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Organic and Biomolecular Chemistry

The secondary structure of a heptapeptide containing trifluoromethyl-λ^6-tetrafluorosulfanyl substituted amino acids

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Abstract: Site specific introduction of the polar hydrophobic trifluoromethyl-λ^6-tetrafluorosulfanyl (CF_3SF_4) group can effectively control the secondary structure of a heptapeptide, the minimum repeat unit of an α-helix. The structural influence of CF_3SF_4-containing amino acid on the heptapeptide was established using NMR methods.

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1. General information

Unless otherwise noted, all chemical reagents were purchased from commercial suppliers and used without further purification. All solvents were freshly distilled with standard methods. Chloroform-d (D, 99.8%) + (0.05% v/v TMS) was purchased from Cambridge Isotope Laboratories, Inc. Reactions were followed by analytical thin later chromatography (TLC), performed with silica get F254 as the adsorbent on 0.2 mm thick, plastic-backed plates. The chromatograms were visualized by fluorescence quenching with UV light (254 nm) or by staining with either potassium permanganate followed by heating. Flash column chromatography was performed on Silica gel 60, 70-230 mesh (Solvent Technologies, LLC.). Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on a 400 Ultrashield spectrometer (Bruker, Billerica, MA, USA) operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C, 376 MHz for ^{19}F, and or a Bruker 600 Advance III HD with QCI Cryoprobe operating at 600 MHz for ^{1}H. The chemical shifts (δ) of the signals in ^{1}H and ^{13}C NMR were reported in parts per million (ppm) relative to residual standard $CHCl_3$ in $CDCl_3$ (7.26 ppm and 77.0 ppm, respectively). The chemical shifts of the signals in ^{19}F NMR were given in ppm relative to $CFCl_3$ (0.00 ppm) as an internal standard. All ^{13}C NMR spectra were acquired in the proton-decoupled mode. The values of coupling constants (*J*) are quoted in Hertz (Hz). The multiplicities were assigned as a s (singlet), d (doublet), t (triplet), q (quartet), p (pentet) and m (multiplet). A DART-AccuTOF (JEOL USA, Inc., Peabody, MA, USA) time-of-flight mass spectrometer operating in positive and negative ion mode was performed with a polyethylene glycol spectrum as reference standard for exact mass measurements (PEG average molecular weight: 600). Melting points (mp) were obtained using MET-TEMP (Laboratory Devices, Auburn, CA, USA).

Infrared spectra (IR) were recorded on a UATR 2 FTIR (Perkin Elmer, Boston, MA, USA).

2. Experimental details

2-1. Synthesis of the CF_3SF_4-substituted amino acid, *Boc*-NLe(Tts)-OH (**2**)

Ethyl (*RS*)-*N*-(*tert*-butoxycarbonyl)-2-aminopent-4-enoate (**A**)

According to the reference,[1] to a mixture of *N,N*-diisopropylamine (3.7 mL, 25.3 mmol, 1.05 eq.) in THF (50.0 mL) was added *n*-BuLi (2.5 M solution in hexane) (11.0 mL, 26.3 mmol, 1.05 eq.) slowly at 0 °C. After stirring for 30 minutes, ethyl 2-(diphenylmethylamino)acetate (6.6 g, 25.0 mmol, 1.0 eq.) was added to the reaction mixture at –78 °C. The reaction mixture was stirred for two hours at the same temperature, and then allyl bromide (2.3 mL, 26.3 mmol, 1.05 eq.) was added to the mixture. After stirring for an additional hour, the reaction mixture was stirred at 0 °C for overnight. When the reaction was completed, the mixture was quenched with water (80 mL) and extracted with ethyl acetate (AcOEt) (3 x 20.0 mL). The organic phase was dried over magnesium sulfate ($MgSO_4$), and filtered and concentrated *in vacuo*. The product (A) was obtained as a yellow oil in 100 % crude yield (7.7 g, 25.0 mmol). To a mixture of A (7.7g, 25.0 mmol, 1.0 eq.) in THF (150.0 mL) was added 15 % solution of citric acid (150.0 mL, 112.5 mmol, 4.5 eq.) at room temperature. After 11 hours, the reaction mixture was treated with acid-base work up with 10 % HCl, saturated $NaHCO_3$ solution and dichloromethane (DCM). The combined organic phase was dried over $MgSO_4$, and filtered and concentrated *in vacuo*. The product (B) was obtained as a yellow oil in 73 % crude yield (2.6 g, 18.3 mmol). To a mixture of B (2.6 g, 18.3 mmol) in DCM (36 mL) was added Et_3N (7.7 mL, 54.9 mmol, 3.0 eq.) and di-*tert*-butyl dicarbonate (6.3 mL, 27.5 mmol, 1.5 eq.) at room temperature. After

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stirring for overnight, the reaction mixture was quenched with water (25.0 mL) and then extracted with DCM (3 x 15.0 mL). The crude product was purified by flash column chromatography (AcOEt:hexane = 5:95) to afford the title product (C) as a colorless oil in 89 % yield (4.0 g, 16.3 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.75–5.65 (m, 1H, Hγ), 5.15–5.11 (m, 2H, Hδ), 5.04 (d, 2H, J = 6.75 Hz, NH), 4.38–4.33 (m, 1H, Hα), 4.26–4.14 (m, 2H, OCH_2), 2.59–2.44 (m, 2H, Hβ), 1.44 (s, 9H, *Boc*), 1.28 (t, 3H, J = 7.24 Hz, OCH_2CH_3), which was similar to the literature data.[1,2,3]

(*S*)-*N*-(*tert*-butylcarbonyl)-2-aminopent-4-enoic acid (**C**)

According to the reference,[2] to a solution of (*RS*)-**A** (730.0 mg, 3.0 mmol, 1.0 eq.) in DMF (3.0 mL) and distilled water (8.0 mL) was added subtilisin (10 units/mg) (3.5 mg, 35units, 11.7 eq.), and then few drops of aqueous NH_3.to maintain the pH of the reaction mixture at 8.0. After stirring at 40 °C for three hours, the mixture was extracted with diethyl ether (Et_2O) (3 x 5.0 mL). And the aqueous phase was acidified with citric acid (pH 3.0), then extracted with AcOEt (3 x 5.0 mL). The combined organic phase was dried over $MgSO_4$, and filtered and concentrated *in vacuo*. The title product (D) was obtained as a colorless oil in 100 % yield (322.0 mg, 3.0 mmol). The purity of the product was sufficient to proceed to the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.75–5.64 (m, 1H, Hγ), 5.15–5.11 (m, 2H, Hδ), 5.04 (d, 1H, J = 5.38 Hz, NH), 4.38–4.33 (m, 1H, Hα), 2.59–2.44 (m, 2H, Hβ), 1.44 (s, 9H, *Boc*), which was similar to the literature data.[2,4]

Methyl (*S*)-*N*-(*tert*-butoxycarbonyl)-2-aminopent-4-enoate (**3**)

According to the reference,[2] to a solution of **D** (172.0 mg, 0.8 mmol. 1.0 eq.) in DMF (1.6 mL) was added potassium carbonate (166.0 mg, 1.2 mmol, 1.5 eq.) at room temperature. After stirring for 10 minutes, methyl iodide (0.1 mL, 1.6 mmol, 2.0 eq.) at the same temperature. When the reaction was completed, the mixture was quenched with water (5.0 mL) and then extracted with AcOEt (3 x 5.0 mL). The combined organic phase was dried over $MgSO_4$, and filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (AcOEt:hexane = 10:90) to afford the title product (E) as a colorless oil in 93% yield (170.0 mg, 0.74 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.71–5.67 (m, 1H, Hγ), 5.16–5.11 (m, 2H, Hδ), 5.02 (br s, 1H, NH), 4.42–4.35 (m, 1H, Hα), 3.75 (s, 3H, OCH_3), 2.60–2.42 (m, 2H, Hβ), 1.45 (s, 9H, *Boc*); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.55 (ester C=O), 155.19 (amide C=O), 132.30 (Cδ), 119.10 (Cγ), 79.92 (*Boc*-C), 52.92 (Cα), 52.23 (OCH_3), 36.81 (Cβ), 28.30 (3C, C1, *Boc*); HRMS (DART-ESI, m/z) Calcd. For $C_{11}H_{20}NO_4$+: 230.1387(M+), Found: 230.1377, which was similar to the literature data.[5]

Methyl (2*S*,4*RS*)-*N*-(*tert*-butoxycarbonyl)-2-amino-4-chloro-6-trifluoro-λ^6-tetrafluorosulfanylpentanoate (**4**)

According to the reference,[2] to a mixture of **3** (23.0 mg, 0.1 mmol, 1.0 eq.) in pentane (1.6 mL) was added CF_3SF_4Cl (0.32 M solution in pentane) (0.38 mL, 0.12 mmol, 1.2 eq.) at 0 °C, and then was added triethylborane (1.0 M solution in hexane) (0.01 mL, 0.01 mmol, 0.1 eq.) slowly. After stirring for one hour, the reaction mixture was quenched with saturated $NaHCO_3$ solution and extracted with Et_2O (3 x 5.0 mL). The combined organic phase was dried over $MgSO_4$, and filtered and concentrated *in vacuo*. The crude

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product was purified by flash column chromatography (AcOEt:hexane = 5:95) to afford the title product (F) as a colorless oil in 91 % yield (40.0 mg, 0.09 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.43 (d, 1H, J = 7.47 Hz, NH), 4.60–4.50 (m, 2H, Hα and γ), 4.22–4.01 (m, 2H, Hδ), 3.76 (s, 3H, OCH_3), 2.61 (ddd, 1H, J = 14.53, 7.08, 2.87 Hz, Hβ), 2.23 (ddd, 1H, J = 14.53, 9.94, 4.53 Hz, Hβ), 1.42 (a, 9H, *Boc*); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.73 (ester C=O), 154.96 (amide C=O), 123.14 (qp, J = 328.19, 49.50 Hz, CF_3), 80.34 (*Boc*-C), 77.97 (p, J = 15.77 Hz, Cδ), 52.57 (C6, OCH3), 51.74 (p, J = 4.87 Hz, Cγ), 50.84 (Cα), 39.79 (Cβ), 28.05 (3C, *Boc*); ^{19}F NMR ($CDCl_3$, 376 MHz) δ: 43.03 (pt, J = 25.06, 8.18 Hz, SF4), –64.28 (p, J = 25.09 Hz, CF_3); HRMS (DART-ESI, m/z) Calcd. For $C_{12}H_{20}ClF_7NO_4S$+: 442.0684(M+), Found: 442.0709 for methyl (2*S*,4*S*)-*N*-(*tert*-butoxycarbonyl)-2-amino-4-chloro-6-trifluoro-λ^6-tetrafluorosulfanylpentanoate. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.24 (d, 1H, J = 8.09Hz, NH), 4.55–4.49 (m, 2H, Hα and γ), 4.24–4.10 (m, 1H, Hδ), 4.10–3.96 (m, 1H, Hδ), 3.72 (s, 3H, OCH_3), 2.34 (ddd, 1H, J = 16.87, 10.58, 2.22 Hz, Hβ), 2.23–2.17 (m, 1H, Hβ), 1.40 (s, 9H, *Boc*); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.06 (ester C=O), 155.57 (amide C=O), 123.14 (qp, J = 328.37, 49.04 Hz, CF_3), 80.37 (*Boc*-C), 78.07 (p, J = 15.69 Hz, Cδ), 52.52 (C6, OCH_3), 52.32 (p, J = 4.95 Hz, Cγ), 51.23 (Cα), 40.09 (Cβ), 28.03 (3C, Boc); ^{19}F NMR ($CDCl_3$, 376 MHz) δ: 43.07 (qt, J = 25.01, 8.42 Hz, 4F, SF_4), –64.25 (p, J = 25.21 Hz, 3F, CF_3); HRMS (DART-ESI, m/z) Calcd. For $C_{12}H_{20}ClF_7NO_4S$+: 442.0684 (M+), Found: 442.0701 for methyl (2*S*,4*R*)-*N*-(*tert*-butoxycarbonyl)-2-amino-4-chloro-6-trifluoro-λ^6-tetrafluorosulfanylpentanoate.

(*S*,*E*)-*N*-(*tert*-butoxycarbonyl)-2-amino-6-trifluoromethyl-λ^6-tetrafluorosulfanylpent-4-enoic acid (**2**)

To a mixture of **4** (22.0 mg, 0.05 mmol, 1.0 eq.) in ethanol (0.25 mL) and water (0.25 mL) was added lithium hydroxide monohydrate (6.0 mg, 0.15 mmol, 3.0 eq.) at room temperature. After stirring for overnight, the reaction mixture was quenched with 10% HCl solution (3.0 mL) and extracted with Et_2O (3 x 5.0 mL). The combined organic phase was dried over $MgSO_4$, and filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (AcOEt:hexane = 10:90) to afford the title product (G) as a colorless oil in 76 % yield (15.0 mg, 0.038 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.64–6.54 (m, 1H, Hγ), 6.54 (br s, 1H, OH), 6.54–6.42 (m, 1H, Hδ), 5.25–5.01 (br s, 1H, NH), 4.61–4.42 (m, 1H, Hα), 2.85–2.51 (m, 2H, Hβ), 1.46 (s, 9H, *Boc*); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.90 (acid C=O), 155.25 (amide C=O), 144.23 (p, J = 21.69 Hz, Cδ), 132.73 (p, J = 7.36 Hz, CF_3), 123.38 (qp, J = 327.19, 48.09 Hz, Cγ), 80.91 (*Boc*-C), 52.13 (Cα), 33.52 (Cβ), 28.15 (3C, *Boc*); ^{19}F NMR ($CDCl_3$, 376 MHz) δ: 39.95 (qd, J = 24.97, 5.45 Hz, 4F, SF_4), –64.13 (p, J = 24.52 Hz, 3F, CF_3); HRMS (DART-ESI, m/z) Calcd. For $C_{11}H_{17}F_7NO_4S$+: 392.0761 (M+), Found: 392.0760.

2-2. Synthesis of the CF_3SF_4-substituted heptapeptide

2-2-1. General procedure

General procedure for the coupling reaction[1]

To a reaction mixture of an amino acid A (0.5 mmol, 1.0 eq.) in THF (5.0 mL) was added another amino acid B (0.5 mmol, 1.0 eq.), 1-hydroxybenzotriazole (92.0 mg, 0.6 mmol, 1.2 eq.), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (115.0 mg, 0.6 mmol, 1.2 eq.) and *N*-methylmorpholine (0.093 mL, 0.85 mmol, 1.7 eq.) at room temperature. After monitoring with TLC until the reaction was completed, the reaction mixture was quenched with water (10.0 mL) and extracted with

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DCM (3 x 10.0 mL). The combined organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography to obtain the coupling peptide.

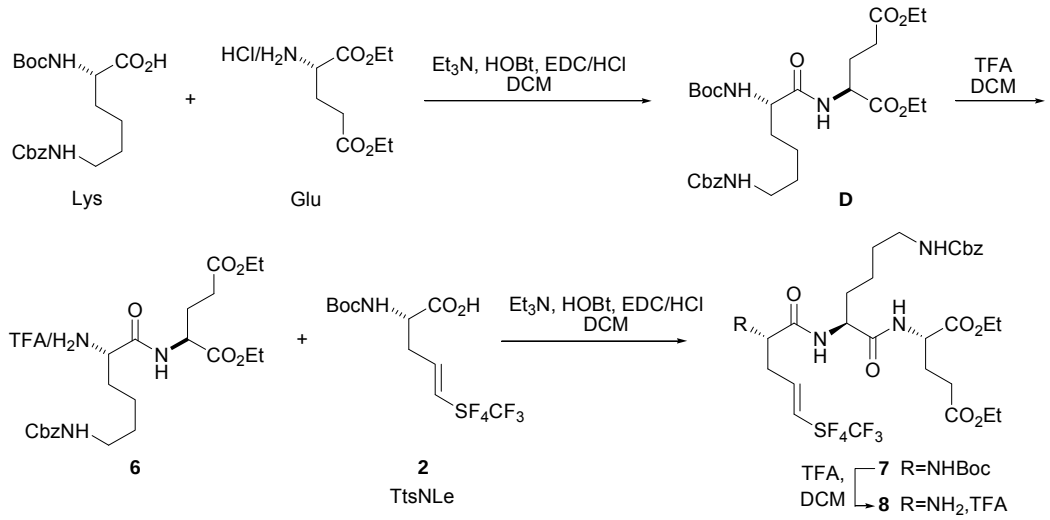
General procedure for the deprotection reaction of *Boc* group[1]

To a mixture of an amino acid (0.5 mmol, 1.0 eq.) in DCM (3.0 mL) was added trifluoroacetic acid (0.2 mL, 2.5 mmol, 5.0 eq.) at 0 °C. The reaction mixture was allowed to increase to room temperature. After the reaction was completed with monitoring with TLC, the mixture was concentrated *in vacuo*. The purity of the product was enough sufficient to proceed to the next step without further purification.

General procedure for the deprotection reaction of *Fmoc* group

To a mixture of an amino acid (0.5 mmol, 1.0 eq.) in DCM (1.5 mL) was added piperidine (0.3 mL, 3.0 mmol, 6.0 eq.) at 0 °C. After stirring for 10 minutes, the reaction mixture was concentrated *in vacuo*. The crude product was purified by flash column chromatography to afford the deprotected peptide.

2-2-2. Product data



Boc-Lys(*Z*)-Glu(OEt)-OEt (**D**)

According to the method showed before, the title product was obtained as a white solid in 98 % yield (277.0 mg, 0.49 mmol) with flash column chromatography (AcOEt:hexane = 20:80). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.33–7.30 (m, 5H, *Cbz* of Lys), 7.30–8.28 (m, 1H, amide NH of Lys), 6.95–6.83 (m, 1H, amide NH of Glu), 5.31–5.20 (m, 1H, Boc NH of Lys), 5.20–5.10 (m, 1H, *Cbz*-NH of Lys), 5.08–5.06 (m, 2H, Cbz-CH_2 of Lys), 4.58–4.52 (m, 1H, Hα of Glu), 4.16–4.07 (m, 5H, Hα of Lys and OCH_2 of Glu), 3.18–3.17 (m, 2H, Hε of Lys), 2.48–2.29 (m, 2H, Hγ of Glu), 2.25–1.91 (m, 2H, Hβ of Glu), 1.85–1.57 (m, 2H, Hδ of Lys), 1.54–1.47 (m, 2H, Hγ of Lys), 1.42 (s, 9H, *Boc* of Lys), 1.40–1.35 (m, 2H, Hβ of Lys), 1.22 (t, 6H, OCH_2CH_3),); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 172.87 (ester C=O of Glu), 172.48 (ester C=O of Glu), 171.63 (amide C=O of NLe), 156.78 (*Cbz* C=O of Lys), 155.81 (*Boc* C=O of NLe), 136.74 (*Cbz* of Lys), 128.50 (2C, *Cbz* of Lys), 128.09 (*Cbz* of Lys), 128.06 (2C, *Cbz* of Lys), 79.99 (*Boc*-C of Lys), 66.61 (*Cbz*-CH_2 of Lys), 61.66 (OCH_2 of Glu), 60.73 (OCH_2 of Lys), 54.32 (Cα of Glu), 51.80 (Cα of Lys), 40.44 (Cε of Lys), 31.98 (Cδ of

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Lys), 30.32 (Cγ of Glu), 29.33 (Cγ of Lys), 28.34 (3C, *Boc* of Lys), 27.10 (Cβ of Glu), 22.38 (Cβ of Lys), 14.18 (OCH_2CH_3 of Glu), 14.12 (OCH_2CH_3 of Glu), which was similar to the literature data.1

TFA. Lys(*Z*)-Glu(OEt)-OEt (**6**)

According to the method showed before, the title product was obtained as a yellow oil in 100 % yield (290.0 mg, 0.5mmol). The purity of the product was sufficient for the next step.

Boc-NLe(Tts)-Lys(*Z*)-Glu(OEt)-OEt (**7**)

According to the method showed before, the title product was obtained as a white solid in 76 % yield (319.0 mg, 0.38 mmol) with flash column chromatography (MeOH:DCM = 2:98). Mp.: 113–114 °C.: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.38–7.35 (m, 5H, *Cbz* of Lys), 7.17–7.06 (m, 2H, amide NH of Lys and Glu), 6.61–6.55 (m, 1H, Hγ of NLe), 6.52–6.46 (m, 1H, Hδ of NLe), 5.47 (d, J = 18.55 Hz, 1H, *Boc*-NH of NLe), 5.33–5.26 (m, 1H, *Cbz*-NH of Lys), 5.11 (s, 2H, *Cbz*-CH_2 of Lys), 4.54 (td, J = 12.44, 3.76 Hz, 1H, Hα of Glu), 4.51–4.45 (m, 1H, Hα of Lys), 4.44–4.38 (m, 1H, Hα of NLe), 4.17 (q, J = 7.08 Hz, 2H, OCH_2 of Glu), 4.15 (q, J = 7.08 Hz, 2H, OCH_2 of Glu), 3.23–3.17 (m, 2H, Hε of Lys), 2.75–2.46 (m, 2H, Hβ of NLe), 2.45–2.36 (m, 2H, Hγ of Glu), 2.11 (dddd, J = 124.50, 26.20, 13.40, 6.17 Hz, 2H, Hβ of Glu), 1.91–1.68 (m, 2H, Hδ of Lys), 1.57–1.50 (m, 2H, Hγ of Lys), 1.45 (s, 9H, *Boc* of NLe), 1.42–1.37 (m, 2H, Hβ of Lys), 1.28–1.23 (m, 6H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.10 (ester C=O of Glu), 171.52 (ester C=O of Glu), 171.13 (amide C=O of Lys), 170.48 (amide C=O of NLe), 156.76 (*Cbz* C=O of Lys), 155.40 (*Boc* C=O of NLe), 144.02 (p, J = 22.00 Hz, Cδ of NLe), 136.53 (*Cbz* of Lys), 133.49 (p, J = 6.68 Hz, Cγ of NLe), 128.48 (2C, *Cbz* of Lys), 128.07 (*Cbz* of Lys), 128.06 (2C, *Cbz* of Lys), 123.39 (pq, J = 328.47, 50.67 Hz, CF_3 of NLe), 80.62 (*Boc*-C of NLe), 66.69 (*Cbz*-CH_2 of Lys), 61.73 (OCH_2 of Glu), 60.88 (OCH_2 of Glu), 52.93 (Cα of Lys), 52.02 (Cα of Glu), 51.93 (Cα of NLe), 40.05 (Cε of Lys), 33.51 (Cβ of NLe), 31.73 (Cδ of Lys), 30.31 (Cγ of Glu), 29.12 (Cγ of Lys), 28.18 (3C, *Boc* of NLe), 26.66 (Cβ of Glu), 21.75 (Cβ of Lys), 14.08 (OCH_2CH_3 of Glu), 14.05 (OCH_2CH3 of Glu); ^{19}F NMR ($CDCl_3$, 376 MHz) δ: 40.00 (qd, J = 24.57, 5.65 Hz, 4F, SF_4), –64.13 (p, J = 24.05 Hz, 3F, CF_3); HRMS (DART-ESI, m/z) Calcd. For $C_{34}H_{50}F_7N_4O_{10}S$+: 839.3130(M+), Found: 839.3145.

NLe(Tts)-Lys(*Z*)-Glu(OEt)-OEt TFA (**8**)

According to the method shown before, the title product was obtained quantitatively as a yellow oil (361.0 mg, 0.5 mmol). The product was sufficient for the next step without further purification.

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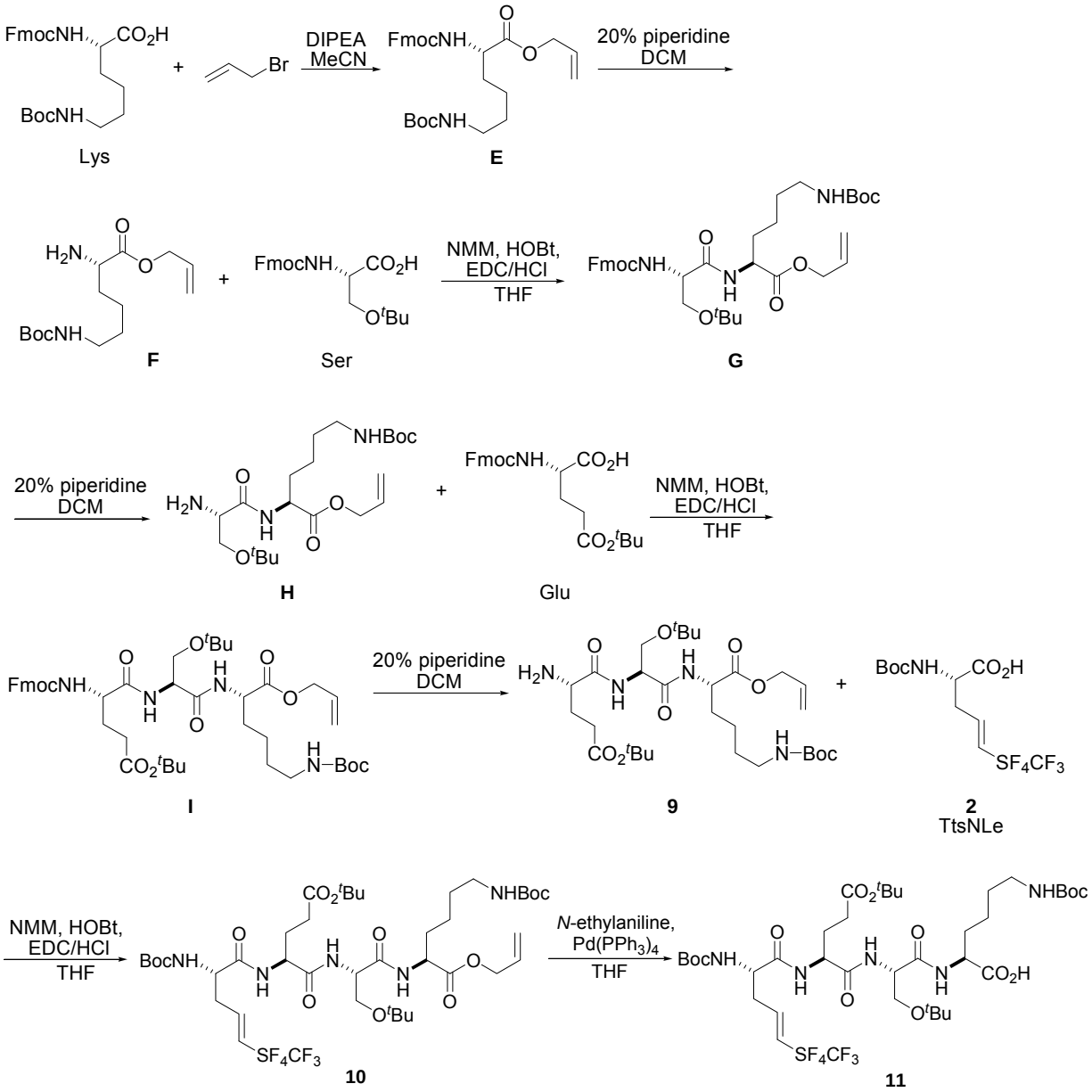

Fmoc-Lys(*Boc*)-OAll (**E**)

To a mixture of *Fmoc*-Lys(Boc)-OH (2.3g, 5.0 mmol, 1.0 eq.) in acetonitrile (10.0 mL) was added allyl bromide (12.0 mL, 140 mmol, 28.0 eq.) and diisopropylethylamine (1.8 mL, 10.0 mmol, 2.0 eq.) at 40 °C. After stirring for 4 hours, the reaction mixture was quenched with 10 % HCl solution (20.0 mL) and extracted with DCM (3 x 15.0 mL). The combined organic phase was dried over $MgSO_4$, and filtered and evaporated *in vacuo*. The title product was obtained as a white solid in 97 % yield (247.0 mg, 0.49 mmol) with flash column chromatography (AcOEt:hexane = 10:90). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.77 (d, J = 7.57 Hz, 2H, *Fmoc*), 7.61 (d, J = 7.19 Hz, 2H, *Fmoc*), 7.41 (dd, J = 7.43, 7.43 Hz, *Fmoc*), 7.32 (dd, J = 7.43,

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7.43 Hz, 2H, *Fmoc*), 5.97–5.87 (m, 1H, OCH_2CH), 5.42–5.41 (m, 1H, *Boc*-NH), 5.36–5.26 (m, 2H, OCH_2CHCH_2), 4.66 (d, J = 5.17 Hz, 2H, *Fmoc*-CH_2), 4.60–4.56 (m, 1H, Hα), 4.45–4.36 (m, 2H, OCH_2), 3.16–3.07 (m, 2H, Hε), 1.93–1.65 (m, 2H, Hδ), 1.55–1.48 (m, 2H, Hγ), 1.44 (s, 9H, *Boc*), 1.41–1.35 (m, 2H, Hβ), which was similar to the literature data.[12]

Lys(*Boc*)-OAll (**F**)

According to the method showed before, the title product was obtained as a yellow oil in 98 % yield (140.0 mg, 0.49 mmol) with flash column chromatography (MeOH:DCM = 1:99). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.97–5.88 (m, 1H, OCH_2CH), 5.36–5.25 (m, 2H, OCH_2CHCH_2), 4.62 (d, J = 6.14 Hz, OCH_2), 4.59–4.52 (m, 1H, Hα), 3.13–3.10 (m, 2H, Hε), 1.81–1.57 (m, 2H, Hδ), 1.55–1.47 (m, 4H, Hγ and Hβ), 1.44 (s, 9H, *Boc*), which was similar to the literature data.[1]

Fmoc-Ser(*tBu*)-Lys(*Boc*)-OAll (**G**)

According to the method showed before, the title product was obtained as a white solid in 99 % yield (323.0 mg, 0.5 mmol) with flash column chromatography (AcOEt:hexane = 10:90). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.77 (d, J = 7.76 Hz, 2H, *Fmoc* of Ser), 7.61 (d, J = 6.68 Hz, 2H, Fmoc- of Ser), 7.40 (dd, J = 7.22, 7.22 Hz, 2H. *Fmoc* of Ser), 7.41–4.39 (m, 1H, amide NH of Lys), 7.32 (dd, J = 7.22, 7.22 Hz, 2H, *Fmoc* of Ser). 5.97–5.84 (m, 1H, OCH_2CH of Lys), 5.83–5.73 (m, 1H, *Boc*-NH of Lys), 5.36–5.25 (m, 2H, OCH_2CHCH_2 of Lys), 4.64 (d, J = 5.14 Hz, *Fmoc*-CH_2 of Ser), 4.63–4.59 (m, 1H, Hα of Lys), 4.59–4.53 (m, 1H, Hα of Ser), 4.40 (d, J = 7.03 Hz, OCH_2 of Lys), 3.85–3.38 (m, 2H, Hβ of Ser), 3.15–3.04 (m, 2H, Hε of Lys), 1.95–1.68 (m, 2H, Hδ of Lys), 1.55–1.47 (m, 2H, Hγ of Lys), 1.44 (s, 9H, *Boc* of Lys), 1.40–1.30 (m, 2H, Hβ of Lys), 1.24 (s, 9H, *Boc* of Ser), which was similar to the literature data.[1]

Ser(*tBu*)-Lys(*Boc*)-OAll (**H**)

According to the method showed before, the title product was obtained as a yellow oil in 85 % yield (183.0 mg, 0.43 mmol) with flash column chromatography (MeOH:DCM = 1:99). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.92 (d, J = 8.06 Hz, 1H, amide NH of Lys), 5.96–5.86 (m, 1H, OCH_2CH of Lys), 5.36–5.24 (m, 2H, OCH_2CHCH_2 of Lys), 4.63 (d, J = 6.23 Hz, OCH_2 of Lys), 4.62–4.58 (m, 1H, Hα of Lys), 4.58–4.53 (m, 1H, Hα of Ser), 3.59–3.50 (m, 2H, Hβ of Ser), 3.15–3.03 (m, 2H, Hε of Lys), 1.92–1.65 (m, 2H, Hδ of Lys), 1.54–1.47 (m, 2H, Hγ of Lys), 1.43 (s, 9H, *Boc* of Lys), 1.39–1.31 (m, 2H, Hβ of Lys), 1.19 (s, 9H, *Boc* of Ser), which was similar to the literature data.[1]

Fmoc-Glu(O*tBu*)-Ser(*tBu*)-Lys(*Boc*)-OAll (**I**)

According to the method showed before, the title compound was obtained as a white solid in 83 % yield (347.0 mg, 0.42 mmol) with flash column chromatography (MeOH:DCM = 1:99). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.76 (d, J = 7.17 Hz, Fmoc of Glu), 7.60 (d, J = 7.17 Hz, *Fmoc* of Glu), 7.40 (dd, J = 7.17, 7.17 Hz, *Fmoc* of Glu), 7.37–7.33 (m, 1H, amide NH of Lys), 7.31 (dd, J = 7.17, 7.17 Hz, *Fmoc* of Glu), 7.09 (d, J =

