Supplementary Material (ESI) for Molecular BioSystems
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Supporting Information

For

Heterologous production of thiostrepton A and biosynthetic engineering of thiostrepton analogs

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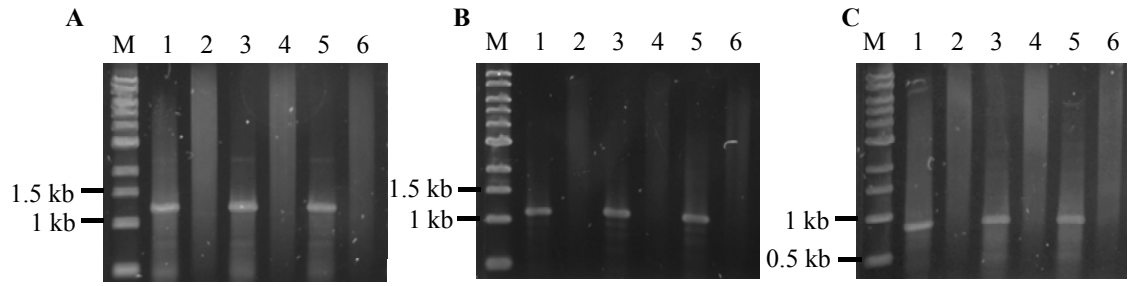


Figure S1. PCR analysis of *S. actuosus* FZ1 and *S. lividans* FZ1. (**A**) Amplification of *tsrK* using primers TSRK-F and TSRK-R. (**B**) Amplification of *tsrV* using primers TSRV-F and TSRV-R. (**C**) Amplification of *tsrN* using primers TSRN-F and TSRN-R. Lanes: (*M*) 1 kb ladder; (*1*) JA3A10; (*2*) pCC1FOS; (*3*) *S. actuosus* FZ1; (*4*) *S. actuosus* FZ2; (*5*) *S. lividans* FZ1; (*6*) *S. lividans* FZ2.

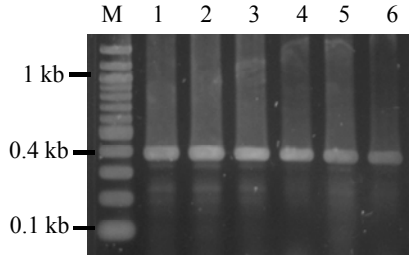
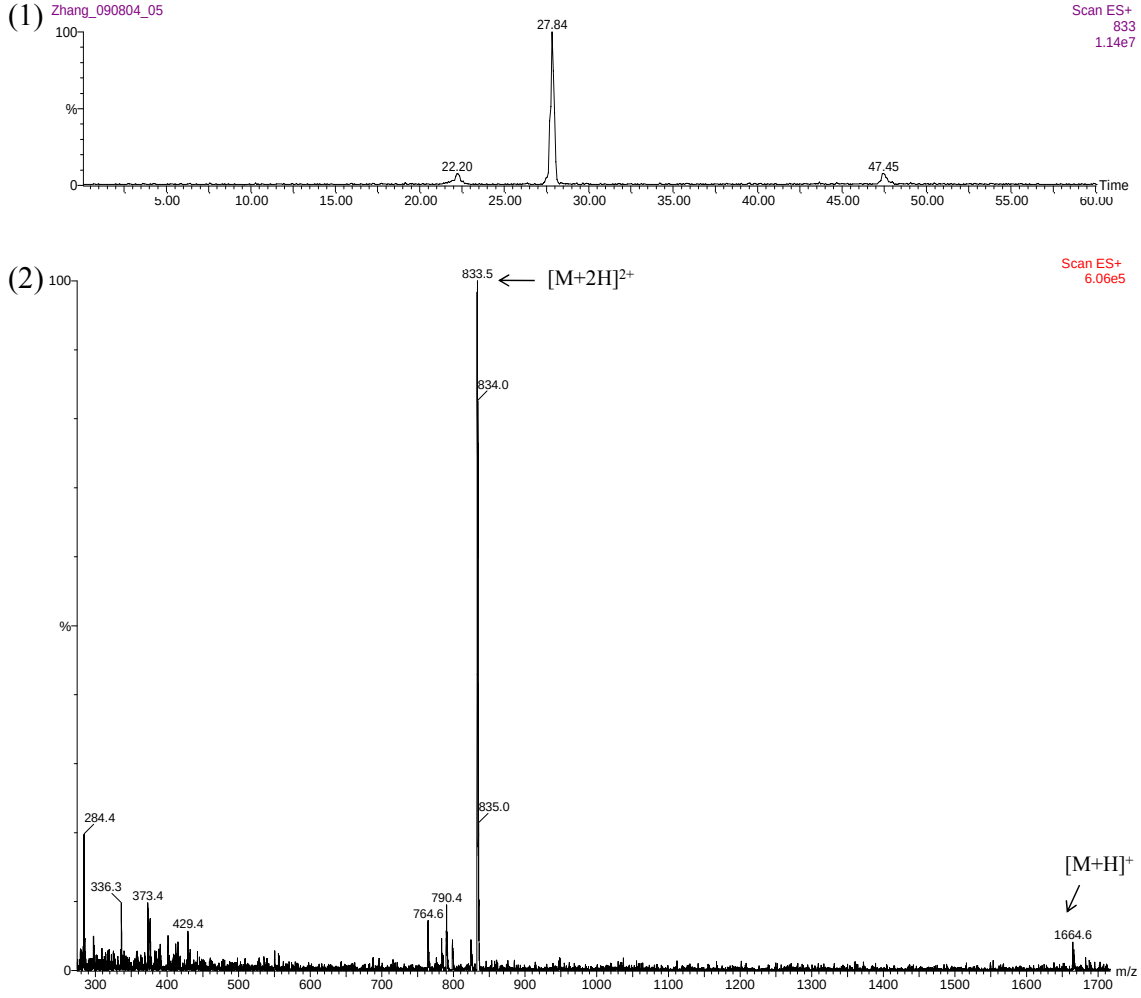


Figure S2. PCR analysis of *S. actuosus* FZ2 and *S. lividans* FZ2 using primers CTSR3-F and CTSR3-R. Lanes: (*M*) 1 kb ladder; (*1*) JA3A10; (*2*) pCC1FOS; (*3*) *S. actuosus* FZ1; (*4*) *S. actuosus* FZ2; (*5*) *S. lividans* FZ1; (*6*) *S. lividans* FZ2.

Figure S3. HPLC-MS of culture extracts from *S. actuosus* FZ1 and *S. actuosus* FZ2.

(**A**) HPLC-MS of the culture extract from *S. actuosus* FZ1. (*1*) Chromatogram extracted for *m*/*z* 833 $[M+2H]^{2+}$ from *S. actuosus* FZ1. (*2*) Mass spectrum of thiostrepton A from *S. actuosus* FZ1 eluting at t_R = 27.8 min.



(**B**) HPLC-MS chromatogram extracted for *m*/*z* 833 $[M+2H]^{2+}$ from the *S. actuosus* FZ2 culture extract.

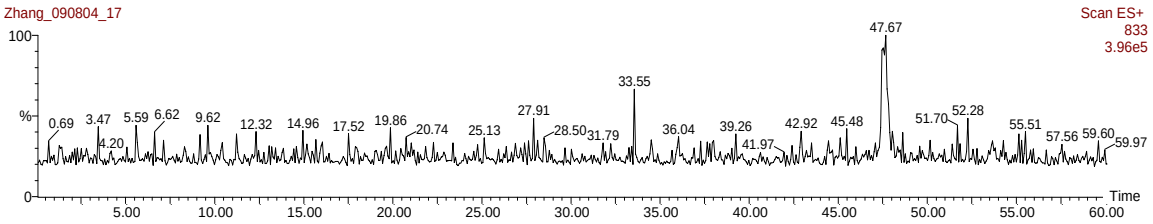
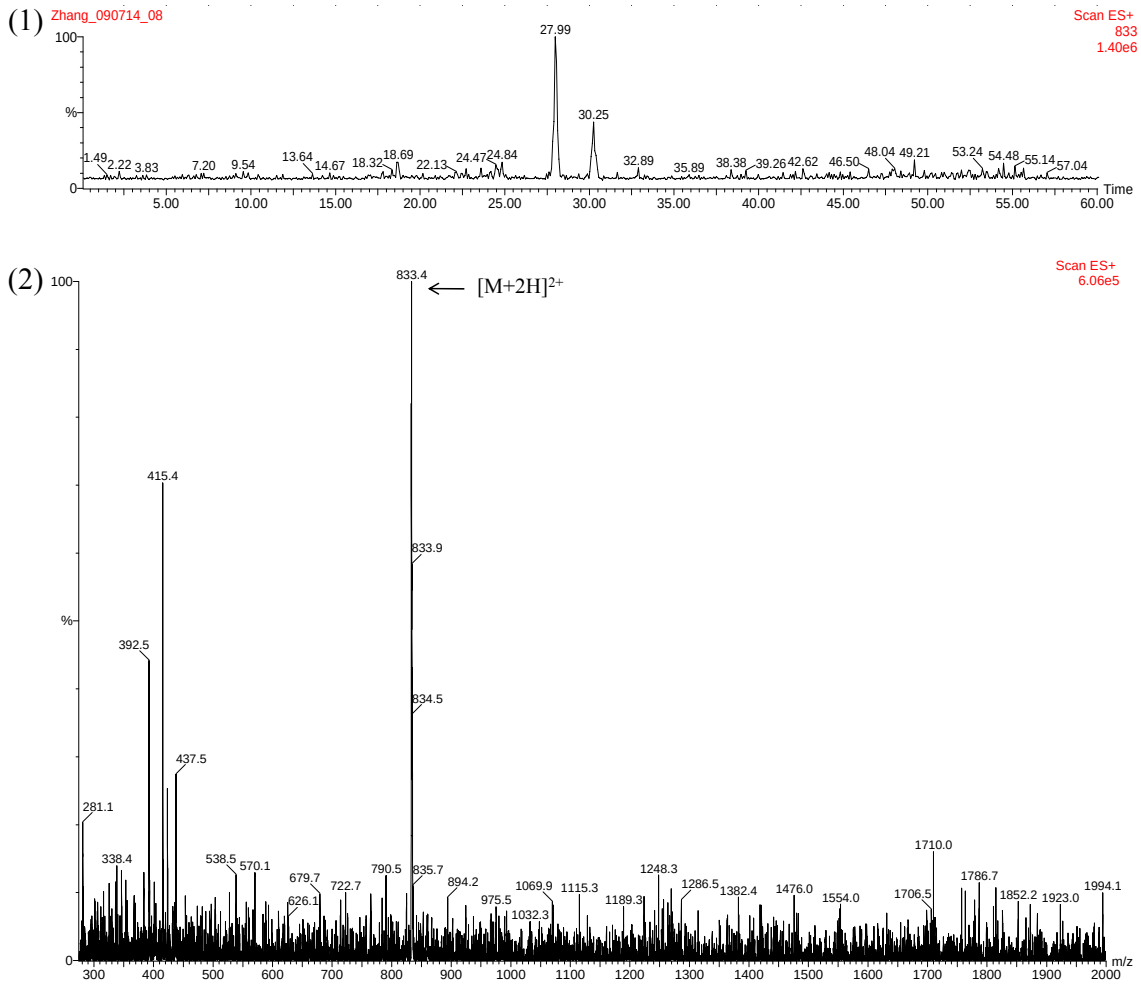
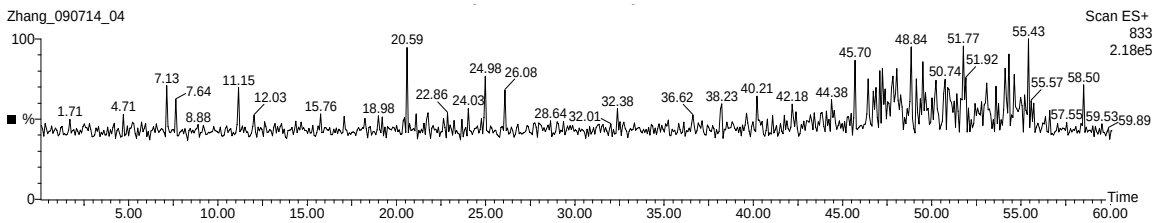


Figure S4. HPLC-MS of culture extracts from *S. lividans* FZ1 and *S. lividans* FZ2.

(**A**) HPLC-MS of the culture extract from *S. lividans* FZ1. (*1*) HPLC-MS chromatogram extracted for *m*/*z* 833 $[M+2H]^{2+}$ from *S. lividans* FZ1. (*2*) Mass spectrum of thiostrepton A from *S. lividans* FZ1 eluting at t_R = 27.8 min.



(**B**) HPLC-MS chromatogram extracted for *m*/*z* 833 $[M+2H]^{2+}$ from the *S. lividans* FZ2 culture extract.



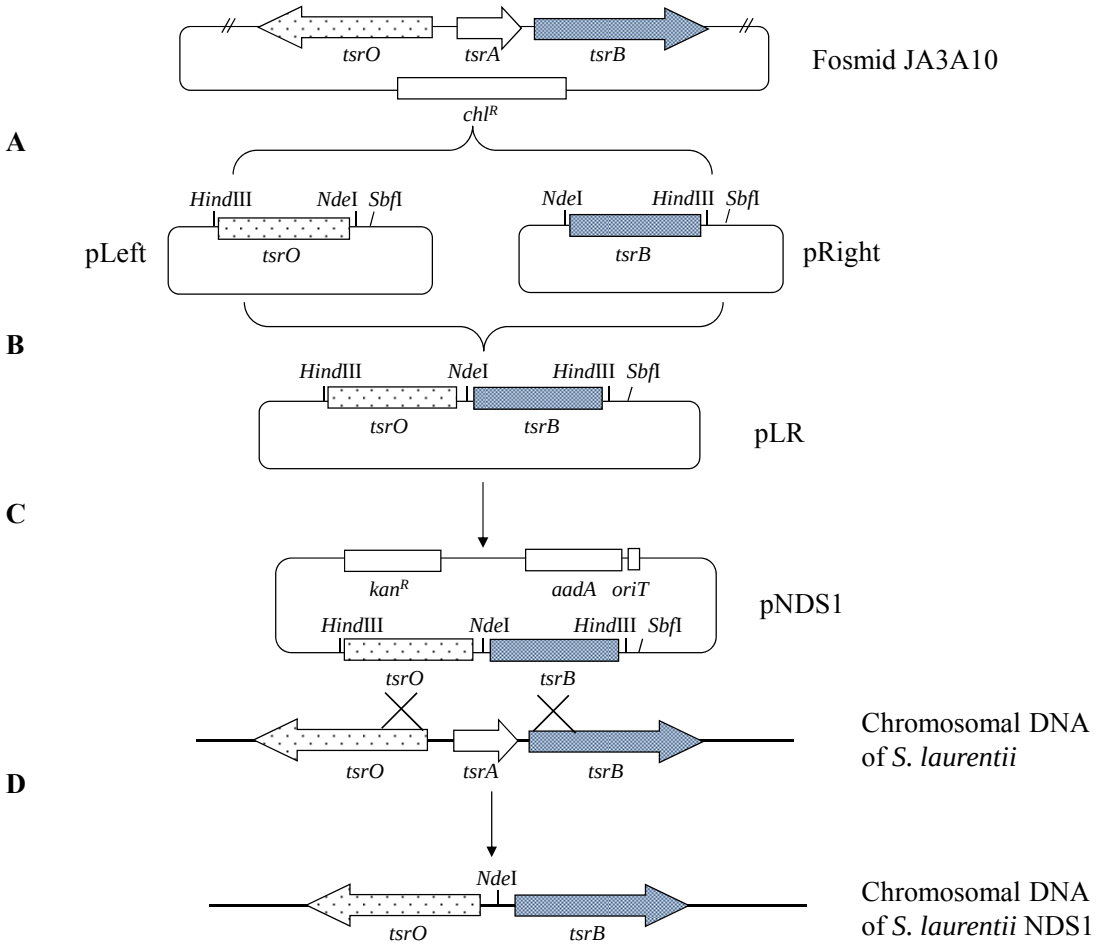


Figure S5. Strategy used to construct *S. laurentii* NDS1, an in-frame deletion mutant of *tsrA*. (*A*) The regions flanking *tsrA* were amplified by PCR from fosmid JA3A10, generating pLeft and pRight. The indicated restriction sites were engineered into the PCR primers. (*B*) The *Nde*I-*Sbf*I fragment from pRight was ligated into pLeft, generating pLR. (*C*) The *Hind*III fragment from pLR was ligated into pGM160HKss, generating pNDS1. (*D*) pNDS1 was introduced into *S. laurentii* by intergeneric conjugation. Homologous recombination between pNDS1 and the chromosome of *S. laurentii* gave rise to *S. laurentii* NDS1.

