Supporting Information

Target profiling of 4-hydroxyderricin in *S. aureus* reveals seryl-tRNA synthetase binding and inhibition by covalent modification

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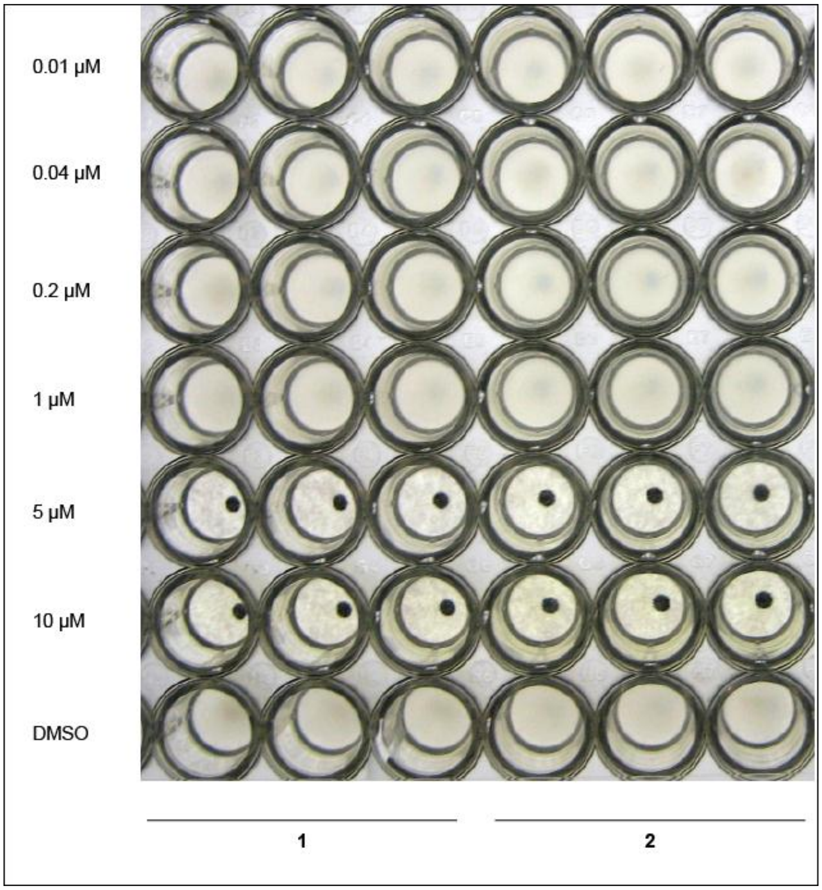
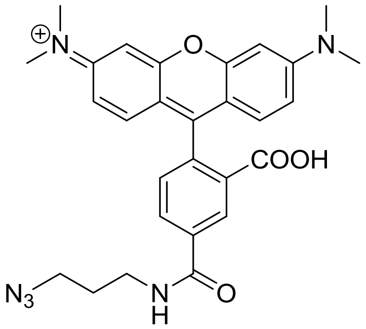
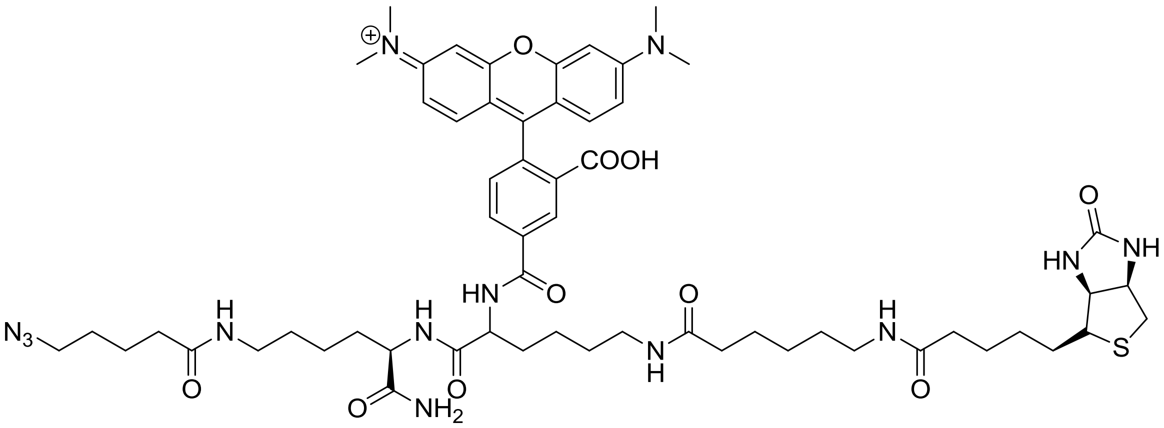


Supporting Figure 1. Comparative MIC of **1** and **2** in *S. aureus* NCTC 8325.

