Supporting information

Pimarane-derived diterpenoids with anti-*Helicobacter pylori* activity from the tuber of *Icacina trichantha*†

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Fig. S1 Flow chart of extraction and isolation.

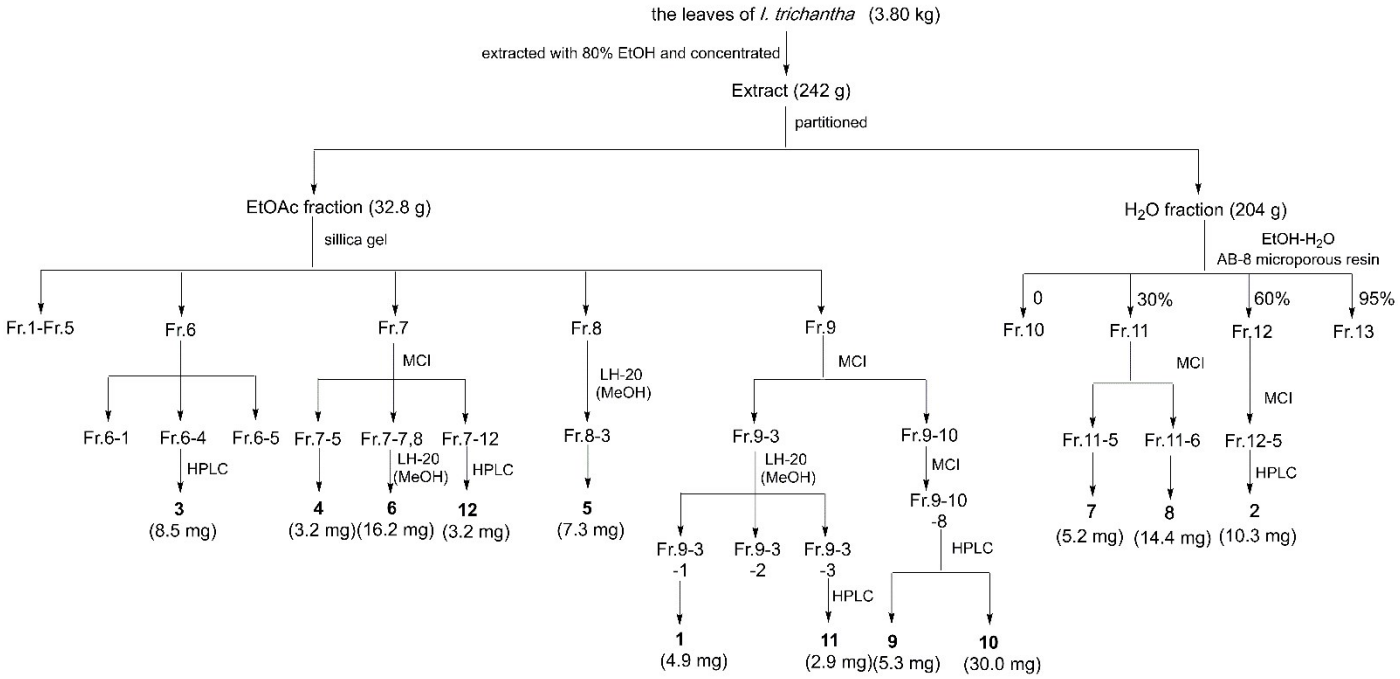

Table S1 Antibacterial activity results of EtOAc and H_2O fractions and the main subfractions from tubers of *I. trichantha* against twelve bacterial species (MIC: μg/ml)

Fractions	MTZ	EtOAc Fr.	H_2O Fr.	Fr. 1	Fr. 2	Fr. 3	Fr. 4	Fr. 5	Fr. 6	Fr. 7	Fr. 8	Fr. 9
G27	1	16	256	32	16	16	32	64	16	16	32	16
HP159	64	32	256	64	32	16	32	64	32	32	64	32
MG1655	4	NA	NA									
PAO1	>128	NA	NA									
ATCC19606	64	NA	NA									
ATCC35657	>128	NA	NA									
ATCC13047	>128	NA	NA									
MC2 155	16	NA	NA									
ATCC19434	2	NA	NA									
FA2-2	4	NA	NA									
168	4	NA	NA									
ATCC6538	0.5	NA	NA									

[a] MICs of MTZ are determined against *Helicobacter* and non-*Helicobacter* species, respectively.

[b] MTZ, metronidazole.

NA: No Inhibition.

Table S2 Antibacterial activity results of compounds **1**−**12** isolated from tubers of *I. trichantha* against a series of bacterial species (MIC: μg/ml)

Compounds	MTZ	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
G27	2	> 64	32	32	32	16	8	32	32	32	16	32	16
26695	1	64	32	32	16	8	8	32	32	32	32	32	32
HP159	16	64	32	32	32	8	8	32	32	32	32	64	32
HP129	32	64	64	64	32	16	16	64	64	64	32	32	32
MG1655	4	/	/	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
PAO1	>128	/	/	128	128	128	128	128	128	128	>128	128	128
ATCC19606	64	/	/	128	128	128	128	128	128	128	128	128	128
ATCC35657	>128	/	/	128	>128	128	128	>128	128	128	128	128	128
ATCC13047	>128	/	/	128	128	>128	128	128	128	128	128	128	128

MC2 155	16	/	/	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
ATCC1943 4	2	/	/	>128	/	/	>128	/	/	/	/	/	/
FA2-2	4	/	/	>128	/	/	>128	/	/	/	/	/	/
168	4	/	/	128	/	/	>128	/	/	/	/	/	/
ATCC6538	0.5	/	/	>128	/	/	>128	/	/	/	/	/	/

[a] MICs of MTZ are determined against *Helicobacter* and non-*Helicobacter* species, respectively.

[b] MTZ, metronidazole.

NA: No Inhibition.

Table S3 ^{1}H [δ, mult (J in Hz)] and ^{13}C NMR spectroscopic data for **1** in chloroform-*d* and **2** in DMSO-d_6

	1		**2**	
no.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1α	2.38, ddd (13.4, 9.8, 3.3)	40.8	1.66, m	36.4
1β	2.54, dd (13.8, 2.8)		1.44, m	
2α	4.38 dd (7.2, 9.6)	65.9	2.17, m	28.7
2β			1.68, m	
3		96.8	3.42, dd (10.9, 5.6)	84.3
4		48.4		45.9
5	2.26, m	51.7	1.52, d (3.0)	45.7
6	5.13, ddd (9.8, 7.9, 5.7)	74.7	4.01, t (3.6)	62.6
7α	4.11, m	25.7	1.57, td (13.6, 2.4)	33.3
7β	2.97, dd (17.2, 5.7)		1.89, m	
8		109.2	3.06, m	28.9
9		132.7	1.43, m	37.8
10		36.0		33.2
11α	6.56, s	100.1	1.80, dd (11.5, 5.7)	17.0
11β			1.16, m	
12α		151.8	2.17, m	17.8
12β			1.97, ddt (18.7, 11.5, 6.0)	
13		118.0		111.3
14		153.8		192.1
15α	6.34, s	100.6		200.1
15β				
16		161.1	4.57, d (5.5)	74.9
18	1.45, s	19.6	1.29, s	17.4
19		179.6		171.8
20α	3.90, d (2.6)	70.7	4.51, d (11.3)	77.3
20β	4.16, dd (6.8, 2.6)		3.87, d (11.3)	
12-OCH3	3.93, s	55.7		
1'α	1.88, m	36.6	4.19, d (7.8)	104.5
1'β	1.10, m			
2'α	1.52, m	19.4	3.06, m	76.8
2'β	1.85, m			
3'α	2.13, m	37.1	3.06, m	76.9
3'β	0.96, m			
4'		43.3	2.94, td (8.5, 4.2)	73.9
5'α	1.60, dd (11.8, 2.6)	48.3	3.06, m	70.0
6'α	2.22, m	26.2	3.64, m	61.1
6'β			3.42, dd (10.9, 5.6)	
7'	3.45, d (5.8)	41.6		
8'		122.9		
9'		140.3		
10'		38.9		
11'α	0.93, m	20.8		
11'β	2.13, m			

12'α	1.77, m	30.5
12'β	1.69, m	
13'		44.3
14'α	2.26, m	37.5
14'β	1.92, m	
15'		214.8
16'	4.33, brd (16.8)	64.1
17'	1.14, s	20.7
18'	0.97, s	28.5
19'		183.2
20'	0.93, s	17.5

Table S4 GIAO ^{13}C NMR calculation of structure **1a** and **1b**

no.	exptl. δ_C	calcd δ_C **1a**	deviation	calcd δ_C **1b**	deviation
1	40.8	40.8	0.0	41.0	0.2
2	65.9	66.1	0.2	66.1	0.2
3	96.8	96.3	−0.5	96.7	−0.1
4	48.4	50.2	1.8	50.1	1.7
5	51.7	53.5	1.8	53.7	2.0
6	74.7	75.3	0.6	75.3	0.6
7	25.7	25.3	−0.4	23.8	−1.9
8	109.2	108.5	−0.7	107.2	−2.0
9	132.7	136.3	3.6	135.4	2.7
10	36.0	35.5	−0.5	34.7	−1.3
11	100.1	97.1	−3.0	100.5	0.4
12	151.8	148.9	−2.9	148.5	−3.3
13	118.0	115.8	−2.2	113.2	−4.8
14	153.8	152.6	−1.2	152.3	−1.5
15	100.6	102.2	1.6	103.7	3.1
16	161.1	162.6	1.5	160.6	−0.5
18	19.6	17.4	−2.2	17.8	−1.8
19	179.6	178.6	−1.0	177.1	−2.5
20	70.7	68.7	−2.9	68.7	−2.9
12-OCH_3	55.7	56.1	0.4	56.3	0.6
1'	36.6	36.6	0.0	35.5	−1.1
2'	19.4	16.9	−2.5	16.6	−2.8
3'	37.1	37.9	0.8	39.6	2.5
4'	43.3	44.5	1.2	43.4	0.1
5'	48.3	49.8	1.5	49.7	1.4
6'	26.2	26.2	0.0	25.4	−0.8
7'	41.6	42.4	0.8	41.8	0.2
8'	122.9	125.8	2.9	124.4	1.5
9'	140.3	144.0	3.7	146.9	6.6
10'	38.9	40.0	1.1	40.5	1.6
11'	20.8	21.6	0.8	23.0	2.2
12'	30.5	31.2	0.7	32.7	2.2
13'	44.3	45.3	1.0	47.5	3.2
14'	37.5	39.4	1.9	40.2	2.7
15'	214.8	214.5	−0.3	218.0	3.2
16'	64.1	62.4	−1.7	61.8	−2.3
17'	20.7	21.0	0.3	19.1	−1.6

18’	28.5	26.8	−1.7	26.5	−2.0
19’	183.2	181.3	−1.9	179.9	−3.3
20’	17.5	14.5	−3.0	15.1	−2.4
		MAE	1.4	MAE	1.9
		RMS	1.8	RMS	2.3
		sDP4+	100%	sDP4+	0%

Table S5 Fractional inhibitory concentration (FIC) and interaction style of icacinlactone B (**6**) combined with four antibiotics against *H. pylori* strain G27

	MIC of **6** (μg/mL)		MIC of MTZ (μg/mL)		MIC of CLR (μg/mL)		MIC of AMX (μg/mL)		MIC of LVX (μg/mL)		FICI	Interaction
	Alone	Combination	Alone	Combination	Alone	Combination	Alone	Combination	Alone	Combination		
6 + MTZ	8	4	2	0.5							0.75	additive effect
6 + CLR	8	2			0.008	0.004					0.75	additive effect
6 + AMX	8	2					0.125	0.125			1.25	indifference
6 + LVX	8	4							0.25	0.25	1.5	indifference

FIC of **6** = MIC of **6** in combination with antibiotic/MIC of **6** alone.

FIC of antibiotic = MIC of antibiotic in combination with **6**/MIC of antibiotic alone.

FIC index = FIC of **6**+ FIC of antibiotic.

FICI ≤ 0.5, synergy; 0.5 < FICI ≤ 1, additive effect; 1 < FICI ≤ 2, indifference; FICI > 4.0, antagonism.

(CLR: clarithromycin, AMX: amoxicillin, MTZ: metronidazole; LVX: levofloxacin)

Table S6 Fractional inhibitory concentration (FIC) and interaction style of icacinlactone B (**6**) combined with four antibiotics against of *H. pylori* strain HP159

	MIC of **6** (μg/mL)		MIC of MTZ (μg/mL)		MIC of CLR (μg/mL)		MIC of AMX (μg/mL)		MIC of LVX (μg/mL)		FICI	Interaction
	Alone	Combination	Alone	Combination	Alone	Combination	Alone	Combination	Alone	Combination		
6 + MTZ	8	4	16	1							0.56	additive effect
6 + CLR	8	2			2	1					0.75	additive effect
6 + AMX	8	4					0.125	0.008			0.56	indifference
6 + LVX	8	2							8	8	1.25	indifference

FIC of **6** = MIC of **6** in combination with antibiotic/MIC of **6** alone.

FIC of antibiotic = MIC of antibiotic in combination with **7**/MIC of antibiotic alone.

FIC index = FIC of **6** + FIC of antibiotic.

FICI ≤ 0.5, synergy; 0.5 < FICI ≤ 1, additive effect; 1 < FICI ≤ 2, indifference; FICI > 4.0, antagonism.

(CLR: clarithromycin, AMX: amoxicillin, MTZ: metronidazole; LVX: levofloxacin)

Table S7 Cartesian coordinate for the lowest energy conformer of **1** calculated at TD/B3LYP/6-311++G(d,p) level of theory in gas phase

Center number	Atomic number	Atomic type	Coordinates (Angstroms)		
			X	Y	Z
1	6	0	6.829779	−1.77288	1.554311
2	6	0	6.075429	−2.96861	0.966137
3	6	0	5.354024	−2.63176	−0.37258
4	6	0	4.48147	−1.34189	−0.15448
5	6	0	5.204936	−0.09872	0.456368
6	6	0	6.252689	0.579895	−0.47069
7	6	0	3.664225	−0.93802	−1.38853
8	6	0	2.546942	0.058417	−1.01977
9	6	0	2.98682	1.078739	0.038455
10	6	0	4.13336	0.982014	0.742511
11	6	0	2.012621	2.233031	0.225803
12	6	0	2.648605	3.487657	0.849337
13	6	0	3.392462	3.039371	2.115738
14	6	0	4.487818	2.008432	1.810704
15	6	0	1.557077	4.526167	1.202456
16	6	0	3.618198	4.192816	−0.10867
17	6	0	3.350322	4.219227	−1.61506
18	8	0	4.259748	5.056333	−2.27228
19	8	0	4.598445	4.79804	0.300425
20	1	0	3.765578	−1.64064	0.620675
21	6	0	5.910942	−0.5603	1.760922
22	6	0	6.356814	−2.55542	−1.53981
23	6	0	−2.27294	−0.35503	−0.34684
24	6	0	−3.16033	−1.3393	0.135953
25	6	0	−0.92733	−0.71053	−0.34715
26	6	0	−0.421	−1.94304	0.091172
27	6	0	−1.32806	−2.90309	0.565017
28	6	0	−2.68568	−2.5864	0.58225
29	8	0	0.107632	0.081474	−0.77817
30	6	0	1.276361	−0.64592	−0.60502
31	6	0	1.008831	−1.87299	−0.08465
32	8	0	−0.94047	−4.13283	1.01544
33	6	0	−2.75528	0.985792	−0.85404
34	1	0	0.024604	−4.20757	0.948981
35	6	0	−5.23076	−1.07797	−1.27833
36	6	0	−4.77743	0.472346	0.699867
37	6	0	−5.55152	−1.85349	1.038446
38	1	0	−3.37051	−3.33766	0.957947
39	1	0	−1.92623	1.699118	−0.87554
40	1	0	−3.10986	0.906263	−1.88949
41	1	0	−4.48953	0.434261	1.75549
42	6	0	−6.22792	0.980195	0.539459
43	6	0	−3.91459	1.540767	−0.00736
44	8	0	−4.8205	2.301221	−0.85409
45	1	0	−3.52532	2.247901	0.732907
46	6	0	−7.03104	−1.43336	0.839462
47	6	0	4.352812	−3.7778	−0.59981
48	8	0	3.458047	−4.07718	0.170577
49	8	0	4.540019	−4.46627	−1.74759
50	1	0	−5.25325	−1.76787	2.089461
51	1	0	−5.48154	−2.90838	0.7588
52	1	0	7.681004	−1.5044	0.916484
53	1	0	7.261551	−2.06526	2.519639
54	1	0	6.758846	−3.81344	0.809569
55	1	0	5.318045	−3.30044	1.685978

56	1	0	1.740016	−2.63581	0.144436
57	1	0	6.530956	1.557682	−0.06503
58	1	0	4.314155	−0.47437	−2.1376
59	1	0	3.218176	−1.81331	−1.87417
60	1	0	2.281948	0.624322	−1.92596
61	1	0	1.546356	2.471177	−0.73835
62	1	0	1.175194	1.92217	0.865876
63	1	0	2.649038	2.603658	2.79601
64	1	0	3.833887	3.900903	2.625993
65	1	0	5.404844	2.537816	1.520033
66	1	0	4.735669	1.488655	2.743465
67	1	0	2.001522	5.427976	1.63658
68	1	0	0.972857	4.820637	0.322916
69	1	0	0.86477	4.098484	1.936643
70	1	0	3.420547	3.184637	−1.99066
71	1	0	2.315309	4.548583	−1.79861
72	1	0	4.88206	5.345016	−1.57478
73	1	0	5.147158	−0.82353	2.506702
74	1	0	6.495547	0.263251	2.18552
75	1	0	5.873545	−2.2699	−2.47837
76	1	0	6.827503	−3.52957	−1.69926
77	1	0	7.149999	−1.83523	−1.33722
78	6	0	−4.63804	−0.97706	0.149969
79	6	0	−7.08178	−0.1768	−0.05107
80	6	0	−6.11496	2.044604	−0.55443
81	6	0	−6.82472	1.609372	1.808916
82	8	0	−8.41333	0.171625	−0.24657
83	8	0	−6.53233	−0.48321	−1.33626
84	8	0	−7.7227	−2.49669	0.205939
85	1	0	−5.30248	−2.13713	−1.55877
86	1	0	−7.50484	−1.19715	1.803269
87	1	0	−4.61107	−0.57482	−2.02531
88	1	0	−8.40943	0.979143	−0.79974
89	1	0	−8.51415	−2.10277	−0.20092
90	1	0	−7.82315	2.004533	1.60688
91	1	0	3.854173	−5.16167	−1.75696
92	1	0	−6.1942	2.431184	2.167973
93	1	0	−6.90139	0.871572	2.613876
94	8	0	−7.0373	2.605833	−1.10206
95	1	0	5.863033	0.750949	−1.4779
96	1	0	7.17599	0.003279	−0.56038

Table S8 Cartesian coordinate for the lowest energy conformer of the aglycon of **2** calculated at B3LYP/6-31+G(d) level of theory in gas phase

Center number	Atomic number	Atomic type	Coordinates (Angstroms)		
			X	Y	Z
1	6	0	−2.83256	2.128613	0.264403
2	6	0	−3.39871	1.145552	−0.76727
3	6	0	−2.78713	−0.2977	−0.59803
4	6	0	−1.24051	−0.18657	−0.73413
5	6	0	−0.68312	0.688788	0.421339
6	6	0	−1.29684	2.106734	0.27493
7	6	0	−0.54171	−1.54937	−0.90266
8	6	0	0.978748	−1.38935	−0.97652
9	6	0	1.533706	−0.64717	0.259584
10	6	0	0.889805	0.761918	0.447419
11	6	0	3.017361	−0.512	0.171859
12	6	0	3.712198	0.628719	−0.06159
13	6	0	3.050591	1.957085	−0.25954
14	6	0	1.539289	1.782357	−0.52324
15	8	0	3.767524	−1.63598	0.324511
16	6	0	5.152507	−1.23625	0.18346
17	6	0	5.124284	0.279805	−0.07255
18	8	0	6.111844	0.981933	−0.24715
19	8	0	−4.81827	1.150287	−0.76831
20	6	0	−1.10741	0.071034	1.773338
21	8	0	−2.46295	−0.41947	1.864513
22	6	0	−3.22371	−0.74046	0.799701
23	8	0	−4.32196	−1.22471	1.002988
24	6	0	−3.39588	−1.22988	−1.65952
25	1	0	1.158092	1.10008	1.461808
26	1	0	1.317695	−1.27351	1.133861
27	1	0	−1.04478	0.346056	−1.67781
28	8	0	−0.93005	−2.39315	0.191428
29	1	0	−3.18858	3.134963	0.015525
30	1	0	−3.23874	1.893973	1.255738
31	1	0	−3.11841	1.480684	−1.77537
32	1	0	−0.91649	2.757332	1.074602
33	1	0	−0.9529	2.540738	−0.67119
34	1	0	−0.88973	−1.99746	−1.84271
35	1	0	1.451958	−2.37611	−1.05646
36	1	0	1.235952	−0.84496	−1.89487
37	1	0	3.519856	2.495733	−1.09221
38	1	0	3.203373	2.588885	0.628195
39	1	0	1.385222	1.467137	−1.56434
40	1	0	1.0662	2.761606	−0.42143
41	1	0	5.591142	−1.7862	−0.65429
42	1	0	5.682773	−1.48872	1.106278
43	1	0	−1.03372	0.823975	2.565642
44	1	0	−0.47533	−0.77583	2.041994
45	1	0	−3.13322	−2.27423	−1.47233
46	1	0	−4.48346	−1.15463	−1.66035
47	1	0	−3.03429	−0.94412	−2.65561
48	1	0	−0.67548	−3.30944	0.002801
49	1	0	−5.14178	0.591711	−0.03735

