Supporting Information:

Edonamides, the first secondary metabolites from the recently described Myxobacterium *Aggregicoccus edonensis*

Sabrina Karwehl,[†,‡] Kathrin I. Mohr,[†,‡] Rolf Jansen,[†,‡] Sakshi Sood,[††] Steffen Bernecker,[†,‡] and Marc Stadler*[,†,‡]

[†]Department of Microbial Drugs, Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany

[‡]German Centre for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Braunschweig, Germany

[††]current address: Mycobacterial division, The Francis Crick Institute, The Ridgeway, Mill Hill, London UK, NW7 1AA

* To whom correspondence should be addressed. Tel.: +49-531-61844240. Fax: +49-531-61819499. E-Mail: marc.stadler@helmholtz-hzi.de.

General Experimental Procedure. UV data were recorded on a Shimadzu UV/Vis-2450 spectrophotometer in methanol (UVASOL, Merck). IR data was recorded on a Bruker IR Tensor 27 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ascend 700 NMR spectrometer, locked to the deuterium signal of the solvent. Data acquisition, processing, and spectral analysis were performed with standard Bruker software and ACD/NMRSpectrus. Chemical shifts are given in parts per million (ppm), and coupling constants in hertz (Hz). HRESIMS data were recorded on a MaXis ESI TOF mass spectrometer (Bruker Daltonics), and molecular formulas were calculated including the isotopic pattern (Smart Formula algorithm). Analytical RP HPLC was carried out with an Agilent 1260 HPLC system equipped with a diode-array UV detector (DAD) and a Corona Ultra detector (Dionex) or a Maxis ESI TOF mass spectrometer (Bruker Daltonics). HPLC conditions: XBridge C18 column 100×2.1 mm, 3.5 µm, solvent A: H_2O/acetonitrile (95/5), 5 mmol NH_4OAc, 0.04 mL/L CH_3COOH; solvent B: H_2O/acetonitrile (5/95), 5 mmol NH_4OAc, 0.04 mL/L CH_3COOH; gradient: 10% B increasing to 100% B in 30 min; flow rate 0.3 mL/min; 40 °C.

Myxobacterial strain. Strain MCy1366 was isolated in 1981 from a soil sample collected from a region near Tokyo, Japan, and is the type-strain of the recently described novel myxobacterial genus and species, *Aggregicoccus edonensis* (DSM 27872[T; 14]). The 16S rRNA sequence of strain MCy1366 was deposited at the National Center for Biotechnology Information (NCBI) under the number KF767690.

Cultivation of MCy1366. The strain was activated from –80 °C in 20 mL of CY/H medium consisting of 0.15% casitone, 0.15% yeast extract, 0.1% soymeal extract, 0.1% glucose, 0.4% starch (Cerestar), 0.05% $MgSO_4$×7 H_2O, 0.1% $CaCl_2$×2 H_2O, 50 mM HEPES, and 4 mg/L Fe-EDTA at pH 7.3. The culture was scaled up to 4.5 L of medium M7-2 consisting of 0.5% probion (single cell protein), 2.0% starch, 0.2% glucose, 0.1% yeast extract, 0.1% $CaCl_2$, 0.1% $MgSO_4$×7 H_2O and used as inoculum for the fermentation of strain MCy1366 that was performed in 70 L of the same media as above but supplemented with 2% Amberlite XAD-16 resin in a 100 L bioreactor. Fermentation was conducted for 460 h at 37 °C with submerged fumigation (0.05 vvm), pO_2-regulation 20%, speed > 100 rpm, pH 6.4-6.6 (regulated with 5% KOH and 5% H_2SO_4). At the end of fermentation the XAD resin (450 g) was recovered from the culture by sieving.

Extraction and Isolation. The XAD resin was eluted in a glass column with 1.5 L of methanol and 1.5 L of acetone. The methanol eluate was concentrated in vacuo to yield an

aqueous residue, which was diluted with water to 300 mL, and extracted three times with 300 mL ethyl acetate (EA). The combined EA extract was dried with Na_2SO_4 and evaporated to give 13.9 g of crude extract.

To isolate edonamides A (**1**) and B (**2**) 4.5 g of the crude extract were further separated by Si-Flash chromatography [column: Reveleris Silica 40 g; solvent A: dichloromethane; solvent B: acetone; gradient system: holding 0% B for 12 min, increasing to 5% B in 1 min, holding at 5% B for 6 min, increasing to 15% B in 0.3 min, holding at 15% B for 6 min, increasing to 100% B in 0.3 min; flow rate 40 mL/min; detection: ELS and UV at 220, 280 and 300 nm] yielding 1.0 g of an enriched fraction with amides **1** and **2**. Pure amides **1** and **2** were isolated from the mixture by RP-MPLC [column: Kronlab ODS-AQ C18, 480×30 mm, 15 µm; solvent A: methanol/H_2O (2/8); solvent B: methanol; gradient system: holding 65% B for 5 min, increasing to 100% B in 80 min; flow rate 30 mL/min, UV detection at 300 nm] to give 18.6 mg of edonamide A (**1**) and 1.4 mg of edonamide B (**2**), corresponding to 57.5 and 4.3 mg from 70 L culture, respectively.

Cytotoxicity assay. In vitro cytotoxicity (IC_{50}) was determined with 3.0 µL (30 µg/ml) of compounds **1** and **2** against a number of mammalian cell lines by serial dilution (60 µl) in 96 well plates for tissue cultures (Falcon). The assay included the mouse fibroblast cell line L929, breast cancer cell line MCF-7, and epidermoid carcinoma cell line A431. Line L929 was cultured in DMEM (Lonza), MCF-7 and A431 were cultured in RPMI (Gibco), all supplemented with 10% of fetal bovine serum (Gibco) and incubated under 5% CO_2 at 37 °C for five days. Methanol was used as the negative control.

Table S1: NMR Data of edonamide A (**1**) in CD_3OD (700.4 MHz; 125.8 MHz).

position	δ_C, mult	δ_H, mult (*J* in Hz)	COSY	H in HMBC	ROESY
1	171.0, C			12, 11	
2		9.22, br d (8.4)	3		
3	124.5, CH	7.53, d (14.6)	4, 2	4	6/10, 4
4	111.8, CH	6.15, d (14.8)	3	6/10	6/10, 3
5	138.1, C			7/9, 3	
6/10	126.1, CH	7.33, dd (8.0, 1.3)	7/9	4, 8	4, 3
7/9	129.5, CH	7.27, t (7.5)	8, 6/10	8	
8	126.9, CH	7.13, tt (7.3, 1.2)	7/9	7/9	
11	36.7, CH_2	2.29, t (7.4)	12	12	13, 12
12	26.2, CH_2	1.65, quin (7.1)	13, 11	11	15, 13, 11
13	30.3, CH_2	1.32, m	15, 12	15, 12, 11	17/18, 15, 16, 12, 11
14	28.0, CH_2	1.32, m		15, 13, 16, 12	
15	39.7, CH_2	1.18, m	13, 16	17/18, 16	13, 16, 12, 17/18
16	28.7, CH	1.53, dquin (13.3, 6.7)	17/18, 15	17/18	15, 13, 17/18
17/18	23.0, CH_3	0.87, d (6.5)	16	15, 16	15, 16, 13

Table S2: NMR Data of edonamide B (**2**) in CD_3OD (700.4 MHz; 125.8 MHz).

position	δ_C, mult	δ_H, mult (*J* in Hz)	COSY	H in HMBC	N in HMBC	ROESY
1	171.1, C			12, 11		
2		9.22, br d (8.2)	3	3, 4, 11		3
3	124.5, CH	7.53, dd (14.6, 10.5)	4, 2		2	6/10, 2
4	111.8, CH	6.15, d (14.6)	3		2	
5	138.1, C			7/9		
6/10	126.1, CH	7.33, dd (8.2, 1.1)	7/9	4, 8		3
7/9	129.5, CH	7.27, t (7.4)	8, 6/10			8
8	126.9, CH	7.13, tt (7.3, 1.3)	7/9	6/10		7/9
11	36.7, CH_2	2.28, t (7.4)	12	12	2	12
12	26.2, CH_2	1.64, quin (7.4)	11	11		13, 11
13	29.9, CH_2	1.32, m		12, 11		12
14	29.9, CH_2	1.29, m		13, 12, 11		
15	32.6, CH_2	1.28, br s	17	17, 16		16
16	23.4, CH_2	1.29, m	17	17		17, 15
17	14.4, CH_3	0.88, br t (7.1)	15, 16			16

