

Supporting Information

Lyngbyoic Acid, a “Tagged” Fatty Acid from a Marine Cyanobacterium, Disrupts Quorum Sensing in *Pseudomonas aeruginosa*

Jason C. Kwan, Theresa Meickle, Dheran Ladwa, Max Teplitski, Valerie Paul, and Hendrik Luesch

Department of Medicinal Chemistry, University of Florida, 1600 SW Archer Road, Gainesville, Florida
32610, Smithsonian Marine Station, 701 Seaway Drive, Fort Pierce, Florida 34949, Department of
Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, U. K., and Soil and Water Science
Department, Genetics Institute, University of Florida-Institute of Food and Agricultural Sciences,
Gainesville, Florida 32610

Contents	Page Number
Figure S1. Treatment of the <i>lacZ</i> -based <i>A. tumefaciens</i> TraR reporter with lyngbyoic acid (4)	S 3
Figure S2. Treatment of constitutively active reporter pTIM2442 with lyngbyoic acid (4)	S 3
Figure S3. Growth curves of <i>P. aeruginosa</i> PAO1 treated with EtOH and lyngbyoic acid (4)	S 3
Figure S4. Lineweaver-Burke plot of LasB inhibition by lyngbyoic acid (4)	S 4
Scheme S1. Effects of lyngbyoic acid (4) on selected genes	S 5
Table S1. Strains and plasmids used in this study	S 7
Table S2. Iron-regulated genes that are affected by lyngbyoic acid (4)	S 8
Table S3. Primers and probes used for RT-qPCR in this study	S 9
Table S4. Validation of GeneChip experiment by RT-qPCR	S 9
Supplementary References	S 10
¹ H NMR spectrum of lyngbyoic acid (4) in CDCl ₃ , 500 MHz	S 11
APT spectrum of lyngbyoic acid (4) in CDCl ₃ , 100 MHz	S 12
COSY spectrum of lyngbyoic acid (4) in CDCl ₃ , 500 MHz	S 13
HMQC spectrum of lyngbyoic acid (4) in CDCl ₃ , 500 MHz	S 14
HMBC spectrum of lyngbyoic acid (4) in CDCl ₃ , 500 MHz (Aliphatic region)	S 15
HMBC spectrum of lyngbyoic acid (4) in CDCl ₃ , 500 MHz (Carbonyl region)	S 16
TOCSY spectrum of lyngbyoic acid (4) in CDCl ₃ , 500 MHz	S 17

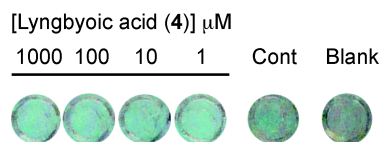


Figure S1. Treatment of the *lacZ*-based *A. tumefaciens* TraR reporter with **4** in the presence of 1 nM 3-oxo- C_8 -HSL. “Cont” refers to wells treated with 3-oxo- C_8 -HSL alone, and “Blank” refers to untreated wells.

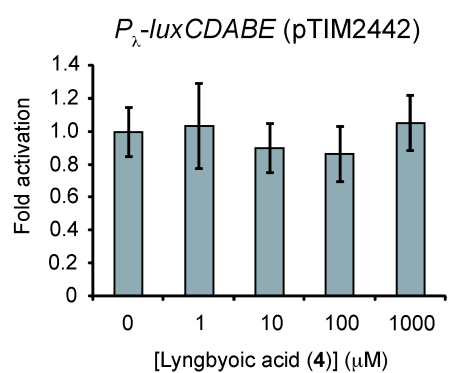


Figure S2. Treatment of constitutively active reporter pTIM2442 with **4**.

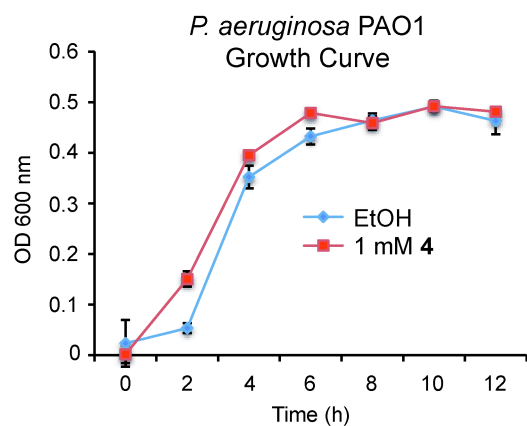


Figure S3. Growth curves of *P. aeruginosa* PAO1 treated with EtOH and lyngbyoic acid (**4**).

