Electronic Supplementary Information

The structure and biosynthesis of heinamides A1-A3 and B1-B5, antifungal members of lipopeptide family laxaphycins

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Table S1. Laxaphycin structures

	Amino acid residue												Ref.
11-residue laxaphycins	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**		
Laxaphycin A	Aoa	HSe	Dhb	OHPro	HSe	Phe	Leu	Ile	Ile	Leu	Gly		27,29,33,11
Laxaphycin A2	Aoa	HSe	Dhb	OHPro	HSe	Phe	Leu	Val	Ile	Leu	Gly		8
Laxaphycin E	Ada	HSe	Dhb	OHPro	HSe	Phe	Leu	Ile	Ile	Leu	Gly		27
Hormothamnin A	Aoa	HSe	Dhb	OHPro	HSe	Phe	Leu	Ile	Ile	Leu	Gly		30
Lobocyclamide A	Aoa	Ser	Dhb	OHPro	HSe	Tyr	Leu	Ile	Ile	Leu	Gly		31
Trichormamide A	Ada	Ser	Ser	Pro	Ser	Tyr	Leu	Ile	Ile	Pro	Gly		38, 35
Trichormamide D	Ada	Gln	Dhb	Pro	Ser	Tyr	Leu	Val	Phe	Leu	Gly		9
Scytocyclamide A	Aoa	Gln	Dhb	OHPro	HSe	Phe	Leu	Ile	Ile	Leu	Gly		32
Scytocyclamide A2	Aoa	Gln	Dhb	Pro	HSe	Phe	Leu	Ile	Ile	Leu	Gly		10
[l-Val^{8}]laxaphycin A	Aoa	HSe	Dhb	OHPro	HSe	Phe	Leu	Val	Ile	Leu	Gly		28
[d-Val^{9}]laxaphycin A	Aoa	HSe	Dhb	OHPro	HSe	Phe	Leu	Ile	Val	Leu	Gly		28
Acyclolaxaphycin A [des-Gly^{11}]	H-Aoa	HSe	Dhb	OHPro	HSe	Phe	Leu	Ile	Ile	Leu	Gly-OH		28
Acyclolaxaphycin A [des-(Leu^{10}-Gly^{11})]	H-Aoa	HSe	Dhb	OHPro	HSe	Phe	Leu	Ile	Ile	Leu-OH			28
acyclolaxaphycin A	H-Aoa	HSe	Dhb	OHPro	HSe	Phe	Leu	Ile	Ile-OH				28
Heinamide A1	Aoa	Ser	Dhb	OHPro	Ser	Tyr	Leu	Ile	Phe	OHPro	Gly		TS
Heinamide A2	Aoa	Ser	Dhb	Pro	Ser	Tyr	Leu	Ile	Phe	OHPro	Gly		TS
Heinamide A3	Aoa	Ser	Dhb	Pro	Ser	Tyr	Leu	Ile	Phe	Pro	Gly		TS
12-residue laxaphycins	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Ref**
Laxaphycin B	Ada	Val	OHLeu	Ala	OHLeu	Gln	NMe-Ile	OHAsn	Thr	Pro	Leu	Thr	27,29,33,34,37
Laxaphycin B2	Ada	Val	OHLeu	Ala	Leu	Gln	NMe-Ile	OHAsn	Thr	Pro	Leu	Thr	33
Laxaphycin B3	Ada	Val	OHLeu	Ala	OHLeu	Gln	NMe-Ile	OHAsn	Thr	OHPro	Leu	Thr	33,37
Laxaphycin B4	Ada	Val	OHLeu	Hse	OHLeu	Gln	NMe-Ile	OHAsn	Thr	OHPro	Leu	Thr	8
Laxaphycin B5	Ada	Ile	OHLeu	Val	OHLeu	Gln	NMe-Ile	Asn	Thr	Pro	Tyr	Thr	11
Laxaphycin B6	Ada	Ile	OHLeu	Val	Leu	Gln	NMe-Ile	Asn	Thr	Pro	Tyr	Thr	11
Laxaphycin D	Aoa	Val	OHLeu	Ala	OHLeu	Gln	NMe-Ile	OHAsn	Thr	Pro	Leu	Thr	27
Lobocyclamide B	Ada	Val	OHLeu	Ala	OHLeu	Gln	NMe-Ile	OHHSe*	Thr	OHPro	Leu	Thr	31
Lobocyclamide C	Aoa	Val	OHLeu	Ala	OHLeu	Gln	NMe-Ile	OHHSe*	Thr	OHPro	Leu	Thr	31
Lyngbyacyclamide A	Ada	Val	OHLeu	Hse	Leu	Gln	NMe-Ile	OHAsn	Thr	Pro	Phe	Thr	12,34
Lyngbyacyclamide B	Ada	Val	OHLeu	Hse	Leu	Gln	NMe-Ile	OHAsn	Thr	OHPro	Phe	Thr	12
Trichormamide B	Ada	Ile	OHLeu	Hse	OHLeu	Gln	NMe-Ile	Ser	Thr	Pro	Tyr	Thr	38
Trichormamide C	Ada	Val	OHLeu	Ala	OHLeu	Gln	NMe-Ile	D-Asn	Thr	Pro	Leu	Thr	9
Acyclolaxaphycin B	Ada	Val	OHLeu-OH	H-Ala	OHLeu	Gln	NMe-Ile	OHAsn	Thr	Pro	Leu	Thr	36,37
Acyclolaxaphycin B3	Ada	Val	OHLeu-OH	H-Ala	OHLeu	Gln	NMe-Ile	OHAsn	Thr	OHPro	Leu	Thr	36,37
Scytocyclamide B	Aoa	Val	OHLeu	Ala	OHLeu	Gln	NMe-Ile	OHAsn	Thr	Pro	Leu	Thr	32
Scytocyclamide B2	Aoa	Val	OHLeu	Ala	OHLeu	Gln	NMe-Ile	Asn	Thr	Pro	Leu	Thr	10
Scytocyclamide B3	Aoa	Val	OHLeu	Ala	Leu	Gln	NMe-Ile	Asn	Thr	Pro	Leu	Thr	10
Scytocyclamide C	Aoa	Val	OHLeu	Ala	Leu	Gln	NMe-Ile	OHAsn	Thr	Pro	Leu	Thr	32
Heinamide B1	OHAoa	Ile	OHLeu	Carb-Ser	Leu	Gln	NMe-Ile	OHHSe	Val	OHMePro	Tyr	Thr	TS
Heinamide B2	OHAoa	Ile	OHLeu	Carb-Ser	Leu	Gln	NMe-Ile	OHHSe	Val	Pro	Tyr	Thr	TS
Heinamide B3	Aoa	Ile	OHLeu	Carb-Ser	Leu	Gln	NMe-Ile	OHHSe	Val	Pro	Tyr	Thr	TS
Heinamide B4	Aoa	Ile	OHLeu	Carb-Ser	Leu	Gln	NMe-Ile	OHHSe	Val	OHMePro	Tyr	Thr	TS
Heinamide B5	OHAoa	Ile	OHLeu	Carb-Ser	Leu	Gln	NMe-Ile	OHHSe	Val	MePro	Tyr	Thr	TS

TS = this study.
Aoa – β-aminooctanoic acid, Ada – β-aminodecanoic acid, Hse – Homoserine, Dhb – Dehydrobutyrine, NMe-Ile – N-Methyl Isoleucine, OHPro – 4-hydroxyproline, OHAsn – 3-hydroxyasparagine, OHLeu – 3-hydroxyleucine, OHThr – 4-hydroxythreonine, OHHse – 3-hydroxy homoserine, OHAoa – 5-hydroxyl β-amino octanoic acid, Carb-Ser – O-Carbamoyl homoserine
*Reported as 4-OHThr by MacMillan et al. 2002, but interpreted here as OHHse according to information of adenylation domain specificity of heinamides in *Nostoc* sp. UHCC0702

Table S2. Strains used in bioassays.

Fungi	
Candida albicans	FBCC 2462
Candida guillermondi	FBCC 2457
Candida krusei	FBCC 2464
Candida parapsilosis	FBCC 2465
Filobasidiella neoformans	FBCC 2466
Aspergillus niger	FBCC 2467
Aspergillus parasiticus	FBCC 2500
Aspergillus flavus	FBCC 2467

Bacteria	
Staphylococcus aureus	HAMBI 66
Enterococcus faecium	HAMBI 1821
Bacillus cereus	HAMBI 1881
Micrococcus luteus	HAMBI 2688
Pseudomonas aeruginosa	HAMBI 25
Escherichia coli	HAMBI 1723
Actineobacter baumannii	HAMBI 1760
Enterobacter aerogenes	HAMBI 1898
Salmonella enterica	HAMBI 2331

Table S3. Solvent gradients used in the identification of peptides.

	Stage 1 Gradient		Stage 2 Gradient		Stage 3 Isocratic	Stage 4 Gradient		Stage 5 Isocratic
	Solvent B (%)	Time (min)	Solvent B (%)	Time (min)	Time (min)	Solvent B (%)	Time (min)	Time (min)
1	5-100	5	-	-	2	100-5	0.5	2.5
2	10-70	5	70-95	0.01	1.99	95-10	0.5	2.5
3	30-50	12	50-100	0.01	3.99	100-30	0.01	3.99
4	5-60	6	60-100	0.01	1.99	100-5	0.01	1.99
5	5-25	12	25-100	0.01	3.99	100-5	0.01	3.99

Gradients 3-5 were used for amino acid analysis.

Table S4. Parameters for NMR experiments used for structural characterization of heinamides.

Experiment	Complex points in (t_1) t_2	Acquisition time in (t_1) t_2 [s]	Number of scans
^{1}H	32k	2.0	8
^{13}C	48k	1.0	2048
2D TOCSY	(256) 2048	(0.0231) 0.183	8
2D DQF-COSY	(256) 1024	(0.0199) 0.0799	16
2D EASY-ROESY	(256) 1024	(0.0199) 0.0799	16
2D ^{13}C HSQC	(128) 1024	(0.0035) 0.0799	32
2D edited ^{13}C HSQC	(128) 1024	(0.0035) 0.0799	32
2D ^{13}C HMBC	(512) 1024	(0.0115) 0.1278	24
2D ^{15}N HSQC	(128) 1024	(0.0128) 0.0799	32

Table S5. UPLC-QTOF analysis of unlabeled (^{14}N) and ^{15}N-labeled heinamides.

Without N-labeling (^{14}N cultivation)

		$[M+H]^+$				$[M+Na]^+$	
Heinamide	t_R	**Formula**	**RI (%)**	**Exp (*m/z*)**	**Δ (ppm)**	**Exp (*m/z*)**	**Δ (ppm)**
A1	3.04	$C_{60}H_{88}N_{11}O_{16}^+$	58	1218.6415	0.8	1240.6230	0.4
A2	3.10	$C_{60}H_{88}N_{11}O_{15}^+$	40	1202.6467	0.9	1224.6291	1.2
A3	3.20	$C_{60}H_{88}N_{11}O_{14}^+$	2	1186.6496	-0.9	1208.6323	-0.3
B1	2.82	$C_{71}H_{119}N_{14}O_{22}^+$	62	1519.8653	2.3	1541.8485	3.1
B2	2.81	$C_{70}H_{117}N_{14}O_{21}^+$	15	1489.8562	3.3	1511.8384	3.4
B3	3.04	$C_{70}H_{117}N_{14}O_{20}^+$	11	1473.8594	2.1	1495.8408	1.7
B4	3.06	$C_{71}H_{119}N_{14}O_{21}^+$	7	1503.8677	0.5	1525.8513	1.6
B5	2.87	$C_{71}H_{119}N_{14}O_{21}^+$	4	1503.8591	-5.2	1525.8476	-0.8

N-labeling (^{15}N cultivation)

		$[M+H]^+$				$[M+Na]^+$	
Heinamide	t_R	**Δ^{15}N-^{14}N (*m/z*)**	**No of N**	**Exp (*m/z*)**	**Δ (ppm)**	**Exp (*m/z*)**	**Δ (ppm)**
A1	3.01	10.9679	11	1229.6094	1.2	1251.5897	-0.1
A2	3.08	10.9686	11	1213.6153	1.9	1235.5975	2.0
A3	3.16	10.9692	11	1197.6188	0.6	1219.6007	0.5
B1	2.79	13.9567	14	1533.8220	1.1	1555.8022	0.0
B2	2.78	13.9574	14	1503.8136	2.6	1525.7963	3.0
B3	3.00	13.9594	14	1487.8188	2.7	1509.8005	2.5
B4	3.03	13.9580	14	1517.8257	0.2	1539.8009	-4.2
B5	2.84	13.9508	14	1517.8099	-10.2	1539.8005	-4.4

Experimental (Exp) ion masses, error (Δ) in ppm to theoretical ion mass, relative intensity (RI) and formula calculated from $[M+H]^+$ signal of heinamides, mass difference of unlabeled and ^{15}N-labeled heinamides (Δ^{15}N-^{14}N) and number of nitrogens (No of N) in heinamides.

Table S6. NMR data of HA A1 (from mixture of HA A1, B3 and B4 [7:2:1] in DMSO-d6).

Unit	No	$\delta_{C/N}$[a]	δ_H	mult, *J*	COSY	HMBC	ROESY[b]
Aoa[1]	1	169.0	-		-	-	
	2	40.5	1.62		2', 3	-	Ser[2]-2-NH
	2'		1.90		2, 3	1	Ser[2]-2-NH
	3	45.1	4.19		2, 2', 3-NH, 4'	-	2, 2', 3-NH, 4, 4'
	4	34.8	1.36		3, 5	2, 3, 5, 6	
	4'		1.41		3, 5	2, 3, 5, 6	
	5	25.3	1.26			3, 6	
	6	30.9	1.21			4, 5, 7, 8	
	6'		1.27			4, 5, 7	
	7	22.1	1.28		8	4, 5, 6	
	8	13.9	0.87	t, 7.1	7	6, 7	
	3-NH	122.6	7.08	d, 9.3	3	3, Gly[11]-1	
Ser[2]	1	172.5	-		-	-	
	2	54.0	4.45		2-NH, 3, 3'	1, 3, Aoa[1]-1	2-NH, 3, 3', Dhb[3]-2-NH
	3	60.8	3.75		2, 3', 3-OH	-	
	3'		3.85		2, 3, 3-OH	1	
	3-OH		5.27		3, 3'	-	
	2-NH	117.4	6.57	brs	2	-	
E-Dhb[3]	1	167.4	-		-	-	
	2	131.0	-		-	-	
	3	119.7	5.57	q, 7.3	4	1, 2, 4	4
	4	12.2	1.70	d, 7.3	3	1, 2, 3	
	2-NH	140.5	10.89	s	-	1, Ser[2]-1	3, Ser[2]-2
Hyp[4]	1	170.5	-		-	-	
	2	59.4	4.49		3, 3'	1, 3, Dhb[3]-1	3, 3', Ser[5]-2-NH
	3	37.7	1.85		2, 3', 4	1, 2	2, 3', 4, 5'
	3'		2.23		2, 3, 4	4, 5	2, 3, 4, 5'
	4	68.1	4.23		3, 3', 5'	-	
	4-OH	-	5.12		4	-	
	5	57.0	3.22		3', 5'	2, 3, 4, Dhb[3]-1	Dhb[3]-4
	5'		3.93		4, 5	-	
Ser[5]	1	169.7	-		-	-	
	2	55.1	4.15		2-NH, 3, 3'	1, 3, Hyp[4]-1	2-NH, 3, 3', Tyr[6] - 2-NH
	3	61.1	3.69		2, 3'	-	
	3'		3.77		2, 3	-	
	3-OH	-	-		-	-	
	2-NH	107.5	7.19	brs	2	Hyp[4]-1	

Tyr[6]	1	172.0	-		-	-	
	2	57.1	4.05		2-NH, 3, 3'	1, 3	2-NH, 3, 3', Leu[7]-2-NH
	3	36.2	2.80		2, 3'	1, 2, 4, 5/9	
	3'		2.93		2, 3	1, 2, 4, 5/9	
	4	128.1	-		-	-	
	5/9	130.0	7.13	d, 8.3	6/8	3, 5/9, 6/8, 7	
	6/8	115.0	6.61	d, 8.3	5/9	4, 6/8, 7	
	7	155.7	-		-	-	
	7-OH		-		-	-	
	2-NH	115.0	7.82	d, 7.3	2	2, 3, Ser[5]-1	
Leu[7]	1	171.3	-		-	-	
	2	51.3	4.21		2-NH, 3, 3'	1, 3, Tyr[6]-1	2-NH, 3, 3', Ile[8]-2-NH
	3	38.7	1.04		2, 3', 4	2, 4, 5	
	3'		1.22		2, 3, 4	4	
	4	24.0	1.49		3, 3', 5, 6	3, 5, 6	
	5	20.3	0.69	d, 6.8	4	3, 4, 6	
	6	22.9	0.77	d, 6.4	4	3, 4, 5, 6	
	2-NH	112.1	7.21	brs	2	-	
Ile[8]	1	171.7	-		-	-	
	2	55.6	4.45		2-NH, 3	-	2-NH, 3, Phe[9]-2-NH
	3	38.3	1.50		2, 3-Me	-	
	3-CH_3	15.3	0.56	d, 6.8	3	2, 3, 4	
	4	21.9	0.64		3, 5	-	
	4'		0.67		5	-	
	5	11.3	0.40	t, 7.3	4, 4'	3, 4	
	NH	107.1	6.49	brs	2	-	
Phe[9]	1	170.9	-		-	-	
	2	53.3	4.60		2-NH, 3, 3'	1, 3	2-NH, 3, 3', Hyp[10]-5,5'
	3	36.3	2.80		2, 3'	1, 2, 4, 5/9	
	3'		3.02		2, 3	1, 2, 4, 5/9	
	4	137.2	-		-	-	
	5/9	128.8	7.34			3, 5/9, 7	
	6/8	128.2	7.30		7	4, 6/8	
	7	126.6	7.22		6/8	5/9	
	2-NH	120.9	8.74	d, 7.3	2	2, 3, Ile[8]-1	
Hyp[10]	1	172.1	-		-	-	
	2	59.5	4.28		3, 3'	1, 3, 4, Phe-1	3,3', Gly[11]-2-NH
	3	36.9	1.93		2, 3', 4	1, 4, 5	2, 4, 5,5'
	3'		1.97		2, 3, 4	4, 5	2, 4, 5,5'
	4	68.6	4.47		3, 5	-	
	4-OH	-	5.31		4	-	
	5	55.2	3.60		4, 5'	2, 3, 4	
			3.75		5	3	
Gly[11]	1	166.8	-		-	-	
	2	42.6	3.28		2', 2-NH	1, Hyp[10]-1	2', 2-NH, Aoa[1]-3-NH
	2'		3.71		2, 2-NH	1, Hyp[10]-1	2, 2-NH, Aoa[1]-3-NH
	2-NH	110.8	8.80	brs	2	-	

[a] = values from ^{13}C HMBC if ^{13}C signals not available

[b] = Most important signals for the sequence

Table S7. NMR data of HA A2 in DMSO-d6.

Unit	No	$\delta_{C/N}$[a]	δ_H	mult, *J*	COSY	HMBC	ROESY[b]
Aoa[1]	1	169.1	-		-	-	
	2	40.6	1.66		2', 3	-	Ser[2]-2-NH
	2'		1.93		2	-	Ser[2]-2-NH
	3	45.2	4.16		2, 2', 3-NH, 4'	-	2, 2', 3-NH, 4, 4'
	4	34.7	1.36		3	5, 6	
	4'		1.41		3, 5	3, 5, 6	
	5	25.3	1.26			3, 6, 7	
	6	30.9	1.20			5, 7, 8	
	6'		1.26			8	
	7	22.1	1.27		8	5, 6	
	8	13.9	0.87	t, 7.1	7	6, 7	
	3-NH	122.4	7.10	d, 8.8	3	Gly[11]-1	
Ser[2]	1	172.1	-		-	-	
	2	54.0	4.47		2-NH, 3, 3'	1, 3, Aoa[1]-1	2-NH, 3, 3', Dhb[3]-2-NH
	3	60.7	3.72		2, 3', 3-OH	-	
	3'		3.83		2, 3, 3-OH	-	
	3-OH		5.26		3, 3'	-	
	2-NH	116.6	6.66	brs	2	-	
E-Dhb[3]	1	166.6	-		-	-	
	2	131.1	-		-	-	
	3	118.6	5.54	q, 7.3	4	1, 2, 4	4
	4	12.3	1.69	d, 7.3	3	1, 2, 3	
	2-NH	139.7	10.86	s	-	-	3, Ser[2]-2, 3'
Pro[4]	1	170.6	-		-	-	
	2	60.7	4.41		3, 3'	1, 3, 4	3, 3', Ser[5]-2-NH
	3	29.4	1.81		2, 3'	1, 2, 4	2, 3', 5'
	3'		2.28		2, 3	1, 4, 5	2, 3
	4	24.4	1.82		5, 5'	3	
	5	48.1	3.30		4, 5'	3, 4	
	5'		3.86		4, 5	3	
Ser[5]	1	169.7	-		-	-	
	2	55.4	4.16		2-NH, 3, 3'	1, 3	2-NH, 3, 3', Tyr[6] - 2-NH
	3	61.1	3.69		2, 3'	-	
	3'		3.77		2, 3	-	
	3-OH	-	-		-	-	
	2-NH	108.5	7.26	brs	2	-	

Tyr[6]	1	171.9	-	-	-	
	2	56.9	4.07	2-NH, 3, 3'	1	2-NH, 3, 3', Leu[7]-2-NH
	3	36.2	2.80	2, 3'	4, 5/9	
	3'		2.90	2, 3	2, 4, 5/9	
	4	128.1	-	-	-	
	5/9	130.0	7.11 d, 8.8	6/8	3, 5/9, 6/8, 7	
	6/8	115.0	6.61 d, 8.3	5/9	4, 6/8, 7	
	7	155.8	-	-	-	
	7-OH		-	-	-	
	2-NH	114.7	7.80 d, 7.8	2	2, 3, Ser[5]-1	
Leu[7]	1	171.4	-	-	-	
	2	51.4	4.21	2-NH, 3, 3'	1, 3	2-NH, 3, 3', Ile[8]-2-NH
	3	38.9	1.07	2, 3', 4	-	
	3'		1.22	2, 3, 4	-	
	4	24.0	1.50	3, 3', 5, 6	3, 5, 6	
	5	20.4	0.69 d, 6.8	4	3, 4, 6	
	6	22.9	0.77 d, 6.4	4	3, 4, 5	
	2-NH	112.2	7.25 brs	2	-	
Ile[8]	1	171.6	-	-	-	
	2	55.7	4.40	2-NH, 3	-	2-NH, 3, Phe[9]-2-NH
	3	38.2	1.51	2, 3-Me	-	
	3-CH_3	15.3	0.56 d, 6.8	3	2, 3, 4	
	4	22.1	0.65	3, 5	-	
	4'		0.72	5	-	
	5	11.3	0.42 t, 7.1	4, 4'	3, 4	
	NH		6.59	2	-	
Phe[9]	1	170.9	-	-	-	
	2	53.1	4.62	2-NH, 3, 3'	1, 3	2-NH, 3, 3', Hyp[10]-5,5'
	3	36.3	2.80	3'	1, 2, 4, 5/9	
	3'		3.02	3	1, 4, 5/9	
	4	137.2	-	-	-	
	5/9	128.9	7.33		3, 5/9, 7	
	6/8	128.2	7.30	5/9	4, 6/8	
	7	126.6	7.22	6/8	5/9	
	2-NH	120.4	8.72 d, 7.3	2	Ile[8]-1	
Hyp[10]	1	172.0	-	-	-	
	2	59.5	4.28	3	1, 3, 4, Phe-1	3,3', Gly[11]-2-NH
	3	37.0	1.92	2, 4	1, 2, 5	2, 4, 5,5'
	3'		1.97	2, 3, 4	4, 5	2, 4, 5,5'
	4	68.6	4.45	3, 5	-	
	4-OH		5.32	4	-	
	5	55.1	3.59	5'	3, 4	
	5'		3.74	4, 5	-	
Gly[11]	1	166.9	-	-	-	
	2	42.6	3.29	2'	1, Hyp[10]-1	2', 2-NH
	2'		3.71	2, 2-NH	1, Hyp[10]-1	2, 2-NH, Aoa[1]-3-NH
	2-NH	109.8	8.72	2	-	

[a] = values from ^{13}C HMBC if ^{13}C signals not available

[b] = Most important signals for the sequence

Table S8. NMR data of HA B1 in DMSO-d6.

Unit	No	$\delta_{C/N}$ [a]	δ_H	mult, *J*	COSY	HMBC	ROESY[b]
5-OHAoa[1]	1	170.9	-		-	-	
	2	40.4	2.31		2', 3	1, 3, 4	
	2'		2.55		2, 3	1, 3, 4	
	3	44.1	4.20		2, 2', 3-NH, 4, 4'	-	
	3-NH	126.2	7.75	brs	3	-	Thr[12]-2
	4	41.6	1.38		3, 4', 5	5	
	4'		1.47		3	-	
	5	66.2	3.46		4, 6, 6'	-	
	6	39.4	1.24		5	5, 7, 8	
	7	18.4	1.23		7', 8	5, 6, 8	
	7'		1.34		7, 8	5, 6, 8	
	8	14.0	0.82		7, 7'	7, 6	
Ile[2]	1	171.7	-		-	-	
	2	58.2	4.12		2-NH, 3	1, 3, 3-Me, 4	
	2-NH	123.3	8.13	brs	2	-	5-OHAoa[1]-2,2
	3	35.9	1.80		2, 3-Me, 4, 4'	-	
	3-CH_3	15.2	0.84		3	2, 3, 4	
	4	24.4	1.15		3, 4', 5	3, 3-Me, 5	
	4'		1.46		3, 4, 5	-	
	5	10.9	0.81		4, 4'	3, 4	
3-OHLeu[3]	1	171.6	-			-	
	2	56.3	4.33		2-NH, 3	1, 3	
	2-NH	-*	8.71	brs	2	-	Ile[2]-2
	3	77.2	3.43		2, 3-OH	4, (5, 6)	
	3-OH		5.29		3	-	
	4	30.6	1.63		3, 5, 6	3, 5, 6	
	5	18.8	0.75		4	3, 4, 6	
	6	19.3	0.94		4	3, 4, 5	
carbHSe[4]	1	169.7	-		-	-	
	2	50.3	4.30		2-NH, 3, 3'	1	
	2-NH	126.7	8.15	brs	2	-	
	3	30.8	1.81		2, 3', 4, 4'	2, 4	
	3'		2.08		2, 3, 4, 4'	4	
	4	60.5	3.76		3, 3', 4'	2, 3, 5	
	4'		3.90		3, 3', 4	2, 3, 5	3-OHLeu[3]-6
	5	156.6	-			-	
	5-NH_2	-*	6.40	brs	-	-	
Leu[5]	1	172.1	-		-	-	
	2	51.9	4.15		2-NH, 3, 3'	1	
	2-NH	117.5	7.97	d, 5.9	2	-	2, carbHSe[4]-2
	3	40.5	1.32		2, 3', 4	4, 5, 6	
	3'		1.49		2, 3	2, 4, 5	
	4	23.9	1.53		3, 5, 6	2, 3, 5, 6	
	5	21.1	0.78		4	3, 4, 6	
	6	22.9	0.84		4	3, 4, 5	

Gln[6]	1	172.4	-		-	-	
	2	48.7	4.56		2-NH, 3, 3'	1, 3, Leu[5]-1	
	2-NH	117.6	8.16	brs	2	-	2, Leu[5]-2
	3	26.1	1.76		2, 3'	2, 4, 5	
	3'		1.82		2, 3	4, 5	
	4	30.8	2.07		3, 3'	2, 3, 5	
	5	174.1	-		-	-	
	5-NH_2	108.9	6.71		5-NH_2	4, 5'	
			7.14		5-NH_2	5	
NMe-Ile[7]	1	170.6	-		-	-	
	2	60.0	4.66		3	1, 3	
	3	31.7	1.91		2, 4'	-	
	3-CH_3	15.2	0.83		3	2, 3, 4	
	4	24.1	0.91		3, 4', 5	3, 3-Me, 5	
	4'		1.29		3, 4, 5	3, 3-Me, 5	
	5	10.2	0.77		4, 4'	3, 4	
	N-CH_3	30.1	2.98	s	-	2, Gln-1	
3-OHHse[8]	1	170.0	-		-	-	
	2	54.6	4.36		2-NH, 3	1, 3, NMe-Ile[7]-1	
	2-NH	114.9	7.71	brs	2	-	NMe-Ile[7]-2
	3	71.2	3.87		2, 3-OH, 4	-	
	3-OH	-	4.90		3	-	
	4	62.5	3.26		3, 4'	2, 3	
	4'		3.30		3, 4	2, 3	
Val[9]	1	169.6	-		-	-	
	2	55.7	4.30		2-NH, 3	1, 3, 4, 5	
	2-NH	117.1	7.28	brs	2	-	4-OHThr[8]-2
	3	30.0	1.92		2, 4, 5	1, 2, 4, 5	
	4	19.1	0.88		3	2, 3, 5	
	5	18.1	0.78		3	2, 3, 4	
OHMePro[10]	1	167.7	-			-	
	2	65.6	4.39		3	1, Val[9]-1	
	3	71.9	4.15		2, 3-OH, 4	-	
	3-OH		4.57		3	-	
	4	38.3	2.06		3, 4-Me, 5	-	
	4-CH_3	10.6	0.94		4	3, 4, 5	
	5	51.0	3.25		4, 5'	-	
	5'		3.90		5	2, 3, 4	
Tyr[11]	1	171.0	-		-	-	
	2	54.6	4.45		2-NH, 3, 3'	-	
	2-NH	118.0	8.08	brs	2	-	OHMePro[10]-2
	3	36.9	2.82		2, 3'	1, 2, 4, 5/9	
	3'		2.88		2, 3	1, 2, 4, 5/9	
	4	127.8	-		-	-	
	5/9	130.0	7.01		6/8	3, 5/9, 6/8, 7	
	6/8	114.8	6.61		5/9	4, 6/8, 7	
	7	155.7	-		-	-	
Thr[12]	1	169.6	-			-	
	2	58.3	4.09		2-NH, 3	1, 3	
	2-NH	112.8	7.82	d, 7.3	2	Tyr[11]-1	2, Tyr[11]-2
	3	66.4	3.86		2, 4	-	
	4	19.2	0.88		3	2, 3	

[a] = values from ^{13}C HMBC if ^{13}C signals not available. [b] = Most important signals for the sequence

* = NH signal in ^{15}N HSQC unclear/inaccurate

Table S9. NMR data of HA B2 (from mixture of HA B1 and B2 [1:1] in DMSO-d6).

