Supporting Information

Selective geranylation of biflavonoids by *Aspergillus terreus* aromatic prenyltransferase AtaPT

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Experimental Procedures

Chemicals

Geranyl diphosphate (GPP) was prepared according to the method described by Woodside.[1]

Plasmid construction, protein overproduction in *E.coli* and purification

The PCR fragment containing the entire coding sequence of *ataPT* was amplified using primers ataPT_F_NdeI (5′-ATACATATGCTCCCCCCATCAGACA-3′) and ataPT_R_XhoI (5′-ATTCTCGAGTTAGTGGTGATGGTGATGATGCACACG TGCGACATTTCCC -3′), and then digested by restriction enzymes NdeI and XhoI. The NdeI-XhoI fragment was ligated into the NdeI-XhoI linearized pCDFDuet™-1 (Novagen) to yield the *E.coli* expression vector pCDFDuet-ataPT. *E.coli* BL21 (DE3) cells harboring the plasmid pCDFDuet-ataPT were cultivated in a 2000 ml Erlenmeyer flask containing 500 ml liquid TB medium supplemented with 100 μg/ml streptomycin and grown at 37 °C to the OD value at 600 nm of 0.6. Isopropyl thiogalactoside (IPTG) was added into the culture to a final concentration of 1.5 mM, and the cells were induced at 37°C for 30 h. The bacterial cells were centrifuged and the pellets were resuspended in lysis buffer (10 mM imidazole, 50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0) at 2-5 mL per gram wet weight. After sonication on ice, the lysate was centrifuged at 13,000 x g for 30 min at 4 ◦C to remove the cellular debris. One-step purification of the soluble his_6-tagged fusion protein by affinity chromatography with Ni-NTA agarose resin (Qiagen) was performed according to the manufacturer's instructions. To change the buffer, the purified protein was passed through a PD-10 column (GE Healthcare), which had been equilibrated with 50 mM Tris-HCl, pH 7.5, previously. AtaPT was eluted with the 50 mM Tris-HCl, 15 % glycerol, pH 7.5. The purified enzyme was checked by SDS-PAGE (Figure S1). Bradford Protein Assay was used for protein quantification.

Enzyme assays

The reaction mixtures (100μL) for determination of enzymatic activities contained 0.5 mM biflavonoid, 10 mM $CaCl_2$, 10% DMSO (v/v), 0.8 mM DMAPP or GPP and 100 μg purified recombinant protein AtaPT in 50 mM Tris–HCl, pH 7.5. After incubation for 16 h at 37 °C, the reaction was terminated by the addition of 100 μl of MeOH and vortexing for 1 min. The protein was removed by centrifugation at 12,000 g, 4 ℃ for 20 min. The supernatant was directly analyzed by HPLC. For kinetic parameter determination, the enzyme assays (50μL) contained 10 mM $CaCl_2$, 10% DMSO (v/v), 2 mM GPP and substrates at final concentrations of 0.02, 0.05, 0.1, 0.2, 0.5 and 1 mM. The used protein amounts and incubation time were 12.5 ug/60 min for substrates **1**-**3** and 25 ug/90 min for substrate **4**.

For enzymatic product isolation, assays were carried out under the same condition described above in large scales (30-60 ml). The reactions were incubated at 37 °C for overnight and then extracted by ethyl acetate for 3 times. The residues were dissolved in methanol and injected to a semi-preparative HPLC for purification.

HPLC conditions for analysis and isolation

The enzymatic products were analyzed by high performance liquid chromatography (HPLC Agilent 1100) with a Venusll MP C18 4.6ID ×10cm column. Water (solvent A) and acetonitrile (solvent B) were used at a flow rate of 1 ml/min. The analysis started with a linear gradient of 30-100 % (solvent B, v/v) for 12 minutes. The column was then washed with 100% (v/v) solvent B for 5 minutes, and equilibrated with 30% (v/v) solvent B for 5 minutes.

For compound isolation, semi-preparative HPLC using semi-preparative column (YMC-Pack, ODS-A 250×10 mm) was eluted at a flow rate of 3.0 ml/min. A linear gradient of 35-95 % (v/v) solvent B for 40 min was used to give compounds.

Protein sequence of his_6-tagged AtaPT

MLPPSDSKDPRPWQILSQALGFPNYDQELWWQNTAETLNRVLEQCDYSVHL
QYKYLAFYHKYILPSLGPFRRPGVEPEYISGLSHGGHPLEISVKIDKSKTICRL

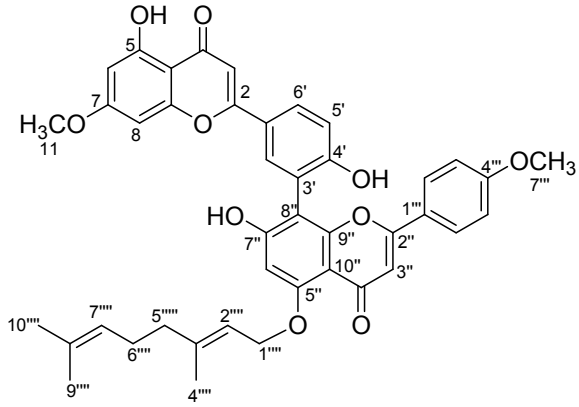
GLQAIGPLAGTARDPLNSFGDRELLKNLATLLPHVDLRLFDHFNAQVGLDRA
QCAVATTKLIKESHNIVCTSLDLKDGEVIPKVYFSTIPKGLVTETPLFDLTFAA
IEQMEVYHKDAPLRTALSALKDFLRPRVPTDASITPPLTGLIGVDCIDPMLSRL
KVYLATFRMDLSLIRDYWTLGGLLKDEGTMKGLEMVETLAKTLKLGDEAC
ETLDAERLPFGINYAMKPGTAELAPPQIYFPLLGINDGFIADALVEFFQYMGW
EDQASRYKDELKAKFPNVDISQTKNVHRWLGVAYSETKGPSMNIYYDVVA
GNVARVHHHHHH*

Supplementary Tables and Figures

Table S1: HR-ESI-MS data of compounds **1a-4a**.

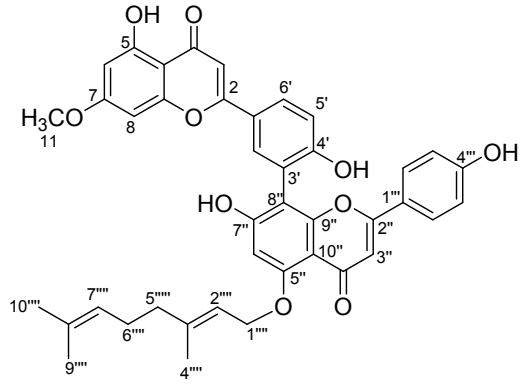
Compounds	Chemical formula	HR-ESI-MS data		Deviation (ppm)
		Calculated	Measured	
1a	$C_{42}H_{38}O_{10}$	703.2543 $[M+H]^+$	703.2545	0.3
2a	$C_{41}H_{36}O_{10}$	689.2387 $[M+H]^+$	689.2382	-0.7
3a	$C_{40}H_{34}O_{10}$	675.2230 $[M+H]^+$	675.2229	-0.1
4a	$C_{40}H_{34}O_{10}$	675.2230 $[M+H]^+$	675.2229	-0.1

Table S2: ^{1}H NMR and ^{13}C NMR data of **1a** in DMSO-d_6.

Compd	 **1a**	
Pos.	δ_H, multi, J	δ_C
2	-	165.0
3	6.75, s	102.7
4	-	181.9
5	-	161.1
6	6.28, s	98.0
7	-	164.5
8	6.36, s	92.5
9	-	157.3
10	-	104.6
11 (OCH_3)	3.74, s	56.0
1′	-	117.2
2′	8.23, bd	131.5
3′	-	123.9
4′	-	166.6
5′	6.86, d, 8.6	119.9
6′	7.87, dd, 2.0, 8.6	126.5
2′′	-	159.0
3′′	6.52, s	106.2
4′′	-	176.0
5′′	-	158.5
6′′	6.25, s	101.4
7′′	-	169.5
8′′	-	108.5
9′′	-	156.4
10′′	-	106.3
1′′′	-	123.4
2′′′	7.69, d, 8.7	127.3

3′′′	6.65, d, 8.7	114.2
4′′′	-	161.4
5′′′	6.65, d, 8.7	114.2
6′′′	7.69, d, 8.7	127.3
7′′′(OCH_3)	3.54, s	55.3
1′′′′	4.59, d, 5.7	65.5
2′′′′	5.46, t, 5.7	120.7
3′′′′	-	139.6
4′′′′	1.74, s	16.5
5′′′′	2.07, m	39.0
6′′′′	2.10, m	25.9
7′′′′	5.12, t, 6.5	123.8
8′′′′	-	131.1
9′′′′	1.65, s	25.5
10′′′′	1.59, s	17.6

Table S3: ^{1}H NMR and ^{13}C NMR data of **2a** in DMSO-d_6.

Compd	 **2a**	
Pos.	**δ_H, multi, *J***	**δ_C**
2	-	165.1
3	6.80, s	103.0
4	-	181.9
5	-	161.1
6	6.31,overlaps	98.1
7	-	165.1
8	6.55, overlaps	92.7
9	-	157.4
10	-	104.7
11 (OCH_3)	3.77, s	56.1
1′	-	117.4
2′	8.19 , d, 2.4	131.4
3′	-	123.9
4′	-	164.2
5′	6.91, d, 8.7	119.7
6′	7.92, dd, 2.4, 8.7	127.5
2′′	-	159.7
3′′	6.45, s	105.4
4′′		176.0
5′′	-	158.6
6′′	6.31, s	97.7
7′′	-	167.1
8′′	-	107.4
9′′	-	156.3
10′′	-	105.6
1′′′	-	121.7

2‴	7.55, d, 8.8	127.8
3‴	6.54, d, 8.7	115.6
4‴	-	160.2
5‴	6.54, d, 8.7	115.6
6‴	7.55, d, 8.8	127.8
1⁗	4.60, d, 5.9	65.6
2⁗	5.46, t, 6.2	120.5
3⁗	-	139.9
4⁗	1.74, s	16.6
5⁗	2.06, m	38.9
6⁗	2.11, m	25.5
7⁗	5.11, t, 6.1	123.8
8⁗	-	131.1
9⁗	1.65, s	25.9
10⁗	1.58, s	17.6

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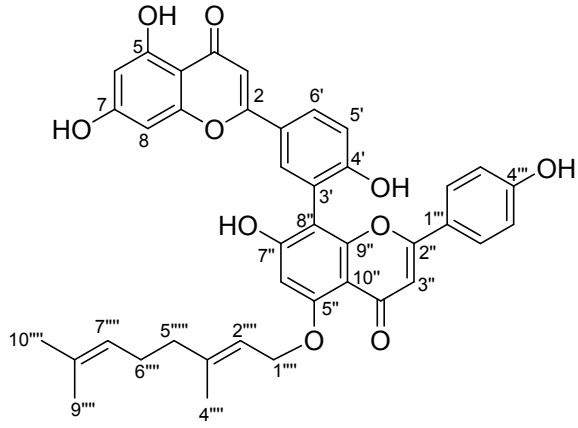
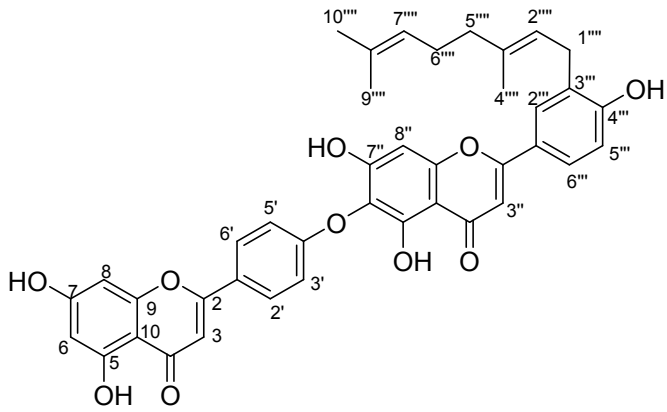
Table S4: ^{1}H NMR data of **3a** in acetone-d_6 and DMSO-d_6.

