Supporting Information

Methionine-Containing Rhabdopeptide/Xenortide-like Peptides from Heterologous Expression of the Biosynthetic Gene Cluster *kj12ABC* in *Escherichia coli*

Lei Zhao,[†,‡] Xiaofeng Cai,[†] Marcel Kaiser,[§,⊥] and Helge B. Bode[†,‖,*]

[†]Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany

[‡]Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, 210014 Nanjing, China

[§]Parasite Chemotherapy, Swiss Tropical and Public Health Institute, 4051 Basel, Switzerland

[⊥]University of Basel, 4003 Basel, Switzerland

[‖]Buchmann Institute for Molecular Life Sciences (BMLS), Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany

*Corresponding author: E-mail: h.bode@bio.uni-frankfurt.de

Supplementary Methods

Chemical synthesis

The synthesis was performed manually using stepwise solid phase peptide synthesis (SPPS) method.[1,2] For a schematic overview see Scheme 1. Synthesis of compound **1** was shown as an example. At step **a**, the attachment of the C-terminal amine phenylethylamine (PEA) on the 2-(3,5-dimethoxy-4-formylphenoxy)ethyl (DFPE) resin was carried out. A mixture of PEA (126 μL, 1.0 mmol, 10 eq.) in 1.4 mL *N*,*N*-dimethylformamide (DMF)/MeOH/AcOH (80:19:1), $NaBH_3CN$ (62.8 mg, 1.0 mmol, 10 eq.) and DFPE resin (95.2 mg, 0.1 mmol, 1 eq.) were placed in a 2 mL Eppendorf tube and incubated in a thermoshaker at 60 °C overnight. The resin was filtered, and subsequently washed with DMF (5 ×) and dichloromethane (DCM) (5 ×), and dried. At step **b**, acylation of *N*-Me-L-Val was conducted. A solution of Fmoc-*N*-Me-Val-OH (353.4 mg, 1.0 mmol, 10 eq.), *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-b]-pyridino-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate (HATU) (381 mg, 1.0 mmol, 10 eq), 1-hydroxy-7-azabenzotriazole (HOAt) (136 mg, 1.0 mmol, 10 eq) and *N*,*N*-diisopropylethylamine (DIPEA) (0.34 mL, 2.0 mmol, 20 eq.) in 2.0 mL DMF was added to resin. The resulting mixture was incubated in a plastic reactor vessel equipped with a Teflon frit at room temperature overnight. The resin was washed with DMF (5 ×) and DCM (5 ×) and treated with 20% piperidine in DMF (3 × 10 min, 2 mL) to remove the Fmoc protecting group. Afterwards the resin was washed with DCM (5 ×) and dried. At step **c**, *N*-Me-L-Met was coupled to peptide sequence. The coupling of Fmoc-*N*-Me-Met-OH was mediated by using the efficient and rapid BTC coupling reagent. The dried peptidyl resin (25 μmol) was swollen with dry tetrahydrofuran (THF) (1 mL) for 15 min; meantime, in a separate flask, bis-(trichloromethyl)carbonate (BTC) (8.5 mg, 28.3 μmol, 1.15 eq.) was dissolved in dry THF (0.7 mL), and the Fmoc-*N*-Me-Met-OH (28.9 mg, 75 μmol, 3 eq.) was added to it, which resulted in a clear amino acid solution. Collidine (34 μL, 250 μmol, 10 eq.) was added to this clear solution, and a precipitate was immediately formed. This precipitate was added to the resin beads, which were pre-mixed with DIPEA (35 μL, 200 μmol, 8 eq.), and the whole reaction mixture was shaken at room temperature for 2 h. The resin was washed with DMF (5 ×) and DCM (5 ×) and treated with 20% piperidine in DMF (3 × 10 min, 2 mL) to remove the Fmoc protecting group. Afterwards the resin was washed with DCM (5 ×) and dried. At step **d**, The L-Val was coupled by using the HATU/HOAt coupling reagent. The dried peptidyl resin (25 μmol) was swollen in DMF. A solution of Fmoc-Val-OH (25.5 mg, 75 μmol, 3 eq.), HATU (28.6 mg, 75 μmol,

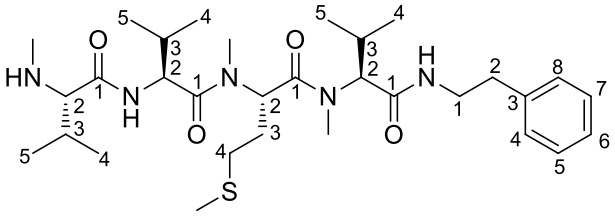
3 eq.), HOAt (10.2 mg, 75 μmol, 3 eq.) and DIPEA (25.5 μL, 150 μmol, 6 eq.) in 0.5 mL DMF was added to resin and shaken at room temperature overnight. The resin was washed with DMF (5 ×) and DCM (5 ×) and treated with 20% piperidine in DMF (3 × 10 min, 2 mL) to remove the Fmoc protecting group. Afterwards the resin was washed with DCM (5 ×) and dried. At step **e**, *N*-Me-L-Val was coupled to peptide sequence using the same method as step **c**. At the final step **f**, the peptide was cleaved from the resin. A total of 1 mL trifluoroacetic acid (TFA)/triisopropylsilane (TIS)/water (95:2.5:2.5) was added to the peptidyl resin (25 μmol) and the mixture was agitated for at least 2 h at room temperature. The resin was removed by filtration and washed twice with TFA. The solution was concentrated *in vacuo*. The residue was purified by Agilent HPLC system. The structures of pure compounds were confirmed by HR-MS, 1D and 2D NMR.

Supplementary Tables

Table S1. HR-MS data of natural and synthetic **1**–**7**, **10** and **12**

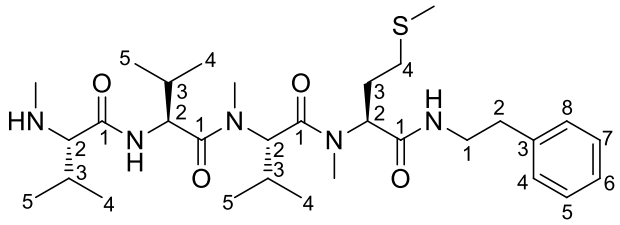
compound	sum formula	*m/z* calcd. $[M + H]^+$	natural		synthetic	
			m/z found $[M + H]^+$	Δ ppm	*m/z* found $[M + H]^+$	Δ ppm
1	$C_{31}H_{53}N_5O_4S$	592.3891	592.3887	0.7	592.3878	2.2
2	$C_{31}H_{53}N_5O_4S$	592.3891	592.3887	0.7	592.3886	0.8
3	$C_{31}H_{53}N_5O_4S$	592.3891	592.3882	1.5	592.3885	1.0
4	$C_{36}H_{62}N_6O_5S$	691.4575	691.4559	2.3	691.4564	1.6
5	$C_{37}H_{64}N_6O_5S$	705.4732	705.4717	2.1	705.4717	2.1
6	$C_{37}H_{64}N_6O_5S$	705.4732	705.4717	2.1	705.4723	1.2
7	$C_{37}H_{64}N_6O_5S$	705.4732	705.4727	0.7	705.4723	1.2
10	$C_{31}H_{53}N_5O_4$	560.4170	560.4164	1.2	560.4165	0.9
12	$C_{37}H_{64}N_6O_5$	673.5011	673.5000	1.7	673.5003	1.2

Table S2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **1** in Methanol-d_4 (δ in ppm, J in Hz)



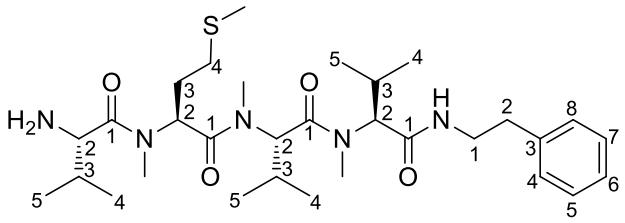
subunit	position	δc	δ_H
N-Me-L-Val	1	168.1 (C)	
	2	68.2 (CH)	3.68, d (5.5)
	3	31.7 (CH)	2.24–2.14, m
	4	18.8 (CH_3)	1.07, d (6.3)
	5	18.7 (CH_3)	1.03, d (6.9)
	N-CH_3	33.3	2.65, s
L-Val	1	173.8 (C)	
	2	56.9 (CH)	4.70, d (7.6)
	3	31.6 (CH)	2.13–2.04, m
	4	18.6 (CH_3)	1.01, d (6.8)
	5	19.9 (CH_3)	0.99, d (6.8)
N-Me-L-Met	1	172.8 (C)	
	2	53.8 (CH)	5.64, dd (8.4, 6.1)
	3	29.5 (CH_2)	2.04–1.96, 1.96–1.88, m
	4	31.4 (CH_2)	2.39, t (7.2)
	S-CH_3	15.4	2.07, s
	N-CH_3	31.7	3.11, s
N-Me-L-Val	1	171.7 (C)	
	2	64.0 (CH)	4.53, d (11.1)
	3	27.6 (CH)	2.24–2.14, m
	4	19.9 (CH_3)	0.87, d (6.4)
	5	19.2 (CH_3)	0.76, d (6.6)
	N-CH_3	31.5	2.95, s
PEA	1	41.6 (CH_2)	3.54–3.47, 3.41–3.33, m
	2	36.5 (CH_2)	2.84–2.72, m
	3	140.5 (C)	
	4	130.0 (CH)	7.19, overlap
	5	129.7 (CH)	7.27, overlap
	6	127.6 (CH)	7.19, overlap
	7	129.7 (CH)	7.27, overlap
	8	130.0 (CH)	7.19, overlap

Table S3. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **2** in Methanol-d_4 (δ in ppm, J in Hz)



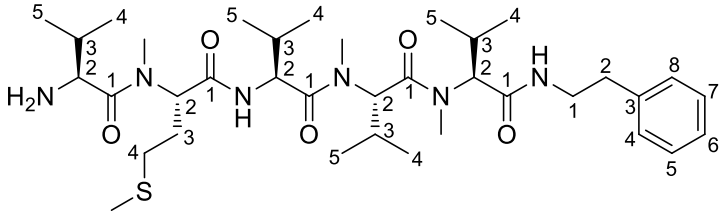
subunit	position	δ_c	δ_H
N-Me-L-Val	1	168.0 (qC)	
	2	68.2 (CH)	3.67, d (5.4)
	3	31.7 (CH)	2.20–2.12, m
	4	18.7 (CH_3)	1.03, d (7.0)
	5	18.5 (CH_3)	1.00, overlap
	N-CH_3	33.3 (CH_3)	2.63, s
L-Val	1	174.1 (qC)	
	2	56.7 (CH)	4.72, d (7.9)
	3	31.8 (CH)	2.11–2.01, m
	4	18.8 (CH_3)	1.00, overlap
	5	19.8 (CH_3)	0.95, d (6.8)
N-Me-L-Val	1	173.0 (qC)	
	2	59.7 (CH)	5.18, d (10.8)
	3	28.7 (CH)	2.45–2.25, m
	4	19.9 (CH_3)	0.88, d (6.4)
	5	19.2 (CH_3)	0.79, d (6.6)
	N-CH_3	31.4 (CH_3)	3.12, s
N-Me-L-Met	1	172.1 (qC)	
	2	57.3 (CH)	5.09, dd (9.7, 5.8)
	3	29.3 (CH_2)	2.11–2.01, 1.92–1.82, m
	4	31.5 (CH_2)	2.29, t (7.4)
	S-CH_3	15.4 (CH_3)	2.04, s
	N-CH_3	32.2 (CH_3)	2.97, s
PEA	1	41.9 (CH_2)	3.42, t (7.2)
	2	36.6 (CH_2)	2.77, t (7.2)
	3	140.5 (qC)	
	4	130.0 (CH)	7.19, overlap
	5	129.7 (CH)	7.26, overlap
	6	127.6 (CH)	7.19, overlap
	7	129.7 (CH)	7.26, overlap
	8	130.0 (CH)	7.19, overlap

Table S4. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **3** in Methanol-d_4 (δ in ppm, J in Hz)



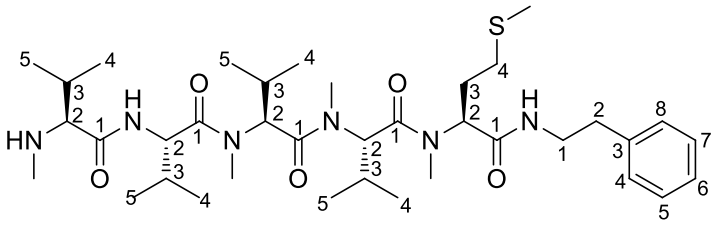
subunit	position	δ_c	δ_H
L-Val	1	171.0 (qC)	
	2	57.1 (CH)	4.27, d (4.5)
	3	30.9 (CH)	2.24–2.16, m
	4	19.6 (CH_3)	1.11, d (7.0)
	5	16.9 (CH_3)	0.99, d (6.9)
N-Me-L-Met	1	172.6 (qC)	
	2	53.7 (CH)	5.73, t (7.3)
	3	29.0 (CH_2)	2.16–2.09, 2.00–1.91, m
	4	31.4 (CH_2)	2.54–2.41, m
	S-CH_3	15.2 (CH_3)	2.07, s
	N-CH_3	31.7 (CH_3)	3.08, s
N-Me-L-Val	1	172.5 (qC)	
	2	60.1 (CH)	5.09, d (10.8)
	3	28.7 (CH)	2.35–2.27, m
	4	20.0 (CH_3)	0.88, d (6.5)
	5	19.0 (CH_3)	0.79, d (6.7)
	N-CH_3	31.1 (CH_3)	3.03, s
N-Me-L-Val	1	171.8 (qC)	
	2	63.9 (CH)	4.57, d (11.1)
	3	27.8 (CH)	2.24–2.16, m
	4	19.9 (CH_3)	0.86, d (6.6)
	5	19.2 (CH_3)	0.76, d (6.7)
	N-CH_3	31.5 (CH_3)	2.99, s
PEA	1	41.7 (CH_2)	3.50–3.35, m
	2	36.6 (CH_2)	2.79–2.74, m
	3	140.4 (qC)	
	4	129.9 (CH)	7.19, overlap
	5	129.7 (CH)	7.26, overlap
	6	127.6 (CH)	7.19, overlap
	7	129.7 (CH)	7.26, overlap
	8	129.9 (CH)	7.19, overlap

Table S5. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **4** in Methanol-d_4 (δ in ppm, J in Hz)



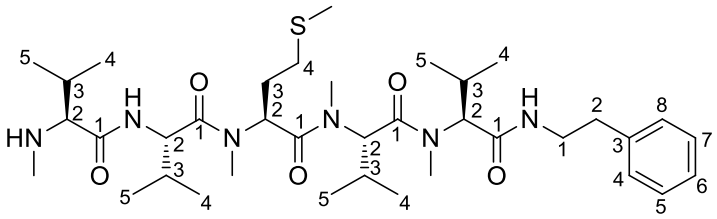
subunit	position	δ_C	δ_H
L-Val	1	171.5 (qC)	
	2	57.1 (CH)	4.22, d (5.0)
	3	31.1 (CH)	2.25–2.13, m
	4	19.5 (CH_3)	1.11, d (7.0)
	5	17.1 (CH_3)	1.01, d (6.9)
N-Me-L-Met	1	172.0 (qC)	
	2	57.5 (CH)	5.20, d (7.1)
	3	29.7 (CH_2)	2.25–2.13, 2.00–1.91, m
	4	31.4 (CH_2)	2.43, t (7.4)
	S-CH_3	15.4 (CH_3)	2.09, s
	N-CH_3	32.0 (CH_3)	3.09, s
L-Val	1	174.6 (qC)	
	2	56.5 (CH)	4.60, d (8.4)
	3	31.7 (CH)	2.08–2.00, m
	4	18.8 (CH_3)	0.93, overlap
	5	19.9 (CH_3)	0.91, overlap
N-Me-L-Val	1	172.8 (qC)	
	2	59.8 (CH)	5.18, d (10.7)
	3	29.0 (CH)	2.36–2.27, m
	4	19.9 (CH_3)	0.87, overlap
	5	18.9 (CH_3)	0.81, d (6.7)
	N-CH_3	31.4 (CH_3)	3.11, s
N-Me-L-Val	1	171.8 (qC)	
	2	63.8 (CH)	4.58, d (11.1)
	3	27.7 (CH)	2.25–2.13, m
	4	19.8 (CH_3)	0.87, overlap
	5	19.0 (CH_3)	0.74, d (6.7)
	N-CH_3	31.6	3.03, s
PEA	1	41.7 (CH_2)	3.49–3.36, m
	2	36.7 (CH_2)	2.77, t (7.2)
	3	140.4 (qC)	
	4	129.9 (CH)	7.19, overlap
	5	129.7 (CH)	7.26, overlap
	6	127.6 (CH)	7.19, overlap
	7	129.7 (CH)	7.26, overlap
	8	129.9 (CH)	7.19, overlap

Table S6. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **5** in Methanol-d_4 (δ in ppm, J in Hz)



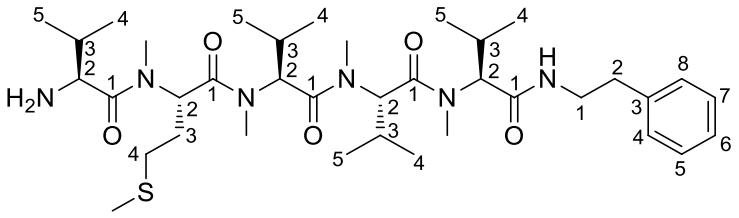
subunit	position	δ_c	δ_H
N-Me-L-Val	1	168.0 (qC)	
	2	68.3 (CH)	3.68, d (5.3)
	3	31.7 (CH)	2.21–2.12, m
	4	18.7 (CH_3)	1.05, d (7.0)
	5	18.5 (CH_3)	1.02, d (6.9)
	N-CH_3	33.3 (CH_3)	2.65, s
L-Val	1	174.1 (qC)	
	2	56.7 (CH)	4.76, d (7.6)
	3	31.7 (CH)	2.11–2.03, m
	4	18.7 (CH_3)	1.02, d (6.9)
	5	19.9 (CH_3)	0.99, d (6.7)
N-Me-L-Val	1	172.3 (qC)	
	2	59.8 (CH)	5.20, d (10.8)
	3	28.7 (CH)	2.37–2.25, m
	4	20.0 (CH_3)	0.88, d (6.4)
	5	19.1 (CH_3)	0.81, overlap
	N-CH_3	31.3 (CH_3)	3.16, s
N-Me-L-Val	1	172.8 (qC)	
	2	59.9 (CH)	5.17, d (10.8)
	3	28.7 (CH)	2.37–2.25, m
	4	20.1 (CH_3)	0.92, d (6.4)
	5	18.8 (CH_3)	0.79, overlap
	N-CH_3	31.5 (CH_3)	3.02, s
N-Me-L-Met	1	172.2 (qC)	
	2	57.5 (CH)	5.10, dd (9.8, 5.6)
	3	29.2 (CH_2)	2.11–2.03, 1.96–1.80, m
	4	31.5 (CH_2)	2.37–2.25, m
	S-CH_3	15.4 (CH_3)	2.06, s
	N-CH_3	32.2 (CH_3)	2.96, s
PEA	1	42.0 (CH_2)	3.46-3.41, m
	2	36.6 (CH_2)	2.79, dd (8.8, 5.5)
	3	140.5 (qC)	
	4	130.0 (CH)	7.20, overlap
	5	129.7 (CH)	7.28, overlap
	6	127.6 (CH)	7.20, overlap
	7	129.7 (CH)	7.28, overlap
	8	130.0 (CH)	7.20, overlap

Table S7. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **6** in Methanol-d_4 (δ in ppm, J in Hz)



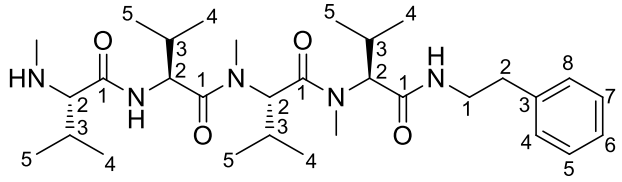
subunit	position	δ_c	δ_H
N-Me-L-Val	1	169.1 (qC)	
	2	68.6 (CH)	3.55, d (5.6)
	3	31.9 (CH)	2.24–2.01, m
	4	19.0 (CH_3)	1.04, overlap
	5	18.8 (CH_3)	1.02, overlap
	N-CH_3	33.5 (CH_3)	2.60, s
L-Val	1	174.0 (qC)	
	2	56.8 (CH)	4.70, d (8.0)
	3	31.6 (CH)	2.24–2.01, m
	4	18.8 (CH_3)	1.02, overlap
	5	19.8 (CH_3)	0.99, overlap
N-Me-L-Met	1	172.6 (qC)	
	2	53.3 (CH)	5.76, t (7.3)
	3	29.2 (CH_2)	2.24–2.01, 1.93–1.84, m
	4	31.3 (CH_2)	2.47–2.36, m
	S-CH_3	15.2 (CH_3)	2.03, s
	N-CH_3	31.8 (CH_3)	3.17, s
N-Me-L-Val	1	172.5 (qC)	
	2	60.1 (CH)	5.10, d (10.7)
	3	28.7 (CH)	2.36–2.27, m
	4	20.1 (CH_3)	0.89, d (6.4)
	5	18.9 (CH_3)	0.80, d (6.8)
	N-CH_3	31.1 (CH_3)	3.01, s
N-Me-L-Val	1	171.8 (qC)	
	2	63.9 (CH)	4.57, d (11.1)
	3	27.8 (CH)	2.24–2.01, m
	4	19.9 (CH_3)	0.86, d (6.5)
	5	19.2 (CH_3)	0.76, d (6.6)
	N-CH_3	31.5 (CH_3)	2.99, s
PEA	1	41.7 (CH_2)	3.51–3.35, m
	2	36.7 (CH_2)	2.80–2.72, m
	3	140.4 (qC)	
	4	129.9 (CH)	7.19, overlap
	5	129.7 (CH)	7.26, overlap
	6	127.6 (CH)	7.19, overlap
	7	129.7 (CH)	7.26, overlap
	8	129.9 (CH)	7.19, overlap

Table S8. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **7** in Methanol-d_4 (δ in ppm, J in Hz)



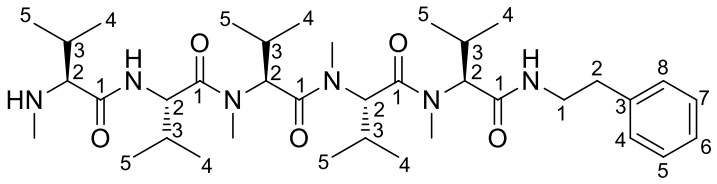
subunit	position	δ_c	δ_H
L-Val	1	171.1 (qC)	
	2	57.1 (CH)	4.26, d (4.5)
	3	30.9 (CH)	2.24–2.04, m
	4	19.6 (CH_3)	1.11, d (7.0)
	5	16.9 (CH_3)	0.99, d (6.9)
N-Me-L-Met	1	172.7 (C)	
	2	53.7 (CH)	5.74, t (7.3)
	3	28.9 (CH_2)	2.24–2.04, 2.02–1.93, m
	4	31.4 (CH_2)	2.55–2.42, m
	S-CH_3	15.2 (CH_3)	2.08, s
	N-CH_3	31.7 (CH_3)	3.09, s
N-Me-L-Val	1	172.0 (qC)	
	2	60.1 (CH)	5.12, d (10.8)
	3	28.5 (CH)	2.37–2.27, m
	4	20.0 (CH_3)	0.88, overlap
	5	18.9 (CH_3)	0.79, overlap
	N-CH_3	31.0 (CH_3)	3.05, s
N-Me-L-Val	1	172.6 (qC)	
	2	59.9 (CH)	5.15, d (10.8)
	3	28.9 (CH)	2.37–2.27, m
	4	20.0 (CH_3)	0.88, overlap
	5	18.9 (CH_3)	0.79, overlap
	N-CH_3	31.3 (CH_3)	2.99, s
N-Me-L-Val	1	171.8 (C)	
	2	63.8 (CH)	4.57, d (11.1)
	3	27.7 (CH)	2.24–2.04, m
	4	19.9 (CH_3)	0.86, overlap
	5	18.9 (CH_3)	0.73, d (6.7)
	N-CH_3	31.6 (CH_3)	3.02, s
PEA	1	41.7 (CH_2)	3.50–3.35, m
	2	36.7 (CH_2)	2.80–2.72, m
	3	140.4 (qC)	
	4	129.9 (CH)	7.19, overlap
	5	129.7 (CH)	7.26, overlap
	6	127.6 (CH)	7.19, overlap
	7	129.7 (CH)	7.26, overlap
	8	129.9 (CH)	7.19, overlap

