Supplementary Information for

Gambierone, a Ladder-Shaped Polyether from the Dinoflagellate *Gambierdiscus belizeanus*

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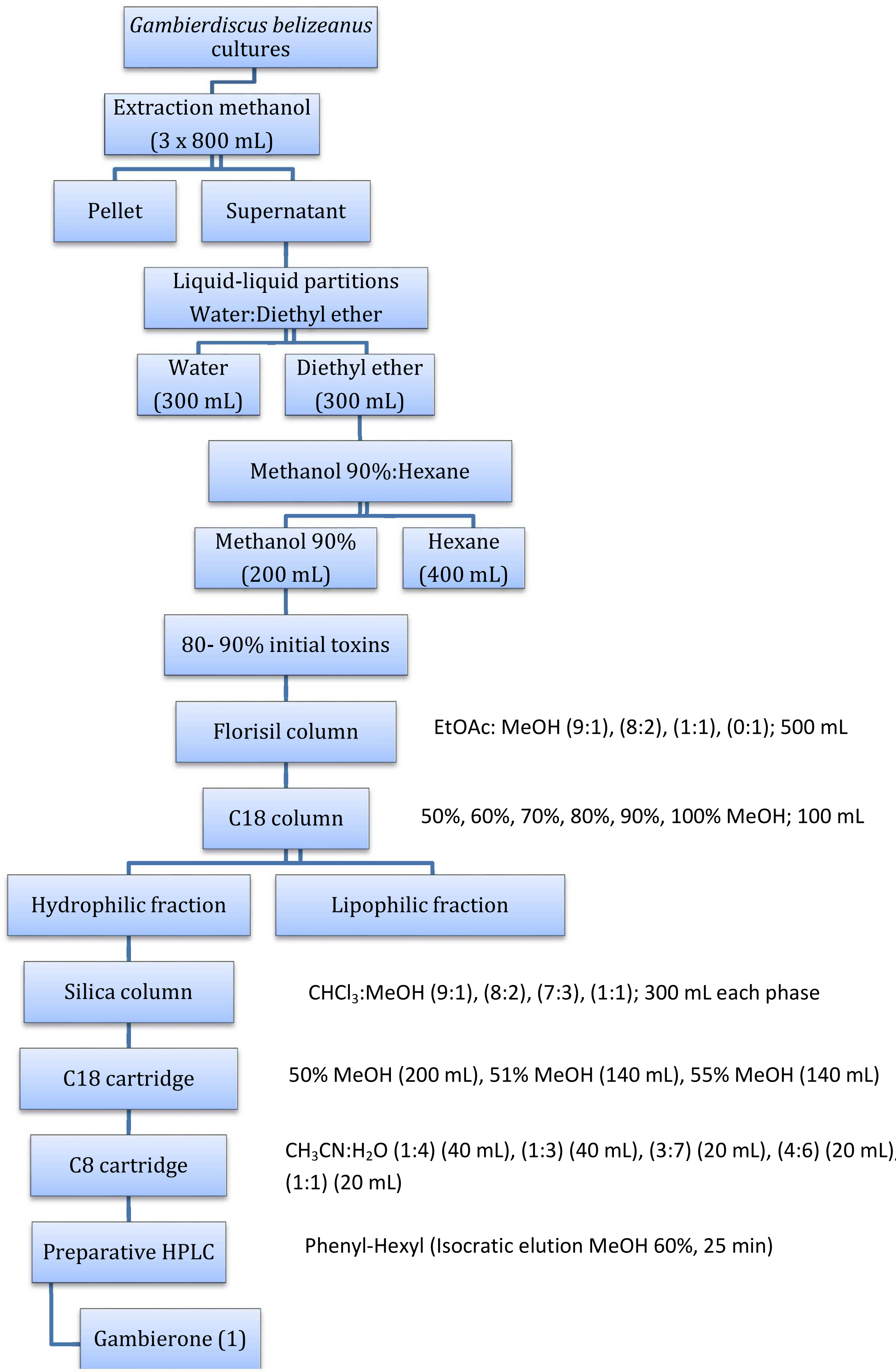
MATERIAL AND METHODS

Chemicals and Solutions

Plastic tissue-culture flasks were purchased from Jet Biofil (Spain). Fetal calf serum, Dulbecco´s modified Eagle medium / F12 nutrient mixture (DMED/F12), Eagles Minimum Essential Medium (EMEM), F12 Medium Gluatamax, Minimum essential medium, non essential amino acids (MEM NEAA), fetal bovine serum, penicillin:streptomycin and G418 were purchased from Gibco (Glasgow, UK).Detachin^{TM} was purchased from Genlantis (USA). FURA-2 AM was obtained from Molecular Probes (The Netherlands). All other chemicals were purchased from Sigma. Acetonitrile, methanol and chloroform were supplied by Panreac (Barcelona, Spain). Formic acid and ammonium formate were purchased from Merck (Darmstadt, Germany). Extraction and purification solvents were analytical or HPLC grade. Water was distilled and passed thought a water purification system (Milli-Q, Millipore, Spain). CTX-3C standard was kindly provided by Dr. Masahiro Hirama, Tohoku University, Sendai, Japan. Maitotoxin was kindly provided by Dr Takeshi Yasumoto, Japan Food Research Laboratories, Tokyo, Japan.

Gambierone purification

Compound **1** was obtained from 460 cultures of *Gambierdiscus belizeanus* CCMP401 (in 20 L bag). The strain was obtained from The Provasoil-Guillard National Center for Marine Algae and Microbiota (NCMA). In the purification process, each fraction was analyzed by the ultra-performance liquid chromatography (UPLC) coupled to mass detection method previously optimized (see below) in order to monitor the presence of Gambierone (**1**), MW 1024.5, as molecule of interest. Purification steps are summarized in scheme 1.



Scheme 1. Purification process of **1**

The CCMP401 strains were cultured and grown in seawater enriched with modified K medium free of silicates [1.764 mM $NaNO_3$, 0.01 mM Na-ß-glycerophosphate, 0.01 nM H_2SeO_3, 1 mM Tris-Base, 0.11 mM Na_2EDTA, 0.01 mM $FeCl_3$-$6H_2O$, 0.9 µM $MnCl_2$-$4H_2O$, 0.08 µM $ZnSO_4$-$7H_2O$, 0.05 µM $CoCl_2$-$6H_2O$, 0.026 µM Na_2MoO_4-$2H_2O$, 0.296 µM thiamine (vit B_1), 2.05 nM biotin and 0.369 nM cyanocobalamin (Vit B_{12})].[1,2] The medium was done in natural seawater., at 33 $^{o}/_{oo}$ salinity level, 24 ºC and photoperiod of 14 hours light/10 hours dark. The cellular growth was divided in steps, increasing the volume progressively until 20 L bags cultures. The cells (19 g wet weight) were harvested by filtration with a 20 µm mesh and suspended in methanol (MeOH). Then the cells were broken by ultrasound (3 cycles) and stored at –20 ºC. The methanolic extracts were centrifuged and the pellets were re-extracted (x2) with MeOH. These extracts were then dried, dissolved in water and lipophilic and hydrophilic contaminants were firstly eliminated by two liquid:liquid partitions, water:diethyl ether and MeOH 90%:hexane. 80-90% of compounds of interest were extracted within the 90% MeOH layer. This fraction was evaporated to dryness, dissolved in ethyl acetate:methanol (EtOAc:MeOH) (9:1) and loaded in a florisil column. This column was eluted with EtOAc:MeOH from 9:1 to 1:1. With these solvents, in the first fractions several coloured contaminants were eliminated, and then, when the percentage of MeOH increased, lipophilic and hydrophilic fractions were collected, mainly with 8:2 and 1:1 EtOAc:MeOH ratio. However, the clean separation of hydrophilic and lipophilic molecules was not fully achieved and additional solid phase extraction (SPE) was performed. All the fractions containing the molecules of interest from the florisil column were collected, evaporated to dryness, dissolved in MeOH 50% and loaded into a reverse phase C_{18} column, eluted with different percentages of MeOH, from 50% to 100%. Using these conditions, two main fractions were obtained: a hydrophilic fraction eluted with 60–70 % MeOH and a lipophilic fraction eluted in 100% MeOH.

Following C_{18} SPE, the hydrophilic fraction was purified in four steps. The first step of this purification was a silica column to remove pigments. The sample was evaporated to dryness, dissolved in chloroform:methanol (9:1) and loaded into the silica column. At the beginning of the elution, green pigments were removed. When the percentage of MeOH was increased, the compounds of interest were collected, mainly with $CHCl_3$: MeOH (7:3). In the second step, all fractions containing the ion at *m/z* 1024.4849 were mixed and evaporated to dryness, dissolved in MeOH 50% and loaded into a C_{18} cartridge. The cartridge was eluted with different percentages of MeOH and the targeted compound was eluted with methanol 50-51%. This fraction was evaporated to dryness, dissolved in acetonitrile:water (1:4) and loaded into a C_8 cartridge. The cartridge was eluted with different percentages of acetonitrile:water (20%, 25%, 30%, 40% and 50% of acetonitrile). Compound **1** was recovered in acetonitrile 25% fractions.

Finally, to complete the purification process, a preparative HPLC method was used to achieve a higher purity of this compound. This method was performed by HPLC using two detectors, mass detector and UV detector. The HPLC system, from Waters, consists of Binary Gradient Module, M515 HPLC Pump, autoinjector and collector 2767 Sample Manager and System Fluidics Organizer. This system is coupled to a MS detector, 3100 Mass Detector, and an UV detector, 2487 2 Channel UV/Vis Detector, both from Waters. The column used was X-

[1] Keller, M.D. and Guillard, R.R.L. 1985. Factors significant to marine diatom culture. pp. 113-6. *In* Anderson, D.M., White, A.W. and Baden, D.G. (eds.) *Toxic Dinoflagellates*. Elsevier, New York.

[2] Keller, M.D., Selvin, R.C., Claus, W. and Guillard, R.R.L. 1987. Media for the culture of oceanic ultraphytoplankton. *J. Phycol.* **23:** 633-638.

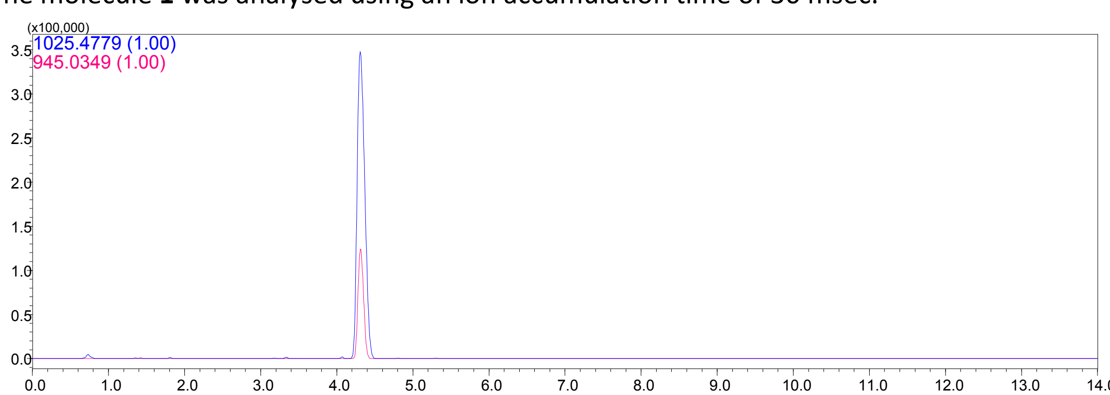
Select Phenyl-Hexyl (10 x 250mm, 5 µm) from Waters, with a flow rate of 4 mL/min and 500 µL of injection volume. The chromatographic separation was performed with an isocratic elution of 60% of MeOH for 25 min. Peaks were collected following the MS signal and UV signal was used to confirm the correct separation. A mass of 2.24 mg (0.012% yield from wet cells) of pure Gambierone (**1**) was finally obtained and analyzed by NMR.

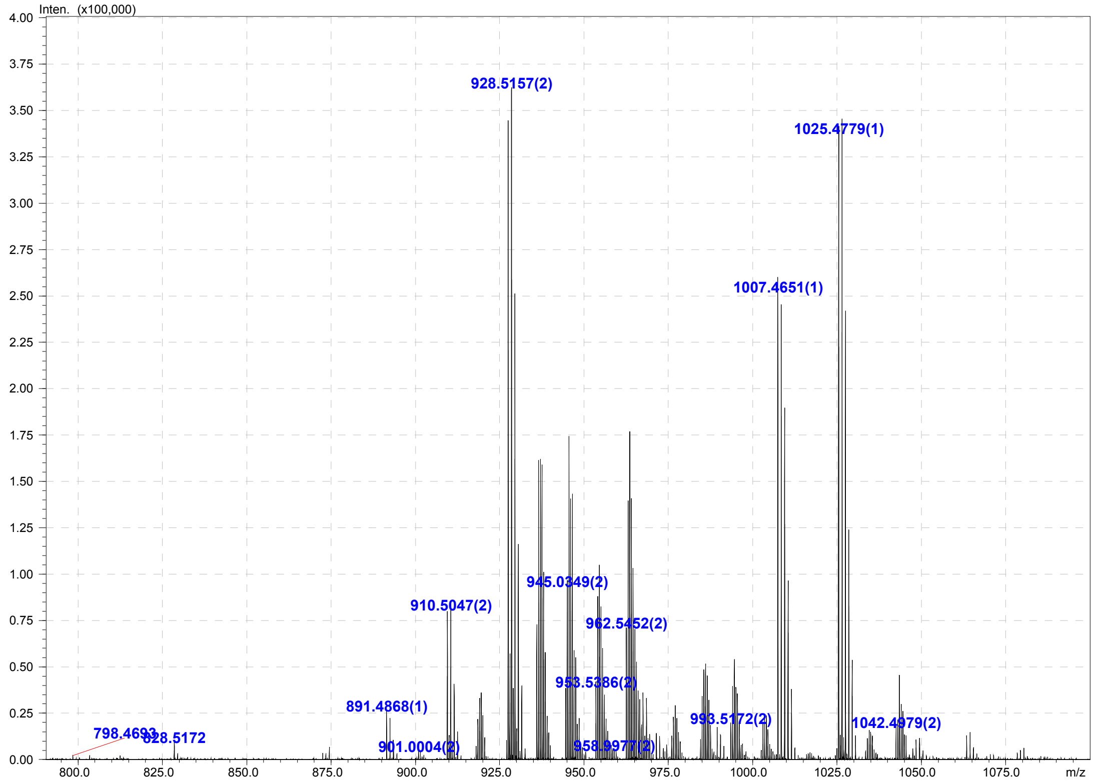
UPLC-MS/MS analysis

The presence of Gambierone, MW 1024.4849, in each fraction was analyzed by UPLC-MS/MS. For this analysis, a 1290 Infinity ultra-high-performance liquid chromatography system coupled to a 6460 Triple Quadrupole mass spectrometer (from Agilent Technologies, Waldbronn, Germany) was used. Chromatographic separation was performed at 35 ºC, the injection volume was 5 µl and flow rate of 0.4 mL/min in a column AQUITY UPLC BEH C18 (2.1x100mm, 1.7 µm, Waters). The nitrogen generator is a Nitrocraft NCLC/MS from Air Liquid. Mobile phases A and B were water and acetronitrile:water (95:5), respectively, both acidified with 50 mM formic acid and 2 mM ammonium formate. Chromatographic separation was performed by gradient elution starting with 50% B for 2.5 min, then increasing to 100%B for 4.5 min., this condition was hold for 4.5 min and decreased afterward to 50% B over 0.1 min. This proportion was maintained 2.4 min. until the next injection to equilibrate the system. Analysis was carried out using electrospray ionisation (ESI) and select ion monitoring (SIM) acquisition. The general source settings were follows: gas temperature, 350 ºC; gas flow, 8 L/min; sheath gas temperature, 400 ºC; sheath gas flow, 11 L/min, nebulizer, 45 psi; capillary voltage, 4000 V (positive and negative); and nozzle voltage 0 V (positive and negative). The scanning quadrupole (MS2) was set to unit resolution. Precursor, fragmentor voltages (180 V for Gambierone) and cell accelerator voltage (7 V for **1**) were performed with MassHunter Data Acquisition software.