Fig. S2 ^{1}H NMR (400 MHz, chloroform-*d*) spectrum of compound **1**

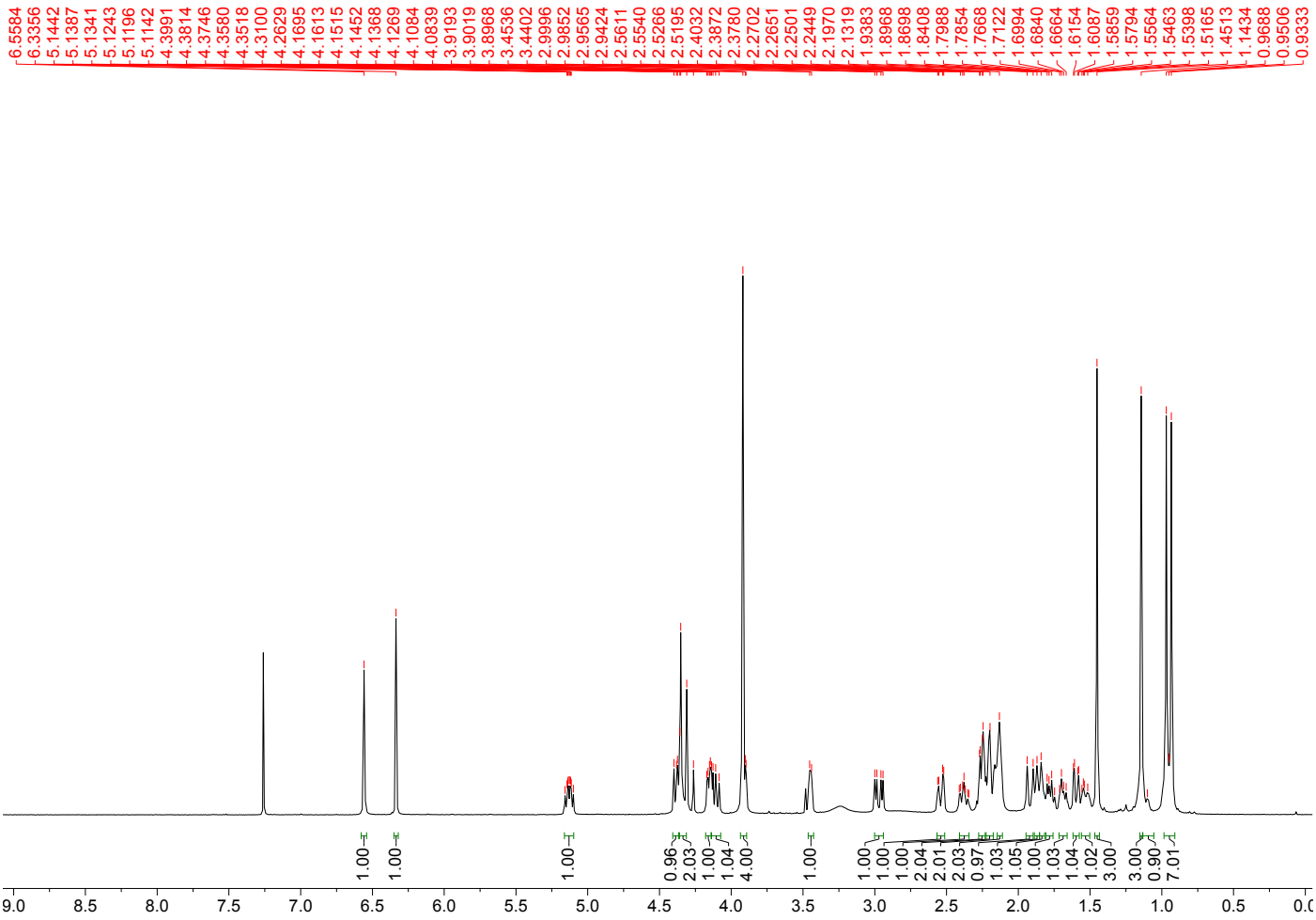

Fig. S3 ^{13}C NMR (100 MHz, chloroform-*d*) spectrum of compound **1**

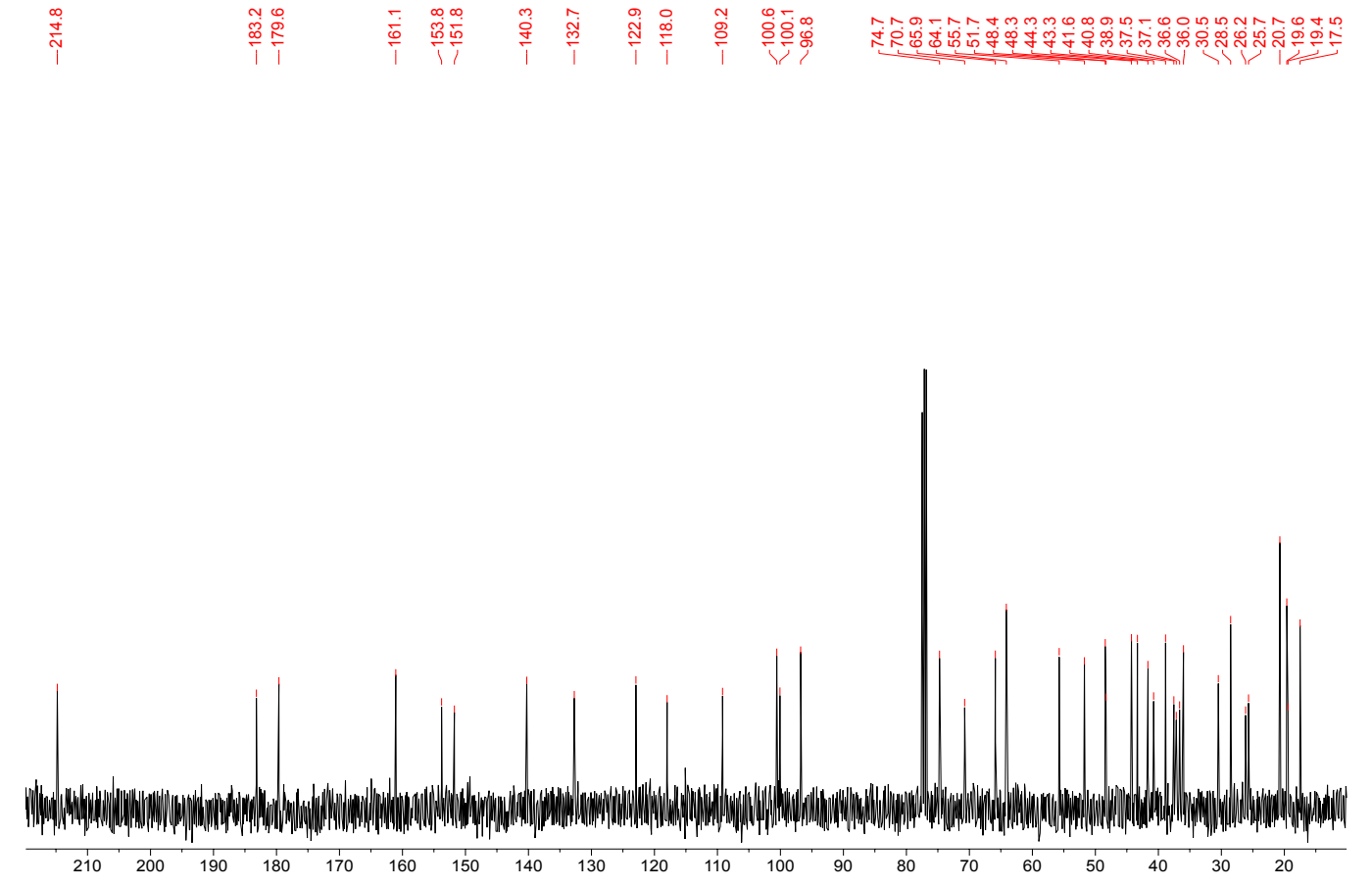

Fig. S4 DEPT135 (100 MHz, chloroform-*d*) spectrum of compound **1**

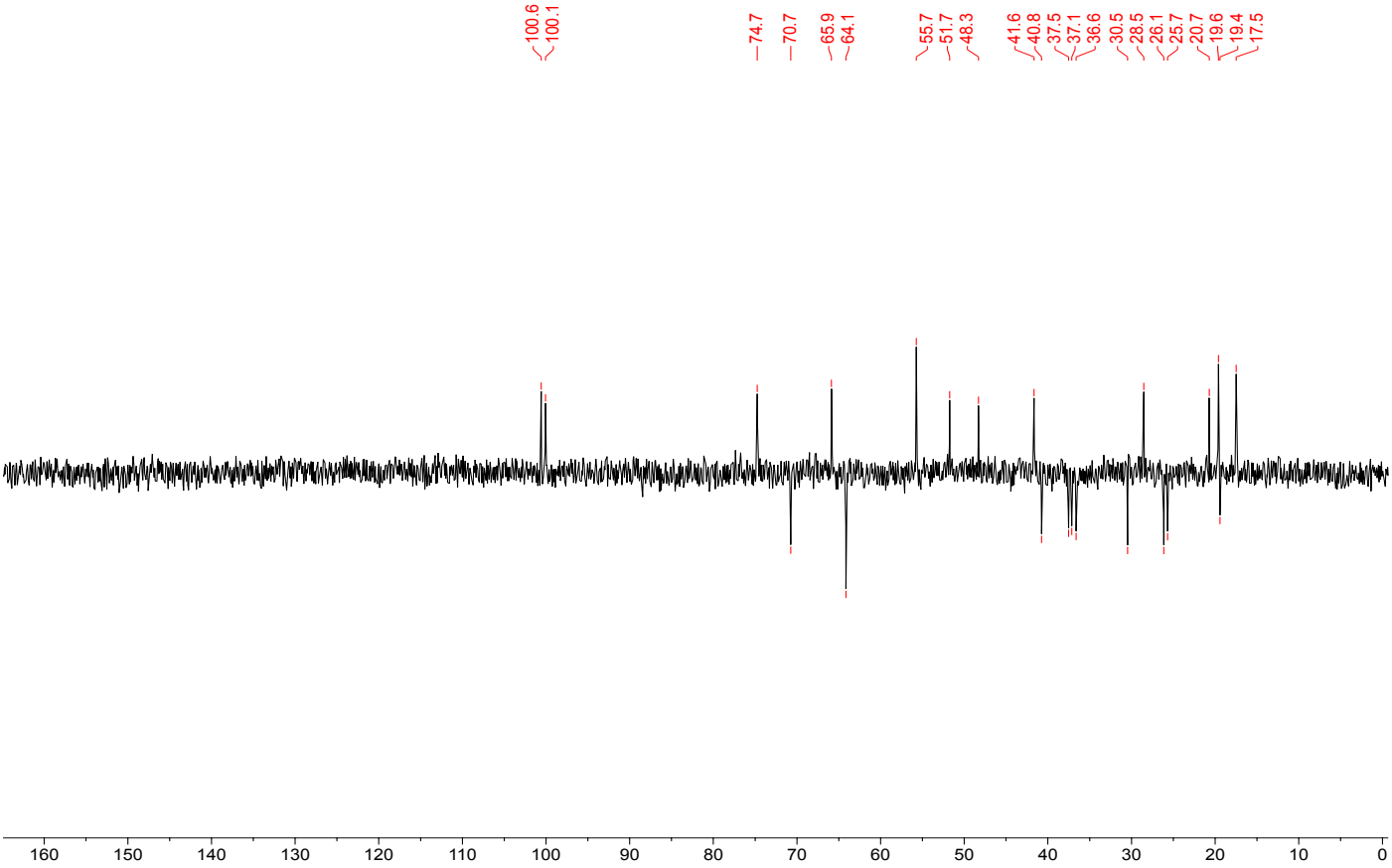

Fig. S5 $^{1}H-^{1}H$ COSY spectrum (400 MHz, chloroform-*d*) of compound **1**

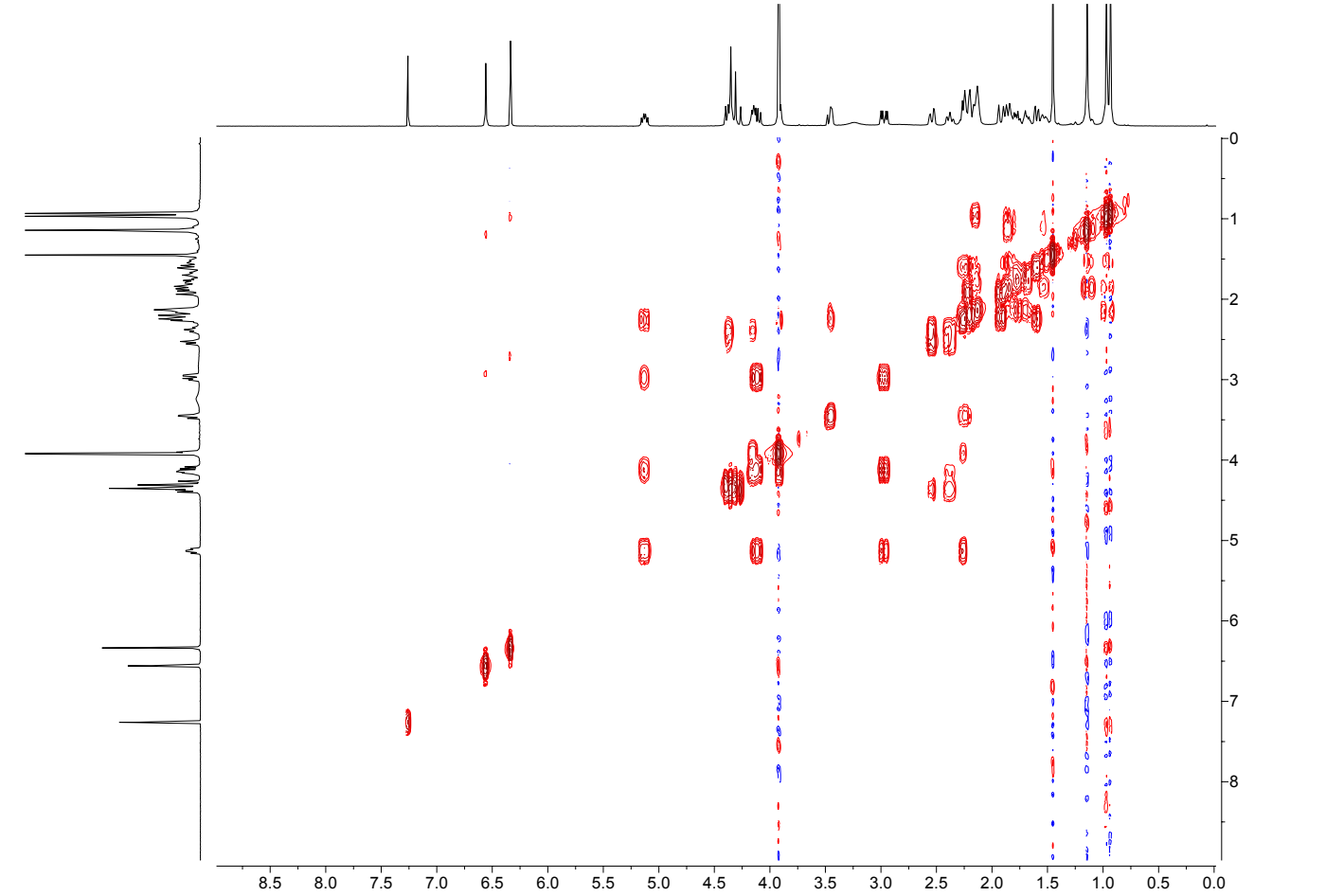

Fig. S6 HSQC spectrum (chloroform-*d*) of compound **1** (^{1}H: 400 MHz, ^{13}C: 100 MHz)

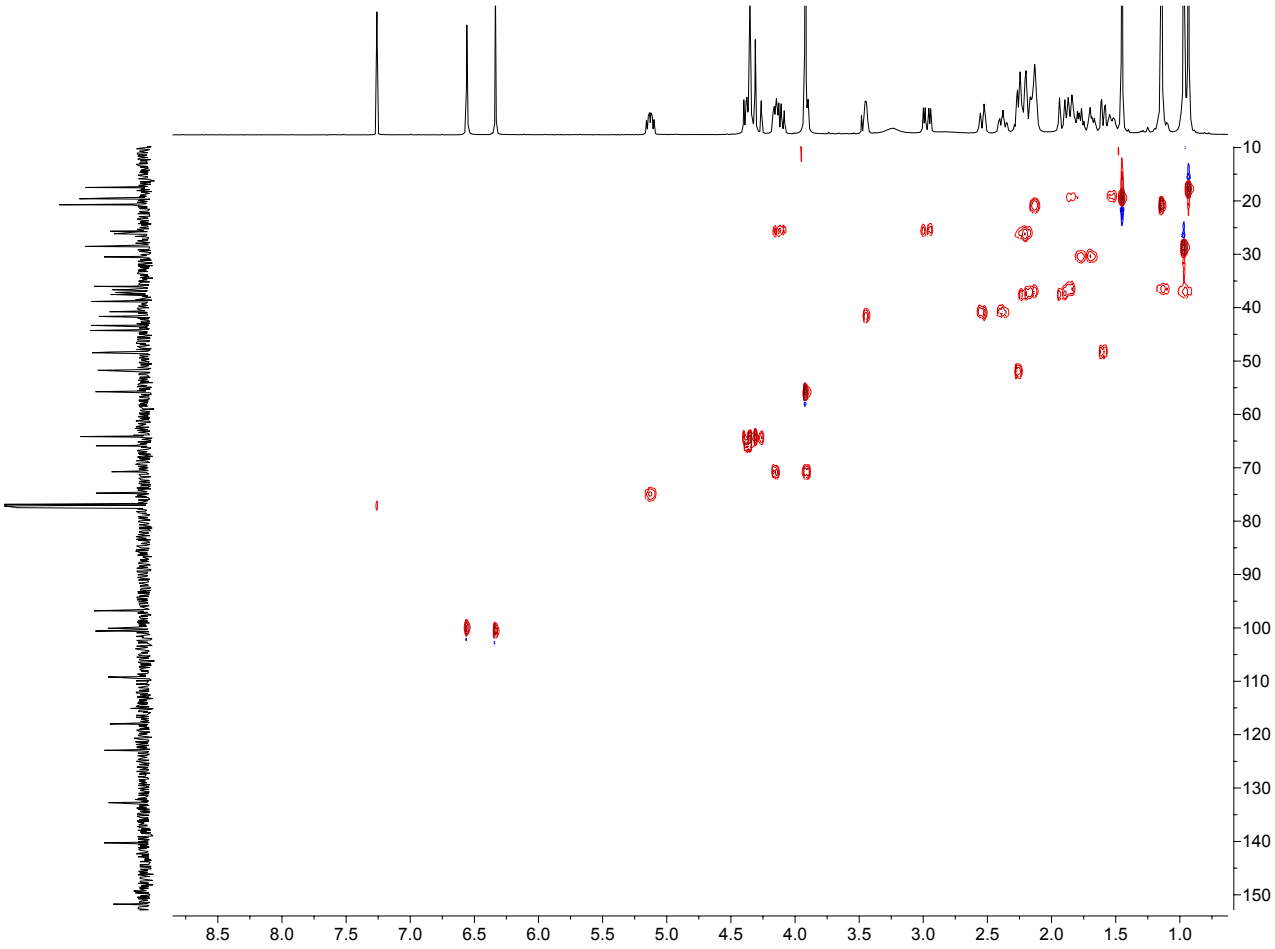

Fig. S7 HMBC spectrum (chloroform-*d*) of compound **1** (^{1}H: 400 MHz, ^{13}C: 100 MHz)

