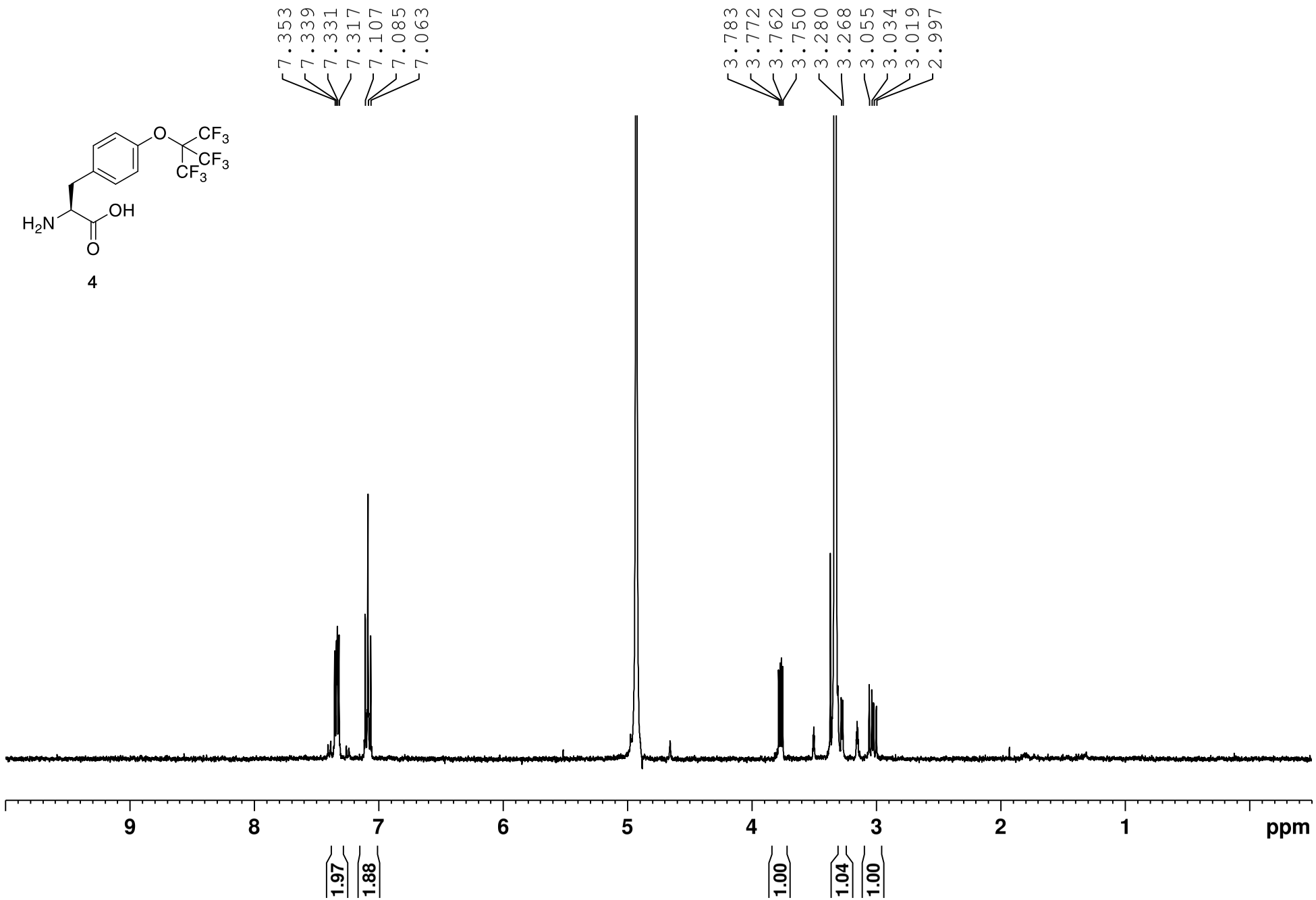
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