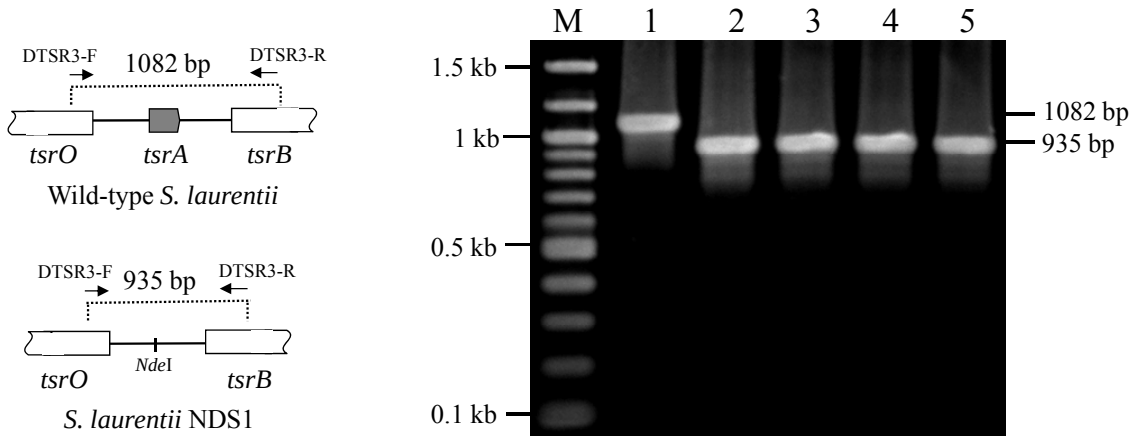


Figure S6. Confirmation of the *tsrA* deletion mutant *S. laurentii* NDS1 by PCR. (*M*) 100 bp DNA ladder; (*1*) genomic DNA of *S. laurentii*; (*2*) pNDS1; (*3*)-(*5*), genomic DNA purified from three colonies of *S. laurentii* NDS1.

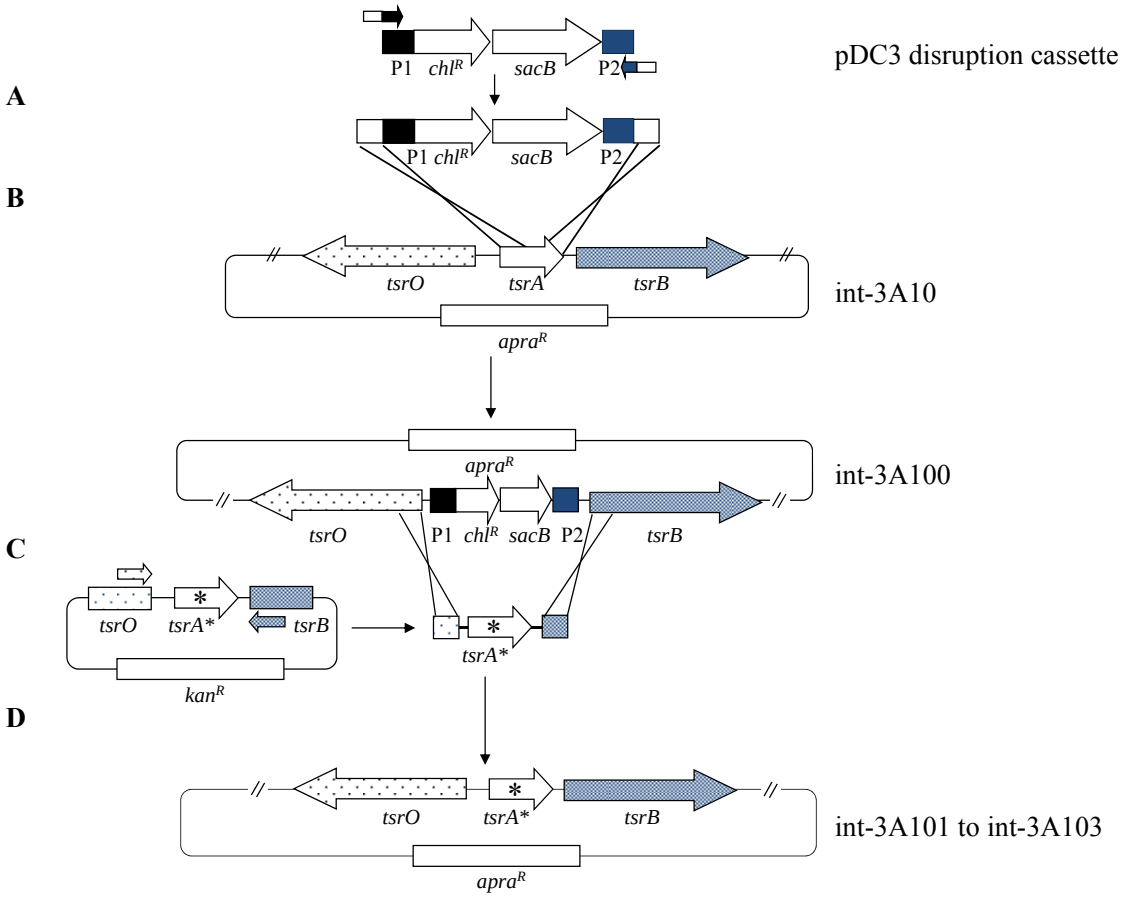
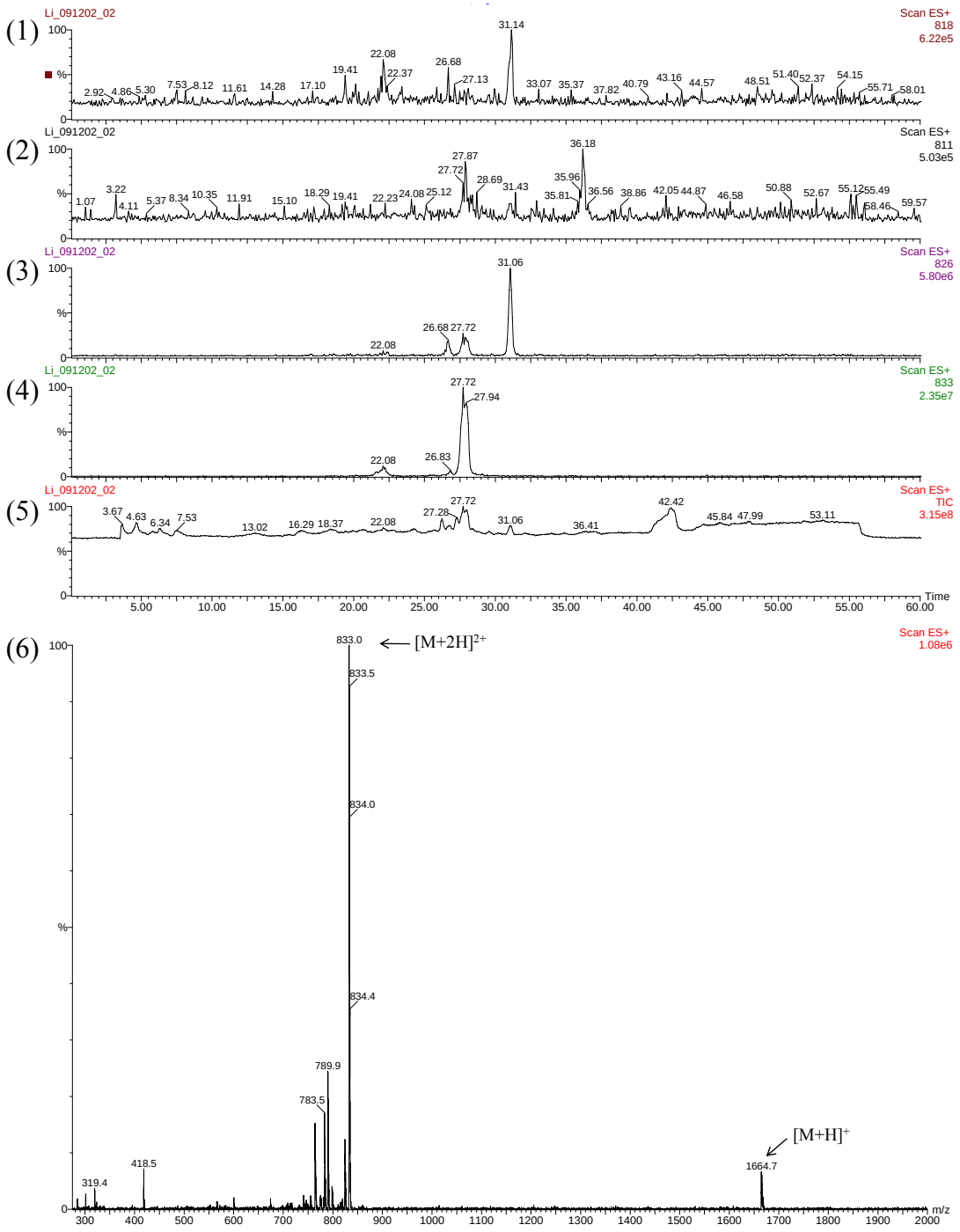


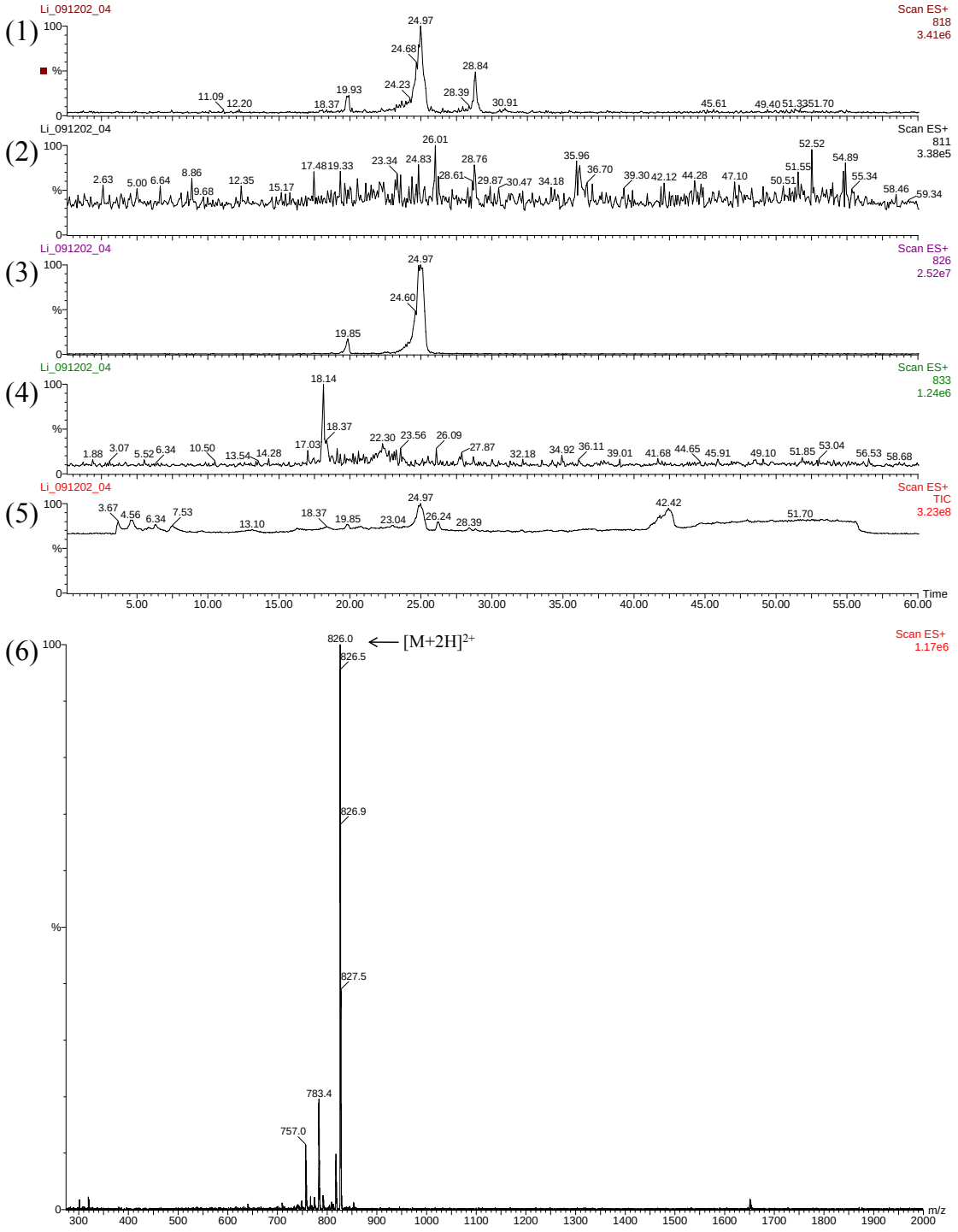
Figure S7. Strategy used to construct *tsrA* site-directed mutants in an integrative *E. coli-Streptomyces* shuttle fosmid. (*A*) The pDC3 disruption cassette was amplified by PCR with primers containing a 39 nt *tsrA* sequence at the 5' end of the primer and a 20 nt P1 or P2 sequence at the 3' end of the primer. (*B*) Homologous recombination between the PCR product and int-3A10 generated int-3A100. (*C*) *tsrA* mutant sequences were amplified by PCR from three plasmids, pCL61 to pCL63, as appropriate. (*D*) Homologous recombination between the PCR product and int-3A100 gave rise to a chloramphenicol-sensitive and sucrose-resistant strain of *E. coli* possessing the *tsrA* site-directed mutant in the fosmid.

Figure S8. HPLC-MS analysis of culture extracts from *S. laurentii tsrA* mutant strains.

