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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

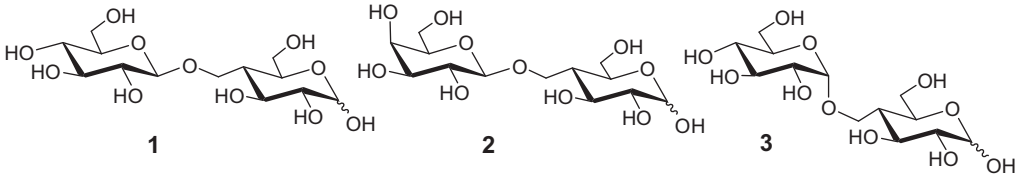
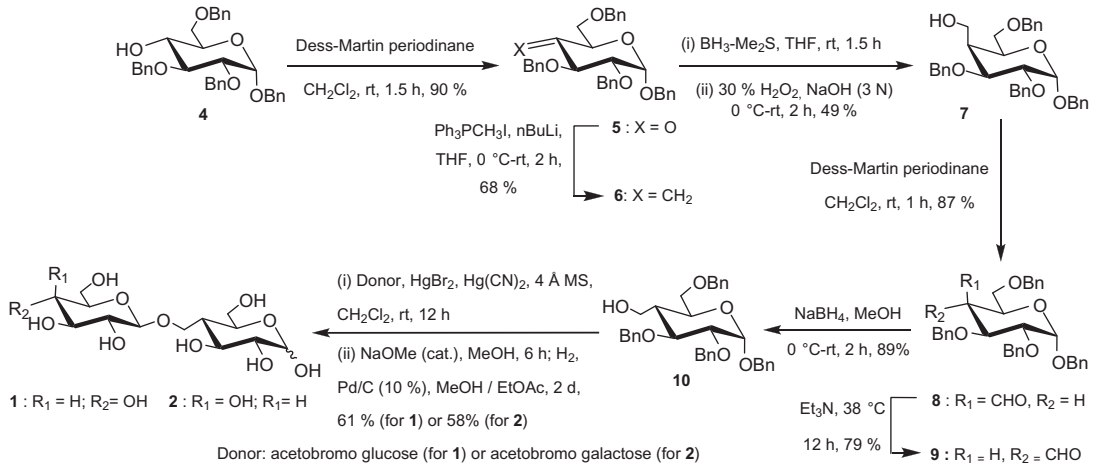
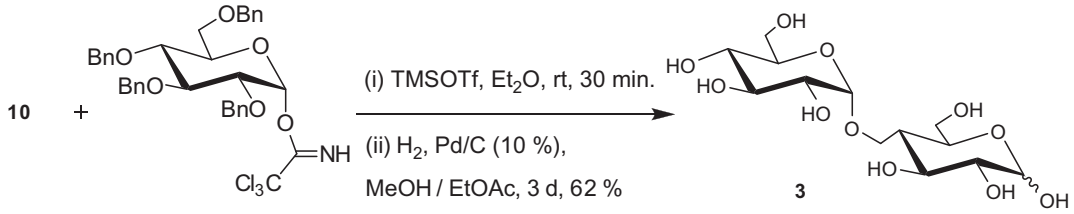


Figure 1. Molecular structures of hydroxylmethyl-linked disaccharides.



Scheme 1.



Scheme 2.

Table 1
First order rate constant for the acid-catalyzed hydrolysis of glycosidic bond in **1**–**3** and **11**–**16**

Compound	Temp (°C)	**1**	**2**	**3**	**11**	**12**	**13**	**14**	**15**	**16**
Rate of hydrolysis (k)[a]	60	0.25	1.37	0.49	4.39	7.85	16.2	2.11	5.60	0.97
	70	1.08	4.78	2.10	19.20	35.80	61.15	12.35	21.04	6.45

[a] $\times 10^5\ s^{-1}\ mol^{-1}$.

