SUPPORTING INFORMATION

The Tyrosine *O*-Prenyltransferase SirD Catalyzes *S*-, *C*-, and *N*-Prenylations on Tyrosine and Tryptophan Derivatives

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Chemicals

N-*t*-Boc-4-iodo-L-phenylalanine, 4-methylindole, 4-methoxyindole, and 7-methylindole were obtained from Chem-Impex International, Inc. Fmoc-OSu was purchased from Novabiochem. Thionyl chloride and lithium chloride were obtained from Mallinckrodt/JT Baker. Chloramphenicol was obtained from MP Biomedicals, LLC. L-tyrosine [^{14}C(U)] and [1-^{14}C]DMAPP were purchased from American Radiolabeled Chemicals, Inc. All other chemicals and reagents were obtained from Sigma-Aldrich.

Bacterial strains, plasmids and culture conditions

tmTrpB, an *E. coli* BL21-(DE3)-RIPL strain containing pET28a-tmTrpB1 was obtained from Professor Reinhard Sterner. tmTrpB1 encodes the β-subunit of tryptophan synthase from the hyperthermophilic bacterium *Thermotoga maritime*.[1] Plasmid pLmSirD is a pET21a(+) construct containing the *L. maculans sirD* gene (see below). Expression strains were grown in liquid LB broth or on solid LB-agar medium at 37 °C. Kanamycin (35 μg mL^{-1}) and chloramphenicol (34 μg mL^{-1}) were used to select vector-containing *E. coli* strains.

The *L. maculans sirD* gene (NCBI GenBank: AY553235.1) was optimized for heterologous expression in *E. coli* and synthesized by Genscript. The gene (Figure S1) was engineered with 5'-BamHI and 3'-HindIII endonuclease restriction sites, an *N*-terminal histidine tag (His_6), and a thrombin cleavage site and placed in the pUC57 vector. The His_6-*sirD* gene in pUC57 and the destination vector pET21a(+) were digested with BamHI and HindIII endonucleases (New England Biolabs, Inc.) as per the manufacturer's instructions. After purification by agarose gel electrophoresis, the His_6-

sirD gene was ligated with T4 DNA Ligase (NEB) into the cut pET-21a(+) vector to produce pLmSirD.

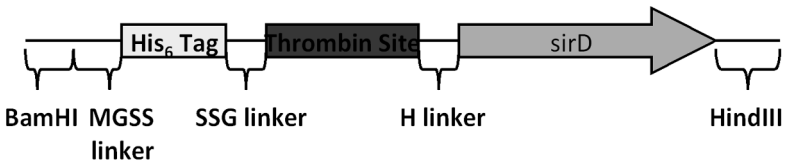

Figure S1. The engineered *sirD* gene construct synthesized by Genscript.

```
   1          M  G  S  S  H  H  H  H  H  H  S  S  G  L  V  P  R  G
   1  GGATCCATGGGCAGCAGCCATCATCATCATCATCATAGCAGCGGCCTGGTGCCGCGTGGC
  21    S  H  M  Q  T  A  R  L  F  Q  G  N  L  N  L  A  A  A  N  I
  61  AGCCATATGCAGACCGCGCGTCTGTTTCAGGGCAACCTGAACCTGGCGGCGGCGAACATT
  41    R  D  Y  E  K  K  E  Q  R  N  L  G  V  W  L  S  L  N  Q  W
 121  CGTGATTATGAGAAGAAAGAACAGCGTAACCTGGGCGTGTGGCTGAGCCTGAACCAGTGG
  61    L  R  L  Y  D  E  D  T  R  F  W  W  T  T  T  A  P  M  L  G
 181  CTGCGTCTGTATGATGAAGATACCCGTTTTTGGTGGACCACCACCGCGCCGATGCTGGGC
  81    R  M  M  E  L  I  G  Y  D  Q  D  A  Q  Q  K  H  L  L  F  Y
 241  CGTATGATGGAACTGATTGGCTATGATCAGGATGCGCAGCAGAAACATCTGCTGTTTTAT
 101    Y  I  Y  V  L  P  S  L  G  R  R  P  S  P  E  G  Y  P  T  G
 301  TATATTTATGTGCTGCCGAGCCTGGGCCGTCGTCCGAGCCCGGAAGGTTATCCGACCGGT
 121    W  N  S  F  M  T  D  D  Y  S  P  L  E  L  S  W  D  W  G  V
 361  TGGAACAGCTTCATGACCGATGATTATAGCCCGCTGGAACTGAGCTGGGATTGGGGCGTG
 141    A  E  G  E  S  S  V  R  F  S  I  E  P  I  G  K  Y  A  G  T
 421  GCGGAAGGCGAAAGCAGCGTGCGTTTTAGCATTGAACCGATTGGCAAATATGCGGGCACC
 161    Q  A  D  P  L  N  Q  K  M  V  Y  Q  L  V  D  G  L  R  P  A
 481  CAGGCAGATCCACTGAACCAGAAAATGGTGTATCAGCTGGTTGATGGCCTGCGTCCGGCG
 181    F  H  H  T  L  D  L  T  L  F  D  V  F  S  E  A  L  T  T  S
 541  TTTCATCATACCCTGGATCTGACCCTGTTTGATGTGTTTAGCGAAGCGCTGACCACCAGC
 201    R  E  K  F  G  T  R  K  L  S  L  E  G  R  S  Q  Y  F  V  A
 601  CGTGAAAAATTTGGCACCCGTAAACTGAGCCTGGAAGGCCGTAGCCAGTATTTTGTGGCG
 221    F  D  L  D  V  G  H  P  R  L  K  A  Y  F  M  P  G  L  K  S
 661  TTTGATCTGGATGTGGGCCATCCGCGTCTGAAAGCGTATTTTATGCCGGGCCTGAAAAGC
 241    I  E  S  N  T  P  V  S  E  L  V  V  K  A  M  D  A  C  E  L
 721  ATTGAAAGCAACACCCCTGTGAGCGAACTGGTGGTGAAAGCGATGGATGCGTGCGAACTG
 261    H  F  G  S  L  F  M  Q  A  F  R  R  L  N  S  D  L  E  A  F
 781  CATTTTGGCAGCCTGTTTATGCAGGCGTTTCGTCGTCTGAACAGCGATCTGGAAGCGTTT
 281    S  A  T  S  Y  H  R  P  E  I  E  I  V  G  I  D  C  V  S  P
 841  AGCGCGACCAGCTATCATCGTCCGGAAATTGAAATTGTGGGCATTGATTGCGTGAGCCCG
 301    V  K  S  R  A  K  I  Y  I  R  H  R  G  T  S  F  D  S  V  C
 901  GTGAAAAGCCGTGCGAAAATTTATATTCGTCATCGTGGCACCAGCTTTGATAGCGTGTGC
 321    R  M  L  S  M  G  A  K  A  P  L  D  A  A  S  V  A  S  L  R
 961  CGTATGCTGAGCATGGGTGCAAAAGCACCGCTGGATGCAGCAAGCGTGGCGAGCCTGCGT
 341    E  L  W  A  L  V  L  G  L  P  K  D  F  P  S  D  Q  E  L  P
1021  GAACTGTGGGCACTGGTGCTGGGTCTGCCGAAAGATTTTCCGAGCGATCAGGAACTGCCG
 361    S  V  P  H  R  T  S  G  V  L  Y  Y  F  E  I  K  P  T  S  D
1081  AGCGTGCCGCATCGTACCAGCGGCGTGCTGTATTATTTTGAAATTAAACCGACCAGCGAT
 381    A  I  V  P  K  V  Y  I  P  V  R  H  Y  A  S  N  D  L  S  I
1141  GCGATTGTGCCGAAAGTGTATATTCCGGTGCGTCATTATGCGAGCAACGATCTGAGCATT
 401    A  Q  G  L  A  T  Y  F  E  R  R  G  Q  T  V  A  A  E  N  Y
1201  GCGCAGGGCCTGGCGACCTATTTTGAACGTCGTGGCCAGACCGTGGCGGCGGAAAACTAT
 421    V  D  A  L  S  D  I  F  S  H  R  S  L  D  S  G  L  G  L  H
1261  GTGGATGCGCTGAGCGATATTTTTAGCCATCGTAGCCTGGATAGCGGCCTGGGCCTGCAT
 441    T  Y  I  S  C  T  F  K  K  T  G  L  S  V  T  S  Y  F  N  P
1321  ACCTATATTAGCTGCACCTTTAAGAAAACCGGCCTGAGCGTGACCAGCTATTTTAACCCG
 461    E  I  Y  H  P  N  R  Y  R  Q  -
1381  GAAATTTATCATCCGAACCGTTATCGTCAGTAAAAGCTT
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Figure S2. *L. maculans sirD* engineered gene and encoded amino acid sequence

Overproduction and purification of tryptophan synthase

Following instructions from the Sterner lab, *E. coli* BL21-(DE3)-RIPL/ pET28a-tmTrpB1 was cultivated in 1 L of LB medium containing kanamycin and chloramphenicol at 37 °C with shaking at 225 rpm to an OD_{600} of 0.5 – 1.0. The culture was then cooled to 20 °C and allowed to incubate for 1 h. Overexpression of the β-subunit of tryptophan synthase was induced with IPTG to a final concentration of 0.5 mM. After 18 h, cells were harvested by centrifugation (2,700 x *g*, 15 min, 4 °C). Pelleted cells were resuspended in 25 mL of lysis buffer (100 mM K_2HPO_4, pH 7.5, containing 300 mM KCl, 10 mM imidazole, and 40 μM PLP) and sonicated (Branson Sonifier 350) for 4 x 30 sec at 4 °C. The lysate was clarified by centrifugation (23,400 x *g*, 15 min, 4 °C). The resulting soluble lysate was heated to 75 °C for 20 min and again clarified by centrifugation (23,400 x *g*, 15 min, 4 °C). The recombinant protein was purified by nickel affinity chromatography (GE Healthcare HisTrap HP) following the manufacturer's instructions. Tryptophan synthase was eluted with elution buffer (100 mM K_2HPO_4, pH 7.5, containing 300 mM KCl and 1 M imidazole). The purified protein was dialyzed 3 x against 4 L of 100 mM K_2HPO_4 buffer, pH 7.5 and stored at –80 °C until use.

Isoprenoid diphosphate synthesis

DMAPP was prepared as described previously.[2]

Amino acid analogue synthesis

N-(tert-Butoxycarbonyl)-4-(S-tert-butylthio)-L-phenylalanine ***(1a).*** The tris(di-benzylideneacetone)dipalladium(0)-chloroform adduct ($Pd_2dba_3 \cdot CHCl_3$, 0.045 g, 0.044 mmol, 1.5 mol%) was added to DMF (15 mL) under nitrogen, followed by 1,1'-Bis(diphenylphosphino)ferrocene (DPPF, 0.111 g, 0.200 mmol, 6.6 mol %). The mixture was allowed to stir at rt for 10 min. A mixture of triethylamine (0.92 mL, 6.60 mmol) and *N-t*-Boc-4-iodo-L-phenylalanine (1.17 g, 3.00 mmol) in DMF (15 mL) was added dropwise and allowed to stir at rt for 10 min before the addition of 2-methyl-2-propanethiol (0.37 mL, 3.30 mmol). After stirring at 75 °C for 3 h, the reaction mixture was concentrated *in vacuo*, diluted with ethyl acetate (40 mL) and water (10 mL), cooled in an ice bath and acidified to pH 2–3 with 50 mM citric acid. The aqueous phase was extracted with EtOAc (25 mL) and the combined organic layers were washed with brine (2 x 20 mL) and dried over Na_2SO_4. The concentrated crude product was purified by silica gel flash chromatography with gradient elution using 0 – 100% EtOAc in hexanes. The resulting yellow oil was re-dissolved in hexanes/EtOAc and filtered to remove a small amount of purple solid. The filtrate was concentrated to yield a thick, yellow oil of **1a** (0.75 g, 2.12 mmol, 71%); R_f 0.41 (DCM:MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 9H), 1.33 (s, 9H), 2.98 (dd, *J* = 6.9, 13.8 Hz, 1H), 3.16 (dd, *J* = 5.1, 13.8 Hz, 1H), 4.55 (q, *J* = 7.2, 13.2 Hz, 1H), 5.09 (d, *J* = 8.1 Hz, 1H), 7.09 (d, *J* = 7.8 Hz, 2H), 7.38 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.1, 28.3, 31.0, 37.7, 45.9, 54.2, 80.2, 129.6, 131.2, 137.1, 137.6, 155.4, 175.1. The ^{1}H NMR matched the literature in DMSO-d_6, however no ^{13}C NMR was previously reported.[3]

*Bis-(4-mercapto-L-phenylalanine) Disulfide (**1**).* Concentrated HCl (8 mL) was added to **1a** (220 mg, 0.62 mmol) and was heated at reflux overnight. After cooling to rt, the solution was diluted with H_2O (20 mL) and washed with EtOAc (3 x 20 mL). The resulting aqueous layer was lyophilized to yield a brown solid of **1** (87 mg, 0.22 mmol, 71%); R_f 0.24 (ACN:H_2O 4:1), 0.34 (thiol) and 0.48 (disulfide) (n-BuOH:AcOH:H_2O:pyridine 15:3:10:6); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.12 (d, 6.5 Hz, 4H), 4.15 (t, 6.5 Hz, 2H), 7.31 (d, 8.5 Hz, 4H), 7.49 (d, 8.0 Hz, 4H), 8.44 (br, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 35.1, 53.0, 127.4, 130.7, 134.6, 134.7, 170.2; UV (ACN:H_2O 1:3) λ_{max} 206.2, 242.8 nm; MS-ESI *m/z* 198.0 [Monomer + H]$^+$, 393.2 [M + H]$^+$, 415.3 [M + Na]$^+$; HRMS-ESI TOF *m/z* [M + H]$^+$ calcd for $C_{18}H_{21}N_2O_4S_2$ 393.0943, found 393.0945.

*L-Tyrosine Methyl Ester (**2a**).* Thionyl chloride (4 mL, 55.0 mmol) was added dropwise to a suspension of L-tyrosine (5.45 g, 30.1 mmol) in MeOH (100 mL, 247 mmol) at 0 °C. The mixture was allowed to warm to rt and stirred overnight. After removing the solvent by rotary evaporation, the residue was washed with diethyl ether (2x50 mL). Compound **2a** was obtained as a white solid (5.7 g, 29.2 mmol, 97%); ^{1}H NMR (300 MHz, D_2O) δ 3.13 (dd, *J* = 7.2, 14.7 Hz, 1H), 3.24 (dd, *J* = 5.7, 14.7 Hz, 1H), 3.82 (s, 3H), 4.36 (dd, *J* = 5.7, 7.2 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 2H), 7.13 (d, *J* = 8.7 Hz, 2H). The ^{1}H NMR data matched the published data,[4] except the α proton (4.36) in our spectrum is resolved from the methyl group (3.82).

*N-(9-Fluorenylmethoxycarbonyl)-L-tyrosine Methyl Ester (**2b**).* A solution of *N*-(9-fluorenylmethoxycarbonyl)succinimide (4.89 g, 14.5 mmol) in 1,4-dioxane (30 mL) was added to a mixture of **2a** (2.50 g, 12.8 mmol) and $NaHCO_3$ (3.11 g, 37.0 mmol) in

H_2O (30 mL). After stirring for 23 h at rt, the mixture was acidified to pH 2–3 with 1 N HCl at 0 °C, extracted with EtOAc (3 x 25 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by silica gel flash chromatography with gradient elution using 0 – 40% EtOAc in hexanes to yield **2b** (4.8 g, 90%) as a colorless sticky solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93-3.09 (m, 2H), 3.70 (s, 3H), 4.18 (t, *J* = 6.9 Hz, 1H), 4.27-4.46 (m, 2H), 4.57-4.67 (m, 1H), 5.35 (d, 8.4 Hz, 1H), 6.72 (d, *J* = 8.4 Hz, 2H), 6.91 (d, *J* = 8.4 Hz, 2H), 7.25-7.43 (m, 4H), 7.51-7.57 (m, 2H), 7.71-7.78 (m, 2H). The ^{1}H NMR matched the published data.[5]

*N-9-Fluorenylmethoxycarbonyl-4-((trifluoromethane-sulfonyl)oxy)-L-phenylalanine Methyl Ester (**2c**).* Trifluoromethanesulfonic anhydride (1.9 mL, 11.3 mmol) was added dropwise to an ice-cold solution of **2b** (4.1 g, 9.82 mmol) and DIPEA (8.6 mL, 49.4 mmol) in DCM (20 mL). Stirring was continued at 0 °C for 2.5 h. The resulting mixture was diluted with H_2O (30 mL) and DCM (50 mL) and washed sequentially with 10% $NaHCO_3$ (25 mL), H_2O (30 mL), 10% citric acid (2 x 30 mL), and H_2O (30 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel flash chromatography using an elution gradient of 0 – 30% EtOAc in hexanes to give **2c** (3.0 g, 5.46 mmol, 56%) as a white solid; R_f 0.43 (hexanes:EtOAc 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.07 (dd, *J* = 6.0, 13.8 Hz, 1H), 3.16 (dd, *J* = 6.0, 14.1 Hz, 1H), 3.70 (s, 3H), 4.19 (t, *J* = 6.6 Hz, 1H), 4.38 (dd, 6.3, 10.5 Hz, 1H), 4.48 (dd, 6.9, 10.8 Hz, 1H), 4.64 (dd, *J* = 6.0, 13.8 Hz, 1H), 5.27 (d, *J* = 7.8 Hz, 1H), 7.12 (d, *J* = 8.7 Hz, 2H), 7.17 (d, *J* = 8.7 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.55 (d, *J* = 7.5 Hz, 2H), 7.76 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 37.8, 47.4, 52.7, 54.8, 67.0, 120.2, 120.3, 121.6, 125.1, 125.2, 127.3, 128.0,

131.3, 136.7, 141.6, 143.8, 143.9, 148.8, 155.6, 171.6; ^{19}F NMR (282 MHz, $CDCl_3$, TFA) δ 73.8; MS-ESI *m/z* 572.1 $[M + Na]^+$, HRMS-ESI TOF *m/z* $[M + Na]^+$ calcd for $C_{26}H_{22}NO_7NaSF_3$ 572.0967, found 572.0970. The ^{1}H NMR matched the published data; however, the ^{13}C NMR had a few differences in the aromatic region and no ^{19}F NMR data were previously reported (Herzner 2007).

*4-Vinyl-L-phenylalanine Methyl Ester (**2d**)*. Under a nitrogen atmosphere, tributyl(vinyl)tin (267 μL, 0.92 mmol) was added to a solution of flame-dried LiCl (270 mg, 6.35 mmol), $PdCl_2(PPh_3)_2$ (12.5 mg, 0.018 mmol), and triflate **2c** (0.5 g, 0.91 mmol) in DMF (8 mL). The yellow-orange solution was then warmed to 90 °C. After stirring for 23 h, the reaction mixture was poured into ice-cold H_2O (15 mL) and extracted with EtOAc (2 x 10 mL). The combined organic extracts were sequentially washed with H_2O (2 x 10 mL) and brine (2 x 10 mL), dried over Na_2SO_4, and concentrated *in vacuo* to give a yellow oil. This oil was partially purified by silica gel flash chromatography with gradient elution using 0 – 100% EtOAc in hexanes, followed by 10% MeOH in EtOAc. Impure 4-vinyl-L-phenylalanine methyl ester (**2d**, 0.13 g, 0.63 mmol, 70%) was recovered from the MeOH fractions; R_f 0.55 (EtOAc:MeOH 4:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.83 (dd, *J* = 8.0, 13.5 Hz, 1H), 3.05 (dd, *J* = 5.5, 13.5 Hz, 1H), 3.61-3.77 (m, 4H), 5.20 (d, *J* = 11.0 Hz, 1H), 5.70 (d, *J* = 17.5 Hz, 1H), 6.67 (dd, *J* = 18.0, 11.0 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H); MS-ESI *m/z* 206.1 $[M + H]^+$, 228.1 $[M + Na]^+$.

*N-9-Fluorenylmethoxycarbonyl-4-vinyl-L-phenylalanine Methyl Ester (**2e**)*. In an attempt to better purify the vinyl amino acid, impure **2d** (0.13 g, 0.63 mmol) was reprotected with Fmoc in a procedure similar to the synthesis of **2b**, using $NaHCO_3$ (0.16

g, 1.91 mmol) and *N*-(9-fluorenylmethoxycarbonyl)succinimide (0.26 g, 0.77 mmol). Silica gel flash chromatography using an elution gradient of 0 – 20% EtOAc in hexanes afforded **14** (0.15 g, 0.35 mmol, 55%). R_f 0.22 (hexanes:EtOAc 4:1), 0.60 (hexanes:EtOAc 3:2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.07 (dd, *J* = 6.0, 13.5 Hz, 1H), 3.12 (dd, *J* = 6.0, 14.0 Hz, 1H) 3.72 (s, 3H), 4.20 (t, *J* = 7.0 Hz, 1H), 4.34 (dd, *J* = 6.5, 10.5 Hz, 1H), 4.42 (dd, *J* = 7.0, 10.5 Hz, 1H), 4.65 (dd, *J* = 5.5, 13.5 Hz, 1H), 5.19-5.26 (m, 2H), 5.71 (d, *J* = 18.0 Hz, 1H), 6.67 (dd, *J* = 11.0, 17.5 Hz, 1H), 7.03 (d, *J* = 7.5 Hz, 2H), 7.27-7.33 (m, 4H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.55 (t, *J* = 7.0 Hz, 2H), 7.75 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 38.1, 47.4, 52.6, 54.9, 67.2, 114.0, 120.1, 120.2, 120.3, 124.9, 125.2, 125.3, 126.7, 127.2, 127.3, 127.8, 127.9, 129.7, 135.5, 136.6, 136.7, 141.5, 141.8, 143.9, 144.1, 155.7, 172.1; MS-ESI *m/z* 450.1 $[M + Na]^+$, HRMS-ESI TOF *m/z* $[M + Na]^+$ calcd for $C_{27}H_{25}NO_4Na$ 450.1681, found 450.1688

*4-Vinyl-L-phenylalanine (**2**).* Protected vinyl amino acid **2e** (0.13 g, 0.30 mmol) was dissolved in a 1:1 mixture of 1 N NaOH (5x mol excess)/THF and allowed to stir at rt for 42 h. The reaction mixture was washed with diethyl ether (3 x 20 mL) and the aqueous layer was subsequently lyophilized to yield an off-white solid (0.056 g, 0.29 mmol, 96%). Compound **2** was dissolved in H_2O and purified by C18 RP-HPLC using a linear elution gradient of 100% H_2O to 100% ACN in a total volume of 30 mL at a flow rate of 1.0 mL/min. Fractions with retention times 9-11 min were collected and lyophilized to afford a white solid; 1H NMR (300 MHz, D_2O/NaOD, DSS) δ 2.92 (dd, J = 7.8, 14.1 Hz, 1H), 3.06 (dd, J = 5.7, 14.1 Hz, 1H), 3.64 (dd, J = 5.7, 7.5 Hz, 1H), 5.28 (d, J = 11.1 Hz, 1H), 5.83 (d, J = 17.7 Hz, 1H), 6.78 (dd, J = 10.8, 17.7 Hz, 1H), 7.26 (d, J = 8.4 Hz, 2H), 7.47 (d, J = 8.4 Hz, 2H); 13C NMR (75 MHz, D_2O/NaOD, DSS) δ 39.2,

57.1, 114.1, 126.5, 129.9, 136.2, 136.5, 137.2, 179.9; UV (ACN:H_2O 1:3) λmax 202.6, 249.9 nm; MS-ESI m/z 214.1 [M + Na]+, 236.1 [M + 2Na+ - H+]+; HRMS-ESI TOF m/z [M + Na]+ calcd for C11H13NO2Na 214.0844, found 214.0838.

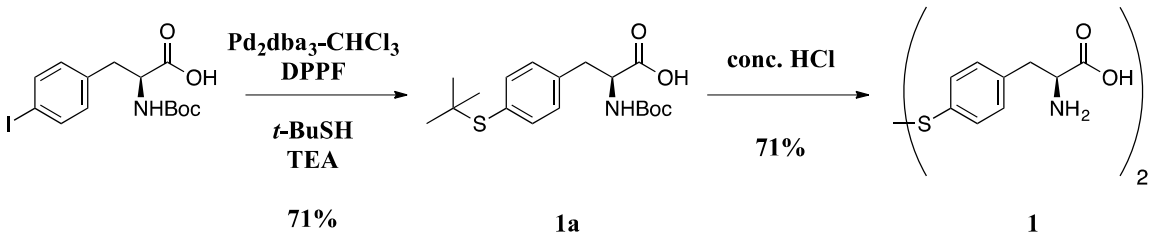
*4-Methyl-L-tryptophan (**3**).* In a nitrogen atmosphere, 4-methylindole (160 mg, 1.22 mmol) and L-serine (250 mg, 2.38 mmol) were incubated at 80 °C with 7.5 mg of recombinant tryptophan synthase from the hyperthermophilic bacterium *Thermotoga maritime*[1] in 25 mL of Buffer TS (100 mM K_2HPO_4, pH 7.5. containing 180 mM KCl, 120 μM PLP and 2% v/v DMSO for 89 h. After incubation, the reaction mixture was cooled to rt and the enzyme was removed by filtration (Millipore Centricon, 10,000 molecular weight cutoff (MWCO)). The filtrate was concentrated in vacuo, the resulting residue was purified by silica gel flash chromatography using a gradient of 0 – 20 % H_2O in ACN to give 175 mg (66%) of an off-white solid; R_f 0.65 (ACN/H_2O 4:1); ^{1}H NMR (300 MHz, D_2O/NaOD) δ 2.19 (s, 3H), 2.44 (dd, *J* = 8.1, 14.4 Hz, 1H), 2.83 (dd, *J* = 5.4, 14.4 Hz, 1H), 2.96 (dd, *J* = 5.7, 7.8 Hz, 1H), 6.38 (d, *J* = 7.5 Hz, 1H), 6.61 (t, *J* = 7.8 Hz, 1H), 6.69 (s, 1H), 6.86 (d, *J* = 8.1 Hz, 1H); UV λ_{max} 219.1, 270.0, 278.4 (shoulder), 289.1 nm (shoulder); MS-ESI *m/z* 219.2 $[M + H]^+$; HRMS-ESI TOF *m/z* $[M + H]^+$ calcd for $C_{12}H_{15}N_2O_2$ 219.1134, found 219.1136. The ^{1}H NMR matched the literature.[6]

*4-Methoxy-L-tryptophan (**4**).* 4-Methoxyindole (160 mg, 1.09 mmol) was incubated with 8 mg of tryptophan synthase for 24 h as described above. Column chromatography yielded 120 mg (47%) of a light yellow solid; R_f 0.44 (ACN:H_2O 4:1); ^{1}H NMR (500 MHz, D_2O) δ 3.23 (dd, *J* = 8.5, 14.5 Hz 1H), 3.67 (dd, *J* = 4.5, 14.5 Hz, 1H), 4.01 (s, 3H), 4.14 (dd, *J* = 4.5, 8.5 Hz, 1H), 6.70 (d, *J* = 7.0 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 1H), 7.18 (s, 1H), 7.22 (t, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, D_2O) δ 28.0, 55.5, 56.5,

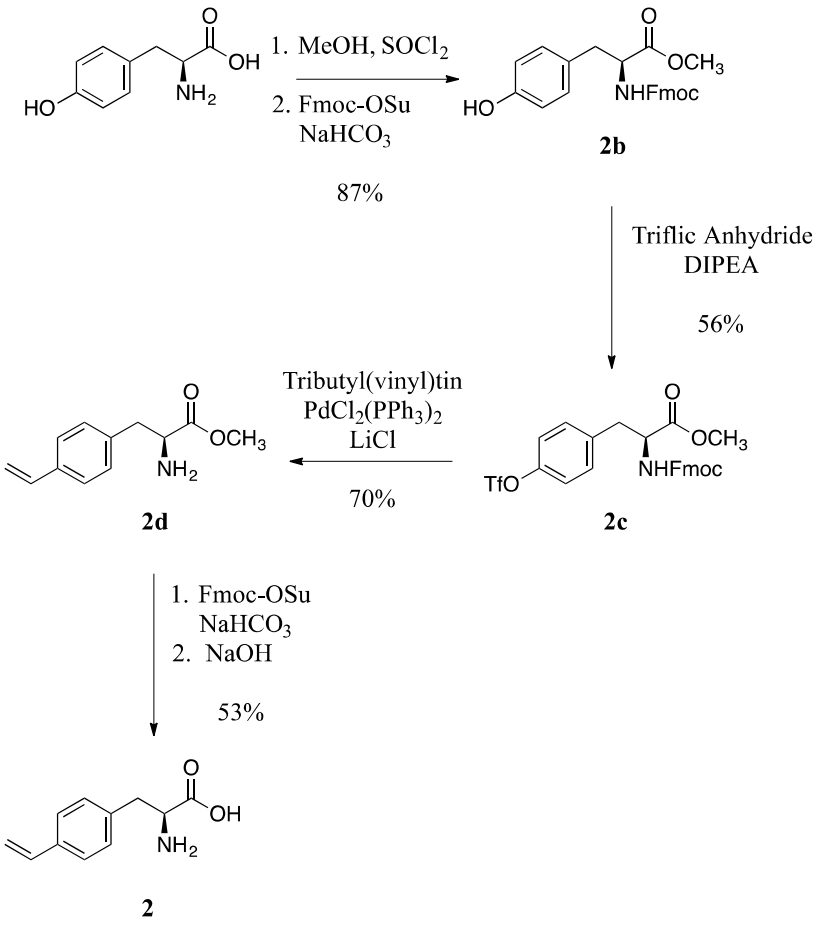
100.1, 105.7, 107.7, 116.6, 123.3, 124.4, 138.3, 153.8, 174.8; UV λ_{max} 217.9, 265.3, 279.6, 289.1 nm; MS-ESI *m/z* 257.1 $[M + Na]^+$, 273.1 $[M + K]^+$; HRMS-ESI TOF *m/z* $[M + H]^+$ calcd for $C_{12}H_{14}N_2O_3Na$ 257.0902, found 257.0903. The ^1H NMR matched the literature for 4-methoxy-D-tryptophan in DMSO-d_6, with the exception of the α proton.[7]

*7-Methyl-L-tryptophan (**5**).* 7-Methylindole (160 mg, 1.22 mmol) was incubated with 2.34 mg tryptophan synthase for 42 h as described above. Column chromatography yielded 230 mg (86%) as a white solid; R_f 0.47 (ACN:H_2O 4:1); ^1H NMR (500 MHz, D_2O) δ 2.57 (s, 3H), 3.36 (dd, *J* = 7.5, 15.0 Hz, 1H), 3.53 (d, *J* = 15.0 Hz, 1H), 4.09-4.15 (m, 1H), 7.15-7.22 (m, 2H), 7.40 (s, 1H), 7.64 (d, *J* = 7.5 Hz, 1H); UV (ACN:H_2O 1:3) λ_{max} 217.9, 270.0, 276.0 nm; HRMS-ESI TOF *m/z* $[M + H]^+$ calcd for $C_{12}H_{14}N_2O_2Na$ 241.0953, found 241.0955. The ^1H NMR matched the literature.[6]

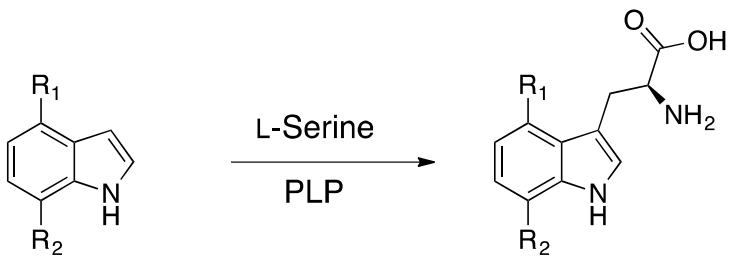
Scheme S1. Synthetic scheme for the preparation of 4-mercapto-L-phenylalanine.



Scheme S2. Synthetic scheme for the preparation of 4-vinyl-L-phenylalanine.



Scheme S3. Biosynthetic scheme for the preparation of L-tryptophan analogues using tryptophan synthase.



NMR spectra of aromatic substrate analogues and precursors

All NMR spectra were recorded on a Varian Unity 300 MHz (300 MHz for ^{1}H) or a Varian VWR 500 MHz (500 MHz for ^{1}H, 125 MHz for ^{13}C) spectrometer in $CDCl_3$, DMSO-d_6, D_2O or D_2O containing minimal NaOD. NMR spectra were visualized with vNMRj and processed with MestReNova 7.1. All chemical shifts are reported in parts per million (ppm). ^{1}H chemical shifts are referenced relative to TMS or DSS at δ 0.00 ppm. ^{13}C chemical shifts are referenced relative to TMS or DSS at δ 0.0 ppm. ^{19}F chemical shifts are referenced relative to trifluoroacetic acid at δ -76.55 ppm.

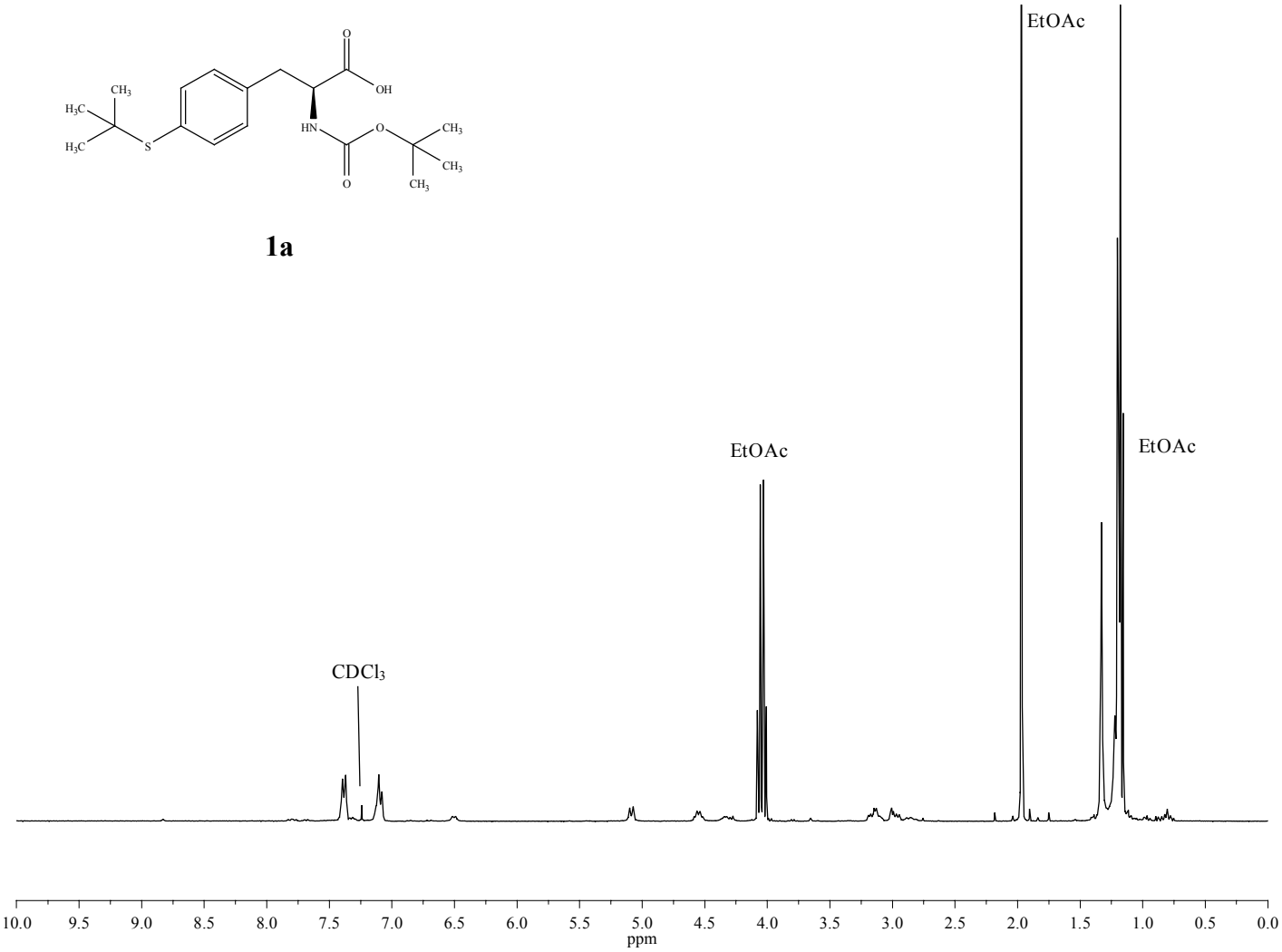


Figure S3. ^{1}H NMR (300 MHz) spectrum of **1a** in $CDCl_3$.

