Sugar Poly(orthoester)

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Synthesis of Highly pH-Responsive Glucose Poly(orthoester)**

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Abstract: *pH-Responsive polymers have great potential in biomedical applications, including the selective delivery of preloaded drugs to tissues with low pH values. These polymers usually contain acid-labile linkages such as esters and acetals/ketals. However, these linkages are only mildly pH-responsive with relatively long half-lives ($t_{1/2}$). Orthoester linkages are more acid-labile, but current methods suffer from synthetic challenges and are limited to the availability of monomers. To address these limitations, a sugar poly(orthoester) was synthesized as a highly pH-responsive polymer. The synthesis was achieved by using 2,3,4-tri-O-acetyl-α-D-glucopyranosyl bromide as a difunctional AB monomer and tetra-n-butylammonium iodide (TBAI) as an effective promoter. Under optimal conditions, polymers with molecular weights of 6.9 kDa were synthesized in a polycondensation manner. The synthesized glucose poly(orthoester), wherein all sugar units were connected through orthoester linkages, was highly pH-responsive with a half-life of 0.9, 0.6, and 0.2 hours at pH 6, 5, and 4, respectively.*

pH-Responsive polymers and materials have demonstrated great potential in biomedical applications, such as their use as micro- and nanocarriers for selective drug delivery.[1] Considering the relatively low extracellular pH values in tumor (ca. pH 6.7)[2] and inflammatory tissues (ca. pH 5),[3] it is advantageous to design pH-responsive materials to selectively deliver drugs to the respective targets. Ideally, these materials should be stable at pH 7.4, but sensitive to acidolysis in environments with lower pH values; thus allowing for rapid degradation and quick release of prepacked drug payloads. pH-Responsive polymers/materials usually contain acid-labile chemical linkages, including esters,[4] hydrazones,[5] phosphoesters,[6] and acetals/ketals.[7] Among them, acetals/ketals are considerably acid-labile, making them particularly attractive for the development of pH-responsive materials. For example, Murthy and co-workers reported the synthesis of polyketal copolymers as a pH-responsive delivery system in the treatment of acute liver failure. Although effectiveness was achieved, these polymers were only mildly sensitive to acidolysis with a half-life ($t_{1/2}$) of 48 hours at pH 4.5.[7c] However, in the treatment of acute inflammatory diseases, rapid degradation of the polymers to release the drugs, usually within several hours, was crucial because of the fast deterioration of tissue and organ functions.[8]

Compared to acetals/ketals, orthoester linkages are more sensitive to acidolysis.[9] However, study on poly(orthoester) is very limited. Thus far, most of the work was reported by Heller and co-workers, who developed four generations of poly(orthoesters) using two major synthetic approaches: 1) transesterification using polyols and alkyl orthoesters,[10] and 2) polycondensation using diols and a diketene acetal.[11] The transesterification method is very challenging, as it requires high vacuum, high temperature, and prolonged reaction time. This approach is also reported to have issues with reproducibility.[9] The polycondensation approach is synthetically more practical, but is limited by the choice of monomer. Currently, only one diketene acetal, 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5,5]undecane (DETOSU), has been reported.[11] This patented monomer[12] is however challenging to prepare and is sensitive to moisture. A few other orthoester-containing polymers have been reported, but the syntheses suffer from similar limitations.[13]

To address these limitations, we pursued the synthesis of a novel class of polymers as highly pH-responsive materials. Herein sugar poly(orthoester) is of special interest, because of efficient synthetic methods, as well as the excellent acid-catalyzed degradability of the orthoester linkages. Furthermore, their feedstocks are abundant, renewable, and sugar-based polymers have exhibited excellent biocompatibility.[14] However, sugar-based orthoester polymers have never been studied before. A few monomeric sugar 1,2-orthoesters have been reported, wherein they served as useful synthetic intermediates or glycosyl donors in Lewis acid promoted glycosylation reactions.[15] The major approach to the synthesis involves the reaction of 2-OAc-containing glycosyl bromides with alcohols in the presence of a promoter, such as tetra-*n*-butylammonium bromide (TBABr),[16] silver triflate (AgOTf),[15a,b] and potassium fluoride.[17] These promoters facilitate the cleavage of the α-bromide to form oxocarbenium and the subsequent acyloxonium ion, which is then attacked by an alcohol to give a sugar 1,2-orthoester (Scheme 1).[16a]

