

Supplementary Material

Lyngbyastatins 8–10, Elastase Inhibitors with Cyclic Depsipeptide Scaffolds Isolated from the Marine Cyanobacterium *Lyngbya semiplena*

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Contents	Page
Figure S1. ESIMS Fragmentation Pattern for Lyngbyastatin 7	S 2
Table S1. NMR Spectral Data for Lyngbyastatin 8 (1) in DMSO- <i>d</i> ₆ at 600 MHz	S 3
Table S2. NMR Spectral Data for Lyngbyastatin 9 (2) in DMSO- <i>d</i> ₆ at 600 MHz	S 4
Table S3. NMR Spectral Data for Lyngbyastatin 10 (3) in DMSO- <i>d</i> ₆ at 600 MHz	S 5
¹ H NMR of Lyngbyastatin 8 (1) in DMSO- <i>d</i> ₆ at 600 MHz	S 6
COSY Spectrum of Lyngbyastatin 8 (1) in DMSO- <i>d</i> ₆ at 600 MHz	S 7
Edited HSQC Spectrum of Lyngbyastatin 8 (1) in DMSO- <i>d</i> ₆ at 600 MHz	S 8
HMBC Spectrum of Lyngbyastatin 8 (1) in DMSO- <i>d</i> ₆ at 600 MHz	S 9
ROESY Spectrum of Lyngbyastatin 8 (1) in DMSO- <i>d</i> ₆ at 600 MHz	S 10
TOCSY Spectrum of Lyngbyastatin 8 (1) in DMSO- <i>d</i> ₆ at 600 MHz	S 11
¹ H NMR of Lyngbyastatin 9 (2) in DMSO- <i>d</i> ₆ at 600 MHz	S 12
COSY Spectrum of Lyngbyastatin 9 (2) in DMSO- <i>d</i> ₆ at 600 MHz	S 13
Edited HSQC Spectrum of Lyngbyastatin 9 (2) in DMSO- <i>d</i> ₆ at 600 MHz	S 14
HMBC Spectrum of Lyngbyastatin 9 (2) in DMSO- <i>d</i> ₆ at 600 MHz	S 15
ROESY Spectrum of Lyngbyastatin 9 (2) in DMSO- <i>d</i> ₆ at 600 MHz	S 16
TOCSY Spectrum of Lyngbyastatin 9 (2) in DMSO- <i>d</i> ₆ at 600 MHz	S 17
¹ H NMR of Lyngbyastatin 10 (3) in DMSO- <i>d</i> ₆ at 600 MHz	S 18
COSY Spectrum of Lyngbyastatin 10 (3) in DMSO- <i>d</i> ₆ at 600 MHz	S 19
Edited HSQC Spectrum of Lyngbyastatin 10 (3) in DMSO- <i>d</i> ₆ at 600 MHz	S 20
ROESY Spectrum of Lyngbyastatin 10 (3) in DMSO- <i>d</i> ₆ at 600 MHz	S 21

Figure S1. ESIMS fragmentation pattern for lyngbyastatin 7. Two series of b ions were observed. In one (top), ring opening at the ester bond occurred, followed by sequential loss of units. In the other (bottom), the side chain was lost, before ring opening at the amide bond between Ahp and Abu. Note: all ions retained Na^+ .

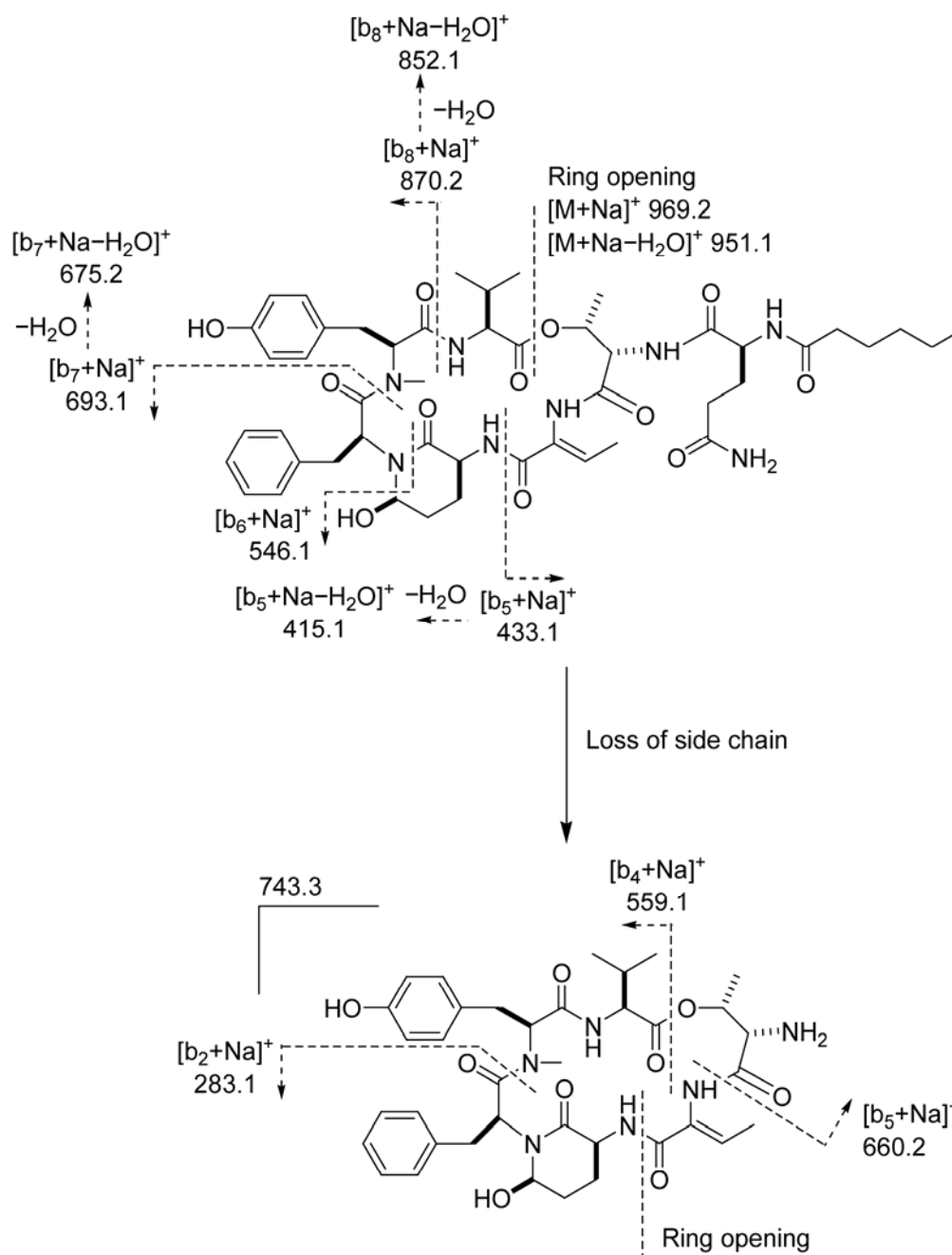


Table S1. NMR Spectral Data for Lyngbyastatin 8 (**1**) in DMSO-*d*₆ at 600 MHz (¹H)

C/H No.	δ _H (J in Hz)	δ _C , mult. ^a	HMBC ^b	Key ROESY
Val-1	1	^c		
	2	4.64, br	55.9, d	
	3	2.05, m	30.4, d	4 ^d , 5
	4	0.86, d (6.6)	19.0, q	2, 3, 5
	5	0.74, d (6.6)	17.2, q	2, 3, 4
	NH	7.50, br d (6.3)		H ₃ -15, OH (Ahp) H ₃ -15, OH (Ahp)
N-Me-Tyr	6	^c		
	7	4.87, dd (11.8, 1.9)	60.6, d	
	8a	3.08, dd (−13.4, 1.9)	32.5, t	10/14
	8b	2.69, dd (−13.4, 11.8)		10/14
	9		127.3, s	
	10/14	6.97, d (8.0)	130.3, d	8, 10/14, 12
	11/13	6.76, d (8.0)	115.2, d	9
	12		156.2, s	
	15	2.74, s	30.2, q	7, 16
	OH	9.38, s		11/13
Phe	16		170.6, s	
	17	4.71, dd (12.1, 3.1)	50.0, d	
	18a	2.86, dd (−12.8, 12.1)	35.0, t	20/24
	18b	1.80, dd (−12.8, 3.1)		
	19		136.8, s	
	20/24	6.82, d (7.2)	129.3, d	18, 20/24, 22
	21/23	7.18, m	127.7, d	19, 21/23
	22	7.14, m	126.1, d	20/24
Ahp	25			
	26	3.77, ddd (12.4, 9.2, 2.4)	47.9, d	
	27a	2.40, dddd (−12.4, 12.4, 11.7, 2.7)	21.7, t	H-27a, H-27b, NH H-26, H-27b, H-28a (w), OH
	27b	1.56, m		H-26, H-27a
	28a	1.70, br d (11.7)	29.1, t	27
	28b	1.56, m		26
	29	5.06, s	73.5, d	
	NH	7.21, d (9.2)		H-17, H-18a, H-20/24, H-28a, H-28b, OH H-26
	OH	6.10, s		H-3, H ₃ -5, H ₃ -15, H-18b, H-27a, H-28a, H-28b, H-29, NH (Val-1)
Abu	30	^c		
	31		130.1, s	
	32	6.49, q (6.8)	131.6, d	
	33	1.47, d (6.8)	12.9, q	31, 32
	NH	6.56, s		H-35, NH H ₃ -33
Thr	34	^c		
	35	4.62, m	55.1, d	
	36	5.53, br	71.4, d	
	37	1.21, d (6.2)	17.6, q	35, 36
	NH	7.91, br		
Val-2	38	^c		
	39	4.36, m	57.0, d	
	40	2.05, m	30.4, d	41 ^d
	41	0.85, d (6.2)	19.0, q	39, 40, 42
	42	0.80, d (6.4)	17.5, q	39, 40, 41
	NH	7.77, br		H ₃ -37 H-44
Ala	43		172.5, s	
	44	4.33, dq (7.4, 6.7)	47.8, d	43, 45
	45	1.17, d (6.7)	17.8, q	43, 44
	NH	8.08, d (7.4)		46
Ac	46		169.0, s	
	47	1.82, s	22.2, q	46

