SUPPORTING INFORMATION

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Microcystins Containing Doubly Homologated Tyrosine Residues from a *Microcystis aeruginosa* Bloom: Structures & Cytotoxicity

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This Supporting Information consists of 27 pages including 8 Figures of NMR spectra, 4 Tables of NMR data, a figure showing a chromatogram of the major microcystin-containing fraction, general procedures used for gathering NMR and HRMS data, cytotoxicity data and procedures, and the isolation procedure and characterization data for microcystin-LR.

NMR Spectroscopic Data

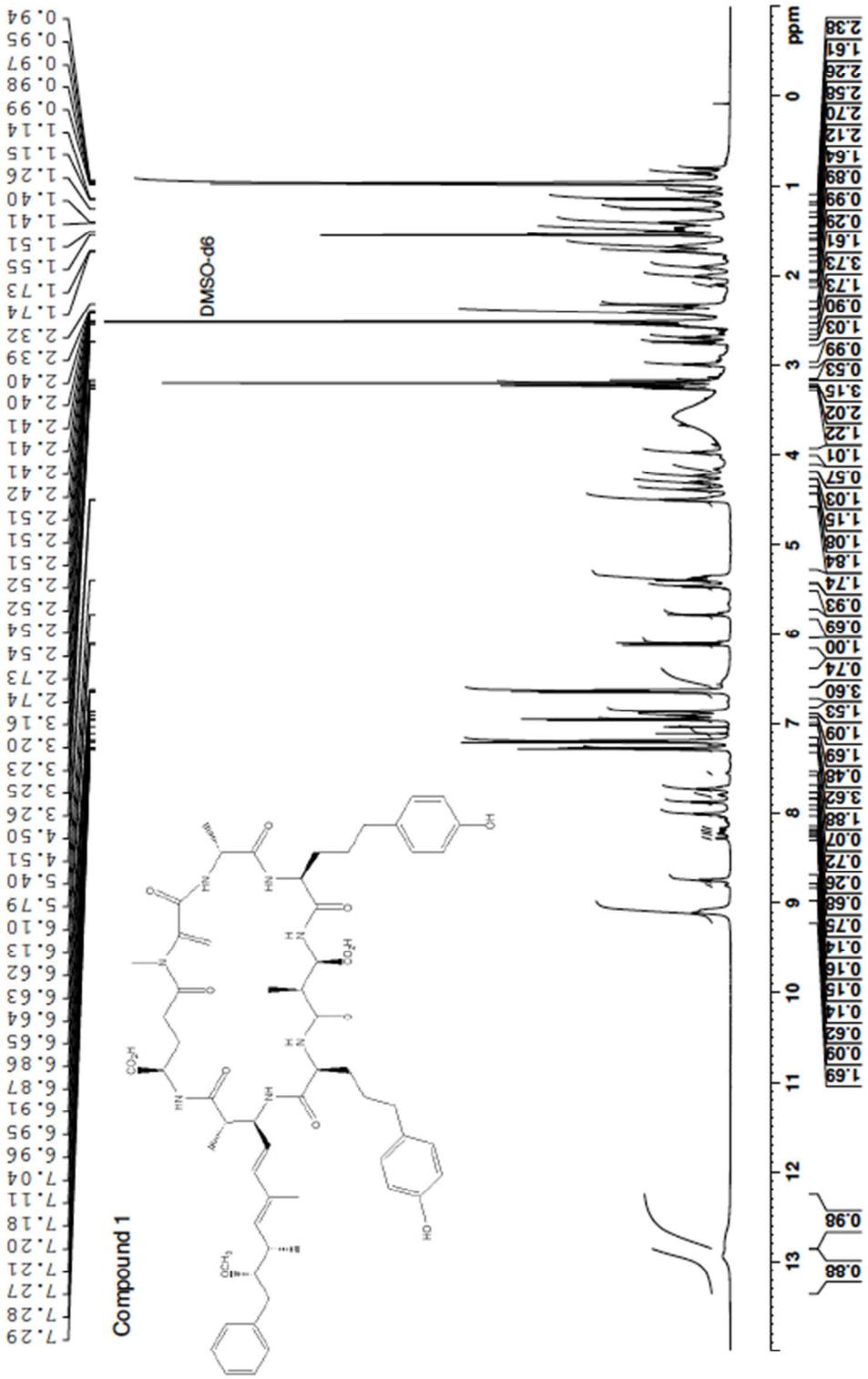
General procedures for NMR and HRMS analyses.

Analytical HPLC Chromatogram of Microcystin-containing Fraction

Cytotoxicity Assay Procedures and Data

Isolation and characterization of microcystin-LR

S1. 1H NMR (700 MHz, DMSO-d6) spectrum of compound 1.



S2. 13C NMR (700 MHz, DMSO-d6) spectrum of compound 1.

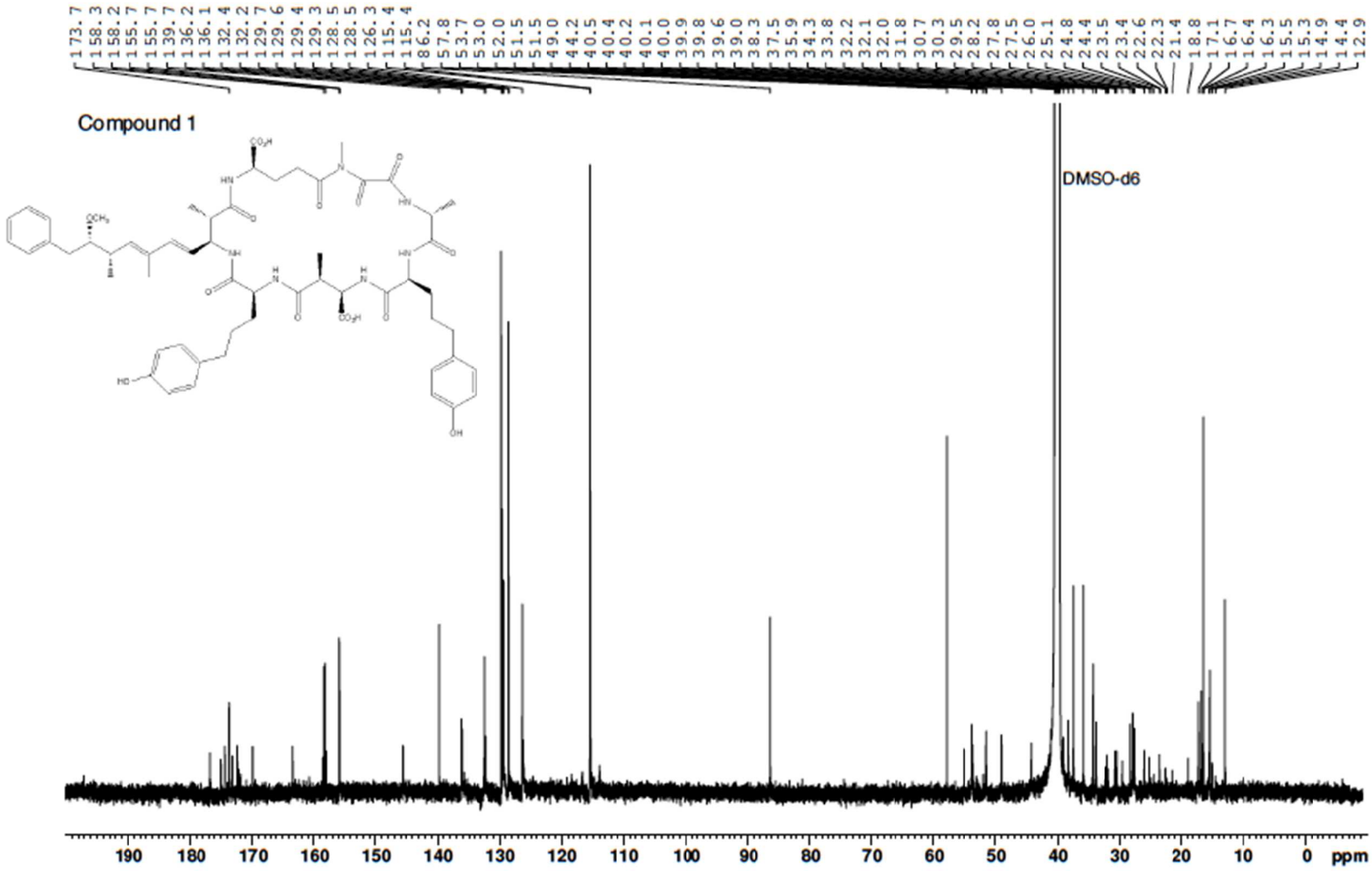


Table S1. NMR Data for Microcystin 1 (1107) in DMSO-d_6 (700 MHz)