Unit	No	$\delta_{C/N}$ [a]	δ_H
5-OHAoa[1]	1	170.9	-
	2	40.4	2.33
	2'		2.55
	3	44.2	4.20
	3-NH	126.4	7.92
	4	41.9	1.43
	5	66.1	3.47
	6	39.5	1.25
	7	18.3	1.24
	7'		1.34
	8	14.0	0.82
Ile[2]	1	171.7	-
	2	58.2	4.09
	2-NH	123.4	8.18
	3	35.8	1.77
	3-CH_3	15.2	0.83
	4	24.5	1.15
	4'		1.46
	5	10.9	0.80
3-OHLeu[3]	1	171.6	-
	2	56.5	4.31
	2-NH	nd	8.77
	3	77.3	3.46
	3-OH		5.39
	4	30.6	1.65
	5	18.7	0.75
	6	19.4	0.94
carbHSe[4]	1	169.7	-
	2	50.3	4.28
	2-NH	nd	8.13
	3	30.7	1.82
	3'		2.07
	4	60.5	3.76
	4'		3.90
	5	156.6	-
	5-NH_2	-*	6.40
Leu[5]	1	172.1	-
	2	52.0	4.13
	2-NH	117.6	8.00
	3	40.4	1.31
	3'		1.49
	4	23.8	1.53
	5	21.1	0.78
	6	22.9	0.84
Gln[6]	1	172.4	-
	2	48.7	4.56
	2-NH	117.6	8.17
	3	26.1	1.77
	3'		1.82
	4	30.7	2.07
	5	174.1	-
	5-NH_2	108.9	6.74
			7.15
NMe-Ile[7]	1	-	-
	2	60.0	4.68
	3	31.7	1.91
	3-CH_3	15.2	0.83
	4	24.1	0.91
	4'		1.29
	5	10.2	0.77
	N-CH_3	30.2	2.98
3-OHHse[8]	1	170.0	-
	2	54.7	4.34
	2-NH	115.2	7.83
	3	71.0	3.87
	3-OH	-	4.90
	4	62.4	3.25
	4'		3.30
Val[9]	1	169.5	-
	2	55.5	4.30
	2-NH	117.3	7.13
	3	30.1	1.90
	4	19.0	0.85
	5	18.0	0.75
Pro[10]	1	167.7	-
	2	59.3	4.31
	3	29.1	1.46
	3'		1.84
	4	24.1	1.72
	5	47.0	3.47
	5'		3.66
Tyr[11]	1	171.0	-
	2	54.1	4.51
	2-NH	117.6	8.26
	3	37.1	2.65
	3'		2.95
	4	127.7	-
	5/9	129.9	7.00
	6/8	114.4	6.62
	7	155.7	-
Thr[12]	1	169.6	-
	2	58.2	4.14
	2-NH	111.4	7.87
	3	66.5	3.97
	3-OH	-	4.90
	4	19.1	0.96

[a] = values from ^{13}C HMBC if ^{13}C signals not available

* = NH signal in ^{15}N HSQC unclear/inaccurate

Table S10. NMR data of HA B3 and B4 (from mixture of HA A1, B3 and B4 [7:2:1] in DMSO-d6).

		HA B3/B4				HA B3/B4		HA B4	
Unit	**No**	**$\delta_{C/N}$ [a]**	**δ_H**	**Unit**	**No**	**$\delta_{C/N}$ [a]**	**δ_H**	**$\delta_{C/N}$ [a]**	**δ_H**
Aoa[1]	1	-	-	NMe-Ile[7]	1	-	-		
	2	40.5	1.62		2	60.0	4.68		
	2'		1.91		3	31.7	1.91		
	3	45.1	4.19		3-CH_3	15.2	0.83		
	3-NH	122.6	7.08		4	24.1	0.92		
	4	34.7	1.36		4'		1.30		
	4'		1.42		5	10.2	0.77		
	5	25.3	1.23		N-CH_3	30.2	2.98		
	6	30.9	1.22	3-OHHse[8]	1	-	-		
	7	22.1	1.24		2	54.7	4.34		
	8	13.8	0.83		2-NH	-	-		
Ile[2]	1	-	-		3	71.1	3.86		
	2	58.1	4.07		3-OH	-	4.90		
	2-NH	-	8.16		4	62.5	3.25		
	3	35.6	1.77		4'		3.30		
	3-CH_3	15.2	0.83	Val[9]	1	-	-		
	4	24.6	1.15		2	55.5	4.30		
	4'		1.46		2-NH	116.7	7.18		
	5	10.8	0.80		3	30.1	1.90		
3-OHLeu[3]	1	171.6	-		4	19.0	0.85		
	2	56.2	4.31		5	18.0	0.75		
	2-NH	-	-	Pro[10] /	1	-	-	-	-
	3	77.1	3.45	OHMePro[10]	2	59.6	4.30	65.6	4.39
	3'				3	29.1	1.46	72.1	4.15
	4	30.6	1.64		3'		1.85		
	5	18.8	0.75		4	24.2	1.72	38.3	2.06
	6	19.3	0.94		4-CH_3	-	-	10.6	0.95
carbHSe[4]	1	-	-		5	47.0	3.47	51.3	3.24
	2	50.4	4.31		5'		3.65		3.88
	2-NH	117.5	8.05	Tyr[11]	1	-	-		
	3	30.8	1.82		2	53.9	4.53		
	3'		2.07		2-NH	-	-		
	4	60.5	3.76		3	37.3	2.64		
	4'		3.90		3'		2.95		
	5	155.7	-		4	127.9	-		
	5-NH_2	-	6.40		5/9	130.0	7.00		
Leu[5]	1	-	-		6/8	114.7	6.62		
	2	51.9	4.17		7	155.7	-		
	2-NH	-	-	Thr[12]	1	-	-	-	-
	3	40.5	1.33		2	58.2	4.14	58.3	4.10
	3'		1.49		2-NH	112.5	7.85		
	4	23.9	1.53		3	66.6	3.90	66.5	3.82
	5	21.2	0.79		3-OH	-	4.87	-	4.85
	6	22.8	0.84		4	19.3	0.94	19.3	0.88
Gln[6]	1	-	-	[a] = values from ^{13}C HMBC if ^{13}C signals not available					
	2	48.7	4.56	* = NH signal in ^{15}N HSQC unclear/inaccurate					
	2-NH	117.5	8.16						
	3	26.2	1.75						
	3'		1.83						
	4	30.8	2.07						
	5	-	-						
	5-NH_2	108.9	6.74						
			7.15						

Table S11. Retention times (t_R) of Marfey derived (with 1-fluoro-2-4-dinitrophenyl-5-L-alanine amide [FDAA] or 1-fluoro-2-4-dinitrophenyl-5-L/D-leucine amide [FDLA] reference amino acids [AA] and of Marfey-derived AAs from isolated and hydrolysed heinamide (HA) mixtures. Gray background reference Aa retention times match with retention times of AAs from HAs or their mixtures.

Marfey derivatives (MD) from reference amino acids	t_R (min) Amino acid		t_R (min) of the Aa's MD-derivatives from the hydrolyzed heinamide (HA) mixtures					
	D-	L-	A1	A2	A1, B3 and B4	B1	B1 and B2	B3 and B4
L-DAA-Ser	2,20	2,17		2,17	2,17			
L-DAA-Pro	2,94	2,86		2,86			2,86	
L-DAA-Pro	2,97	2,88			2,88			
L-DAA-Val	4,05	3,62					3,62	
L-DAA-Val	4,07	3,64			3,64			
L-DAA-Thr	8,39	6,51			6,54		6,52	
L-DAA-*allo*-Thr	7,50	6,77						
L-DAA-Hse	7,13	6,62			6,61		6,61	
L-DAA-(4S)-4-OH-Pro	5,78	6,03						
L-DAA-(4R)-4-OH-Pro	4,46	5,08		5,08	5,08			
L-DAA-Glu	2,76	2,58					2,58	
L-DAA-Glu	2,81	2,61			2,61			
L-DAA-Aoa	5,16	4,77		5,16	5,16			
L-DAA-Phe	4,34	4,00		4,35	4,35			
di-L-DAA-Tyr	4,78	4,44		4,77			4,78	
di-L-DAA-Tyr	4,78	4,45			4,78			
L-DLA-Ile	11,80	6,84				6,84		
L-DLA-Ile	11,86	6,87	6,87					6,87
L-DLA-*allo*-Ile	11,76	6,77						
L-DLA-Leu	11,90	6,97				11,91		
L-DLA-Leu	11,94	7,00	11,94	11,95		11,95		11,94
L-DLA-NMe-Ile	NA	8,91				8,91		8,91
D-DLA-Ile	6,84	11,80				11,83		
D-DLA-Ile	6,86	11,85	11,86	11,85			11,86	11,86
D-DLA-*allo*-Ile	6,79	11,78						
D-DLA-Leu	6,97	11,92				6,97		
D-DLA-Leu	6,99	11,92	7,00	7,00			7,00	7,00
D-DLA-NMe-Ile	NA	11,83					11,84	11,84

DAA = dinitrophenylalanine amide, DLA = dinitrophenylleucine amide, NA = Not Available

Table S12. Predicted and activated substrates of adenylation domains in laxaphycins, with the binding pocket amino acid residues as identified with NRPSpredictor2 in AntiSMASH 5.1.2

AA residue	Domain	Predicted	Activated	A-domain residues	Stachelhaus code % match	Domain	Predicted	Activated	A-domain residues	Stachelhaus code % match
	11-residue heinamides					**11-residue scytocyclamides**				
2	$LxaC1_1$	Ser	Ser	DVWHISLIDK	100	$LxaC_1$	Gln	Gln	DAWQFGLIDK	100
3	$LxaC1_2$	Thr	Thr	DFWNIGMVHK	100	$LxaC_2$	Thr	Thr	DFWNIGMVHK	100
4	$LxaC1_3$	Pro	OHPro/Pro	DAQFIAHVVK	90	$LxaC_3$	Pro	OHPro/Pro	DAHFIAHVVK	80
5	$LxaC1_4$	Ser	Ser	DVWHISLIDK	100	$LxaC_4$	Hse*	HSe	DlknFGSdvK	*
6	$LxaC1_5$	Tyr	Tyr	DASTIAAVCK	100	$LxaC_5$	Phe	Phe	DAWTIAAVCK	90
7	$LxaC1_6$	Leu	Leu	DAWFLGNVVK	100	$LxaC_6$	Leu	Leu	DAWFLGNVVK	100
8	$LxaC1_7$	Ile	Ile	DAFFLGVTFK	100	$LxaD_1$	Ile	Ile	DAFFLGVTFK	100
9	$LxaC1_8$	Phe	Phe	DAWTIAAVCK	90	$LxaD_2$	Ile	Ile	DAFFLGVTFK	100
10	$LxaC1_9$	Pro	OHPro/Pro	DAHFIAHVVK	80	$LxaD_3$	Leu	Leu	DAWFLGNVVK	100
11	$LxaC1_{10}$	Gly	Gly	DILQLGLIWK	100	$LxaD_4$	Gly	Gly	DILQLGLIWK	100
	12-residue heinamides					**12-residue scytocyclamides**				
2	$LxaI1_1$	Ile	Ile	DAFFLGVTFK	100	$LxaI_1$	Val	Val	DAFWLGGTFK	90
3	$LxaI1_2$	Leu	Leu	DAWFLGNVVK	100	$LxaI_2$	Leu	Leu	DAWFLGNVVK	100
4	$LxaI1_3$	Hse*	Hse	DlknFGSdvK	*	$LxaI_3$	Ala	Ala	DLFNNALTYK	100
5	$LxaI1_4$	Leu	Leu	DAWFLGNVVK	100	$LxaJ_1$	Leu	Leu	---FLGNVVK	70
6	$LxaI1_5$	Gln	Gln	DAWQFGLIDK	100	$LxaJ_2$	Gln	Gln	DAWQFGLIDK	100
7	$LxaI1_6$	Ile	Ile	DAFFLGVTFK	100	$LxaJ_3$	Ile	Ile	DAFFLGVTFK	100
8	$LxaJ1_1$	Hse*	Hse	DlknFGSdvK	*	$LxaK_1$	Asn	Asn	DLTKIGEVGK	100
9	$LxaK1_1$	Val	Val	DAFWLGGTFK	90	$LxaK_2$	Thr	Thr	DFWNIGMVHK	100
10	$LxaK1_2$	Pro	OHMePro/Pro	DVQFIAHAAK	90	$LxaK_3$	Pro	Pro	DVQFMAHVVK	90
11	$LxaK1_3$	Tyr	Tyr	DASTIAAVCK	100	$LxaK_4$	Leu	Leu	DAWFLGNVVK	100
12	$LxaK1_4$	Thr	Thr	DFWNIGMVHK	100	$LxaL_1$	Thr	Thr	DFWNIGMVHK	100

*Prediction by AntiSMASH 4.1.0 based on stachelhaus code.

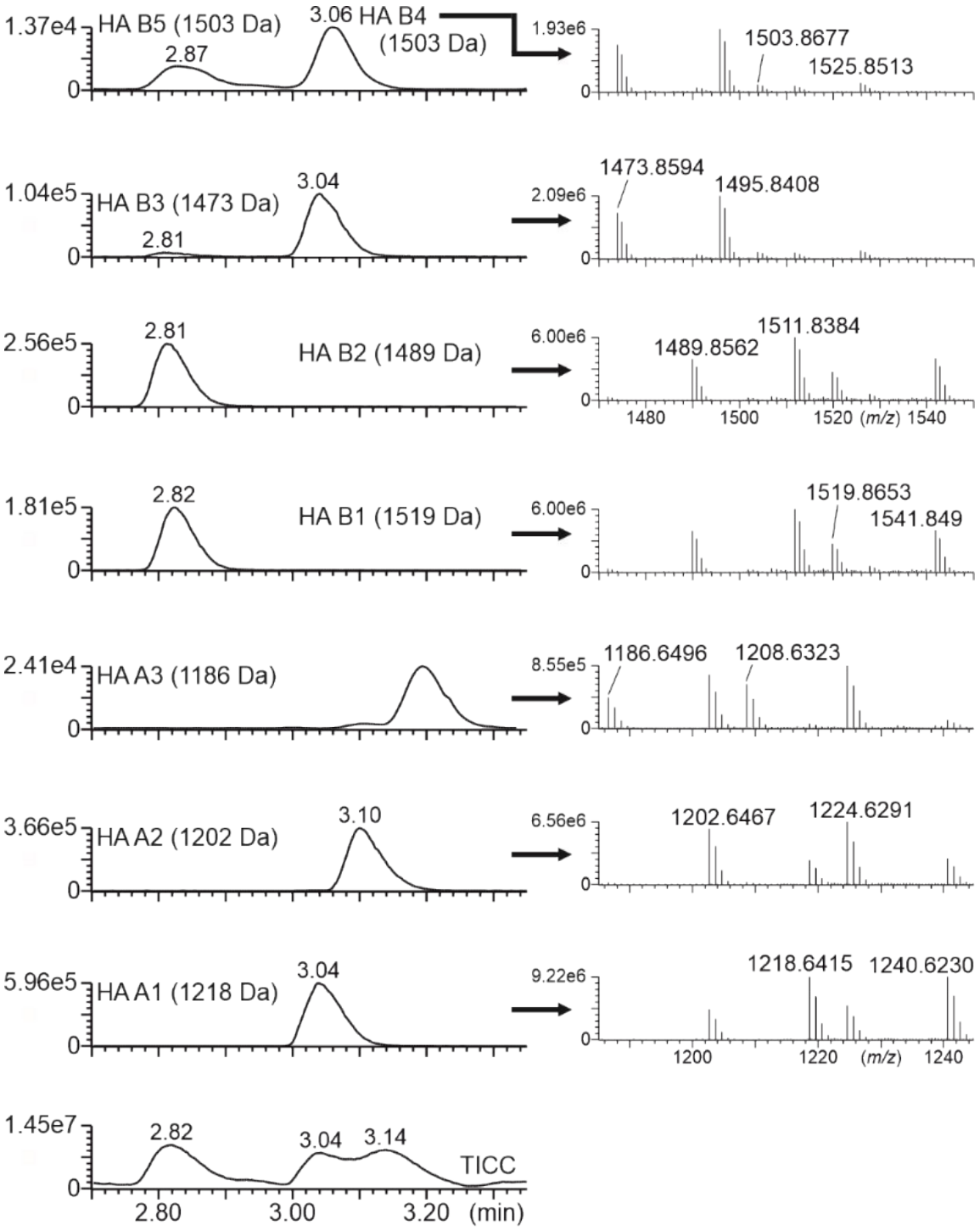


Figure S1. Extracted ion chromatograms of heinamides (HA), total ion current chromatogram (TICC), and mass spectra from heinamide peaks with $[M+H]^+$ and $[M+Na]^+$ ion masses.

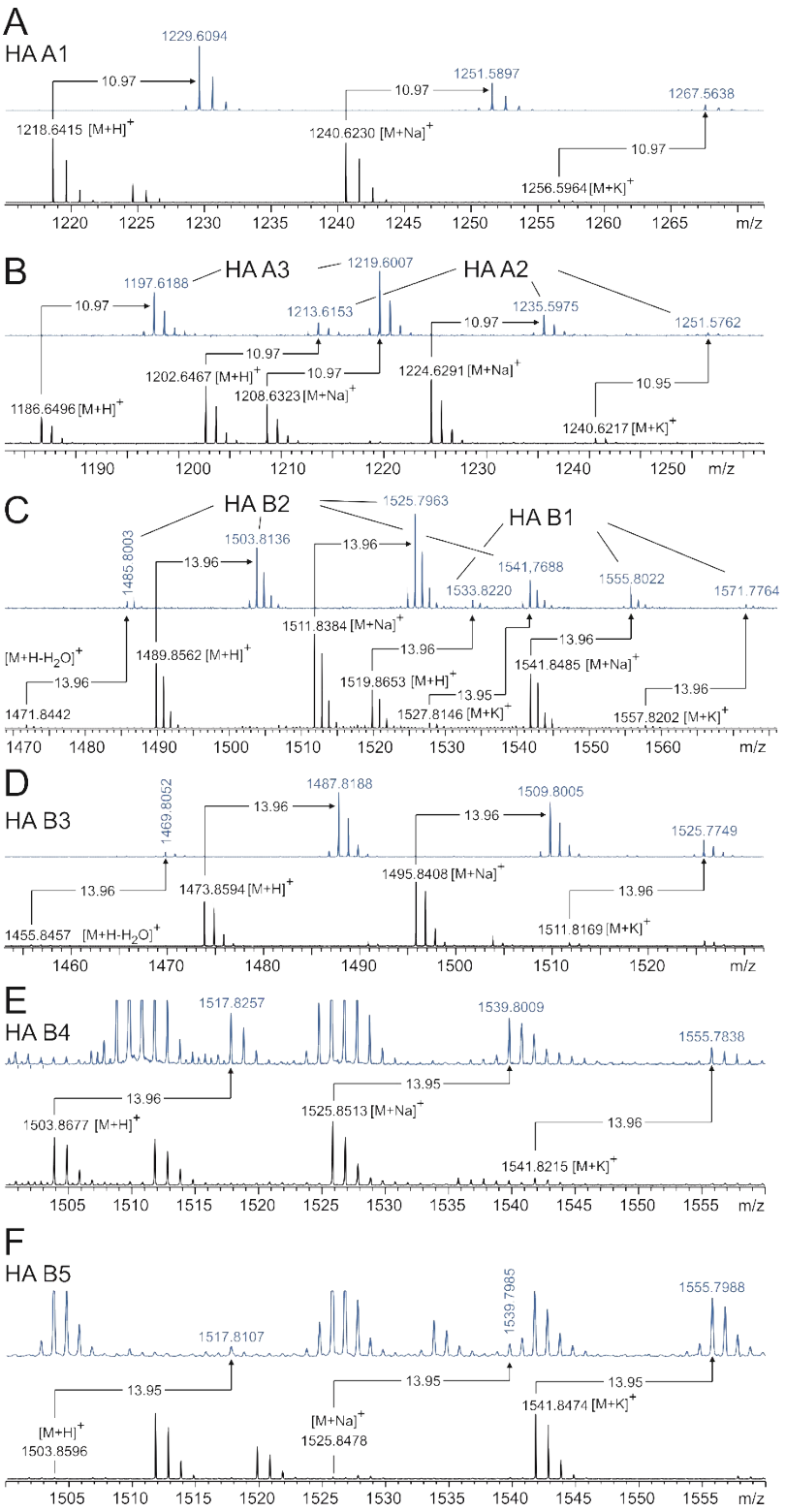


Figure S2. UPLC-QTOF mass spectra of unlabeled (^{14}N, black trace) and nitrogen-labeled (^{15}N, blue trace) heinamides (HA) A1 (A), A3 and A2 (B), B1 and B2 (C), B3 (D), B4 (E), and B5 (F). Mass increase of 10.97 (10.95 in HA B5) and 13.96 (10.95 in HA B5) Da of protonated and sodium/potassium adduct ions after ^{15}N-labeling shows presence of 11 and 14 N atoms in the laxa A group (A1-3) and laxa B group (B1-5) heinamides, respectively.

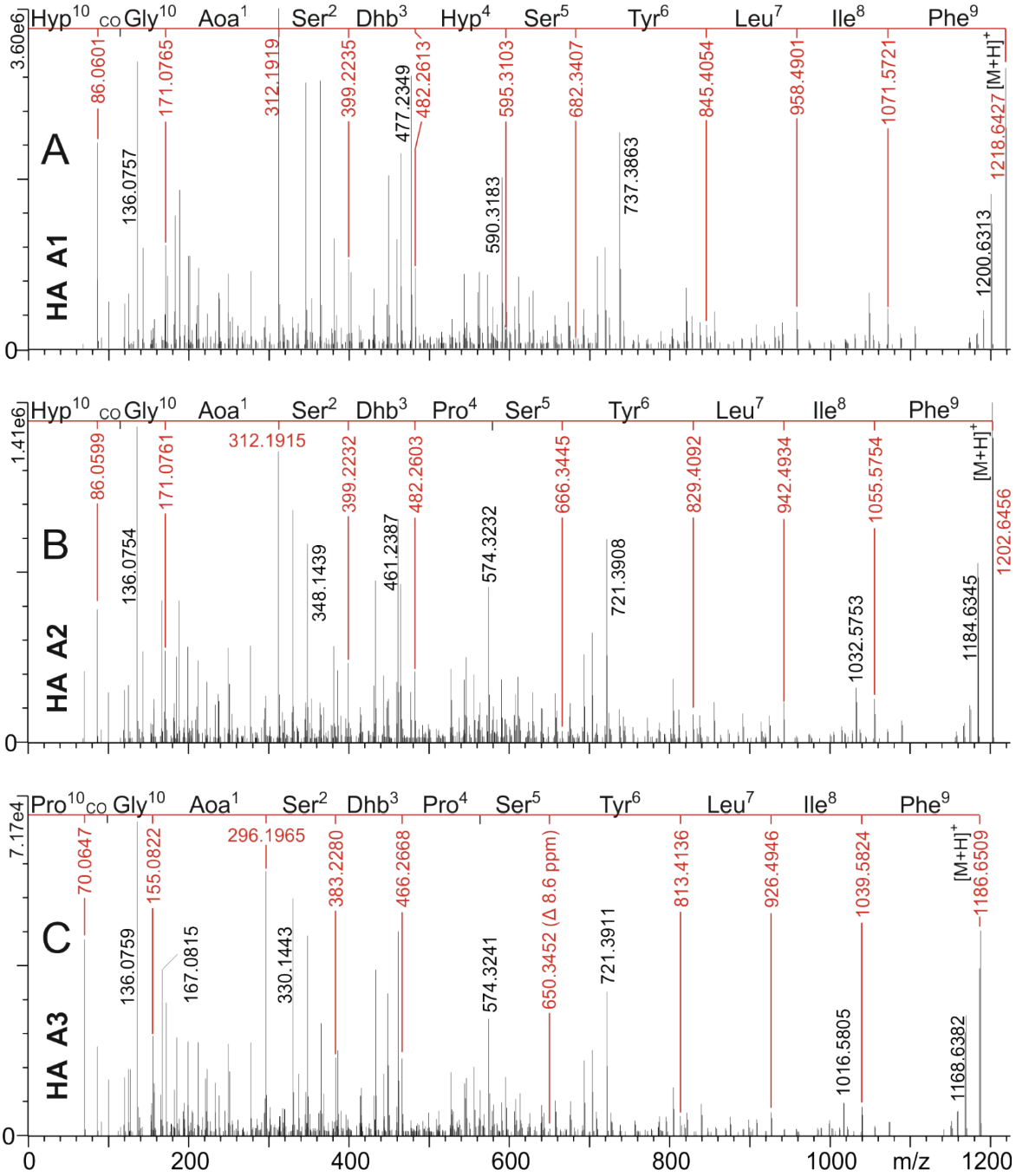


Figure S3. UPLC-QTOF product ion mass spectra of protonated Laxa A group heinamides (HA) A1 (A), A2 (B), and A3 (C). The most complete product ion series showing the amino acid sequences are marked with red numbers and lines. Hyp = OHPro.