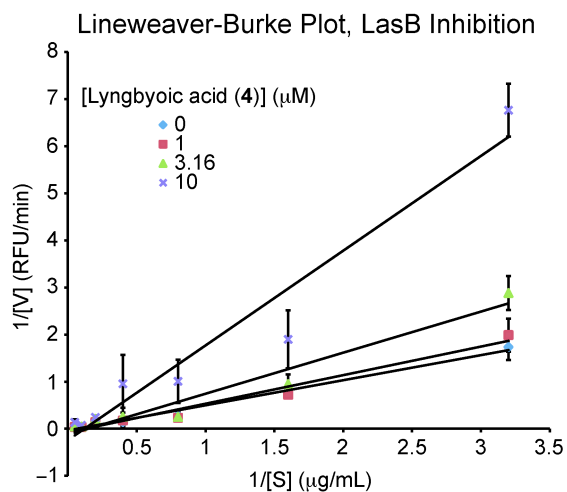
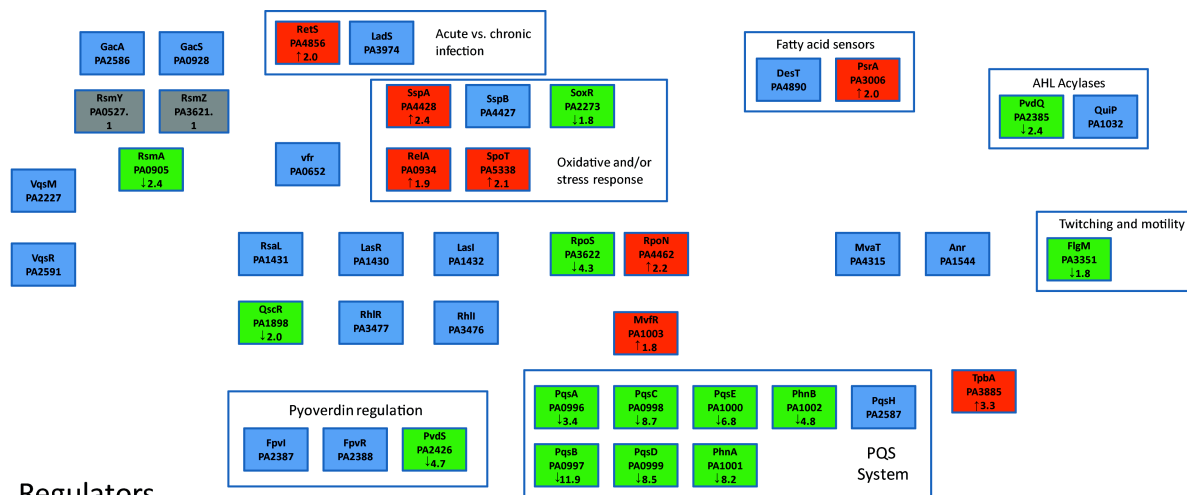
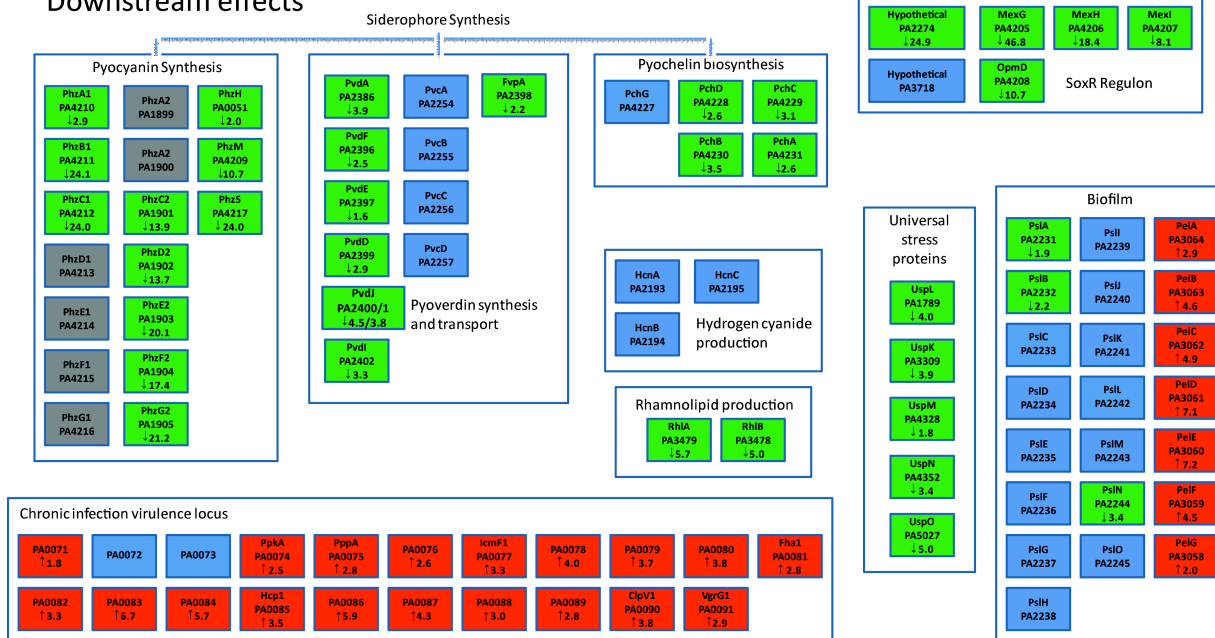


Figure S4. Lineweaver-Burke plot of LasB inhibition by lyngbyic acid (4)



Downstream effects



Scheme S1. Effects of lyngbyoic acid (**4**) on selected genes, categorized according to function. Green boxes depict downregulated transcripts, red boxes depict upregulated transcripts and blue boxes depict genes whose expression level was not significantly changed. Grey boxes depict small non-coding RNAs for which there were no probes on the microarray and also some pyocyanin biosynthetic genes which are identical to other genes (on the *P. aeruginosa* chromosome there are two copies of the *phzABCDEFG* operon).

Lyngbyoic acid (**4**) affected genes under the control of two fatty acid sensors, DesT (PA4890)¹ and PsrA (PA3006),² and also the level of *psrA* transcript was increased (+2.0 fold). Consistent with activation of the repressor DesT, the transcript levels of desaturases *desB* (PA4888) and *desC* (PA4889) were reduced (−3.2 and −2.9 fold, respectively). The fatty acid metabolism genes under control of the activator PsrA were upregulated, except for *lipA*, which could be a result of a direct action on PsrA or of its increased expression.

