SUPPLEMENTAL DATA FOR

Biochemical characterization of indole prenyltransferases: Filling the last gap of prenylation positions by a 5-dimethylallyltryptophan synthase from *Aspergillus clavatus*

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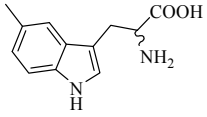
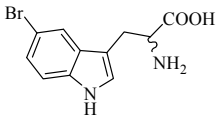
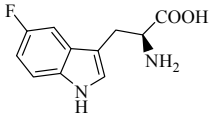
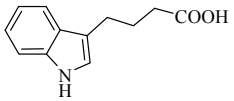
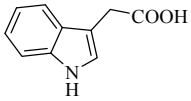
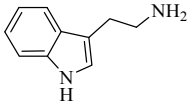
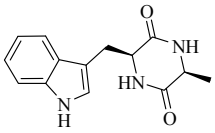
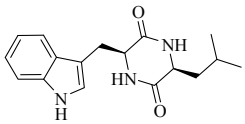
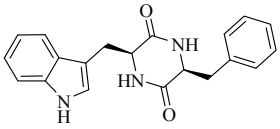
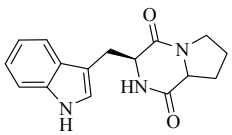
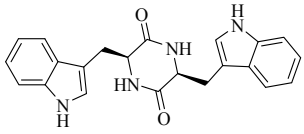
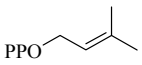
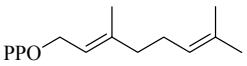
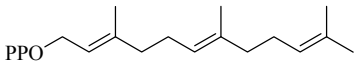
Table S1. Enzyme activity of 5-DMATS towards aromatic compounds, which are not included in Table 1.

Name	Structure	Product yield (%)
5-methyl-DL-tryptophan		1.7
5-bromo-DL-tryptophan		< 0.5
5-fluoro-L-tryptophan		7.4
indole-3-butyric acid		20.2
indole-3-acetic acid		1.8
tryptamine		6.1
cyclo-L-Trp-L-Ala		1.9
cyclo-L-Trp-L-Leu		6.4
cyclo-L-Trp-L-Phe		2.2
cyclo-L-Trp-L-Pro		0.9
cyclo-L-Trp-L-Trp		9.0

The reaction mixtures containing aromatic compounds and DMAPP were incubated with 1 μM protein for 7 h.

Table S2. Enzyme activity of 5-DMATS towards prenyl diphosphates

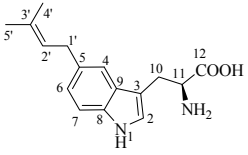
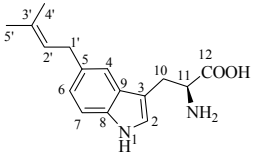
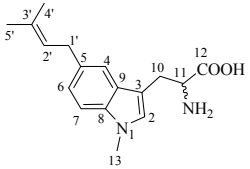
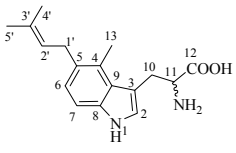
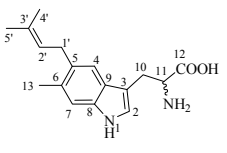
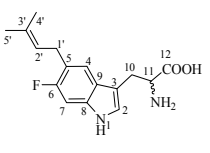
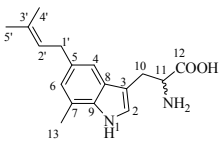
Name	Structure	Product yield (%)		
		1h incubation 0.40 µM 5-DMATS	7h incubation 1 µM 5-DMATS	24h incubation 1 µM 5-DMATS
DMAPP		95.4	100	100
GPP		< 0.5	< 0.5	< 0.5
FPP		< 0.5	< 0.5	< 0.5

L-tryptophan was used as aromatic substrate.

Table S3. HR-ESI-MS data of enzyme products of 5-DMATS

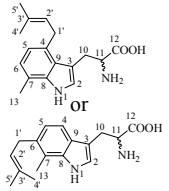
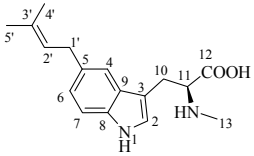
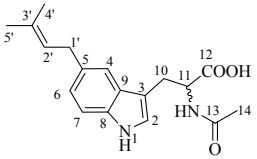
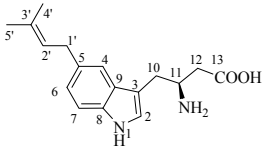
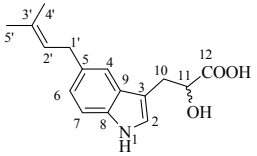
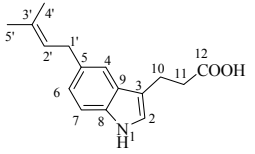
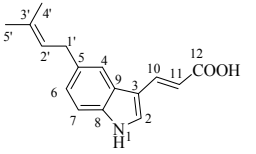
		HR-ESI-MS data		
Comp.	Chemical formula	Calculated	Measured	Deviation (ppm)
1b	$C_{16}H_{20}N_2O_2$	273.1603 $[M+H]^+$	273.1604	-0.3
2b	$C_{17}H_{22}N_2O_2$	287.1760 $[M+H]^+$	287.1746	4.5
3b	$C_{17}H_{22}N_2O_2$	287.1760 $[M+H]^+$	287.1753	2.4
4b	$C_{17}H_{22}N_2O_2$	287.1760 $[M+H]^+$	287.1791	-11.0
5b	$C_{16}H_{19}FN_2O_2$	291.1509 $[M+H]^+$	291.1473	12.4
6b and **6c**	$C_{17}H_{22}N_2O_2$	287.1760 $[M+H]^+$	287.1725	11.9
7b	$C_{17}H_{22}N_2O_2$	287.1760 $[M+H]^+$	287.1750	3.3
8b	$C_{18}H_{22}N_2O_3$	337.1528 $[M+Na]^+$	337.1499	8.7
9b	$C_{17}H_{22}N_2O_2$	287.1760 $[M+H]^+$	287.1744	5.4
10b	$C_{16}H_{19}NO_3$	296.1263 $[M+Na]^+$	296.1245	5.9
11b	$C_{16}H_{19}NO_2$	280.1313 $[M+Na]^+$	280.1346	-11.6
12b	$C_{16}H_{17}NO_2$	278.1157 $[M+Na]^+$	278.1153	1.4

Table S4. ^{1}H- and ^{13}C- NMR data of enzyme products obtained in CD_3OD or DMSO- d_6.

Compd	 **1b** (in DMSO- d_6)		 **1b** (in CD_3OD)	 **2b** (in CD_3OD)	 **3b** (in DMSO- d_6)		 **4b** (in CD_3OD)	 **5b** (in CD_3OD)	 **6b** (in DMSO- d_6)
Pos.	δ_C	δ_H, multi., J	δ_H, multi., J	δ_H, multi.,J	δ_C	δ_H, multi.,J	δ_H, multi., J	δ_H, multi., J	δ_H, multi., J
2	124.1	7.18, s	7.15, s	7.05, s	124.5	7.11, s	7.14, s	7.14, s	7.14, br. s
3	109.3	-	-	-	§	-	-	-	-
4	117.2	7.32, br. s	7.49, d, 1.6	7.49, br. s	126.5	-	7.44, s	7.49, d, 7.3	7.14, br. s
5	131.0	-	-	-	128.9	-	-	-	-
6	121.8	6.88, br. d, 8.3	6.95, dd, 8.4, 1.6	7.02, dd, 8.4, 1.5	123.0	6.85, d, 8.2	-	-	6.69, br. s
7	111.2	7.25, d, 8.3	7.27, d, 8.4	7.25, d, 8.4	108.8	7.08, d, 8.2	7.08, s	7.02, d, 10.8	-
8	134.9	-	-	-	135.6	-	-	-	-
9	127.4	-	-	-	125.5	-	-	-	-
10	27.2	3.30, dd, 15.1, 3.6	3.51, dd, 15.3, 4.0	3.48, dd, 15.3, 4.0	29.2	3.63, d, 15.2	3.50, dd, 15.2, 3.7	3.48, dd, 15.2, 3.8	#
10		2.90, dd, 15.1, 9.3	3.10, dd, 15.2, 9.8	3.09, dd, 15.3, 9.5		2.89, dd, 15.4, 10.6	3.08, dd, 15.2, 9.8	3.09, dd, 15.3, 9.6	2.87, dd, 14.9, 9.5
11	54.8	3.44, dd, 9.3, 3.6	3.86, dd, 9.8, 4.0	3.83, dd, 9.5, 4.0	§	#	3.85, dd, 9.8, 3.7	3.83, dd, 9.6, 3.8	#
12	170.2	-	-	-	§	-	-	-	-
13	-	-	-	3.75, s	14.9	2.55, s	2.34, s	-	2.40, s
14	-	-	-	-	-	-	-	-	-
1'	34.1	3.36, d, 7.4	3.43, d, 7.4	3.44, d, 7.3	31.4	3.34	3.41, d, 6.8	3.41, d, 7.1	#
2'	124.9	5.33, t, 7.4	5.37, m	5.37, m	124.3	5.17, t, 7.1	5.24, m	5.33, t, 7.1	5.32, m
3'	130.4	-	-	-	129.5	-	-	-	
4'	17.7	1.72, s	1.76, s	1.76, s	17.3	1.73, s	1.77, s	1.76, s	1.71, s
5'	25.5	1.70, s	1.74, s	1.74, s	25.1	1.67, s	1.72, s	1.72, s	1.69, s

Chemical shifts (δ) were given in ppm and coupling constants (J) in Hz. #: overlapping signals with those of solvents; §: due to low amount, the signals were not observed.