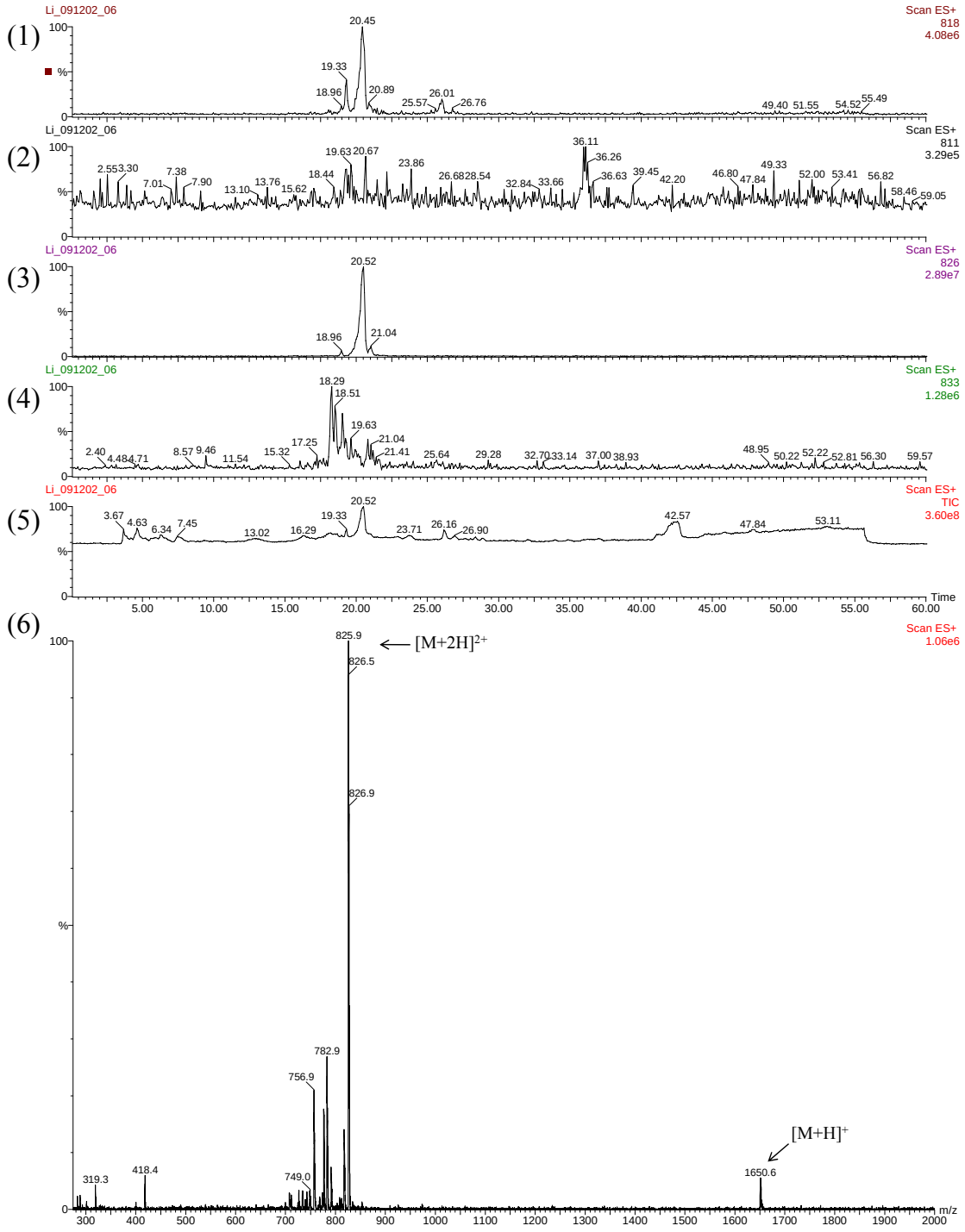
(**A**) HPLC-MS chromatogram of the *S. laurentii* NDS1 int-3A10 (thiostrepton A) culture extract. (*1*) Chromatogram extracted for *m*/*z* 818. (*2*) Chromatogram extracted for *m*/*z* 811. (*3*) Chromatogram extracted for *m*/*z* 826. (*4*) Chromatogram extracted for *m*/*z* 833. (*5*) Total ion chromatogram. (*6*) Mass spectrum of thiostrepton A from *S. laurentii* NDS1 int-3A10 eluting at t_R = 27.7 min (calculated *m/z* 832.7 $[M+2H]^{2+}$, observed *m/z* 833.0 $[M+2H]^{2+}$; calculated *m/z* 1664.5 $[M+H]^{+}$, observed *m/z* 1664.7 $[M+H]^{+}$).



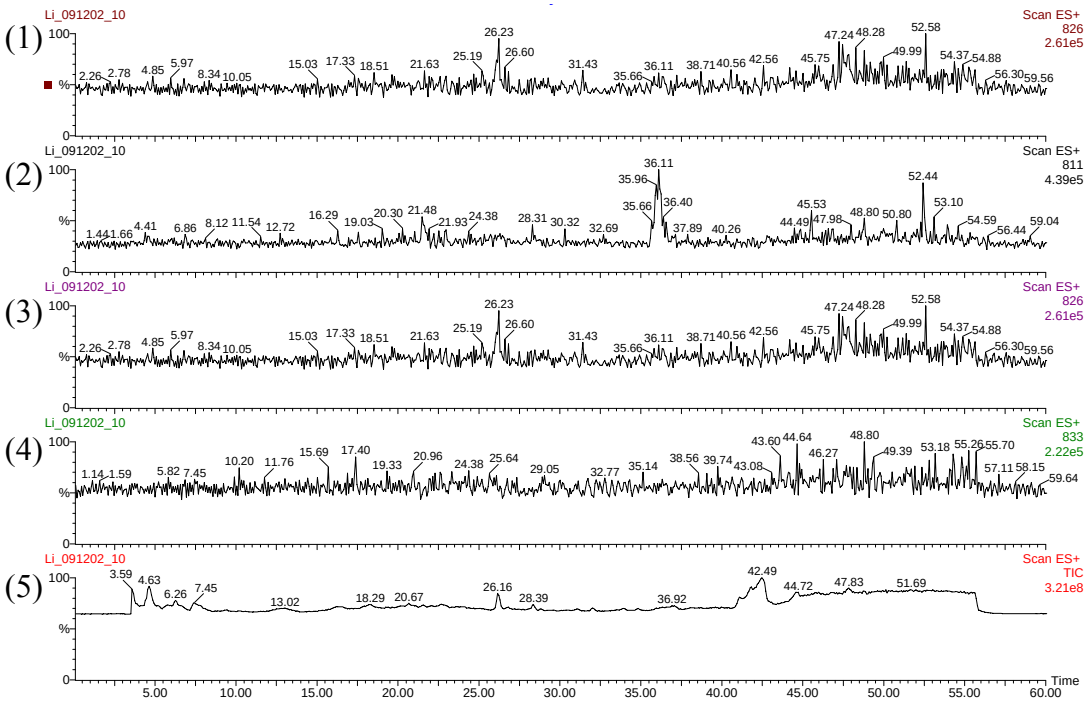
(**B**) HPLC-MS chromatogram of the *S. laurentii* NDS1 int-3A101 (thiostrepton Ala2Gly) culture extract. (*1*) Chromatogram extracted for *m*/*z* 818. (*2*) Chromatogram extracted for *m*/*z* 811. (*3*) Chromatogram extracted for *m*/*z* 826. (*4*) Chromatogram extracted for *m*/*z* 833. (*5*) Total ion chromatogram. (*6*) Mass spectrum of thiostrepton Ala2Gly from *S. laurentii* NDS1 int-3A101 eluting at t_R = 25.0 min (calculated *m*/*z* 825.7 $[M+2H]^{2+}$, observed *m*/*z* 826.0 $[M+2H]^{2+}$).



(**C**) HPLC-MS chromatogram of the *S. laurentii* NDS1 int-3A102 (thiostrepton Ala4Gly) culture extract. (*1*) Chromatogram extracted for *m*/*z* 818. (*2*) Chromatogram extracted for *m*/*z* 811. (*3*) Chromatogram extracted for *m*/*z* 826. (*4*) Chromatogram extracted for *m*/*z* 833. (*5*) Total ion chromatogram. (*6*) Mass spectrum of thiostrepton Ala4Gly from *S. laurentii* NDS1 int-3A102 eluting at t_R = 20.5 min (calculated *m*/*z* 825.7 $[M+2H]^{2+}$, observed *m*/*z* 826.0 $[M+2H]^{2+}$; calculated *m*/*z* 1650.5 $[M+H]^{+}$, observed *m*/*z* 1650.6 $[M+H]^{+}$).



(**D**) HPLC-MS chromatogram of the *S. laurentii* NDS1 int-3A103 (thiostrepton Thr7Gly) culture extract. (*1*) Chromatogram extracted for *m*/*z* 818. (*2*) Chromatogram extracted for *m*/*z* 811. (*3*) Chromatogram extracted for *m*/*z* 826. (*4*) Chromatogram extracted for *m*/*z* 833. (*5*) Total ion chromatogram.



(**E**) HPLC-MS chromatogram of the *S. laurentii* NDS1 culture extract. (*1*) Chromatogram extracted for *m*/*z* 833. (*2*) Total ion chromatogram.

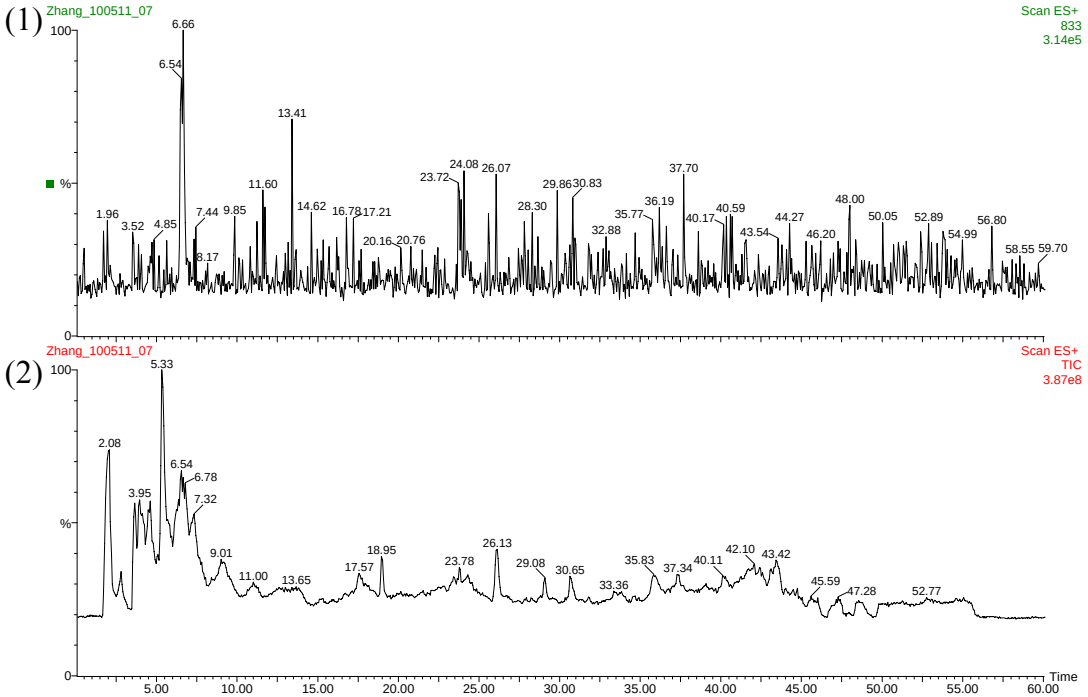
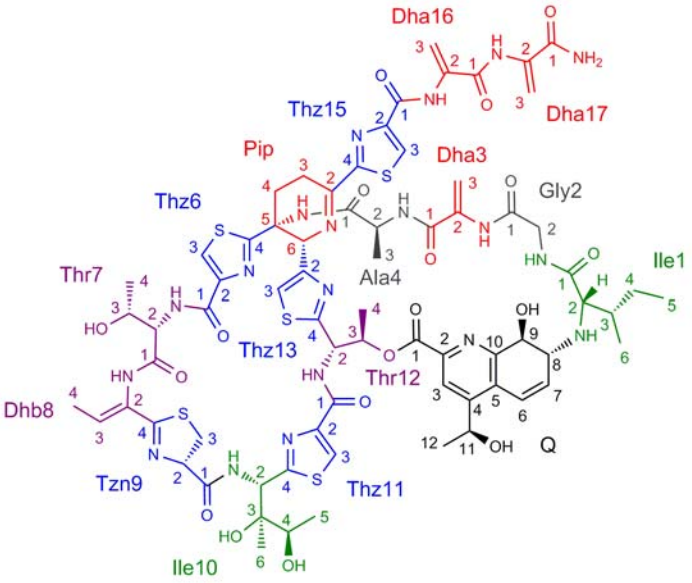


Figure S9. Structures of and numbering systems used for (**A**) thiostrepton Ala2Gly and (**B**) thiostrepton Ala4Gly.

(**A**) Thiostrepton Ala2Gly



(**B**) Thiostrepton Ala4Gly

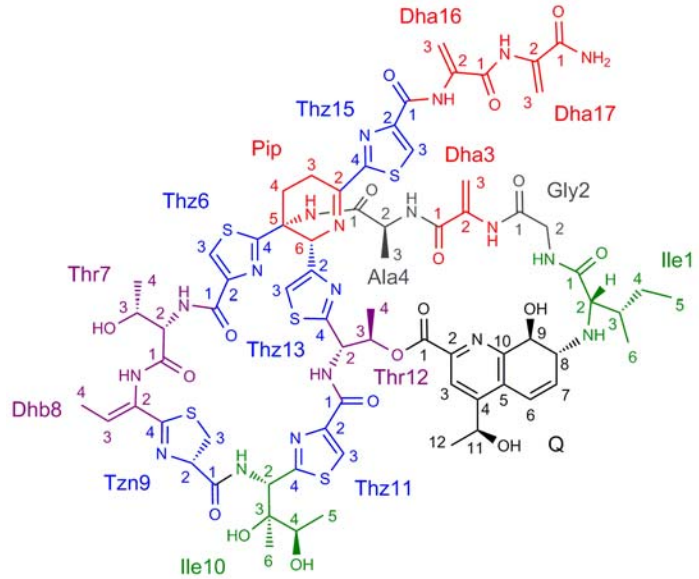
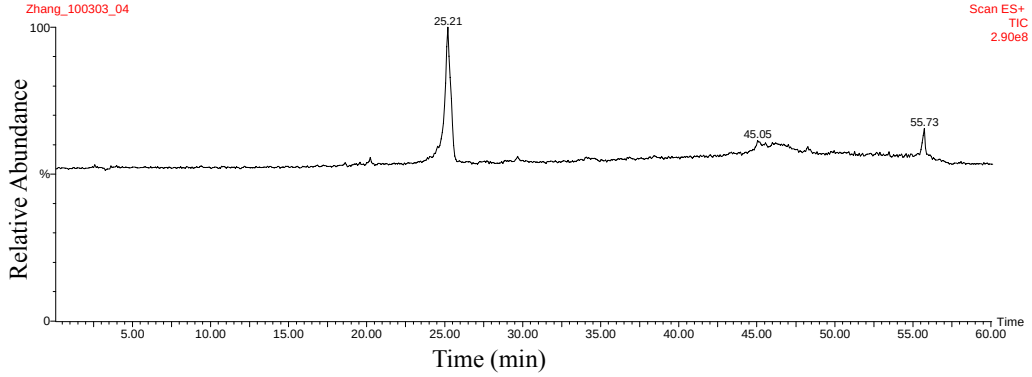
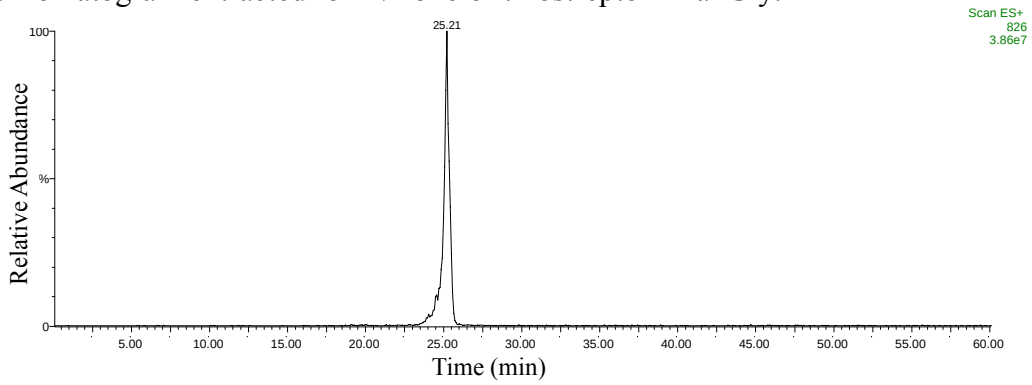


Figure S10. HPLC-MS of thiostrepton Ala2Gly isolated from *S. laurentii* NDS1 int-3A101.