^aDeduced from edited HSQC. ^bProtons showing HMBC correlations to the indicated carbon. ^cCould not be detected due to lack of HMBC correlation. ^dThese correlations overlap closely, and thus the atom pair(s) that give rise to it cannot be determined.

Table S2. NMR Spectral Data for Lyngbyastatin 9 (**2**) in DMSO-*d*₆ at 600 MHz (¹H)

C/H No.	δ _H (J in Hz)	δ _C , mult. ^a	HMBC ^b	Key ROESY
Val-1	1	^c		
	2	4.62, m	55.9, d	
	3	2.05, m	30.3, d	2
	4	0.85 ^c	18.9, q	2, 3, 5
	5	0.74, d (6.6)	17.1, q	2, 3, 4
	NH	7.52, br d (6.8)		H ₃ -15
N-Me-Tyr	6		^c	H ₃ -15
	7	4.87, dd (12.2, 0)	60.5, d	H-7, H ₃ -15
	8a	3.08, dd (−13.0, 0)	32.4, t	H-10/14, H ₃ -15, H-17, H-18a, NH (Val-1)
	8b	2.69, dd (−13.0, 12.2)		H-10/14
	9		127.2, s	H-10/14
	10/14	6.97, d (7.6)	130.2, d	7, 9, 10/14
	11/13	6.76, d (7.6)	115.1, d	7, 9, 10/14
	12		156.0, s	
	15	2.74, s	30.1, q	8, 12, 10/14
	OH	9.41, s		9, 11/13, 12
Phe	16		170.3, s	H ₃ -4, H ₃ -5, H-7, H-10/14, NH (Val-1), OH (Ahp)
	17	4.71, dd (12.1, 3.0)	49.9, d	7, 16
	18a	2.85, dd (−12.8, 12.1)	35.0, t	11/13, 12
	18b	1.80, dd (−12.8, 3.0)		16, 18, 29
	19		136.5, s	17, 19, 20/24
	20/24	6.82, d (7.3)	129.2, d	H-7, H-10/14, H-20/24
	21/23	7.18, m	127.6, d	H-7, H-20/24, OH (Ahp)
	22	7.14, m	126.0, d	19, 20/24, 25 ^d
Ahp	25		168.5, s	H-20/24, H-29, OH (Ahp)
	26	3.77, ddd (14.1, 10.6, 2.3)	47.9, d	H-20/24, H-26, H-28a
	27a	2.40, dddd (14.1, −12.4, 11.3, 4.4)	21.6, t	H-27b, H-29, OH (Ahp)
	27b	1.56, m		H-29
	28a	1.71, br d (11.3)	29.0, t	H-28a, H-28b, OH (Ahp)
	28b	1.55, m		H-26
	29	5.06, s	73.4, s	H-3, H ₃ -5, H ₃ -15, H-18a, H-28a, H-29, NH (Val-1)
	NH	7.21, br		
	OH	6.11, br		
Abu	30		162.7, s	
	31		129.7, s	
	32	6.49, q (6.8)	131.5, d	
	33	1.47, d (6.8)	12.8, q	30, 31, 33
	NH	6.58, s		30 ^d , 31, 32
Thr	34		^c	
	35	4.63, m	55.0, d	H-36, H ₃ -37
	36	5.53, br	71.4, d	H-35
	37	1.21, d (6.2)	17.6, q	H-35
	NH	7.92, br		H-39
Val-2	38		171.7, s	
	39	4.37, m	56.8, d	
	40	2.05, m	30.3, d	38
	41	0.84 ^c	13.3, q	38, 41, 42
	42	0.80, d (6.5)	17.4, q	39, 40, 42
	NH	7.69, br		39, 40
Ala	43		172.3, s	H-44
	44	4.34, dq (7.2, 6.8)	47.7, d	
	45	1.18, d (6.8)	17.6, q	43, 45
	NH	8.04, d (7.2)		43, 44
Ba	46		171.8, s	44, 45, 46
	47	2.07, m (2H)	36.7, t	H ₂ -47
	48	1.48, m (2H)	18.3, t	
	49	0.83 ^c	13.3, q	46, 47, 50
				47

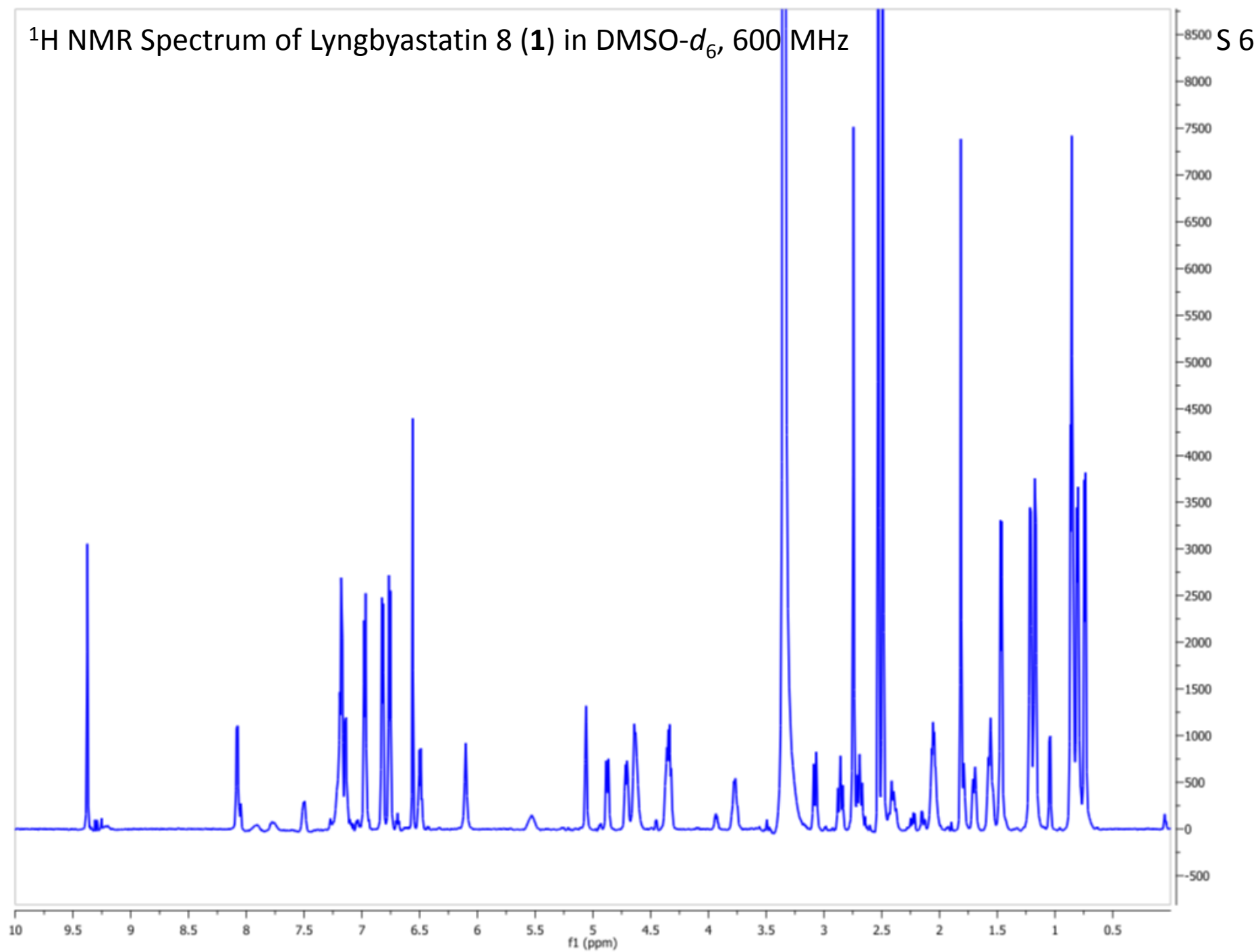
^aDeduced from edited HSQC. ^bProtons showing HMBC correlations to the indicated carbon. ^cCould not be detected due to lack of HMBC correlation. ^dAn unusual 4-bond HMBC correlation. ^eThe multiplicity of these signals could not be deduced due to signal overlap.

