DOI: 10.1002/ejoc.200900148

The Observation of the C–H···O$^{sp^3}$ Hydrogen Bond in Trisialic Acid Lactone and Its Implications for Cooperative Lactonization

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Keywords: Sialic acids / Lactones / Hydrogen bonds / Ab initio calculations / NMR spectroscopy

The C–H···O$^{sp^3}$ hydrogen bond in trisialic acid lactones has been examined by using long-range COSY (LRCOSY) and ab initio calculations. From an analysis of the LRCOSY spectra, ^3J correlations of $^{B}H^{9}_{ax}$/$^{A}H^{8}$ and $^{C}H^{9}_{ax}$/$^{B}H^{8}$ confirmed the existence of hydrogen-bond connections of $^{x+1}C^{9}$–$^{x+1}H^{9}_{ax}$···$^{x}O^{8}$. The theoretical bond energy of the hydrogen bond was estimated to be 1.0 or 1.6 kcal/mol by using two models. The acid-catalyzed cooperative lactonization of oligosialic acids can be understood in terms of the additional C–H···O$^{sp^3}$ stabilization in the lactone product.

Introduction

The acid-catalyzed lactonization of oligo- or polysialic acids [OSA/PSA, α2,8-(NeuAc)$_n$, n = 3–5] has been studied by using high-performance capillary electrophoresis[1–6] (Figure 1). Previous work has shown that this stepwise lactonization is cooperative: once a δ-lactone forms between two saccharides in OSA, the formation of the adjacent δ-lactone is accelerated and successive reactions result in complete lactonization of OSA/PSA. From these studies, a secondary structure of OSA or PSA lactone that allows for increasing cyclization rates has been speculated.[7,8] Cooperative stabilization in the biopolymer has been ascribed to a structural driving force from either O–H···O or N–H···O hydrogen bonds.[9,10] In sialic acid monosaccharide, one carboxylate and five hydroxy groups can form quite a few conventional hydrogen bonds.[11,12] The formation of OSA and PSA and their subsequent lactonization reduces the number of exocyclic torsional degrees of freedom from four to two, and the number of hydroxy groups from five to two, and therefore the numbers of possible conventional hydrogen bonds in the final lactone product are greatly reduced. Consequently, C–H···O hydrogen bonds arise from the more rigid lactone product, which can be considered to be the main force driving cooperative lactonization. On the other hand, the effect of the C–H···O hydrogen bond has been shown to be a factor determining the enantioselectivity of Lewis acid catalyzed reactions of aldehydes.[13–15]

The existence of a hydrogen bond is usually inferred after structure determination by X-ray diffraction[16–19] or NMR spectra[20,21] from the relative orientation of the hydrogen-bonding donor and acceptor pair. In NMR spectroscopy, the temperature coefficient of the chemical shift and the hydrogen-exchange property have often been used as indicators of hydrogen bonds, after their incorporation as constraints in structural simulations. Although hydrogen bond XH···O–CH ^{3h}J coupling constants are usually too small (<0.5 Hz) to be examined as splittings in ^{1}H NMR spectra,[10,22] weak correlations can still be observed in a COSY-like experiment with an extra delay time, known as a LRCOSY experiment.[23–25] In the work reported herein, we observed a C–H···O–CH ^{3h}J correlation of the hydrogen-bond donor and acceptor in trisialic acid lactone using a selective 1D LRNMR pulse sequence qualitatively and used it to explain the possible role of the C–H···O hydrogen bond in the cooperative stabilization of the OSA/PSA lactone.

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200900148.