Table S9. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **10** in Methanol-d_4 (δ in ppm, J in Hz)



subunit	position	δc	δ_H
N-Me-L-Val	1	168.8 (qC)	
	2	68.6 (CH)	3.58, d (5.4)
	3	31.8 (CH)	2.16–2.09, m
	4	18.8 (CH_3)	1.03, d (6.5)
	5	18.7 (CH_3)	1.00, overlap
	N-CH_3	33.5 (CH_3)	2.60, s
L-Val	1	174.2 (qC)	
	2	56.6 (CH)	4.73, d (7.9)
	3	31.8 (CH)	2.09–2.02, m
	4	18.8 (CH_3)	1.00, overlap
	5	19.8 (CH_3)	0.95, d (6.8)
N-Me-L-Val	1	172.9 (qC)	
	2	59.6 (CH)	5.19, d (10.8)
	3	28.9 (CH)	2.35–2.27, m
	4	19.8 (CH_3)	0.86, d (5.9)
	5	19.2 (CH_3)	0.80, d (6.7)
	N-CH_3	31.4 (CH_3)	3.15, s
N-Me-L-Val	1	171.8 (qC)	
	2	63.8 (CH)	4.58, d (11.1)
	3	27.7 (CH)	2.24–2.16, m
	4	19.8 (CH_3)	0.86, d (5.9)
	5	19.0 (CH_3)	0.74, d (6.7)
	N-CH_3	31.7 (CH_3)	3.05, s
PEA	1	41.7 (CH_2)	3.48–3.35, m
	2	36.7 (CH_2)	2.76, t (7.3)
	3	140.4 (qC)	
	4	129.9 (CH)	7.19, overlap
	5	129.7 (CH)	7.26, overlap
	6	127.6 (CH)	7.19, overlap
	7	129.7 (CH)	7.26, overlap
	8	129.9 (CH)	7.19, overlap

Table S10. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of **12** in Methanol-d_4 (δ in ppm, J in Hz)



subunit	position	δ_c	δ_H
N-Me-L-Val	1	168.6 (qC)	
	2	68.5 (CH)	3.61, d (5.3)
	3	31.8 (CH)	2.24–2.12, m
	4	18.7 (CH_3)	1.01, overlap
	5	18.6 (CH_3)	1.01, overlap
	N-CH_3	33.5 (CH_3)	2.61, s
L-Val	1	174.2 (qC)	
	2	56.6 (CH)	4.76, d (7.7)
	3	31.7 (CH)	2.11–2.03, m
	4	18.8 (CH_3)	1.01, overlap
	5	20.0 (CH_3)	0.98, overlap
N-Me-L-Val	1	172.4 (qC)	
	2	59.8 (CH)	5.20, d (10.8)
	3	28.7 (CH)	2.37–2.26, m
	4	19.9 (CH_3)	0.86, overlap
	5	19.0 (CH_3)	0.78, overlap
	N-CH_3	31.3 (CH_3)	3.15, s
N-Me-L-Val	1	172.7 (qC)	
	2	59.7 (CH)	5.18, d (10.8)
	3	28.9 (CH)	2.37–2.26, m
	4	19.9 (CH_3)	0.86, overlap
	5	19.0 (CH_3)	0.78, overlap
	N-CH_3	31.5 (CH_3)	3.04, s
N-Me-L-Val	1	171.8 (qC)	
	2	63.8 (CH)	4.57, d (11.1)
	3	27.7 (CH)	2.24–2.12, m
	4	19.9 (CH_3)	0.86, overlap
	5	19.1 (CH_3)	0.74, d (6.66)
	N-CH_3	31.6 (CH_3)	3.04, s
PEA	1	41.7 (CH_2)	3.49–3.35, m
	2	36.7 (CH_2)	2.76, m
	3	140.4 (qC)	
	4	129.9 (CH)	7.19, overlap
	5	129.7 (CH)	7.26, overlap
	6	127.6 (CH)	7.19, overlap
	7	129.7 (CH)	7.26, overlap
	8	129.9 (CH)	7.19, overlap

Table S11. Bacterial strains used in this study

strain	relevant Genotype	reference
E. coli DH10B MtaA	F– mcrA, Δ(*mrr-hsd*RMS-*mcr*BC), Φ80*lac*ZΔM15, Δ*lac*X74, *rec*A1, *end*A1, *ara*D139, Δ(*ara leu*)7697, *gal*U, *gal*K, *rps*L, *nup*G, λ–, *ent*D::*mtaA*	[3,4]
Xenorhabdus KJ12.1	wild type	[3]

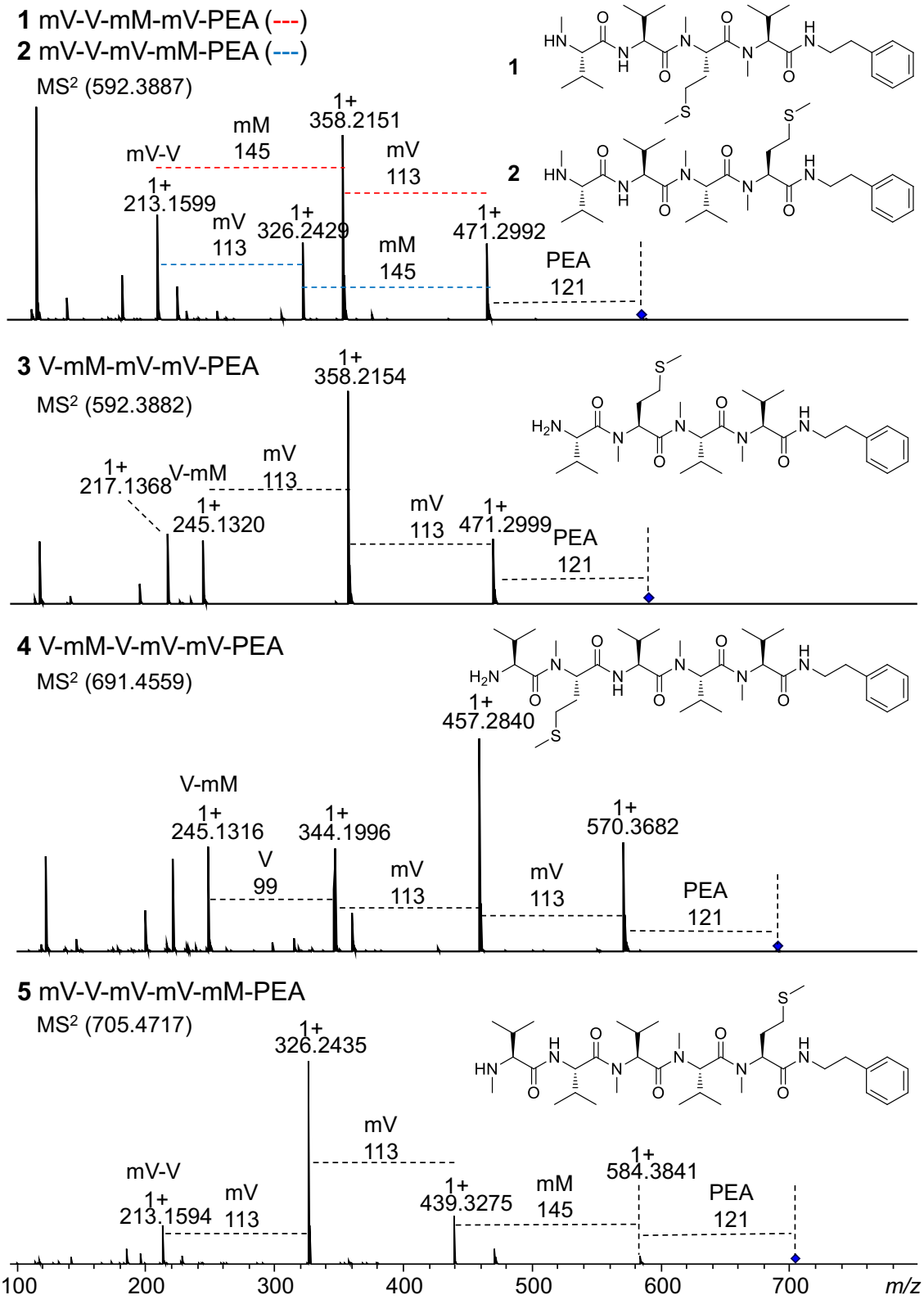
Table S12. Plasmids used in this study

plasmid	description	reference
pCOLA-ara-tacI	3,345 bp, modified from pCOLA_tacI/I that contains arabinose-inducible promotor and kanamycin resistance gene (Km^R)	[3]
pCDF-ara-tacI	3,404 bp, modified from pCDF_tacI/I that contains arabinose-inducible promoter and spectinomycin resistance gene (Sm^R)	[3]
pCX3	16,107 bp, *kj12ABC* gene cluster from *Xenorhabdus* KJ12.1 genomic DNA assembled into pCOLA-ara-tacI, Km^R	[3]
pLZ59	3566 bp, *MbtH* gene from *Xenorhabdus* KJ12.1 genomic DNA assembled into pCDF-ara-tacI, Sm^R	this work
pLZ60	3566 bp, *MbtH* gene from *E. coli* DH10B MtaA genomic DNA assembled into pCDF-ara-tacI, Sm^R	this work

Table S13. Primers used in this study

primer	sequence (5'-3')	targeting DNA fragment	plasmid
XC252-Fw	AATTCCATGGAACAATTAACCGGAAATG	*MbtH* from *Xenorhabdus* KJ12.1 (218 bp)	pLZ59
XC252-Rv	ATGATTAATTGTTAGTGCATATCAGTCTGCTTTTTAG		
XC253-Fw	AGACTGATATGCACTAACAATTAATCATCGGCTCGTATAATG	pCDF-ara-tacI vector backbone (3,403 bp)	
XC253-Fw	ATTTCCGGTTAATTGTTCCATGGAATTCCTCCTGTTAGC		
LZ_160	ATGGCATTCAGTAATCCCTTCGATG	*MbtH* from *E. coli* DH10B MtaA (219 bp)	pLZ60
LZ_161	TCATTGTGCCTCCTGCAACTG		
LZ_162	AATTTTACCCAGTTGCAGGAGGCACAATGACAATTAATCATCGGCTCGTATAATG	pCDF-ara-tacI vector backbone (3,427 bp)	
LZ_163	TGCGGATCATCGAAGGGATTACTGAATGCCATGGAATTCCTCCTGTTAGCCC		

Supplementary Figures



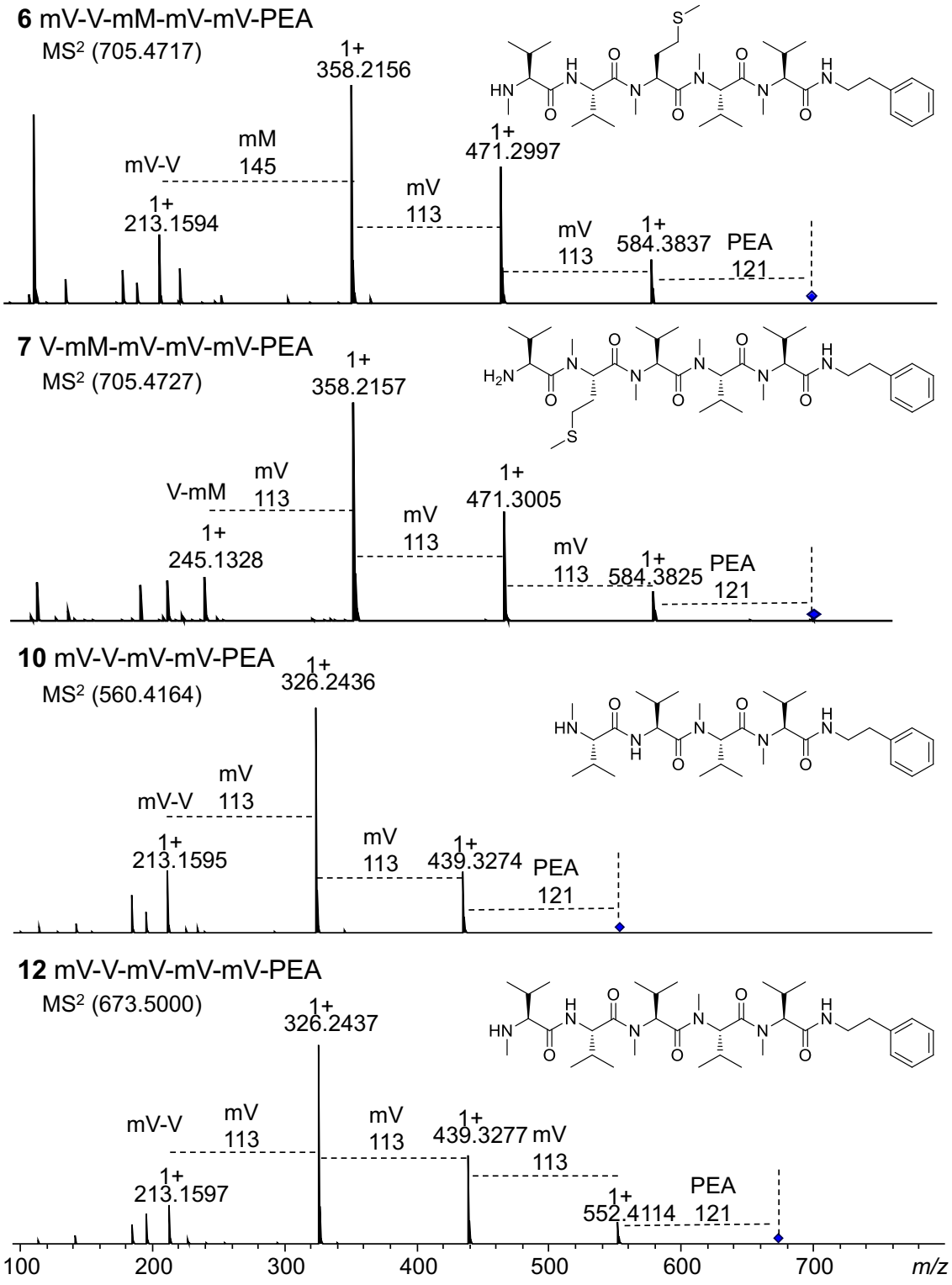
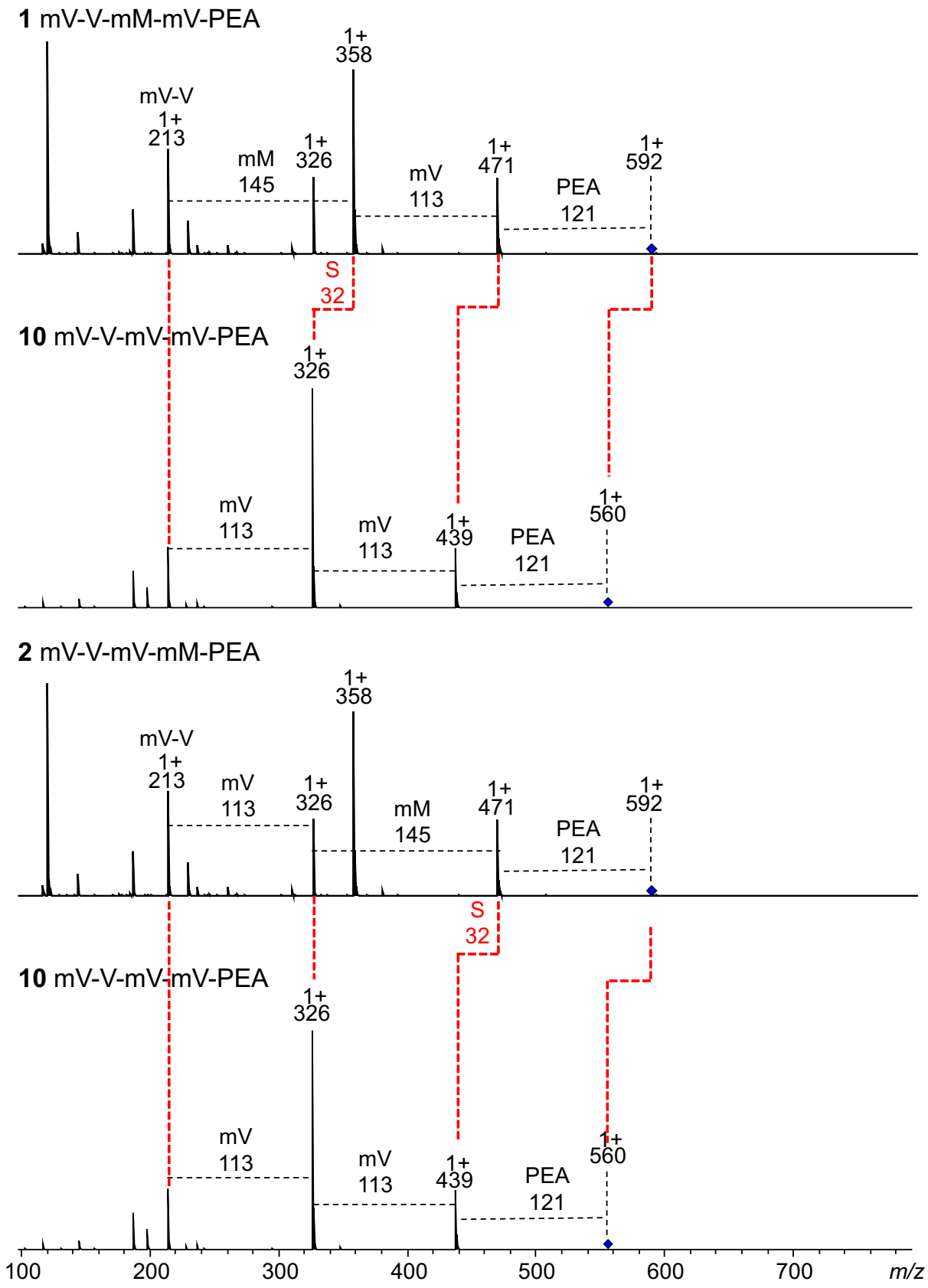


Figure S1. MS^2 fragmentation patterns of natural **1**–**7**, **10** and **12**.


1 mV-V-mM-mV-PEA
1+
358
mV-V
1+
213
mM
145
1+
326
mV
113
1+
471
PEA
121
1+
592
S
32
10 mV-V-mV-mV-PEA
1+
326
mV
113
mV
113
1+
439
PEA
121
1+
560
2 mV-V-mV-mM-PEA
1+
358
mV-V
1+
213
mV
113
1+
326
mM
145
1+
471
PEA
121
1+
592
S
32
10 mV-V-mV-mV-PEA
1+
326
mV
113
mV
113
1+
439
PEA
121
1+
560
100
200
300
400
500
600
700
m/z

