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# Synthesis and conformational analysis of D-*gluco*-pyranosyl-(6,6′)-D-*gluco*-pyranuronate, a model compound for the inter-glycan 6,6′-ester linkage

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ABSTRACT

The synthesis of a 6,6′-ester linked disaccharide analog model compound was achieved in five steps from D-glucose and featured a key oxidative esterification transformation. The synthesized D-*gluco*-pyranosyl-(6,6′)-D-*gluco*-pyranuronate was characterized in $D_2O$ using NMR spectroscopy. Using the experimental data together with molecular dynamics simulations (TIP3P, water), a model of the compound's conformational behavior was established. The effect of the 6,6′-ester linkage on the solution phase structure was compared to that of the previously reported 6,6′-ether linkage in a disaccharide analog. Based on the established models, the ester linkage was found to have a profound effect on the overall shape of the molecule.

## 1. Introduction

It is widely known that the variability in constitution and stereochemistry between different carbohydrates can result in large conformational differences. For example, many glycosidases, such as mammalian muscle acid $\alpha$-glucosidase, show high activity toward maltose and other $\alpha(1 \rightarrow 4)$-glucans, but will have low activity toward isomaltose or cellulose, which are $\alpha(1 \rightarrow 6)$- and $\beta(1 \rightarrow 4)$-linked, respectively [1,2]. For this reason, the conformational analysis of naturally occurring carbohydrates has received considerable attention [3–16]. NMR spectroscopy has been used widely for conformational analyses and to describe the molecular shape of molecules in solution. In particular, NOE experiments and coupling constant analysis generally allow chemists to propose reasonable solution phase structurese [3–6,17]. However, there continues to be a need to supplement the experimental data with computational modeling, particularly in the case of molecules that have large conformational spaces and low rigidity, such as carbohydrates, where more than only a handful of conformers will be accessible to the molecule of interest [3,6–8,18]. Beyond that, molecules designed to act similar to naturally occurring carbohydrates, so called carbohydrate analogs or mimics, have been explored for a variety of reasons, such as therapeutic applications or the elucidation of enzyme mechanisms and biochemical pathways [19–22]. A large number of studies have been published on the conformational analysis of carbohydrate analogs using a combination of NMR and computational modeling [23–28].

The ester linkage is not uncommon in carbohydrate-containing natural products, such as the glycolipid ‘lipid A’ from Gram-negative bacteria, as well as tannin compounds such as gallotannins or ellagitannins, for example [29–32]. However, only a small number of naturally occurring ester-crosslinked di- or polysaccharides have been reported in the literature [33–35]. Ester-linked disaccharides or disaccharides have been studied previously with respect to drug delivery and as intermediates in stereoselective ‘redox glycosylation’ [36–42]. However, the effect of the ester-linkage on the conformational behavior of the aforementioned ester-linked disaccharide analogs was not considered in the respective studies.

As such, the present work aimed to elucidate the influence of the ester functional group on the flexibility of the linkage between two sugar monomers. Based on the general conformational behavior of the ester functional group, it was hypothesized that it would decrease the flexibility of a carbohydrate analog when compared to a more conformationally flexible ether or anomeric

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acetal linkage. This hypothesis was tested on a novel 6,6′-ester linked model disaccharide analog, which was synthesized in five-steps starting from D-glucose and involving a key oxidative esterification step, and studied by NMR and molecular dynamics to determine its solution phase structure.

## 2. Results and discussion

### *2.1. Synthesis of 6,6′-linked ester disaccharides*

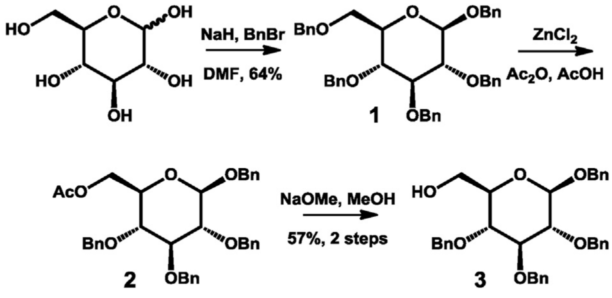
To address the question of the influence of an ester functional group on the linkage between two monosaccharide units, a model 6,6′-linked ester disaccharides was prepared. An oxidative esterification was envisioned to provide convenient access to 6,6′-ester linked disaccharides from 6-unprotected monosaccharide precursors [43–47]. The synthesis of a selectively 6-unprotected monosaccharide precursor was achieved for D-glucose in three steps on the basis of literature procedures, as depicted in Scheme 1 [48]. Perbenzylation of free D-glucose yielded benzyl 2,3,4,6-tetra-*O*-benzyl-*β*-D-*gluco*-pyranoside **1** in 64% yield, followed by selective debenzylation-acetylation at the 6-position mediated by $ZnCl_2$ to give benzyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-*β*-D-*gluco*-pyranoside **2**. In the last step, benzyl 2,3,4-tri-O-benzyl-*β*-D-*gluco*-pyranoside **3** was readily obtained from crude **2,** through deacetylation in methanol with a catalytic amount of sodium, in 57% yield over two steps and after recrystallization from ethanol. This three-step synthetic pathway could be carried out on a multi-gram scale and it was found that sufficient purity (>95% based on $^{1}$H NMR) could be reached without the need for column chromatography, contrary to the description in the literature [48].

