

Sulfated Aeruginosins from Lake Kinneret *Microcystis* bloom, Isolation, Structure Elucidation and Biological Activity

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Table S1. 86 isolated and fully characterized (5 from sponge) aeruginosins + 46 characterized by LCMSMS.

Cyanobacterium	Name/MW	Residue 1 ^a	Residue 2	Residue 3	Residue 4	Activity (μM)
<i>M. aeruginosa</i> NIES-89 ¹	Aeruginosin 89-A 717/719, MH ⁺	<i>m</i> -Cl-D-Hpla-O-sulfate	D-Leu	L-Choi	L-Argal	Trypsin (0.56) Thrombin (0.04)
<i>M. aeruginosa</i> NIES-89 ¹	Aeruginosin 89-B 717/719, MH ⁺	<i>m</i> -Cl-D-Hpla-O-sulfate	D-Leu	L-Choi	D-Argal	Trypsin (9.2) Thrombin (0.07)
<i>M. aeruginosa</i> NIES-98 ²	Aeruginosin 98-A 689/691, MH ⁺	<i>m</i> -Cl-D-Hpla	D- <i>allo</i> lle	L-Choi-6- <i>O</i> -sulfate	Agm	Trypsin (0.87) Thrombin (10.1)
<i>M. aeruginosa</i> NIES-98 ²	Aeruginosin 98-B 655, MH ⁺	D-Hpla	D- <i>allo</i> lle	L-Choi-6- <i>O</i> -sulfate	Agm	Trypsin (0.92) Thrombin (15.3)
<i>M. aeruginosa</i> NIES-98 ¹	Aeruginosin 98-C 731/733, MH ⁺	<i>m</i> -Br-D-Hpla	D- <i>allo</i> lle	L-Choi-6- <i>O</i> -sulfate	Agm	Trypsin (5.3) Thrombin (4.1)
<i>M. aeruginosa</i> NIES-101 ¹	Aeruginosin 101 721/723/725, MH ⁺	<i>m,m</i> -di-Cl-D-Hpla	D- <i>allo</i> lle	L-Choi-6- <i>O</i> -sulfate	Agm	Trypsin (4.1) Thrombin (4.4)
<i>M. viridis</i> NIES-102 ³	Aeruginosin 102-A 733, MH ⁺	D-Hpla-O-sulfate	D-Tyr	L-Choi	L-Argal	Trypsin (0.27) Thrombin (0.05)
<i>M. viridis</i> NIES-102 ³	Aeruginosin 102-B 733, MH ⁺	D-Hpla-O-sulfate	D-Tyr	L-Choi	D-Argal	Trypsin (1.5) Thrombin (0.14)
<i>M. viridis</i> NIES-103 ⁴	Aeruginosin 103-A 681, MH ⁺	D-Hpla	D-Tyr	L-Choi	L-Argal-O-ethyl	Trypsin (75.0) Thrombin (13.2)
<i>O. agardhii</i> NIES-205 ⁵	Aeruginosin 205-A 807/805, MH ⁺	D-Hpla-2-O-sulfate// D-Plac	D-Xyl-3-O-Leu?// 3-Cl-Leu	L-6-Cl-Choi?// D-4'-sulfate-Xyl-6-O-L-Choi	Agm	Trypsin (0.09) Thrombin (1.86)
<i>O. agardhii</i> NIES-205 ⁵	Aeruginosin 205-B 807/805, MH ⁺	L-Hpla-2-O-sulfate// L-Plac	D-Xyl-3-O-Leu?// 3-Cl-Leu	L-6-Cl-Choi?// D-4'-sulfate-Xyl-6-O-L-Choi	Agm	Trypsin (0.09) Thrombin (0.21)
<i>M. aeruginosa</i> NIES-298 ^{6,7}	Aeruginosin 298-A	D-Hpla	D-Leu	L-Choi	L-Argol	Trypsin (1.6) Thrombin (0.49)
<i>M. aeruginosa</i> NIES-298 ^{1,8}	Aeruginosin 298-B 462, MH ⁺	D-Hpla	D-Leu	L-Choi-amide	-	Trypsin (>200) Thrombin (>200)
<i>Microcystis</i> sp. ⁹	Microcin SF608	L-Hpla	L-Phe	L-Choi	Agm	Trypsin (0.82)

<i>M. aeruginosa</i> ^{10,11}	Aeruginosin EI461	L-Hpla	D-Leu	L-diepiChoi-amide	-	Trypsin (>100) Thrombin (?)
<i>O. agardhii</i> ^{12,13}	Oscillarin	D-Plac	D-Phe	L-Choi	Adc	Trypsin (0.56) Thrombin (0.04)
<i>Planktothrix agardhii</i> CYA126 ¹⁴	Aeruginoside 126A	D-Plac	D-Leu	Xyl-6-O-L-Choi	Adc	Trypsin (0.56) Thrombin (0.04)
<i>Planktothrix agardhii</i> CYA126 ¹⁴	Aeruginoside 126A	D-Plac	D-Leu	Xyl-6-O-L-Choi	Agm	Trypsin (0.56) Thrombin (0.04)
<i>Microcystis</i> sp. IL-323 ¹⁵	Aeruginosin KY642	<i>m,m</i> -di-Cl-L-Hpla	D-alloIle	L-Choi	Agm	Trypsin (2.1) Thrombin (?)
<i>Microcystis</i> sp. IL-323 ¹⁵	Aeruginosin KY608	<i>m</i> -Cl-L-Hpla	D-alloIle	L-Choi	Agm	Trypsin (2.8) Thrombin (?)
<i>M. aeruginosa</i> IL-337 ¹⁶	Aeruginosin GH553	L-Hpla	D-Tyr	D-diepiChoi-amide	-	Trypsin (100) Thrombin (>100)
<i>M. aeruginosa</i> IL-347 ¹⁶	Aeruginosin KT608A	L-Hpla	D-Phe	D-diepiChoi	Agm	Trypsin (1.9) Thrombin (enh)
<i>M. aeruginosa</i> IL-347 ¹⁶	Aeruginosin KT608B	D-Hpla	D-Phe	D-diepiChoi	Agm	Trypsin (1.3) Thrombin (100)
<i>M. aeruginosa</i> IL-347 ¹⁶	Aeruginosin KT650	D-Hpla	D-Phe	D-diepiChoi	Agm	Trypsin (19.9) Thrombin (>100)
<i>M. aeruginosa</i> IL-347 ¹⁶	Pseudoaeruginosin KT554	L-Hpla	D-Leu	L-Phe	Agm	Trypsin (100) Thrombin (>100)
<i>M. aeruginosa</i> PCC 7806 ¹⁷	Aeruginosin 686A	<i>m</i> -Cl-L-Hpla	D-Tyr	Choi	Argal	Trypsin (0.56) Thrombin (0.04)
<i>M. aeruginosa</i> PCC 7806 ¹⁷	Aeruginosin 686B	<i>m</i> -Cl-L-Hpla	D-Tyr	Choi	Argal	Trypsin (0.56) Thrombin (0.04)
<i>Lamellodysidea chlorea</i> ¹⁸	Dysinosin A	D-Mgs	D-Leu	5-OH-L-Choi	Adc	Trypsin (0.56) Thrombin (0.04)

<i>Lamellodysidea chlorea</i> ^{19,20}	Chlorodysinosin A	D-Mgs	(3 <i>R</i>)-Cl-D-Leu	5-OH-L-Choi	Adc	Trypsin (0.56) Thrombin (0.04)
<i>Lamellodysidea chlorea</i> ²¹	Dysinosin B	D-Mgs	Val	6-Glu-5-OH-L-Choi	Adc	Trypsin (0.56) Thrombin (0.04)
<i>Lamellodysidea chlorea</i> ²¹	Dysinosin C	D-Mgs	Val	5-OH-L-Choi	Adc	Trypsin (0.56) Thrombin (0.04)
<i>Lamellodysidea chlorea</i> ²¹	Dysinosin D	D-Mgs	Val	5-OH-L-Choi	Adc	Trypsin (0.56) Thrombin (0.04)
<i>Nodularia (sphaerocarpa) spumigena</i> AV1 ²²	Suomilide	D-Mgs	<i>allo</i> -Ile	2,6-di-HA- β -Glu-7-O-Abn	Adc	Trypsin (0.56) Thrombin (0.04)
<i>Nostoc</i> sp. IL-235 ²³	Banyaside A	Mgs	D-Leu	2-HA-3-carbamyl- α -Glu-7-O-Abn	Adc	Trypsin (0.56) Thrombin (0.04)
<i>Nostoc</i> sp. IL-235 ²³	Banyaside B	Mgs	D-Leu	6-HA- α -Glu-7-O-Abn	Adc	Trypsin (0.56) Thrombin (0.04)
<i>M. aeruginosa</i> IL-374 ²⁴	Aeruginosin DA495A	D-Hpla	D-Phe	L-6- <i>epi</i> Choi amide	-	Trypsin (>100) Thrombin (>100)
<i>M. aeruginosa</i> IL-374 ²⁴	Aeruginosin DA511	D-Hpla	D-Tyr	L-6- <i>epi</i> Choi amide	-	Trypsin (>100) Thrombin (>100)
<i>M. aeruginosa</i> IL-374 ²⁴	Aeruginosin DA642A	<i>m</i> -Cl-L-Hpla	L-Phe	L-Choi	Agm	Trypsin (30.8) Thrombin (15.5)
<i>M. aeruginosa</i> IL-374 ²⁴	Aeruginosin DA642B	<i>m</i> -Cl-D-Hpla	L-Phe	L-Choi	Agm	Trypsin (19.0) Thrombin (>100)
<i>M. aeruginosa</i> IL-374 ²⁴	Aeruginosin DA688	<i>m</i> -Cl-D-Hpla	D-Leu	L-Choi-6-sulfate	Agm	Trypsin (9.5) Thrombin (>100)
<i>M. aeruginosa</i> IL-374 ²⁴	Aeruginosin DA722	<i>m,m</i> -di-Cl-D-Hpla	D-Leu	L-Choi-6-sulfate	Agm	Trypsin (7.3) Thrombin (>100)
<i>M. aeruginosa</i> IL-374 ²⁴	Aeruginosin DA495B	<i>m</i> -Cl-D-Hpla	D-Leu	L-Choi amide	-	Trypsin (>100) Thrombin (>100)

<i>M. aeruginosa</i> IL-377 ²⁵	Aeruginosin GE642	<i>m,m</i> -di-Cl-D-Hpla	D-Leu	L-Choi	Agm	Trypsin (8.5) Thrombin (>100)
<i>M. aeruginosa</i> IL-377 ²⁵	Aeruginosin GE686	<i>m,m</i> -Br,Cl-D-Hpla	D- <i>allo</i> Ile	L-Choi	Agm	Trypsin (3.2) Thrombin (12.8)
<i>M. aeruginosa</i> IL-377 ²⁵	Aeruginosin GE766	<i>m,m</i> -Br,Cl-D-Hpla	D- <i>allo</i> Ile	L-Choi-6- <i>O</i> -sulfate	Agm	Trypsin (12.2) Thrombin (>100)
<i>M. aeruginosa</i> IL-377 ²⁵	Aeruginosin GE730	<i>m,m</i> -di-Br-D-Hpla	D- <i>allo</i> Ile	L-Choi	Agm	Trypsin (2.3) Thrombin (12.9)
<i>M. aeruginosa</i> IL-377 ²⁵	Aeruginosin GE810	<i>m,m</i> -di-Br-D-Hpla	D- <i>allo</i> Ile	L-Choi-6- <i>O</i> -sulfate	Agm	Trypsin (18.2) Thrombin (>100)
M. IN-1 ²⁶	Aeruginosin IN608	<i>m</i> -Cl-D-Hpla	D-Leu	L-Choi	Agm	Trypsin (4.3) Thrombin (?)
M. IN-1 ²⁶	Aeruginosin IN652	<i>m</i> -Br-D-Hpla	D-Leu	L-Choi	Agm	Trypsin (4.1) Thrombin (?)
<i>N. spumigena</i> ²⁷	Spumigin A	D-Hpla	D-Hty	L-(4S)-MePro	Argol	
<i>N. spumigena</i> ²⁷	Spumigin B1/B2	D-Hpla	D-Hty	L-(4S)-MePro	Arg	
<i>N. spumigena</i> ²⁷	Spumigin C	D-Hpla	D-Hty	L-Pro	Arg	
<i>N. spumigena</i> ²⁷	Spumigin D	D-Hpla	D-Hty	L-Pro	Argol	
<i>N. spumigena</i> ²⁷	Spumigin E	D-Hpla	D-Hty	L-(4S)-MePro	Argal	
<i>N. spumigena</i> ²⁷	Spumigin F	D-Hpla	D-Hty	L-Pro	Argal	
<i>N. spumigena</i> ²⁷	Spumigin G	D-Hpla	D-Hph	L-(4S)-MePro	Argal	
<i>N. spumigena</i> ²⁷	Spumigin H	D-Hpla	D-Hph	L-Pro	Argal	
<i>N. spumigena</i> ²⁸	Aeruginosin NAL1	Bu	Tyr	Choi	Argal	
<i>N. spumigena</i> ²⁸	Aeruginosin NAL2	Hex	Tyr	Choi	Argal	
<i>N. spumigena</i> ²⁸	Aeruginosin NAL3	Oct	Tyr	Choi	Argal	
<i>N. spumigena</i> ²⁸	Aeruginosin NAL4	Oct	Tyr	Choi-pentose	Argal	

<i>N. spumigena</i> ²⁸	Aeruginosin NOL1	Ac	Tyr	Choi	Argol	
<i>N. spumigena</i> ²⁸	Aeruginosin NOL2	Bu	Tyr	Choi	Argol	
<i>N. spumigena</i> ²⁸	Aeruginosin NOL3	Hex	Tyr	Choi	Argol	
<i>N. spumigena</i> ²⁸	Aeruginosin NOL4	Oct	Tyr	Choi	Argol	
<i>N. spumigena</i> ²⁸	Aeruginosin NOL5	Hex	Tyr	Choi-pentose	Argol	
<i>N. spumigena</i> ²⁸	Aeruginosin NOL6	Oct	Tyr	Choi-pentose	Argol	
<i>N. spumigena</i> ²⁸	Aeruginosin NOL7	Dec	Tyr	Choi-pentose	Argol	
<i>Microcystis</i> sp. ²⁹	Aeruginosin KB676	D-Hpla	D-Phe	L-6- <i>epi</i> -Choi	⁴ N-prenyl-agmatine	Trypsin (40.0)
<i>Microcystis</i> sp. ³⁰	Aeruginosin LH650A	D-Cl-Hpla	D-Leu	L-Choi	L-Amap	Trypsin (37.9) Thrombin (1.8)
<i>Microcystis</i> sp. ³⁰	Aeruginosin LH650B	D-Cl-Hpla	D-Leu	L-Choi	L-Amap	Trypsin (35.3) Thrombin (1.8)
<i>Microcystis</i> sp. ³⁰	Aeruginosin LH606	D-Cl-Hpla	D-Leu	L-Choi	Aap	Trypsin (18.5) Thrombin (2.5)
<i>Nodularia spumigena</i> ³¹	Pseudoaeruginosin NS1	Hexanoate	L-Tyr	L-(4S)-MePro	Argal	Trypsin (0.19)
<i>Nodularia spumigena</i> ³¹	Pseudoaeruginosin NS2	Hexanoate	L-Tyr	L-(4S)-MePro	Argol	
<i>Nostoc</i> sp. CENA543 ³²	Pseudospumigin A 613	Hpla	D-Hty	L-Ile	Argininal	Trypsin (4.5)
<i>Nostoc</i> sp. CENA543 ³²	Pseudospumigin B 613	Hpla	Hty	Leu	Argininal	

<i>Nostoc</i> sp. CENA543 ³²	Pseudospumigin C 599	Hpla	Hty	Val	Argininal	
<i>Nostoc</i> sp. CENA543 ³²	Pseudospumigin D 597	Hpla	Hph	Ile	Argininal	
<i>Nostoc</i> sp. CENA543 ³²	Pseudospumigin E 597	Hpla	Hph	Leu	Argininal	
<i>Nostoc</i> sp. CENA543 ³²	Pseudospumigin F 583	Hpla	Hph	Val	Argininal	
<i>Microcystis</i> sp. ³³	Aeruginosin TR642	D-Hpla	D- <i>allo</i> Ile	(2R,3aR,6R,7aR)-Choi	4,5-didehydroaraginal	Trypsin (3.8) Thrombin (0.85)
<i>M. sp.</i> ³⁴	Aeruginosin BH462A	L-Hpla	D- <i>allo</i> Ile	L-Choi	-	Tryp -
<i>M. sp.</i> ³⁴	Aeruginosin BH462B	L-Hpla	D-Leu	L-Choi	-	Tryp -
<i>M. sp.</i> ³⁴	Aeruginosin BH604	L-Hpla	L- <i>allo</i> Ile	L-Choi	L-Argol	Tryp -
<i>Nostoc</i> sp. UHCC 0870 ³⁵	Varlaxin 1046A	Mgs	D-Ile	L-Choi-a-D-Glu(Hpaa) ₂	Aaep	Tryp (0.62-3.6 nM)
<i>Nostoc</i> sp. UHCC 0870 ³⁵	Varlaxin 1022A	Mgs	D-Ile	L-Choi-a-D-Glu(Hpaa) ₂	Agm	Tryp (97-230 nM)
<i>Nostoc</i> spp. ³⁵	+22 variants by LCMSMS					
<i>Nodularia spumigena</i> ³⁶	9 spumigins + 4 aeruginosins by LCMSMS			Pro/MePro/Choi		
<i>Bloom material</i> ³⁷	11 variants by LCMSMS	Hpla/Pla		5-OH-Choi/Choi-glyco-FA(4-8)	Agm	

^aOrder of the residues from the N-terminus to the C-terminus. Hpla – *p*-hydroxyphenyllactic acid; *m*-Cl-Hpla – *m*-chloro-*p*-hydroxyphenyllactic; *m,m*-di-Cl-L-Hpla – *m,m*-dichloro-*p*-hydroxyphenyllactic; *m*-Br-D-Hpla – *m*-bromo-*p*-hydroxyphenyllactic; Plac – phenyllactic acid; L-Choi – (2S,3aS,6R,7aS)-2-carboxy-6-hydroxyoctahydroindole; L-*diepi*Choi – (2S,3aR,6R,7aR)-2-carboxy-6-hydroxyoctahydroindole; D-*diepi*Choi –

(2R,3aR,6R,7aR)-2-carboxy-6-hydroxyoctahydroindole; Agm – agmatine; Argal – dihydroarginine; Argol – tetrahydroarginine; Adc – 1-(N-amidino- Δ^3 -pyrrolino)ethyl.

