Depsipeptide Companeramides from a Panamanian Marine Cyanobacterium Associated with the Coibamide Producer

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Contents

Table S1. NMR Spectroscopic Data for Companeramide A (**1**) in $CDCl_3$ (700 MHz)

Unit	Position	δ_H, mult. (*J*, Hz)	δ_C, mult.	COSY	HMBC	ROESY
AMOYA	1		174.3, C			
	2	2.51, dq (7.2, 7.2)	46.4, CH	3, 9	1, 3, 4, 9	3, 9
	3	3.97, m	51.2, CH	2, 3-NH, 4		2, 3-NH, 4, 5, 9
	3-NH	6.75, br d, (6.5)		3		11, 17
	4	1.71, obs 1.63, obs	31.3, CH_2	3, 5		3, 5
	5	1.54, m 1.48, obs	25.2, CH_2	4, 6	4, 7 4, 7	4a, 4b, 6
	6	2.17, obs	17.9, CH_2	5, 8	4, 5, 7, 8	5a, 5b, 8
	7		83.8, C			
	8	1.89, t (2.6)	68.6, CH	6	6, 7	6
	9	1.31, d (7.5)	14.2, CH_3	2	1, 2, 3	2, 3
N-Me-Val-1	10		170.1, C			
	11	4.58, d (11.2)	62.8, CH	12	10, 12, 13, 14, 15, 16	12, 13, 15
	12	2.29, obs	25.6, CH	11, 13, 14	10, 11, 13, 14, 15	11, 13, 14, 15
	13	0.95, d (6.4)	19.4, CH_3	12	11, 12, 14	12, 14
	14	0.88, d (6.7)	18.4, CH_3	12	11, 12, 13	11, 12, 14, 15
	15	3.14, s	30.2, CH_3		11, 16	11, 12, 13, 17, 18
Ala	16		174.4, C			
	17	4.72, obs	46.2, CH	17-NH, 18	16, 18, 19	15, 18
	17-NH	8.86, d (6.3)		17	19	17
	18	1.32, d (7.0)	16.4, CH_3	17	16, 17	15, 17
N-Me-Leu	19		169.4, C			
	20	4.67, dd (9.7, 4.9)	58.6, CH	21	19, 21, 22, 23, 24, 25, 26	21, 22, 23, 24, 25, 27
	21	1.83, ddd (14.5, 8.5, 4.9) 1.59, m	36.7, CH_2	20, 22	19, 20, 22, 23, 24 19, 20, 22, 23, 24	20, 22, 23, 24,25
	22	1.41, obs	24.2, CH	21, 23, 24	21	20, 21, 23, 24, 25
	23	0.92, d (6.6)	22.2, CH_3	22	21, 22, 24	20, 21, 22, 24
	24	0.91, d (6.8)	24.3, CH_3	22	21, 22, 23	20, 21, 22, 23
	25	2.69, s	28.8, CH_3		20, 26	20, 21, 22, 27
Pro	26		172.4, C			
	27	4.92, dd (7.4, 6.4)	55.4, CH	28	26, 28, 29, 30, 31	20, 25, 28a, 28b, 29a, 29b
	28	2.11, m 2.00, m	29.6, CH_2	27, 29	26, 29, 30 27, 29, 30	27, 30a, 30b
	29	2.28, obs 1.94, m	25.6, CH_2	28, 30	27, 28 27, 28, 30	27, 30a, 30b
	30	3.84, m 3.76, dt (9.9, 7.5)	48.0, CH_2	29	27, 28, 29 28, 29	28a, 28b, 29a, 29b, 32, 33, 34
Ile-1	31		170.0, C			
	32	4.74, dd (8.9, 6.9)	54.0, CH	32-NH, 33	31, 33, 34, 35	30a, 30b, 32-NH, 33, 34
	32-NH	6.91, d (8.9)		32	37	32, 38
	33	1.69, obs	38.6, CH	32, 34, 35		30b, 32, 34, 35a, 35b, 36
	34	0.92, d (6.5)	15.4, CH_3	33	32, 33, 35	30a, 30b, 32, 33
	35	1.39, m 1.06, obs	24.3, CH_2	33, 36	 33, 34, 36	33, 36
	36	0.83, dd (7.5, 4.8)	11.0, CH_3	35	33, 34, 35	33, 35a, 35b
N-Me-Val-2	37		169.5, C			
	38	4.71, obs	61.7, CH	39	37, 39, 40, 41	32-NH, 39, 40, 41, 42
	39	2.27, obs	25.7, CH	38, 40, 41	37, 38, 40, 41	38, 40, 41, 42
	40	0.94, d (6.3)	19.9, CH_3	39	38, 39, 41	38, 39, 41, 42
	41	0.77, d (6.8)	17.9, CH_3	39	38, 39, 40	38, 39, 40, 42
	42	3.06, s	30.5, CH_3		38, 43	38, 39, 40, 41, 45, 46
Ile-2	43		172.3, C			

	44	4.72, obs	53.0, C	44-NH, 45	43, 45	44-NH, 45, 46
	44-NH	6.92, d (8.8)		44	49	44, 50
	45	1.74, obs	37.3, CH	44, 46, 47	44	42, 44, 46, 47a, 47b, 48
	46	0.84, d (6.7)	15.0, CH_3	45	44, 45, 47	42, 44, 45, 47, 48
	47	1.48, obs 1.07, obs	23.4, CH_2	45, 48	44, 45, 48 45, 46	45, 46, 48
	48	0.82, dd (7.4, 5.0)	11.0, CH_3	47		45, 46, 47
N-Me-Ala	49		170.1, C			
	50	5.26, q (7.3)	51.7, CH	51	49, 51, 52, 53	44-NH, 51, 52
	51	1.31, d (7.3)	13.5, CH_3	50	49, 50	50, 52
	52	2.92, s	30.5, CH_3		50, 51, 53	50, 51, 54, 55, 56, 57
Hiva	53		171.1, C			
	54	4.80, d (8.3)	75.4, CH	55	1, 53, 55, 56, 57	52, 55, 56, 57
	55	2.19, obs	30.1, CH	54, 56, 57	53, 54, 56, 57	52, 54, 56, 57
	56	1.04, d (6.6)	18.6, CH_3	55	54, 55, 57	52, 54, 55, 57
	57	0.93, d (5.7)	17.8, CH_3	55	54, 55, 56	52, 54, 55, 56

Table S2. NMR Spectroscopic Data Companeramide B (**2**) in $CDCl_3$ (700 MHz)

Unit	Position	δ_H, mult. (*J*, Hz)	δ_C, mult.	COSY	HMBC	ROESY
AMO	1		175.4, C			
	2	2.63, m	46.0, CH	3, 9	1, 3, 9	3, 3-NH, 4, 9
	3	3.81, m	52.4, CH	2, 3-NH, 4		2, 3-NH, 4, 5, 9, 11
	3-NH	7.44, d (6.0)		3	10	2, 3, 4b, 9, 11, 13
	4	1.93, m 1.73, m	30.2, CH_2	3, 5	8	2, 3, 3-NH, 13
	5	1.53, m 1.45, m	25.5, CH_2	4, 6	3, 4, 6, 7	3, 6
	6	2.18, m	18.0, CH_2	5, 8	4, 7, 8	4, 5
	7		83.4, C			
	8	1.91, obs	69.4, CH	6	7	
	9	1.36, obs	14.2, CH_3	2	1, 2	2, 3, 3-NH, 11
N-Me-Val-1	10		170.2, C			
	11	4.72, d (11.2)	62.4, CH	12	12, 13, 15	3, 3-NH, 9, 12, 13, 14
	12	2.27, m	26.5, CH	11, 13, 14	11	11, 13, 14, 15
	13	0.96, obs	18.5, CH_3	12	11	11, 12, 14, 15
	14	0.81, obs	19.9, CH_3	12	11	11, 12, 13, 15
	15	3.23, s	30.9, CH_3		11, 16	12, 13, 14, 17, 18, 19, 20
Val-1	16		173.4, C			
	17	4.58, dd (8.3, 7.8)	55.5, CH	17-NH, 18	16, 18, 21	15, 17-NH, 18, 19, 20
	17-NH	8.84, d (7.8)		17	17, 18	17, 18, 19, 20, 22, 23, 26
	18	2.13, m	30.5, CH	17, 19, 20	17, 21	15, 17, 17-NH, 19, 20
	19	0.91, obs	18.8, CH_3	18		15, 17, 17-NH, 18, 20
	20	0.93, obs	18.7, CH_3	18		15, 17, 17-NH, 18, 19
N-Me-Ala-1	21		169.8, C			
	22	4.96, q (6.8)	56.1, CH	23	21, 23, 24, 25	17-NH, 23, 24
	23	1.32, d (6.8)	15.3, CH_3	22	21, 22, 25	17-NH, 22, 24
	24	2.74, s	29.1, CH_3		22, 25	17-NH, 22, 23
Pro	25		171.8, C			
	26	4.83, obs	55.4, CH	27	25, 27, 28, 29, 30	17-NH, 27, 28
	27	2.10, m 2.04, m	29.8, CH_2	26, 28	26, 29	26, 29
	28	2.29, m 1.97, m	25.6, CH_2	27, 29	26, 29	26, 29
	29	3.83, m 3.72, m	48.0, CH_2	28	26, 27, 28	27, 28, 31, 32, 33

Ile	30		170.2, C			
	31	4.76, m	54.4, CH	31-NH, 32	30, 32, 33, 34	31-NH, 32, 33, 34, 35
	31-NH	7.48, d (8.5)		31	31, 36	21, 32, 33, 34, 37
	32	1.70, obs	39.0, CH	31, 33, 34	31, 33	29, 31, 31-NH, 33, 35
	33	0.93, obs	15.5, CH_3	32	31, 32, 34, 35	29, 31, 31-NH, 32, 34, 25
	34	1.39, m 1.06, m	24.7, CH_2	32, 35	31, 32, 35	31, 31-NH, 33, 35
	35	0.87, obs	11.7, CH_3	34	32	31, 32, 33, 34
N-Me-Val-2	36		169.3, C			
	37	4.90, d (11.1)	62.0, CH	38	36, 38, 39, 40, 41	31-NH, 38, 39, 40, 41
	38	2.33, m	25.8, CH	37, 39, 40	37	37, 39, 40, 41
	39	0.98, obs	19.9, CH_3	38	37	37, 38, 40, 41
	40	0.78, d (6.5)	18.7, CH_3	38	37	37, 38, 39
	41	3.06, s	30.8, CH_3		37, 42	31-NH, 37, 38, 39
Val-2	42		172.4, C			
	43	4.67, dd (9.3, 8.9)	54.7, CH	43-NH, 44	42, 44, 45, 46, 47	41, 43-NH, 44, 45, 46
	43-NH	6.80, d (9.3)		43	43, 47	41, 43, 44, 45, 46, 48, 50
	44	2.00, m	31.1, CH	43, 45, 46	43	43, 43-NH, 44, 45, 46
	45	0.93, obs	19.2, CH_3	44	43	43, 44, 46
	46	0.91, obs	18.4, CH_3	44		43, 44, 45
N-Me-Ala-2	47		170.3, C			
	48	5.40, q (7.0)	52.2, CH	49	47, 49, 50	43-NH, 49, 50
	49	1.35, obs	13.8, CH_3	48	48	48, 50
	50	2.89, s	30.7, CH_3		48, 51	43-NH, 48, 49, 52, 54, 55
Hiva	51		170.8, C			
	52	4.80, d (7.7)	75.5, CH	53	1, 53, 54, 55	50, 53, 54, 55
	53	2.19, m	30.1, CH	52, 54, 55	52, 54, 55	50, 52, 54, 55
	54	1.08, d (6.9)	18.2, CH_3	53	52, 53, 55	50, 52, 53
	55	1.00, d (6.5)	18.2, CH_3	53	52, 53, 54	50, 52, 53

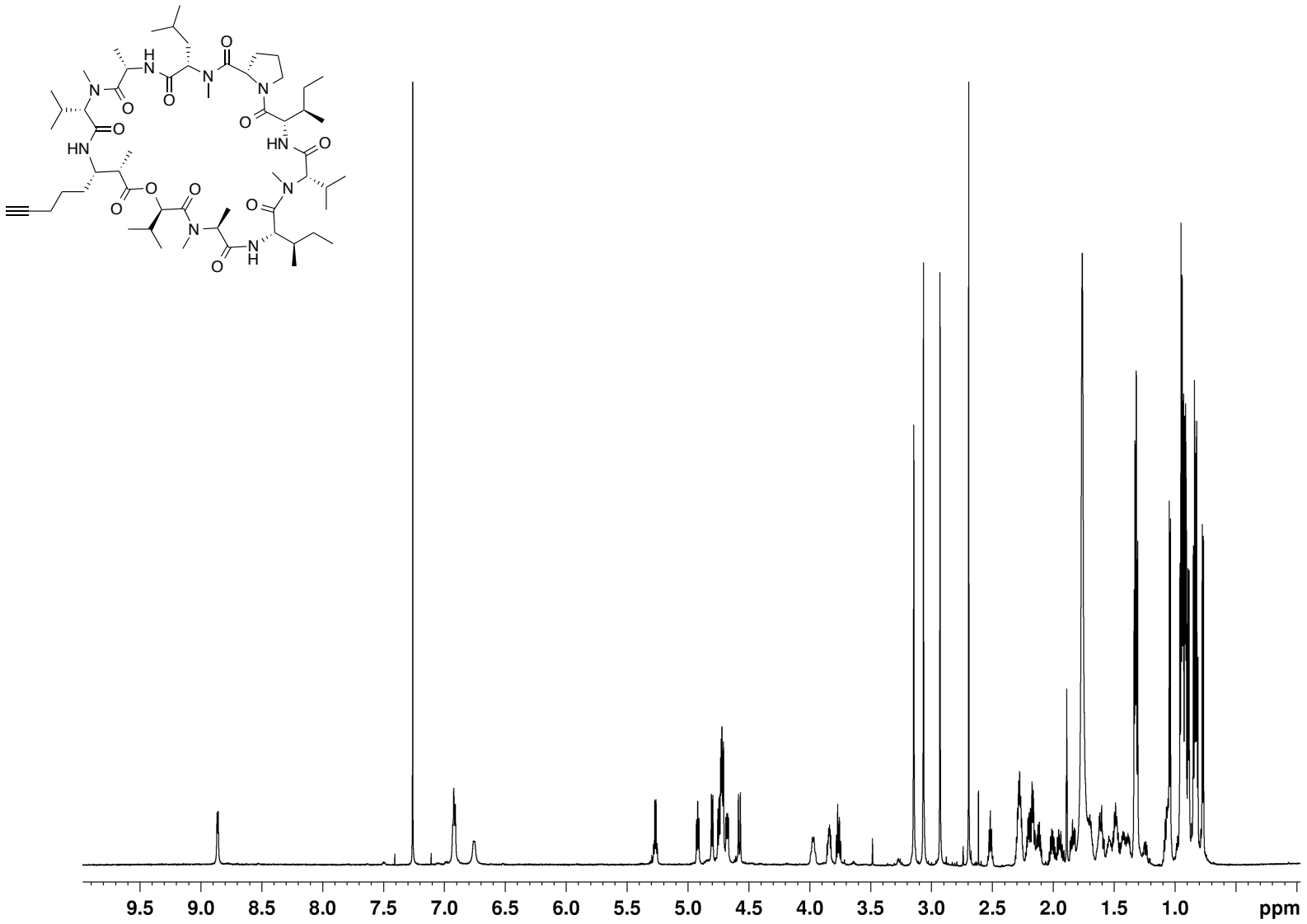

Figure S3. ^{1}H NMR spectrum for companeramide A (**1**) in $CDCl_3$ (700 MHz)