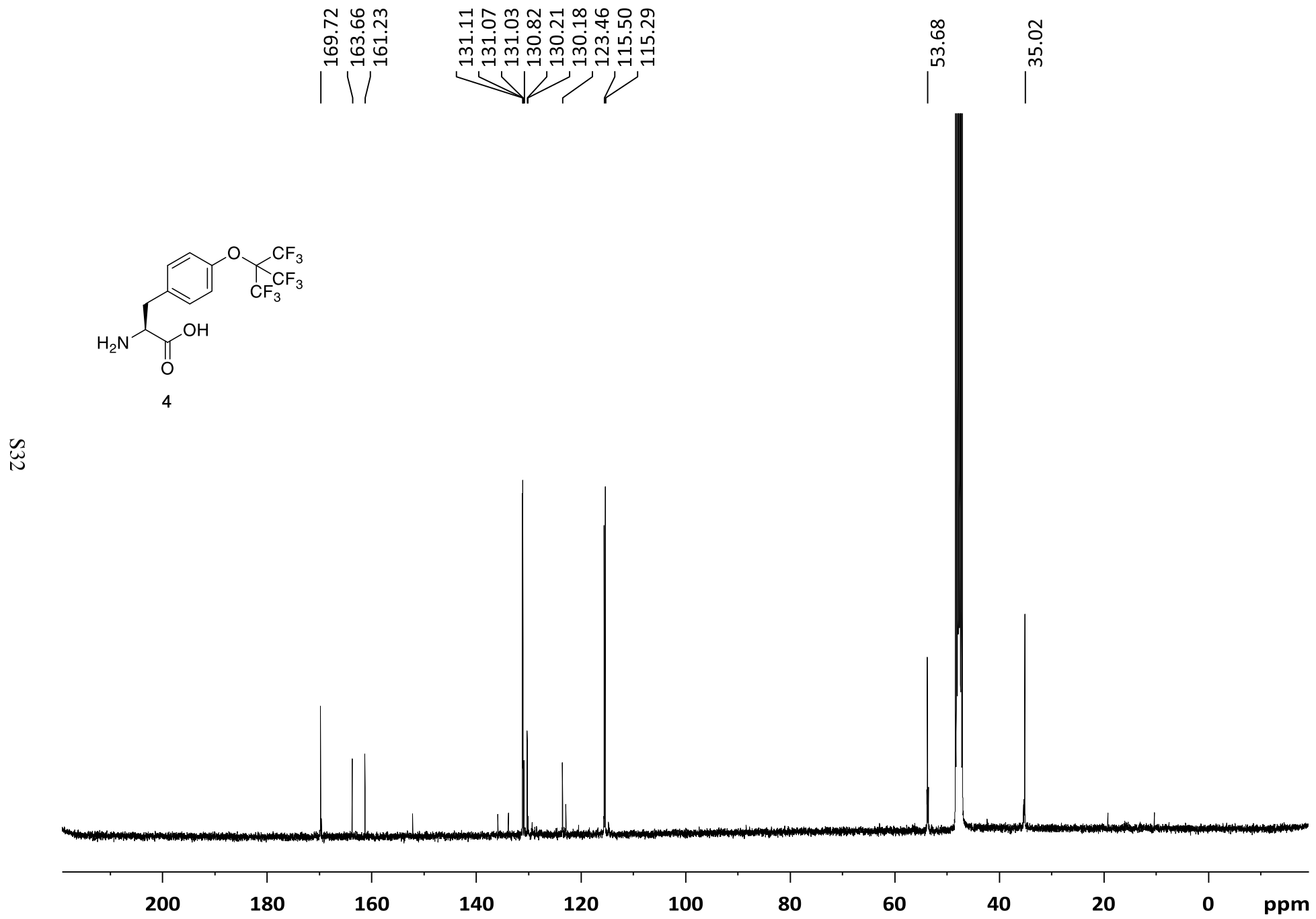
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