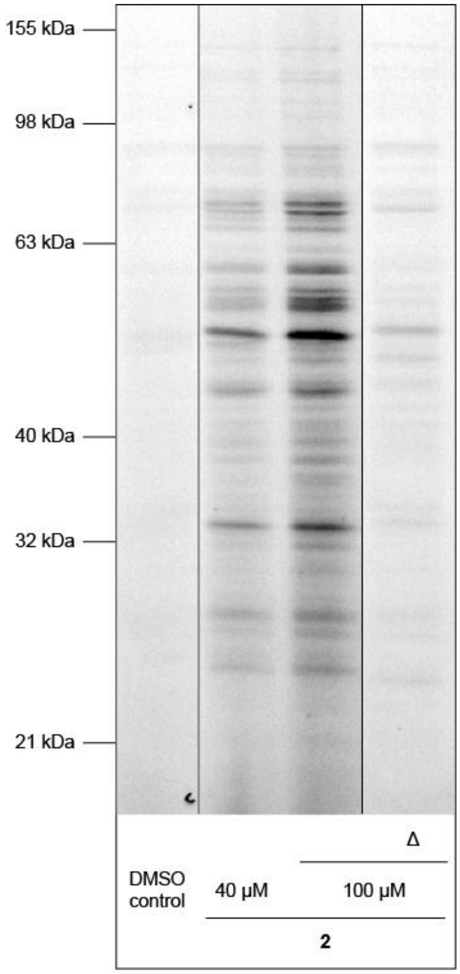


Rh-N_3

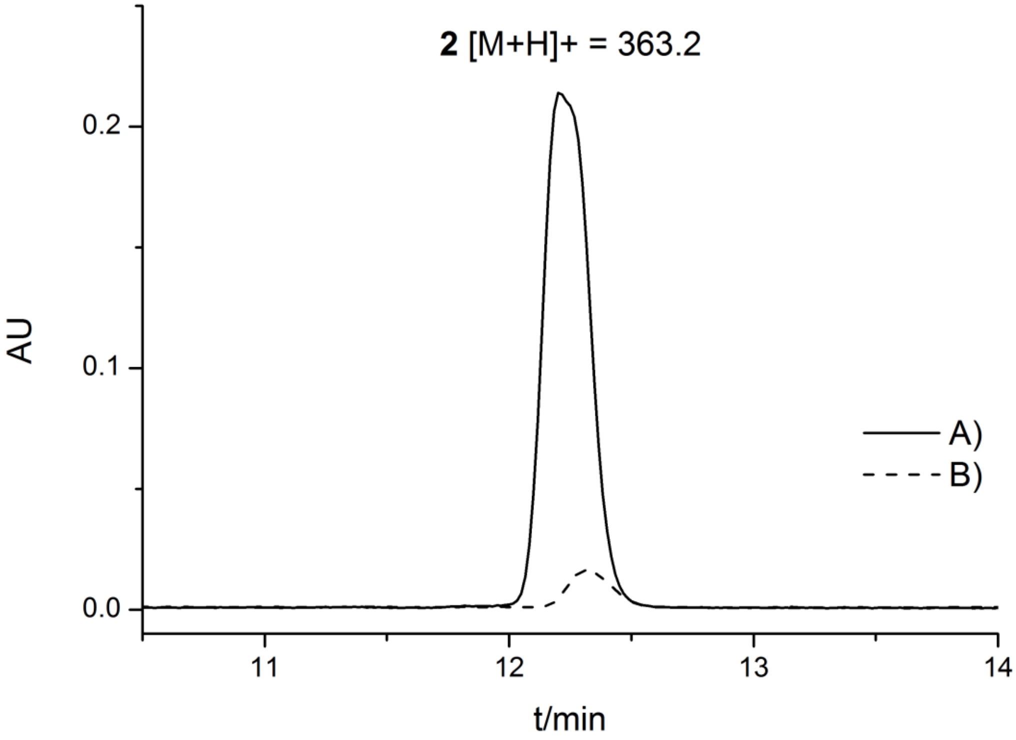


Rh-biotin-N_3

Supporting Figure 2. Structures of rhodamine azide Rh-N_3 and trifunctional rhodamine-biotin-N_3.[1]



Supporting Figure 3. Concentration dependent labeling experiments of *S. aureus* NCTC 8325 proteome with probe **2** (Δ: Labeling with heat denatured proteome).



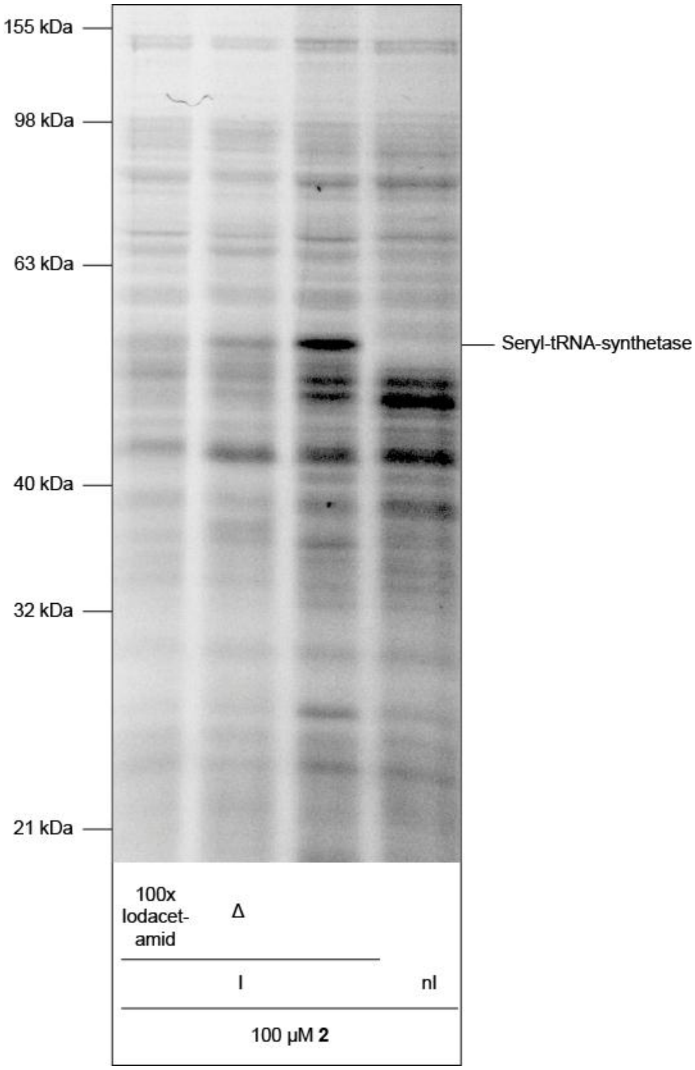
Supporting Figure 4. UV absorption of **2** at 363 nm: A) before and B) after heat decomposition in water at 99 °C for 20 min.