UPLC-IT-TOF-MS analysis

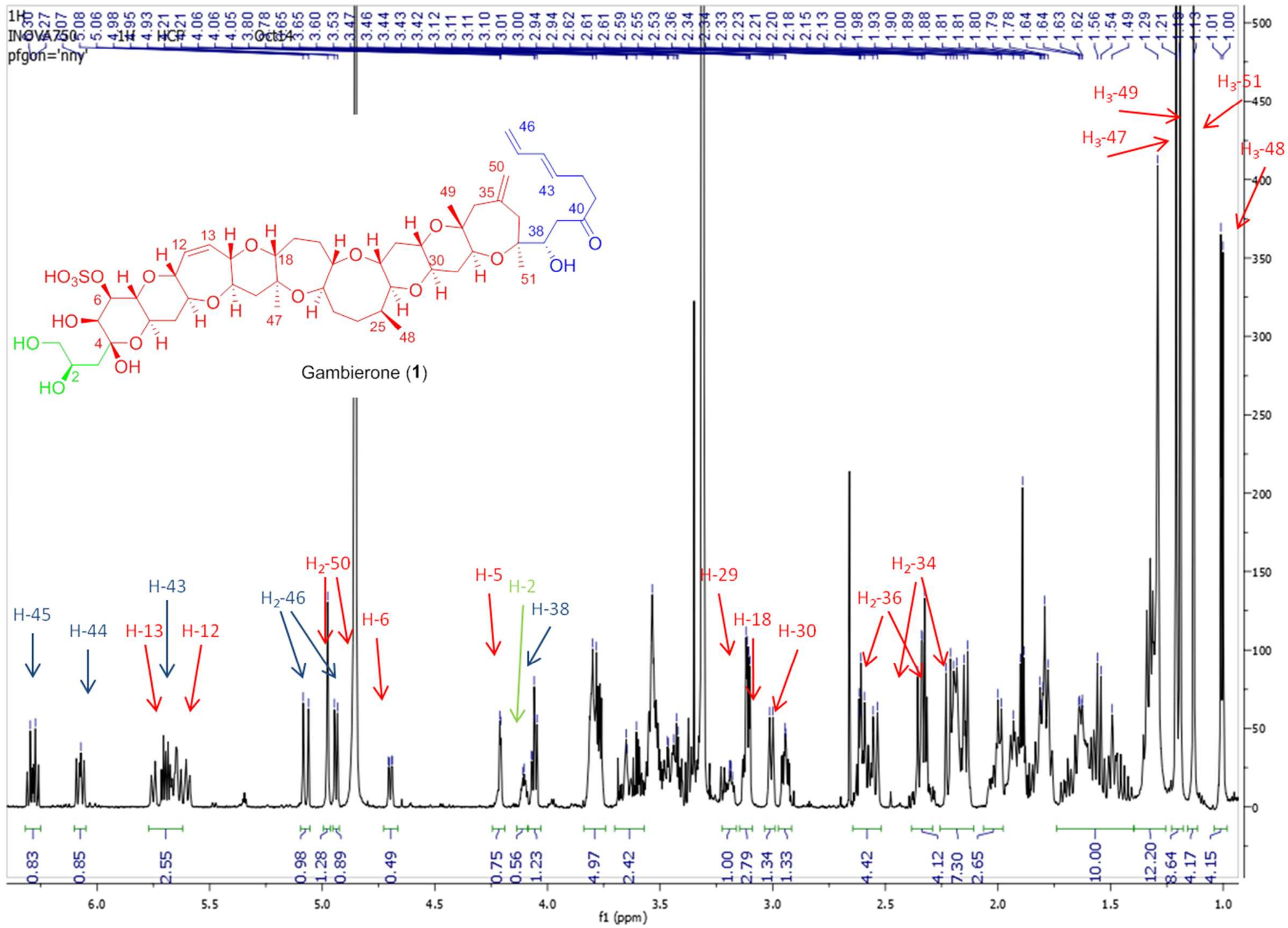
To confirm the gambierone MW an UPLC-IT-TOF analysis was done. The UPLC system, Nexera-X2 from Shimadzu (Kyoto, Japan) consists of two pumps (LC-30AD), autoinjector (SIL-10AC) with refrigerated rack, degasser (DGU-20A), column oven (CTO-10AS) and a system controller (SCL-10Avp). The system is coupled to an ion trap/time-of-flight mass spectrometer (IT-TOF-MS) system with an electrospray ionization (ESI) interface (Shimadzu, Kyoto, Japan). The nitrogen generator is a Nitrocraft NCLC/MS from Air Liquide (Spain). The separation was performed with a column, ACQUITY UPLC Phenyl-Hexyl (2.1x100 mm, 1.7 µm particle size, Waters, Spain). Mobile phases A and B were water and acetronitrile:water (95:5), respectively, both acidified with 50mM formic acid and 2 mM ammonium formate. Chromatographic separation was performed by gradient elution starting with 50% B for 2.5 min, then increasing to 100%B for 4.5 min., this condition was hold for 4.5 min and reduced afterward to 50% B over 0.1 min. This proportion was maintained 2.4 min. until the next injection to equilibrate the system. The mobile phase flow rate was 0.4 mL/min, the injection volume was 5 µl and the temperature was maintained at 35 ºC. The MS method was operated in positive mode with the following ESI source conditions: nebulizing gas flow, 1.5 L/min, heat block temperature and CDL temperature, 200 ºC and detector voltage, 1.65 kV. The molecule **1** was analysed using an ion accumulation time of 30 msec.



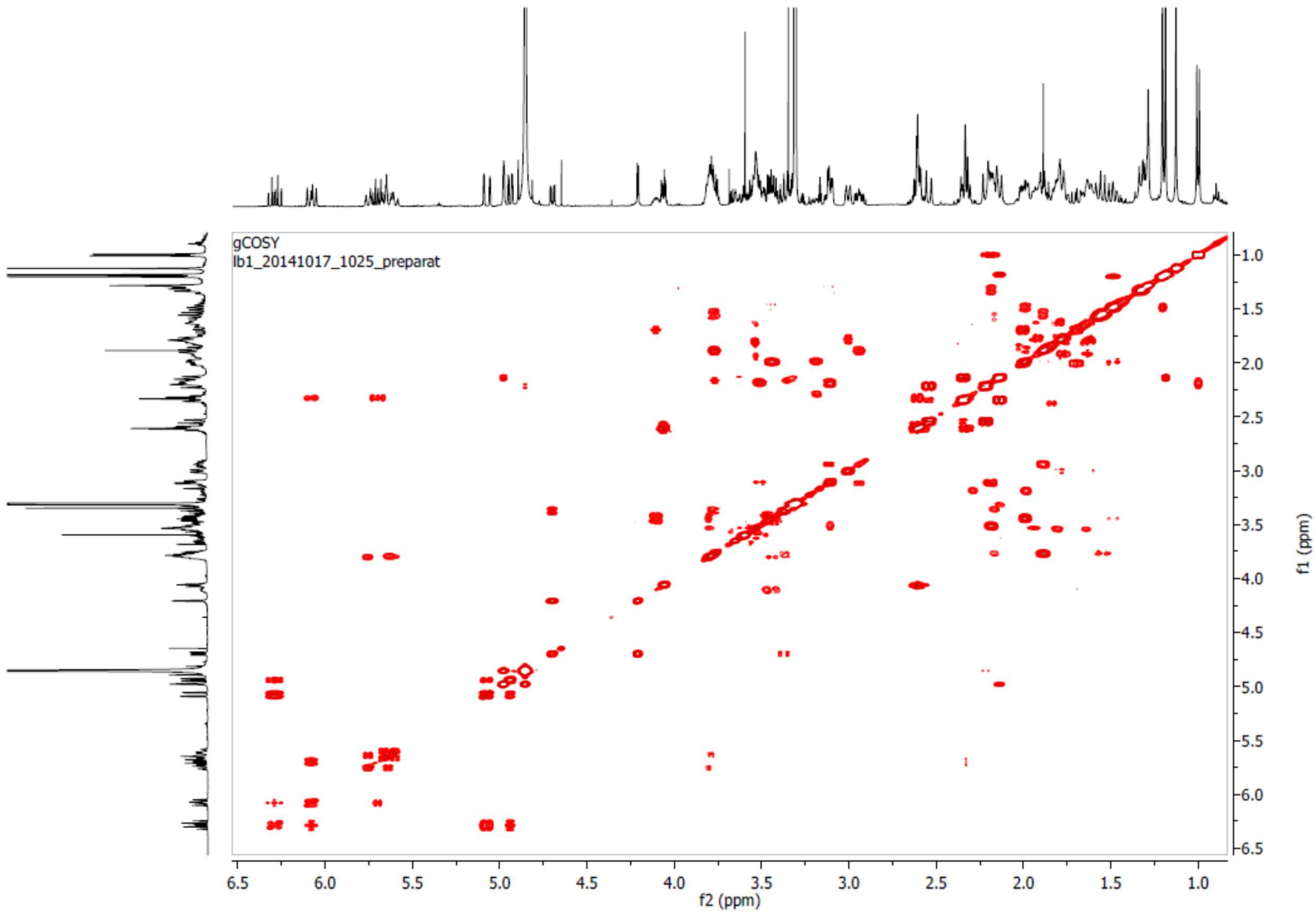


NMR analyses

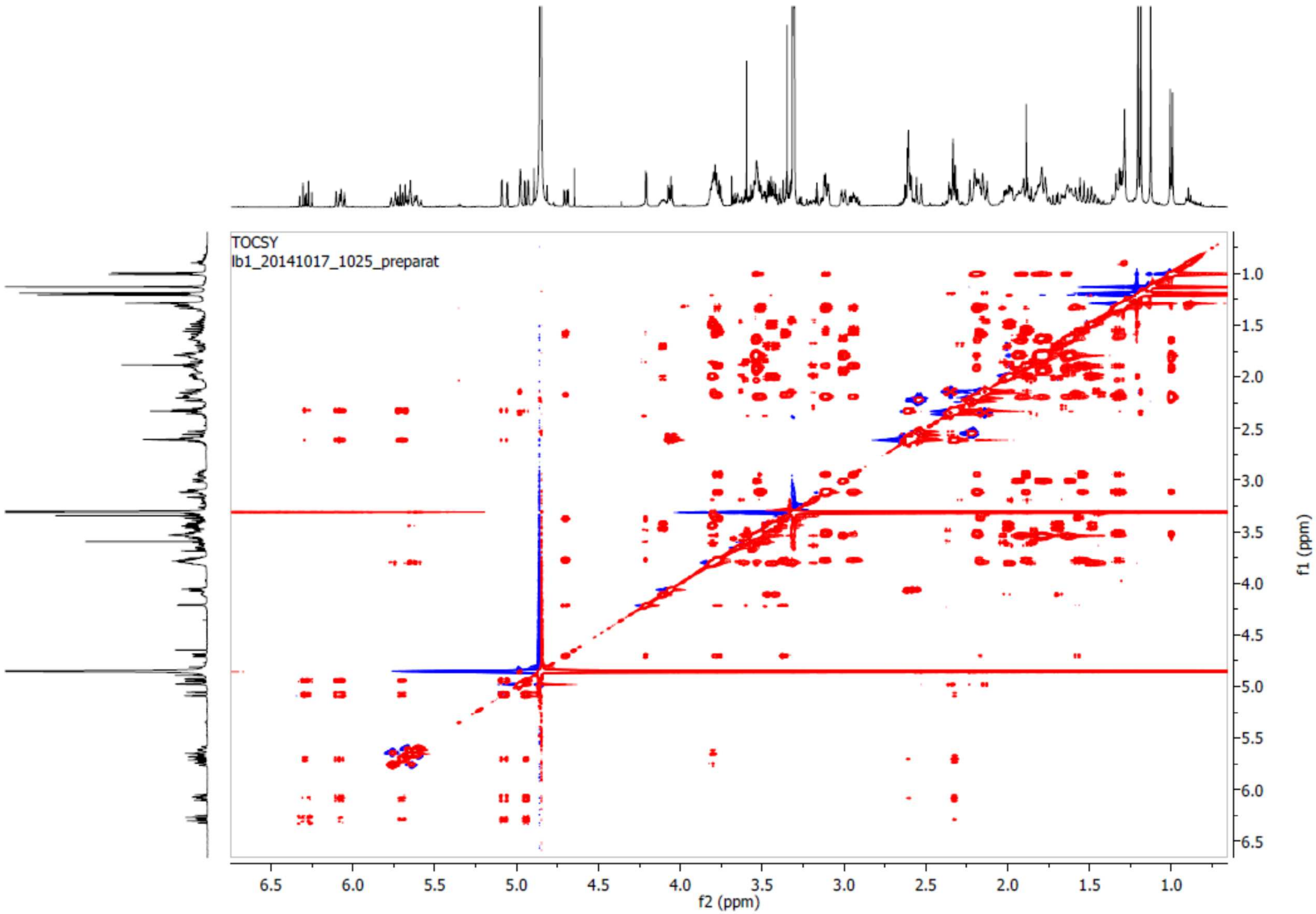
NMR analyses were performed on a Varian Inova 750 MHz equipment for ^{1}H and 2D experiments while the ^{13}C spectrum was obtained with the more sensitive Varian VNMRS-500-WB (OneNMR^{TM} Probe with two canals ^{1}H, X.). Residual peaks of CD_3OD were used as references at δ_H 3.31 for ^{1}H NMR and δ_C 49.0 for ^{13}C NMR.



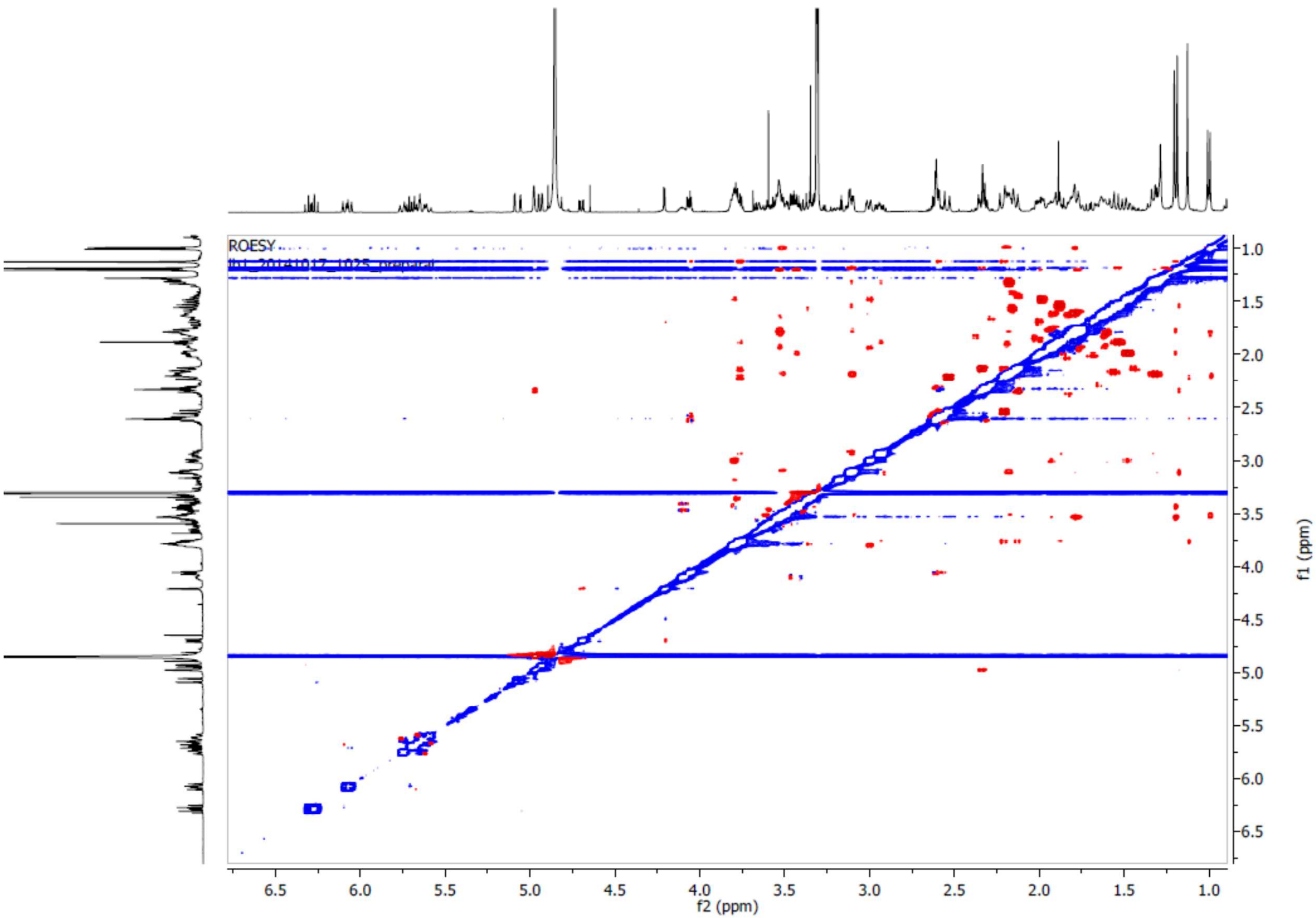
[1]H NMR spectrum of **1** at 750 MHz in CD_3OD



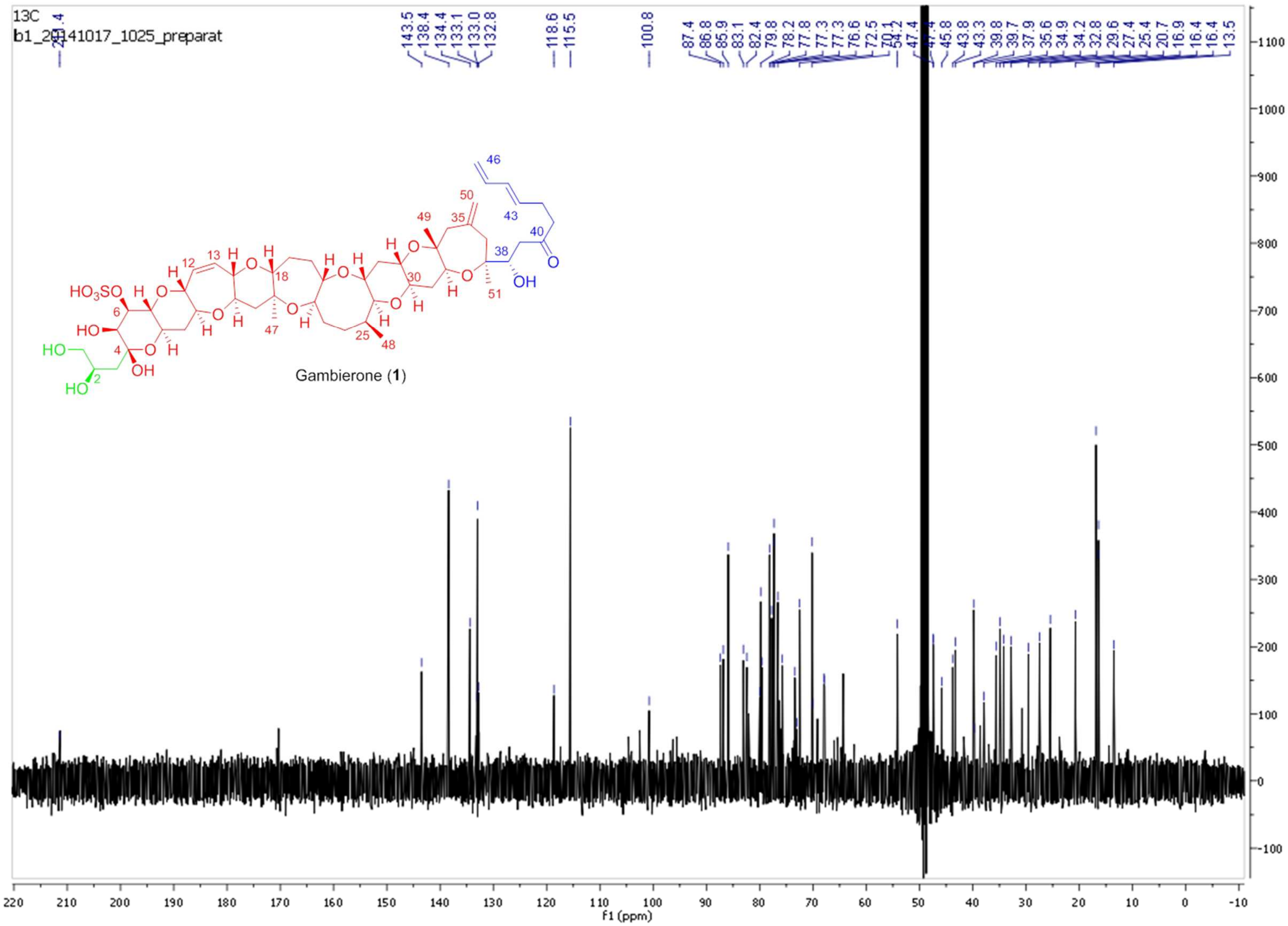
COSY NMR spectrum of **1** at 750 MHz in CD_3OD



