A Glycosidic Linkage Constrained to the "Anti" Conformation

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Received January 29, 1999

The glycosidic linkages of oligopyranoses populate the so-called "syn" conformation which is characterized by a close-to-parallel orientation of the transglycosidic C−H bonds.[1] Until now, no glycosidic bond was described which is constrained in solution entirely to the "anti" conformation, i.e., with the transglycosidic C−H bonds turned from each other by ∼180°. "Anti" rotamers were identified as transient intermediates of *O*-glycosides[2] and they are discussed for *C*-glycosides;[3] populations higher than 10% are found for *S*-glycosides.[4] "Band flips" are observed in solid-state structures of cycloamyloses where fast conformational equilibria dominate in solution.[5] "Anti" rotamers are also well-known from computational studies in which they are observed as secondary energy minima for several types of glycosidic linkages.[6] Secondary energy minima are of importance for the discussion of receptor-bound conformations of oligosaccharides,[7] and constrained carbohydrate analogues are of interest for medicinal chemistry.

A *m*-xylylene bridge constrains the D-Glcβ(1→3)D-Glc disaccharide unit of **1** within a 14-membered ring. Synthetic details of the intramolecular glycosylation by activation of the anomeric position of ring b were described.[8] Compound **1** was investigated by homo- and heteronuclear NMR methods. Figure 1 shows two expansions from a compensated ROESY spectrum[9] (O1 = 6.9 ppm, 4 kHz pulsed spin lock, 200-ms mixing time) of **1**. Spin diffusion is negligible under these experimental conditions, and the volume integral of each cross-peak correlates to a single interproton distance (two-spin approximation).[10] Conformational homogeneity of **1** is indicated by the well-separated ROE cross-peak intensities of the three methylene groups within the 14-membered macrocycle. The ROESY expansions in Figure 1 show an intense through-space correlation between H7x^{proS} and H1a, while the cross signals between H7x^{proR} and glucose a are very weak. Cross signals of similar intensity are detected between H8x^{proR} and both 6b protons, while H8x^{proS} shows no correlation to glucose b. The exocyclic methylene group of ring b exclusively populates the *gg* conformation with ω (H5−C5−C6−O6) = 180° [^3J(H5b−H6b^{proR}) = 2.1 Hz, ^3J(H5b−H6b^{proS}) = 1.7 Hz]. H2x points into the 14-membered ring and acts as a well-separated NMR probe at 8.04 ppm. Four ROEs are visible between H2x and protons of the macrocycle; an expansion of this region is