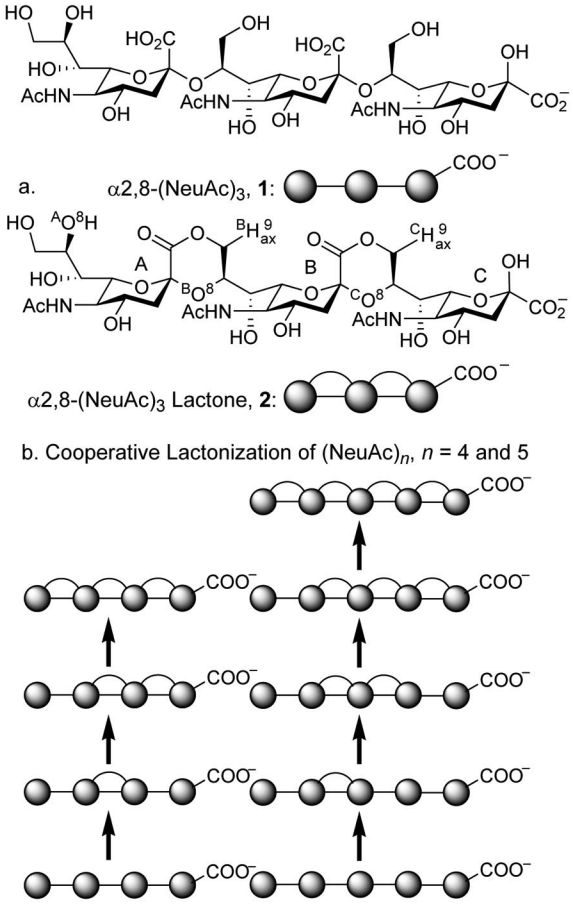

Figure 1. (a) Structures of the α2,8-(NeuAc)$_3$ lactones **1** and **2**. The monosaccharide NeuAc is represented as a sphere and the δ-lactone is represented as a curve between two adjacent spheres. The α2,8-(NeuAc)$_3$ lactone **2** consists of three spheres and two curves, and the reducing terminus is always on the right-hand side. (b) Cooperative lactonization of α2,8-(NeuAc)$_n$ (n = 4 and 5). Once a δ-lactone is formed between two monosaccharide residues in glacial acetic acid, the vicinal carboxylic acid tends to react to form another six-membered δ-lactone.

Results and Discussion

We have previously reported the helical structure of OSA/PSA lactones with a rotation angle of 240° per residue, with 1.5 residues per turn, with each lactone ring in a skewed twist–boat $^{C2}S^{C9}$ conformer.[26] With this helical structure, we attempted to explore the formation of possible hydrogen bonds. The geometrical criteria of the C–H···O hydrogen bond in the carbohydrates employed are as follows:[19,27] 1) A C–H···O angle greater than 90° and 2) a H···O distance less than the van der Waals contact distance of 2.7 Å. We found that an inter-residue $^{x+1}C^9$–$^{x+1}H^9_{ax}$···$^xO^8$ hydrogen bond (x = A, B, C) might exist in OSA lactone. Another hydrogen bond is the intra-residue hydrogen bond $^xO^{10}$···^xH–$^xO^7$. We studied the latter hydrogen bond in **2** through a molecular simulation (see the Supporting Information), and the result was similar to previous reports[11] for OSA.

The C–H···O hydrogen bonds of the α2,8-(NeuAc)$_3$ lactone **2** were detected by 2D LRCOSY (Figure 2). The observed inter-residue ^{3h}J correlations of $^BH^9_{ax}$/$^AH^8$ and $^CH^9_{ax}$/$^BH^8$ confirmed the anticipated existence of weak $^{x+1}C^9$–$^{x+1}H^9_{ax}$···$^xO^8$ hydrogen bonds, in this case the $^BC^9$–$^BH^9_{ax}$···$^AO^8$ and $^CC^9$–$^CH^9_{ax}$···$^BO^8$ connections, respectively. The LRCOSY spectra also provided evidence for the ^4J correlations ($^xH^9_{ax}$–$^xC^9$–$^xC^8$–$^xC^7$–$^xH^7$) of residues B and C. Relative to the hydrogen-bond cross-peak between two residues, the intensities of the inter-residue ^4J correlations of $^BH^9_{ax}$/$^BH^7$ and $^CH^9_{ax}$/$^CH^7$ was much stronger. In addition, the ^4J cross-peaks are triplets in the F2 projection and are split by passive couplings[28,29] from H^9_{eq} and H^8. The polarization from H^9_{ax} to H^7 is modulated by the H^9_{eq} and H^8 magnetizations, and both contribute equally a coupling of 11 Hz to the ^4J cross-peak. This result could be applied to the ^{3h}J couplings of $^{x+1}H^9_{ax}$/$^xH^8$ and $^xH^9_{ax}$/$^xH^8$, which were observed as twice the intensity owing to the larger passive couplings from $^xH^9_{eq}$.

