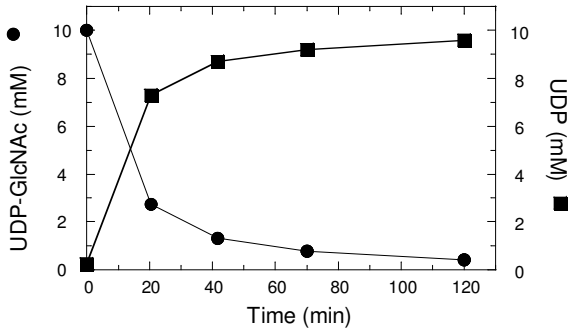
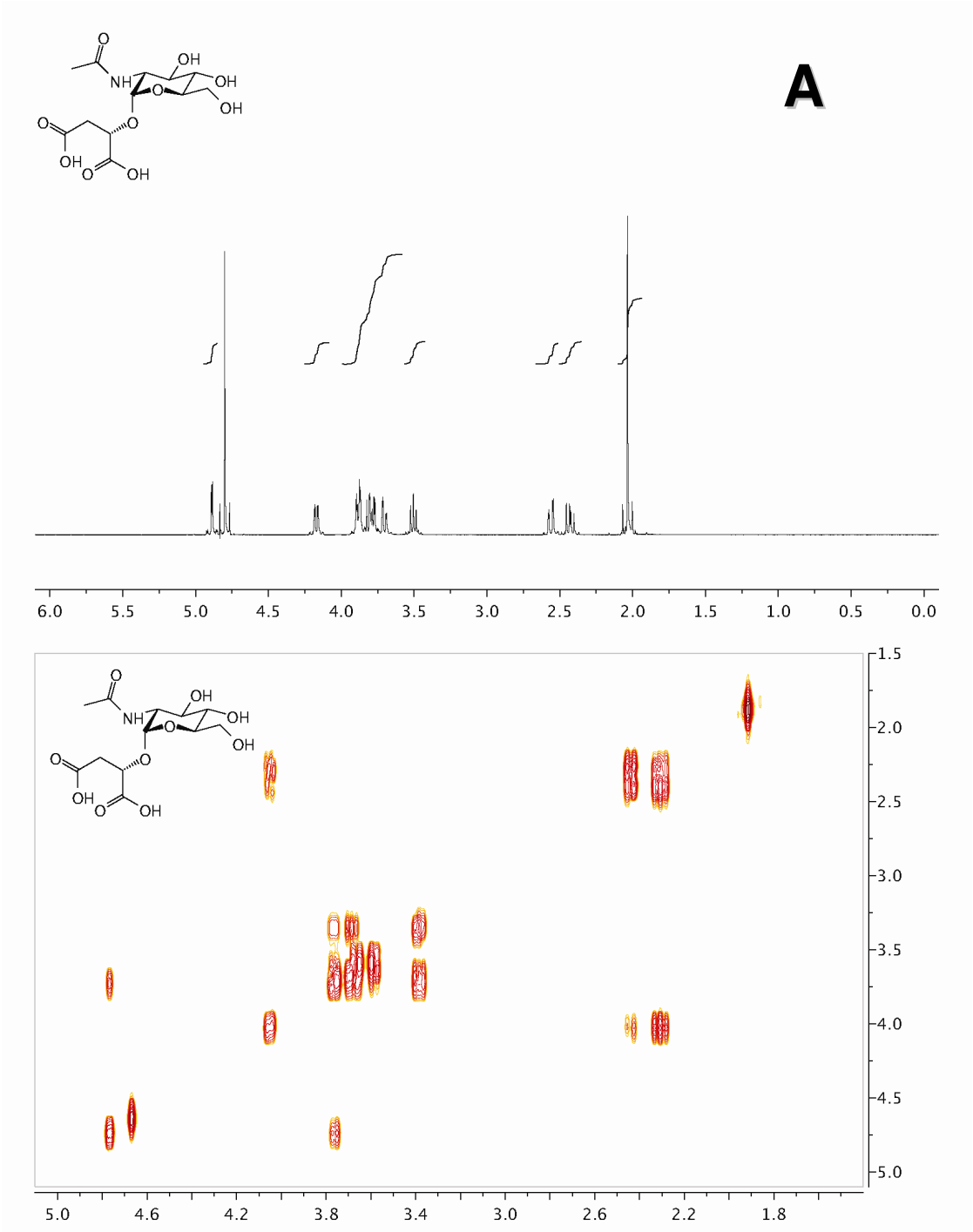


