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From Glucose to Polymers----A Continuous Chemoenzymatic Process

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Abstract: *Aiming for sustainable development, the syntheses of degradable polymers from renewable resources are highly encouraging. Unfortunately, such efforts are deterred by technical and economic challenges; especially, the conversion of natural building blocks to polymerizable monomers is inefficient, requiring multistep synthesis and chromatographic purification. Herein we report a chemoenzymatic process to address these challenges. In this work, a novel enzymatic reaction system was designed, which allowed for regioselective functional group transformation efficiently, converting glucose to a polymerizable monomer in quantitative yield, thus removing the need for chromatographic purification. With this key success, we further designed a continuous, 3-step process, which enabled the synthesis of a sugar polymer [sugar poly(orthoester)] directly from glucose in high yield (73% from glucose). The work reported here may provide a proof-of-concept in developing technically and economically viable approaches to address the many issues associated with current petroleum-based polymers.*

With an annual production of *ca*. 400 million tons, polymers play indispensable roles in our daily lives.[1-3] Yet, there are environmental, economic, and social concerns, especially the feedstock origins and end-of-use options. Currently, about 6% of the world's total petroleum output is used to produce polymers,[2] and most of which are non-degradable, ending up in landfills, and even in oceans. It is estimated that by 2050, there will be more polymers in oceans than fish by mass.[4] Although there is no panacea to address these complex problems, one promising strategy is to develop polymers from natural building blocks,[3-6] such as fatty acids[7, 8] and carbohydrates.[9, 10] However, despite their abundance, converting pristine building blocks to polymerizable monomers has been challenging; primarily due to the need for multistep chemical synthesis and chromatographic purification in each step, current syntheses are neither readily scalable nor economically viable.

Amongst the many natural building blocks, glucose is one of the most readily available, with the current price of roughly \$0.4-\$0.6/kg.[11] With the advancement in new bioprocesses that use cellulose/lignocellulose as the feedstock;[12, 13] the price is expected to decrease further, and there are hopes that one day this class of polymer may potentially replace certain polymers arising from petroleum. Indeed, there is an increasing number of studies on the synthesis of glucose-based polymers,[14-21] as a few of these examples shown in Figure 1, including sugar polyamide (**IIIa**),[15] sugar polycarbonate (**IIIb**)[16] and sugar poly(orthoester) (**IIIc**).[19] However, in all of these studies, glucose must be first converted to a polymerizable monomer, often, in a stepwise manner, and requiring chromatographic purification in each step.

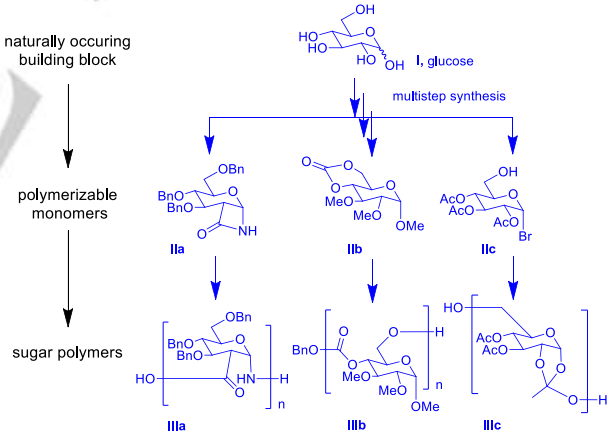

Figure 1. Examples of monomers (**IIa-IIc**) derived from glucose (**I**); and the corresponding sugar polymers (**IIIa-IIIc**).

We were very interested in the sugar orthoester linkage (Figure 1, structure **IIIC**), which exhibited favorable biocompatibility that may enable further development as biomaterials.[22] From the perspective of polymer degradation, the orthoester was highly acid-sensitive and could be conveniently degraded into non-toxic glucose derivatives under acidic conditions.[19, 22] For example, at pH = 5, the half-life of the sugar poly(orthoester) was only 0.6 h, allowing for efficient degradation without the use of toxic reagents or energy input for degradation.[19, 22] However, despite these advantages, the synthesis of the polymer, like every other sugar polymer reported in the literature, was inefficient and tedious, involving many

