Supporting Information

Flavipesides A-C, PKS-NRPS Hybrids as Pancreatic Lipase Inhibitors from a Marine Sponge Symbiotic Fungus *Aspergillus flavipes* 164013

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Contents

Figure S47. The ^{13}C NMR spectrum of flavipeside C (**3**) in DMSO-d_6

Figure S48. The ^{13}C NMR spectrum of flavipeside C (**3**) in DMSO-d_6

Figure S49. The DEPT135 NMR spectrum of flavipeside C (**3**) in DMSO-d_6

Figure S50. The ^{1}H-^{1}H COSY spectrum of flavipeside C (**3**) in DMSO-d_6

Figure S51. The HSQC spectrum of flavipeside C (**3**) in DMSO-d_6

Figure S52. The HMBC spectrum of flavipeside C (**3**) in DMSO-d_6

Figure S53. The ROESY spectrum of flavipeside C (**3**) in DMSO-d_6

Figure S54. The HRESIMS spectrum of flavipeside C (**3**)

Figure S55. The UV spectrum of flavipeside C (**3**) in MeOH

Figure S56. The CD spectrum of flavipeside C (**3**) in MeCN

Experimental Section

General Experimental Procedures

Optical rotations were measured on an Autopol VI (No. 91003, Rudolph Research Analytical, Hackettstown, NJ, USA). High-resolution ESIMS spectra were obtained on a Waters Xevo G2-SQTOF spectrometer. ECD spectra were collected on a Jasco J-810 spectropolarimeter. ID and 2D NMR spectra were recorded on Bruker AV 600 MHz spectrometer, operating at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR in DMSO-d_6 with calibration at δ_H 2.50 and δ_c 39.5, respectively. Semi-preparative HPLC was performed with a Waters 2535 pump and a 2998 photodiode array detector, equipped with a reversed-phase YMC-Pack Pro C18 RS column (250 × 10 mm, 5μm). All the semi-preparative HPLC experiments used 2 mL/min flow rate and fraction collection based on detected peak. Analytical TLC was performed with silica gel 60 (Merck) plates and visualized with UV lamp (254 and 365 nm) and anisaldehyde/sulfuric acid reagent. All organic solvents and chemicals were of analytical and HPLC grade. Water was purified by PALL Life Science water purification system.

Fungal Material, Fermentation, and Isolation

The fungal strain *Aspergillus flavipes* 164013 was firstly isolated from the marine sponge *Dysidea* sp. XD130608 and latterly isolated from the cyanobacterium *Lyngbya majuscula* collected in South China Sea. 164013 was cultured in potato dextrose agar plate and supplemented with 3% sea salt at 28 °C for three days, and then was transferred into a 2 L Erlenmeyer flask containing solid rice medium (300 g of rice, 0.2% yeast extract, 0.2% bacterial peptone, and 3% sea salt). The flasks were cultured statically at room temperature for 28 days. A total of 15.0 kg harvested medium and mycelium were extracted with 5 L MeOH for five times to afford an extract following solvent evaporation. The extract was suspended in water and successively partitioned by the same volume of petroleum ether and EtOAc. The EtOAc part was divided into 11 fractions (A-K) by silica gel CC using a gradient elution of petroleum ether/EtOAc (10:1, 5:1, 3:1, 1:1, v/v) and CH_2Cl_2/MeOH (10:1, 5:1, 0:1, v/v). All the 11 fractions were analyzed by LC-DAD/MS, and the tenth fraction J

(5.4 g) were found to containing interesting metabolites with molecular weights more than 750 Da and UV absorptions at 205, 240, and 370 nm. Therefore, the fraction J was separated into four fractions J1-J4 by Sephadex LH-20 using CH_2Cl_2/MeOH (1:1, v/v). Subsequent LC-DAD/MS chemical profile analysis showed that the fraction J1 contained the target metabolites. J1 was subjected to an ODS-MPLC column eluted by gradient CH_3CN/H_2O (50:50, 60:40, 70:30, 80:20, 90:10, 100:0) to afford 10 fractions, J1A-J1K. The first fraction J1A was purified by semi-preparative HPLC to afford flavipeside A (**1**) (t_R = 24.6 min; 23 mg) eluted by 65% MeOH/H_2O with an isocratic 0.01% trifluoroacetic acid (TFA) modifier. While semi-preparative HPLC separation of the second fraction J1B afforded flavipeside B (**2**) (t_R = 26.1 min; 5.6 mg) and flavipeside C (**3**) (t_R = 23.5 min; 3.6 mg) and using 60% MeOH/H_2O as eluent with anisocratic 0.01% TFA modifier.

Flavipeside A (**1**): yellowish neddles; $[\alpha]_D^{23}$ -11.5 (*c* 0.2, MeOH); UV (MeOH): λ_{max} (log ε) 207 (4.19), 245 (4.13), 370 (3.95) nm; CD (MeCN): λ ($\Delta\varepsilon$) 194 (-6.8), 218 (+7.31), 247 (-1.92), 273 (+0.54) nm; ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) in DMSO-d_6 see Table S1 and S2; ESI-MS: *m*/*z* 814.3 $[M]^+$/*m*/*z* 816.3 $[M + 2]^+$/*m*/*z* 818.3 $[M + 4]^+$ (9:6:1); HR-ESI-MS: *m*/*z* 814.2419 $[M + H]^+$ (calcd for $C_{42}H_{42}O_8N_5Cl_2$, 814.2405).

Flavipeside B (**2**): yellowish powders; $[\alpha]_D^{23}$ -14.7 (*c* 0.1, MeOH); UV (MeOH): λ_{max} (log ε) 202 (4.29), 242 (4.05), 312 (3.45), 364 (3.69) nm; CD (MeCN): λ ($\Delta\varepsilon$) 193 (-13.6), 220 (+8.23) nm; ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) in DMSO-d_6 see Table S1 and S3; ESI-MS: *m*/*z* 780.3 $[M]^+$/*m*/*z* 782.3 $[M + 2]^+$ (3:1); HR-ESI-MS: *m*/*z* 780.2818 $[M + H]^+$ (calcd for $C_{42}H_{43}O_8N_5Cl$, 780.2800).

Flavipeside C (**3**): yellowish powders; $[\alpha]_D^{23}$ -15.6 (*c* 0.6, MeOH); UV (MeOH): λ_{max} (log ε) 202 (4.23), 240 (3.99), 369 (3.79) nm; CD (MeCN): λ ($\Delta\varepsilon$) 194 (-11.2), 218 (+6.72) nm; ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) in DMSO-d_6 see Table S1 and S4; ESI-MS: *m*/*z* 780.3 $[M]^+$/*m*/*z* 782.3 $[M + 2]^+$ (3:1); HR-ESI-MS: *m*/*z* 780.2795 $[M + H]^+$ (calcd for $C_{42}H_{43}O_8N_5Cl$, 780.2800).

X-ray crystallographic analysis data of flavipeside A (1)

The data of flavipeside A (**1**) was collected on a Bruker D8 Venture diffractometer using Cu Kα radiation (λ = 1.54178 Å) at 297.14 K in the ω/2θ scan mode. Using Olex2, the structure was solved with the ShelXD structure solution program using Dual Space and refined with the ShelXL refinement package using Least Squares minimization. Crystal Data: $2(C_{42}H_{41}Cl_2N_5O_8)NO_3$, M = 1691.40, triclinic, space group P1, a = 9.5610(12) Å, b = 12.0187(16) Å, c = 20.258(3) Å, α = 80.118(7) °, β = 81.340(7) °, γ = 76.779(7) °, V = 2217.5(5) Å^3, Z = 1, T = 297.14 K, Dx = 1.267 mg/m^3, F(000) = 883.0, μ(CuKα) = 1.815 mm^{-1}, *Dcalc* = 1.267 g/cm^3, 23776 reflections measured (4.458° ≤ 2Θ ≤ 133.28 °), 13934 unique (R_{int} = 0.1008, Rsigma = 0.1141) which were used in all calculations. The final $R1$ was 0.0986 (I > 2σ(I)) and wR_2 was 0.2972 (all data). Flack parameter was 0.14(2). Crystallographic Data for **1** has been deposited at the Cambridge Crystallographic Data Center as supplementary publication (CCDD 1976121). Copies of the data can be obtained free of charge by application to the CCDD, 12 Union Road, Cambridge CB21EZ, UK. Tel: (+44) 1223-336-408; Fax: (+44) 1223-336-033; Email: deposit@cccdd.cam.ac.uk.

Enzyme inhibition assays

The inhibitory effects against pancreatic lipase (PL, Sigma type II) were performed as described previously.[1] 4-Methylumbelliferyl oleate (4-MUO) was used as substrate. Briefly, the incubation mixture with a total volume of 200 μL was consisted of lipase solution (10 μg/mL, final concentration), 0.1 M citrate phosphate buffer (0.1 M citrate-Na_2HPO4, pH 7.4), and each test compounds, using kaempferol as the positive control. After pre-incubation at 37 ℃ for 10 min, the reaction was started by the addition of 4-methylumbelliferyl oleate (2.5 μM, final concentration), with the final concentration of DMSO at 1% (v/v, without loss of the catalytic activity). All incubations with or without inhibitor were performed and 4-MU (the hydrolytic metabolite of 4-MUO) was further analyzed by a multi-Mode microplate reader (Molecular Devices SpectraMax iD3, USA). The florescence signals of the hydrolytic metabolite (4-MU) were real-time monitored under physiological conditions (pH 7.4 at 37 ℃) within 30 min incubation, with an interval of 60 s.

The excitation wavelength of the hydrolytic metabolite (4-MU) was set at 340 nm, while the emission wavelength was 460 nm. The PMT gain value was set at 100 V and integration time was set 10 ms. The residual activities of lipase were calculated with the following formula:

The residual activity (%) = (the florescence intensity in the presence of inhibitor)/the florescence intensity in negative control (DMSO only) × 100%.

The inhibitory activity against pancreatic lipase of flavipesides A–C (**1–3**) was shown (Figure S1), using kaempferol as the positive control with IC_{50} value of 1.50 ± 0.21 μM.

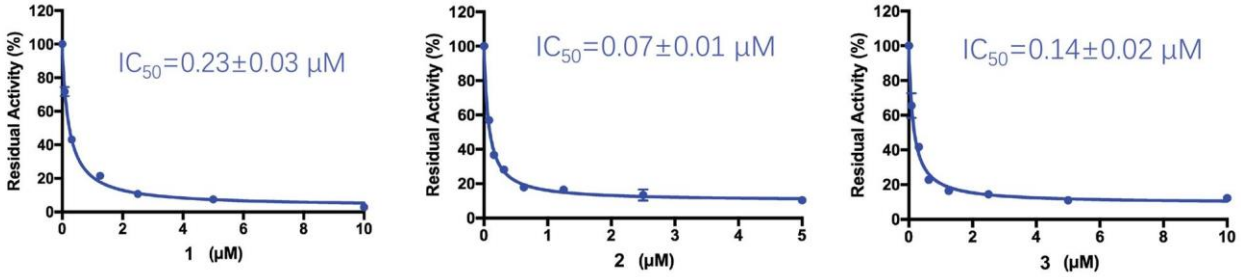

Figure S1. The inhibitory activities of flavipesides A–C (**1–3**) and their IC_{50} values.

Cytotoxicity Bioassay

Cytotoxicity of flavipesides A–C (**1–3**) against the six human cancer cell lines and three normal human cell lines were measured using CCK8 assay as described previously.[2] Six human cancer cell lines include non-small-cell lung cancer PC-9, cervical cancer HeLa, melanoma A375, hepatoma HepG2, colon cancer SW480 and SW620 cell lines, while three normal human cell lines include bronchial eptithelial 16HBE, colon mucosal epithelial NCM460, and keratinocyte HaCaT cell lines. The cell lines were cultured in RPMI-1640 or DMEM medium (Hyclone), and supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37 ℃. Test compounds were solubilized in medium at eight different concentrations. The cells were seeded into 96-well microplates at a density of 4000 cells per well and allowed to grow undisturbed for 24 h before the treatment of various concentrations of test compounds. After 72 h of incubation with the test substances, 10 μL per well of CCK-8 solution was added and the cells were incubated at 37 ℃ for 30 min. The cell viability was assayed by reading the absorbance at 450 nm using a multifunctional microplate

reader (Thermo Scientific, Waltham, USA). IC_{50} values were derived from the mean OD values of the quadruplicate tests versus drug concentration curves.

References

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2. Li, J.; Yang, F.; Wang, Z.; Wu, W.; Liu, L.; Wang, S. P.; Zhao, B. X.; Jiao, W. H.; Xu, S. H.; Lin, H. W. Unusual anti-inflammatory meroterpenoids from the marine sponge *Dactylospongia* sp. *Org. Biomol. Chem.* **2018**, *16*, 6773−6782.

Table S1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for flavipesides A–C (**1–3**) in DMSO-d_6.[a]

	1		**2**		**3**	
Position	δ_C, mult	δ_H (J in Hz)	δ_C, mult	δ_H (J in Hz)	δ_C, mult	δ_H (J in Hz)
1	154.8, C		155.7, C		158.4, C	
2	113.6, C		114.3, C		112.9, CH	6.91, s
3	140.4, C		146.4, C		146.5, C	
4	109.8, C		109.1, CH	7.20, s	110.0, C	
5	148.6, C		153.2, C		150.5, C	
6	158.0, C		157.4, C		157.1, C	
7	103.6, CH	6.20, s	104.4, CH	7.23, s	104.4, CH	7.26, s
8	173.0, C		160.8, C		160.8, C	
9	126.3, C		120.0, C		120.0, C	
10	129.9, C		132.1, C		132.1, C	
11	98.5, C		108.1, C		108.2, C	
12	175.5, C		178.8, C		178.7, C	
13	106.2, C		106.6, C		107.0, C	
14	18.2, CH_3	2.53, s	21.0, CH_3	2.48, s	20.7, CH_3	2.46, s
15	166.9, C		165.1, C		165.0, C	
16	52.0, CH_3	3.64, s	53.1, CH_3	3.75, s	53.1, CH_3	3.73, s
17	137.9, CH	7.35, d (1.2)	138.0, CH	7.04, s	138.0, CH	8.90, s
18	118.0, CH	6.67, d (1.2)	120.1, CH	7.38, s	120.2, CH	7.43, s
19	137.2, C		134.1, C		134.1, C	
20	27.7, CH_2	2.49, m	25.6, CH_2	2.68, m	25.4, CH_2	2.72, t (8.0)
21a	38.6, CH_2	3.25, ddd (16.2, 7.8, 7.8)	37.6, CH_2	3.22, m	37.5, CH_2	3.26, m
21b		3.08, d (16.2)				
22	169.0, C		169.5, C		169.6, C	
23	53.5, CH	4.64, ddd (11.4, 10.8, 6.0)	53.5, CH	4.63, m	53.5, CH	4.65, m
24a	38.0, CH_2	3.00, d (18.0, 6.0)	38.1, CH_2	2.93, dd (16.8, 6.6)	38.1, CH_2	2.94, dd (16.2, 6.0)
24b		2.65, m		2.65, m		2.67, dd (16.2, 12.6)
25	137.2, C		137.1, C		137.1, C	

26	129.4, CH	7.18, d (8.4)	129.4, CH	7.16, d (7.2)	129.4, CH	7.16, d (7.2)
27	128.0, CH	7.23, t (8.4)	128.5, CH	7.24, t (7.2)	128.5, CH	7.22, t (7.2)
28	126.5, CH	7.23, t (8.4)	126.6, CH	7.15, t (7.2)	126.6, CH	7.15, t (7.2)
29	128.0, CH	7.23, t (8.4)	128.5, CH	7.24, t (7.2)	128.5, CH	7.22, t (7.2)
30	129.4, CH	7.18, d (8.4)	129.4, CH	7.16, d (7.2)	129.4, CH	7.16, d (7.2)
31	164.3, C		164.2, C		164.2, C	
32	74.2, CH	4.17, dd (11.4, 3.0)	74.4, CH	4.09, dd (13.8, 3.6)	74.3, CH	4.10, dd (13.8, 3.6)
33a	31.7, CH_2	3.31, dd (16.2, 3.0)	31.8, CH_2	3.32, dd (14.4, 3.6)	31.8, CH_2	3.30, m
33b		3.03, d (16.2)		3.02, t (14.4)		3.01, t (14.4)
34	134.0, C		134.1, C		134.1, C	
35	129.1, CH	7.12, d (7.2)	129.3, CH	7.13, d (7.2)	129.3, CH	7.13, d (7.2)
36	128.6, CH	7.27, t (7.2)	128.1, CH	7.23, t (7.2)	128.1, CH	7.18, t (7.2)
37	127.2, CH	7.27, t (7.2)	127.2, CH	7.22, t (7.2)	127.2, CH	7.22, t (7.2)
38	128.6, CH	7.27, t (7.2)	128.1, CH	7.23, t (7.2)	128.1, CH	7.18, t (7.2)
39	129.1, CH	7.12, d (7.2)	129.3, CH	7.13, d (7.2)	129.3, CH	7.13, d (7.2)
40	51.5, CH_3	2.91, s	51.5, CH_3	2.89, s	51.5, CH_3	2.91, s
41	51.5, CH_3	2.91, s	51.5, CH_3	2.89, s	51.5, CH_3	2.91, s
42	51.5, CH_3	2.91, s	51.5, CH_3	2.89, s	51.5, CH_3	2.91, s
1-OH		14.25, s		12.76, s		12.06, s
21-NH		7.43, m		8.15, t (6.6)		8.13, t (6.6)
23-NH		8.74, d (10.8)		8.72, d (10.8)		8.75, d (10.8)

[a]600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR. [b]The number of attached protons was determined by analysis of DEPT135 and 2D NMR spectroscopic data.

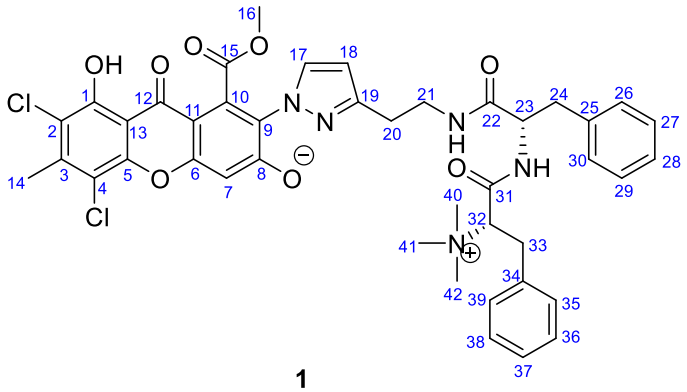

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Table S2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for flavipeside A (**1**) in DMSO-d_6.[a]

Position	δ_C, mult	δ_H (J in Hz)	COSY	HMBC (H→C)	NOESY
1	154.8, C				
2	113.6, C				
3	140.4, C				
4	109.8, C				
5	148.6, C				
6	158.0, C				
7	103.6, CH	6.20, s		C-6, 8, 9, 11, 12	40, 41, 42
8	173.0, C				
9	126.3, C				
10	129.9, C				
11	98.5, C				
12	175.5, C				
13	106.2, C				
14	18.2, CH_3	2.53, s		C-2, 3, 4	
15	166.9, C				
16	52.0, CH_3	3.64, s		C-15	17
17	137.9, CH	7.35, d (1.2)	18	C-18, 19	16
18	118.0, CH	6.67, d (1.2)	17	C-17, 19	20
19	137.2, C				
20	27.7, CH_2	2.49, m		C-17, 18, 21	18, 21-NH
21a	38.6, CH_2	3.25, ddd (16.2, 7.8, 7.8)	21-NH, 21b	C-19, 20, 22	
21b		3.08, d (16.2)	21-NH, 21a	C-19, 20, 22	
22	169.0, C				
23	53.5, CH	4.64, ddd (11.4, 10.8, 6.0)	23-NH, 24	C-22, 25, 31	21-NH
24a	38.0, CH_2	3.00, d (18.0, 6.0)	23, 24b	C-22, 25	23-NH
24b		2.65, m	23, 24a	C-23, 26, 30	
25	137.2, C				
26	129.4, CH	7.18, d (8.4)	27	C-24, 28, 20	23

27	128.0, CH	7.23, t (8.4)	26, 28	C-27, 28, 29	
28	126.5, CH	7.23, t (8.4)	27, 29	C-26, 30	
29	128.0, CH	7.23, t (8.4)	28, 30	C-25, 27	
30	129.4, CH	7.18, d (8.4)	29	C-24, 28	23
31	164.3, C				
32	74.2, CH	4.17, dd (11.4, 3.0)	33a, 33b		23-NH, 32 35, 40
33a	31.7, CH_2	3.31, dd (16.2, 3.0)	32	C-31, 32, 34, 35, 39	
33b		3.03, d (16.2)	32		
34	134.0, C				
35	129.1, CH	7.12, d (7.2)	36	C-33	32
36	128.6, CH	7.27, t (7.2)	35, 37	C-34, 38	
37	127.2, CH	7.27, t (7.2)	36, 38	C-35, 39	
38	128.6, CH	7.27, t (7.2)	37, 39	C-34, 36	
39	129.1, CH	7.12, d (7.2)	38	C-33, 35	32
40	51.5, CH_3	2.91, s		C-32, 41, 42	7, 32
41	51.5, CH_3	2.91, s		C-32, 40, 42	7, 32
42	51.5, CH_3	2.91, s		C-32, 40, 41	7, 32
1-OH		14.25, s		C-1, 2, 13	
21-NH		7.43, m	21		20, 23, 23-NH
23-NH		8.74, d (10.8)	23	C-23, 31	21-NH, 24, 32

[a]600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR. [b]The number of attached protons was determined by analysis of DEPT135 and 2D NMR spectroscopic data.

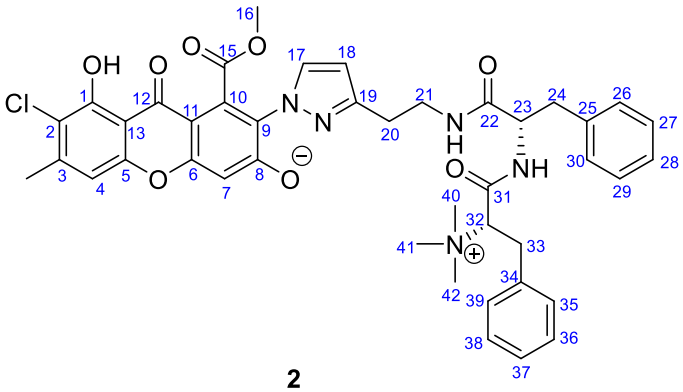

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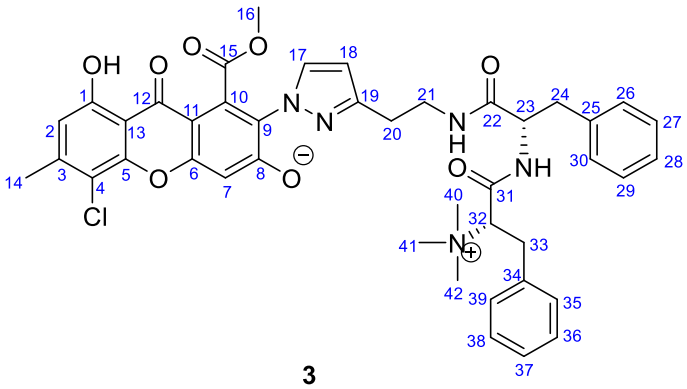
Table S3. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for flavipeside B (**2**) in DMSO-d_6.[a]

Position	δ_C, mult	δ_H (J in Hz)	COSY	HMBC (H→C)	NOESY
1	155.7, C				
2	114.3, C				
3	146.4, C				
4	109.1, CH	7.20, s		C-2, 5, 13, 14	14
5	153.2, C				
6	157.4, C				
7	104.4, CH	7.23, s		C-6, 8, 9, 11	40, 41, 42
8	160.8, C				
9	120.0, C				
10	132.1, C				
11	108.1, C				
12	178.8, C				
13	106.6, C				
14	21.0, CH_3	2.48, s		C-2, 3, 4	4
15	165.1, C				
16	53.1, CH_3	3.75, s		C-15	17
17	138.0, CH	7.04, s			
18	120.1, CH	7.38, s	17		20
19	134.1, C				
20	25.6, CH_2	2.68, m	21	C-18, 19, 21	18, 21-NH
21	37.6, CH_2	3.22, m	20, 21-NH	C-22	
22	169.5, C				
23	53.5, CH	4.63, m	23-NH, 24	C-22, 24, 25, 31	21-NH
24a	38.1, CH_2	2.93, dd (16.8, 6.6)	23, 24b		23-NH
24b		2.65, m	23, 24a	C-22, 23, 25, 26	
25	137.1, C				
26	129.4, CH	7.16, d (7.2)	27	C-24, 28, 20	23
27	128.5, CH	7.24, t (7.2)	26, 28	C-27, 28, 29	

28	126.6, CH	7.15, t (7.2)	27, 29	C-26, 30	
29	128.5, CH	7.24, t (7.2)	28, 30	C-25, 27	
30	129.4, CH	7.16, d (7.2)	29	C-24, 28	23
31	164.2, C				
32	74.4, CH	4.09, dd (13.8, 3.6)	33	C-31, 33, 34, 40, 41, 42	23-NH, 32 35, 40
33a	31.8, CH_2	3.32, dd (14.4, 3.6)	32	C-31, 32, 34, 35, 39	
33b		3.02, t (14.4)	32		
34	134.1, C				
35	129.3, CH	7.13, d (7.2)	36	C-33, 37	32
36	128.1, CH	7.23, t (7.2)	35, 37	C-34, 38	
37	127.2, CH	7.22, t (7.2)	36, 38	C-35, 39	
38	128.1, CH	7.23, t (7.2)	37, 39	C-34, 36	
39	129.3, CH	7.13, d (7.2)	38	C-33, 35	32
40	51.5, CH_3	2.89, s		C-32, 41, 42	7, 32
41	51.5, CH_3	2.89, s		C-32, 40, 42	7, 32
42	51.5, CH_3	2.89, s		C-32, 40, 41	7, 32
1-OH		12.76, s		C-1, 2, 13	
21-NH		8.15, t (6.6)	21	C-21, 22	20, 23, 23-NH
23-NH		8.72, d (10.8)	23	C-23, 31	21-NH, 24, 32

[a]600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR. [b]The number of attached protons was determined by analysis of DEPT135 and 2D NMR spectroscopic data.



3

Table S4. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for flavipeside C (**3**) in DMSO-d_6.[a]

Position	δ_C, mult	δ_H (J in Hz)	COSY	HMBC (H→C)	NOESY
1	158.4, C				
2	112.9, CH	6.91, s	14	C-1, 4, 13, 14	14
3	146.5, C				
4	110.0, C				
5	150.5, C				
6	157.1, C				
7	104.4, CH	7.26, s		C-6, 8, 9, 11	40, 41, 42
8	160.8, C				
9	120.0, C				
10	132.1, C				
11	108.2, C				
12	178.7, C				
13	107.0, C				
14	20.7, CH_3	2.46, s	2	C-2, 3, 4	2
15	165.0, C				
16	53.1, CH_3	3.73, s		C-15	17
17	138.0, CH	8.90, brs			
18	120.2, CH	7.43, s			
19	134.1, C				
20	25.4, CH_2	2.72, t (8.0)	21	C-18, 19, 21	18, 21-NH
21	37.5, CH_2	3.26, m	20, 21-NH	C-19, 20, 22	
22	169.6, C				
23	53.5, CH	4.65, m	23-NH, 24	C-22, 24, 31	21-NH
24a	38.1, CH_2	2.94, dd (16.2, 6.0)	23	C-22, 25	23-NH
24b		2.67, dd (16.2, 12.6)	23	C-23, 26, 30	
25	137.1, C				
26	129.4, CH	7.16, d (7.2)	27	C-24, 28, 20	23

27	128.5, CH	7.22, t (7.2)	26, 28	C-27, 28, 29	
28	126.6, CH	7.15, t (7.2)	27, 29	C-26, 30	
29	128.5, CH	7.22, t (7.2)	28, 30	C-25, 27	
30	129.4, CH	7.16, d (7.2)	29	C-24, 28	23
31	164.2, C				
32	74.3, CH	4.10, dd (13.8, 3.6)	33		23-NH, 32 35, 40
33a	31.8, CH_2	3.30, m	32	C-31, 32, 34, 35, 39	
33b		3.01, t (14.4)	32		
34	134.1, C				
35	129.3, CH	7.13, d (7.2)	36	C-33	32
36	128.1, CH	7.18, t (7.2)	35, 37	C-34, 38	
37	127.2, CH	7.22, t (7.2)	36, 38	C-35, 39	
38	128.1, CH	7.18, t (7.2)	37, 39	C-34, 36	
39	129.3, CH	7.13, d (7.2)	38, 40	C-33, 35	32
40	51.5, CH_3	2.91, s	39	C-32, 41, 42	7, 32
41	51.5, CH_3	2.91, s		C-32, 40, 42	7, 32
42	51.5, CH_3	2.91, s		C-32, 40, 41	7, 32
1-OH		12.06, s		C-1, 2, 13	
21-NH		8.13, t (6.6)	21	C-21, 22	20, 23, 23-NH
23-NH		8.75, d (10.8)	23	C-23, 24, 31	21-NH, 24, 32

[a]600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR. [b]The number of attached protons was determined by analysis of DEPT135 and 2D NMR spectroscopic data.