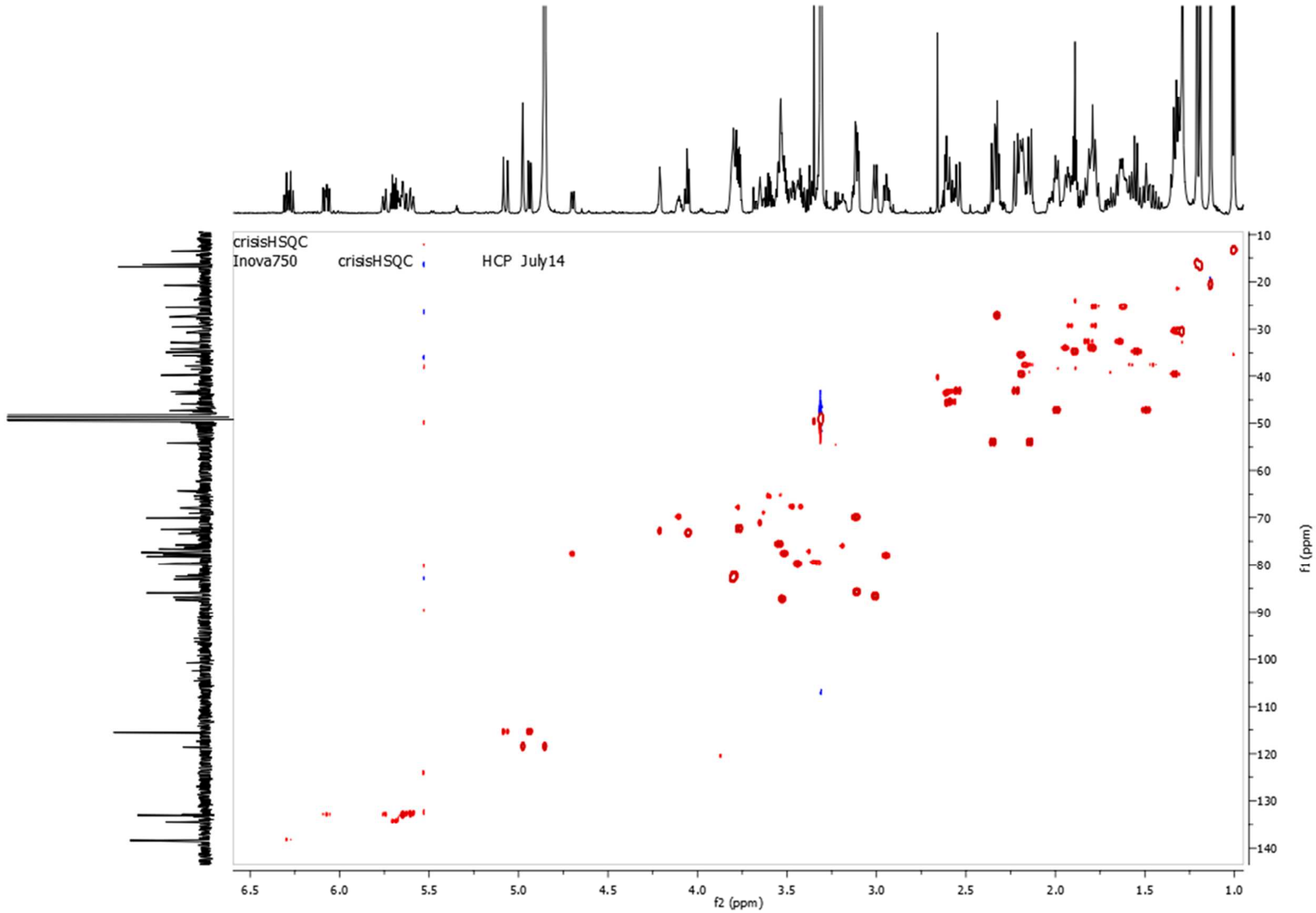
TOCSY NMR spectrum of **1** at 750 MHz in CD_3OD



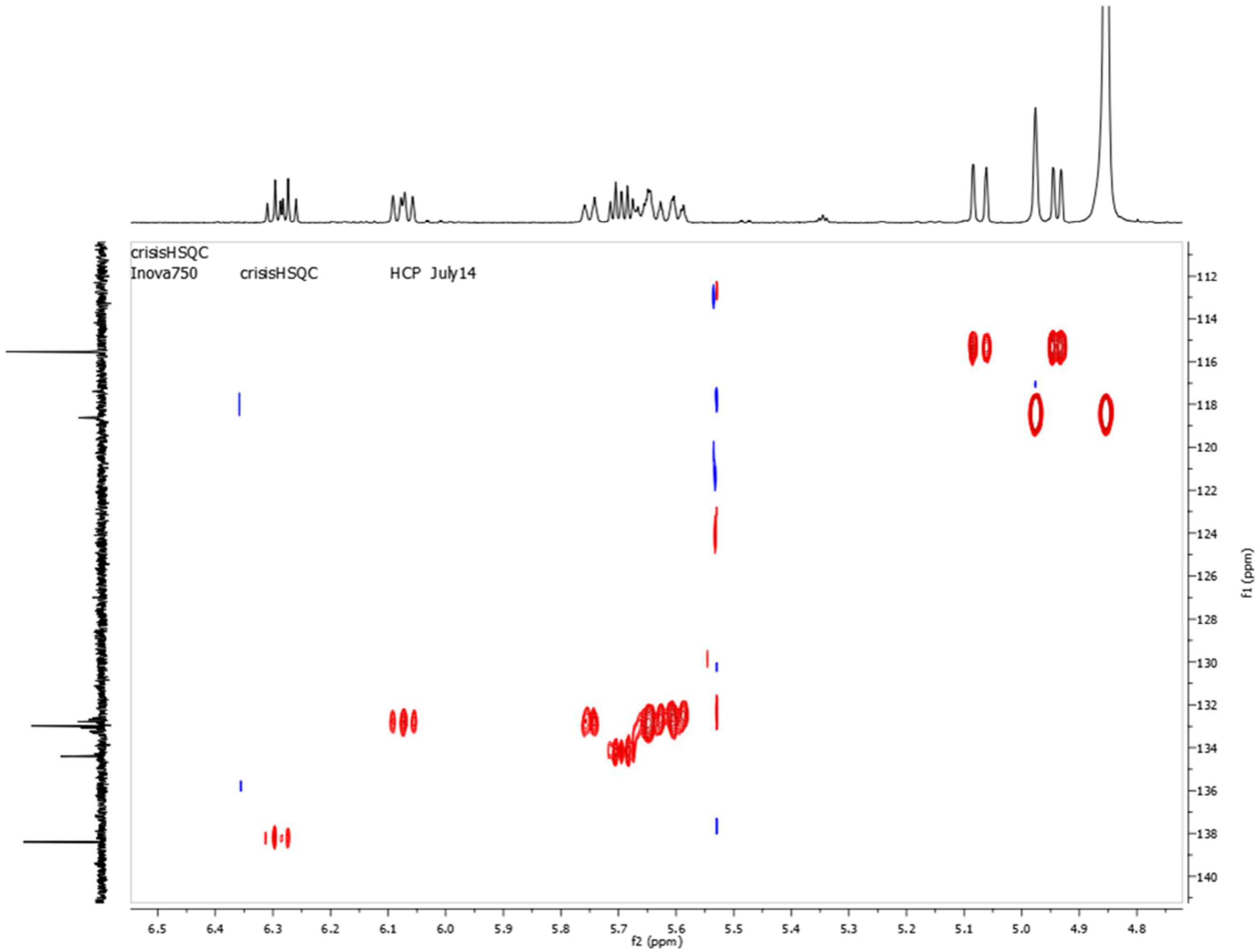
ROESY NMR spectrum of **1** at 750 MHz in CD_3OD



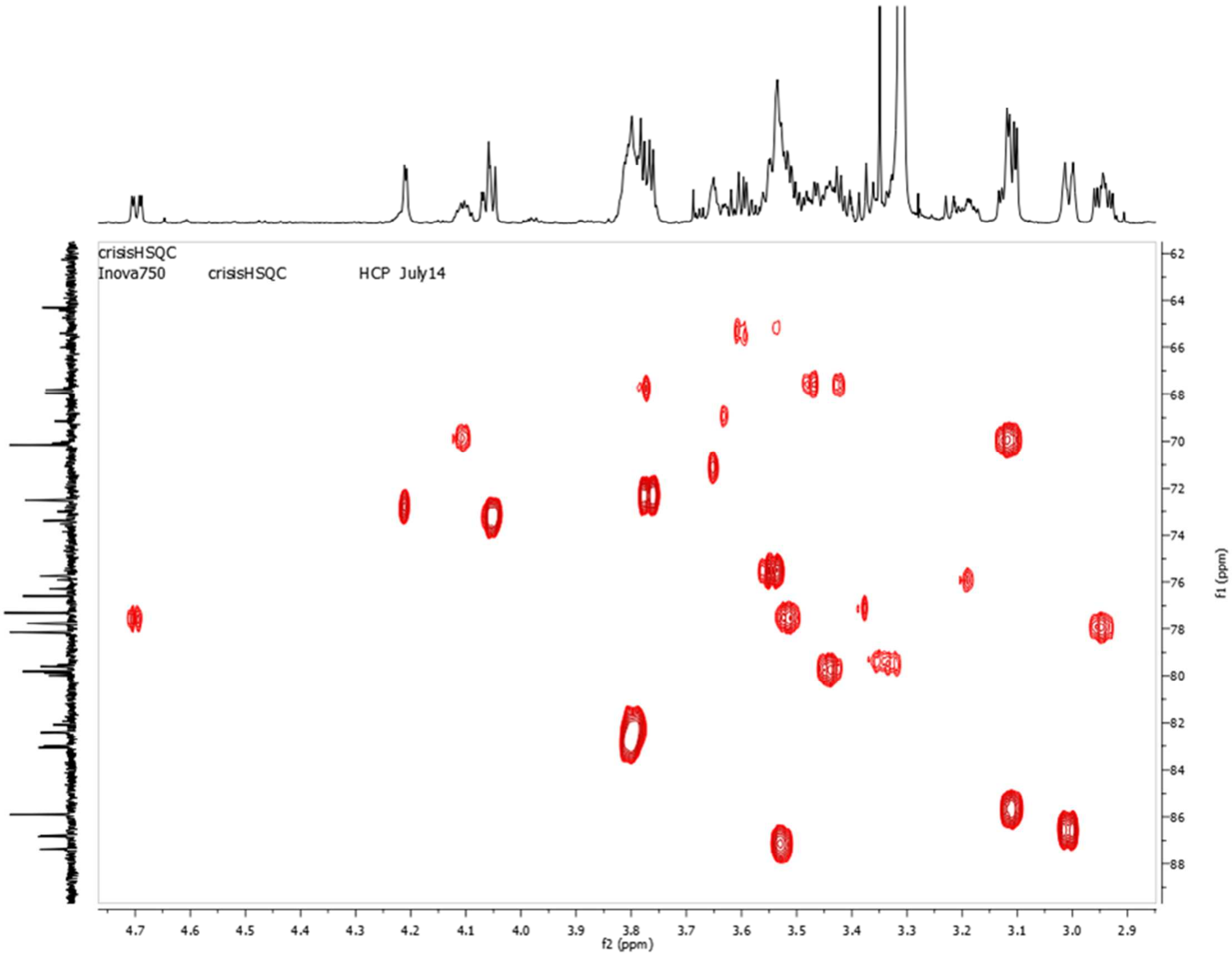
^{13}C NMR spectrum of **1** at 125 MHz in CD_3OD



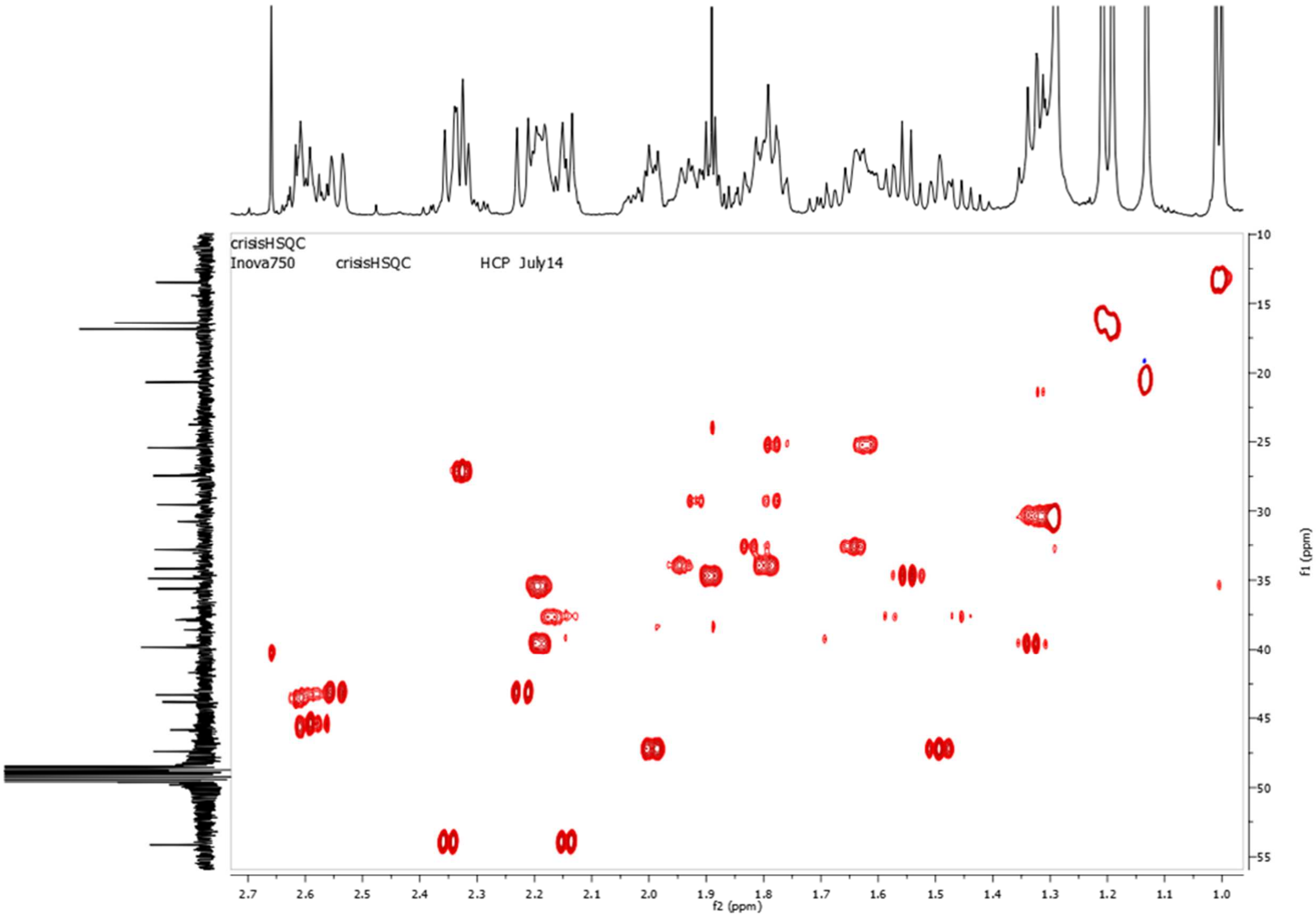
HSQC NMR spectrum of **1** at 750 MHz in CD_3OD