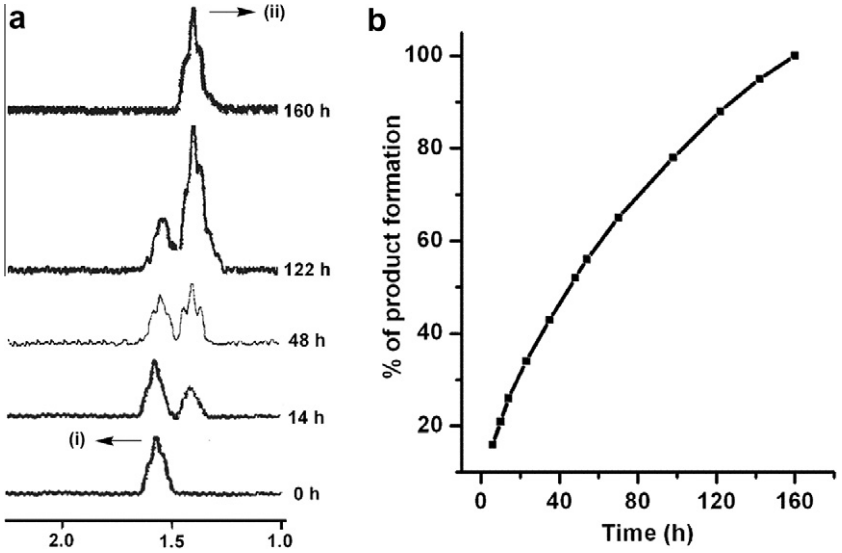


Figure 2. (a) Stack plot of few representative temperature dependent ^{1}H NMR spectra of hydrolysis of **1**, wherein (i) corresponds to H-4 of **1** and (ii) H-4 of 4-*C*-hydroxymethyl D-glucopyranose. (b) Kinetic plot of formation of 4-*C*-hydroxymethyl D-glucopyranose as a function of time, monitored by the evolution of resonance (ii) in (a).

shift the transition state of *C*1–*O*1 heterolysis toward the oxocarbenium ion conformation. Thus, when comparing maltose (**13**) and cellobiose (**11**), maltose hydrolyze faster. An opposite trend of β-methyl glucopyranoside **14** hydrolyze faster than the α-anomer **16**

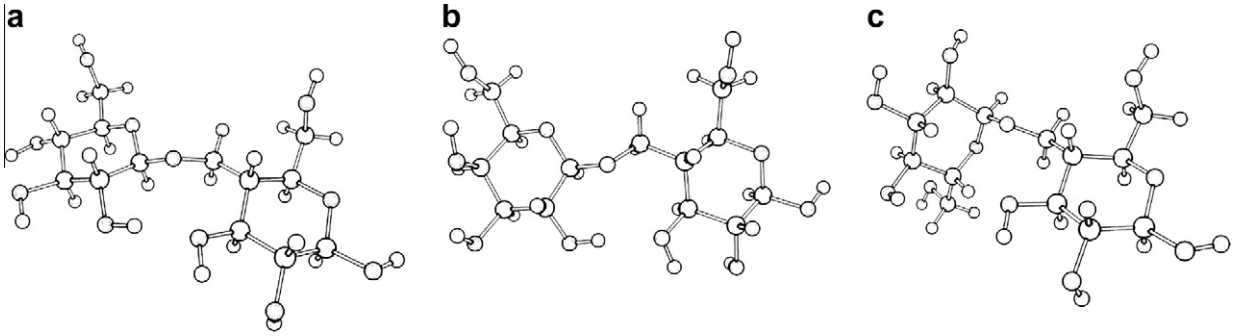


Figure 3. Structures of **1**–**3** derived from energy minimization protocol.

was thought to arise through an effect of SLPH (vide supra), wherein synpreiplanar arrangement of β-anomer is as favorable for bond reorganization as the antiperiplanar arrangement of the α-anomer. The trend of α-anomer **3** undergoing faster hydrolysis than β-anomer **1** is analogous to α-anomer **13** undergoing faster hydrolysis than β-anomer **11**. An effect resulting from ALPH would thus be appropriate for the faster hydrolysis of **3** as much as with **13**. The observed rate differences between natural disaccharides **11–13** and non-natural disaccharide analogues **1–3** may arise from altered rates protonation of glycosidic oxygen, even when the ALPH accounts the trend of hydrolysis rates. Axially oriented hydroxyl group in **2** contributes to the highest observed rate of hydrolysis, possibly through the recently established hyperconjugation effect (vide supra).[22] Activation energies and entropies[30] remain to be assessed so as to assess how the pre-equilibration step of protonation of anomeric oxygen and anomeric C–O bond heterolysis is affected in the disaccharide analogues **1–3**. Further studies are required to verify these and more issues concerned with the hydrolysis trends in **1–3**.

In order to derive low energy structures of **1–3** and to compare with structures of disaccharides **11–13**, molecular modeling of new disaccharide analogues were performed using Gaussian 09 software at B3LYP/6-31G* level theory. The structures derived from the modeling are shown in Figure 3, wherein pyranose were seen to retain $^4C_1$ conformation. The dihedral angles around glycosidic linkage and the anomeric bond length were, respectively, **1**: −83.658, 1.3887 Å; **2**: −81.580, 1.3882 Å, and **3**: 67.463, 1.4025 Å. The corresponding values for disaccharides were, respectively, **11**: −75.837, 1.3952 Å[31]; **12**: −93.413, 1.3980 Å[32], and **13**: 116.099, 1.4151 Å.[33] These values indicate that the presence of additional methylene group in **1–3** did not deviate the interglycosidic linkage conformation greatly from that of natural disaccharides **11–13**.