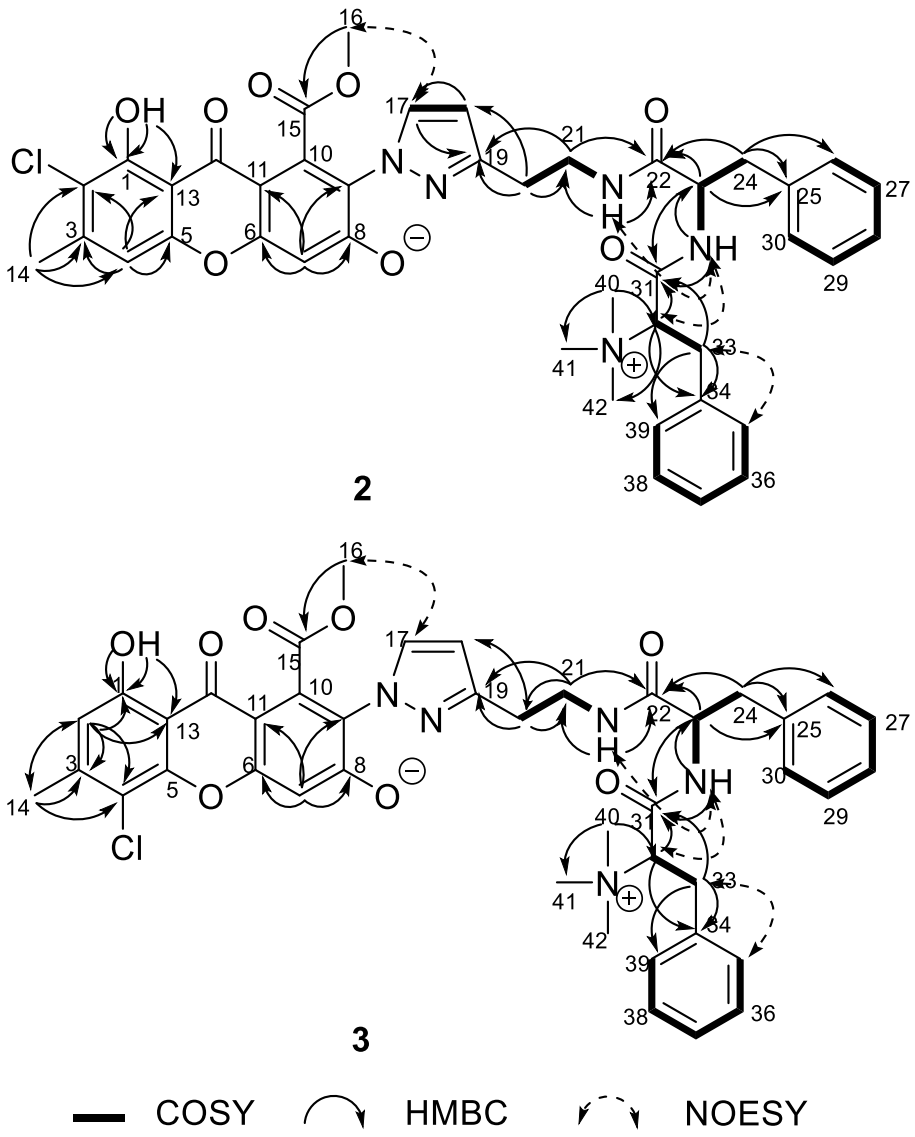


Figure S2. Key 2D NMR correlations for flavipesides B (**2**) and C (**3**)

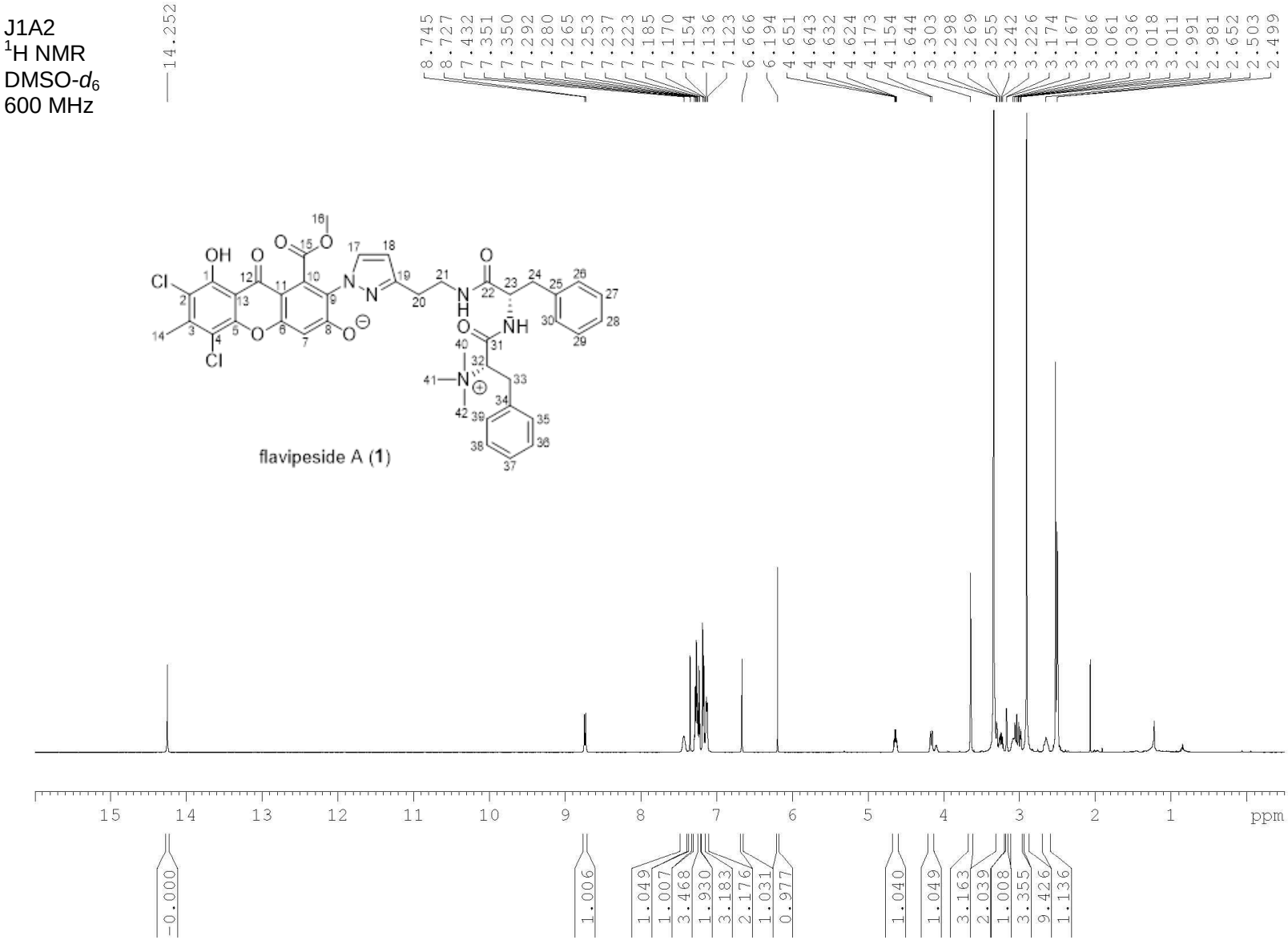


Figure S3. The ^{1}H NMR (600 MHz) spectrum of flavipeside A (**1**) in DMSO-d_6.

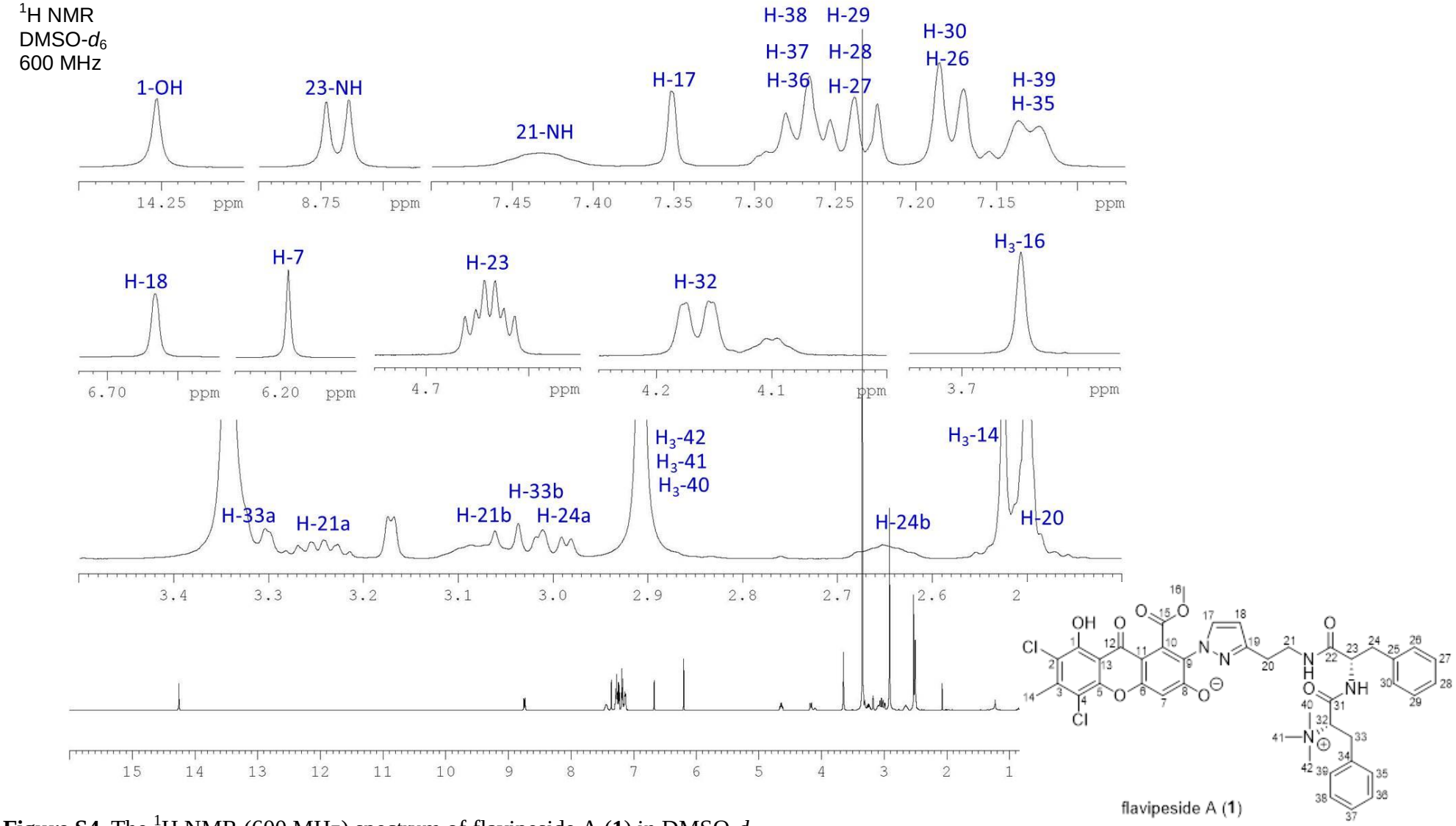


Figure S4. The ^{1}H NMR (600 MHz) spectrum of flavipeside A (**1**) in DMSO-d_6.

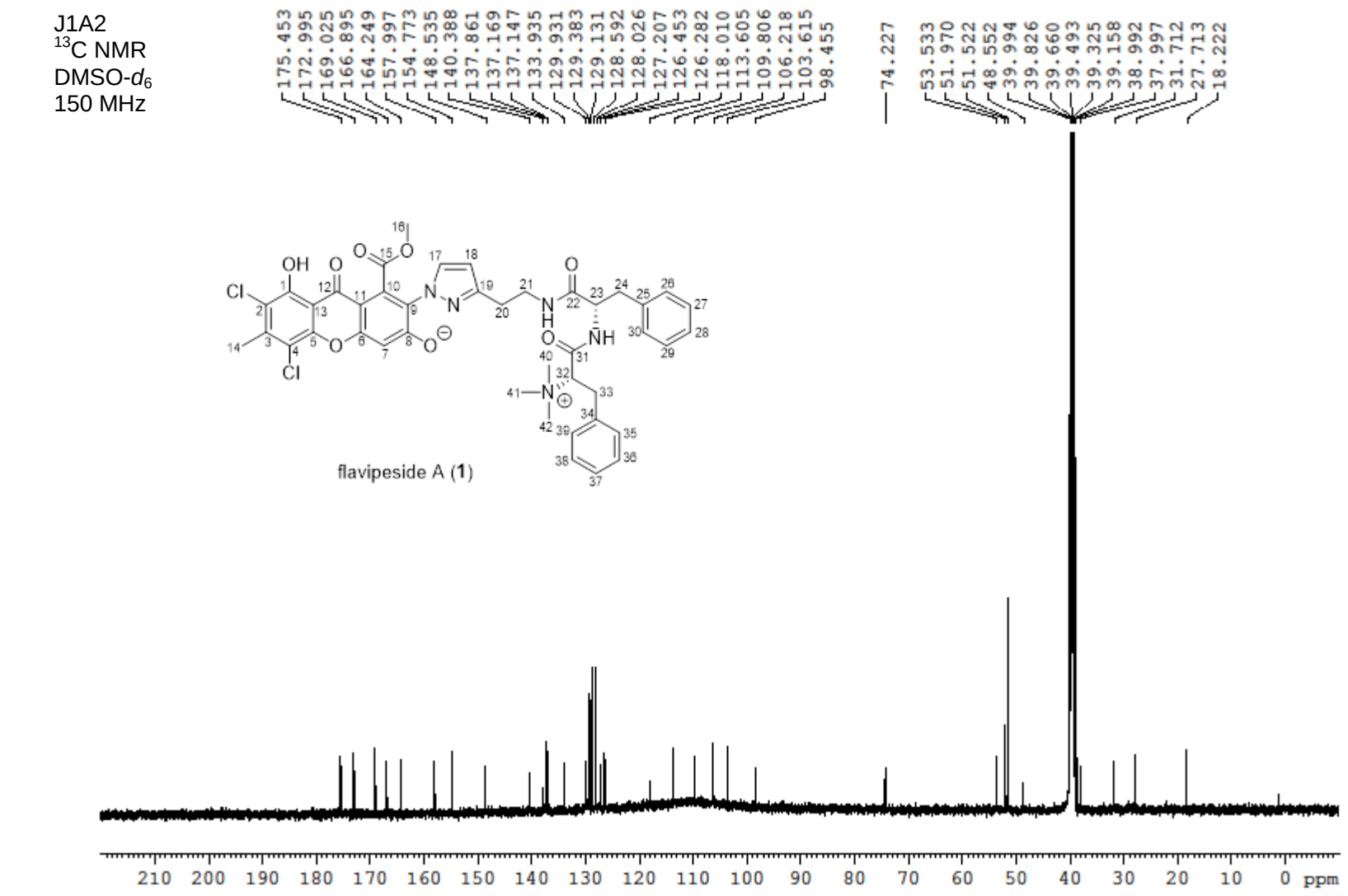


Figure S5. The ^{13}C NMR (150 MHz) spectrum of flavipeside A (**1**) in DMSO-d_6.

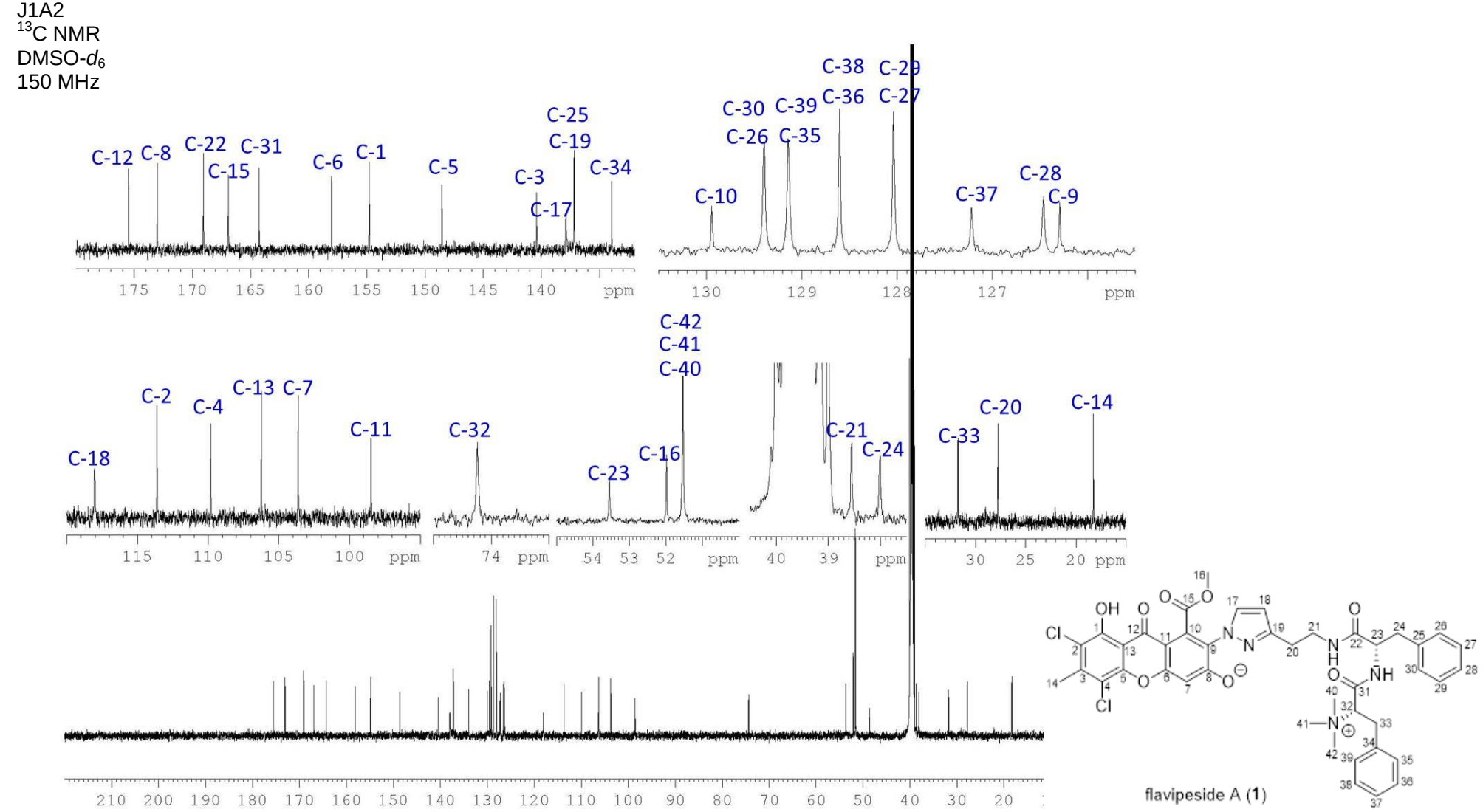


Figure S6. The ^{13}C NMR spectrum of flavipeside A (**1**) in DMSO-d_6.

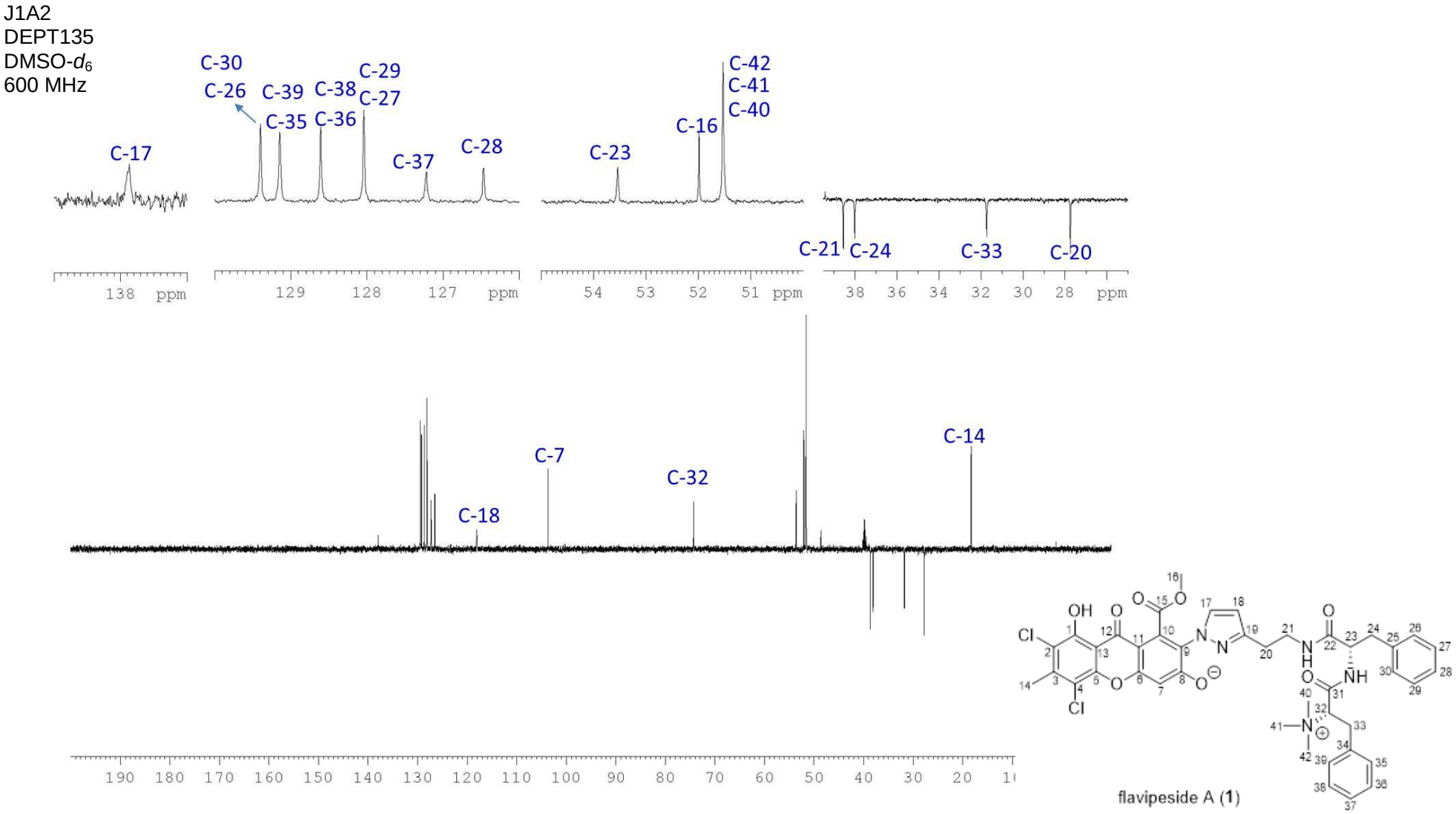


Figure S7. The DEPT135 NMR spectrum of flavipeside A (**1**) in DMSO-d_6.

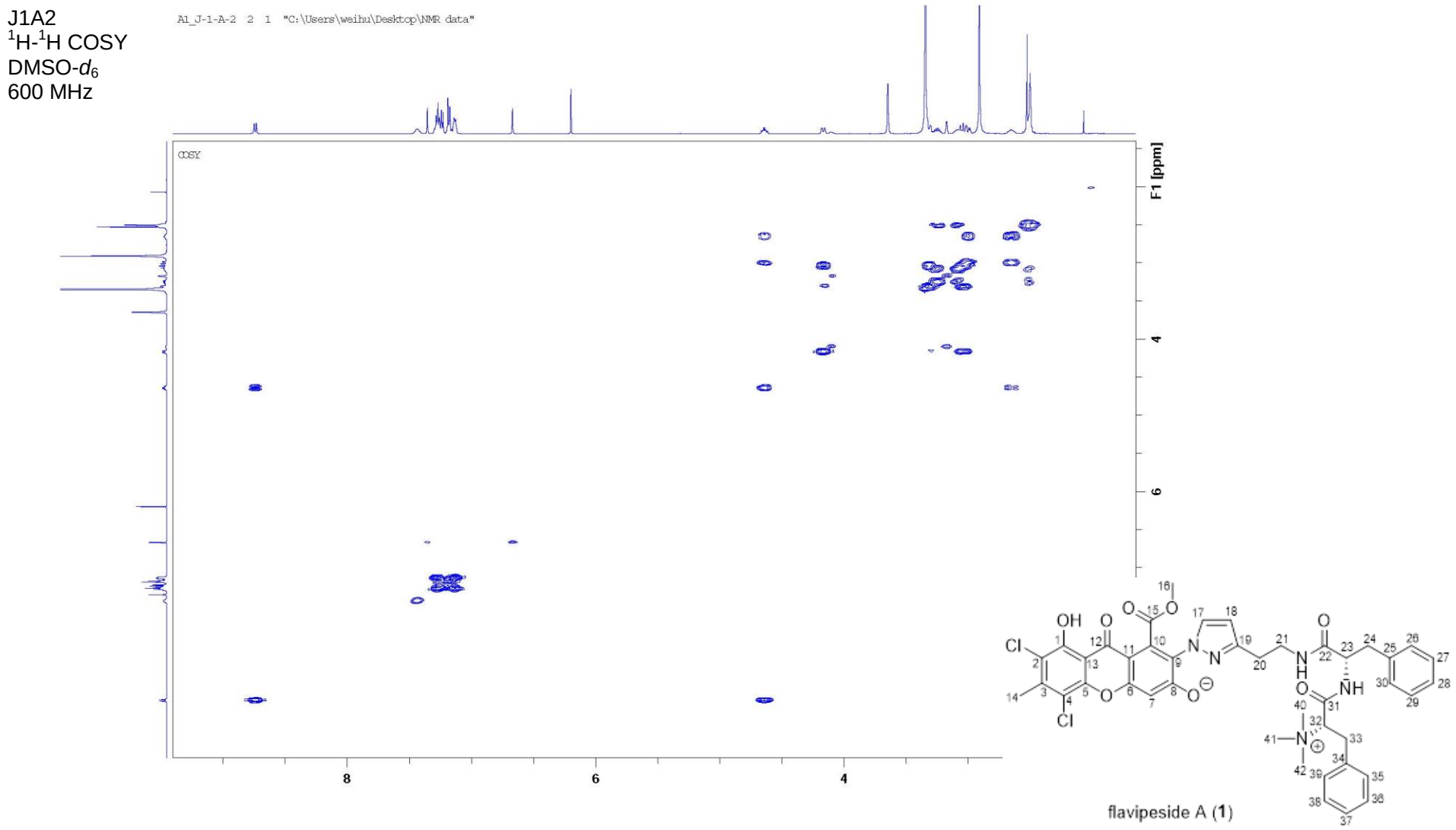


Figure S8. The ^{1}H-^{1}H COSY spectrum of flavipeside A (**1**) in DMSO-d_6.

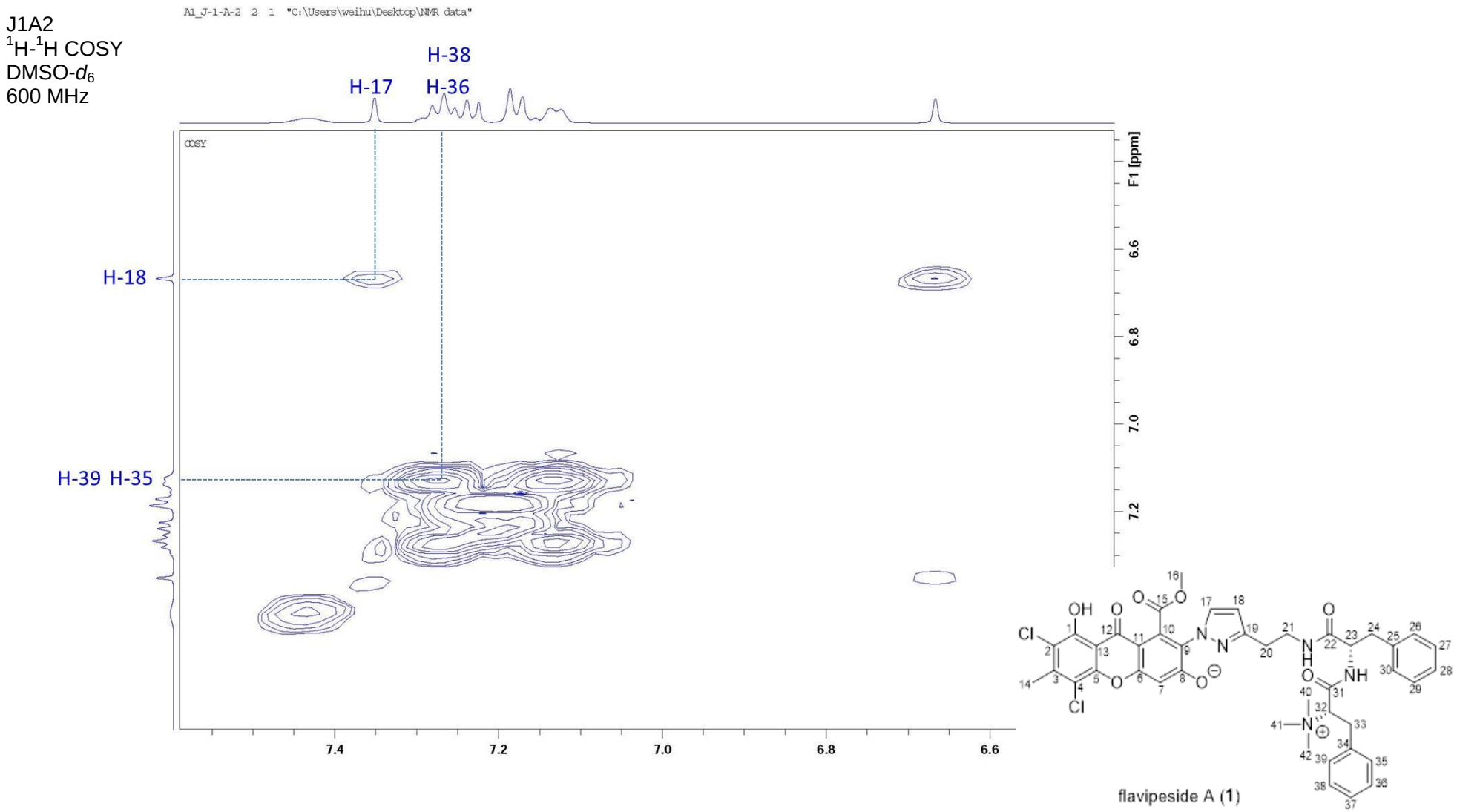


Figure S9. The ^{1}H-^{1}H COSY spectrum of flavipeside A (**1**) in DMSO-d_6.

