Supplementary Materials

Metabolomic Characterization of a cf. *Neolyngbya* Cyanobacterium from the South China Sea Reveals Wenchangamide A, a Lipopeptide with In Vitro Apoptotic Potential in Colon Cancer Cells

Lijian Ding [1,2,†], Rinat Bar-Shalom [3,†], Dikla Aharonovich [2], Naoaki Kurisawa [4], Gaurav Patial [1], Shuang Li [1], Shan He [1], Xiaojun Yan [1], Arihiro Iwasaki [4,5], Kiyotake Suenaga [4,5], Chengchong Zhu [5], Haixi Luo [5], Fuli Tian [5], Fuad Fares [3], C. Benjamin Naman [1,5,*] and Tal Luzzatto-Knaan [2,*]

[1] Li Dak Sum Yip Yio Chin Kenneth Li Marine Biopharmaceutical Research Center, Department of Marine Pharmacy, College of Food and Pharmaceutical Sciences, Ningbo University, Ningbo 315800, China;
[2] Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa, Haifa 3498838, Israel;
[3] Department of Human Biology, Faculty of Life Sciences, University of Haifa, Haifa 31905, Israel;
[4] Department of Chemistry, Keio University, 3-14-1, Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan;
[5] Key Laboratory of Medicinal and Edible Plant Resources of Hainan Province, Hainan Vocational University of Science and Technology, Haikou 571126, China;
* Correspondence: bnaman@nbu.edu.cn (C.B.N.); tluzzatto@univ.haifa.ac.il (T.L.-K.)
† These authors contributed equally to the work.

Table of Contents

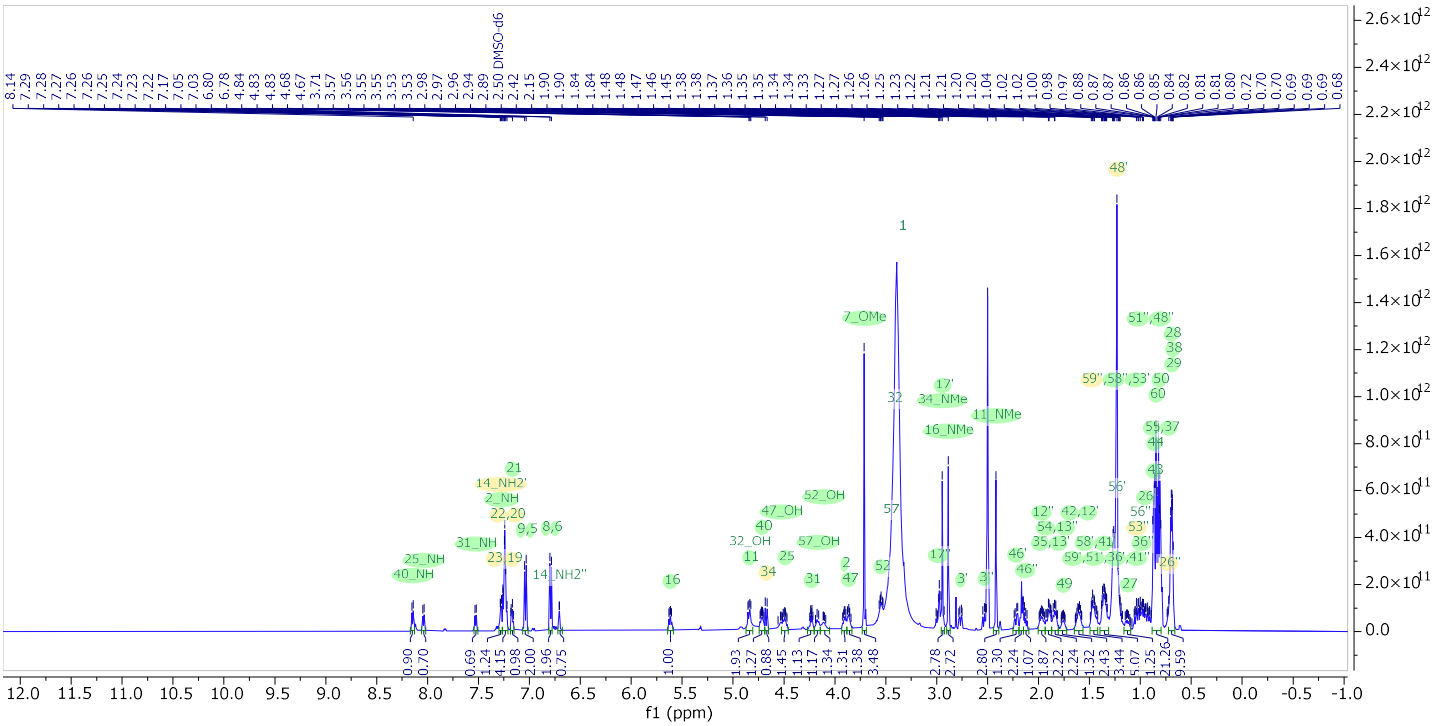


Figure S1. [1]H NMR spectrum of **1** in DMSO-d_6.

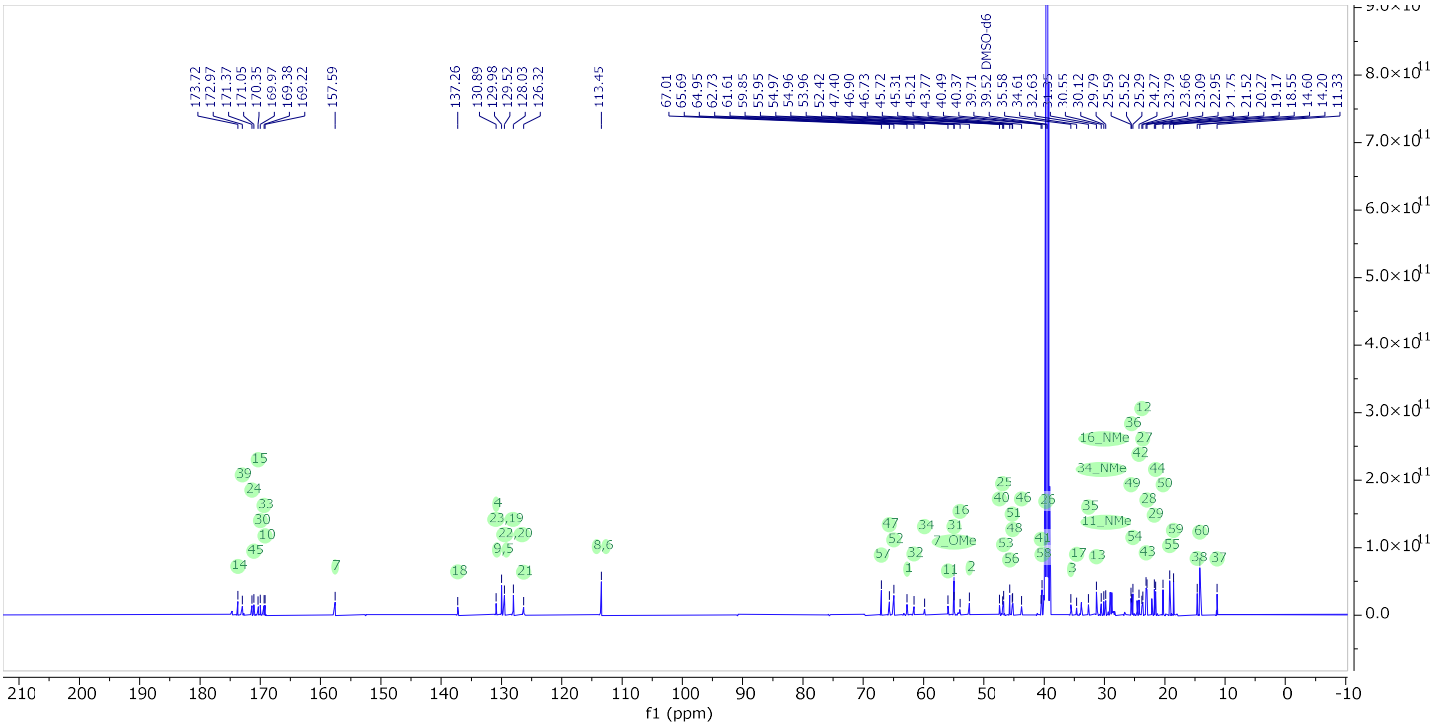


Figure S2. [13]C NMR spectrum of **1** in DMSO-d_6.

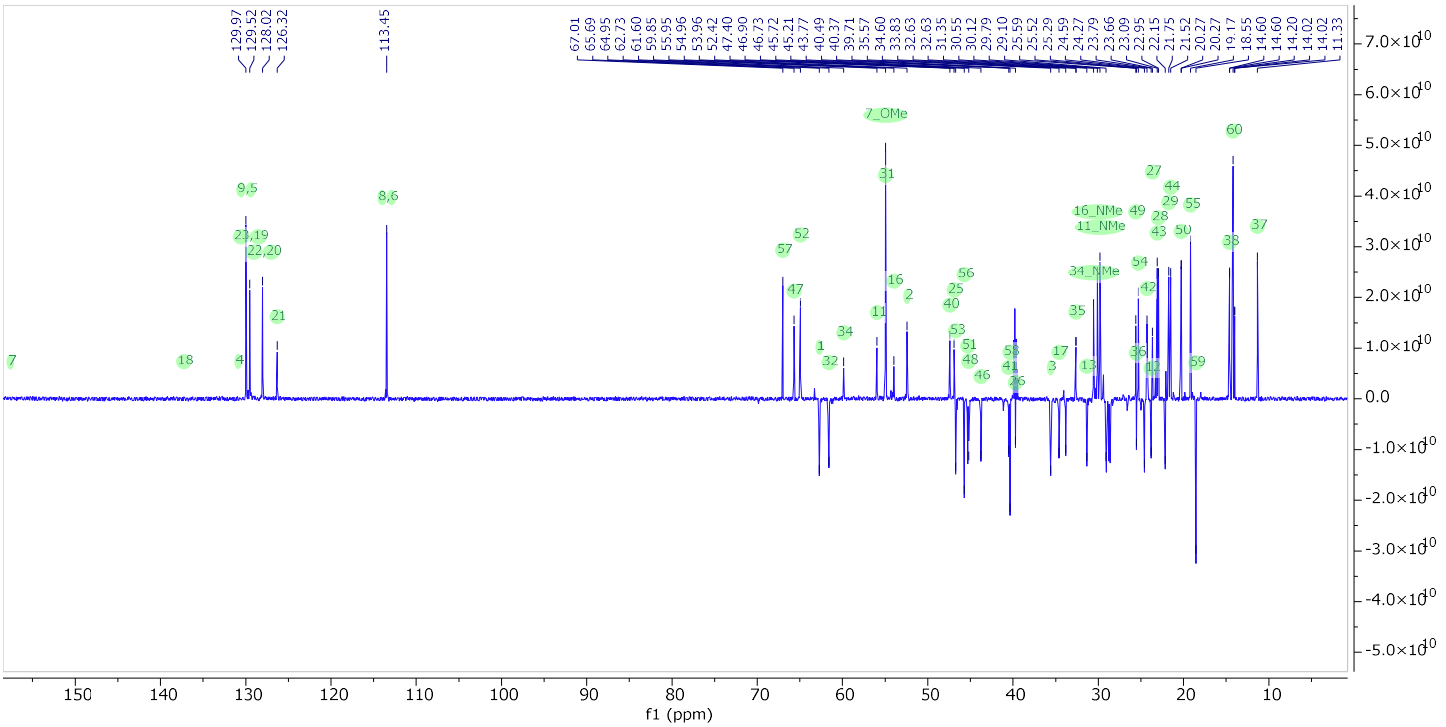


Figure S3. ^{13}C DEPT135 NMR spectrum of **1** in DMSO-d_6.

