support, and we are grateful to Kirin Breweries, Co., Ltd. for ongoing support of this research.

Supplementary Material Available: Experimental details, elemental analysis data, and ^1H NMR, ^{13}C NMR, and IR spectral data, figures showing the inhibition of SE and OSC plus SE by dodecanol, dodecanethiol, **4a,c-g,j-l**, and **5**, and a plot of the time dependency of SE inactivation by **4i** (33 pages). Ordering information is given on any current masthead page.

Hydroxyl and Amido Groups as Long-Range Sensors in Conformational Analysis by Nuclear Overhauser Enhancement: A Source of Experimental Evidence for Conformational Flexibility of Oligosaccharides

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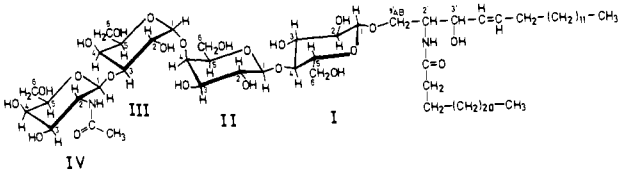
The three-dimensional (3D) structure, or conformation, of biooligomers is known to play a decisive role in their biological activity. One of the most important methods for the determination of the 3D structure of biomolecules is nuclear Overhauser enhancement (NOE) spectroscopy, which enables one to detect proximity in space between protons located in different, yet spatially neighboring, parts of the molecule. Although formally this applies to all classes of molecules, the analysis of oligosaccharide conformation is heavily handicapped as compared to that for proteins,[1] for example, because the number of NOE contacts observed is smaller by almost one order of magnitude. These contacts are usually restricted to interactions between the protons linked to the two carbon atoms at the glycosidic bridge (the anomeric and the aglyconic one), other contacts being rare. An important unfavorable consequence is that, in most cases, the amount of experimental data available for an adequate interactive fit of the theoretically calculated conformation(s) is insufficient to warrant a reliable description of a conformation or a possible conformational equilibrium. The problems arising in this connection have been discussed in detail by several research groups.[2]

We show here a way of supplementing this source of structural information by investigating NOE contacts with unexchanged hydroxyl and amido groups. Protons of these groups protrude farther from the carbon skeleton than the C-linked protons and provide a great number of additional distance constraints that may confirm or disprove hypothetical conformers obtained by energy minimum calculations. We illustrate this approach by analyzing the spectra of native (unexchanged) globoside.



The resonances of the C-linked protons of D_2O-exchanged globoside have been assigned previously[2c,3] and, allowing for their small temperature shifts, confirmed here by one-dimensional (1D) total correlation spectroscopy (TOCSY;[4a] synonymous with homonuclear Hartmann-Hahn spectroscopy—HOHAHA[4b]). Since the deuterium isotope effect on protons separated by three bonds is small, these CH resonances remain practically unchanged in the native globoside, and their scalar coupling connectivities with the OH and NH resonances provide unequivocal assignments of the latter (Figure 1a,b and Table I).

The dipolar coupling connectivities were obtained by rotating frame NOE experiments (ROESY;[5a-c] synonymous with CAMELSPIN[5d]). A z-filter was added for suppression of scalar coupling cross peaks, and the rf carrier frequency was offset away from the region of sugar proton resonances during the spin lock time and then returned to the middle of the spectrum during acquisition for better digital resolution[5c] (Figure 1c). ROESY