Compound **4** appeared to have complex effects on oxidative and/or stress response regulators. Transcripts of *relA* (PA0934), *spoT* (PA5338), and *sspA* (PA4428) were all upregulated (+1.9 to +2.4-fold). Also downregulated are the universal stress proteins *uspK* (PA3309, −3.9-fold), *uspN* (PA4352, −3.4-fold), *uspL* (PA1789, −4-fold), *uspM* (PA4328, −1.8-fold) and *uspO* (PA5027, −5-fold). PA3309 was shown to be under control of the oxygen-sensing regulator Anr³ (PA1544, no change in expression), while the other *usp* genes are dependent on SpoT.⁴ Two genes encoding arginine fermentation genes, ArcB and C (PA5172–PA5173), were also downregulated (−1.9 and −2.4-fold, respectively). These genes were previously shown to be upregulated during anaerobic growth.³ Another oxidative stress regulator, *soxR* (PA2273), was downregulated (−1.8-fold), as was the majority of its known regulon,⁵ the *mexGHID* efflux pump (PA4205–8, −8.1 to −46.8-fold) and the hypothetical protein PA2274 (−24.9-fold). This regulon has previously been identified as QS-controlled,⁶ and thus downregulation could be a result of general QS inhibition as well as reduction of *soxR*. Additionally, it has been shown that pyocyanin itself is able to activate SoxR,⁷ and thus some of the effect could be accounted for by reduced amounts of this metabolite. Overall, it would seem that **4** negatively regulates stress responses in PAO1.

Table S1. Strains and plasmids used in this study

Strain	Relevant Characteristics	Receptor	Cognate AHL	Source
<i>Escherichia coli</i> JM109				
pSB401	<i>luxR</i> ⁺ P _{luxI} - <i>luxCDABE</i> ; Tet ^r p15A origin	LuxR	3-oxo-C ₆ -HSL	Winson et al. 1998 ⁸
pSB536	<i>ahyR</i> ⁺ P _{ahyI} - <i>luxCDABE</i> ; Amp ^r ColE1 origin	AhyR	C ₄ -HSL	Winson et al. 1998 ⁸
pSB1075	<i>lasR</i> ⁺ P _{lasI} - <i>luxCDABE</i> ; Amp ^r ColE1 origin	LasR	3-oxo-C ₁₂ -HSL	Winson et al. 1998 ⁸
pTIM505	P _{lasI} - <i>luxCDABE</i> ; Amp ^r ColE1 origin	-		Rajamani et al. 2008 ⁹
pTIM5211	<i>lasR</i> ⁺ , Kan ^r ColE1 origin	LasR		Alagely et al. 2010 ¹⁰
pTIM5319	<i>lasR</i> ⁺ (ΔS13-S172), P _{lasI} - <i>luxCDABE</i> ; Amp ^r ColE1 origin	Truncated LasR, lacks AHL-binding domain		Rajamani et al. 2008 ⁹
<i>Escherichia coli</i> DH5α				
pTIM2442	P _λ - <i>luxCDABE</i> ; Amp ^r ColE1 origin Contains nt770-893 of pWD42-09, locus AF129072 cloned upstream of promoterless <i>luxCDABE</i> cassette. Constitutively luminescent construct	-		Alagely et al. 2010 ¹⁰
<i>Agrobacterium tumefaciens</i> NT1				
pZLR4	<i>traR</i> ⁺ <i>traG</i> :: <i>lacZ</i> ; Gm ^r	TraR	3-oxo-C ₈ -HSL	Shaw et al. 1997 ¹¹
<i>Pseudomonas aeruginosa</i> PAO1				
	Wild-type <i>P. aeruginosa</i>	LasR	3-oxo-C ₁₂ -HSL	Holloway et al. 1979 ¹²
		RhlR	C ₄ -HSL	
<i>Pseudomonas aeruginosa</i> PAO-JP2				
	Δ <i>lasI</i> Δ <i>rhlI</i>	LasR	3-oxo-C ₁₂ -HSL	Pesci et al. 1997 ¹³
		RhlR	C ₄ -HSL	

Table S2. Iron-regulated genes^a that are affected by lynnbyoic acid (4)

Gene	Gene name	Fold Change	Annotation
PA0266	gabT	-1.9	4-aminobutyrate aminotransferase
PA0471		-1.9	Probable transmembrane sensor
PA0500	bioB	2.9	Biotin synthase
PA0672	hemO	-4.2	Heme oxygenase
PA1003	mvfR	1.8	Transcriptional regulator
PA1249	aprA	-2.9	Alkaline metalloproteinase precursor
PA2112		36.1	Conserved hypothetical protein
PA2384		-2.4	Hypothetical protein
PA2385	pvdQ	-2.4	3-oxo-C ₁₂ -homoserine lactone acylase
PA2386	pvdA	-3.9	L-ornithine N5-oxygenase
PA2394	pvdN	-2.5	
PA2395	pvdO	-3.8	
PA2396	pvdF	-2.5	Pyoverdine synthetase
PA2398	fpvA	-2.2	Ferripyoverdine receptor
PA2399	pvdD	-2.9	Pyoverdine synthetase
PA2400	pvdJ	-4.5	
PA2401	pvdJ	-3.4	
PA2402		-3.3	Probable NRPS
PA2403		-2.2	Hypothetical protein
PA2411		-7.5	Probable thioesterase
PA2413	pvdH	-9.4	L-2,4-diaminobutyrate:2-ketoglutarate 4-aminotransferase
PA2424	pvdL	-2.3	
PA2426	pvdS	-4.7	Sigma factor
PA3165	hisC2	2.2	Histidinol-phosphate aminotransferase
PA3530		-5.7	Conserved hypothetical protein
PA4175	prpL	-2.7	PvdS regulated endoprotease (keratitis etc.)
PA4228	pchG	-2.6	Pyochelin biosynthetic protein
PA4229	pchC	-3.1	Pyochelin biosynthetic protein
PA4230	pchB	-3.5	Salicylate biosynthesis protein
PA4231	pchA	-2.6	Salicylate biosynthesis isochorismate synthase
PA4357	ssb	-3.4	Conserved hypothetical protein
PA4359		-5.0	Probable MFS transporter
PA4468	sodM	-4.1	Superoxide dismutase
PA4469		-6.3	Hypothetical protein
PA4470	fumC1	-4.4	Fumarate hydratase
PA4471		-2.0	Hypothetical protein
PA4500		5.2	Probable binding component of ABC transporter
PA4570		-3.9	Hypothetical protein
PA4708	phuT	-2.3	Heme transport protein
PA4709		-2.4	Probable hemin degrading factor
PA5314		-2.6	Hypothetical protein
PA5531	tonB	1.8	ton B