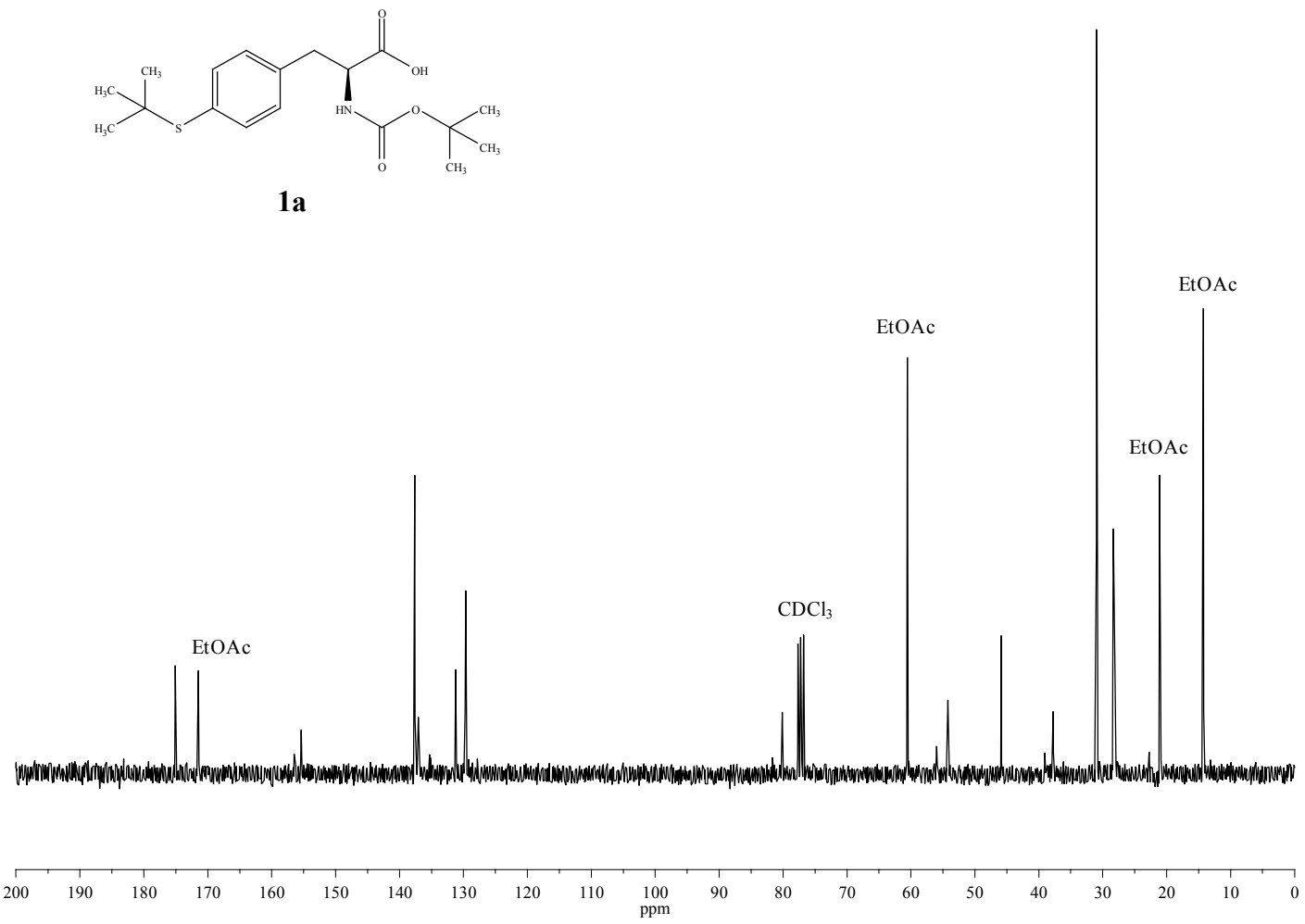


Figure S4. ^{13}C NMR (75 MHz) spectrum of **1a** in $CDCl_3$.

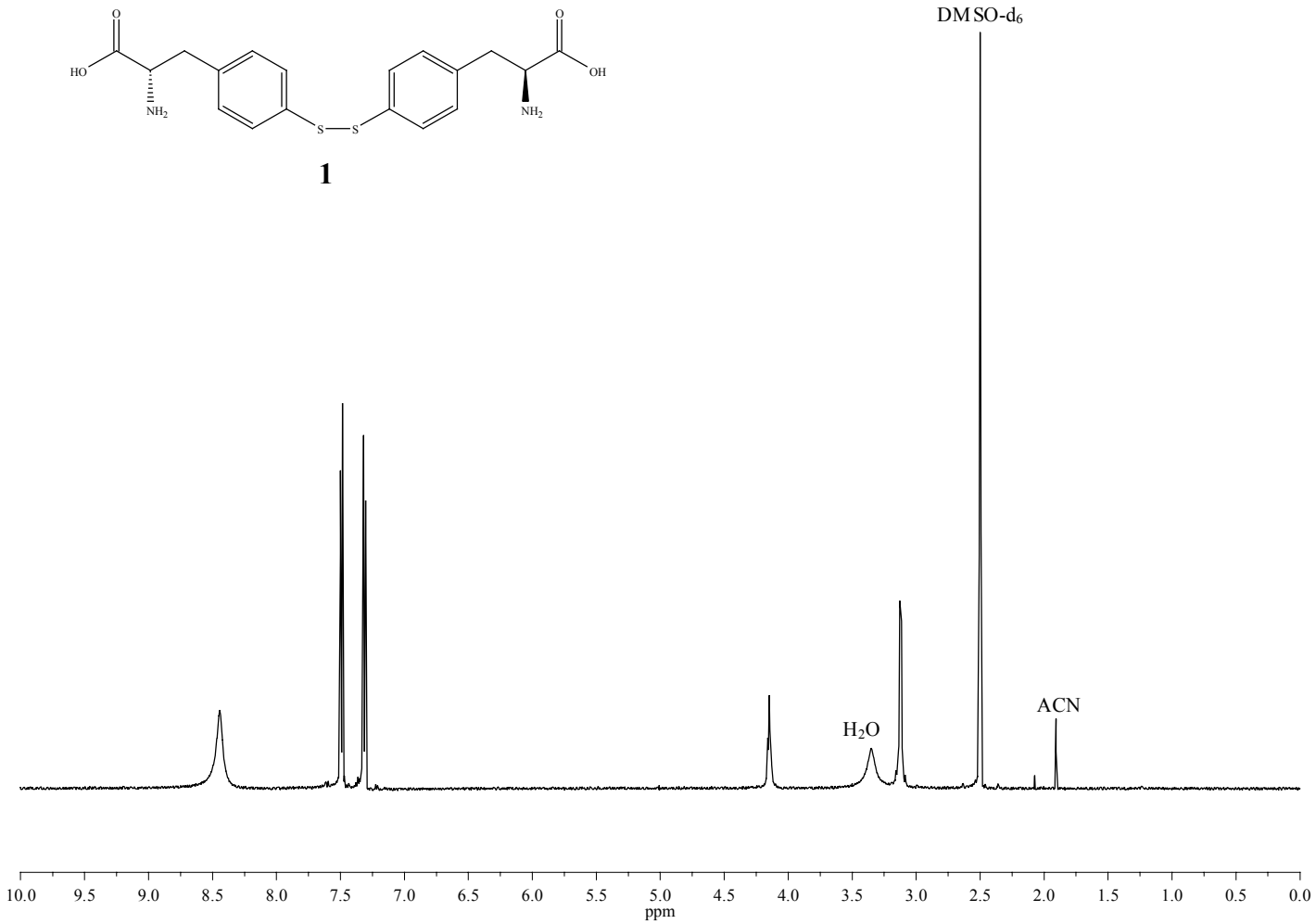


Figure S5. ^{1}H NMR (500 MHz) spectrum of **1** in DMSO-d_6.

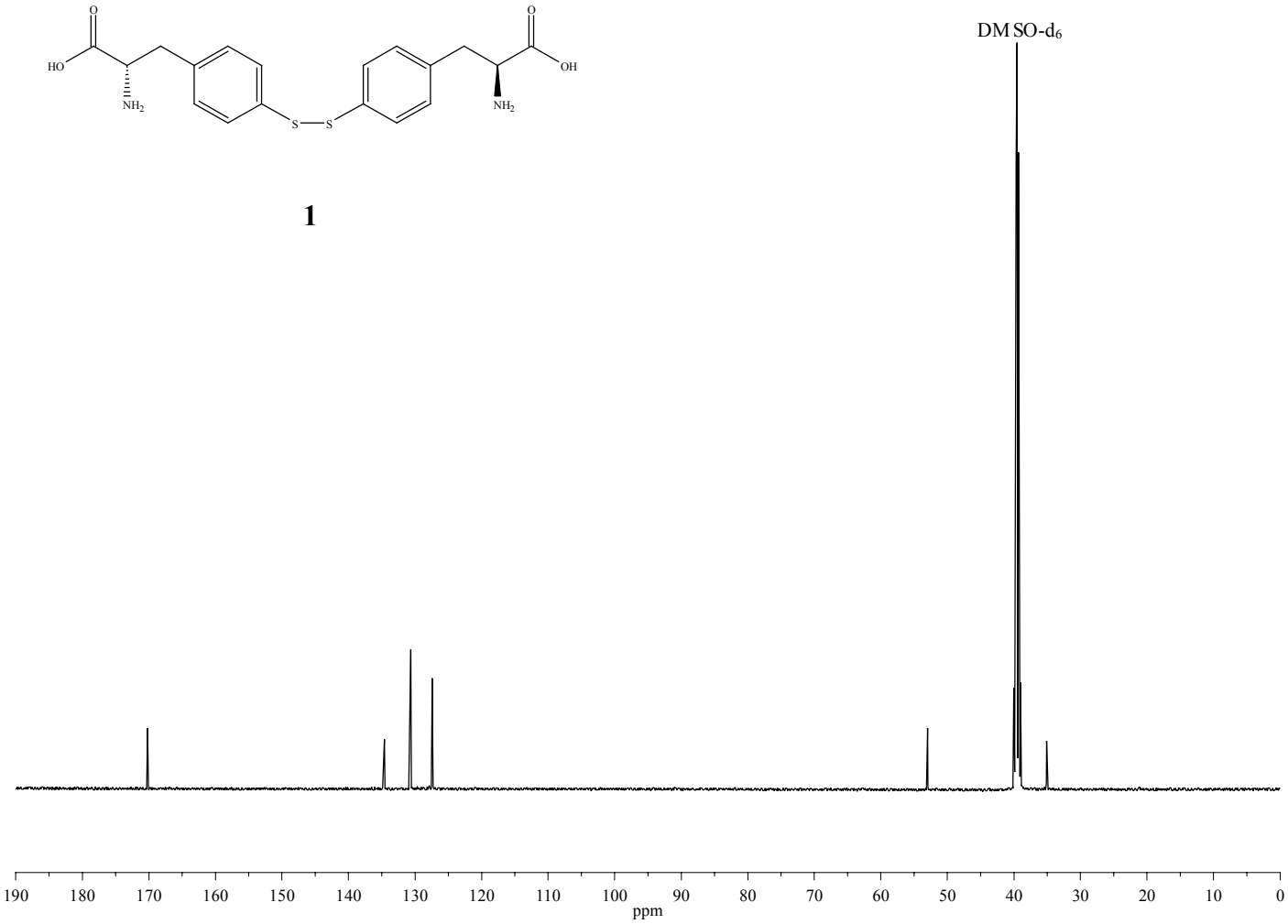


Figure S6. ^{13}C NMR (125 MHz) spectrum of **1** in DMSO-d_6.

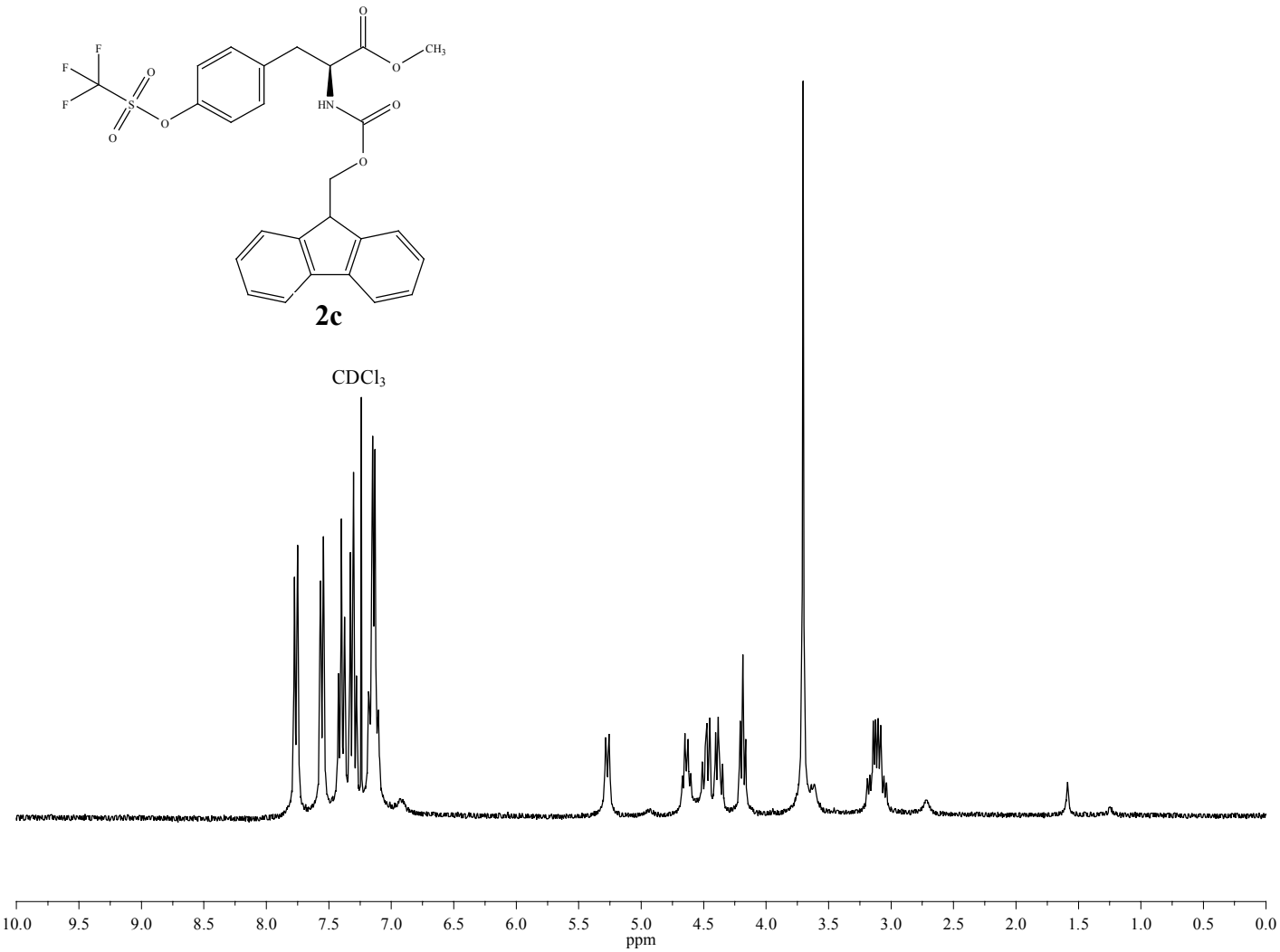


Figure S7. ^{1}H NMR (300 MHz) spectrum of **2c** in $CDCl_3$.

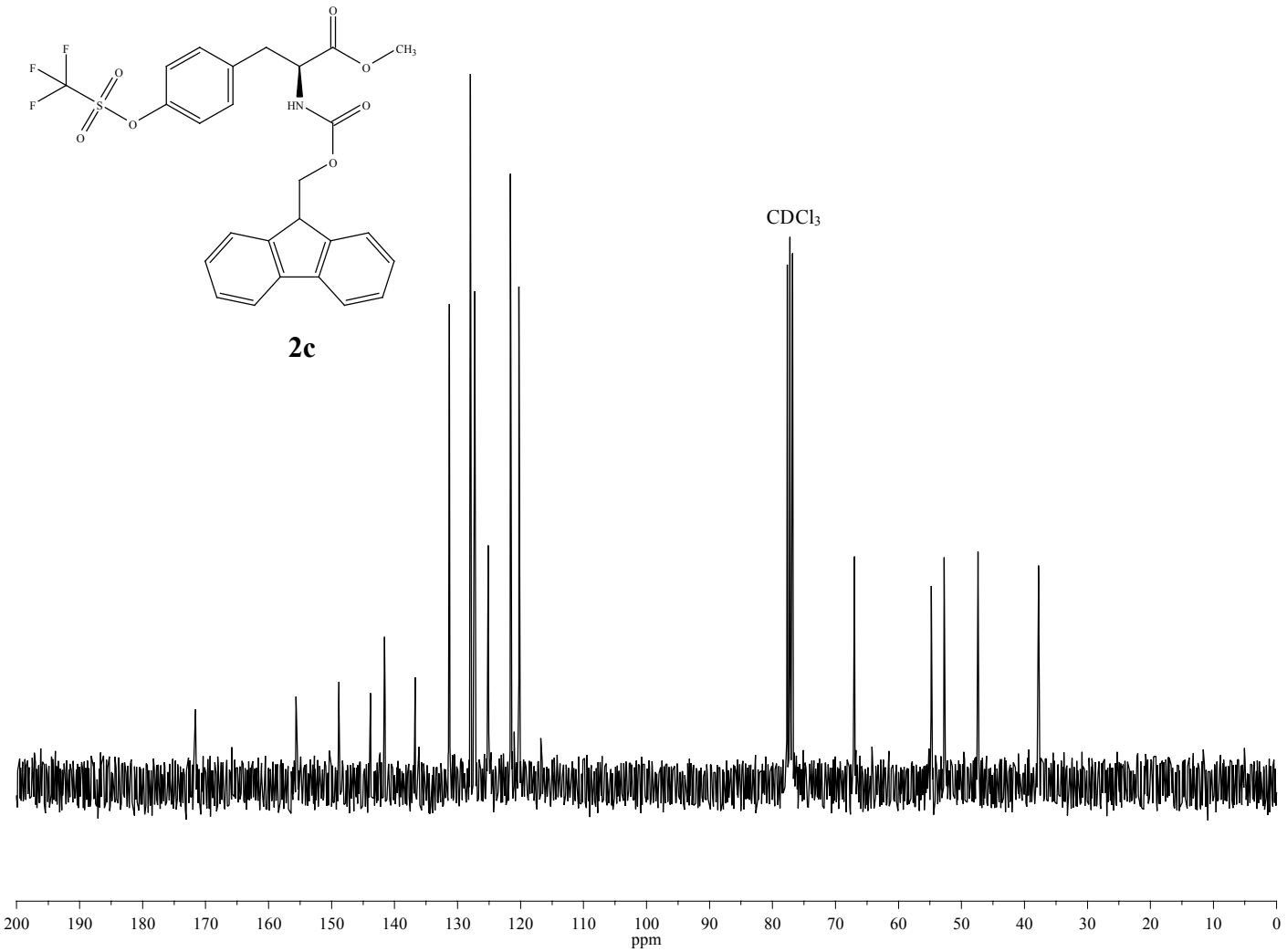


Figure S8. ^{13}C NMR (75 MHz) spectrum of **2c** in $CDCl_3$.

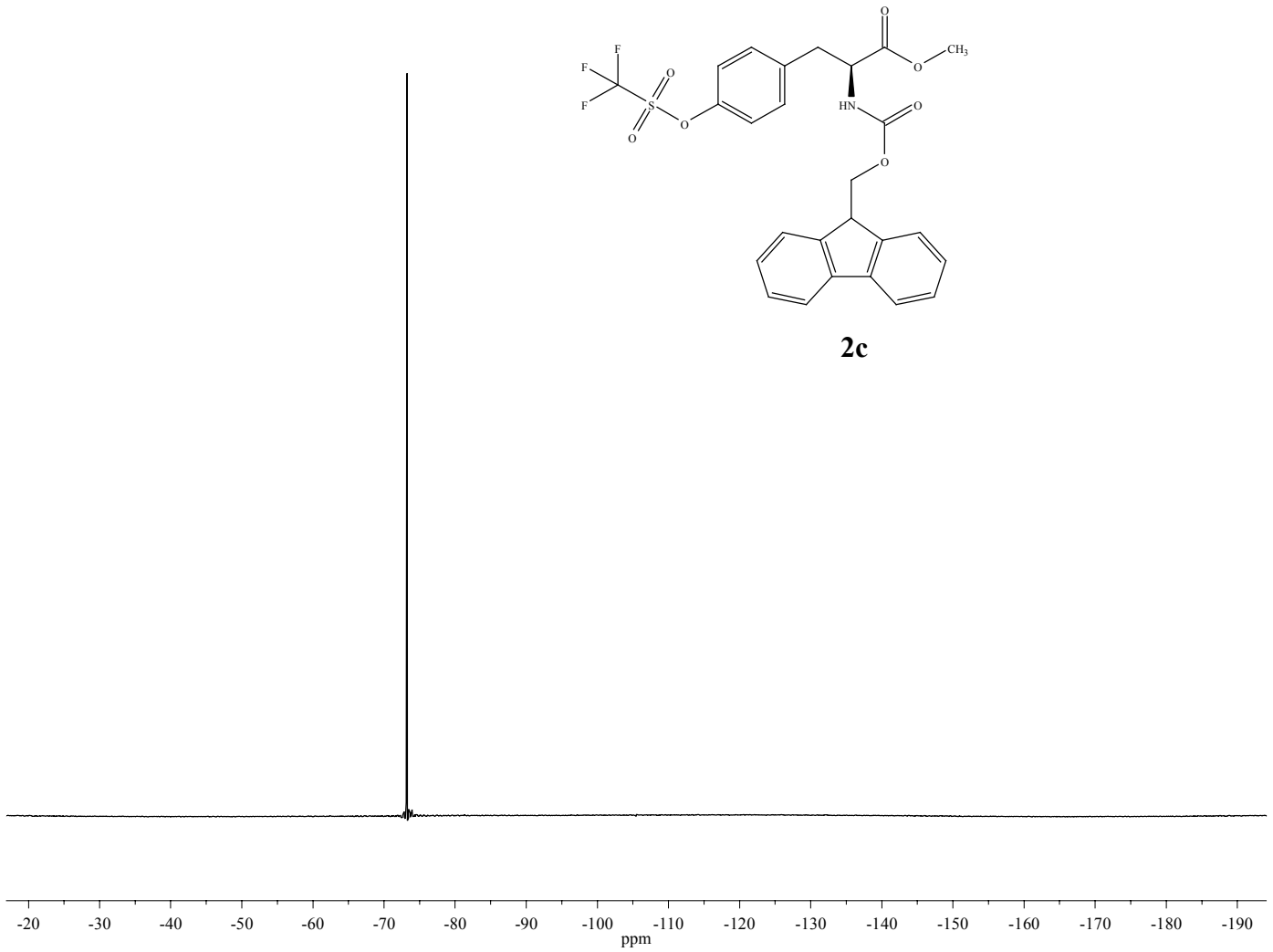


Figure S9. ^{19}F NMR (282 MHz) spectrum of **2c** in $CDCl_3$.

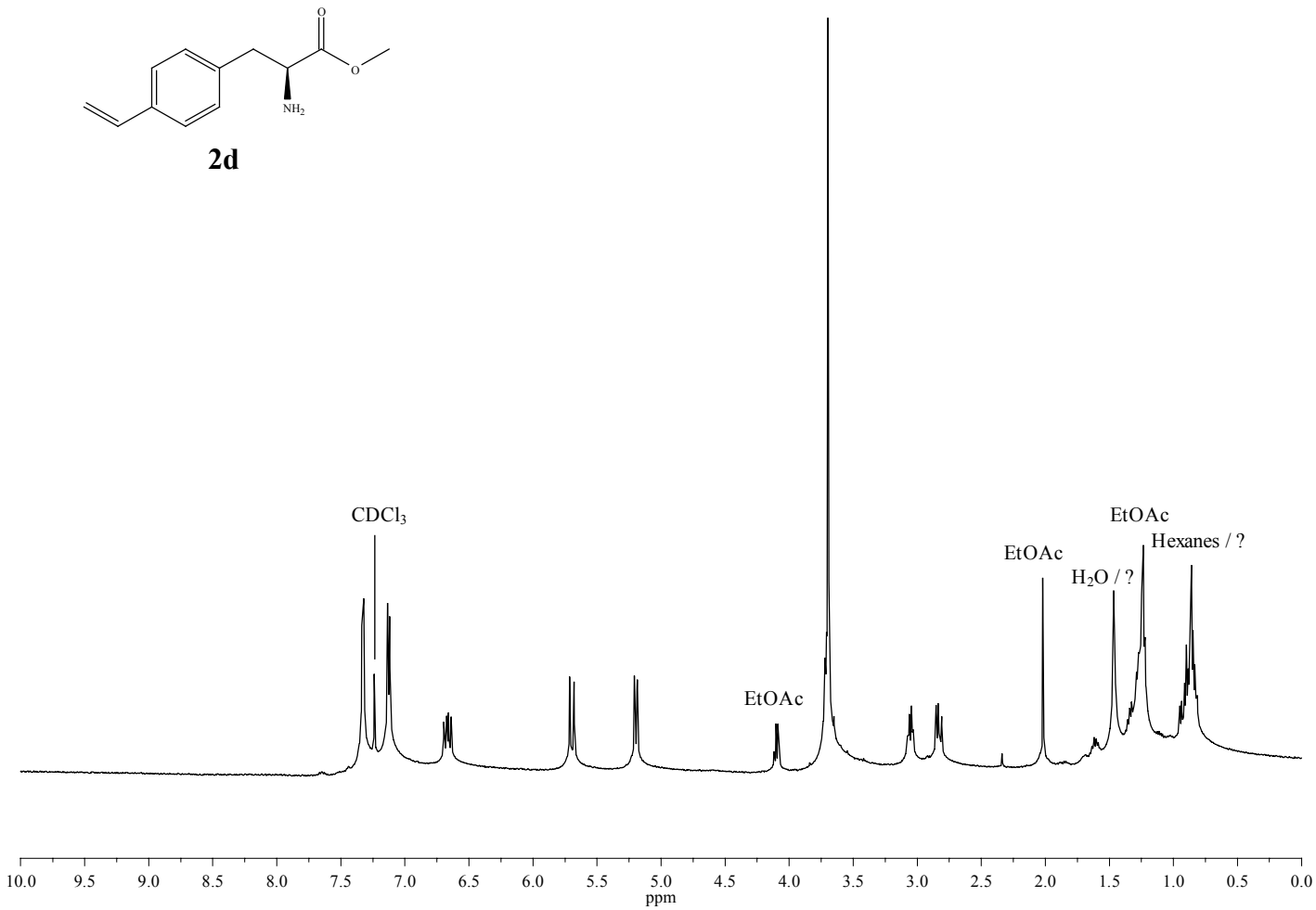


Figure S10. ^{1}H NMR (500 MHz) spectrum of **2d** in $CDCl_3$.

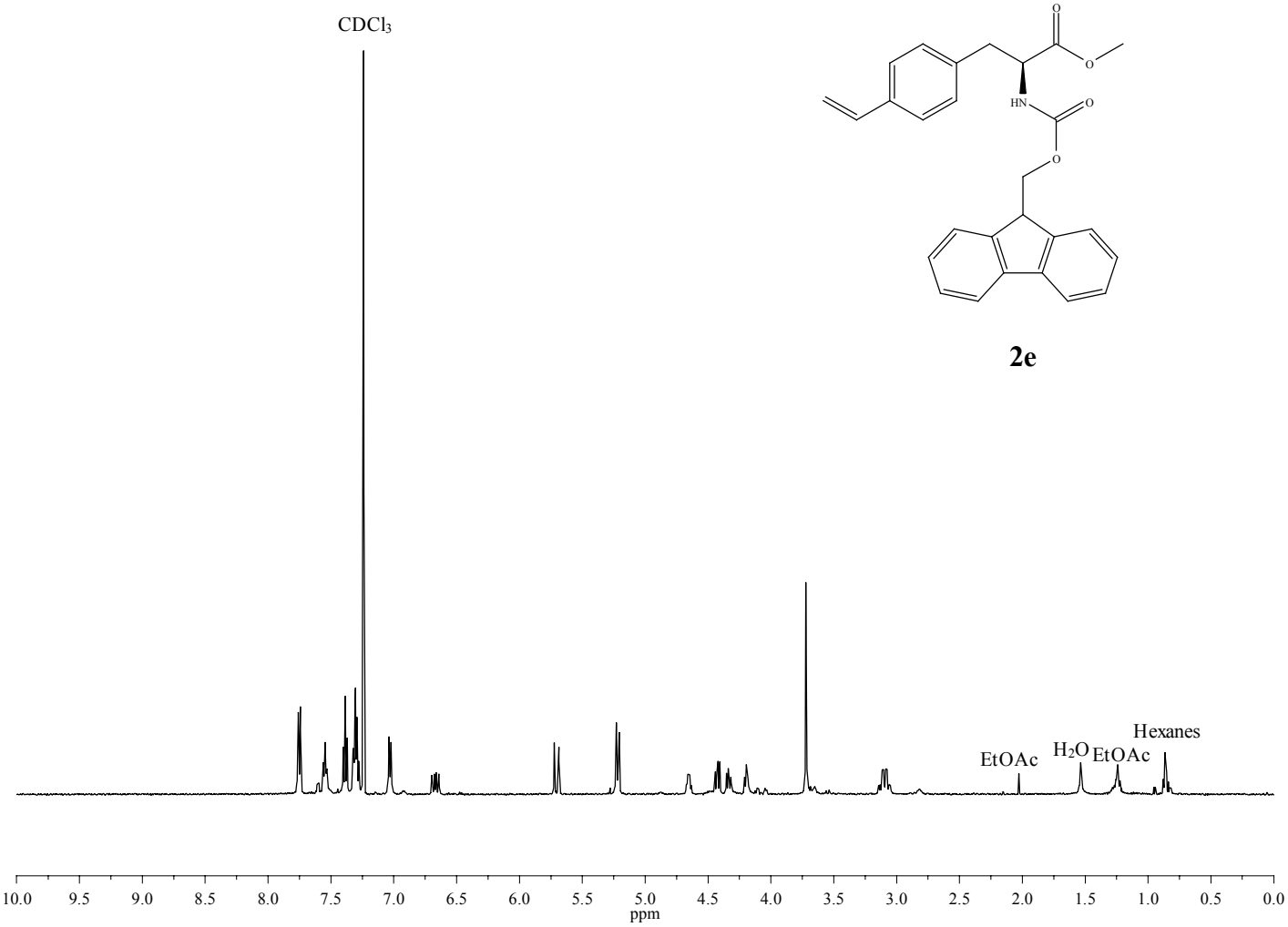


Figure S11. ^{1}H NMR (500 MHz) spectrum of **2e** in $CDCl_3$.

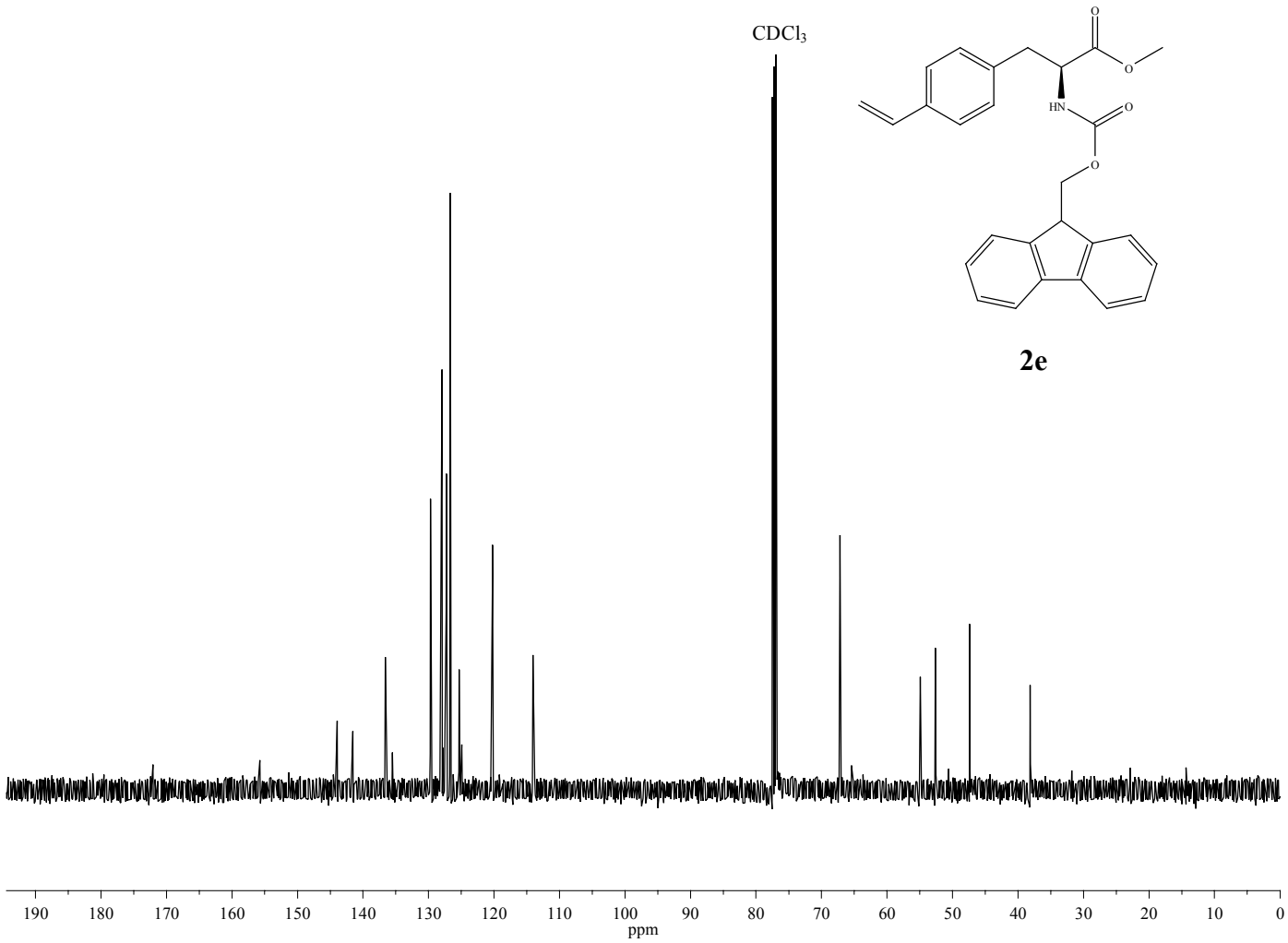


Figure S12. ^{13}C NMR (125 MHz) spectrum of **2e** in $CDCl_3$.

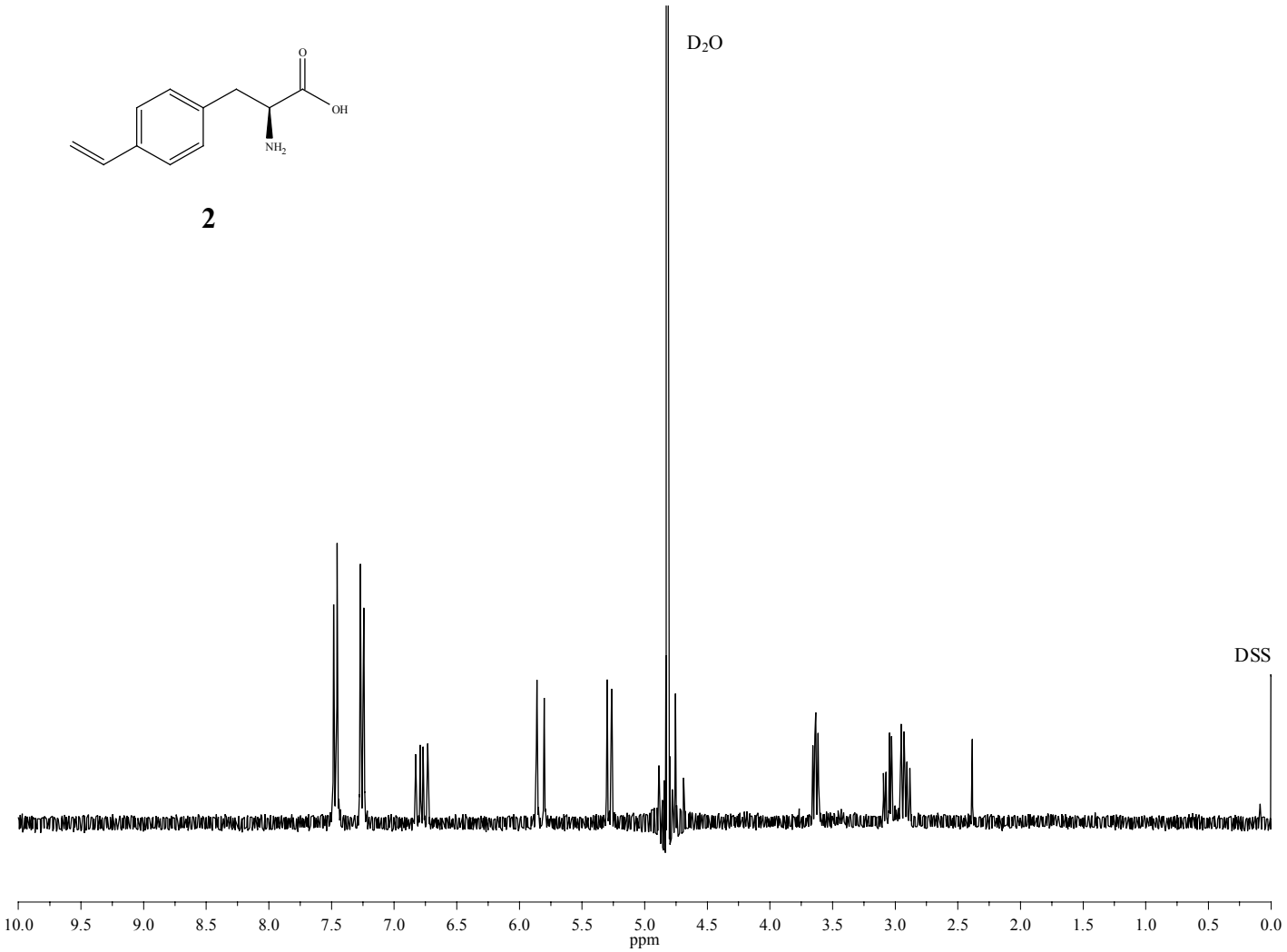


Figure S13. ^{1}H NMR (300 MHz) spectrum of **2** in D_2O with DSS.