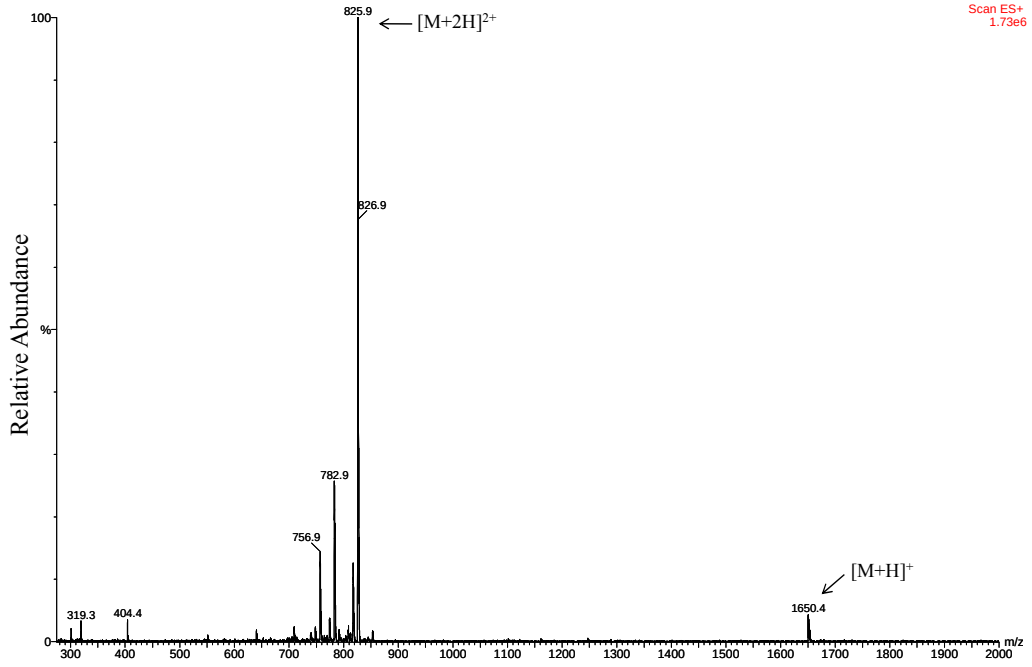
(**A**) Total ion chromatogram of thiostrepton Ala2Gly.



(**B**) Chromatogram extracted for *m*/*z* 826 of thiostrepton Ala2Gly.



(**C**) Mass spectrum of thiostrepton Ala2Gly eluting at t_R = 25.2 min.



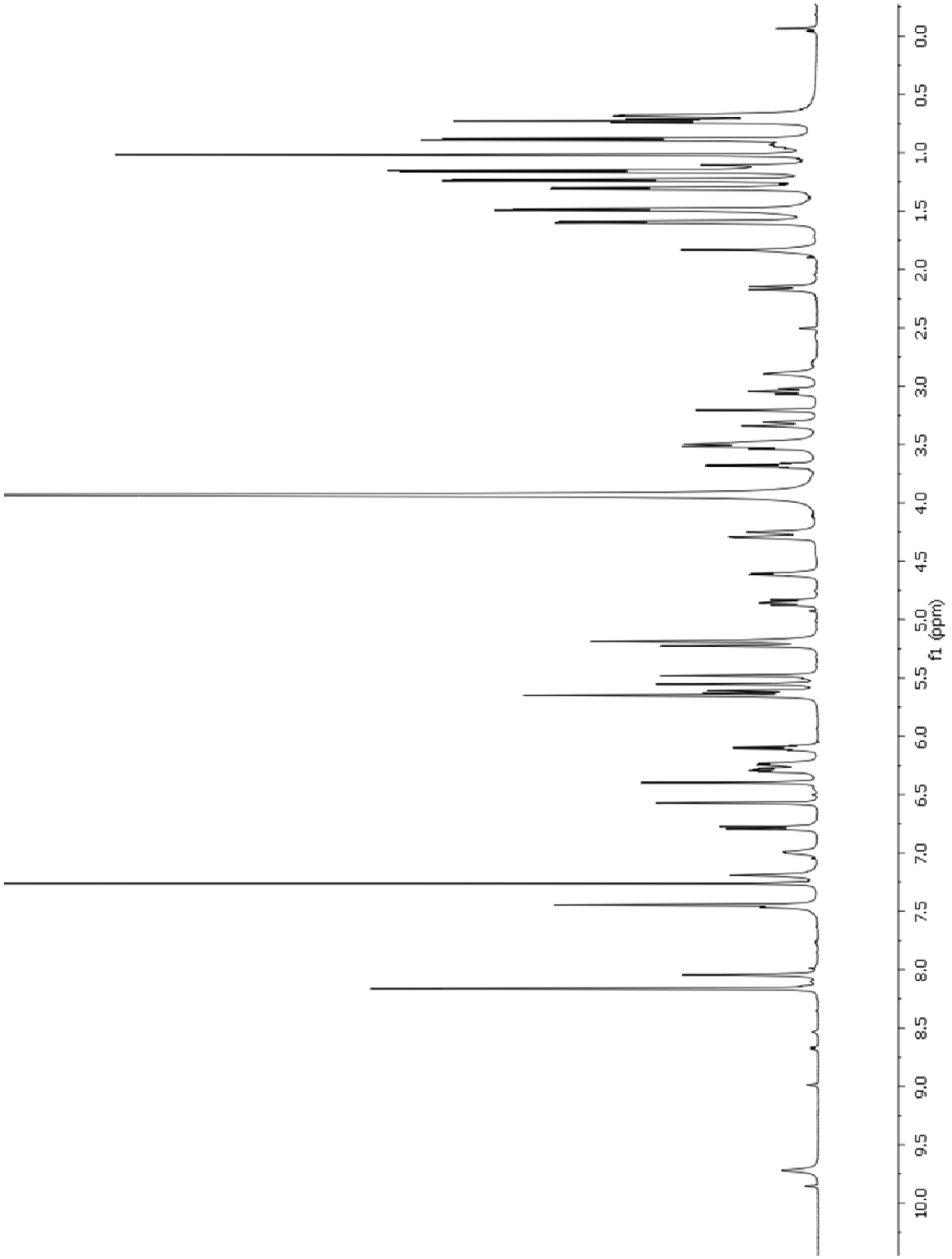


Figure S11. ^{1}H NMR spectrum of thiostrepton Ala2Gly (500 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

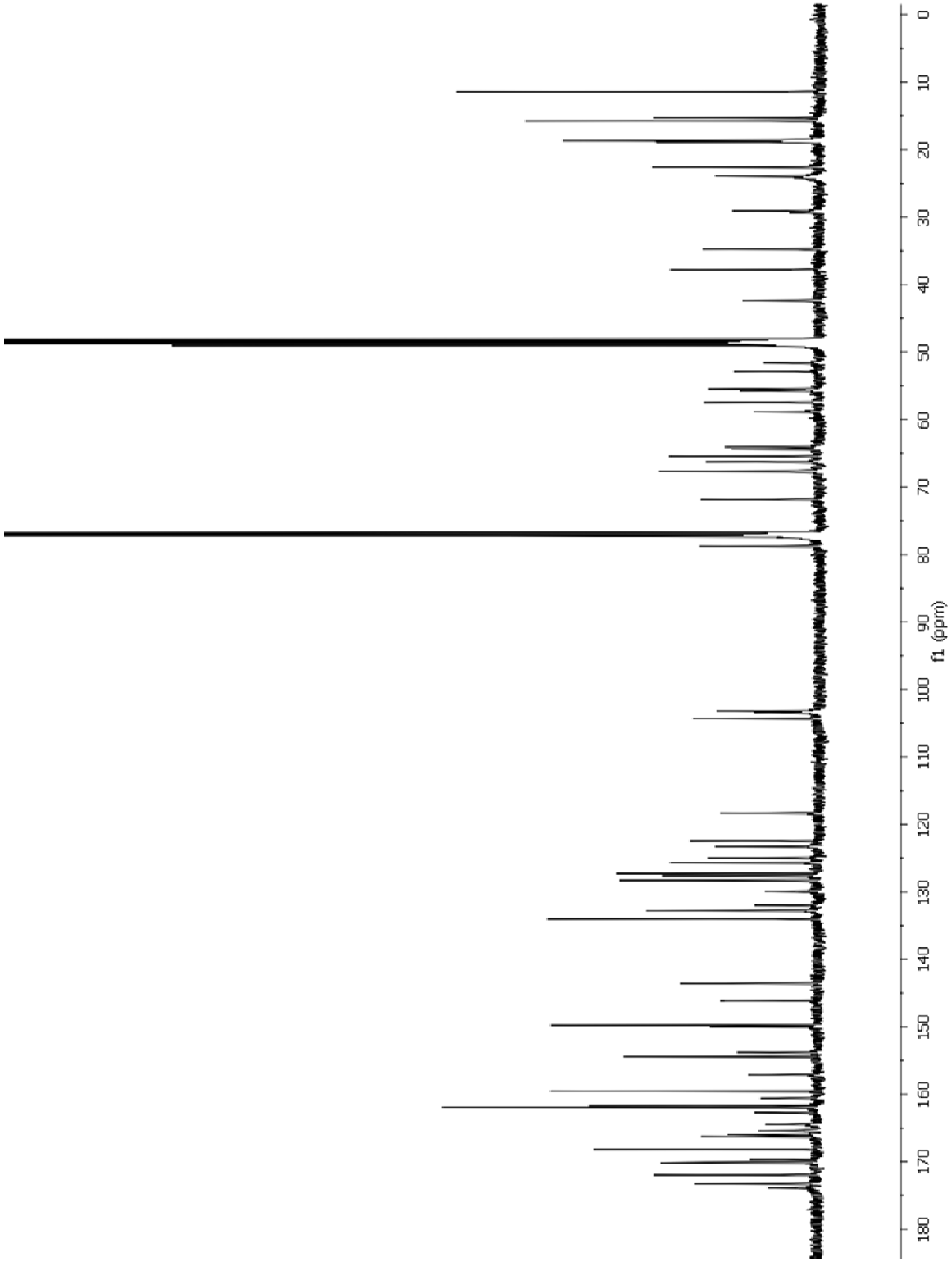


Figure S12. ^{13}C NMR spectrum of thiostrepton Ala2Gly (125 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

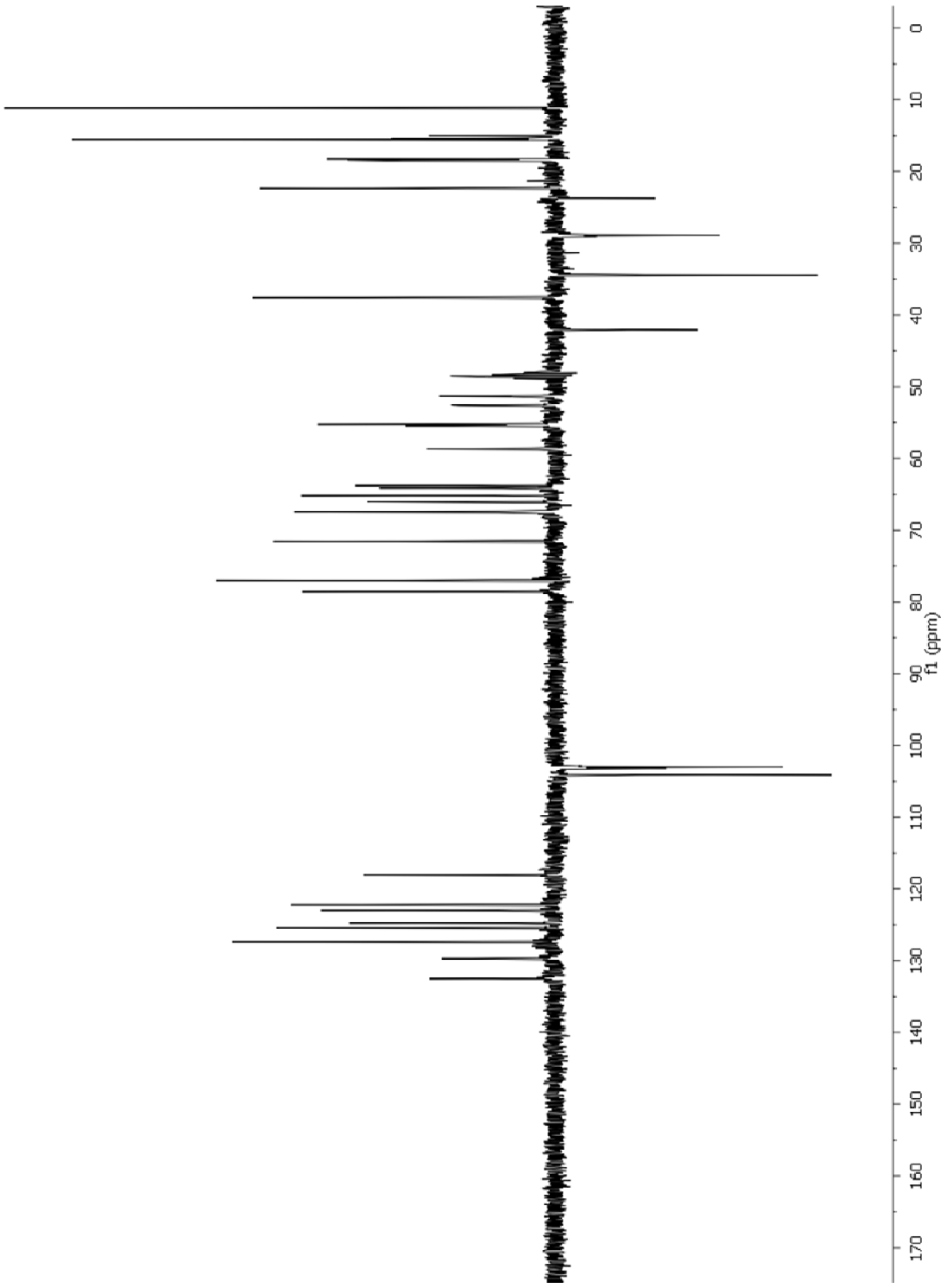


Figure S13. DEPT-135 NMR spectrum of thiostrepton Ala2Gly (125 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