Protein	**Protein ID**	**MW [kD]**	**Exp. #**	**Score**	**# Peptides**	**# Unique Peptides**	**PSM**
Seryl-tRNA synthetase	P95689	48,6	**1**	7,99	2	2	2
			2	5,65	2	2	2
			3	37,18	9	9	12
			4	–	–	–	–
			5	–	–	–	–
Glycyl-tRNA synthetase	Q2FY08	53,6	**1**	12,05	4	4	4
			2	72,31	16	16	22
			3	71,07	15	15	21
			4	41,81	10	10	13
			5	–	–	–	–
Enolase	Q2G028	47,1	**1**	8,60	3	3	3
			2	46,24	10	10	13
			3	55,76	12	12	16
			4	68,37	13	13	20
			5	5,94	2	2	2
Elongation factor Tu	Q2G0N0	43,1	**1**	83,09	9	9	25
			2	197,96	17	17	61
			3	247,81	17	17	77
			4	247,02	17	17	73
			5	18,84	3	3	6

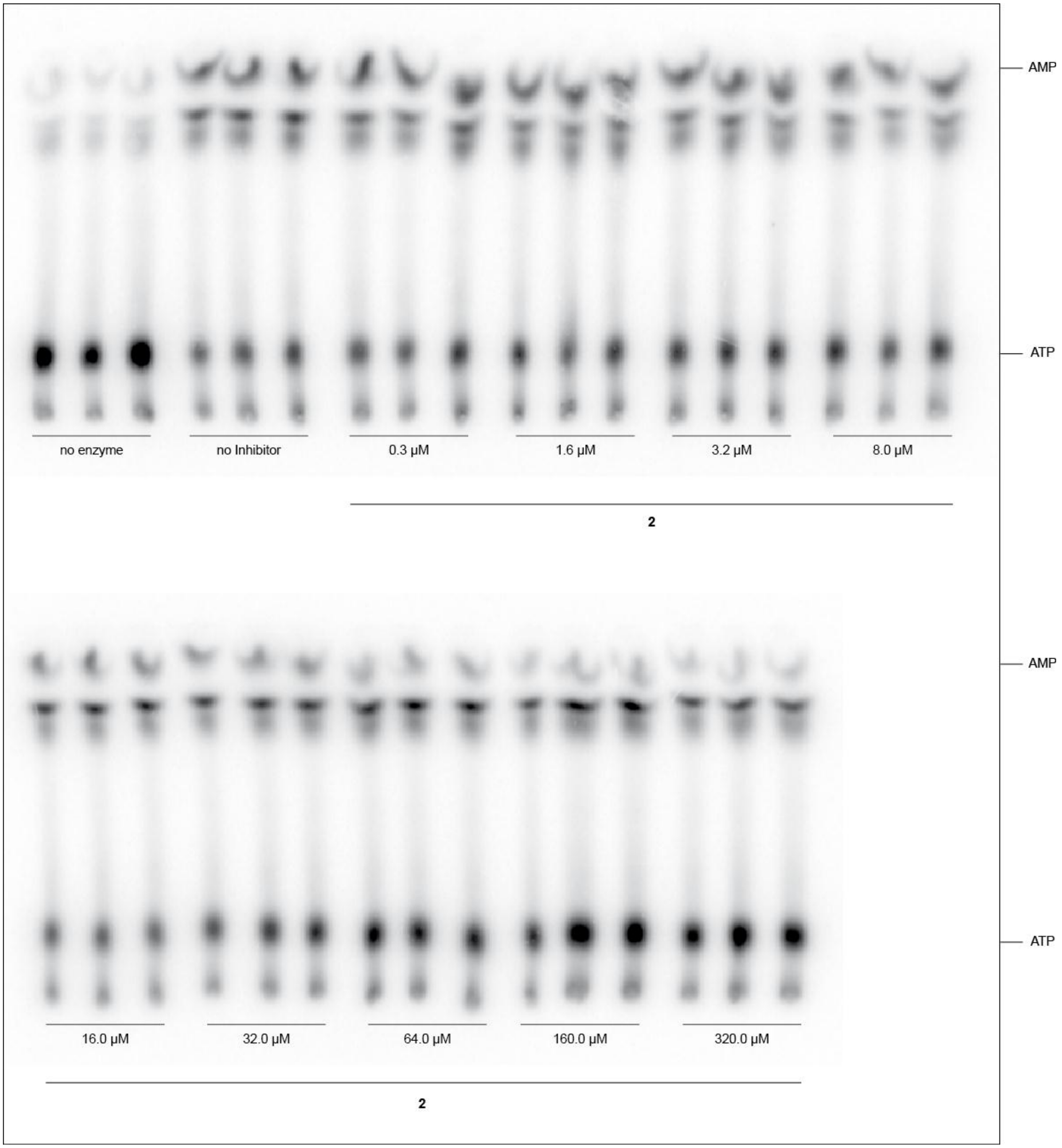
Supporting Table 1. Probabilities for protein hits identified by MS in three independent enrichment experiments (Exp. 1-3) in comparison with two DMSO control experiments (Exp. 4-5) in *S. aureus*.