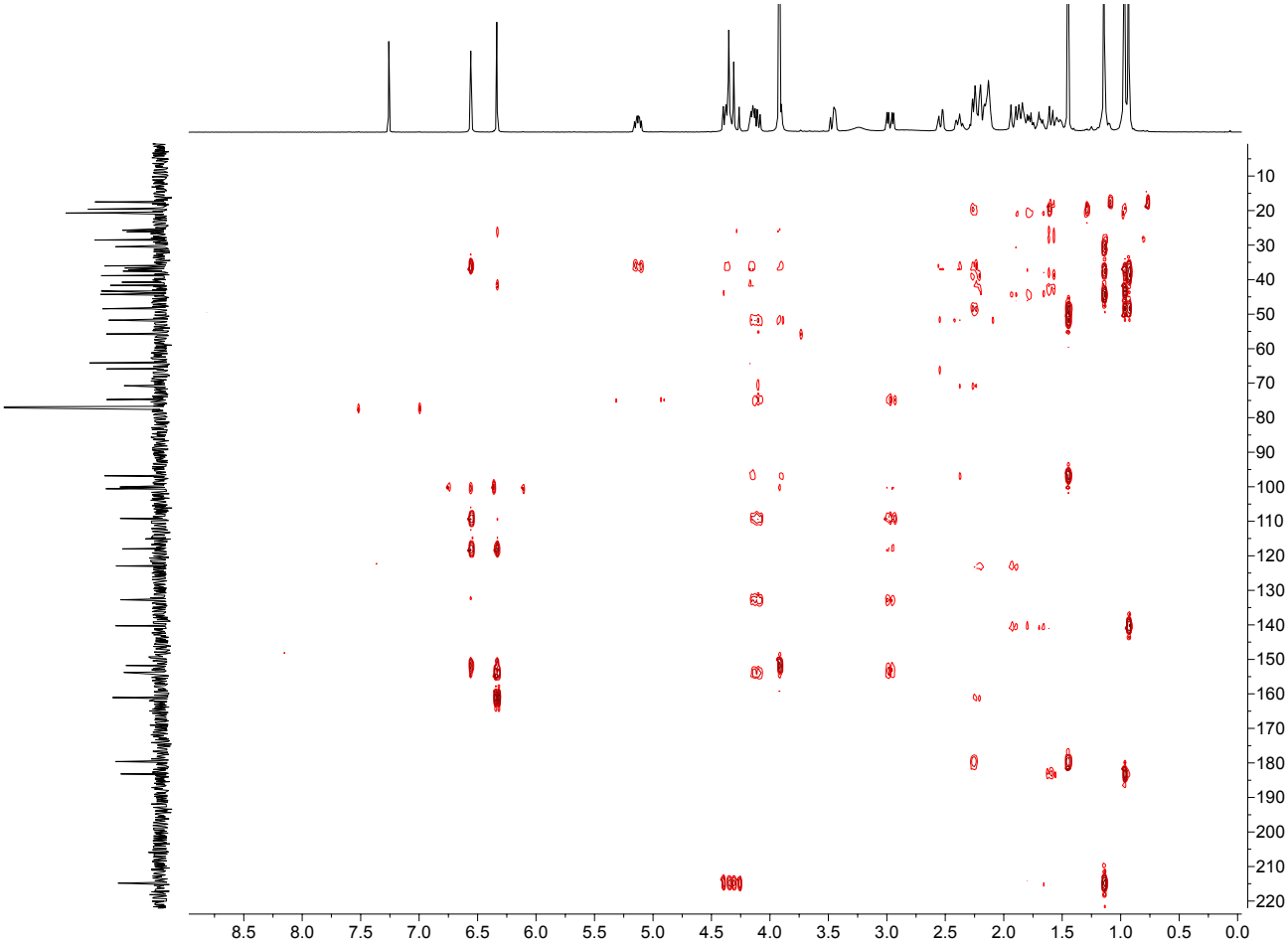

Fig. S8 NOESY spectrum (400 MHz, chloroform-*d*) of compound **1**

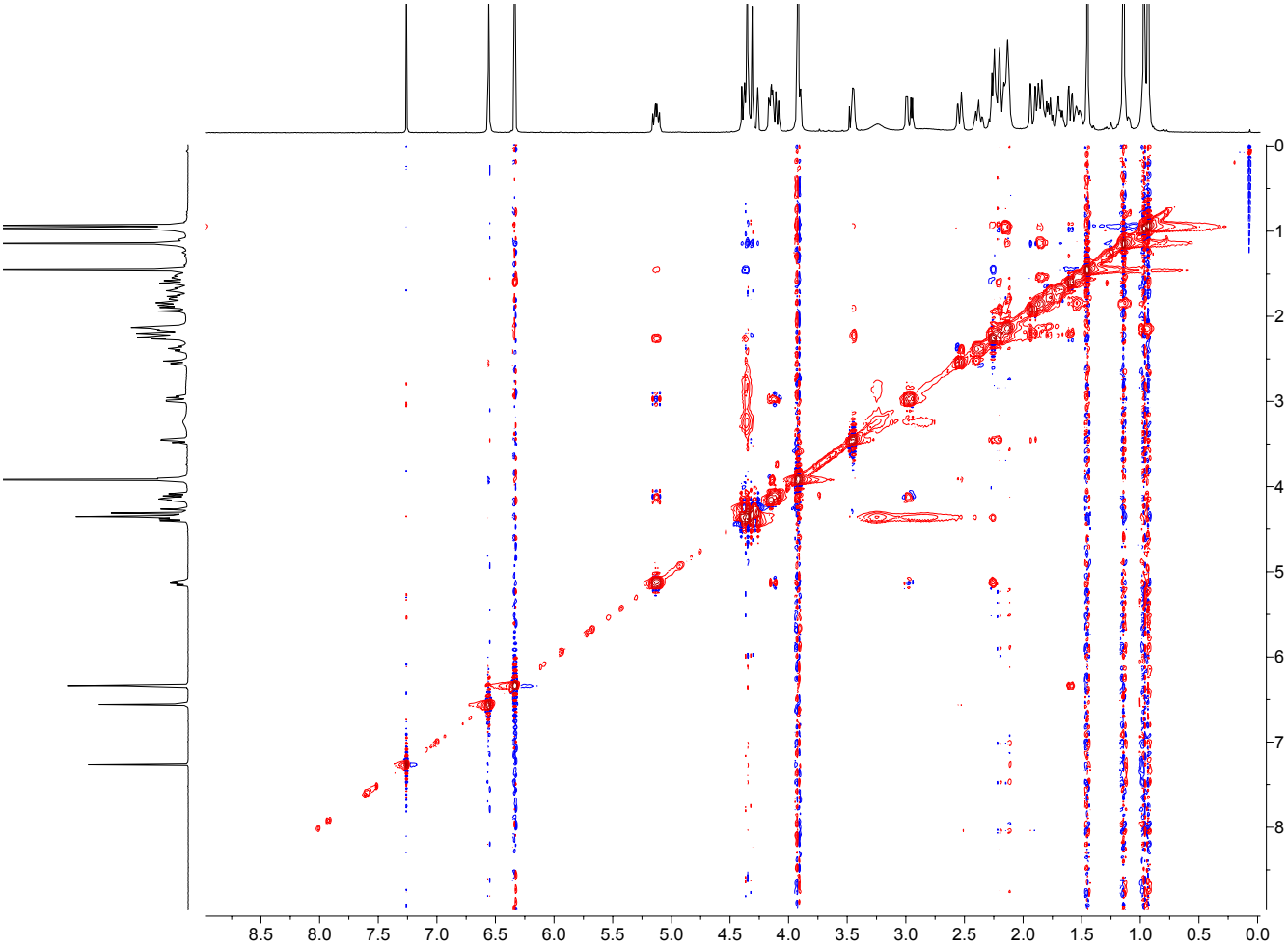

Fig. S9 IR spectrum of compound **1**

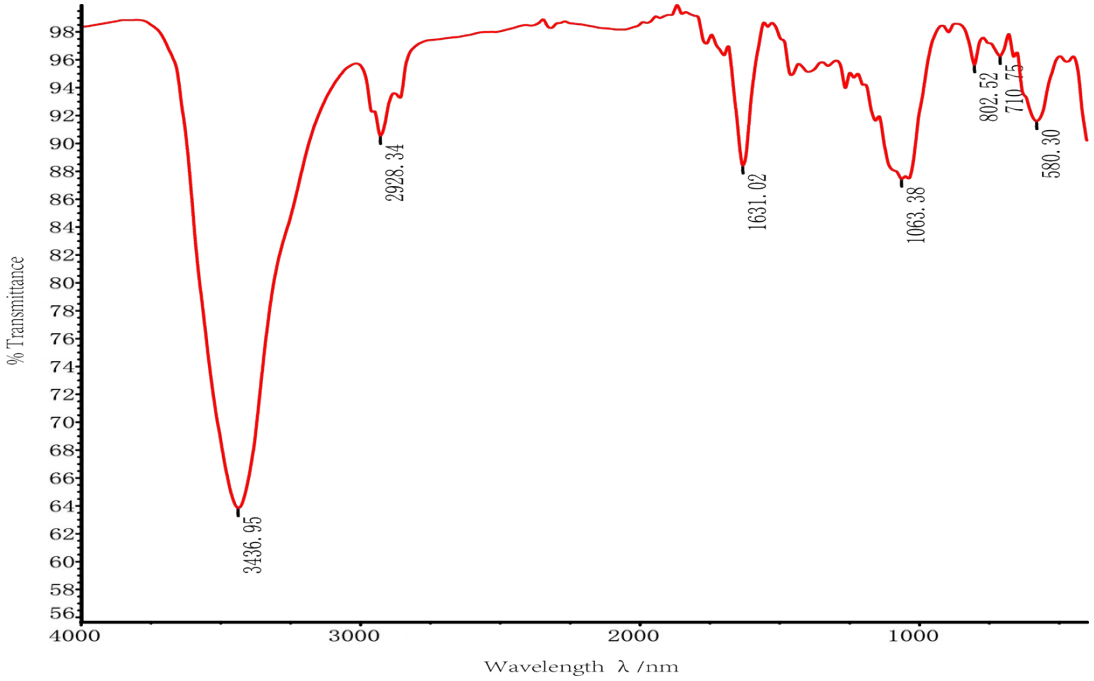

Fig. S10 (+)-HR-ESI-MS spectrum of compound **1**

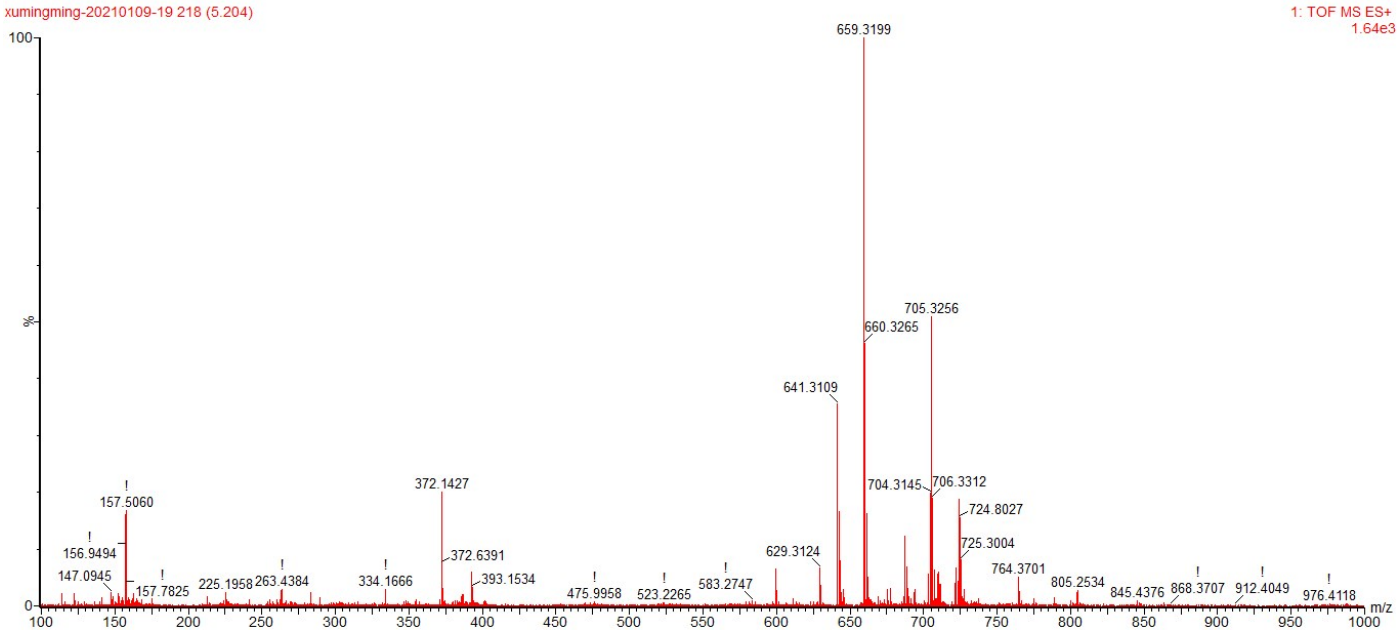

Fig. S11 UV spectrum of compound **1**

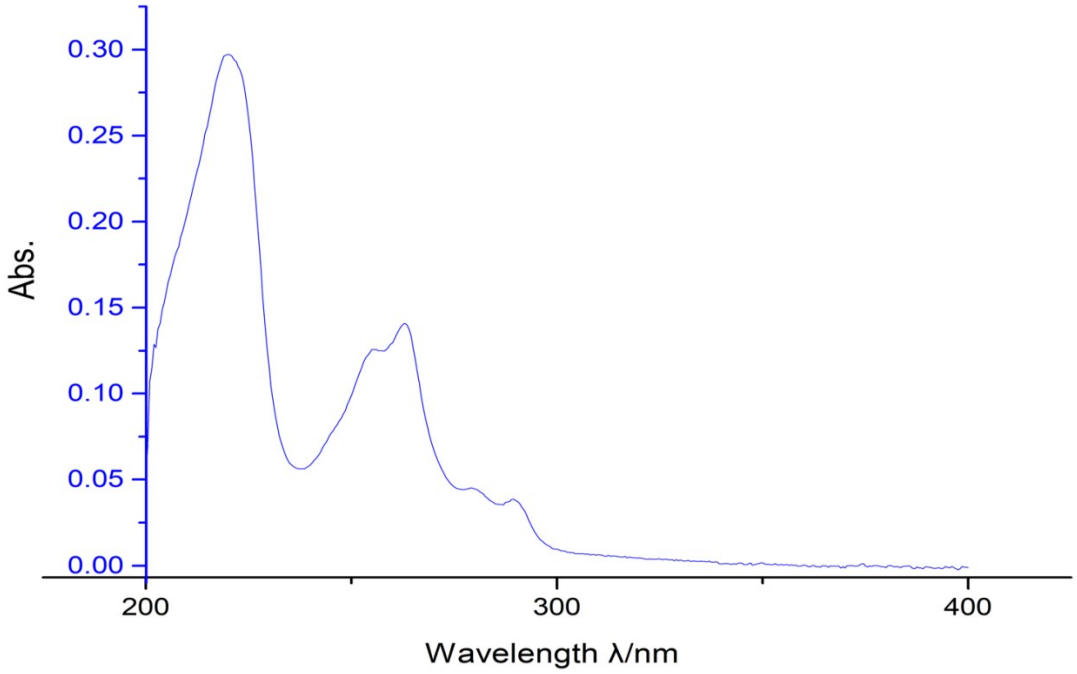

Fig. S12 ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **2**

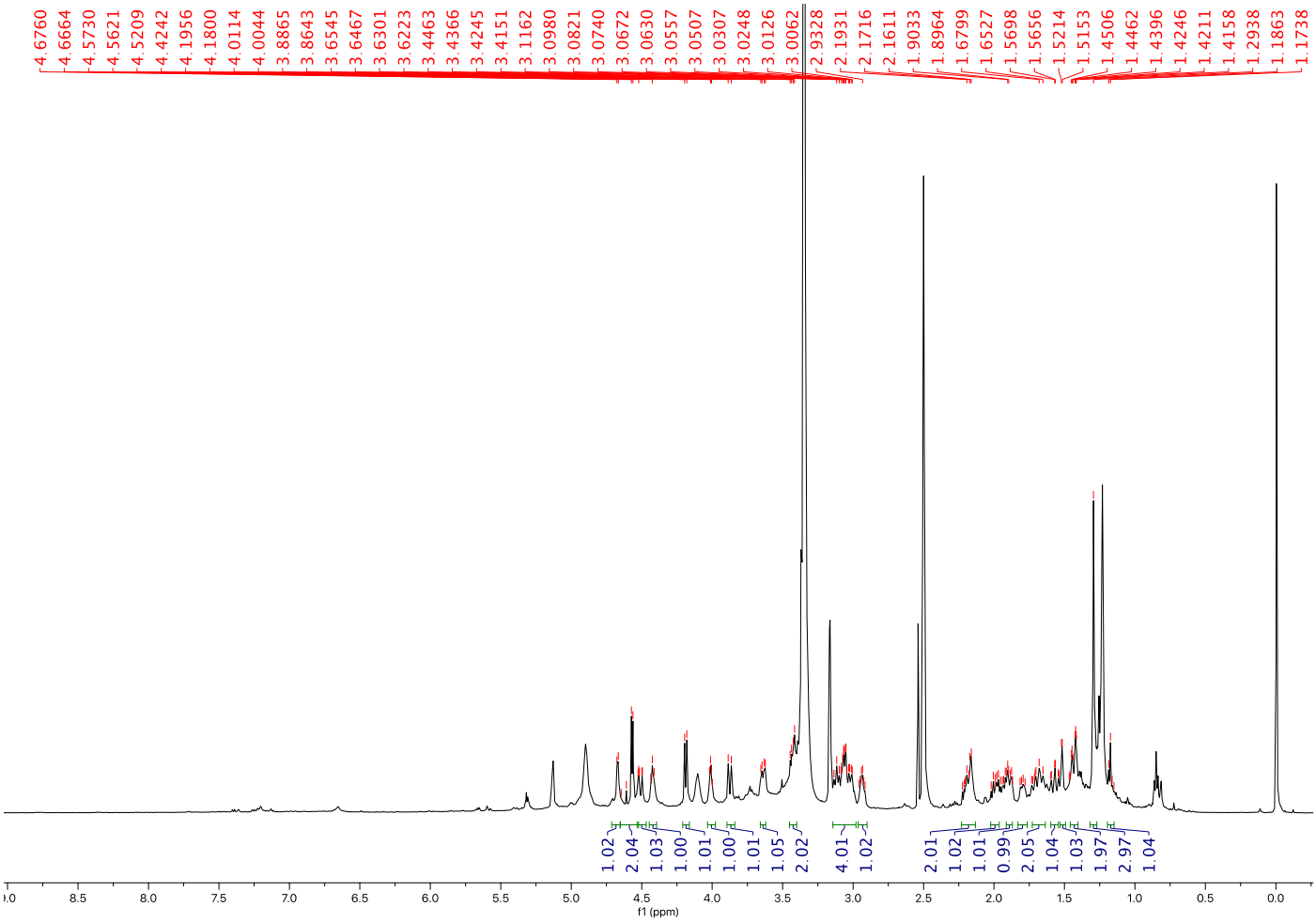

