

## Supporting Information

*for*

### Biochemical Studies of Inositol *N*-Acetylglucosaminyltransferase Involved in Mycothiol Biosynthesis in *Corynebacterium diphtheria*

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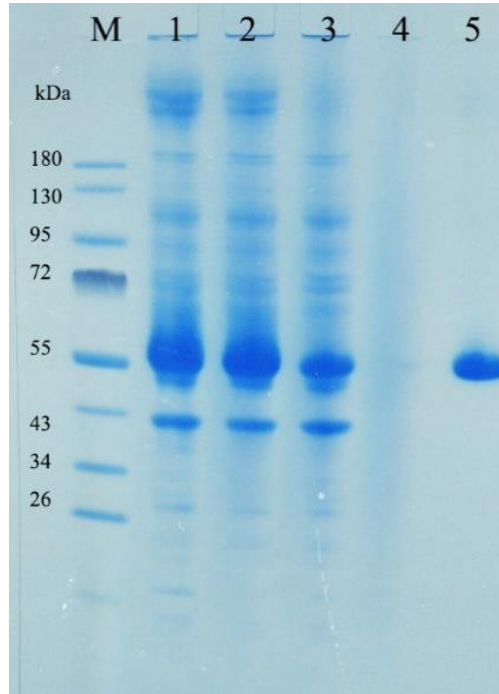
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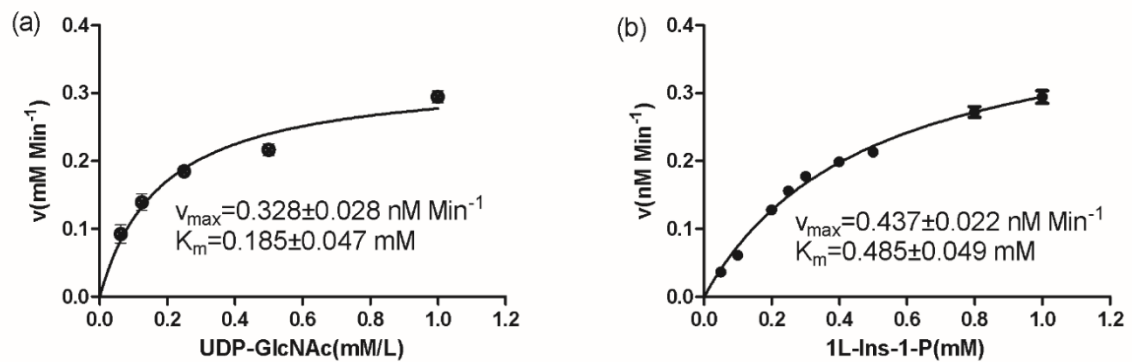
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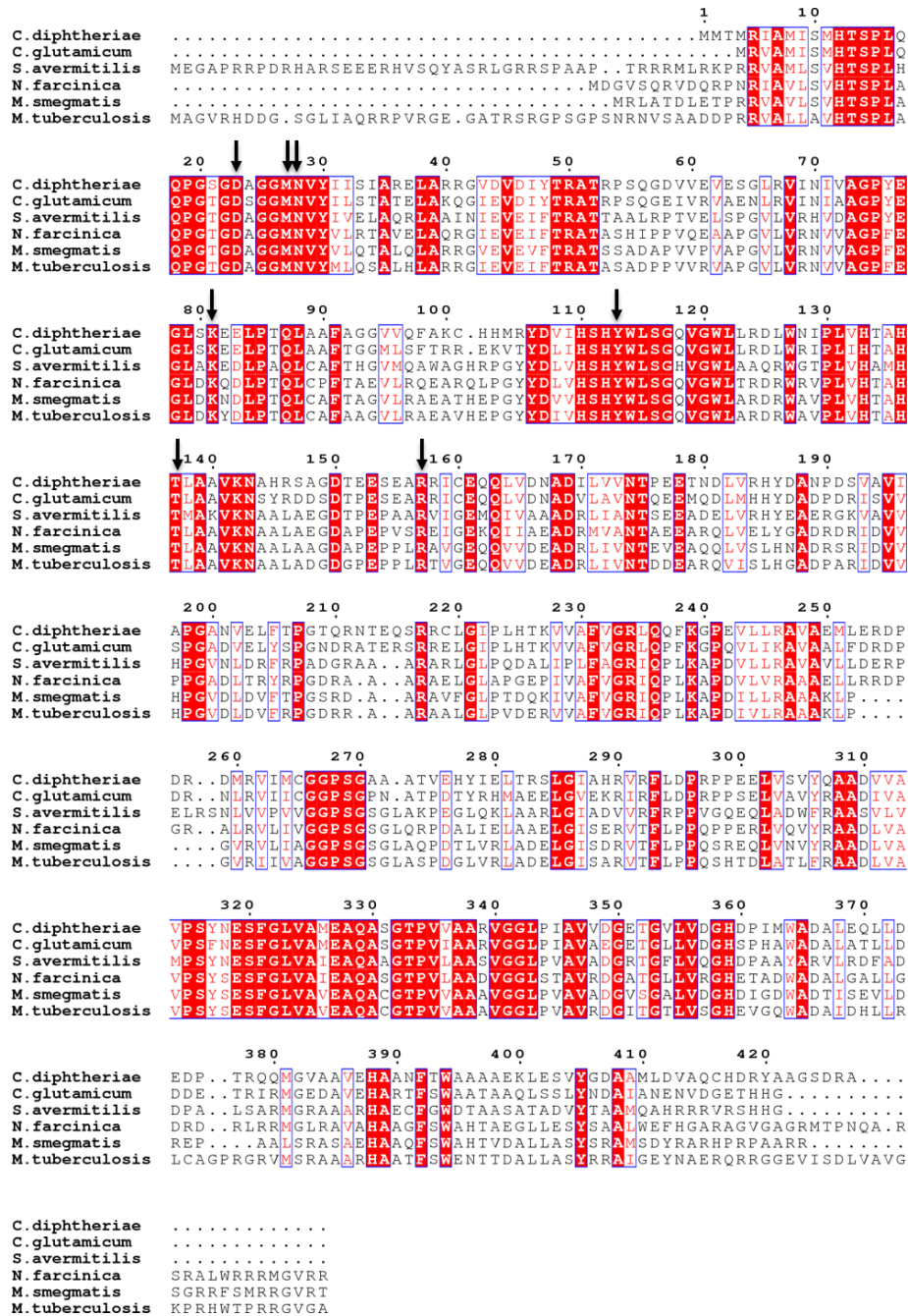
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**Figure S1.** SDS-PAGE results of CdMshA purification. Lane M: molecular marker; Lane 1: whole cell lysate; Lane 2: supernatant; Lane 3: flow through fraction; Lane 4: eluent with 50 mM of imidazole; Lane 5: eluent with 100 mM of imidazole



