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CONFORMATIONAL ANALYSES OF α AND β(1-6) MANNODISACCHARIDES BY DEUTERIUM SUBSTITUTION EFFECT ON RELAXATION RATE AND NOE

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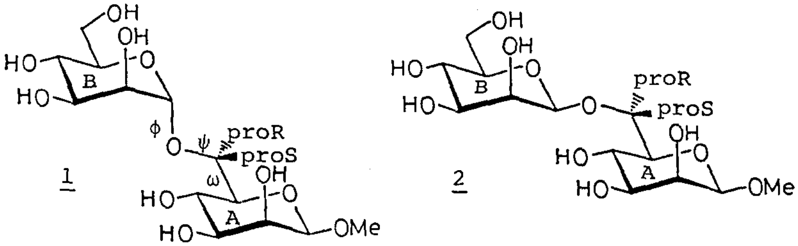
Summary:The study of ^{1}H differential relaxation rate and NOE using chirally deuterated sugars, revealed the preferred conformation of each α and β (1-6) linkage of oligomannoses in solution.

Conformational analyses of oligosaccharides in solution are important to rationalize their biological properties. Since the ring conformations of each pyranose units are rigid chair form with a few exception, overall conformation of an oligosaccharide is governed by torsion angles (ϕ and ψ) about the glycosidic linkage (Fig. 1). In the case of (1-6) linkages, an additional C5-C6 bond (ω angle), which is not glycosidic linkage, further complicate the conformational analyses. The origin of this complication is the difficulty to discreminate H-6<u>proS</u> and H-6<u>proR</u> protons on these ^{1}H-NMR spectra. We have already settled this problem by using hexoses chirally deuterated at C-6[1-3] and unequivocally elucidated the rotamer distributions about C5-C6 bonds (ω angle) on the basis of $J_{5,6\underline{proS}}$ and $J_{5,6\underline{proR}}$ values[4,5]. In this paper, we wish to describe the use of deuterium substitution effect on relaxation time (DESERT)[6-8] as well as NOE for the elucidation of the preferential ϕ (H1'-C1'-O1'(6)-C6) and ψ(C1'-O1'(6)-C6-C5) angles for the both α and β(1-6) mannobioses.

Methyl 6-O-α-D-mannopyranosyl-β-D-mannopyranoside <u>1</u>, which is an model of core portion of N-linked oligosaccharide and its isomer methyl 6-O-β-D-mannopyranosyl-β-D-mannopyranoside <u>2</u> and the corresponding chirally deuterated compounds <u>S1</u>, <u>R1</u>, <u>S2</u> and <u>R2</u> were prepared to be analyzed[11] (Fig. 1).



prefix	proS	proR
–	H	H
S	D	H
R	H	D

Fig. 1 Three torsion angles about (1-6) linkage and structures of the disaccharides <u>1</u>, <u>S1</u>, <u>R1</u>, <u>2</u>, <u>S2</u> and <u>R2</u>.

The signals of H-6proS and H-6proR protons of 1 and 2 were unambiguously assigned by comparison with the spectra of S1, R1, S2 and R2 (Fig. 2). The chemical shift of H-6proR (3.98 ppm) was more deshielded than that of H-6proS (3.84 ppm) in the α(1-6) biose 1 and the relation inversed in the β(1-6) biose 2 (3.88 and 4.25 ppm, respectively). This unequivocal assignment is the foundation for the following disscusion. Similar change of the chemical shift of the two protons according to the anomeric configulation of the (1-6) linkages had been found for α and β(1-6) gluco- and galactobioses by the use of chirally deuterated sugars by us5). The effect of ring A on the chemical shifts of the H-6 in 1 should be equal to that in 2 because similar rotamer distributions about the ω angles for 1 (gg:gt:tg=58:42:0) and 2 (gg:gt:tg=56:44:0) calculated from $J_{5,6}$ ($J_{5,6proS}$=1.9 and $J_{5,6proR}$=5.6 Hz for 1 and 1.9 and 5.8 Hz, respectively, for 2) were found. Hence, the difference of the chemical shifts of these protons between 1 and 2 could be ascribed to the effect of ring B and reflected the φ and ψ angles which determined the orientation of the ring B.