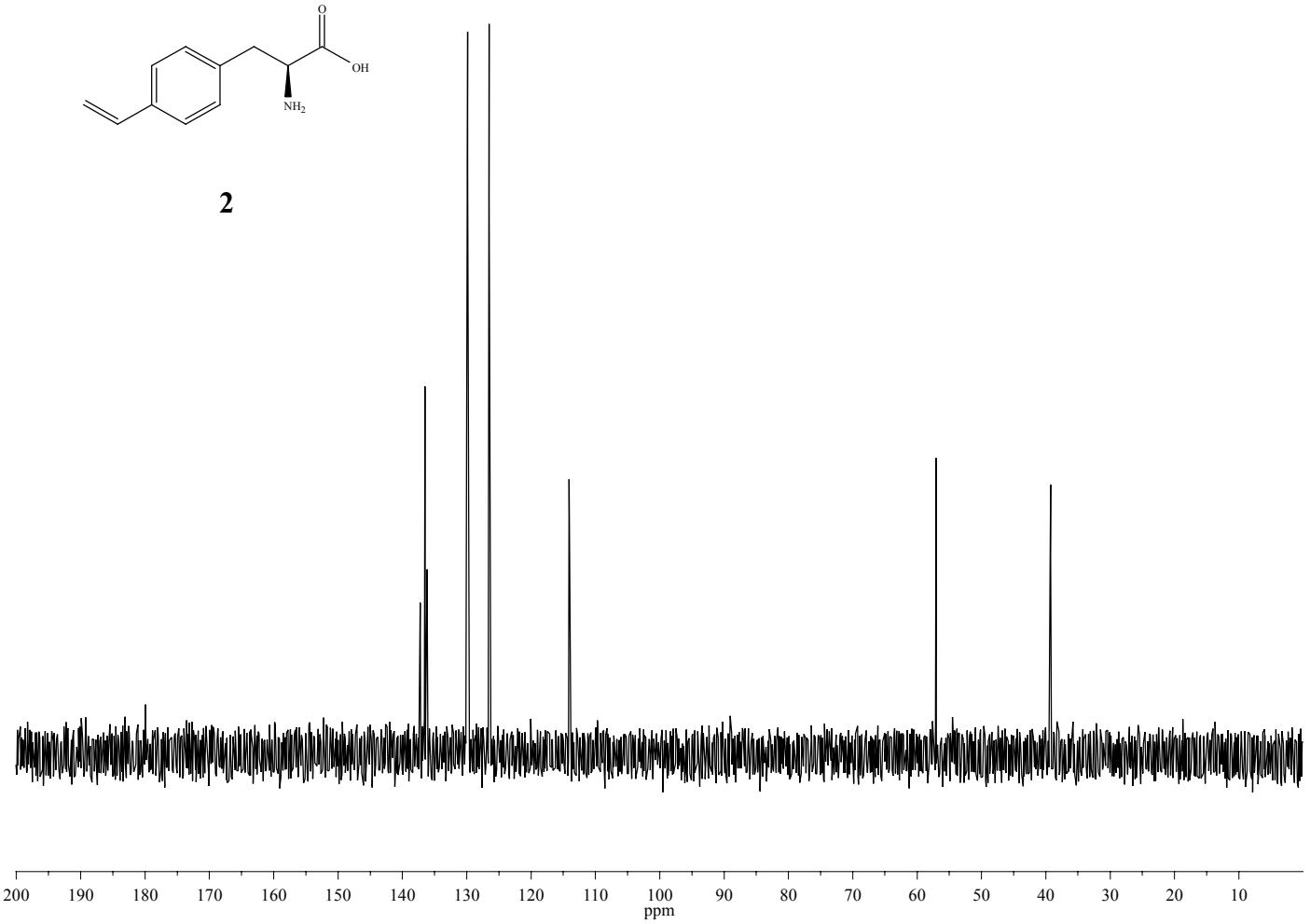


Figure S14. ^{13}C NMR (75 MHz) spectrum of **2** in D_2O.

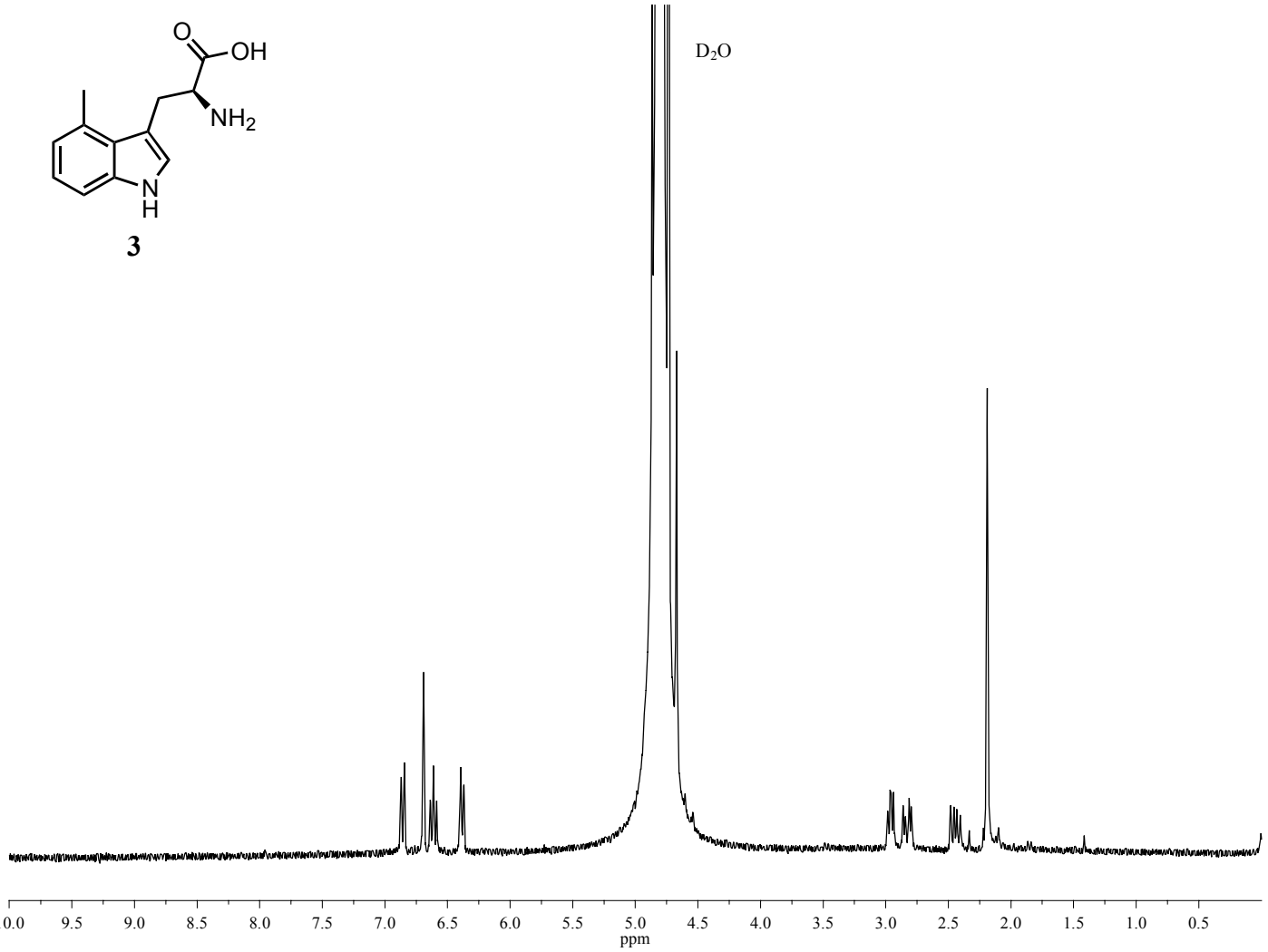


Figure S15. ^{1}H NMR (300 MHz) spectrum of 4-methyl-L-trytpophan (**3**) in D_2O/NaOD.

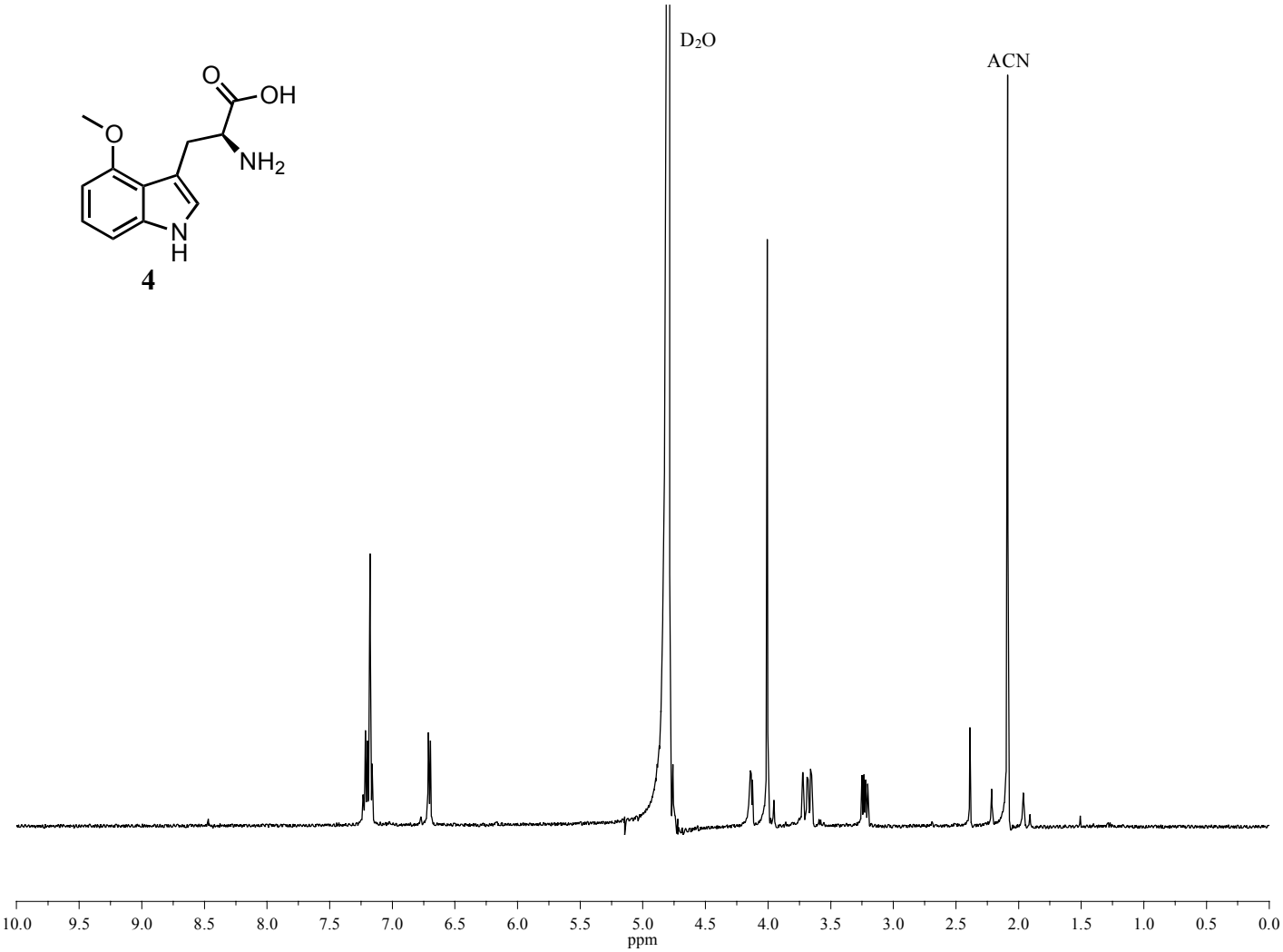


Figure S16. ^{1}H NMR (500 MHz) spectrum of 4-methoxy-L-trytpophan (**4**) in D_2O.

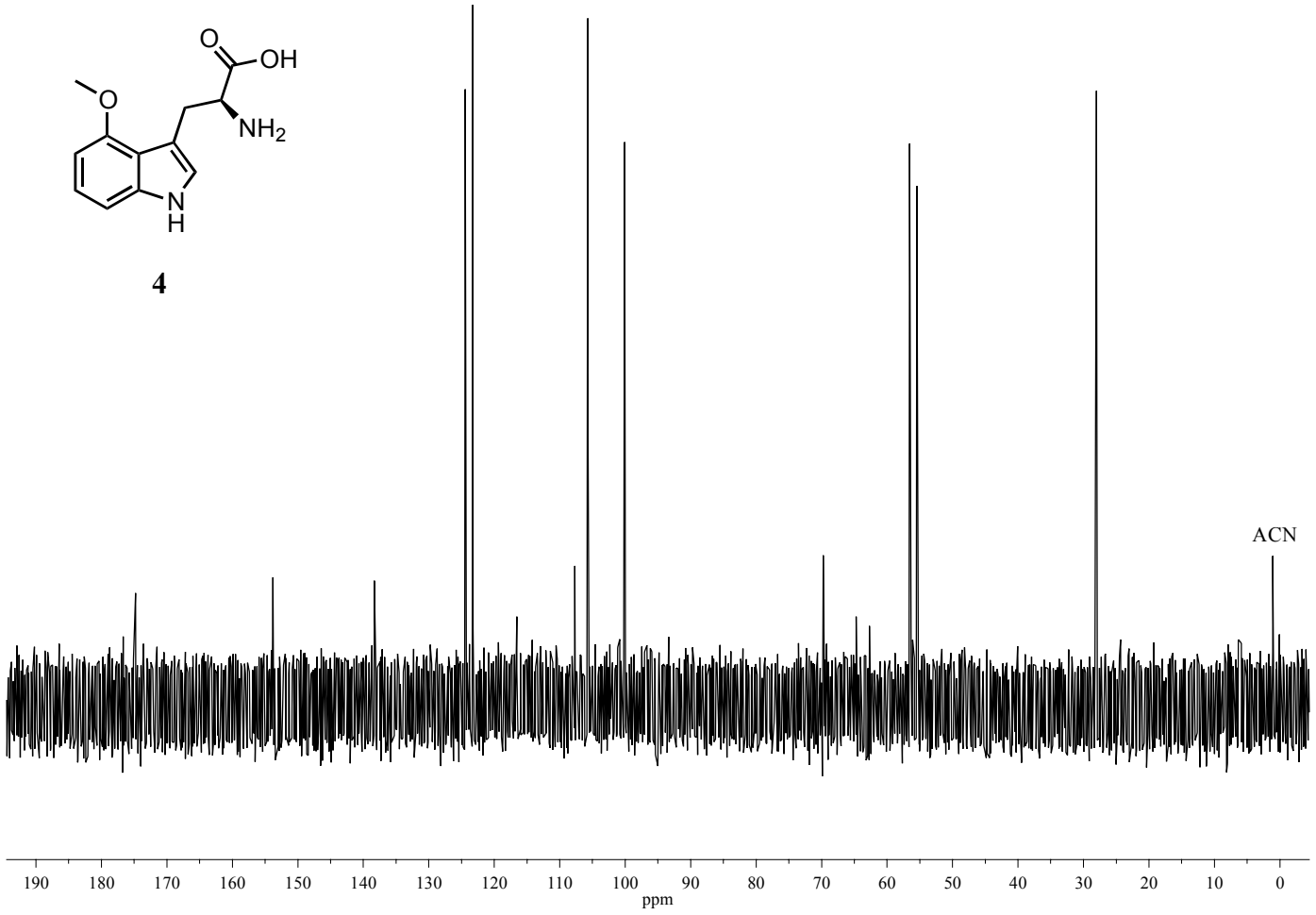


Figure S17. ^{13}C NMR (125 MHz) spectrum of 4-methoxy-L-trytpophan (**4**) in D_2O.

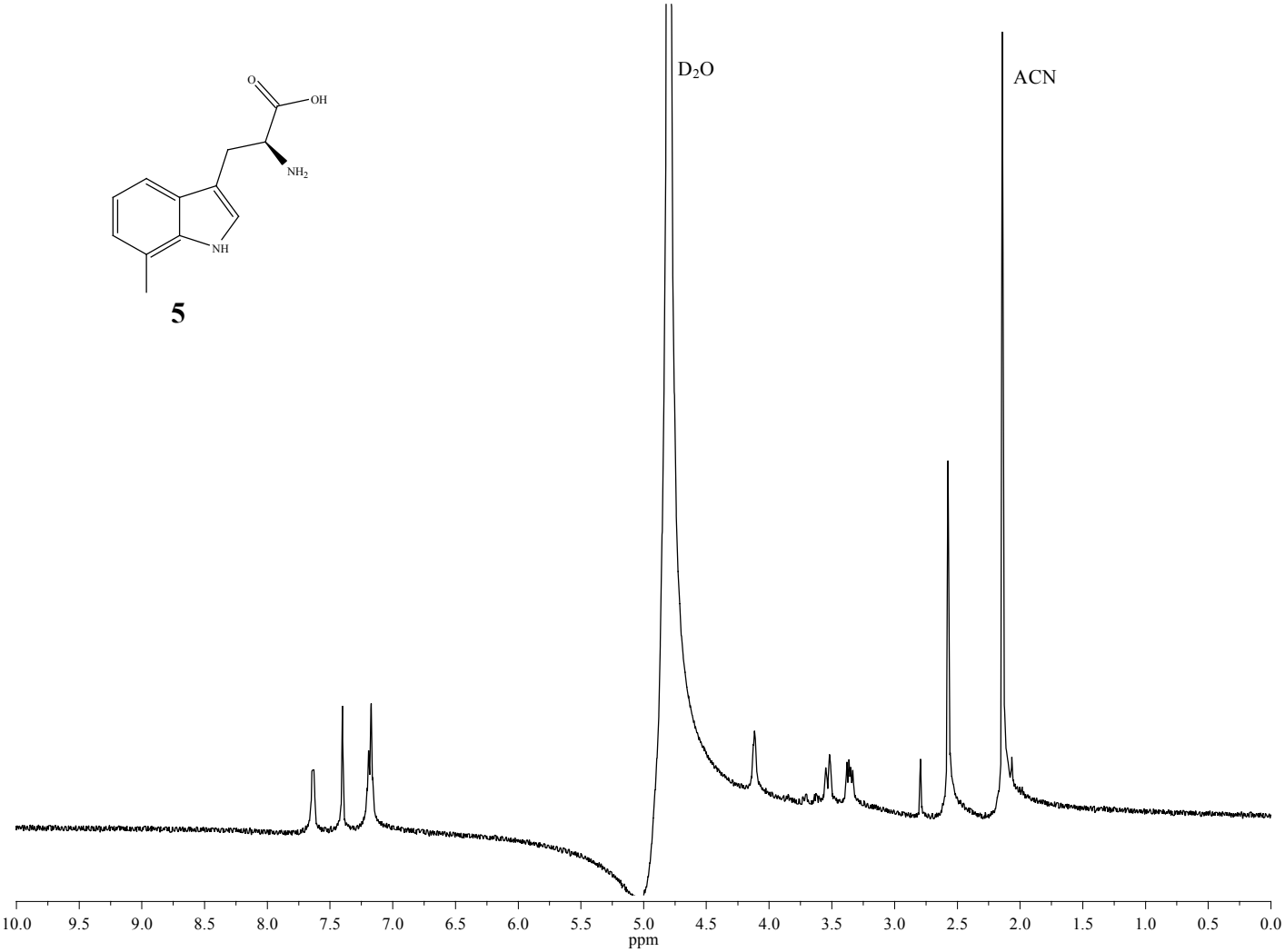


Figure S18. ^{1}H NMR (125 MHz) spectrum of 7-methyl-L-trytpophan (**5**) in D_2O

NMR spectra of prenylated tyrosine and tryptophan products

After HPLC purification, each prenylated product was lyophilized to dryness, redissolved in D_2O (D, 99.9%), re-lyophilized, dissolved in DMSO-d_6 (99.96%) or D_2O (D, 99.96%) (Cambridge Isotope Laboratories, Inc.; Andover, MA) and placed in a Norell 3 mm NMR tube (Sigma). NMR experiments were recorded on an INOVA 600 NMR spectrometer equipped with a HCN cryogenic probe. All spectra were visualized with vNMRj and processed with MestReNova 7.1. Any peaks listed in NMR tables, but not seen in spectra shown below (usually HSQC/HMQC or HMBC spectra) were visualized with vNMRj. Methanol was present in all samples due to solvent contamination during HPLC purification.

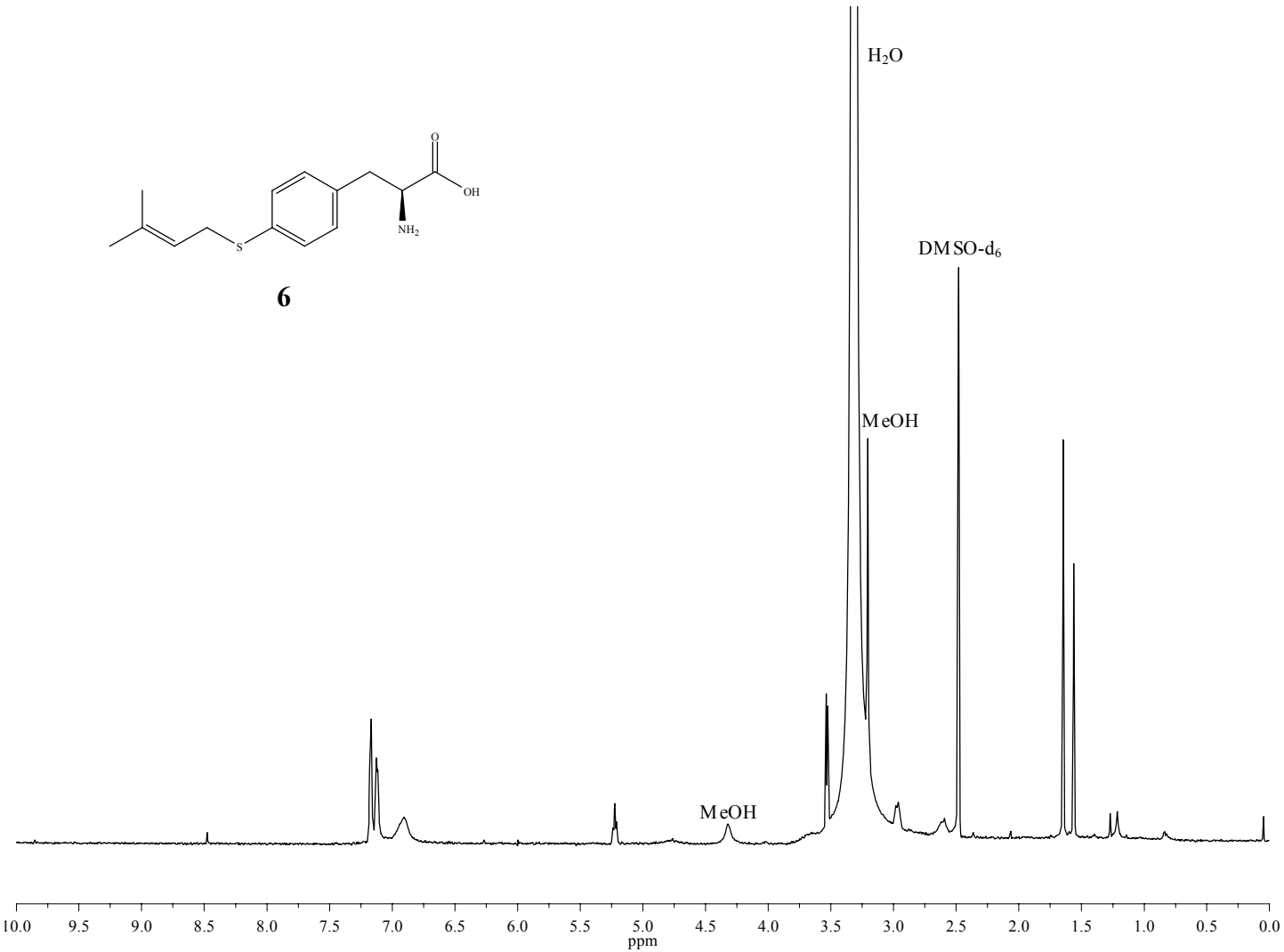


Figure S19. ^{1}H NMR (600 MHz) spectrum of **6** in DMSO-d_6.

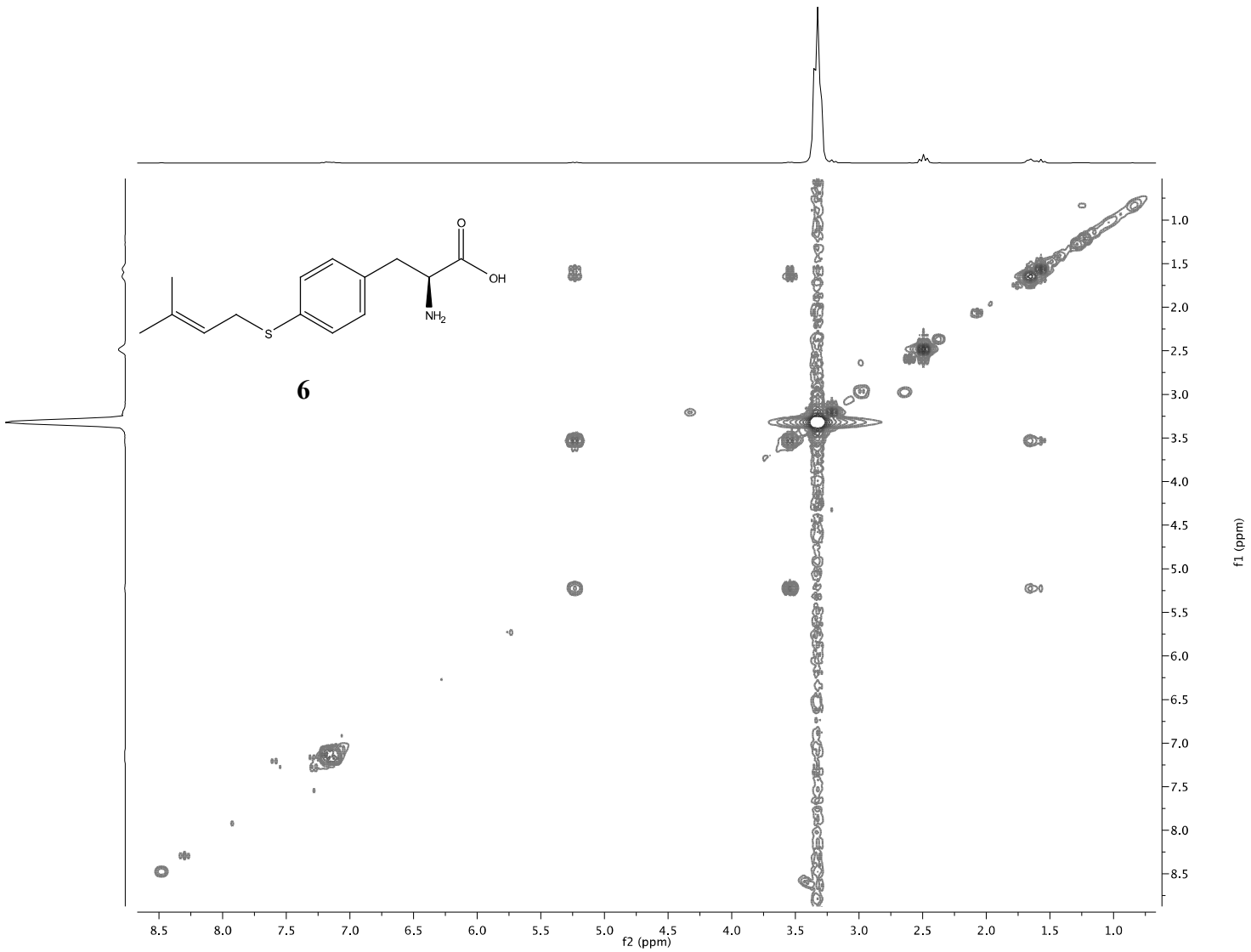


Figure S20. 2D ^{1}H-^{1}H COSY NMR spectrum of **6** in DMSO-d_6.

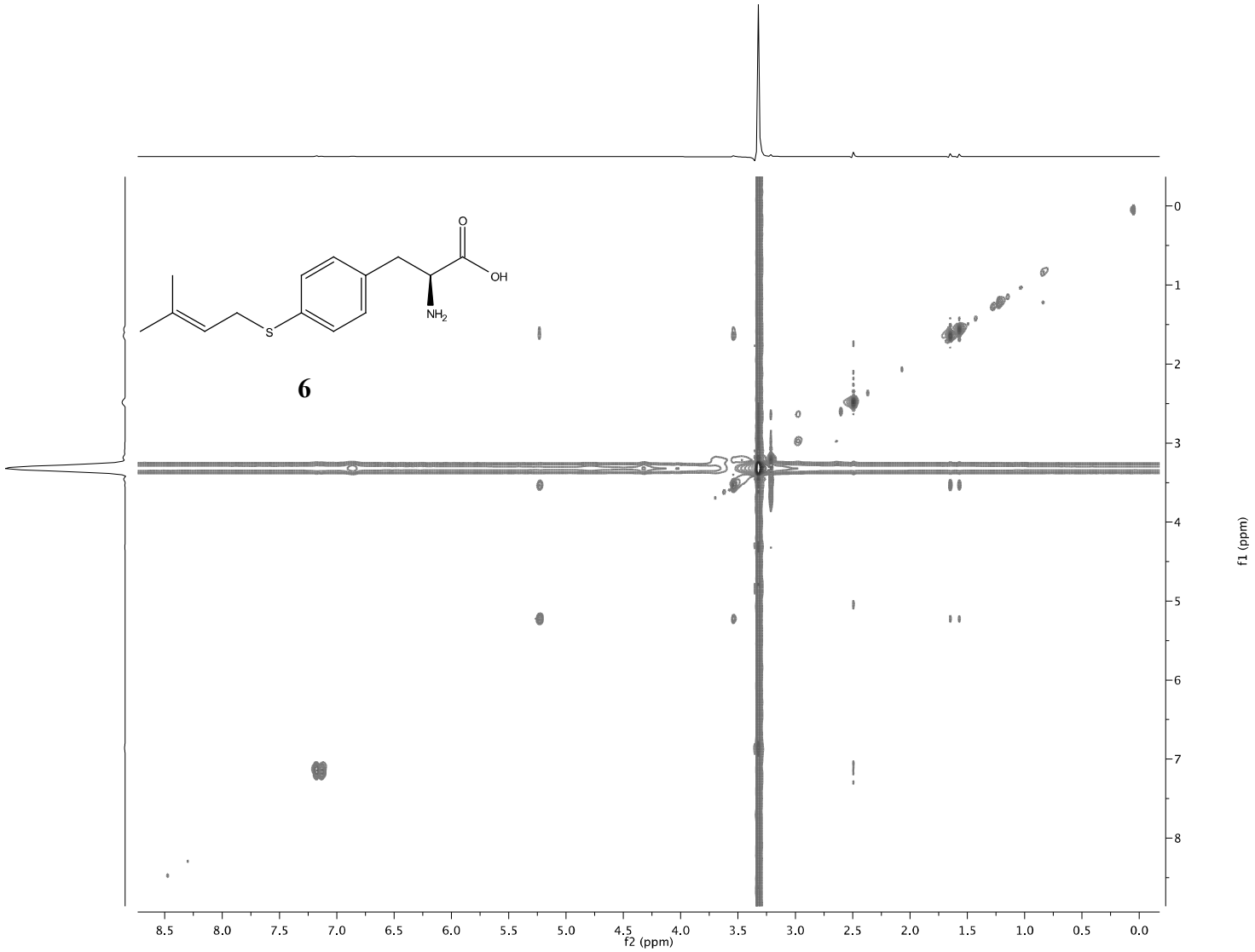


Figure S21. 2D ^{1}H-^{1}H TOCSY NMR spectrum of **6** in DMSO-d_6.

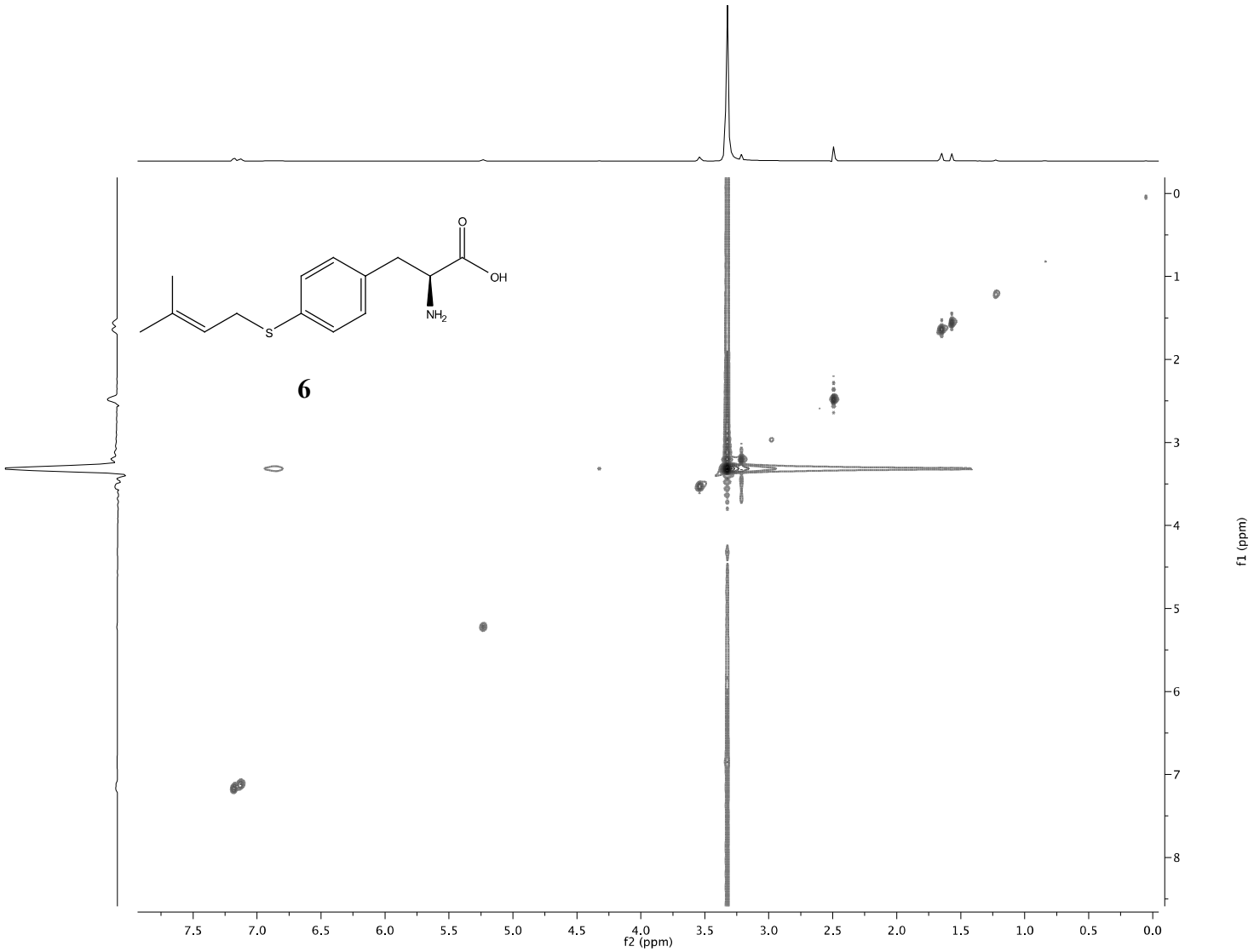


Figure S22. 2D ^{1}H-^{1}H ROESY NMR spectrum of **6** in DMSO-d_6.

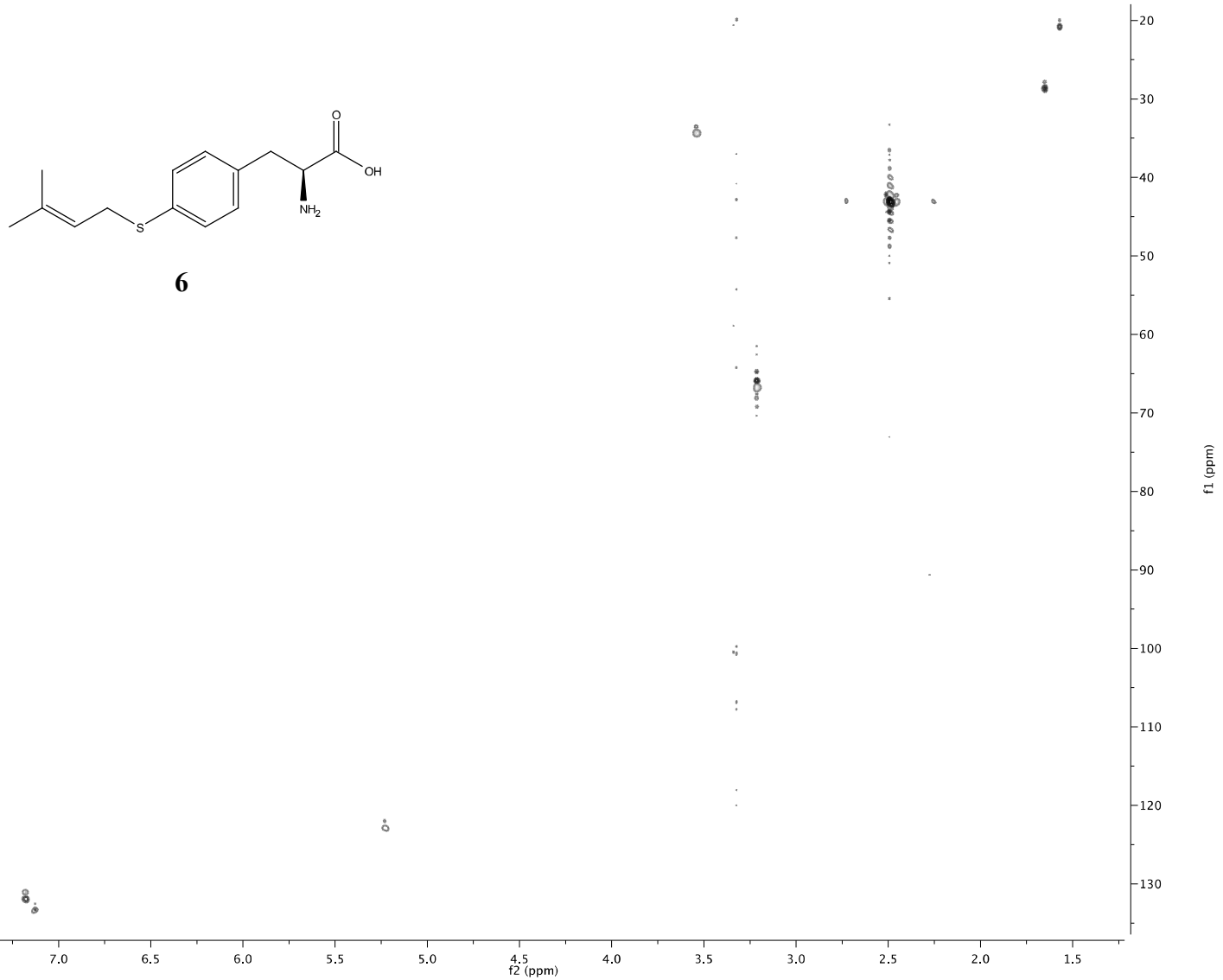


Figure S23. 2D ^{1}H-^{13}C HSQC NMR spectrum of **6** in DMSO-d_6.