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Figure S1. ^1H NMR Spectrum of Aeruginosin KT688 (**1**) in $\text{DMSO}-d_6$

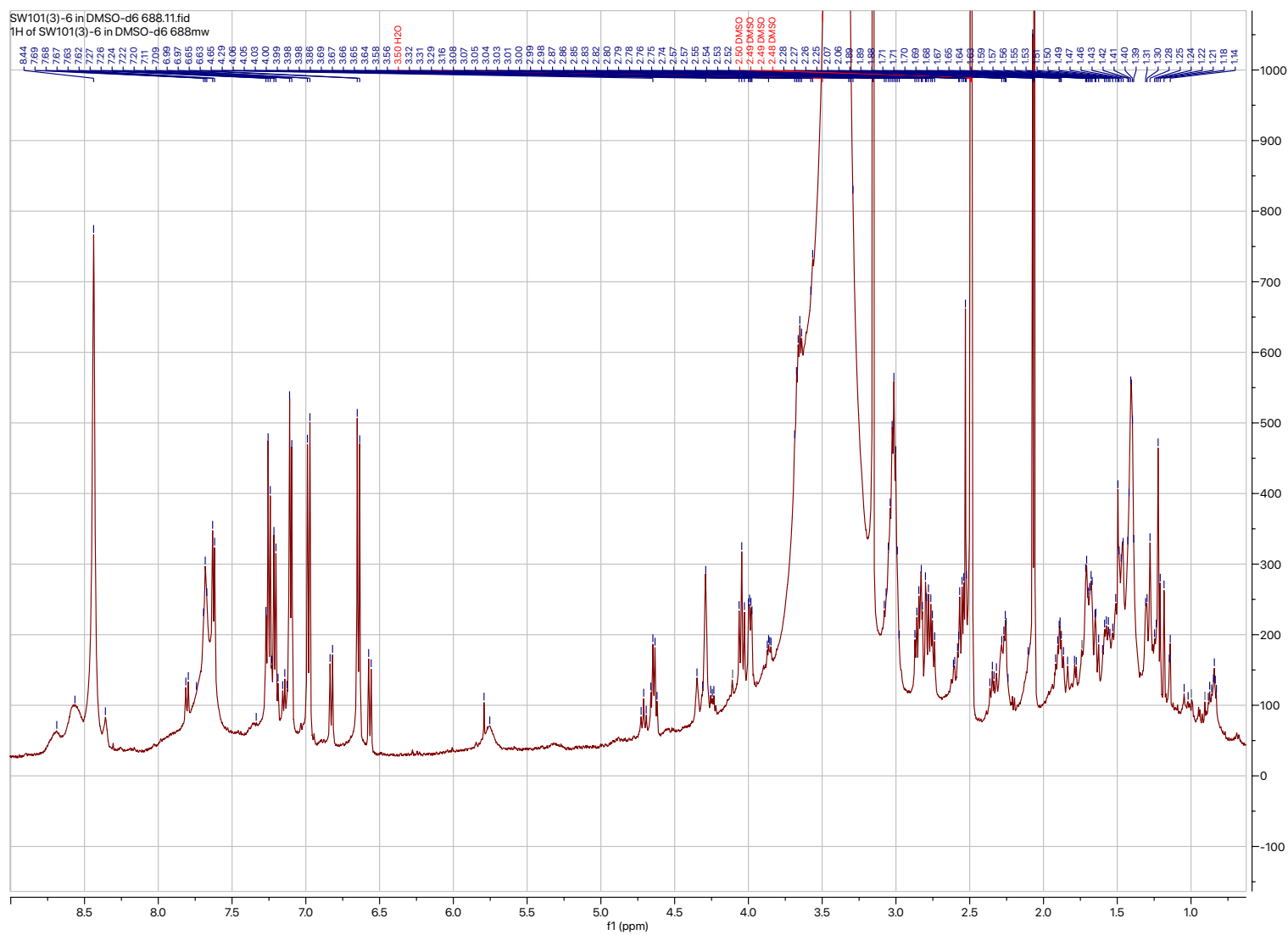


Figure S2. ^{13}C NMR Spectrum of Aeruginosin KT688 (**1**) in $\text{DMSO}-d_6$

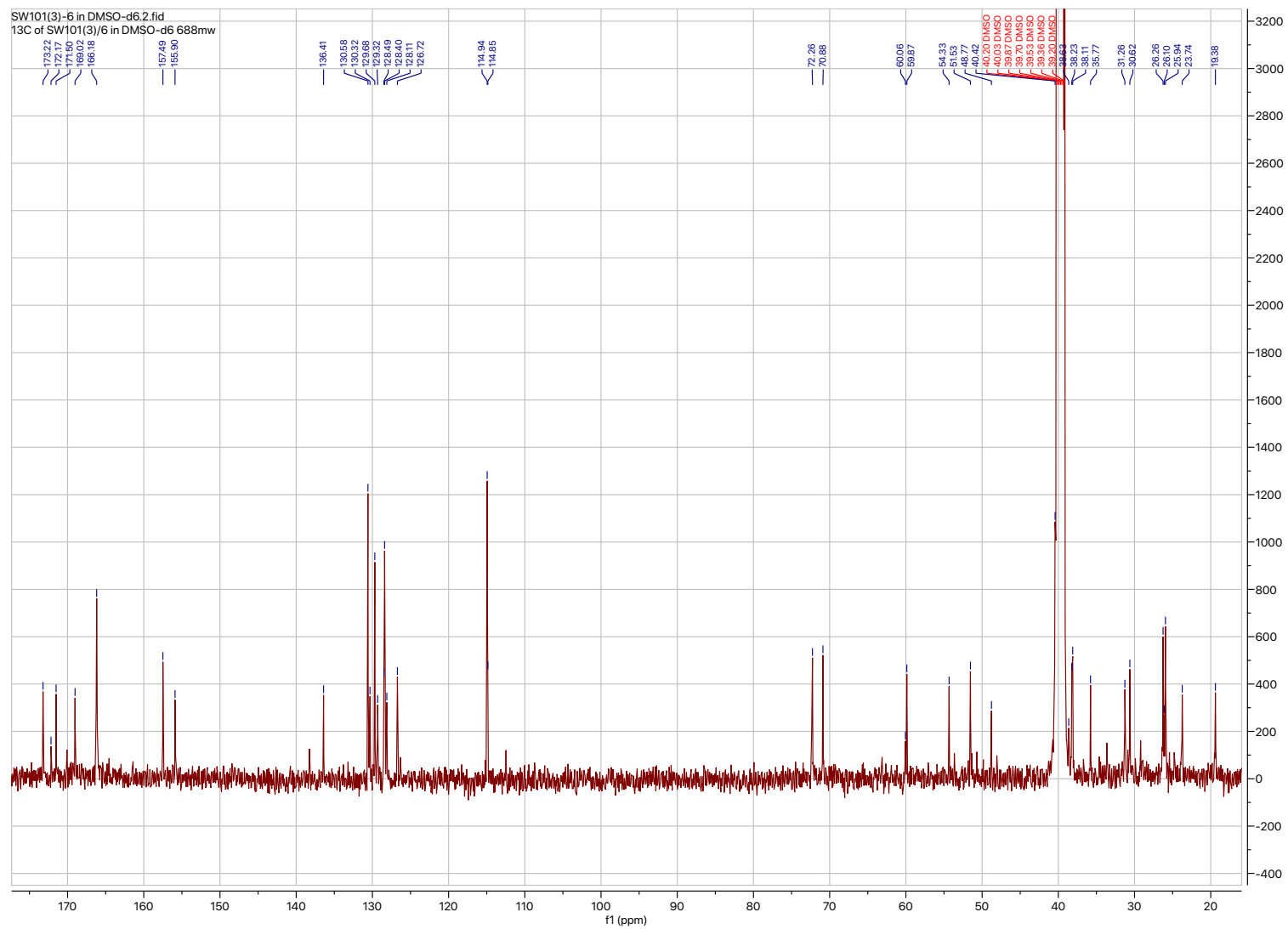


Figure S3. HSQC Spectrum of Aeruginosin KT688 (**1**) in DMSO- d_6

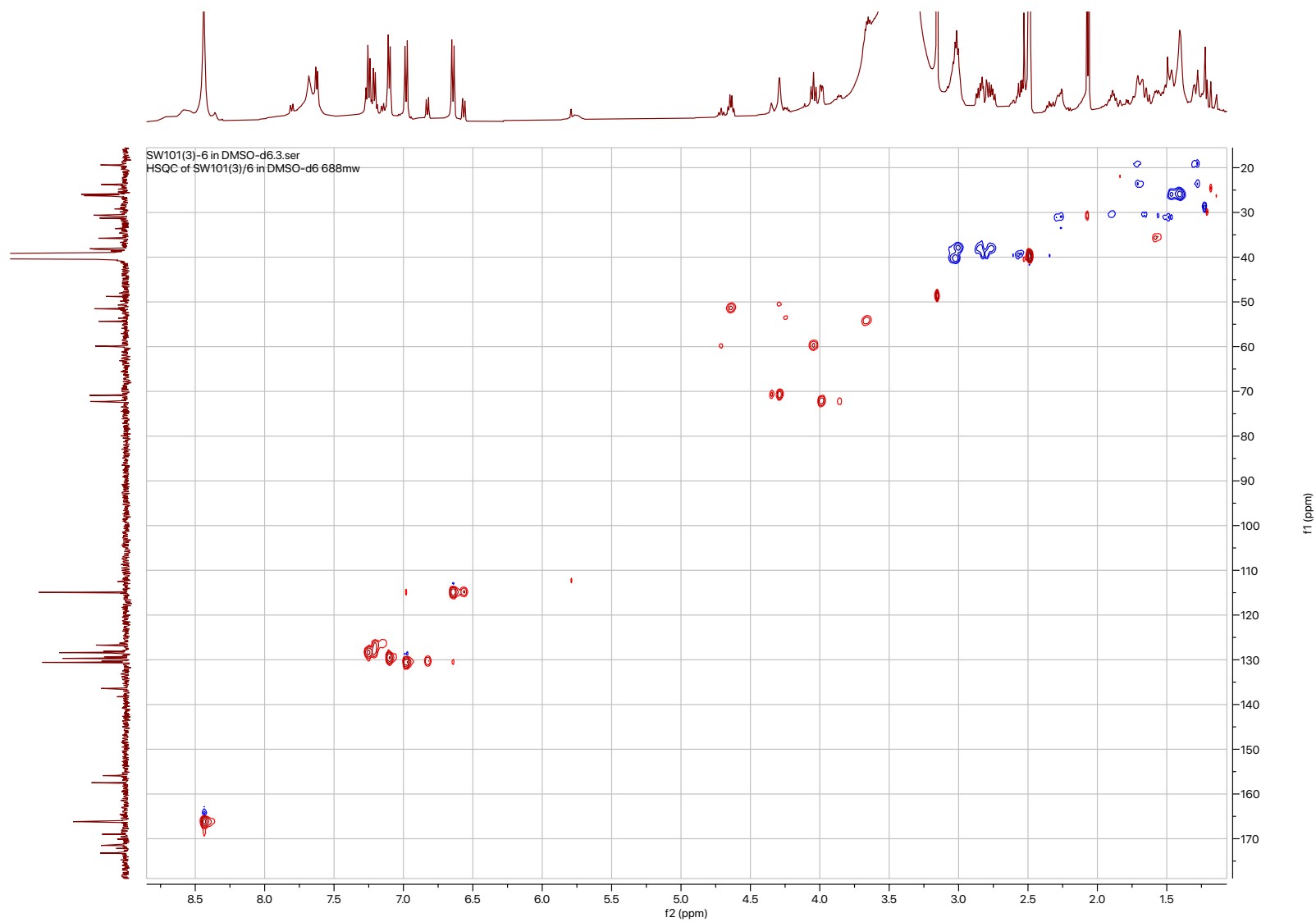


Figure S4. HMBC Spectrum of Aeruginosin KT688 (**1**) in DMSO-*d*₆

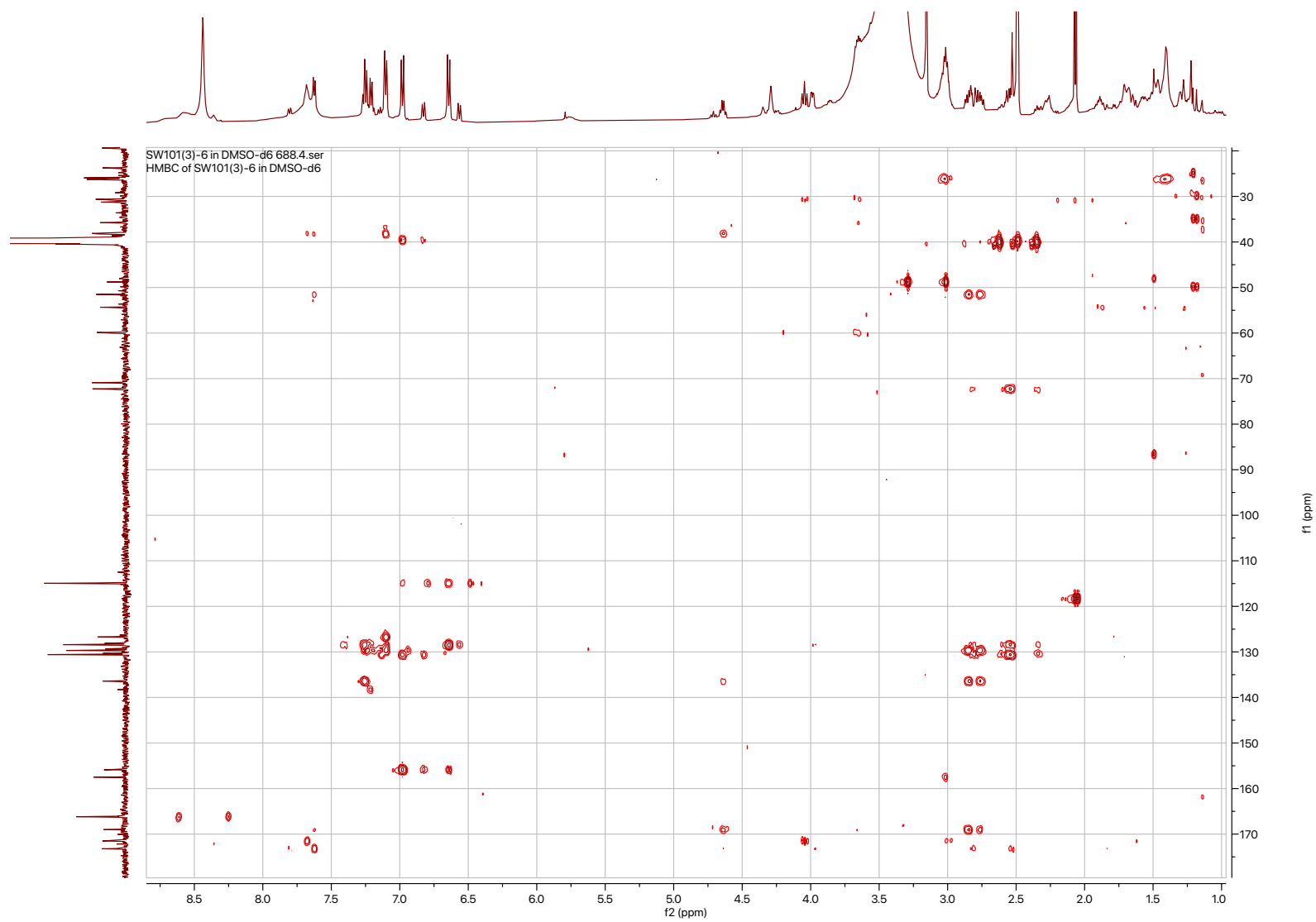


Figure S5. COSY Spectrum of Aeruginosin KT688 (**1**) in DMSO- d_6

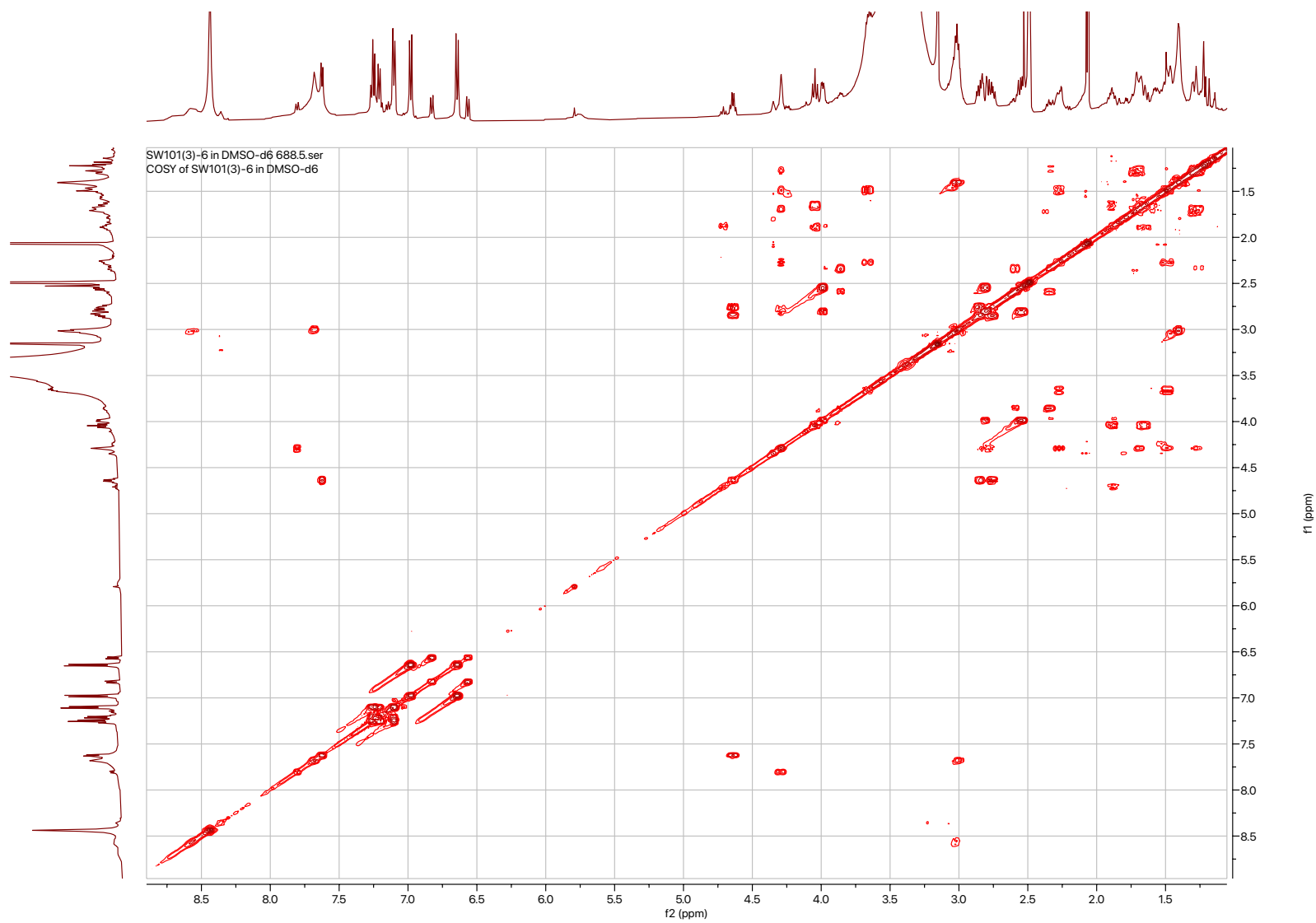


Figure S6. TOCSY Spectrum of Aeruginosin KT688 (**1**) in DMSO- d_6

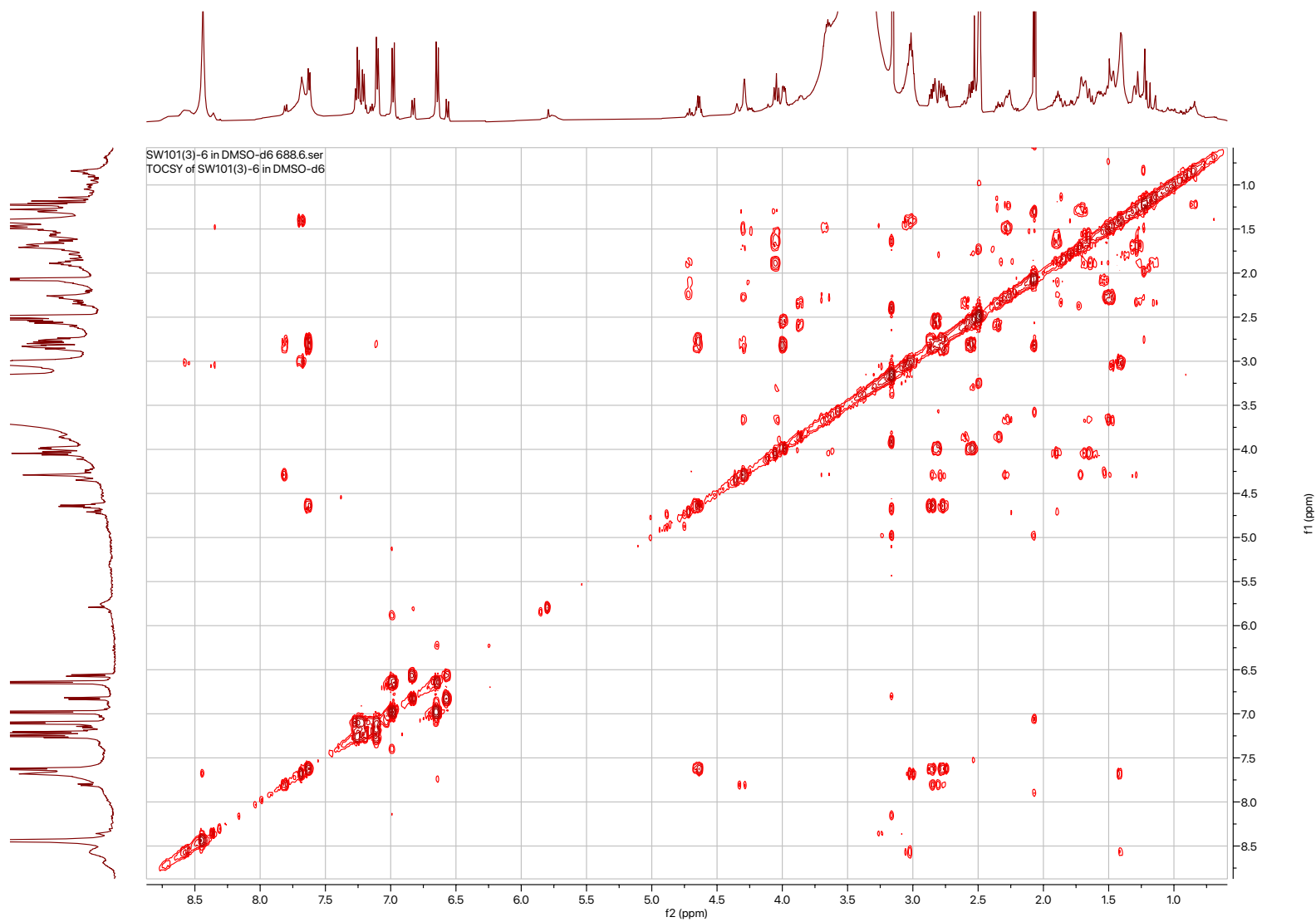


Figure S7. ROESY Spectrum of Aeruginosin KT688 (**1**) in DMSO- d_6

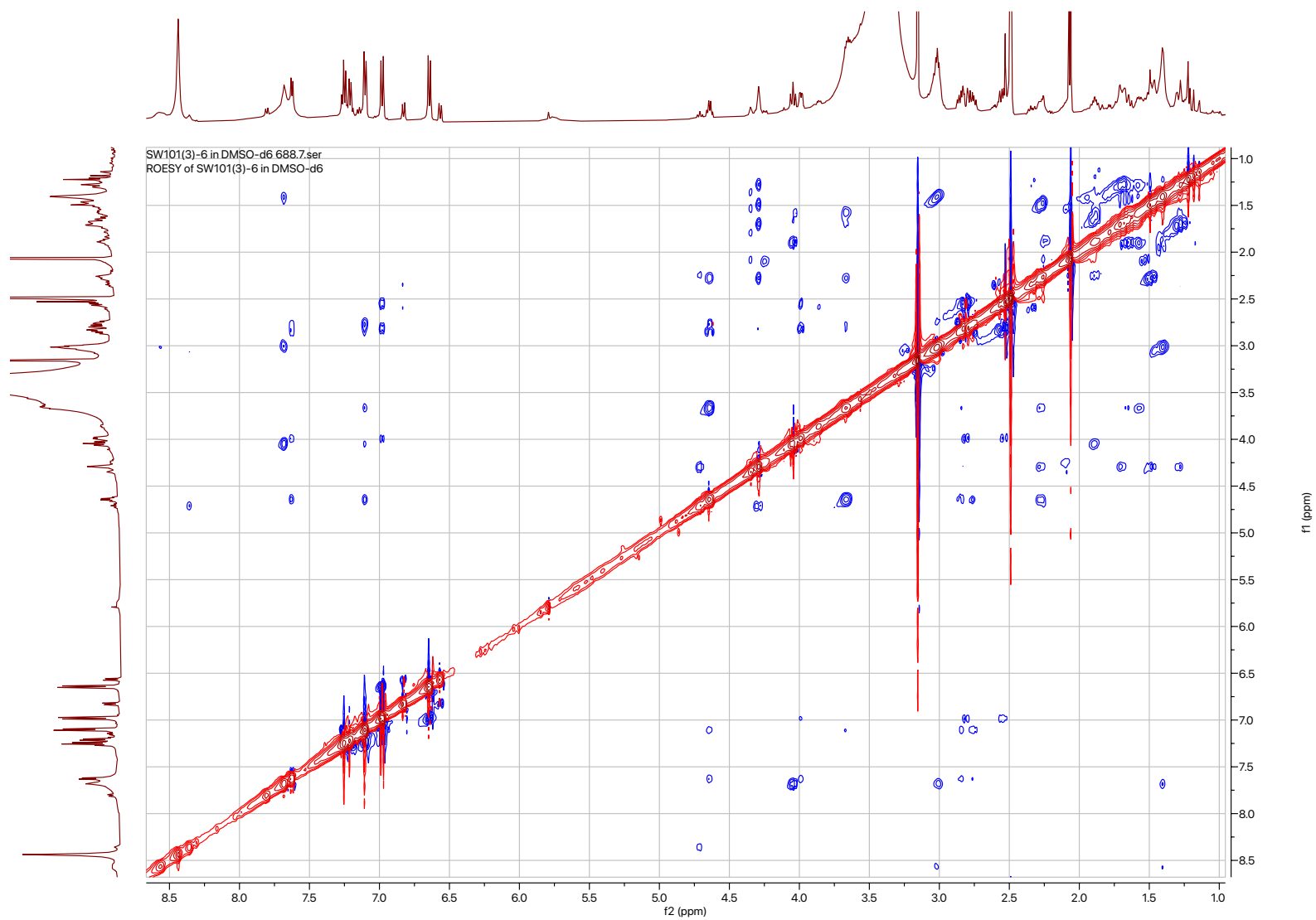


Table S2. NMR Data of the Minor *cis* Rotamer of Aeruginosin KT688 (1) in DMSO-*d*₆^a

Position	δ_C mult.	δ_H mult. (<i>J</i> in Hz)	HMBC Correlation	COSY Correlation	TOCSY Correlation	ROESY Correlation
Hpla 1	173.1, C		Phe-2-NH, Hpla-3a			
2	72.4, CH	3.86, dd (7.8, 4.2)	Hpla-3a,3b	Hpla-3a,3b	Hpla-3a,3b	Phe-2-NH, Hpla-3a,3b,5,5'
2-OH		5.75, brm				
3a	39.7, CH ₂	2.59, m	Hpla-5,5'	Hpla-2,3b	Hpla-2,3b	Hpla-2,5,5' Hpla-5,5'
3b		2.34, m		Hpla-2,3a	Hpla-2,3a	
4	128.4, C		Hpla-3a,3b,6,6'			
5,5'	130.3, CH x2	6.83, d (8.2)	Hpla-3a,3b,6,6'	Hpla-6,6'	Hpla-6,6'	Hpla-2,3a,3b
6,6'	114.8, CH x2	6.56, d (8.2)	Hpla-6',6	Hpla-5,5'	Hpla-5,5'	Phe-5,5'
7	155.8, C		Hpla-5,5',6,6'			
Phe 1	170.1, C		Phe-2-NH			
2	50.7, CH	4.29, m		Phe-2-NH, 3a,3b	Phe-2-NH, 3a,3b	Phe-2-NH, 3a,3b,5,5', ChoiSul-2
2-NH		7.81, d (8.5)		Phe-2,	Phe-2,3a,3b,	Phe-2, Hpla-2