Table S4 (continued)

Compd	 **6c** (in DMSO- d_6)	 **7b** (in CD_3OD)		 **8b** (in CD_3OD)	 **9b** (in CD_3OD)	 **10b** (in CD_3OD)	 **11b** (in CD_3OD)	 **12b** (in CD_3OD)
Pos.	$\delta_{H,}$ multi., *J*	δ_C	$\delta_{H,}$ multi., *J*	$\delta_{H,}$ multi., *J*	$\delta_{H,}$ multi., *J*	$\delta_{H,}$ multi., *J*	$\delta_{H,}$ multi., *J*	$\delta_{H,}$ multi., *J*
2	7.12, d, 2.2	125.3	7.18, s	7.05, s	7.13, s	7.09, s	7.00, s	7.60, s
3	-	108.6	-	-	-	-	-	-
4	7.29, d, 8.1	118.3	7.47, br. s	7.35, br. s	7.36, d, 1.6	7.45, br. s	7.33, br. s	7.61, br. s
5	6.80, d, 8.1	133.8	-	-	-	-	-	-
6		123.8	6.95, dd, 8.4, 1.5	6.88, dd, 8.3, 1.5	6.95, dd, 8.4, 1.6	6.88, dd, 8.3, 1.5	6.89, dd, 8.3, 1.5	7.05, dd, 8.3, 1.5
7	-	112.3	7.26, d, 8.4	7.19, d, 8.3	7.27, d, 8.4	7.19, d, 8.3	7.20, d, 8.3	7.33, d, 8.3
8	-	136.9	-	-	-	-	-	-
9	-	128.7	-	-	-	-	-	-
10	#	27.6	3.46, dd, 15.5, 4.6	3.34, dd, 14.6, 4.8	2.54, dd, 16.8, 3.9	3.26, dd, 14.8, 3.4	2.57, m	6.34, d, 15.9
10	2.90, dd, 14.3, 9.3		3.23, dd, 15.5, 8.2	3.13, dd, 14.6, 6.7	2.35, dd, 16.8, 9.2	2.92, dd, 14.8, 8.2	-	-
11	#	65.4	3.77, dd, 8.2, 4.6	4.58, dd, 6.7, 4.8	3.66, m	4.21, dd, 8.2, 3.4	3.01, m	7.90, d, 15.9
12	-	173.4	-	-	3.05, d, 7.2	-	-	-
13	2.36, s	33.1	2.56, s	-	-	-	-	-
14	-	-	-	1.86, s	-	-	-	-
1'	#	35.6	3.43, d, 7.3	3.40, d, 7.3	3.42, d, 6.9	3.40, d, 7.3	3.40, d, 7.2	3.47, d, 7.3
2'	5.18, m	126.0	5.37, m	5.37, m	5.37, m	5.38, m	5.37, m	5.40, m
3'	-	132.0	-	-	-	-	-	-
4'	1.72, s	17.9	1.76, s	1.76, s	1.76, s	1.76, s	1.76, s	1.78, s
5'	1.67, s	25.9	1.74, s	1.74, s	1.74, s	1.74, s	1.75, s	1.77, s

#: overlapping signals with those of solvents; §: due to low amount, the signals were not observed.

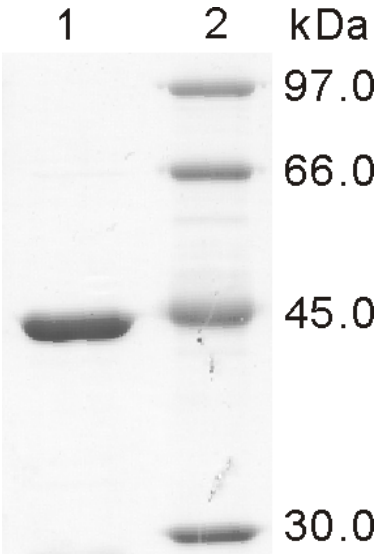


Figure S1. Analysis of purified His_6-5-DMATS on SDS-PAGE. Lane 1: purified His_6-5-DMATS; 2: molecular mass standard.

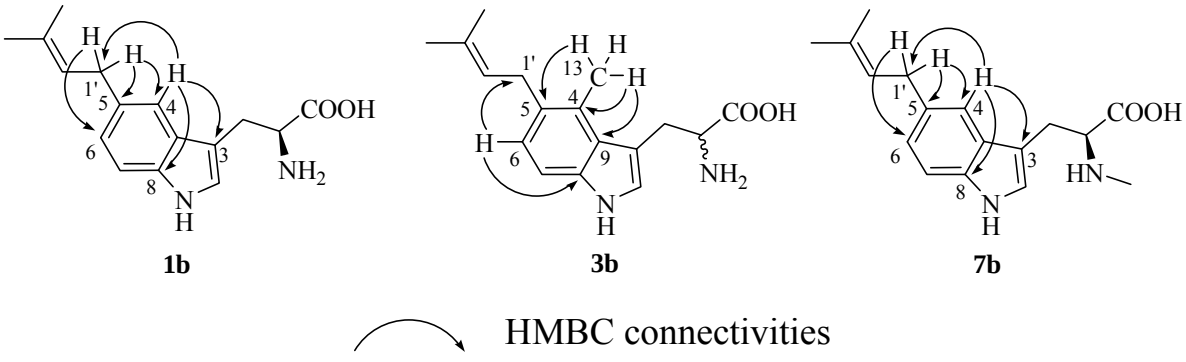


Figure S2. Summary of the HMBC connectivities of enzyme products **1b**, **3b** and **7b**.

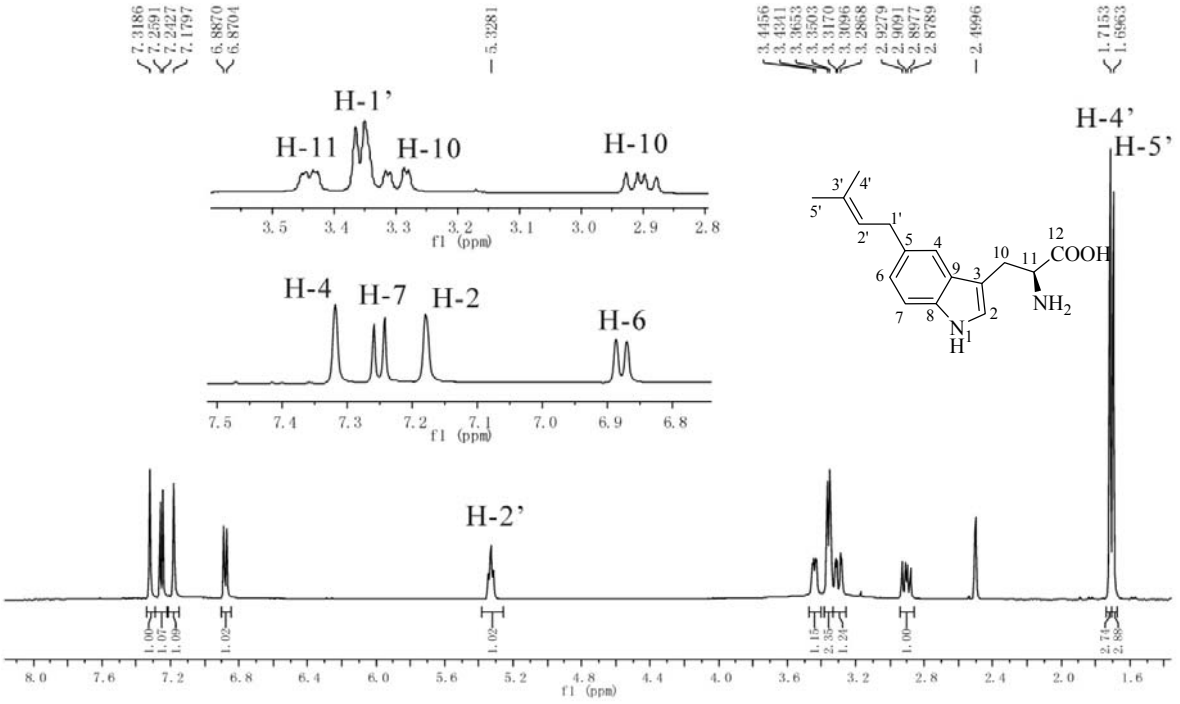


Figure S3.1. ^{1}H-NMR spectrum of **1b** in DMSO-d_6 (500 MHz)