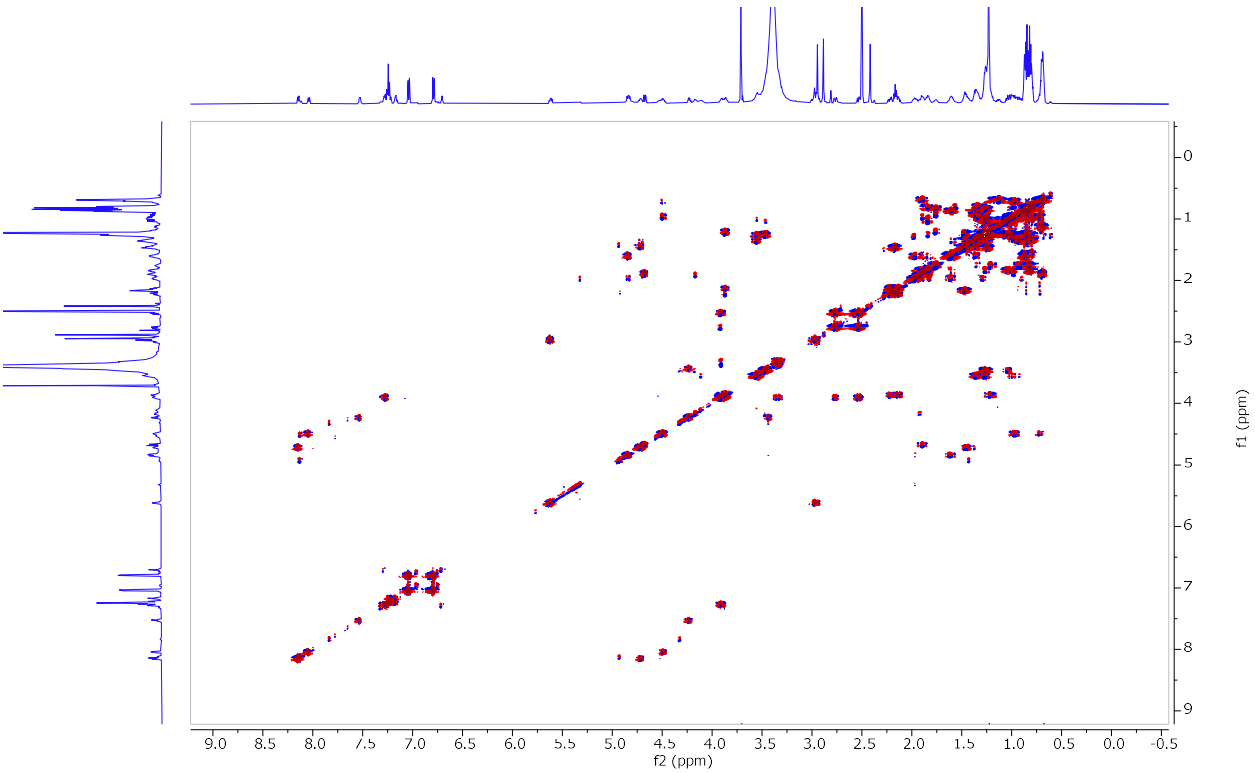


Figure S4. ^{1}H-^{1}H COSY NMR spectrum of **1** in DMSO-d_6.

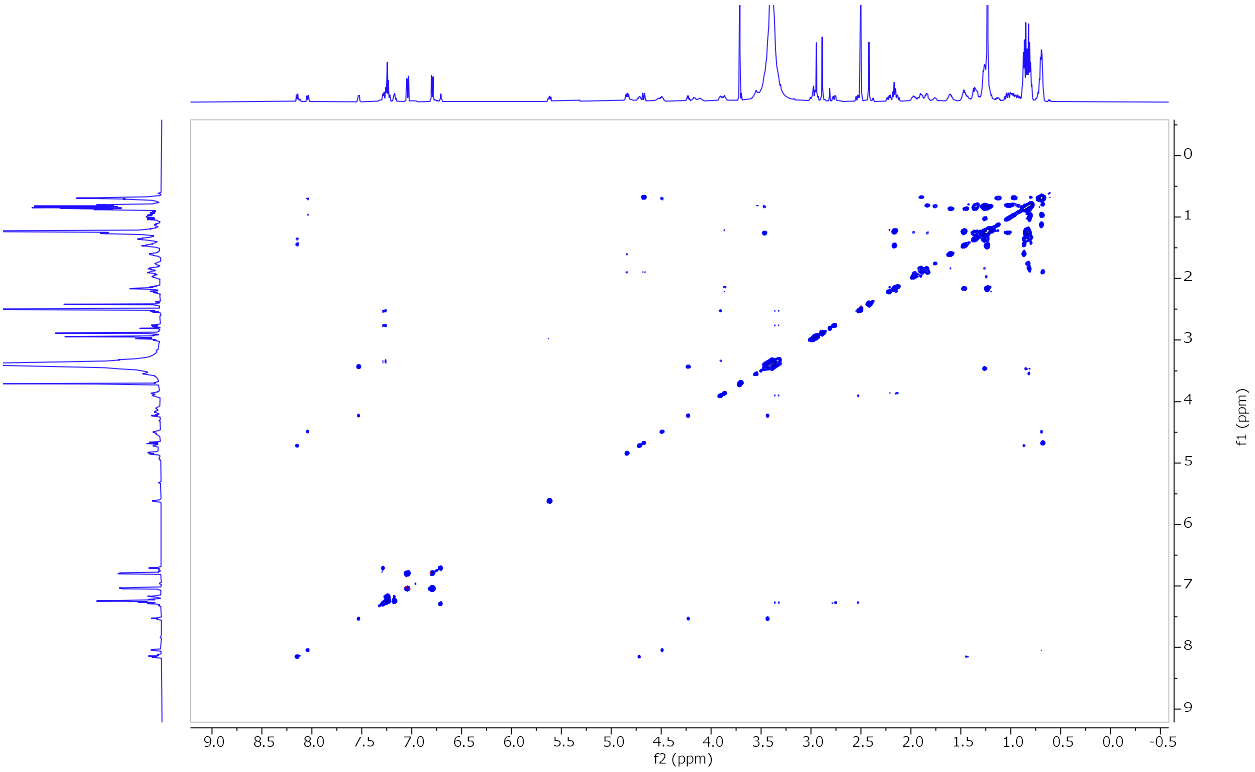


Figure S5. ^{1}H-^{1}H TOCSY NMR spectrum of **1** in DMSO-d_6.

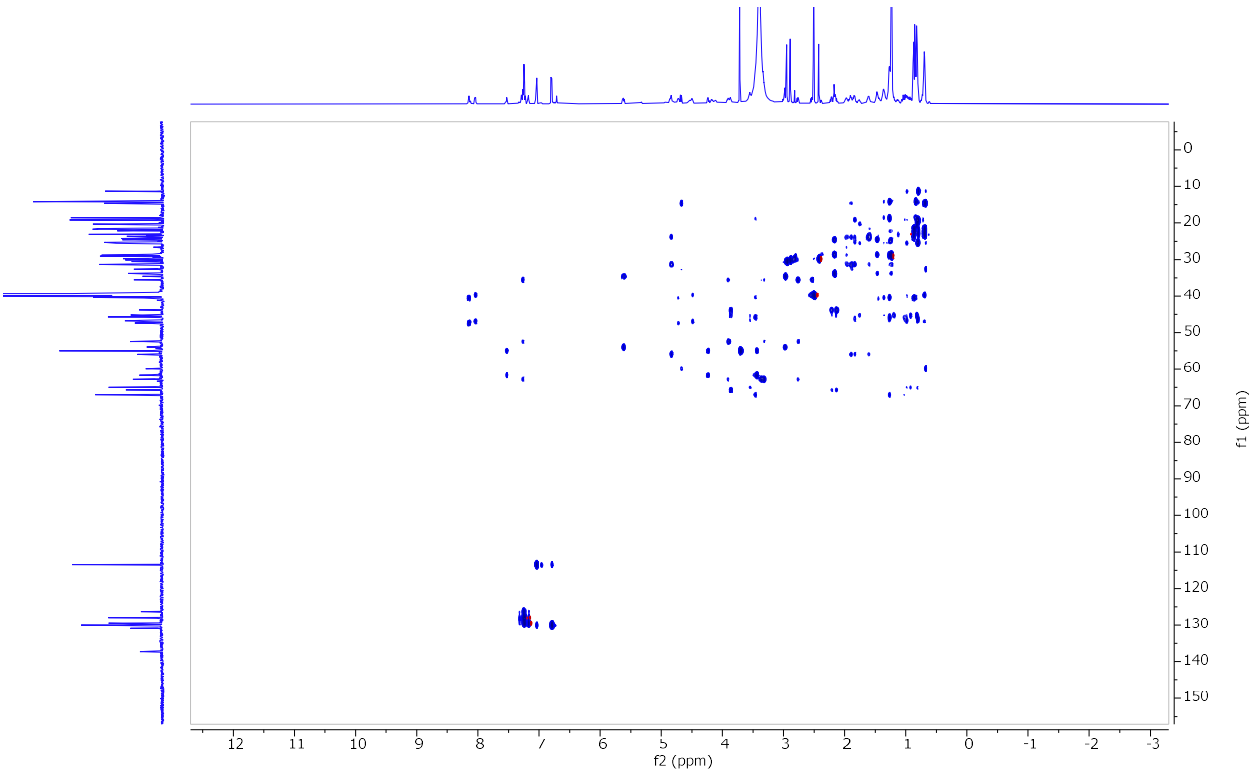


Figure S6. ^{1}H-^{13}C HSQC-TOCSY NMR spectrum of **1** in DMSO-d_6.