3a	36.7, CH ₂	2.83, m	Phe-2		Phe-2-NH,	Phe-2
3b		2.78, m	Phe-2		Phe-2-NH,	Phe-2
4	138.3, C		Phe-2-NH,3a,3b			
5,5'	129.3, CH x2	7.10, d (7.3)	Phe-3a,3b,5',5	Phe-6,6'	Phe-6,6'	Phe-2
6,6'	128.1, CH x2	7.23, m	Phe-6,6,	Phe-5,5'	Phe-5,5'	ChoiSul-2
7	126.3, CH	7.14, t (7.3)	Phe-5,5'	Phe-6,6'	Phe-6,6'	
ChoiSul 1	172.2, C		Agm-1-NH			
2	60.1, CH	4.71, t (8.9)		ChoiSul-3pe,3pa	ChoiSul-3pe,3pa,7a	Phe-2,6,6', ChoiSul-3pe,7a

3pe ^b	33.6, CH ₂	2.25, m		ChoiSul-2,3pa		ChoiSul-2,3pe,3pa, Agm-2 ChoiSul-3a
3pa ^b		1.88, m		ChoiSul-2,3pe		
3a	34.5, CH	2.12, m				
4a	19.5, CH ₂	1.95, m		ChoiSul-5a,5b, ChoiSul-4a,5a		
4b		1.41, m		ChoiSul-5b,6	ChoiSul-5b	ChoiSul-5a,6,
5a	23.9, CH ₂	1.80, m		ChoiSul-5a,6	ChoiSul-5a	
5b		1.37, m		ChoiSul-5a,5b, 7pa,7pe	ChoiSul-7pa	ChoiSul-5a,5b,7pe,7pa
6	70.9, CH	4.34, brs		ChoiSul-5a,7a	ChoiSul-5a,7pa	ChoiSul-7pa,7a
7pe	34.6, CH ₂	2.10, m			ChoiSul-7pa	ChoiSul-7pe
7pa		1.53, m			ChoiSul-7pa,7pe	
7a	53.7, CH	4.25, dt (11.0, 6.5)				
Agm-1-NH		8.36, t (5.2)		Agm-1a,1b	Agm-1a,1b,2	Agm-1a,1b,2
1a	38.4, CH ₂	3.24, m		Agm-1b,2	Agm-1-NH,1b,2 Agm-1-NH,1a,2	Agm-1-NH,1b,2
1b		3.06, m		Agm-1a,2		Agm-1-NH,1a,2
2	26.1, CH ₂	1.47, m	Agm-1a,3		Agm-1-NH	Agm-1-NH
3	26.4, CH ₂	1.41, m	Agm-2			
4	40.4 CH ₂	3.02		Agm-4-NH	Agm-4-NH	
4-NH		8.72, brs		Agm-4	Agm-4	
5	157.6, C					
5-NH,NH ₂		8.69, brs 8.72, brs				

^a500 MHz for ¹H, 125 MHz for ¹³C. ^bpe: pseudo-equatorial, pa: pseudo-axial.

Figure S8. Positive HR ESI MS/MS Spectrum of Aeruginosin KT688 (1)

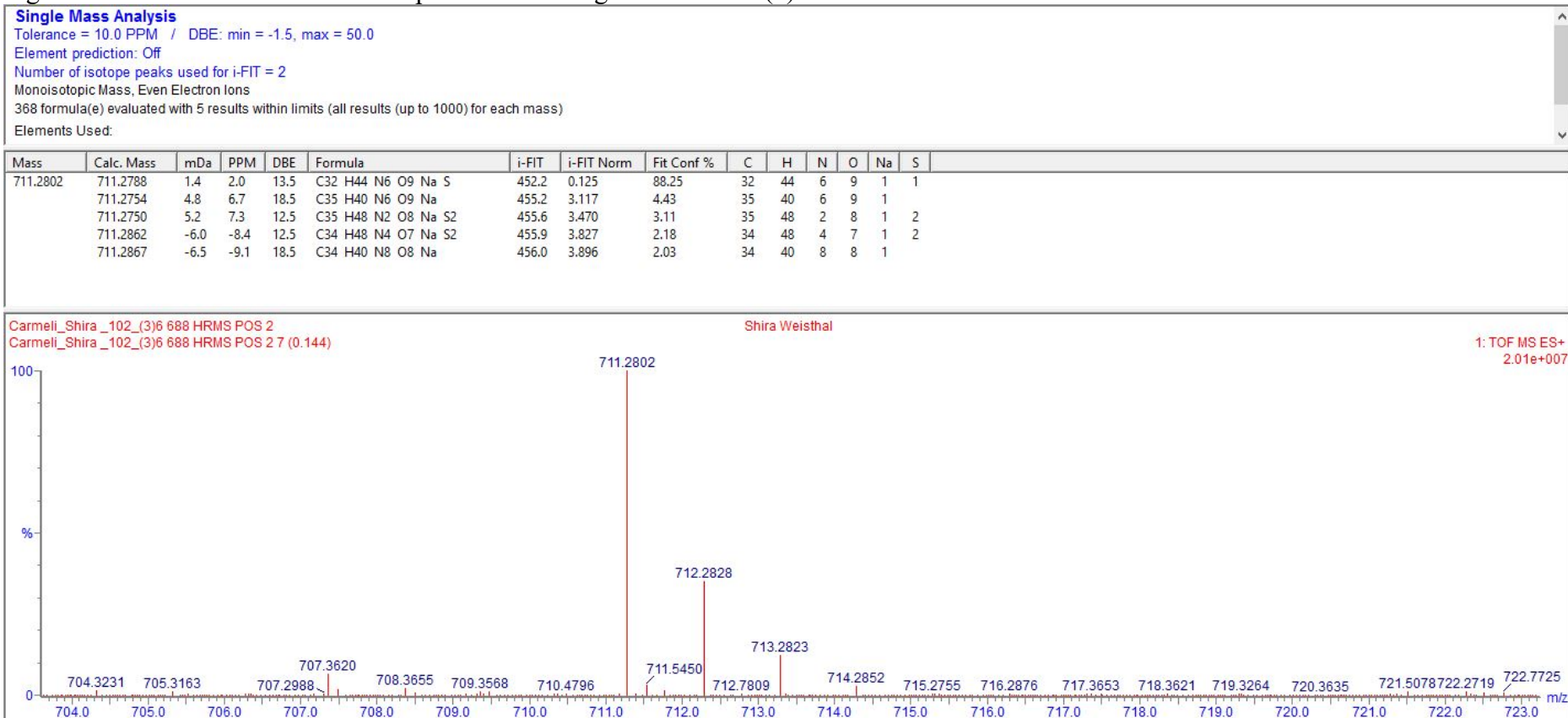


Table S3. Comparison of the chemical shifts of the Choi-6-sulfate moiety of the *trans* rotamer of Aeruginosins KT688 (**1**) and GE766.