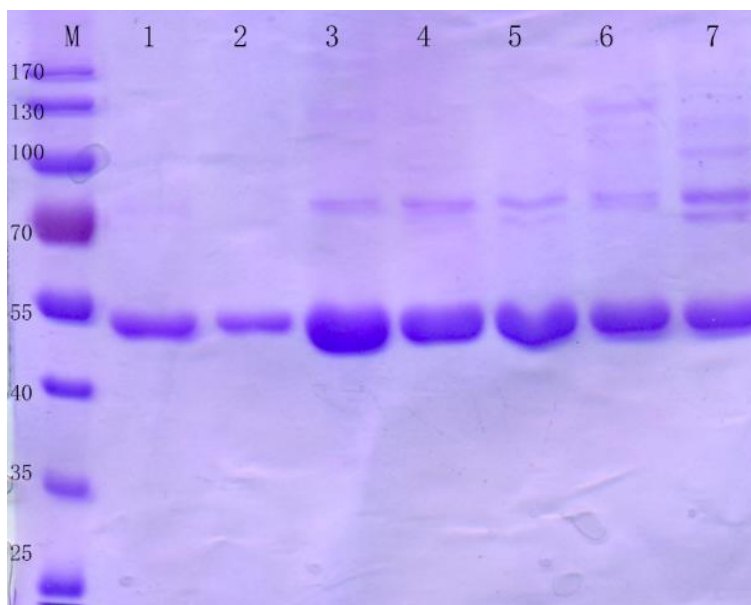
**Figure S2.** Results of kinetics studies on CdMshA-catalyzed reaction. The kinetics parameters for for UDP-GlcNAc (a) and 1L-Ins-1-P (b)