Fig. S13 ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **2**

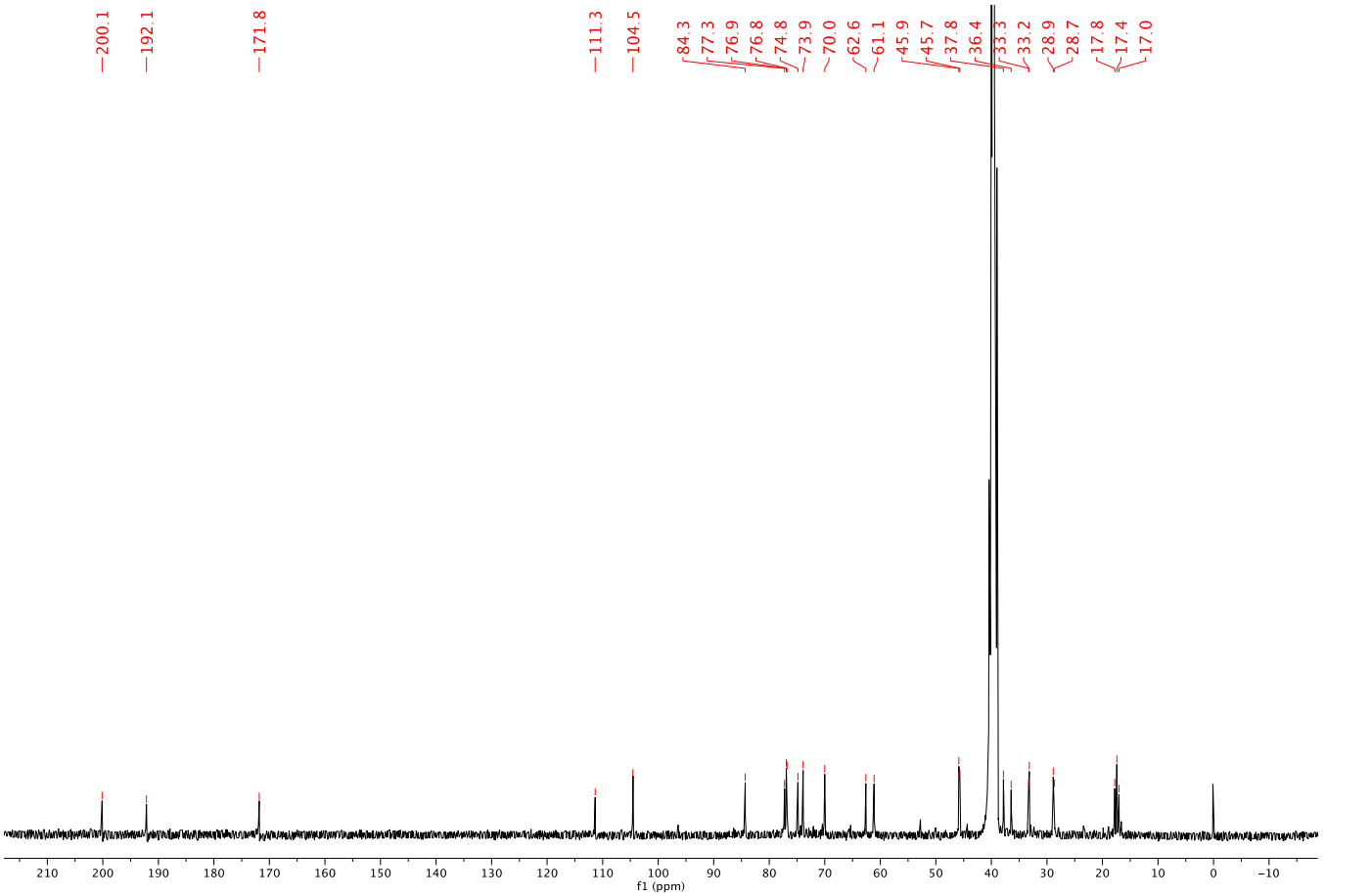

Fig. S14 DEPT135 (125 MHz, DMSO-d_6) spectrum of compound **2**

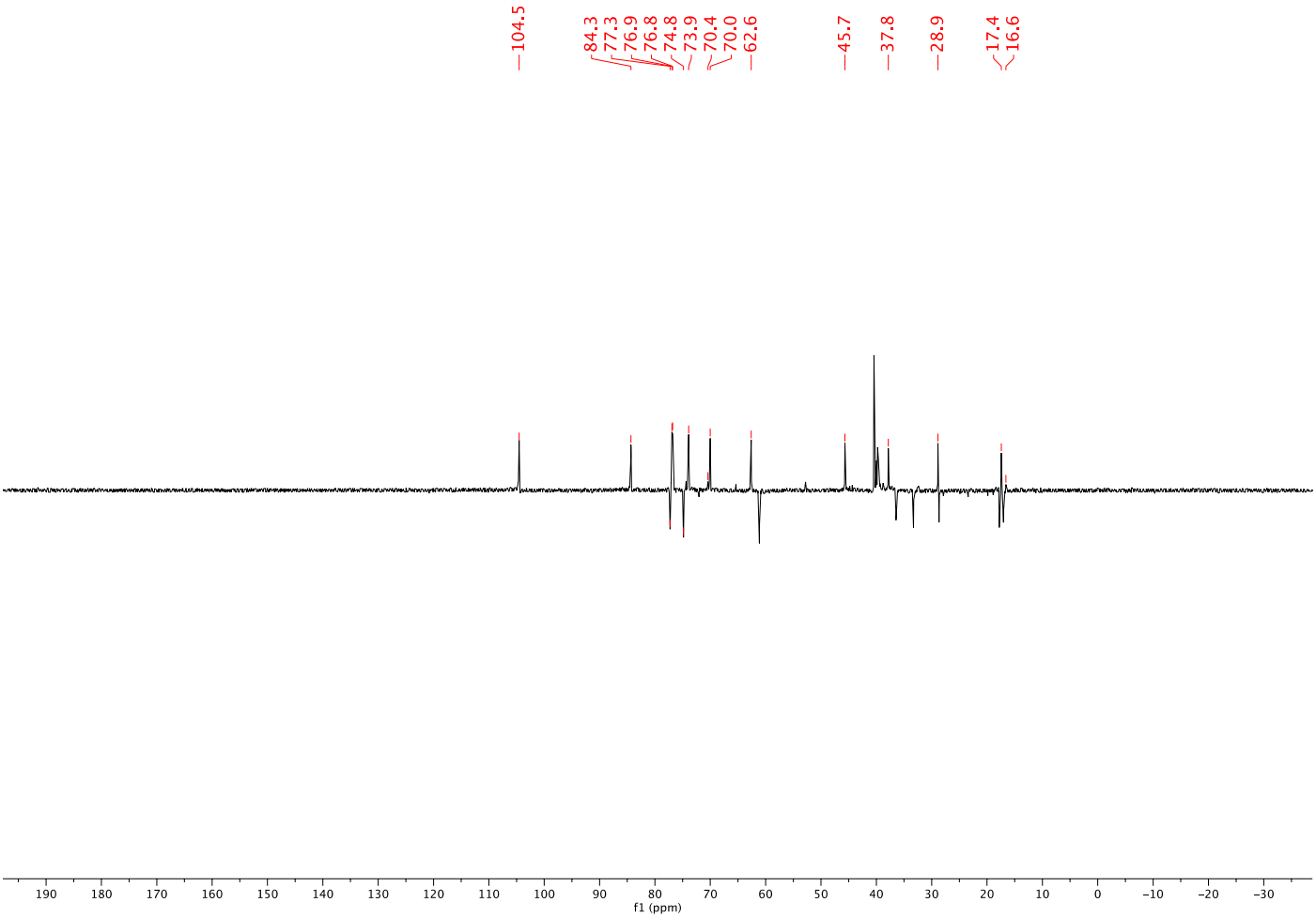

Fig. S15 ^{1}H−^{1}H COSY spectrum (500 MHz, DMSO-d_6) of compound **2**

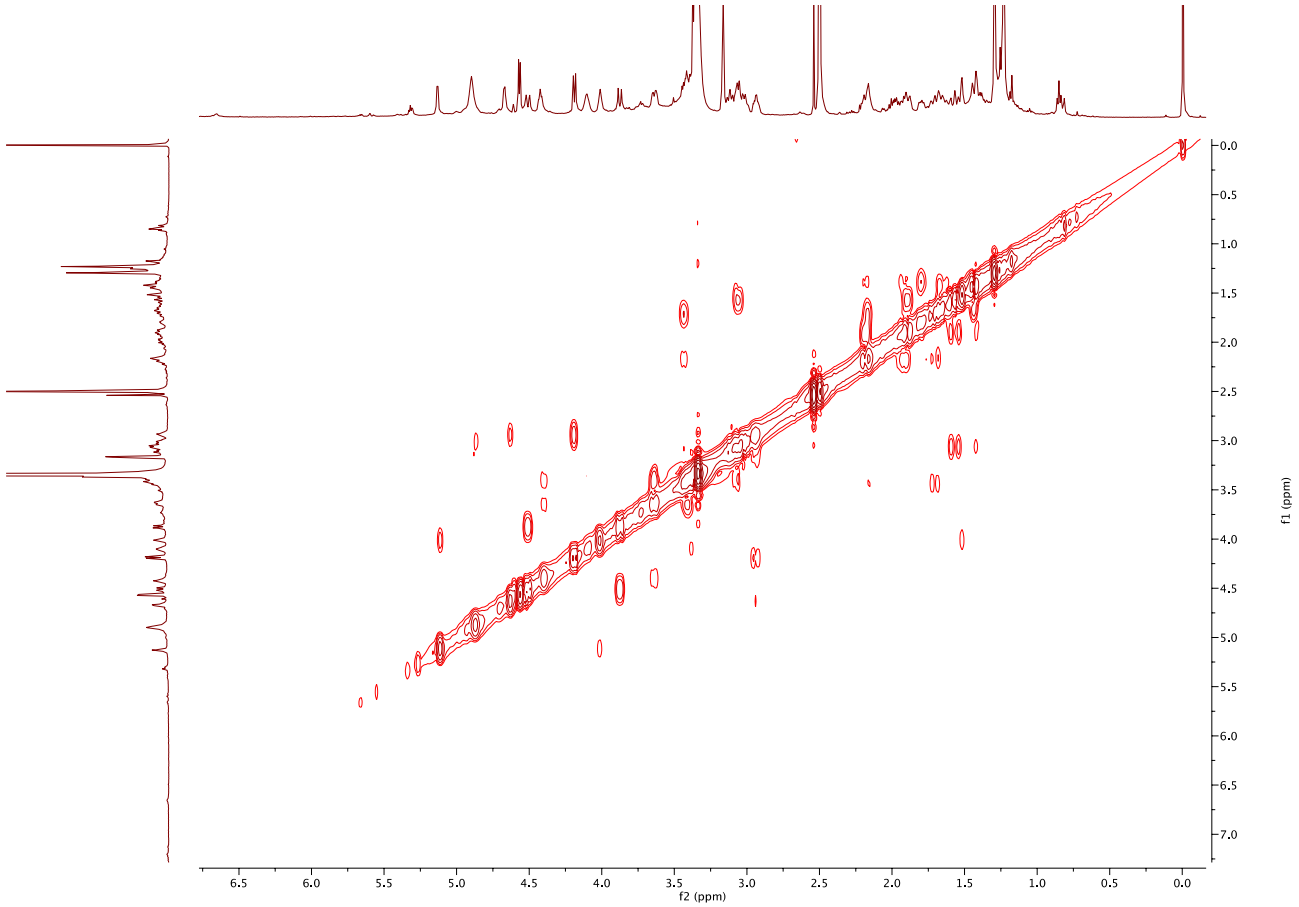

Fig. S16 HSQC spectrum (DMSO-d_6) of compound **2** (^{1}H: 500 MHz, ^{13}C: 125 MHz)

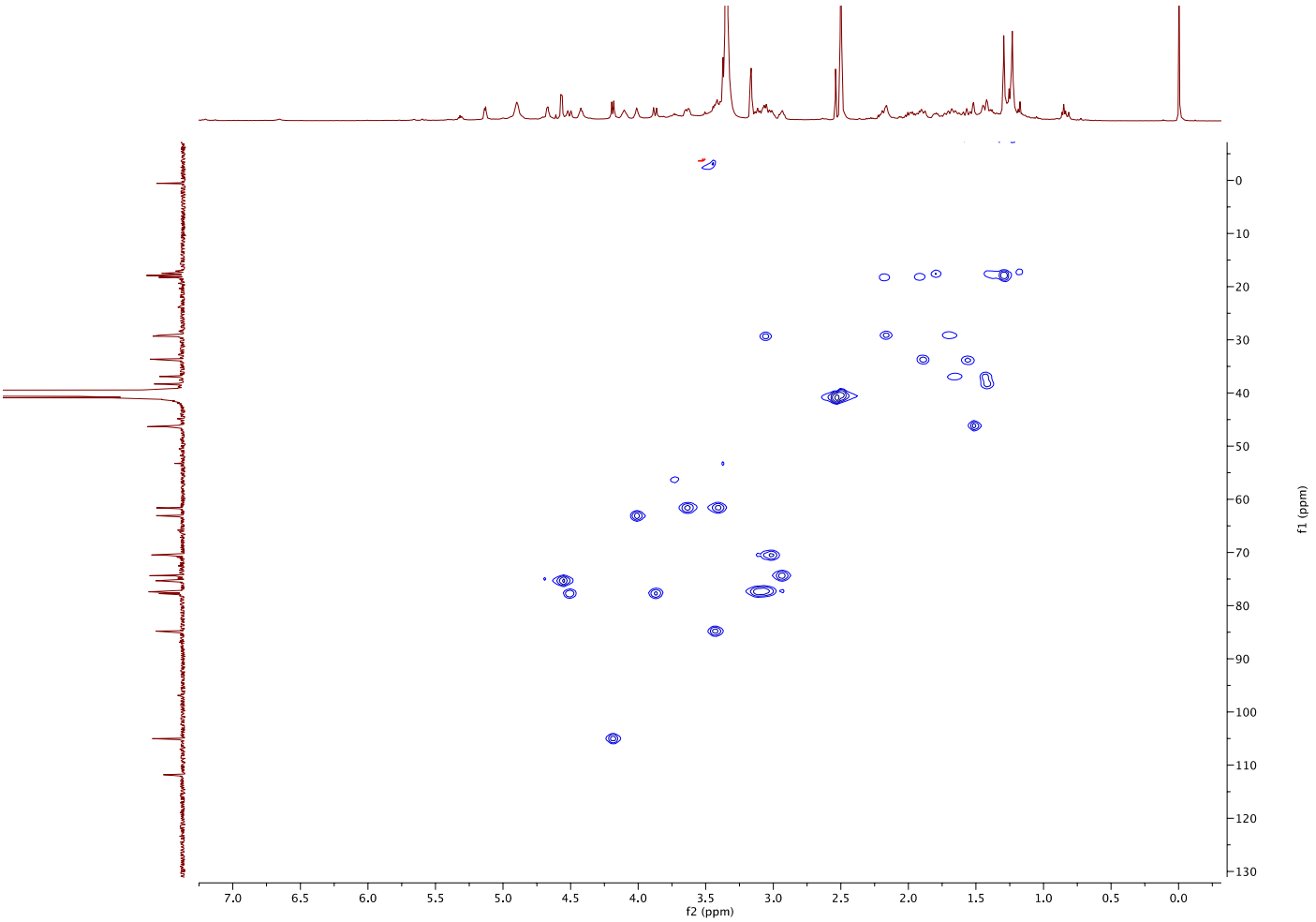

Fig. S17 HMBC spectrum (DMSO-d_6) of compound **2** (^{1}H: 500 MHz, ^{13}C: 125 MHz)

