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High-yield synthesis of glucooligosaccharides (GlOS) as potential prebiotics from glucose via non-enzymatic glycosylation

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Abstract

This study demonstrated a high-yield process to synthesize glucooligosaccharides (GlOS) from glucose via non-enzymatic glycosylation in an acidic lithium bromide trihydrate (ALBTH, a concentrated aqueous solution of LiBr containing a small amount of acid) system. The single-pass yield of the GlOS was up to 75%, which was the highest yield ever reported. The synthesized GlOS consisted of 2-9 glucose units linked predominantly via α/β–1,6 glycosidic bonds (69%), followed by α/β–1,3, α/β–1,2, α/β–1,1, and α–1,4 glycosidic bonds. Preliminary *in-vitro* fermentation tests indicated that GlOS could be utilized by select gut probiotic strains, suggesting that GlOS had the potential as prebiotics. The enhanced glucose glycosylation in

ALBTH was attributed to the unique properties of the solvent system, including water-deficient nature, extra-high capacity of dissolving glucose, and enhanced acid catalysis. The LiBr salt could be recovered after separating the GlOS by anti-solvent precipitation and directly reused. This process provided a new approach for valorizing the biomass derived glucose into high-value oligosaccharides.

Keywords: molten salt hydrate, lignocellulose valorization, oligosaccharide synthesis, acid catalysis

Introduction

Lignocellulose, which is composed of 50-80% polysaccharides (mostly polymers of glucose and xylose), is the most abundant source of natural carbohydrates on earth.[1] Valorization of the renewable carbohydrates has been extensively studied via chemical, biological or the combined approaches to platform chemicals and fuels (such as furan derivatives, organic acids, polyols, and fuel-grade ethanol and butanol).[1-3] Selective production of value-added oligosaccharides with low degrees of polymerization (*DP*, commonly 2-10) from natural carbohydrates is also attractive, since certain types of oligosaccharides exhibit prebiotic activities, such as fructooligosaccharides (FOS), xylooligosaccharides (XOS), isomaltooligosaccharides (IMO), and gentiooligosaccharides (GenOS).[4-6] The prebiotic oligosaccharides can survive (not digested) from the upper gastrointestinal tract but be selectively metabolized by beneficial bacteria (probiotics) in the colon, thus modulating the composition and/or activity of the gut microbiota and improving host health.[7-9] It has demonstrated that intake of the prebiotics (oligosaccharides) confers direct physiological benefits by stimulating the growth of probiotics such as *Bifidobacteria* and *Lactobacilli*, leading to increased production of short-chain fatty acids

(SCFA) in the colon. The microbiome modulation has also been linked to a range of systemic health implications, including inhibition of pathogenic microorganisms, constipation alleviation, obesity reduction, improvement of mineral absorption, repression of allergic symptoms, and enhancement of immune system.[5,9-11] Not surprisingly, there is emerging interest in large-scale production of the oligosaccharides as supplementary ingredients in the food, beverage, and other related industries. The global prebiotics market is expected to expand from ~$3.5 billion in 2017 to ~6.0 billion in 2022.[12]

Oligosaccharides can be produced by either a top-down approach (hydrolyzing parent polysaccharides to oligosaccharides) or a bottom-up approach (synthesizing oligosaccharides from simple sugars). The top-down approach partially cleaves the glycosidic bonds of polysaccharides using enzymes or acids to get oligosaccharides, such as FOS and XOS from controlled hydrolysis of inulin and xylan, respectively.[13] The resultant oligosaccharides inherit the glycosidic linkages and sugar profile from the parent polysaccharides, and usually no new glycosidic linkage is formed. The oligosaccharides are commonly produced from the hydrolysis of hemicellulose-like polysaccharides but not cellulose, because the glucose units are linked by the β-1,4 glycosidic bond in cellulose, and unfortunately, the intestinal microbiota in humans and non-ruminant animals are unable to utilize β-1,4 linked glucose polymers and oligomers.[7] In the bottom-up approach, oligosaccharides are synthesized from monosaccharides or disaccharides via glycosylation and transglycosylation reactions. Enzymes (primarily glycosyl hydrolases and glycosyl transferases) are commonly used to catalyze the oligosaccharide synthesis. For example, IMO (α-1,6 linked glucose units), GnOS (β-1,6 linked glucose units), and FOS (β-1,2 linked fructose units) have been synthesized from maltose, gentiobiose/sucrose, and sucrose by α-glucosidase from *Microbacterium* sp.,[14] dextransucrase from *Leuconostoc mesenteroides*,[15] and

inulosucrase from *Lactobacillus gasseri*,[16] respectively, with yield from 14 to 45%. Low activity and high price of the enzymes are the limitations of the enzymatic synthesis.[5]

Acids can also catalyze the glycosylation of glucose, which was described as early as in 1954.[17] For example, disaccharides with 1→1, 1→2, 1→3, 1→4, and 1→6 glycosidic bonds were detected when treating monosaccharides (e.g., glucose and xylose) in the dilute acid systems.[18,19] However, the yield of the disaccharides barely exceeded 20%, and no oligosaccharides with *DP*>2 were detected. In addition, the acid-catalyzed processes generate undesired side-products such as furfural, hydroxymethylfurfural (HMF) and organic acids due to the degradation of the monosaccharides.[18] In lab, some oligosaccharides can be precisely designed and synthesized by chemical glycosylation, in which desired glycosidic bonds could be formed from selected monosaccharides with controlled regio- and stereo-selectivity.[20] However, the glycosylation process involves expensive and hazardous chemicals and tedious synthetic procedures for protecting, deprotecting, and activating the glycosides, which is not attractive and feasible for large-scale production of oligosaccharides.[21]

While investigating the saccharification/hydrolysis of cellulose and lignocellulosic biomass in the acidic lithium bromide trihydrate (ALBTH, $LiBr{\cdot}3H_2O$) system, a molten salt hydrate (also called inorganic ionic liquid), we detected oligosaccharides in the hydrolysates.[22,23] It was conceived as a consequence of incomplete hydrolysis of cellulose. However, after further examining chemical structures of the resultant oligosaccharides, we realized that glycosylation reaction occurred in the system, because new glycosidic bonds rather than the β-1,4 bond, which is the only glycosidic bond in cellulose or its hydrolysis products, were identified in the oligosaccharides. This observation suggested that the synthesis of oligosaccharides from simple sugars was viable in the ALBTH system. Our following-up investigation led to a high-yield

process to synthesize glucooligosaccharides (GlOS) directly from glucose which can be readily obtained from cellulose hydrolysis. This is what we will report in the present study. The synthesized GlOS were identified and characterized using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), gel permeation chromatography (GPC), and nuclear magnetic resonance (NMR) spectroscopy, respectively. The mechanisms underlying the enhanced glycosylation of glucose in the ALBTH system were investigated. The prebiotic activity of the GlOS as potential prebiotics to support the growth of gut probiotics was evaluated and confirmed via the *in-vitro* fermentation.

Results and discussion

1. Synthesis of GlOS from glucose via glycosylation in ALBTH

New glycosidic bonds are formed in the acid-catalyzed glucose glycosylation via a nucleophilic substitution pathway, as illustrated in Fig. 1A. Protonation of C1 hydroxyl results in a C1 carbocation intermediate (glycosyl donor) after releasing a water molecule. The C1 carbocation is stabilized by an oxocarbenium resonance structure, and trapped by a hydroxyl group of adjacent glucose molecules (glycosyl acceptor).[24] The subsequent deprotonation releases a proton back to the solvent medium, generating a new glycosidic bond. Regio- and stereo-selection of the hydroxyl groups that trap the C1 carbocation, results in different glycosylation products. The glycosylation is a reversible reaction, and acid-catalyzed cleavage (hydrolysis) of glycosidic bonds also occurs. As hydrolysis outweighed glycosylation in traditional dilute acidic systems, the acid-catalyzed glycosylation usually gave a low yield (<20%) of oligosaccharides.[18,19] As discussed below, the chemistry of glucose glycosylation in the ALBTH

is the same as in traditional acid systems. However, because of the unique properties of the ALBTH system, the yield of oligosaccharides was much higher.