The ^{1}H spin-lattice relaxation rates (R_1 values) of the deuterated and the non-deuterated bioses were measured by non-selective inversion recovery method in 0.1M D_2O solution at 270 and 400 MHz at 298 K. The results were summarised in Table where ΔR_1 values was defined as the difference between a R_1 value for a certain proton of non-deuterated compound and that of the corresponding deuterated compound. This parameter is proportional to inverse sixth power of internuclear distance between a certain proton and one exchanged with a deuteron under appropriate condition6-8). Since the difference of R_1 values measured at 270 and 400 MHz were little, these measurements were under extreme narrowing condition. The deuterium substitution at C-6 did not effect on the relaxation rate (ΔR_1=-0.02∼+0.05 sec^{-1}) for H-1 of both α and β(1-6) bioses because of long distance between H-1 and H-6, while did cause significant effect on the relaxation rate for H-1'; deuteration at 6proS gave larger ΔR_1 value(-0.29 sec^{-1}) of H-1' than that (-0.10 sec^{-1}) obtained by deuteration at 6proR for 1, on the other hand deuteration at 6proR caused larger ΔR_1 value (0.23 sec^{-1}) than deuteration at 6proS (-0.16 sec^{-1}) for 2 (Table). These results suggested that H-6proS was closer proximity to H-1' than H-6proR at the α(1-6) linkage of 1, whereas H-6proR was in closer proximity to H-1' than H-6proS at the β(1-6) linkage of 2.

Further, in three staggered conformers about φ angle, the facts that deuteration at either 6proS and 6proR caused little effect on the R_1 values of H-2' and H-3' of 1 and H-2', 3' and 5' (ΔR_1=-0.01∼+0.04 sec^{-1}) of 2 excluded the conformers (φ=+60° and 180° for 1 and φ=-60° for 2. The R_1 values of H-5' of 1, S1 and R1 could not be obtained due to peak overlap.) in which H-6 and H -2' or 3' were in close proximity with each other. Therefore the preferred rotamer about φ angle was near to -60° for 1 and +60° for 2, which supported exo-anomeric effect10).

Similar conformations were also deduced by NOE experiments on saturation

Table R_1 and ΔR_1 values of the α and β(1-6) mannodisaccharides.

compd.	R_1 (sec^{-1})					$\Delta R_1{}^c$ (sec^{-1})				
	H-1	H-1′	H-2′	H-3′	H-5′	H-1	H-1′	H-2′	H-3′	H-5′
1	1.57^a(1.64)^b	0.97(0.93)	0.89^d	0.99	e					
S1	1.60(1.72)	0.68(0.70)	0.88^d	1.03	e	+0.03	-0.29	-0.01	+0.04	e
R1	1.55	0.87	0.89^d	0.97	e	-0.02	-0.10	0.00	-0.02	e
2	1.50	1.97	0.85	1.18	1.28					
S2	1.54	1.81	0.87	1.20	1.27	+0.04	-0.16	+0.02	+0.02	-0.01
R2	1.55	1.58	0.87	0.86	1.31	+0.05	-0.23	+0.02	-0.01	+0.03

a) measured at 400 MHz. b) measured at 270 MHz. c) on the basis of R_1 values measured at 400 MHz. d) average of R_1 and ΔR_1 value for H-2 and 2′ which overlapped each other. e) could not be obtained due to peak overlap.