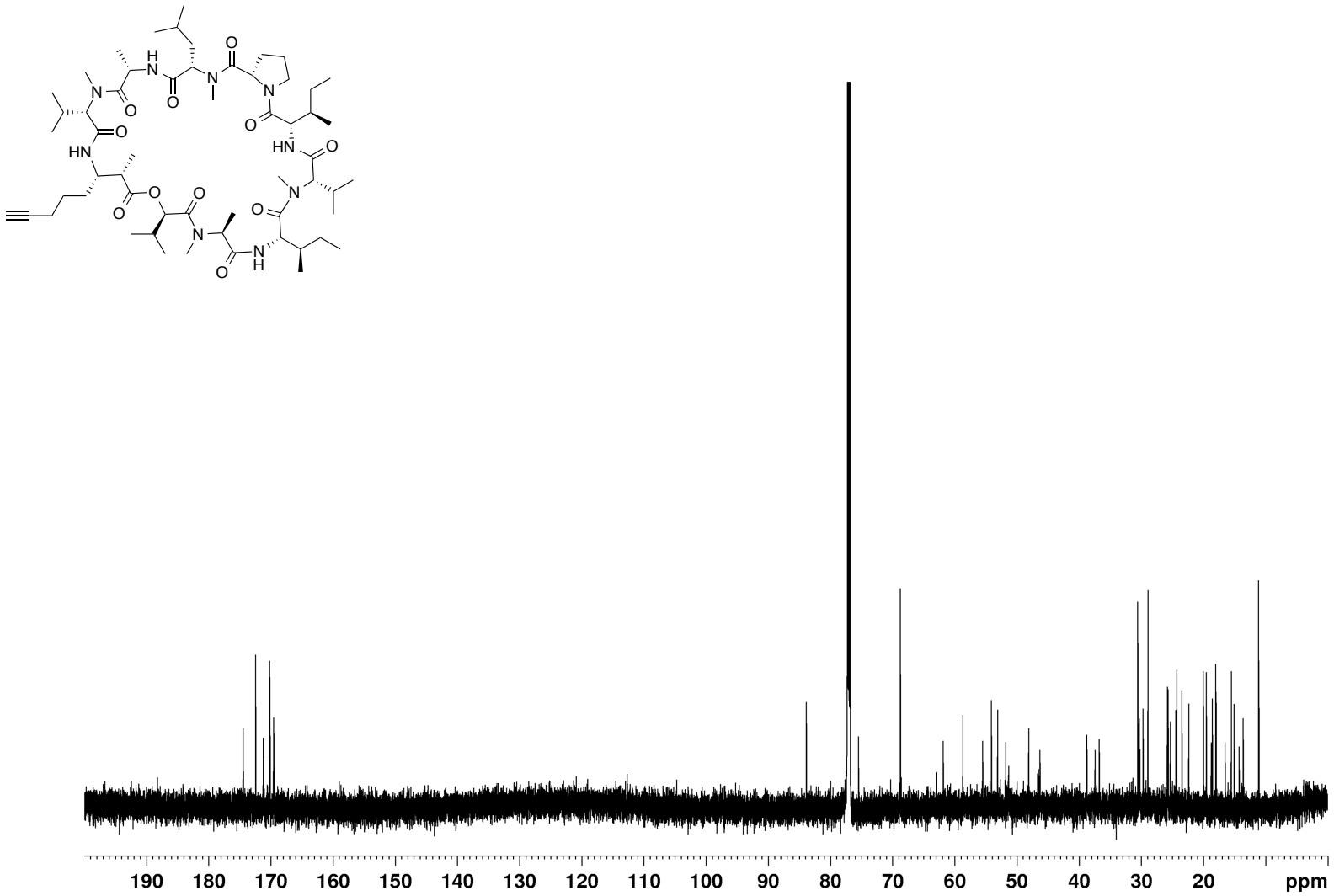

Figure S4. ^{13}C NMR spectrum for companeramide A (**1**) in $CDCl_3$ (175 MHz)

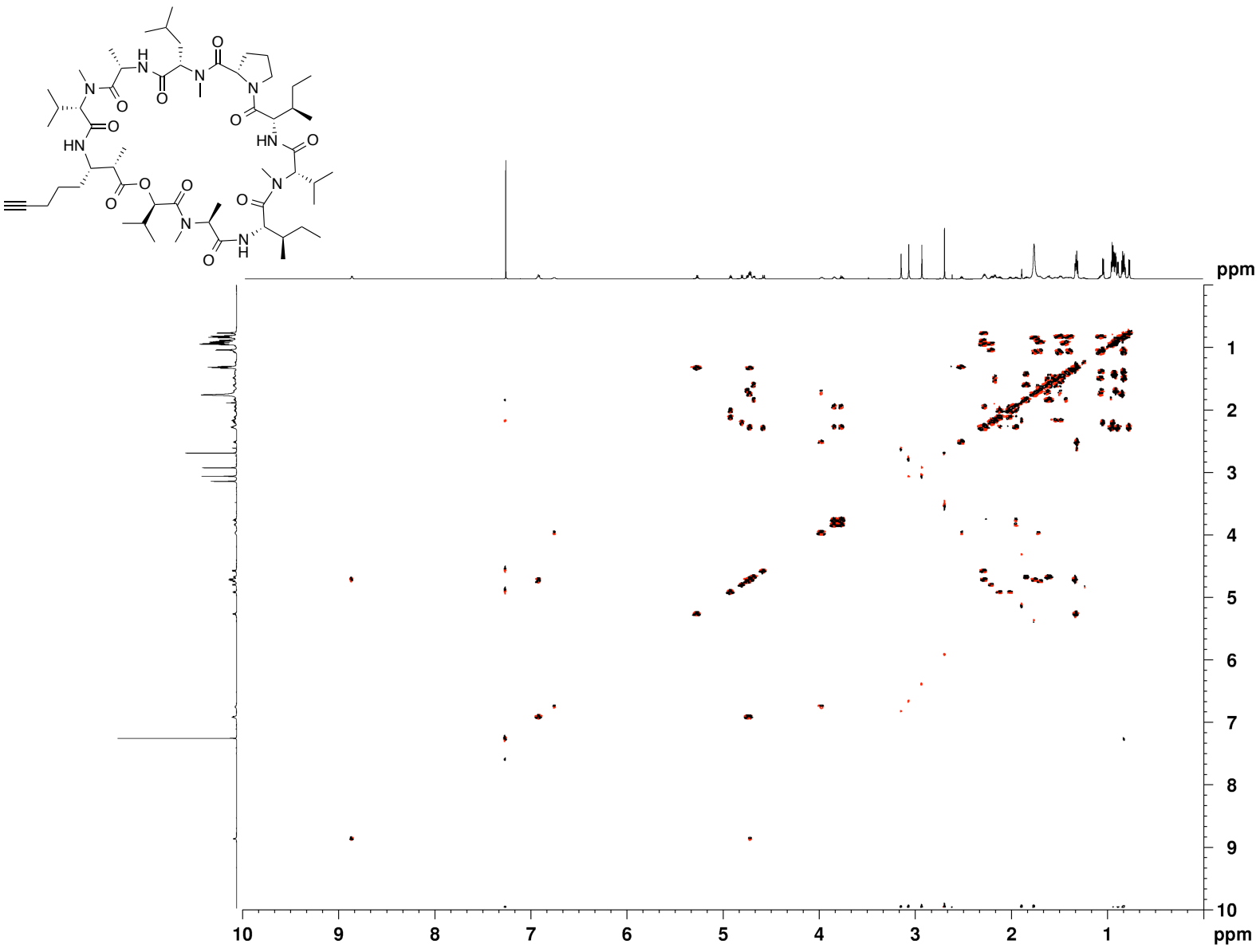

Figure S5. DQF COSY spectrum for companeramide A (**1**) in $CDCl_3$ (700 MHz)

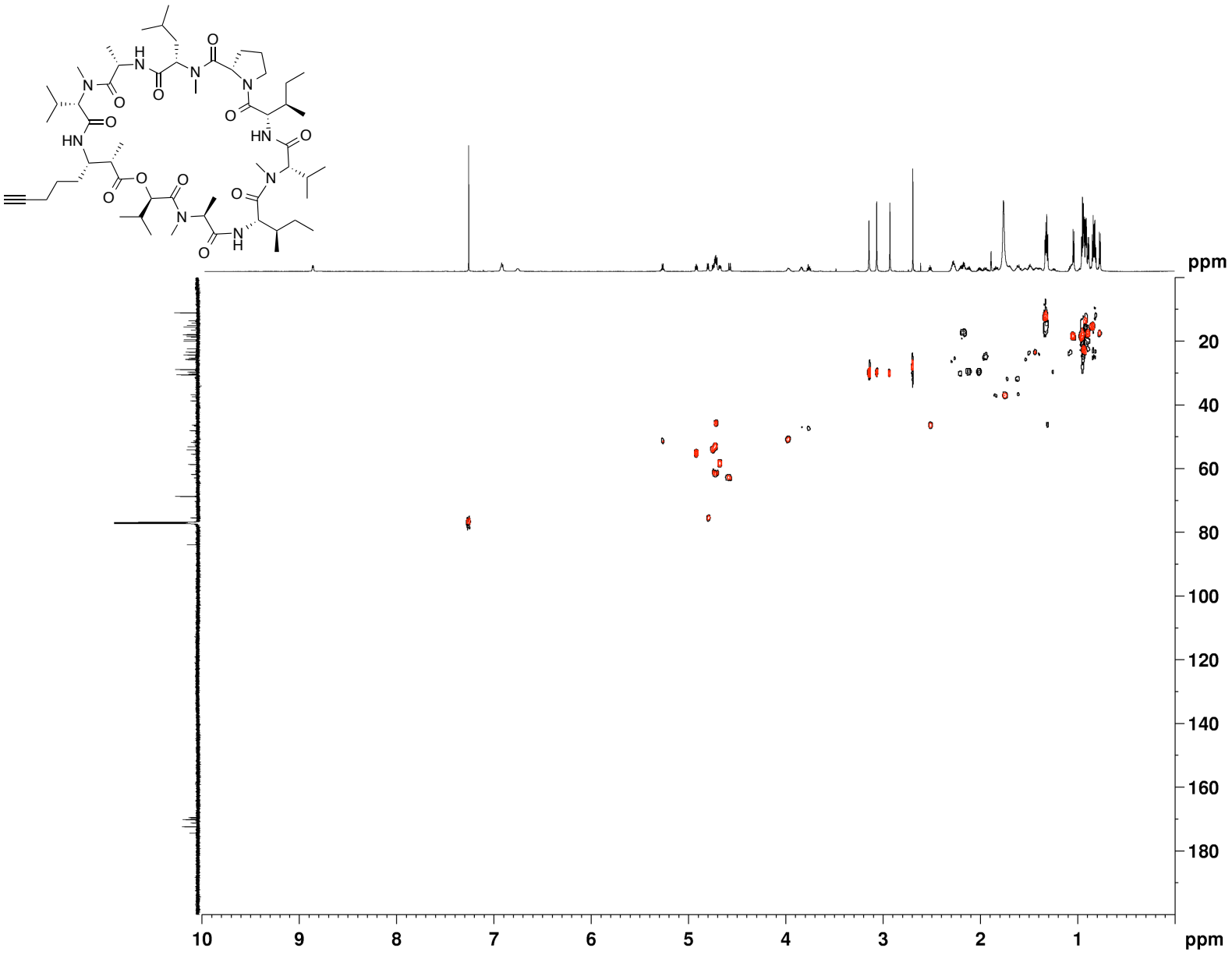

Figure S6. Multiplicity-edited HSQC spectrum for companeramide A (**1**) in $CDCl_3$ (700 MHz)

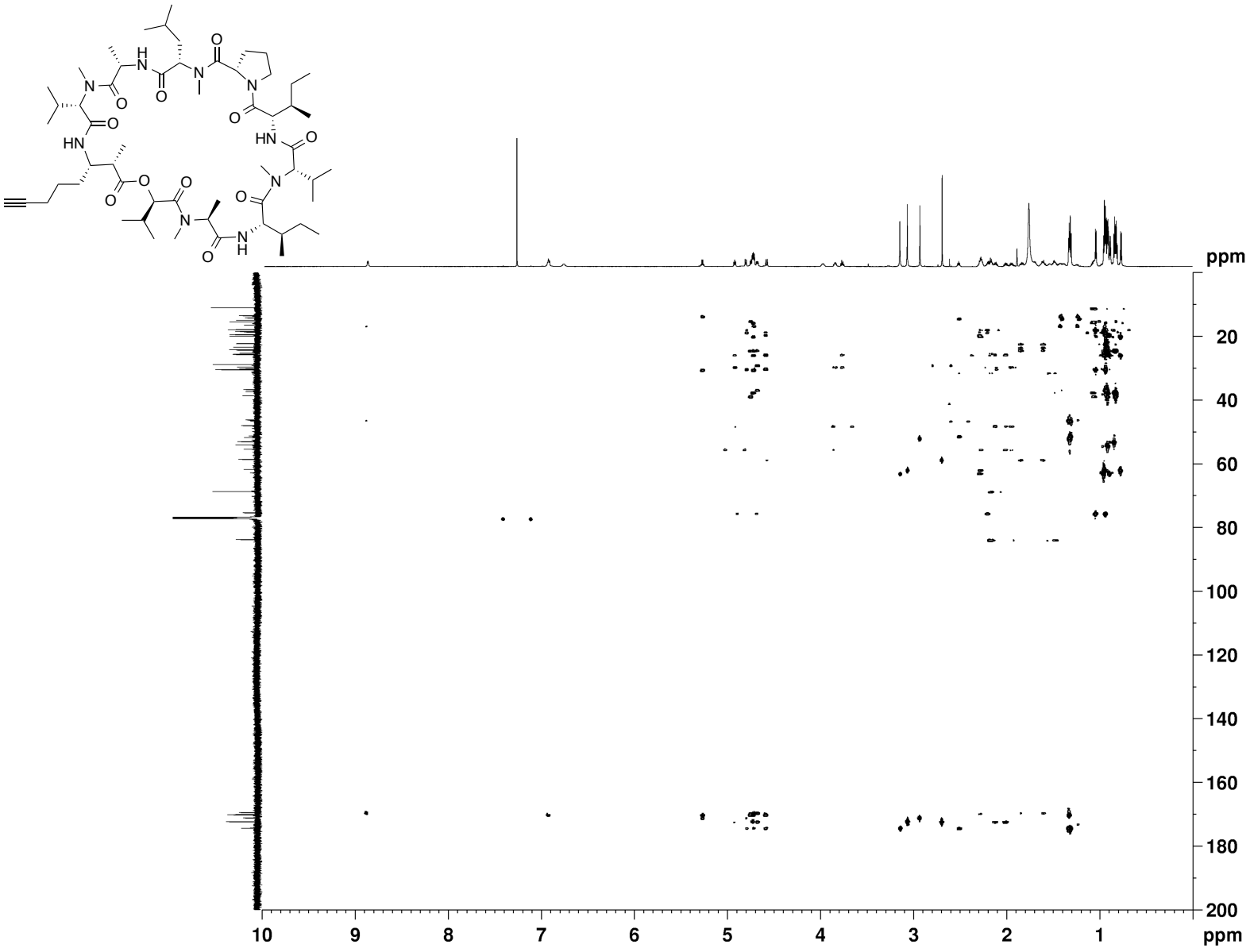

Figure S7. HMBC spectrum for companeramide A (**1**) in $CDCl_3$ (700 MHz)

