Preferred Conformation of *C*-Lactose at the Free and Peanut Lectin Bound States

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Abstract: A conformational analysis of methyl α-*C*-lactoside (**5**) is carried out (Table 1), which suggests the preferred solution conformation of **5** to be described as a mixture of conformers **A** ($\phi = 300°$; $\psi = 60°$) and **B** ($\phi = 300°$; $\psi = 300°$) to a first approximation. Applying a modified Karplus equation for the vicinal coupling constants observed for the H.a and H.1′ protons of **5**, the dihedral angle (ϕ = O.5′−C.1′−C.a−C.4) was deduced to be approximately +280°. Nuclear Overhauser effect (NOE) experiments were then conducted on **5-D$_2$** and its parent methyl α-*O*-lactoside in a 7:3 mixture of pyridine-d_5 and methanol-d_4, qualitatively demonstrating that the conformational characteristics of both methyl α-*C*- and α-*O*-lactosides at the free states are represented as a mixture of two conformers **A** and **B**, but their relative population may be different. Through X-ray analysis, it has been definitively established that the conformation ($\phi = 297(7)°$ and $\psi = 120(2)°$) of *C*-lactose bound to peanut lectin is practically identical to the conformation ($\phi = 291(6)°$ and $\psi = 118(9)°$) of its parent *O*-lactose bound to the same protein.

Previous work[1] from one of these laboratories has experimentally established that the *C*-glycosidic bond of a *C*-glycoside preferentially adopts the *exo*-anomeric conformation[2] like the *O*-glycosidic bond of its parent *O*-glycoside does. The conformational preference of *C*-aglyconic bond can be predicted primarily on the basis of steric considerations. To a first approximation, the *C*-monosaccharides, *C*-disaccharides, and *C*-trisaccharides have been shown to have conformational properties close to those of the corresponding parent *O*-glycosides.

There has been disagreement over whether *C*- and *O*-lactosides share the same conformational characteristics. Using nuclear Overhauser effect (NOE) experiments coupled with force-field calculations, Jiménez-Barbero and co-workers[3] compared the conformation of *C*-lactose with that of methyl α-*O*-lactoside. They concluded that (1) *C*-lactose preferentially adopts the *exo*-anomeric conformation around the *C*-glycosidic bond and (2) *C*-lactose exhibits different conformational properties around the *C*-aglyconic bond from those of methyl α-*O*-lactoside. On the other hand, Berthault and co-workers[4] used a strong off-resonance rf irradiation as a spin-lock in the mixing time of ROESY experiments and found that methyl β-*C*-lactoside shares the same conformational characteristics, including those around the aglyconic bond, with its parent methyl β-*O*-lactoside.

In this paper, we report the conformational properties of methyl α-*C*-lactoside with CH_2- and CD_2-bridges in reference to its parent methyl α-*O*-lactoside and present definitive evidence that *C*-lactose bound to peanut lectin adopts a virtually identical conformation to the one observed for *O*-lactose.

Conformational Analysis of Methyl α-*C*-Lactoside. As mentioned, the *C*-glycosidic bond of all the *C*-monosaccharides, *C*-disaccharides, and *C*-trisaccharides exhibits a clear preference for the *exo*-anomeric conformation like the *O*-glycosidic bond of their parent *O*-saccharides.[1] This preference is more pronounced for the axial *C*-glycosidic bond than for the corresponding equatorial *C*-glycosidic bond.[5]

Accepting this conformational preference of the *C*-glycosidic bond, the conformational analysis of methyl α-*C*-lactoside can be done, to a first approximation, by focusing only on the *C*-aglyconic bond. This implies that the same analysis can be applied to *C*-lactose and also to methyl α-*C*-cellobioside and *C*-cellobiose, because the C.1 and C.4′ substituents of these saccharides are not directly involved in this analysis. To clearly evaluate and present through-space steric interactions, a diamond-lattice analysis was introduced.[6] For example, in the analysis of methyl α-*C*-lactoside (**5**), only the three ideal staggered conformers around the *C*-aglyconic bond, **A** ($\phi = 300°$; $\psi = 60°$),[7] **B** ($\phi = 300°$; $\psi = 300°$), and **C** ($\phi = 300°$; $\psi = 180°$),

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(1) Wei, A.; Boy, K. M.; Kishi, Y. *J. Am. Chem. Soc.* **1995**, *117*, 9432 and references therein.

(2) The term *exo*-anomeric conformation is used to describe the conformation of *O*- or *C*-glycosidic bond with the glycosidic oxygen or carbon being anti-periplanar to the C.1−C.2 bond.

(3) (a) Espinosa, J.-F.; Martín-Pastor, M.; Asensio, J. L.; Dietrich, H.; Martín-Lomas, M.; Schmidt, R. R.; Jiménez-Barbero, J. *Tetrahedron Lett.* **1995,** *36*, 6329. (b) Espinosa, J.-F.; Cañada, F. J.; Asensio, J. L.; Dietrich, H.; Martín-Lomas, M.; Schmidt, R. R.; Jiménez-Barbero, J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 303. (c) Espinosa, J.-F.; Cañada, F. J.; Asensio, J. L.; Martín-Pastor, M.; Dietrich, H.; Martín-Lomas, M.; Schmidt, R. R.; Jiménez-Barbero, J. *J. Am. Chem. Soc.* **1996**, *118*, 10862. (d) Espinosa, J.-F.; Montero, E.; Vian, A.; García, J. L.; Dietrich, H.; Schmidt, R. R.; Martín-Lomas, M.; Imberty, A.; Cañada, F. J.; Jiménez-Barbero, J. *J. Am. Chem. Soc.* **1998**, *120*, 1309.

(4) Rubinstenn, G.; Sinäy, P.; Berthault, P. *J. Phys. Chem. A* **1997**, *101*, 2536.

(5) (a) Goekjian, P. G.; Wu, T.-C.; Kishi, Y. *J. Org. Chem.* **1991**, *56*, 6412. (b) Wei, A.; Kishi, Y. *J. Org. Chem.* **1994**, *59*, 88.

(6) Babirad, S. A.; Wang, Y.; Goekjian, P. G.; Kishi, Y. *J. Org. Chem.* **1987**, *52*, 4825.

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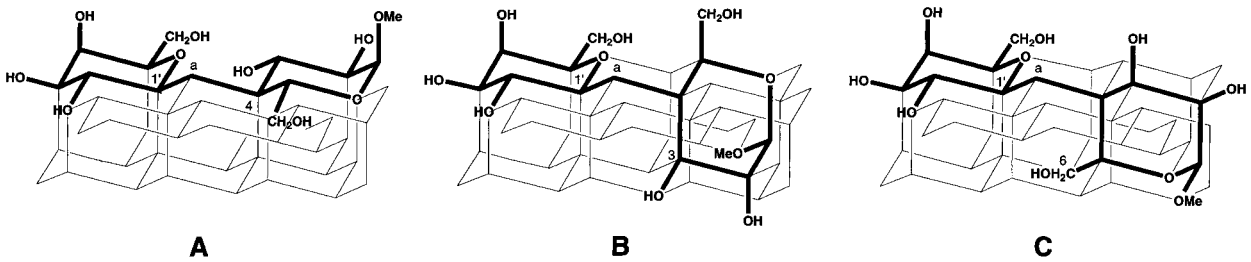


Figure 1. Three ideal staggered conformers of methyl α-*C*-lactoside. The *C*-glycosidic and *C*-aglyconic bonds are $\phi = 300°$ and $\psi = 60°$ for **A**, $\phi = 300°$ and $\psi = 300°$ for **B**, and $\phi = 300°$ and $\psi = 180°$ for **C**, respectively.

