Supplementary Information

Figure S1. Detector response from HPLC purification of ngercheumicins, where fractions 6, 8, 9 and 11 gave ngercheumicin F, G, H and I, respectively. Fraction size is not shown, as these are pooled fractions from the automatic fractionation. Fraction 7 and 10 contains isomers of ngercheumicin F and H.

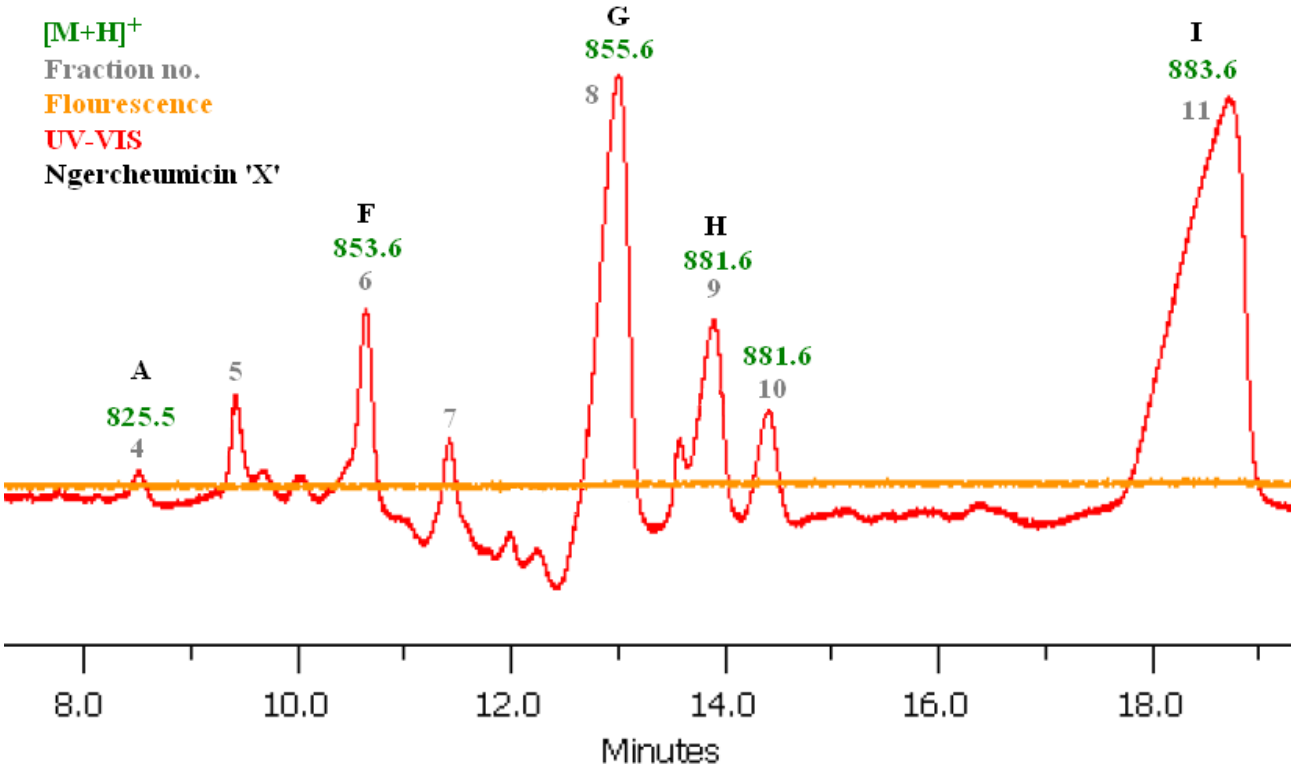

Figure S2. Diode array detected (200–700 nm) chromatograms for purified samples of ngercheumicin F, G, H and I (from the top).

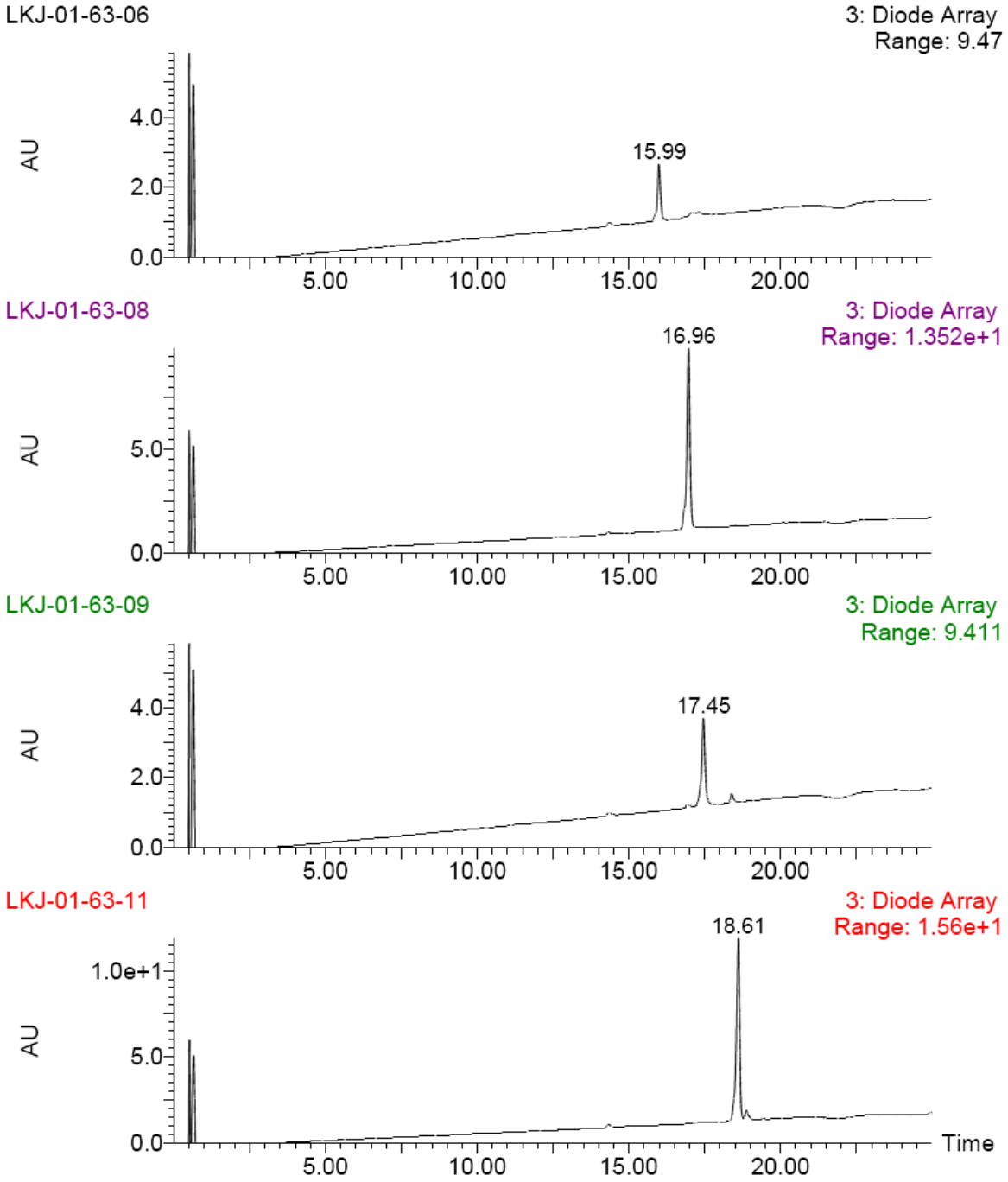

Figure S3. Selected region of the total ion chromatograms (TIC) of ngercheumicin F, G, H and I (from the top), showing $[M + H]^+$, $[M + NH_4]^+$ and weak $[M + Na]^+$ adducts. Outside the range are also $[2M + NH_4]^+$ at *m/z* 1723–1784.

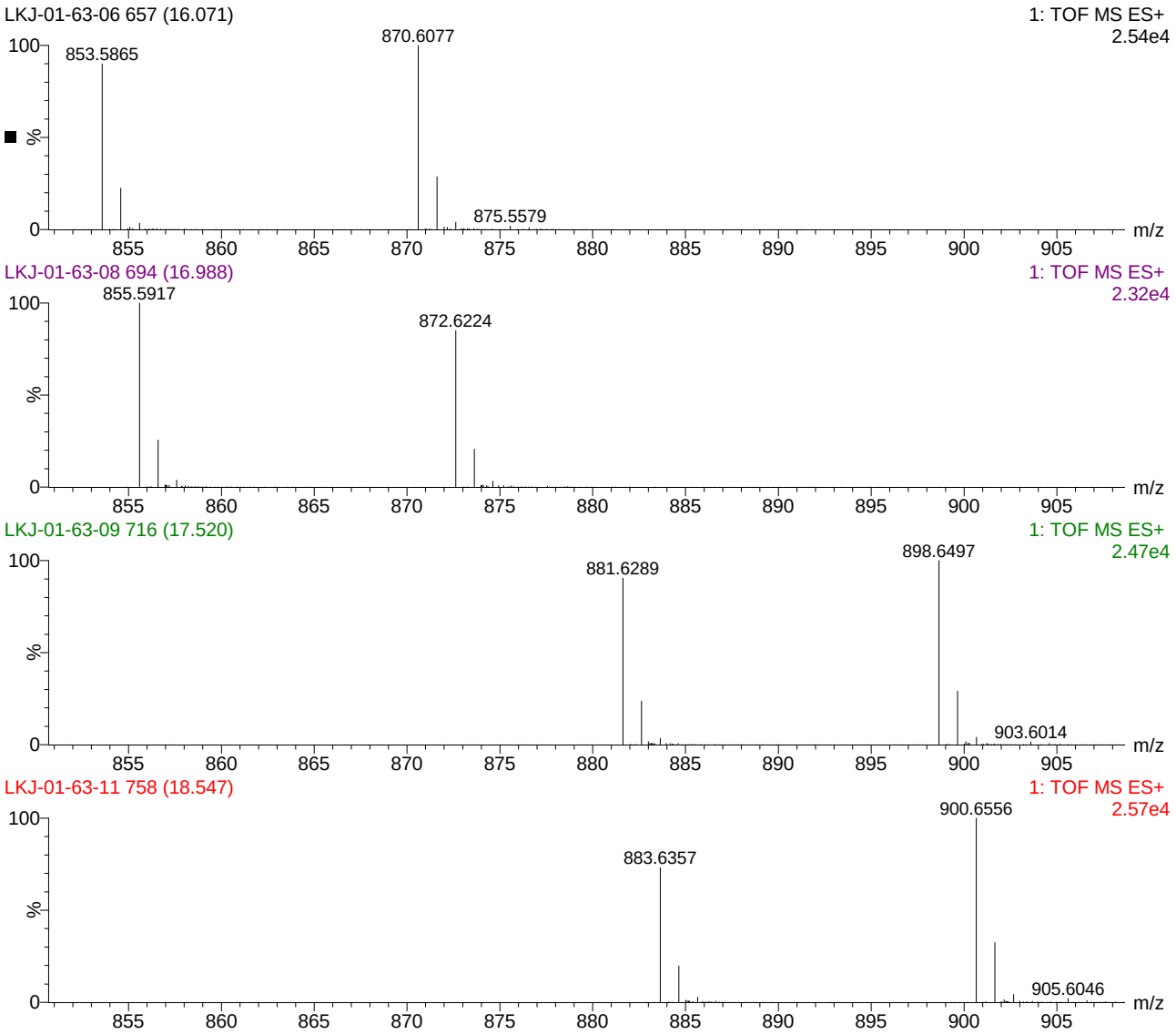

Figure S4. ^{1}H spectrum for ngercheumicin F.