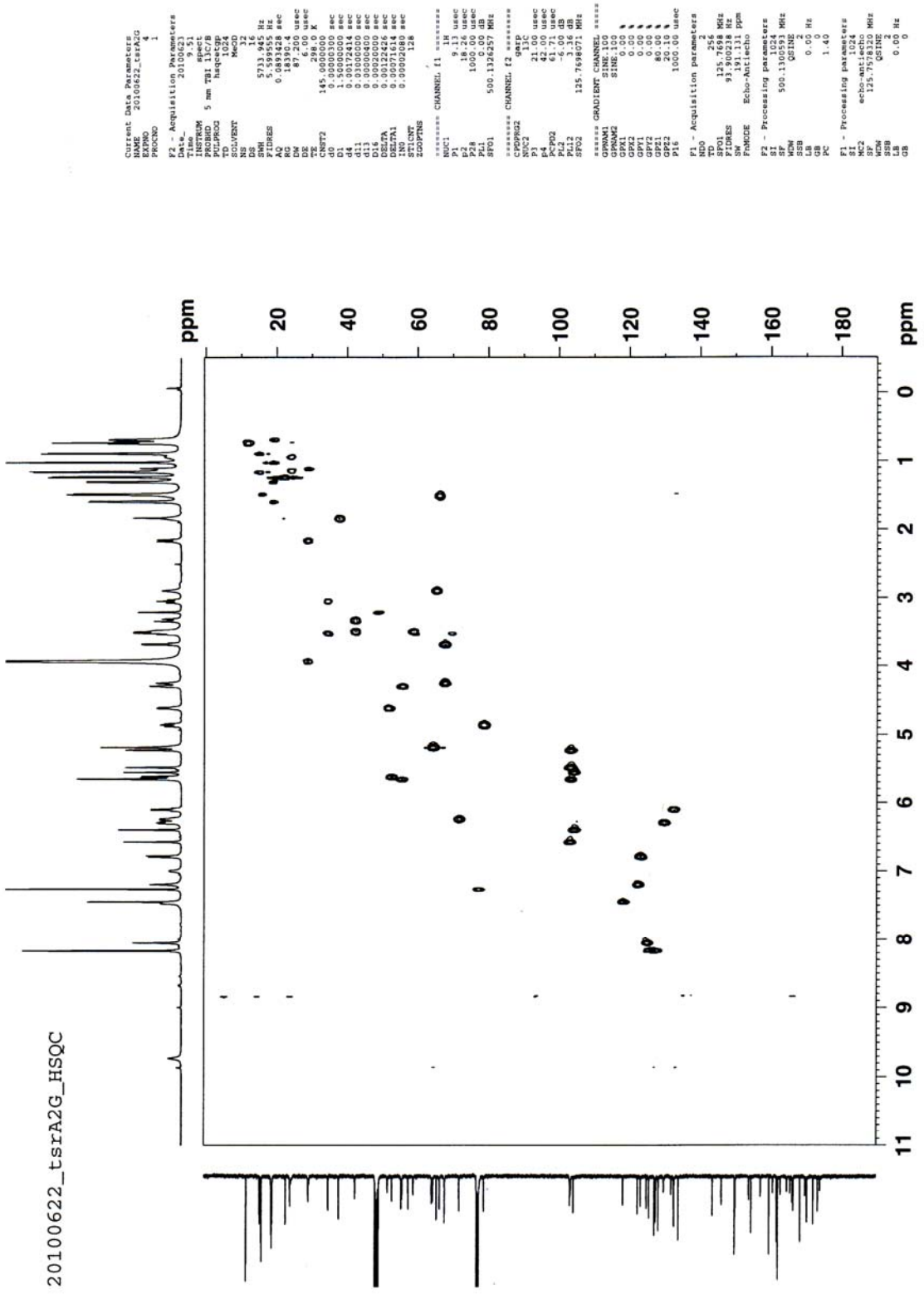


Figure S14. gHSQC spectrum of thiostrepton Ala2Gly (500 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

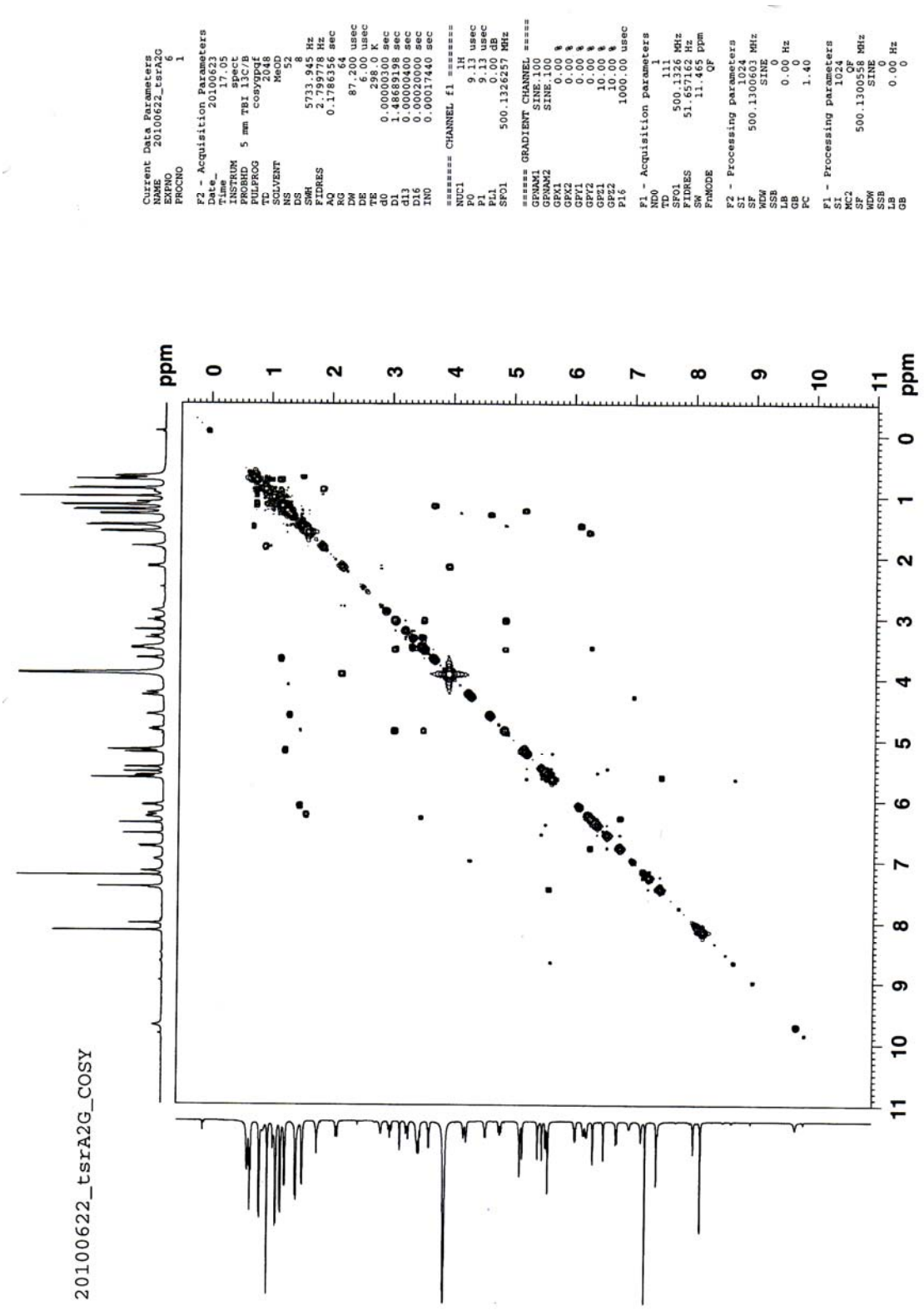


Figure S15. gCOSY spectrum of thiostrepton Ala2Gly (500 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

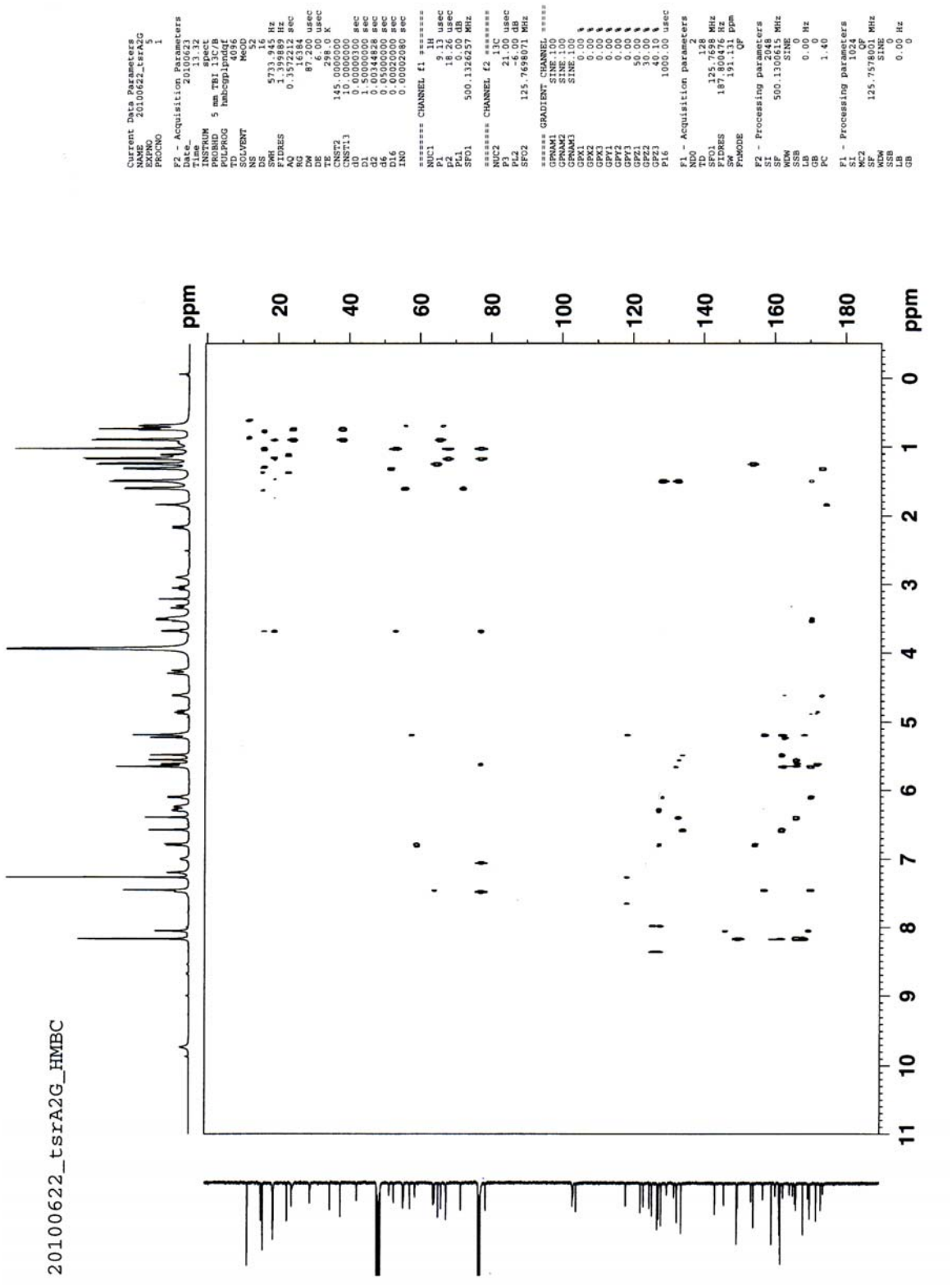
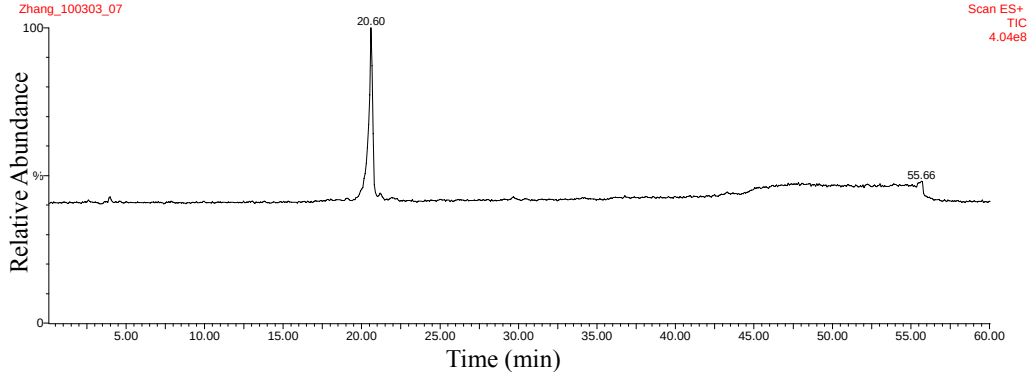


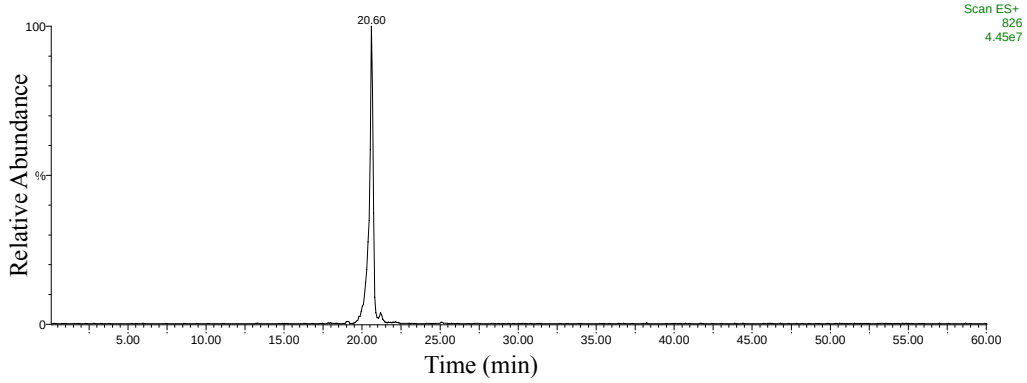
Figure S16. gHMBC spectrum of thiostrepton Ala2Gly (500 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

Figure S17. HPLC-MS of thiostrepton Ala4Gly isolated from *S. laurentii* NDS1 int-3A102.

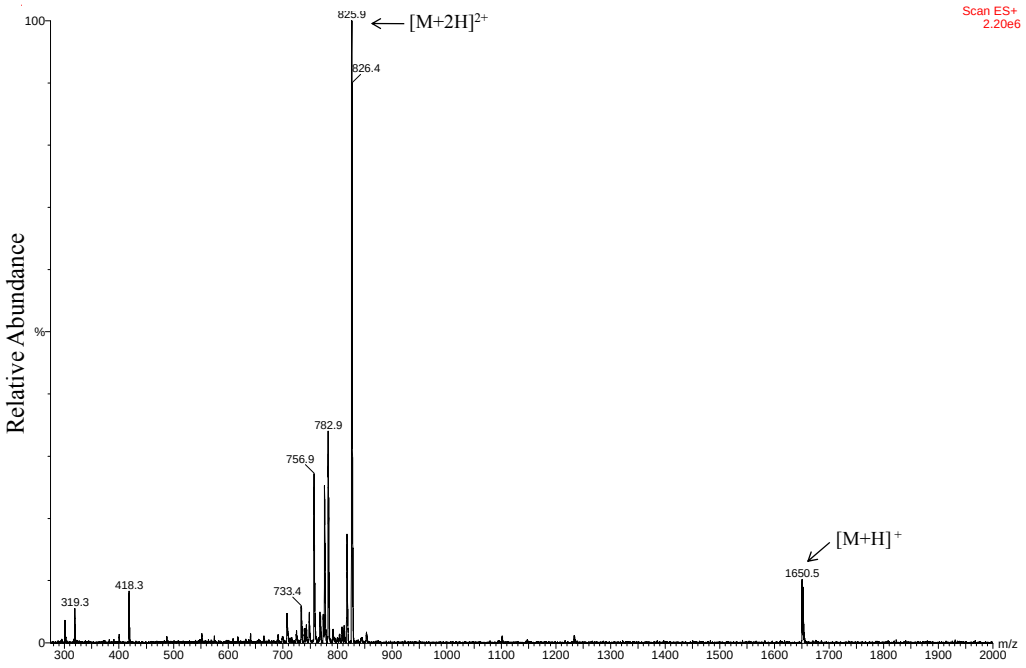
(**A**) Total ion chromatogram of thiostrepton Ala4Gly.



(**B**) Chromatogram extracted for *m*/*z* 826 for thiostrepton Ala4Gly.



(**C**) Mass spectrum of thiostrepton Ala4Gly eluting at t_R = 20.6 min.