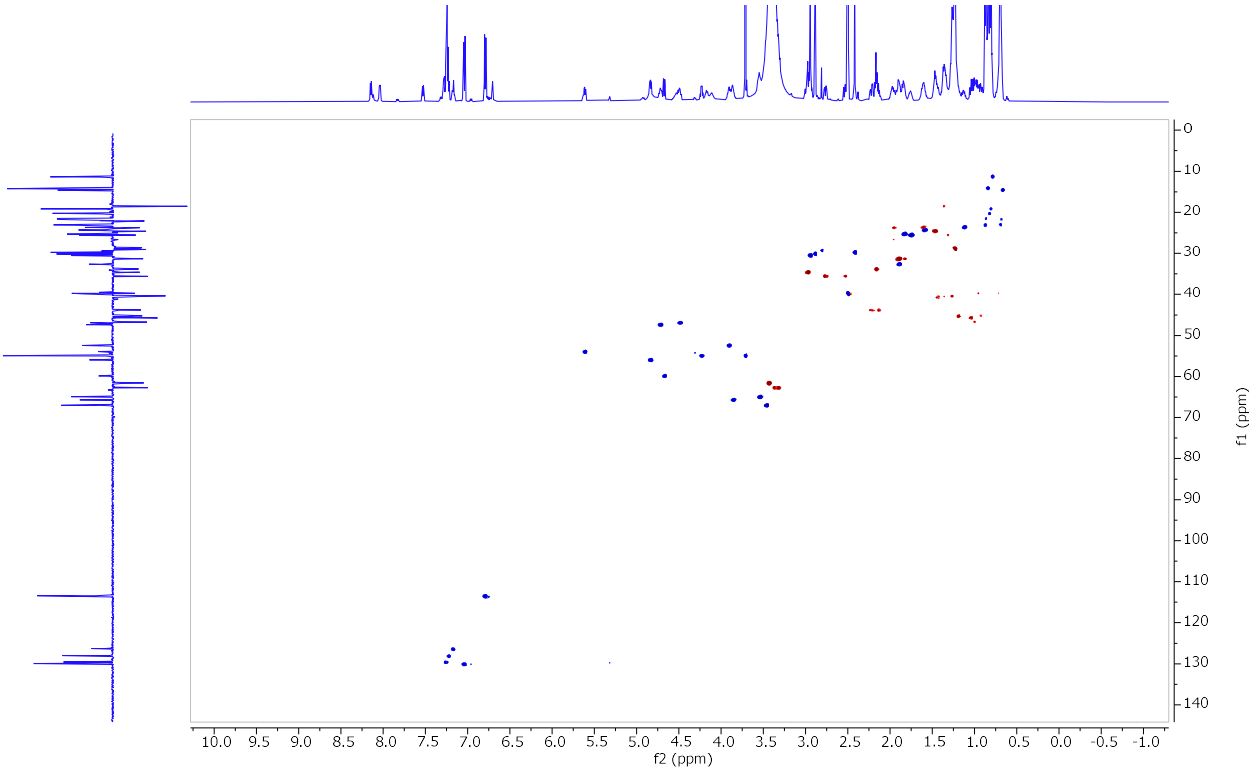


Figure S7. ^{1}H-^{13}C HSQC NMR spectrum of **1** in DMSO-*d*$_6$.

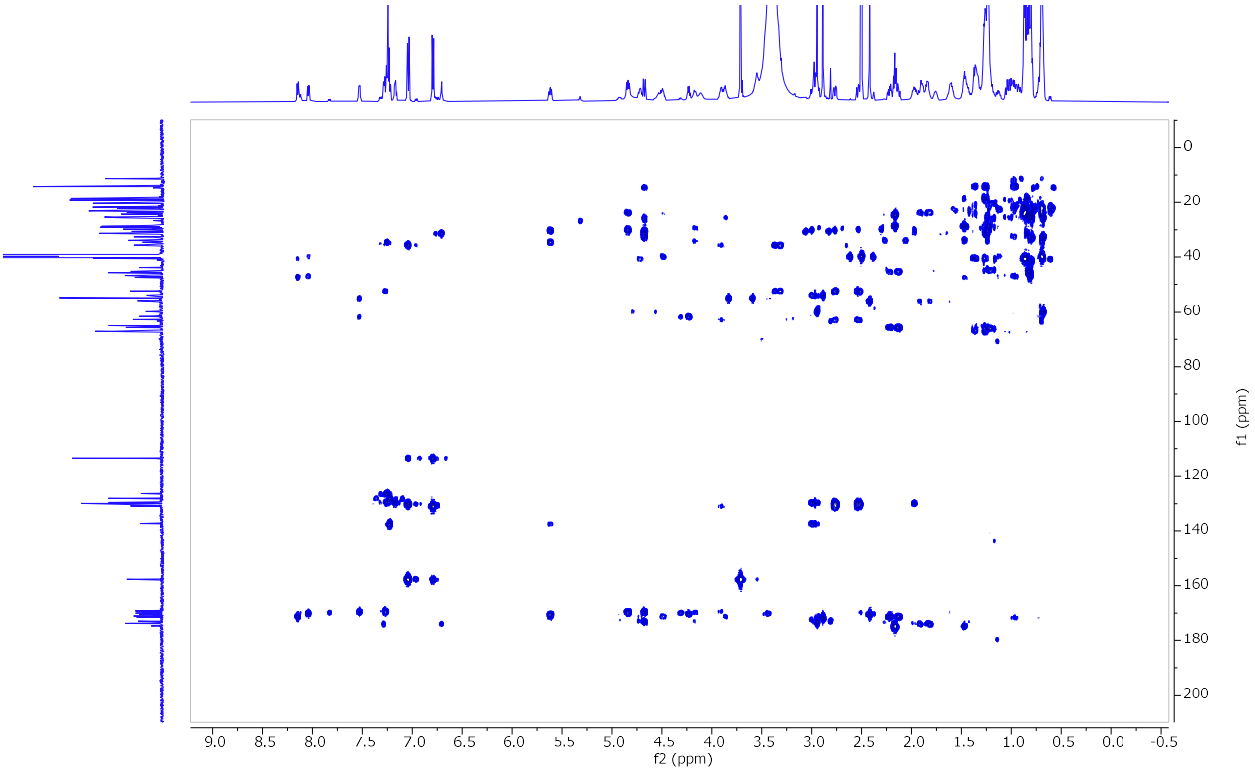


Figure S8. ^{1}H-^{13}C HMBC NMR spectrum of **1** in DMSO-*d*$_6$.

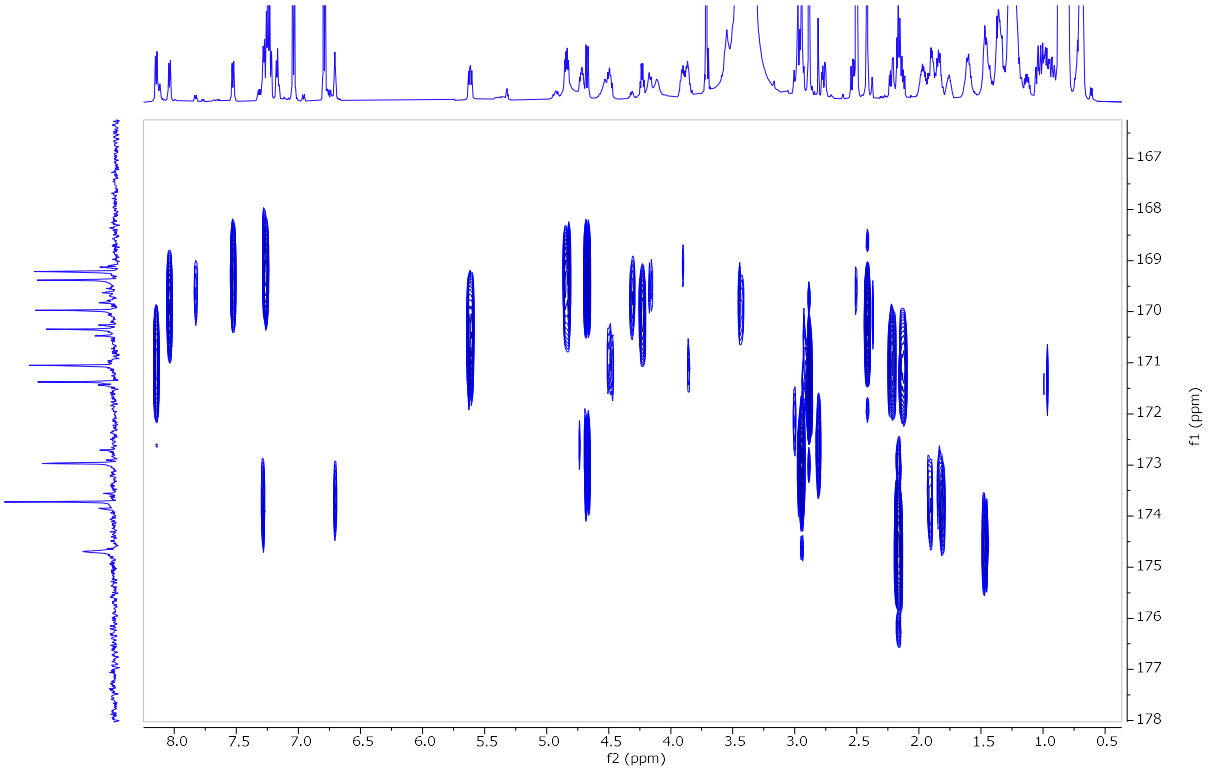


Figure S9. ^{1}H-^{13}C HMBC NMR spectrum of **1** in DMSO-*d*$_6$ (expanded ester carbonyl region).

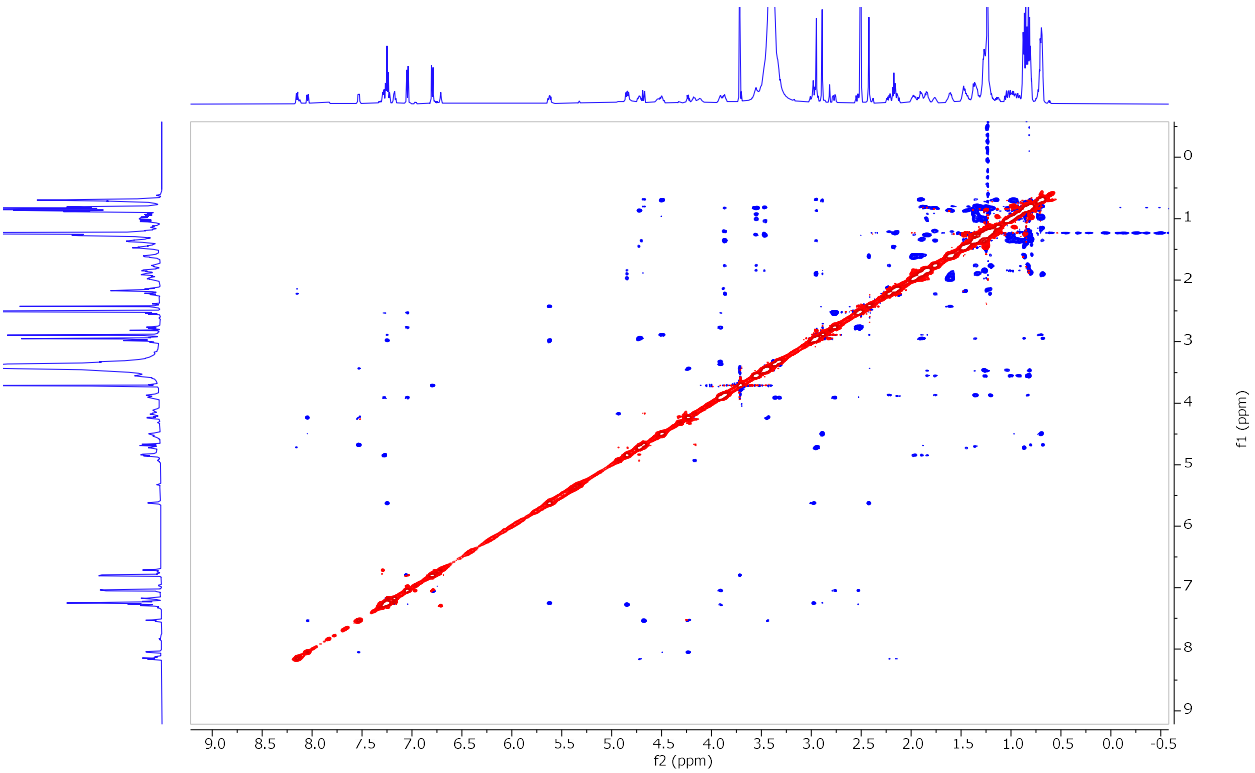


Figure S10. ^{1}H-^{1}H ROESY NMR spectrum of **1** in DMSO-*d*$_6$.