Gene, organism	Forward primer	Reverse primer
Seryl-tRNA synthetase (P95689), *S. aureus* NCTC 8325	5’-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT TAT GTT AGA CAT TAG ATT AT TC	5’-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTG TTA TTT AAC TGG TTT TGA AAT TT

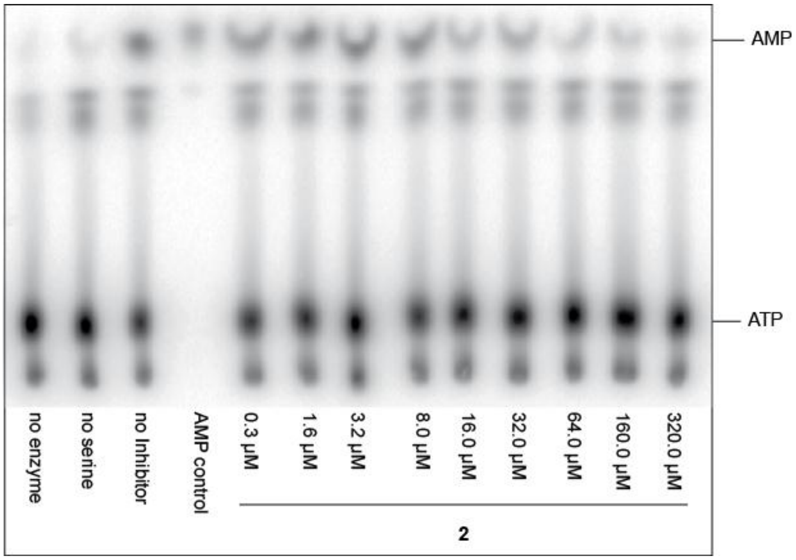
Supporting Table 2. Gateway PCR primers.



Supporting Figure 5. Labeling of recombinant Seryl-tRNA synthetase in *E. coli* proteome. I: induced nI: non-induced.



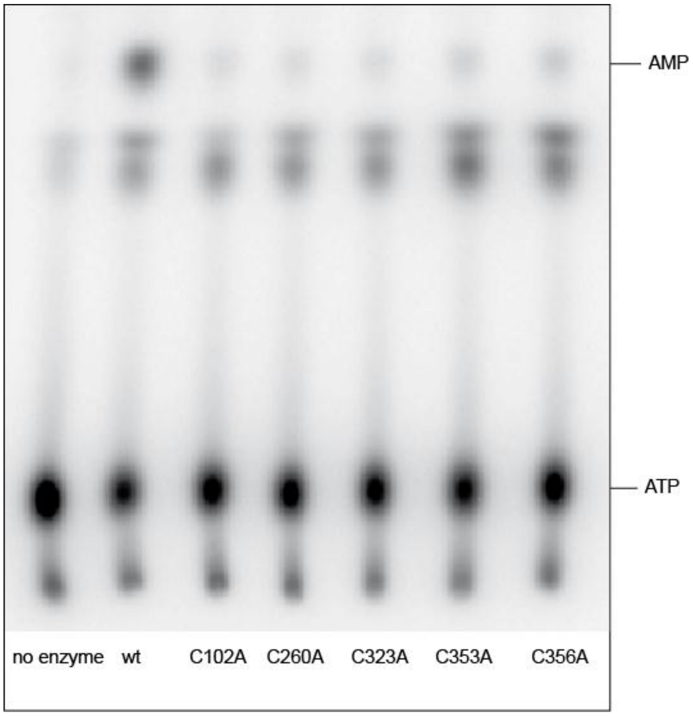
Supporting Figure 6. TLC read out for Seryl-tRNA-Synthetase catalyzed AMP quantification.