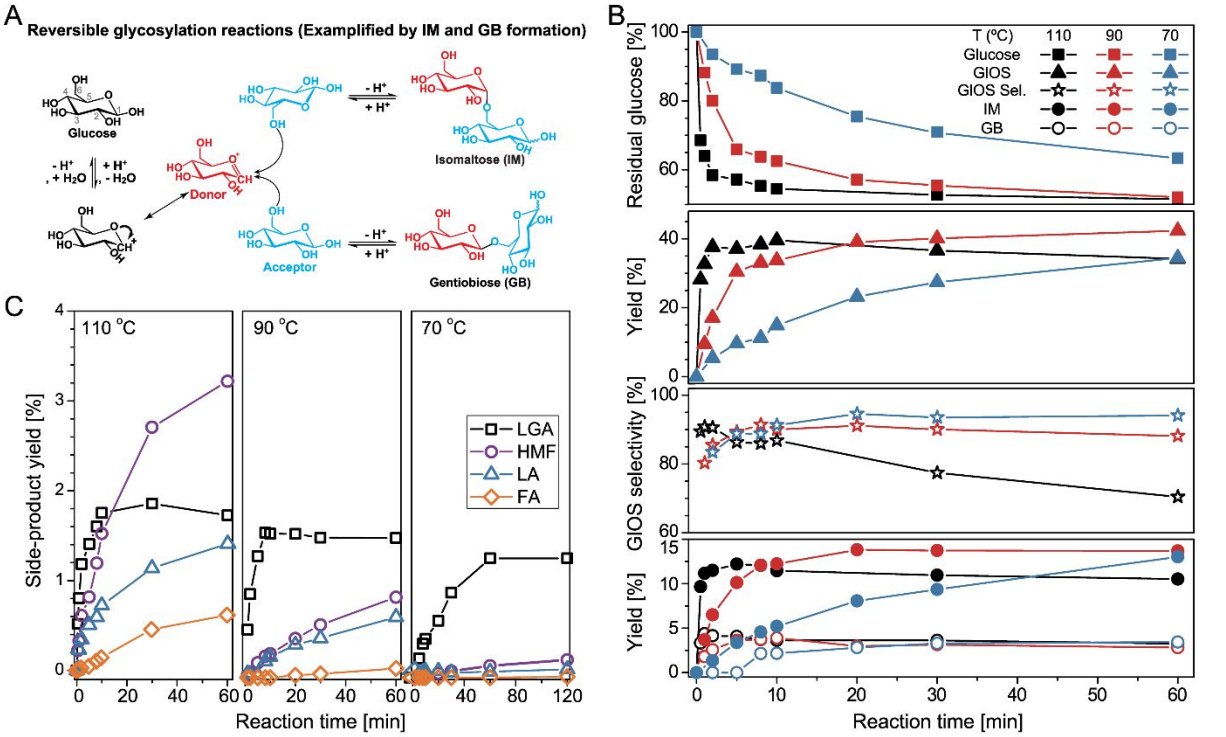

Fig. 1 Synthesis of GlOS in ALBTH at three temperatures (70, 90, and 110 °C). A. Chemistry of acid-catalyzed glycosylation of glucose (e.g., formation of α/β–1,6-glycosidic bonds); B. GlOS yield and selectivity as a function of reaction time; C. Yield of side-products as a function of reaction time. The glycosylation reaction was conducted at 19% (w/w) initial glucose concentration in 60% LiBr (LiBr trihydrate) with 40 mM HCl. GlOS, IM, GB, LGA, HMF, LA, and FA denote total glucooligosaccharides, isomaltose, gentiobiose, levoglucosan, hydroxymethylfurfural, levulinic acid, and formic acid, respectively.

The results of glucose glycosylation in the ALBTH system at varied temperatures (70-110 °C) are summarized in Fig. 1B and 1C. As shown in Fig. 1B, over 41.6 mol% of glucose was consumed in less than 2 min at 110 °C. The turnover frequency (TOF) of glucose consumption exceeded 20.0 $mol_{glucose} \cdot mol_{HCl}^{-1} \cdot min^{-1}$. The glucose concentration then gradually reached an asymptote with time on stream, indicating that the glucose conversion was mostly equilibrium-controlled. The GlOS yield ascended to a maximum (39.5%) at 10 min with 87% GlOS

selectivity. The selectivity then decreased with reaction time to 70% at 60 min because the GlOS yield decreased slightly and more undesired side-products were formed (Fig. 1C). For example, glucose could undergo reversible intramolecular dehydration to form 1,6–anhydro–β–D–glucopyranose (levoglucosan, LGA) (Fig. S1 in the Supplementary Materials, Reaction 1). Irreversible dehydration of glucose formed 5-hydroxymethylfurfural (HMF), followed by subsequent rehydration of HMF to form levulinic acid (LA) and formic acid (FA) (Fig. S1 in the Supplementary Materials, Reaction 2 and 3). The yields of HMF and organic acids (LA and FA) increased from 1.5% and 0.9% at 10 min to 3.2% and 2.0% at 60 min, respectively (Fig. 1C).

Lowering the reaction temperature from 110 °C to 70 °C reduced the rate of glucose conversion (expressed as TOF) from 20.0 $mol_{glucose} \cdot mol_{HCl}^{-1} \cdot min^{-1}$ to 1.8 $mol_{glucose} \cdot mol_{HCl}^{-1} \cdot min^{-1}$. However, the maximum GlOS yield increased with lowering temperature. For example, the GlOS yield increased from 39.5% at 110 °C and 10 min to 42.3 % at 90 °C and 60 min (Fig. 1B) and 47.5% at 70 °C and 240 min (Fig. S2 in the Supplementary Materials), respectively. The glucose degradation reactions were attenuated at lower temperatures. As shown in Fig. 1C, 3.2% HMF was detected after 60 min reaction at 110 °C, while only 0.9% and 0.1% HMF at 90 °C and 70 °C, respectively. LA and FA followed the same trend and became negligible at 70 °C. The LGA yield also decreased to less than 1.3% with decreasing temperature. In addition, lower temperature gave higher GlOS selectivity (up to 94%) (Fig. 1B). However, further lowering temperature to 50 °C significantly slowed down the glycosylation reaction. For example, only less than 25% glucose was converted to GlOS at 50 °C in 12 h. Isomaltose (IM, an α–1,6 linked dimer of glucose) and gentiobiose (GB, a β–1,6 linked dimer) were identified as the most abundant disaccharides in the GlOS. The highest IM and GB yields were 12.2% and 4.1 % at 110 °C and 13.8% and 2.7% at 70 °C, respectively (Fig. 1B). Gentiobiose reached the highest yield

earlier than isomaltose, suggesting that the β–glycosidic bonds might be kinetically more favorable to form than the α–glycosidic bonds during the glycosylation.

The results above indicated that the GlOS could be synthesized from glucose with good yield and selectivity in ALBTH, compared to those in acidic water.[18,25] As will be further discussed in Section 3 below, tuning and optimizing operation and reaction variables could bring the GlOS yield and selectivity up to 75% and 99%, respectively.