In a typical 2D COSY assignment, a spin system can be assigned from diagonal line to cross-peak. However, a wrong assignment might arise from differentiation of the overlapping chemical shifts from either the nuclear sequencing or the through-space correlation. Moreover, the distinguishable contour must be cut at a higher level as the weaker hydrogen bond ^{3h}J correlation and the cross-peak overlapping with the diagonal of the 2D LRCOSY would be observed as a broad intensity band. In this case, the 1D LRCOSY spectrum can be used to further confirm the existence of the weak hydrogen bond ^{3h}J correlation (Figure 3). In a typical ^{1}H NMR experiment, the distinguishable proton signals of the sialyl lactone **2** were selected as irradiated frequencies using an 80 ms delay in selective 1D LRNMR spectroscopy. Starting from the irradiated $^CH^9_{ax}$ signal (Figure 3, d), 1D LRCOSY can provide the geminal $^CH^9_{ax}$/$^CH^9_{eq}$, vicinal $^CH^9_{ax}$/$^CH^8$, long-range $^CH^9_{ax}$/$^CH^7$ and $^CH^9_{ax}$/$^CH^6$, and hydrogen-bonded $^CH^9_{ax}$/$^BH^8$ couplings. The assignment of $^BH^8$ from $^CH^8$ can be achieved by comparison of the residual spin-correlation in selective 1D TOCSY starting from irradiated $^BH^9_{ax}$ and $^CH^9_{ax}$ in Figure 3 (c and e). Therefore, the corresponding $^CH^9_{ax}$/$^BH^8$ signal in Figure 3 (f) is an asymmetric signal on the right side of the vicinal $^CH^9_{ax}$/$^CH^8$ coupling and can be differentiated as the ^{3h}J correlation. In the same way, from the irradiated $^CH^9_{ax}$ signal in Figure 3 (b), the long-range experiment can provide the geminal $^BH^9_{ax}$/$^BH^9_{eq}$, vicinal $^BH^9_{ax}$/$^BH^8$, long-range $^BH^9_{ax}$/$^BH^7$, and hydrogen-bonded $^BH^9_{ax}$/$^AH^8$ couplings. The $^BH^9_{ax}$/$^AH^8$ ^{3h}J correlation is distinguished from the residual $^BH^9_{ax}$/$^BH^6$ correlation by comparison of the different chemical shifts, which are 3.296 and 3.268 ppm for $^AH^8$ and $^BH^6$, respectively.

Provided by the observed hydrogen bonds, two additional distance restraints of $^BH^9_{ax}$···$^AO^8$ and $^CH^9_{ax}$···$^BO^8$ in the range 2.0–2.7 Å were combined with the NOE-based distance restraints[26] of the α2,8-(NeuAc)$_3$ lactone **2** for use in simulations performed with the annealing program Xplor NIH[30] to generate 100 different structures (see Table S1 in the Supporting Information). Of these, 10 candidates that