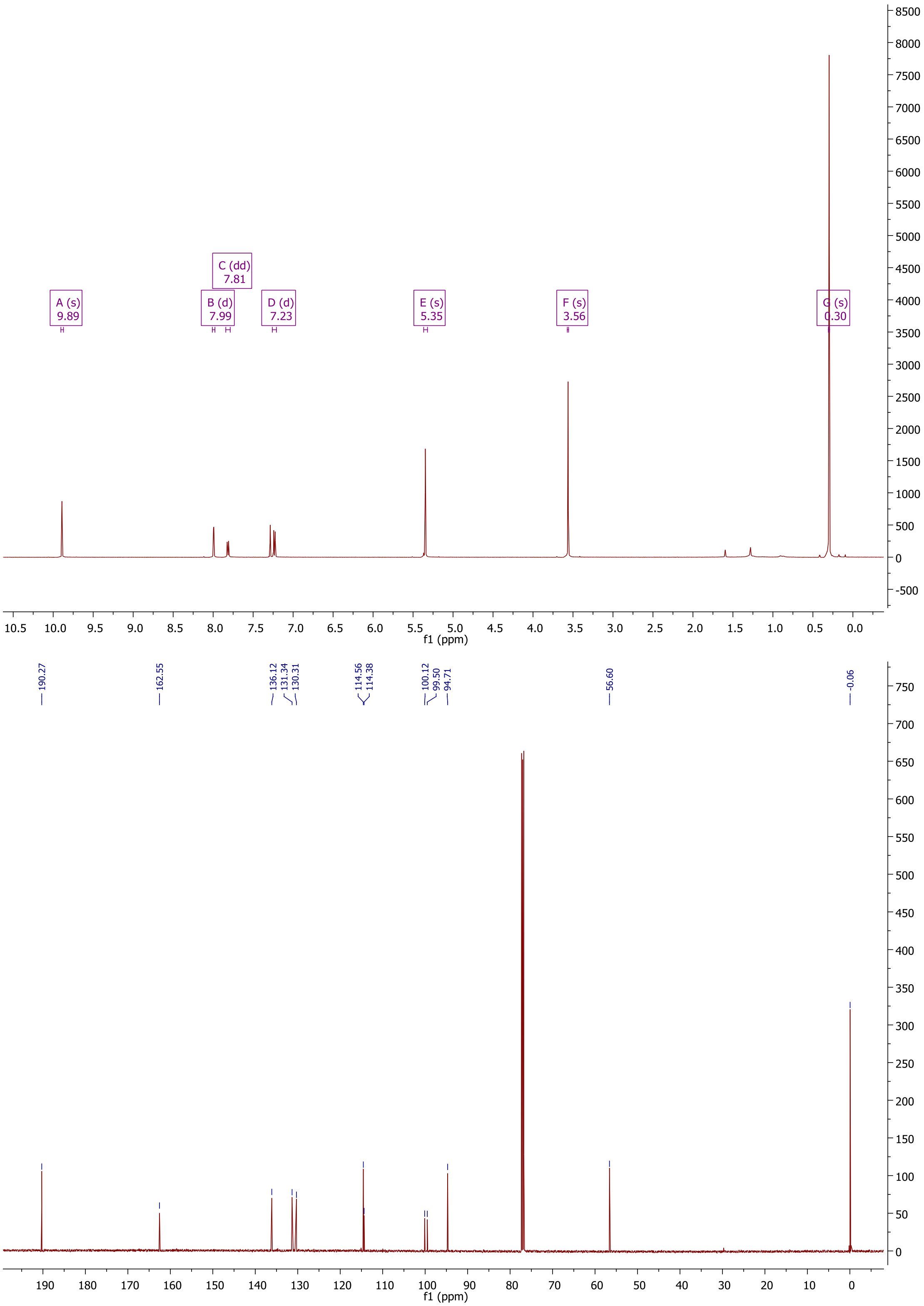
Supporting Figure 7. TLC read out for Seryl-tRNA-Synthetase catalyzed AMP quantification with “no serine” and AMP control.

Seryl-tRNA synthetase (P95689), *S. aureus* NCTC 8325 mutants	Forward primer	Reverse primer
C102A	5’-TAA ATG AAA TTG ATA ATA AAA TGA CAG GTA TCC TTG CTC GTA TTC CAA ATT TAA TAA GTG ATG A	5’-TCA TCA CTT ATT AAA TTT GGA ATA CGA GCA AGG ATA CCT GTC ATT TTA TTA TCA ATT TCA TTT A
C260A	5’-ATT CAC TGG TCA ATC TGC AGC TTT CCG TAG TGA AGC AGG A	5’-TCC TGC TTC ACT ACG GAA AGC TGC AGA TTG ACC AGT GAA T
C323A	5’-GTT AGG TTT ACC ATA CCG TCG TGT TAT TTT AGC TAC AGG TGA TAT TGG AT	5’-ATC CAA TAT CAC CTG TAG CTA AAA TAA CAC GAC GGT ATG GTA AAC CTA AC
C353A	5’-TAC AAT GAT TAT AAA GAA ATT AGT TCA GCC TCA AAC TGT ACG GAT TTC CAA GC	5’-GCT TGG AAA TCC GTA CAG TTT GAG GCT GAA CTA ATT TCT TTA TAA TCA TTG TA
C356A	5’-AAA TTA GTT CAT GCT CAA ACG CTA CGG ATT TCC AAG CGC GTC	5’-GAC GC GCT TGG AAA TCC GTA GCG TTT GAG CAT GAA CTA ATT T