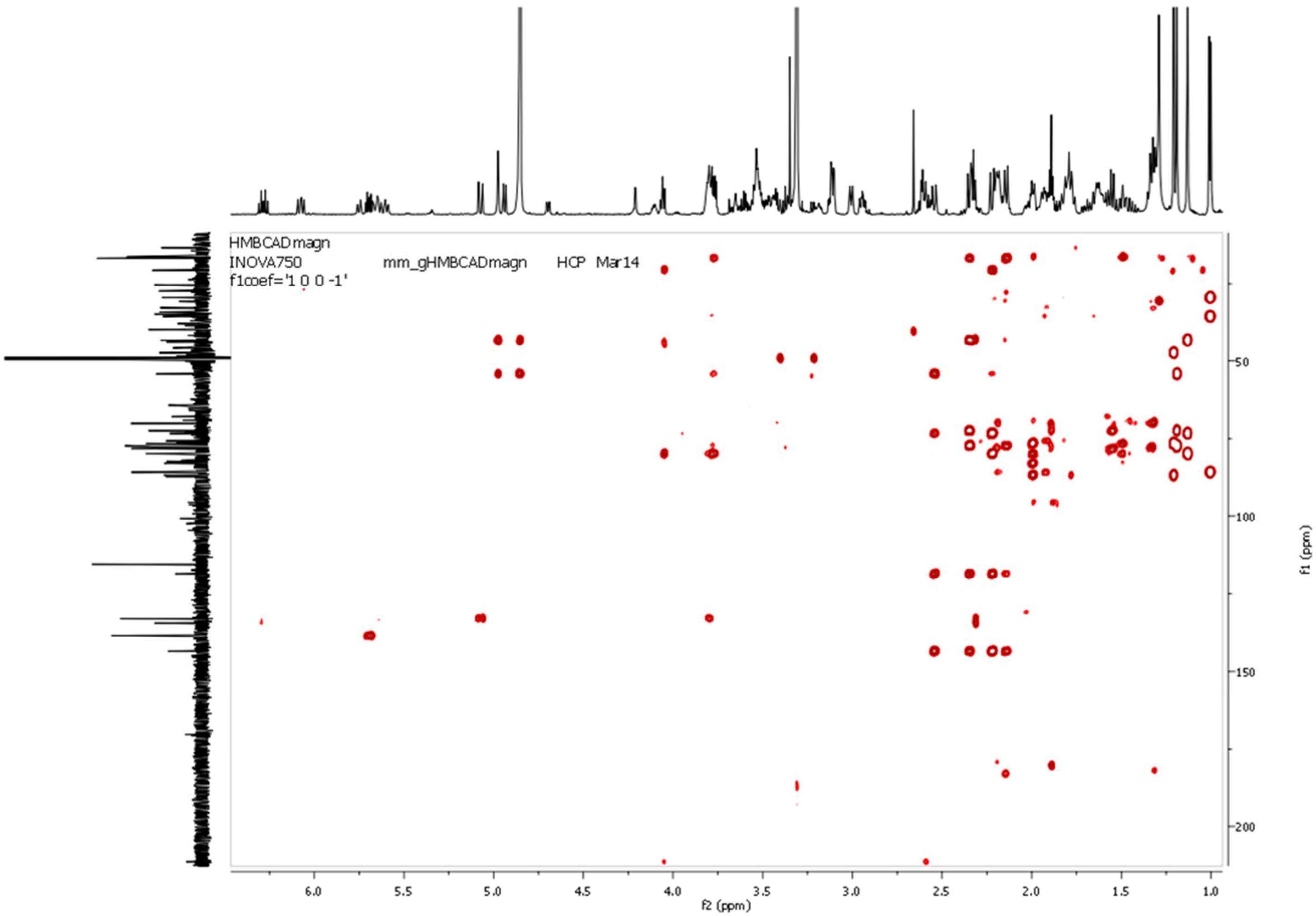
Expansion of HSQC NMR spectrum of **1** at 750 MHz in CD_3OD



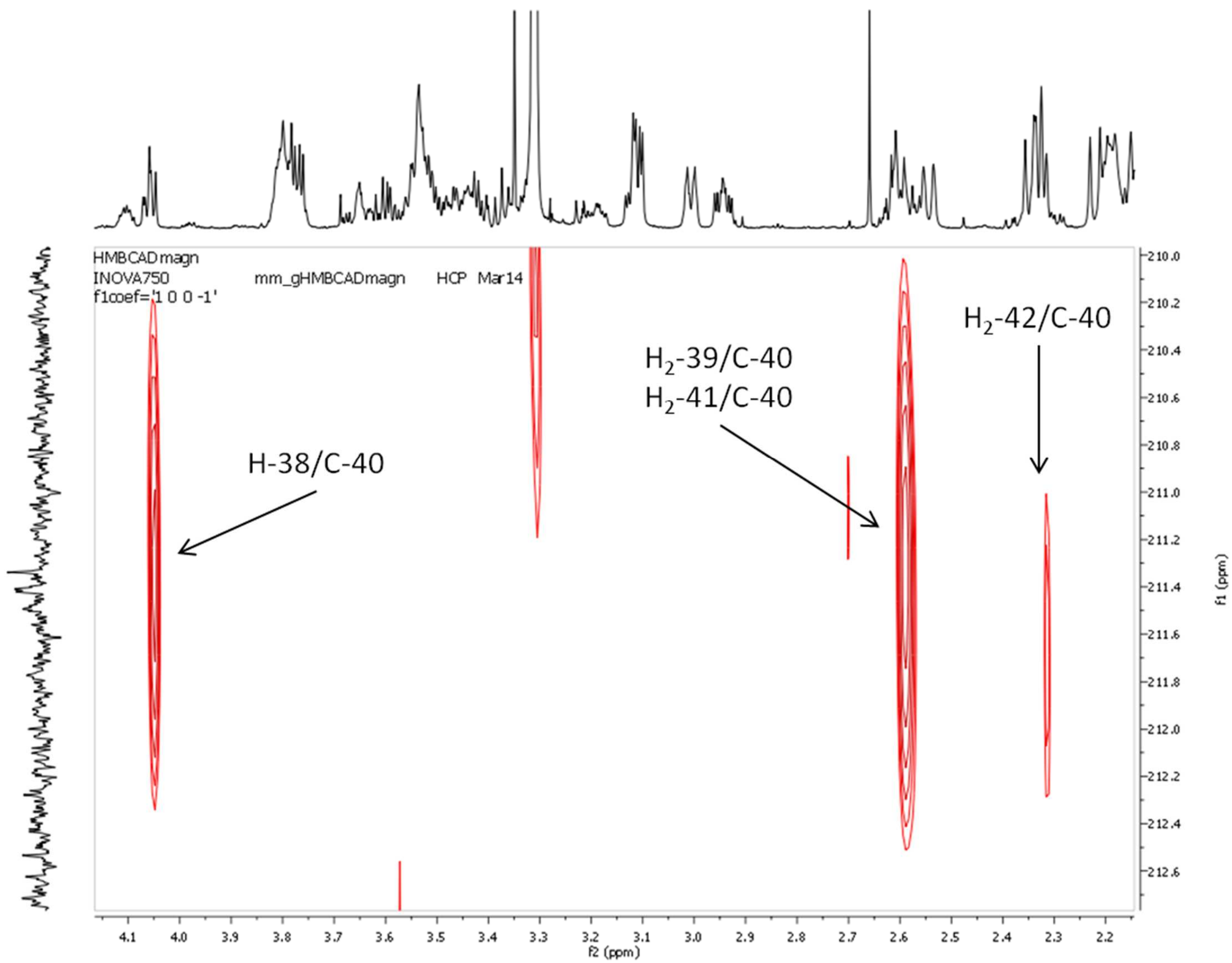
Expansion of HSQC NMR spectrum of **1** at 750 MHz in CD_3OD



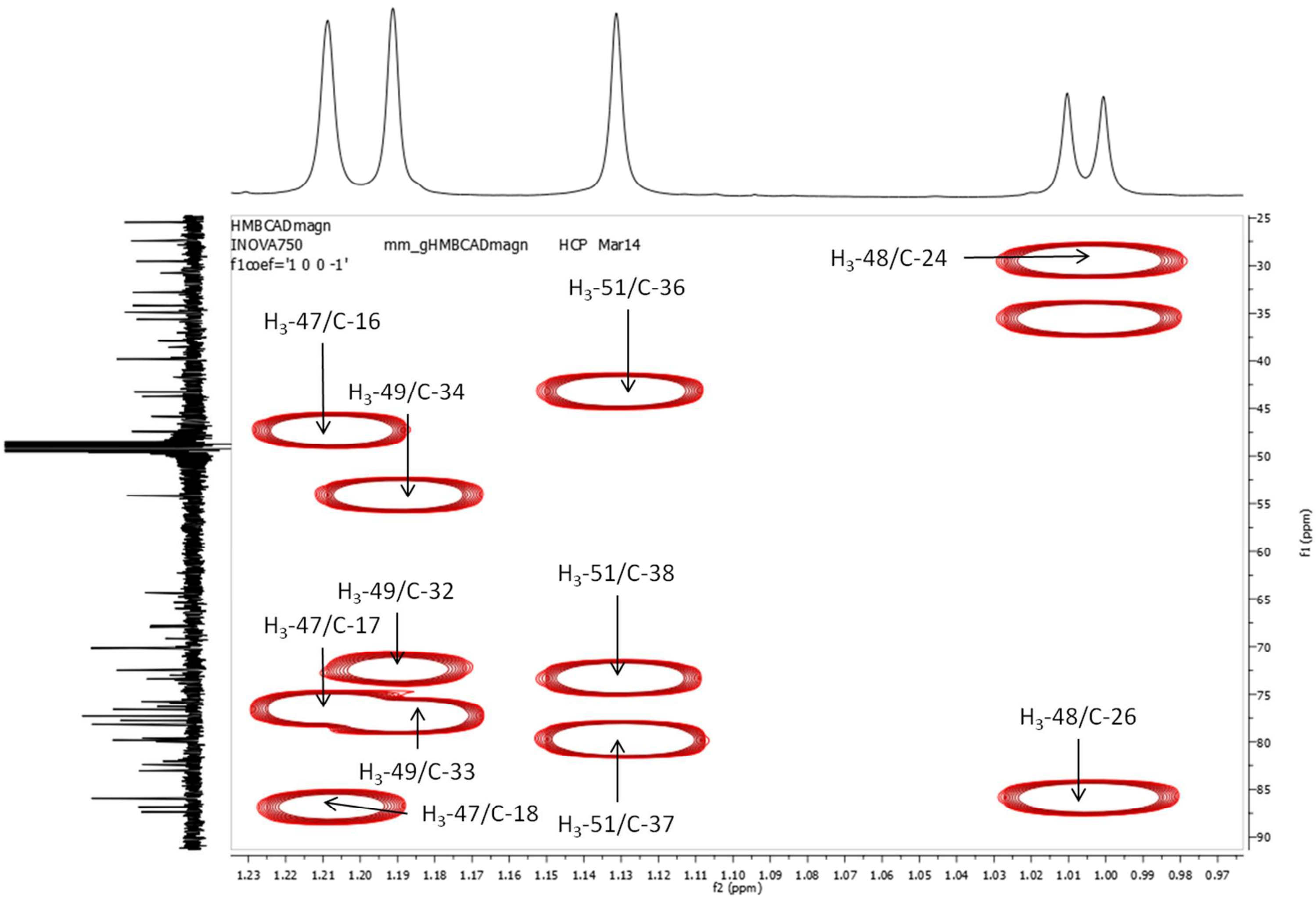
Expansion of HSQC NMR spectrum of **1** at 750 MHz in CD_3OD



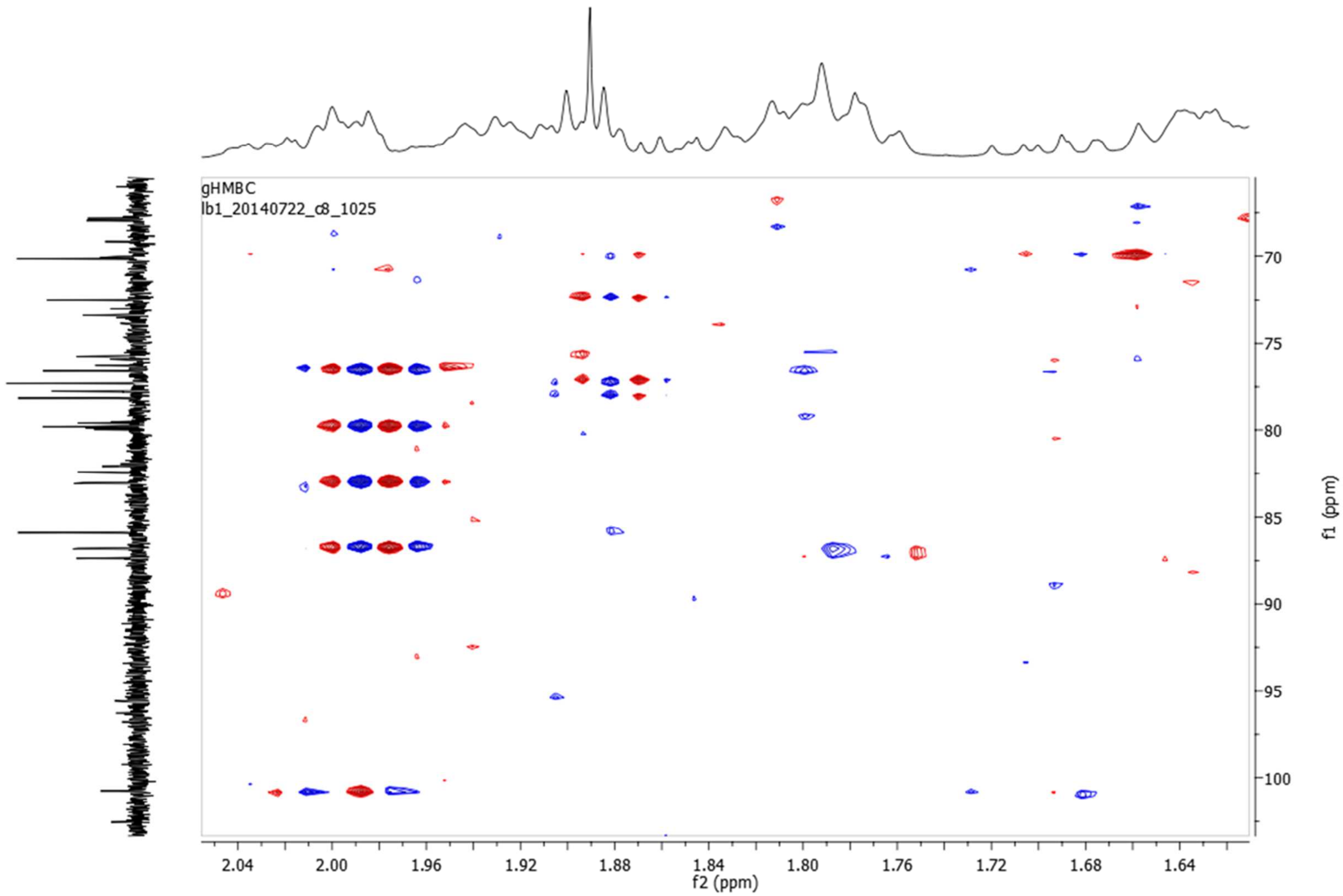
HMBC spectrum of **1** at 750 MHz in CD_3OD



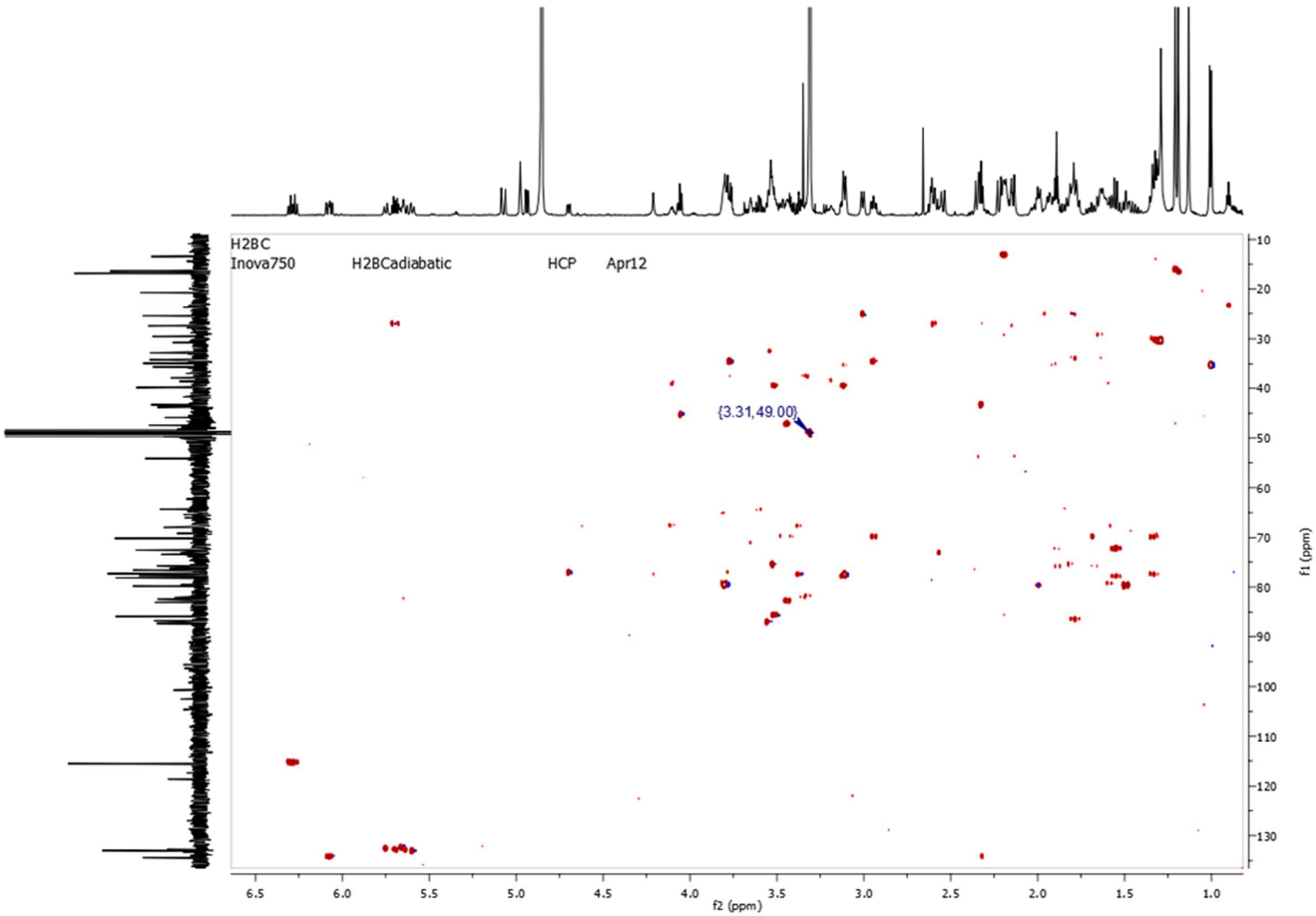
Expansion of HMBC spectrum of **1** at 750 MHz in CD_3OD