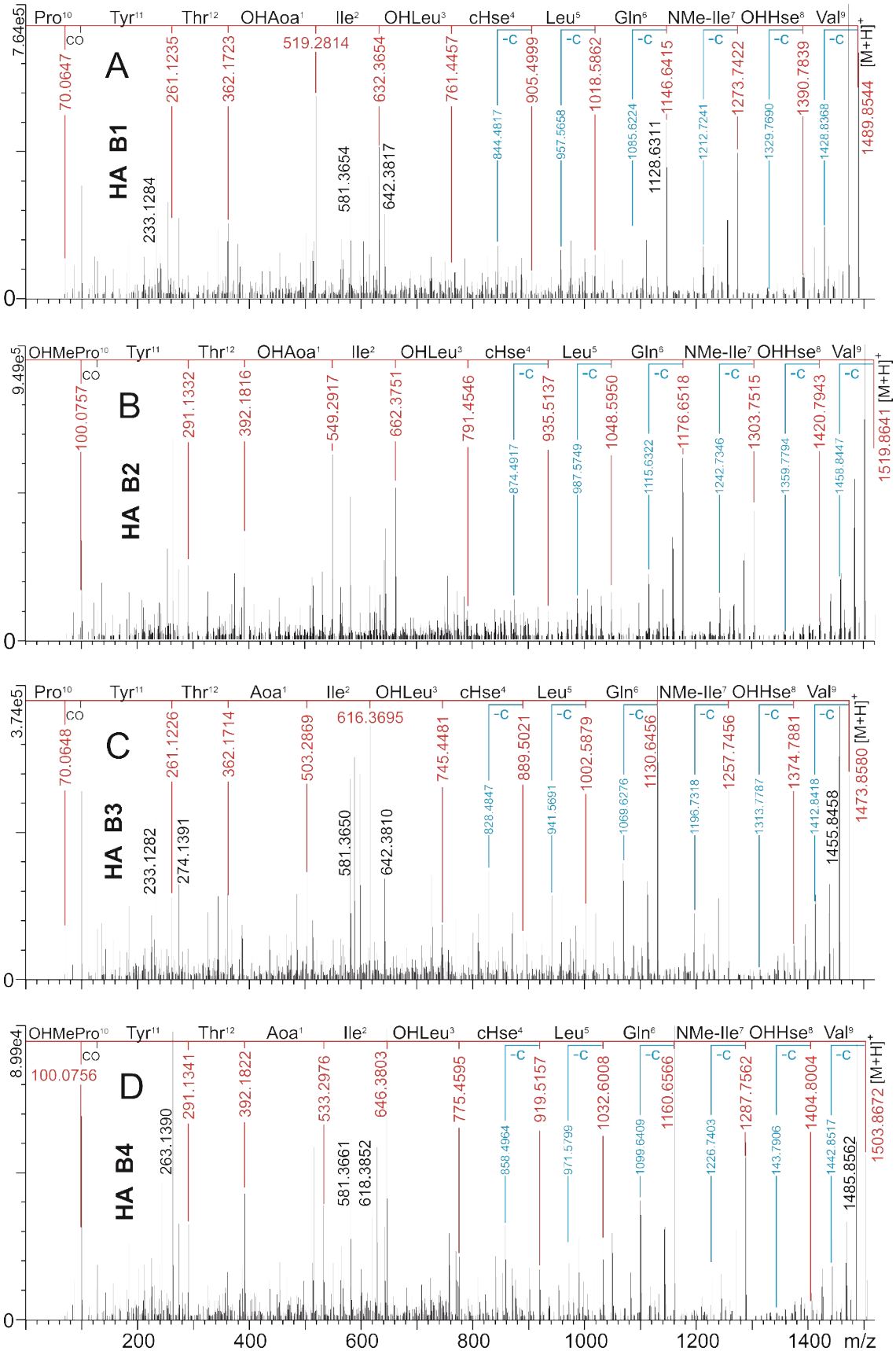


Figure S4. UPLC-QTOF product ion mass spectra of protonated Laxa B group heinamides (HA) B1 (A), B2 (B), B3 (C), and B4 (D). The most complete product ion series showing the amino acid sequences are marked with red numbers and lines. cHse = O-carbamoyl-Hse, -C = loss of carbamoyl group as NH_2COOH (61.02 Da, marked with blue numbers and lines).

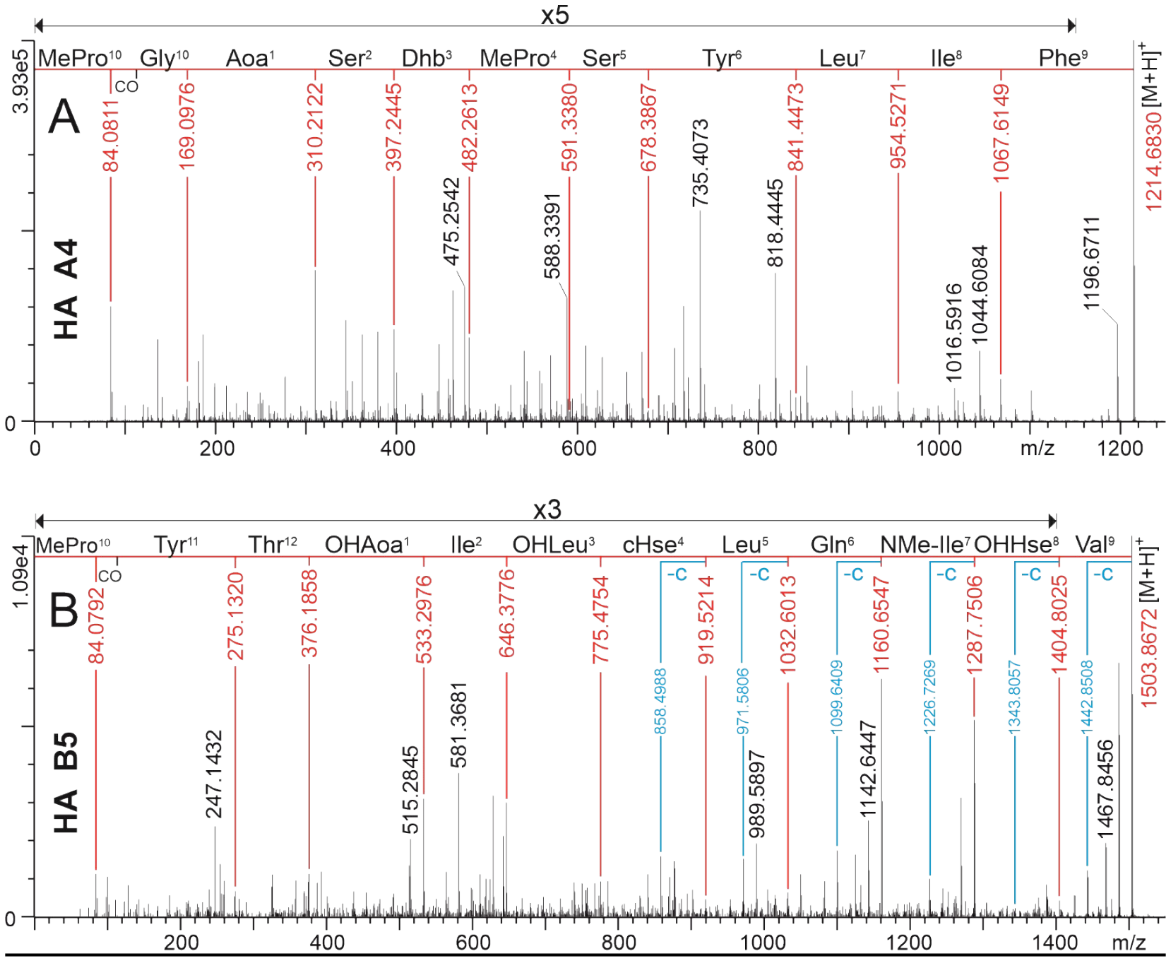


Figure S5. UPLC-QTOF product ion mass spectra of protonated 11-residue laxaphycin heinamide HA A4 (A) and 12-residue laxaphycin heinamide HA B5 (B) obtained by feeding *Nostoc* sp. UHCC 0702 with (2S,4R)-MePro or (2S,4S)-MePro, respectively. The most complete product ion series showing the amino acid sequences are marked with red numbers and lines. Hyp = OHPro. cHse = O-carbamoyl-Hse, -C = loss of carbamoyl group as NH_2COOH (61.02 Da, marked with blue numbers and lines). Line above the spectra shows the range of magnified (X5, x3) ion intensities.

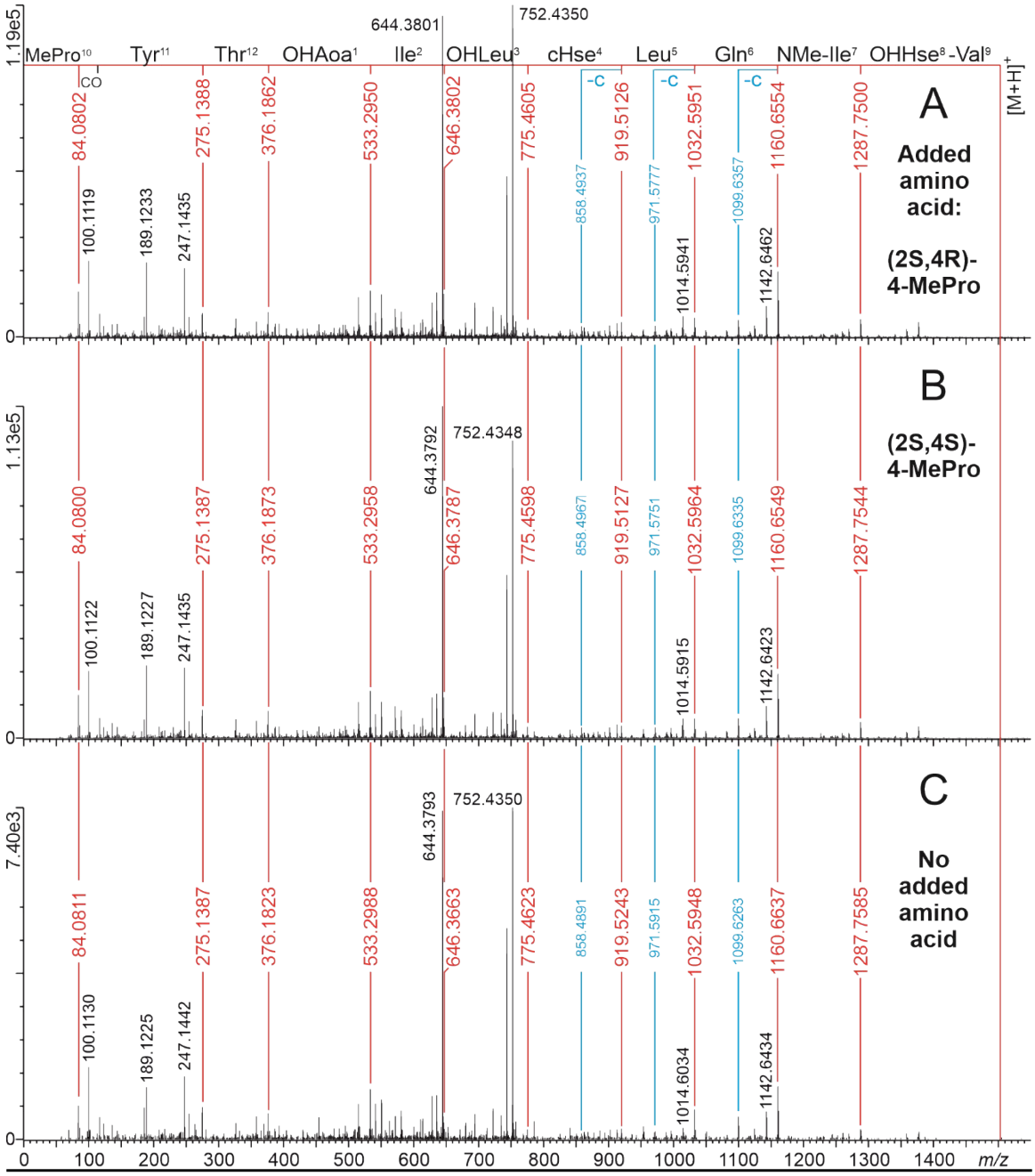
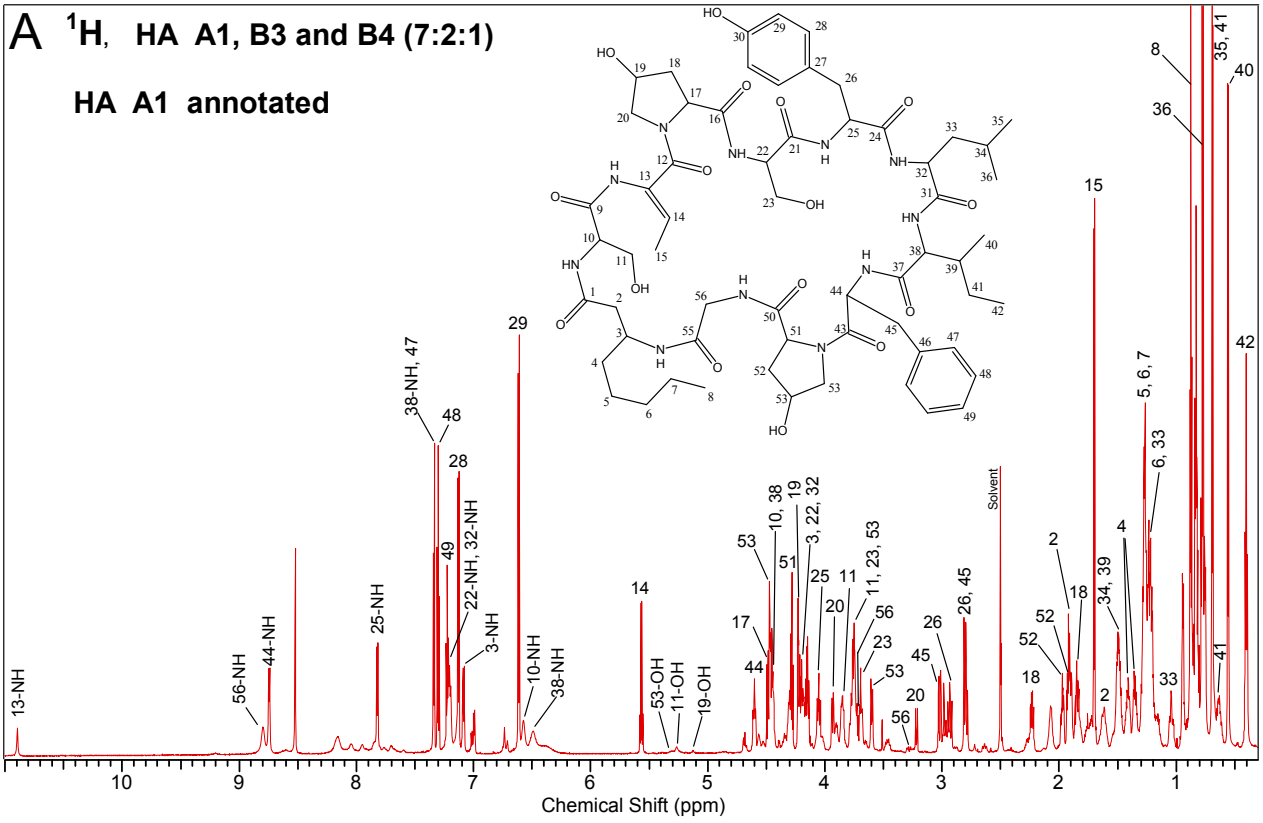
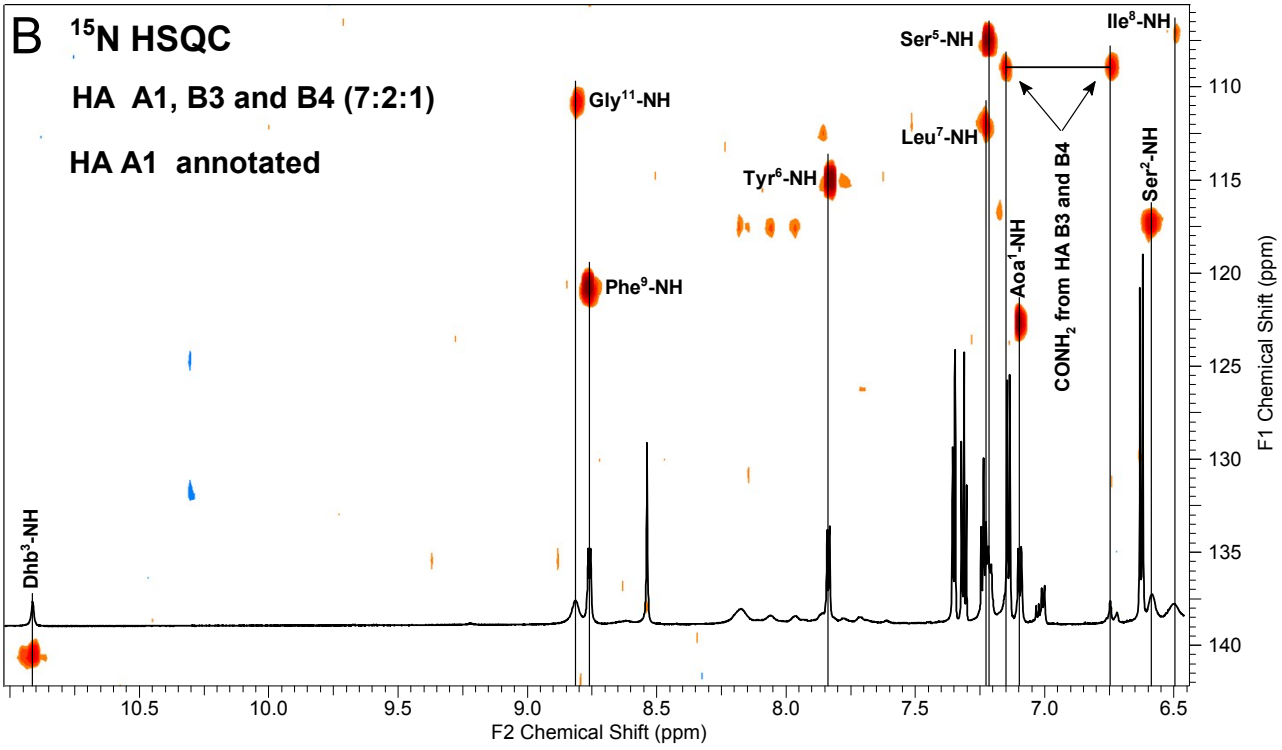


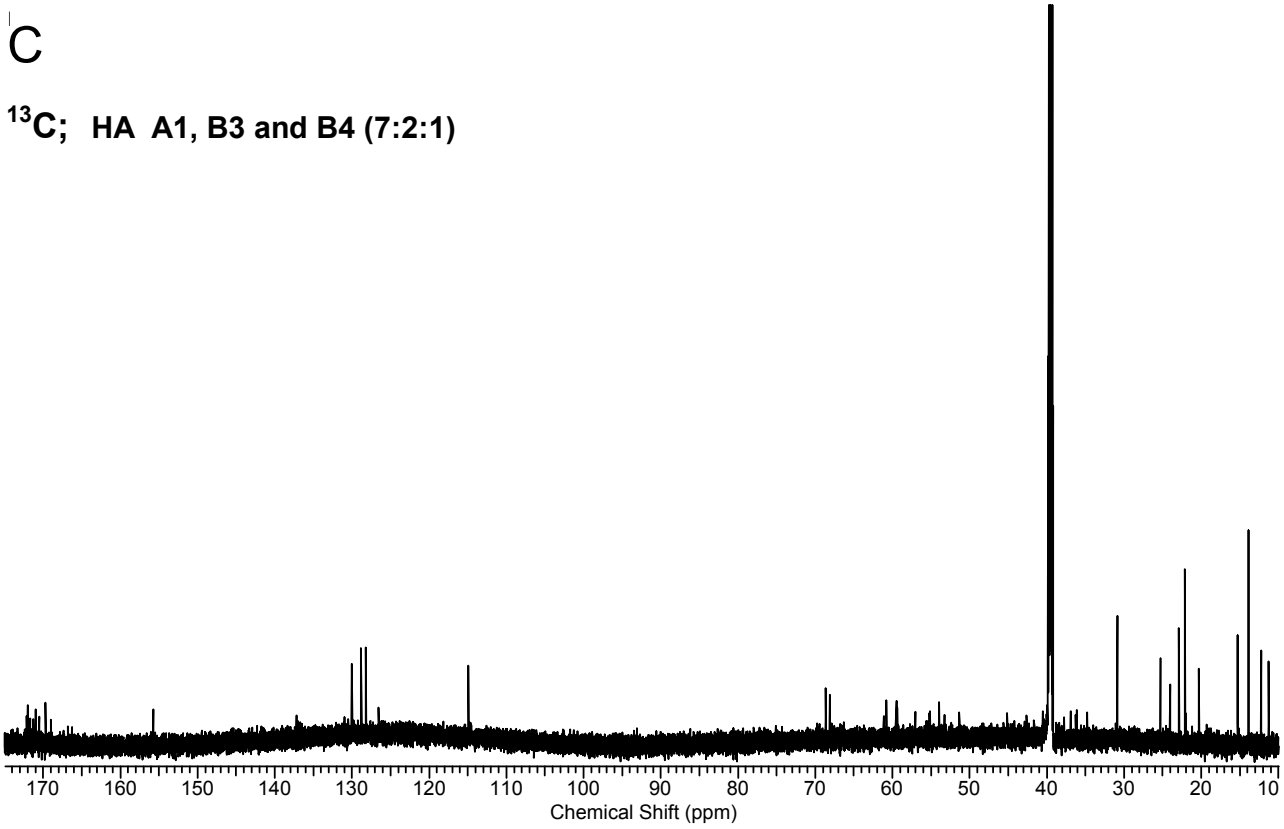
Figure S6. UPLC-QTOF product ion mass spectra of doubly protonated 12-residue laxaphycin heinamide HA B5 obtained by feeding *Nostoc* sp. UHCC 0702 with (2S,4R)-4-MePro (A) or (2S,4S)-4-MePro (B) or without feeding of extra amino acid. Product ion series showing the amino acid sequence are marked with red numbers and lines. cHse = O-carbamoyl-Hse, -C = loss of carbamoyl group as NH_2COOH (61.02 Da, marked with blue numbers and lines).


A
1H, HA A1, B3 and B4 (7:2:1)
HA A1 annotated
13-NH
56-NH
44-NH
25-NH
38-NH, 47
48
28
49
22-NH, 32-NH
3-NH
29
10-NH
38-NH
14
53-OH
11-OH
19-OH
53
17
44
51
10, 38
19
3, 22, 32
25
20
11
11, 23, 53
56
23
53
20
56
26
45
26, 45
Solvent
52
18
2
52
18
2
34, 39
4
6, 33
5, 6, 7
33
41
15
8
36
35, 41
40
42
10
9
8
7
6
5
4
3
2
1
Chemical Shift (ppm)


B
15N HSQC
HA A1, B3 and B4 (7:2:1)
HA A1 annotated
Dhb3-NH
Phe9-NH
Gly11-NH
Tyr6-NH
Leu7-NH
Ser5-NH
Ile8-NH
Aoa1-NH
CONH2 from HA B3 and B4
Ser2-NH
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
F2 Chemical Shift (ppm)
110
115
120
125
130
135
140
F1 Chemical Shift (ppm)

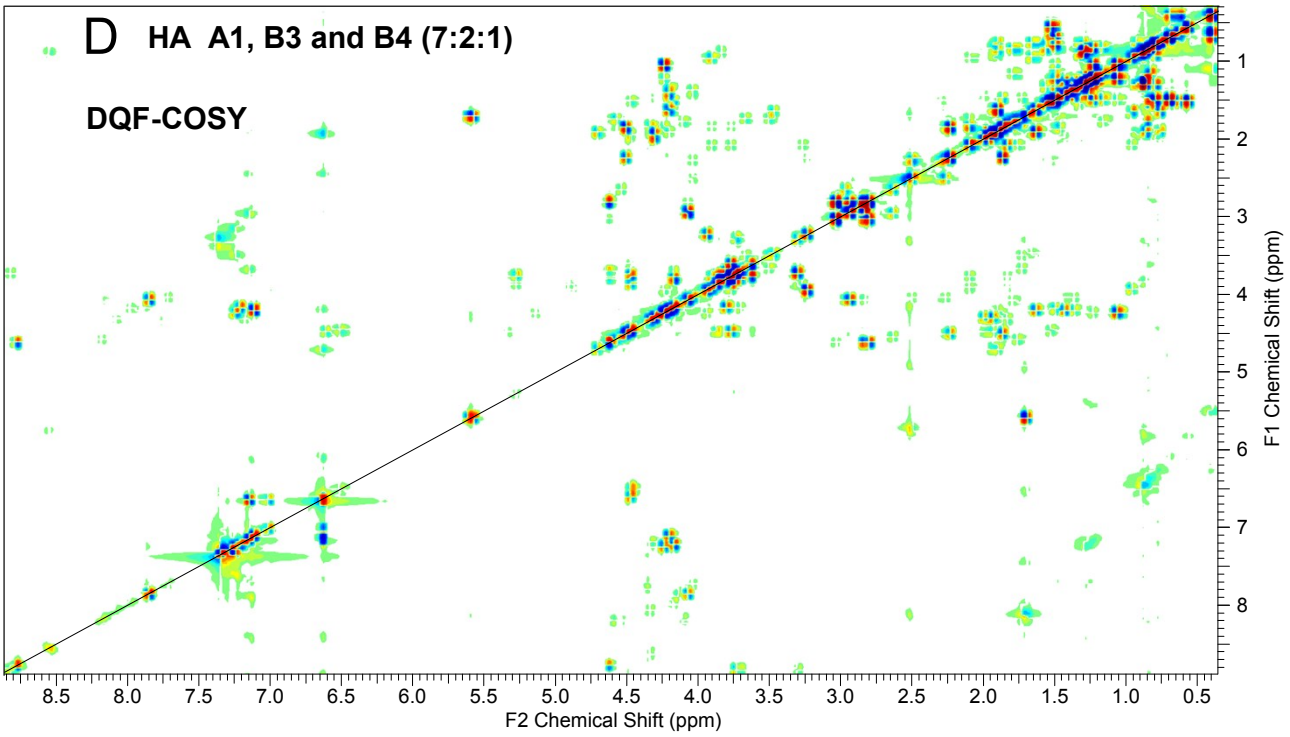
C

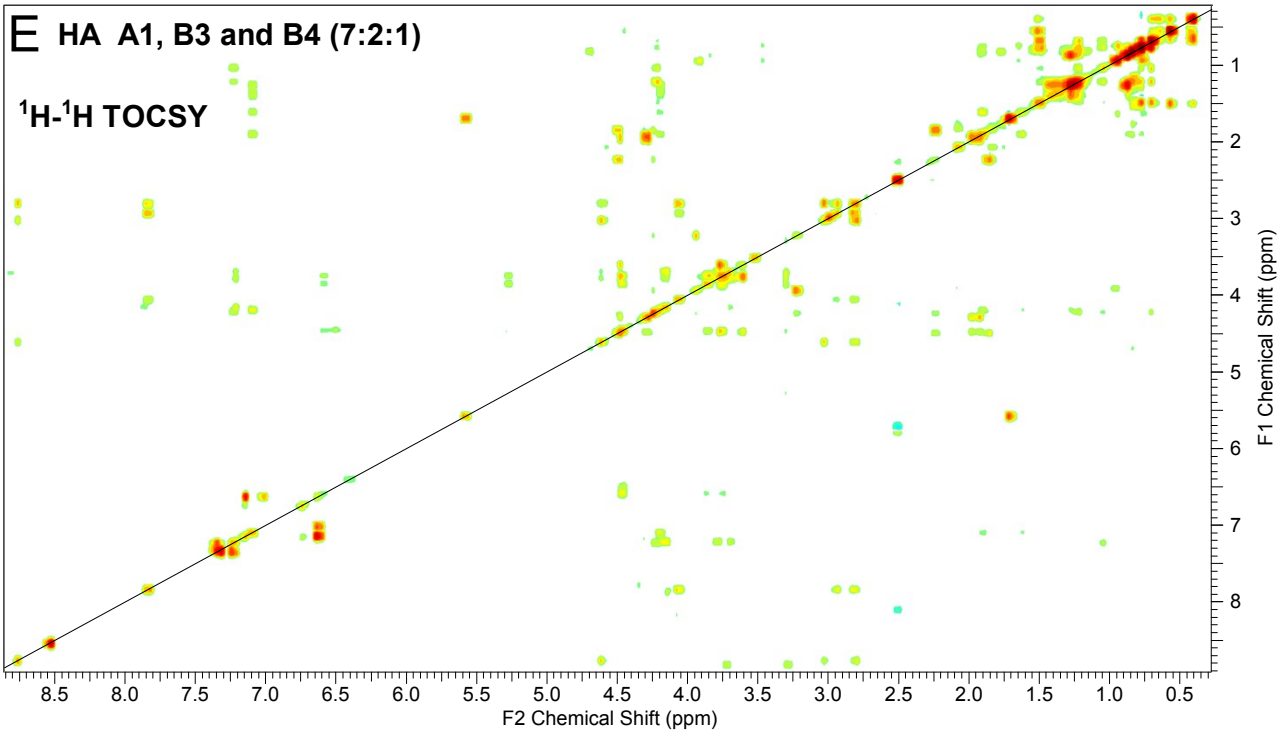
^{13}C; HA A1, B3 and B4 (7:2:1)