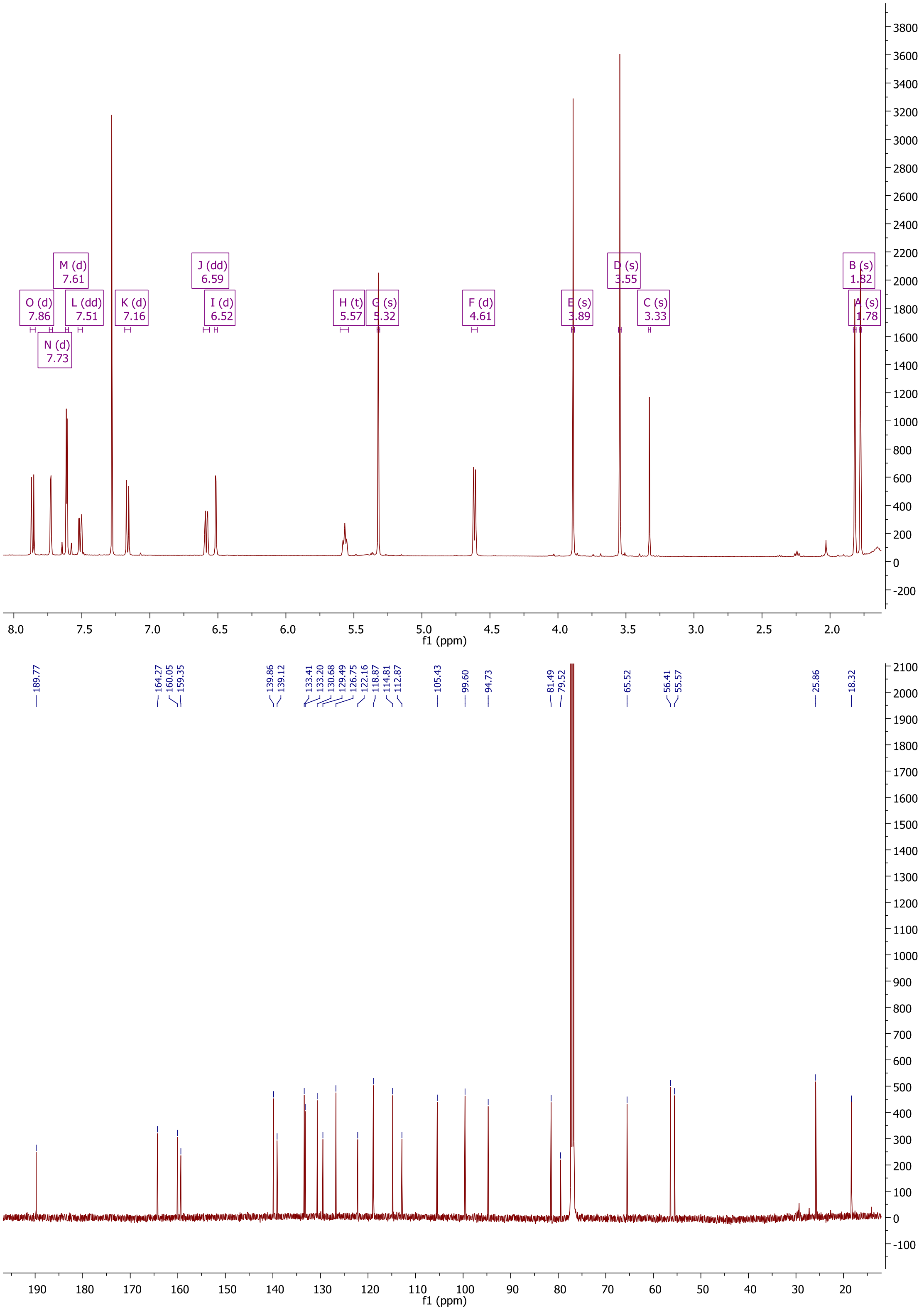
Supporting Table 3. Missmatch primers for cysteine to alanine mutations.



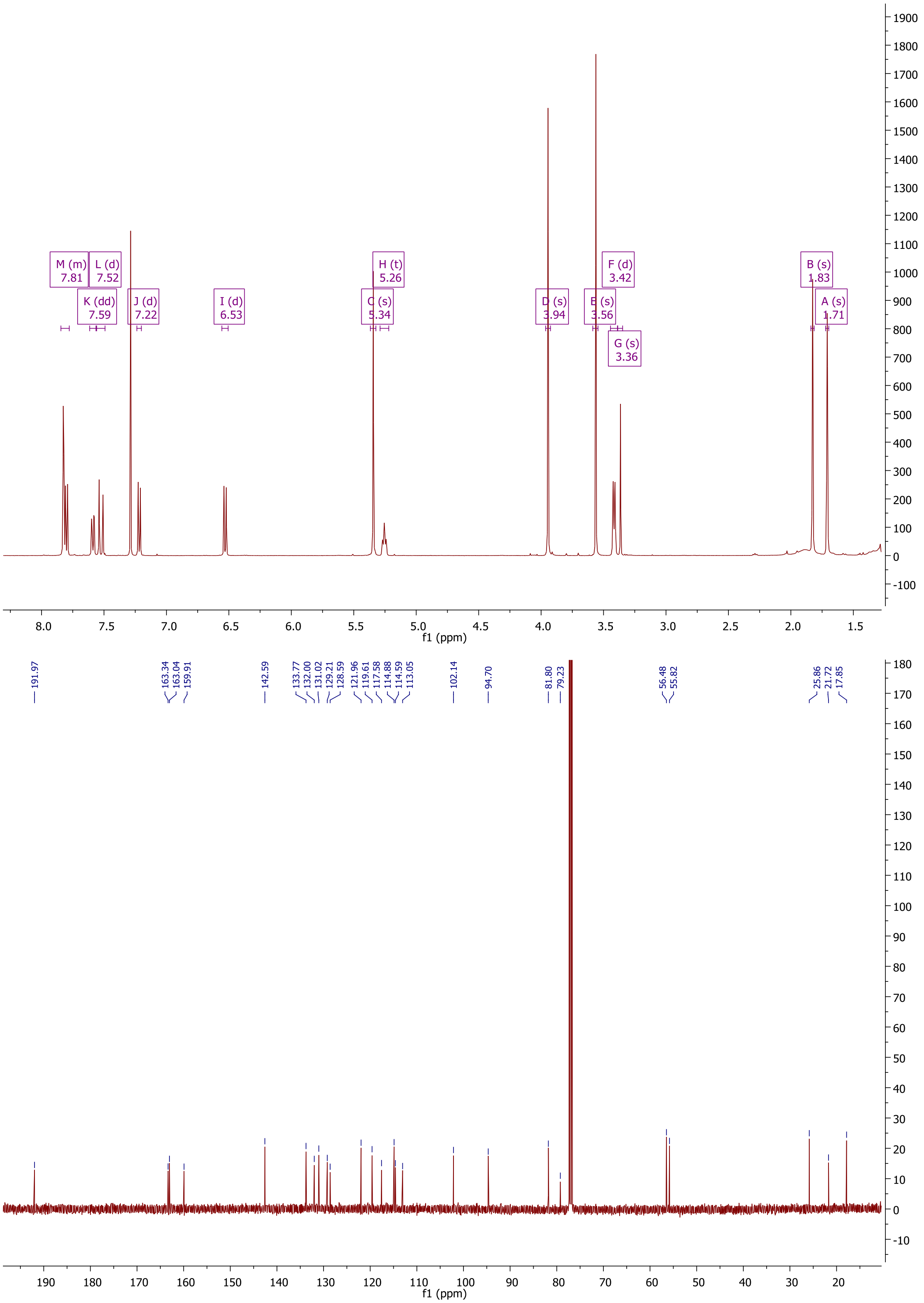
Supporting Figure 8. TLC activity read out for STS wt and STS C to A mutants.