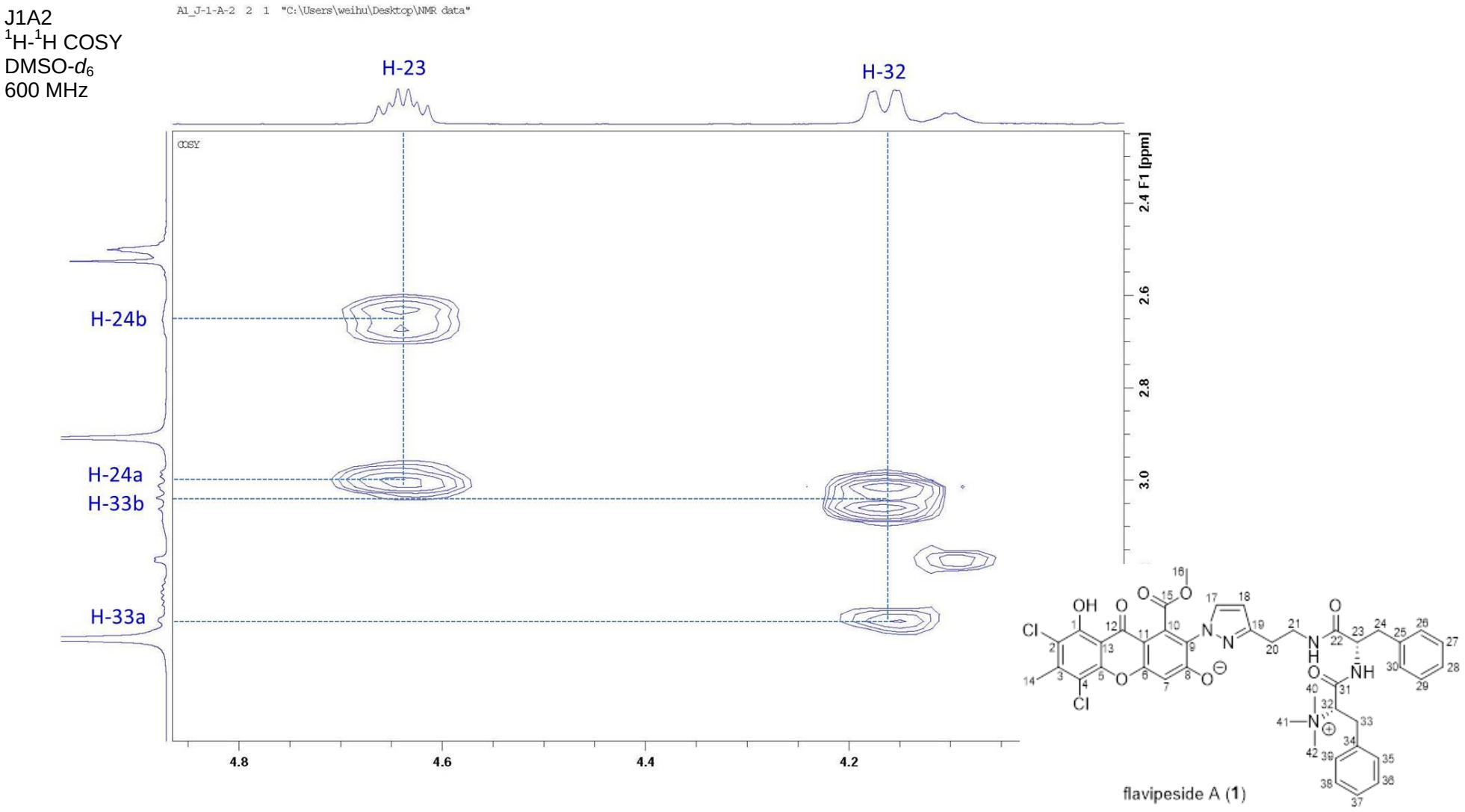


Figure S10. The ^{1}H-^{1}H COSY spectrum of flavipeside A (**1**) in DMSO-d_6.

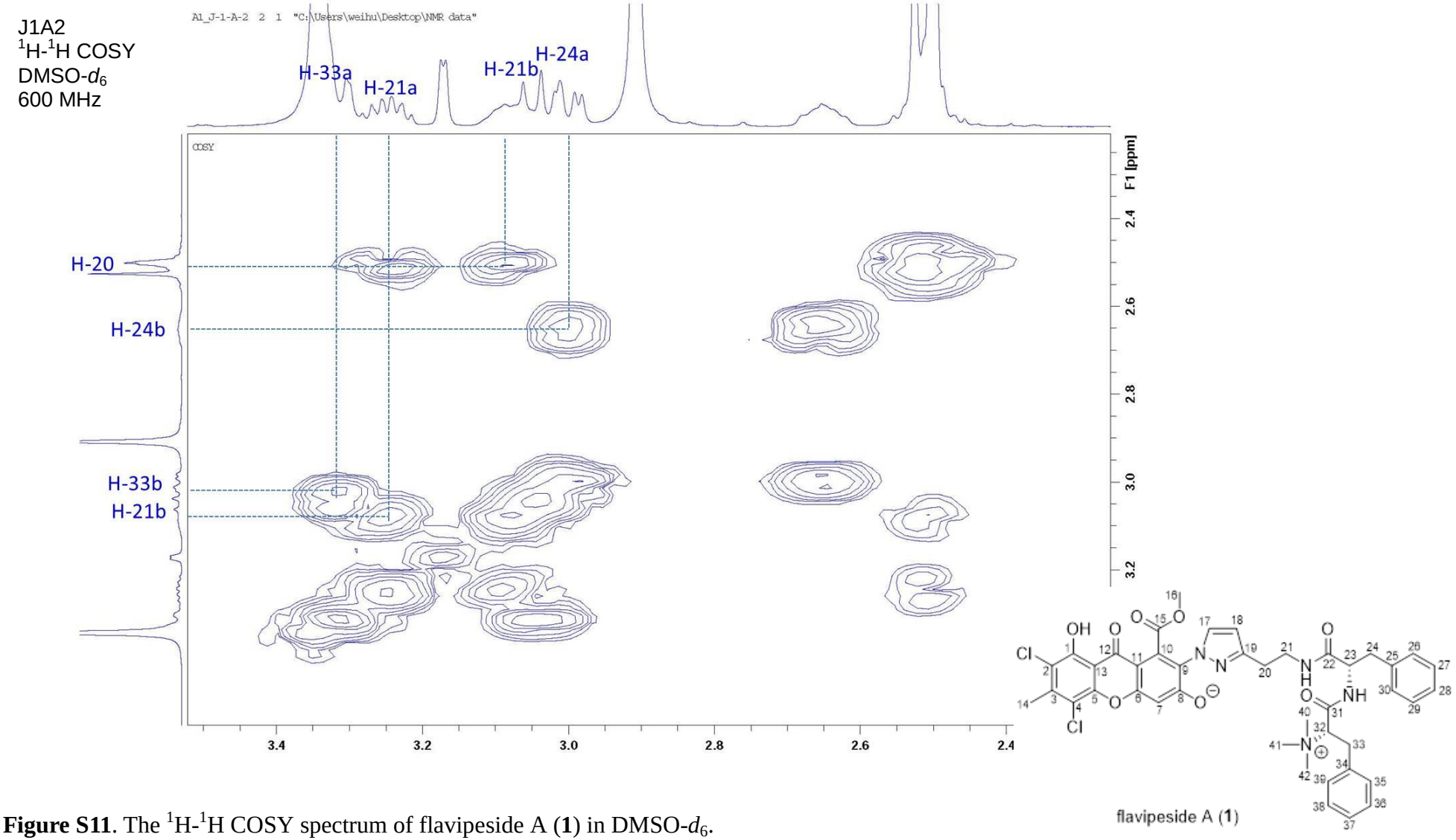


Figure S11. The ^{1}H-^{1}H COSY spectrum of flavipeside A (**1**) in DMSO-d_6.

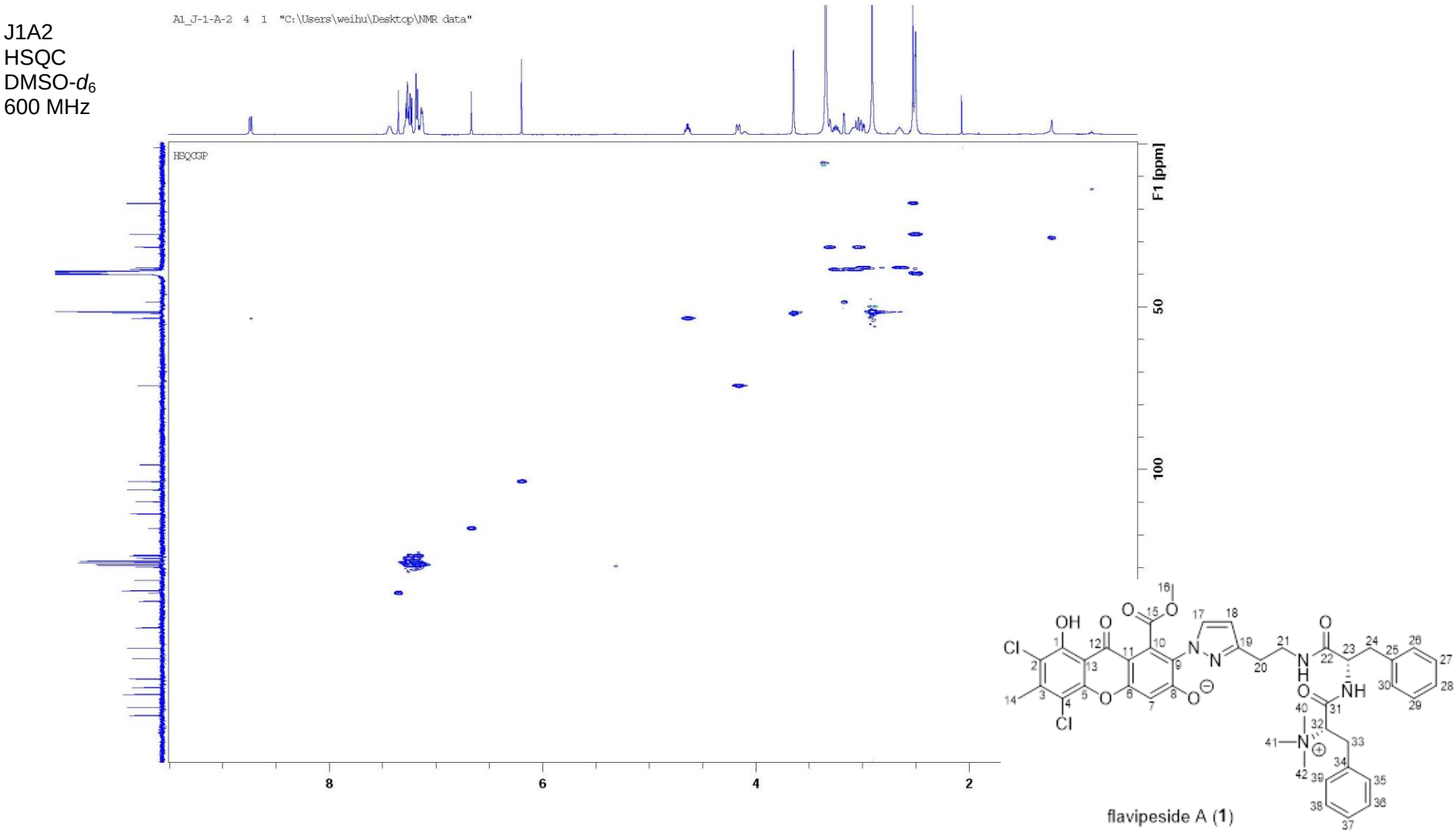


Figure S12. The HSQC spectrum of flavipeside A (**1**) in DMSO-d_6.

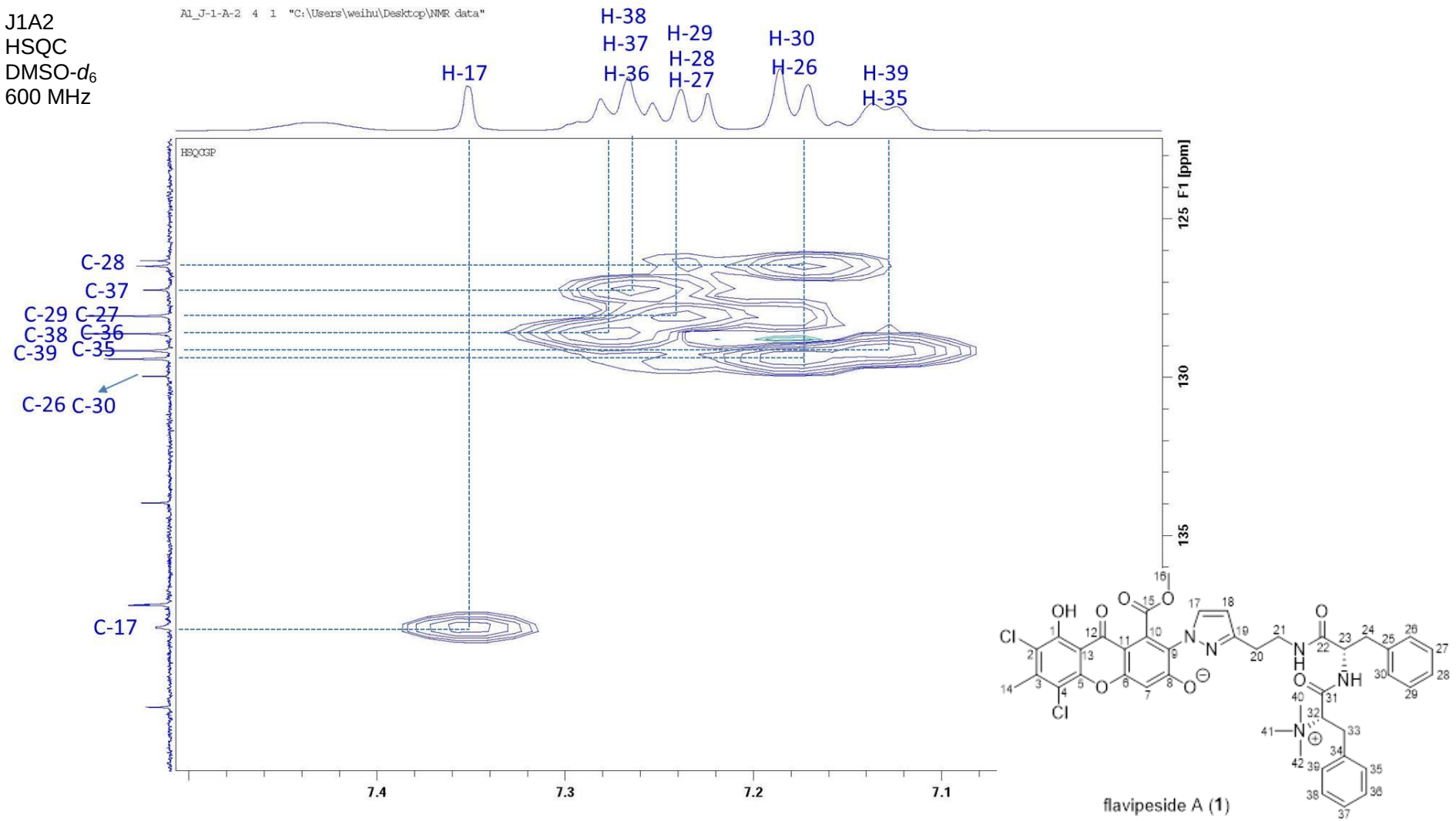


Figure S13. The HSQC spectrum of flavipeside A (**1**) in DMSO-d_6.

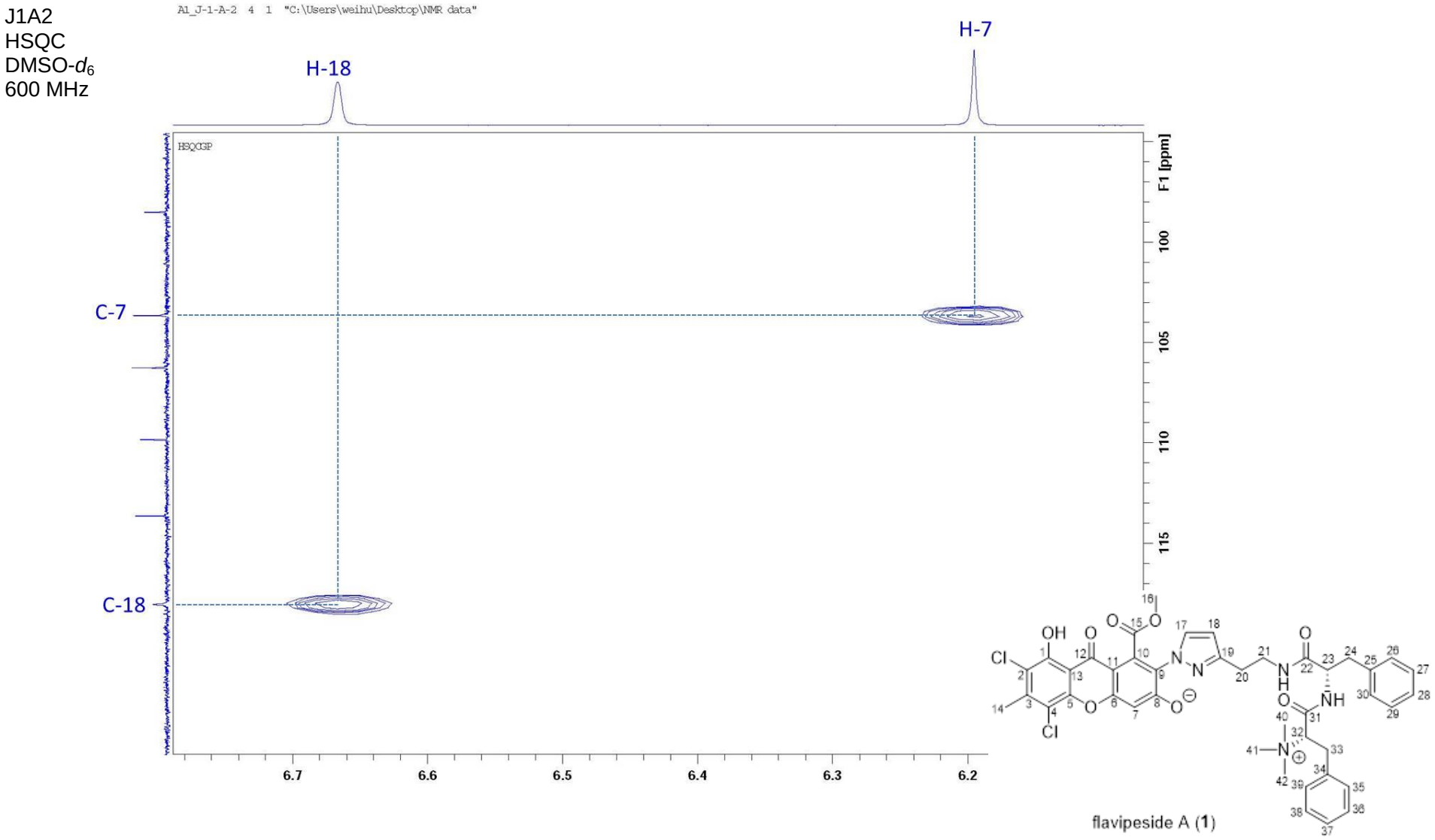


Figure S14. The HSQC spectrum of flavipeside A (**1**) in DMSO-d_6.

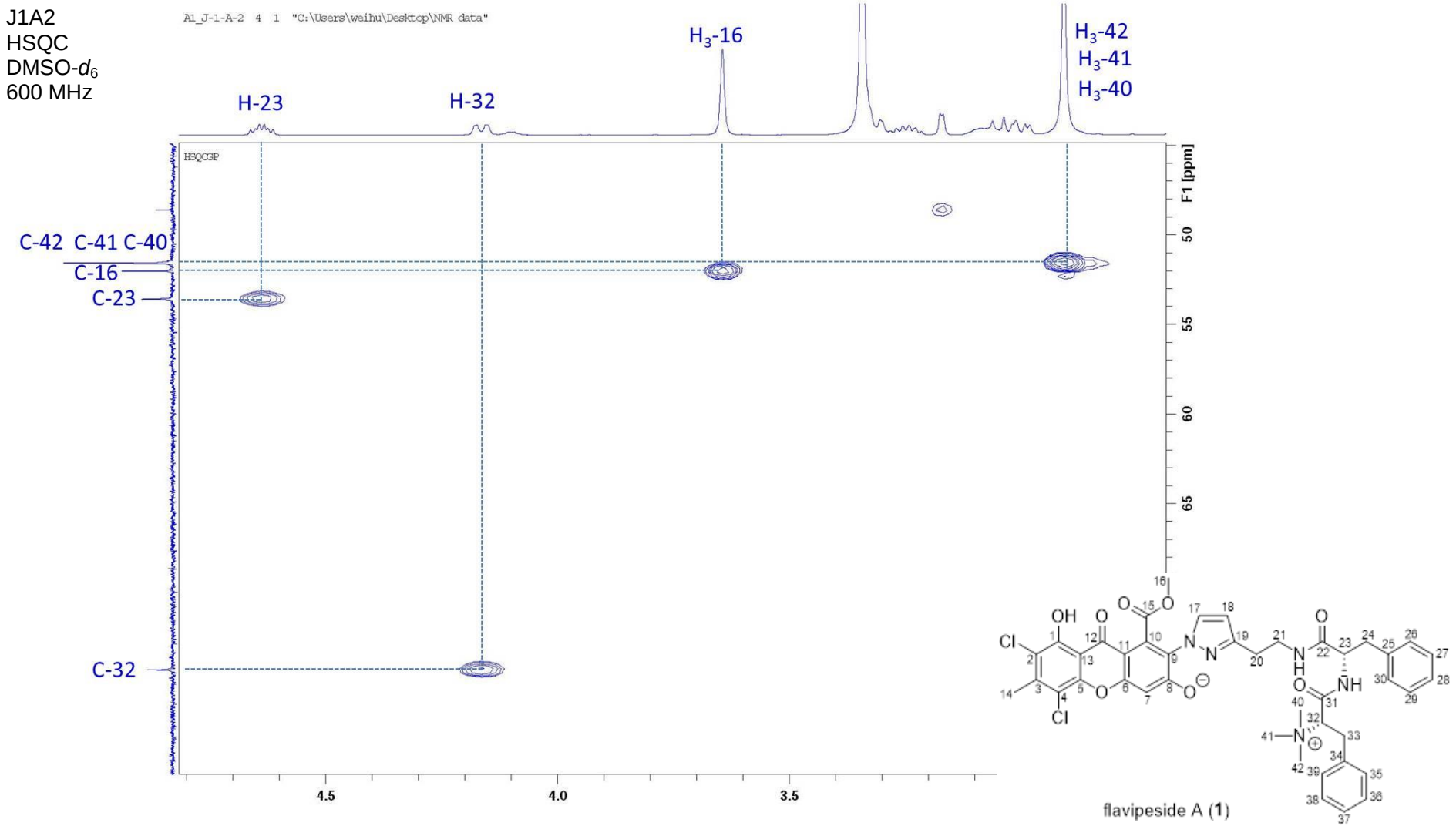


Figure S15. The HSQC spectrum of flavipeside A (**1**) in DMSO-d_6.

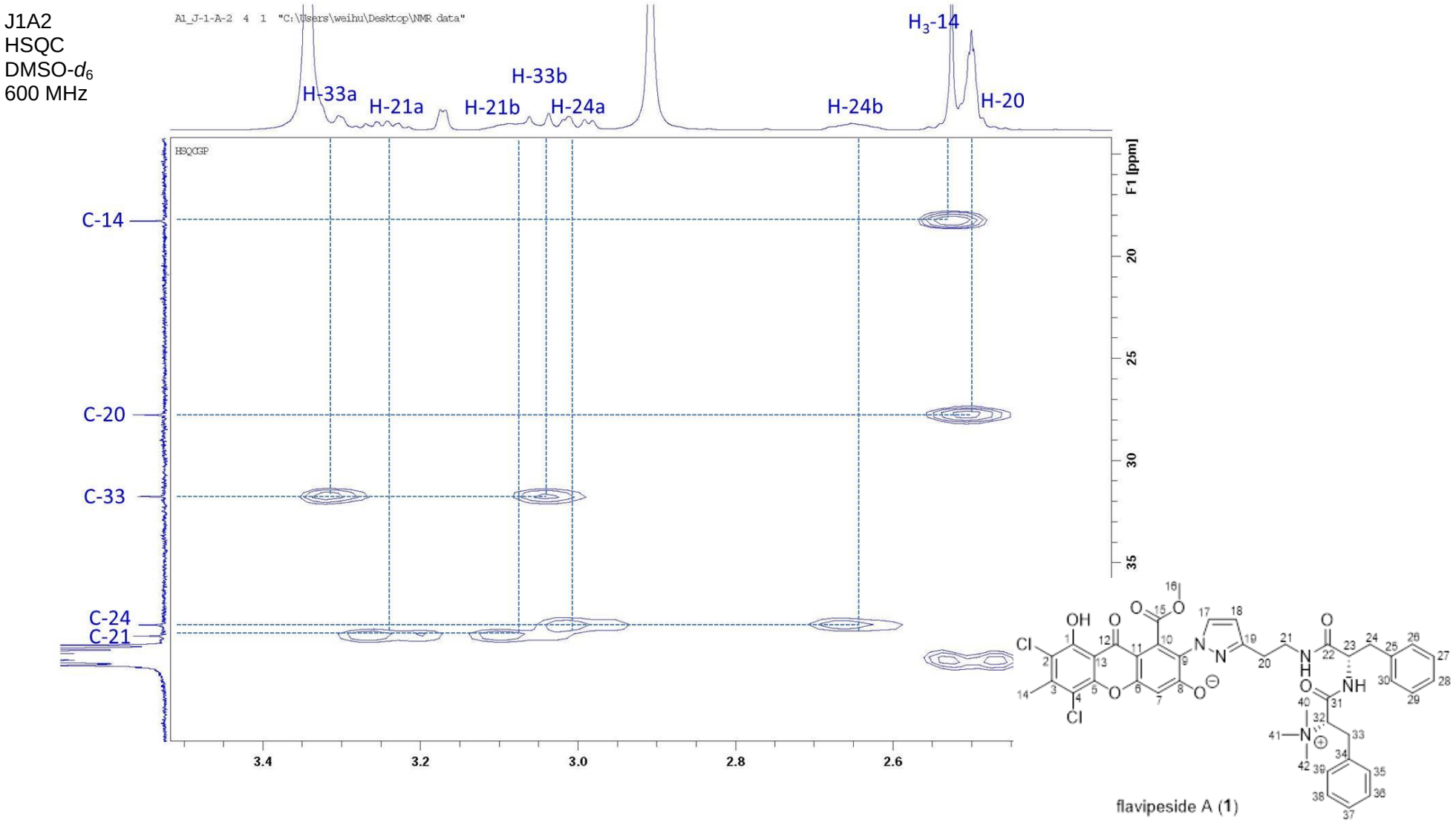


Figure S16. The HSQC spectrum of flavipeside A (**1**) in DMSO-d_6.

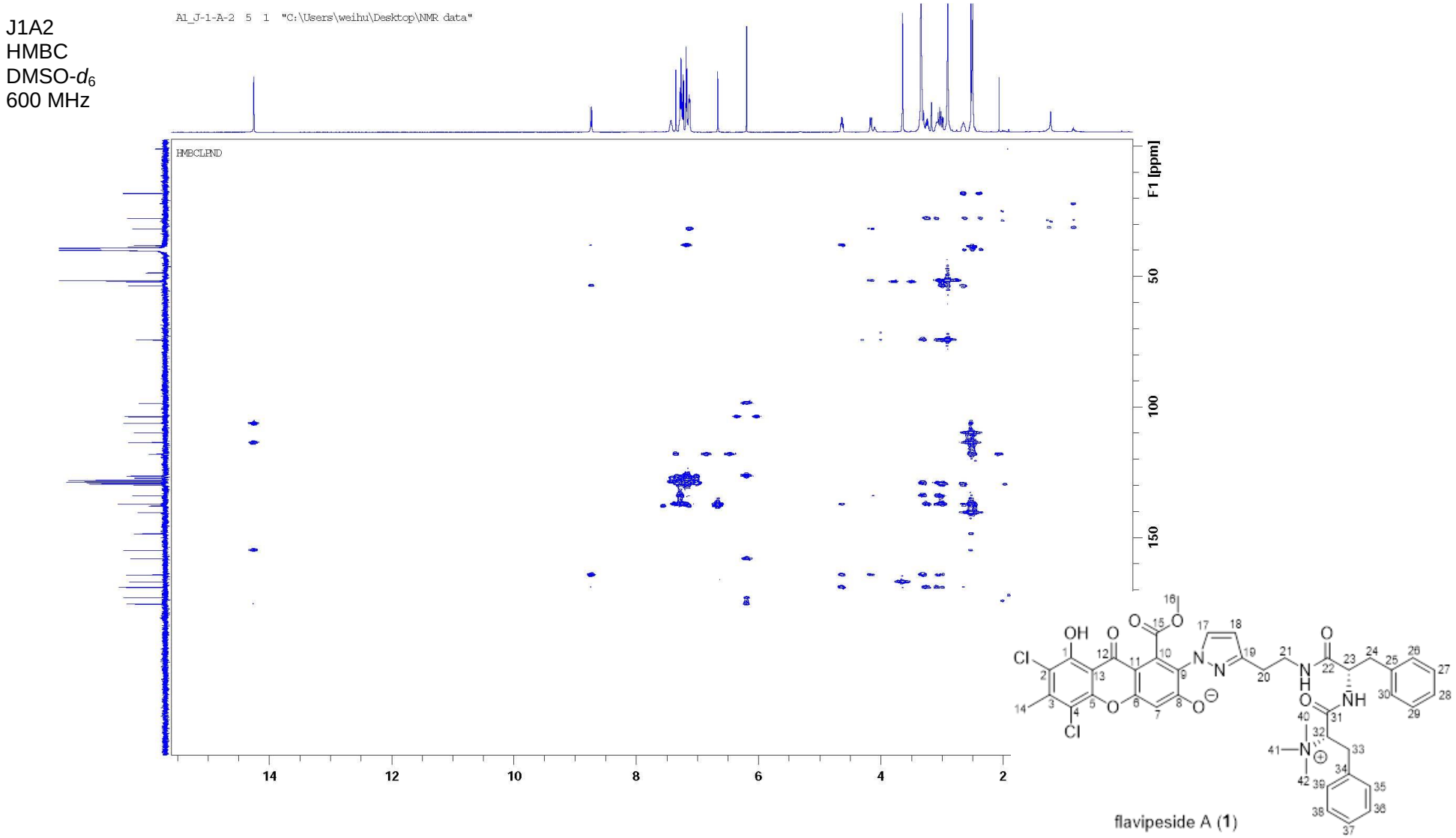


Figure S17. The HMBC spectrum of flavipeside A (**1**) in DMSO-d_6.