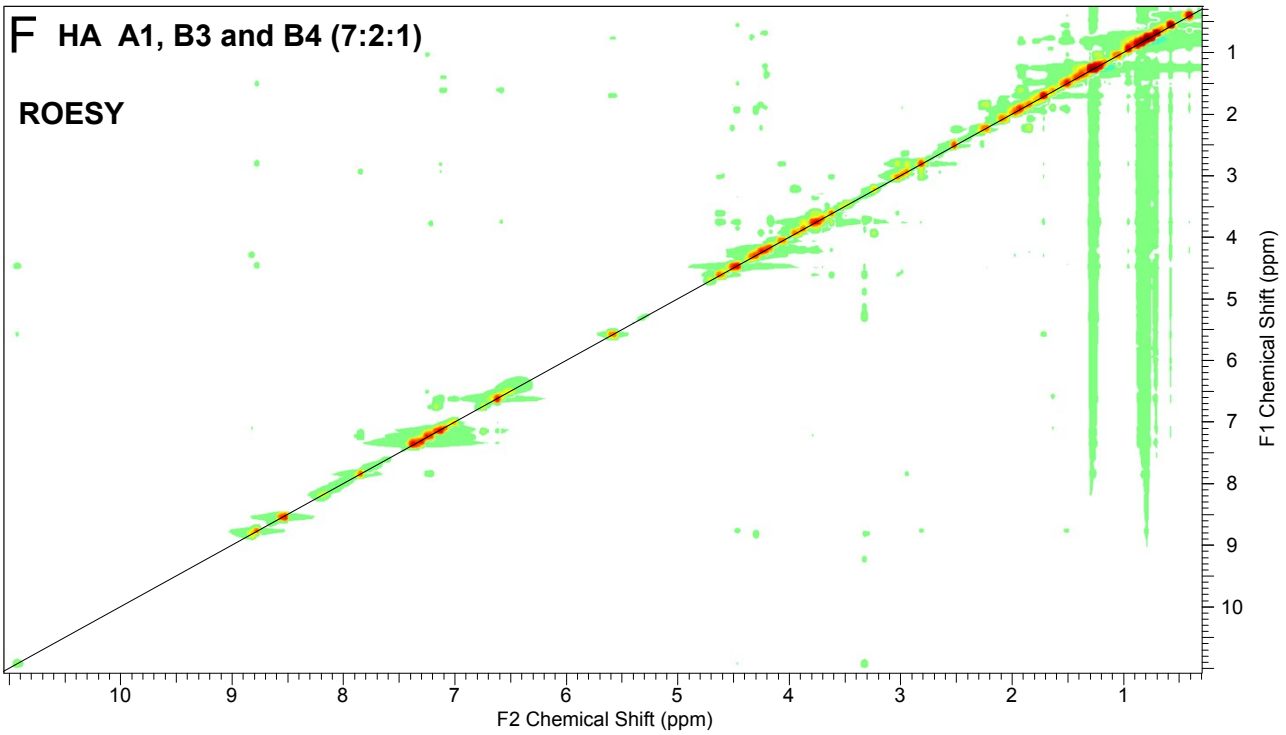


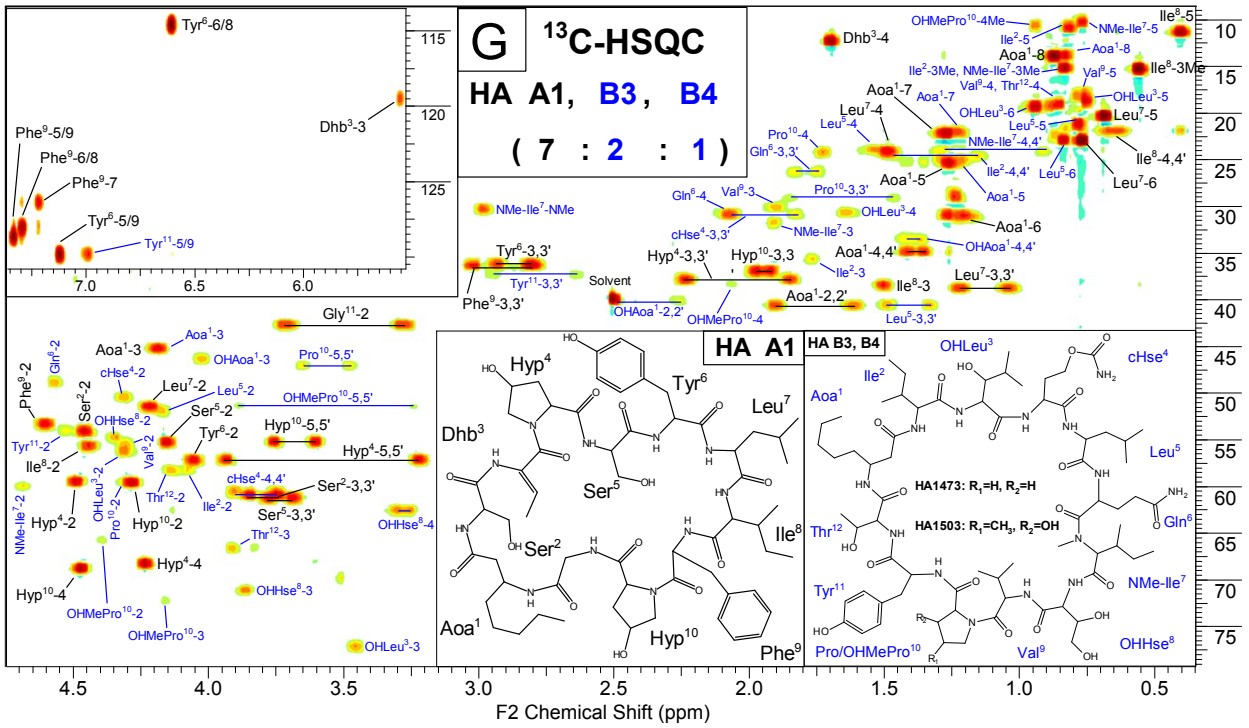
D **HA A1, B3 and B4 (7:2:1)**

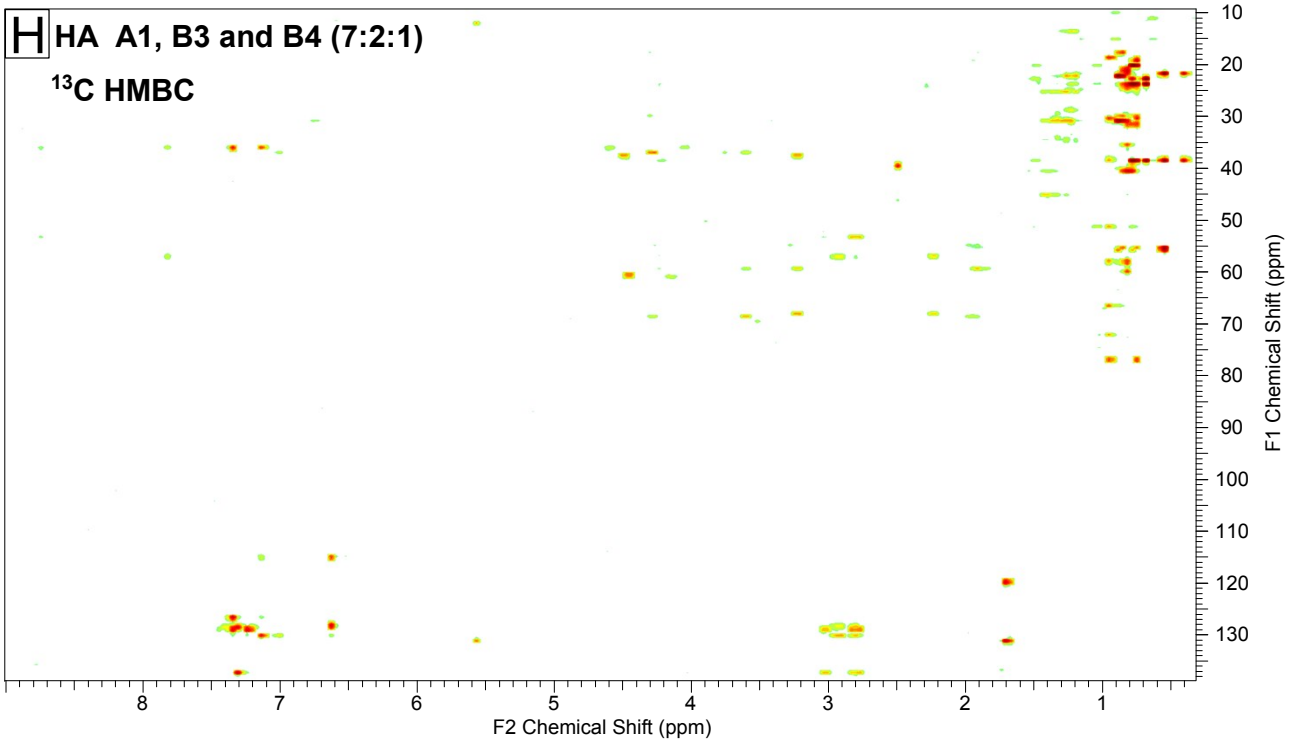
DQF-COSY




E
HA A1, B3 and B4 (7:2:1)
^{1}H-^{1}H TOCSY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


F
HA A1, B3 and B4 (7:2:1)
ROESY
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


G
13C-HSQC
HA A1, B3, B4
(7 : 2 : 1)
HA A1
HA B3, B4
HA1473: R1=H, R2=H
HA1503: R1=CH3, R2=OH
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


H
HA A1, B3 and B4 (7:2:1)
13C HMBC
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

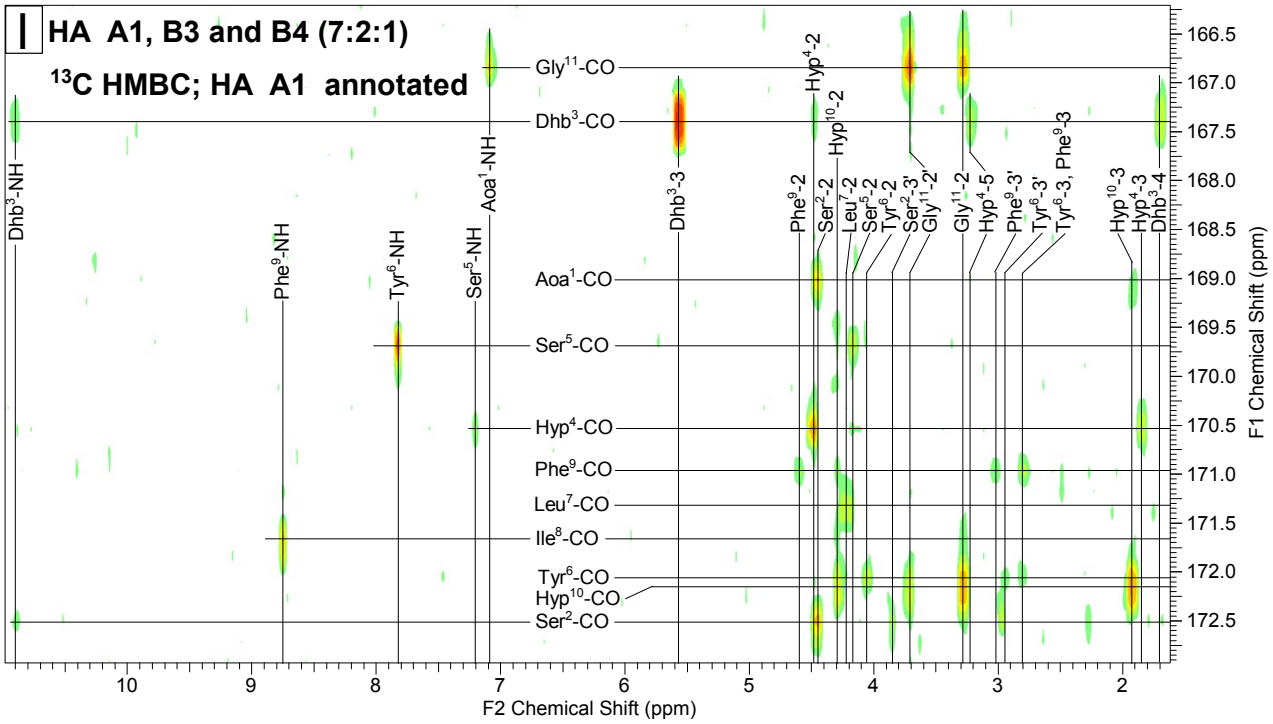
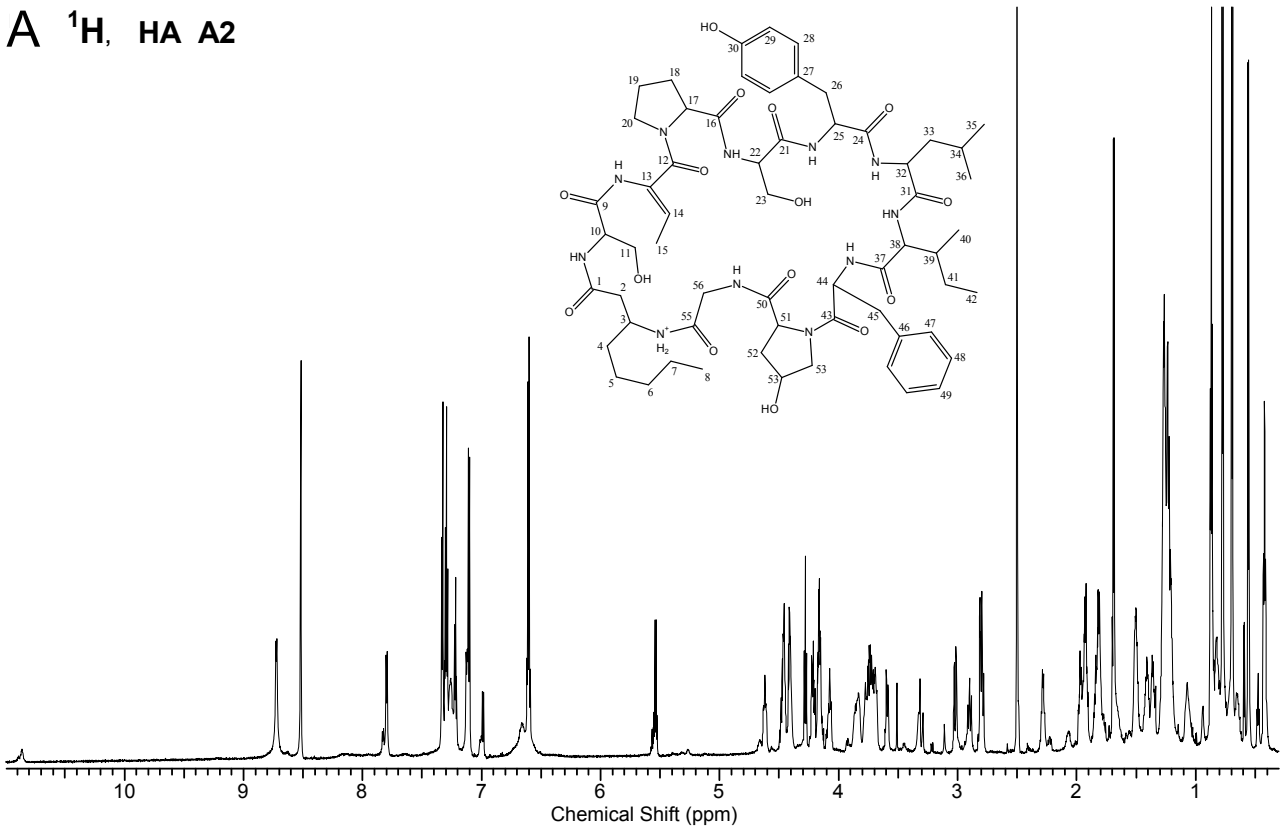
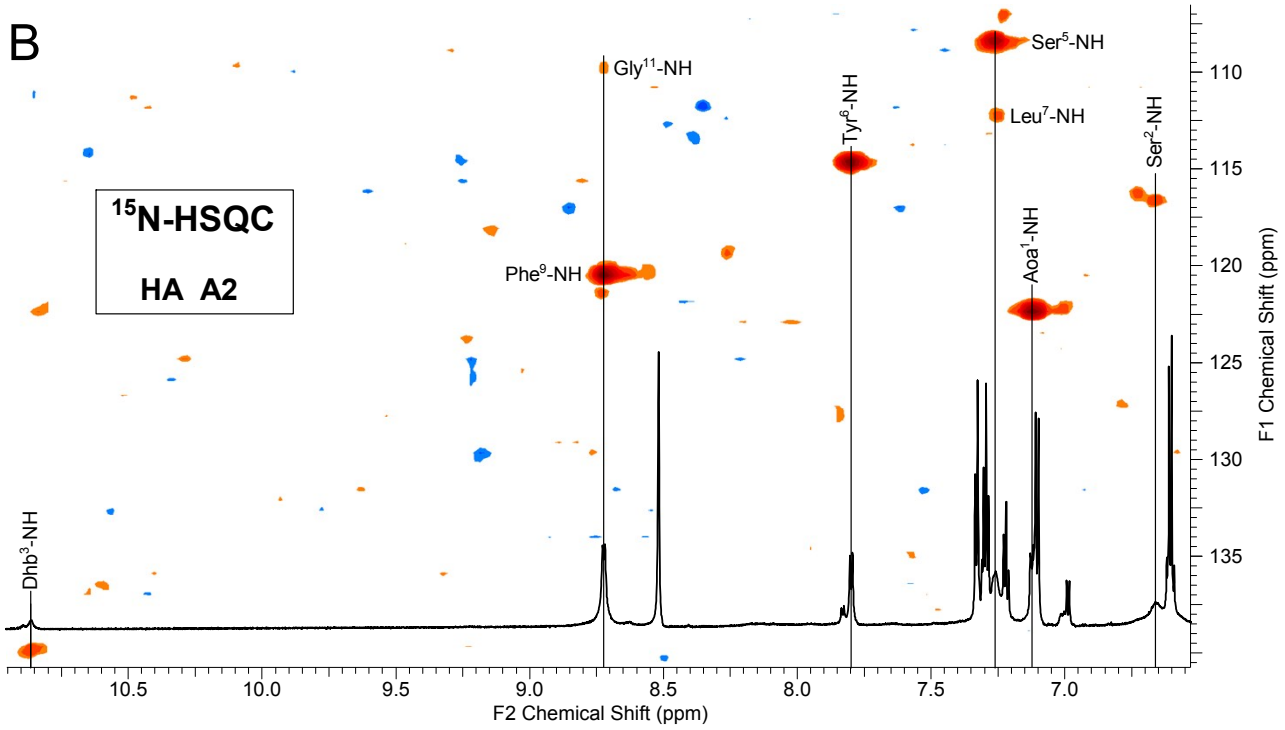


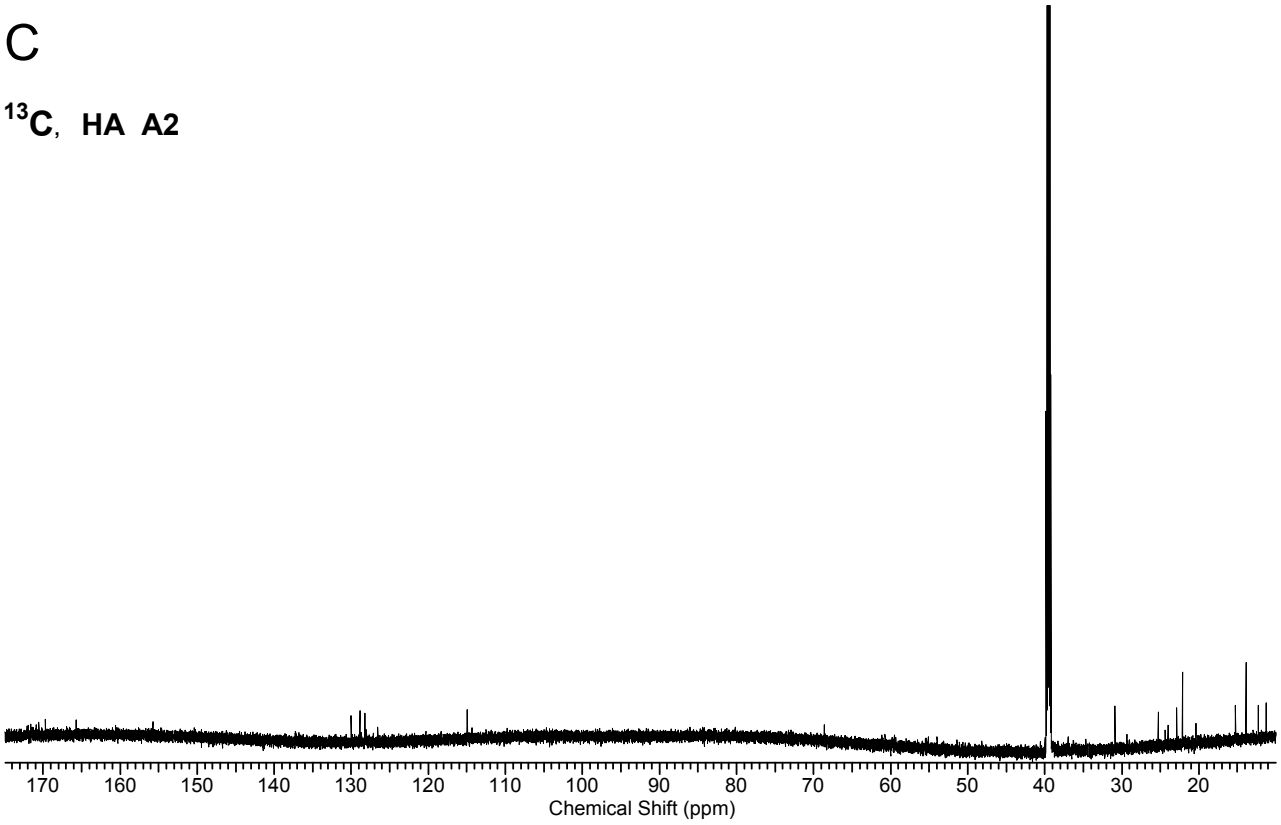
Figure S7. NMR spectra from heinamide (HA) A1, B3 and B4 (7:2:1) mixture. A: ^{1}H (HA A1 annotated), B: ^{1}H+^{15}N-HSQC (HA A1 annotated), C: ^{13}C, D: DQF-COSY, E: ^{1}H-^{1}H TOCSY, F: ROESY, G: ^{13}C-HSQC (HA A1, B3, and B4 annotated), H: ^{13}C-HMBC, I: annotated ^{13}C-HMBC carbonyl region.

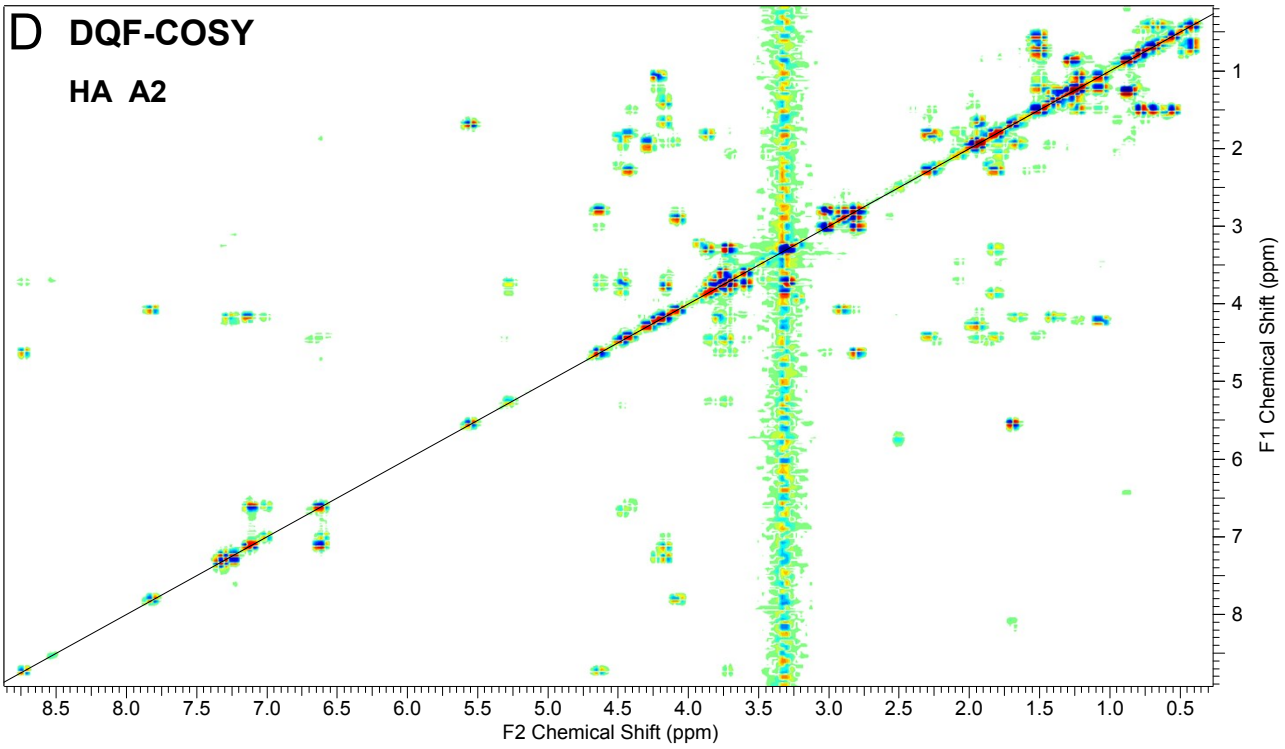


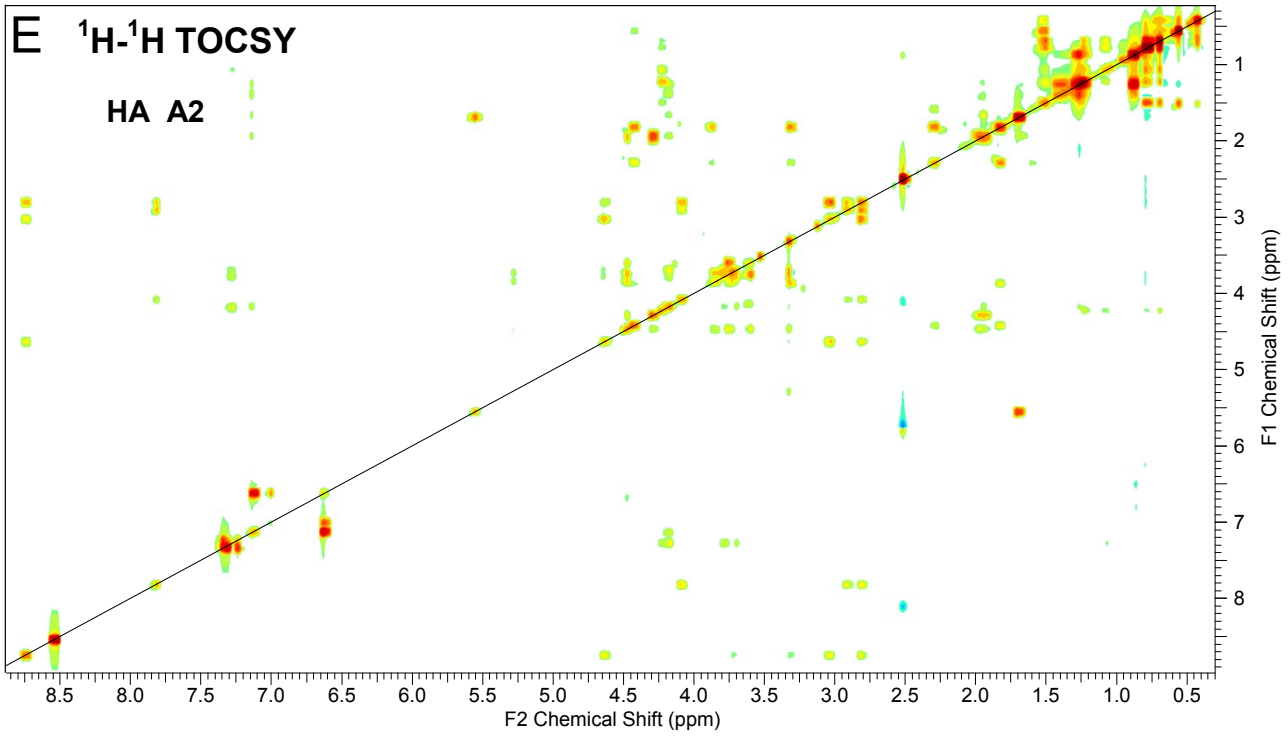

B
15N-HSQC
HA A2
Dhb3-NH
Phe9-NH
Gly11-NH
Tyr6-NH
Ser5-NH
Leu7-NH
Aoa1-NH
Ser2-NH
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
F2 Chemical Shift (ppm)
110
115
120
125
130
135
F1 Chemical Shift (ppm)