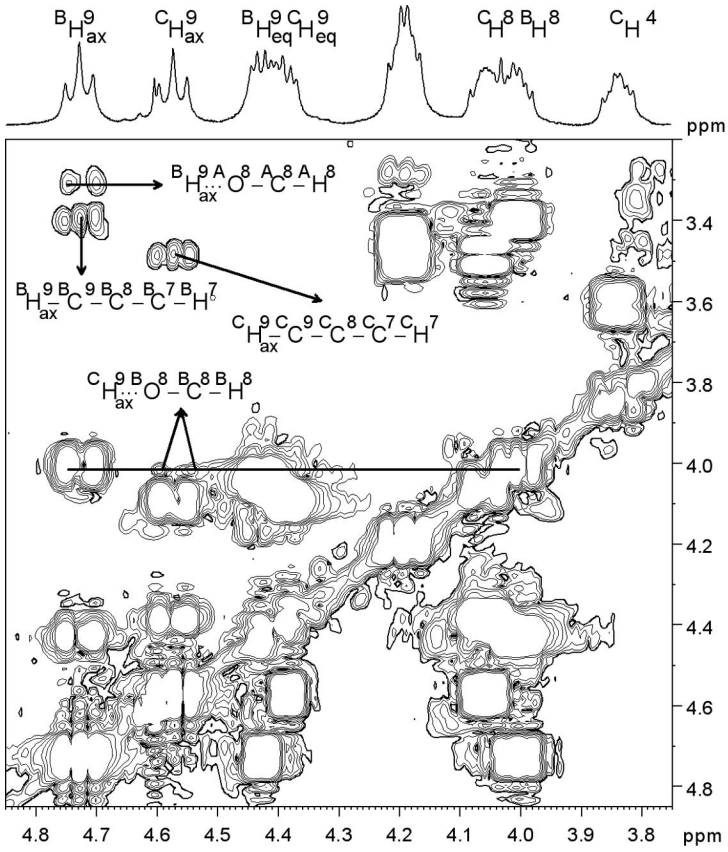

Figure 2. Partial 2D LRCOSY spectrum of the α2,8-(NeuAc)$_3$ lactone **2** in [D$_6$]DMSO solutions at 305 K using an 80-ms extra delay. The cross-peaks of $^{B}H^{9}_{ax}/^{A}H^{8}$ and $^{C}H^{9}_{ax}/^{B}H^{8}$ indicate ^{3h}J correlations through C–H···O hydrogen bonds. Two additional ^{4}J correlations of the H^{9}_{ax}/H^{7} cross-peaks of residues B and C were assigned as long-range couplings.

met the criterion of a total energy lower than 32 kcal/mol were selected (Figure 4). The heavy atoms of the resulting structures in the mean conformation yielded average root-mean-square deviations (RMSD) of 0.59 Å. The structure of the lactone I is different to that of the α2,8-(NeuAc)$_3$ lactone reported previously.[6] The $^{C2}S^{C9}$ conformer of lactone I is the same for lactone II in this report. This can be explained by the additional C–H···O hydrogen-bond restraints that stabilize the skewed twist–boat $^{C2}S^{C9}$ conformation of lactone I. We note that the simulated structure of the α2,8-(NeuAc)$_3$ lactone at the global minimum has appropriate bond lengths and angles[7] for the formation of intramolecular $^{x+1}C^{9}\_^{x+1}H^{9}_{ax}\cdots^{x}O^{8}$ hydrogen bonds.

In proteins, the C–H···O$^{sp^2}$ hydrogen-bonding energy was suggested to be around –3 kcal/mol,[31] which is approximately half the energy of a conventional hydrogen bond. The binding energy of a C–H···O$^{sp^3}$ hydrogen bond has theoretically been estimated to be –1.0 kcal/mol or less.[32–34] To evaluate the binding energy of the $^{x+1}C^{9}\_^{x+1}H^{9}_{ax}\cdots^{x}O^{8}$ hydrogen bond in an α2,8-(NeuAc)$_3$ lactone, we used two simplified models, the spiro[5,5]lactone dimer (SLD) **3** and the 4-oxolactone dimer (OLD) **4**, created from the NMR solution structures (Figure 5). Compared with the non-hydrogen-bonded $^{B}C^{9}–^{B}H^{9}_{ax}$, the bond length of the hydrogen-bonded $^{C}C^{9}–^{C}H^{9}_{ax}$ in SLD **3** is calculated to be slightly lower by 3–6 mÅ at the B3LYP/6-31G* and MP2/6-31G* levels of theory,[35] as shown in Table 1. This is because of a formal charge redistribution leading to slightly more positive charges on the C^9 and O^8 atoms and negative charge on H^{9}_{ax}, for example, $^{\delta+}C^{9}–^{\delta-}H^{9}_{ax}\cdots^{\delta+}O^{8}$, and this change in C–H bond length is consistent with previous reports[36,37] on weak C–H···O$^{sp^3}$ interactions.

