



Supporting Information

for

New variochelins from soil-isolated *Variovorax* sp. H002

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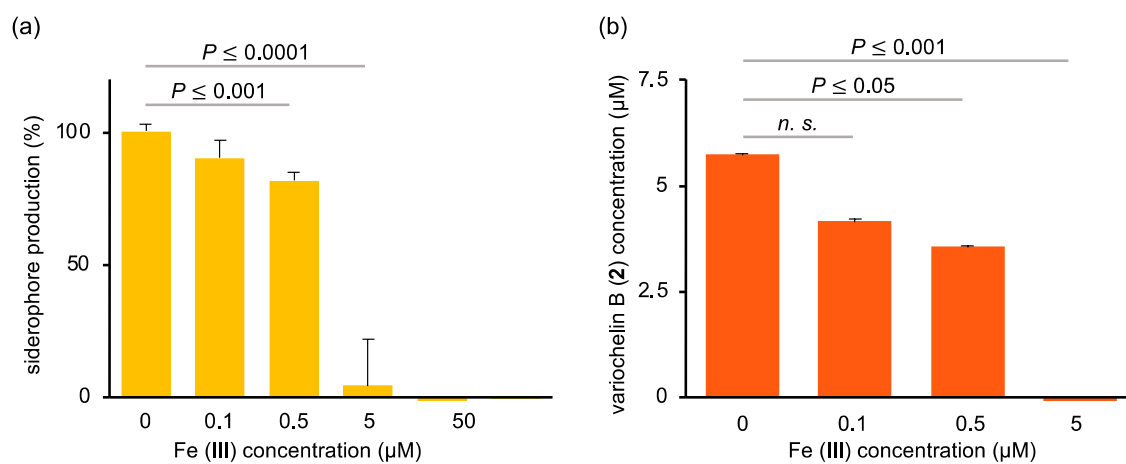


Figure S1. The effect of various iron concentrations on (a) total siderophore production indicated by CAS assay activity ($n = 3$), and (b) production of variochelin B (2) indicated by calculation of total area under curve ($n = 3$).

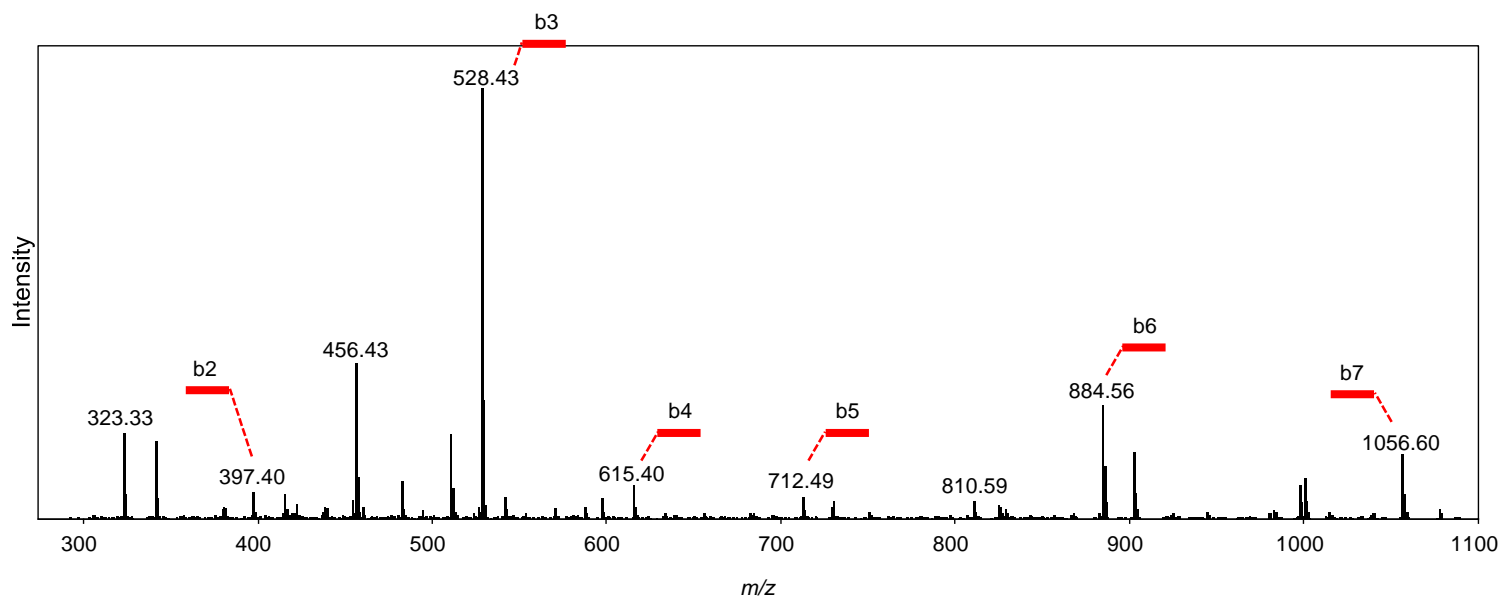
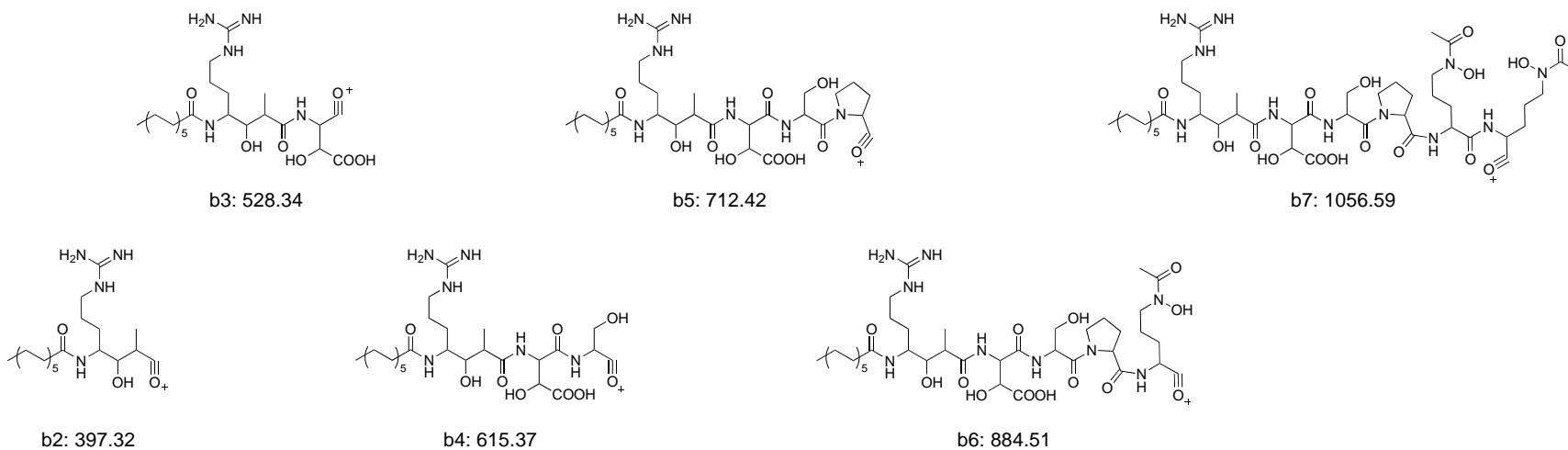


Figure S2. ESIMS/MS analysis of variochelin A (**1**). Structures and m/z of predicted fragmentation ions were described the top. An ion peak of $[M + H]^+$ at m/z 1075.08 was chosen as a parent ion.

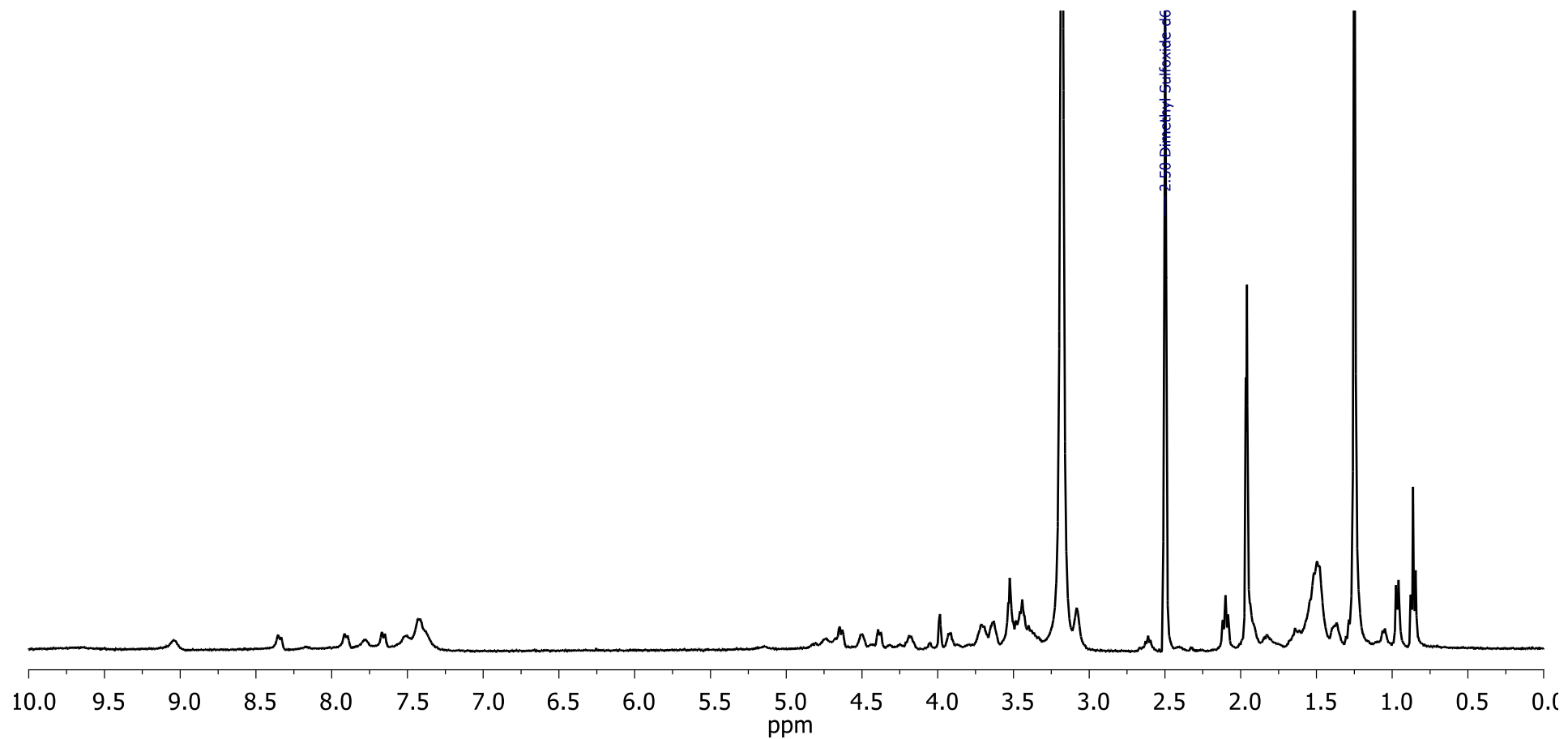


Figure S3. ^1H NMR spectrum of variochelin A (**1**) (500 MHz, $\text{DMSO-}d_6$).

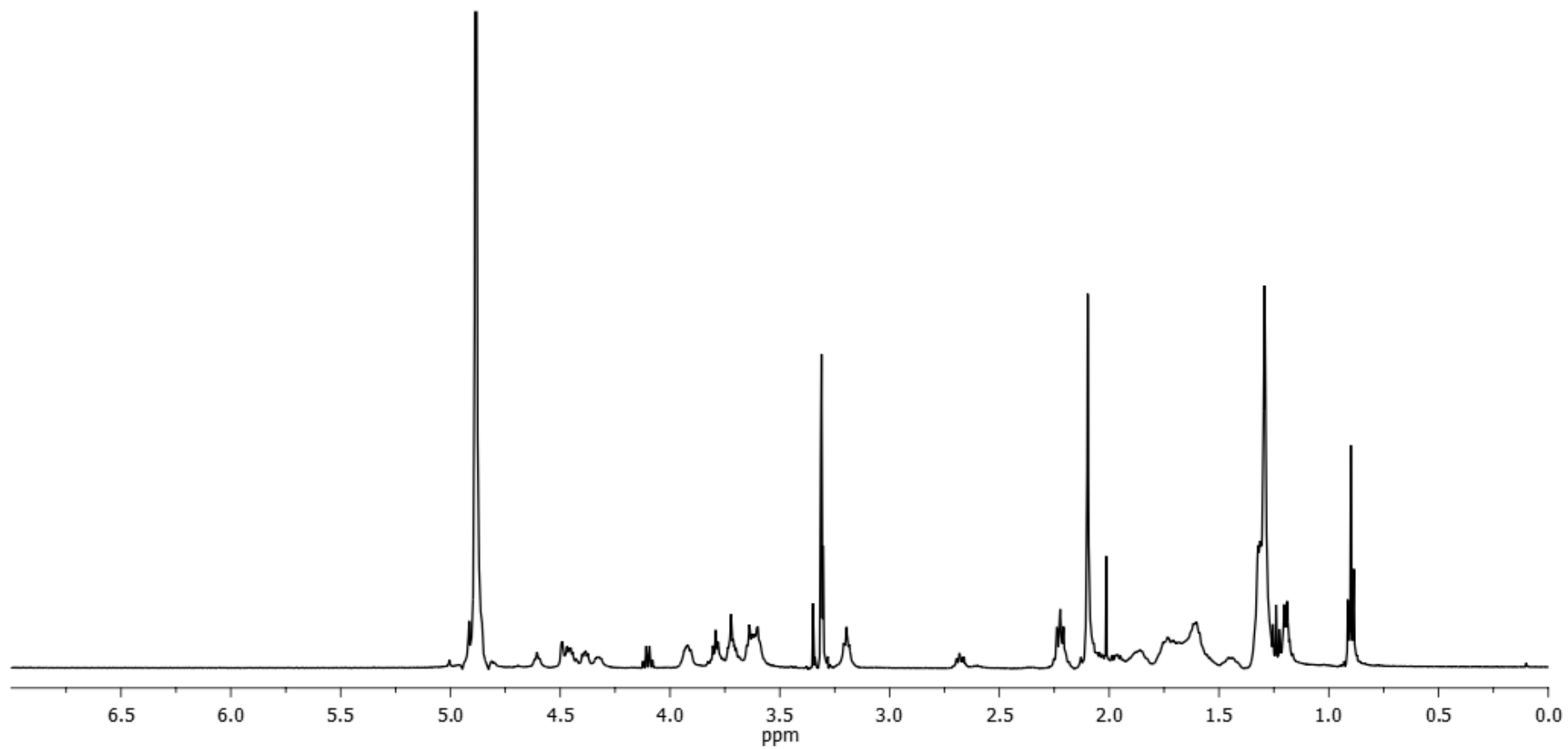


Figure S4. ^1H NMR spectrum of variochelin A (**1**) (500 MHz, methanol- d_4).

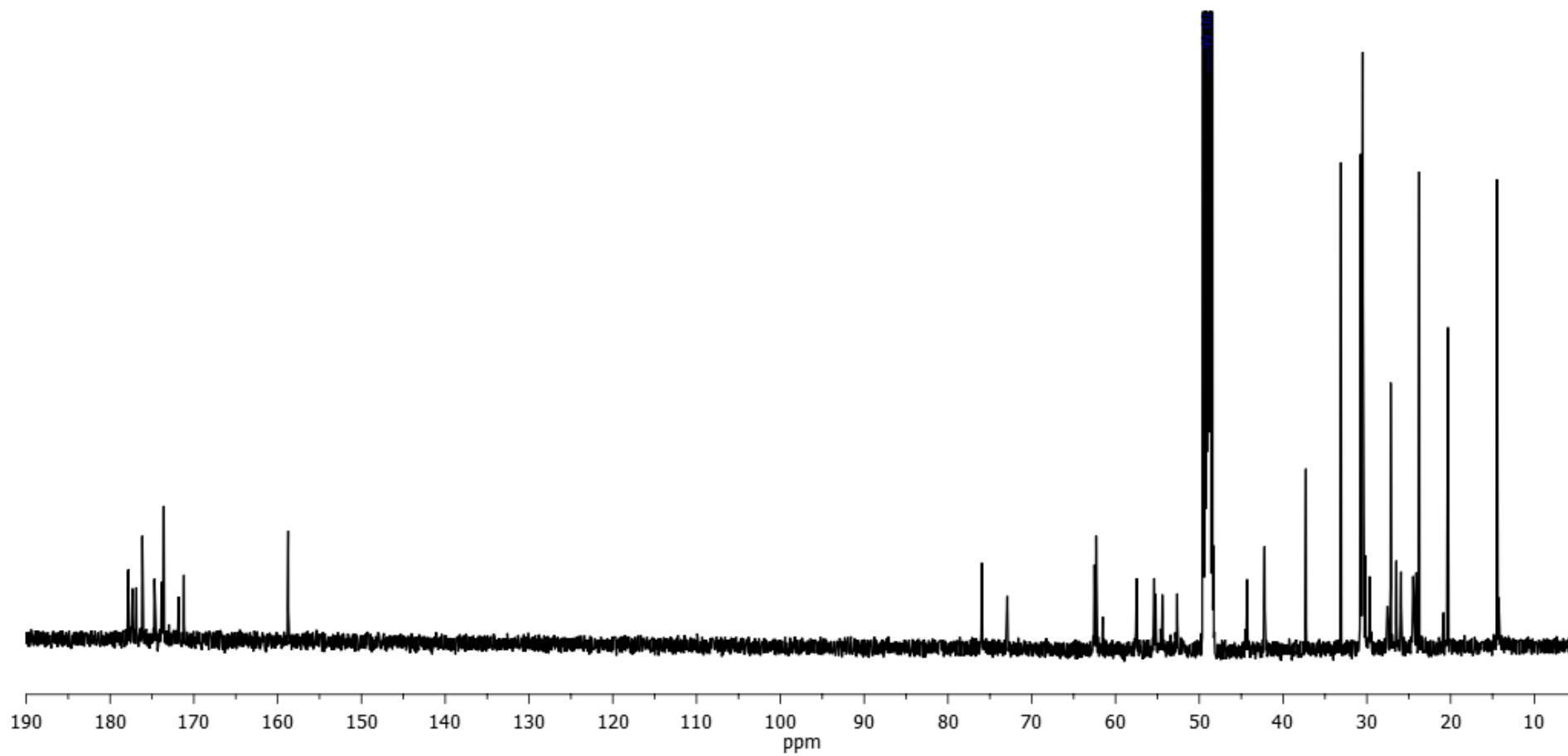


Figure S5. ^{13}C NMR spectrum of variochelin A (**1**) (125 MHz, $\text{methanol-}d_4$).

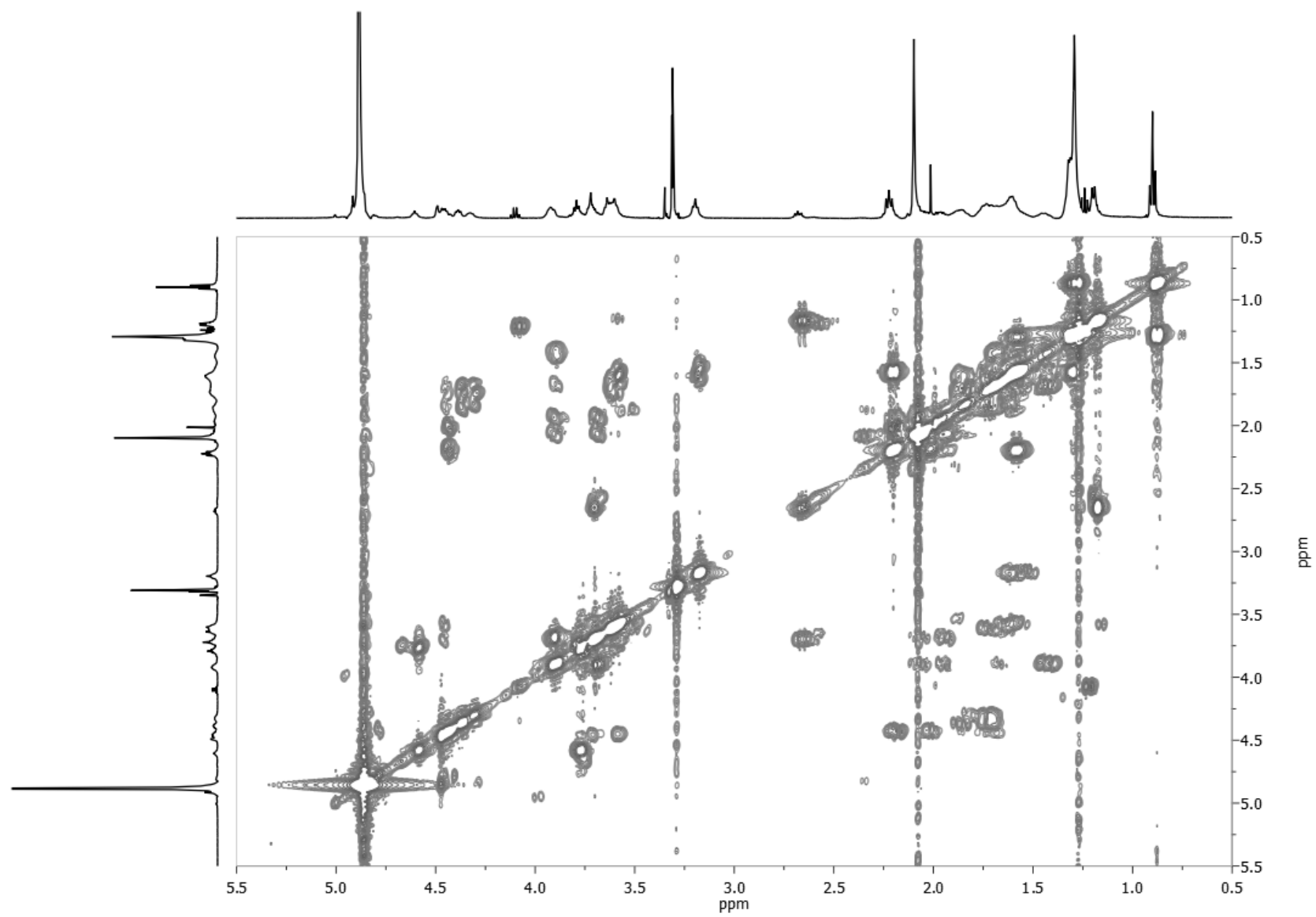


Figure S6. COSY spectrum of variochelin A (**1**) (500 MHz, methanol- d_4).