Table S3: Antimicrobial Minimum Inhibitory Concentrations (MIC) in µg/mL of edonamides A (**1**) and B (**2**). 20 µL and 2 µl of a 1 mg/mL concentration solved in MeOH (67 µg/mL) was tested. 20 µL MeOH was tested as negative control and showed no activity against the selected test organisms. n.i. no inhibition

	µg/mL	DSM No.	1	2	reference
Gram +	*Bacillus subtilis*	10	/	/	8.3[b]
	Staphylococcus aureus	346	/	/	0.21[b]
	Mycobacterium sp.	43270	/	/	0.83[b]
	Micrococcus luteus	1790	/	/	0.83[b]
Gram -	*Pseudomonas aeruginosa* PA14	/	/	/	0.1[c]
	*Chromobacterium violaceum**	30191	/	/	0.83[b]
	Escherichia coli	1116	/	/	3.3[b]
yeasts	*Schizosaccharomyces pombe*	70572	/	/	33.3[a]
	Rhodotorula glutinis	10134	/	/	8.3[a]
	Candida albicans	1665	/	/	33.3[a]
	Pichia anomala	6766	/	/	33.3[a]
fungi	*Mucor hiemalis*	2656	/	/	16.6[a]

[a]Nystatin; [b]Oxytetracyclin; [c]Gentamycin

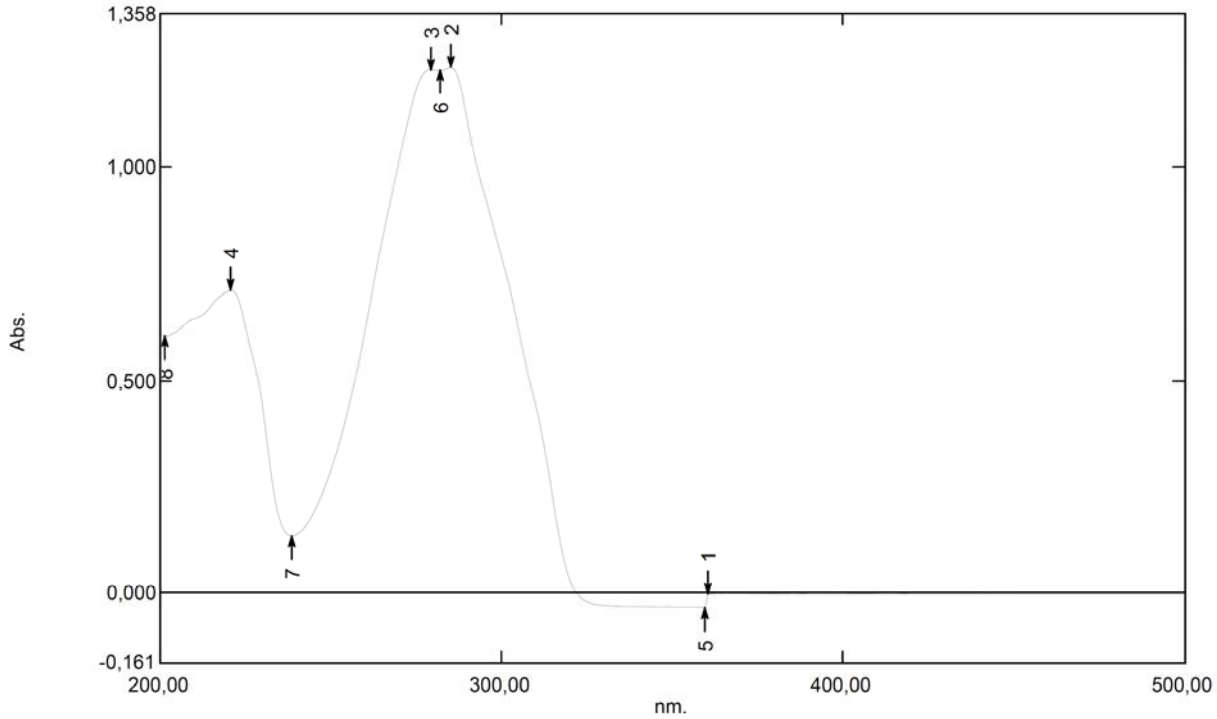


Figure S1: UV spectrum of Edonamide A (**1**) in MeOH.

Figure S2: IR spectrum of Edonamide A (**1**) (diamond ATR).