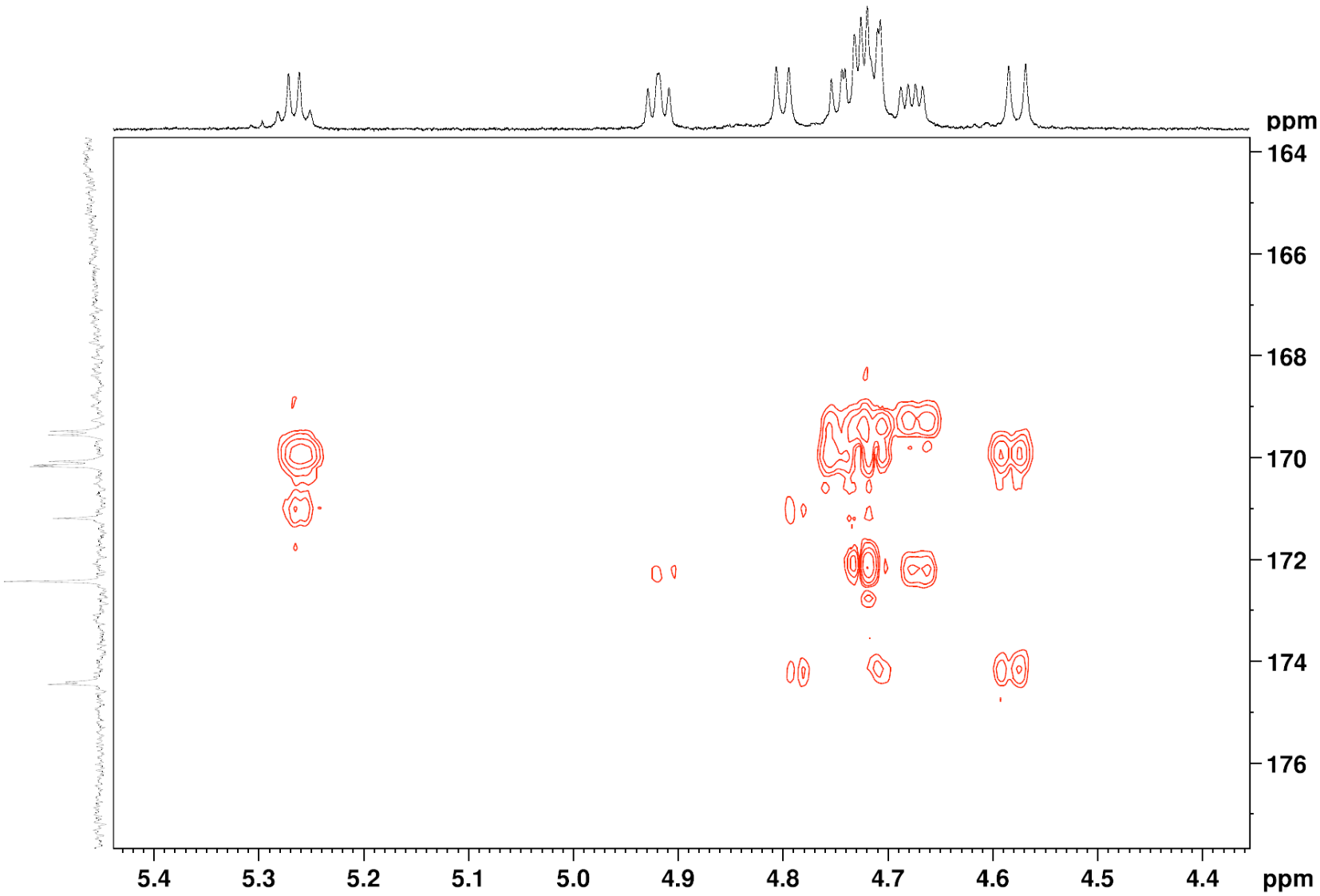

Figure S7A. Expanded partial HMBC spectrum for companeramide A (**1**) in $CDCl_3$ (700 MHz)

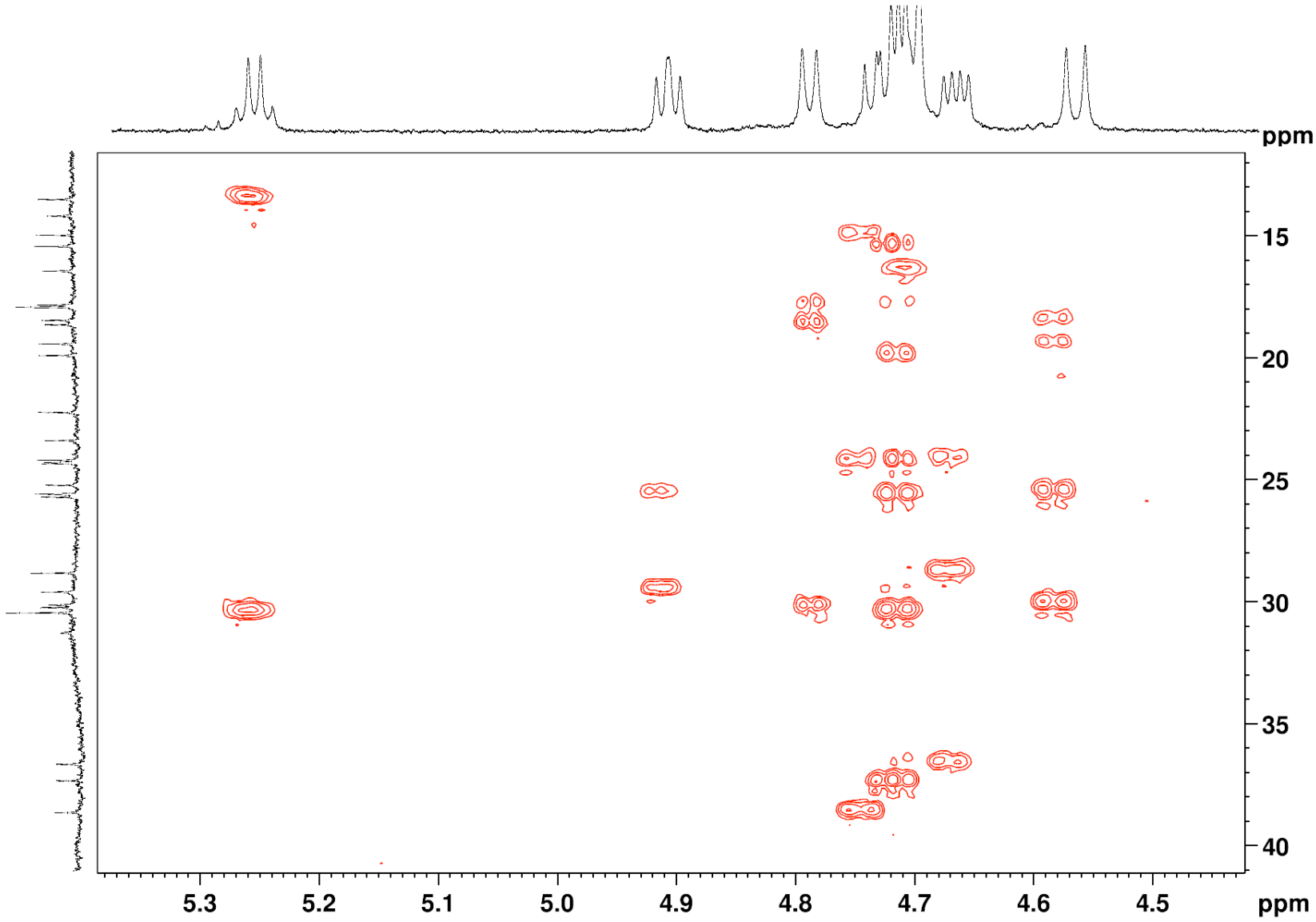

Figure S7B. Expanded partial HMBC spectrum for companeramide A (**1**) in $CDCl_3$ (700 MHz)

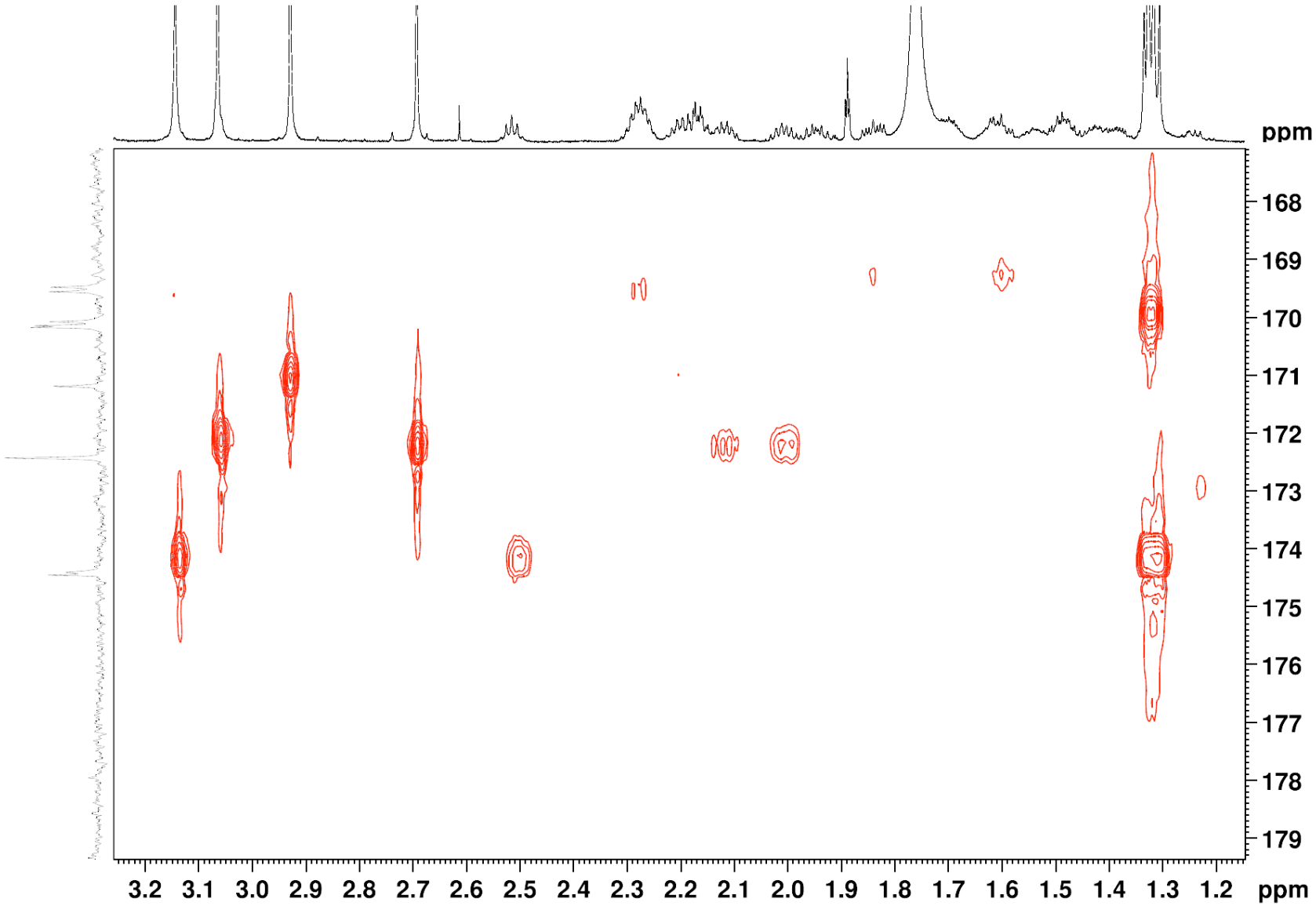

Figure S7C. Expanded partial HMBC spectrum for companeramide A (**1**) in $CDCl_3$ (700 MHz)

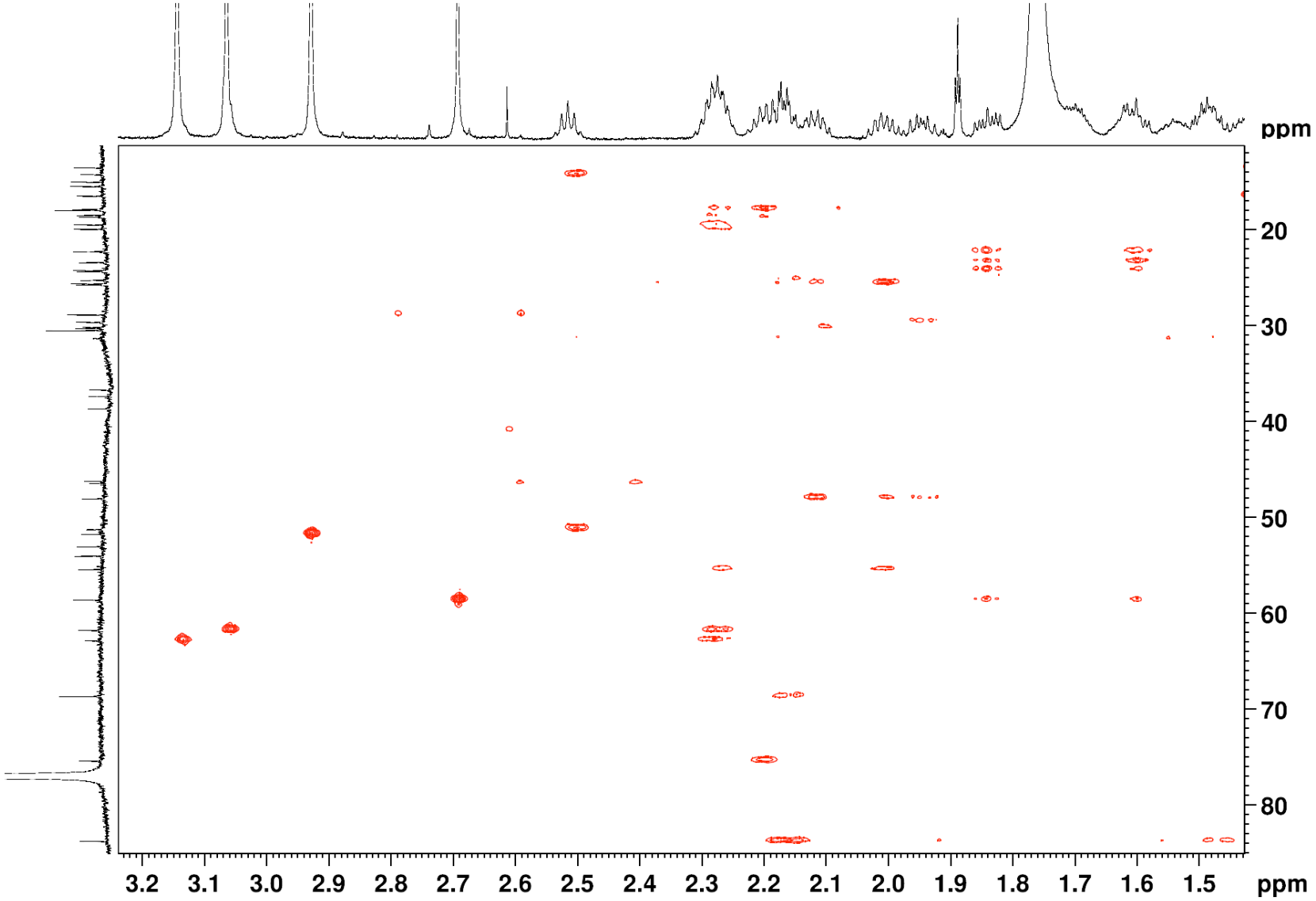

Figure S7D. Expanded partial HMBC spectrum for companeramide A (**1**) in $CDCl_3$ (700 MHz)