Studies on carbohydrate mimetics assume importance in order to understand several biological recognition processes mediated by carbohydrates. An approach to such mimetics is to replace the glycosidic oxygen with a hetero-atom so as to endow increased hydrolytic stabilities toward enzymatic degradations. Sulfur, selenium, carbon, and nitrogen links at the anomeric carbon emerged as viable options toward this objective.[34] To the extent that increased hydrolytic stabilities were observed for the disaccharide analogues presented herein, the analogues open up possibilities of newer mimetics that are retained with the glycosidic oxygen.

3. Conclusion

A study of glycosidic bond hydrolysis of disaccharide analogues presenting an additional methylene group in between the glycoside bond was accomplished. Three new disaccharide analogues having hydroxymethyl glycosidic linkage were prepared, by utilizing a 4-*C*-hydroxymethyl D-glucopyranoside as the glycosyl acceptor. Hydrolytic stabilities of these new disaccharide analogues, studied under an acid catalyzed condition, showed that the glycosidic bond was hydrolytically more stable than naturally-occurring disaccharides and methyl glycopyranosides, as judged from hydrolysis rates assessed at two different temperatures. The rate of hydrolysis followed a trend observed normally for the naturally-occurring disaccharides. Energy minimized structures, derived through molecular modeling, revealed that the disaccharides analogues possessed dihedral angles around the glycosidic linkage nearly similar to that of naturally-occurring disaccharides. The study shows the possibility to derive hydrolytically more stable disaccharides, retained with O-glycosidic linkage for the first time.

4. Experimental

4.1. General methods

Solvents were dried and distilled according to literature procedures.[35] All chemicals were purchased from commercial sources and were used without further purification. Silica gel (100–200 mesh) was used for column chromatography and TLC analysis was performed on commercial plates coated with Silica Gel 60 F_{254}. Visualization of the spots on TLC plates was achieved by UV radiation or spraying 5% sulfuric acid in ethanol. High resolution mass spectra were obtained from Q-TOF instrument by electrospray ionization (ESI). ^{1}H and ^{13}C NMR spectral analyses were performed on a spectrometer operating at 300 MHz, 400 MHz, and 75 MHz, 100 MHz, respectively, in $CDCl_3$, D_2O solutions, unless otherwise stated. Chemical shifts are reported with respect to tetramethylsilane (TMS) for ^{1}H NMR and the central line (77.0 ppm) of $CDCl_3$ for ^{13}C NMR. Coupling constants (*J*) are reported in Hz. Standard abbreviations s, d, t, dd, br s, m refer to singlet, doublet, triplet, doublet of doublet, broad singlet, multiplet. For disaccharide derivatives **1–3**, H-1 denotes anomeric proton of hydroxymethyl containing sugar moiety and H-1′ denotes anomeric proton of pyranoside moiety.

4.2. Synthesis and characterization

4.2.1. Benzyl 2, 3, 6-*tri*-*O*-benzyl-α-D-xylo-hexulopyranoside (5)

Dess-Martin periodinane (2.94 g, 6.94 mmol) was added to a solution of **4**[25] (2.5 g, 4.62 mmol) in CH_2Cl_2 (25 mL) and stirred at room temperature for 1.5 h under N_2 atmosphere. The reaction mixture was diluted with water (100 mL), extracted with $CHCl_3$ (3x60 mL), washed with aq $NaHCO_3$ (2x50 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified (hexane/EtOAc = 9:1) to afford **5** (2.25 g, 90%), as a gummy oil. R_f = 0.33 (hexane/EtOAc = 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43–7.23 (band, 20H, aromatic), 5.01 (d, 1H, *J* = 4.4 Hz, PhCH_2), 4.96 (d, 1H, *J* = 3.6 Hz, H-1), 4.81–4.53 (m, 7H, PhCH_2), 4.48 (d, 1H, *J* = 10.8 Hz, H-3), 4.34 (dd, 1H, *J* = 3.4, 5.8 Hz, H-5), 3.88 (dd, 1H, *J* = 3.4, 10.8 Hz, H-6_a), 3.80 (dd, 1H, *J* = 3.4, 10.8 Hz, H-6_b), 3.67 (dd, 1H, *J* = 3.6, 10.8 Hz, H-2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.1, 137.7, 137.8, 137.8,

136.5, 128.4, 128.3, 128.3, 128, 127.9, 127.8, 127.7, 127.6, 95.7, 82.6, 80.2, 74.4, 73.6, 73.6, 73.0, 69.8, 67.5. HRMS *m/z*: $[M+Na]^+$ calcd for $C_{34}H_{34}O_6Na$, 561.2253; found 561.2255.