To gain a better understanding of the intramolecular C–H···O$^{sp^3}$ hydrogen bond of SLD **3**, we performed a second-order perturbation theory analysis of the Fock matrix within the natural bond orbital (NBO) basis[38,39] derived by employing B3LYP/6-31G*. The NBO result showed that SLD **3** consists of one $^{x+1}C^{9}\_^{x+1}H^{9}_{ax}\cdots^{x}O^{8}$ hydrogen-bond equivalence. The nonbonding n orbital of $^{B}O^{8}$ is the donor molecular orbital, whereas δ^* of $^{C}C^{9}–^{C}H^{9}_{ax}$ is the acceptor molecular orbital. The second-order interaction energy is 1.05 kcal/mol,[38] similar to previously calculated values of the C–H···O hydrogen-bonding systems.[40] Furthermore, the binding energy of the C–H···O$^{sp^3}$ hydrogen bond in the two-fragment model OLD **4** can be directly calculated by using a standard procedure to remove basis set superposition errors (BSSE), and the binding energy was estimated to be 1.61 kcal/mol at the MP2/6-31G* level of theory. The MP2 binding energy was further checked by using a mixed basis set in which extra sets of diffuse and polarized basis functions for the two atoms $^{C}H^{9}_{ax}$ (6-311++G**) and $^{B}O^{8}$ (6-31+G*) were used. With this mixed basis the polariza-

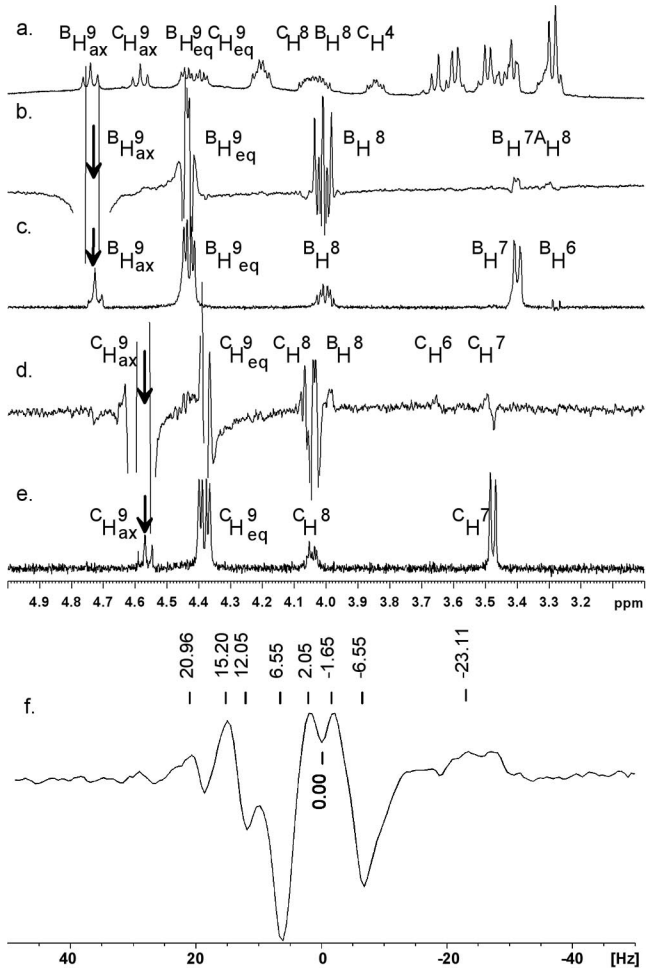

Figure 3. Partial 1D NMR spectra of the α2,8-$(NeuAc)_3$ lactone **2** in [D_6]DMSO solutions at 305 K. Arrows indicate the irradiated proton signals in the selective 1D LRCOSY spectra (b, d) using an 80 ms extra delay time and 1D TOCSY (c, e) experiments. f) The spectrum from 4.2 to 3.9 ppm in Figure 3d is expanded and reprinted in frequency units (Hz) using the proton $^{C}H^{8}$ at 4.036 ppm as the reference. The clear difference between $^{C}H^{8}$ and $^{B}H^{8}$ was used to explain the ^{3h}J correlation of the $^{C}H^{9}_{ax}/^{B}H^{8}$ pair by reference to the $^{C}C^{9}$–$^{C}H^{9}_{ax}\cdots{}^{B}O^{8}$ hydrogen bond.

