SUPPORTING INFORMATION

Multi-Site Prenylation of 4-Substituted Tryptophans by Dimethylallyltryptophan Synthase

Jeffrey D. Rudolf[1], Hong Wang[2], and C. Dale Poulter[1,3]

[1]Department of Chemistry, University of Utah, 315 South 1400 East, Salt Lake City, Utah 84112

[2]Division of Endocrinology, Metabolism and Diabetes, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado 80045

[3]Corresponding author: poulter@chem.utah.edu

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Chemicals

4-Methylindole and 4-methoxyindole were obtained from Chem-Impex International, Inc. (Wood Dale, IL). Chloramphenicol was obtained from MP Biomedicals, LLC (Solon, OH). [1-^{14}C]DMAPP was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). All other chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO).

Isoprenoid diphosphate synthesis

DMAPP was prepared as described previously.[1]

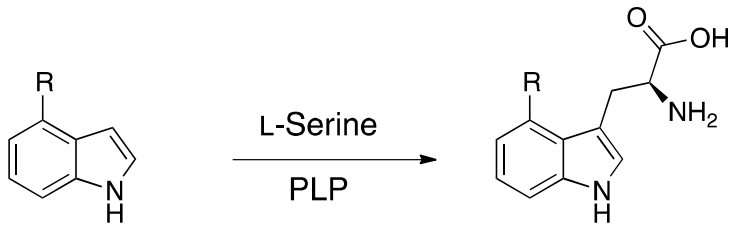
Bacterial strains, plasmids and culture conditions

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*.[2] Plasmid pHDMAT is a pET28a construct containing the *C. purpurea* dmaW, which encodes 4-DMATS. Plasmid pGroESL is a construct containing the *E. coli* groE operon[3] and was obtained from Anthony Gatenby. The expression strain *E. coli* BL21 Star^{TM} (DE3) was obtained from Stratagene. Expression strains were grown in liquid LB broth or on solid LB-agar medium at 37 °C. Kanamycin (35 μg/mL) and chloramphenicol (34 μg/mL) were used to select vector-containing *E. coli* strains.

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.

Scheme S1. Biosynthetic scheme for the preparation of L-tryptophan analogues using tryptophan synthase.



^{1}H and ^{13}C NMR spectra of 4-CH_3-L-TRP, 4-OCH_3-L-TRP, and 4-NH_2-L-TRP

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 D_2O (4-OCH_3-TRP, 4-NH_2-TRP) or D_2O containing minimal NaOD (4-CH_3-TRP). 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 DSS at δ 0.00 ppm. ^{13}C chemical shifts are referenced relative to DSS at δ 0.0 ppm.

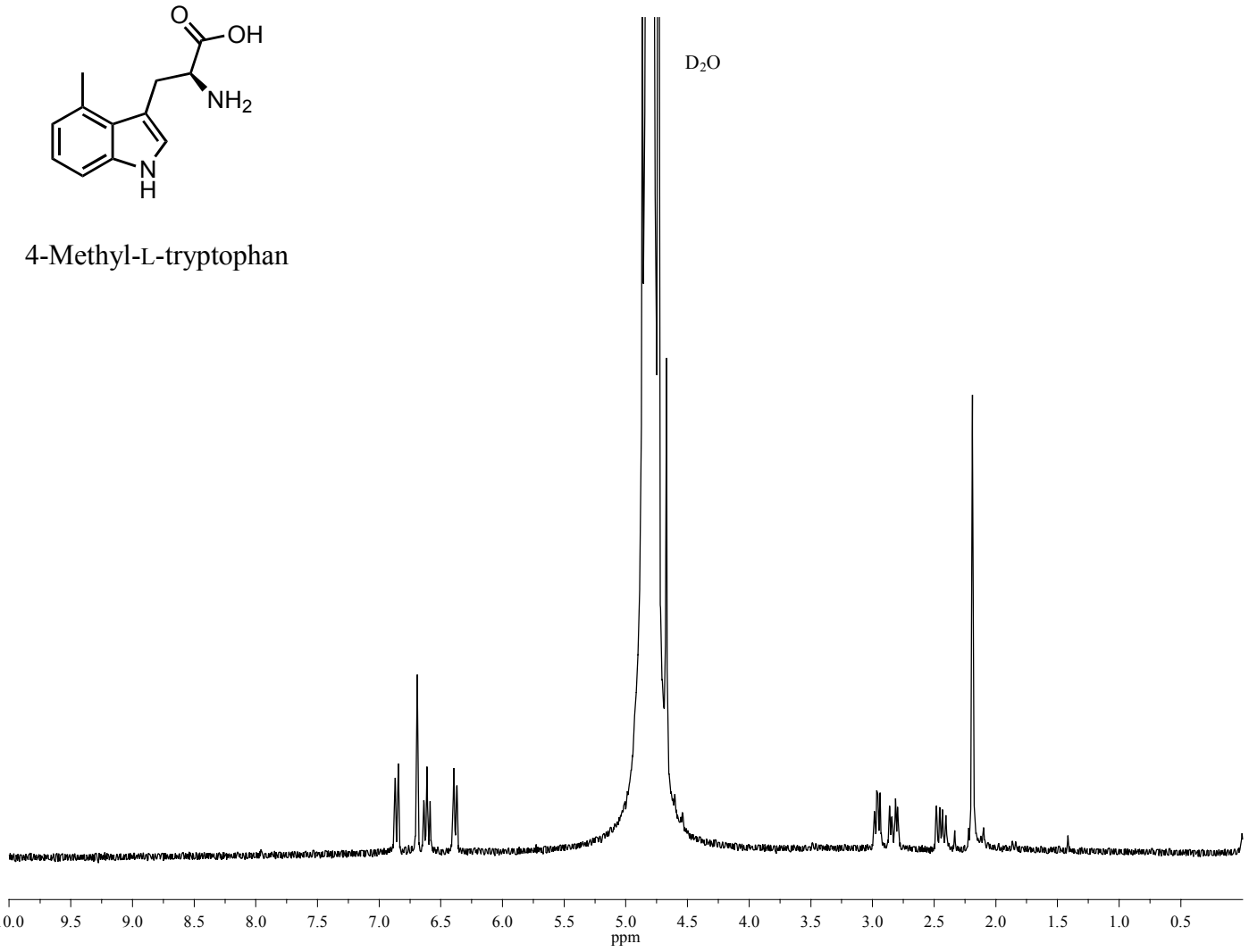


Figure S1. ^{1}H NMR (300 MHz) spectrum of 4-methyl-L-trytpophan in D_2O/NaOD.

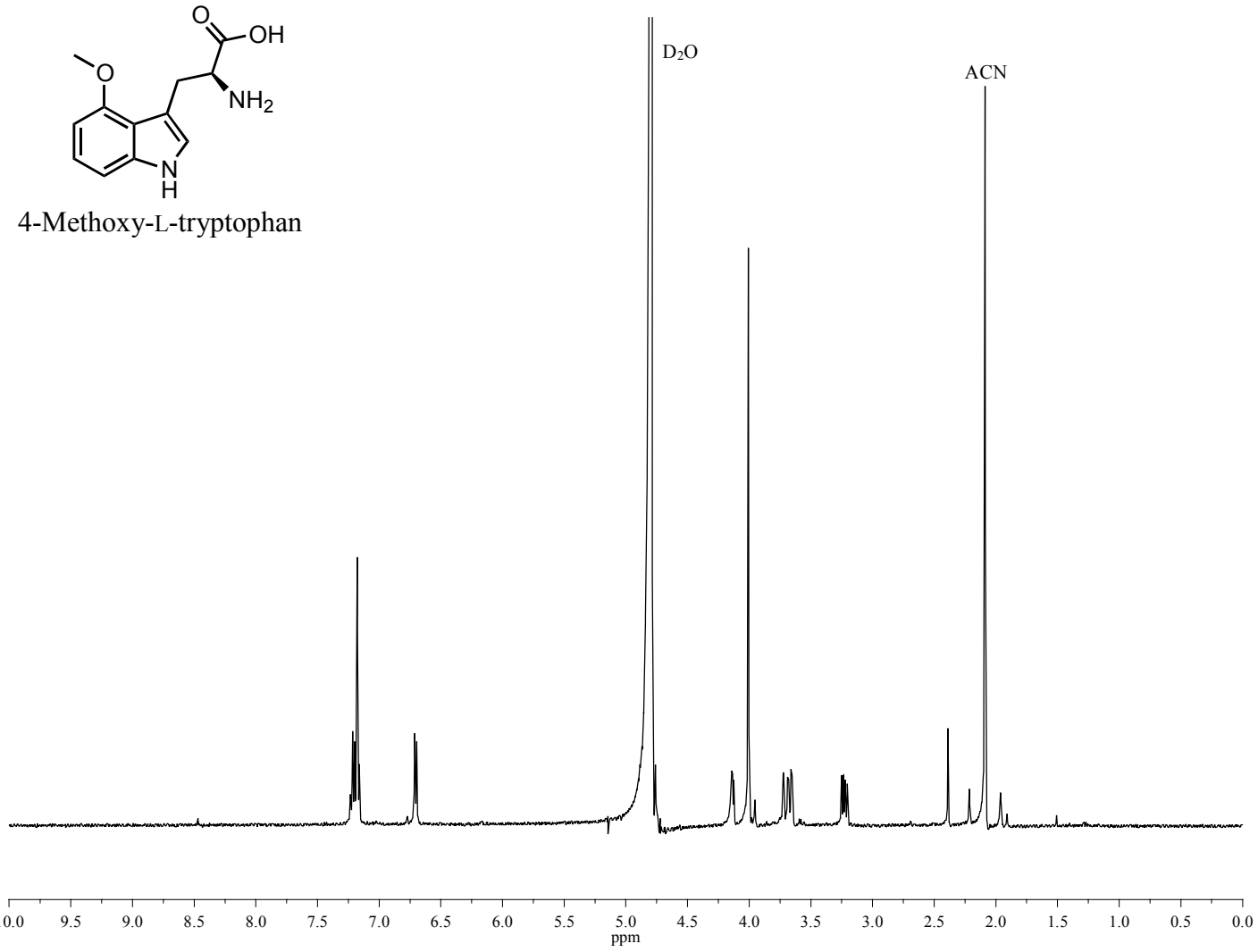


Figure S2. ^{1}H NMR (500 MHz) spectrum of 4-methoxy-L-trytpophan in D_2O.

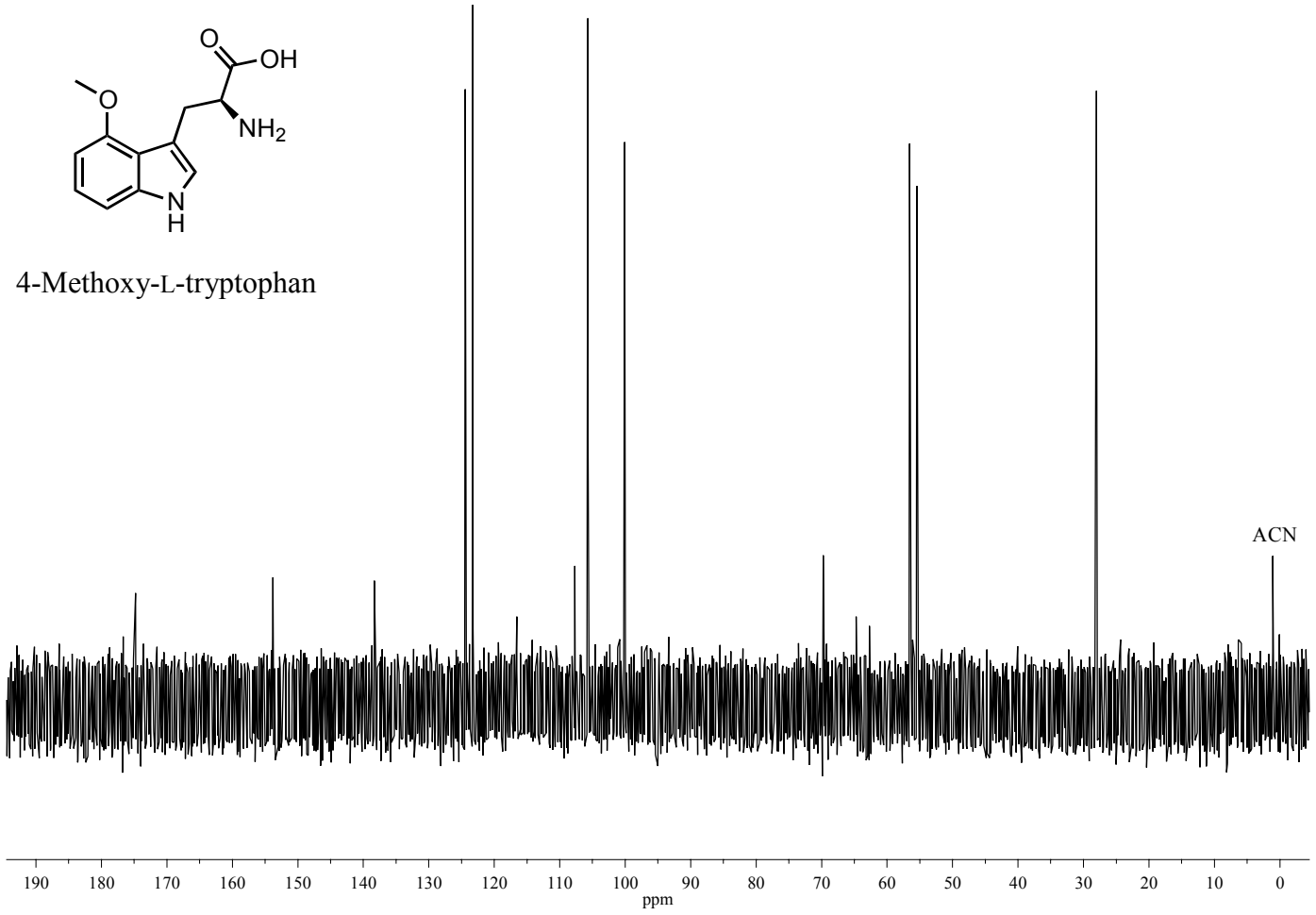


Figure S3. ^{13}C NMR (125 MHz) spectrum of 4-methoxy-L-trytpophan in D_2O.

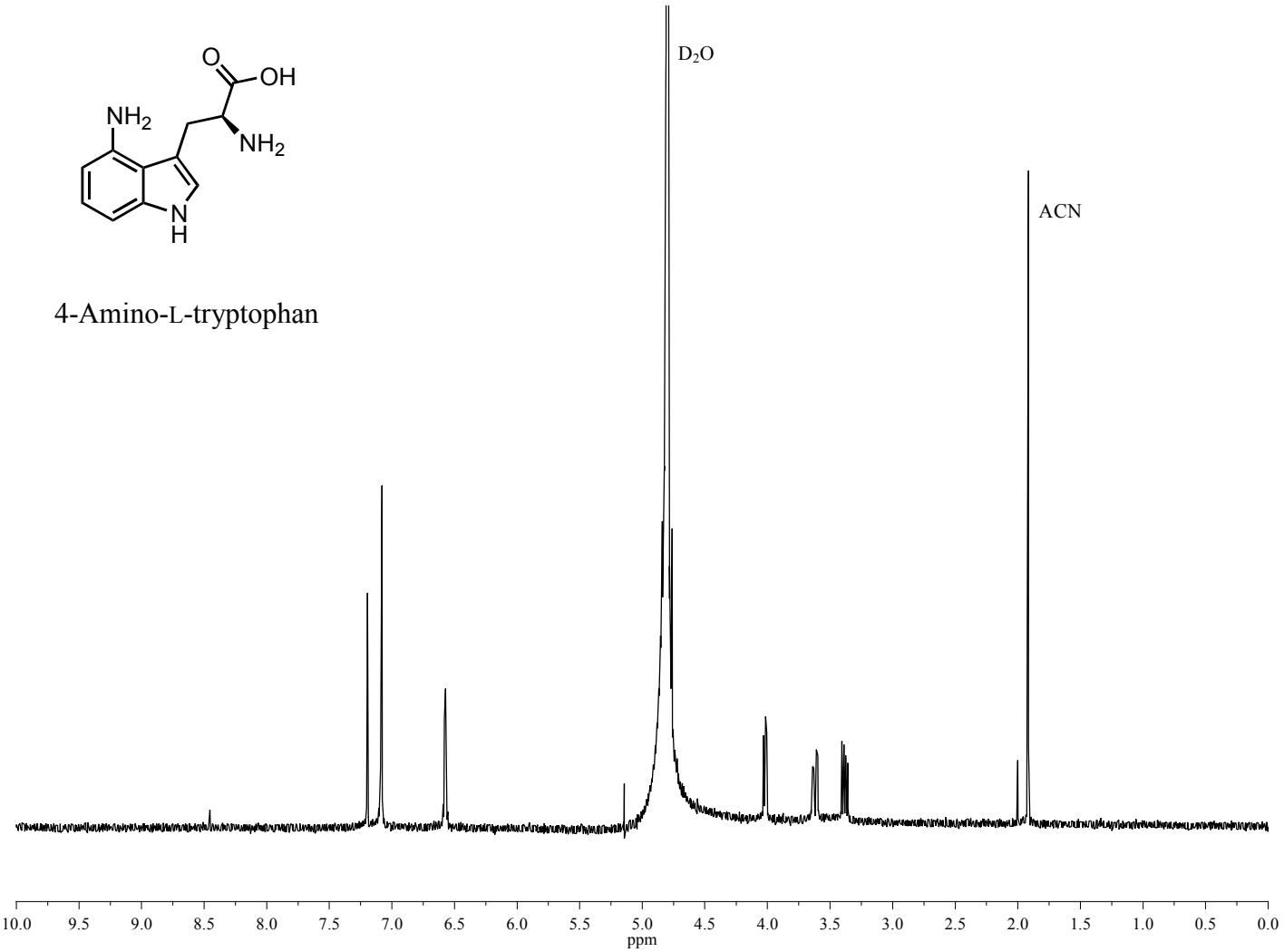
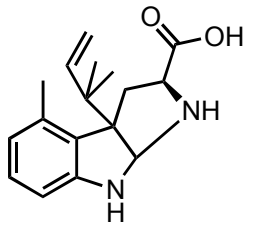


Figure S4. ^{1}H NMR (125 MHz) spectrum of 4-amino-L-trytpophan in D_2O.

NMR tables and spectra of prenylated tryptophan analogues

After HPLC purification, each prenylated product was lyophilized to dryness, redissolved in D_2O (D, 99.9%), re-lyophilized, dissolved in 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.

Table S1. NMR data table and structure of **1**.



1

Carbon	**^{13}C (ppm)**	**^{1}H (ppm)**	**Multiplicity**	**^{1}H-^{1}H**	**HMBC**
α	63.5	3.49	t: 7.8 Hz	COSY: βa, βb, C2	MeOH? NH?
β	38.0	a: 2.34	dd: 8.4, 13.8 Hz	COSY: βb, α ROESY: βb	α, C3', C3, C4, COOH
		b: 2.72	dd: 7.8, 13.8 Hz	COSY: βa, α, C2 ROESY: βa	C2, C3, C4
COOH	179.5	-	-	-	-
C2	83.3	5.06	s	COSY: βb, α	C4, C7a, C3'
C3	68.1	-	-	-	-
C3a	138.9	-	-	-	-
C4	130.9	-	-	-	-
4-CH_3	23.1	2.29	s	COSY: C5, C6, C7 ROESY: C5, C6, C7	C3a, C4, C5
C5	125.8	6.61	d: 7.8 Hz	COSY: 4-CH_3, C6 ROESY: 4-CH_3, C6	C4, C7, 4-CH_3
C6	131.5	7.00	t: 7.8 Hz	COSY: 4-CH_3, C5, C7 ROESY: 4-CH_3, C5, C7	C3a, C7a
C7	110.8	6.48	d: 7.8 Hz	COSY: 4-CH_3, C6 ROESY: 4-CH_3, C6	C4, C5
C7a	152.7	-	-	-	-
C1'	116.2	a: 4.95	dd: 0.6, 10.2 Hz	COSY: C2' ROESY: C2'	C2', C3'
		b: 4.94	dd: 0.6, 17.4 Hz		
C2'	147.6	5.85	dd: 10.8, 17.4 Hz	COSY: C1' ROESY: C1'	C3', C5'
C3'	44.7	-	-	-	-
C4'	26.1	0.93	s	COSY: C1' ROESY: C5, C6, C7	C3, C2', C3', C5'
C5'	25.7	0.85	s		C3, C2', C3', C4'

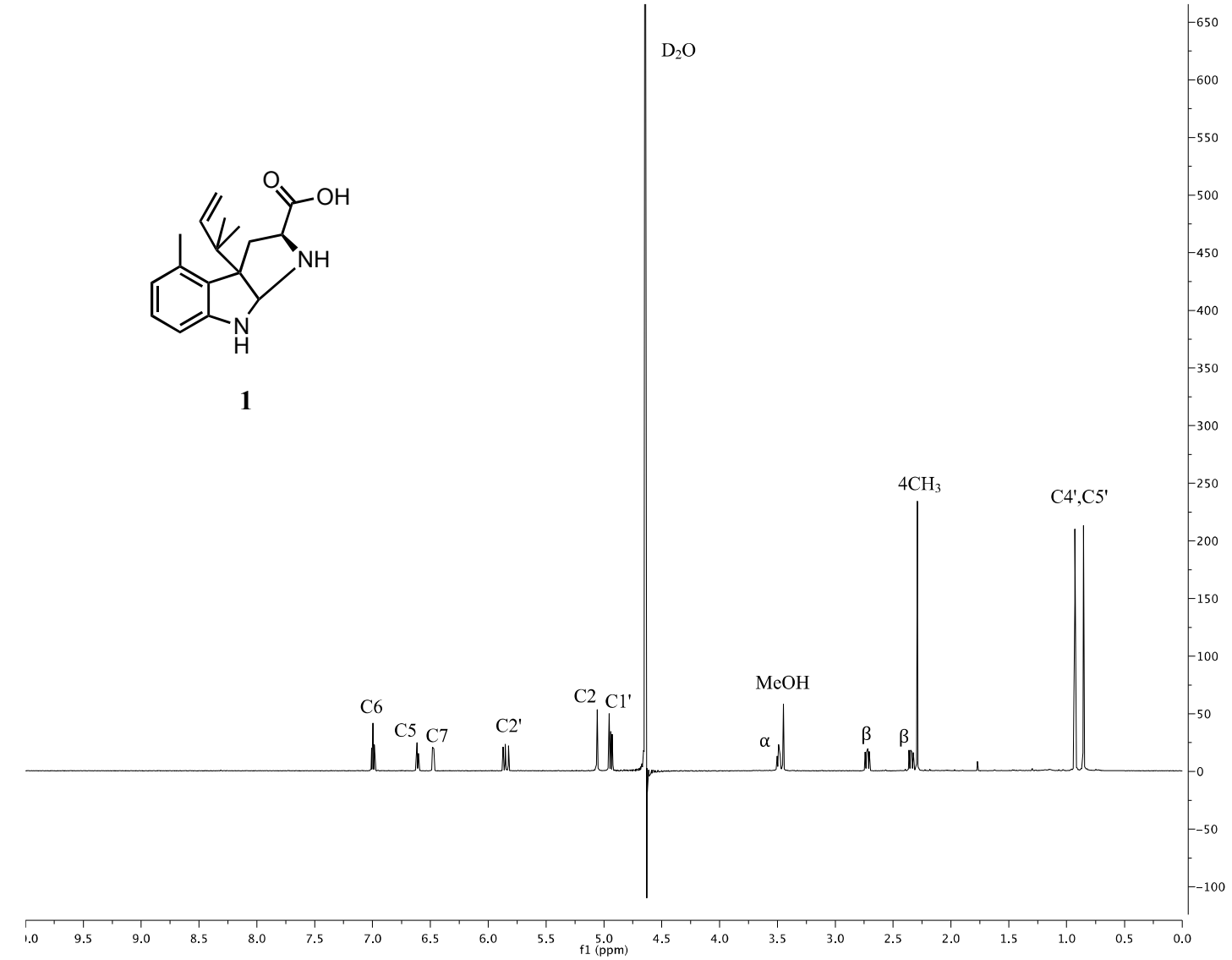


Figure S5. ^{1}H NMR spectrum of **1** in D_2O.