Table S3. NMR Spectral Data for Lyngbyastatin 10 (**3**) in DMSO-*d*₆ at 600 MHz (¹H)

C/H No.	δ_{H} (J in Hz)	δ_{C} , mult. ^a	¹ H- ¹ H COSY	Key ROESY
Val-1	1			
	2	4.60, m	56.2, d	H-3, NH
	3	2.03, m	30.4, d	H-2, H ₃ -4, H ₃ -5
	4	0.86, d (7.0)	19.0, q	H-3
	5	0.74, d (7.0)	17.2, q	H-3
	NH	7.52, br d (7.3)	H-2	H ₃ -15
N-Me-3'-Br-Tyr	6			
	7	4.86, dd (12.0, 2.4)	60.6, d	H-8a, H-8b
	8a	3.08, dd (-13.6, 2.4)	32.2, t	H-7, H-8b
	8b	2.72, dd (-13.6, 12.0)		H-7, H-8a
	9			
	10	7.25, s	133.4, d	
	11			
	12			
	13	6.93, d (8.2)	116.5, d	H-14
	14	6.97, d (8.2)	130.0, d	H-13
	15	2.75, s	30.3, q	
	OH	8.48, s		
Phe	16			
	17	4.70, dd (11.7, 3.2)	50.2, d	H-18a, H-18b
	18a	2.88, dd (-13.7, 11.7)	35.2, t	H-17, H-18b
	18b	1.87, dd (-13.7, 3.2)		H-17, H-18a
	19			
	20/24	6.78, d (7.4)	129.5, d	H-21/23
	21/23	7.16, dd (7.4, 7.3)	128.0, d	H-20/24, H-22
	22	7.13, t (7.3)	126.4, d	H-21/23
Ahp	25			
	26	3.78, ddd (11.6, 8.8, 2.2)	48.2, d	H-27a, H-27b, NH
	27a	2.40, m	21.8, t	H-26
	27b	1.56, m		H-26
	28a	1.71, br d (12.4)	29.2, t	H-29
	28b	1.57, m		H-29
	29	5.07, s	73.7, d	H-28a, H-28b
	NH	7.21, br		H-26
	OH	3.15, s ^c		
Abu	30			
	31			
	32	6.50, q (7.1)	132.1, d	H ₃ -33
	33	1.47, d (7.1)	12.9, q	H-32
	NH			
Thr	34			
	35	4.61, m	55.7, d	
	36	5.50, br		
	37	1.21, d (6.7)	17.8, q	
	NH	7.92, br		
Val-2	38			
	39	4.36, dd (8.8, 6.1)	57.1, d	H-40
	40	2.03, m	30.4, d	H-39, H ₃ -41, H ₃ -42
	41	0.85, d (6.1)	19.0, q	H-40
	42	0.80, d (6.6)	17.5, q	H-40
	NH	7.68, br		
Ala	43			
	44	4.32, dq (7.6, 7.0)	47.9, d	H ₃ -45, NH
	45	1.18, d (7.0)	17.8, q	H-45
	NH	8.01, d (7.6)		H-44
Ba	46			
	47	2.07, m (2H)	36.8, t	H ₂ -48
	48	1.48, m (2H)	18.5, t	H ₂ -47, H ₃ -49
	49	0.83, t (7.3)	13.4, q	H ₂ -48

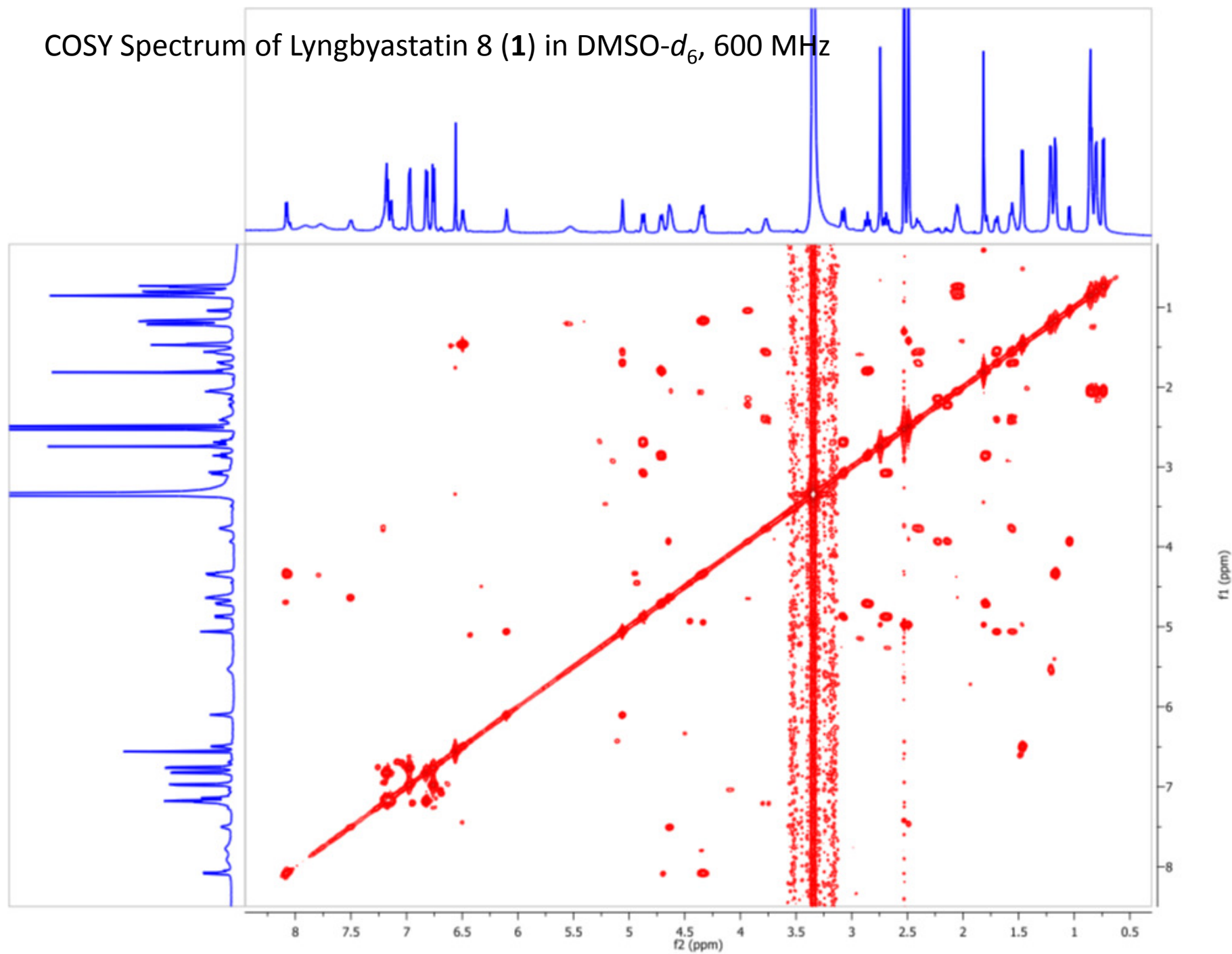
^aDeduced from edited HSQC. ^bHMBC spectrum could not be acquired due to lack of material, and chemical shift could not be determined. ^cAssigned by default as no COSY correlations were observed. Chemical shift is different to **1** and **2** probably because of different sample concentrations (data for **1** and **2** were acquired with a 1-mm probe and therefore they were much more concentrated).