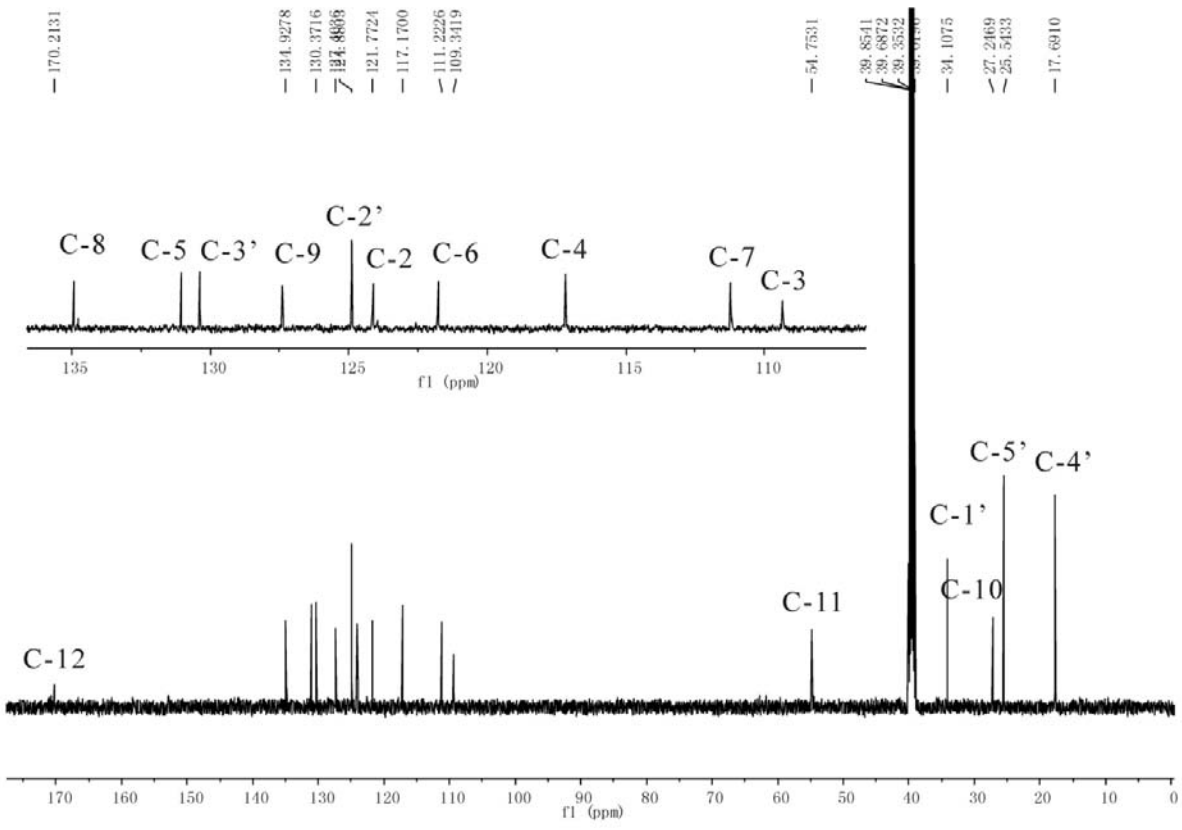


Figure S3.2. ^{13}C-NMR spectrum of **1b** in DMSO-d_6 (500 MHz)

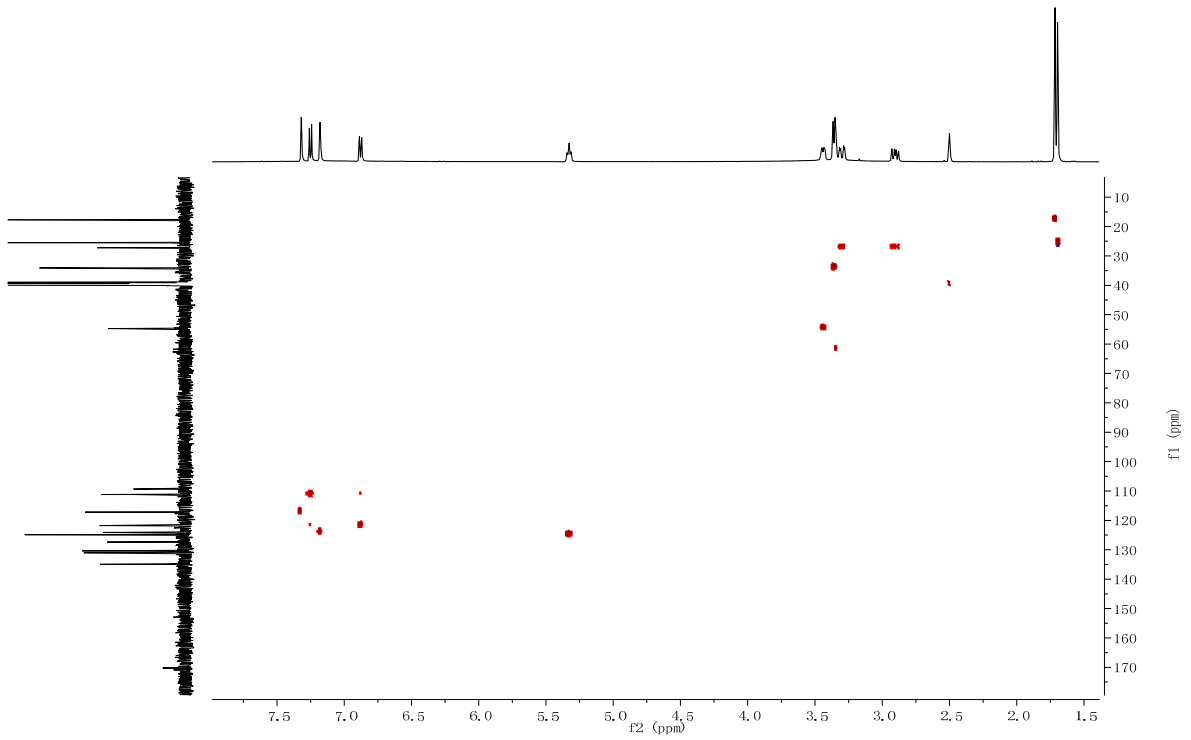


Figure S3.3. HSQC spectrum of **1b** in DMSO-d_6 (500 MHz)

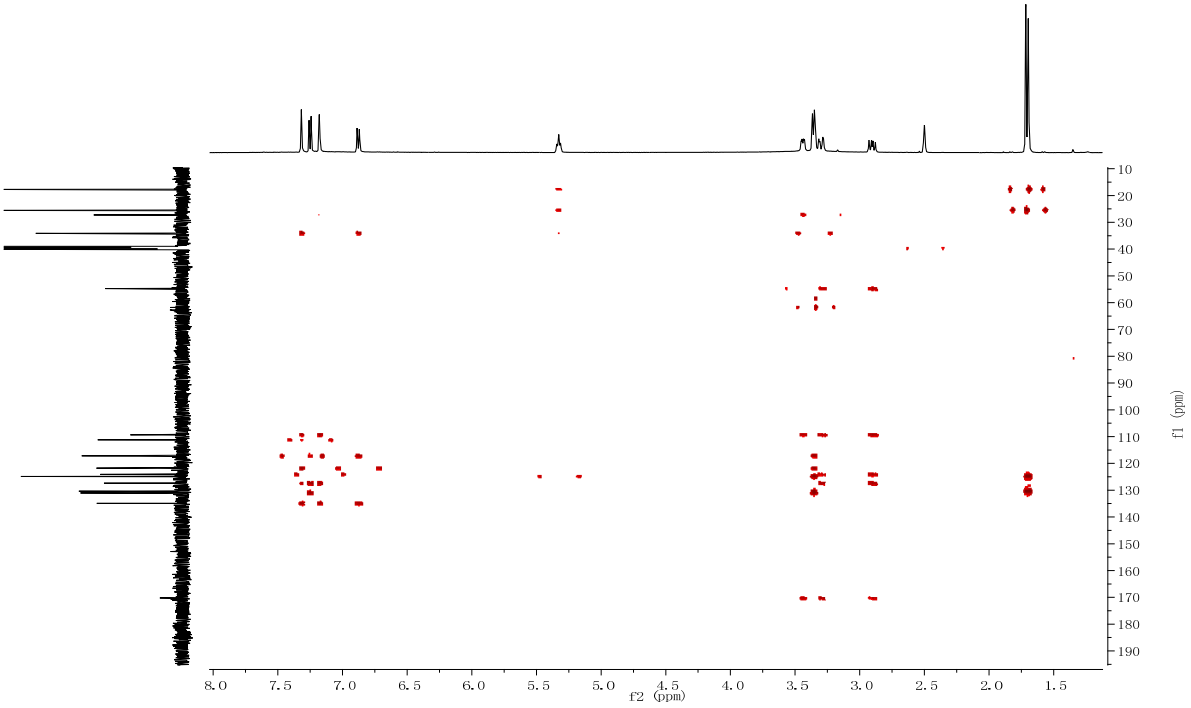


Figure S3.4. HMBC spectrum of **1b** in DMSO-d_6 (500 MHz)

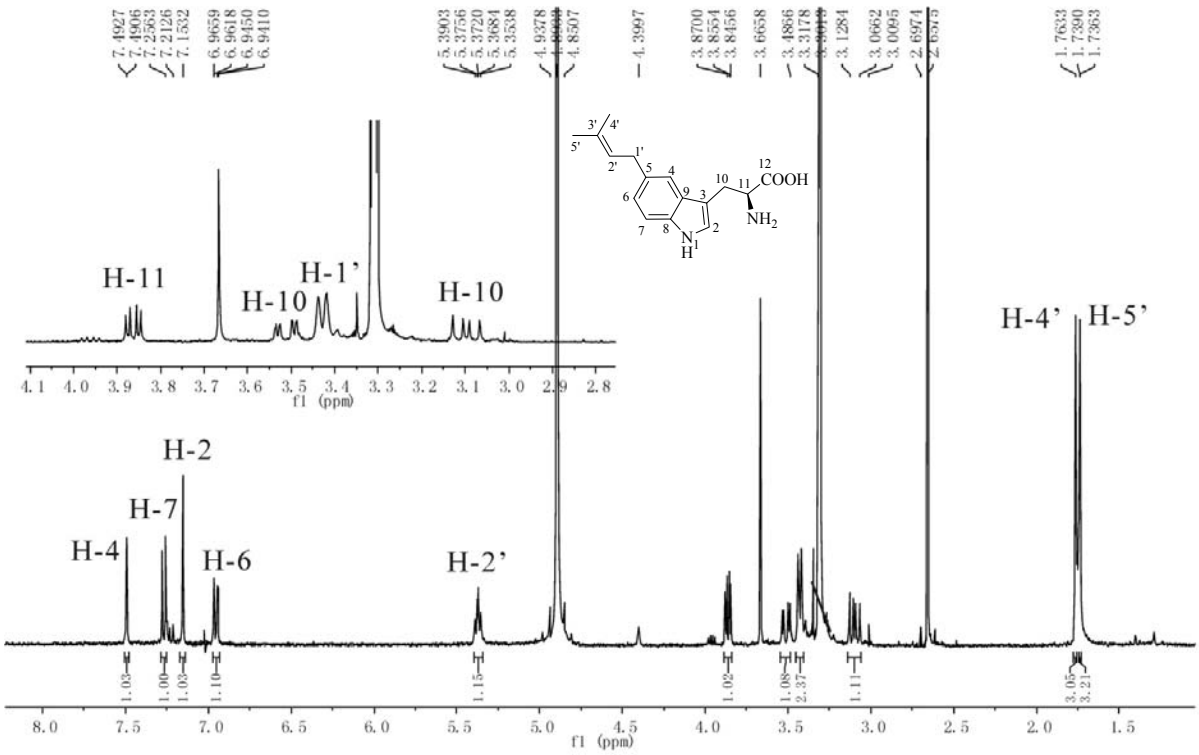


Figure S3.5. ^{1}H-NMR spectrum of **1b** in CD_3OD (400 MHz)