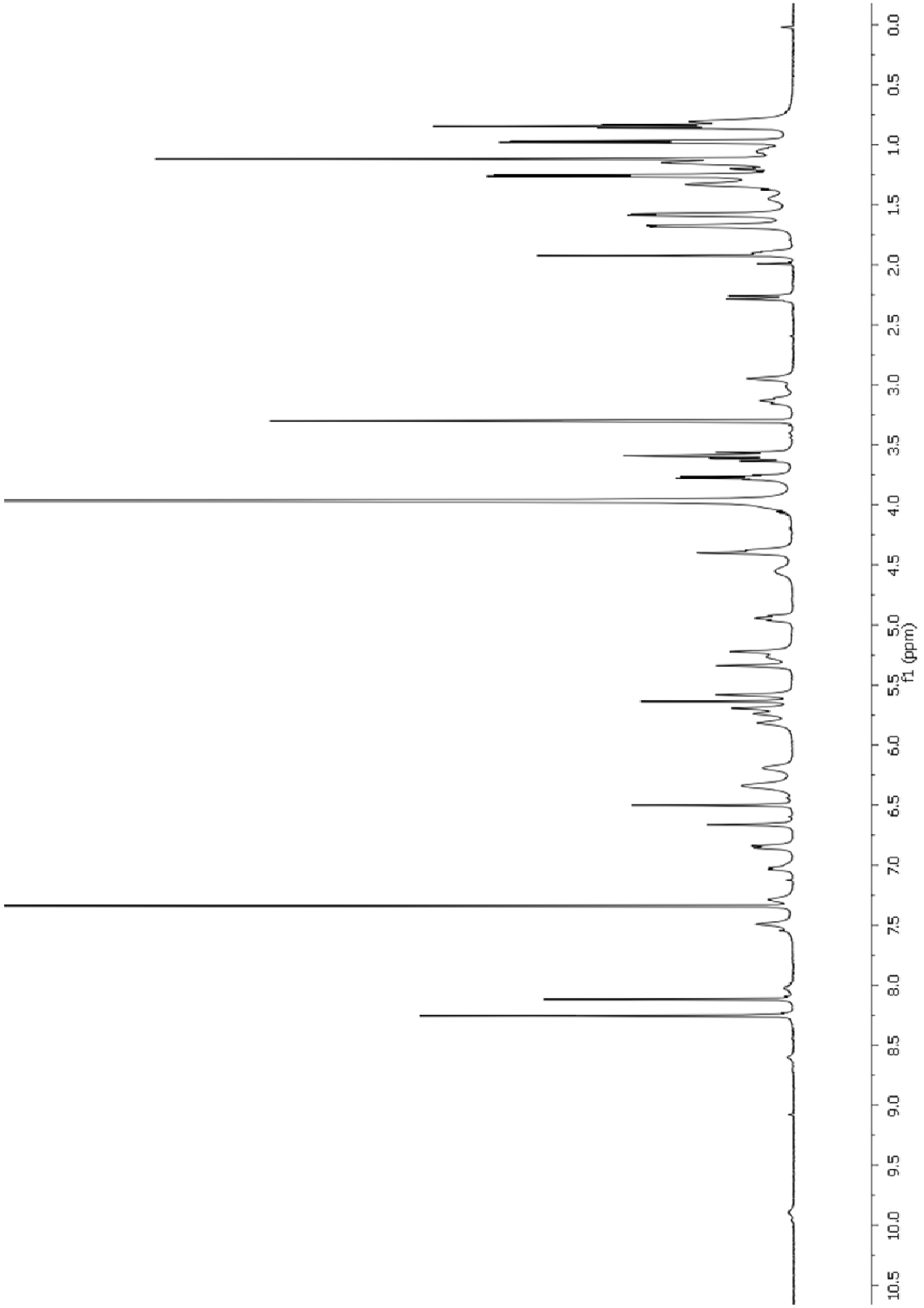


Figure S18. ^{1}H NMR spectrum of thiostrepton Ala4Gly (500 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

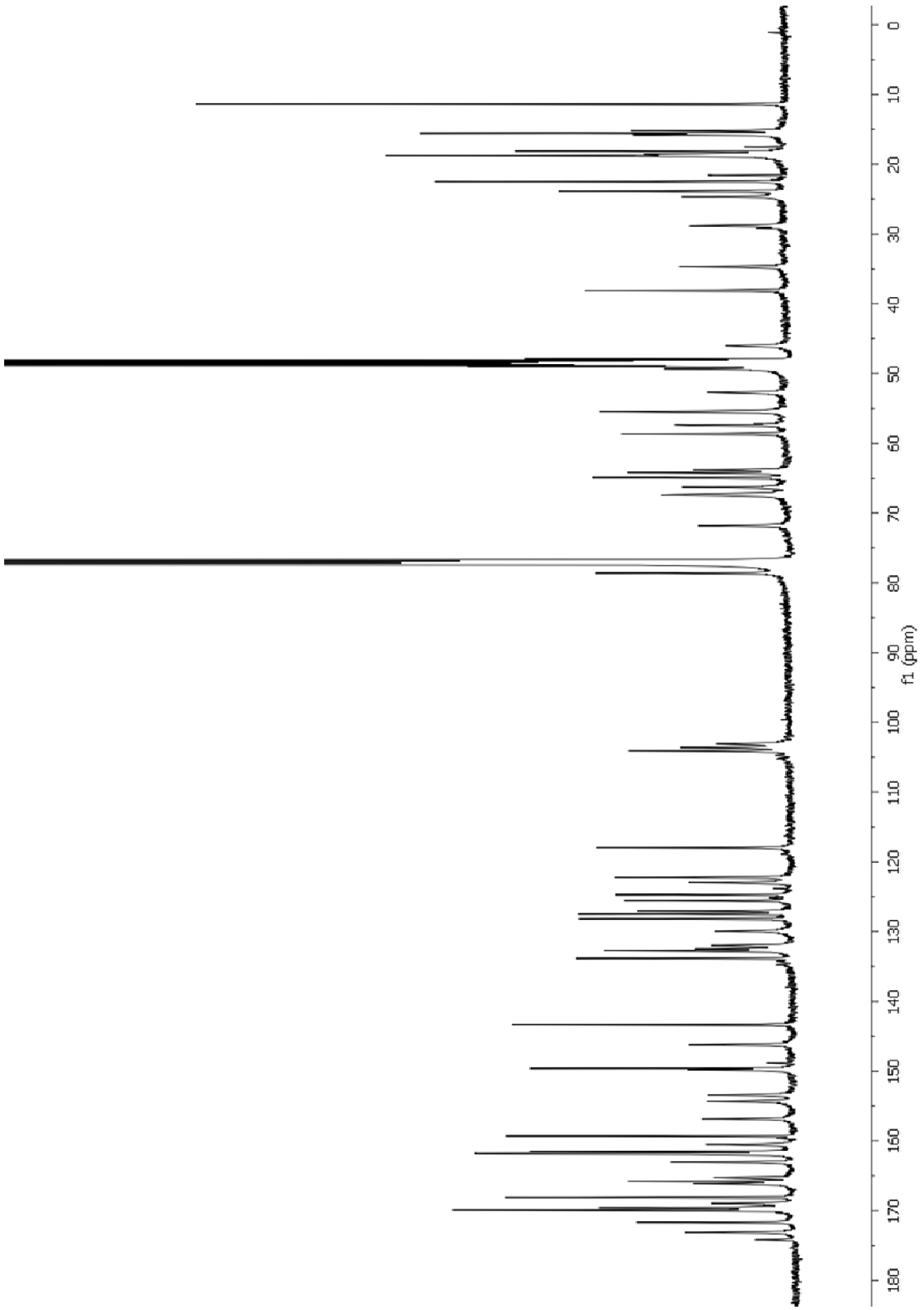


Figure S19. ^{13}C NMR spectrum of thiostrepton Ala4Gly (125 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

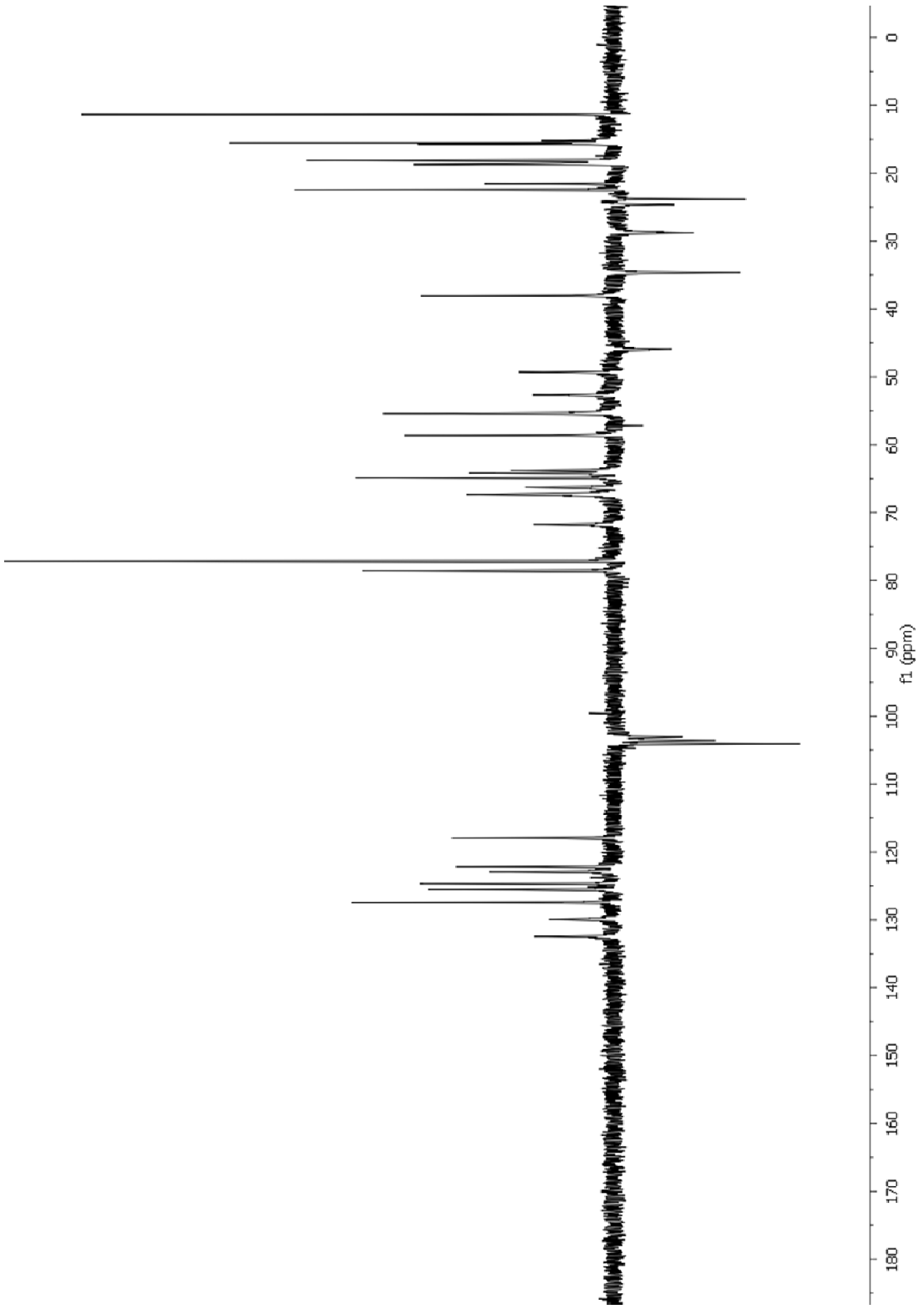


Figure S20. DEPT-135 NMR spectrum of thiostrepton Ala4Gly (125 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

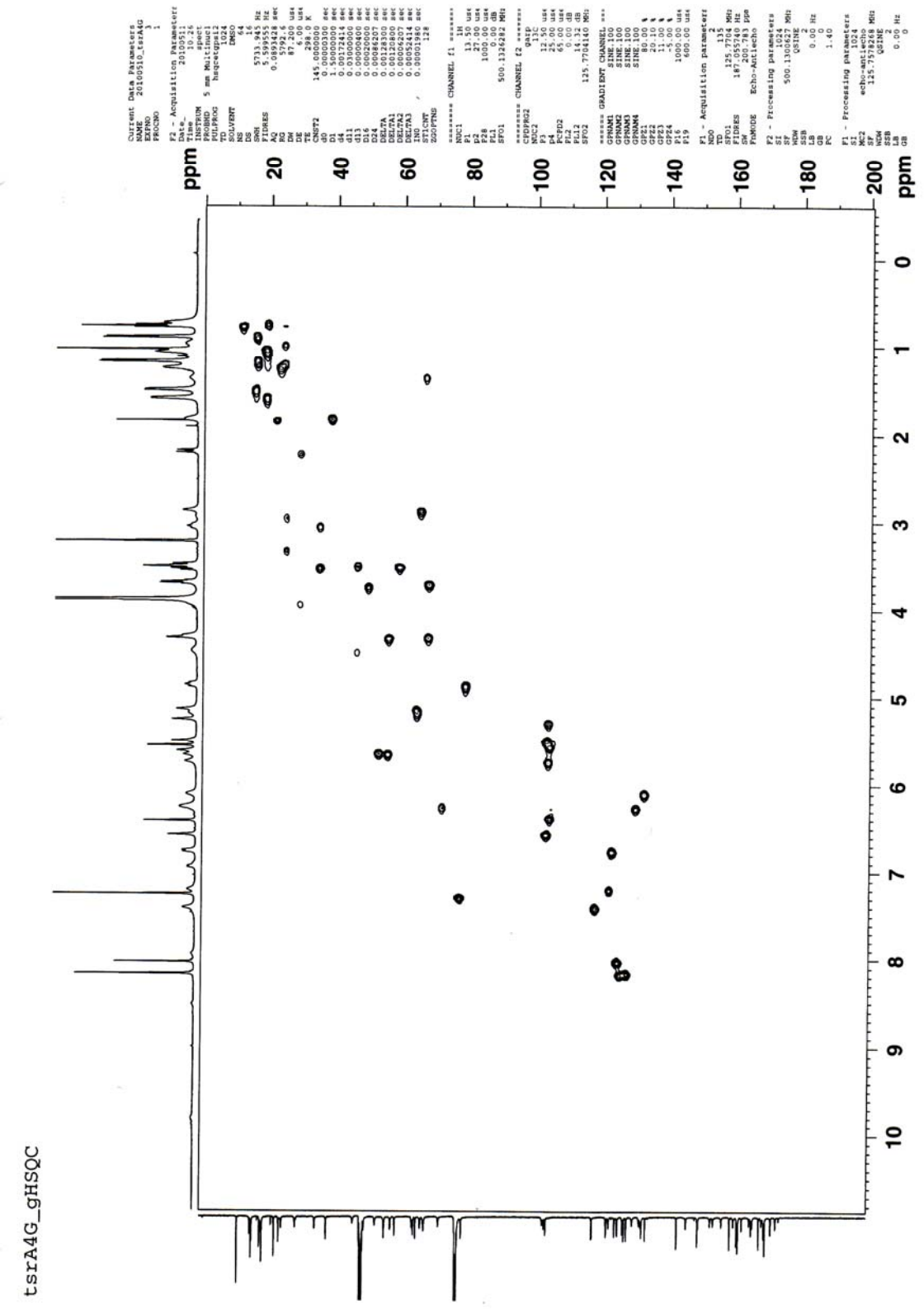


Figure S21. gHSQC spectrum of thiostrepton Ala4Gly (500 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

