

Supplemental Material for “Metabolomics Analysis Identifies D-Alanine-D-alanine Ligase as the Primary Lethal Target of D-cycloserine in Mycobacteria” by Steven Halouska, Robert J. Fenton, Denise K. Zinniel, Darrell D. Marshall, Raul G. Barletta, Robert Powers

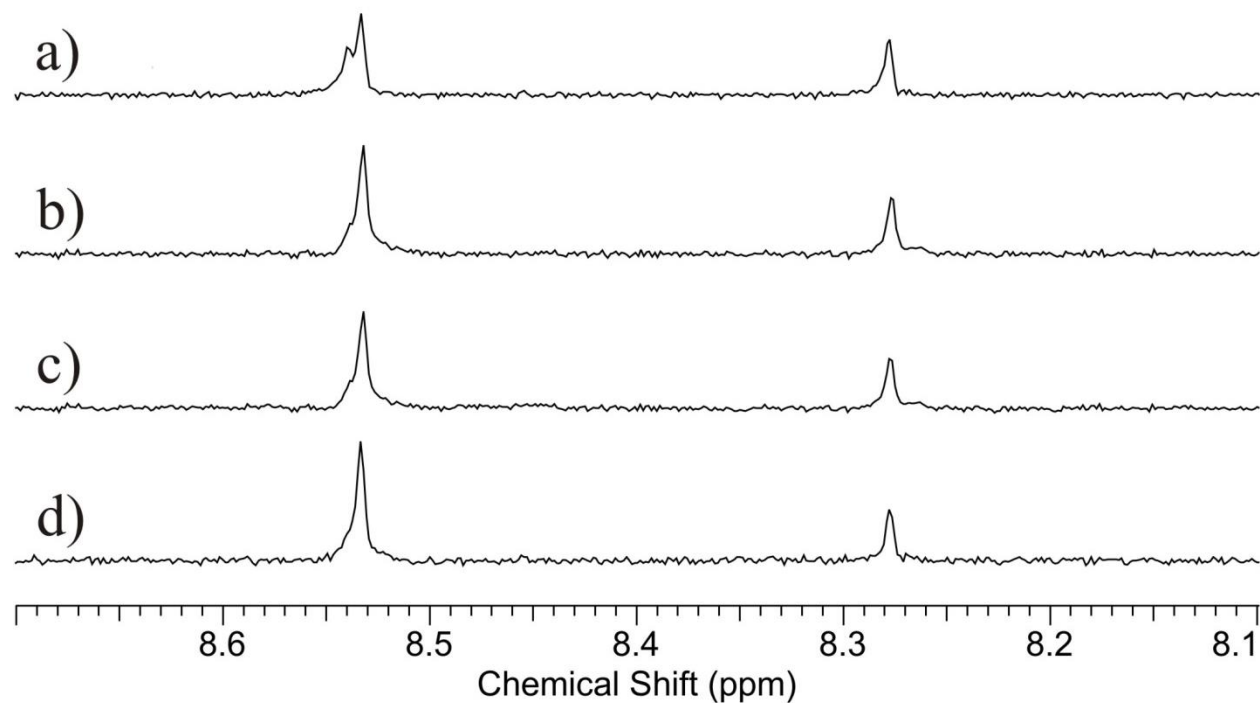


Figure 1S: Ligand binding assays comparing the intensities of the ATP peaks (8.28 and 8.54ppm) in mixtures consisting of: a) 100 μ M ATP, D-alanine, DCS and 25 μ M Ddl; b) 100 μ M ATP and D-alanine and 25 μ M Ddl; c) 100 μ M ATP and 25 μ M Ddl; and d) 100 μ M ATP as a control.