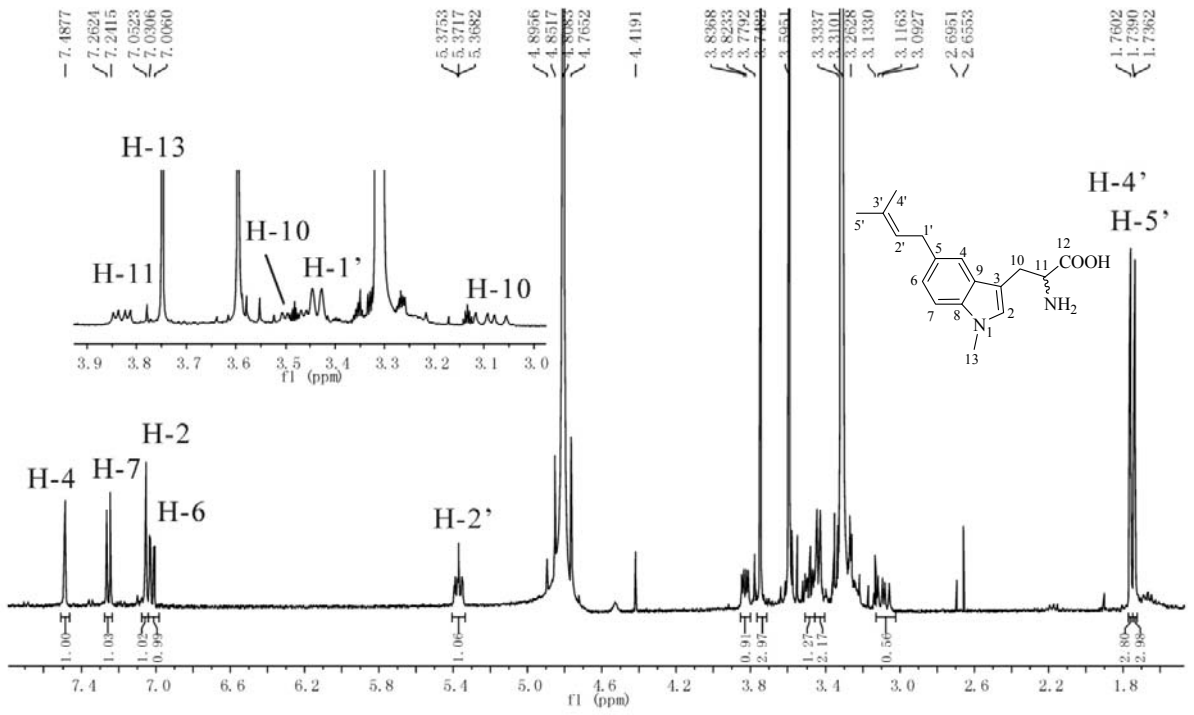


Figure S4. ^{1}H-NMR spectrum of **2b** in CD_3OD (400 MHz)

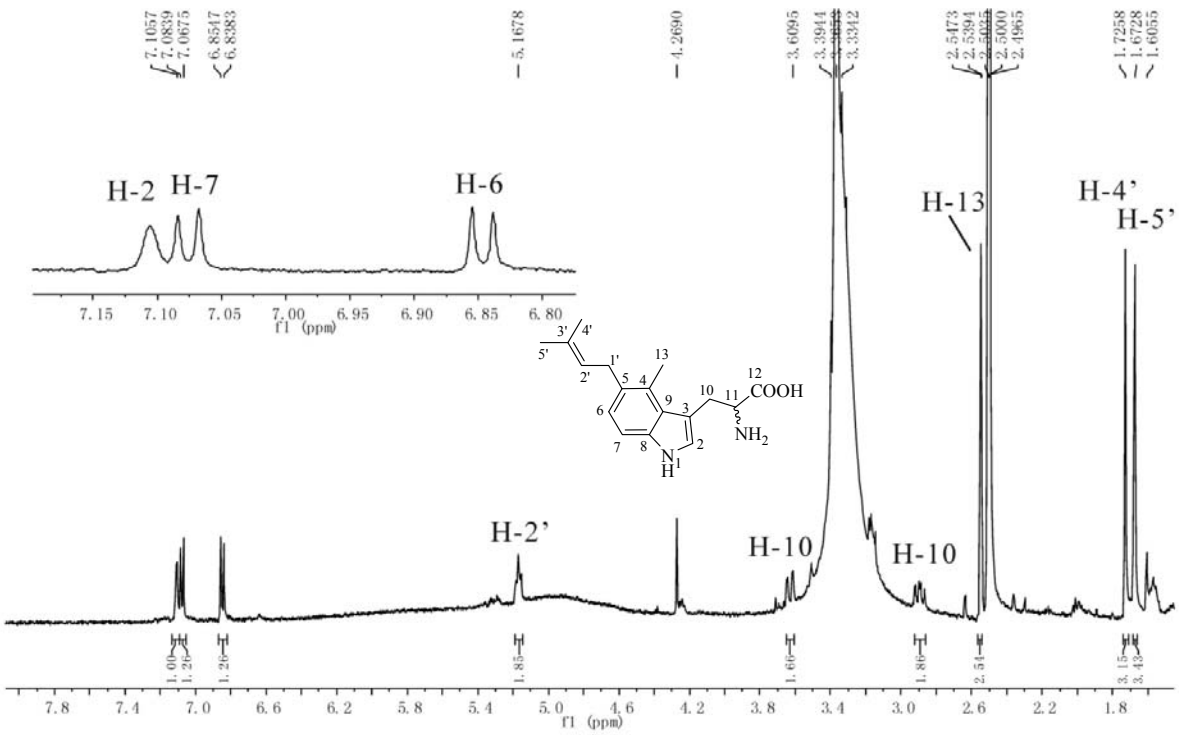


Figure S5.1. ^{1}H-NMR spectrum of **3b** in DMSO-d_6 (500 MHz)

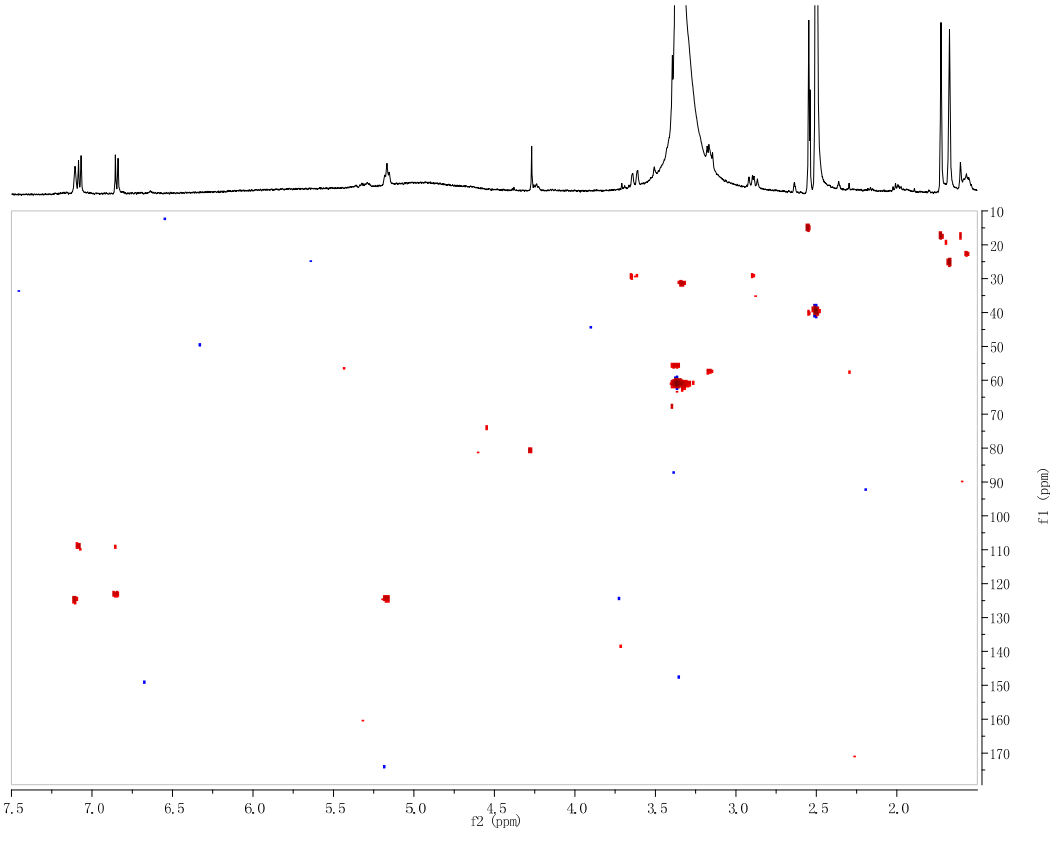


Figure S5.2. HSQC spectrum of **3b** in DMSO-d_6 (500 MHz)