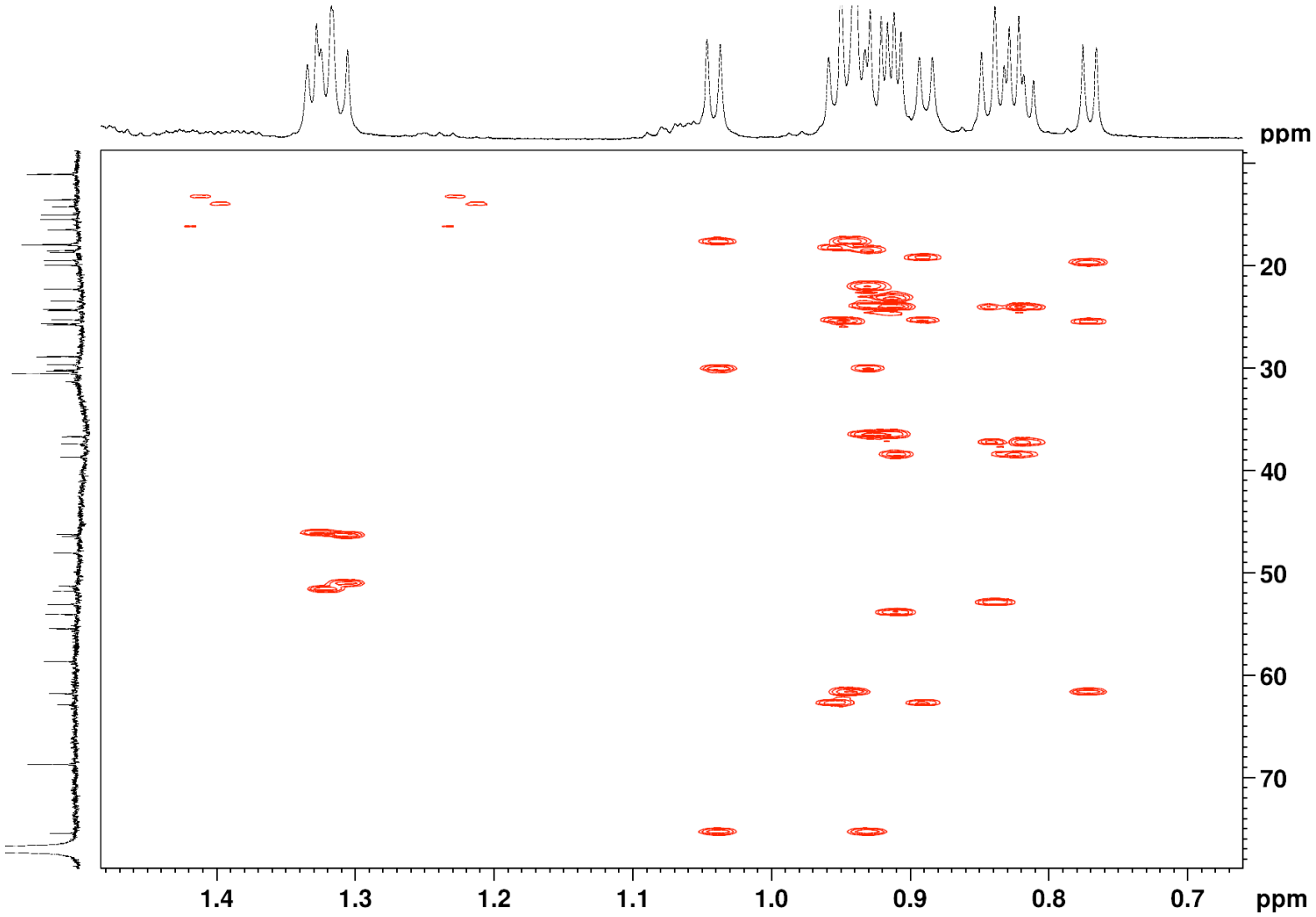

Figure S7E. Expanded partial HMBC spectrum for companeramide A (**1**) in $CDCl_3$ (700 MHz)

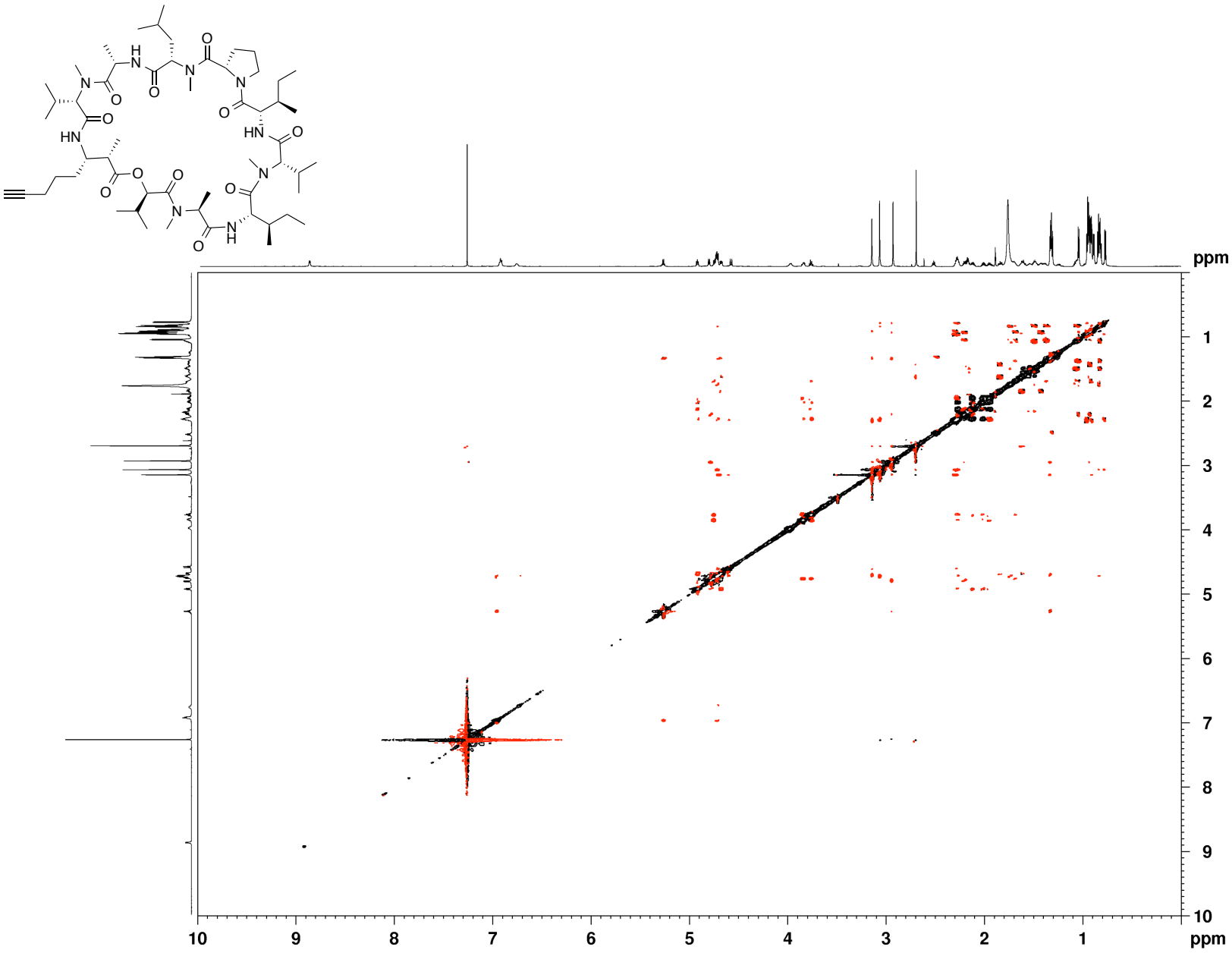

Figure S8. ROESY spectrum for companeramide A (**1**) in $CDCl_3$ (700 MHz)

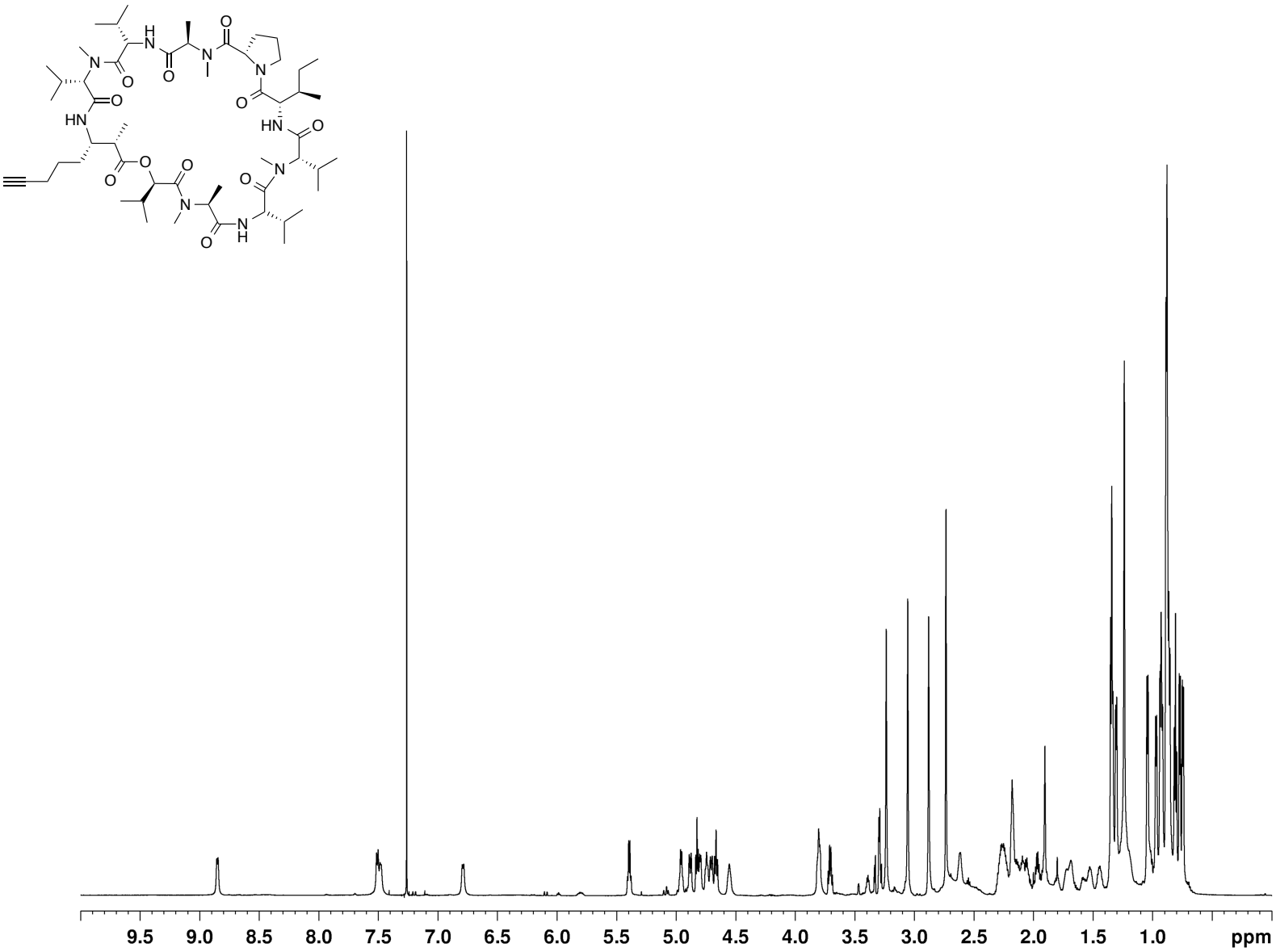

Figure S9. ^{1}H NMR spectrum for companeramide B (**2**) in $CDCl_3$ (700 MHz)

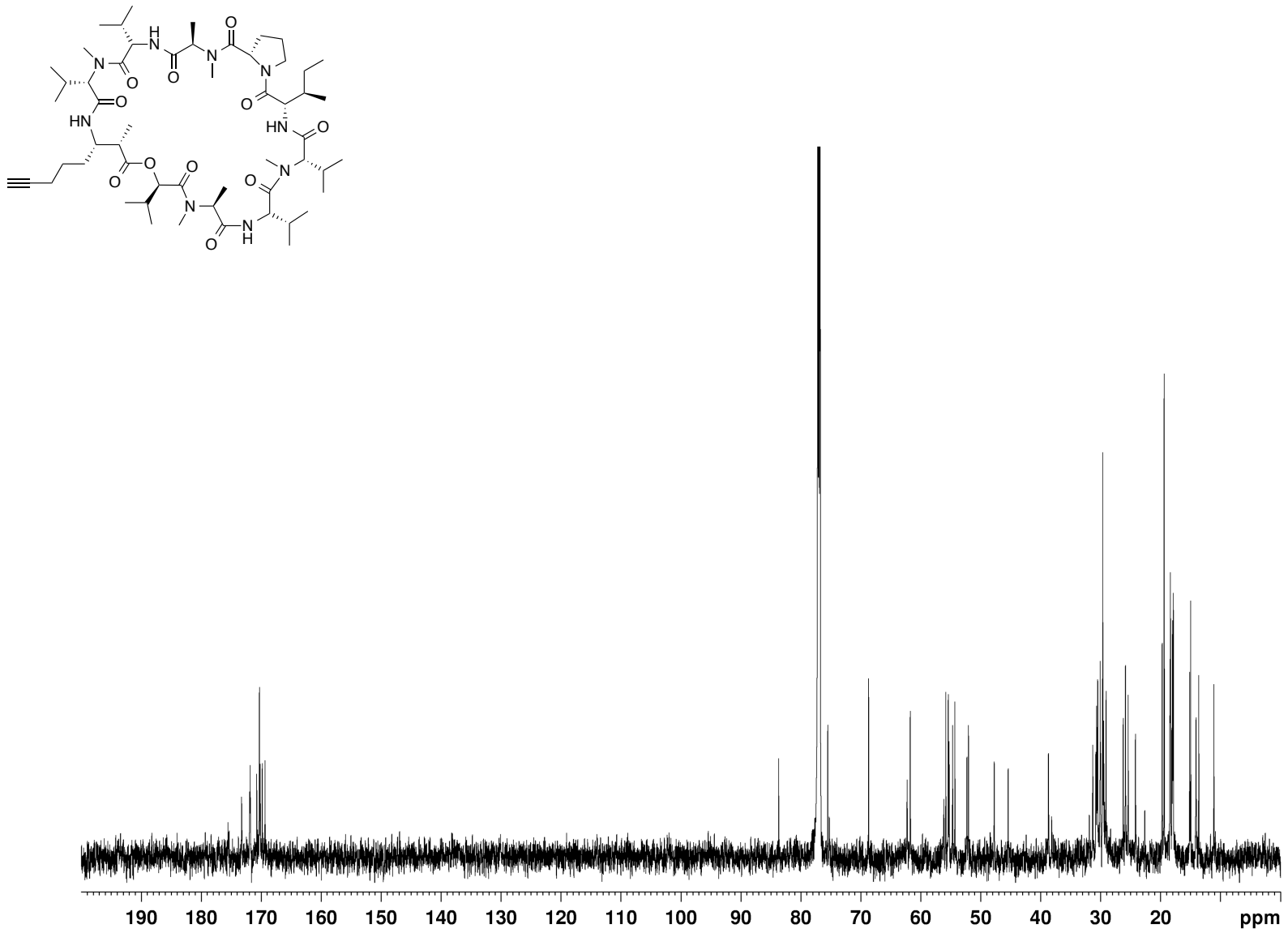

Figure S10. ^{13}C NMR spectrum for companeramide B (**2**) in $CDCl_3$ (175 MHz)