^aGenes previously identified as upregulated under iron-starvation in a microarray study.¹⁴

Table S3. Primers and probes used for RT-qPCR in this study

Gene	Forward	Reverse	Probe
<i>lasB</i> (PA3724)	5'-GCCTATTCGCCGCTGAAC-3'	5'-AGTCCCGGTACAGTTGAACAC-3'	5'-ACGCGCATTTCCTC-3'
<i>phzG1</i> (PA4216)	5'-CCCGCCGGGCTACTG-3'	5'-GCTTCCAGCCTCCTTCGT-3'	5'-ACTCCAGGCACAGTTC-3'
<i>retS</i> (PA4856)	5'-ACGCCAGCGGCTGAT-3'	5'-TCGCTGGTGCCTGTT-3'	5'-CCAGCAGCTCAACCTG-3'
<i>fadA5</i> (PA3013)	5'-GGCATGATGGGCCTGACT-3'	5'-CGCCTCACGGCTGATACC-3'	5'-TCTTGCCGAGCATTTC-3'
<i>lasR</i> (PA1430)	5'-TCCATCTACCAGACGCGAAAG-3'	5'-CGGCCGAGGCTTCCT-3'	5'-CAGCACGAGTTCTTCG-3'
<i>rpoD</i> (PA0576)	5'-GCGAGCGCATGGACATG-3'	5'-GGCTCTTTGGCGATCTTCAGT-3'	5'-ACCTTGCGGATCTTGT-3'
<i>rpsL</i> (PA4268)	5'-CTGCGTAAGGTATGCCGTGTA-3'	5'-CACCGATGTACGAGGAAACCT-3'	5'-CTGACCAACGGTTTCG-3'

Table S4. Validation of GeneChip experiment by RT-qPCR

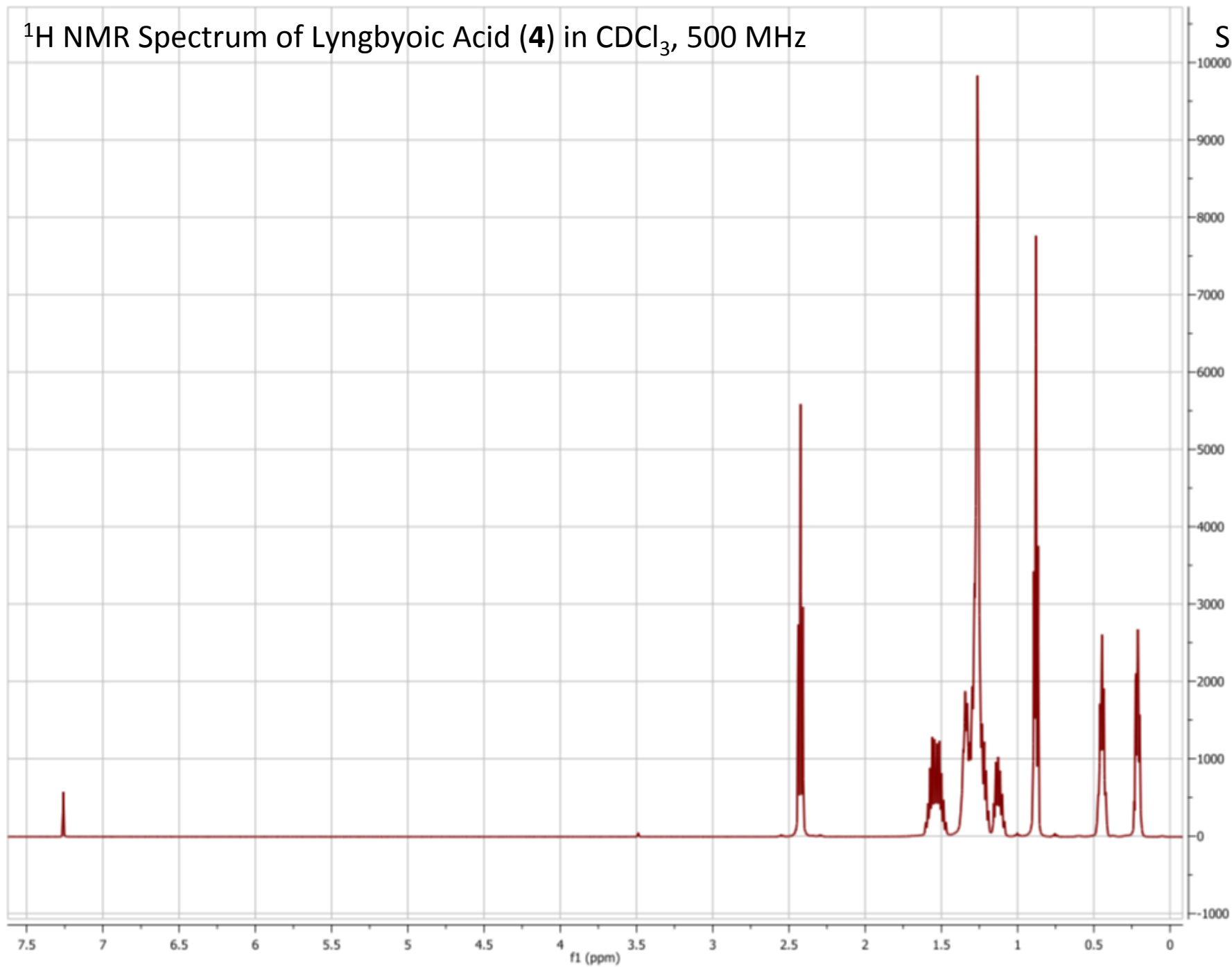
Gene	Fold change (RT-qPCR)	Fold change (GeneChip)
<i>lasB</i> (PA3724)	-34.9	-13.8
<i>phzG1</i> (PA4216)	-13.5	-21.2
<i>retS</i> (PA4856)	+2.2	+2.0
<i>fadA5</i> (PA3013)	+28.7	+18.2
<i>lasR</i> (PA1430)	+1.05	+1.02