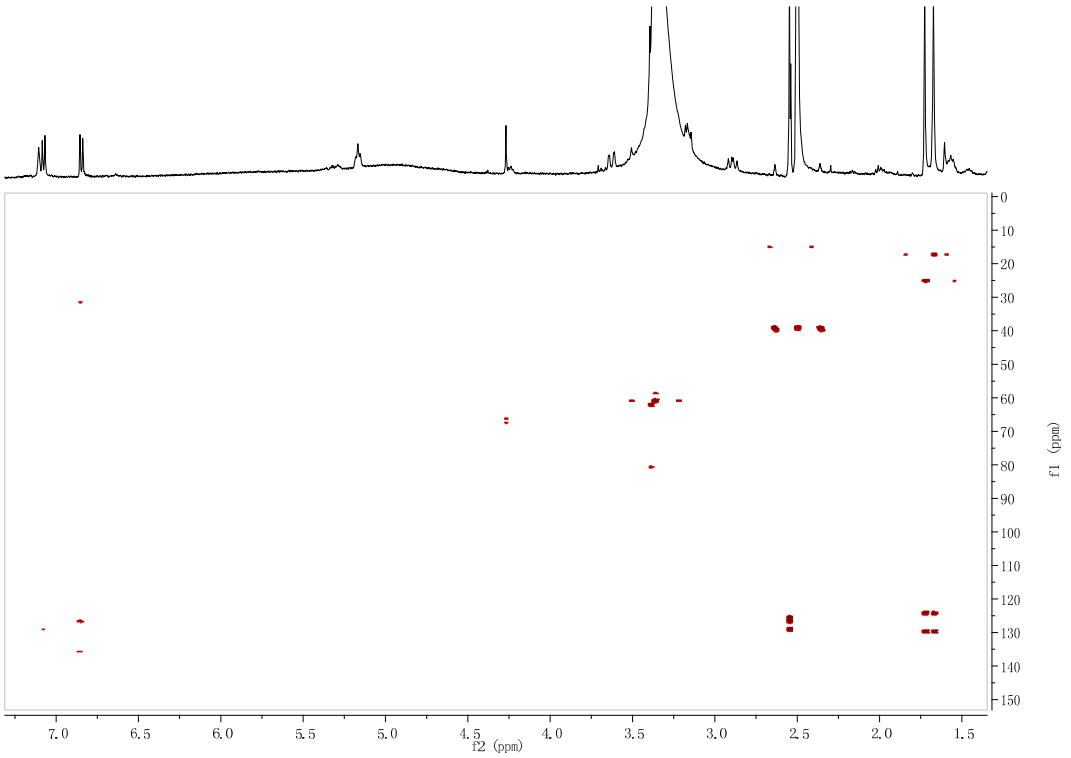


Figure S5.3. HMBC spectrum of **3b** in DMSO-d_6 (500 MHz)

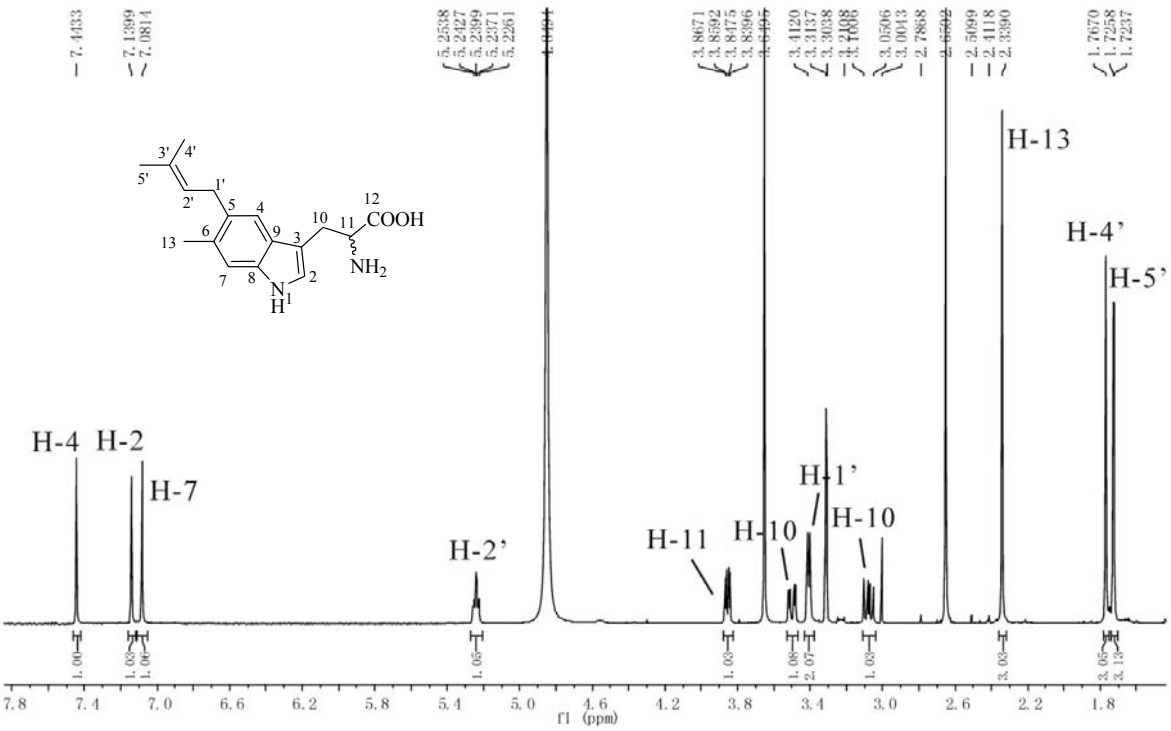


Figure S6. ^{1}H-NMR spectrum of **4b** in CD_3OD (500 MHz)

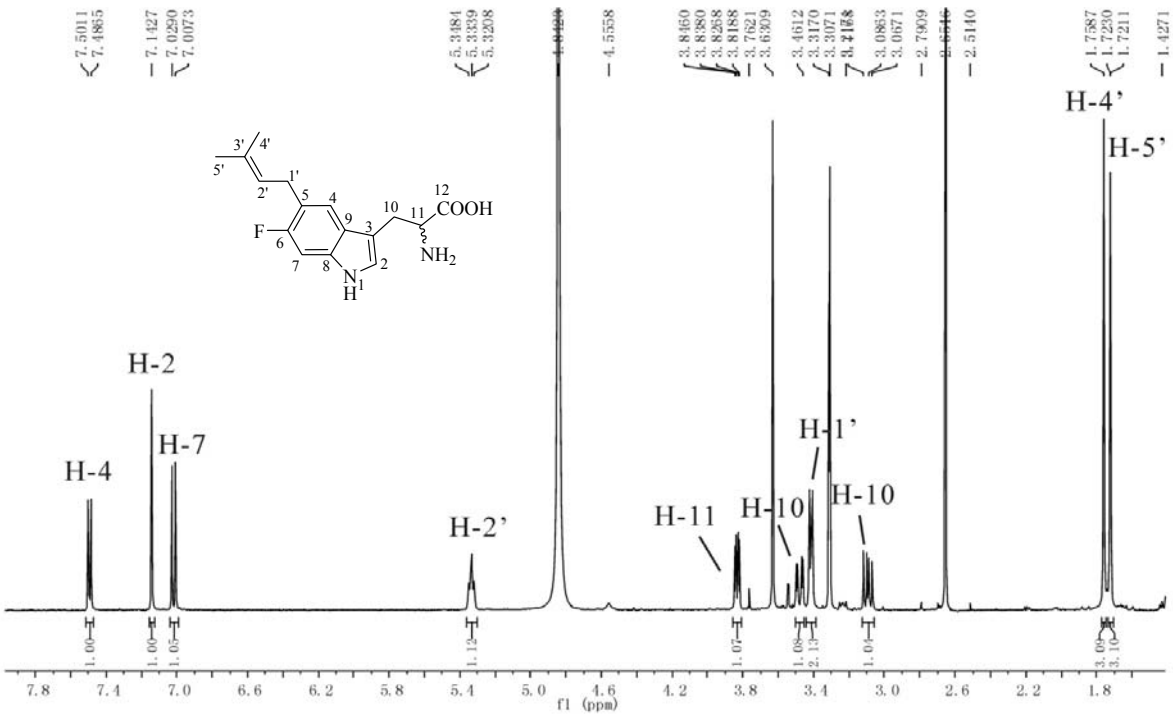


Figure S7. ^{1}H-NMR spectrum of **5b** in CD_3OD (500 MHz)