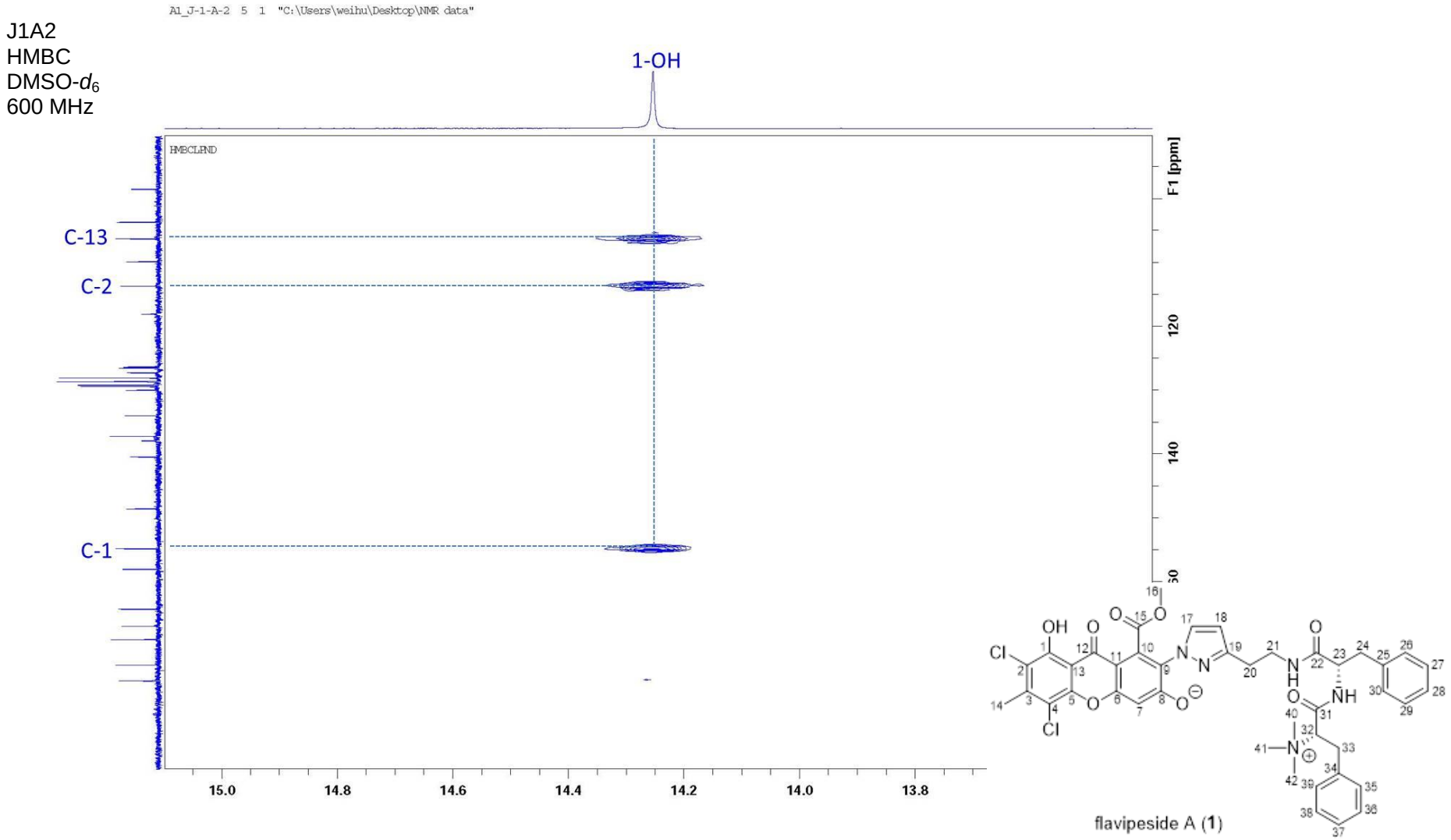


Figure S18. The HMBC spectrum of flavipeside A (**1**) in DMSO-d_6.

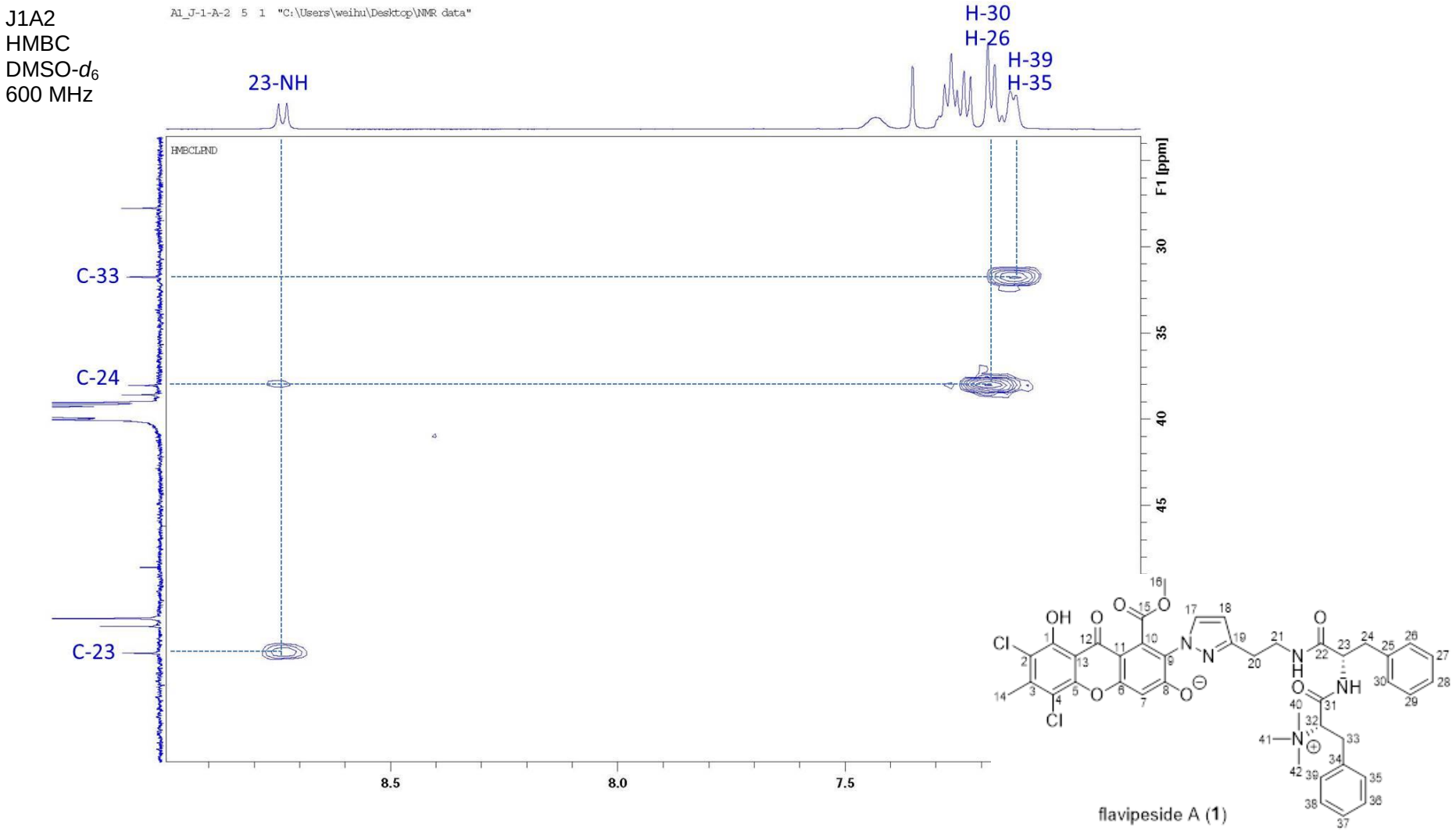


Figure S19. The HMBC spectrum of flavipeside A (**1**) in DMSO-d_6.

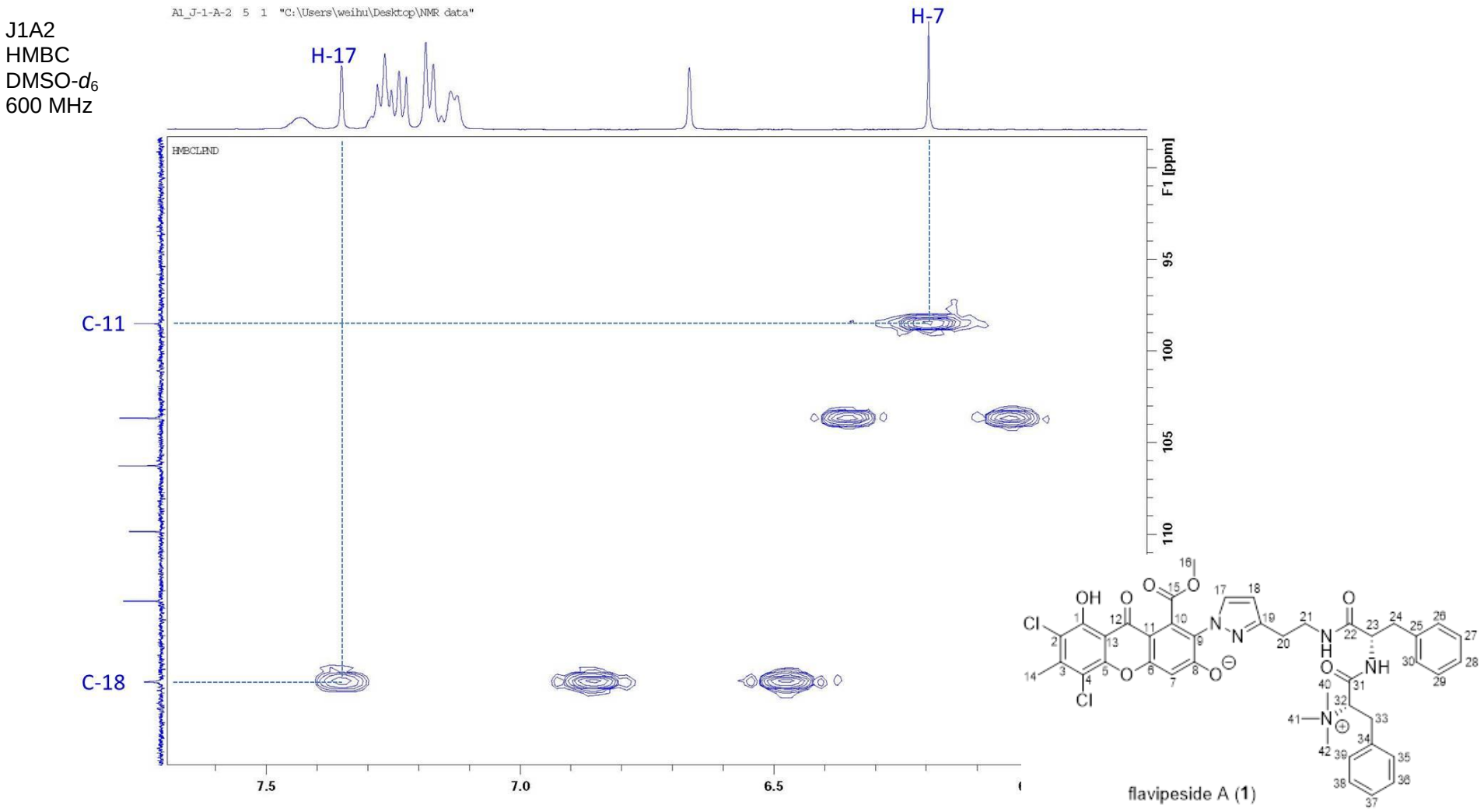


Figure S20. The HMBC spectrum of flavipeside A (**1**) in DMSO-d_6.

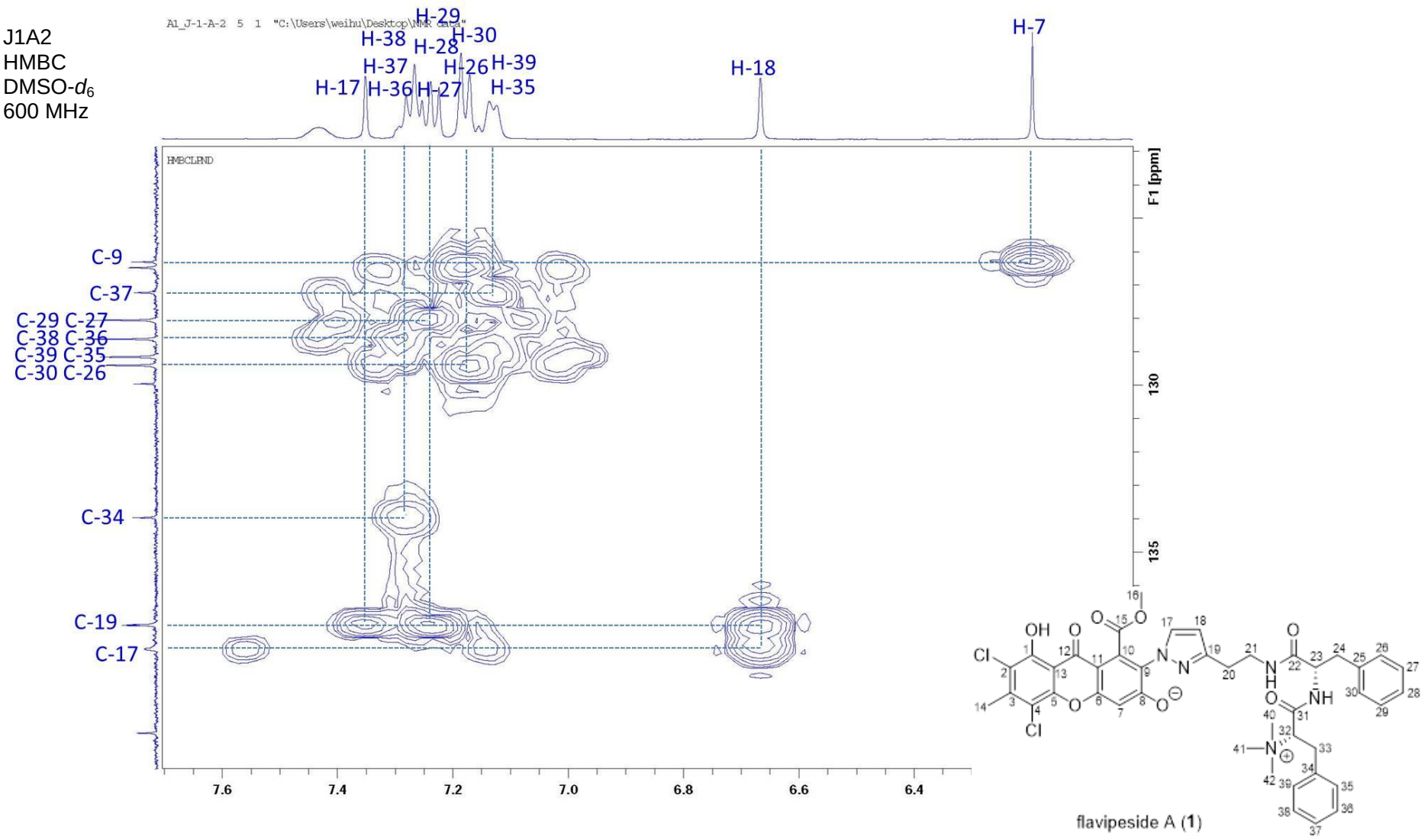


Figure S21. The HMBC spectrum of flavipeside A (**1**) in DMSO-d_6.

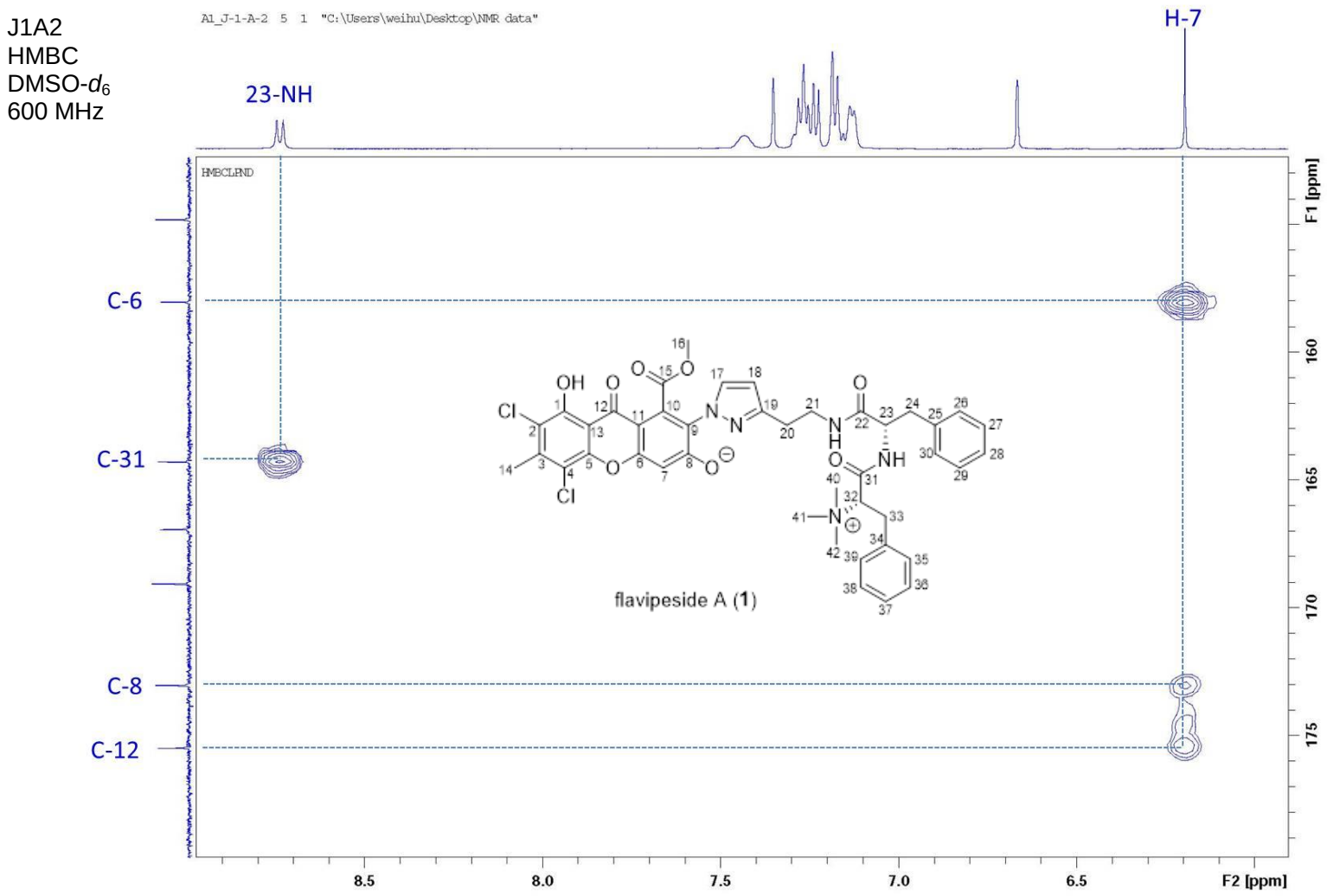


Figure S22. The HMBC spectrum of flavipeside A (**1**) in DMSO-d_6.

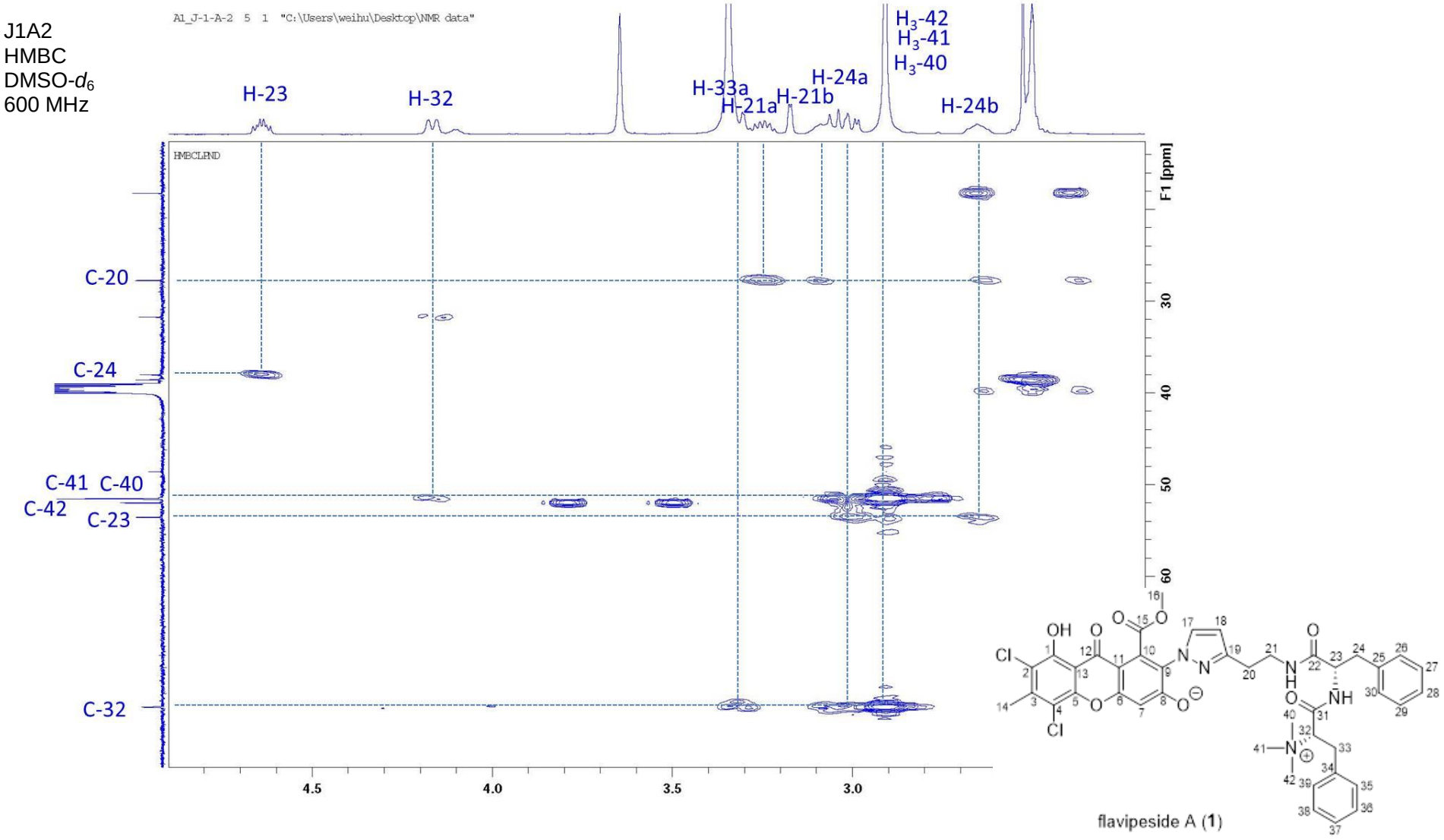


Figure S23. The HMBC spectrum of flavipeside A (**1**) in DMSO-d_6.

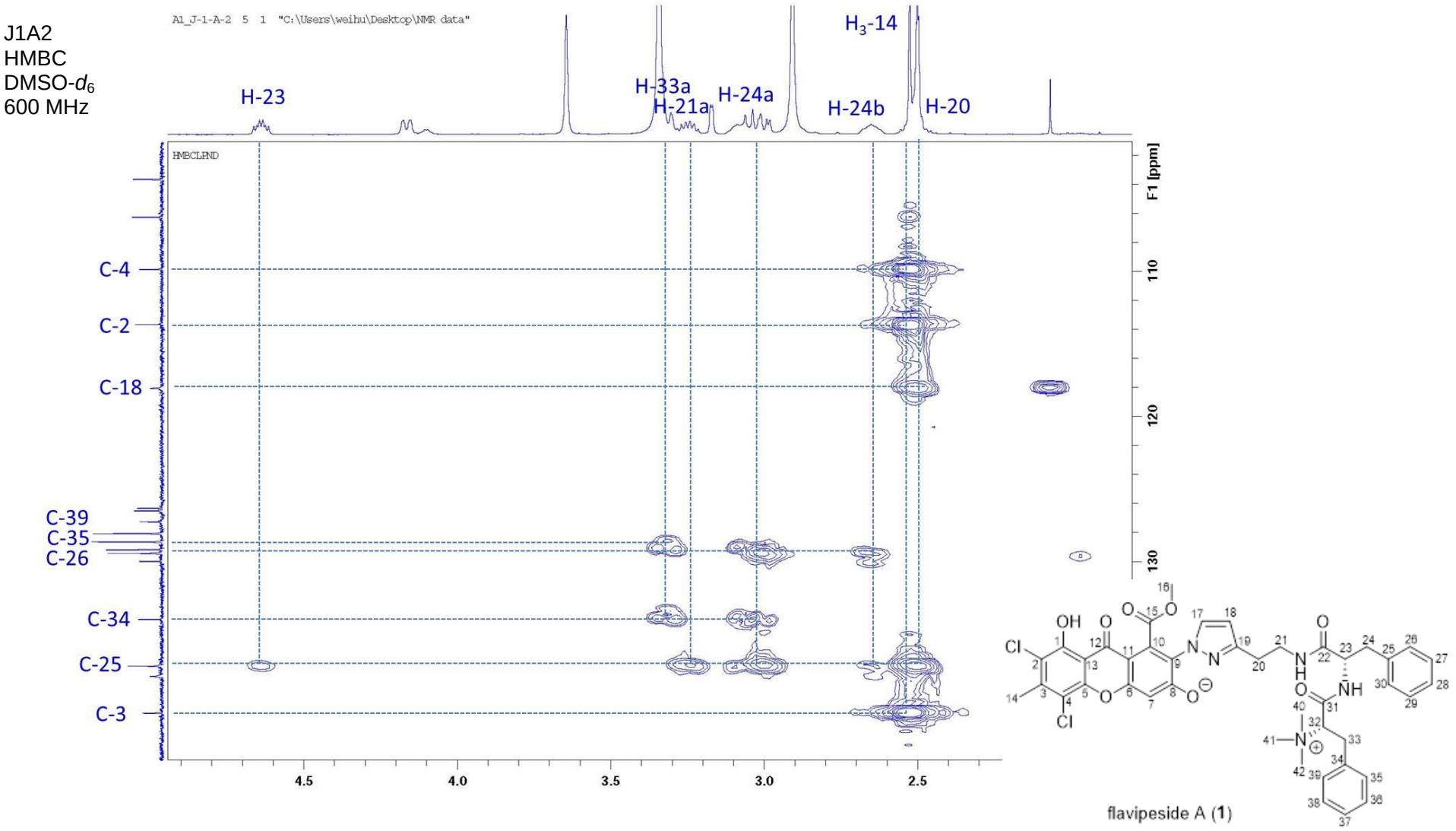


Figure S24. The HMBC spectrum of flavipeside A (**1**) in DMSO-d_6.

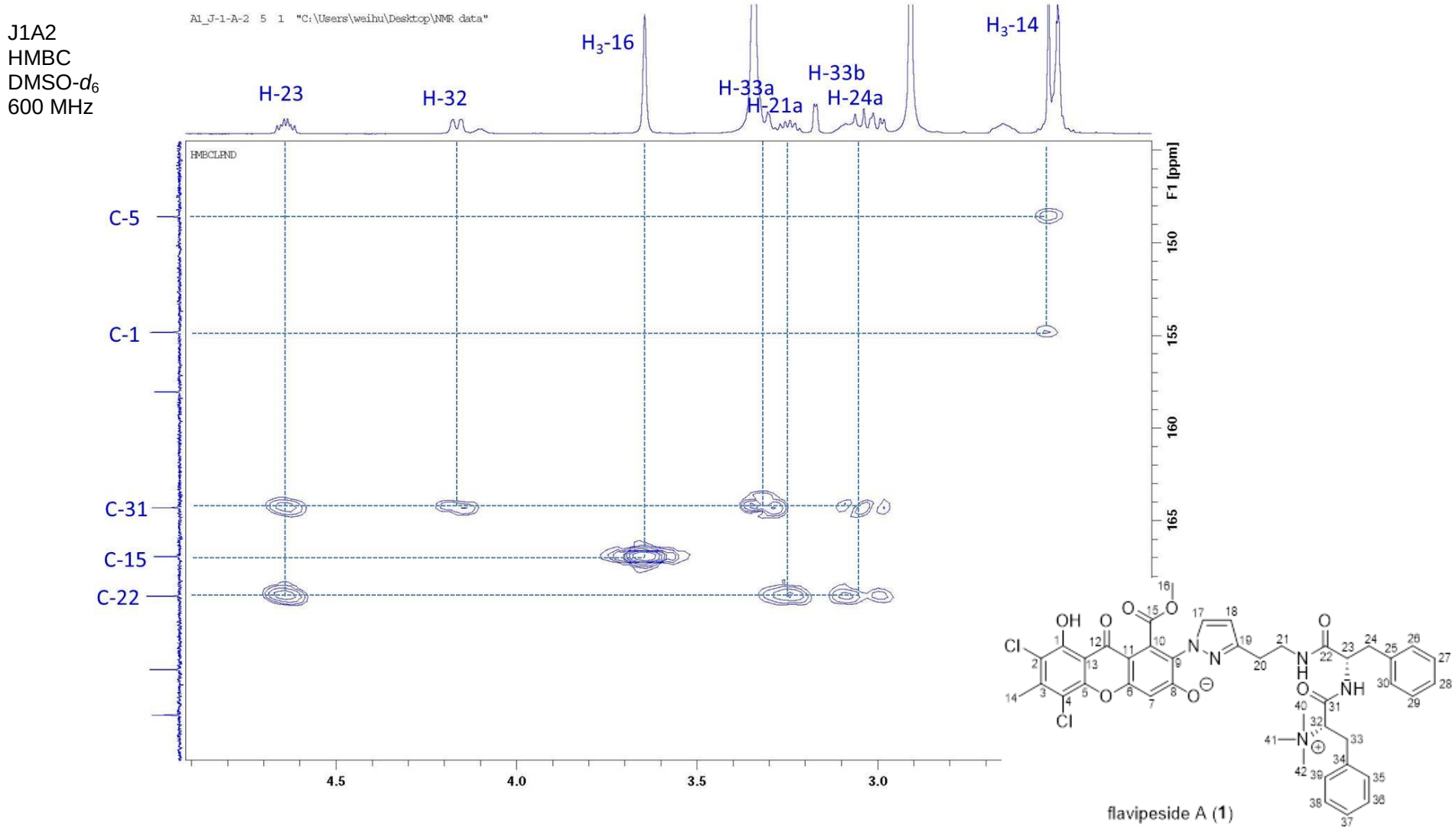


Figure S25. The HMBC spectrum of flavipeside A (**1**) in DMSO-d_6.