Compd	 **3a**	
Pos.	**δ_H, multi, *J* in Acetone-d_6**	**δ_H, multi, *J* in** DMSO-d_6
2	-	-
3	6.74, s	6.63, s
4	-	-
5	-	-
6	6.23, d, 2.0	6.05, br.s
7	-	-
8	6.53, d, 2.0	6.14, br.s
9	-	-
10	-	-
5-OH	13.04	-
1′	-	-
2′	8.10, d, 2.3	8.22, d, 1.4
3′	-	-
4′	-	-
5′	7.23, d, 8.7	6.78, d, 6.8
6′	8.03, dd, 2.3, 8.6	7.80, dd, 1.6, 6.8
2′′	-	-
3′′	6.62, s	6.39, s
4′′	-	-
5′′	-	-
6′′	6.45, s	6.16, s
7′′	-	-
8′′	-	-
9′′	-	-
10′′	-	-

1‴	-	-
2‴	7.54, d, 8.8	7.61, d, 7.2
3‴	6.78, d, 8.8	6.48, d, 7.2
4‴	-	-
5‴	6.78, d, 8.8	6.48, d, 7.2
6‴	7.54, d, 8.8	7.61, d, 7.2
1⁗	4.70, d, 6.0	4.57, d, 6.7
2⁗	5.56, t, 5.9	5.46, t, 6.8
3⁗	-	-
4⁗	1.77, s	1.73, s
5⁗	2.08, m	2.06, m
6⁗	2.14, m	2.11, m
7⁗	5.13, t, 6.8	5.12, t, 6.1
8⁗	-	-
9⁗	1.66, s	1.65, s
10⁗	1.60, s	1.59, s

Table S5. ^{1}H NMR and ^{13}C NMR data of **4a** in DMSO-d_6.

Compd	 **4a**	
Pos.	δ_H, multi, J	δ_C
2	-	163.2
3	6.86, s	103.9
4	-	181.9
5	-	161.5
6	6.20, d, 2.0	99.0
7	-	164.5
8	6.49, d, 2.0	94.1
9	-	157.4
10	-	104.0
5-OH	12.89, s	-
7-OH	10.93, s	-
1′	-	124.3
2′	8.02, d, 8.9	128.4
3′	7.03, d, 9.0	115.4
4′	-	160.7
5′	7.03, d, 9.0	115.4
6′	8.02, d, 8.9	128.4
2′′	-	164.3
3′′	6.81, s	102.5
4′′	-	182.1
5′′	-	153.2
6′′	-	124.7
7′′	-	153.8
8′′	6.71, s	94.6
9′′	-	157.2
10′′	-	104.2

5′′-OH	13.21, s	-
1′′′	-	121.0
2′′′	7.79, overlap	128.0
3′′′	-	128.7
4′′′	-	159.2
5′′′	6.96, d, 8.2	115.5
6′′′	7.81, overlaps	126.1
4′′′-OH	10.41, s	-
1′′′′	3.31, d, 7.4	28.0
2′′′′	5.34, t, 6.8	122.2
3′′′′	-	135.6
4′′′′	1.72, s	16.0
5′′′′	2.01, m	39.3
6′′′′	2.07, m	26.3
7′′′′	5.08, t, 5.6	124.1
8′′′′	-	130.9
9′′′′	1.58, s	25.5
10′′′′	1.54, s	17.6

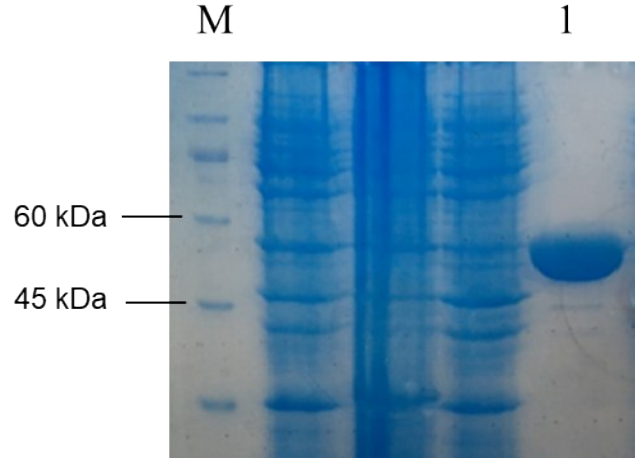


Figure S1: Analysis of purified his_6-AtaPT on SDS-PAGE.
Lane M: molecular mass standard; lane 1: purified his_6-AtaPT.

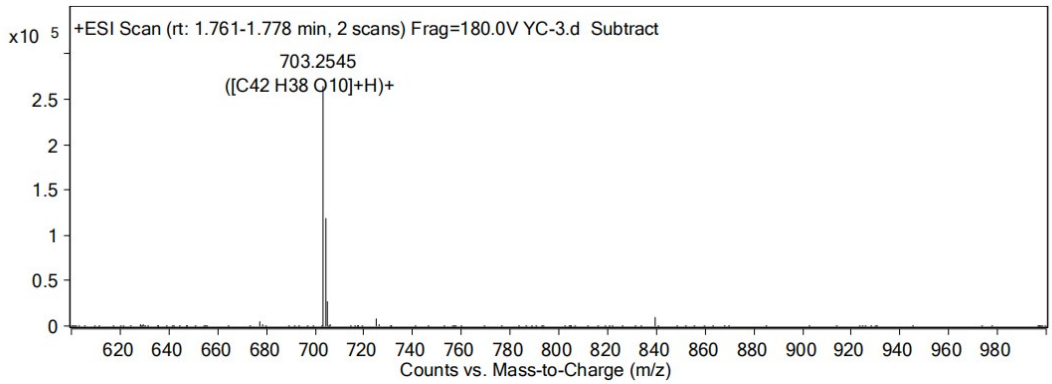


Figure S2: HR-ESI-MS spectrum of compound **1a**.

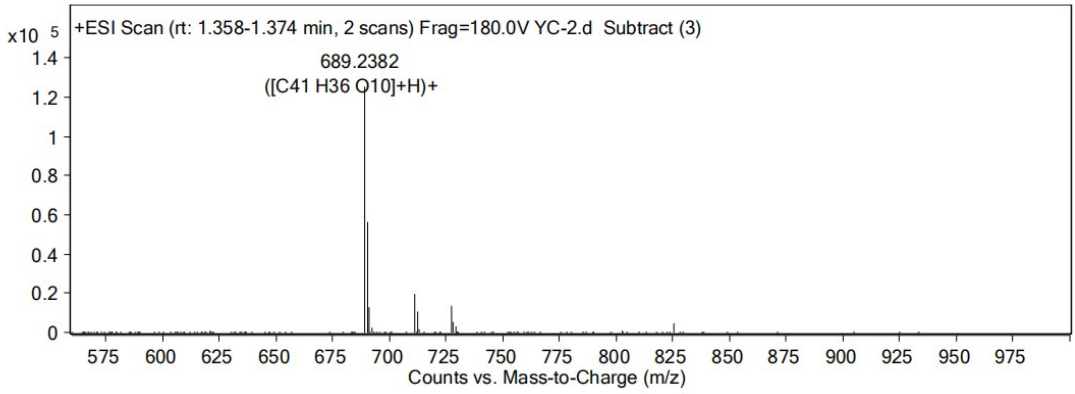


Figure S3: HR-ESI-MS spectrum of compound **2a**.

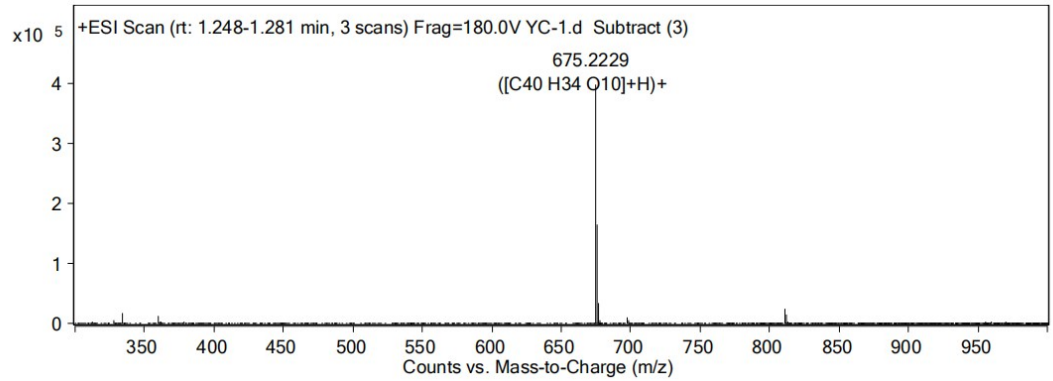


Figure S4: HR-ESI-MS spectrum of compound **3a**.

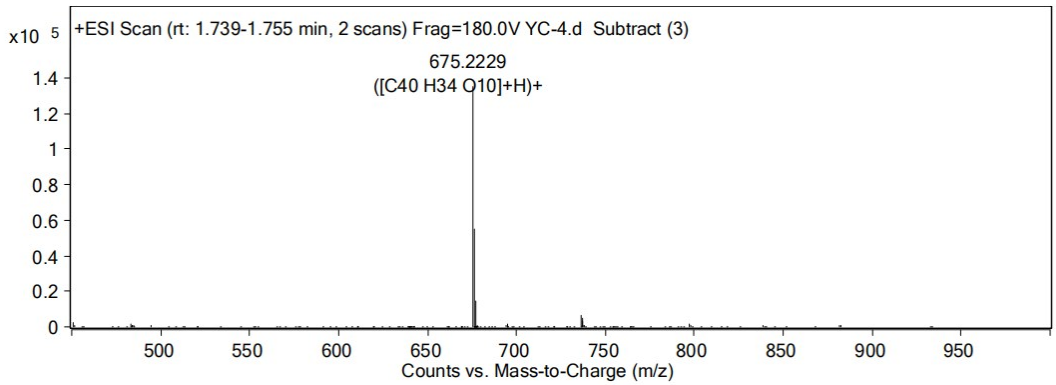


Figure S5: HR-ESI-MS spectrum of compound **4a**.

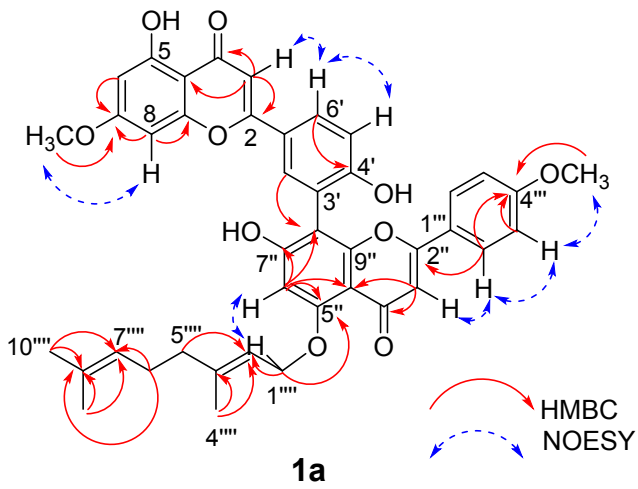


Figure S6: Key HMBC and NOESY correlations of AtaPT product **1a**.

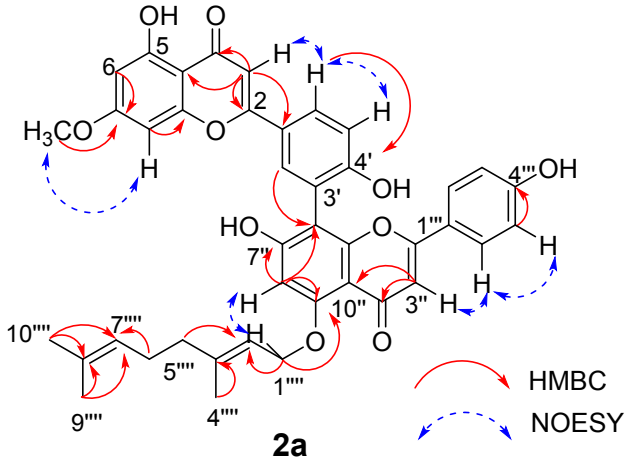


Figure S7: Key HMBC and NOESY correlations of AtaPT product **2a**.

