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Increased glycosidic bond stabilities in 4-*C*-hydroxymethyl linked disaccharides

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ABSTRACT

Three new hydroxymethyl-linked non-natural disaccharide analogues, containing an additional methylene group in between the glycosidic linkage, were synthesized by utilizing 4-*C*-hydroxymethyl-α-D-glucopyranoside as the glycosyl donor. A kinetic study was undertaken to assess the hydrolytic stabilities of these new disaccharide analogues toward acid-catalyzed hydrolysis, at 60 °C and 70 °C. The studies showed that the disaccharide analogues were stable, by an order of magnitude, than naturally-occurring disaccharides, such as, cellobiose, lactose, and maltose. The first order rate constants were lower than that of methyl glycosides and the trend of hydrolysis rate constants followed that of naturally-occurring disaccharides. α-Anomer showed faster hydrolysis than the β-anomer and the presence of axial hydroxyl group also led to faster hydrolysis among the disaccharide analogues. Energy minimized structures, derived through molecular modeling, showed that dihedral angles around the glycosidic bond in disaccharide analogues were nearly similar to that of naturally-occurring disaccharides.

1. Introduction

Studies on understanding the hydrolytic stabilities of glycosidic bonds occupy a significant place in sugar chemistry.[1–5] The relevance of glycosidic bonds to glycosidase activities is an example *par excellence* to illustrate rich chemistry surrounding glycosidic bonds in biological recognition processes.[6,7] Chemical and enzymatic studies to assess the glycosidic bond stabilities are well-established, allowing early postulations and later refinements to evolve in order to account for the observed hydrolytic stabilities of glycosidic bonds more accurately.[8–13] Differences in the rates of hydrolysis of anomeric and epimeric glycosides were the subject of sustained introspection. Added with the fact that levels of computational studies were not accessible few decades ago, refinements of rationale behind differing hydrolytic rates of glycosidic bonds continue to evolve with advancements in computational studies. More than a century-old observation that methyl β-D-glucopyranoside[14] undergoes nearly twice faster hydrolysis than the α-anomer is a case in point. Early suggestions regarding this rate difference pertained to steric hindrance in protonating glycosidic oxygen of α-anomer and lesser free energy of activation to protonate the same oxygen in the case of the β-anomer.[15–17] The first step of protonation and subsequent heterolysis of *C*1–*O*1 bond, leading to the formation of positively charged oxocarbenium ion, are major events preceding a hydrolysis. Protonation of *exocyclic O*-1 is well resolved for pyranosides as the first step. The stabilities, bond reorganizations, and conformational changes of the protonated species were determined to be important, prior to heterolysis of *C*1–*O*1 bond. Further advancements elucidate a stereoelectronic interpretation for acid-catalyzed hydrolysis, wherein α-anomeric *O*5*C*1–*O*1R bond in the antiperiplanar orientation facilitates protonation, governed by the so-called 'antiperiplanar lone pair hypothesis' (ALPH). Whereas, when the β-anomer undergoes faster hydrolysis, a possible effect through 'synperiplanar lone pair hypothesis' (SLPH) appeared to operate.[11–13,18,19] Both these hypotheses refer to orientation of the leaving group *O*1R with respect to lone pair electrons on *O*5 upon protonation of *O*-1 and the attendant bond reorganizations toward favorable ring conformations before heterolysis. In accounting reactivities of the anomers, it was advocated that the energetically accessible half-chair conformation of the β-anomer resulting through a synperiplanar interaction, toward attaining a boat conformation, is equivalent to half-chair conformation of α-anomer resulting from an antiperiplanar interaction. Rationale contradicting ALPH were also put forth on the basis of preferred alternate conformations of reactive ground-state of the glycosides.[20] Whereas experiments and theories evolved for the rate of hydrolysis of anomeric glycosides, such is also the case to account differences observed with epimeric glycosides. Faster hydrolysis of glycosidic bonds on glycosides with axial hydroxyl groups than in equatorial hydroxyl groups is an excellent case to illustrate this phenomenon.[21] The early reasoning that axial hydroxyl groups relieve ring strain during formation of oxocarbenium ion was refined later, rather conclusively, as arising due to hyperconjugative and field effects of axial and equatorial hydroxyl group. Recent studies of Bols and co-workers,[22,23] and Withers and co-workers[24] establish the role of hyperconjugative effects and field effects, in order to account influence of substituents on glycosidic bond stabilities. Bols put