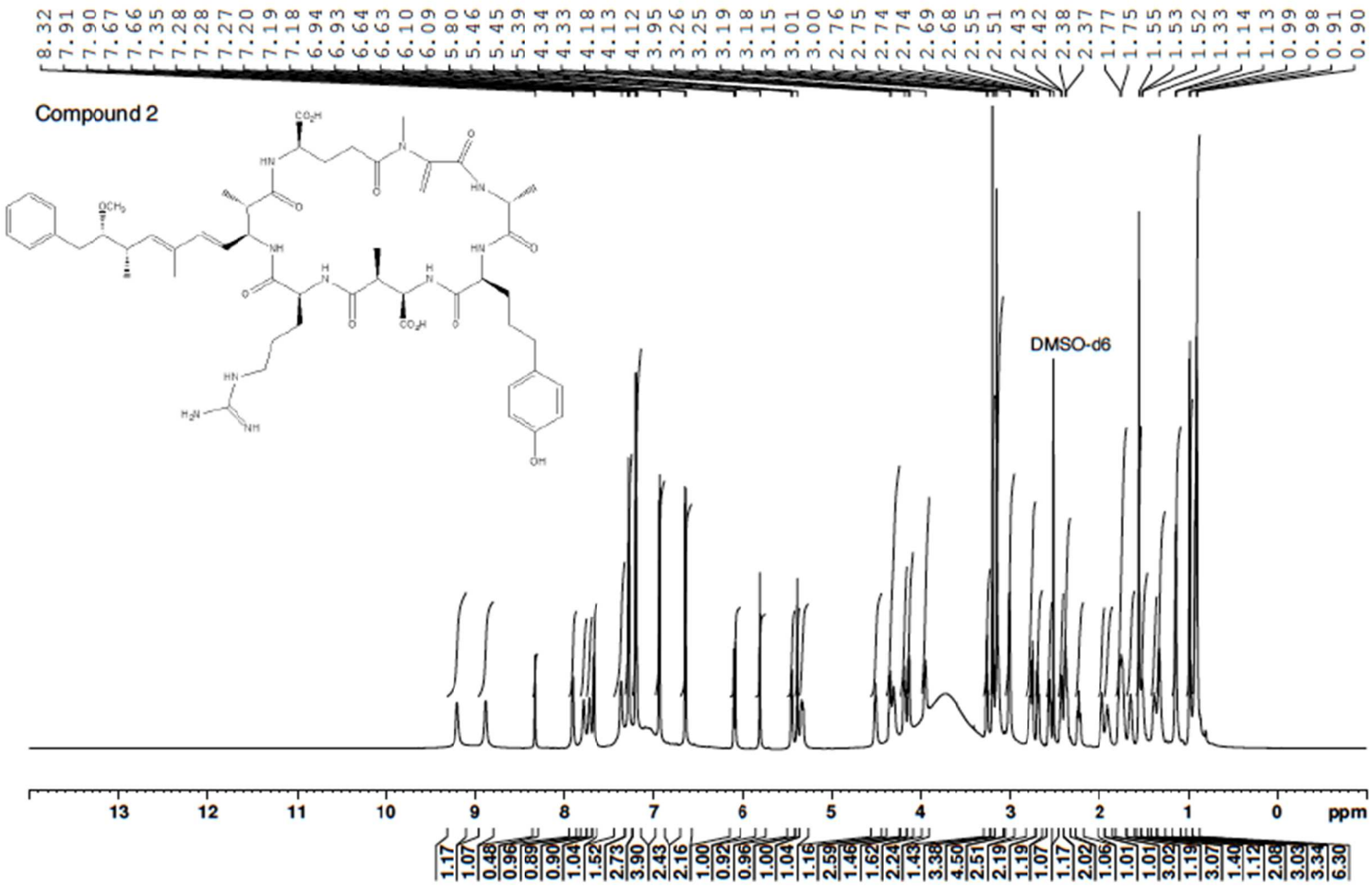
Unit	C/H #	δ_C, mult.	δ_H (*J* in Hz)	^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC	^{1}H-^{1}H NOESY
Ala	1	173.2, qC	-			
	2	49.0, CH	4.31, m	H_3-3, NH	1,3	NH(Ahppa-2)
	3	17.1, CH_3	1.14, d (6.8)	H-2	1,2	NH
	NH		7.72, d (6.2)	H-2		H_3-3
Ahppa-2	1	172.3, qC				
	2	55.0, CH	3.97, m	H-3a, H-3b, NH	3,4	NH(Masp)
	3a	30.7, CH_2	1.73, m	H-2, H-4a, H-4b	2	NH
	3b		1.72, m	H-2, H-4a, H-4b		NH
	4a	28.2, CH_2	1.68, m	H-3a, H-3b, H-5a, H-5b	2, 3, 5, 6	
	4b		1.49, m	H-3a, H-3b, H-5a, H-5b	2, 3, 5, 6	NH
	5a	34.3, CH_2	2.39, m	H-4a, H-4b	3, 4, 6, 7/11	
	5b		2.38, m	H-4a, H-4b	3, 4, 6, 7/11	
	6	132.4, qC				
	7/11	129.6, CH	6.96, d (8.3)	H-8, H-10	5, 6, 8, 9	H-4b
	8/10	115.4, CH	6.64, d (8.3)	H-7, H-11	6, 9	9-OH
	9	155.7, qC				
	9-OH		9.1, brs		9	
	NH		7.87, d (8.8)	H-2	1(Ala)	H_2-3, H-4b
Masp	1	169.7, qC				
	2	53.7, CH	4.50, m	H-3, NH	3, 4	NH(Ahppa-4)
	3	39.1, CH	2.99, m	H_3-5, H-2	4	NH(Ahppa-4)
	4	176.7, qC				
	5	15.3, CH_3	0.97, d (7.4)	H-3	2, 3, 4	NH, NH(Ahppa-4)
	NH		8.02, d (8.4)	H-2	1(Ahppa-2)	H_3-5

	1-OH		12.9, br			
Ahppa-4	1	172.4, qC				
	2	51.5, CH	4.23, m	H-3a, H-3b, NH		H-5b, NH(Adda)
	3a	30.4, CH_2	1.90, m	H-2, H-3b, H-4a, H-4b		
	3b		1.40, m	H-2, H-3a, H-4a, H-4b		
	4a	27.8, CH_2	1.46, m	H-3a, H-3b, H-5a, H-5b	2, 3, 5, 6	NH
	4b		1.40, m	H-3a, H-3b, H-5a, H-5b	2, 3, 5, 6	
	5a	33.8, CH_2	2.40, m	H-4a, H-4b	3, 4, 6	
	5b		2.33, m	H-4a, H-4b	3, 4, 6	
	6	132.2, qC				
	7/11	129.3, CH	6.86, brd (8.0)	H-8, H-10	5, 9	H-4a
	8/10	115.4, CH	6.63, d (8.0)	H-7, H-11	6, 9	9-OH
	9	155.6, qC				H-8/10
	9-OH		9.1, brs			
	NH		8.76, d (8.4)	H-2		H_2-3a, H-3(Masp), H_3-5(Masp), NH(Adda)
Adda	1	174.4, qC				
	2	44.2, CH	2.53, m	H-3, H_3-17	3, 17	NH(Glu)
	3	53.6, CH	4.51, m	H-2, H-4, NH	1, 2, 4, 5, 1(Ahppa-4)	
	4	126.2, CH	5.36, dd (16, 7.8)	H-3, H-5	3, 6	NH, H_3-17, H_3-18
	5	136.2, CH	6.12, d (16)	H-4	3, 4, 6, 7, 18	NH
	6	132.4, qC				
	7	136.1, CH	5.47, d (9.0)	H-8	5, 6, 8, 18, 19	H-9
	8	35.9, CH	2.57, m	H-7, H-9, H_3-19	6, 7, 9, 19	
	9	86.2, CH	3.26, m	H-8, H-10a, H-10b	7, 8, 10, 11, 19, 20	H-7
	10a	37.5, CH_2	2.74, dd (5.0, 14.0)	H-9, H-10b	8, 9, 11, 12/16	
	10b		2.69, dd (7.0, 14.0)	H-9, H-10a	8, 9, 11, 12/16	

	11	139.7, qC				
	12/16	129.7, CH	7.20, m	H-13, H-15	10, 11, 13/15, 14	
	13/15	128.6, CH	7.28, m	H-12, H-14, H-16	11, 12/16, 14	
	14	126.3, CH	7.19, m	H-13, H-15	12/16, 13/15	
	17	16.7, CH_3	0.97, d (7.5)	H-2	1, 2, 3	H-4, H-5, H-7, NH
	18	13.0, CH_3	1.56, s		5, 6, 7	H-5
	19	16.4, CH_3	0.98, d (7.4)	H-8	7, 8, 9	
	20	57.8, OCH_3	3.2, s		9	
	NH		6.91, m	H-3		H-2, H-2(Ahppa-4), NH(Ahppa-4)
D-Glu	1	173.7, qC				
	2	51.5, CH	4.38, m	H-3a, H-3b	1, 3, 4	H-4a
	3a	27.5, CH_2	2.00, m	H-2, H-4a, H-4b	1, 2	NH
	3b		1.67, m	H-2, H-4a, H-4b	1, 2	NH
	4a	32.0, CH_2	2.54, m	H-3a, H-3b		H-2
	4b		2.42, m	H-3a, H-3b	5	
	5	175, qC				
	NH		7.19, m	H-2		H_3-17(Adda), H-3a(Glu), H-3b(Glu), H-2(Adda)
	1-OH		12.9, br			
Mdha	1	163.4, qC				
	2	145.6, qC				
	3a	113.9, CH_2	5.78, s	H-3b	1,2	H_3-4
	3b		5.40, s	H-3a	1	H_3-4, NH(Ala), H-5(Adda)
	4	36.3, NCH_3	3.23, s		2, 5 (Glu)	H-3a, NH(Ala)

*Direct ^{1}H-^{13}C correlations and the multiplicities of the ^{13}C signals were assigned by a phase-sensitive HSQC spectrum.

S3. 1H NMR (800 MHz, DMSO-d6) spectrum of compound 2.



S4. 13C NMR (800 MHz, DMSO-d6) spectrum of compound 2.

