Supporting Material

For

Efficient and regioselective synthesis of β-GalNAc/GlcNAc-lactose by a bifunctional transglycosylating β-*N*-acetylhexosaminidase from *Bifidobacterium bifidum*

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Table of Contents

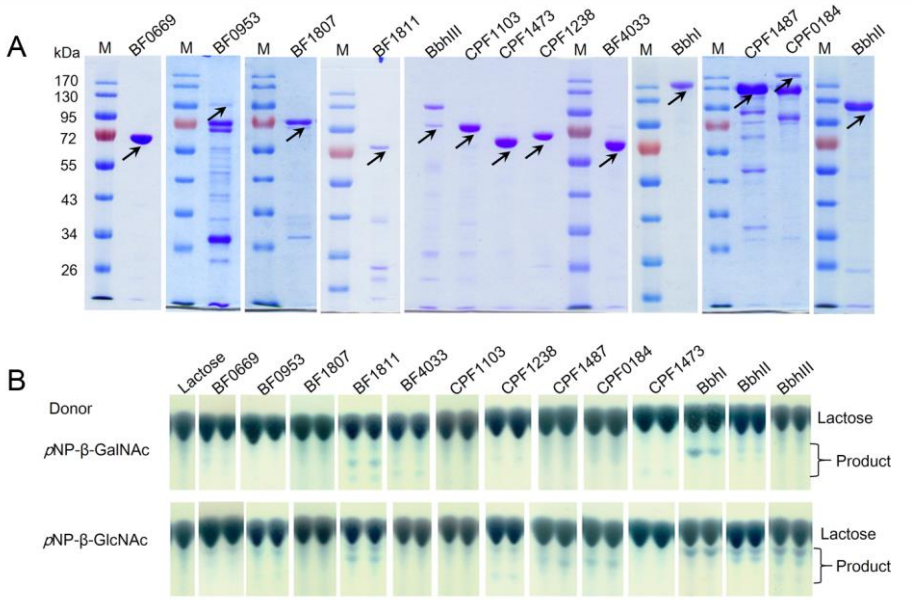

Fig. S1 SDS-PAGE analysis of thirteen recombinant β-*N*-acetylhexosaminidases purified from *E. coli* (A) and TLC analysis of the reactions by incubation of these enzymes with lactose as acceptor and *p*NP-β-GalNAc or *p*NP-β-GlcNAc as donors (B). Transglycosylation activities of the purified recombinant enzymes were screened by incubation of 0.5 mg/mL enzymes with 20 mM *p*NP-β-GalNAc or *p*NP-β-GlcNAc as donors and 200 mM lactose as acceptor at 37 ℃ for 10 min to 6 h. The reactions were stopped by heating at 100 ℃ for 10 min and then detected by TLC.

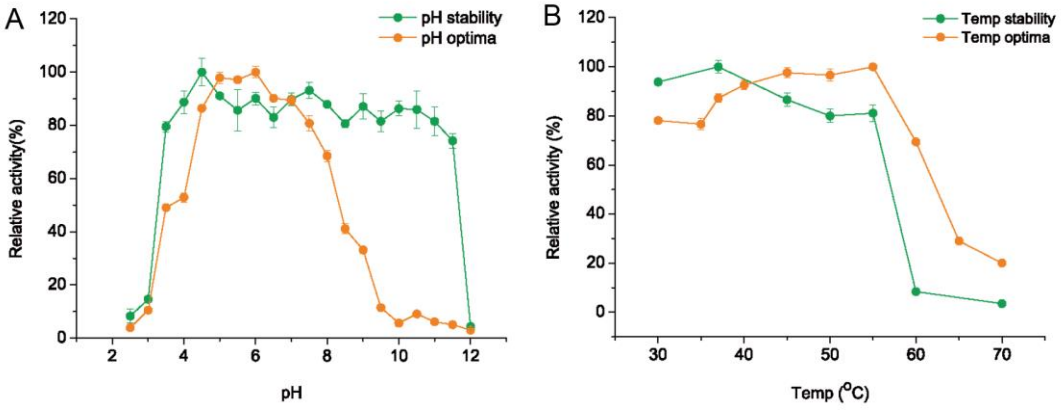

Fig. S2 Effects of pH (A) and temperature (B) on BbhI activity and stability. The optimal pH was assayed by incubating the enzymes with *p*NP-β-GlcNAc in 30 mM buffers from pH 2.5 to 12.0. The effect of pH on enzyme stability was determined by incubation in the same range at 4 ℃ overnight. The optimal temperature was measured at 30 to 70 ℃ for 10 min. Thermal stability was studied by assessing enzyme activity after incubation at 30 to 70 ℃ for 30 min. Data points represent the means ± S.D. of three replicates.