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5.53 Hz, amide NH of Ser), 6.04–5.84 (m, 2H, *Boc*-NH of Lys and OCH_2CH of Lys), 5.35–5.23 (m, 2H, OCH_2CHCH_2), 4.62 (d, J = 5.18 Hz, *Fmoc*-CH_2 of Lys), 4.60–4.56 (m, 1H, Hα of Lys), 4.51–4.44 (m, 1H, Hα of Ser), 4.43–4.42 (m, 2H, OCH_2 of Lys), 4.29–4.24 (m, 1H, Hα of Glu), 3.86–3.37 (m, 2H, Hβ of Ser), 3.10–3.01 (m, 2H, Hε of Lys), 2.52–2.32 (m, 2H, Hγ of Glu), 2.21–1.93 (m, 2H, Hβ of Glu), 1.91–1.64 (m, 2H, Hδ of Lys), 1.47 (s, 9H, *Boc* of Glu), 1.44 (s, 9H, Boc of Lys), 1.40–1.25 (m, 4H, Hγ and Hβ of Lys), 1.19 (s, 9H, *Boc* of Ser); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.16 (ester C=O of Glu), 171.61 (ester C=O of Lys), 171.30 (amide C=O of Ser), 169.98 (amide C=O of Glu), 156.06 (*Boc* C=O og Lys), 143.90 (2C, *Fmoc* of Glu), 141.37 (2C, *Fmoc* of Glu), 131.72 (OCH_2CH of Lys), 127.82 (2C, *Fmoc* of Glu), 127.18 (2C, *Fmoc* of Glu), 125.22 (2C, *Fmoc* of Glu), 120.07 (2C, *Fmoc* of Glu), 118.94 (OCH_2CHCH_2 of Lys), 81.24 (*Boc*-C of Lys), 79.27 (*Boc*-C of Glu), 74.26 (*tBu*-C of Ser), 67.35 (*Fmoc*-CH2 of Glu), 65.91 (OCH_2 of Lys), 61.22 (Cβ of Ser), 54.91 (Cα of Ser), 53.26 (Cα of Glu), 52.30 (Cα of Lys), 47.21 (Cε of Lys), 32.06 (Cδ of Lys), 31.94 (Cγ of Glu), 31.78 (Cγ of Lys), 29.55 (Cβ of Glu), 28.51 (3C, Boc of Lys), 28.16 (3C, *Boc* of Glu), 27.44 (3C, *tBu* of Ser), 22.50 (Cβ of Lys), which was similar to the literature data.[1]

Glu(O*tBu*)-Ser(*tBu*)-Lys(*Boc*)-OAll (**9**)

According to the method showed before, the title compound was obtained as a yellow oil in 90 % yield (277.0 mg, 0.45 mmol) with flash column chromatography (MeOH:DCM = 1:99). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.86–7.80 (m, 1H, amide NH of Lys), 7.32 (d, J = 6.89 Hz, amide NH of Ser), 5.89–5.76 (m, 1H, OCH_2CH of Lys), 5.28–5.17 (m, 2H, OCH_2CHCH_2 of Lys), 4.77–4.71 (m, 1H, Hα of Glu), 4.55 (d, J = 5.02 Hz, OCH_2 of Lys), 4.54–4.46 (m, 1H, Hα of Lys), 4.44–4.33 (m, 1H, Hα of Ser), 3.74–3.30 (m, 2H, Hβ of Ser), 3.06–2.94 (m, 2H, Hε of Lys), 2.41–1.97 (m, 2H, Hβ of Glu), 2.32–2.25 (m, 2H, Hγ of Glu), 1.85–1.60 (m, 2Hm Hδ of Lys), 1.46–1.40 (m, 2H, Hγ of Lys), 1.36 (s, 18H, *Boc* of Lys and Glu), 1.31–1.26 (m, 2H, Hβ of Lys), 1.15 (s, 9H, *Boc* of Ser); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 174.80 (ester C=O of Glu), 172.68 (ester C=O of Lys), 171.66 (amide C=O of Ser), 170.34 (amide C-O of Glu), 156.00 (*Boc* C=O of Lys), 131.61 (OCH_2CH of Lys), 118.91 (OCH_2CHCH_2 of Lys), 80.49 (*Boc*-C of Lys), 78.95 (*Boc*-C of Glu), 74.13 (*tBu*-C of Ser), 65.83 (OCH_2 of Lys), 61.38 (Cβ of Ser), 54.73 (Cα of Ser), 52.77 (Cα of Glu), 52.20 (Cα of Lys), 40.23 (Cε of Lys), 32.06 (Cδ of Lys), 31.92 (Cγ of Glu), 30.32 (Cγ of Lys), 29.52 (Cβ of Glu), 28.44 (3C, *Boc* of Lys), 28.09 (3C, *Boc* of Glu), 27.40 (3C, *Boc* of Ser), 22.43 (Cβ of Lys), which was similar to the literature data.[1]

Boc-NLe(Tts)-Glu(O*tBu*)-Ser(*tBu*)-Lys(*Boc*)-OAll (**10**)

According to the method showed before, the title product was obtained as a white solid in 93 % yield (459.0 mg, 0.47 mmol) with flash column chromatography (MeOH:DCM = 2:98). Mp.: 164–166 °C.: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.42–7.28 (m, 2H, amide NH of Glu and Lys), 7.06 (d, J = 5.19 Hz, 1H, amide NH of Ser), 6.60–6.53 (m, 1H, Hγ of NLe), 6.53–6.46 (m, 1H, Hδ of NLe), 5.99 (ddt, J = 17.02, 10.56, 5.60 Hz, 1H, OCH_2CH of Lys), 5.69–5.55 (m, 1H, *Boc*-NH of Lys), 5.33 (dd, J = 17.24, 0.55 Hz, 1H, OCH_2CHCH_2 of Lys), 5.27–5.24 (m, 1H, *Boc*-NH of NLe), 5.26 (dd, J = 10.48, 1.13 Hz, 1H, OCH_2CHCH_2 of Lys), 4.63 (d, J = 4.88 Hz, 2H, OCH_2 of Lys), 4.61–4.57 (m, 1H, Hα of Lys), 4.47–4.37 (m, 2H, Hα of Ser and Glu), 4.37–4.23 (m, 1H, Hα of NLe), 3.83–3.36 (m, 2H, Hβ of Ser), 3.14–3.03 (m, 2H, Hε of Lys), 2.77–2.49 (m, 2H, Hβ of NLe), 2.48–2.35 (m, 2H, Hγ of Glu), 2.17–1.92 (m, 2H, Hβ of Glu), 1.91–1.58 (m, 2H, Hδ of Lys), 1.52–1.48 (m, 2H, Hγ of Lys), 1.45 (s, 9H, *Boc* of Lys), 1.44 (s, 9H, *Boc* of Glu), 1.43 (s, 9H, *Boc* of NLe),

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1.37–1.31 (m, 2H, Hβ of Lys), 1.21 (s, 9H, *Boc* of Ser); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.22 (ester C=O of Glu), 171.56 (ester C=O of Lys), 170.53 (amide C=O of Ser), 170.47 (amide C=O of Glu), 169.85 (amide C=O of NLe), 155.94 (*Boc* C=O of Lys), 155.23 (*Boc* C=O of NLe), 144.08 (p, J = 23.66 Hz, Cδ of NLe), 133.38 (p, J = 3.37 Hz, Cγ of NLe), 131.55 (OCH_2CH of Lys), 123.39 (qp, J = 327.36, 49.70 Hz, CF3 of NLe), 118.87 (OCH_2CHCH_2 of Lys), 81.23 (*Boc*-C of Lys), 80.52 (*Boc*-C of NLe), 79.07 (*Boc*-C of Glu), 74.20 (*tBu*-C of Ser), 65.84 (OCH_2 of Lys), 61.07 (Cβ of Ser), 53.38 (Cα of Ser), 53.17 (Cα of Glu), 53.14 (Cα of NLe), 52.20 (Cα of Lys), 40.26 (Cε of Lys), 33.70 (Cβ of NLe), 31.98 (Cδ of Lys), 31.68 (Cγ of Glu), 29.50 (Cγ of Lys), 28.39 (3C, *Boc* of Lys), 28.20 (*Boc* of Glu), 28.01 (*Boc* of NLe), 27.81 (Cβ of Glu), 27.29 (*tBu* of Ser), 22.43 (Cβ of Lys); ^{19}F NMR (CDCl3, 376 MHz) δ: 40.06 (qd, J = 24.49, 4.08 Hz, 4F, SF_4), –64.12 (p, J = 24.25 Hz, 3F, CF_3); HRMS (DART-ESI, m/z) Calcd. For $C_{41}H_{69}F_7N_5O_{12}S$+: 990.4703(M+), Found: 990.4695.

Boc-NLe(Tts)-Glu(O*tBu*)-Ser(*tBu*)-Lys(*Boc*)-OH (**11**)

To a mixture of R (110.0 mg, 0.11 mmol) and $Pd(PPh_3)_4$ (25.0 mg, 0.022 mmol, 20 mol%) in THF (4.0 mL) was added *N*-ethylaniline (0.14 mL, 1.1 mmol, 10.0 eq.) at room temperature. After stirring for one hour, the reaction mixture was quenched with sat. NH_4Cl solution (5.0 mL), and extracted with AcOEt (3 x 5.0 mL). The combined organic phase was dried over $MgSO_4$, and filtered and evaporated *in vacuo*. The title product was obtained as a white solid in 95 % yield (99.0 mg, 0.10 mmol) with flash column chromatography (MeOH:DCM = 2:98). Mp.: 109–111 °C.: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.51–7.39 (m, 2H, amide NH of Glu and Lys), 7.12 (d, J = 7.90 Hz, 1H, amide NH of Ser), 6.53–6.54 (m, 1H, Hγ of NLe), 6.54–6.42 (m, 1H, Hδ of NLe), 6.63–6.42 (m, 4H, Hα of Lys, Ser, Glu and NLe), 3.80–3.46 (m, 2H, Hβ of Ser), 3.13–3.02 (m, 2H, Hε of Lys), 2.77–2.48 (m, 2H, Hβ of NLe), 2.44–2.33 (m, 2H, Hγ of Glu), 2.14–1.87 (m, 2H, Hβ of Glu), 1.78–1.57 (m, 2H, Hδ of Lys), 1.52–1.47 (m, 2H, Hγ of Lys), 1.44 (s, 18H, Boc of Lys and Glu), 1.43 (s, 9H, *Boc* of NLe), 1.28–1.23 (m, 2H, Hβ of Lys), 1.18 (s, 9H, *Boc* of Ser); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.38 (ester C=O of Glu), 171.74 (amide C=O of Ser), 171.45 (amide C=O of Glu), 170.46 (amide C=O of NLe), 156.23 (acid C=O of Lys), 156.13 (*Boc* C-O of Lys), 155.52 (*Boc* C=O of NLe), 140.84 (p, J = 24.16 Hz, Cδ of NLe), 133.71 (p, J = 4.31 Hz, Cγ of NLe), 123.40 (qp, J = 329.27, 51.31 Hz, CF_3 of NLe), 81.29 (*Boc*-C of Lys), 80.44 (*Boc*-C of NLe), 79.06 (*Boc*-C of Glu), 74.09 (*tBu*-C of Ser), 61.02 (Cβ of Ser), 53.67 (Cα of Ser), 53.18 (Cα of Glu), 53.06 (Cα of NLe), 52.79 (Cα of Lys), 40.30 (Cε of Lys), 33.62 (Cβ of NLe), 31.89 (Cδ of Lys), 31.70 (Cγ of Glu), 29.67 (Cγ of Lys), 28.39 (3C, *Boc* of Lys), 28.22 (3C, *Boc* of Glu), 28.03 (Cβ of Glu), 28.00 (3C, *Boc* of Nle), 27.30 (3C, *tBu* of Ser), 22.66 (Cβ of Lys); ^{19}F NMR ($CDCl_3$, 376 MHz) δ: 40.09 (qd, J = 24.68, 4.84 Hz, 4F, SF_4), –64.16 (p, J = 24.35 Hz, 3F, CF_3); HRMS (DART-ESI, m/z) Calcd. For $C_{38}H_{65}F_7N_5O_{12}S$+: 948.4233(M+), Found: 948.4350.

Boc-NLe(Tts)-Glu(O*tBu*)-Ser(*tBu*)-Lys(Boc)-NLe(Tts)-Lys(Z)-Glu(OEt)-OEt (**12**)

For this coupling reaction, DMF was used as a solvent instead of THF, and the scale of the reaction was changed to 0.01 mmol. The title compound was obtained as a white solid in 78 yield (13.0 mg, 0.0078 mmol) with flash column chromatography (MeOH:DCM = 2:98).

HRMS (DART-ESI, m/z) Calcd. For $C_{67}H_{104}F_{14}N_9O_{19}S_2$+: 1668.6661(M+), Found: 1668.6654.

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NLe(Tts)-Glu-Ser-Lys-NLe(Tts)-Lys-Glu(OEt)-OEt (**5**)

To a mixture of TFA/triisopropylsilane/H_2O (95:2.5:2.5) was added S (8.0 mg, 0.0048 mmol, 1.0 eq.) at room temperature. After stirring for overnight, the mixture was evaporated in vacuo. The crude product was purified with flash column chromatography (MeOH:DCM = 1:10) to afford the title product (**5**) in 91 % yield (5.3 mg, 0.0044 mmol.

HRMS (DART-ESI, m/z) Calcd. For $C_{41}H_{66}F_{14}N_9O_{13}S_2$+: 1222.3992(M+), Found: 1222.3395.

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2.3 Determination of the enantiomeric purity for the CF_3SF_4-substituted amino acid, *Boc*-NLe(Tts)-OH (**3**)

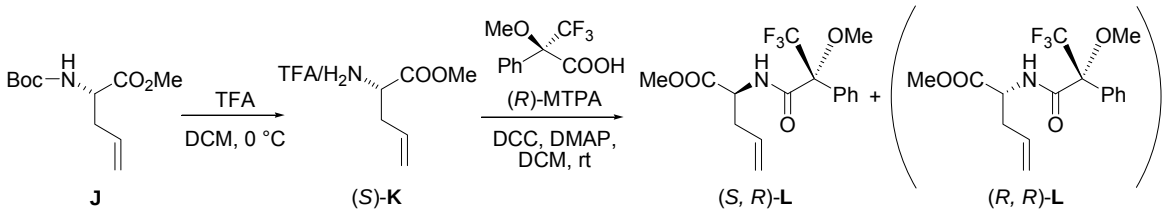
For determination of the enantiomeric purity, Mosher acid was used for the coupling reaction with the amino acid (**G**), *Boc*-NLe(Tts)-Lys(*Z*)-Glu(OEt)-OEt (**J**) and *Boc*-NLe(Tts)-Glu(O*tBu*)-Ser(*tBu*)-Lys(*Boc*)-OAll (**R**). The general procedure is below.

2-3-1. General procedure

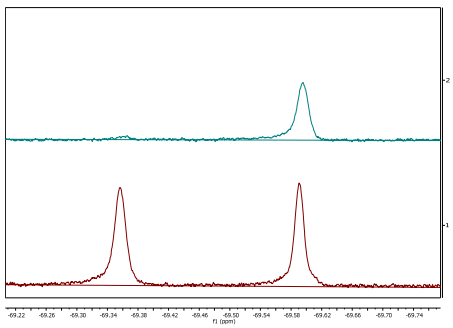
To a mixture of an amino acid or peptide (0.1 mmol, 1.0 eq.) in DCM (0.6 mL) was added TFA (0.04 mL, 0.5 mmol, 5.0 eq.) at 0 °C. The reaction mixture was allowed to increase to room temperature. After the reaction was completed with monitoring with TLC, the mixture was concentrated *in vacuo*. To the round-bottom-flask contained the crude product was added DCM (1.0 mL), and then added (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (Mosher acid) (28.0 mg, 0.12 mmol, 1.2 eq.), *N*,*N*'-dicyclohexylcarbodiimide (DCC) (24.0 mg, 0.12 mmol, 1.2 eq.) and 4-dimethylaminopyridine (DMAP) (2.0 mg, 0.015 mmol, 0.15 eq.) at room temperature. After stirring for overnight, the reaction mixture was quenched with sat. $NaHCO_3$ solution (3.0 mL) and extracted with AcOEt (3 x 3.0 mL). The combined organic phase was dried over Mg_2SO_4, and filtered and concentrated *in vacuo*. The crude product was compared with racemic product respectively in ^{19}F NMR.

2-3-2. Analysis for the enantiomeric purity

The ^{19}F NMR data for the amino acid methyl ester (**L**).



J (*S*)-**K** (*S*, *R*)-**L** (*R*, *R*)-**L**



The ee of **L** is 88 % (ratio is 6:94).

The ^{19}F NMR data for the *Boc*-NLe(Tts)-Lys(*Z*)-Glu(OEt)-OEt (L)

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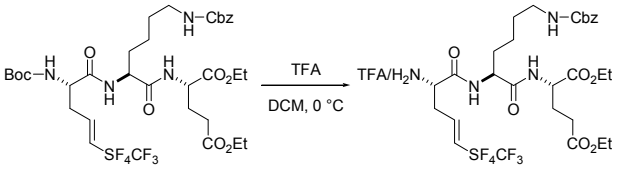
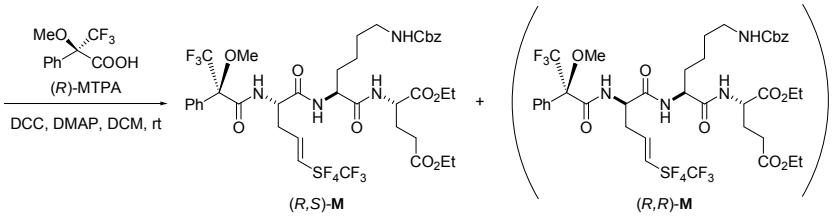
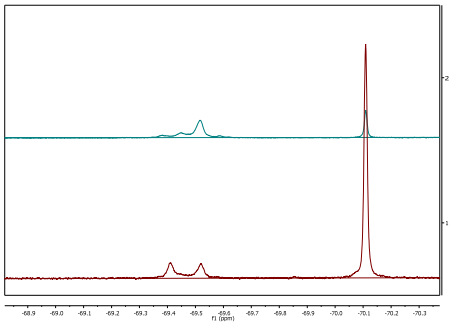
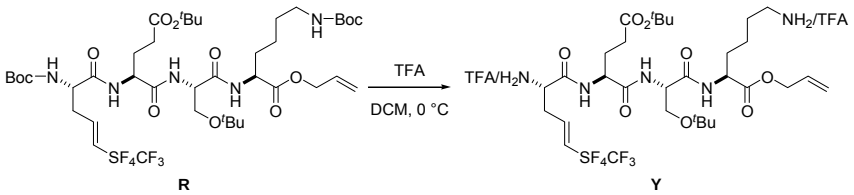


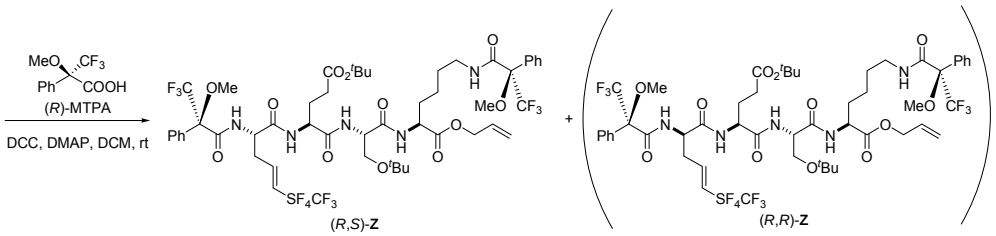




The ee of **M** is 84 % (ratio is 8:92).

The ^{19}F NMR data for the *Boc*-NLe(Tts)-Glu(O^t*Bu*)-Ser(^{t}*Bu*)-Lys(*Boc*)-OAll (Z)





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References

1) Lim, D. S.; Lin, J.-H.; Welch, J. T. *Eur. J. Org. Chem.*, *2012*, **21**, 3964-3954.
2) Krishnamurthy, S.; Arai, T.; Nakanishi, K.; Nishino, N. *RSC Adv.*, 2014, **4**, 2482-2490.
3) Schneider, H.; Sigmund, G.; Schricker, B.; Thirring, K.; Berner, H. *J. Org. Chem*., 1993, **58**, 683-689.
4) (a) Myers, A. G.; Gleason, J. L. *Org. Synth*., 1999, **76**, 57-66. (b) Saniere, L.; Leman, L.; Bourguignon, J.-J.; Dauban, P.; Dodd, R. H. *Tetrahedron*, 2004, **60**(28), 5889-5897.
5) (a) Luebke, M.; Jung, M.; Haufe, G. *J. Fluorine Chem*., 2013, **152**, 144-156. (b) Allen, N. E.; Boyd, D. B.; Campbell J. B.; Deeter, J. B.; Elzey, T. K.; Foster, B. J.; Hatfield, L. D.; Hobbs, J. N., Jr.; Hormback, W. J. *Tetrahedron*, 1989, **45**, 1905-1928.
6) (a) Li, S.-G.; Portela-Cubillo, F.; Zard, S.Z. *Org. Lett*., 2016, **18**, 1888-1891. (b) Dondoni, A.; Massi, A.; Minghini, E.; Bertolasi, V. *Tetrahedron*, 2004, **60**, 2311-2326.

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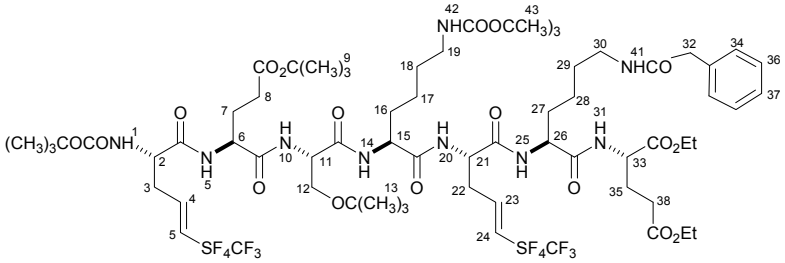
2.4 NMR structure determination

2-4-1a *Boc*-NLe(Tts)-Glu(O*tBu*)-Ser(*tBu*)-Lys(*Boc*)-NLe(Tts)-Lys(*Z*)-Glu(OEt)-OEt



Proton assignments

Resonance	Shift[a]	Residue
1	7.645	TtsV1
2	4.799	TtsV1
3	2.834	TtsV1
3	2.651	TtsV1
4	6.599	TtsV1
5	6.481	TtsV1
5	7.577	E2
6	4.787	E2
7	2.416	E2
7	2.202	E2
8	3.087	E2
8	2.981	E2
9	1.278	E2
9	1.278	E2
9	1.278	E2
9	1.278	E2
9	1.278	E2
9	1.278	E2
9	1.278	E2
9	1.278	E2
10	7.144	S3
11	4.554	S3
12	3.799	S3
12	3.426	S3
13	1.234	S3
13	1.234	S3
13	1.234	S3
13	1.234	S3
14	7.359	K4
15	4.417	K4
16	2.736	K4
16	2.566	K4
17	1.558	K4
17	1.436	K4
18	1.894	K4
18	1.791	K4
19	3.212	K4
20	7.949	TtsV5
21	4.796	TtsV5
22	2.654	TtsV5
22	2.834	TtsV5
23	6.601	TtsV5
24	6.481	TtsV5
25	7.219	K6
26	4.475	K6
27	2.439	K6
27	2.114	K6
28	1.427	K6
28	1.553	K6
29	1.784	K6
29	1.861	K6
30	3.201	K6
31	7.725	E7
32	5.152	K6
32	5.116	K6
33	4.749	E7
34	7.372	K6
34	7.372	K6
35	2.031	E7
35	2.199	E7
36	7.372	K6
36	7.372	K6
38	2.774	E7
38	2.619	E7
37	7.372	K6
39	4.180	E7
39	4.180	E7
40	1.173	E7
40	1.173	E7
40	1.173	E7
43	1.278	K4
43	1.278	K4
43	1.278	K4
43	1.278	K4
43	1.278	K4
43	1.278	K4
43	1.278	K4
43	1.278	K4
41	5.277	K6
42	5.333	K4

[a] in ppm.

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2-4-1b Backbone torsional angles from TALOS

Amino acid	Backbone angle	Torsional angle range	
2 E2	PHI	-106.6	-82.4
2 E2	PSI	96.9	151.4
3 S3	PHI	-140.2	-31.0
3 S3	PSI	73.8	192.2
4 K4	PHI	-104.0	-65.0
4 K4	PSI	-48.7	-26.4
5 TTSV2	PHI	-180.2	-89.1
5 TTSV2	PSI	118.3	185.6
6 K6	PHI	-105.5	-42.4
6 K6	PSI	-50.1	-13.4

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2-4-1c Sidechain torsional angles from Cyana.

Torsional angles	Amino acid residue	angle	Torsional angle range	
1	1 TTSV1	CHI1	-90.0	210.0
2	1 TTSV1	CHI1	-330.0	-30.0
3	1 TTSV1	CHI1	-210.0	90.0
4	2 E2	CHI1	-90.0	210.0
5	2 E2	CHI1	-330.0	-30.0
6	2 E2	CHI1	-210.0	90.0
7	2 E2	CHI2	-90.0	210.0
8	2 E2	CHI2	-330.0	-30.0
9	2 E2	CHI2	-210.0	90.0
10	3 S3	CHI1	-90.0	210.0
11	3 S3	CHI1	-330.0	-30.0
12	3 S3	CHI1	-210.0	90.0
13	4 K4	CHI1	-90.0	210.0
14	4 K4	CHI1	-330.0	-30.0
15	4 K4	CHI1	-210.0	90.0
16	4 K4	CHI2	-90.0	210.0
17	4 K4	CHI2	-330.0	-30.0
18	4 K4	CHI2	-210.0	90.0
19	4 K4	CHI3	-90.0	210.0
20	4 K4	CHI3	-330.0	-30.0
21	4 K4	CHI3	-210.0	90.0
22	4 K4	CHI4	-90.0	210.0
23	4 K4	CHI4	-330.0	-30.0
24	4 K4	CHI4	-210.0	90.0
25	5 TTSV2	CHI1	-90.0	210.0
26	5 TTSV2	CHI1	-330.0	-30.0
27	5 TTSV2	CHI1	-210.0	90.0
28	6 K6	CHI1	-90.0	210.0
29	6 K6	CHI1	-330.0	-30.0
30	6 K6	CHI1	-210.0	90.0
31	6 K6	CHI2	-90.0	210.0
32	6 K6	CHI2	-330.0	-30.0
33	6 K6	CHI2	-210.0	90.0
34	6 K6	CHI3	-90.0	210.0
35	6 K6	CHI3	-330.0	-30.0
36	6 K6	CHI3	-210.0	90.0
37	6 K6	CHI4	-90.0	210.0
38	6 K6	CHI4	-330.0	-30.0
39	6 K6	CHI4	-210.0	90.0
40	7 E7	CHI1	-90.0	210.0
41	7 E7	CHI1	-330.0	-30.0
42	7 E7	CHI1	-210.0	90.0
43	7 E7	CHI2	-90.0	210.0
44	7 E7	CHI2	-330.0	-30.0
45	7 E7	CHI2	-210.0	90.0

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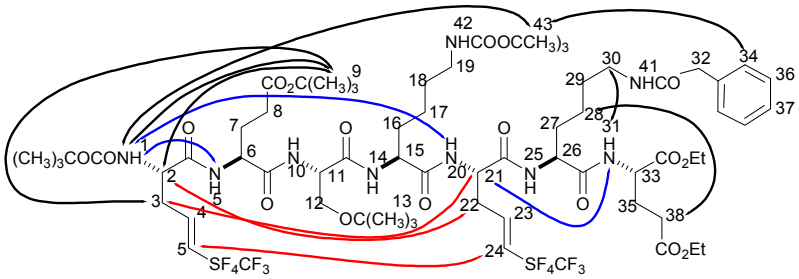
2-4-1d Significant NOE Relationships

Blue-amide proton interactions

Red-CF_3SF_4 side chain interactions

Black-other significant interactions



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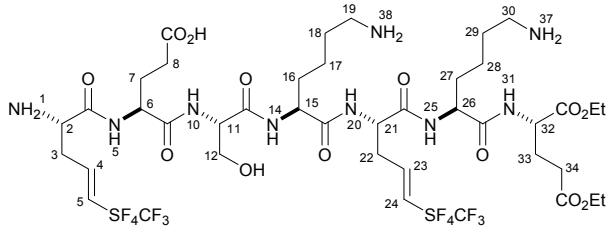
2-4-2a. NLe(Tts)-Glu-Ser-Lys-NLe(Tts)-Lys-Glu(OEt)-OEt

Proton assignments



Resonance	Shift[a]	Residue	17	1.287	K4
1	9.700	TtsV1	16	1.528	K4
2	4.563	TtsV1	16	1.654	K4
3	2.710	TtsV1	20	9.603	TtsV5
3	2.547	TtsV1	21	4.568	TtsV5
4	6.590	TtsV1	22	2.714	TtsV5
5	6.973	TtsV1	22	2.549	TtsV5
6	7.351	E2	24	6.951	TtsV5
7	5.330	E2	25	8.281	K6
9	2.743	E2	26	4.231	K6
8	1.986	E2	27	1.647	K6
8	1.275	E2	27	1.531	K6
10	8.367	S3	30	2.361	K6
11	4.232	S3	29	1.994	K6
12	1.980	S3	28	1.818	K6
12	1.821	S3	31	7.716	E7
14	8.294	K4	32	4.889	E7
15	4.236	K4	33	3.003	E7
19	2.963	K4	34	1.355	E7
18	1.401	K4	34	1.233	E7

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2-4-2b. Sidechain torsional angles from Cyana

Torsional angles	Amino acid residue	angle	Torsional angle range	
1	1 TtsV1	CHI1	-90.0	210.0
2	1 TtsV1	CHI1	-330.0	-30.0
3	1 TtsV1	CHI1	-210.0	90.0
4	2 E2	CHI1	-90.0	210.0
5	2 E2	CHI1	-330.0	-30.0
6	2 E2	CHI1	-210.0	90.0
7	2 E2	CHI2	-90.0	210.0
8	2 E2	CHI2	-330.0	-30.0
9	2 E2	CHI2	-210.0	90.0
10	3 S3	CHI1	-90.0	210.0
11	3 S3	CHI1	-330.0	-30.0
12	3 S3	CHI1	-210.0	90.0
13	4 K4	CHI1	-90.0	210.0
14	4 K4	CHI1	-330.0	-30.0
15	4 K4	CHI1	-210.0	90.0
16	4 K4	CHI2	-90.0	210.0
17	4 K4	CHI2	-330.0	-30.0
18	4 K4	CHI2	-210.0	90.0
19	4 K4	CHI3	-90.0	210.0
20	4 K4	CHI3	-330.0	-30.0
21	4 K4	CHI3	-210.0	90.0
22	4 K4	CHI4	-90.0	210.0
23	4 K4	CHI4	-330.0	-30.0
24	4 K4	CHI4	-210.0	90.0
25	5 TtsV5	CHI1	-90.0	210.0
26	5 TtsV5	CHI1	-330.0	-30.0
27	5 TtsV5	CHI1	-210.0	90.0
28	6 K6	CHI1	-90.0	210.0
29	6 K6	CHI1	-330.0	-30.0
30	6 K6	CHI1	-210.0	90.0
31	6 K6	CHI2	-90.0	210.0
32	6 K6	CHI2	-330.0	-30.0
33	6 K6	CHI2	-210.0	90.0
34	6 K6	CHI3	-90.0	210.0
35	6 K6	CHI3	-330.0	-30.0
36	6 K6	CHI3	-210.0	90.0
37	6 K6	CHI4	-90.0	210.0
38	6 K6	CHI4	-330.0	-30.0
39	6 K6	CHI4	-210.0	90.0
40	7 E7	CHI1	-90.0	210.0
41	7 E7	CHI1	-330.0	-30.0
42	7 E7	CHI1	-210.0	90.0
43	7 E7	CHI2	-90.0	210.0
44	7 E7	CHI2	-330.0	-30.0
45	7 E7	CHI2	-210.0	90.0

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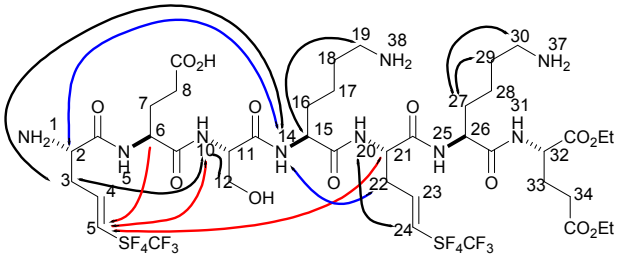
2-4-2c NOE Relationships

Blue-amide proton interactions

Red-CF_3SF_4 side chain interactions



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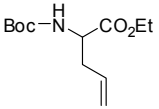

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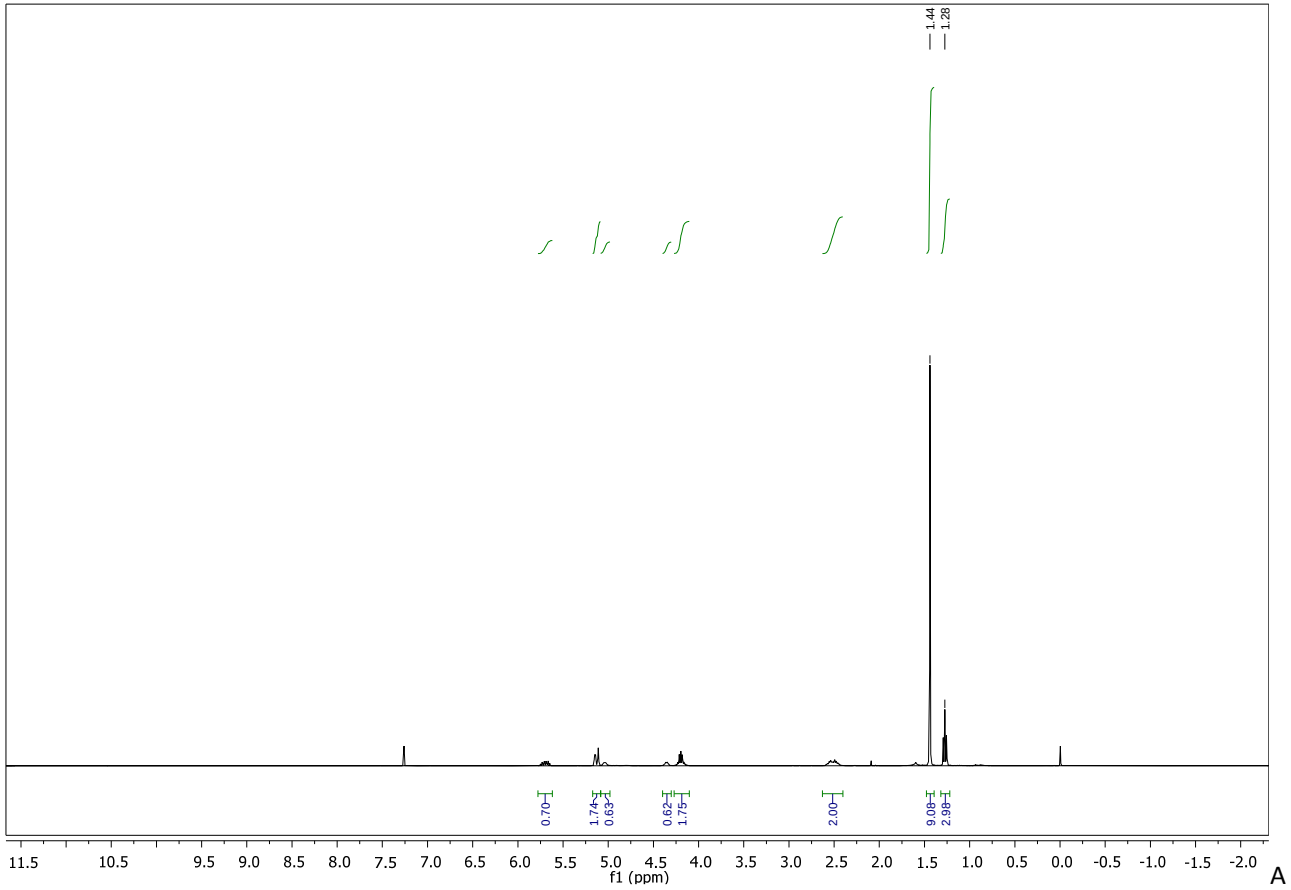