We hypothesized that if a glycosyl bromide, containing an OH group at the C6 position and an OAc group at the C2 position, was used as a difunctional AB monomer, poly(orthoester) may be synthesized in a polycondensation manner. Herein, we report the first synthesis of glucose poly(orthoester) using 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide (**3**) as such a monomer (Scheme 2).

The synthesis of the monomer started from glucose (**1**), which was converted to **2** according to a procedure reported previously.[18] The subsequent conversion from **2** to **3** has been

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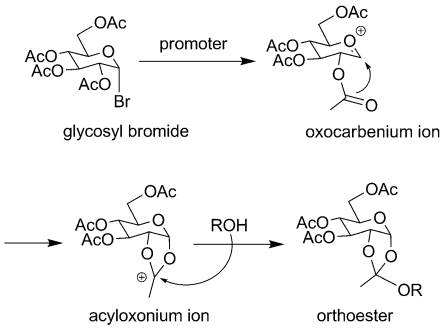
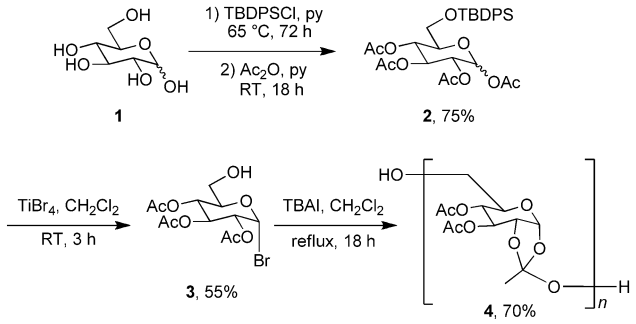


Scheme 1. Synthesis of monomeric sugar 1,2-orthoester.[16a]



Scheme 2. Synthesis of monomer **3** and polymer **4**.

reported, which was achieved through a three-step process: 1) selective anomeric deacetylation using cyclohexylamine, 2) conversion of 1-OH to 1-Br using triphenyl phosphine/carbon tetrabromide (Ph_3P/CBr_4), and 3) deprotection of 6-OTBDPS (*tert*-butyldiphenylsilyl) using HF/pyridine (HF/py).[18] This synthesis was however tedious with a low overall yield. Herein we pursued a more efficient approach, wherein the conversion **2** to **3** was achieved in a one-step manner using titanium tetrabromide ($TiBr_4$), which selectively converted 1-OAc to 1-Br, while simultaneously deprotecting the 6-OTBDPS group to give monomer **3** in 55% yield. This is an important improvement, considering that convenient access to monomer is essential for polymer synthesis.

With monomer **3** in hand, promoters were then screened to effectively induce the polycondensation. Initially, the polymerization was conducted using TBABr as the promoter,[16a] which however only produced oligomers with molecular weights (M_n) of 1.2 kDa, as monitored by gel permeation chromatography (GPC; Figure 1 and Table 1, entry 1). Similarly, when AgOTf[15b,c] was used, only oligomers were produced (Figure 1 and Table 1, entries 2 and 3).

In polymer synthesis, the total number of collision is significantly lower (compared to small-molecule synthesis) because of the limited accessibility to the chain ends. Thus, highly reactive chain ends are desired to increase the number of successful collisions, and therefore the molecular weights of the resulting polymer. Among the many reactive glycosyl donors, glycosyl iodide is probably the most reactive thus far.[19] We hypothesized that if an OH-containing glycosyl iodide could be generated in situ from monomer **3** by TBAI, a high-molecular-weight poly(orthoester) may be produced.

