Bending Trisaccharides by a Chelation-Induced Ring Flip of a Hinge-Like Monosaccharide Unit

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Chelation-induced conformational change of polypeptides is a useful strategy in de novo protein design,[1] which ultimately aims at the construction of artificial proteins with tailor-made structures and functionalities. Employing this strategy with oligosaccharides may lead to the development of novel sugar-based architectures. Although the addition of metal ions to solutions of some natural polysaccharides causes coil−helix transitions[2,3] and some monosaccharides undergo shifts in the ring conformation equilibrium by the addition of metal ions,[2,4] the extent to which metal ions influence these conformational properties is usually small, and no extraordinary conformations are created.

In this study, we created a novel turn structure of the trisaccharides **2** and **3** by a hinge-like $^4C_1$-to-$^1C_4$ ring flip of the 2,4-diamino-2,4-dideoxy-β-D-xylopyranoside unit (Figure 1). This ring flip was enabled by the flexible ring structure[5] and the strong chelating ability of diamino groups in the 1,3-diaxial orientation.[6] Conformational behavior of the methyl glycoside **1** and that of the hinge unit on addition of metal ions were investigated by ^{1}H NMR.

The methyl glycoside **1** was synthesized in five steps from the known compound **4**[7] (Scheme 1). The methods for synthesizing 2,4-diazido-2,4-dideoxy derivatives of glucopyranoside[8] and α-xylopyranoside[9] were employed for the synthesis of the key intermediate **7**. Birch reduction of **7** gave the compound **1**. The trisaccharides **2** and **3** were designed to mimic the trisaccharide sequence (Galβ1−3GlcNAcβ1−2Manα)[10] of complex *N*-linked glycans, in which the central sugar, GlcNAc, is replaced with the hinge unit. Introduction of the mannose units at the reducing end of **1** was accomplished by conversion of **7** into the 1-*O*-trichloroacetimidate **10** and then conversion of **10** by the Schmidt method[11] to give disaccharides **11** and **12**. Galactose residues were incorporated into **11** and **12** by the trichloroacetimidate method to give trisaccharides **13** and **14**, which were subjected to deprotection and reduction of the azido groups to give the desired trisaccharides **2** and **3**.

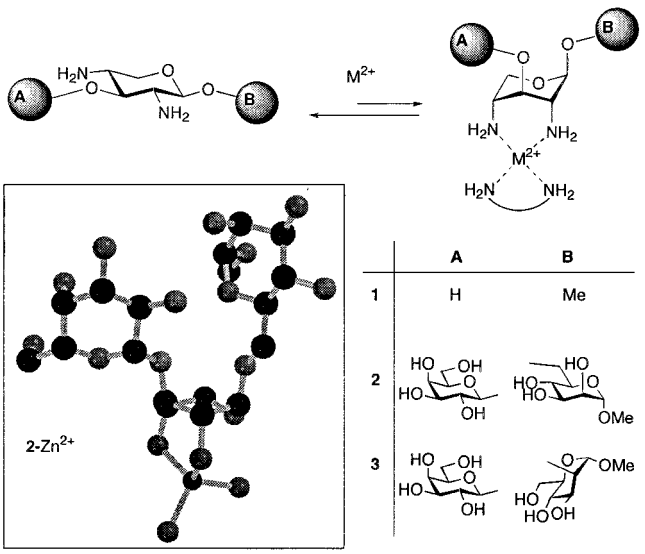
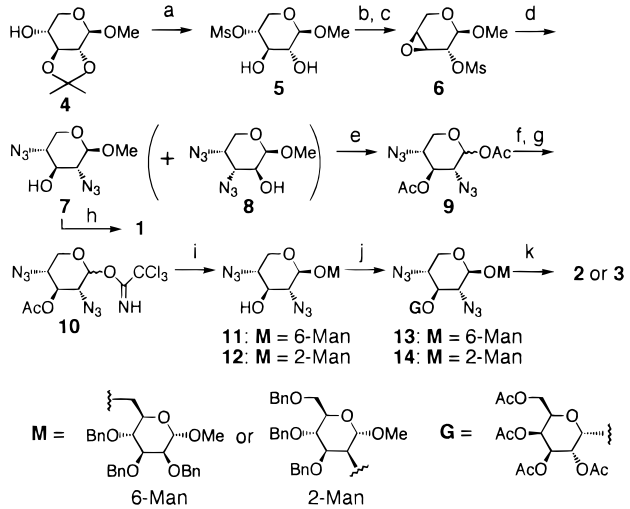


Figure 1. Structures of hinge sugar **1** and the hinged trisaccharides **2** and **3**. Upon chelation of a metal ion (Hg^{2+} or Zn^{2+}), a ring flip of the hinge unit is induced to give the turn structure shown in the upper right. In the frame is the global minimum of the turn structure of **2**−Zn^{2+}, preliminarily calculated by molecular dynamics simulation (MM2 force field, 300 K); all hydrogen atoms are omitted for clarity.