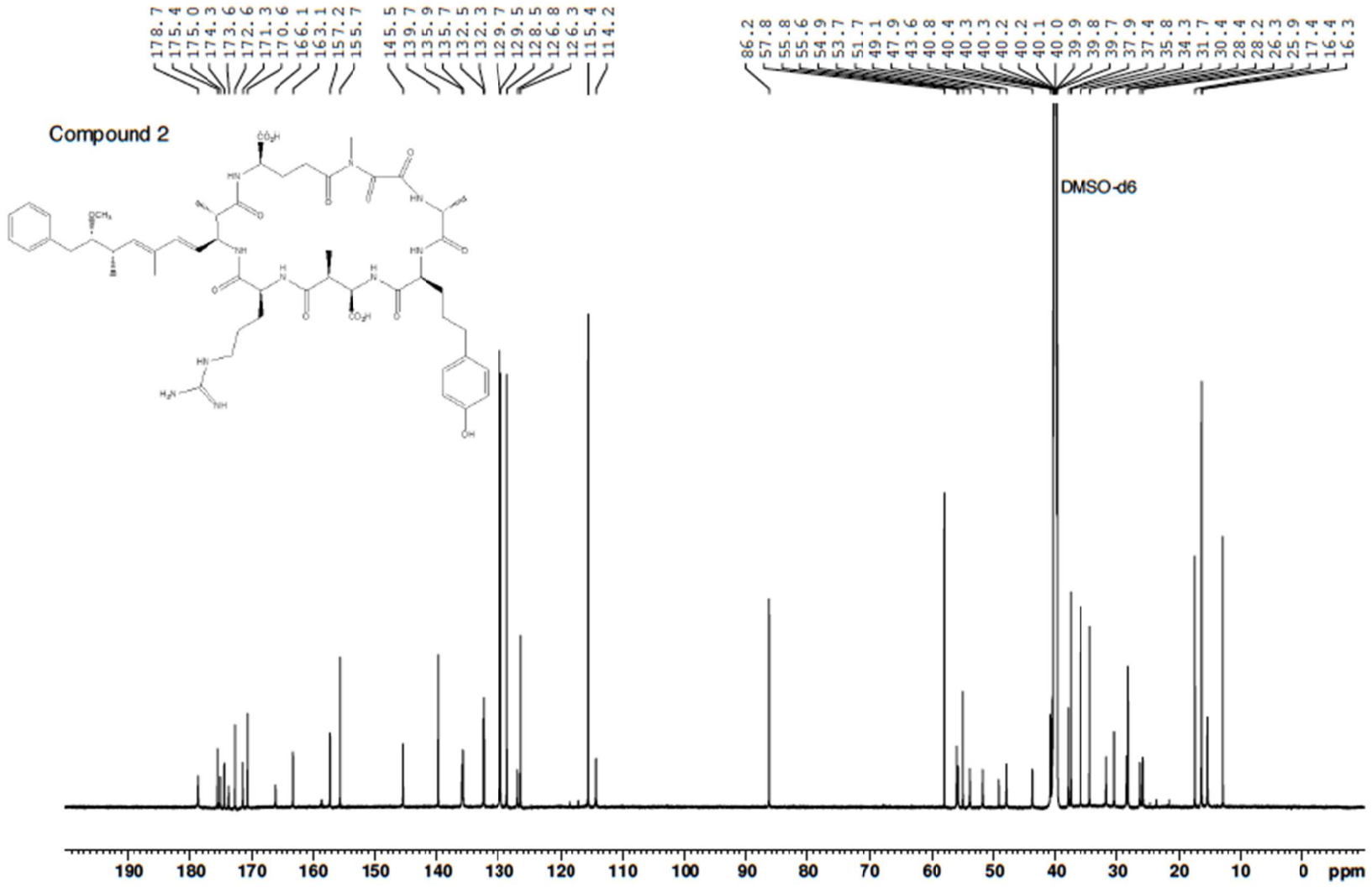


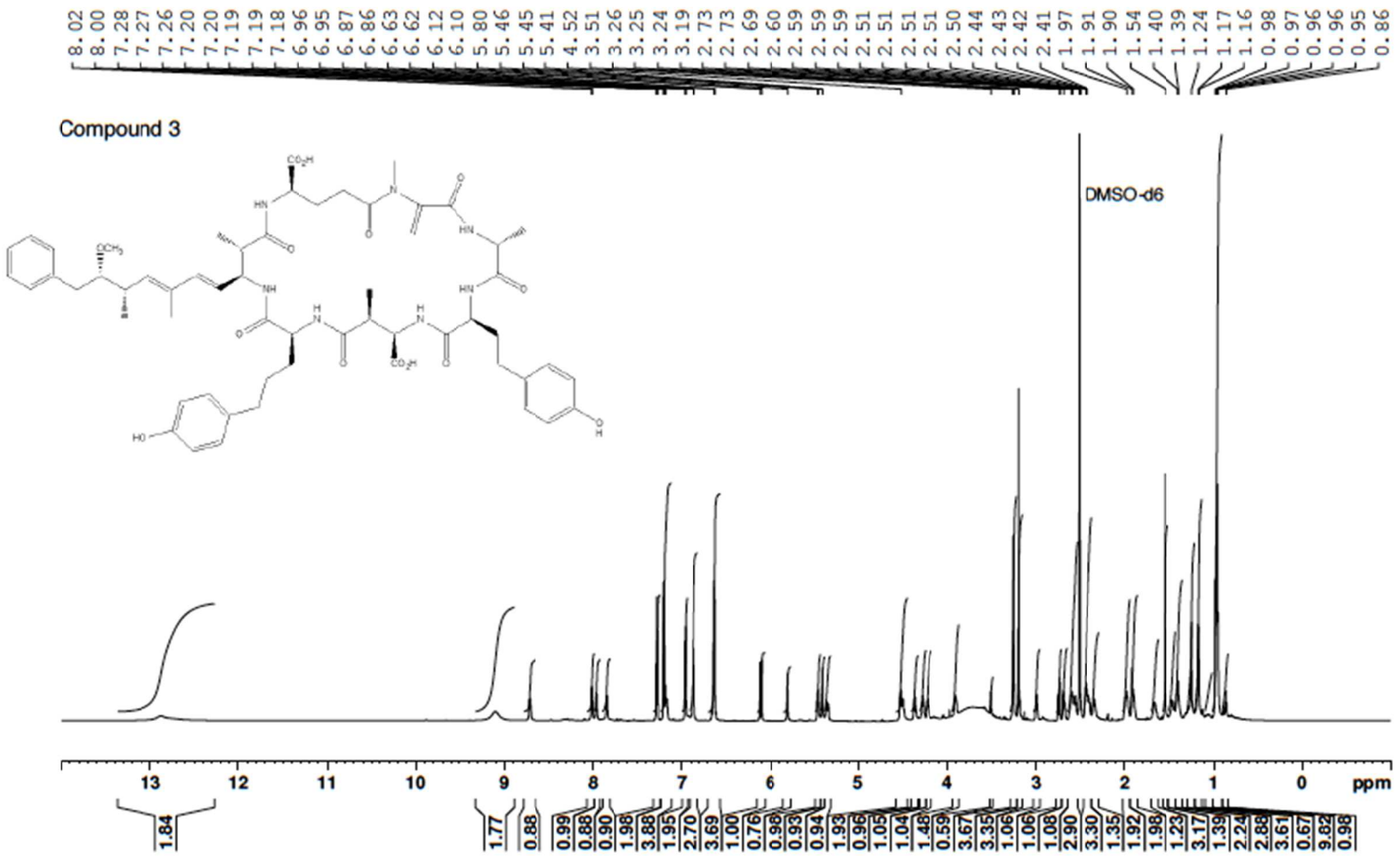
Table S2. NMR Data for Microcystin 2 (1072) in DMSO-d_6 (700 MHz)

Unit	C/H #	δ_C, mult.	δ_H (*J* in Hz)	^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC	^{1}H-^{1}H NOESY
Ala	1	172.6, qC	-			
	2	47.9, CH	4.51, m	H_3-3, NH	1, 3, 1(Mdha)	NH(Ahppa), H_3-5(Masp), NH(Masp)
	3	17.4, CH_3	1.14, d (7.2)	H-2	1, 2	NH, NH(Ahppa), H_3-5(Masp), NH(Masp)
	NH		7.36, m	H-2		H_3-3, NH(Ahppa), H-3(Masp), NH(Masp), H-2(Arg)
Ahppa	1	171.3, qC				
	2	55.6, CH	3.95, m	H_2-3, NH	1, 3	H-4a, H-5a, H-5b
	3	30.4, CH_2	1.74, m	H-2, H-4a, H-4b	4, 5	NH, NH(Masp)
	4a	28.2, CH_2	1.65, m	H_2-3, H-5a, H-5b	2, 3, 5, 6	H-2, NH
	4b		1.52, m	H_2-3, H-5a, H-5b	2, 3, 5, 6/10	H-2, NH
	5a	34.3, CH_2	2.42, m	H-4a, H-4b	3, 4, 6, 7/11	H-2, H-7/11
	5b		2.38, m	H-4a, H-4b	3, 4, 6, 7/11	H-2, H-7/11
	6	132.3, qC				
	7/11	129.5, CH	6.94, d (8.3)	H-8, H-10	5, 8/10, 9	H_2-3, H-4a, H-4b, H-5a, H-5b
	8/10	115.4, CH	6.64, d (8.4)	H-7, H-11	6, 9	H-5a, H-5b
	9	155.7, qC				
	9-OH		9.2, brs			
	NH		7.67, d (7.2)	H-2	2, 3, 1(Ala)	H_2-3, H-4a, H-4b, H-2(Ala), H_3-3(Ala), NH(Ala), H_3-5(Masp), NH(Masp)
Masp	1	174.4, qC				
	2	55.9, CH	4.13, m	H-3, NH	1, 3, 4, 5, 1(Ahppa)	NH (Arg)
	3	40.0, CH	3.15, m	H_3-5, H-2	1	NH, NH(Arg), H-3a(Arg), NH(Adda)
	4	178.7, qC				
	5	15.4, CH_3	0.90, d (7.2)	H-3	2, 3, 4	H-2, NH, NH(Ahppa), NH(Arg)