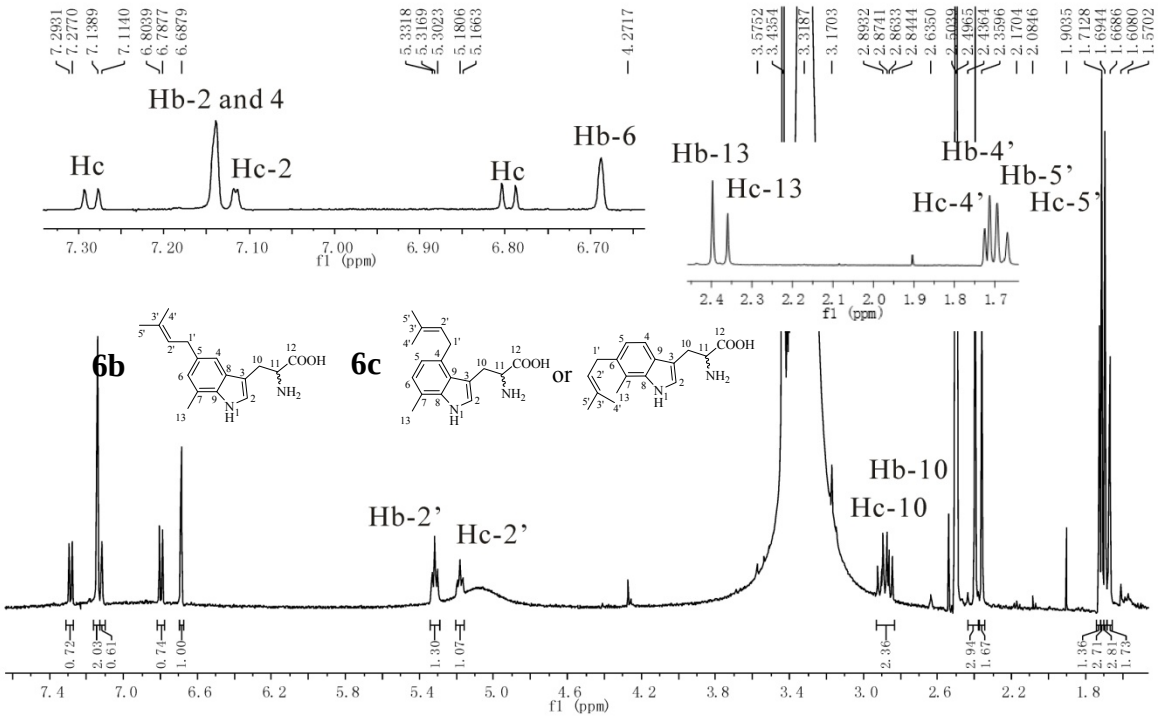


Figure S8. ^{1}H-NMR spectrum of **6b** and **6c** in DMSO-d_6 (500 MHz). The protons of **6b** and **6c** were labeled with Hb and Hc, respectively.

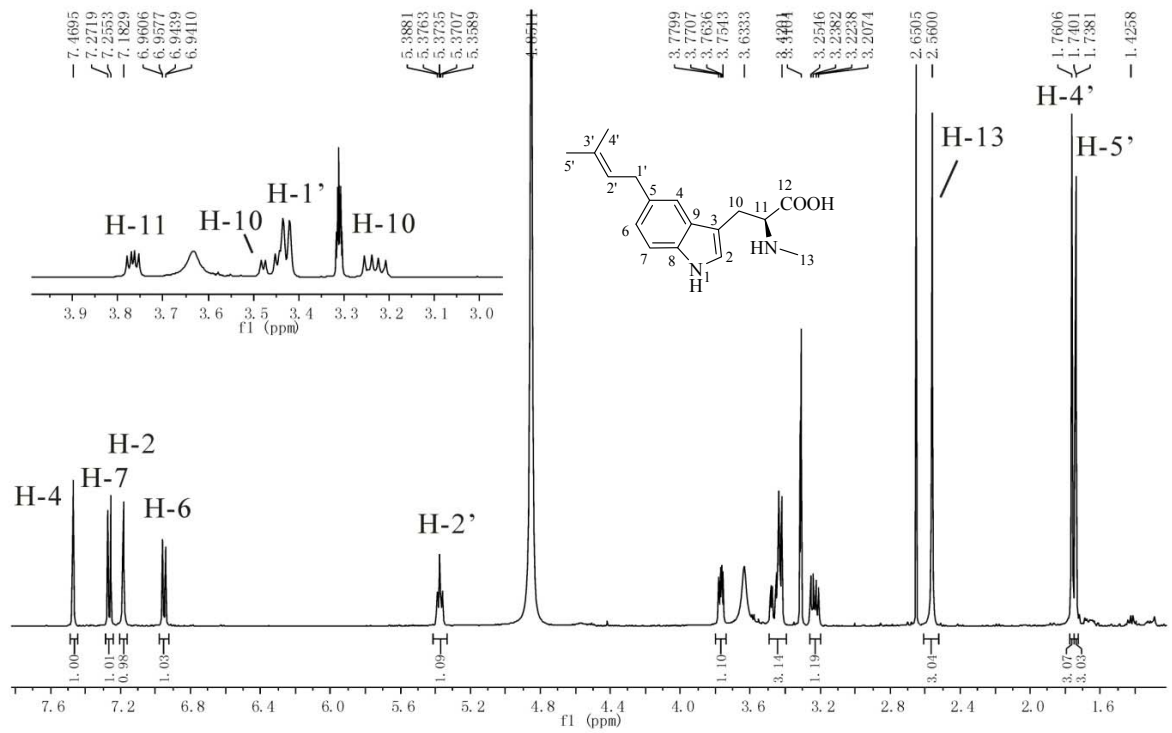


Figure S9.1. ^{1}H-NMR spectrum of **7b** in CD_3OD (500 MHz)

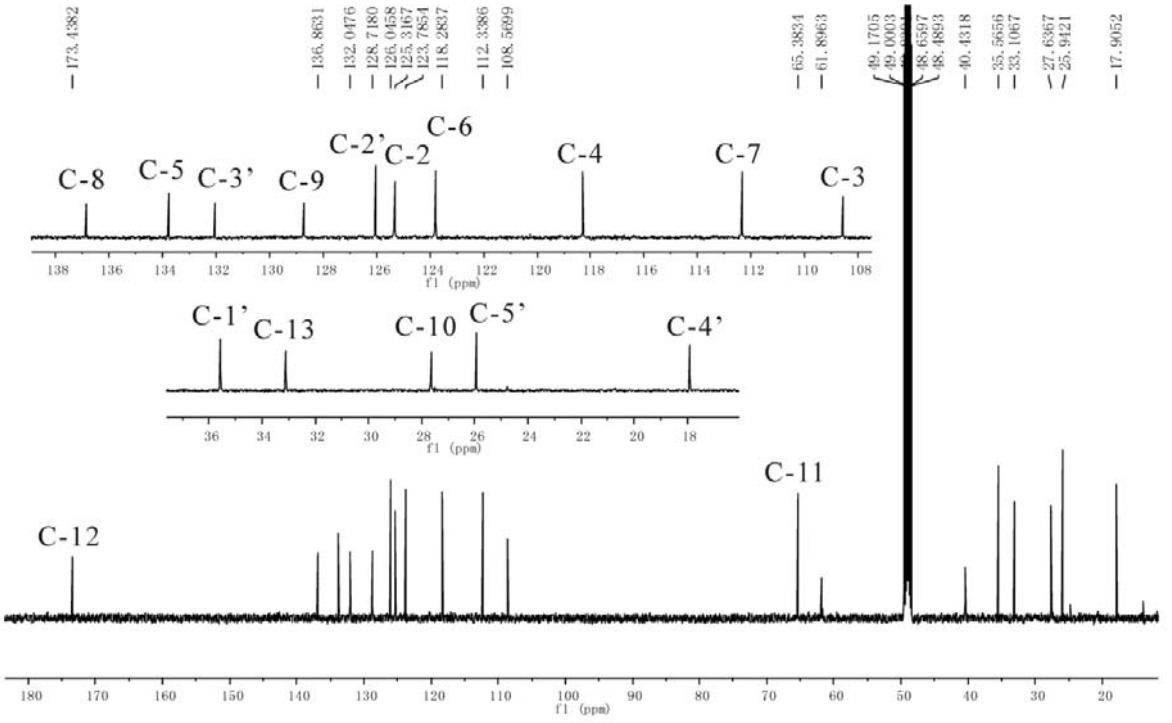


Figure S9.2. ^{13}C-NMR spectrum of **7b** in CD_3OD (500 MHz)