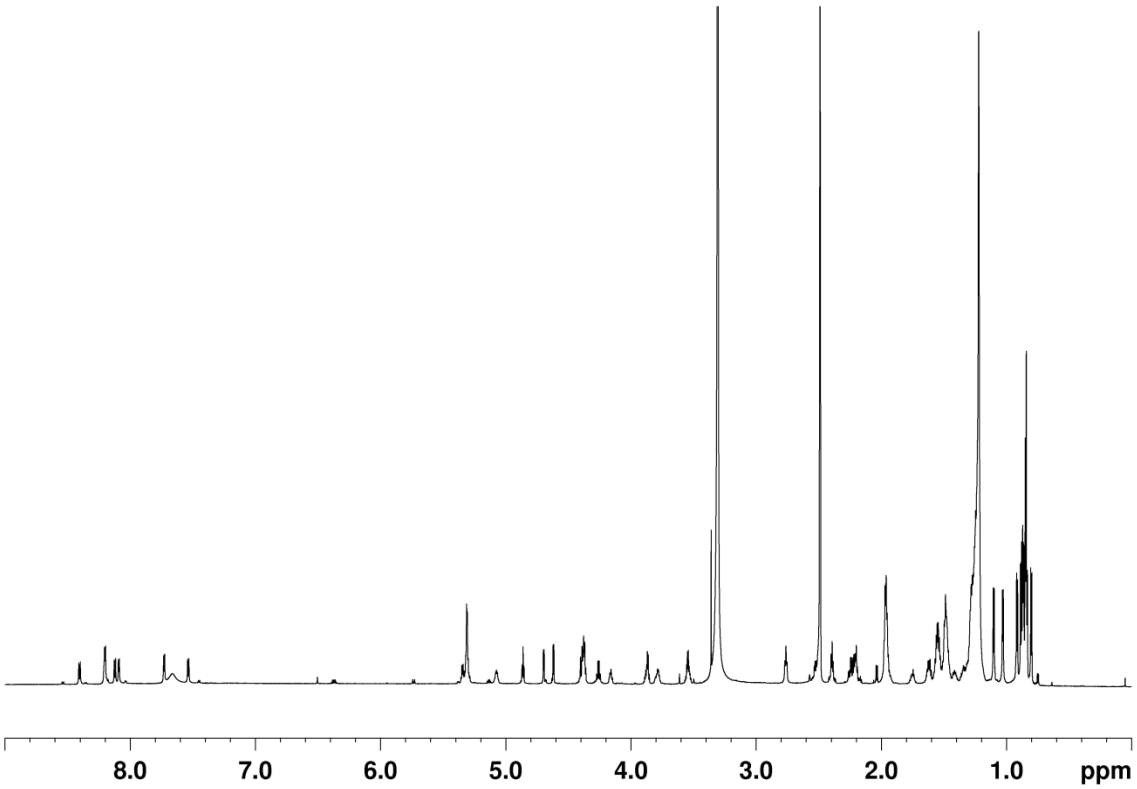

Figure S5. DQF-COSY for ngercheumicin F.

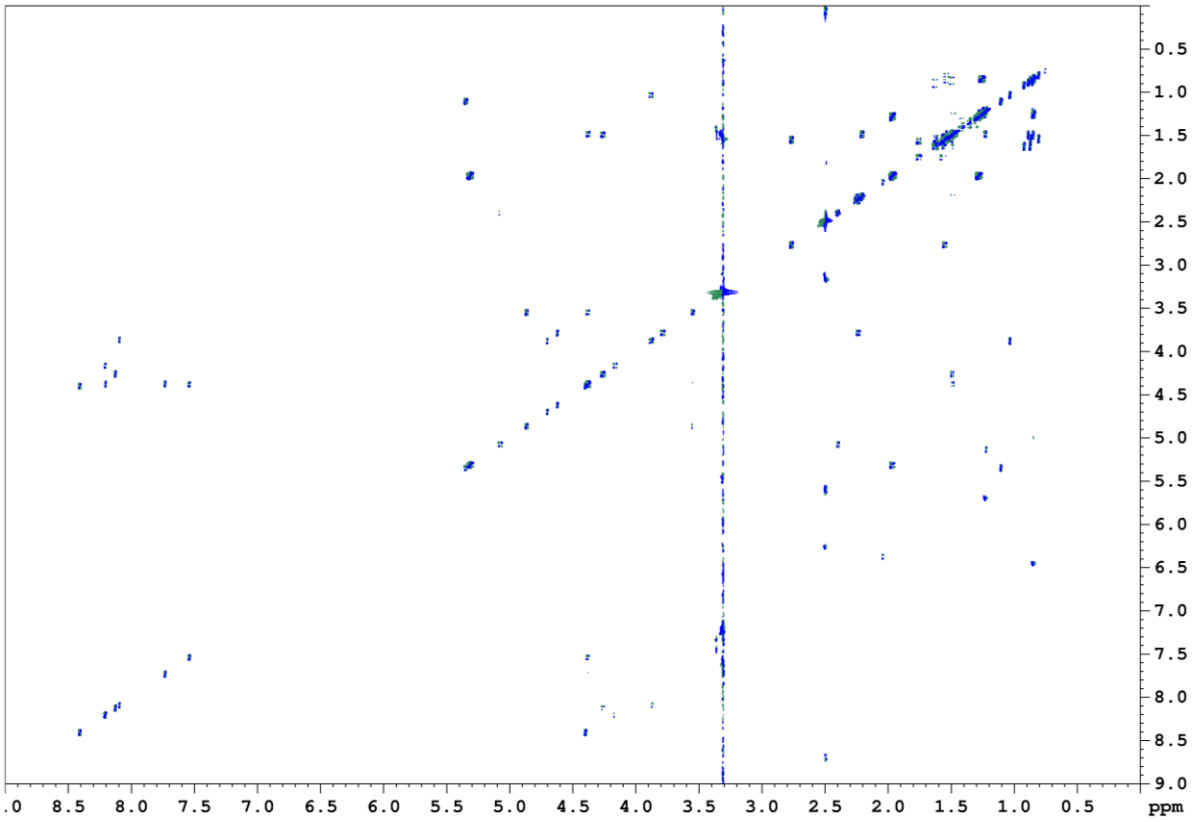

Figure S6. Multiplicity edited gHSQC for ngercheumicin F.

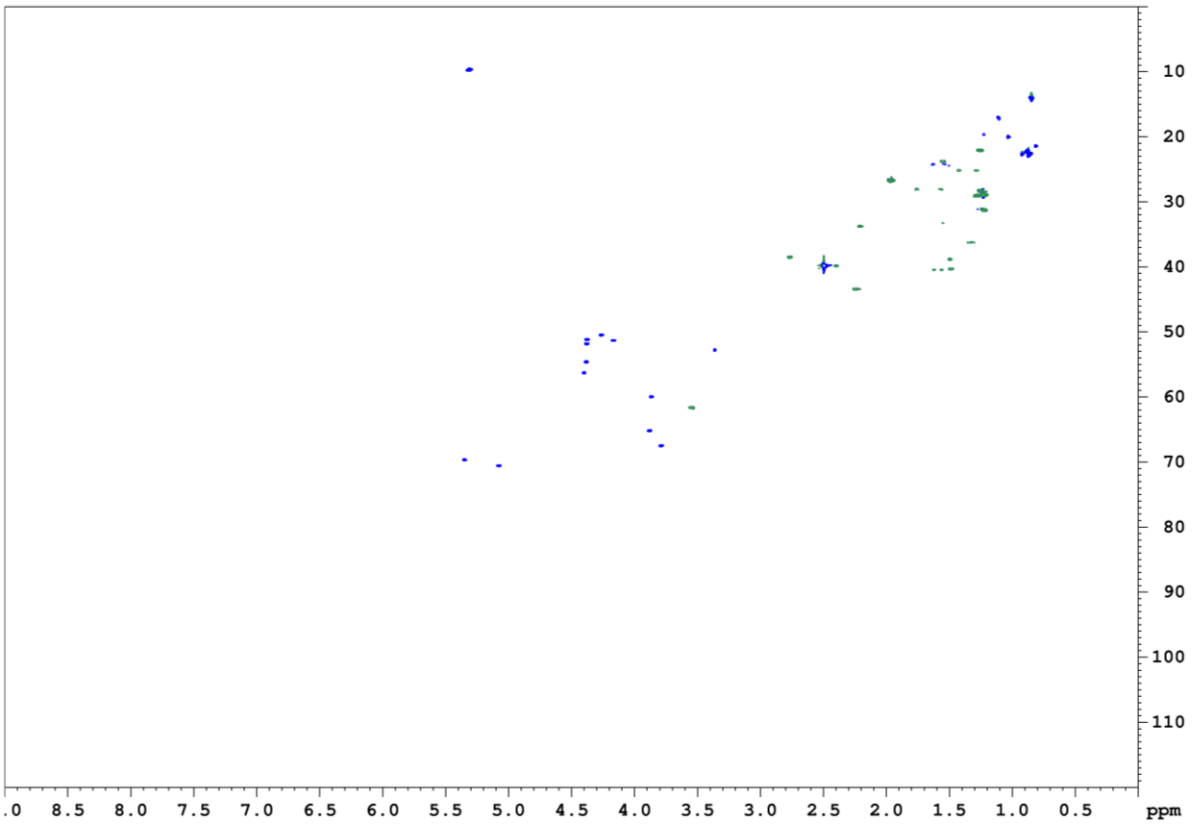

Figure S7. gHMBC for ngercheumicin F.

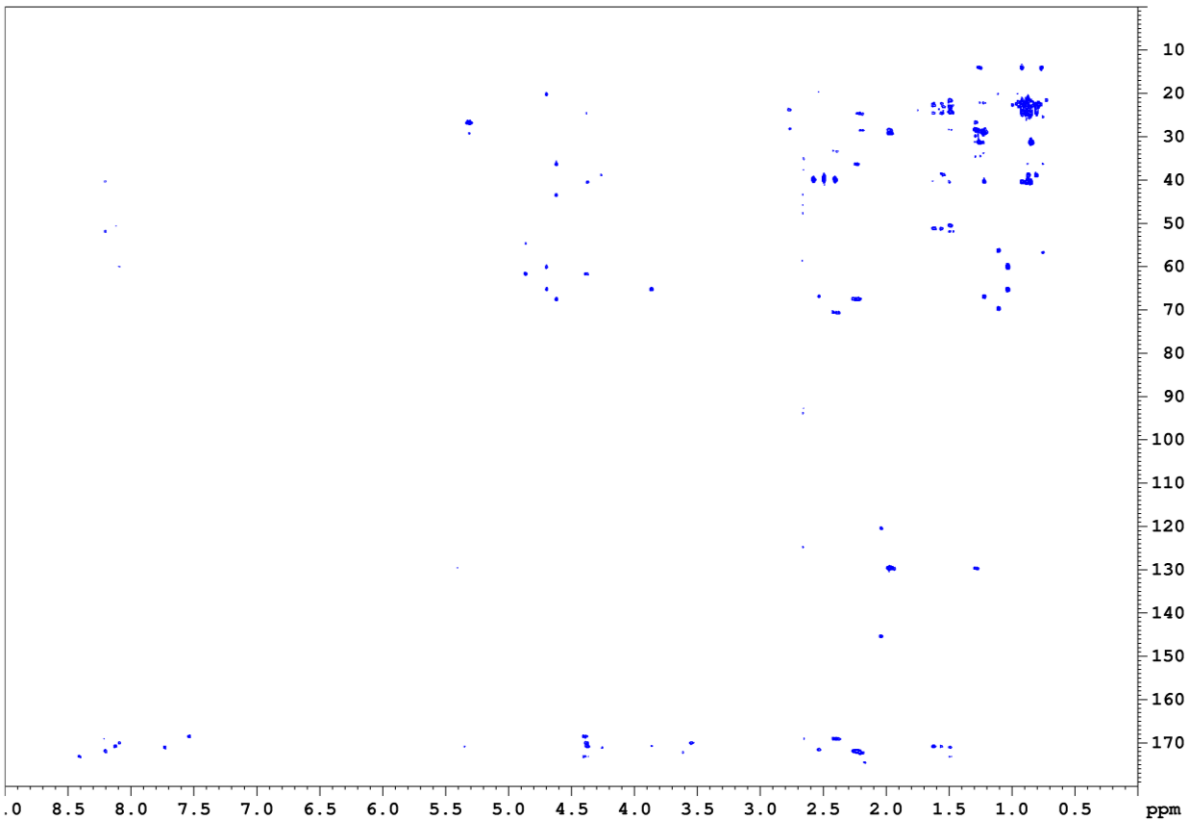

Figure S8. ^{1}H spectrum for ngercheumicin G.