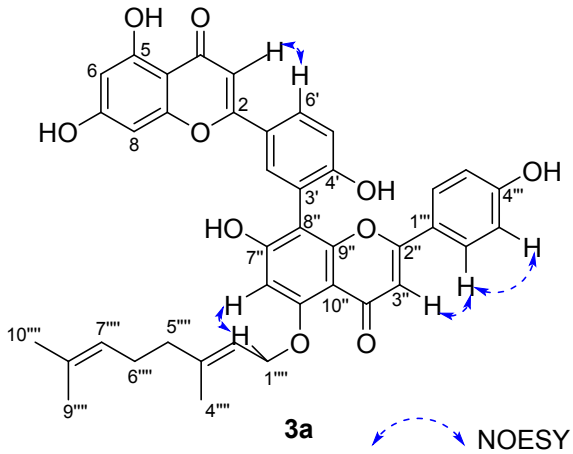


Figure S8: Key NOESY correlations of AtaPT product **3a**.

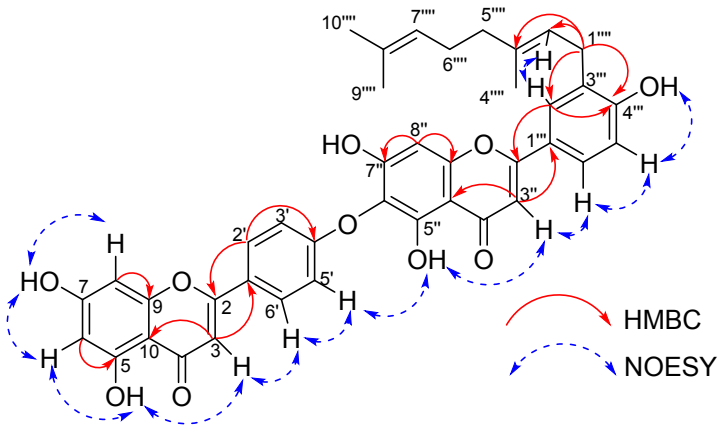


Figure S9: Key HMBC and NOESY correlations of AtaPT product **4a**.

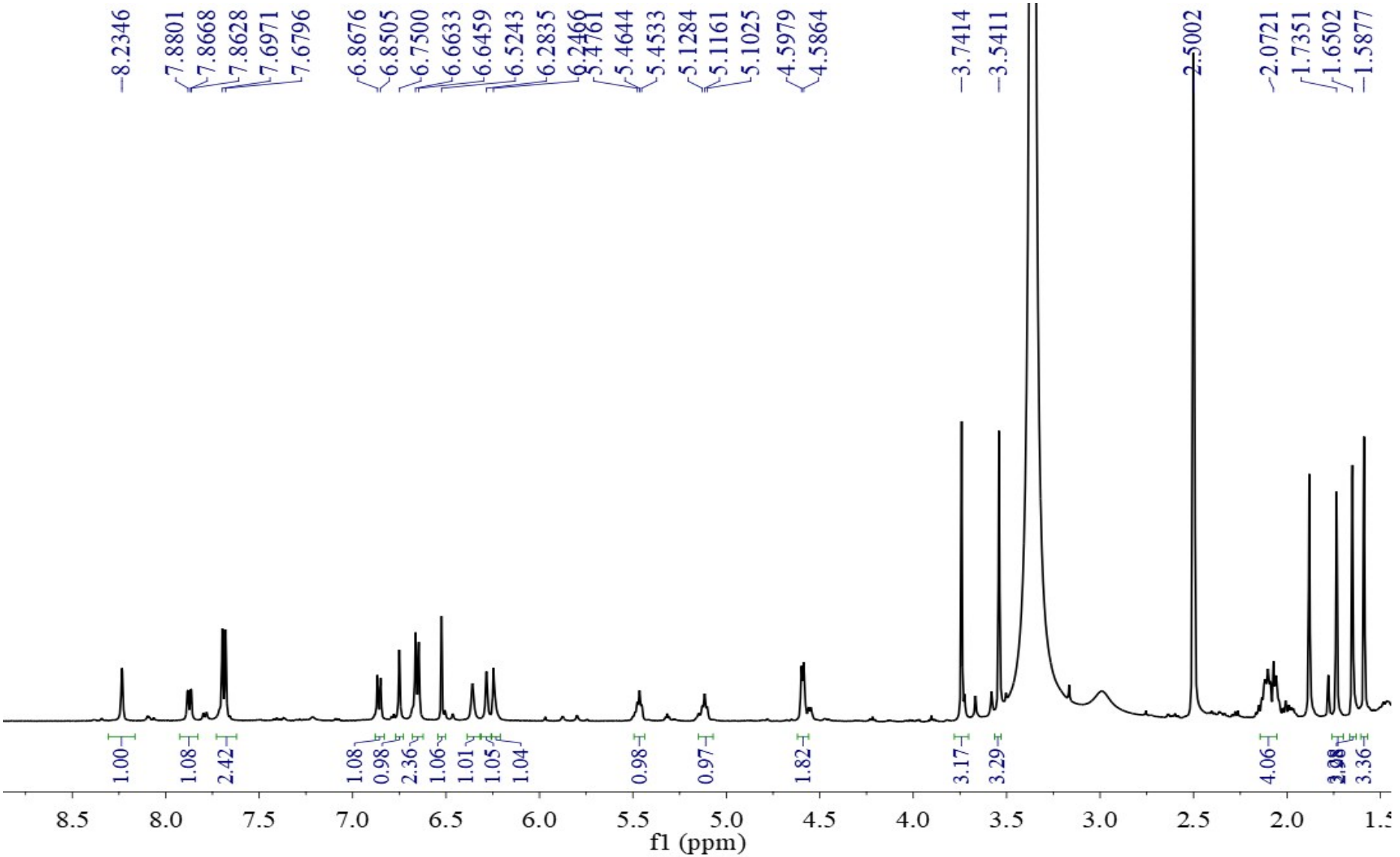


Figure S10: ^{1}H NMR spectrum of **1a** in DMSO-d_6 (500 MHz).

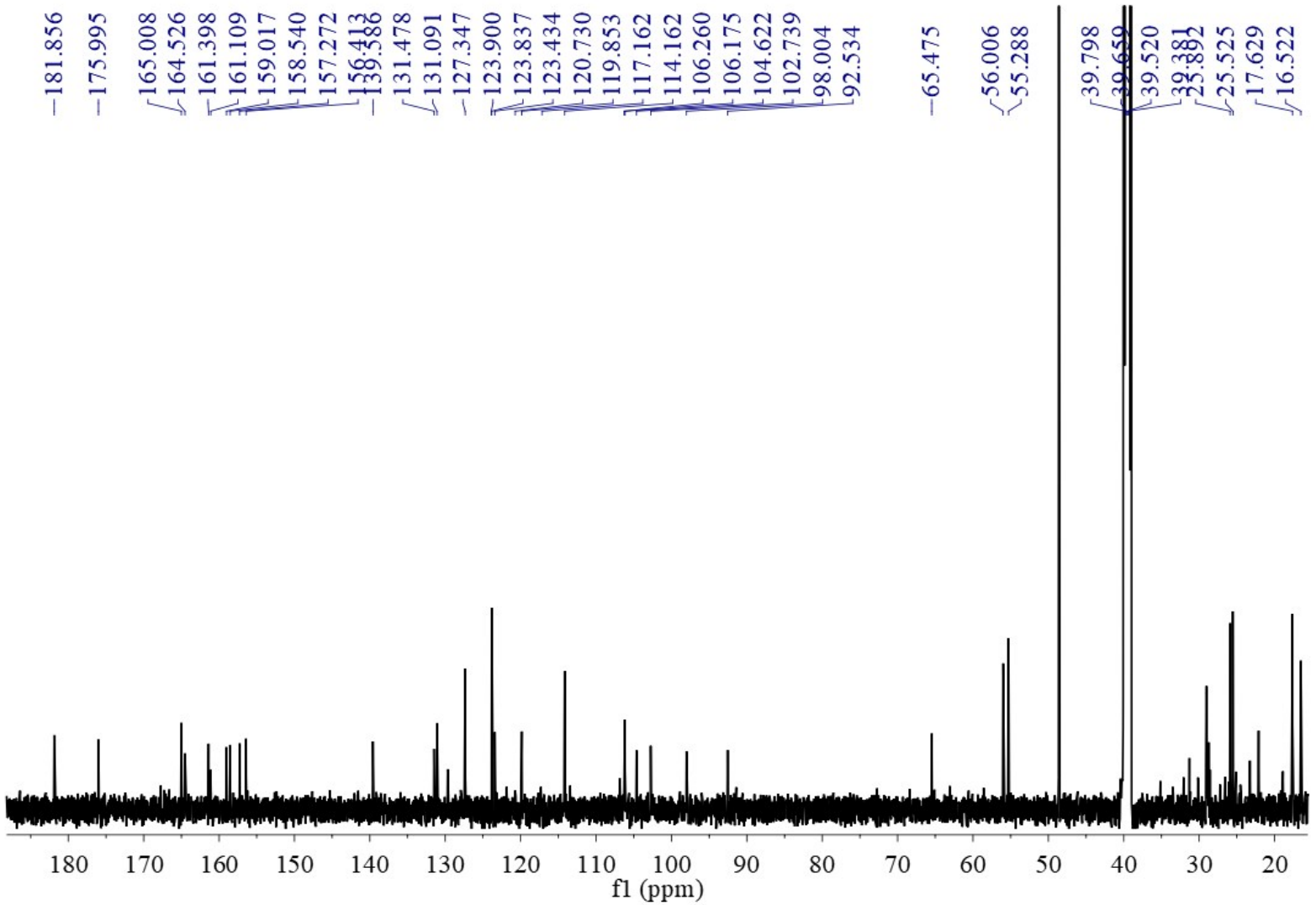


Figure S11: ^{13}C NMR spectrum of **1a** in DMSO-d_6 (600 MHz).