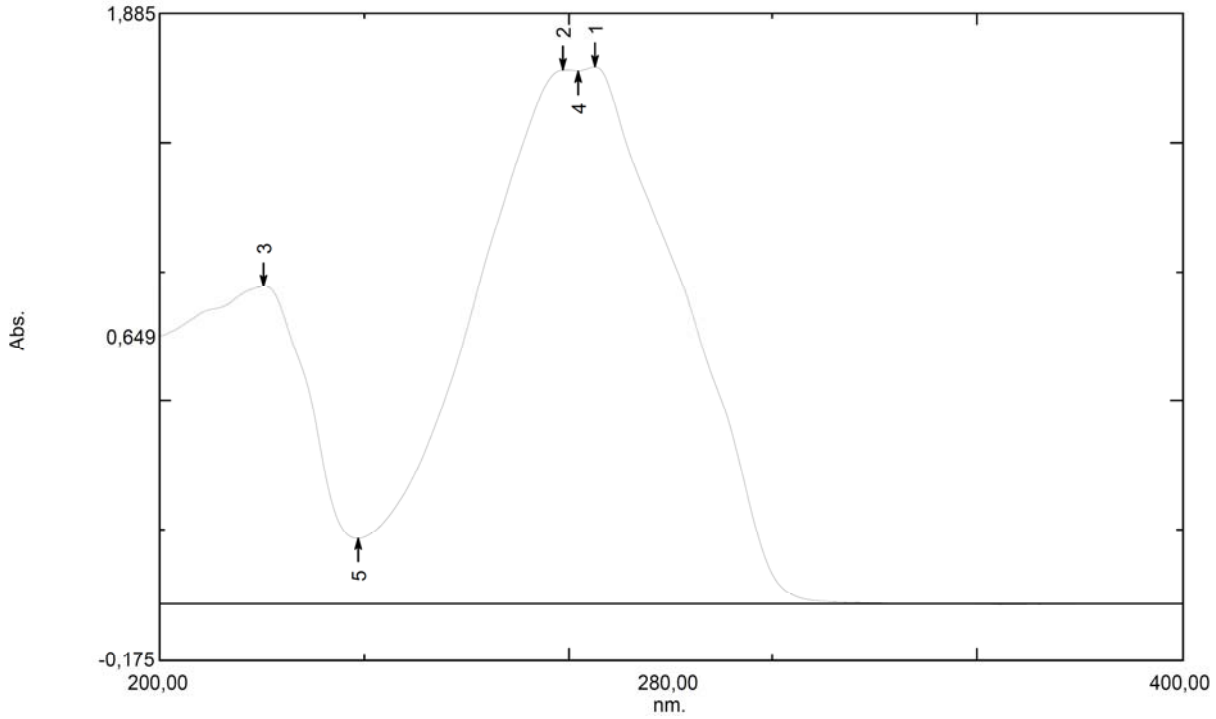
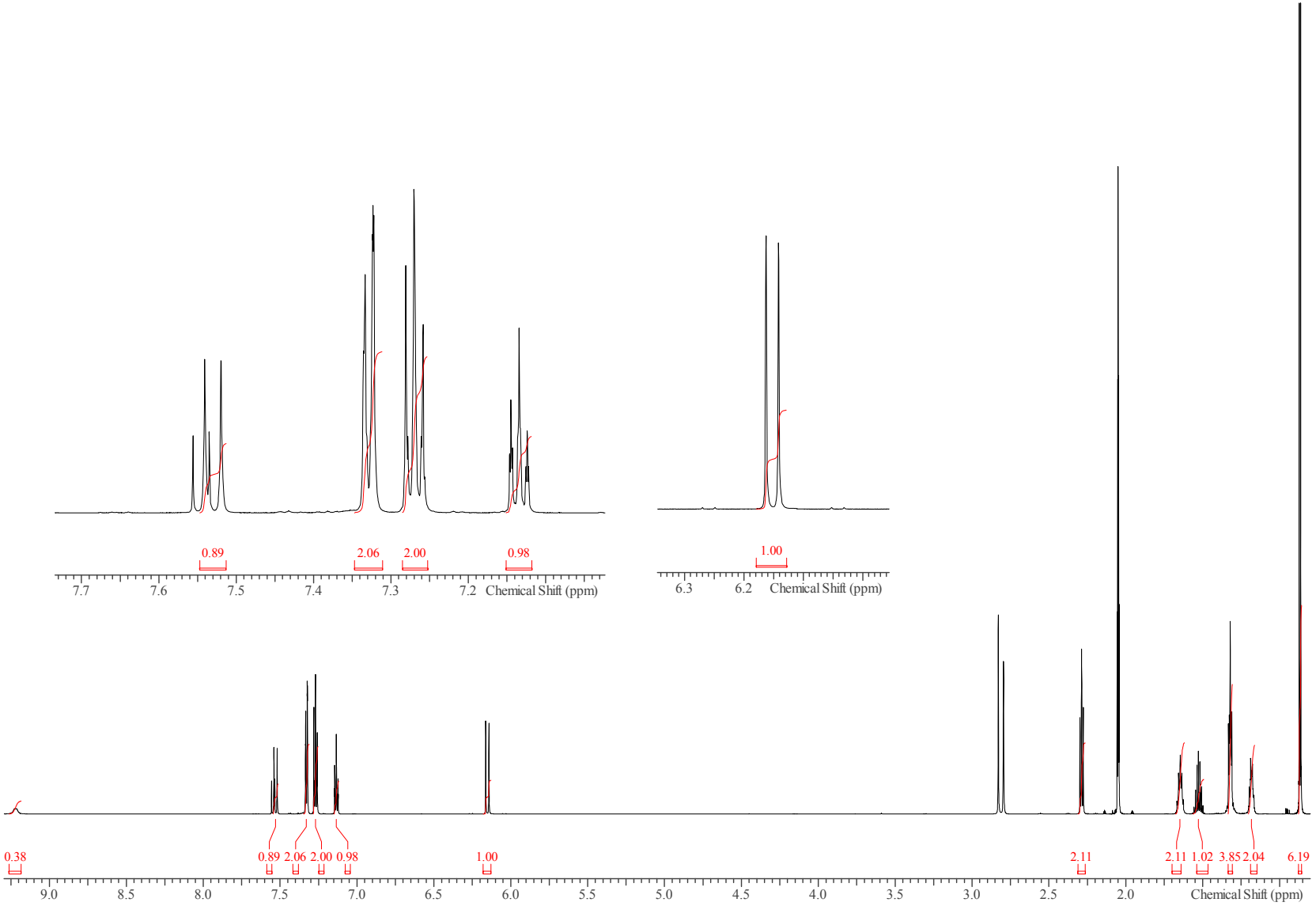
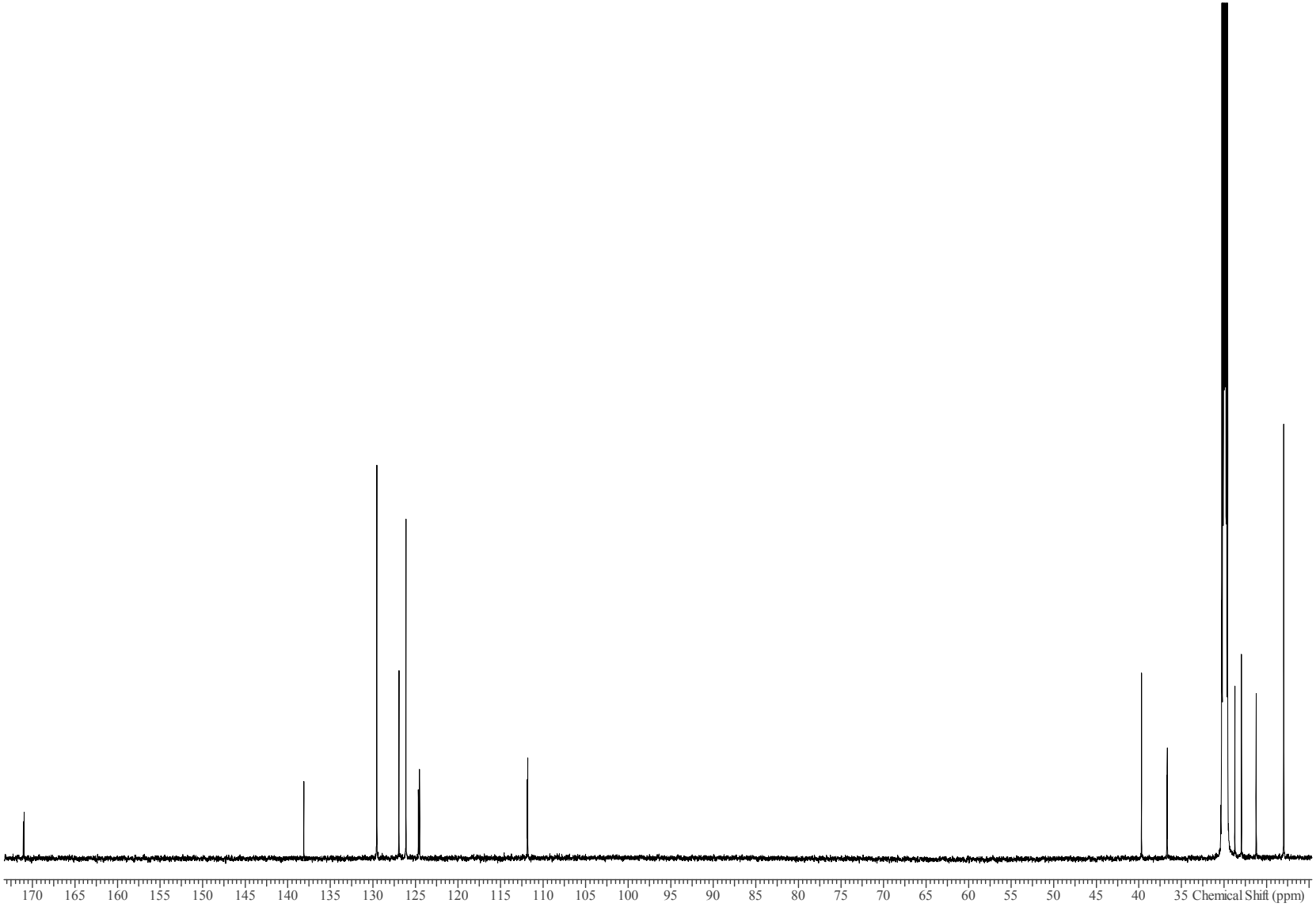


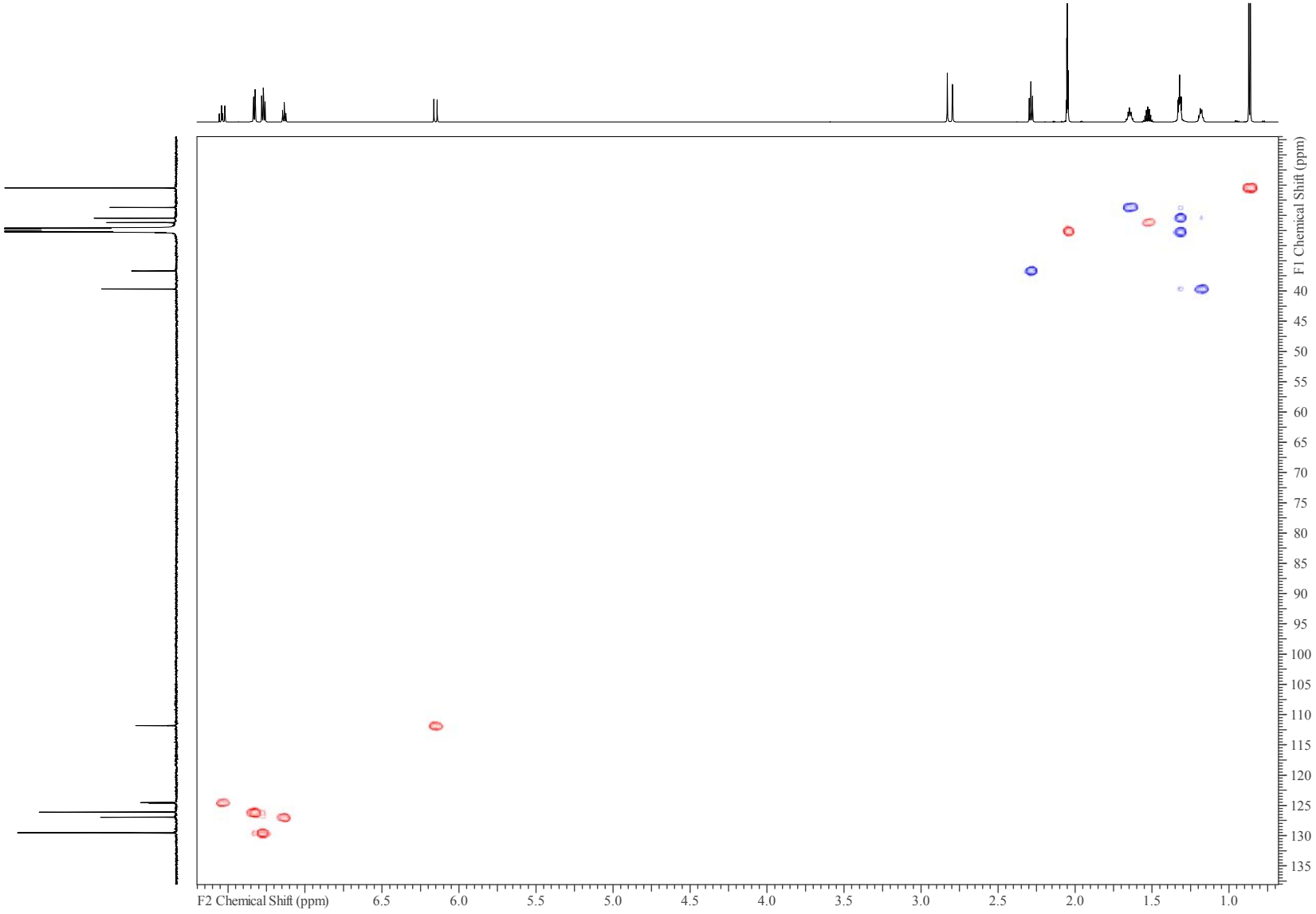
Figure S3: UV spectrum of Edonamide B (**2**) in MeOH.