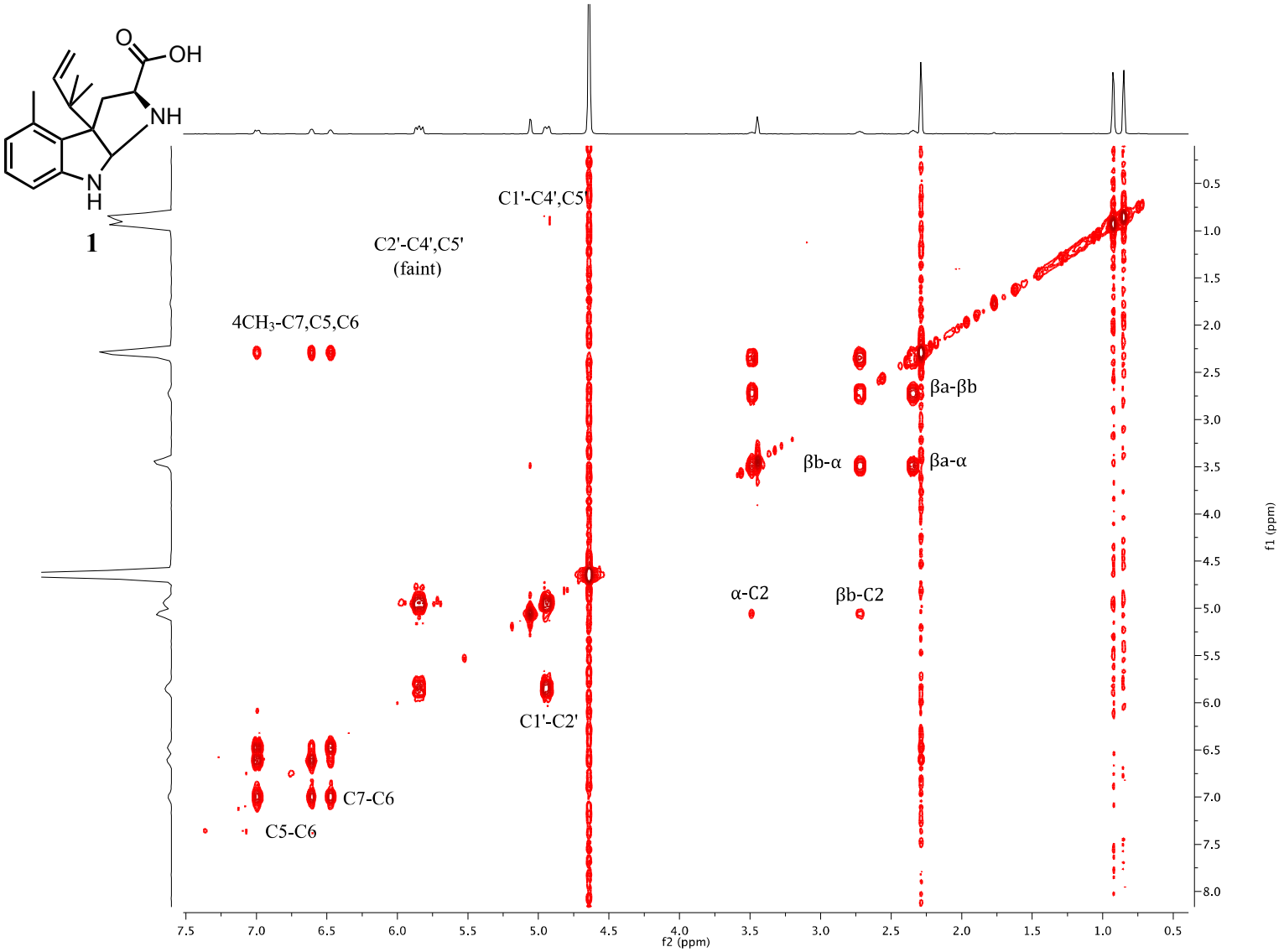


Figure S6. 2D ^{1}H-^{1}H COSY NMR spectrum of **1** in D_2O.

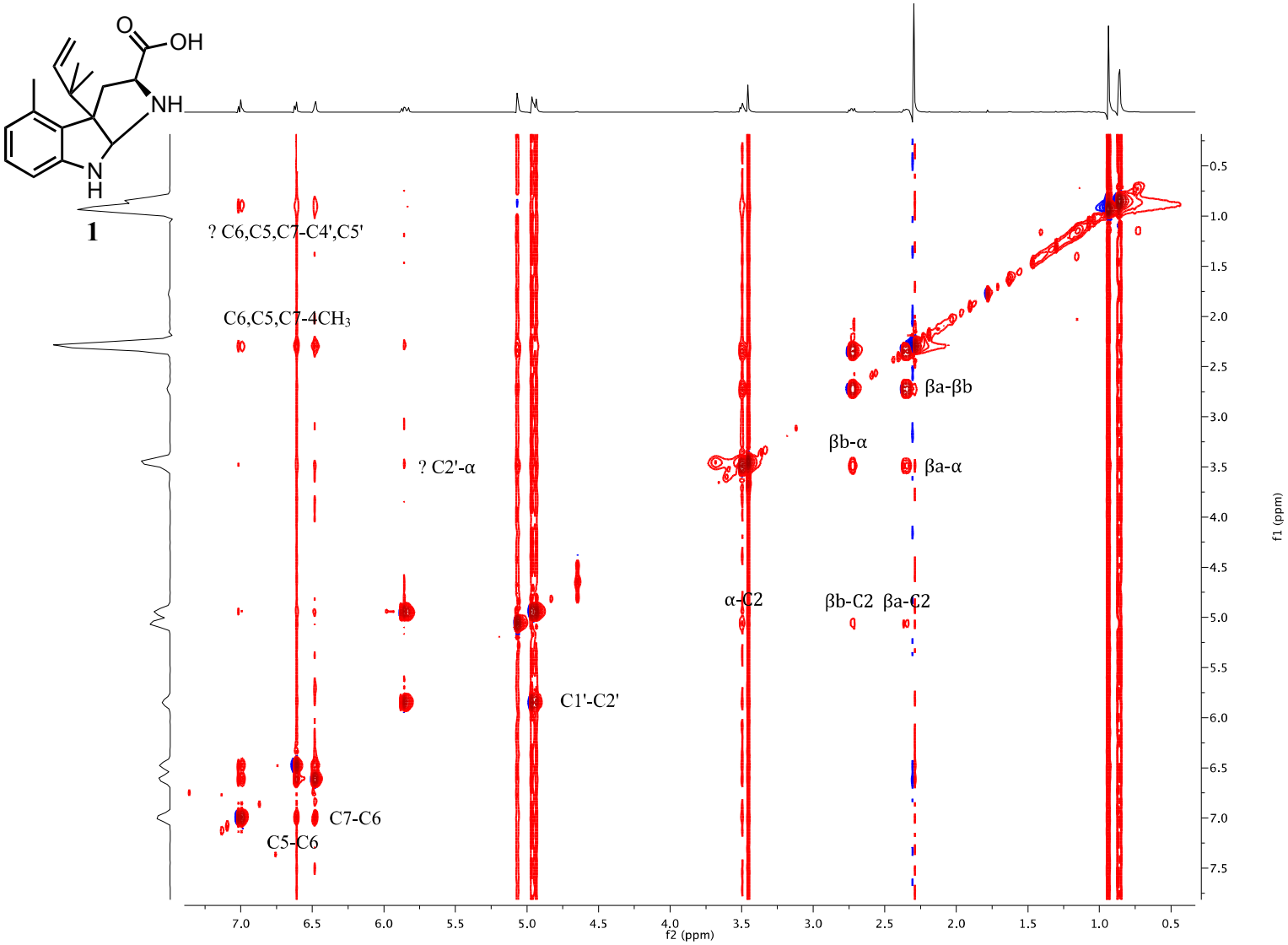


Figure S7. 2D ^{1}H-^{1}H TOCSY NMR spectrum of **1** in D_2O.

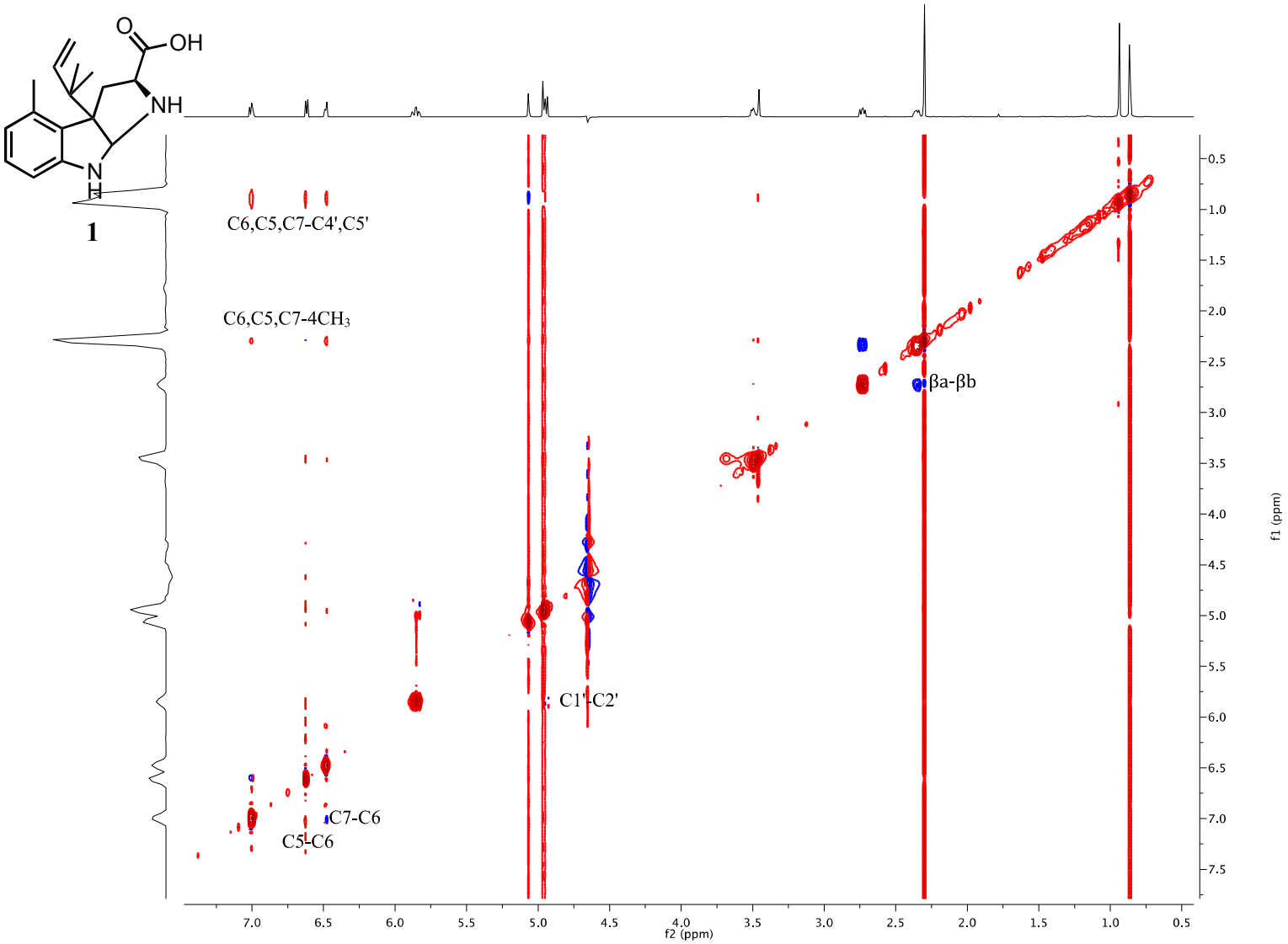


Figure S8. 2D ^{1}H-^{1}H ROESY NMR spectrum of **1** in D_2O.

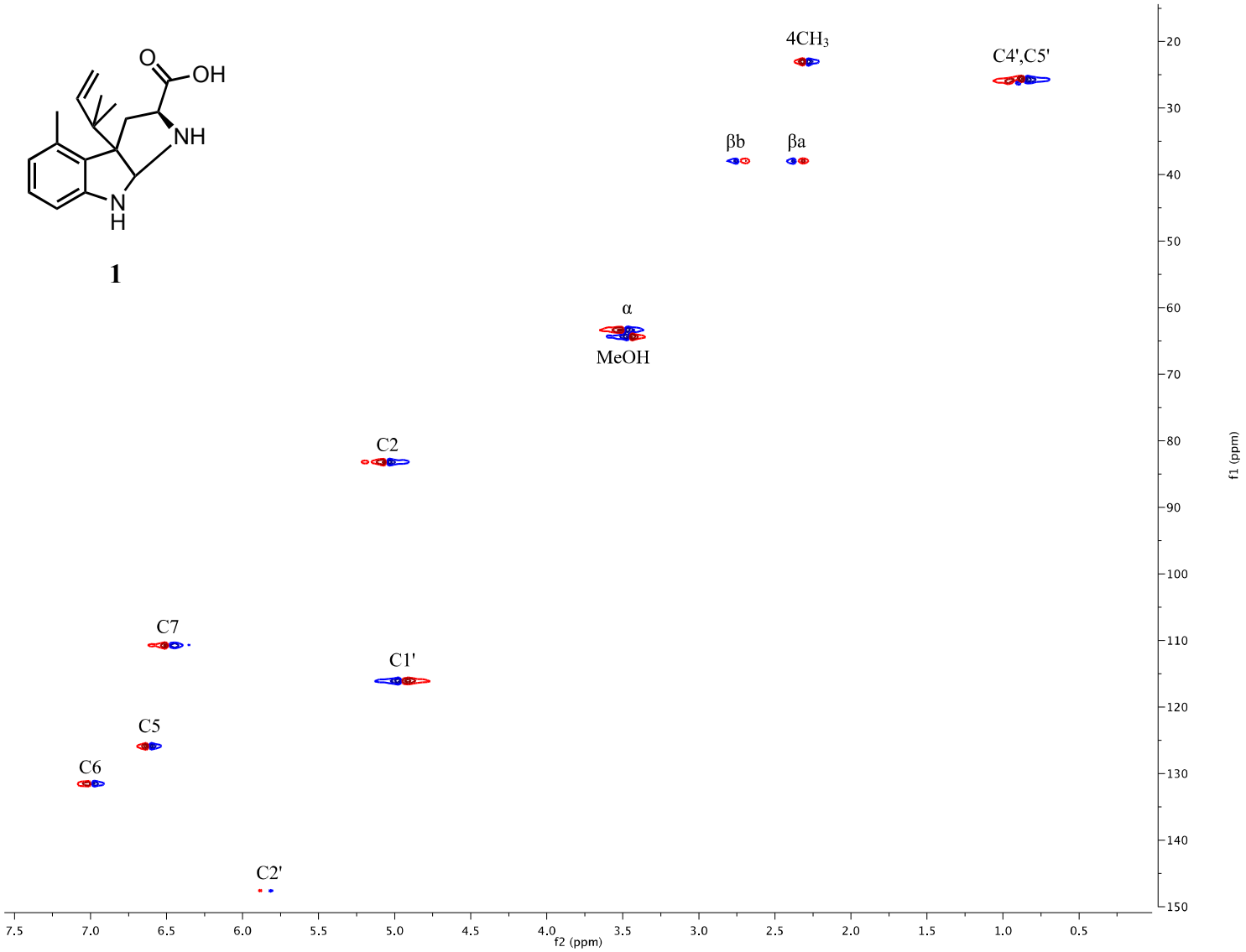


Figure S9. 2D ^{1}H-^{13}C HSQC NMR spectrum of **1** in D_2O.

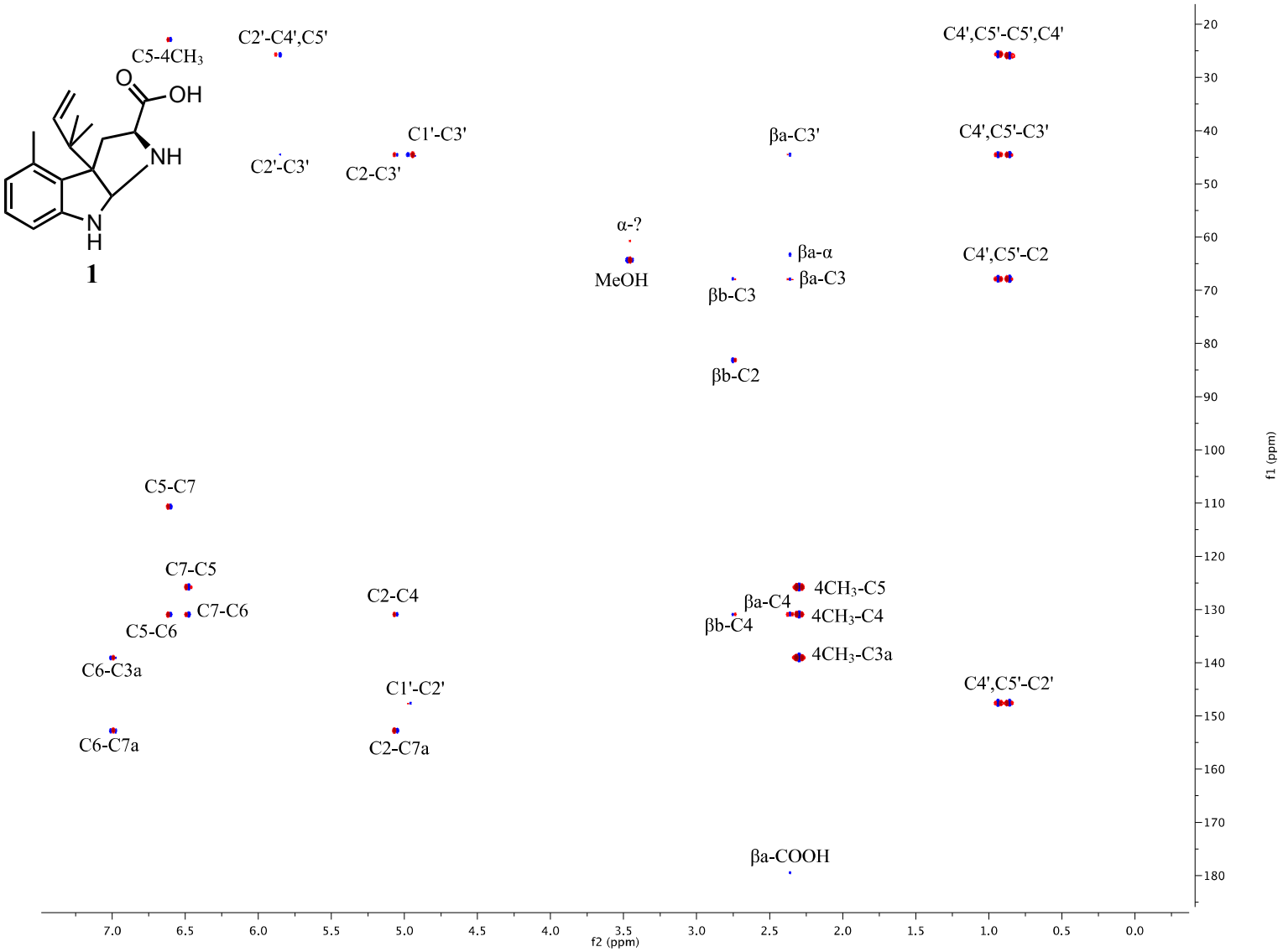
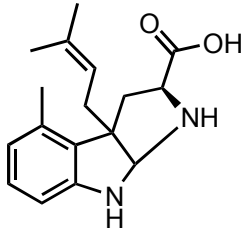


Figure S10. 2D ^{1}H-^{13}C HMBC NMR spectrum of **1** in D_2O.

Table S2. NMR data table and structure of **2**. Except where mentioned below, TOCSY peaks were identical to COSY peaks.

Carbon	**^{13}C (ppm)**	**^{1}H (ppm)**	**Multiplicity**	**^{1}H-^{1}H**	**HMBC**
α	63.4	3.40	m (MeOH obscures)	COSY: βa, βb	MeOH?
β	44.2	a: 2.00	dd: 8.4, 12.6 Hz	COSY: βb, α ROESY: βb	N/A
		b: 2.51	dd: 6.6, 12.6 Hz	COSY: βa, α ROESY: βa, α	N/A
COOH	n.f.	-	-	-	-
C2	84.2	4.84	s	ROESY: C1'b (faint)	C7a, C1', C4?
C3	61.1	-	-	-	-
C3a	137.7	-	-	-	-
C4	133.7	-	-	-	-
4-CH_3	20.0	2.22	s	COSY: C5, C7 (faint) ROESY: C5	C3a, C4 (faint), C5
C5	124.5	6.57	d: 7.8 Hz	COSY: 4-CH_3, C6 ROESY: 4-CH_3	C4, C7, 4-CH_3
C6	130.8	6.94	t: 7.8 Hz	COSY: C5, C7 ROESY: 4-CH_3, C5, C7	C3a, C7a
C7	110.5	6.45	d: 7.8 Hz	COSY: 4-CH_3, C6	C5, C6
C7a	152.2	-	-	-	-
C1'	37.4	a: 2.34	dd: 8.4, 15.0 Hz	COSY: C1'b, C2' TOCSY: C4', C5' ROESY: C1'b	C2', C3', C3
		b: 2.55	dd: 6.6, 15.0 Hz	COSY: C1'a, C2' TOCSY: C4', C5' ROESY: C1'a	C2'
C2'	121.7	4.77	t: 7.2 Hz	COSY: C1'a, C1'b, C4', C5' ROESY: C1'a, C4', C5'	C3', C4', C5'
C3'	138.6	-	-	-	-
C4'	19.8	1.47	s	COSY: C1'b, C2' TOCSY: C1'a, C1'b, C2'	C2', C3', C5'
C5'	27.4	1.46	s		



2

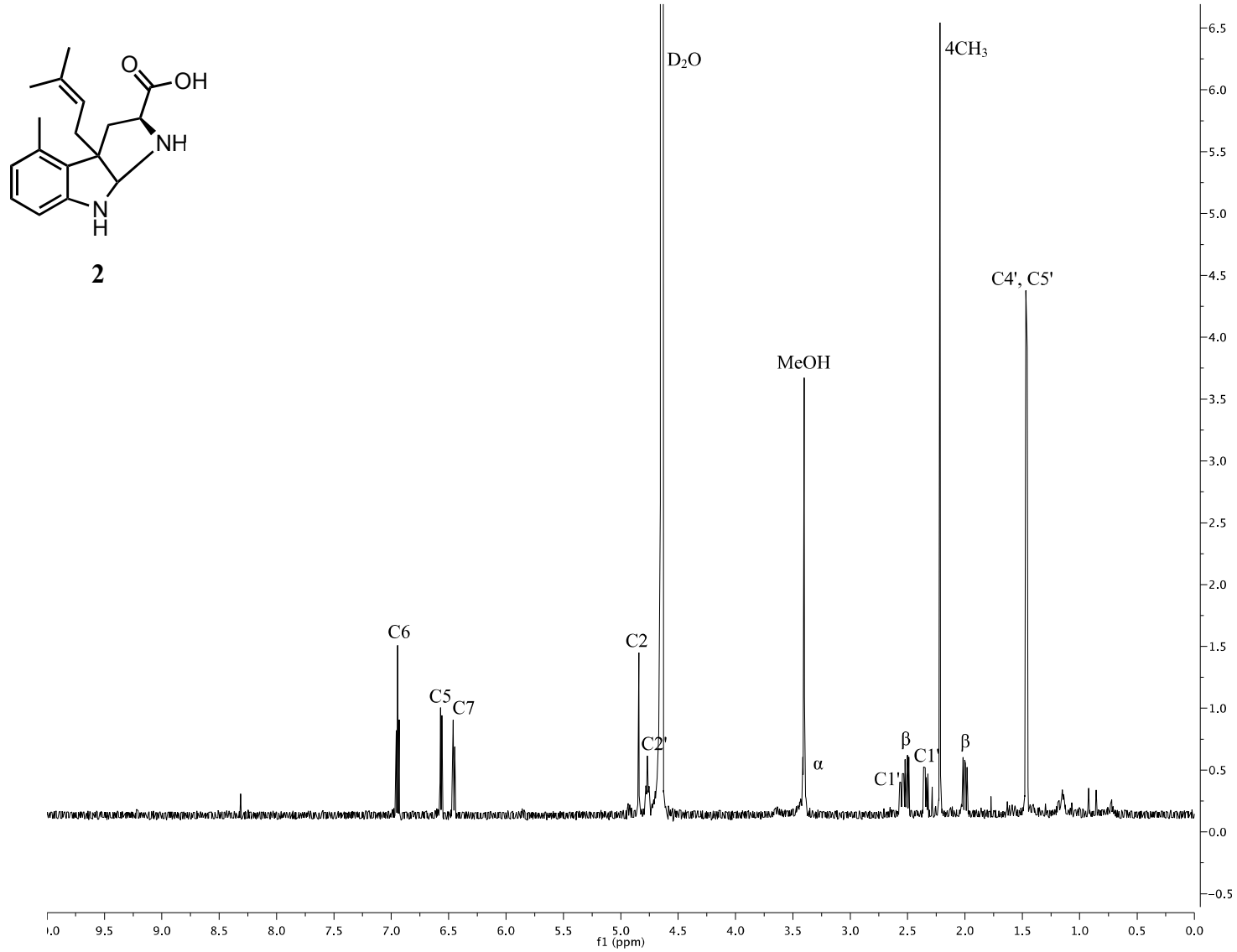


Figure S11. ^{1}H NMR spectrum of **2** in D_2O.