^1H NMR Spectrum of Lyngbyastatin 8 (**1**) in $\text{DMSO}-d_6$, 600 MHz



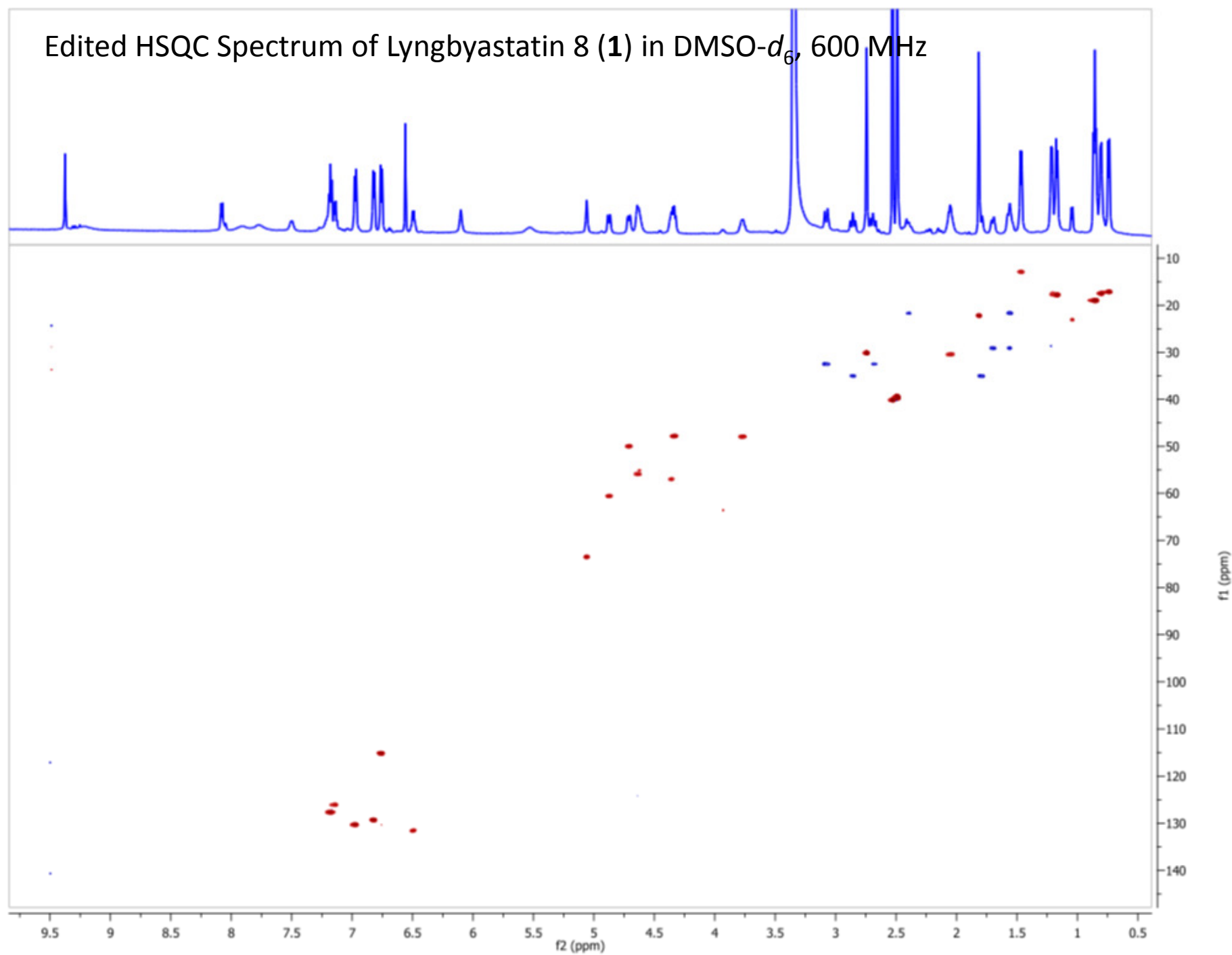
COSY Spectrum of Lyngbyastatin 8 (**1**) in DMSO- d_6 , 600 MHz

S 7



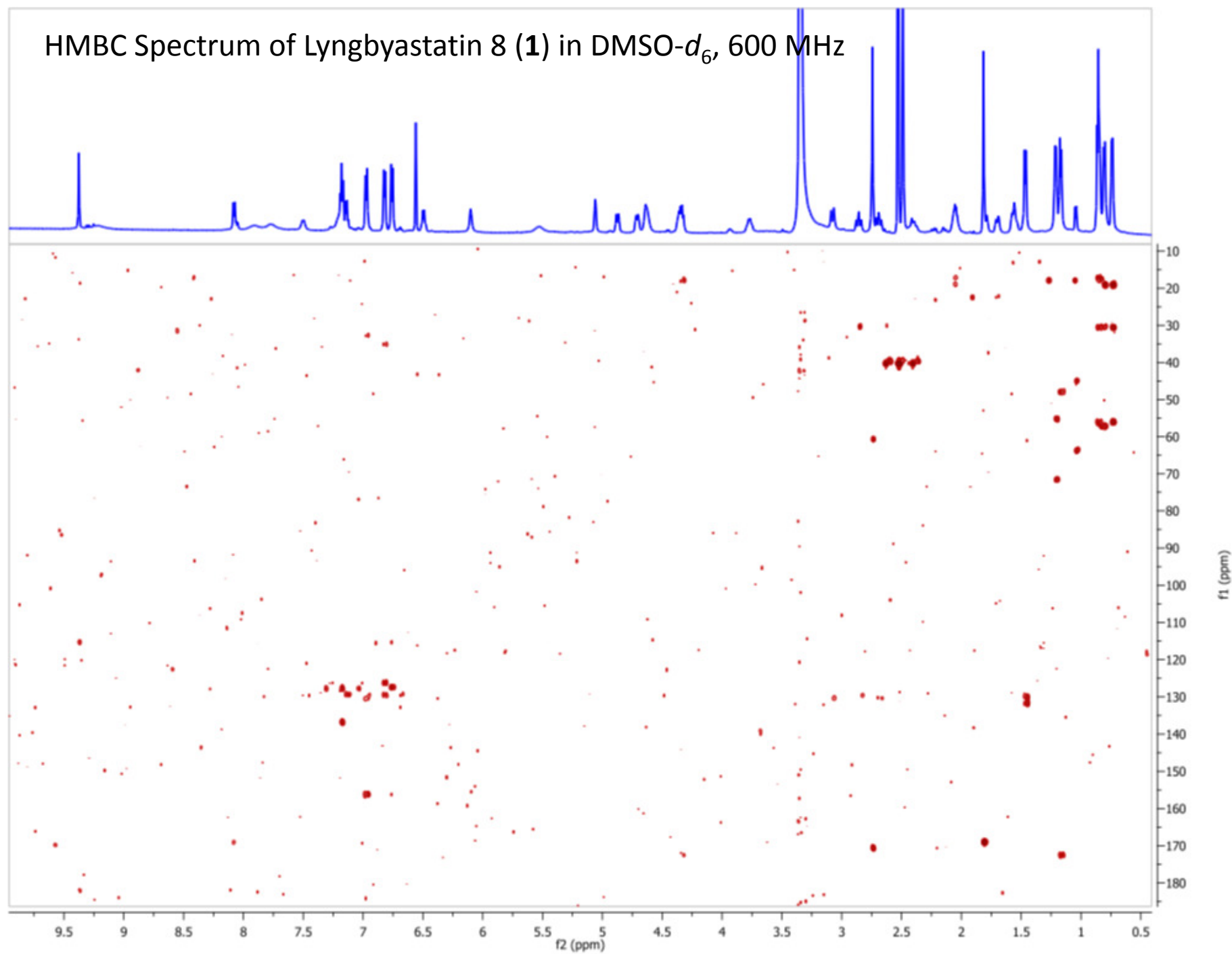
Edited HSQC Spectrum of Lyngbyastatin 8 (**1**) in DMSO- d_6 , 600 MHz

S 8



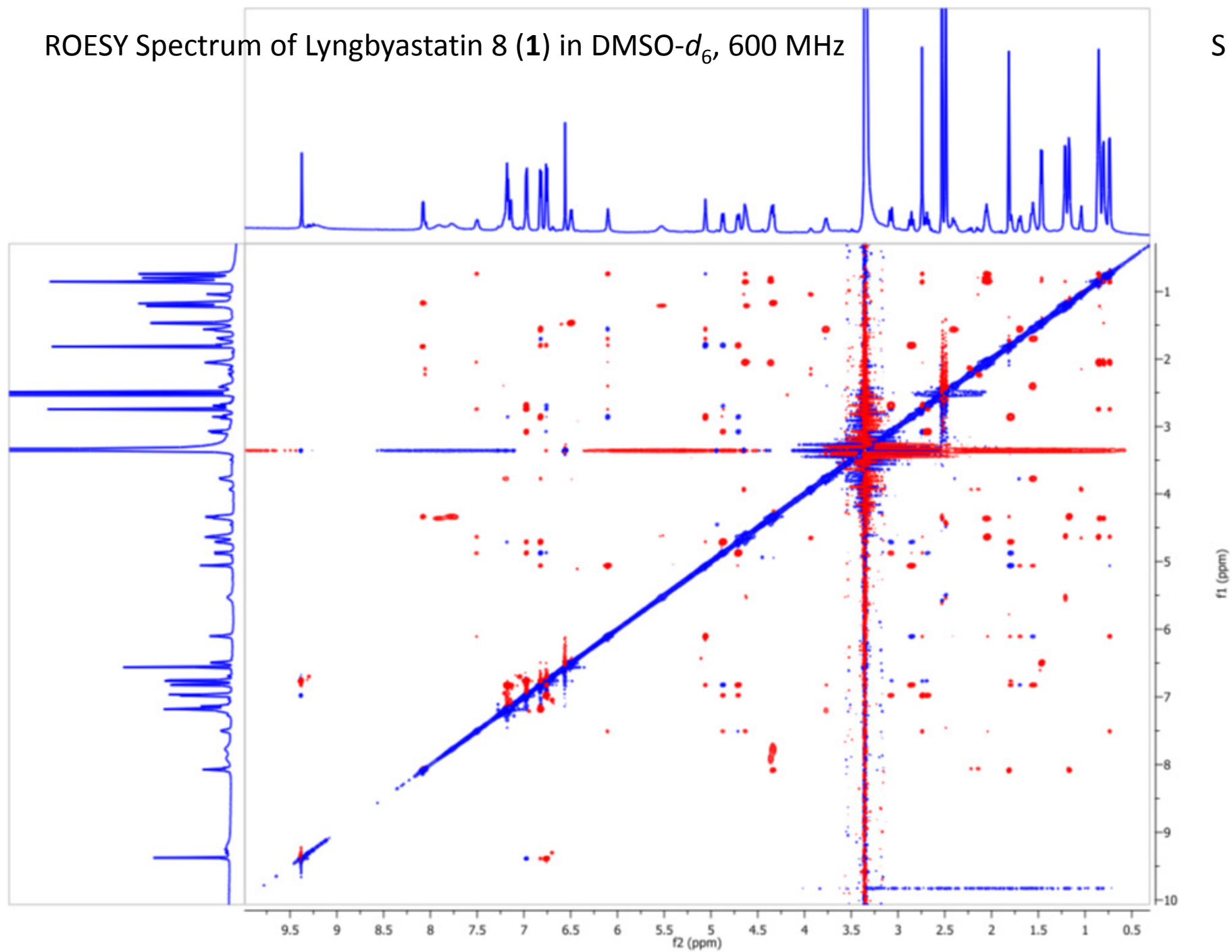
HMBC Spectrum of Lyngbyastatin 8 (**1**) in DMSO- d_6 , 600 MHz