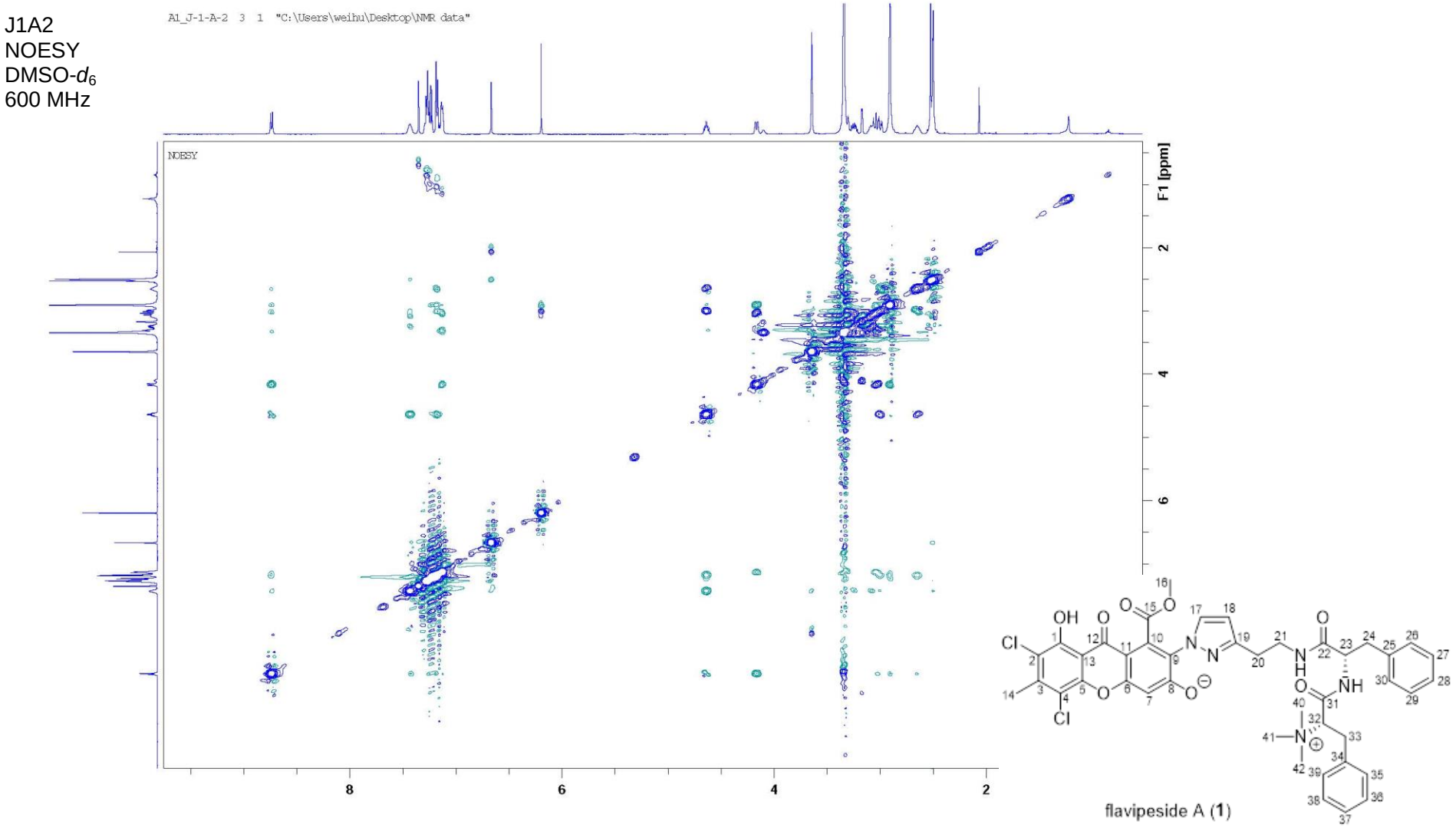


Figure S26. The NOESY spectrum of flavipeside A (**1**) in DMSO-d_6.

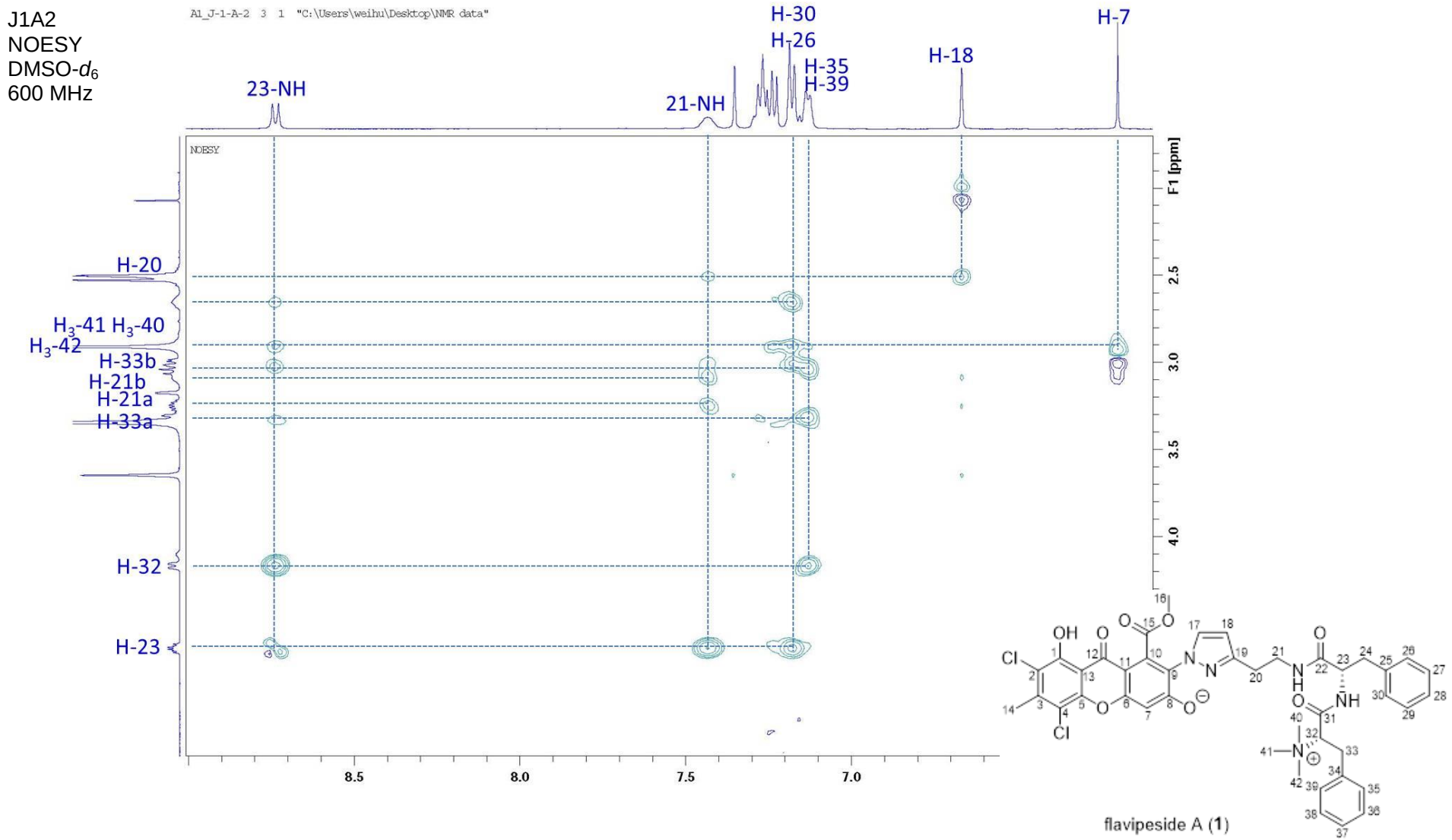


Figure S27. The NOESY spectrum of flavipeside A (**1**) in DMSO-d_6.

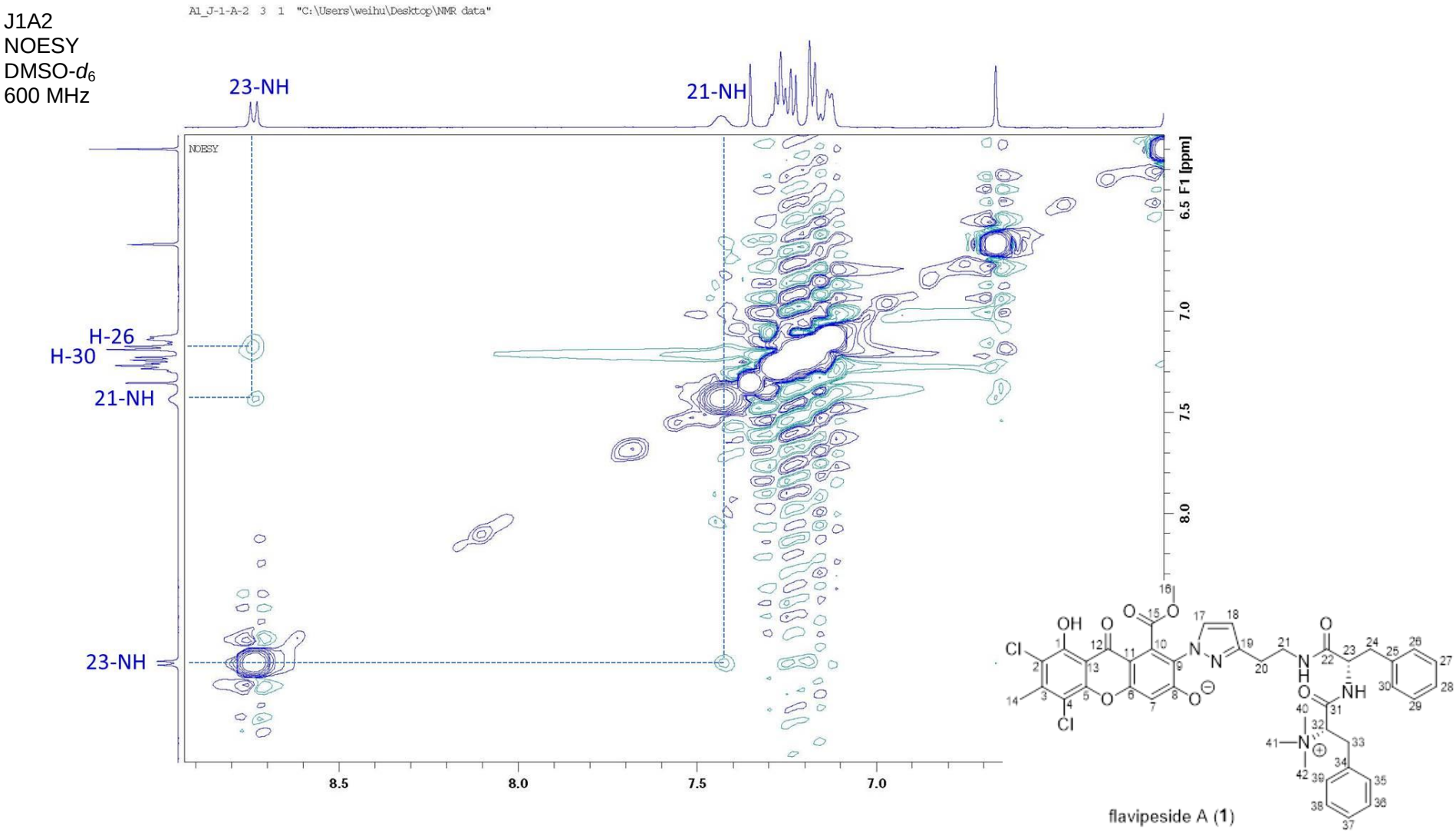


Figure S28. The NOESY spectrum of flavipeside A (**1**) in DMSO-d_6.

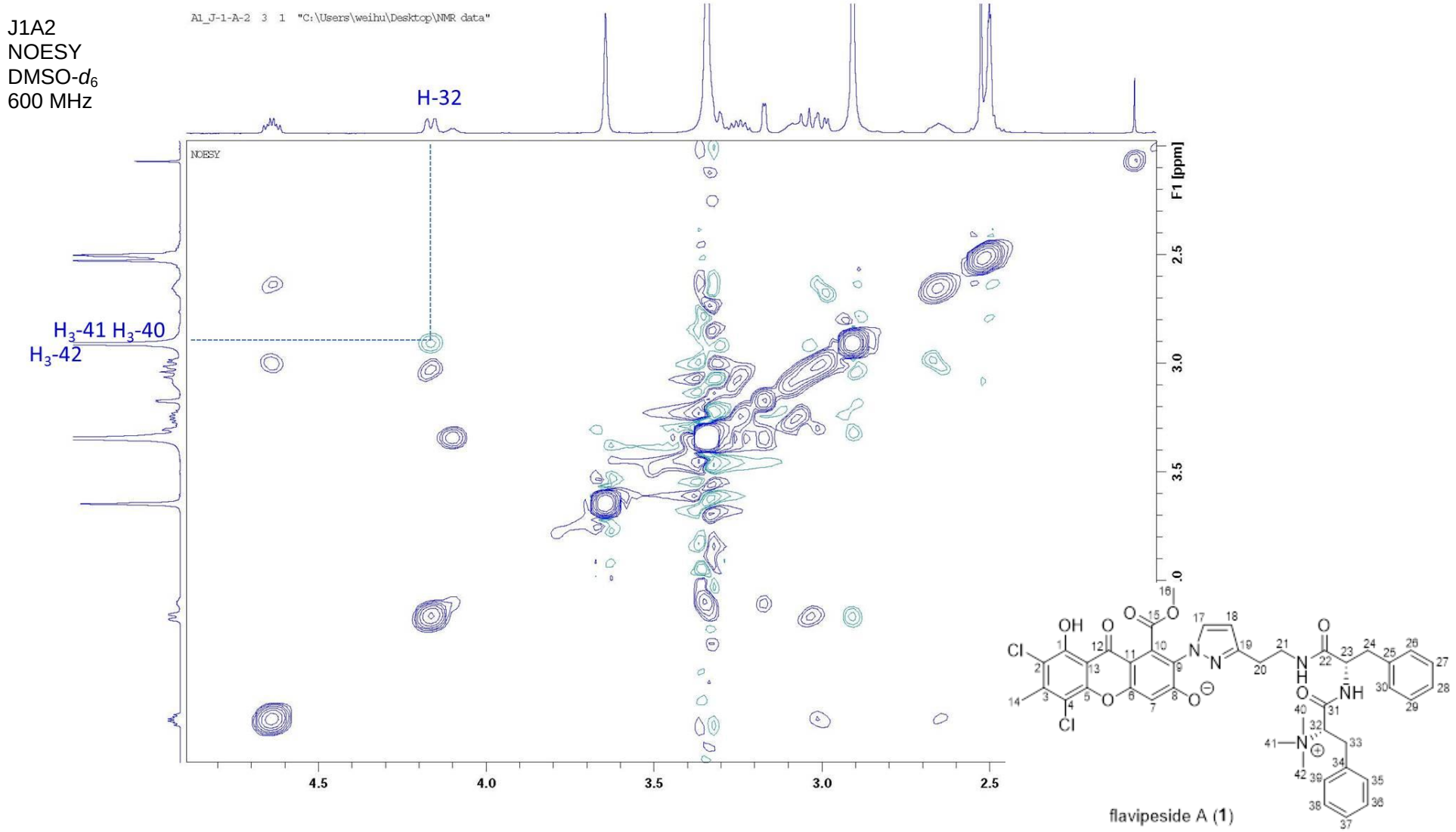


Figure S29. The NOESY spectrum of flavipeside A (**1**) in DMSO-d_6.

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Cl
814.2419	814.2419	0.0	0.0	27.5	C47 H41 N O10 Cl	785.7	7.691	0.05	47	41	1	10	1
	814.2420	-0.1	-0.1	14.5	C36 H48 N7 O6 Cl4	785.4	7.462	0.06	36	48	7	6	4
	814.2414	0.5	0.6	37.5	C49 H32 N7 O6	794.4	16.472	0.00	49	32	7	6	
	814.2424	-0.5	-0.6	28.5	C43 H38 N9 O4 Cl2	780.0	2.049	12.89	43	38	9	4	2
	814.2410	0.9	1.1	23.5	C42 H42 N5 O8 Cl2	779.1	1.106	33.08	42	42	5	8	2
	814.2429	-1.0	-1.2	18.5	C41 H47 N3 O8 Cl3	783.4	5.383	0.46	41	47	3	8	3
	814.2432	-1.3	-1.6	32.5	C48 H37 N5 O6 Cl	786.4	8.412	0.02	48	37	5	6	1

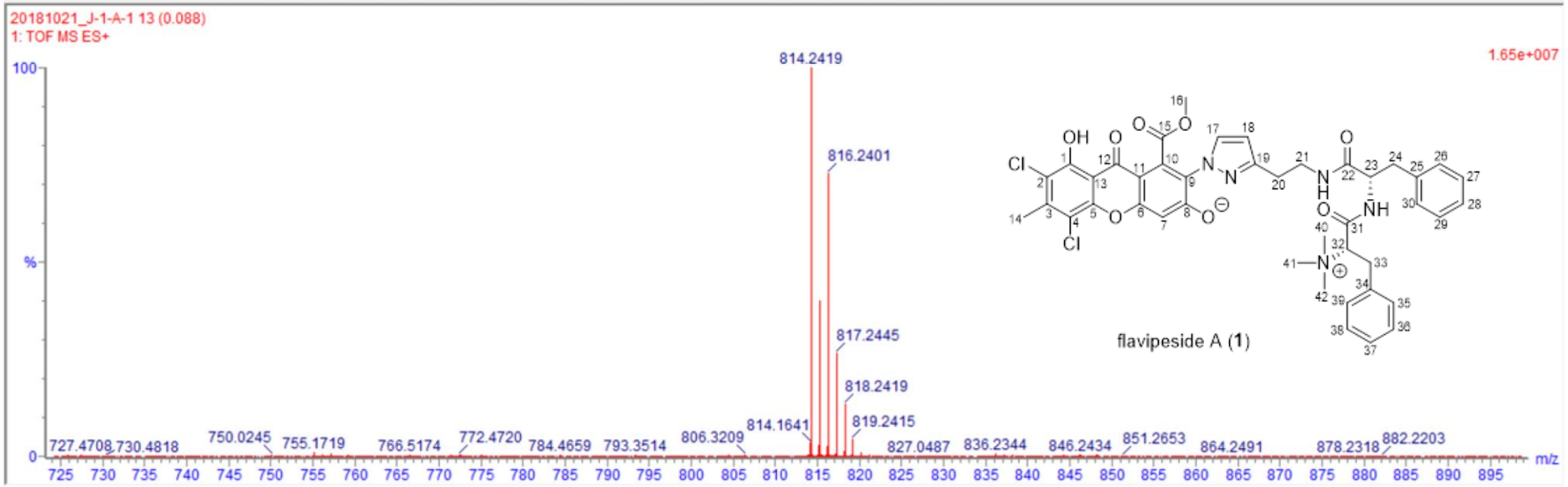
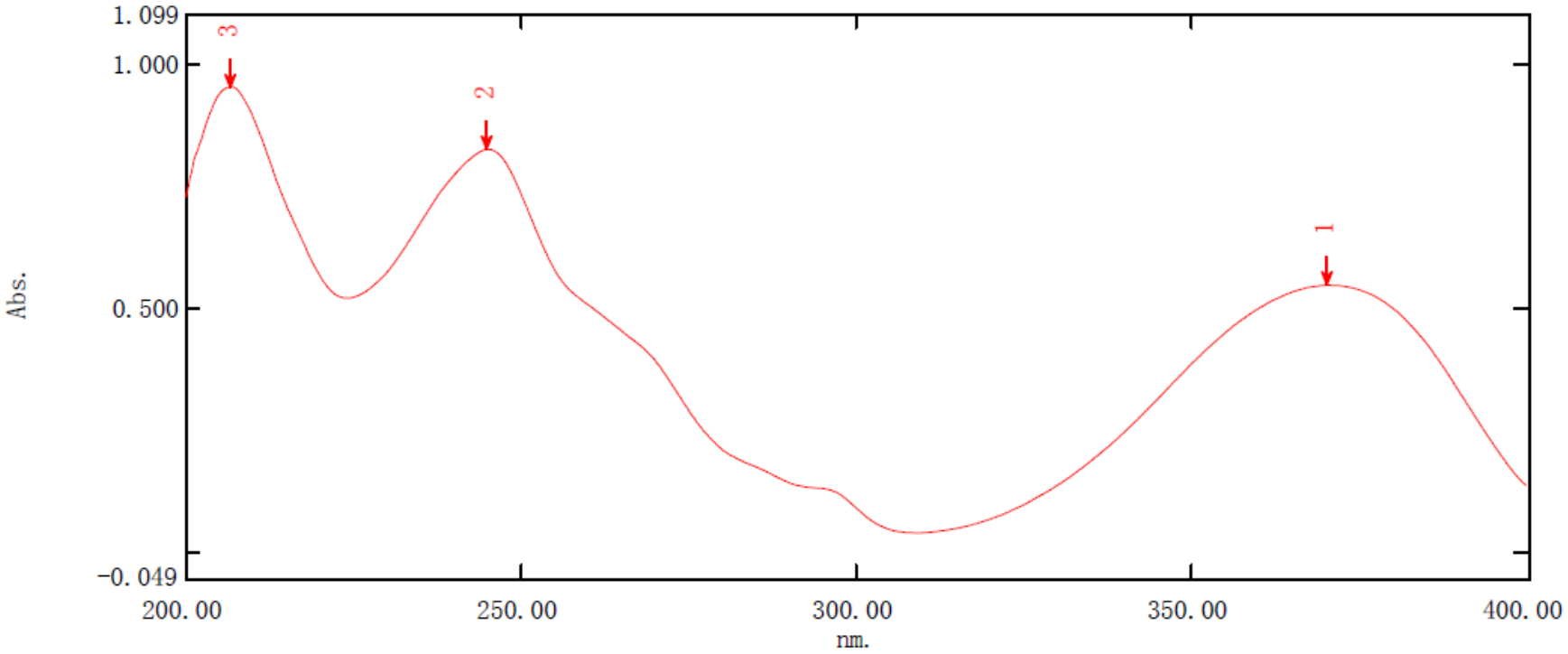


Figure S30. The HRESIMS spectrum of flavipeside A (**1**).



No.	P/V	波长(nm)	Abs.	描述
1	↑	370.20	0.549	
2	↑	245.00	0.826	
3	↑	206.80	0.954	
4	↓	308.80	0.042	
5	↓	224.00	0.522	

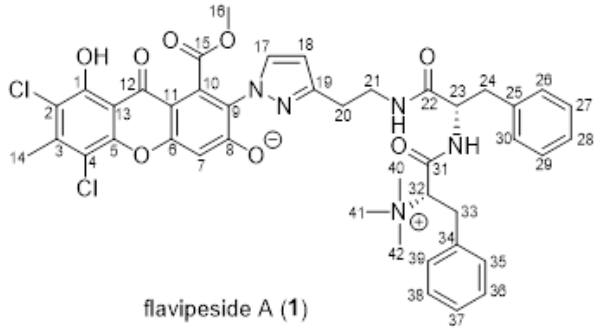


Figure S31. The UV spectrum of flavipeside A (**1**) in MeOH.

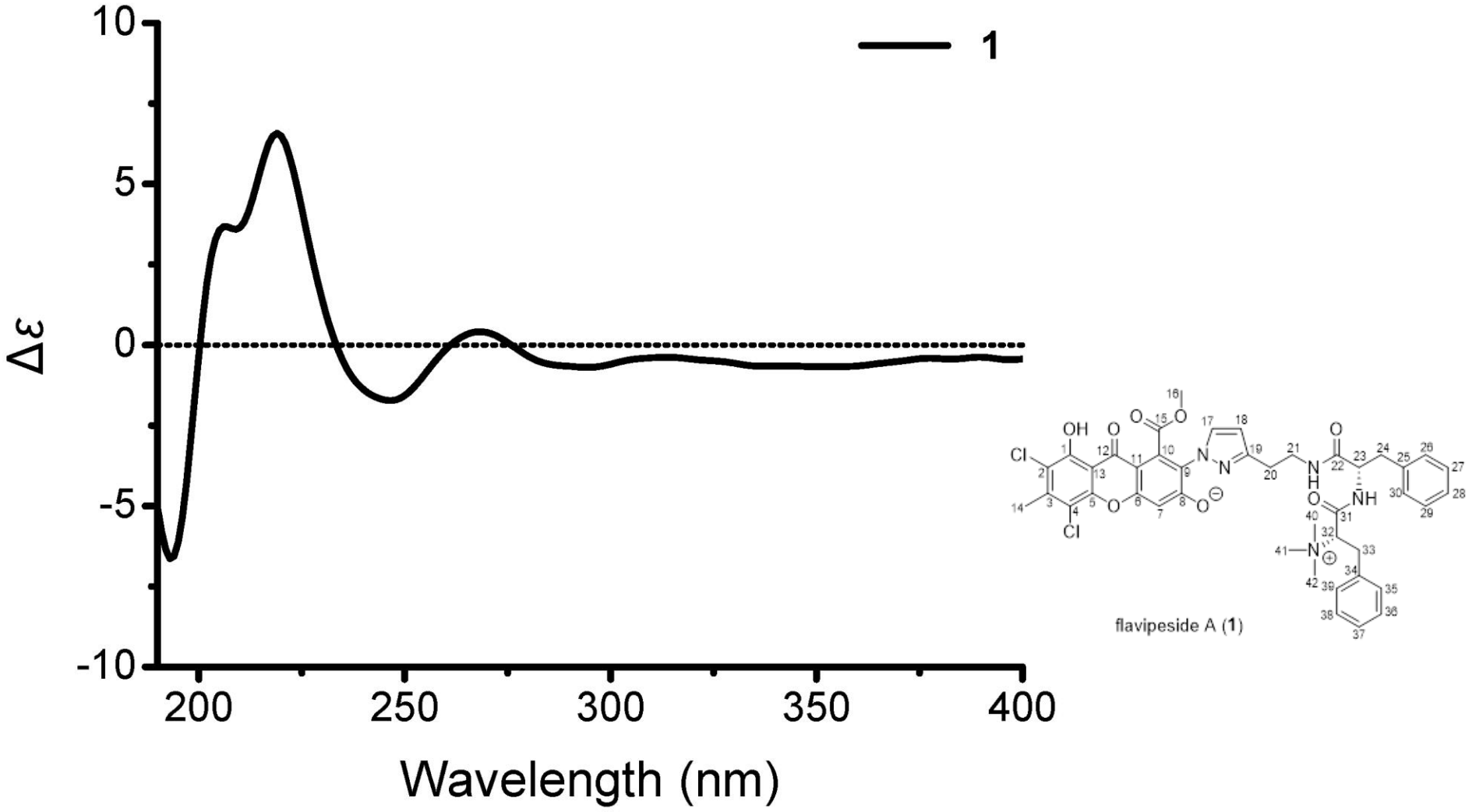


Figure S32. The CD spectrum of flavipeside A (**1**) in MeCN.

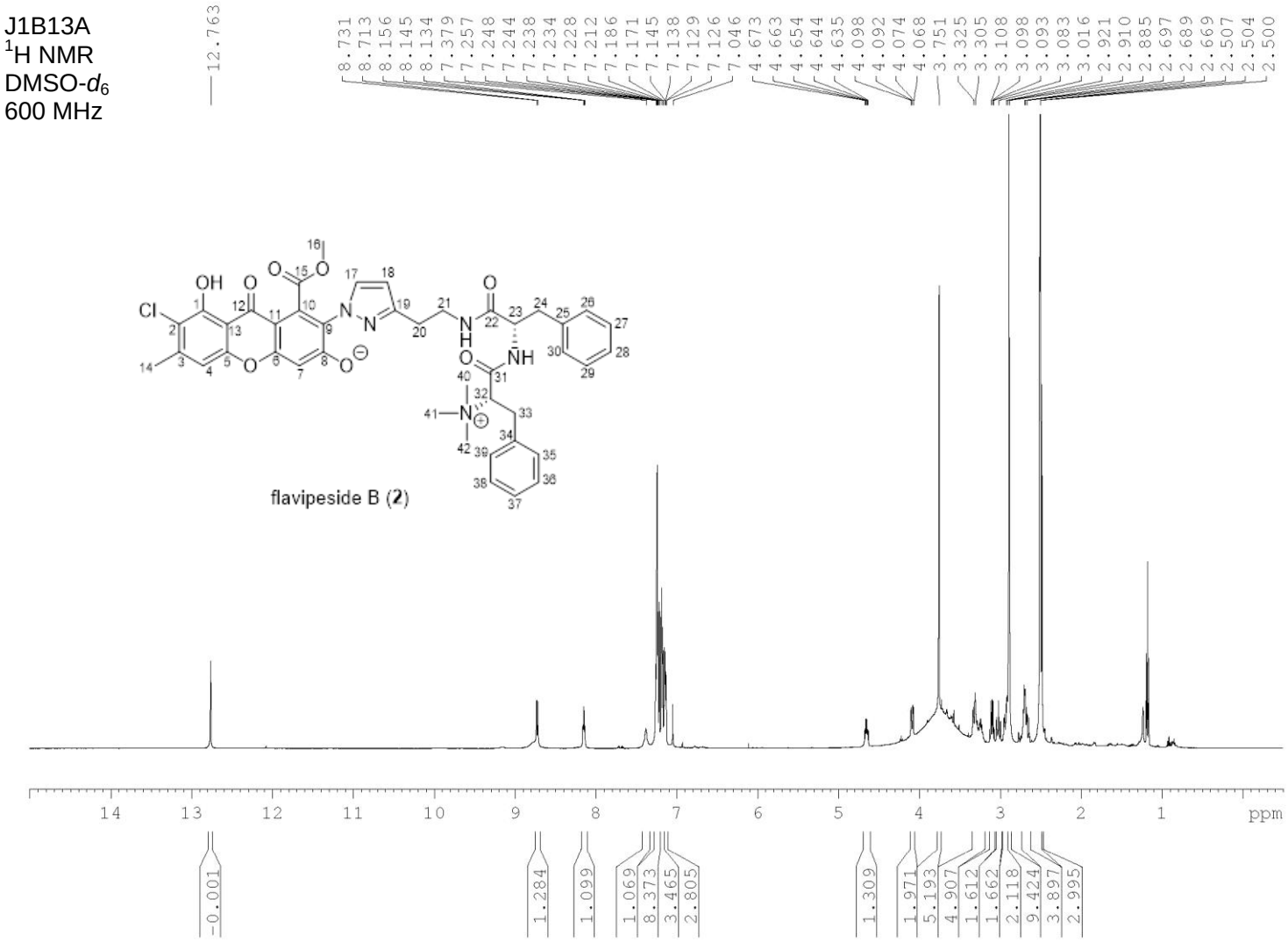


Figure S33. The ^{1}H NMR spectrum of flavipeside B (**2**) in DMSO-d_6.