2. Identification and characterization of the synthesized GlOS

The synthesized GlOS from glucose in ALBTH appeared as colorless crystals (Fig. 2A). MALDI-TOF MS analysis indicated that the GlOS were a mixture of oligosaccharides with 2 to 9 recurring glucose units (Fig. 2A). The peaks observed in the spectrum had an interval of 162, suggesting that the oligosaccharides were exclusively derived from glucose, and the sugar degradation products (such as LGA, HMF, and organic acids) did not incorporate into the GlOS. The relative intensity of the GlOS peaks in the MALDI-TOF MS spectrum suggested that the majority of the GlOS had *DP* 2-5 along with a small portion of *DP* 6-9. The *DP* of the GlOS was further estimated by GPC, as described in the experimental section. As shown in Fig. 2B, the GlOS had an average *DP* 2.9, including approximately 35% disaccharides, 45% trisaccharides, 15% tetrasaccharides and 4% pentasaccharides, along with a small amount (~1%) of larger oligosaccharides (*DP*>5). These results agreed with the MALDI-TOF MS spectrum (Fig. 2A), suggesting that the GlOS from glucose glycosylation in ALBTH were abundant in the short-chain oligomers (*DP* 2-5). The glycosylation in ALBTH was distinct from those in the traditional dilute acid processes where the glycosylation products with *DP*>2 were negligible.[18,19] The short-chain GlOS (*DP* 2-5) synthesized in the ALBTH meet the general *DP* requirement for

prebiotic functions (longer oligosaccharides have poorer water solubility and function more like diet fibers rather than prebiotics),[26] and are similar to those synthesized by enzymes.[14,15]

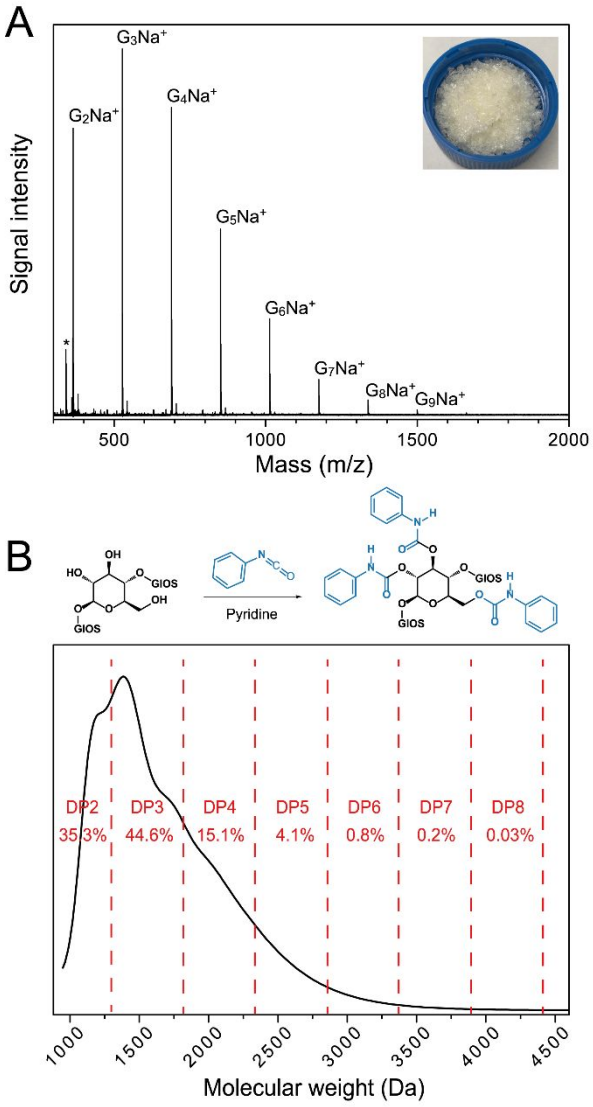

Fig. 2 Molecular weight distribution of GlOS synthesized from glucose. A. MALDI-TOF MS spectrum showing the mass peak assignment based on their corresponding m/z values (G_nNa^+ represents the GlOS containing "n" units of glucose "G".); B. GPC chromatogram of derivatized GlOS. Note: The glycosylation reaction was conducted at 19% (w/w) initial glucose concentration and 70 °C in ALBTH containing 40 mM HCl for 2 h.

The glycosidic bonds formed in the GlOS were identified using 2D heteronuclear single quantum correlation (HSQC) NMR in D_2O (Fig. 3 and Fig. S3 in the Supplementary Materials). The ^{1}H-^{13}C correlation contours of the GlOS were assigned according to the references of di- and oligosaccharide standards.[27] There glycosidic linkages identified in the GlOS samples included α/β–1,1, α/β–1,2, α/β–1,3, α–1,4, and α/β–1,6 glycosidic bonds. No β–1,4 glycosidic bond, exclusive in cellulose, was detected with the characteristic contour of C4–H4 correlation at δ_C/δ_H 81.1/3.65 ppm. Neither was cellobiose (a β–1,4 glycosidic dimer) detected in the high performance anion exchange chromatography (HPAEC) analysis of GlOS samples (Fig. S4 in the Supplementary Materials), confirming that no β–1,4 glycosidic bond was formed from the glucose glycosylation in the ALBTH system.

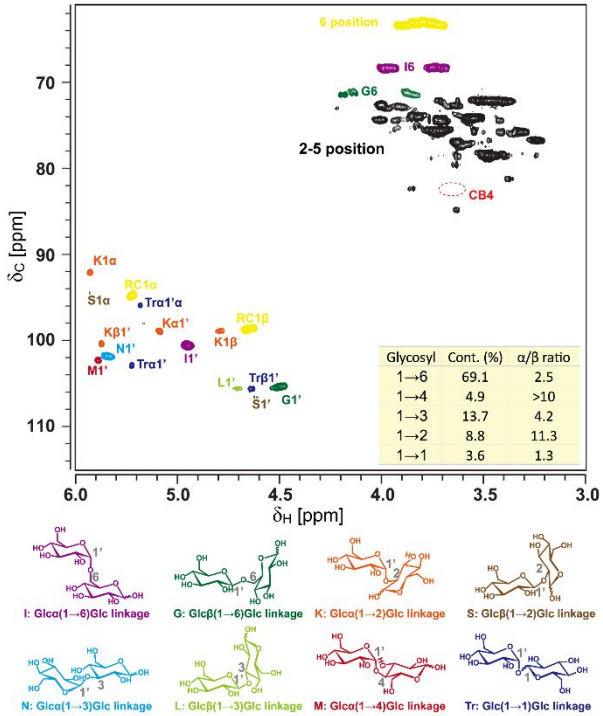

Fig. 3 Identification and relative quantitation of glycosidic bonds in GlOS by 2D ^{1}H-^{13}C HSQC NMR. Note: The GlOS was synthesized by at 19% (w/w) initial glucose concentration and 70 °C in ALBTH containing 40 mM HCl for 2 h.

Semi-quantitative HSQC NMR analysis was conducted to investigate the regio- and stereo-selectivity of the glucose glycosylation in ALBTH (Fig. 3 and Table S1 in the Supplementary Materials). Although the standard "hsqcetgpsisp 2.2" pulse program is theoretically non-quantitative, a relative comparison of the anomeric integrals is valid because of the insignificant T2 effect on the GlOS (molecular weight less than 1500 Da) and the comparable bond coupling constants ($^1J_{C\text{-}H}$) among various anomeric C1-H1 correlations (158-172 Hz for β–anomers and 10-15 Hz lower for α–anomers).[28,29] The semi-quantitative results indicated that the 1→6 glycosidic linkages were the most abundant (69.1%), followed by other glycosidic linkages in the order of 1→3 (13.7%) > 1→2 (8.8%) > 1→4 (4.9%) ≈ 1→1 (3.6%). The observation suggested that the 1→6 glycosidic linkages were the most regio-selective in the glucose glycosylation reaction. In terms of the stereo-selectivity, it is apparent that the α–anomeric linkages were more favorable. For example, the (1→6), (1→3), and (1→2) glycosidic linkages had α to β conformation ratios of 2.5, 4.2, and 11.3, respectively. Considering the fact that the abundance of the α–1,4 glycosidic bond was 4.9% and no β–1,4 glycosidic bond was observed, the α/β ratio of (1→4) glycosidic linkages was even higher than others. The predominance of the α-glycosidic bonds (axial orientation) over the β-glycosidic bonds (equatorial orientation) was due to the anomeric effect. Although the anomeric carbocation (sp^2 hybridized) had no spatial disparity to trap a glycosyl acceptor either from the bottom (α-glycoside) or from the top (β–glycoside), the α-anomers were thermodynamically more favorable because of the electron repulsive interaction and hyperconjugation effect.[24] The possible reasons why 1→6 glycosidic bonds are less stereo-selective than 1→2, 1→3, and 1→4 glycosidic ones are 1) the primary C6 hydroxyl group is the most accessible one, compared to the secondary hydroxyls (C2–OH, C3–OH, and C4–OH) and the hemiacetal hydroxyl (C1–OH), which make the activation energies of

forming α– and β–1,6 glycosidic bonds have a marginal difference; and 2) the methylene group reduces the steric hindrance of C6–OH where the two bulky glucose units form a glycosidic bond, which makes the α and β conformations of 1→6 glycosidic bond have comparable thermodynamic stability and thereby less stereo-selective. Similar observations were reported in the glucose glycosylation catalyzed by glycosidases and H_2SO_4.[18,30] Increasing the glycosylation temperature from 70 to 110 °C (Table S1 in the Supplementary Materials) led to marginal changes in the relative abundance of the glycosidic bonds and the ratio of α/β conformations, indicating reaction temperature barely affected the regio- and stereo-selectivity of the glycosylation under the conditions investigated.