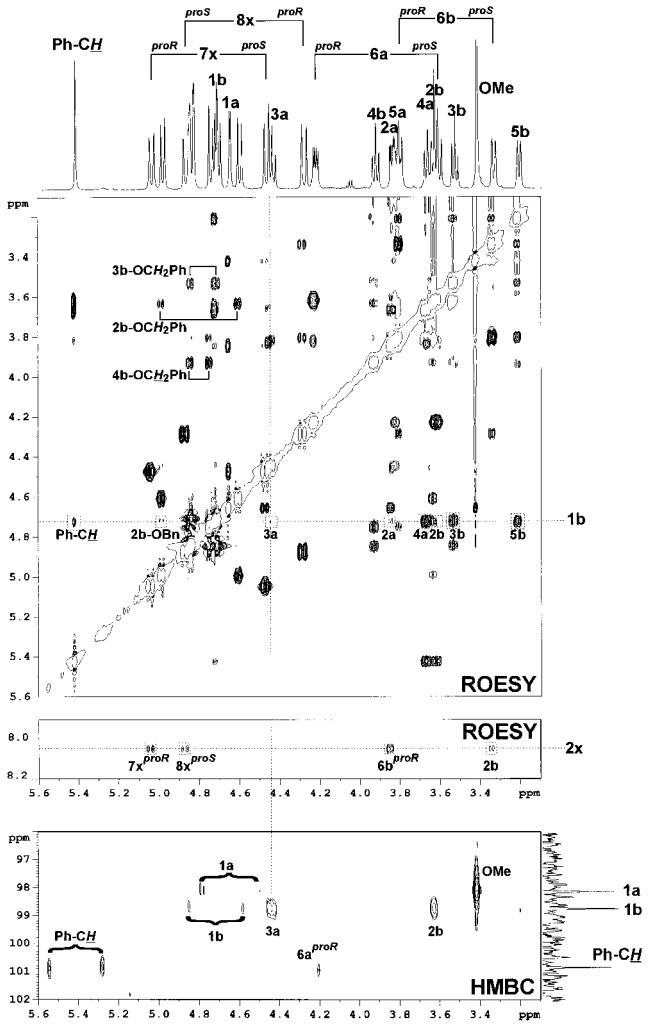

Figure 1. ^{1}H NMR of **1** (600 MHz, 300 K, $CDCl_3$) showing a single well-dispersed signal set. The cross signal intensities in the ROESY spectrum of **1** correspond to interproton distances between 1.8 and 4 Å. The absence of a transglycosidic ROE between H1b and H3a (upper ROESY expansion, crossing of broken lines) indicates the "anti" conformation of the glycosidic linkage. H1b and the high-field resonance of 3b-OCH_2Ph are overlapping in the ROESY, therefore, cross signals to H2b and to H3b are the sums of both ROEs. The anomeric region of the gradient-selected HMBC spectrum displays a transglycosidic $^3J_{C,H}$ correlation between C1b and H3a, proving the D-Glcβ(1→3)D-Glc glycosidic linkage. Brackets indicate the residual $^1J_{C,H}$ couplings.

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10.1021/ja990294s CCC: $18.00
Published on Web 06/16/1999

Table 1. Average Interproton Distances (ROE) Which Served as Experimental Restraints for the Molecular Dynamics Simulation of **1**

	ROE[a]		ROE[a]
H1a−H7x^{proS}	230	H6b^{proS}−H2x	310
H2a−H7x^{proR}	365	H6b^{proS}−H8x^{proS}	450
H2a−H2x	265	H6b^{proS}−H8x^{proR}	265
H2x−H7x^{proS}	400	H6b^{proR}−H8x^{proR}	270
H2x−H7x^{proR}	290	H4a−CH−Ph	225
H6x−H7x^{proS}	255	H6a^{proS}−CH−Ph	235
H6x−H7x^{proR}	360	H1b−CH−Ph	330
H2x−H8x^{proS}	300	H1b−H2a	315
H2x−H8x^{proR}	410	H1b−H4a	205
H4x−H8x^{proS}	330	H2b−H2x	385
H4x−H8x^{proR}	260	H4b−H2x	360

[a] Picometers.

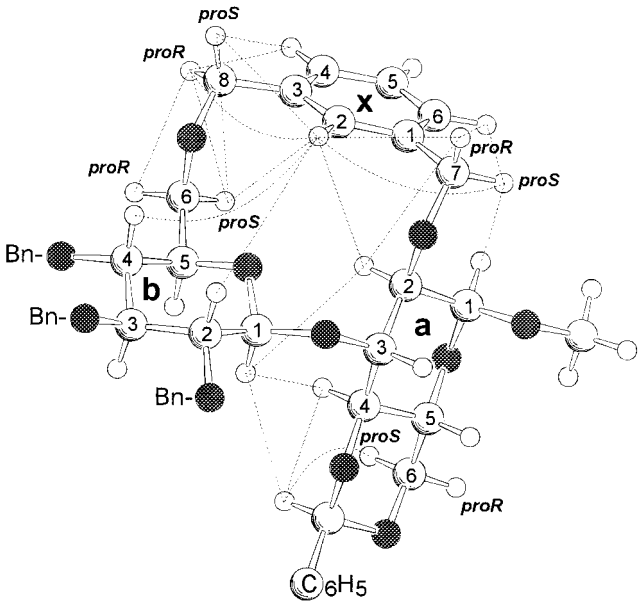


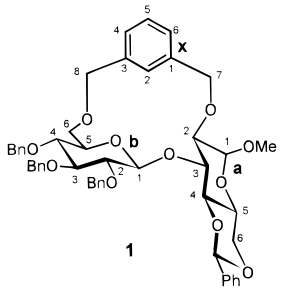
Figure 2. Energy-minimized average conformation of a 100-ps molecular dynamics simulation of **1**. ROE-derived proton−proton distances which served as experimental distance restraints are included as broken lines. The benzyl and benzylidene protecting groups are not shown.

included in Figure 1. The unusual relative orientation of the pyranose rings is also directly visible in the ROESY spectrum. The very weak transglycosidic ROE (H1b to H3a) and the strong ROE between H1b and H4a indicate the "anti" conformation. The HMBC spectrum[11] (Figure 1) proves the proper D-Glcβ(1→3)D-Glc connectivity of disaccharide **1**.