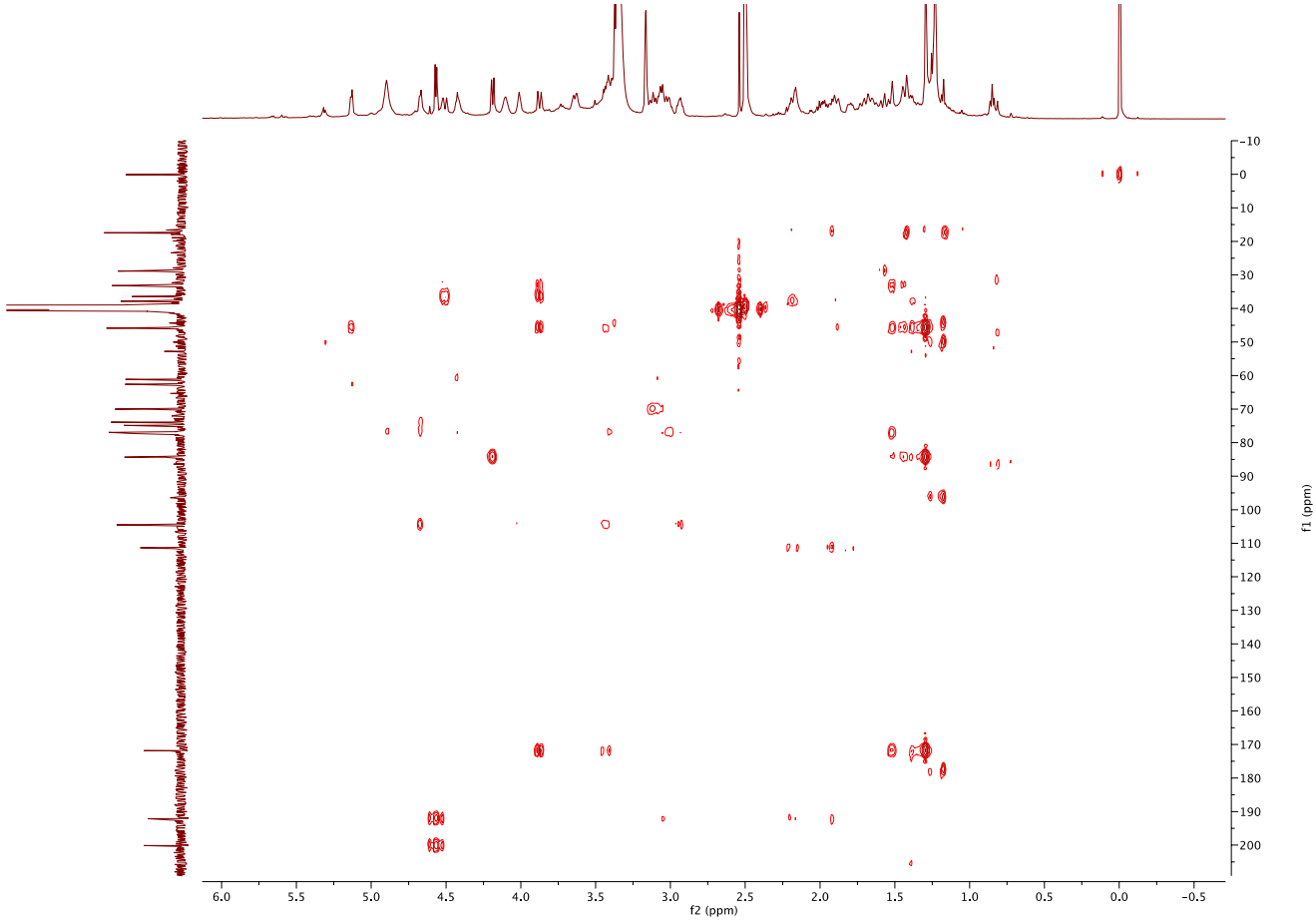

Fig. S18 NOESY spectrum (500 MHz, DMSO-d_6) of compound **2**

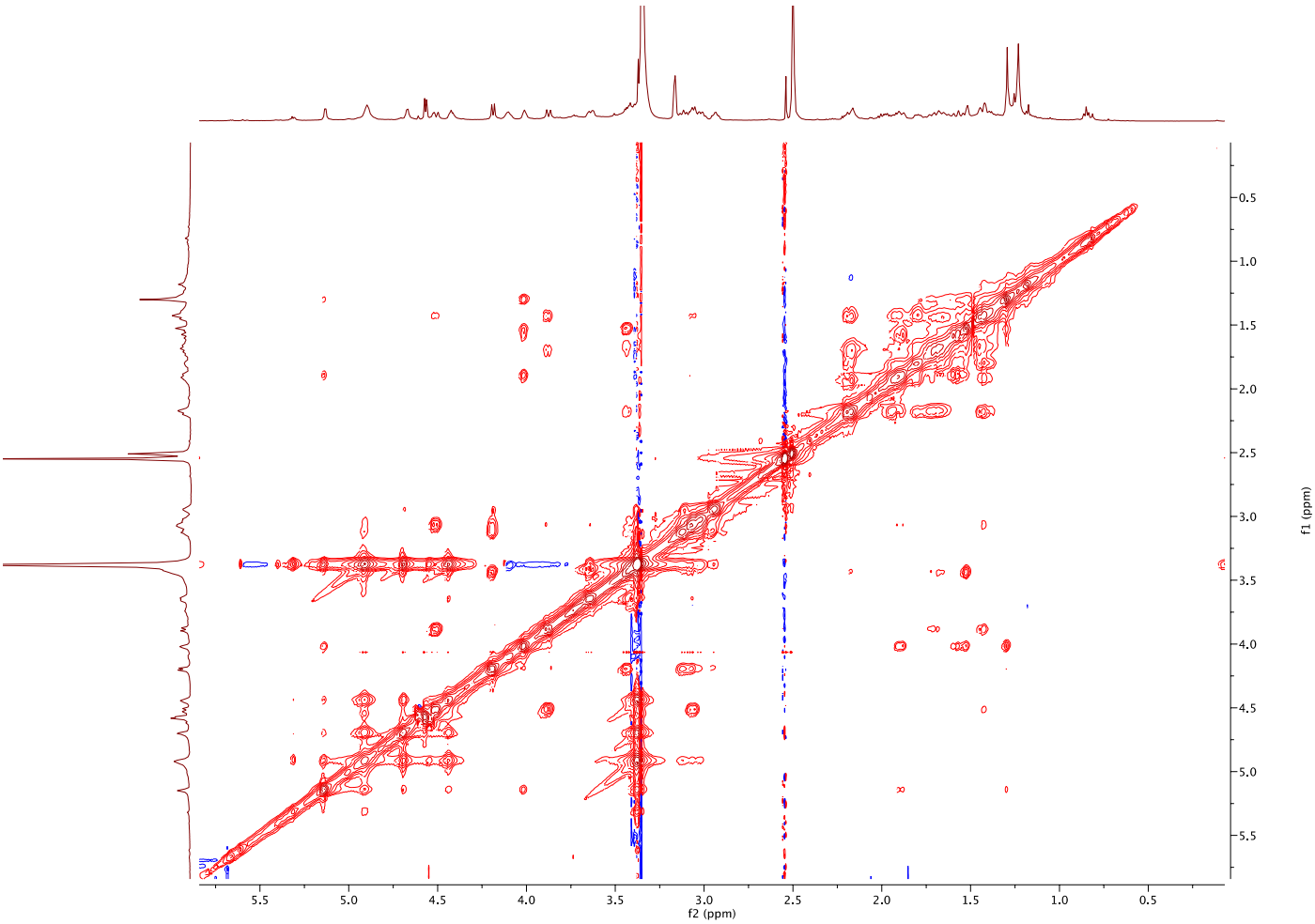

Fig. S19 IR spectrum of compound **2**

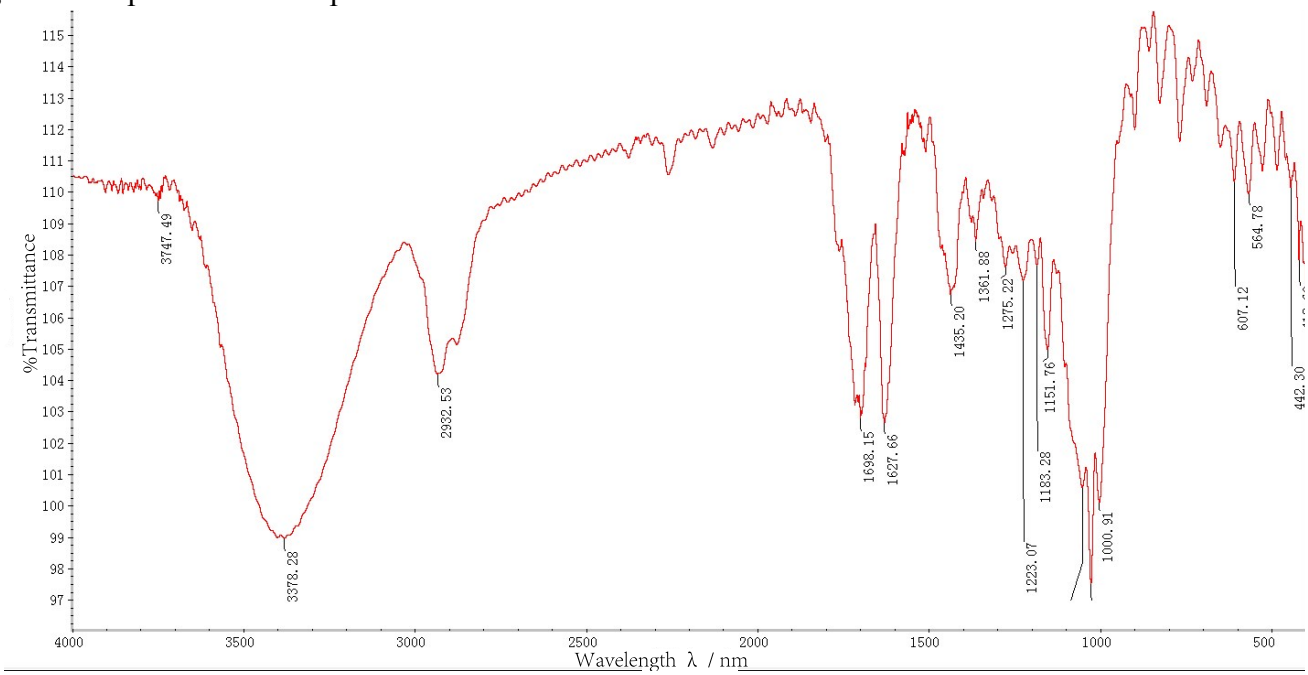

Fig. S20 (−)-HR-ESI-MS spectrum of compound **2**

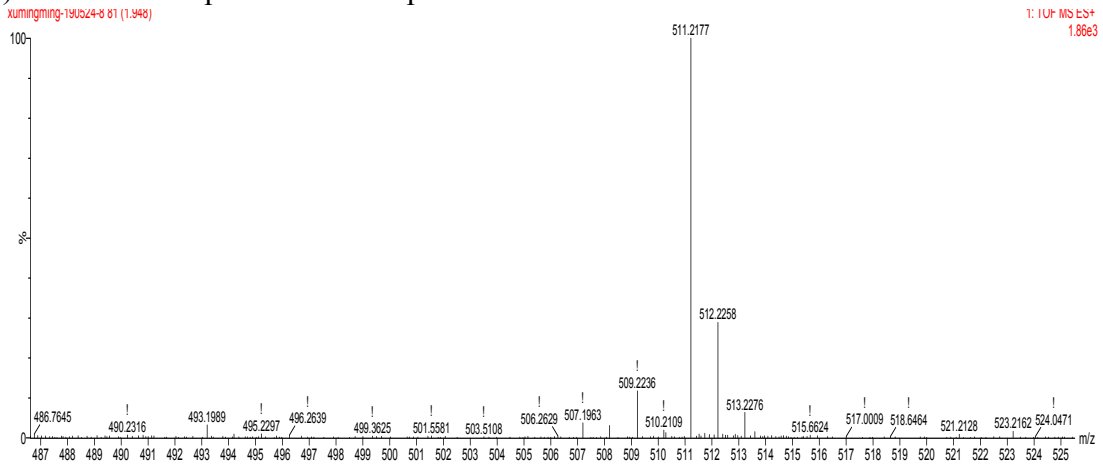

Fig. S21 UV spectrum of compound **2**

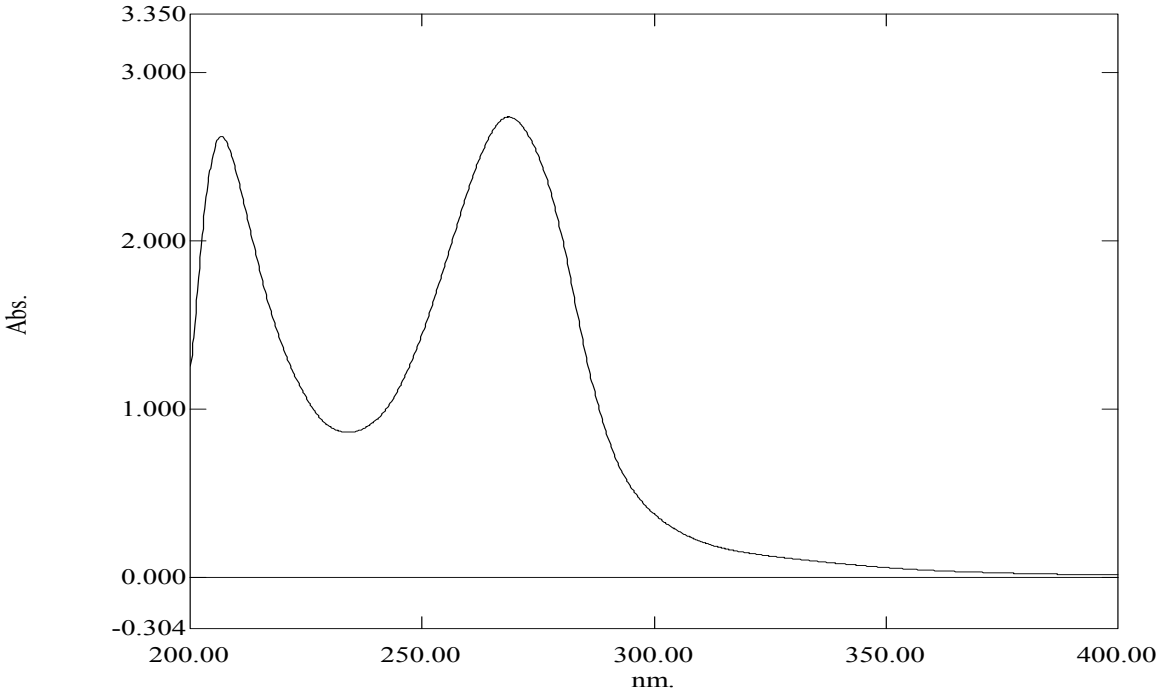

Fig. S22 ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **3**

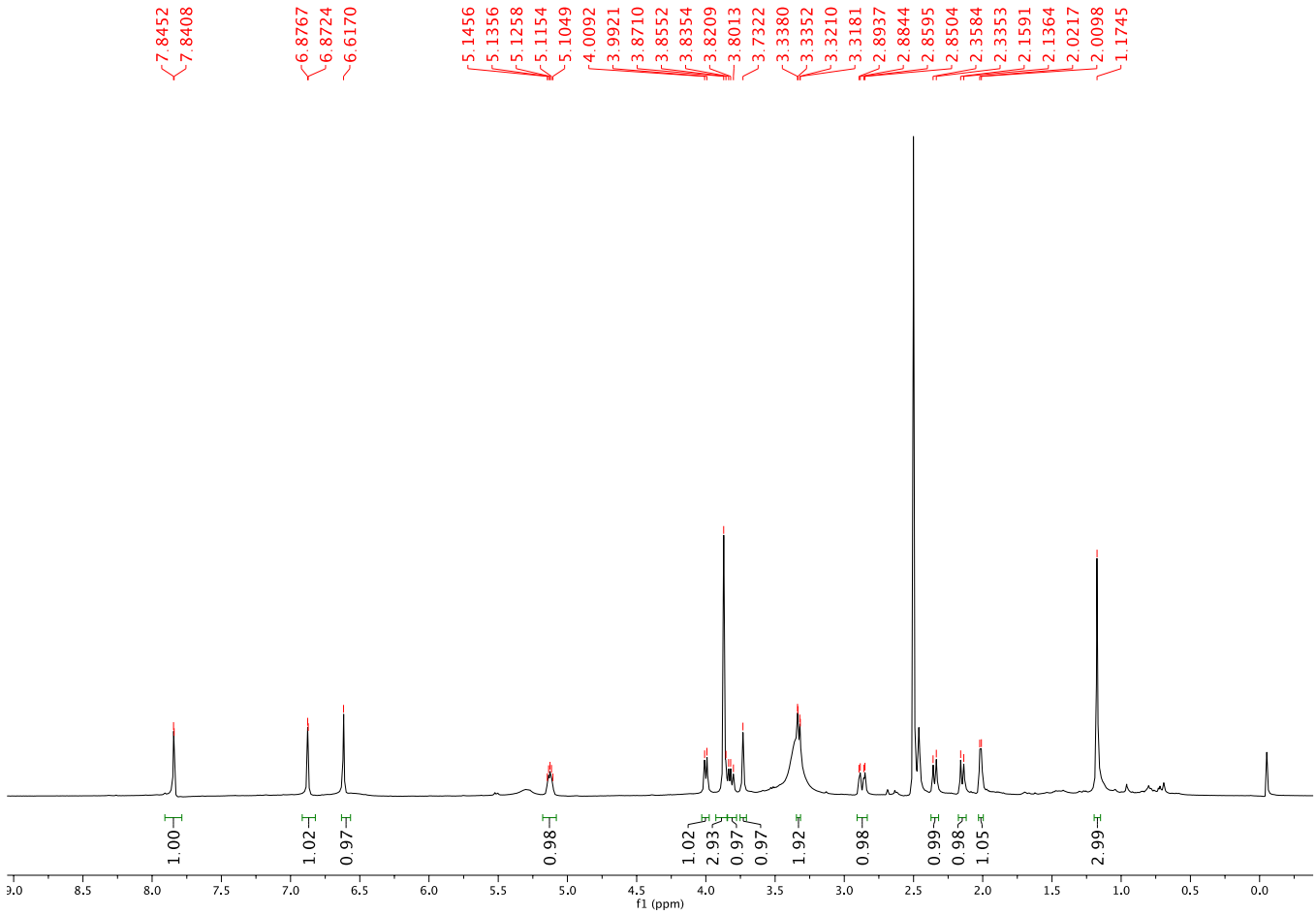

Fig. S23 ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **3**

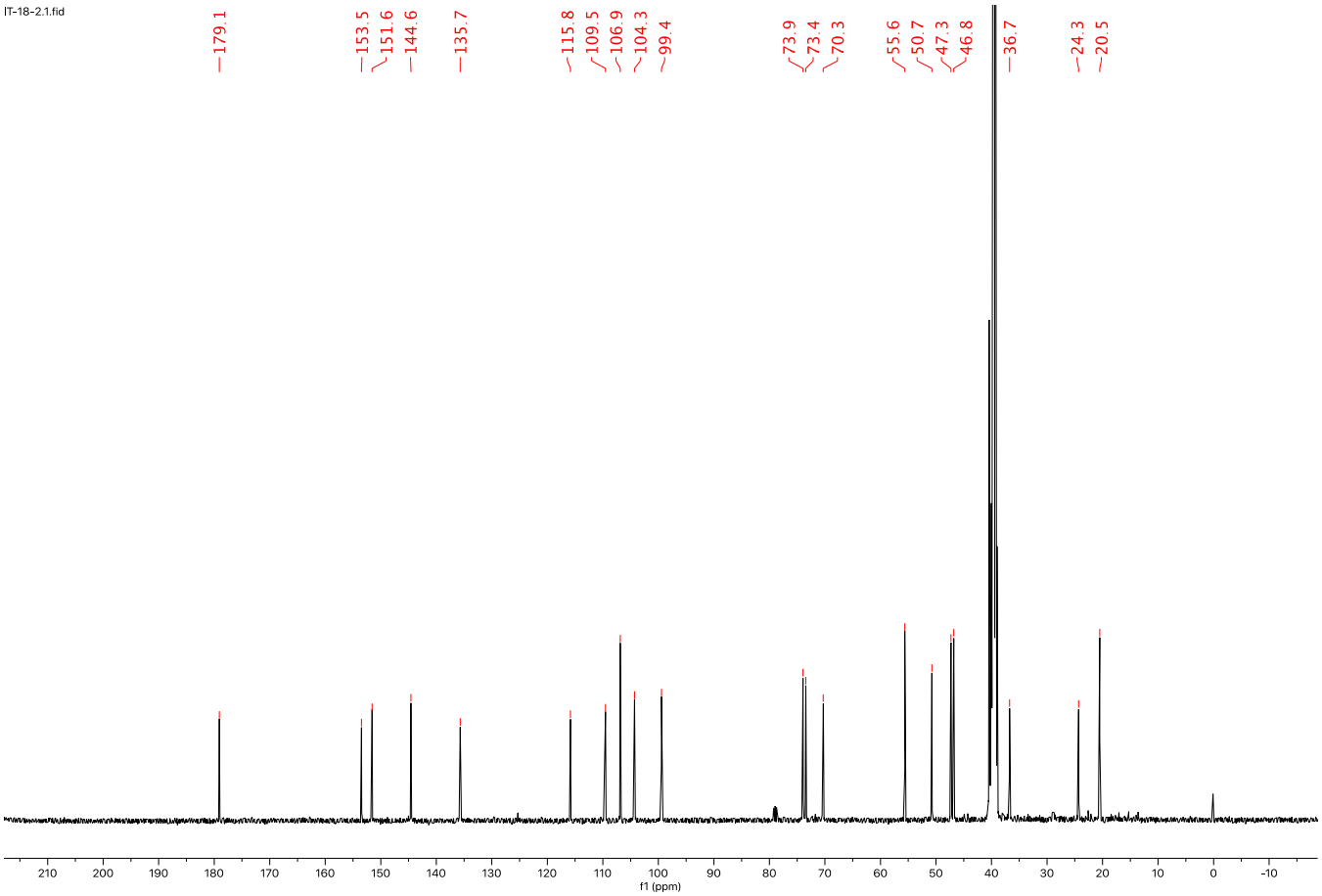

Fig. S24 ^{1}H NMR (500 MHz, CD_3OD-d_4) spectrum of compound **4**

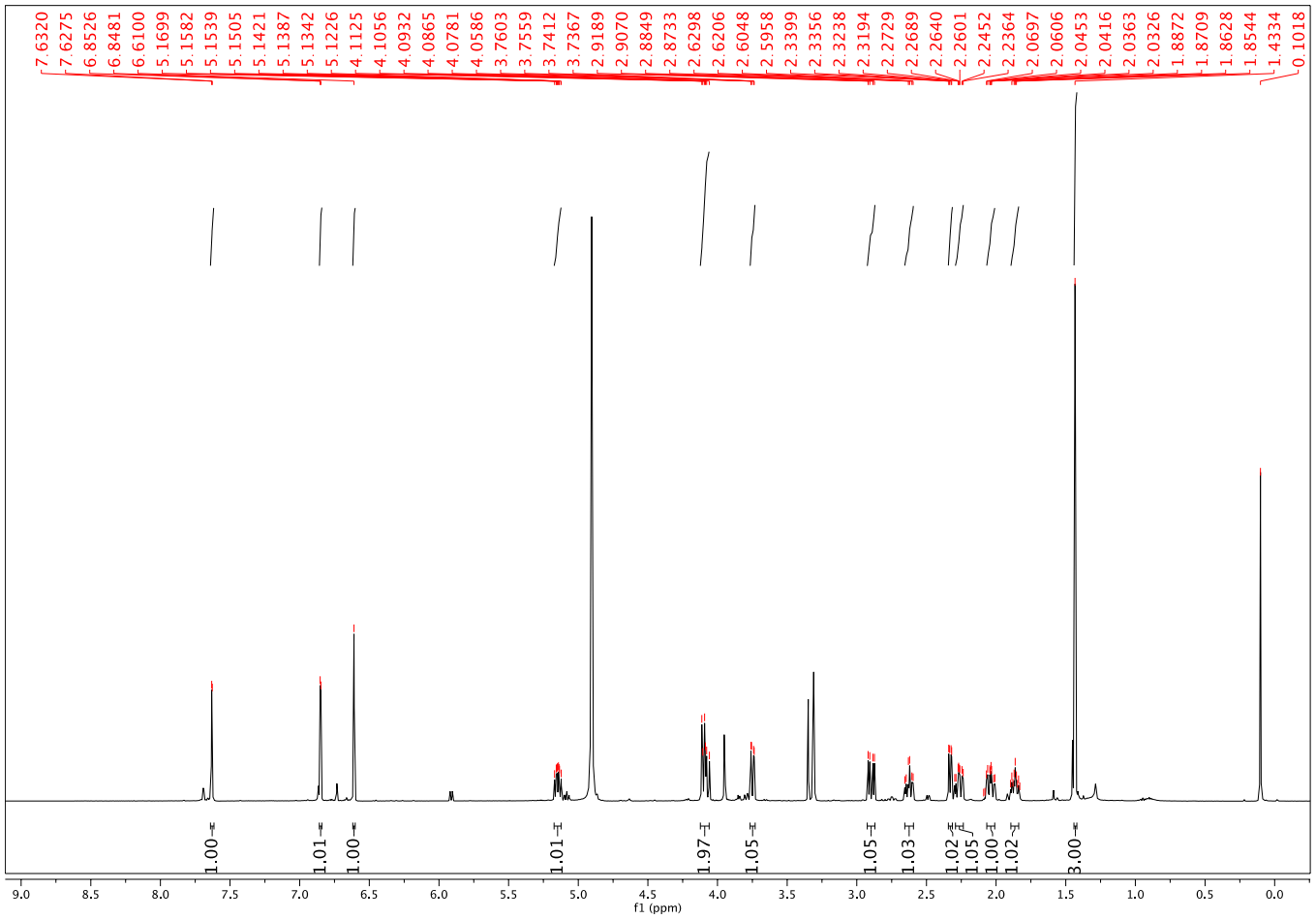