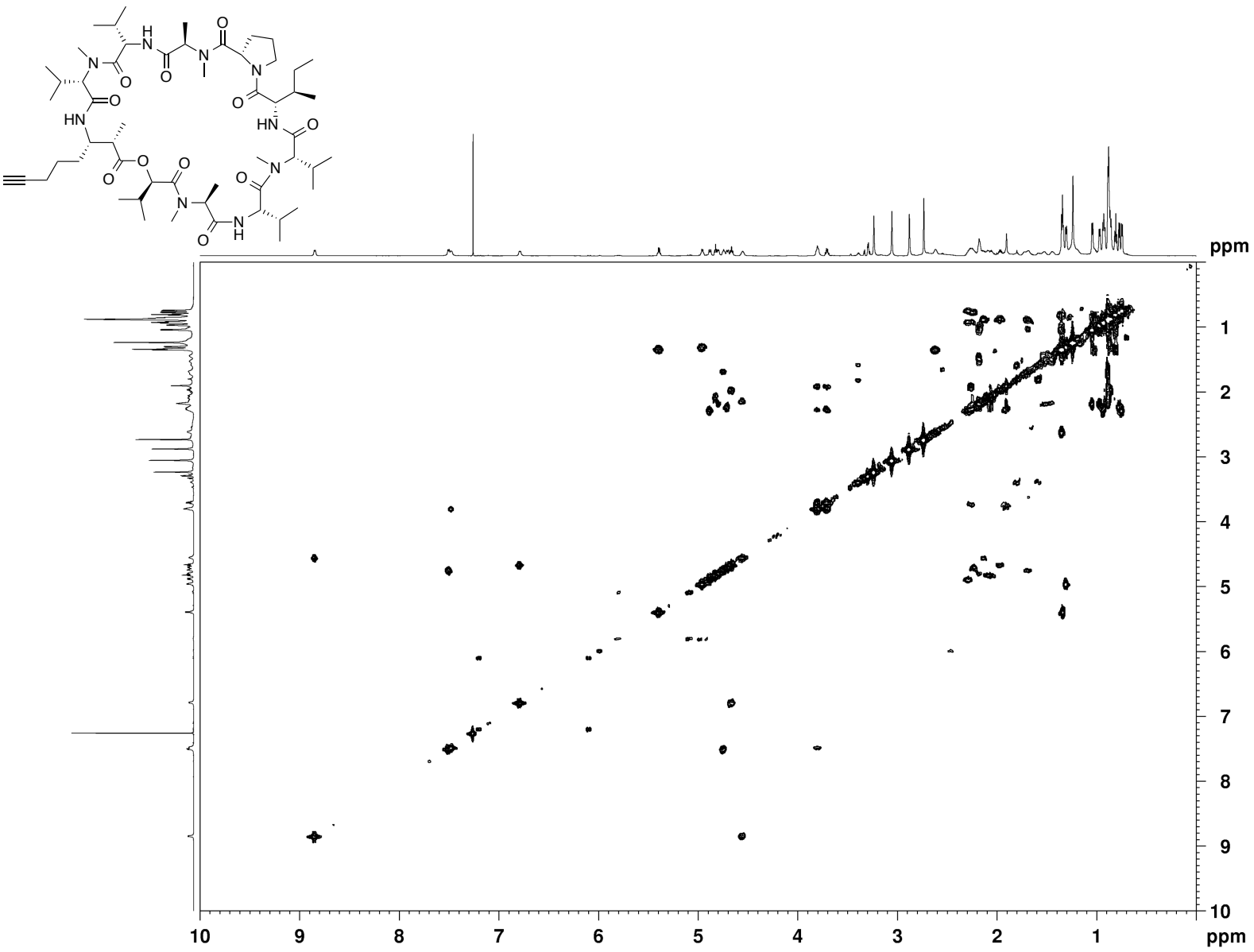

Figure S11. COSY spectrum for companeramide B (**2**) in $CDCl_3$ (700 MHz)

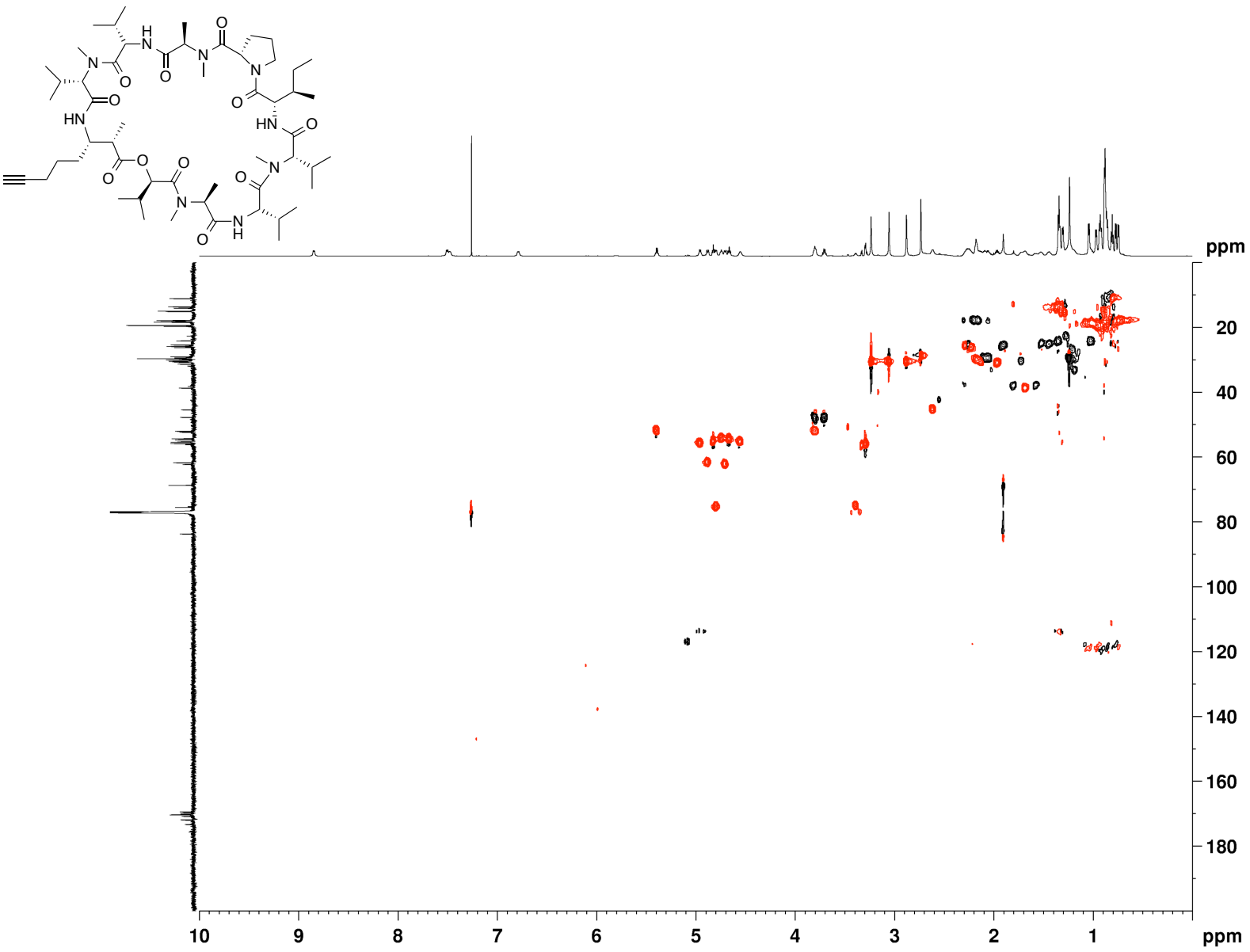

Figure S12. Multiplicity-edited HSQC spectrum for companeramide B (**2**) in $CDCl_3$ (700 MHz)

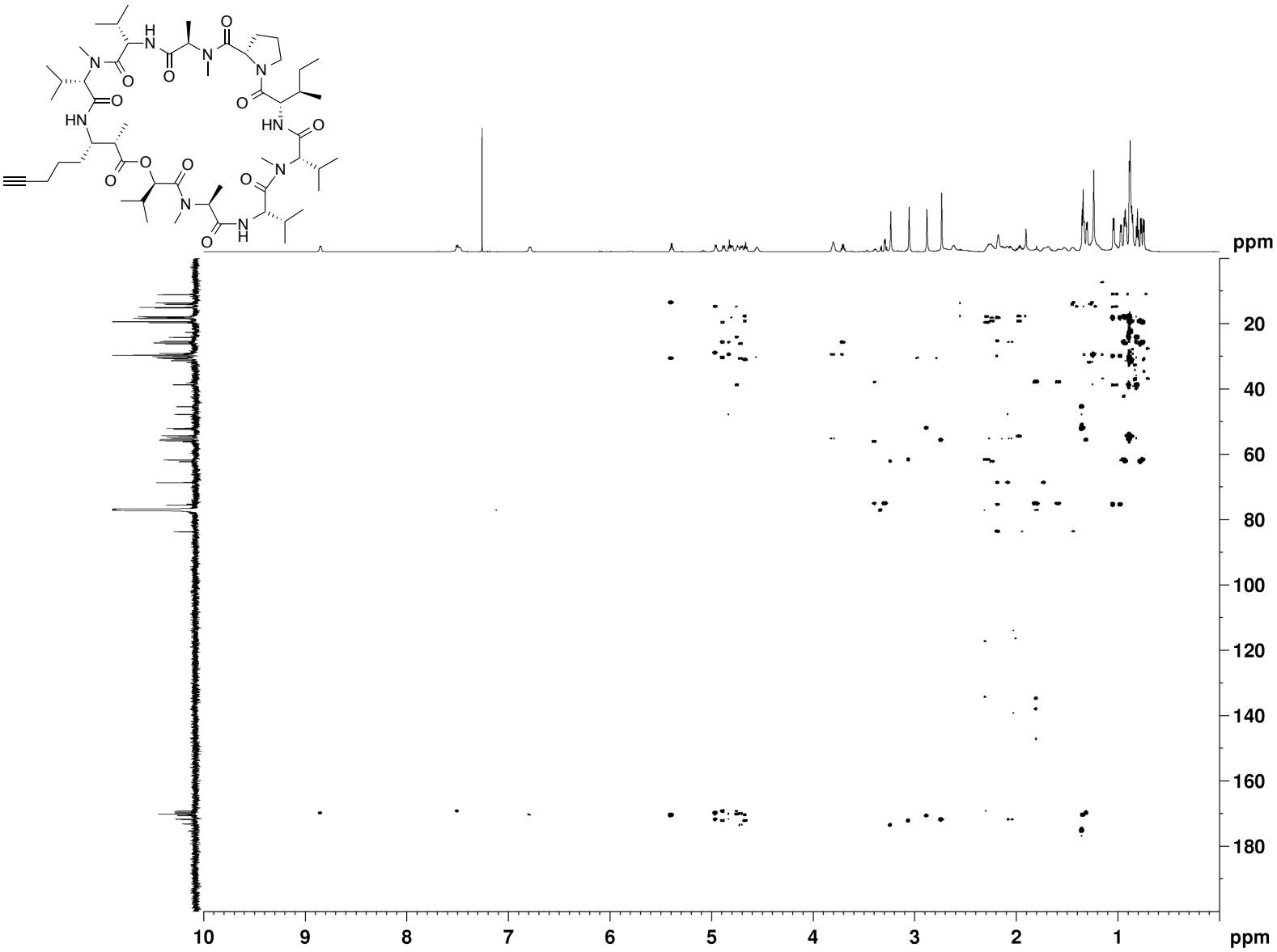

Figure S13. HMBC spectrum for companeramide B (**2**) in $CDCl_3$ (700 MHz)

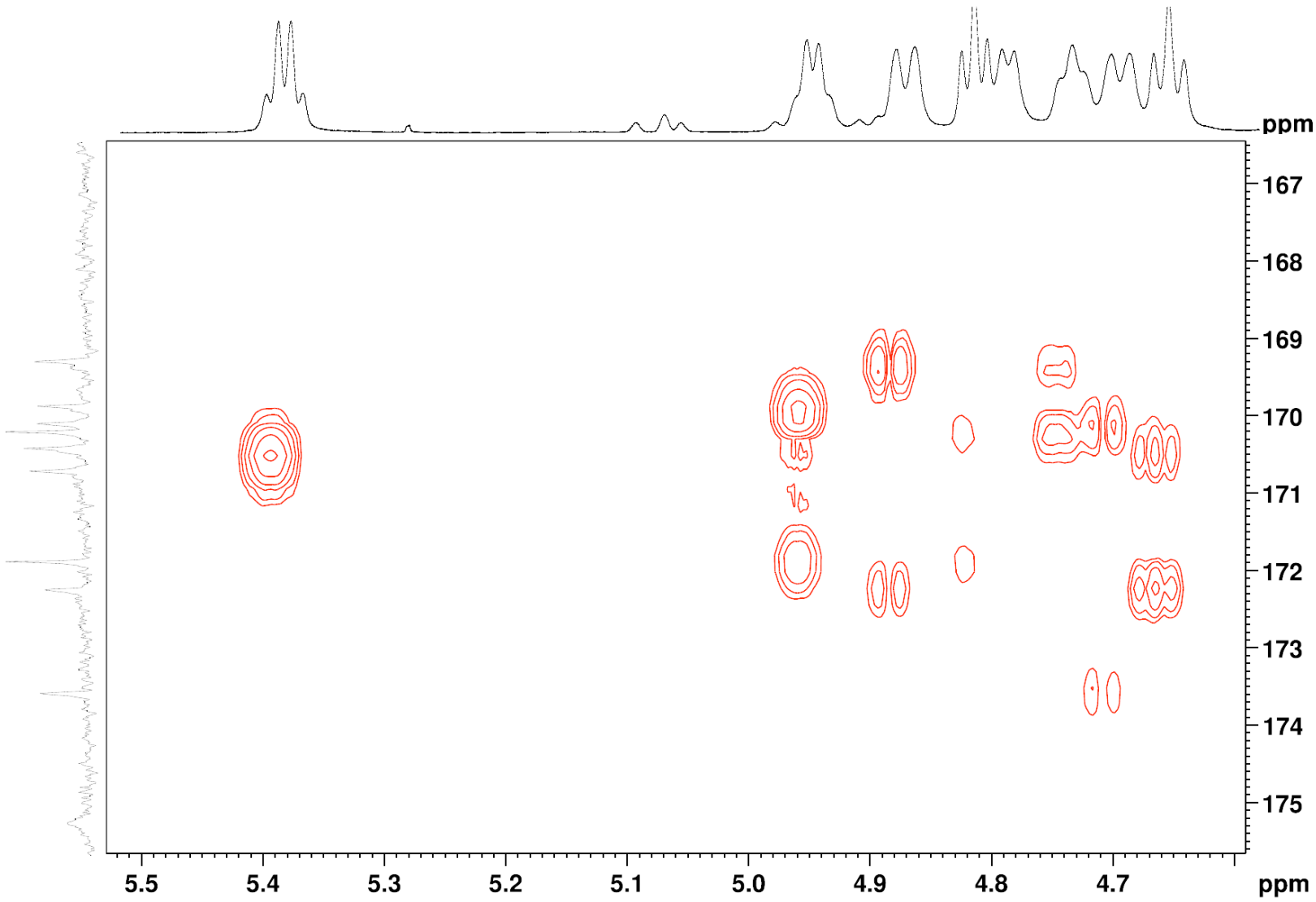

Figure S13A. Expanded partial HMBC spectrum for companeramide B (**2**) in $CDCl_3$ (700 MHz)