Supplementary References

- 1 K. Zhu, K.-H. Choi, H. P. Schweizer, C. O. Rock, Y.-M. Zhang, *Mol. Microbiol.* 2006, **60**, 260–273.
- 2 Y. Kang, D. T. Nguyen, M. S. Son, T. T. Hoang, *Microbiol.* 2008, **154**, 1584–1598.
- 3 K. Schreiber, N. Boes, M. Eschbach, L. Jaensch, J. Wehland, T. Bjarnsholt, M. Givskov, M. Hentzer, M. Schobert, *J. Bacteriol.* 2006, **188**, 659–668.
- 4 N. Boes, K. Schreiber, M. Schobert, *J. Bacteriol.* 2008, **190**, 7189–7199.
- 5 M. Palma, J. Zurita, J. A. Ferreras, S. Worgall, D. H. Larone, L. Shi, F. Campagne, L. E. N. Quadri, *Infect. Immun.* 2005, **73**, 2958–2966.
- 6 M. Schuster, C. P. Lostroh, T. Ogi, E. P. Greenberg, *J. Bacteriol.* 2003, **185**, 2066–2079.; V. E. Wagner, D. Bushnell, L. Passador, A. I. Brooks, B. H. Iglewski, *J. Bacteriol.* 2003, **185**, 2080–2095.
- 7 L. E. P. Dietrich, A. Price-Whelan, A. Petersen, M. Whiteley, D. K. Newman, *Mol. Microbiol.* 2006, **61**, 1308–1321.
- 8 M. K. Winson, S. Swift, L. Fish, J. P. Throup, F. Jørgensen, S. R. Chhabra, B. W. Bycroft, P. Williams, G. S. A. B. Stewart, *FEMS Microbiol. Lett.* 1998, **163**, 185–192.
- 9 S. Rajamani, W. D. Bauer, J. B. Robinson, J. M. Farrow, E. C. Pesci, M. Teplitski, M. Gao, R. T. Sayre, D. A. Phillips, *Mol. Plant-Microbe Interact.* 2008, **21**, 1184–1192.
- 10 A. Alagely, S. Rajamani, M. Teplitski, *Methods Mol. Biol.* 2011, **692**, 113–130.
- 11 P. D. Shaw, G. Ping, S. L. Daly, C. Cha, J. E. Cronan, K. L. Rinehart, S. K. Farrand, *Proc. Natl. Acad. Sci. U. S. A.* 1997, **94**, 6036–6041.
- 12 B. W. Holloway, V. Krishnapillai, A. F. Morgan, *Microbiol. Rev.* 1979, **43**, 73–102.
- 13 E. C. Pesci, J. P. Pearson, P. C. Seed, B. H. Iglewski, *J. Bacteriol.* 1997, **179**, 3127–3132.
- 14 M. Palma, S. Worgall, L. E. N. Quadri, *Arch. Microbiol.* 2003, **180**, 374–379.

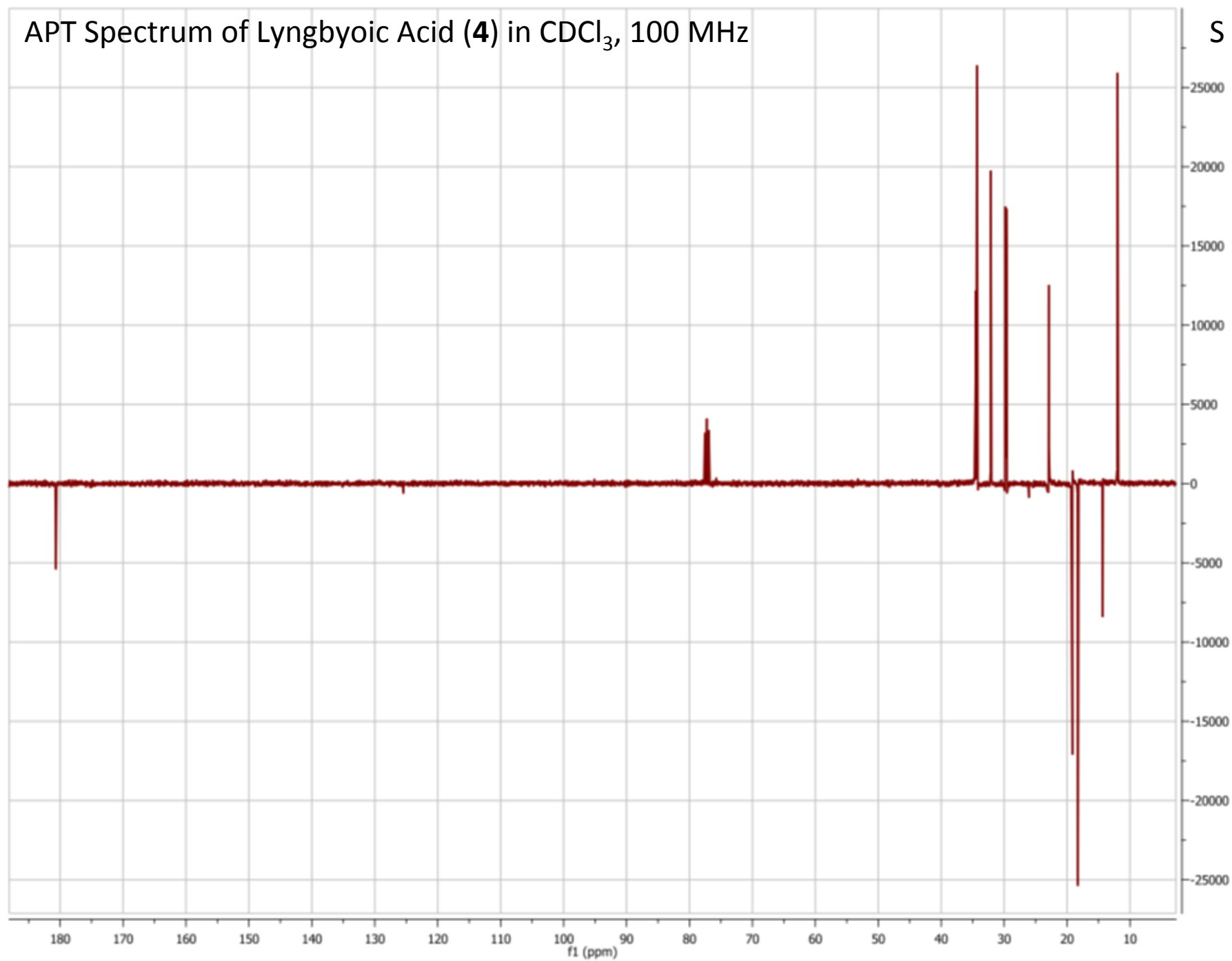
^1H NMR Spectrum of Lyngbyoic Acid (**4**) in CDCl_3 , 500 MHz