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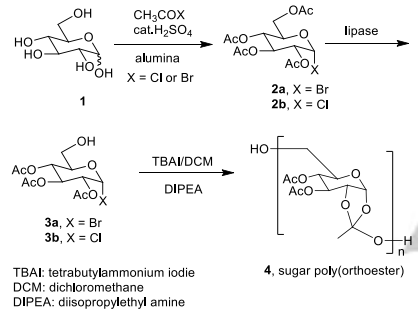
selective protection and deprotection steps in the preparation of a polymerizable monomer.[19, 23]

In light of this, enzymatic reactions, which are especially advantageous for chemo- and regioselective functional group transformations, have attracted our attention. We hypothesized that by applying a right enzymatic reaction and condition, it would greatly simplify the synthesis. Furthermore, if high conversion could be obtained, *e.g.* quantitative conversion, it may also allow for the removal of the need for chromatographic purification, affording a highly efficient and even a continuous synthesis that could potentially be economically-viable. With this goal, herein we report a highly efficient chemo-enzymatic process to synthesize sugar polymers directly from naturally occurring glucose.

To apply an enzymatic reaction, we first converted glucose (**1**) to a monomer precursor (**2**), of which, the 6-OAc can then be enzymatically deprotected to give the monomer (Scheme 1). There have been studies on the synthesis of **2**, *e.g.* from glucosyl hemiacetal,[24], or glucose pentaacetate.[25] However, these methods did not give 100% conversion, and consequently required further purification. Herein, we designed an acetyl halide/H_2SO_4/alumina system that could achieve the quantitative synthesis. Under this condition, glucose was first converted to glucose pentaacetate, following that the anomeric –OAc was transformed to an anomeric halide, giving compounds **2a/2b (**Scheme 1) quantitatively, without any detectable by-product(s) (Figure 3A and SI for details).



Scheme 1. The synthesis of sugar polymer **4** (same structure as that of **IIIc** shown in Figure 1) from glucose (**1**).

With **2a/2b** in hand, we were excited to explore the enzymatic reaction. A variety of lipase has been reported to selectively deprotect the 6-OAc of glucose derivatives (*e.g.* glucose pentaacetate) with yields in the range of 80%-95%.[26-28] Encouraged by these results, we first tested the deprotection of compound **2a** using lipase from *Candida rugosa*. However, when the reaction was performed in THF/water (50/50, v/v), the anomeric –Br was quickly hydrolyzed, *e.g.* within minutes (determined by NMR analysis). The rate of hydrolysis was much faster than that of 6-OAc deprotection, resulting in an undesired by-product. Reducing the water content to 20% did not mitigate this issue. Realizing that glycosyl bromide was highly sensitive to hydrolysis, we then employed a more stable glucosyl chloride precursor (**2b**, Scheme 1). Unfortunately, significant hydrolysis was still observed, although at a much slower rate (*ca.* 20% hydrolysis within 1 hour). Other efforts have been invested to optimize the conditions, including using other sources of lipase and varying the pH values. However, glycosyl halides are extremely sensitive to hydrolysis and these efforts failed to solve the problem.

To address this issue, we then conceived a system to "lock" the water molecule. In this context, highly hydrophilic polymers, such as alginate, [29] with the capability to "lock" water molecules through a massive number of hydrogen bonding, may prevent the hydrolysis. Therefore, we fabricated enzyme-encapsulated alginate beads at varying concentrations (*e.g.* 2.0 g lipase in 10.0 mL of 2% alginate solution) using $CaCl_2$ or $BaCl_2$ solutions (2%). Unfortunately, this strategy did not work, either. Possibly, within this system, there was sufficient freedom for water movement due to alginate's egg-box gelling mechanism.[30, 31] With this in mind, we next sought unconventional methods, such as using an organic solvent, *e.g.* tetrahydrofuran (THF), acetone, and methanol as the gelling agent. The less polar organic solvent (compared to alginate) may push alginate, as well as water molecules to pack tightly to form a stable gel.[32] In such a case, we envisioned that there would be little freedom for water movement, creating a "water locking solid system" (WLSS).