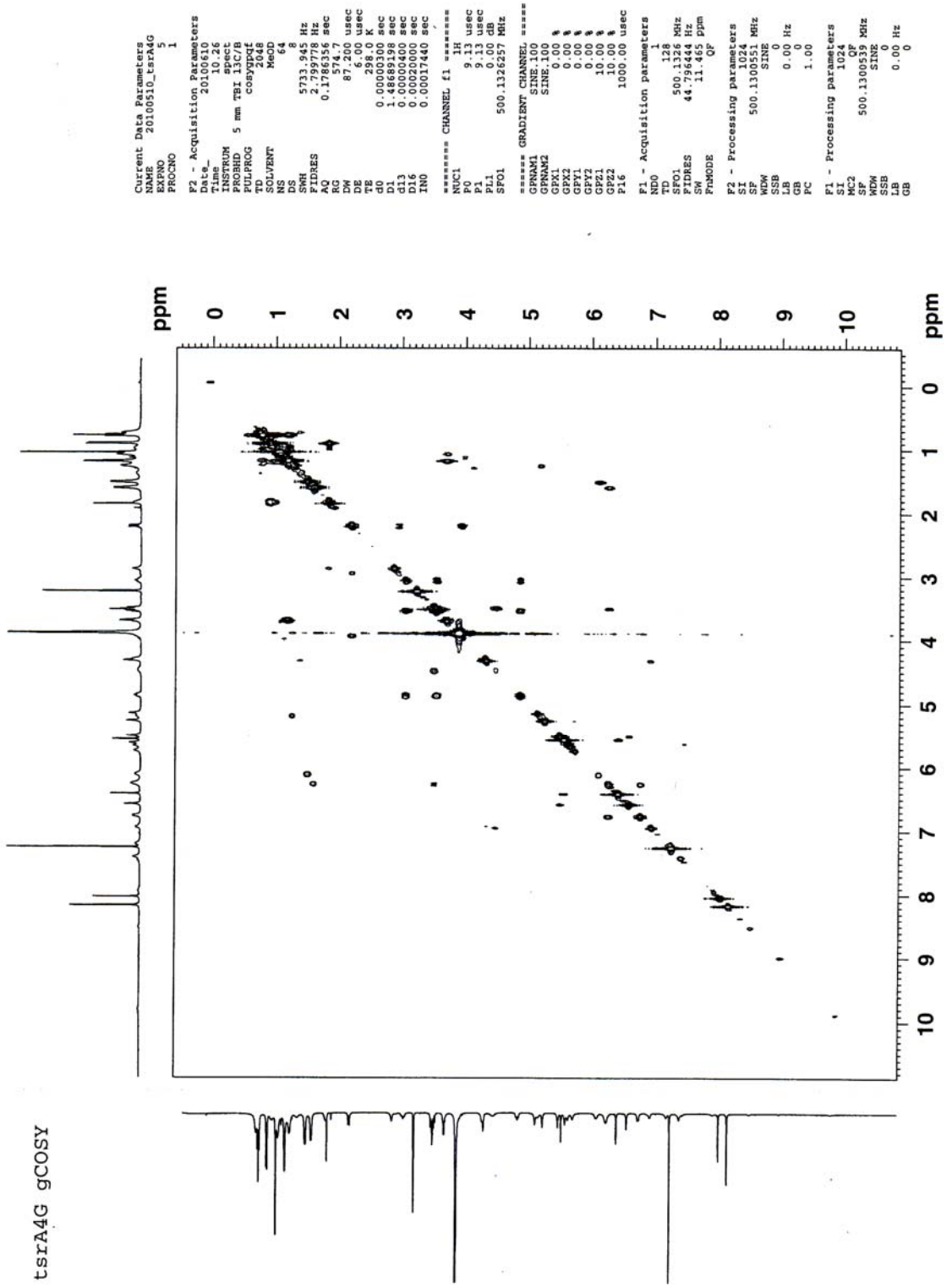


Figure S22. gCOSY spectrum of thiostrepton Ala4Gly (500 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

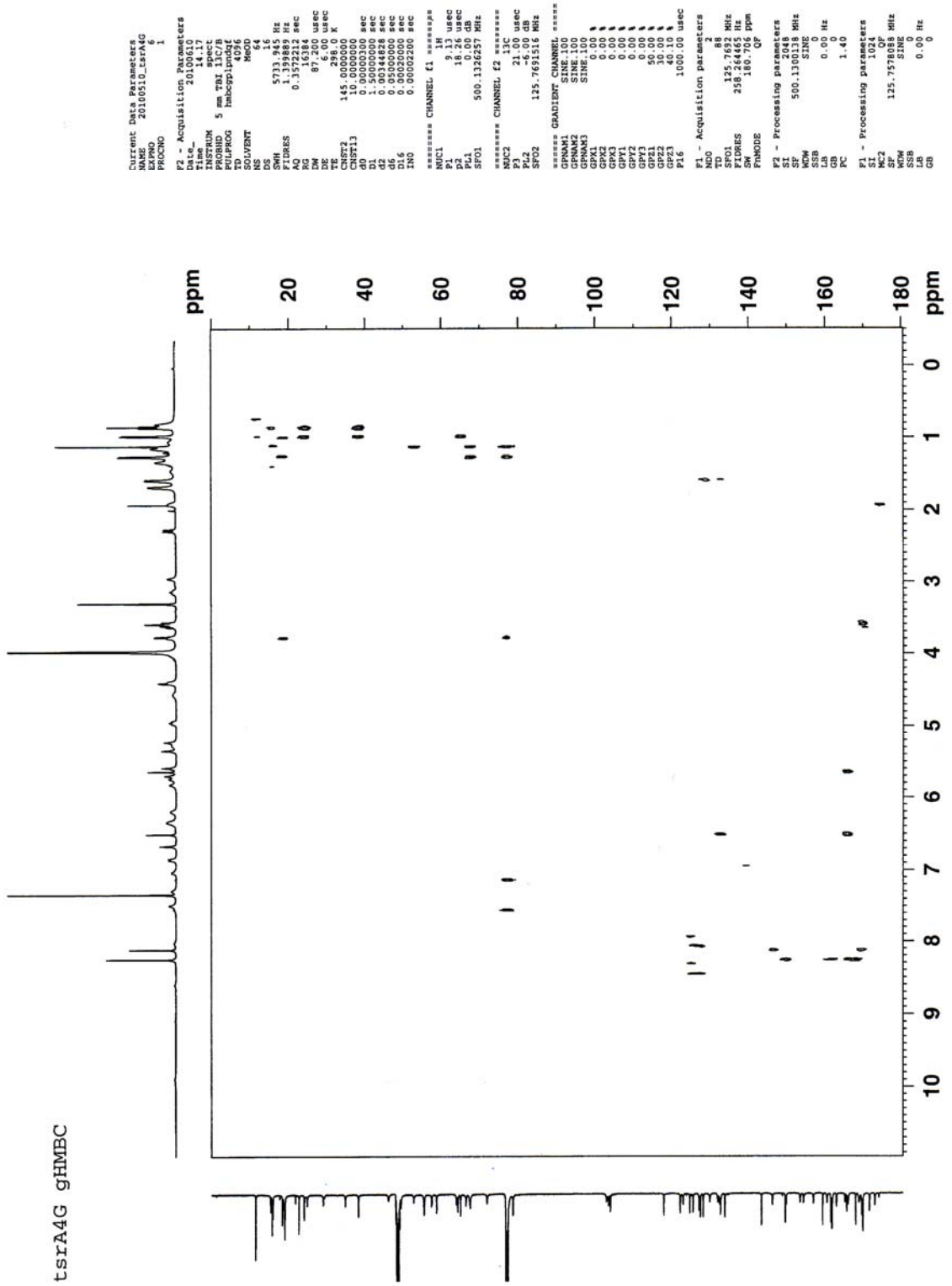


Figure S23. gHMBC spectrum of thiostrepton Ala4Gly (500 MHz, $CDCl_3$-CD_3OD 4:1, 25 °C).

Table S1. ^{1}H and ^{13}C NMR assignments of thiostrepton Ala2Gly

Position	δ_C [ppm]; mult	δ_H [ppm] (mult, J in Hz)	HMBC[a]	COSY[b]
Ile1				
Ile1-1	173.9; C q			
Ile1-2	65.4; CH	2.89 (s)		
Ile1-3	37.8; CH	1.83 (m)	Ile1-1	Ile1-6, Ile1-4-H_B
Ile1-4	23.9; CH_2	H_A: 1.16-1.15 (m)		Ile1-4-H_A, Ile1-5
		H_B: 0.93 (m)		Ile1-3, Ile1-4-H_B, Ile1-5
Ile1-5	11.4; CH_3	0.73 (t, 7.3)	Ile1-3, Ile1-4	Ile1-4-H_A, Ile1-4-H_B
Ile1-6	15.3; CH_3	0.88 (d, 6.9)	Ile1-2, Ile1-3, Ile1-4	Ile1-3
Gly2				
Gly2-1	164.4; C q			
Gly2-2	42.4; CH_2	H_A: 3.55-3.46 (m)		Gly2-2-H_B
		H_B: 3.33 (d, 17.2)	Gly2-1	Gly2-2-H_A
Dha3				
Dha3-1	162.7; C q			
Dha3-2	132.0; C q			
Dha3-3	103.4; CH_2	H_A: 5.65 (s)	Dha3-1, Dha3-2	Dha3-3-H_B
		H_B: 5.23 (bs)	Dha3-1	Dha3-3-H_A
Ala4				
Ala4-1	173.3; C q			
Ala4-2	51.6; CH	4.61 (q, 6.2)	Dha3-1, Ala4-1	Ala4-3
Ala4-3	18.6; CH_3	1.30 (d, 6.4)	Ala4-1, Ala4-2	Ala4-2
Pip				
Pip-2	162.0; C q			
Pip-3	23.9; CH_2	2.82-2.77 (m)		Pip-4-H_B
Pip-4	29.1; CH_2	H_A: 3.95[c],		Pip-4-H_B
		H_B: 2.16 (d, 13.1)		Pip-4-H_A, Pip-3
Pip-5	57.5; C q			
Pip-6	64.1; CH	5.19 (s)	Pip-2, Pip-5, Thz13-2, Thz13-3, Thz15-4	
Thz6				
Thz6-1	161.7; C q			
Thz6-2	146.1; C q			
Thz6-3	125.0; CH	8.04 (s)	Thz6-2, Thz6-4	
Thz6-4	169.7; C q			
Thr7				
Thr7-1	165.4; C q			
Thr7-2	55.7; CH	4.29 (d, 3.0)	Thz6-1	Thr7-3, Thr7-NH
Thr7-3	66.3; CH	1.53-1.45 (m)		Thr7-4
Thr7-4	18.9; CH_3	0.68 (d, 4.7)	Thr7-2, Thr7-3	Thr7-3
Thr7-NH[d]		7.00 (d, 6.2)		Thr7-2
Dhb8				
Dhb8-2	128.3; C q			
Dhb8-3	132.7; CH	6.10 (q, 6.9)	Dhb8-2, Tzn9-4	Dhb8-4
Dhb8-4	15.7; CH_3	1.49 (d, 7.0)	Dhb8-2, Dhb8-3, Tzn9-4	Dhb8-3
Tzn9				
Tzn9-1	172.0; C q			
Tzn9-2	78.8; CH	4.85 (dd, 12.3, 9.4)	Tzn9-1, Tzn9-4	Tzn9-3-H_A, Tzn9-3-H_B
Tzn9-3	34.8; CH_2	H_A: 3.55-3.46 (m)	Tzn9-2, Tzn9-4	Tzn9-2, Tzn9-3-H_B,
		H_B: 3.04 (t, 12.1)	Tzn9-1, Tzn9-2	Tzn9-2, Tzn9-3-H_A
Tzn9-4	170.2; C q			
Ile10				
Ile10-2	52.9; CH	5.62 (d, 9.9)	Tzn9-1, Ile10-3, Thz11-4	Ile10-NH
Ile10-3	77.2; C q			
Ile10-4	67.7; CH	3.68 (q, 6.4)	Ile10-2, Ile10-3, Ile10-5, Ile10-6	Ile10-5
Ile10-5	15.3; CH_3	1.16 (d, 6.4)	Ile10-3, Ile10-4, Ile10-6	Ile10-4
Ile10-6	18.7; CH_3	1.02 (s)	Ile10-2, Ile10-3, Ile10-4, Ile10-5	
Ile10-NH[d]		7.48-7.43 (m)		Ile10-2
Thz11				
Thz11-1	161.7; C q			
Thz11-2	150.0; C q			
Thz11-3	125.7; CH	8.16 (s)	Thz11-1, Thz11-2, Thz11-4	
Thz11-4	166.3; C q			

Position	δ_C [ppm]; mult	δ_H [ppm] (mult, J in Hz)	HMBC[a]	COSY[b]
Thr12				
Thr12-2	55.5; CH	5.65 (s)	Thz11-1, Thz13-4	Thr12-NH
Thr12-3	71.8; CH	6.24 (m)	Q-1	Thr12-4
Thr12-4	18.7; CH_3	1.59 (d, 6.4)	Thr12-2, Thr12-3	Thr12-3
Thr12-NH[d]		8.67 (d, 8.8)		Thr12-2
Thz13				
Thz13-2	157.1; C q			
Thz13-3	118.3; CH	7.44 (s)	Pip-6, Thz13-2, Thz13-4	
Thz13-4	170.1; C q			
Thz15				
Thz15-1	159.5; C q			
Thz15-2	149.8; C q			
Thz15-3	127.6; CH	8.16 (s)	Thz15-1, Thz15-2, Thz15-4	
Thz15-4	168.2; C q			
Dha16				
Dha16-1	161.9; C q			
Dha16-2	134.0; C q			
Dha16-3	103.2; CH_2	H_A: 6.57 (d, 1.8)	Dha16-1, Dha16-2	Dha16-3-H_B
		H_B: 5.48 (d, 1.7)	Dha16-1, Dha16-2	Dha16-3-H_A
Dha17				
Dha17-1	166.0; C q			
Dha17-2	132.8; C q			
Dha17-3	104.3; CH_2	H_A: 6.40 (s)	Dha17-1, Dha17-2	Dha17-3-H_B
		H_B: 5.55 (s)	Dha17-1, Dha17-2	Dha17-3-H_A
Q				
Q-1	160.6; C q			
Q-2	143.6; C q			
Q-3	122.5; CH	7.19 (s)	Q-5	
Q-4	153.8; C q			
Q-5	127.3; C q			
Q-6	123.3; CH	6.78 (d, 9.9)	Q-5, Q-8, Q-10	Q-7
Q-7	129.9; CH	6.29 (dd, 9.5, 5.7)	Q-5	Q-6, Q-8
Q-8	58.9; CH	3.55-3.46 (m)		Q-7
Q-9	67.7; CH	4.25 (s)		
Q-10	154.4; C q			
Q-11	64.4; CH	5.21-5.15 (m)		Q-12
Q-12	22.6; CH_3	1.23 (d, 6.6)	Q-4, Q-11	Q-11

[a] HMBC correlations are from the proton to the indicated carbon.
[b] COSY correlations are from the proton to the proton attached to the indicated position.
[c] The δ of this resonance was determined by HSQC due to overlap with the methanol-d_4 peak.
[d] Only those amide resonances demonstrating COSY correlations to neighboring protons were assigned.