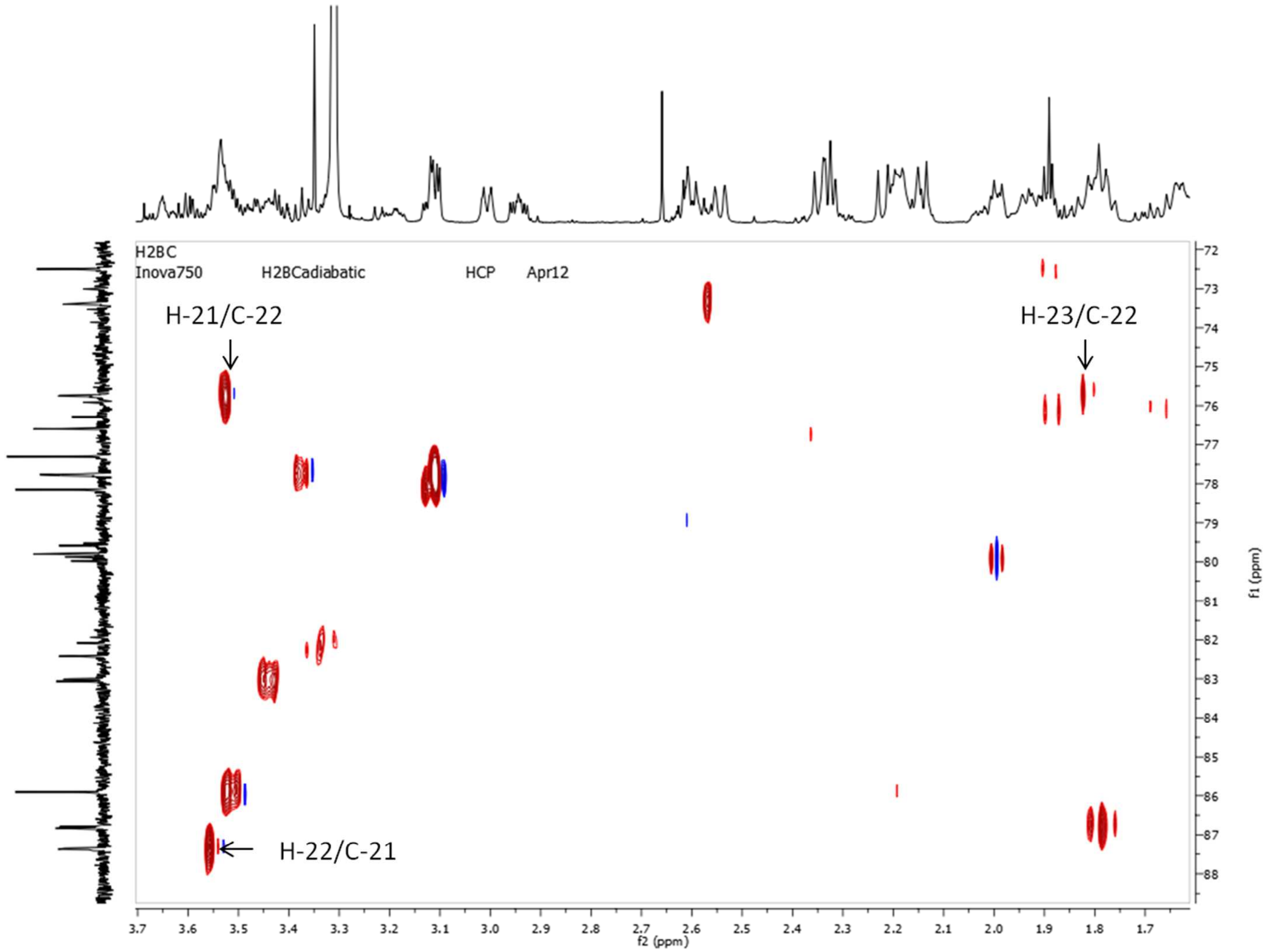
Expansion of HMBC spectrum of **1** at 750 MHz in CD_3OD



Expansion of HMBC spectrum of **1** at 500 MHz in CD_3OD

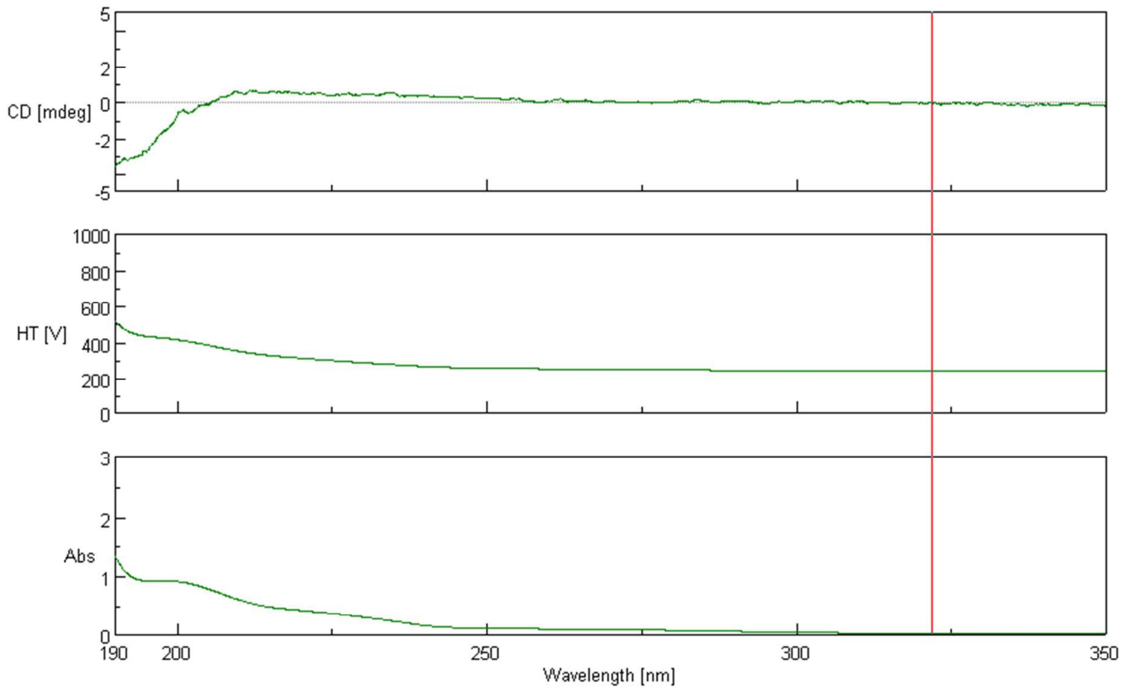


H2BC spectrum of **1** at 750 MHz in CD_3OD



Expansion of the H2BC spectrum of **1** at 750 MHz in CD_3OD

ECD and UV spectra of 1 at 10 µg.mL^{-1} in CH_3CN



Computational Methods

Geometry optimization was performed using GAMESS VERSION = 1 MAY 2013 (R1) and the B3LYP functional at the STO-3G level. Due to the size and the high flexibility of the structure, the NMR calculations have been performed on the lowest conformer only. After a frequency calculation at the same level of theory in order to check that the studied structure was not presenting any negative (imaginary) frequencies, the shielding constants have been calculated. For the NMR calculations Gaussian 09, Revision D.01 was used with the GIAO method at the B3LYP/6-31G(d) level. The enantiopodes of the expected absolute configurations are drawn below.

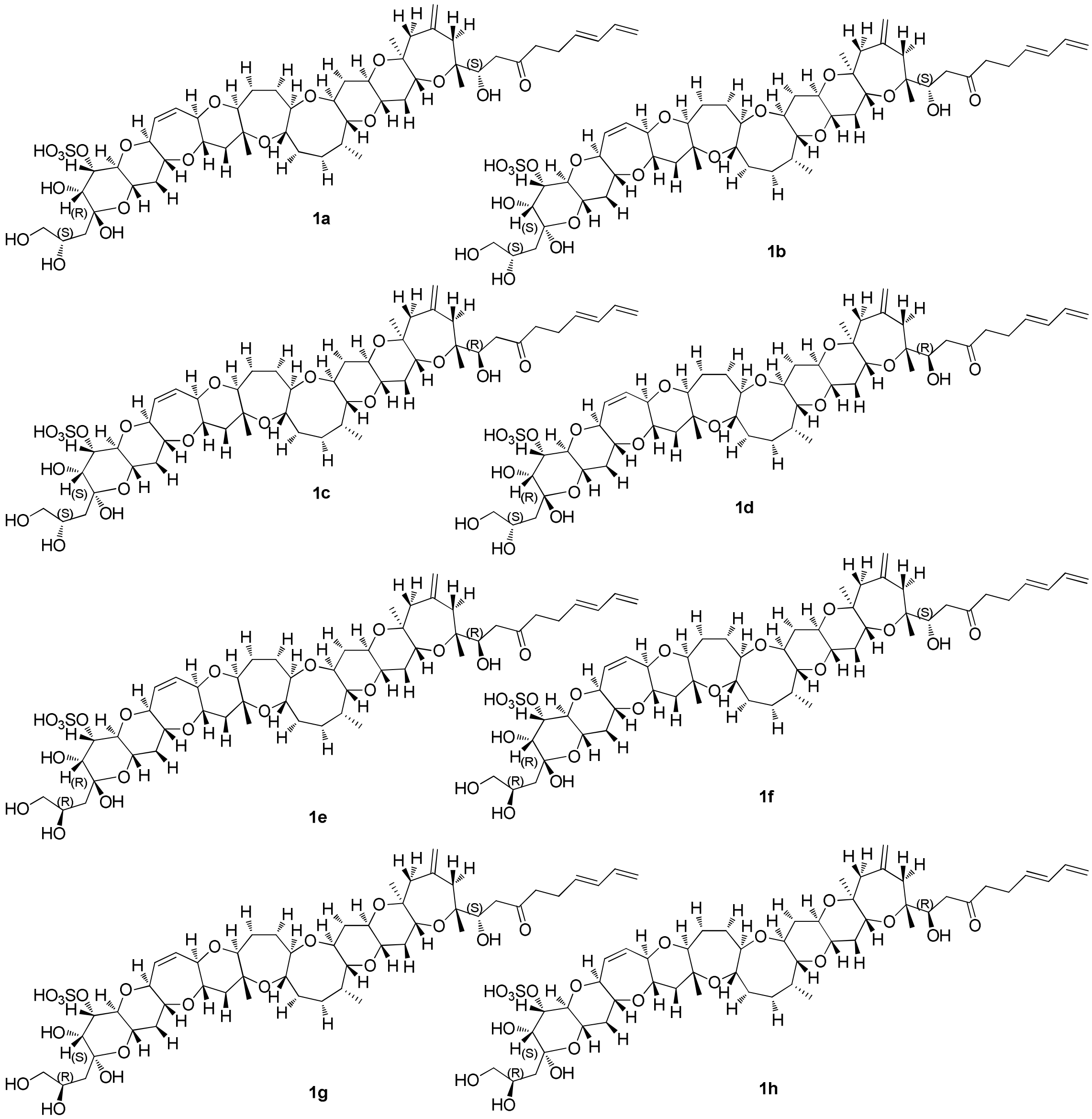


Figure 1: Structures of all studied diastereoisomers.

Table 1: Calculated ^{13}C chemical shifts.

Position	1a	1b	1c	1d	1e	1f	1g	1h
1	49.5	55.1	52.5	52.8	50.2	56.5	55.4	49.5
2	92	92.9	92.3	92.1	91.9	93.1	93	91.7
3	87.6	85.8	85.3	85.1	85.2	85.6	85.9	85.9
4	97.2	93.4	94	94	94.5	93.4	93.2	95
5	50	51.2	49.3	49.2	49.4	50.8	50.7	49
6	146.1	145.4	145.1	144.7	145.1	145.6	145.2	145
7	117	117.3	116.6	116.8	116.6	117.7	117.3	117
8	82.7	82.6	83	82.8	83	82.3	82.5	82.8
9	90.3	90.6	91.4	91.3	90.3	89.4	90.6	91.6
10	39.4	39.9	39.4	39.7	39.5	40.2	39.9	39.1
11	88.8	90.4	86.2	86.5	86.6	90.6	90.5	86.8
12	24.3	23.6	23.5	23.6	23.5	24.4	23.8	23.5
13	49.4	49.5	52.9	53.1	53.4	49.4	49.4	53.2
14	229.3	230.6	224.7	224	225.7	230.3	230.7	225
15	53.6	55.3	53.1	53.8	51.4	55.4	55.4	53.7
16	38.7	39.3	38.3	38.4	37.4	39	39	37.4
17	133.9	134.9	133.6	134.1	133	135.4	135.2	133.2
18	133.3	133.1	134.2	133.7	133.6	132.4	132.9	134.4
19	136.9	137	136.9	137	136.5	137.2	137.1	136.6
20	116.3	115.7	116.9	116.5	117	115.4	115.6	117.1
21	46.7	47.1	47.4	47.2	47	46.6	47.1	47.5
22	86.1	86.3	86.4	86.2	86.3	86.1	86	86.1
23	93.6	94.1	94.6	94.5	93.5	93.4	94.1	94.7
24	93.5	93.7	93	93.5	92.9	93.3	93.2	92.8
25	87.5	87.6	86.8	86.8	88.1	88.2	87.6	87.4
26	41	41.3	41.3	41.1	41.5	41.5	41.4	41.2
27	34.4	34.1	34	34.3	34.4	33.8	34.2	34.2
28	46.2	46.2	46	46.2	46.3	46.4	46.2	46.3
29	42.8	42.7	41.8	42.3	42.7	42.9	42.9	42.6
30	36.6	36.4	35.5	36.5	35.7	36	36.2	35.7
31	92.7	93	93.4	92.5	93.1	93.1	92.6	92.5
32	90.6	90.5	90.4	90.7	90.3	90.2	90.4	90.3
33	91.9	91.7	92	91.8	91.2	91.4	91.6	91.6
34	91	91.5	92.2	92.2	91.2	91.3	91.4	90.6
35	52.8	52.7	52.6	52.9	52.1	52.8	52.2	52.2
36	23.2	23.6	23.8	23.3	23.3	23.3	23.4	23.3
37	134.5	137.6	137	134.5	138.2	138	135	135
38	133.3	128.6	128.3	132.6	129.2	128.3	132	132.5
39	93.7	95	95	92	92.9	94.3	92.2	92
40	91.2	89.7	90.8	90.8	89.2	89.3	89.6	89.6
41	93.3	93.4	94	85.7	89.2	90.5	89	89.3
42	79.7	78.7	78.6	81.3	79.5	80.9	81.7	81.5
43	44.2	45.7	45.5	41.8	40.7	41.5	44.1	44.2
44	112.6	89.5	89.7	103.9	107.8	109.1	114.4	113.8
45	101	85.5	85.4	87.4	89.4	89	88.4	88.5
46	127.1	124.1	123.9	121.4	121.5	122	127.1	126.9
47	73.5	71.6	71.8	72.6	74.4	78.5	80.3	80.5
48	85.6	90	90	85.2	80.6	74.8	75.8	75.7
49	44.6	40.5	40.5	49.3	54.8	54.1	53.4	53.7
50	29.4	23.5	26.9	25.9	28.4	23.2	23.6	29.5
51	19.2	19.1	19.4	19	19.2	19	19	19

Table 2: Coordinates (Ångstroms) for 1a

Atom	x	y	z
C	8.8666	-21.5608	7.4207
C	9.4675	-20.8158	8.6934
C	9.8551	-19.2982	8.3644
O	10.9431	-19.1748	7.3324
C	10.4177	-18.9102	5.953
C	9.5994	-20.1458	5.3543
C	8.4438	-20.6282	6.2618
C	7.1435	-20.313	6.0148
O	8.2948	-20.7619	9.6596
C	8.6891	-20.091	10.944
C	9.1482	-18.5905	10.6959
C	10.3344	-18.5452	9.667
C	11.7366	-18.6826	5.0681
C	10.6782	-21.6493	9.2614
C	12.7157	-19.9237	5.0661
O	12.5437	-17.5086	5.5356
C	13.0779	-20.4475	6.5096
C	13.9282	-19.4647	7.3963
O	12.7769	-21.659	6.875
C	15.4779	-19.6812	7.129
C	16.3196	-18.6868	7.9539
C	17.2188	-19.0644	8.9149
C	18.0294	-18.0791	9.6991
C	18.9392	-18.4426	10.6532
C	7.4332	-20.085	11.8899
C	7.8361	-19.3507	13.2394
C	8.4022	-17.8835	12.9196
O	9.5988	-17.9952	12.0011
O	6.8108	-19.4273	14.3344
C	5.4653	-18.7713	14.1498
C	5.5007	-17.2051	13.7925
C	6.4256	-16.3284	14.7412
C	7.7677	-16.9507	15.2706
C	8.9048	-17.1096	14.196
C	4.5682	-19.6033	13.1412
C	3.0294	-19.61	13.4565
C	2.292	-18.224	13.657
C	3.0141	-16.8913	13.1399
O	4.1656	-16.555	14.0473
O	0.9813	-18.4075	12.9276
C	0.0124	-17.3069	13.2739
C	0.6042	-15.8889	12.834
C	2.0313	-15.6889	13.4602
C	3.3359	-16.915	11.5976