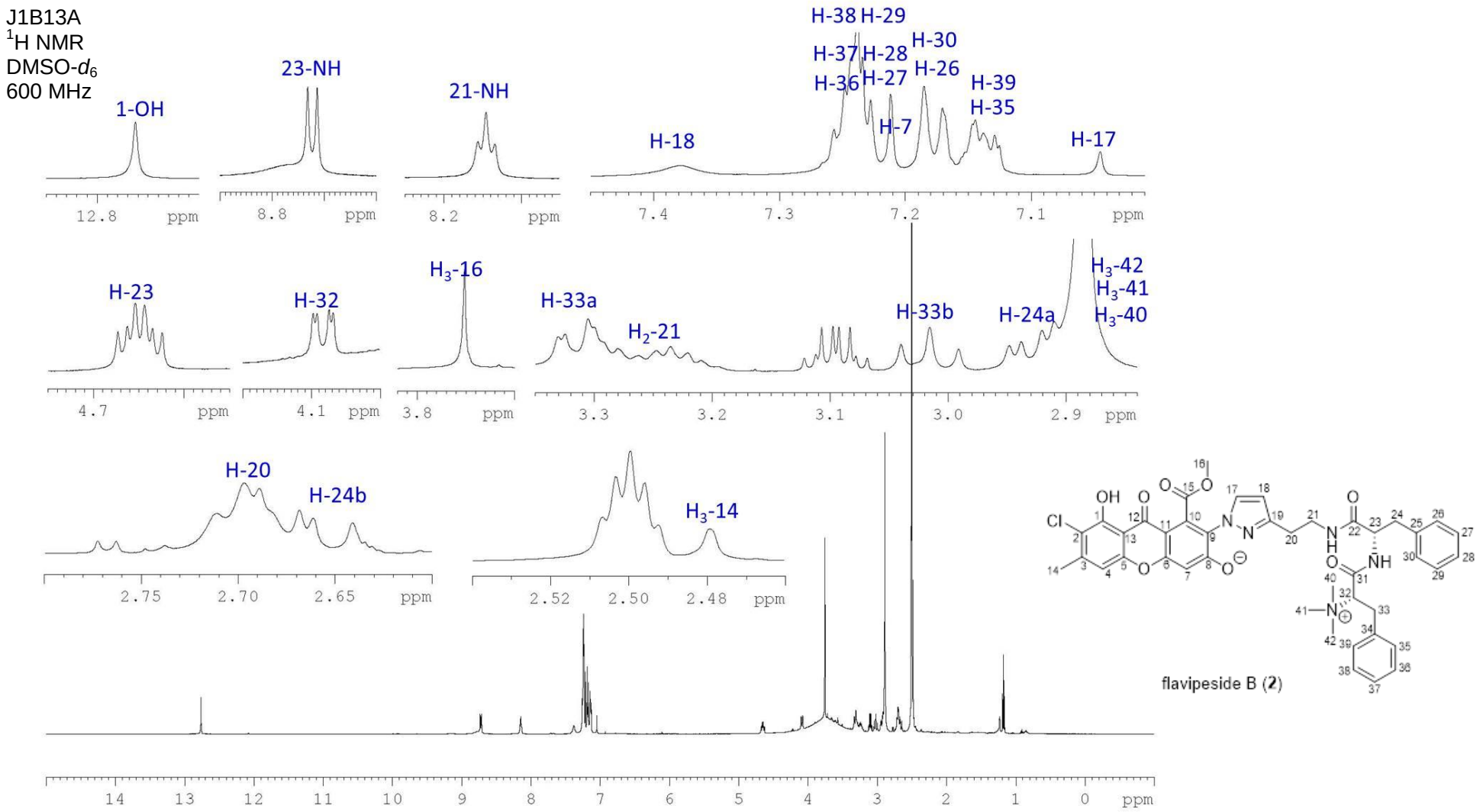


Figure S34. The ^{1}H NMR spectrum of flavipeside B (**2**) in DMSO-d_6.

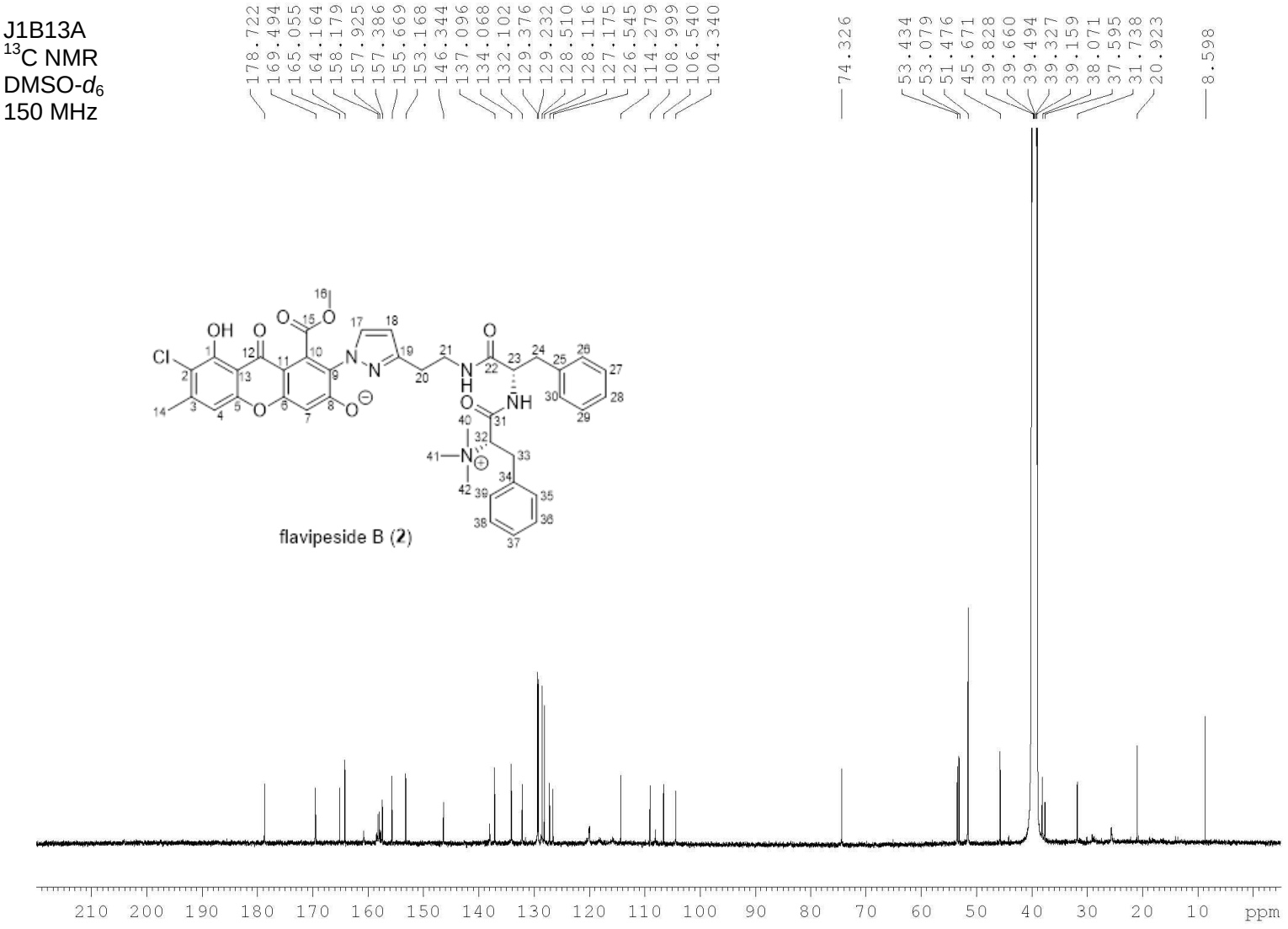


Figure S35. The ^{13}C NMR spectrum of flavipeside B (**2**) in DMSO-d_6.

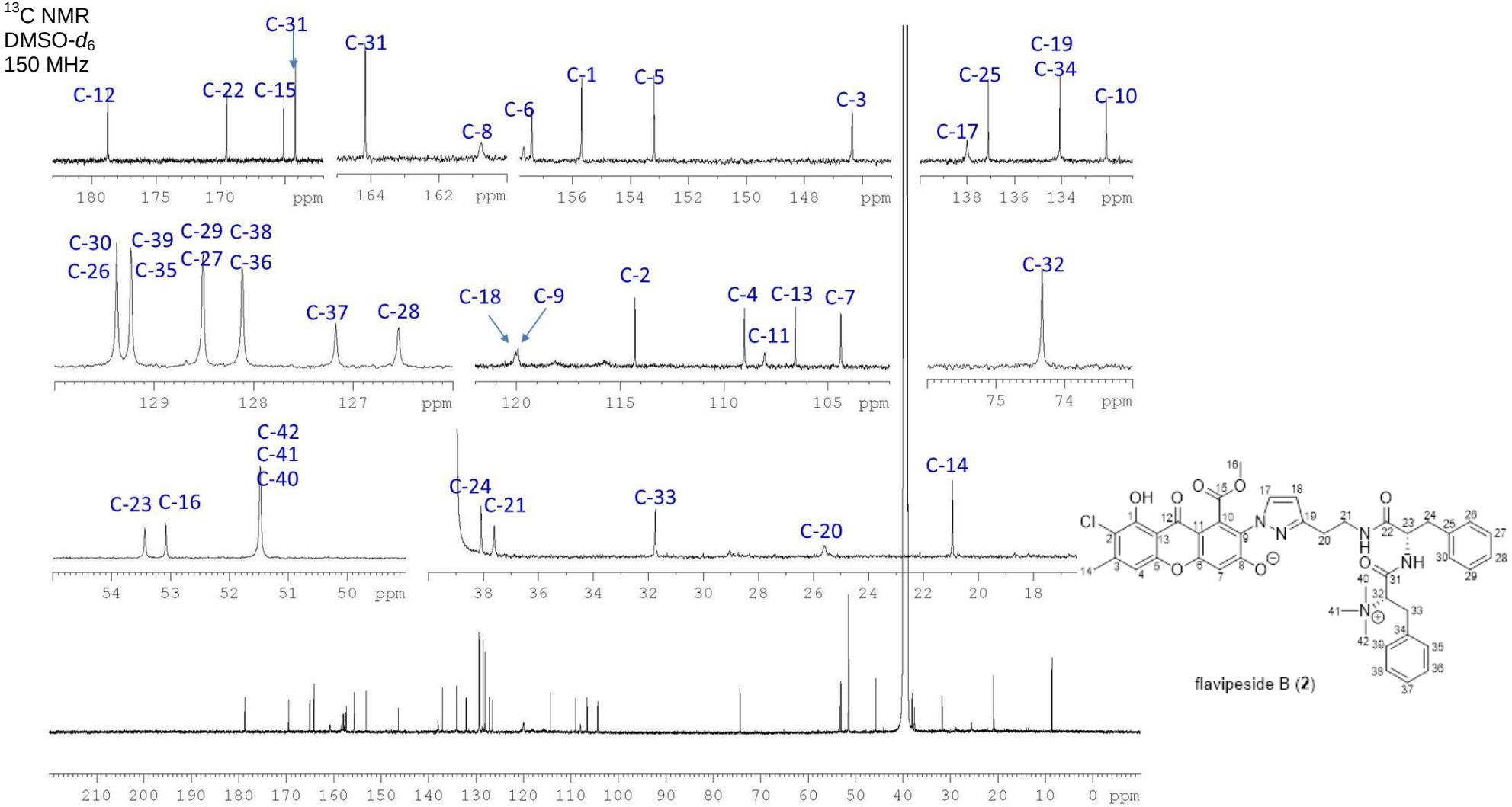


Figure S36. The ^{13}C NMR spectrum of flavipeside B (**2**) in DMSO-d_6.

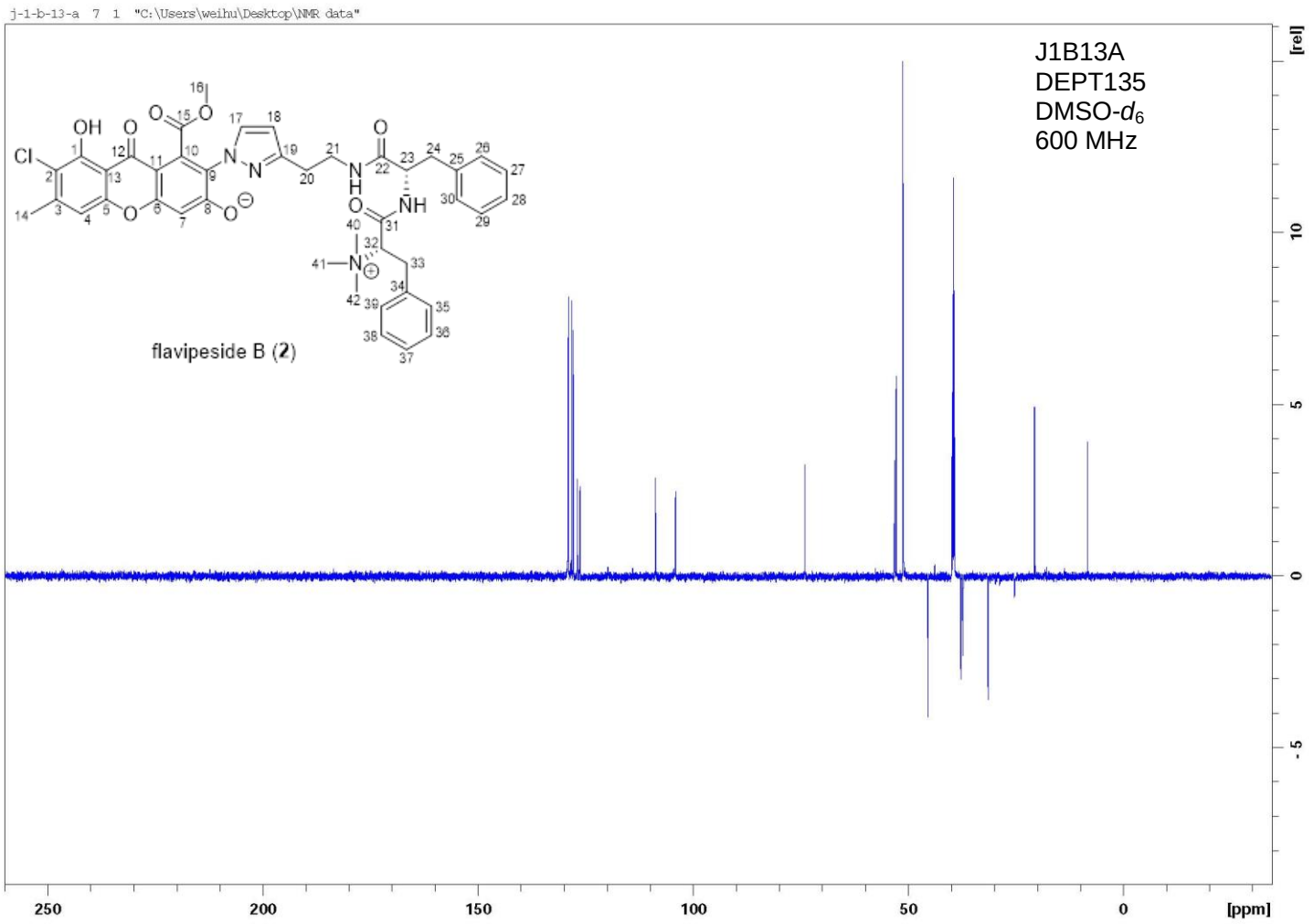


Figure S37. The DEPT135 NMR spectrum of flavipeside B (**2**) in DMSO-d_6.

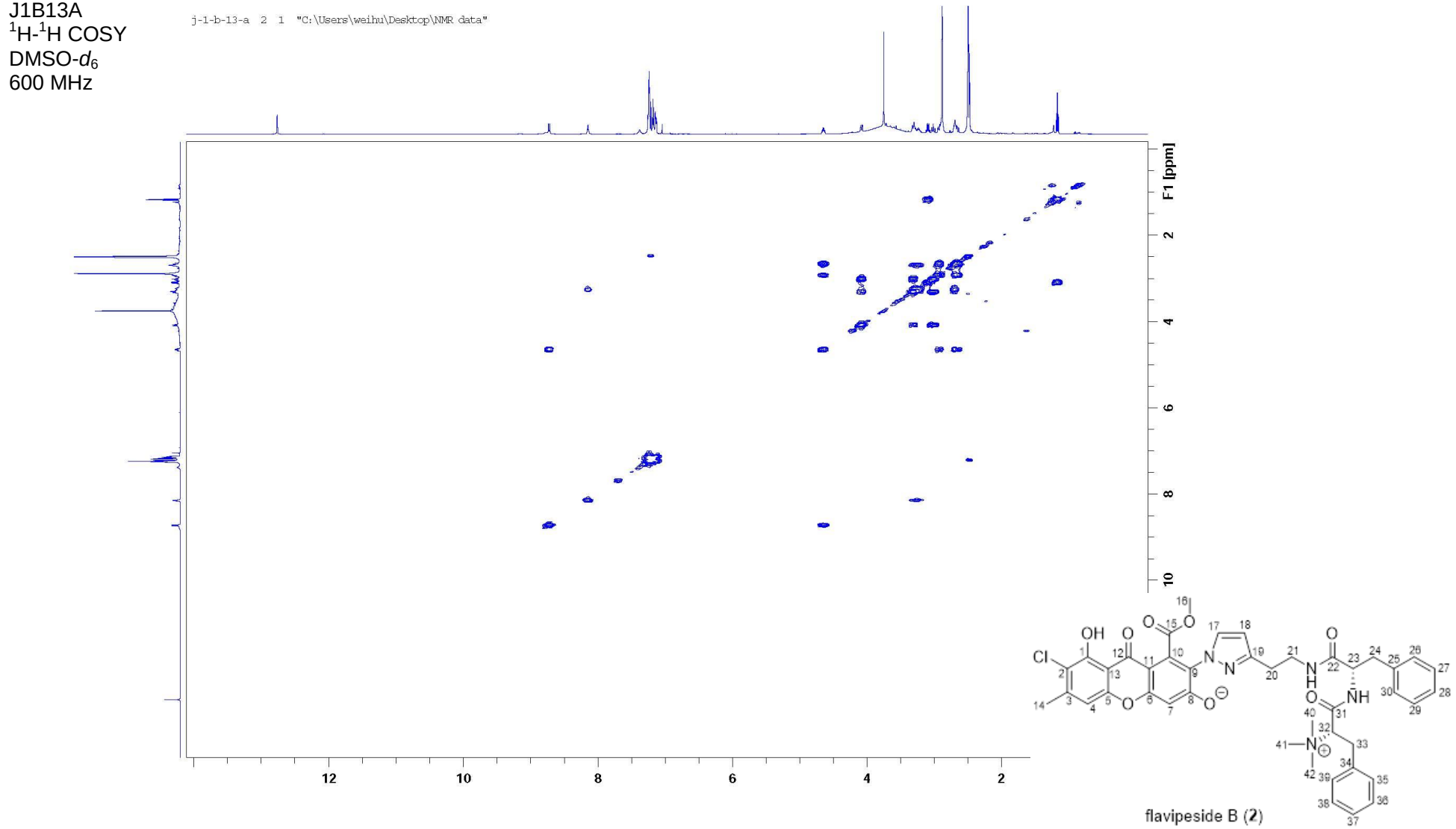


Figure S38. The ^{1}H-^{1}H COSY spectrum of flavipeside B (**2**) in DMSO-d_6.

J1B13A
HSQC
DMSO-d_6
600 MHz

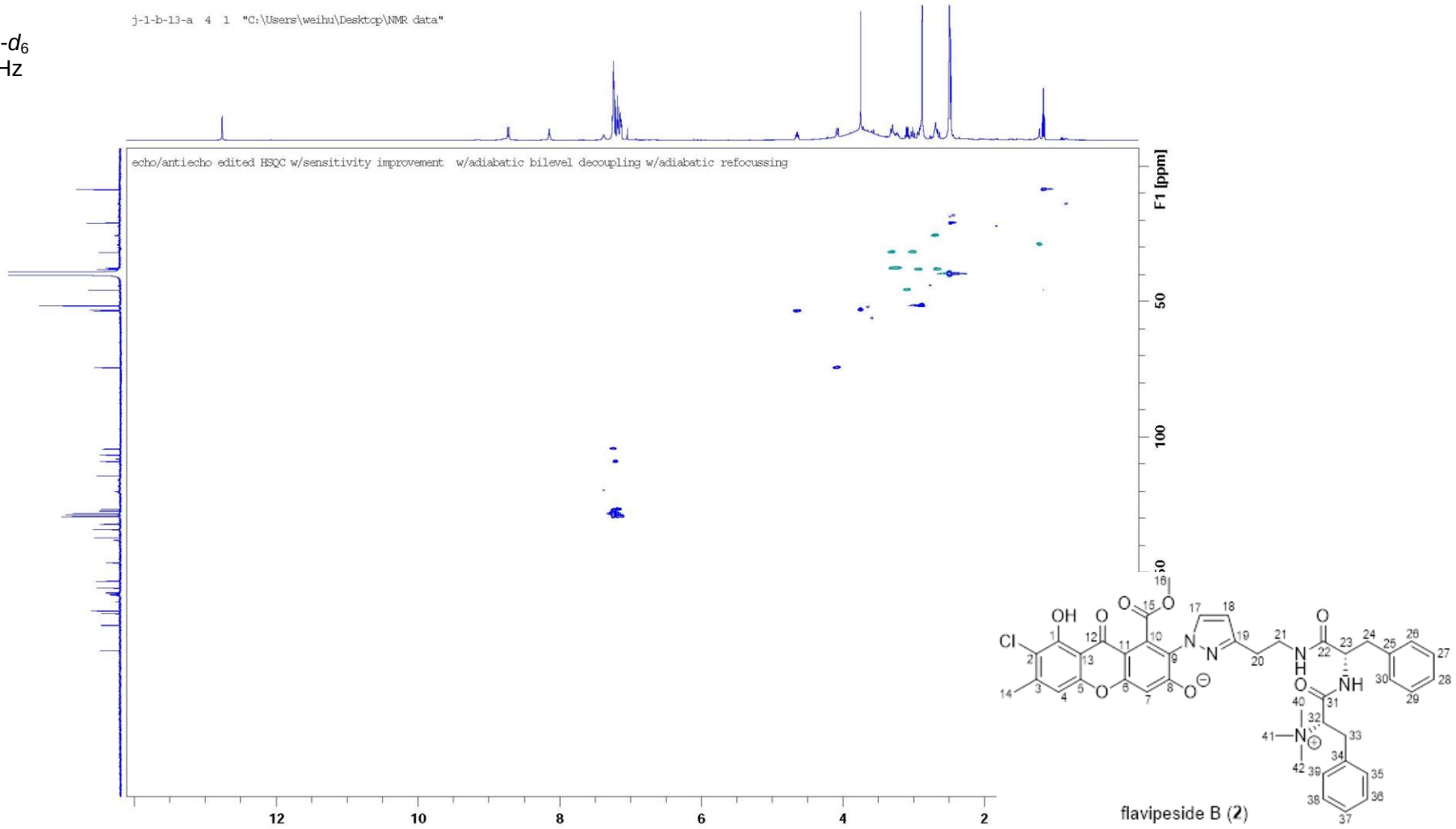


Figure S39. The HSQC spectrum of flavipeside B (**2**) in DMSO-d_6.

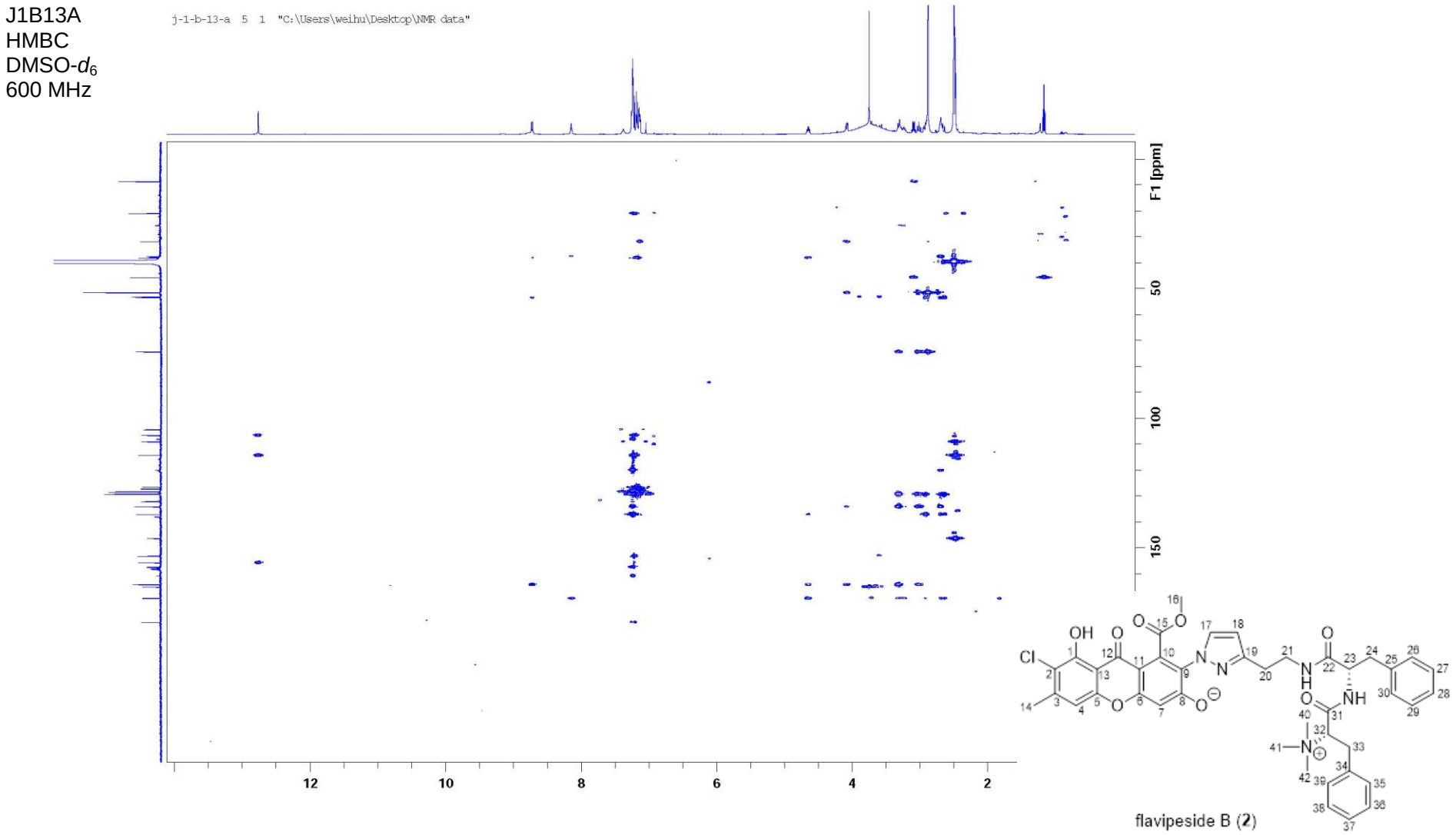


Figure S40. The HMBC spectrum of flavipeside B (**2**) in DMSO-d_6.