3. Mechanisms underlying the enhanced glucose glycosylation in ALBTH

The forementioned results have demonstrated that glucose glycosylation in ALBTH occurred with high GlOS yields and selectivity. Evidence below suggested that the excellent glycosylation performance be attributed to the unique properties of the ALBTH system and that tuning the reaction condition and operation be able to further improve and enhance the performance: 1) the water-deficient nature, 2) extremely high capacity of dissolving glucose, and 3) enhanced acidity for C1-OH protonation and anomeric carbocation formation.

3.1 Effect of the available water in the ALBTH system on glycosylation

Glucose glycosylation is a dehydration reaction, and the free water molecule in the system can compete with glucose as a glycosyl donor to terminate the glycosylation reaction. Thereby a water-deficient system such as the ALBTH is supposed to favor the glycosylation. This hypothesis is supported by the observation that the oligosaccharide yield was 4 times higher in

the ALBTH system than in an aqueous dilute sulfuric acid under similar conditions (Fig. S5 in the Supplementary Materials). Intuitively, the ALBTH system ($LiBr{\cdot}3H_2O$) had much less water (38.4 wt%) than the dilute aqueous acid solution (nearly 100 wt% water). In addition, in the ALBTH system ($LiBr{\cdot}3H_2O$), one Li^+ coordinates with three water molecules, and all water molecules in $LiBr{\cdot}3H_2O$ are confined in the lithium hydrate sphere. In other words, the water in the ALBTH system is not "freely" available (uncoordinated).[23]

The influence of water content on glucose glycosylation was further investigated by changing the water content in LiBr solutions (29.3-71.3 wt%, from $LiBr{\cdot}2H_2O$ to $LiBr{\cdot}12H_2O$) (Fig. 4A and 4B). The GlOS yield increased from 9% to 39.7% as the water content reduced from 71.3wt% ($LiBr{\cdot}12H_2O$) to 38.4wt% ($LiBr{\cdot}3H_2O$). Further decreasing the water content to 29.3wt% ($LiBr{\cdot}2H_2O$) led to an extra 5.8% increase in GlOS yield. These results verified that the glycosylation of glucose was favorable when low or no "free water" was present. The crux of water deficiency in the glycosylation reaction was further verified by the following experiment, in which extra anhydrous LiBr was supplemented during the glucose glycosylation reaction with an aim to confining the water freshly released from the glycosylation. The supplementary quantity of anhydrous LiBr was equivalent to one-third of the released water in mole, as one mole LiBr was expected to coordinate with three moles of water. It turned out that the anhydrous LiBr addition after 10 min led to an increase in GlOS yield by 3.6% at 20 min (Table 1 and Section S1 in the Supplementary Materials). The additional anhydrous LiBr introduced new hydrate coordination sites, thus reducing the free water in the system and driving the equilibrium toward the side of GlOS formation. Introducing lithium salt to reduce the water activity was also reported in the enzymatic glycosylation of maltose, which resulted in ~15% increase in IMO yield.[14]

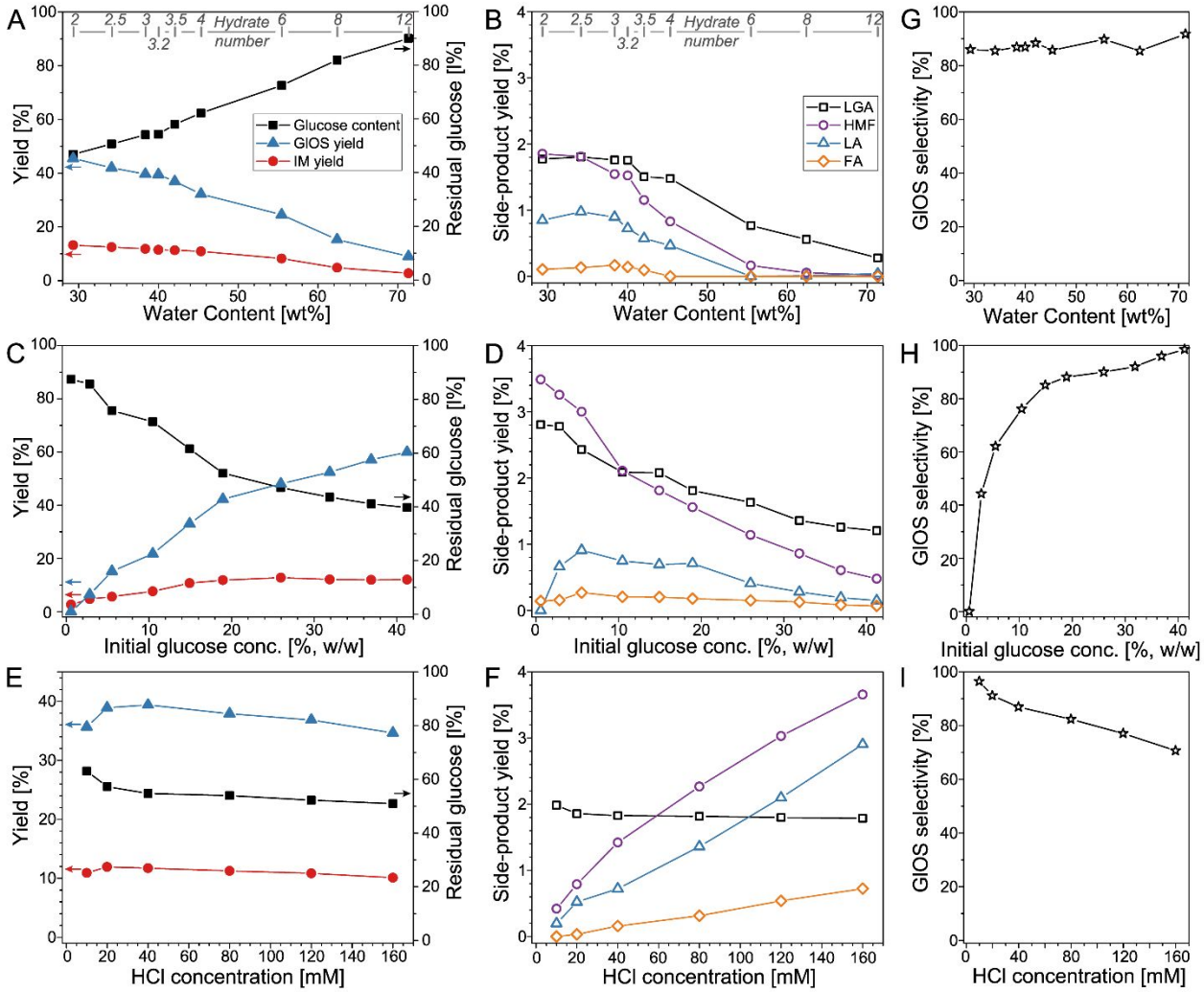

Fig. 4 Factors influencing the glucose glycosylation in acidic LiBr solutions. Effects of water content in LiBr solutions on GlOS yield (A), side-product yield (B) and GlOS selectivity (G); C, D, and H: Effects of initial glucose concentration on GlOS yield (C), side-product yield (D) and GlOS selectivity (H); Effects of HCl dosage on GlOS yield (E), side-product yield (F) and GlOS selectivity (I). Other conditions were kept constant (19% (w/w) initial glucose concentration, 40wt% water content in LiBr solution, 40 mM HCl) unless being investigated; all the reactions were conducted at 110 °C and quenched after 10 min. Note: GlOS, IM, LGA, HMF, LA, and FA denote total glucooligosaccharides, isomaltose, levoglucosan, hydroxymethylfurfural, levulinic acid, and formic acid, respectively; hydrate number of the LiBr solutions equals the molar ratio of water to LiBr.