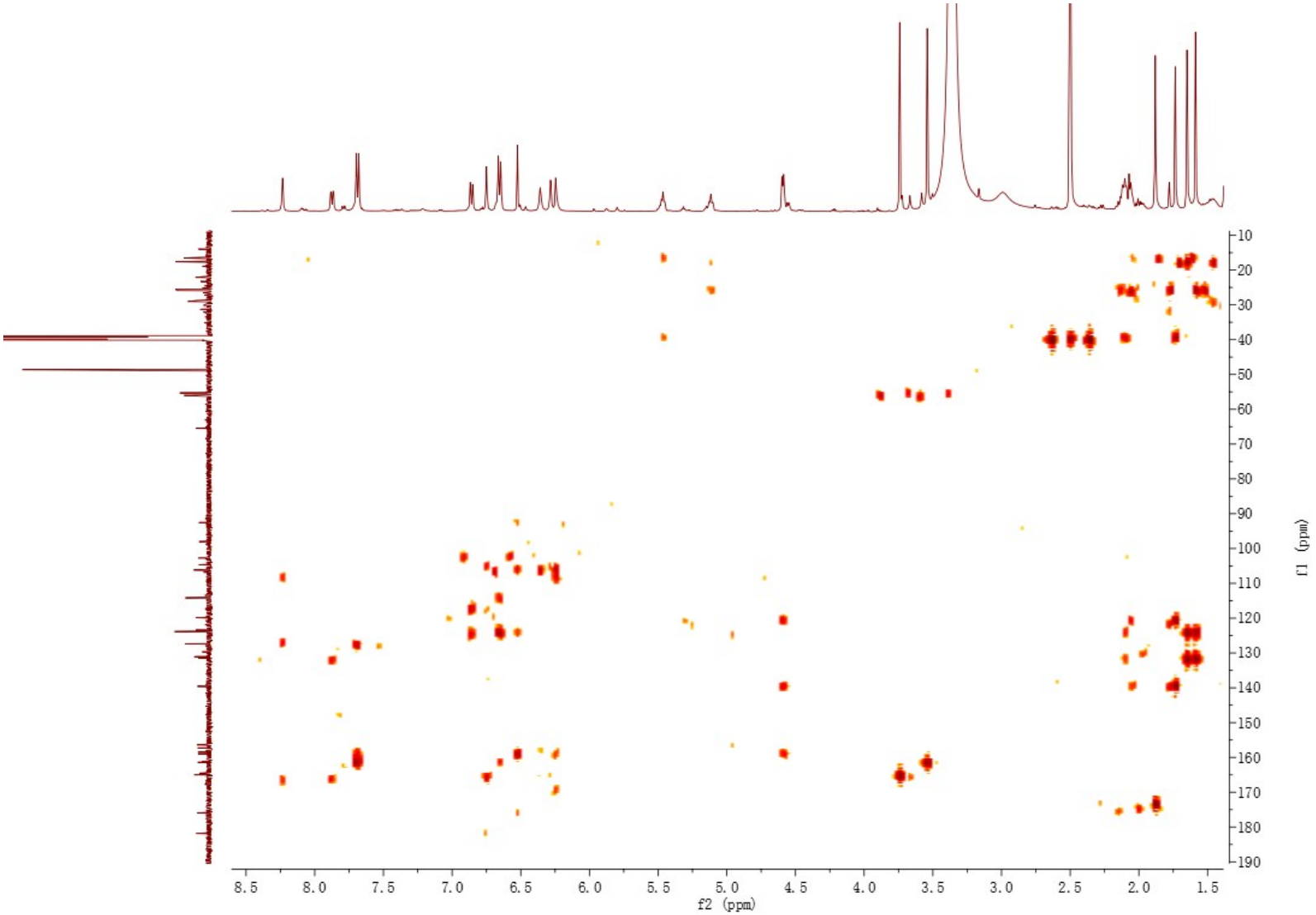


Figure S12: HMBC spectrum of **1a** in DMSO-d_6 (500 MHz).

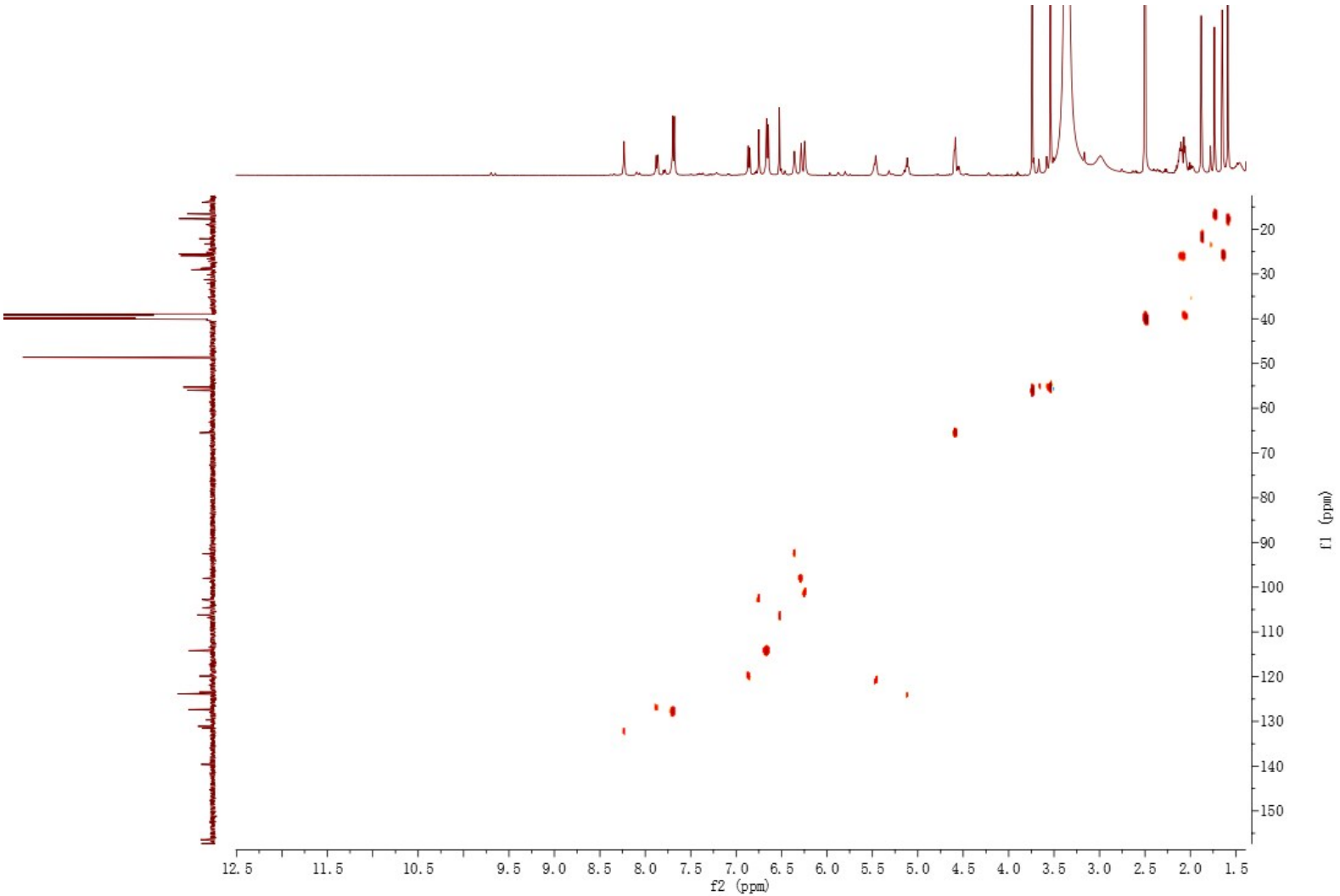


Figure S13: HSQC spectrum of **1a** in DMSO-d_6 **(**(400 MHz).

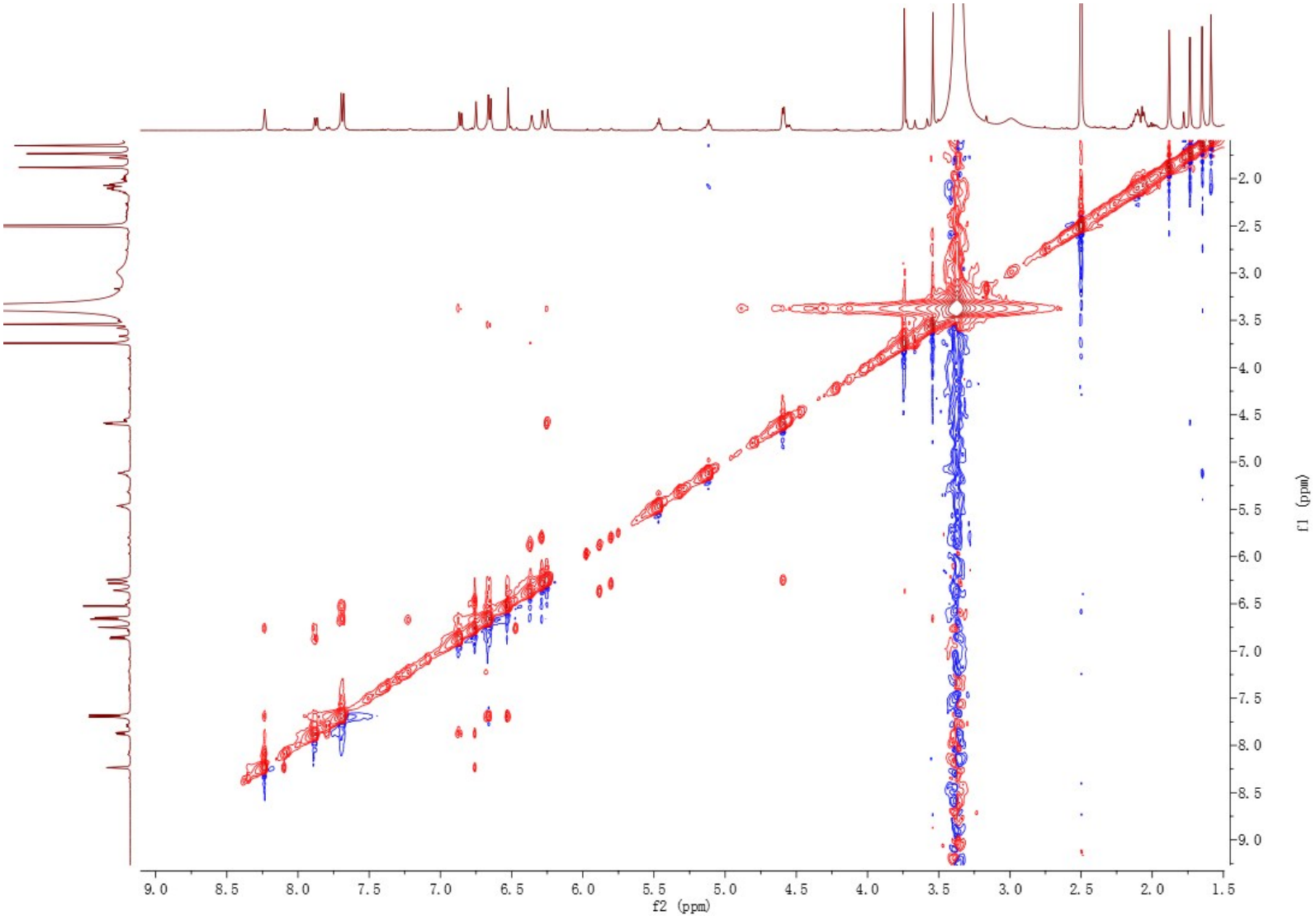


Figure S14: NOESY spectrum of **1a** in DMSO-d_6 (500 MHz).

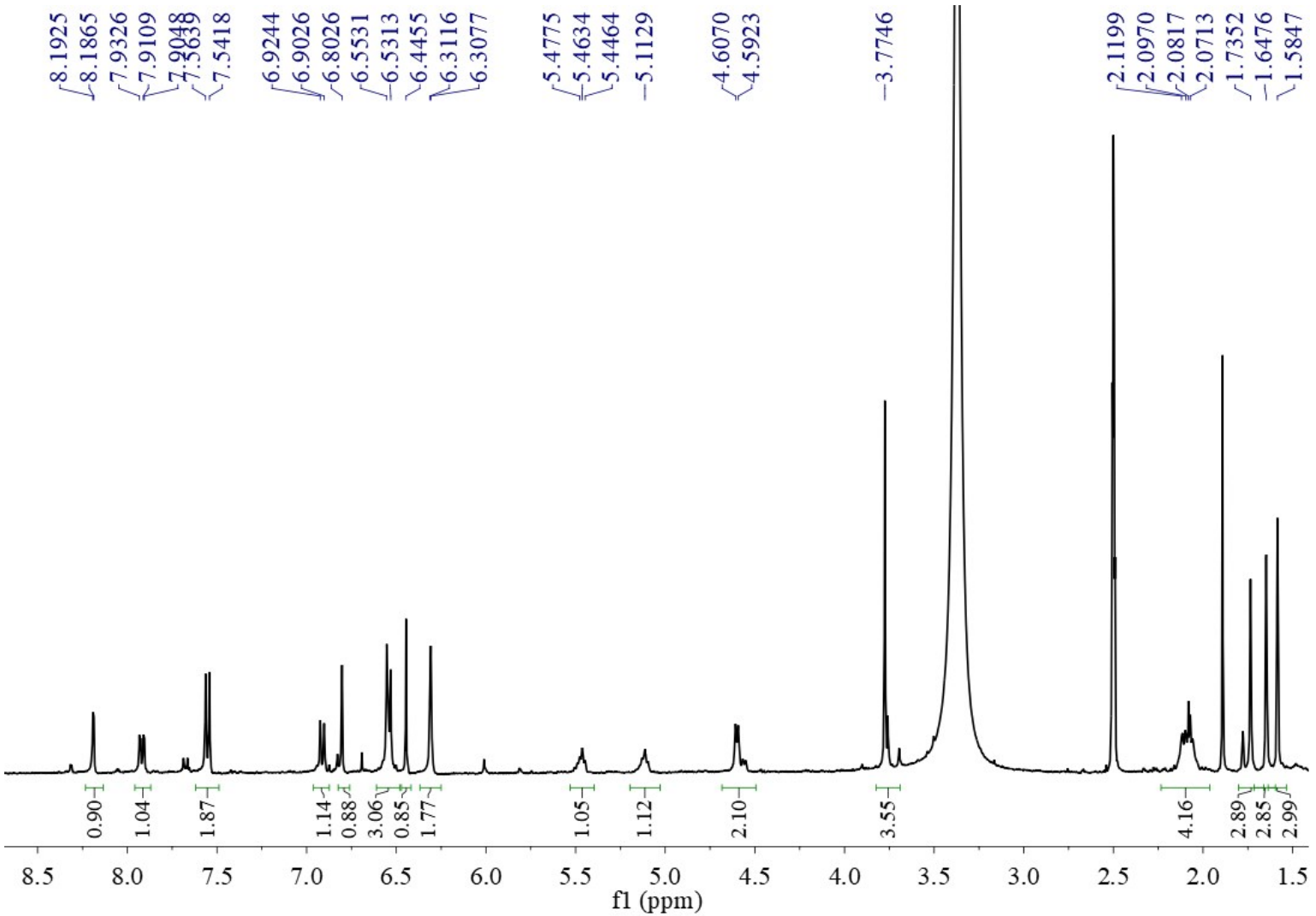


Figure S15: ^{1}H NMR spectrum of **2a** in DMSO-d_6 (400 MHz).