The oxidative esterification of **3** with TEMPO in anhydrous acetonitrile and with TCCA as a soluble terminal oxidant yielded the desired benzyl 2,3,4-tri-*O*-benzyl-*β*-D-*gluco*-pyranosyl-(6 → 6′)-(benzyl 2,3,4-tri-*O*-benzyl-*β*-D-*gluco*-pyranuronate) **4**, albeit in relatively low 26% yield after column chromatography (Scheme 2). Based on analysis of the observed $^{3}J_{HH}$ couplings in the $^{1}$H NMR spectrum, **4** was determined to retain the $^{4}C_{1}$ conformation for both monosaccharide units.

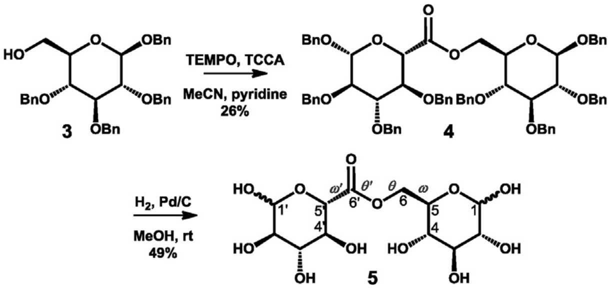
In the final step, the reductive deprotection of **4** with hydrogen gas and catalytic Pd/C afforded the desired D-*gluco*-pyranosyl-6,6′-D-*gluco*-pyranuronate **5** in 49% yield, after column chromatography with DCM:MeOH (gradient elution 95:5–7:3).

### *2.2. NMR spectral analysis of D-gluco-pyranosyl-6,6′-D-gluco-pyranuronate (5)*

The $^{1}$H NMR spectrum of the purified **5** measured in $D_2O$ indicated the presence of several different sugar units, as was expected due to the different anomers combinations. Using 1D TOCSY experiments, the spin systems corresponding to both *α*- and *β*-D-*gluco*-pyranuronate (*α*/*β*-GlcA) and *α*- and *β*-D-*gluco*-pyranosyl (*α*/*β*-Glc*p*) could be elucidated, as shown in Fig. 1. The proton signals of H-$6_{R/S}$ of both *α*/*β*-Glc*p* were found to be significantly deshielded when compared to free *α*/*β*-D-glucose, which was indicative of the ester linkage. The assignment of the respective proton signals could be achieved from a 2D COSY experiment and the corresponding carbon signals were assigned from HMQC correlations (See Supplemental Information for their spectra and chemical shift assignments in Table 4.). The coupling constants of all four spin systems were indicative of $^{4}C_{1}$ ring conformation for **5**.



**Scheme 1.** Synthesis of the 6-unprotected precursor **3** from D-glucose in 3 steps and 36% overall yield [48].



**Scheme 2.** Oxidative esterification [47] of **3** and subsequent debenzylation leading to the desired 6,6′-ester linked glucose dimer **5**.

Additionally, an HMBC experiment confirmed the ester linkage. As shown in Fig. 2, a total of four carbonyl carbon signals appeared in the $^{13}$C spectrum. Based on the two-bond correlations to H-5 of the glucuronates, these could be assigned pairwise to the *α*-GlcA and *β*-GlcA subunits, respectively. Notably, the HMBC spectrum revealed three-bond correlations from both *α*-Glc*p*-H-$6_{R/S}$ and *β*-Glc*p*-H-$6_{R/S}$ to *α*-GlcA-C-6. The same was the case for *β*-GlcA-C-6. Additionally, the signals of *α*/*β*-GlcA-H-5 appeared to be two overlapping doublets. Taken together, these observations revealed that the four identified spin systems were present in duplicate. Thus, it was concluded that all four possible anomeric pairs of **5**, namely *αα*-**5**, *αβ*-**5**, *βα*-**5** and *ββ*-**5**, were present in solution and the 1D TOCSY spectra resulted from the overlap of two virtually identical spin systems, that only differed in the chemical shift of H-5 of the *α*/*β*-GlcA subunits, as seen in Figs. 1 and 2.

The complexity of the NMR spectrum of **5**, with a mixture of four diastereomers present due to the two anomeric centers, could have been reduced by fixation of the anomeric position, for example by starting the synthesis from methyl-*α*-D-glucopyranoside. However, as work from this laboratory and others has shown, methylation of the anomeric hydroxyl can significantly alter the dihedral distribution of the *ω* and *θ* angles in glucose [49–51]. To allow for the elucidation of the conformational effect of the ester functional group on the linkage between the two monosaccharides, the aforementioned methylation was omitted in order to avoid confounding changes to the conformational behavior of **5**.

Additional measurements of the $^{1}$H NMR spectrum of the NMR sample of **5**, taken 24 h after preparation and storage at RT, indicated the increased abundance of other anomeric signals in the spectrum, as highlighted in Fig. 3. One of these could be clearly identified as free *β*-D-glucose through 1D TOCSY experiments on the basis of chemical shift data. Although no thorough experiments were performed on the stability of **5** under different acidic or basic conditions, the above findings suggest the relative instability of the ester linkage in **5**.

### *2.3. Conformational analysis of D-gluco-pyranosyl-6,6′-D-gluco-pyranuronate (5)*

The basis for molecular modeling of the saccharides was the