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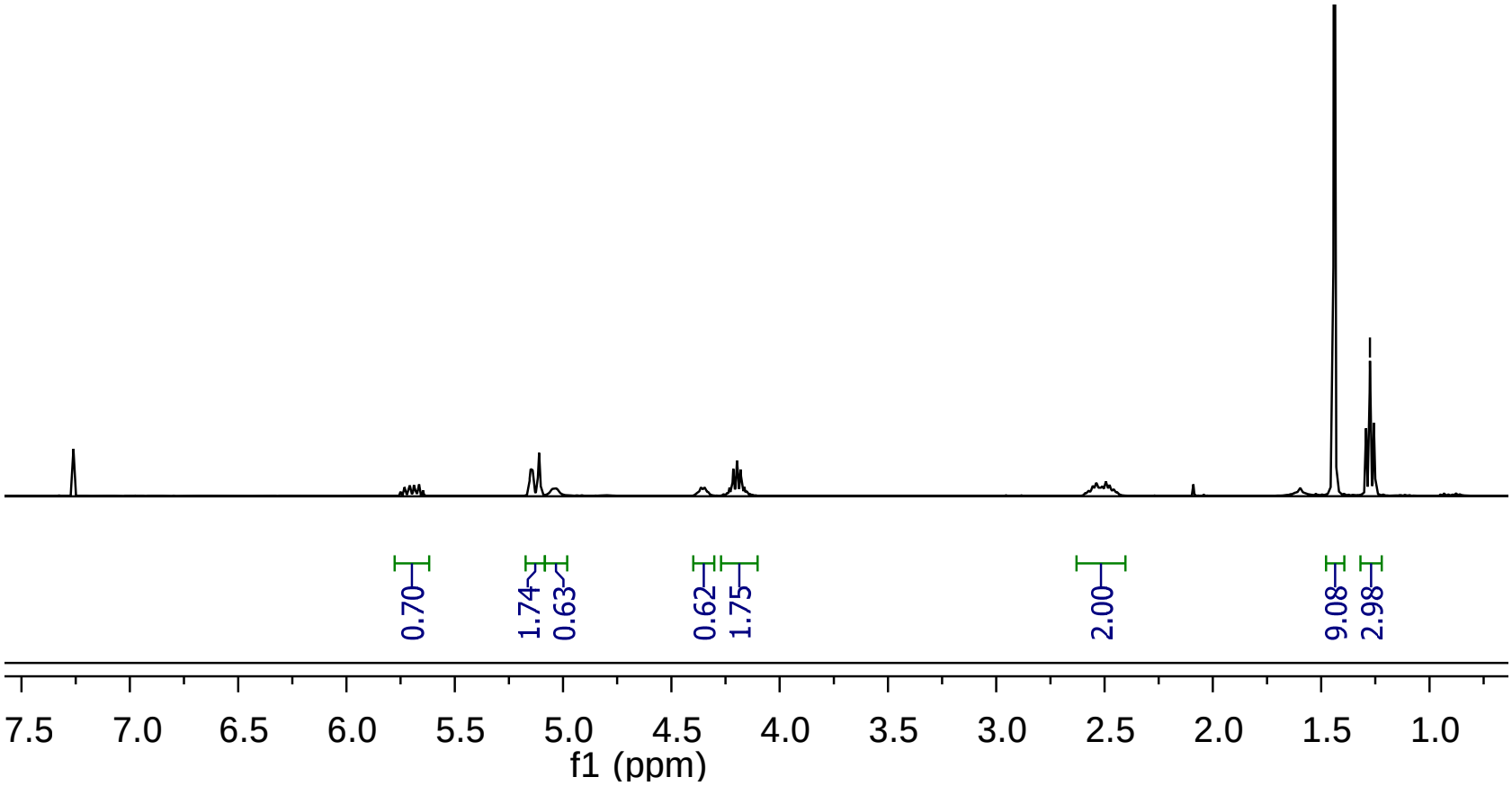

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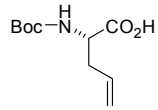

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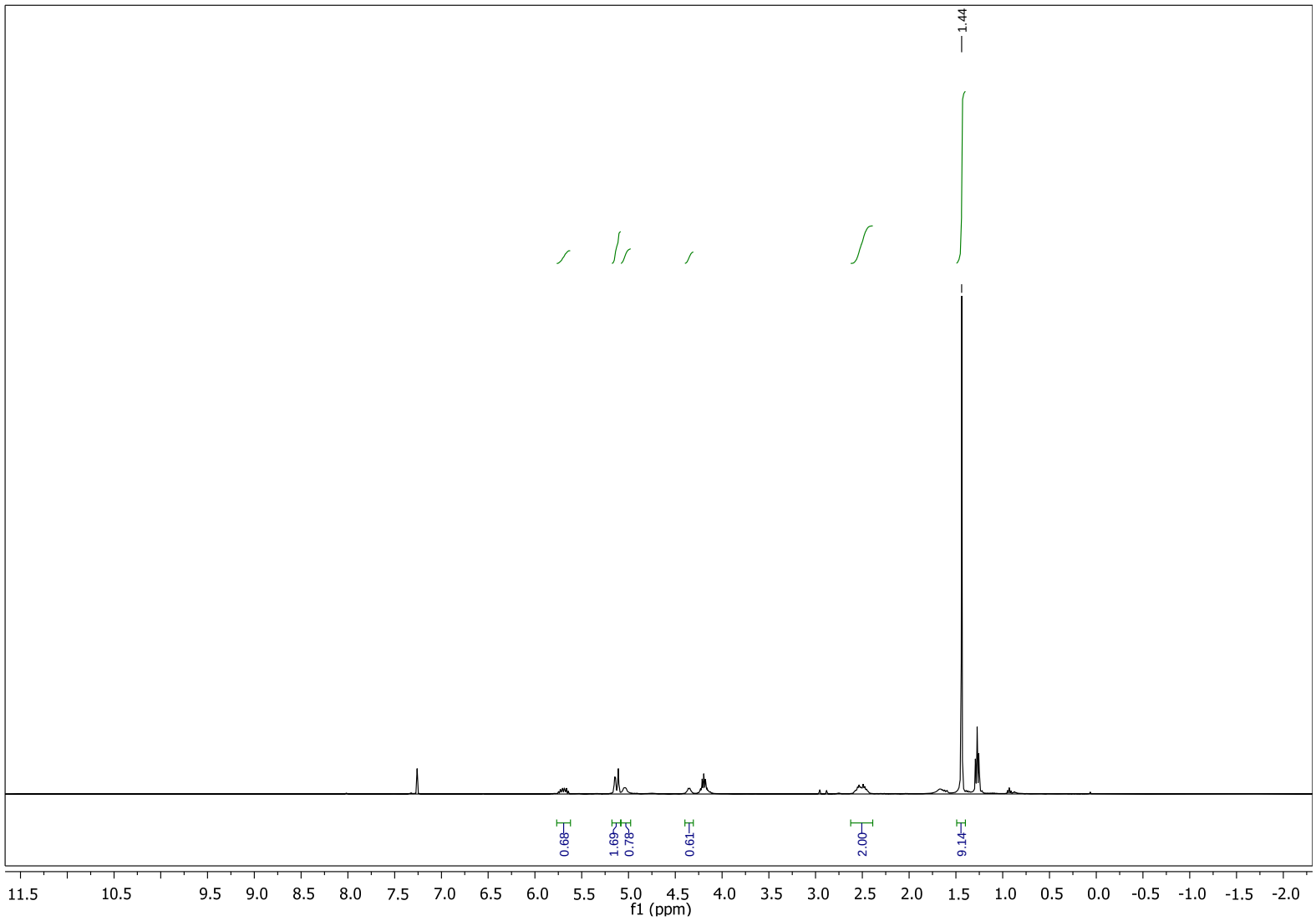

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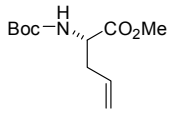

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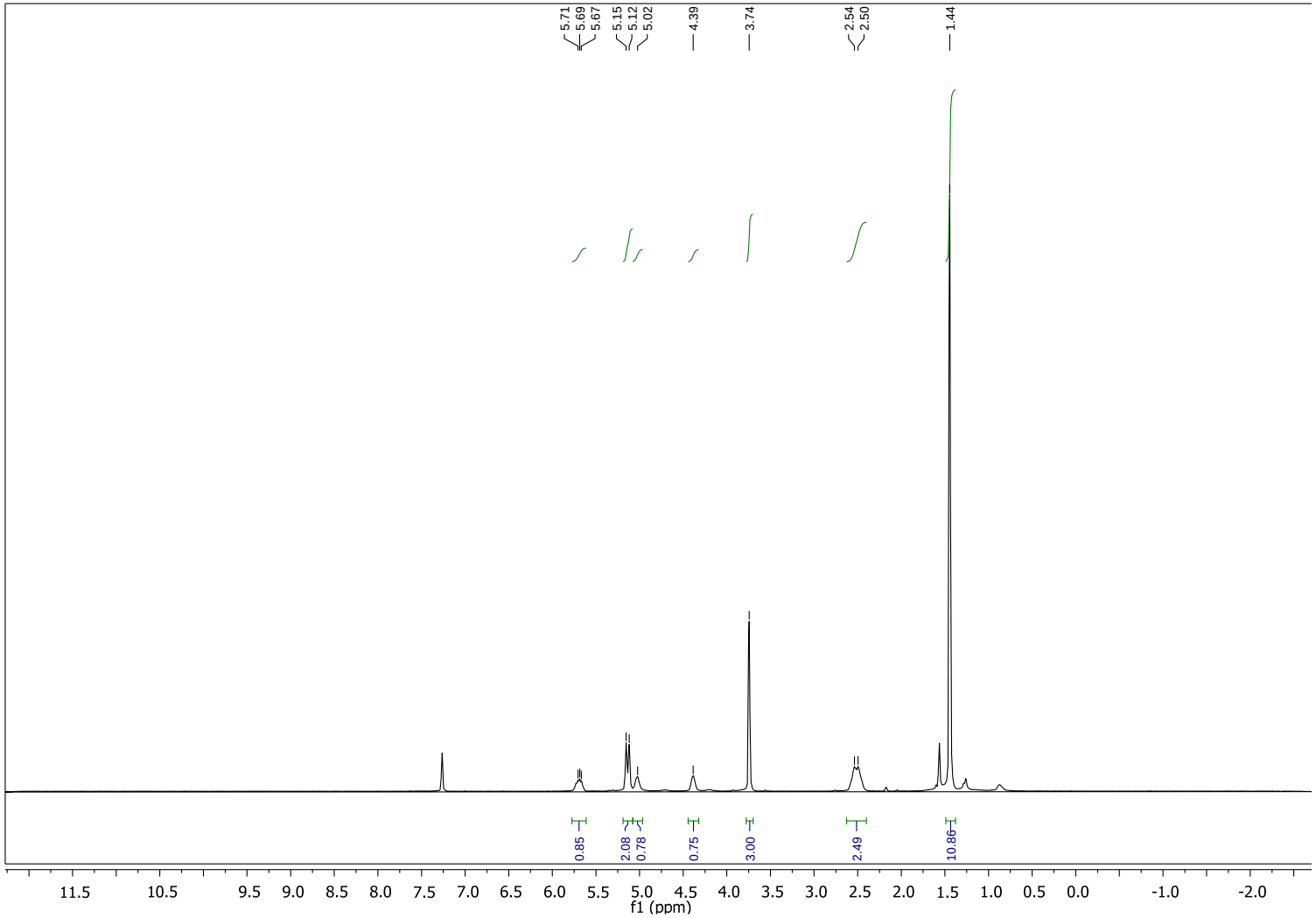

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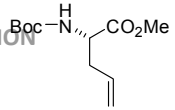

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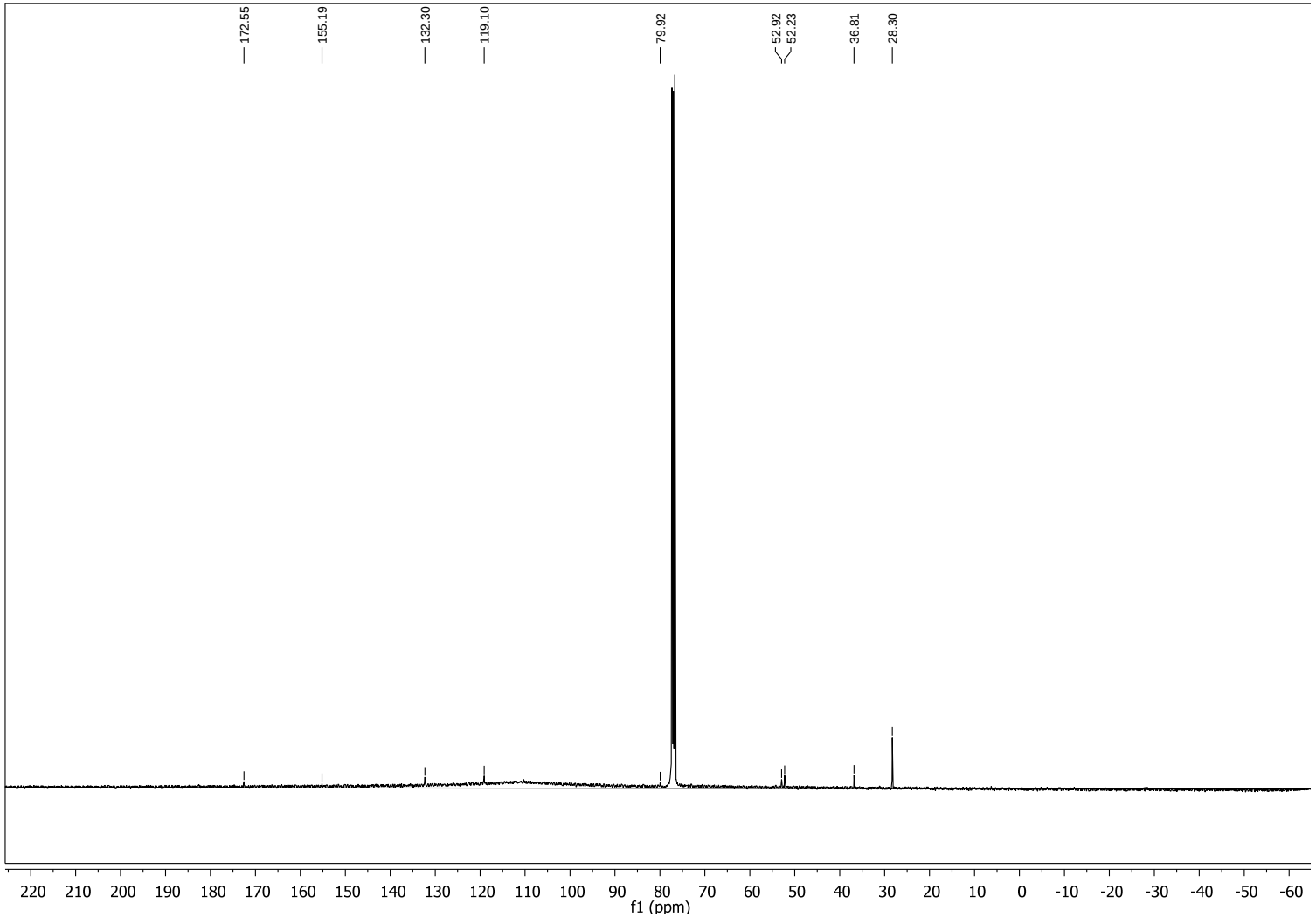

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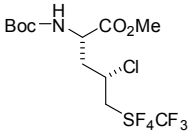
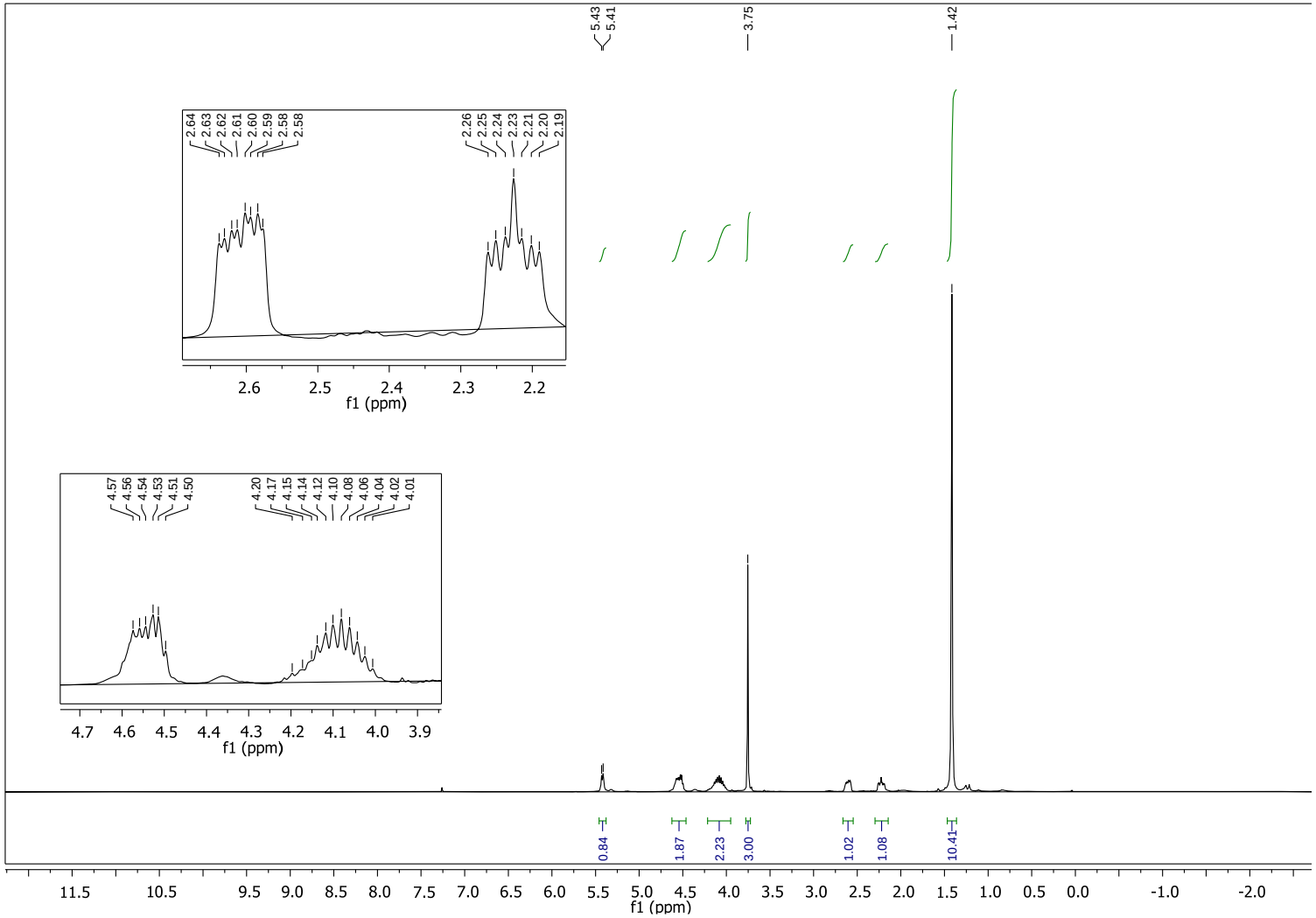


4 (2*S*,4*S*)



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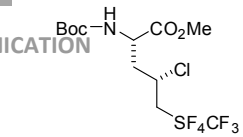

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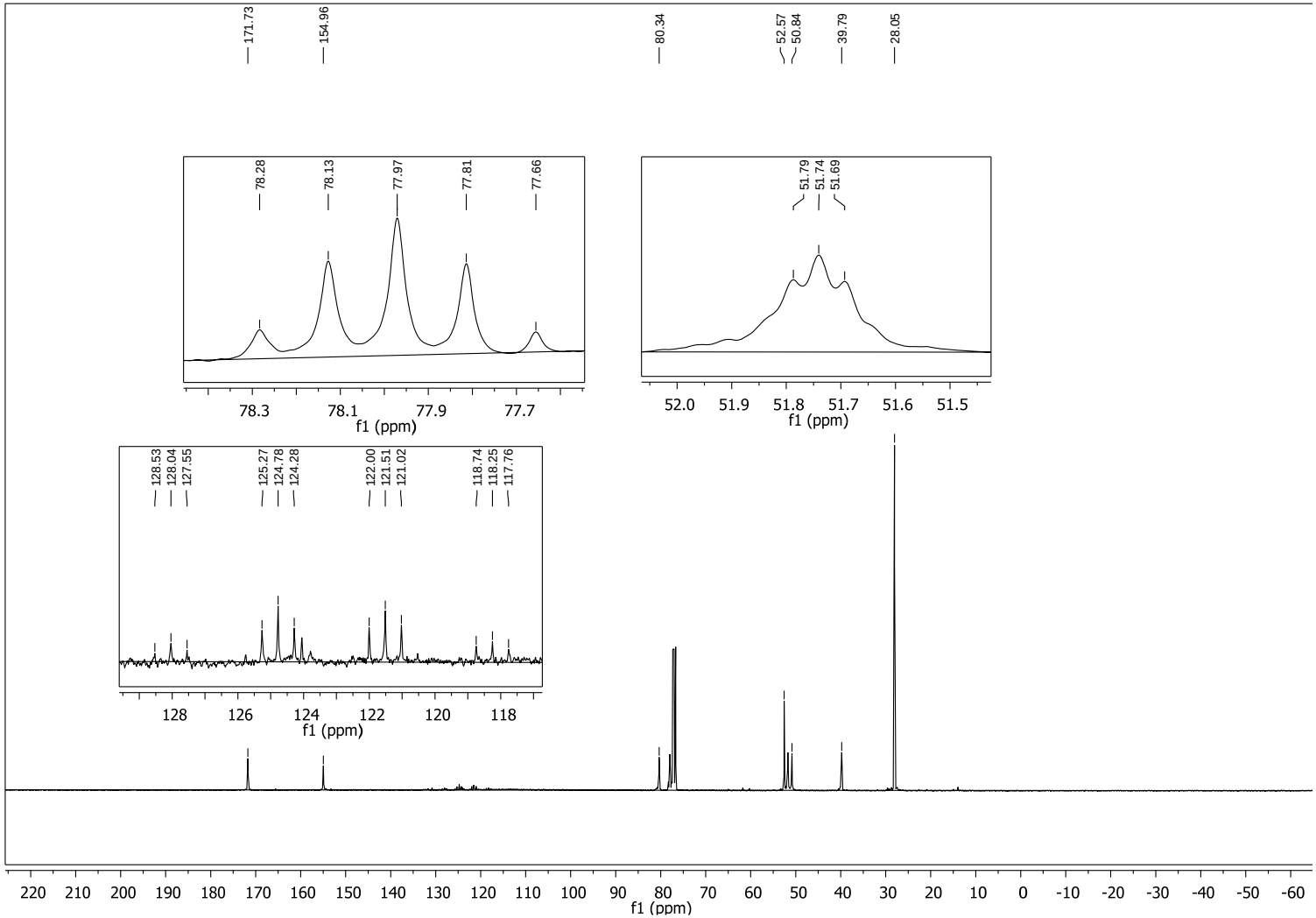

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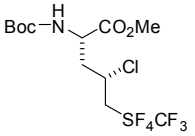
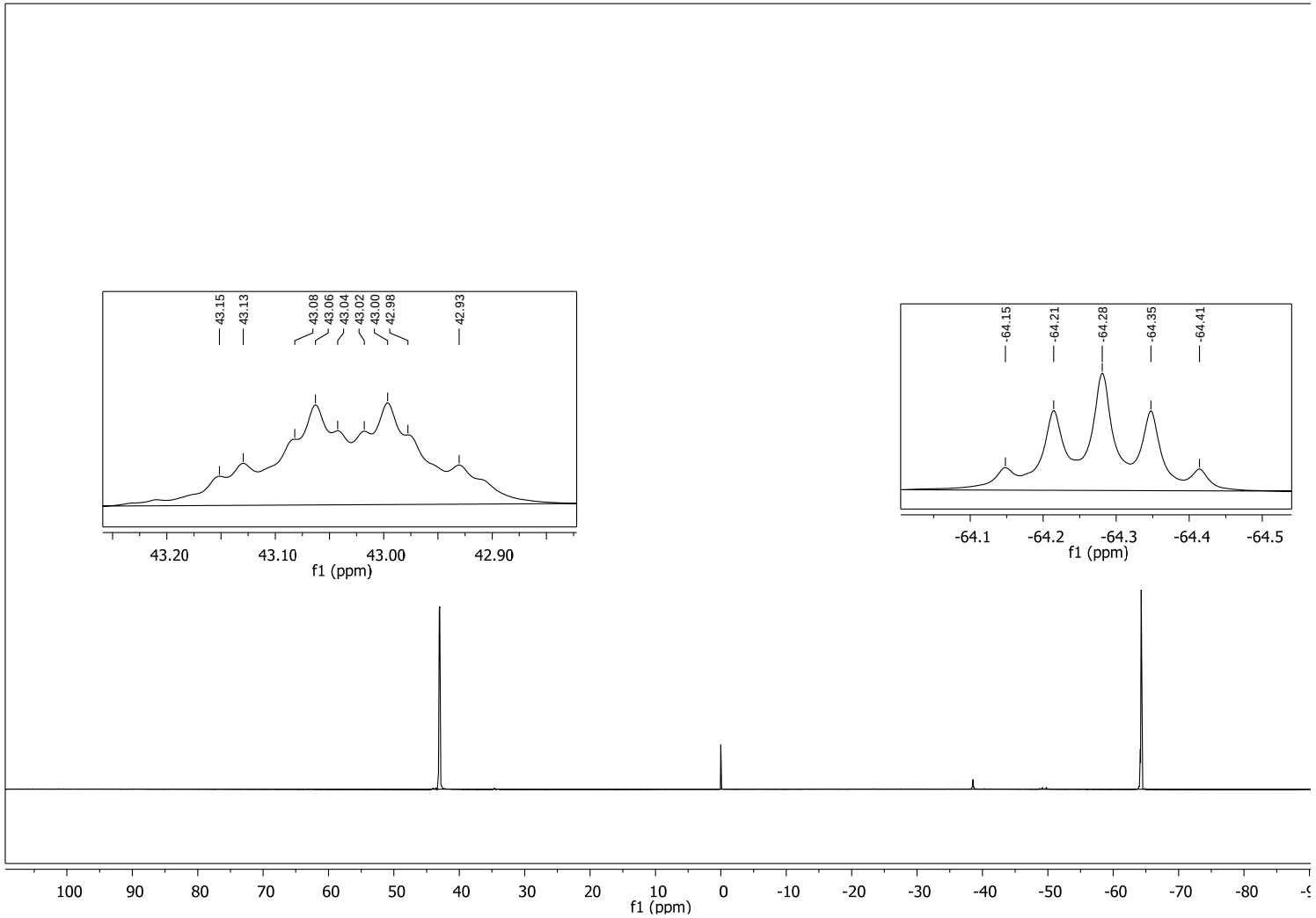


4 (2*S*,4*S*)



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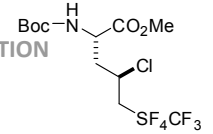
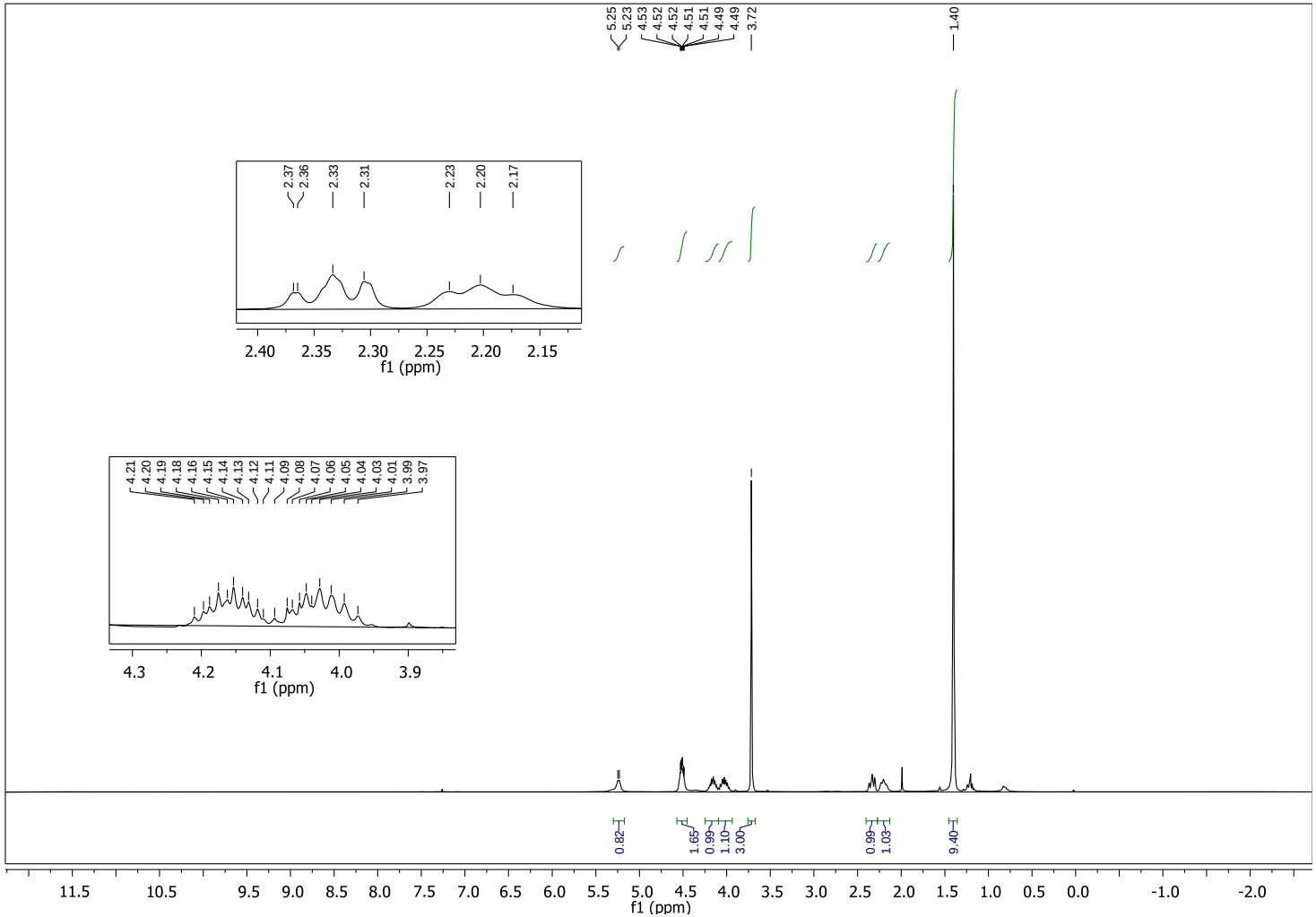

Boc
H
N
CO2Me
Cl
SF4CF3

4 (2S4*R*)


5.25
5.23
4.53
4.52
4.52
4.51
4.51
4.49
4.49
3.72
1.40
2.37
2.36
2.33
2.31
2.23
2.20
2.17
2.40
2.35
2.30
2.25
2.20
2.15
f1 (ppm)
4.21
4.20
4.19
4.18
4.16
4.15
4.14
4.13
4.12
4.11
4.09
4.08
4.07
4.06
4.05
4.04
4.03
4.01
3.99
3.97
4.3
4.2
4.1
4.0
3.9
f1 (ppm)
0.82
1.65
0.99
1.10
3.00
0.99
1.03
9.40
11.5
10.5
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
0.0
-1.0
-2.0
f1 (ppm)

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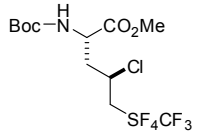