Table 1. Major Steric Interactions and Estimated Destabilization Energy for the Conformers **A**−**C** of Methyl α-*C*- and *O*-Lactosides

conformer A			conformer B			conformer C		
1,3-diaxial-like interaction								
	C[a]	O[b]		C[a]	O[b]		C[a]	O[b]
C.4−C.3/C.1′−O.5′	1.9[c]	1.9[c]	C.4−C.5/C.1′−O.5′	1.9[c]	1.9[c]	C.5−C.6/C.a−C.1′	3.7[c]	1.9[c]
C.3−O.3/C.a−C.1′	1.9[c]	1.9[c]						
single-axial-like interaction								
C.a−C.4/C.6−H	0.9[d,e]	0.4[d,e]	C.4−C.3/C.1′−H	0.9[d,f]	0.9[d,f]	C.4−C.5/C.1′−H	2.4[g]	2.4[g]
			C.4−C.a/C.6−H	0.9[h]	0.4[h]	C.3−O.3/C.a−H	0.4[d]	0
			C.a−C.1′/C.5-H	0.9[d]	0.9[d]	C.1′−O.5′/C.4-H	0.4[d]	0.4[d]
			C.3−O.3/C.a−H	0.4[d]	0	C.1′−O.5′/C.6-H	0.4[d]	0.4[d]
Total[i]	4.7	4.2		5.0	4.1		7.3	6.7

[a] Methyl α-*C*-lactoside. [b] Methyl α-*O*-lactoside. [c] 1,3-Diaxial destabilization energy reported for OMe/CH_2R (1.9 kcal/mol) and Me/Me (3.7 kcal/mol) substituted cyclohexanes was adopted.[10] [d] One-half of *A*-value reported for CH_2R (1.8 kcal/mol) and OR (0.8 kcal/mol) substituted cyclohexanes was used.[10] [e] With an imaginative six-membered ring including the C.a−C.4−C.5, it is seen that the single-axial-like interaction of C.5−C.6/C.a−H is already counted in the single-axial-like interaction of C.a−C.4/C.6−H. The destabilization energy is estimated to be 0.9 for both in the *C*-series but to be 0.4 for the former and 0.0 for the latter in the *O*-series, and 0.4 is used for this estimation. [f] With an imaginative six-membered ring including the O.5′−C.1′−C.a−C.4, it is seen that the single-axial-like interaction of C.a−C.1′/C.3−H is already counted in the single-axial-like interaction of C.4−C.3/C.1′−H. [g] One-half of *A*-value (4.9 kcal/mol) reported for C(Me)$_3$-cyclohexane was used.[9] The single-axial-like interaction of C.4−C.a/C.6−H is the same type of interaction as this, but involves the same protons as the C.4−C.5/C.1′. [h] With an imaginative six-membered ring including the C.4−C.5−C.6, it is seen that the single-axial-like interaction of C.5−C.6/C.a−H is already counted in the single-axial-like interaction of C.4−C.a/C.6−H. For estimation of the destabilization energy, see the footnote e. [i] A total destabilization energy (kcal/mol) estimated.

are considered (Figure 1). By focusing on 1,3-diaxial-like and single-axial-like interactions, the steric destabilization of these conformers can then be analyzed (Table 1).[8] Clearly, the conformer **C** suffers from several steric destabilizations. Among the single-axial-like interactions, the interaction between the C.1′ hydrogen and one of the C.6 hydrogens (or C.6 oxygen) is assumed to be most significant; the C.1′ and C.6 hydrogens need to occupy the same point in a diamond lattice, and this steric situation can be compared with that of *tert*-butylcyclohexane.[9] The conformer **B** suffers from steric congestion due to one 1,3-diaxial-like and four single-axial-like interactions, whereas the conformer **A** suffers steric congestion due to two 1,3-diaxial-like and one single-axial-like interactions. Overall, none of the three ideal staggered conformers is free of unfavorable steric interactions.

The relative stability among these three ideal staggered conformers is not predictable in a straightforward manner. Using (1) 1,3-diaxial interaction energy reported for Me/Me- and OMe/CH_2R-substituted cyclohexanes,[10] (2) $^1/_2$ of the *A*-value reported for *tert*-butylcyclohexane,[9] and (3) $^1/_2$ of the *A*-values reported for CH_2R- and OR-cyclohexanes,[10] the relative stability of **A**, **B**, and **C** is estimated to be 4.7, 5.0, and 7.3 kcal/mol, respectively (Table 1). However, as a hydrogen bond between the C.3-OH and the O.5′ may provide some stabilization, the total destabilization of **A** may be less than 4.7 kcal/mol. Overall, this analysis qualitatively suggests that the preferred conformation of methyl α-*C*-lactoside (**5**) is predominantly a mixture of conformers **A** and **B**.[11] It should be noted that this conformational analysis provides only a rough gross picture for the conformational characteristics of these substrates, and the actual low-energy conformers are likely to deviate from the conformers **A** and **B**.

The conformational analysis of methyl α-*O*-lactoside and *O*-lactose, and methyl α-*O*-cellobioside and *O*-cellobiose, can be done in a similar manner (Table 1). Thus, the relative stability of the three conformers corresponding to **A**, **B**, and **C** is estimated to be 4.2, 4.1, and 6.7 kcal/mol, respectively. Once again, a hydrogen bond between the C.3-OH and the O.5′ might make the total destabilization of **A** less than 4.2 kcal/mol. In addition, the C.3−O.3/O.a−C.1′ 1,3-diaxial-like steric destabilization of **A** might not be as pronounced as it would be in a general case because of the antiparallel orientation of the polar carbon−oxygen bonds involved.

Overall, this analysis qualitatively suggests that the preferred conformation of methyl α-*C*- and α-*O*-lactosides, as well as

(7) All dihedral angles are defined according to the IUPAC Joint Commission on Biochemical Nomenclature (JCBN), symbols for specifying the conformation of polysaccharide chains, 1981 recommendation. Reproduced in *Eur. J. Biochem.* **1983**, *131*, 5; ϕ = O.5′−C.1′−O.4−C.4 and ψ = C.1′−O.4−C.4−C.3.