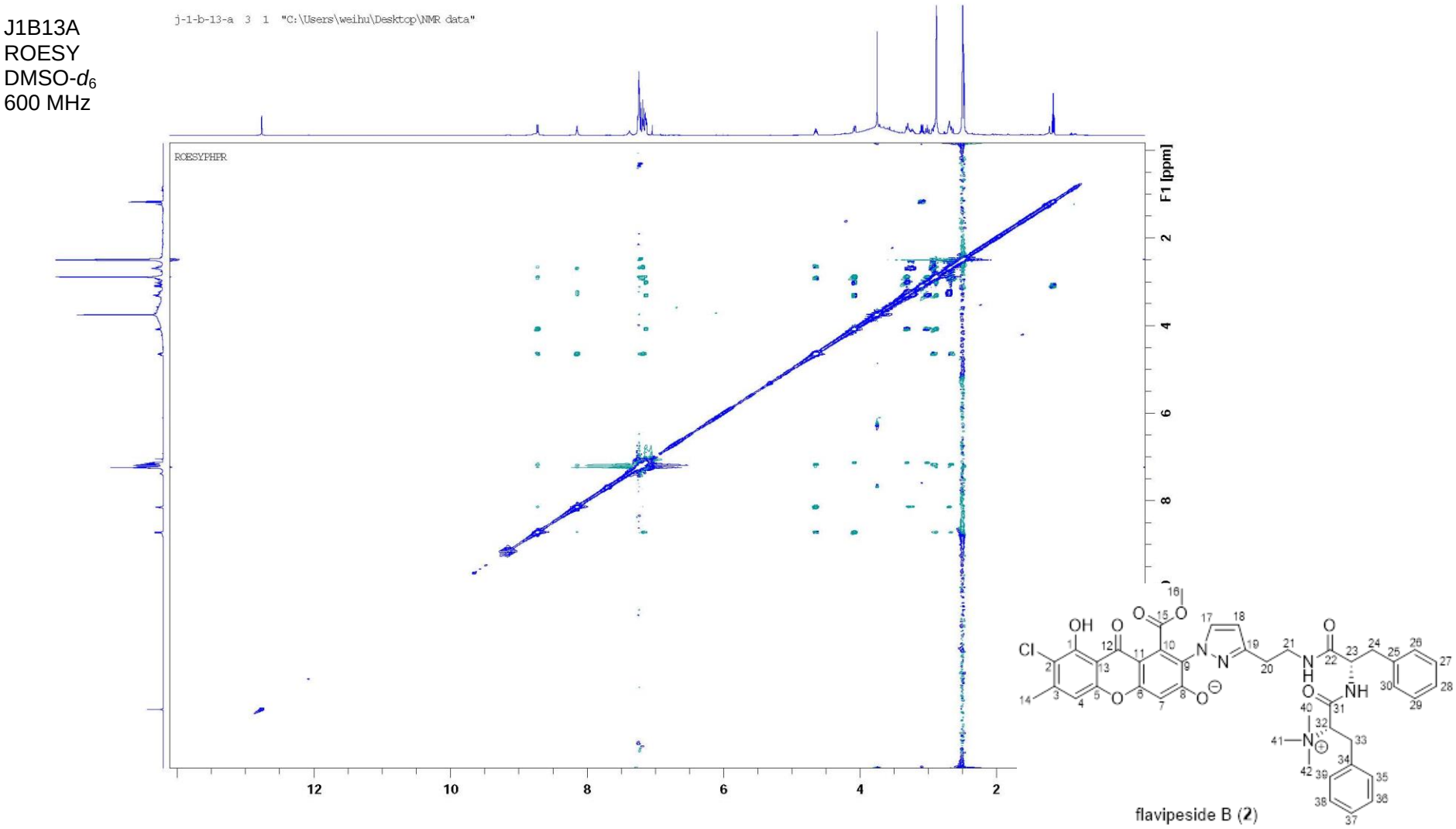


Figure S41. The ROESY spectrum of flavipeside B (**2**) in DMSO-d_6.

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

1207 formula(e) evaluated with 8 results within limits (all results (up to 1000) for each mass)

Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Cl
780.2818	780.2800	1.8	2.3	23.5	C42 H43 N5 O8 Cl	222.2	0.669	51.21	42	43	5	8	1
	780.2814	0.4	0.5	28.5	C43 H39 N9 O4 Cl	222.9	1.363	25.60	43	39	9	4	1
	780.2840	-2.2	-2.8	27.5	C47 H43 N3 O6 Cl	223.4	1.877	15.31	47	43	3	6	1
	780.2854	-3.6	-4.6	32.5	C48 H39 N7 O2 Cl	224.1	2.557	7.75	48	39	7	2	1
	780.2809	0.9	1.2	27.5	C47 H42 N O10	229.4	7.926	0.04	47	42	1	10	
	780.2822	-0.4	-0.5	32.5	C48 H38 N5 O6	229.5	8.011	0.03	48	38	5	6	
	780.2782	3.6	4.6	28.5	C43 H38 N7 O8	229.5	8.017	0.03	43	38	7	8	
	780.2835	-1.7	-2.2	37.5	C49 H34 N9 O2	229.6	8.096	0.03	49	34	9	2	

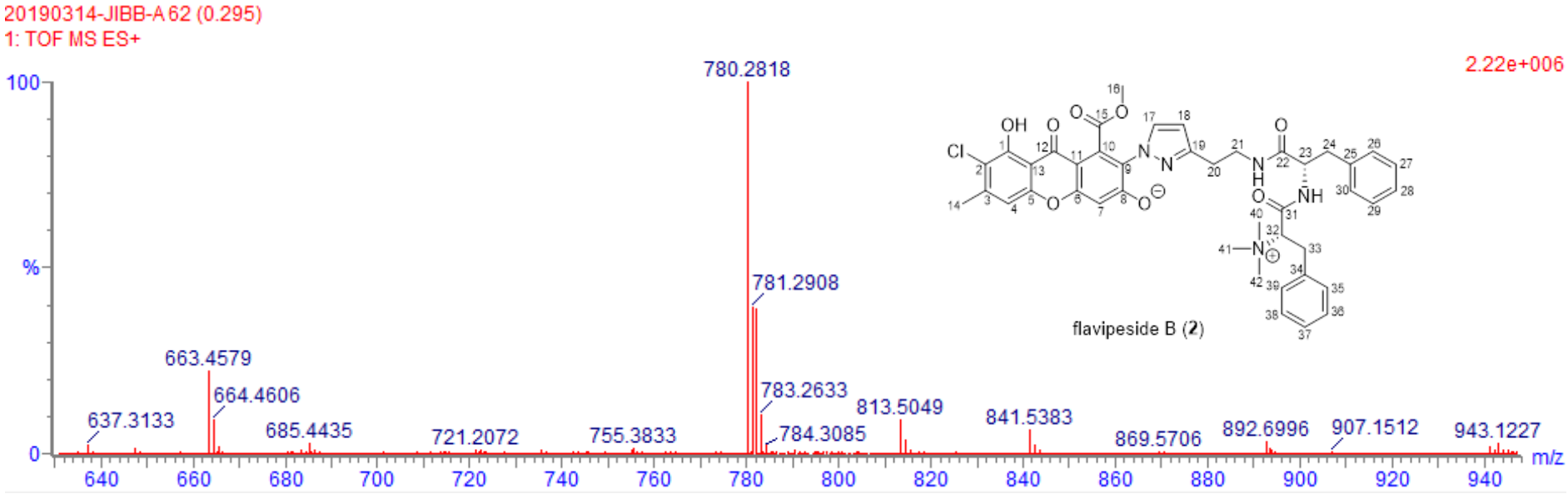


Figure S42. The HRESIMS spectrum of flavipeside B (**2**).

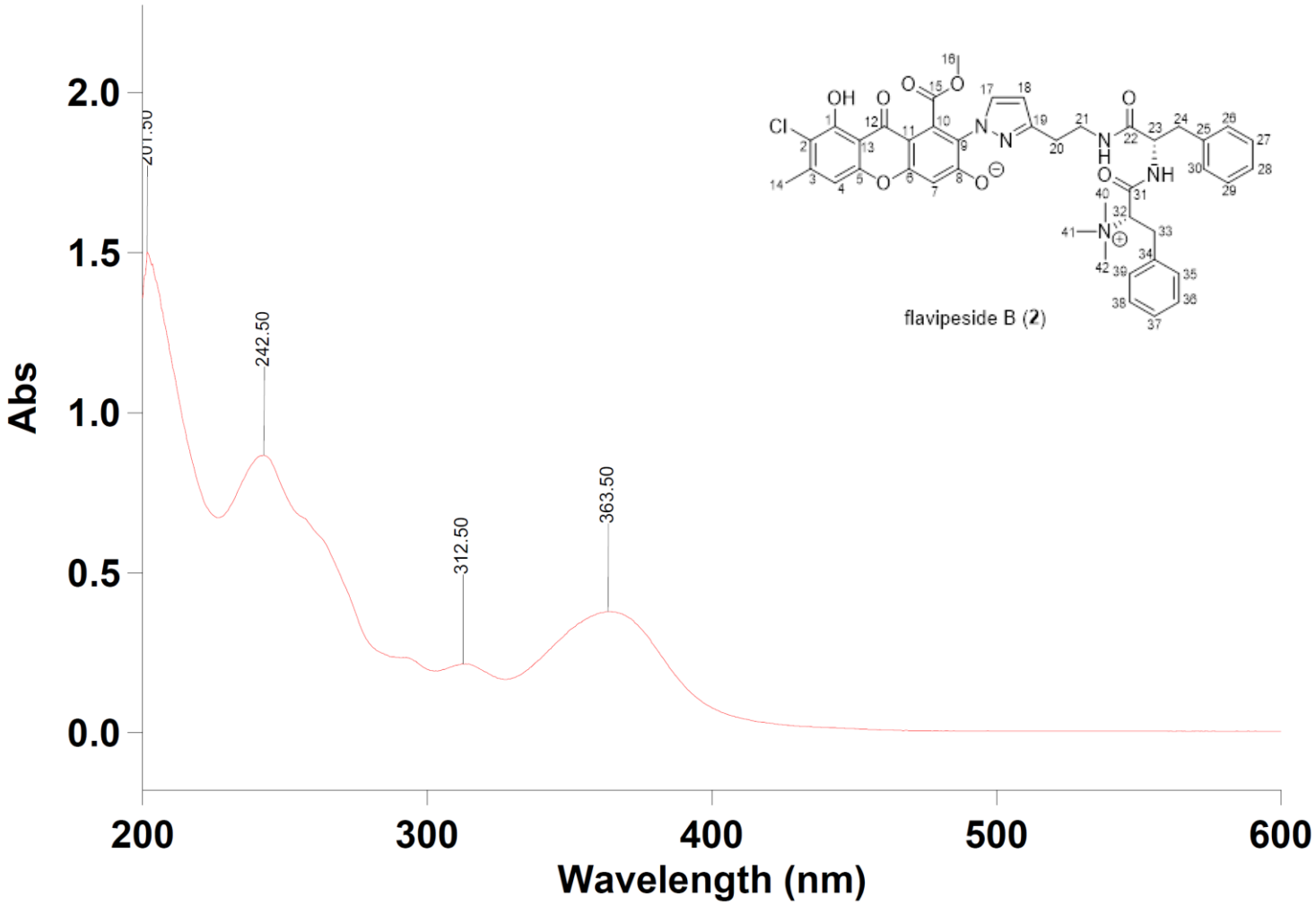


Figure S43. The UV spectrum of flavipeside B (**2**) in MeOH.

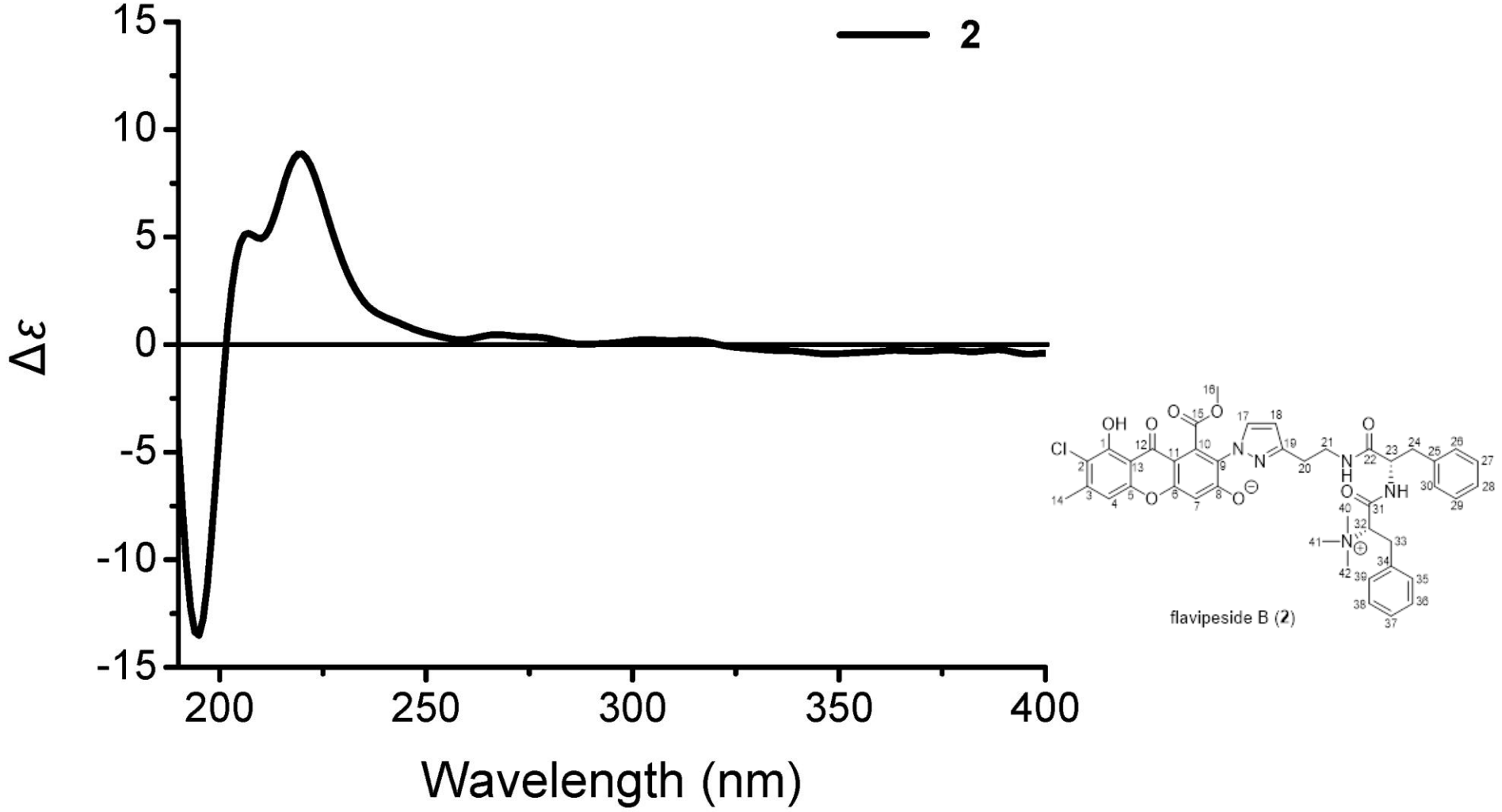


Figure S44. The CD spectrum of flavipeside B (**2**) in MeCN.

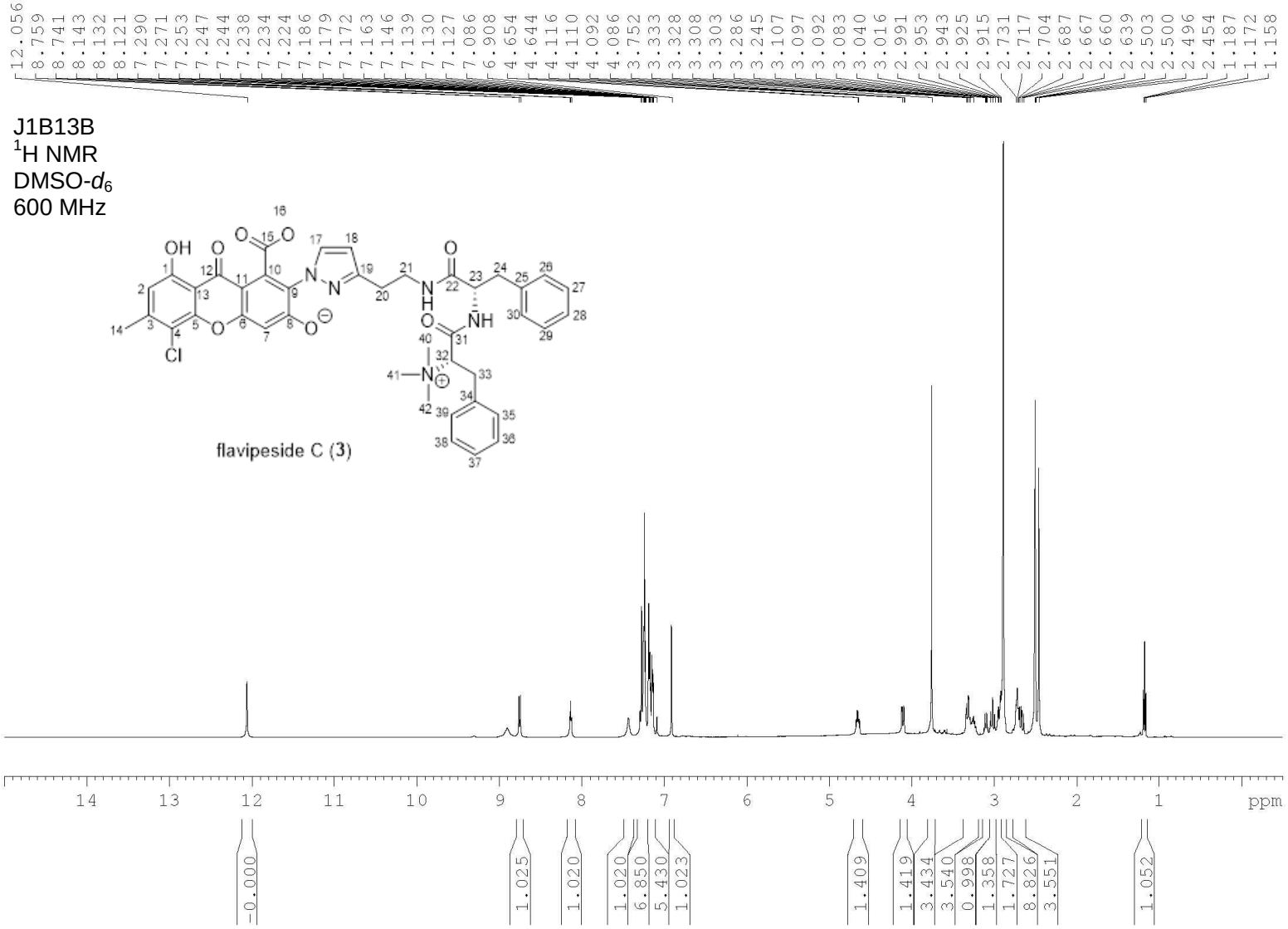


Figure S45. The ^{1}H NMR spectrum of flavipeside C (**3**) in DMSO-d_6.

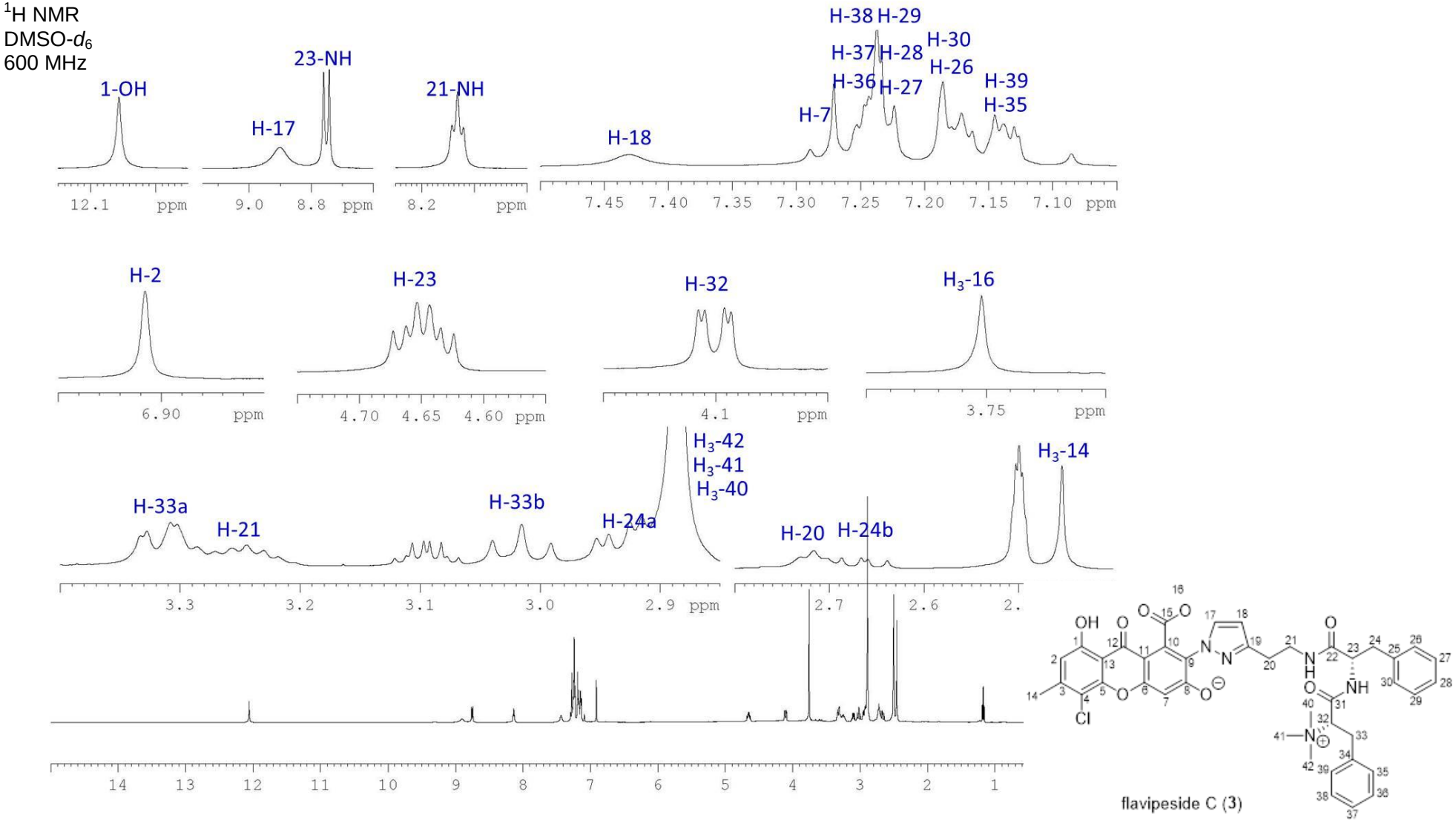


Figure S46. The ^{1}H NMR spectrum of flavipeside C (**3**) in DMSO-d_6.

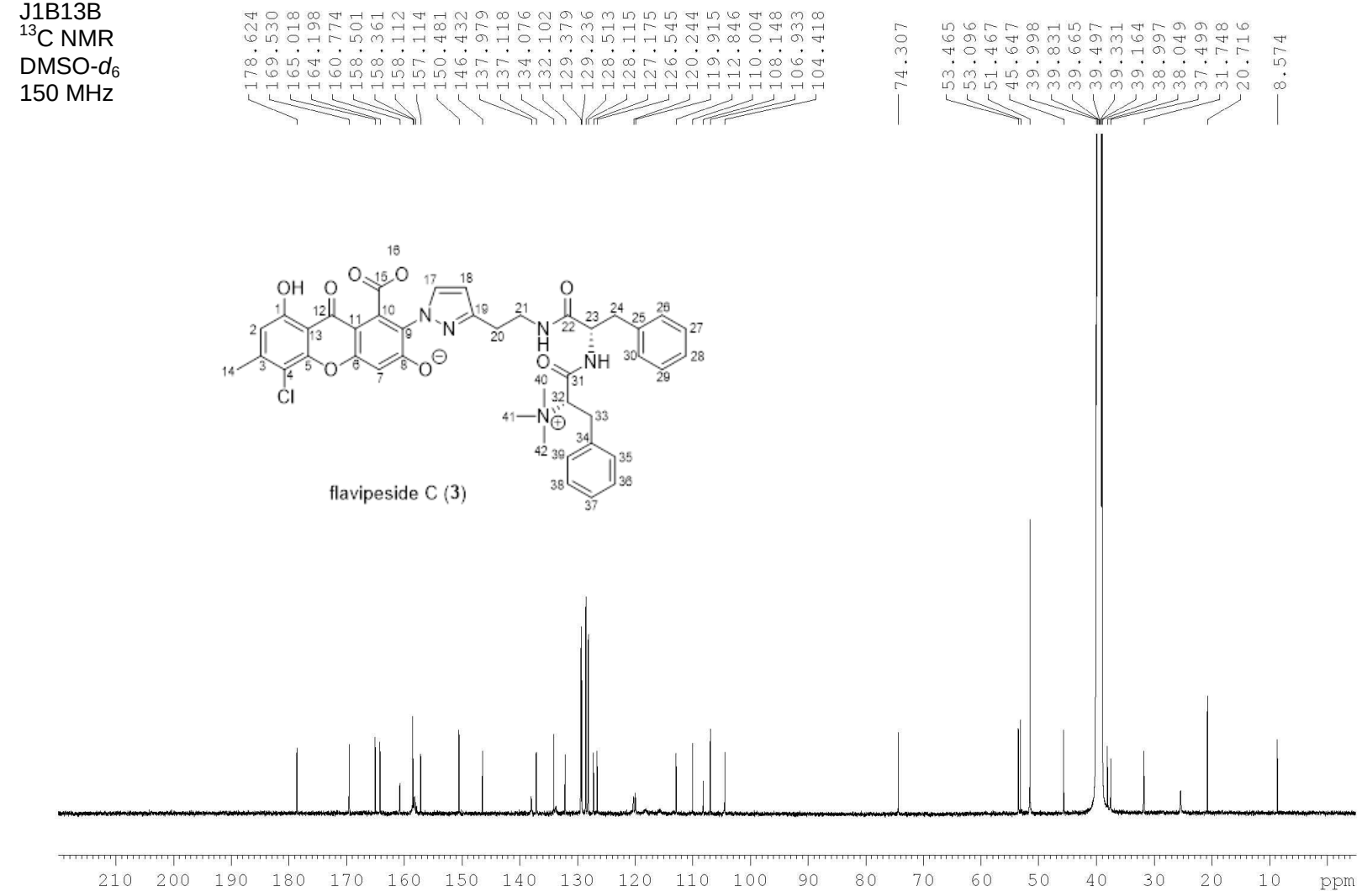


Figure S47. The ^{13}C NMR spectrum of flavipeside C (**3**) in DMSO-d_6.

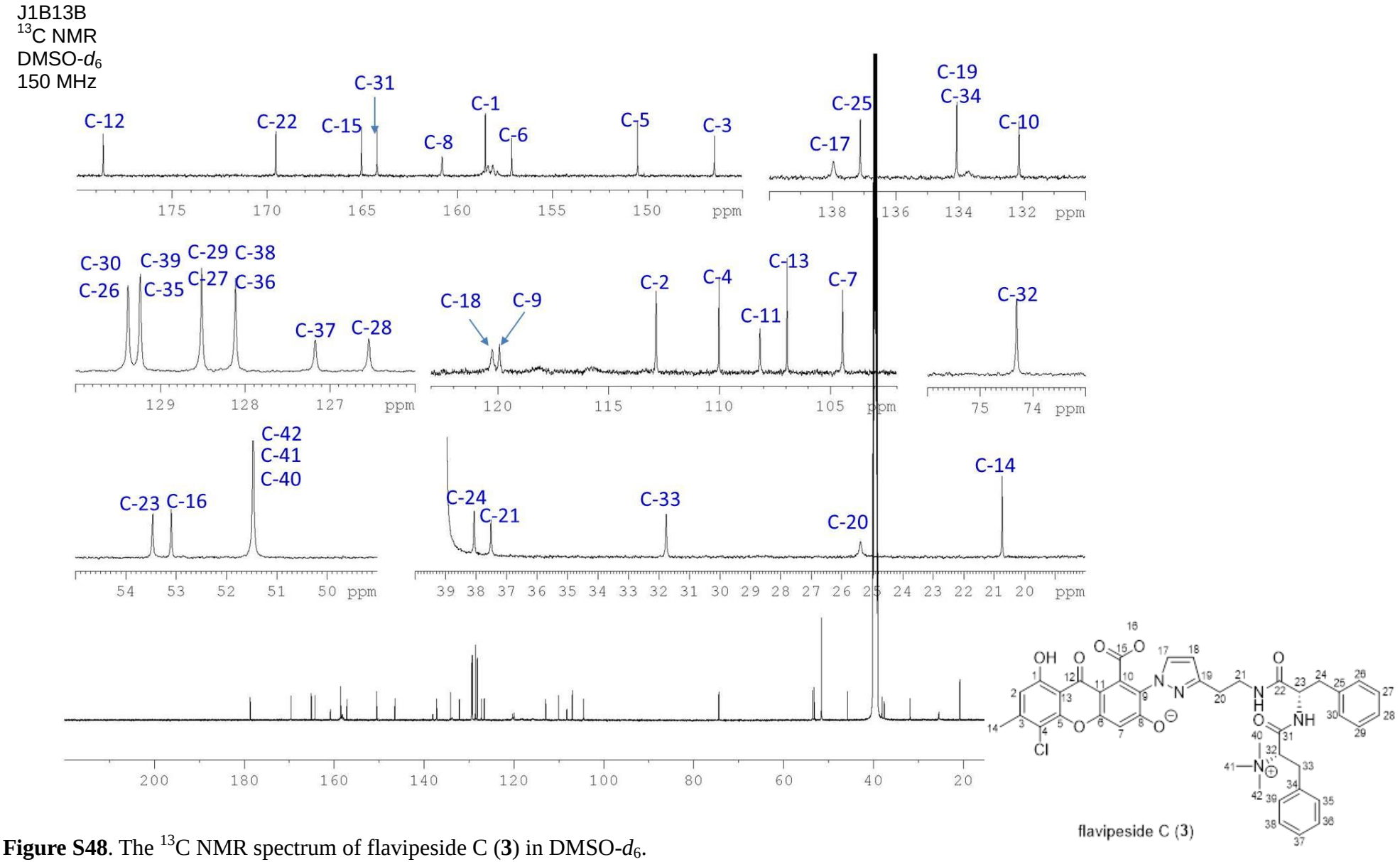


Figure S48. The ^{13}C NMR spectrum of flavipeside C (**3**) in DMSO-d_6.