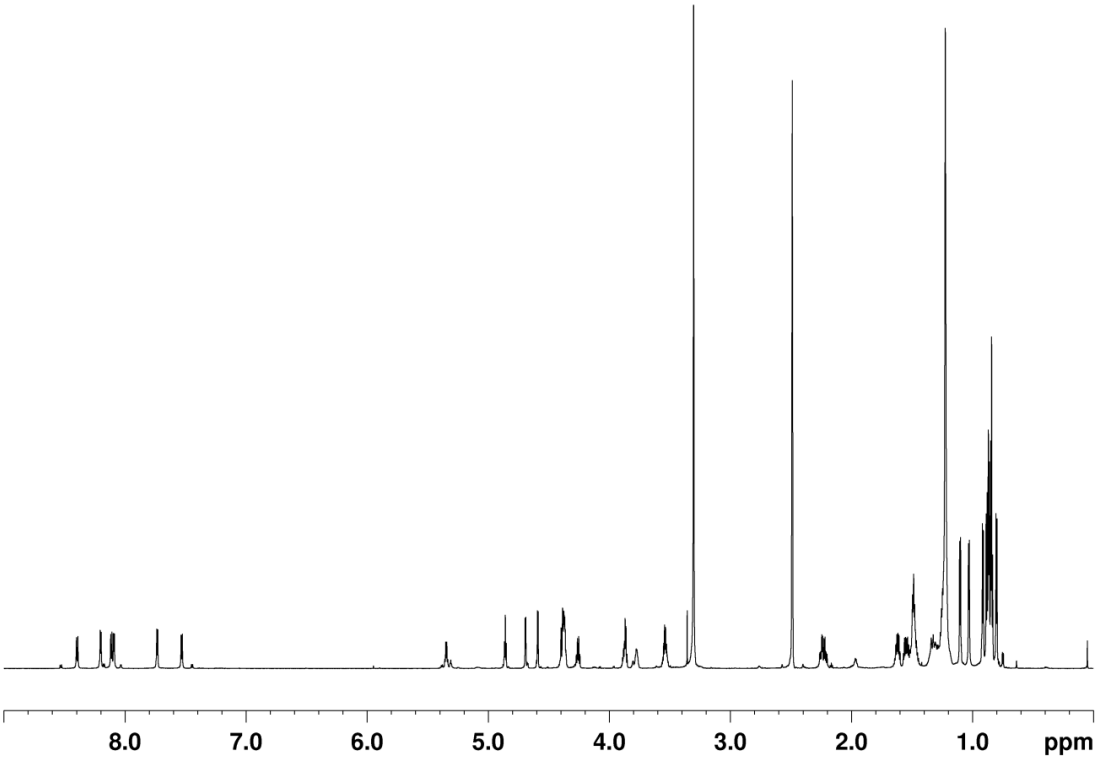

Figure S9. ^{13}C spectrum for ngercheumicin G.

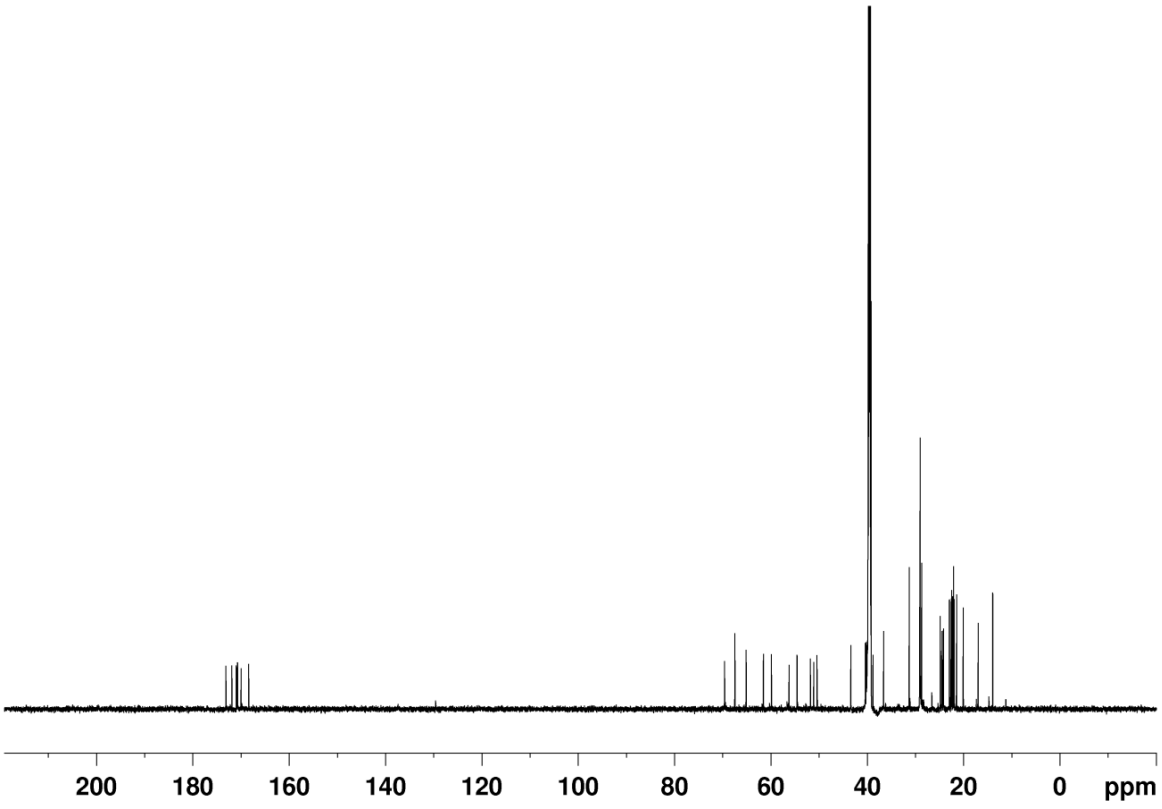

Figure S10. DQF-COSY for ngercheumicin G.

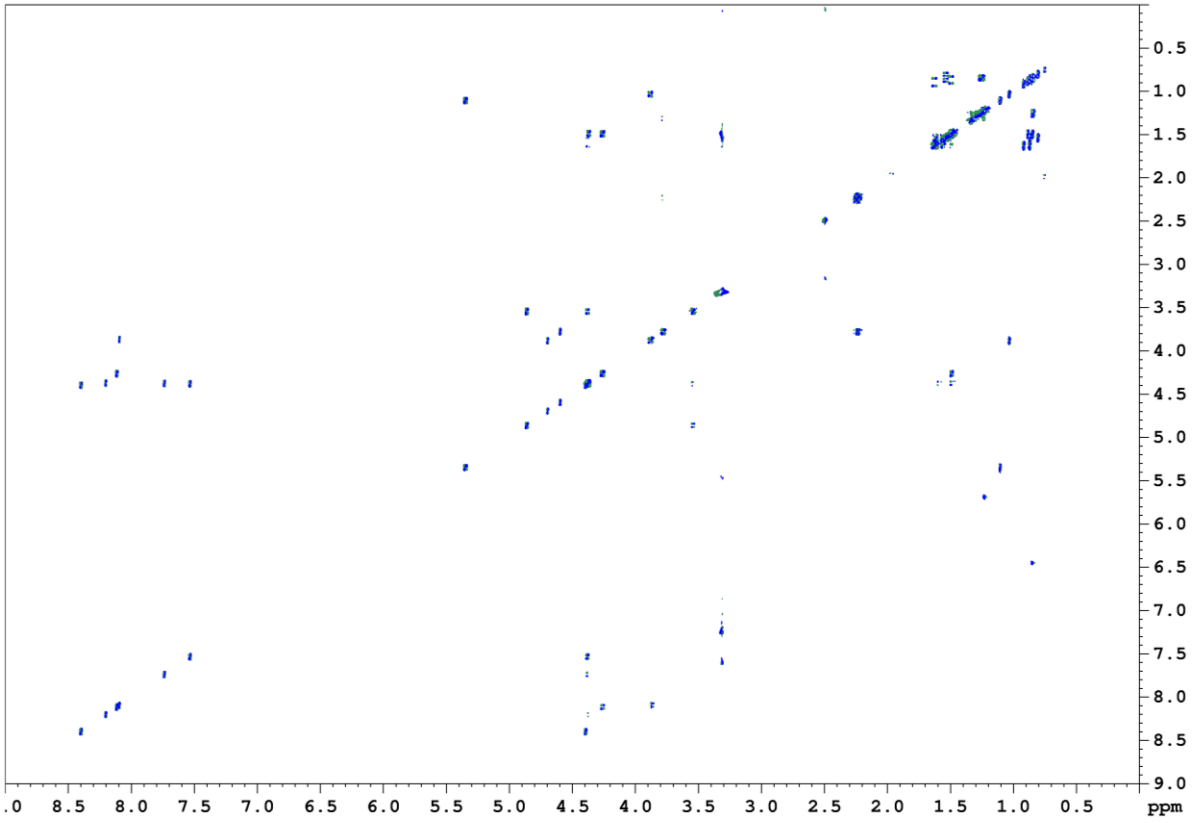

Figure S11. Multiplicity edited gHSQC for ngercheumicin G.

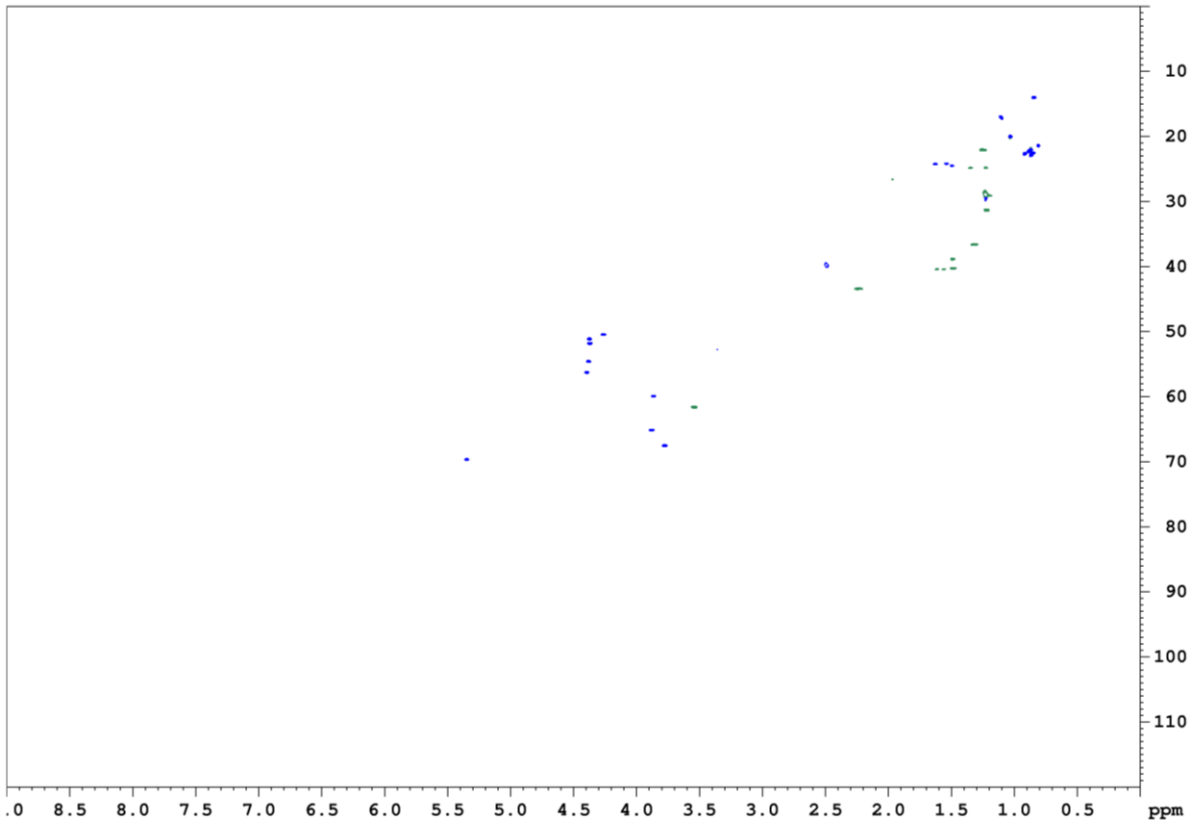

Figure S12. gHMBC for ngercheumicin G.