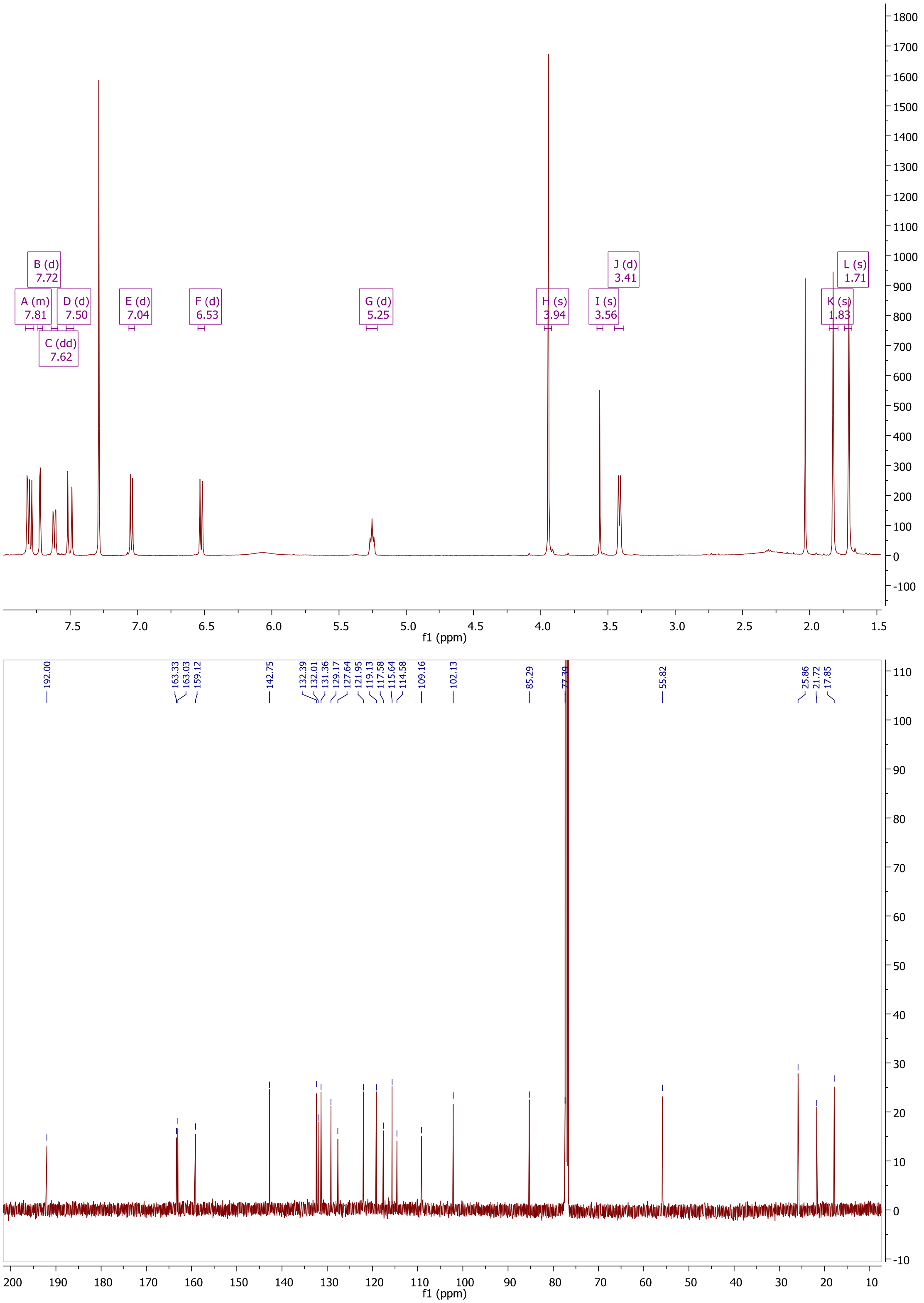
Supporting Figure 9: ^{1}H and ^{13}C NMR spectra of **5**.