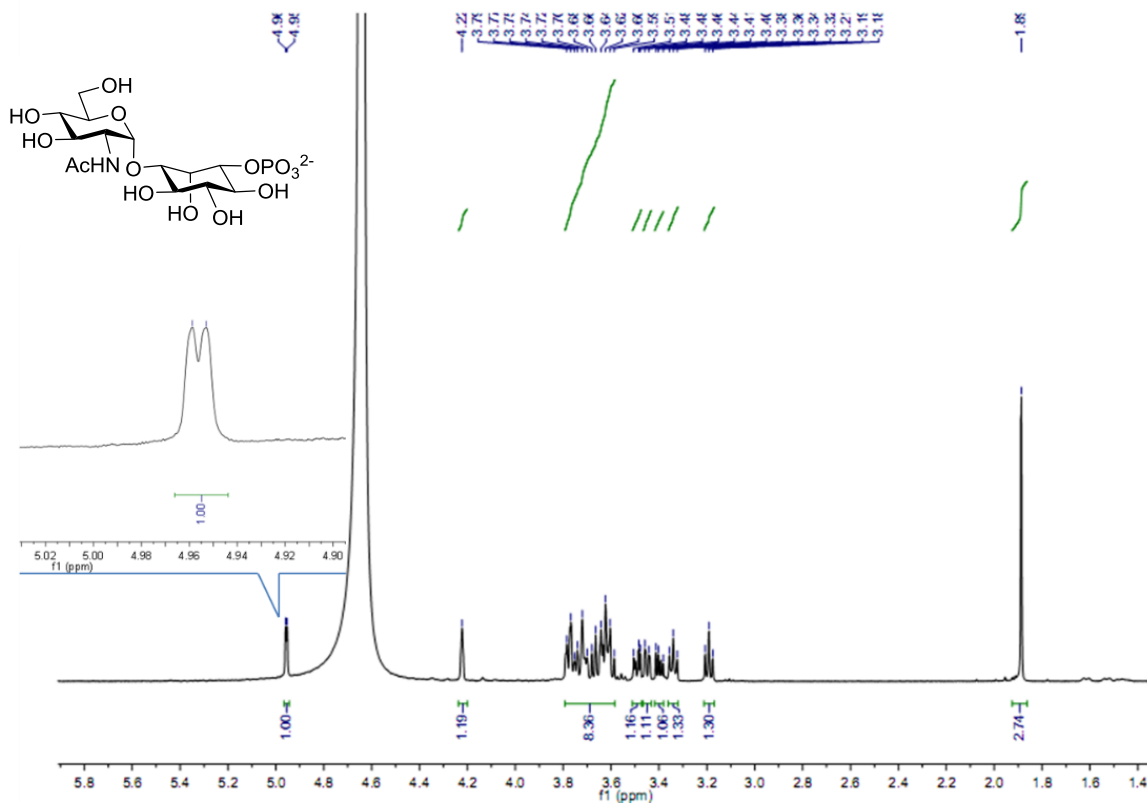
**Figure S3.** Sequence alignment of the *mshA* genes from various mycothiol-producing organisms: *C. diphtheriae* (WP\_014316335.1); *C. glutamicum* (BAB97794.1); *Streptomyces avermitilis* (WP\_010985435.1); *Nocardia farcinica* (BAD60044.1); *Mycobacterium smegmatis* (WP\_011727296.1); *M. tuberculosis* (NP\_215000.1). Red and pink boxes represent conserved and semi-conserved AA residues, respectively, among the orthologs analyzed. The sites studied by mutagenesis were indicated with arrows. Sequence alignment and comparison were conducted with Clustal Omega program (<http://www.ebi.ac.uk/Tools/msa/clustalo/>) and the figure was prepared with ESPrnt 3 (<http://esprnt.ibcp.fr/ESPrnt/cgi-bin/ESPrnt.cgi>)

**Table S2.** Primers used for site-directed mutagenesis of CaMshA

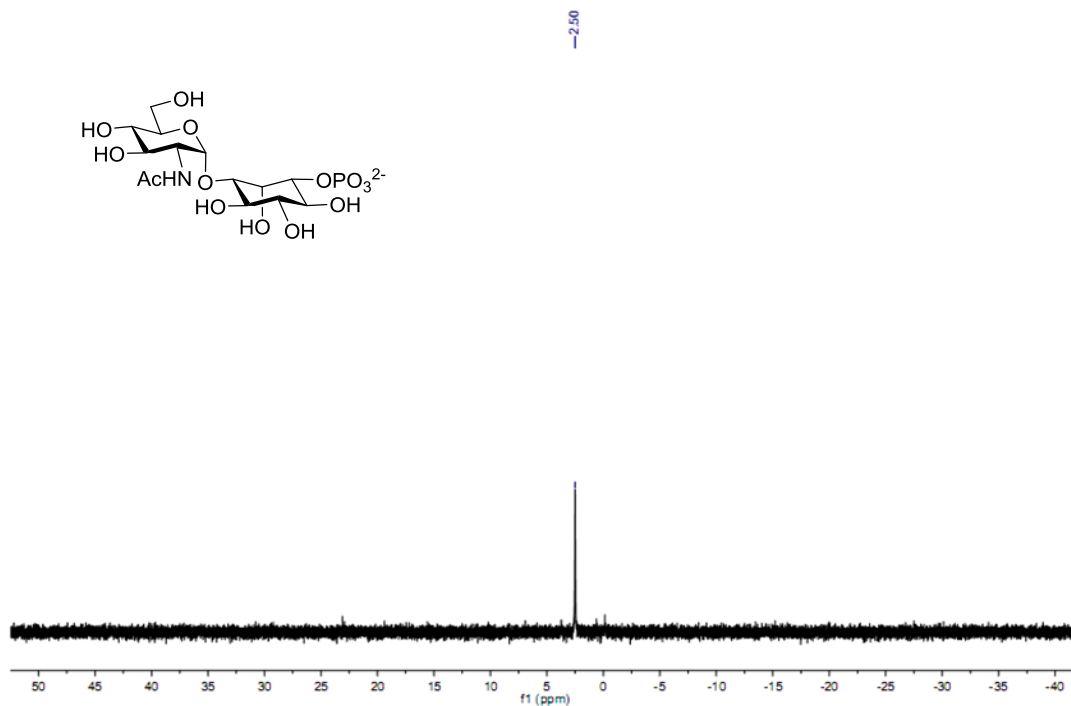
Mutants	Primers
D23A-F	CCAGGCAGCGGAGCCGCAGGTGGAATG
D23A-R	GGCTCCGCTGCCTGGTTGCTGCAGG
M27A-F	GACGCCGGTGGTGCGAACGTGTACATC
M27A-R	CGCACCACCGGCGTCACCGCTACCT
N28A-F	GCCGGTGGTATGGCCGTGTACATCATT
N28A-R	GGCCATAACCACCGGCGTCACCGCTA
K81A-F	GAGGGCCTGAGCGCAGAAGAAGACTGCCG
K81A-R	TGCGCTCAGGCCCTCATACGGGCCT
Y113A-F	ATCCATTCCCACGCCTGGCTGTCCGGC
Y113A-R	GGCGTGGGAATGGATCACGTCGTA
T137A-F	CACACCGCGCACGCCCTGGCGGCGGTA
T137A-R	GGCGTGCGCGGTGTGTACCAGTGGG
R157A-F	GAATCTGAGGCTGCTCGTATTTGTGAG
R157A-R	AGCAGCCTCAGATTCTTCGGTATCG