C	-1.2998	-17.6904	12.5374
C	-2.4081	-16.9203	12.3785
C	-2.627	-15.4748	12.9025
C	-1.4196	-14.4758	12.5204
O	-0.2275	-14.7616	13.384
O	-3.9533	-15.0496	12.3008
C	-4.2279	-13.6534	12.7865
C	-3.1685	-12.6906	12.1163
C	-1.7835	-12.9582	12.7957
C	-5.7122	-13.1997	12.4385
O	-3.6479	-11.2707	12.2533
C	-5.7635	-12.2534	11.0577
C	-4.6538	-11.0366	11.143
O	-3.9543	-10.9419	9.8189
O	-7.09	-11.8413	10.6894
C	-5.7818	-9.4891	13.9644
C	-6.2499	-9.2189	12.4471
C	-5.2119	-9.5603	11.316
O	-7.0836	-9.7961	14.6547
O	-7.5246	-9.9933	12.3051
C	9.5201	-17.6036	5.8954
H	8.9256	-18.8125	8.0014
H	8.2833	-18.0152	10.2932
H	9.5242	-20.6351	11.4389
H	8.6809	-19.9195	13.6873
H	7.6211	-17.2862	12.4055
H	5.7799	-17.0792	12.7255
H	2.0959	-18.0558	14.7413
H	5.0286	-18.86	15.1702
H	0.6403	-15.8356	11.7231
H	-0.154	-17.2728	14.3804
H	-4.1188	-13.6078	13.9
H	-2.7131	-15.4694	14.0192
H	-1.1713	-14.6138	11.4433
H	-3.0789	-12.9583	11.0412
H	-6.373	-14.0794	12.2344
H	-5.4034	-12.9834	10.2886
C	10.1571	-17.8159	14.8252
O	-6.2487	-12.3271	13.5095
S	-6.5718	-13.2154	15.2828
O	-6.9448	-11.8232	16.2669
O	-7.5346	-14.6195	15.3103
O	-4.7865	-13.6888	15.8702
H	8.0006	-22.1469	7.7793
H	9.6348	-22.2603	7.0436
H	10.3027	-20.9858	5.2172

H	9.2063	-19.8416	4.3679
H	6.8543	-19.6805	5.1628
H	6.3342	-20.6938	6.6556
H	11.2281	-19.0359	10.0896
H	10.5919	-17.4984	9.4255
H	11.3991	-18.515	4.0153
H	11.4865	-21.6807	8.4979
H	10.3406	-22.6776	9.475
H	11.0801	-21.204	10.1837
H	12.2759	-20.7546	4.4863
H	13.6492	-19.592	4.5685
H	11.86	-16.7777	5.7434
H	13.7072	-19.6589	8.461
H	13.6294	-18.4323	7.1167
H	15.6782	-19.5248	6.0505
H	15.7586	-20.7178	7.387
H	16.1704	-17.6167	7.7367
H	17.3761	-20.1308	9.1394
H	17.8627	-17.0143	9.4689
H	19.1201	-19.5006	10.897
H	19.52	-17.6907	11.2085
H	7.1187	-21.1221	12.1041
H	6.6097	-19.5611	11.3807
H	5.791	-16.0876	15.6145
H	6.6319	-15.3768	14.2195
H	7.5621	-17.9402	15.7128
H	8.1491	-16.2857	16.0665
H	9.2115	-16.1073	13.8447
H	4.9187	-20.6505	13.1961
H	4.7356	-19.2577	12.1114
H	2.8528	-20.2185	14.3613
H	2.511	-20.1151	12.6188
H	1.928	-15.6075	14.5575
H	2.4733	-14.7489	13.086
H	3.6452	-15.9103	11.2628
H	2.4197	-17.2164	11.0585
H	4.1283	-17.6367	11.3546
H	-1.2883	-18.7179	12.133
H	-3.2848	-17.3294	11.8469
H	-1.8403	-12.7831	13.8853
H	-0.9964	-12.3074	12.3715
H	-3.8827	-11.8931	9.4503
H	-7.3747	-11.0371	11.3969
H	-5.0797	-10.3569	13.9911
H	-5.3083	-8.5841	14.4092
H	-6.4911	-8.1337	12.3431

H	-5.6729	-9.2965	10.3431
H	-4.3161	-8.92	11.4465
H	-6.9715	-10.6189	15.3298
H	-7.7191	-10.1097	13.3773
H	8.4971	-17.832	6.2392
H	9.4558	-17.2408	4.8545
H	9.9376	-16.81	6.5391
H	9.8759	-18.7954	15.2449
H	10.574	-17.1953	15.6363
H	10.9269	-17.9618	14.0478
H	-4.6894	-13.0247	16.6665

Table 3: Coordinates (Ångstroms) for 1b

Atom	x	y	z
C	8.7504	-21.652	7.7807
C	9.3686	-20.8075	8.9824
C	9.7066	-19.3131	8.5279
O	10.7546	-19.2546	7.4504
C	10.1745	-19.0772	6.0792
C	9.3521	-20.3556	5.5885
C	8.2543	-20.8085	6.582
C	6.9362	-20.5372	6.3802
O	8.2287	-20.7115	9.982
C	8.6407	-19.9261	11.1947
C	9.0556	-18.4396	10.8119
C	10.2124	-18.4532	9.7499
C	11.4605	-18.8782	5.1403
C	10.6176	-21.5747	9.5615
C	12.4462	-20.1129	5.1423
O	12.2782	-17.6898	5.5485
C	12.8505	-20.5946	6.5896
C	13.742	-19.5935	7.4122
O	12.5346	-21.7841	7.0098
C	15.2581	-19.6739	6.9511
C	16.1139	-18.6562	7.7333
C	17.1175	-19.0115	8.5939
C	17.9311	-18.0084	9.3523
C	18.9324	-18.3514	10.218
C	7.4104	-19.8678	12.1729
C	7.8308	-19.0127	13.4447
C	8.3486	-17.5651	12.9864
O	9.5251	-17.7283	12.0495
O	6.8336	-19.0213	14.5686
C	5.471	-18.4036	14.3702
C	5.4646	-16.8705	13.8908
C	6.3827	-15.9042	14.7536
C	7.7578	-16.4569	15.2738
C	8.8602	-16.663	14.172
C	4.56	-19.3327	13.4626
C	3.0298	-19.3241	13.8169
C	2.2631	-17.9396	13.8233
C	2.9867	-16.6511	13.2043
O	4.1133	-16.2424	14.1128
O	0.9849	-18.2073	13.0646
C	-0.0085	-17.1064	13.3213
C	0.557	-15.7136	12.7832
C	1.9832	-15.4361	13.3913
C	3.3401	-16.8041	11.6759

C	-1.3065	-17.5716	12.6092
C	-2.4369	-16.8456	12.4066
C	-2.6752	-15.3757	12.8486
C	-1.51	-14.359	12.4217
O	-0.2907	-14.5667	13.2673
O	-3.9604	-14.9545	12.1669
C	-4.4022	-13.5716	12.5731
C	-3.332	-12.5336	12.0345
C	-1.9382	-12.8635	12.6949
C	-5.8301	-13.3202	11.9436
O	-3.6874	-11.1046	12.3595
C	-6.1107	-11.7412	11.8451
C	-5.1253	-10.9678	12.856
O	-5.2959	-11.5436	14.1981
O	-7.5314	-11.4799	12.1908
C	-3.1517	-8.5303	13.7809
C	-4.7369	-8.5758	13.9258
C	-5.4621	-9.4168	12.8034
O	-2.5711	-8.0471	15.0706
O	-4.9407	-9.2144	15.2992
C	9.2582	-17.7852	5.9805
H	8.7563	-18.8775	8.1574
H	8.1665	-17.9218	10.3845
H	9.5042	-20.4071	11.7069
H	8.7014	-19.5231	13.9133
H	7.5404	-17.0353	12.4416
H	5.7253	-16.8201	12.8138
H	2.015	-17.6631	14.8739
H	5.0656	-18.4157	15.4071
H	0.5897	-15.734	11.6707
H	-0.1878	-16.9945	14.4211
H	-4.494	-13.4764	13.6835
H	-2.8214	-15.305	13.9572
H	-1.2861	-14.5132	11.3406
H	-3.2611	-12.6356	10.9256
H	-5.8968	-13.793	10.9338
H	-5.9084	-11.3728	10.8127
C	10.1635	-17.2598	14.8139
O	-6.9095	-13.8336	12.8609
S	-7.3211	-15.768	12.4861
O	-7.0596	-16.5156	13.9993
O	-8.8068	-15.8685	11.6516
O	-5.9815	-16.185	11.2441
H	7.9182	-22.2449	8.2025
H	9.5316	-22.3454	7.419
H	10.064	-21.1897	5.4581

H	8.8978	-20.1134	4.6113
H	6.5903	-19.9673	5.5056
H	6.1694	-20.8909	7.0855
H	11.1265	-18.8981	10.1789
H	10.4432	-17.4243	9.4205
H	11.0852	-18.7421	4.0958
H	11.3867	-21.6633	8.7634
H	10.3041	-22.5855	9.8736
H	11.0564	-21.0527	10.4253
H	11.9953	-20.9626	4.5993
H	13.3621	-19.792	4.6071
H	11.6014	-16.9406	5.7087
H	13.6644	-19.8401	8.4859
H	13.343	-18.576	7.2117
H	15.3136	-19.4455	5.8687
H	15.6497	-20.694	7.1126
H	15.8715	-17.5919	7.5824
H	17.3596	-20.0735	8.7545
H	17.6833	-16.9478	9.1833
H	19.1951	-19.4043	10.402
H	19.5086	-17.5868	10.7609
H	7.122	-20.888	12.4831
H	6.5634	-19.4051	11.6431
H	5.7679	-15.6296	15.6311
H	6.542	-14.9814	14.1676
H	7.5905	-17.4203	15.7847
H	8.152	-15.7328	16.0093
H	9.1125	-15.6847	13.7234
H	4.9239	-20.3669	13.6087
H	4.6966	-19.0875	12.4003
H	2.8933	-19.788	14.8098
H	2.5042	-19.9647	13.0825
H	1.8737	-15.2397	14.4737
H	2.4109	-14.5337	12.9203
H	3.6032	-15.8205	11.2515
H	2.4508	-17.2057	11.157
H	4.1742	-17.5006	11.5113
H	-1.2674	-18.6199	12.262
H	-3.2963	-17.321	11.9028
H	-2.004	-12.7024	13.787
H	-1.1612	-12.1887	12.2899
H	-5.0864	-10.7464	14.8489
H	-7.7679	-12.3637	12.6963
H	-2.8011	-9.557	13.4961
H	-2.8626	-7.812	12.9819
H	-5.1443	-7.5387	13.9574

H	-6.5649	-9.3384	12.8966
H	-5.1668	-9.0169	11.8137
H	-3.1863	-8.5191	15.7507
H	-5.8455	-8.8868	15.6491
H	8.2462	-18.0086	6.3579
H	9.1669	-17.4727	4.9255
H	9.6826	-16.958	6.5754
H	9.9305	-18.1799	15.3743
H	10.6094	-16.5291	15.5092
H	10.8904	-17.4939	14.0167
H	-5.0698	-15.7794	11.702

Table 4: Coordinates (Ångstroms) for 1c

Atom	x	y	z
C	8.7451	-21.6812	7.9526
C	9.3132	-20.9278	9.2283
C	9.6965	-19.4293	8.8403
O	10.7197	-19.3795	7.7385
C	10.1057	-19.0717	6.3965
C	9.1141	-20.1891	5.8448
C	8.1166	-20.734	6.8998
C	6.7831	-20.473	6.8482
O	8.1473	-20.8344	10.1983
C	8.5771	-20.1242	11.4517
C	9.0689	-18.6376	11.154
C	10.2282	-18.6326	10.0936
C	11.396	-18.9437	5.4686
C	10.5149	-21.7547	9.8273
C	12.2646	-20.2693	5.4455
O	10.8985	-18.5934	4.0977
C	12.7549	-20.7287	6.876
C	13.7042	-19.7526	7.6733
O	12.476	-21.9221	7.3114
C	15.0858	-19.5387	6.924
C	16.0139	-18.6031	7.7261
C	16.3317	-17.3324	7.3261
C	17.2182	-16.4264	8.1232
C	17.54	-15.1556	7.7319
C	7.3392	-20.0545	12.4223
C	7.7993	-19.2884	13.737
C	8.3735	-17.84	13.3573
O	9.5603	-18.0242	12.4364
O	6.8232	-19.3441	14.8761
C	5.466	-18.6932	14.7435
C	5.4882	-17.1665	14.246
C	6.4086	-16.219	15.1274
C	7.7611	-16.8051	15.67
C	8.8878	-17.0081	14.5915
C	4.5083	-19.6244	13.8908
C	2.9756	-19.551	14.229
C	2.2638	-18.1383	14.2288
C	3.0167	-16.9038	13.5377
O	4.1555	-16.4931	14.4267
O	0.9345	-18.3862	13.5571
C	0.0097	-17.223	13.8008
C	0.6087	-15.9138	13.119
C	2.0581	-15.6454	13.6693
C	3.3627	-17.138	12.0183

C	-1.3669	-17.6785	13.2463
C	-2.465	-16.9207	12.9836
C	-2.6152	-15.3852	13.1617
C	-1.3815	-14.5222	12.611
O	-0.1843	-14.6902	13.4938
O	-3.8556	-15.011	12.3748
C	-4.2176	-13.5481	12.4811
C	-3.0459	-12.6954	11.8384
C	-1.7259	-12.984	12.6479
C	-5.571	-13.3519	11.6875
O	-3.3141	-11.2145	11.8743
C	-5.7236	-11.822	11.22
C	-4.7834	-10.8974	12.1426
O	-5.1394	-11.1696	13.5449
O	-7.1464	-11.4186	11.3509
C	-2.7572	-8.4252	12.8171
C	-4.3462	-8.3537	12.7437
C	-4.9889	-9.3792	11.7279
O	-2.3063	-7.6898	14.0378
O	-4.7726	-8.6725	14.1747
C	9.3882	-17.6621	6.3959
H	8.7579	-18.9523	8.49
H	8.2001	-18.0596	10.7638
H	9.3998	-20.6792	11.9546
H	8.6602	-19.8529	14.1596
H	7.6016	-17.2584	12.8128
H	5.7848	-17.1352	13.1772
H	2.0939	-17.8162	15.2824
H	5.1182	-18.6893	15.8012
H	0.6169	-16.0478	12.0141
H	-0.0781	-17.0258	14.9
H	-4.3758	-13.2446	13.5453
H	-2.7857	-15.1201	14.2372
H	-1.1661	-14.8522	11.5685
H	-2.9222	-13.0192	10.7776
H	-5.5887	-14.0336	10.8027
H	-5.4042	-11.7087	10.158
C	10.1366	-17.6992	15.2472
O	-6.7569	-13.5968	12.5818
S	-7.279	-15.5392	12.5305
O	-7.1654	-16.0186	14.1668
O	-8.7089	-15.6899	11.6092
O	-5.889	-16.2489	11.488
H	8.0142	-22.4303	8.3066
H	9.5938	-22.2047	7.476
H	9.7054	-21.0434	5.4706

H	8.5609	-19.7438	4.9985
H	6.3533	-19.8219	6.0729
H	6.0926	-20.9056	7.5876
H	11.1279	-19.1202	10.5065
H	10.4881	-17.5975	9.8081
H	12.0068	-18.1129	5.8951
H	11.281	-21.9006	9.037
H	10.1432	-22.7368	10.1661
H	10.9765	-21.2326	10.6784
H	11.673	-21.0948	5.012
H	13.1547	-20.1067	4.8062
H	11.741	-18.5115	3.5227
H	13.8796	-20.19	8.6732
H	13.1889	-18.7823	7.8081
H	14.9039	-19.1045	5.9263
H	15.5754	-20.5242	6.7939
H	16.4163	-18.9905	8.6759
H	15.9451	-16.9412	6.3726
H	17.6162	-16.8352	9.0661
H	17.1427	-14.7284	6.7987
H	18.2036	-14.5222	8.3403
H	7.008	-21.0743	12.6882
H	6.5144	-19.5322	11.9125
H	5.7835	-15.9288	15.992
H	6.6019	-15.3016	14.5432
H	7.5674	-17.778	16.1528
H	8.1488	-16.108	16.4344
H	9.2045	-16.0254	14.1967
H	4.8319	-20.6644	14.0861
H	4.6613	-19.4442	12.8177
H	2.8094	-20.0022	15.2233
H	2.4347	-20.1781	13.4943
H	1.9848	-15.3772	14.7391
H	2.5014	-14.7915	13.128
H	3.6851	-16.1889	11.5574
H	2.4531	-17.5043	11.5089
H	4.1544	-17.8884	11.8828
H	-1.4397	-18.7746	13.123
H	-3.3875	-17.4298	12.6541
H	-1.8714	-12.6747	13.6994
H	-0.8822	-12.4051	12.2284
H	-4.969	-10.2571	14.0352
H	-7.4911	-12.1545	12.0079
H	-2.4602	-9.5067	12.8238
H	-2.3089	-7.9304	11.9274
H	-4.6642	-7.3146	12.4956

H	-6.0844	-9.2207	11.651
H	-4.5409	-9.2214	10.7274
H	-3.0258	-7.9789	14.7174
H	-5.6872	-8.2353	14.32
H	8.4027	-17.7192	6.8866
H	9.2474	-17.3547	5.3437
H	10.0169	-16.9202	6.9162
H	9.85	-18.6632	15.6979
H	10.5545	-17.0532	16.0376
H	10.9079	-17.8739	14.4772
H	-4.9826	-15.8403	11.9561

Table 5: Coordinates (Ångstroms) for 1d

Atom	x	y	z
C	8.8619	-21.7537	7.9464
C	9.4122	-20.9723	9.2142
C	9.7897	-19.4719	8.8231
O	10.81	-19.397	7.7241
C	10.2013	-19.1101	6.3758
C	9.2675	-20.2835	5.8269
C	8.2557	-20.826	6.8658
C	6.928	-20.5355	6.8124
O	8.2312	-20.8696	10.1614
C	8.6363	-20.1625	11.424
C	9.1345	-18.6783	11.128
C	10.3091	-18.6755	10.0819
C	11.4944	-18.9467	5.4619
C	10.6156	-21.7813	9.8346
C	12.4189	-20.2337	5.4738
O	10.99	-18.6586	4.0775
C	12.8611	-20.6863	6.9234
C	13.7547	-19.6887	7.7592
O	12.6047	-21.8904	7.3423
C	15.2502	-19.6679	7.2269
C	16.0909	-18.6489	8.024
C	16.5247	-17.4552	7.5129
C	17.3267	-16.4743	8.3126
C	17.7637	-15.279	7.8124
C	7.3814	-20.0896	12.3684
C	7.818	-19.326	13.6919
C	8.4044	-17.8789	13.3207
O	9.6027	-18.0477	12.4108
O	6.8196	-19.3602	14.8125
C	5.4683	-18.7142	14.6224
C	5.5048	-17.1807	14.1499
C	6.4281	-16.2428	15.0398
C	7.7612	-16.8401	15.6215
C	8.9091	-17.0624	14.5688
C	4.5424	-19.6274	13.7148
C	3.0094	-19.5726	14.0499
C	2.2749	-18.1713	14.0086
C	3.0518	-16.9083	13.4041
O	4.1659	-16.518	14.3383
O	1.028	-18.4214	13.1935
C	0.0449	-17.2922	13.3702
C	0.6819	-15.9201	12.8533
C	2.0698	-15.6714	13.5529
C	3.4456	-17.0826	11.8876