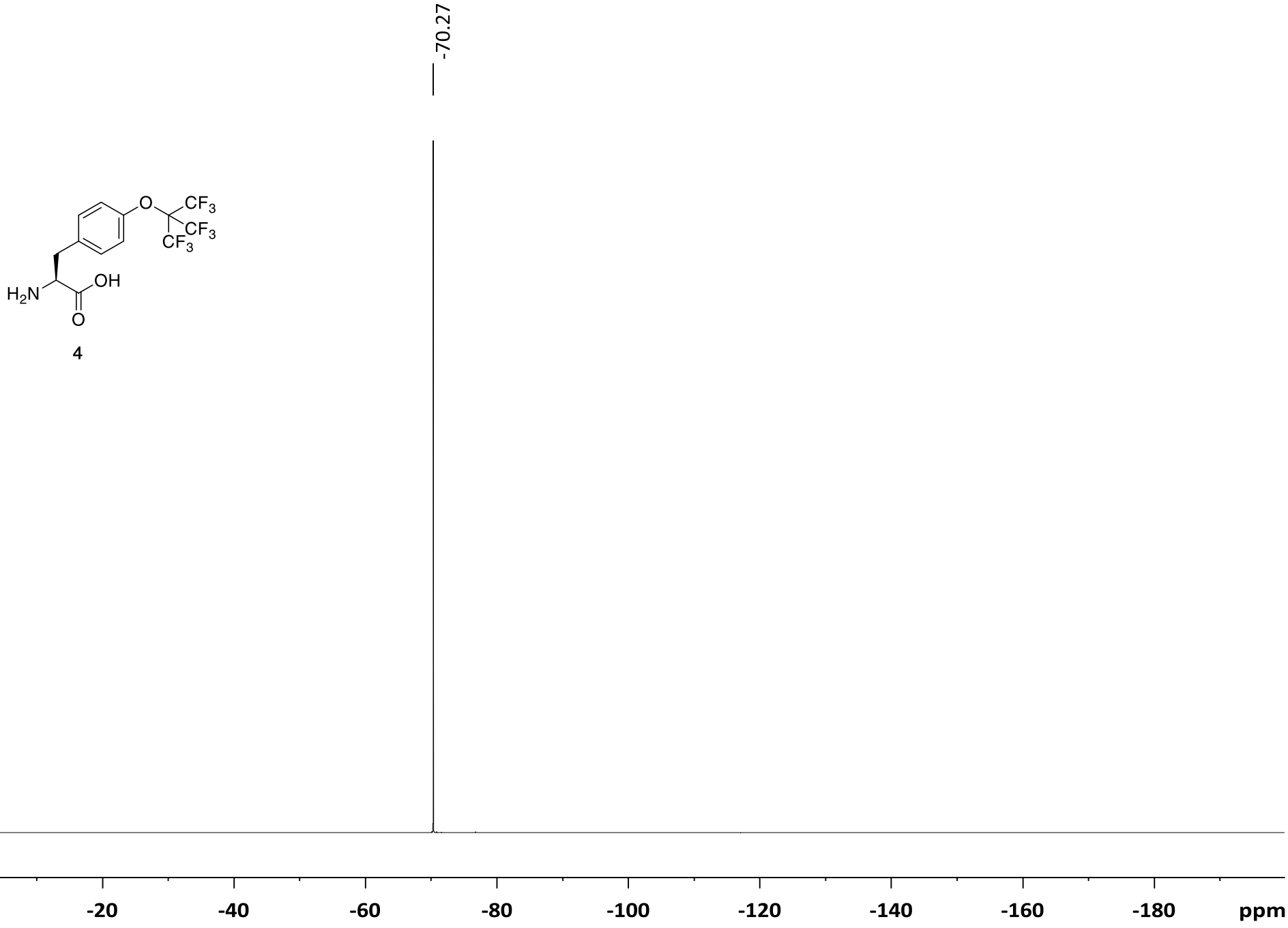

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