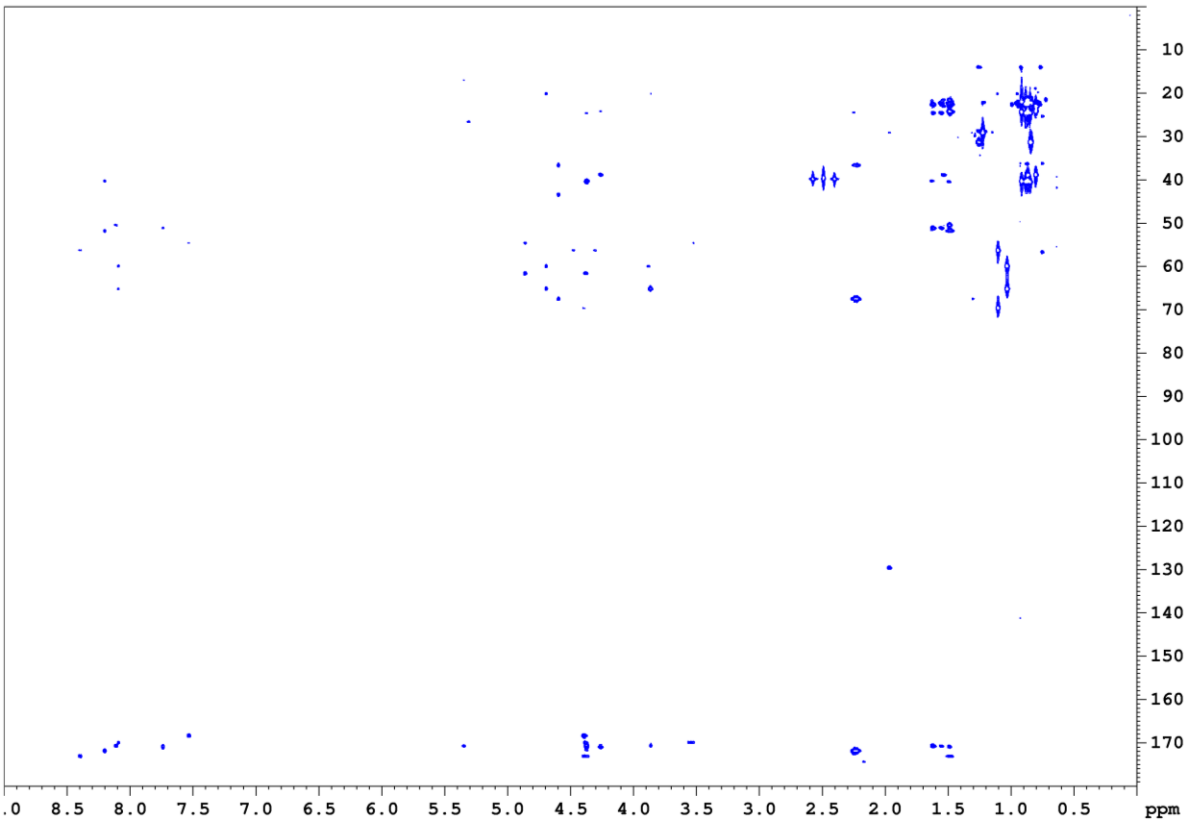

Figure S13. ^{1}H spectrum for ngercheumicin H.

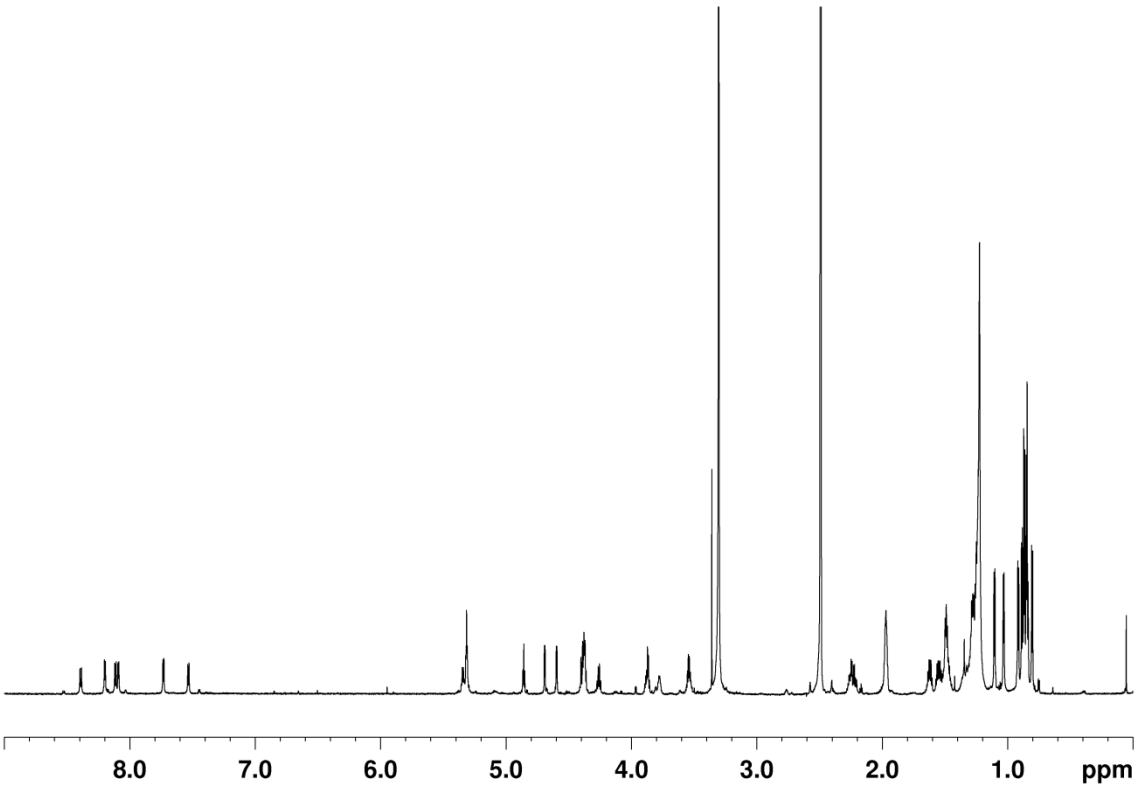

Figure S14. ^{13}C spectrum for ngercheumicin H.

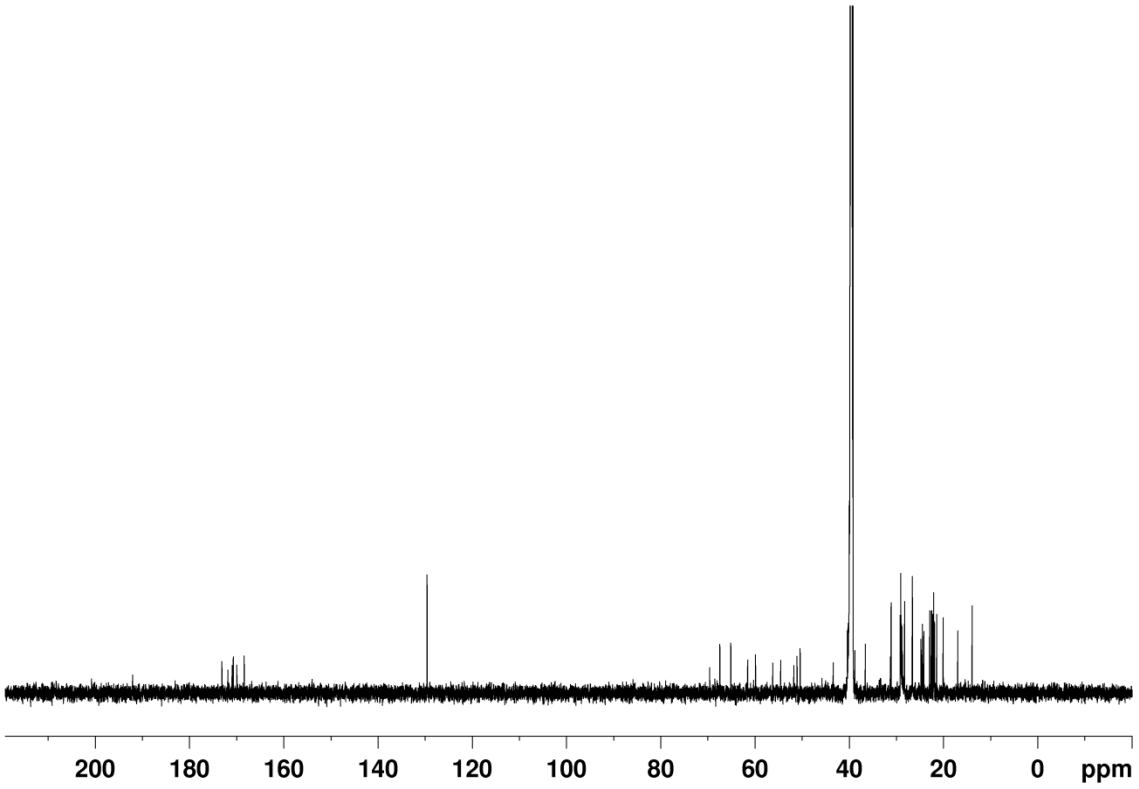

Figure S15. DQF-COSY for ngercheumicin H.

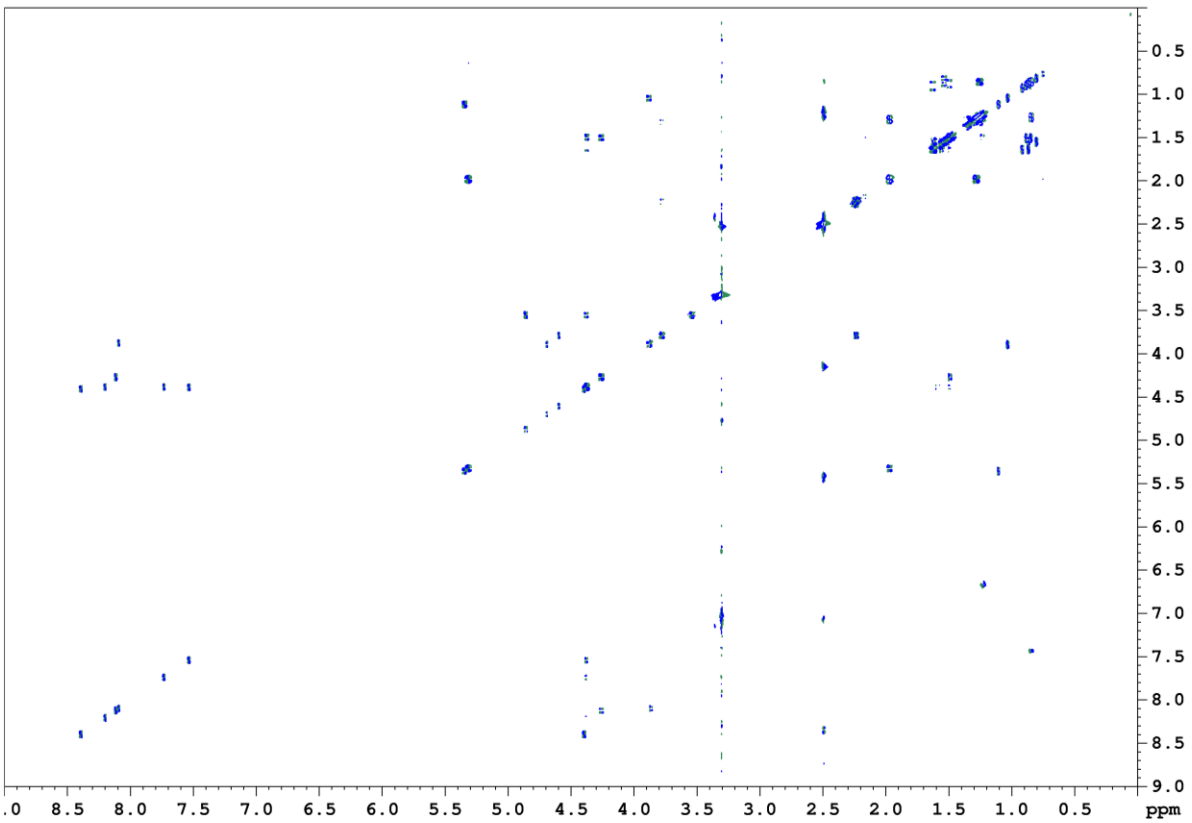

Figure S16. Multiplicity edited gHSQC for ngercheumicin H.