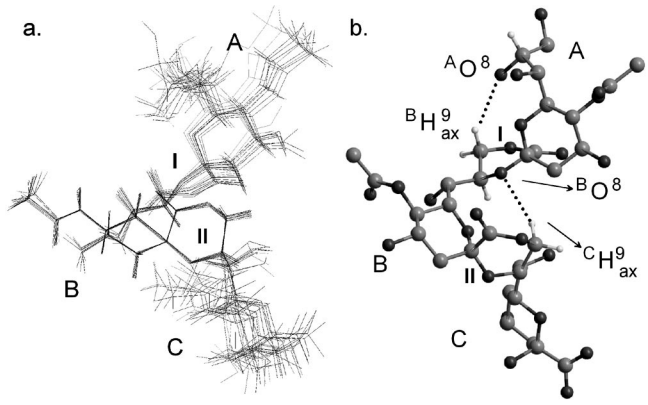

Figure 4. NMR solution structures of the α2,8-$(NeuAc)_3$ lactone **2**: a) Superimposition of the heavy atoms of the 10 lowest-energy structures derived from Xplor NIH simulations. The structures are best fitted to residue B. b) Ball-and-stick model of heavy atoms with the lowest energy. The $^{B}H^{9}_{ax}/^{A}H^{8}$ and $^{C}H^{9}_{ax}/^{B}H^{8}$ correlations of the weak $^{x+1}C^{9}$–$^{x+1}H^{9}_{ax}\cdots{}^{x}O^{8}$ hydrogen bonds are indicated.

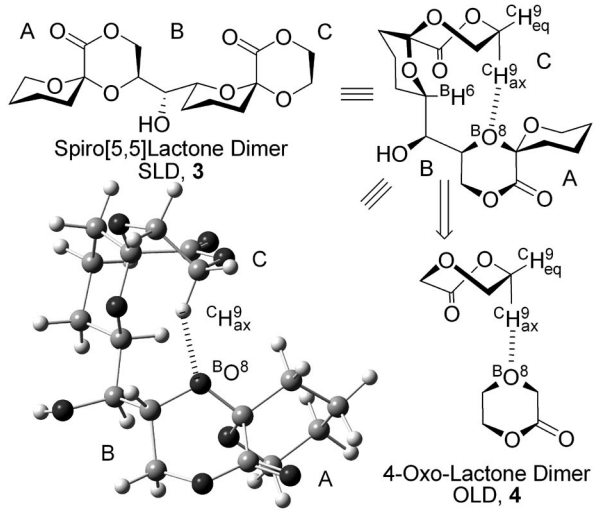

Figure 5. Reducing strategy from the α2,8-$(NeuAc)_3$ lactone **2** to the spiro[5,5]lactone dimer (SLD) **3** for NBO analysis and to 4-oxolactone dimer (OLD) **4** for ab initio calculation with BSSE correction. The optimized geometry of SLD **3** at the B3LYP/6-31G* level of theory is given as a ball-and-stick structure.

tion effect can be better accounted and the MP2 binding energy is essentially the same. The presence of the inter-residue C–H···O^{sp^3} hydrogen bond in SLD **3** and OLD **4** was verified by theoretical calculations, and the results can be generalized to other similar hydrogen bonds, denoted as $^{x+1}C^{9}$–$^{x+1}H^{9}_{ax}\cdots{}^{x}O^{8}$, in OSA/PSA lactones.

According to previous ab initio calculations of δ-valerolactone,[41] the total energy of the *S*-type conformer is 1–1.5 kcal/mol higher than that of the half-chair conformer. In OSA/PSA lactones, the glycosidic oxygen O^{8} as a hydrogen-bond acceptor contributes approximately 1.0–1.6 kcal/mol of the binding energy of two lactone moieties. This energy is considered to stabilize the conformation of the skewed twist–boat $^{C2}S^{C9}$ conformers as each *S* conformer encircling the helical axis serves both as hydrogen-bond donor (H^{9}_{ax}) and acceptor (O^{8}) for extra stabilization.