4.2.2. Benzyl 2,3,6-*tri*-*O*-benzyl-4-deoxy-4-*C*-methylene-α-D-hexopyranoside (6)

^{n}BuLi (2.6 mL, 1.6 M, 4.18 mmol) was added dropwise over a period of 10 min. to a solution of Ph_3PMeI (2.5 g, 6.27 mmol) in THF (25 mL) at 0 °C. After 15 min, a solution of **5** (2.25 g, 4.18 mmol) in THF (25 mL) was added dropwise and stirring was continued for 2 h. The reaction mixture was diluted with H_2O (150 mL) and extracted with $CHCl_3$ (3 × 50 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo, and purified (hexane/EtOAc = 8:1) to afford **6** (1.54 g, 68%), as a colorless oil. R_f = 0.45 (hexane/EtOAc = 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43–7.26 (band, 20H, aromatic), 5.36 (s, 1H, methylene), 4.99 (s, 1H, methylene), 4.90 (d, 1H, J = 3.6 Hz, H-1), 4.86–4.71 (m, 4H, PhCH_2), 4.60–4.56 (m, 4H, PhCH_2), 4.39–4.42 (m, 2H, H-3, H-5), 3.78 (dd, 1H, J = 4.8, 10.0 Hz, H-6_a), 3.66 (dd, 1H, J = 6.0, 10.0 Hz, H-6_b), 3.48 (dd, 1H, J = 3.6, 9.6 Hz, H-2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.6, 138.5, 138.4, 138.0, 137.3, 128.4, 128.3, 128.2, 127.8, 127.1, 127.6, 127.5, 127.5, 107.7, 95.9, 81.6, 79.2, 73.8, 73.4, 73.2, 69.6, 68.9, 68.1. HRMS *m/z*: $[M+Na]^+$ calcd for $C_{35}H_{36}O_5Na$, 559.2460; found 559.2469.

4.2.3. Benzyl 2,3,6-*tri*-*O*-benzyl-4-*C*-hydroxymethyl-α-D-galactopyranoside (7)

A solution of BH_3–Me_2S (2 M) (1.8 mL, 3.6 mmol) was added dropwise to a solution of **6** (1.54 g, 2.87 mmol) in THF (25 mL) at room temperature, under N_2 atmosphere and stirred for 1.5 h. The reaction mixture was cooled at 0 °C, aq NaOH (3 N) (1 mL) and aq. H_2O_2 (30%) (1 mL) were added dropwise subsequently. After 2 h, the reaction mixture was diluted with H_2O (100 mL) and extracted with Et_2O (3 × 60 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The resulting residue was purified (hexane/EtOAc = 4:1) to afford **7** (0.79 g, 49%), as a gummy oil. R_f = 0.37 (hexane/EtOAc = 4:1); $[\alpha]_D$ +62.7 (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.4–7.24 (band, 20H, aromatic), 4.86 (d, 1H, J = 3.8 Hz, H-1), 4.77–4.67 (m, 4H, PhCH_2), 4.60–4.53 (m, 4H, PhCH_2), 4.14–4.10 (m, 2H, H-3, H-5), 3.95 (dd, 1H, J = 5.6, 11.4 Hz, H-7_a), 3.78 (dd, 1H, J = 5.6, 11.4 Hz, H-7_b), 3.68 (dd, 1H, J = 3.8, 10.2 Hz, H-2), 3.57–3.50 (m, 2H, H-$6_{a,b}$), 2.36–2.38 (m, 1H, H-4); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.3, 137.4, 137.2, 128.4, 128.3, 128.2, 127.7, 127.7, 127.6, 95.9, 78.6, 76.6, 76.5, 73.6, 73, 70.3, 68.9, 68.1, 57.8, 43.5. HRMS *m/z*: $[M+Na]^+$ calcd for: $C_{35}H_{38}O_6Na$, 577.2566; Found 577.2556.

4.2.4. Benzyl 2,3,6-*tri*-*O*-benzyl-4-deoxy-4-*C*-formyl-α-D-galactopyranoside (8)

Dess–Martin periodinane was added to a solution of **7** (0.79 g, 1.42 mmol) in CH_2Cl_2 (20 mL), stirred at room temperature for 1 h under N_2 atmosphere. The reaction mixture was diluted with H_2O (150 mL), extracted with $CHCl_3$ (2 × 50 mL), washed with aq $NaHCO_3$ (2x50 mL) dried (Na_2SO_4), filtered, and concentrated in vacuo. The resulting residue was purified (hexane/EtOAc = 8:1) to afford **8** (0.69 g, 87%), as an oil. R_f = 0.25 (hexane/EtOAc = 9:1); $[\alpha]_D$ +75.5 (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.91 (d, 1H, J = 5.2 Hz, C*H*O), 7.40–7.25 (band, 20H, aromatic), 5.0 (d, 1H, J = 3.8 Hz, H-1), 4.79–4.43 (m, 8H, PhCH_2), 4.22–4.18 (m, 2H, H-3, H-5), 3.97 (dd, 1H, J = 3.8, 10.0 Hz, H-2), 3.53 (dd, 1H, J = 4.2, 10.2 Hz, H-6_a), 3.46 (dd, 1H, J = 5.2, 10.2 Hz, H-6_b), 3.02 (dt, 1H, J = 2.6, 5.2 Hz, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.3, 138.1, 137.5, 136.9, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 96.4, 76.6, 76.5, 73.3, 72.2, 73.2, 70.1, 69.3, 68.0, 54.5. HRMS *m/z*: $[M+Na]^+$ calcd for: $C_{35}H_{36}O_6Na$, 575.2410; Found 575.2403.