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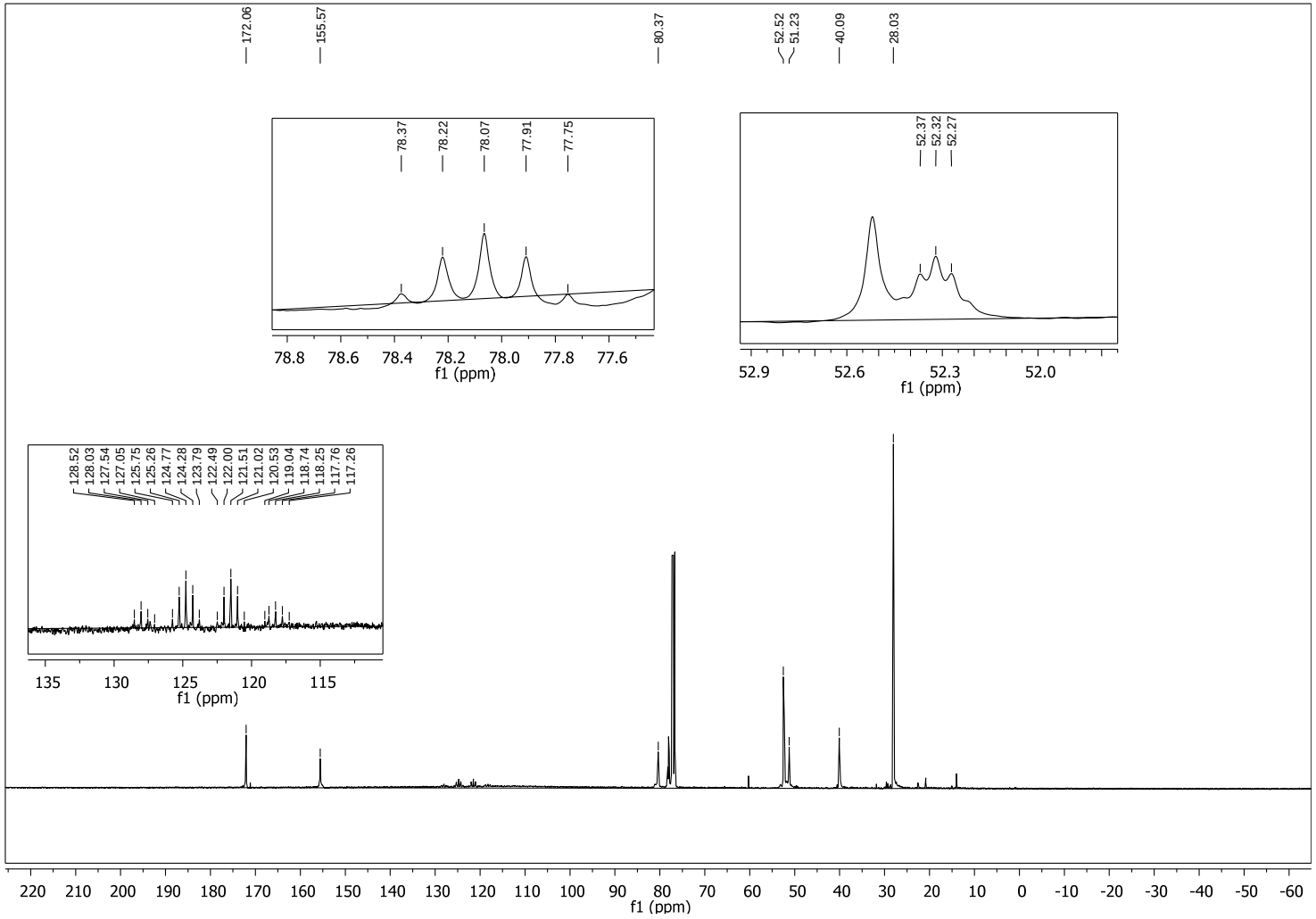

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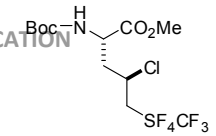
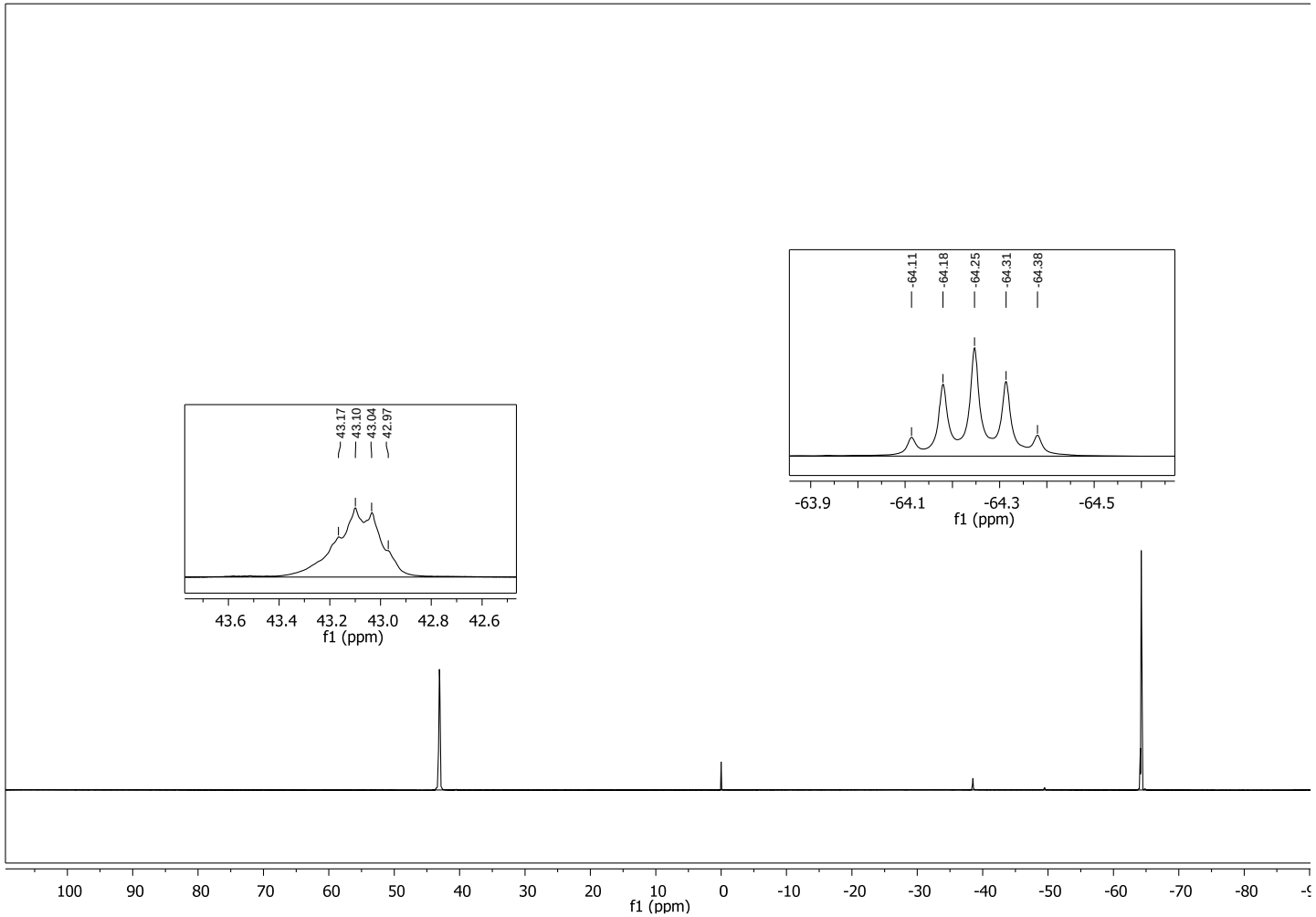


4 (2S4*R*)



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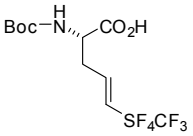

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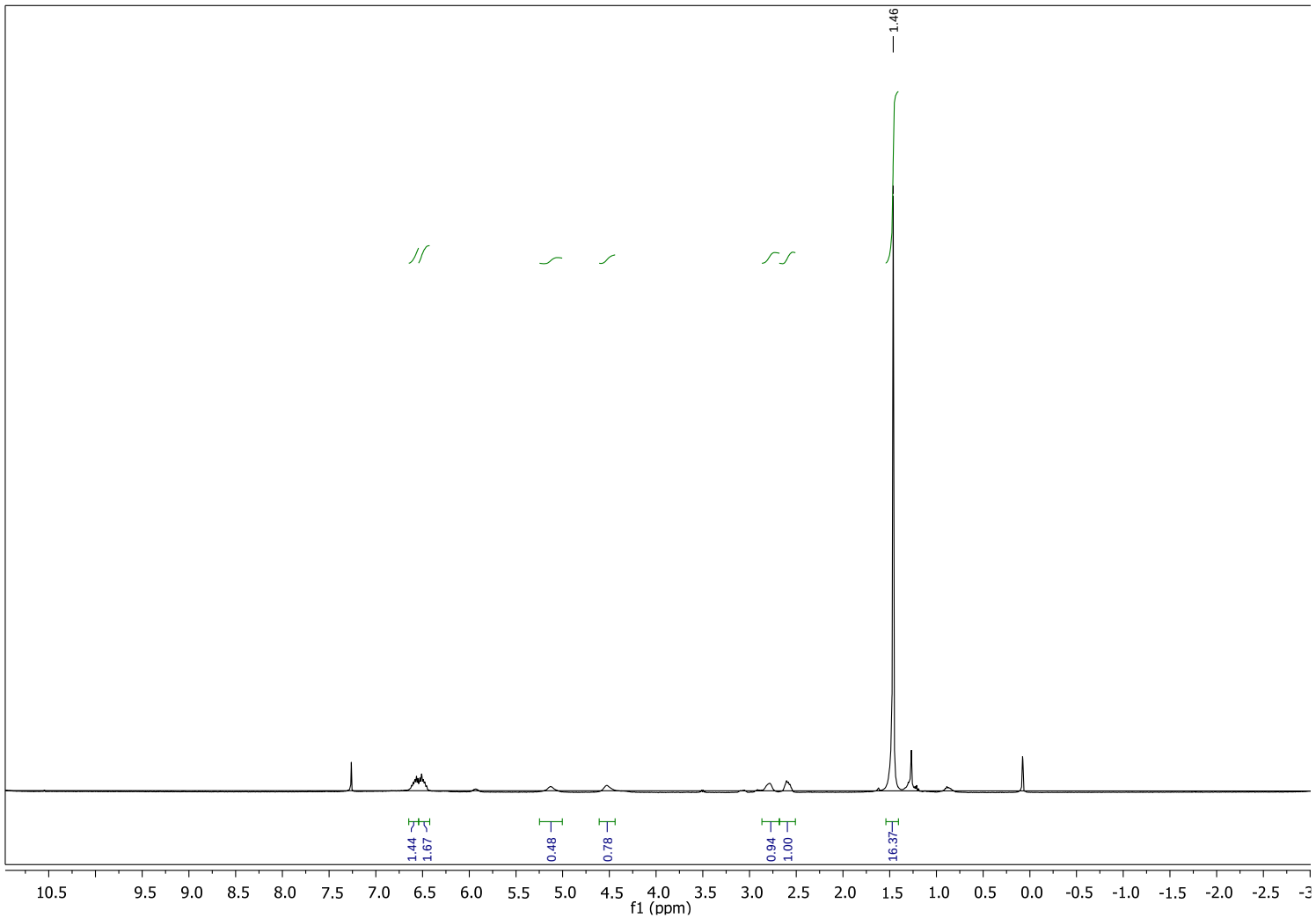

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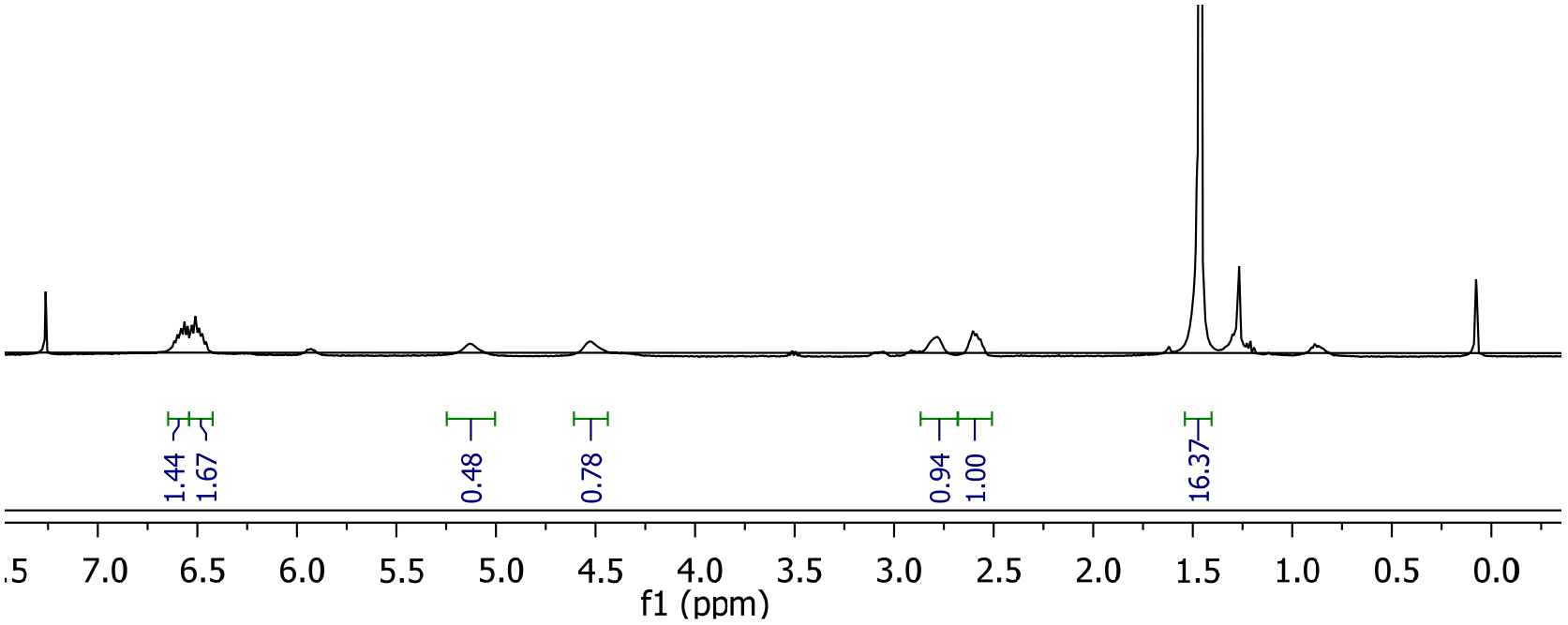

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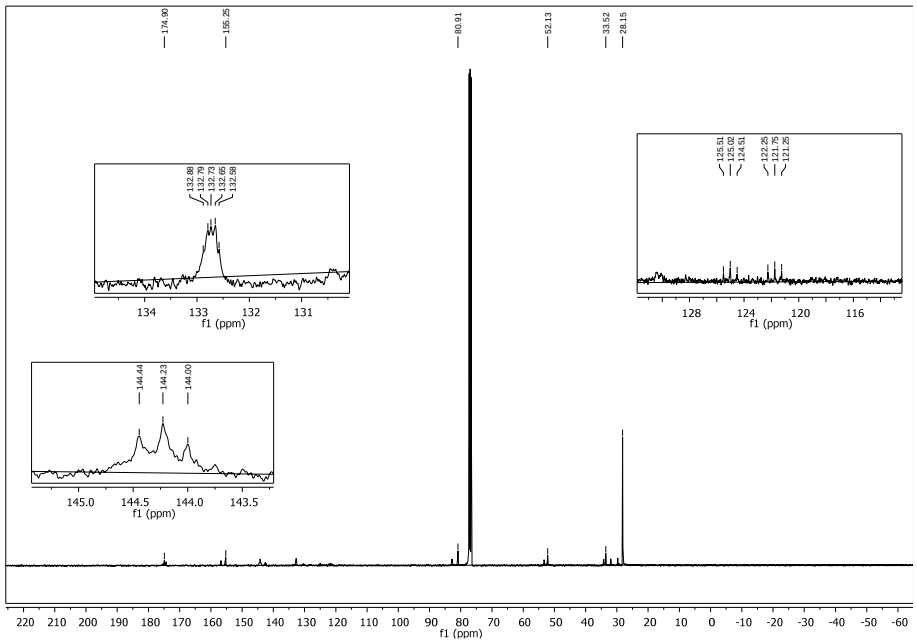

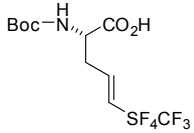

2

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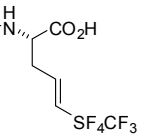

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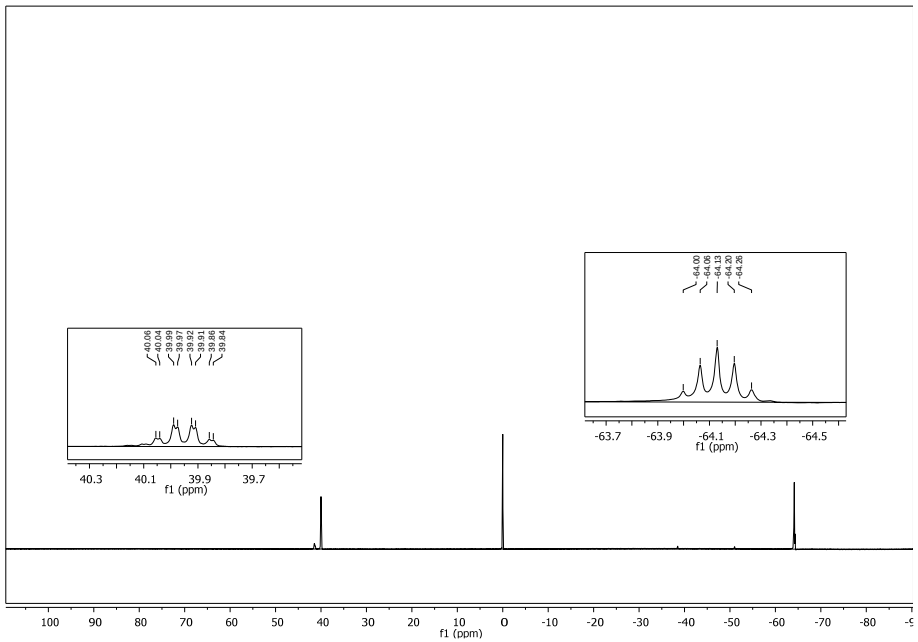

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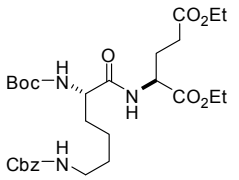

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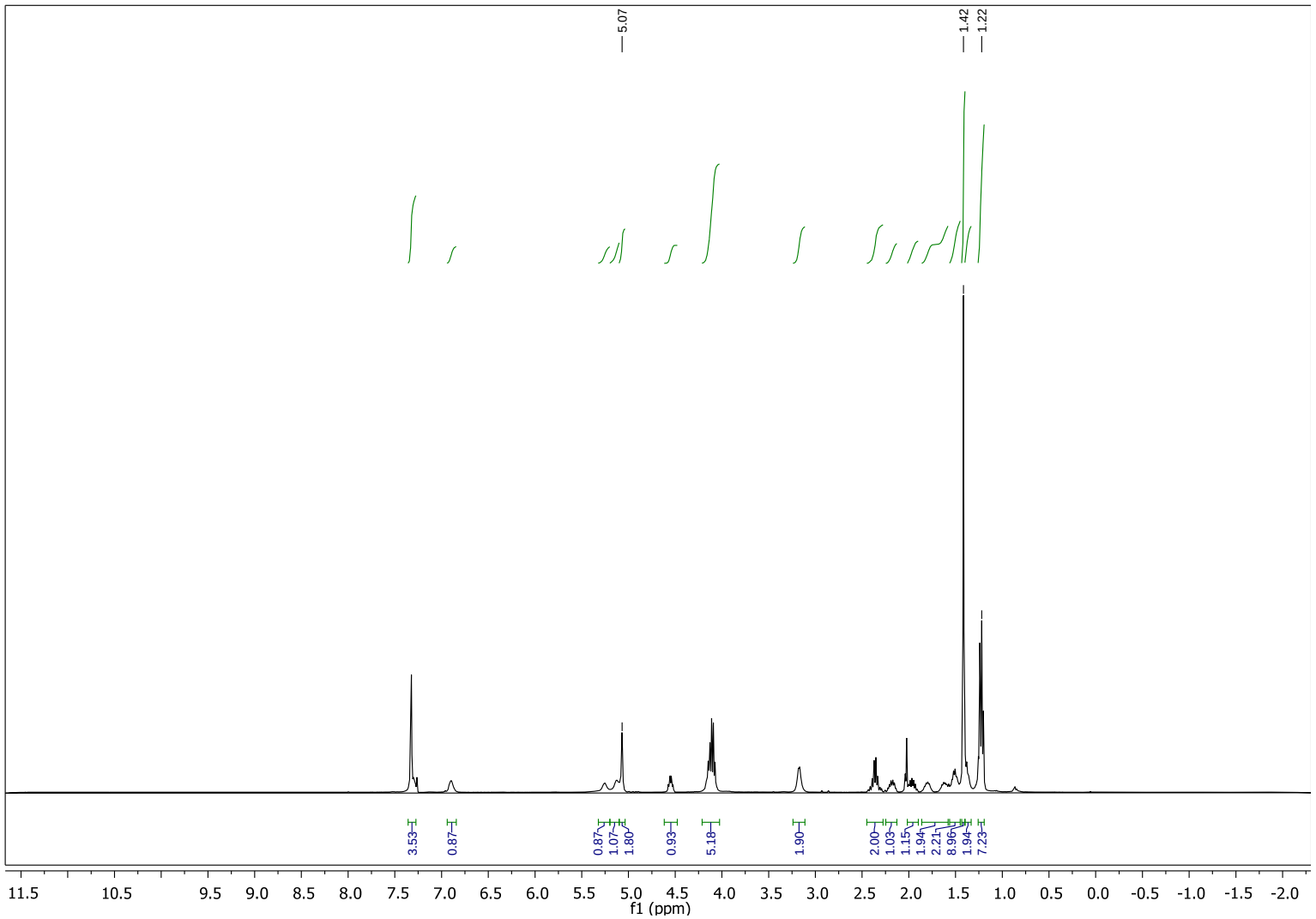

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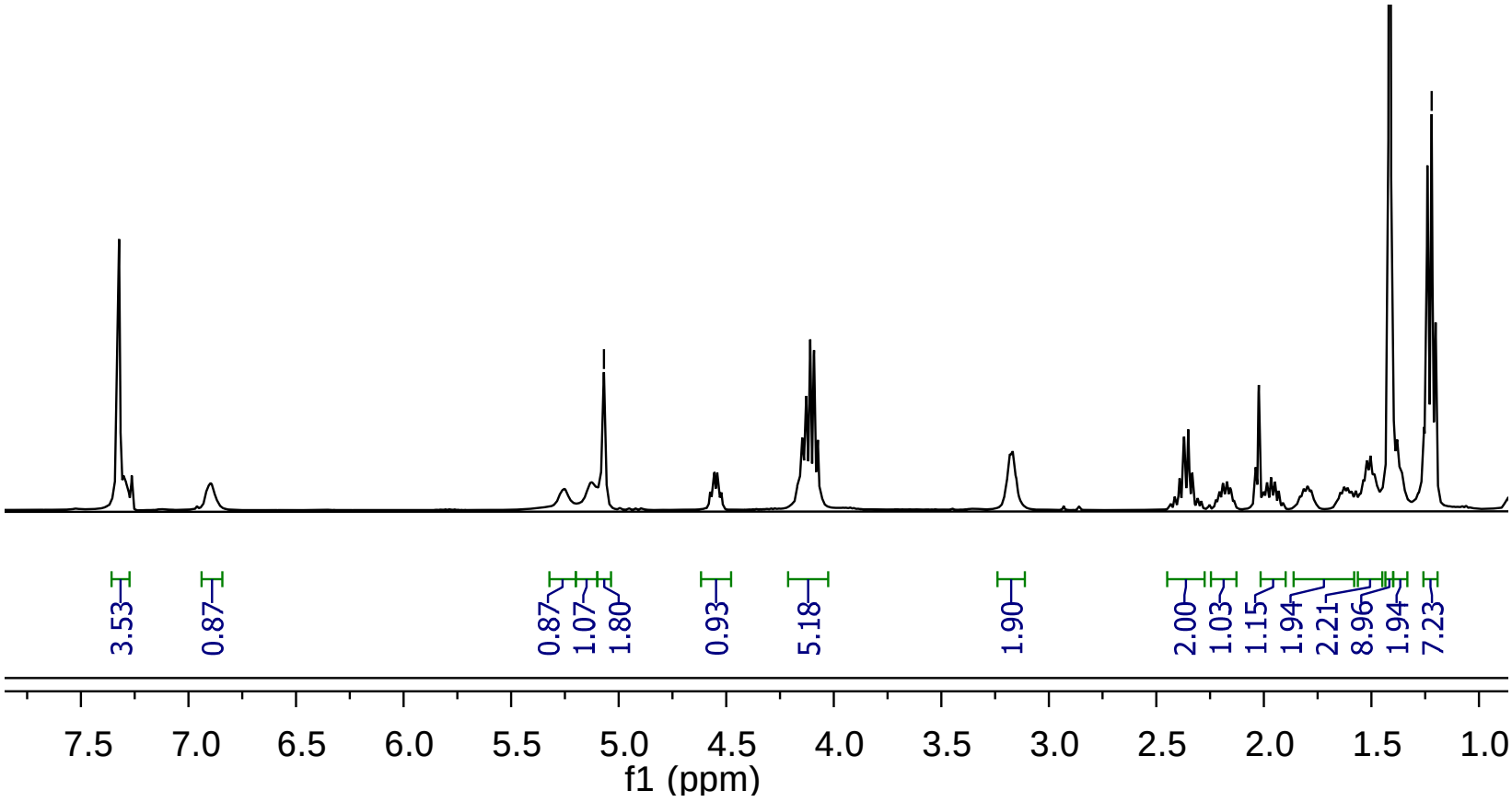

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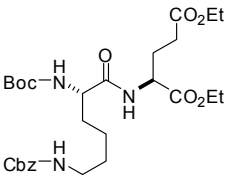

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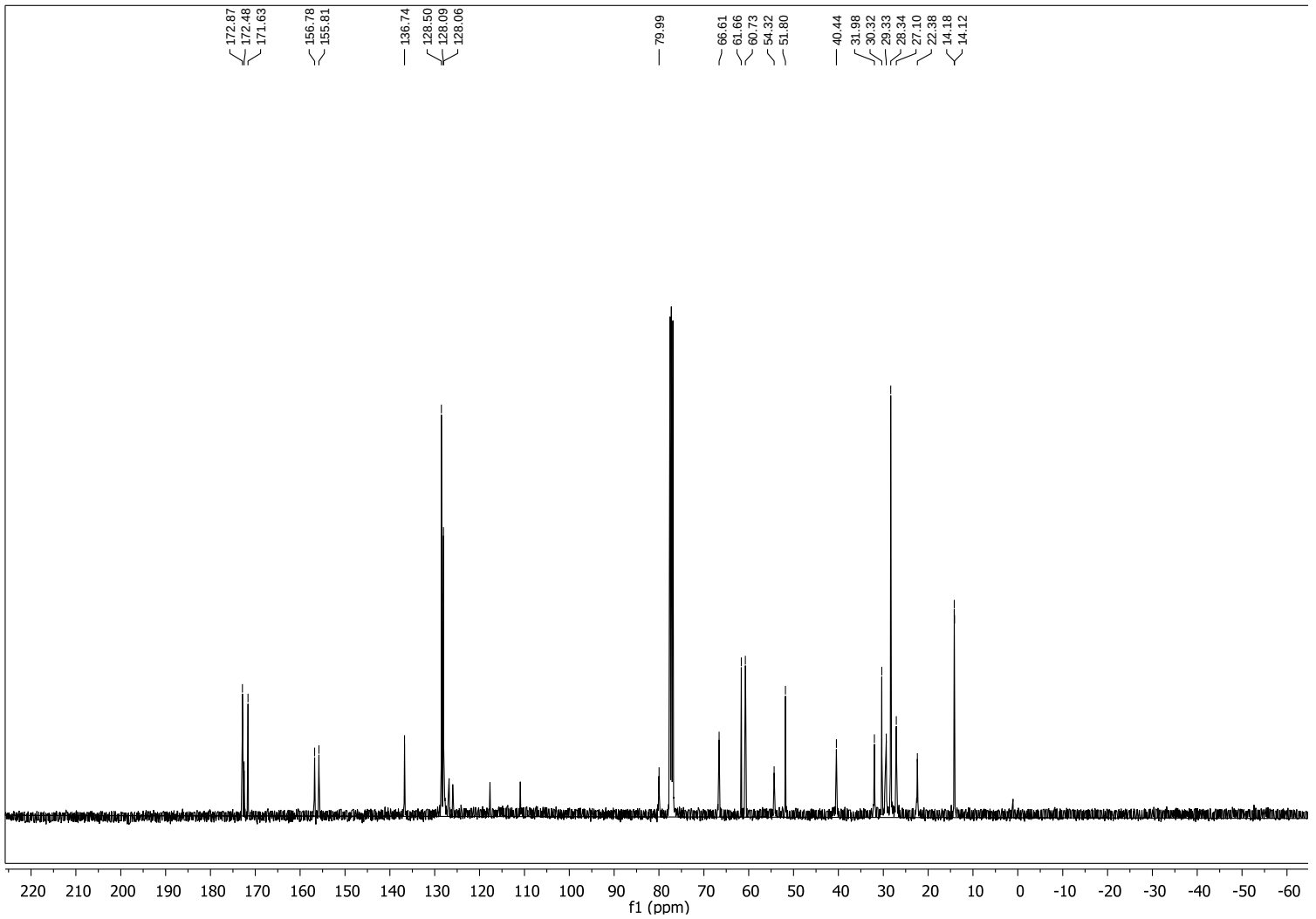

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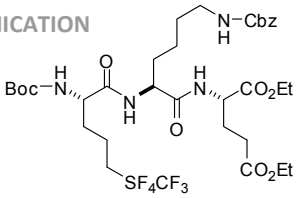

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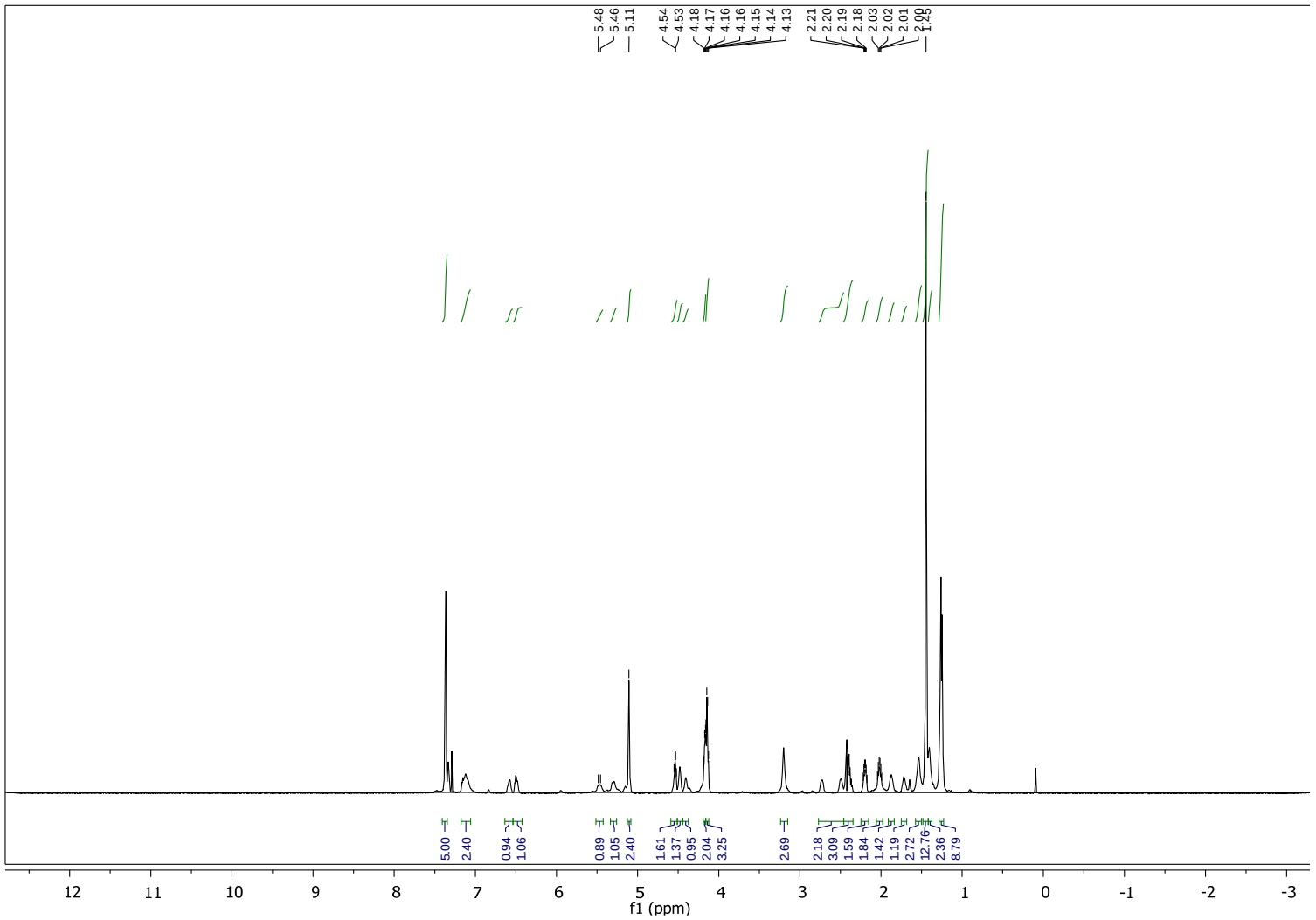