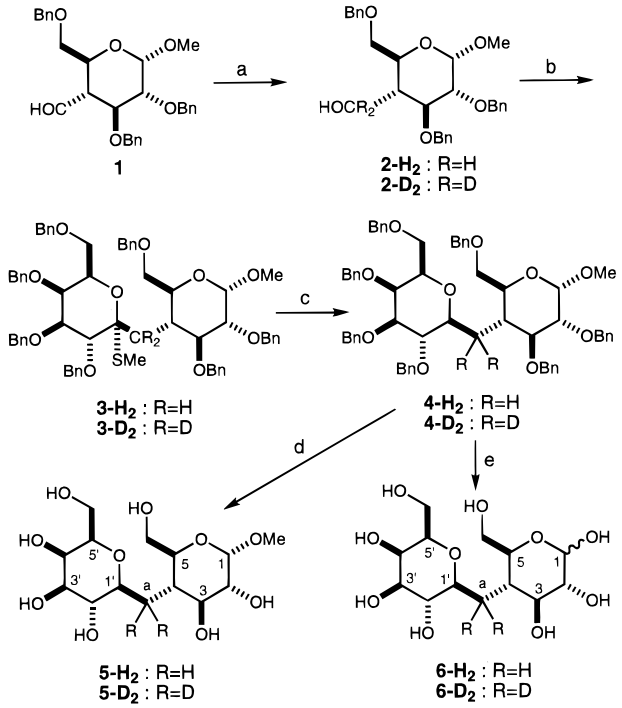
(8) The single-axial-like interaction of C.2′−O.2′/C.a−H is not included in Table 1 because it exists in all the three staggered conformers **A**−**C**.

(9) With an imaginative six-membered ring including O.5′−C.1′−C.a−C.4 on the conformer **C**, it is seen that the C.5 and C.6 carbons correspond to the quaternary and primary carbons of the *tert*-butyl group of *tert*-butylcyclohexane. Thus, the single-axial-like interaction between the C.1′−H and the C.4−C.5−C.6−C.6−H (or OH) is assumed to be roughly represented by $^1/_2$ of the *A*-value (4.9 kcal/mol) of *tert*-butylcyclohexane reported by Manoharan, M.; Eliel, E. L. *Tetrahedron Lett.* **1984**, *25*, 3267. The single-axial-like interaction of C.4−C.a−C.1′−C.1′−H/C.6−H is the same type of interaction as this. However, since this interaction involves the same protons as the single-axial-like interaction of C.4−C.5/C.1′−H, the steric destabilization is counted only once.

(10) Corey, E. J.; Feiner, N. F. *J. Org. Chem.* **1980**, *45*, 765.

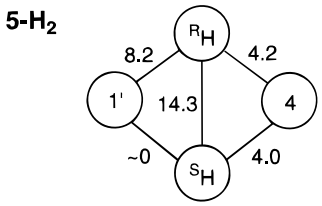
(11) Extensive variable-temperature ^{1}H NMR experiments were performed on *C*-monoglycosides to demonstrate that these substrates exist as mixture of conformers.[5a]

Scheme 1. Synthesis of Methyl α-*C*-Lactoside and *C*-Lactose with the CH_2- and CD_2-Bridges[a]



[a] Reagents and yields: (a) (1) $NaClO_2$, NaH_2PO_4, $(Me)_2C{=}CHMe$, *t*-BuOH; (2) $LiAlD_4$, ether, 63% overall yield. (b) (1) Ph_3P, Im, I_2, 88%; (2) *n*-BuLi, −90 °C, followed by addition of galactolactone, 81%; (3) MeSH, CH_2Cl_2, $BF_3{\cdot}OEt_2$, dropwise, 71% (17% of SM recovered). (c) Ph_3SnH, AIBN, 96%. (d) $H_2/Pd(OH)_2$ on C, MeOH/EtOAc, 85%. (e) (1) allyl alcohol, $Sc(OTf)_3$, 100 °C; DMSO, KOBu-*t*, 95 °C; acetone, 1N HCl, 80%; (2) $H_2/Pd(OH)_2$ on C, MeOH/EtOAc, 87%.

Chart 1. Vicinal Coupling Constants Observed for Methyl α-*C*-Lactoside in a Mixture of Pyridine-d_5 and Methanol-d_4



that of *C*- and *O*-lactoses, is described primarily as a mixture of conformers **A** and **B**.

Substrates for NMR Studies. Methyl α-*C*-lactoside (**5**) and *C*-lactose (**6**)[12] were synthesized as shown in Scheme 1. $LiAlD_4$ or $LiAlH_4$ reduction of the carboxylic acid derived from the aldehyde **1**,[13] followed by the succeeding steps, furnished methyl α-*C*-lactoside (**5**) and *C*-lactose (**6**) with the CD_2- or CH_2-bridge.

NMR Analysis. The NMR studies were performed on methyl α-*C*-lactoside (**5**) in D_2O and a mixture of pyridine-d_5 and methanol-d_4, respectively, at approximately 20 °C.

The vicinal coupling constants ($J_{1,2} = 3.6$ Hz, $J_{2,3} = 9.5$, $J_{3,4} = 10.5$, $J_{4,5} = 10.6$, $J_{1',2'} = 9.3$, $J_{2',3'} = 9.2$, $J_{3',4'} = 3.4$, $J_{4',5'} = 0$) observed for the ring protons clearly show that both tetrahydropyran rings of **5** adopt a chair conformation, as do the corresponding rings of parent methyl α-*O*-lactoside and *O*-lactose.

The vicinal coupling constants observed for the C.a and C.1′ protons of **5-H$_2$** (Chart 1) experimentally demonstrate that the *C*-glycosidic bond of **5** preferentially adopts the *exo*-anomeric conformation. This conformation can be refined further; using a modified Karplus equation[14] and coupling constants of 8.2 and 0 Hz yields a dihedral angle (ϕ = O.5′−C.1′−C.a−C.4) of approximately +280°. On the other hand, the vicinal coupling constants observed for the C.4 and C.a protons of **5** (Chart 1) experimentally demonstrate that the *C*-aglyconic bond does not preferentially adopt any one of the three ideal staggered conformers **A**−**C**. This observation implies that the steric repulsion existing in the conformers **A**−**C** is avoided by rotating primarily the *C*-aglyconic bond away from the ideal staggered position rather than the *C*-glycosidic bond. This phenomenon, i.e., the steric destabilization present in the ideal staggered conformers that primarily results in the deformation of the *C*-aglyconic bond over the *C*-glycosidic bond, is uniformly observed for all the *C*-saccharides studied thus far and importantly, the same phenomenon is known for the parent *O*-saccharides as well.[15] It is worthwhile to note that the vicinal coupling constants, including the bridging methylene protons, are remarkably close to those observed for methyl α-*C*-cellobioside,[16] as expected from the conformational analysis given in the previous section.

NOE experiments are expected to provide more defined information on these characteristics. Namely, an NOE between the C.1′ and C.4 protons is expected for the conformer **A**, between the C.1′ and C.3 protons for the conformer **B**, and between the C.1′ and C.6 protons for the conformer **C**. However, considering the previous observations made on methyl α-*C*-maltoside and its analogues,[16] we opted to use the bis-deuterated substrate **5-D$_2$**. NMR experiments were first attempted in D_2O, but many resonances were severely overlapped, and a definitive conclusion on the absence or presence of NOEs between the C.1′ proton and the C.3, C.4, and C.6 protons could not be drawn. Therefore, we searched for a suitable solvent system in which all the C.1′, C.3, C.4, and C.6 protons of both methyl α-*C*- and α-*O*-lactosides were separated, and a 7:3 mixture of pyridine-d_5 and methanol-d_4 met this need.[17] As seen in the spectrum of methyl α-*C*-lactoside shown in Figure 2,[18] a pronounced cross-peak between the C.1′ and C.4 protons, as well as between the C.1′ and C.3 protons, was observed. The resonances of C.6 protons were well separated in this solvent system, and the NOE between the C.1′ and each of the C.6 protons could be examined; there

(12) (a) Preuss, R.; Jung, K.-H.; Schmidt, R. R. *Liebigs Ann. Chem.* **1992**, 377. (b) Dietrich, H.; Schmidt, R. R. *Liebigs Ann. Chem.* **1994**, 975.