Fig. S25 ^{13}C NMR (125 MHz, CD_3OD-d_4) spectrum of compound **4**

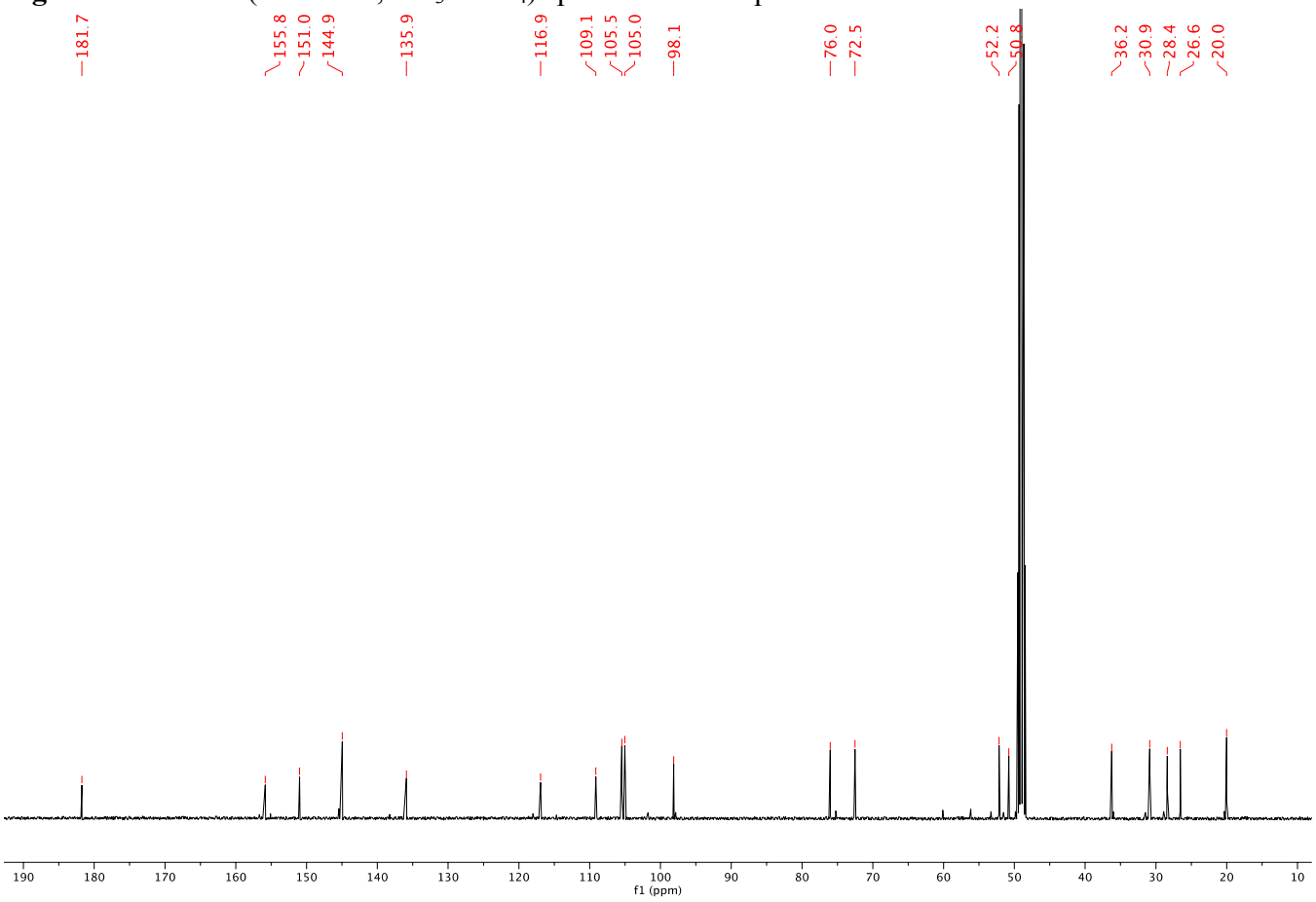

Fig. S26 ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **5**

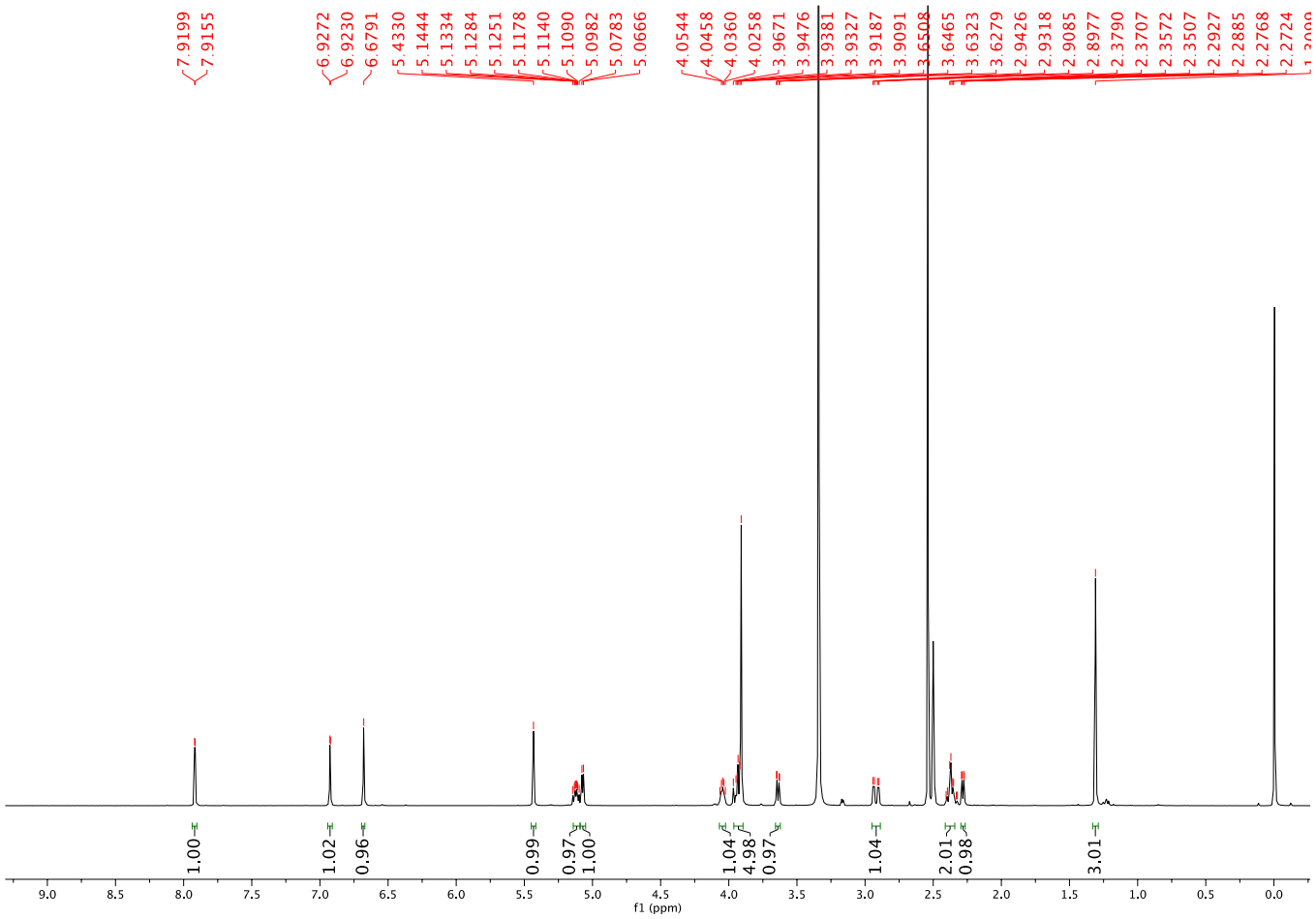

Fig. S27 ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **5**

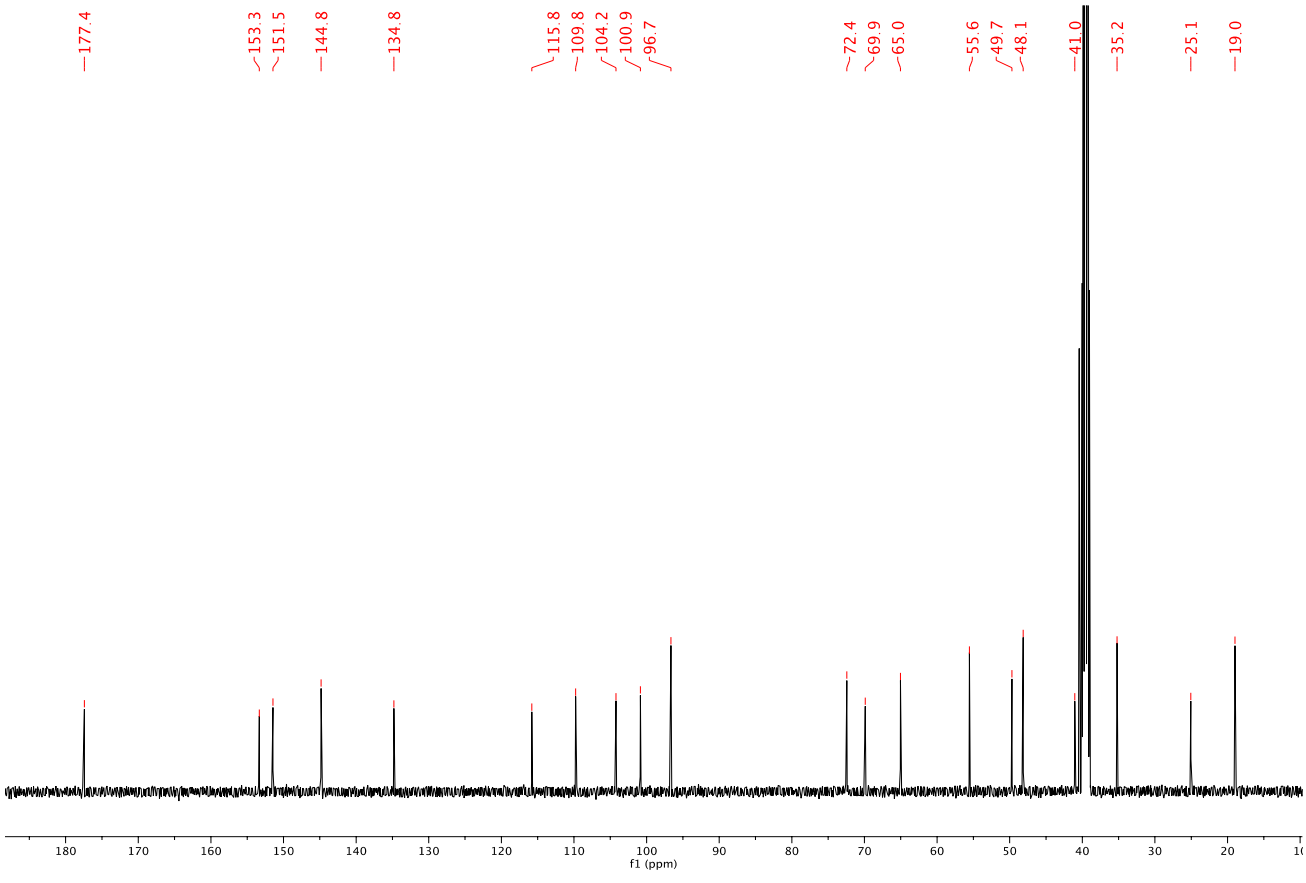

Fig. S28 ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **6**

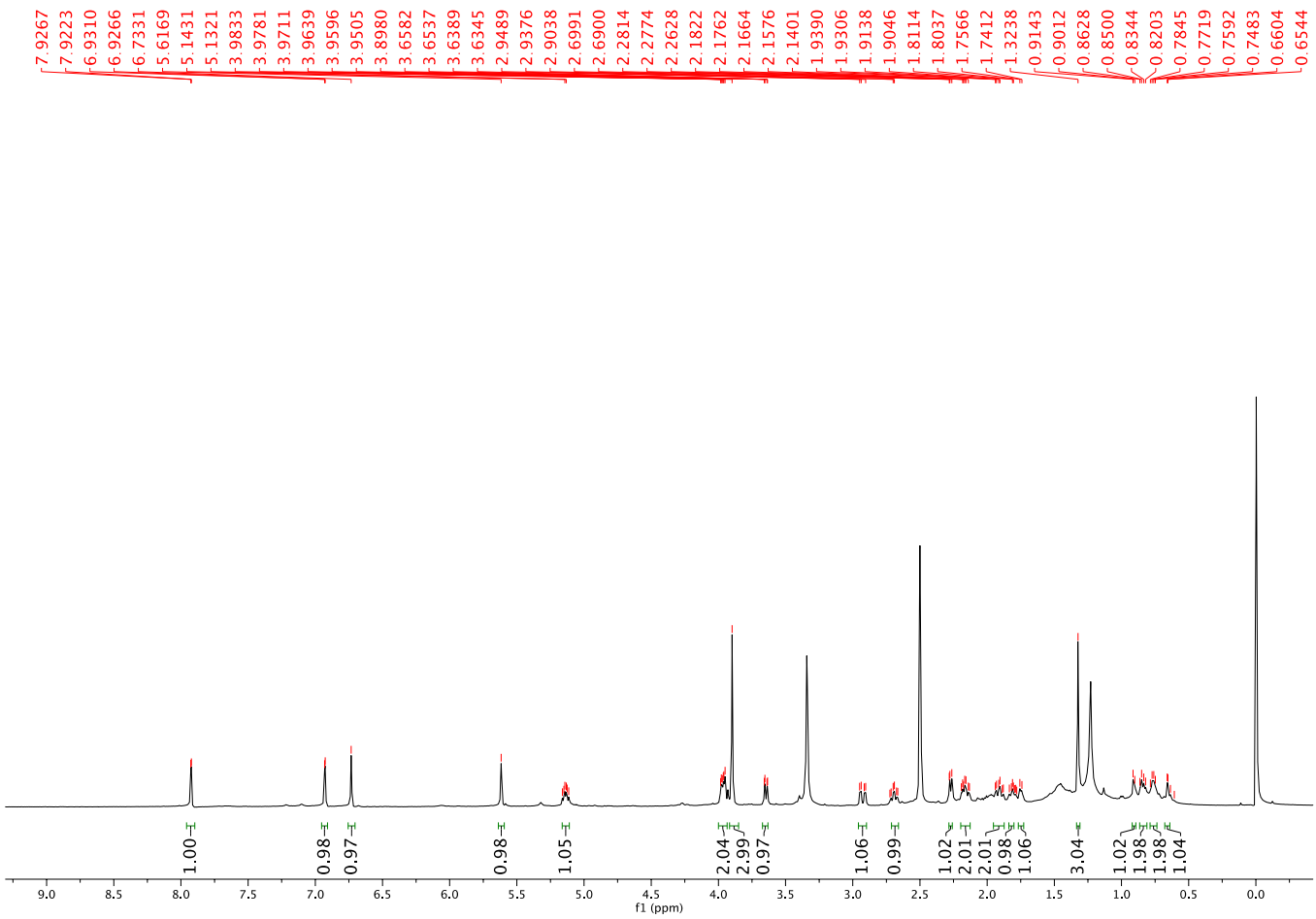

Fig. S29 ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **6**

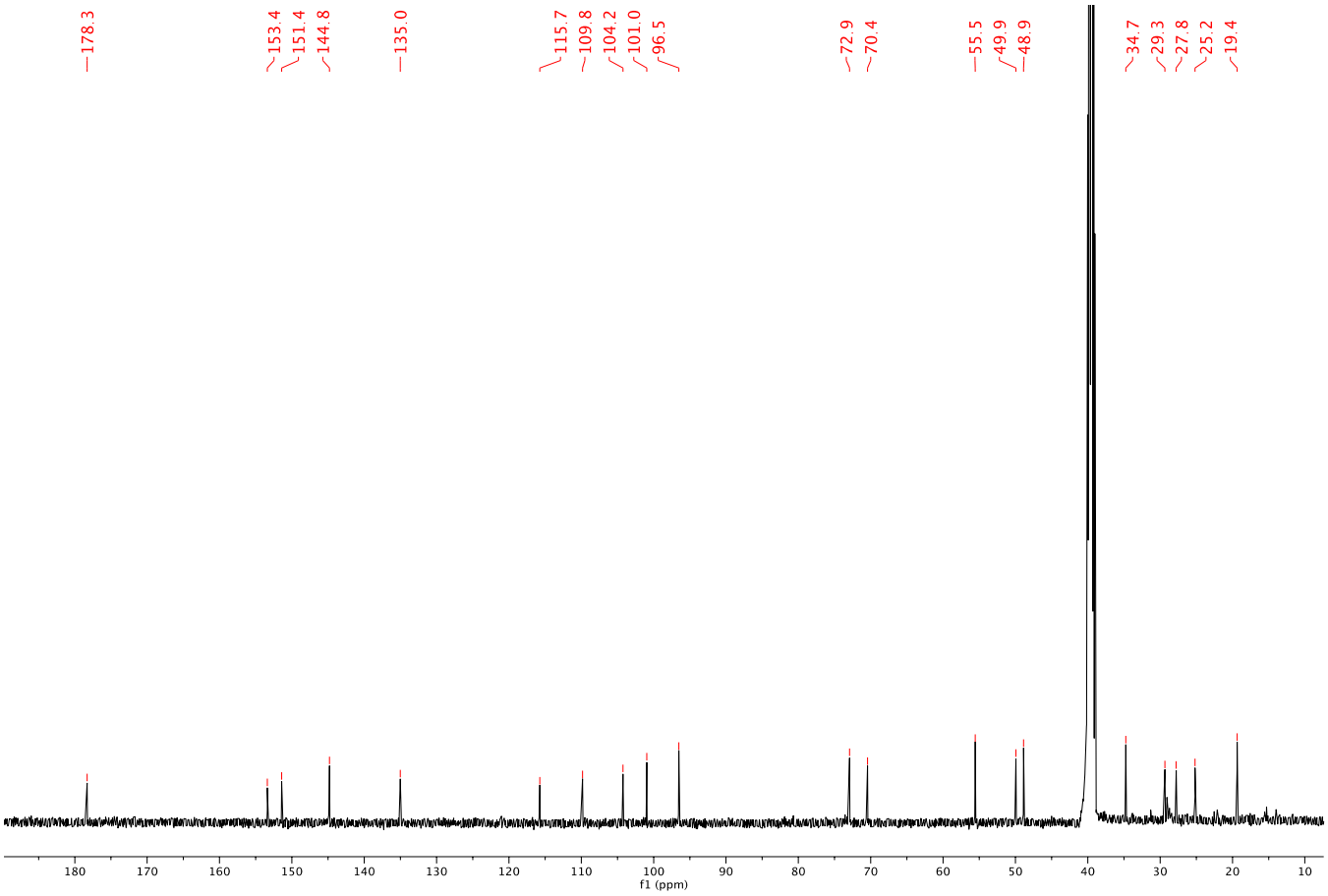

Fig. S30 ^{1}H NMR (500 MHz, Pyridine-d_5) spectrum of compound **7**

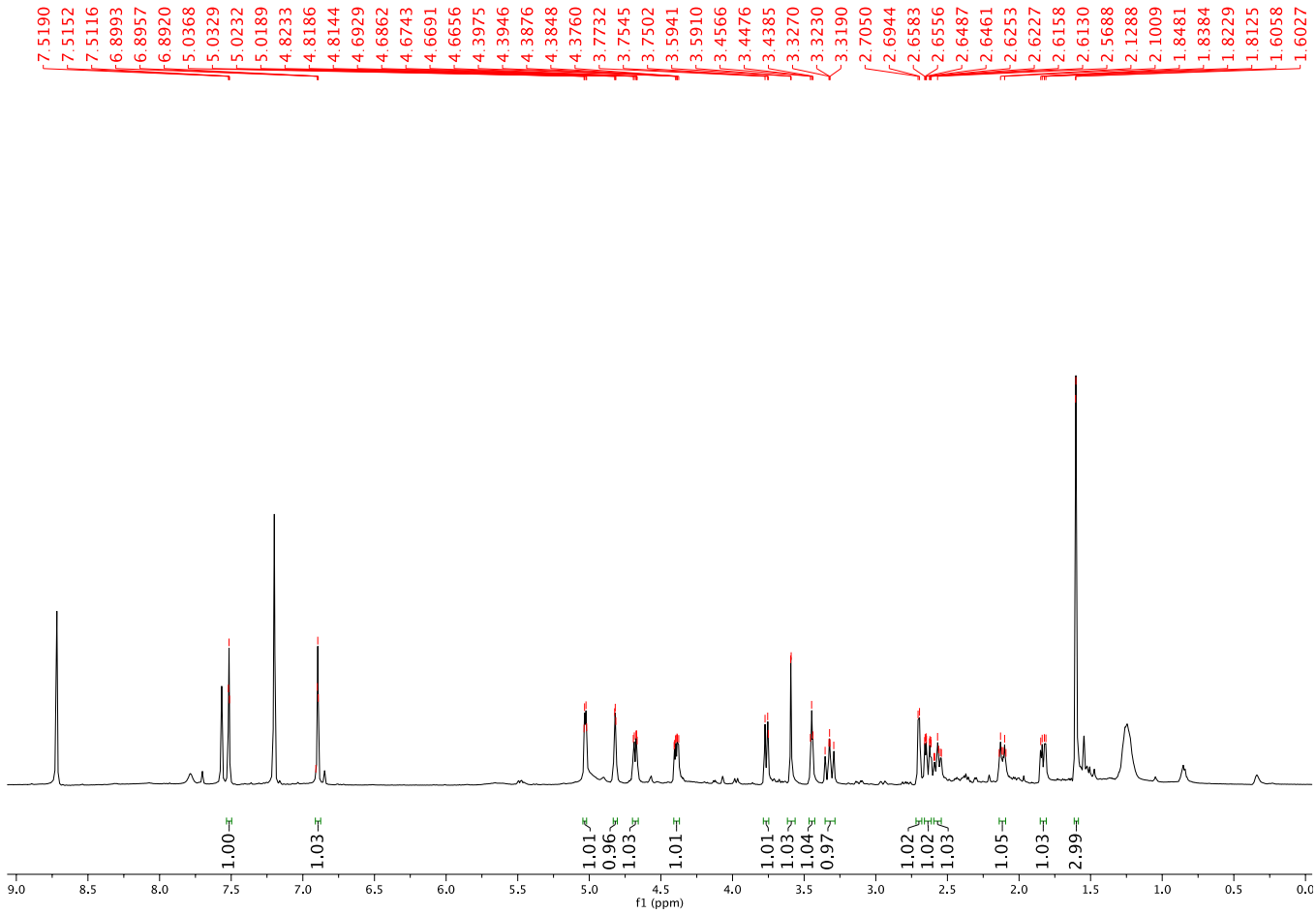

Fig. S31 ^{13}C NMR (125 MHz, pyridine-d_5) spectrum of compound **7**