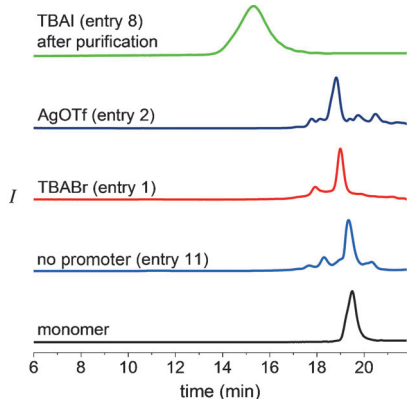


Figure 1. GPC curves of the polymerization under varying reaction conditions (oligomers from Table 1, entries 1, 2, and 11 were not purified by precipitation because of their low molecular weights).

Table 1: Polymerization conditions and results.

Entry	Promoter (equiv)	Base	Solvent	*T*	M_n [kDa][a]	PDI	Yield
1	TBABr (3.0)	sym-collidine	CH_2Cl_2	reflux	1.2	1.5	n.a.[c]
2	AgOTf (3.0)	lutidine	CH_2Cl_2	reflux	1.5	1.8	n.a.[c]
3	AgOTf (3.0)	DIPEA	CH_2Cl_2	reflux	1.7	1.6	n.a.[c]
4	TBAI (0.1)	DIPEA	toluene	65°C	1.7	1.7	n.a.[c]
5	TBAI (3.0)	DIPEA	toluene	65°C	1.6	1.7	n.a.[c]
6	TBAI (0.1)	DIPEA	benzene	65°C	1.7	1.6	n.a.[c]
7	TBAI (0.1)	pyridine	pyridine	65°C	1.6	1.7	n.a.[c]
8	TBAI (0.1)	DIPEA	CH_2Cl_2	reflux	6.6	1.3[b]	70%
9	TBAI (1.0)	DIPEA	CH_2Cl_2	reflux	6.8	1.3[b]	72%
10	TBAI (3.0)	DIPEA	CH_2Cl_2	reflux	6.9	1.3[b]	70%
11	–	DIPEA	CH_2Cl_2	reflux	0.9	1.6	n.a.[c]

[a] Measured by GPC, calibrated using polystyrene standards. [b] Measured after purification. [c] The oligomers were not purified by precipitation because of their low molecular weights. n.a. = not applicable. PDI = polydispersity index.

The reaction was then conducted using 0.1 equivalent TBAI as the promoter and toluene as the solvent, according to a reported procedure for small-molecule synthesis.[20] *N*,*N*-Diisopropylethylamine (DIPEA) was chosen because it has been reported to be an effective acid scavenger in glycosyl iodide chemistry.[19] Unfortunately, when the reaction was conducted at 65°C, the polymerization only gave oligomers with molecular weights of about 1.7 kDa (Table 1, entry 4). Neither increasing the amount of TBAI to 3.0 equivalents, nor changing the solvent to benzene or pyridine increased the molecular weights of the oligomers (Table 1, entries 5–7).

However, when the solvent was changed to refluxing dichloromethane (CH_2Cl_2), polymers with molecular weights of 6.6 kDa were produced (Figure 1, Table 1, entry 8). The remarkable increase in molecular weight is possibly a result of the good solubility of the polymers in CH_2Cl_2. The reaction likely occurs by in situ conversion of the α-bromide to a highly reactive β-iodide,[19] thus setting the stage for the subsequent intramolecular attack by the 2-OAc to form the acyloxonium ion.[20] Thereafter, intermolecular attack by the 6-OH afforded the desired sugar poly(orthoester) **4**. Herein

the conversion of α-bromide to β-iodide is crucial; when no TBAI was used, only low-molecular-weight oligomers were produced (Figure 1, Table 1, entry 11).

Next, we studied the effect of higher TBAI concentrations on the molecular weights. However, when the amount of TBAI was increased to 1.0 equivalent and even to 3.0 equivalents, the molecular weights only slightly increased to 6.8–6.9 kDa (Table 1, entries 9 and 10). Possibly, the accessibility of the reactive chain end became a limiting factor on the growth of the polymer when its size reached a certain value.