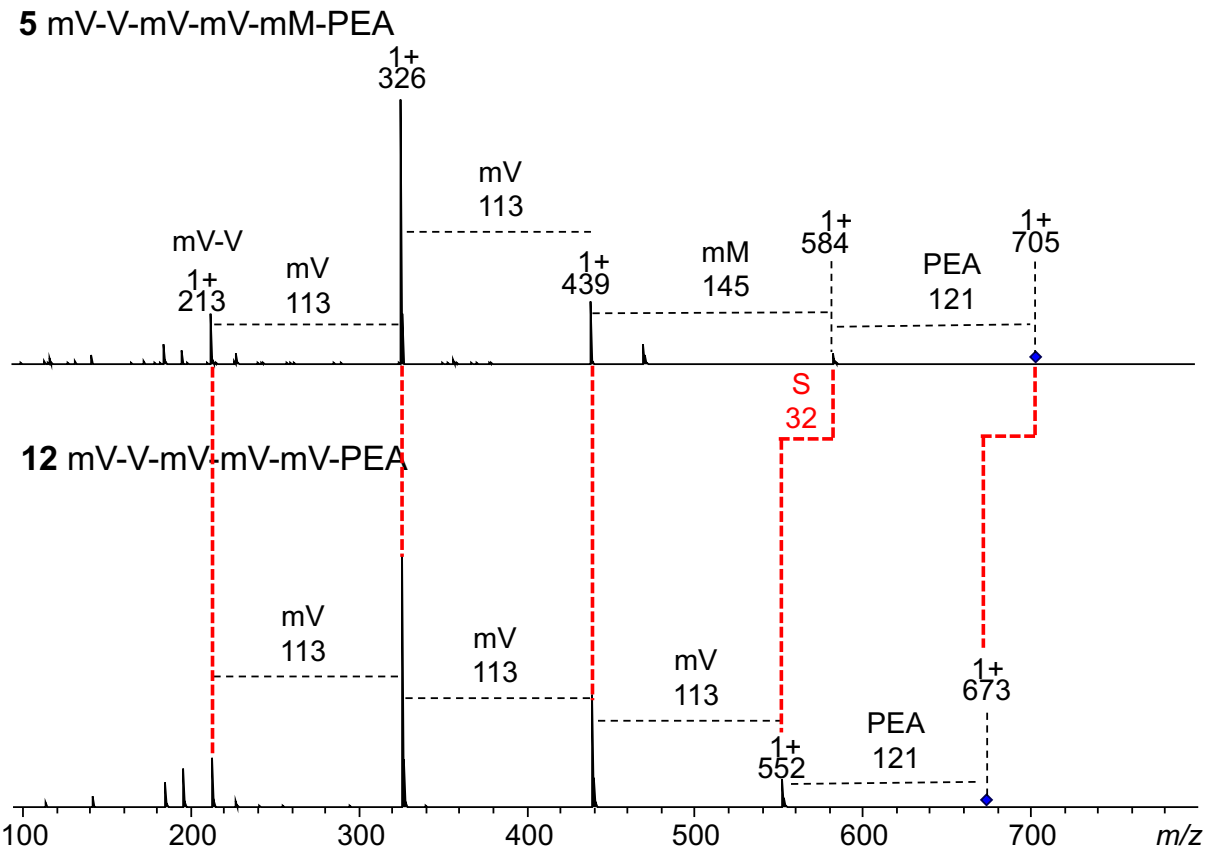
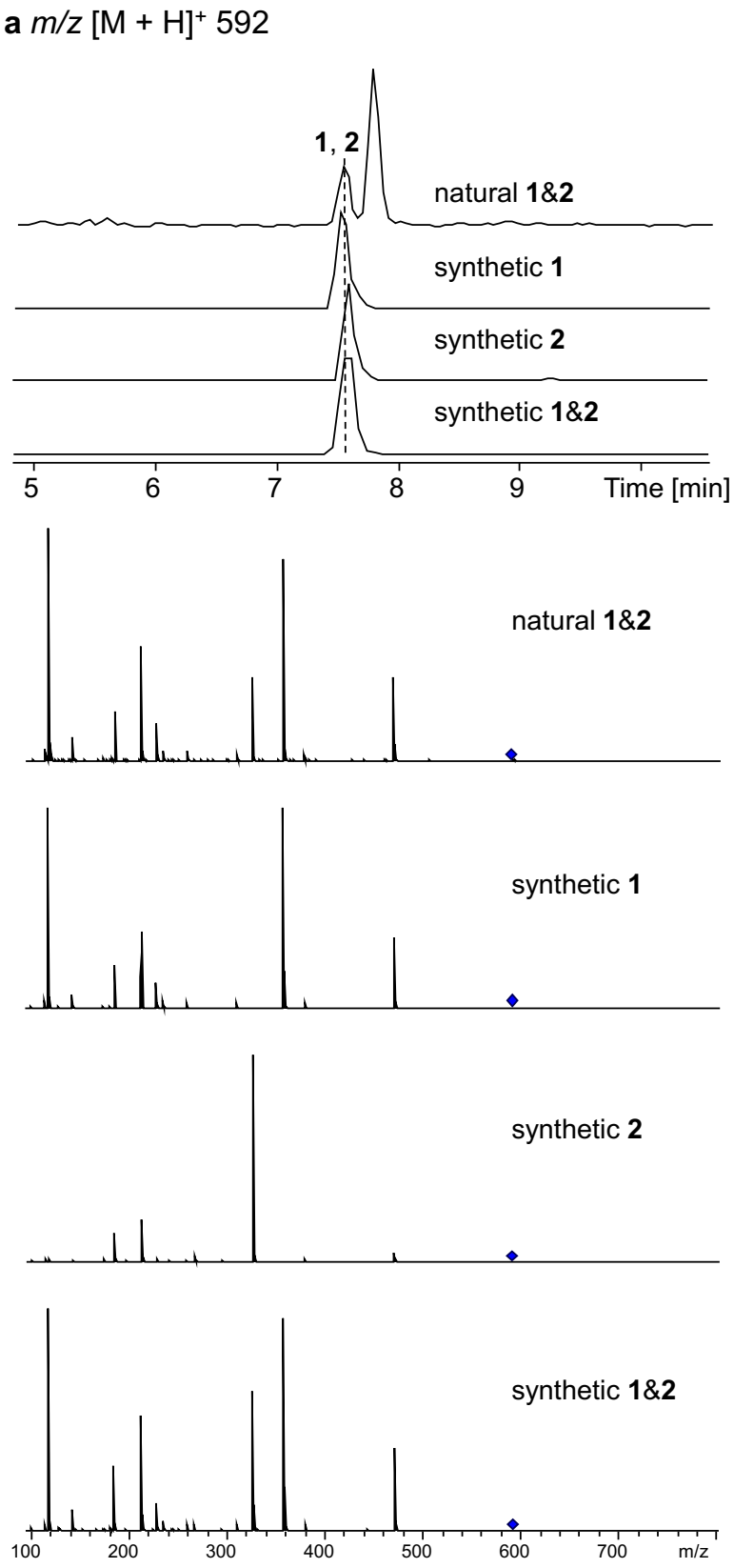
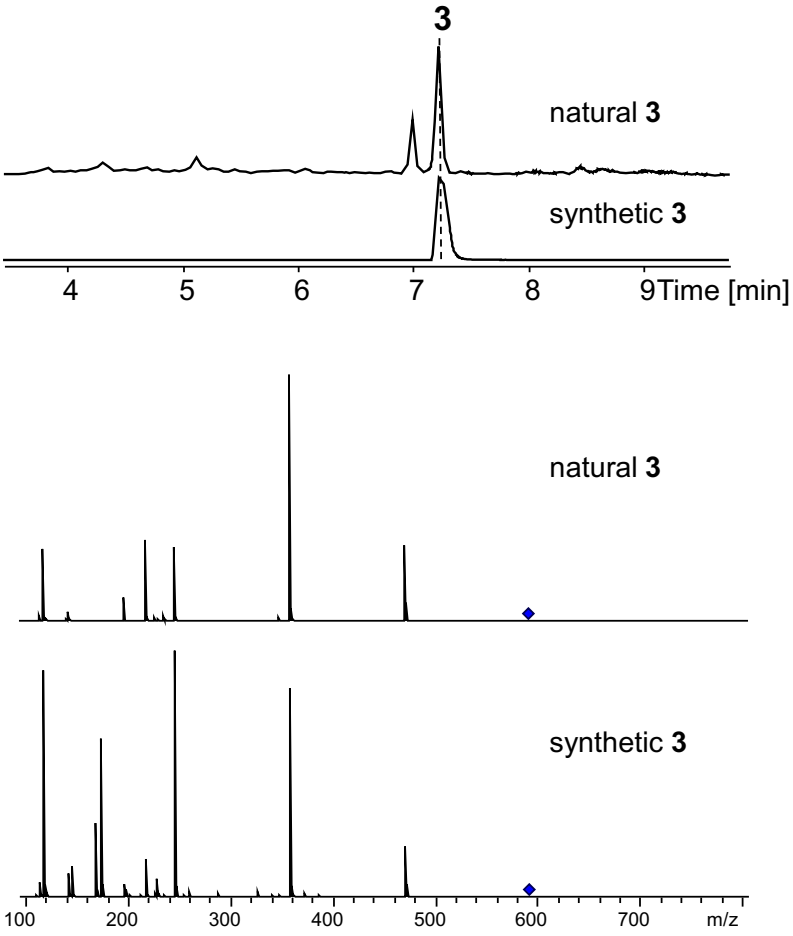
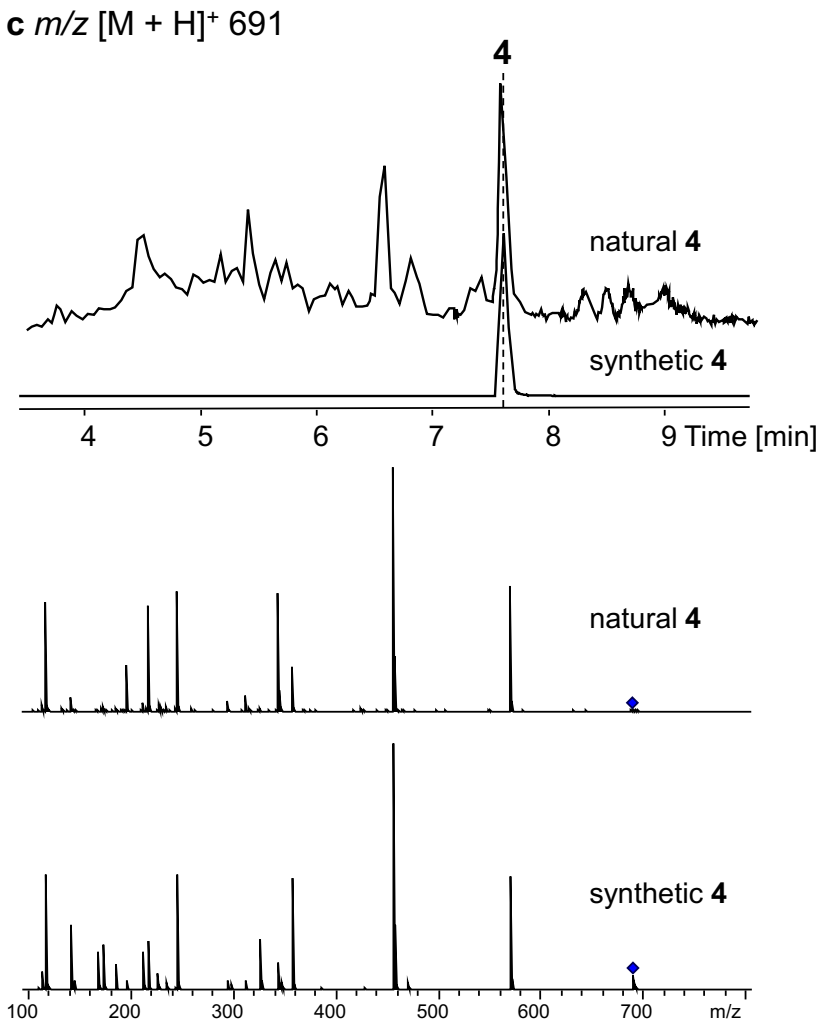


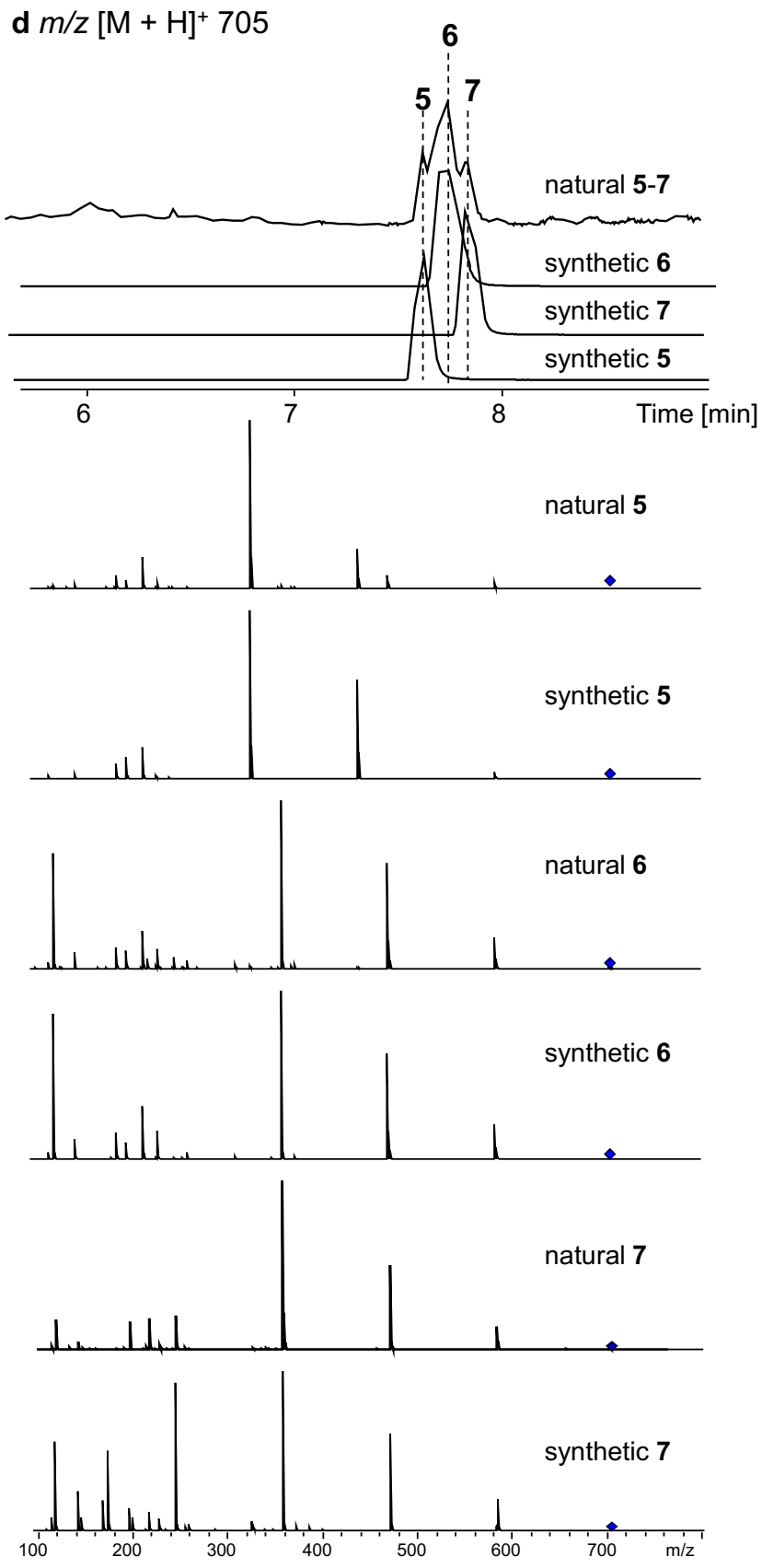
Figure S2. Comparative MS^2 fragmentation patterns of methionine-containing compounds **1**, **2** and **5** with corresponding valine-substituted compounds **10** and **12**.


a m/z [M + H]+ 592
1, 2
natural 1&2
synthetic 1
synthetic 2
synthetic 1&2
5
6
7
8
9
Time [min]
natural 1&2
synthetic 1
synthetic 2
synthetic 1&2
100
200
300
400
500
600
700
m/z

b *m/z* $[M + H]^+$ 592


3
natural 3
synthetic 3
4
5
6
7
8
9Time [min]
natural 3
synthetic 3
100
200
300
400
500
600
700
m/z


c m/z [M + H]+ 691
4
natural 4
synthetic 4
4
5
6
7
8
9 Time [min]
natural 4
synthetic 4
100
200
300
400
500
600
700
m/z


d m/z [M + H]+ 705
6
5
7
natural 5-7
synthetic 6
synthetic 7
synthetic 5
6
7
8
Time [min]
natural 5
synthetic 5
natural 6
synthetic 6
natural 7
synthetic 7
100
200
300
400
500
600
700
m/z

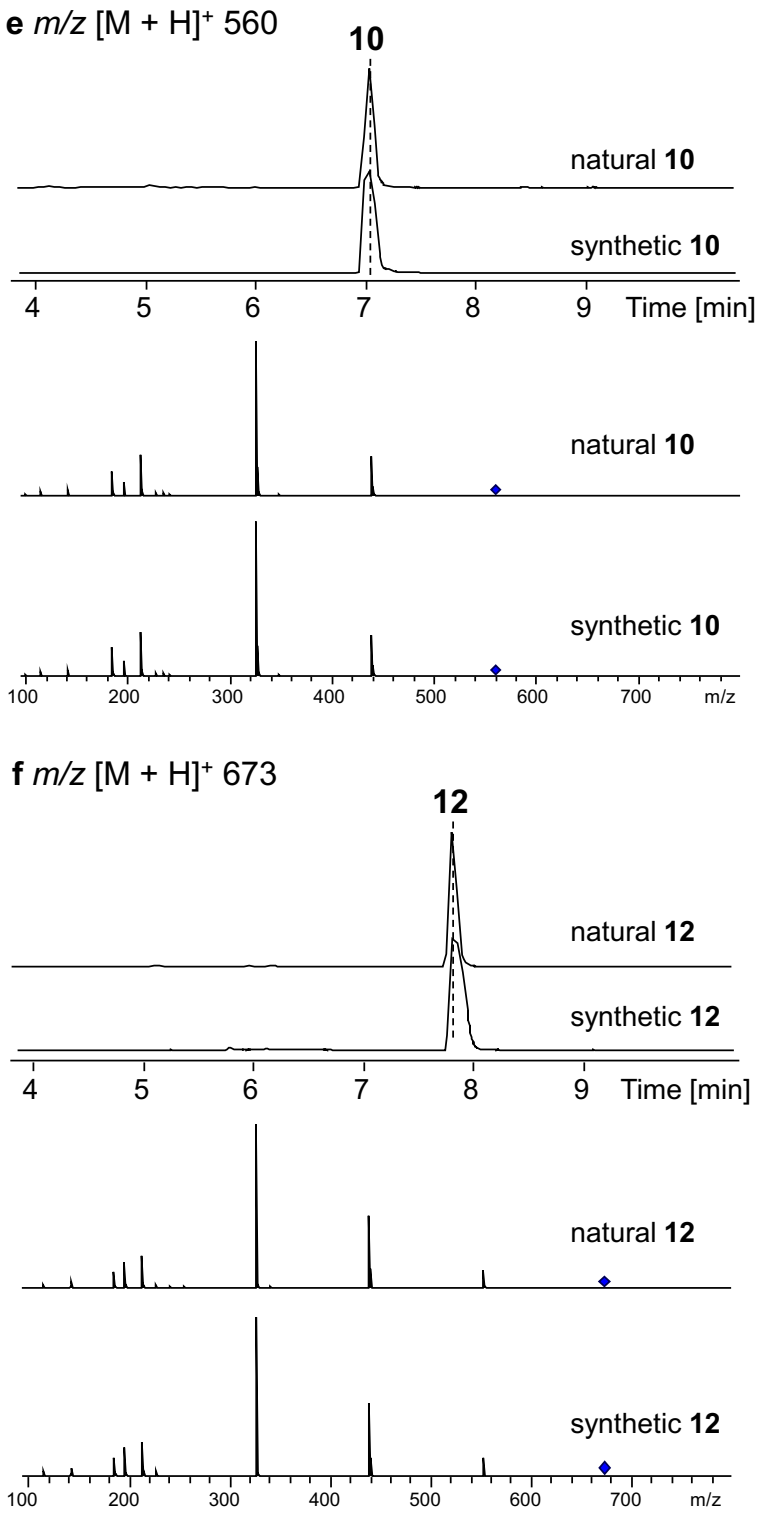


Figure S3. Extracted ion chromatograms (EICs) and the corresponding MS^2 fragmentation patterns of natural and synthetic **1**–**7**, **10** and **12** by HPLC-HR-MS/MS analysis. (**a**) *m/z* $[M + H]^+$ 592 for compounds **1** and **2** (EIC from HPLC-ESI-MS analysis). (**b**) *m/z* $[M + H]^+$ 592 for compound **3**; (**c**) *m/z* $[M + H]^+$ 691 for compound **4**; (**d**) *m/z* $[M + H]^+$ 705 for compounds **5**–**7**; (**e**) *m/z* $[M + H]^+$ 560 for compound **10**; (**f**) *m/z* $[M + H]^+$ 673 for compound **12**.

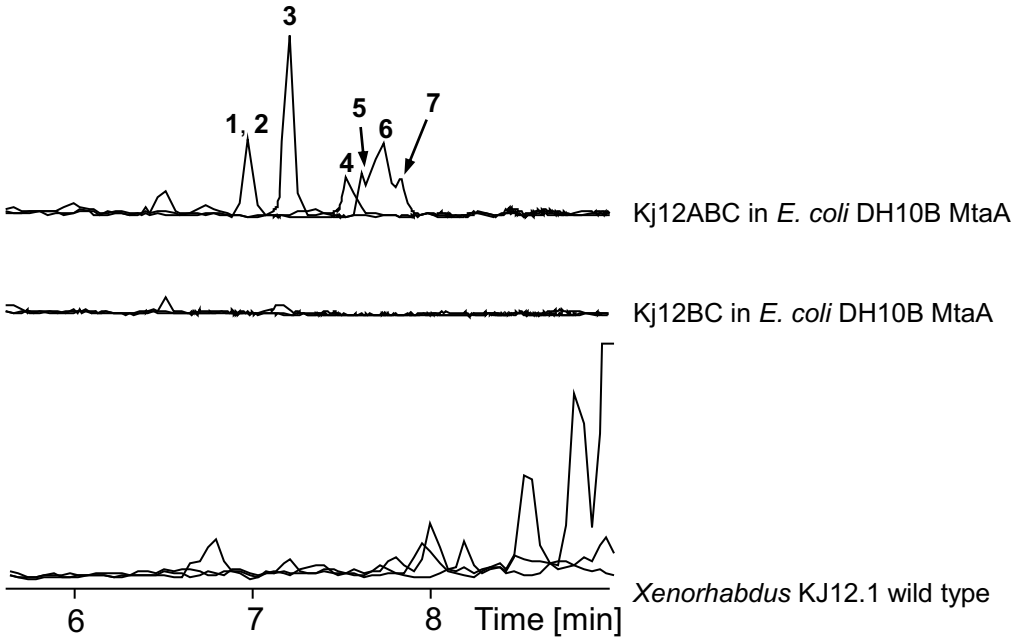


Figure S4. Extracted ion chromatograms (EICs) for methionine-containing rhabdopeptide/xenortide-like peptides (**1**–**7**) produced from the expression of Kj12ABC and Kj12BC in *E. coli* DH10B MtaA and wild type strains by HPLC-HR-MS analysis.

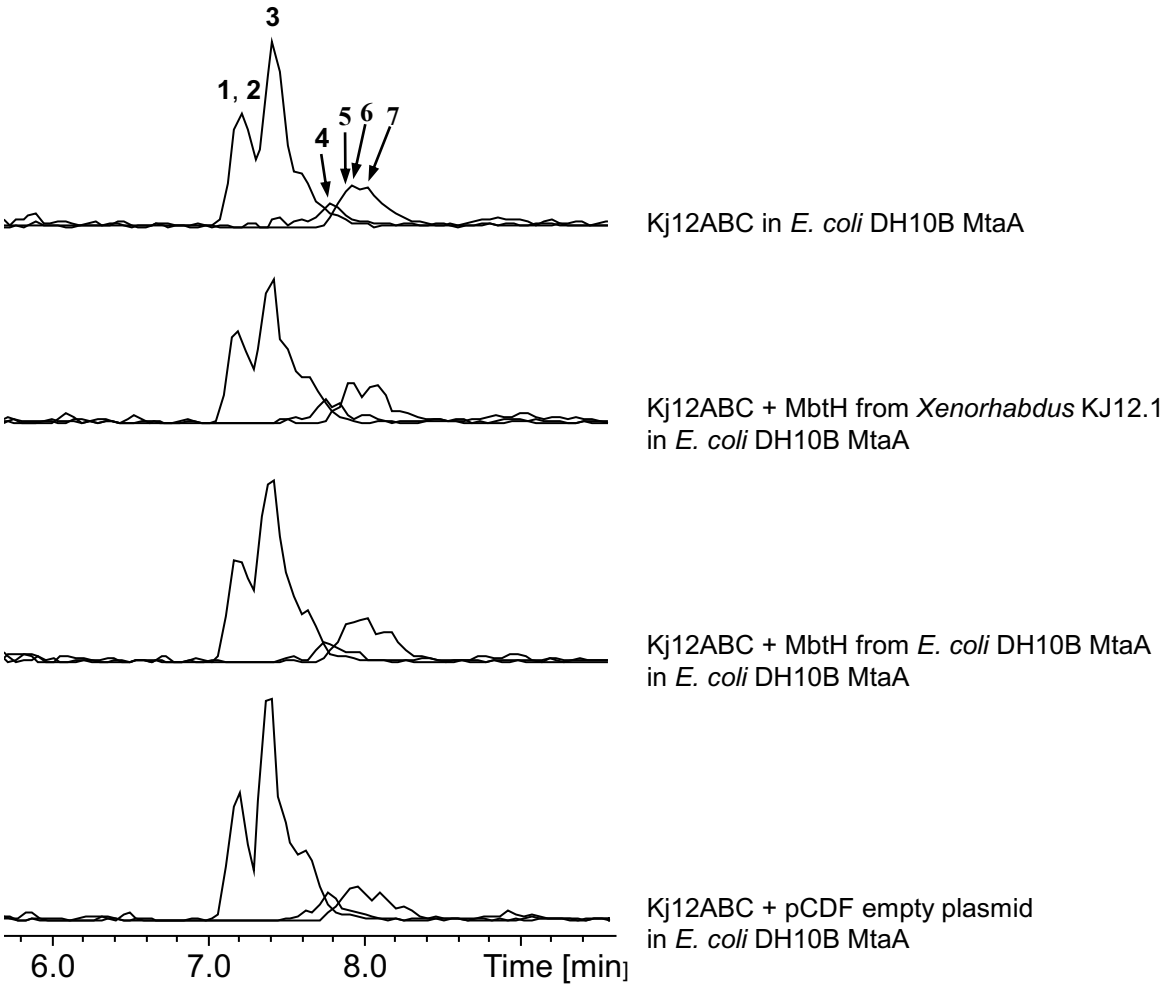


Figure S5. Extracted ion chromatograms (EICs) for methionine-containing rhabdopeptide/xenortide-like peptides (**1**–**7**) produced from the overexpression of *mbtH* of *Xenorhabdus* KJ12.1 and *E. coli* DH10B MtaA in *E. coli* DH10B MtaA strain by HPLC-ESI-MS analysis. All chromatograms were drawn to the same scale.