Table S1. ^{1}H and ^{13}C NMR Spectroscopic Data for **1** in pyridine-d_5.[a,b]

Moiety	Position	δ_C	Type	δ_H, mult (*J* in Hz)	Moiety	Position	δ_C	Type	δ_H, mult (*J* in Hz)
AMP	1	63.6	CH_2	3.92, m	*N*-Me-Ile	33	170.0	C	
	2	53.2	CH	4.65, m		34	61.2	CH	5.24 d (10.6)
	3	36.7	CH_2	2.77, dd (13.9, 5.8);		35	32.7	CH	2.23, m
				2.53, dd (13.9, 8.8)					
	4	131.4	C			36	26.9	CH_2	1.67, m; 1.01, m
	5, 9	130.7	CH	7.04, d (8.5)		37	11.6	CH_3	0.81, t (7.2)
	6, 8	114.3	CH	6.79, d (8.5)		38	14.9	CH_3	0.79, d (6.5)
	7	158.7	C			34-*N*-Me	31.3	CH_3	3.26, s
	7-*O*-Me	55.3c	CH_3	3.71, s					
	2-NH			7.85, d (8.8)	Leu-2	39	171.5	C	
						40	48.7	CH	5.22, m
N-Me-Gln	10	170.8	C			41	41.4	CH_2	1.81, m
	11	57.3	CH	5.49, dd (11.7, 4.3)		42	25.2	CH	1.87, m
	12	24.9	CH_2	2.67, m; 2.51, m		43	23.0	CH_3	0.87, d (6.3)
	13	32.5	CH_2	2.53, m; 2.33, m		44	22.4	CH_3	0.94, d (6.1)
	14	176.1	C			40-NH			9.28, d (7.2)
	14-NH_2			8.26, m; 7.52, m					
	11-*N*-Me	30.8	CH_3	3.10, s	FA	45	173.4	C	
						46	44.5	CH_2	2.81, dd (13.7, 3.6)
N-Me-Phe	15	172.0	C						2.76, dd (13.7, 8.3)
	16	54.6	CH	6.15, dd (10.1, 6.1)		47	66.8	CH	4.63, m
	17	35.6	CH_2	3.29, dd (13.9, 6.1)		48	46.6	CH_2	1.81, m; 1.67, m
				3.20, dd (13.9, 10.1)		49	26.8	CH	2.41, m
	18	138.0	C			50	20.6	CH_3	1.10, d (6.5)
	19, 23	130.3	CH	7.37, d (7.2)		51	46.2	CH_2	1.91, m; 1.29, m
	20, 22	128.7	CH	7.27, dd (7.4, 7.2)		52	66.5	CH	4.13, m
	21	126.9	CH	7.17, t (7.4)		53	46.9	CH_2	1.78, m; 1.36, m
	16-*N*-Me	30.7	CH_3	3.25, s		54	26.6	CH	2.57, m
						55	19.6	CH_3	1.11, d (6.5)
Leu-1	24	172.7	C			56	48.0	CH_2	1.76 m; 1.37 m
	25	47.9	CH	5.03, m		57	68.5	CH	3.97, m
	26	40.4	CH_2	1.37, m; 1.07, m		58	41.6	CH_2	1.65, m; 1.55, m
	27	24.5	CH	1.20, m		59	19.6	CH_2	1.66, m; 1.51, m
	28	22.9	CH_3	0.63, d (6.5)		60	14.6	CH_2	0.92, t (7.0)
	29	22.4	CH_3	0.70, d (6.5)		47-OH			6.48, d (4.7)
	25-NH			9.83, d (7.9)		52-OH			5.64, d (5.8)
						57-OH			5.69, d (5.8)
Ser	30	171.7	C						
	31	55.6c	CH	5.17, m					
	32	63.0	CH_2	4.14, m; 4.07, m					
	31-NH			7.86, d (8.8)					
	32-OH			6.17, m					

[a] Data recorded at 298 K, 600 MHz (^{1}H) and 150 MHz (^{13}C). [b] Assignments supported by 2D NMR.

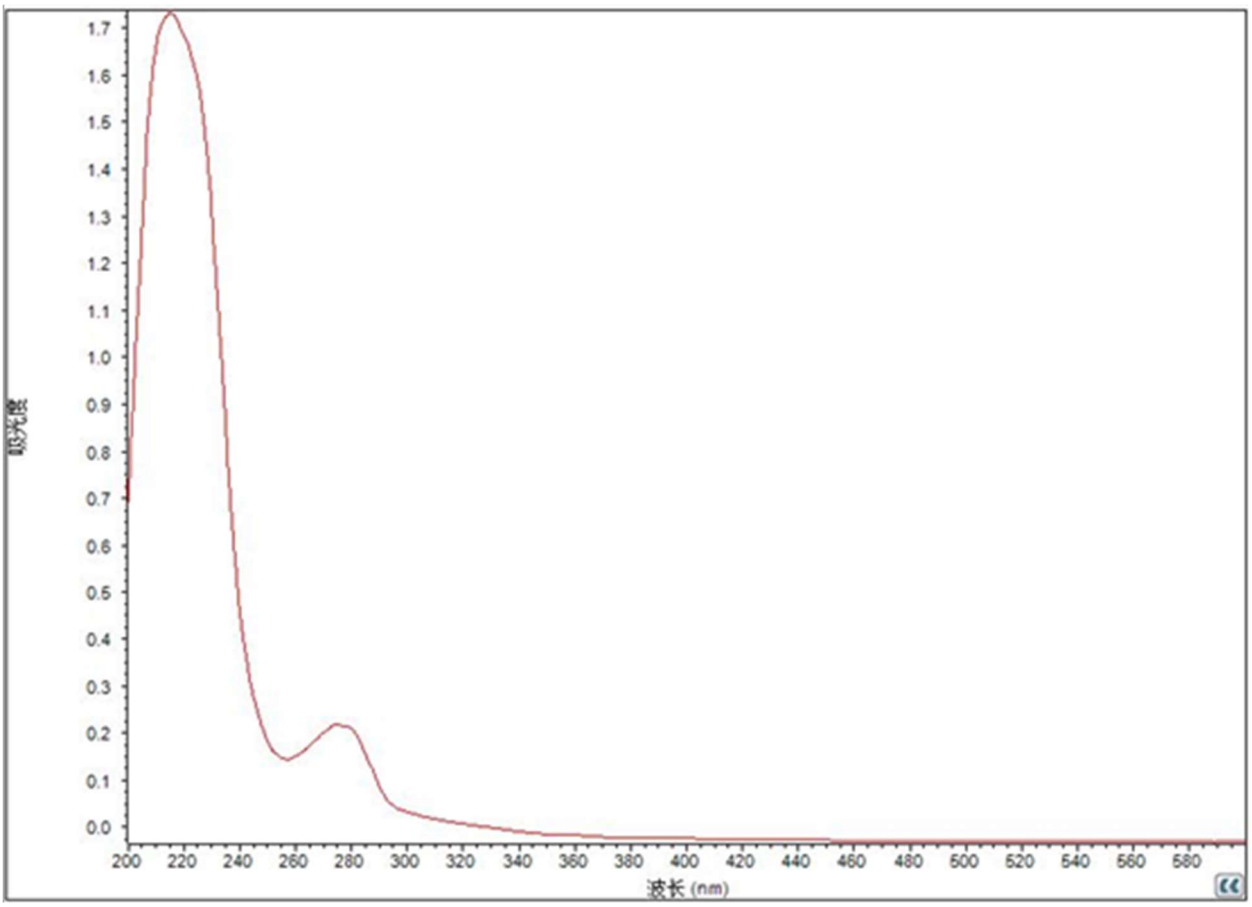


Figure S11. UV spectrum of **1** in MeOH at 0.25 mg/mL.

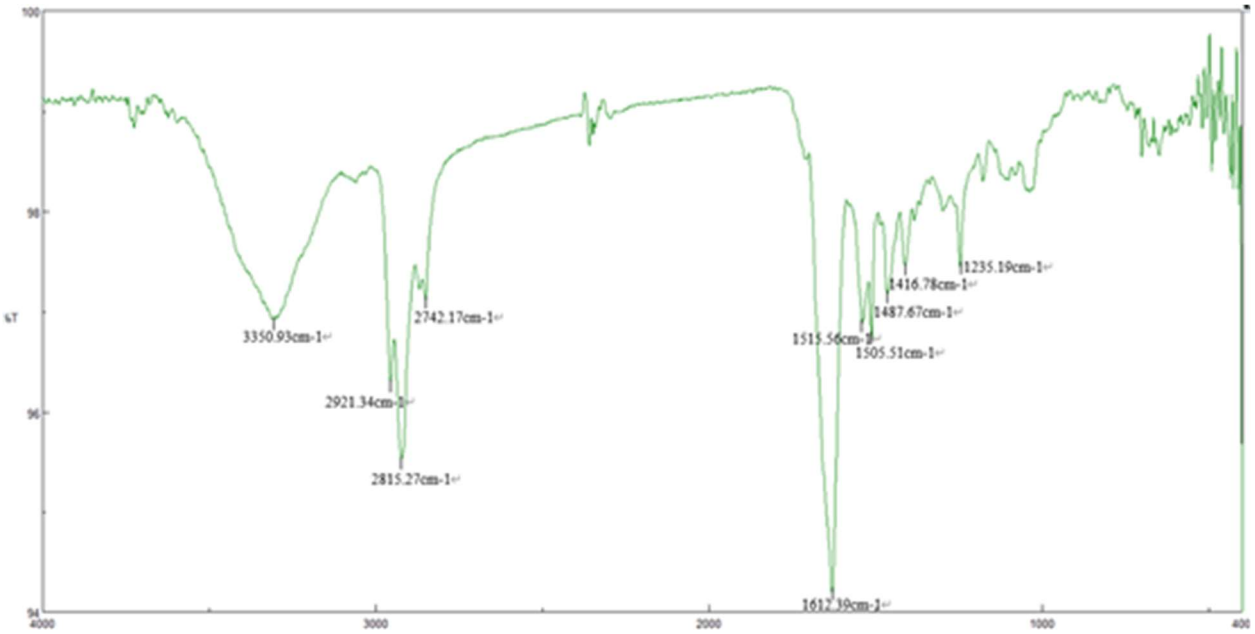


Figure S12. IR spectrum of **1** in KBr.