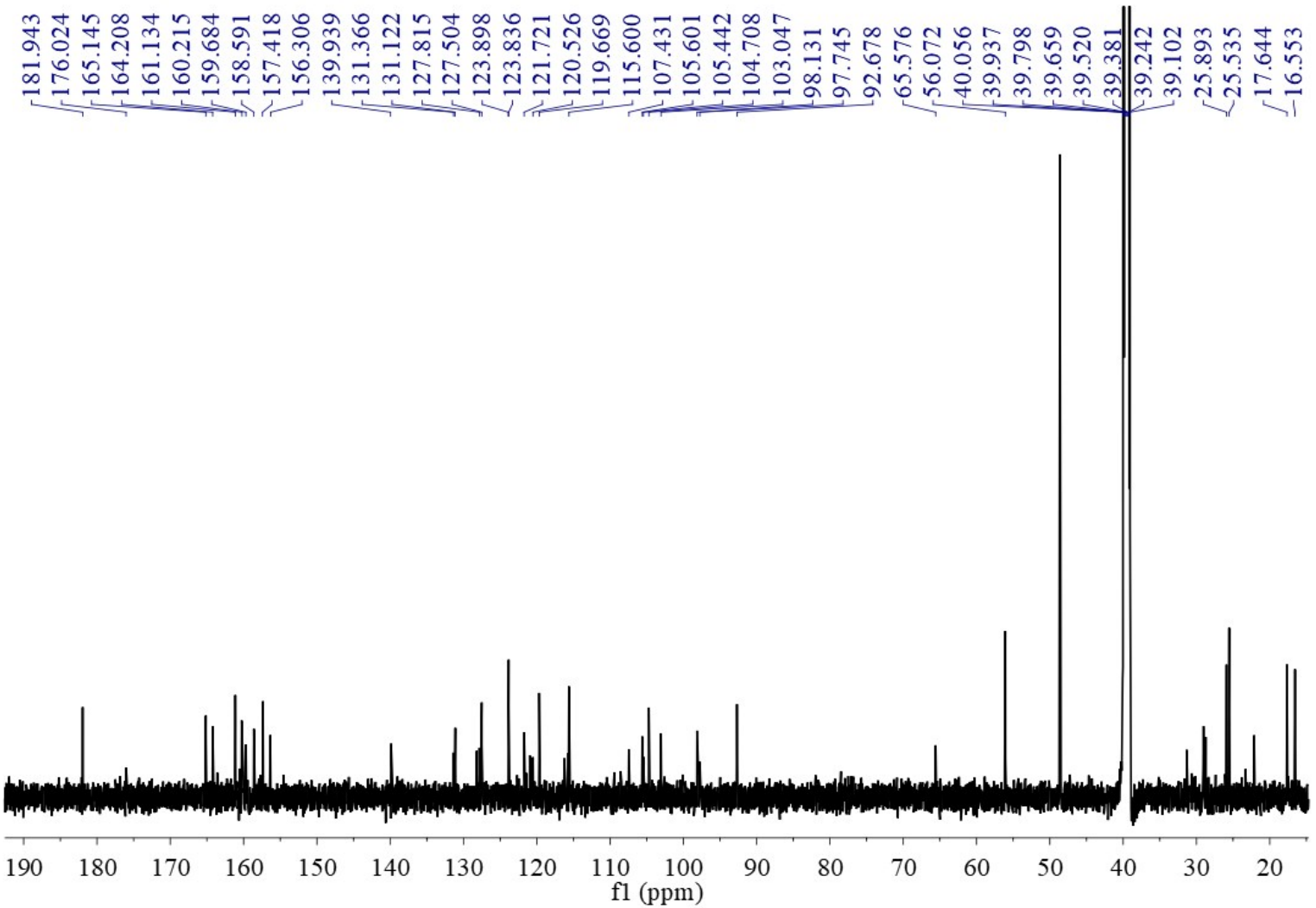


Figure S16: ^{13}C NMR spectrum of **2a** in DMSO-d_6 (600 MHz).

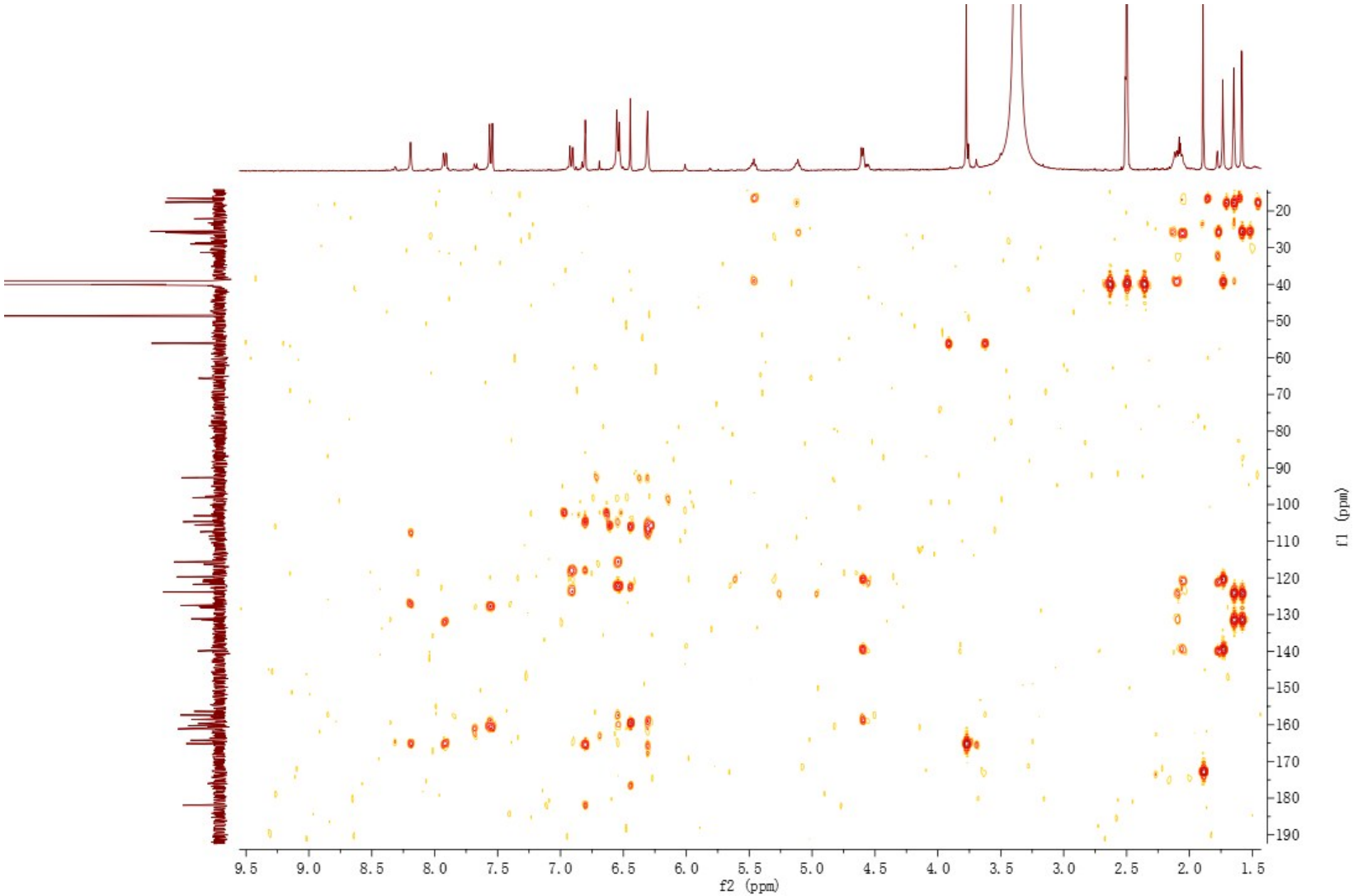


Figure S17: HMBC spectrum of **2a** in DMSO-d_6 (500 MHz).

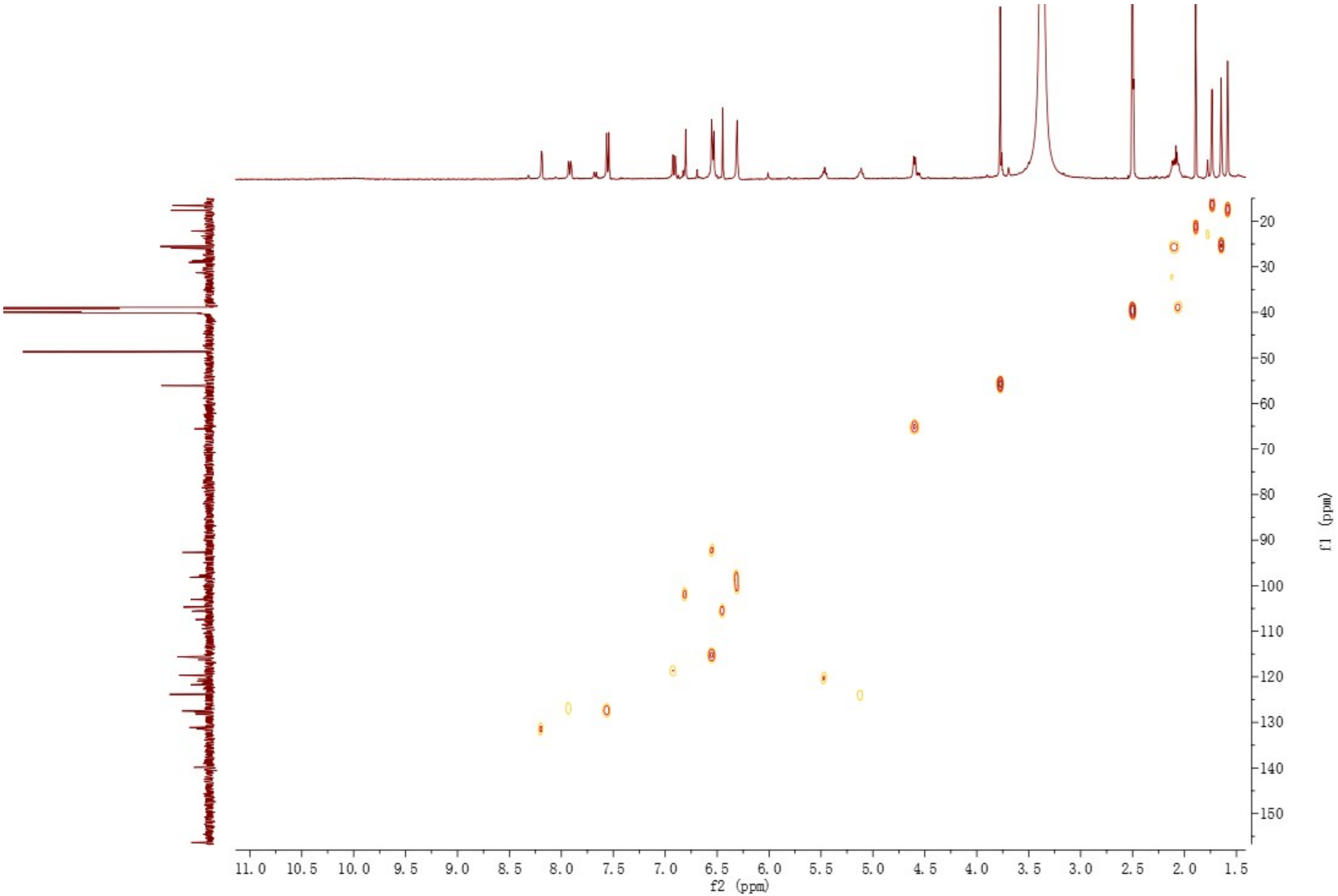


Figure S18: HSQC spectrum of **2a** in DMSO-d_6 (400 MHz).