	NH		7.90, d (9.5)	H-2	1(Ahppa)	H-3, H_3-5, H_3-3(Ala), NH(Ala), H_2-3(Ahppa), H-2(Ahppa), NH(Ahppa)
Arg	1	170.6, qC				
	2	51.7, CH	4.18, m	H-3a, H-3b, NH	1, 3, 4, 4(Masp)	H_2-5, H_3-3(Ala), NH(Ala), H_3-5(Masp), NH(Adda), H-4a(Glu), H-4b(Glu)
	3a	28.4, CH_2	1.97, m	H-2, H-3b, H-4a, H-4b	4	H_2-5, NH-2, H-3(Masp)
	3b		1.33, m	H-2, H-3a, H-4a, H-4b	4	H_2-5, NH-2, H-3(Masp)
	4a	25.9, CH_2	1.39, m	H-3a, H-3b, H_2-5	3, 5	NH-2, H_3-5(Masp)
	4b		1.33, m	H-3a, H-3b, H_2-5	3, 5	NH-2, H_3-5(Masp)
	5	40.8, CH_2	3.0, m	H-4a, H-4b, NH-5	3, 4, 6	H-2, H-3b, NH, H_3-3(Ala), H_5-5(Masp)
	6	157.2, qC				
	2-NH		8.9, m	H-2		H-3a, H-3b, H_2-4, H-3(Masp), H_3-5(Masp), NH(Adda)
	5-NH		7.78 br			H-3a, H-3b, H_2-4
	6-NH					
	6-NH_2					
Adda	1	174.3, qC				
	2	43.7, CH	2.77, m	H-3, H_3-17		H-4, H_3-18, NH, NH(Arg), NH(Glu)
	3	54.9, CH	4.34, m	H-2, H-4, NH	1, 2, 4, 5, 17, 1(Arg)	H-3(Masp), NH(Arg)
	4	126.8, CH	5.34, m	H-3, H-5	3, 6	H-2, NH, NH(Glu)
	5	135.9, CH	6.09 d (15.5)	H-4	3, 4, 6, 7, 18	H-2, H-8, H-10a, H-10b, H_3-17, H_3-19, NH, NH(Arg)
	6	132.5, qC				
	7	135.7, CH	5.45, d (9.7)	H-8	5, 8, 9, 18, 19	H-2, H-9 H-9, NH
	8	35.8, CH	2.56, m	H-7, H-9, H_3-19	6, 7, 9, 19	H-12/16
	9	86.2, CH	3.26, m	H-8, H-10a, H-10b	7, 8, 10, 11, 19, 20	H-12/16
	10a	37.5, CH_2	2.75, dd (5.0, 14.0)	H-9, H-10b	8, 9, 11, 12/16	H-7, H_3-18, H-12/16

	10b		2.68, dd (7.0, 14.0)	H-9, H-10a	8, 9, 11, 12/16	H-7, H_3-18
	11	139.7, qC				
	12/16	129.7, CH	7.19, m	H-13, H-15	10, 14	H-8, H-10a, H-10b, H_3-18, H_3-19
	13/15	128.5, CH	7.27, m	H-12, H-14, H-16	11, 12/16, 14	
	14	126.3, CH	7.19, m	H-13, H-15	12/16, 13/15	
	17	16.3, CH_3	0.92, d (6.8)	H-2	1, 2, 3	H-3, H-4, H-5, H-7, NH, NH(Glu)
	18	13.0, CH_3	1.56, s		5, 6, 7	H-10a
	19	16.4, CH_3	0.98, d (6.7)	H-8	7, 8, 9	H-4, H-7, H-9
	20	57.8, OCH_3	3.19, s		9	
	NH		7.72, brd (6.7)	H-3		H-2, H-4, H-5, H_3-17, H-3(Masp), H-2(Arg), H_2-3(Arg), H_2-4(Arg), NH(Arg), NH(Glu), H-4b(Glu)
D-Glu	1	173.6, qC				
	2	53.7, CH	4.30, m	H-3a, H-3b, NH	1, 3, 4, 1(adda)	H-4a, H-4b, H_3-17(Adda)
	3a	26.3, CH_2	1.90, m	H-2, H-4a, H-4b	4	NH, H-2(Adda), NH(Ahppa), NH(Masp)
	3b		1.78, m	H-2, H-4a, H-4b	1	NH, H-2(Adda), NH(Ahppa), NH(Masp)
	4a	31.7, CH_2	2.37, m	H-3a, H-3b	3, 5	H-2, H-2(Arg), NH
	4b		2.23, m	H-3a, H-3b	3, 5	H-2, H-2(Arg), NH, NH(Masp)
	5	175.4, qC				
	NH		9.2 br	H-2	2, 1(Adda)	H-3a, H-3b, H-2(Adda), H_3-17(Adda), NH(Adda)
Mdha	1	163.1, qC				
	2	145.5, qC				
	3a	114.2, CH_2	5.80, s	H-3b	1, 2	NH(Ala)
	3b		5.39, s	H-3a	1	NH(Ala)
	4	37.9, NCH_3	3.15, s		2, 5 (Glu)	NH(Adda)

S5. 1H NMR (800 MHz, DMSO-d6) spectrum of compound 3.



S6. 13C NMR (800 MHz, DMSO-d6) spectrum of compound 3.