Position	Aeruginosins KT688 (1)		Aeruginosins GE766	
	δ_C , mult.	δ_H , mult. (<i>J</i> in Hz)	δ_C , mult.	δ_H , mult. (<i>J</i> in Hz)
Choi-1	171.5, C		171.5, C	
Choi-2	59.9, CH	4.05, t (9.1)	59.9, CH	4.16, t (9.2)
Choi-3pe ^a	30.6, CH ₂	1.89, m*	30.6, CH ₂	2.02, m
Choi-3pa ^a		1.66, m		1.74, m
Choi-3a	35.8, CH	1.58, m*	35.9, CH	2.28, m
Choi-4a	19.4, CH ₂	1.71, m	19.3, CH ₂	1.98, m
4b		1.29, m		1.42, m
Choi-5a	23.7, CH ₂	1.70, m	23.3, CH ₂	1.85, m
5b		1.28, m		1.32, m
Choi-6-sulfate	70.9, CH	4.29, brs	70.8, CH	4.35, brs
Choi-7pe	31.3, CH ₂	2.27, m	31.6, CH ₂	2.24, m
7pa		1.49, m		1.68, m
Choi-7a	54.3, CH	3.66, dt (11.1, 5.8)	54.0, CH	4.00, dt (10.0, 4.8)

*The anisotropic effect on these protons is most probably due to the proximity of the phenyl moiety of the adjacent Phenylalanyl residue. ^a pe: pseudo-equatorial, pa: pseudo-axial.

Table S4. NMR Data of the Major *cis* Rotamer of Aeruginosins KT608A, KT608B and Minor *cis* KT688 (**1**) in DMSO-*d*₆^a

Position	Aeruginosin KT608A		Aeruginosins KT608B		Aeruginosins KT688 (1)	
	δ_{C} , mult. ^b	δ_{H} , mult., <i>J</i> (Hz)	δ_{C} , mult. ^b	δ_{H} , mult., <i>J</i> (Hz)	δ_{C} , mult. ^b	δ_{H} , mult., <i>J</i> (Hz)
Agm 1	38.6, CH ₂	3.18, m 3.06, m	38.6, CH ₂	3.07, m 3.19, m	38.4, CH ₂	3.24, m 3.06, m
2	26.2, CH ₂	1.45, m	26.2, CH ₂	1.45, m	26.1, CH ₂	1.47, m
3	26.4, CH ₂	1.41, m	26.4, CH ₂	1.41, m	26.4, CH ₂	1.41, m
4	40.6, CH ₂	3.08, m	40.5, CH ₂	3.07, m	40.4, CH ₂	3.02, m
5	156.9, C		156.9, C		157.6, C	
1-NH		8.29, t (5.5)		8.20, t (5.2)		8.36, t (5.2)
4-NH		7.52, t (5.5)		7.62, brs		8.72, brs
Choi 1	172.3, C		172.3, C		172.2, C	
2	59.6, CH	4.82, d (9.5)	59.5, CH	4.80, d (8.8)	60.1, CH	4.71, t (8.9)
3	33.6, CH ₂	2.38, m (ax) 1.70, dd (12.0,6.0)	33.8, CH ₂	2.35, m (ax) 1.70, dd (12.0,6.0)	33.6, CH ₂	2.25, m 1.88, m
3a	32.7, CH	2.23, m	32.7, CH	2.22, m	34.5, CH	2.12, m
4	22.7, CH₂	1.63, m (2H)	22.7, CH₂	1.60, m (2H)	19.5, CH₂	1.95, m 1.41, m
5	29.9, CH₂	1.56, m (eq) 1.19, m (ax)	29.9, CH₂	1.55, m (eq) 1.17, m (ax)	23.9, CH₂	1.80, m 1.37, m
6	67.0, CH	3.30, m	67.0, CH	3.30, m	70.9, CH	4.34, m
7	36.1, CH₂	2.37, m (eq) 0.83, q (12.0)	36.2, CH₂	2.35, m (eq) 0.83, q (12.0)	34.6, CH₂	2.10, m 1.53, m

7a	56.8, CH	4.04, ddd (12.0,8.0,6.5)	56.8, CH	4.05, ddd (12.0,8.2,5.6)	53.7, CH	4.25, dt (11.0,6.5)
Phe 1	170.0, C		170.0, C		170.1, C	
2	51.8, CH	4.20, m	51.4, CH	4.26, m	50.7	4.29, m
3	36.5, CH ₂	2.84, m 2.81, m	36.5, CH ₂	2.80, m 2.81, m	36.7, CH ₂	2.83, m 2.78, m
4	138.4, C		138.2, C		138.3, C	
5,5'	129.2, CH	7.12, d (7.0)	129.2, CH	7.10, d (7.0)	129.3, CH	7.10, d (7.3)
6,6'	128.2, CH	7.19, t (7.0)	128.2, CH	7.22, m	128.1, CH	7.23, m
7	126.4, CH	7.14, t (7.0)	126.4, CH	7.16, m	126.3, CH	7.14, t (7.3)
NH		7.97, d (8.0)		7.97, d (8.0)		7.81, d (8.5)
Hpla 1	173.5, C		173.4, C		173.1, C	
2	72.0, CH	3.85, dd (9.0,3.5)	72.3, CH	3.88, m	72.4, CH	3.86, dd (7.8, 4.2)
3	39.5, CH ₂	2.33, m 2.55, dd (14.0,3.5)	39.7, CH ₂	2.29, m 2.60, dd (14.0,3.6)	39.7, CH ₂	2.34, m 2.59, m
4	128.5, C		128.4, C		128.4, C	
5,5'	130.4, CH	6.82, d (8.4)	130.3, CH	6.85, d (8.4)	130.3, CH x2	6.83, d x2 (8.2)
6,6'	114.9, CH	6.57, d (8.4)	114.9, CH	6.58, d (8.4)	114.8, CH x2	6.56, d x2 (8.2)
7	155.7, C		155.7, C		155.8, C	
2-OH		5.05, brs		5.31, d 6.4		5.75, brm
7-OH		9.06, brs		9.10, brs		-

^a400 MHz for ¹H, 100 MHz for ¹³C for aeruginosins KT608A&B, 500 MHz for ¹H, 125 MHz for ¹³C for aeruginosin KT688 (1).

Figure S9. LCMS Traces of Marfey's Method Amino Acids Analysis of Aeruginosin KT688 (1)

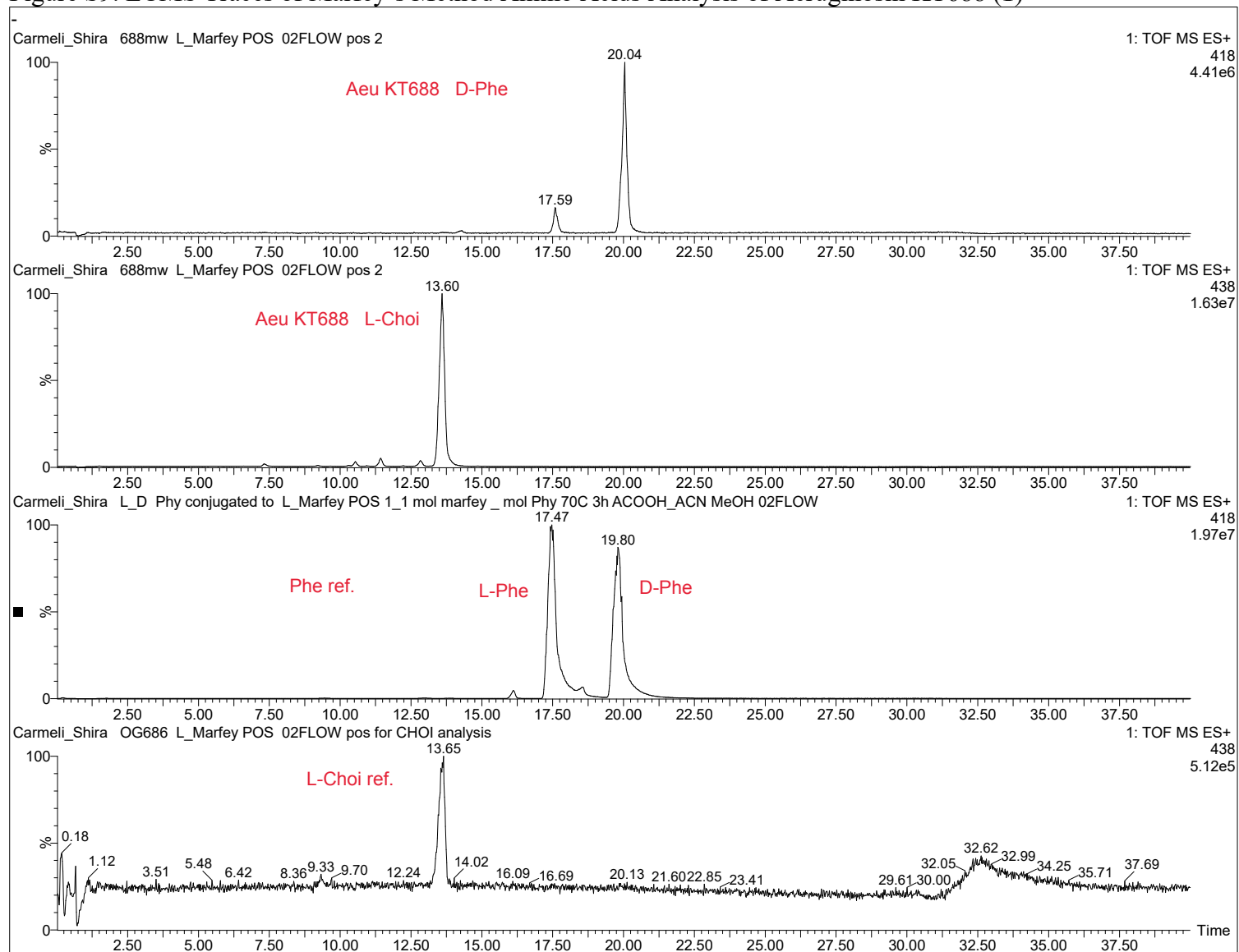


Figure S10. HPLC Traces of Chiral Chromatography of HPLA Analysis of Aeruginosin KT688 (1)