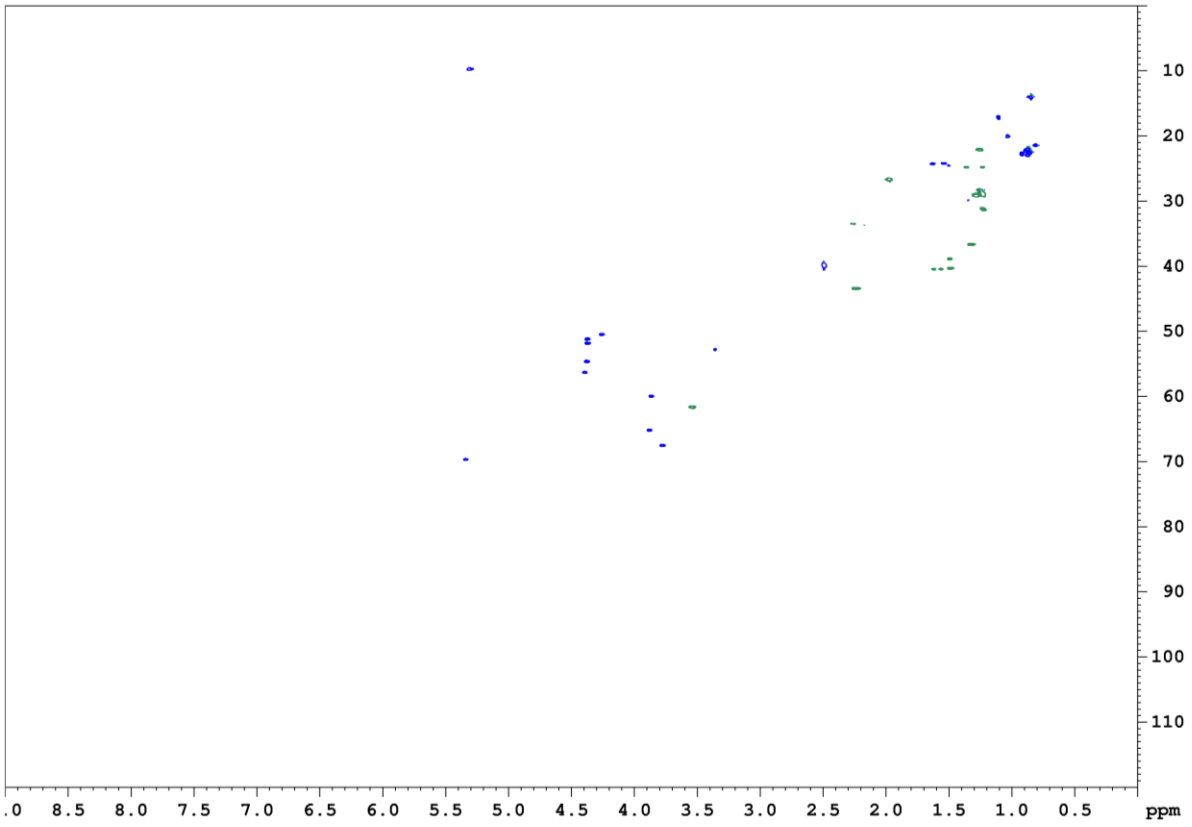

Figure S17. gHMBC for ngercheumicin H.

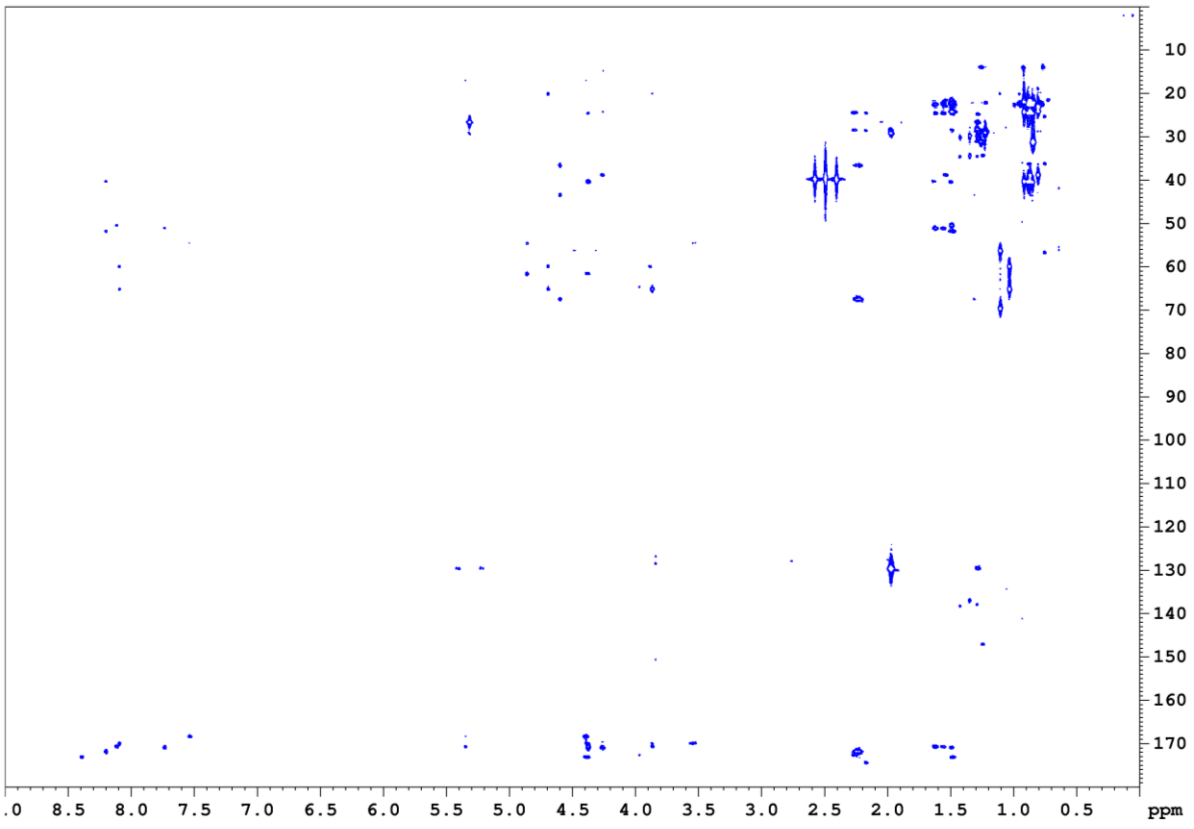

Figure S18. ^{1}H spectrum for ngercheumicin I.

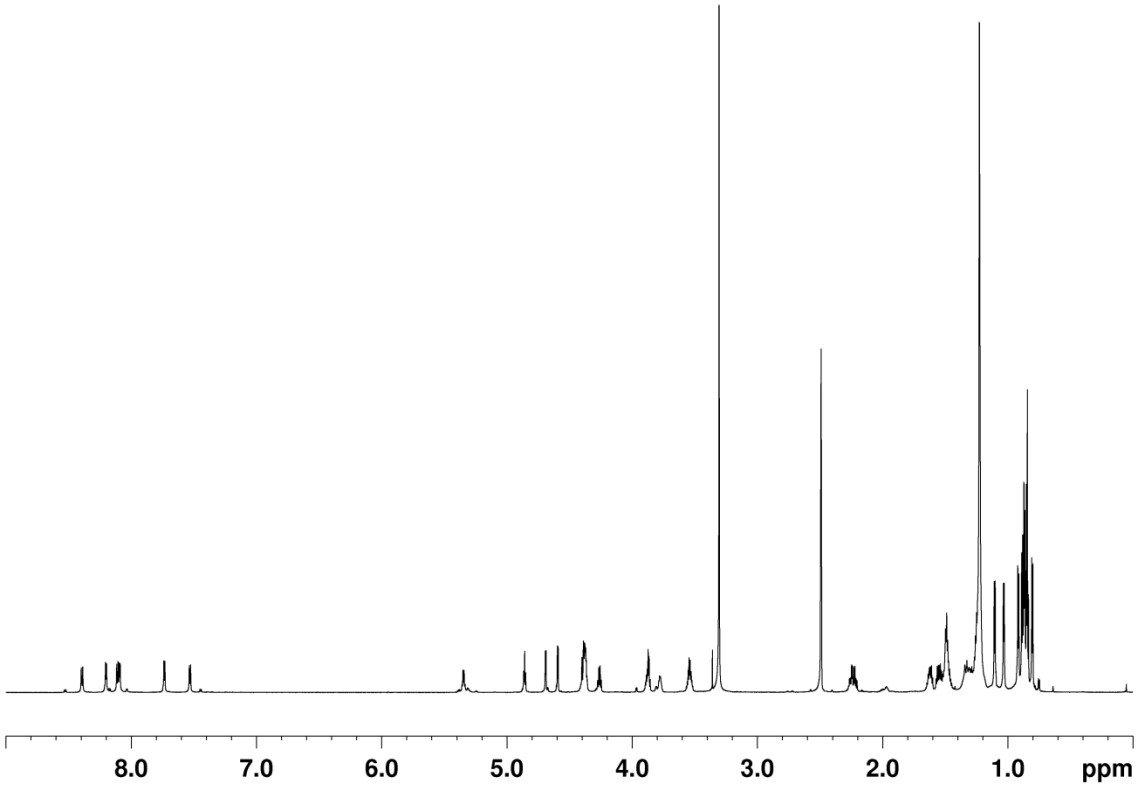

Figure S19. ^{13}C spectrum for ngercheumicin I.

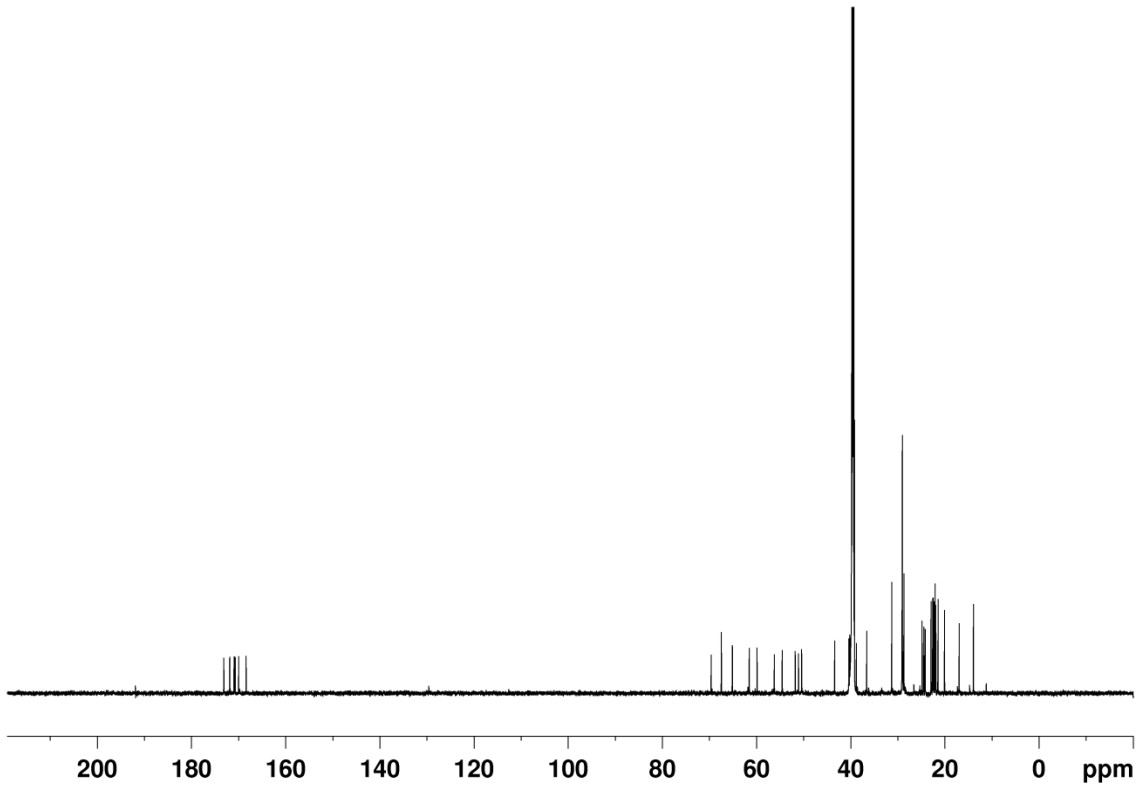

Figure S20. DQF-COSY for ngercheumicin I.