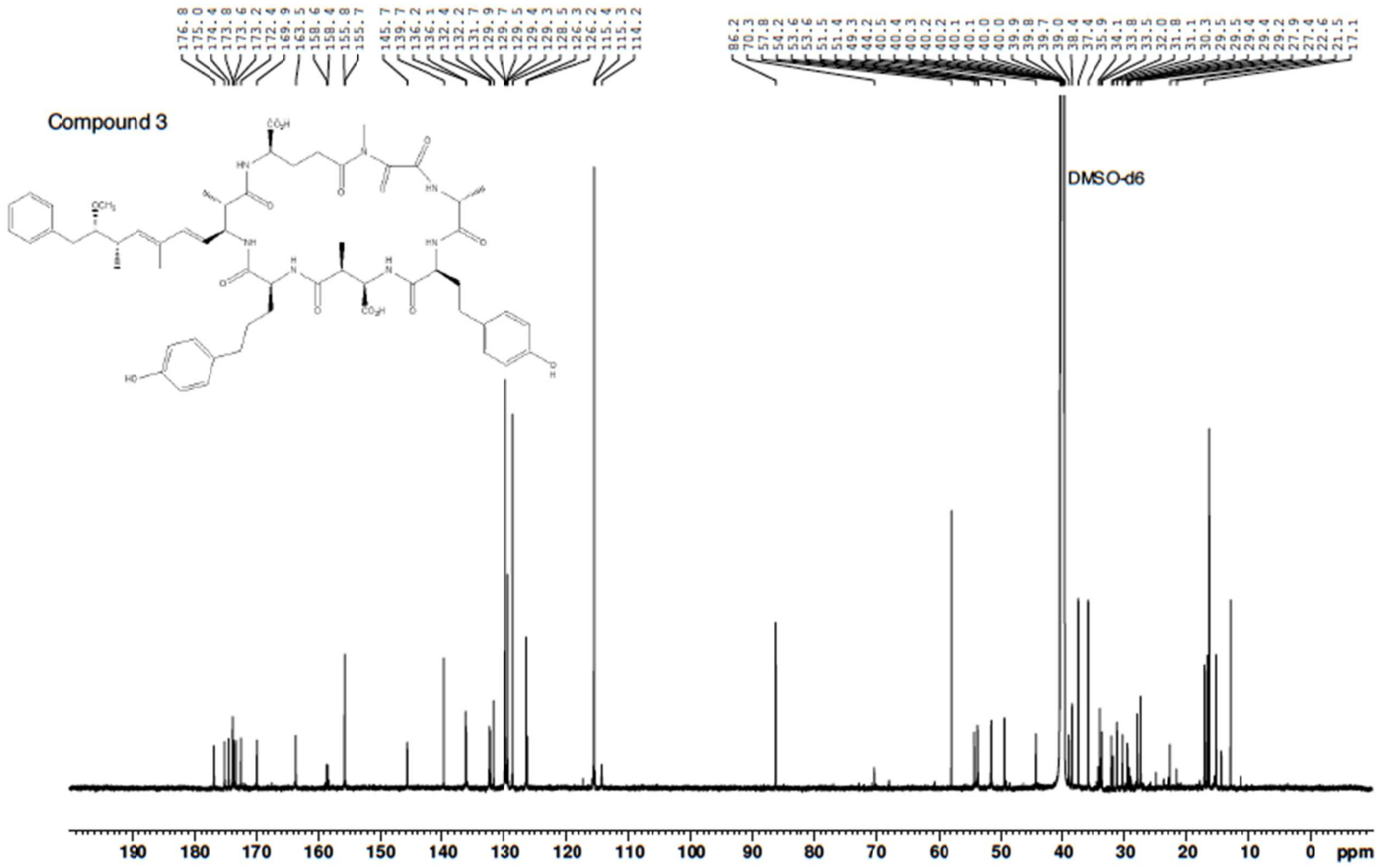


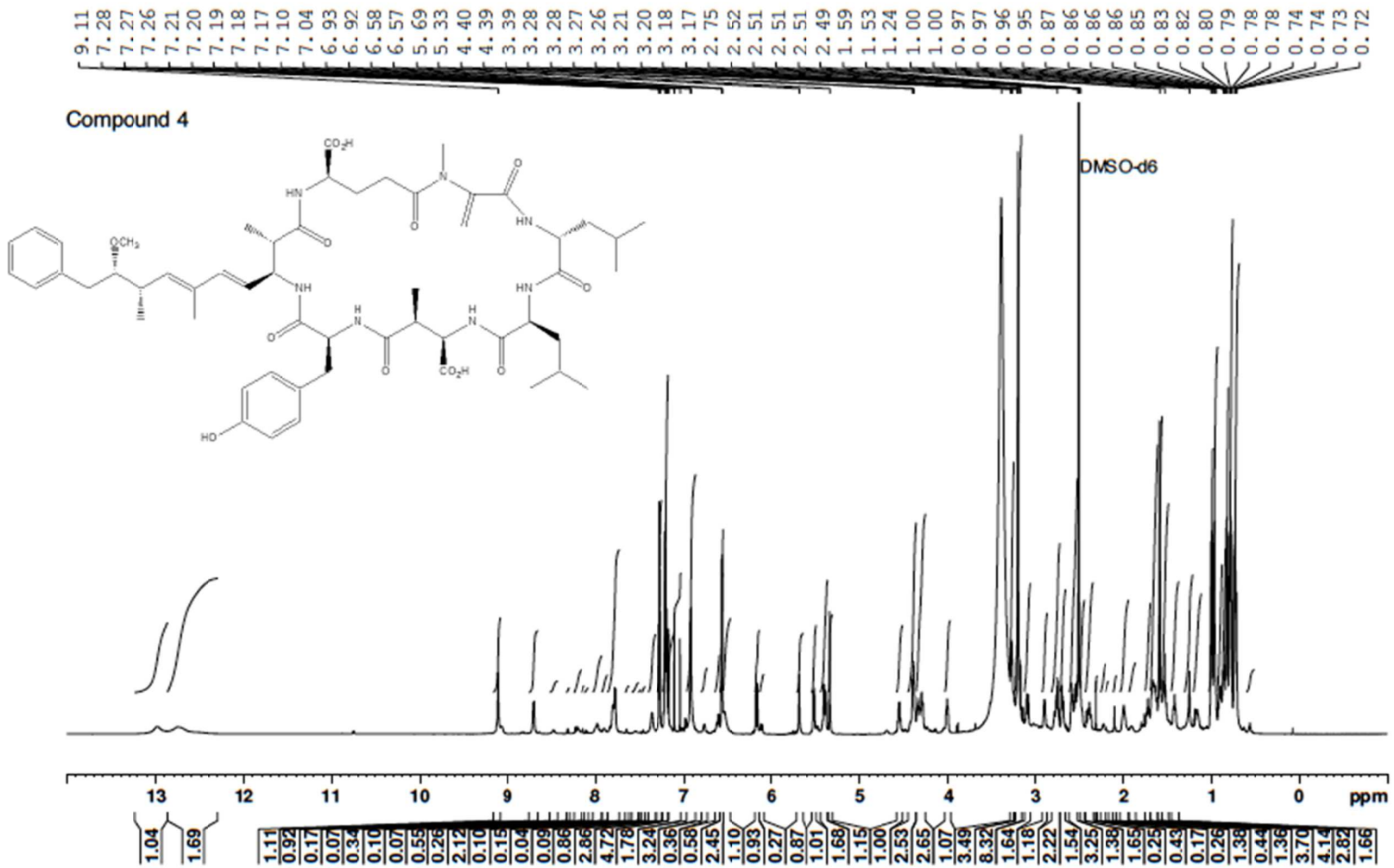
Table S3. NMR Data for Microcystin 3 (1093) in DMSO-d_6 (700 MHz)

Unit	C/H #	δ_C, mult.	δ_H (*J* in Hz)	^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC	^{1}H-^{1}H NOESY
Ala	1	173.2, qC	-			
	2	49.3, CH	4.28, m	H_3-3, NH	1, 3, 1(Mdha)	NH(Htyr)
	3	17.1, CH_3	1.17, d (7.1)	H-2	1, 2	NH
	NH		7.84, brd (6.3)	H-2		H_3-3
Htyr	1	172.4, qC				
	2	54.2, CH	3.90, m	H-3a, H-3b, NH	1, 3, 4	H-4a, H-6/10
	3a	33.5, CH_2	1.97, m	H-2, H-4a, H-4b	1, 2, 4, 5	H-6/10
	3b		1.90, m	H-2, H-4a, H-4b	1, 2, 4, 5	H-6/10
	4a	31.1, CH_2	2.60, m	H-3a, H-3b	2, 3, 5, 6/10	H-2, H-6/10
	4b		2.43, m	H-3a, H-3b	2, 3, 5, 6/10	H-2, H-6/10
	5	132.2, qC				
	6/10	129.9, CH	6.96, d (8.2)	H-7, H-8	4, 7, 8, 9	H-2, H-3a, H-3b, H-4a, H-4b
	7/9	115.4, CH	6.63, d (8.1)	H-6, H-10	5, 8	
	8	155.8, qC				
	8-OH		9.1, brs			
	NH		7.96, brd	H-2	2, 1(Ala)	H-3b, H-4a, H-4b, H-2(Ala)
Masp	1	174.4, qC				
	2	53.6, CH	4.51, m	H-3, NH	1, 3, 4, 1(Htyr)	H_3-5
	3	39.0, CH	3.0, m	H_3-5, H-2	2, 4, 5	NH(Ahppa)
	4	176.8, qC				
	5	15.2, CH_3	0.95, d (7.0)	H-3	2, 3, 4	H-2
	NH		8.01, d (9.0)	H-2	2, 3, 1(Htyr)	H_3-5, H-2(Htyr)
Ahppa	1	169.9, qC				
	2	51.5, CH	4.22, m	H-3a, NH	1, 3, 4	H-5a, H-5b, H-7/11, NH(Adda), H_3-4(Mdha)
	3a	30.3, CH_2	1.90, m	H-2, H-3b, H-4a, H-4b	4	NH

	3b		1.39, m	H-2, H-3a, H-4a, H-4b	4	
	4a	27.9, CH_2	1.45, m	H-3a, H-3b,H-4b, H-5a, H-5b	2, 3, 5, 6	H-2, H-7/11
	4b		1.40, m	H-3a, H-3b,H-4a, H-5a, H-5b	2, 3, 5, 6	H-2, H-7/11
	5a	33.9, CH_2	2.40, m	H-4a, H-4b	3, 4, 6, 7, 9	H-2, H-3b, H-7/11
	5b		2.33, m	H-4a, H-4b	3, 4, 6, 7, 9	H-2, H-7/11
	6	132.2, qC				
	7/11	129.3, CH	6.86, brd (7.8)	H-8, H-10	5, 8, 9, 10	H-2, H-4a, H-4b, H-5a, H-5b, NH, H-2(Adda), H-4(Adda), H-5(Adda)
	8/10	115.4, CH	6.62, d (7.8)	H-7, H-11	6, 9	
	9	155.7, qC				
	9-OH		9.1, brs			
	NH		8.72, brd (8.6)	H-2	2, 4(Masp)	H-3a, H-7/11, H-3(Masp), H-3(Adda), NH(Adda)
Adda	1	173.6, qC				
	2	44.2, CH	2.52, m	H-3, H_3-17	1, 3, 17	H-4, NH(Glu)
	3	53.6, CH	4.50, m	H-2, H-4, NH	1, 2, 4, 5	H_3-17, H-5, H-7/11(Ahppa)
	4	126.2, CH	5.35, dd (15.4, 7.5)	H-3, H-5	2, 3, 5, 6	H-2, H_3-17, H_3-18, H-7/11(Ahppa)
	5	136.2, CH	6.11 d (15.4)	H-4	2, 3, 4, 6, 7, 18	H-3, H-7, H_3-17, H_3-18, H-7/11(Ahppa)
	6	132.4, qC				
	7	136.1, CH	5.46, d (9.8)	H-8	5, 8, 9, 18, 19	H-5, H-9, H-10a, H-10b, H_3-18, H_3-19
	8	35.9, CH	2.56, m	H-7, H-9, H_3-19	6, 7, 9, 10, 19	H_3-18
	9	86.2, CH	3.25, m	H-8, H-10a, H-10b	7, 8, 10, 11, 19, 20	H-7, H_3-18
	10a	37.5, CH_2	2.74, dd (5.0, 14.0)	H-9, H-10b	8, 9, 11, 12/16	H-7
	10b		2.68, dd (7.0, 14.0)	H-9, H-10a	8, 9, 11, 12/16	H-7
	11	139.7, qC				
	12/16	129.7,	7.20, m	H-13, H-15	10, 11,	H-10a, H-10b, H_3-

		CH			13/15, 14	18, H_3-19
	13/15	128.5, CH	7.27, m	H-12, H-14, H-16	11, 12/16, 14	H_3-18, H-9
	14	126.3, CH	7.20, m	H-13, H-15	12/16, 13/15	
	17	16.7, CH_3	0.96, brd (6.9)	H-2	1, 2, 3	H-2, H-4, H-5, H_3-18
	18	12.9, CH_3	1.54, s		5, 6, 7	H-4, H-5, H-8, H_3-19
	19	16.4, CH_3	0.98, d (7.4)	H-8	7, 8, 9	
	20	57.8, OCH_3	3.19, s		9	H-10a, H-10b, H_3-18, H_3-19
	NH		6.90, m	H-3	1(Ahppa)	H-2, H-4, H-5, H-2(Ahppa), NH(Ahppa)
D-Glu	1	173.8, qC				
	2	51.4, CH	4.37, m	H-3a, H-3b, NH	1, 3, 4, 1(adda)	H-4a, H-4b
	3a	27.4, CH_2	1.98, m	H-2, H-4a	1, 2, 4	
	3b		1.66, m	H-2, H-4a, H-4b	1, 2, 4	
	4a	32.0, CH_2	2.60, m	H-3a, H-3b	3, 5	H-2, H_3-4(Mdha)
	4b		2.43, m	H-3a, H-3b	3, 5	H-2, H_3-4(Mdha)
	5	175.1, qC				
	NH		7.16, brd	H-2	2, 1(Adda)	H-3a, H-2(Adda)
	1-OH		12.9, br			
Mdha	1	163.5, qC				
	2	145.7, qC				
	3a	114.1, CH_2	5.80, s	H-3b	1, 2	H_3-4, H-5(Adda), H-4a(Glu), H-4b(Glu)
	3b		5.41, s	H-3a	1	NH(Ala)
	4	38.1, NCH_3	3.25, s		2, 5 (Glu)	H-3a, H-3b, NH(Ala), H-2(Ahppa)

S7. 1H NMR (800 MHz, DMSO-d6) spectrum of compound 4.



S8. 13C NMR (800 MHz, DMSO-d6) spectrum of compound 4.