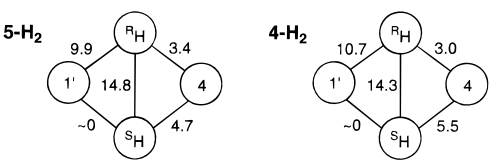
(13) Daly, S. M.; Armstrong, R. W. *Tetrahedron Lett.* **1989**, *30*, 5713.

(14) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.

(15) Lemieux, R. U.; Koto, S. *Tetrahedron* **1974**, *30*, 1933.

(16) Wang, Y.; Goekjian, P. G.; Ryckman, D. M.; Miller, W. H.; Babirad, S. A.; Kishi, Y. *J. Org. Chem.* **1992**, *57*, 482.

(17) The conformational preference of *C*-glycosidic and *C*-aglyconic bonds in D_2O is well compared to those in a mixture of pyridine-d_5 and methanol-d_4; cf. the chart shown below with the one in Chart 1.



This chart shows vicinal coupling constants observed for **5** in D_2O and for **4** in benzene-d_6. It was noted in many cases that the conformational preference of *C*-glycosidic and *C*-aglyconic bonds was not affected significantly with the protecting groups on the hydroxy groups;[1] for example, the vicinal coupling constants observed for **4-H$_2$** are an additional example for this general observation.

(18) Chemical shifts of methyl α-*C*-lactoside were assigned by a COSY experiment: ^{1}H NMR (pyridine-d_5/CD_3OD, 7/3) δ 2.37 (H.4), 3.84 (H.1′), 3.9 (H.5′), 3.96 (H.2), 3.99 (H.3′), 4.11 (H.6), 4.17 (H.6′), 4.20 (H.5), 4.21 (H.2′), 4.29 (H.3), 4.34 (H.6), 4.37 (H.6′), 4.40 (H.4′), 5.11 (H.1).

was no cross-peak detected between the C.1′ and one of the two C.6 protons, but there was a faint, barely detectable cross-peak between the C.1′ and the other C.6 proton. Overall, this result points out that the conformational characteristics of methyl α-*C*-lactoside should be described primarily as a mixture of conformers **A** and **B**. It is worth noting that the conformational characteristics of both glycosidic and aglyconic bonds of *C*-glycoside **7** have been shown to correspond nicely to those of **5**.[19]

The conformational characteristics of methyl α-*O*-lactoside and *O*-lactose,[20] as well as those of methyl α-*O*-cellobioside and *O*-cellobiose,[21] have been studied using NMR spectroscopy, primarily NOE, coupled with force-field calculations, and it is generally agreed that these glycosides exist as a mixture of conformers corresponding primarily to **A** and **B**. However, to compare the conformational characteristics of methyl α-*C*-lactoside with those of its parent methyl α-*O*-lactoside in the same environment, an NOE experiment of methyl α-*O*-lactoside was done in a 7:3 mixture of pyridine-d_5 and methanol-d_4 (Figure 3[22]). All of the C.1′, C.3, C.4, and C.6 protons of methyl α-*O*-lactoside were separated, and a pronounced cross-peak was clearly detected between the C.1′ and C.4 protons, as well as between the C.1′ and C.3 protons, but not between the C.1′ and C.6 protons.

The relative population of conformers **A** and **B** could be estimated from the magnitude of NOE observed. However, correlation of the magnitudes of NOE with internuclei distance is not straightforward.[23] Nevertheless, it is worthwhile to note that the cross-peak observed between C.1′ and C.3 protons was more intense than the cross-peak observed between C.1′ and C.4 protons for methyl α-*C*-lactoside, whereas the cross-peak observed between the C.1′ and C.3 protons was less intense than the cross-peak observed between C.1′ and C.4 protons for its parent methyl α-*O*-lactoside. These results may give a qualitative, rather than a quantitative, indication that the conformational characteristics of both methyl α-*C*- and α-*O*-lactosides could be described as a mixture of conformers **A** and **B**, but their relative population may be different.

Protein-Bound Conformation of *C*-Lactose. Many crystal structures have been reported for the bound form of *O*-lactose and plant or animal lectins.[24] In these X-ray crystal structures, *O*-lactose was shown to bind through the galactose moiety and leave the glucose moiety outside the binding pocket.[25] A large number of hydrogen bonds to the protein and to the bound water molecules, as well as hydrophobic interactions with the side chains of some amino acids, hold the galactose inside the binding pocket. Interestingly, even though the glucose residue resides outside the binding pocket, the conformational preference around the aglyconic bond appears to be shared by all of the protein-bound *O*-lactoses studied.

Jiménez-Barbero and co-workers used TR-NOESY and TR-ROESY NMR experiments to study the bound-state conforma-

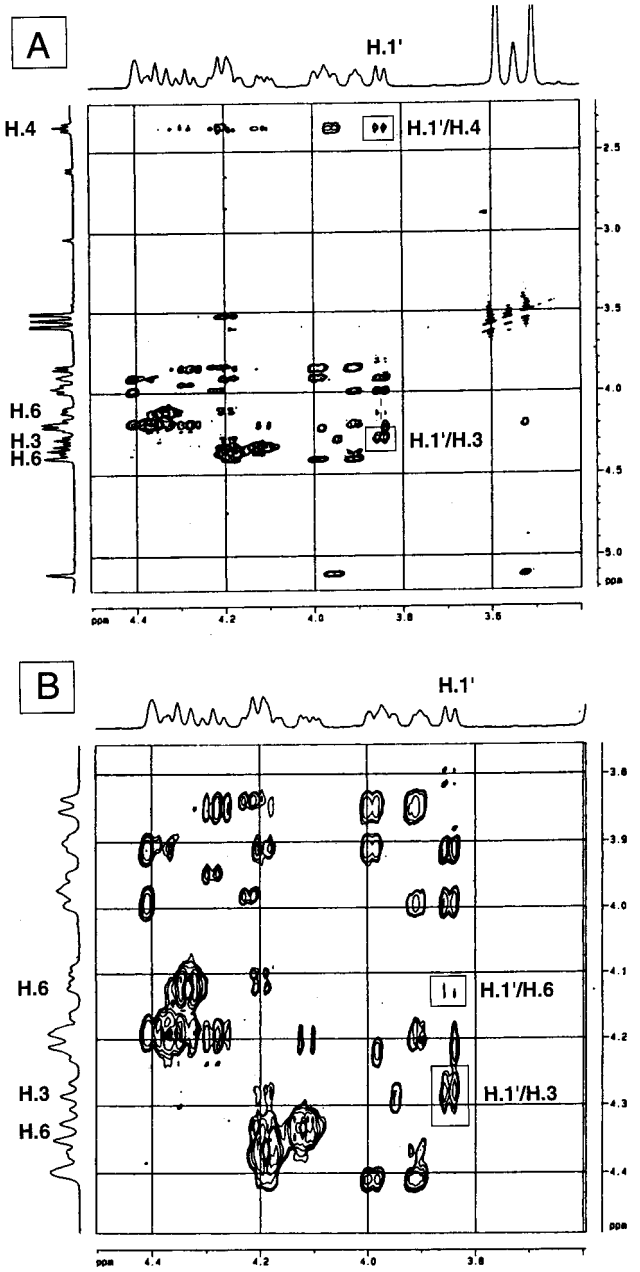
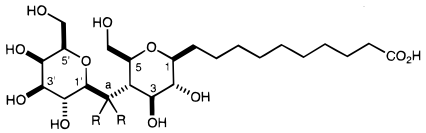


