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CONFORMATIONAL ANALYSES OF O-4,O-6-BRANCHING TRI-D-GLUCOPYRANOSIDES; INFLUENCE OF O-4 LINKED RESIDUES ON SOLUTION CONFORMATIONS ABOUT C5-C6 BONDS AT (1-6)-LINKAGES

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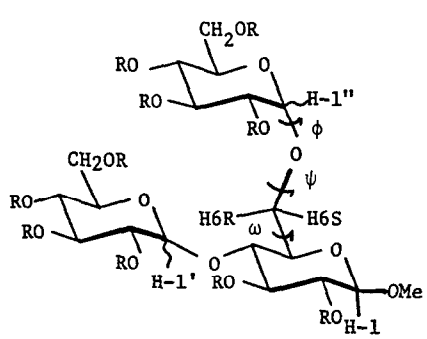
Summary: Preferred conformations about α and β(1-6)-linkages of four types of O4,O6-branching trisaccharides **I-IV** were studied to examine the influence of the possible interaction between the O-4 and O-6 linked residues on the solution conformations.

In the course of our studies with chirally deuterated sugars,[1,2] C6-specifically deuterated hexoses[1] have been successfully used to determine the conformational properties (ϕ, ψ, ω angles, Fig. 1) about the C5-C6 bonds of hexoses[3-6] (ω on the basis of $J_{H5,H\text{-}6proR}$ and $J_{H5,H\text{-}6proS}$) and of (1-6)-linked disaccharides[7,8] (ϕ, ψ, ω on the basis of DESERT,[8] NOE, chemical shifts of the H-6proR and H-6proS and the $J_{H5,H6}$ values). Here, our method was applied to the conformational analysis of O4, O6-branching tri-D-glucopyranosides **I-IV** and their per-O-acylated derivatives[9] (Fig. 1) and we would like to describe the results, especially the new NOE enhancements on saturation at H-1' which are useful for the conformational analysis of O4,O6-branching trisaccharides, the influence of O-4 linked residues on the conformations of the (1-6)-linkages and the comparison of our results with those by HSEA (Hard-Sphere exoanomeric) calculations[10] carried out by GESA program.[11]

The general features of the relative chemical shifts of the two prochiral protons (table 1) were consistent with our previous results[7,8] δH-6proR > δH-6proS for **I-Ib** and **III-IIIb** on the α(1-6)-linkage and δH-6proS > δH-6proR for **II-IIb** and **IV-IVb** on the β(1-6)-linkage. This strongly indicated that the trisaccharides prefer conformations in which the downfield protons are in close proximity to the ring oxygen of the O-6 residue[8] (Figs. 2a and 2b).



R=H	Acetyl	Benzoyl	Configurations (1-4)	(1-6)
I	**Ia**	**Ib**	α	α
II	**IIa**	**IIb**	α	β
III	**IIIa**	**IIIb**	β	α
IV	**IVa**	**IVb**	β	β

Fig. 1 Model compounds I - IV and their per-O-acylated derivatives. Three anomeric protons H-1, H-1' and H-1", two prochiral protons H-6proR and H-6proS, and three angles ϕ, ψ and ω angles about (1-6)-linkages are defined as previously.[8]

Table 1 NMR Data of H-6proR and H-6proS Signals [9] and Rotamer Populations of C5-C6 Bonds.