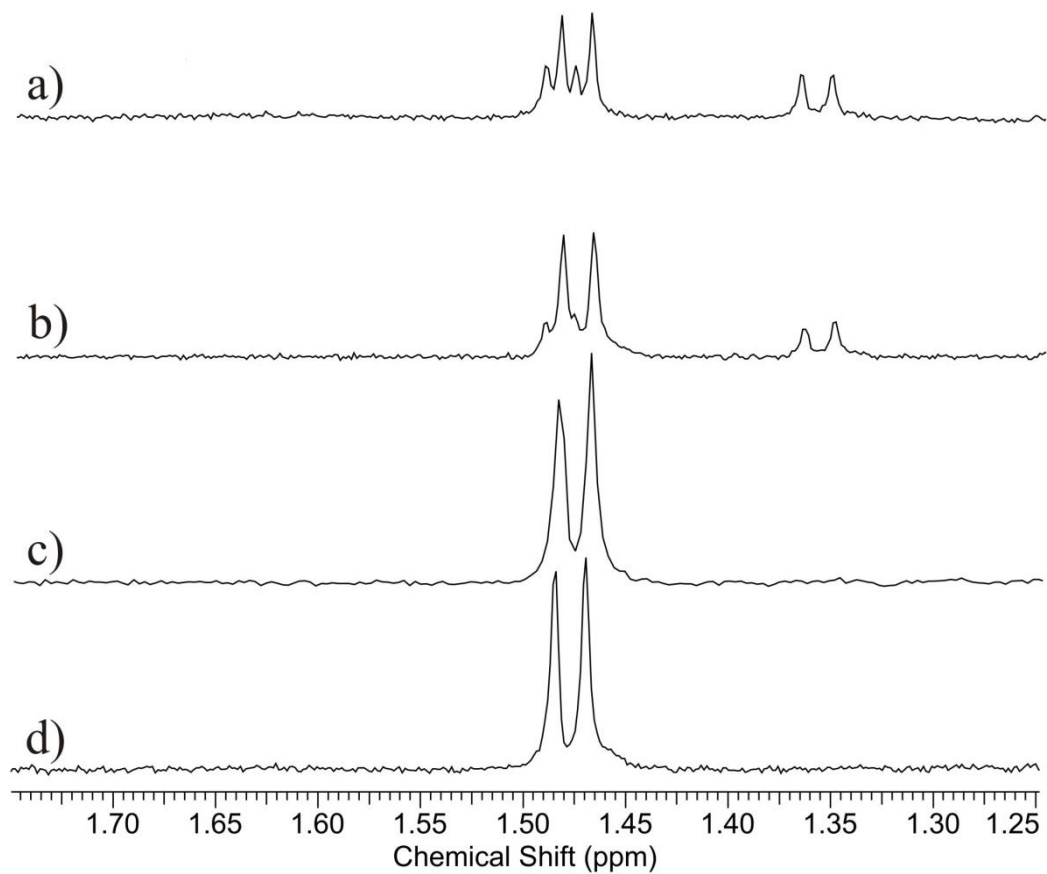


Figure 2S: Ligand binding assays comparing the intensities of the D-alanine peaks (1.45-1.50) in mixtures consisting of: a) 100 μ M ATP, D-alanine, DCS and 25 μ M Ddl; b) 100 μ M ATP and D-alanine and 25 μ M Ddl; c) 100 μ M D-alanine and 25 μ M Ddl; and d) 100 μ M D-alanine as a control. Formation of D-alanyl-D-alanine (1.33-1.38ppm) is formed when ATP, D-alanine, and Ddl are in the same mixture.