Figure 2. NOESY spectrum of methyl α-*C*-lactoside in a 7:3 mixture of pyridine-d_5 and methanol-d_4 at approximately 20 °C with (A) 2.2−5.2/3.4−4.5 ppm region and (B) 3.6−4.5/3.6−4.5 ppm region.

(19) As the case of **5**, the vicinal spin-coupling constants were used for the conformational study for the glycosidic bond of **7-H$_2$**, whereas the NOE experiment was conducted on the corresponding bis-deuterated substrate **7-D$_2$**



7-H$_2$: R=H
7-D$_2$: R=D

in a mixture of pyridine-d_5 and methanol-d_4 for the conformational study of the aglyconic bond. The synthesis of these substrates and the NOESY spectrum are included in Supporting Information.

(20) For example, see: Fernández, P.; Jiménez-Barbero, J. *Carbohydr. Res.* **1993**, *248*, 15.

(21) For example, see: Peters, T.; Meyer, B.; Stuike-Prill, R.; Somorjai, R.; Brisson, I.-R. *Carbohydr. Res.* **1993**, *243*, 49.

(22) Chemical shifts of methyl α-*O*-lactoside were assigned by a COSY experiment: ^{1}H NMR (pyridine-d_5/CD$_3$OD, 7/3) δ 4.02 (H.2), 4.06 (H.5), 4.07 (H.3′), 4.08 (H.5′), 4.15 (H.4), 4.29 (H.6′), 4.35 (H.6), 4.38 (H.6′), 4.4 (H.2′), 4.41 (H.3), 4.43 (H.6), 4.43 (H.4′), 5.0 (H.1′), 5.04 (H.1).

(23) For example, see the discussion given in ref 4.

(24) (a) Banerjee, R.; Das, K.; Ravishankar, R.; Suguna, K.; Surolia, A.; Vijayan M. *J. Mol. Biol.* **1996**, *259*, 281. (b) Liao, D. I.; Kapadia, G.; Ahmed, H.; Vasta, G. R.; Herzberg, O. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1428. (c) Lobsanov, Y. D.; Gitti, M. A.; Leffler, H.; Barondes, S. H.; Rini, J. M. *J. Biol. Chem.* **1993**, *268*, 27034. (d) Sixma, T. K.; Pronk, S. E.; Kalk, K. H.; van Zanten, B. A. M.; Berghuis, A. M.; Hol, W. G. J. *Nature* **1992**, *355*, 561. (e) Shaanan, B.; Lis, H.; Sharon, N. *Science* **1991**, *254*, 862. (f) Montfort, W.; Villafranca, J. E.; Monzingo, A. F.; Ernst, S. R.; Katzin, B.; Rutenber, E.; Xuong, N. H.; Hamlin, R.; Robertus, J. D. *J. Biol. Chem.* **1987**, *262*, 5398.

(25) Interestingly, S-lac lectins, the lactose-binding animal lectins, are reported to interact with more than one monosaccharide residue through hydrogen bonding, unlike the C-type lectin family.[24b]

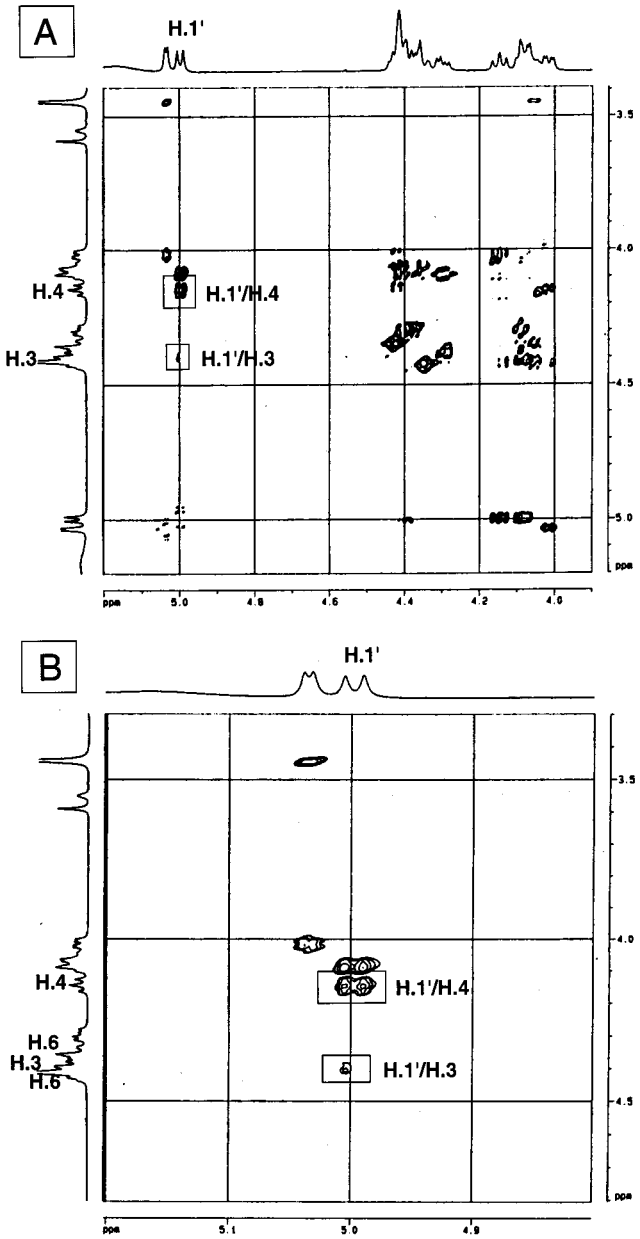


Figure 3. NOESY spectrum of methyl α-*O*-lactoside in a 7:3 mixture of pyridine-d_5 and methanol-d_4 at approximately 20 °C with (A) 3.4−5.2/3.9−5.4 ppm region and (B) 3.3−4.8/4.8−5.2 ppm region.

tion of *C*- and *O*-lactose with a galactose-binding protein ricin-B[3c] and, more recently, with an *Escherichia coli* β-galactosidase[3d] and concluded that the conformation of the *C*-glycoside is different from its corresponding *O*-glycoside in bound states. In light of the conformational characteristics of methyl α-*C*- and α-*O*-lactosides described in the previous section, coupled with the information available through several X-ray structures, Jiménez-Barbero's conclusion is rather surprising.