S 9



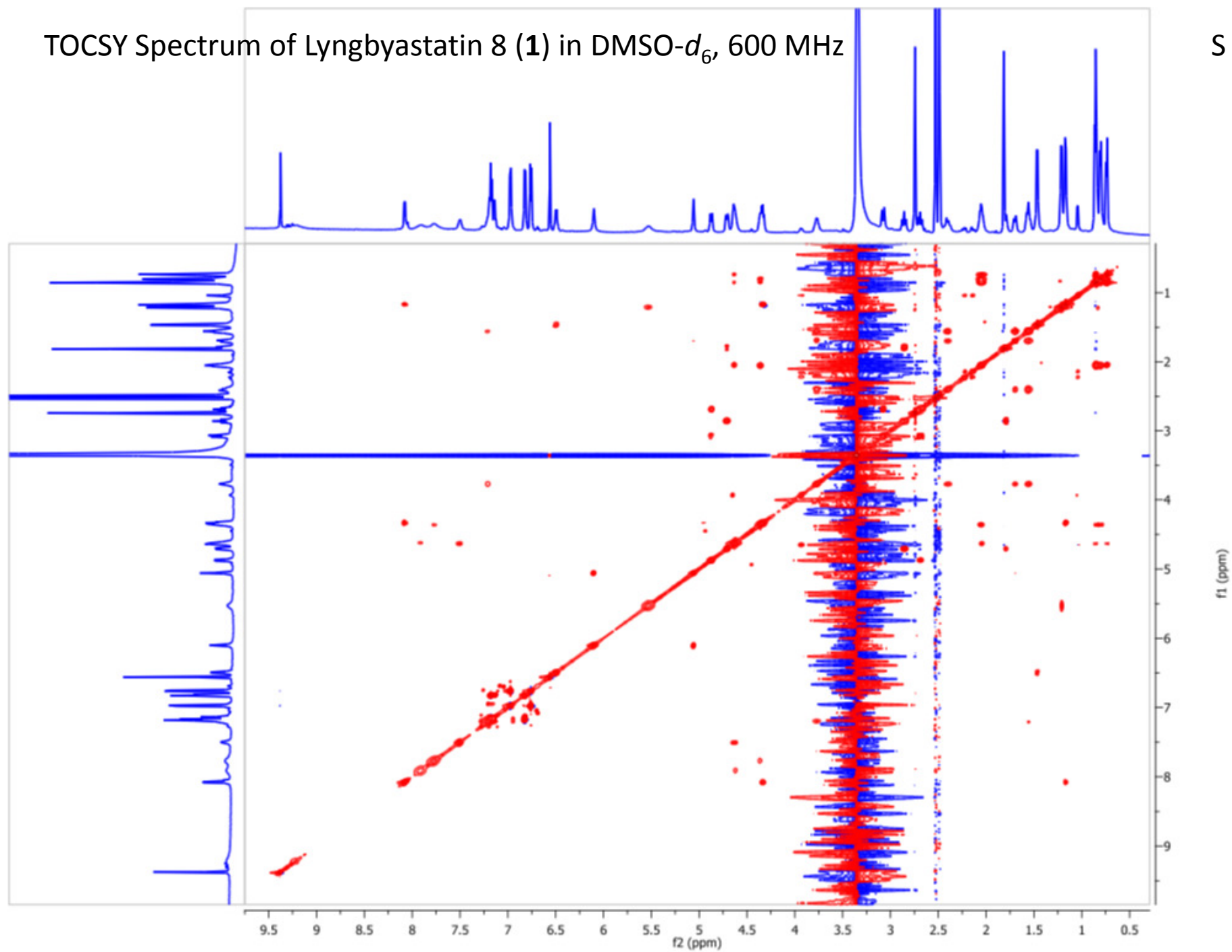
ROESY Spectrum of Lyngbyastatin 8 (**1**) in DMSO- d_6 , 600 MHz