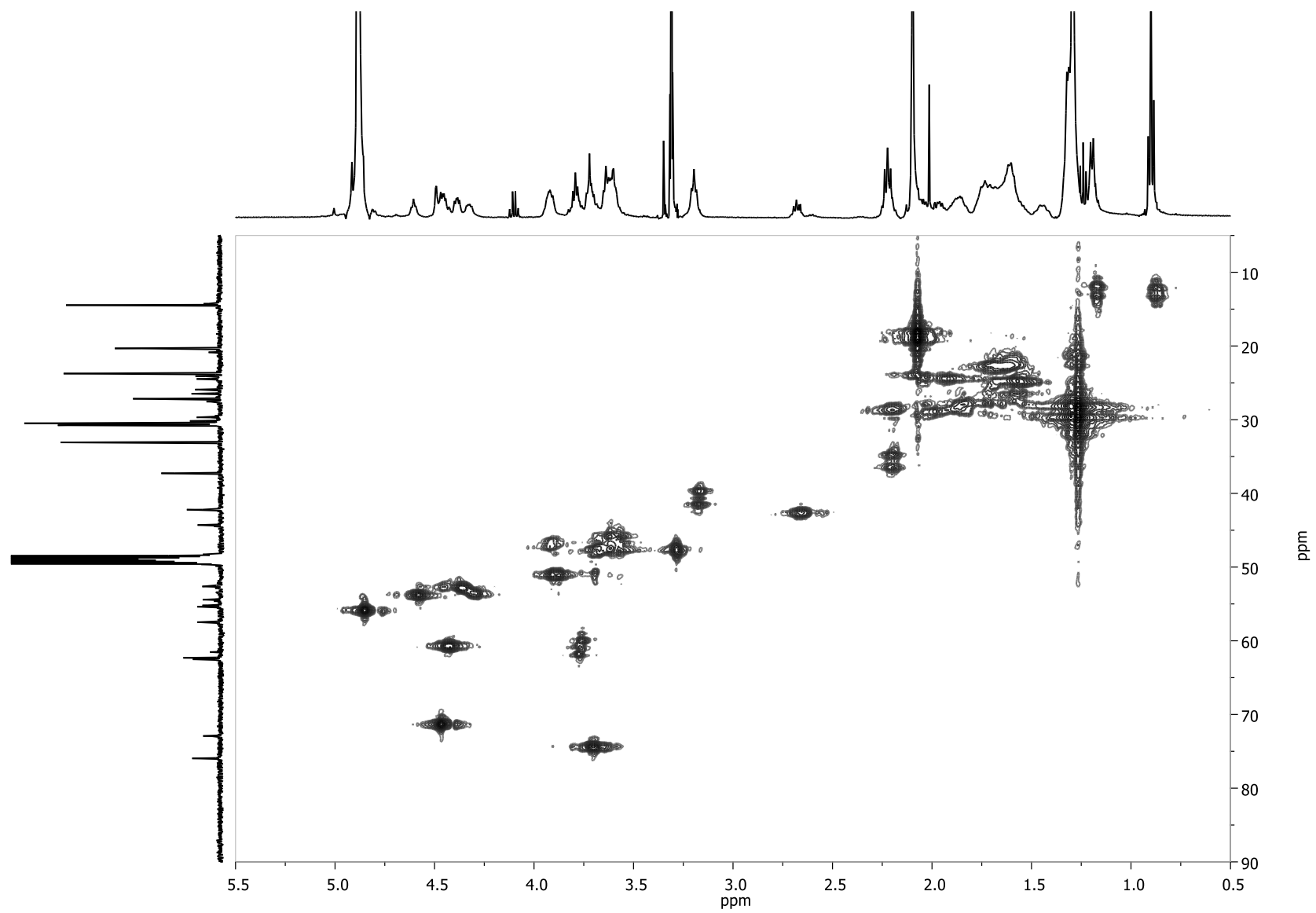


Figure S7. HSQC spectrum of variochelin A (**1**) (500 MHz, methanol- d_4).

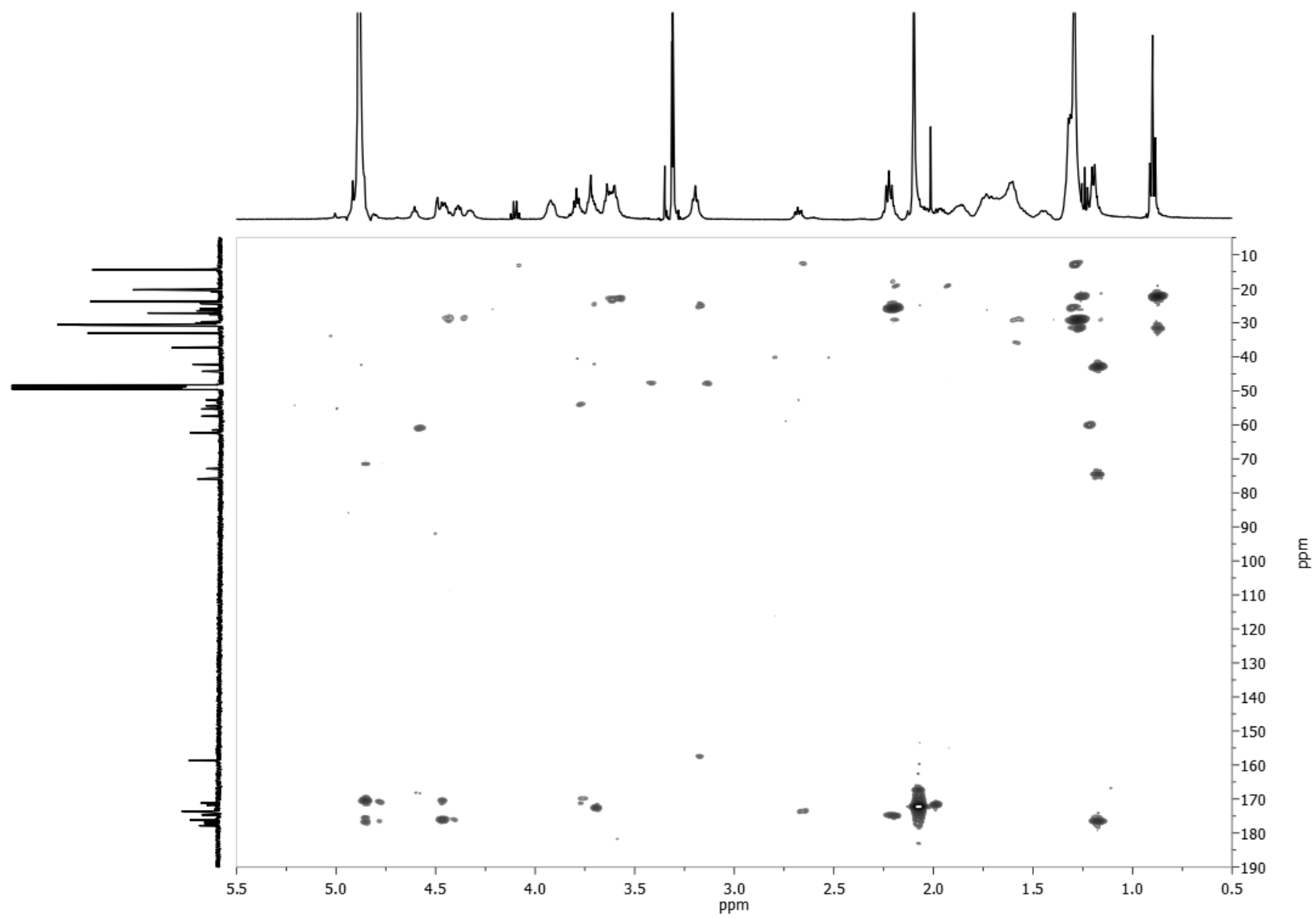


Figure S8. HMBC spectrum of variochelin A (**1**) (500 MHz, methanol- d_4).

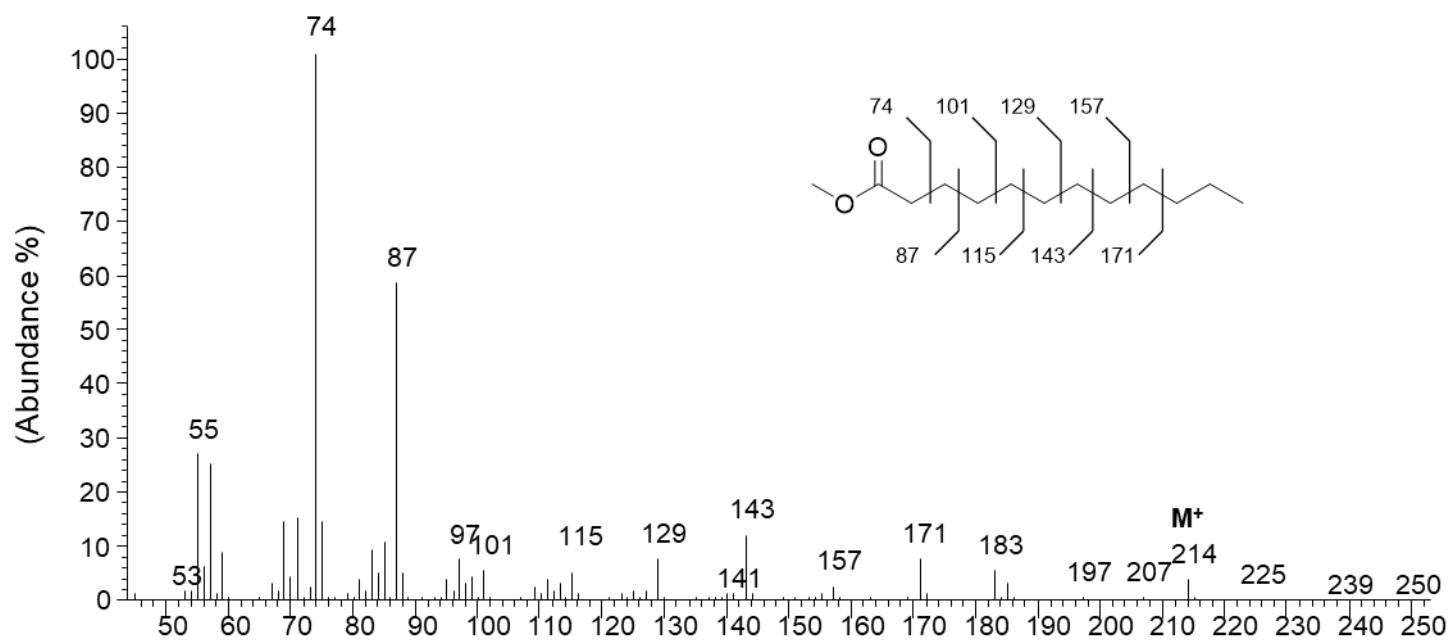


Figure S9. EIMS of dodecanoic acid methyl ester in variochelin A (**1**).

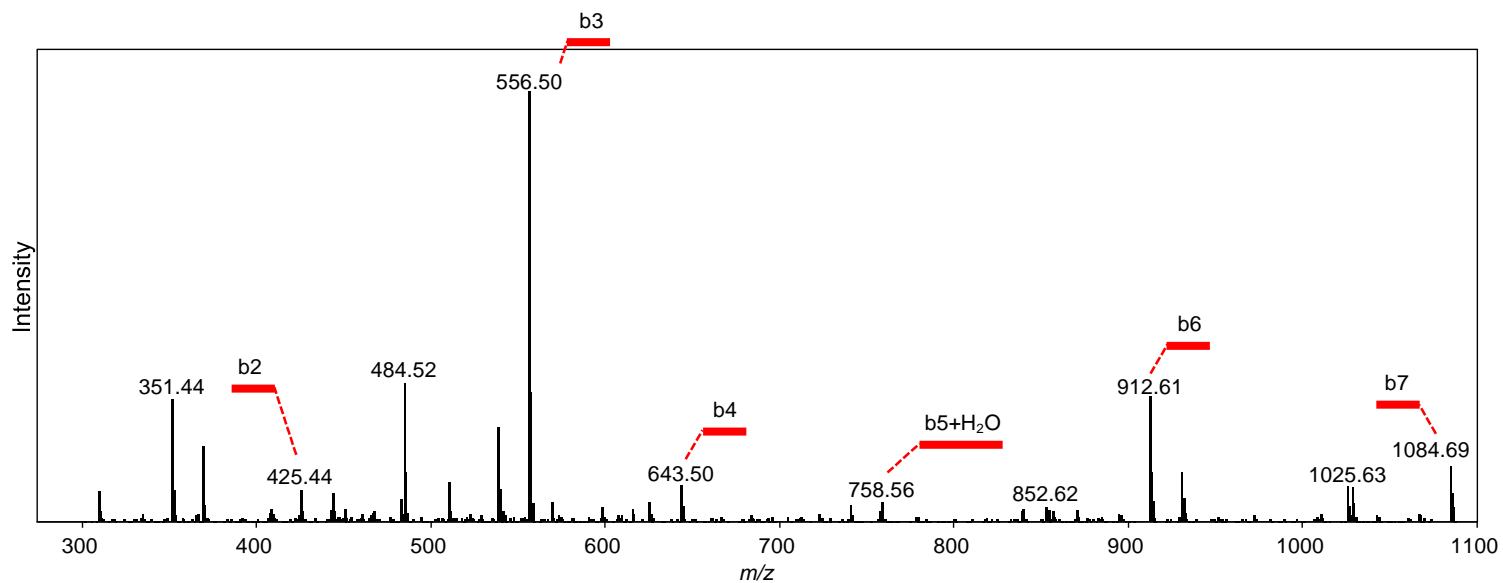
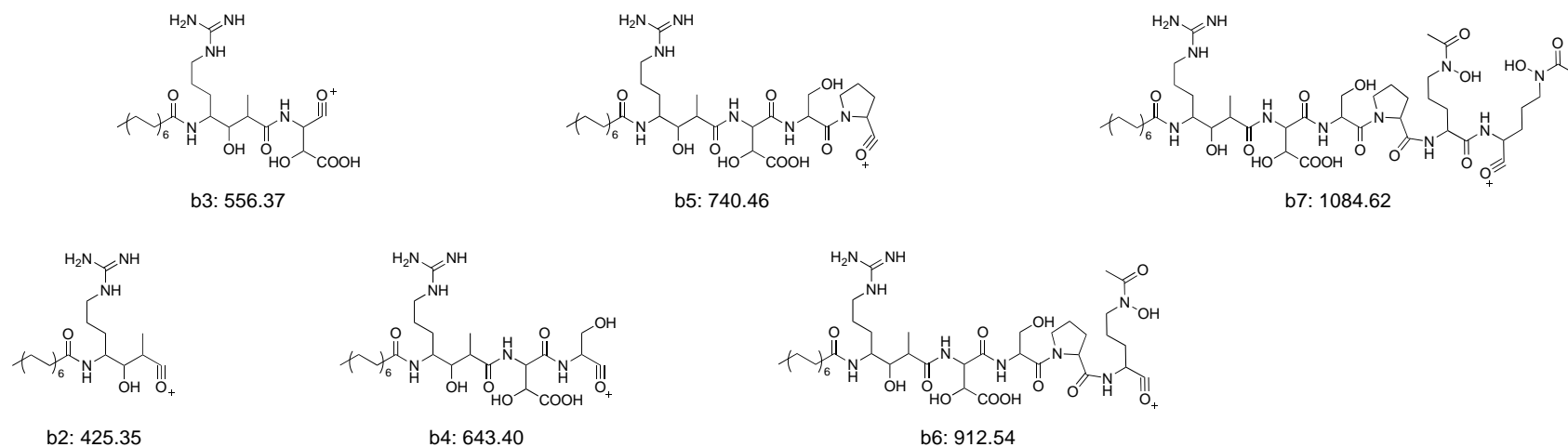


Figure S10. ESIMS/MS analysis of variochelin B (**2**). Structures and m/z of predicted fragmentation ions were described the top. An ion peak of $[M + H]^+$ at m/z 1103.18 was chosen as a parent ion.

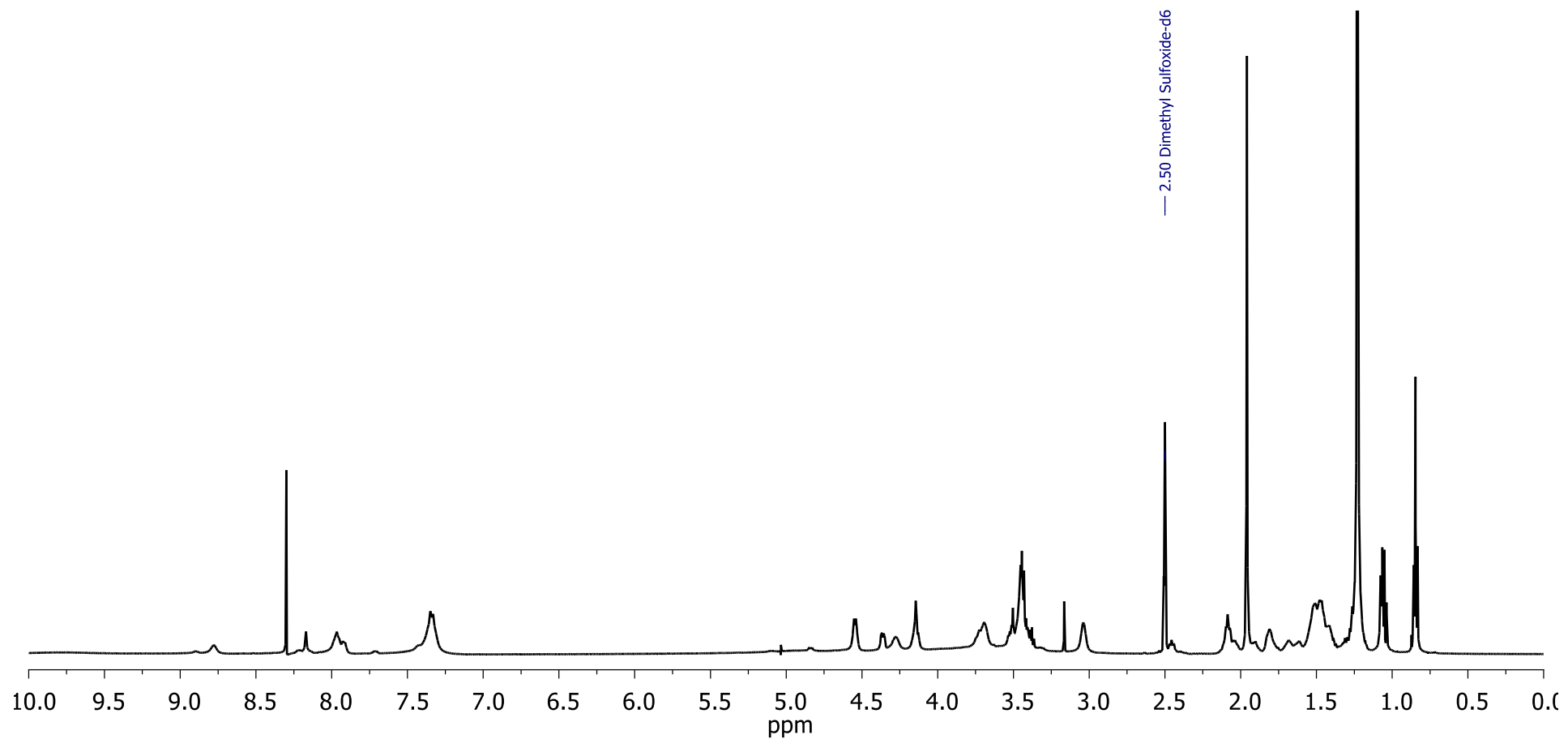


Figure S11. ^1H NMR spectrum of variochelin B (2) (500 MHz, $\text{DMSO-}d_6$).

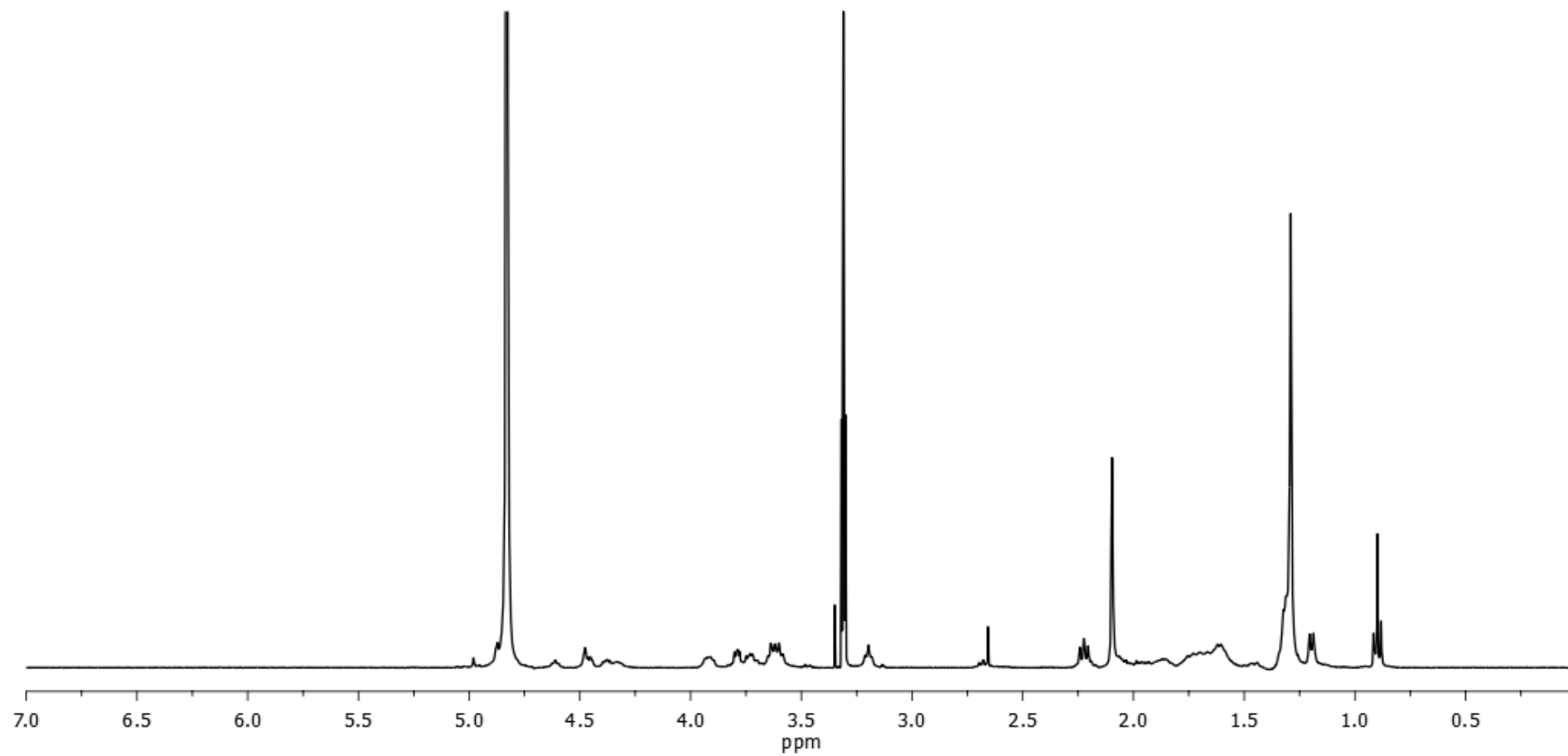


Figure S12. ^1H NMR spectrum of variochelin B (**2**). (500 MHz, methanol- d_4).