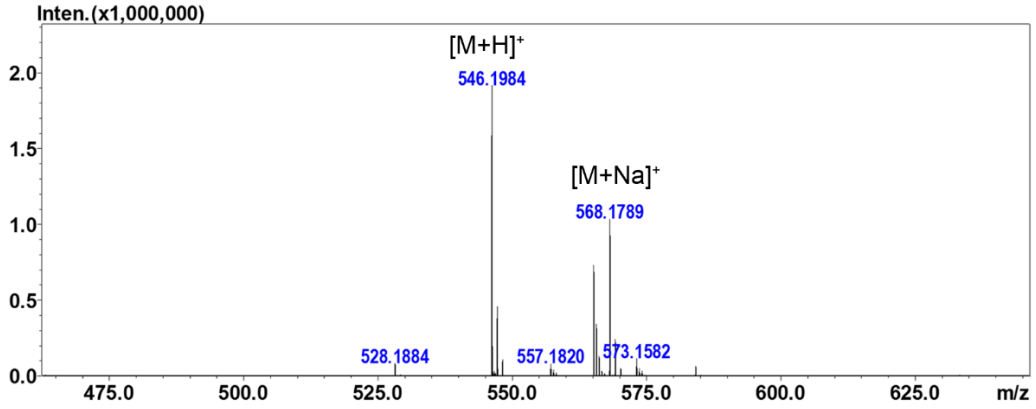

Fig. S3 MS spectrum of GalNAcβ1-3Galβ1-4Glc (*M_r 545*).

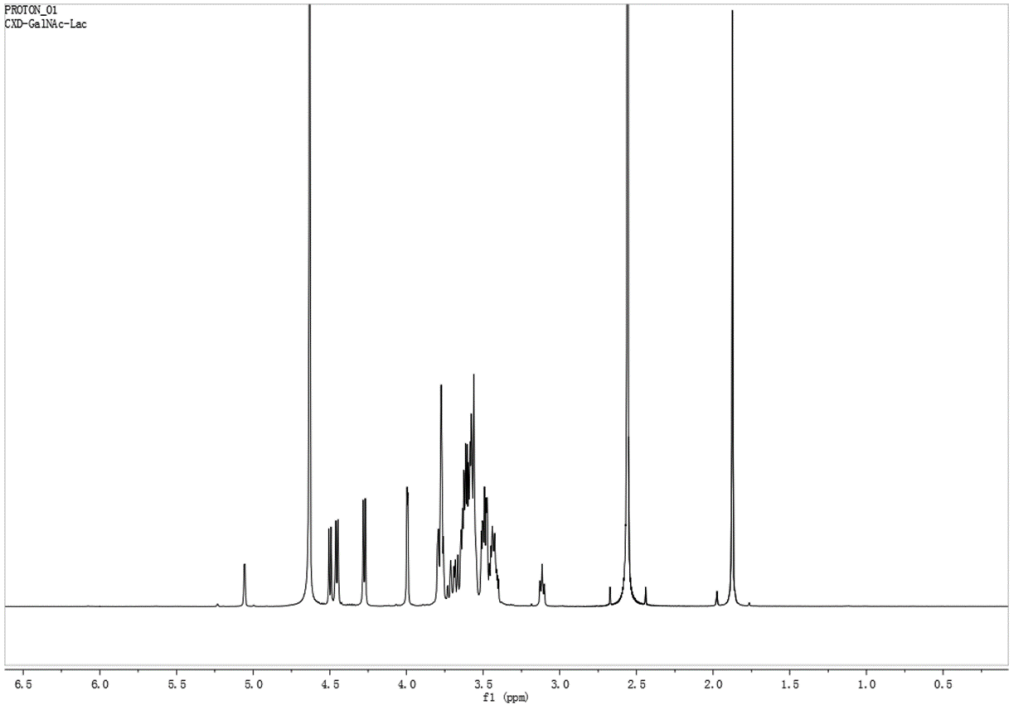

Fig. S4 ^{1}H NMR spectrum of GalNAcβ1-3Galβ1-4Glc.