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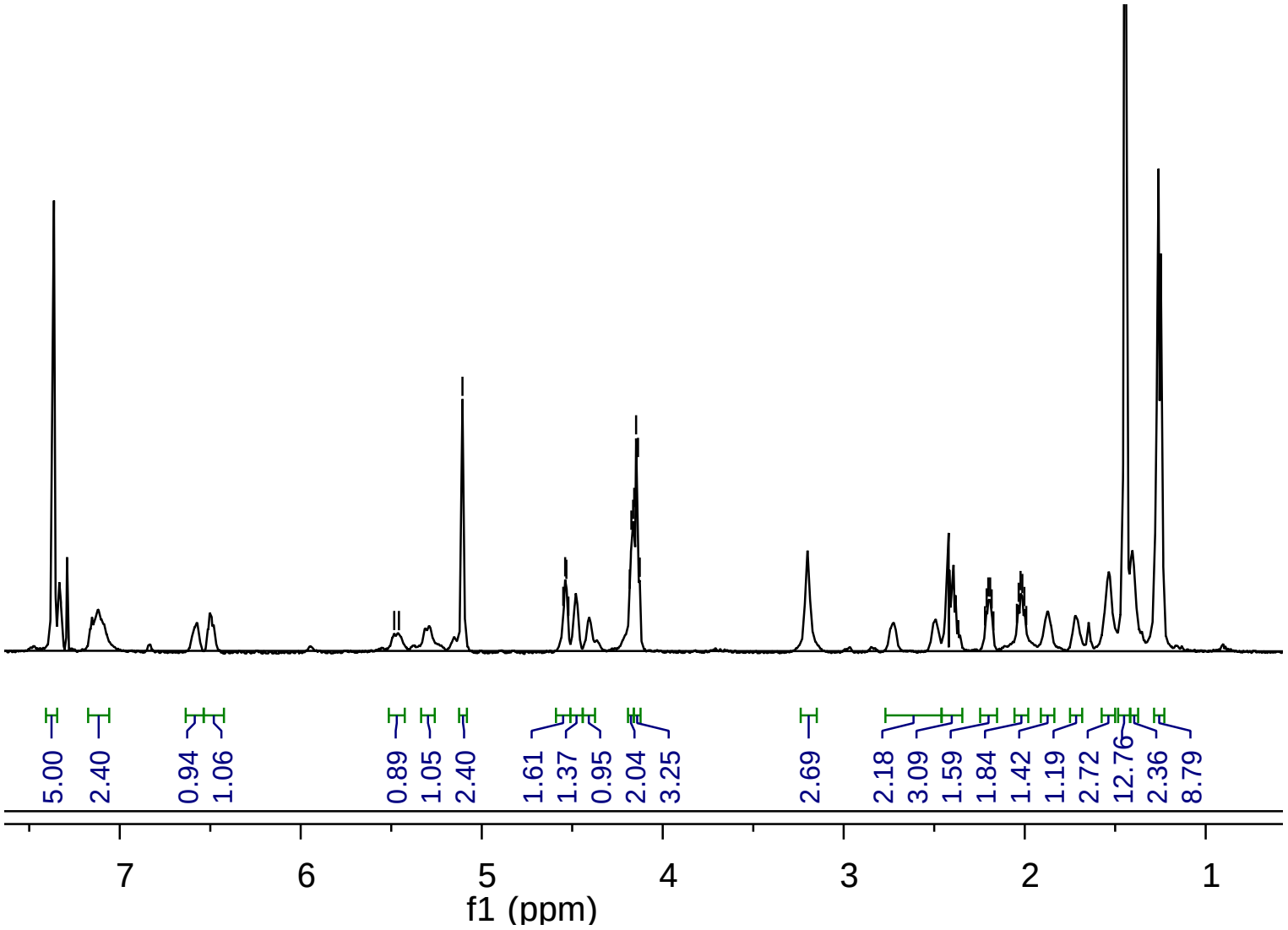

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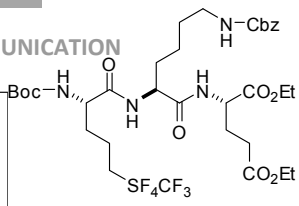
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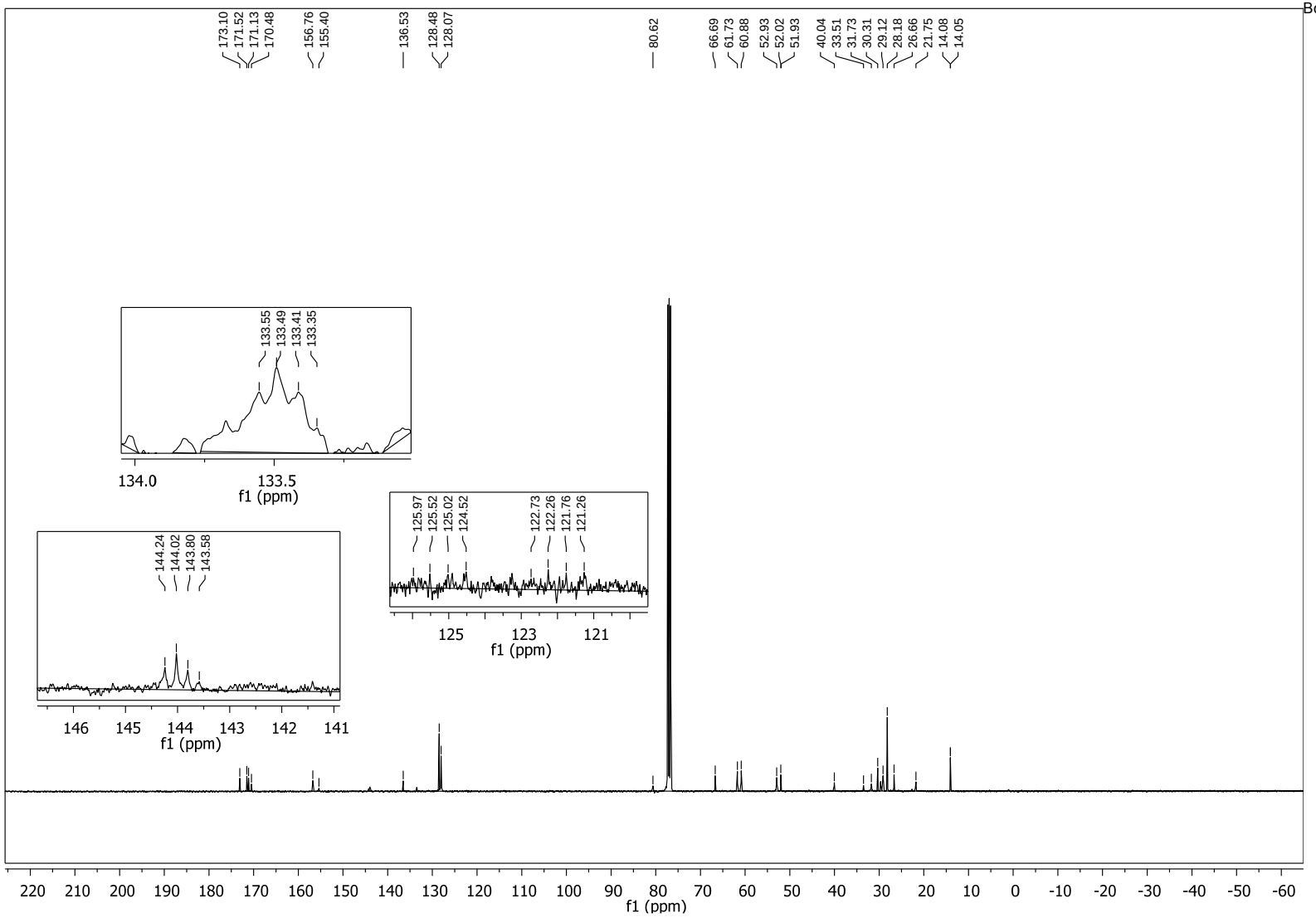
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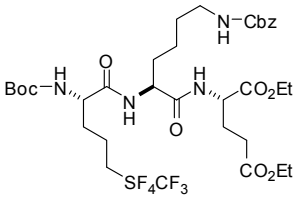

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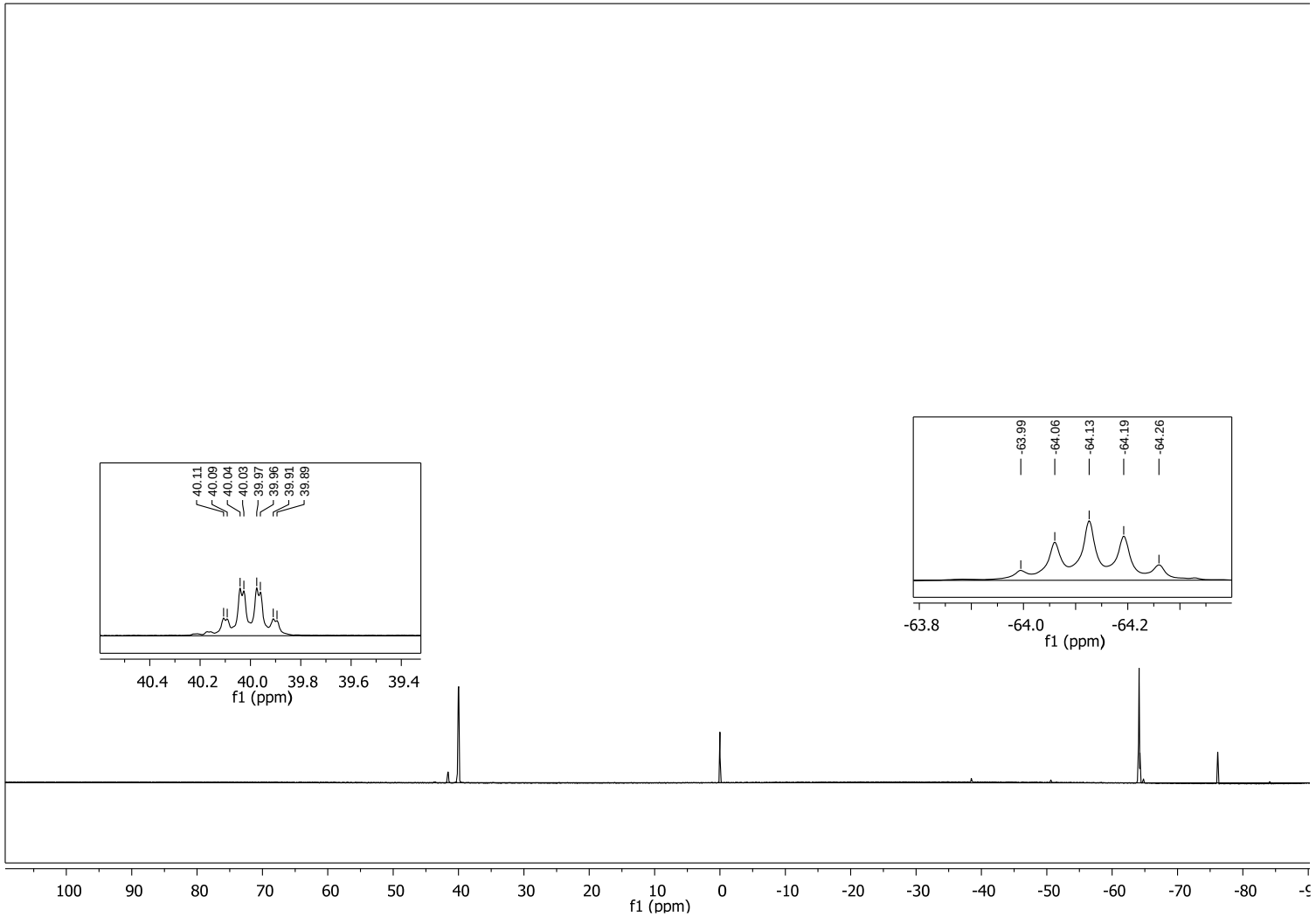

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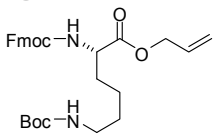

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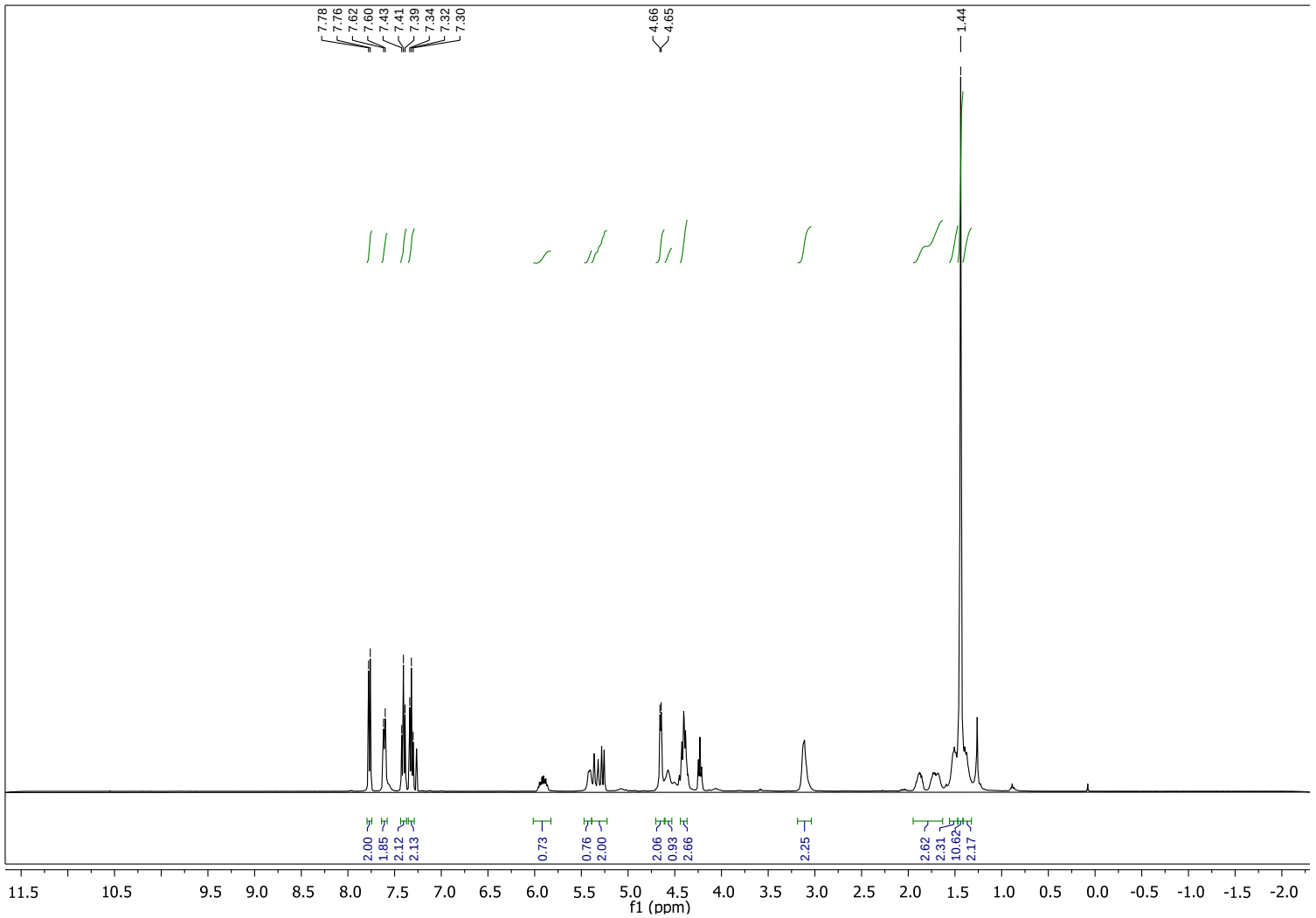

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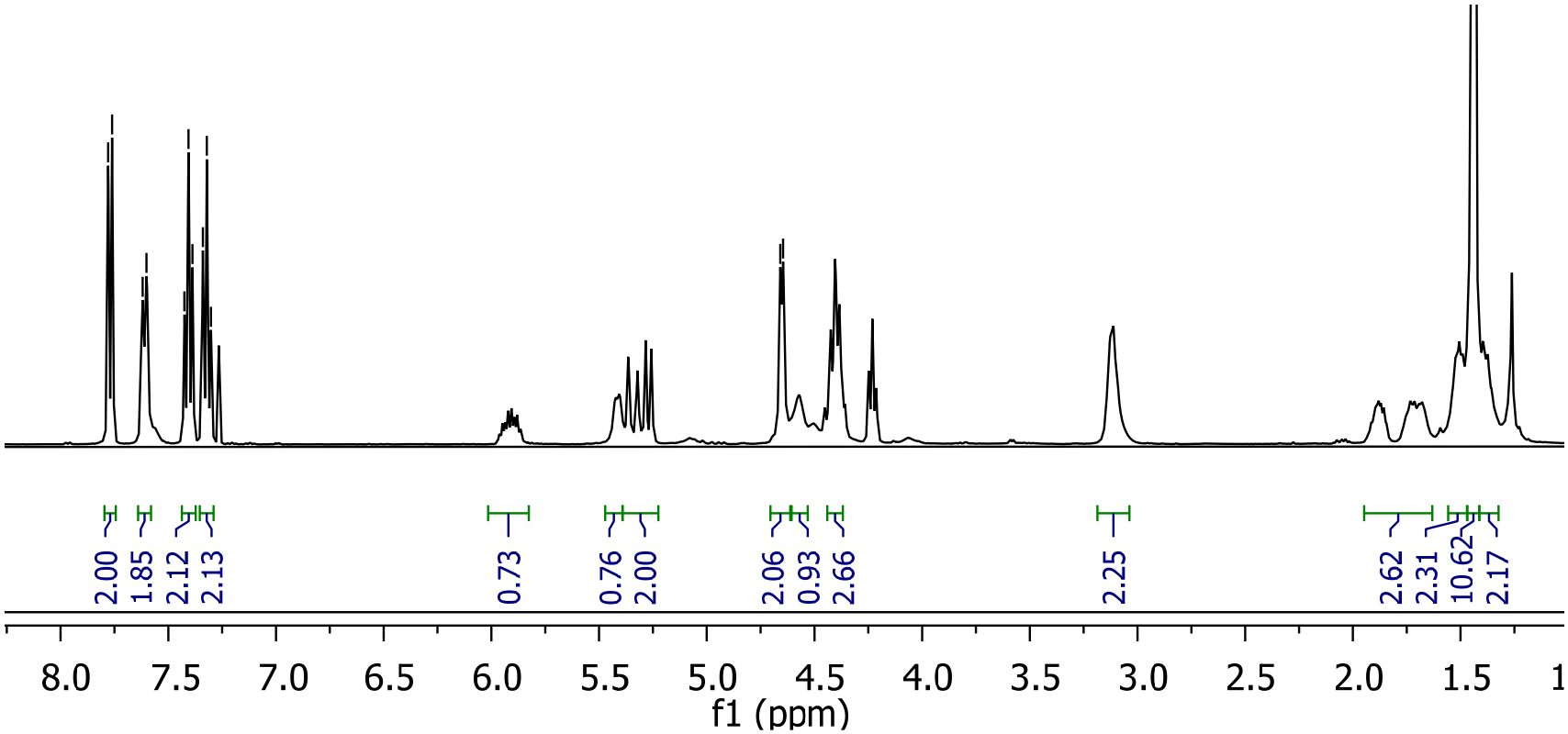

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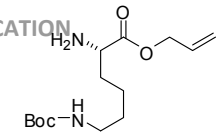

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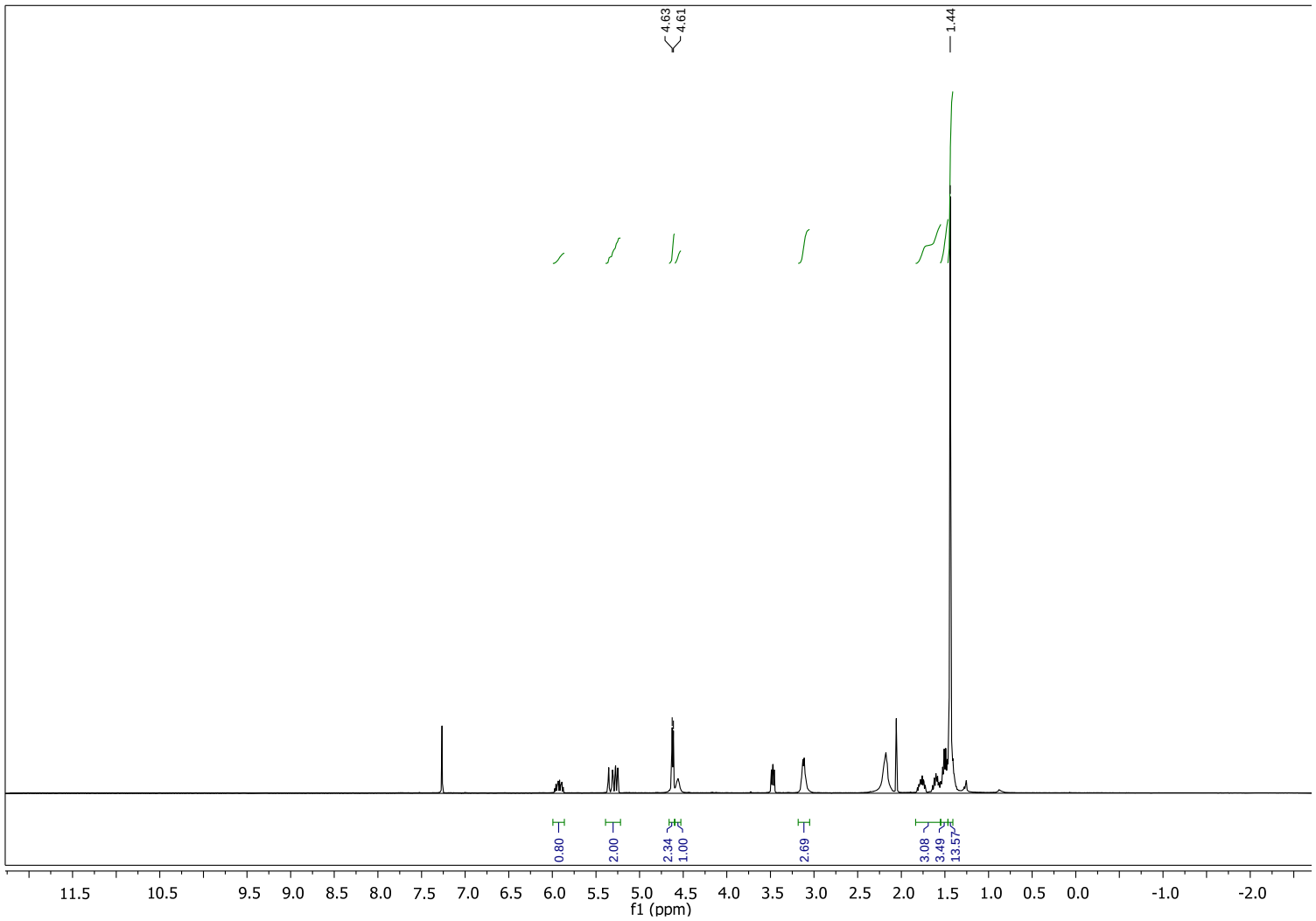

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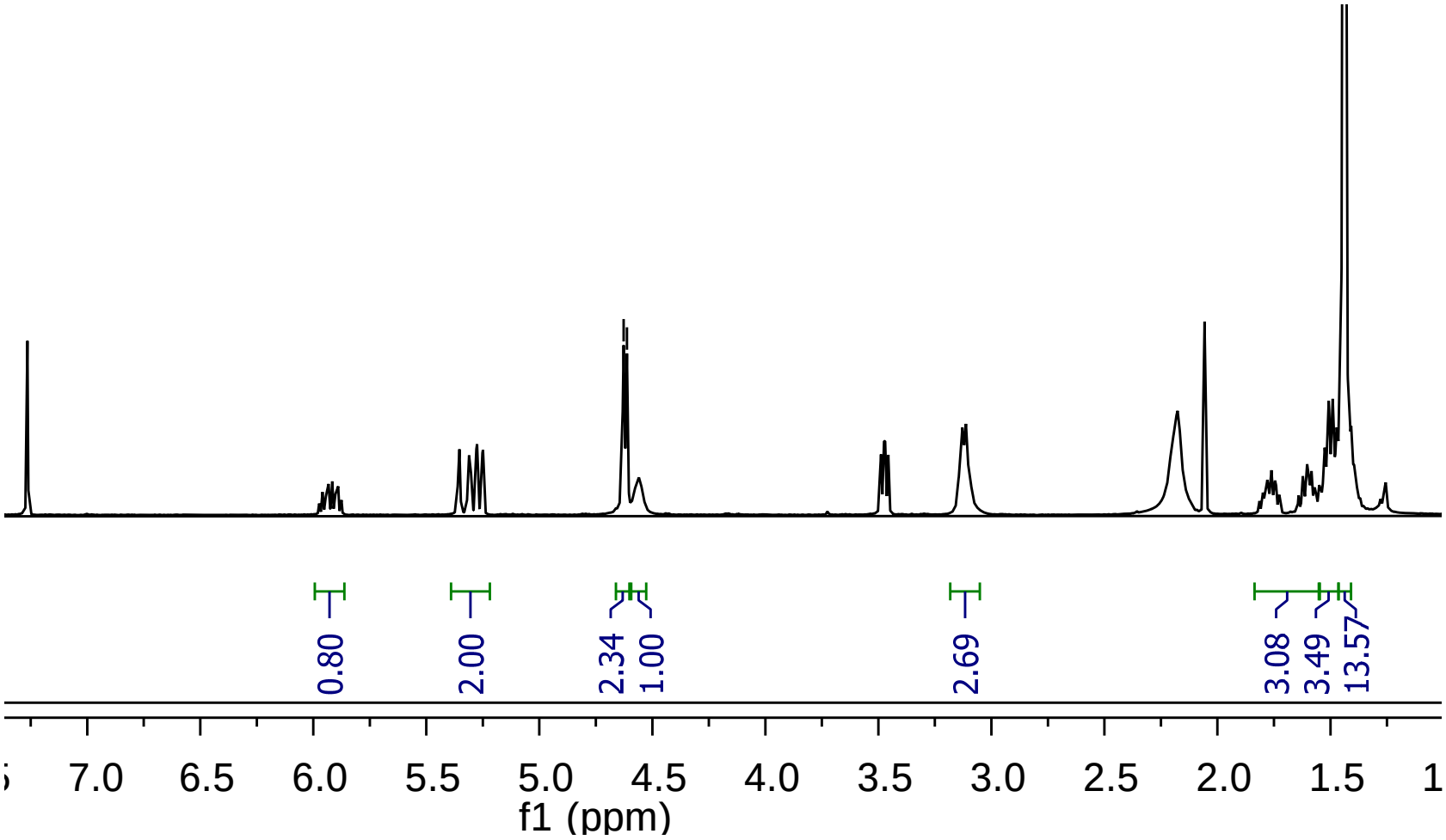

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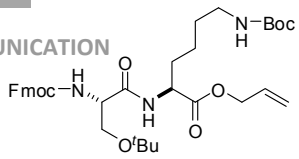

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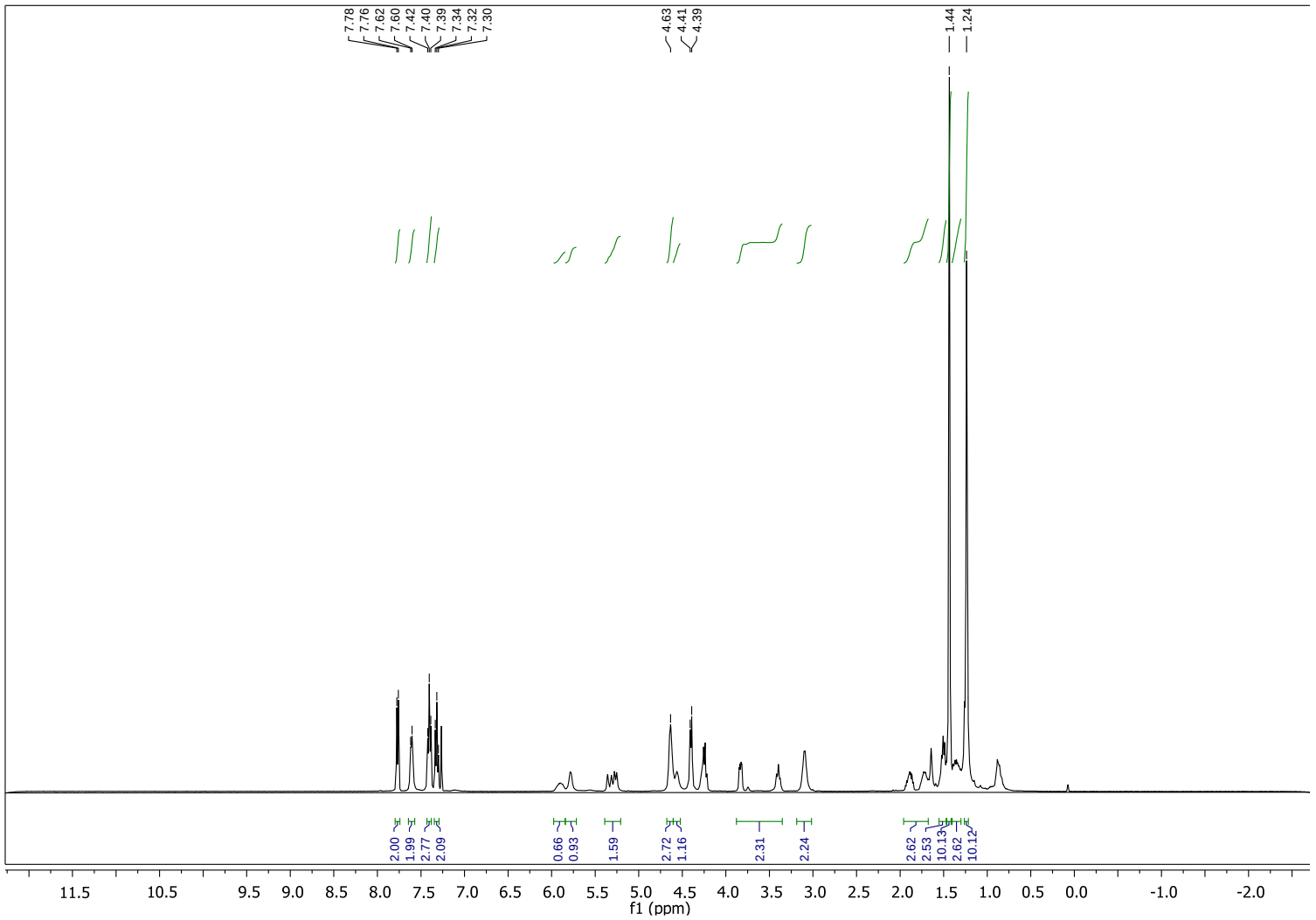

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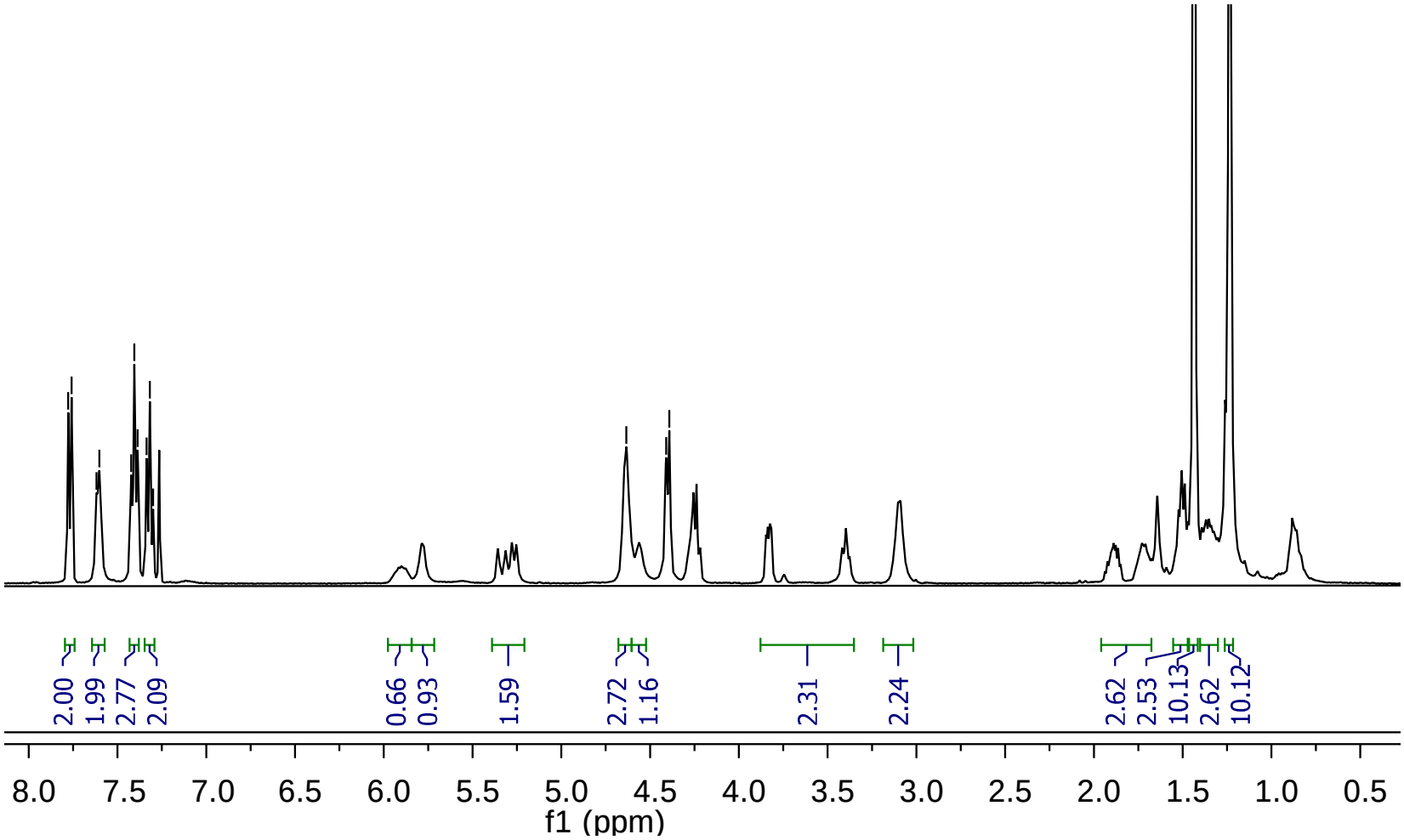