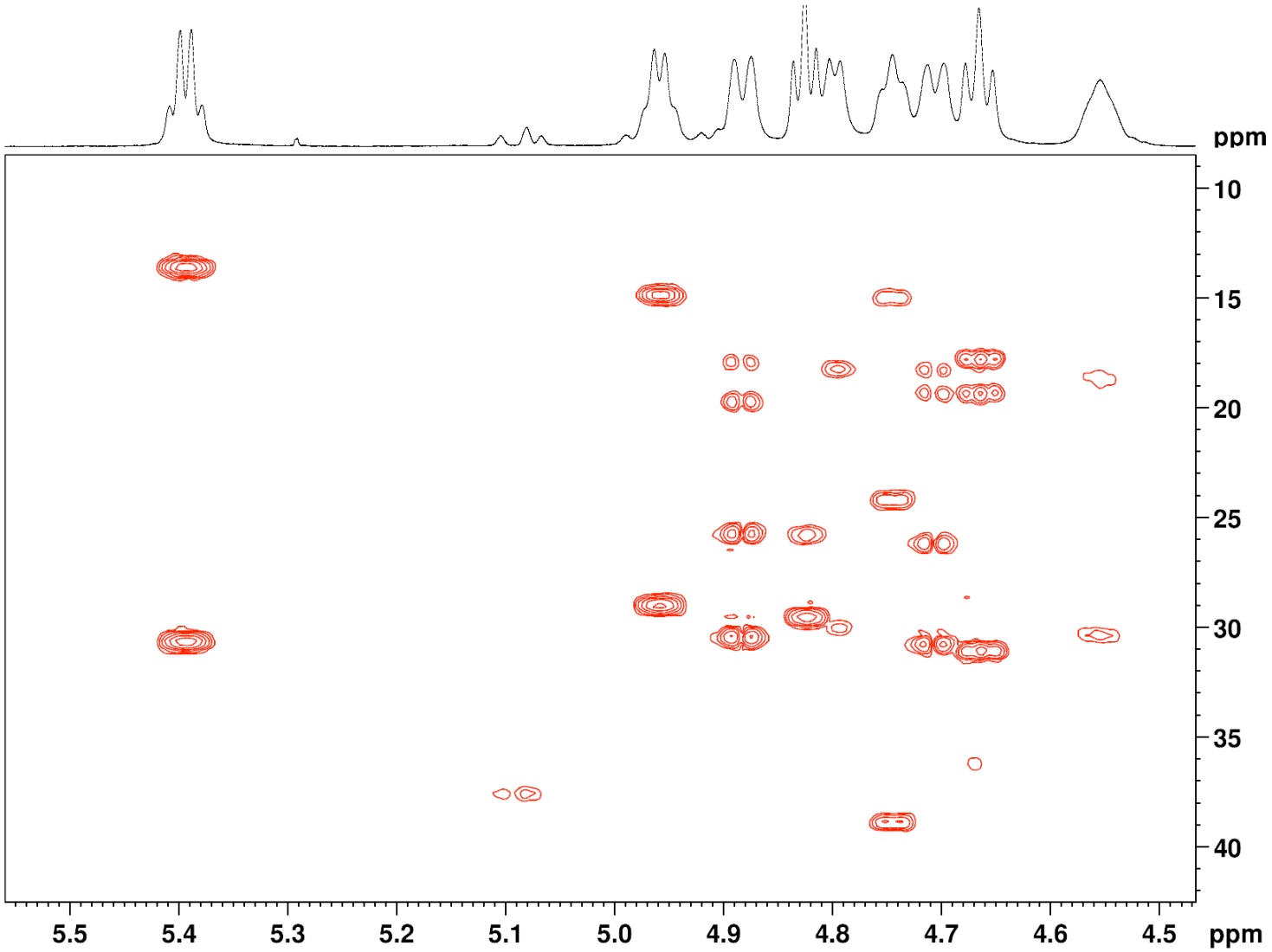

Figure S13B. Expanded partial HMBC spectrum for companeramide B (**2**) in $CDCl_3$ (700 MHz)

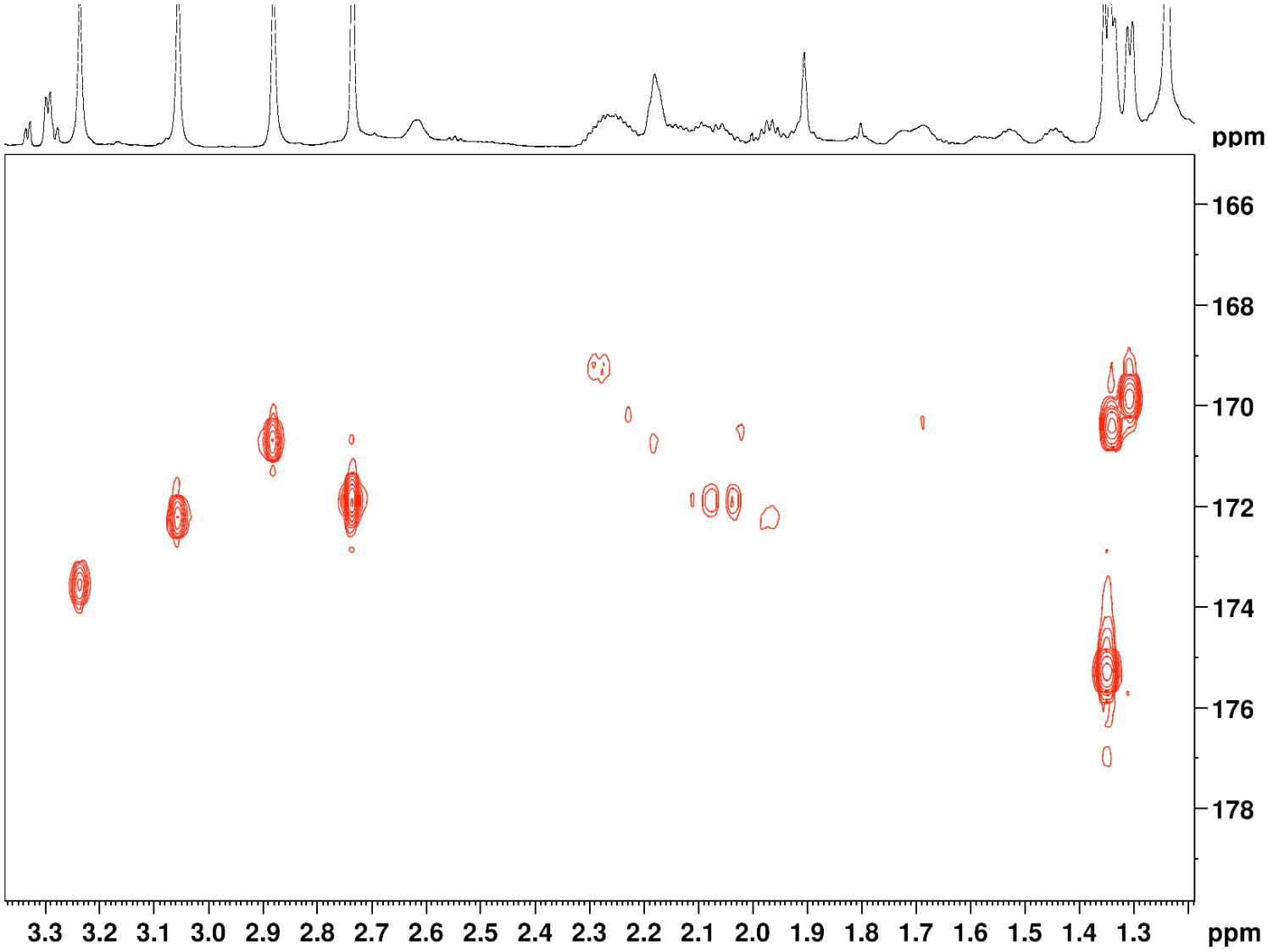

Figure S13C. Expanded partial HMBC spectrum for companeramide B (**2**) in $CDCl_3$ (700 MHz)

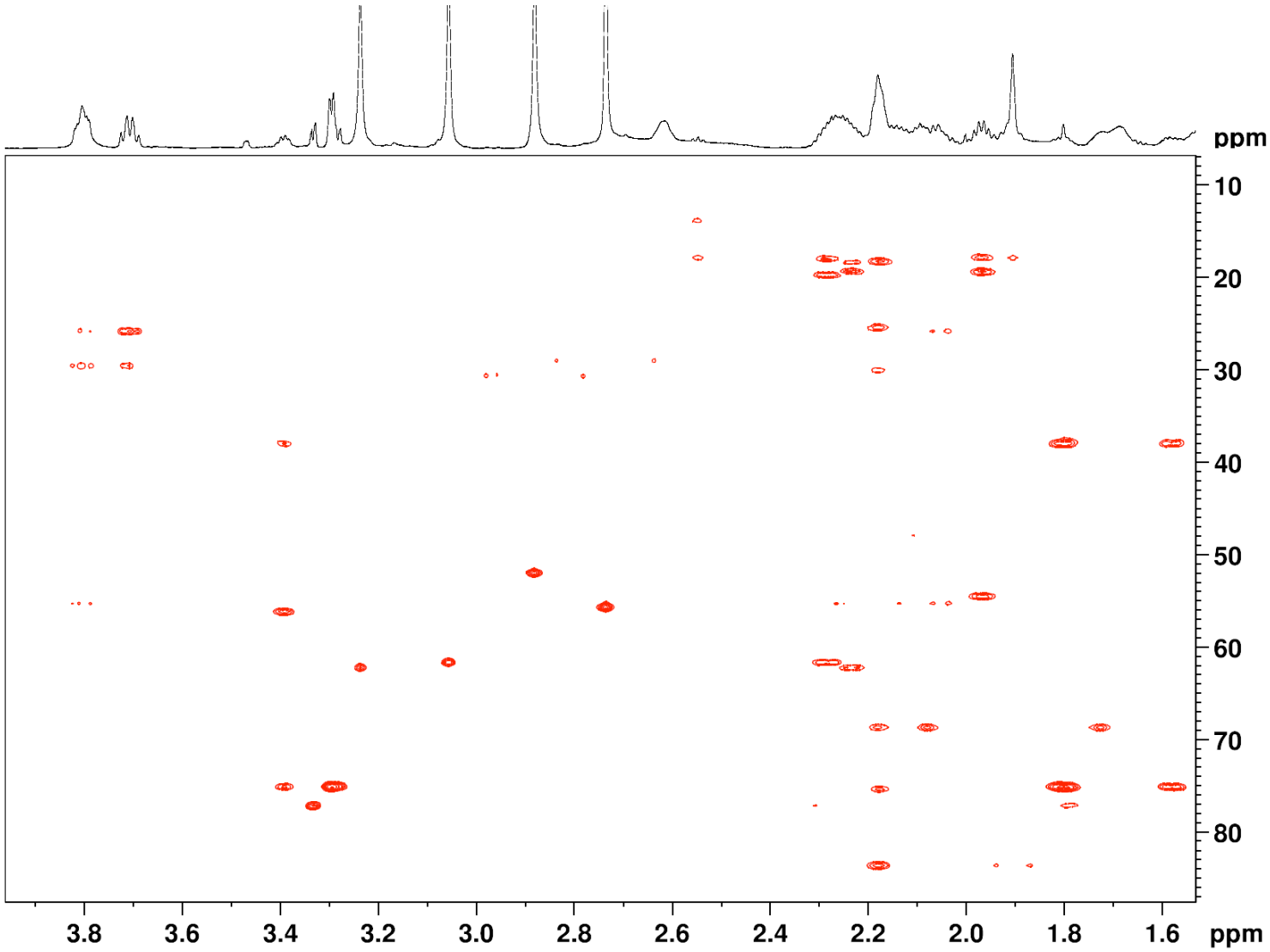

Figure S13D. Expanded partial HMBC spectrum for companeramide B (**2**) in $CDCl_3$ (700 MHz

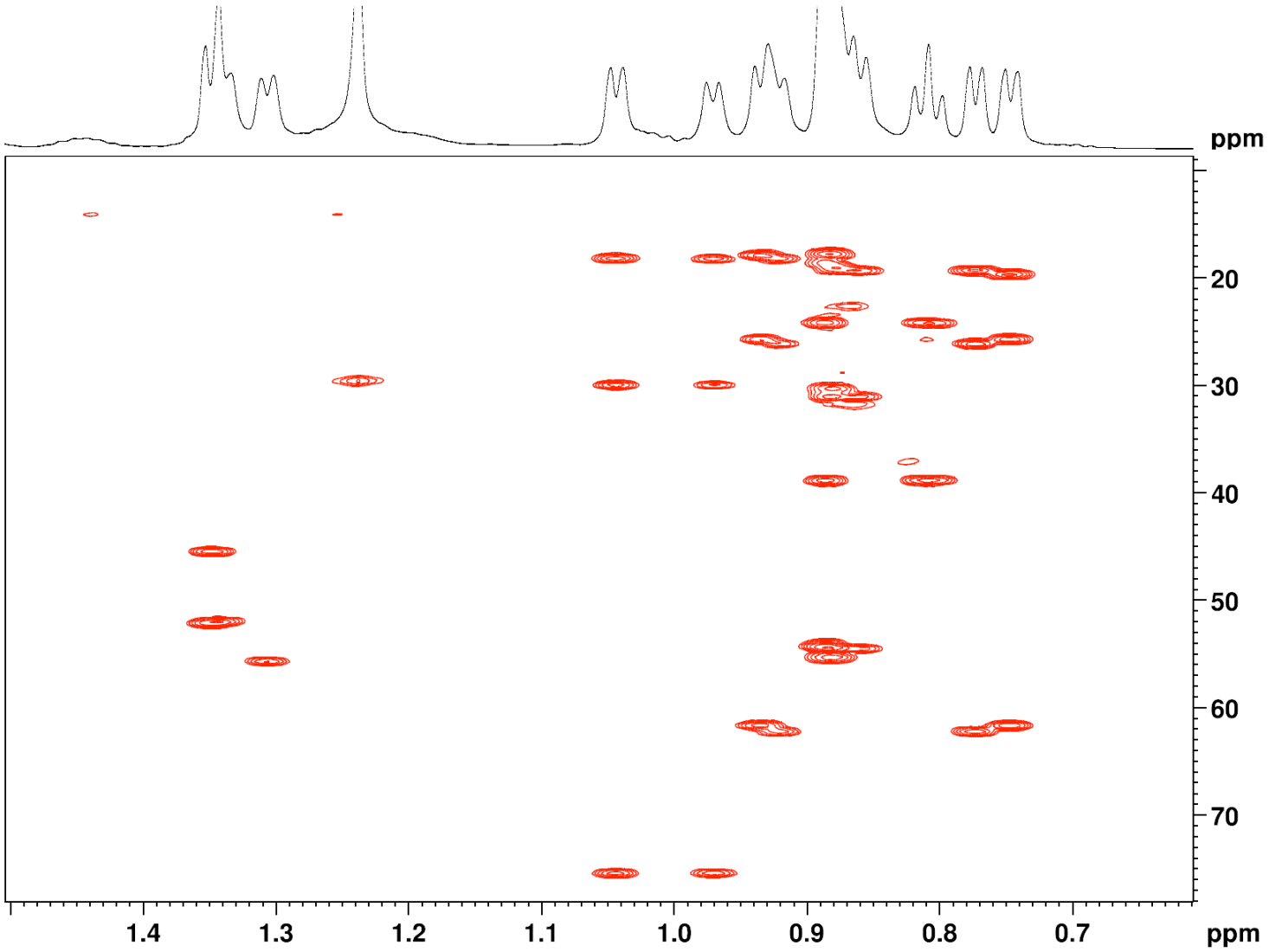

Figure S13E. Expanded partial HMBC spectrum for companeramide B (**2**) in $CDCl_3$ (700 MHz)