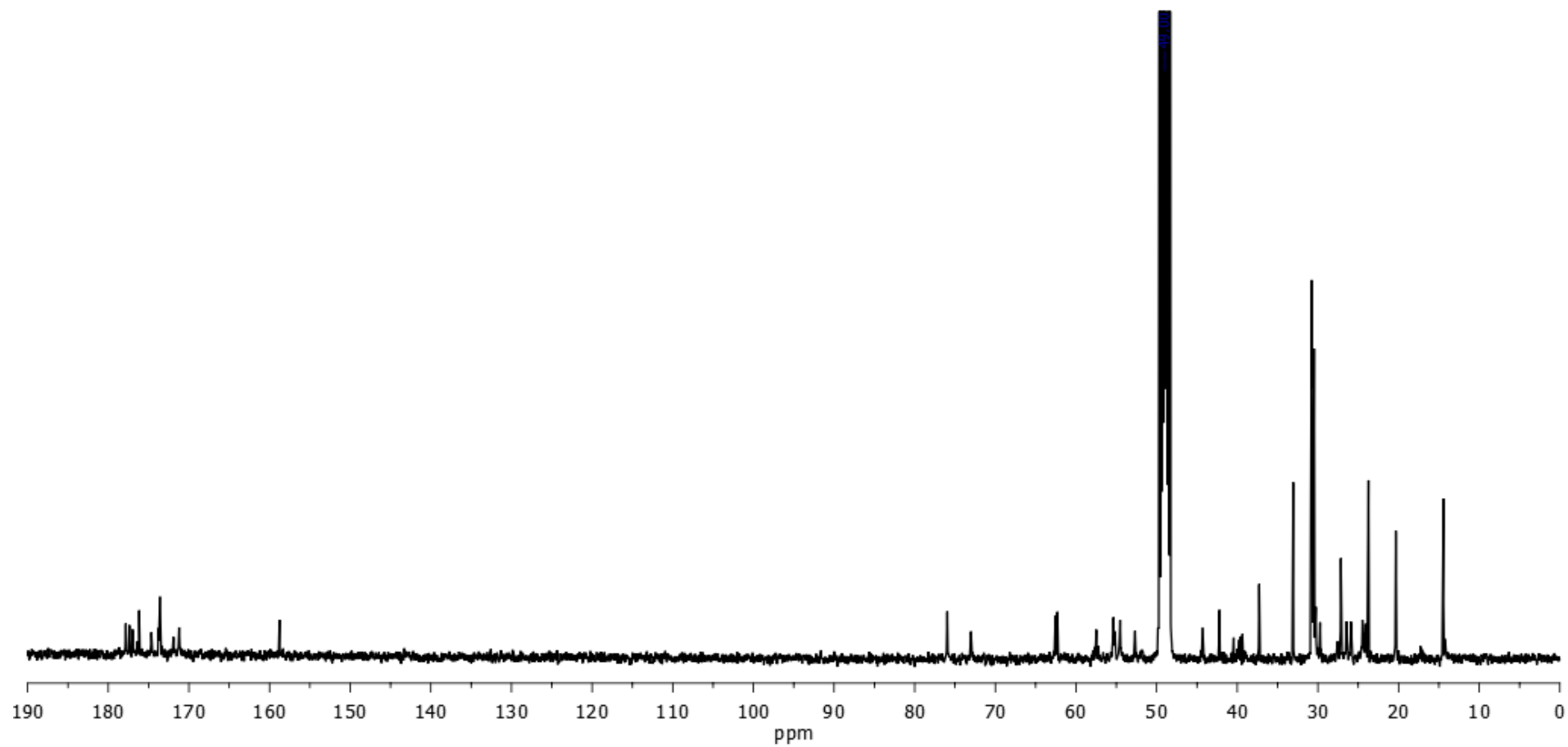


Figure S13. ^{13}C NMR spectrum of variochelin B (**2**). (125 MHz, $\text{methanol-}d_4$).

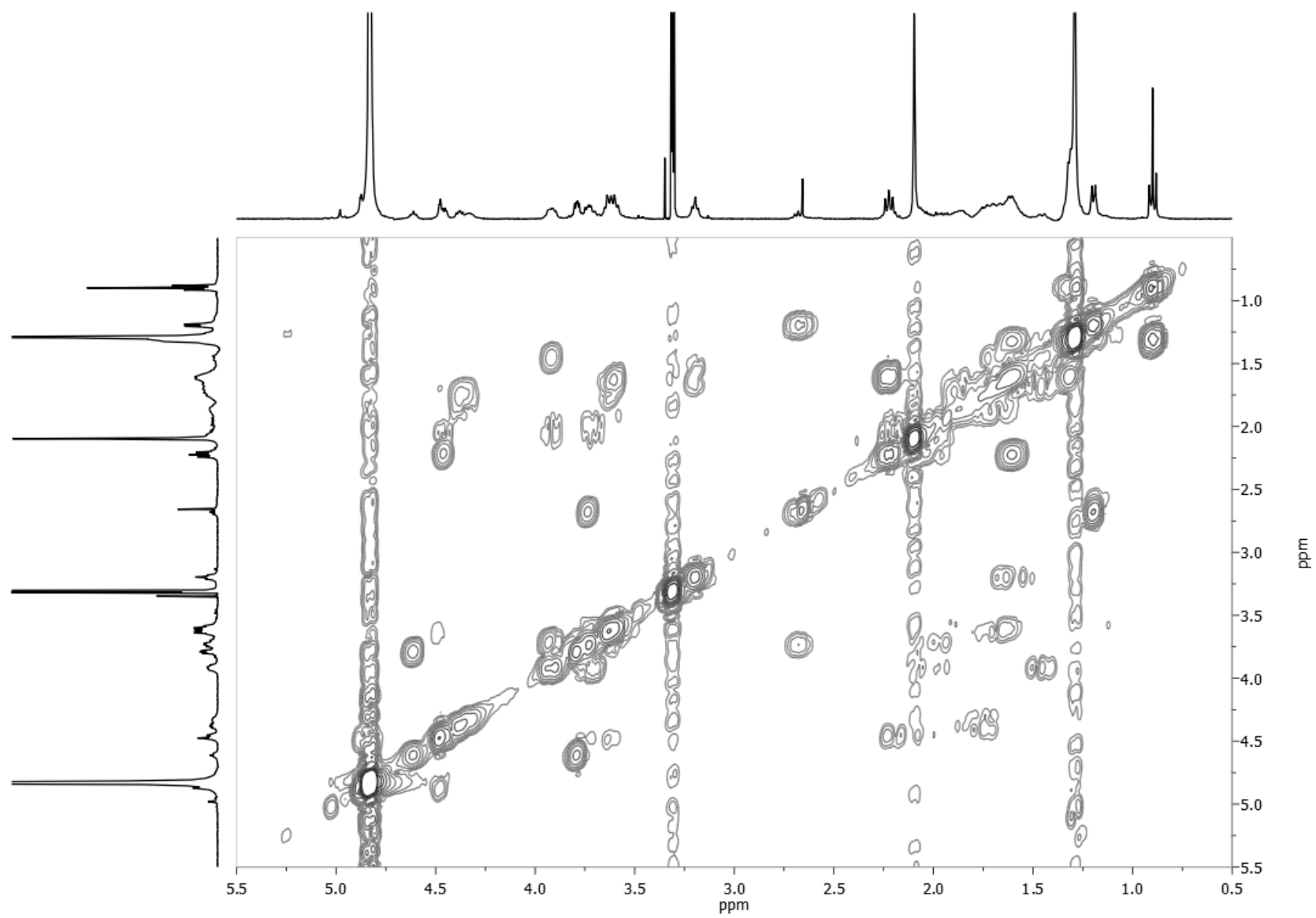


Figure S14. COSY spectrum of variochelin B (**2**). (500 MHz, methanol- d_4).

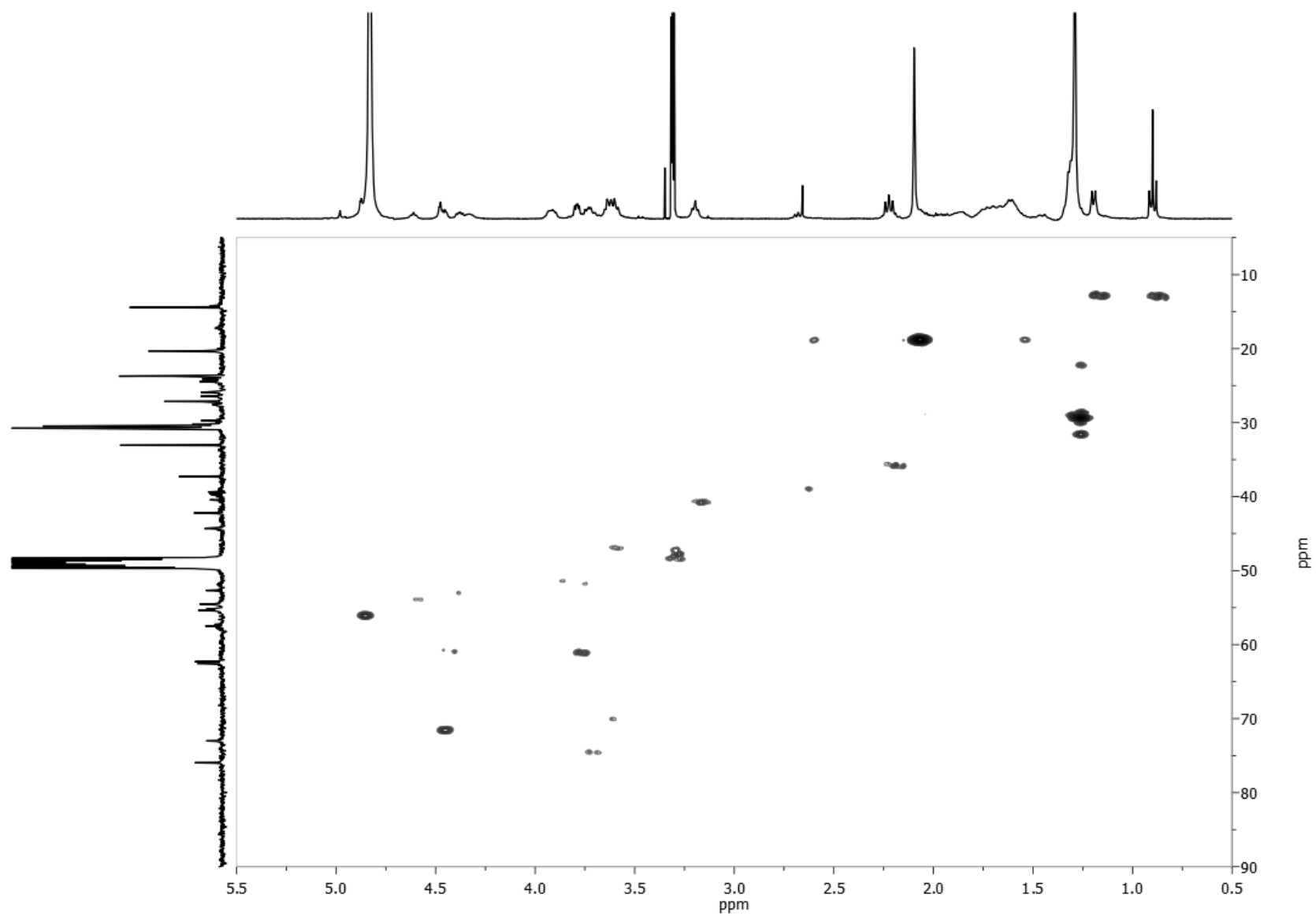


Figure S15. HSQC spectrum of variochelin B (**2**). (500 MHz, methanol- d_4).

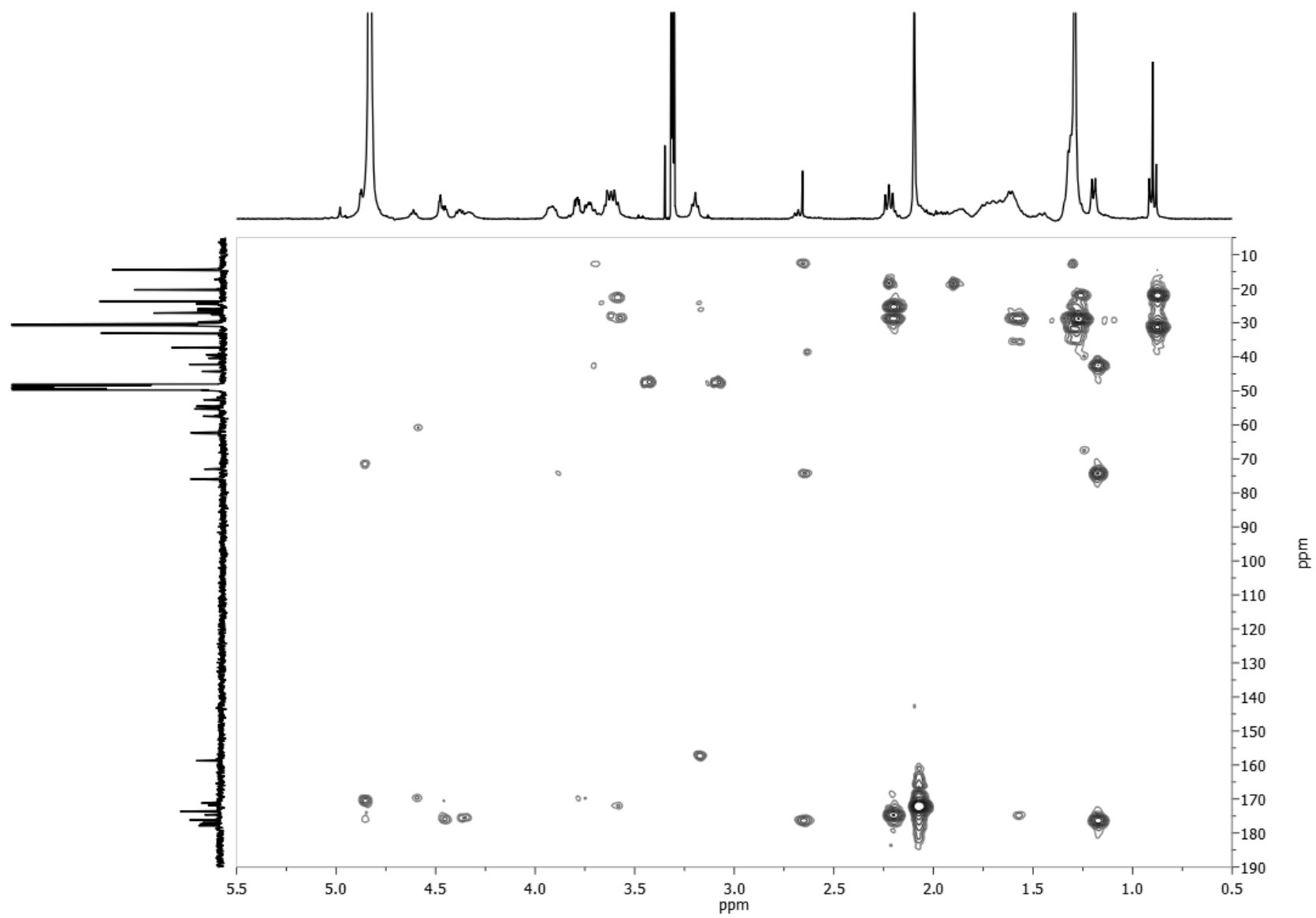


Figure S16. HMBC spectrum of variochelin B (**2**). (500 MHz, methanol-*d*₄).

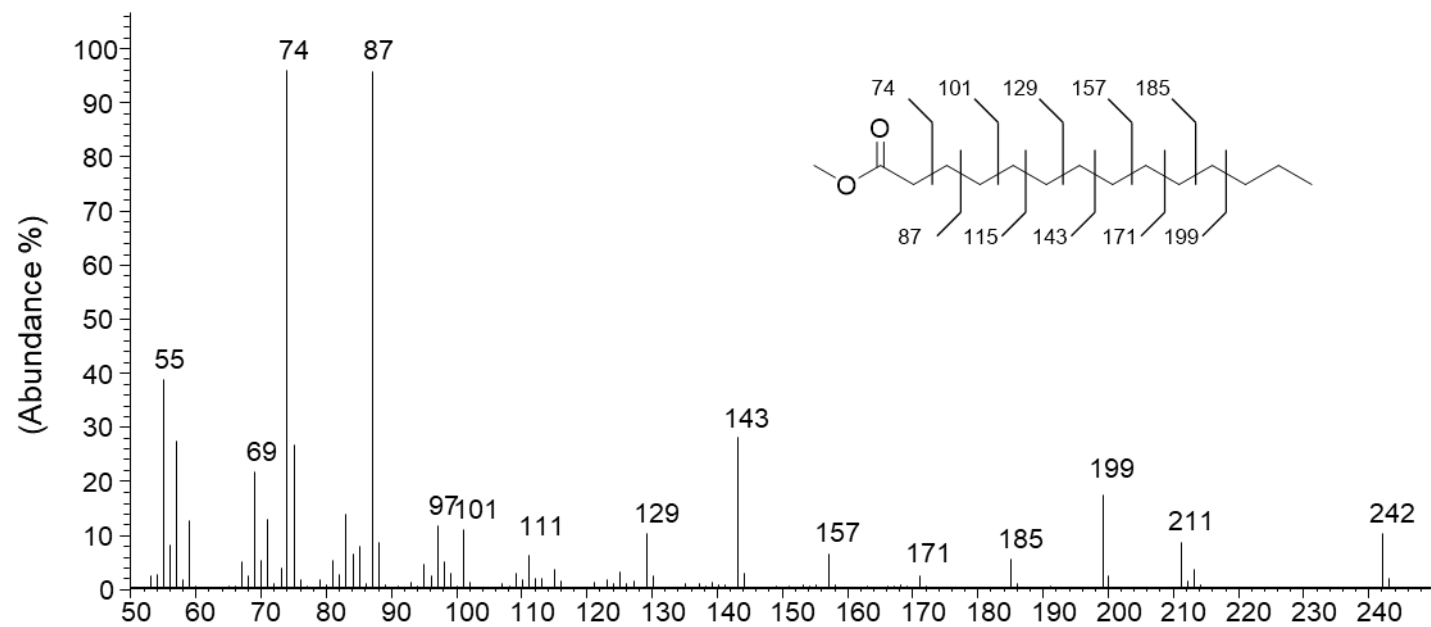


Figure S17. EIMS of tetradecanoic acid methyl ester in variochelin B (**2**).

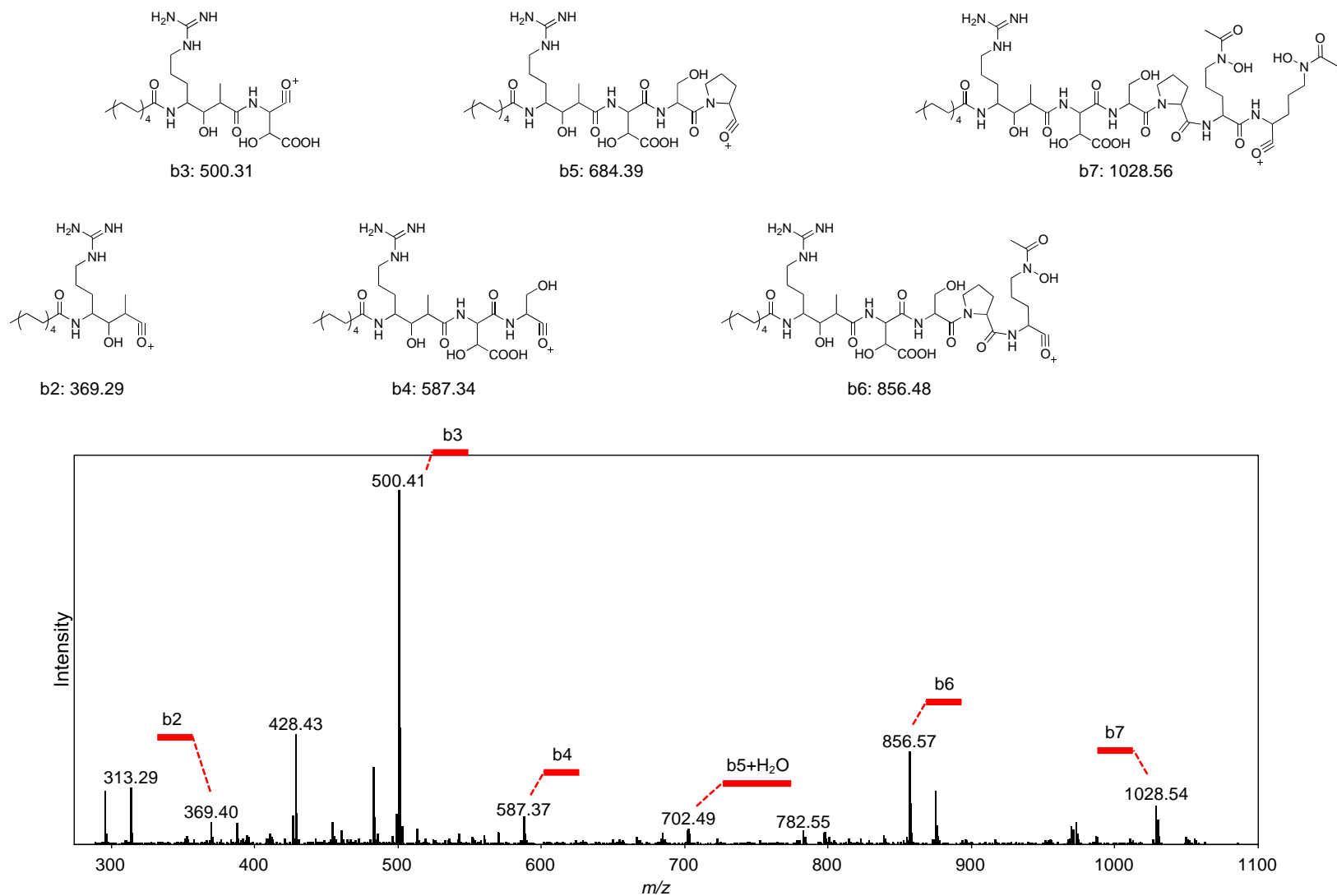


Figure S18. ESIMS/MS analysis of variochelin C (**3**). Structures and m/z of predicted fragmentation ions were described the top. An ion peak of $[M + H]^+$ at m/z 1047.06 was chosen as a parent ion.