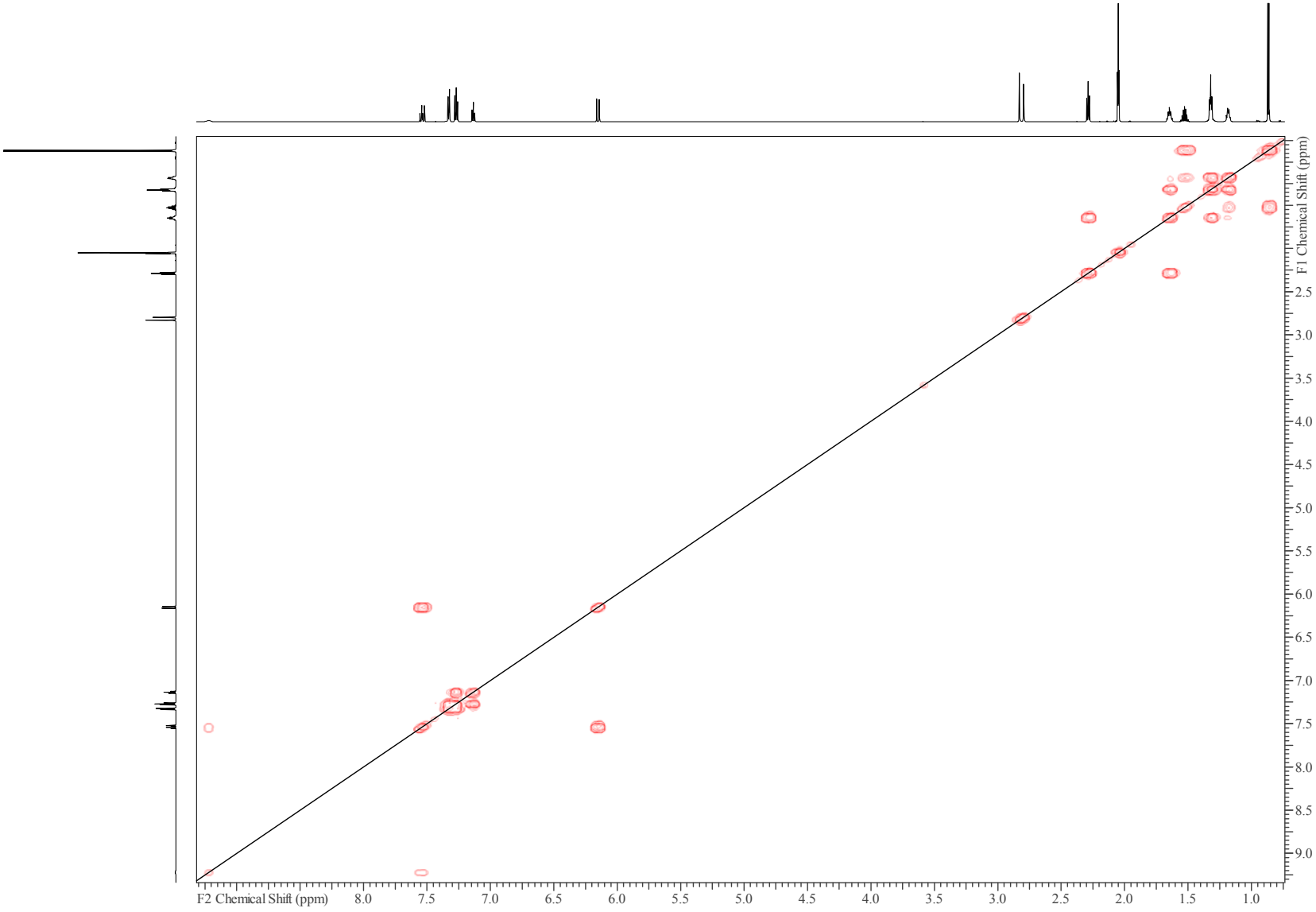
Spectrum S1: ^{1}H NMR spectrum of edonamide A (**1**) in acetone-d_6 (700 MHz).



Spectrum S2: ^{13}C NMR spectrum of edonamide A (**1**) in acetone-d_6 (176 MHz).