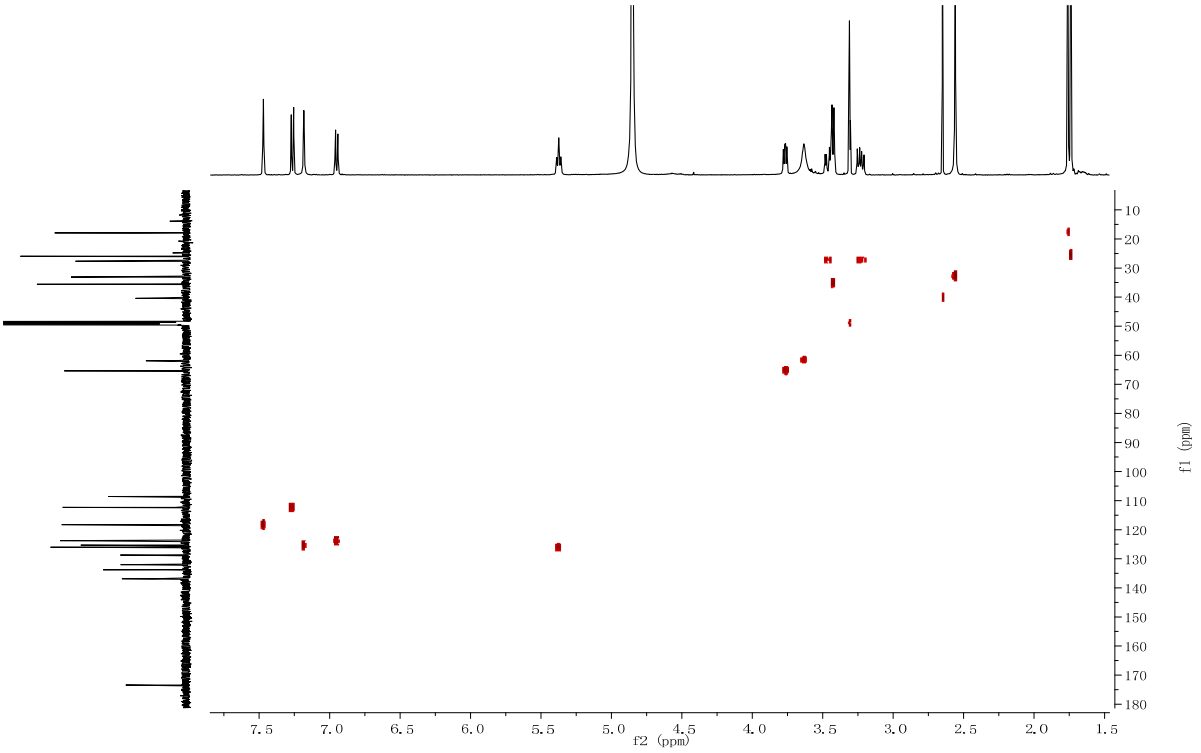


Figure S9.3. HSQC spectrum of **7b** in CD_3OD (500 MHz)

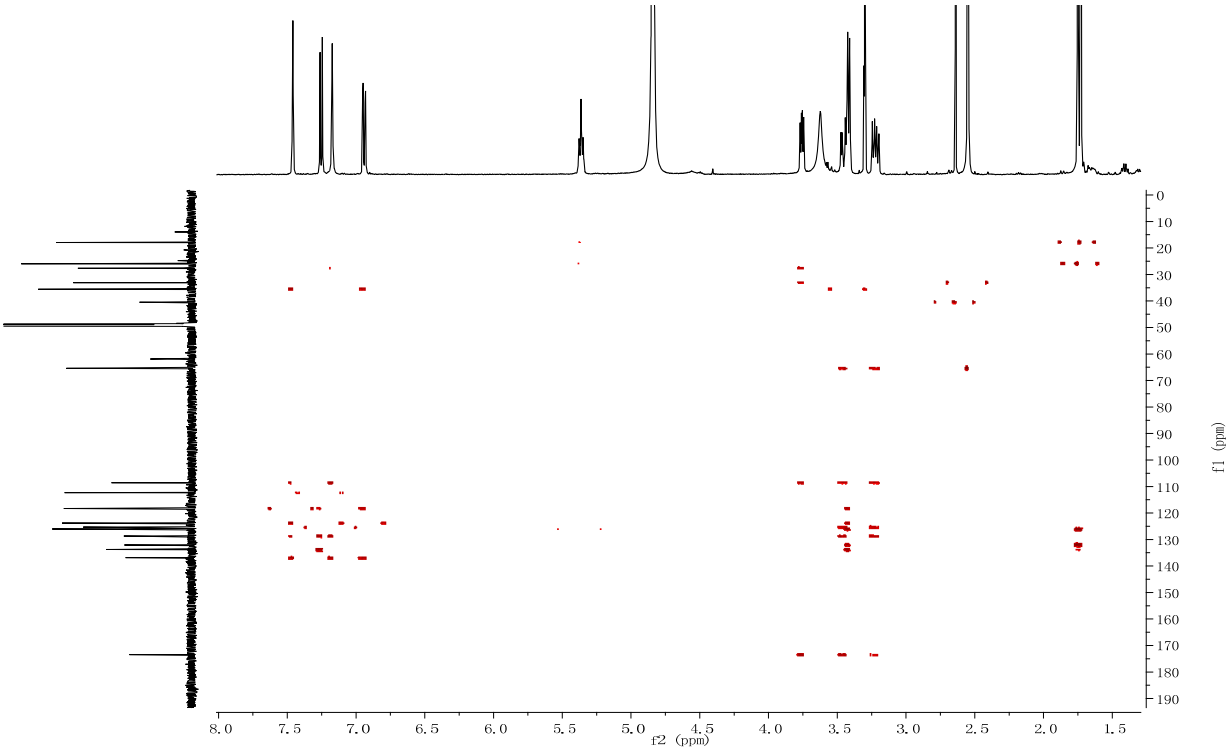


Figure S9.4. HMBC spectrum of **7b** in CD_3OD (500 MHz)

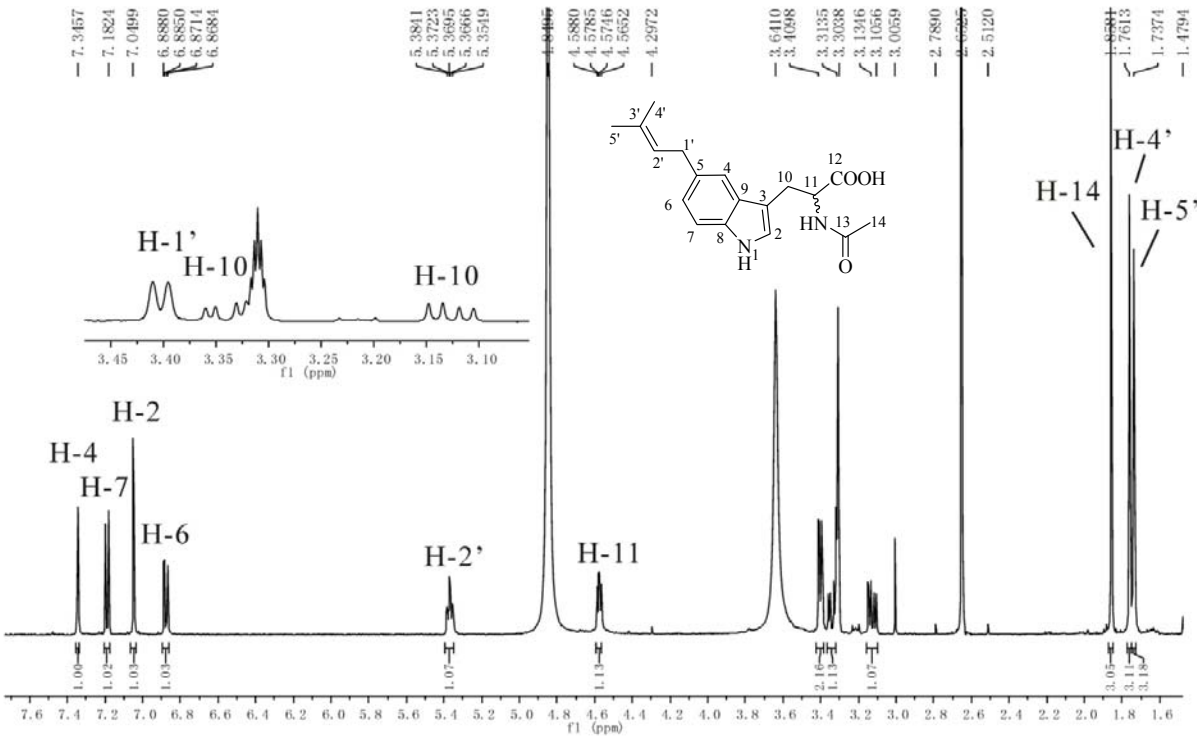


Figure S10. ^{1}H-NMR spectrum of **8b** in CD_3OD (500 MHz)

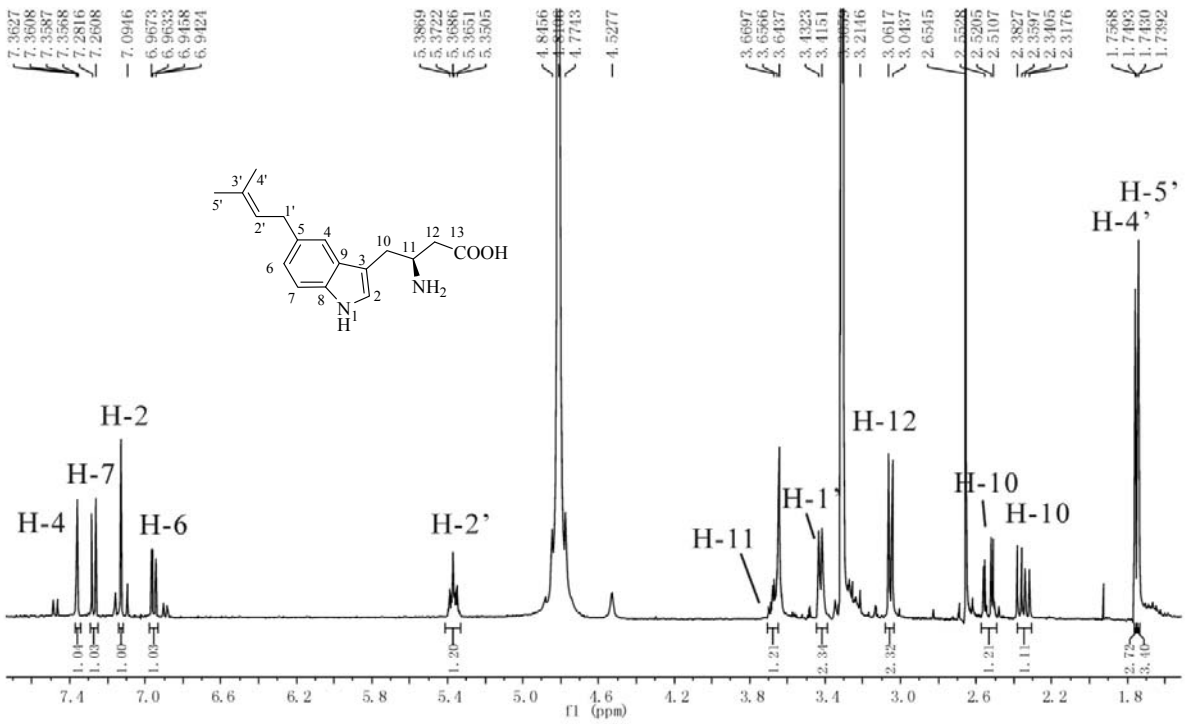


Figure S11. ^{1}H-NMR spectrum of **9b** in CD_3OD (400 MHz)