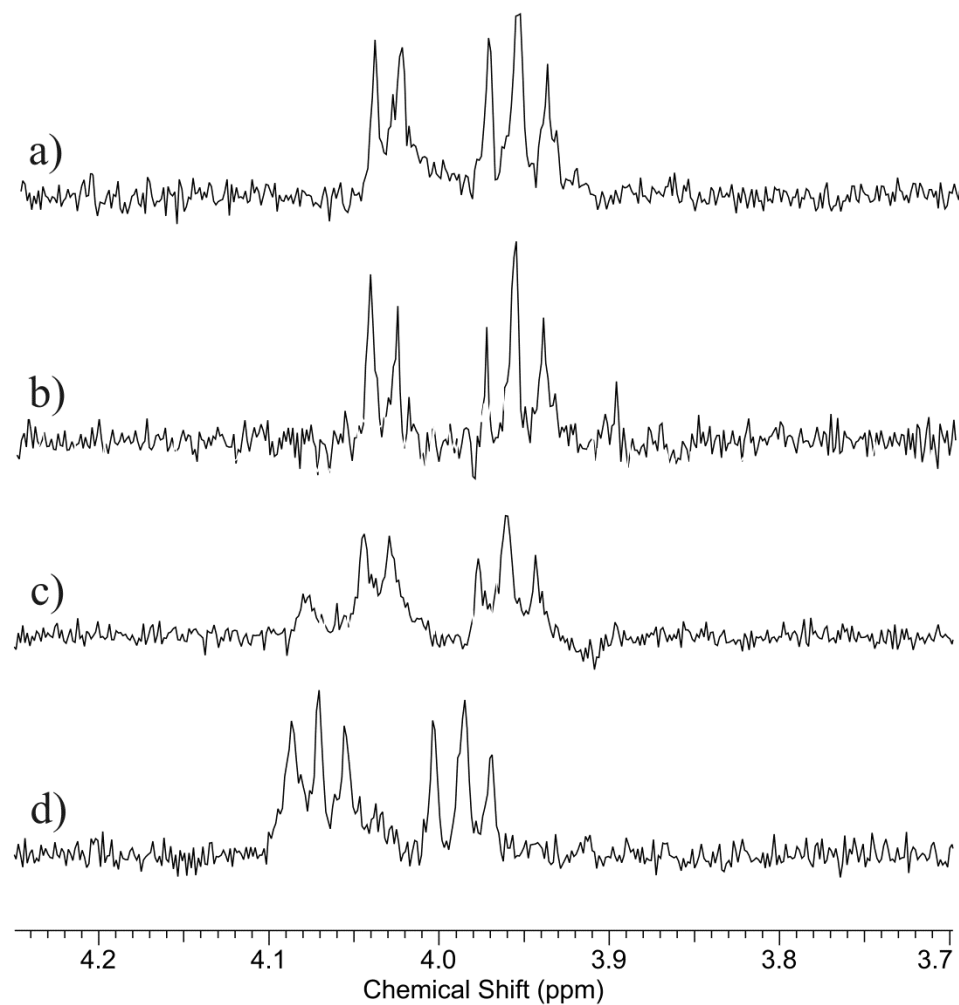
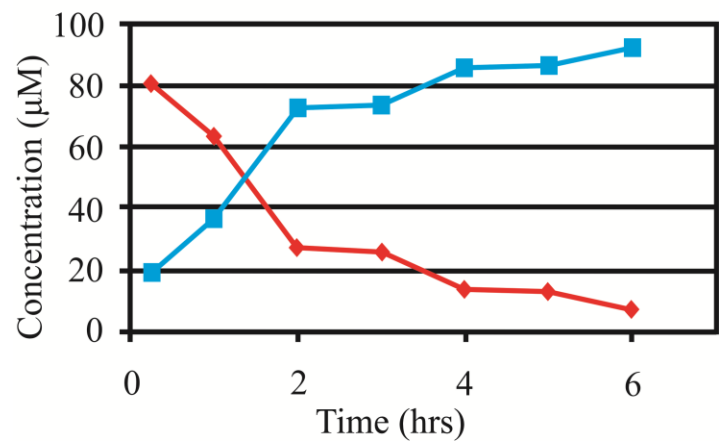


Figure 3S: Ligand binding assays comparing the intensities of the DCS peaks (3.95-4.10 ppm) in mixtures consisting of: a) 100 μ M ATP, D-alanine, DCS and 25 μ M Ddl; b) 100 μ M ATP, DCS and 25 μ M Ddl; c) 100 μ M DCS and 25 μ M Ddl; and d) 100 μ M DCS as a control.

a)



b)

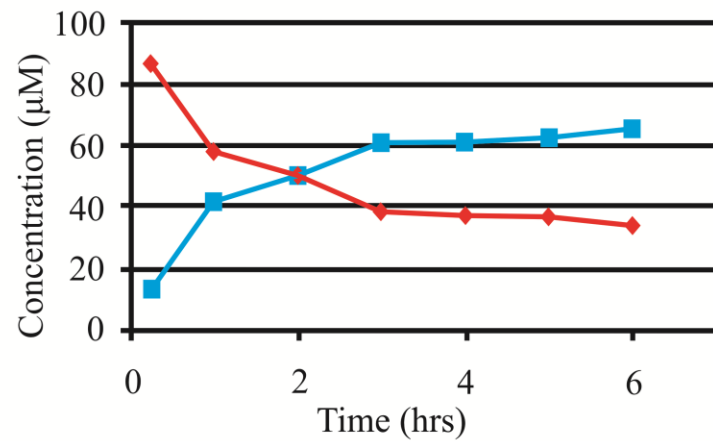


Figure 4S: 1D ^1H NMR was used to monitor the conversion of (a) ATP (blue) to ADP (red) and (b) D-alanine (red) to D-alanyl-D-alanine (blue) by Ddl. The initial concentrations were fixed at Ddl (25 μM) D-alanine (100 μM) and ATP (100 μM).