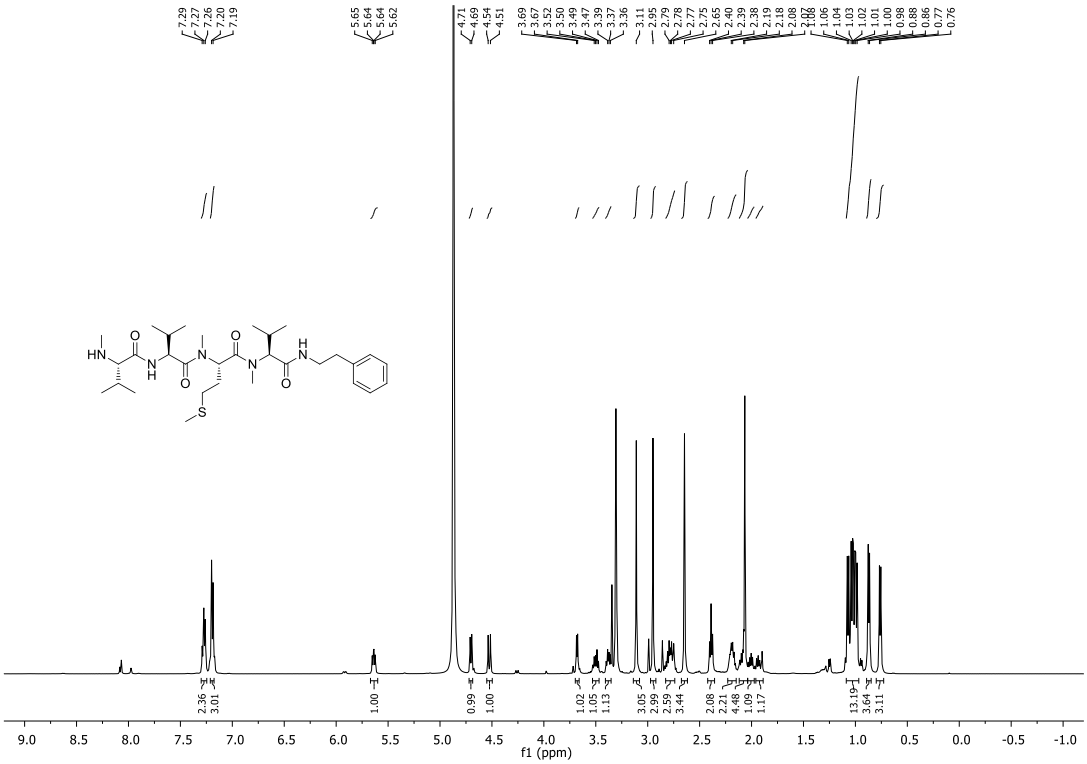


Figure S6. ^{1}H NMR (500 MHz, methanol-d_4) spectrum of **1**

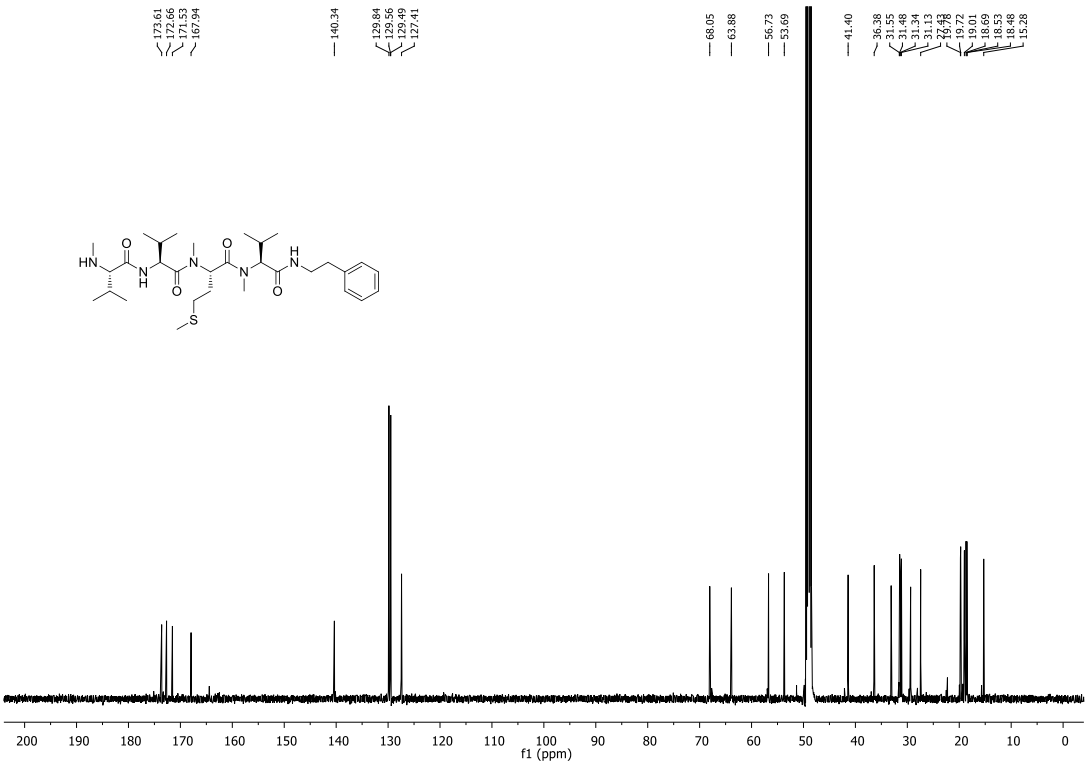


Figure S7. ^{13}C NMR (125 MHz, methanol-d_4) spectrum of **1**

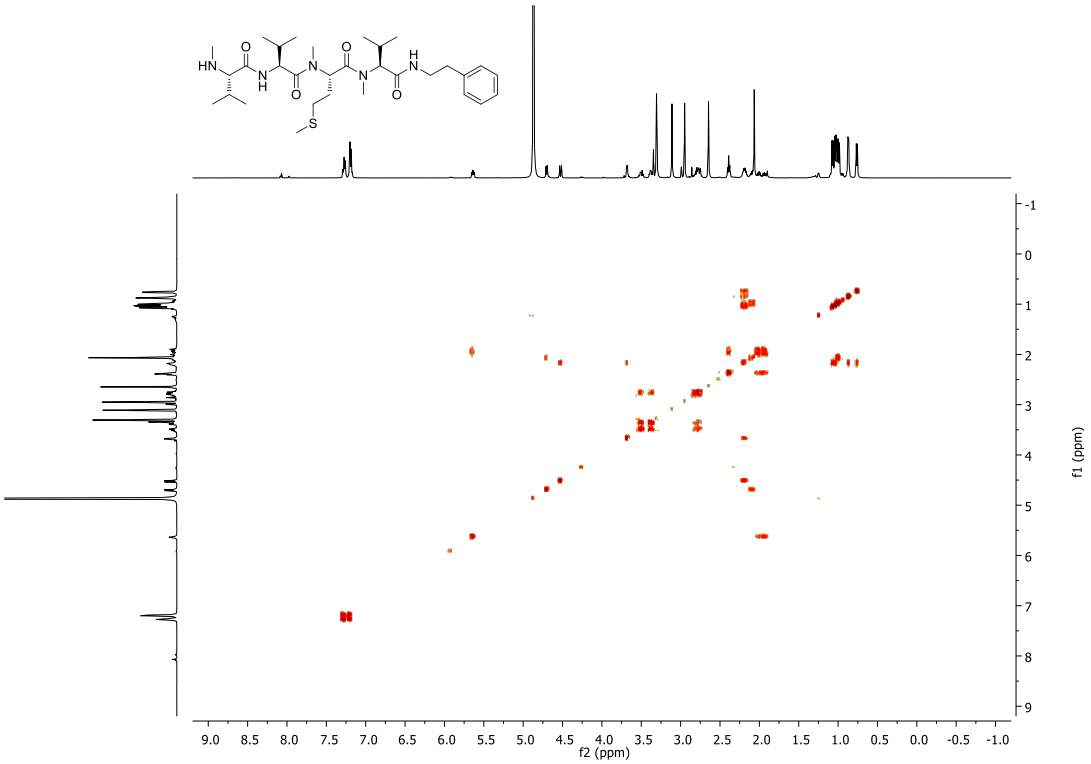


Figure S8. COSY (methanol-d_4) spectrum of **1**

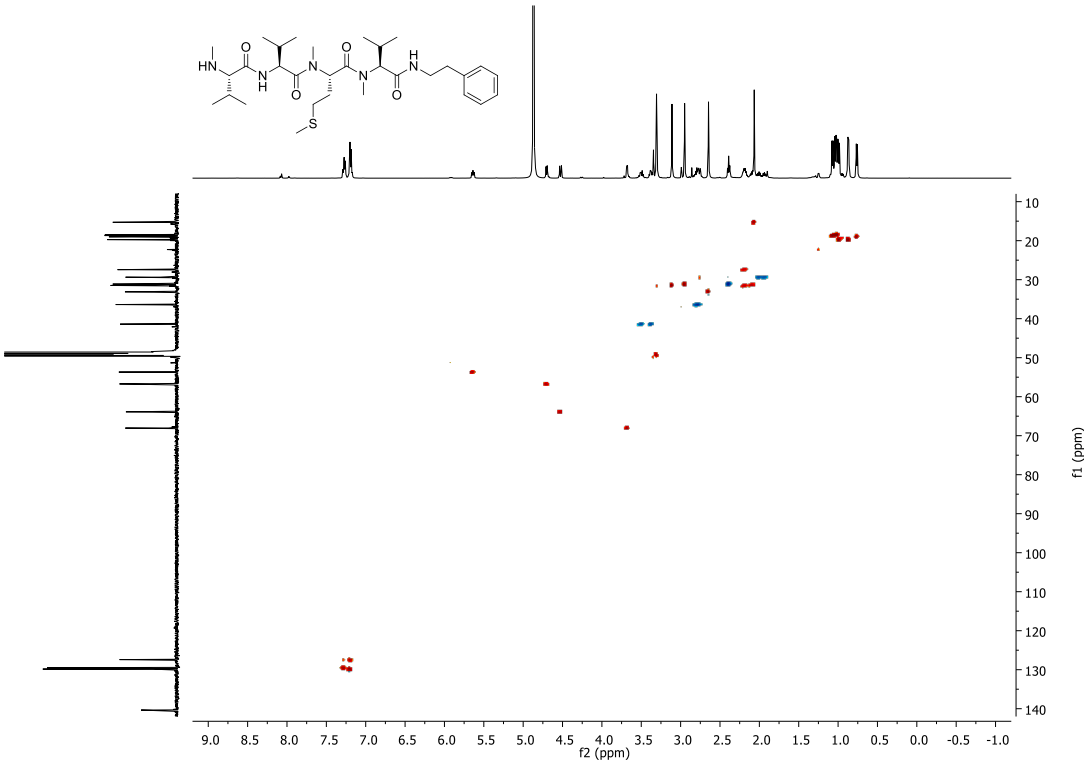


Figure S9. HSQC (methanol-d_4) spectrum of **1**

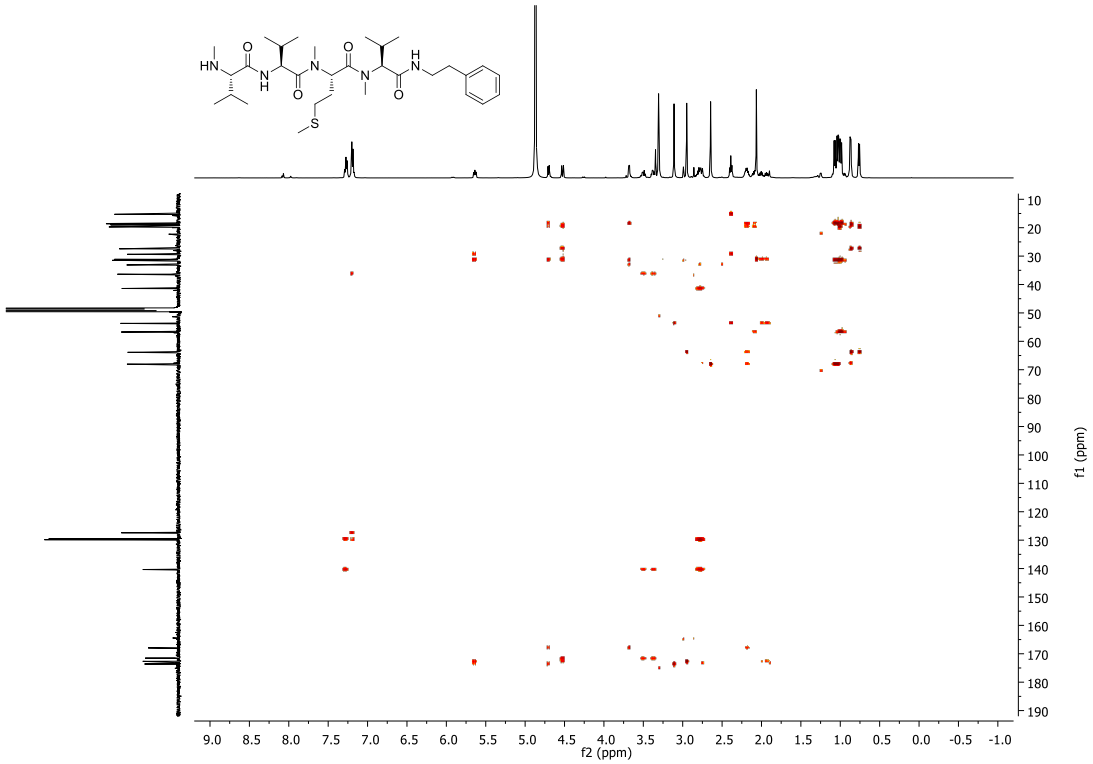


Figure S10. HMBC (methanol-d_4) spectrum of **1**

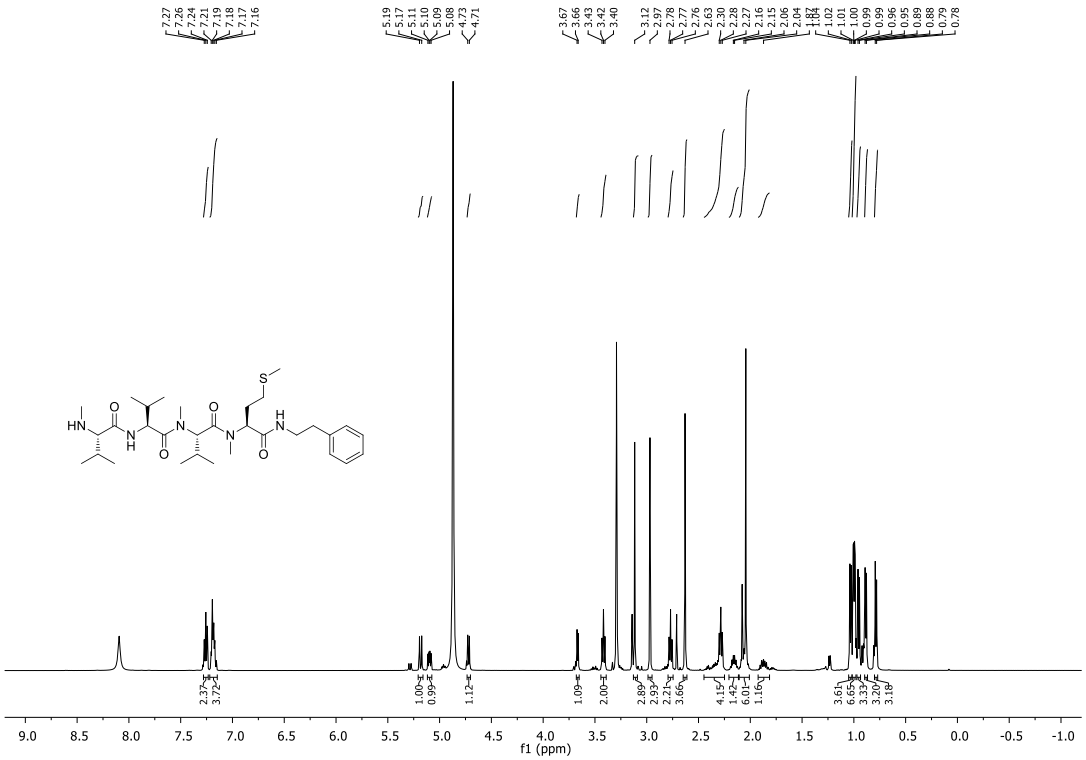


Figure S11. ^{1}H NMR (500 MHz, methanol-d_4) spectrum of **2**

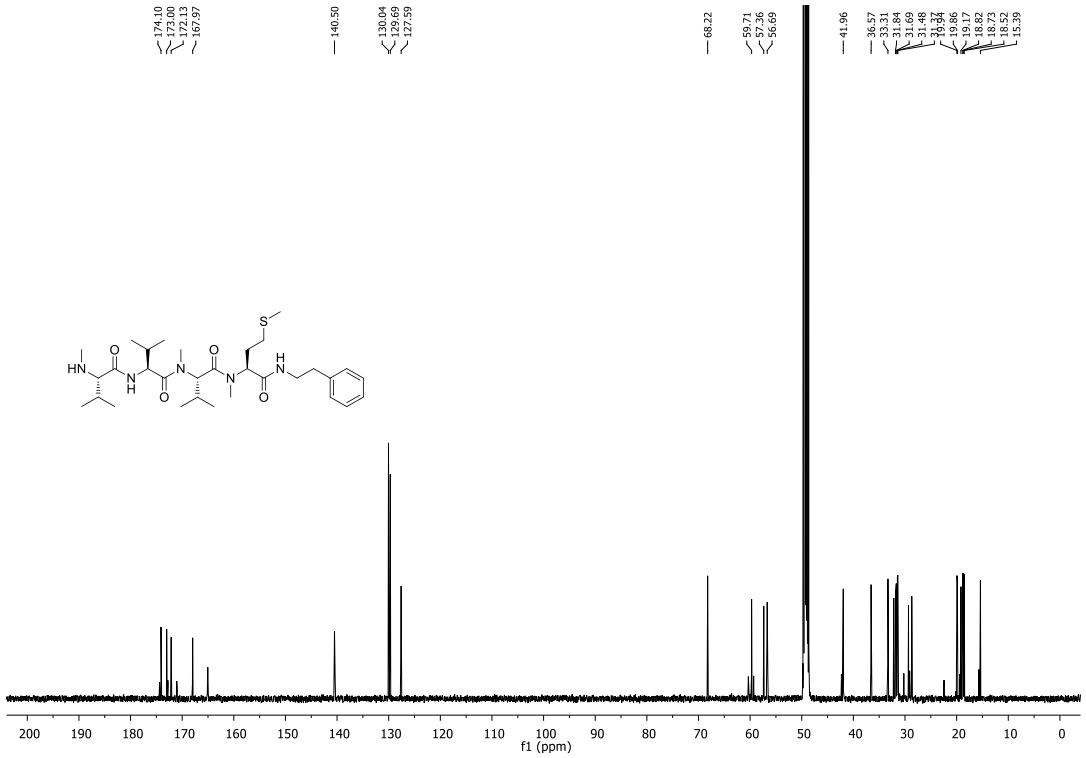


Figure S12. ^{13}C NMR (500 MHz, methanol-d_4) spectrum of **2**

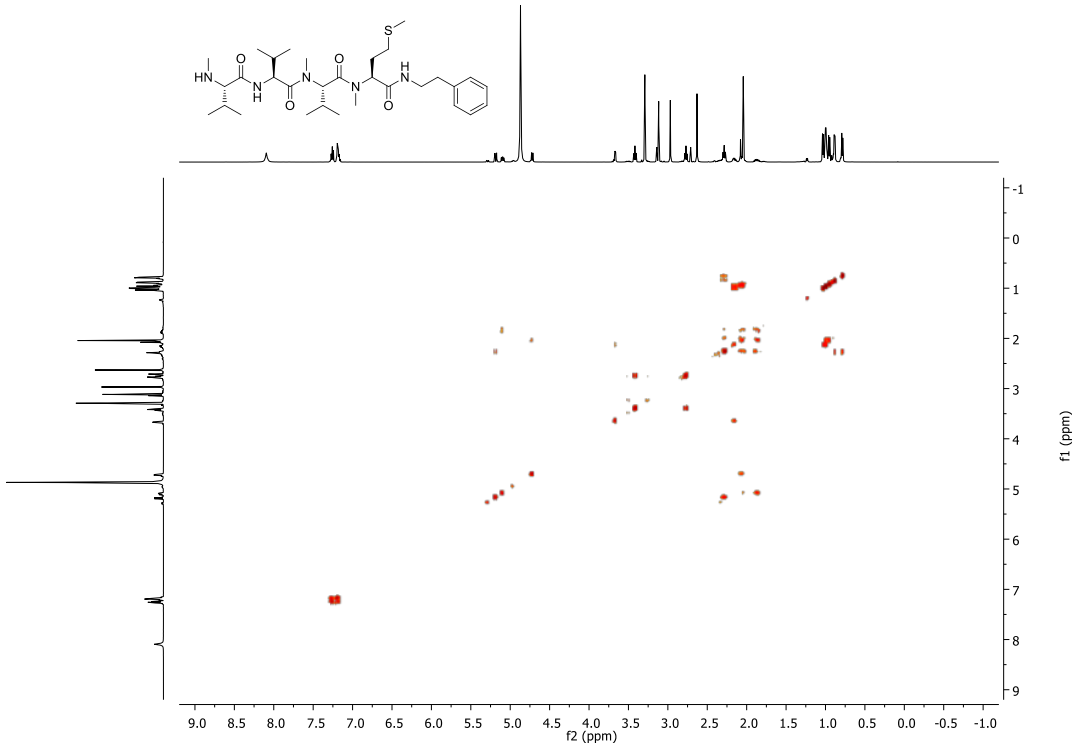


Figure S13. COSY (methanol-d_4) spectrum of **2**

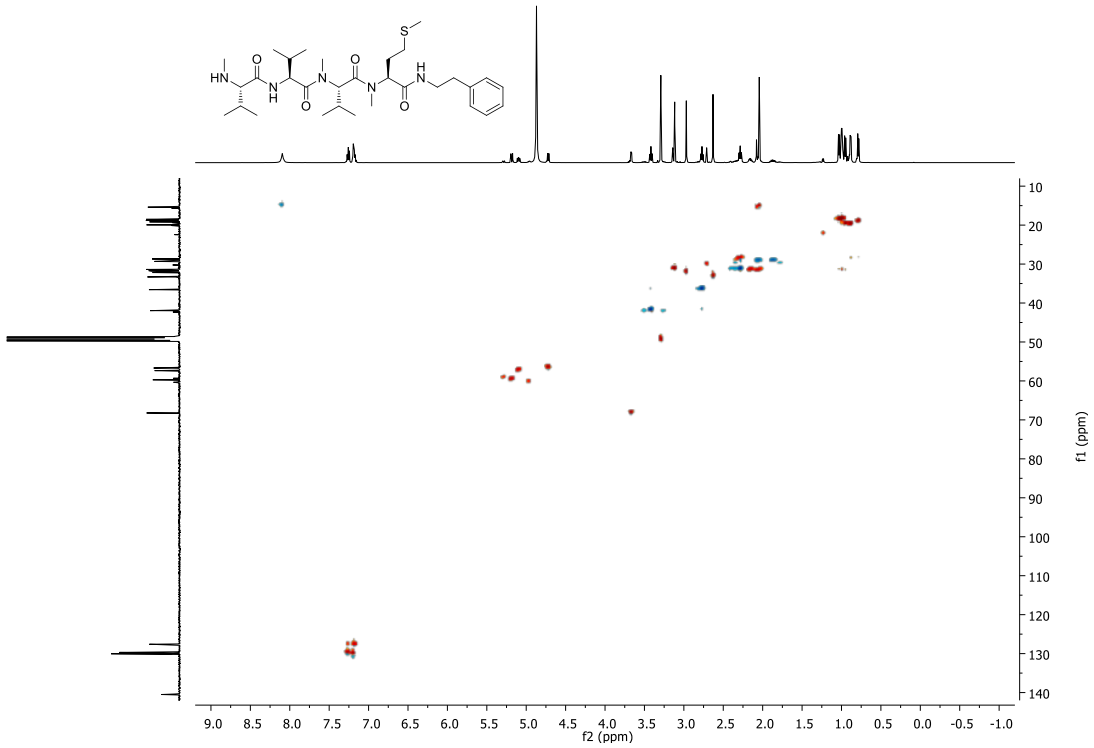


Figure S14. HSQC (methanol-d_4) spectrum of **2**

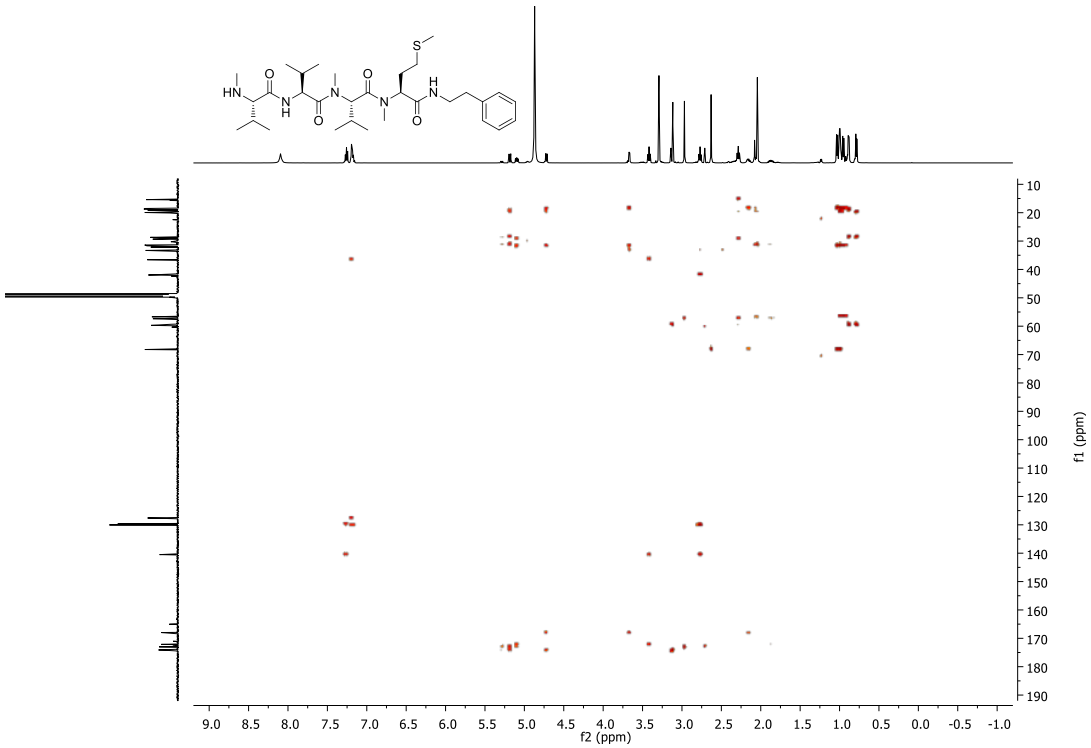


Figure S15. HMBC (methanol-d_4) spectrum of **2**