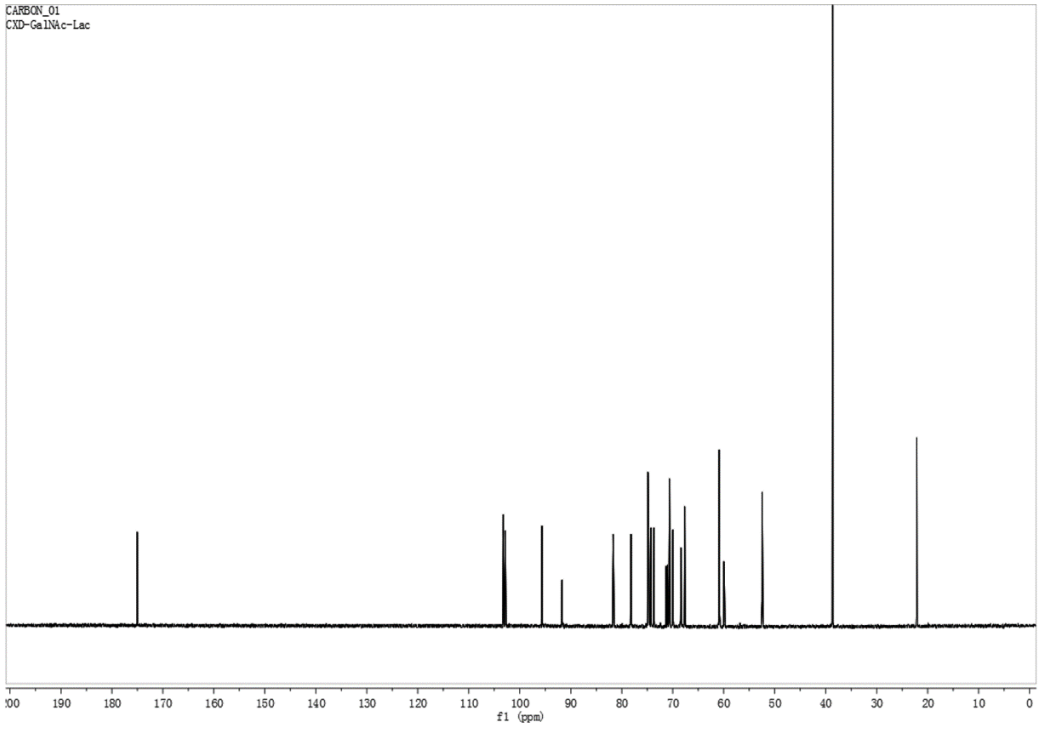

Fig. S5 ^{13}C NMR spectrum of GalNAcβ1-3Galβ1-4Glc.

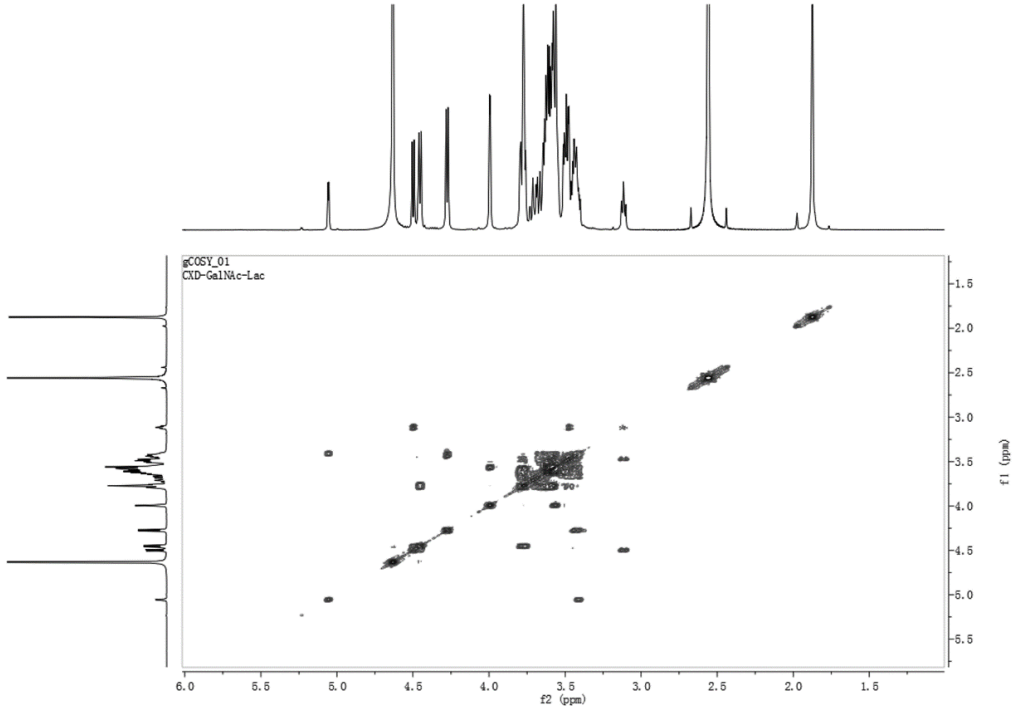

Fig. S6 COSY spectrum of GalNAcβ1-3Galβ1-4Glc.