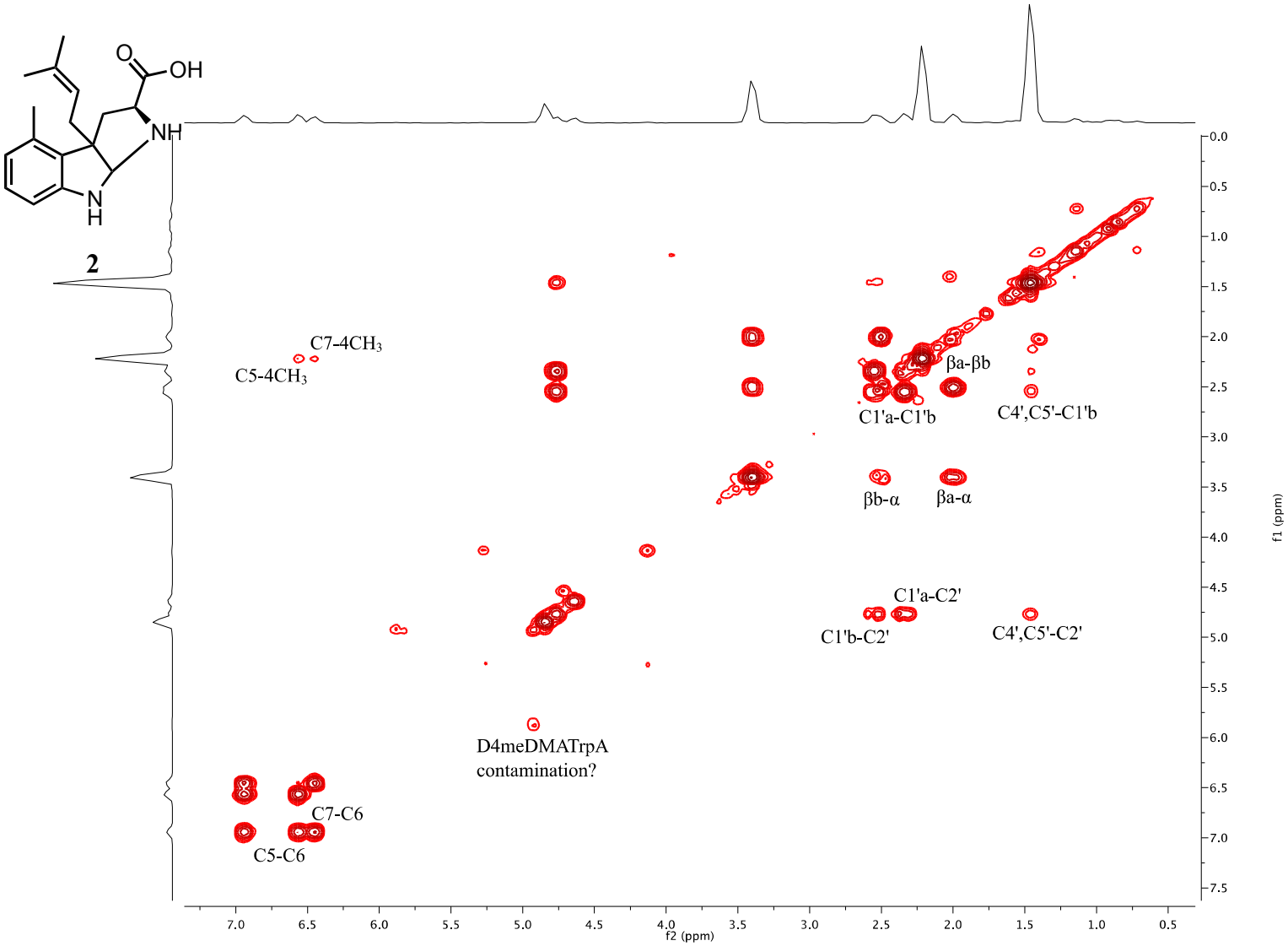


Figure S12. 2D ^{1}H-^{1}H COSY NMR spectrum of **2** in D_2O.

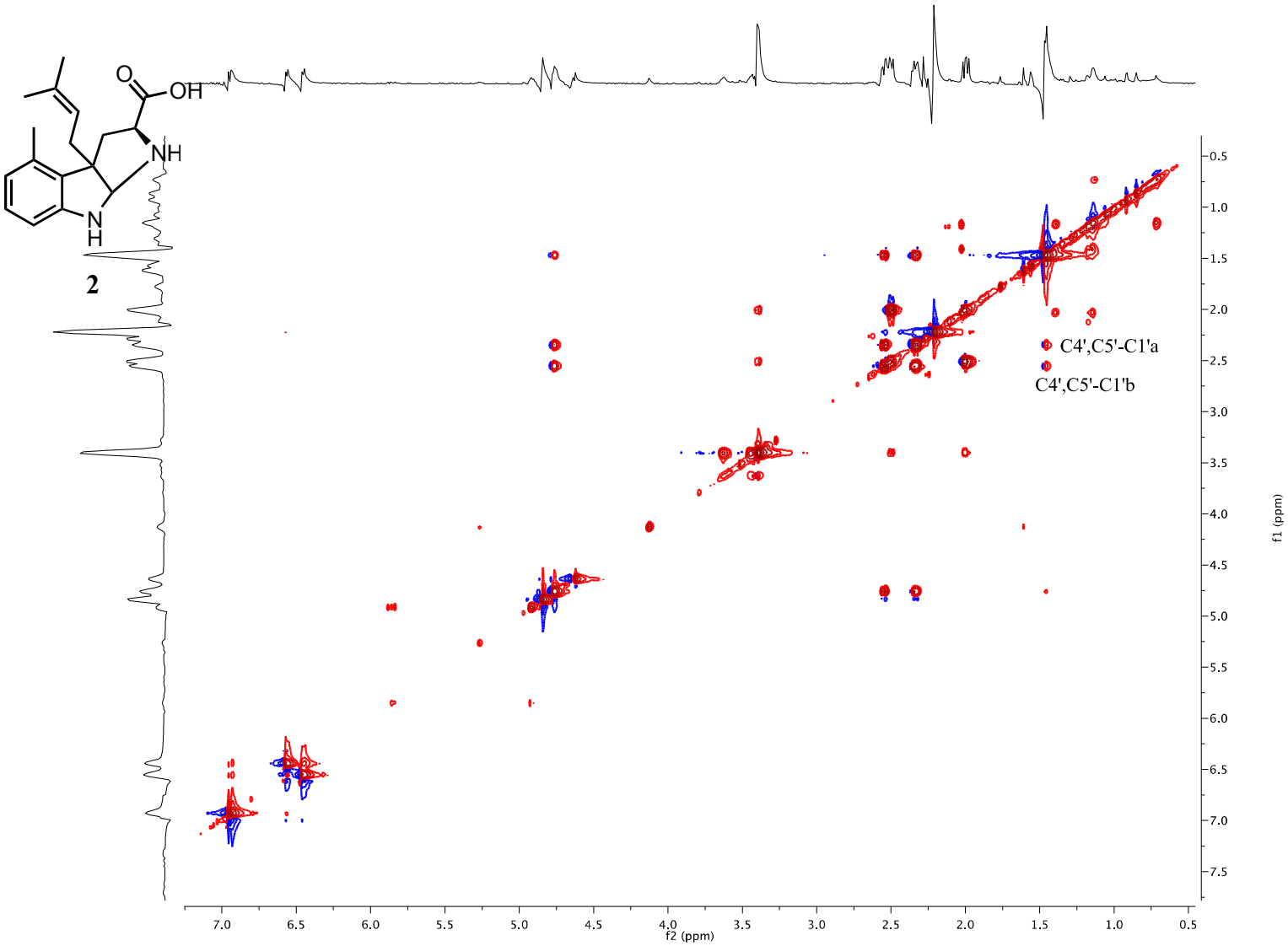


Figure S13. 2D ^{1}H-^{1}H TOCSY NMR spectrum of **2** in D_2O. Unlabeled crosspeaks are equivalent crosspeaks found in the COSY spectrum (Figure S12).

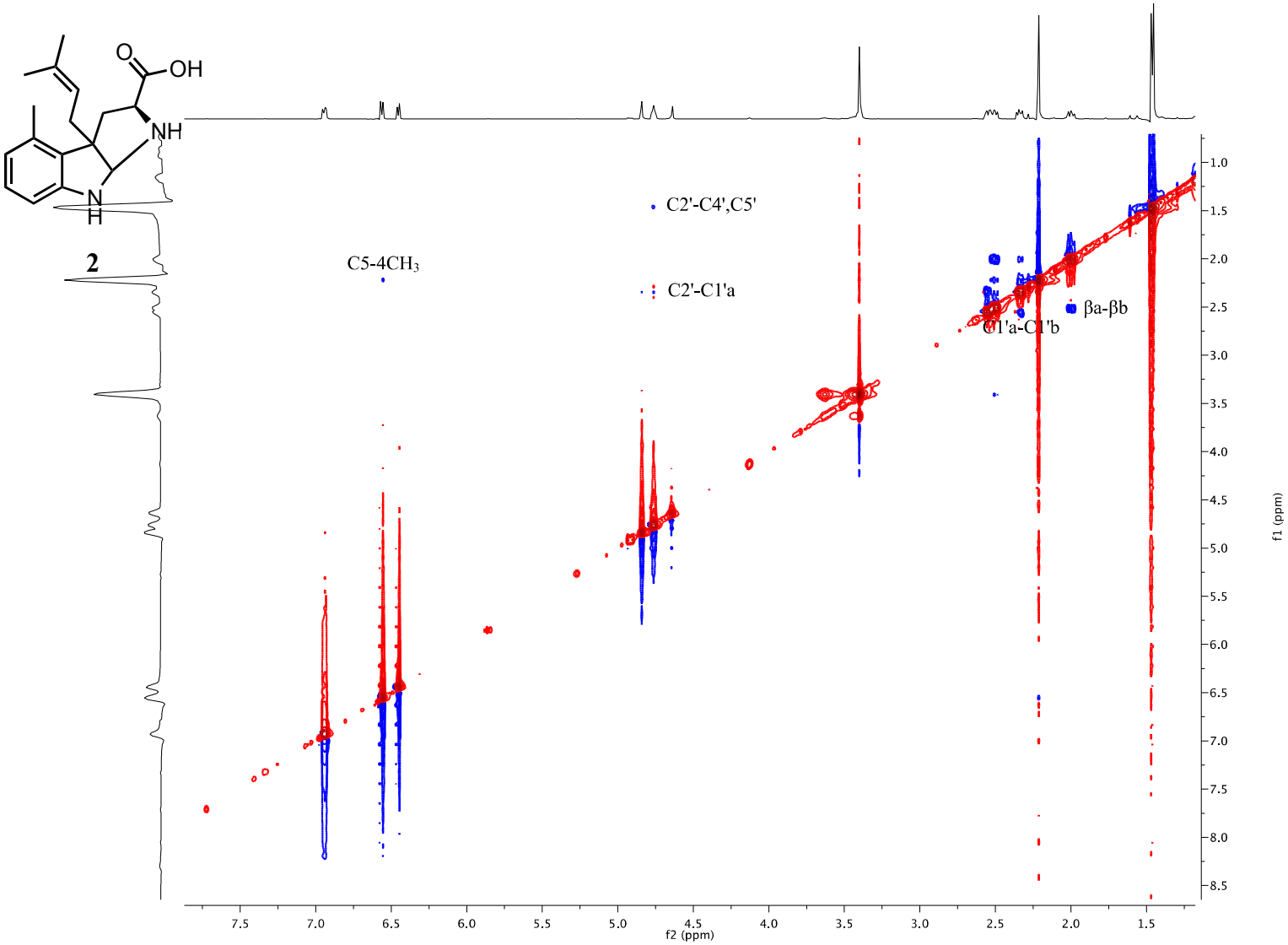


Figure S14. 2D ^{1}H-^{1}H ROESY NMR spectrum of **2** in D_2O.

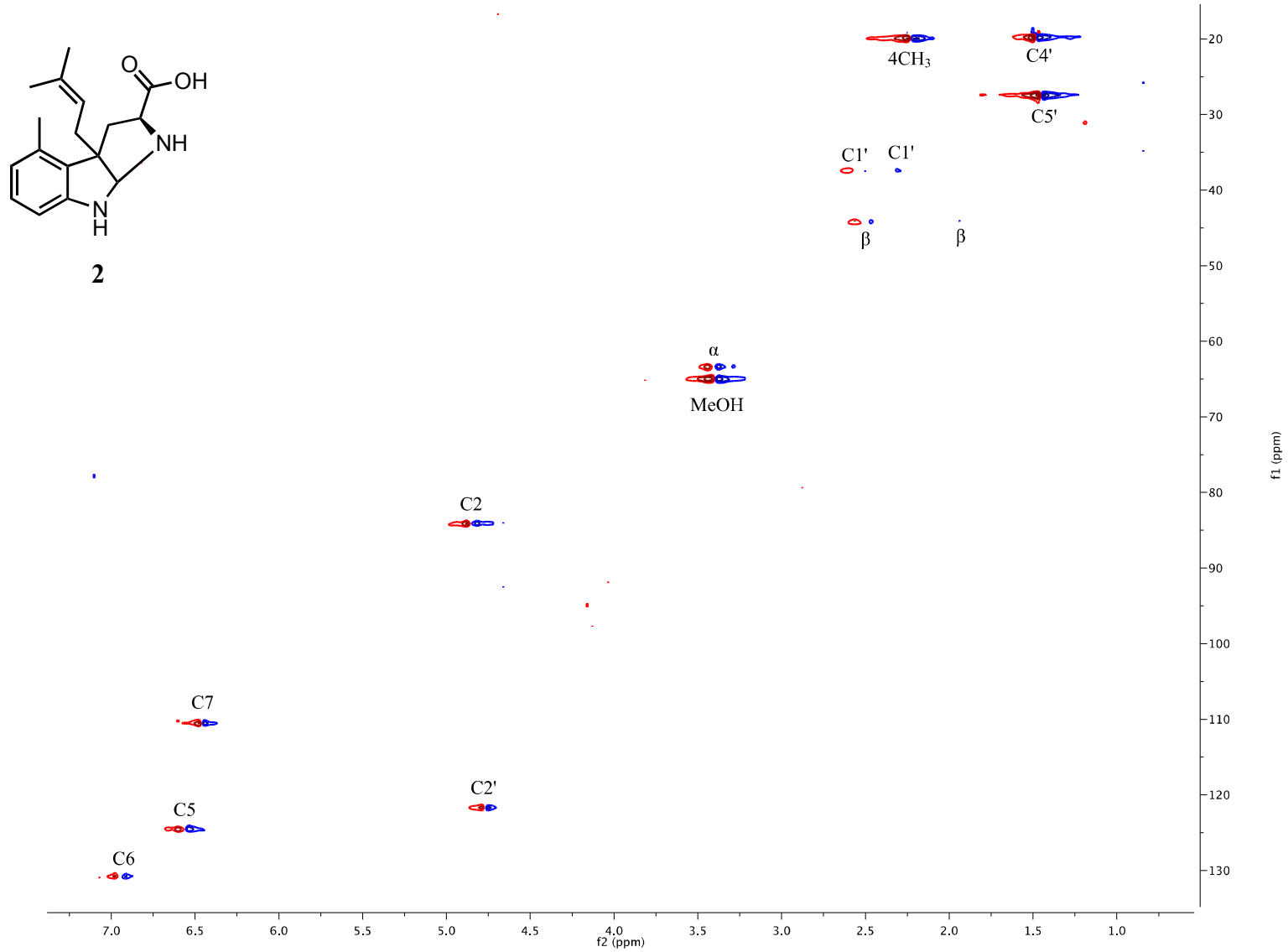


Figure S15. 2D ^{1}H-^{13}C HMQC NMR spectrum of **2** in D_2O.

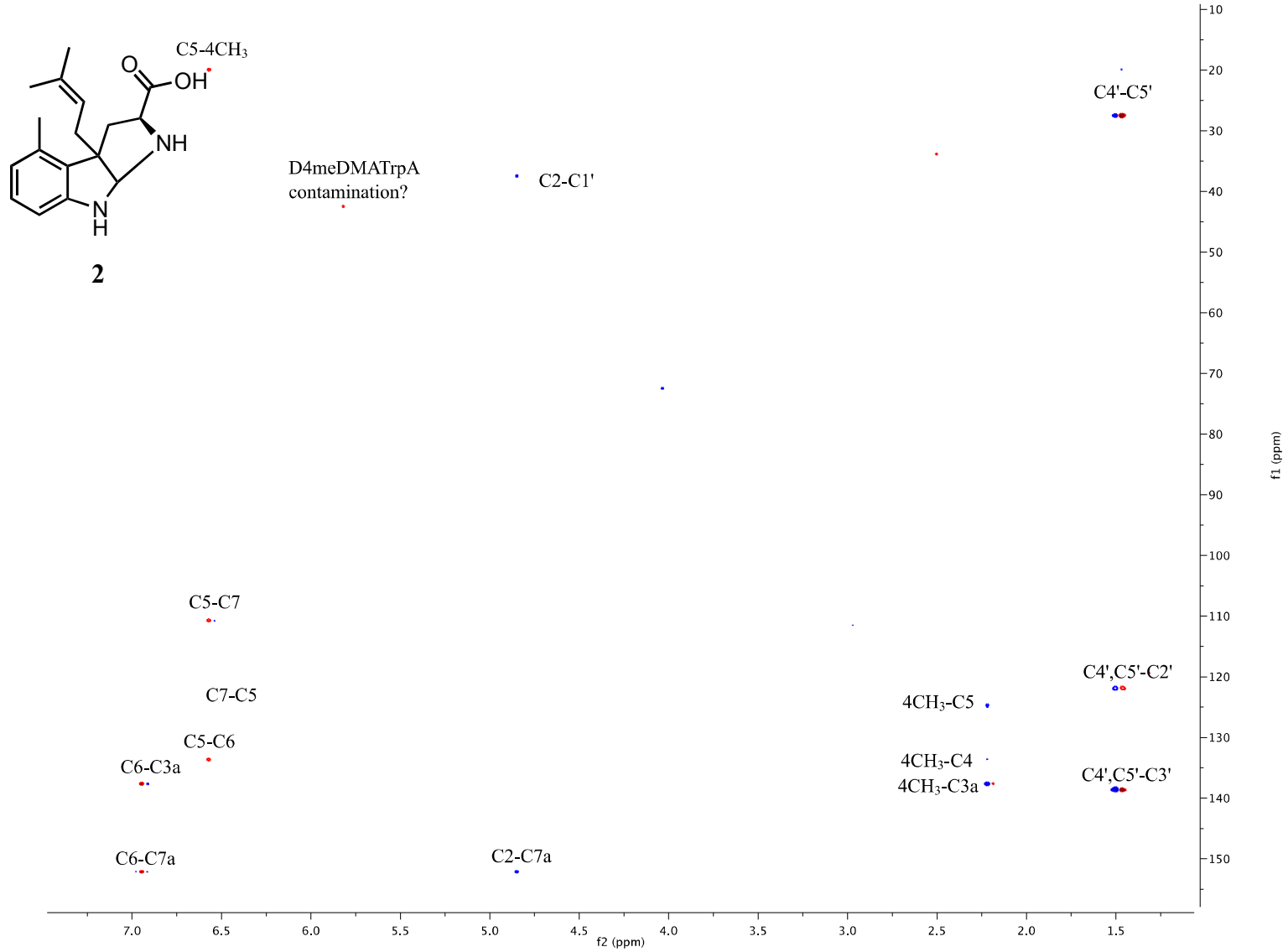
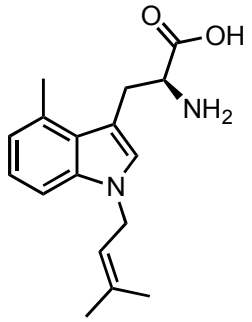


Figure S16. 2D ^{1}H-^{13}C HMBC NMR spectrum of **2** in D_2O.

Table S3. NMR data table and structure of **4**.



4

Carbon	**^{13}C (ppm)**	**^{1}H (ppm)**	**Multiplicity**	**^{1}H-^{1}H**	**HMBC**
α	59.6	3.61	dd: 4.8, 9.0 Hz	COSY: βa, βb	
β	32.4	a: 2.98	dd: 9.0, 15.0 Hz	COSY: βb, α TOCSY: βb, α ROESY: βb	α, C2
		b: 3.42	dd: 4.8, 15.0 Hz	COSY: βa, α TOCSY: βb, α ROESY: βa	C2, C3a, C7a (maybe)
COOH	n.f.	-	-	-	-
C2	130.6	7.07	s	TOCSY: βa and βb (maybe)	C3, C3a, C7a
C3	112.0	-	-	-	-
C3a	128.2	-	-	-	-
C4	133.8	-	-	-	-
4-CH_3	22.2	2.58	s	COSY: C5	C3a, C4, C5
C5	123.3	6.80	d: 7.2 Hz	COSY: 4-CH_3, C6 TOCSY: C6, C7, 4-CH_3 ROESY: C6	C3a, C7
C6	124.4	7.03	dd: 7.2, 8.4 Hz	COSY: C5, C7 TOCSY: C5, C7	C4, C7a
C7	110.7	7.19	d: 8.4 Hz	COSY: 4-CH_3, C6 TOCSY: C5, C6	C3a, C5
C7a	138.9	-	-	-	-
C1'	46.3	4.60	d: 6.6 Hz	COSY: C2' TOCSY: C2', C4', C5'	C2, C2', C3'
C2'	121.9	5.24	t: 6.6 Hz	COSY: C1', C4', C5' TOCSY: C1', C4', C5'	
C3'	140.4	-	-	-	-
C4'	19.8	1.70	s	COSY: C1', C2' TOCSY: C1', C2'	C2', C3', faint C5'
C5'	27.2	1.60	s		C2', C3', C4'

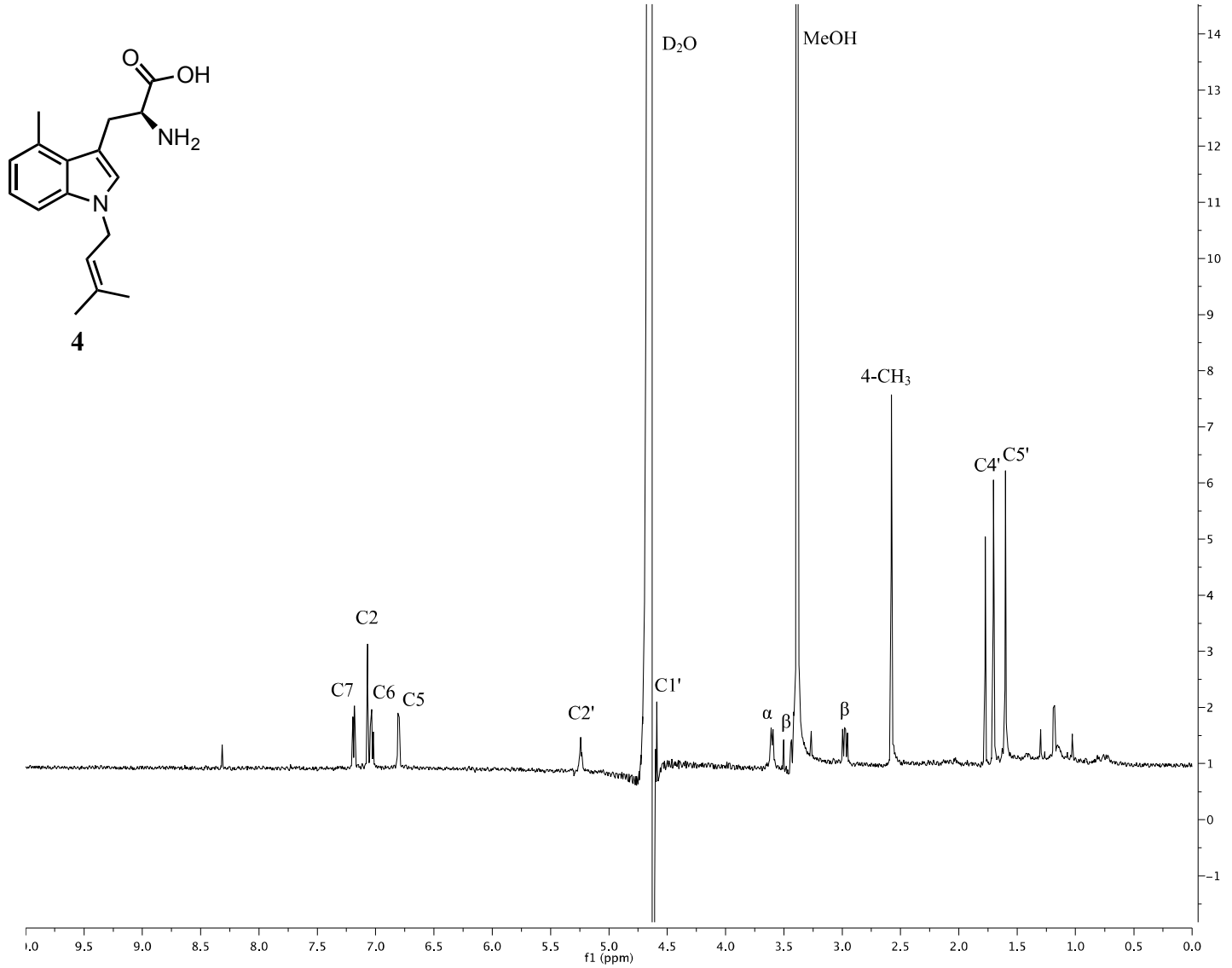


Figure S17. ^{1}H NMR spectrum of **4** in D_2O.