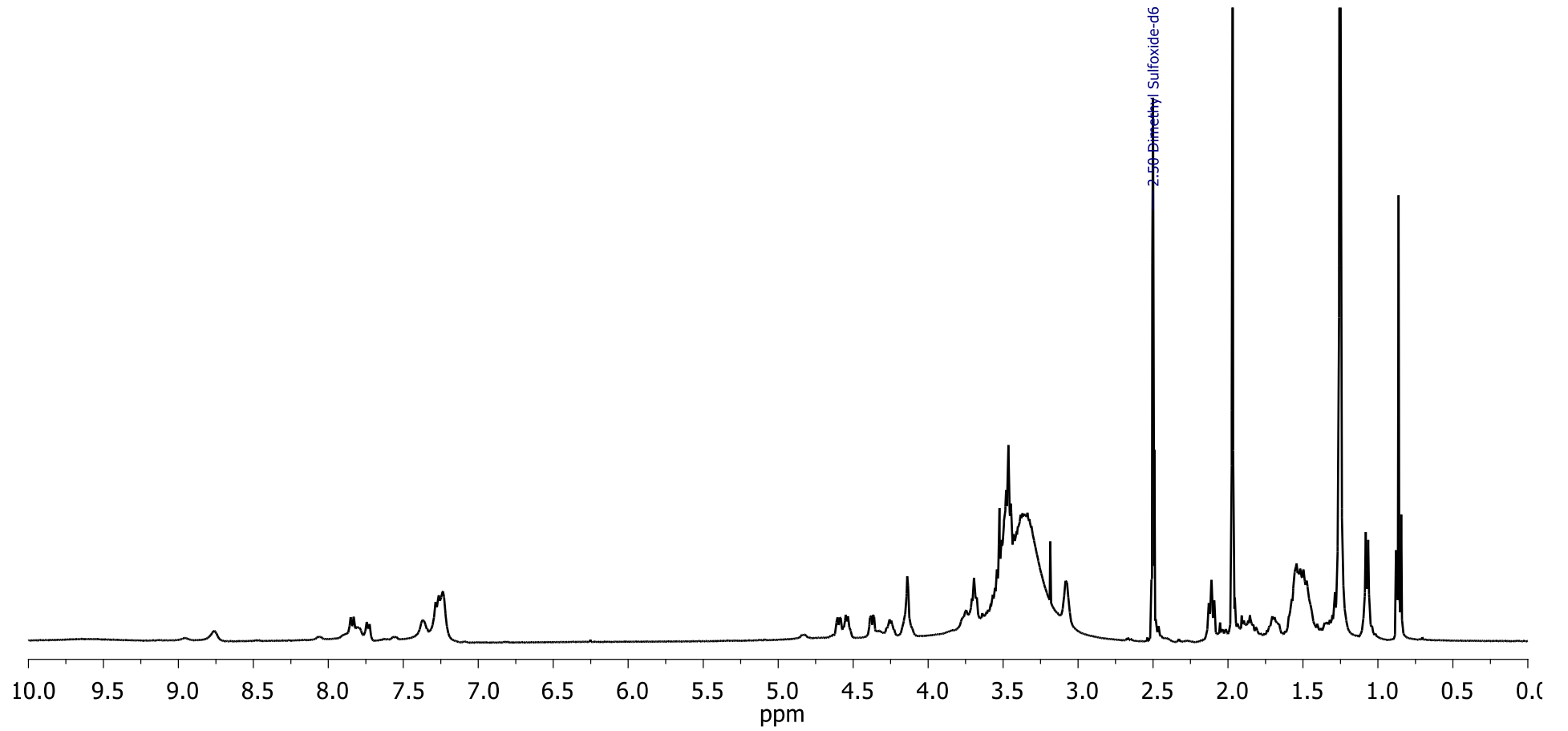


Figure S19. ^1H NMR spectrum of variochelin C (3) (500 MHz, $\text{DMSO-}d_6$).

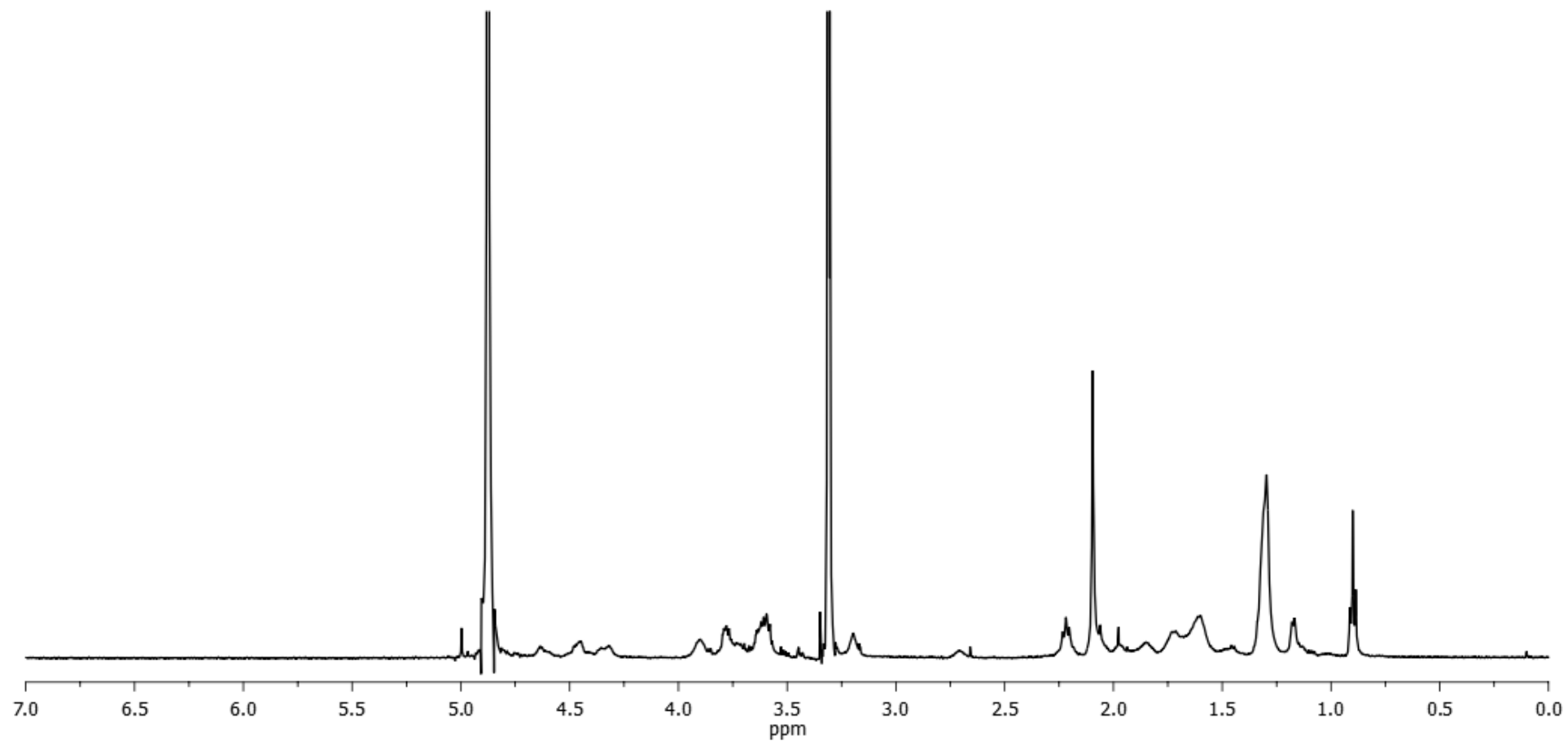


Figure S20. ^1H NMR spectrum of variochelin C (**3**). (500 MHz, methanol- d_4).

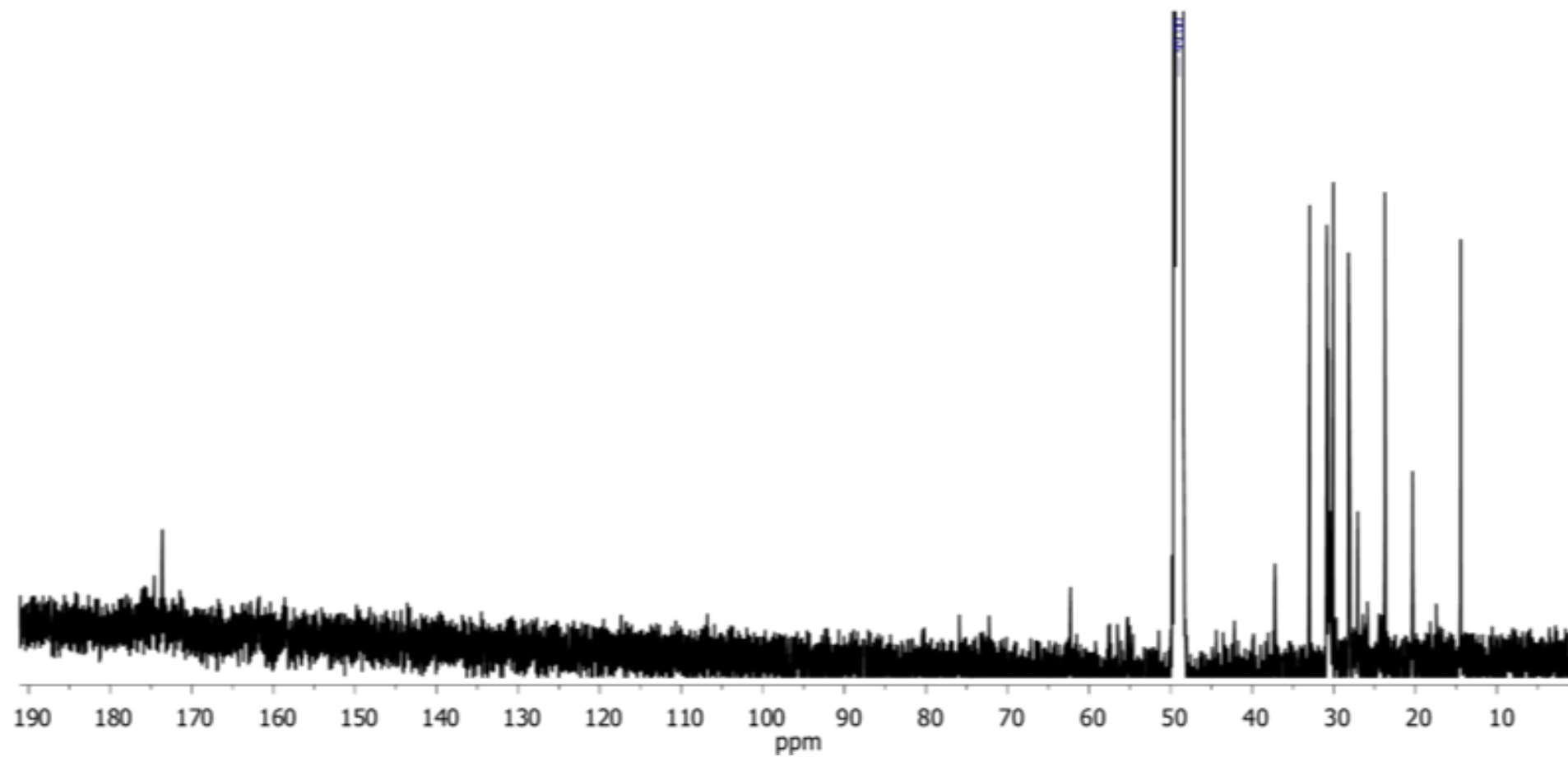


Figure S21. ^{13}C NMR spectrum of variochelin C (**3**). (125 MHz, methanol- d_4).

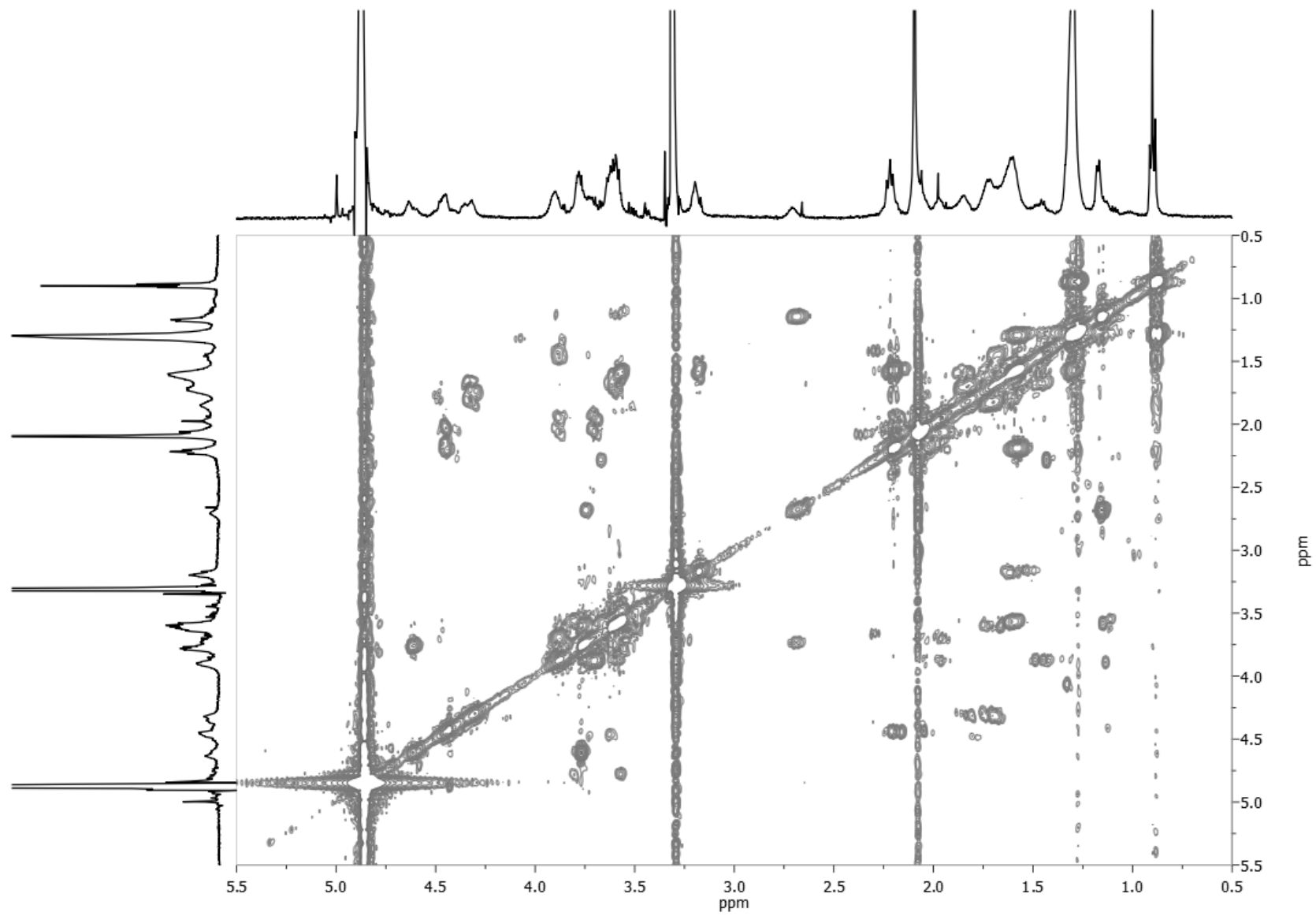


Figure S22. COSY spectrum of variochelin C (**3**). (500 MHz, methanol- d_4).

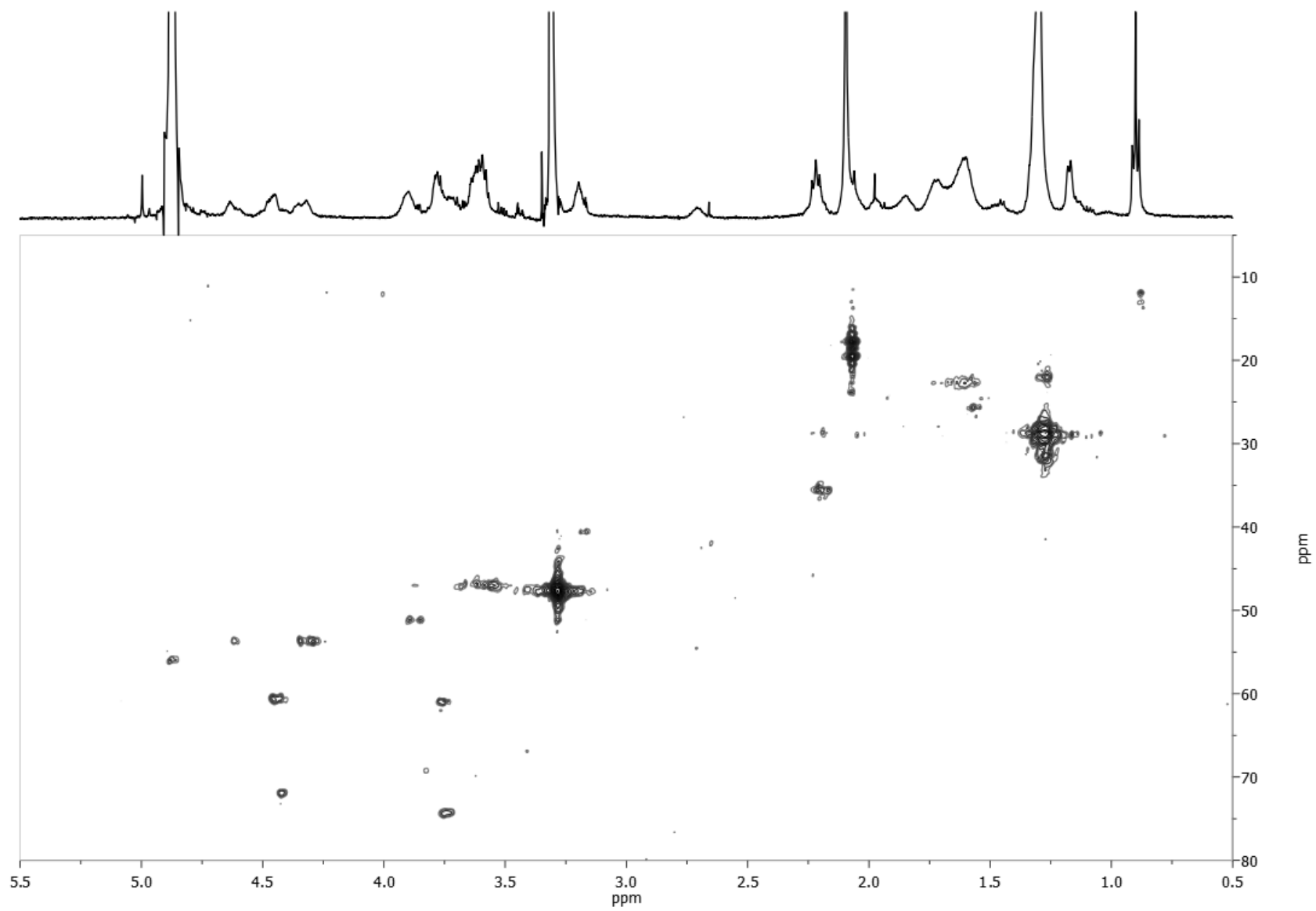


Figure S23. HSQC spectrum of variochelin C (**3**). (500 MHz, methanol- d_4).

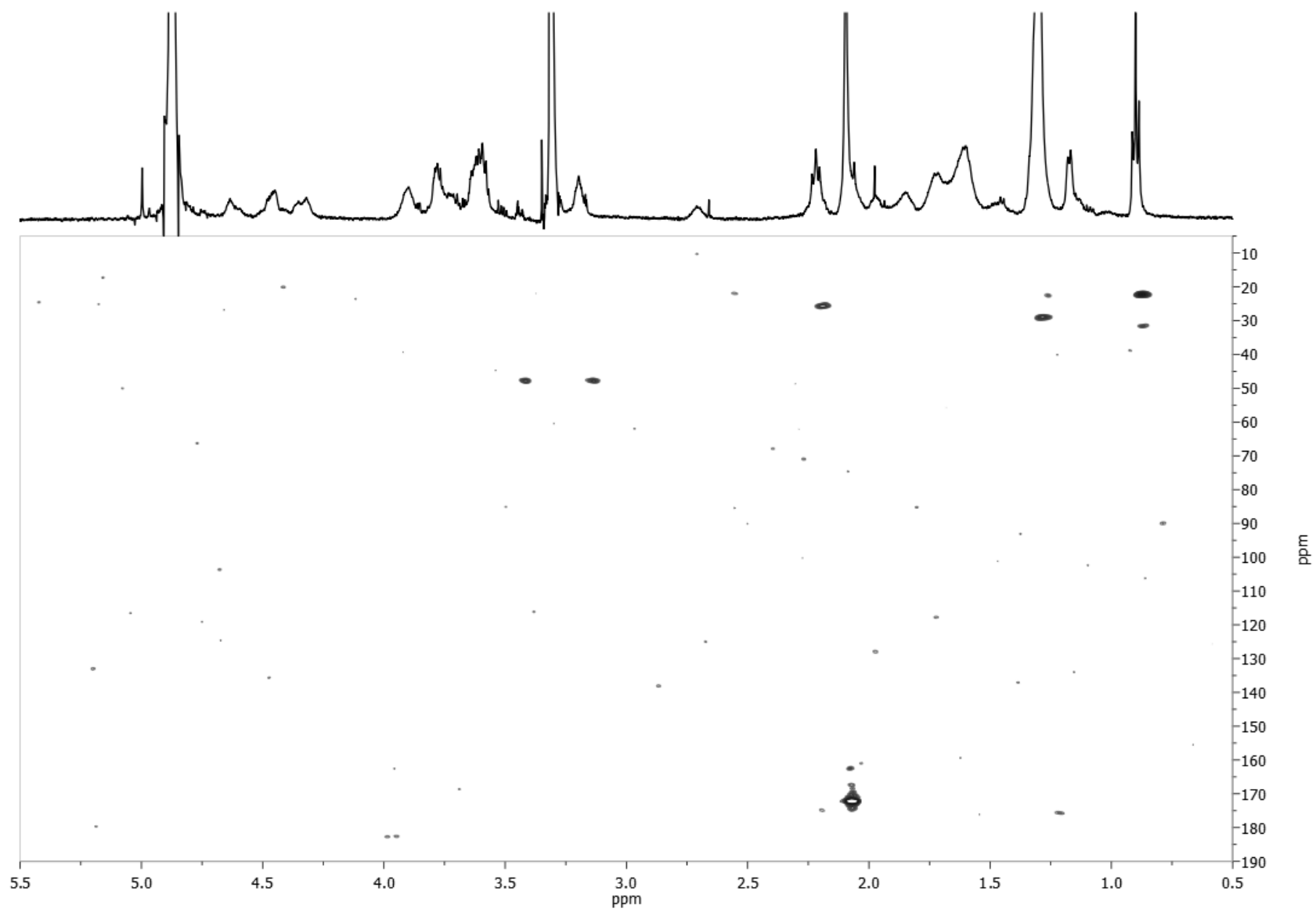


Figure S24. HMBC spectrum of variochelin C (**3**). (500 MHz, methanol-*d*₄).