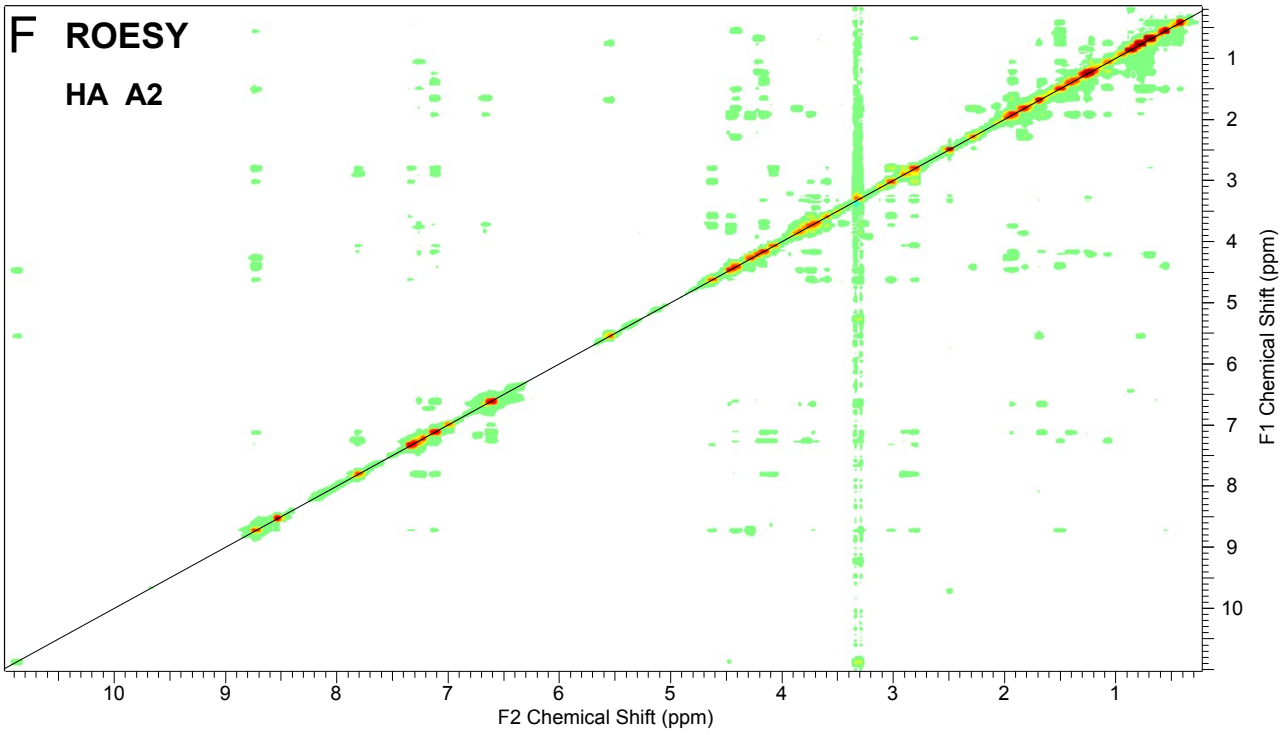
C

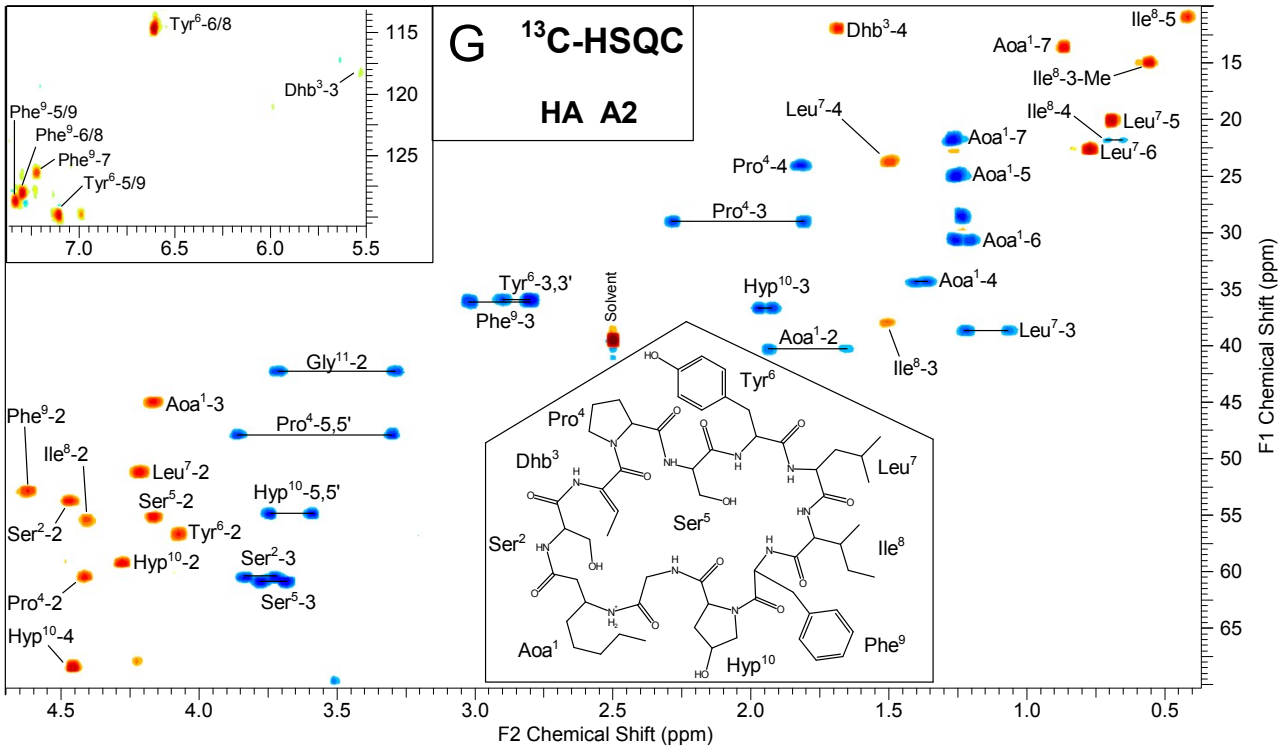
^{13}C, HA A2

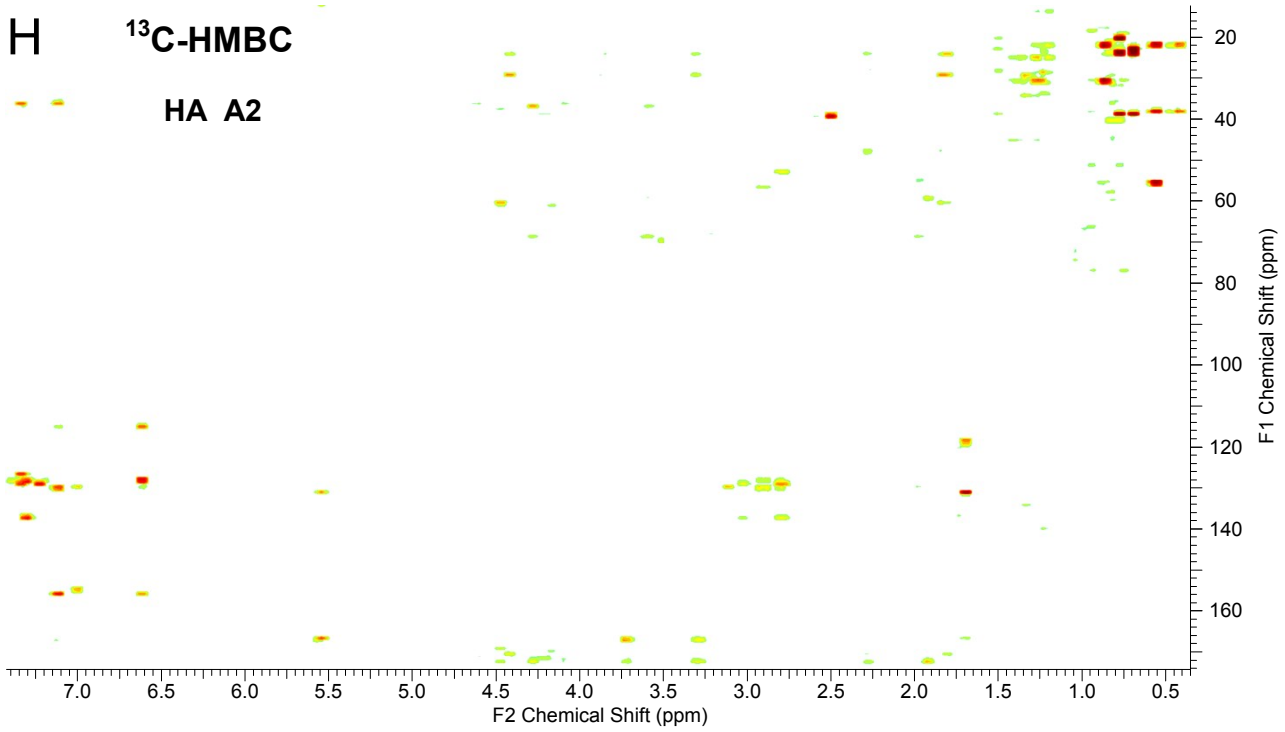

170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
Chemical Shift (ppm)


D DQF-COSY
HA A2
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F2 Chemical Shift (ppm)
1
2
3
4
5
6
7
8
F1 Chemical Shift (ppm)


E
^{1}H-^{1}H TOCSY
HA A2
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


F
ROESY
HA A2
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


G
13C-HSQC
HA A2
Tyr6-6/8
Dhb3-3
Phe9-5/9
Phe9-6/8
Phe9-7
Tyr6-5/9
Dhb3-4
Ile8-5
Aoa1-7
Ile8-3-Me
Leu7-4
Ile8-4
Leu7-5
Leu7-6
Aoa1-7
Pro4-4
Aoa1-5
Pro4-3
Aoa1-6
Tyr6-3,3'
Solvent
Hyp10-3
Aoa1-4
Phe9-3
Aoa1-2
Leu7-3
Ile8-3
Gly11-2
Tyr6
Aoa1-3
Pro4
Phe9-2
Pro4-5,5'
Dhb3
Leu7
Ile8-2
Leu7-2
Ser5-2
Hyp10-5,5'
Ser5
Ser2
Ser2-2
Tyr6-2
Ile8
Hyp10-2
Ser2-3
Ser5-3
Pro4-2
Aoa1
Phe9
Hyp10
Hyp10-4
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


H
13C-HMBC
HA A2
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

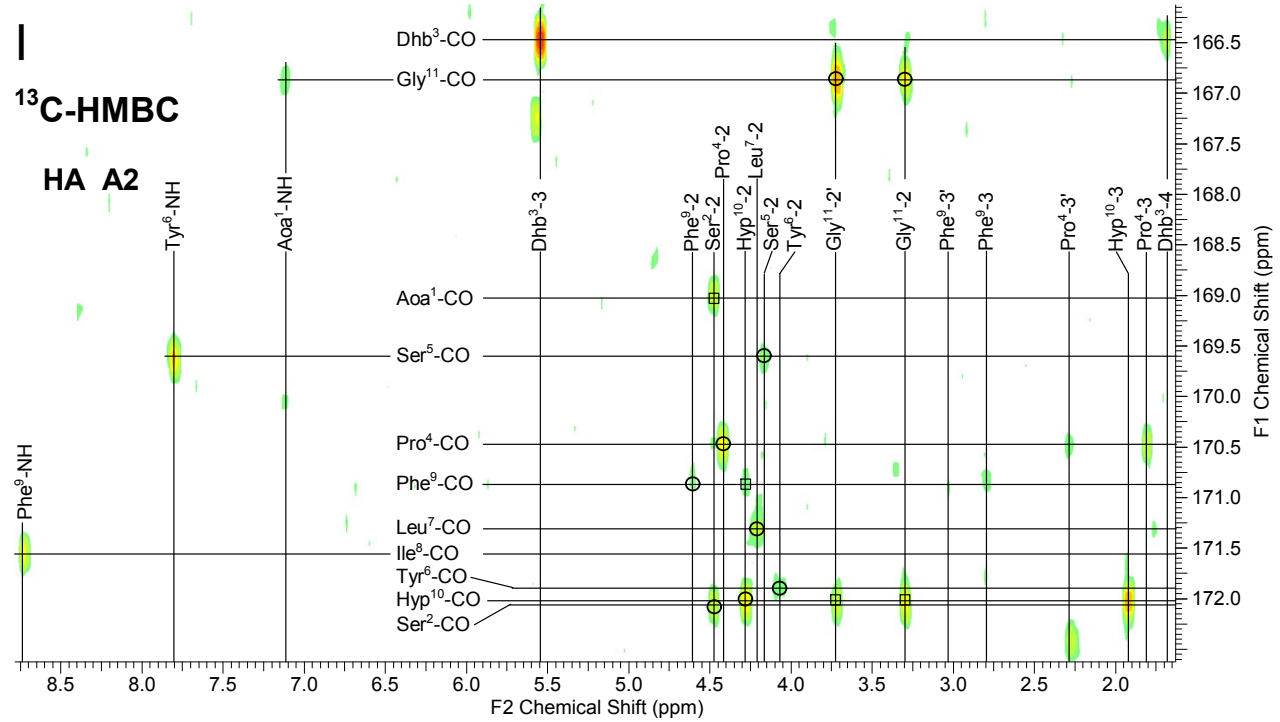
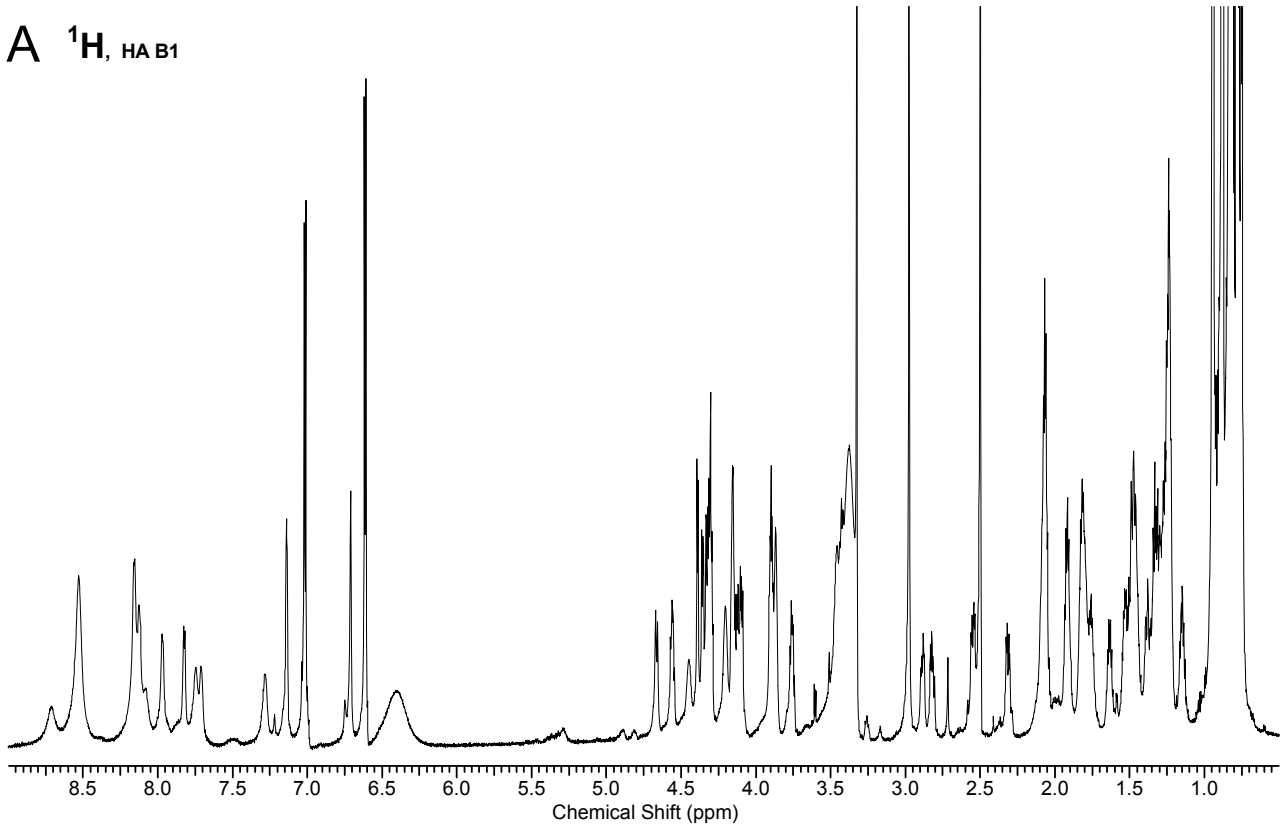
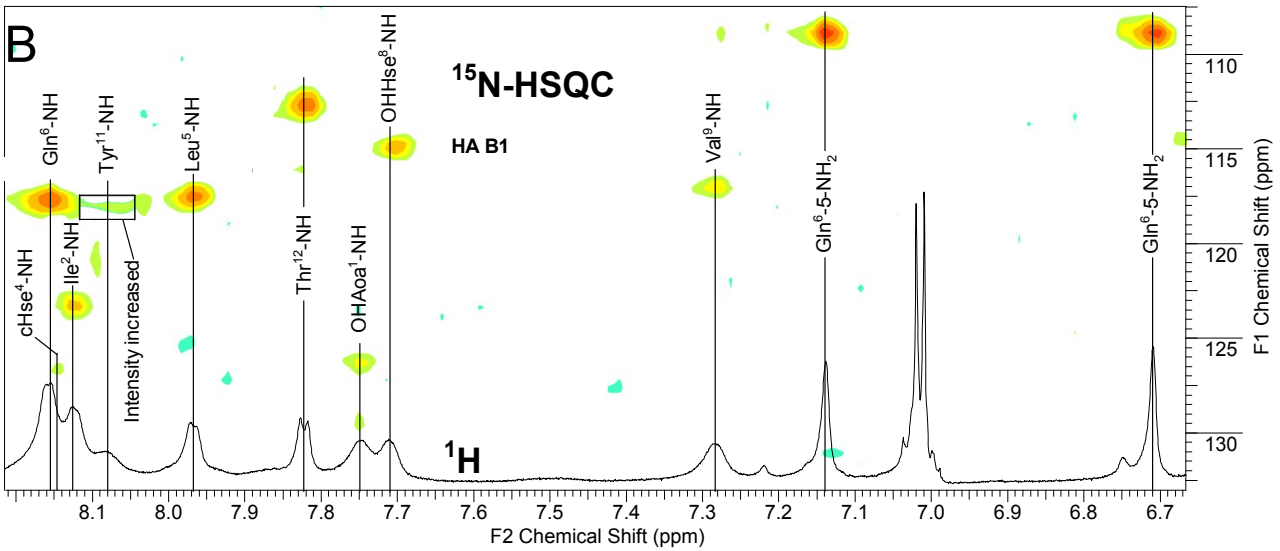
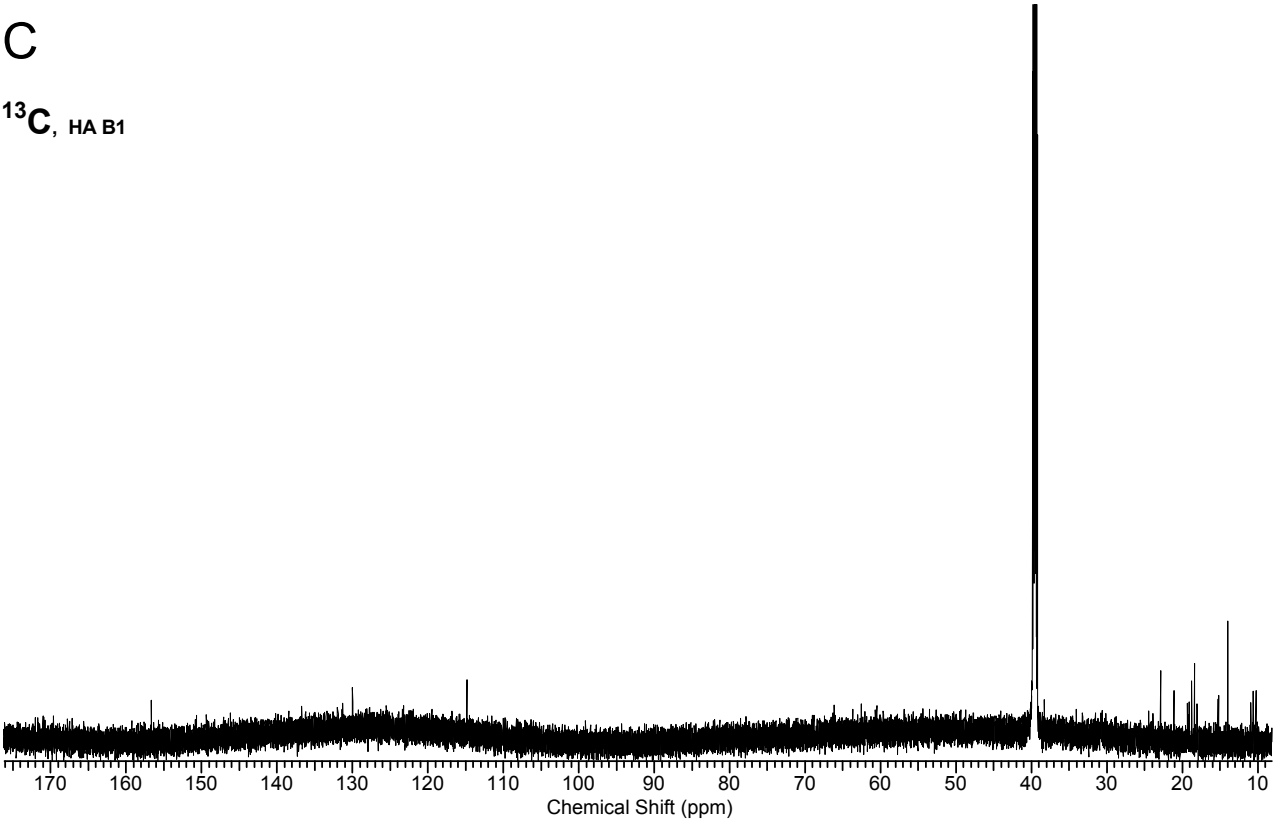
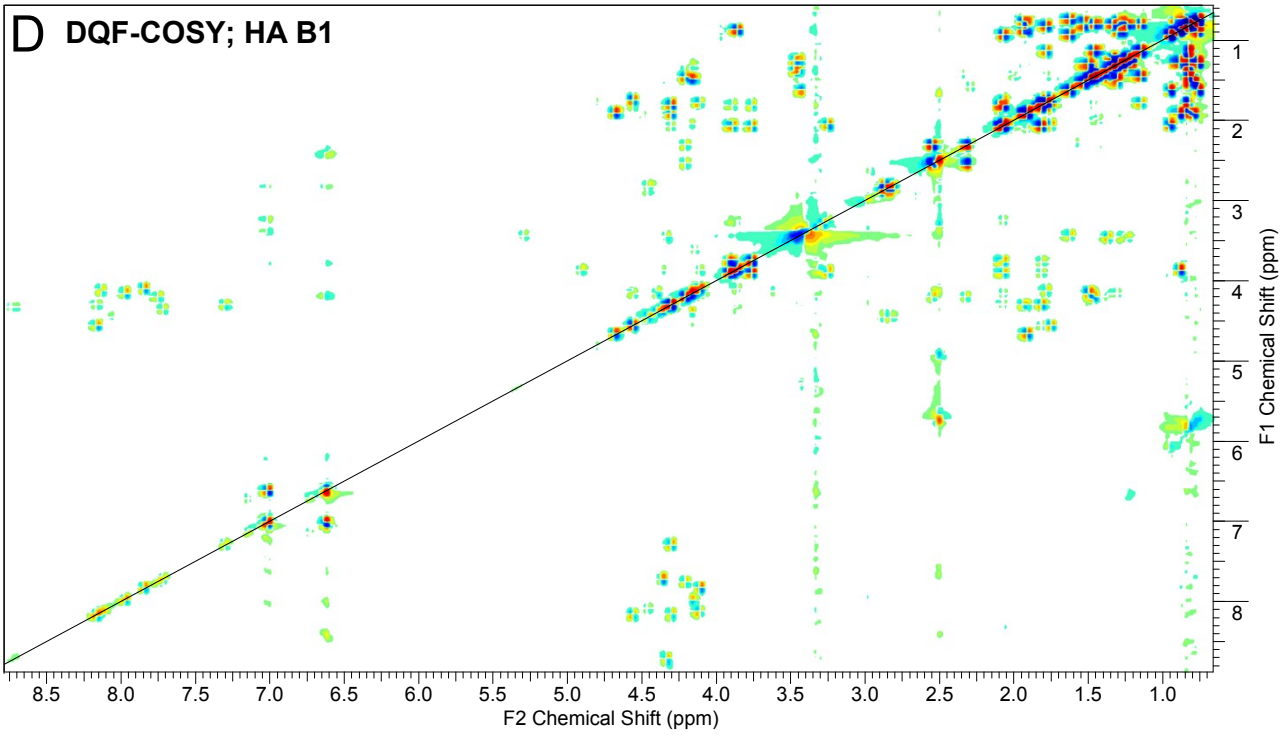


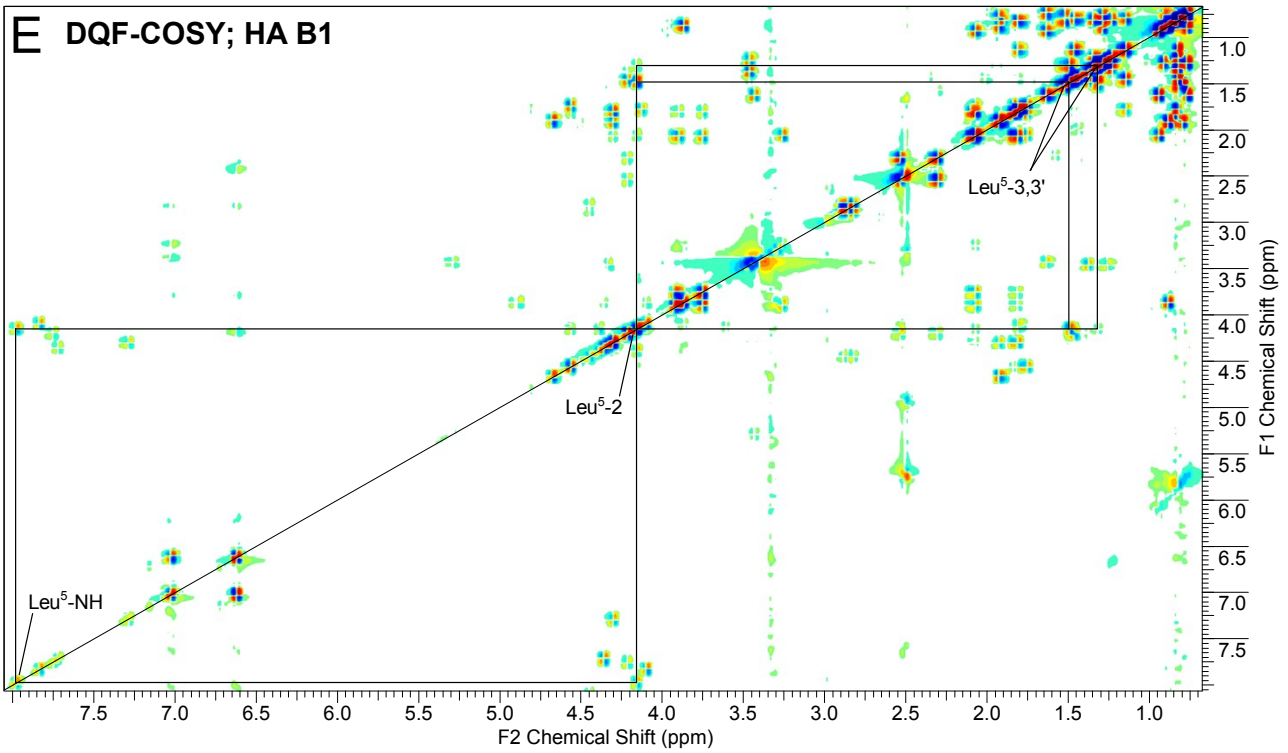
Figure S8. Laxa A group heinamide (HA) A2 NMR spectra. A: ^{1}H, B: annotated ^{1}H+^{15}N-HSQC, C: ^{13}C, D: DQF-COSY, E: ^{1}H-^{1}H TOCSY, F: ROESY, G: annotated ^{13}C-HSQC, H: ^{13}C-HMBC, I: annotated ^{13}C-HMBC carbonyl region.