The yields of levoglucosan, HMF, and organic acids were inversely correlated to the water content in the LiBr solution (Fig. 4B). This is in agreement with our previous study that the acid-

catalyzed glucose dehydration to furans was promoted in the ALBTH system.[31] However, the yields of these degradation side-products were less than 2% under the investigated conditions (Fig. 4B).

3.2 Ultra-high capacity of dissolving glucose of ALBTH

During the glucose glycosylation, glucose acts as both the glycosyl donor and the glycosyl acceptor. Therefore, increasing the reactant (glucose) concentration in the system should favor GlOS formation according to the Le Chatelier's principle. This was verified by the experiments shown in Fig. 4C, in which increasing the initial glucose concentration from 0.6% to 41% (w/w) significantly increased the GlOS yield. At a low glucose concentration (≤3%, w/w), only 14.4% glucose was converted, and the GlOS yield was less than 6.4% (Fig. 4C). Meanwhile, more side-products were formed, including levoglucosan (2.8%), HMF (3.3%), levulinic acid (0.7%), and formic acid (0.2%) (Fig. 4D), respectively. When glucose concentration was elevated to 26% and 41% (w/w), the GlOS yield reached 48.0% and 60.0%, respectively. The GlOS selectivity was over 95% at 41% glucose concentrations (Fig. 4H). The observations above proved that high initial glucose concentration was crucial to increase the yield and selectivity of GlOS.

It was found that glucose had a very high solubility in ALBTH. For example, 40 g glucose was able to readily dissolve in 10 mL of ALBTH (equivalent to a concentration of 70%, w/w) at 110 °C, which was a transparent solution (Fig. S6) with medium viscosity agitable by a magnetic stirring bar. At this concentration, 71% of the glucose was converted to GlOS in 20 min. The glucose loading concentration could be elevated to as high as 85% (w/w, 100 g glucose in 10 mL ALBTH consisting of 10.3 g LiBr, 6.8 g water, 14.6 mg HCl) using a fed-batch method. In other words, 100 g glucose was actually in only 6.8 g water. At 85% glucose concentration, the GlOS

yield was further increased up to 75% within 70 min reaction (Table 1). The yields of HMF, levoglucosan, and total organic acids were negligible (<1%) at the ultra-high glucose concentrations (70-85%, w/w). As far as we know, it has not been reported that a glycosylation reaction could be conducted effectively at such high glucose concentrations (up to 85%, w/w). For comparison, previous studies on acidic glycosylation were conducted at much lower sugar concentrations of 20-40% (w/w).[18,19] The results above suggested that the ultra-high glucose concentration in ALBTH was indispensable for achieving the GlOS yields up to 75%.

Table 1. Glucose glycosylation at ultra-high glucose concentration in ALBTH (40 mM HCl) at 110 °C

Loading (%, w/w)	Time (min)	Content (%)	Yield (%)							Selectivity (%)
		Glucose	IM	GB	GlOS	LGA	HMF	LA	FA	GlOS
54[a]	10	30.6	13.5	4.3	65.9	1.2	0.2	0.1	<0.1	94.9
	20	27.9	9.2	3.4	69.1	0.9	0.3	0.1	<0.1	95.9
	20[c]	26.2	9.3	2.8	69.2	1.1	0.4	0.1	0.1	93.8
70[a]	10	39.5	12.3	4.0	58.2	0.9	0.1	0.1	<0.1	96.1
	20	27.0	8.3	4.3	71.1	0.9	0.1	0.1	<0.1	97.5
	20[c]	22.8	7.6	3.1	74.7	1.0	0.1	0.1	<0.1	96.8
75[a]	20	29.1	9.6	3.9	68.8	0.8	0.1	0.1	<0.1	97.0
80[b]	21	42.7	11.5	3.7	56.5	0.6	0.1	0.1	<0.1	98.6
85[b]	70	23.3	7.6	3.3	75.0	0.6	0.1	0.1	<0.1	97.9

Note: (a) A one-pot approach was applied to reach 54%, 70%, and 75% (w/w) initial glucose concentration by adding 20 g, 40 g and 50 g of glucose, respectively, in 10 mL of ALBTH; (b) a fed-batch strategy was applied to reach 80.4% (40 g +10 g +10 g of glucose in 10 mL of ALBTH) and 85.4% (50 g +10 g +10 g +10 g +10 g +10 g of glucose in 10 mL of ALBTH) glucose loading concentration. (c) LiBr anhydrous (one-third of the released water from glycosylation in mole) was added after 10 min.

3.3 Enhanced acidity in ALBTH for glycosylation

The glycosylation in ALBTH is catalyzed by acid, and protonation of the glucose anomeric hydroxyl group is a prerequisite to forming an anomeric carbocation intermediate, as illustrated in Fig. 1A.[24] The *ab initio* molecular dynamics simulation indicated that glucose protonation to the anomeric carbocation was the rate-limiting step in acid-catalyzed glycosylation of glucose, and the major energy barrier was to overcome the strong proton affinity for water.[32] Therefore, the acid concentration and acidity was supposed to be an imperative factor for the high-yield and high-selectivity synthesis of GlOS. The effect of acid concentration on the glycosylation is shown in Fig. 4E. The GlOS yield first increased from 35.7% to 39.4% with HCl concentration from 10 to 40 mM and then gradually decreased to 34.6% at 160 mM HCl. Meanwhile, the glucose degradation products (HMF, LA, and FA) kept increasing with the acid concentration, but less than 2% yield at 40 mM HCl. (Fig. 4F). These results suggested that very low HCl concentration (20-40 mM) was able to provide sufficient protons to catalyze the glycosylation in the ALBTH without causing significant sugar degradation. For comparison, glycosylation in a dilute sulfuric acid solution (no LiBr salt) at the same acid concentration (40 mM H^+) generated limited oligosaccharides (~8%) under a similar glycosylation condition (140 °C, and 30 min).[18] The high-yield glycosylation was achieved at low acid concentrations in the ALBTH system because (1) Hammett acidity (activity of proton) is higher in the ALBTH system than in aqueous solution at the same acid concentration,[22,33] resulting in more active protons to catalyze the glucose protonation, and (2) low water content and reduced water activity in the ALBTH limites the competition of water for proton and thereby favors the proton to catalyze glycosylation.

Table 2. Comparison of the acids (40 mM) with varied p*K*a values in catalyzing the glucose glycosylation in ALBTH at 110 °C

Acids	pKa	Glucose content (%)	Yield (%)							GlOS selectivity (%)
			IM	GB	GlOS	LGA	HMF	LA	FA	
HCl	-4.00	55.6	11.7	2.6	38.0	1.6	1.8	0.7	0.2	85.8
H_2SO_4	-3.00	55.7	12.9	2.5	38.5	1.6	2.0	0.9	0.2	87.0
TsOH	-2.80	55.4	12.9	2.1	38.1	1.4	1.9	0.8	0.1	85.3
Oxalic acid	1.25	54.0	12.5	2.1	39.4	1.7	1.8	0.4	0.2	85.5
DCA	1.35	57.9	12.5	3.1	35.9	0.9	2.0	0.3	0.1	85.2
H_3PO_4	2.16	56.9	13.2	2.4	39.1	1.1	1.8	0.4	0.1	90.9
Citric acid	3.13	64.7	11.5	2.9	31.6	0.5	2.2	0.2	0.0	89.6
Formic acid	3.75	88.3	4.0	1.5	9.5	0.1	1.0	0.1	0.3	81.1
Acetic acid	4.76	100.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	--
Acetic acid[a]	4.76	95.7	2.6	0.0	3.1	0.0	0.5	0.1	0.0	70.8
Control[b]	7.00	87.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--

Note: (a) Acid concentration: 200 mM; (b) LiBr trihydrate (60 wt%) without acid catalyst. GlOS, IM, GB, LGA, HMF, LA, and FA denote total glucooligosaccharides, isomaltose, gentiobiose, levoglucosan, hydroxymethylfurfural, levulinic acid, and formic acid, respectively.