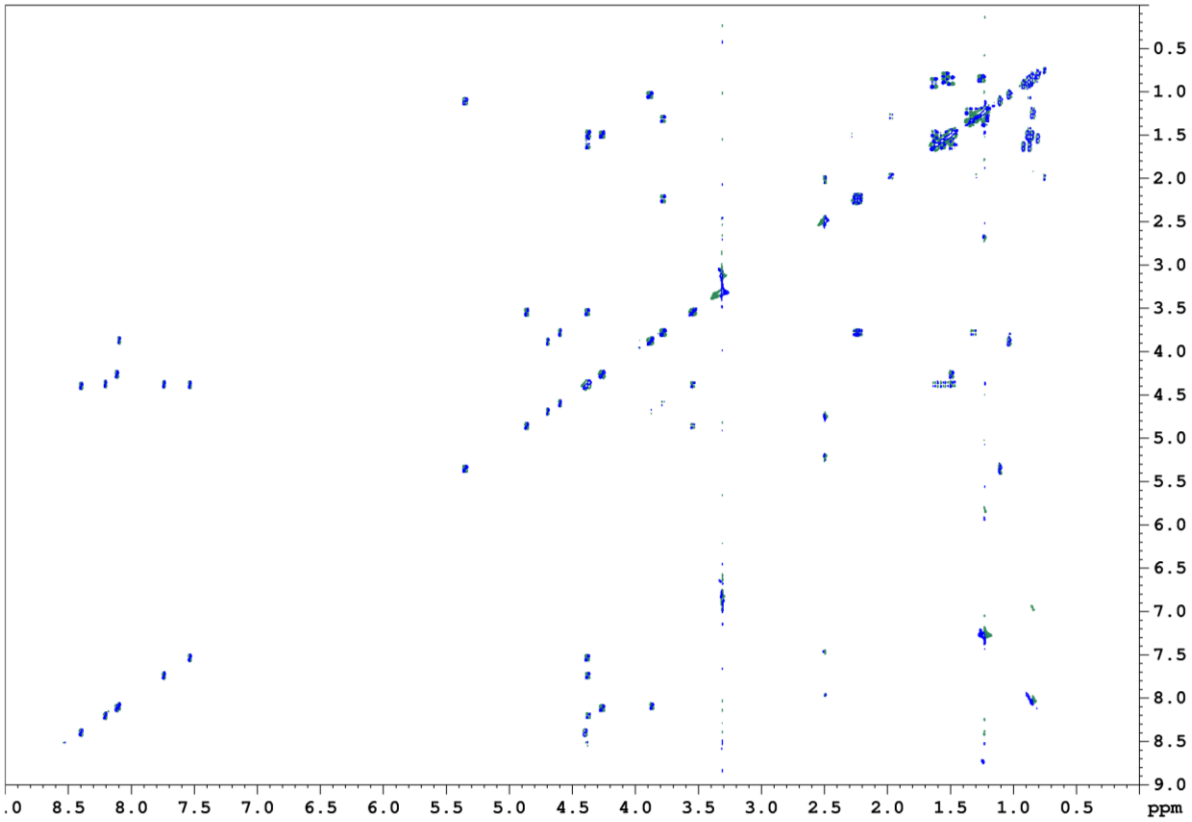

Figure S21. Multiplicity edited gHSQC for ngercheumicin I.

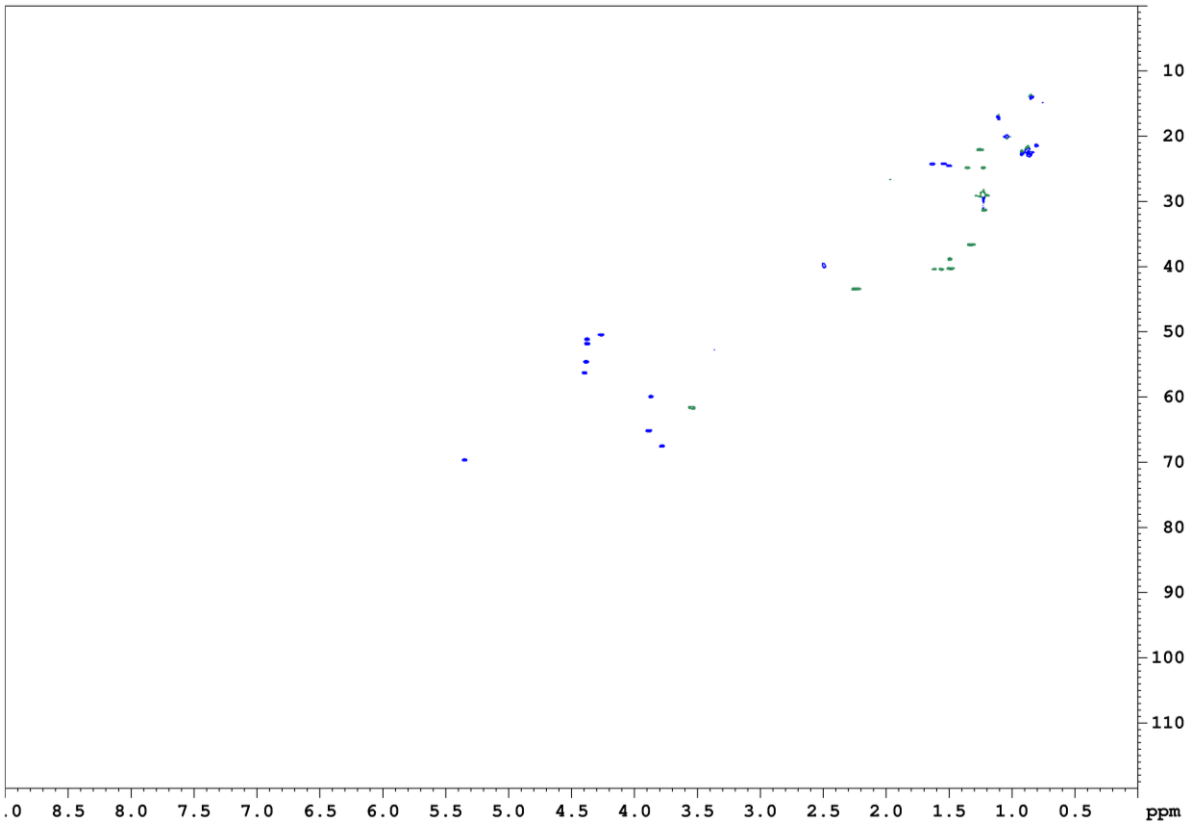

Figure S22. gHMBC for ngercheumicin I.

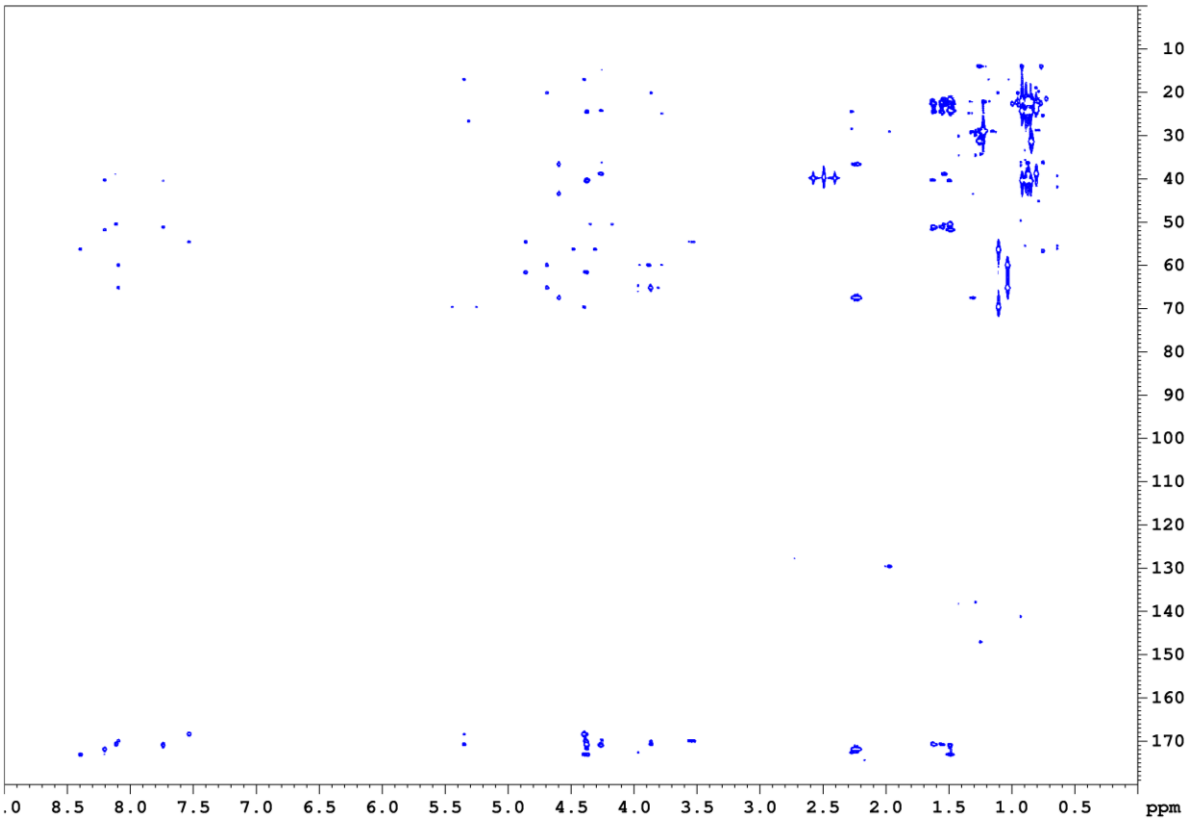

Figure S23. Key HMBC and H2BC correlations in the fatty acid chain of Ngercheumicin F.

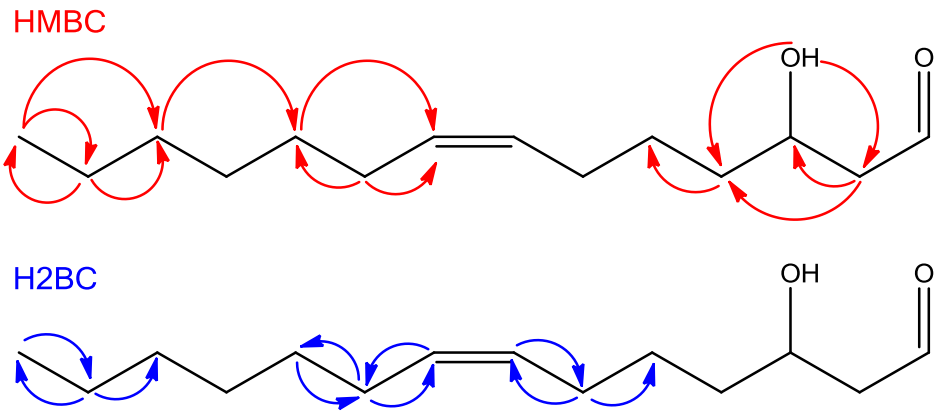

Figure S24. Northern blot results for ngercheumicin F, G, H and I in DMSO at 5 μg/mL (≈6 μM) and 20 μg/mL (≈23 μM) against *S. aureus* USA300/FPR3757 at OD_{600} = 0.8 and OD_{600} = 3.0. DMSO is used as negative control.