Figure S13. HRESIMS/MS of **1**.

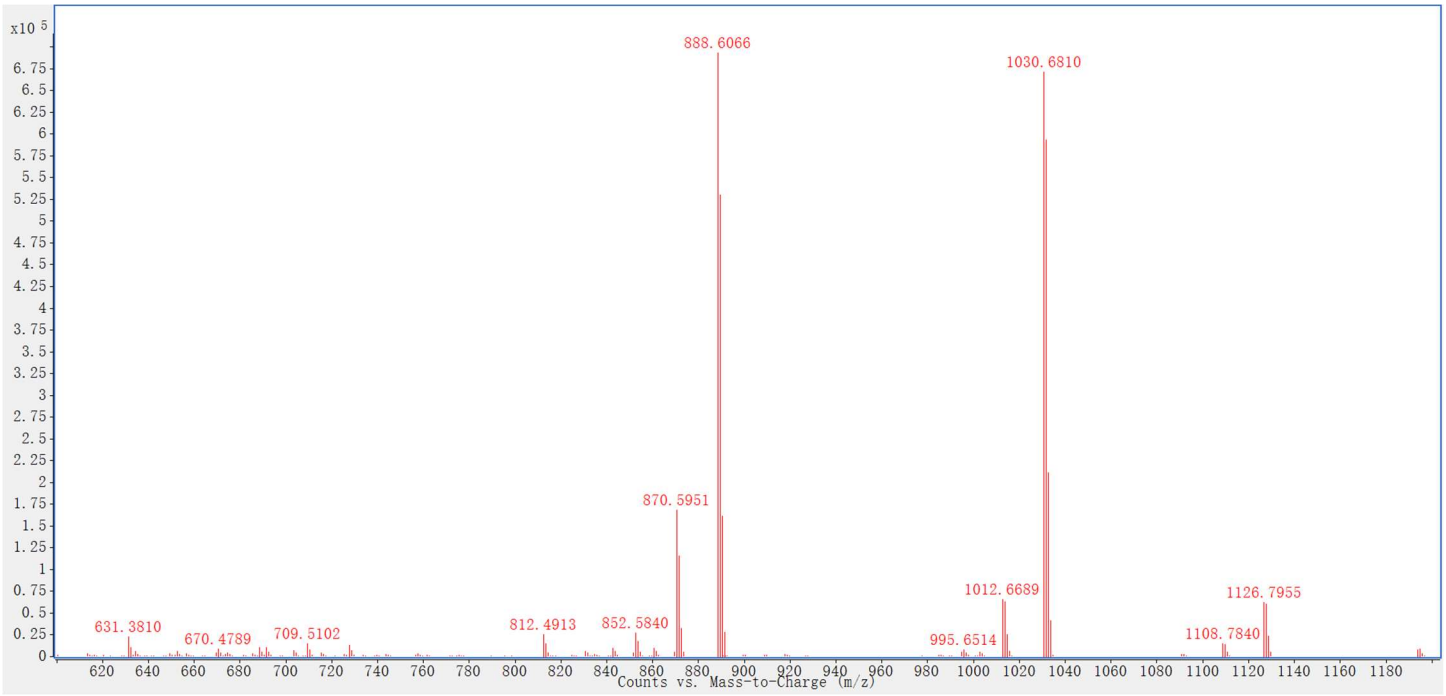


Figure S14. HRESIMS/MS spectrum of **1** (expanded view *m*/*z* 600-1200).

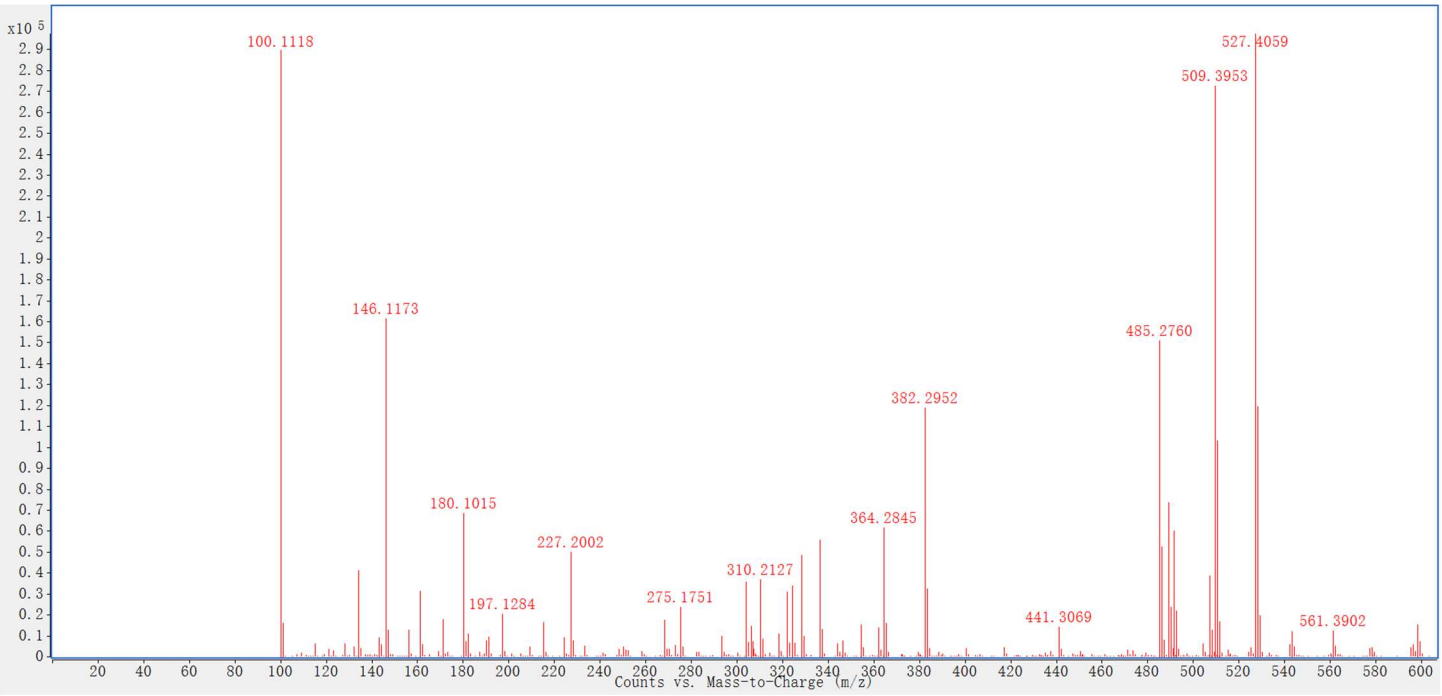


Figure S15. HRESIMS/MS spectrum of **1** (expanded view *m*/*z* 0-600).

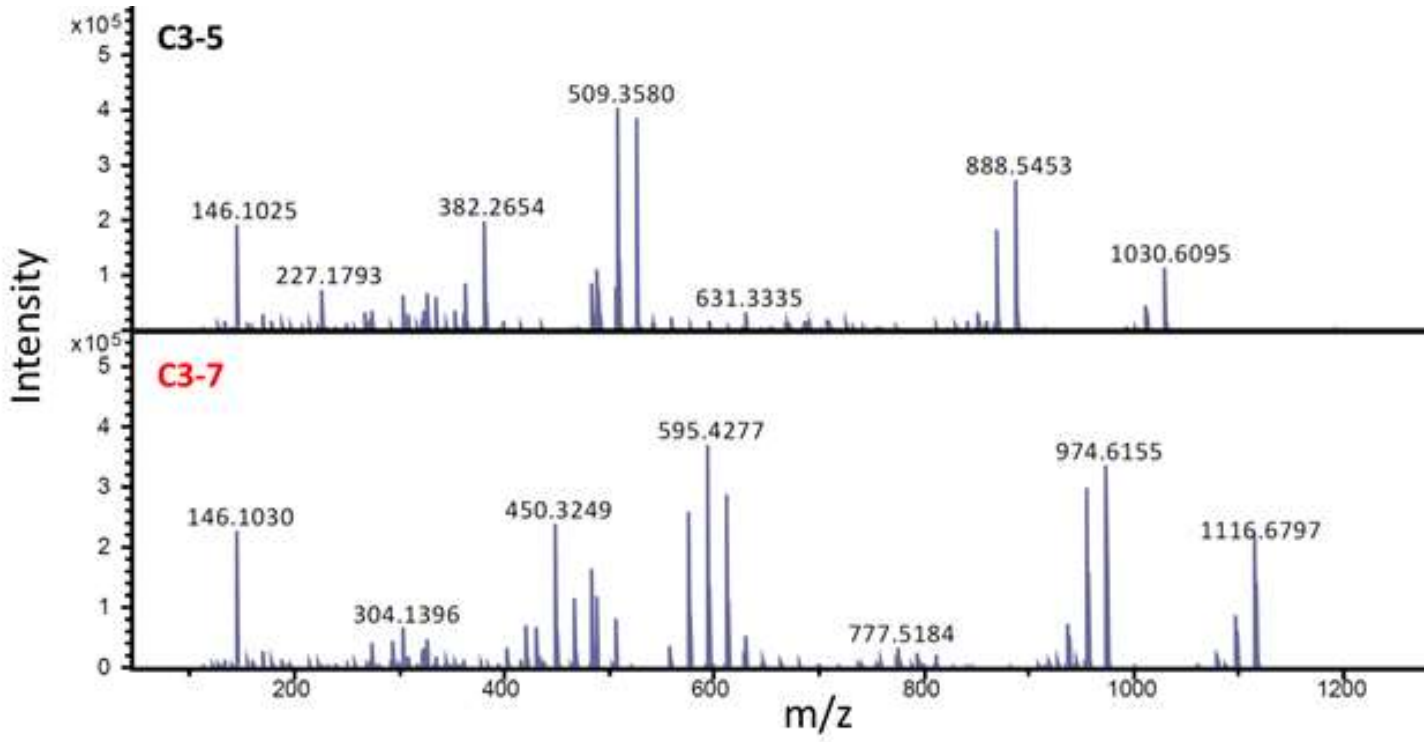


Figure S16. MS/MS spectra of wenchangamide A (**1;** *m*/*z* 1211) in C3-5 and wenchangamide B (proposed; *m*/*z* 1297) in C3-7.