Table 1. Important geometrical and bonding parameters for the intra-residue $^{C}C^{9}$–$^{C}H^{9}_{ax}\cdots{}^{B}O^{8}$ hydrogen bond in the α2,8-$(NeuAc)_3$ lactone **2** and SLD **3** by different calculation methods.[a]

Molecule	$^{C}C^{9}$–$^{C}H^{9}_{ax}$	$^{C}H^{9}_{ax}\cdots{}^{B}O^{8}$	$\angle{}^{C}C^{9}$–$^{C}H^{9}_{ax}\cdots{}^{B}O^{8}$	$^{B}C^{9}$–$^{B}H^{9}_{ax}$ [b]
2[c]	1.098	2.593	140.07	1.099
SLD **3**[d]	1.090	2.605	142.92	1.093
SLD **3**[e]	1.085	2.365	131.90	1.091

[a] Distances are given in Ångströms, angles in degrees. [b] Not in the C–H···O hydrogen bond. [c] Averaged results from 10 final data sets of the Xplor NIH simulation. [d] Optimized at the B3LYP/6-31G* level of theory. The resulting structure was used for the subsequent NBO analysis. [e] Optimized at the MP2/6-31G* level of theory. The resulting structure was used to build the two-fragment model OLD **4** to directly calculate the binding energy.

A sequential x and x+1 relationship in the $^{x+1}C^9$–$^{x+1}H^9_{ax}$···$^xO^8$ hydrogen-bonding cooperativity between two sialic acids or lactone moieties in OSA/PSA is suggested to be an important factor in cooperative acid-catalyzed lactonization. This suggestion stems from Hammond's postulate.[42,43] Additional stabilization in the lactonized product could increase the stabilization energy in the transition state and thus lead to the observed cooperative lactonization in OSA.

Conclusions

This study by LRCOSY provides direct evidence for C–H···O$^{sp^3}$ hydrogen-bonding cooperativity in the α2,8-(NeuAc)$_3$ lactone **2**, and the C–H···O hydrogen bond is confirmed with the structure and binding energy estimated by using ab initio calculations. The $^{x+1}C^9$–$^{x+1}H^9_{ax}$···$^xO^8$ hydrogen bond not only stabilizes the skewed twist–boat conformers in lactones, but also serves as the driving force for cooperative lactonization in OSA/PSA lactone. The C–H···O hydrogen bond described herein has been deduced from a simulated rigid structure, as the essential donor and acceptor are located in the groove and encircled in a relatively hydrophobic environment, which further prevents its direct contact with solvent. Incidentally, the structures of oligosaccharides with C–H···O hydrogen-bonding could not be resolved efficiently due to overlapping proton signals and structural variations, preventing a distinguishable anomeric proton. In spite of the hydrophilic nature and the overlapping of signals from saccharide hydroxy groups and water molecules, these findings indicate that LRCOSY is a useful technique for studying X–H···O hydrogen bonds in oligosaccharides or glycopeptides.

Experimental Section

General: Tri-*N*-acetylneuraminic acid [α2,8-(NeuAc)$_3$] was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). The synthesis and analysis of the α2,8-(NeuAc)$_3$ lactone **2** by capillary electrophoresis has been reported previously.[26] NMR spectra were recorded with a Bruker Avance II 500 spectrometer and the 1D and 2D LRCOSY pulse sequences therein were analyzed by the Topspin 2.1 software. NMR samples comprised the α2,8-(NeuAc)$_3$ lactone **2** (8 mg) with [D$_6$]DMSO (300 μL) placed in a Shigemi NMR tube. ^{1}H and ^{13}C NMR chemical shifts are reported in ppm relative to external *tert*-butyl alcohol as the reference (1.24 and 31.6 ppm for ^{1}H and ^{13}C nuclei, respectively). For 2D LRCOSY spectra, a 512 (F1) × 1024 (F2) data matrix with an extra delay 80, 100, or 120 ms was used in acquisition, and the processing was zero-filling to 2048 (F1) × 2048 (F2) with a linear prediction of 2048 (F1) before Fourier transform.