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forward the rationale of charge-dipole interaction being the driving force for observed differences in the hydrolysis rates of epimeric methyl glycosides.[22] An equatorial hydroxyl group at β- or γ-position in methyl glycosides exerts higher electron withdrawing effect than an axial hydroxyl group, leading to different rates at which charge development occurs in the transition state of anomeric C–O bond heterolysis. This explanation provides the first possibility wherein differing rates of hydrolysis does not necessarily depend on the conformational change as the driving force.

In the studies so far, acid-catalyzed glycosidic bond hydrolyses were performed on stereoisomeric glycosides, having sugar, as well as, non-sugar moieties at the reducing end. The effect of *exocyclic* methyl *vs* sugar substituent is that the β-anomer undergoes faster hydrolysis in the former and that of α-anomer in the latter case. Propensities to undergo required bond reorganizations upon conjugate acid formation and favorable conformations of reactive intermediates during hydrolysis lead to the observed differences between methyl glycosides and disaccharides. An objective of the present work is to assess glycosidic bond stability of 4-*C*-hydroxymethyl-linked disaccharides (Fig. 1), wherein the glycosidic bond is interrupted with a methylene moiety. Glycosidic bond expanded disaccharides might be considered analogues to naturally-occurring disaccharides, in which case, it was of interest to identify whether the glycosidic bond stability would match that of normal disaccharides. Following synthesis of new disaccharide analogues **1**–**3**, their acid-catalyzed hydrolytic stabilities were studied, details of which are presented herein.

2. Results and discussion

Synthesis of non-natural disaccharides **1**–**3** was accomplished through a glycosylation of a glycosyl donor with 4-*C*-hydroxymethyl α-D-glucopyranoside acceptor as shown in Scheme 1. Synthesis was initiated from benzyl 2,3,6-*tri*-*O*-benzyl-α-D-glucopyranoside **4**,[25] which upon oxidation with Dess–Martin periodinane provided carbonyl compound **5**. Subsequent Wittig methylenation afforded derivative **6**, in 68% yield. Borane addition, followed by an oxidation afforded **7** with *galacto*-configuration at the newly generated hydroxymethyl group. Subsequent oxidation of **7** with Dess–Martin periodinane led to *galacto*-configured 4-*C*-aldehyde **8**, which upon treatment with Et_3N led to epimerization[26] to *gluco*-configuration at C-4 (**9**), in a moderate yield. The presence of *gluco*-configuration in **9** was confirmed through *J*-coupled correlation (COSY) spectroscopy, in which the H-4 nucleus resonated at ~3.0 ppm as a doublet of an apparent triplet with J = 2.6, 10.8 Hz. Aldehyde **9** was reduced subsequently to alcohol **10** with $NaBH_4$ in MeOH, in a good yield (Scheme 1).

Synthesis of new disaccharides was accomplished through a glycosylation of glycosyl donor and **10**. Glycosylation of acetobromo glucose[27] with acceptor **10**, in the presence of $Hg(CN)_2$ and $HgBr_2$, afforded protected disaccharide **1**, in a moderate yield. The appearance of H-1 nucleus corresponding to glucopyranoside residue of protected derivative **1** at ~4.30 ppm, as a doublet with J = 8.0 Hz, confirmed the presence of the β-glycosidic bond. The protected derivative **1** was O-deacetylated under Zemplén condition, followed by removal of benzyl group, using Pd/C and H_2 to afford free hydroxyl-group containing disaccharide analogue **1**, in a good yield. On the other hand, a glycosylation of acetobromo galactose and **10**, in the presence of $HgBr_2/Hg(CN)_2$, followed by removal of protecting groups afforded **2**, in a moderate yield. Appearance of an H-1′ signal at ~4.26 ppm, as a doublet with J = 8.0 Hz, confirmed β-anomeric glycosidic bond of protected derivative **2** (Scheme 1).