Compound	Solvent	Chemical Shift (ppm) [a] H-6proR (J_{vic})	H-6proS (J_{vic}) [b]	Populations (%) A [c] gg	gt	tg	B [c] gg	gt	tg	C [c] gg	gt	tg
I	D_2O	3.98 (5.2)	3.92 (1.8)	62	38	0	67	46	-14	66	40	-5
Ia	$CDCl_3$	3.93 (4.5)	3.89 (2.1)	67	30	4	72	38	-10	71	31	-2
Ib	$CDCl_3$	3.90 (5.6)	3.90 (1.8)	58	43	-1	63	50	-14	61	44	-5
II	D_2O	3.90 (5.4)	4.25 (1.8)	60	40	0	65	48	-14	63	42	-5
IIa	$CDCl_3$	3.82 (6.2)	4.07 (2.0)	52	48	0	56	55	-12	53	50	-3
IIb	$CDCl_3$	3.93 (6.7)	4.29 (1.6)	50	55	-5	54	62	-16	51	57	-8
III	D_2O	4.03 (4.8)	3.92 (1.8)	66	34	0	71	43	-14	70	35	-5
IIIa	$CDCl_3$	3.99 (2.9)	3.78 (1.6)	84	15	1	91	25	-16	92	15	-7
IIIb	$CDCl_3$	3.73 (3.5)	3.65 (1.5)	79	22	-1	86	31	-17	86	22	-8
IV	D_2O	3.96 (3.8)	4.31 (2.1)	73	22	5	79	31	-10	78	24	-2
IVa	$CDCl_3$	3.81 (5.7)	4.04 (1.8)	57	44	-1	62	51	-14	60	45	-5
IVb	$CDCl_3$	3.56 (4.9)	3.85 (1.7)	65	36	-1	71	44	-15	70	36	-6

a) Measured at 400 MHz using the C-1 - OMe signal as a reference (3.580 ppm) for OH sugars in D_2O, as reference (3.155 ppm) for Bz sugars in $CDCl_3$, and using the $CHCl_3$ signal as reference (7.250 ppm) for Ac sugars.

b) Obtained by first-order analysis for Ac and Bz sugars (ref. 9) and by iterative spin-simulations for OH sugars. ($\pm$ 0.2 Hz).

c) Equation A: As/Ar = 1.3/1.3 Bs/Br = 2.7/11.5 Cs/Cr = 11.7/5.8 (ref. 13)
B: 2.9/1.0 3.0/11.2 11.2/4.9 (ref. 13, 14, 15)
C: 2.2/1.7 2.4/10.8 11.1/4.1 (ref. 6, 14)

for general form

$$As\underline{gg} + Bs\underline{gt} + Cs\underline{tg} = J_{H5,H\text{-}6proS} \quad --(1)$$

$$Ar\underline{gg} + Br\underline{gt} + Cr\underline{tg} = J_{H5,H\text{-}6proR} \quad --(2)$$

$$\underline{gg} + \underline{gt} + \underline{tg} = 1 \quad --(3)$$

On saturation at H-1", more intensive NOE enhancements (Table 2) were observed at H-6proS than H-6proR for **I** and **III** with α(1-6)-linkage and at H-6proR than H-6proS for **II** and **IV** with β(1-6)-linkage. The NOE results indicated that **I-IV** took the conformation in which the more enhanced protons were in close proximity to H-1" [8] (Fig. 2a and 2b). The above results strongly suggested that the φ and ψ angles of (1-6)-linkages of **I-IV** were very similar to those proposed in our preceding paper [8] and consistent with exo-anomeric effect. [10]

NOE enhancements on saturation at H-1' (Table 2) were found to be also useful for the conformational analysis of (1-6)-linkage as well as (1-4)-linkage. The strong enhancements at H-4 for **I-IV** indicated that H-1' and H-4 were situated in near 1,3-synperiplanar disposition with each other. The enhancements at H-3" and H-5" for III suggested the proximity of the O-4 and O-6 residues (Fig. 2a). Further the enhancements at H-6proR for **IV** informed its high gg preference (Fig. 2b).