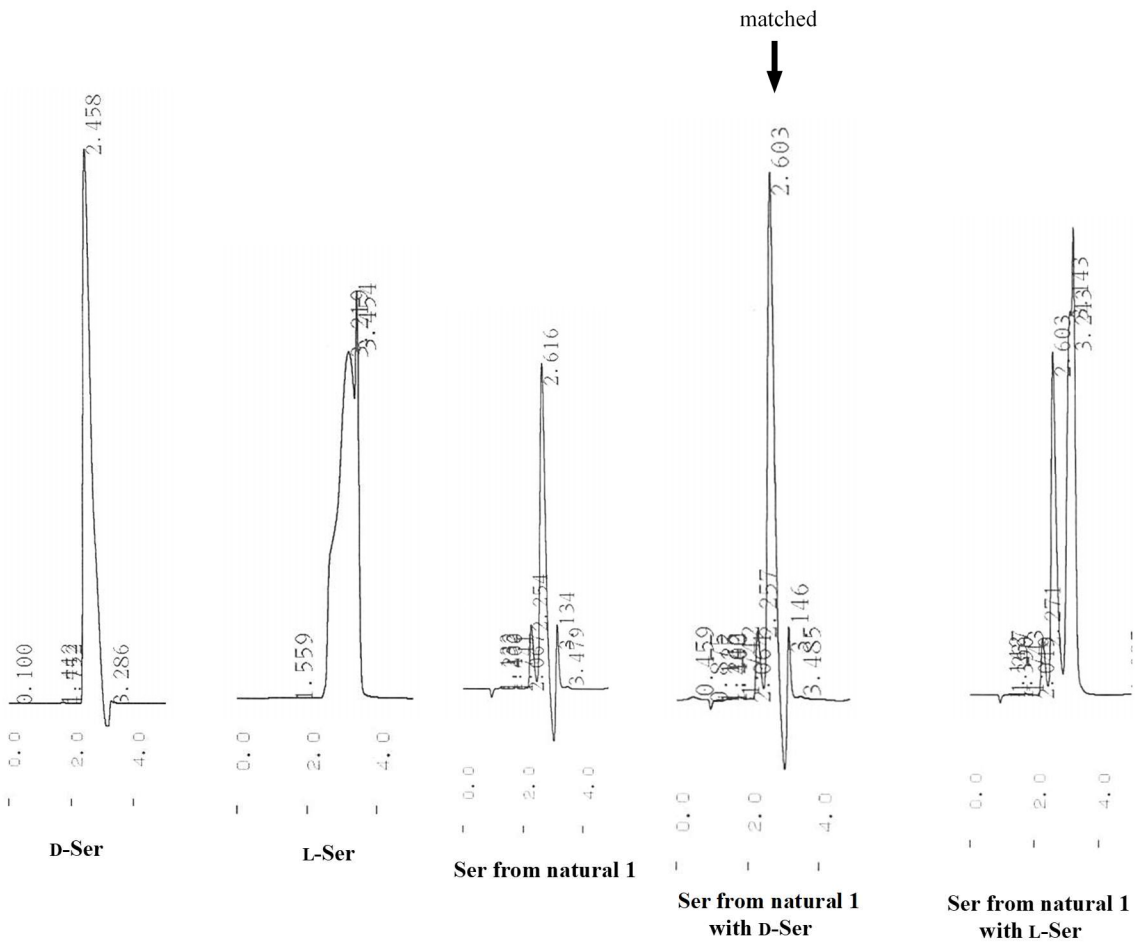


Figure S17. Chiral HPLC analysis of hydrosylates of **1** (Ser).

Ser: column, DAICEL CHIRALPAK MA(+) (4.6 × 50 mm × 2 in series); flow rate 1 mL/min; detection, UV 254 nm; solvent 2.0 mM $CuSO_4$

t_R (min): Authentic samples D-Ser (2.5), L-Ser (3.2)

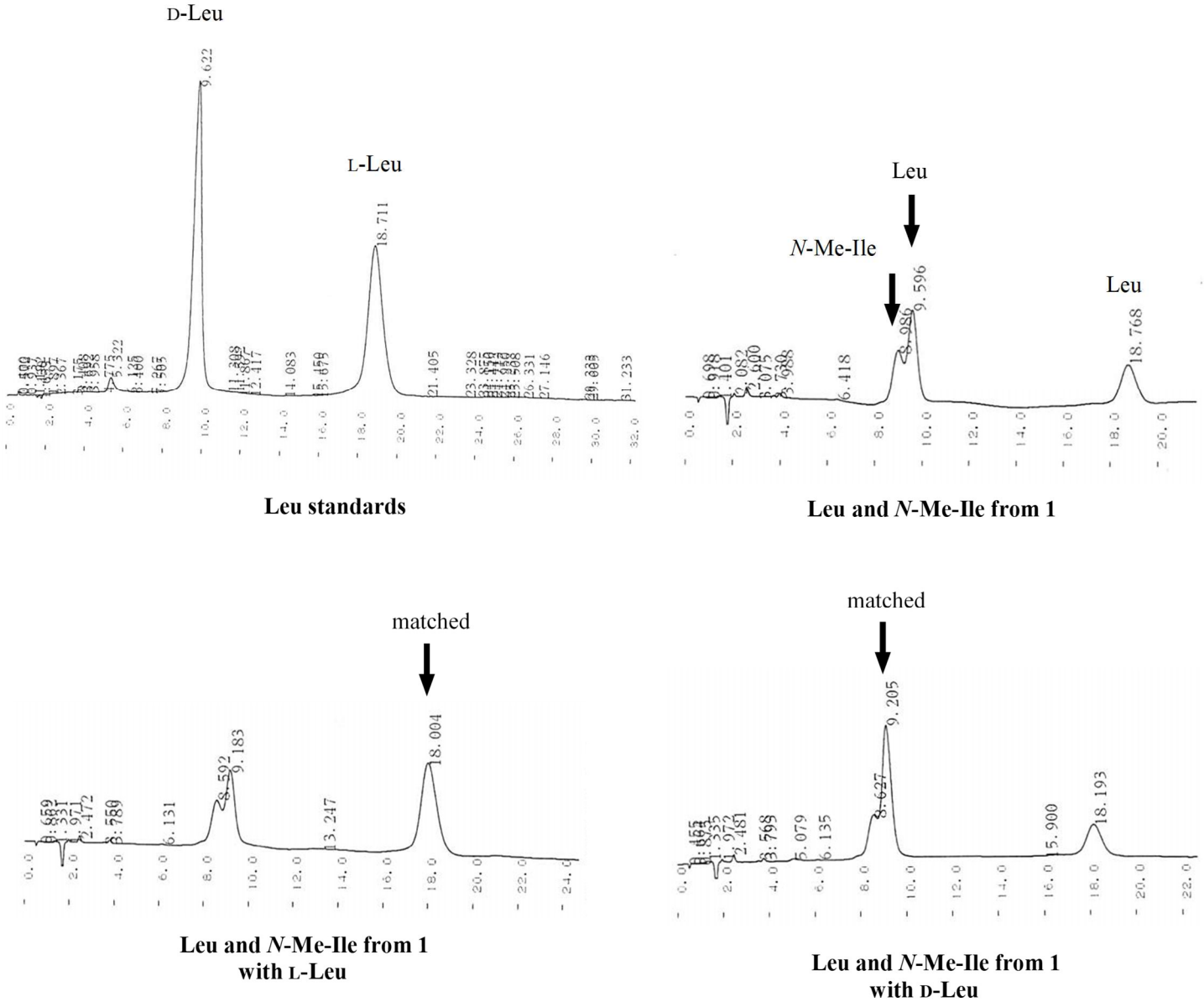


Figure S18. Chiral HPLC analysis of hydrosylates of **1** (Leu).

Leu: column, DAICEL CHIRALPAK MA(+) (4.6 × 50 mm); flow rate 1 mL/min; detection, UV 254 nm; solvent 2.0 mM $CuSO_4$

t_R (min): Authentic samples D-Leu (9.6), L-Leu (18.7)

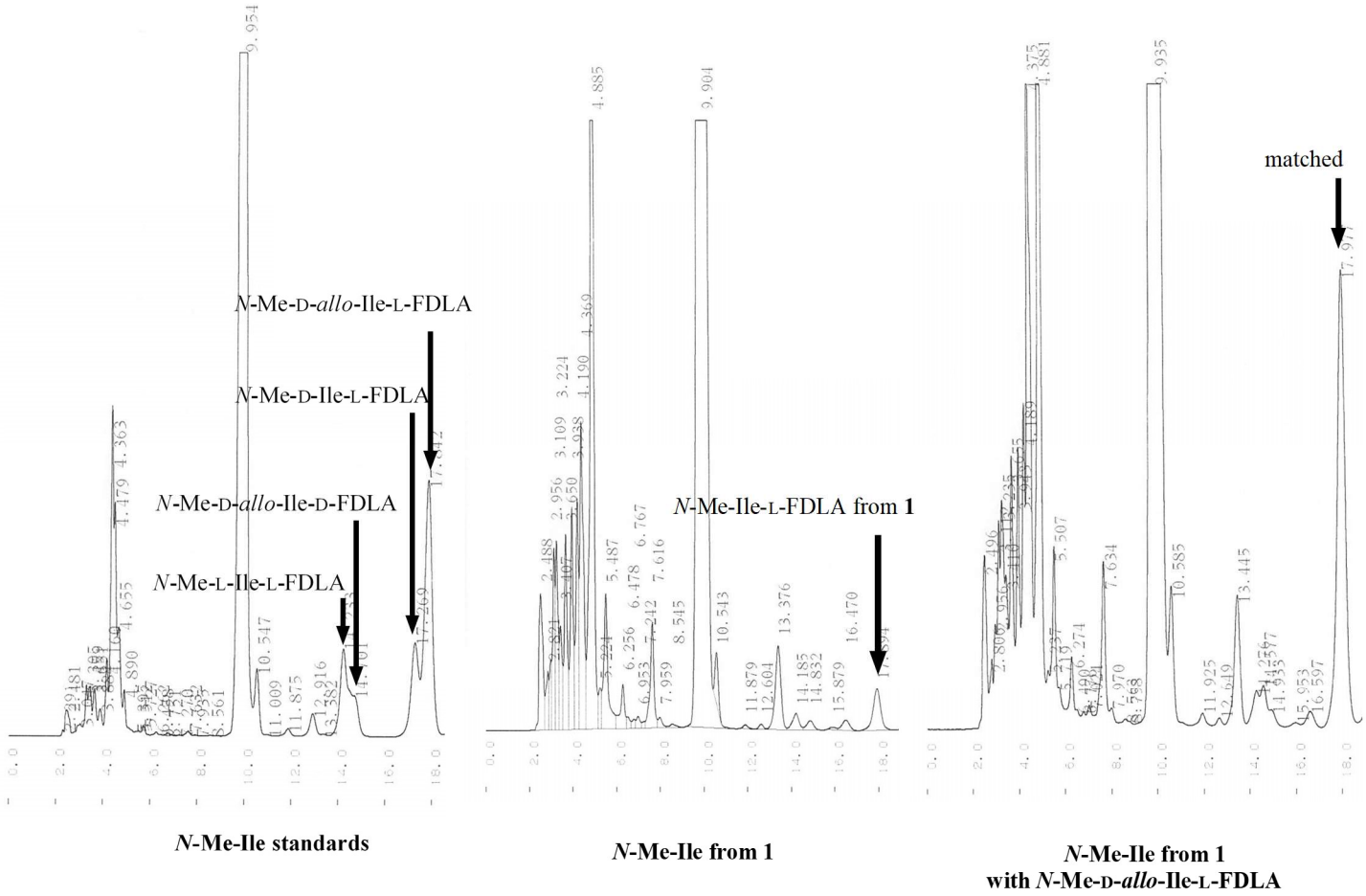
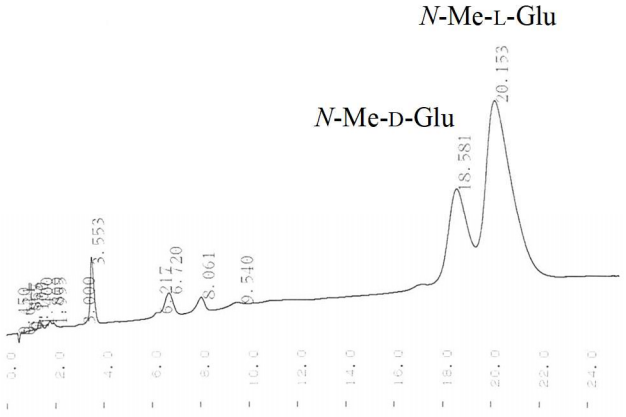


Figure S19. Chiral HPLC analysis of hydrosylates of **1** (*N*-Me-Ile).