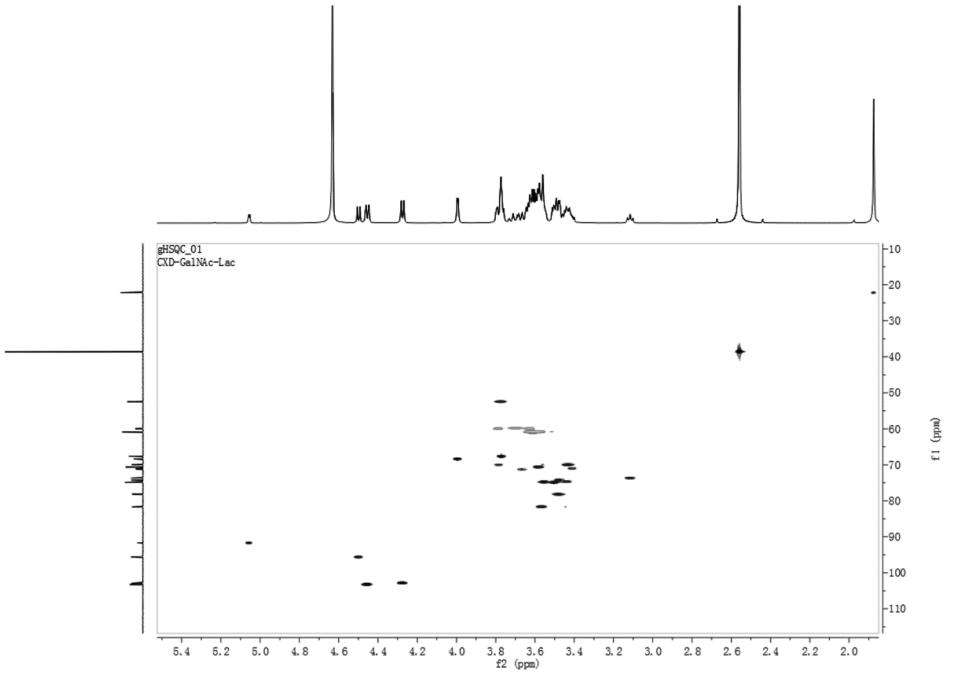

Fig. S7 HSQC spectrum of GalNAcβ1-3Galβ1-4Glc

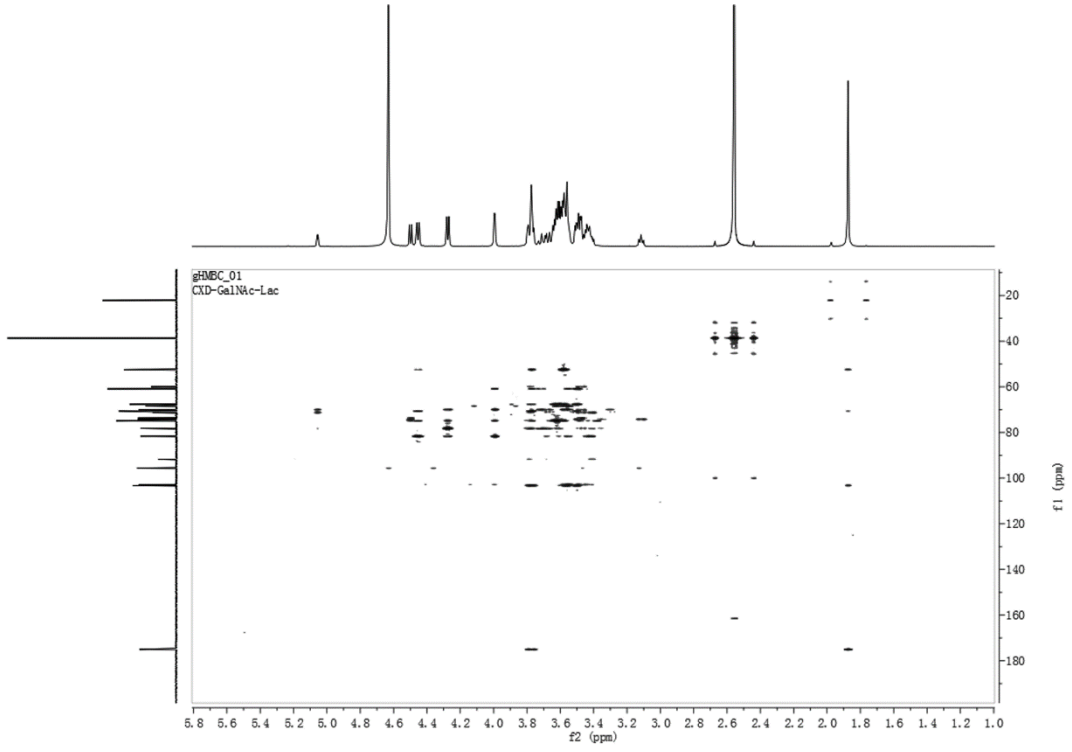