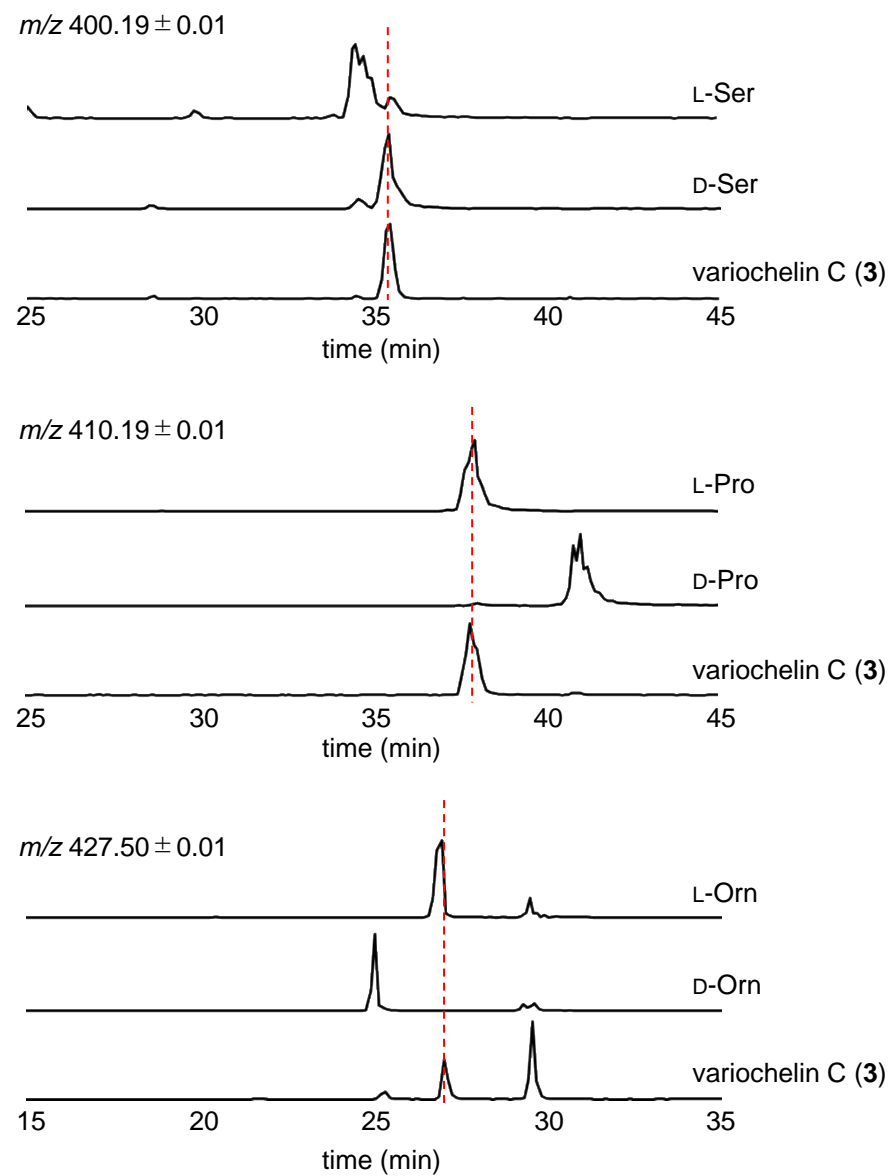


Figure S25. Extracted ion chromatograms from advanced Marfey's analyses of variochelin C (3).

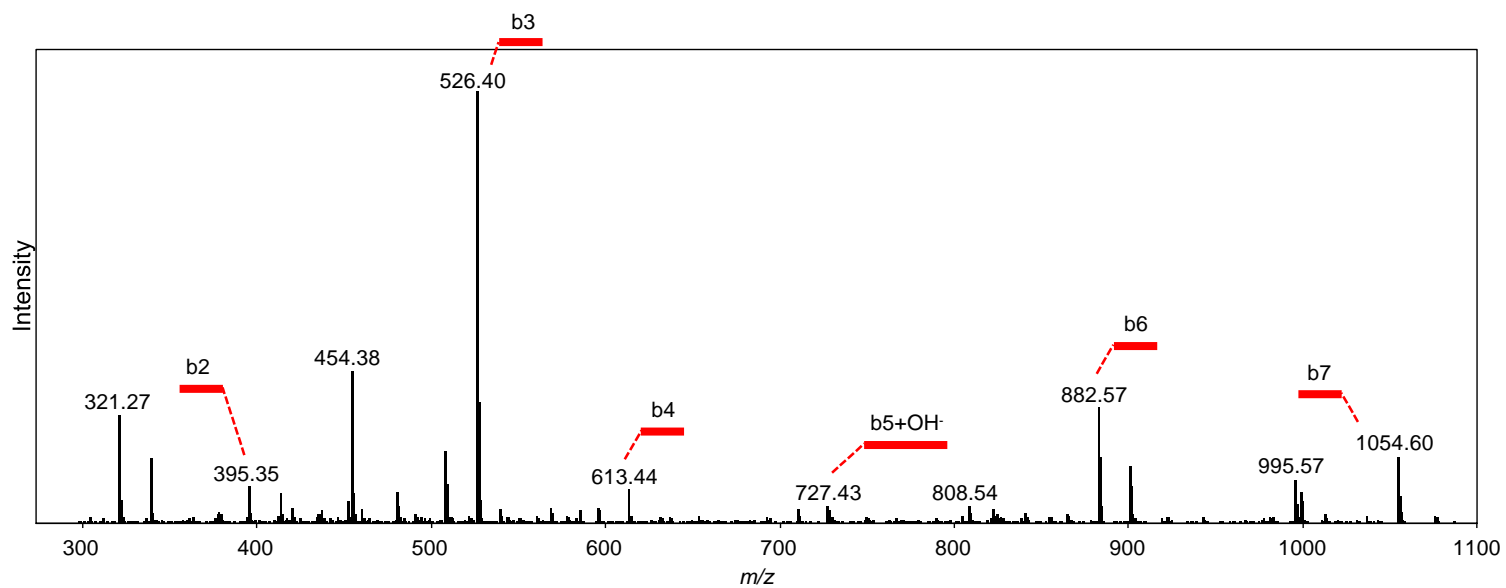
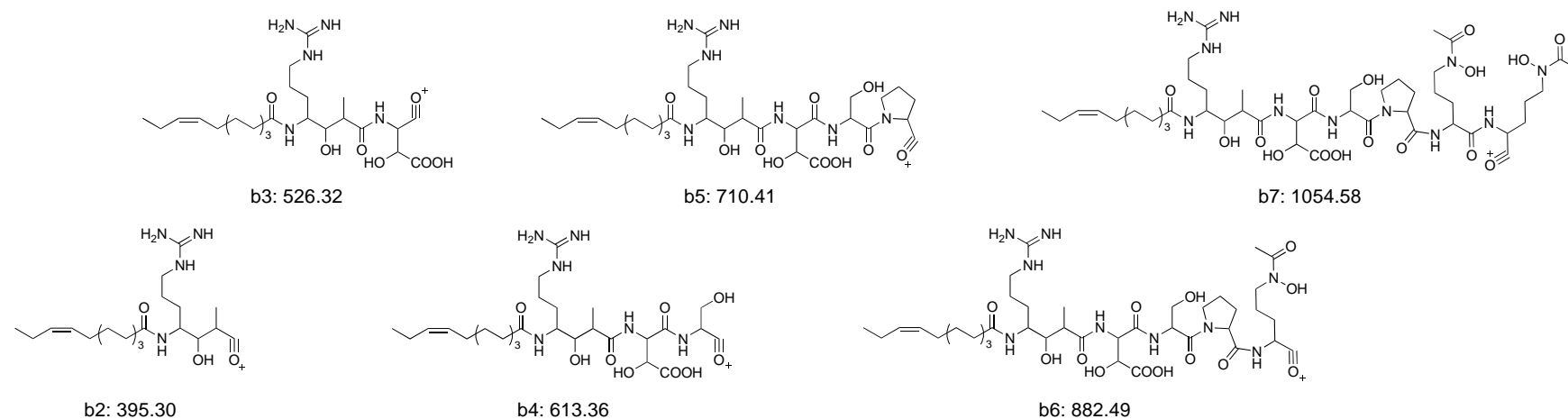


Figure S26. ESIMS/MS analysis of variochelin D (**4**). Structures and m/z of predicted fragmentation ions were described the top. An ion peak of $[M + H]^+$ at m/z 1072.95 was chosen as a parent ion.

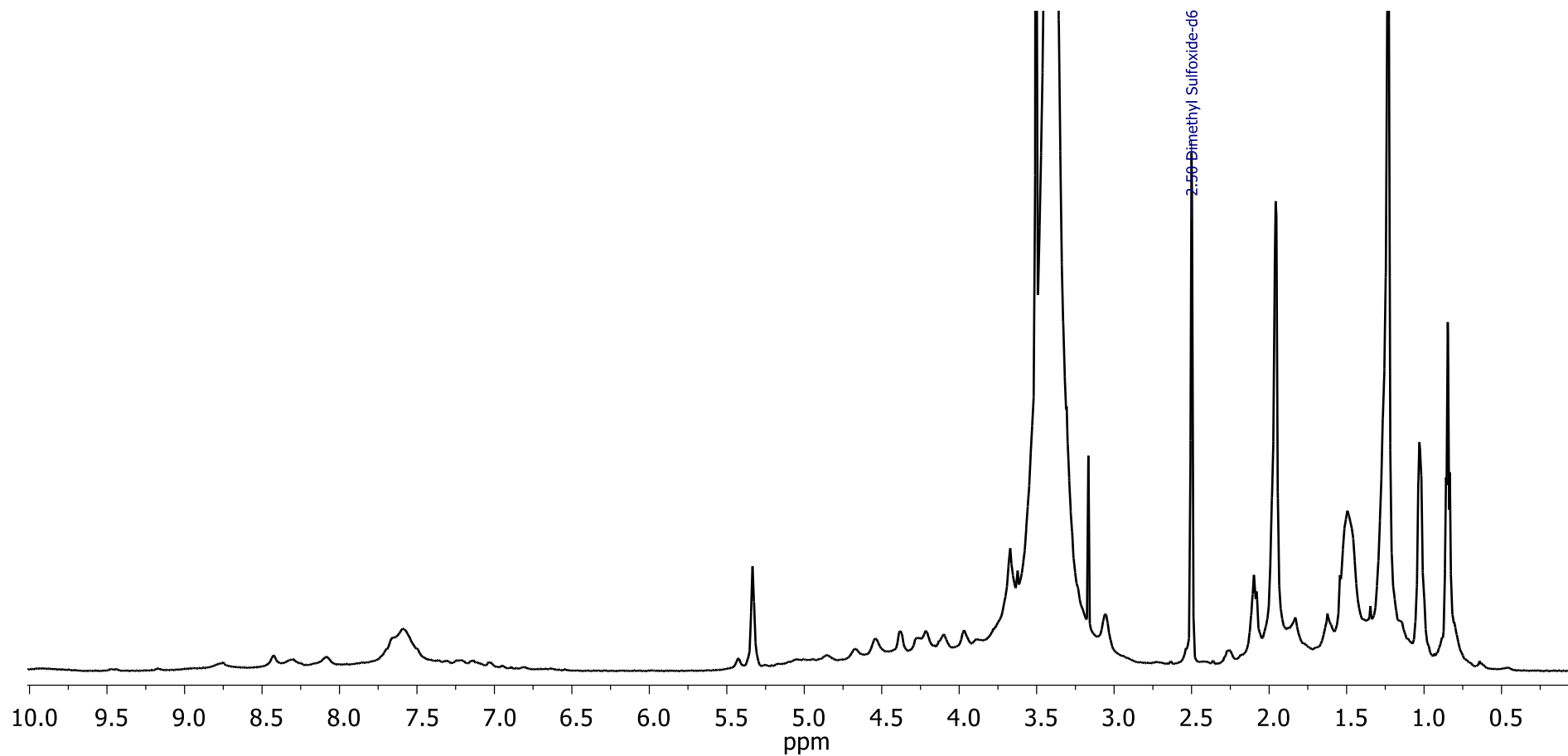


Figure S27. ¹H NMR spectrum of variochelin D (**4**) (500 MHz, DMSO-*d*₆).

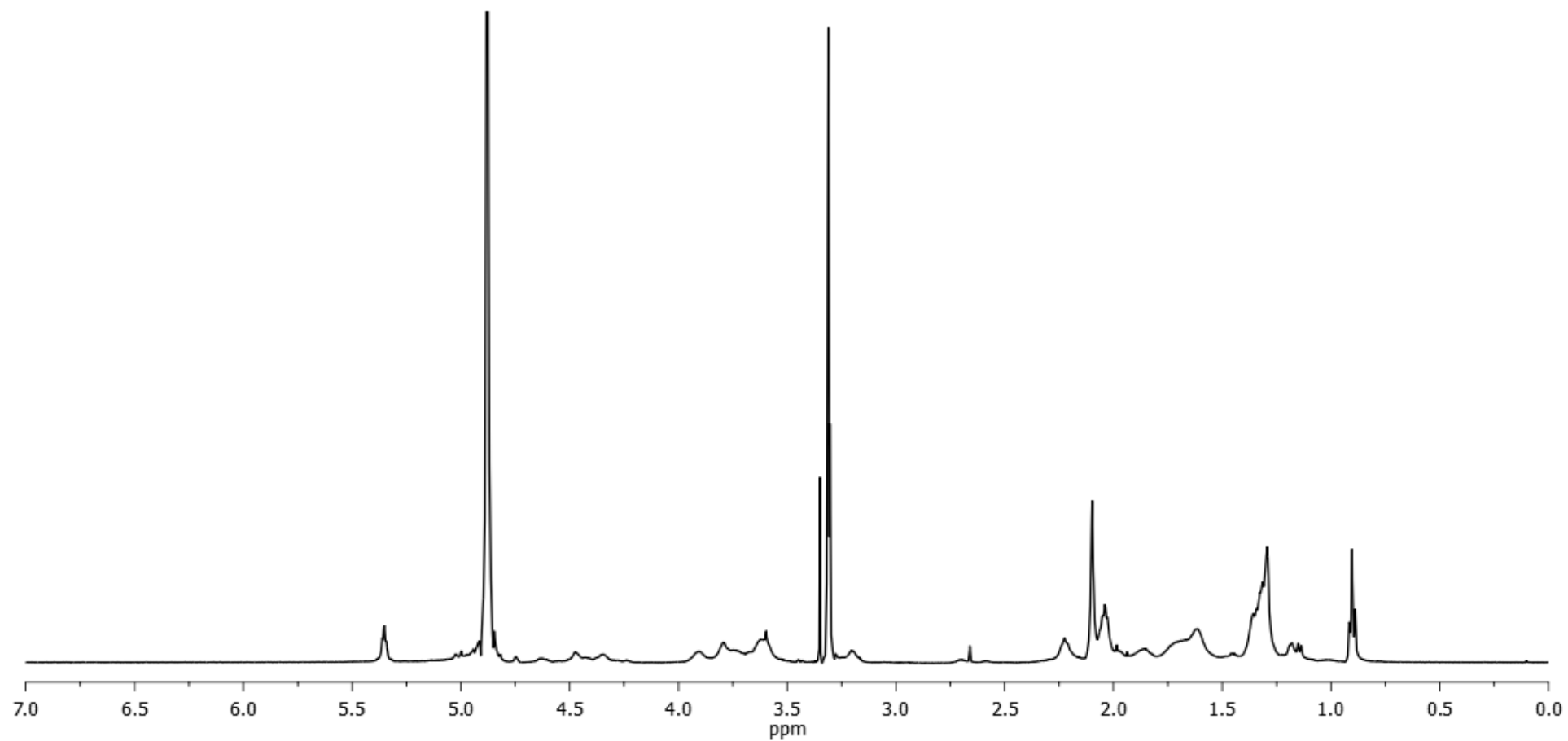


Figure S28. ^1H NMR spectrum of variochelin D (**4**). (500 MHz, methanol- d_4).

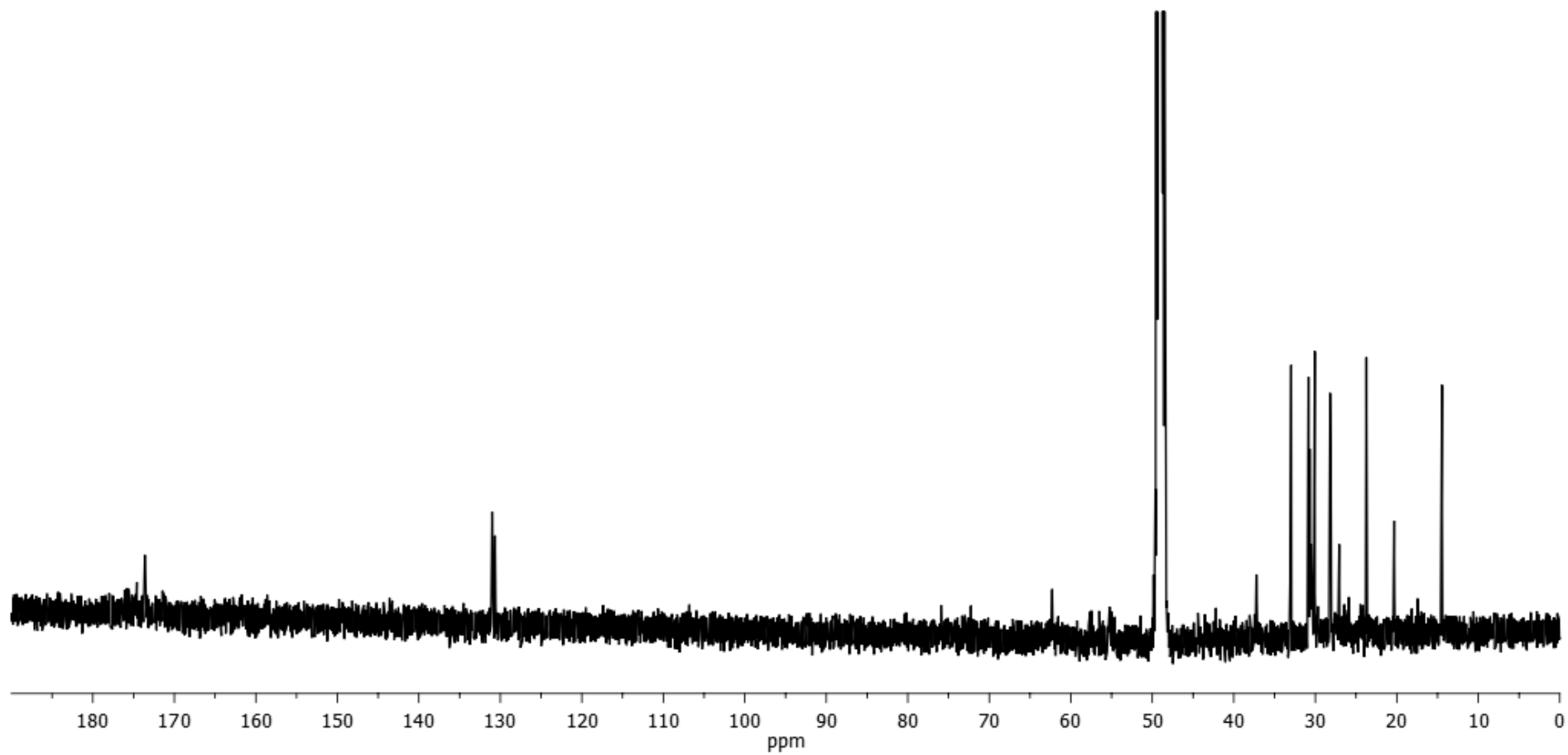


Figure S29. ^{13}C NMR spectrum of variochelin D (**4**). (125 MHz, methanol- d_4).

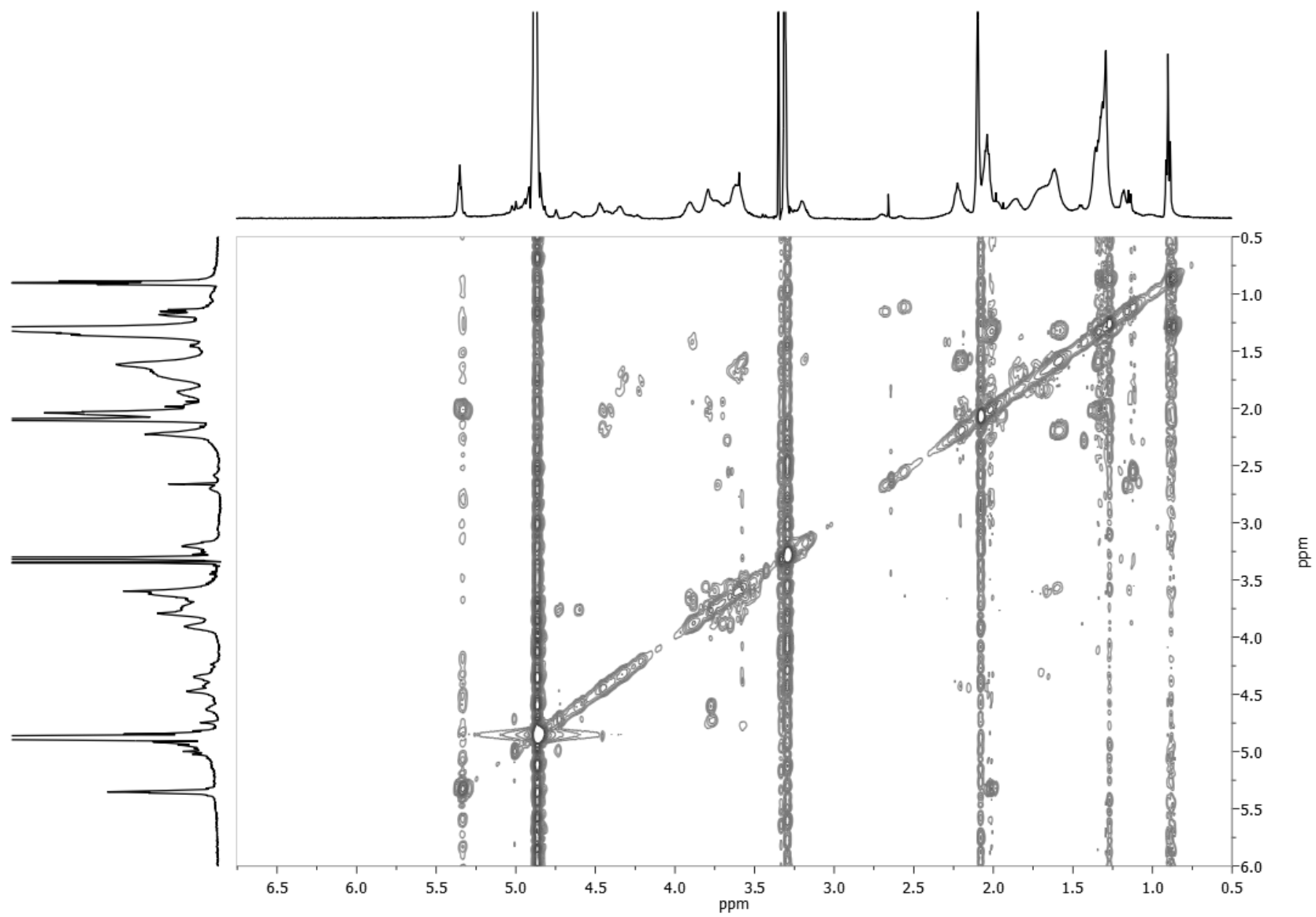


Figure S30. COSY spectrum of variochelin D (**4**). (500 MHz, methanol-*d*₄).