Thereby, lipase from varying sources (*Aspergillus niger, Aspergillus oryzae, Candida rugosa,* and porcine pancreas) were suspended in alginate solution (2% in phosphate buffer) and was subsequently added to the organic solvent through a syringe pump (at the rate of *ca.* 1 mL/min.). However, THF, acetone, and methanol did not give stable WLSS, possibly, the enzyme may adversely affect the packing between each alginate chains. Fortunately, when 1,4-dioxane was used, stable and robust WLSS beads were successfully fabricated. By changing the gauge of the syringe needle, varying sizes of the beads could be obtained. We later determined that beads of 0.5 mm ± 0.2 mm provide both good handling (*e.g.* allow for easy cannulation in the later step), high loading efficiency (66%) and loading capacity (40% by mass) (Figures 2A, 2B and SI for details of the protocol and measurement). The high loading capacity is essential in terms of providing high enzyme/substrate ratio, thereby high reaction efficiency.

With this success, we then tested its "water locking" and catalytic capability. We envisioned that the pre-formed enzyme encapsulated beads may be suspended in an organic solvent which would provide good solubility to help transport the substrate and the product in and out (of the beads), accomplishing the regioselective 6-OAc deprotection, as schematically illustrated in Figure 2A.

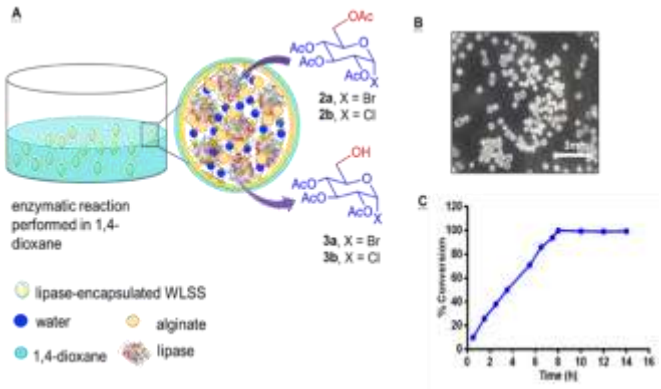


Figure 2. A) Schematic illustration of the enzymatic reaction system, wherein WLSS was suspended in 1,4-dioxane; compounds **2a** or **2b** were introduced to the solution, transported into the beads wherein it was subject to 6-OAc deprotection. B) Camera photo images of the enzyme-encapsulated WLSS hydrogel beads, with sizes of 0.5 mm ± 0.2 mm; C) Kinetic study of the 6-OAc deprotection of **2b** (to give **3b**) using WLSS system; the conversion values were calculated from NMR analysis (Figure S8).

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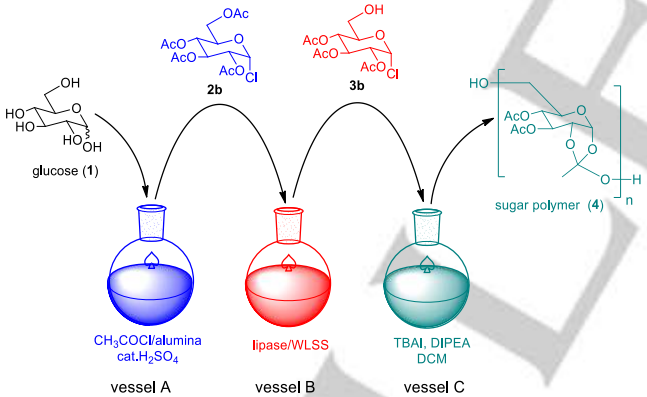
Not surprisingly, within this WLSS reaction system, there was insignificant hydrolysis for **2a** (*ca.* 10% hydrolysis after 3 h); and essentially no detectable hydrolysis for **2b** even after 15 h reaction, indicating a successful "lock" of the water molecules. It should be noted that many factors could also affect the efficiency and regioselectivity, including the source of enzyme,[33] and pH value.[34] After screening these parameters (see Table S1) we found that at pH = 4, lipase from *Candida rugosa* performed the best, giving **3b** in quantitative yield, as shown in the reaction kinetics and NMR analysis (Figures 2C, 3B and Figure S8). It is worth mentioning that under this condition, the WLSS was very robust, staying effective for up to 7 cycles, without noticeable loss of enzyme activity. Furthermore, such a system could be scaled up to 1.0 g, with about the same reaction efficiency.