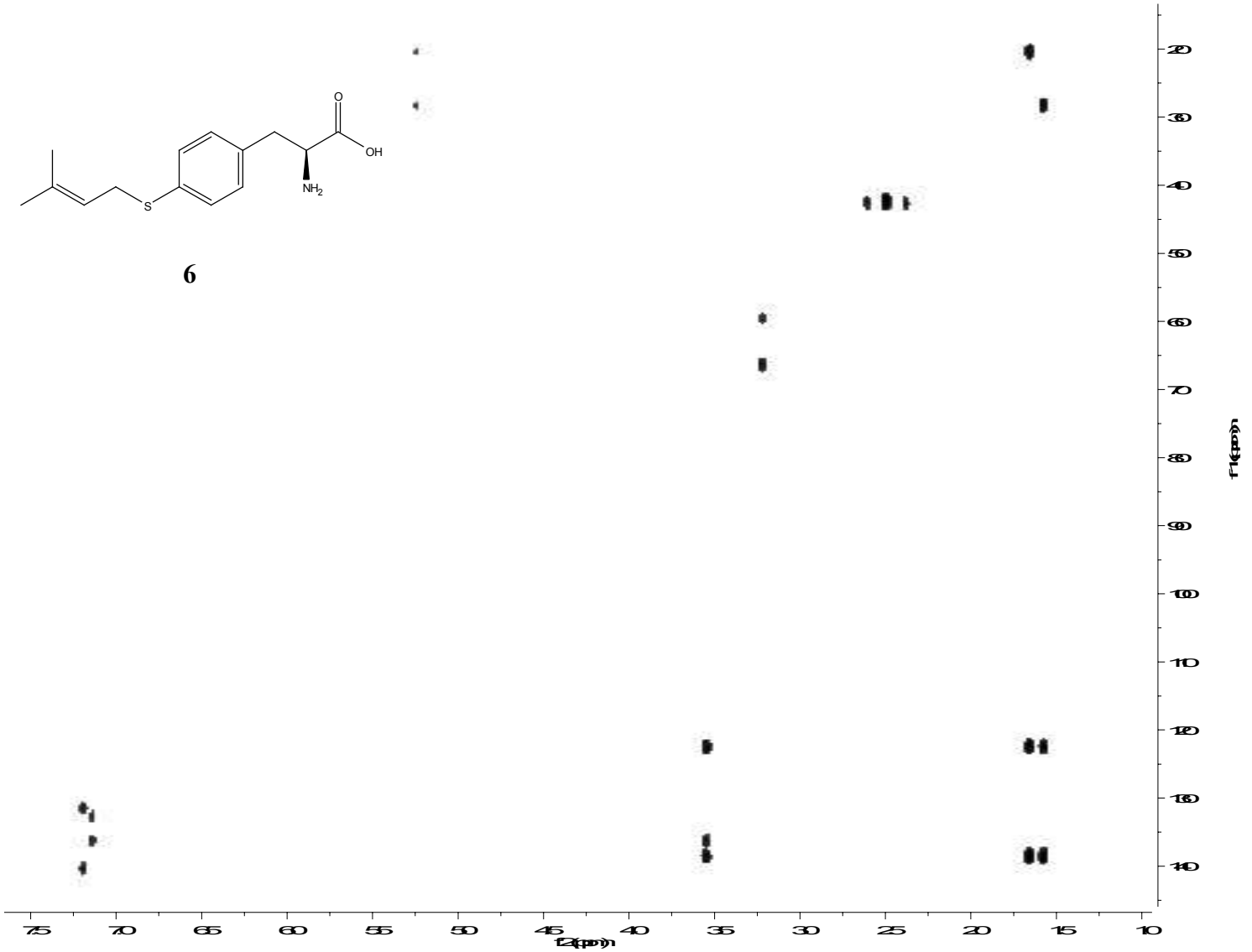


Figure S24. 2D ^{1}H-^{13}C HMBC NMR spectrum of **6** in DMSO-d_6.

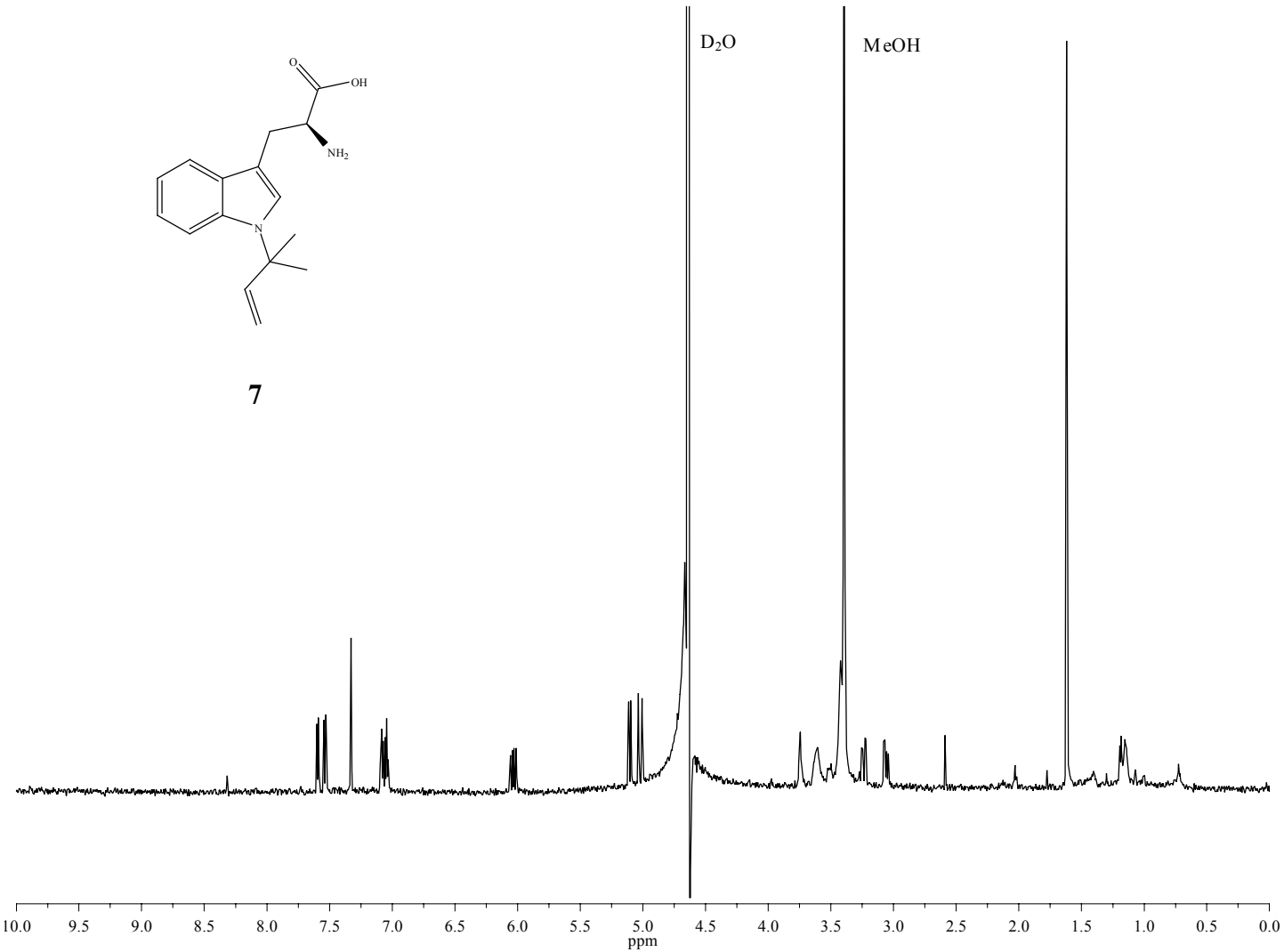


Figure S25. ^{1}H NMR (600 MHz) spectrum of **7** in D_2O.

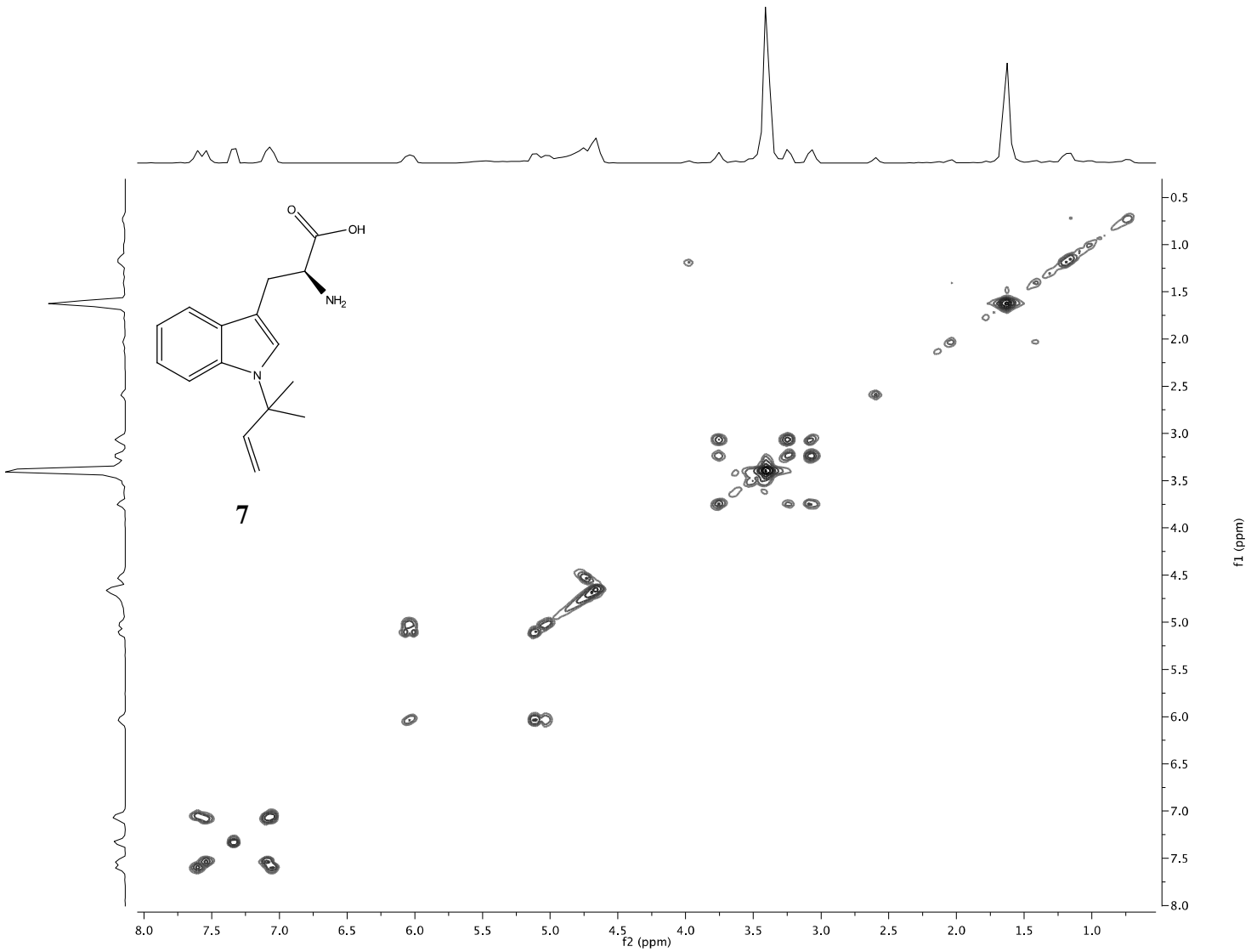


Figure S26. 2D ^{1}H-^{1}H COSY NMR spectrum of **7** in D_2O.

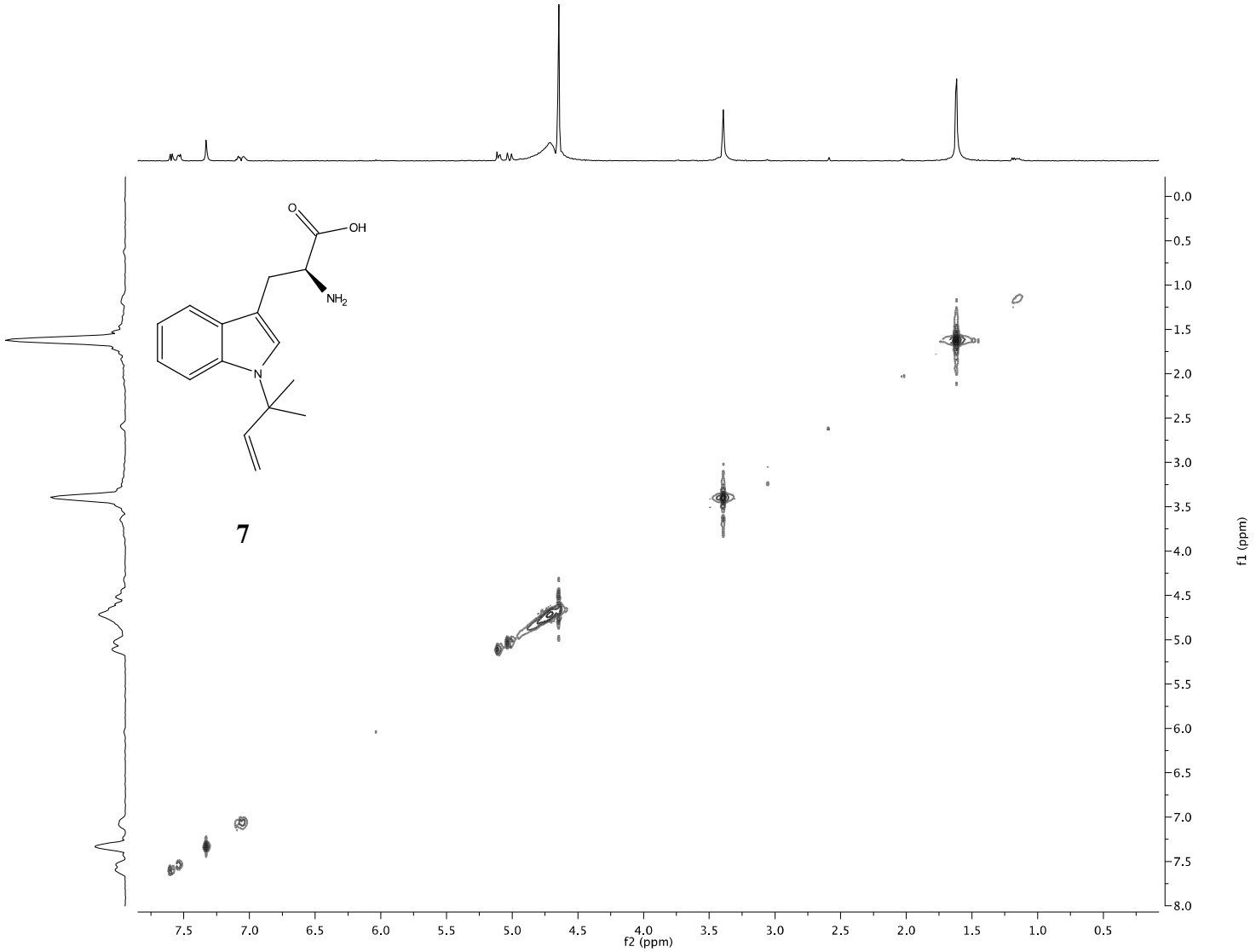


Figure S27. 2D ^{1}H-^{1}H TOCSY NMR spectrum of **7** in D_2O.

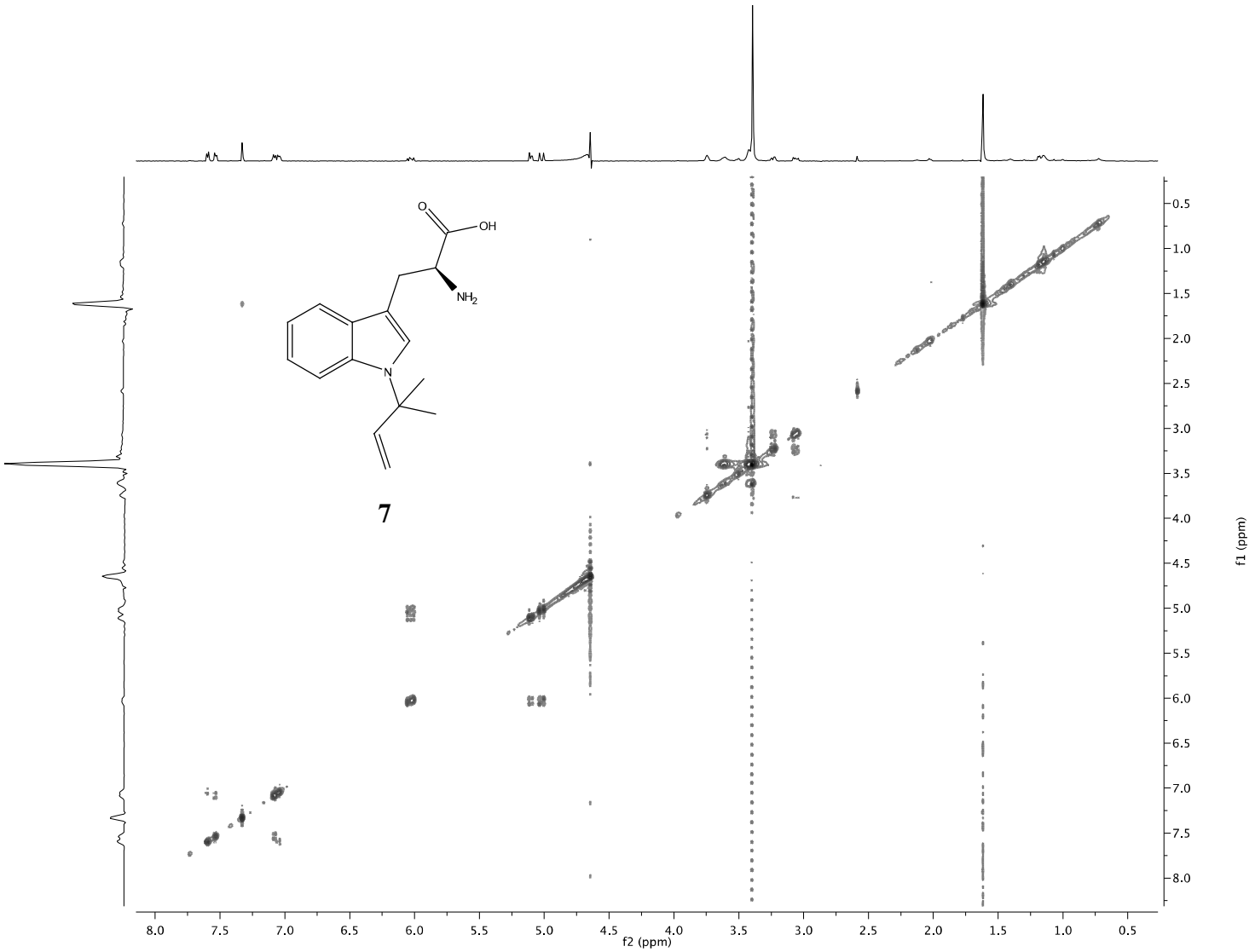


Figure S28. 2D ^{1}H-^{1}H ROESY NMR spectrum of **7** in D_2O.

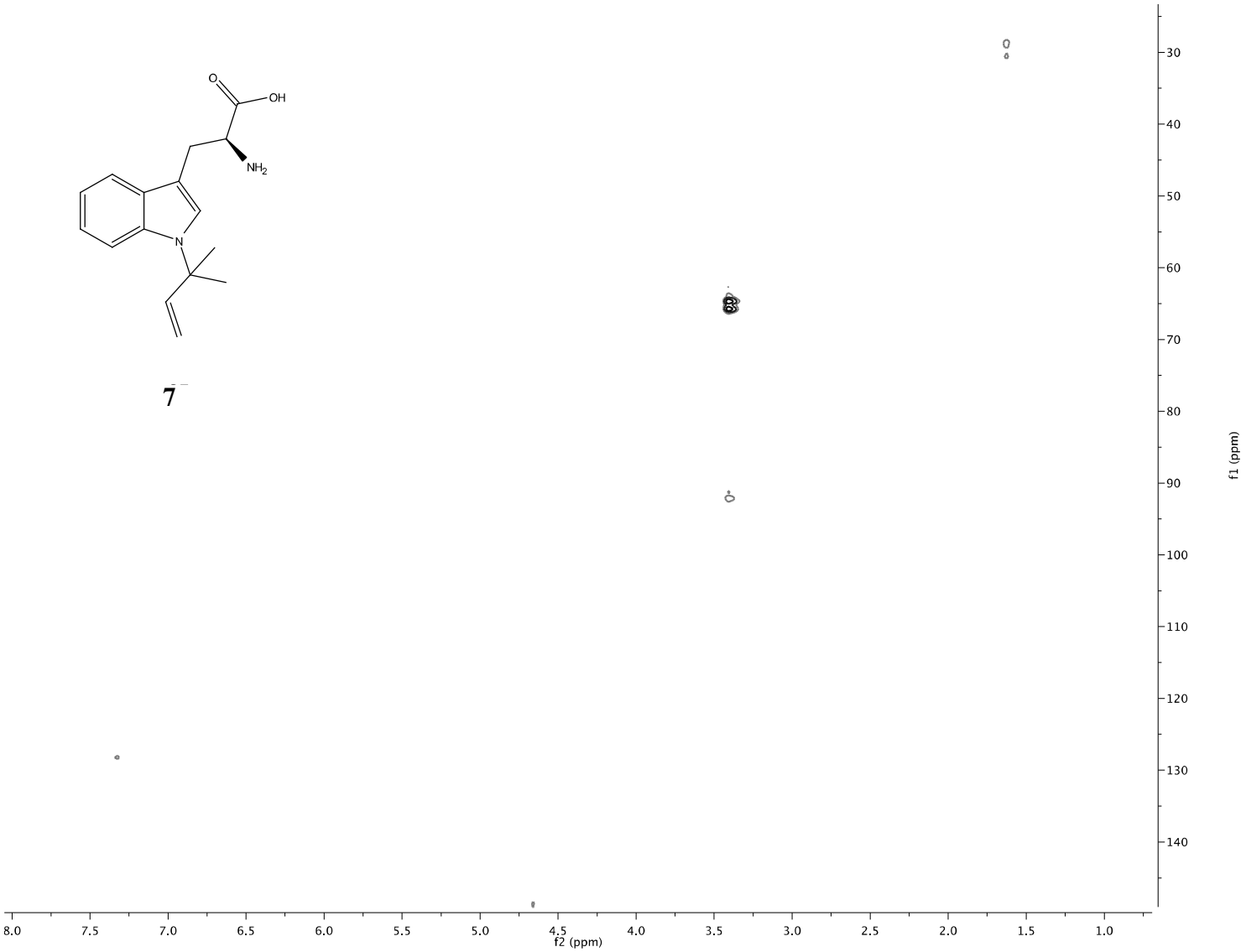


Figure S29. 2D ^{1}H-^{13}C HMQC NMR spectrum of **7** in D_2O.

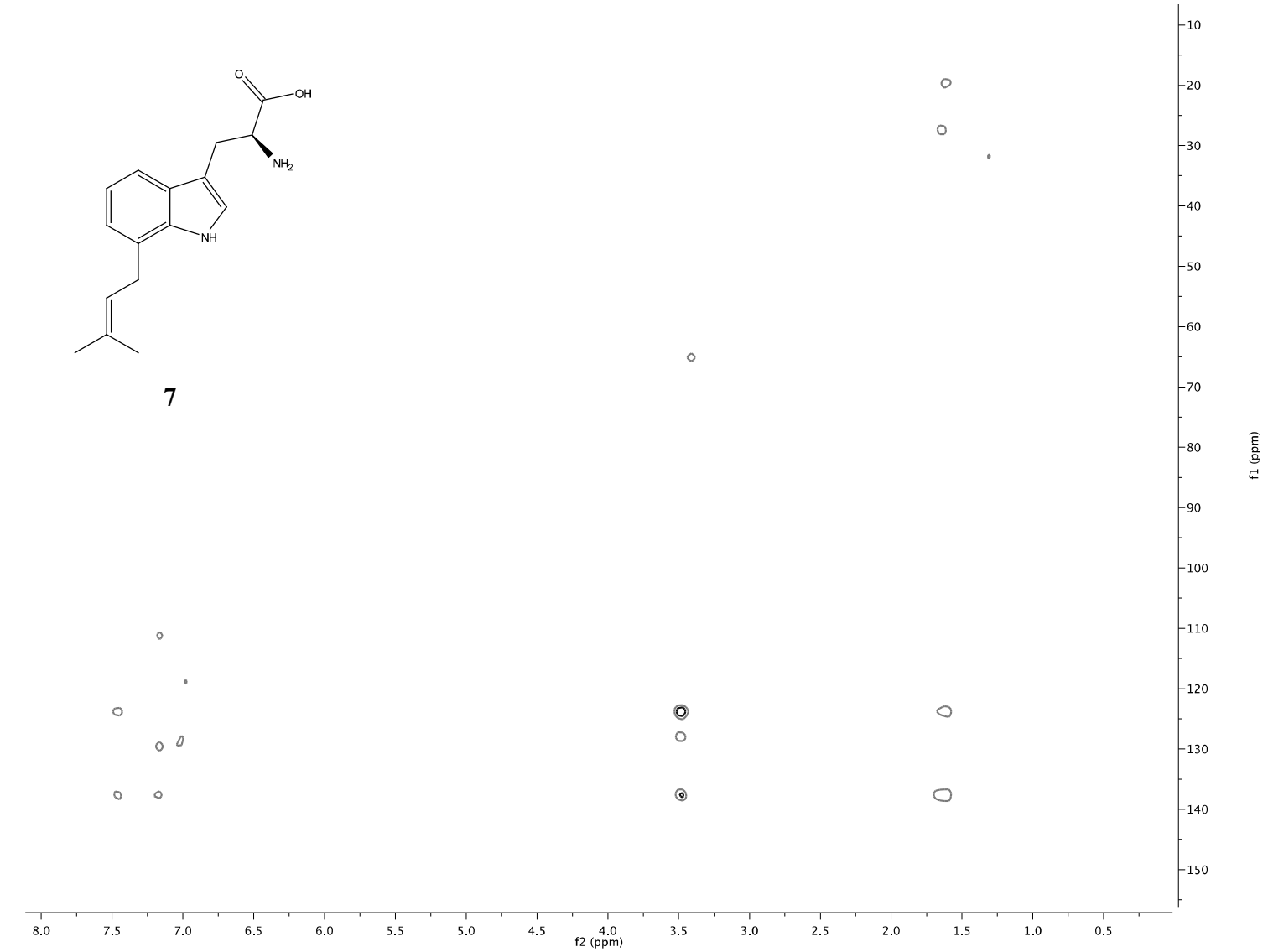


Figure S30. 2D ^{1}H-^{13}C HMBC NMR spectrum of **7** in D_2O.

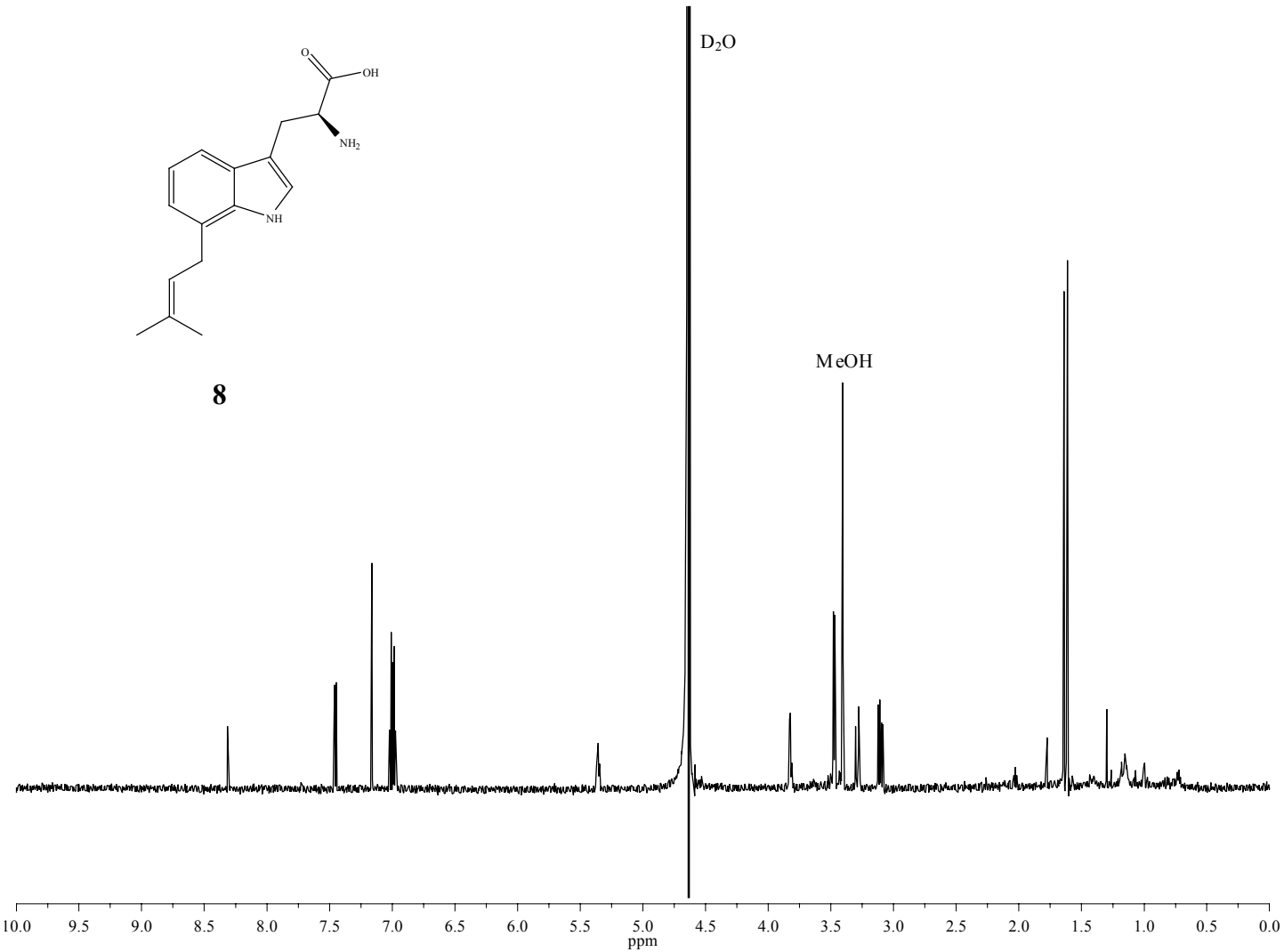


Figure S31. ^{1}H NMR (600 MHz) spectrum of **8** in D_2O.

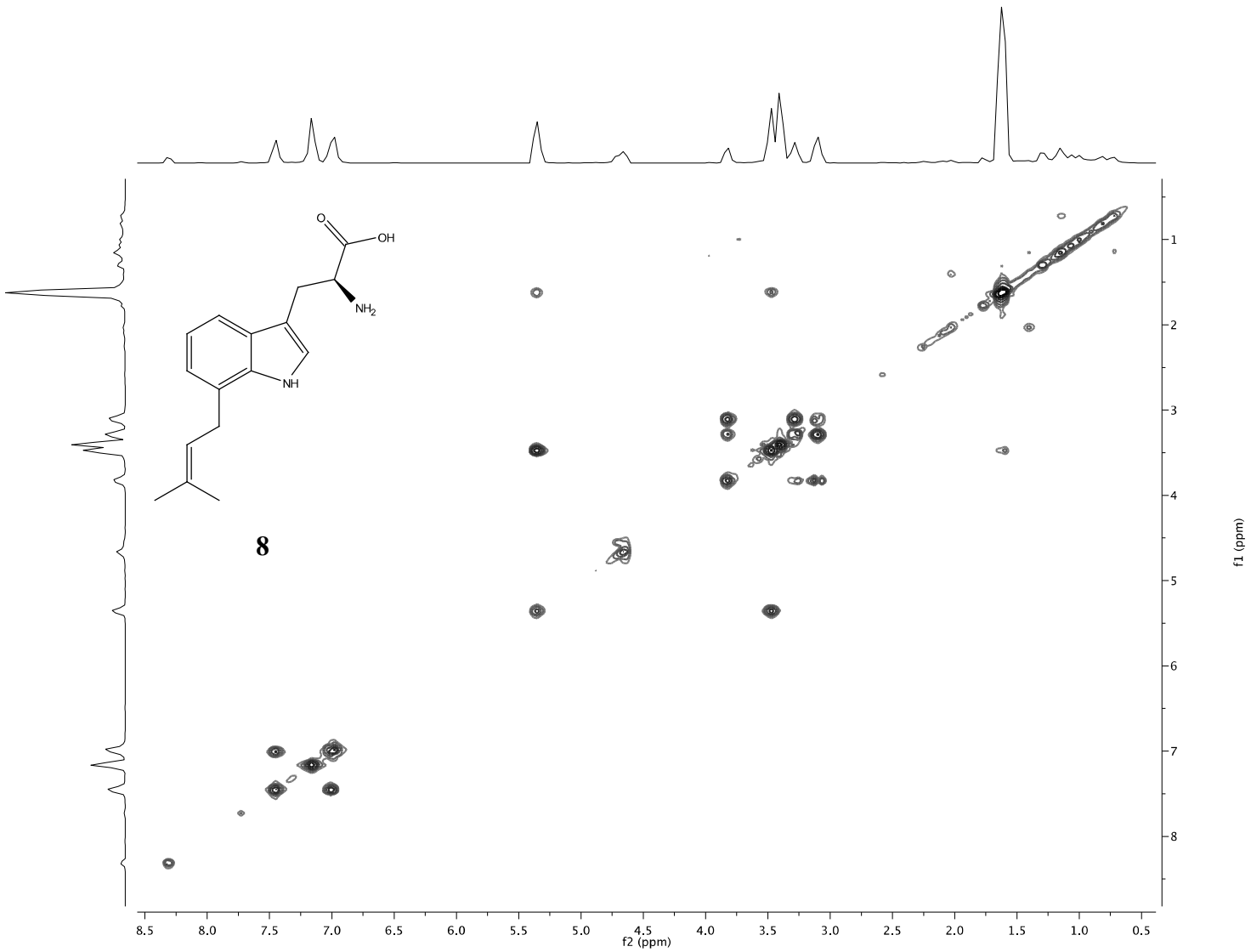


Figure S32. 2D ^{1}H-^{1}H COSY NMR spectrum of **8** in D_2O.

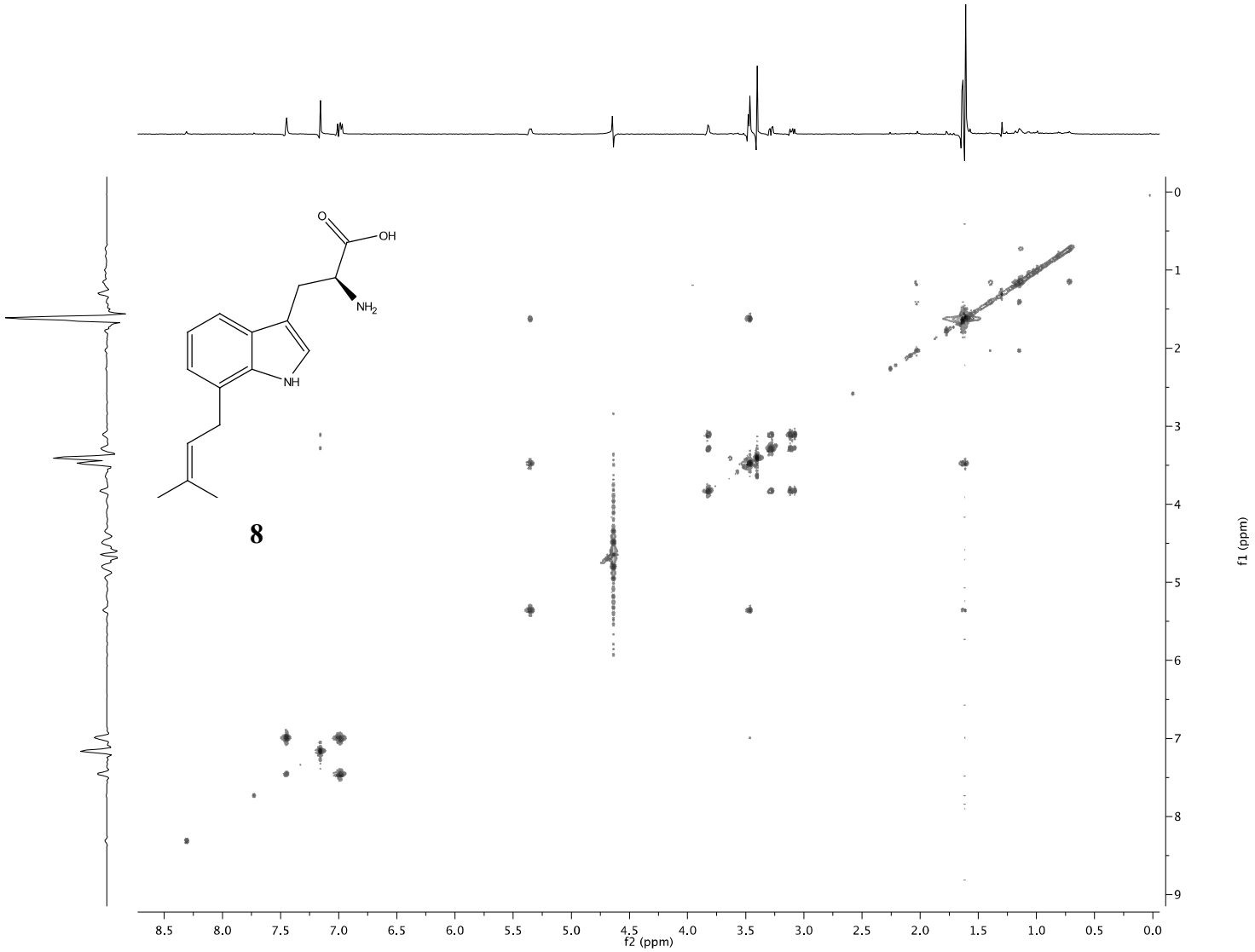


Figure S33. 2D ^{1}H-^{1}H TOCSY NMR spectrum of **8** in D_2O.