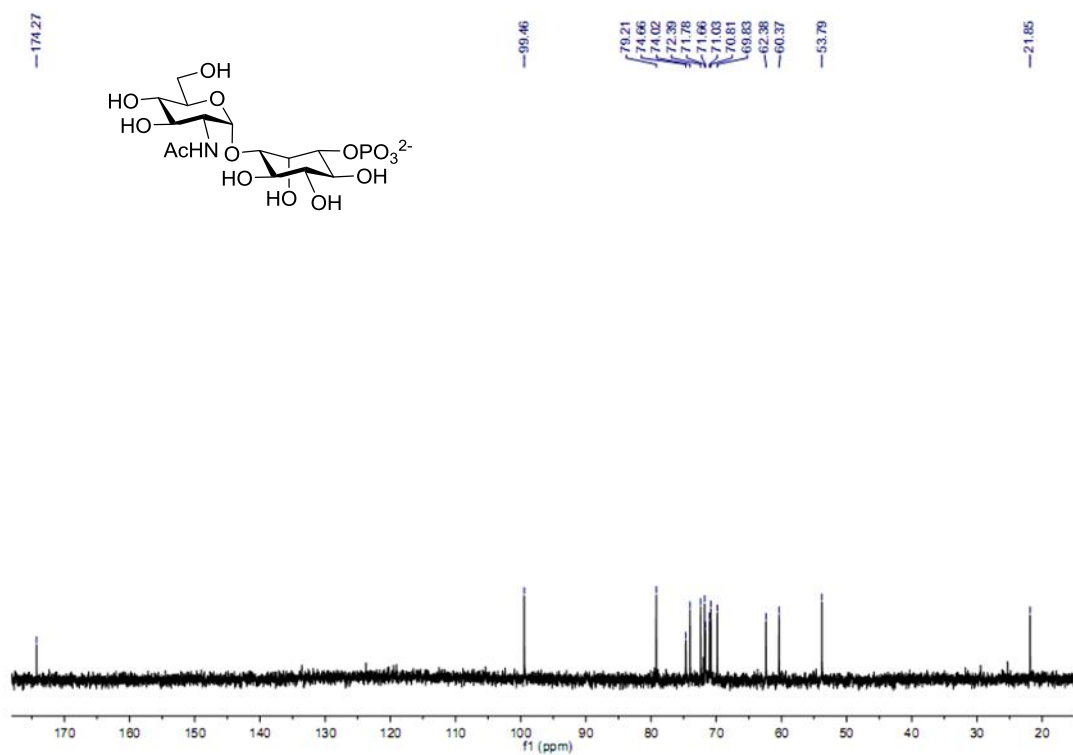
**Figure S4.** SDS-PAGE results of various CdMshA mutants. Lane M: molecular marker; Lane 1: D23A; Lane 2: M27A; Lane 3: N28A; Lane 4: K81A; Lane 5: Y113A; Lane 6: T137A; Lane 7: R157A