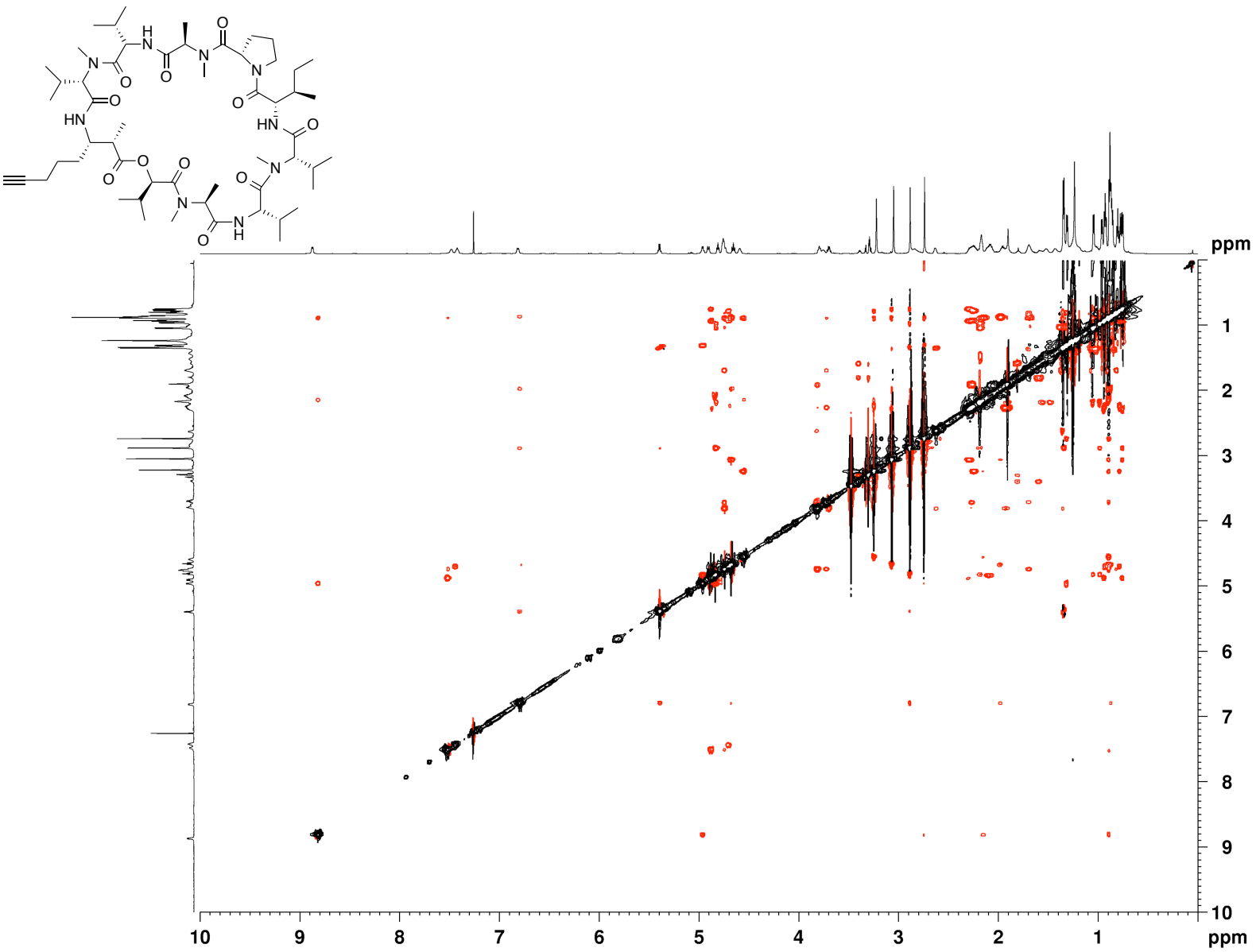

Figure S14. ROESY spectrum for companeramide B (**2**) in $CDCl_3$ (700 MHz)

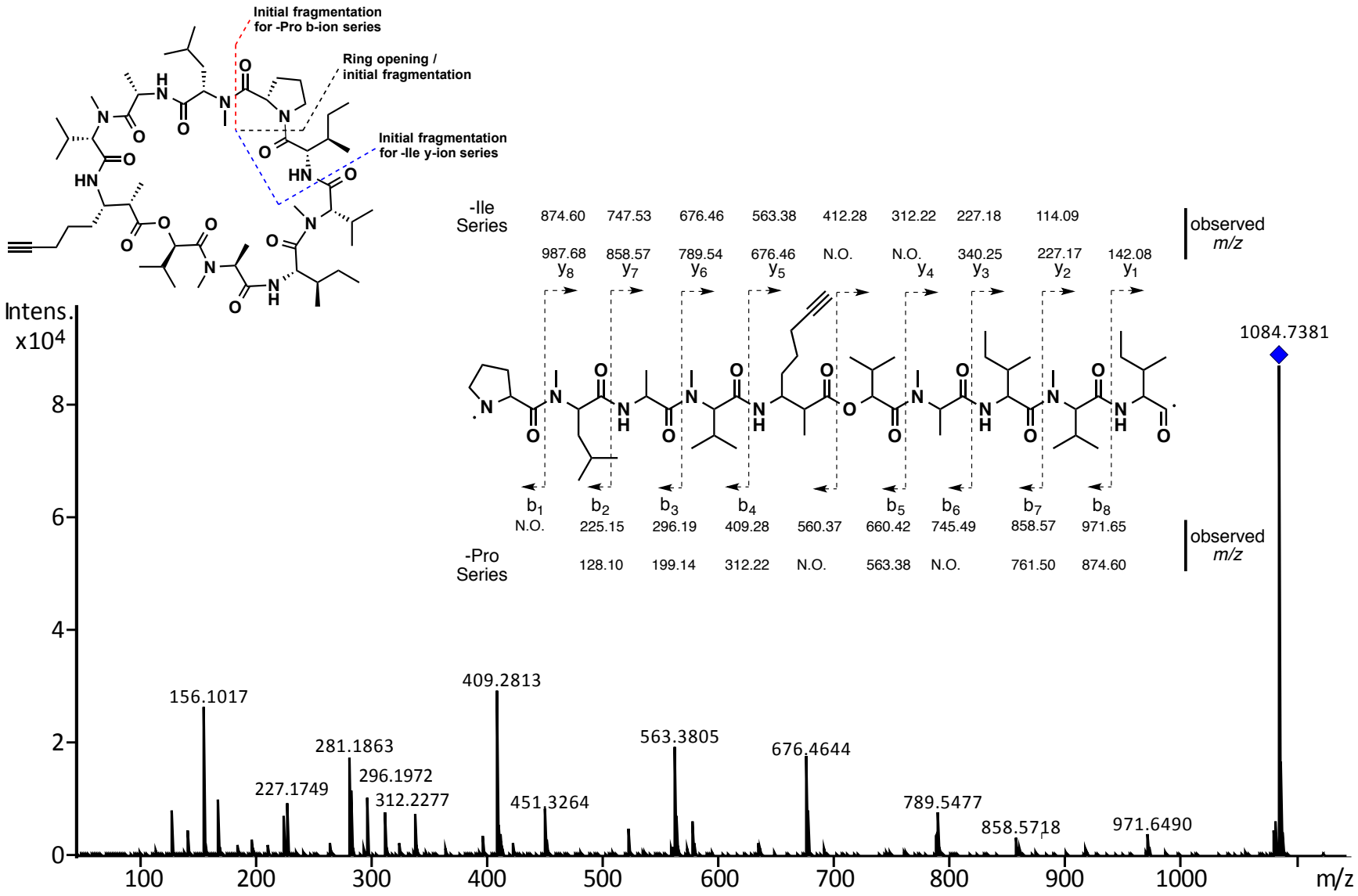

Figure S15. Annotated MS/MS spectrum for companeramide A (**1**) indicating $[M + H]^+$ ions arising from three alternate initial fragmentation events. N.O. designates ions that were not observed.

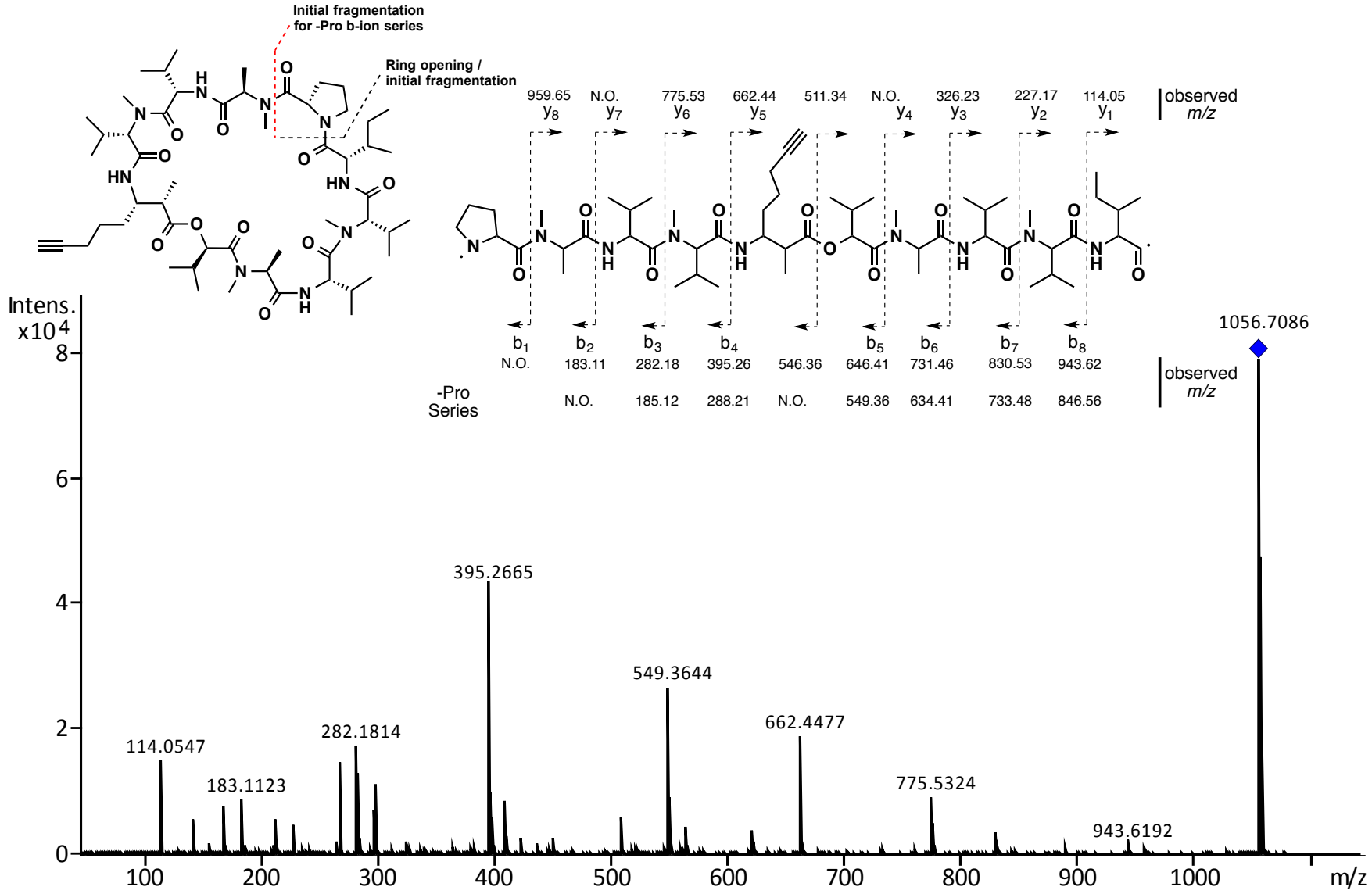

Figure S16. Annotated MS/MS spectrum for companeramide B (**2**) indicating $[M + H]^+$ ions arising from two alternate initial fragmentation events. N.O. designates ions that were not observed.

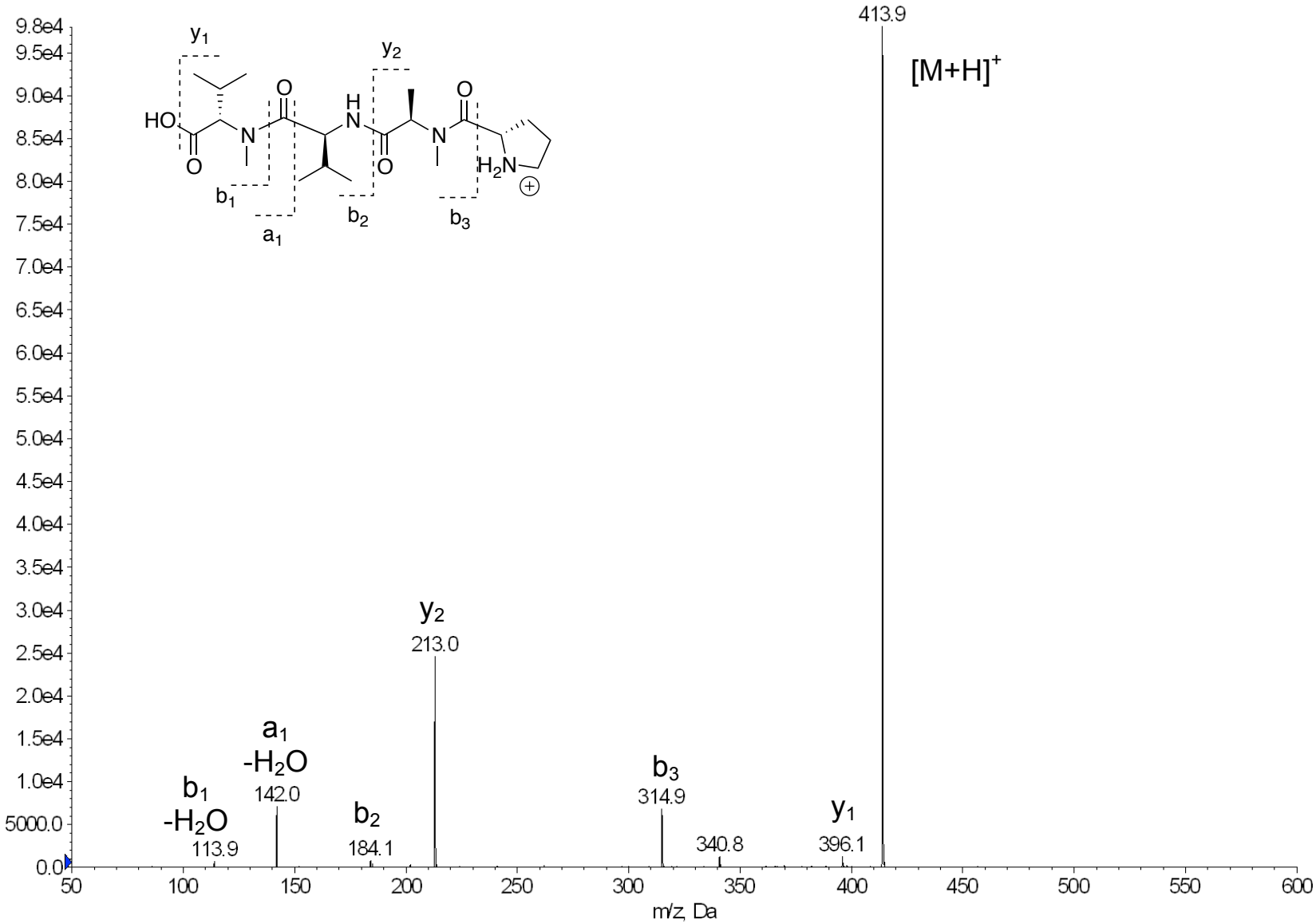

Figure S17. Annotated MS/MS spectrum for companeramide B (**2**) tetrapeptide (fragment 1)