Acids with different p*Ka* values were compared to catalyze the glycosylation in the ALBTH system. As shown in Table 2, the acids with p*Ka* values from -4.00 to 2.16 all gave similar GlOS yields, indicating that weak Brønsted acids, such as phosphoric acid and oxalic acid, had comparable catalytic performance to strong Brønsted acids. A weak acid such as formic acid (p*Ka* = 3.75) was still capable of catalyzing the glycosylation reaction, though both glucose conversion and GlOS yield were low. These results further confirmed that the acid acidity was enhanced in the ALBTH system. The presence of acid catalyst (even a weak acid) was essential for the glycosylation. When glucose was treated in LiBr trihydrate without an acid catalyst (Table 2), approximately 12% of glucose was consumed. However, no glycosylation product was generated, and only fructose and mannose were detected from the glucose isomerization and epimerization, respectively, which was consistent with the observation in our previous study.[34]

The results above demonstrated the synergy between LiBr and acid, which contributed to the enhanced glycosylation of glucose. A similar synergy effect was observed during the lignin depolymerization in the ALBTH system.[35]

In summary, the ALBTH system has unique features, the water-deficient system, the ultra-high capacity of dissolving glucose, and the enhanced catalytic activity of acids. It is the uniqueness of the system that allows the glucose glycosylation to achieve very high GlOS yield (up to 75%) and selectivity (~99%) under mild conditions in ALBTH.

It was found in our previous studies that the ALBTH system had an excellent capability of directly hydrolyzing cellulose and hemicelluloses in lignocellulose to monosaccharides.[23] All these findings including those from the present study suggest the viability to synthesize the prebiotic oligosaccharides directly from the non-edible lignocellulosic feedstock by simultaneous saccharification and glycosylation in the ALBTH system. Since cellulose and hemicellulose are composed of multiple sugars (primarily glucose, xylose, mannose, galactose, and arabinose), it is also possible to synthesize the prebiotic oligosaccharides with different sugar profile directly for lignocellulose. An explicit study is under investigation and will be reported soon.

Some organic solvent systems, such as organic ionic liquids (e.g., 1-ethyl-3-methylimidazolium chloride [EMIM]Cl and 1-butyl-3-methylimidazolium sulfate [BMIM][HSO_4]) and polar aprotic solvents (e.g., γ-valerolactone and dioxane), have similar properties with the ALBTH system, such as low moisture content and enhanced acidity of Brønsted acids. However, no success has been reported to synthesize oligosaccharides in these solvents. Although it was found that glycosylation occurred in [EMIM]Cl,[36] only disaccharides from glucose were produced with less than 20% yield, and no oligosaccharide with *DP*>2 was

detected, which was probably attributed to the limited glucose solubility and the preferential formation of HMF from glucose dehydration in the organic solvents.

4. Separation and purification of GlOS and recovery and reuse of LiBr

A conceptual flowchart of the GlOS production from glucose in the ALBTH is presented in Fig. 5. Fresh glucose is mixed with the ALBTH in the presence of 20-40 mM HCl under mild conditions (70-110 °C and ambient pressure). The glycosylation reaction leads to a viscous syrup-like mixture including the generated GlOS, unreacted glucose, HCl, the LiBr solution, and a small amount of sugar degradation products. The mixture is then diluted with methanol (a diluting solvent) to reduce viscosity. Acetone (an anti-solvent of GlOS) is added into the diluted mixture to homogeneously precipitate/crystallize GlOS. The GlOS separation processes, including the selection of the dilution solvent and the anti-solvent, are described in detail in Section S2 of the Supplementary Materials. After centrifugation, the crude GlOS crystals are collected. If necessary, further purification can be applied by repeating the methanol-dissolution and acetone-precipitation to remove glucose and LiBr, both of which co-precipitated with GlOS. For example, two purification rounds can bring the purity of GlOS crystals to 98%. The supernatant after separating GlOS crystals contains acetone, methanol, unreacted glucose, and LiBr, together with a small amount of un-precipitated GlOS and sugar degradation products. Acetone and methanol can be easily evaporated in a flash tank due to their distinct volatilities to LiBr trihydrate. A lab test using a rotary evaporator removed over 99.9% acetone and methanol, which can be subsequently separated by distillation[37] and reused in the next batch. The quantities of residual glucose and GlOS in the recovered LiBr stream are dependent on the methanol dilution factor (Table S2 in the Supplementary Materials). The presence of glucose and GlOS did

not affect the reusability of the recovered LiBr solution. A glycosylation reaction was conducted in the recovered LiBr solution, which gave a comparable yield of GlOS to fresh LiBr solution. The GlOS synthesized in the recovered LiBr solution had the identical chemical structures as those synthesized in fresh LiBr solution, as verified with HSQC NMR (Fig. S7 in the Supplementary Materials). These results suggested that the recovered LiBr solution can be directly used for the next batch reaction. The accumulated sugar degradation side-products in the system after multiple-time recycles could be optionally removed/separated through, for example, solvent extraction or a simulated moving bed ion exclusion chromatographic system.

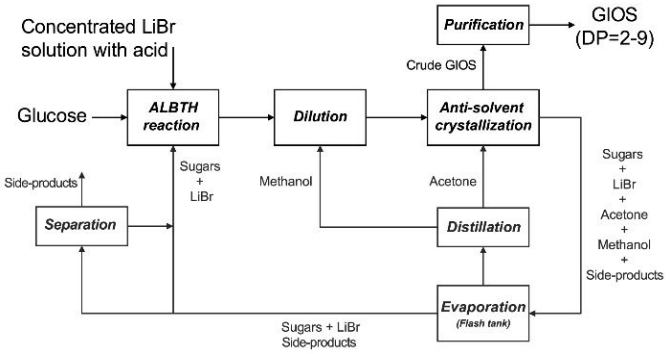

Fig. 5 Conceptual workflow of the GlOS production process. The ALBTH solvent after glucose glycosylation can be separated from GlOS and recycled in a closed-circle.

5. Preliminary evaluation of the GlOS as potential prebiotics *in vitro*

It has been reported that isomalto-oligosaccharides and gentio-oligosaccharides (linked via α–1,6 and β–1,6 glycosidic bonds, respectively) were of prebiotic functions,[15,38] and the disaccharides with α/β–1,2, α/β–1,3, and α–1,6 glycosidic bonds promoted the growth of probiotic strains.[39] Since the GlOS synthesized from glucose in this study had abundant α–1,6 and β–1,6 glycosidic bonds as well as a small amount of α/β–1,1, α–1,2, and α–1,3 glycosidic bonds, they were expected to exhibit prebiotic properties.

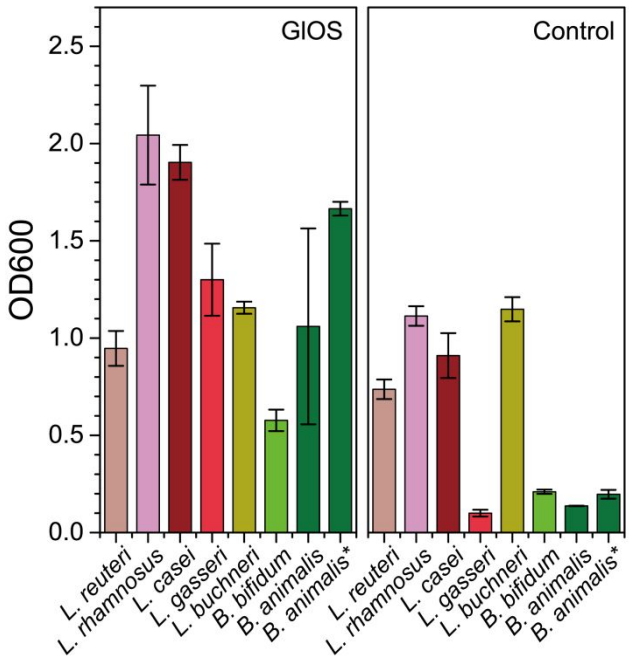

Fig. 6 Growth of select *Lactobacillus* and *Bifidobacterium* strains on GlOS. (Note: The control media contained 0.5 g/L glucose for *Lactobacillus* strains except *L. buchneri* and 0.0 g/L glucose for *Bifidobacterium* strains and *L. buchneri*; *B. animals** had 48-h incubation time.)