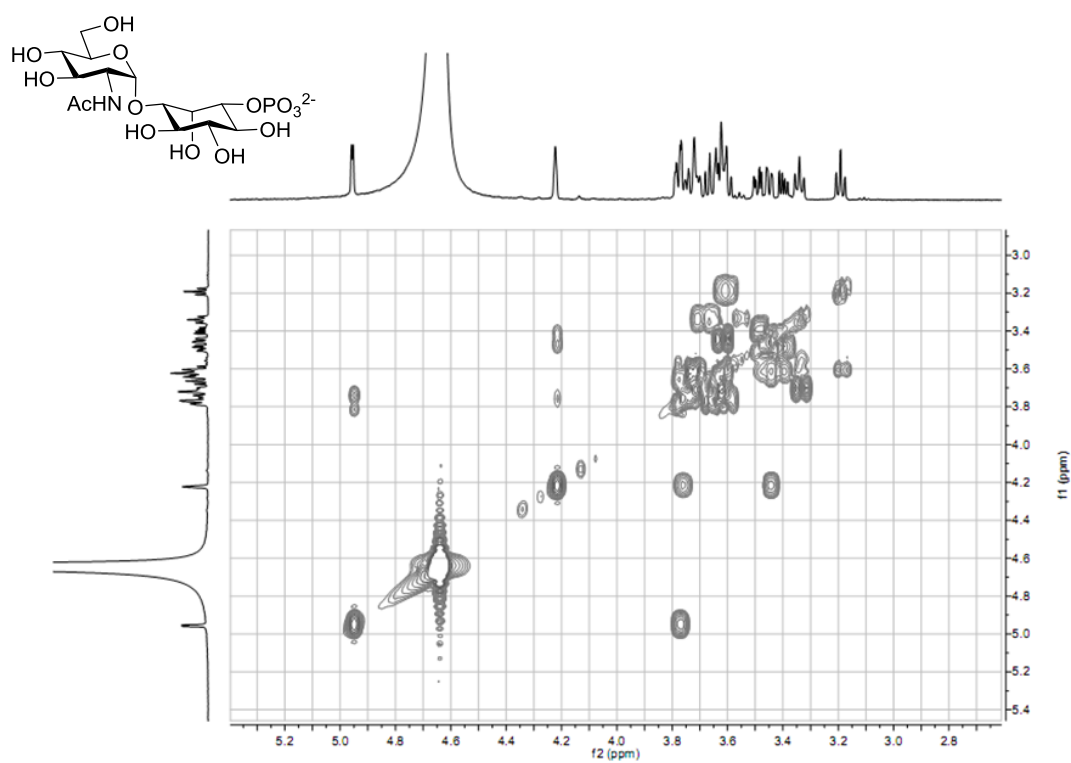
**Figure S5:** <sup>1</sup>H NMR spectrum (600 MHz, D<sub>2</sub>O, 25 °C) of GlcNAc-Ins-3-P



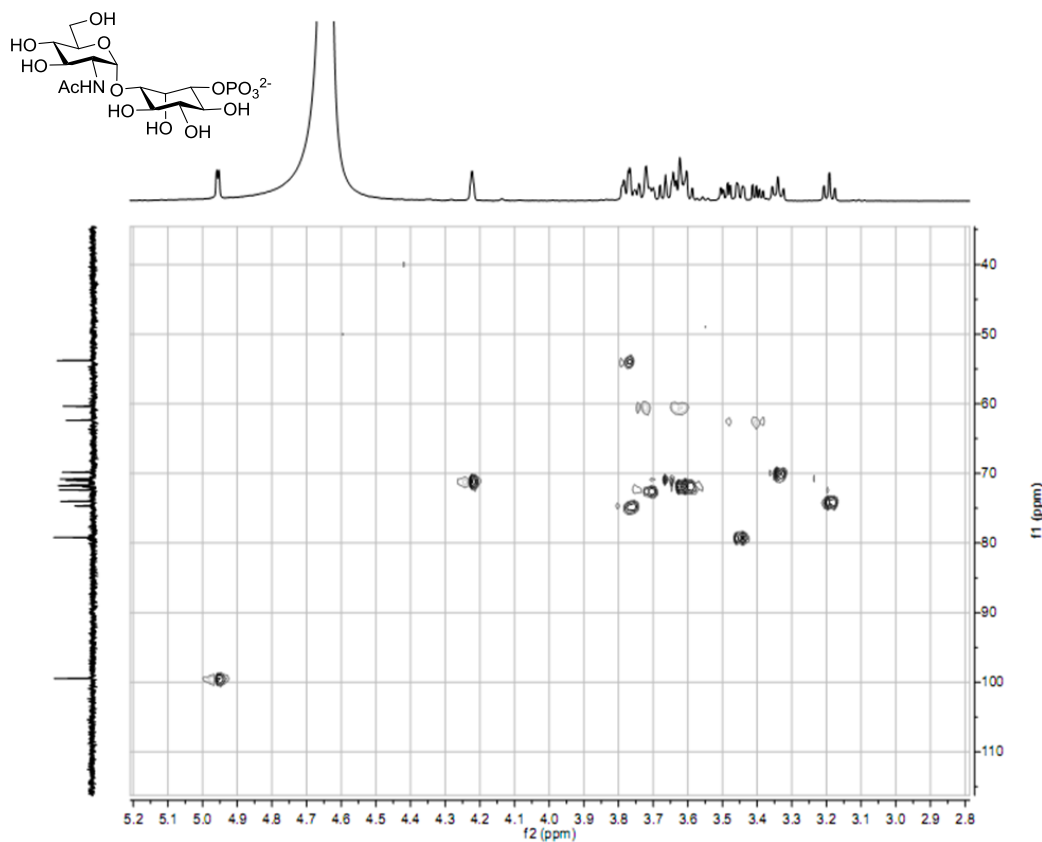
**Figure S6:**  $^{31}\text{P}$  NMR spectrum (243.9 MHz,  $\text{D}_2\text{O}$ , 25 °C) of GlcNAc-Ins-3-P