Table S2. ^{1}H and ^{13}C NMR assignments of thiostrepton Ala4Gly

Position	δ_C [ppm]; mult	δ_H [ppm] (mult, J in Hz)	HMBC[a]	COSY[b]
Ile1				
Ile1-1	174.1; C q			
Ile1-2	64.9; CH	2.87 (s)		Ile1-3
Ile1-3	38.1; CH	1.83 (m)	Ile1-1	Ile1-4-H_B, Ile1-6
Ile1-4	23.9; CH_2	H_A: 1.18-1.17 (m)		Ile1-4-H_B, Ile1-5
		H_B: 0.97 (m)		Ile1-3, Ile1-4-H_A, Ile1-5
Ile1-5	11.4; CH_3	0.77 (t, 7.3)	Ile1-3, Ile1-4, Ile1-6	Ile1-4-H_A, Ile1-4-H_B
Ile1-6	15.6; CH_3	0.90 (d, 6.9)	Ile1-1, Ile1-2, Ile1-3, Ile1-4, Ile1-5	Ile1-3
Ala2				
Ala2-1	168.9; C q			
Ala2-2	49.3; CH	3.69 (q, 6.3)		Ala2-3
Ala2-3	18.6; CH_3	1.07 (br)		Ala2-2
Dha3				
Dha3-1	163.0; C q			
Dha3-2	132.0; C q			
Dha3-3	103.6; CH_2	H_A: 5.74 (s)		
		H_B: 5.26 (s)		Dha3-3- H_A
Gly4				
Gly4-1	173.1; C q			
Gly4-2	46.0; CH_2	H_A: 4.47 (br)		Gly4-2-H_B
		H_B: 3.56-3.48 (m)		Gly4-2-H_A
Gly4-NH[d]		6.95 (d, 8.2)		Gly4-2-H_A
Pip				
Pip-2	161.6; C q			
Pip-3	24.7; CH_2	H_A: 3.34-3.30 (m)		
		H_B: 2.94 (m)		Pip-4-H_B
Pip-4	28.8; CH_2	H_A: 4.00-3.93 (m)[c]		Pip-4-H_B
		H_B: 2.21-2.18 (m)		Pip-4-H_A, Pip-3-H_B
Pip-5	57.4; C q			
Pip-6	63.8; CH	5.14 (s)		
Thz6				
Thz6-1	161.9; C q	Thz6-1		
Thz6-2	146.2; C q			
Thz6-3	124.7; CH	8.04 (s)	Thz6-2, Thz6-4	
Thz6-4	169.6; C q			
Thr7				
Thr7-1	165.3; C q			
Thr7-2	55.5; CH	4.32 (d, 2.6)		Thr7-3
Thr7-3	66.3; CH	1.37 (br)		Thr7-4
Thr7-4	18.8; CH_3	0.73 (br s)		Thr7-3
Dhb8				
Dhb8-2	128.2; C q			
Dhb8-3	132.5; CH	6.11 (s)		Dhb8-4
Dhb8-4	15.2; CH_3	1.50 (d, 6.4)	Dhb8-2, Dhb8-3	Dhb8-3
Tzn9				
Tzn9-1	171.7; C q			
Tzn9-2	78.6; CH	4.86 (t, 10.7)		Tzn9-3-H_A, Tzn9-3-H_B
Tzn9-3	34.7; CH_2	H_A: 3.56-3.48 (m)	Tzn9-4	Tzn9-2, Tzn9-3-H_B
		H_B: 3.06 (t, 11.2)		Tzn9-2, Tzn9-3-H_A
Tzn9-4	169.9; C q			
Ile10				
Ile10-2	52.7; CH	5.61 (s)		
Ile10-3	77.2; C q			
Ile10-4	67.4; CH	3.69 (q, 6.3)	Ile10-3, Ile10-6	Ile10-5
Ile10-5	15.7; CH_3	1.18 (d, 6.4)	Ile10-3, Ile10-4, Ile10-6	Ile10-4
Ile10-6	18.1; CH_3	1.04 (s)	Ile10-2, Ile10-C3, Ile10-4, Ile10-5	
Thz11				
Thz11-1	159.3; C q			
Thz11-2	149.8; C q			
Thz11-3	125.5; CH	8.18 (s)	Thz11-2, Thz11-4	
Thz11-4	168.1; C q			

Position	δ_C [ppm]; mult	δ_H [ppm] (mult, J in Hz)	HMBC[a]	COSY[b]
Thr12				
Thr12-2	55.5; CH	5.66 (s)		
Thr12-3	71.8; CH	6.26 (s)		Thr12-4
Thr12-4	18.8; CH_3	1.60 (d, 5.6)		Thr12-3
Thz13				
Thz13-2	156.8; C q			
Thz13-3	118.0; CH	7.41 (s)		
Thz13-4	170.0; C q			
Thz15				
Thz15-1	161.5; C q			
Thz15-2	149.6; C q			
Thz15-3	127.5; CH	8.17 (s)	Thz15-1, Thz15-C2, Thz15-C4	
Thz15-4	166.1; C q			
Dha16				
Dha16-1	161.7; C q			
Dha16-2	133.8; C q			
Dha16-3	103.1; CH_2	H_A: 6.58 (s)		Dha3-16- H_B
		H_B: 5.50 (s)		Dha3-16- H_A
Dha17				
Dha17-1	165.7; C q			
Dha17-2	132.7; C q			
Dha17-3	104.1; CH_2	H_A: 6.42 (s)	Dha17-1, Dha17-2	Dha3-17- H_B
		H_B: 5.56 (s)	Dha17-1	Dha3-17- H_A
Q				
Q-1	160.5; C q			
Q-2	143.3; C q			
Q-3	122.2; CH	7.21 (s)		
Q-4	153.4; C q			
Q-5	127.1; C q			
Q-6	123.0; CH	6.77 (d, 9.7)		Q-7
Q-7	129.9; CH	6.26 (s)		Q-6, Q-8
Q-8	58.7; CH	3.56-3.48 (m)		Q-7
Q-9	67.4; CH	4.30 (m)		Q-9-OH
Q-10	154.3; C q			
Q-11	64.2; CH	5.19 (br)		Q-12
Q-12	22.5; CH_3	1.25 (br)		Q-11
Q-9-OH		6.95 (d, 8.2)		Q-9

[a] HMBC correlations are from the proton to the indicated carbon.
[b] COSY correlations are from the proton to the proton attached to the indicated position.
[c] The δ of this resonance was determined by HSQC due to overlap with the methanol-d_4 peak.
[d] Only this amide resonance demonstrating COSY correlation to neighboring proton was assigned.

Table S3. Strains and plasmids used in this study

Strain/Plasmid	Description	Reference or source
Streptomyces strains		
S. laurentii ATCC 31255	Wild-type, thiostrepton producer	ATCC
S. actuosus ATCC 25421	Wild-type, nosiheptide producer	ATCC
S. lividans TK24	A common host for *Streptomyces* gene expression and manipulation	[1]
S. coelicolor CH999	A common host for *Streptomyces* gene expression and manipulation	[2]
S. actuosus FZ1	*S. actuosus* containing int-3A10	This study
S. actuosus FZ2	*S. actuosus* containing int-pCC1FOS	This study
S. lividans FZ1	*S. lividans* containing int-3A10	This study
S. lividans FZ2	*S. lividans* containing int-pCC1FOS	This study
S. laurentii NDS1	*S. laurentii* containing an in-frame deletion of *tsrA*	This study
S. laurentii NDS1/int-3A10	*S. laurentii* NDS1 containing int-3A10	This study
S. laurentii NDS1/int-3A101	*S. laurentii* NDS1 containing int-3A101	This study
S. laurentii NDS1/int-3A102	*S. laurentii* NDS1 containing int-3A102	This study
S. laurentii NDS1/int-3A103	*S. laurentii* NDS1 containing int-3A103	This study
E. coli strains		
BW25113/pKD46	Host for PCR-targeted disruption of a gene from a fosmid or plasmid	[3]
ET12567/pUZ8002	Host for conjugation with *Streptomyces* species	[4]
Strains used for antimicrobial assays		
Bacillus sp. ATCC 27859	Wild-type	ATCC
Escherichia coli ATCC 27856	Wild-type	ATCC
Staphylococcus aureus ATCC 10537	Methicillin-resistant	ATCC
Enterococcus faecium ATCC 12952	Vancomycin-resistant	ATCC
Plasmids		
pCC1FOS	Fosmid used for genomic library construction	Epicentre Inc.
pSC-B-amp/kan	A routine vector from StrataClone Blunt PCR Cloning Kit, for cloning blunt-end PCR product	Agilent Technologies
JA3A10	Fosmid containing the *tsr* biosynthetic gene cluster	[5]
int- pCC1FOS	A fosmid containing all essential genes for the conjugal transfer and integration into a streptomycete chromosome	This study
int-3A10	A fosmid containing the entire *tsr* gene cluster and all essential genes for the conjugal transfer and integration into a streptomycete chromosome	This study
pSET152	An integrative plasmid containing the apramycin resistance gene and all essential genes for the conjugal transfer and integration into a streptomycete chromosome	[6]
pSE34	Plasmid containing the thiostrepton resistance gene	Pfizer
pIJ778	A plasmid containing the spectinomycin/streptomycin resistance cassette	[4]
pLeft	pSC-B-amp/kan vector harboring the 1022 nt sequence upstream of *tsrA*	This study
pRight	pSC-B-amp/kan vector harboring the 1003 nt sequence downstream of *tsrA*	This study
pLR	pLeft containing a 1 kb insert from the *Nde*I-*Sbf*I digestion of pRight	This study
pGM160HK	A derivative of pGM160K[5] in which a 1.6 kb *Hind*III-digested fragment was deleted	This study
pGM160HKss	A derivative of pGM160HK, in which the ampicillin resistance gene was replaced by the spectinomycin-streptomycin resistance cassette. It is a conjugal and temperature-sensitive *E. coli*-*Streptomyces* shuttle vector	This study
pNDS1	pGM160HKss containing a 2 kb insert from *Hind*III digestion of pLR	This study
pDC1	Vector pSC-B-amp/kan containing a 0.9 kb chl^R gene	This study
pDC2	Vector pSC-B-amp/kan containing a 1.8 kb *sacB* gene amplified from pEX100T with primers: SacB-F and SacB-R	This study
pDC3	Plasmid containing the dual-marker cassette (chl^R and *sacB*)	This study
pJP11	pCR4-Blunt vector harboring a 1.8 kb fragment PCR amplified from *S. laurentii* genomic DNA, which contains the 0.2 kb *tsrA* gene and its two flanking 0.8 kb region	[5]

pCL61	Plasmid containing the *tsrA* variant encoding the Ala2Gly mutation	This study
pCL62	Plasmid containing the *tsrA* variant encoding the Ala4Gly mutation	This study
pCL63	Plasmid containing the *tsrA* variant encoding the Thr7Gly mutation	This study
int-3A100	Derived from int-3A10. *tsrA* is replaced by chl^R–*sacB* cassette	This study
int-3A101	Derived from int-3A100. The chl^R–*sacB* cassette is replaced by the *tsrA* variant encoding the Ala2Gly mutation	This study
int-3A102	Derived from int-3A100. The chl^R–*sacB* cassette is replaced by the *tsrA* variant encoding the Ala4Gly mutation	This study
int-3A103	Derived from int-3A100. The chl^R–*sacB* cassette is replaced by the *tsrA* variant encoding the Thr7Gly mutation	This study
pEX100T	Plasmid containing *sacB* gene	ATCC

Table S4. Primers used in this study

Primer	Sequence	Description
CTSR1-F	5'-TTTGAGTTATCGAGATTTTCAGGAGCTAAGG AAGCTAAAGCGGTGGTTTTTTTGTTTGCAAGC-3'	Primers for the replacement of *chl*^{R} in pCC1FOS, or fosmids based on it, with genes *aac(3)IV*, *int*, *attP* and *oriT* amplified from pSET152. pSET152 sequence is underlined.
CTSR1-R	5'-ACCAGGCGTTTAAGGGCACCAATAACTGCCTT AAAAAAACCGATGCAAAGTGCCGATCA-3'	
CTSR2-F	5'-GCGGTGGTTTTTTTGTTTGCAAGC-3'	Primers for the confirmation of int-pCC1FOS and int-3A10.
CTSR2-R	5'-CCGATGCAAAGTGCCGATCA-3'	
CTSR3-F	5'- GCGGTGGTTTTTTTGTTTGCAAGC-3'	Primers for the confirmation of int-pCC1FOS and int-3A10.
CTSR3-R	5'- CTACGGAAGGAGCTGTGGAC-3'	
TSRK-F	5'-CCGATGCAAAGTGCCGATCA-3'	Primers for the amplification of *tsrK*.
TSRK-R	5'-TCGCTCGAGGCGCAGCACCTTGCC-3'	
TSRV-F	5'-TGCTGCATATGACGGGAGTCACCGAACCG-3'	Primers for the amplification of *tsrV*.
TSRV-R	5'-TTCCTCGAGTCAGTCTCCCGGCGCCTC -3'	
TSRN-F	5'-AGTATTCATATGACCGCCCCCGCGCTCCCGCTC-3'	Primers for the amplification of *tsrN*.
TSRN-R	5'- ACCCTCGAGTCAGACGGCGAGCCGCCGTTC-3'	
DTSR1-F	5'-AAGCTTGTGAGGGTCACCACGGATCC-3'	Primers for the amplification of the region upstream of *tsrA*. Underlined regions are the restriction sites of *Hind*III, *Nde*I and *Sbf*I.
DTSR1-R	5'-CCTGCAGGCATATGCTCCAGGGCGGCATTGCT CAT-3'	
DTSR2-F	5'-CATATGTGAGGTAACACCCGGCGCGGA-3'	Primers for the amplification of the region downstream of *tsrA*. Underlined regions are restriction sites of *Hind*III, *Nde*I and *Sbf*I.
DTSR2-R	5'-CCTGCAGGAAGCTTGCTCCAGGTCCGCGACGC CG-3'	
DTSR3-F	5'-ATCGTGTTGGGCTTGACG-3'	Primers used to confirm the construction of *S. laurentii* NDS1.
DTSR3-R	5'-CGCGGTGCAATAGGACAT-3'.	
Chl-F	5'-ATTCCGGGGATCCGTCGACCAGATCT*GCCGCTC CATGAGCTTATCG*-3'	Primers for the amplification of *chl*^{R} from pCC1FOS. Underlined regions are restriction sites of *Bgl*II, *Nde*I and *Sbf*I. pCC1FOS sequence is italicized.
Chl-R	5'-CCTGCAGGCATATG*AATTACGCCCCGCCCTGCC*-3'	
SacB-F	5'-CATATG*AACTTTATGCCCATGCAACAG*-3'	Primers for the amplification of *sacB* gene from pEX100T. Underlined regions are restriction sites of *Bgl*II, *Nde*I and *Sbf*I. pEX100T sequence is italicized.
SacB-R	5'-CCTGCAGGTGTAGGCTGGAGCTGCTTCAGATCT*G AGAGTGCACCATAATCGGC*-3'	
A2G-F	5'-GTCACGATGATCGGCTCCGCCTCCTGC-3'	Primers to generate TsrA Ala2Gly.
A2G-R	5'-GCAGGAGGCGGAGCCGATCATCGTGAC-3'	
A4G-F	5'-GATGATCGCGTCCGGCTCCTGCACCACC-3'	Primers to generate TsrA Ala4Gly.
A4G-R	5'-GGTGGTGCAGGAGC CGGACGCGATCATC-3'	
T7G-F	5'-GTCCGCC TCCTGCGGCACCTGCATCTGC-3'	Primers to generate TsrA Thr7Gly.
T7G-R	5'-GCAGATGCAGGTGCCGCAGGA GGCGGAC-3'	
AmpSS-F	*5'-CCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATC TG*GGAATAGGAACTTCATGAGC-3'	Primers for the amplification of the spectinomycin-streptomycin resistance cassette from pIJ778 to replace ampicillin resistance gene in pGM160HK. Underlined regions are sequence of pIJ778. pGM160HK sequence is in italics.
AmpSS-R	5'-*ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTT T*GAAGTTCCCGCCAGCCTCGC-3'	
DC1-F	5'-ATTCCGGGGATCCGTCGACCAGATCTGCCG CTCCATGAGCTTATCG-3'	Primers for the amplification of *chl*^{R} from pCC1FOS. The *Sbf*I and *Nde*I restriction sites are underlined and in italics, respectively.
DC1-R	5'-CCTGCAGG*CATATG*AATTACGCCCCGCCCTG CC-3'	
DC2-F	5'-*CATATG*AACTTTATGCCCATGCAACAG-3'	Primers for the amplification of *sacB* from pEX100T. The *Sbf*I and *Nde*I restriction sites are underlined and in italics, respectively.
DC2-R	5'-CCTGCAGGTGTAGGCTGGAGCTGCTTCAGA TCTGAGAGTGCACCATAATCGGC-3'	
SD1-F	5'-GCGCGATCGACGCGACCGCAGACTTGCCGA AAGGTTGTGATTCCGGGGATCCGTCGACC-3'	Primers for the disruption of *tsrA* in int-3A10. The underlined regions are homologous to *tsrA*.
SD1-R	5'-GGCGGGGAGGAACAGTCCTCCGCGCCGGGT GTTACCTCATGTAGGCTGGAGCTGCTTC-3'	
SD2-F	5'-GCGCGATCGACGCGACCGCAG-3'	Primers used in the amplification of *tsrA* variants from pCL61-63.
SD2-R	5'-GGCGGGGAGGAACAGTCCTCC-3'	
SD3-F	5'-ATCGTGTTGGGCTTGACG-3'	Primers used in DNA sequencing to confirm int-3A101 to 103.
SD3-R	5'-CGCGGTGCAATAGGACAT-3'	

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