S 11



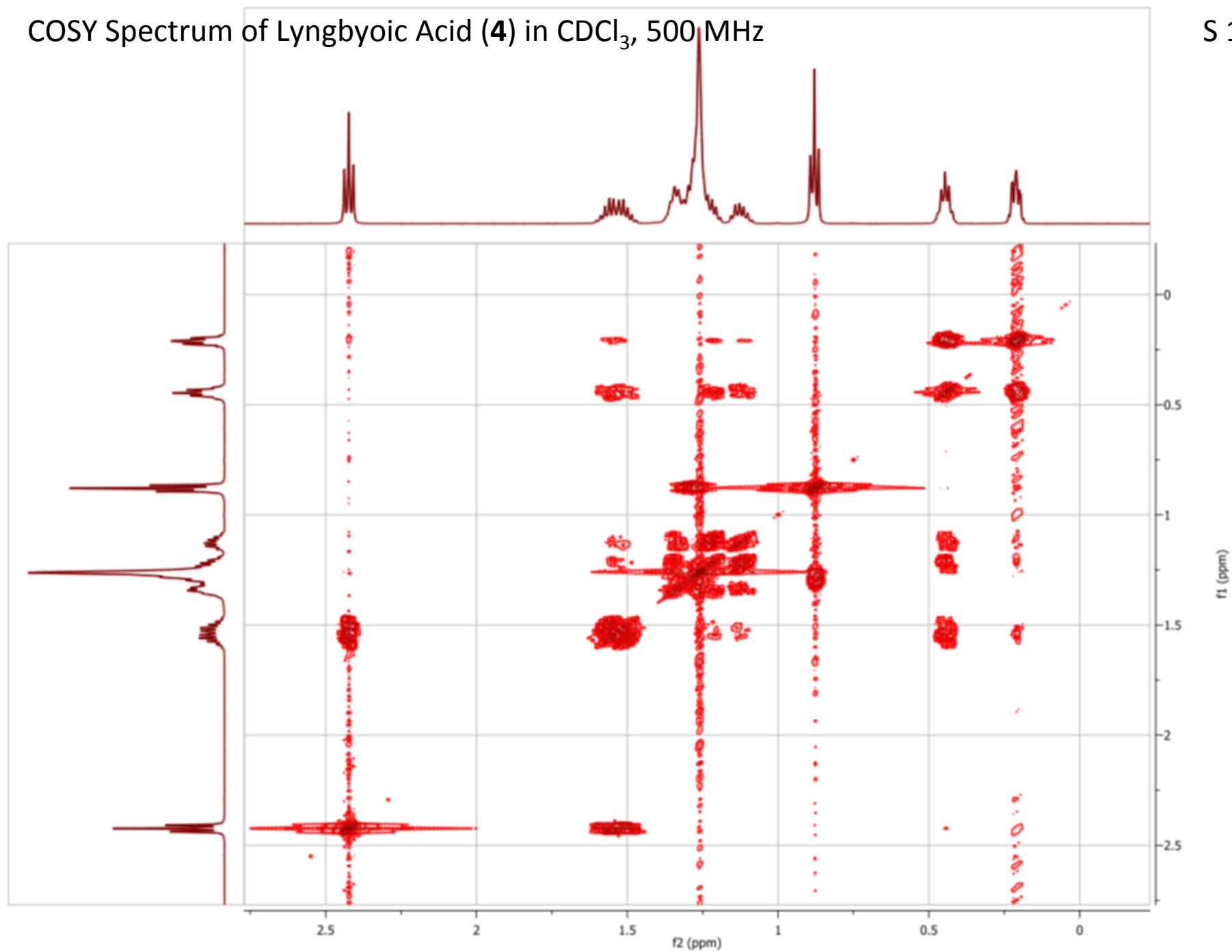
APT Spectrum of Lyngbyoic Acid (**4**) in CDCl₃, 100 MHz