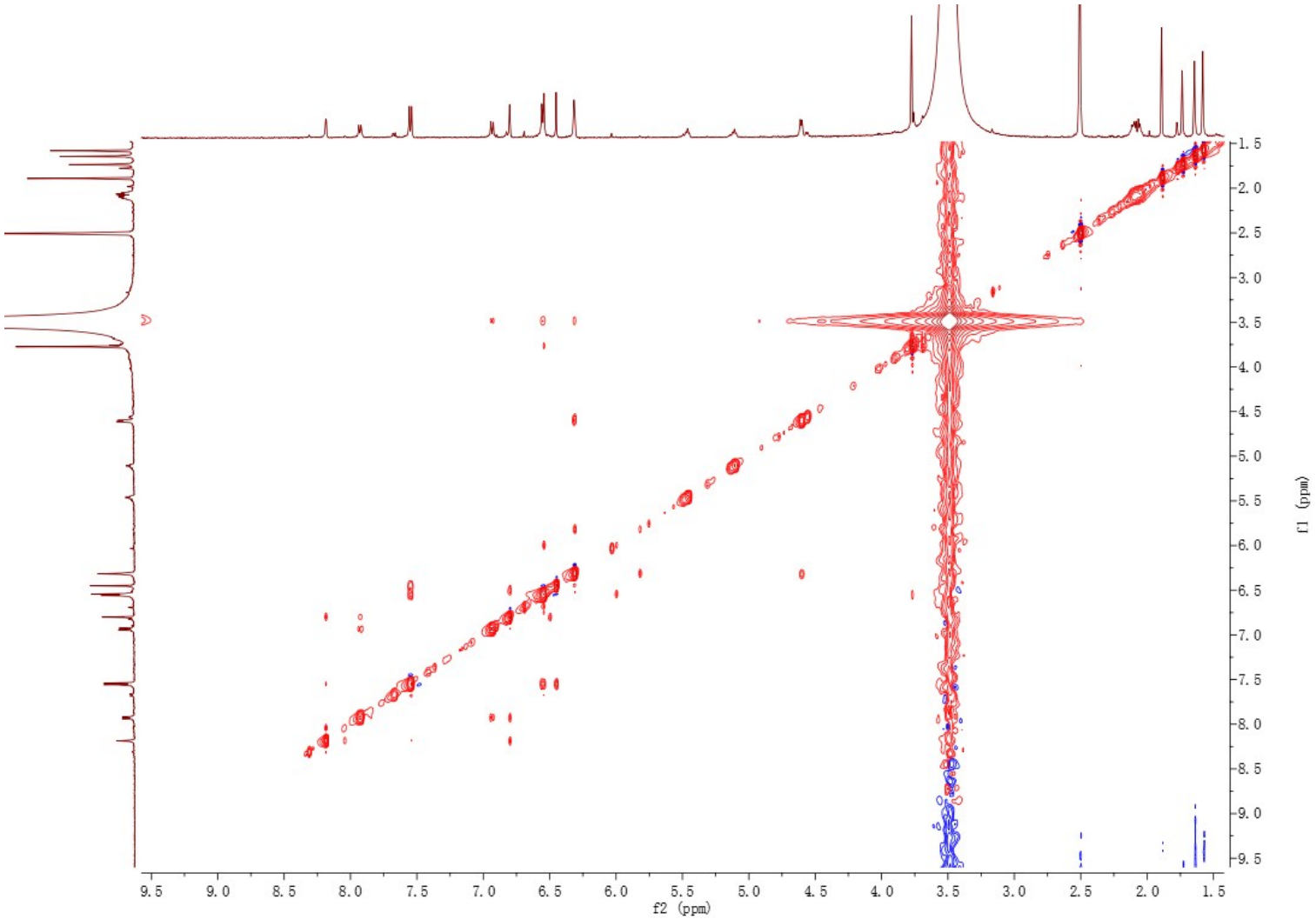


Figure S19: NOESY spectrum of **2a** in DMSO-d_6 (500 MHz).

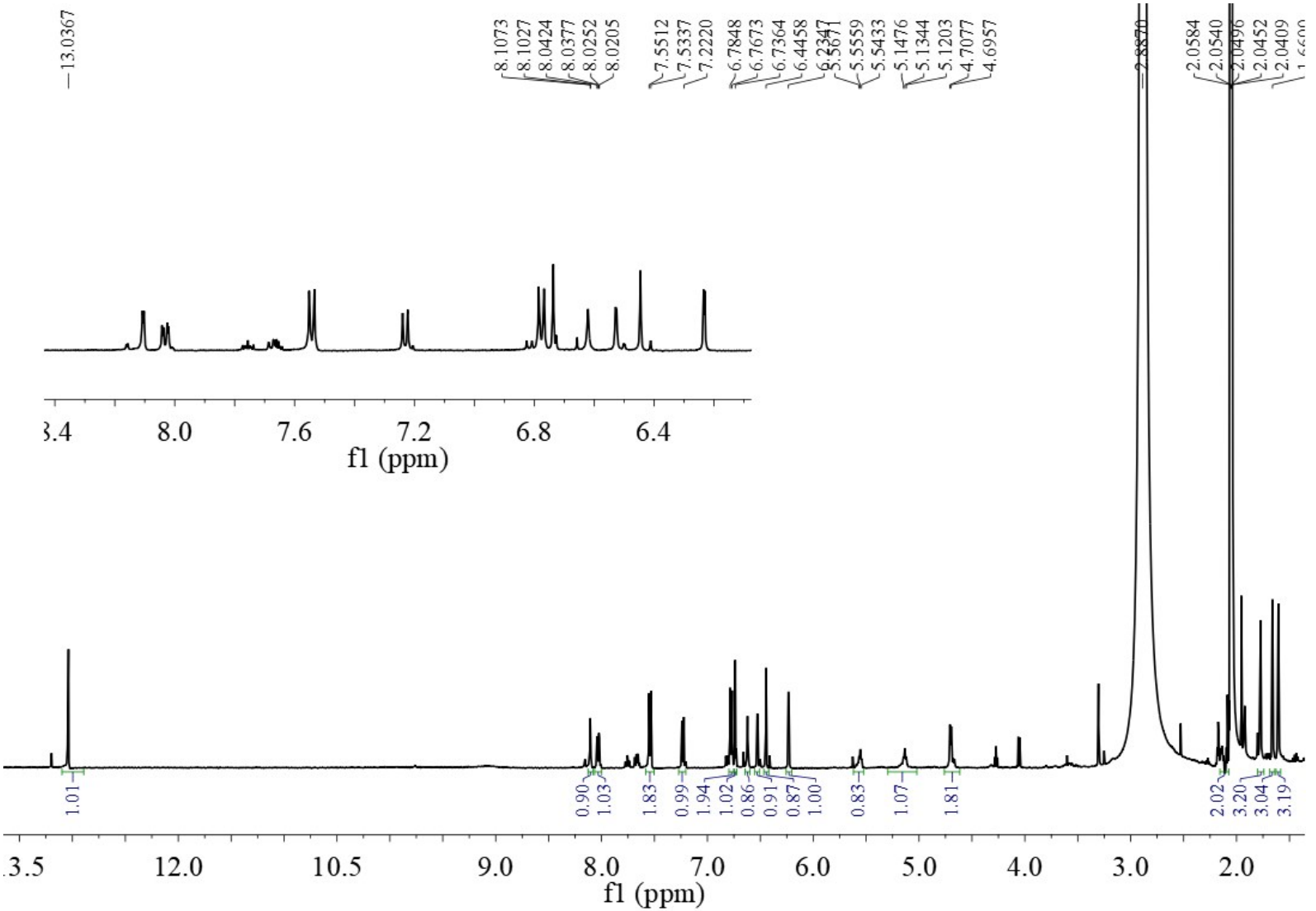


Figure S20: ^{1}H NMR spectrum of **3a** in acetone-d_6 (500 MHz).

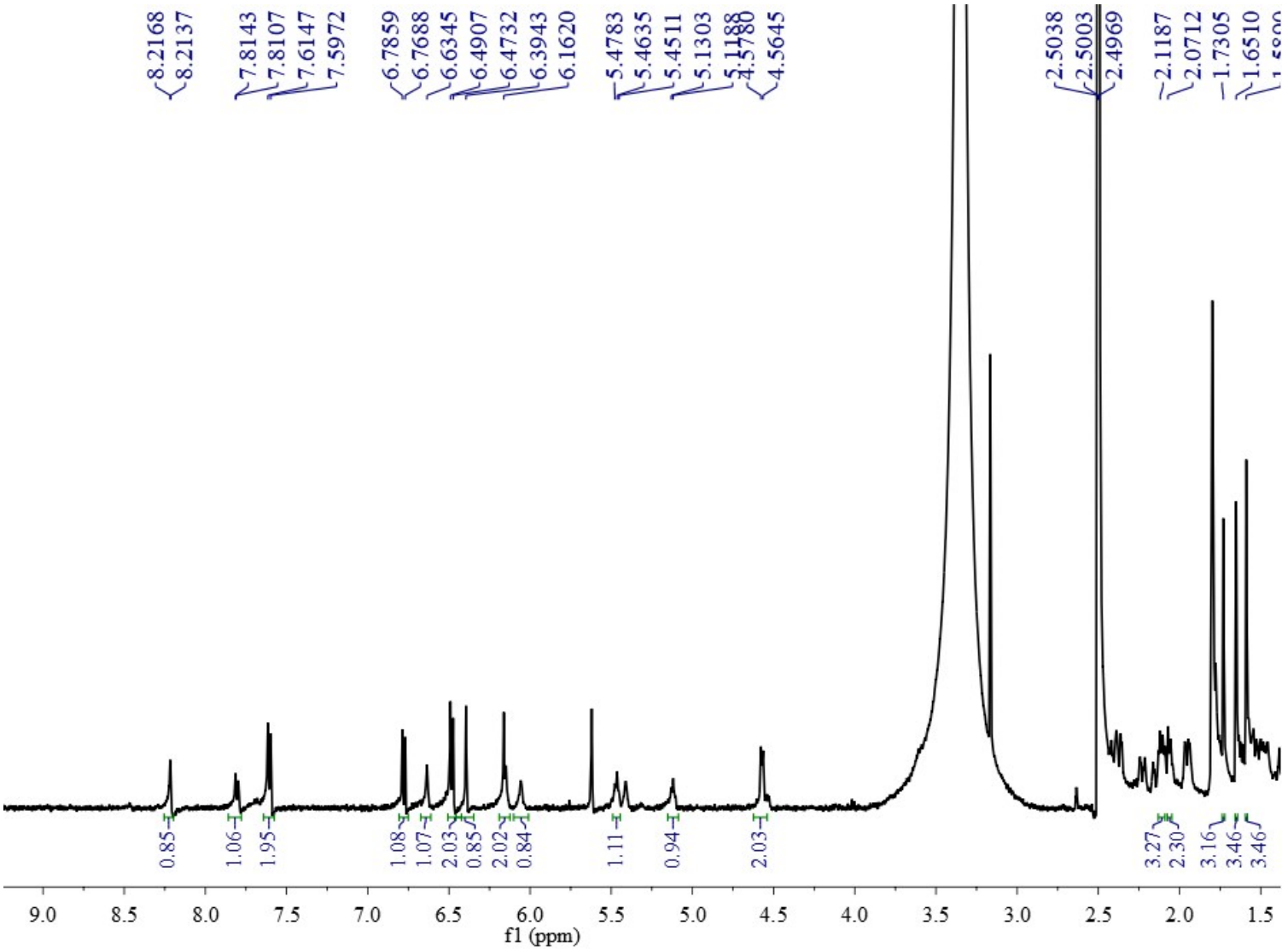


Figure S21: ^{1}H NMR spectrum of **3a** in DMSO-d_6 (500 MHz).