C	-1.2035	-17.737	12.5634
C	-2.2774	-16.9797	12.2193
C	-2.535	-15.4866	12.5585
C	-1.2893	-14.5199	12.2668
O	-0.1727	-14.7454	13.244
O	-3.7019	-15.1077	11.6758
C	-4.1411	-13.7109	12.0049
C	-2.9895	-12.6907	11.625
C	-1.7073	-13.0111	12.4721
C	-5.4174	-13.421	11.1168
O	-3.4332	-11.288	11.9637
C	-5.8545	-11.8715	11.1767
C	-4.5781	-10.8862	11.0838
O	-4.1866	-10.9391	9.6391
O	-6.6789	-11.6236	12.3754
C	-3.2413	-8.7802	13.4326
C	-4.771	-9.0294	13.0335
C	-4.9882	-9.3968	11.5091
O	-3.1055	-8.9391	14.9107
O	-5.2565	-10.1621	13.9341
C	9.4159	-17.74	6.364
H	8.8426	-19.0044	8.4835
H	8.2774	-18.0979	10.716
H	9.4549	-20.7094	11.9426
H	8.667	-19.8926	14.1347
H	7.6402	-17.2859	12.7776
H	5.7934	-17.1335	13.0801
H	1.9864	-17.8745	15.0431
H	5.0641	-18.7238	15.6597
H	0.796	-15.9716	11.7476
H	-0.205	-17.1626	14.4538
H	-4.4068	-13.6082	13.0896
H	-2.8177	-15.3727	13.6361
H	-0.9443	-14.6946	11.2218
H	-2.7793	-12.7639	10.5325
H	-5.1877	-13.6765	10.0523
H	-6.4849	-11.6767	10.2762
C	10.1463	-17.7556	15.2396
O	-6.5747	-14.2914	11.484
S	-7.2549	-13.8794	13.3261
O	-6.0252	-13.4552	14.4476
O	-8.8576	-13.2966	13.4227
O	-7.4013	-15.7788	13.4019
H	8.1231	-22.4916	8.3075
H	9.7128	-22.2978	7.4969
H	9.9157	-21.1173	5.5066

H	8.737	-19.8853	4.9436
H	6.5112	-19.8874	6.0277
H	6.2272	-20.9447	7.5554
H	11.2079	-19.1522	10.5077
H	10.5614	-17.6384	9.7961
H	12.0672	-18.0765	5.862
H	11.3881	-21.9254	9.0495
H	10.2538	-22.7666	10.175
H	11.0713	-21.251	10.6837
H	11.8734	-21.0813	5.0221
H	13.3274	-20.0403	4.8703
H	11.8322	-18.5301	3.5105
H	13.7332	-20.0187	8.8139
H	13.3249	-18.6719	7.7032
H	15.2615	-19.3989	6.1567
H	15.6841	-20.6808	7.3407
H	16.3274	-18.9091	9.0683
H	16.298	-17.1781	6.472
H	17.55	-16.7594	9.3536
H	17.5409	-14.9763	6.7778
H	18.3511	-14.5794	8.4266
H	7.0372	-21.1098	12.615
H	6.5734	-19.5535	11.8468
H	5.792	-15.935	15.8904
H	6.6488	-15.3346	14.4514
H	7.5471	-17.8015	16.1179
H	8.1367	-16.1299	16.3801
H	9.2316	-16.0789	14.1802
H	4.8762	-20.6688	13.8808
H	4.7017	-19.4001	12.6519
H	2.8475	-20.0118	15.0501
H	2.4784	-20.2155	13.3213
H	1.9002	-15.4895	14.6297
H	2.5457	-14.7734	13.1222
H	3.7045	-16.1032	11.4514
H	2.576	-17.5077	11.354
H	4.2942	-17.7684	11.7573
H	-1.1715	-18.799	12.2632
H	-3.1001	-17.4384	11.6437
H	-1.9234	-12.8418	13.5435
H	-0.8685	-12.3541	12.1776
H	-3.3687	-10.325	9.5678
H	-6.0527	-11.1677	13.1074
H	-2.6158	-9.5196	12.8745
H	-2.9387	-7.7394	13.1813
H	-5.3708	-8.1187	13.2638

H	-6.055	-9.2624	11.2457
H	-4.3923	-8.6837	10.9062
H	-3.5675	-9.8484	15.0556
H	-5.9104	-9.7474	14.6052
H	8.4406	-17.8385	6.8678
H	9.2494	-17.455	5.3088
H	10.0159	-16.9565	6.8569
H	9.8534	-18.7176	15.691
H	10.557	-17.1081	16.0326
H	10.9252	-17.9348	14.4784
H	-6.9299	-15.9453	12.4831

Table 6: Coordinates (Ångstroms) for 1e

Atom	x	y	z
C	9.3259	-21.7279	7.8376
C	9.6769	-20.964	9.1829
C	10.1437	-19.4883	8.8071
O	11.3124	-19.5311	7.8614
C	10.9102	-19.1858	6.4488
C	9.8444	-20.1787	5.7806
C	8.7844	-20.7834	6.7379
C	7.4536	-20.5562	6.5712
O	8.3715	-20.8193	9.943
C	8.624	-20.0725	11.2253
C	9.1587	-18.5981	10.9519
C	10.4755	-18.6345	10.0894
C	12.2972	-19.2671	5.6683
C	10.7432	-21.7939	9.9936
C	12.989	-20.6905	5.7715
O	11.9859	-18.9296	4.2396
C	13.3379	-21.1256	7.2473
C	14.3199	-20.2063	8.0717
O	12.8871	-22.2477	7.7266
C	15.7247	-20.0469	7.3647
C	16.6404	-19.0641	8.1287
C	17.4237	-18.1337	7.5008
C	18.3285	-17.1924	8.2346
C	19.1091	-16.2643	7.6028
C	7.272	-19.9832	12.0204
C	7.5646	-19.207	13.3765
C	8.1817	-17.7633	13.0497
O	9.4636	-17.9326	12.2654
O	6.4547	-19.2339	14.3861
C	5.1373	-18.5686	14.0783
C	5.2144	-17.0144	13.6752
C	6.0731	-16.1148	14.6635
C	7.3663	-16.7308	15.3092
C	8.5781	-16.9314	14.3279
C	4.3144	-19.434	13.035
C	2.7544	-19.4173	13.2211
C	2.022	-18.0215	13.3446
C	2.7843	-16.71	12.8366
O	3.8642	-16.3589	13.8248
O	0.7524	-18.2153	12.5458
C	-0.2241	-17.1071	12.826
C	0.3896	-15.7028	12.3684
C	1.7921	-15.49	13.0483
C	3.2244	-16.7821	11.3266

C	-1.5041	-17.4991	12.0415
C	-2.6179	-16.7375	11.892
C	-2.8122	-15.3133	12.4831
C	-1.6599	-14.2974	12.0297
O	-0.445	-14.5526	12.8723
O	-4.2017	-14.8983	11.9977
C	-4.5351	-13.4573	12.3476
C	-3.4558	-12.4673	11.7444
C	-2.0455	-12.8021	12.3393
C	-5.9485	-13.1164	11.7094
O	-3.7895	-11.0611	12.1579
C	-6.345	-11.6024	12.0702
C	-5.1188	-10.6565	11.574
O	-5.1152	-10.8564	10.0874
O	-6.7413	-11.3585	13.4734
C	-3.8024	-8.7847	14.0946
C	-5.2779	-8.8555	13.5015
C	-5.3339	-9.1293	11.9421
O	-3.8767	-8.0646	15.4028
O	-5.831	-7.4801	13.8078
C	10.3897	-17.6964	6.3586
H	9.272	-19.023	8.3034
H	8.3705	-18.0282	10.4076
H	9.3783	-20.6043	11.8459
H	8.3577	-19.7721	13.9148
H	7.4662	-17.1786	12.4333
H	5.5614	-16.918	12.6253
H	1.7587	-17.8251	14.4097
H	4.6261	-18.622	15.0659
H	0.4615	-15.6735	11.2578
H	-0.44	-17.039	13.9226
H	-4.5728	-13.3438	13.4607
H	-2.813	-15.333	13.6035
H	-1.4448	-14.444	10.9461
H	-3.4635	-12.5452	10.6317
H	-5.8567	-13.1754	10.5946
H	-7.2053	-11.3338	11.409
C	9.7812	-17.5949	15.0882
O	-7.085	-14.0799	11.9905
S	-7.0293	-14.7952	13.8121
O	-6.2584	-13.5873	14.7984
O	-8.5637	-15.4266	14.1858
O	-5.8469	-16.2086	13.4971
H	8.5933	-22.5206	8.0743
H	10.2608	-22.1992	7.4818
H	10.3925	-21.0126	5.3067

H	9.3391	-19.612	4.9794
H	7.0763	-19.8947	5.7779
H	6.7114	-21.0303	7.2307
H	11.2963	-19.0882	10.6712
H	10.7711	-17.6097	9.8009
H	12.9715	-18.5008	6.1199
H	11.5877	-22.0517	9.3208
H	10.2729	-22.7239	10.3571
H	11.1387	-21.2313	10.8526
H	12.3346	-21.4672	5.3386
H	13.9294	-20.6577	5.187
H	12.8859	-18.9767	3.7548
H	14.448	-20.6643	9.0696
H	13.838	-19.217	8.2043
H	15.596	-19.6901	6.3289
H	16.2125	-21.0428	7.3211
H	16.6522	-19.142	9.2278
H	17.4103	-18.0508	6.4031
H	18.3429	-17.2771	9.3335
H	19.1107	-16.1684	6.5062
H	19.7648	-15.5837	8.1677
H	6.8998	-20.9999	12.2397
H	6.5267	-19.4534	11.4071
H	5.3788	-15.845	15.4811
H	6.3272	-15.1809	14.131
H	7.1194	-17.7026	15.7686
H	7.6954	-16.0395	16.1059
H	8.9074	-15.9425	13.9589
H	4.6423	-20.481	13.1758
H	4.577	-19.147	12.0074
H	2.4975	-20.0085	14.118
H	2.2998	-19.9305	12.3519
H	1.6354	-15.3623	14.1352
H	2.2571	-14.5682	12.6566
H	3.5799	-15.7925	10.9921
H	2.3489	-17.0797	10.7214
H	4.0189	-17.5246	11.1656
H	-1.4574	-18.5018	11.5794
H	-3.4705	-17.1138	11.3009
H	-2.0602	-12.65	13.4352
H	-1.2813	-12.129	11.9076
H	-4.4264	-10.1862	9.7316
H	-6.4975	-12.2015	14.056
H	-3.3959	-9.8066	14.2596
H	-3.1414	-8.2478	13.3722
H	-5.8865	-9.6596	13.9923

H	-6.3366	-8.8493	11.5658
H	-4.5732	-8.5156	11.4253
H	-4.6468	-7.404	15.1861
H	-6.8386	-7.6077	13.9312
H	9.3773	-17.607	6.7852
H	10.363	-17.4048	5.2927
H	11.0786	-17.0264	6.9005
H	9.478	-18.5601	15.5256
H	10.116	-16.9319	15.9035
H	10.6158	-17.7616	14.3856
H	-5.1497	-15.7439	12.7844

Table 7: Coordinates (Ångstroms) for 1f

Atom	x	y	z
C	8.8465	-21.7282	7.6006
C	9.4469	-20.9257	8.8428
C	9.913	-19.4517	8.4283
O	11.1023	-19.4761	7.5064
C	10.7391	-19.3239	6.0605
C	9.934	-20.5907	5.5108
C	8.6459	-20.8955	6.3122
C	7.4118	-20.5187	5.8802
O	8.2516	-20.786	9.7726
C	8.6269	-20.0387	11.0212
C	9.089	-18.5621	10.6665
C	10.3229	-18.6053	9.6958
C	12.1399	-19.2114	5.2858
C	10.6139	-21.7588	9.4994
C	13.0786	-20.4668	5.4729
O	12.938	-18.017	5.7106
C	13.3569	-20.8704	6.9723
C	14.0809	-19.8252	7.8965
O	13.0402	-22.0608	7.3903
C	15.4415	-19.3061	7.2765
C	16.1582	-18.3661	8.2664
C	17.3977	-18.6115	8.7935
C	18.0738	-17.6763	9.7483
C	19.3055	-17.9173	10.2914
C	7.3547	-19.97	11.9434
C	7.7184	-19.1095	13.2283
C	8.2682	-17.6648	12.7985
O	9.4876	-17.8399	11.9212
O	6.6736	-19.1207	14.3074
C	5.3267	-18.489	14.0648
C	5.3513	-16.9497	13.6007
C	6.2541	-15.9962	14.4939
C	7.5935	-16.5781	15.0707
C	8.7379	-16.777	14.0129
C	4.4444	-19.3883	13.0997
C	2.9046	-19.3914	13.4089
C	2.1511	-18.0022	13.4791
C	2.8767	-16.7054	12.8805
O	4.0035	-16.3042	13.7912
O	0.8569	-18.2432	12.7353
C	-0.1213	-17.1372	13.0206
C	0.4508	-15.7399	12.5011
C	1.885	-15.4859	13.095
C	3.2236	-16.833	11.3481

C	-1.4331	-17.5611	12.3082
C	-2.5593	-16.8157	12.1583
C	-2.7685	-15.3583	12.6547
C	-1.6078	-14.35	12.2036
O	-0.379	-14.5972	13.0226
O	-4.1177	-14.9469	12.0582
C	-4.4877	-13.5282	12.4493
C	-3.392	-12.5158	11.8992
C	-1.9976	-12.8571	12.5253
C	-5.8702	-13.1331	11.7748
O	-3.7258	-11.1092	12.3006
C	-6.2247	-11.6608	12.317
C	-5.0645	-10.7071	11.7244
O	-5.0948	-10.9454	10.246
O	-6.2559	-11.5162	13.7865
C	-3.9072	-9.0105	14.3269
C	-5.3101	-8.801	13.5791
C	-5.252	-9.1684	12.0453
O	-3.8323	-7.965	15.4174
O	-5.6508	-7.3474	13.6868
C	9.8922	-18.0065	5.7953
H	9.0484	-18.9661	7.9312
H	8.2408	-18.0363	10.1701
H	9.4541	-20.5512	11.5608
H	8.5656	-19.6216	13.7367
H	7.4938	-17.1242	12.2152
H	5.6395	-16.899	12.5301
H	1.9257	-17.7501	14.5409
H	4.8796	-18.5184	15.0839
H	0.4635	-15.7363	11.388
H	-0.2919	-17.0398	14.123
H	-4.5617	-13.4511	13.5615
H	-2.8444	-15.3111	13.7715
H	-1.4072	-14.4851	11.1155
H	-3.3716	-12.6039	10.7869
H	-5.711	-13.0252	10.6697
H	-7.1893	-11.359	11.8337
C	10.0141	-17.3851	14.6975
O	-7.0103	-14.1216	11.8189
S	-7.1888	-15.0278	13.5431
O	-6.6306	-13.9272	14.7633
O	-8.7488	-15.7049	13.6292
O	-5.9361	-16.3726	13.2163
H	7.8784	-22.1446	7.9343
H	9.5262	-22.5689	7.3719
H	10.5983	-21.4701	5.5778

H	9.687	-20.407	4.4498
H	7.2702	-19.9646	4.9407
H	6.5076	-20.7734	6.4529
H	11.1896	-19.0691	10.1979
H	10.6012	-17.5813	9.3886
H	11.8948	-19.1359	4.1971
H	11.4226	-21.9112	8.7538
H	10.2228	-22.7409	9.816
H	11.0399	-21.2431	10.3733
H	12.6307	-21.344	4.9717
H	14.0425	-20.2358	4.9767
H	12.265	-17.2494	5.7614
H	14.2401	-20.2908	8.8859
H	13.3851	-18.966	7.9976
H	15.1846	-18.7413	6.356
H	16.1027	-20.1513	7.0188
H	15.6168	-17.4484	8.5475
H	17.9417	-19.5306	8.527
H	17.5236	-16.7566	10.0046
H	19.868	-18.8306	10.0454
H	19.7739	-17.2087	10.991
H	7.0556	-20.988	12.2511
H	6.529	-19.5167	11.3723
H	5.609	-15.6989	15.3414
H	6.4609	-15.0857	13.9038
H	7.3929	-17.5495	15.5534
H	7.9607	-15.8736	15.8386
H	9.0162	-15.7955	13.5871
H	4.8058	-20.4277	13.2082
H	4.6116	-19.0931	12.0543
H	2.7318	-19.9103	14.3685
H	2.3985	-19.9831	12.6218
H	1.7861	-15.304	14.1809
H	2.3178	-14.5814	12.633
H	3.4652	-15.8395	10.9342
H	2.3394	-17.2457	10.8289
H	4.0723	-17.5088	11.1709
H	-1.4146	-18.5955	11.9193
H	-3.4341	-17.2559	11.6489
H	-2.0317	-12.7227	13.6225
H	-1.2251	-12.1812	12.1134
H	-4.6444	-10.1275	9.8281
H	-6.5189	-12.4527	14.1975
H	-3.8033	-10.0458	14.7233
H	-3.0614	-8.7839	13.6434
H	-6.0788	-9.4549	14.0627