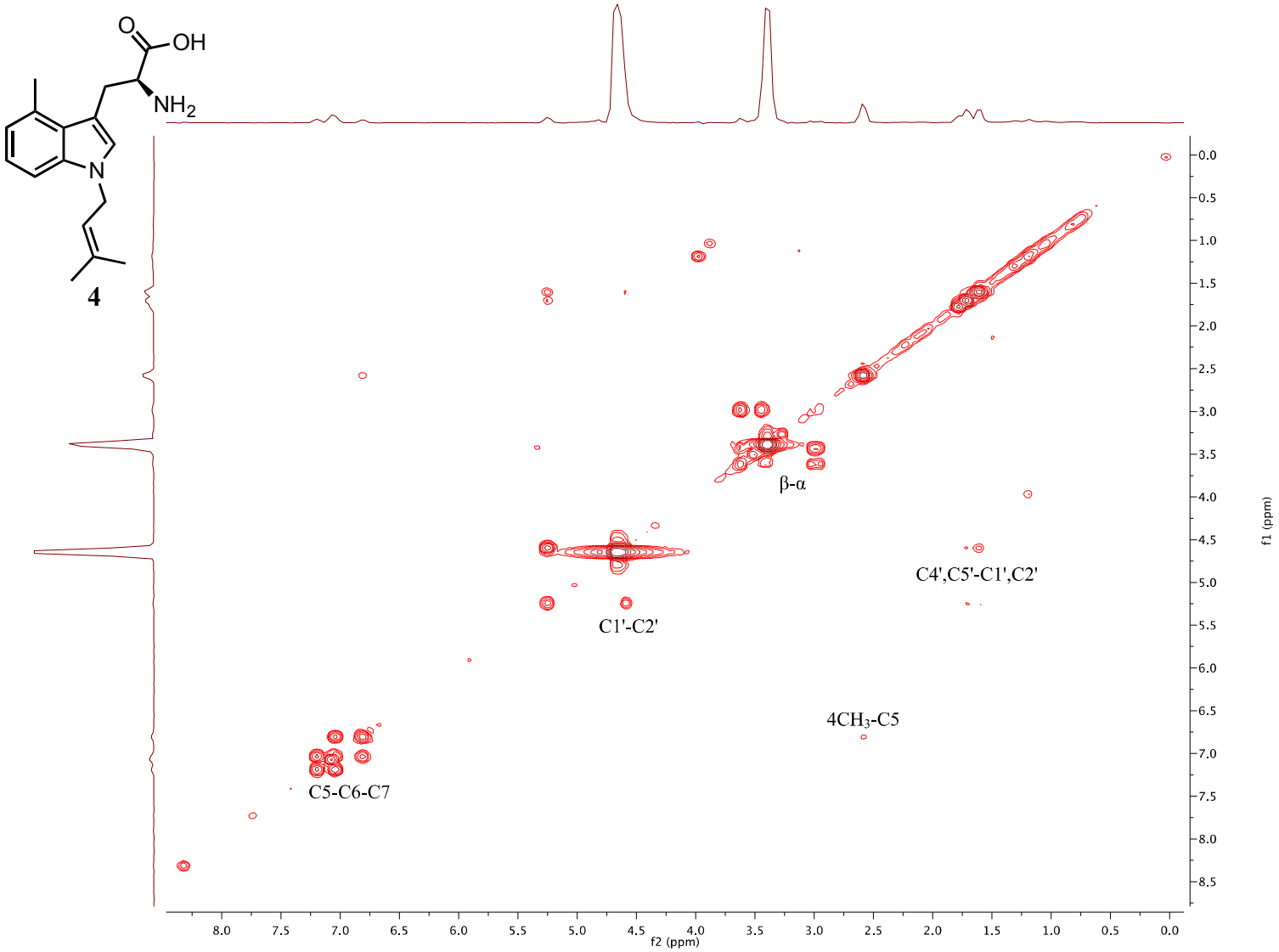


Figure S18. 2D ^{1}H-^{1}H COSY NMR spectrum of **4** in D_2O.

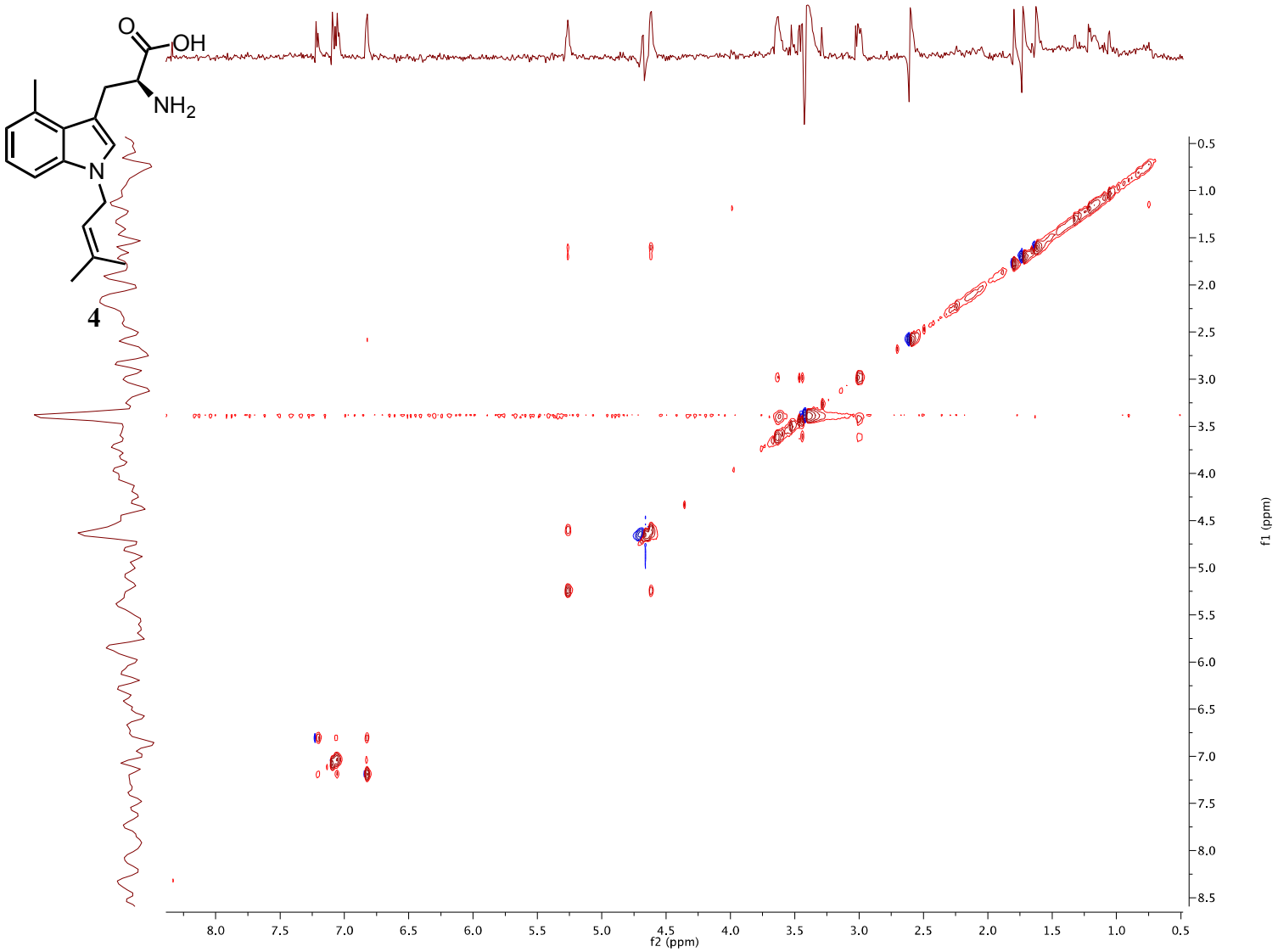


Figure S19. 2D ^{1}H-^{1}H TOCSY NMR spectrum of **4** in D_2O. Unlabeled crosspeaks are equivalent crosspeaks found in the COSY spectrum (Figure S18).

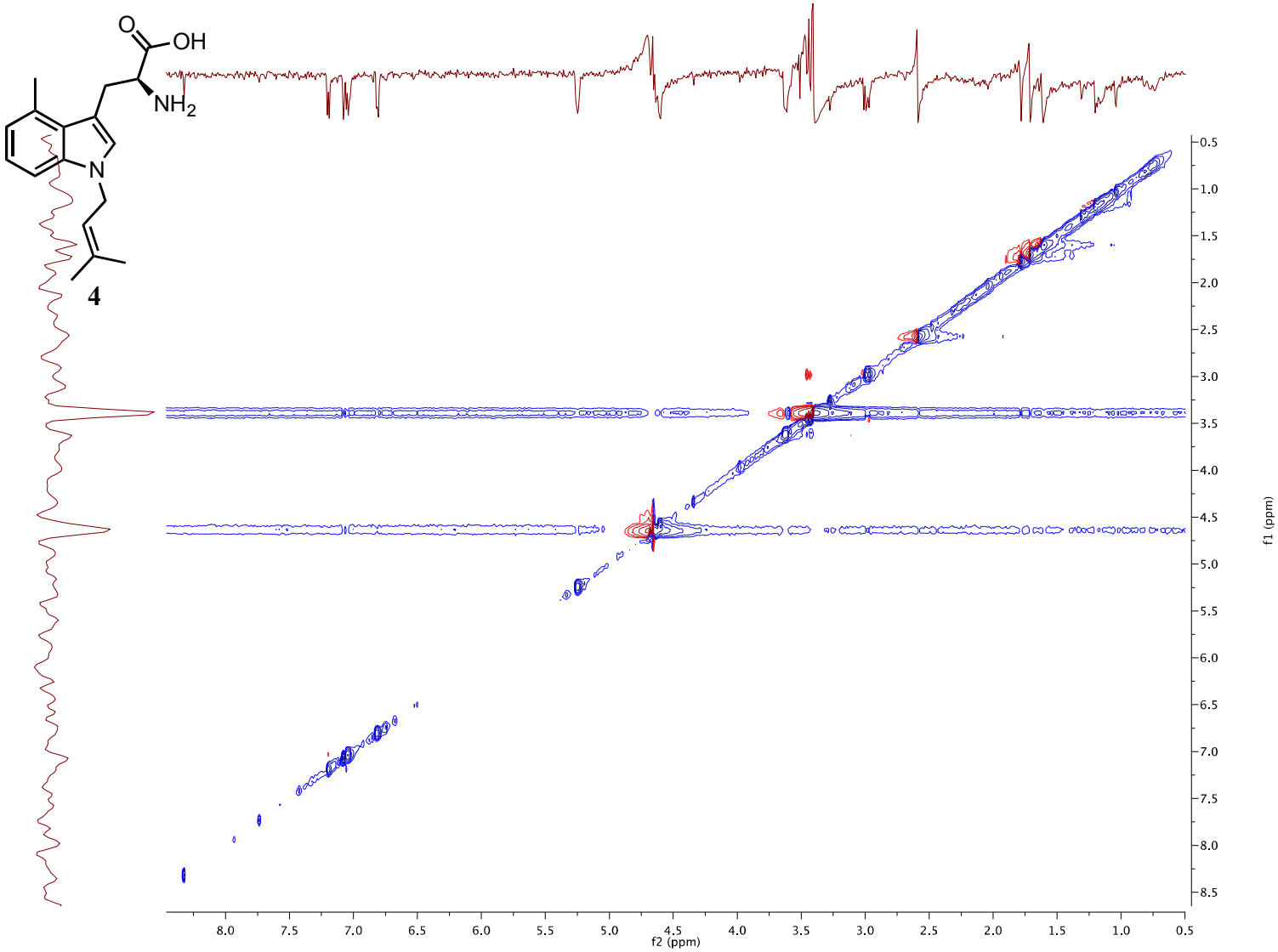


Figure S20. 2D ^{1}H-^{1}H ROESY NMR spectrum of **4** in D_2O.

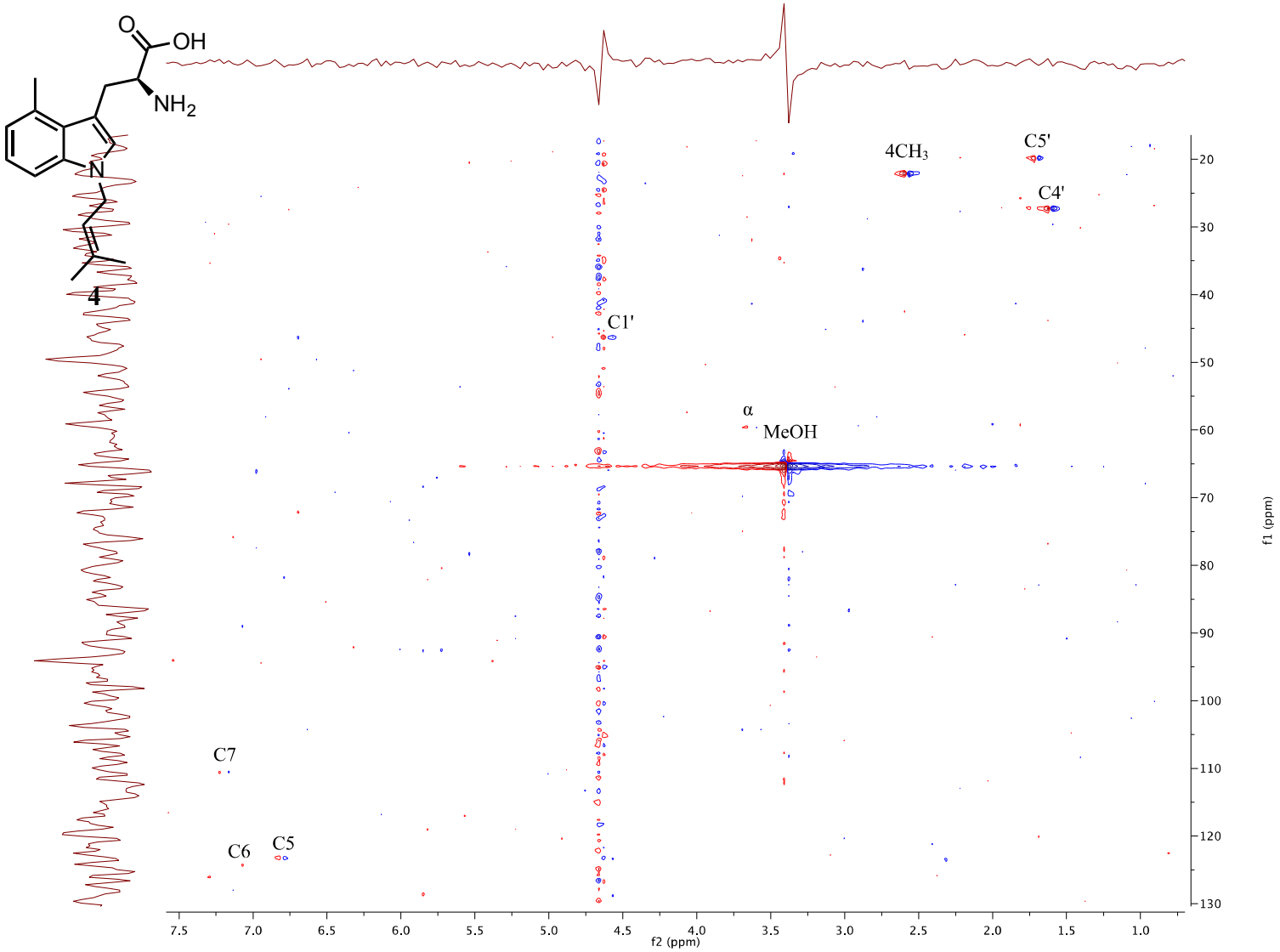


Figure S21. 2D ^{1}H-^{13}C HMQC NMR spectrum of **4** in D_2O.

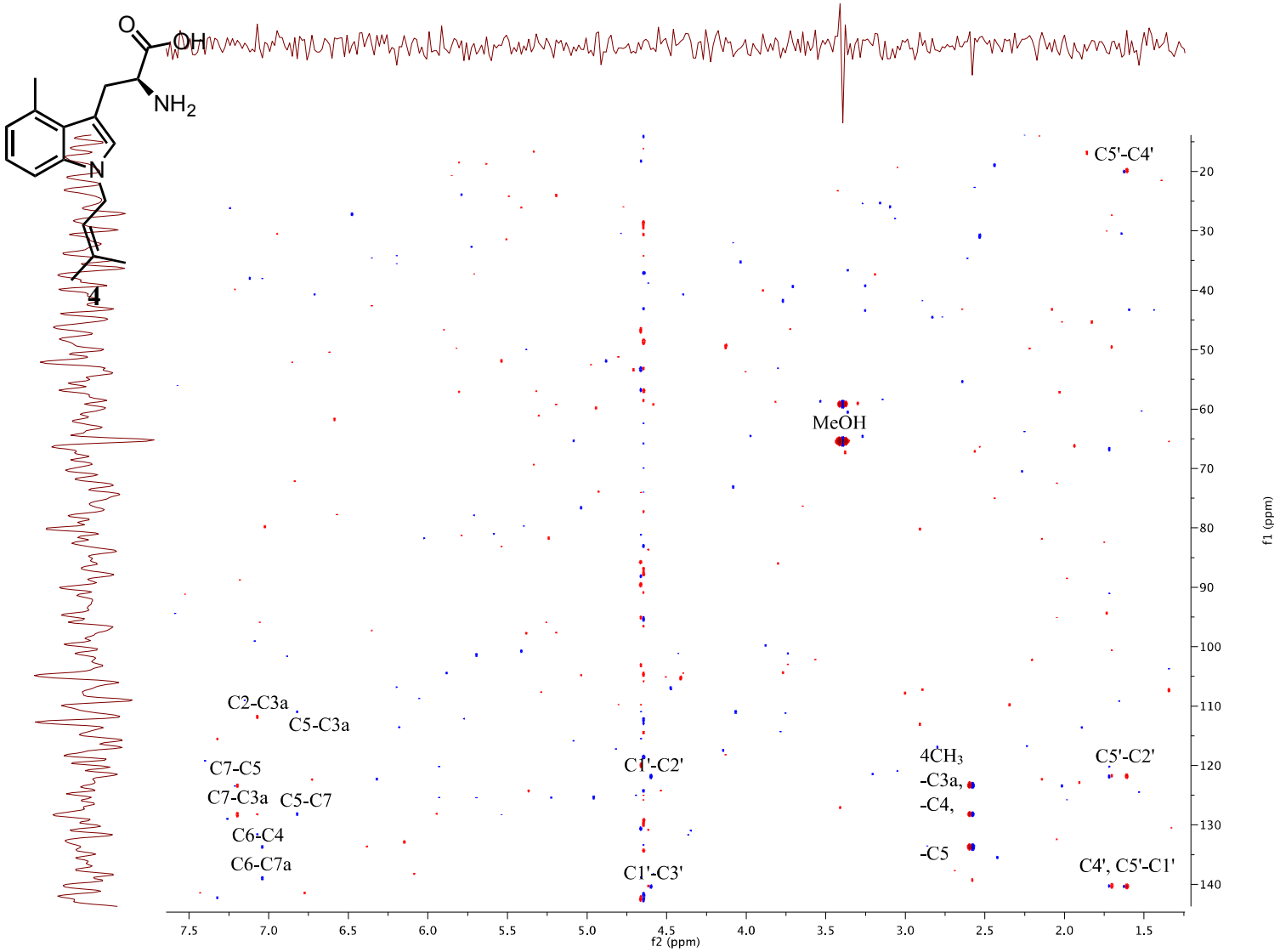
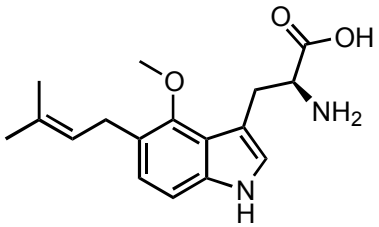


Figure S22. 2D ^{1}H-^{13}C HMBC NMR spectrum of **4** in D_2O.

Table S4. NMR data table and structure of **7**.



7

Carbon	**^{13}C (ppm)**	**^{1}H (ppm)**	**Multiplicity**	**^{1}H-^{1}H**	**HMBC**
α	40.34?	3.87	dd: 4.8, 9.0 Hz	COSY: βa, βb TOCSY: βa, βb	N/A
β	39.18?	a: 3.06	dd: 9.0, 15.0 Hz	COSY: α, βb TOCSY: α	N/A
		b: 3.41-3.44	m (shielded by MeOH)	N/A	N/A
COOH	n.f.	-	-	-	-
C2	127.8	7.10	s	TOCSY: βa	N/A
C3	n.f.	-	-	-	-
C3a	n.f.	-	-	-	-
C4	n.f.	-	-	-	-
4-OCH_3	65.1	3.79	s	N/A	N/A
C5	n.f.	-	-	-	-
C6	126.8	6.98	d: 8.4 Hz	COSY: C7 TOCSY: C7	N/A
C7	111.7	7.15	d: 8.4 Hz	COSY: C6 TOCSY: C6	N/A
C7a	n.f.	-	-	-	-
C1'	29.7	3.35	d: 7.2 Hz	COSY: C2', C4', C5' TOCSY: C2', C4', C5'	C2'
C2'	126.2	5.25	t: 7.2 Hz	COSY: C1', C4', C5' TOCSY: C1', C4', C5'	N/A
C3'	136.3	-	-	-	-
C4'	19.8	1.67	s	COSY: C1', C2' TOCSY: C1', C2'	C2', C3', C5'
C5'	27.5	1.60	s		C2, C3'

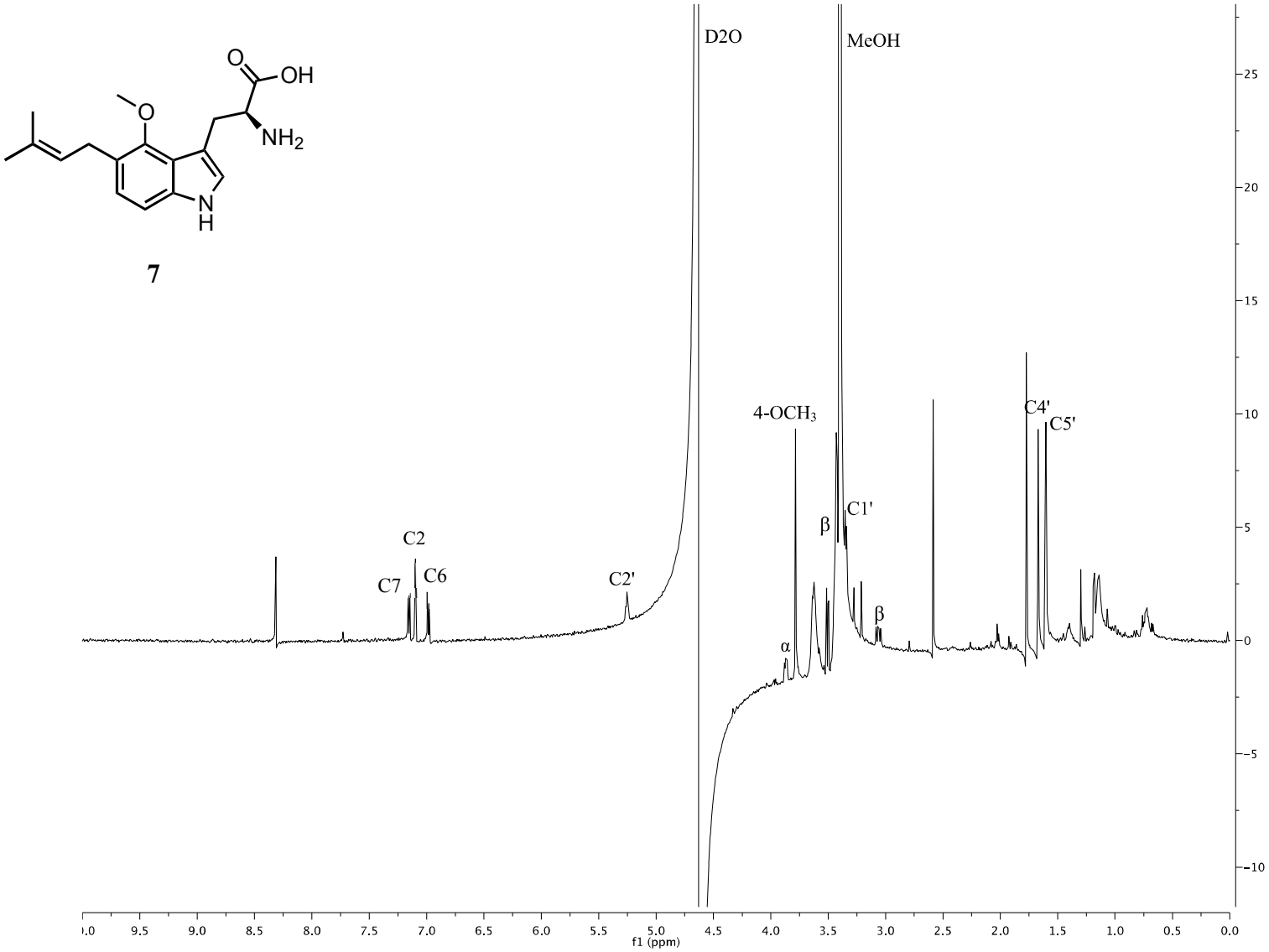


Figure S23. ^{1}H-NMR spectrum of **7** in D_2O.