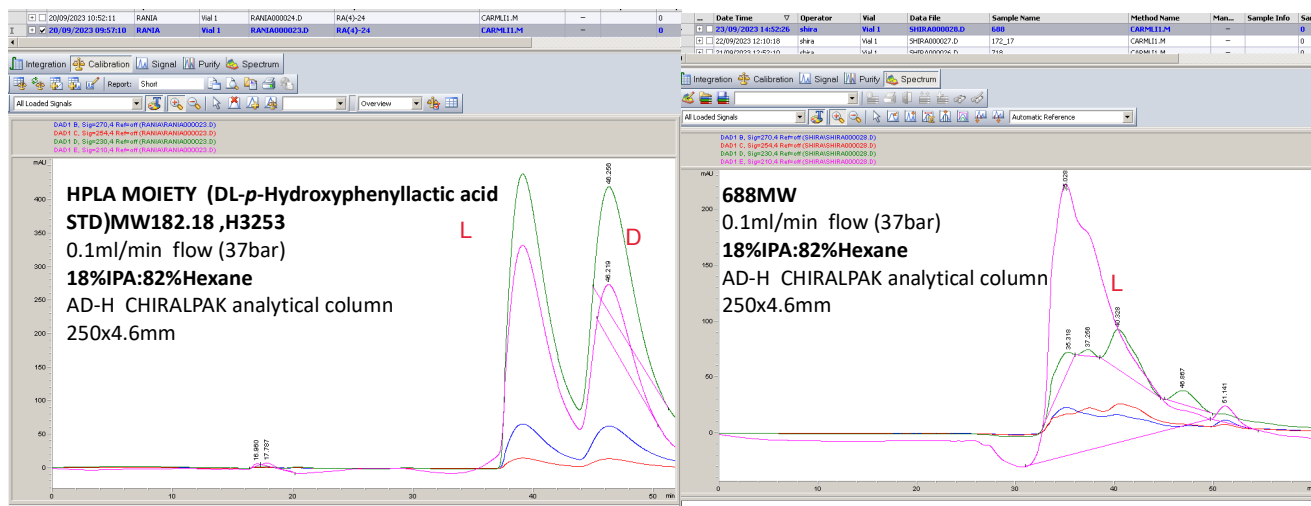


Figure S11. ^1H NMR Spectrum of Aeruginosin KT718 (**2**) in $\text{DMSO}-d_6$

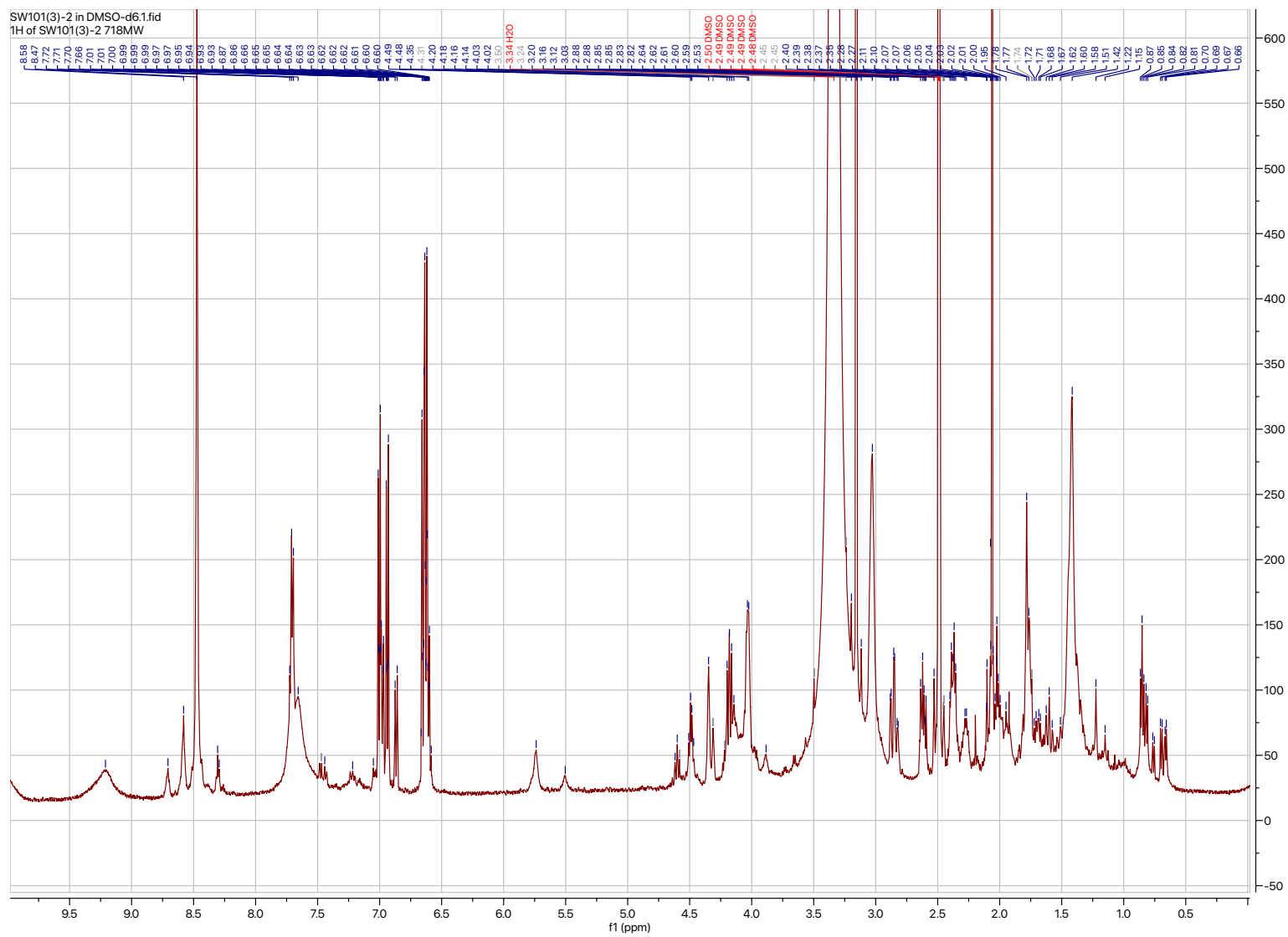


Figure S12. ^{13}C NMR Spectrum of Aeruginosin KT718 (**2**) in DMSO- d_6

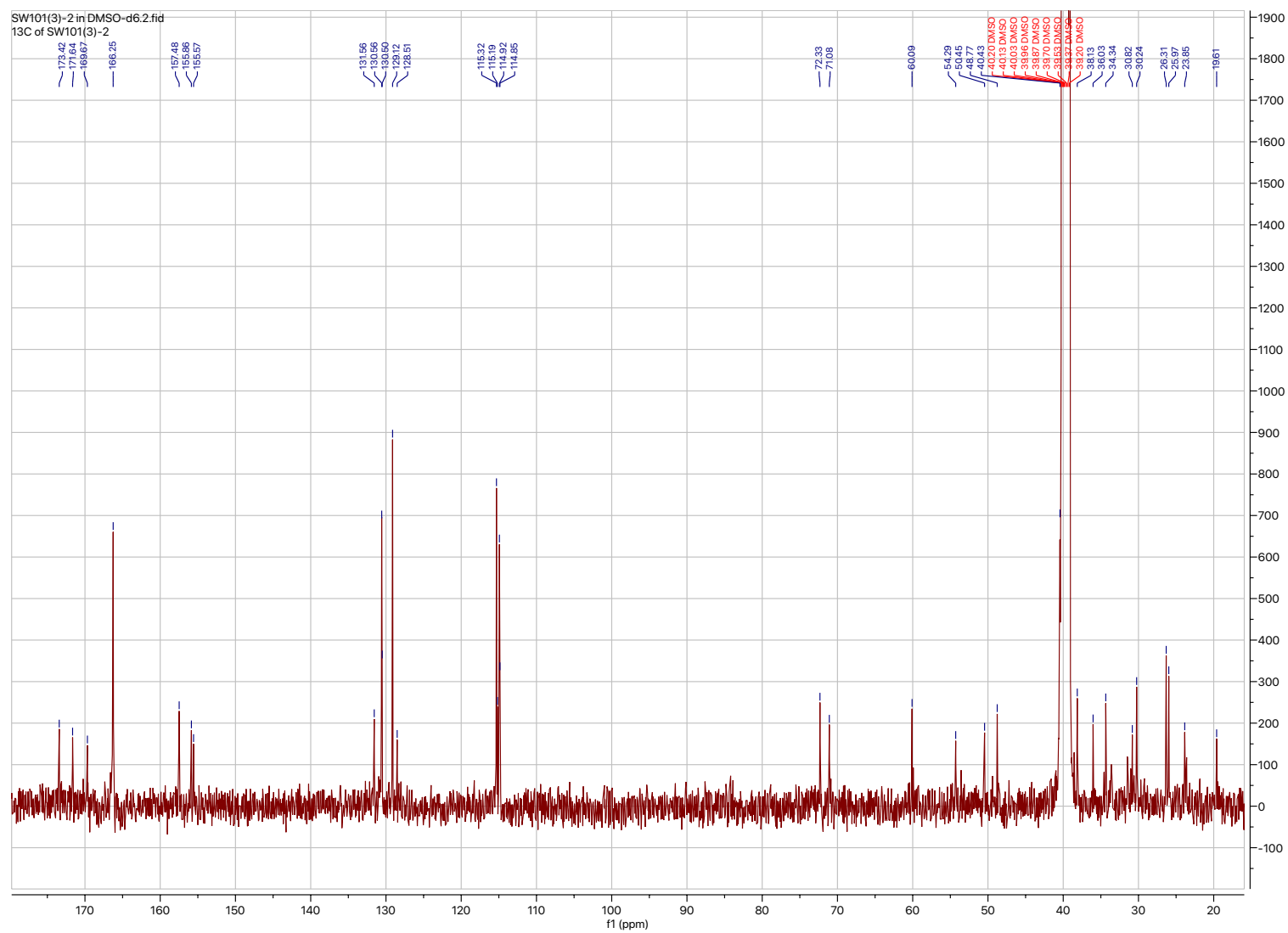


Figure S13. HSQC Spectrum of Aeruginosin KT718 (**2**) in DMSO- d_6

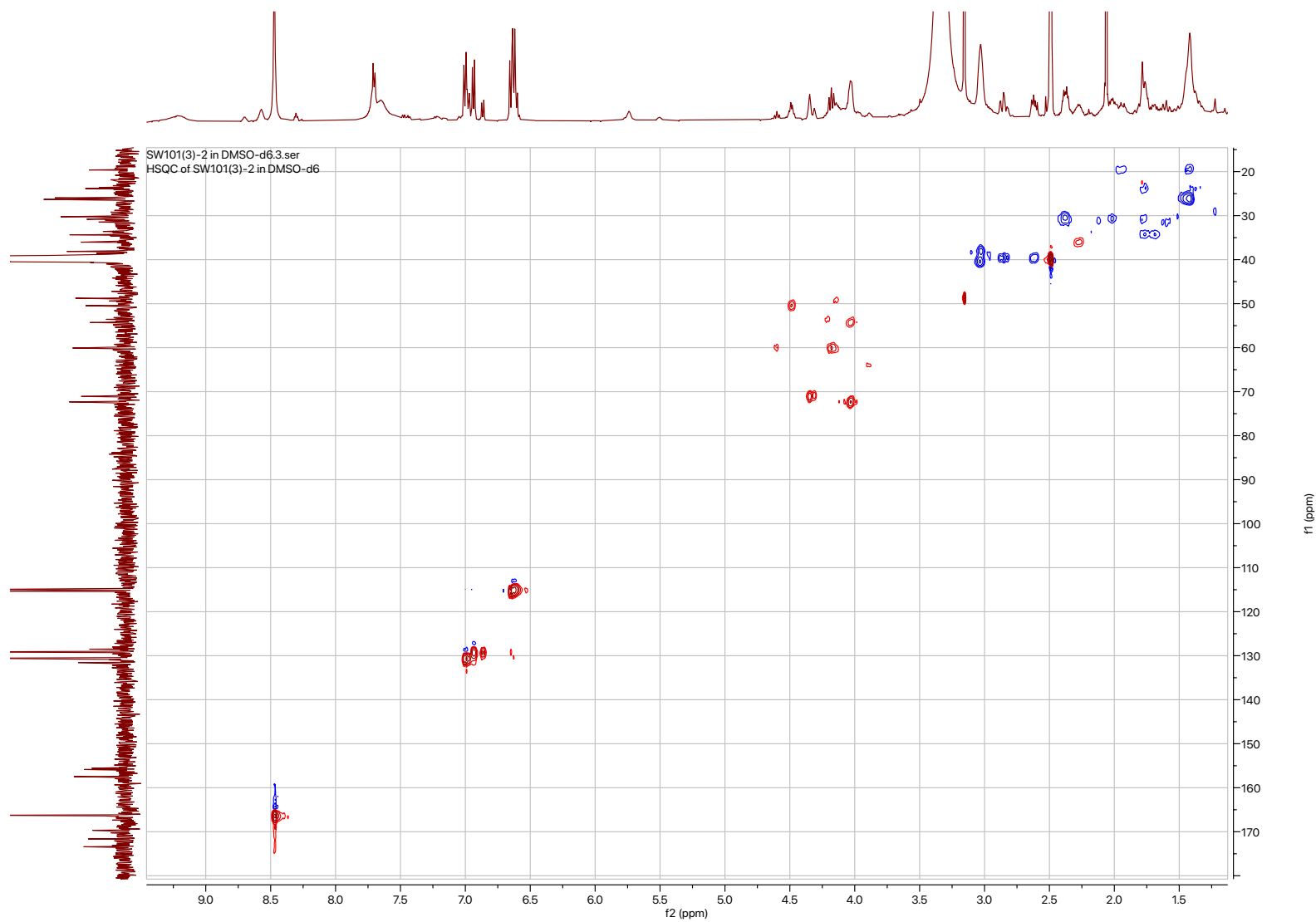


Figure S14. HMBC Spectrum of Aeruginosin KT718 (**2**) in DMSO-*d*₆

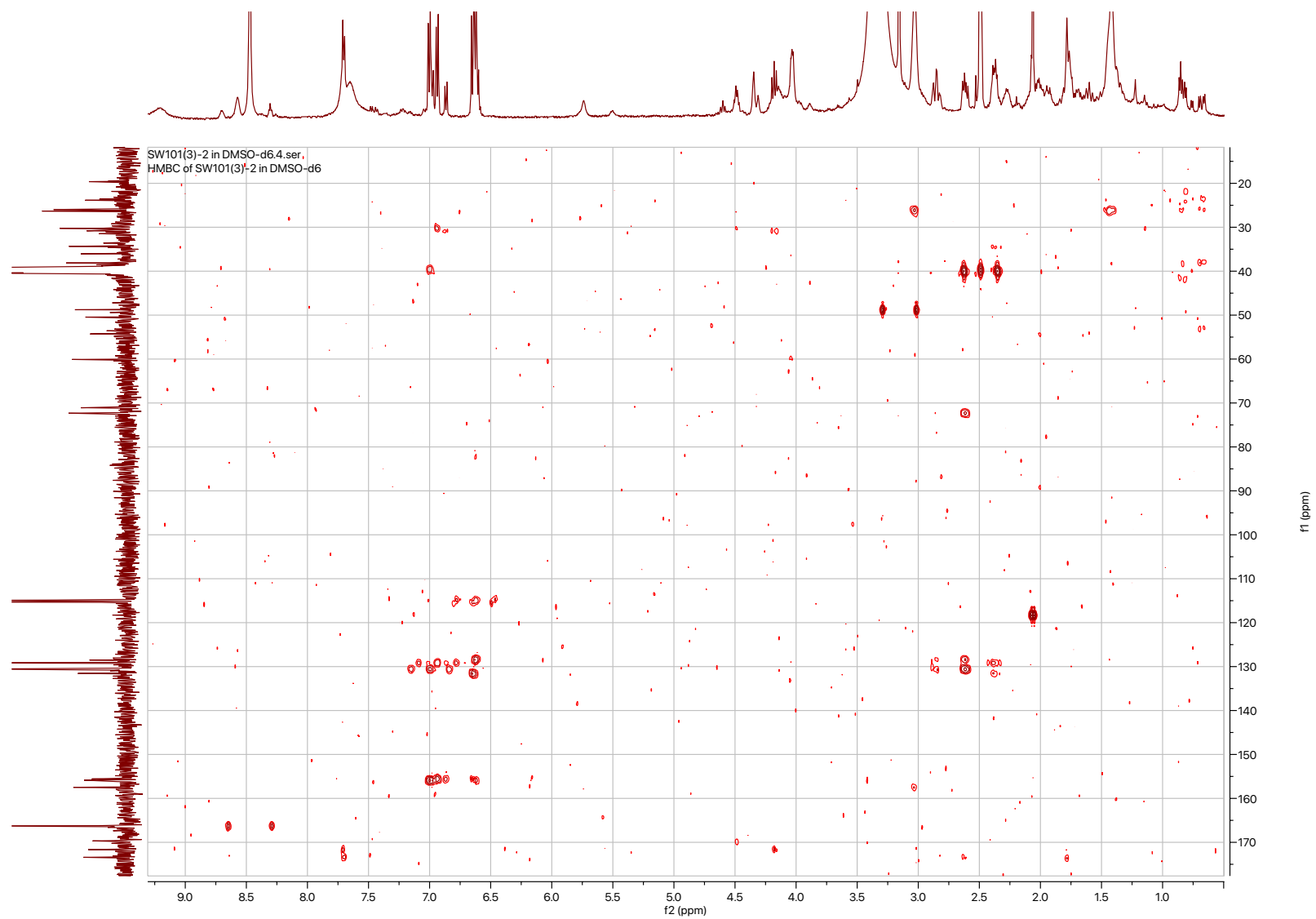


Figure S15. COSY Spectrum of Aeruginosin KT718 (**2**) in DMSO- d_6

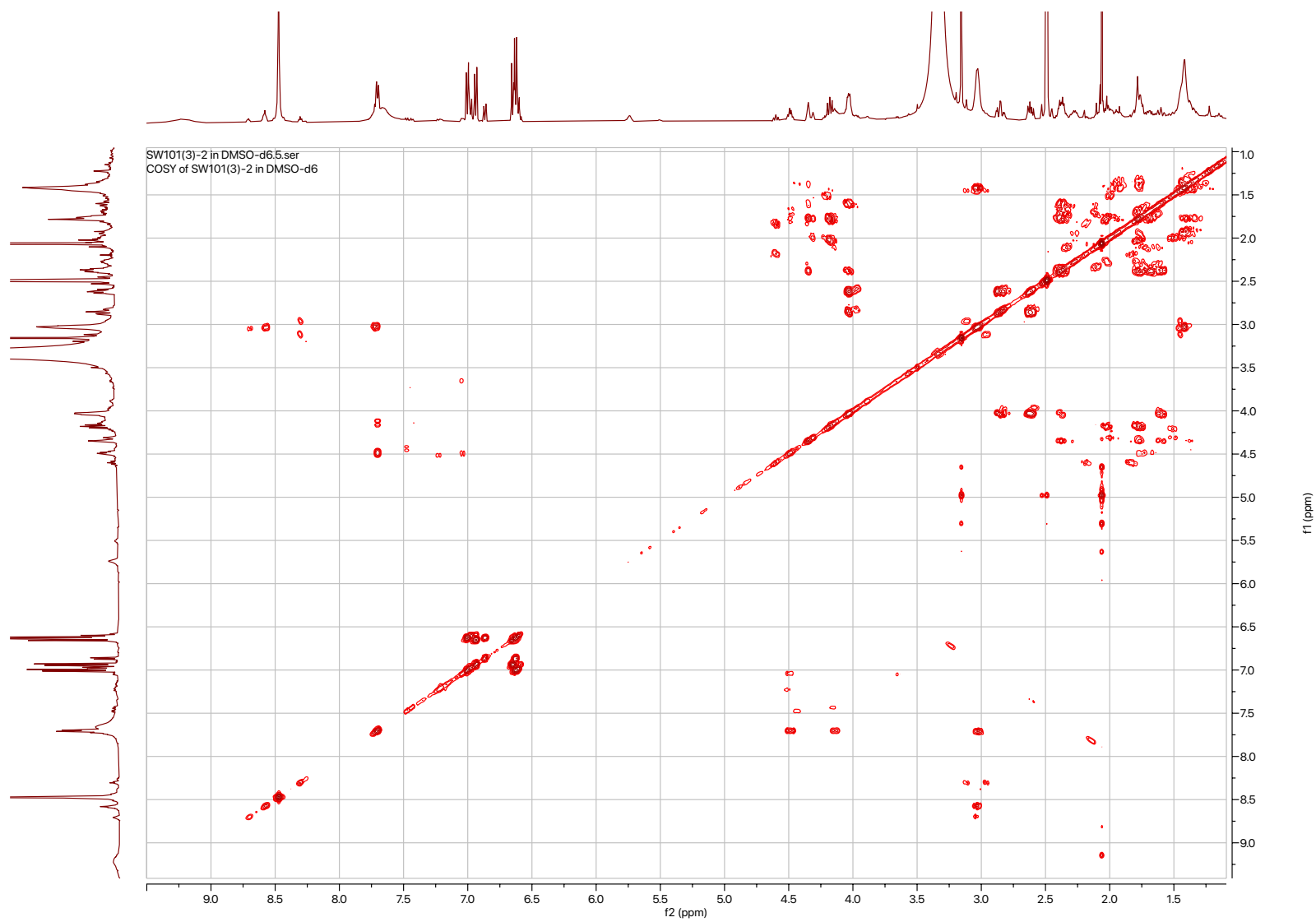


Figure S16. TOCSY Spectrum of Aeruginosin KT718 (2) in DMSO- d_6

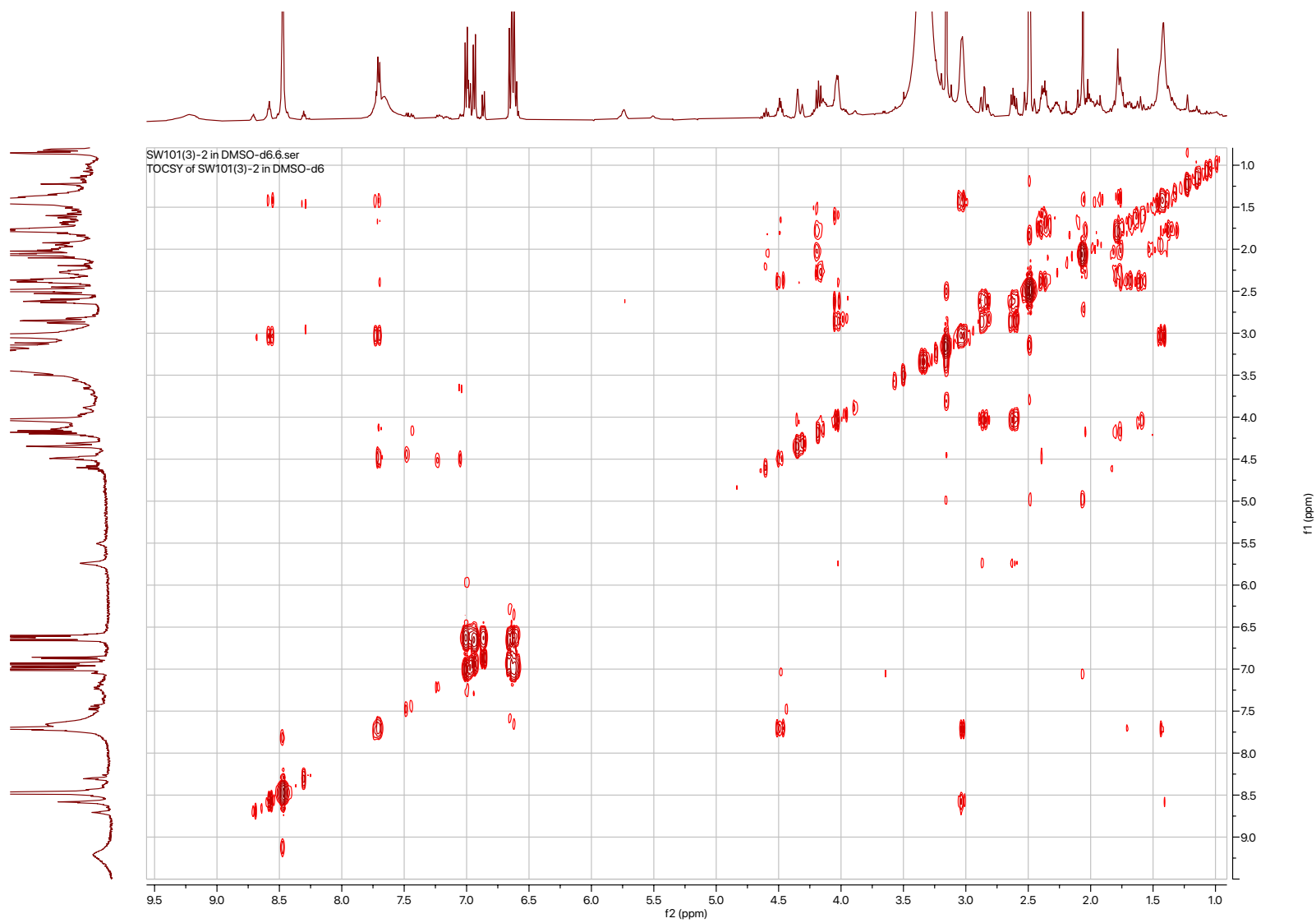


Figure S17. ROESY Spectrum of Aeruginosin KT718 (2) in DMSO- d_6

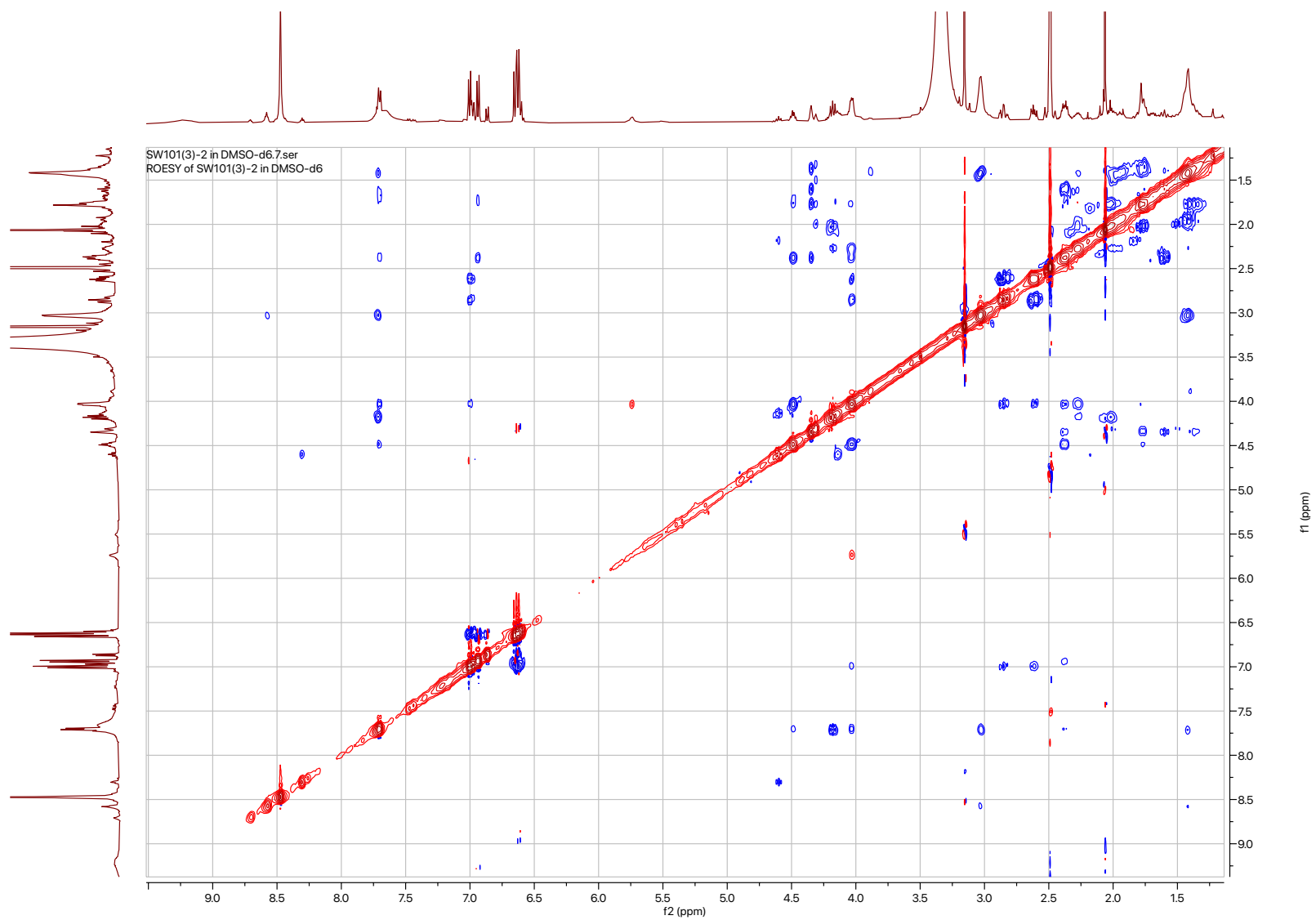


Table S5. NMR Data of Aeruginosin KT718 (**2**) minor *cis* rotamer in DMSO-*d*₆^a

Position	δ _C , mult.	δ _H , mult. (<i>J</i> in Hz)	HMBC Correlation	COSY Correlation	TOCSY Correlation	ROESY Correlation
Hpla 1	173.0, C		Hty-2-NH, Hpla-3a,3b			
2	72.3, CH	3.98, m		Hpla-3a,3b		Hpla-3a,3b
2-OH		5.50, brs			Hpla-2,3a,3b	
3a	39.3, CH ₂	2.84, dd		Hpla-2,3b Hpla-2,3a		
3b		(14.3,4.4) 2.61, dd (14.3,7.5)				
4	128.0, C		Hpla-3b			
5,5'	130.5, CH x2	6.98, d (8.6)	Hpla-3a,3b,5,5'	Hpla-6,6'		Hpla-2,3a,3b
6,6'	114.9, CH x2	6.61, d (8.6)	Hpla-6',6	Hpla-5,5'		
7	155.9, C		Hpla-5,5'			
Hty 1	169.6, C					
2	49.4, CH	4.14, m		Hty-3eq,3ax		ChoiSul-2,3eq,3ax
2-NH		7.71, d (7.7)		Hty-2	Hty-2,3ax,4	