NMR analyses supported a successful synthesis of **4.** As shown in the ^{1}H NMR spectra of monomer **3** (Figure 2, top) and polymer **4** (bottom), the polymer possesses characteristic

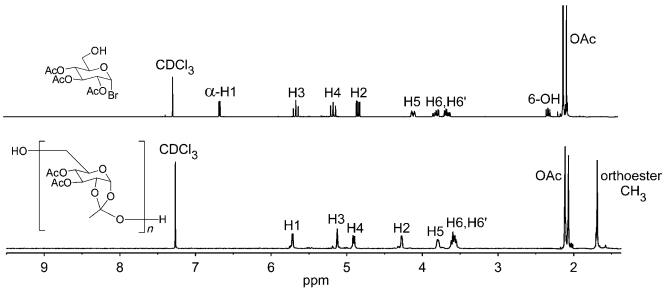


Figure 2. ^{1}H NMR spectra of monomer **3** (top) and polymer **4** (bottom). All NMR signals were assigned based on 2D COSY NMR analyses (see the Supporting Information).

orthoester methyl protons, which resonate at 1.69 ppm, and anomeric protons, which resonate at 5.71 ppm. Likewise, the ^{13}C NMR spectroscopy analyses supported the structural assignment, with characteristic orthoester carbon atoms resonating at 121.3 ppm and anomeric carbon atoms at 97.3 ppm (see the Supporting Information).

The NMR studies indicated that there was no glycosylation product, suggesting that once the acyloxonium was formed, the intermolecular attack of the 6-OH predominately occurred at the acyloxonium carbon atom, instead of at the anomeric carbon atom (Scheme 1). This is understandable, as the formation of the orthoester is a kinetically favored process; in the absence of an acid catalyst, orthoester is the predominant product. The NMR studies also suggested that other side reactions, such as the rearrangement of the orthoester linkages[15a] and the formation of 1,6-anhydroglucose, which could undergo ring-opening polymerization to give polysaccharides,[21] were minimal.

The synthesized sugar poly(orthoester) **4** was very stable at pH 7.4. When monitored by ^{1}H NMR spectroscopy, no significant degradation was detected for 12 hours (Figure 3). However, when the pH value was decreased to pH 6, acidolysis occurred and the ^{1}H NMR signal intensity of the orthoester (-CH_3 at 1.69 ppm) gradually decreased over a period of 12 hours (Figure 3). When the pH value dropped lower, the acidolysis progressed even faster and it took 6 and 2 hours to fully degrade all orthoester linkages at pH 5 and 4, respectively (Figure 3). In all acidolysis studies, the kinetics could be described by the first-order Arrhenius equation (see the Supporting Information), with half-lives of 0.9, 0.6, and

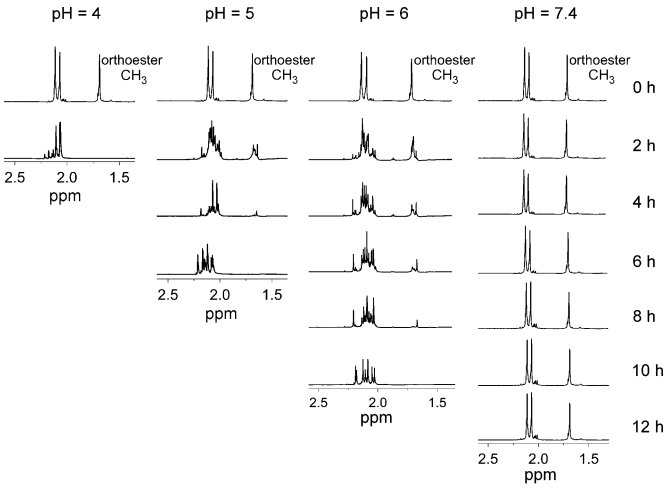


Figure 3. Progress of acidolysis of **4** at varying pH values, as monitored by ^{1}H NMR spectroscopy.