S 10

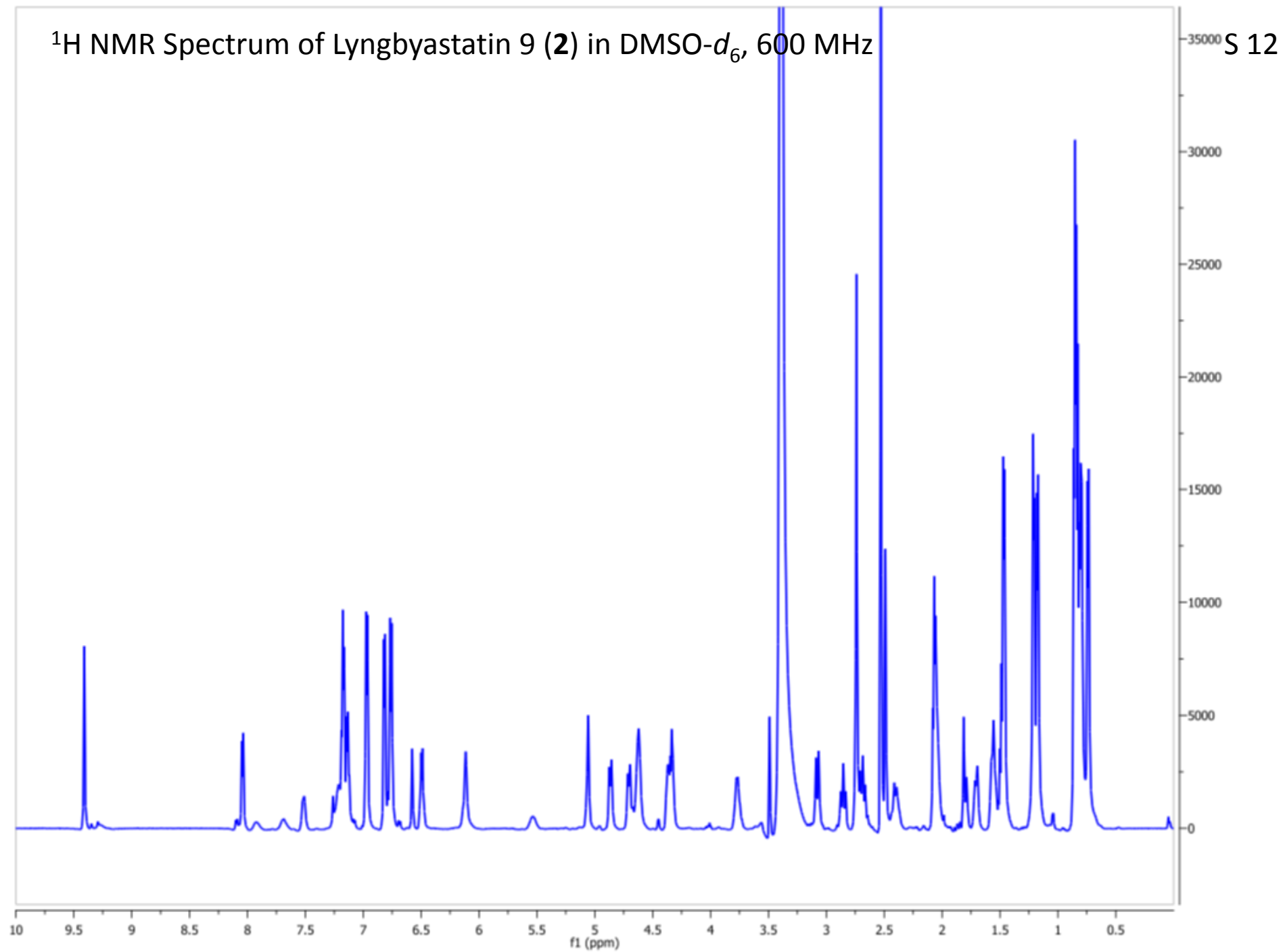


TOCSY Spectrum of Lyngbyastatin 8 (**1**) in DMSO- d_6 , 600 MHz

S 11

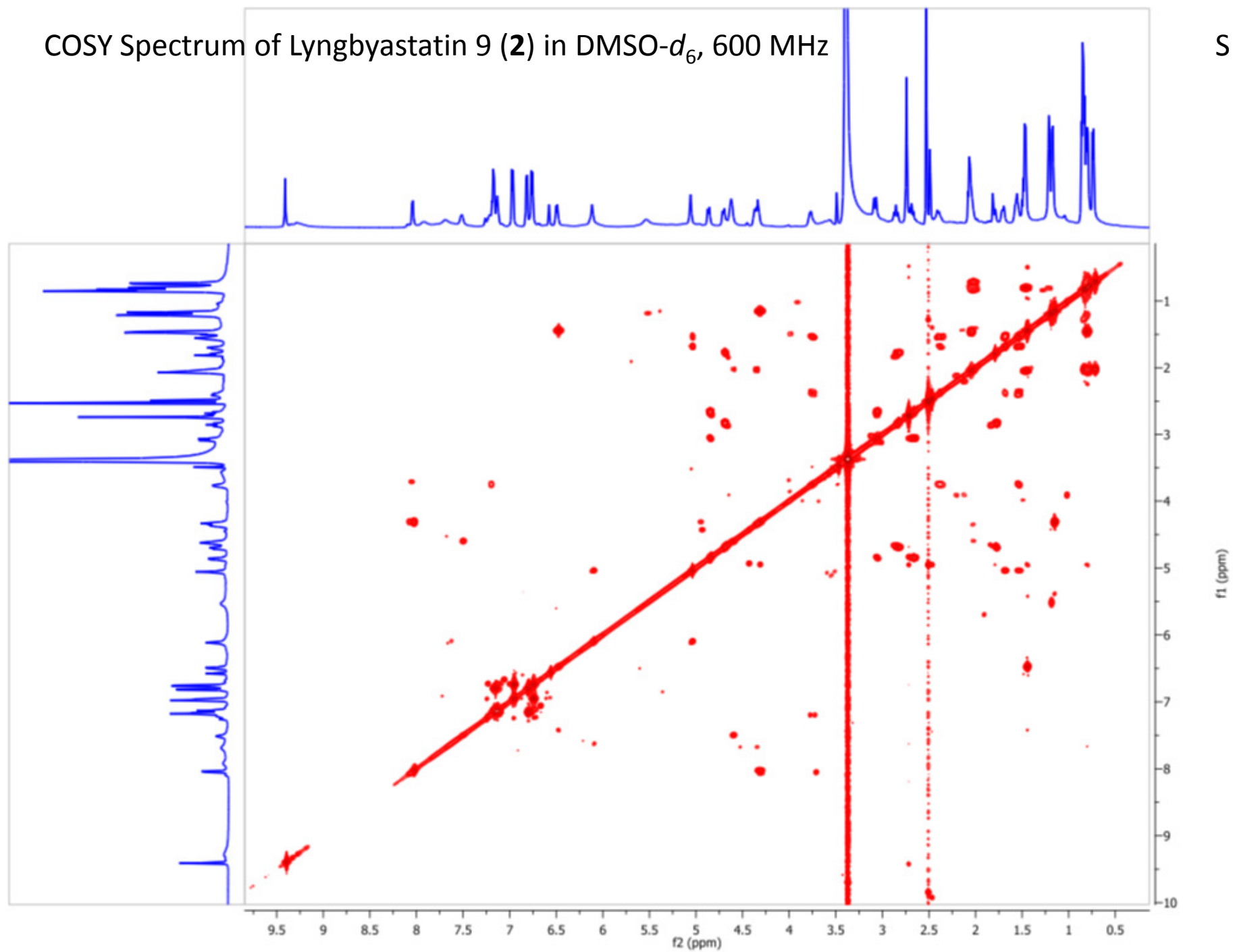


^1H NMR Spectrum of Lyngbyastatin 9 (**2**) in $\text{DMSO}-d_6$, 600 MHz



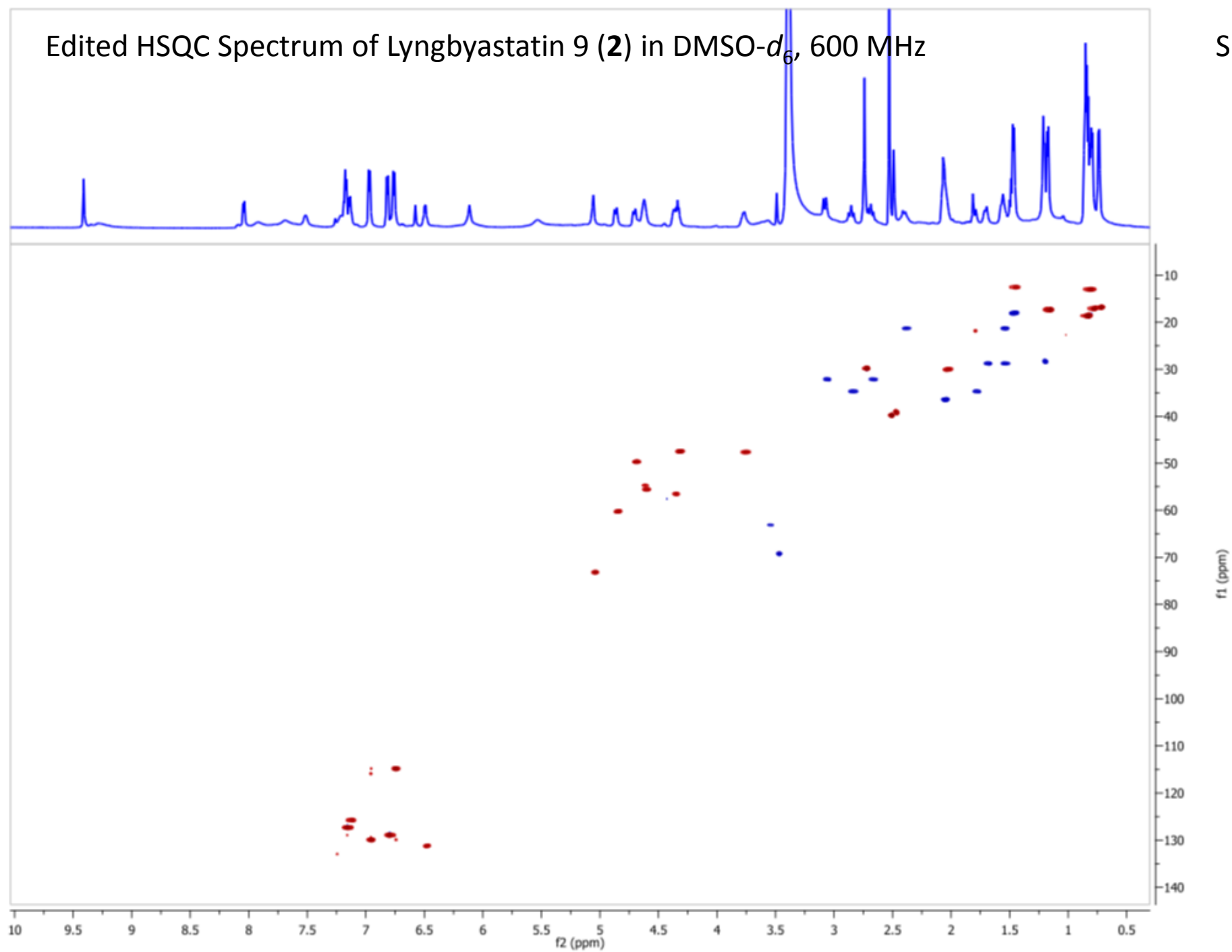
COSY Spectrum of Lyngbyastatin 9 (**2**) in DMSO- d_6 , 600 MHz