These results were encouraging, we then envisioned that we could possibly design a continuous process to produce the polymer directly from glucose. As illustrated in Scheme 2, starting in reaction vessel A, glucose was first added in a slurry of acetyl chloride/alumina, with a few drops of conc. H_2SO_4 as a catalyst (see SI for experimental details). After determining that glucose had been completely converted to **2b** (monitored by NMR analysis), ethyl acetate (EtOAc) and water were added and the top layer (EtOAc solution) was cannulated into reaction vessel B. To accommodate a continuous process, the solvent should be removed and recycled. Thereby we further developed a Vortex-Assisted Vacuum Evaporation System (VAVES) [35] for in-vessel solvent removal. This self-designed system, by creating a vortex thereby increasing the surface area, allowed for removal of the solvent (EtOAc) at a rate of about 50 mL/h (depending on the airflow rate). After applying the VAVES, **2b** was obtained as a pure compound, without any trace of solvent or impurity, as determined by NMR analysis (Figure 3A).



Scheme 2. Schematic illustration of the continuous chemoenzymatic process to synthesize sugar polymer (**4**) directly from glucose.

In reaction vessel B, lipase-loaded WLSS beads suspended in 1,4-dioxane were then added and the reaction mixture was gently stirred (at *ca.* 250 rpm). The reaction was monitored by TLC and NMR and the percentage of conversion was calculated from the NMR analysis (Figure S8 and Figure 2C). After determining that the reaction was completed, the solution was cannulated into reaction vessel C, in which, the solvent (1,4-dioxane) was removed through VAVES, leaving **3b** as a pure compound, as determined by NMR analysis (Figure 3B).

With monomer **3b** in hand, we then tested the polymerization. Pre-calculated amount of TBAI, DIPEA in DCM was added and the polymerization was performed according to the literature.[19]

After determining successful polymerization (by NMR and GPC), the solvent was removed by VAVES; and the sugar polymer was precipitated in a mixture solvent of methanol/water, giving a polymer at 73% yield (based on glucose). The synthesized polymer **4** was structurally identical to that reported before[19] (Figure 3C), indicating a successful synthesis.

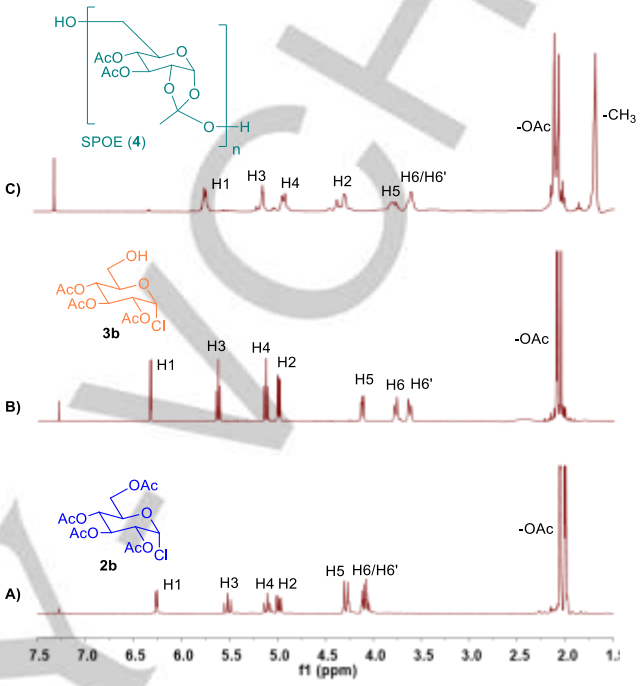


Figure 3. The ^{1}H NMR spectra of A) precursor **2b**; B) monomer **3b**; and C) sugar polymer **4.** Both **2b** and **3b** were obtained by simple extraction of the reaction mixture, without any chromatographic purification.

However, the monomer gave the sugar polymer with only moderate molecular weights (M_n^{GPC} = 4.2 kDa, Figure 4A). Efforts have been invested, *e.g.* increasing the reaction time/catalyst, even the use of reverse-anomeric-effect (RAE)-enabling catalysts such as DMAP (N,N-dimethylamino pyridine) or TPP (triphenyl phosphine).[23] Unfortunately, these efforts did not improve the molecular weights, possibly, due to the low reactivity of the glycosyl chloride, (as compared to the glycosyl bromide monomer **3a**, which gave a molecular weight of *ca.* 7.0 kDa[19]).