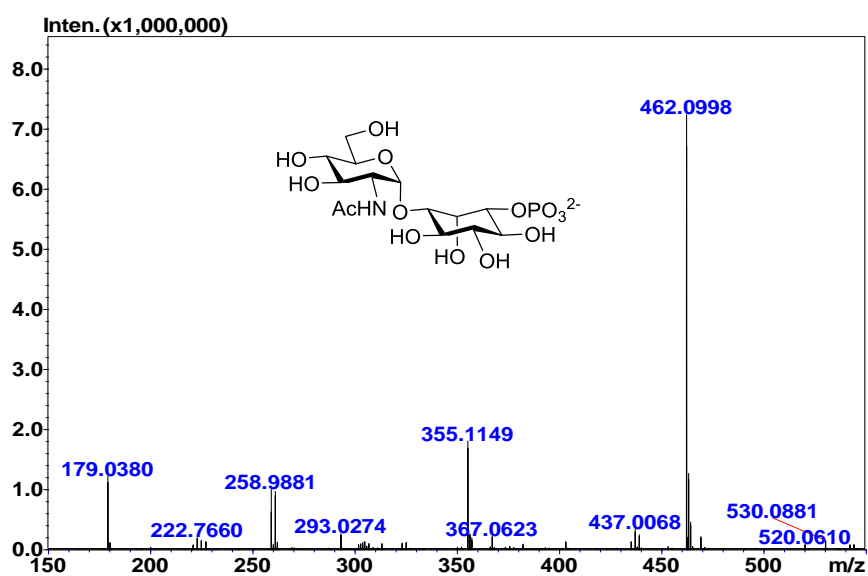
**Figure S7:**  $^{13}\text{C}$  NMR spectrum (125 MHz,  $\text{D}_2\text{O}$ , 25 °C) of GlcNAc-Ins-3-P



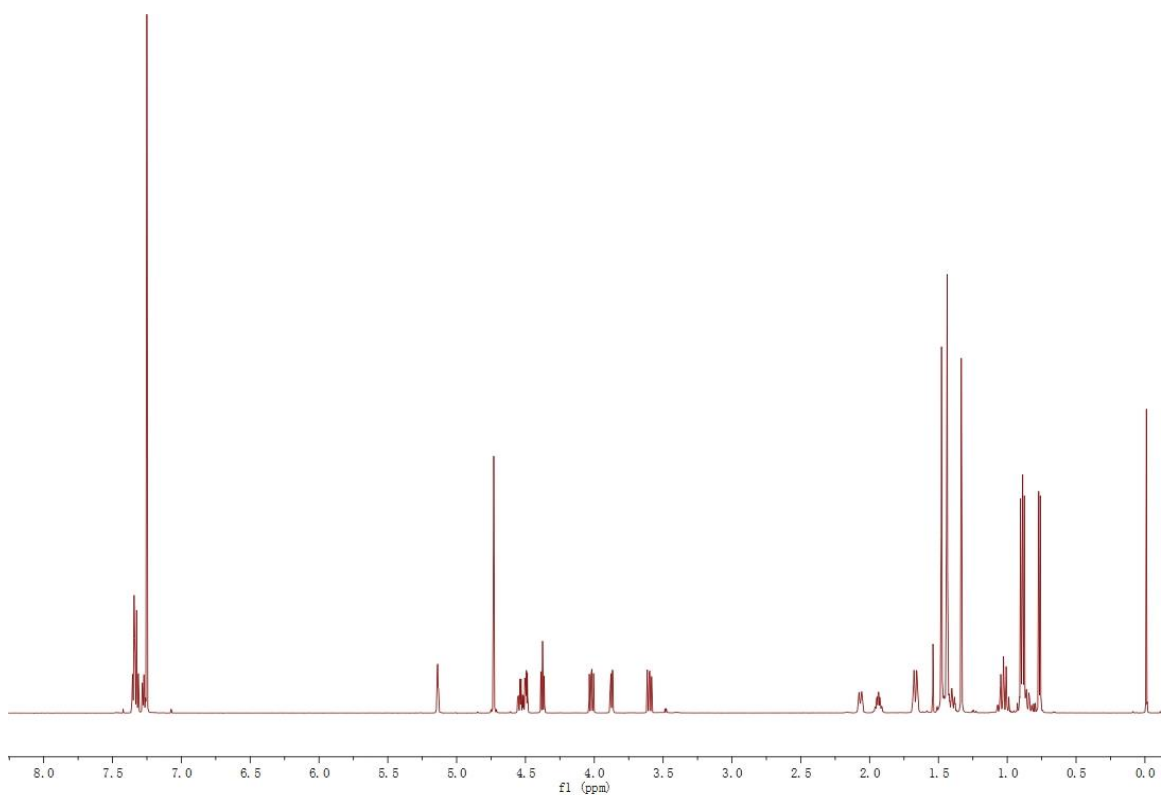
**Figure S8:**  $^1\text{H}$ - $^1\text{H}$  COSY NMR spectrum (600 MHz,  $\text{D}_2\text{O}$ , 25 °C) of GlcNAc-Ins-3-P