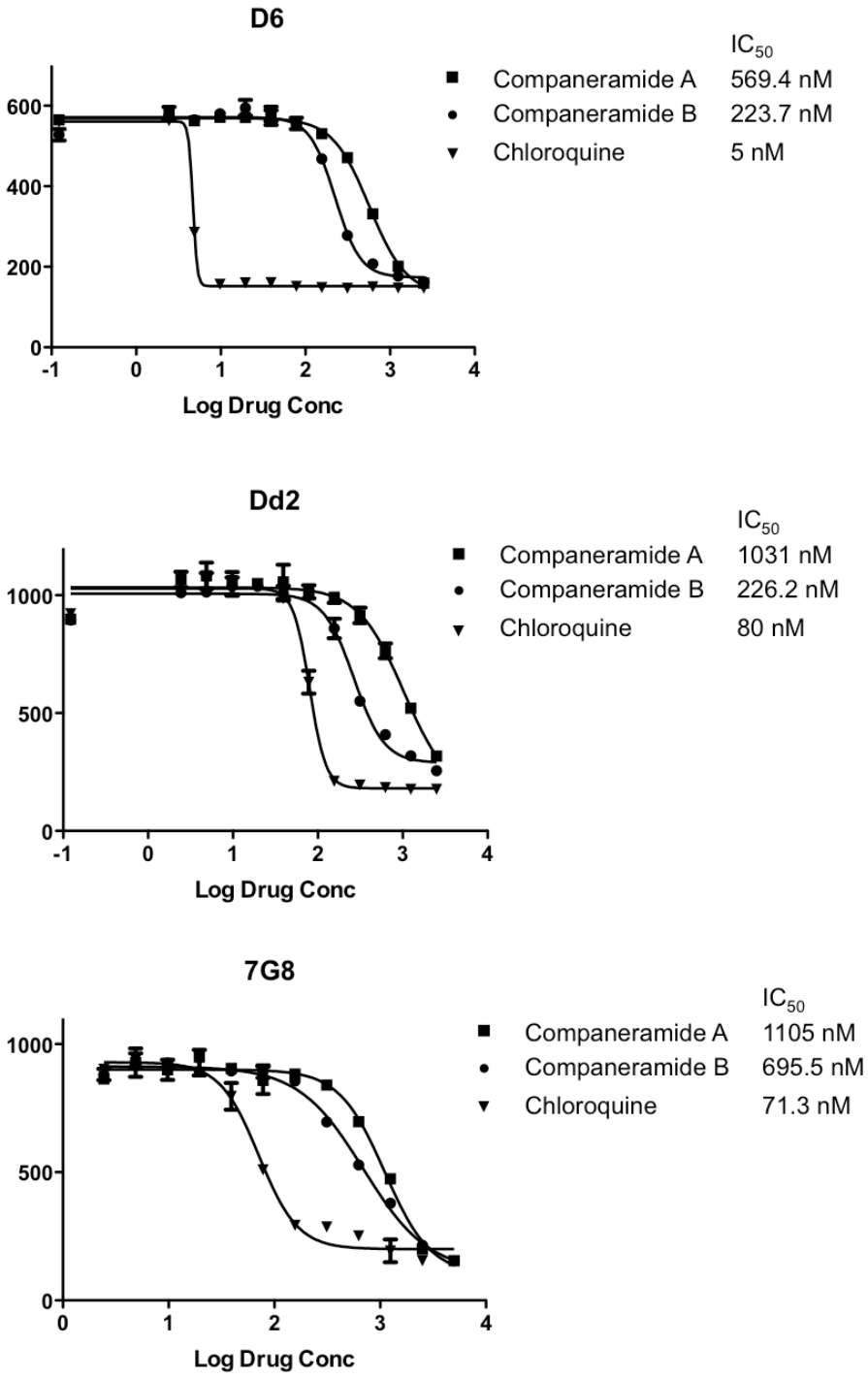

Figure S18. Concentration-response profiles for companeramides A (**1**) and B (**2**) against three strains of *Plasmodium falciparum*

S19. DNA Extraction and Amplification of Cyanobacterial 16S rRNA. A monoculture of the small filament cyanobacterium associated with companeramide production was lysed by mechanical bead beating as previously described[1] and total genomic DNA was purified by phenol-chloroform extraction. The 16S rRNA gene was amplified by PCR using PrimeSTAR GXL polymerase (Takara Bio) from total DNA with the primers CYA106F (5'-CGGACGGGTGAGTAACGCGTGA-3')[2] and 1513R (5'-TACIGITACCTTGTTACGACTT-3').[3] PCR products were cloned into the *Eco*RV site of pBlueScript SK- (Stratagene) and were sequenced with M13 primers that anneal outside of the multiple cloning site.[4] Desired sequences were aligned with MAFFT using the L-INS-i method.[5]

Phylogenetic Analysis. Phylogenetic analysis utilized bayesian inference with MrBayes software[6] using the GTR+I+G model[7] and was run for 6 million generations with *Gloeobacter violaceous* PCC 7412 (NR074282) as the outgroup. The 16S rRNA gene sequence of this small filament cyanobacterium (GenBank Accession number: KM882611) was found to be 99% similar to the filamentous cyanobacterium FLK9 isolated from the surface mat of the coral *Colpophyllia natans* from the Florida Keys. Construction of a phylogenetic tree showed that our small filament cyanobacterium PAC-10-3 csf1 clustered together with other filamentous marine strains (Figure S20), some of which are associated with Black Band Disease in coral.[8] Interestingly, unicellular *Synechococcus* strains (clustered with PAC-10-3 csf1) cluster with *Prochlorothrix* and *Geitlerinema* in a similar arrangement to that generated by a full genome phylogenetic comparison.[9]

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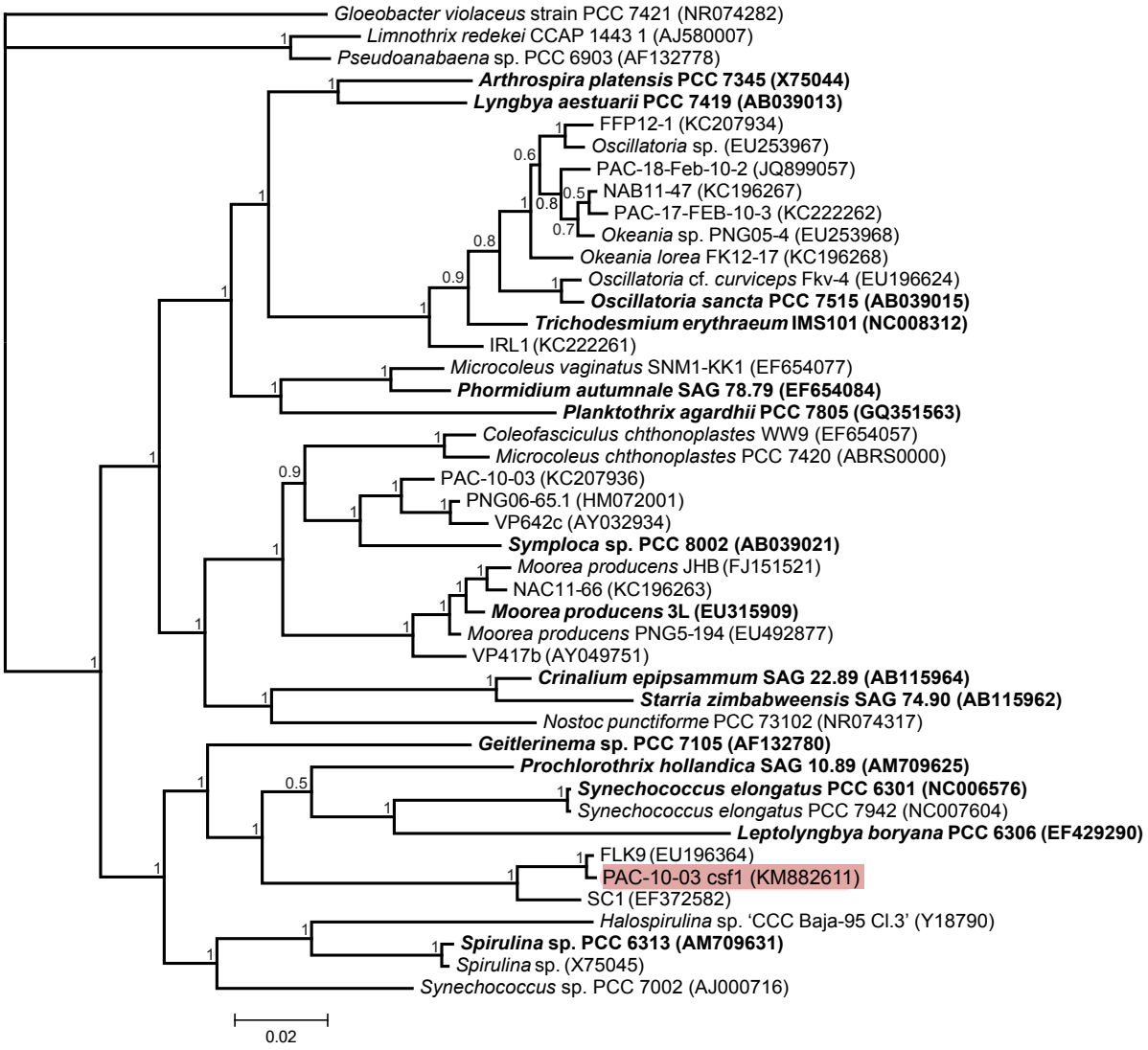

Figure S20. Phylogenetic tree of known natural product-producing marine cyanobacteria in relation to strain PAC-10-03 csf1 (highlighted in red), and its two closest BLAST hits FLK9 and SC1. Reference strains are in bold. *Gloeobacter violaceus* strain PCC 7412 was used as an outgroup. 16S rRNA analysis is based on Bayesian inference using the MrBayes software package and node values indicate posterior probability of occurrence. Scale bar indicates 0.05 nucleotide substitutions per site.

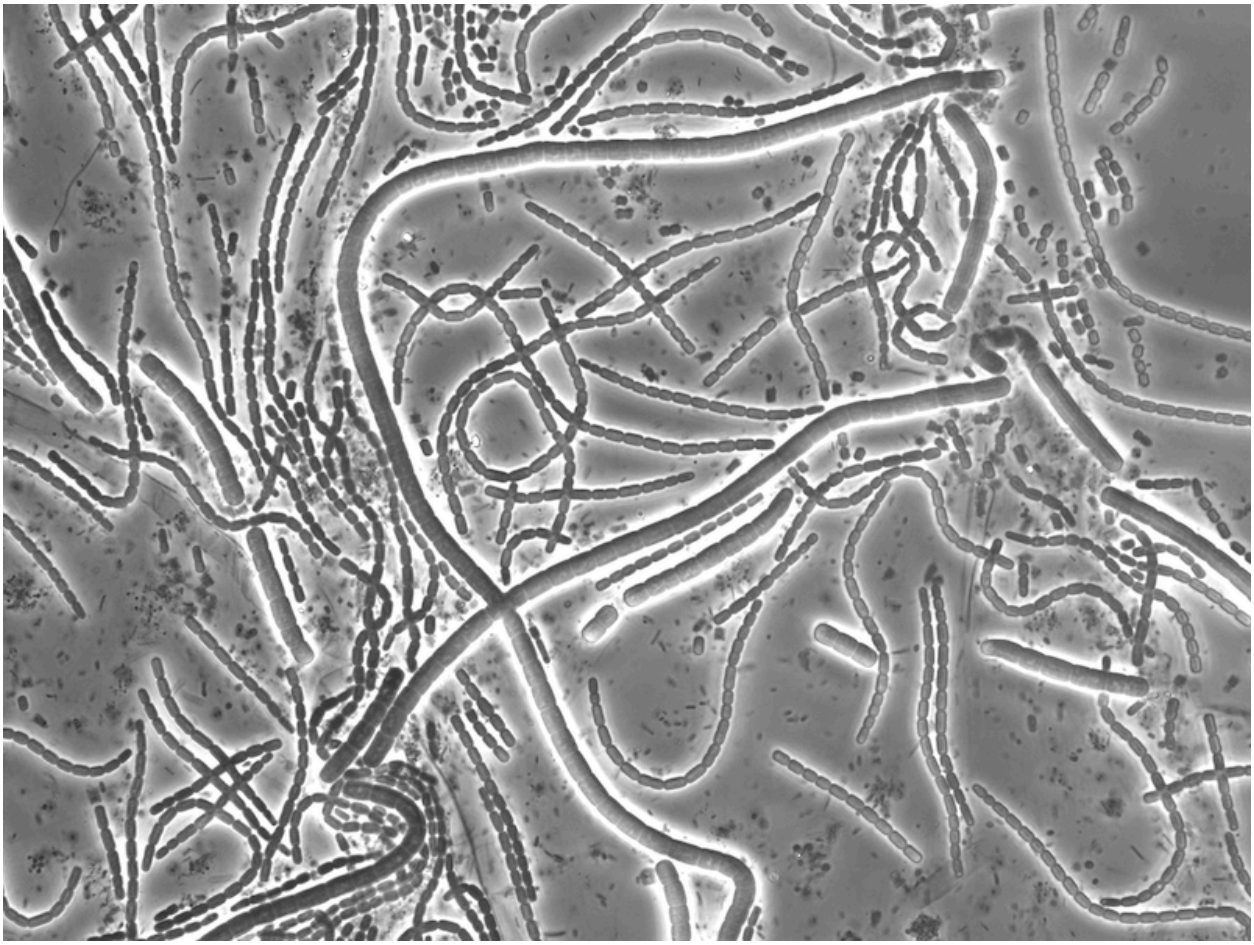

Figure S21. Phase contrast micrograph (60 X magnification) of the laboratory cultured cyanobacterial assemblage from field collection PAC-10-3, showing relative abundance of the large filament *Symploca*-type (GenBank # KC207936) and small filament (GenBank # KM882611) cyanobacteria.