Fig. S8 HMBC spectrum of GalNAcβ1-3Galβ1-4Glc

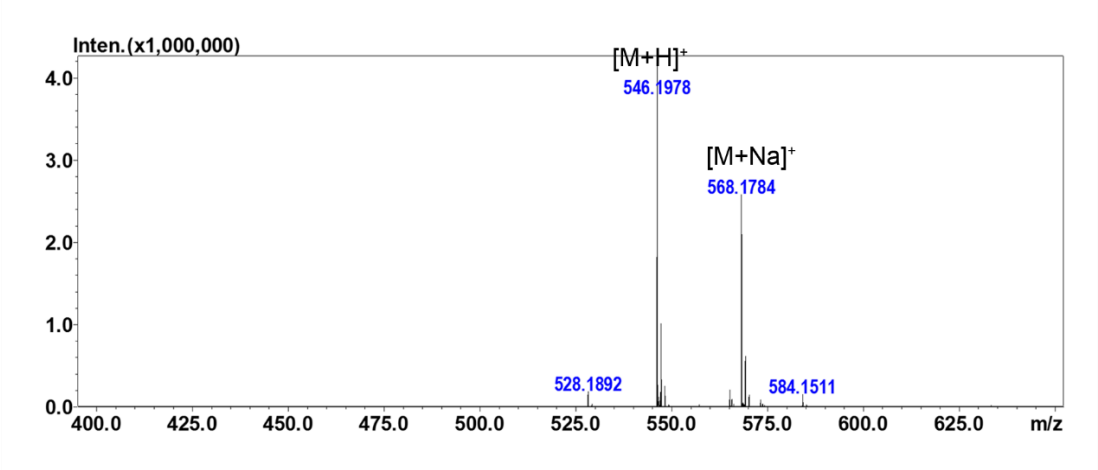

Fig. S9 MS spectrum of GlcNAcβ1-3Galβ1-4Glc (*M_r 545*).