Table 2 NOE data of **I-IV** on Saturation of H-1" (Q-6 residue) and H-1' (Q-4 residue)

Compound	MHz	Saturation of H-1" (Relative Enhancement (%))					Saturation of H-1'							
		H-6proR	H-6proS	H-2"	H-3"	H-5"	H-4	H-6proR	H-6proS	H-2'	H-3'	H-5'	H-3"	H-5"
I	400	1.2	2.0	4.4*	-	-	6.1	-	-	5.2*	2.5	-	-	-
(D_2O)	270	1.2	2.6	7.0*	-	-	7.5	-	-	6.0*	-	-	-	-
II	400	2.7	1.6	- * (7.5)			5.5	-	-	3.8*	-	-	-	-
(D_2O)	270	3.0	-	- * (9.5)			9.3	-	-	5.5*				
III	400	-	1.9	5.8	-	-	(4.4)[a]	-	-	-*	(4.8)		(4.4)[a]	
(D_2O)	270	-	2.7	5.8	-	-	(8.5)[a]	-	-	-*	(9.2)		(8.5)[a]	
IV	400	2.7	1.0	2.3	2.9	3.3	2.2	0.8	-	-*	2.5	2.2	-	-
(D_2O)	270	3.6	1.0	-*	(8.6)		8.2	2.0	-	-*	(9.2)		-	-

* partially or totally dispersed.
a) enhancement of (H-4 + H-3" + H-5")

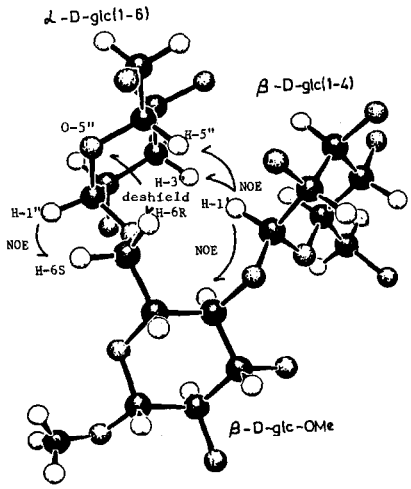


Fig. 2a Inter-residue NOE enhancement for **III** in the D_2O solution and its preferred conformation about (1-4) and (1-6)-linkages.

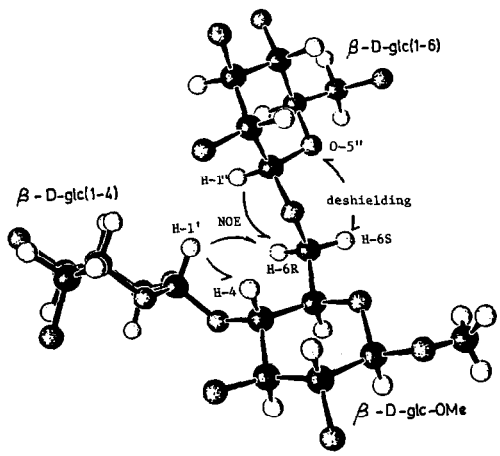


Fig. 2b Inter-residue NOE enhancement for **IV** in the D_2O solution and its preferred conformation about (1-4) and (1-6)-linkages.

From the vicinal coupling constants $J_{5,6proR}$ and $J_{5,6proS}$ the rotamer populations at the C5 - C6 bond can be estimated (table 1). In general, the major conformers are gg and gt with the amount gg exceeding gt in each case. For instance the trisaccharide **I** which is a model of the branching point of amylopectin[12] shows gg and gt in the ratio of ca. 60/40 suggesting two types of three dimensional structures of amylopectin. Except for **I** trisaccharides show higher gg population than the corresponding (1-6)-linked di-Glcp.[7] Here it should be noted that very high gg population ($\geq$ 70%) were observed with **III** and **IV** having β(1-4)-Glcp and this reminds one of the substitution effects of β-D-GlcNAc (bisecting GlcNAc) at Q-4 of D-Manp residue at the branching point of N-linked oligosaccharides to stabilize the gg conformation of their (1-6)-linkages.[11,16-18]