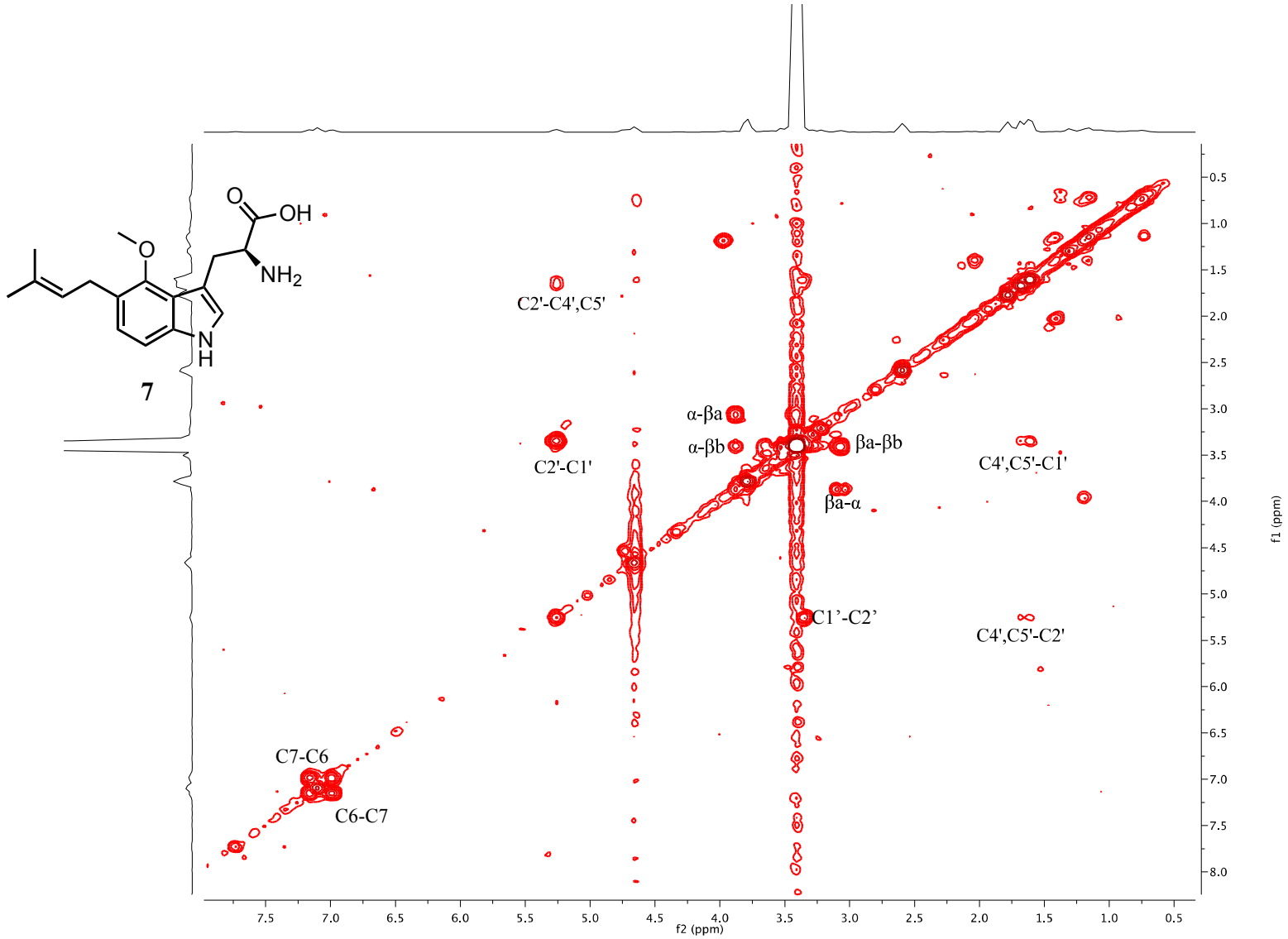


Figure S24. 2D ^{1}H-^{1}H COSY NMR spectrum of **7** in D_2O.

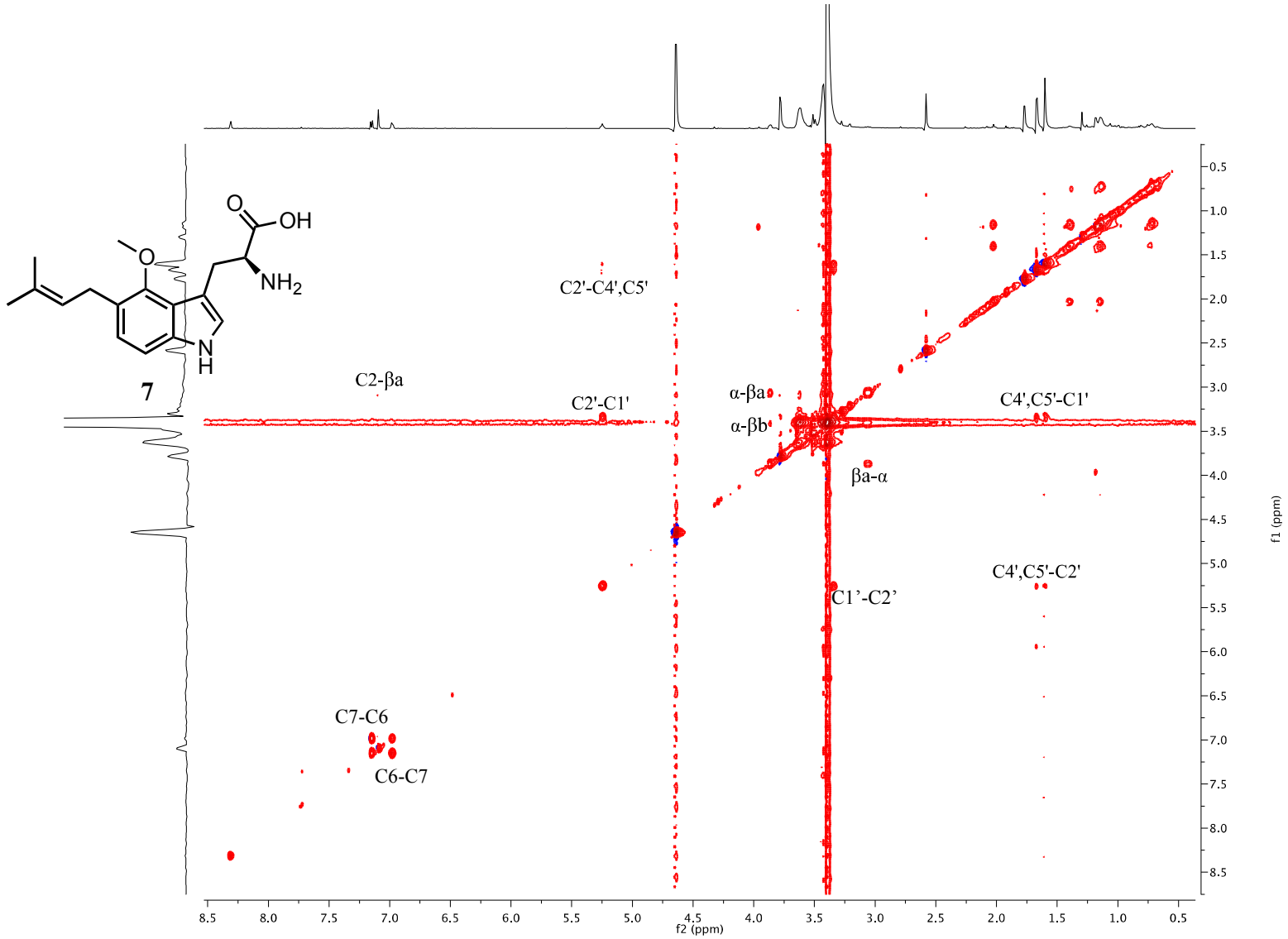


Figure S25. 2D ^{1}H-^{1}H TOCSY NMR spectrum of **7** in D_2O.

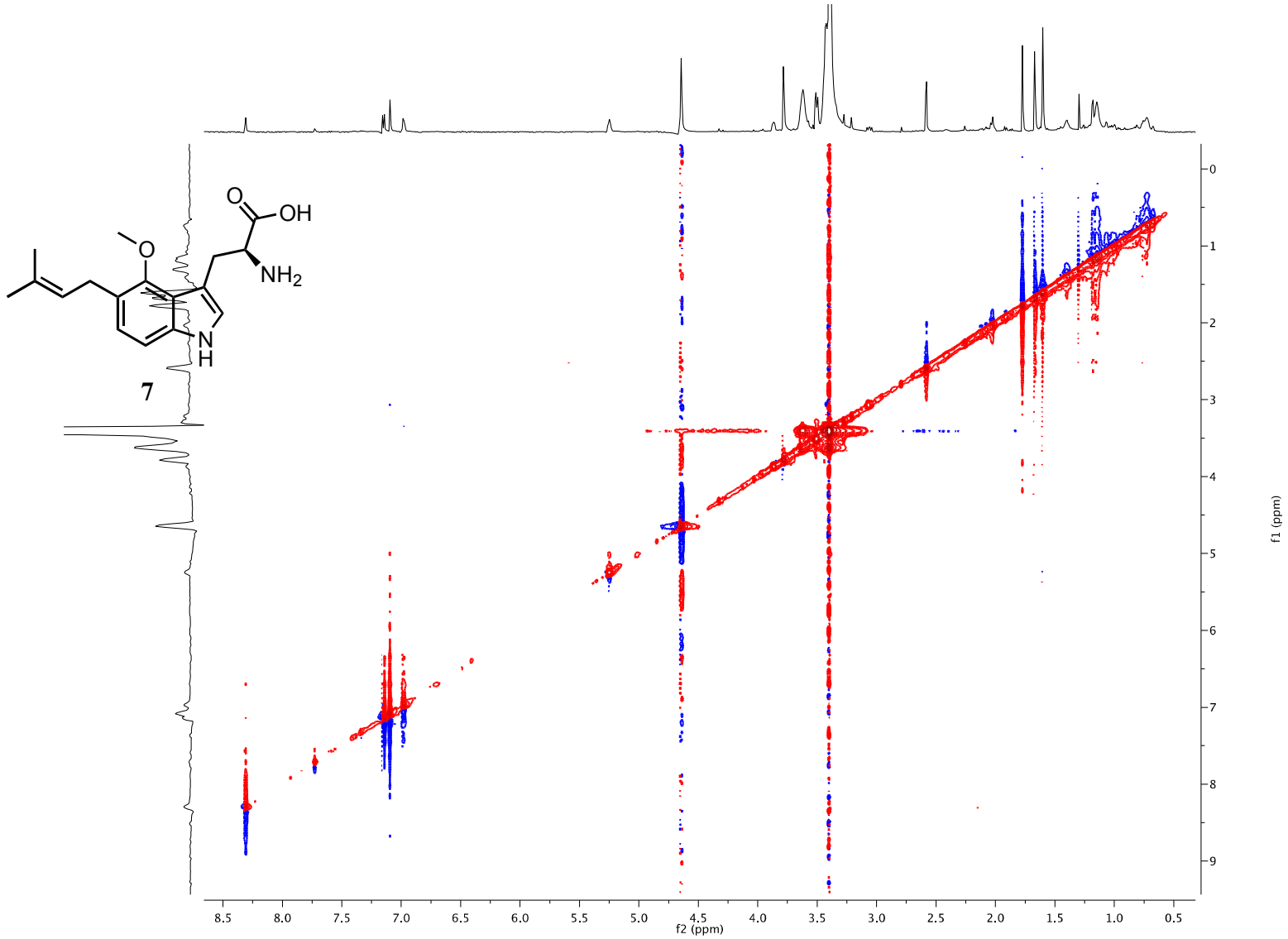


Figure S26. 2D ^{1}H-^{1}H ROESY NMR spectrum of **7** in D_2O.

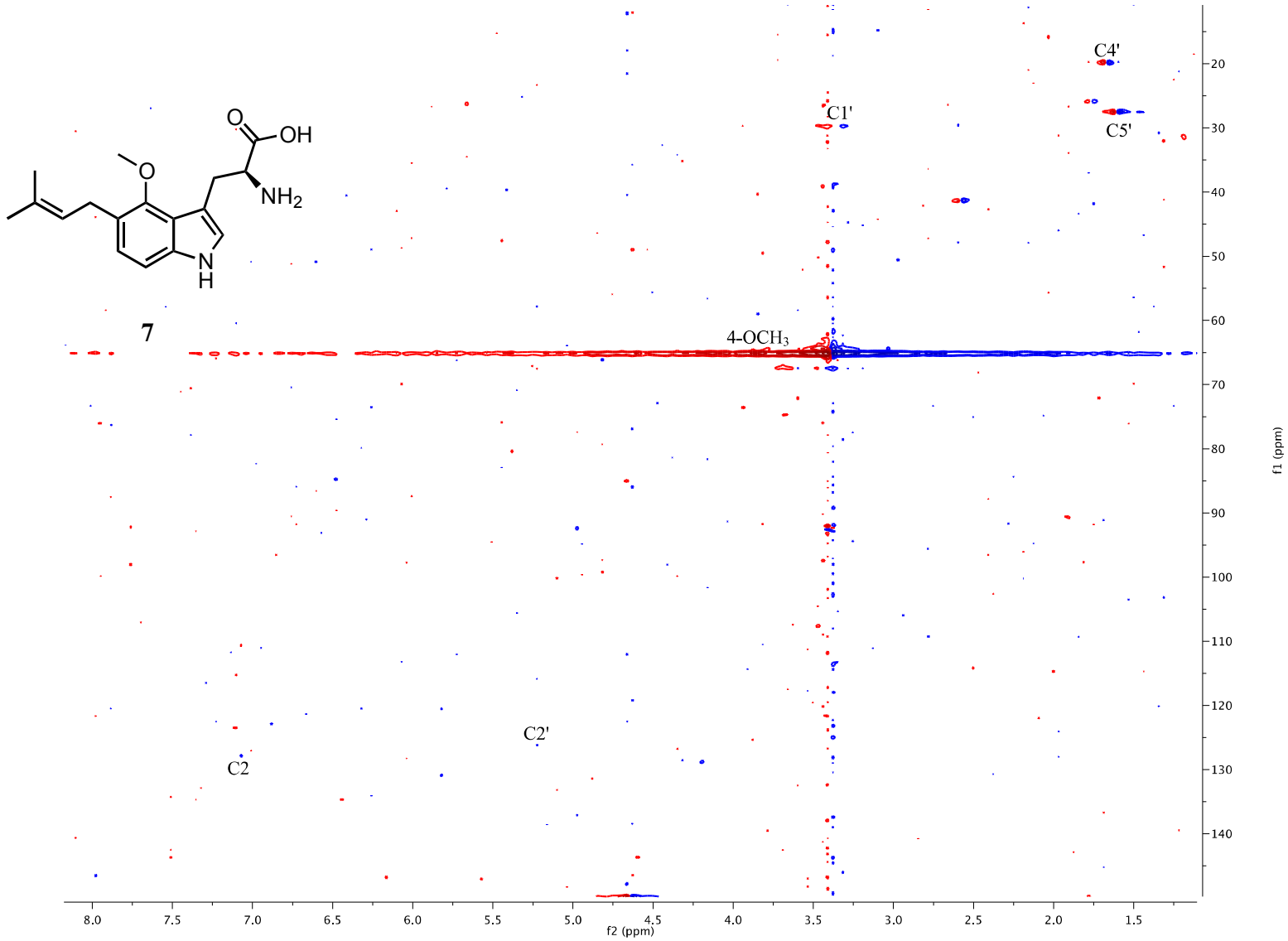


Figure S27. 2D ^{1}H-^{13}C HMQC NMR spectrum of **7** in D_2O.

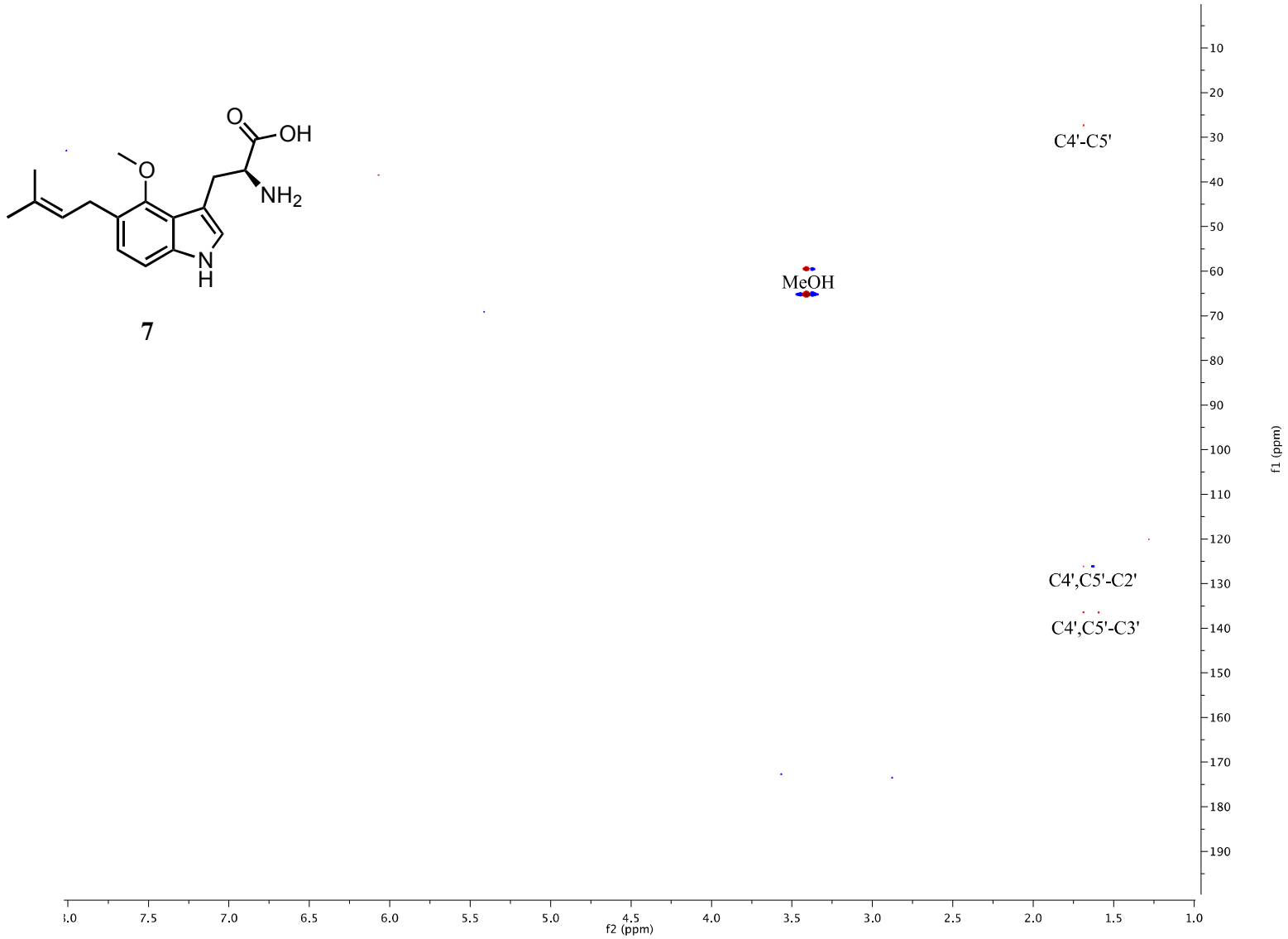
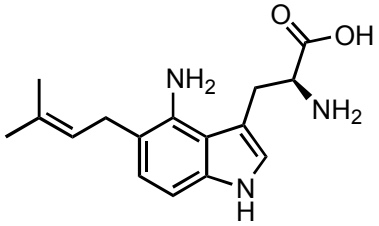


Figure S28. 2D ^{1}H-^{13}C HMBC NMR spectrum of **7** in D_2O.

Table S5. NMR data table and structure of **9**. Except where mentioned below, TOCSY peaks were identical to COSY peaks.



9

Carbon	**^{13}C (ppm)**	**^{1}H (ppm)**	**Multiplicity**	**^{1}H-^{1}H**	**HMBC**
α	58.7	3.77-3.82	m	COSY: βa, βb	N/A
β	31.0	a: 3.17	dd: 8.4, 15.6 Hz	COSY: α, βb	α, C2, C3, C3a
		b: 3.42-3.47	m (shielded by MeOH)	COSY: α, βa	α
COOH	n.f.	-	-	-	-
C2	127.3	7.02	s	COSY: βb TOCSY: α, βa, βb	C3, C3a, C7a
C3	109.9	-	-	-	-
C3a	120.0	-	-	-	-
C4	138.4	-	-	-	-
C5	n.f.	-	-	-	-
C6	127.0	6.87	d: 8.4 Hz	ROESY: C1'	C3a, C4, C7a
C7	107.0	6.89	d: 8.4 Hz	N/A	
C7a	139.4	-	-	-	-
C1'	31.9	3.24	d: 7.2 Hz	COSY: C2', C4', C5'	C3a, C4, C6, C2', C3'
C2'	125.0	5.19	t: 7.2 Hz	COSY: C1', C4', C5'	C4', C5'
C3'	136.8	-	-	-	-
C4'	19.6	1.66	s	COSY: C1', C2'	C2', C3', C5'
C5'	27.4	1.61	s		C2', C3', C4'

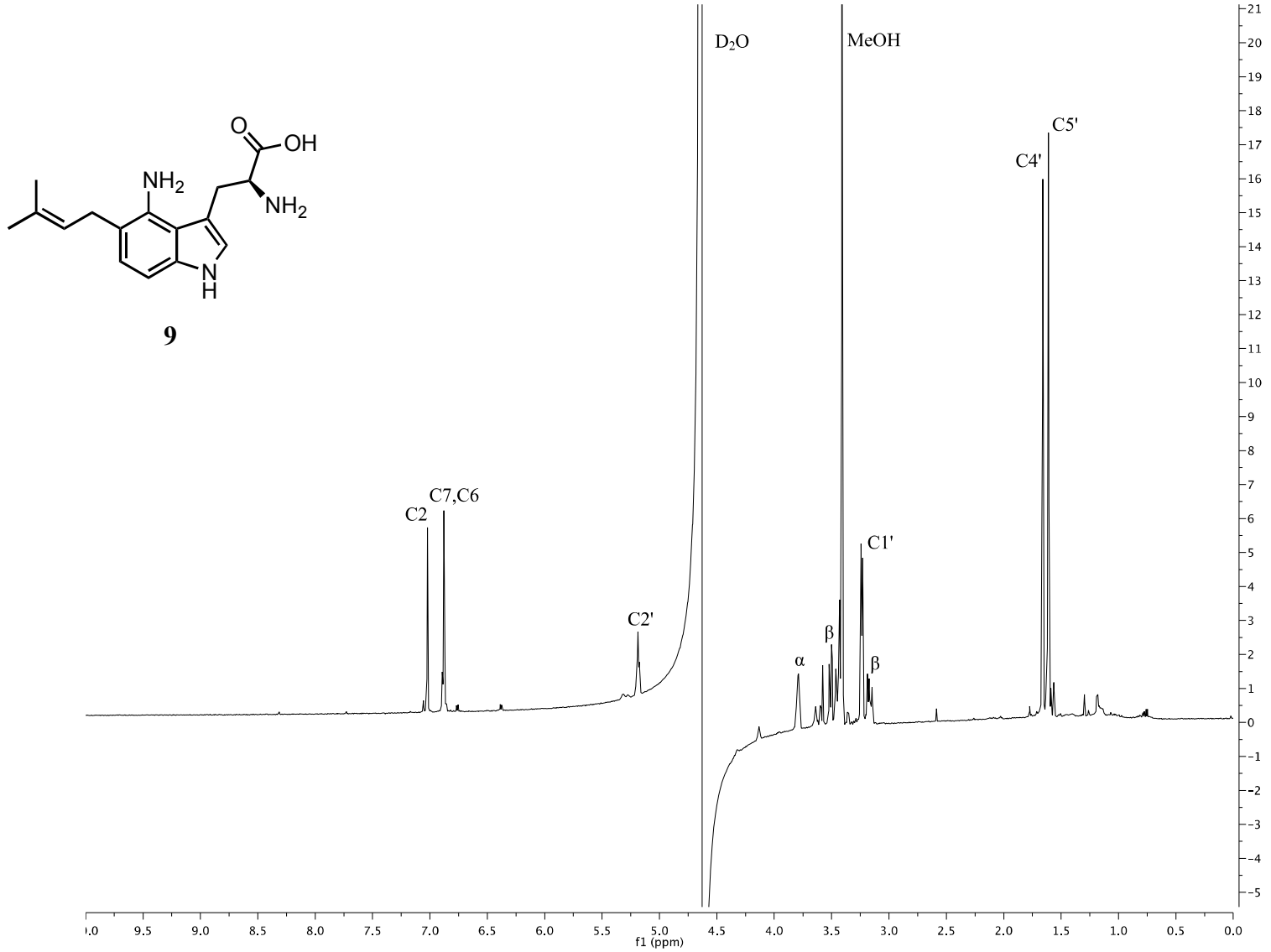


Figure S29. ^{1}H NMR spectrum of **9** in D_2O.