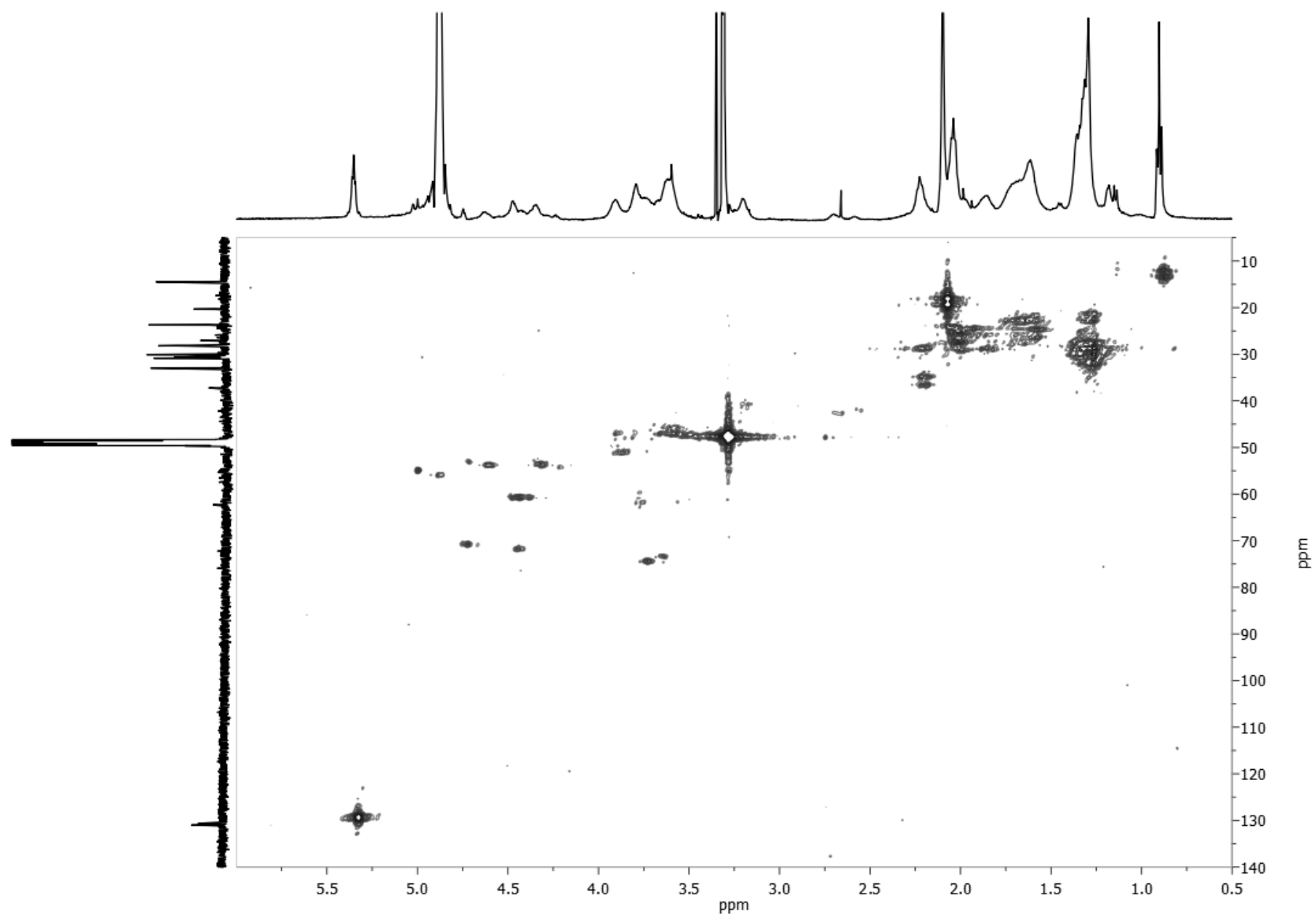


Figure S31. HSQC spectrum of variochelin D (**4**). (500 MHz, methanol- d_4).

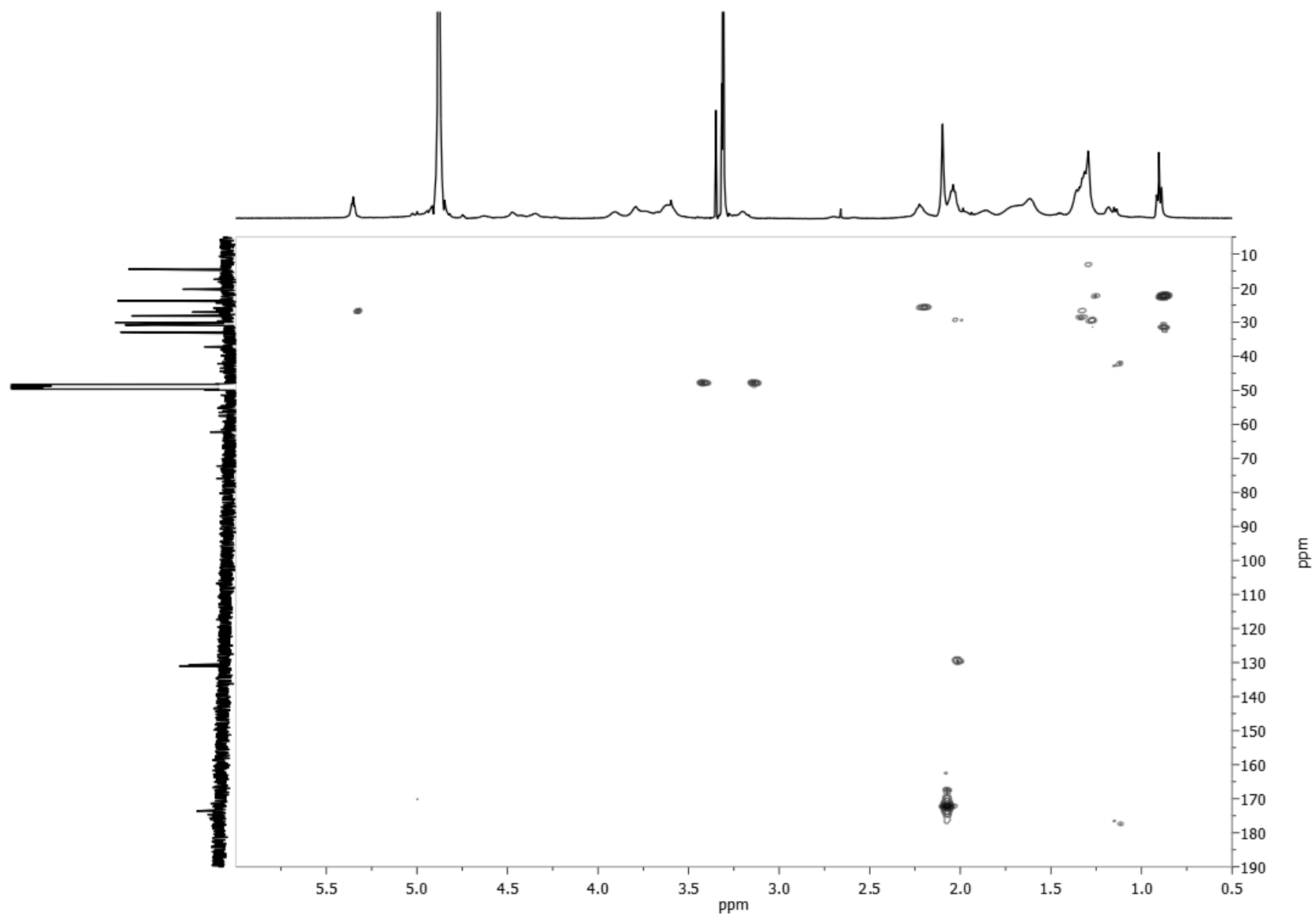


Figure S32. HMBC spectrum of variochelin D (**4**). (500 MHz, methanol- d_4).

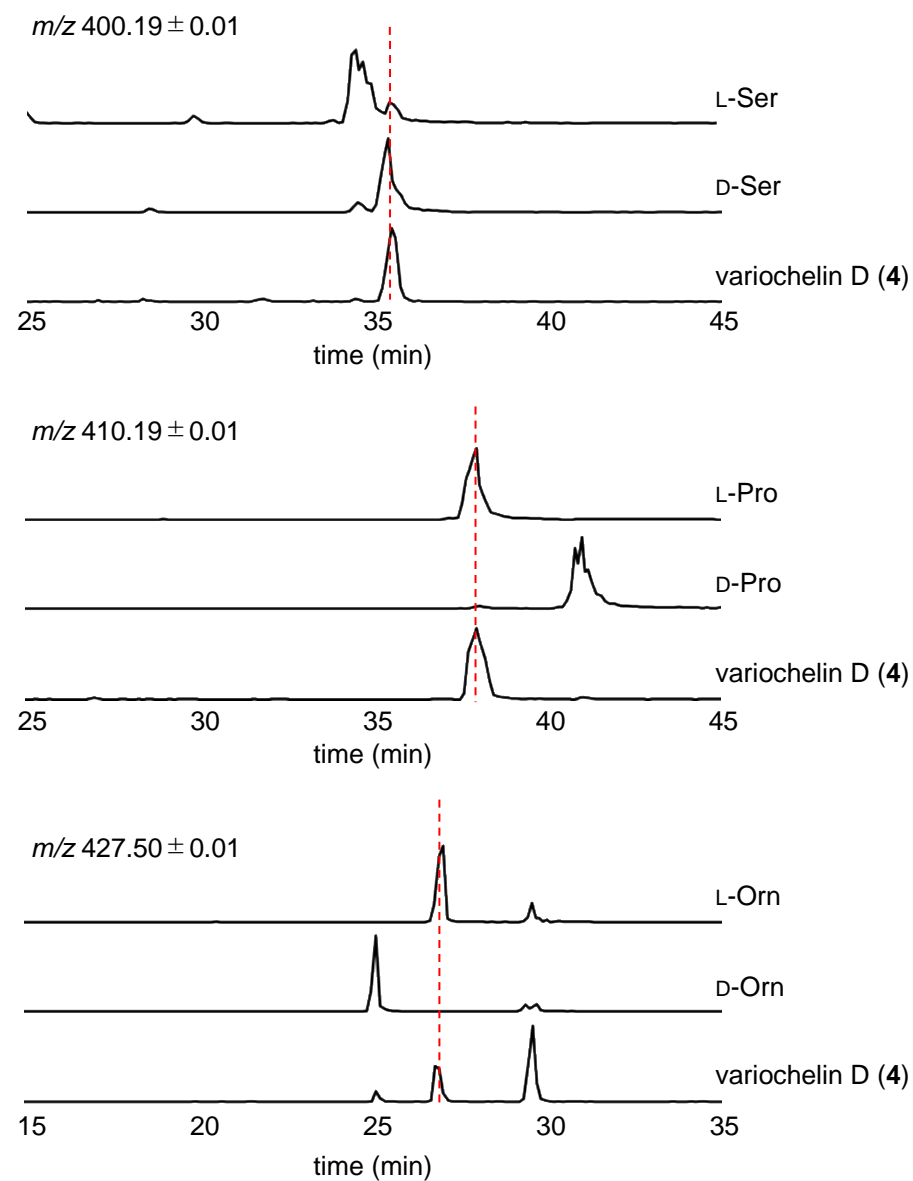


Figure S33. Extracted ion chromatograms from advanced Marfey's analyses of variochelin D (4).

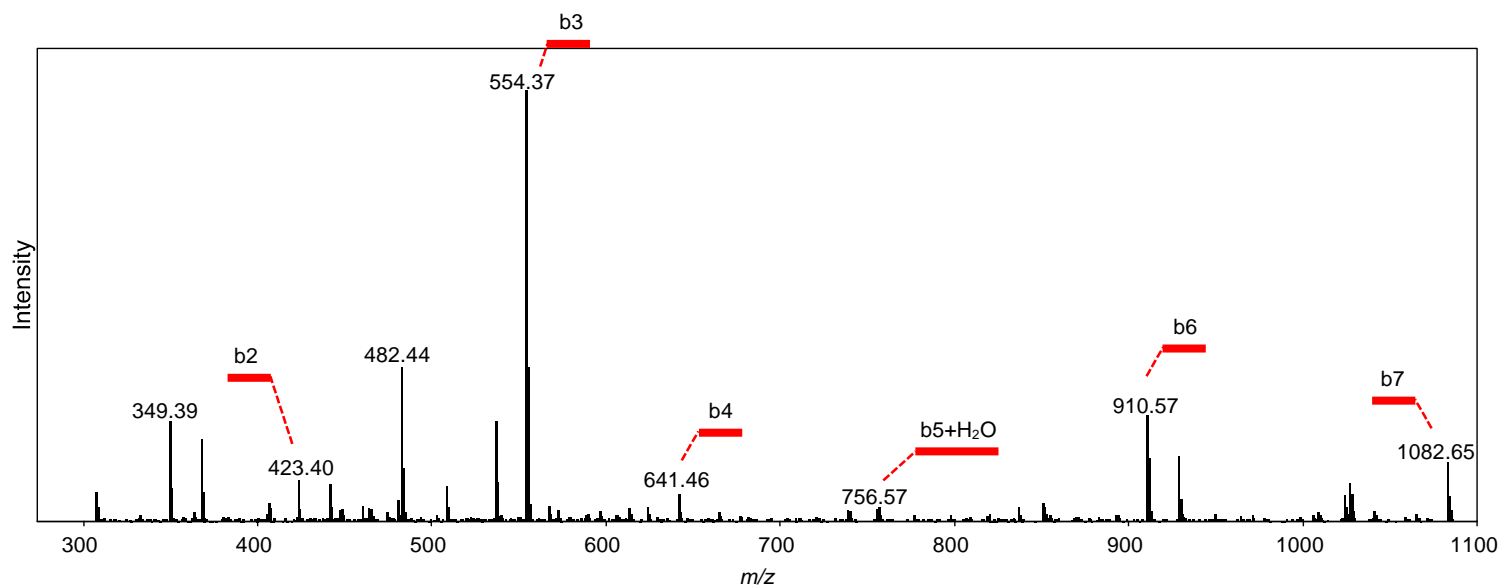
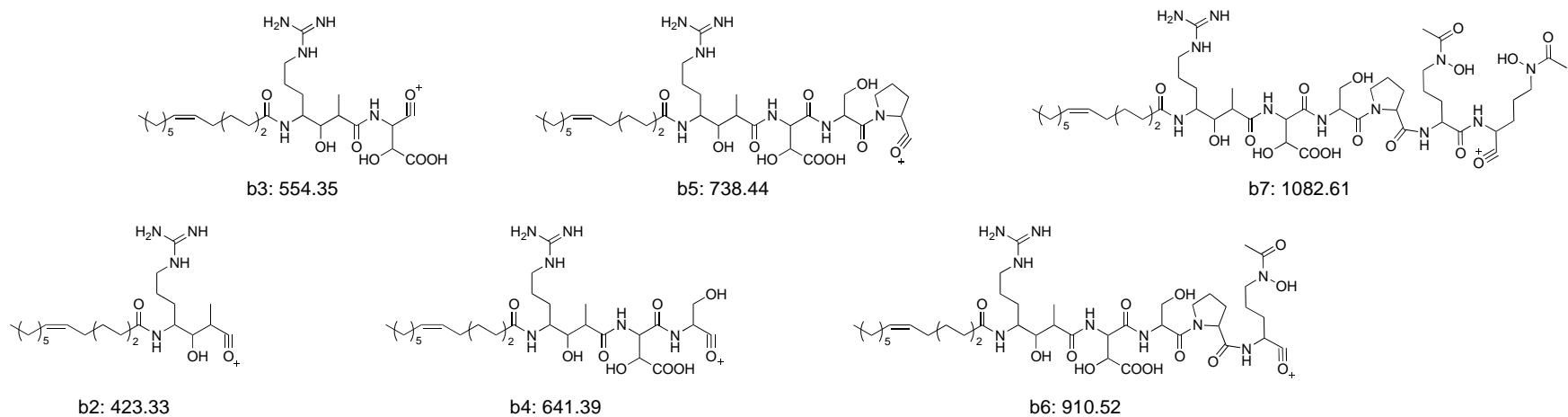


Figure S34. ESIMS/MS analysis of variochelin E (**5**). Structures and m/z of predicted fragmentation ions were described the top. An ion peak of $[M + H]^+$ at m/z 1101.14 was chosen as a parent ion.

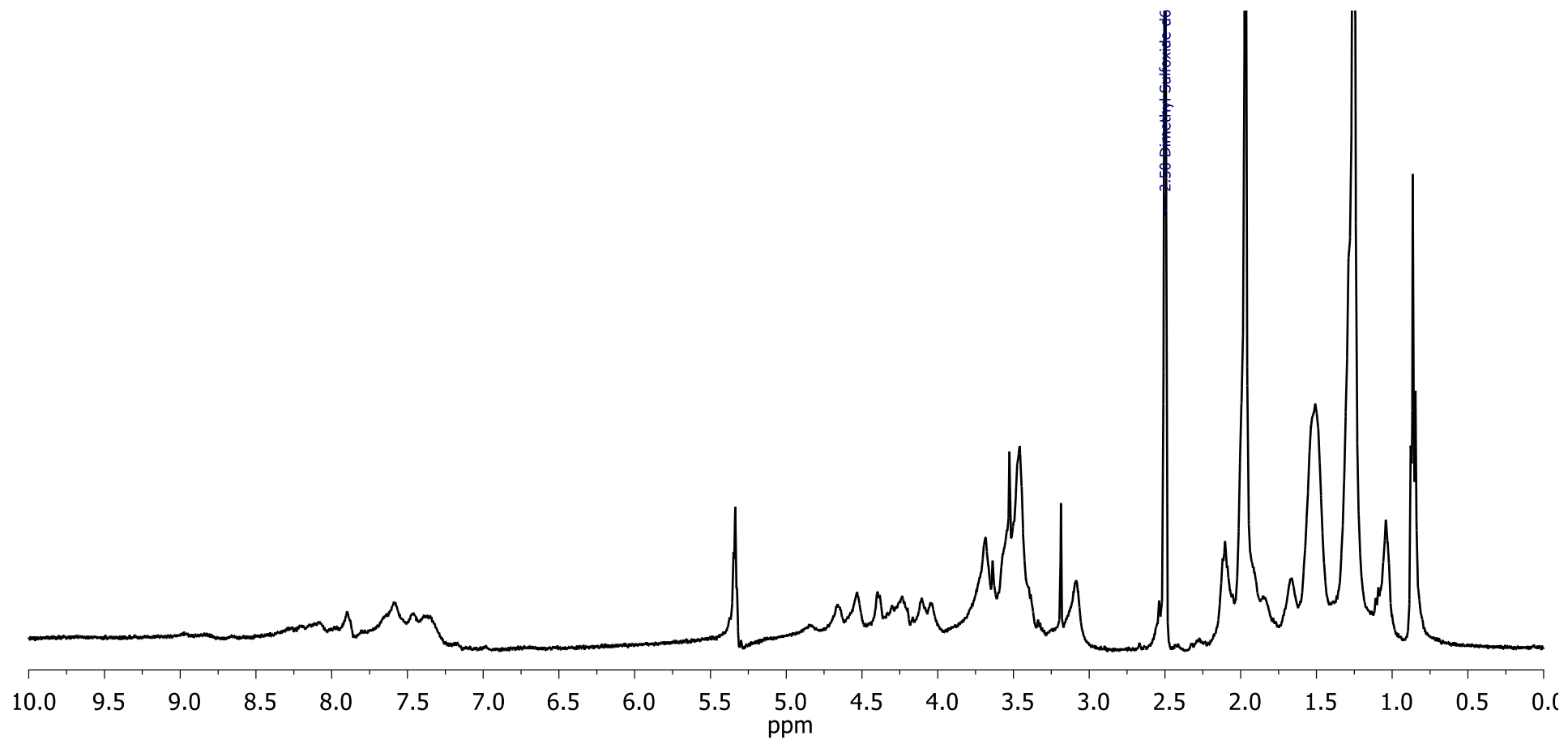


Figure S35. ^1H NMR spectrum of variochelin E (**5**) (500 MHz, $\text{DMSO-}d_6$).

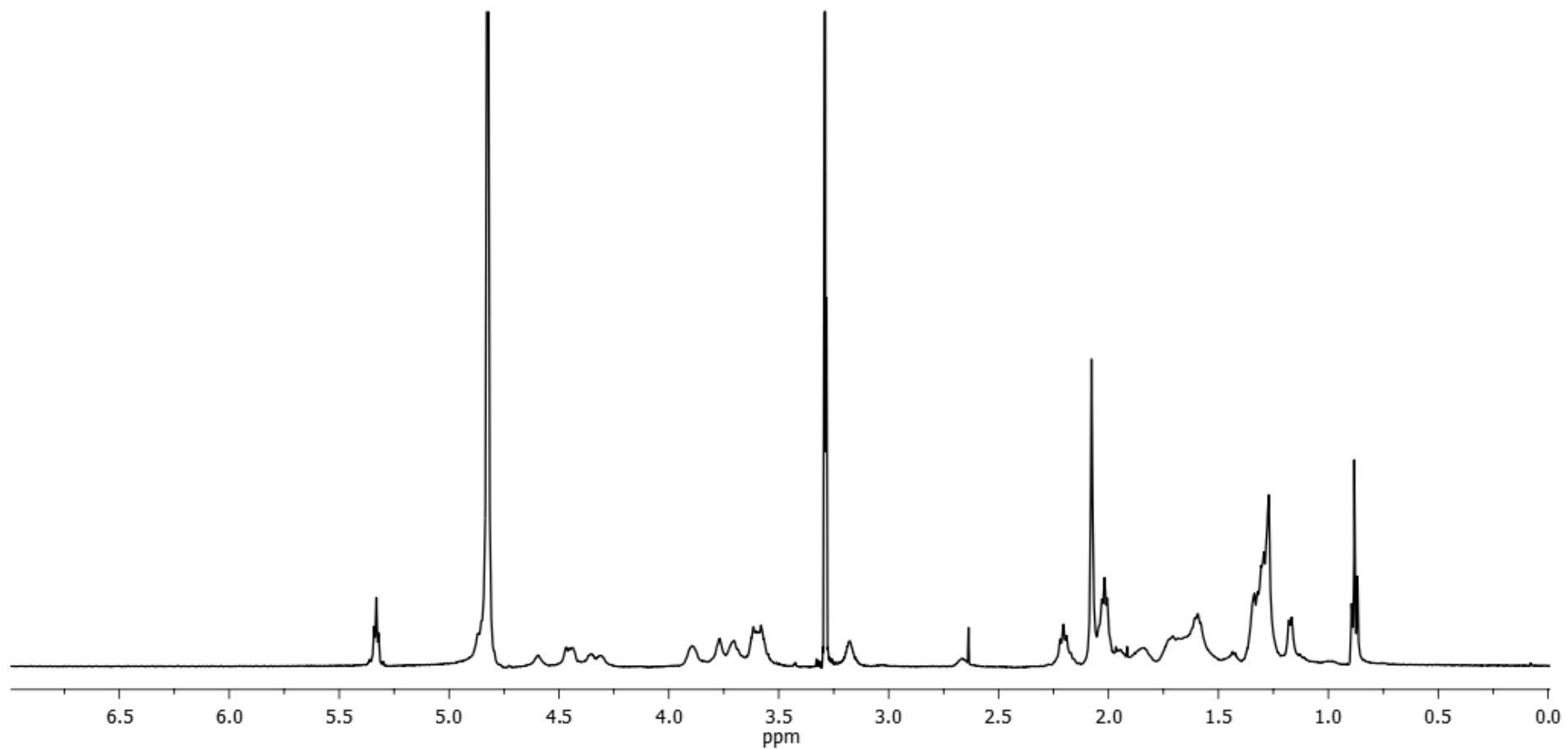


Figure S36. ^1H NMR spectrum of variochelin E (**5**). (500 MHz, methanol- d_4).