S 12



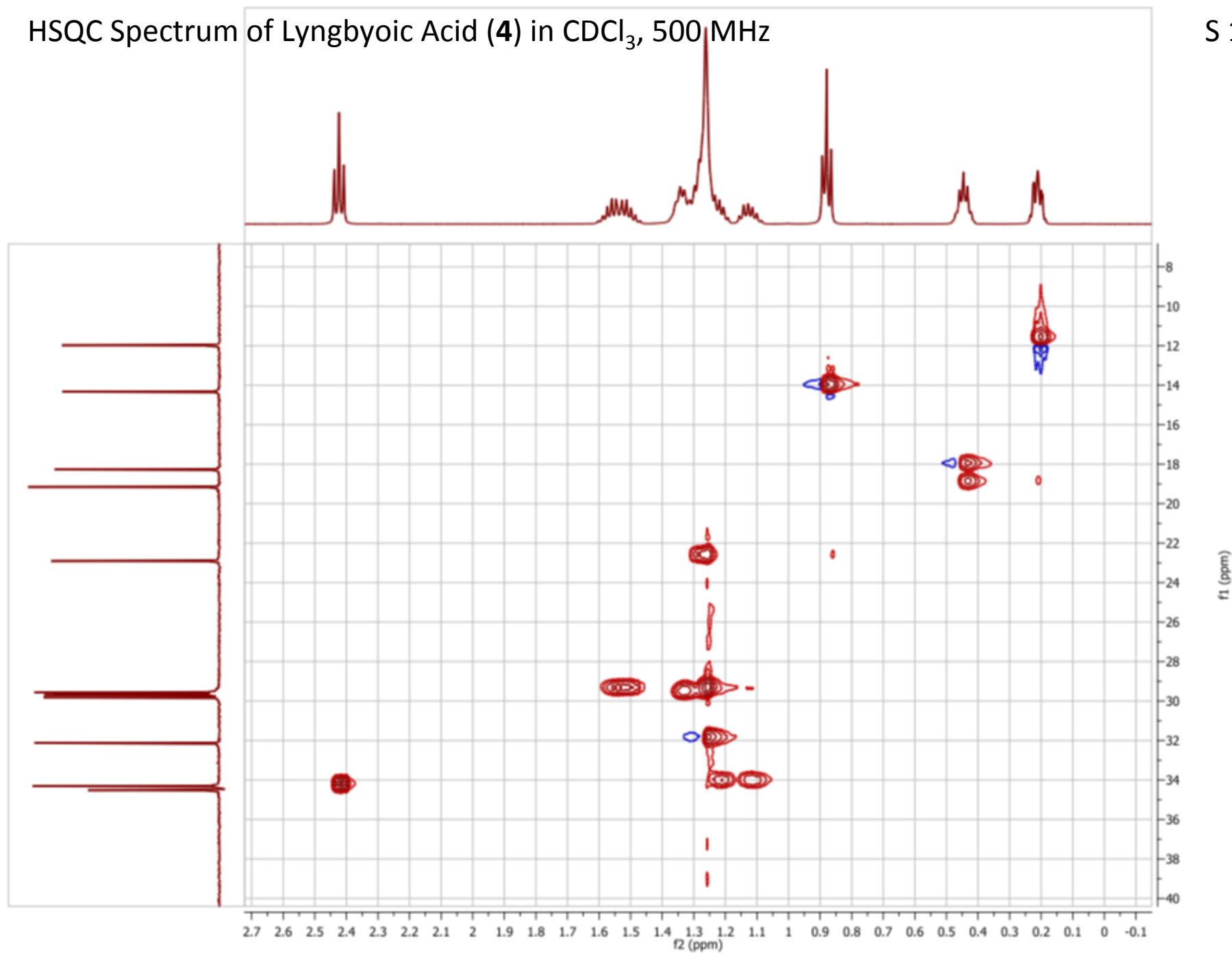
COSY Spectrum of Lyngbyoic Acid (**4**) in CDCl₃, 500 MHz

S 13



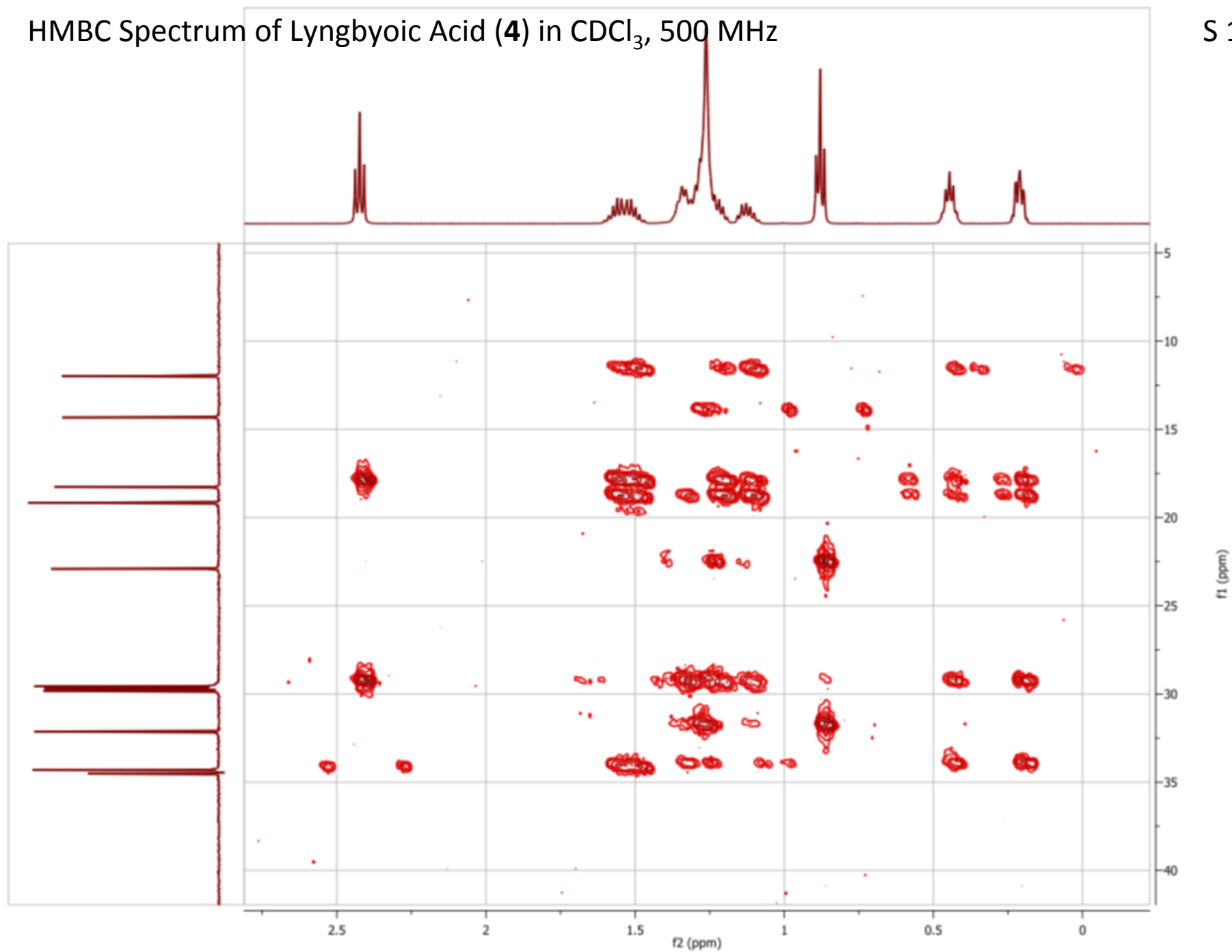
HSQC Spectrum of Lyngbyoic Acid (**4**) in CDCl₃, 500 MHz