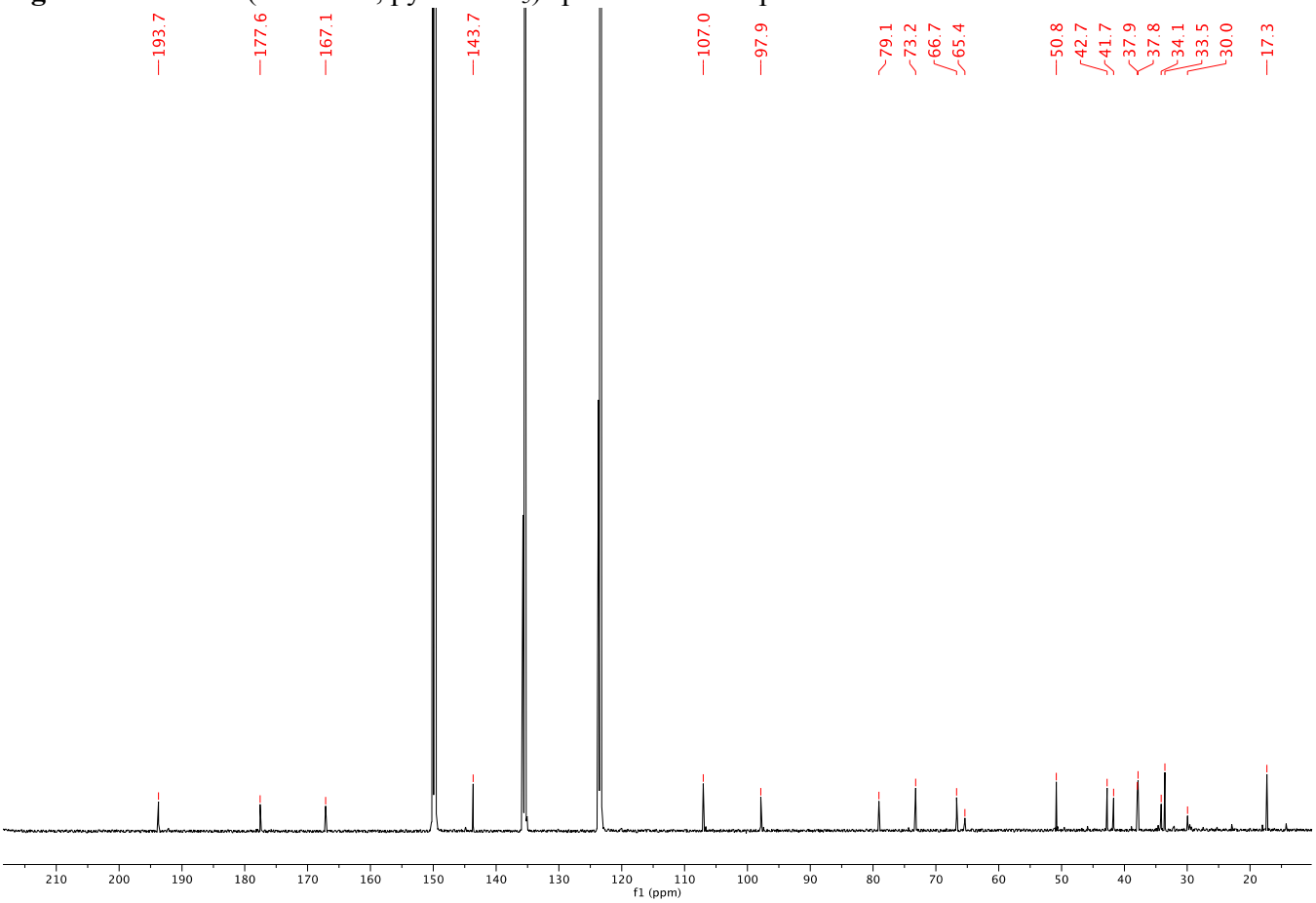

Fig. S32 ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **8**

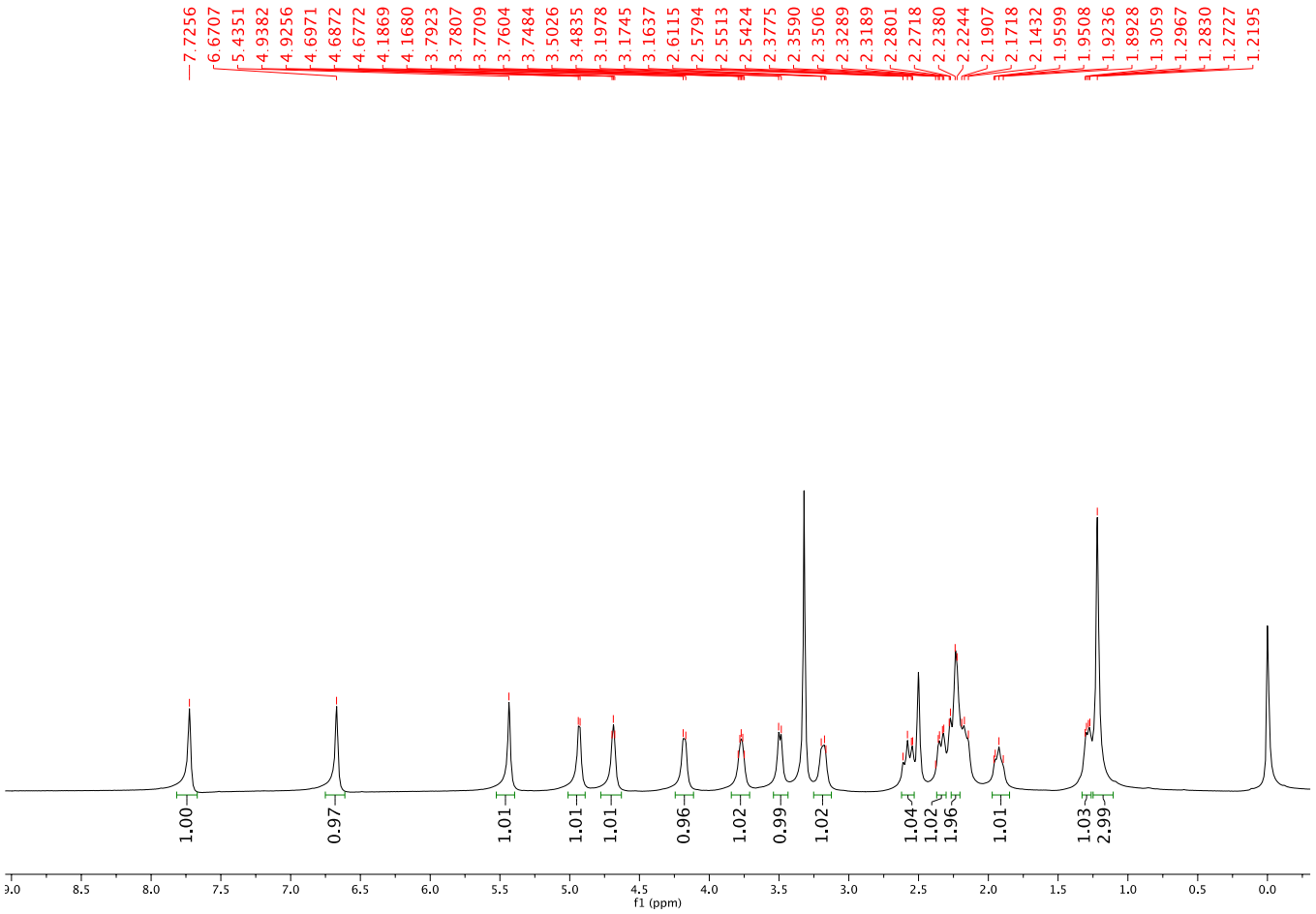

Fig. S33 ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **8**

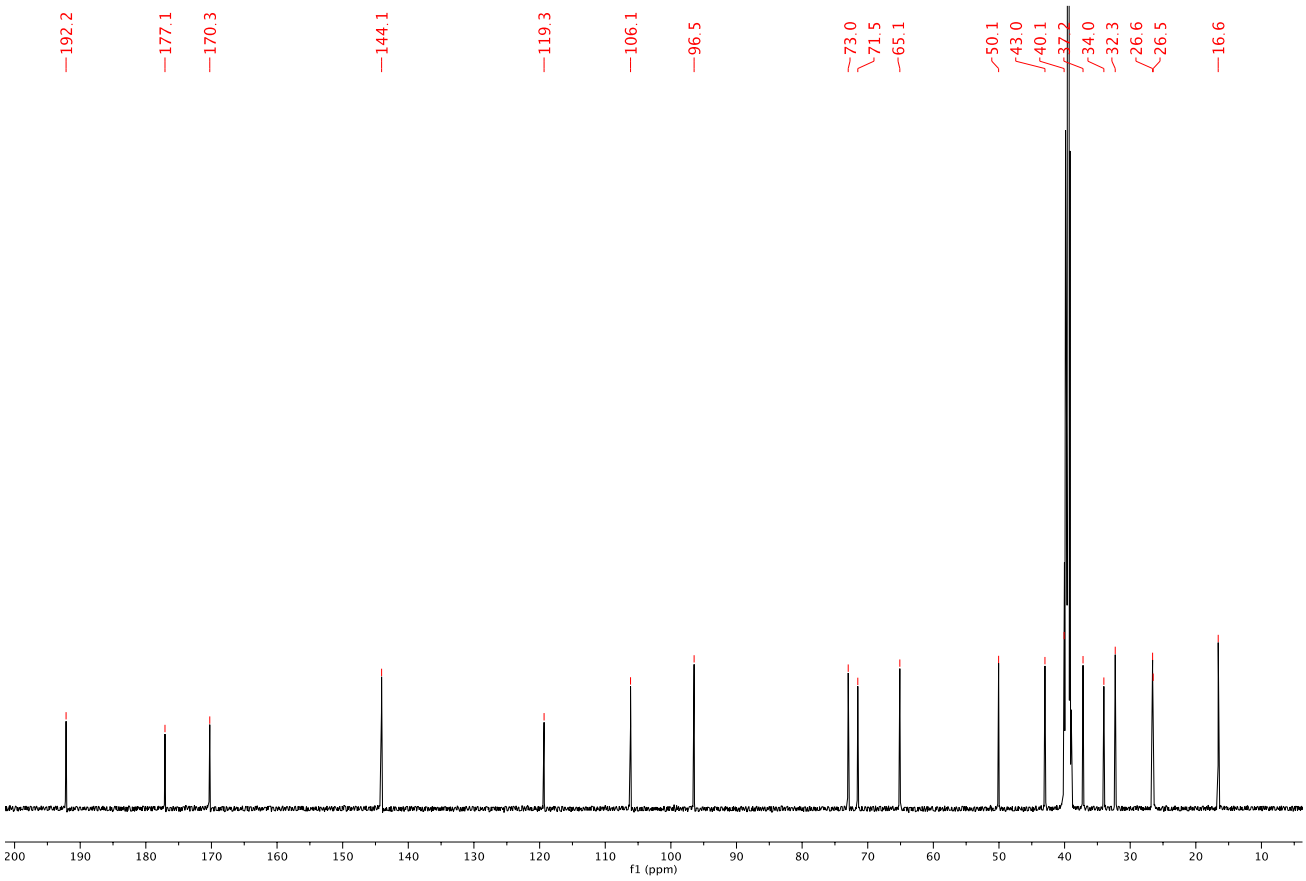

Fig. S34 ^{1}H NMR (500 MHz, Pyridine-d_5) spectrum of compound **9**

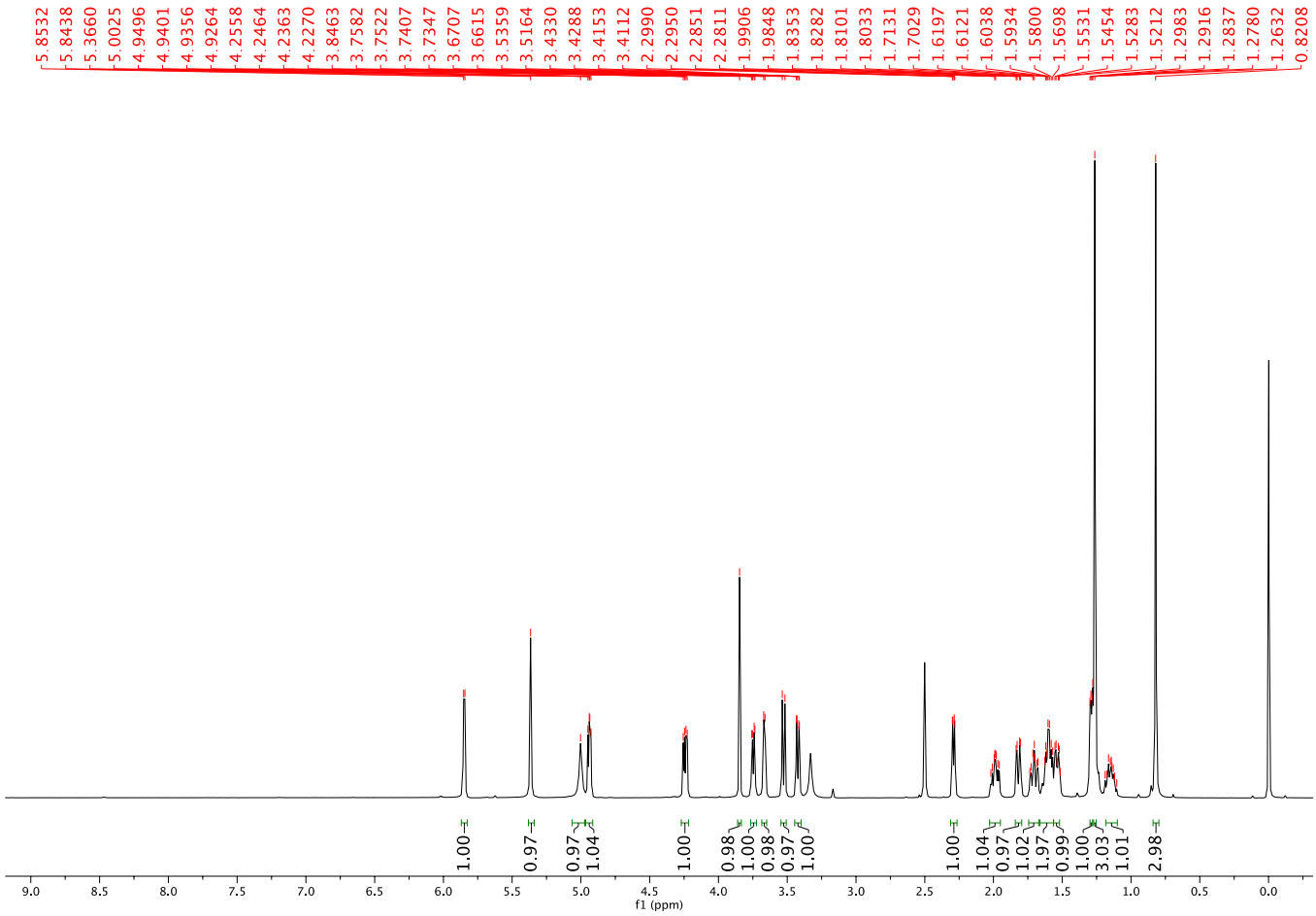

Fig. S35 ^{13}C NMR (125 MHz, Pyridine-d_5) spectrum of compound **9**

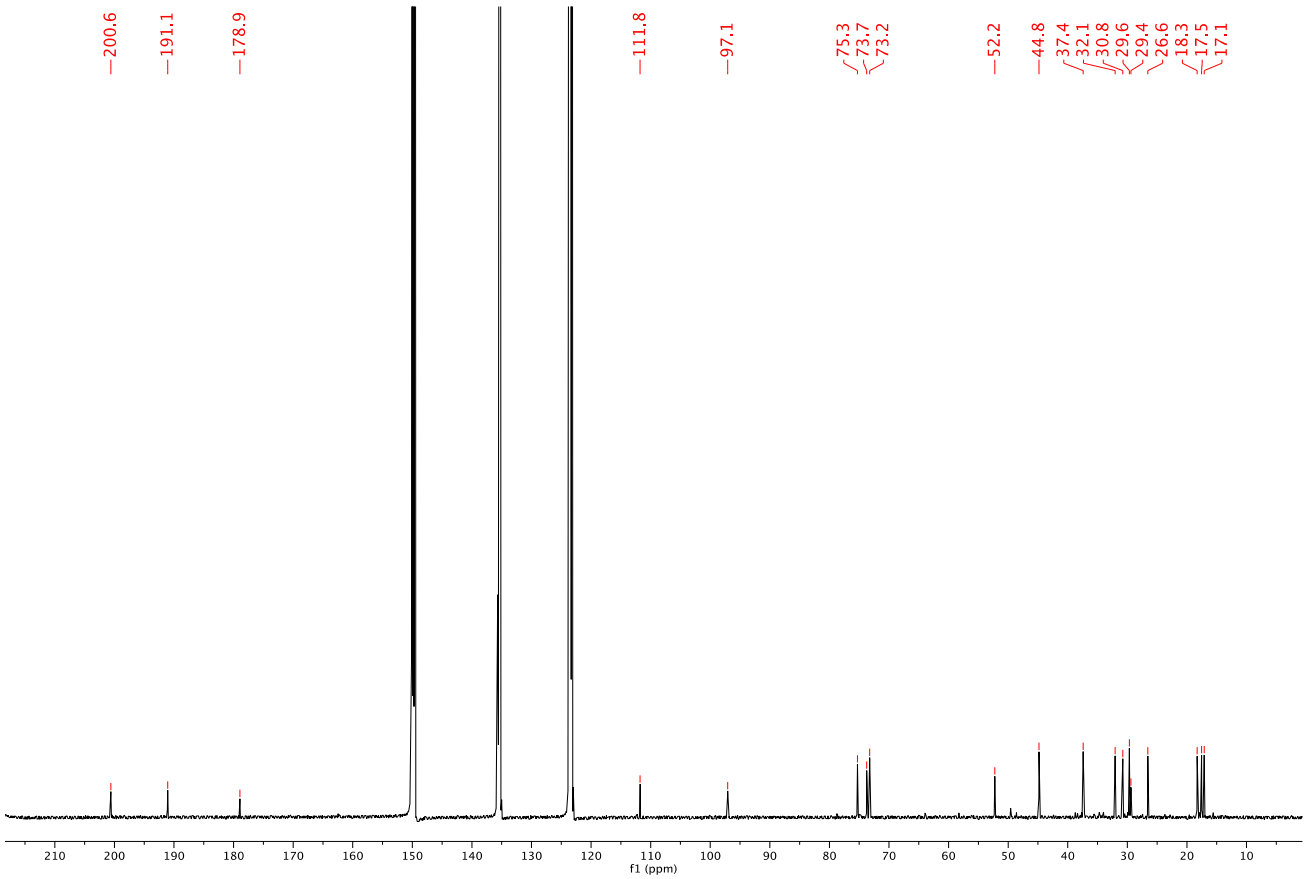

Fig. S36 ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **10**

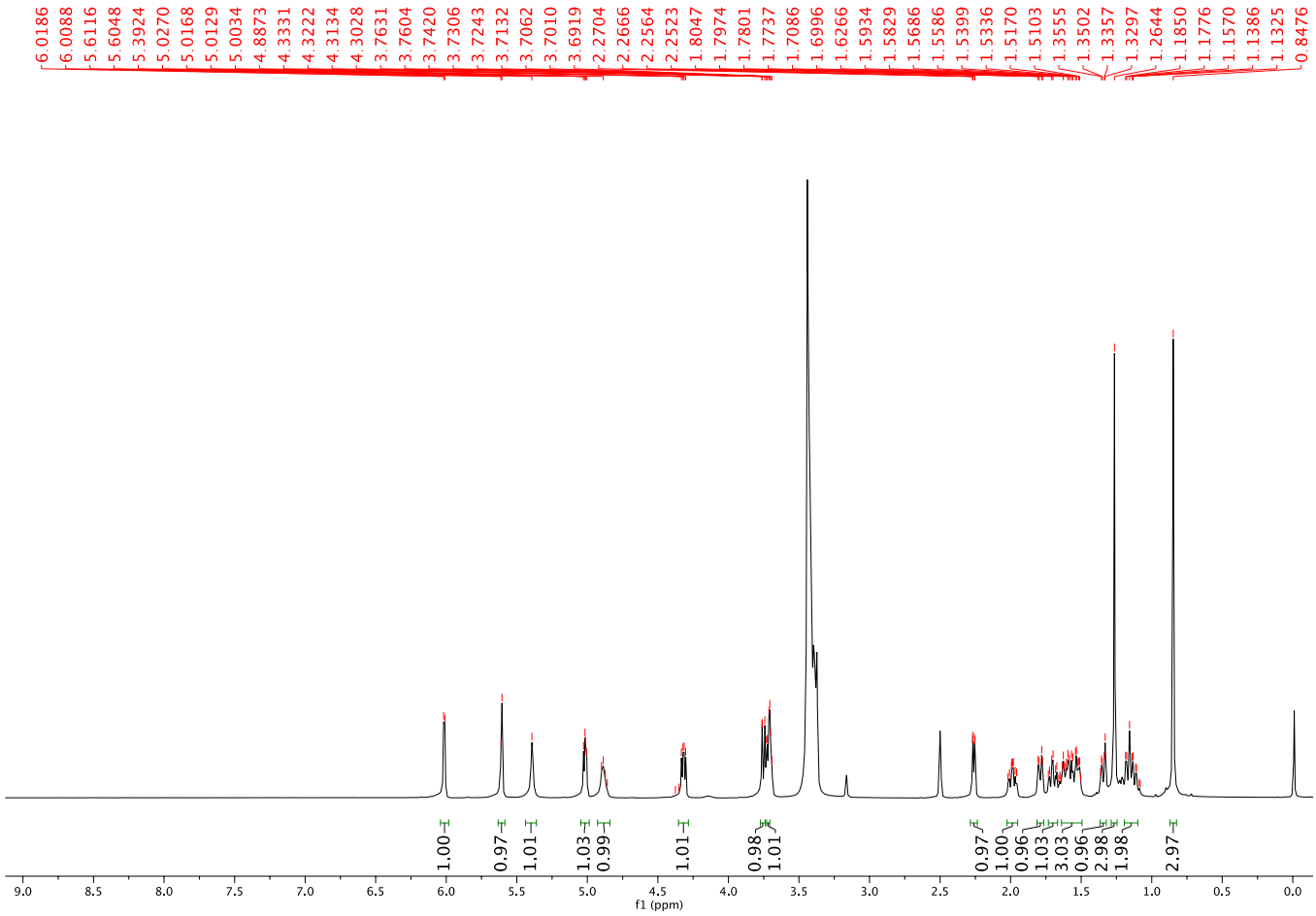

Fig. S37 ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **10**

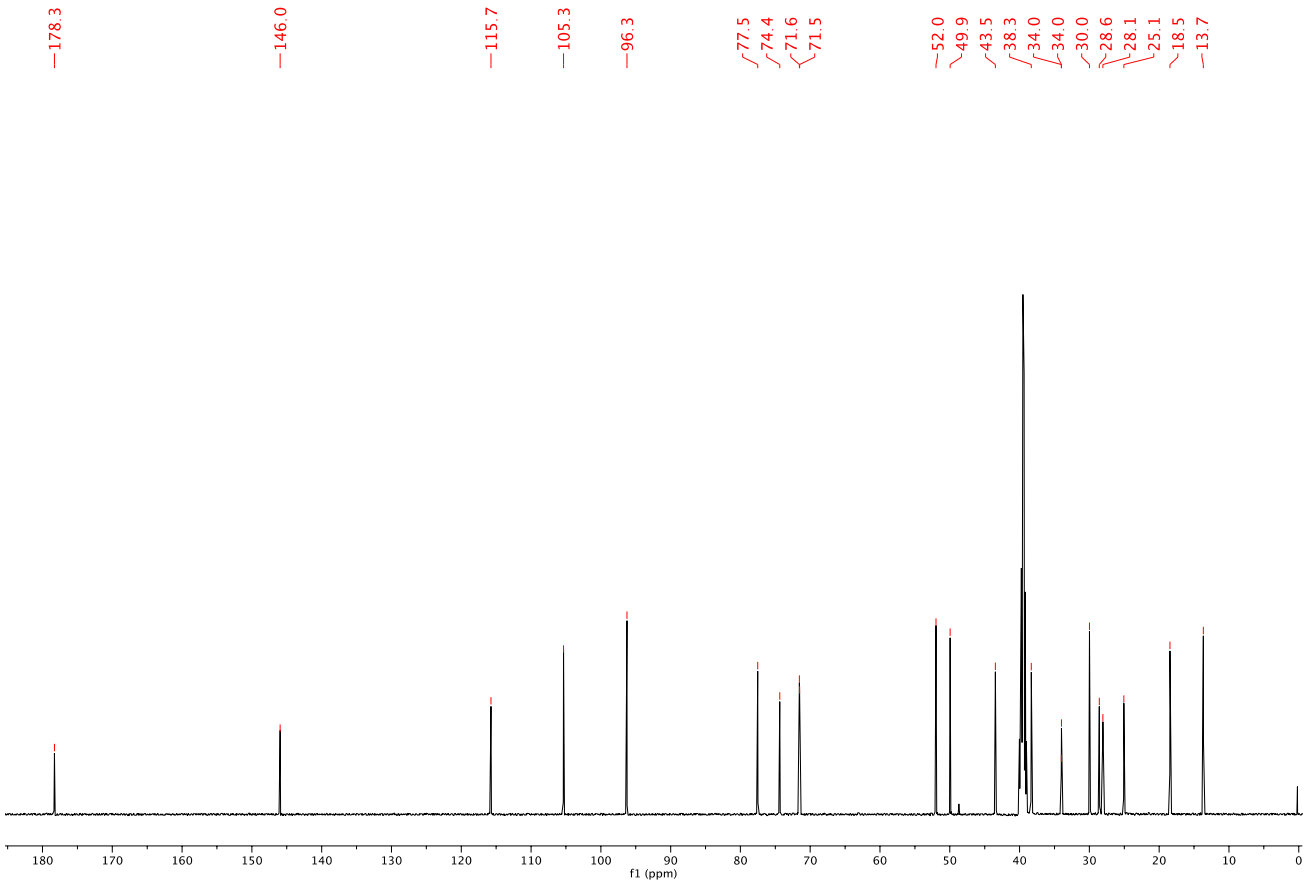

Fig. S38 ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **11**