3eq	31.0, CH ₂	2.11, m		Hty-2,3ax,4 Hty-2,3eq,4		
3ax		1.62, m				
4	30.5, CH ₂	2.34, m		Hty-3eq,3ax		
5	132.1, C		Hty-4,7,7'			
6,6'	129.1, CH x2	6.86, d (8.6)	Hty-4,6',6	Hty-7,7'		
7,7'	115.3, CH x2	6.63, d (8.6)	Hty-7',7	Hty-6,6'		
8	155.7, C		Hty-6,6'			
ChoiSul 1	171.9, C		Hty-2-NH, ChoiSul-2,3ax			

2	60.0, CH	4.60, dd (9.1,8.6)		ChoiSul-3eq	ChoiSul-3eq	Hty-2, ChoiSul-3eq, Agm-1-NH
3eq	33.6, CH ₂	2.18, m		ChoiSul-2,3ax,4 ChoiSul-2,3eq,4		Hty-2
3ax		1.82, m				
3a	34.6, CH	2.09, m		ChoiSul-3eq,3ax		
4eq	19.5, CH ₂	1.93, m		ChoiSul-4ax,5eq,5ax ChoiSul-4eq,5eq,5ax		
4ax		1.42, m				
5eq	23.6, CH ₂	1.76, m		ChoiSul-4eq,4ax,5ax,6 ChoiSul-4eq,4ax,5eq,6		
5ax		1.33, m				
6	70.8, CH	4.31, brs		ChoiSul-5eq, 5ax,7eq,7ax		ChoiSul-5ax,7eq,7ax
7eq	30.2, CH ₂	2.00, m		ChoiSul-6,7ax,7a ChoiSul-6,7eq,7a		
7ax		1.51, m				
7a	53.6 CH	4.21, m		ChoiSul-7eq,7ax		
Agm-1-NH		8.31, t (5.5)		Agm-1a,1b	Agm-3	ChoiSul-2, Agm-1b,3
1a	38.5, CH ₂	3.10, m		Agm-1-NH,1b,2 Agm-1-NH,1a,2		
1b		2.97, m				
2	26.1, CH ₂	1.42, m	Agm-3,4	Agm-4		
3	26.0, CH ₂	1.43, m	Agm-2,4			
4	40.4, CH ₂	3.03, m		Agm-3		Agm-3
4-NH		8.71, dd (5.9,4.8)		Agm-4	Agm-4	
5	157.5, C		Agm-4			
5-NH ₂ ,NH		7.65, brs				

^a500 MHz for ¹H, 125 MHz for ¹³C.

Figure S18. Positive HR ESI MS/MS Spectrum of Aeruginosin KT718 (2)

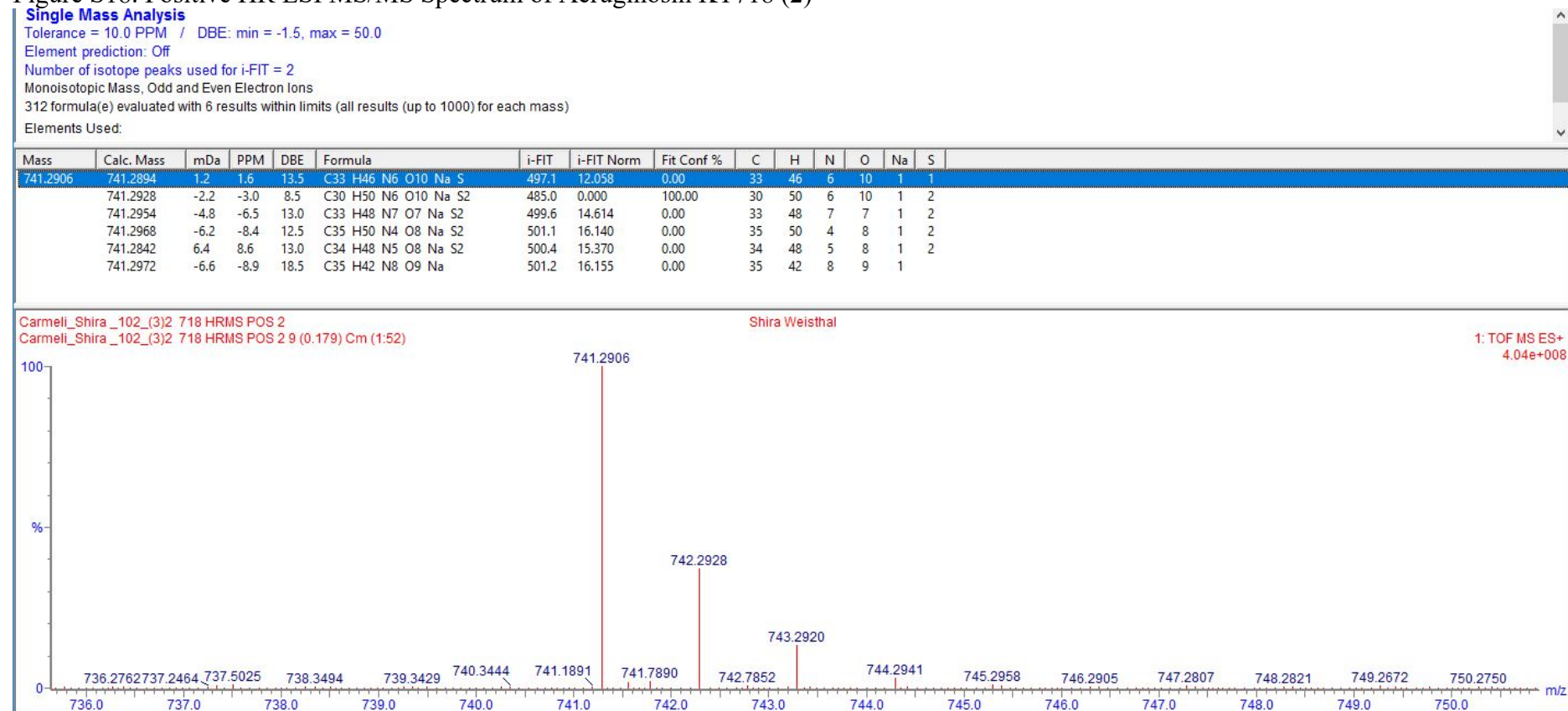


Figure S19. LCMS Traces of Marfey's Method Amino Acids Analysis of Aeruginosin KT718 (2)

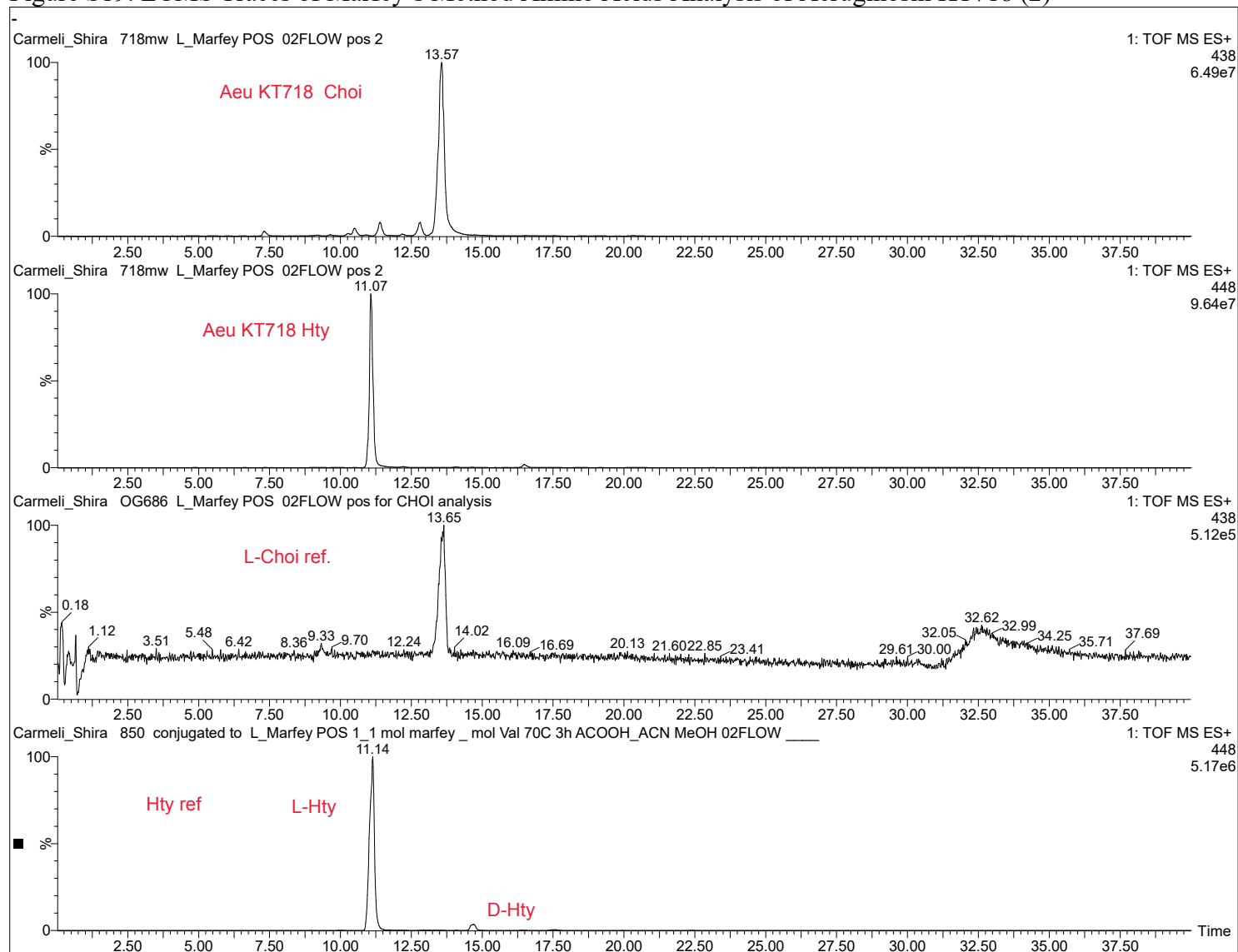


Figure S20. HPLC Traces of Chiral Chromatography of HPLA Analysis of Aeruginosin KT718 (2)