Even with a small molecular weight, the polymer exhibited a high glass transition temperature (T_g = 93.3 °C, Figure 4B). The T_g value is comparable to other sugar polymers with much higher molecular weights (*e.g.* 15-16 kDa).[17] The relatively high T_g value is possibly due to the "locked" sugar orthoester bond resulting in a rigid backbone, and allowing for little rotation. Additionally, the unique structure of the sugar unit may lead to close stacking among the polymer chains, as reported in several sugar molecules by Seeberger and co-workers.[36] On the other hand, the polymer exhibited high thermal stability (stable at 180 °C, Figure 4C), indicating strong orthoester bonds within the polymer. The two peaks suggested a two-stage thermal degradation, with the orthoester being degraded first, giving a ketal structure, which degraded subsequently. The high T_g and high thermal stability suggested that the polymer could be a useful material, although its mechanical properties and processability need to be further studied.

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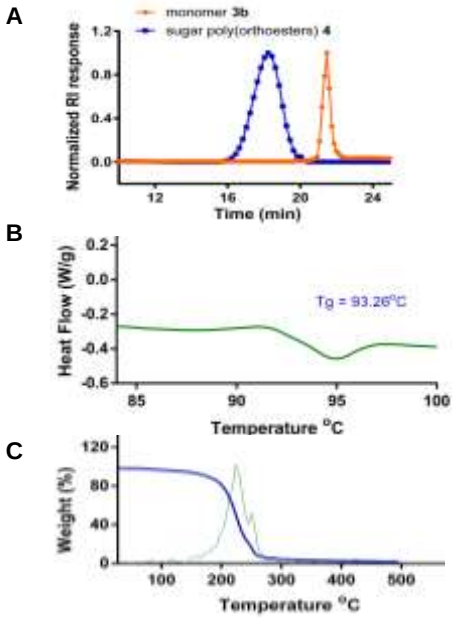

Figure 4. Gel Permeation Chromatography (GPC) analysis (A); Differential Scanning Calorimetry analysis (DSC) (B); and Thermogravimetric analysis (TGA) (C) of sugar polymer **4.**

In conclusion, we have successfully developed a chemoenzymatic synthesis of a sugar polymer using natural building block glucose as the feedstock. We attributed the success to the development of a WLSS enzymatic reaction system that solved the hydrolysis problem during the enzymatic reaction, allowing for efficient synthesis of a polymerizable monomer. It should be noted that as most of the current enzymatic reactions involve the use of water as a co-solvent, the development of the WLSS could serve as a successful model to expand the applications of enzymatic reactions to a large number of organic substrates that bear water-sensitive functional groups.

With the successful development of the WLSS system, the synthesis became efficient, without the use of chromatographic purification, and thus allowing for the development of a continuous process. We demonstrated here as a proof-of-concept, yet we envisioned that such a process may be conveniently translated into an automated version to make an economically viable synthesis to address the sustainability and environmental pollutions issues associated with current petroleum-polymers.

This type of sugar polymer, considering its favorable biocompatibility, low immunogenicity,[21] and non-toxic degraded products,[22] may one day be developed as advanced biomaterials for biomedical and medical applications. Yet, the current polymers, with low molecular weights and high T_g, may give a brittle material with poor mechanical properties.[37] Efforts to improve the molecular weights and to tune the T_g values, including the use of a varying length of fatty acid chains to replace the –OAc groups,[17] or (partially) degradation of the –OAc groups to improve hydrogen-bond interactions among the polymer chains, are currently underway in our lab.

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Keywords: Polymer • synthesis, chemoenzymatic • continuous; sustainable • glucose • renewable • poly(orthoester)

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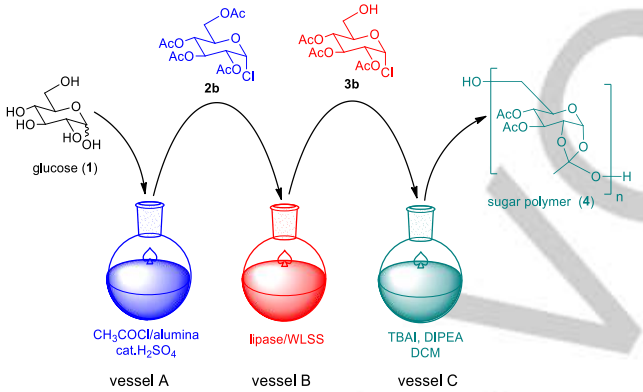

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