S 14



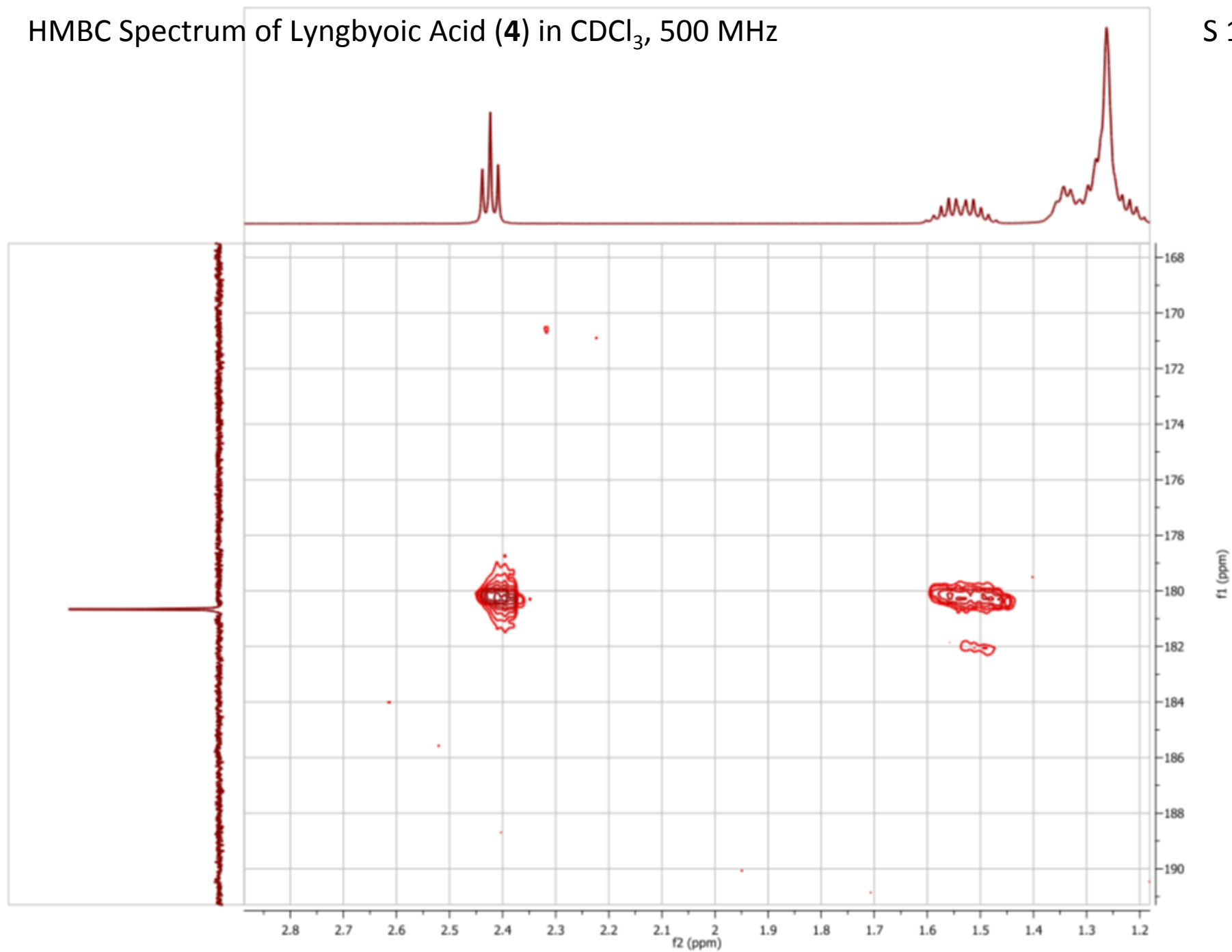
HMBC Spectrum of Lyngbyoic Acid (**4**) in CDCl₃, 500 MHz

S 15



HMBC Spectrum of Lyngbyoic Acid (**4**) in CDCl₃, 500 MHz

S 16



TOCSY Spectrum of Lyngbyoic Acid (4) in CDCl₃, 500 MHz

S 17