In order to evaluate a model for conformational behaviour of the compounds under investigation, HSEA calculations of low energy conformations with the program GESA[11] were performed. In the case of the trisaccharides II and III the population distribution established by these empirical energy calculations turned out to be in quite well agreement with the experimentally derived coupling constants. The HSEA method predicts the gg states to be most populated. Nevertheless difficulties arise in the case of substances I and IV. For I the gg/gt ratio is calculated to be of reversed order and for IV the tg conformers are found to be unrealistically low energy. This disagreement may occur in other cases as well. Probably it originates from electrostatic interactions between O6 and O4 (Hassel-Ottar-effect) [18,20] and stereoelectronic interactions between O6 and O5, which are not taken into account yet by the GESA calculations. Moreover a comparison would require motional averaging for the flexible (1-6)-linkage as this has been pointed out recently.[20]

As a major conclusion which is based on the experimental data given in this paper it can be stated that the D-Glcp residue at the O-4 position stabilized the gg conformations at the (1-6)-linkage.

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REFERENCES

1) H. Hori, T. Nakajima, Y. Nishida, H. Ohrui and H. Meguro, J. Carbohydrate Chem., 5, 585 (1986) and references therein.
2) H. Ohrui, T. Misawa, Y. Nishida, H. Hori and H. Meguro, Agric. Biol. Chem., 51, 81 (1987)
3) Y. Nishida, H. Ohrui and H. Meguro, Tetrahedron Lett., 25, 1575 (1984).
4) H. Ohrui, Y. Nishida, H. Higuchi, H. Hori and H. Meguro, Can. J. Chem., 65, 1145 (1987).
5) Y. Nishida, H. Hori, H. Ohrui and H. Meguro, Carbohydrate Res., 170, 106 (1987).
6) Y. Nishida, H. Hori, H. Ohrui and H. Meguro, J. Carbohydrate Chem., 7, 239 (1988).
7) H. Ohrui, Y. Nishida, M. Watanabe, H. Hori and H. Meguro, Tetrahedron Lett., 26, 3251 (1985).
8) H. Hori, Y. Nishida, H. Ohrui, H. Meguro and J. Uzawa, preceding paper.
9) Y. Nishida, H. Hori, H. Ohrui, H. Meguro, S. Zushi, J. Uzawa and T. Ogawa, Agric. Biol. Chem., 52, 1003 (1988).
10) R. U. Lemieux, K. Bock, L. T. Delbaere, S. Koto, V. S. Rao, Can. J. Chem., 58, 631 (1980); K. Bock, B. Meyer, H. Thogersen, R. U. Lemieux, Can. J. Chem., 60, 44 (1982).
11) H. Paulsen, T. Peters, V. Sinnwell, R. Lebuhn and B. Meyer, Liebigs Ann. Chem., 489 (1985); H. Paulsen, T. Peters, V. Sinnwell, H. Heume and B. Meyer, Carbohydrate Res., 156, 87 (1986).
12) K. Bock and H. Pederson, J. Carbohydrate Chem., 3, 591 (1984).
13) P. C. Manor, W. Saenger, D. B. Davies, K. Jankowski and A. Rabczenko, Biochim. Biophysi. Acta, 340, 472 (1974).
14) L. H. Koole, H. D. Boer, J. W.-Haan, C. A. G. Haasnoot, P. Dael and H. M. Buck, J. Chem. Soc., Chem. Commun., 362 (1986) and references therein.
15) Application of equation B should be approached cautiously because it gives large negative population of tg for D-Glcp and D-Manp and their (1-6)-linked derivatives as shown in Table 1. The modified equation C for D-Glcp by taking possible deviations of the dihedral angles about the C5-C6 bond into account affords more suitable results (ref. 6).
16) J. R. Brisson and J. P. Carver, Biochemistry, 22, 3671 and 1680 (1983).
17) S. W. Homans, R. A. Dwek and T. W. Rademacher, Biochemistry, 26, 6571 (1987).
18) S. W. Homans, R. A. Dwek, J. Boyd, M. Mahmoudian, W. G. Richard and T. M. Rademacher, Biochemistry, 25, 6342 (1986).
19) W. T. Wiesler, J. T. Vazquez and K. Nakanishi, J. Am. Chem. Soc., 109, 5586 (1987).
20) D. A. Cumming and J. P. Carver, Biochemistry, 26, 6664 (1987).

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