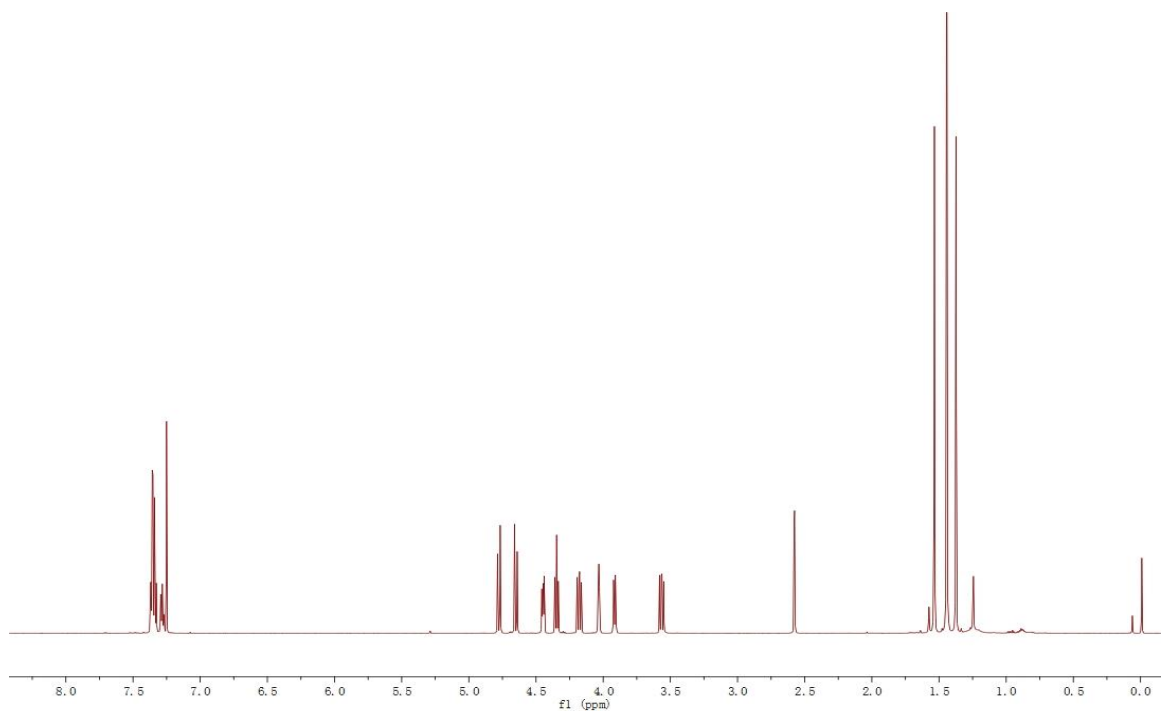
**Figure S9:**  $^1\text{H}$ - $^{13}\text{C}$  HSQC NMR spectrum (600/150 MHz,  $\text{D}_2\text{O}$ , 25 °C) of GlcNAc-Ins-3-P