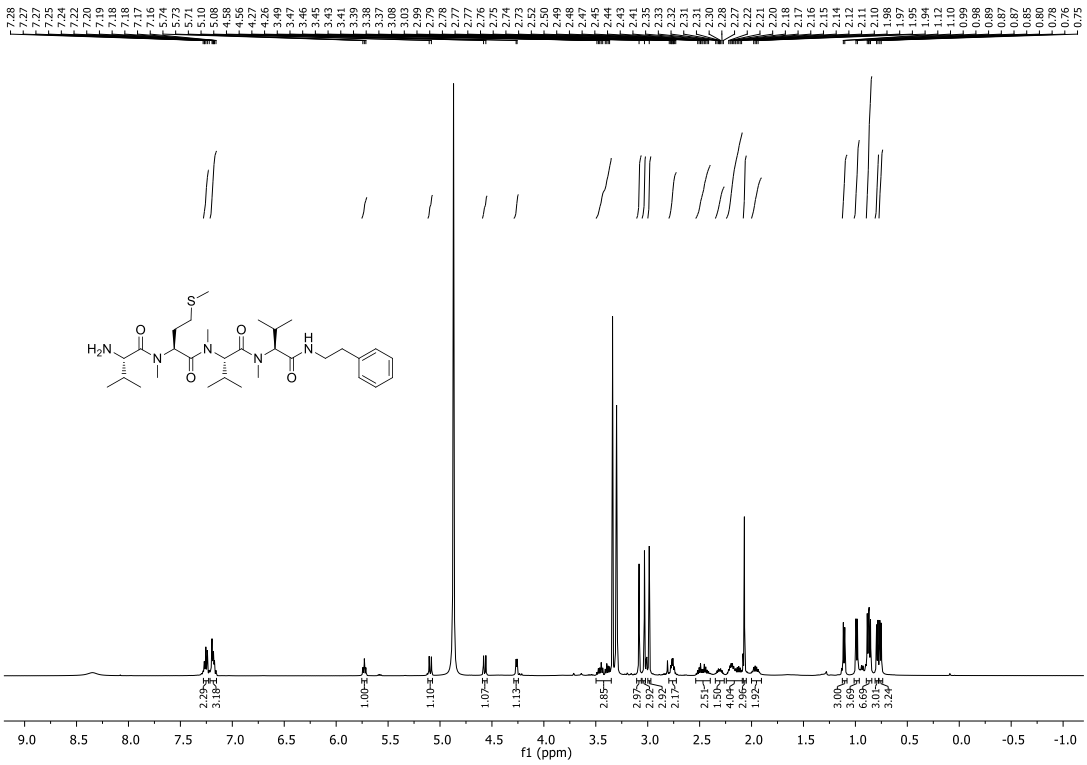


Figure S16. ^{1}H NMR (500 MHz, methanol-d_4) spectrum of **3**

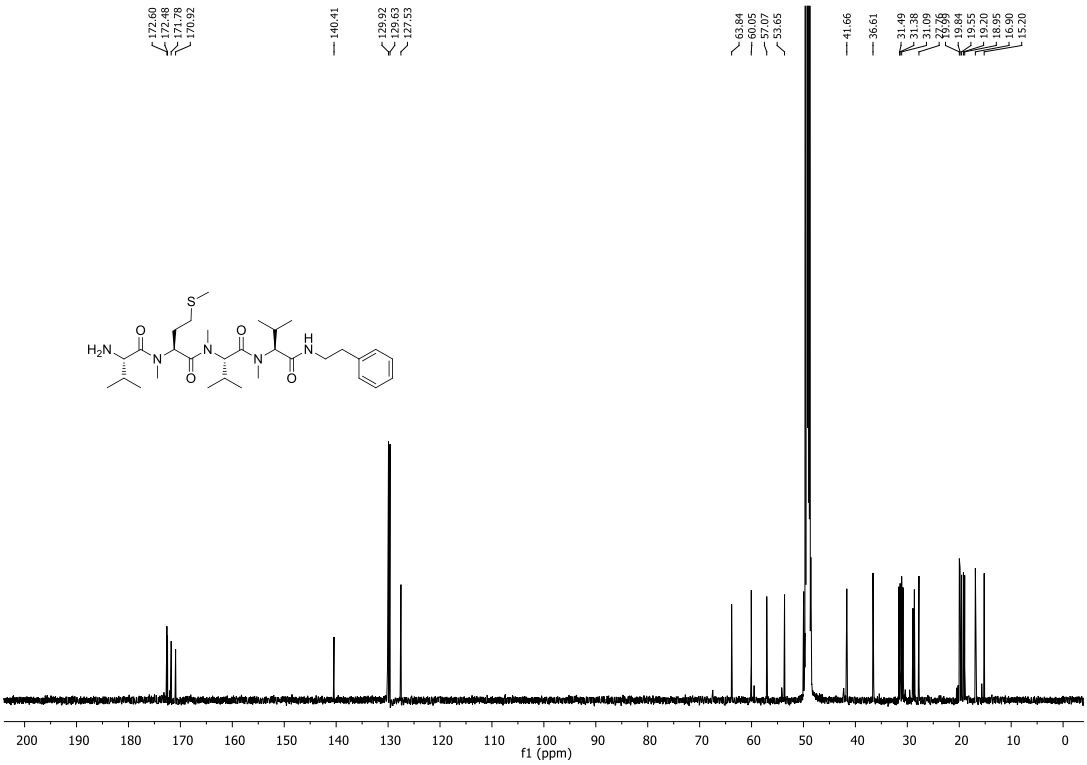


Figure S17. ^{13}C NMR (125 MHz, methanol-d_4) spectrum of **3**

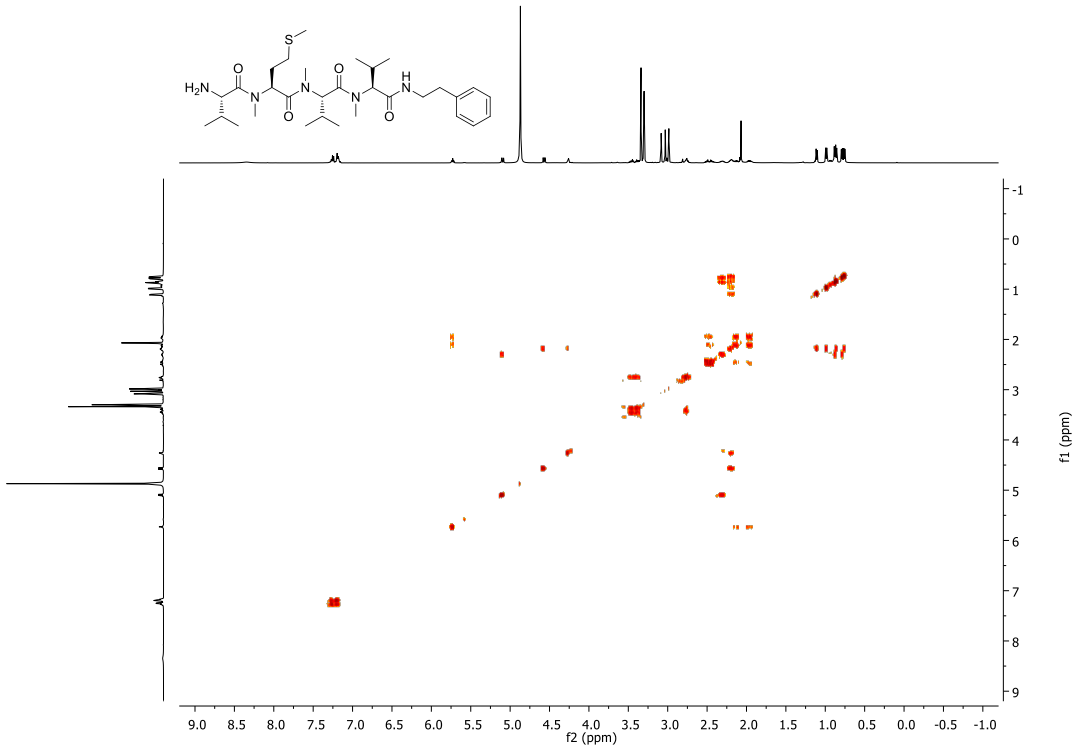


Figure S18. COSY (methanol-d_4) spectrum of **3**

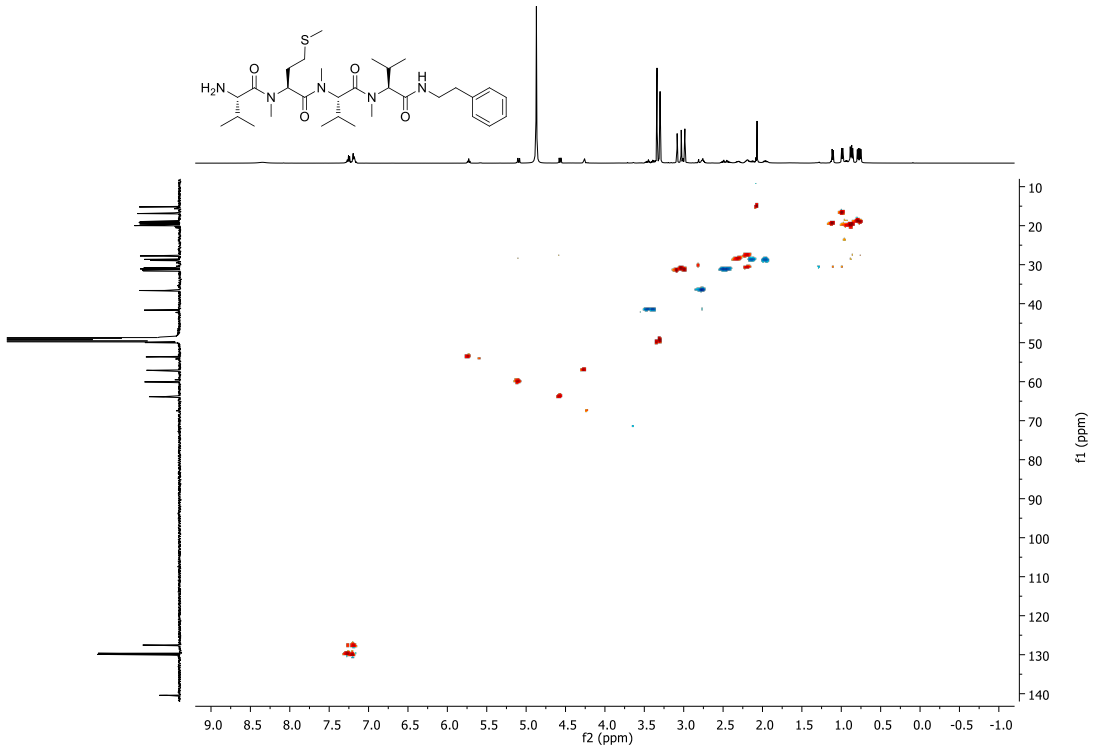


Figure S19. HSQC (methanol-d_4) spectrum of **3**

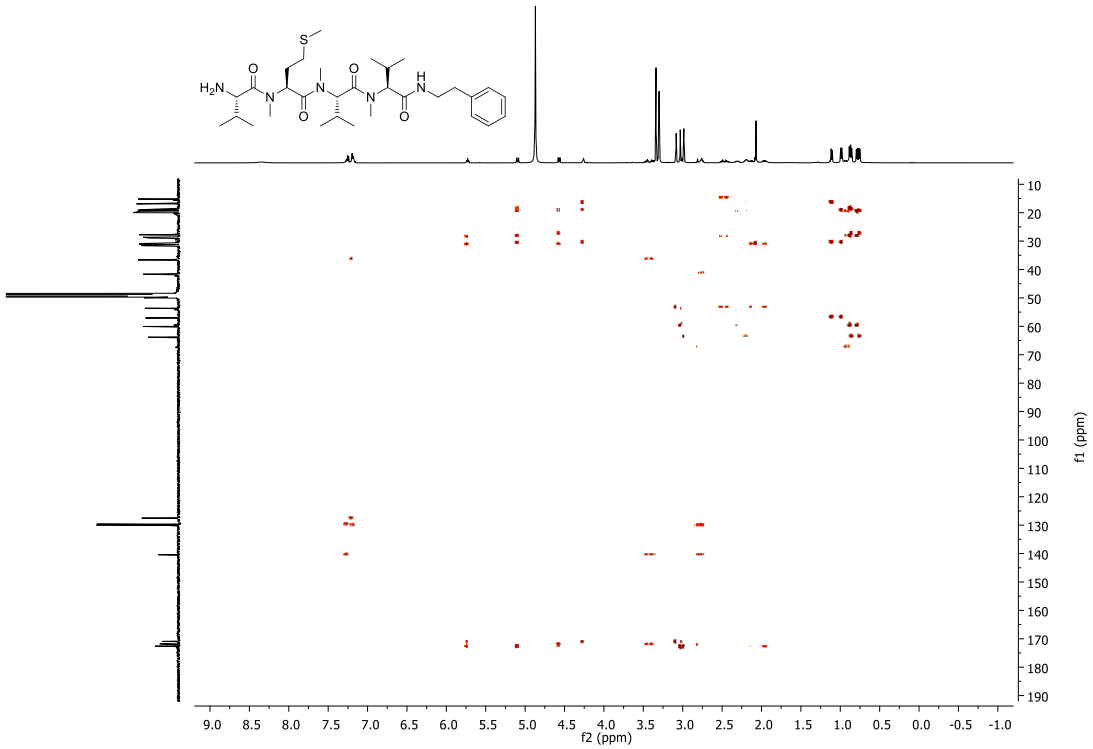


Figure S20. HMBC (methanol-d_4) spectrum of **3**

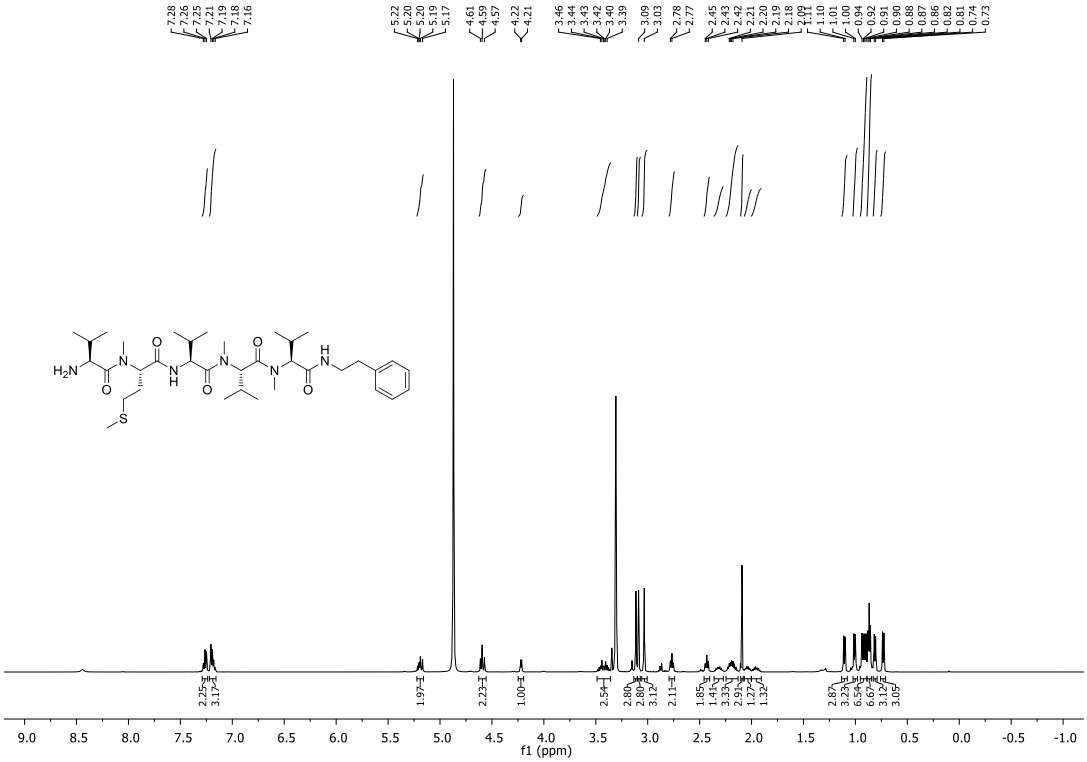


Figure S21. ^{1}H NMR (500 MHz, methanol-d_4) spectrum of **4**

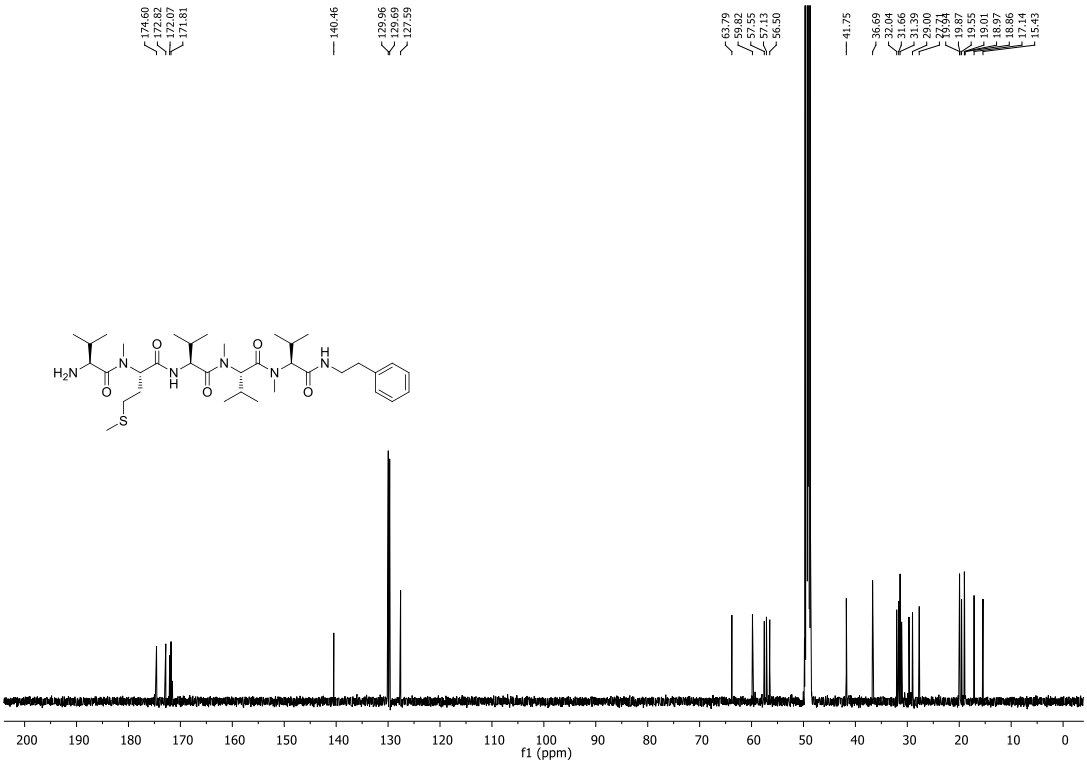


Figure S22. ^{13}C NMR (125 MHz, methanol-d_4) spectrum of **4**

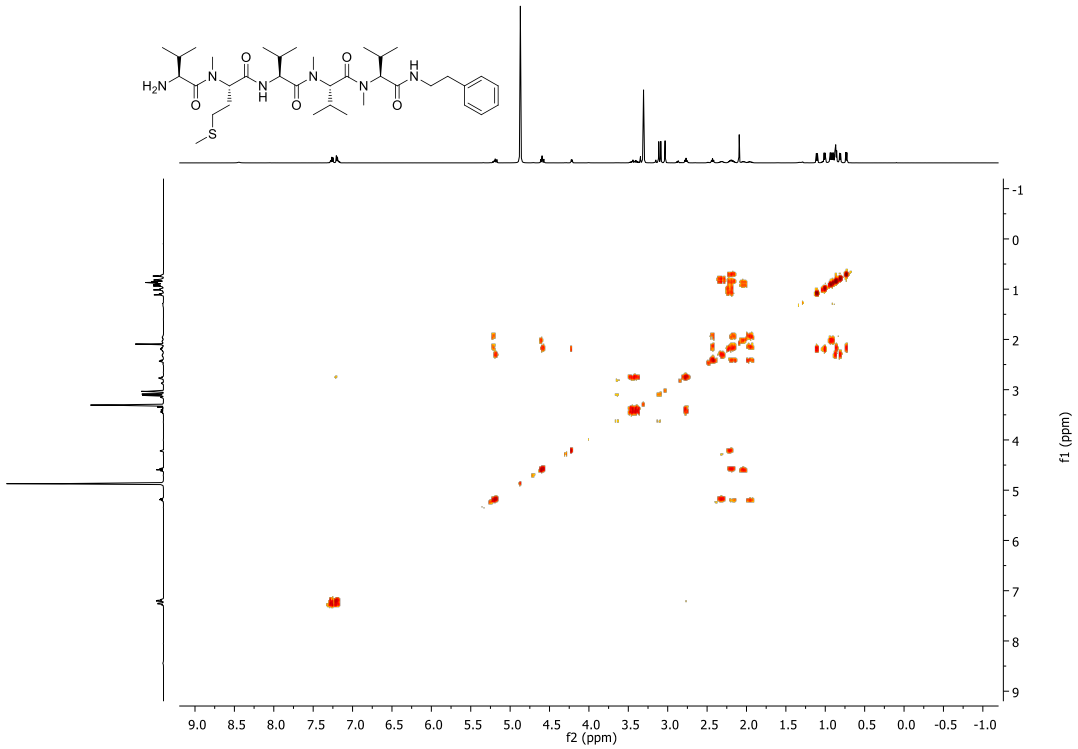


Figure S23. COSY (methanol-d_4) spectrum of **4**

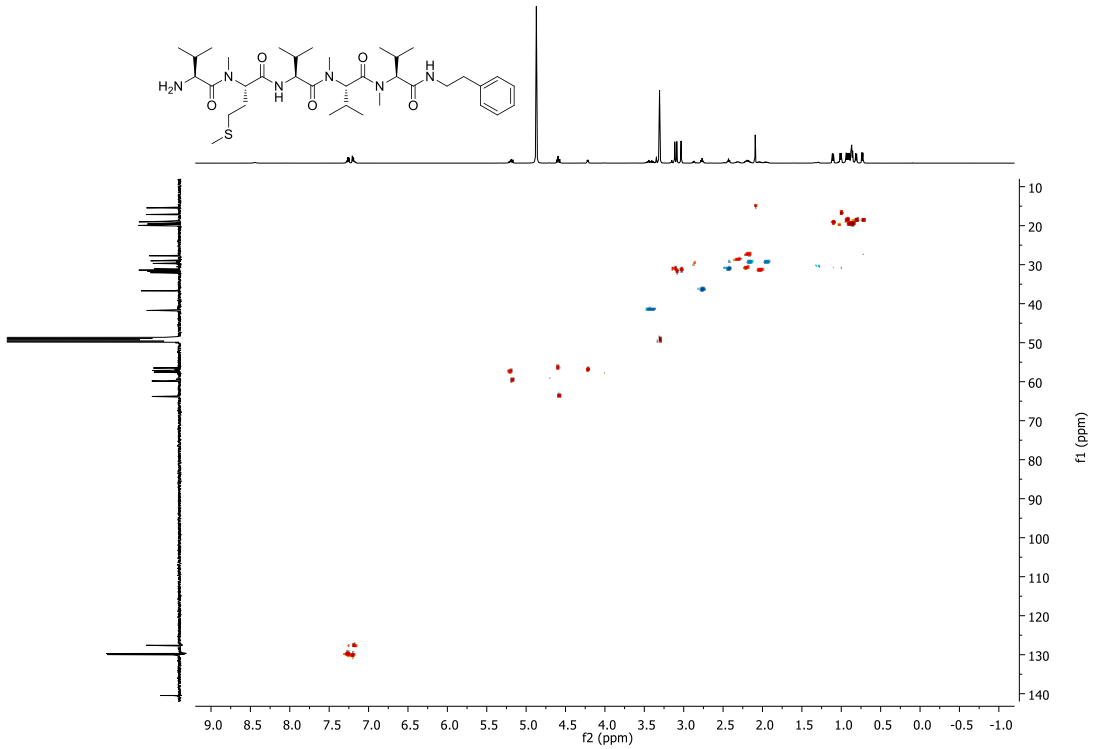


Figure S24. HSQC (methanol-d_4) spectrum of **4**

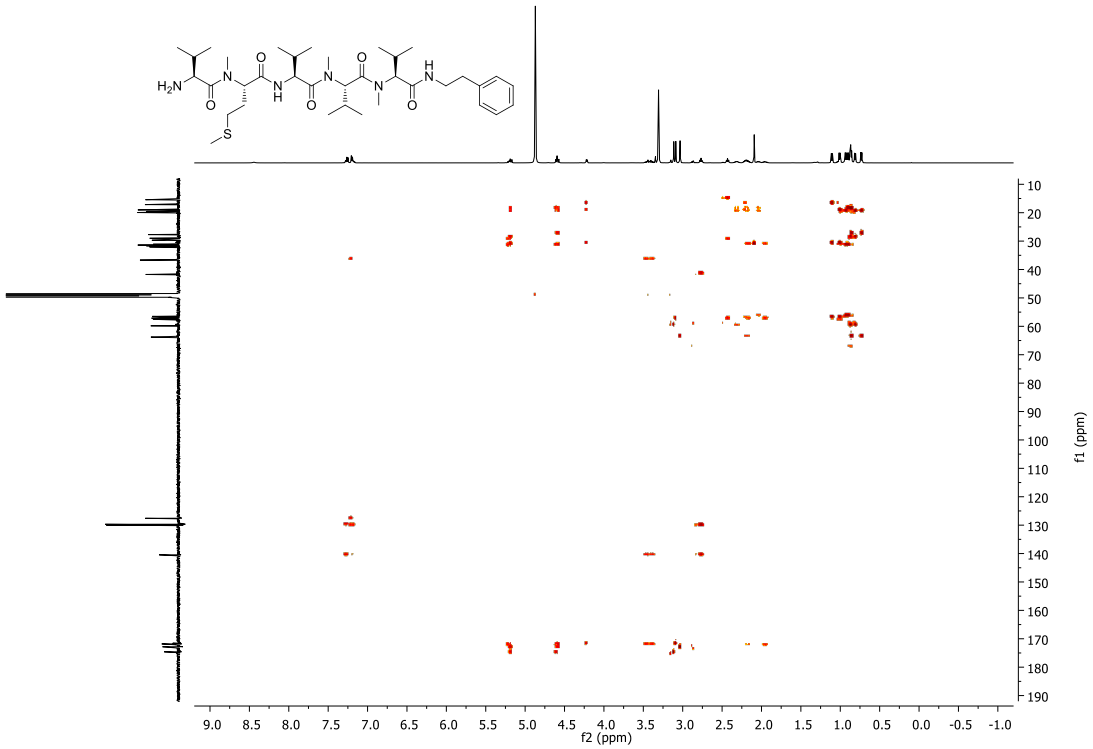


Figure S25. HMBC (methanol-d_4) spectrum of **4**