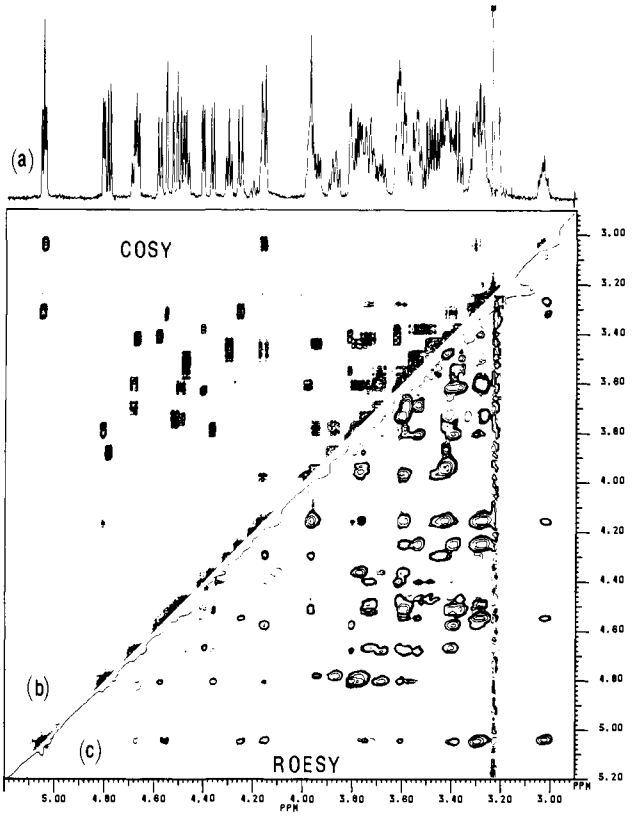


Figure 1. Partial 500 MHz ^1H NMR spectra of globoside in Me_2SO-d_6 at 315 K. (a) 1D spectrum; (b) scalar coupling autocorrelated (COSY) spectrum; (c) ROESY spectrum obtained with a mixing time of 200 ms. The diagonal and the exchange cross peaks are drawn with a single contour line; NOE cross peaks are filled.

Table I. Chemical Shifts for Globoside in Me_2SO-d_6 at 315 K

residue		1	2	3	4	5	6a	6b
GalNAcβ-IV	CH	4.53	3.75	3.43	3.63	3.39	3.55	3.51
	OH		7.62[a]	4.67	4.41		4.48	
Galα-III	CH	4.81	3.79	3.61	3.99	4.17	3.49	3.45
	OH		4.37		3.78		4.31	
Galβ-II	CH	4.26	3.32	3.42	3.83	3.56	3.61	3.70
	OH		5.05	4.58			4.69	
Glcα-I	CH	4.16	3.05	3.32	3.32	3.29	3.62	3.75
	OH		5.04	4.55			4.50	

[a] NH.

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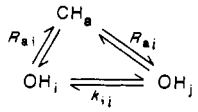
Table II. Interresidue NOE Contacts Observed at Several Temperatures in the 290–333 K Range in the ROESY Spectrum of Globoside, Dissolved in Me_2SO-d_6

IV-H1/III-H3	IV-H1/III-OH2	IV-NH/III-OH2
IV-H1/III-H4	IV-H1/III-OH4	
	IV-OH6/III-H4	
III-H1/II-H4	III-H1/II-OH3	III-OH2/II-OH6[a]
III-H1/II-H6a	III-H1/II-OH6	III-OH6/II-OH3
III-H1/II-H6b	III-H5/II-OH3	
III-H5/II-H2	III-OH2/II-H6b[b]	
III-H5/II-H4	III-OH6/II-H2	
	III-OH6/II-H4	
II-H1/I-H4	II-OH2/I-H6a	II-OH2/I-OH3
	II-OH2/I-H6b	II-OH2/I-OH6
	II-H1/I-OH6	II-OH6/I-OH3
	II-H1/I-OH3	
	II-H6R/I-OH3	
	II-H6S/I-OH3	

[a] We also found a hydrogen bonding between these two protons for the 50% deuteriated globoside, by using the method developed by Lemieux and Bock[2e,11] and Christofides et al.[12] [b] II-H6a was overlapped.

is preferable in this case to conventional (laboratory frame) NOESY, since the ROESY cross peaks due to cross relaxation are negative, whereas those arising from proton exchange or indirect NOEs are of the same sign as the diagonal peaks (positive) and can readily be distinguished. Besides, spin diffusion is no problem with ROESY, whereas NOE spectra, particularly those of higher oligomers, can be completely misleading by exhibiting spin diffusion signals simulating nonexistent spatial proximities. These two characteristics, which are of critical significance to the method suggested here, had not been duly taken into account in a similar study of amylose and nigeran by conventional NOESY.[6] Furthermore, it is important for the quantitative interpretability of ROESY spectra[5b] that rotating frame cross relaxation is relatively insensitive to the anisotropy of molecular tumbling.[8]