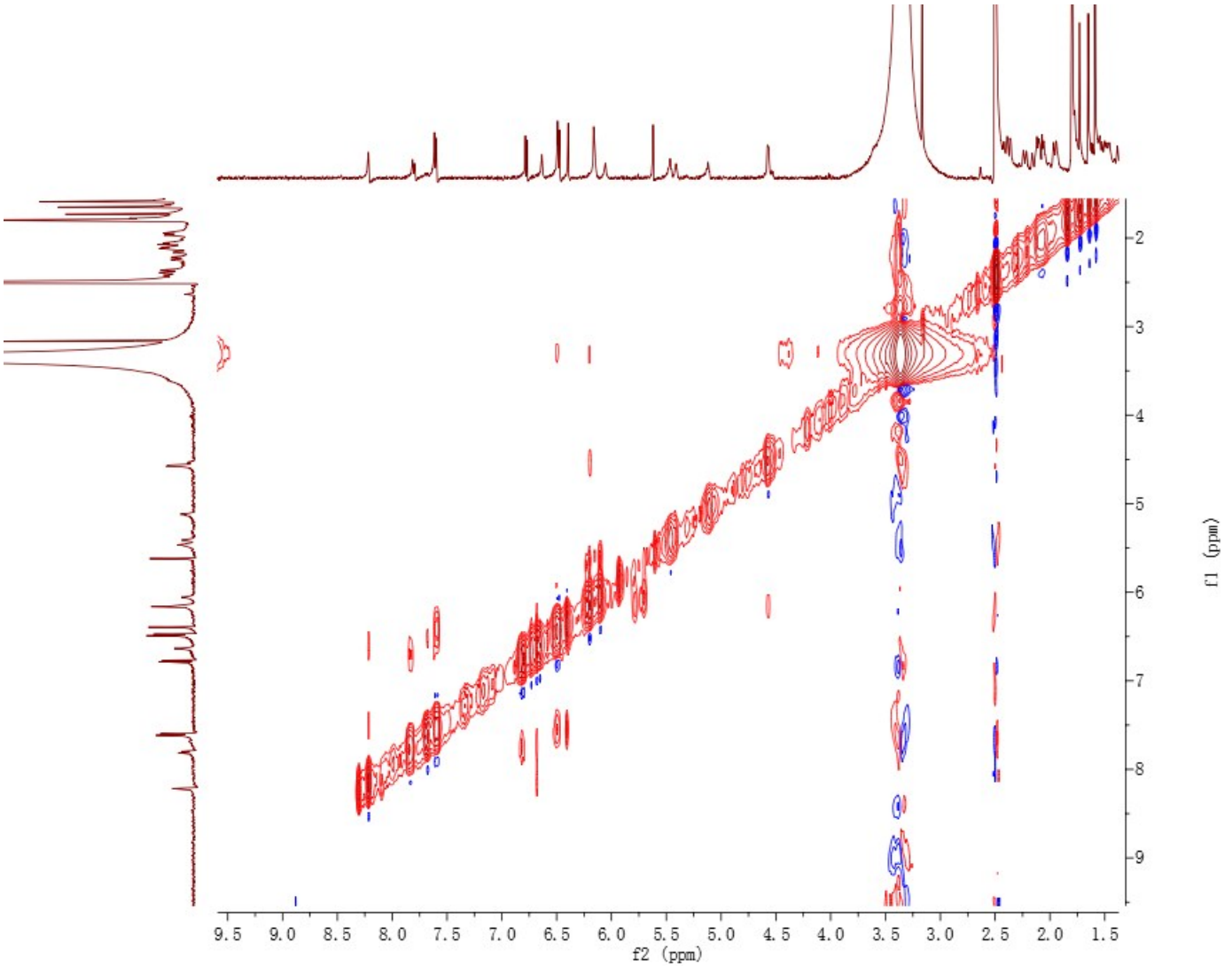


Figure S22: NOESY spectrum of **3a** in DMSO-d_6 (400 MHz).

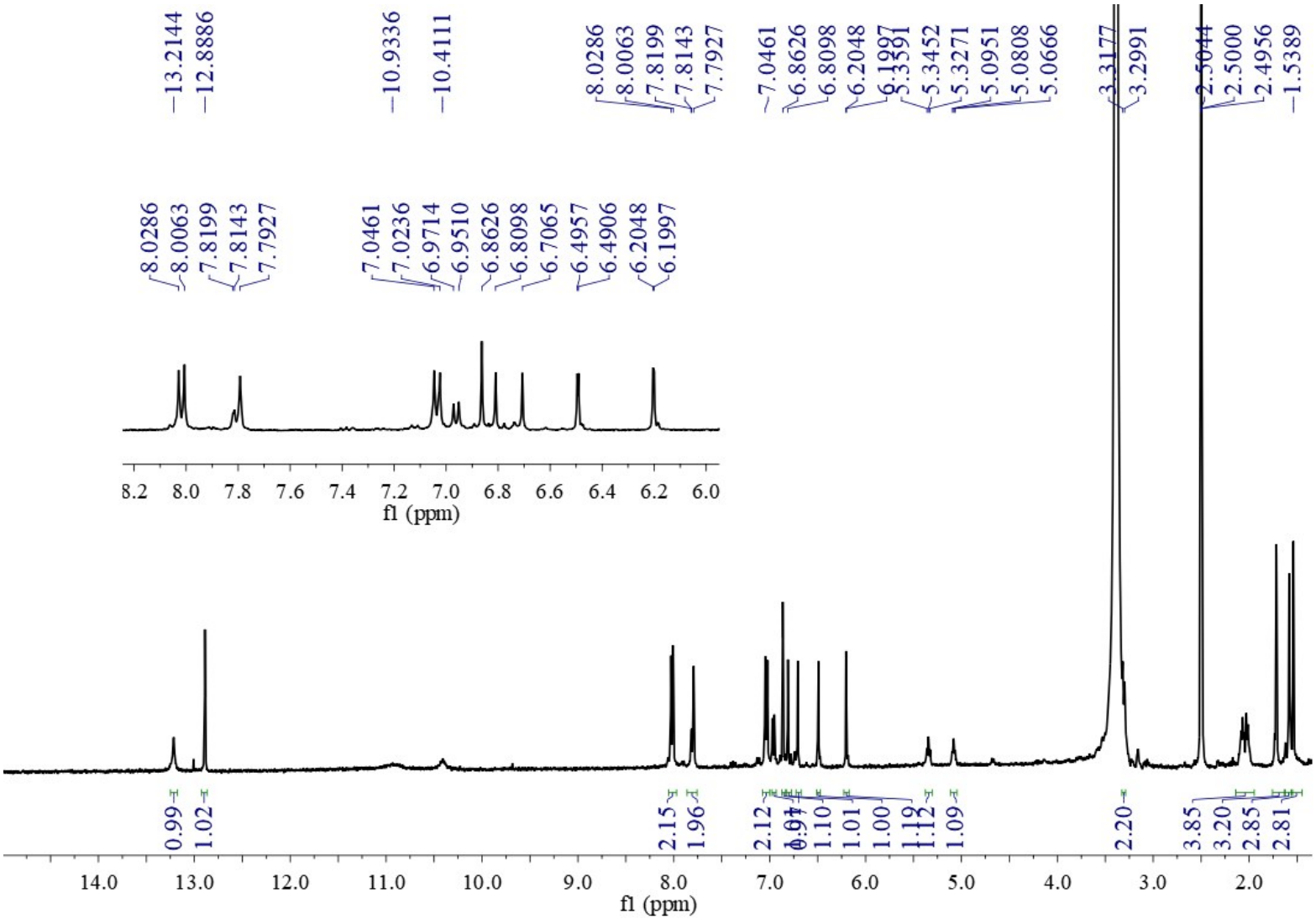


Figure S23: ^{1}H NMR spectrum of **4a** in DMSO-d_6 (400 MHz).

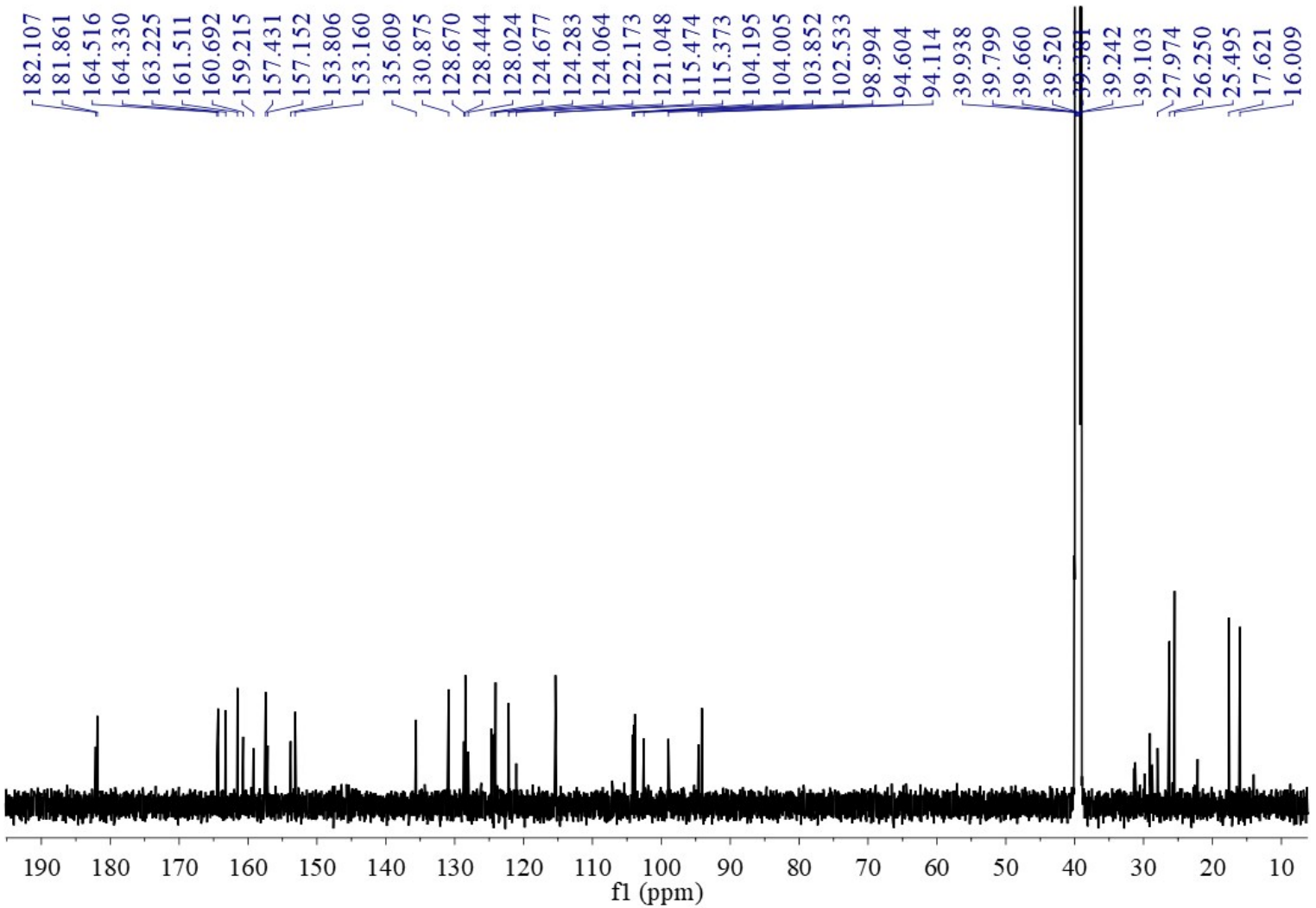


Figure S24: ^{13}C NMR spectrum of **4a** in DMSO-d_6 (600 MHz).