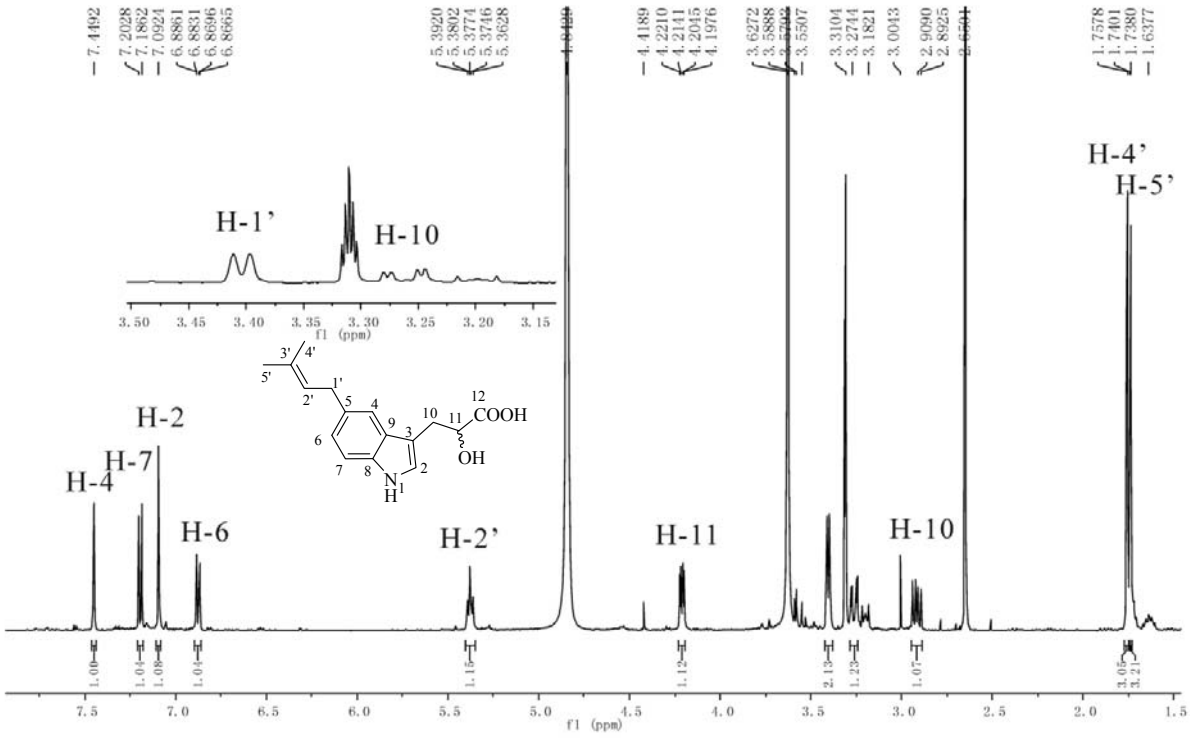


Figure S12. ^{1}H-NMR spectrum of **10b** in CD_3OD (500 MHz)

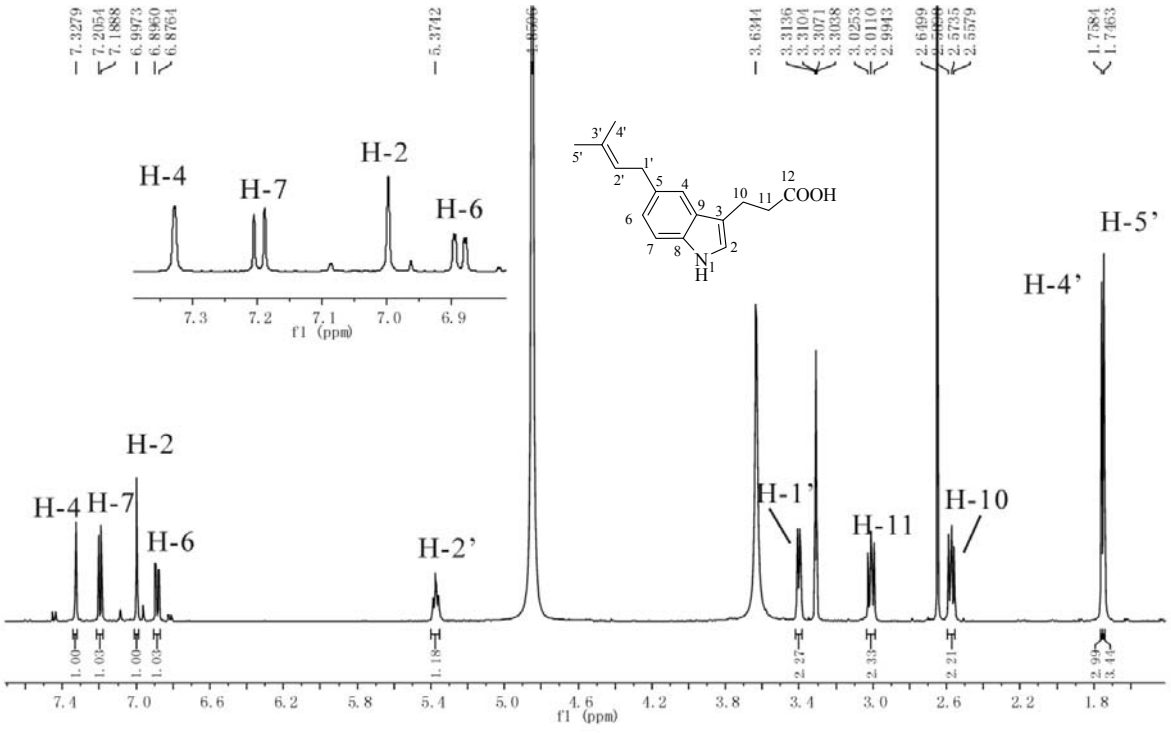


Figure S13. ^{1}H-NMR spectrum of **11b** in CD_3OD (500 MHz)

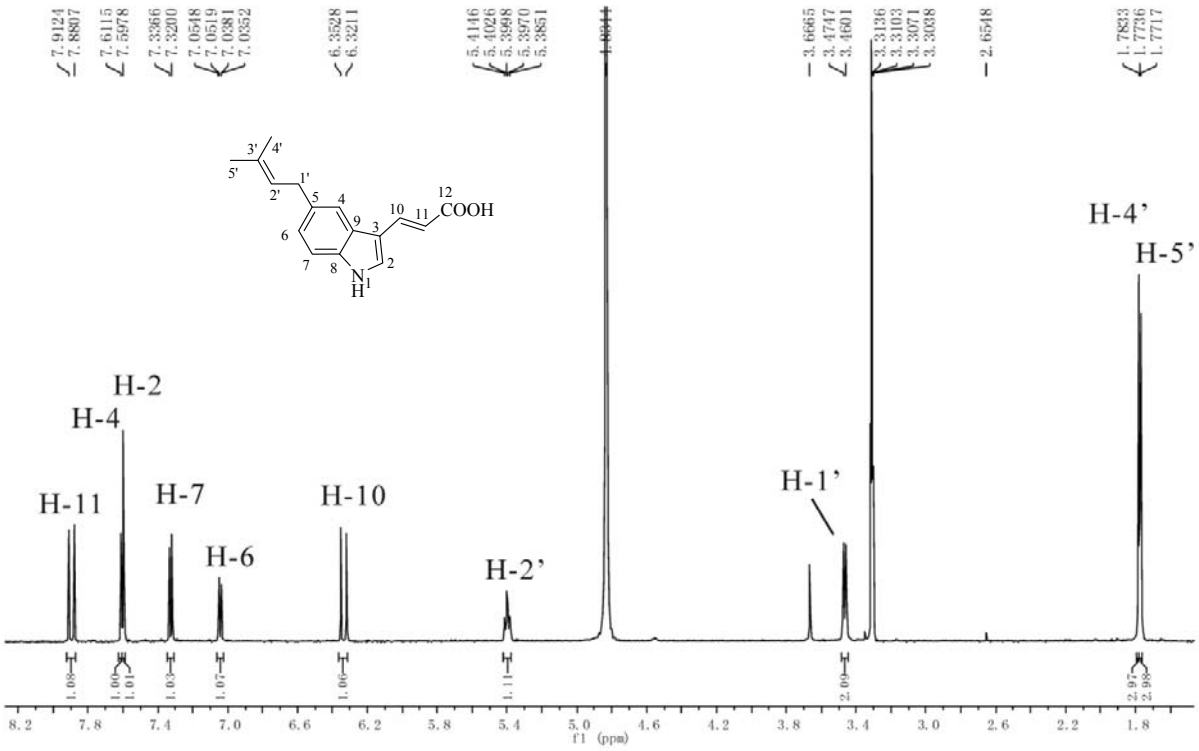


Figure S14. ^{1}H-NMR spectrum of **12b** in CD_3OD (500 MHz)