Supplementary Information

UDP-(5F)-GlcNAc acts as a slow-binding inhibitor of MshA, a retaining glycosyltransferase.

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Experimental Procedures

Purification of MshA. The expression and purification of MshA from *Corynebacterium glutamicum* was performed as previously described (Vetting, Frantom, and Blanchard *J. Biol. Chem.* **2008**, *23*, 15834-15844). A plasmid carrying the *mshA* gene was introduced into Rosetta2 cells (Invitrogen). The cells were grown at 23 ºC in ZYP5052 media for 36-48 hours. The cells were then lysed, and the supernatant was passed over a nickel-Sepharose HP (GE-Healthcare) column. Eluant fractions containing the enzyme were made 1 M in $(NH_4)_2SO_4$ and applied to a Phenyl-Sepharose HP (GE-Healthcare) column. Eluant fractions containing the enzyme were concentrated by ultrafiltration and stored at -80 ºC.

Kinetic Assays. The production of UDP was measured using a coupled assay system and monitoring the reaction at 340 nm. Standard conditions were 30 nM enzyme, 50 mM TAE, pH 7.8, 200 μM NADH, 500 μM PEP, 10 mM $MgCl_2$, 20 units of pyruvate kinase, and 55 units of lacate dehydrogenase in a 1 mL reaction at 25 ˚C. Except for the enzyme, all components were mixed in the cuvette and allowed to equilibrate for two minutes. Reactions were initiated by the addition of enzyme. In experiments where the inhibitor was pre-incubated with the enzyme, the reactions were initiated by the addition of UDP-GlcNAc. The amount of coupling enzymes was sufficient to not limit the rate of reaction and required to minimize a lag in the assays.

Data Analysis. Initial velocities from the inhibition studies of UDP-(5F)-GlcNAc versus UDP-GlcNacData were fit to equation S1 where V_{max} is the maximal velocity, S is the concentration of UDP-GlcNAc, K_m is the Michaelis constant for UDP-GlcNAc, I is the concentration of UDP-(5F)-GlcNAc, and K_i is the inhibition constant for UDP-(5F)-GlcNAc.

$$v = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_i}\right)+[S]} \qquad \text{Equation S1}$$

Data described with single variable equations were analyzed using KaleidaGraph (Synergy Software). Data described using equations with multiple independent variables were analyzed using GraFit (Erithacus Software).

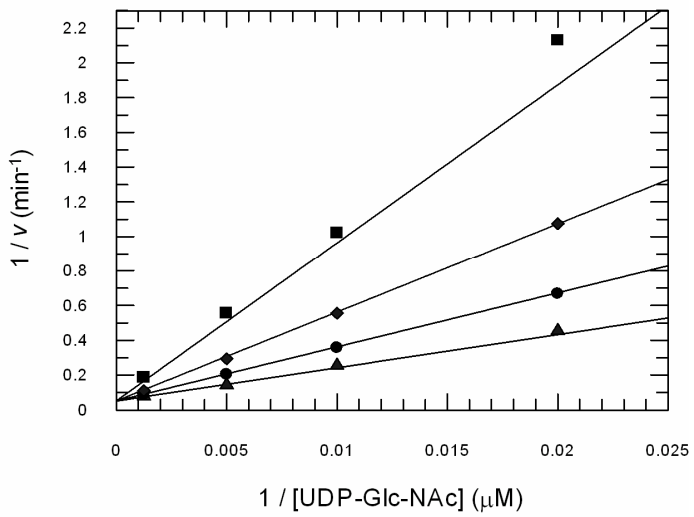

Figure S1. Double-reciprocal plot for UDP-(5F)-GlcNAc versus UDP-GlcNAc. Enzymatic reactions were performed as described in Materials and Methods with the addition of 2 mM L-I1P and varying amounts of UDP-GlcNAc. Concentrations of UDP-(5F)-GlcNAc used were 1 μM (▲), 2.5 μM (●), 5 μM (♦), and 10 μM (■). Solid lines are from a fit to equation S1. The results of the fit are V_{max} = 19.2 ± 0.8 s^{-1}, $K_{UDP\text{-}GlcNAc}$ = 212 ± 29 μM, and K_i = 1.4 ± 0.2 μM.