Obviously, the definitive answer should be obtained through an X-ray analysis of a protein-bound *C*-lactose. For this purpose, we chose peanut lectin because a high-resolution X-ray structure was already available for *O*-lactose bound to peanut lectin in one of these laboratories.[24a]

Crystals of the peanut lectin/*C*-lactose complex were grown under the conditions similar to those used for the corresponding *O*-lactose complex.[24a] The structure of the crystals of peanut lectin/*C*-lactose complex was solved and refined using 2.7 Å resolution X-ray intensity data. The four subunits in the molecule have almost identical structures. The peanut lectin tetramer in the two complexes has the same structure, with an rms deviation of 0.28 Å in C^{α} positions.

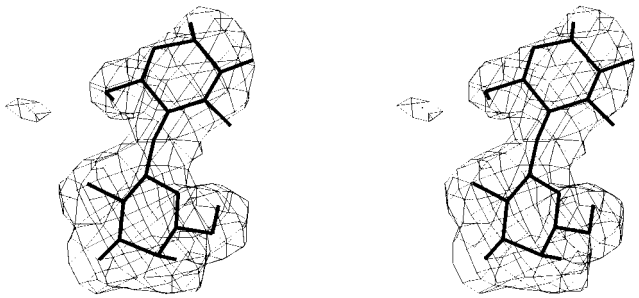


Figure 4. Stereoview of the $F_o - F_c$ electron density corresponding to the *C*-lactose molecule. The map is contoured at 2.5 σ level.

The electron density corresponding to the sugar molecules is well defined (Figure 4), as indeed was the case with the crystals of the *O*-lactose complex.[26] A representation of the location of the *C*-lactose molecule in a subunit is illustrated in Figure 5. A close-up of the sugar binding site with the C-lactose molecule is shown in Figure 6. For comparison, the location of the *O*-lactose molecule is also shown in this figure. The mode of binding in the two complexes is the same with an rms deviation as low as 0.61 among all of the atoms in the two sugar molecules. The peanut lectin/*C*-lactose interactions are given in Table 2. They are identical to those in the *O*-lactose case.

In summary, the first X-ray crystal structure of *C*-lactose bound to peanut lectin has definitely established the protein-bound conformation ($\phi = 297(7)°$, $\psi = 120(2)°$)[27] of *C*-lactose. The *C*-glycosidic bond adopts the almost perfect *exo*-anomeric conformation, whereas the *C*-aglyconic bond exists in the midpoint between the conformers **A** and **C**. Most importantly, the conformation of *C*-lactose bound to peanut lectin is virtually identical to the conformation of its parent *O*-lactose ($\phi = 291(6)°$, $\psi = 118(9)°$) bound to the same protein. Interestingly, the conformation of *C*-lactose at the protein-bound state is remarkably similar to one of the two major conformations at the free state described by Berthault and co-workers.[4,28,29] Strictly speaking, the present study is only applied for the case of *C*-lactose bound to peanut lectin. The conclusion gained in the present studies, i.e., that the conformation of *C*- and *O*-lactoses bound to peanut lectin are virtually identical, is perfectly consistent with the conclusions derived from our previous work on the H-type II blood group[1], and it is tempting to claim that this is a general phenomenon unless an unusual steric or electronic factor(s) exists within molecules.

Experimental Section

Experiments for the Synthesis Shown in Scheme 1. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, and chemical shifts are reported in parts per million. The residual solvent

(26) The C.1 OH group of *C*-lactose in the subunits 1, 2, and 4 was found to adopt the α configuration, whereas that in the subunit 3 was found to adopt the β configuration. Interestingly, the same phenomenon was observed for the parent *O*-lactose case.[24a]

(27) The ϕ and ψ values given here are averaged over the four subunits of the protein.

(28) With the fixed angle ϕ, Berthault and co-workers[4] searched for the best agreement between experimental and back-calculated NOE distances, resulting in two refined structures with $\phi = 295°/\psi = 130°$ and $\phi = 295°/\psi = 300°$.

(29) The conformation of *O*-lactose bound to peanut lectin is well compared with the crystal structure ($\phi = 266°$; $\psi = 95°$) of *O*-lactose monohydrate: Beevers, C. A.; Hans, N. H. *Acta Crystallogr.* **1971**, *B27*, 1323−1325.

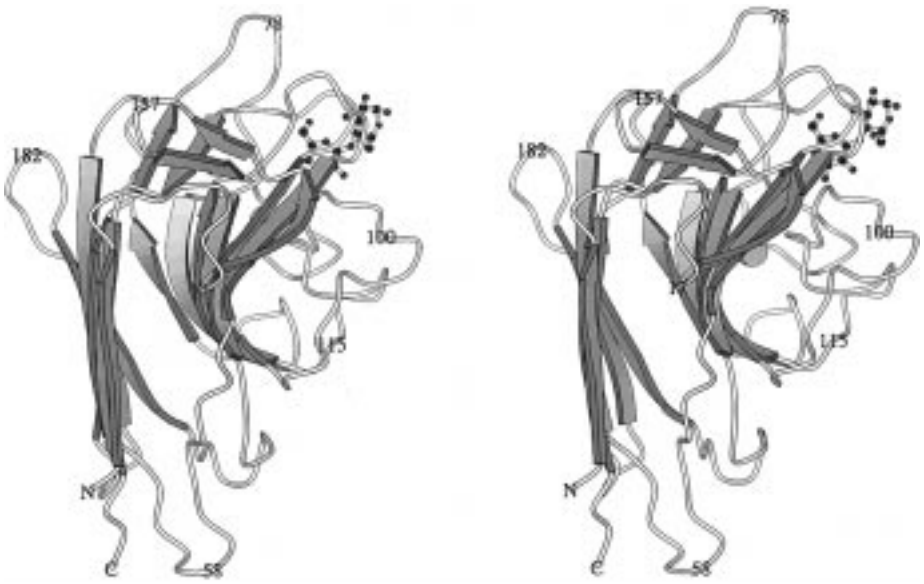


Figure 5. Stereoview of the peanut lectin monomer. The *C*-lactose molecule is in ball-and-stick representation. The figure was generated using MOLSCRIPT.[38]

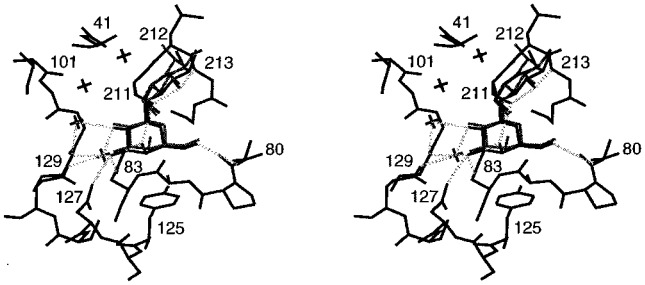


Figure 6. Stereoview of the superposition of the peanut lectin/*C*-lactose interactions. The crosses represent water molecules and the broken lines, hydrogen bonds. The *O*-lactose molecule (light lines) is also shown for comparison.