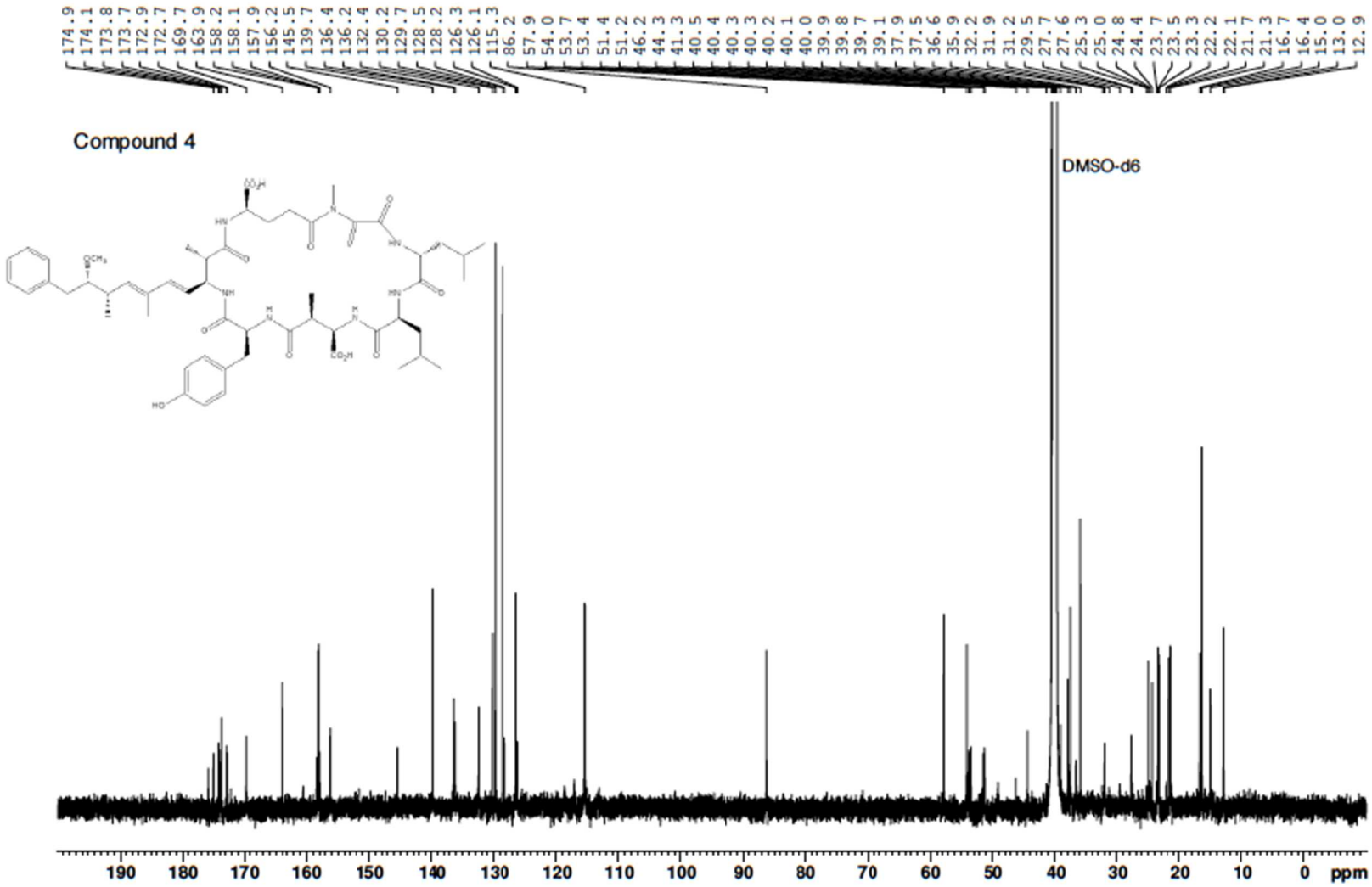


Table S4. NMR Data for Microcystin 4 (1043) in DMSO-d_6 (700 MHz)

Unit	C/H #	δ_C, mult.	δ_H (*J* in Hz)	^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC	^{1}H-^{1}H NOESY
Leu	1	172.7, qC	-			
	2	51.2, CH	4.34, m	H-3a, H-3b, NH	1	H-3b, H-4, H_3-5, NH(Leu-2), H-3(Adda)
	3a	39.6, CH_2	1.64, m	H-2, H-3b, H-4	5, 6	H_3-5, NH, NH(Leu-2)
	3b		1.16, m	H-2, H-3a, H-4	2, 4, 5, 6	NH(Leu-2)
	4	25.0, CH	1.53, m	H-3a, H-3b, H_3-5, H_3-6		H-2, NH
	5	21.7, CH_3	0.74, d(6.5)	H-4	3, 4, 6	H-2, H-3a
	6	23.3, CH_3	0.80, d(6.5)	H-4	3, 4, 5	
	NH		7.37, m	H-2		H-3a, H-3b, H-4, NH(Leu-2)
Leu	1	172.9, qC				
	2	53.4, CH	4.01, m	H-3a, H-3b, NH	1, 1(Leu-1)	H_3-5
	3a	39.8, CH_2	1.72, m	H-2, H-3b, H-4	5, 6	H_3-6, NH
	3b		1.43, m	H-2, H-3a, H-4	2, 4, 5, 6	NH
	4	24.4, CH	1.67, m	H-3a, H-3b, H_3-5, H_3-6	5	H-2, NH
	5	21.3, CH_3	0.78, d (6.2)	H-4	3, 4, 6	H-2, NH
	6	23.5, CH_3	0.83, d (6.2)	H-4	3, 4, 5	H-2, NH
	NH		7.78, brd (6.4)	H-2		H-3a, H-3b, H-2(Leu-1), NH(Leu-1)
Masp	1	175.9, qC				
	2	53.7, CH	4.39, m	H-3, NH		H_3-5, NH(Tyr)
	3	39.1, CH	2.90, m	H_3-5, H-2	5	NH, NH(Tyr), NH(Adda)
	4	175.9, qC				
	5	15.0, CH_3	0.73 d (7.0)	H-3	2, 3, 4	H-2, NH, NH(Tyr), NH(Adda)
	NH		7.80, m	H-2		H-3, H_3-5, NH(Leu-1)
Tyr	1	169.7, qC				
	2	54.0, CH	4.30, m	H-3a, H-3b, NH	1, 3, 4	NH(Adda), H_3-4(Mdha)
	3a	36.6, CH_2	3.09, m	H-2, H-3b	2	H-5, H-9

	3b		2.50, m	H-2, H-3a	2	H-5, H-9, NH
	4	128.2, qC				
	5/9	130.2, CH	6.92, d (8.0)	H-6, H-8	3, 6, 7, 8	H-3a, H-3b, H_3-5(Masp)
	6/8	115.3, CH	6.57, d (8.0)	H-5, H-9	4, 5, 7, 9	OH-9, H_3-5(Masp)
	7	156.2, qC				
	NH		8.70, d (8.8)			H-3a, H-3b, H-3(Masp), H_3-5(Masp), NH(Adda)
	9-OH		9.11 brs			H-6/8
Adda	1	173.8, qC				
	2	44.3, CH	2.54, m	H-3, H_3-17	1, 3, 17	H-4, H-5, NH, NH(Glu)
	3	54.03, CH	4.55, m	H-2, H-4, NH	2, 4, 5	H-5, H-7, H_3-17, H-2(Leu-1)
	4	126.1, CH	5.39, m	H-3, H-5	2, 3, 6	H_3-17, H_3-18, NH
	5	136.4, CH	6.17 d (15.5)	H-4	3, 6, 7, 18	H-3, H_3-17, NH
	6	132.4, qC				
	7	136.2, CH	5.51, d (9.6)	H-8	5, 8, 9, 18, 19	H-5, H-9, H_3-19
	8	35.9, CH	2.59, m	H-7, H-9, H_3-19	6, 9, 19	H_3-19
	9	86.2, CH	3.27, m	H-8, H-10a, H-10b	7, 8, 10, 11, 19, 20	H-5, H-7, H_3-19
	10a	37.5, CH_2	2.76, dd (4.8, 13.8)	H-9, H-10b	8, 9, 11, 12/16	H-7
	10b		2.70, dd (7.1, 13.8)	H-9, H-10a	8, 9, 11, 12/16	H_3-19, H-7
	11	139.7, qC				
	12/16	129.7, CH	7.21, m	H-13, H-15	10, 14	H-9
	13/15	128.6, CH	7.27, m	H-12, H-14, H-16	11, 12/16, 14	
	14	126.3, CH	7.19, m	H-13, H-15	12/16, 13/15	
	17	16.7, CH_3	0.97, d (6.6)	H-2	1, 2, 3	H-3, H-4, H-5, H-2(Leu-1)
	18	12.98, CH_3	1.59, s		5, 6, 7	H-5, H_3-19, NH, NH(Glu)
	19	16.4, CH_3	1.00, d (6.6)	H-8	7, 8, 9	H-4, H-5, H-7, H-9
	20	57.9, OCH_3	3.20, s		9	H-7, H_3-20
	NH		6.92, brd (7.9)	H-3		H-4, H-5, H_3-17, H-3(Masp), H_3-

						5(Masp) H-2(Tyr), H-3b(Tyr), NH(Tyr)
D-Glu	1	173.7, qC				
	2	51.4, CH	4.39, m	H-3a, H-3b, NH	1, 2, 3	H-4a, H-4b, NH (Adda)
	3a	27.7, CH_2	1.99, m	H-2, H-3b, H-4a		NH
	3b		1.67, m	H-2, H-3a, H-4b		NH
	4a	31.9, CH_2	2.48, m	H-3a, H-3b		H-2, H-3a(Mdha)
	4b		2.40, m	H-3a, H-3b		
	5	174.9, qC				
	NH		7.2, m	H-2		H-3a, H-3b, H-2(Adda), H_3-17(Adda)
Mdha	1	163.9, qC				
	2	145.5, qC				
	3a	113.0, CH_2	5.69, s	H-3b	1, 2	H_3-4, H-4a (Glu), NH(Leu-1)
	3b		5.33, s	H-3a	1	H_3-4, NH(Leu-1)
	4	37.9, NCH_3	3.20, s		2, 5 (Glu)	NH(Leu-1)

General procedures for NMR analyses. 1D and 2D NMR data were acquired at 298 K on a Bruker 700 or 800 MHz NMR spectrometer equipped with a triple resonance (TXI) cryoprobe. Samples were dissolved in ca. 0.6 ml of deuterated dimethylsulfoxide (DMSO-d_6) with deuterium serving as the lock nucleus. The NMR experiments were performed using Bruker's pulse programs at their default settings, but when necessary, some parameters including number of scans (NS), spectral width (SW), transmitter frequency offset (O1P), size of fid (TD), and delays (D[#]) were modified. ^{1}H-^{1}H geminal and vicinal coupling were obtained using double quantum filtered COSY (DQF-COSY). Heteronuclear single quantum correlation (HSQC) data were acquired with $^{1}J_{CH}$ optimized for 145 Hz. The mixing time for 2D Total Correlation Spectroscopy (2D-TOCSY) was 60 ms while that for 2D Nuclear Overhauser Effect (2D-NOESY) was 500 ms. For Heteronuclear Multiple Bond Correlation (HMBC) experiments, long range ^{1}H-^{13}C coupling was optimized for 8 Hz, and ^{13}C data were acquired in proton decoupled mode. The acquired NMR data were processed using Topspin software (version 2.1, 3.2 or 3.5).