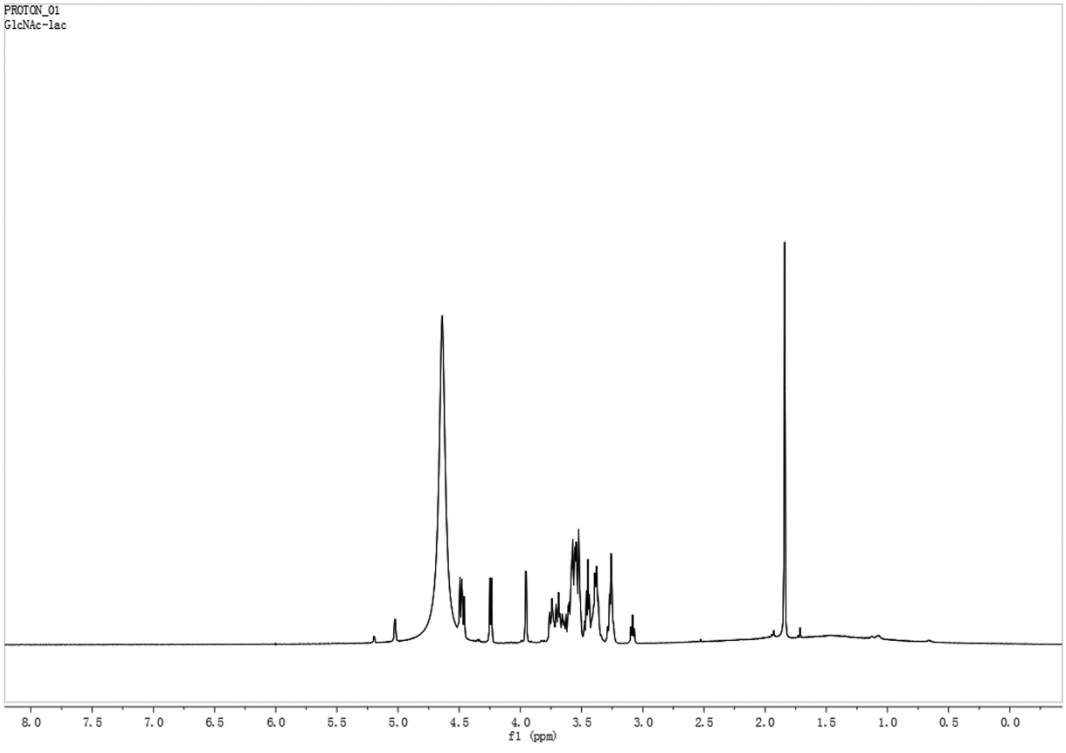

Fig. S10 ^{1}H NMR spectrum of GlcNAcβ1-3Galβ1-4Glc.

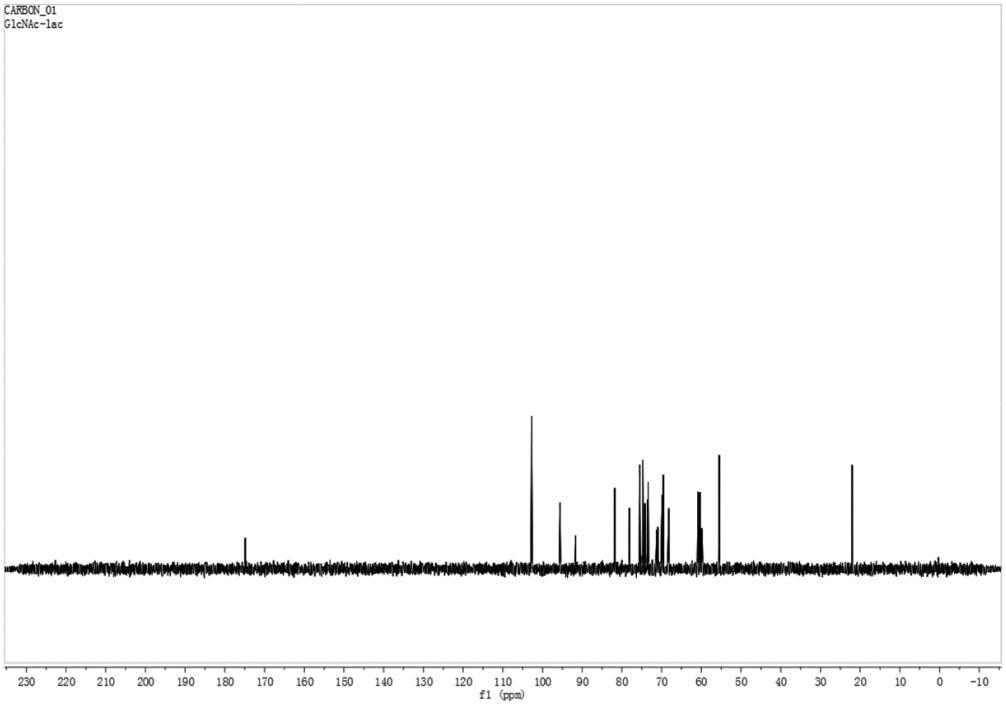

Fig. S11 ^{13}C NMR spectrum of GlcNAcβ1-3Galβ1-4Glc.