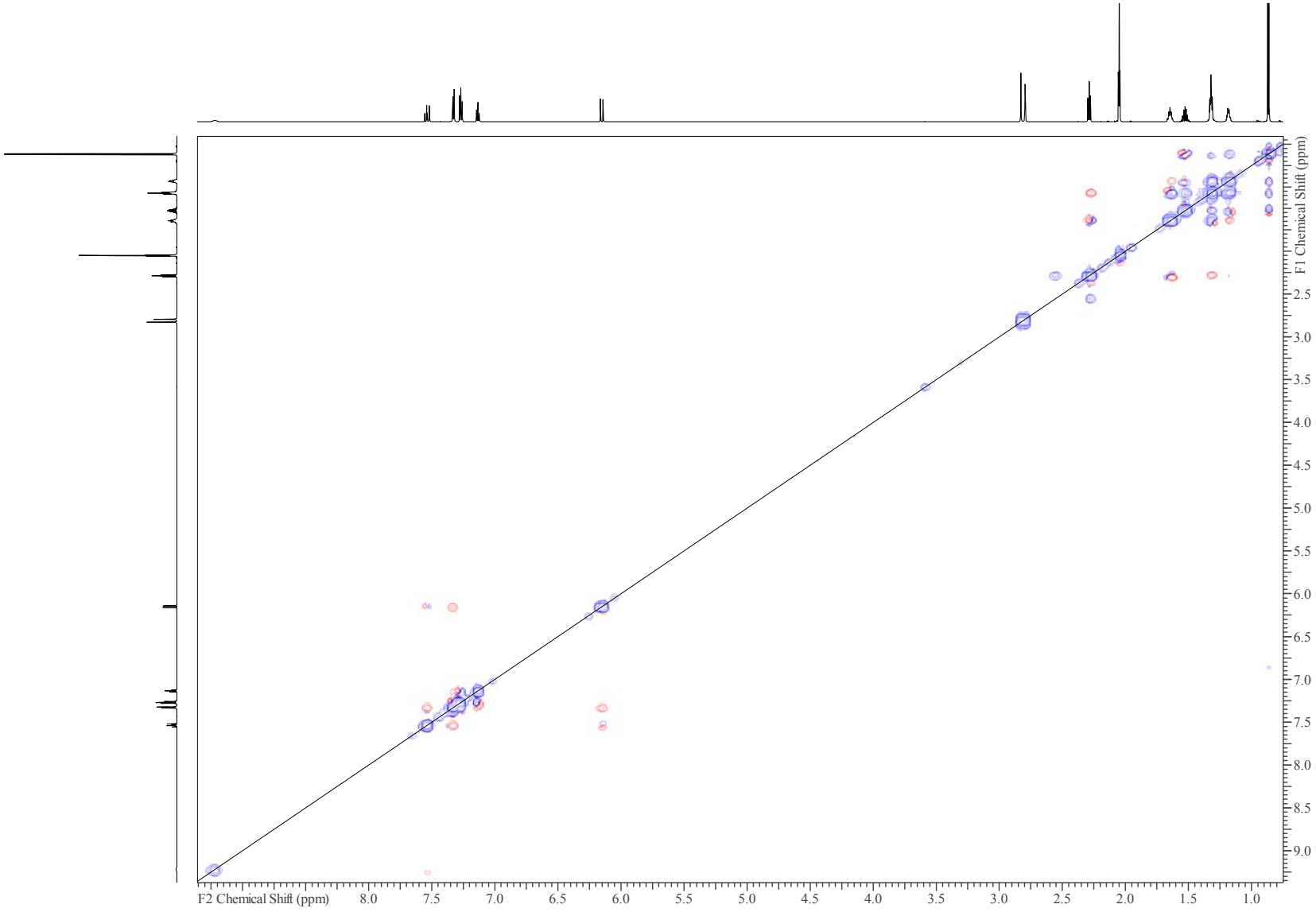


Spectrum S3: $^{1}H,^{13}C$ HSQC-DEPT NMR spectrum of edonamide A (**1**) in acetone-d_6 (700 MHz, 176 MHz).

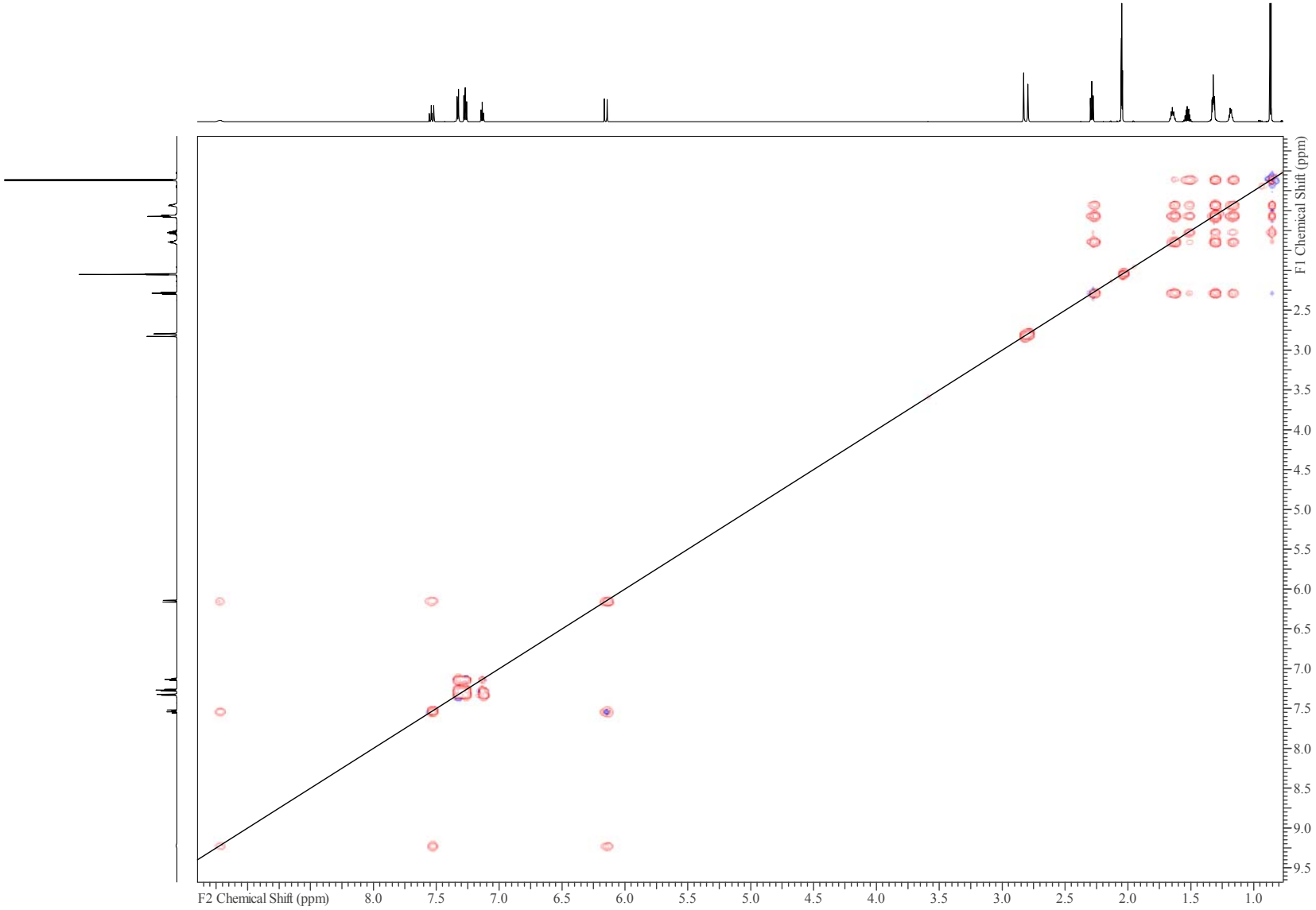


Spectrum S4: ^{1}H,^{1}H COSY NMR spectrum of edonamide A (**1**) in acetone-d_6 (700 MHz).

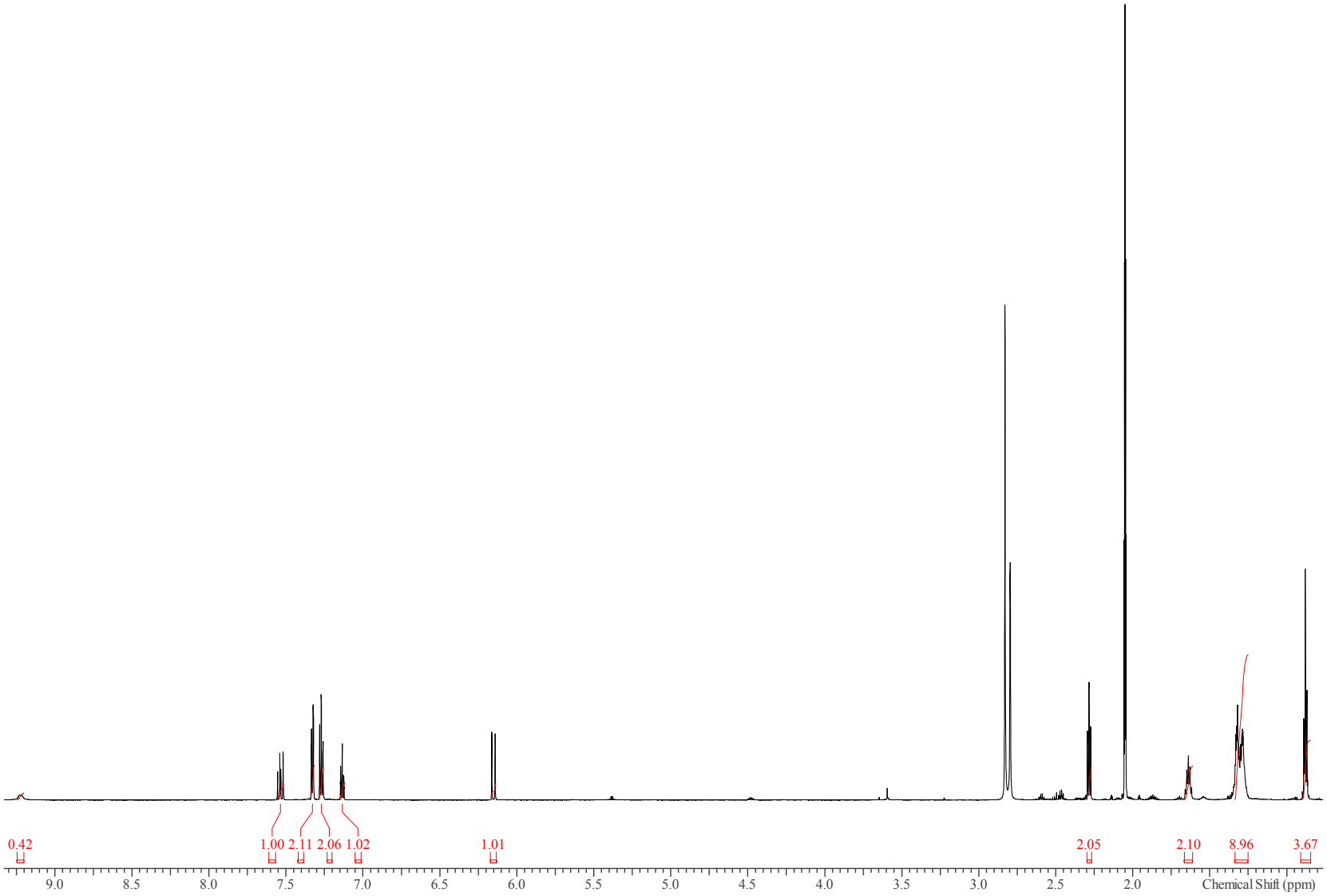
Spectrum S5: $^{1}H,^{13}C$ HMBC NMR spectrum of edonamide A (**1**) in acetone-d_6 (700 MHz, 176 MHz).