General procedures for HRMS analyses. HRMS Analysis. High resolution mass spectrometric data were obtained using an Acquity UPLC system (Waters Corporation, Milford, MA, USA) coupled with a QTOF-MS (Xevo G2 QTOF, Waters MS Technologies, Manchester, UK), controlled by MassLynx v4.1 software. Mass spectra were acquired in both positive and negative modes over the range m/z 100–2000 Da in two channels with scan time 1s. The capillary voltages were set at 3000 V (positive mode) and 2500 V (negative mode), respectively, and the cone voltage was 20V. Nitrogen gas was used both for the nebulizer and in desolvation. The desolvation and cone gas flow rates were 600 and 20 L/h, respectively. The desolvation temperature was 250°C, and the source temperature was 100 °C. A solution of Leucine Enkephalin (1µg/mL) in acetonitrile/water (1:1) containing 0.1% formic acid was utilized as the lock mass at a flow rate 10 µL/mL, m/z 556.2771 was used for the positive mode and m/z 554.2615 for the negative mode.

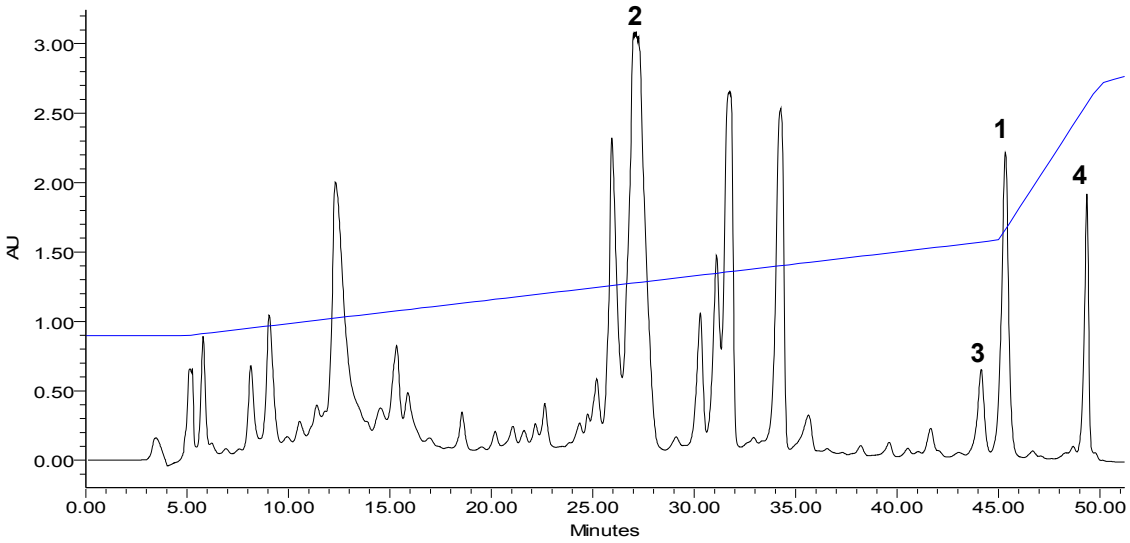


Figure S9. HPLC Chromatogram of fraction C18-B.

New microcystins eluted as indicated on the chromatogram: **2** (27 min), **3** (44 min), **1** (45.5 min), **4** (49.2 min)

A sample of fraction C18-B (1 mg/mL methanol) was separated on HPLC. Column: Phenomenex Synergi C18 column (250 x 10 mm, 4 uM particle size, 80 A pore size). The column was eluted with acetonitrile/water (0.1 % TFA) at 3 mL/min as follows: 30% acetonitrile for 5 min; gradient elution from 30% to 52% acetonitrile from 5 to 45 min; gradient from 52% to 88% acetonitrile from 45 to 50 min. Detection: UV absorbance at 220 nm.

Figure S10. Cytotoxicity Data

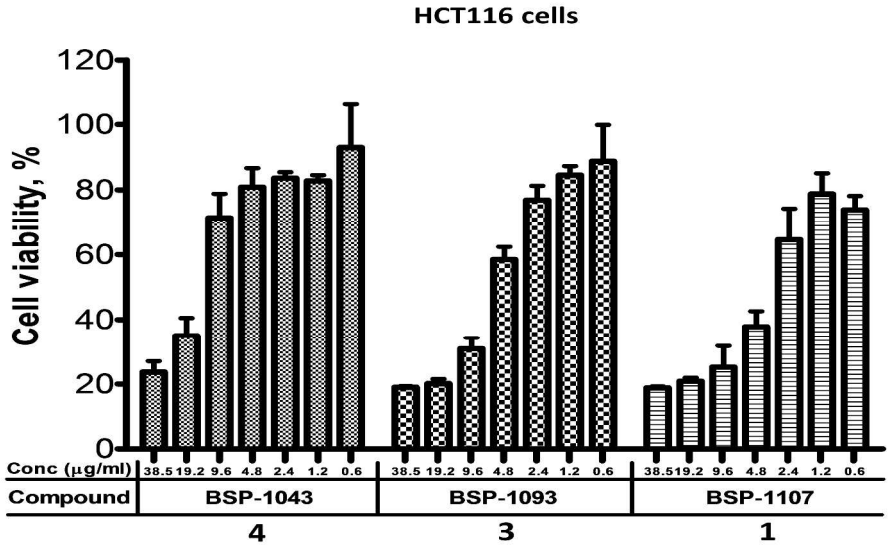


Figure S10A. HCT-116 Cell viability dilution series for microcystins 1 (BSP-1107), 3 (BSP-1093) & 4 (BSP-1043)

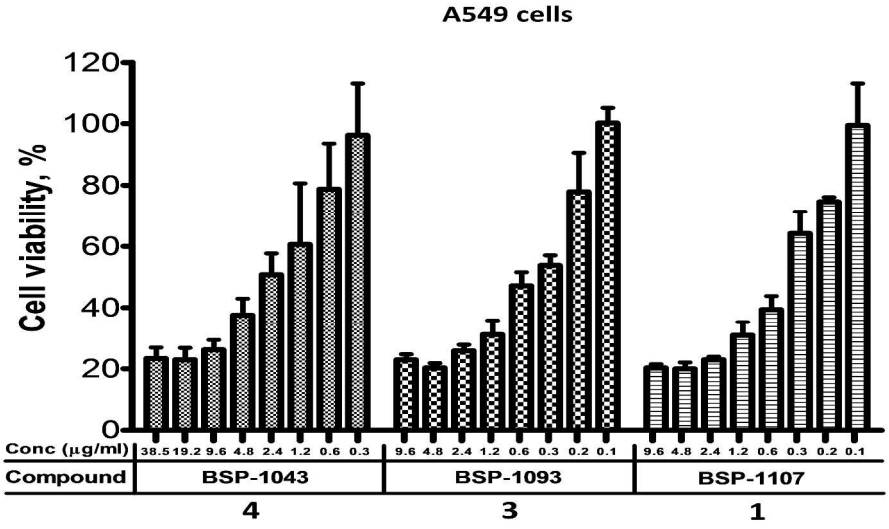


Figure S10B. A549 Cell viability dilution series for microcystins 1 (BSP-1107), 3 (BSP-1093) & 4 (BSP-1043)

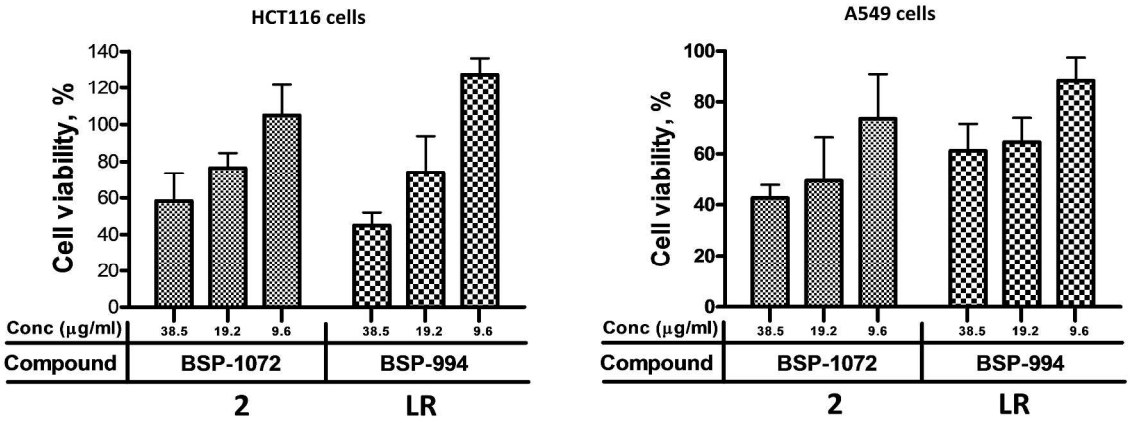


Figure S10C. HCT-116 Cell viability for microcystins 2 (BSP-1072) & 5 LR (BSP-994)