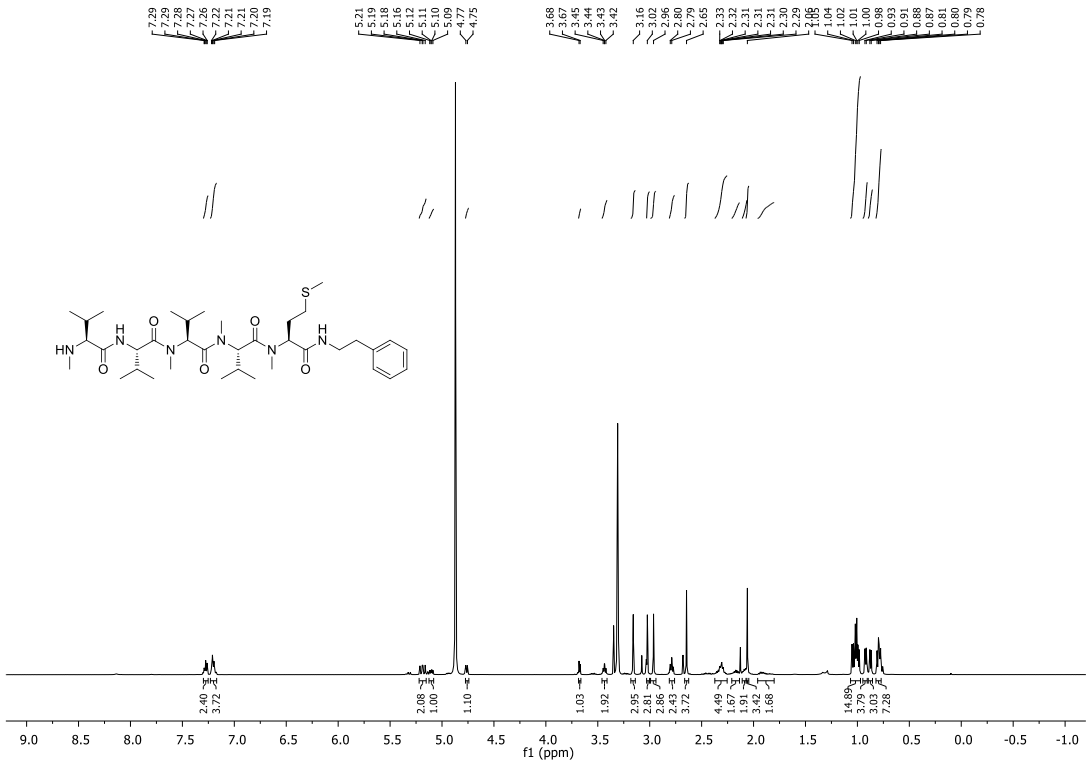


Figure S26. ^{1}H NMR (500 MHz, methanol-d_4) spectrum of **5**

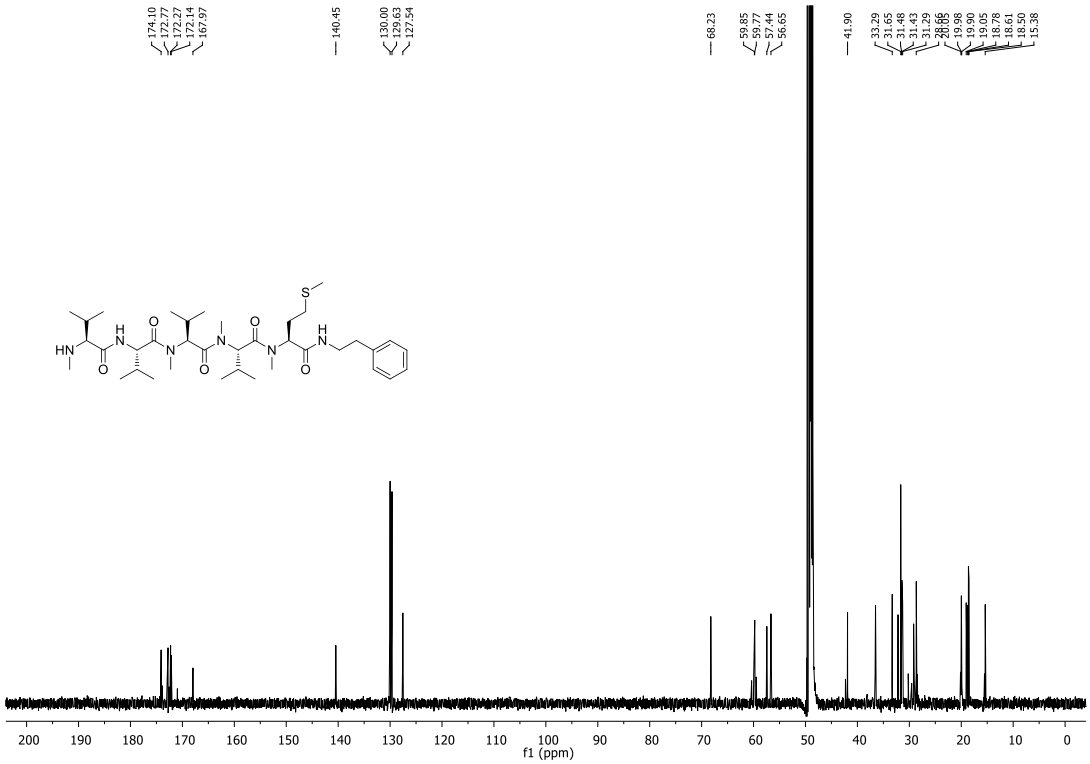


Figure S27. ^{13}C NMR (125 MHz, methanol-d_4) spectrum of **5**

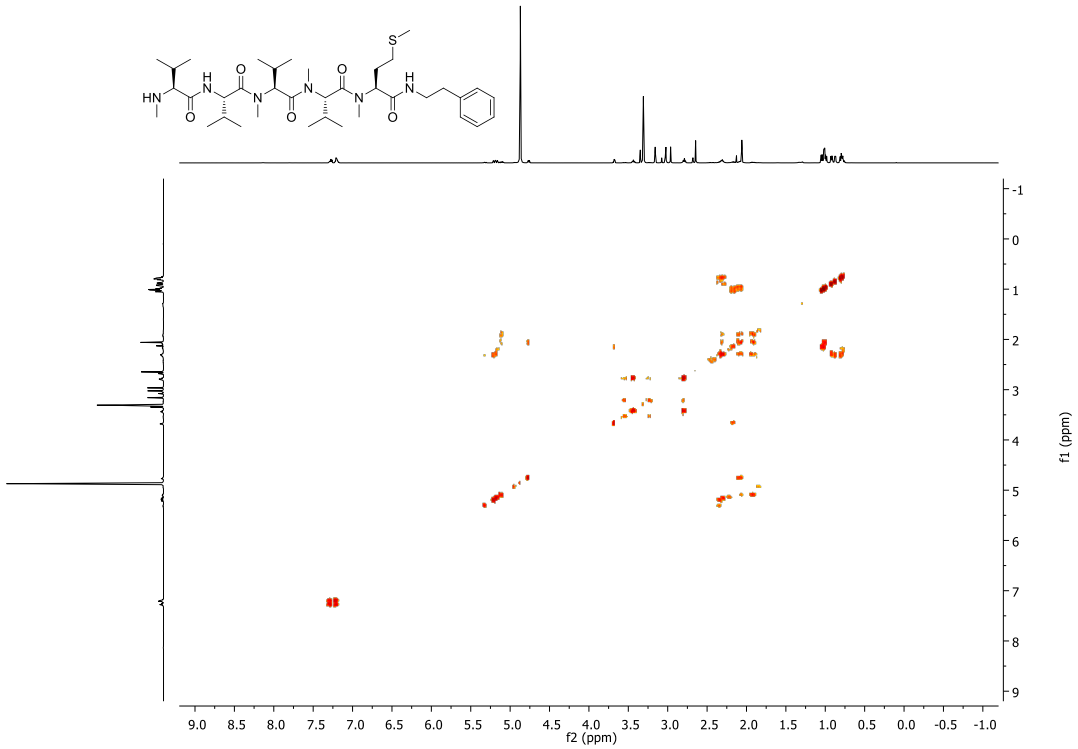


Figure S28. COSY (methanol-d_4) spectrum of **5**

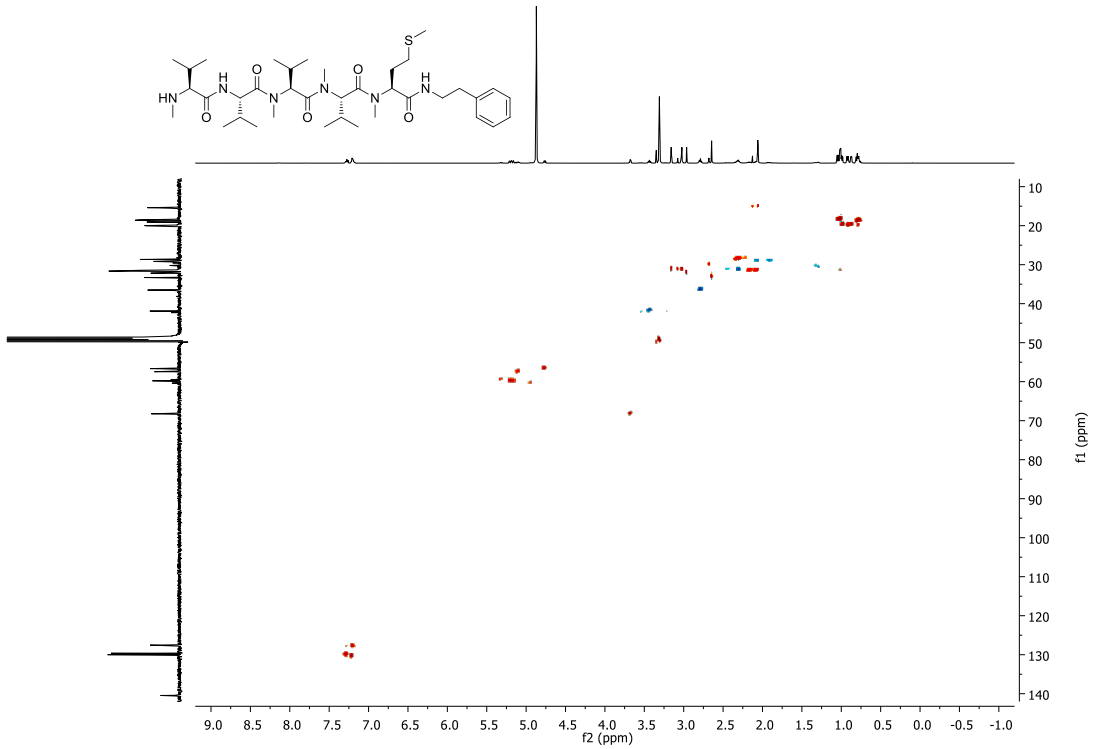


Figure S29. HSQC (methanol-d_4) spectrum of **5**

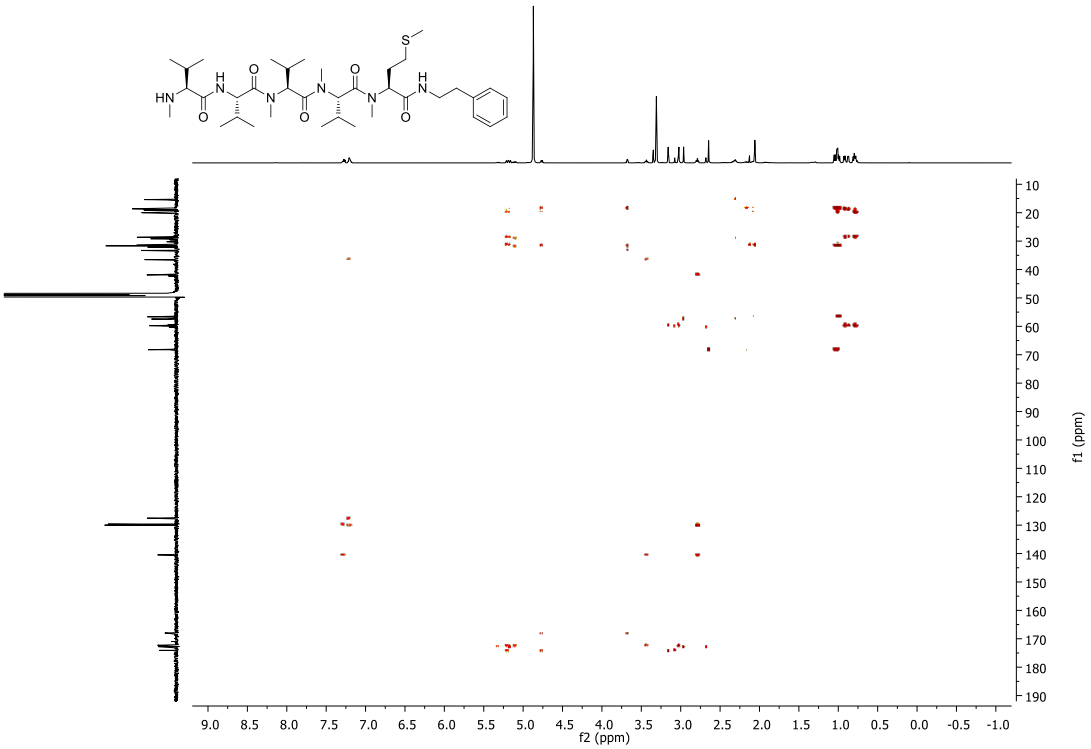


Figure S30. HMBC (methanol-d_4) spectrum of **5**

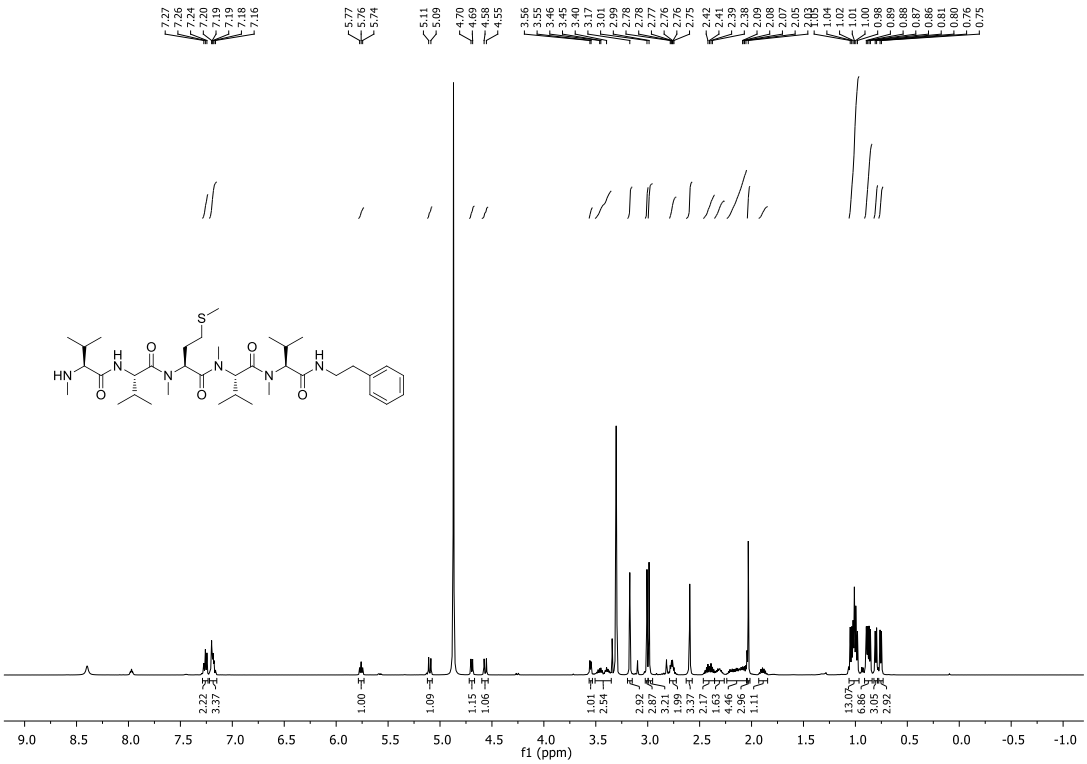


Figure S31. ^{1}H NMR (500 MHz, methanol-d_4) spectrum of **6**

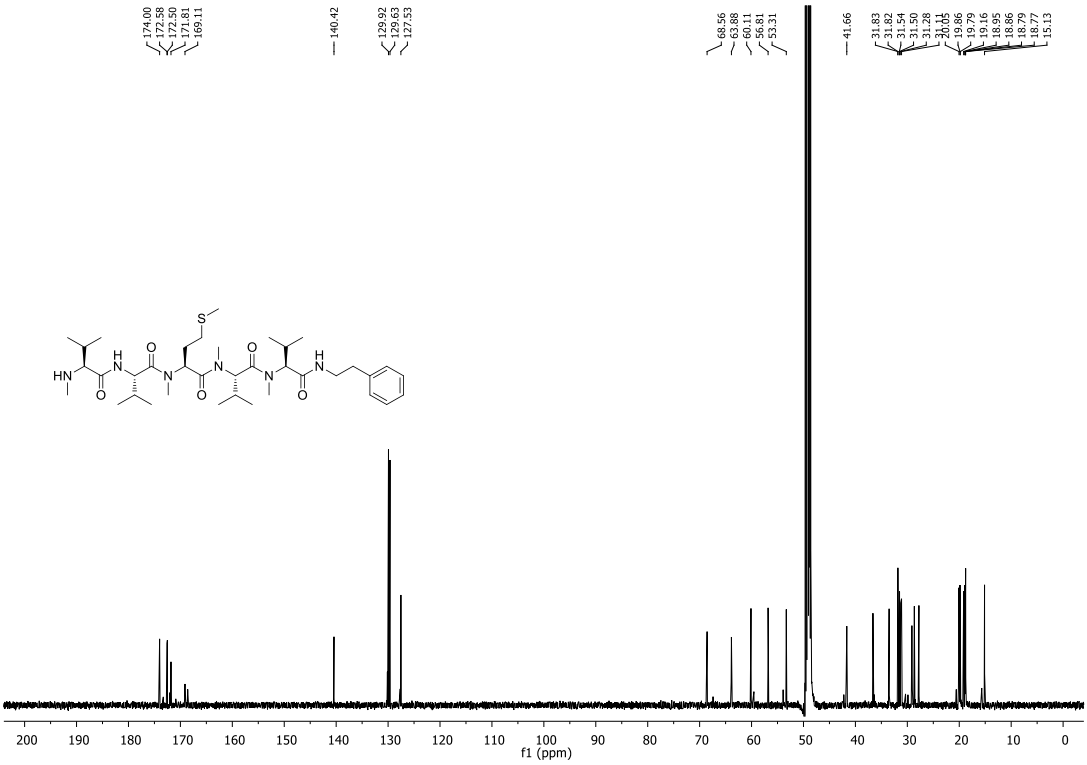


Figure S32. ^{13}C NMR (125 MHz, methanol-d_4) spectrum of **6**

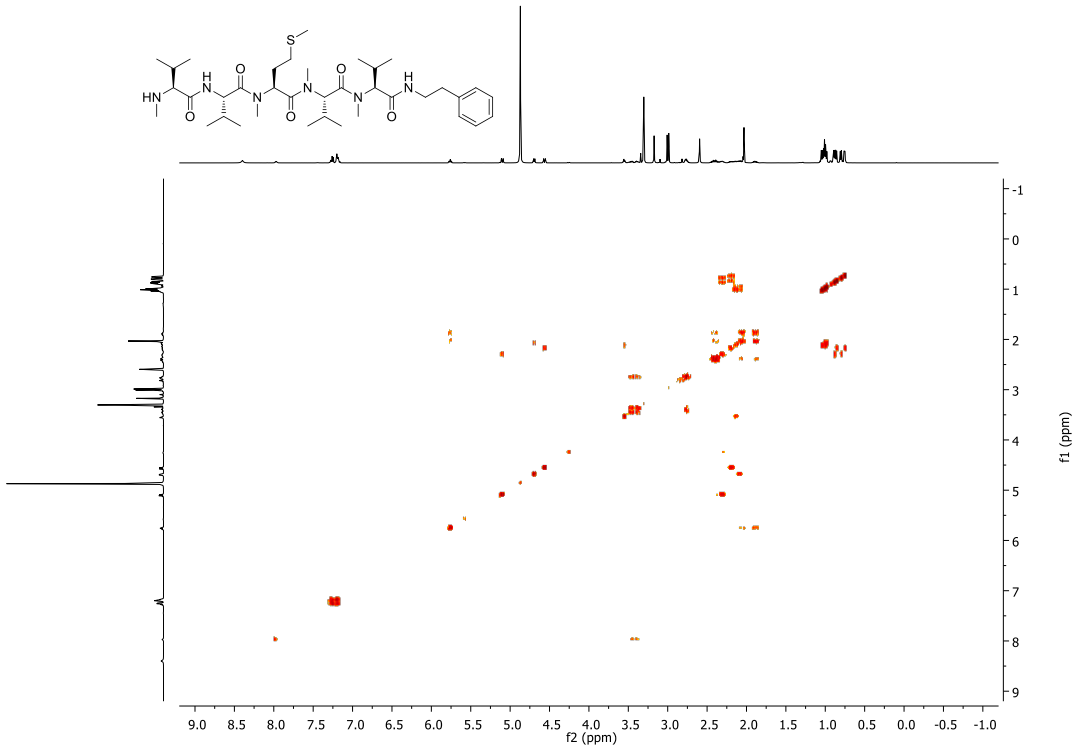


Figure S33. COSY (methanol-d_4) spectrum of **6**

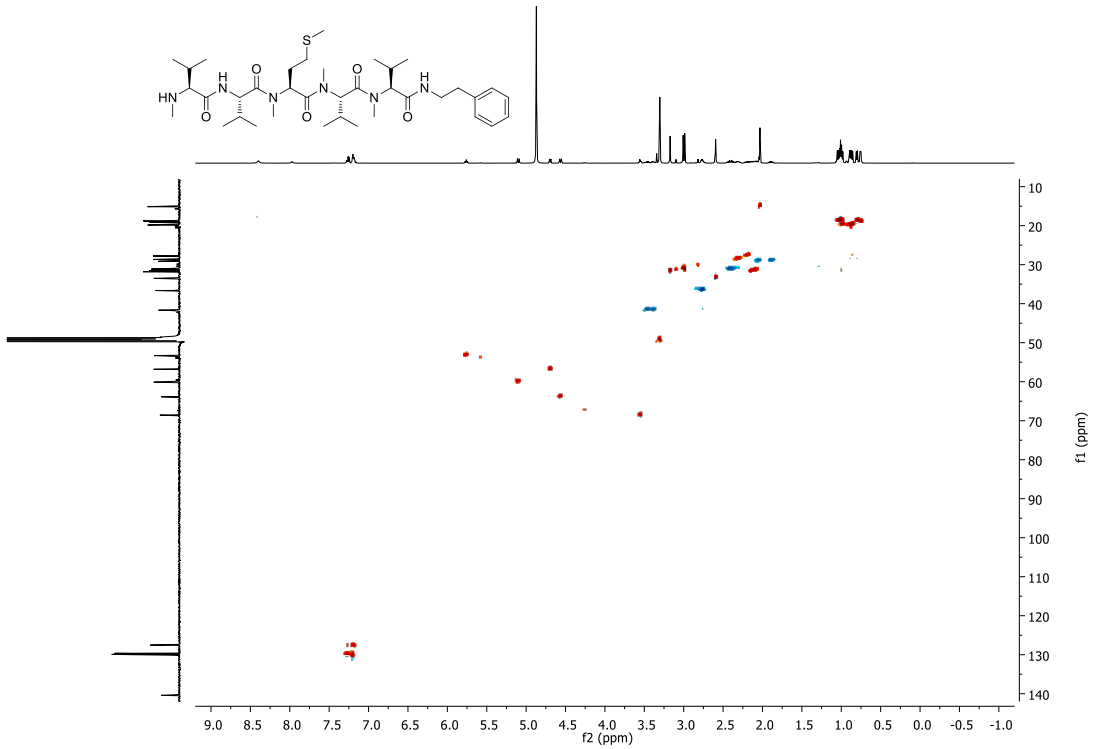


Figure S34. HSQC (methanol-d_4) spectrum of **6**

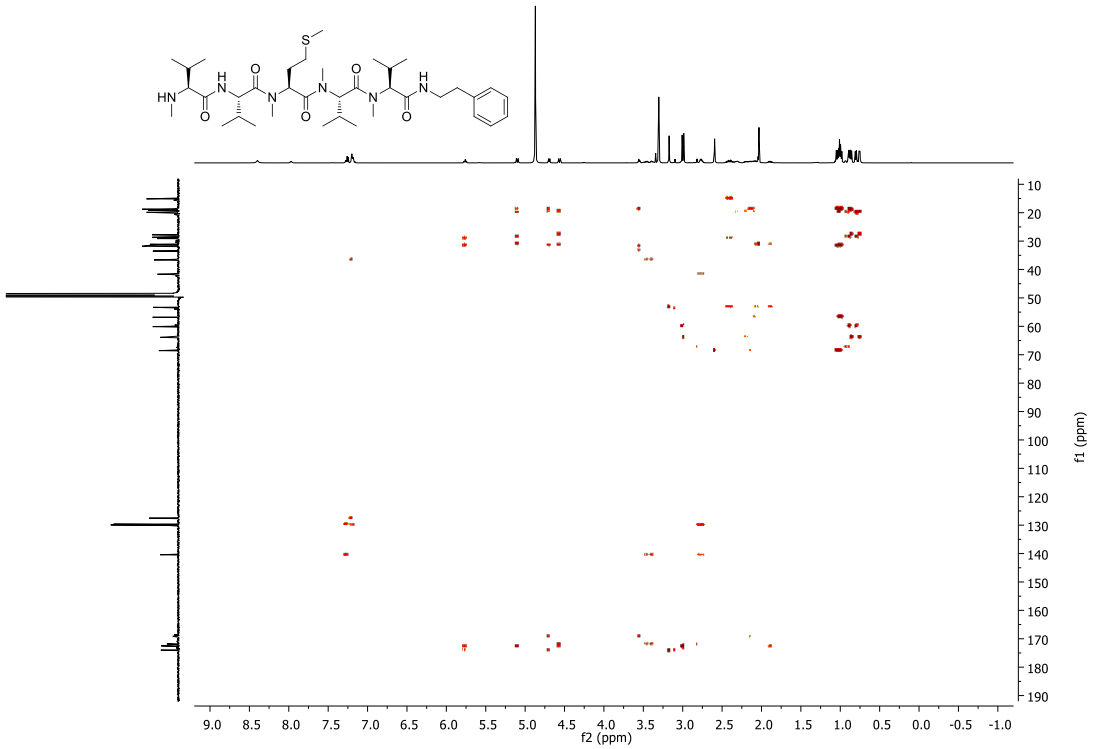


Figure S35. HMBC (methanol-d_4) spectrum of **6**