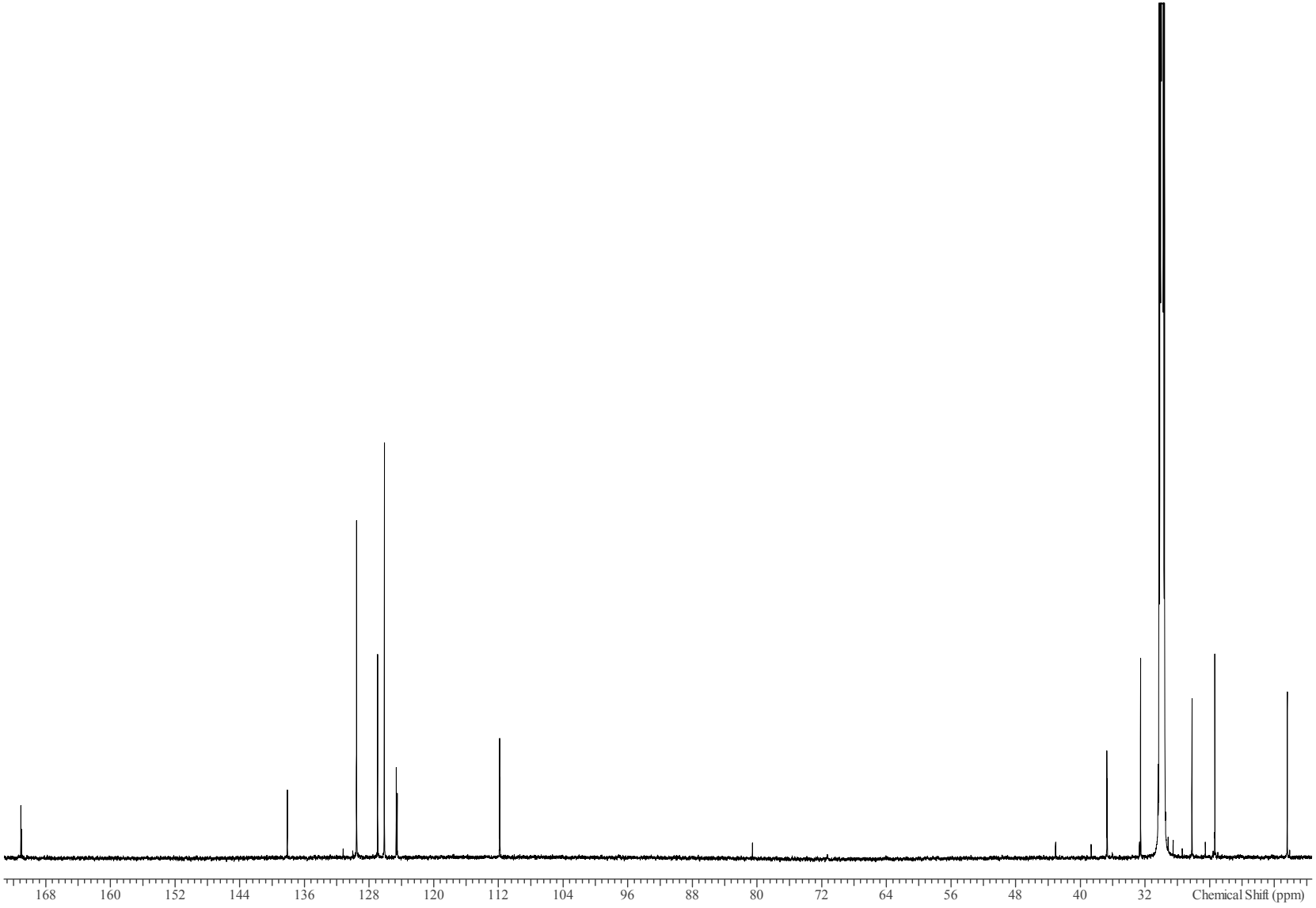
Spectrum S6: $^{1}H,^{1}H$ ROESY NMR spectrum of edonamide A (**1**) in acetone-d_6 (700 MHz).



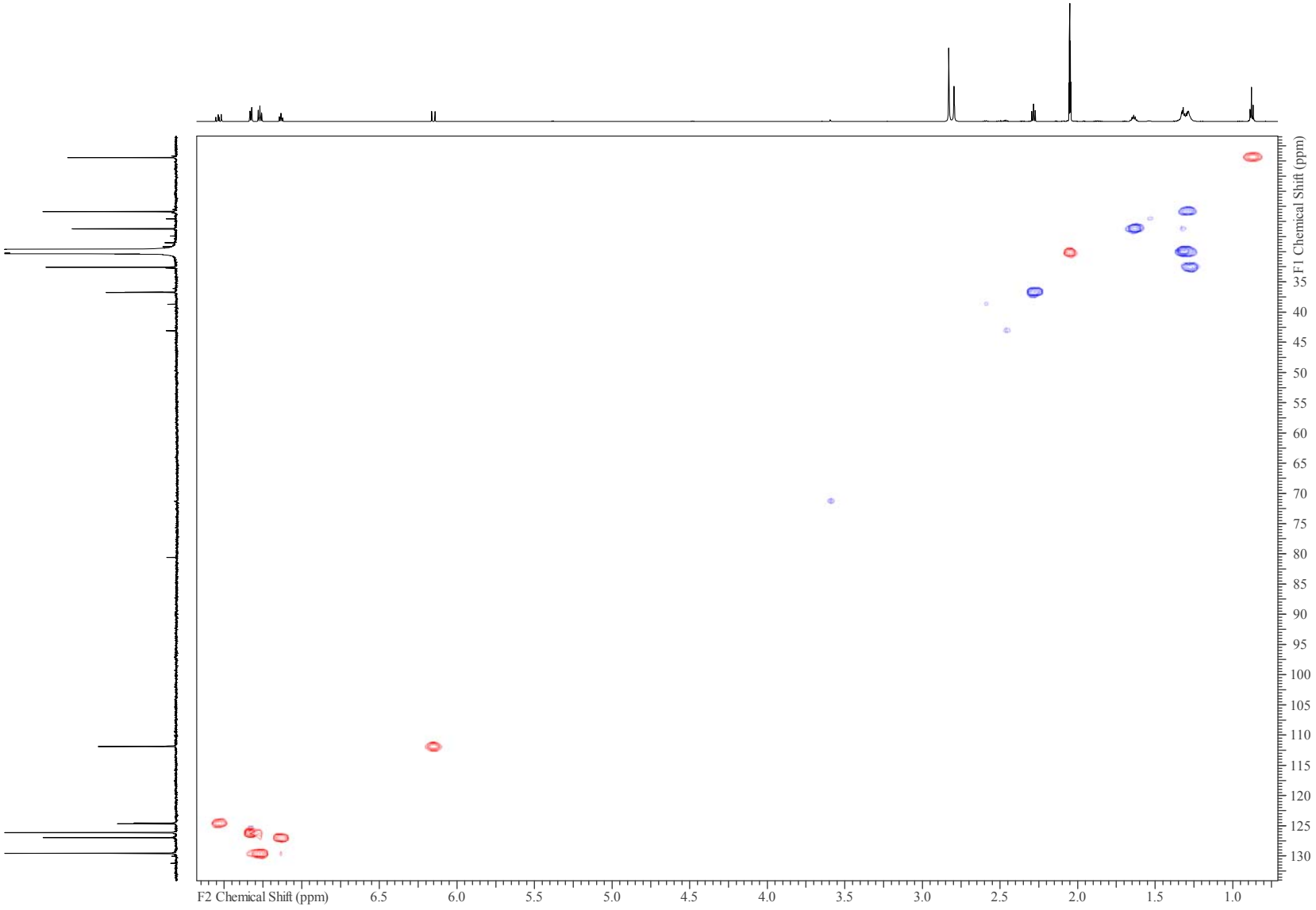
Spectrum S7: $^{1}H,^{1}H$ TOCSY NMR spectrum of edonamide A (**1**) in acetone-d_6 (700 MHz).