S 13



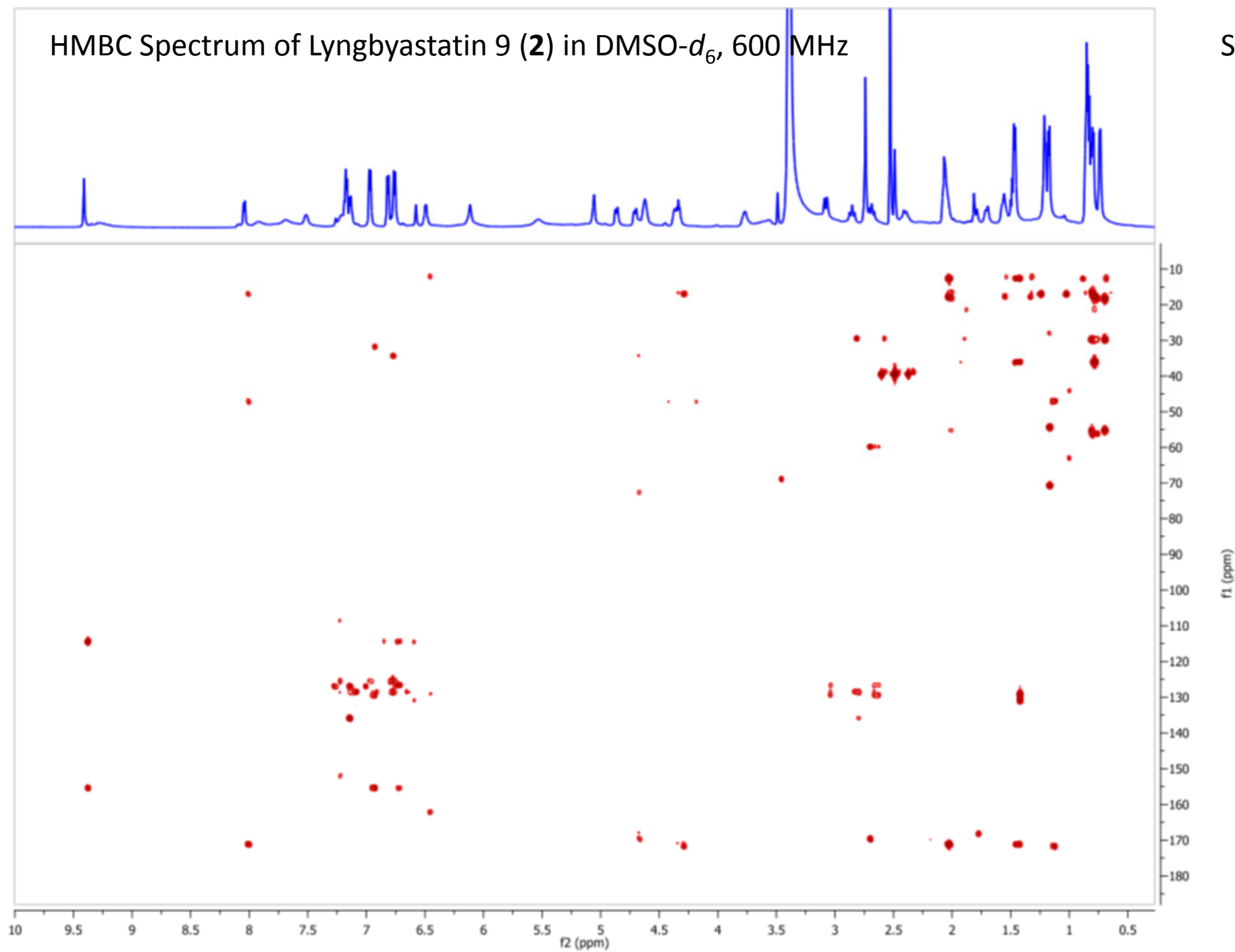
Edited HSQC Spectrum of Lyngbyastatin 9 (**2**) in DMSO- d_6 , 600 MHz

S 14



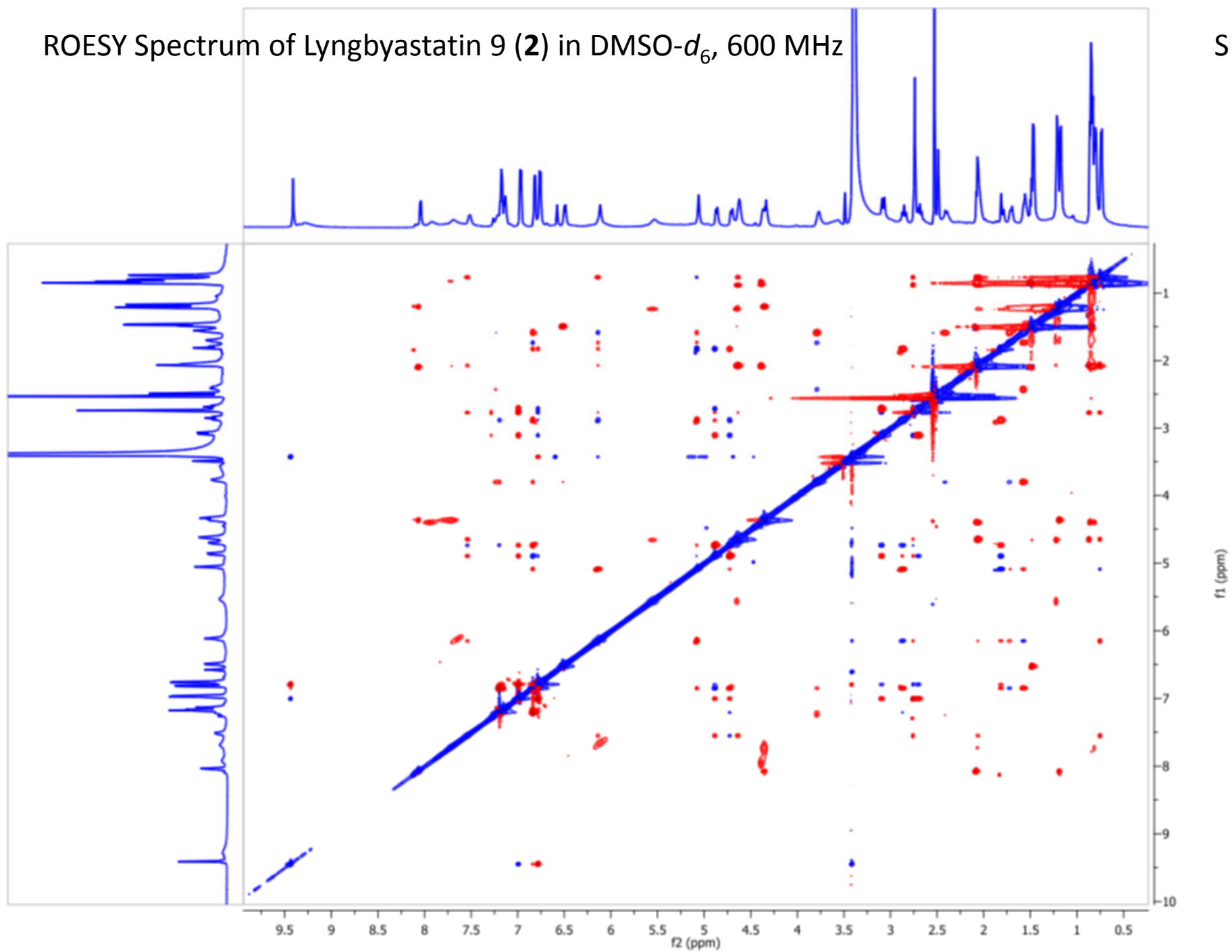
HMBC Spectrum of Lyngbyastatin 9 (**2**) in DMSO- d_6 , 600 MHz

S 15



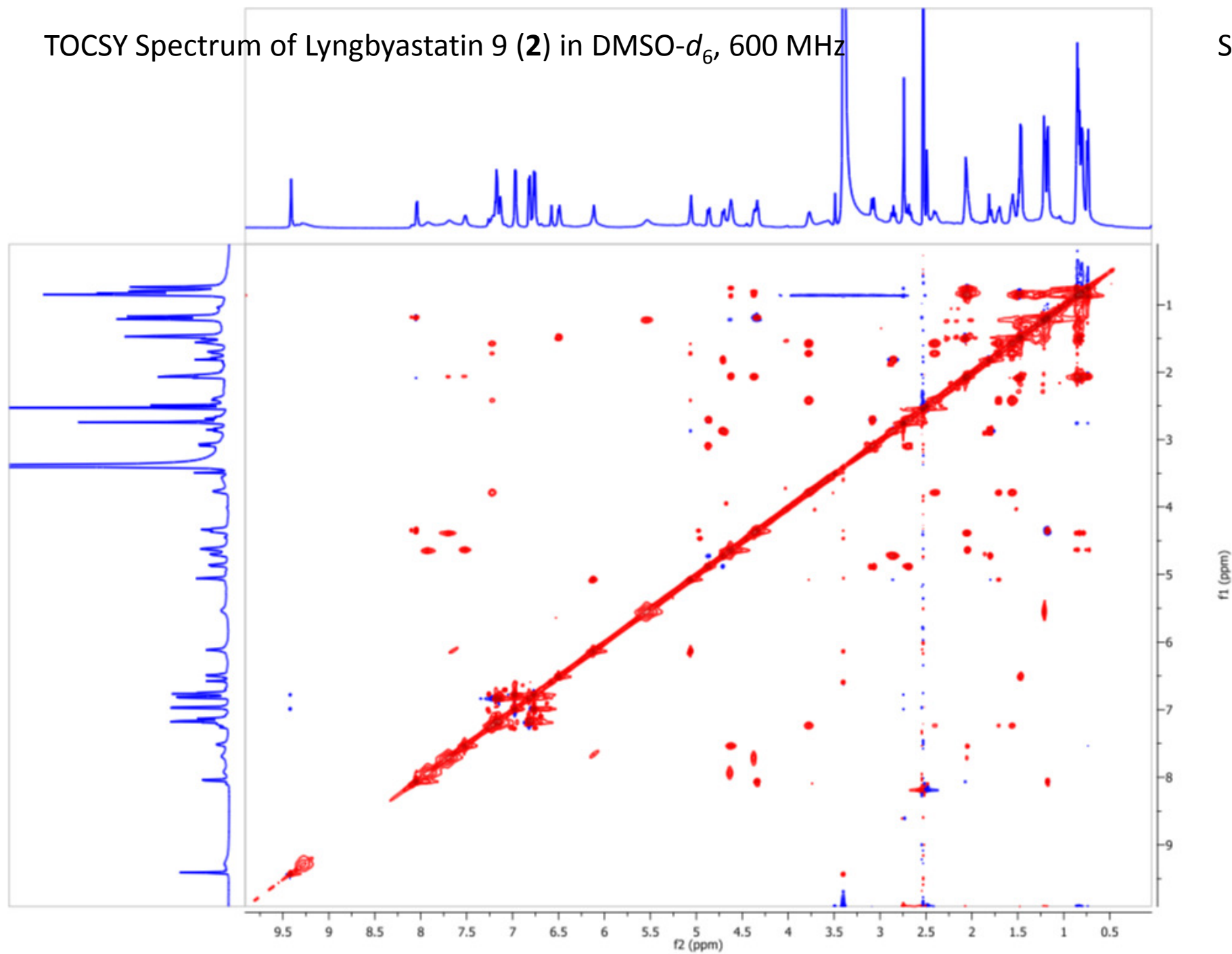
ROESY Spectrum of Lyngbyastatin 9 (**2**) in DMSO- d_6 , 600 MHz