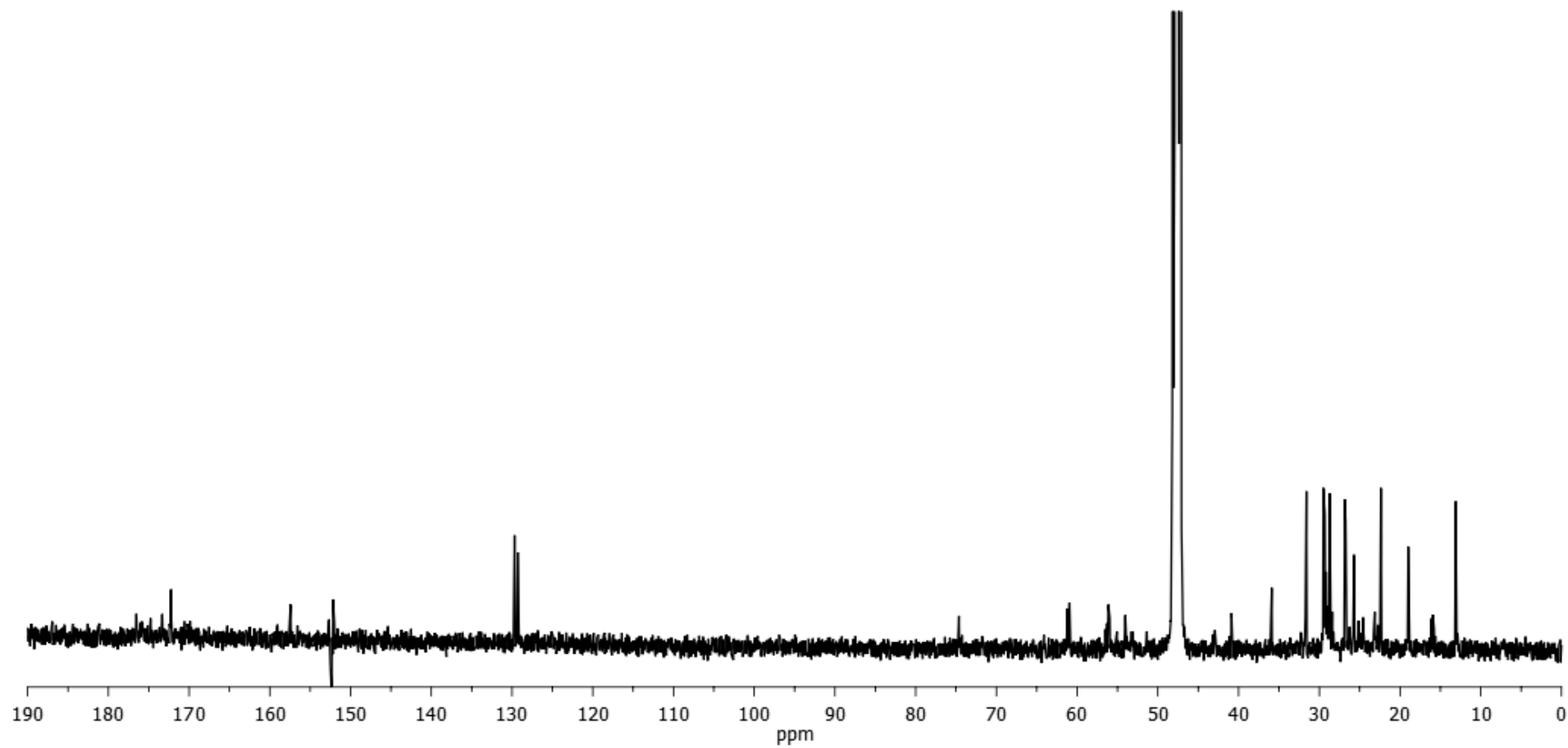


Figure S37. ^{13}C NMR spectrum of variochelin E (**5**). (125 MHz, CD_3OD).

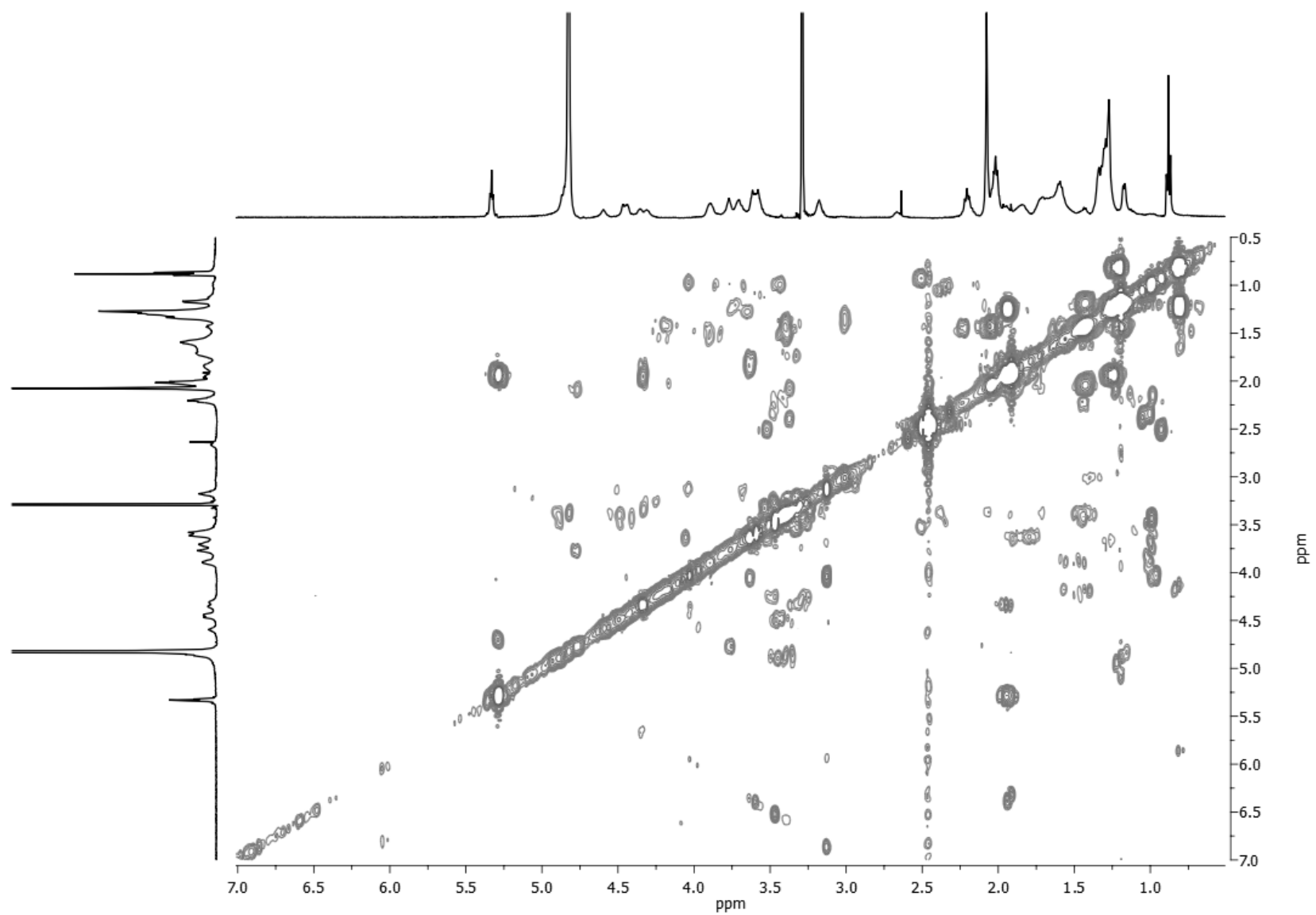


Figure S38. COSY spectrum of variochelin E (**5**). (500 MHz, methanol-*d*₄).

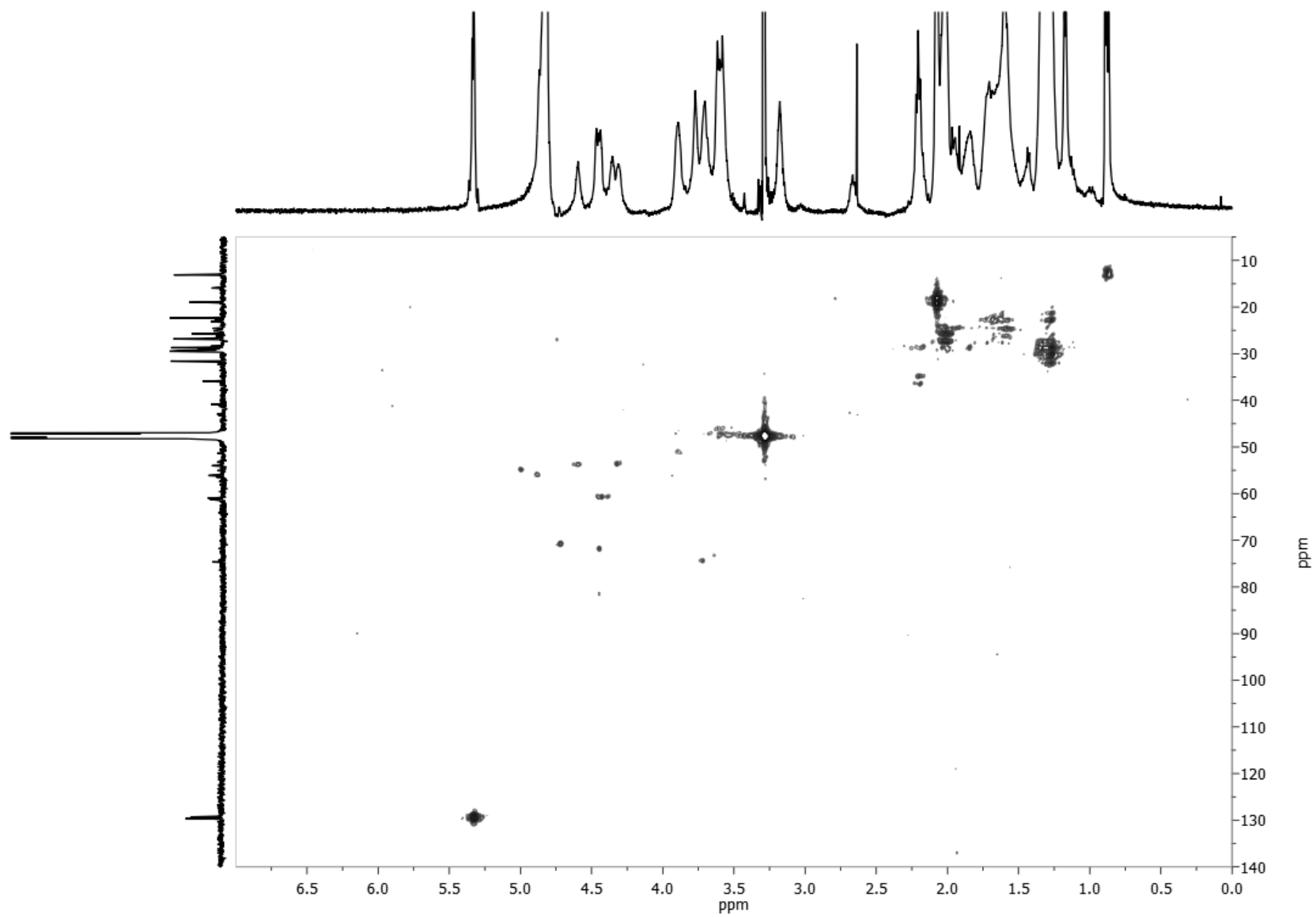


Figure S39. HSQC spectrum of variochelin E (**5**). (500 MHz, methanol- d_4).

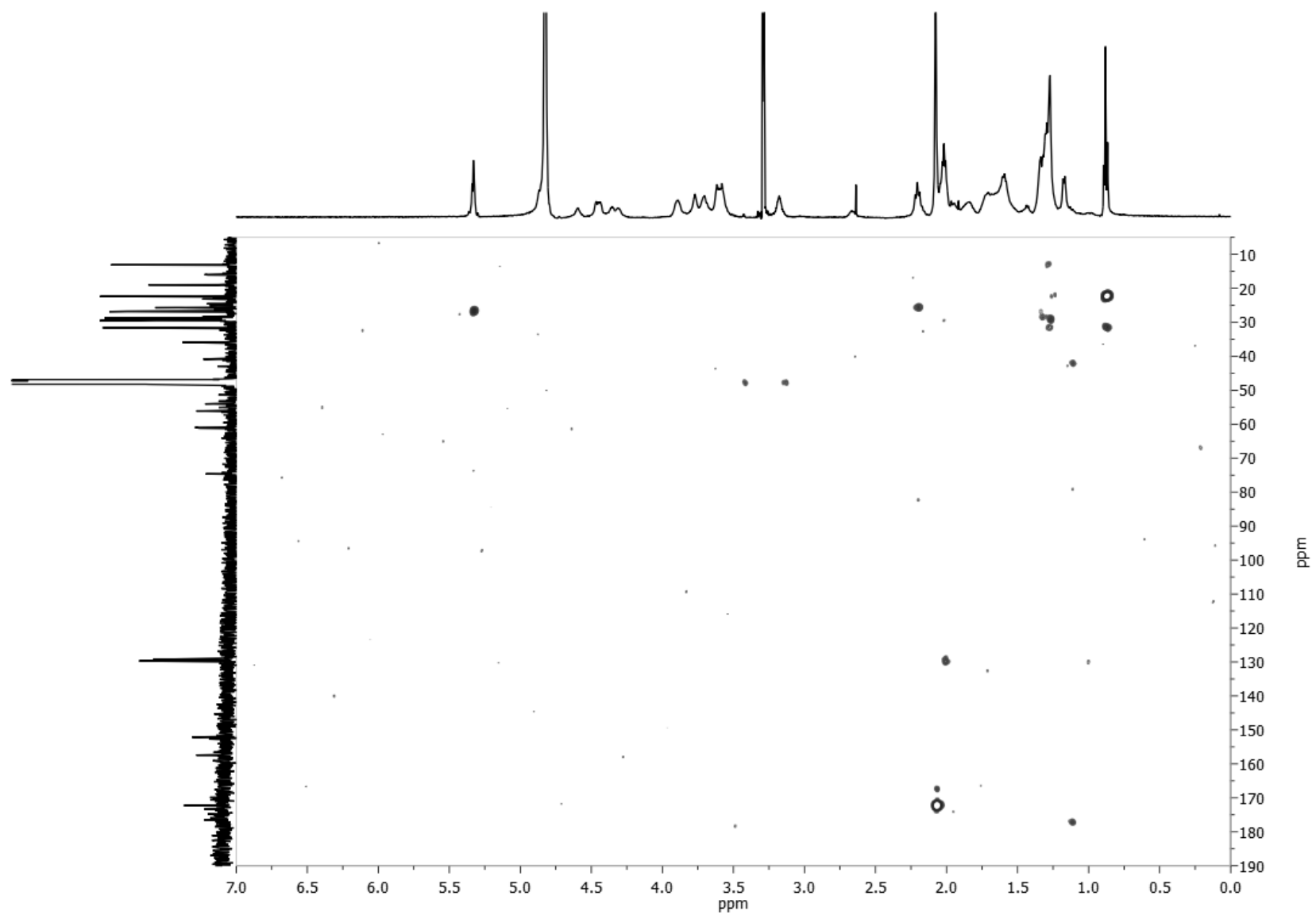


Figure S40. HMBC spectrum of variochelin E (5). (500 MHz, methanol-*d*₄).

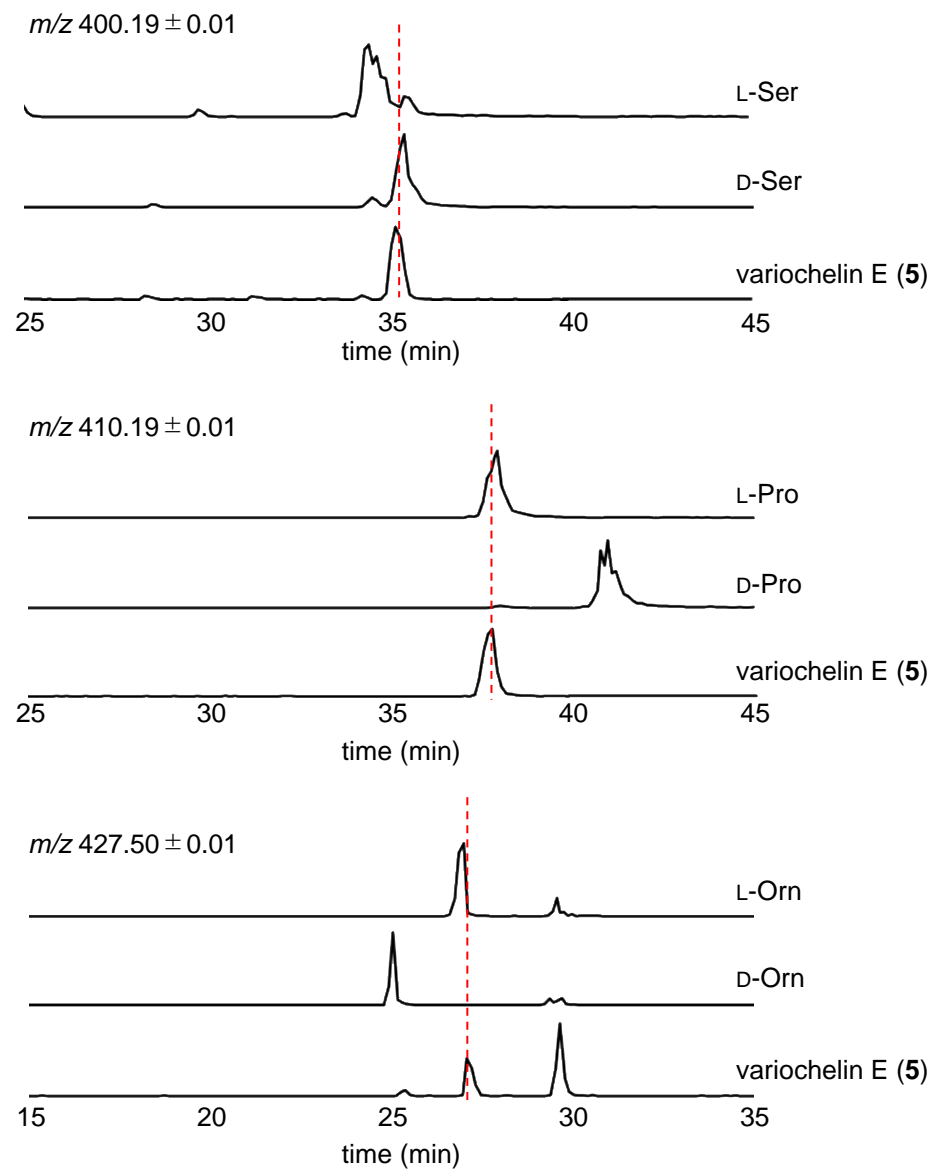


Figure S41. Extracted ion chromatograms from advanced Marfey's analyses of variochelin E (5).

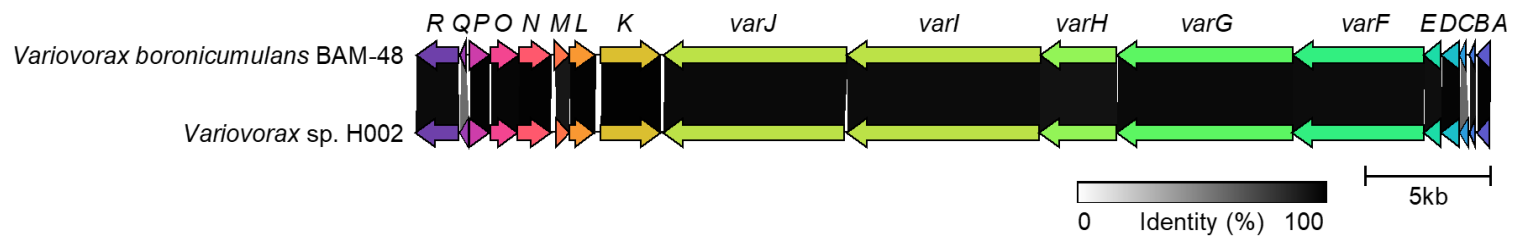
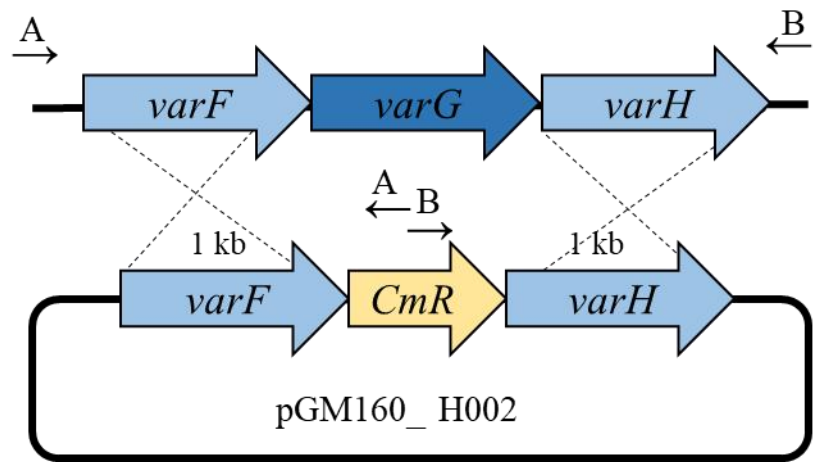
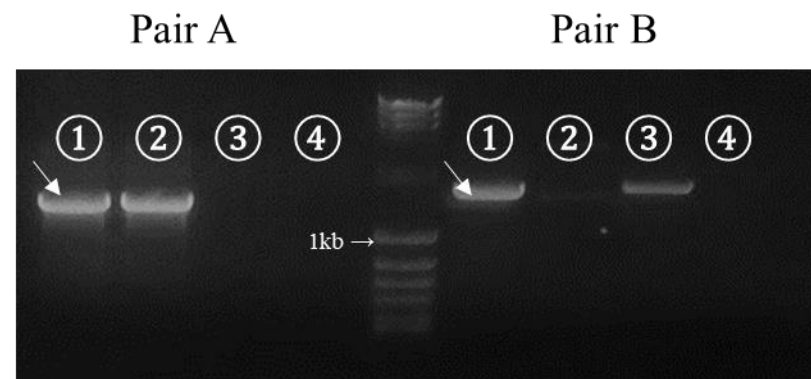


Figure S42. Variochelin BGCs comparison identified in *Variovorax boronicumulans* BAM-48 (up) and *Variovorax* sp. H002 (bottom) generated by clinker. Links between homologous genes are shown according to the percentage identity (see identity scale bar).