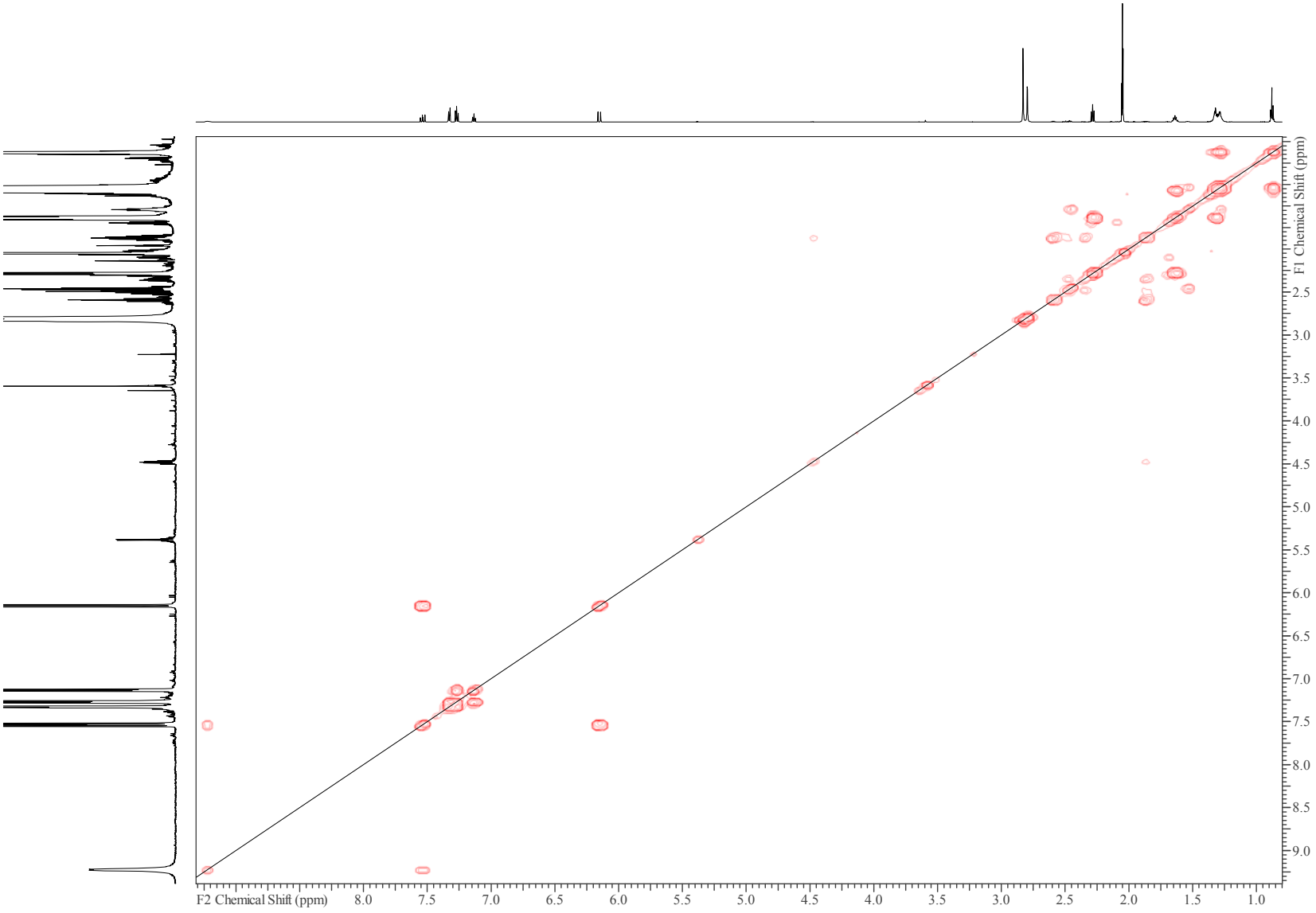
Spectrum S8: ^{1}H NMR spectrum of edonamide B (**2**) in acetone-d_6 (700 MHz).



Spectrum S9: ^{13}C NMR spectrum of edonamide B (**2**) in acetone-d_6 (176 MHz).

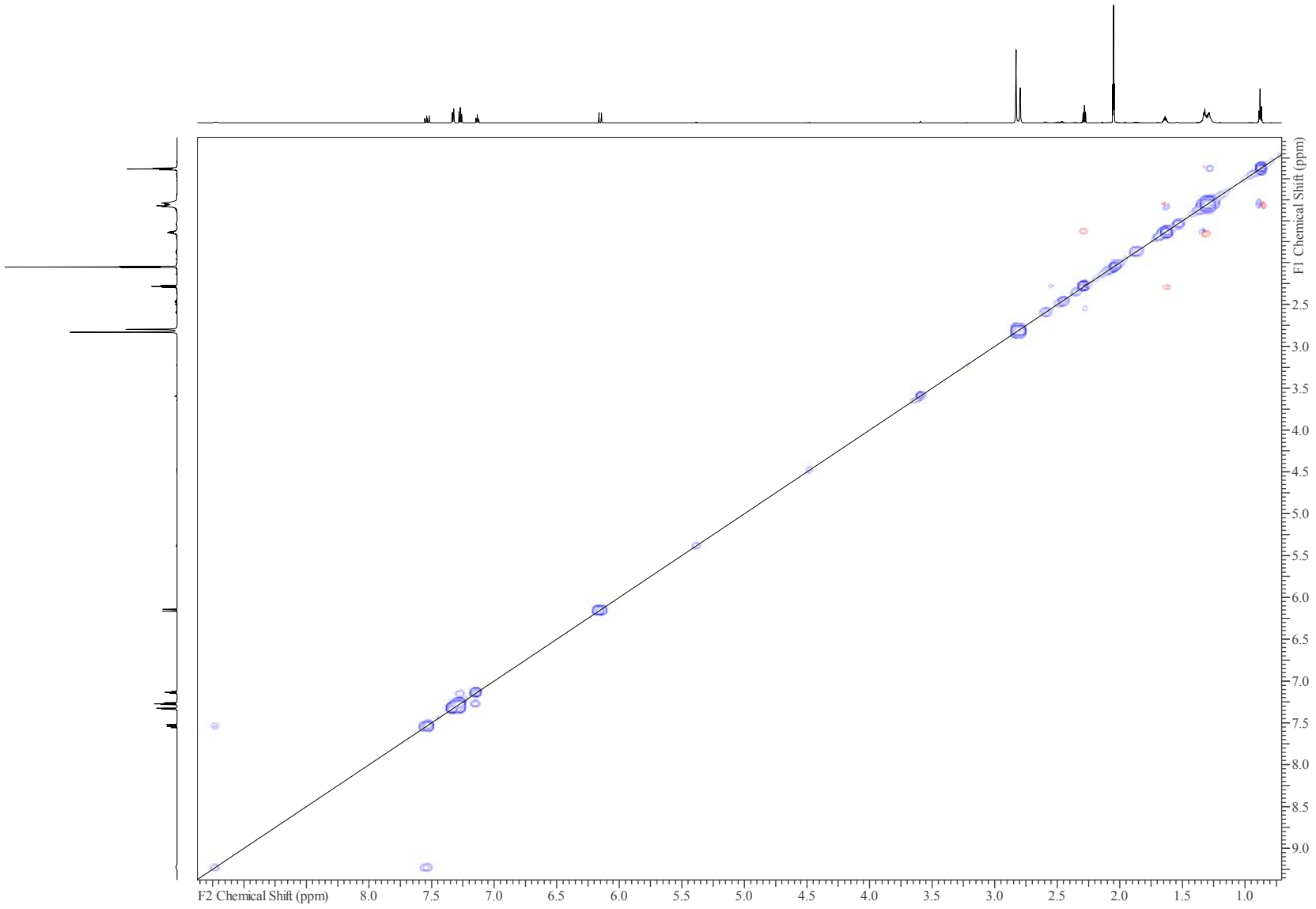


Spectrum S10: $^{1}H,^{13}C$ HSQC-DEPT NMR spectrum of edonamide B (**2**) in acetone-d_6 (700 MHz, 176 MHz).