To assess whether the GlOS can be utilized by select probiotics strains, we performed an *in vitro* growth experiment under anaerobic conditions with five *Lactobacilli* and two *Bifidobacteria*. In the modified medium containing GlOS, probiotic bacteria proliferated after 24-h incubation with a final optical density (OD600) ranging from 0.6 (*B. bifidum*) up to 2.0 (*L. rhamnosus GG*) (Fig. 6). Since the GlOS contained a small amount of glucose (~5 wt%), it was assessed if the supported growth could be attributed to the residual glucose rather than GlOS utilization. The results showed that glucose was predominately metabolized in the first 3-4 h, and the GlOS contributed to the subsequent growth of the probiotic bacteria, which were distinct from the limited glucose control (Fig. S8 in the Supplementary Materials). This indicated that the select probiotics could utilize GlOS for growth. The carbohydrate analysis of the post-fermentation broth (Table S3 in the Supplementary Materials) indicated that GlOS were partially

consumed by both *Lactobacillus* and *Bifidobacterium* strains. For example, *L. rhamnosus GG* utilized gentiobiose completely, and *L. buchneri* consumed more than 90% isomaltose, but not gentiobiose. *B. animalis* consumed more than 70% disaccharides and up to 43% total oligosaccharide. The metabolic activities of the probiotic strains produced short-chain fatty acids and lactic acids as fermentation products. The production of formic acid (0.2-0.6 g/L), acetic acid (0.6-2.3 g/L) and lactic acid (0.3-2.2 g/L) on GlOS was strain-dependent. Direct production of propionate or butyrate was negligible by *Lactobacillus* and *Bifidobacterium* strains in this study, although both lactate and acetate could be utilized by a group of bacteria such as *Eubacterium hallii* and *Anaerostipes caccae* to produce propionate and/or butyrate.[40]

The preliminary results above demonstrated the prebiotic potential of the GlOS by probiotic strains. These studies provide us an impetus to conduct more comprehensive studies of the GlOS prebiotic performance according to the criterions stated by the International Scientific Association for Probiotics and Prebiotics (ISAPP).[7] In addition, the animal and human dietary intervention experiments in the future work would be helpful to provide a comprehensive evaluation of the prebiotic influence on the overall ecosystem of the gut.

Conclusion

Acidic lithium bromide trihydrate system (ALBTH) was demonstrated to be an efficient medium for glucose glycosylation under mild conditions (moderate temperature and low acid dosage) to synthesize GlOS with high yield (up to 75%). The GlOS were composed of 2–9 glucose units linked dominantly by α/β–1,6 glycosidic bonds along with a small portion of α/β–1,1, α/β–1,2, α/β–1,3, and α–1,4 glycosidic bonds. Several unique properties of the ALBTH system contributed to the enhanced glycosylation of glucose, including the water-deficient nature, the

ultra-high capacity of dissolving glucose, and the enhanced catalysis activity of acids in this solvent system. After separating the synthesized GlOS by precipitation in the anti-solvent (acetone), the recovered ALBTH solution could be directly reused for the next batch glycosylation. Select *Lactobacillus* and *Bifidobacterium* strains utilized GlOS, which exemplified the potential to exploit GlOS as prebiotics. This non-enzymatic glycosylation method provides a new approach for producing high-value and functional oligosaccharide prebiotics directly from inexpensive and abundant monosaccharides.

Experimental

Chemicals

D-Glucose (98%), levoglucosan (99%), levulinic acid (98%), phosphoric acid (85wt%), and aminopyrazine (98%) were purchased from Acros Organics (Pittsburgh, PA). D-Gentiobiose (98%), lithium bromide (99%), formic acid (97%), oxalic acid (98%), and 5-hydroxymethyl-2-furaldehyde (HMF, 98%) were purchased from Alfa Aesar (Tewksbury, MA). D-Maltose (94%), isomaltose (97%), acetone (99.5%), acetonitrile (HPLC grade), and acetic acid (99.8%) were purchased from Fisher Scientific (Pittsburgh, PA). Methanol (99.8%), ethyl alcohol (anhydride), sodium hydroxide (50%), hydrochloric acid (37%), sulfuric acid (98%), and citric acid (99%) were purchased from VWR (Radnor, PA). *p*-Toluenesulfonic acid monohydrate (TsOH, 98.5%), dichloroacetic acid (DCA, 99%), 2,5-hydroxybenzonic acid (DHB, 98%), and deuterium oxide (D_2O, 99.9 atom% D with 1% 3-(trimethylsilyl)-1-propanesulfonic acid, DSS) were purchased from Sigma Aldrich (St. Louis, MO). All the chemicals were used as received without further purification.

Glucose glycosylation to GlOS

Synthesis of GlOS from glucose was carried out in a 40-mL glass reactor heated in an oil bath under atmospheric pressure. Unless specified, glucose (0.05-20.0 g) was initially mixed with 5 mL of acidic LiBr solution using a Teflon-coated magnetic stirring bar, and the mixture was heated up to the reaction temperature (50-110 °C) within 2 min and maintained at the temperature for the rest of reaction duration. Then the glycosylation was quenched by immersing the glass reactor in ice water. GlOS were recovered and purified by the operation of anti-solvent precipitation.

Anti-solvent precipitation and GlOS purification

The GlOS from the glycosylation reaction were separated and recovered by precipitation in acetone as an anti-solvent. In brief, the syrup-like mixture after the glycosylation reaction was first diluted with methanol (equivalent to 2, 5, 15 folds of the volume of the reaction mixture) and subsequently transferred dropwise to 300 mL acetone in a centrifuge bottle immersed in ice water with vigorous agitation to precipitate the GlOS. The acetone insoluble GlOS fraction was collected by centrifugation at 4500 rpm for 20 min, further purified by repeating the procedure of dissolution in water and precipitation in acetone, and finally freeze-dried to yield the colorless GlOS crystal for subsequence characterization.

Fermentability of the GlOS synthesized from glucose glycosylation

Lactic acid bacteria (LAB) from humin intestine including *Lactobacillus reuteri* (ATCC 6475), *Lactobacillus rhamnonsus* GG, *Lactobacillus casei* BFLM 218, and *Lactobacillus gasseri* ATCC 33323 were kindly provided from Dr. Pijkeren's lab and *Lactobacillus buchneri* (ATCC 4005), *Bifidobacterium bifidum* (ATCC 29521), and *Bifidobacterium animalis* (DSM 10140) were generously provided by USDA ARS culture collection (NRRL). The strains were reactivated at

37 °C under anaerobic conditions on MRS medium (ATCC medium 416) for LAB and on Reinforcement Clostridial Medium for *Bifidobacteria* (ATCC medium 2107).

Fermentability of the GlOS by the probiotic strains was evaluated by the anaerobic fermentation experiment in a Hungate tube at 37 °C. The modified MRS fermentation broth and Reinforcement Clostridial Medium fermentation broth were reconstituted without glucose, as described below. Modified MRS medium for LAB stains: dissolve peptone (1.0 g), beef extractive (1.0 g), yeast extractive (0.5 g), NaCl (0.4 g), dipotassium phosphate (0.4 g), ammonium citrate (0.4 g), manganese sulfate (0.01 g), magnesium sulfate (0.02 g), Tween 80 (0.2 g), and L-cysteine-HCl (0.1 g) in 200 mL deionized (DI) water and adjust the pH to 6.80 ± 0.05 under anaerobic condition. Modified Reinforcement Clostridial broth (pre-reduced) for *Bifidobacteria*: dissolve peptone (1.0 g), beef extractive (1.0 g), yeast extractive (0.5 g), dipotassium phosphate (0.4 g), sodium chloride (0.4 g), ammonium citrate (0.4 g), manganese sulfate (0.01 g), magnesium sulfate (0.02 g), ferrous sulfate (0.002 g), Tween 80 (0.2 g), resazurin (0.0002 g), and L-cysteine-HCl (0.1 g) in 200 mL DI water and adjust the pH to 6.80 ± 0.05 under anaerobic condition. The filter-sterilized GlOS solution was mixed with the fermentation broth to yield a final carbohydrate concentration of 10 g/L. The pre-cultured cells were washed twice with PBS (phosphate-buffered saline) buffer, and a cell suspension of approximately O.D. = 0.1 was inoculated anaerobically for 24-48 h. The cell growth was monitored by the changes in optical density at 600 nm (OD) from duplicated fermentation experiments.