Scheme 1[a]



[a] (a) MsCl, pyr; then 88% AcOH, 80%. (b) NaOMe. (c) MsCl, pyr, 90% in 2 steps. (d) NaN_3, Bu_4NBr, toluene−H_2O (1:1), 140 °C, **7**, 51%; **8**, 21%. (e) Ac_2O, H_2SO_4, 89%. (f) H_2NNH_2·AcOH, DMF, 50 °C. (g) Cl_3CCN, Cs_2CO_3, $ClCH_2CH_2Cl$, 58% in 2 steps. (h) Na, liq. NH_3, 88%. (i) **M**−OH, TMSOTf, CH_3CN, −40 °C; then NaOMe. **11**, 63%; **12**, 53% (α-isomer: 23%). (j) **G**−OC(=NH)CCl_3, BF_3·OEt_2, MS4A, CH_2Cl_2, −78 °C → rt. **13**, 71%; **14**, 86%. (k) NaOMe; then H_2S, pyr−H_2O (1:1); then Na, liq. NH_3, −78 °C. **2**, 50%; **3**, 63%.

The ^{1}H NMR spectrum of **1** showed the *J*-values characteristic of the $^4C_1$ chair conformation (Figure 2a), and these did not change within the temperature range 298−353 K. Addition of diamagnetic metal ions, $Hg(OAc)_2$ and $Zn(OAc)_2$, caused a line-broadening at 298 K (Figure 2b). This line-broadening can be explained by the relatively slow exchange process between different structures when there are more than two structures, because acceleration of the process by increasing the temperature up to 348 K resulted

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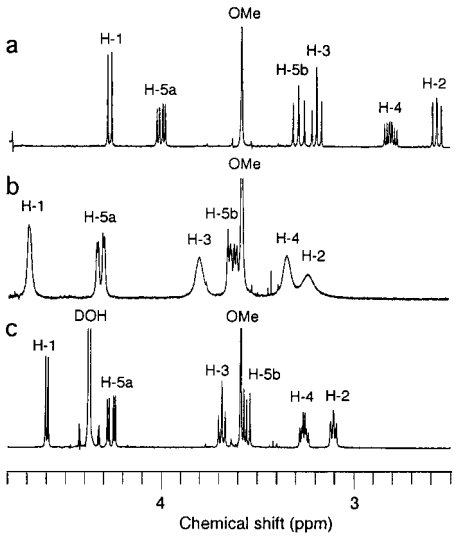

Figure 2. ^{1}H NMR spectra (400 MHz) of compound **1** (26 mM) in the absence and presence of $Hg(OAc)_2$ (0.5 equiv) in 50 mM AcONa-d_3 buffer (pH 7.0). Methyl signals were adjusted to 3.584 ppm. (a) **1** at 298 K. (b) **1** with $Hg(OAc)_2$ at 298 K. (c) **1** with $Hg(OAc)_2$ at 348 K.

Table 1. *J*-Values (Hz)[a] for the Vicinal Ring Protons of the Xylose Unit and the Calculated $^1C_4$ Populations (%) of Compounds **1**, **2**, **3**, and Their Complexes with Metal Ions[b]

compd	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5a}$	$J_{4,5b}$	$^1C_4$
1	8.1	9.6	9.6	5.1	10.7	0
1−Hg^{2+}	5.0	6.4	6.6	4.0	6.9	41
1−Zn^{2+}	5.6	7-8[c]	7.5	4.0	7.5	33
2	8.2	9.6	9.5	5.2	10.8	0
2−Hg^{2+}	5.3	6.9	7.2	4.3	7.0	39
2−Zn^{2+}	6.1	8.1	8.1	4.0	9.0	21
3	8.1	9.8	9.6	5.3	10.8	0
3−Hg^{2+}	7.2	8.7	9.0	4.7	9.0	17
3−Zn^{2+}	6.6	7.9	8.1	4.5	8.5	23