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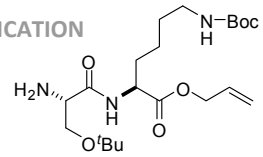

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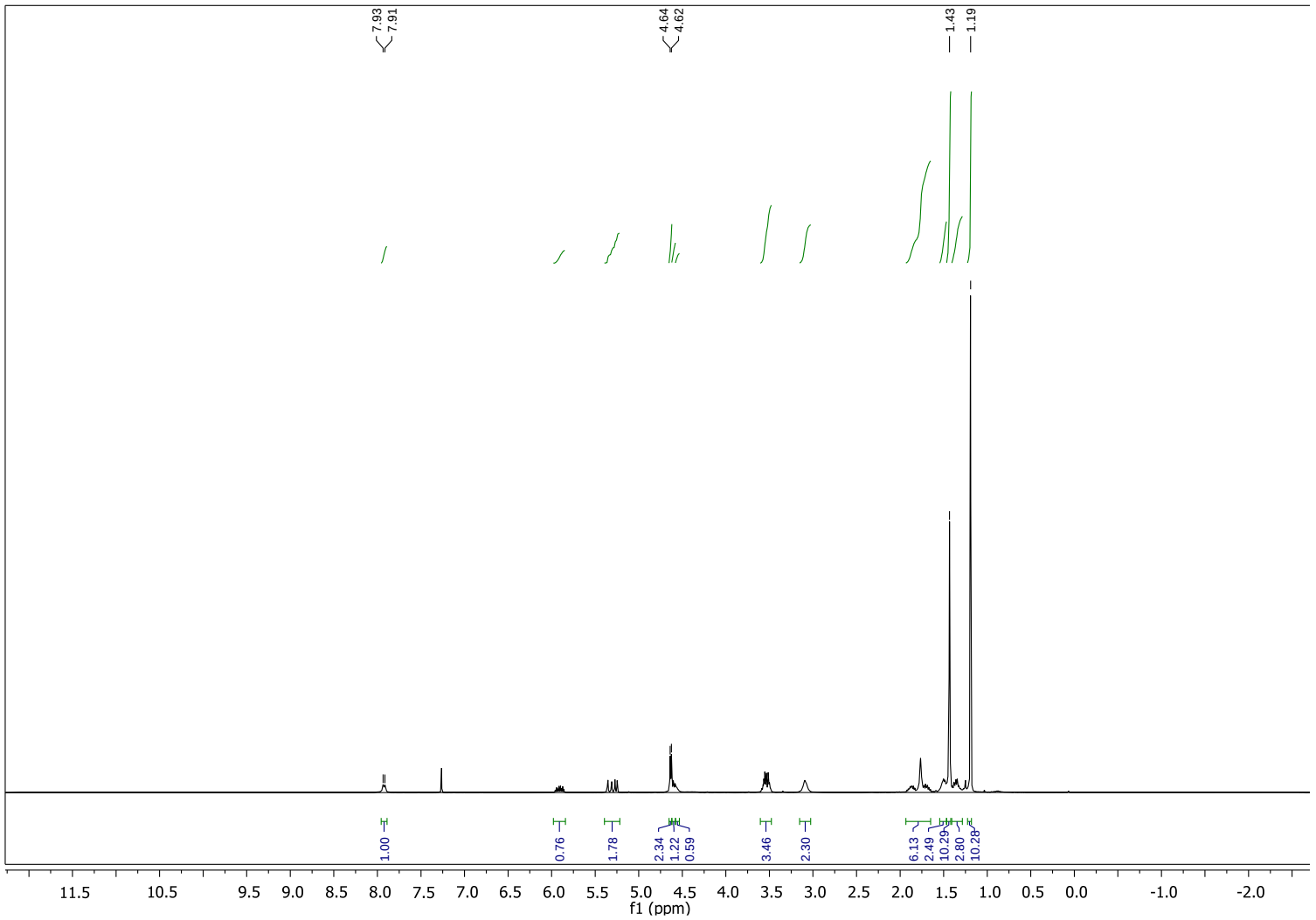

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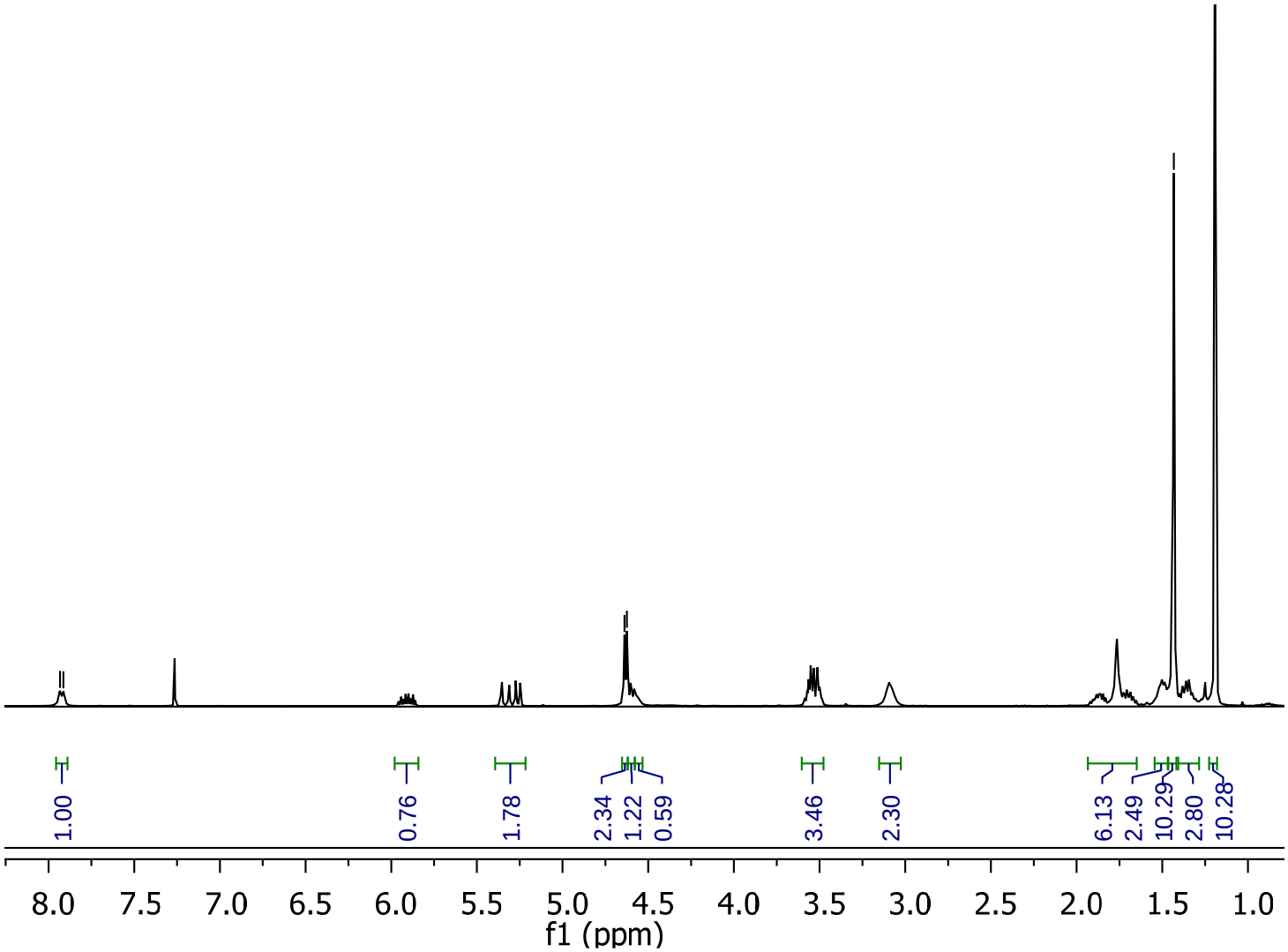

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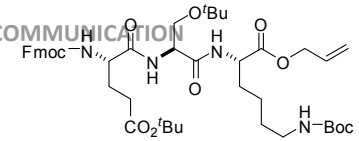

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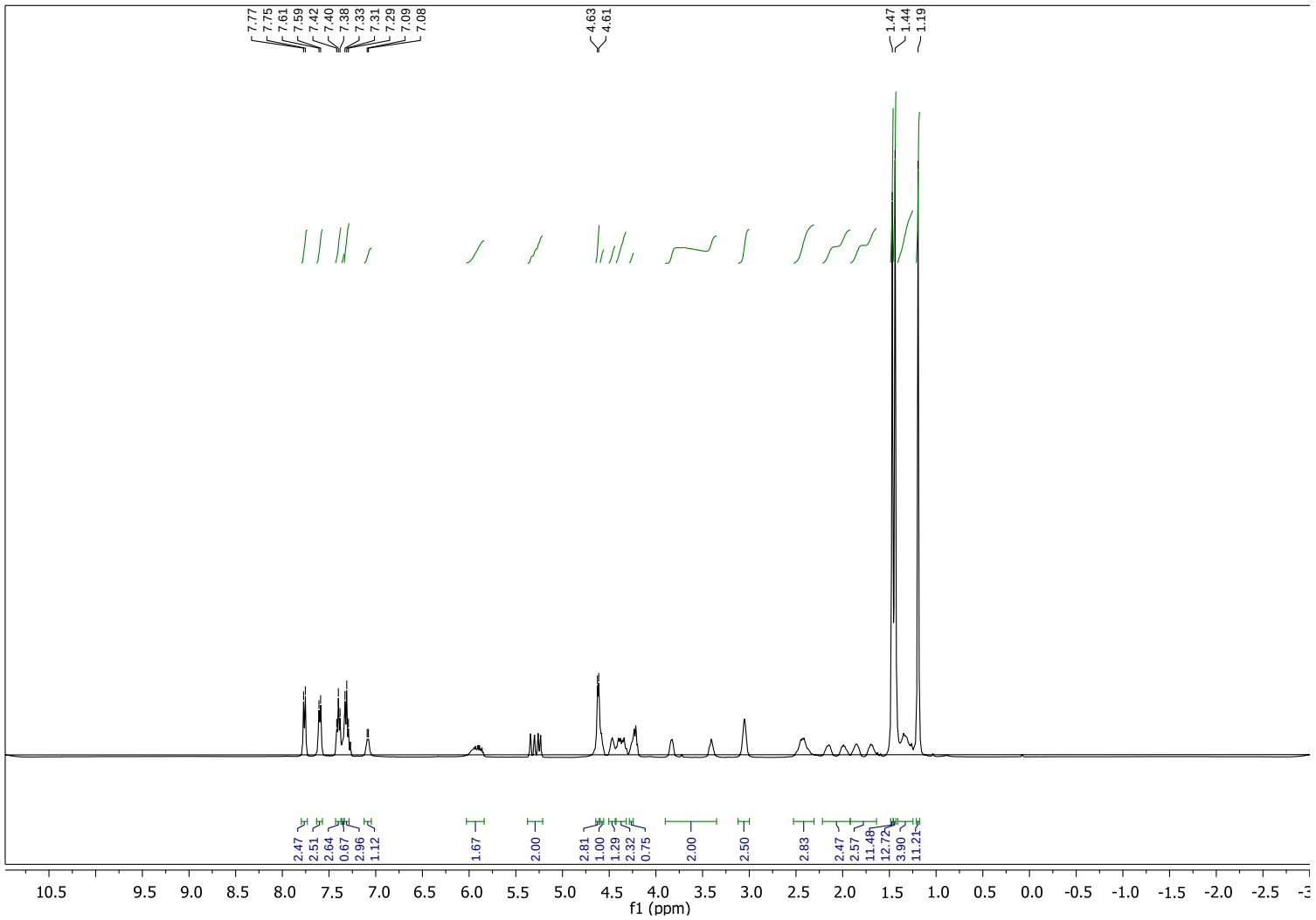

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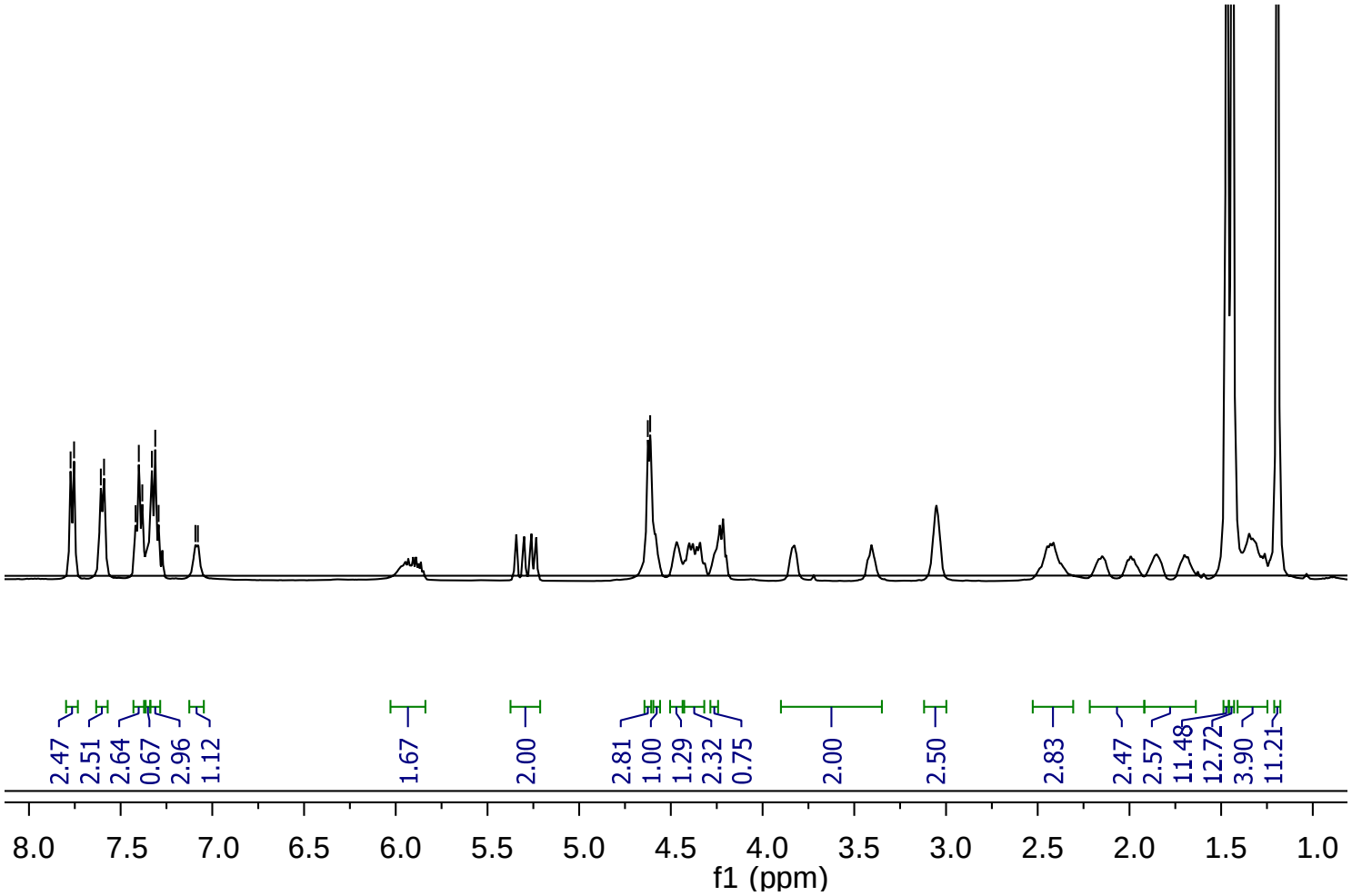

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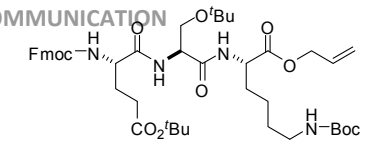

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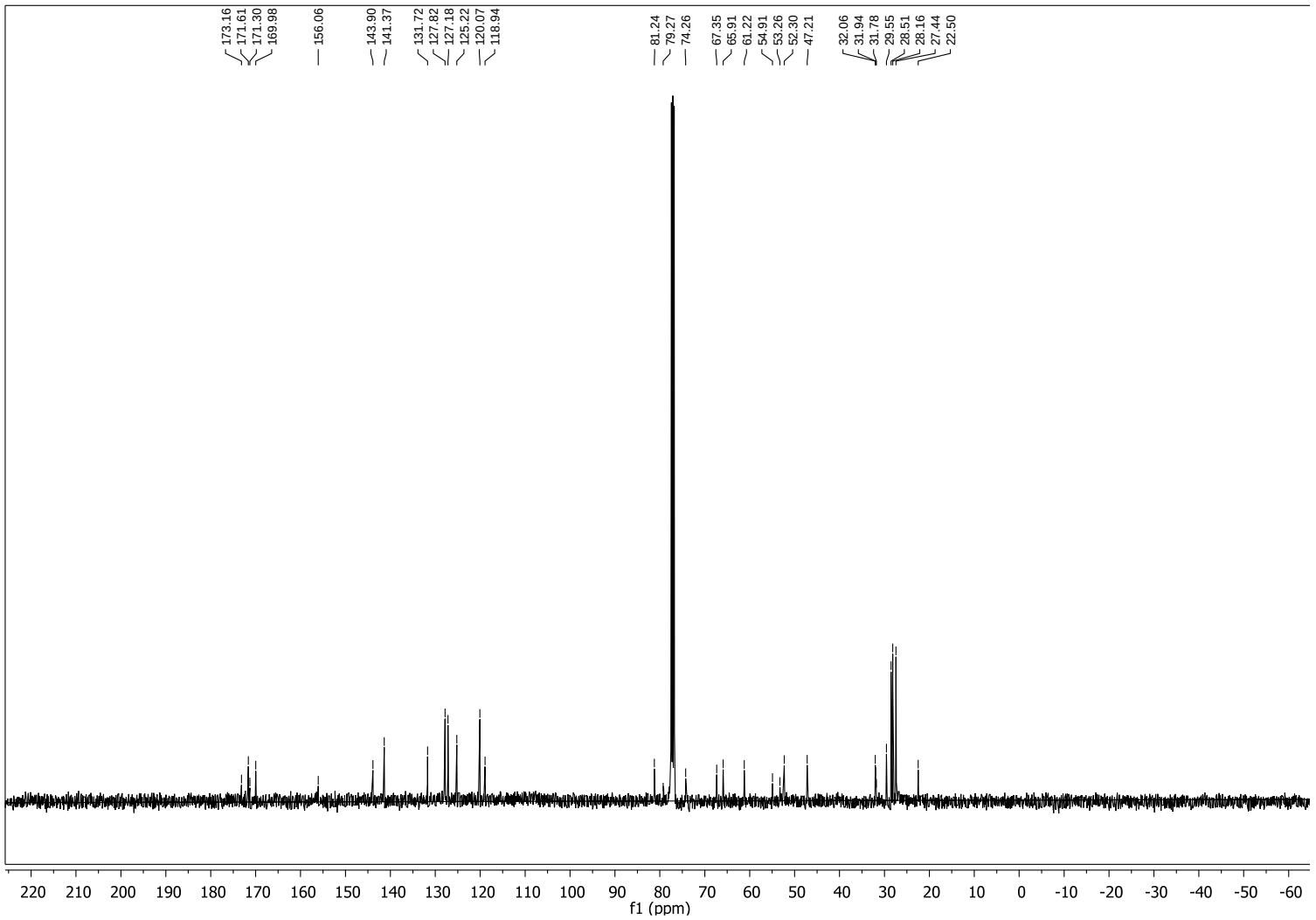

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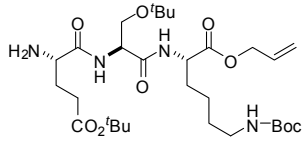

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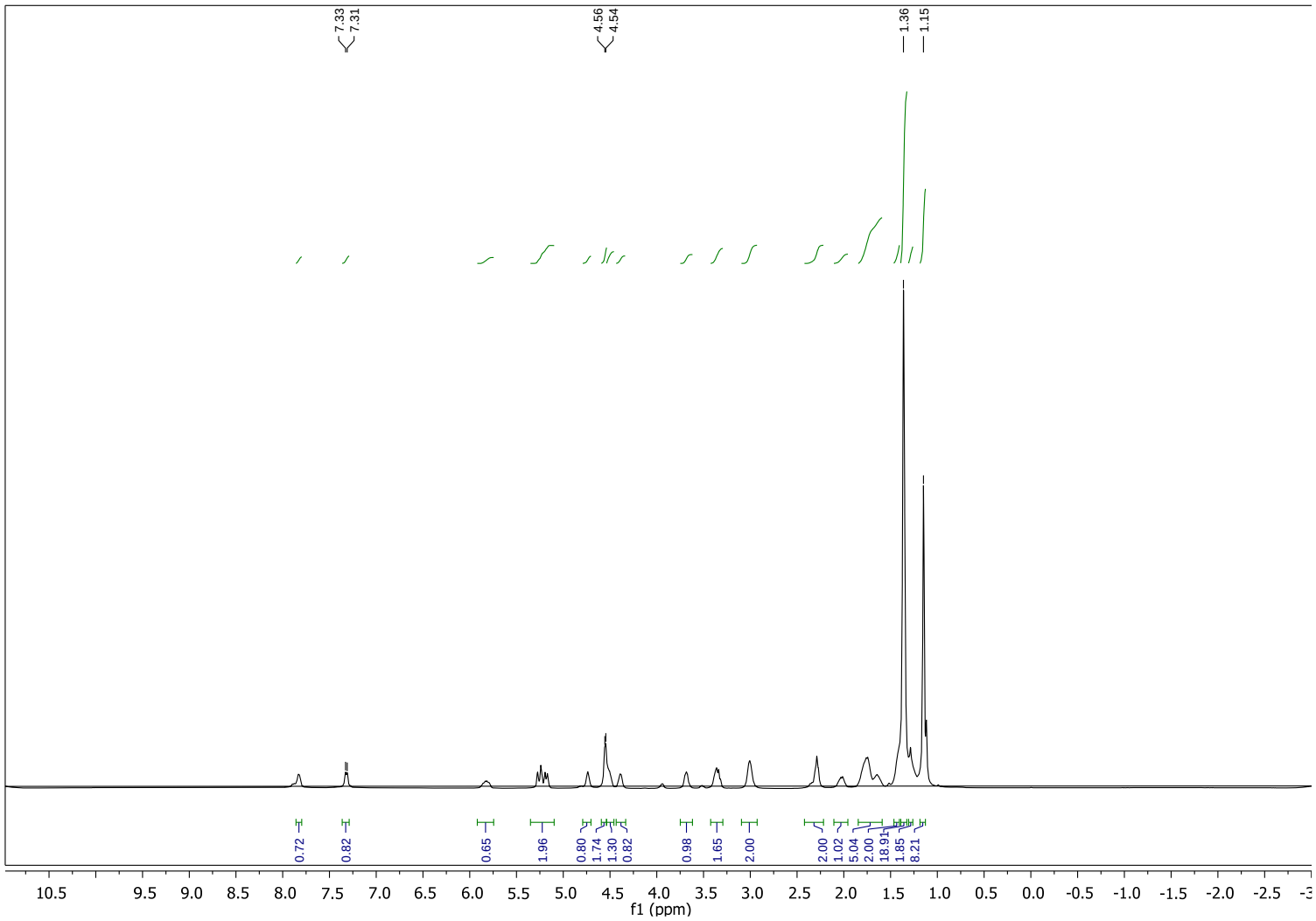

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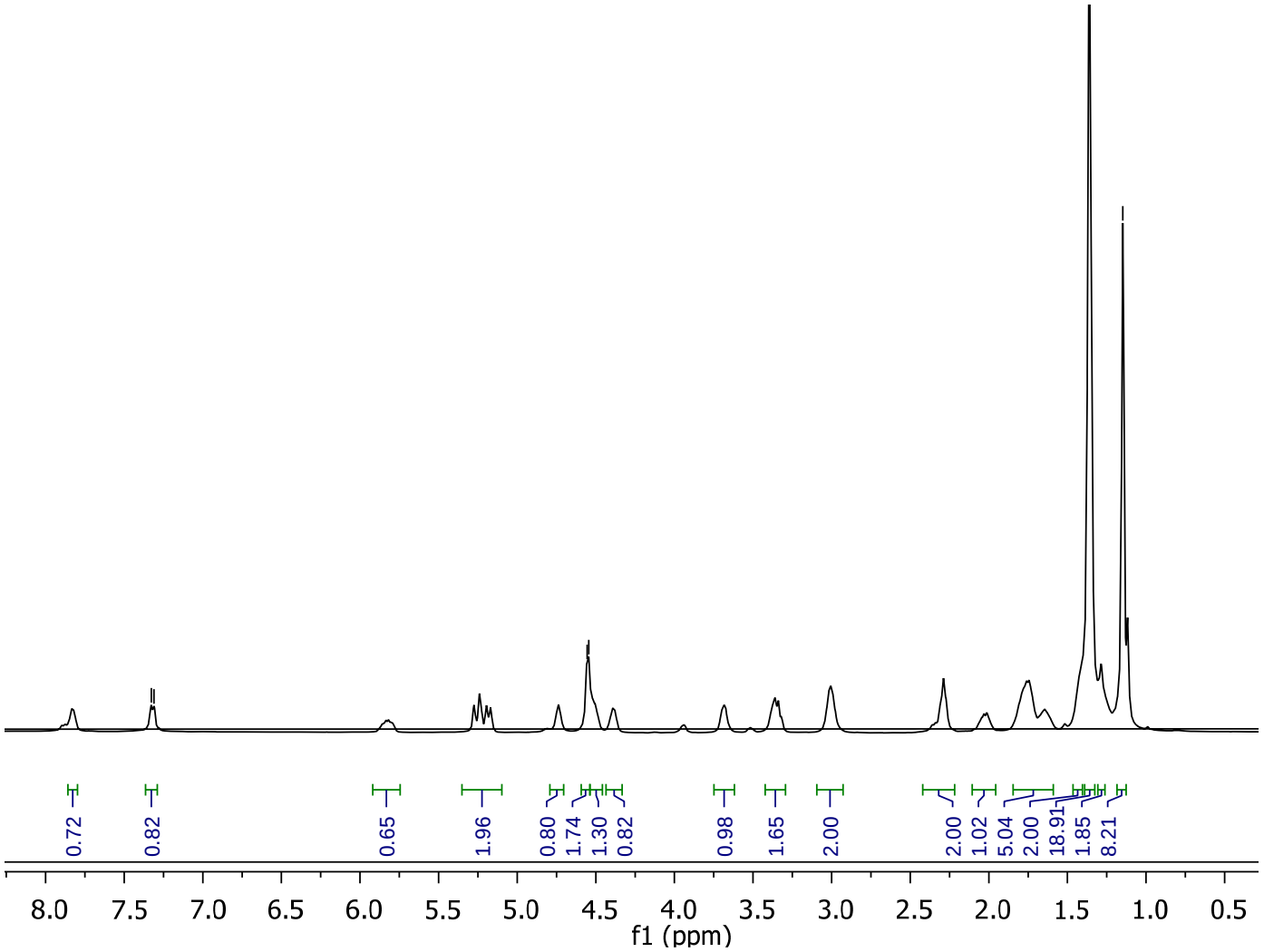

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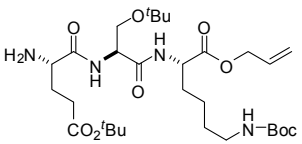

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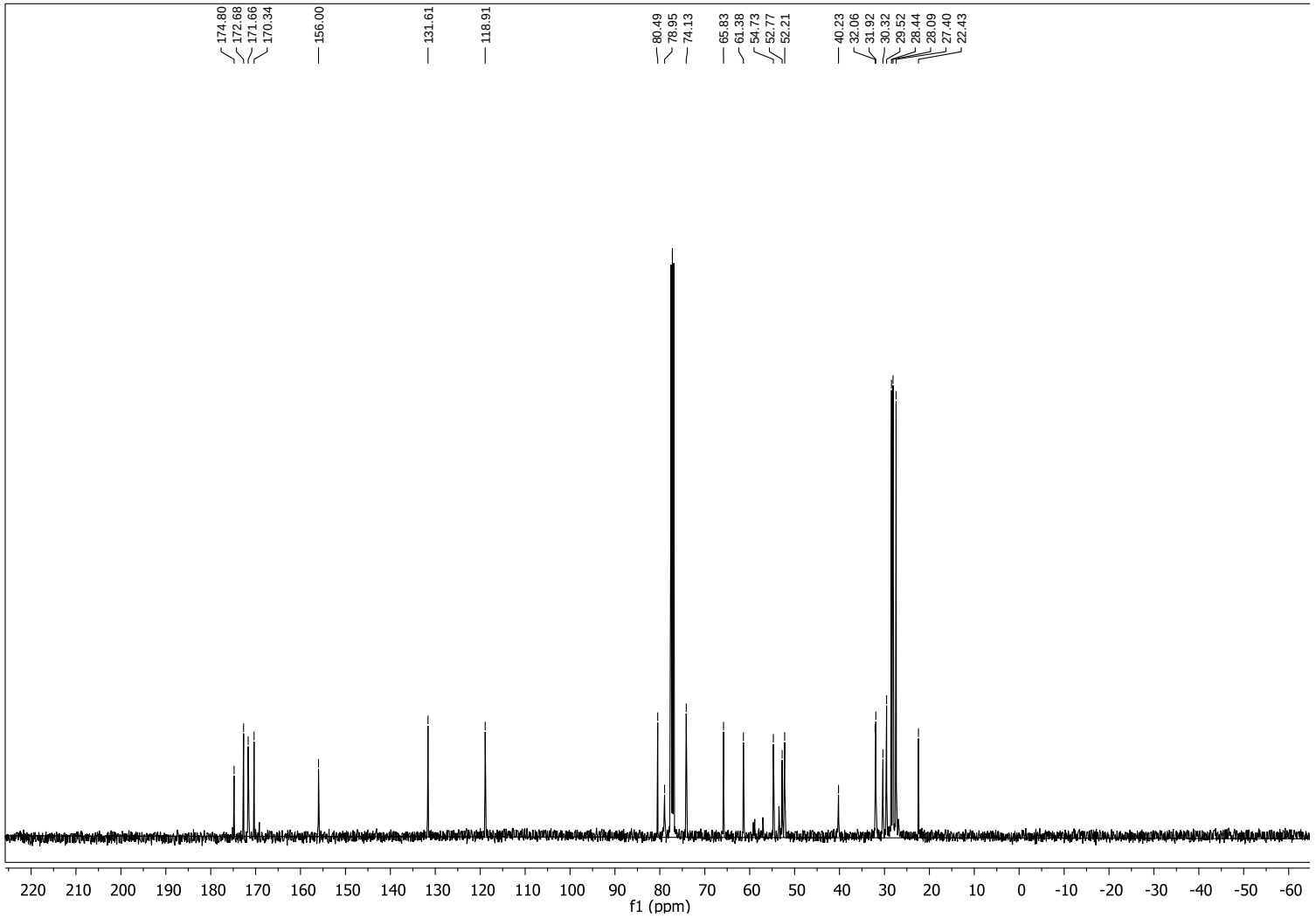

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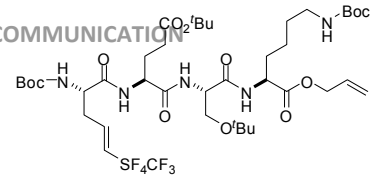