The solution conformation of **1** is solvent-independent, since neither ROE intensities nor $J_{H,H}$ couplings change upon the addition of a second solvent like DMSO-d_6. $J_{H,H}$ couplings are in full accordance with $^4C_1$ conformations of the pyranose rings.

All ROEs were integrated and offset-corrected, and average cross-peak intensities of the three well-separated geminal proton pairs 6a, 7x, and 8x were calibrated to 1.8 Å. The isotropic tumbling assumption is reasonable for **1** since the six cross-peak intensities deviate less than 10% from the average value. The proton−proton distances listed in Table 1 were calculated according to the r^{-6} dependence (r = interproton distance) of the NOE.[12] These average distances served as restraints in a molecular dynamics simulation. Weak torsional restraints were included for the *gg* rotamer about ω and for the exoanomeric conformation[13] of pyranose b.[14] Glycosidic angles of ring b were

Scheme 1



determined to $\phi = 47°$ ($\phi = H^{1'}-C^{1'}-O^3-C^3$) and $\psi = -161°$ ($\psi = C^{1'}-O^3-C^3-H^3$). An average structure of **1** is shown in Figure 2. The 22 nontrivial distance restraints for **1** (Table 1) are in accordance with a single conformation of the disaccharide moiety, and conformational averaging can be excluded,[15] the conformational homogeneity of **1** being a requirement for the direct quantification of NOEs.[16]

The *m*-xylylene bridge spans an O−O distance of about 5 Å within 14-membered macrocycles,[17] but the distance between O6b and O2a in a low-energy "syn" conformation of the D-Glcβ-(1→3)D-Glc disaccharide unit is in the region of 7−8 Å. Therefore, the intramolecular glycosylation must result in a distorted disaccharide structure. The −161° ψ angle of the glycosidic linkage in **1** allows the ring closure by intramolecular glycosylation and at the same time maintains a spacing of 5.1 Å between O6b and O2a.

Several synthetic bridged di- and trisaccharide analogues have been described in the literature.[17,18] Compound **1** is the first example where a scarcely populated high-energy disaccharide conformation is "frozen" in a macrocycle so it can be studied as the main conformation in solution. This concept is not restricted to the D-Glcβ(1→3)D-Glc linkage but should be applicable to identify and characterize secondary energy minima of other types of glycosidic linkages.

Acknowledgment. This research was supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie.

Supporting Information Available: ^{1}H NMR and ^{13}C NMR chemical shifts of **1**, DQF-COSY, TOCSY, and HMQC spectra, and the average solution conformation (PDB). NMR investigation of the benzylidene deprotected compound. This material is available free of charge via the Internet at http://pubs.acs.org.

JA990294S

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(14) A 100-ps molecular dynamics simulation with the experimental proton−proton distances included as weak (7 kcal mol^{-1} Å^{-2}) additional restraints was performed with the MM+ force field which is included in the *HyperChem* program package (Hypercube Inc.). Benzyl protecting groups were exchanged for methyl groups for the simulation. Structure sampling and energy minimization were carried out according to ref 17.

(15) The benzylidene group was selectively removed, and the deprotected compound was investigated in the solvent mixture D_2O/DMSO-d_6 (1/1; mole/mole). This solvent mimics an amphiphilic environment (Fesik, S. W.; Olejniczak, E. T. *Magn. Reson. Chem.* **1987**, *25*, 1046−1048; Motta, A.; Picone, D.; Tancredi, T.; Temussi, P. A. *J. Magn. Reson.* **1987**, *75*, 364−370; Geyer, A.; Müller, G.; Kessler, H. *J. Am. Chem. Soc.* **1994**, *116*, 7735−7743). As a result of to the rigidity of the macrocycle, no changes in the relative ROE intensities were observed. The anti conformation is neither influenced by the protecting groups nor by the solvent polarity (details are in the Supporting Information).

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