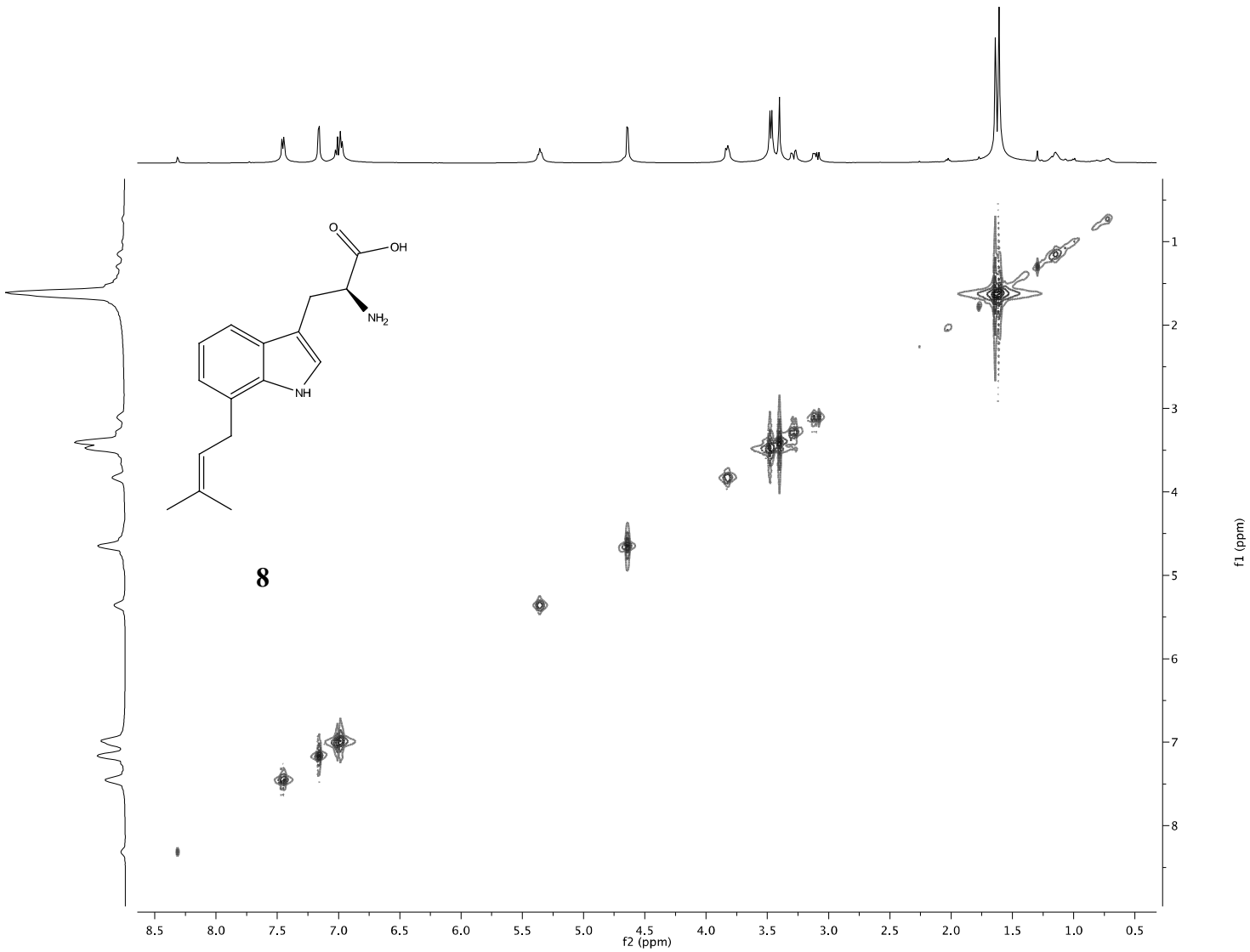


Figure S34. 2D ^{1}H-^{1}H ROESY NMR spectrum of **8** in D_2O.

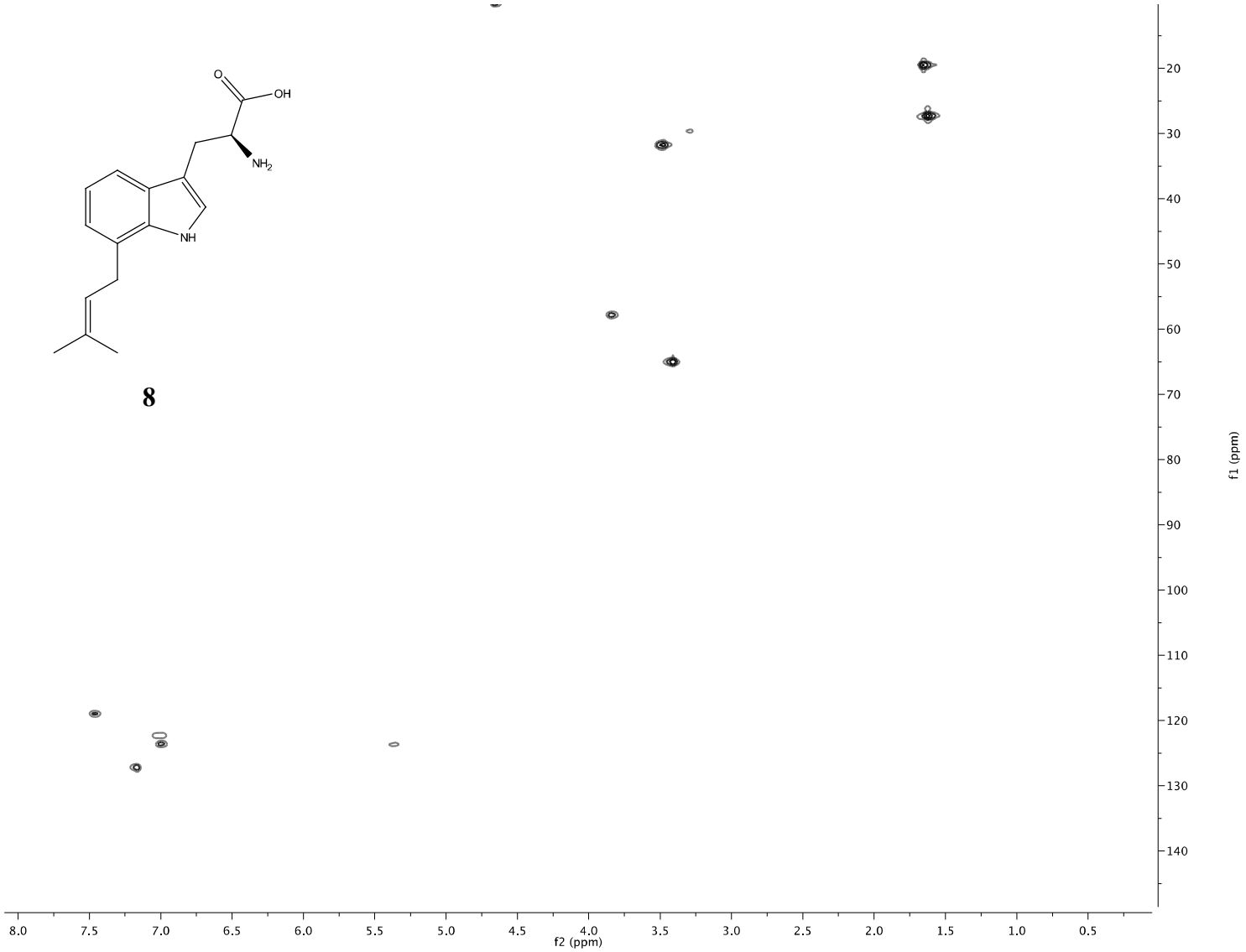


Figure S35. 2D ^{1}H-^{13}C HMQC NMR spectrum of **8** in D_2O.

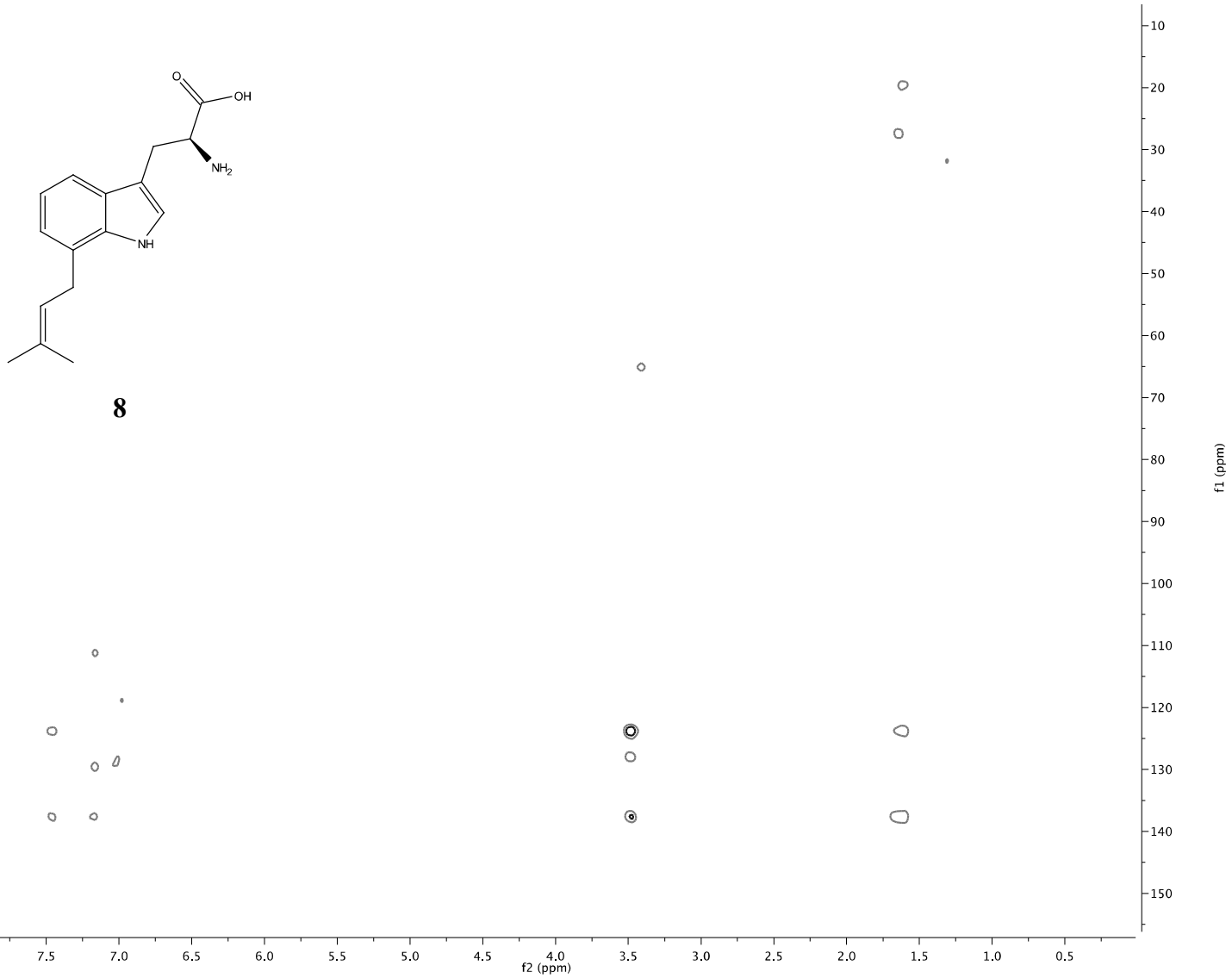


Figure S36. 2D ^{1}H-^{13}C HMBC NMR spectrum of **8** in D_2O.

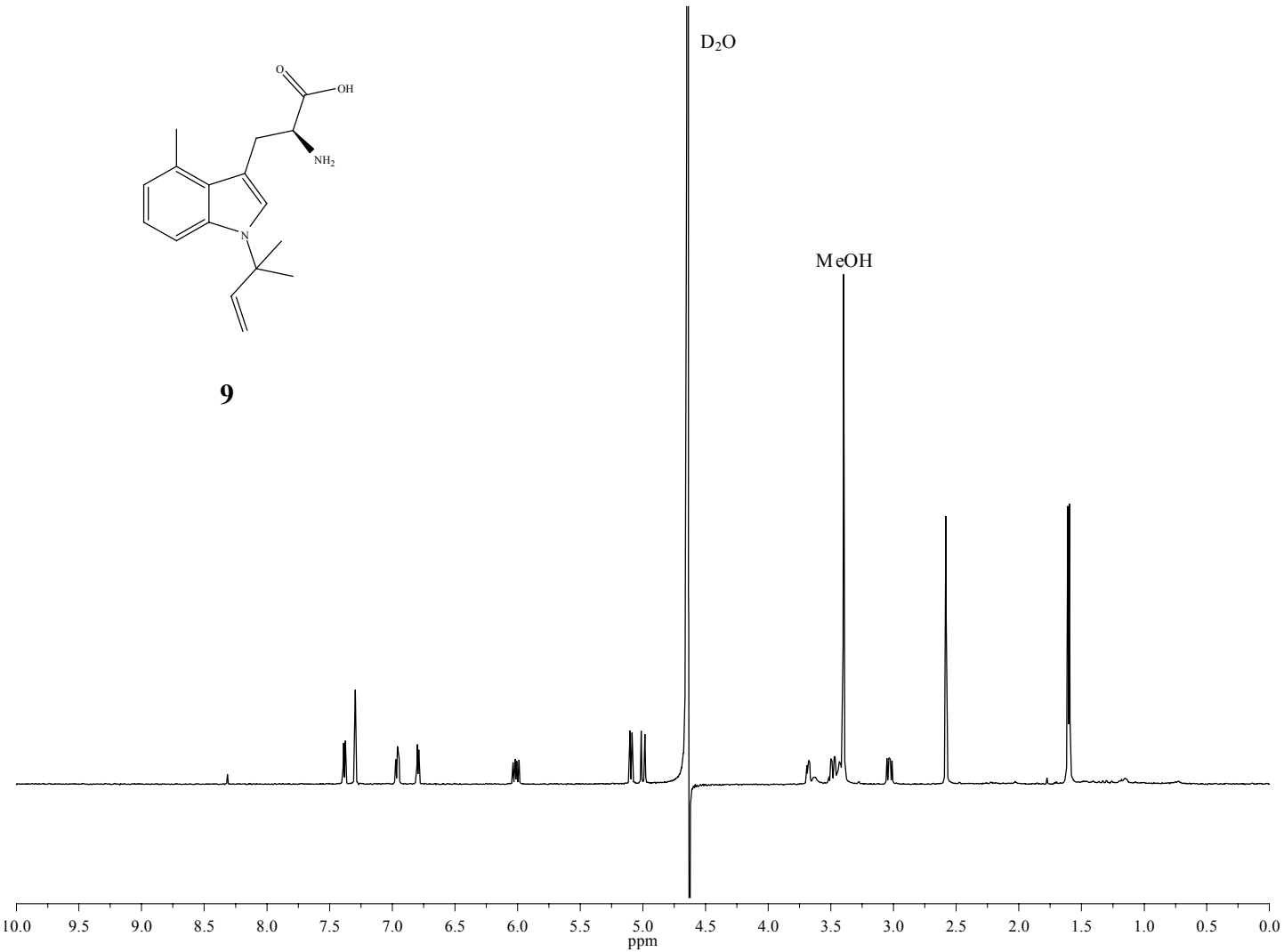


Figure S37. ^{1}H-NMR (600 MHz) spectrum of **9** in D_2O.

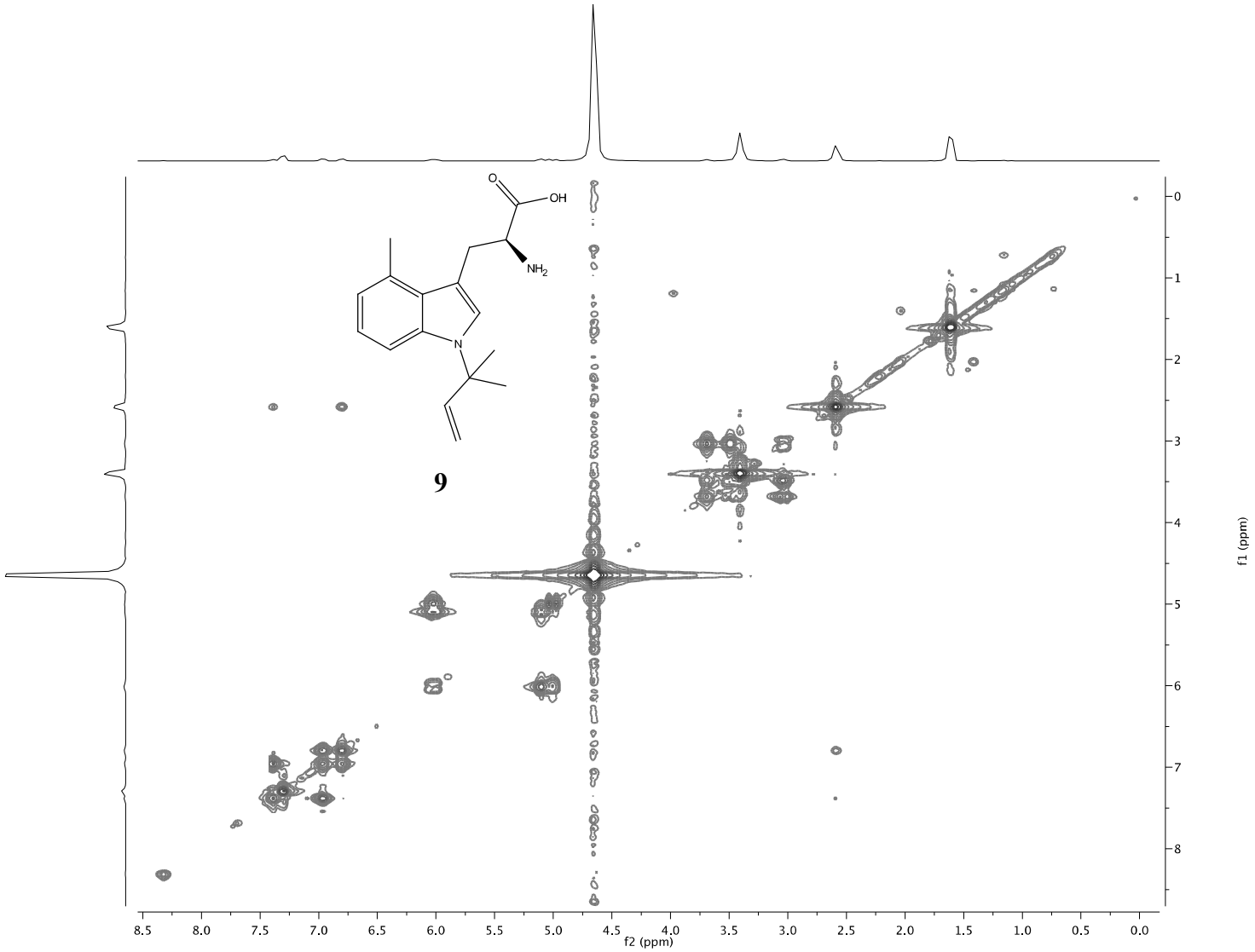


Figure S38. 2D ^{1}H-^{1}H COSY NMR spectrum of **9** in D_2O.

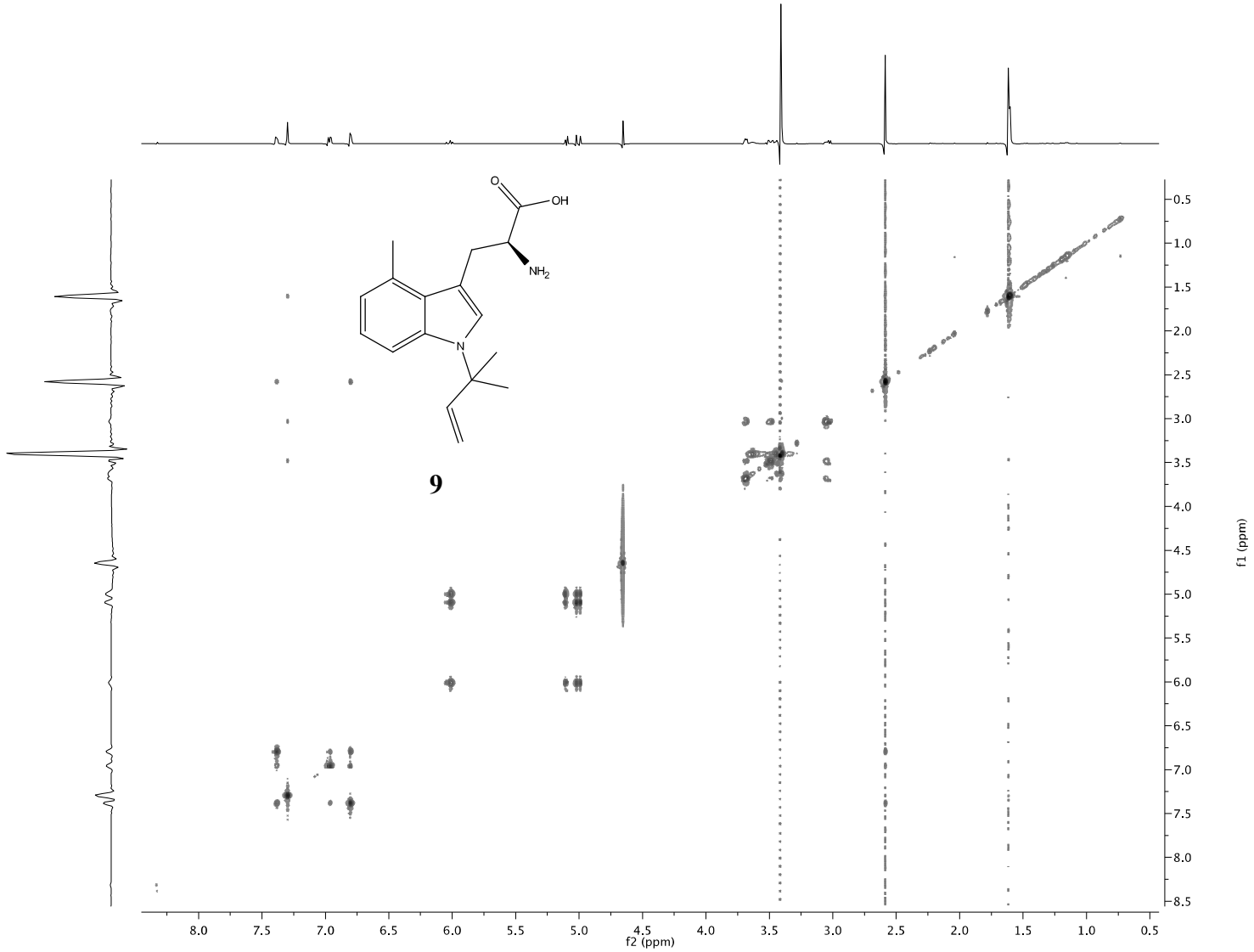


Figure S39. 2D ^{1}H-^{1}H TOCSY NMR spectrum of **9** in D_2O.

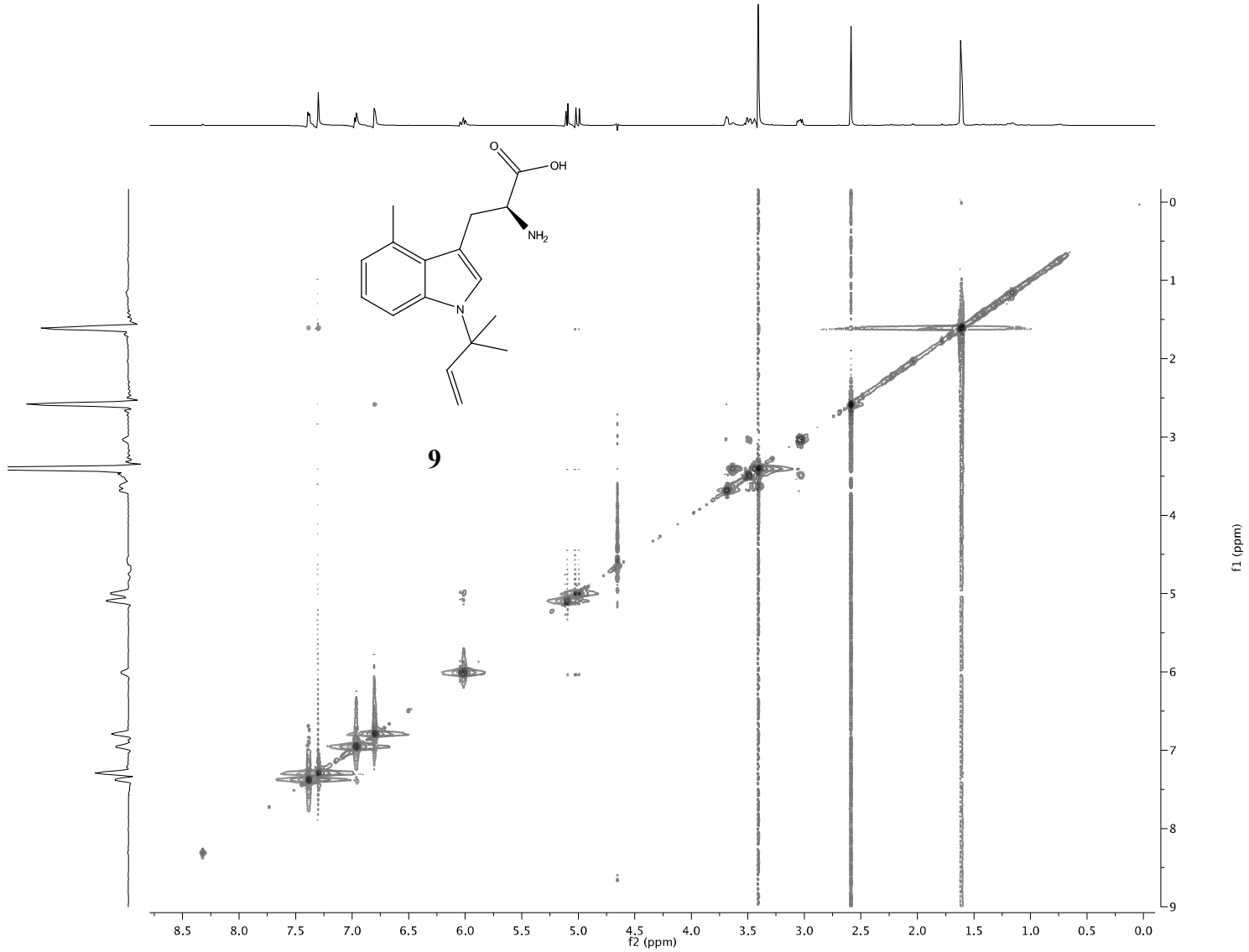


Figure S40. 2D ^{1}H-^{1}H ROESY NMR spectrum of **9** in D_2O.

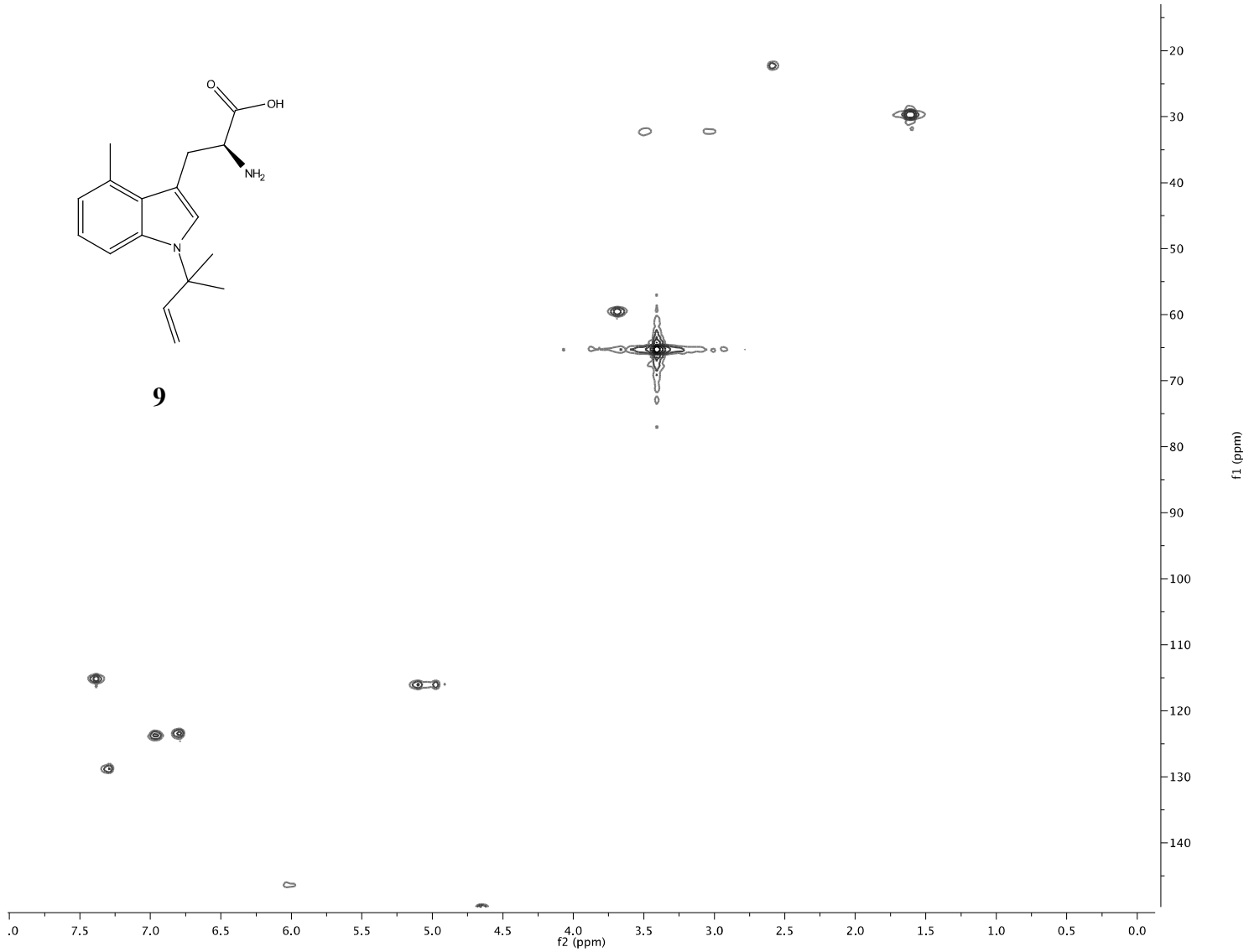


Figure S41. 2D ^{1}H-^{13}C HSQC NMR spectrum of **9** in D_2O.

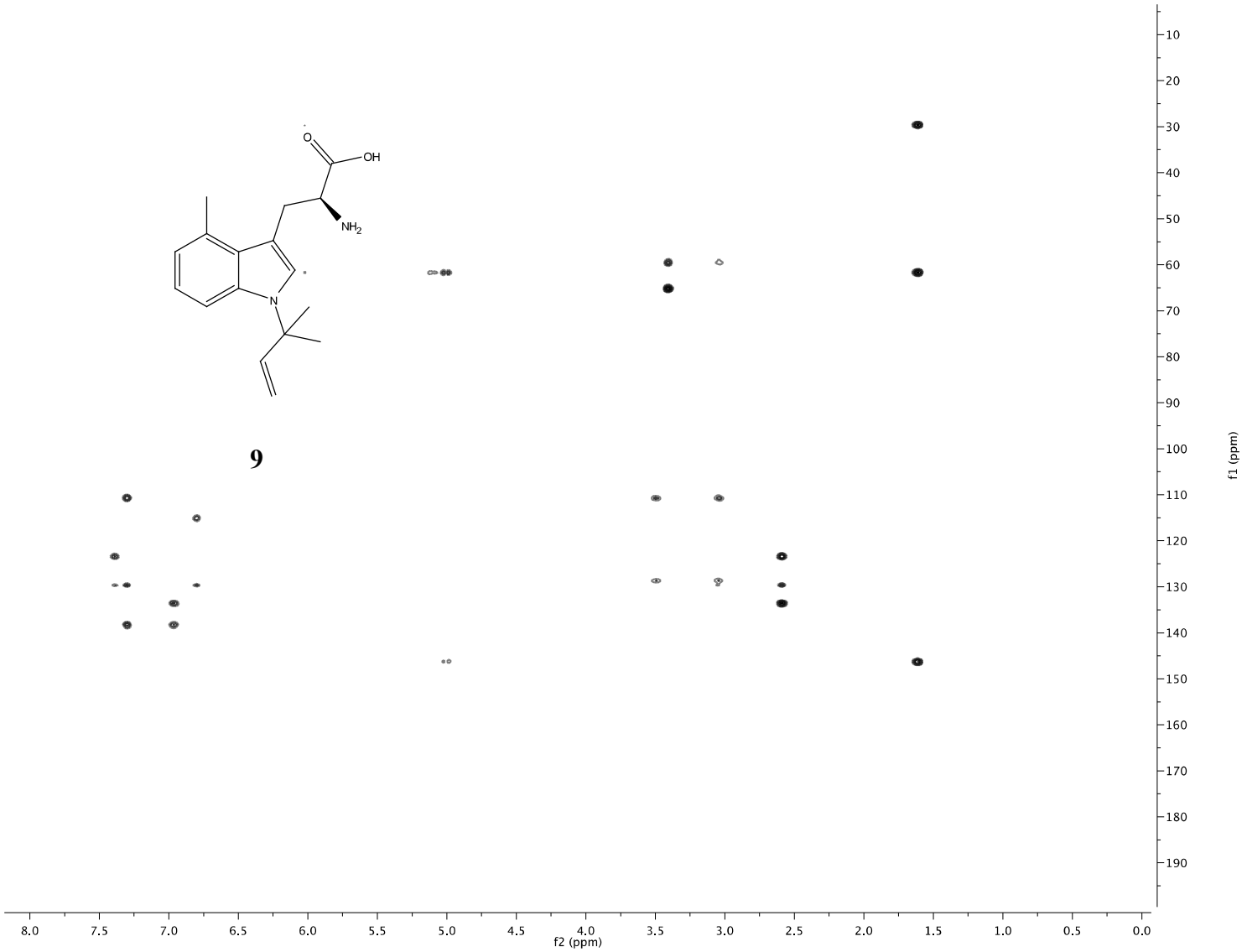
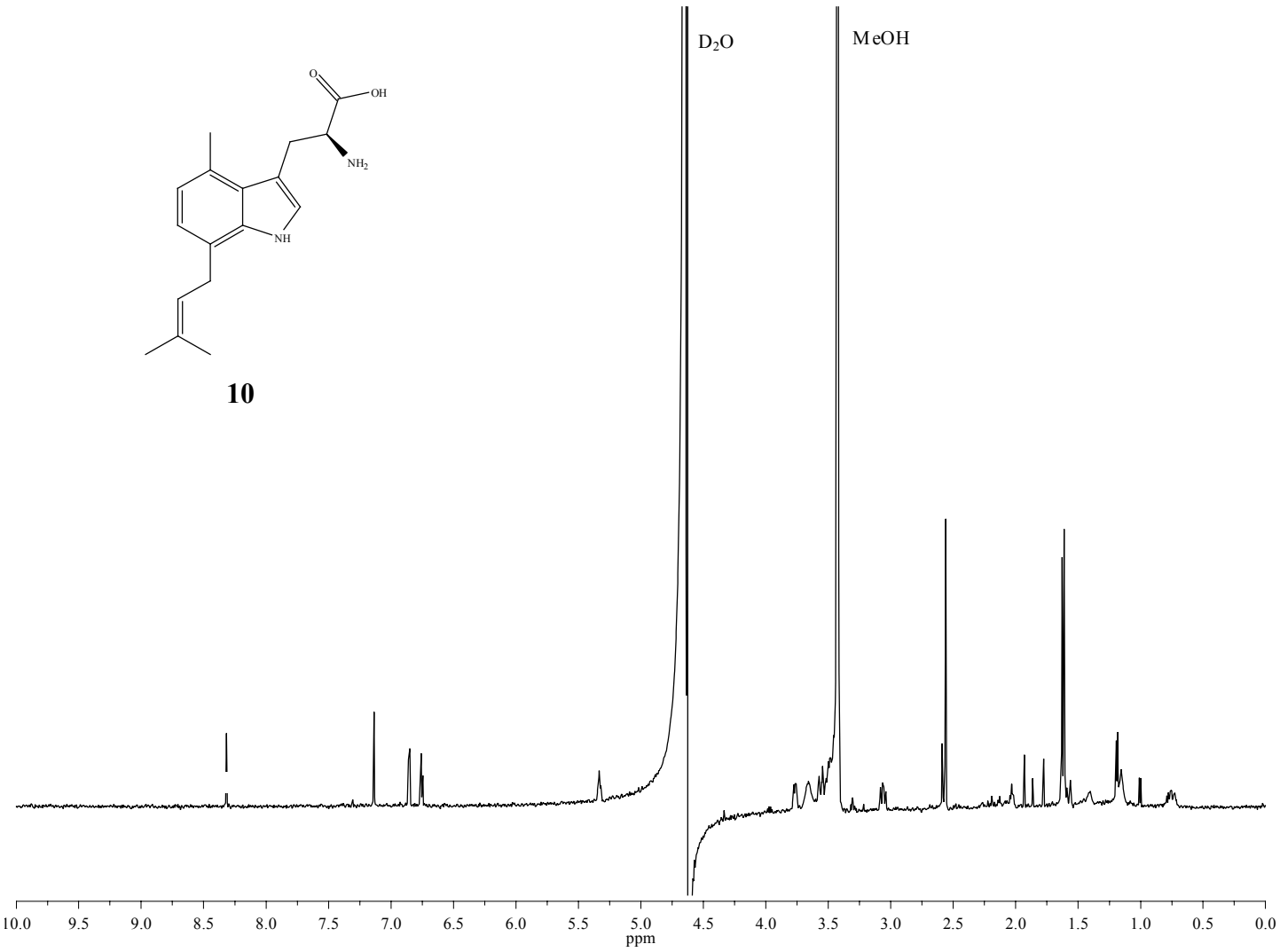


Figure S42. 2D ^{1}H-^{13}C HMBC NMR spectrum of **9** in D_2O.



10

Figure S43. ^{1}H NMR (600 MHz) spectrum of **10** in D_2O.