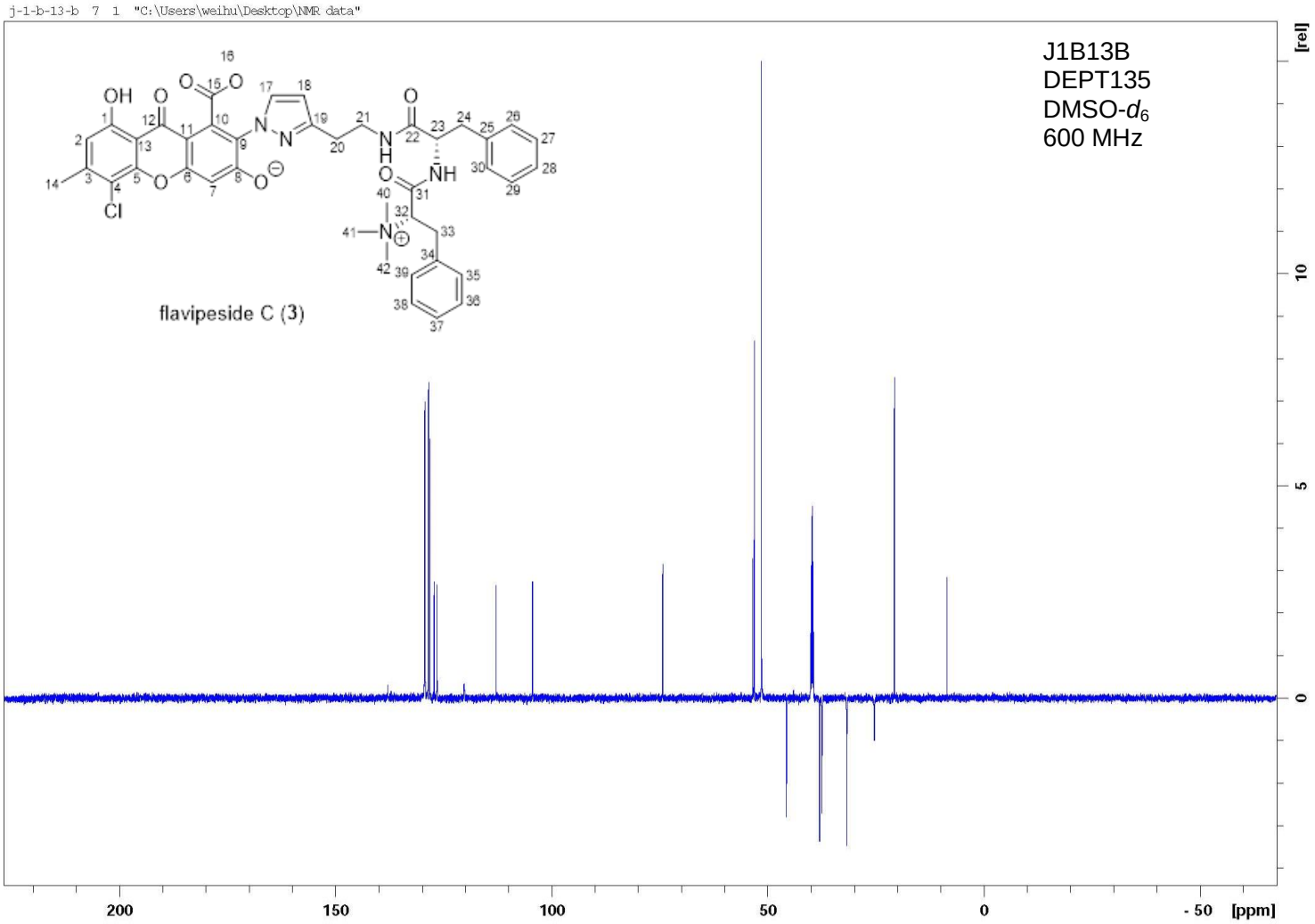


Figure S49. The DEPT135 NMR spectrum of flavipeside C (**3**) in DMSO-d_6.

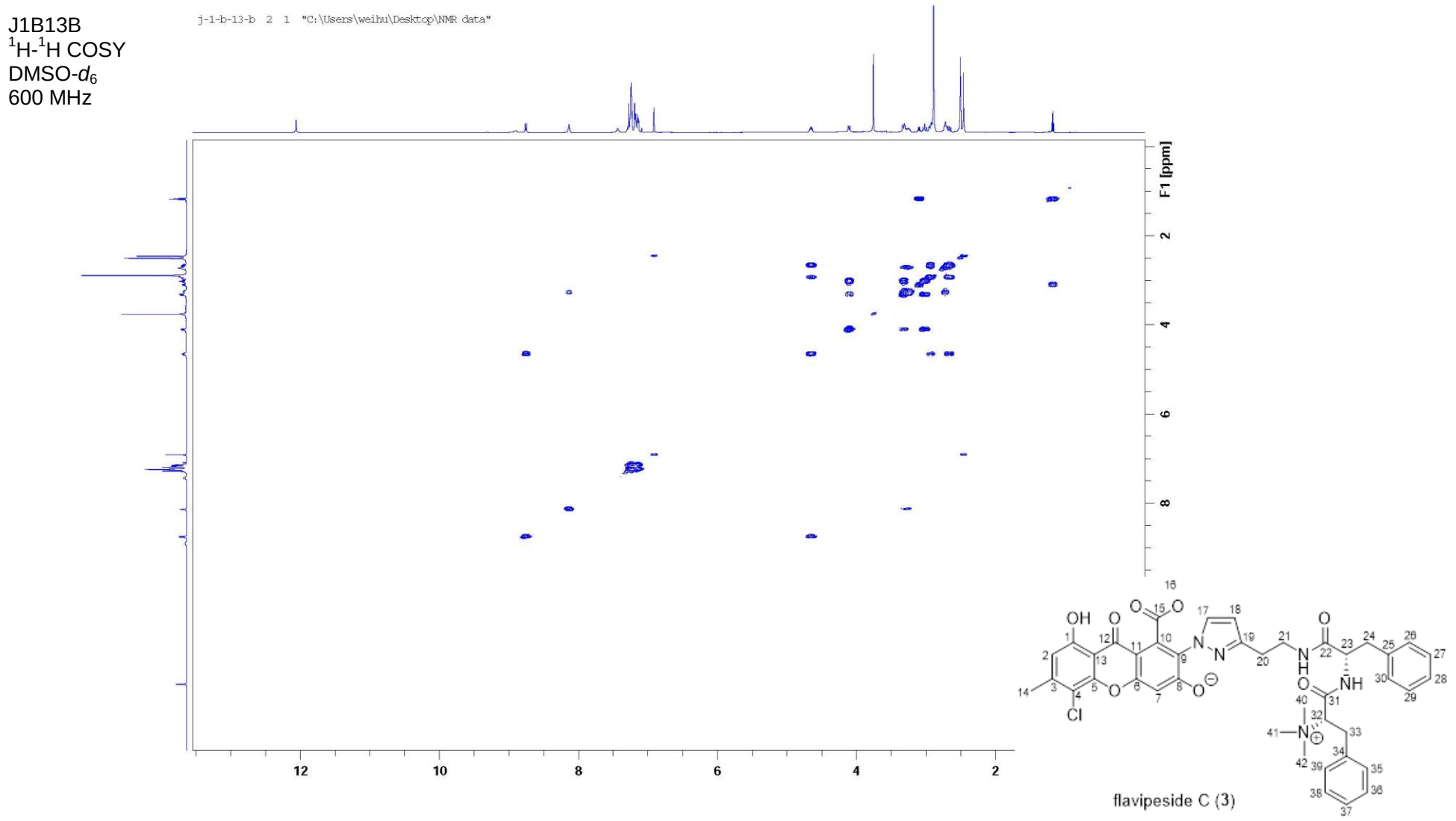


Figure S50. The ^{1}H-^{1}H COSY spectrum of flavipeside C (**3**) in DMSO-d_6.

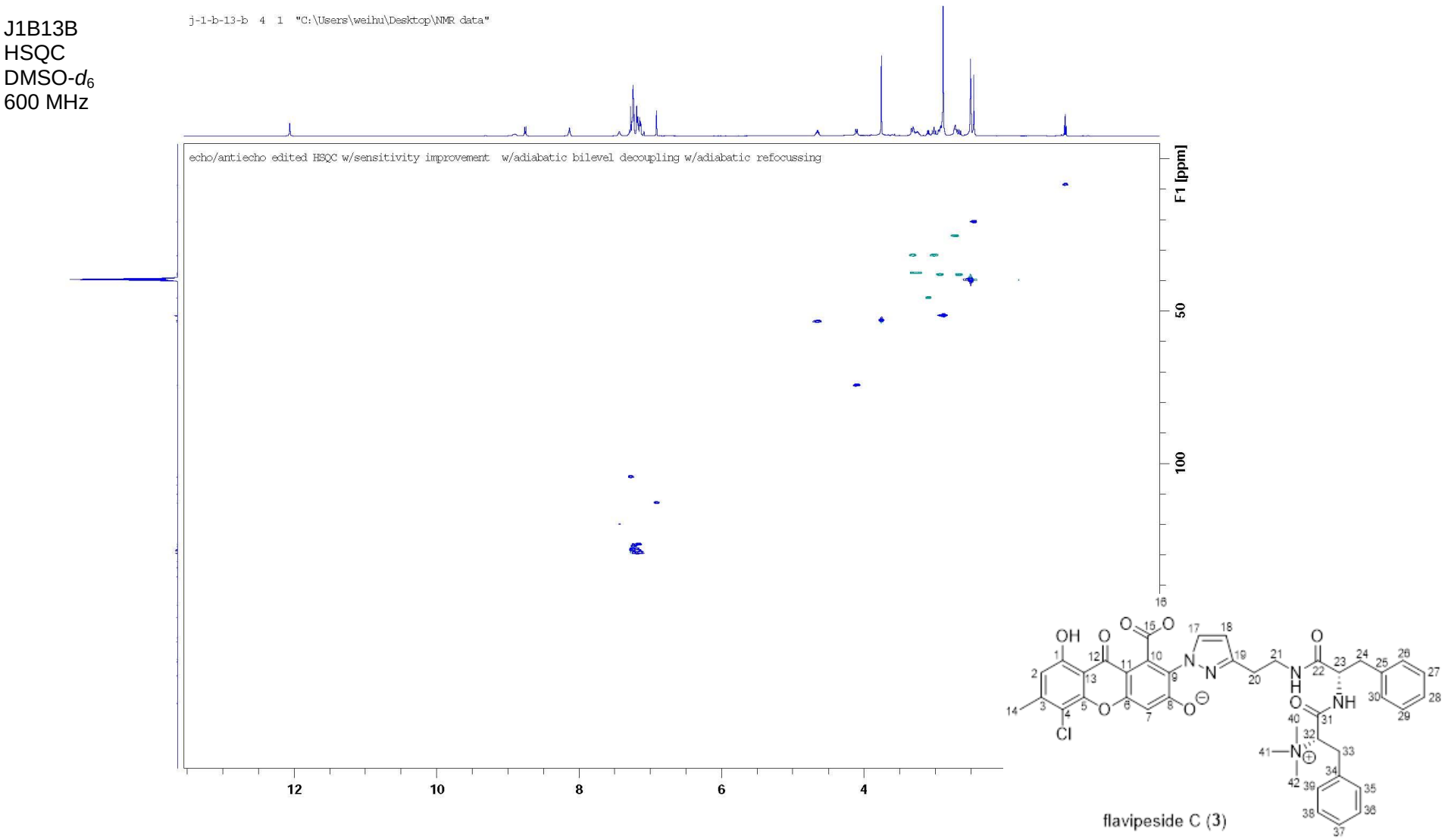


Figure S51. The HSQC spectrum of flavipeside C (**3**) in DMSO-d_6.

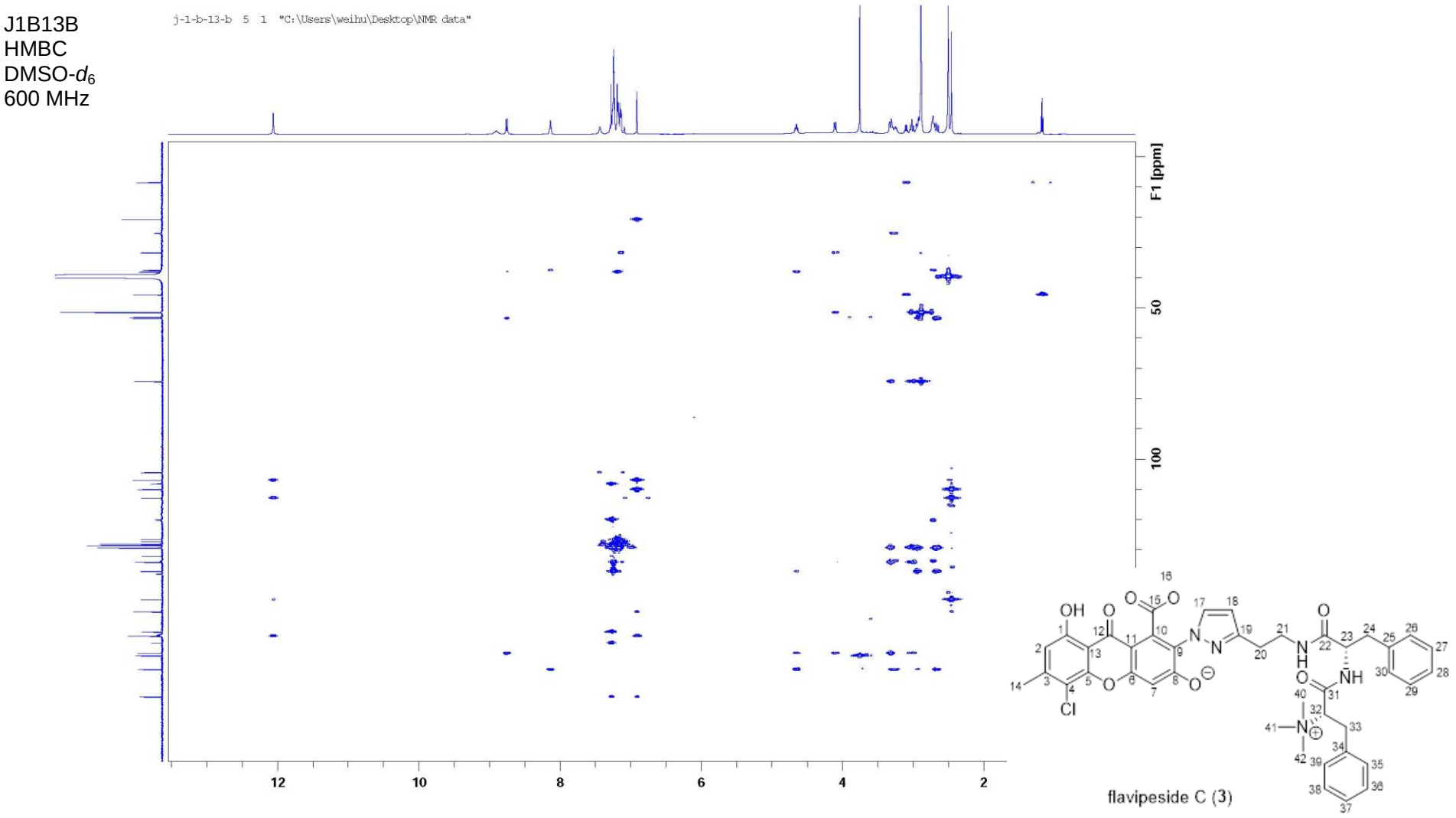


Figure S52. The HMBC spectrum of flavipeside C (**3**) in DMSO-d_6.

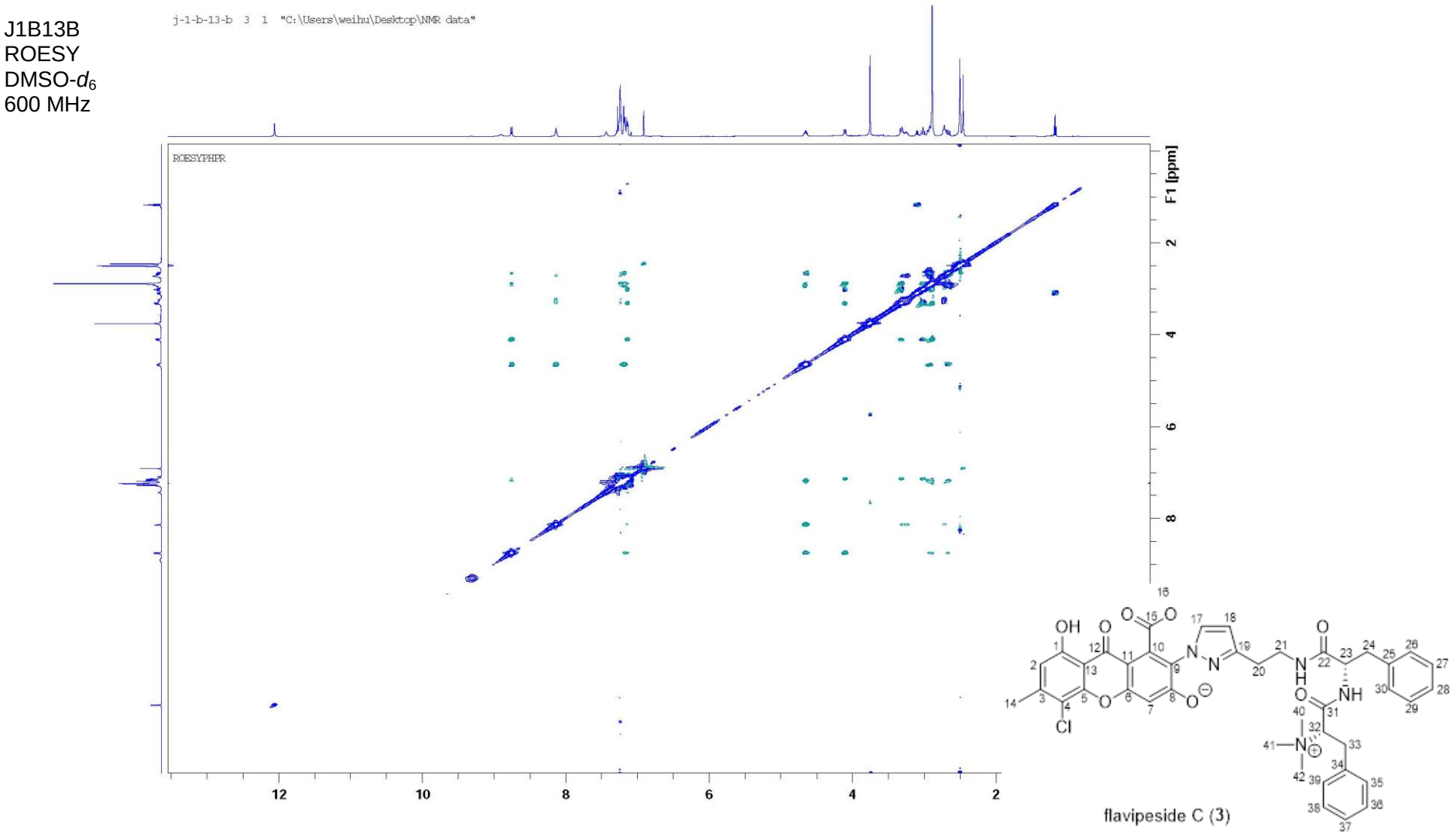


Figure S53. The ROESY spectrum of flavipeside C (**3**) in DMSO-d_6.

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
1207 formula(e) evaluated with 5 results within limits (all results (up to 1000) for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Cl
780.2795	780.2800	-0.5	-0.6	23.5	C42 H43 N5 O8 Cl	356.9	0.693	49.99	42	43	5	8	1
	780.2814	-1.9	-2.4	28.5	C43 H39 N9 O4 Cl	357.2	0.964	38.15	43	39	9	4	1
	780.2809	-1.4	-1.8	27.5	C47 H42 N O10	359.4	3.192	4.11	47	42	1	10	
	780.2782	1.3	1.7	28.5	C43 H38 N7 O8	359.4	3.223	3.98	43	38	7	8	
	780.2822	-2.7	-3.5	32.5	C48 H38 N5 O6	359.5	3.281	3.76	48	38	5	6	

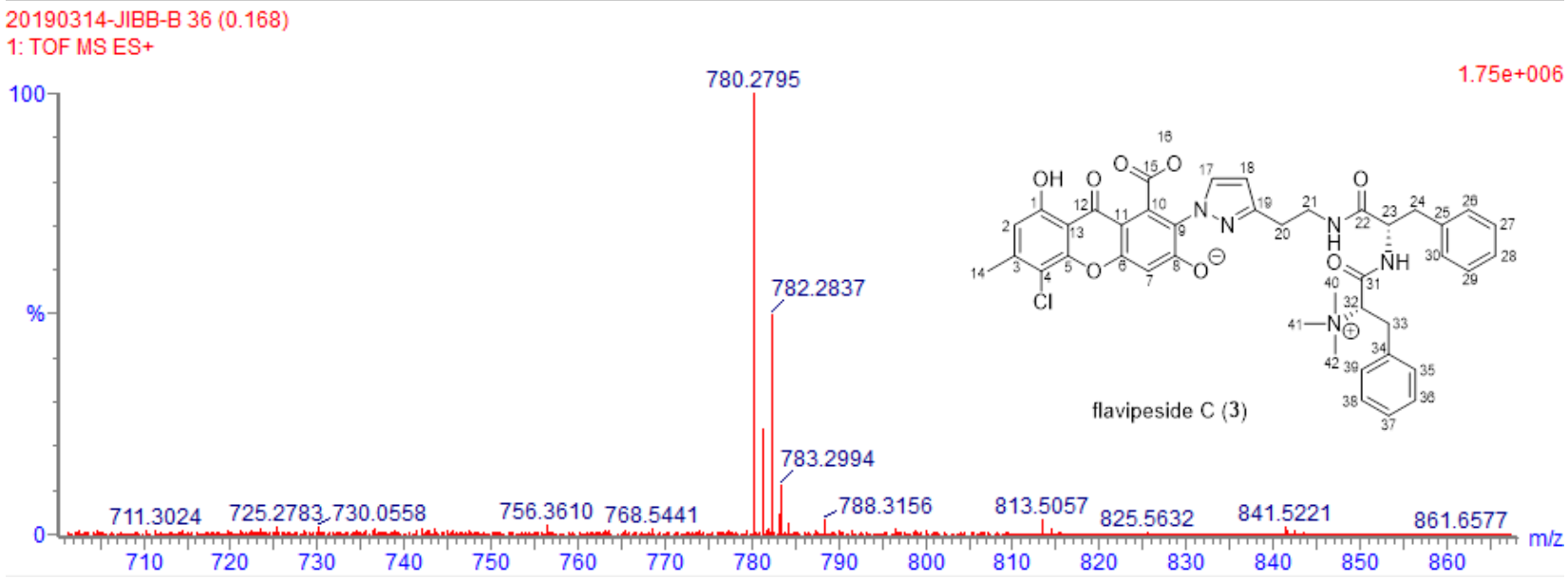


Figure S54. The HRESIMS spectrum of flavipeside C (**3**).

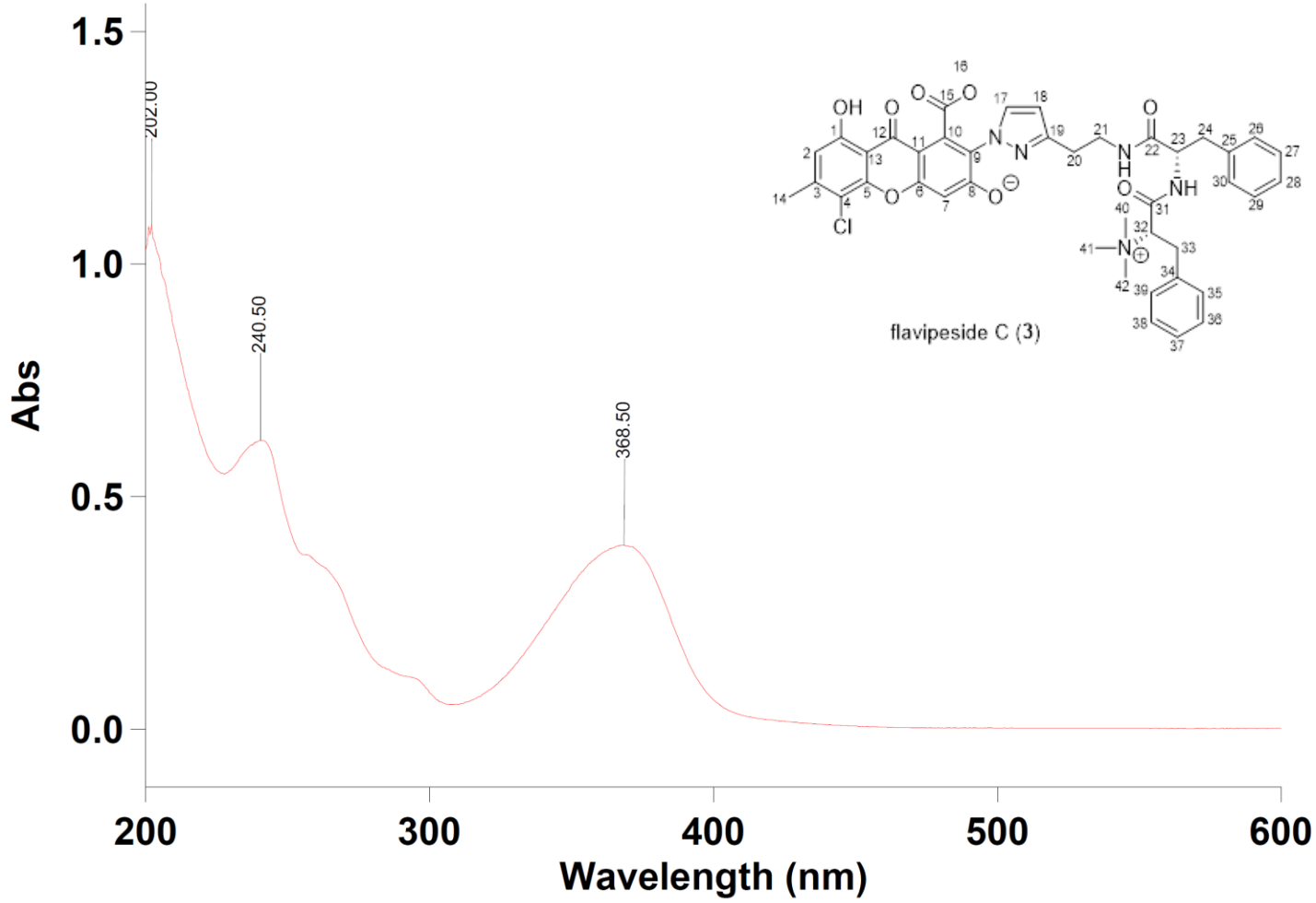


Figure S55. The UV spectrum of flavipeside C (**3**) in MeOH.

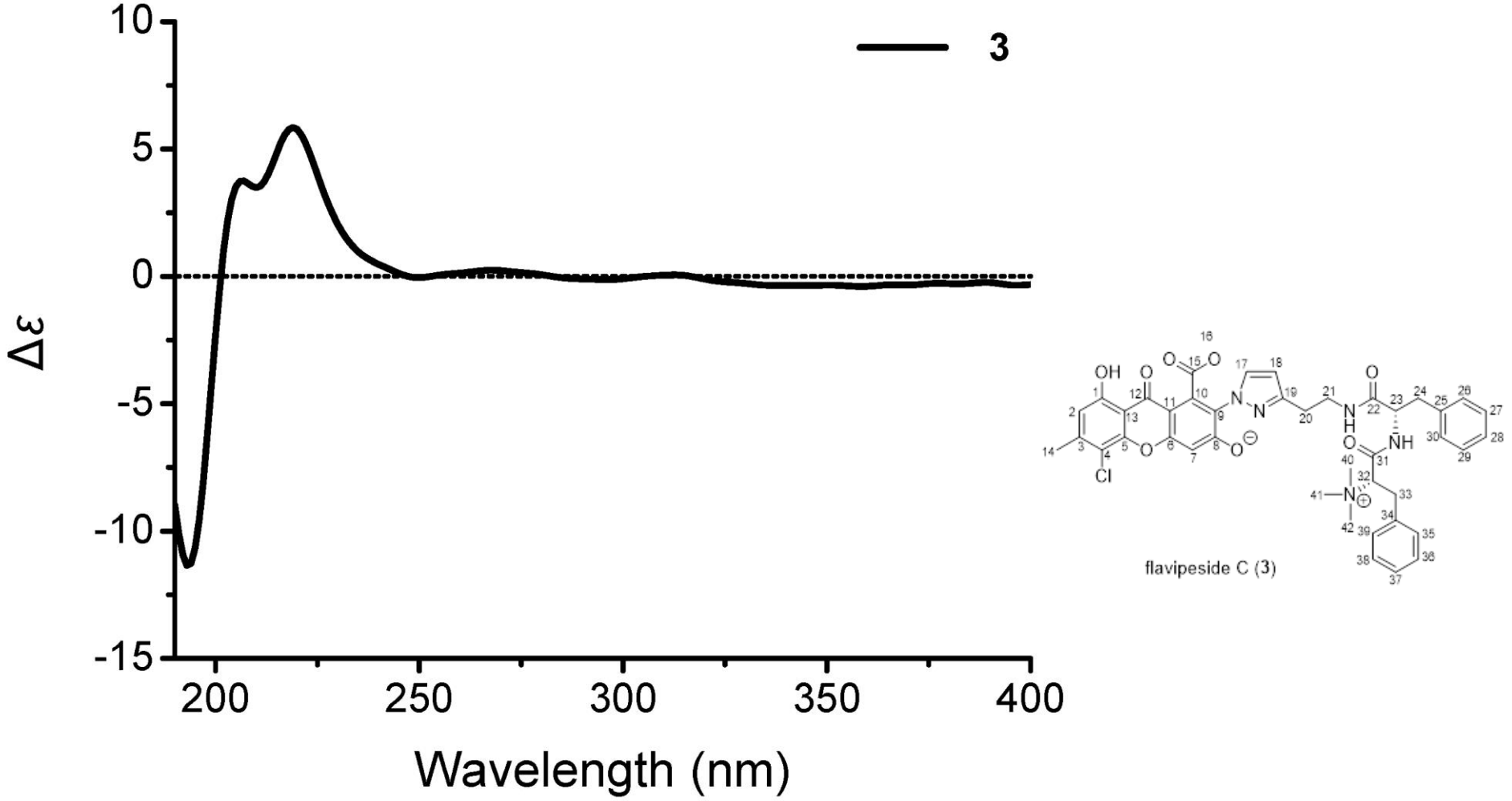


Figure S56. The CD spectrum of flavipeside C (**3**) in MeCN.