Figure S1. Preparative conversion of UDP-GlcNAc to GlcNAc-malate by *Ba*BshA (ORF BA1558). Product was confirmed independently by NMR (^{1}H, gCOSY, and ^{13}C; Figure S2) and high resolution mass spectrometry.

Figure S2. NMR spectra of purified GlcNAc-malate (structure given in Figure S2A) synthesized enzymatically with *Ba*BshA as described in "Experimental Procedures" and Figure S1. (A) 500 MHz proton NMR spectrum and gradient COSY NMR correlation of GlcNAc-malate at pH 6-7. (B) 125 MHz carbon NMR spectrum at pH 6-7. ^{1}H NMR (500 MHz, D_2O, trace sodium trifluoroacetate) δ ppm; 4.89 (d, *J* 3.8 Hz, 1H), 4.17 (dd, *J* 2.6, 11.1 Hz, 1H), 3.86-3.9 (m, 2H), 3.76-3.83 (m, 2H), 3.70 (dd, *J* 2.3, 12.5 Hz, 1H), 3.50 (dd, *J* 10.0, 8.8 Hz, 1H), 2.56 (dd, *J* 2.5, 15.0 Hz, 1H), 2.43 (dd, *J* 11.1, 15.0 Hz, 1H,), 2.04 (s, 3H); ^{13}C NMR (125 MHz, D_2O, trace sodium trifluoroacetate) δ ppm; 179.4, 178.3, 174.3, 99.0, 79.4, 72.2, 71.1, 69.4, 59.8, 53.5, 41.6, 21.9.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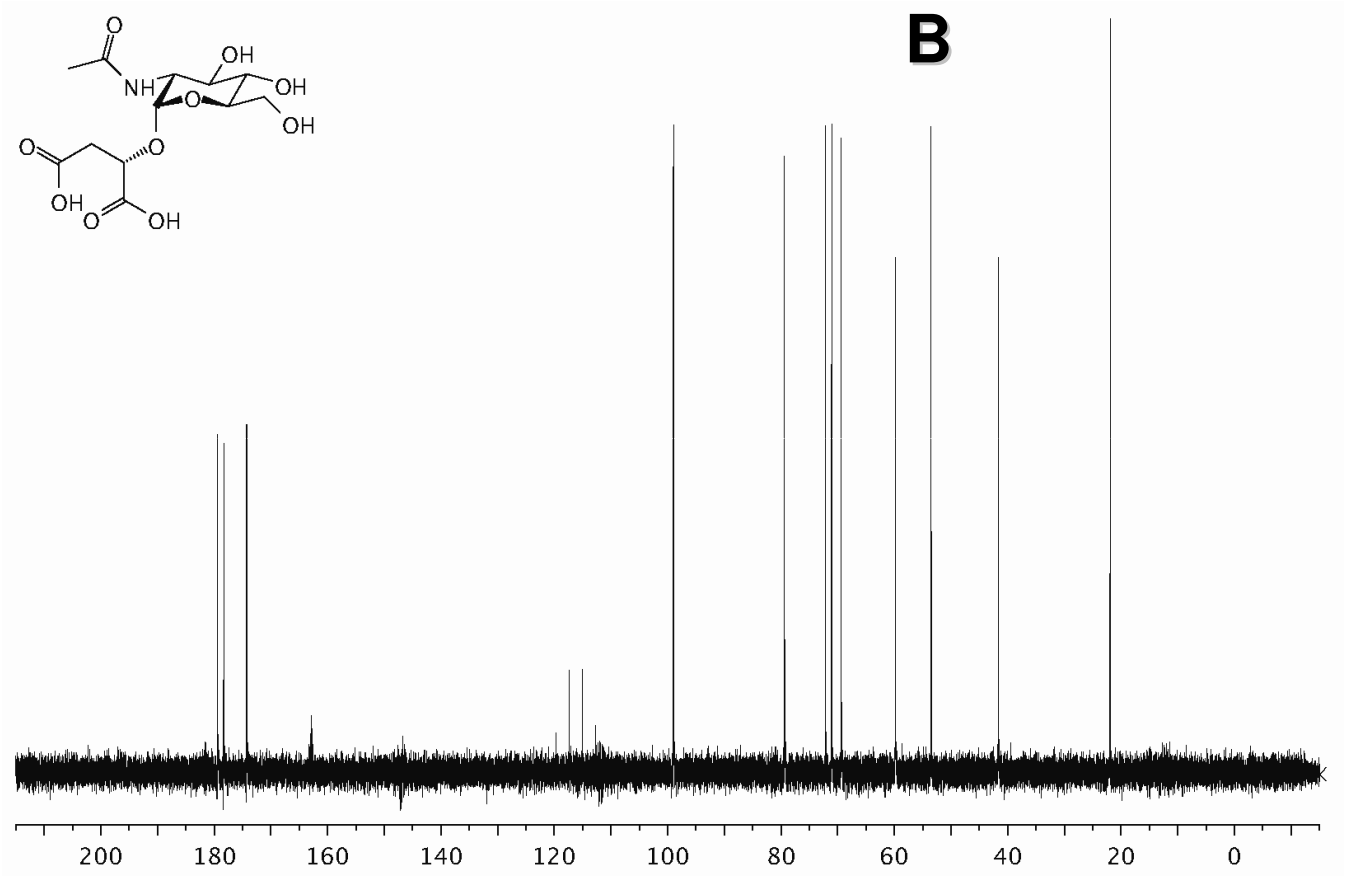
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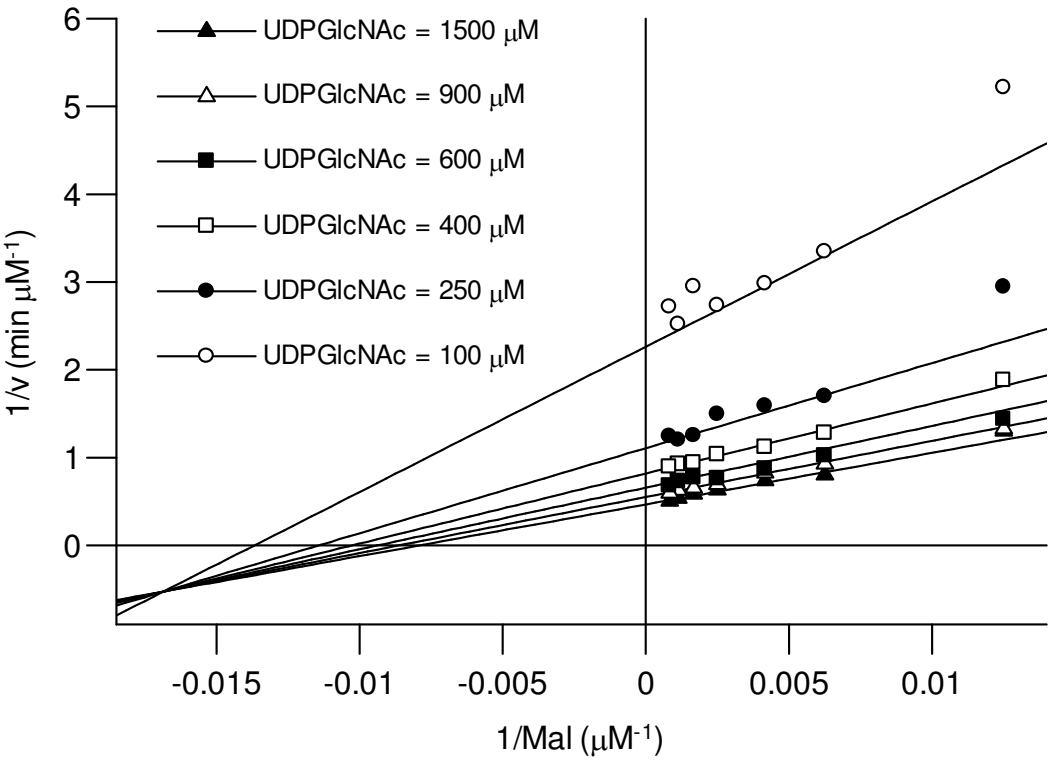