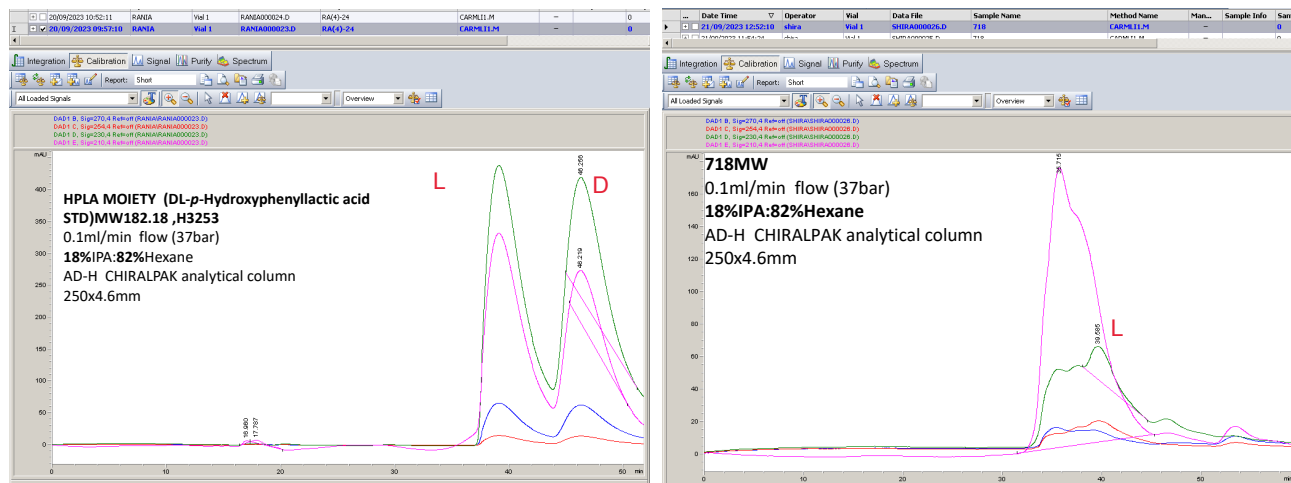


Figure S21. ^1H NMR Spectrum of Aeruginosin KT575 (**3**) in $\text{DMSO}-d_6$

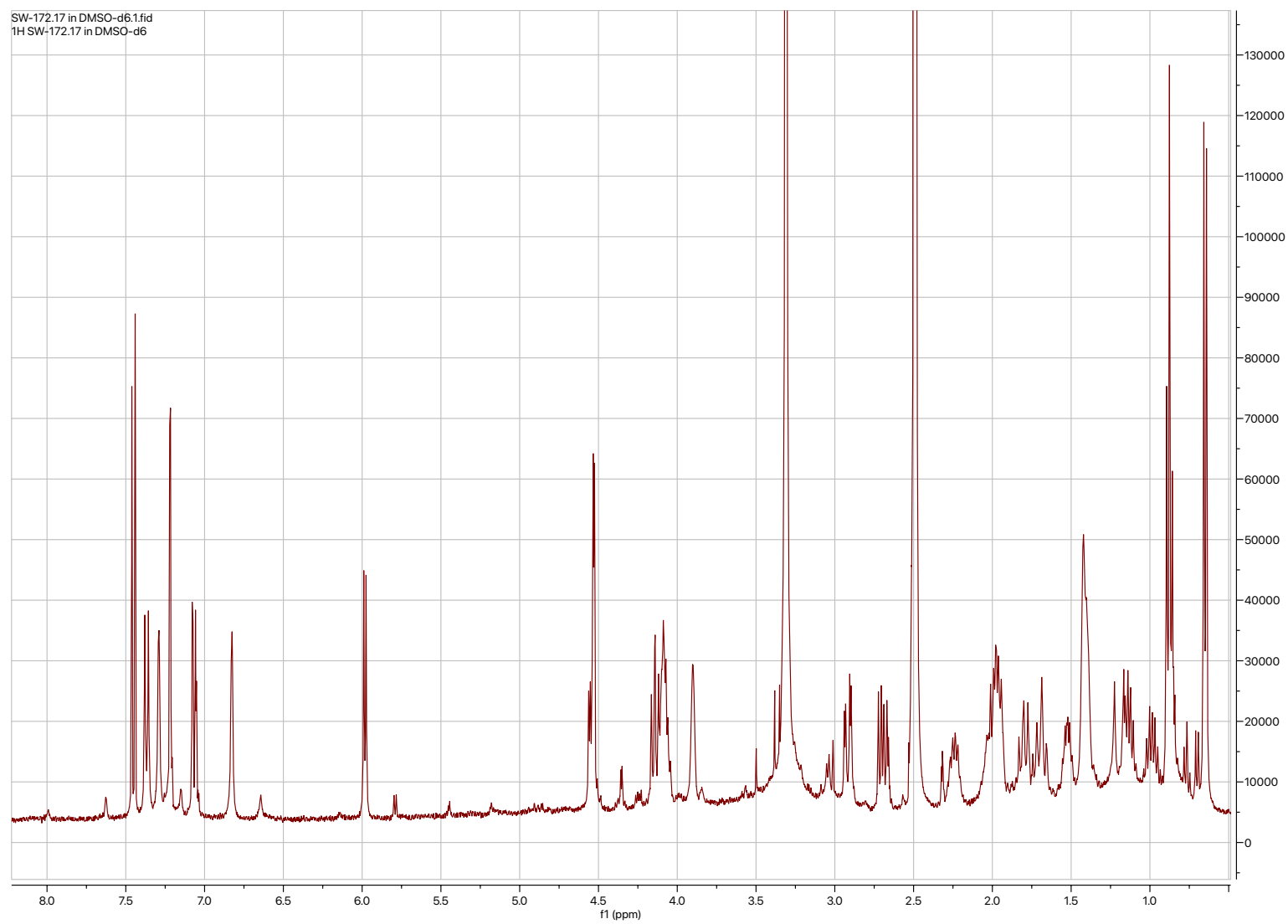


Figure S22. ^{13}C NMR Spectrum of Aeruginosin KT575 (**3**) in $\text{DMSO-}d_6$