4.2.5. Benzyl 2,3,6-*tri*-*O*-benzyl-4-deoxy-4-*C*-formyl-α-D-glucopyranoside (9)

A solution of **8** (0.69 g, 1.25 mmol) in Et_3N (10 mL) was stirred at 38 °C for 12 h. The reaction mixture was concentrated in vacuo and crude reaction mixture purified (hexane/EtOAc = 1:8) to afford **9** (0.55 g, 79%), as a gummy oil. R_f = 0.30 (9:1 hexane/EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.65 (d, 1H, J = 2.6 Hz, C*H*O), 7.40–7.25 (band, 20H, aromatic), 4.93 (d, 1H, J = 10.8 Hz, PhCH_2), 4.87 (d, 1H, J = 3.4 Hz, H-1), 4.73–4.43 (m, 7H, PhCH_2), 4.31 (dd, 1H, J = 9.4, 10.8 Hz, H-3), 4.13–4.06 (m, 1H, H-5), 3.59 (dd, 1H, J = 3.4, 9.4 Hz, H-2), 3.51 (d, 2H, J = 4.2 Hz, H-$6_{a,b}$), 3.0 (app. dt, 1H, J = 2.6, 10.8 Hz, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.4, 138.2, 137.9, 137.6, 137.1, 128.4, 128.0, 127.8, 127.7, 95.7, 80.6, 75.4, 75.2, 73.5, 72.6, 70.2, 69.2, 67.4, 56.7. HRMS *m/z*: $[M+Na]^+$ calcd for: $C_{35}H_{36}O_6Na$, 575.2410; found 575.2422.

4.2.6. Benzyl 2,3,6-*tri*-*O*-benzyl-4-*C*-hydroxymethyl-α-D-glucopyranoside (10)

$NaBH_4$ (0.05 g, 1.4 mmol) was added to a solution of **9** (0.55 g, 0.99 mmol) in MeOH (18 mL) at 0 °C and stirred for 2 h. Solvents were removed in vacuo and the resulting residue dissolved in EtOAc (3 × 45 mL), diluted with H_2O (100 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was purified (hexane/EtOAc = 4:1) to afford **10** (0.49 g, 89%), as a colorless oil. R_f = 0.39 (4:1 hexane/EtOAc); $[\alpha]_D$ +49.1 (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42–7.27 (band, 20H, aromatic), 5.0 (d, 1H, J = 10.8 Hz, PhCH_2), 4.88 (d, 1H, J = 3.6 Hz, H-1), 4.72–4.45 (m, 7H, PhCH_2), 3.97 (dd, 1H, J = 9.6, 10.4 Hz, H-3), 3.91–3.87 (m, 1H, H-5), 3.70 (dd, 1H, J = 3.6, 11.4 Hz, CH_2^aOH), 3.61–3.58 (m, 4H, H-2, CH_2^bOH, H-$6_{a,b}$), 1.91–1.85 (m, 1H, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.5, 138.1, 137.6, 137.3, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 95.6, 81.4, 75.7, 75.2, 73.5, 72.3, 70.5, 68.8, 68.6, 59.7, 46.2. HRMS *m/z*: $[M+Na]^+$ calcd for: $C_{35}H_{38}O_6Na$, 577.2566; Found 577.2572.

4.2.7. (4-Deoxy-4-methyl-(1,2,3,6-*tetra*-*O*-benzyl-α-D-glucopyranosyl)-(2,3,4,6-*tetra*-*O*-acetyl)-β-D-glucopyranoside (protected derivative of 1)