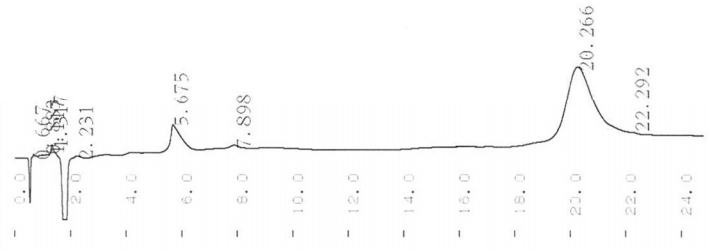
N-Me-Ile: column, Cosmosil PBr (4.6 × 250 mm); flow rate 1 mL/min; detection, UV 340 nm; solvent 55% MeCN, 0.1% TFA

t_R (min): Authentic samples *N*-Me-L-Ile-L-FDLA (14.2), *N*-Me-D-Ile-L-FDLA (17.3), *N*-Me-D-*allo*-Ile-D-FDLA (14.7), *N*-Me-D-*allo*-Ile-L-FDLA (17.8)

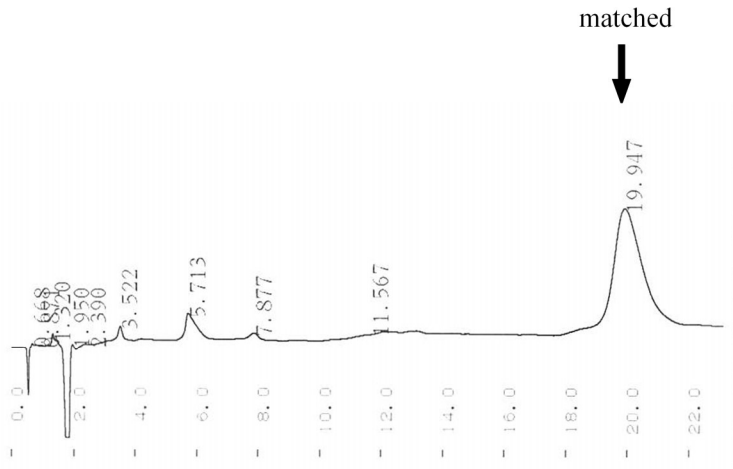
N-Me-D-*allo*-Ile-D-FDLA was used as a substitute of *N*-Me-L-*allo*-Ile-L-FDLA



***N*-Me-Glu standards**



***N*-Me-Glu from 1**

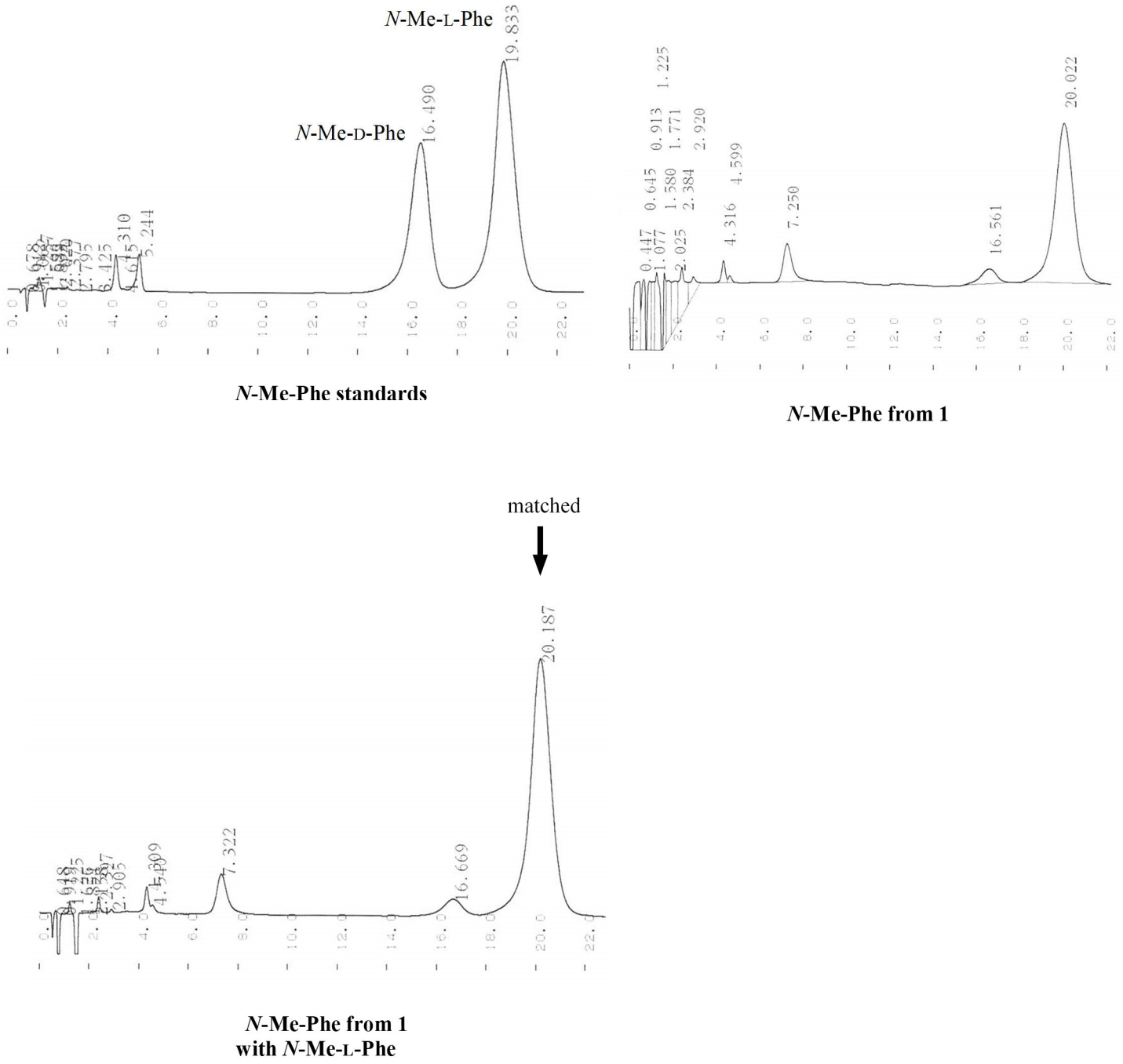


***N*-Me-Glu from 1**
with *N*-Me-L-Glu

Figure S20. Chiral HPLC analysis of hydrosylates of **1** (*N*-Me-Glu).

N-Me-Glu: column, DAICEL CHIRALPAK MA(+) (4.6 × 50 mm); flow rate 1 mL/min; detection, UV 254 nm; solvent 2.0 mM $CuSO_4$

t_R (min): Authentic samples *N*-Me-D-Glu (18.6), *N*-Me-L-Glu (20.2)



***N*-Me-Phe standards**

***N*-Me-Phe from 1**

***N*-Me-Phe from 1 with *N*-Me-L-Phe**

Figure S21. Chiral HPLC analysis of hydrosylates of **1** (*N*-Me-Phe).

N-Me-Phe: column, DAICEL CHIRALPAK MA(+) (4.6 × 50 mm); flow rate 1 mL/min; detection, UV 254 nm; solvent 2.0 mM $CuSO_4$

t_R (min): Authentic samples *N*-Me-D-Phe (16.5), *N*-Me-L-Phe (19.8)

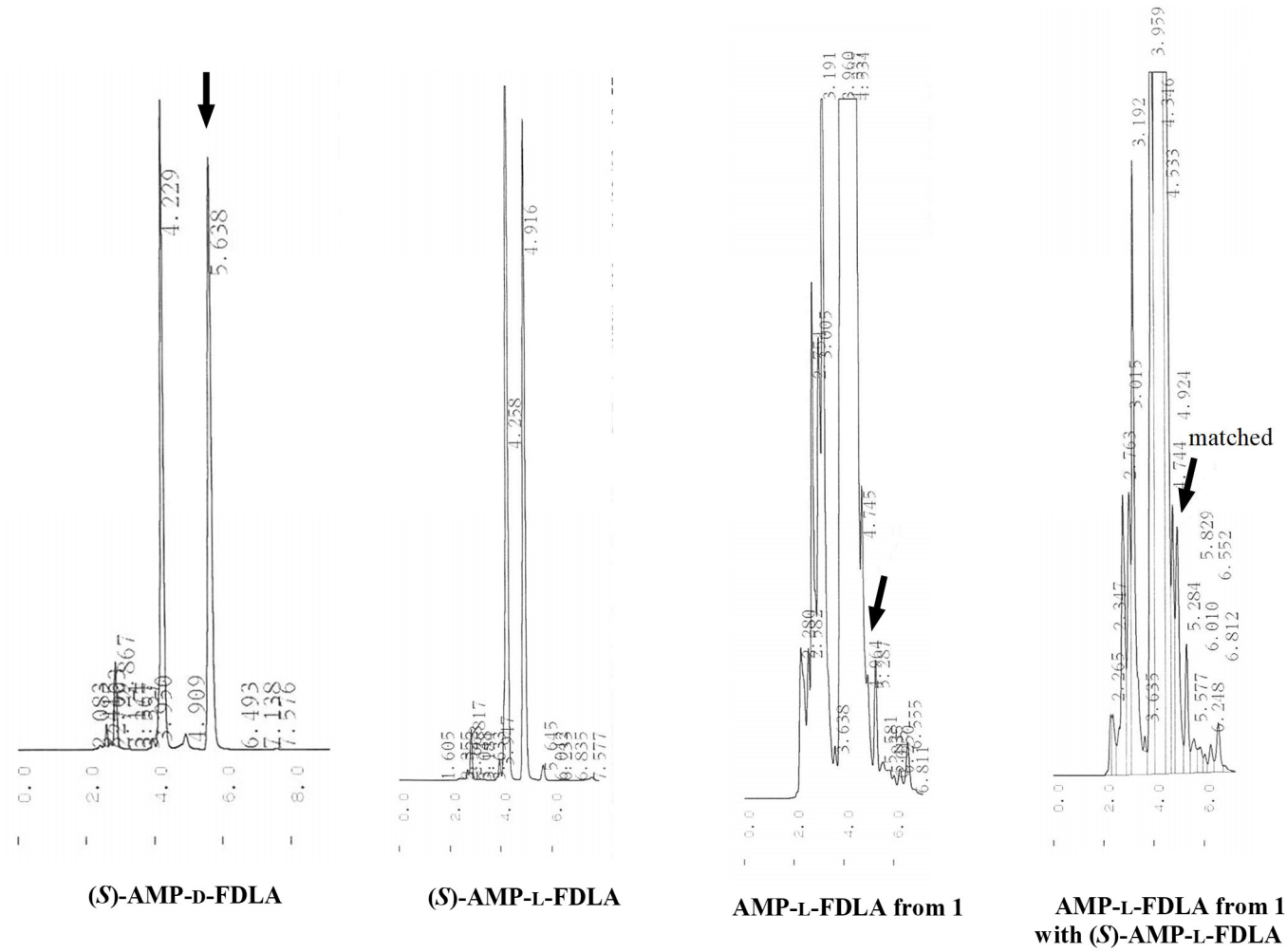


Figure S22. Chiral HPLC analysis of hydrosylates of **1** (AMP).