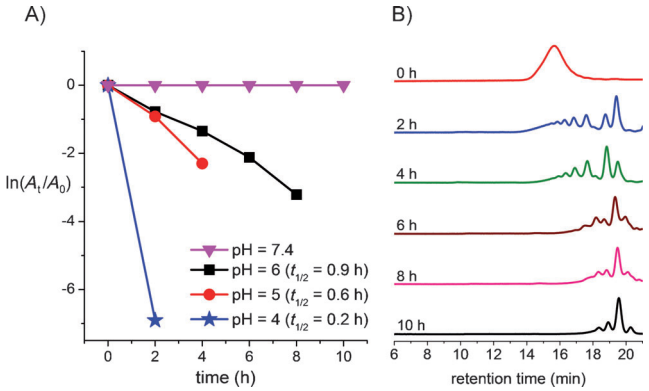


Figure 4. A) First-order Arrhenius plot of the acidolysis of **4** at pH 7.4, 6, 5, and 4. B) Progress of acidolysis of **4** at pH 6, as monitored by GPC.

0.2 hours at pH 6, 5, and 4, respectively (Figure 4 A). As expected, when monitored by GPC, partial degradation of the orthoester linkages resulted in a sharp decrease in molecular weights. Within only 2 hours, low-molecular-weight oligomers were produced (Figure 4 B). This is very attractive in the formation of pH-responsive materials for drug delivery, as partial degradation of the polymer is sufficient for rapid release of the drugs.

In conclusion, we have demonstrated the successful synthesis of a new class of sugar-based polymers, in which the sugar units are connected through orthoester linkages. To the best of our knowledge, this is the first report describing a sugar poly(orthoester). Considering the efficient and practical syntheses of the monomer and the polymer, the availability and abundance of monosaccharides, and numerous methods for carbohydrate functionalization, this method may have broad applications for the synthesis of diverse sugar-based polymers. Because of its high sensitivity to acidolysis, the reported sugar poly(orthoester) may be useful in the synthesis of highly pH-responsive materials, which could selectively and rapidly deliver drugs to malign

tissues that have lower extracellular pH values. Future studies, such as controlling the rate of acidolysis and synthesizing nanostructures, are currently under way in our laboratory.