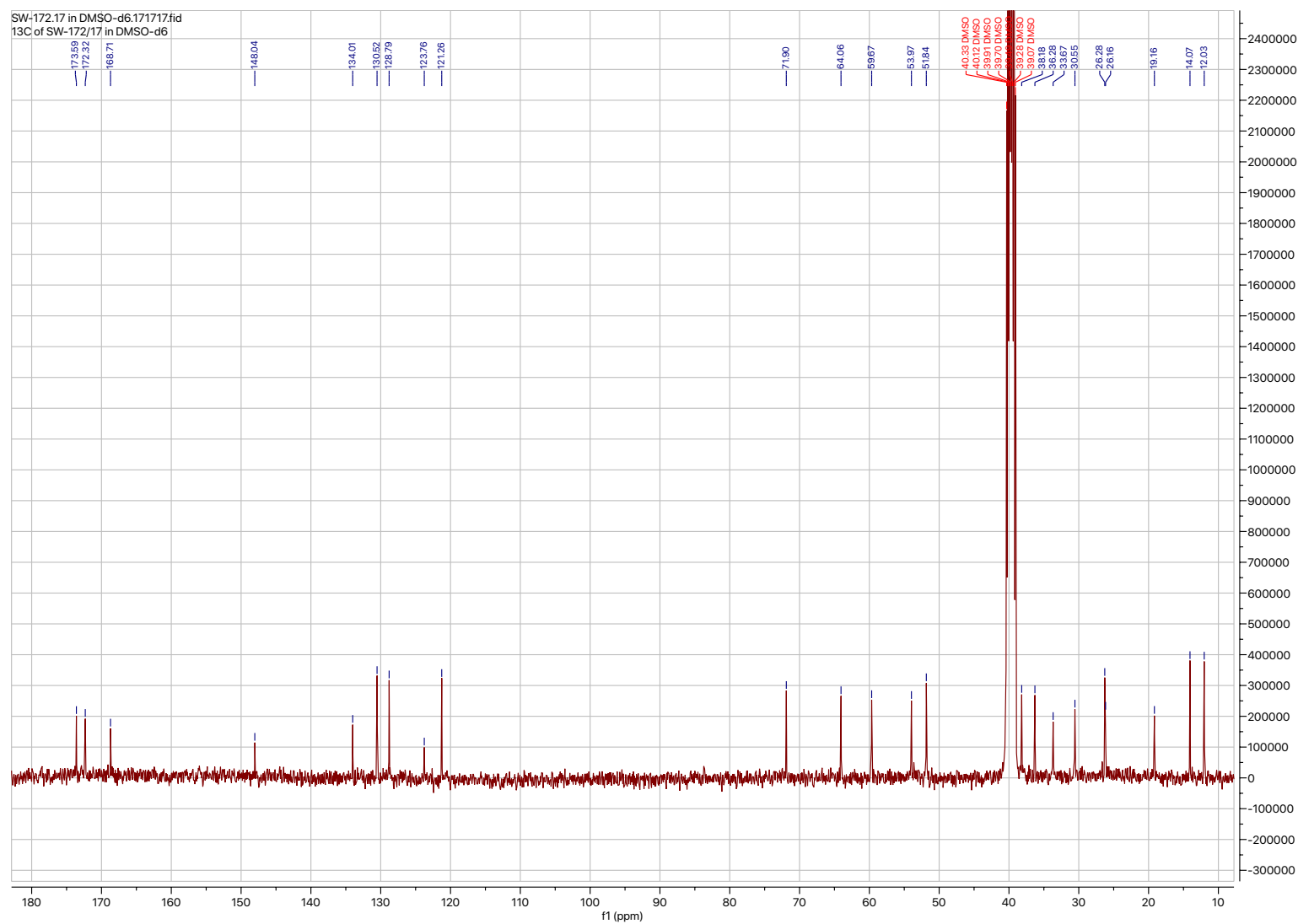


Figure S23. HSQC Spectrum of Aeruginosin KT575 (**3**) in DMSO- d_6

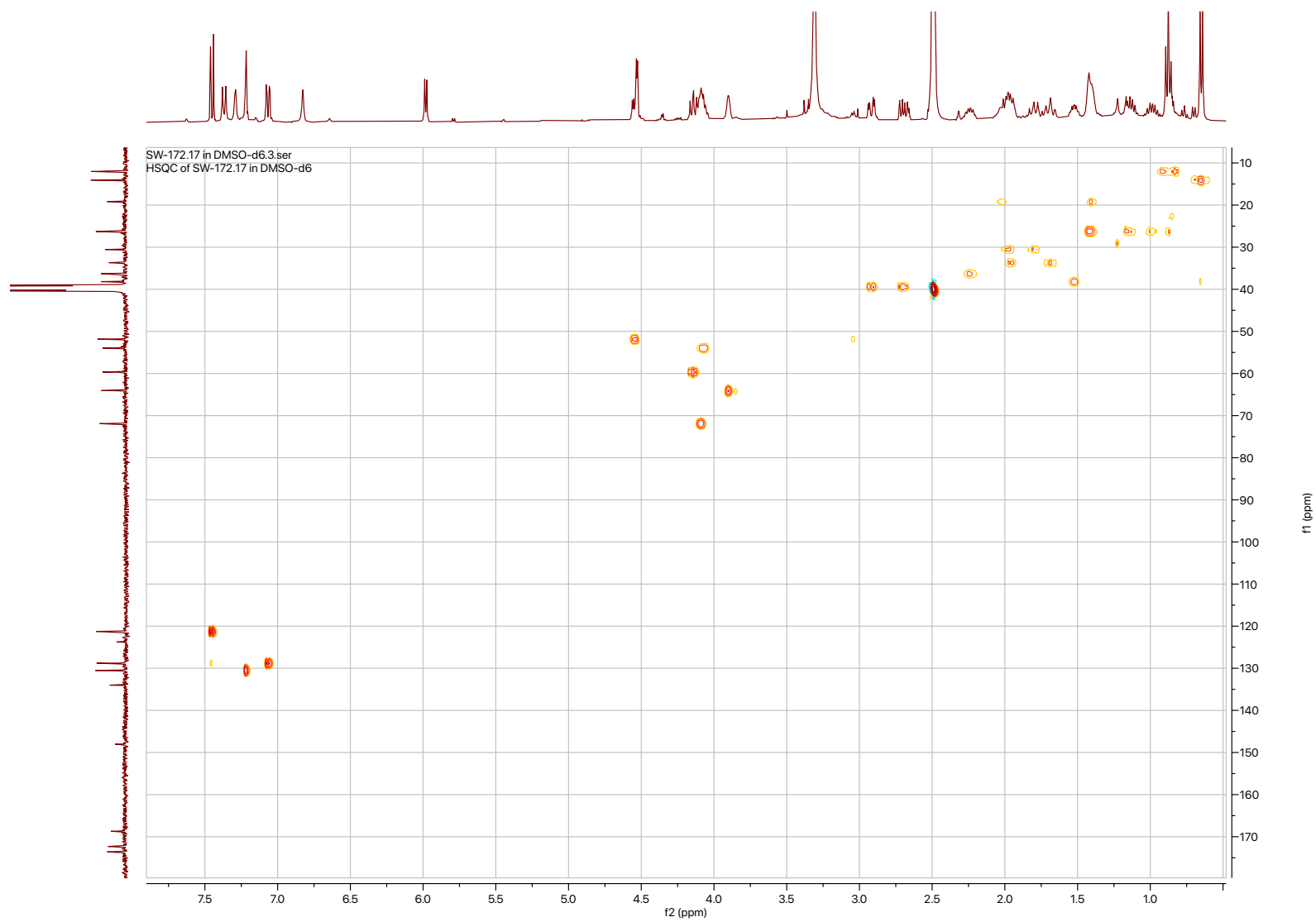


Figure S24. HMBC Spectrum of Aeruginosin KT575 (**3**) in DMSO- d_6

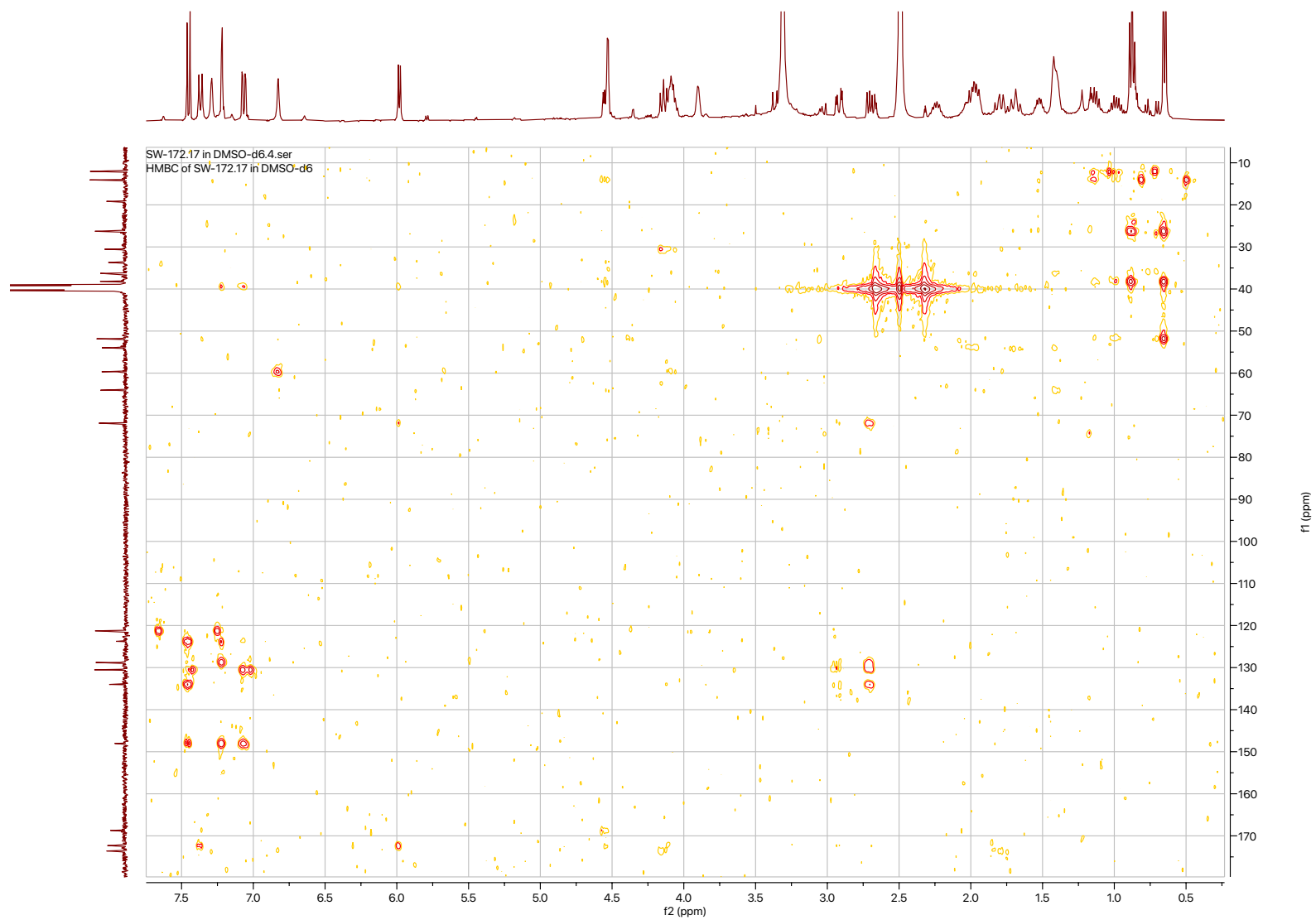


Figure S25. COSY Spectrum of Aeruginosin KT575 (**3**) in DMSO-*d*₆

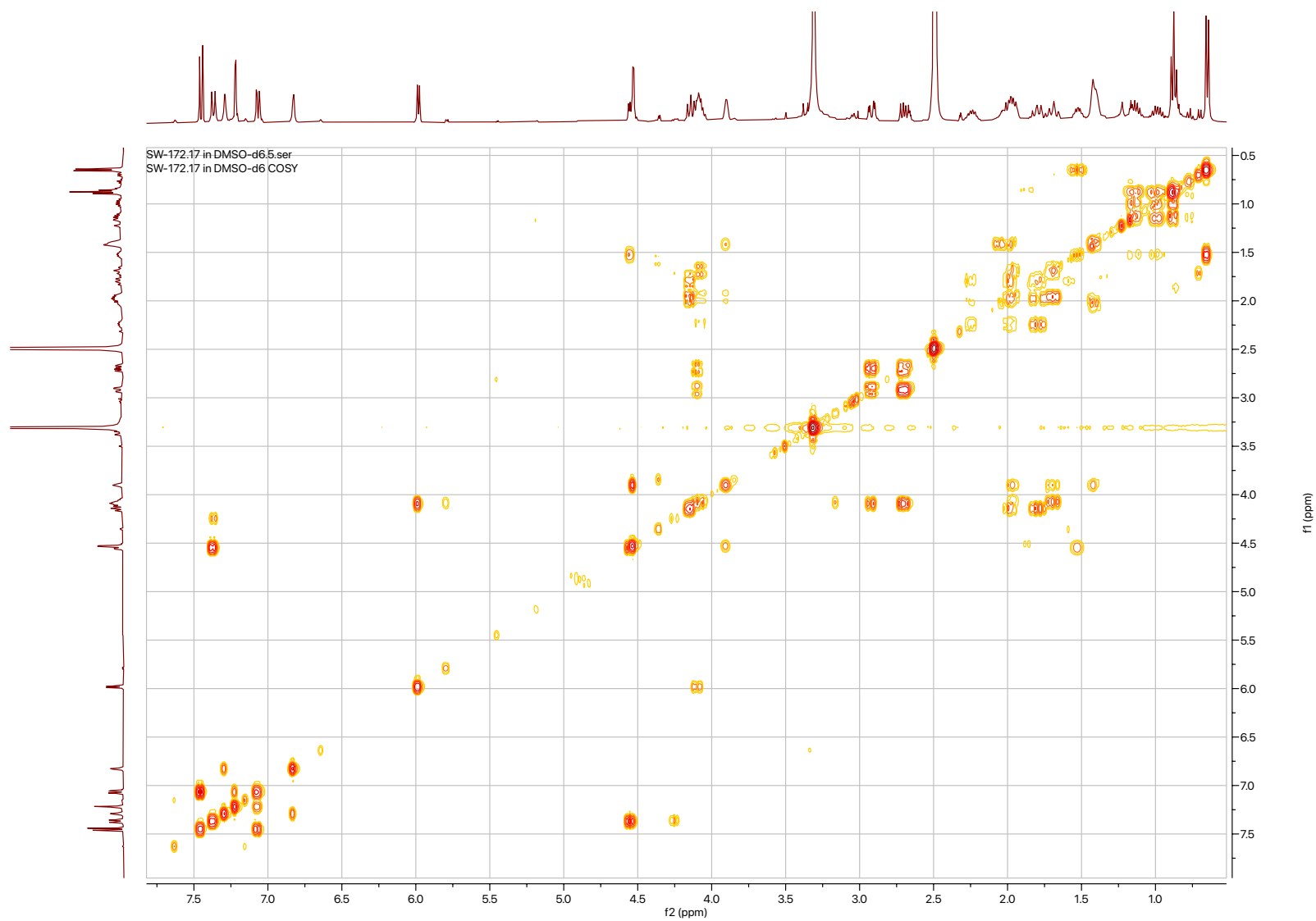


Figure S26. TOCSY Spectrum of Aeruginosin KT575 (**3**) in DMSO-*d*₆

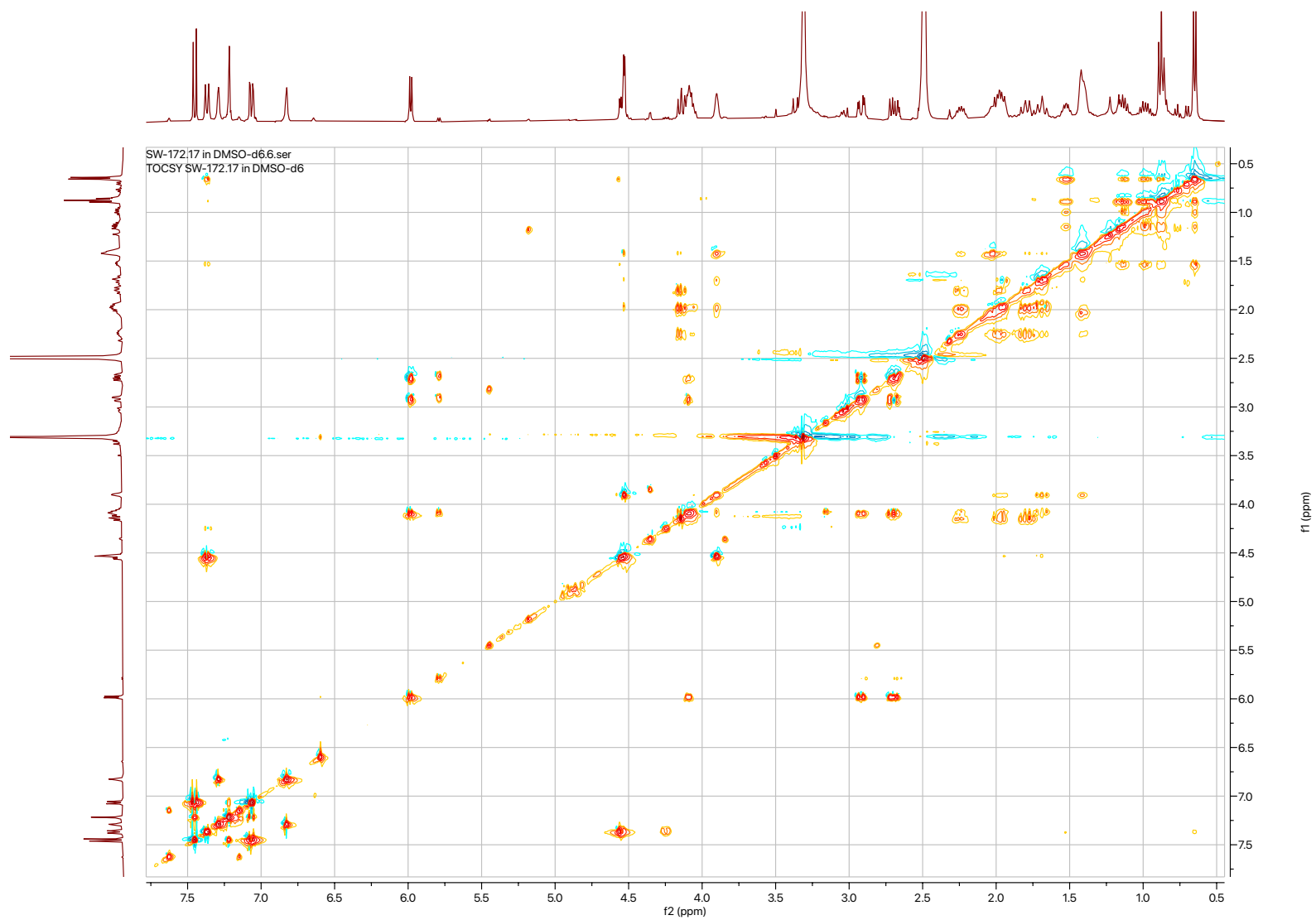


Figure S27. ROESY Spectrum of Aeruginosin KT575 (**3**) in DMSO-*d*₆

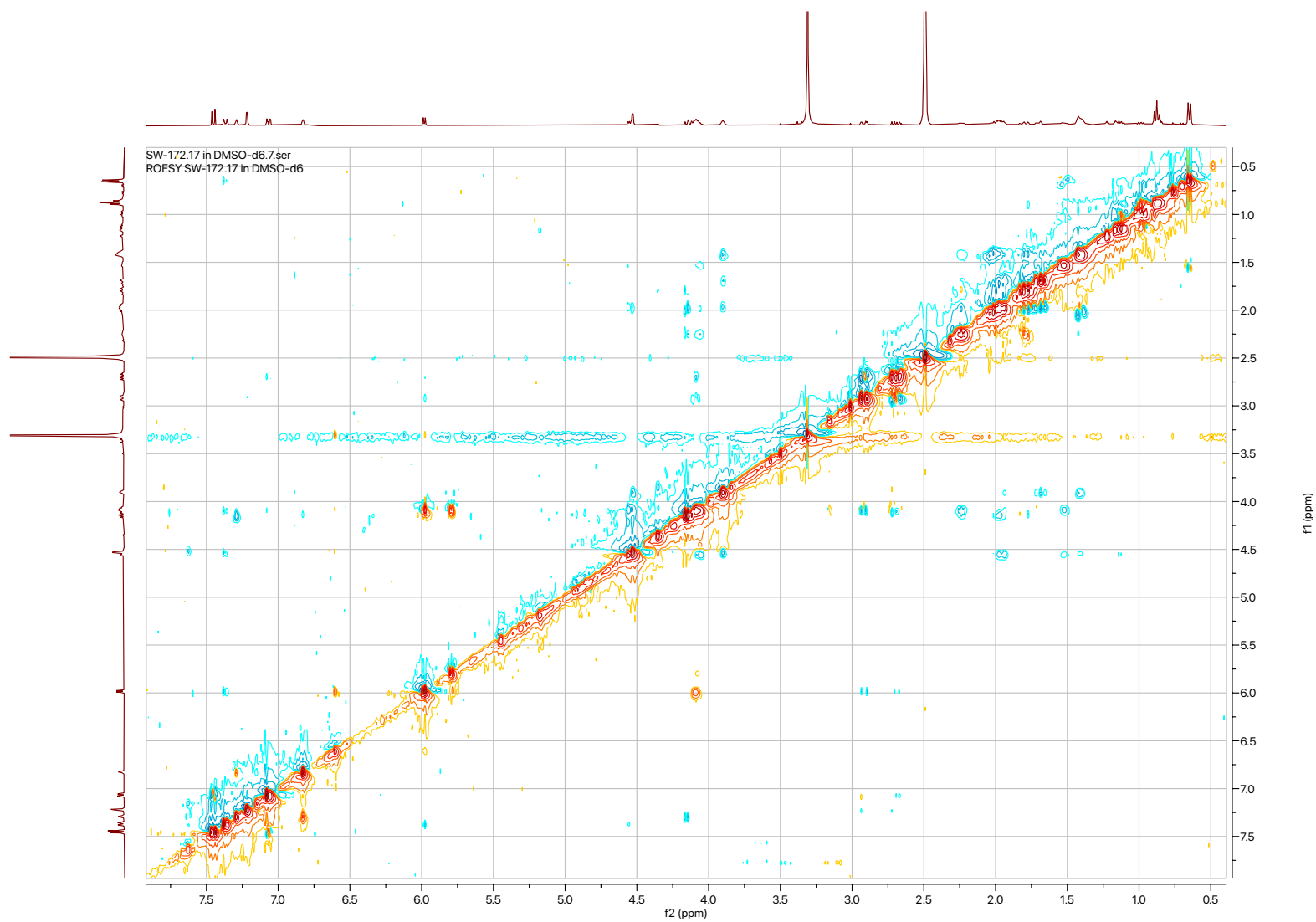


Figure S28. Negative HR ESI MS/MS Spectrum of Aeruginosin KT575 (**3**)

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Odd and Even Electron Ions

877 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)

Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	S	Cl
574.1647	574.1626	2.1	3.7	9.5	C ₂₄ H ₃₃ N ₃ O ₉ S Cl	570.6	0.246	78.18	24	33	3	9	1	1
	574.1687	-4.0	-7.0	9.0	C ₂₄ H ₃₅ N ₄ O ₆ S ₂ Cl	572.6	2.247	10.57	24	35	4	6	2	1
	574.1691	-4.4	-7.7	15.0	C ₂₄ H ₂₇ N ₈ O ₇ Cl	572.6	2.184	11.26	24	27	8	7		1

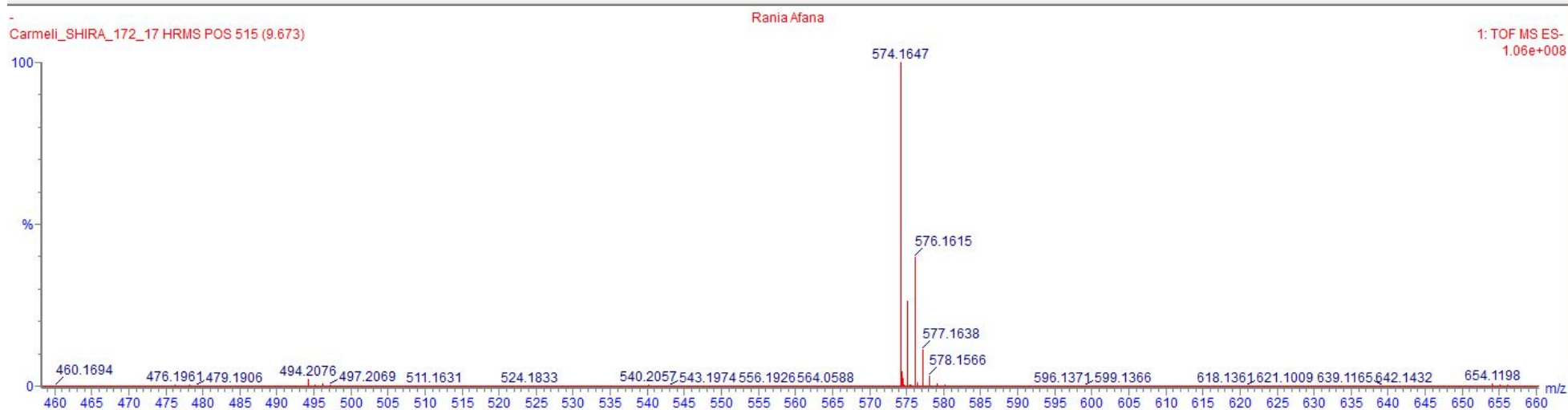


Figure S29. LCMS Traces of Marfey's Method Amino Acids Analysis of Aeruginosin KT575 (3)

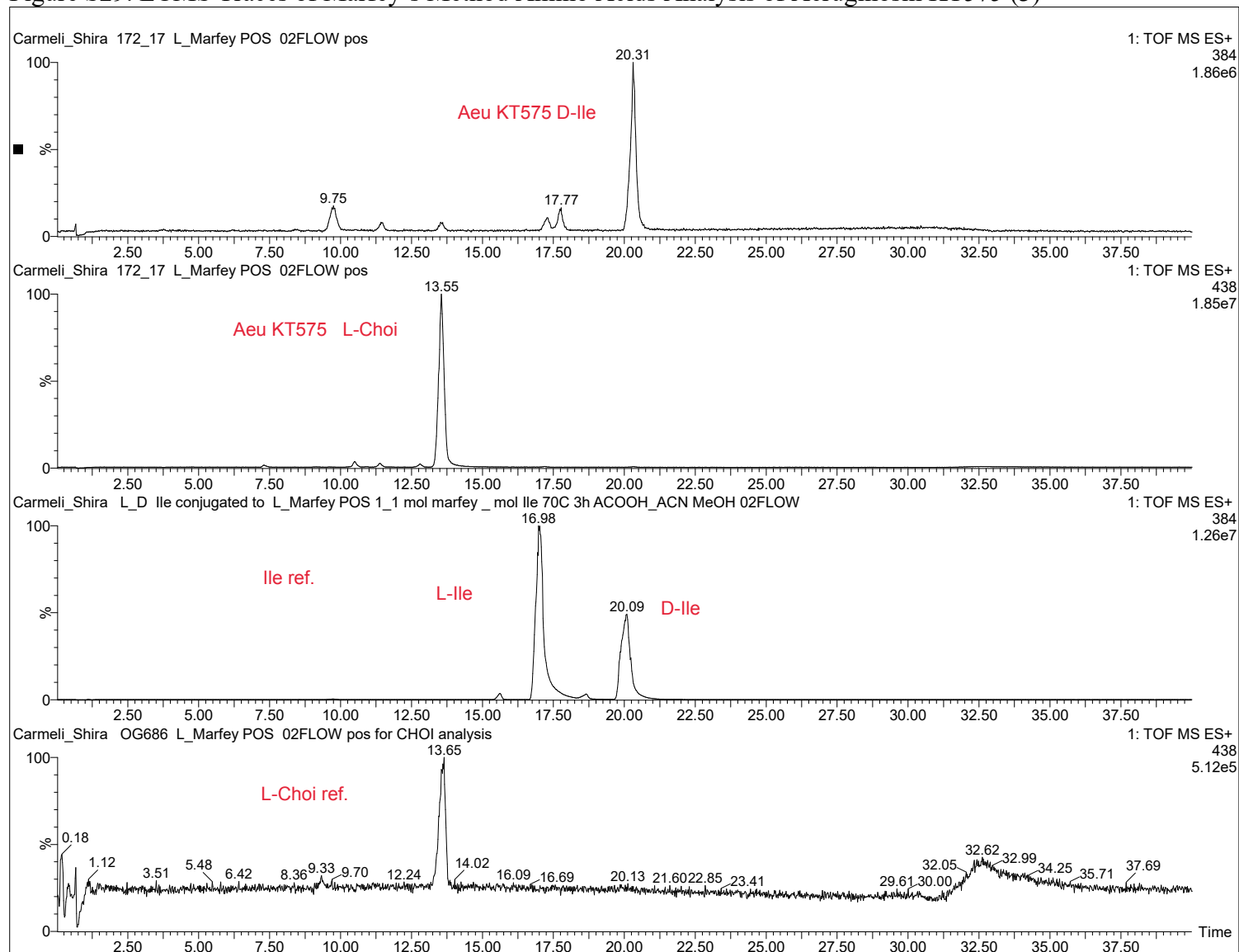


Table S6. Comparison of the ^1H and ^{13}C NMR data of the 6-Cl-Hpla-7-sulfate moiety in Aeruginosin KT575 (**3**) and Aeruginosin 89-B in DMSO- d_6

Aeruginosin KT575 (3) ^a			Aeruginosin 89-B ^b		
Position	δ_{C} , Mult.	δ_{H} , Mult. (J in Hz)	Position	δ_{C} , Mult.	δ_{H} , Mult. (J in Hz)
1	172.3, C		1	172.0, C	
2	71.9, CH	4.09, m	2	71.6, CH	4.08, dd (7.3,3.9)
2-OH		5.98, d (5.8)	2-OH		-
3pe	39.4, CH ₂	2.92, dd (14.0,3.4)	3a	39.0, CH ₂	2.93, dd (13.7,3.9)
3pa		2.70, dd (14.0,7.6)	3b		2.70, dd (13.7,7.3)
4	134.0, C		4	133.8, C	
5	130.5, CH	7.22, d (2.1)	5	130.3, CH	7.20, d (1.7)
6	123.8, C		6	123.6, C	
7	148.0, C		7	147.8, C	
8	121.3, CH	7.45, d (8.9)	8	121.2 CH	7.46, d (8.6)
9	128.8, CH	7.07, dd (8.9,2.1)	9	128.6 CH	7.05, dd (8.6,1.7)

^a400 MHz for ^1H , 100 MHz for ^{13}C . ^b600 MHz for ^1H , 150 MHz for ^{13}C .

Table S7. Comparison of the ^1H and ^{13}C NMR data of the Choi moiety of Aeruginosin KT575 (**3**) and Aeruginosin DA495B in DMSO- d_6 ^a

Aeruginosin KT575 (3)			Aeruginosin DA495B		
Position	δ_{C} , Mult.	δ_{H} , Mult. (J in Hz)	Position	δ_{C} , mult.	δ_{H} , mult., J (Hz)
Choi-1	173.6, C		Choi-1	173.5, C	
1- NH ₂		7.29, d (1.0)	1- NH ₂		7.23, s 6.83, s
		6.83, d (1.0)			
2	59.7, CH	4.14, dd (9.6,8.4)	2	59.7, CH	4.12, dd (9.3,8.2)
3pe	30.6, CH ₂	1.97, m	3	30.6, CH ₂	1.97, m 1.79, m
3pa		1.79, ddq (12.5, 9.8, 7.8)			
3a	36.3, CH	2.24, ddq (12.9, 7.1, 6.0)	3a	36.3, CH	2.23, m
4pe	19.2, CH ₂	2.02, m	4	19.2, CH ₂	2.02, m 1.39, m
4pa		1.41, m			
5	26.2, CH ₂	1.42, m	5	26.1, CH ₂	1.40, m
6	64.1, CH	3.90, brs	6	64.0, CH	3.91, brs
6-OH		4.53, d (3.1)	6-OH		4.48, d (2.8)
7pe	33.7, CH ₂	1.96, m	7	33.7, CH ₂	1.96, m
7pa		1.69, brtd (11.8, 1.9)			1.65, brt (12.0)
7a	54.0, CH	4.07, m	7a	54.1, CH	4.00, ddd (11.2, 6.5,5.9)

^a400 MHz for ^1H , 100 MHz for ^{13}C .