A negative cross peak produced by a C-linked proton (CH_a) and an exchangeable one (OH_i) can only be caused by their direct mutual cross relaxation. In order to decide whether this cross peak is diagnostic of the spatial proximity of these protons, let us consider a possible proton exchange with another hydroxy group (OH_j) that is distant enough for the cross relaxation rate R_{aj} to be negligible. With short mixing time applied and low proton



exchange rate, $k_{ij} \lesssim R_{ai}$, the CH_a/OH_i cross peak intensity will practically depend on R_{ai} alone and be diagnostic of the H_a–H_i distance. With more rapid exchange, $k_{ij} \gg R_{ai}$, this cross peak will diminish, and one for CH_a/OH_j will arise at its expense, thereby simulating a CH_a–OH_j proximity. Since we could have begun the analysis at OH_j, this uncertainty will apply to both of these cross peaks. Fortunately, it is easy to avoid a misinterpretation of this kind, since a positive OH_i/OH_j cross peak would betray the misleading exchange. In practice, only few exchange cross peaks were observed at 315 K (Figure 1c), and only one (IV-OH4/IV-OH6) was left at 298 K, whereas the intensities of the NOE-type cross peaks remained practically unaffected. Under these conditions of frozen exchange, the negative NOE cross peaks produced by protons of two exchangeable groups also became diagnostic of spatial proximity.

The interresidue NOE and exchange contacts derived from the ROESY spectrum are summarized in Table II. The contacts between C-linked protons, grouped in the left column, are the same, as those found by Scarsdale et al.[2c] (III-H5/II-H2 and III-H5/II-H4 are additional). We could not confirm, however, the contacts between II-H1 and either of the two methylene protons of the Glc-I residue. These authors concluded that the II-I and III-II glycosidic linkages are adequately described by the one-state model by using the pseudoenergy approach (i.e., that each linkage is represented by one conformation). For the IV-III linkage, indications of conformational averaging between two states were presented, and this was considered as possibly connected with the terminal position of the GalNAc-IV residue.[9]

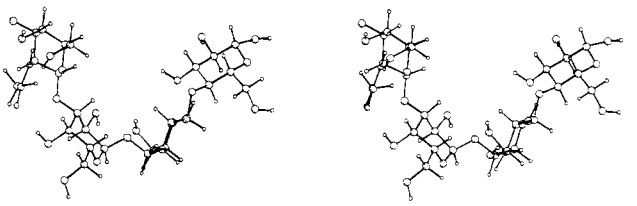


Figure 2. Stereo plot (PLUTO) of the conformation of globoside (without the ceramide) at its energy minimum, characterized by the following value of the Φ and Ψ angles for the particular linkages: IV-III, 55, 0; III-II, −41, −13; II-I, 49, 0.

The interresidue NOE cross peaks referring to hydroxyl and amido protons provide an additional set of data for the determination of 3D structure in a similar way as is currently being done for proteins[1] and oligonucleotides.[1,10] In this communication we shall restrict the discussion to the III/II linkage, for which the conformational implications of these contacts are obvious without a quantitative treatment (cf. Figure 2). Thus, all NOEs between C-linked protons and some of the NOEs between O- and C-linked protons, III-H1/II-OH6, III-H5/II-OH3, III-OH2/II-H6b, and III-OH6/II-H4 as well as those between hydroxyl protons, III-OH2/II-OH6 and III-OH6/II-OH3, are fully compatible with the conformation calculated here with the aid of the SUGAR program[2l] and with the slightly different conformations obtained by other authors with the HSEA program.[2c,e] At the same time, the III-H1/II-OH3 and III-OH6/II-H2 NOEs require a significant increase of the Ψ angle and probably a slight increase (decrease of the absolute value) of the Φ angle. Thus, conformational averaging was found here for the Gal-III residue, for which a single state was diagnosed by using the pseudoenergy method[2c] (cf. ref[9]). One conformation was also obtained for galabiose,[2e] a disaccharide identical with the III-II fragment of globoside. It should be emphasized that residue III is an internal one, i.e., flexibility does not seem to depend on the terminal position in the oligosaccharide chain. This example shows that by putting to use the interatomic contacts revealed by long-range "sensors" such as protons of hydroxy and amido groups, new structural information can be gained, and this may necessitate a revision of conformation(s) based on a smaller number of interactions between C-linked protons alone. Our forthcoming quantitative analysis of this problem will show how the two approaches compare.

Finally, another very helpful use of native oligosaccharides should be mentioned. The signal of an O-linked proton of a hydroxymethyl group is readily recognizable by its quasi-triplet fine structure, and its connectivity cross peaks with the usually strongly overlapped signals of C-linked protons of this group are capable of furnishing the $J(5,6)$ and $J(5,6')$ coupling constants, which are indispensible for conformational analysis. We obtained the best results by applying the 1D HOHAHA[4b] technique for this purpose.

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