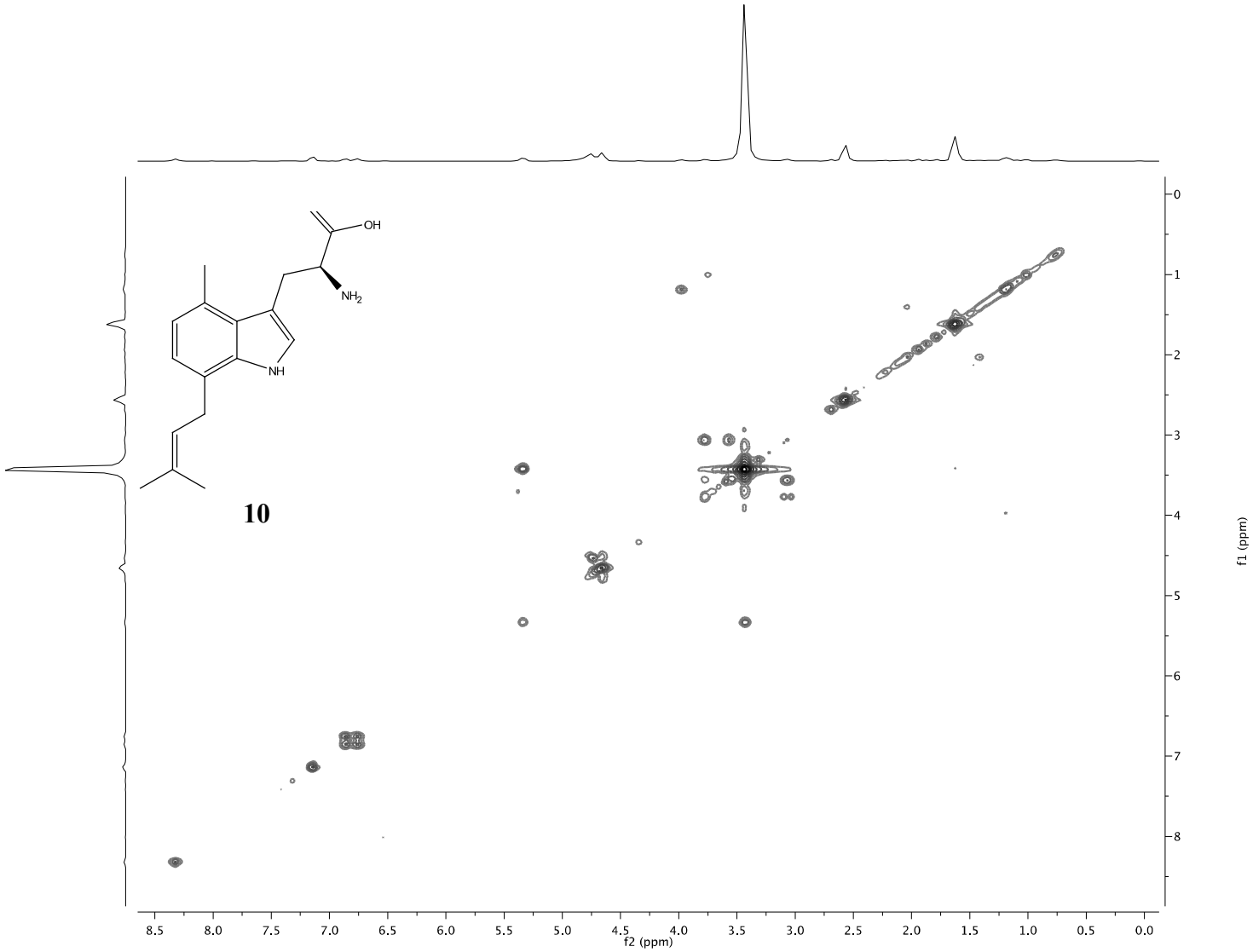


Figure S44. 2D ^{1}H-^{1}H COSY NMR spectrum of **10** in D_2O.

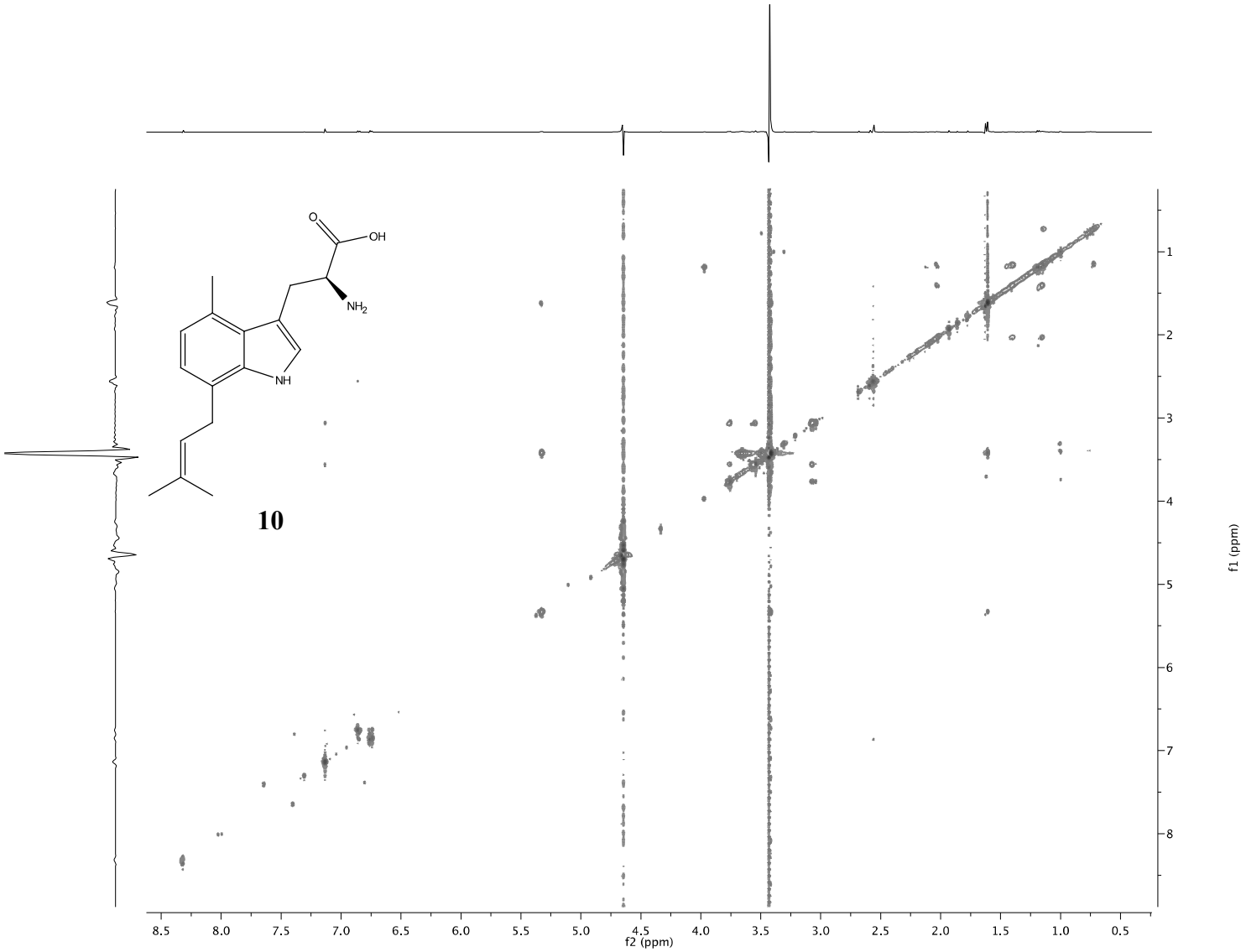


Figure S45. 2D ^{1}H-^{1}H TOCSY NMR spectrum of **10** in D_2O.

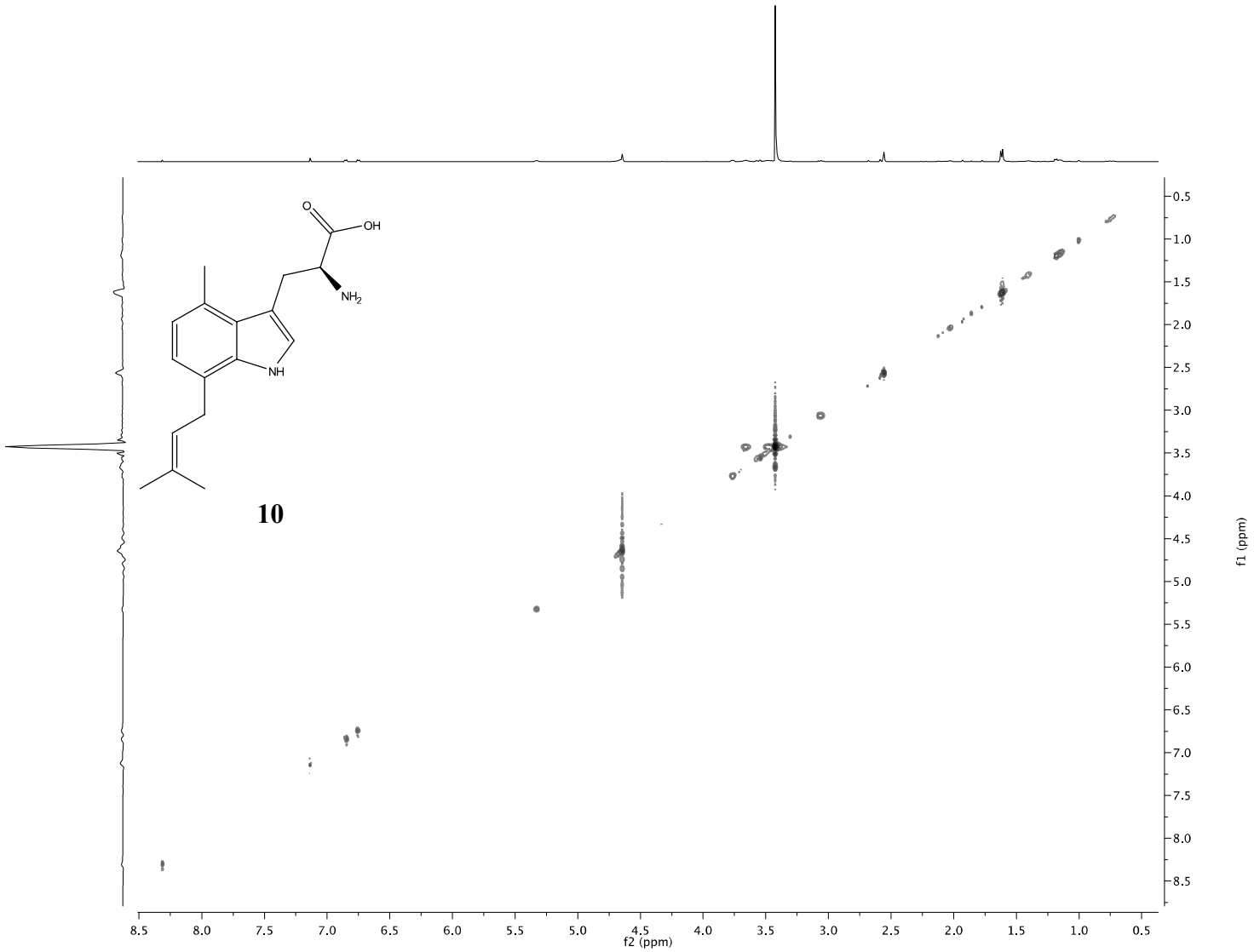


Figure S46. 2D ^{1}H-^{1}H ROESY NMR spectrum of **10** in D_2O.

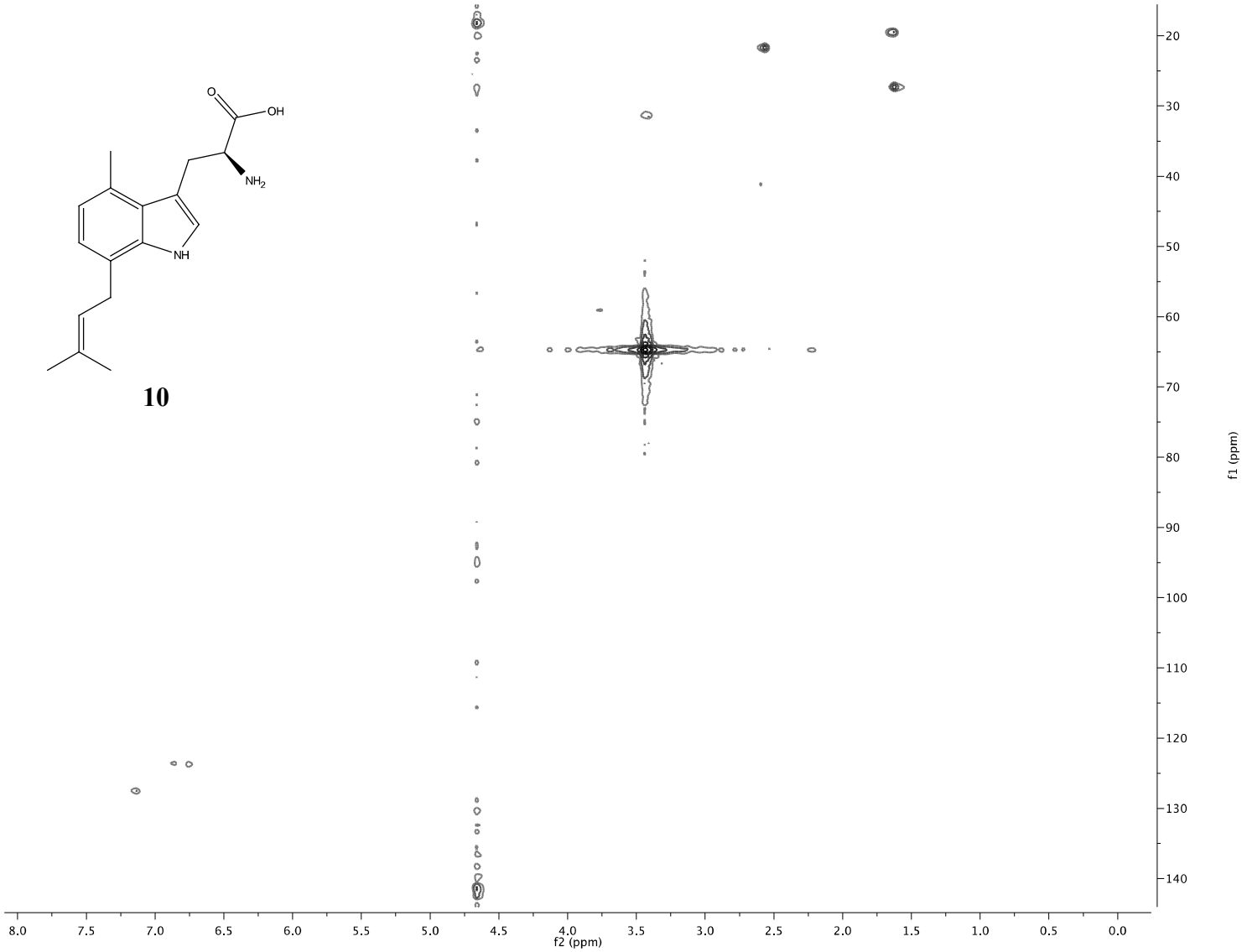


Figure S47. 2D ^{1}H-^{13}C HMQC NMR spectrum of **10** in D_2O.

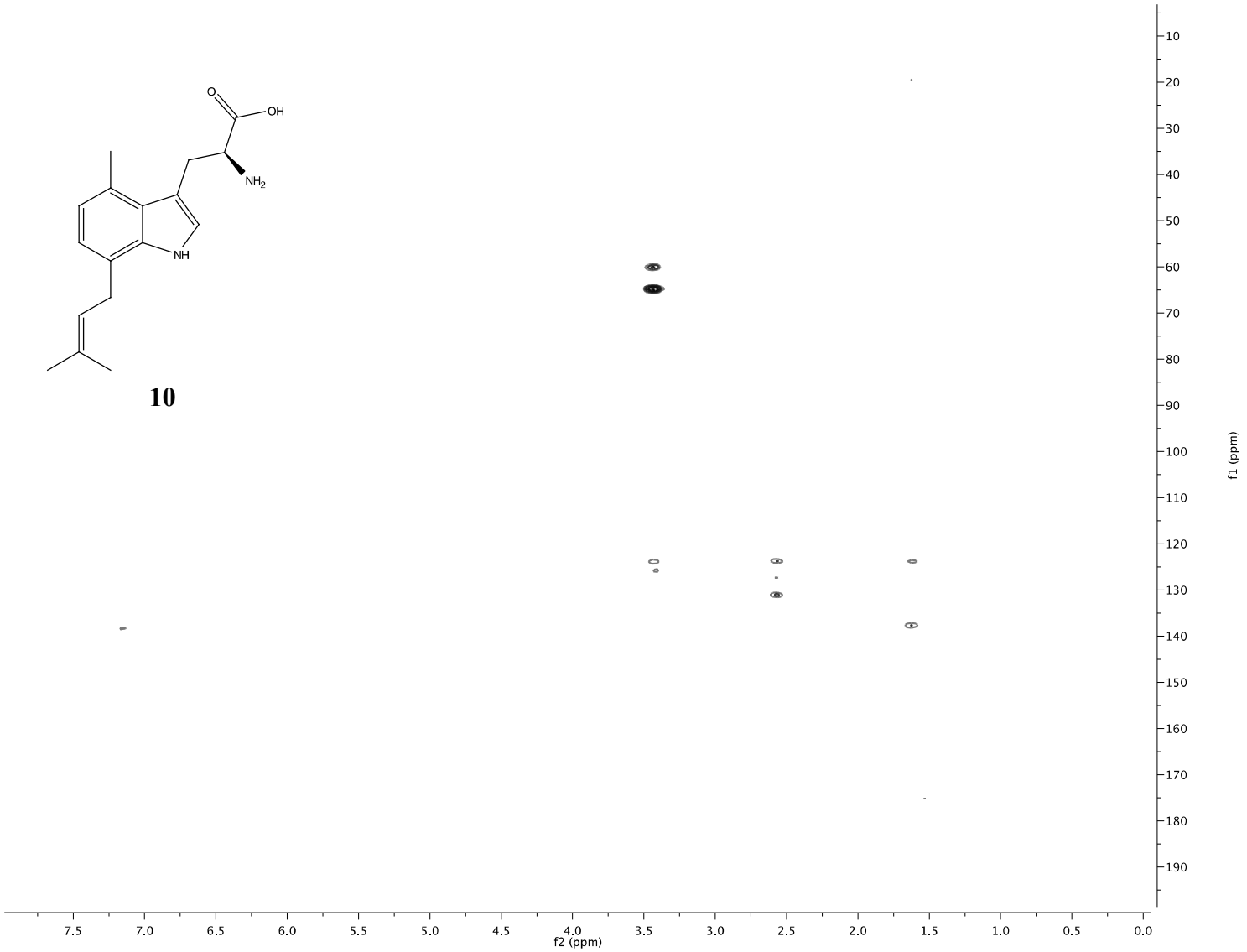


Figure S48. 2D ^{1}H-^{13}C HMBC NMR spectrum of **10** in D_2O.

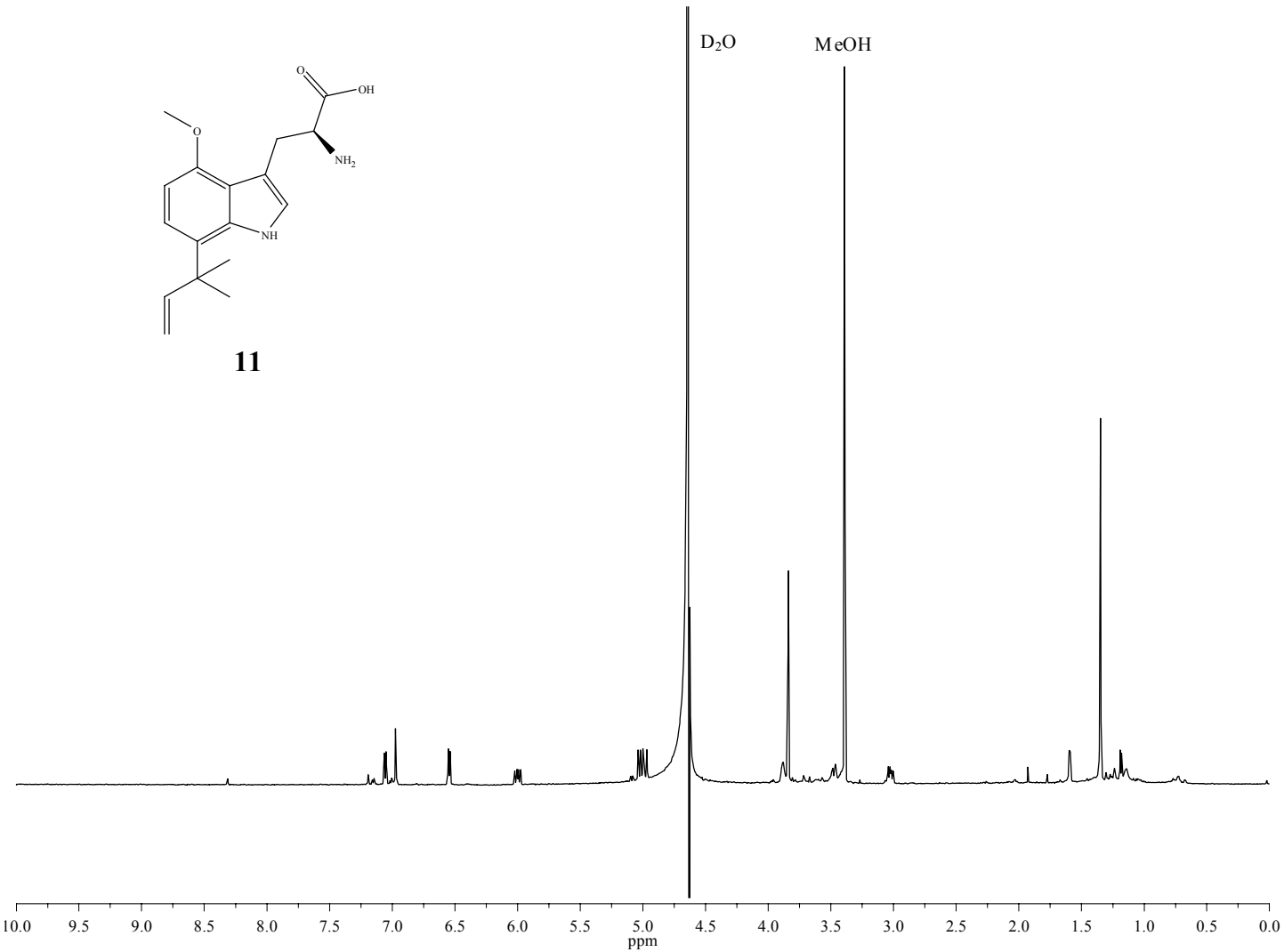


Figure S49. ^{1}H NMR (600 MHz) spectrum of **11** in D_2O.

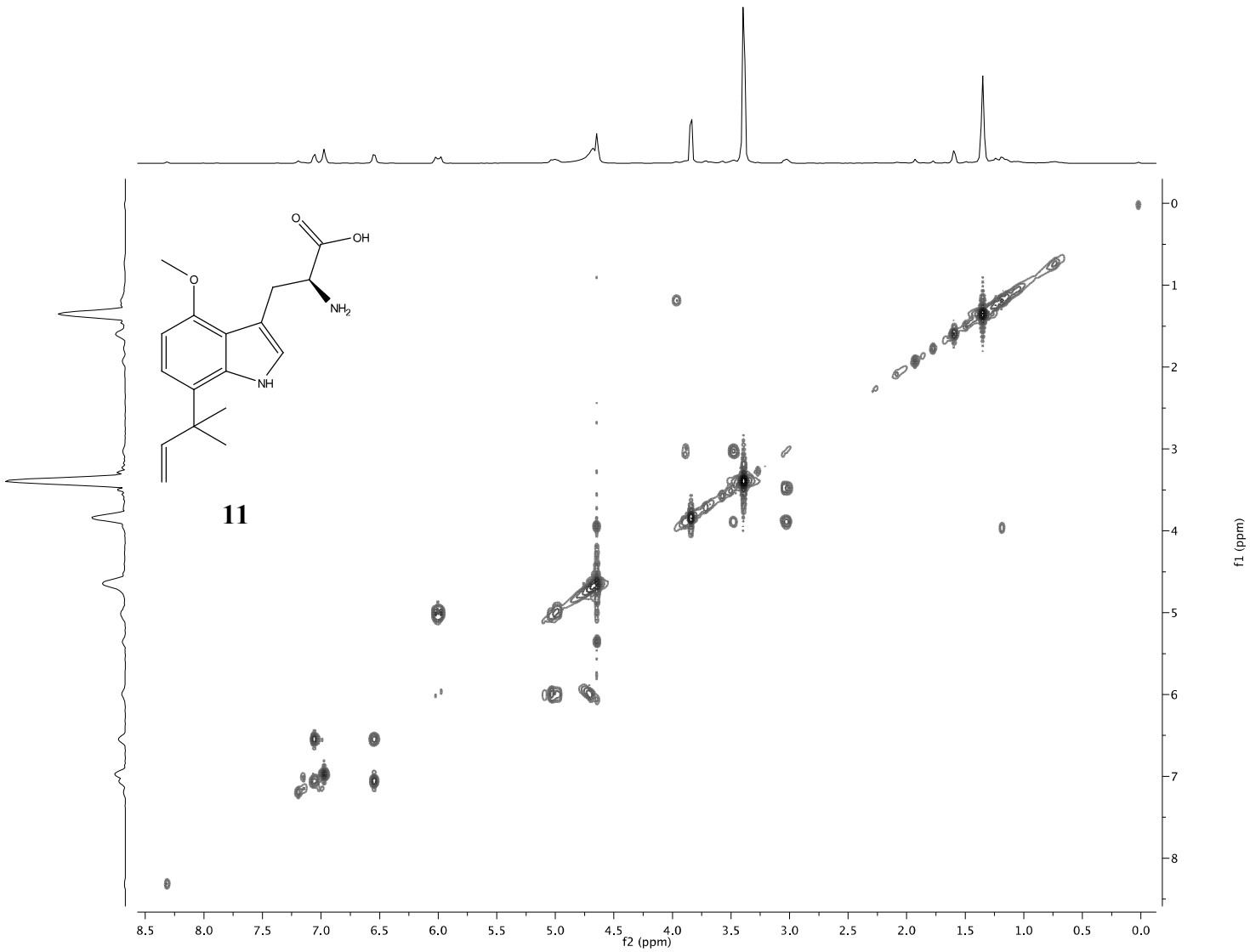


Figure S50. 2D ^{1}H-^{1}H COSY spectrum of **11** in D_2O.

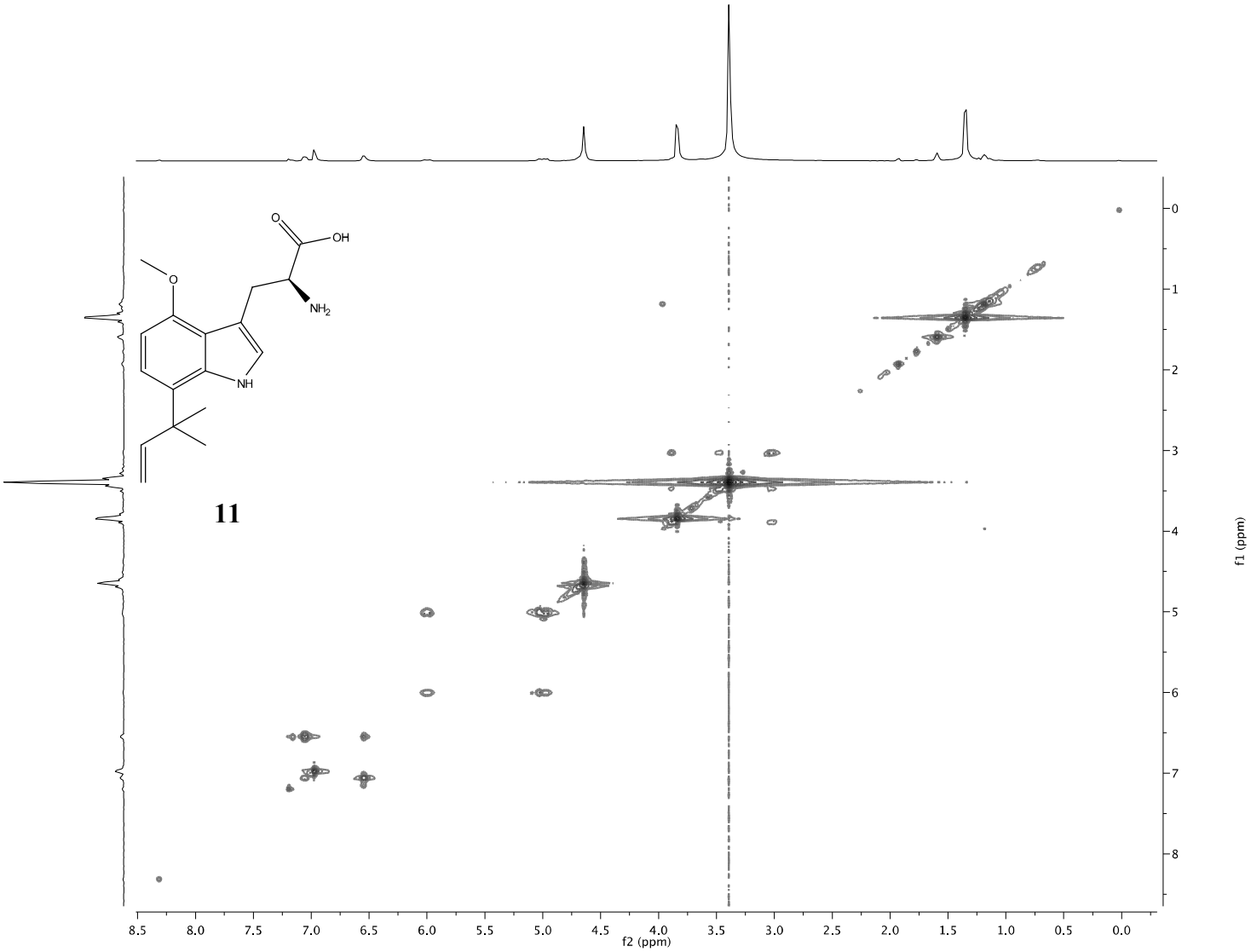


Figure S51. 2D ^{1}H-^{1}H TOCSY spectrum of **11** in D_2O.

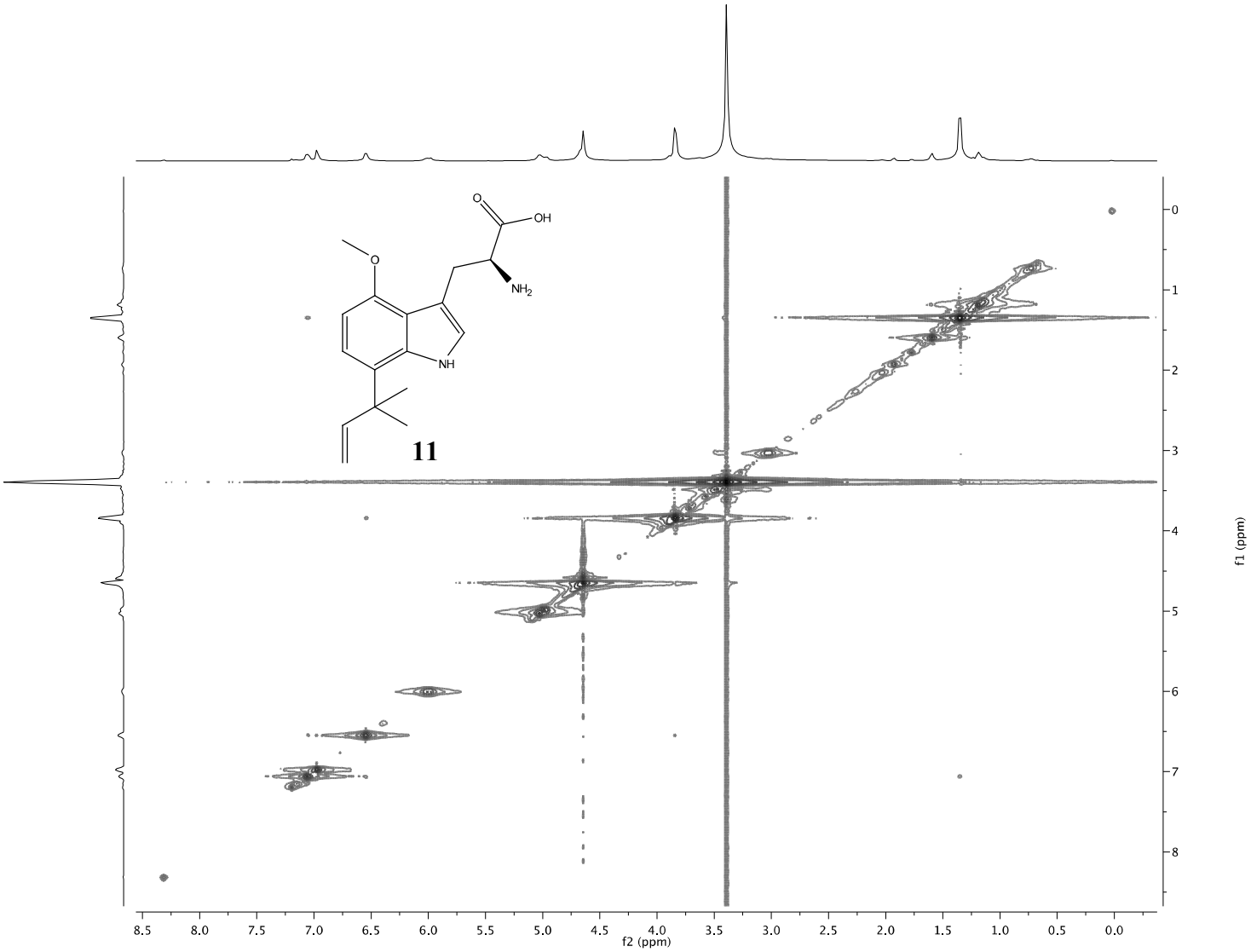


Figure S52. 2D ^{1}H-^{1}H ROESY spectrum of **11** in D_2O.

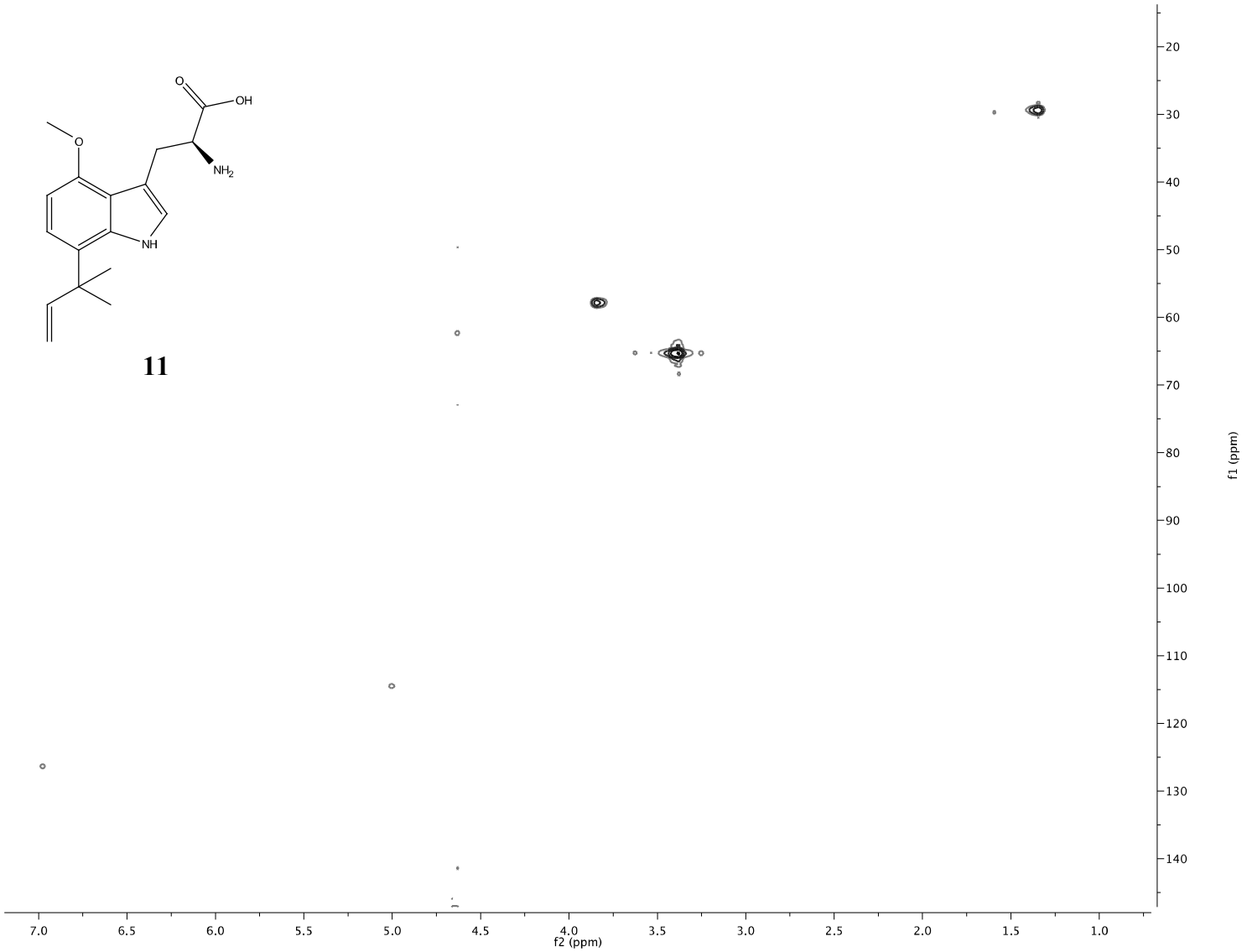


Figure S53. 2D ^{1}H-^{13}C HMQC spectrum of **11** in D_2O.