A mixture of **10** (0.16 g, 0.28 mmol), $Hg(CN)_2$ (0.11 g, 0.43 mmol), $HgBr_2$ (0.10 g, 0.28 mmol), and molecular sieves 4 Å (1 g) in CH_2Cl_2 (20 mL) was stirred at room temperature under N_2 atmosphere. After 10 min, acetobromo glucose[27] (0.29 g, 0.72 mmol) in CH_2Cl_2 (15 mL) was added dropwise and stirred for 12 h at room temperature, under N_2 atmosphere. The reaction mixture was neutralized with Et_3N (1 mL), filtered through celite pad, filtrate washed with H_2O (150 mL) and aq. $Na_2S_2O_3$ (10%). The organic layer was dried (Na_2SO_4) concentrated in vacuo and purified (hexane/EtOAc = 1:4) to afford protected derivative of **1** (0.18 g, 68%), as an oil. R_f = 0.23 (hexane/EtOAc = 3:1); $[\alpha]_D$ +34.9 (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43–7.25 (band, 20H, aromatic), 5.17–4.93 (band, 6H), 4.89 (d, 1H, J = 3.6 Hz, H_α-1), 4.71 (q, 1H, J = 12.0 Hz), 4.63 (dd, 2H, J = 2.6, 12.0 Hz), 4.53 (q, 1H, J = 6 Hz), 4.48–4.45 (m, 2H), 4.30 (d, 1H, J = 8.0 Hz, H_β-1′), 4.21–4.16 (m, 2H), 4.06–4.03 (m, 1H), 3.99 (dd, 1H, J = 2.6, 12.8 Hz), 3.94 (t, 1H, J = 3.0 Hz), 3.59–3.50 (m, 3H), 3.38 (dd, 1H, J = 2.6, 10.0 Hz), 2.01 (s, 3H), 1.99 (s, 3H), 1.98–1.97 (m, 1H, H-4), 1.95 (s, 3H), 1.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6, 170.2, 169.3, 168.7, 138.9, 138.1, 138, 137.2, 128.5, 128.4, 128.3, 127.9, 127.8, 127.6, 100.6, 95.7, 81.3, 75.5, 75.3, 73.4, 72.7, 72.6, 71.7, 71.2, 69.6, 68.8, 68.6, 68.3, 65.4, 61.8, 43.8, 20.7, 20.5, 20.3, 20.2. HRMS *m/z*: $[M+Na]^+$ calcd for: $C_{49}H_{56}O_{15}Na$, 907.3517; found 907.3511.

4.2.8. (4-Deoxy-4-methyl glucopyranosyl)-β-D-glucopyranoside (1)

A solution of protected derivative of **1** (0.18 g, 0.19 mmol) in MeOH (10 mL) was admixed with NaOMe in MeOH (0.3 mL,

0.5 M), stirred for 6 h, neutralized with amberlite IR-120 resin (H^+) and solvents evaporated in vacuo. The crude residue was dissolved in MeOH/EtOAc (1:1, 30 mL), added with Pd/C (10%, 0.11 g) and stirred under a positive pressure of H_2 gas for 2 days, filtered through celite and solvents evaporated in vacuo to afford **1** (0.068 g, 90%, α/β = 1:1.5), as a foamy solid; R_f = 0.18 (6:2:1 $CHCl_3$/MeOH/H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 5.19 (d, 1H, J = 3.6 Hz, H_α-1), 4.51 (d, 1.5H, J = 7.8 Hz, H_β-1), 4.35 (d, 2.5H, J = 8.0 Hz, H_β-1′), 4.06–4.03 (m, 2.5H) 3.85 (ddd, 5H, J = 2, 4.4.0, 12.0 Hz), 3.80–3.56 (m, 10H), 3.50–3.38 (m, 5H), 3.44–3.38 (m, 2.5H), 3.32 (dt, 2.5H, J = 1.6, 10.4 Hz), 3.21 (dt, 2.5H, J = 1.6, 9.6 Hz), 3.17 (q, 2.5H, J = 7.2 Hz), 1.76–1.71 (m, 2.5H, H-4); ^{13}C NMR (D_2O, 100 MHz) δ 102.4, 102.3, 95.7, 75.8, 75.6, 75.5, 74.2, 72.9, 72.6, 72.5, 70.5, 69.6, 69.5, 66.9, 65.8, 65.7, 61.4, 60.6, 46.5, 43.2. HRMS *m/z*: $[M+Na]^+$ calcd for: $C_{13}H_{24}O_{11}Na$ = 379.1216; found 379.1226.

4.2.9. (4-Deoxy-4-methyl (1,2,3,6-*tetra*-*O*-benzyl-α-D-glucopyranosyl)-(2,3,4,6-*tetra*-*O*-acetyl)-β-D-galactopyranoside (protected derivative of 2)