Table 2. Protein−Sugar Interactions

protein atom	sugar atom	subunit 1[a]	subunit 2[a]	subunit 3[a]	subunit 4[a]
A. Hydrogen Bonds					
Asp83 OD1	galactose O3	2.85	2.60	2.64	2.47
Gly104 N	galactose O3	3.19	3.06	2.97	2.84
Asn127 ND2	galactose O3	2.68	2.81	2.67	2.79
Asp83 OD2	galactose O4	2.73	2.69	2.85	2.65
Ser211 OG	galactose O4	2.83	2.76	2.75	2.89
Ser211 OG	galactose O5	3.00	3.16	3.42	3.08
Asp80OD2	galactose O6	3.14	3.30	3.26	3.49
Ser211 OG	glucose O3	3.45	3.06	3.16	2.77
Gly213 N	glucose O3	2.62	2.62	3.03	2.78
B. Water bridges galactose O2 connected to N Gly 104 and OE2 Glu 129 through water bridges					
C. Residues within 4 Å from the Sugar Asp80, Ala82, Asp83, Gly103, Gly104, Tyr125, Asn127, Ser211, Leu212, and Gly214					

[a] Distance in Å.

peak was used as an internal reference. Fast atom bombardment (FAB) mass spectra were obtained using 3-nitrobenzyl alcohol or glycerol as the matrix. Sodium iodide was added when indicated. Fourier transform infrared spectra were obtained as films on sodium chloride plates.

Analytical thin-layer chromatography (TLC) was performed with E. Merck precoated TLC plates, silica gel 60F-254, layer thickness 0.25 mm. Preparative TLC (PTLC) separations were carried out on E. Merck precoated plates, silica gel 60F-254, layer thickness 0.50 mm. Flash chromatography separations were performed using E. Merck Kieselgel 60 (230−400 mesh) silica gel.

Reagents and solvents are commercial grade and were used as supplied, with the following exceptions: benzene, ether, and THF were distilled from sodium benzophenone ketyl. Methylene chloride was distilled from phosphorus pentoxide. Toluene was distilled from sodium.

All reactions sensitive to air or moisture were conducted under argon or nitrogen. Reaction vessels for moisture-sensitive reactions were flame-dried or oven-dried and allowed to cool under inert atmosphere.

2-D$_2$. To a solution of aldehyde **1**[13] (0.55 g, 1.15 mmol) and 2-methyl-2-butene (5 mL) in *t*-BuOH (15 mL) was added a solution of $NaClO_2$ (1.1 g) and NaH_2PO_4 (1.0 g) in H_2O (8 mL). After 2 h at 21 °C, the mixture was concentrated and extracted with ether. The extracts were dried ($MgSO_4$), filtered, and concentrated to afford the crude acid.

A solution of the acid (0.57 g, 1.15 mmol) in ether (30 mL) was treated at 0 °C with 0.19 g (4.5 mmol) of $LiAlD_4$. After 2 h at 40 °C, the mixture was quenched with 10% NaOH solution, dried ($MgSO_4$), filtered (Celite), and concentrated. The residue was purified by chromatography on SiO_2 (hexanes/EtOAc, 2:1) to give 0.35 g (63%) of the alcohol **2-D$_2$** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.37−7.20 (15H, m), 5.01 (1H, d, J = 11.2), 4.8−4.42 (6H, m), 3.89 (1H, t, J = 9.5), 3.75−3.68 (1H, m), 3.7−3.57 (3H, m), 3.37 (3H, s), 1.84 (1H, t, J = 10.7). Note: the corresponding **2-H$_2$** is known in the literature.[12,13]

3-D$_2$. To a solution of alcohol **2-D$_2$** (0.26 g, 0.54 mmol), triphenylphosphine (0.42 g, 1.62 mmol), and imidazole (0.11 g, 1.62 mmol) in benzene (10 mL) was added iodine (0.41 g, 1.62 mmol) at 21 °C. After 1 h at 80 °C, the mixture was cooled and sequentially treated with saturated $NaHCO_3$ solution, iodine, and saturated $Na_2S_2O_3$ solution. The organic layer was separated, dried ($MgSO_4$), and concentrated. Chromatography on SiO_2 (hexanes/EtOAc, 8:1) gave 0.28 g (88%) of the iodide as an oil.

A solution of the iodide (0.28 g, 0.47 mmol) in THF (10 mL) was deoxygenated three times by freeze−pump−thaw cycle and cooled to −90 °C, followed by the addition of *n*-BuLi (0.28 mL, 0.61 mmol, 2.2 M in hexanes). The solution was stirred for 45 min at −90 °C before a solution of the galactolactone (0.39 g, 0.71 mmol) in THF (2 mL) was added. The mixture was allowed to warm to −50 °C over 1 h, quenched with aqueous NH_4Cl, and extracted with ether. The extracts were dried ($MgSO_4$), concentrated, and chromatographed on SiO_2 (hexanes/EtOAc, 7:1 to 3:1) to afford 0.38 g (81%) of the hemiketal as an oil.

A solution of the hemiketal (0.38 g, 0.38 mmol) in CH_2Cl_2 (15 mL) was cooled to −78 °C and methanethiol (10 mL) was condensed into the reaction mixture. A solution of $BF_3{\cdot}OEt_2$ (9 mL, 0.27 mmol, 0.03

M in CH_2Cl_2) was added at −30 °C via a syringe pump over a period of 2 h. The reaction mixture was quenched with $NaHCO_3$ before excess methanethiol was removed in vacuo. The residue was extracted with CH_2Cl_2, and the extracts were dried ($MgSO_4$) and concentrated. Chromatography on SiO_2 (hexanes/EtOAc, 7:1 to 3:1) provided 0.28 g (71%) of methyl thioketal **3-D₂** and 0.064 g (17%) of the recovered hemiketal as oils.

4-D₂. A stirred solution of thioketal **3-D₂** (0.28 g, 0.27 mmol), AIBN (4.4 mg, 0.03 mmol), and triphenyltin hydride (0.24 g, 0.68 mmol) in toluene (15 mL) was immersed in a preheated oil bath (110 °C). After 1 h at 110 °C, the reaction mixture was concentrated and chromatographed on SiO_2 (hexanes/EtOAc, 20:1 to 3:1) to give 0.26 g (96%) of the disaccharide **4-D₂** as an oil: ^{1}H NMR (C_6D_6) δ 7.48−7.05 (35H, m), 5.18−4.27 (14H, m), 4.78 (1H, d, J = 3.3), 4.15 (1H, dd, J = 10.9, 2.7), 4.06 (1H, dd, J = 10.2, 9.6), 3.92−3.88 (2H, m), 3.81−3.66 (4H, m), 3.67 (1H, dd, J = 9.1, 3.4), 3.56−3.53 (2H, m), 3.41 (1H, dd, J = 9.2, 2.8), 3.23 (3H, s), 2.50 (1H, t, J = 11.0); ^{13}C NMR ($CDCl_3$) δ 139.06, 138.63, 138.48, 138.38, 138.31, 137.97, 128.40, 128.23, 128.08, 127.92, 127.88, 127.81, 127.74, 127.58, 127.55, 127.47, 127.40, 127.19, 96.16, 84.94, 81.91, 79.24, 77.27, 76.57, 75.27, 74.84, 74.60, 73.92, 73.46, 73.07, 72.81, 72.09, 70.57, 70.05, 68.66, 54.81, 40.1; HRMS (FAB, NaI) calculated for $C_{63}H_{66}D_2O_{10}Na$ ($[M + Na]^+$) 1009.4834, found 1009.4836.