Cytotoxicity (MTT) Assay

To establish whether the new microcystins **1-4** that were isolated in this work are cytotoxic, we tested the compounds for cytotoxicity on human cancer cell lines HCT-116 (colon) and A549 (lung). Each cell line was grown in its respective growth medium and allowed to reach >80% confluence before harvesting for cytotoxicity testing. Harvesting of the cells involved removal of the spent growth medium, rinsing the cells with 10 ml PBS, trypsinization, and suspending the loosened cells in the appropriate growth medium. The harvested cells were then counted and used in the preparation of a suspension with known cell density (20,000 cells/well). Subsequently, the cells were seeded in 96-well microplates at a density of 2000 cells/well and incubated overnight at 37°C to allow them to bind to the bottom of the wells and equilibrate. Test compounds were individually suspended in 50% aqueous methanol to make 1 mg/ml test solutions. These test solutions were then serially diluted (2-fold) in 50% aqueous methanol to prepare 7 additional test solutions for each compound. Test solutions (4µl) were transferred in triplicate to distinct wells containing HCT116 or A549 cells. For the positive and negative control experiments, the cells were treated with 30 µl of methanol (100%) or 4 µl of 50% aqueous methanol, respectively. The cells were subsequently incubated at 37°C for ~72 hr before their viability was assessed by the MTT method as described previously, but with minor modifications. Briefly, 20 µl of 2.5 mg/ml MTT in PBS was added to each test well followed by incubation at 37°C for 4 hr before the growth medium was removed and 170 µl of DMSO (100%) added. After a further 5 min incubation at 37°C, optical density 540 nm was measured on a SpectraMax Plus 384 Microplate Reader integrated with SpectraMax® Pro software (Molecular Devices LLC, Sunnyvale, CA, USA) for recording and processing data. Further data processing and analysis were performed using Microsoft Excel 2010 (Redmond, WA, USA), and GraphPad Prism 5 (La Jolla, CA, USA) software.

Isolation and characterization of microcystin-LR

The mixture (61 mg) containing microcystin-LR, which was described in the isolation of **2** in the Materials and Methods section, was further separated by preparative reverse phase chromatography. A solution of the material in methanol:DMF 2:1 (1.5 mL) was separated in three equal portions on a YMC Pack ODSA column 250 X 30 mm, 10 uM column developed with a gradient of methanol: water from 65 to 70% over 30 minutes at 20 ml/min. Microcystin-LR eluted at 14.5 minutes. Evaporation of the solvents yielded a residue of 15 mg microcystin-LR.

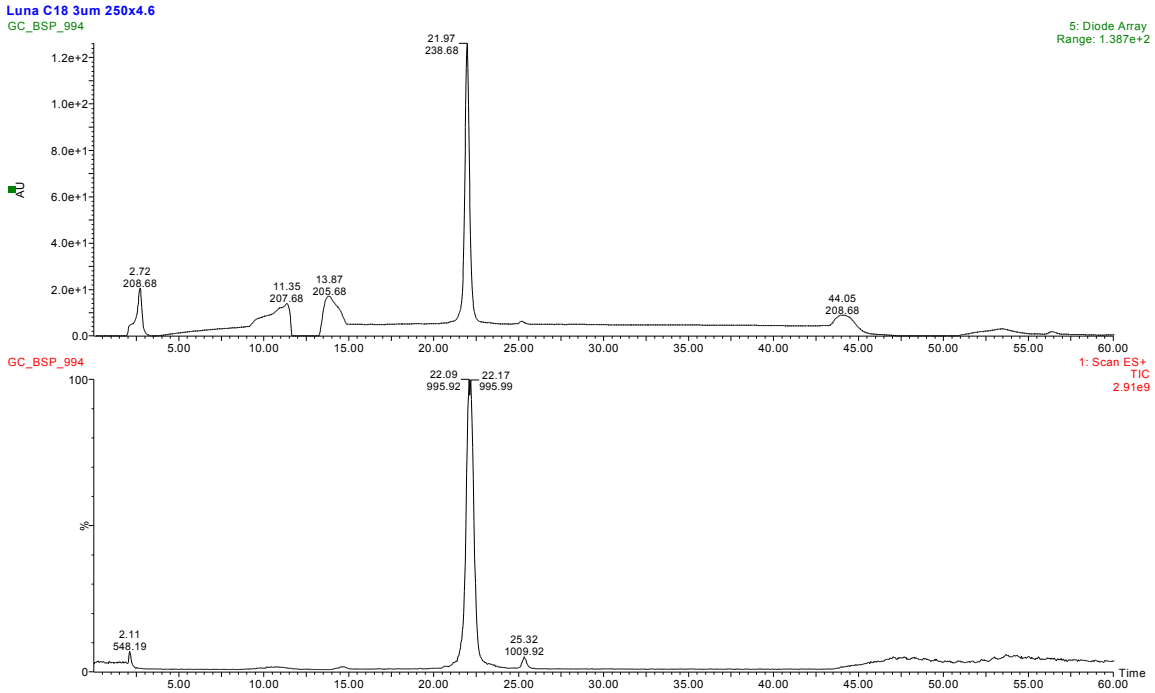


Figure S11. LC/MS Chromatogram of isolated Microcystin-LR.
Top panel: UV detection. Bottom panel: ESI positive ion detection.

LC/MS Analysis of isolated microcystin-LR. The sample (1 mg/mL) was separated on a Luna C18 column (250 x 4.6 mm, 3uM) at 0.4 mL/min with a solvent gradient of acetonitrile in water (each with 0.1 % Formic acid) as follows: 25% to 35% acetonitrile from 0 to 35 minutes, 35% to 100% acetonitrile from 36 to 46 min; return to 25% acetonitrile from 47 to 60 min. Microcystin-LR eluted at 22 min as shown in Figure S11.

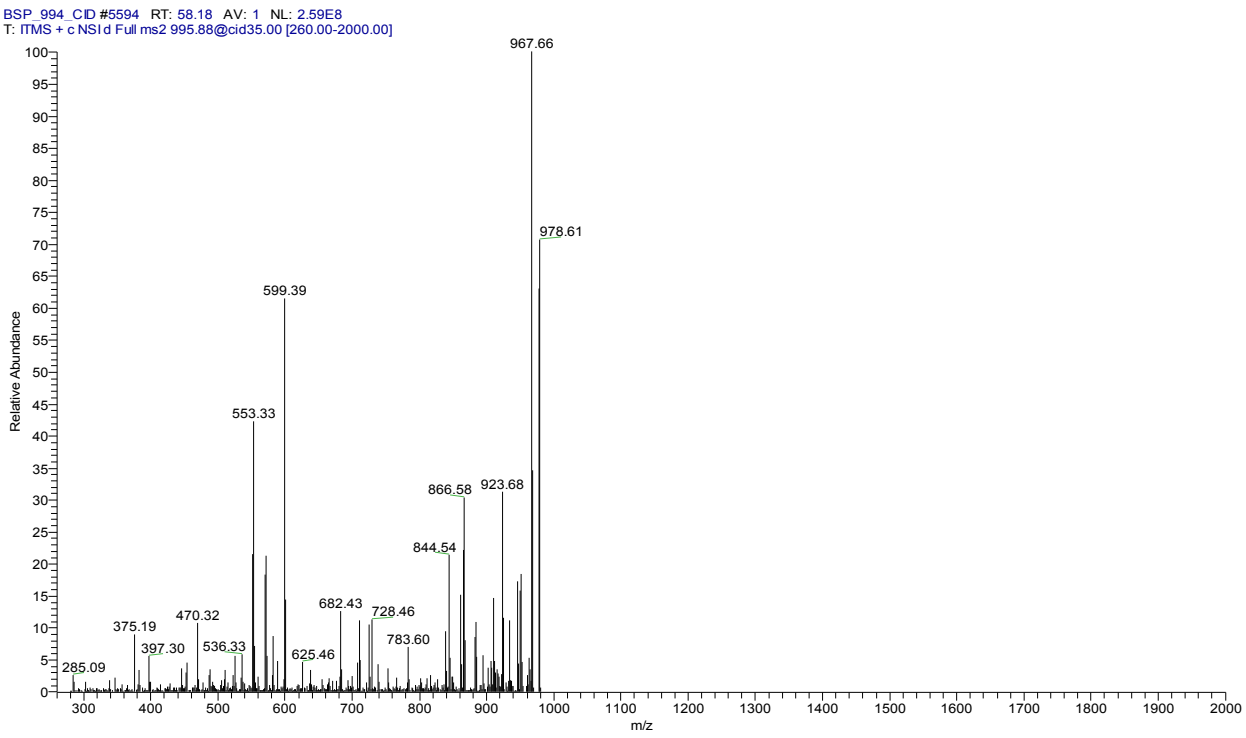


Figure S12. MS/MS Spectrum of isolated microcystin-LR. CID of $(M+H)^+$ at M/Z 995

MS/MS Analysis of isolated microcystin-LR. The isolated sample was subjected to MS/MS analysis for confirmation of structure. The CID of the parent ion at M/Z 995 (M+H)+ is shown in Figure S12. The fragment ions observed correlate very well with published MS/MS data: Tsuyoshi Mayumi, Hajime Kato, Susumu Imanishi, Yoshito Kawasaki, Masateru Hasegawa, Ken-ichi Harada. Structural Characterization of Microcystins by LC/MS/MS under Ion Trap Conditions. J. Antibiot. 59(11): 710–719, 2006