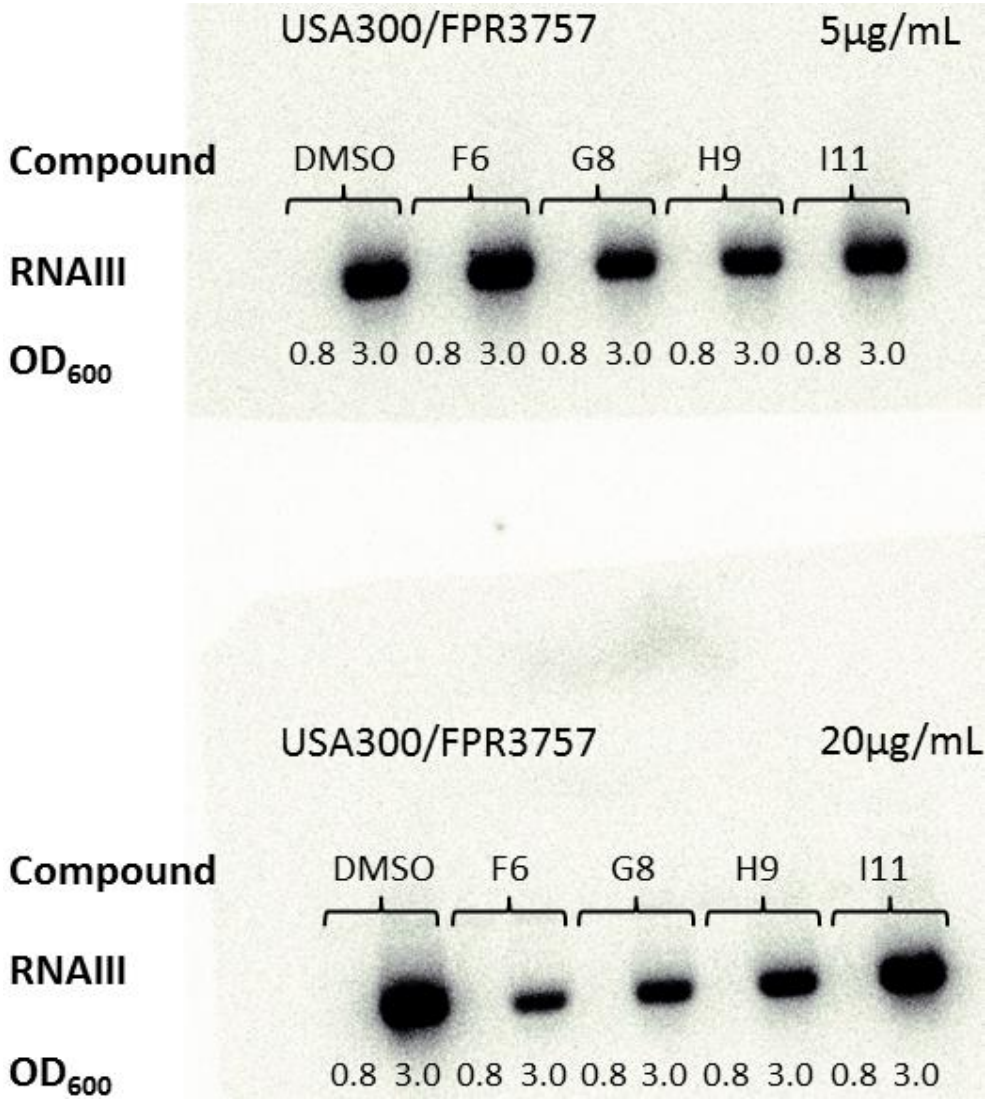

Figure S25. Plate assay results in the colorimetric *S. aureus lacZ* reporter assay with ngercheumicins F, G, H and I in DMSO at 1 mg/mL against *S. aureus* 8325-4. DMSO and water is used as negative controls. Inhibitors of the *agr* locus enhance transcription of *spa* and reduce expression of *hla* and *rnaIII*.

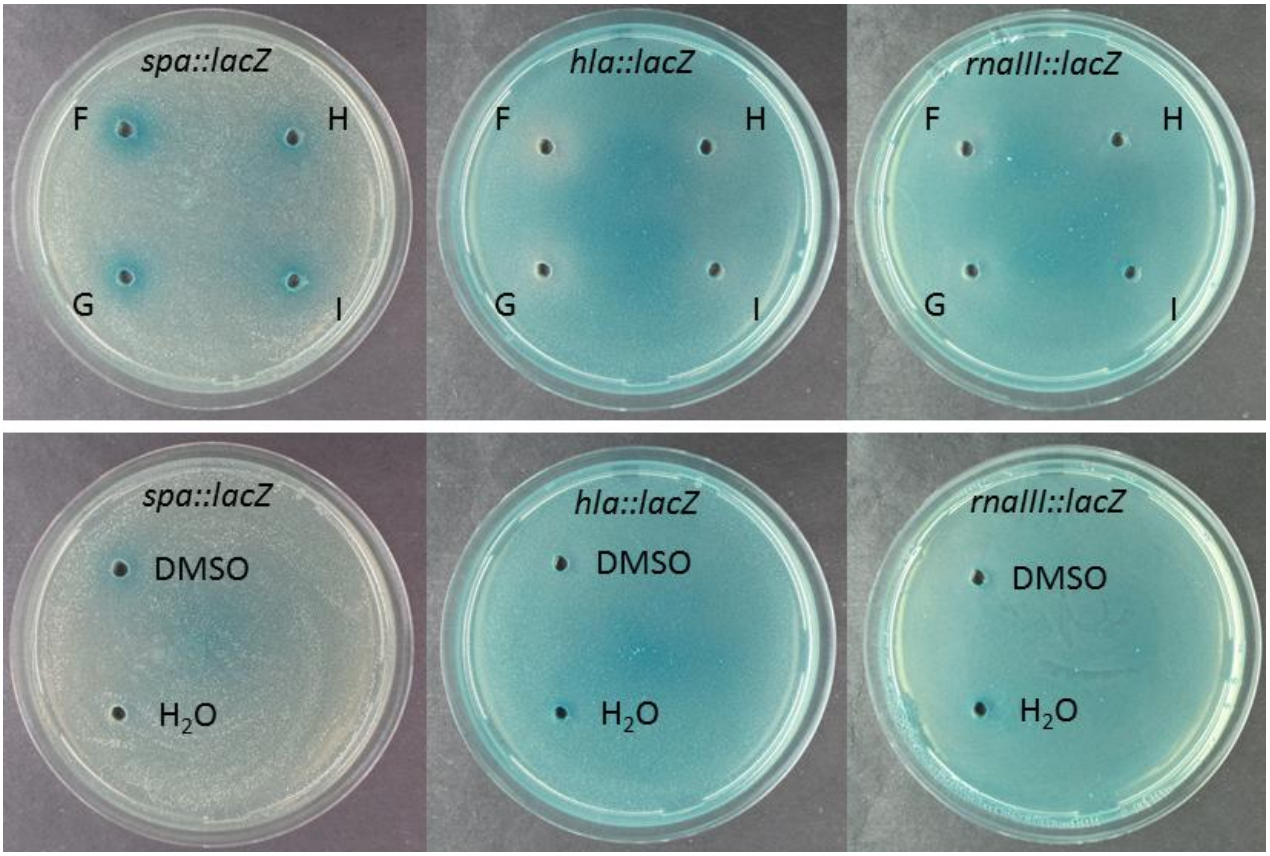

Table S1. NMR table for Ngercheumicin F.

Position, Type	δ_H (ppm), #H, Multiplicity, *J* (Hz)	δ_C (ppm)	HMBC Connectivity	H2BC Connectivity
Leu[1]				
1—CO	–	170.8	–	
2—CH_α	4.37, 1H, m	51.1	1, 3	
3—CH_β	1.62, 1H, m	40.4	1, 2, 4, 5, 6	
	1.55, 1H, m			
4—CH_γ	1.49, 1H, m	24.5	3	
5—$CH_{\delta,1}$	0.88, 3H, d, 6.5	22.3	3, 4, 6	4
6—$CH_{\delta,2}$	0.85, 3H, d, 6.5	22.5	3, 4, 5	4
7—NH	7.73, 1H, d, 6.5	–	(2), 8	2
Leu[2]				
8—CO	–	171.0	–	
9—CH_α	4.26, 1H, q, 8.0	50.4	8, 10	10
10—CH_β	1.49, 2H, m	38.8	9, 11, 13	9
11—CH_γ	1.53, 1H, m	24.2		10
12—$CH_{\delta,1}$	0.87, 3H, d, 6.5	22.9	10, 11, 13	11
13—$CH_{\delta,2}$	0.80, 3H, d, 6.5	21.4	10, 11, 12	11
14—NH	8.12, 1H, d, 8.5	–	(9), 15	9

Table S1. *Cont.*

L-*allo-Thr*[1]				
15—CO	–	170.7	–	
16—CH_{α}	3.86, 1H, m	59.9	15, 17	17
17—CH_{β}	3.88, 1H, m	65.1	(16)	16, 18
17—OH	4.70, 1H, d, 4.6	–	16, 17, 18	17
18—CH_{γ}	1.03, 3H, d, 6.0	20.0	16, 17	17
19—NH	8.09, 1H, d, 6.0	–	(16), 20	16
D-Ser				
20—CO	–	170.0	–	
21—CH_{α}	4.38, 1H, m	54.5	20, 22, 24	22
22—CH_{β}	3.54, 2H, m	61.6	20, (21)	21
22—OH	4.86, 1H, t, 6.0	–	(21), 22	22
23—NH	7.53, 1H, d, 8.0	–	24	21
L-*allo-Thr*[2]				
24—CO	–	168.4	–	
25—CH_{α}	4.40, 1H, d, 9.0	56.2	24, 29	
26—CH_{β}	5.35, 1H, qd, 6.0, <1	69.6	1	27
27—CH_{γ}	1.10, 3H, d, 6.5	17.0	25, 26	26
28—NH	8.40, 1H, d, 9.5		29	25
Leu[3]				
29—CO	–	173.1	–	
30—CH_{α}	4.37, 1H, m	51.7	29, 31	31
31—CH_{β}	1.48, 2H, m	40.3	29, 30, 32	
32—CH_{γ}	1.62, 1H, m	24.2	33	
33—$CH_{\delta,1}$	0.92, 3H, d, 6.5	22.6	31, 32, 34	32
34—$CH_{\delta,2}$	0.87, 3H, d, 6.5	21.9	31, 32, 33	32
35—NH	8.20, 1H, d, 6.5	–	30, 31, 36	30
Fatty acid				
36—CO	–	171.8	–	
37	~2.24, 2H, m	43.3	36, 38, (39)	38
38	3.78, 1H, m	67.4		37
38—OH	4.62, 1H, d, 5.0	–	37, 38, 39	38
39	1.34, 1H, m	36.2	(38), 40	(38), 40
	1.30, 1H, m			
40	1.42, 1H, m	25.1	41, 42	41
	1.28, 1H, m			
41	1.96, 2H, m	26.6	43	(40), 42
42	5.31, 1H, m	129.6	44	41, 43
43	5.31, 1H, m	129.6	43	42, 44
44	1.96, 2H, m	26.5	42	43, 45
45	1.28, 2H, m	29.0		44
46	1.24, 2H, m	28.3		47
47	1.22, 2H, m	31.1	45	46
48	1.25, 2H, m	22.1	47, 49	47, 49
49	0.84, 3H, t, 7.0	13.9	47, 48	48

Table S2. NMR table for Ngercheumicin G.