H	-6.2101	-8.8462	11.5915
H	-4.4308	-8.6038	11.5651
H	-4.3813	-8.344	16.1953
H	-5.0011	-7.0688	14.4527
H	8.8265	-18.1938	6.0084
H	9.9819	-17.7107	4.7352
H	10.2424	-17.1813	6.4393
H	9.7581	-18.3012	15.2546
H	10.4452	-16.6551	15.4028
H	10.7621	-17.628	13.9227
H	-5.1591	-15.8173	12.6688

Table 8: Coordinates (Ångstroms) for 1g

Atom	x	y	z
C	8.9969	-21.4588	7.4749
C	9.5655	-20.7122	8.762
C	9.9336	-19.1846	8.4546
O	11.0435	-19.0347	7.4505
C	10.5467	-18.7749	6.0589
C	9.7776	-20.0269	5.4325
C	8.6032	-20.5299	6.3031
C	7.308	-20.2151	6.0295
O	8.3757	-20.6866	9.7074
C	8.7305	-20.0192	11.0055
C	9.1624	-18.5076	10.7734
C	10.3707	-18.4344	9.7731
C	11.8855	-18.4997	5.2203
C	10.7832	-21.5284	9.3412
C	12.8766	-19.7297	5.169
O	12.6707	-17.3502	5.7775
C	13.1984	-20.3396	6.5878
C	14.0314	-19.4125	7.5466
O	12.8409	-21.5525	6.8911
C	15.574	-19.4406	7.1754
C	16.3593	-18.4695	8.0803
C	17.3154	-18.8617	8.9784
C	18.045	-17.8931	9.857
C	19.0001	-18.2662	10.7619
C	7.454	-20.0441	11.9224
C	7.8115	-19.3067	13.2829
C	8.3423	-17.8235	12.975
O	9.5654	-17.9016	12.0891
O	6.7735	-19.415	14.3615
C	5.4087	-18.807	14.1513
C	5.3969	-17.2491	13.7646
C	6.2675	-16.323	14.7186
C	7.6113	-16.8958	15.2987
C	8.7851	-17.0279	14.2605
C	4.5532	-19.6955	13.154
C	3.0095	-19.7367	13.4409
C	2.2167	-18.3698	13.5326
C	2.9295	-17.0301	13.0228
O	4.0304	-16.6493	13.9761
O	0.9691	-18.6083	12.7127
C	-0.0583	-17.5433	12.9928
C	0.5019	-16.0988	12.596
C	1.9011	-15.8495	13.2699
C	3.3196	-17.0784	11.4976

C	-1.3034	-17.9656	12.1666
C	-2.4379	-17.2422	11.9789
C	-2.7438	-15.8347	12.5566
C	-1.5832	-14.7606	12.2911
O	-0.3833	-15.016	13.1533
O	-4.0148	-15.4063	11.8582
C	-4.5035	-14.1151	12.4471
C	-3.4583	-12.9803	12.0679
C	-2.0639	-13.3179	12.7124
C	-5.9026	-13.8238	11.7534
O	-3.8311	-11.5965	12.5516
C	-6.2035	-12.2266	11.6303
C	-5.2929	-11.3376	12.6375
O	-5.7643	-11.6173	14.06
O	-7.6166	-11.8448	11.6806
C	-3.1019	-8.7075	12.4344
C	-4.5263	-8.8623	13.1604
C	-5.5168	-9.795	12.3528
O	-2.5917	-7.3223	12.7544
O	-5.1205	-7.4965	13.254
C	9.6186	-17.4897	5.9885
H	9.0049	-18.7133	8.0717
H	8.2953	-17.9536	10.3463
H	9.5676	-20.5516	11.5101
H	8.6662	-19.8529	13.7401
H	7.5593	-17.2499	12.4374
H	5.6949	-17.1313	12.7021
H	1.9314	-18.1768	14.5924
H	4.9648	-18.8892	15.1691
H	0.5638	-16.0187	11.4876
H	-0.3008	-17.5181	14.0855
H	-4.6306	-14.2013	13.5559
H	-2.918	-15.8914	13.661
H	-1.3142	-14.7879	11.2099
H	-3.3688	-12.9523	10.9561
H	-5.8302	-14.2144	10.7061
H	-5.8462	-11.934	10.6144
C	10.0387	-17.6908	14.9329
O	-7.019	-14.6936	12.2715
S	-7.6346	-14.2889	14.0901
O	-8.5537	-12.8214	13.9418
O	-8.328	-15.7275	14.6786
O	-6.0732	-13.9166	15.0505
H	8.1189	-22.0404	7.8107
H	9.7722	-22.1625	7.12
H	10.5021	-20.8524	5.315

H	9.4088	-19.7301	4.4344
H	7.0345	-19.5841	5.1713
H	6.4863	-20.6005	6.6514
H	11.2648	-18.9106	10.2105
H	10.6127	-17.3818	9.5408
H	11.5725	-18.2731	4.171
H	11.5828	-21.5768	8.5699
H	10.4501	-22.5523	9.5818
H	11.1927	-21.06	10.2487
H	12.466	-20.5244	4.5209
H	13.8196	-19.3556	4.7229
H	11.9749	-16.6385	6.0101
H	13.8895	-19.7475	8.5894
H	13.6349	-18.3832	7.4099
H	15.6865	-19.1252	6.1195
H	15.9743	-20.4645	7.2813
H	16.1006	-17.4021	7.9896
H	17.5763	-19.9264	9.0825
H	17.7722	-16.8311	9.7461
H	19.2875	-19.3209	10.8902
H	19.512	-17.5243	11.3933
H	7.1511	-21.0869	12.1253
H	6.634	-19.5314	11.3962
H	5.6021	-16.0823	15.5687
H	6.4621	-15.3774	14.1818
H	7.4238	-17.8882	15.7426
H	7.9449	-16.2121	16.0999
H	9.0714	-16.0192	13.9104
H	4.9313	-20.7299	13.2557
H	4.7333	-19.3861	12.1149
H	2.8379	-20.2886	14.3821
H	2.5269	-20.3161	12.6301
H	1.7498	-15.7447	14.3597
H	2.3313	-14.906	12.8912
H	3.6091	-16.0724	11.1499
H	2.4381	-17.4257	10.9284
H	4.1447	-17.7787	11.3069
H	-1.215	-18.9707	11.7183
H	-3.2663	-17.662	11.3818
H	-2.1495	-13.2834	13.8141
H	-1.3103	-12.5738	12.3958
H	-5.5364	-10.8053	14.6341
H	-8.0156	-12.1918	12.6028
H	-2.3847	-9.5058	12.7348
H	-3.2378	-8.7242	11.3313
H	-4.3582	-9.2757	14.1851

H	-6.5644	-9.543	12.6181
H	-5.3955	-9.6143	11.269
H	-2.1403	-7.3939	13.6719
H	-4.2704	-6.917	13.0776
H	8.5848	-17.7537	6.2683
H	9.6054	-17.0938	4.9579
H	9.98	-16.706	6.6767
H	9.7733	-18.671	15.3614
H	10.4159	-17.0452	15.7438
H	10.8322	-17.8271	14.1778
H	-5.8959	-12.8859	14.6929

Table 9: Coordinates (Ångstroms) for 1h

Atom	x	y	z
C	9.2338	-21.4684	7.8089
C	9.6758	-20.8381	9.1942
C	10.094	-19.3234	8.9369
O	11.1756	-19.2368	7.8957
C	10.6374	-18.7425	6.5768
C	9.4769	-19.6354	5.9163
C	8.5584	-20.4362	6.8757
C	7.206	-20.2874	6.8454
O	8.4319	-20.8058	10.0609
C	8.7434	-20.159	11.3842
C	9.2555	-18.6619	11.2012
C	10.525	-18.6116	10.2745
C	11.9356	-18.7523	5.6549
C	10.813	-21.7216	9.8365
C	12.5951	-20.1877	5.5434
O	11.4882	-18.2555	4.3118
C	13.0989	-20.7575	6.9267
C	14.1576	-19.9142	7.7373
O	12.7183	-21.9342	7.3298
C	15.5425	-19.8195	6.9637
C	16.5621	-18.9799	7.7622
C	16.8668	-17.6782	7.4671
C	17.8598	-16.8743	8.2474
C	18.167	-15.5749	7.9507
C	7.4122	-20.1301	12.2207
C	7.7138	-19.4206	13.6084
C	8.3078	-17.9529	13.3404
O	9.5898	-18.0862	12.5497
O	6.6231	-19.5113	14.6348
C	5.2776	-18.8897	14.3535
C	5.3153	-17.338	13.9409
C	6.1506	-16.411	14.9236
C	7.4409	-16.9982	15.6034
C	8.6799	-17.1592	14.6491
C	4.4634	-19.7808	13.3254
C	2.9004	-19.7794	13.4926
C	2.1376	-18.3944	13.532
C	2.907	-17.0802	13.0434
O	3.9524	-16.7103	14.0609
O	0.9244	-18.618	12.6603
C	-0.0926	-17.5292	12.8787
C	0.5164	-16.1027	12.4826
C	1.8909	-15.8742	13.2146
C	3.3869	-17.158	11.5457

C	-1.3083	-17.9456	12.0073
C	-2.4429	-17.2227	11.823
C	-2.7277	-15.8162	12.4126
C	-1.5601	-14.7573	12.1097
O	-0.357	-14.981	12.9797
O	-4.0079	-15.3795	11.7405
C	-4.4697	-14.0767	12.324
C	-3.4355	-12.9642	11.8607
C	-2.0245	-13.2922	12.4753
C	-5.9033	-13.8085	11.6955
O	-3.7789	-11.5595	12.3009
C	-6.1947	-12.217	11.4971
C	-5.236	-11.2741	12.4082
O	-5.6597	-11.4438	13.8619
O	-7.6018	-11.822	11.5838
C	-3.0532	-8.659	11.7469
C	-4.3593	-8.7983	12.6646
C	-5.4492	-9.7508	12.0254
O	-2.4857	-7.2813	12.0075
O	-4.9398	-7.4291	12.8044
C	10.1436	-17.2444	6.6953
H	9.1811	-18.82	8.5577
H	8.4382	-18.0706	10.7285
H	9.5233	-20.7346	11.9312
H	8.5293	-19.994	14.1029
H	7.5892	-17.3539	12.7425
H	5.6778	-17.2564	12.8949
H	1.8141	-18.1791	14.5765
H	4.7837	-18.9544	15.3491
H	0.6301	-16.0488	11.3764
H	-0.3826	-17.4834	13.9588
H	-4.5412	-14.1291	13.44
H	-2.8764	-15.8667	13.521
H	-1.2896	-14.8243	11.0305
H	-3.3898	-12.9899	10.7461
H	-5.8926	-14.259	10.6699
H	-5.8703	-11.986	10.4544
C	9.8719	-17.8436	15.407
O	-6.9945	-14.6301	12.3284
S	-7.5013	-14.1157	14.1512
O	-8.4211	-12.6549	13.952
O	-8.1623	-15.5145	14.8576
O	-5.8856	-13.6892	14.9865
H	8.5419	-22.303	8.0221
H	10.1436	-21.8737	7.3263
H	9.9505	-20.3564	5.2248

H	8.8612	-18.9507	5.3086
H	6.7186	-19.5635	6.1766
H	6.5569	-20.8935	7.4947
H	11.3784	-19.127	10.7463
H	10.8068	-17.561	10.0794
H	12.6692	-18.0493	6.116
H	11.6042	-21.9035	9.0798
H	10.3799	-22.6884	10.1462
H	11.2646	-21.2307	10.7115
H	11.8684	-20.9108	5.132
H	13.4534	-20.1386	4.8437
H	12.3358	-18.2385	3.7386
H	14.3092	-20.4178	8.7095
H	13.7557	-18.8999	7.9226
H	15.3871	-19.3755	5.9658
H	15.9329	-20.8474	6.8333
H	17.0524	-19.4764	8.615
H	16.3795	-17.1753	6.6177
H	18.3516	-17.3816	9.0933
H	17.6802	-15.0495	7.1148
H	18.9108	-15.0109	8.5344
H	7.0478	-21.1591	12.3904
H	6.6519	-19.5771	11.6471
H	5.4422	-16.1296	15.7251
H	6.4033	-15.4841	14.3785
H	7.2074	-17.9802	16.0479
H	7.727	-16.3087	16.4177
H	9.0053	-16.1567	14.3157
H	4.8048	-20.8222	13.4756
H	4.7244	-19.5031	12.2948
H	2.6359	-20.3388	14.4072
H	2.4746	-20.3371	12.6359
H	1.6947	-15.7417	14.2941
H	2.3604	-14.9496	12.8357
H	3.7352	-16.1665	11.2096
H	2.528	-17.4741	10.9262
H	4.1957	-17.89	11.4091
H	-1.2059	-18.9439	11.5475
H	-3.2648	-17.6221	11.204
H	-2.0792	-13.2124	13.5768
H	-1.2734	-12.5677	12.1113
H	-5.3518	-10.6173	14.3759
H	-7.9604	-12.114	12.5409
H	-2.309	-9.4661	11.9381
H	-3.341	-8.6559	10.6729
H	-4.0496	-9.1895	13.6646

H	-6.4596	-9.443	12.3652
H	-5.4317	-9.6592	10.9239
H	-1.8739	-7.3779	12.8238
H	-4.1062	-6.8552	12.5496
H	9.1963	-17.1837	7.2553
H	9.9902	-16.8523	5.6734
H	10.9102	-16.6344	7.2031
H	9.5579	-18.8159	15.8204
H	10.2074	-17.2	16.2374
H	10.7102	-18.0021	14.7066
H	-5.7431	-12.6753	14.567

Stable mammalian cell line expressing hNa_v.

HEK-293 cell line stably transfected with $hNa_v1.3$ and 1.6 were kindly provided by Dr Andrew Powell (GlaxoSmithKline R&D, Stevenage, UK). Cells were cultured in DMEM/F12 medium supplemented withGlutamax, MEM NEAA (1% w/v) and 10% of fetal bovine serum. A final concentration of G418 0.4 mg/ml was added to the culture medium. Cells were incubated in a humidified 5% CO_2/95% air atmosphere at 37ºC until reach a 80 % of confluence. Then, cells are incubated at 30ºC for 24-48 hours before electrophysiological measurements. Medium was replaced every 2-3 days and split once a week.

Neuroblastoma cell line

Neuroblastoma cell line SH-SY5Y was from American Type Culture Collection (ATCC) Number CRL-2266. The cells were plated in 25-cm^2 flasks. The cultures were maintained in EMEM and F12 Medium. This medium was enriched with 10% fetal bovine serum (FBS), 100 UI/ml penicillin and 100μg/ml streptomycin.

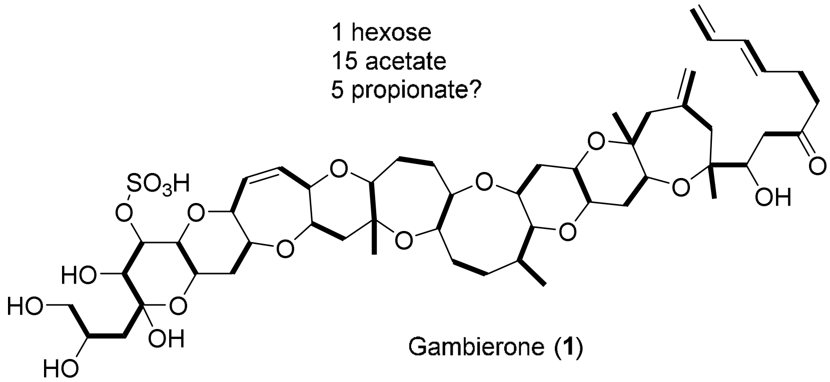
Automated Patch clamp electrophysiological recordings

HEK-293 cells were recorded in whole-cell patch clamp configuration using an IonFlux 16 system (Fluxion, California, USA) and the corresponding Ionflux 16 software for cell capture, seal formation, whole cell obtaining, data acquisition and analysis. After cell incubation at 30 ºC for 24-48 hours, cells were washed twice with Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) and harvested with 5 ml of Detachin™ solution. Then, cells were re-suspended in extracellular solution containing (mM): 2 $CaCl_2$, 1 $MgCl_2$, 100 Hepes, 4 KCl, 145 NaCl, 10 TEA-Cl and 10 Glucose. pH7.4 and 320 mOsm. Electrophysiological recordings were carried out at room temperature (±22ºC) in a 96-well IonFlux microfluidic plate. The intracellular solution composition for I_{Na}recordings was (in mM): 100 CsF, 45 CsCl, 10 Hepes, 5 NaCl, 5 EGTA corrected to pH7.1 using CsOH. The contaminating effects of resistance and capacitance currents were compensated electronically by the software. Leak resistance is measured by introducing a short 20 mV pulse at the beginning of each sweep and measuring the current difference [Spencer, 2012 #3]. The holding potential (V_h) was set at -90 mV and a sampling frequency of 10 kHz was used.

For voltage-dependent activation studies in hNa_v channels, Na^+ currents (I_{Na}) were activated by a voltage-step protocol that depolarized cells from the complete inactivation, at -120 mV, to different voltages in 10 mV steps from -100 mV to 20 mV. The peak amplitude of I_{Na} was determined for each step and then the current-voltage relationship (I-V curve) was done.

Cytosolic Ca^{2+} measurements

Neuroblastoma cells were seeded onto 18-mm glass coverslips. Cells were washed twice with saline solution plus 0.1% BSA, final pH 7.2-7.4 (equilibrated with CO_2). Cells attached to coverslips were loaded with the Fura-2 AM (0.5 μM) for 10 min at 37 ºC in saline solution plus 0,1% BSA. Loaded cells were washed twice with saline solution and coverslips were placed in a thermostatic chamber. Cells were viewed using a Nikon Diphot 200 microscope equipped with epifluorescent optics. Cytosolic Ca^{2+} levels were measured as Fura-2 ratio from the images collected by fluorescent (Lambda-DG4). The Light source was a xenon lamp and the wavelengths used were 340 and 380 nm for excitation and 510 nm for emission.



Gambierone (**1**)

Biosynthetic hypothesis for gambierone (**1**)