S 16



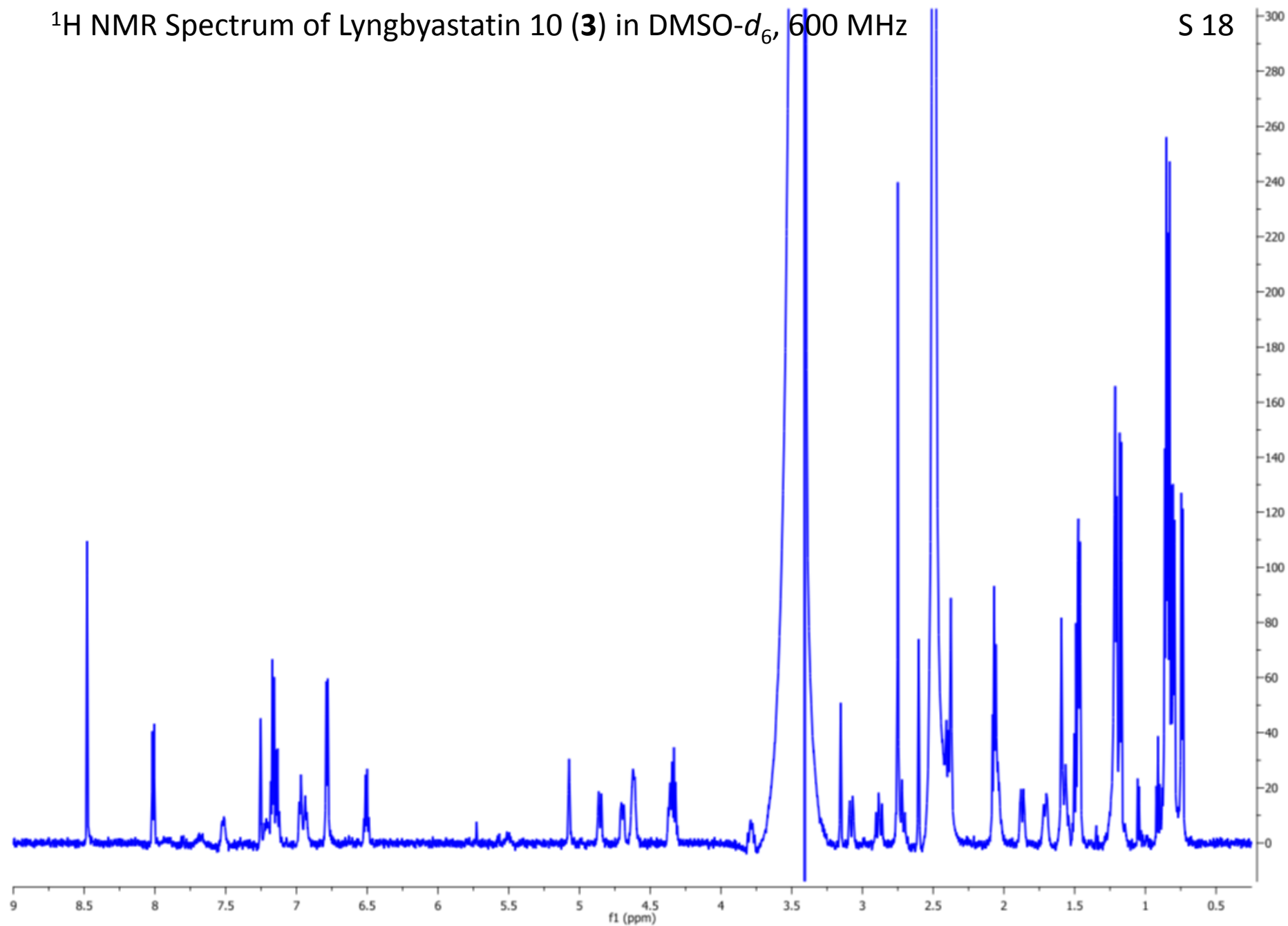
TOCSY Spectrum of Lyngbyastatin 9 (**2**) in DMSO- d_6 , 600 MHz

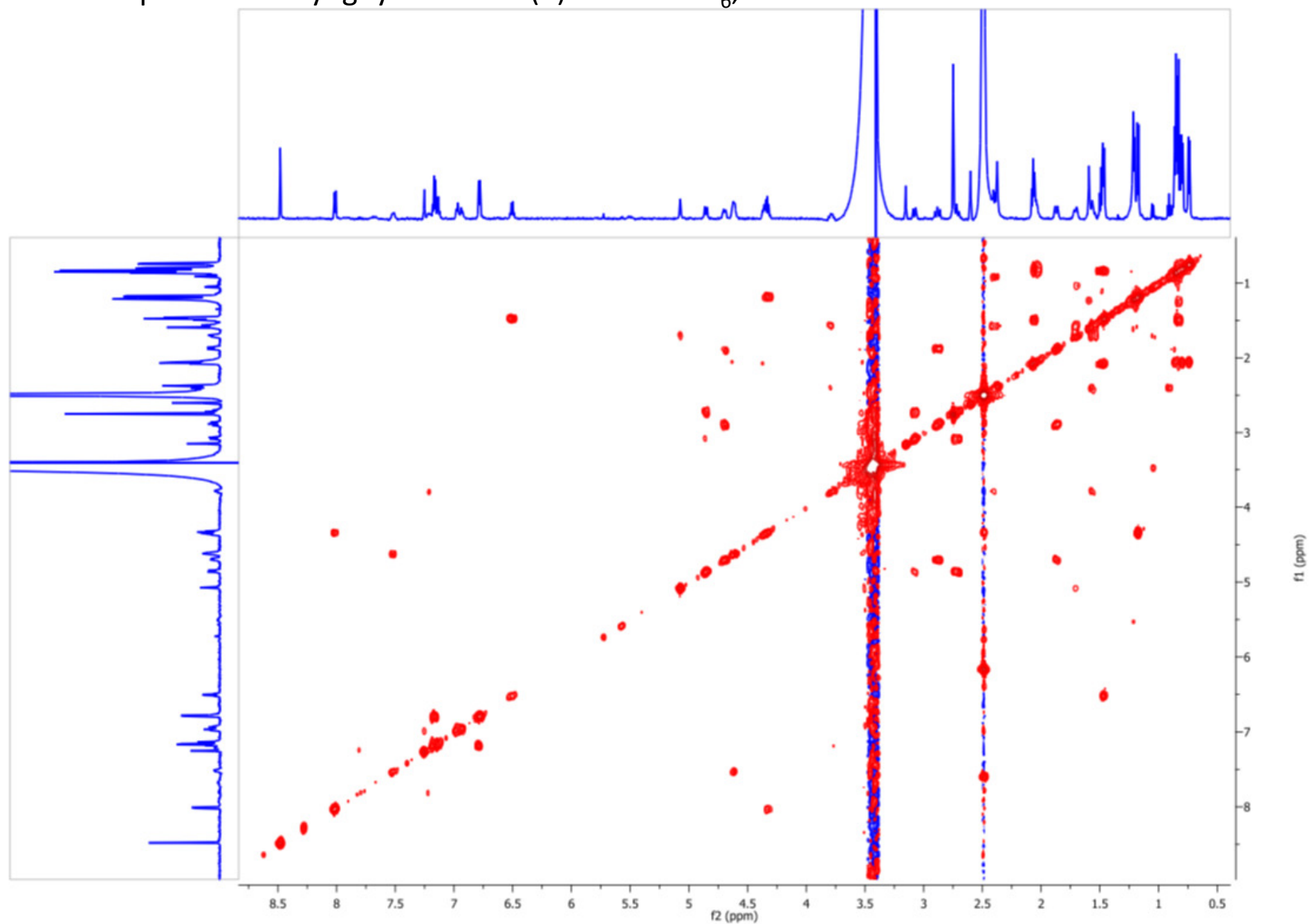
S 17



^1H NMR Spectrum of Lyngbyastatin 10 (**3**) in $\text{DMSO}-d_6$, 600 MHz

S 18





Edited HSQC Spectrum of Lyngbyastatin 10 (**3**) in DMSO- d_6 , 600 MHz

S 20