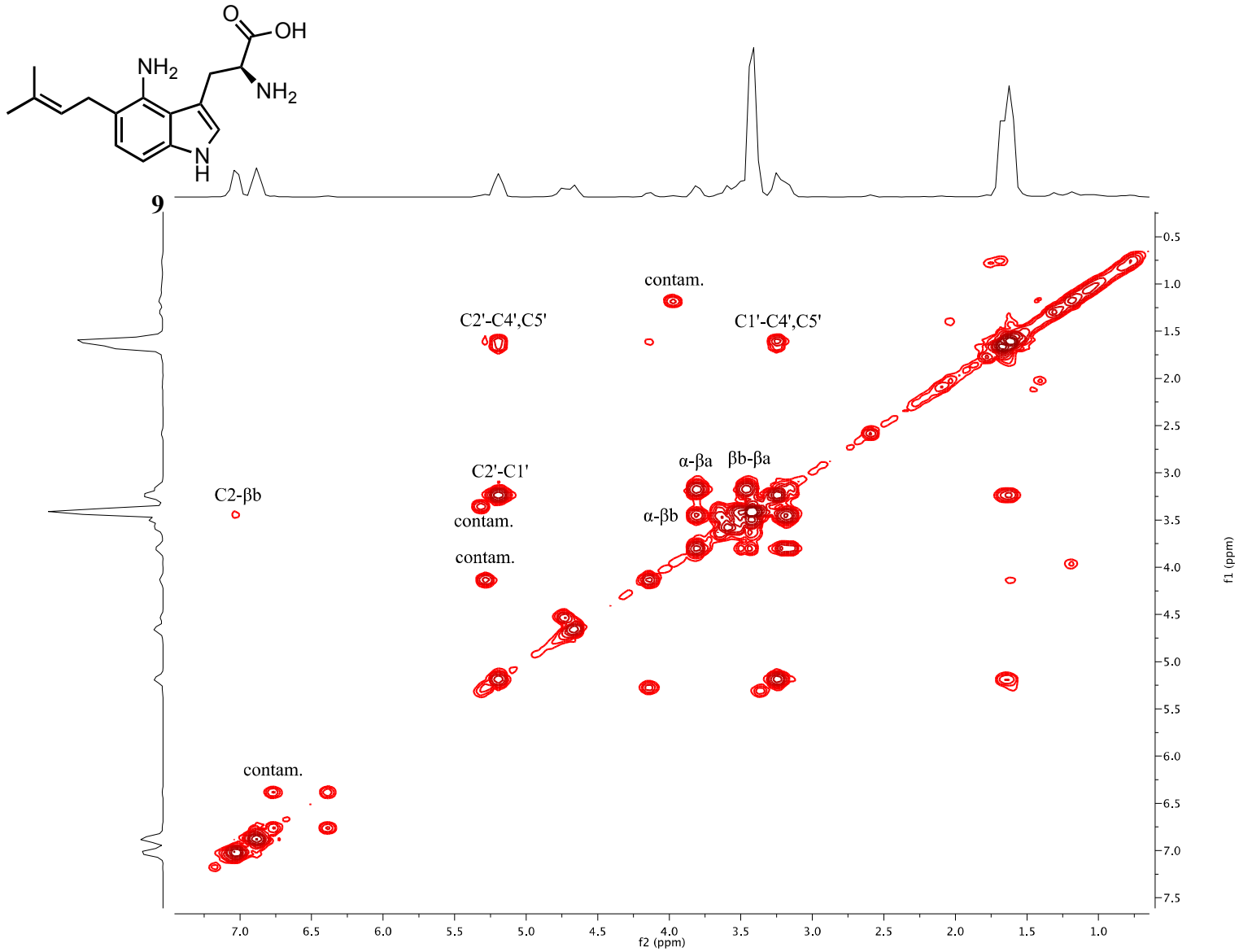


Figure S30. 2D ^{1}H-^{1}H COSY NMR spectrum of **9** in D_2O. Crosspeaks labeled "contam." were determined to be **10**.

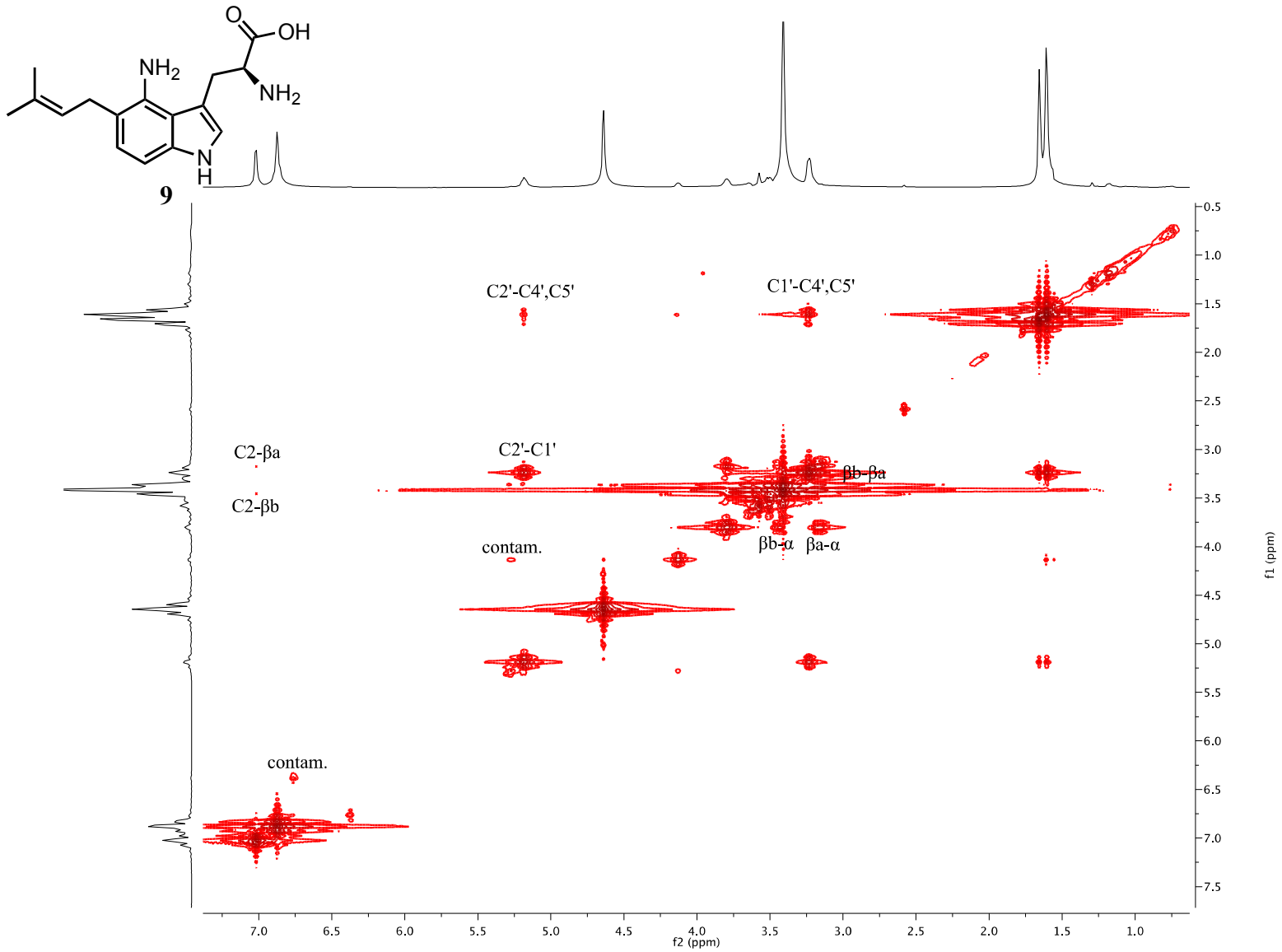


Figure S31. 2D ^{1}H-^{1}H TOCSY NMR spectrum of **9** in D_2O. Crosspeaks labeled "contam." were determined to be **10**.

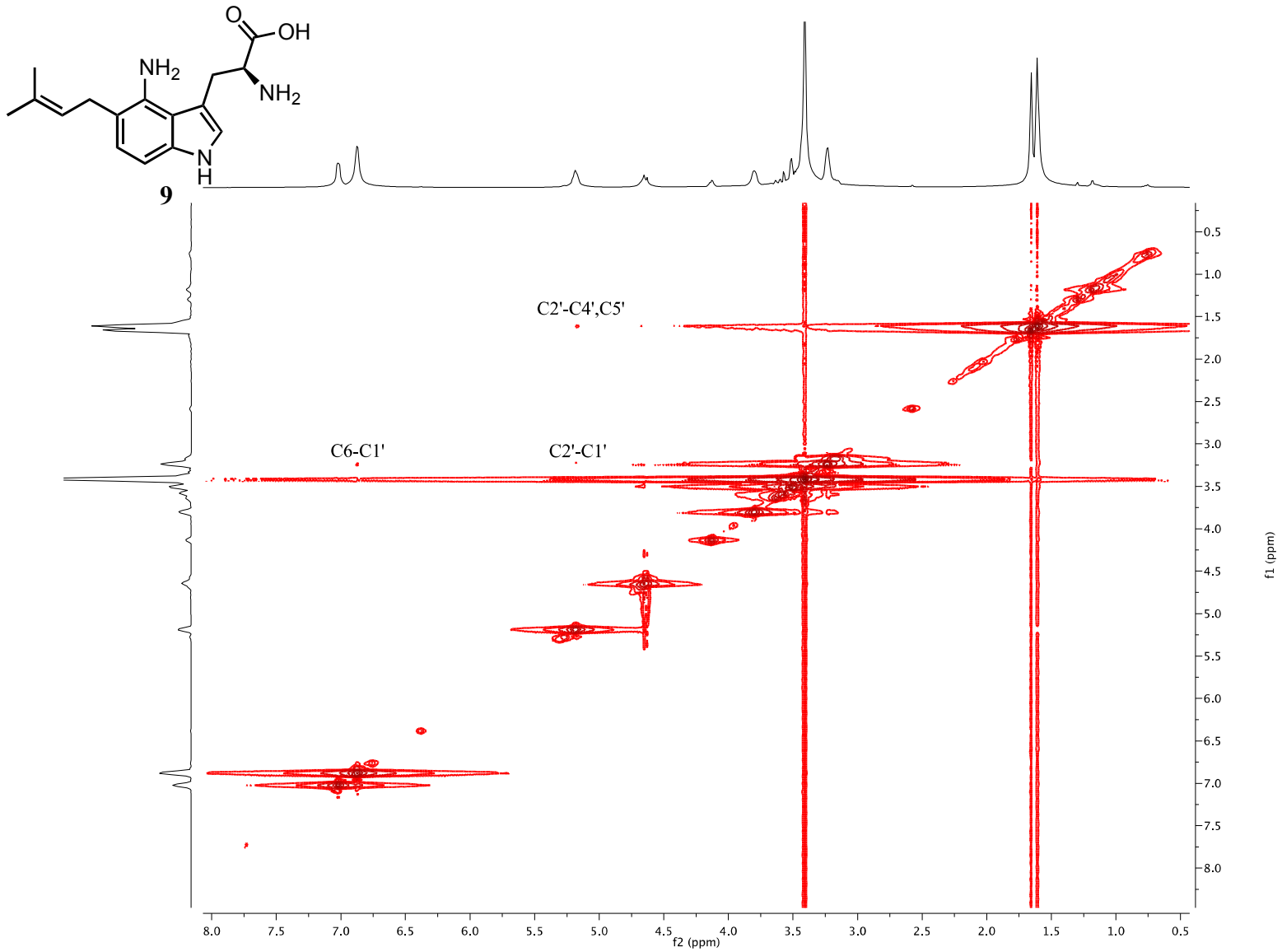


Figure S32. 2D ^{1}H-^{1}H ROESY NMR spectrum of **9** in D_2O.

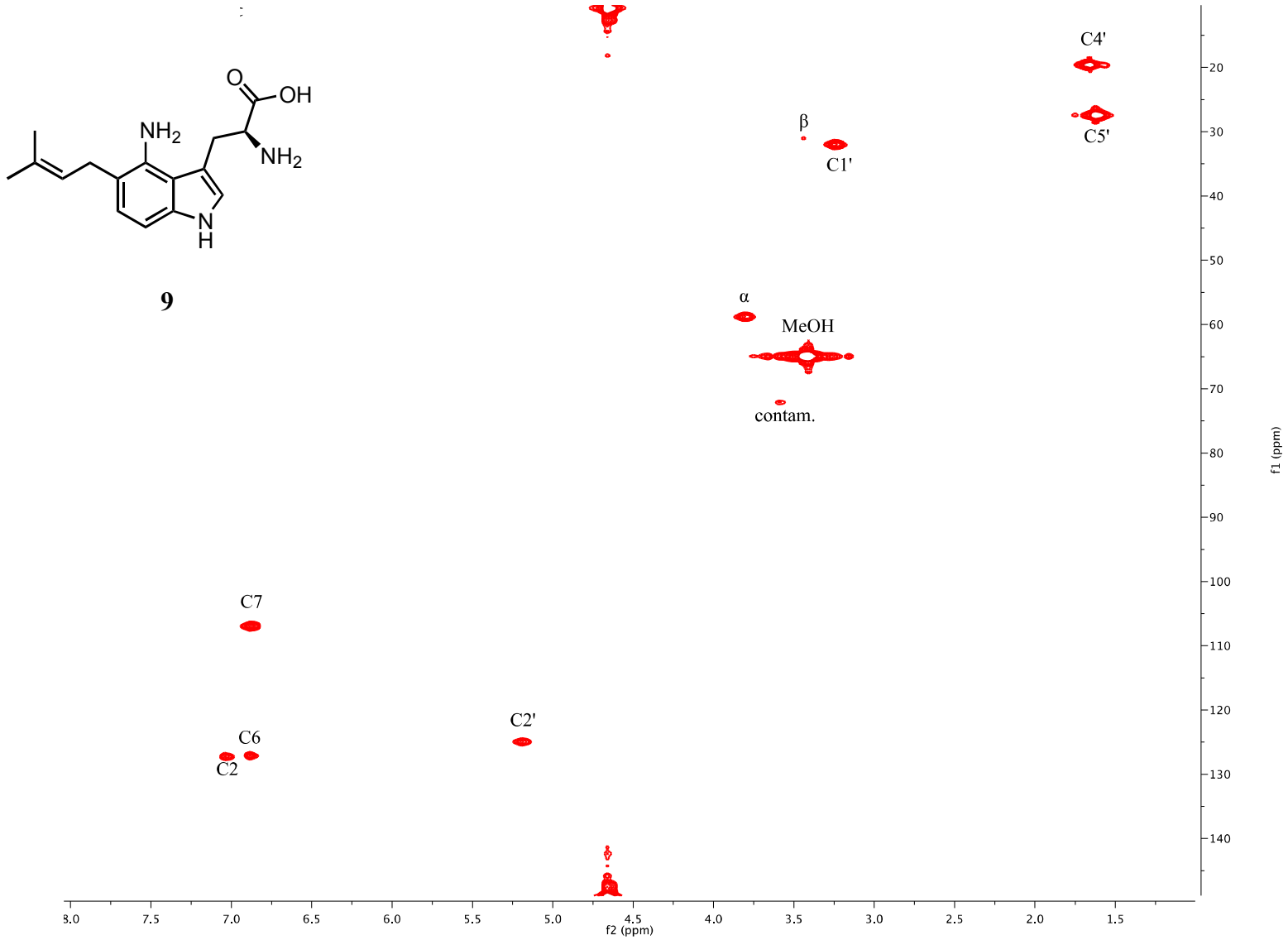


Figure S33. 2D ^{1}H-^{13}C HMQC NMR spectrum of **9** in D_2O.

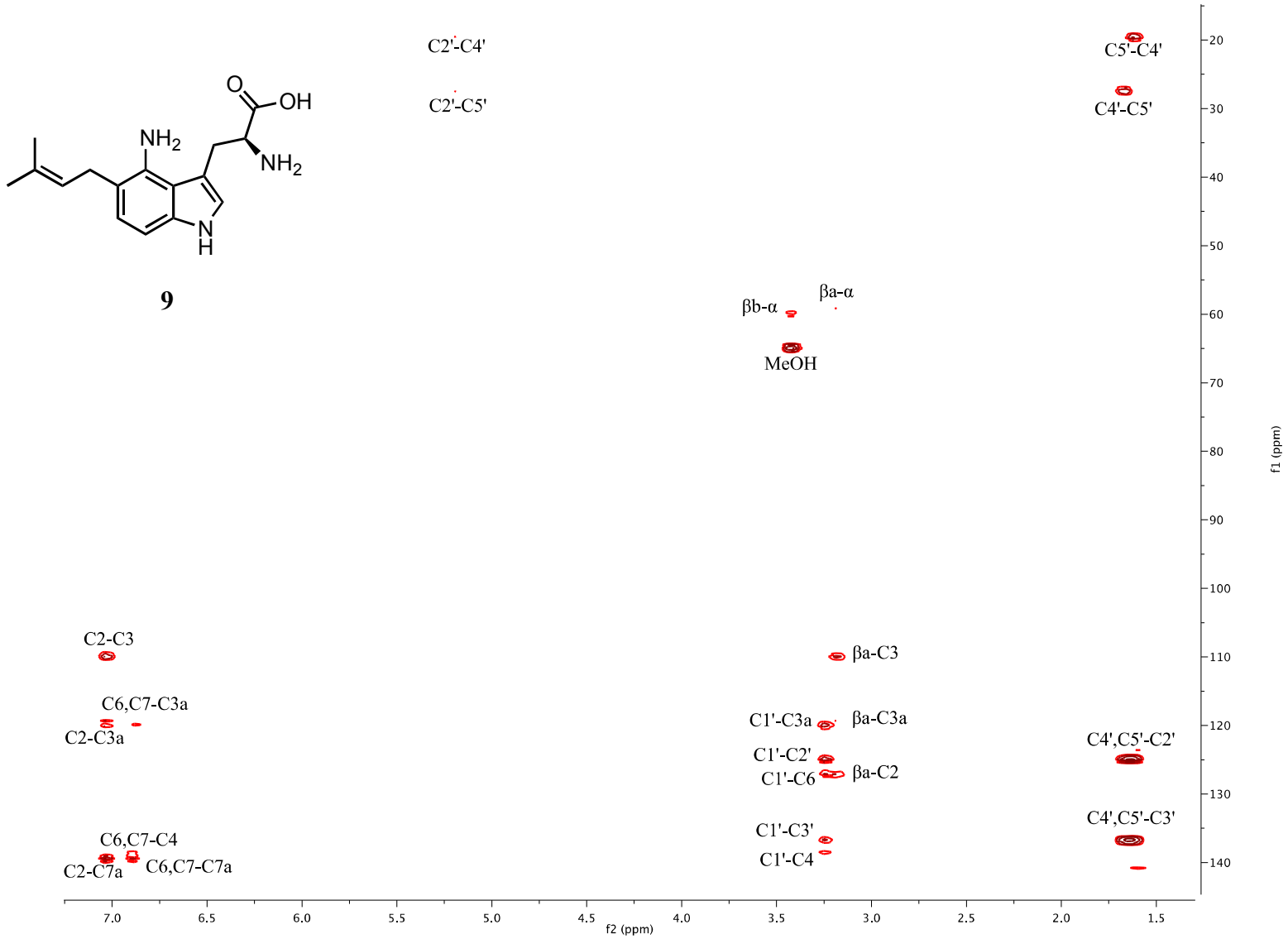
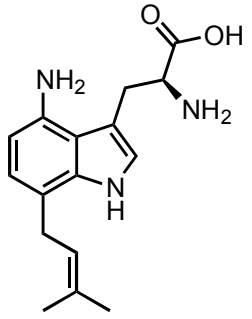


Figure S34. 2D ^{1}H-^{13}C HMBC NMR spectrum of **9** in D_2O.

Table S6. NMR data table and structure of **10**. Except where mentioned below, TOCSY peaks were identical to COSY peaks.

Carbon	**^{13}C (ppm)**	**^{1}H (ppm)**	**Multiplicity**	**^{1}H-^{1}H**	**HMBC**
α	58.9	3.68-3.72	m	COSY: βa, βb	N/A
β	31.4	a: 3.15	dd: 7.2, 15.0 Hz	COSY: α, βb	α, C2, C3, C3a
		b: 3.34-3.40	m (shielded by MeOH)	COSY: α, βa	N/A
COOH	n.f.	-	-	-	-
C2	126.6	7.05	s	TOCSY: α, βa	C3, C3a, C7a
C3	111.3	-	-	-	-
C3a	119.9	-	-	-	-
C4	139.9	-	-	-	-
C5	110.1	6.36	d: 7.2 Hz	COSY: C6	C3a, C7
C6	124.1	6.75	d: 7.2 Hz	COSY: C5, C1'	C4, C7a, C1'
C7	120.3	-	-	-	-
C7a	138.9	-	-	-	-
C1'	31.2	3.35	d: 7.8 Hz	COSY: C2', C4', C5'	C7, C7a, C2', C3'
C2'	124.2	5.31	t: 7.8 Hz	COSY: C1', C4', C5'	C4'
C3'	137.3	-	-	-	-
C4'	19.4	1.62	s	COSY: C1', C2'	C2', C3', C5'
C5'	27.3	1.61	s		C2', C3', C4'



10

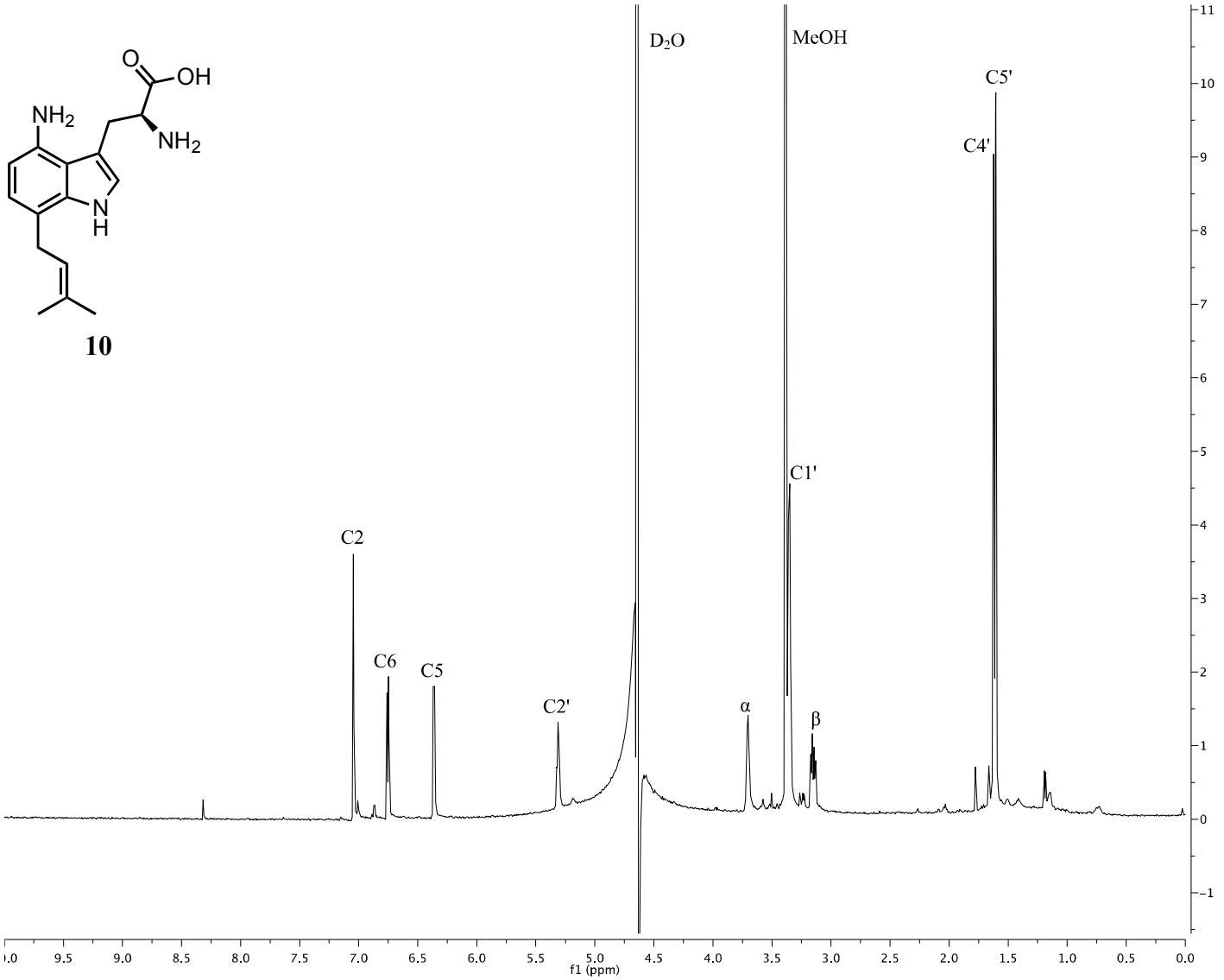


Figure S35. ^{1}H NMR spectrum of **10** in D_2O.