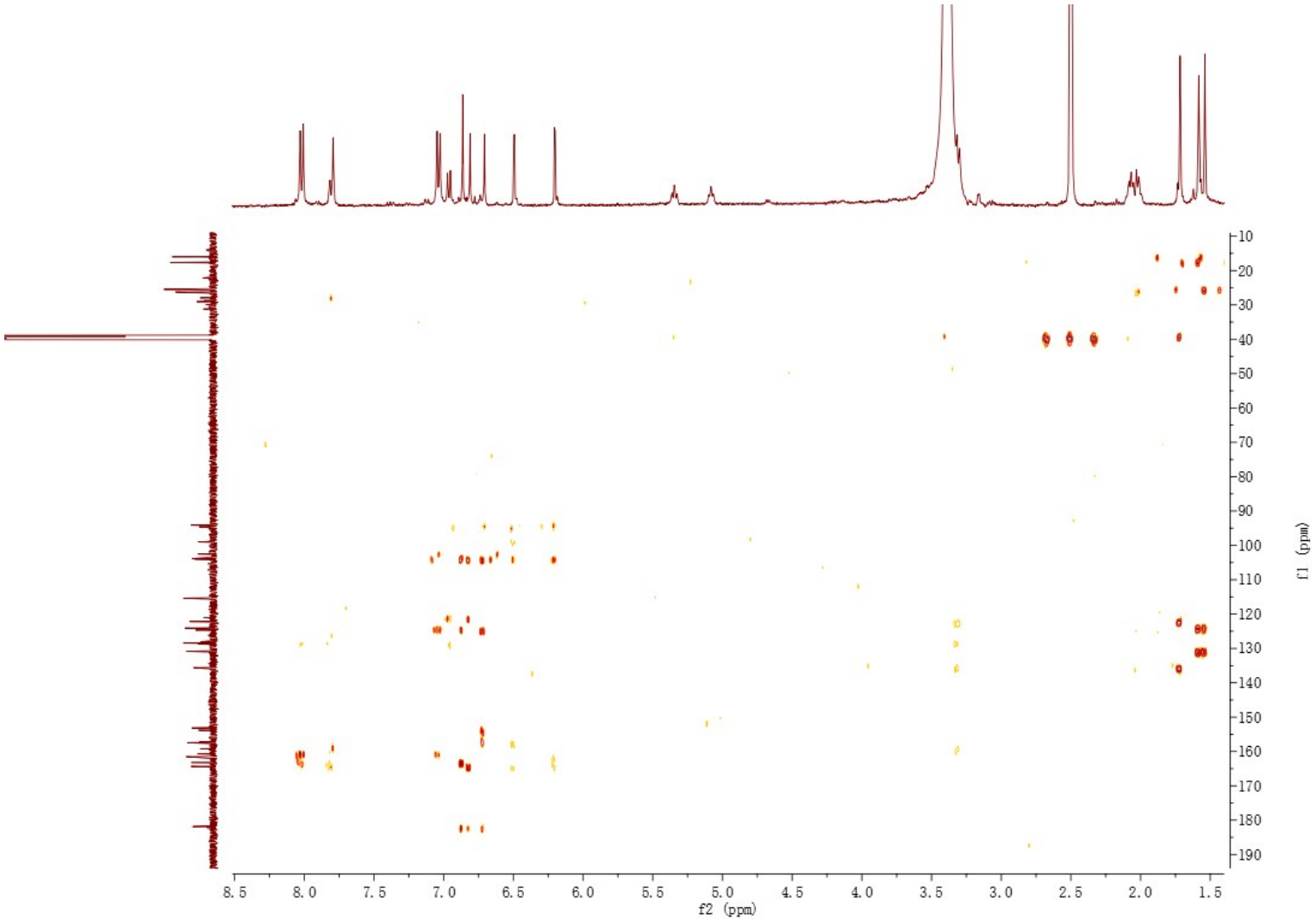


Figure S25: HMBC spectrum of **4a** in DMSO-d_6 (400 MHz).

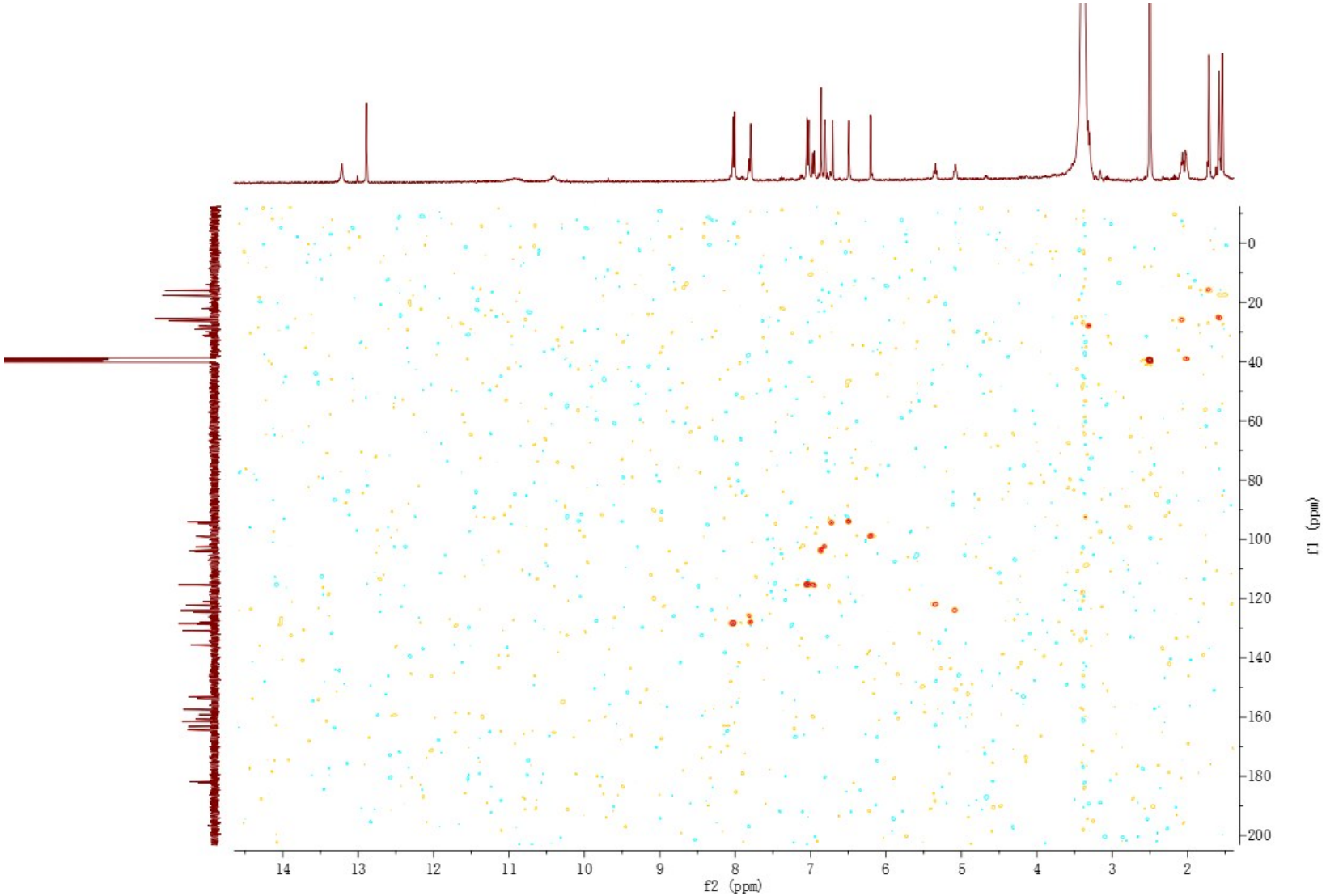


Figure S26: HSQC spectrum of **4a** in DMSO-d_6 (500 MHz).

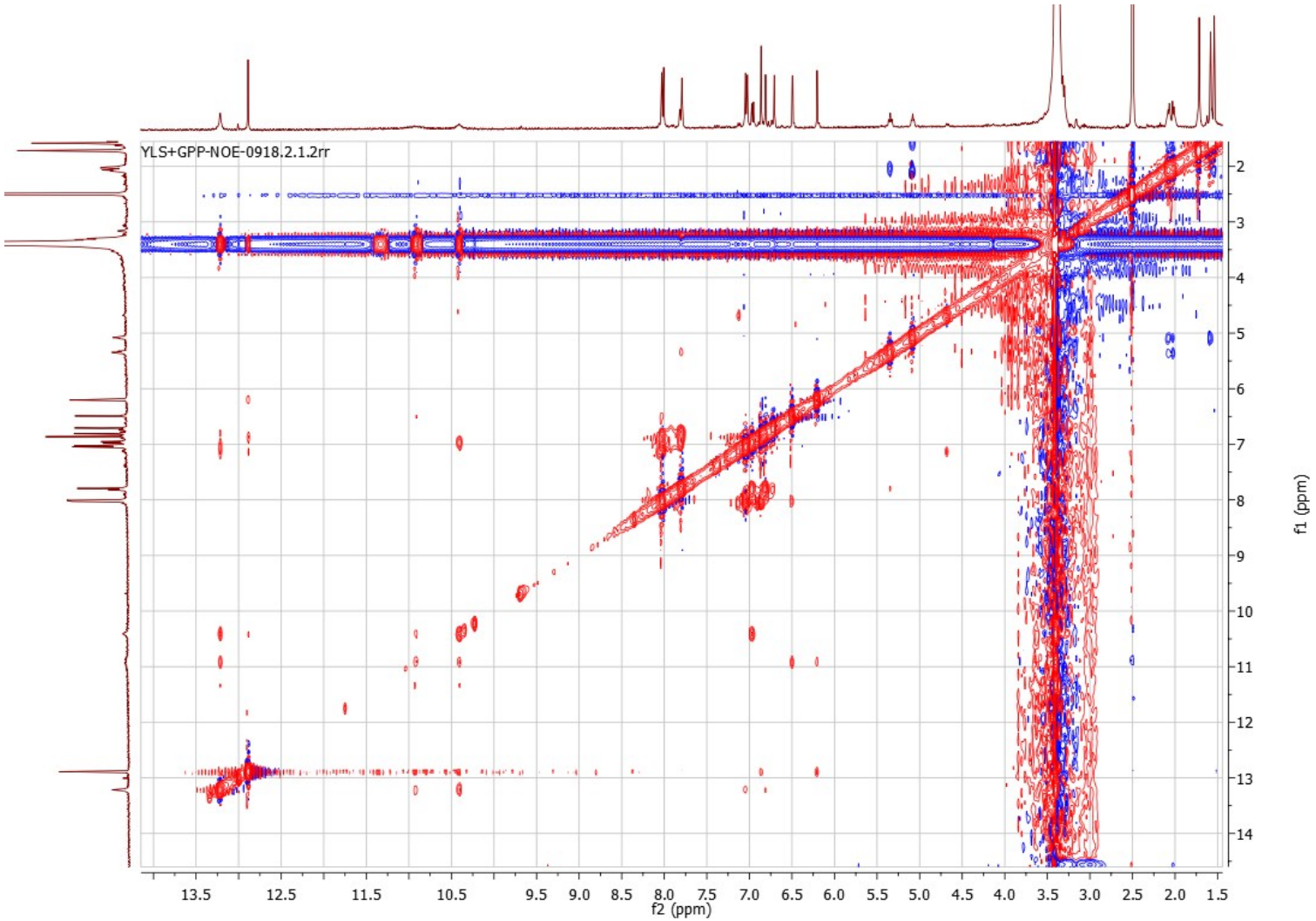


Figure S27: NOESY spectrum of **4a** in DMSO-d_6 (500 MHz).

Reference

1. A. B. Woodside, Z. Huang and C. D. Poulter, *Org. Synth.*, 1988, **66**, 211-215.