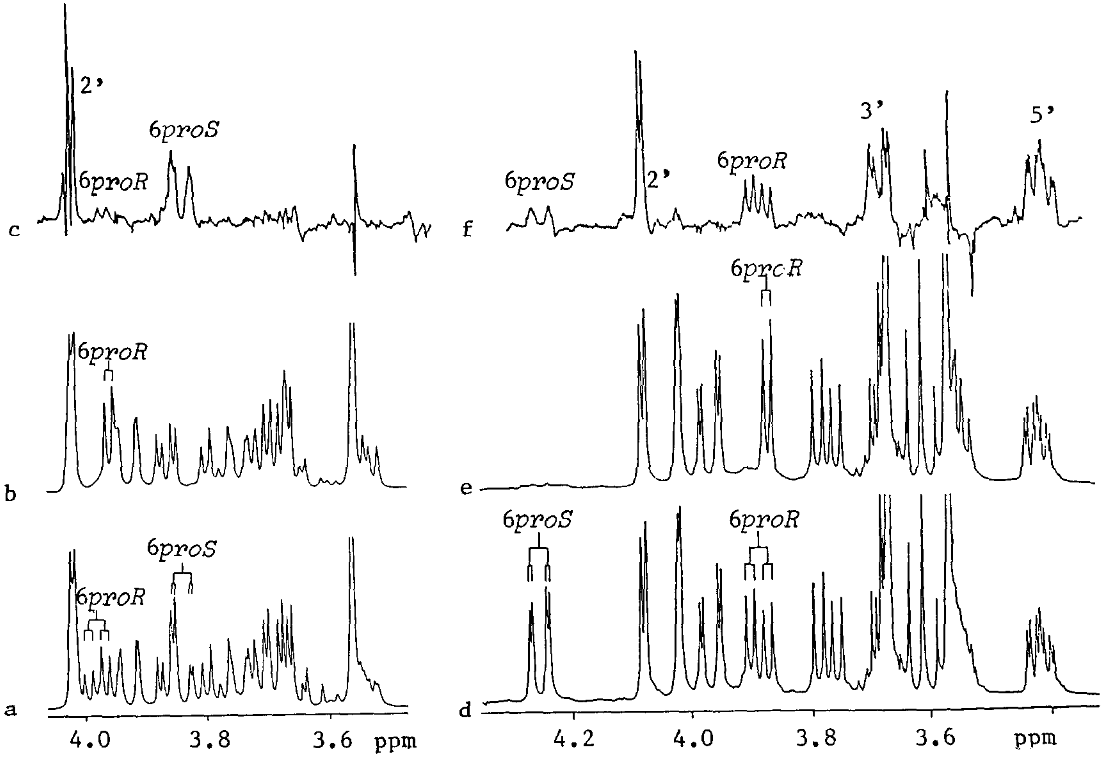


Fig. 2 ^{1}H-NMR spectra of a) 1, b) S1, d) 2 and e) S2 and differential NOE spectra of c) 1 and f) 2 saturated at H-1′ in D_2O at 400 MHz.

at H-1′ of 1 and 2. While the NOE was observed more intensively at H-6proS (4.9%) than at H-6proR (~1%) for 1, it was observed more intensively at H-6proR (5.8%) than at H-6proS (1.9%) for 2. Other strong enhancements were also observed at H-2′ (7.0%) of 1 and H-2′, 3′ and 5′ (8~9%) of 2 because of their close proximity to H-1′.

We concluded that the preferred conformations about ϕ and ψ angles could be assigned near -60° and 180° for 1, and +60° and 180° for 2, respectively (Fig. 3) on the basis of the NOE and the ΔR_1 values together with the consideration of down-field shifts of H-6proR of 1 and H-6proS of 2 which would be ascribed to approach of O-5′ to these protons. Although the similar conformations have been posturated for the related compounds 9), our chirally deuterated disaccharides gave for the first time unequivocal experimental evidences for them.

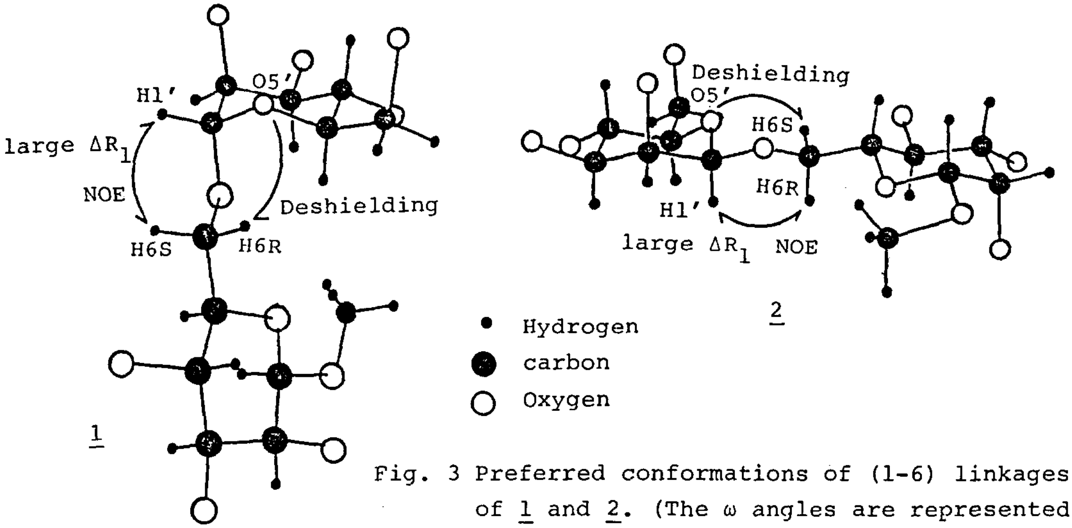


Fig. 3 Preferred conformations of (1-6) linkages of 1 and 2. (The ω angles are represented as -60°)

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