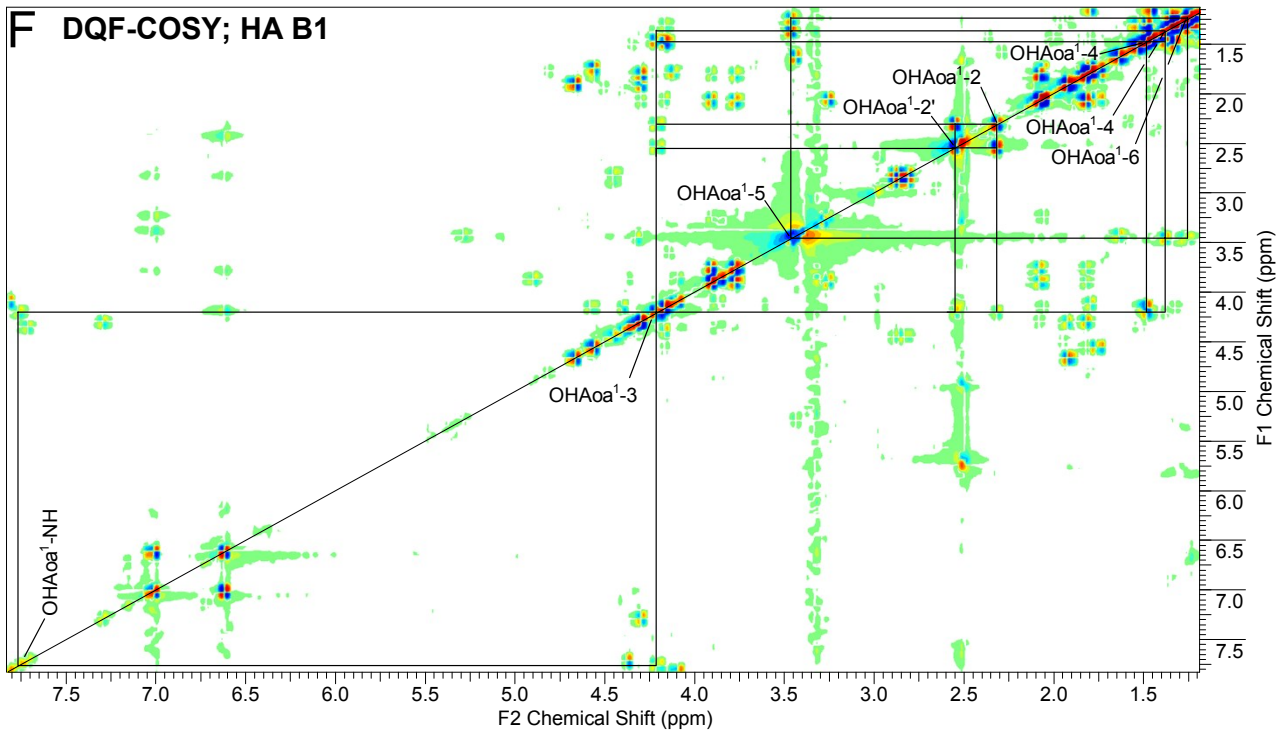


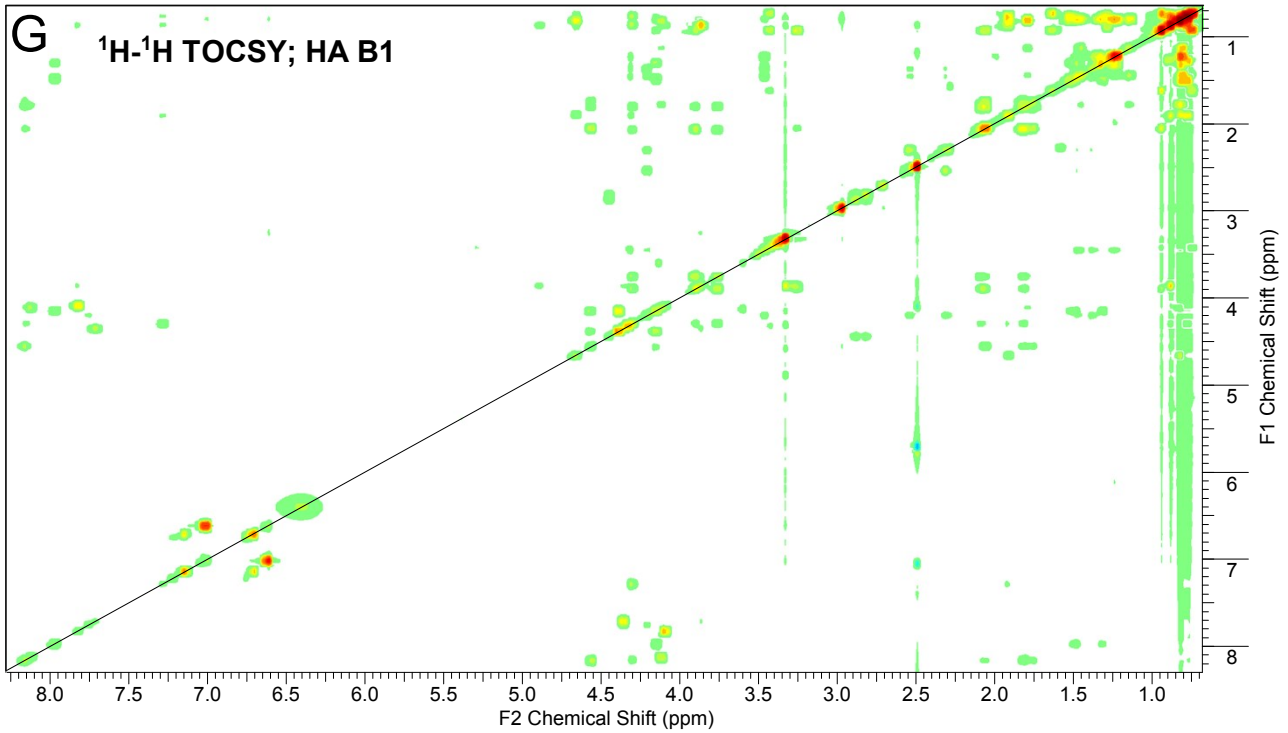

B
15N-HSQC
HA B1
1H
Gln6-NH
Tyr11-NH
Leu5-NH
OHHse8-NH
Val9-NH
Gln6-5-NH2
Gln6-5-NH2
cHse4-NH
Ile2-NH
Intensity increased
Thr12-NH
OHAoa1-NH
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

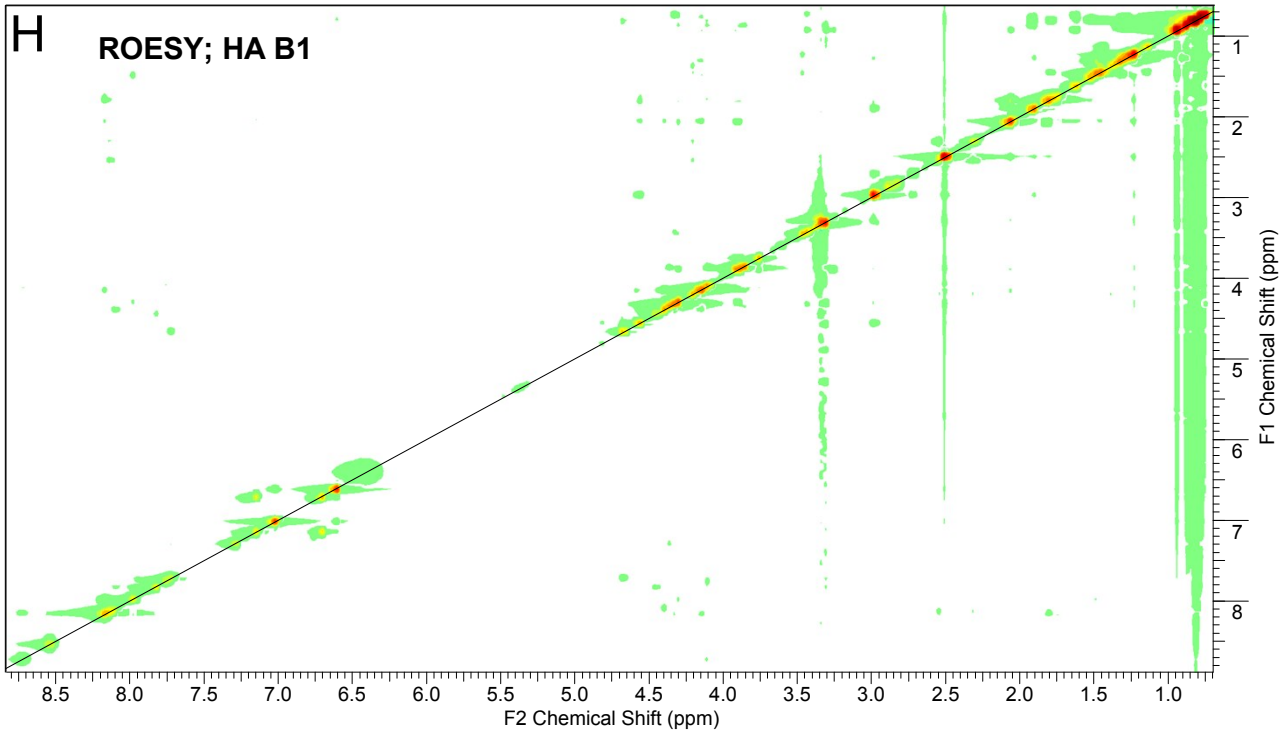

C
13C, HA B1
Chemical Shift (ppm)

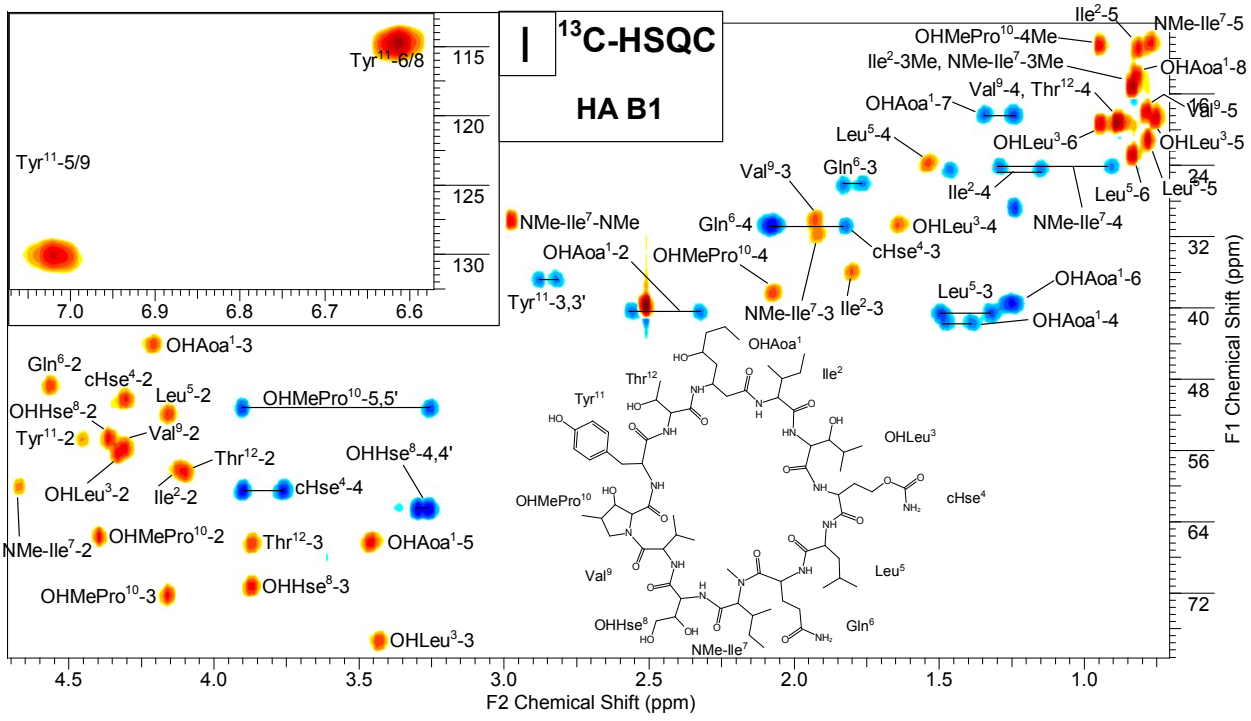

D
DQF-COSY; HA B1
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)

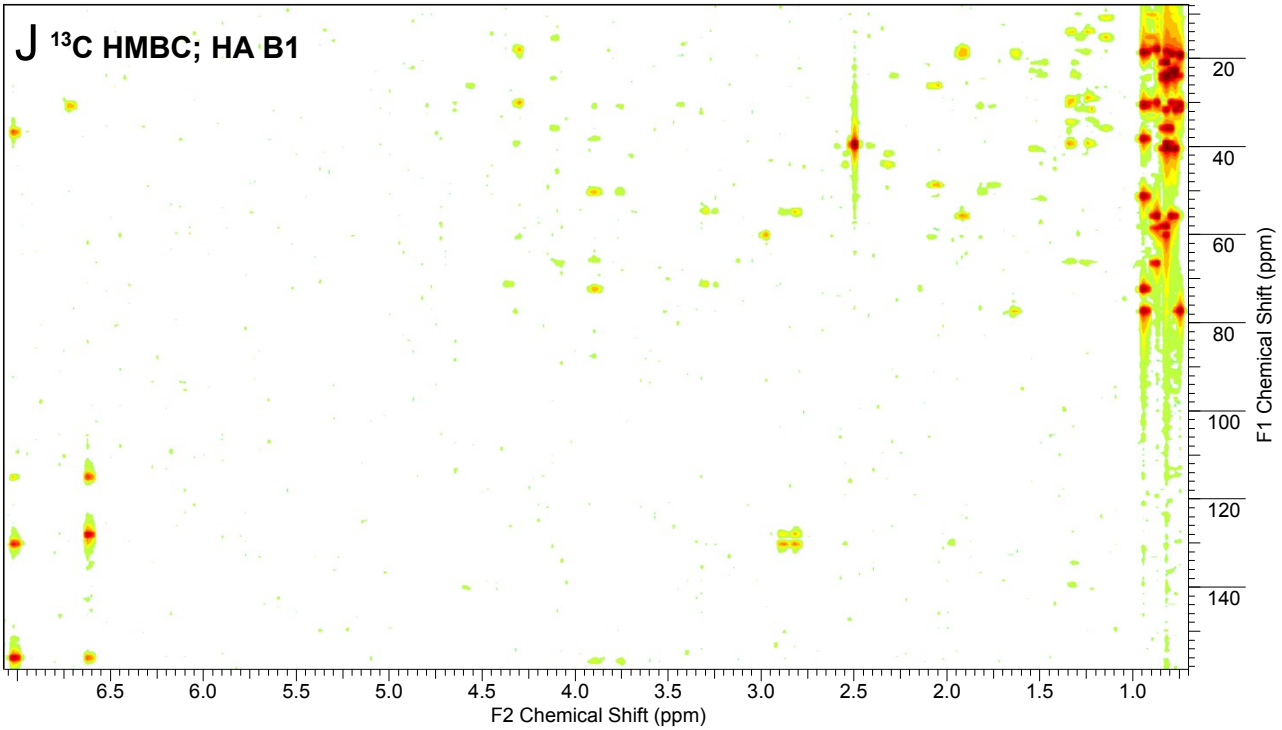

E
DQF-COSY; HA B1
Leu5-3,3'
Leu5-2
Leu5-NH
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)

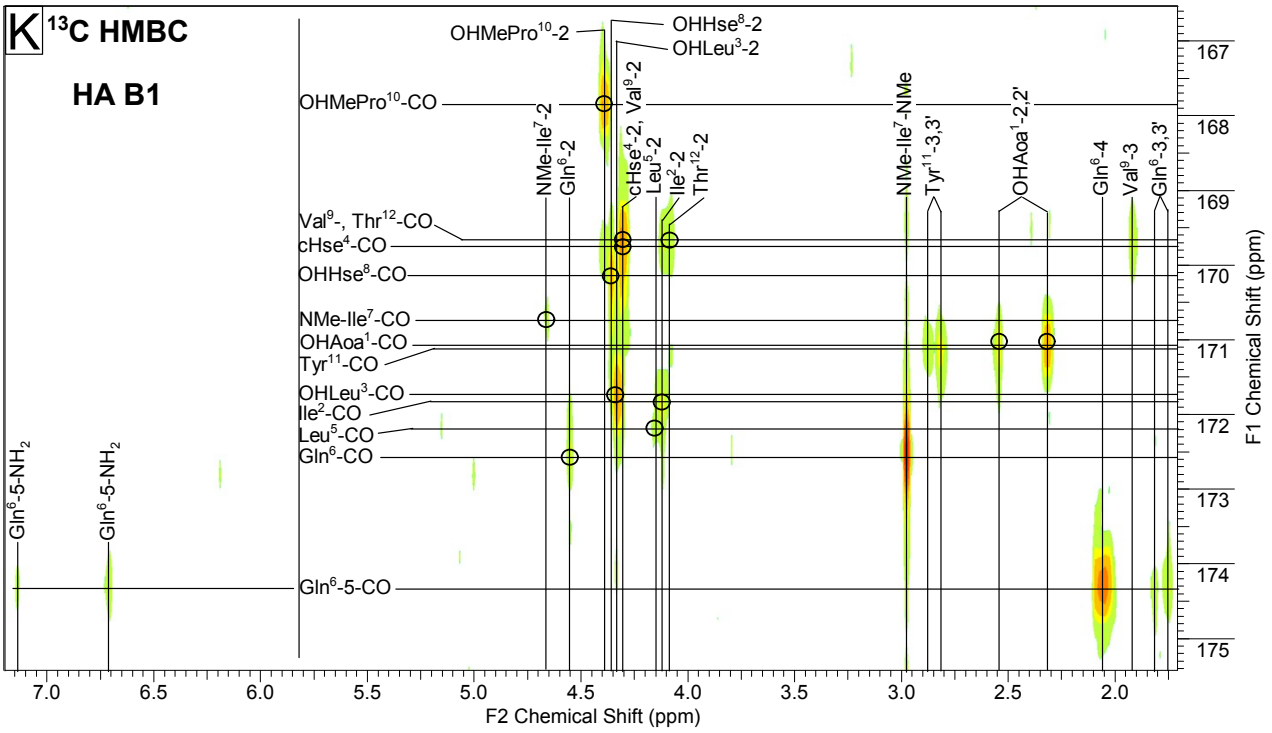

F
DQF-COSY; HA B1
OHAoa1-4
OHAoa1-2
OHAoa1-2'
OHAoa1-4
OHAoa1-6
OHAoa1-5
OHAoa1-3
OHAoa1-NH
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

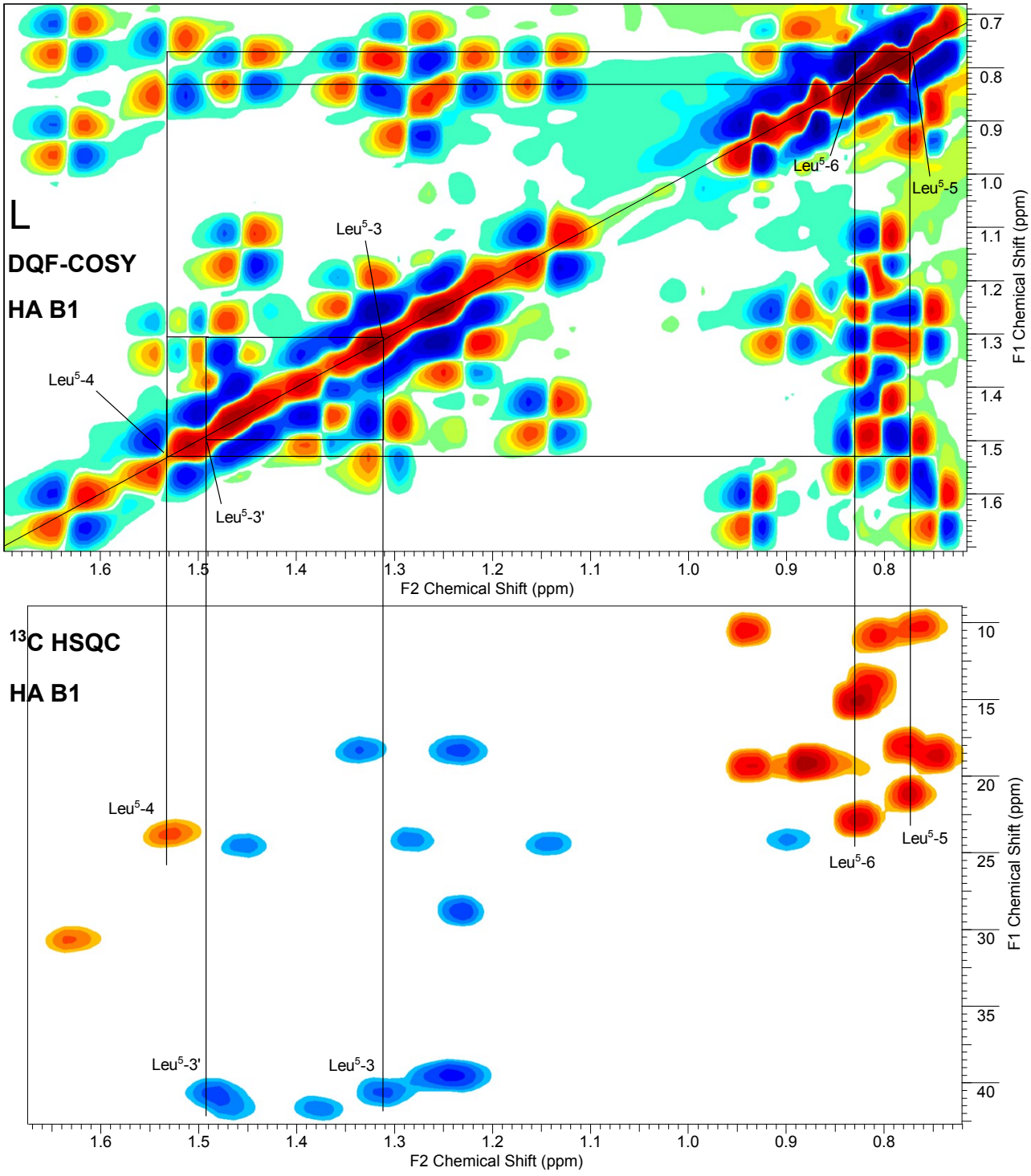

G
1H-1H TOCSY; HA B1
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

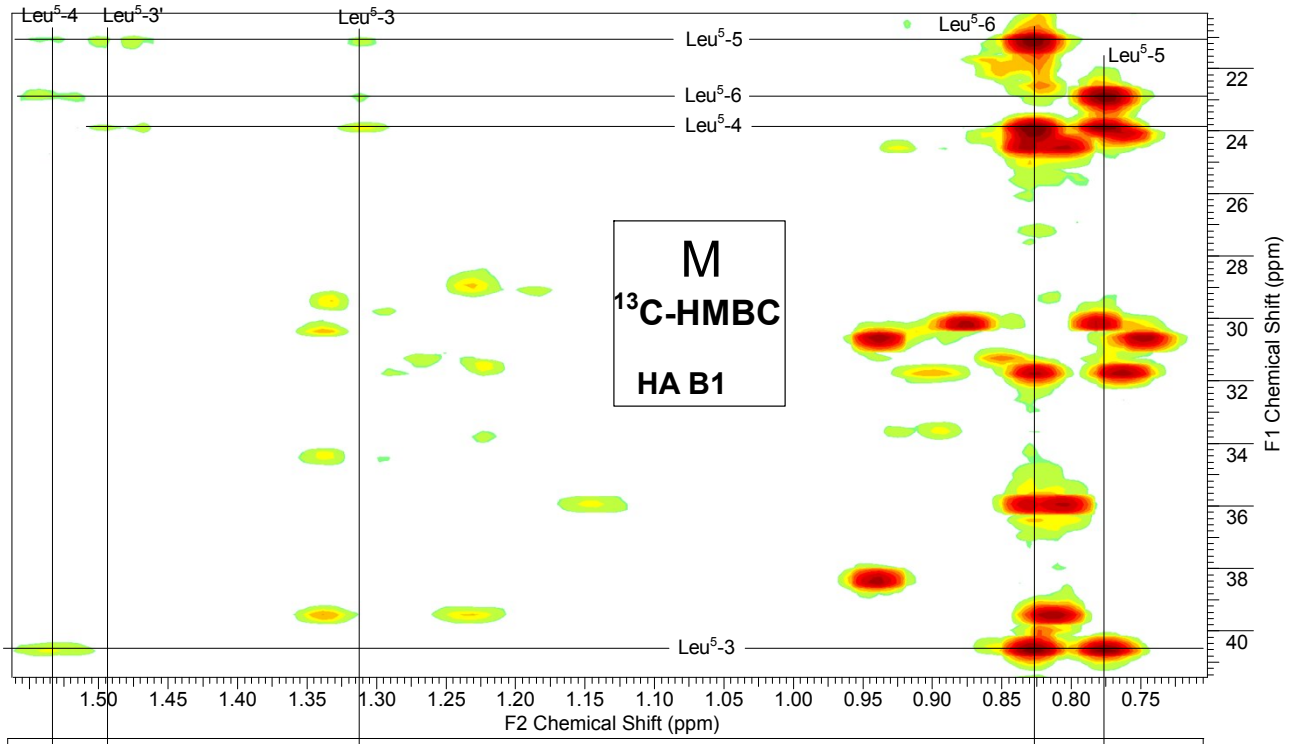

H
ROESY; HA B1
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

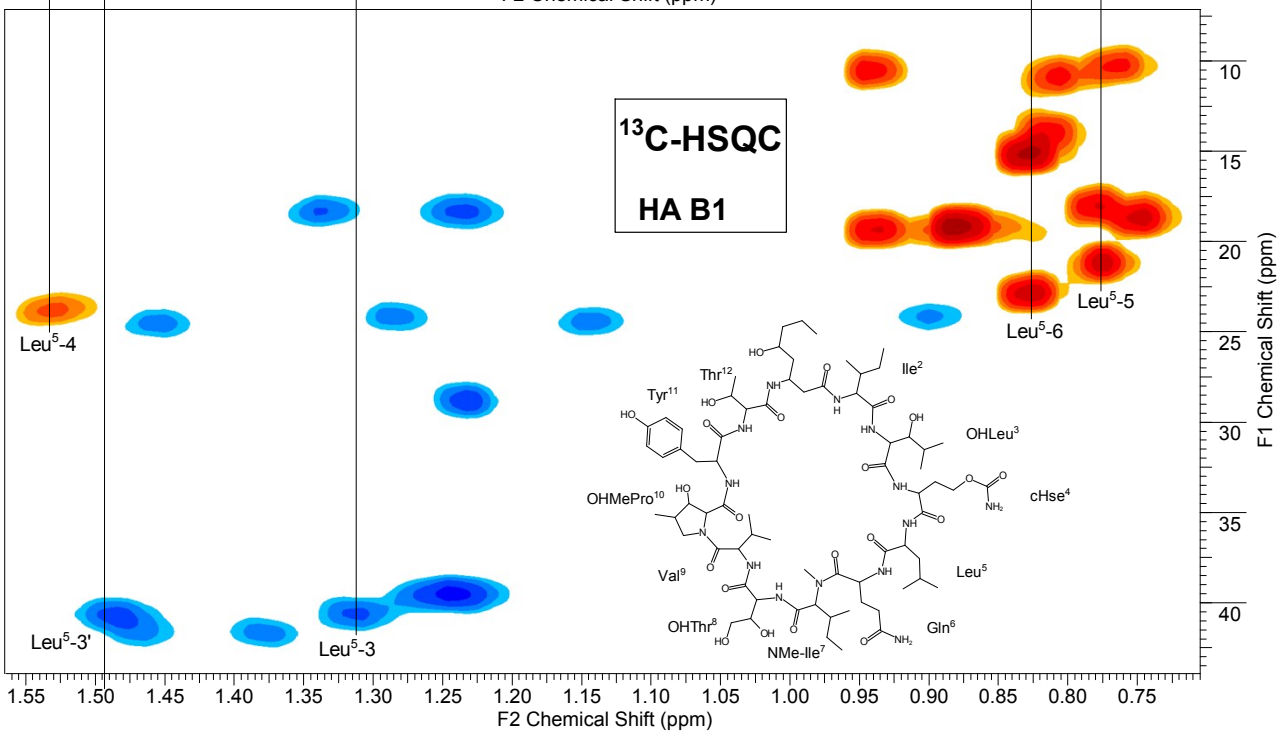

I
13C-HSQC
HA B1
Tyr11-6/8
Tyr11-5/9
OHAoa1-3
Gln6-2
cHse4-2
Leu5-2
OHHse8-2
Tyr11-2
Val9-2
OHLeu3-2
Ile2-2
Thr12-2
cHse4-4
OHHse8-4,4'
NMe-Ile7-2
OHMePro10-2
Thr12-3
OHAoa1-5
OHMePro10-3
OHHse8-3
OHLeu3-3
OHMePro10-5,5'
NMe-Ile7-NMe
OHAoa1-2
Tyr11-3,3'
OHMePro10-4
Val9-3
Gln6-4
NMe-Ile7-3
Gln6-3
cHse4-3
Ile2-3
OHLeu3-4
Ile2-4
Leu5-3
OHAoa1-6
OHAoa1-4
NMe-Ile7-4
Leu5-6
Leu5-4
OHLeu3-6
OHAoa1-7
Val9-4, Thr12-4
Ile2-3Me, NMe-Ile7-3Me
OHMePro10-4Me
Ile2-5
NMe-Ile7-5
OHAoa1-8
Val9-5
OHLeu3-5
Leu5-5
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