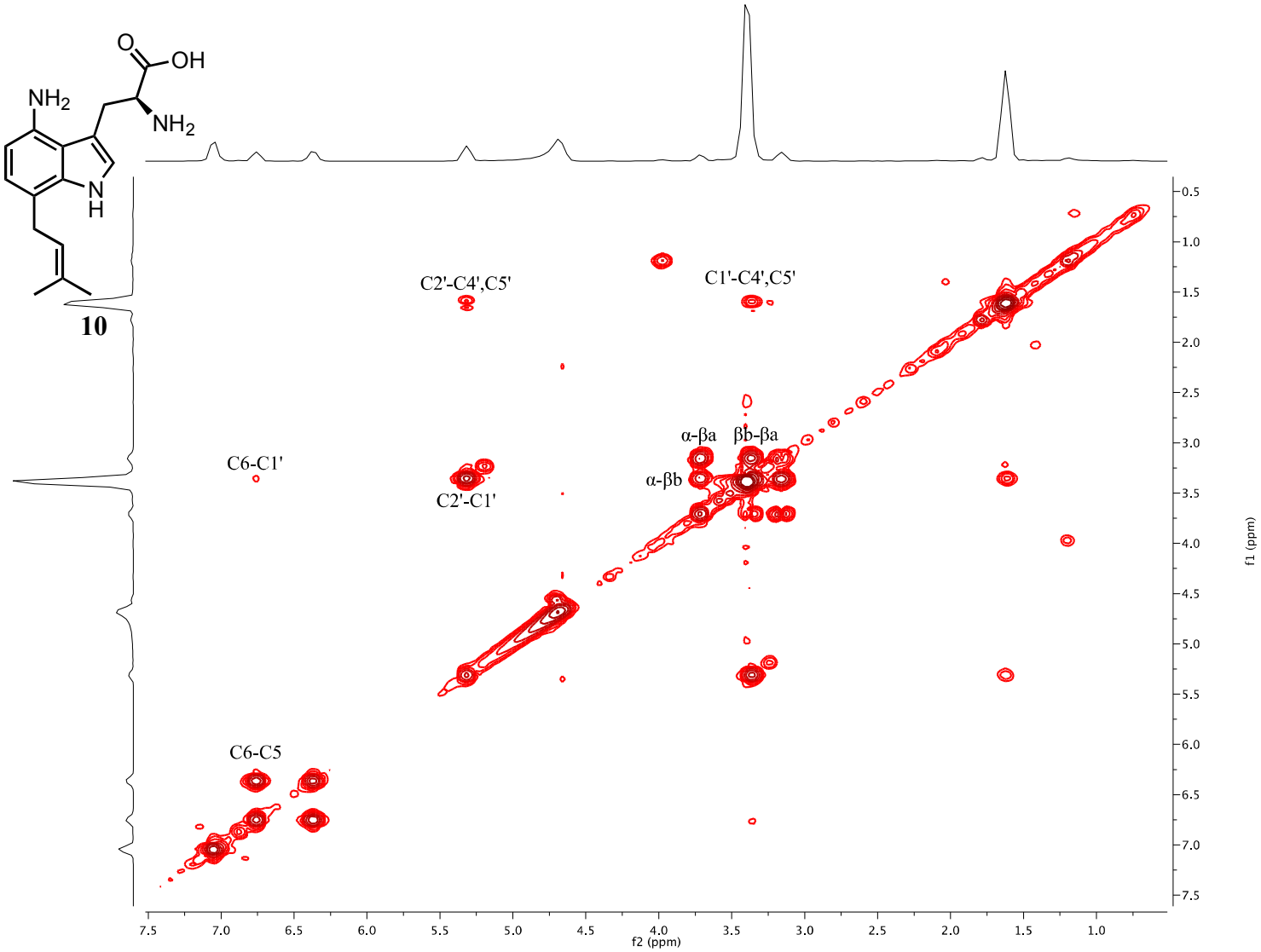


Figure S36. 2D ^{1}H-^{1}H COSY spectrum of **10** in D_2O.

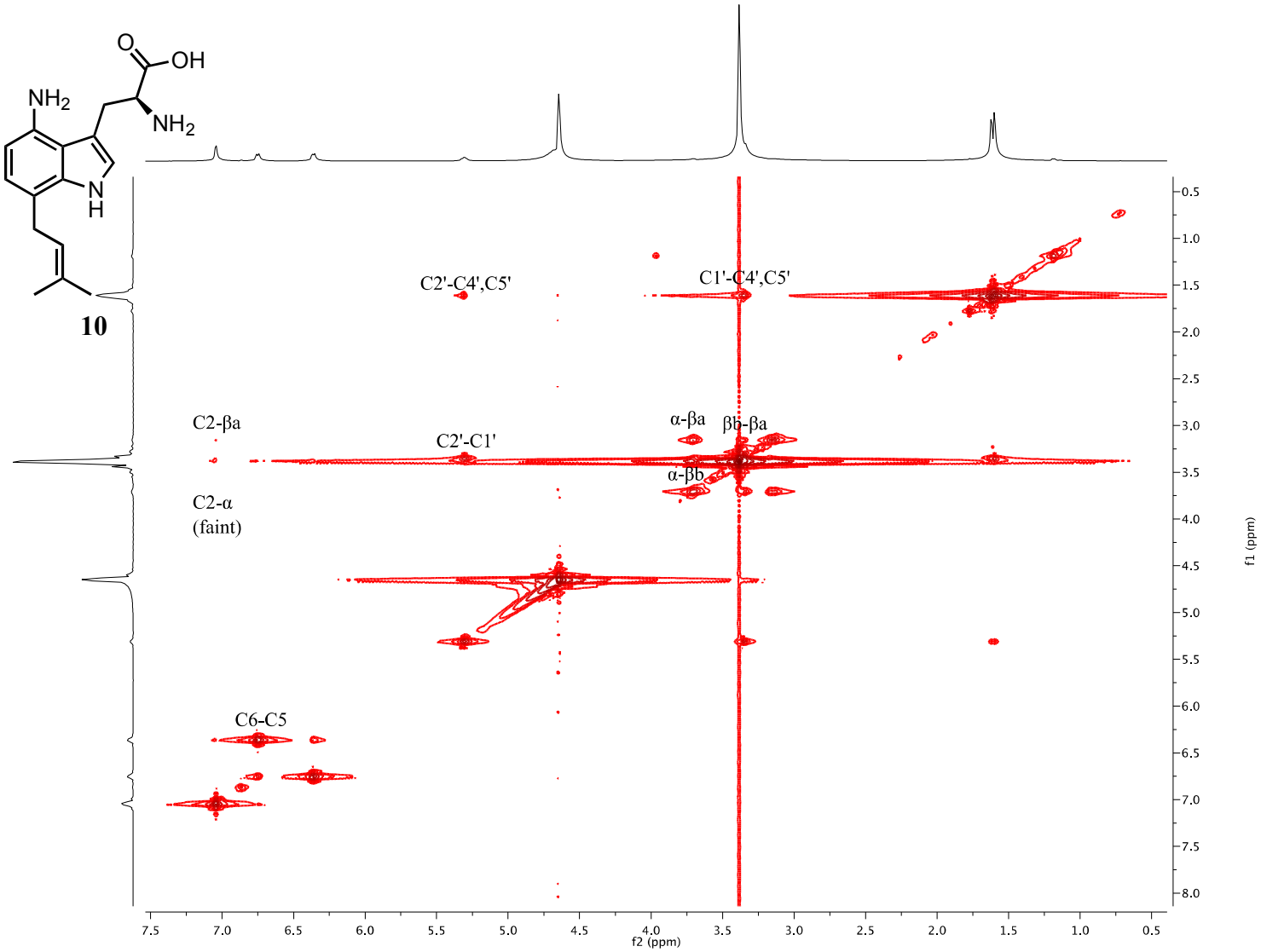


Figure S37. 2D ^{1}H-^{1}H TOCSY spectrum of **10** in D_2O.

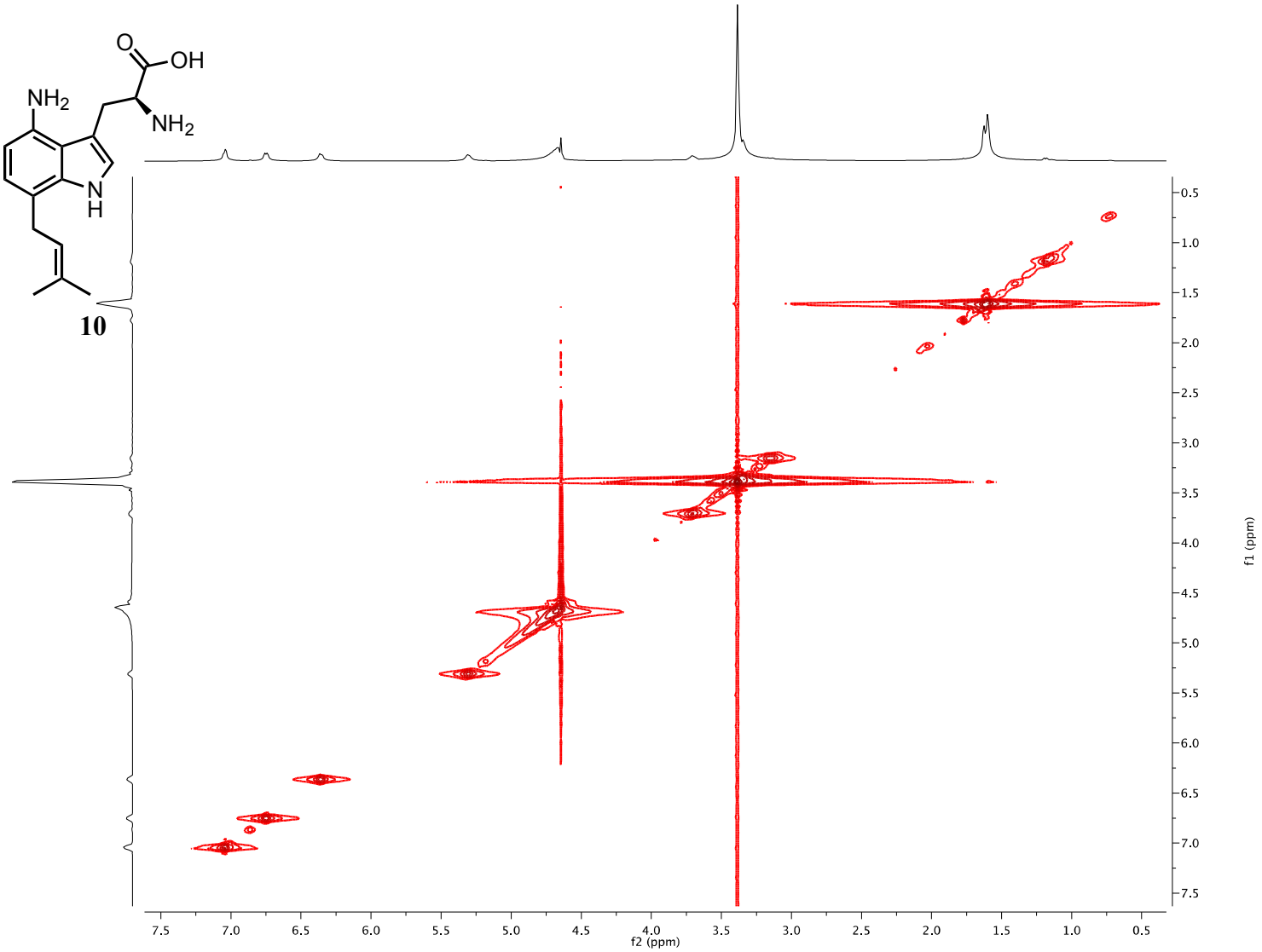


Figure S38. 2D ^{1}H-^{1}H ROESY spectrum of **10** in D_2O.

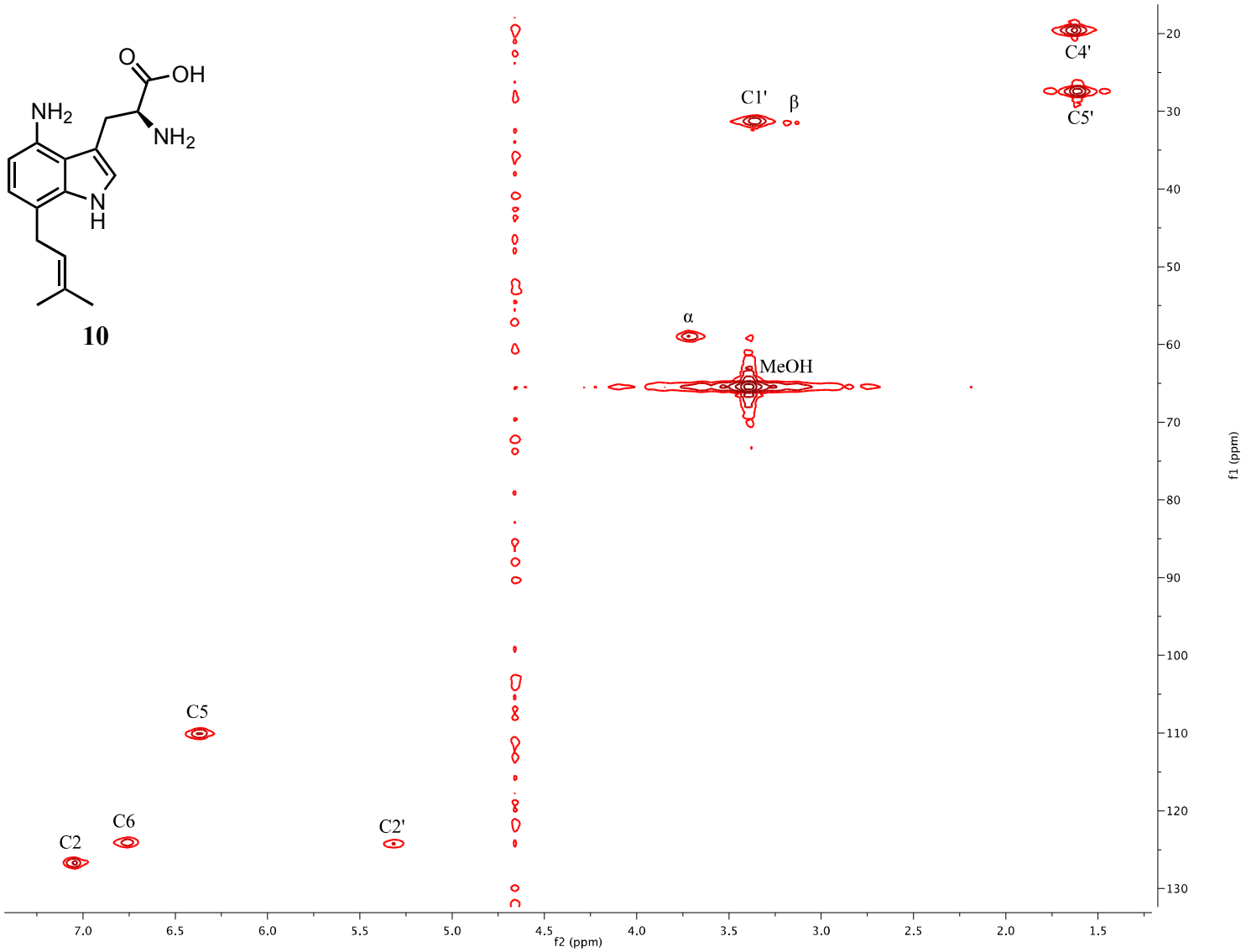


Figure S39. 2D ^{1}H-^{13}C HMQC spectrum of **10** in D_2O.

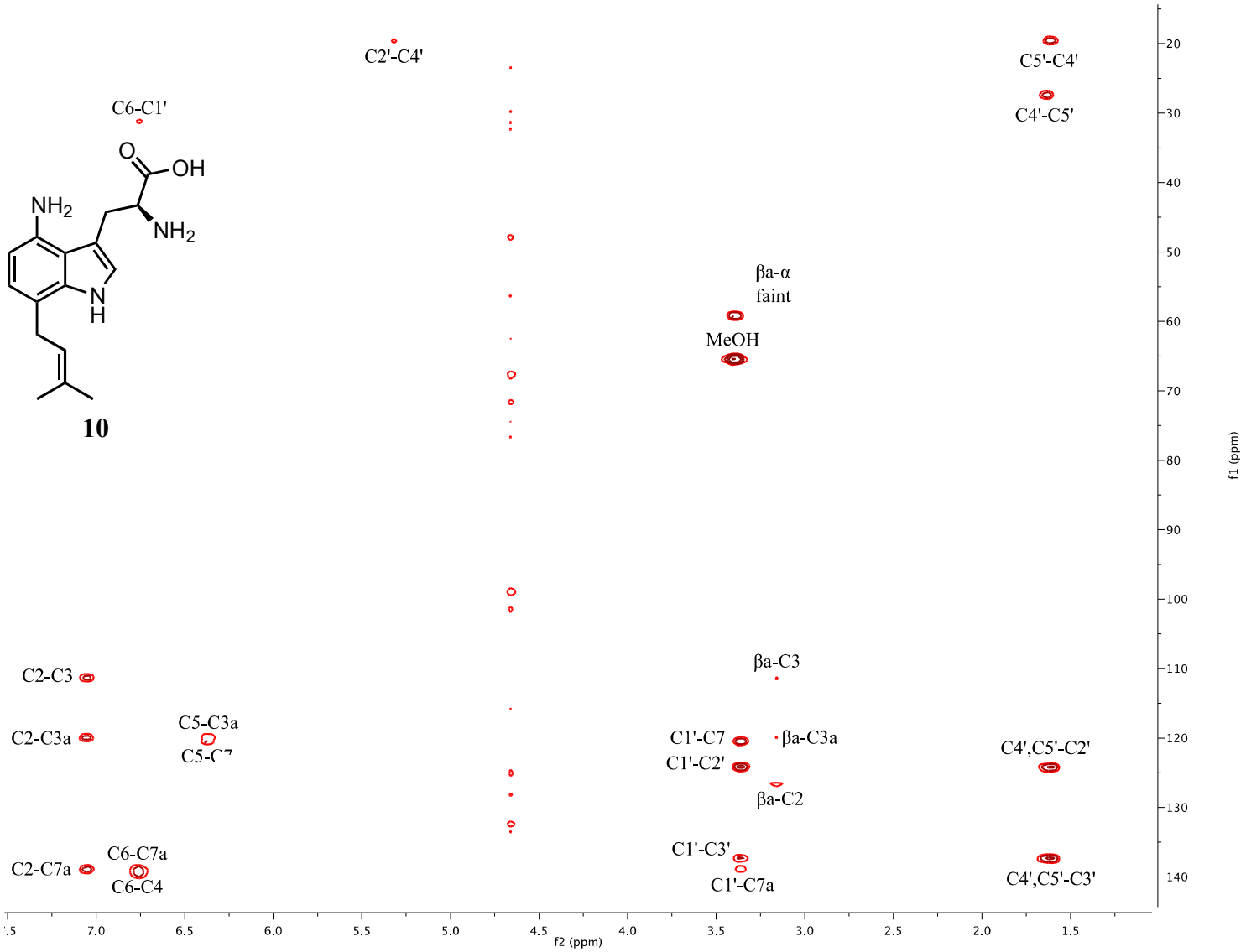


Figure S40. 2D ^{1}H-^{13}C HMBC spectrum of **10** in D_2O.

Enzymatic prenylation product structures with pertinent 2D NMR correlations

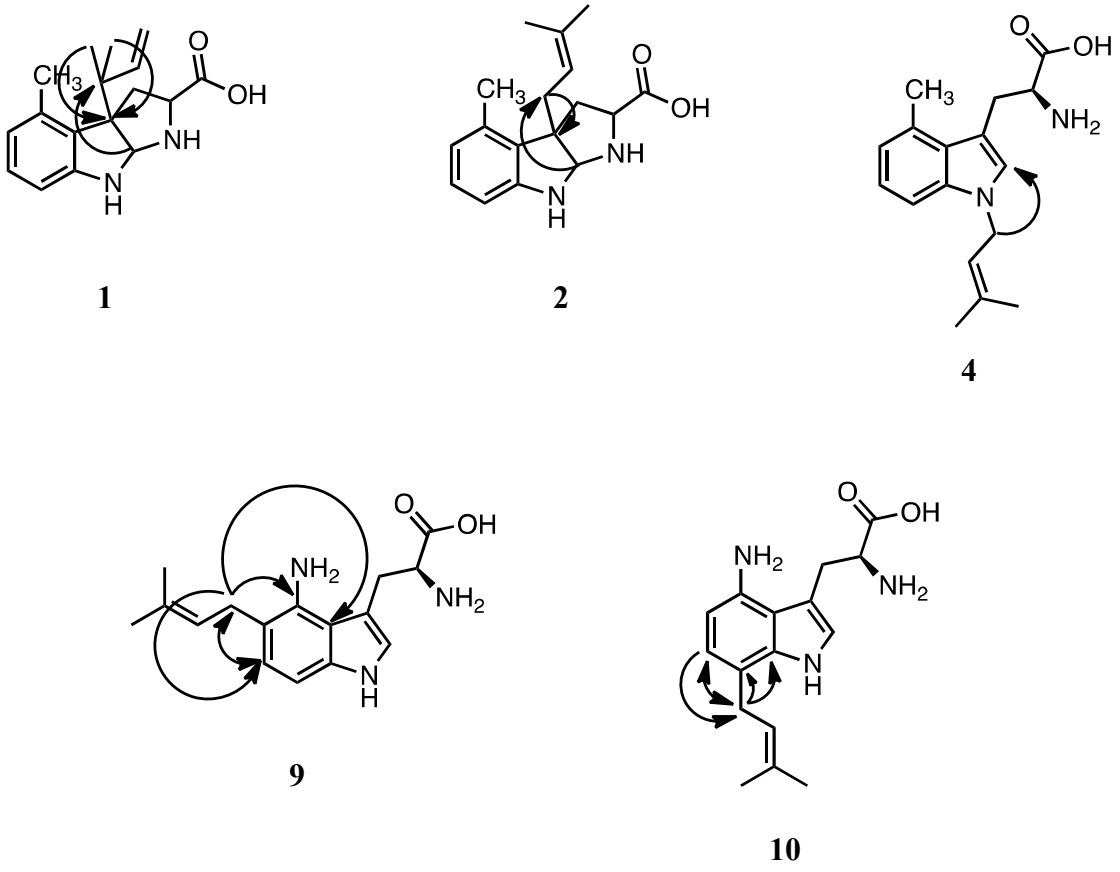


Figure S41. Structures of enzymatic products **1**, **2**, **4**, **9** and **10** showing the pertinent HMBC (single arrow) and COSY/ROESY (double arrow) correlations.

Table S7. HRMS data of prenylated tryptophan analogues

Compound	Chemical Formula	Calculated	Found	Deviation (ppm)
1	$C_{17}H_{22}N_2O_2Na$	309.1579 $(M + Na)^+$	309.1581	0.6
2	$C_{17}H_{22}N_2O_2$	287.1760 $(M + H)^+$	287.1755	-1.7
3	$C_{17}H_{22}N_2O_2Na$	309.1579 $(M + Na)^+$	309.1594	4.9
4	$C_{17}H_{22}N_2O_2$	287.1760 $(M + H)^+$	287.1760	0.0
5	$C_{17}H_{22}N_2O_3Na$	325.1528 $(M + Na)^+$	325.1539	3.4
6	$C_{17}H_{22}N_2O_3Na$	325.1528 $(M + Na)^+$	325.1524	-1.2
7	$C_{17}H_{22}N_2O_3Na$	325.1528 $(M + Na)^+$	325.153	0.6
8	$C_{17}H_{22}N_2O_3Na$	325.1528 $(M + Na)^+$	325.1534	1.8
9	$C_{16}H_{22}N_3O_2$	288.1712 $(M + H)^+$	288.1715	1.0
10	$C_{16}H_{22}N_3O_2$	288.1712 $(M + H)^+$	288.1717	1.7

Negative ion FABMS of 4 fully exchanged in basic D_2O

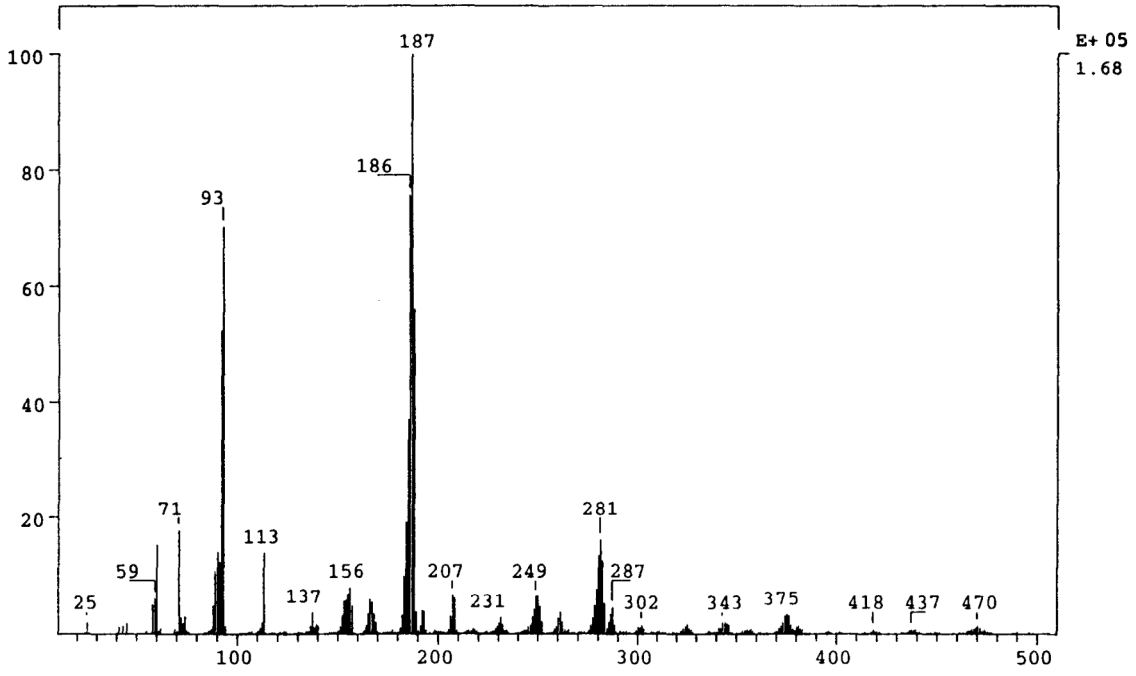


Figure S42. Negative ion fast atom bombardment mass spectrometry (FABMS) spectrum of **4** fully exchanged in basic D_2O. The high intensity peaks around 93, 186, and 281 are from deuterated glycerol. A peak with *m/z* of 287 (M-1) was present for deuterated **4**.