Figure S3. Initial velocity reciprocal plots of *Ba*BshA activity. The [L-malate] was varied while [UDP-GlcNAc] was held constant at 1.5 mM (▲), 0.9 mM (△), 0.6 mM (■), 0.4 mM (□), 0.25 mM (●), or 0.1 mM (○). Solid lines are from fits of the data to equation 2.

Table S1: Primers Used in the Study

Name	Sequence	Use
BAS1444up FWD	CACACACAGCGGCCGCAAAGCAGCAAGGTCATCC	Construction and verification of Δ*bshA*
BAS1444up REV	CTCATAAATCCTTCGGCATACCCGGGCACTCATAATTGTTCCCCTC	
BAS1444dwn FWD	CCCGGGTATGCCGAAGGATTTATGAG	
BAS1444dwn REV	CACACACAGCGGCCGCCAACATCAGATTGCTCAATACC	
BAS1444out FWD	CAGTAGGAACAATTATCGCTCC	
BAS1444out REV	CACAACATCTTGCACACG	
BAS1445up FWD	CACACACAGCGGCCGCGAGGGGAACAATTATGAGTG	Construction and verification of Δ*bshB*
BAS1445up REV	CTGCTCGTAAACACTTTCTCTTCCCGGGCCAGAACCACCTACAGAAGG	
BAS1445dwn FWD	CCCGGGAAGAGAAAGTGTTTACGAGCAG	
BAS1445dwn REV	TATATATAGCGGCCGCCTCCACTGTAATTCTCTCAATTGC	
BAS1445out FWD	CAATCTGGTCCTCTTGGC	
BAS1445out REV	CCCATGCCTCAATGTCTG	
CoADR5f	ATAAGAATGCGGCCGCGTGGTACGACTATTTTGAGCGCATGATCTG	Construction and verification of Δ*cdr*
CoADR5r	CCGCTCGCCTCCCGGGCACTTTACCACTCCTTCTTTTTTACATACTCTTTACG	
CoADR3f	GTGGTTAAAGTGCCCGGGAGGCGAGCGGAATAGCACTTTTTAAGTGC	
CoADR3r	CGCGGATCCCATCAAATTCGATGTTTAGCTCCCTATATACTTTCTTCC	
CDR-F	CCGTATTAGACAGGAAAGCTAACGGC	
CDR-R	GCCCCCTGGTTCGTTCATTGCTTTG	
CDRRHD1	TAATAGTGAGGATCCCCCGGGACAGTCTTACCAGAACGTATTGTAT	Construction and verification of Δ*cdr2*
CDRRHD2	CCCGGGGGATCCTCACTATTATCCGCCTACTACAACTATTTTTCTG	
CDRRHD3	CGCGCGGCCGCGTACAGAGAATATTGTGTGTACTGA	
CDRRHD4	CGCGCGGCCGCATACATATACCTGTAGGGGTATAAT	
CDRRHD5	GTTCTGTGAATAAAAGAACAGGAAA	
CDRRHD6	CTTTTATACTCATAGTCGATTCCTC	

Table S2: *Ba*BshA (ORF BA1558) Acceptor Substrate Specificity

Substrate[a]	Specific activity (nmol/min/mg)	Relative rate
L-Malic acid	4800 ± 400	100
D-Malic acid	19 ± 4	0.4
Inositol 1-L-phosphate	14 ± 4	0.29
D-Glyceric acid	20 ± 1	0.42
Glycolic acid	22 ± 2	0.46
D,L-Isocitric acid	22 ± 3	0.46
Citric acid	22 ± 2	0.46
D-Lactic acid	17 ± 2	0.35

[a]0.1 mM Acceptor substrate with 3 mM UDP-GlcNAc, using *Ba*BshA assay conditions described in "Experimental Procedures" (n = 3).