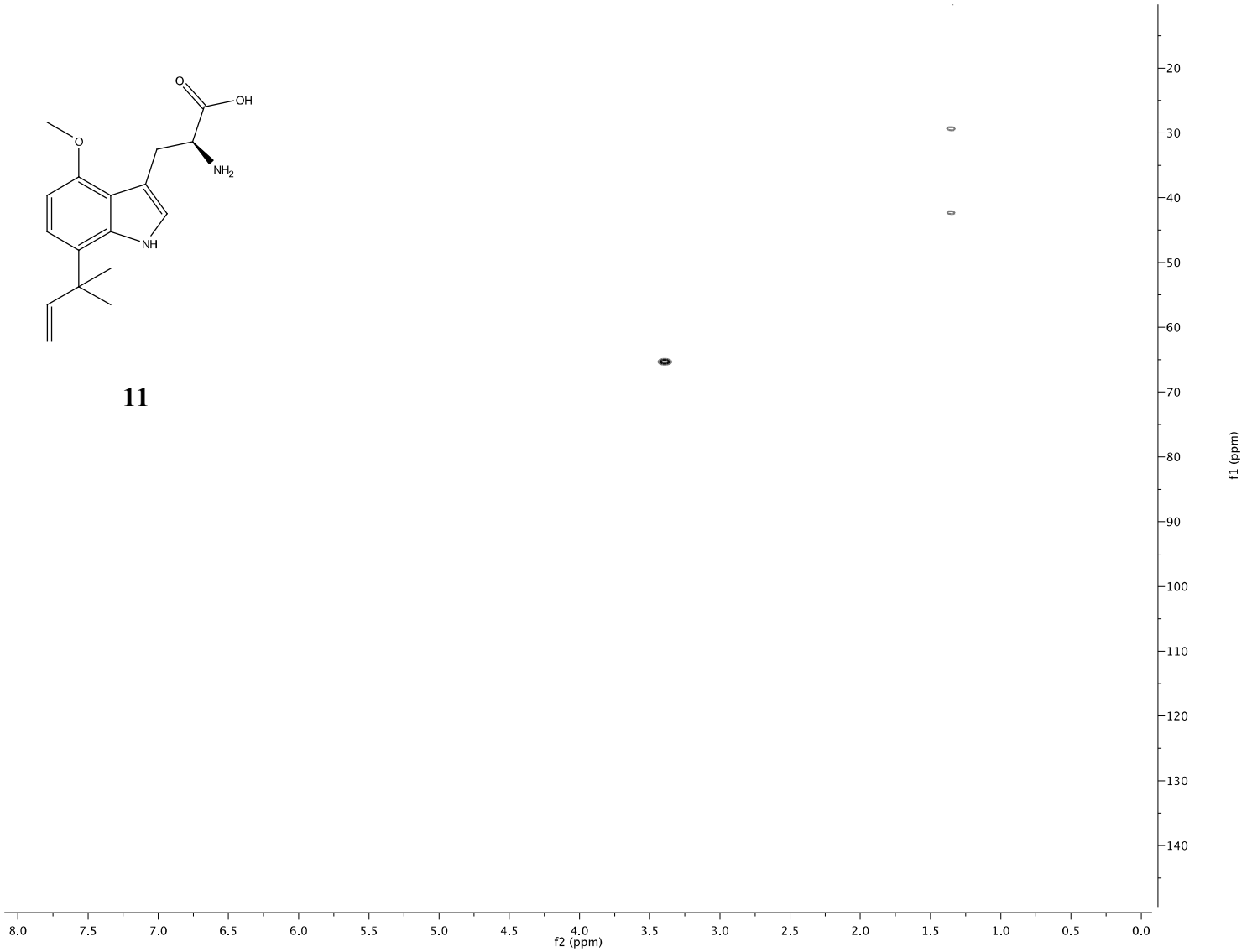


Figure S54. 2D ^{1}H-^{13}C HMBC spectrum of **11** in D_2O.

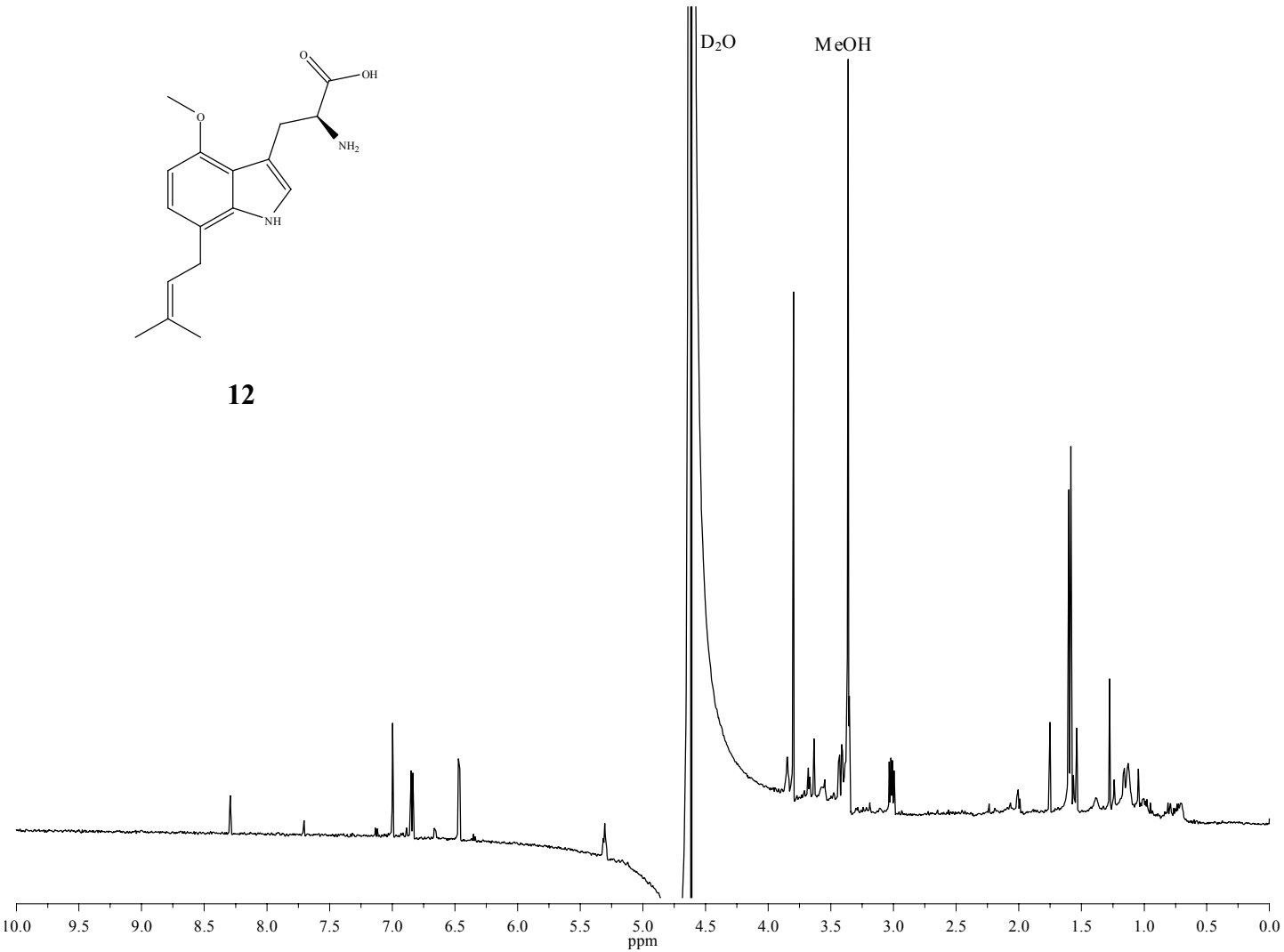


Figure S55. ^{1}H NMR (600 MHz) spectrum of **12** in D_2O.

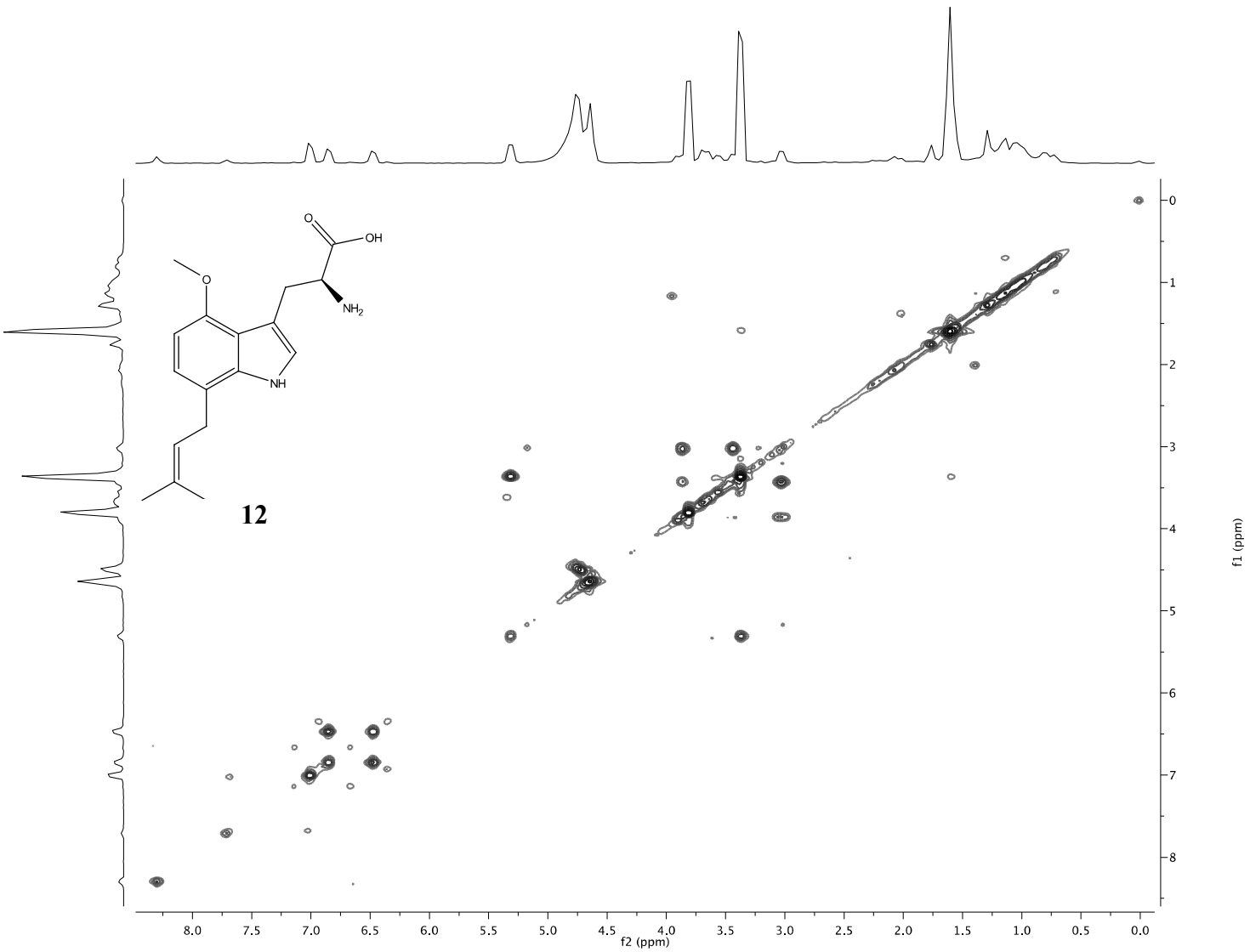


Figure S56. 2D ^{1}H-^{1}H COSY NMR spectrum of **12** in D_2O.

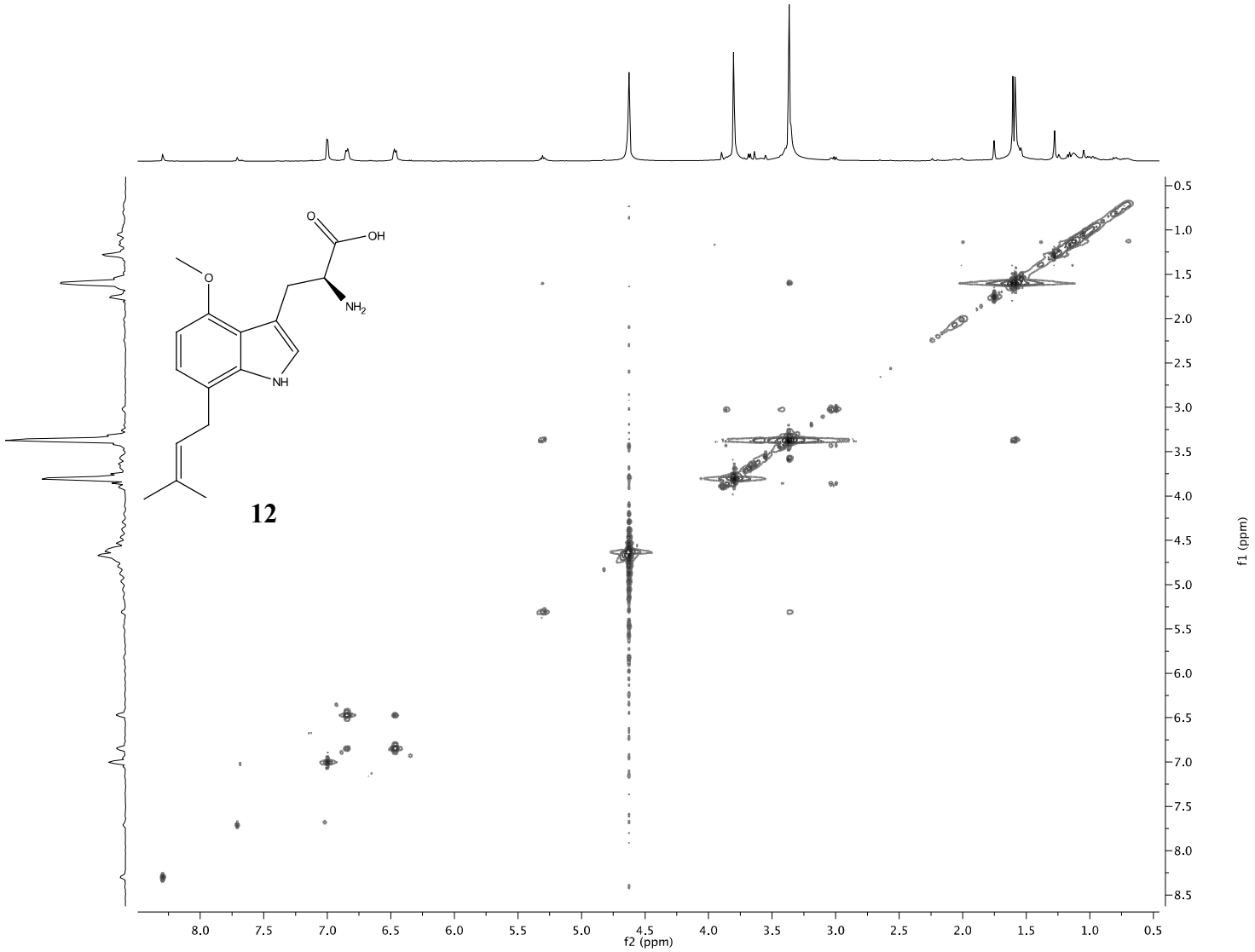


Figure S57. 2D ^{1}H-^{1}H TOCSY NMR spectrum of **12** in D_2O.

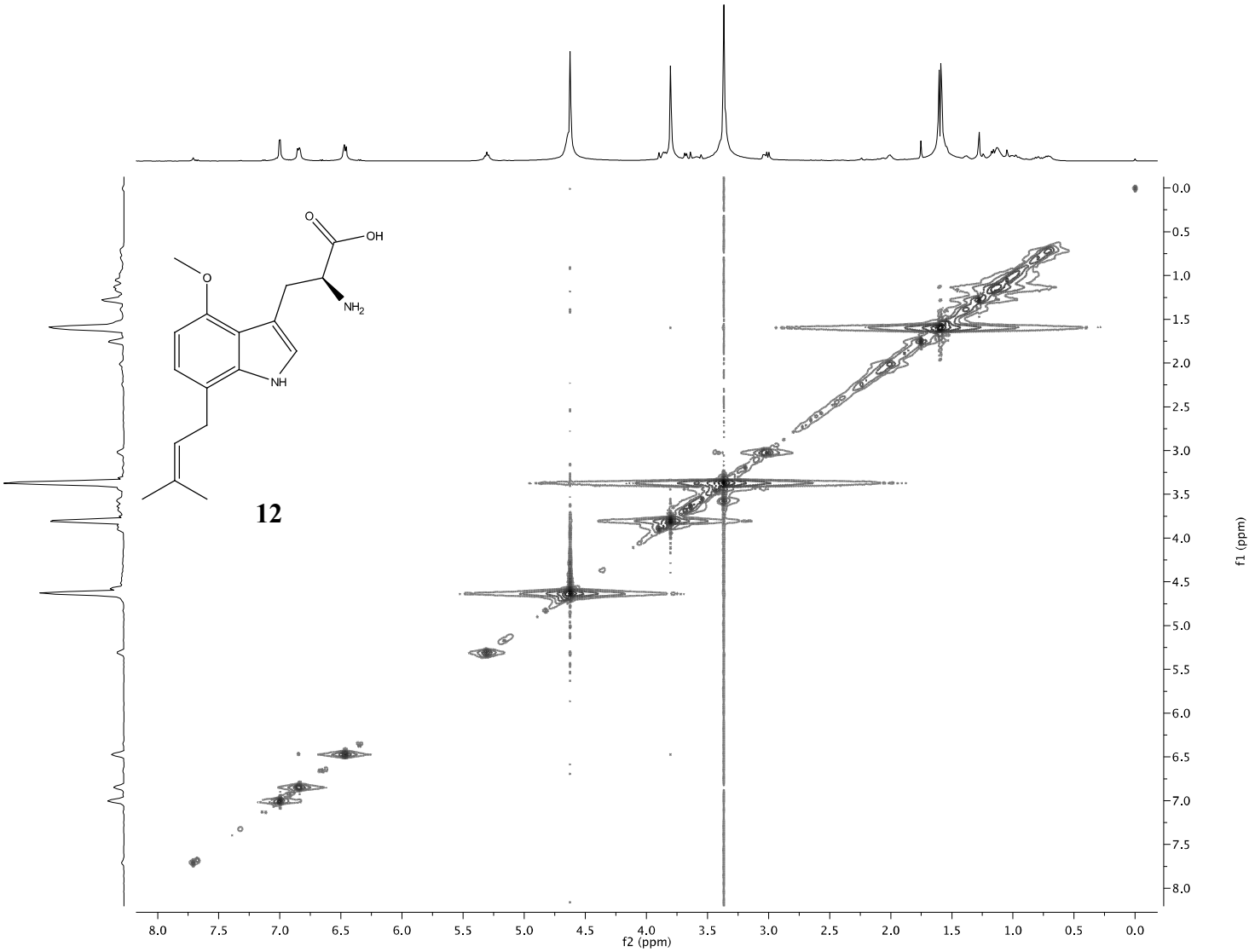


Figure S58. 2D ^{1}H-^{1}H ROESY NMR spectrum of **12** in D_2O.

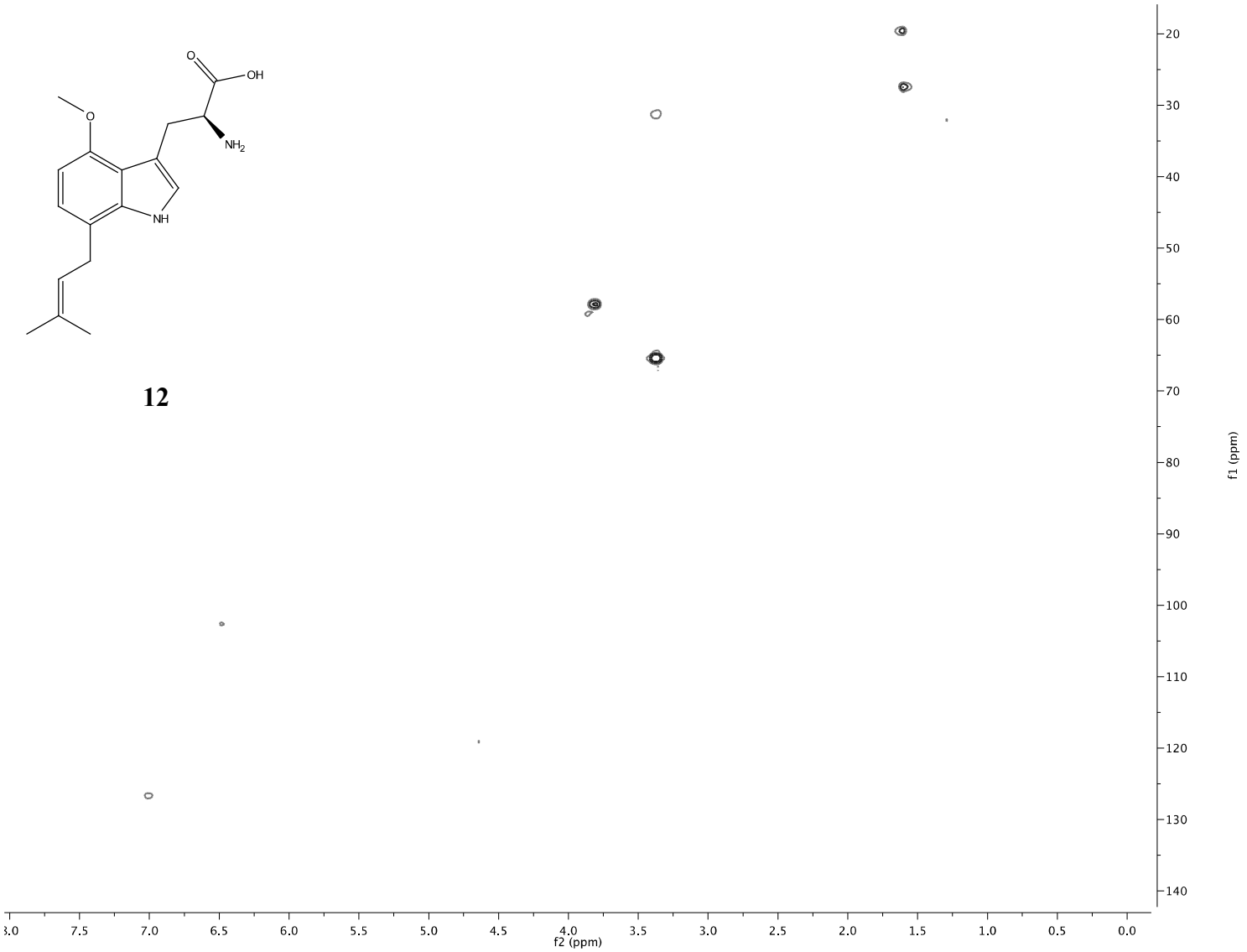


Figure S59. 2D ^{1}H-^{13}C HMQC NMR spectrum of **12** in D_2O.

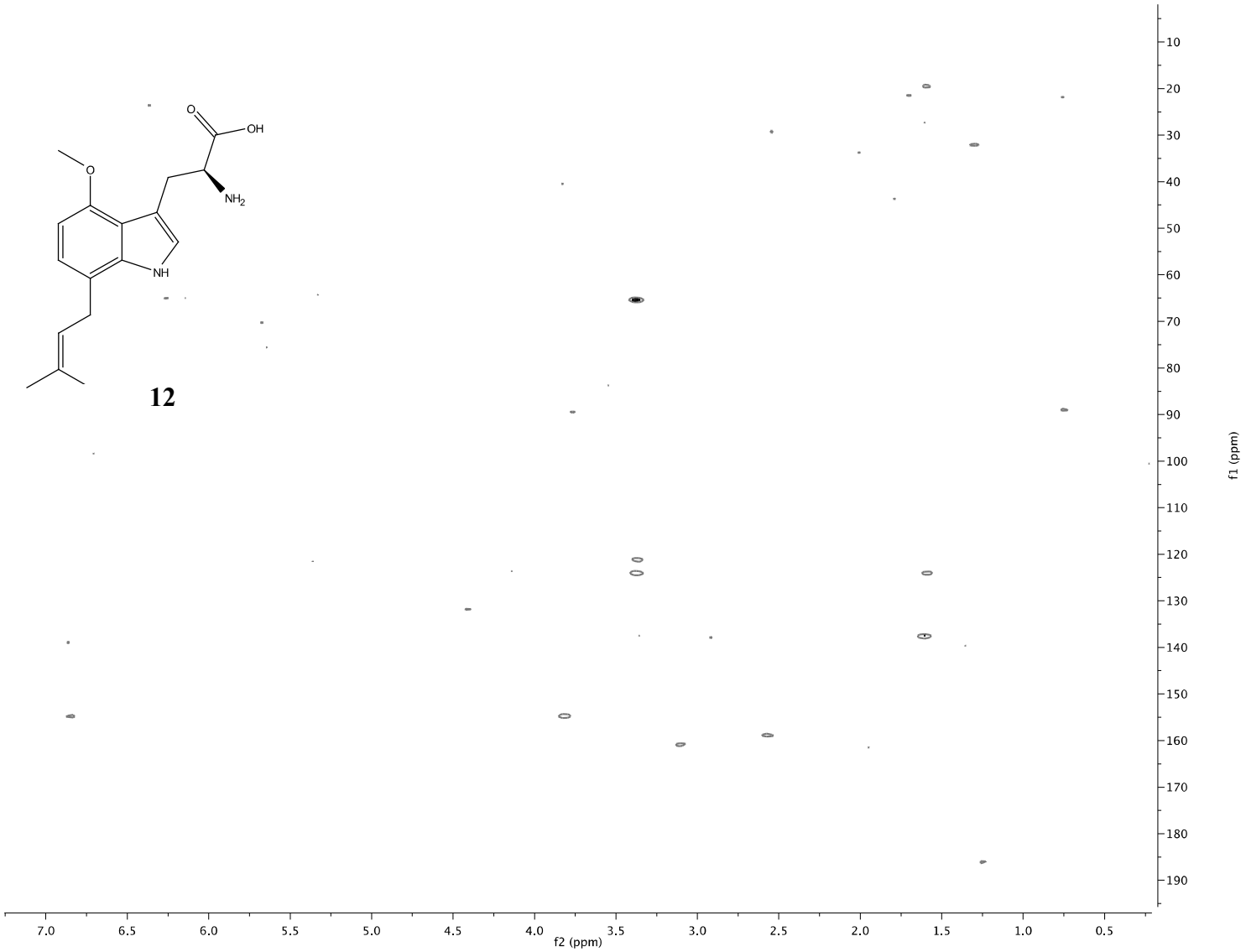


Figure S60. 2D ^{1}H-^{13}C HMBC NMR spectrum of **12** in D_2O.

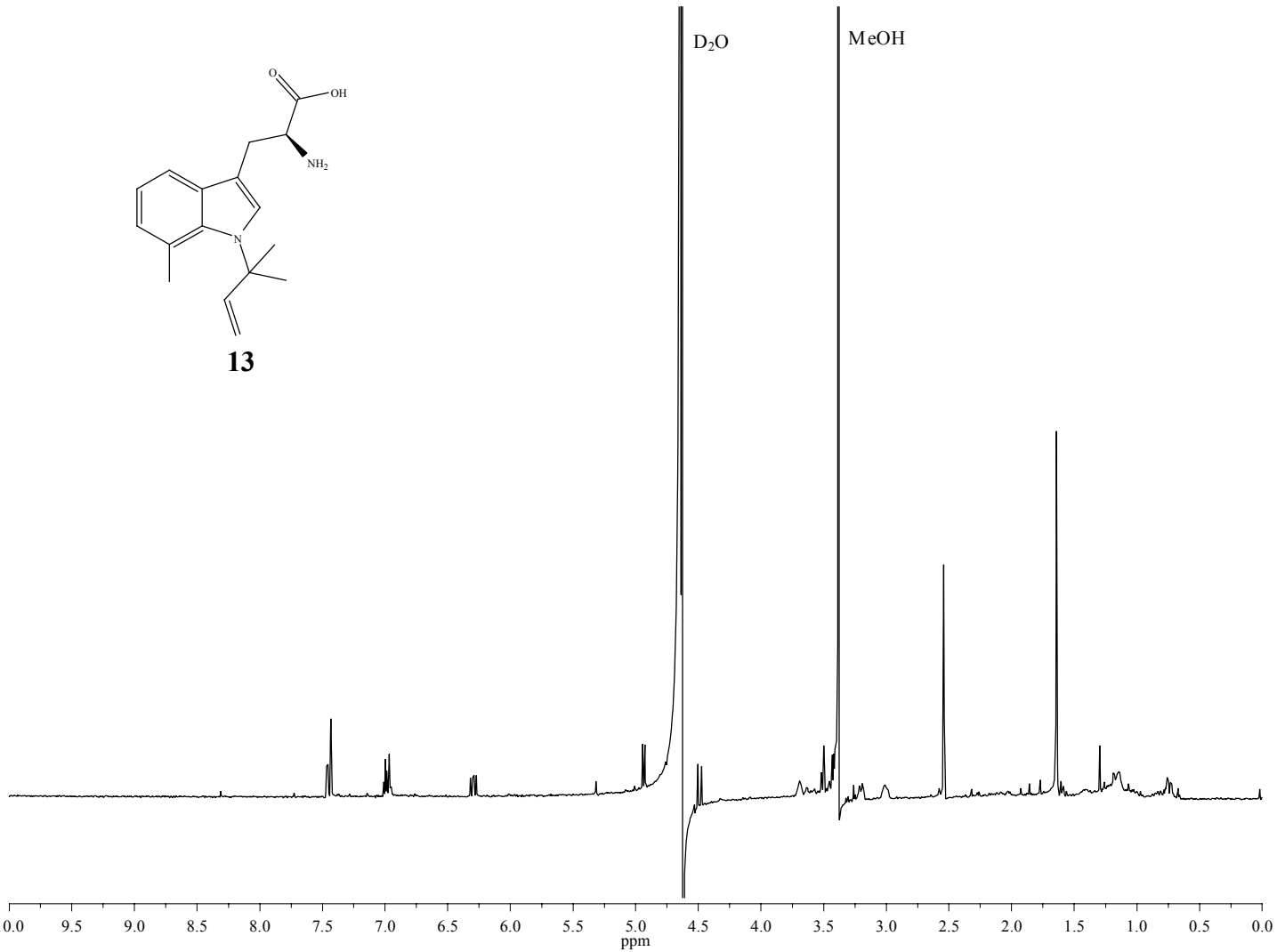


Figure S61. ^{1}H NMR (600 MHz) spectrum of **13** in D_2O.

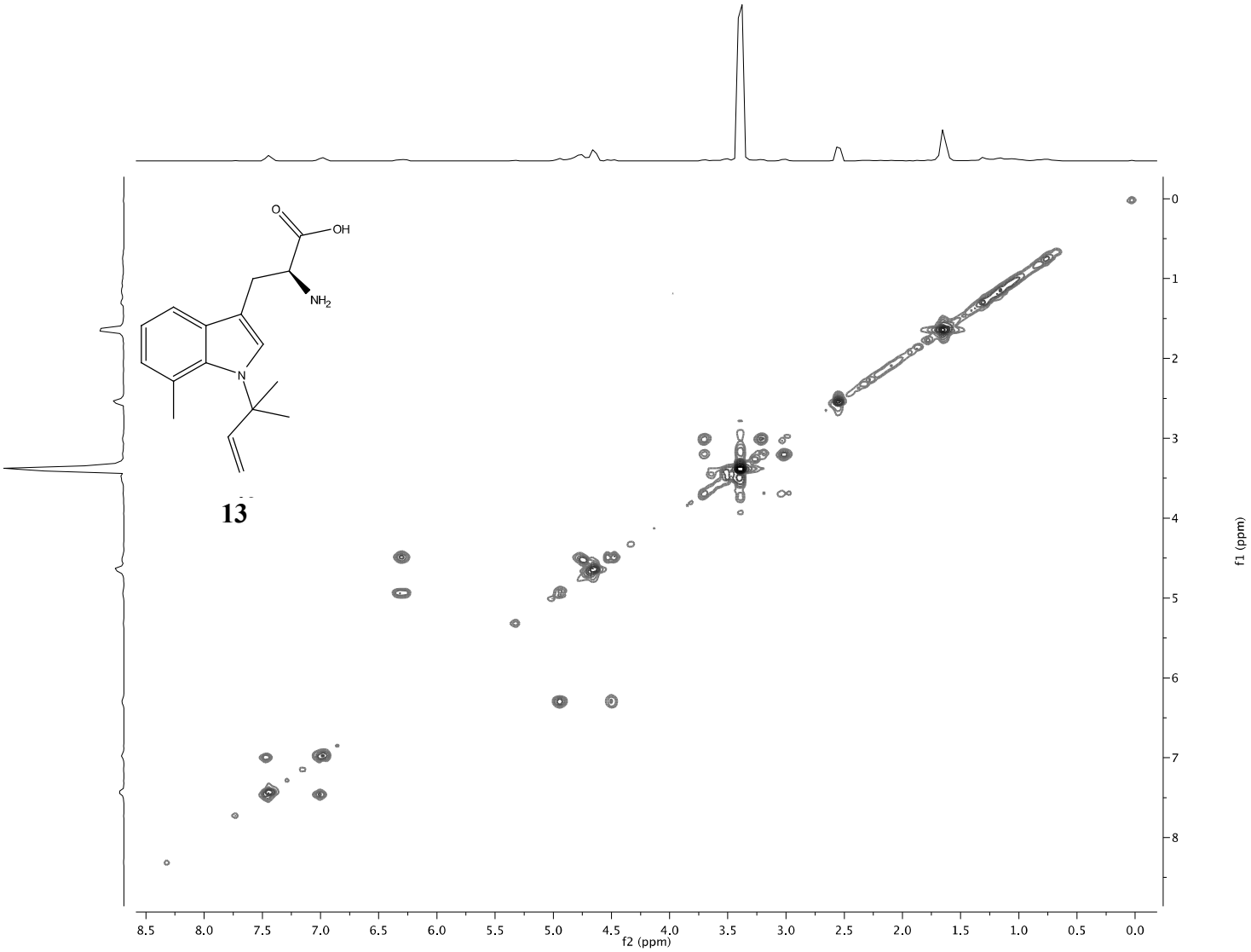


Figure S62. 2D ^{1}H-^{1}H COSY spectrum of **13** in D_2O.

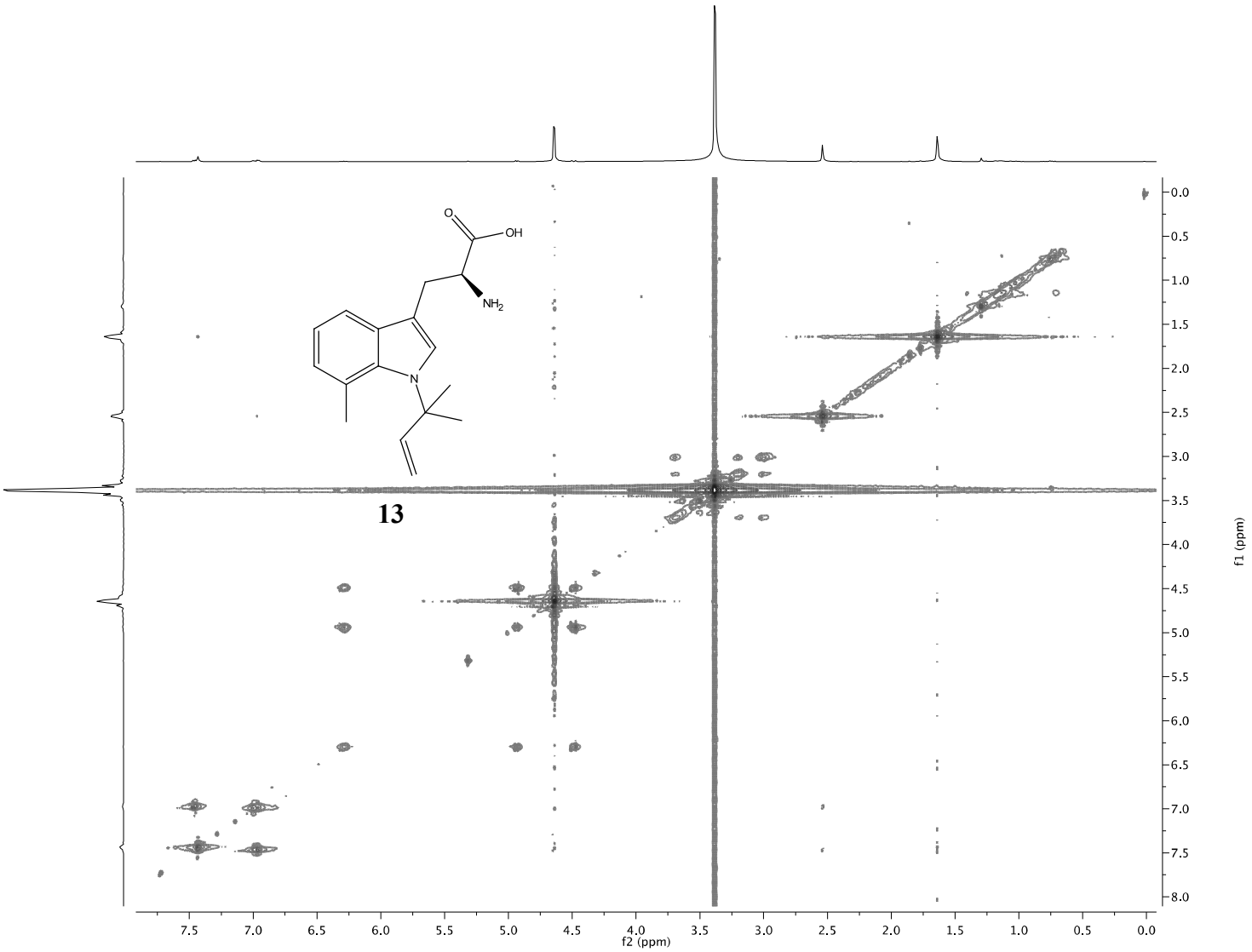


Figure S63. 2D ^{1}H-^{1}H TOCSY spectrum of **13** in D_2O.

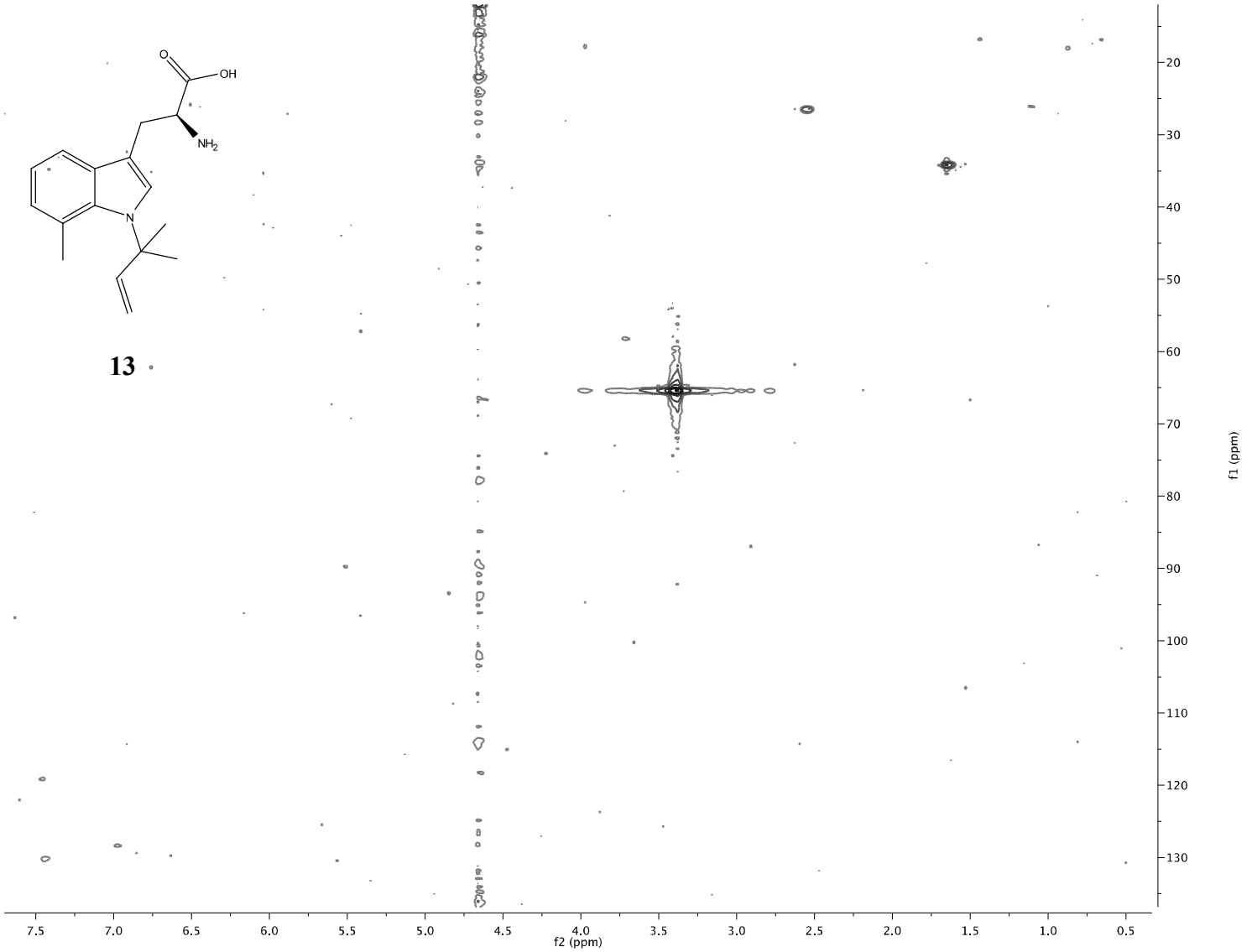


Figure S64. 2D ^{1}H-^{13}C HMQC spectrum of **13** in D_2O.