Consumption of carbohydrate substrates and production of short-chain fatty acid (SCFA) in the fermentation broths after 24-48 h growth at 37 °C were quantitated using chromatographic methods, as described below.

Chromatographic quantitation of saccharides

Glucose, disaccharides (isomaltose and gentiobiose), and levoglucosan were quantitated using a high performance anion exchange chromatography (HPAEC) on an ICS-3000 system (Dionex, Sunnyvale, CA) equipped with a pulsed amperometric detector and a 250 mm × 4 mm (length × inner diameter) CarboPac PA1 column (Thermo Scientific, Sunnyvale, CA) at 30 °C. A gradient eluent containing A: deionized water (18 MΩ) and B: 100 mM NaOH was programed as 0-40 min, 80% A and 20% B; 40-49 min, 30% A and 70% B; and 49-58 min, 80% A and 20% B. An isocratic post-column eluent of 0.5 M NaOH was used at a flow rate of 0.3 mL/min to ensure the baseline stability and to enhance the detector sensitivity.

Quantitation of GlOS

Quantitation of the total GlOS was conducted following a post-hydrolysis procedure that converted all the GlOS (DP ≥ 2) to glucose before HPAEC analysis. Briefly, the syrup-like mixture (including residual glucose and GlOS) after the glycosylation reaction was diluted with 4% sulfuric acid to a total sugar concentration ≤ 5 g/L and hydrolyzed at 121 °C for 1h in an autoclave unit to convert GlOS to glucose. After neutralization, glucose in the hydrolysate was quantitated using the HPAEC method described above. The free glucose in the syrup-like mixture before the hydrolysis was quantitated using the same HPAEC method. The yield and selectivity of the GlOS was then calculated following Equation 1 and 2.

$$\text{GlOS yield (\%)} = \frac{\text{Glu(mol, post - hydrolysis)} - \text{Glu(mol, after glycosylation)} - \text{LGA (mol, after glycosylation)}}{\text{Glu(mol, before glycosylation)}} \quad (1)$$

$$\text{GlOS selectivity (\%)} = \frac{\text{Glu(mol, post - hydrolysis)} - \text{Glu(mol, after glycosylation)} - \text{LGA (mol, after glycosylation)}}{\text{Glu(mol, before glycosylation)} - \text{Glu(mol, after glycosylation)}} \quad (2)$$

Quantitation of sugar degradation products and SCFA

The sugar degradation products including formic acid, levulinic acid, 5-hydroxymethyl furfural (HMF), and SCFA (acetic acid, propionic acid, and butyric acid) were quantitated using a high performance liquid chromatography (HPLC) on an ICS-3000 system (Dionex, Sunnyvale, CA) equipped with a 300 mm × 7.8 mm (length × inner diameter) C-610H column (Supelco, Bellefonte, PA) at 30 °C and a VWD detector at 210 nm. Isocratic 0.1% phosphoric acid was used as the mobile phase at a flow rate 0.6 mL/min.

Characterization of GlOS

MALDI-TOF MS analysis

The molecular weight distribution of the GlOS was estimated by mass spectrometry in a positive ionization mode using an AB Sciex 4800 MALDI TOF/TOF mass spectrometer (Foster City, CA) equipped with Nd: YAG_200Hz laser at 355 nm. To attenuate the background signals, in particular those below m/z 500, a binary matrix mixture was applied.[41] The matrix containing aminopyrazine (AP, 2.5 mg/mL) with 2,5 dihydroxybenzoic acid (DHB, 7.5 mg/mL) in acetonitrile was combined with an equal volume of a GlOS sample (2 mg/mL) and then placed on a stainless steel target. After air-drying, the sample spot was exposed to an accumulation of one thousand laser shots to record a MS spectrum.

GPC analysis

The degree of polymerization of GlOS was estimated using GPC after derivatizing the hydroxyl groups of the GlOS with phenylisocyanate to form GlOS tricarbanilates. First, 30 mg GlOS was dried in a Duran bottle (100 mL) in a vacuum oven at 50 °C for 12 h. Then anhydrous pyridine (6 mL) and phenyl isocyanate (2 mL) were added. The bottle was sealed with a screw thread cap with a PTFE faced silicone liner, and the mixture was reacted in an incubating shaker at 70 °C and 80 rpm for 48 h. The derivatization reaction was quenched by adding methanol (5 mL) and

then cooling in an ice bath. The mixture was transferred dropwise to a mixture of methanol/water (35 mL, 7/3, v/v) and glacial acetic acid (1 mL). The precipitates of GlOS derivatives were collected by centrifugation at 8000 rpm, washed twice with the methanol/water mixture, and then vacuum-dried at 50 °C.

GPC analysis was carried out on a Dionex ICS-3000 system (Dionex, Sunnyvale, CA) equipped with three tandem 300 mm × 7.8 mm (l × i.d.) Phenogel 5U columns (10000, 500, and 50 Å, respectively) and a 50 mm × 7.8 mm (l × i.d.) Phenogel 5U guard column (Phenomenex, Torrance, CA). The eluent was an isocratic 100% THF (HPLC grade without stabilizer) at a flow rate of 1.0 mL/min, and the column temperature was kept at 30 °C. The derivatized GlOS (10 mg/mL in THF) was injected after passing through a 0.45 μm syringe filter and detected with a variable wavelength detector (VWD) at 270 nm. The apparent weight average molecular weight (M_w) was calibrated using polystyrene standards. The weight average degree of polymerization (*DP*) of the GlOS was calculated using Equation 3.

$$DP = M_w/519 \quad (3)$$

where 519 g/mol is the molecular weight of the repeating unit of the derivatized GlOS.

NMR analysis

The glycosidic linkages of the GlOS from the glucose glycosylation reaction were identified using NMR spectroscopy. The GlOS were dissolved in D_2O with 1% DSS as a reference. ^{1}H-^{13}C HSQC spectra were recorded on a Brucker AVANCE 500 MHz instrument (Billerica, MA) equipped with a cryoprobe. Bruker pulse program "hsqcetgpsisp 2.2 (adiabatic-pulse fashion)" was used with spectral widths of 10 ppm (from 9 to -1 ppm) and 160 ppm (from 155 to -5 ppm) for the ^{1}H and ^{13}C dimensions, respectively. The acquisition time for ^{1}H and ^{13}C dimensions was 200 ms and 8 ms, respectively, with the relaxation delay of 1-10 s. The spectra were processed

using Topspin 3.5 software with a final 2D data matrix size of 2k × 1k data points. To estimate the regio- and stereo- selectivity, the anomeric correlation contours of α/β–1,1, α/β–1,2, α/β–1,3, α–1,4, and α/β–1,6 glycosidic linkages were integrated for relative comparison due to the similar C-H environment and distinguishable chemical shifts. The anomeric integral of α/β–1,1 glycosidic linkages were halved in the calculation.

Quantitation of acetone, methanol, and LiBr

Acetone and methanol were quantitated using a gas chromatography (GC-2014, Shimadzu, MD) with a flame ionization detector (FID) and a 30 m × 0.32 mm × 0.5 μm (length × inner diameter × film thickness) ZB-Wax Plus column. The oven temperature was kept at 100 °C for 5 min and gradually increased to 180 °C in 20 min. LiBr was titrated using $AgNO_3$ of known concentration based on the Mohr's method.

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US patent application related to this work (P180109US01, Dec. 26, 2017). All other authors declare no conflict interest.

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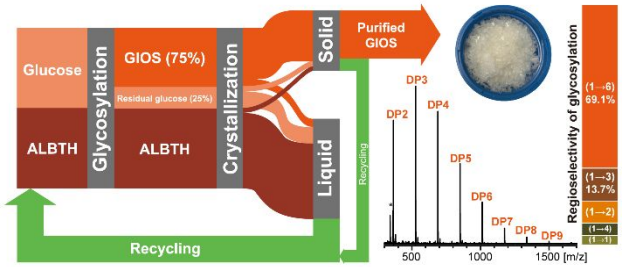
Graphic TOC



Glucooligosaccharides with potential prebiotic functions were synthesized from glucose in high yield and selectivity in acidic lithium bromide trihydrate medium.