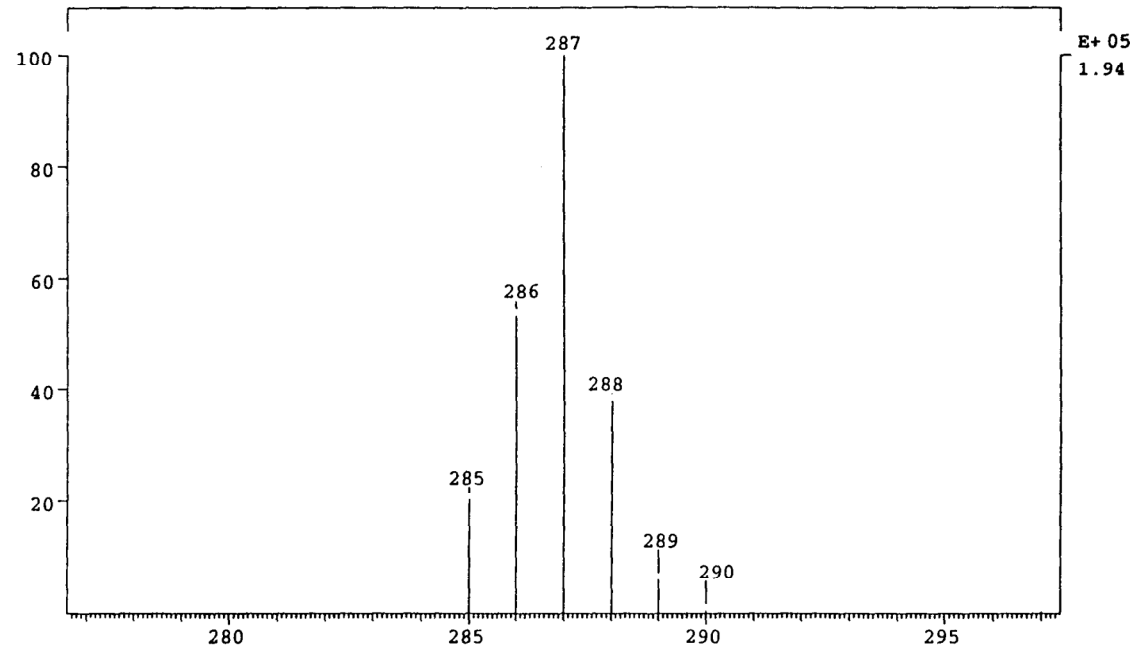


Figure S43. Negative ion FABMS difference spectrum of **4** fully exchanged in basic D_2O. The deuterated glycerol background peaks were subtracted from the spectrum in Figure S42. Deuterated **4** is clearly present (*m/z* 287).

HPLC chromatograms of prenylation product co-injection assay

HPLC was performed as described in the article. Prenylated 4-methyltryptophan products were co-injected to determine if retention time was identical. The concentrations of prenylated products were qualitatively determined to be equimolar to ensure all injected products were visualized at 225 nm.

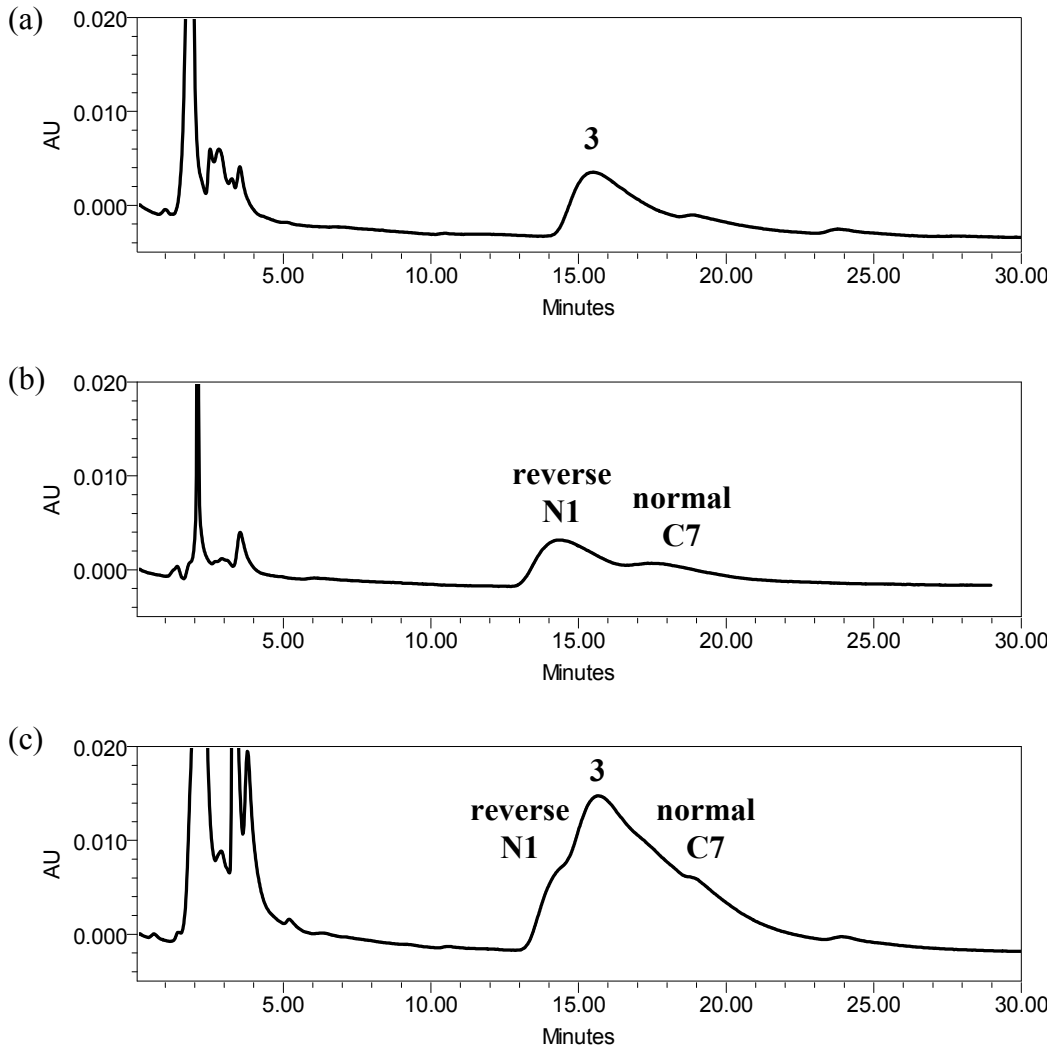


Figure S44. HPLC chromatograms of co-injection assays for comparison of **3** and reverse *N*-dimethylallyl-4-methyl-L-tryptophan and normal 7-dimethylallyl-4-methyl-L-tryptophan. (a) Isolated **3** injection, (b) reverse N1 and normal C7 prenylation products, (c) **3** co-injection with reverse N1 and normal C7 prenylation products.

RadioTLCs and corresponding Michaelis-Menten curves

All kinetic assays were performed as described in the article. Reaction mixtures were spotted and developed by RP-C8 TLC (Silicycle) using a solvent system of 4:6 25 mM NH_4HCO_3/methanol. The developed TLC plates were then imaged on a storage phosphor screen (Molecular Dynamics), scanned by a Typhoon 8600 Variable Mode Imager (GE Healthcare), and the data was visualized and processed with ImageQuant 5.2. Product turnovers were blanked with a 0 μM 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 duplicate or triplicate. L-tryptophan concentrations of 3, 10, 20, 30 and 60 μM were used. 4-methyltryptophan concentrations of 30, 100, 300 and 600 μM were used. 4-methoxytryptophan concentrations of 30, 100, 300, 600, 1000 and 3000 μM were used. 4-aminotryptophan concentrations of 3, 10, 20, 30, 60, and 100 μM were used. Specific activities of DMATS vs. substrate concentration were plotted and fit using a non-linear regression enzyme kinetic plot by GraFit 5.0.11.

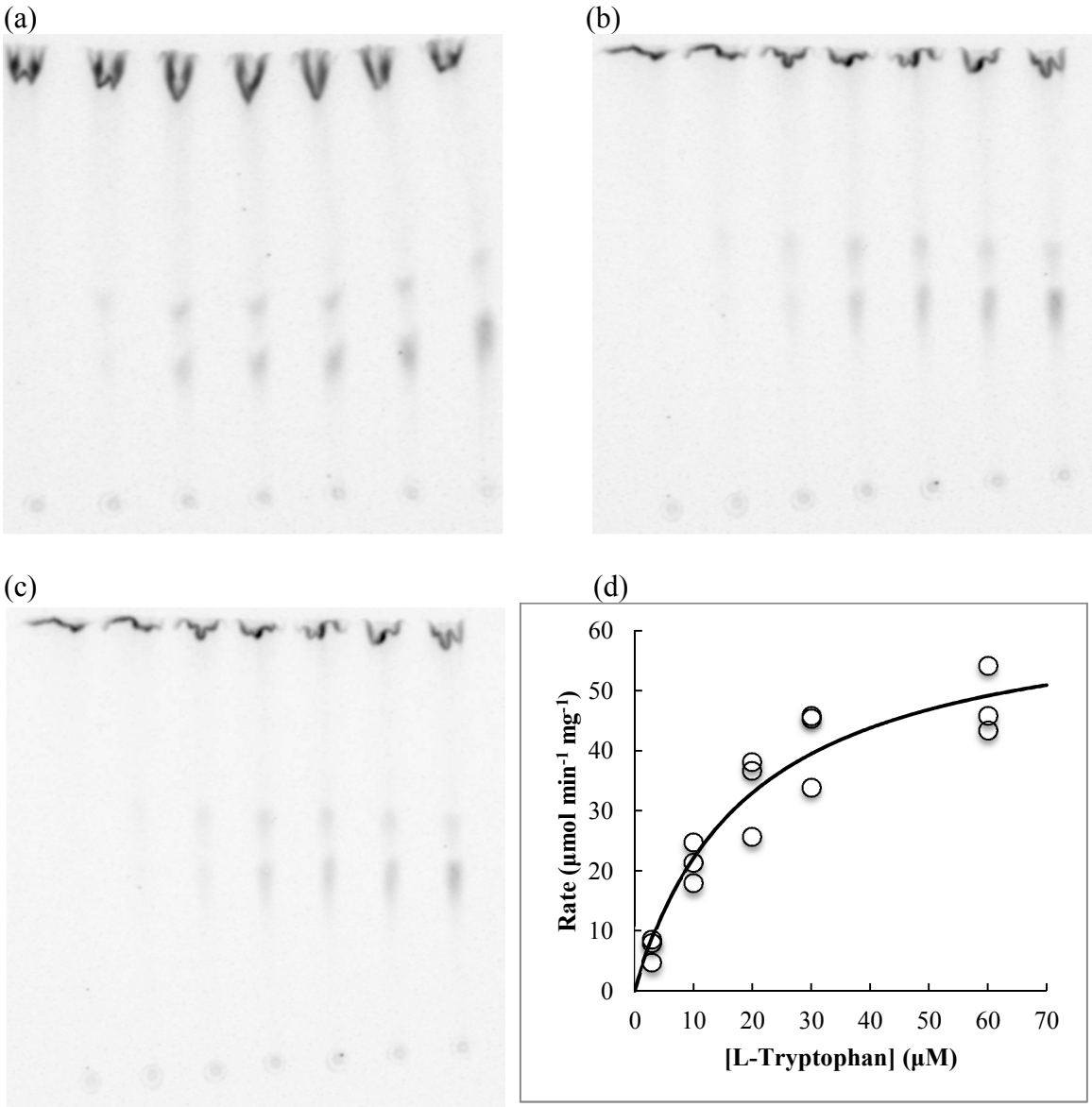


Figure S45. RadioTLCs and Michaelis-Menten curve for L-TRP. (a-c) Autoradiography TLCs for triplicate experiments. In each TLC, L-TRP concentrations from left to right are 0, 3, 10, 20, 30, 60, and 100 μM. (d) Michaelis-Menten curve for L-TRP generated by GraFit 5.0.11 including L-TRP concentrations of 3, 10, 20, 30, and 60 μM.

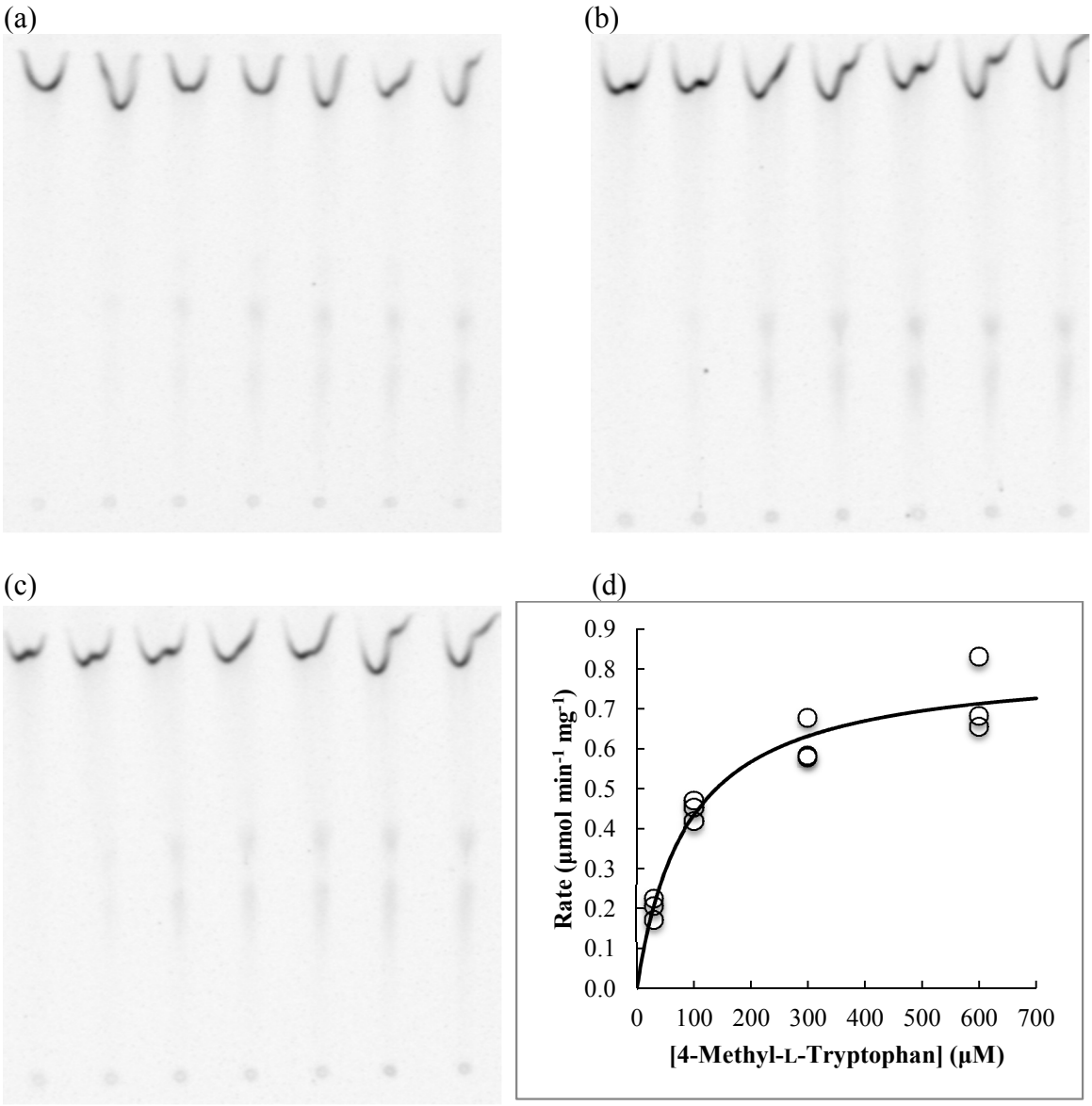


Figure S46. RadioTLCs and Michaelis-Menten curve for 4-CH_3-L-TRP. (a-c) Autoradiography TLCs for triplicate experiments. In each TLC, 4-CH_3-L-TRP concentrations from left to right are 0, 30, 100, 300, 600, 1000, and 3000 μM. (d) Michaelis-Menten curve for 4-CH_3-L-TRP generated by GraFit 5.0.11 including 4-CH_3-L-TRP concentrations of 30, 100, 300, and 600 μM.

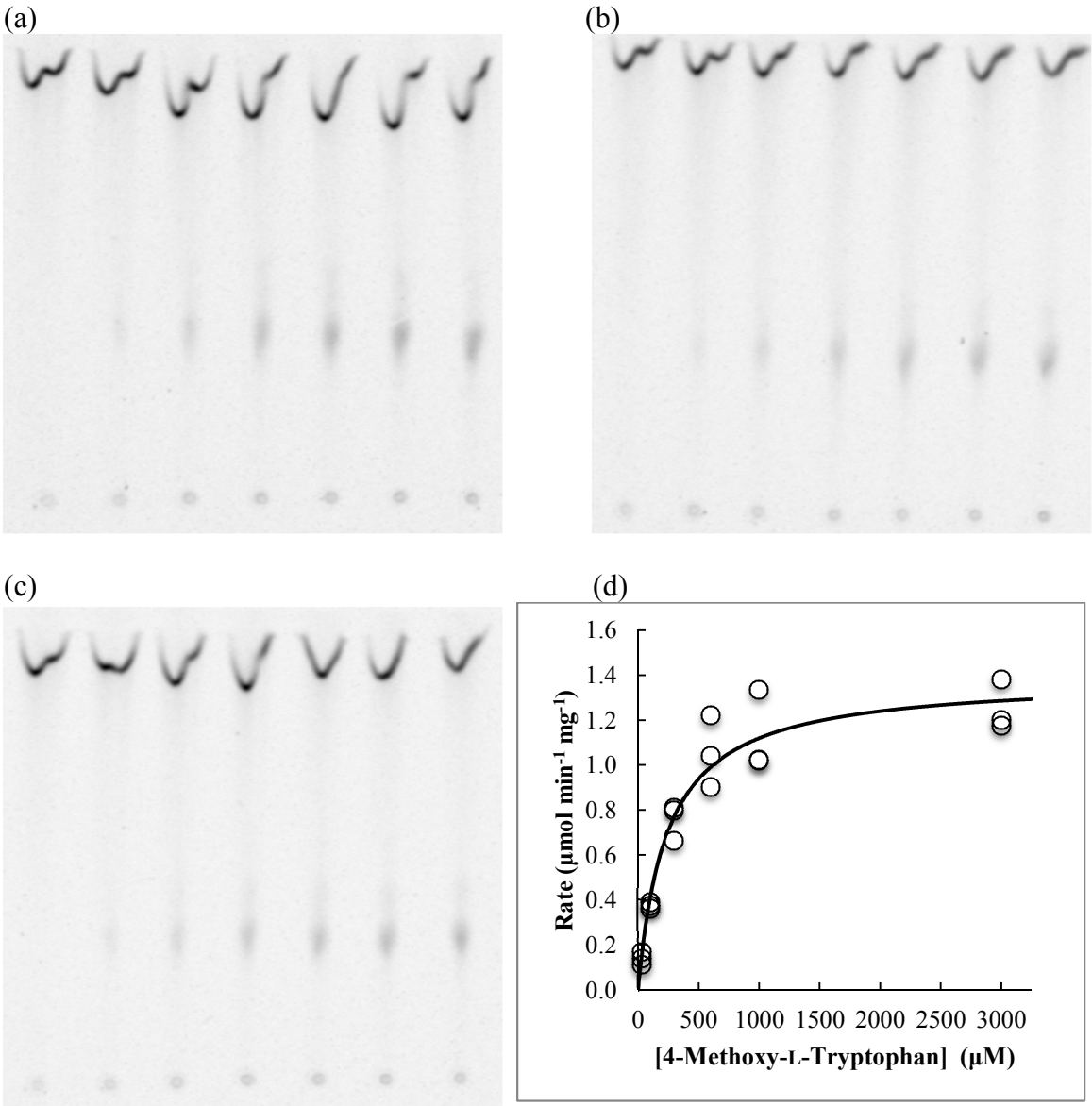
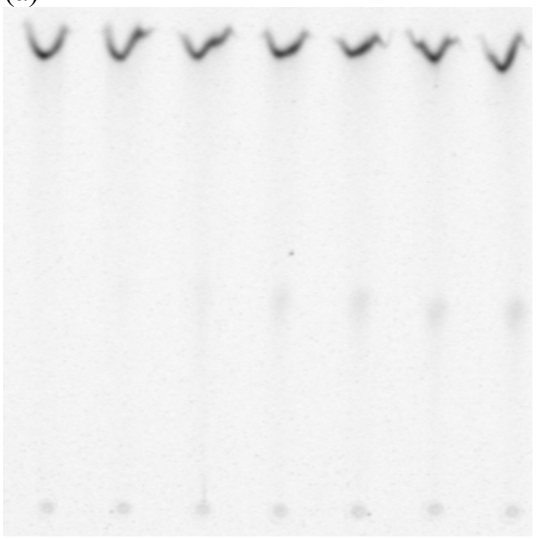
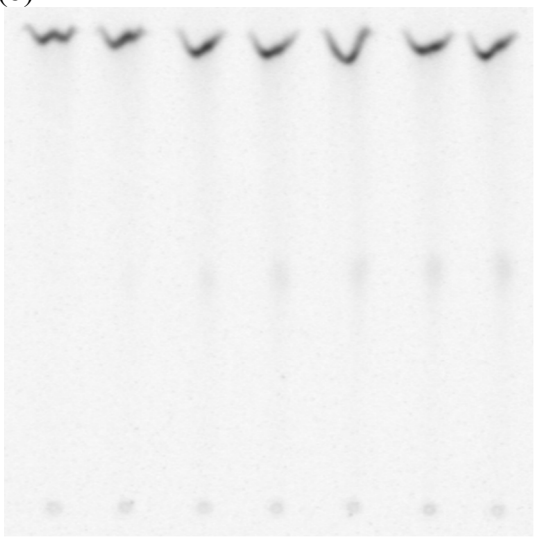


Figure S47. RadioTLCs and Michaelis-Menten curve for 4-OCH_3-L-TRP. (a-c) Autoradiography TLCs for triplicate experiments. In each TLC, 4-OCH_3-L-TRP concentrations from left to right are 0, 30, 100, 300, 600, 1000, and 3000 μM. (d) Michaelis-Menten curve for 4-OCH_3-L-TRP generated by GraFit 5.0.11 including 4-OCH_3-L -TRP concentrations of 30, 100, 300, 600, 1000, and 3000 μM.

(a)



(b)



(c)

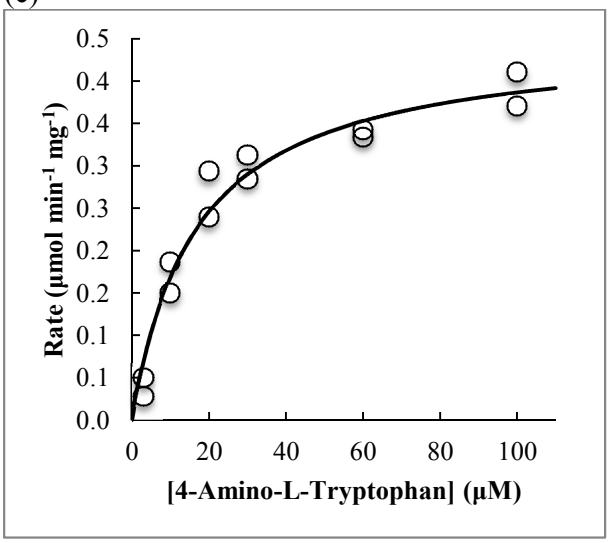


Figure S48. RadioTLCs and Michaelis-Menten curve for 4-NH_2-L-TRP. (a-b) Autoradiography TLCs for duplicate experiments. In each TLC, 4-NH_2-L-TRP concentrations from left to right are 0, 3, 10, 20, 30, 60, and 100 µM. (c) Michaelis-Menten curve for 4-NH_2-L-TRP generated by GraFit 5.0.11 including 4-NH_2-L -TRP concentrations of 3, 10, 20, 30, 60, and 100 µM.

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(2) Hettwer, S.; Sterner, R. *Journal of Biological Chemistry* **2002**, *277*, 8194.

(3) Goloubinoff, P.; Gatenby, A. A.; Lorimer, G. H. *Nature (London)* **1989**, *337*, 44.