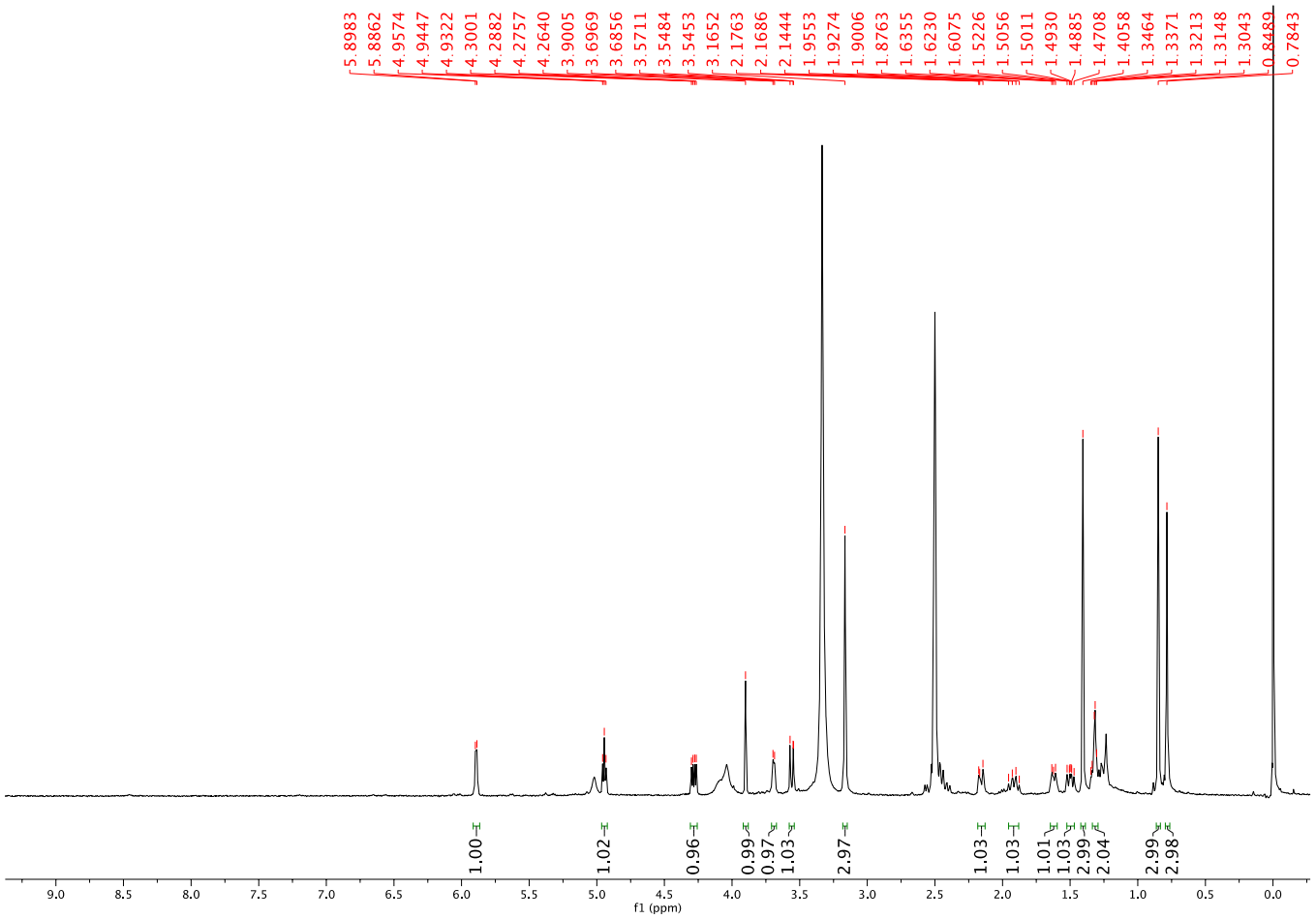

Fig. S39 ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **11**

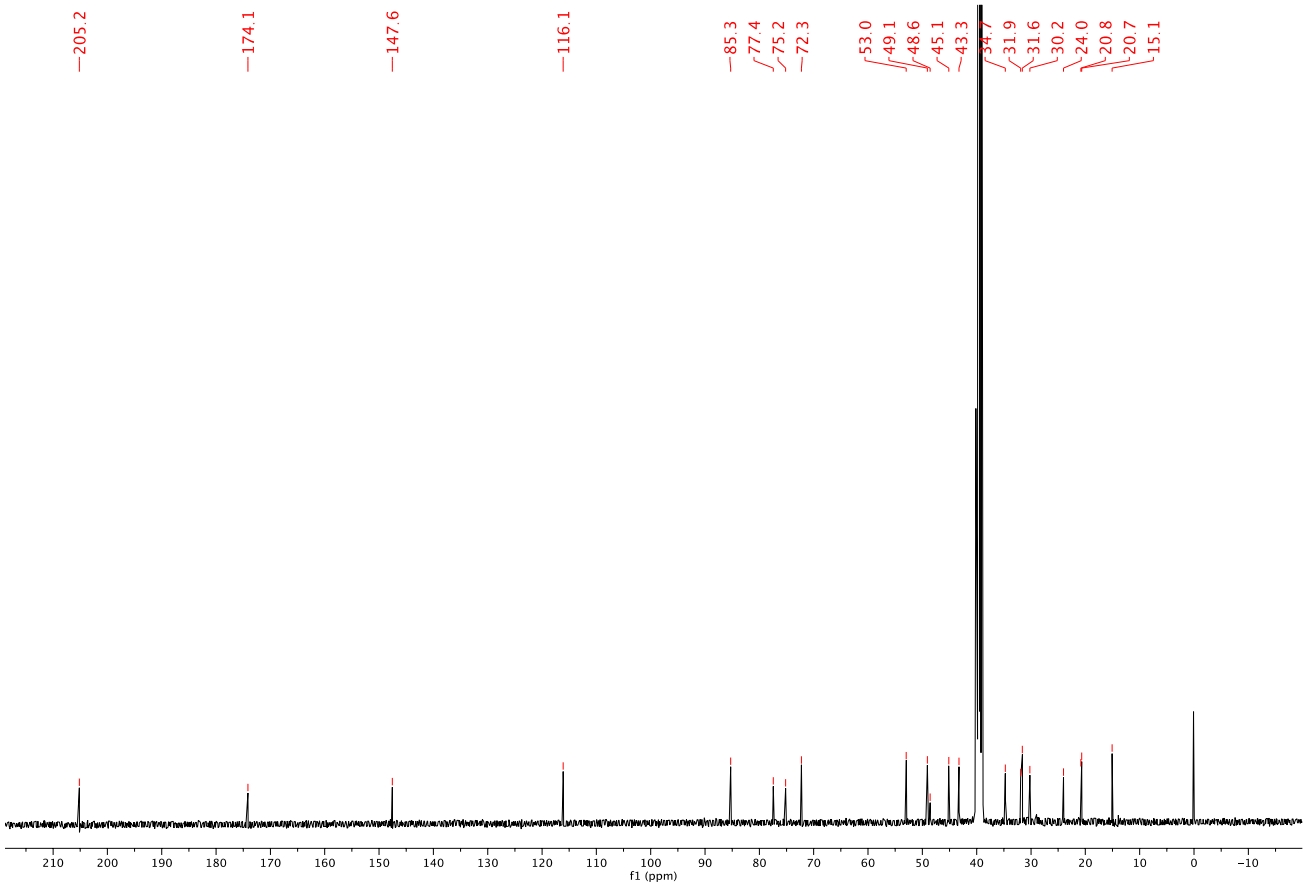

Fig. S40 ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of compound **12**

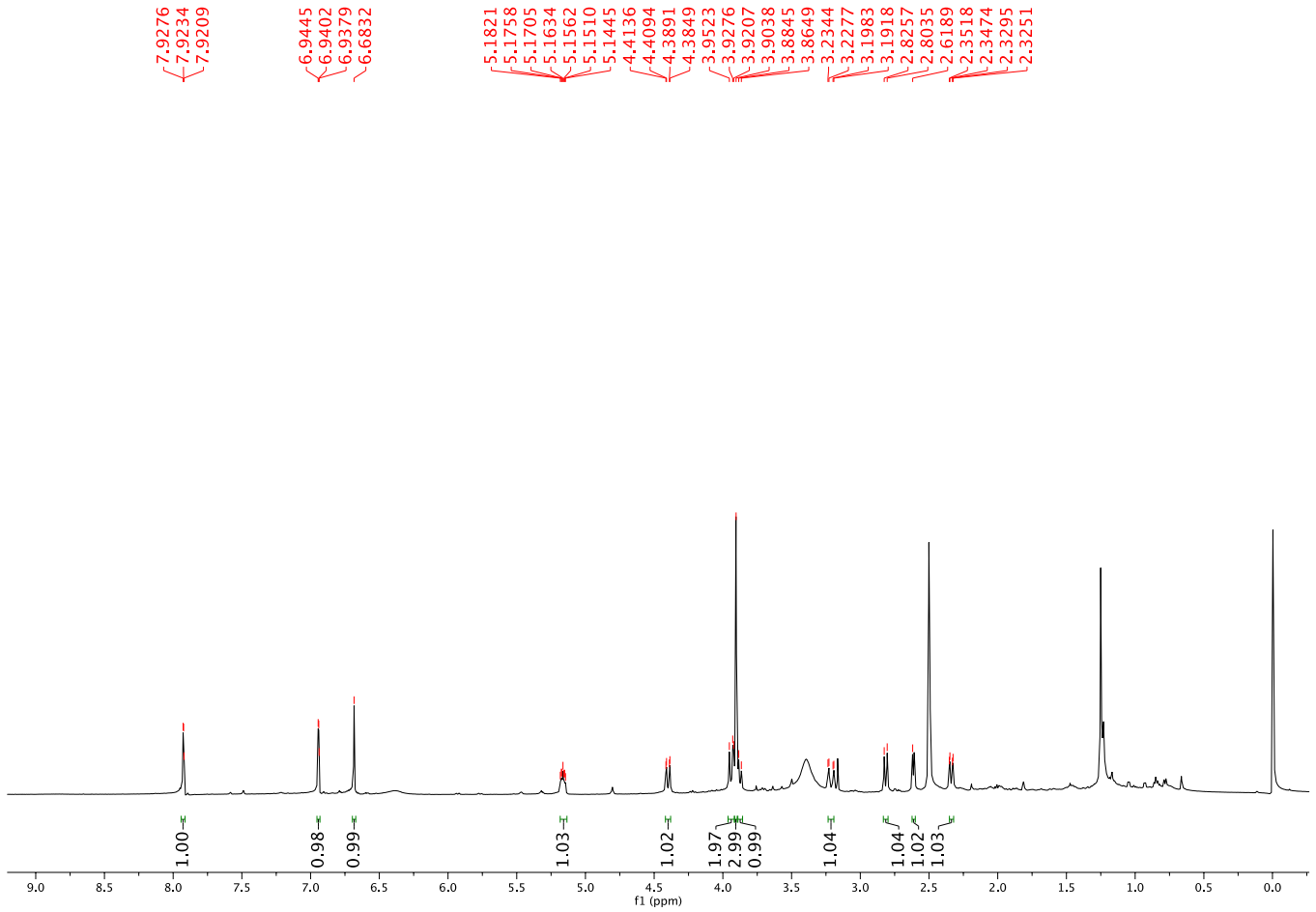

Fig. S41 ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of compound **12**

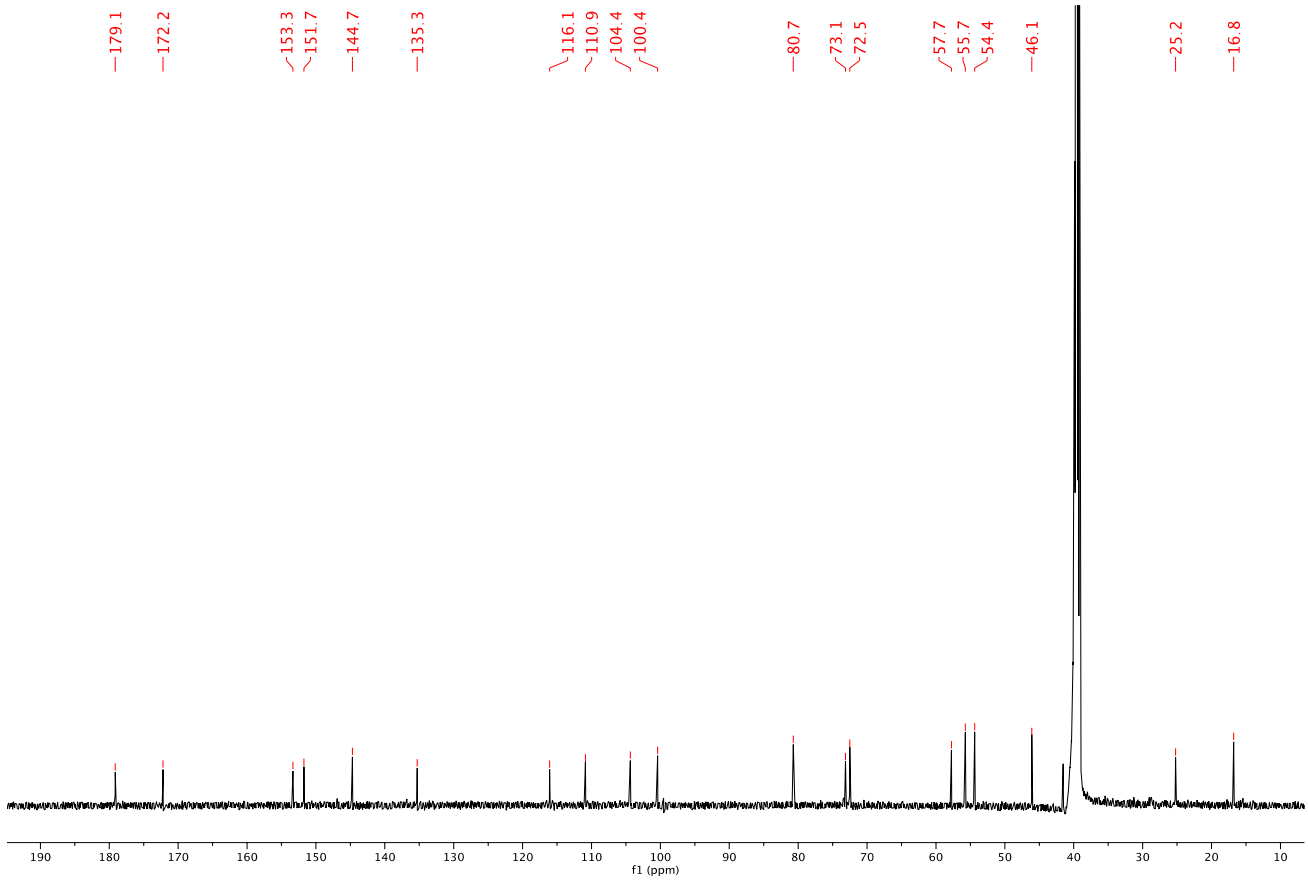

Fig. S42 HPLC chromatogram of the derivative of the standard L-Glucose

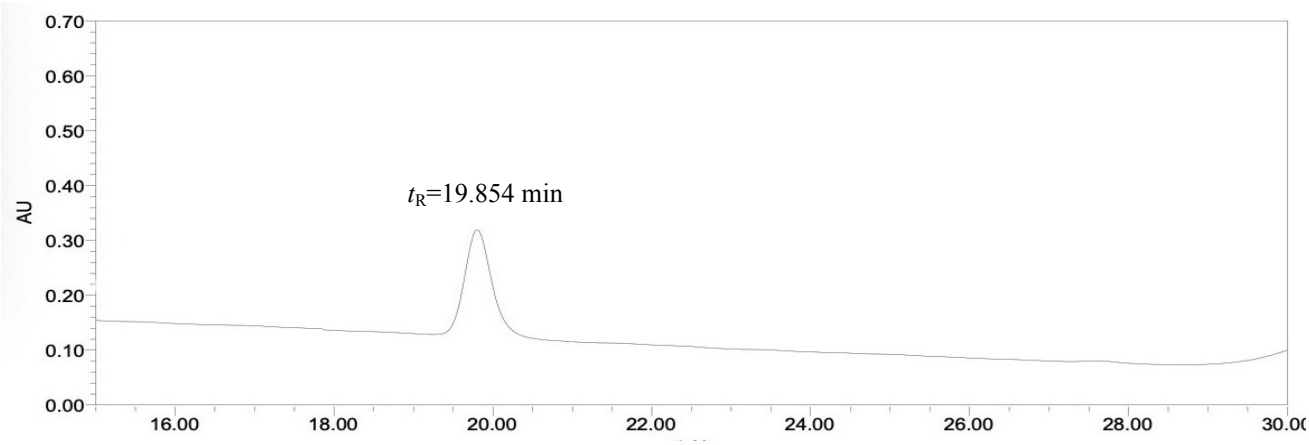

Fig. S43 HPLC chromatogram of the derivative of the standard D-Glucose

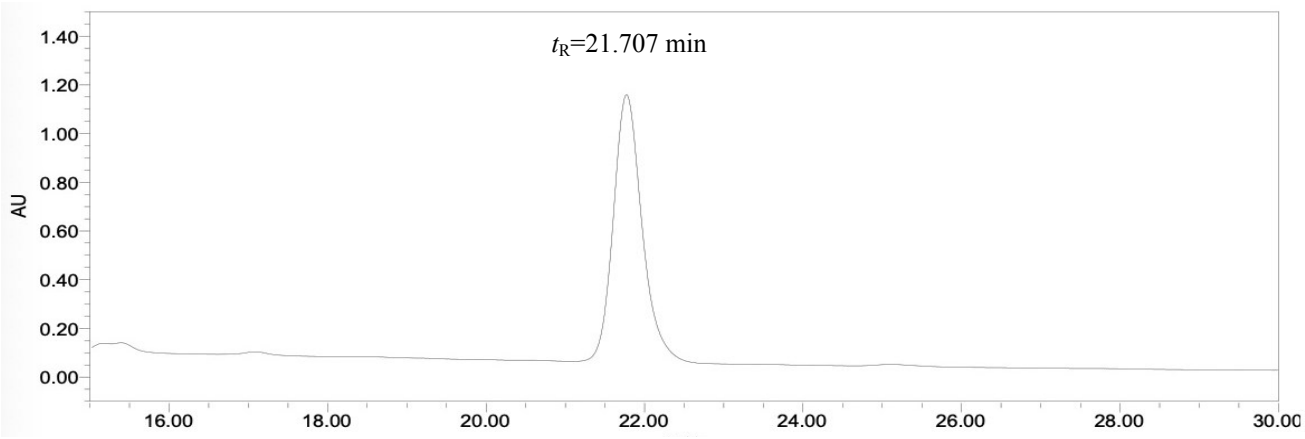

Fig. S44 HPLC chromatogram of derivative of the hydrolysate of compound **2**.