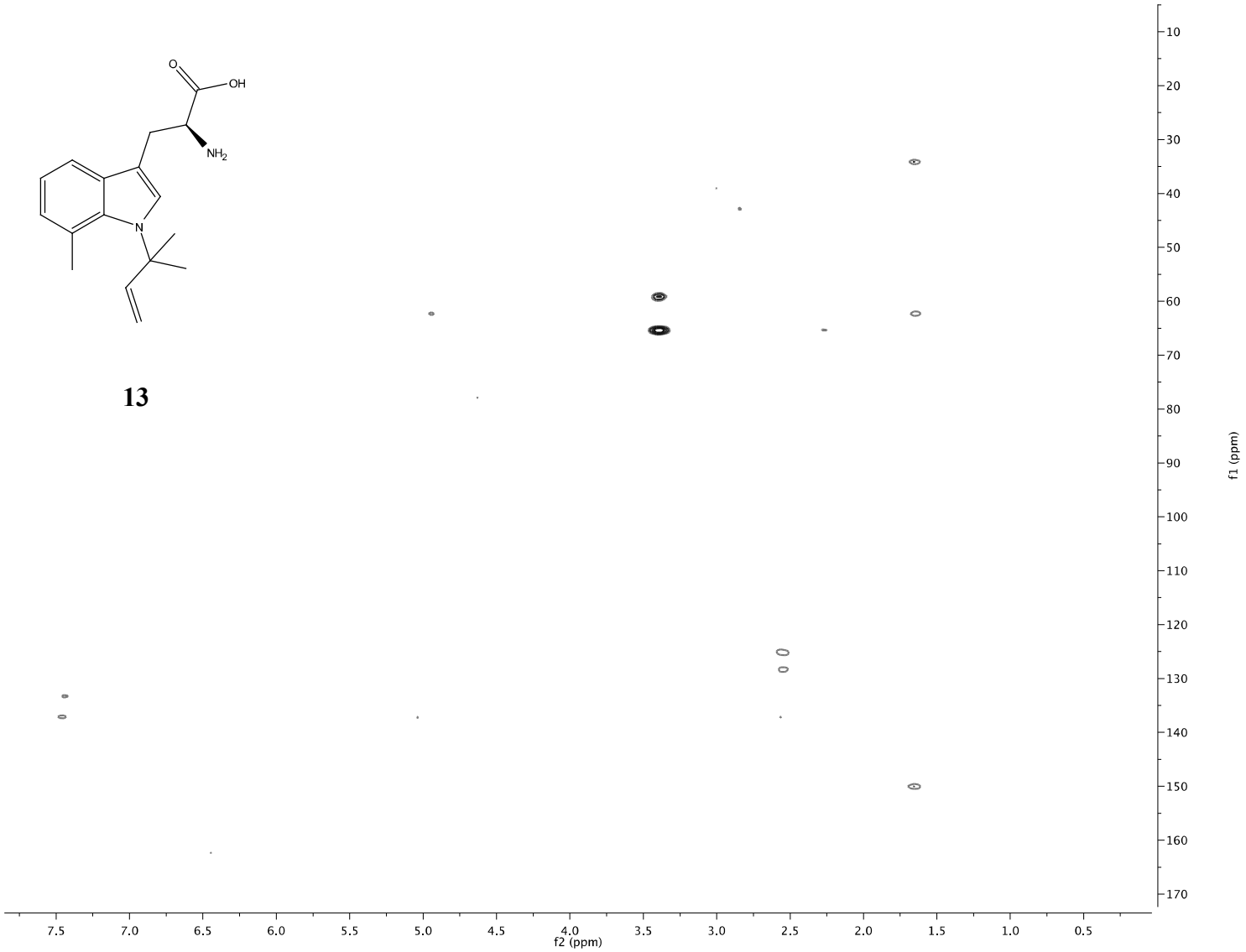


Figure S65. 2D ^{1}H-^{13}C HMBC spectrum of **13** in D_2O.

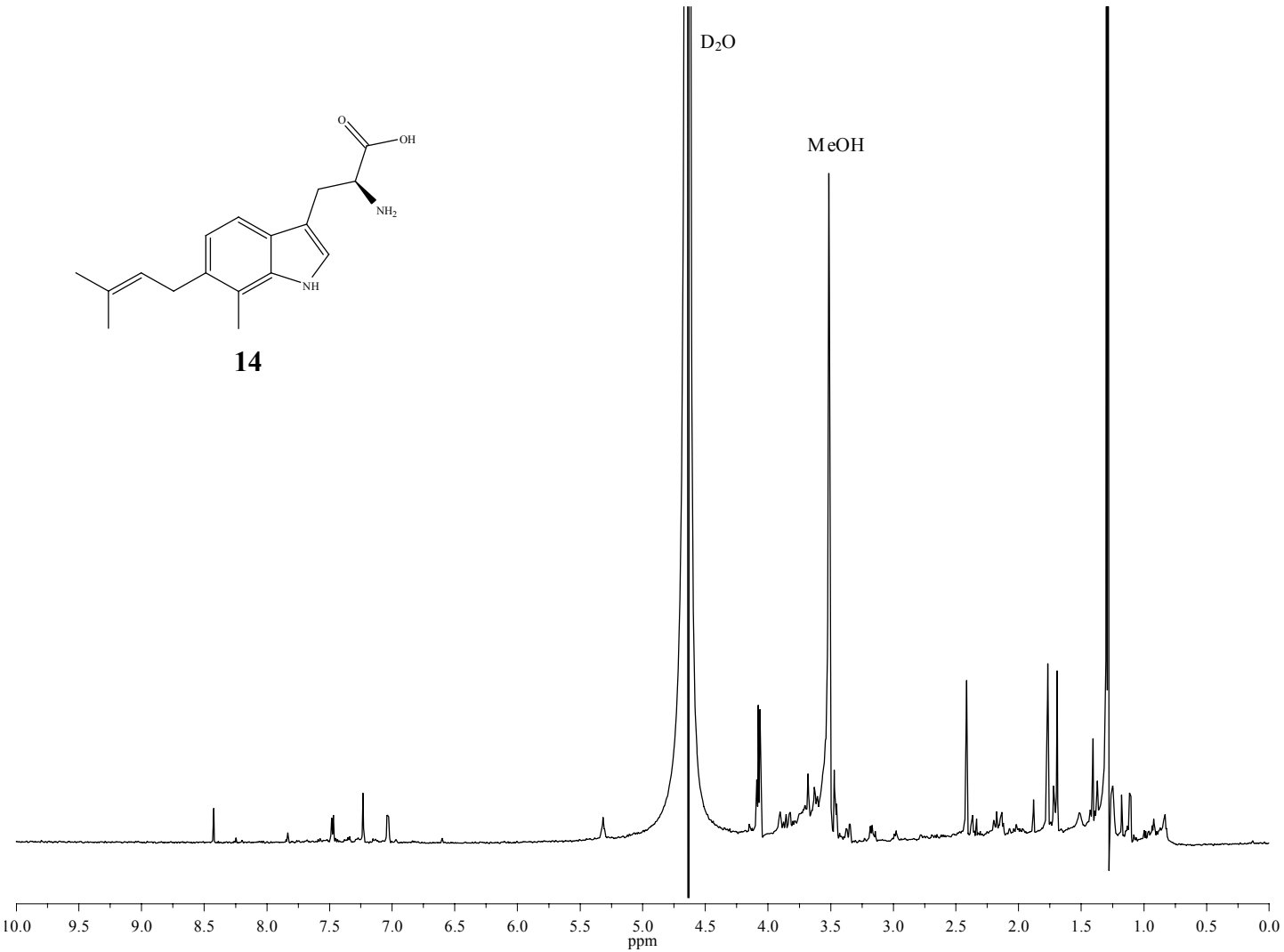


Figure S66. ^{1}H NMR (600 MHz) spectrum of **14** in D_2O.

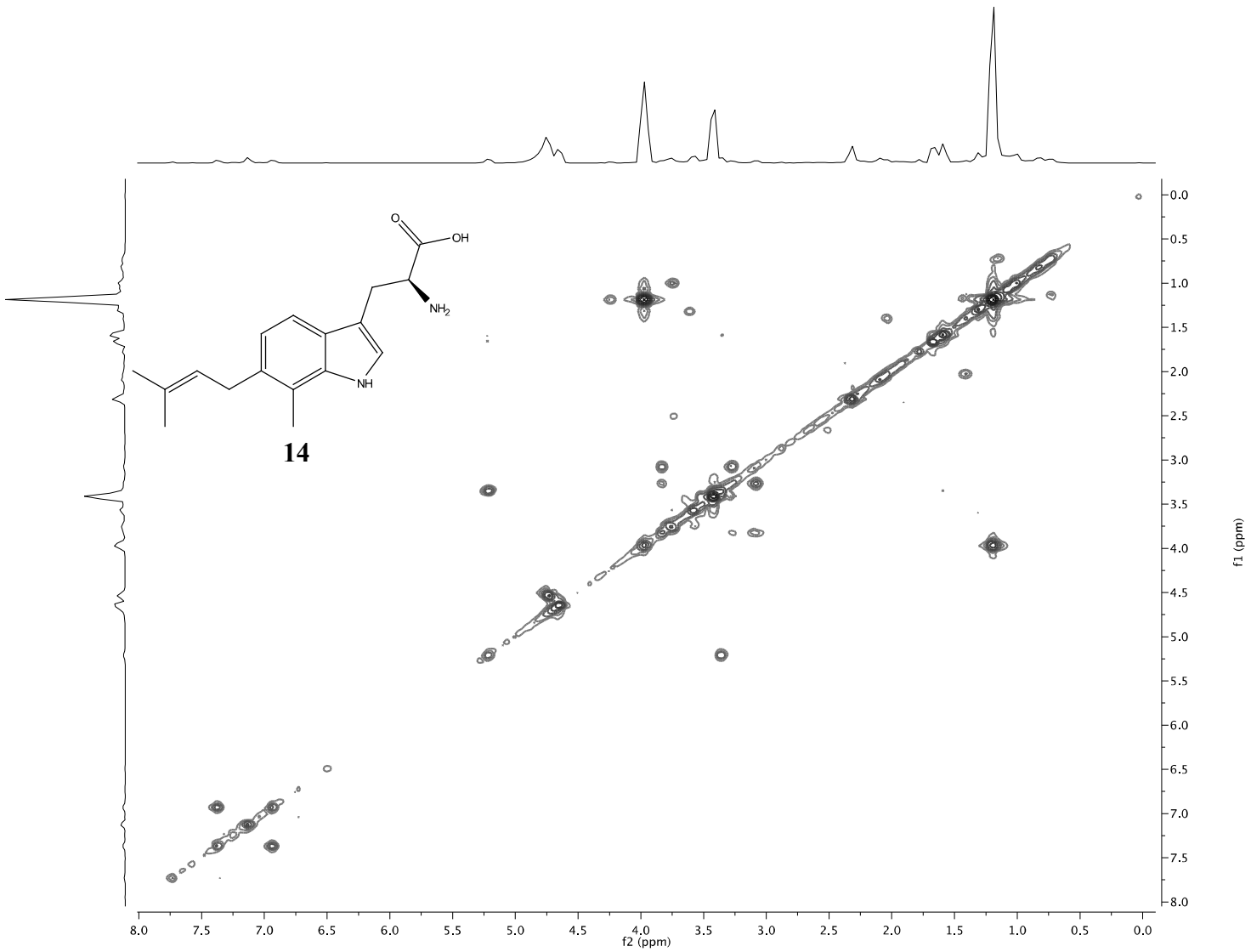


Figure S67. 2D ^{1}H-^{1}H COSY spectrum of **14** in D_2O.

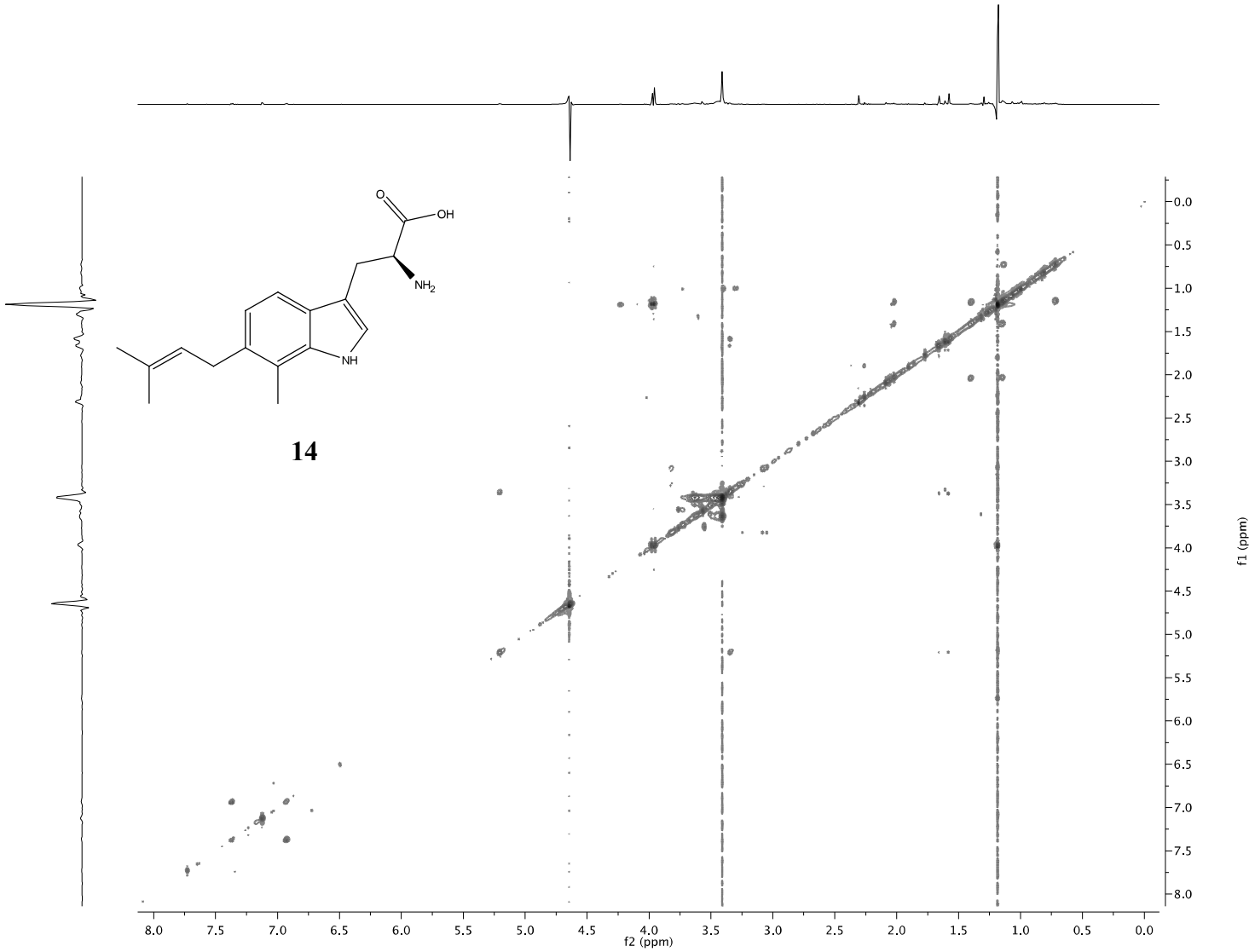


Figure S68. 2D ^{1}H-^{1}H TOCSY spectrum of **14** in D_2O.

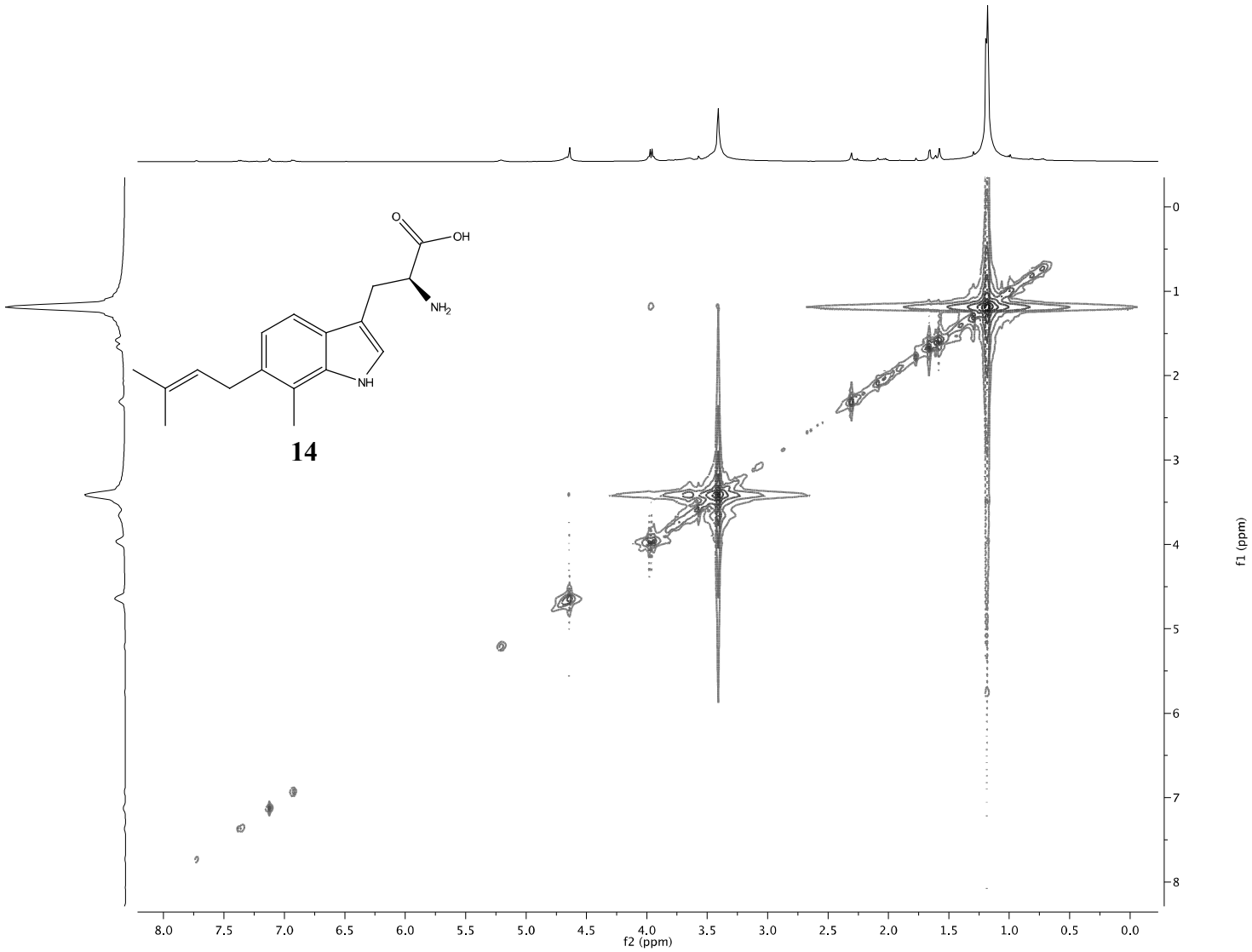


Figure S69. 2D ^{1}H-^{1}H ROESY spectrum of **14** in D_2O.

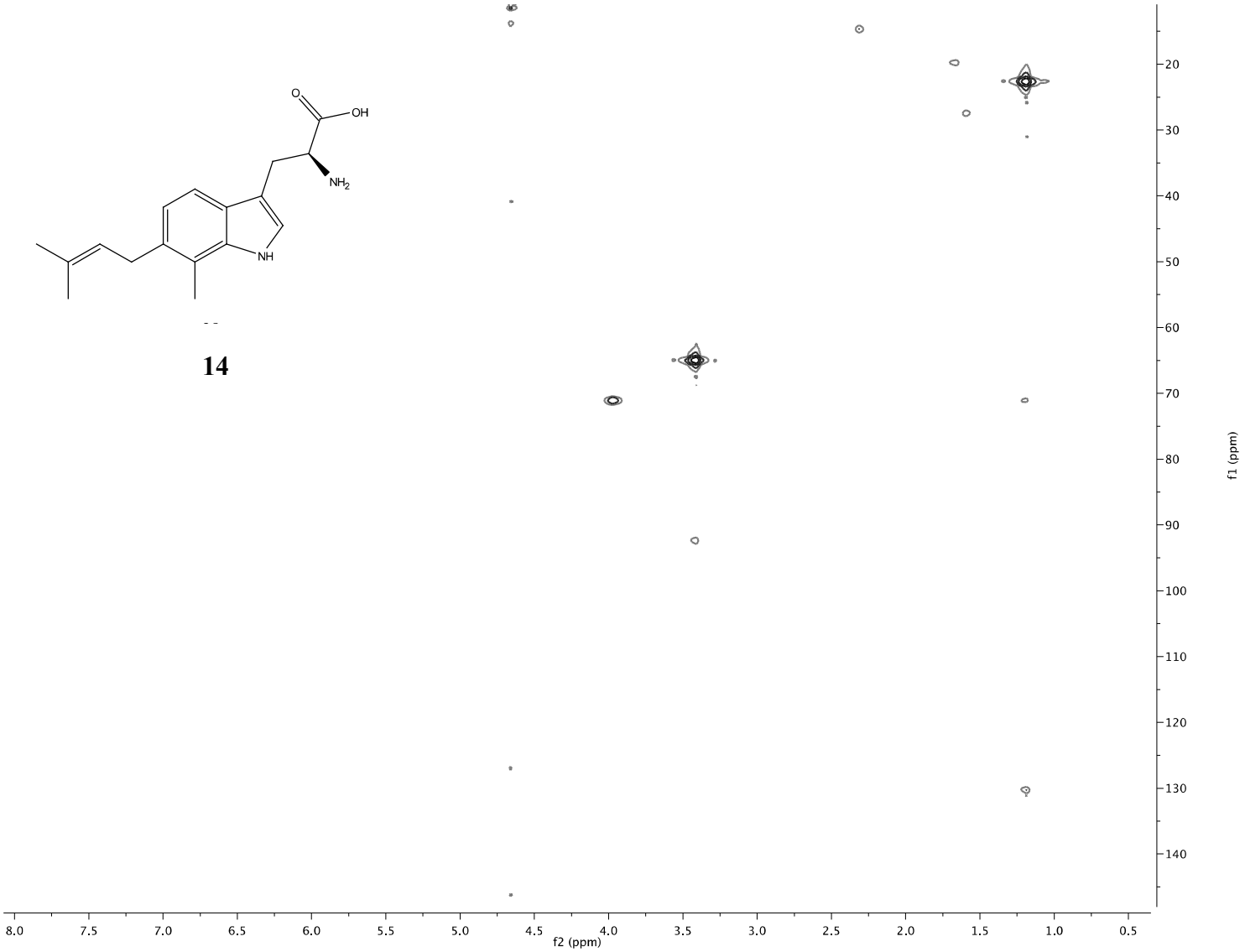


Figure S70. 2D ^{1}H-^{13}C HMQC spectrum of **14** in D_2O.

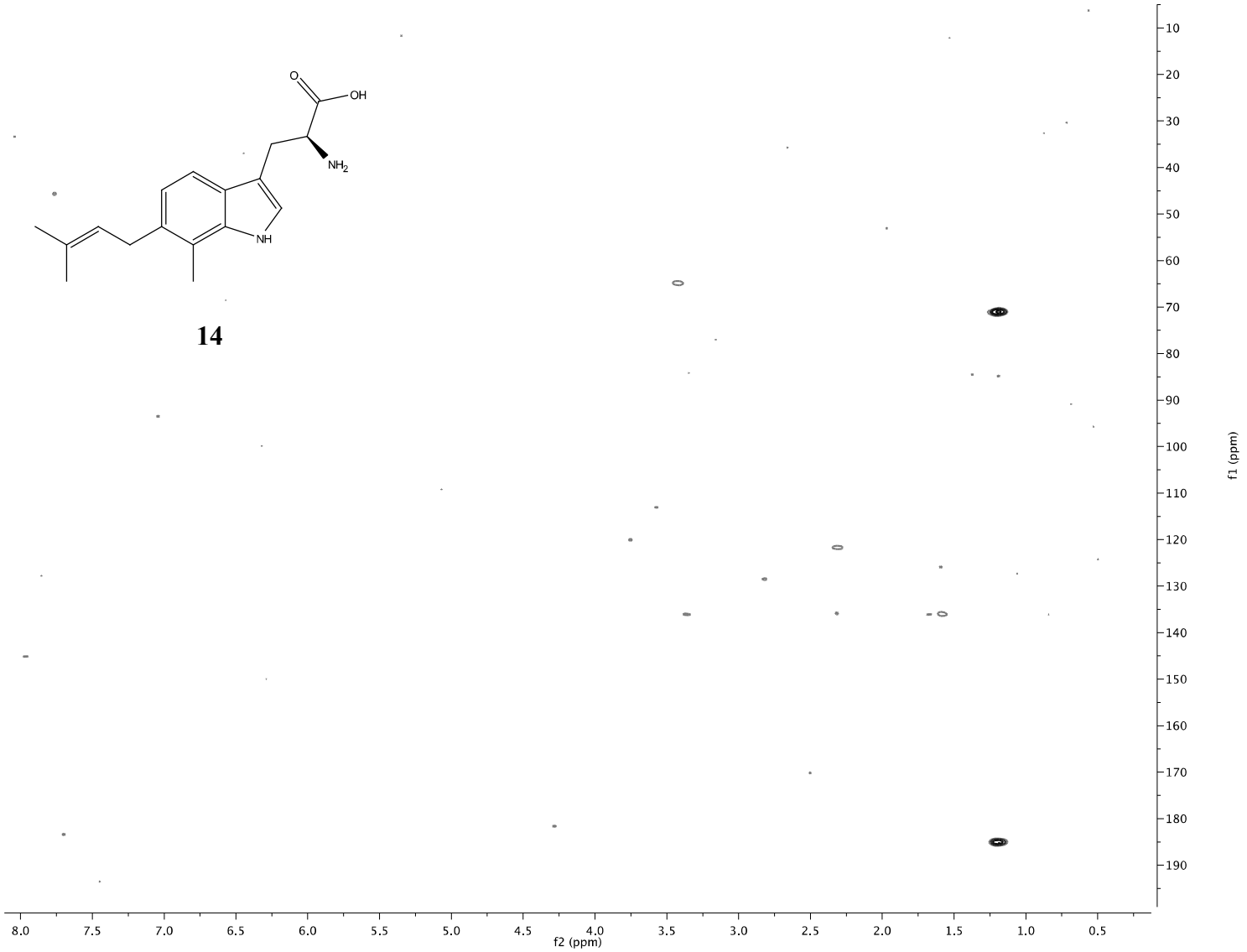


Figure S71. 2D ^{1}H-^{13}C HMBC spectrum of **14** in D_2O.

Structures of products with pertinent 2D NMR correlations

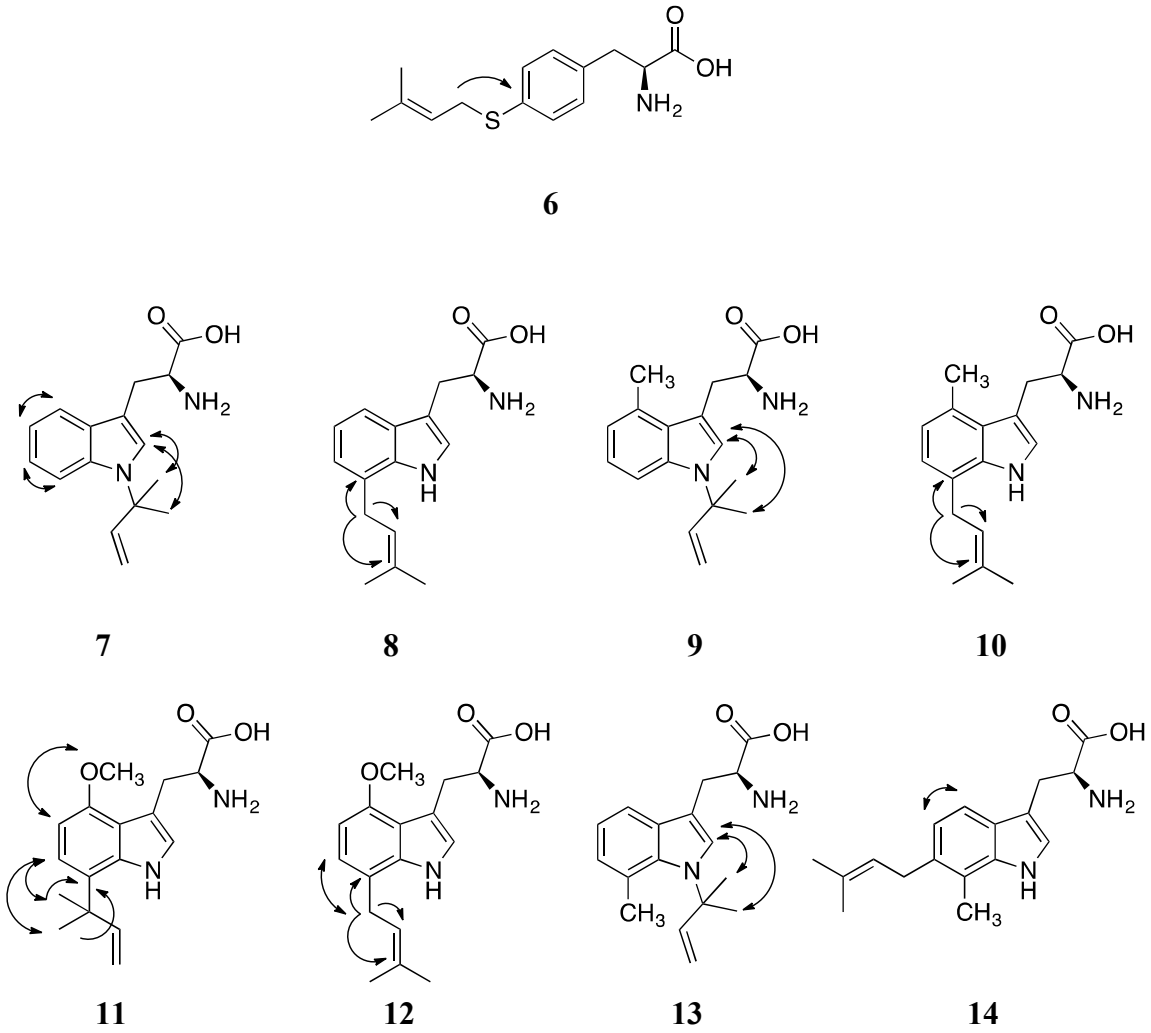


Figure S72. Structures of enzymatic products **6-14** showing the pertinent HMBC (single arrow) and COSY/ROESY (double arrow) correlations.

Table S1. HRMS data of synthetic and enzymatic products

Compound	Chemical Formula	Calculated	Found	Deviation (ppm)
1	$C_{18}H_{21}N_2O_4S_2$	393.0943 $(M + H)^+$	393.0945	0.5
2c	$C_{26}H_{22}NO_7NaSF_3$	572.0967 $(M + Na)^+$	572.0970	0.5
2e	$C_{27}H_{25}NO_4Na$	450.1681 $(M + Na)^+$	450.1688	1.6
2	$C_{11}H_{13}NO_2Na$	214.0838 $(M + Na)^+$	214.0838	-2.8
6	$C_{14}H_{19}NO_2NaS$	288.1034 $(M + Na)^+$	288.1041	2.4
7	$C_{16}H_{20}N_2O_2Na$	295.1422 $(M + Na)^+$	295.1428	2.0
8	$C_{16}H_{21}N_2O_2$	273.1603 $(M + H)^+$	273.1609	2.2
9	$C_{17}H_{23}N_2O_2$	287.1760 $(M + H)^+$	287.1762	0.7
10	$C_{17}H_{23}N_2O_2$	287.1760 $(M + H)^+$	287.1763	1.0
11	$C_{17}H_{22}N_2O_3Na$	325.1528 $(M + Na)^+$	325.1532	1.2
12	$C_{17}H_{22}N_2O_3Na$	325.1528 $(M + Na)^+$	325.1532	1.2
13	$C_{17}H_{22}N_2O_2Na$	309.1579 $(M + Na)^+$	309.1579	0.0
14	$C_{17}H_{22}N_2O_2Na$	309.1579 $(M + Na)^+$	309.1576	-1.0

Michaelis-Menten analysis of kinetic data

All kinetic assays were performed as described in the article. Product turnovers were blanked with a 0 mM substrate assay and were calculated from the reaction completion percentage. Concentrations resulting in ≥ 15% reaction completion were not used in kinetic calculations and plots. Each kinetic assay was performed in triplicate or quadruplicate. Specific activities of SirD vs. substrate concentration were plotted and fit using a nonlinear regression enzyme kinetic plot by GraFit 5.0.11.

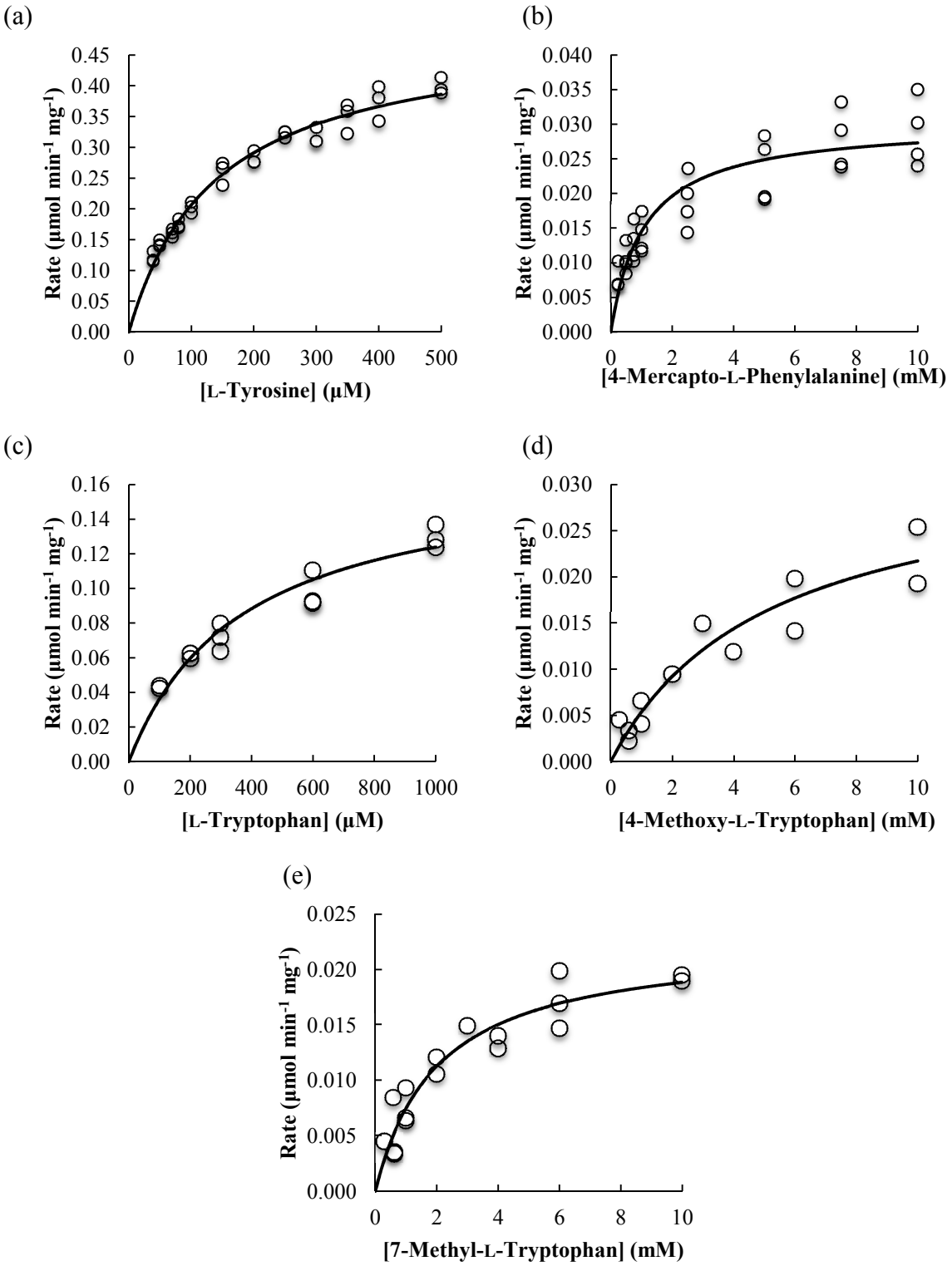


Figure S73. Michaelis-Menten curves for (a) L-tyrosine; (b) 4-mercapto-L-phenylalanine; (c) L-tryptophan; (d) 4-methoxy-L-tryptophan; and (e) 7-methyl-L-tryptophan. All curves were generated by GraFit 5.0.11.

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