(A)



(B)

Figure S43. Generation of *Variovorax* sp. H002 *varG* null-mutant; (a) Illustration of *varG* knock-out using homolog recombination technology; dark blue-colored arrow is target gene to be replaced by yellow-colored chloramphenicol resistant gene (*cmR*) embedded in plasmid pGM160_H002; light blue-colored arrows are homologous flanking genes; small black arrows, pair A and pair B, represent primer sites employed for confirmation of successful recombination. (b) Confirmation of successful recombination in four selected mutants; PCR amplification followed by visualization on 1% Agarose S gel showed mutant number 1 generated positive band (white arrows) in both primers pair A and B, while other mutants only give one band of either primers (number 2 and 3) or no band detected (number 4). The mutant number 1 is used for further experiments.

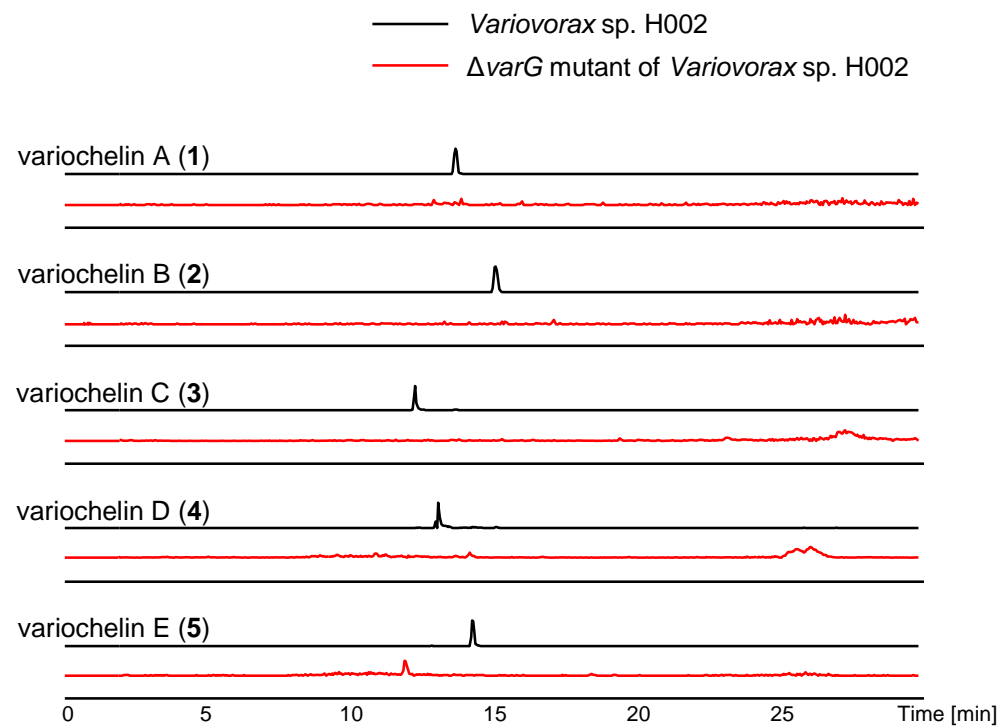


Figure S44. Extracted ion chromatogram showing deletion of *varG* abolishes variochelin A–E (1–5) production in *Variovorax* sp. H002. LC–MS-monitored production of variochelin A (m/z 1074.59 [M + H]⁺), variochelin B (m/z 1102.62 [M + H]⁺), variochelin C (m/z 1046.56 [M + H]⁺), variochelin E (m/z 1072.58 [M + H]⁺), and xenomicin E (m/z 1100.61 [M + H]⁺).

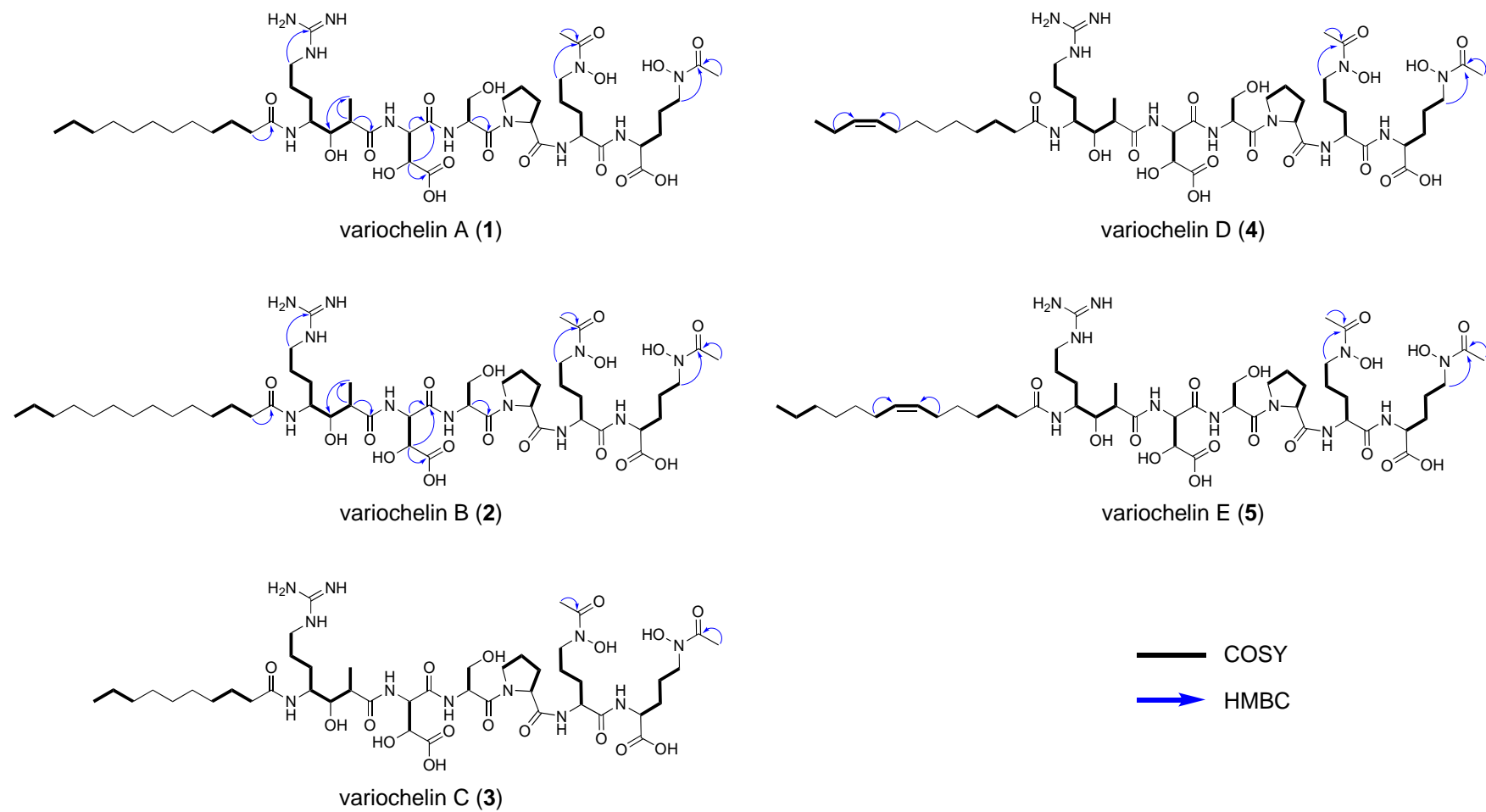
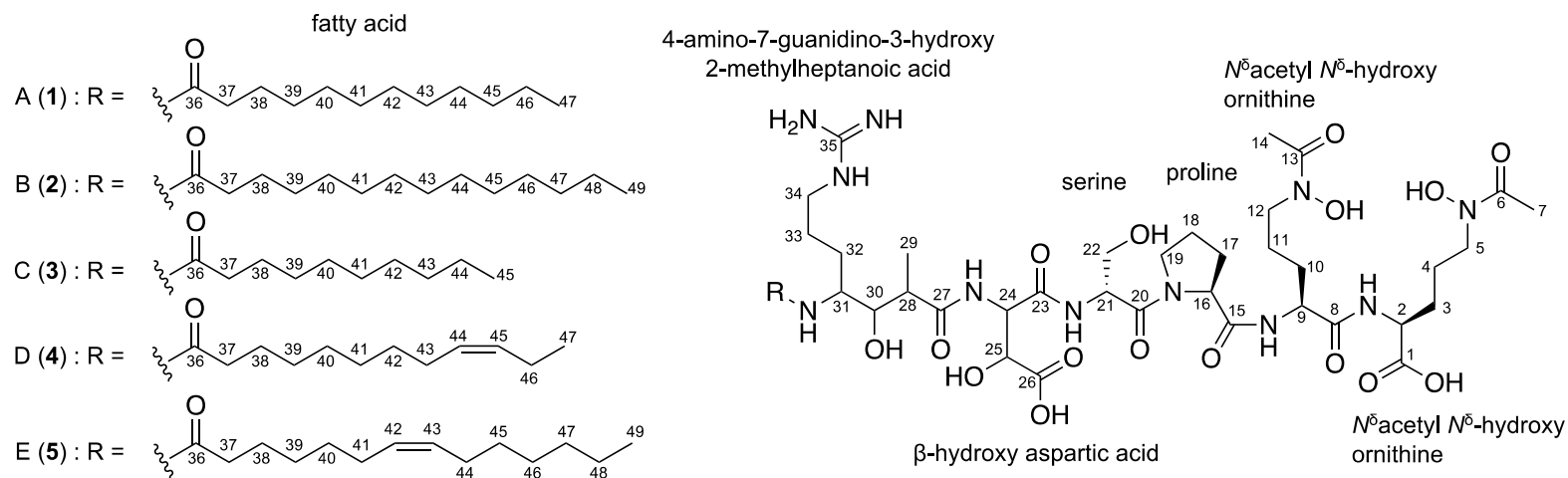


Figure S45. Selected COSY and HMBC correlations of variochelin A–E (1–5) in methanol- d_4 .

Table S1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of variochelins A–E (**1–5**) in methanol- d_4 (δ in ppm).



Pos	variochelin A (1)		variochelin B (2)		variochelin C (3)		variochelin D (4)		variochelin E (5)	
	δ_{H} , M (J in Hz)	δ_{C}	δ_{H} , M (J in Hz)	δ_{C}	δ_{H} , M (J in Hz)	δ_{C}	δ_{H} , M (J in Hz)	δ_{C}	δ_{H} , M (J in Hz)	δ_{C}
N^6 -acetyl N^6 -hydroxy ornithine										
1		n.r.		n.r.		n.r.		n.r.		n.r.
2	4.33 (m)	55.2	4.33 (m)	55.1	4.33 (m)	55.3	4.32 (m)	55.3	4.34 (m)	55.1
3	1.72-1.88 (br)	29.6	1.64-1.86 (br)	29.7	1.70-1.88 (br)	29.7	1.66-1.85 (br)	29.7	1.66-1.85 (br)	29.6
4	1.59-1.71 (br)	25.9	1.59-1.71 (br)	25.9	1.59-1.71 (br)	25.9	1.62 (br)	25.9	1.62 (br)	25.7
5	3.71 (br)	48.9*	3.73 (br)	48.9*	3.59 (br)	48.4*	3.59 (br)	48.4*	3.59 (br)	48.4*
6		173.6		173.7		n.r.		173.6		173.6
7	2.09 (s)	20.3	2.09 (s)	20.3	2.09 (s)	20.1	2.10 (s)	20.5	2.10 (s)	20.3
N^6 -acetyl N^6 -hydroxy ornithine										
8		n.r.		n.r.		n.r.		n.r.		n.r.

	9	4.39 (m)	54.4	4.39 (m)	54.5	4.36 (m)	55.1	4.36 (m)	55.1	4.38 (m)	55.1
	10	1.72-1.88 (br)	29.6	1.64-1.86 (br)	29.7	1.70-1.88 (br)	29.7	1.66-1.85 (br)	29.7	1.66-1.85 (br)	29.6
	11	1.53-1.81 (br)	25.9	1.52-1.71 (br)	25.9	1.65 (br)	25.9	1.65 (br)	25.9	1.65 (br)	25.7
	12	3.61 (br)	48.4*	3.61 (br)	48.4*	3.60 (br)	48.4*	3.60 (br)	48.4*	3.60 (br)	48.4*
	13		173.6		173.5		n.r.		173.6		173.6
	14	2.09 (s)	20.3	2.09 (s)	20.3	2.09 (s)	20.1		20.5	2.10 (s)	20.3
Proline (Pro)	15		n.r.		n.r.		n.r.		n.r.		n.r.
	16	4.46 (m)	62.3	4.46 (m)	62.3	4.47 (m)	62.0	4.46 (m)	62.1	4.46 (m)	62.1
	17	a. 2.22 (m) b. 2.06 (m)	30.1	a. 2.23 (m) b. 2.07 (m)	30.2	a. 2.22(m) b. 2.06 (m)	30.2	a. 2.20(m) b. 2.04 (m)	30.2	a. 2.20(m) b. 2.04 (m)	30.1
	18	1.62 br	26.4	1.62 br	26.4	1.61 (br)	26.5	1.61 (br)	26.5	1.61 (br)	26.6
	19	3.61 (br)	48.7*	3.62 (br)	48.7*	3.60 (br)	48.4*	3.59 (br)	48.4*	3.59 (br)	48.4*
Serine (Ser)	20		171.2		171.2		n.r.		n.r.		n.r.
	21	4.61 (m)	55.3	4.62 (m)	55.3	4.64 (m)	55.0	4.60 (m)	55.0	4.62 (m)	55.2
	22	3.79 (m)	62.5	3.79 (m)	62.5	3.78 (m)	63.4	3.78 (m)	62.6	3.79 (m)	64.5
β -hydroxy aspartic acid (Hya)	23		171.8		171.9		n.r.		n.r.		n.r.
	24	4.89 (br)	57.4	4.89 (br)	57.4	4.90 (m)	57.2	4.91 (m)	57.5	4.91 (m)	57.3
	25	4.49 (br)	72.9	4.49 (br)	73.0	4.44 (m)	73.4	4.46 (m)	73.1	4.49 (m)	73.2
	26		177.3		177.3		n.r.		n.r.		n.r.
4-amino-7- guanidino -3-hydroxy	27		177.8		177.8		n.r.		n.r.		n.r.

2-
methylheptano
ic acid

28	2.69 (m)	44.3	2.67 (m)	44.3	2.70 (m)	43.9	2.69 (m)	44.1	2.69 (m)	44.5
29	1.19 (d, 6.8)	14.4	1.20 (d, 6.8)	14.4	1.18 (d, 6.4)	n.r.	1.17 (br)	14.4	1.2 (br)	n.r.
30	3.72 (m)	75.9	3.74 (m)	75.9	3.78 (m)	75.7	3.72 (m)	75.7	3.73 (m)	75.8
31	3.92 (m)	52.6	3.92 (m)	52.6	3.90 (m)	52.6	3.89 (m)	52.6	3.91 (m)	52.5
32	a. 1.45 (m)	29.8	a. 1.46 (m)	29.8	1.48-1.54 (br)	29.8	1.44-1.97 (br)	29.8	1.44-1.97 (br)	29.8
	b. 1.71 (m)		b. 1.71 (m)							
33	1.61 (m)	24.1	1.61 (m)	24.1	1.54-1.70 (br)	24.2	1.58 (br)	24.2	1.58 (br)	24.2
34	3.19 (m)	42.2	3.19 (m)	42.2	3.20 (m)	42.1	3.21 (m)	42.5	3.21 (m)	41.9
35		158.7		158.7		n.r.		n.r.		n.r.
fatty acid		176.1		176.4		n.r.		n.r.		n.r.
36		176.1		176.4		n.r.		n.r.		n.r.
37	2.22 (m)	37.2	2.22 (m)	37.2	2.22 (m)	37.1	2.20 (m)	37.3	2.23 (m)	37.2
38	1.62 (br)	23.7	1.61 (br)	23.7	1.62 (br)	24.1	1.61 (br)	24.1	1.61 (br)	24.3
39	1.33 (br)	29.7-30.8	1.31 (br)	29.8-30.7	1.32 (br)	29.7-30.8	1.33 (br)	30.0-30.8	1.29 (br)	29.7-30.8
40	1.33 (br)	29.7-30.8	1.31 (br)	29.8-30.7	1.32 (br)	29.7-30.8	1.33 (br)	30.0-30.8	1.29 (br)	29.7-30.8
41	1.33 (br)	29.7-30.8	1.31 (br)	29.8-30.7	1.32 (br)	29.7-30.8	1.33 (br)	30.0-30.8	2.04 (br)	28.1
42	1.33 (br)	29.7-30.8	1.31 (br)	29.8-30.7	1.32 (br)	29.7-30.8	1.33 (br)	30.0-30.8	5.35 (m, 4.6)	131.1
43	1.33 (br)	29.7-30.8	1.31 (br)	29.8-30.7	1.32 (br)	29.7-30.8	2.04 (br)	28.1	5.35 (m, 4.6)	130.6
44	1.33 (br)	29.7-30.8	1.31 (br)	29.8-30.7	1.32 (br)	29.7-30.8	5.35 (m, 4.6)	131.1	2.04 (br)	28.1

45	1.33 (br)	29.7-30.8	1.31 (br)	29.8-30.7	0.90 (t, 6.9)	14.1	5.35 (m, 4.6)	130.6	1.29 (br)	29.7-30.8
46	1.33 (br)	29.7-30.8	1.31 (br)	29.8-30.7			2.04 (br)	28.1	1.29 (br)	29.7-30.8
47	0.90 (t, 7.0)	14.4	1.31 (br)	29.8-30.7			0.90 (t, 6.9)	14.1	1.29 (br)	29.7-30.8
48			1.31 (br)	29.8-30.7					1.29 (br)	29.7-30.8
49			0.90 (t, 7.0)	14.4					0.90 (t, 6.9)	14.4

n.r. (not resolved due to broadening signals)

* assigned chemical shifts according to HSQC coupling

Table S2. List of primers, strains and plasmids used for generation of *varG* null-mutant.

Primers	Sequence (5' → 3'):
varG-up_fw	gccaaaagtggcccagggcTCAGAGGGTTCTTTCCGTGGG
varG-up_rv	tcttctggcagataagcttCGTGAACGCCTACGGCCC
CmR_fw	CACCAATAACTGCCTTAAAAAATTACG
CmR_rv	GCCCTGGGCCAACTTTTG
varG-down_fw	cggatgaatgggataagcttTGGGCGGCGCCTCGACGA
varG-down_rv	ttttaaggcagttattggtgATGACGACCATGACCGACCACTTCGAC
H002_checkA_fw	TCCAGCAGGCAGAGGTATTG
H002_checkA_rv	GCAAGATGTGGCGTGTTACG
H002_checkB_fw	CACCAGCTCACCGTCTTTCA
H002_checkB_rv	ACAGCTCAGTGAACGGAACC
pRED_fw	ATCTGCCAGGAAGATACTTAAC
pRED_rv	ATCCCATTCATCCGCTTATTATC
Strains and plasmids	Genotype or description
<i>E. coli</i> DH5a	F ⁻ φ80 <i>lacZ</i> ΔM15 Δ(<i>lacZYA-argF</i>) U169 <i>endA1 recA1 hsdR17</i> (rk ⁻ , mk ⁺) <i>supE44 thi</i> ⁻¹ <i>gyrA996 relA1 phoA</i>
<i>E. coli</i> S17-1 λ <i>pir</i>	λ <i>pir hsdR pro thi</i> ; chromosomal integrated RP4-2 Tc::Mu m::Tn7
<i>Variovorax</i> sp. H002	Wild type
pRED	Cloning vector; Cm ^r
pGM160	Conjugation vector; Amp ^r , Tsr ^r , Neo ^r , Kan ^r
pRED_H002	H002 Knock-out cassette on pRED
pGM160_H002	H002 Knock-out cassette on pGM160

Table S3. Annotation of *var* biosynthetic gene cluster in *Variovorax* sp. H002.

■ regulatory genes
 ■ core BGC genes
 ■ accessory BGC genes
 ■ transport genes
 ■ others

ORF	Nucl.	AA	Homolog	Cov./Id.	Accession
A	534	177	RNA polymerase sigma factor	99/83.5	WP_092757814.1
B	246	81	FecR/PupR family sigma factor regulator	91/59.5	WP_166300416.1
C	249	82	MbtH family	98/82.7	WP_211958485.1
D	747	248	Thioesterase	92/70.4	SDO97538.1
E	702	233	4'-phosphopantetheinyl transferase	95/57.7	WP_272548812.1
F	5265	1754	NRPS (FAAL-A-PCP)	-	-
G	7056	2351	PKS (KS-AT-KR-ACP-C)	-	-
H	3105	1034	NRPS (C-A-PCP)	-	-
I	7764	2587	NRPS (C-A-PCP-E-C-A-PCP)	-	-
J	7377	2458	NRPS (C-A-PCP-C-A-PCP-TE)	-	-
K	2448	815	TonB-dependent siderophore receptor	96/60.3	WP_198971676.1
L	1029	342	Iron dicitrate transport regulator FecR	97/66.3	WP_121454243.1
M	579	192	RNA polymerase sigma factor	99/78.4	WP_092759873.1
M	1320	439	L-Ornithine N(5)-oxygenase	99/71.5	ODT73388.1
O	1107	368	Acetyltransferase	94/58.0	WP_090907594.1
P	786	261	Siderophore-iron reductase	99/89.6	RIX80240.1
Q	234	77	Hypothetical protein	77/75.4	WP_092759869.1
R	1680	559	Cyclic peptide export ABC transporter	98/74.73	WP_211956633.1