In order to synthesize α-linked disaccharide **3**, 2,3,4,6-*tetra*-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate[28] was used as the glycosyl donor. TMSOTf-mediated glycosylation of trichloroacetimidate and **10**, followed by O-deacetylation led to the formation of protected derivative of **3** (Scheme 2). The anomeric configuration of newly formed glycosidic bond was confirmed by ^1H and ^{13}C spectroscopies. Anomeric proton corresponding to glucopyranoside residue of protected derivative of **3**, at ~4.92 ppm, as a doublet with J = 3.6 Hz, indicated α-anomeric configuration of glycosidic bond.

Toward assessing hydrolytic stabilities of disaccharide analogues **1–3**, acid-catalyzed hydrolysis was undertaken. The studies were conducted in comparison to that of naturally-occurring disaccharides, namely, cellobiose (**11**), lactose (**12**), and maltose (**13**). Prior to hydrolysis studies, verification of aqueous solubilities of **1–3** showed following solubilization in water: **1**: 0.065 g mL^{-1}; **2**: 0.12 g mL^{-1}, and **3**: 0.24 g mL^{-1}. These aqueous solubilities are lower than that for disaccharides:[29] **11**: 0.14 g mL^{-1}; **12**: 0.19 g mL^{-1}, and **13**: 0.42 g mL^{-1}. In order to evaluate the kinetics of acid-catalyzed hydrolysis, ^1H NMR spectroscopy was used. Hydrolysis of **1–3** and **11–13** was performed with DCl in D_2O (2 N), at 60 and 70 °C and ^1H NMR spectrum was recorded periodically. The analysis was facilitated through assigning distinct protons. Progress of the glycosidic bond cleavage was monitored by the appearance of new H-4 of 4-*C*-hydroxymethyl D-glucopyranose. The hydrolysis data obtained from ^1H NMR spectra were plotted as a function of time and fitted to an exponential decay curve. The observed first order rate constants are summarized in Table 1. Representative ^1H NMR profiles corresponding to H-4 resonance of **1** and that of hydrolysis product 4-*C*-hydroxymethyl D-glucopyranose and the associated exponential decay curve are shown in Figure 2. For comparison, hydrolysis data of methyl glycosides, namely, methyl-β-D-glucopyranoside (**14**), methyl-β-D-galactopyranoside (**15**), and methyl-α-D-glucopyranoside (**16**), obtained under identical conditions, are also given.

The kinetic data showed that among three new disaccharide analogues **1–3**, the rate of hydrolysis was least for **1** and highest for **2**, thereby following the trend that glycosides with one or more axial hydroxyl groups tend to undergo faster the hydrolysis than those with only equatorial hydroxyl groups. On the other hand, hydrolysis rate of **2** was 5.7 times slower than **12**, **1** was 17.5 times slower than **11**, and **3** was 33 times slower than **13** at 60 °C. Differences in pairwise rates of hydrolysis of **11** and **14**, **12,** and **15** were not as high as that seen with **1–3**, except **13** and **16** pair. Similar differences among the glycosides could also be observed at 70 °C. The presence of hydroxymethyl-linkage in the glycosidic bond of **1–3** reduced the hydrolysis rate significantly when compared to **11–16** in general. Faster rates of protonation and stabilities of conjugate acid lead to faster hydrolyses of disaccharides, when compared to methyl glycosides **14–16**. Bond reorganization occurs upon protonation, so as to

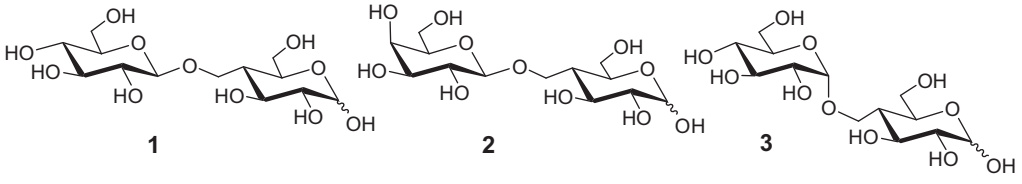

Figure 1. Molecular structures of hydroxylmethyl-linked disaccharides.