Glycosylation of **10** (0.19 g, 0.34 mmol) with acetobromo galactose[27] (0.35 g, 0.85 mmol) in CH_2Cl_2 (25 mL) in presence of $Hg(CN)_2$ (0.13 g, 0.51 mmol), $HgBr_2$ (0.12 g, 0.34 mmol) and molecular sieves 4 Å (1 g) was performed as described above for the synthesis of protected derivative of **1**. Purification (hexane/EtOAc = 3:1) of reaction mixture afforded protected derivative of **2** (0.2 g, 66%), as an oil. R_f = 0.2 (hexane/EtOAc = 4:1); $[\alpha]_D$ +32.5 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43–7.25 (band, 20H, aromatic), 5.34 (dd, 1H, J = 1.0, 3.6 Hz), 5.13 (dd, 1H, J = 8.0, 10.4 Hz), 4.99 (d, 1H, J = 10.8 Hz, PhCH_2), 4.94 (dd, 1H, J = 3.6, 10.4 Hz,), 4.89 (d, 1H, J = 3.6 Hz, H_α-1), 4.77–4.44 (band, 7H, PhCH_2), 4.26 (d, 1H, J = 8.0 Hz, H $_\beta$-1′), 4.20 (dd, 1H, J = 2.8, 9.6 Hz), 4.12–3.96 (m, 4H), 3.77 (dt, 1H, J = 1.0, 7.2 Hz,), 3.59 (dd, 1H, J = 2.4, 10.4 Hz), 3.56 (d, 1H, J = 3.6 Hz) 3.53 (t, 1H, J = 3.4 Hz), 3.40 (dd, 1H, J = 2.4, 9.6 Hz), 2.14 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 1.94–1.93 (m, 1H, H-4), 1.89 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4, 170.3, 170.1, 168.8, 139.0, 138.2, 138.0, 137.3, 128.5, 128.3, 127.9, 127.8, 127.6, 101.0, 95.4, 81.3, 75.4, 75.2, 72.4, 72.3, 70.8, 70.5, 69.6, 68.8, 68.7, 66.5, 66.9, 65.3, 61.2, 43.7, 20.7, 20.6, 20.5, 20.4. HRMS *m/z*: $[M+Na]^+$ calcd for: $C_{49}H_{56}O_{15}Na$, 907.3517; found 907.3514.

4.2.10. (4-Deoxy-4-methyl glucopyranosyl)-β-D-galactopyranoside (2)

A solution of protected derivative of **2** (0.2 g, 0.22 mmol) in MeOH (15 mL) was admixed with NaOMe/MeOH (0.4 mL, 0.5 M), the reaction mixture stirred for 6 h, neutralized with amberlite IR-120 resin (H^+), solvents evaporated in vacuo. The crude residue was dissolved in MeOH/EtOAc (1:1, 30 mL), added with Pd/C (10%, 0.11 g) and stirred under a positive pressure of H_2 gas for 2 days, filtered through Celite and solvents evaporated in vacuo to afford **2** (0.07 g, 88%, α/β = 1:2), as a foamy solid. R_f = 0.20 ($CHCl_3$/MeOH/H_2O = 6:2:1); ^{1}H NMR (D_2O, 400 MHz) δ 5.08 (d, 1H, J = 3.2 Hz, H_α-1), 4.41 (d, 2H, J = 8.0 Hz, H_β-1), 4.17 (d, 3H, J = 7.8 Hz, H_β-1′), 3.98–3.92 (m, 6H), 3.74–3.72 (m, 9H), 3.65–3.45 (m, 15H), 3.39–3.31 (m, 6H), 3.07–3.03 (t, 3H, J = 8.8 Hz), 1.61–1.59 (m, 3H, H-4); ^{13}C NMR ($CDCl_3$,100 MHz) δ 103.0, 102.9, 95.8, 92.3, 75.5, 75.1, 74.3, 72.6, 72.5, 72.4, 70.6, 70.5, 68.8, 68.6, 67.0, 65.7, 61.5, 61.0, 43.3, 43.2. HRMS *m/z*: $[M+Na]^+$ calcd for: $C_{13}H_{24}O_{11}Na$ = 379.1216; found 379.1208.

4.2.11. (4-Deoxy-4-methyl-(1,2,3,6-*tetra*-*O*-benzyl-α-D-glucopyranosyl)-(2,3,4,6-*tetra*-*O*-benzyl)-α-D-glucopyranoside (protected derivative of 3)

A mixture of **10** (0.15 g, 0.27 mmol) and 2,3,4,6-*tetra*-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate[28] (0.25 g, 0.36 mmol) and TMSOTf (6 μL, 0.027 mmol, 0.1 equiv) in Et_2O (15 mL) was stirred for 30 min., at room temperature, the reaction mixture was then quenched with Et_3N (1 mL), solvents removed in vacuo and the residue purified (hexane/EtOAc = 7.5:1) to afford protected compound of **3** (0.198 g, 68%), as an oil. R_f = 0.4 (hexane/EtOAc = 3:0.5); $[\alpha]_D$ +34.9 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31–7.13 (band, 40H, aromatic), 4.92 (d, 1H, J = 3.6 Hz, H_α-1′), 4.9–4.51 (m, 16H, PhCH_2), 4.48–4.29 (m, 6H), 4.22–4.17 (m, 1H), 3.89 (t, 1H, J = 9 Hz), 3.75–3.67 (m, 2H), 3.61–3.54 (m, 2H), 3.50 (dd, 1H, J = 3.6, 9.8 Hz), 3.40–3.33 (m, 1H), 2.13–2.04 (m, 1H, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 138.9, 136.6, 138.3, 138.2, 138.1, 137.9, 137.5, 137.3, 128.2, 127.9, 127.8, 127.6, 127.5, 127.5, 127.4, 127.3, 97.8, 96.1, 83.9, 82.1, 81.3, 79.5, 77.7, 75.5, 75.2, 75.0, 73.5, 73.2, 73.0, 72.5, 70.7, 69.7, 69.3, 69.0, 68.3, 64.6, 43.2. HRMS *m/z*: $[M+Na]^+$ calcd for: $C_{69}H_{72}O_{11}Na$ = 1099.4972; found 1099.4978.