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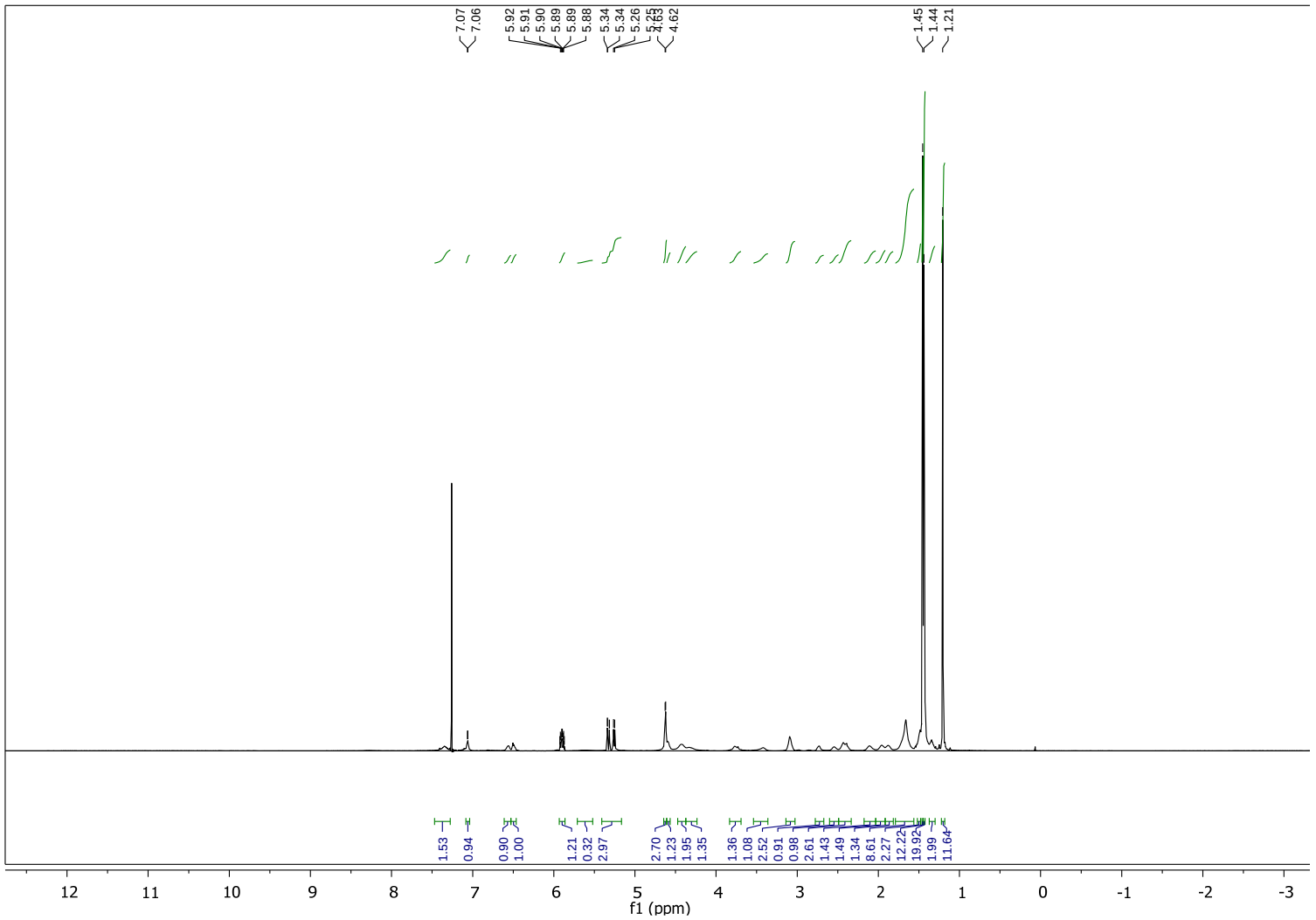

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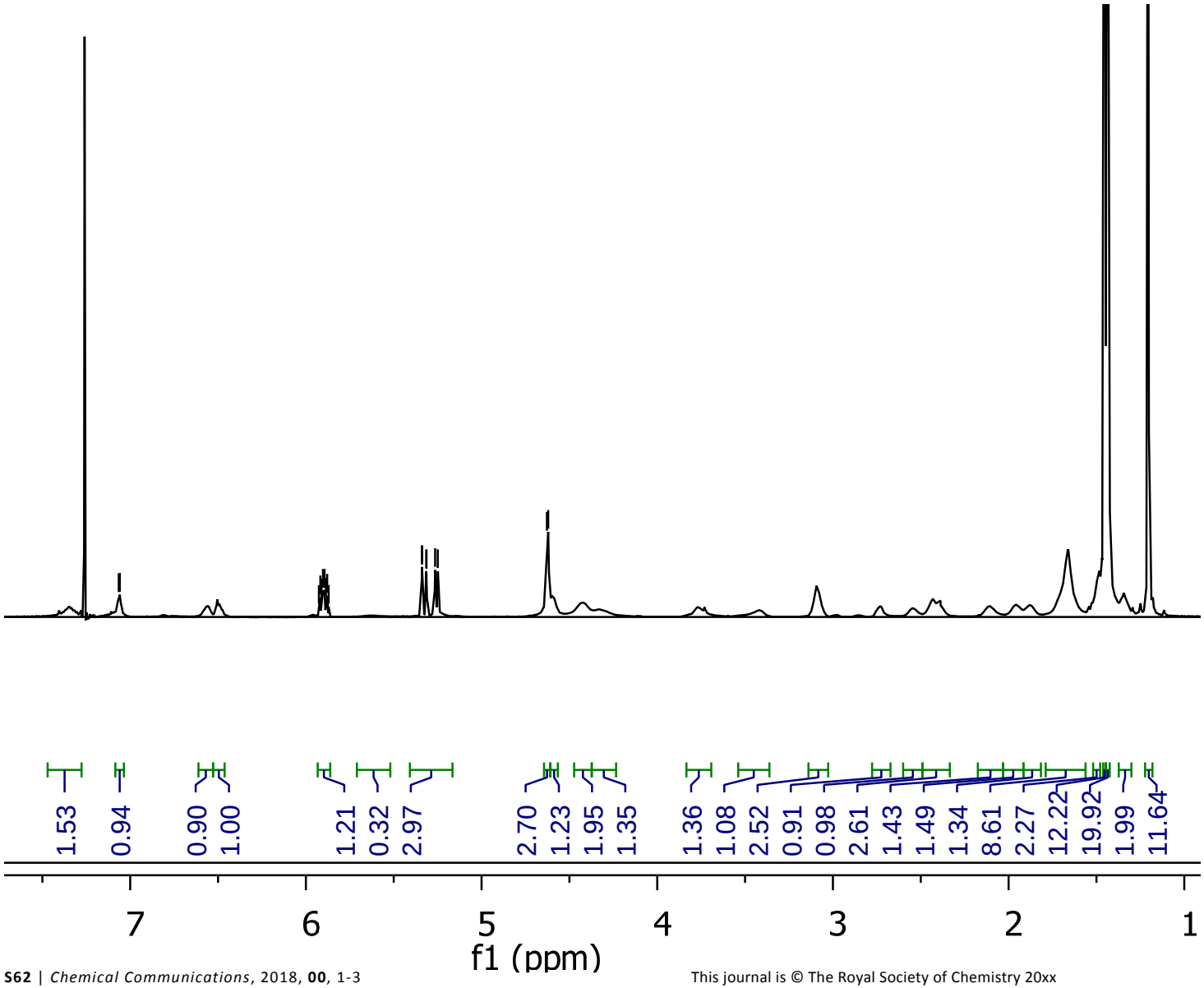

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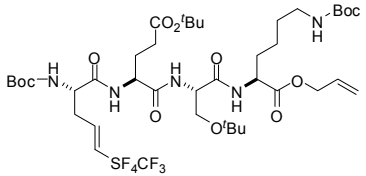

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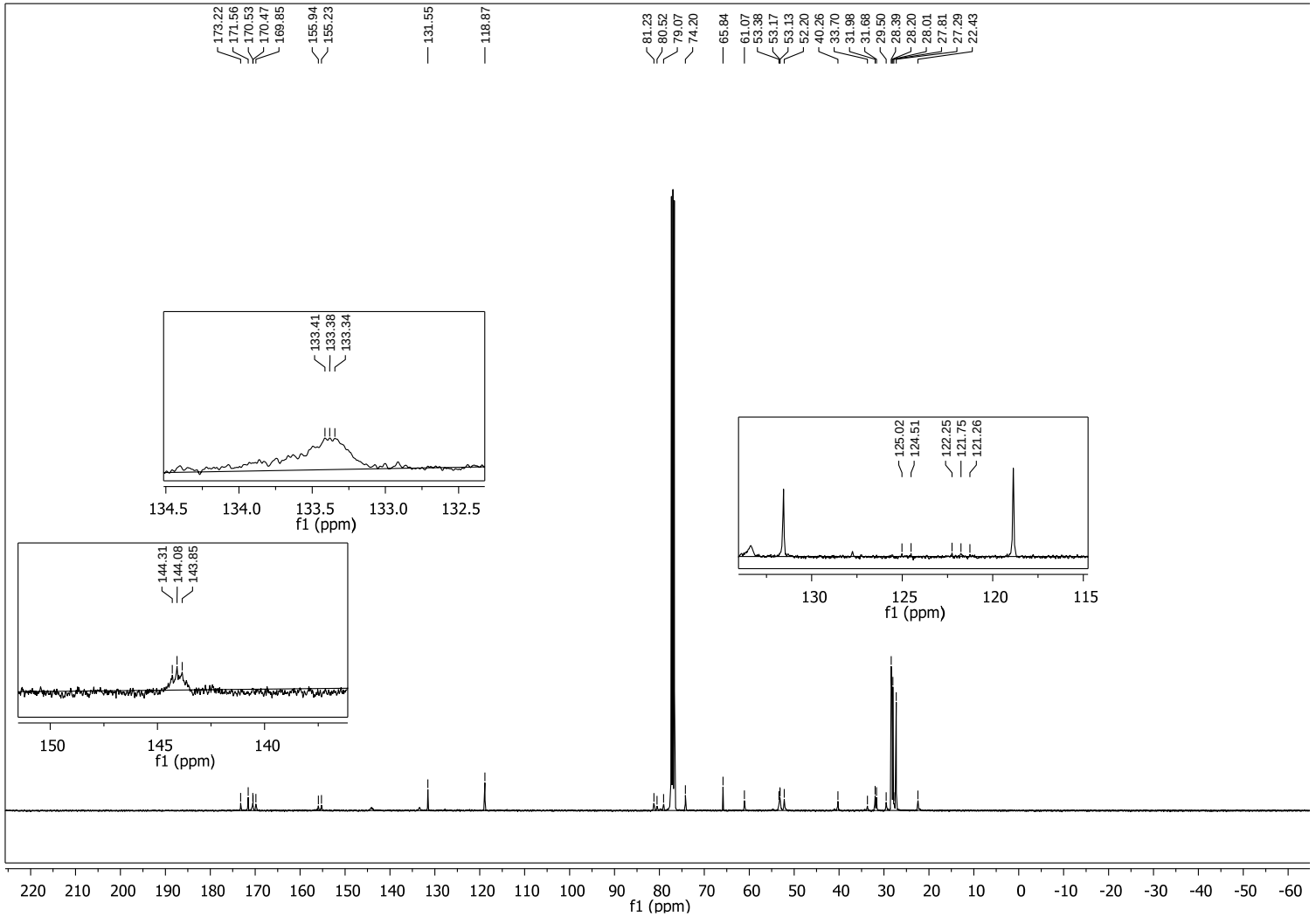

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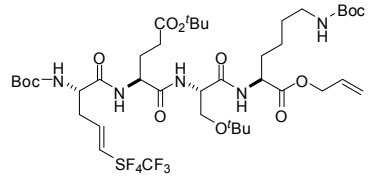

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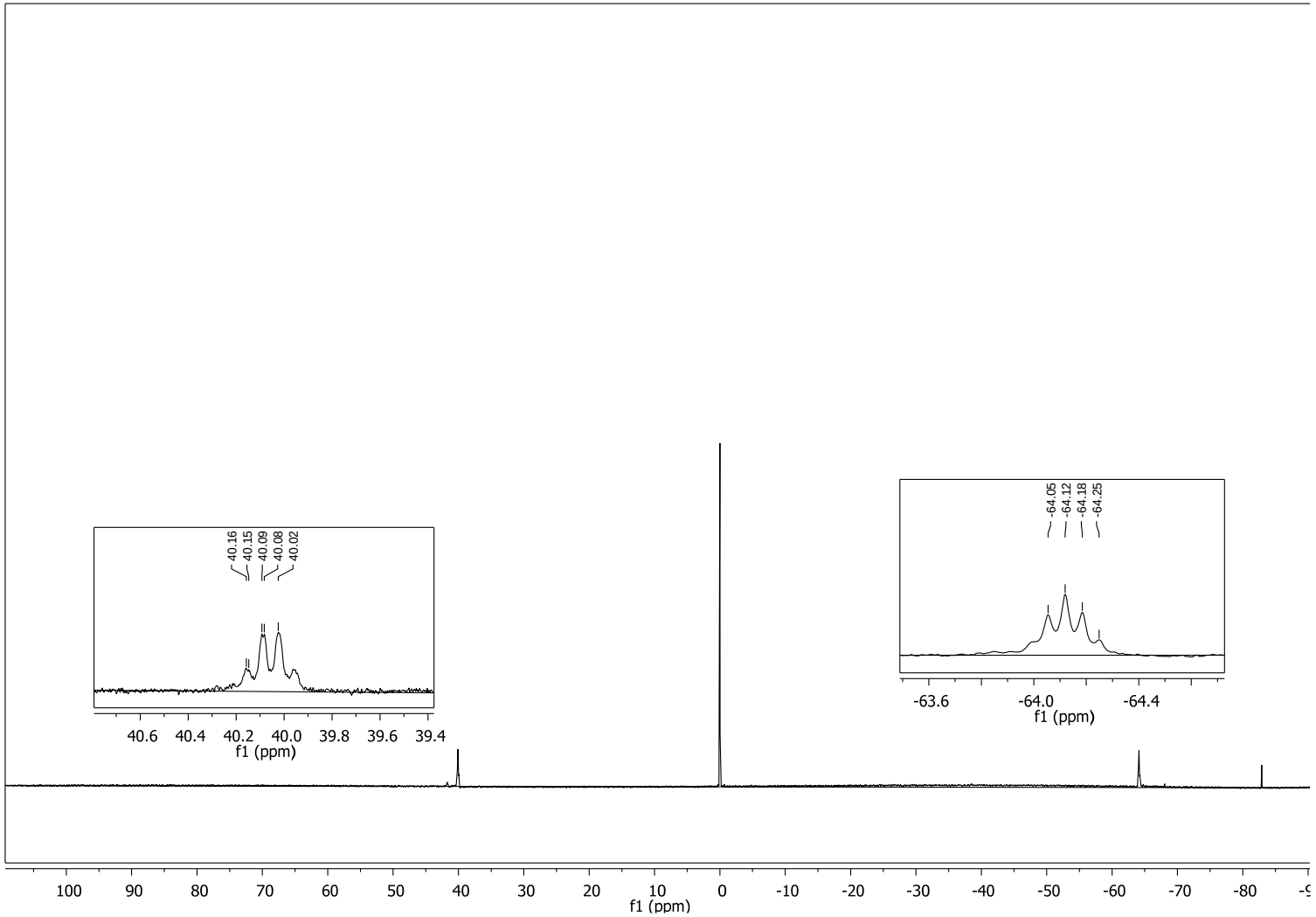

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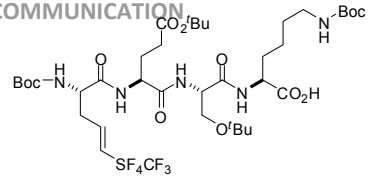

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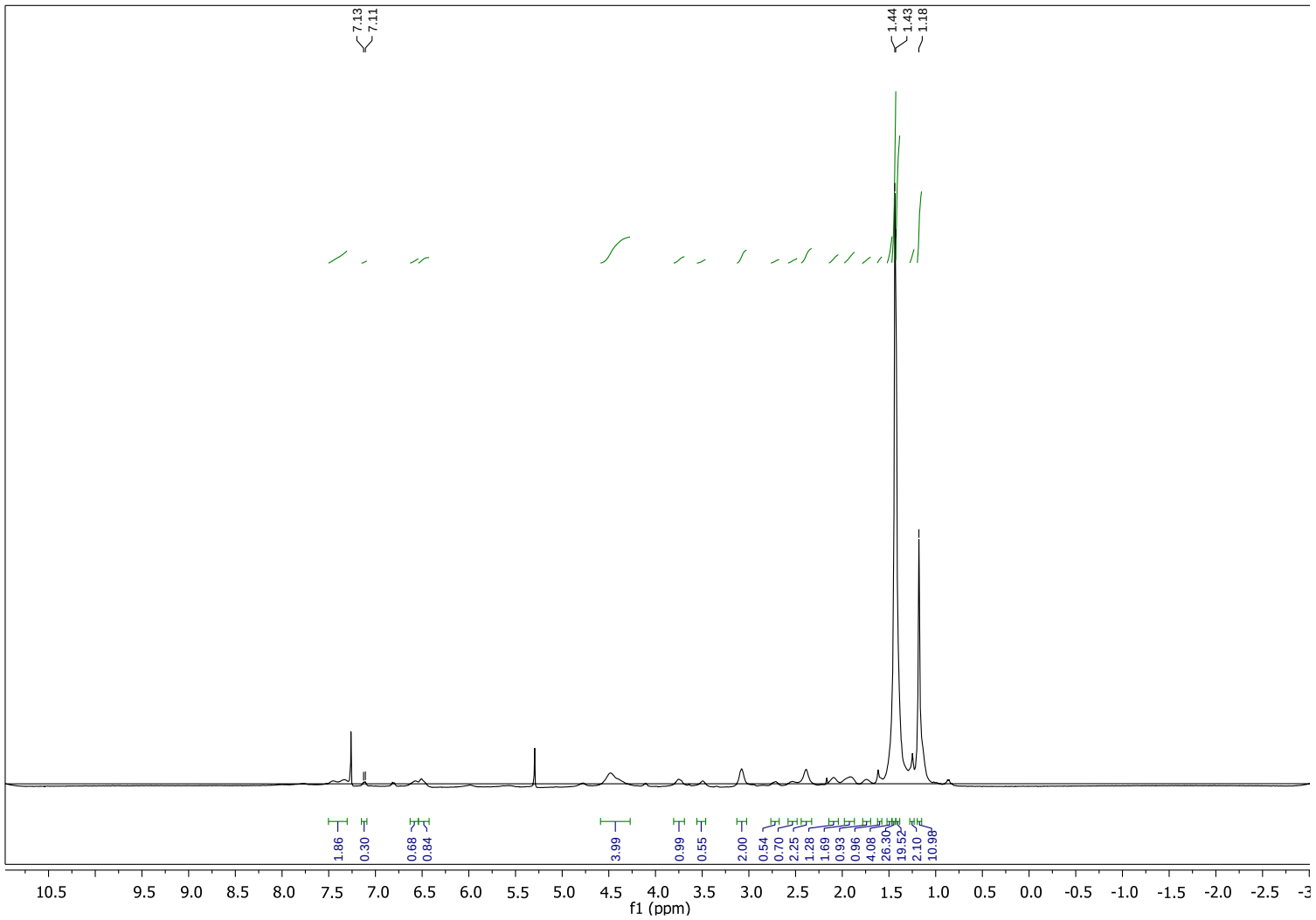

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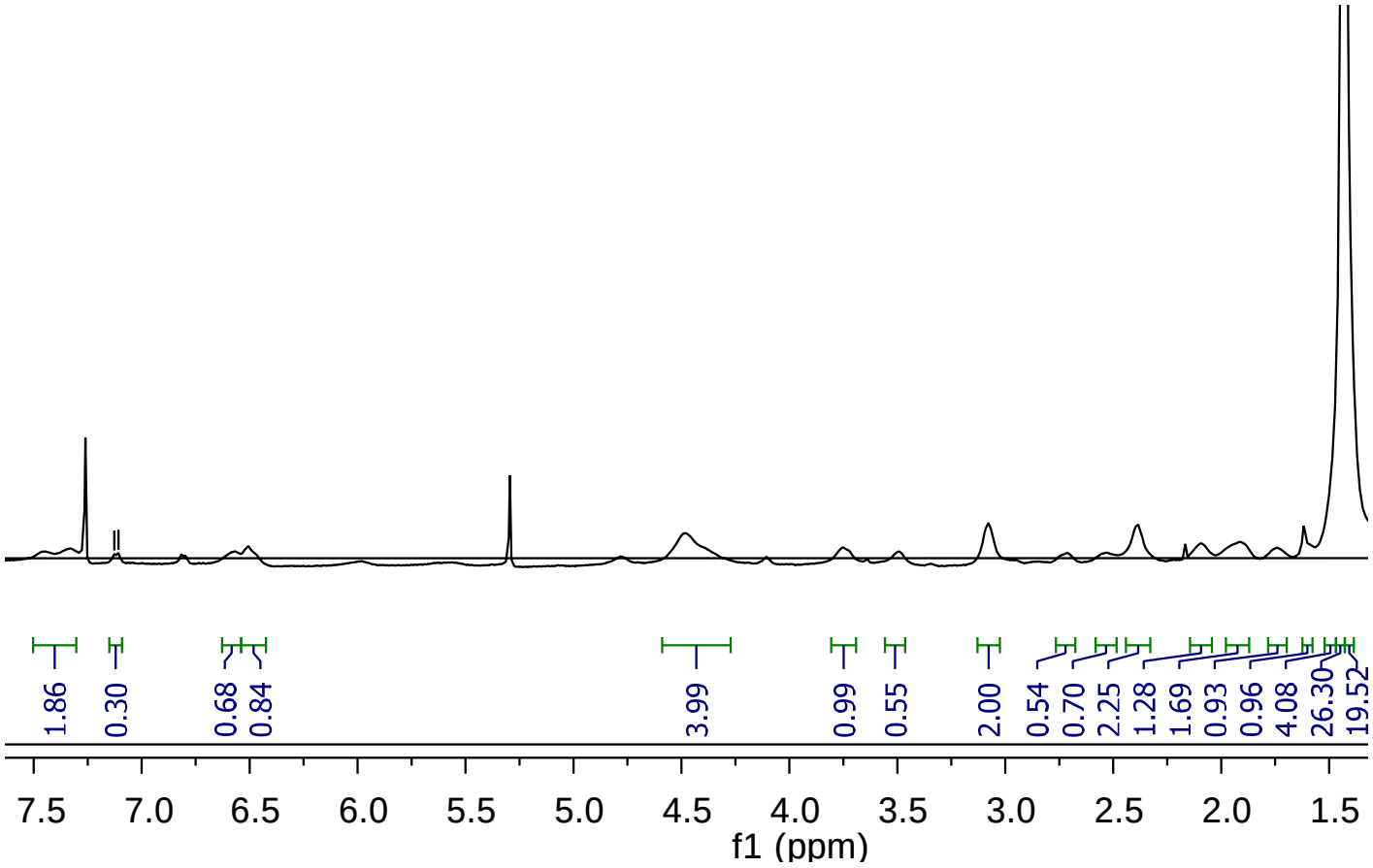

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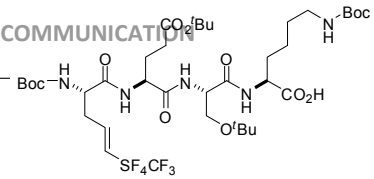

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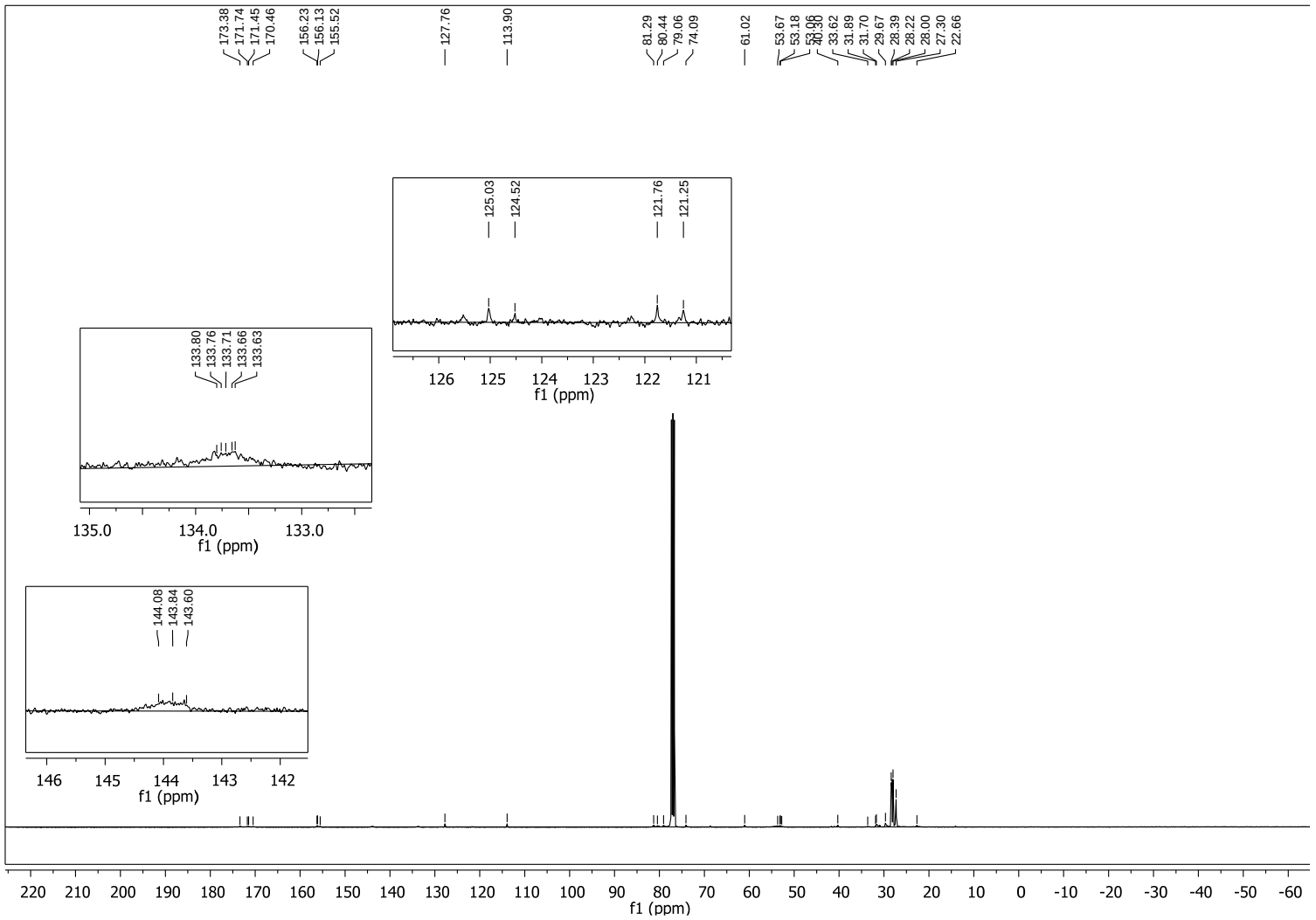

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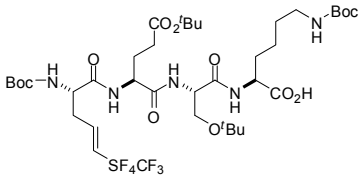

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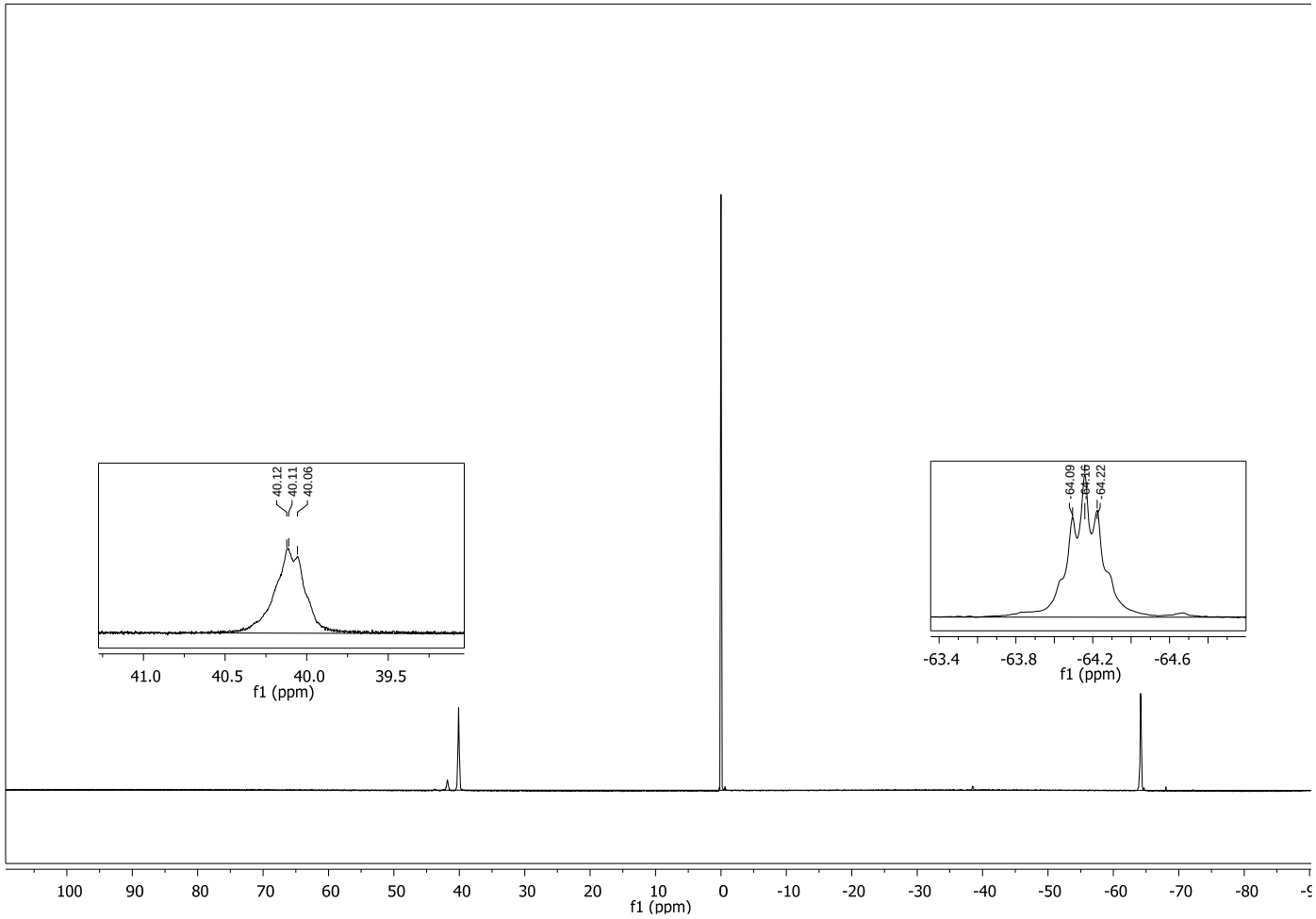

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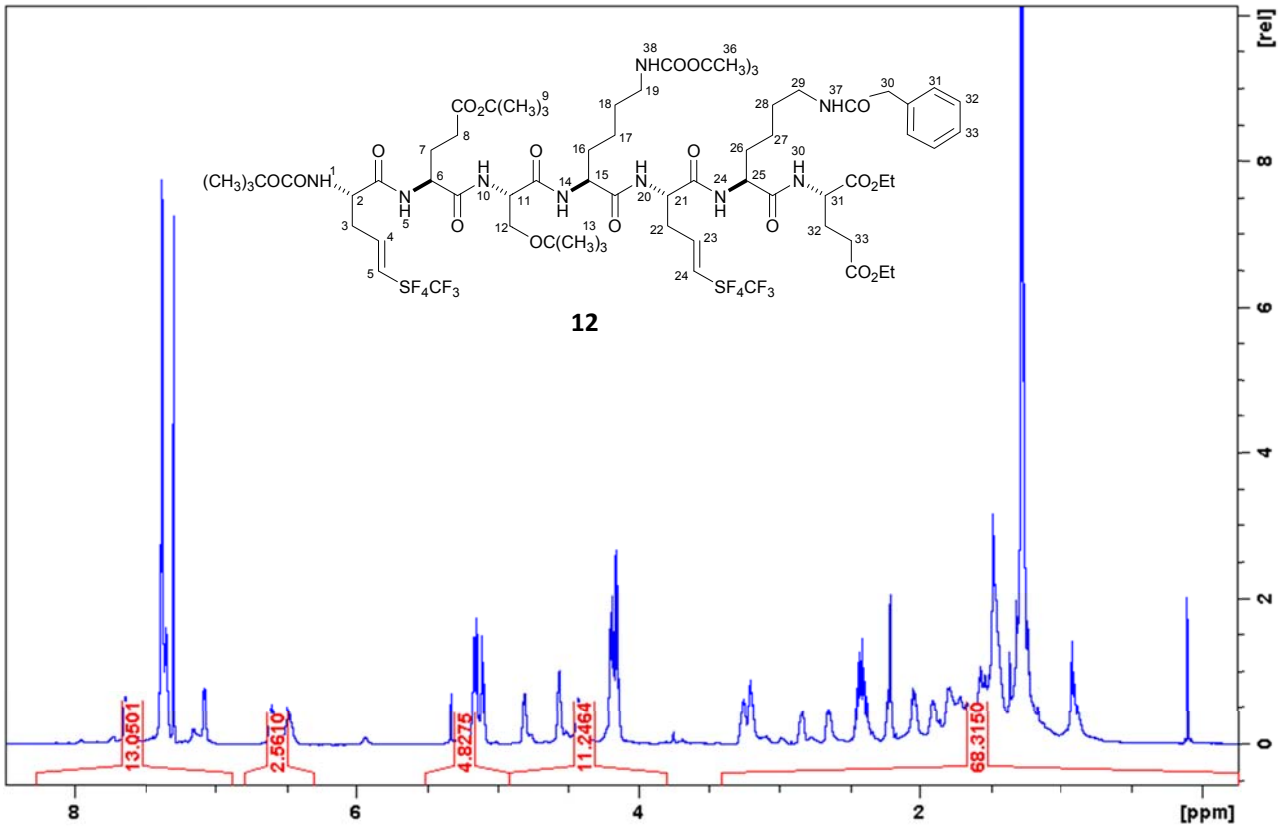