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[1] a) S. Binauld, M. H. Stenzel, *Chem. Commun.* **2013**, *49*, 2082 – 2102; b) W. W. Gao, J. M. Chan, O. C. Farokhzad, *Mol. Pharm.* **2010**, *7*, 1913 – 1920.
[2] L. E. Gerweck, S. Vijayappa, S. Kozin, *Mol. Cancer Ther.* **2006**, *5*, 1275 – 1279.
[3] K. H. Steen, A. E. Steen, P. W. Reeh, *J. Neurosci.* **1995**, *15*, 3982 – 3989.
[4] a) S. Sengupta, D. Eavarone, I. Capila, G. Zhao, N. Watson, T. Kiziltepe, R. Sasisekharan, *Nature* **2005**, *436*, 568 – 572; b) R. Tong, J. Cheng, *J. Am. Chem. Soc.* **2009**, *131*, 4744 – 4754; c) C.-C. Lee, Y. Liu, T. M. Reineke, *ACS Macro Lett.* **2012**, *1*, 1388 – 1392.
[5] R. M. Sawant, J. P. Hurley, S. Salmaso, A. Kale, E. Tolcheva, T. S. Levchenko, V. P. Torchilin, *Bioconjugate Chem.* **2006**, *17*, 943 – 949.
[6] L. Zhang, Y.-I. Jeong, S. Zheng, S. I. Jang, H. Suh, D. H. Kang, I. Kim, *Polym. Chem.* **2013**, *4*, 1084 – 1094.
[7] a) M. J. Heffernan, N. Murthy, *Bioconjugate Chem.* **2005**, *16*, 1340 – 1342; b) Y. Li, W. Du, G. Sun, K. L. Wooley, *Macromolecules* **2008**, *41*, 6605 – 6607; c) S. C. Yang, M. Bhide, I. N. Crispe, R. H. Pierce, N. Murthy, *Bioconjugate Chem.* **2008**, *19*, 1164 – 1169; d) J. A. Cohen, T. T. Beaudette, J. L. Cohen, K. E. Broaders, E. M. Bachelder, J. M. J. Fréchet, *Adv. Mater.* **2010**, *22*, 3593 – 3597.
[8] a) M. B. Sintzel, J. Heller, S. Y. Ng, M. S. Taylor, C. Tabatabay, R. Gurny, *Biomaterials* **1998**, *19*, 791 – 800; b) H.-H. Chia, Y.-Y. Yang, T.-S. Chung, S. Ng, J. Heller, *J. Controlled Release* **2001**, *75*, 11 – 25; c) M. Palamoor, M. M. Jablonski, *Mol. Pharm.* **2012**, *10*, 701 – 708; d) M. B. Sintzel, J. Heller, S. Y. Ng, C. Tabatabay, K. Schwach-Abdellaoui, R. Gurny, *J. Controlled Release* **1998**, *55*, 213 – 218; e) J. Heller, J. Barr, S. Y. Ng, H. R. Shen, K. Schwach-Abdellaoui, S. Emmahl, A. Rothen-Weinhold, R. Gurny, *Eur. J. Pharm. Biopharm.* **2000**, *50*, 121 – 128.
[9] R. Williams, S. W. Schalm, J. G. O'Grady, *Lancet* **1993**, *342*, 273 – 275.
[10] a) J. Heller, J. Barr, *Biomacromolecules* **2004**, *5*, 1625 – 1632; b) J. Heller, J. Barr, S. Y. Ng, K. S. Abdellauoi, R. Gurny, *Adv. Drug Delivery Rev.* **2002**, *54*, 1015 – 1039.
[11] A. Merkli, J. Heller, C. Tabatabay, R. Gurny, *J. Biomater. Sci. Polym. Ed.* **1993**, *4*, 505 – 516.
[12] J. Heller, S. Y. Ng, D. W. Penhale, U. S. Patent, 4513143, **1985**.
[13] a) R. Tang, W. Ji, C. Wang, *Polymer* **2011**, *52*, 921 – 932; b) R. Tang, R. N. Palumbo, W. Ji, C. Wang, *Biomacromolecules* **2009**, *10*, 722 – 727.
[14] a) M. Ahmed, B. F. L. Lai, J. N. Kizhakkedathu, R. Narain, *Bioconjugate Chem.* **2012**, *23*, 1050 – 1058; b) N. Xiao, H. Liang, J. Lu, *Soft Matter* **2011**, *7*, 10834 – 10840; c) S. Mizrahy, D. Peer, *Chem. Soc. Rev.* **2012**, *41*, 2623 – 2640.
[15] a) F. Kong, *Carbohydr. Res.* **2007**, *342*, 345 – 373; b) W. Wang, F. Kong, *Angew. Chem.* **1999**, *111*, 1330 – 1333; *Angew. Chem. Int. Ed.* **1999**, *38*, 1247 – 1250; c) W. Wang, F. Kong, *J. Org. Chem.* **1998**, *63*, 5744 – 5745.
[16] a) R. U. Lemieux, A. R. Morgan, *Can. J. Chem.* **1965**, *43*, 2198 – 2204; b) S. Wei, J. Zhao, H. Shao, *Can. J. Chem.* **2009**, *87*, 1733 – 1737.
[17] S. Shoda, M. Moteki, R. Izumi, M. Noguchi, *Tetrahedron Lett.* **2004**, *45*, 8847 – 8848.
[18] L. Li, E. A. Franckowiak, Y. Xu, E. McClain, W. Du, *J. Polym. Sci. Part A* **2013**, *51*, 3693 – 3699.
[19] a) W. Du, J. Gervay-Hague, *Org. Lett.* **2005**, *7*, 2063 – 2065; b) W. Du, S. S. Kulkarni, J. Gervay-Hague, *Chem. Commun.* **2007**, 2336 – 2338.
[20] J. A. Morales-Serna, Y. Díaz, M. I. Matheu, S. Castillón, *Eur. J. Org. Chem.* **2009**, 3849 – 3852.
[21] J. Fréchet, C. Schuerch, *J. Am. Chem. Soc.* **1969**, *91*, 1161 – 1164.