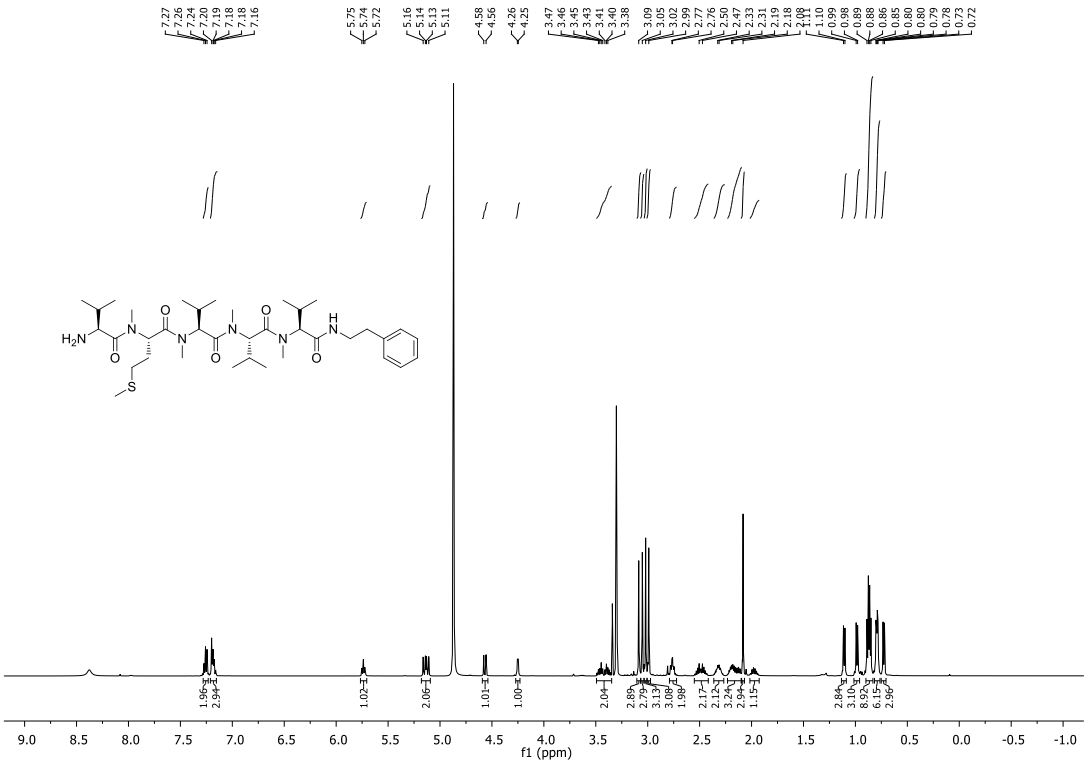


Figure S36. ^{1}H NMR (500 MHz, methanol-d_4) spectrum of **7**

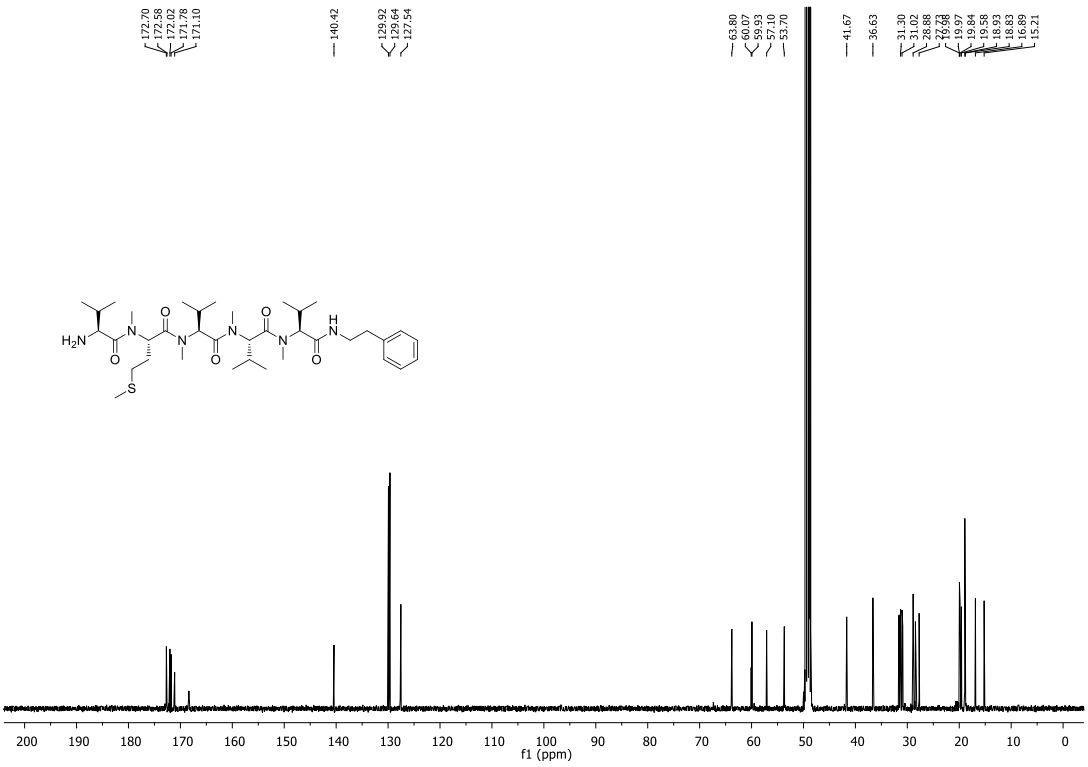


Figure S37. ^{13}C NMR (125 MHz, methanol-d_4) spectrum of **7**

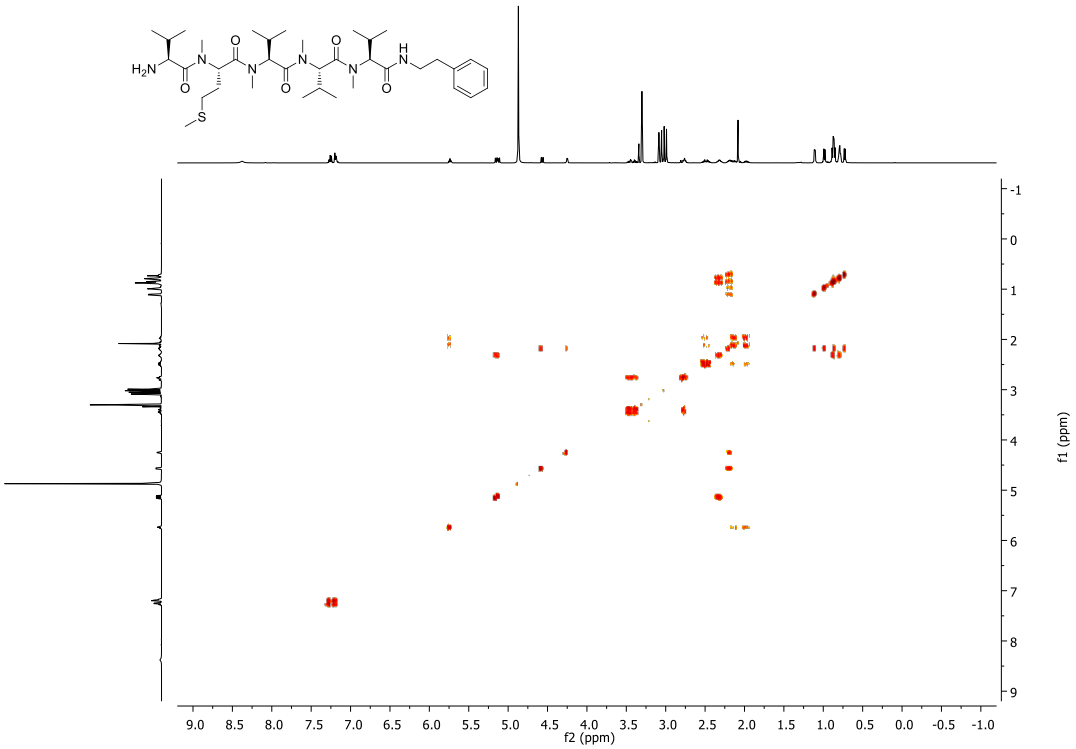


Figure S38. COSY (methanol-d_4) spectrum of **7**

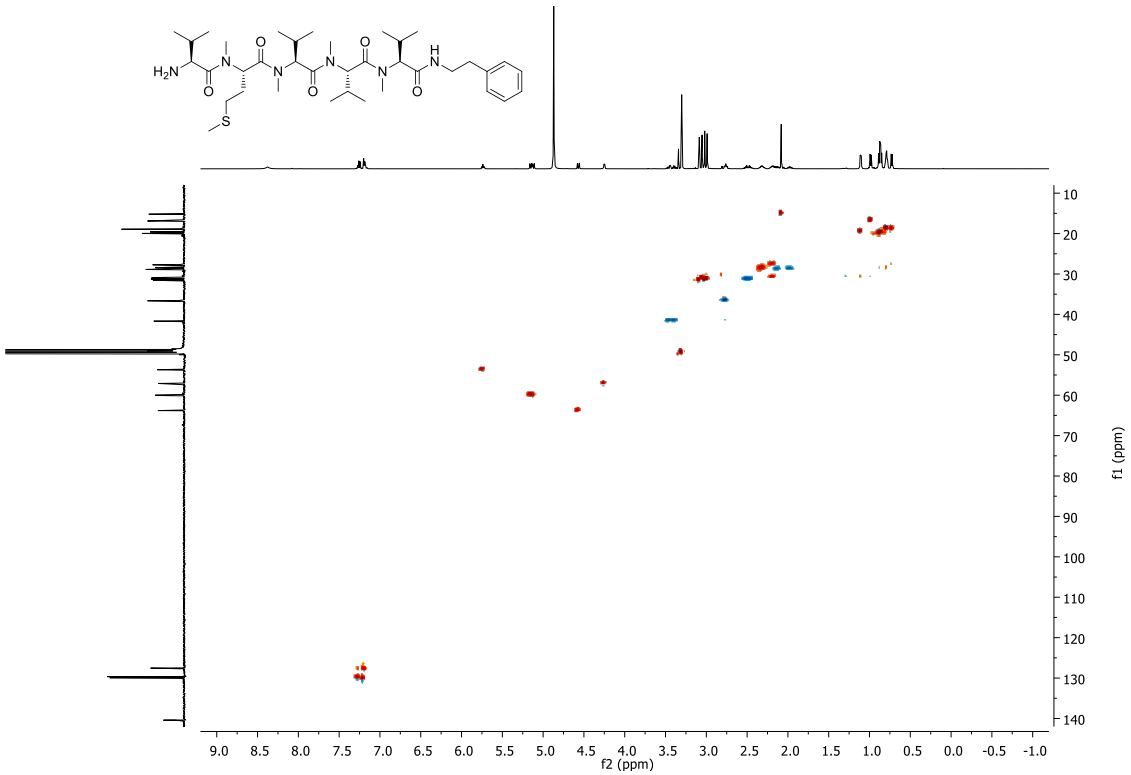


Figure S39. HSQC (methanol-d_4) spectrum of **7**

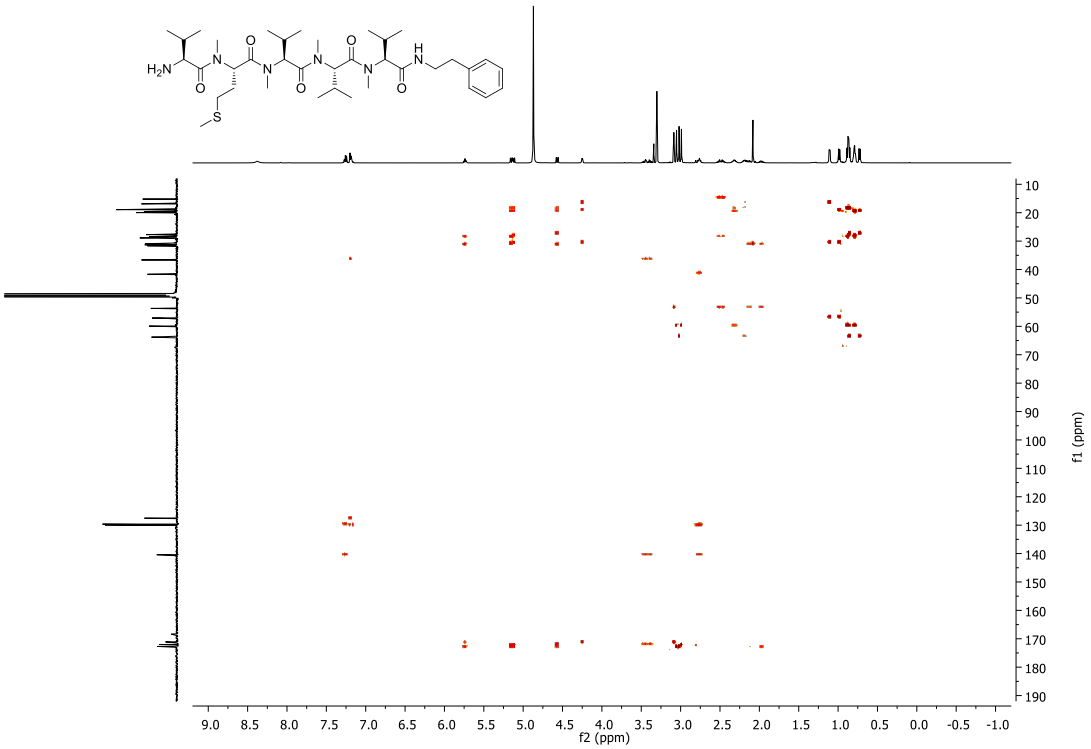


Figure S40. HMBC (methanol-d_4) spectrum of **7**

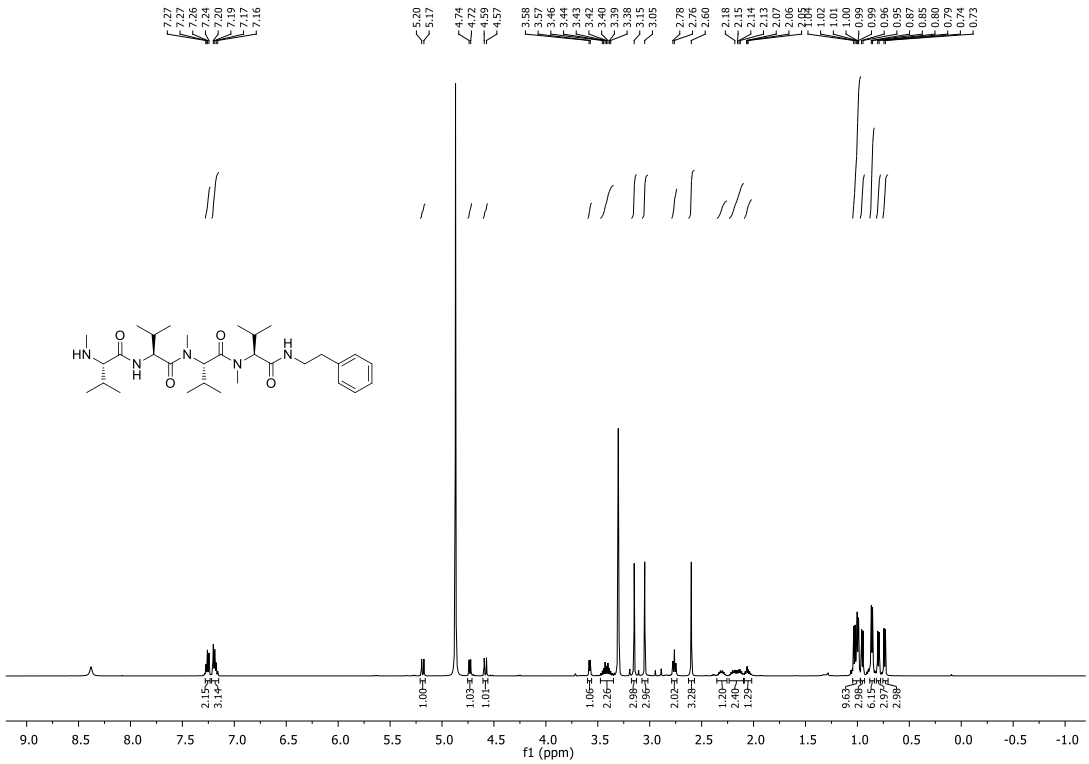


Figure S41. ^{1}H NMR (500 MHz, methanol-d_4) spectrum of **10**

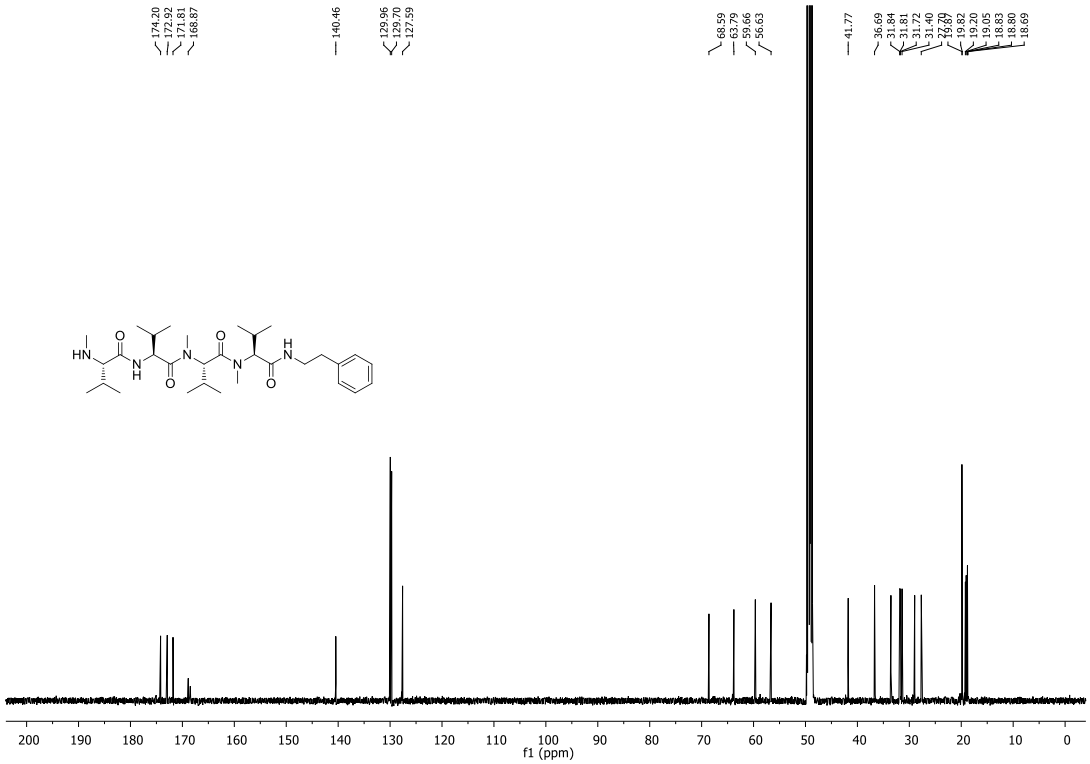


Figure S42. ^{13}C NMR (125 MHz, methanol-d_4) spectrum of **10**

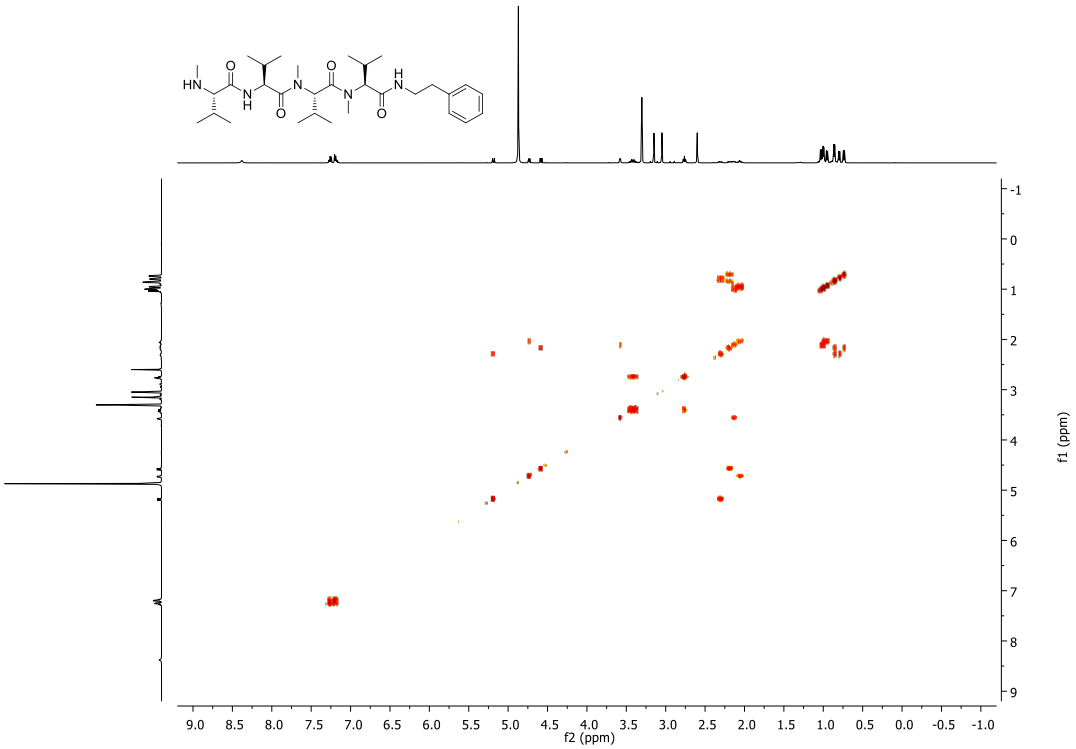


Figure S43. COSY (methanol-d_4) spectrum of **10**

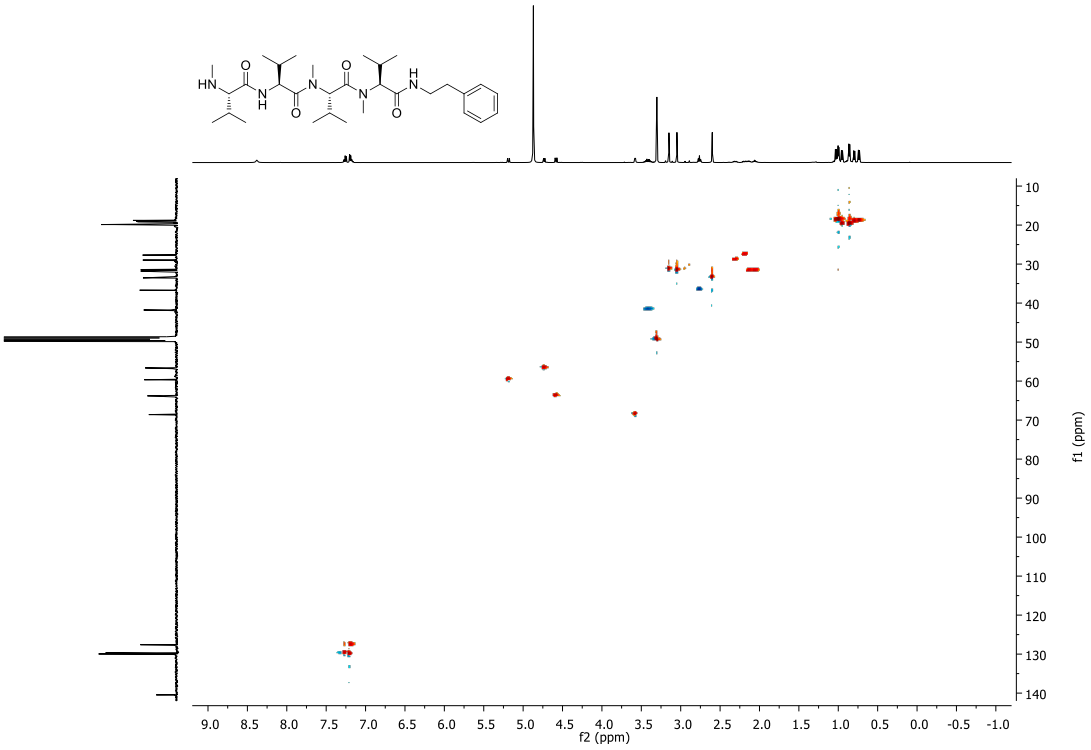


Figure S44. HSQC (methanol-d_4) spectrum of **10**

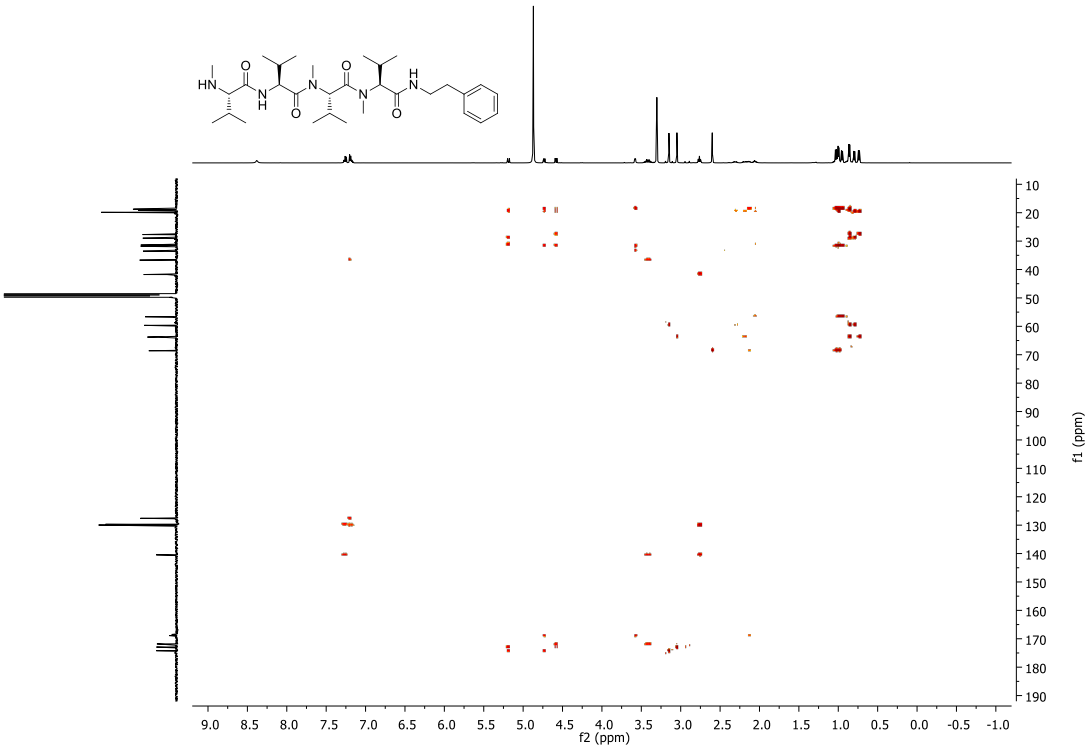


Figure S45. HMBC (methanol-d_4) spectrum of **10**