AMP: column, Cosmosil Cholester (4.6 × 250 mm); flow rate 1 mL/min; detection, UV 340 nm; solvent 70% aqueous MeCN, 0.1% TFA

t_R (min): Authentic samples (*S*)-AMP-D-FDLA (5.6), (*S*)-AMP-L-FDLA (4.9)

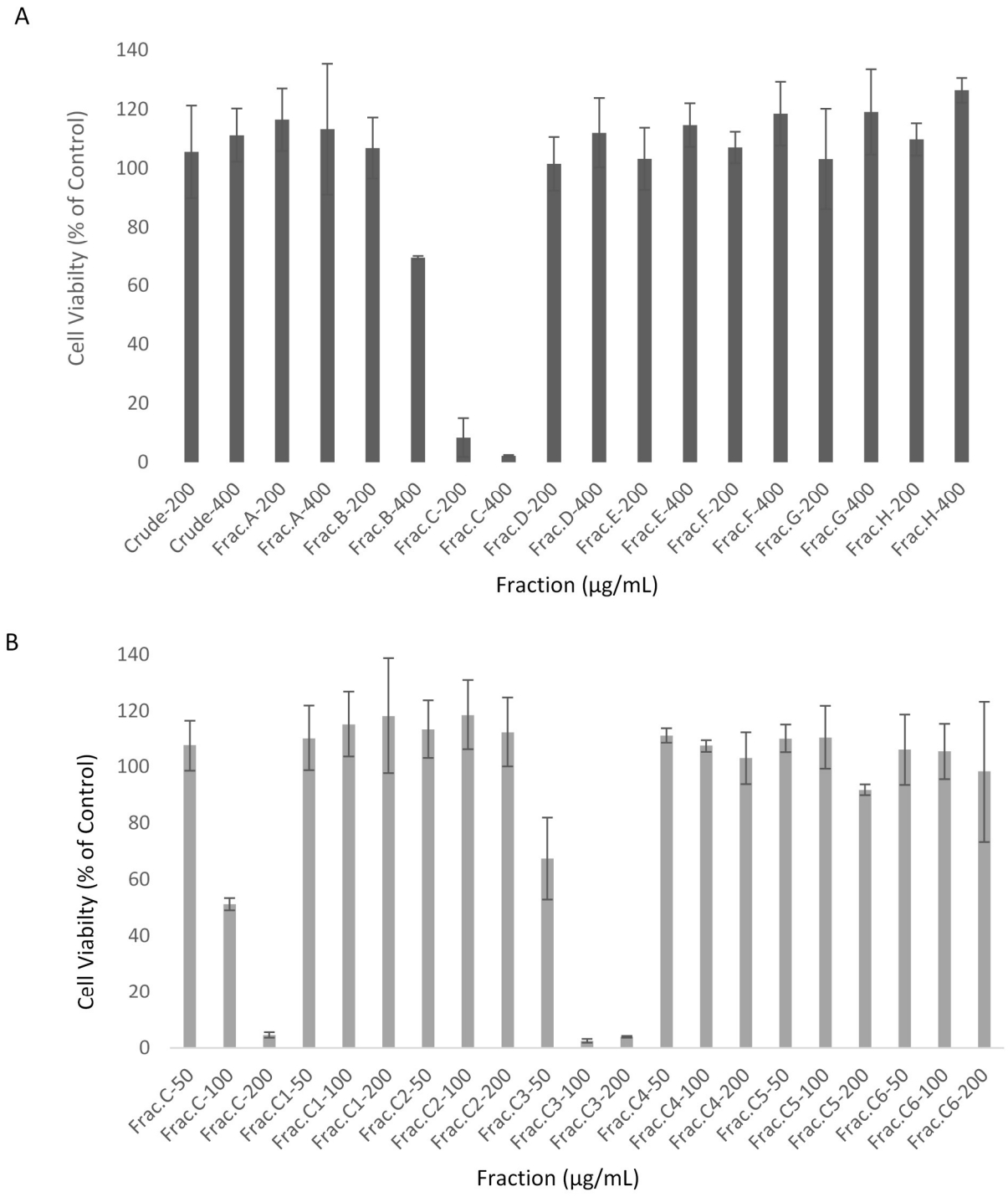


Figure S23. Bioactivity data used to guide fractionation of cf. *Neolyngbya* sp. extract using HCT116 human colorectal cancer cell viability *in vitro*.

Cells were untreated (control) and treated with (A) crude fractions or (B) sub-fractions for 24 h followed by cell viability assessment using XTT assay. Data represent the mean cell proliferation of three trials using the XTT assay, and bars represent the standard deviations.

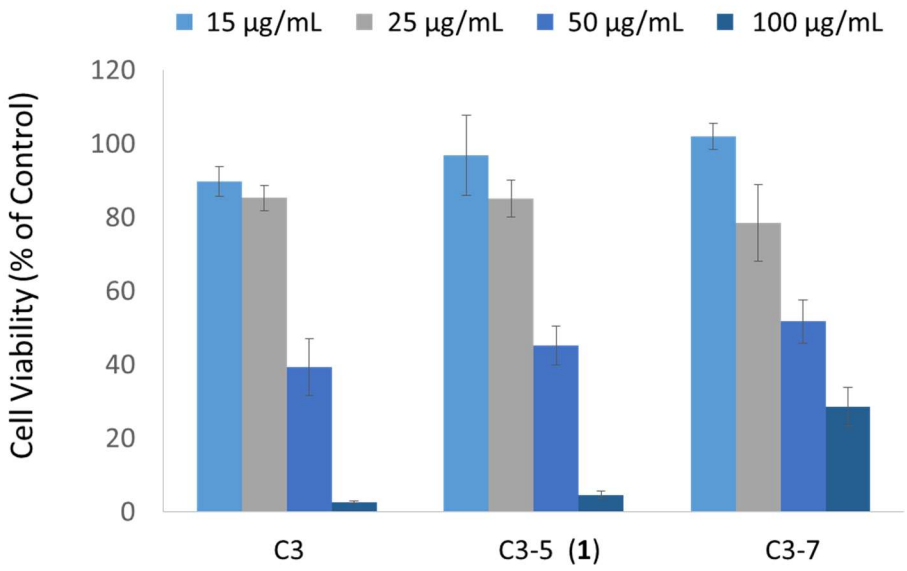


Figure S24. The *in vitro* effect of C3, C3-5 (compound **1**), and C3-7 on HCT116 cells viability, following 24 h of treatment with escalating concentrations.

Cell viability assessment done using the XTT assay. Results are presented as the percentage of control untreated HCT116 cells. Error bars represent the standard deviations.

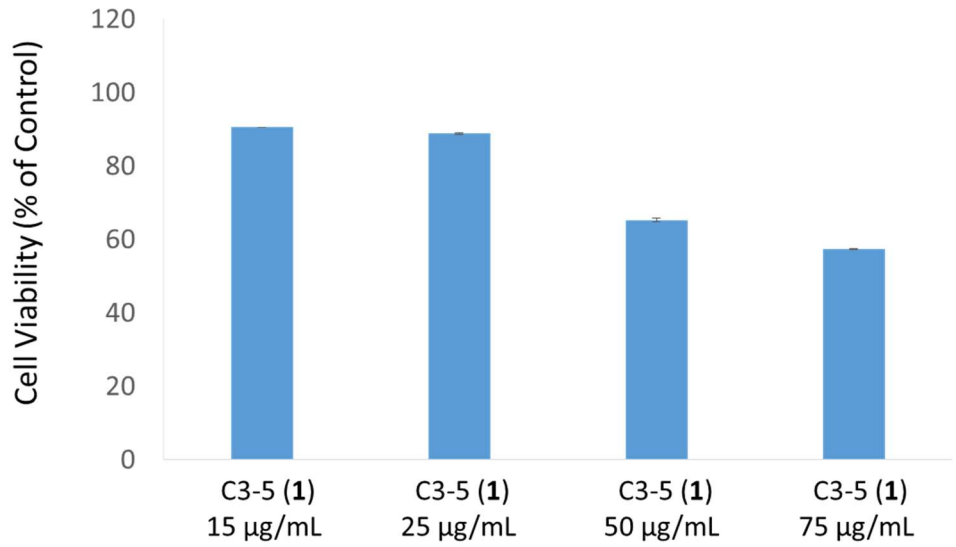


Figure S25. The *in vitro* effect of C3-5 (compound **1**) on HCT116 cells viability, following 8 h of treatment with escalating concentrations.

Cell viability assessment of C3-5 using the XTT assay. Results are presented as the percentage of control untreated HCT116 cells. Error bars represent the standard deviations.

https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=f62b23918fb24bca9f4a234f3555df50

Weblink S1. GNPS metabolomics data used to prepare main text Figure 4.

https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=0e36af9bc15d4d6c901292d5be8ff32b

Weblink S2. GNPS metabolomics data used to prepare main text Figure 9.

UGGGGAAUUUUCCGCAAUGGGCGAAGCCUGACGGAGCAAGACCGCGUGGGGGAGAAAGCCUUUGGGUUGUA
AACCCCUUUUCUGGGAAGAGUGACGGUACCAGGAAUCAGCCUCGGCUAACUCCGUGCCAGCAGCCGCGGUA
AUACGGAGGAGGCAGCGUUAUCCGGAAUGAUUGGGCGUAAAGCGUCCGCAGGUGGAAGUCGUGUUAAACCA
AGCUAACUGAGGGCAUGGAAACUGCUGAGUCGGUAGGGGCAGAGGGAAUUCCUGGUGUAGCGGUGAAAUG
CGUAGAGAUCAGGAAGAACACCGUGGCGAAAGCGCUCUGCUGGGCCACUGACACUCAGGGACGAAAGCUAG
GGGAGCGAAUGGG

Sequence S1. 16S rRNA gene V3-V4 amplicon used to prepare main text Figure 3C.

This was the most prominent sequence obtained from the microbiome analysis of sample HAINAN-19SEP17-3.