**Figure S10:** High resolution ESI-MS spectrum of GlcNAc-Ins-3-P

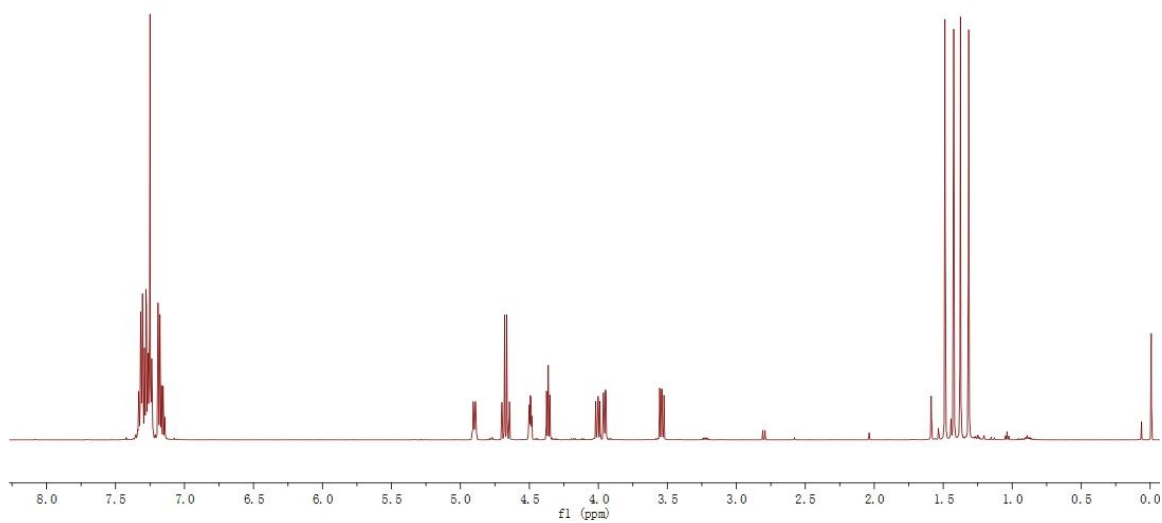


**Figure S11:** <sup>1</sup>H NMR spectrum (600 MHz, CDCl<sub>3</sub>) of (+)-**2**

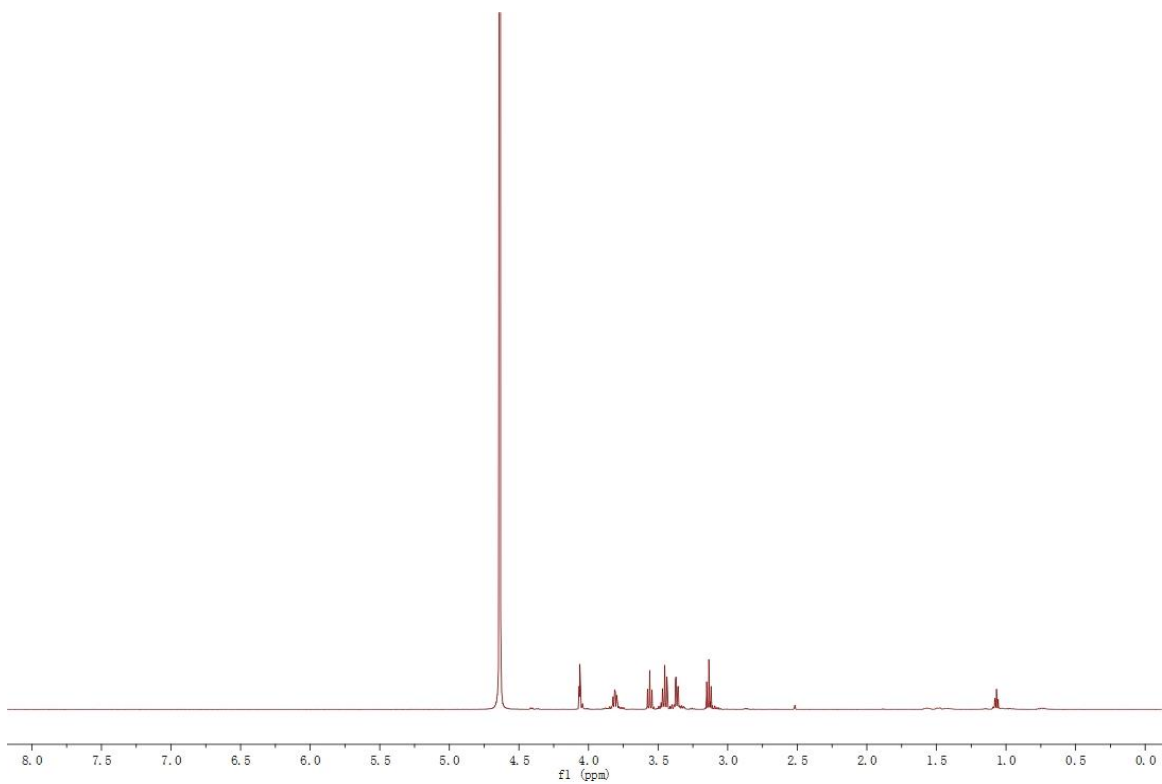


**Figure S12:** <sup>1</sup>H NMR spectrum (600 MHz, CDCl<sub>3</sub>) of (+)-**1**





**Figure S13:** <sup>1</sup>H NMR spectrum (600 MHz, CDCl<sub>3</sub>) of (+)-**3**



**Figure S14:** <sup>1</sup>H NMR spectrum (600 MHz, CDCl<sub>3</sub>) of 1L-Ins-1-P