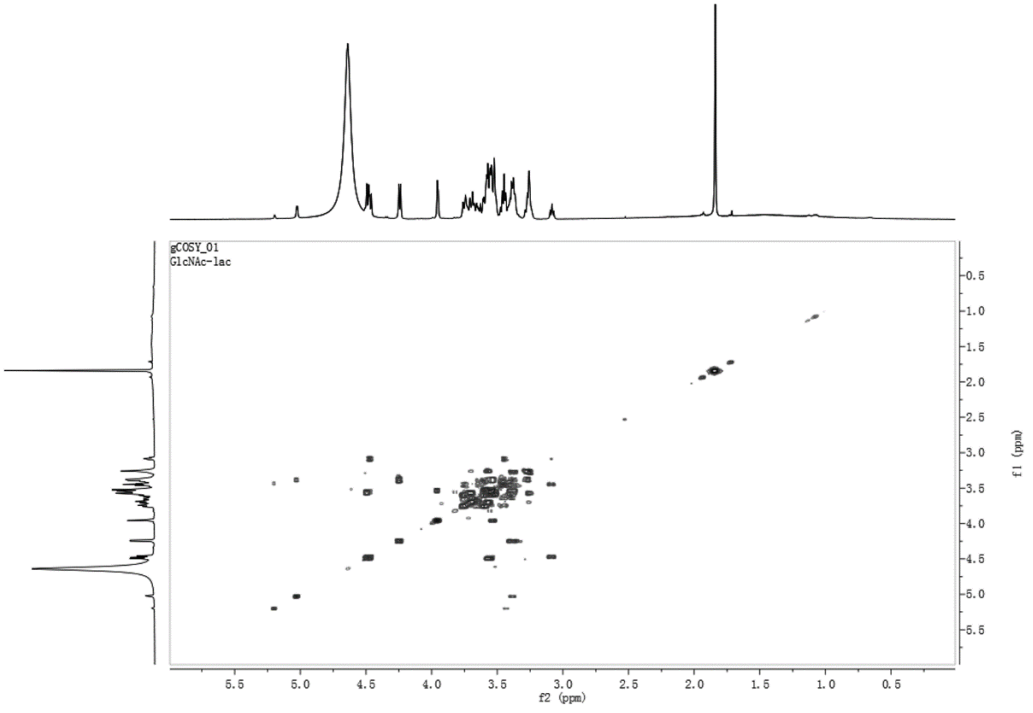

Fig. S12 COSY spectrum of GlcNAcβ1-3Galβ1-4Glc.

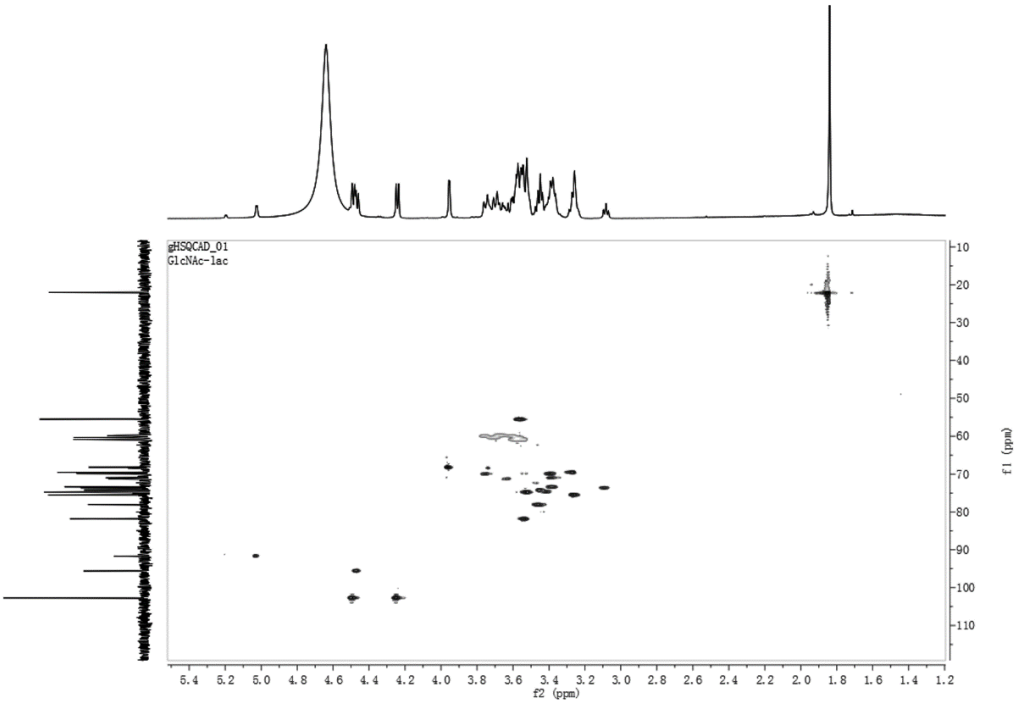

Fig. S13 HSQC spectrum of GlcNAcβ1-3Galβ1-4Glc.