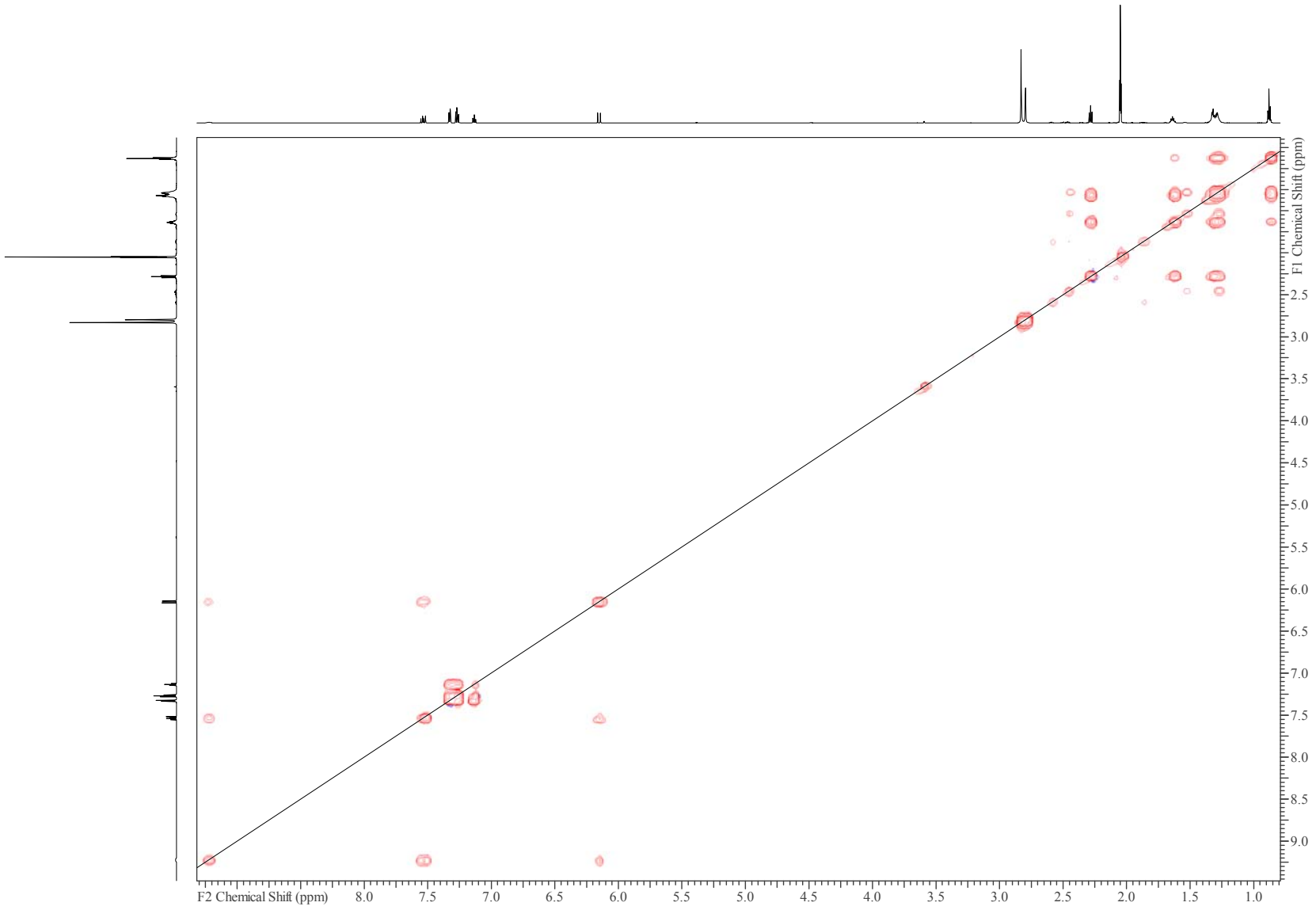


Spectrum S11: $^{1}H,^{1}H$ COSY NMR spectrum of edonamide B (**2**) in acetone-d_6 (700 MHz).

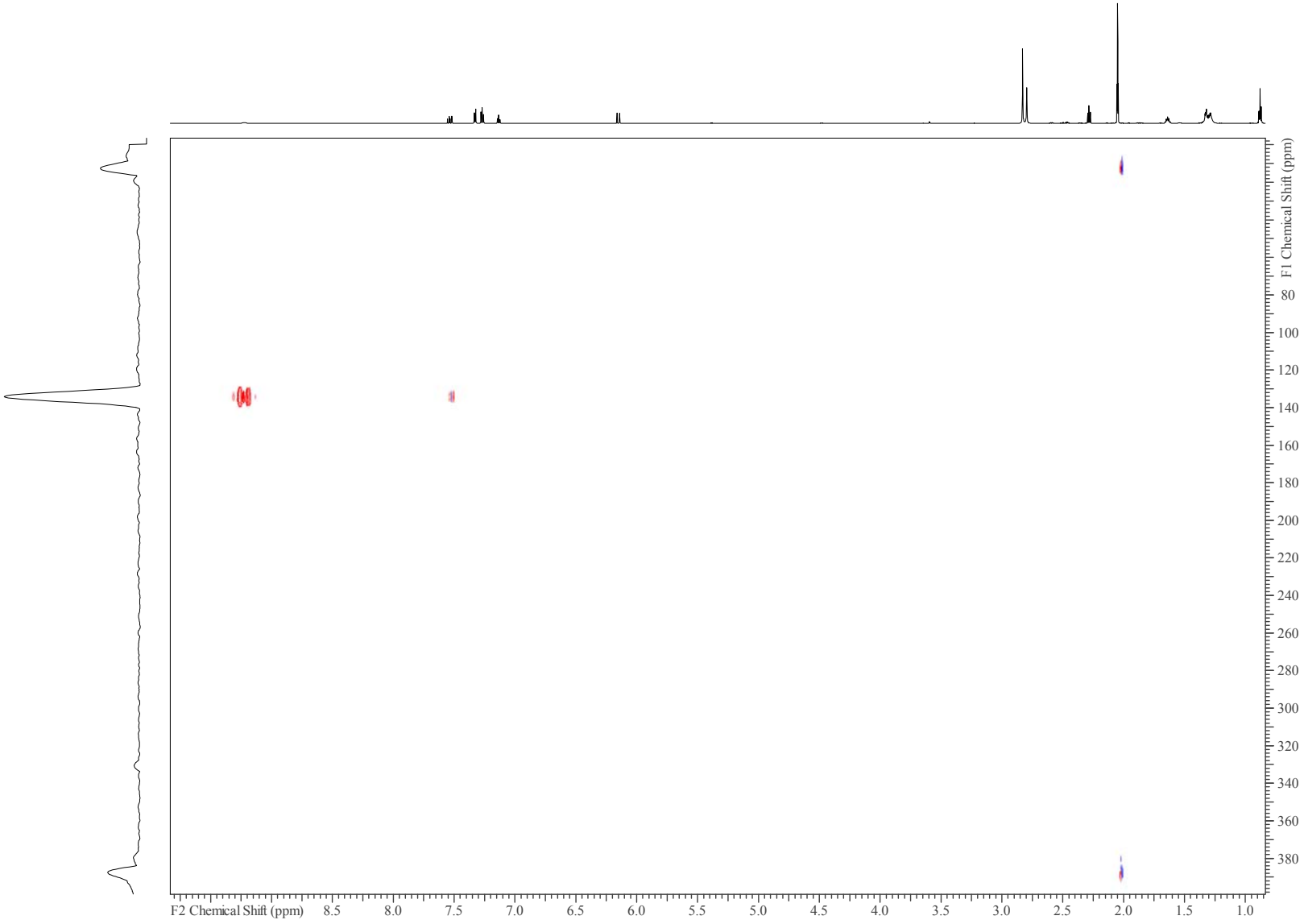
Spectrum S12: $^{1}H,^{13}C$ HMBC NMR spectrum of edonamide B (**2**) in acetone-d_6 (700 MHz, 176 MHz).



Spectrum S13: $^{1}H,^{1}H$ ROESY NMR spectrum of edonamide B (**2**) in acetone-d_6 (700 MHz).



Spectrum S14: $^{1}H,^{1}H$ TOCSY NMR spectrum of edonamide B (**2**) in acetone-d_6 (700 MHz).



Spectrum S15: $^{1}H,^{15}N$ HSQC NMR spectrum of edonamide B (**2**) in acetone-d_6 (700 MHz, 71 MHz).

Spectrum S16: $^{1}H,^{15}N$ HMBC NMR spectrum of edonamide B (**2**) in acetone-d_6 (700 MHz, 71 MHz).