4.2.12. (4-Deoxy-4-methyl glucopyranosyl)-α-D-glucopyranoside (3)

A solution of protected derivative of **3** (0.19 g, 0.17 mmol) in MeOH and EtOAc (1:1 v/v, 35 mL) was hydrogenolyzed over Pd/C (10%, 0.08 g) at room temperature under positive pressure of H_2 gas for 3 days, filtered through Celite and evaporated in vacuo, to afford **3** (0.057 g, 91%, α/β = 1:1.8), as a foamy solid. R_f = 0.4 ($CHCl_3$/MeOH/H_2O = 6:3:1); ^{1}H NMR (D_2O, 400 MHz): δ 5.0 (d, 1H, J = 3.6 Hz, H_α-1′), 4.71 (d, 2.8H, J = 3.6 Hz, H_α-1), 4.43 (d, 1.8H, J = 8.0 Hz, H_β-1), 3.76–3.51 (m, 18.4H), 3.43–3.37 (m, 8H), 3.34–3.32 (m, 7.2H), 3.31–3.10 (m, 2.8H), 1.62–1.58 (m, 2.8H, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 103.1, 98.7, 95.7, 75.9, 75.7, 74.6, 74.1, 72.9, 72.5, 72.2, 71.5, 70.6, 70.4, 69.8, 69.6, 63.7, 61.5, 60.5, 48.8, 43.3. HRMS *m/z*: $[M+Na]^+$ calcd for: $C_{13}H_{24}O_{11}Na$ = 379.1216; found 379.1218.

4.3. Kinetic studies of hydrolysis as monitored by ^{1}H NMR spectroscopy

Kinetic studies of acid-catalyzed hydrolysis of **1–3**, **11–16** (5–6 mg) were performed in DCl/D_2O (2 N) (600 μL), in an NMR tube maintained at 60 ± 1 °C and 70 ± 1 °C, in a thermostated water bath. ^{1}H NMR spectrum was recorded periodically by monitoring the peak intensities of either H-4 proton of 4-*C*-hydroxymethyl glucopyranose formed from **1** to **3** or anomeric proton (**11–16**) of gluco- or galactopyranoside, resulting from hydrolysis. Integration of chemical shift at 1.45 ppm, corresponding to H-4 nucleus of 4-*C*-hydroxymethyl glucopyranose formed upon hydrolysis in **1–3**, was considered for rate constant studies. Average integrations of chemical shifts, corresponding to anomeric H-1 of gluco- or galactopyranose formed upon hydrolysis, were considered for rate constant analysis: **11**, **13**, **14,** and **16**: 4.33 and 3.78 ppm (α- and β-glucopyranose); **12** and **15**: 4.56 and 3.94 ppm (α- and β-galactopyranose). ^{1}H NMR peak intensity profiles as a function of time were secured till complete hydrolysis, plotted and the plots were fit to a first-order exponential decay function, from which first-order rate constants (k) were derived. The fitting curves of all hydrolysis rates showed correlation coefficients (r^2) more than 0.999.

4.4. Molecular modeling procedure

Molecular modeling studies were performed using MacroModel 8.0 software,[36] running on a SGI-Irix machine. The molecules were built using standard template present in the software, in their respective geometries before initiating the minimization. The AMBER* force field was chosen for the minimization, in water using GB/SA continuum solvent model approach and a dielectric constant of 80. Optimizations were performed with the Polak–Ribiere algorithm and the termination condition was RMS gradient

of 0.05 kcal mol^{-1} or a maximum of 10,000 iterations. Monte-Carlo search was conducted for each molecule by varying the dihedral angle around the glycosidic linkage. All optimized conformers within 3 kcal mol^{-1} of the global minimum were saved. The lowest 10 conformers from the conformational search were further optimized at B3LYP/6-31G* level theory in gas phase, using G09 software.[37] The lowest energy conformer from ab initio calculations was used to find out bond lengths and bond angles.

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Supplementary data

Supplementary data (kinetic plots of compounds) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.08.030.

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