[a] 400 MHz NMR at 70−80 °C. [b] Hg^{2+}, 0.5 equiv; Zn^{2+}, 1−3 equiv. [c] Unclear assignment due to slight line-broadening at H-2 and H-4 signals.

in sharp signals that are indicative of time-averaged resonances of each proton (Figure 2c). The line-broadening may be due, in part, to the restricted motions of the whole molecule, if the complex is extremely large.[12]

The *J*-values at 348 K for the mixture of **1** and 0.5 equiv of $Hg(OAc)_2$ are significantly smaller than those for **1** only (Table 1), suggesting structural deformation by chelation. A similar tendency was observed when 1.4 equiv of $Zn(OAc)_2$ were added to **1**. To estimate the conformations of **1** in the presence of these metal ions, least-squares fitting of the *J*-values, computed for all possible conformers, to the observed *J*-values was performed.[13,14] The calculation indicated that, in the presence of metal ions, **1** exists as an equilibrium mixture of the $^4C_1$ and $^1C_4$ conformations only. The $^1C_4$ populations are listed in Table 1. The partial formation of the $^1C_4$ conformation is most likely due to an intramolecular chelation bridge between the two axially oriented amino groups. Indeed, when the amino group at C-4 of **1** was replaced with an acetamido group, metal ion additions caused no signal broadening or decreases in the *J*-values in ^{1}H NMR spectra. Moreover, the titration of **1** with the metal ions afforded hyperbolic curves for the chemical shifts,[16] from which a 2:1 stoichiometry of the **1**−metal ion complex was also deduced.

The ^{1}H NMR spectra of the trisaccharides **2** and **3** showed that the hinge sugar unit maintains a $^4C_1$ chair conformation within the temperature range 298−353 K. Addition of 0.5 equiv Hg^{2+} or 1−3 equiv Zn^{2+} ion caused a line-broadening for the hinge sugar unit at 298 K in a manner similar to that of **1**. On the other hand, signals for Gal and Man underwent comparatively slight line-broadening. The difference in the extent of line-broadening suggests that the deformation of the ring structure occurs only at the hinge sugar unit and this ring flip permits a harmonic movement of the peripheral sugar units. Indeed, decreases of the *J*-values at 353 K compared with those for **2** or **3** was observed only for the hinge sugar unit. The populations of the $^1C_4$ conformation of the hinge sugar unit of **2** or **3** that complexes with a metal ion, estimated from the *J*-values, are shown in Table 1. These results indicate that the trisaccharides **2** and **3** were obviously bent to afford turn structures by the addition of the metal ions. There is likely to be an attractive force such as the van der Waals force between Gal and Man in the turn structure of **3**, because the disaccharide formed by removing Gal from **3** hardly underwent a ring flip at the hinge sugar ($^1C_4$ < 5%) on addition of metal ions.

In conclusion, by adding metal ions, we were able to bend trisaccharides having a hinge diaminosugar unit in the center. In analogy with this hinge sugar, the equilibrium between the $^1C_4$ and $^2S_0$ conformations of the iduronic acid unit of heparin shifts toward the $^1C_4$ conformation on addition of calcium chloride.[16] However, the interconversion of the conformations of the iduronic acid unit hardly affect the end-to-end distance of the heparin chain.[13] In this respect, the hinged trisaccharides dramatically change their shape from straight to bent. This flexibility and the novel turn structure may be applied to the fabrication of novel artificial architectures containing oligosaccharide building blocks.

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Supporting Information Available: Experimental procedures and full characterization of the reported compounds. Plots of the $^1C_4$ populations and chemical shifts of **1** as a function of amounts of added metal ions. 400 MHz ^{1}H NMR spectra of **2** and **2**−Zn^{2+} (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Molecular dynamics simulation was carried out for the compounds **1** and **1**−Zn^{2+} (MM2 force field, 1000 K) by Cache 3.9 software (Oxford Molecular Group, Inc.). The generated ring structures ($^4C_1$, $^1C_4$, $^2S_0$, $^3S_1$, ^{03}B) that exceed 1 ppm in population were further optimized by MM2, and the *J*-values were calculated by the generalized Karplus equation.[15] The least-squares fitting of the calculated *J*-values to the observed ones was performed by Sigma Plot 5.0 software (Jandel Corporation).

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