5-D₂. A solution of benzyl ether **4-D₂** (20 mg, 20 mmol) in MeOH/EtOAc (1:1, 5 mL) was treated with $Pd(OH)_2$ on C (20 mg) under a hydrogen atmosphere for 2 h at 21 °C. The mixture was filtered (Celite) and concentrated. The residue was chromatographed on SiO_2 (CH_3CN/H_2O, 5:1) to give 5.8 mg (85%) of the polyol **5-D₂**: ^{1}H NMR (pyridine-d_5/CD_3OD, 95/5) δ 5.13 (1H, d, J = 3.6), 4.45 (1H, d, J = 3.4), 4.43 (1H, dd, J = 11.4, 4.7), 4.37 (1H, d, J = 11.9), 4.36 (1H, t, J = 10.0), 4.31 (1H, t, J = 9.2), 4.25 (1H, dd, J = 10.6, 5.2), 4.22 (1H, m), 4.14 (1H, dd, J = 11.9, 5.3), 4.03 (1H, dd, J = 9.2, 3.4), 3.99 (1H, dd, J = 9.5, 3.6), 3.93 (1H, t, J = 5.6), 3.91 (1H, d, J = 9.3), 2.40 (1H, t, J = 10.5); ^{13}C NMR (CD_3OD) δ 101.33, 80.53, 79.41, 76.15, 74.84, 74.01, 72.46, 72.29, 71.05, 63.75, 63.21, 55.36, 49.43, 41.57; HRMS (FAB) calculated for $C_{14}H_{25}D_2O_{10}$ ($[M + H]^+$) 357.1727, found 357.1729.

6-H₂. A solution of **4-H₂** (0.56 g, 0.57 mmol) and $Sc(OTf)_3$ (42 mg, 0.09 mmol) in allyl alcohol (20 mL) was heated to 100 °C for 6 h. The mixture was cooled, treated with $NaHCO_3$, and concentrated. The residue was diluted with ether and brine. The organic layer was separated, dried ($MgSO_4$), and concentrated to give 0.65 g of the crude allyl lactoside. To a solution of the above allyl acetal in DMSO (4 mL) was added *t*-BuOK (0.6 g, 5.3 mmol). After 1 h at 90 °C, the mixture was extracted with ether, and the extracts were washed with water. Drying ($MgSO_4$) and concentration afforded 0.53 g of the crude enol ether. A solution of the enol ether in acetone/1N HCl (9:1, 20 mL) was heated to 55 °C for 1 h. The mixture was treated with $NaHCO_3$ before the acetone was evaporated off. The residue was extracted with ether, and the extracts were dried ($MgSO_4$), concentrated, and chromatographed on SiO_2 (hexanes/EtOAc, 4:1 to 2:1) to provide 442 mg (80% from **4-H₂**) of the benzyl protected lactose as an oil.

A solution of the benzyl ether (0.30 g, 0.31 mmol) in MeOH/EtOAc (1:1, 20 mL) was treated with $Pd(OH)_2$ on C (50 mg) under a hydrogen atmosphere for 12 h at 21 °C. The mixture was filtered (Celite), concentrated, and chromatographed on SiO_2 (CH_3CN/H_2O, 5:1) to afford 92 mg (87%) of *C*-lactose **6-H₂**.[12]

Experiments for the X-ray Analysis

Crystallization. The protein was prepared by affinity chromatography on cross-linked arabinogalactan.[30] The crystals of the peanut lectin/*C*-lactose complex were grown from a hanging drop of 5 mg/mL protein in 0.05 M sodium phosphate buffer, pH 7.0, containing 0.2 M sodium chloride, 0.02% sodium azide, 10 mM *C*-lactose, and 12% (w/v) PEG 8000, equilibrated against 40% (w/v) PEG 8000 in the same buffer.

Data Collection. X-ray intensity data were collected and processed to 2.7 Å on a MAR image plate detector system mounted on a Rigaku RU200 X-ray generator from a single crystal (space group $P2_12_12$, a = 129.12, b = 126.75, c = 76.90). The data were processed using the MAR-XDS[31] suite of programs. The data collection statistics are in Table 3.

Table 3. Crystallographic Data and Refinement Statistics

data collection statistics	
resolution (Å)	2.7
no. of observations	124179
no. of unique reflections	33099
data completeness (%)	93.81
R-merge[a] (%)	12.9
refinement statistics	
resolution (Å)	10−2.7
no. of protein atoms	6976
no. of sugar atoms	92
no. of solvent atoms	330
no. of other ions	4 Mn^{2+}, 4 Ca^{2+}
R-cryst ($F > 0\sigma F$) (%)	18.8
R-free ($F > 0\sigma F$) (%)	22.7
root-mean-square deviation from ideal geometry	
bond length	0.008
bond angle	1.619

[a] R-merge = $\Sigma|I - \langle I\rangle|/\Sigma\langle I\rangle$.

Structure Refinement. The coordinates of the peanut lectin tetramer in the *O*-lactose complex[24a] were used as the starting model. Refinement was carried out with data in the range 10−2.7 Å using the program XPLOR.[32] Noncrystallographic restraints were used in all but the final cycles of refinement. A difference map calculated after a few cycles of refinement showed clear density for the *C*-lactose molecules in all of the four subunits. Several rounds of fitting and refinement were done during which extensive use was made of omit-type maps.[33,34] All maps were inspected using FRODO.[35] After the fitting of the sugar in the electron density maps, water molecules were added in steps using $2F_o - F_c$ and $F_o - F_c$ maps. The model converged to a final R-value of 18.8% and R-free of 22.7%. The quality of the model was monitored using PROCHECK.[36] More than 90.0% of the residues are in the most favorable region of the Ramachandran map[37] with no residue in the disallowed region. The relevant geometric parameters are in Table 3. The coordinates and the structure factors have been deposited in the Brookhaven Protein Data Bank.

Acknowledgment. The diffraction data were collected at the National Area Detector supported by the Department of Science and Technology (DST) and the Department of Biotechnology (DBT). Facilities at the Super Computer Education and Research Centre and the DBT supported Interactive Graphics Based Molecular Modeling Facility were used for computations. Financial support from DST (M.V.) and from the National Institutes of Health (NS-12108; Y.K.) is gratefully acknowledged.

Supporting Information Available: Synthesis of **7**, diagram of vicinal coupling constants of **7-H₂**, NOESY spectrum of **7-D₂**, and Ramachandran map of the peanut lectin bound *C*-lactose (5 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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