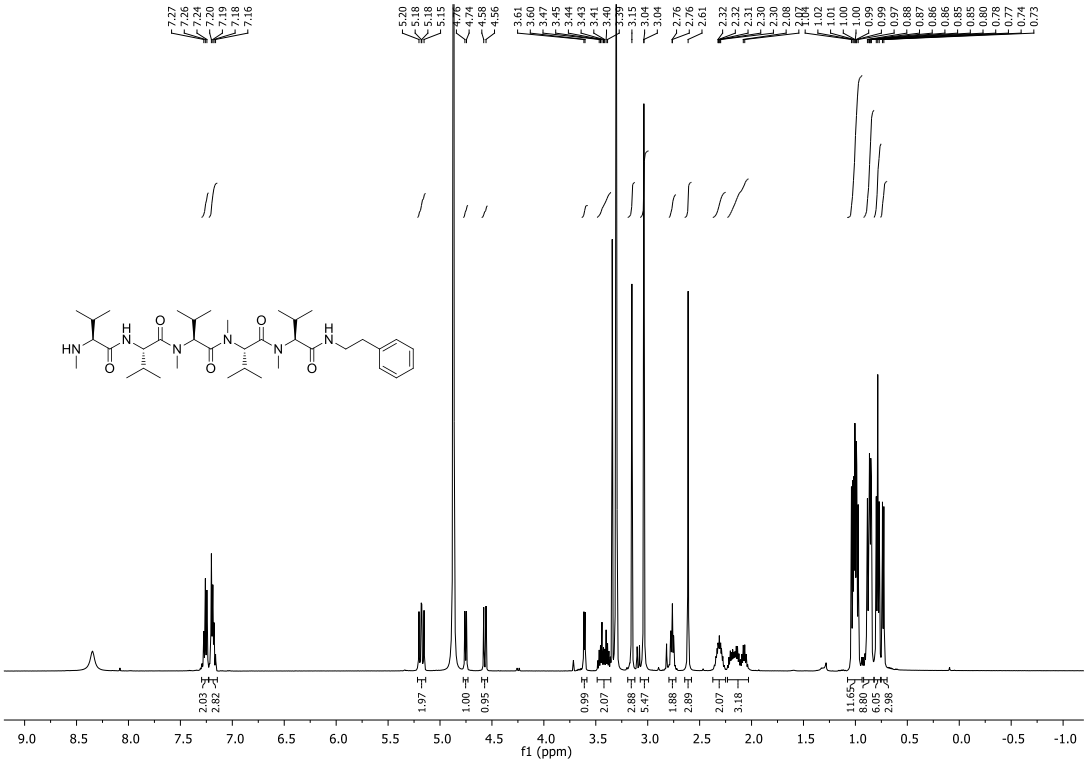


Figure S46. ^{1}H NMR (500 MHz, methanol-d_4) spectrum of **12**

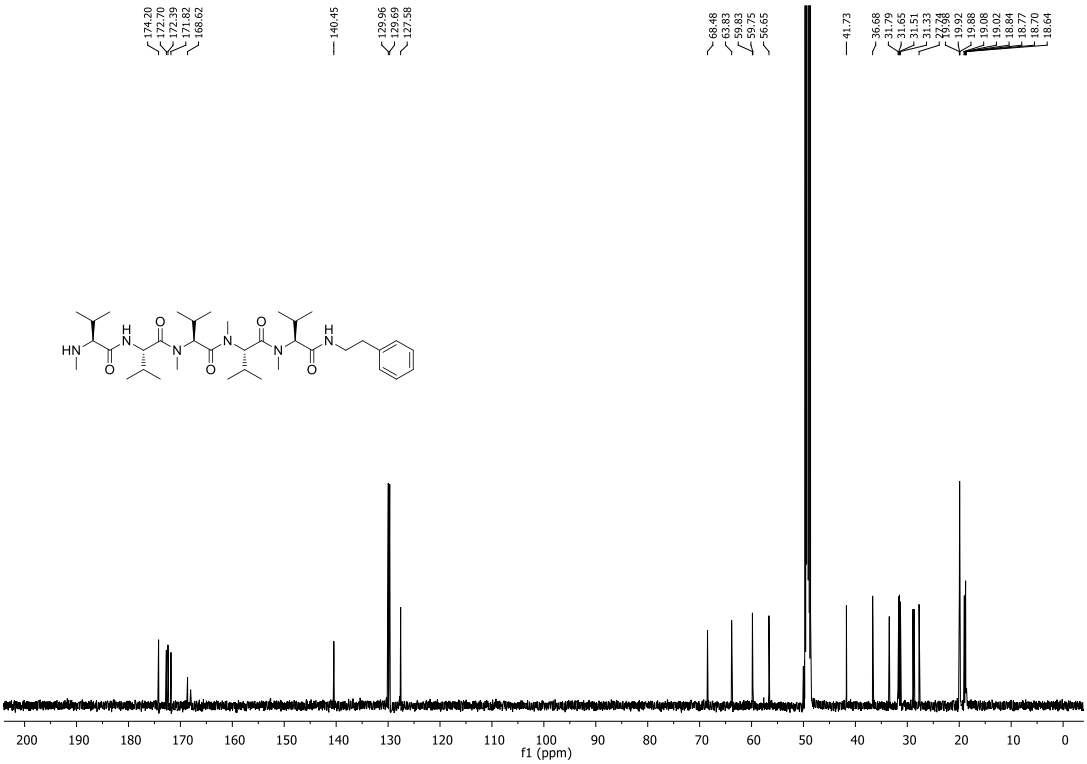


Figure S47. ^{13}C NMR (125 MHz, methanol-d_4) spectrum of **12**

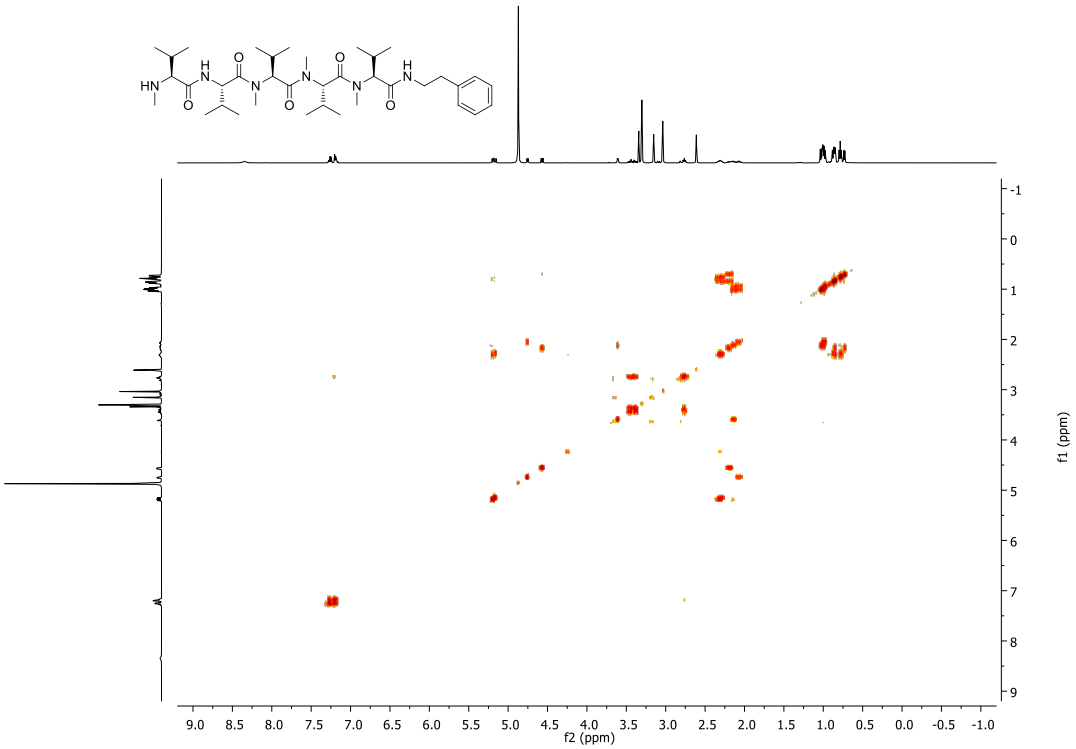


Figure S48. COSY (methanol-d_4) spectrum of **12**

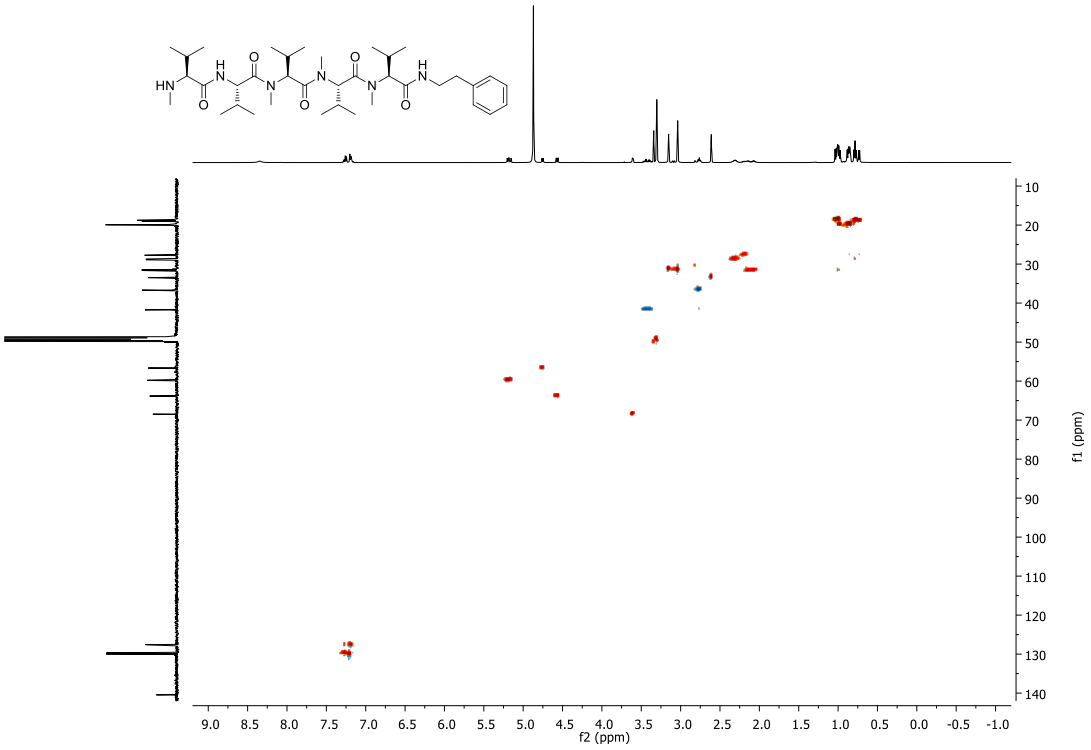


Figure S49. HSQC (methanol-d_4) spectrum of **12**

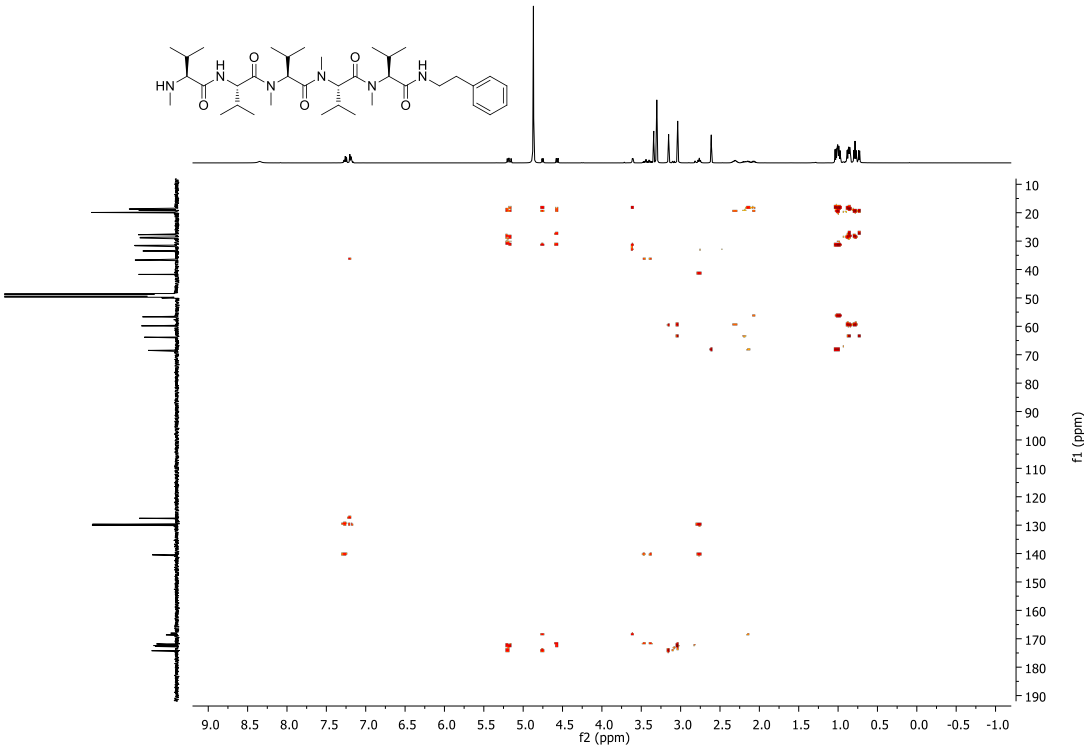


Figure S50. HMBC (methanol-d_4) spectrum of **12**

References

(1) Wesche, F.; Adihou, H.; Kaiser, A.; Wurglics, M.; Schubert-Zsilavecz, M.; Kaiser, M.; Bode, H. B. *J. Med. Chem.* **2018**, *61*, 3930–3938.

(2) Sable, G. A.; Park, J.; Kim, H.; Lim, S. J.; Jang, S.; Lim, D. *Eur. J. Org. Chem.* **2015**, 7043–7052.

(3) Cai, X.; Nowak, S.; Wesche, F.; Bischoff, I.; Kaiser, M.; Fürst, R.; Bode, H. B. *Nat. Chem.* **2017**, *9*, 379–386.

(4) Schimming O., Fleischhacker F., Nollmann F. I., Bode H. B. *Chembiochem* **2014**, *15*, 1290–1294.