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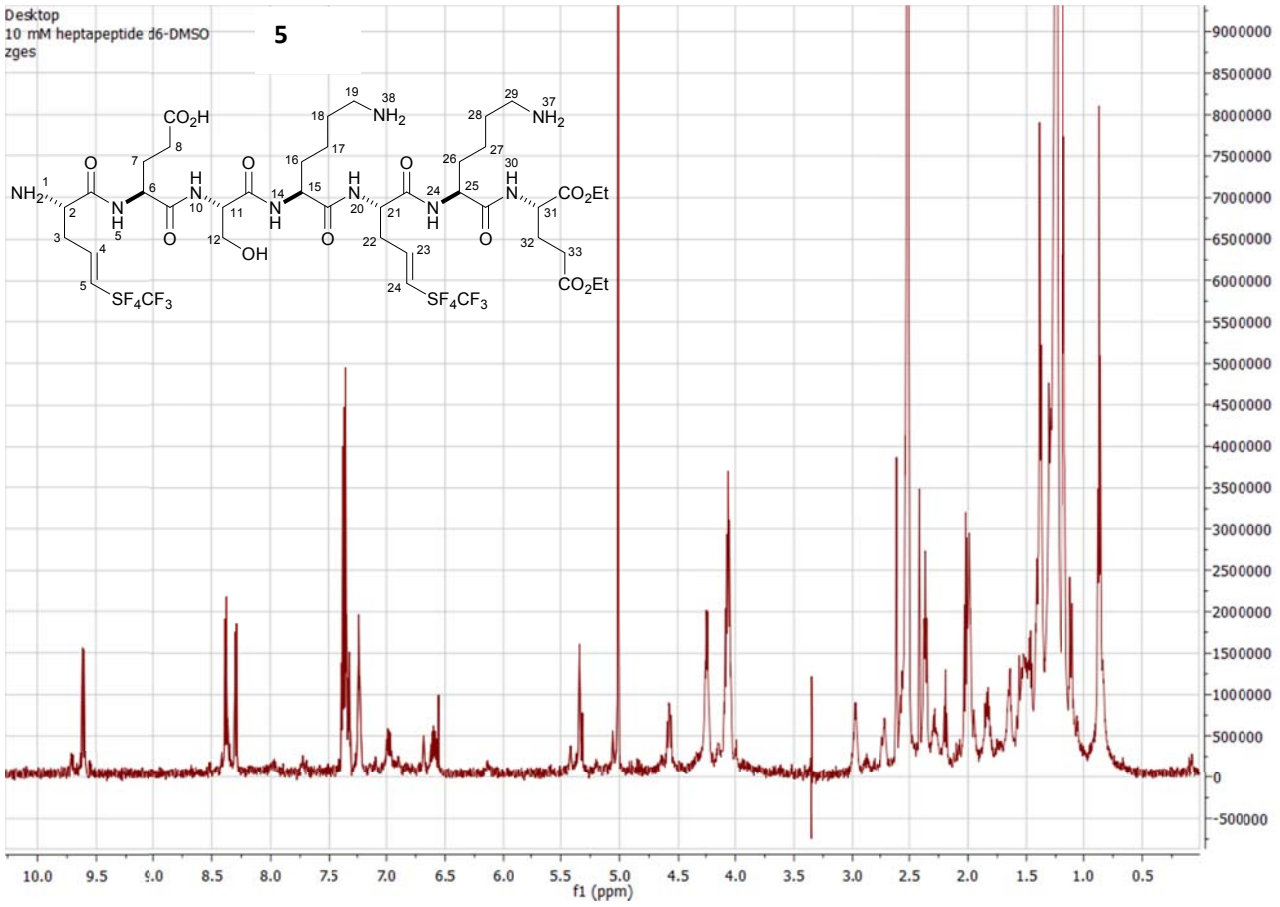

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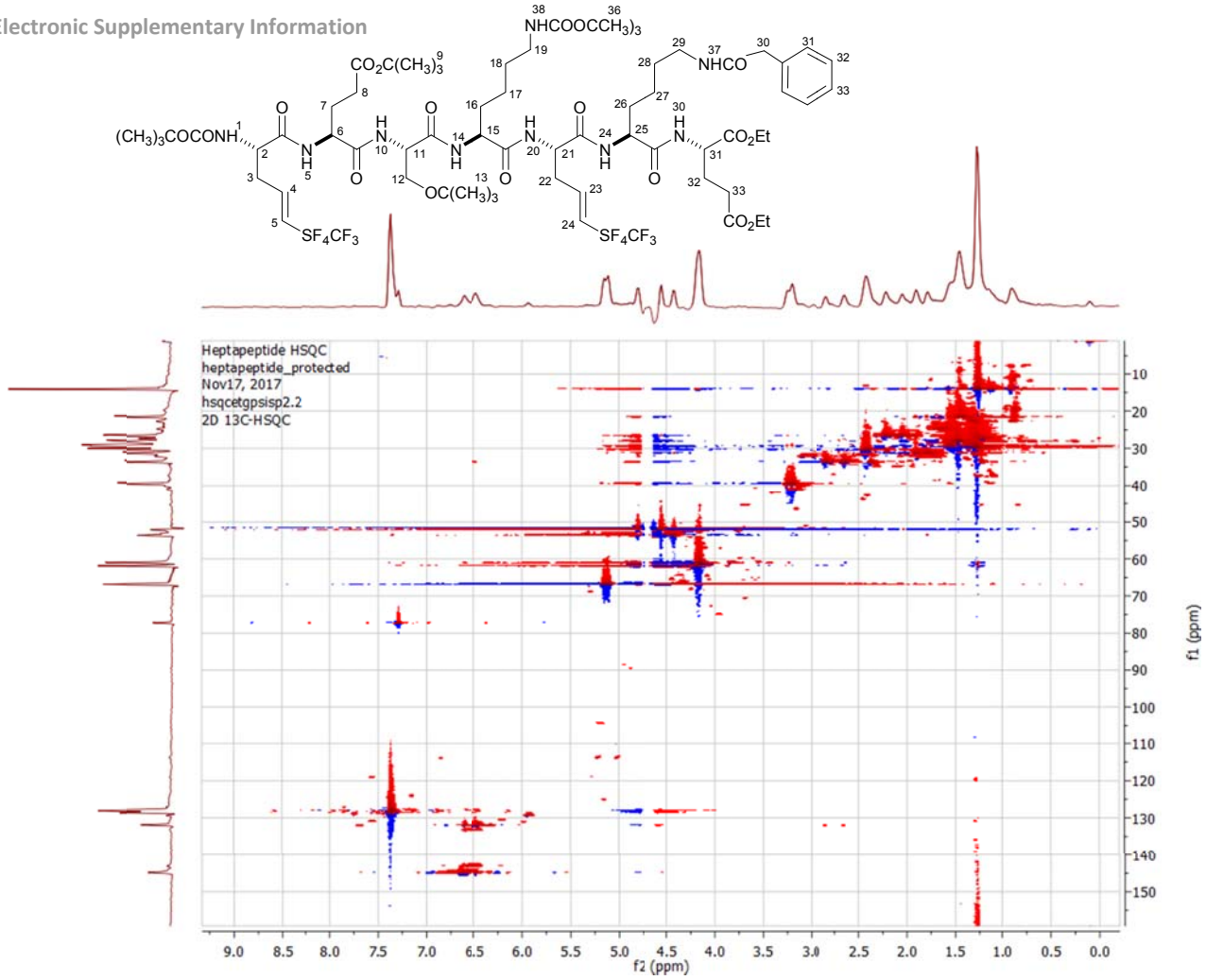

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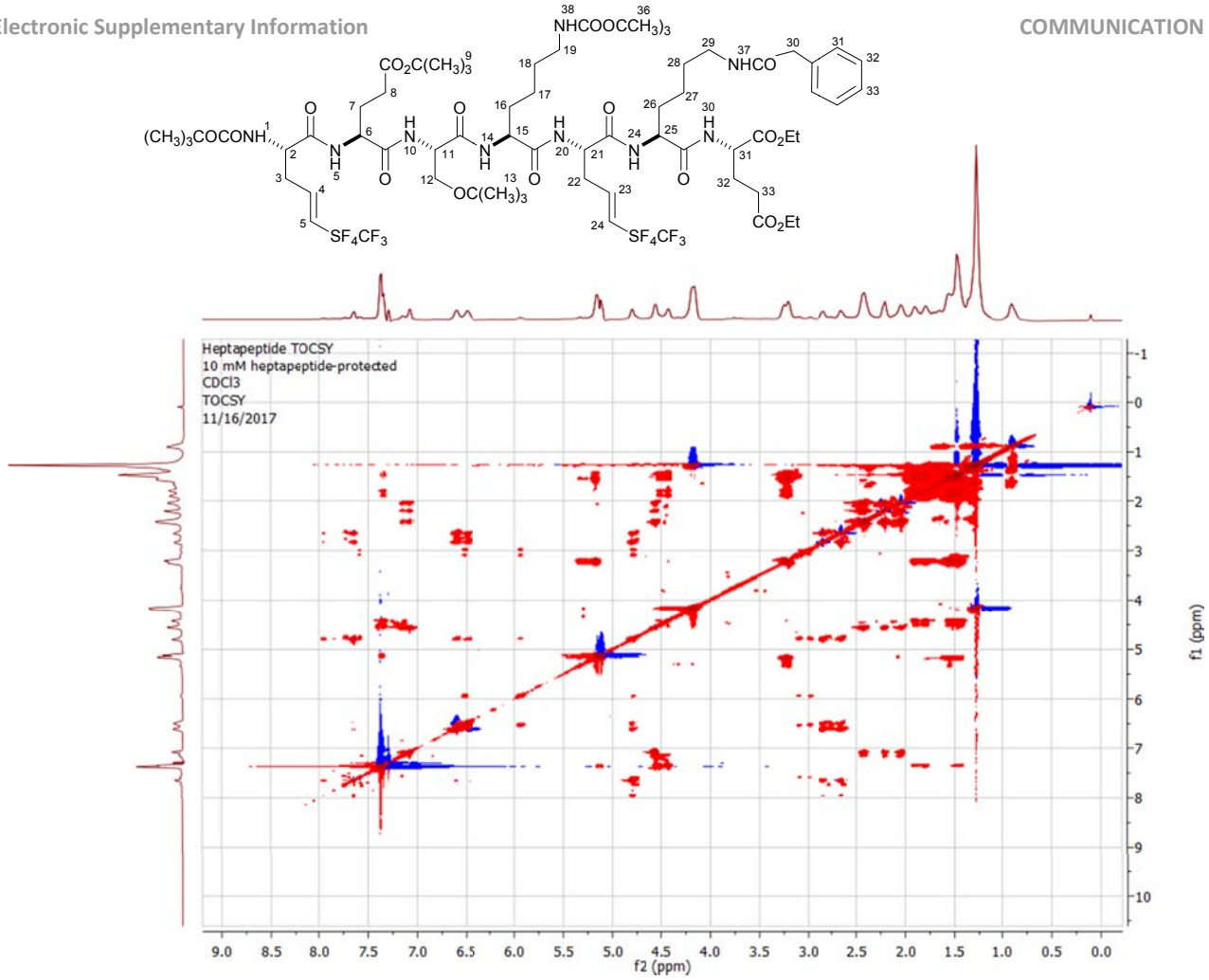

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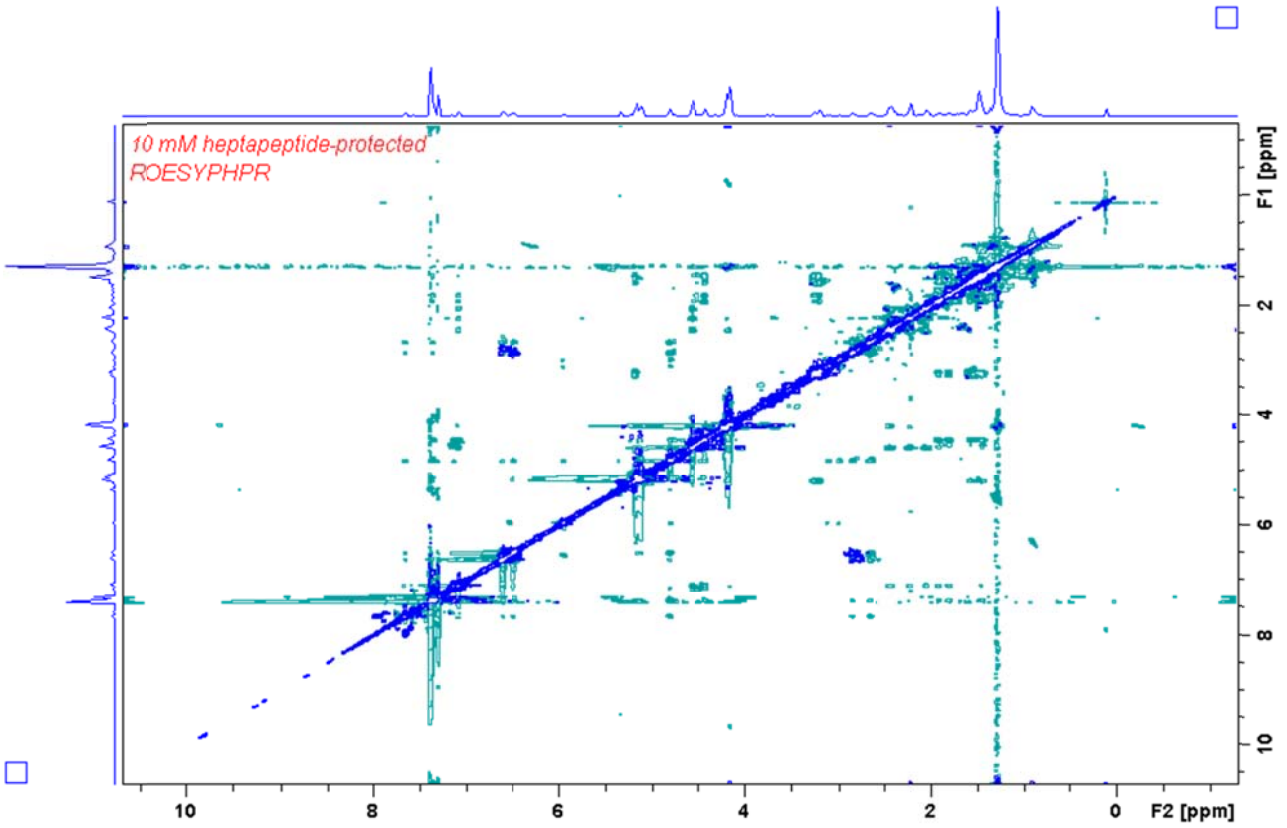

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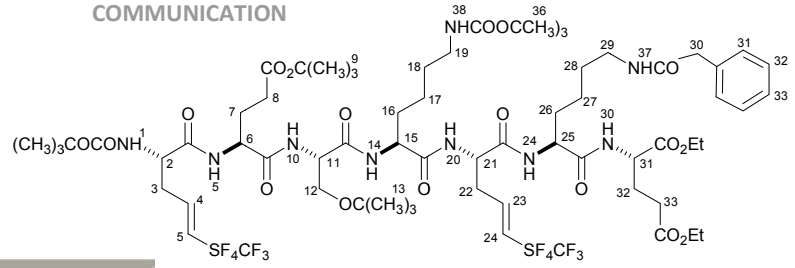
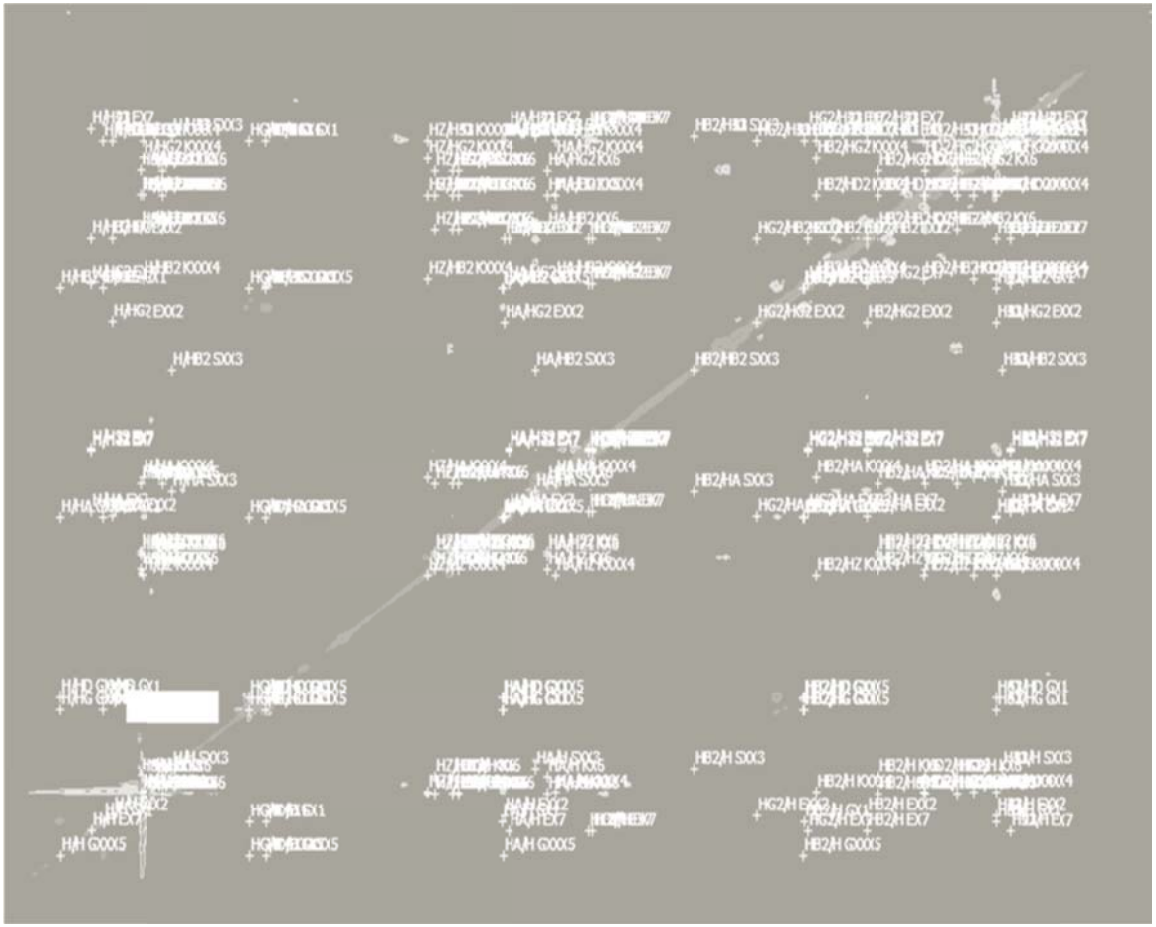
ROESY cross peaks identified in CARA for Protected heptapeptide.





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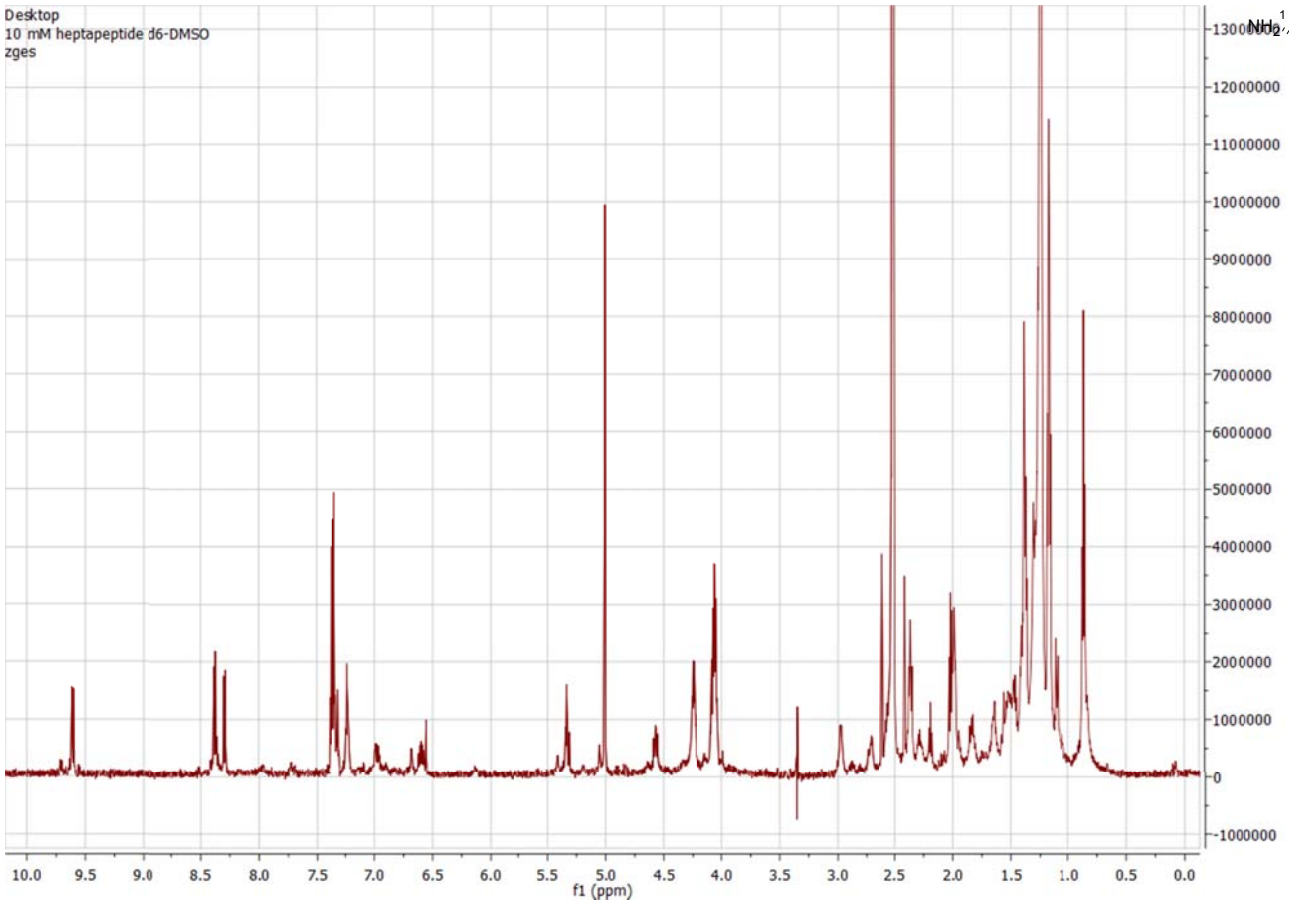

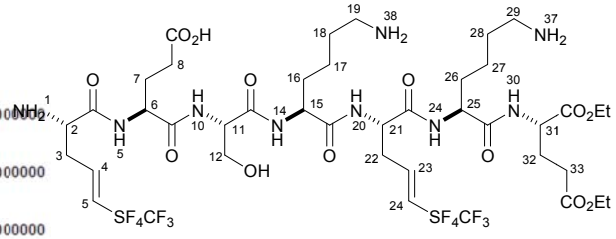

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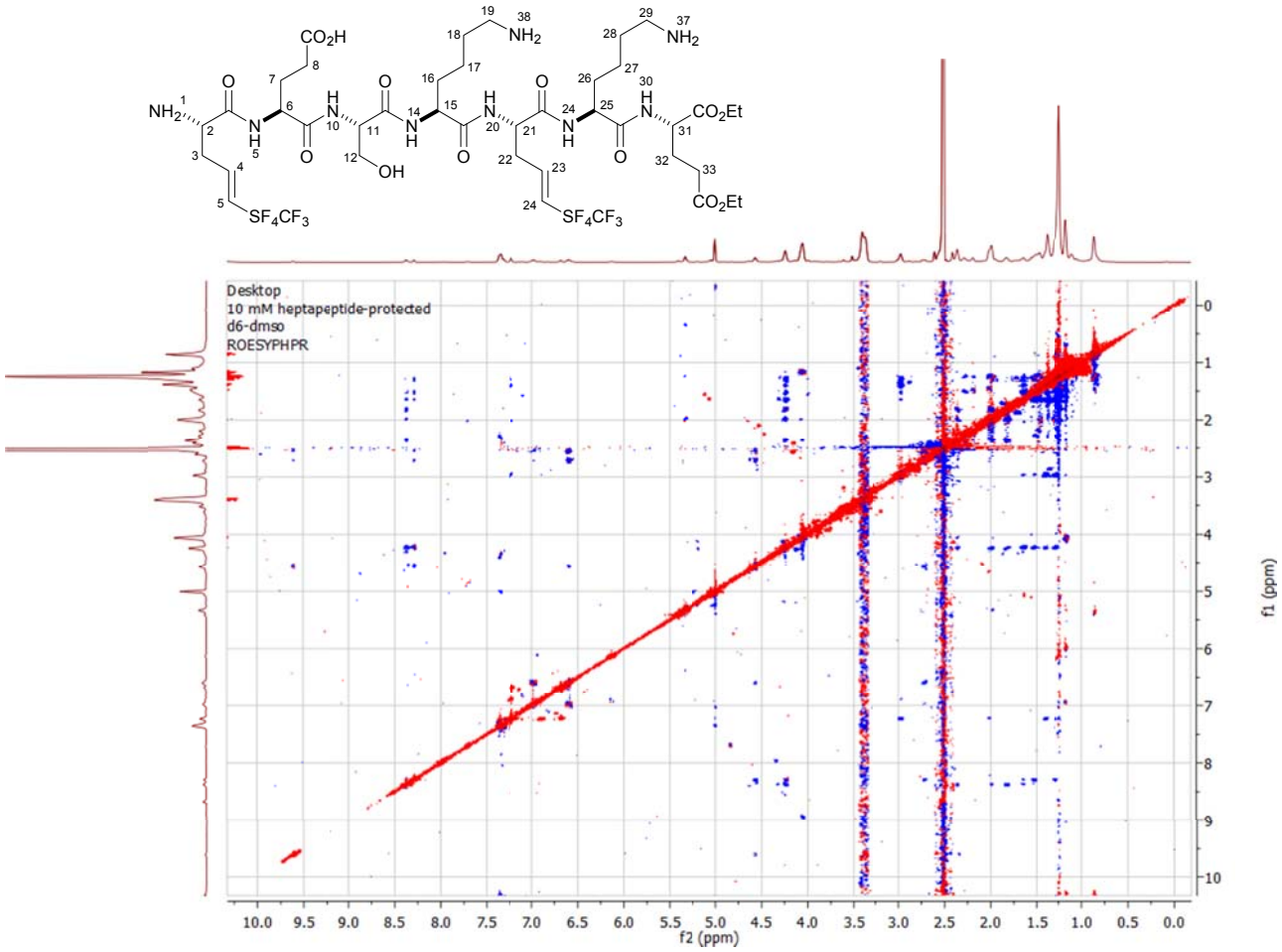

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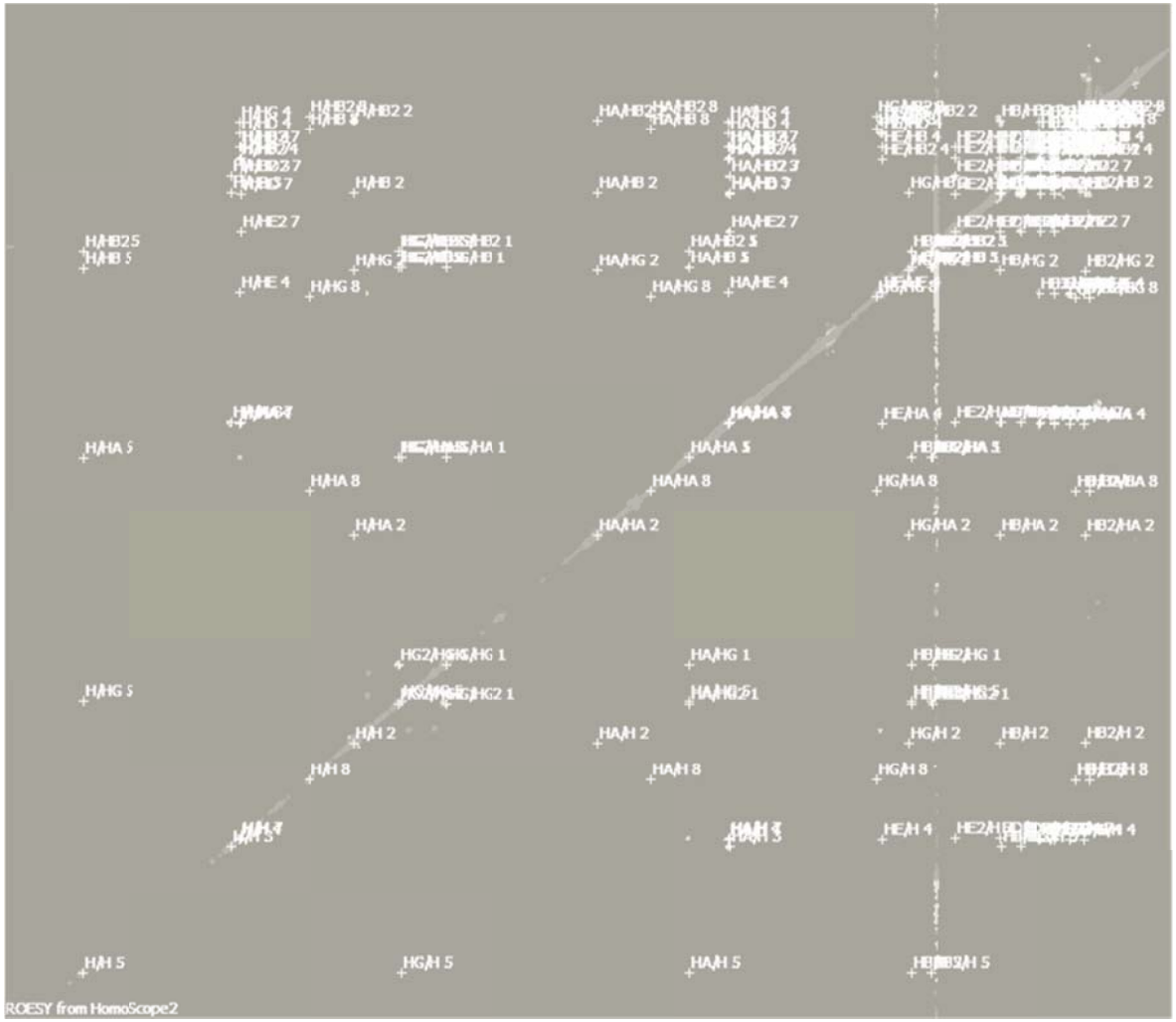
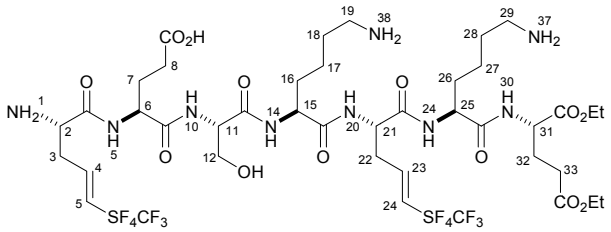
ROESY cross peaks identified in CARA for deprotected heptapeptide





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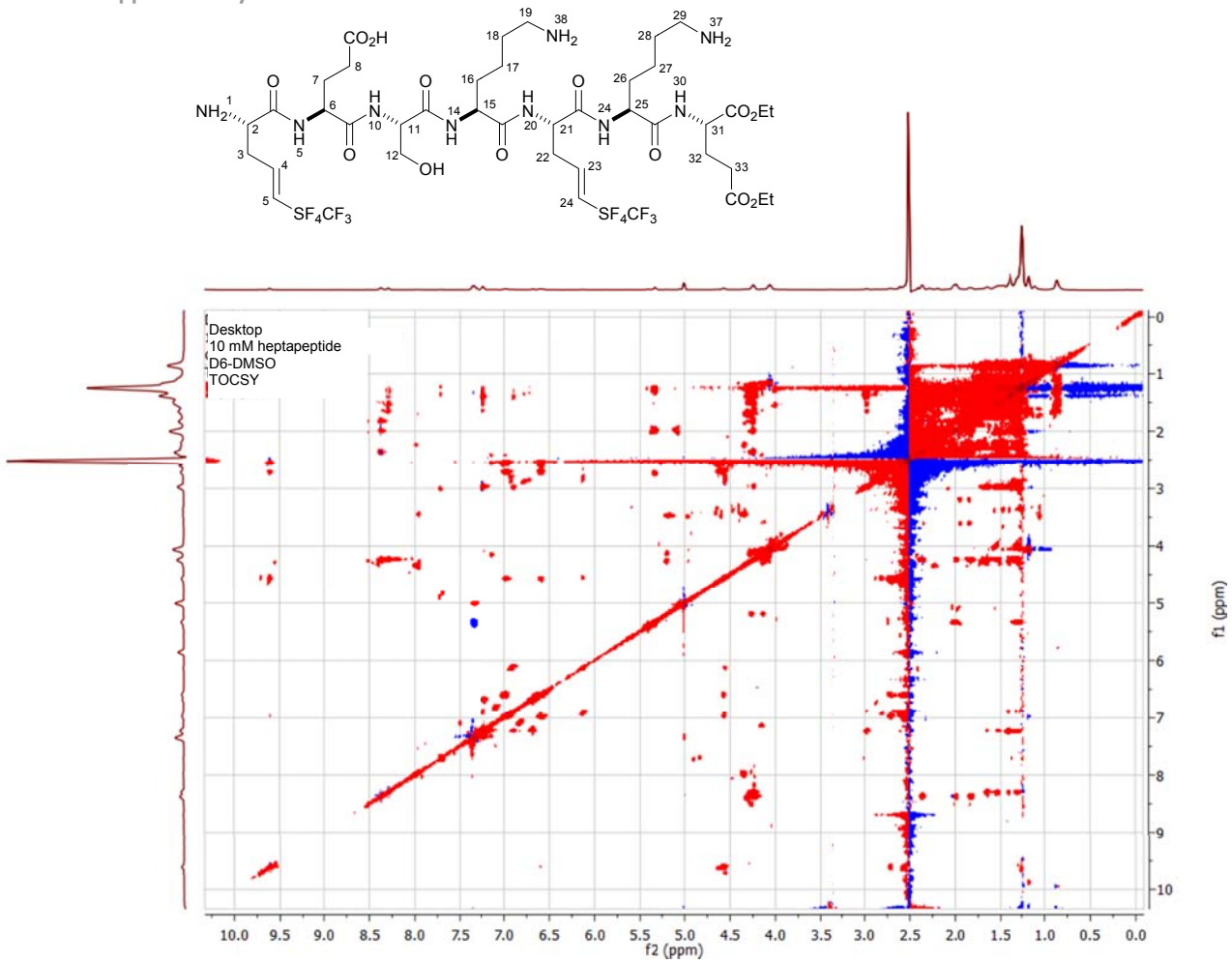

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