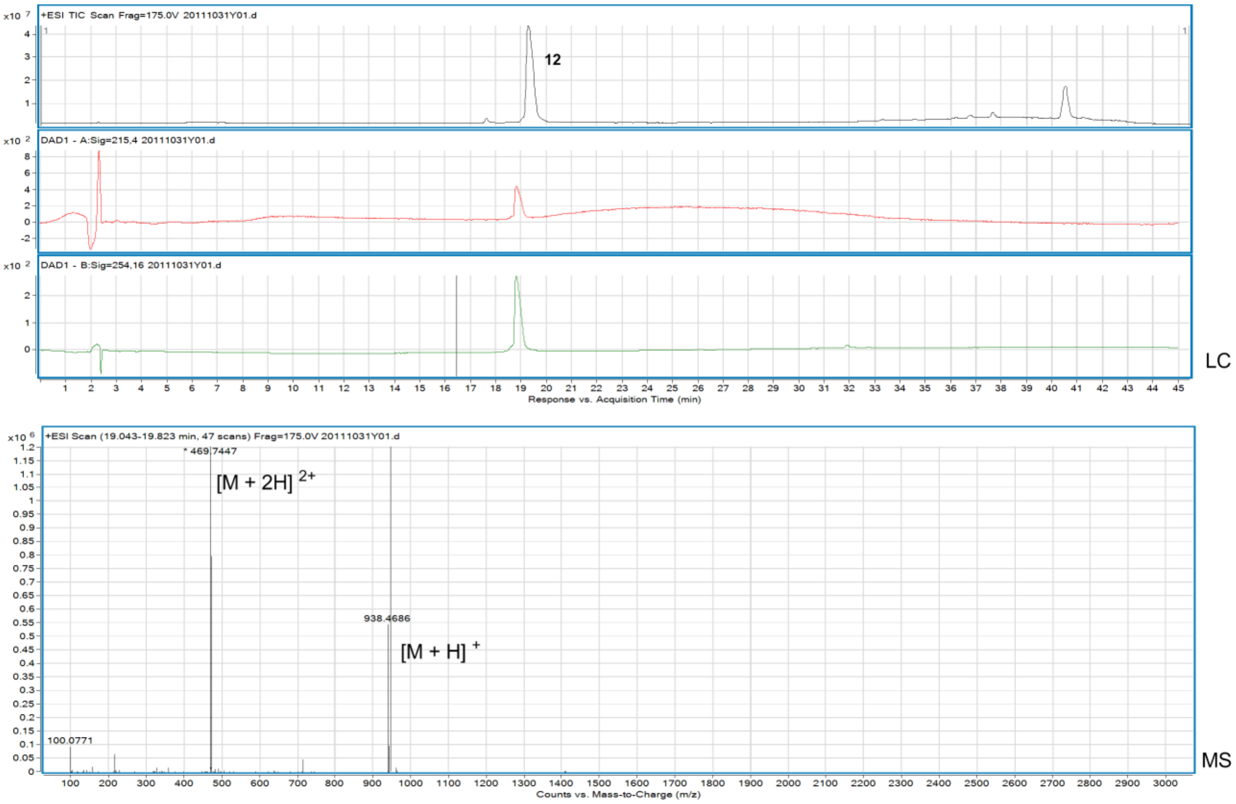
Supporting Figure 10: ^{1}H and ^{13}C NMR spectra of **8**.



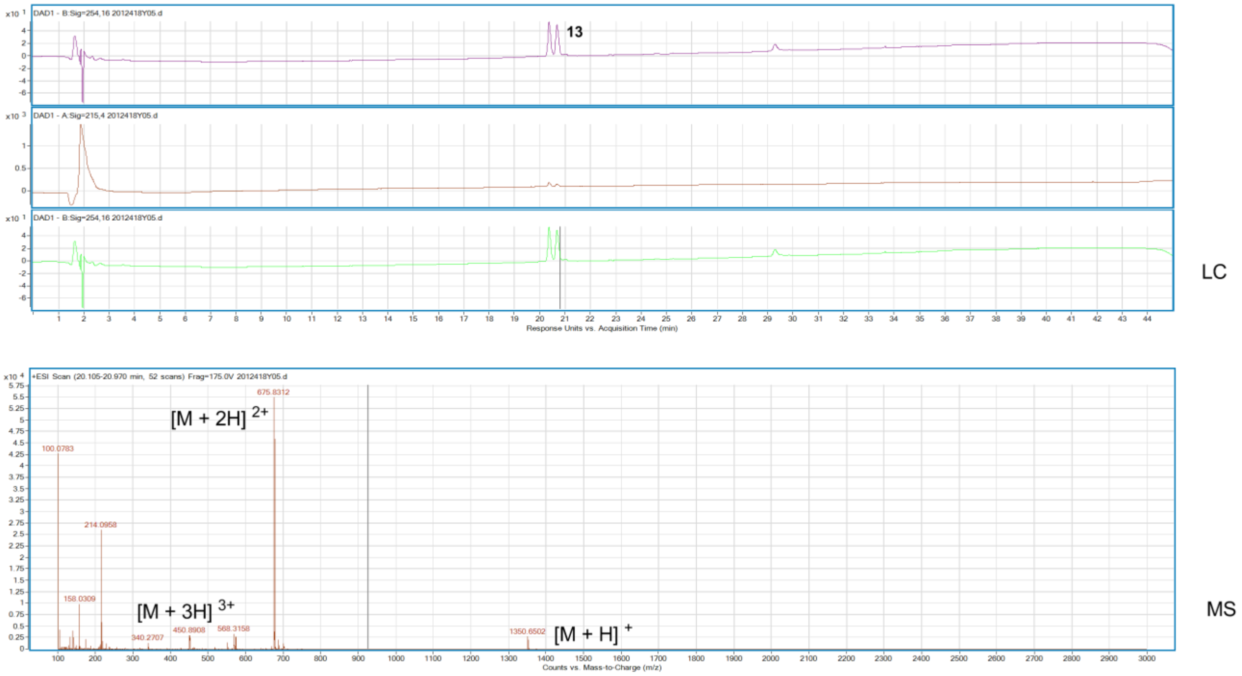
Supporting Figure 11: ^{1}H and ^{13}C NMR spectra of **9**.



Supporting Figure 12: ^{1}H and ^{13}C NMR spectra of **2**.



Supporting Figure 13: LC-MS data for biotin-diazo-N3 linker **12**.



Supporting Figure 14: LC-MS data for Rh-biotin-diazo-N_3 linker **13** (two Rh isomers).

[1] J. Eirich, J. L. Burkhart, A. Ullrich, G. C. Rudolf, A. Vollmar, S. Zahler, U. Kazmaier, S. A. Sieber, *Mol Biosyst* **2012**, *8*, 2067-2075.