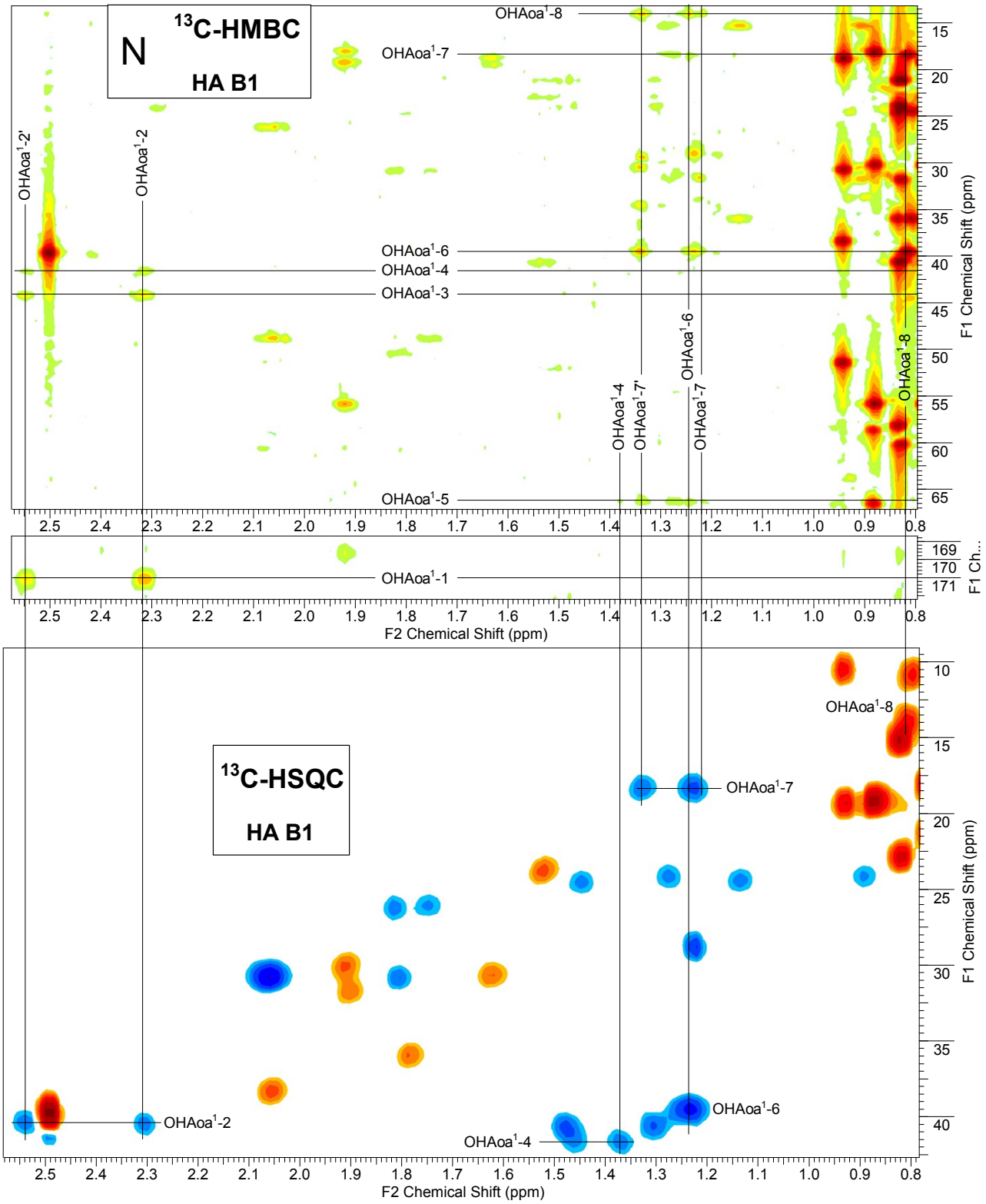

J ¹³C HMBC; HA B1
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


K ¹³C HMBC
HA B1
OHMePro¹⁰-2
OHHse⁸-2
OHLeu³-2
OHMePro¹⁰-CO
NMe-Ile⁷-2
Gln⁶-2
cHse⁴-2, Val⁹-2
Leu⁵-2
Ile²-2
Thr¹²-2
NMe-Ile⁷-NMe
Tyr¹¹-3,3'
OHAoa¹-2,2'
Gln⁶-4
Val⁹-3
Gln⁶-3,3'
Val⁹-, Thr¹²-CO
cHse⁴-CO
OHHse⁸-CO
NMe-Ile⁷-CO
OHAoa¹-CO
Tyr¹¹-CO
OHLeu³-CO
Ile²-CO
Leu⁵-CO
Gln⁶-CO
Gln⁶-5-NH₂
Gln⁶-5-NH₂
Gln⁶-5-CO
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


L
DQF-COSY
HA B1
Leu5-3
Leu5-4
Leu5-3'
Leu5-6
Leu5-5
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)
13C HSQC
HA B1
Leu5-4
Leu5-3'
Leu5-3
Leu5-6
Leu5-5
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)


Leu5-4
Leu5-3'
Leu5-3
Leu5-5
Leu5-6
Leu5-4
Leu5-6
Leu5-5
Leu5-3
M
13C-HMBC
HA B1
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)


13C-HSQC
HA B1
Leu5-4
Leu5-3'
Leu5-3
Leu5-6
Leu5-5
Tyr11
Thr12
Ile2
OHLeu3
cHse4
OHMePro10
Val9
Leu5
OHThr8
NMe-Ile7
Gln6
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)


N
13C-HMBC
HA B1
OHAoa1-8
OHAoa1-7
OHAoa1-2'
OHAoa1-2
OHAoa1-6
OHAoa1-4
OHAoa1-3
OHAoa1-4
OHAoa1-7'
OHAoa1-6
OHAoa1-7
OHAoa1-8
OHAoa1-5
OHAoa1-1
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)
13C-HSQC
HA B1
OHAoa1-8
OHAoa1-7
OHAoa1-2
OHAoa1-6
OHAoa1-4
F1 Chemical Shift (ppm)
F2 Chemical Shift (ppm)

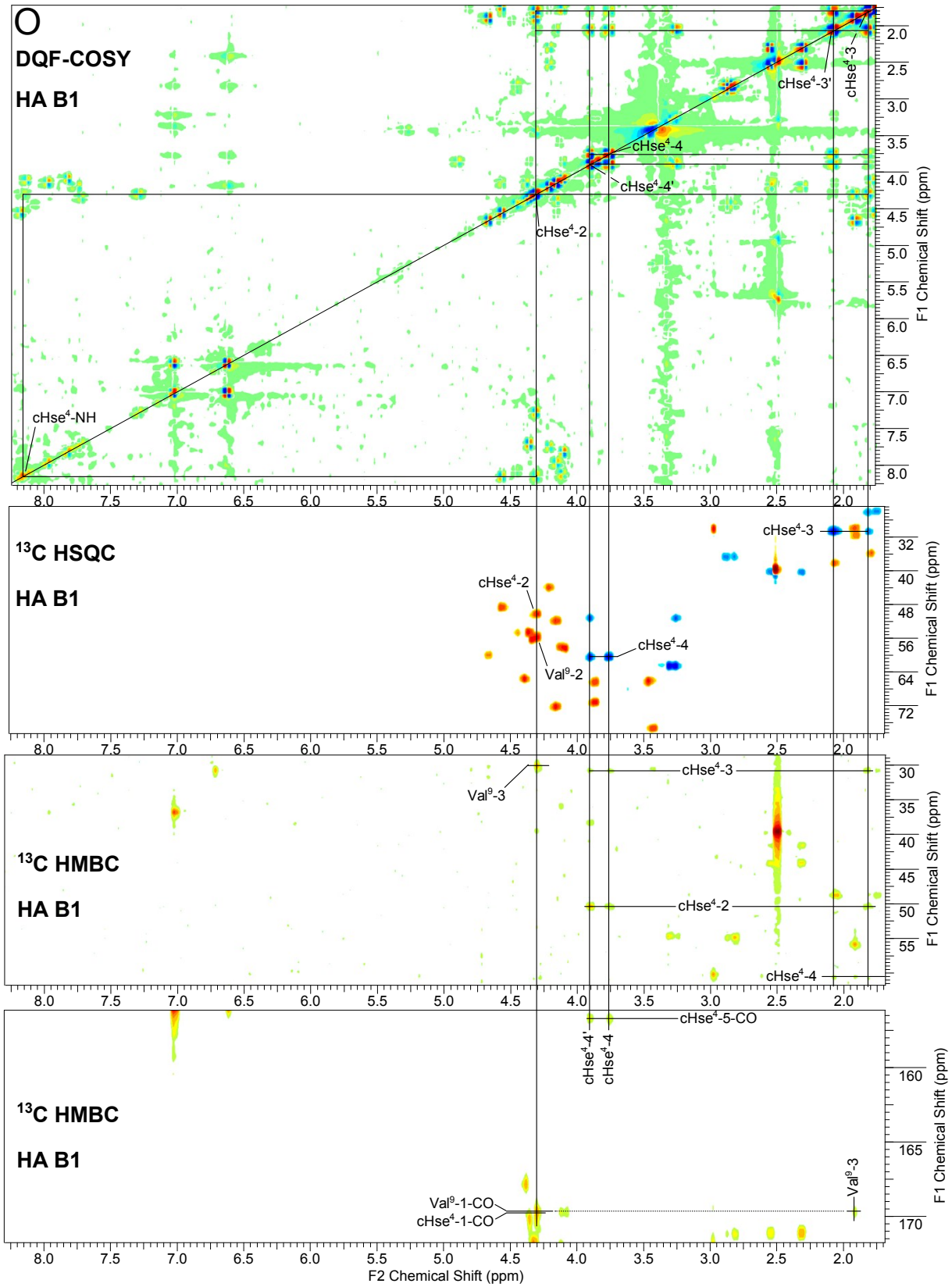
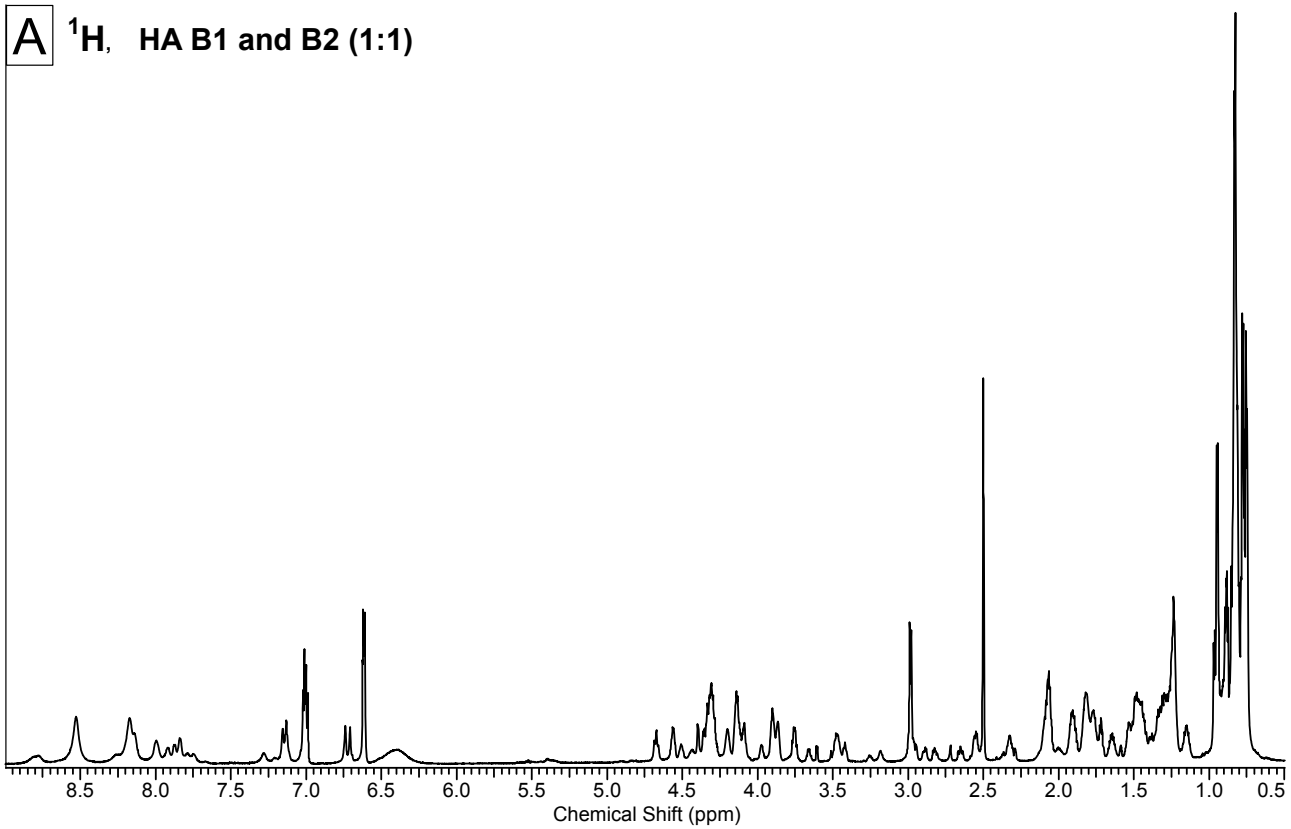
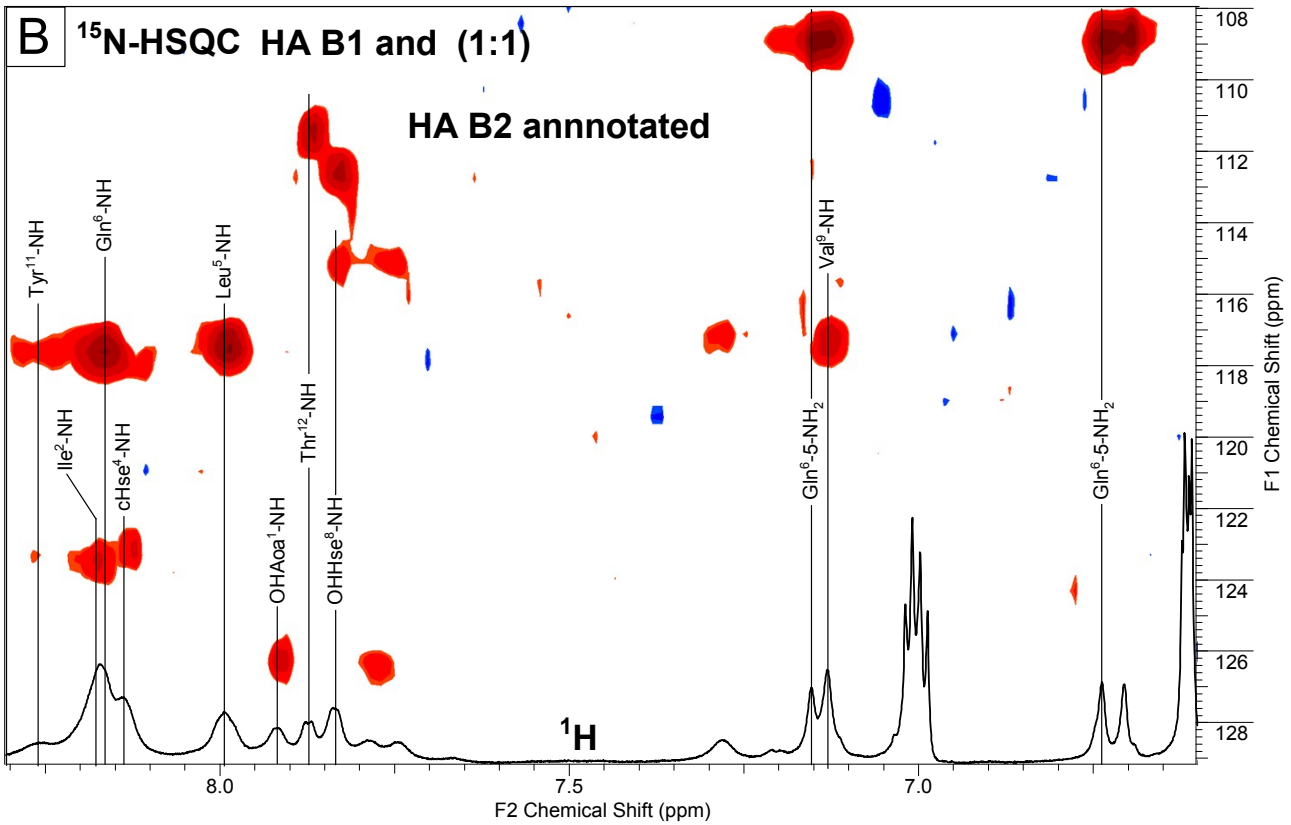


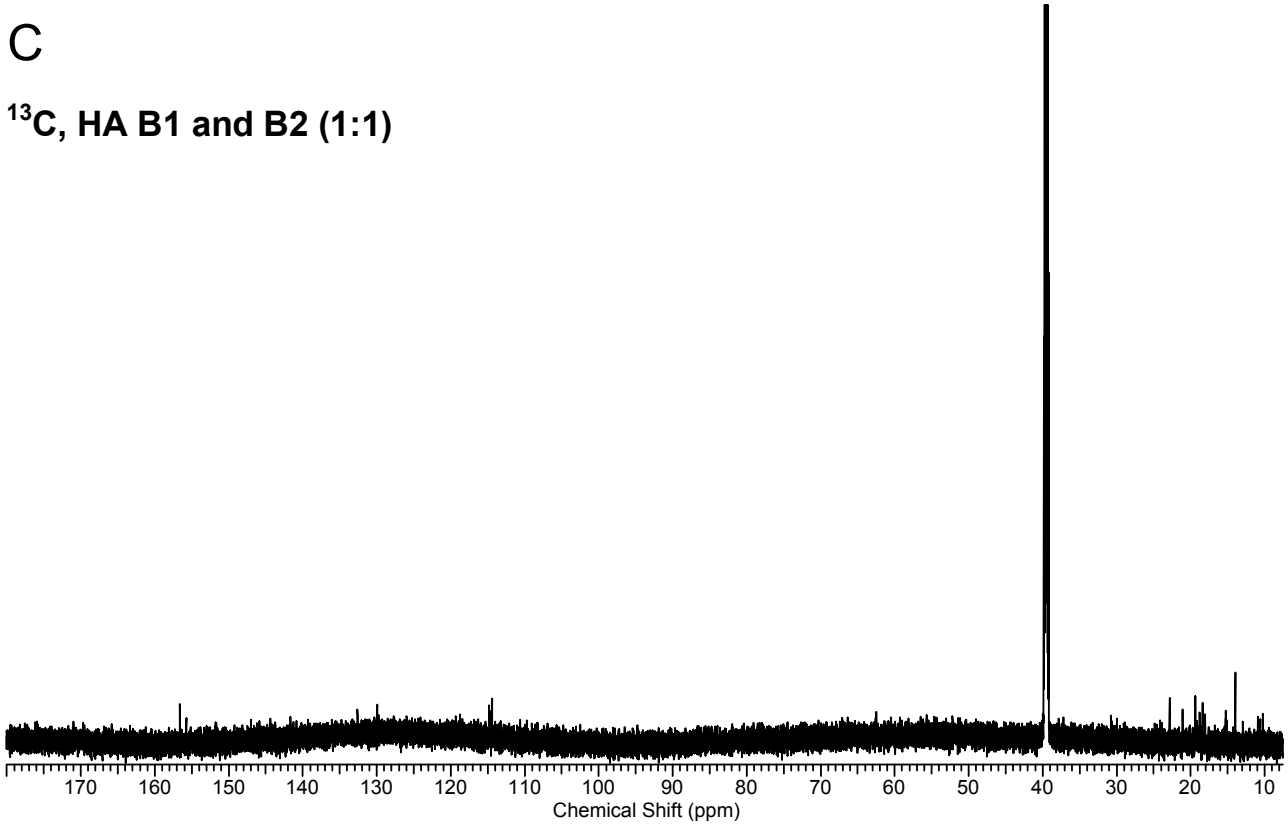
Figure S9. NMR spectra of heinamide (HA) B1 (97% HA B1, 3% HA B2). A: ^{1}H, B: annotated ^{1}H+^{15}N-HSQC, C: ^{13}C, D: DQF-COSY, E: DQF-COSY Leu^{5} correlations, F: DQF-COSY OHAoa^{1} correlations, G: ^{1}H-^{1}H TOCSY, H: ROESY, I: annotated ^{13}C-HSQC, J: ^{13}C-HMBC, K: annotated ^{13}C-HMBC carbonyl region, L: COSY and HSQC Leu^{5} correlations, M: HMBC and HSQC Leu^{5} correlations, N: HMBC and HSQC OHAoa^{1} correlations, O: COSY, HSQC, and HMBC cHse^{4} correlations.