Xplor-NIH Simulation: The distance restraints of the NOE cross-peaks were measured and organized in our previous study.[26] Additionally, two distance restraints of the $^{x+1}C_9$–$^{x+1}H^9_{ax}$···$^xO^8$ hydrogen bond between $^{x+1}H^9_{ax}$ and $^xO^8$, x = A or B, were analyzed by LRCOSY and the distance limit was assigned as 2.0–2.7 Å. Three-dimensional structures were generated by using the simulated annealing and energy minimization protocol in the program X-PLOR NIH. Average structures were calculated by using the final sets of refined structures and their energies were further minimized to ensure correct local geometry. INSIGHT II was used to visualize the final sets of the structures and to draw the electrostatic surface potentials of the final 3-D models. The convergence of the calculated structures was evaluated in terms of the structural parameters, that is, the RMSD of the experimental distance and dihedral constraints, the values of the energy statistics (Fnoe, Ftor, and Frepel), and the RMSD of the idealized geometry (see Table S1 of the Supporting Information).

Ab Initio Calculations

Binding energies were calculated by Q-CHEM, version 3.0,[35,44] in combination with the natural bond orbital (NBO) software package, version 5.0.[38,39] The initial geometry of the simplified model spiro[5,5]lactone dimer (SLD) **3** was modified from the lowest total energy configuration of the α2,8-(NeuAc)$_3$ lactone **2** derived from Xplor-NIH simulations.

In brief, the atoms and relative protons of C7–C8, O4, NHAc, and O7–O9 of residue A, O4 and NHAc of residue B, and C1–C7, O1, O2, O4, NHAc, and O7 of residue C in the α2,8-(NeuAc)$_3$ lactone **2** were deleted. The resulting carbon atoms were defined with sp^3-hybridization, and protons were added by the software spontaneously. The constructed structure, SLD **3**, was judged to have the same inter-residual CHO hydrogen bond as trisialic acid lactone **2**. The initial structure of SLD **3** was optimized at the B3LYP/6-31G* and MP2/6-31G* levels of theory with mixed basis sets. In calculations with mixed basis sets, 6-31+G* and 6-311++G** were respectively used for $^BO^8$ and $^CH^9_{ax}$, and 6-31G* was used for the residual atoms at the MP2 level. These two optimized structures are quite similar, and the variation in the hydrogen bond $^CH^9_{ax}$···$^BO^8$ is less than 0.002 Å.

The hydrogen bond in SLD **3** was estimated by the second-order perturbation theory analysis in the NBO package. The resulting structure of SLD **3** at the MP2/6-31G* level of theory was used as the basis for investigating the nature of the corresponding intramolecular $^CC^9$–$^CH^9_{ax}$···$^BO^8$ hydrogen bond.

The binding energy in 4-oxolactone dimer (OLD) **4** was evaluated at the MP2/6-31G* level of theory with the basis set superposition errors (BSSE). The initial geometry of the simplified model OLD **4** was modified from the SLD **3** structure optimized at the MP2/6-31G* level. In summary, the atoms and protons of C3–C6 and O6 of residue A and C3–C7, O6, and O7 of residue B were deleted. The resulting carbon atoms were defined with sp^3-hybridization, while protons were added by the software. The structure OLD **4** constructed for the binding energy calculation was modified in accord with this structure. For further confirmation, the binding energy in OLD **4** modified with MP2/mixed basis sets with the same treatment was also calculated at the MP2 level of theory with the same specified basis sets. The variation in binding energy was less than 0.01 kcal/mol.

Supporting Information (see also the footnote on the first page of this article): Computational details and structural coordinates for trisialic acid lactone.

Acknowledgments

The authors thank Dr. Chun-Hua Hsu (Department of Agricultural Chemistry) and Prof. Chun-Chung Chan (Department Chemistry, National Taiwan University) for helpful discussions. This work was supported by the National Science Council and Academia Sinica, Taiwan.

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Received: April 20, 2009
Published Online: May 27, 2009