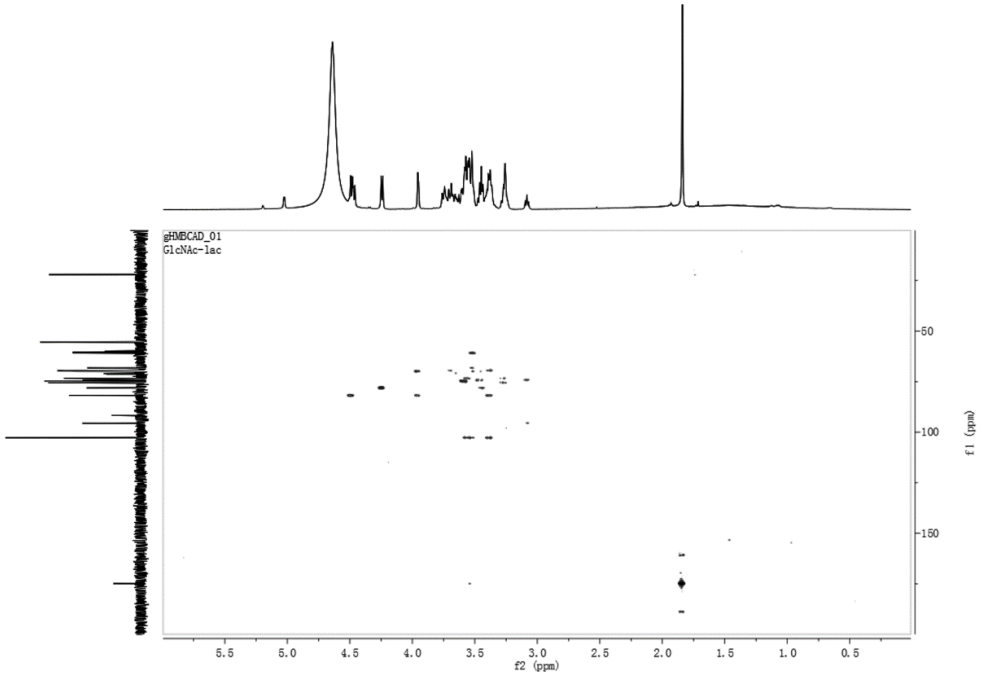

Fig. S14 HMBC spectrum of GlcNAcβ1-3Galβ1-4Glc.

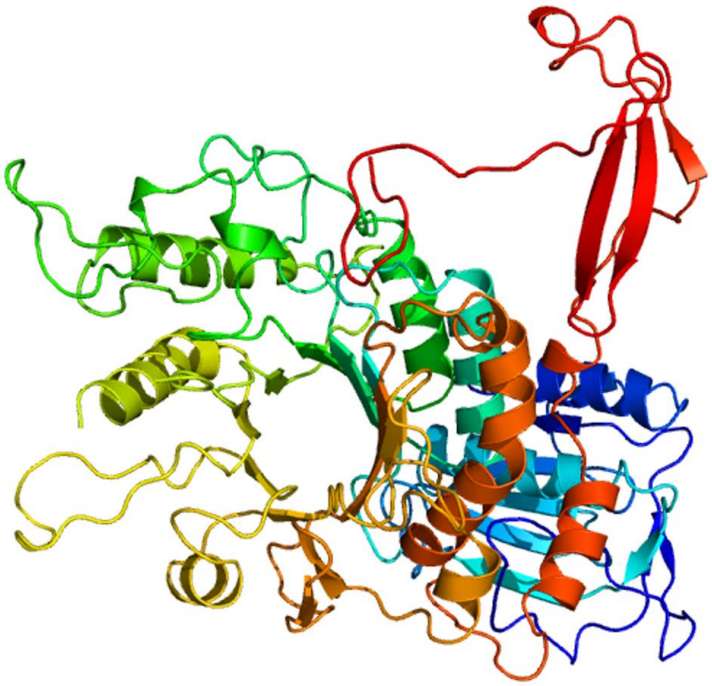

Fig. S15 Putative 3D model of BbhI. Homology modelling of BbhI was performed with PHYRE2 (http://www.sbg.bio.ic.ac.uk/phyre2/), using the structure of *Bb*LNBase from *B. bifidum* JCM 1254 (PDB entry 4h04) as template. Colors from cyan to orange represent *N*-terminus to *C*-terminus.

Table S1 Sequence analysis of thirteen β-*N*-acetylhexosaminidases

Enzyme source	Signal peptide[a] (Amino acid No.)	Transmembrane region[b] (Amino acid No.)
***B. fragilis* ATCC 25285**		
BF0669	1–23	7–26
BF0953	N	N
BF1807	N	7–29
BF1811	N	N
BF4033	1–20	N
***C. perfringens* ATCC 13124**		
CPF1103	N	N
CPF1238	N	N
CPF0184	1–30	13–35
CPF1487	1–35	1145–1167
CPF1473	N	N
***B. bifidum* JCM 1254**		
BbhI	1–32	9–31, 1600–1622
BbhII	1–36	7–29, 1033–1055
BbhIII	N	N

N, no signal peptide or transmembrane region

a, Signal peptide was predicted using online tools (http://www.cbs.dtu.dk/services/SignalP/).

b, Transmembrane region was predicted using online tools (http://www.cbs.dtu.dk/services/TMHMM/)