Position, Type	δ_H (ppm), #H, Multiplicity, *J* (Hz)	δ_C (ppm)	HMBC Connectivity	H2BC Connectivity
Leu[1]				
1—CO	–	170.8	–	
2—CH_α	4.37, 1H, m	51.1	1, 3, 4, 8	3
3—CH_β	1.61, 1H, m	40.4	1, 2, 4, 5, 6	2, 4
	1.55, 1H, m			
4—CH_γ	1.49, 1H, m	24.5	3, 5, 6	3
5—$CH_{\delta,1}$	0.88, 3H, d, 6.5	22.3	3, 4, 6	4
6—$CH_{\delta,2}$	0.85, 3H, d, 6.5	22.5	3, 4, 5	4
7—NH	7.73, 1H, d, 6.5	–	(1), 2, 3, 8	2
Leu[2]				
8—CO	–	171.0	–	
9—CH_α	4.26, 1H, q, 8.0	50.4	8, 10, 11	10
10—CH_β	1.49, 2H, m	38.8	9, 11, 12, 13	9, 11
11—CH_γ	1.53, 1H, m	24.2	(9), 10, 12, 13	10
12—$CH_{\delta,1}$	0.87, 3H, d, 6.5	22.9	10, 11, 13	11
13—$CH_{\delta,2}$	0.80, 3H, d, 6.5	21.4	10, 11, 12	11
14—NH	8.11, 1H, d, 8.5	–	9, (10), 15	9
L-*allo-Thr*[1]				
15—CO	–	170.7	–	
16—CH_α	3.86, 1H, m	59.9	15, 17, (18), 20	17
17—CH_β	3.88, 1H, m	65.1	16	16, 18
17—OH	4.69, 1H, d, 4.6	–	16, 17, 18	17
18—CH_γ	1.03, 3H, d, 6.0	20.1	16, 17	17
19—NH	8.09, 1H, d, 6.0	–	16, 17, 20	16
D-Ser				
20—CO	–	170.0	–	
21—CH_α	4.38, 1H, m	54.6	20, 22, (24)	22
22—CH_β	3.54, 2H, m	61.5	20, 21	21
22—OH	4.86, 1H, t, 6.0	–	21, 22	22
23—NH	7.53, 1H, d, 8.0	–	(20), 21, 22, 24	21
L-*allo-Thr*[2]				
24—CO	–	168.4	–	
25—CH_α	4.39, 1H, d, 9.0	56.2	24, 26, 27, 29	
26—CH_β	5.34, 1H, qd, 6.0, <1	69.6	1, 24, 27	27
27—CH_γ	1.10, 3H, d, 6.5	17.0	25, 26	26
28—NH	8.39, 1H, d, 9.5		25, (26), 29	25
Leu[3]				
29—CO	–	173.2	–	
30—CH_α	4.37, 1H, m	51.8	29, 31, 32, 36	31
31—CH_β	1.48, 2H, m	40.2	29, 30, 32, 33	30
32—CH_γ	1.62, 1H, m	24.2	31, 33	31, 33, 34
33—$CH_{\delta,1}$	0.92, 3H, d, 6.5	22.6	31, 32, 34	32
34—$CH_{\delta,2}$	0.87, 3H, d, 6.5	21.9	31, 32, 33	32
35—NH	8.20, 1H, d, 6.5	–	(29), 30, 31, 36	30
Fatty acid				

Table S2. *Cont.*

36—CO	–	171.9	–	
37	2.24, 1H, dd, 14.0, 6.0	43.4	36, 38, 39	38
	2.22, dd, 14.0, 7.0			
38	3.77, 1H, m	67.5	36, 40	37, 39
38—OH	4.60, 1H, d, 5.0	–	37, 38, 39	38
39	1.31, 2H, m	36.6	37, 38, 41	38, 40
40	1.34, 1H, m	24.8		
	1.22, 1H, m			
41	1.23, 2H, m	~29		
42	~1.2, 2H, m	~29		
43	~1.2, 2H, m	~29		
44	~1.2, 2H, m	~29		
45	~1.2, 2H, m	~29		
46	1.21, 2H, m	29.1		
47	1.22, 2H, m	31.3		46, 48
48	1.25, 2H, m	22.1	47, 49	47, 49
49	0.84, 3H, t, 7.0	13.9	47, 48	48

Table S3. NMR table for ngercheumicin H.

Position, Type	**δ_H (ppm), #H, Multiplicity, *J* (Hz)**	**δ_C (ppm)**	**HMBC Connectivity**	**H2BC Connectivity**
Leu[1]				
1—CO	–	170.8	–	
2—CH_α	4.37, 1H, m	51.1	1, 3, 4, 8	
3—CH_β	1.62, 1H, m	40.4	1, 2, 4, 5, 6	
	1.56, 1H, m			
4—CH_γ	1.50, 1H, m	24.5	2, 3, 5, 6	
5—$CH_{\delta,1}$	0.89, 3H, d, 6.5	22.3	3, 4, 6	4
6—$CH_{\delta,2}$	0.85, 3H, d, 6.5	22.5	3, 4, 5	4
7—NH	7.73, 1H, d, 6.5	–	(1), 2, 3, 8	2
Leu[2]				
8—CO	–	171.0	–	
9—CH_α	4.25, 1H, q, 8.0	50.4	8, 10, 11	10
10—CH_β	1.49, 2H, m	38.8	9, 11, 13	9, 11
11—CH_γ	1.54, 1H, m	24.1	9, 10, 13	10
12—$CH_{\delta,1}$	0.87, 3H, d, 6.5	22.9	10, 11, 13	11
13—$CH_{\delta,2}$	0.80, 3H, d, 6.5	21.4	10, 11, 12	11
14—NH	8.11, 1H, d, 8.5	–	9, 10, 15	9
L-*allo-Thr*[1]				
15—CO	–	170.7	–	
16—CH_α	3.86, 1H, m	59.9	15, 17, 18, 20	17
17—CH_β	3.88, 1H, m	65.1	16	16, 18
17—OH	4.69, 1H, d, 4.6	–	16, 17, 18	17
18—CH_γ	1.03, 3H, d, 6.0	20.1	16, 17	17
19—NH	8.09, 1H, d, 6.0	–	16, 17, 20	16
D-*Ser*				

Table S3. *Cont.*

20—CO	–	170.0	–	
21—CH_{α}	4.38, 1H, m	54.5	20, 22, 24	22
22—CH_{β}	3.54, 2H, m	61.5	20, 21	21
22—OH	4.86, 1H, t, 6.0	–	21, 22	22
23—NH	7.53, 1H, d, 8.0	–	(20), 21, 22, 24	21
L-*allo-Thr*[2]				
24—CO	–	168.4	–	
25—CH_{α}	4.39, 1H, d, 9.0	56.2	24, 26, 27, 29	
26—CH_{β}	5.34, 1H, qd, 6.0, <1	69.6	1, 24, 27	27
27—CH_{γ}	1.10, 3H, d, 6.5	16.9	25, 26	26
28—NH	8.39, 1H, d, 9.5		25, 26, 29	25
Leu[3]				
29—CO	–	173.1	–	
30—CH_{α}	4.37, 1H, m	51.8	29, 31, 32, 36	31
31—CH_{β}	1.48, 2H, m	40.2	29, 30, 32, 33	30
32—CH_{γ}	1.62, 1H, m	24.2	30, 31, 34	31
33—$CH_{\delta,1}$	0.91, 3H, d, 6.5	22.6	31, 32, 34	32
34—$CH_{\delta,2}$	0.87, 3H, d, 6.5	21.9	31, 32, 33	32
35—NH	8.20, 1H, d, 6.5	–	(29), 30, 31, 36	30
Fatty acid				
36—CO	–	171.9	–	
37	2.25, 1H, dd, 14.0, 6.0	43.4	36, 38, 39	38
	2.22, 1H, dd, 14.0, 7.0			
38	3.78, 1H, m	67.5	36, 40	37, 39
38—OH	4.60, 1H, d, 5.0	–	37, 38, 39	38
39	1.32, 2H, m	36.6	38, 41	38, 40
40	1.36, 1H, m	24.8		39
	1.23, 1H, m			
41	~1.2, 2H, m	~29		
42	1.28, 2H, m	~29	44/45	43
43	1.97, 2H, m	26.6	41?	42, 44
44	5.31, 1H, m	129.6	43/46	43, 45
45	5.31, 1H, m	129.6	43/46	44, 46
46	1.97, 2H, m	26.6	48	45, 47
47	1.28, 2H, m	~29	44/45	46
48	1.25, 2H, m	28.2		
49	1.22, 2H, m	31.1	47, (51)	48, 50
50	1.25, 2H, m	22.0	48, 49, 51	49, 51
51	0.84, 3H, t, 7.0	13.9	49, 50	50

Table S4. NMR table for ngercheumicin I.

Position, Type	δ_H (ppm), #H, Multiplicity, *J* (Hz)	δ_C (ppm)	HMBC Connectivity	H2BC Connectivity
Leu[1]				
1—CO	–	170.8	–	
2—CH_α	4.37, 1H, m	51.1	1, 3, 4, 8	
3—CH_β	1.61, 1H, m	40.4	1, 2, 4, 5, 6	
	1.56, 1H, m			
4—CH_γ	1.49, 1H, m	24.5	2, 3, 5, 6	
5—$CH_{\delta,1}$	0.88, 3H, d, 6.5	22.3	3, 4, 6	4
6—$CH_{\delta,2}$	0.85, 3H, d, 6.5	22.5	3, 4, 5	4
7—NH	7.73, 1H, d, 6.5	–	(1), 2, 3, 8	2
Leu[2]				
8—CO	–	171.0	–	
9—CH_α	4.26, 1H, q, 8.0	50.4	8, 10, 11	10
10—CH_β	1.49, 2H, m	38.8	9, 11, 13	9, 11
11—CH_γ	1.54, 1H, m	24.1	9, 10, 13	10
12—$CH_{\delta,1}$	0.87, 3H, d, 6.5	22.9	10, 11, 13	11
13—$CH_{\delta,2}$	0.80, 3H, d, 6.5	21.4	10, 11, 12	11
14—NH	8.11, 1H, d, 8.5	–	9, 10, 15	9
L-*allo-Thr*[1]				
15—CO	–	170.7	–	
16—CH_α	3.86, 1H, m	59.9	15, 17, 18, 20	17
17—CH_β	3.87, 1H, m	65.1	16	16, 18
17—OH	4.69, 1H, d, 4.6	–	16, 17, 18	17
18—CH_γ	1.03, 3H, d, 6.0	20.1	16, 17	17
19—NH	8.09, 1H, d, 6.0	–	16, 17, 20	16
D-*Ser*				
20—CO	–	170.0	–	
21—CH_α	4.38, 1H, m	54.5	20, 22, 24	22
22—CH_β	3.54, 2H, m	61.5	20, 21	21
22—OH	4.86, 1H, t, 6.0	–	21, 22	22
23—NH	7.53, 1H, d, 8.0	–	(20), 21, 22, 24	21
L-*allo-Thr*[2]				
24—CO	–	168.4	–	
25—CH_α	4.39, 1H, d, 9.0	56.2	24, 26, 27, 29	
26—CH_β	5.35, 1H, qd, 6.0, <1	69.6	1, 24, 27	27
27—CH_γ	1.10, 3H, d, 6.5	17.0	25, 26	26
28—NH	8.39, 1H, d, 9.5		25, 26, 29	25
Leu[3]				
29—CO	–	173.2	–	
30—CH_α	4.37, 1H, m	51.8	29, 31, 32, 36	31
31—CH_β	1.48, 2H, m	40.2	29, 30, 32, 33	30
32—CH_γ	1.63, 1H, m	24.2	30, 31, 34	
33—$CH_{\delta,1}$	0.92, 3H, d, 6.5	22.6	31, 32, 34	32

Table S4. *Cont.*

34—$CH_{\delta,2}$	0.87, 3H, d, 6.5	21.9	31, 32, 33	32
35—NH	8.20, 1H, d, 6.5	−	(29), 30, 31, 36	30
Fatty acid				
36—CO	−	171.9	−	
37	2.25, 1H, dd, 14.0, 7.0 2.22, 1H, dd, 14.0, 6.0	43.4	36, 38, 39	38
38	3.78, 1H, m	67.5	36, 40	37, 39
38—OH	4.59, 1H, d, 5.0	−	37, 38, 39	38
39	1.32, 2H, m	36.6	38, 41	38, 40
40	1.35, 1H, m	24.8		
	1.22, 1H, m			
41	1.23, 2H, m	29.1		
42	~1.2, 2H, m	~29		
43	~1.2, 2H, m	~29		
44	~1.2, 2H, m	~29		
45	~1.2, 2H, m	~29		
46	~1.2, 2H, m	~29		
47	~1.2, 2H, m	~29		
48	~1.2, 2H, m	~29		
49	1.22, 2H, m	31.3		48, 50
50	1.25, 2H, m	22.1	48, 49, 51	49, 51
51	0.84, 3H, t, 7.0	13.9	49, 50	50