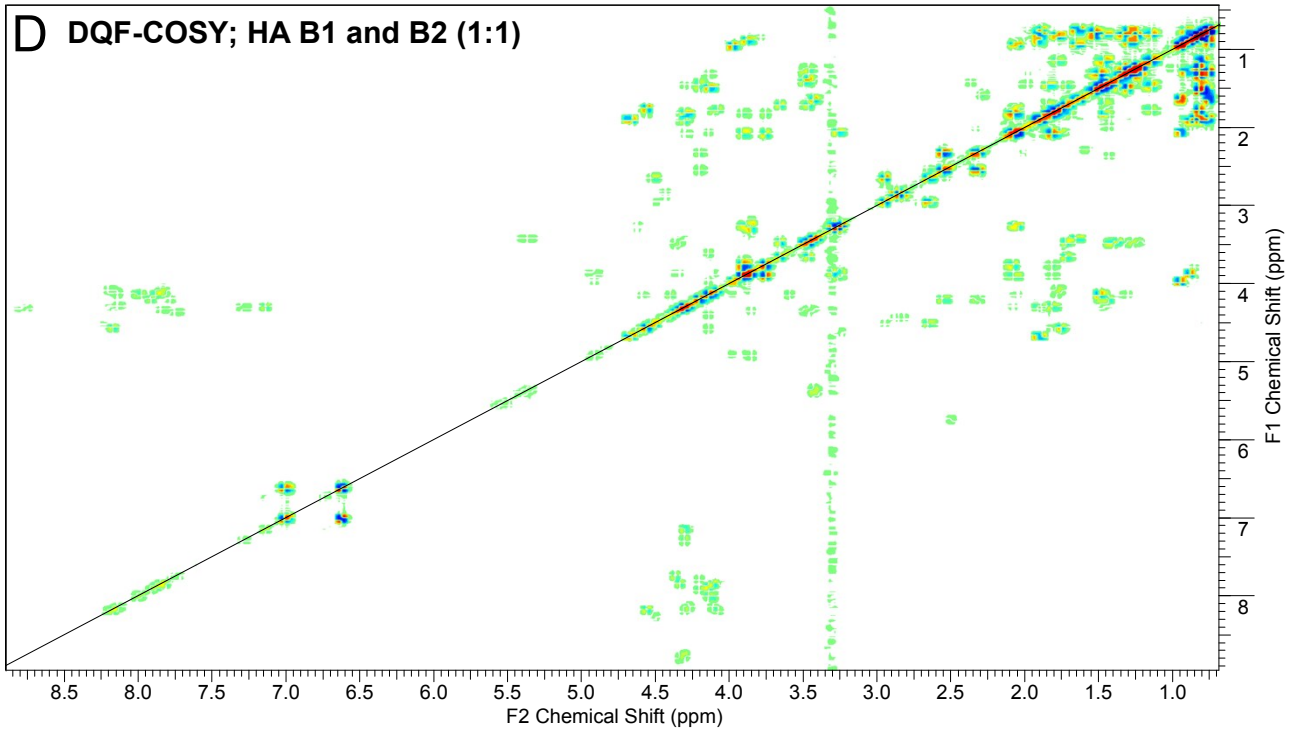

A
^{1}H, HA B1 and B2 (1:1)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)

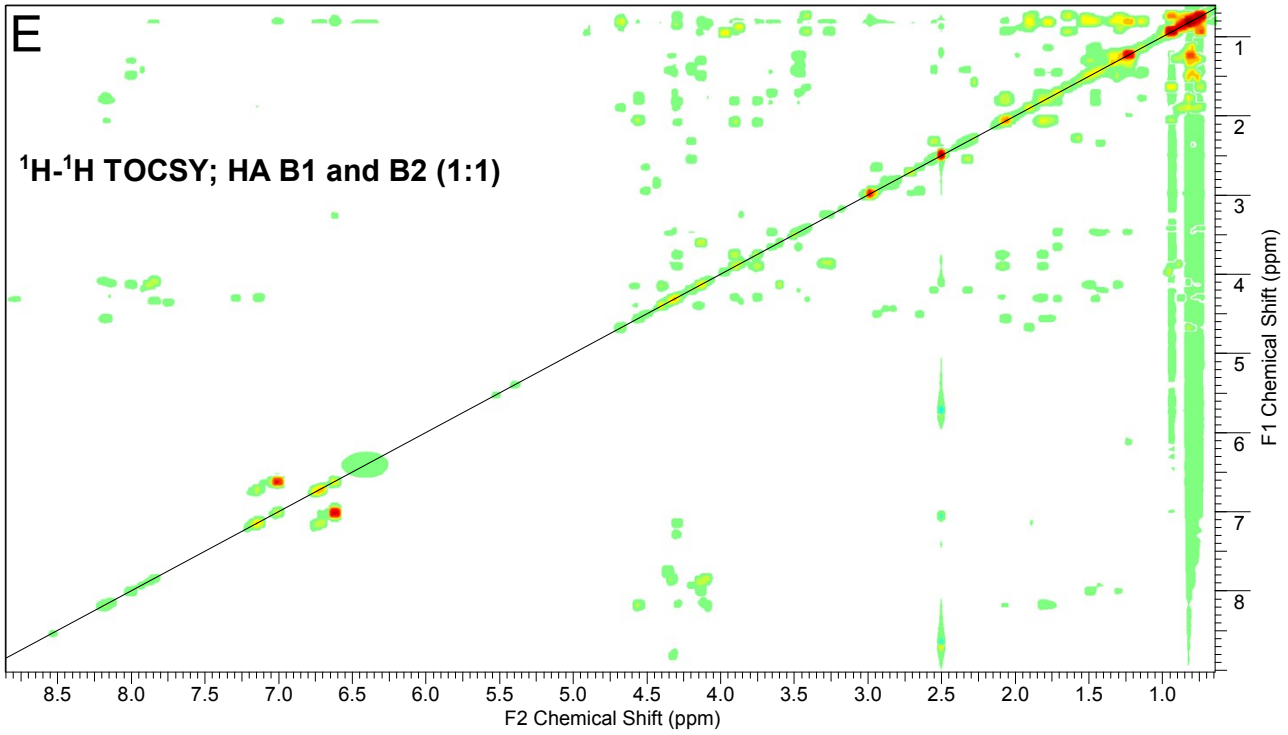

B
^{15}N-HSQC HA B1 and (1:1)
HA B2 annnotated
Tyr^{11}-NH
Gln^{6}-NH
Leu^{5}-NH
Ile^{2}-NH
cHse^{4}-NH
OHAoa^{1}-NH
Thr^{12}-NH
OHHse^{8}-NH
Val^{9}-NH
Gln^{6}-5-NH$_2$
Gln^{6}-5-NH$_2$
^{1}H
8.0
7.5
7.0
F2 Chemical Shift (ppm)
108
110
112
114
116
118
120
122
124
126
128
F1 Chemical Shift (ppm)

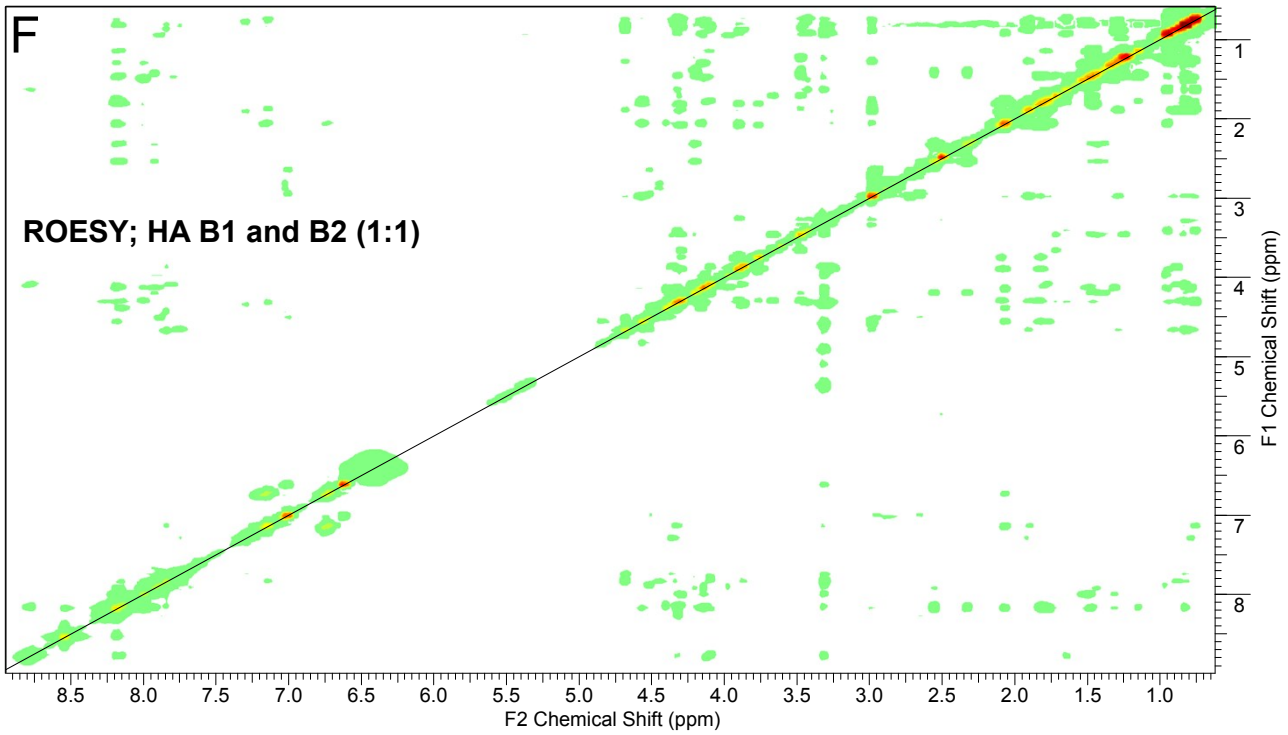
C

^{13}C, HA B1 and B2 (1:1)






E
^{1}H-^{1}H TOCSY; HA B1 and B2 (1:1)
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)


F
ROESY; HA B1 and B2 (1:1)
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

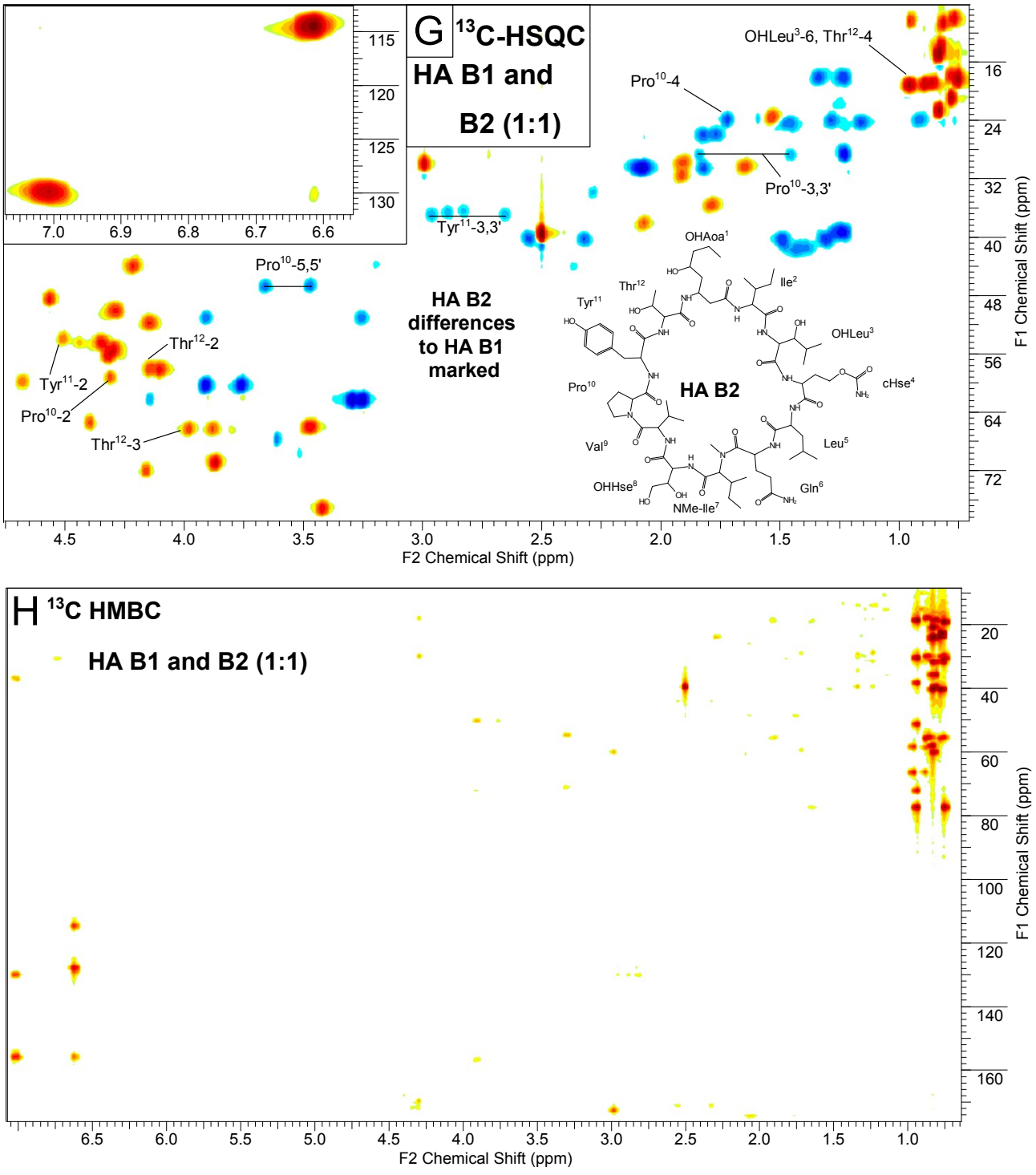


Figure S10. NMR spectra of heinamide (HA) B1 and B2 (1:1) mixture. A: ^{1}H, B: ^{1}H+^{15}N-HSQC (HA B2 annotated), C: ^{13}C, D: DQF-COSY, E: ^{1}H-^{1}H TOCSY, F: ROESY, G: ^{13}C-HSQC (HA B2 differences to HA B1 annotated), H: ^{13}C-HMBC.

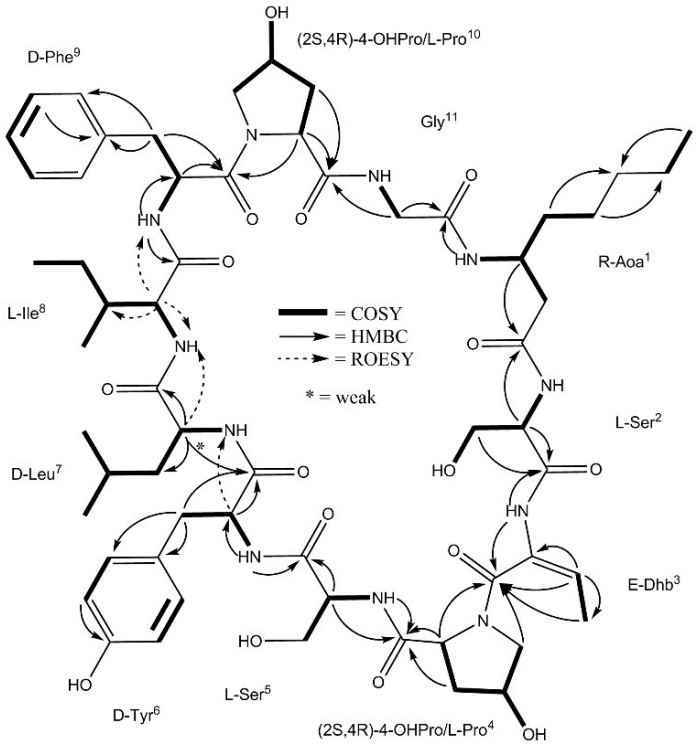


Figure S11. COSY correlations and HMBC/ROESY correlations for subunit sequencing of heinamide A1.

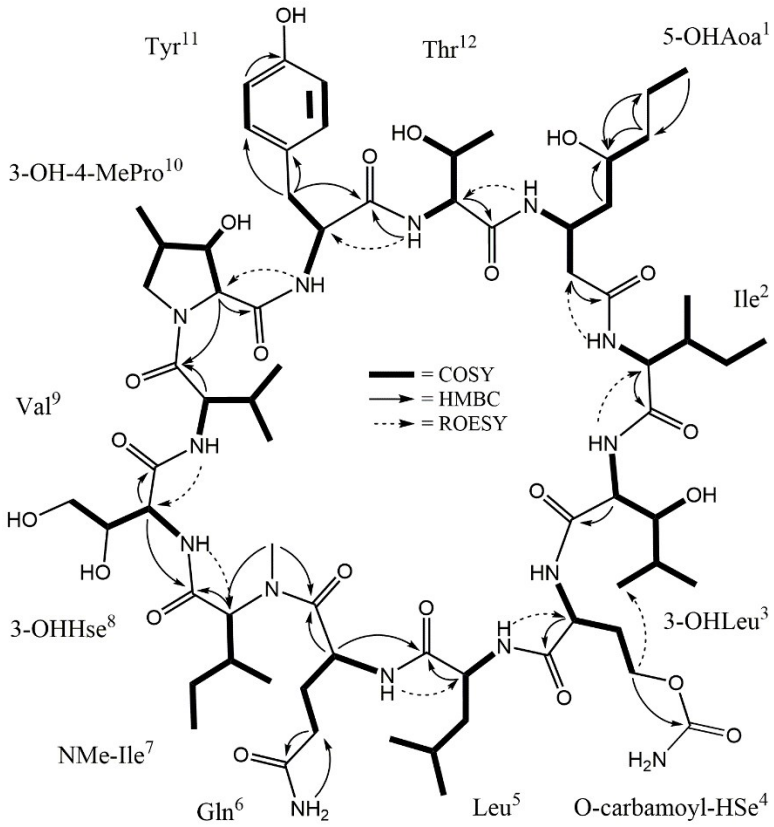


Figure S12. COSY correlations and HMBC/ROESY correlations for subunit sequencing of heinamide B1.

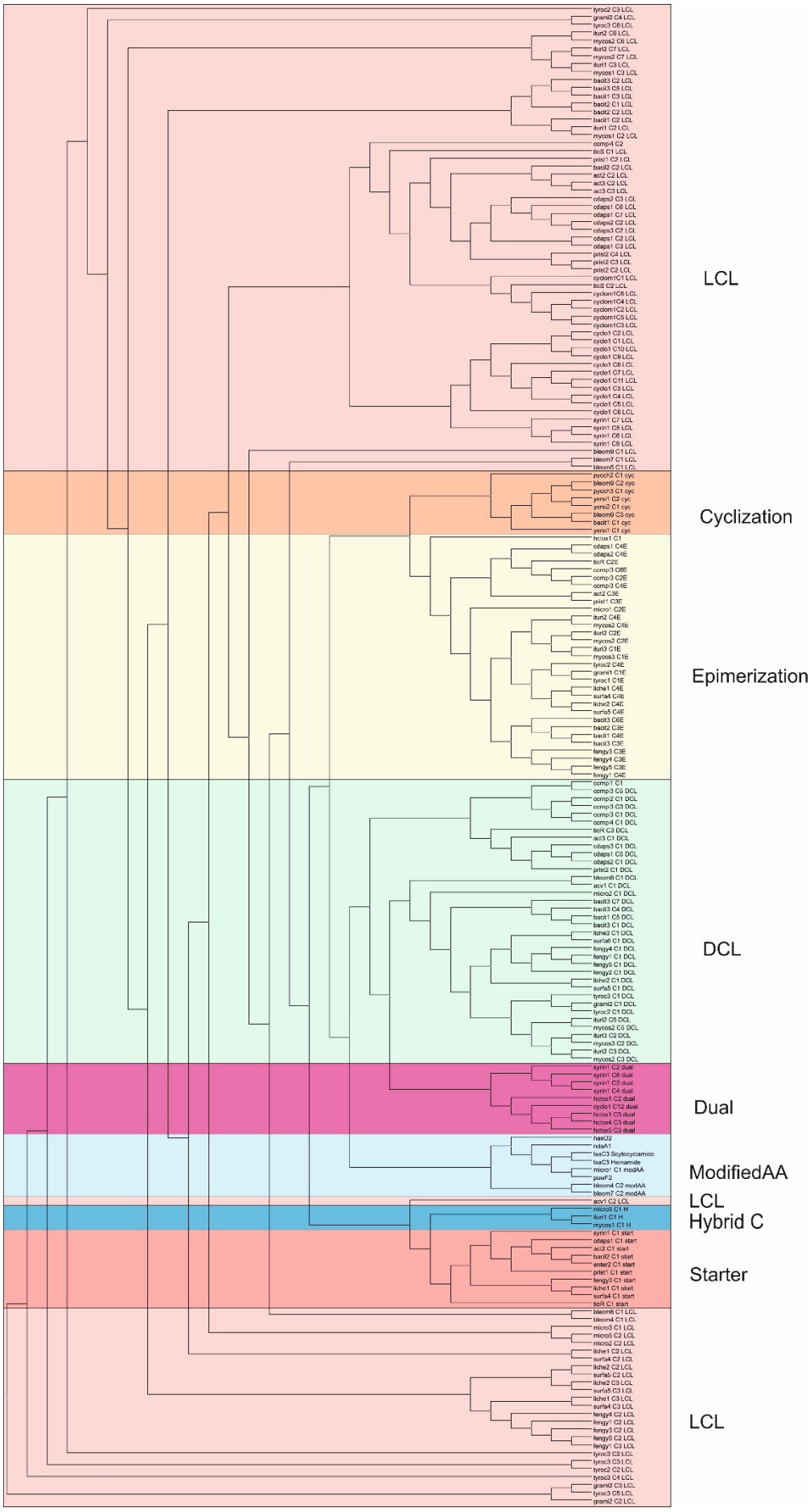

LCL
Cyclization
Epimerization
DCL
Dual
ModifiedAA
LCL
Hybrid C
Starter
LCL

Figure S13. Phylogeny-based C-domain classification showing LxaC3, HasO2, and NdaA1 C-domains clustered in the modified AA clade. The phylogenetic tree was produced with NaPDoS [1]. LCL domains catalyze a peptide bond between two L-amino acids, Cyclization domains catalyze peptide bond formation and cyclization, Epimerization domains change the chirality of the preceding amino acid, DCL domains add an L-amino acid to the peptide ending with a D-amino acid, Dual domains catalyze both epimerization and condensation reactions, ModifiedAA domains are involved in the modification of the incorporated amino acid, Hybrid C domains are involved in the condensation of an amino acid to an aminated polyketide resulting in a hybrid PKS/NRPS product, Starter domains acylate the first amino acid with a β-hydroxy-carboxylic acid.

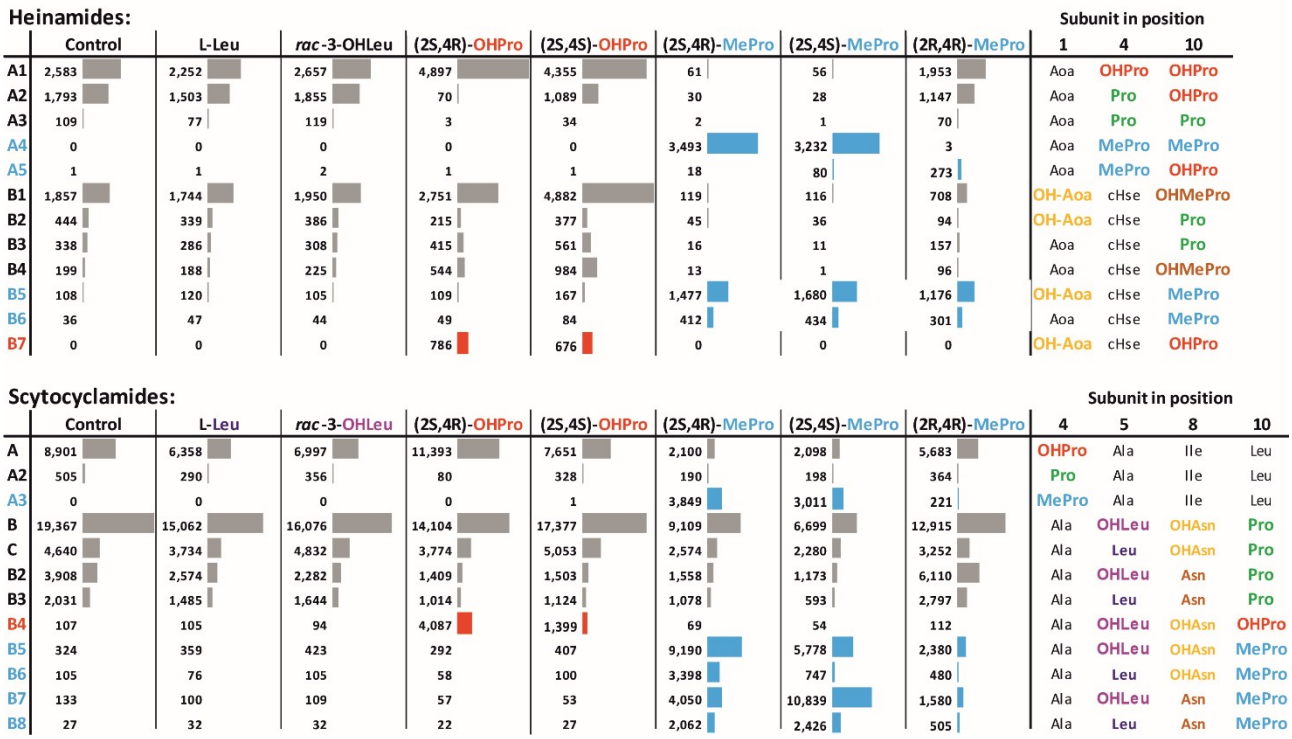


Figure S14. Laxaphycin amounts (sum of single and double protonated and sodiated peak areas) in methanol extracts of cells grown in modified media with added amino acids. Laxaphycin codes and bars marked with blue and red are novel (B5 considerably increased) variants containing added amino acids.

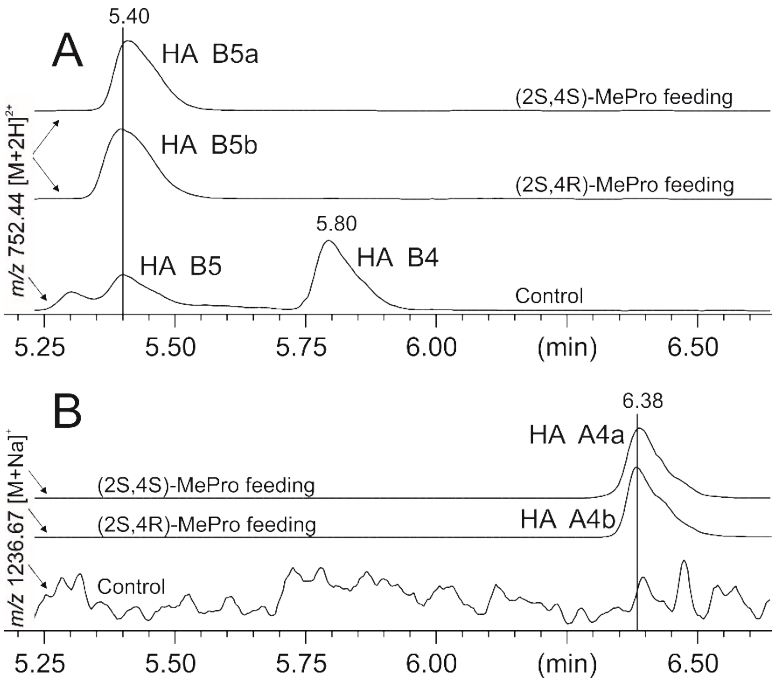


Figure S15. Extracted ion chromatograms *m/z* 752.44 $[M+2H]^{2+}$ and *m/z* 1236.67 $[M+Na]^{+}$ of heinamides HA B5 (A) and HA A4 (B), respectively, from feeding *Nostoc* sp. UHCC 0702 with (2S,4S)-MePro and (2S,4R)-MePro. Control chromatograms are without MePro feeding.

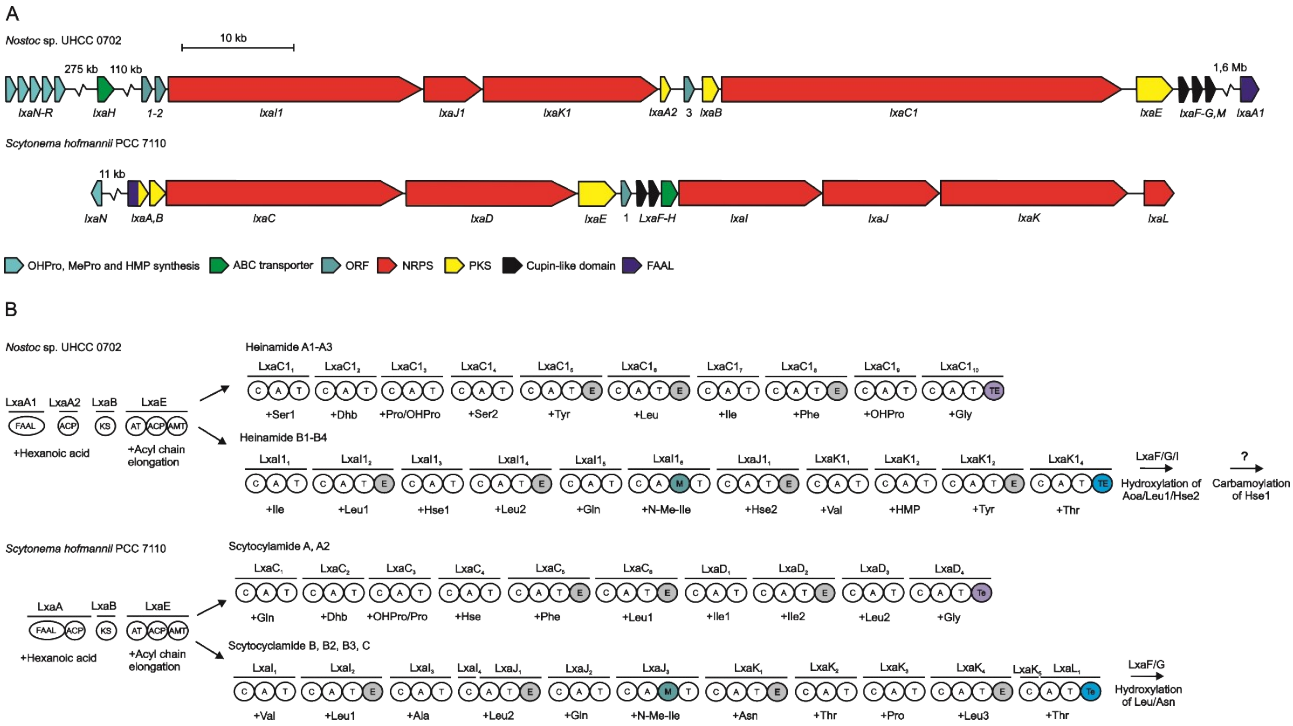


Figure S16. The laxaphycin (*lxa*) biosynthetic gene clusters and putative biosynthetic schemes in *Nostoc* sp. UHCC 0702 and *Scytonema hofmannii* PCC 7110. A: Organization of predicted heinamide and scytocyclamide biosynthetic genes. B: Proposed biosynthetic pathway of heinamides and scytocyclamides. NRPS non-ribosomal peptide synthetase, PKS polyketide synthase, FAAL fatty acyl AMP Ligase, ACP acyl carrier protein, KS ketosynthase, AT acyltransferase, AMT aminotransferase, C condensation domain, A adenylation domain, T thiolation domain, M methylation domain, TE thioesterase domain.