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An NMR spectroscopic and conformational study of 12 pseudo-disaccharides (D-glucopyranosyl-5a-carba-D- and -L-glucopyranoses)

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ABSTRACT

NMR spectroscopic data for 12 pseudo-disaccharides of the general structure: (α or β)-D-glucopyranosyl-(1 → *x*)-5a-carba-(D or L)-glucopyranose, representing analogues of laminaribiose (β-D-Glc*p*, *x* = 3), cellobiose (β-D-Glc*p*, *x* = 4), and maltose (α-D-Glc*p*, *x* = 4) are presented. The assigned NMR chemical shifts together with NOE difference measurements in association with calculations applying the HSEA force field combined with Monte Carlo simulations have been used to assess the conformational preferences of the investigated compounds. The results are correlated with general structural features involved in the interactions between monosaccharide units of oligosaccharides.

INTRODUCTION

Knowledge of the conformational behavior of oligosaccharides is becoming increasingly important, in order to understand the specificity of carbohydrate–protein interaction[1–4]. From an experimental point of view, the assessment of conformational behavior in aqueous solution is performed most efficiently and in detail based on interpretation of NMR spectroscopic data[5–10] even though these parameters inherently are probing average properties of the conformational dynamics[11–13]. Investigations of the three-dimensional structure of oligosaccharides therefore in general rely mainly on NOE measurements as restraints of interunit interactions or on interpretation of the chemical shifts, in combination with appropriate computer modelling. The set of compounds used in the present study are potential probes for biological studies. However, more importantly, they serve as simple model compounds for the interpretation of the NMR parameters in simple disaccharides.

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Thus, it is believed that the data from compounds with different chemical shift distributions than the parent disaccharides can be used to draw general conclusions about interactions between neighboring monosaccharide units. These are of utmost importance for the overall shape of oligosaccharides as earlier addressed in a series of publications by Kenne and co-workers[14–21] and Kochetkov and co-workers[22–30], and more recently in a publication on the structural assignments of pseudo-trehaloses by Bock et al.[31]. The interpretation of the chemical shift differences imposed by the different structural interactions is complex as concluded by the published data mentioned above. However, when a pair of compounds differing only in one parameter is available, the analysis of the chemical shifts together with NOE measurements provides useful information, which can be interpreted based on simple rules discussed earlier[31] and which will be examined in the subsequent discussion.

RESULTS AND DISCUSSION

The synthesis of the octa-acetates of compounds **1–12** has been published[32] and therefore only the assigned NMR data for the deprotected compounds **1–12** (Fig. 1) are presented in Tables I–III (the assigned ^{1}H and ^{13}C NMR data for the

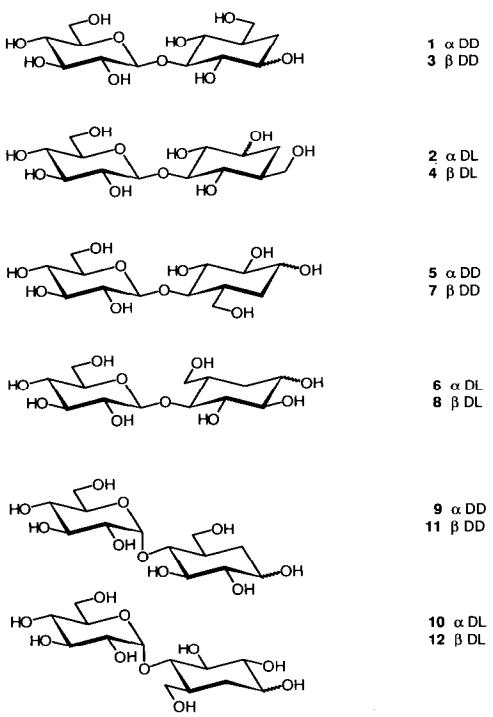

Fig. 1. Structures of compounds **1–12**.

TABLE I

^{13}C NMR chemical shifts of compounds **1–12** [a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	
1 αDD-	104.1	74.4	76.4	70.3	76.8	61.4	
2 αDL-	104.0	74.4	76.4	70.4	76.7	61.5	
3 βDD-	103.9	74.3	76.4	70.3	76.8	61.4	
4 βDL-	103.9	74.4	76.4	70.4	76.9	61.5	
5 αDD-	103.7	74.3	76.4	70.3	76.8	61.4	
6 αDL-	104.0	74.5	76.6	70.6	76.6	61.7	
7 βDD-	103.7	74.2	76.5	70.2	76.9	61.4	
8 βDL-	104.0	74.5	76.6	70.6	76.6	61.7	
9 αDD-	101.0	72.7	73.9	70.2	73.4	61.3	
10 αDL-	100.2	72.4	73.7	70.1	72.8	61.0	
11 βDD-	100.9	72.6	73.8	70.2	73.5	61.3	
12 βDL-	100.3	72.4	73.7	70.1	72.7	60.9	
	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C-7′
1 αDD-	69.6	74.2	86.1	72.2	38.7	63.0	30.8
2 αDL-	69.3	73.2	85.6	73.2	38.8	62.8	30.7
3 βDD-	71.6	77.3	87.2	71.8	40.5	62.8	32.2
4 βDL-	71.7	76.1	87.0	73.1	40.7	62.7	32.3
5 αDD-	69.3	74.5	73.7	84.7	38.3	62.4	30.9
6 αDL-	69.4	74.5	75.0	82.5	37.6	62.5	30.8
7 βDD-	71.6	77.5	76.0	84.0	40.5	62.3	32.4
8 βDL-	71.7	77.6	77.3	81.9	40.0	62.5	32.3
9 αDD-	69.3	74.4	75.6	83.3	37.7	62.8	31.0
10 αDL-	69.2	74.8	73.8	83.9	38.4	63.0	31.0
11 βDD-	71.7	77.5	77.9	82.6	39.8	62.7	32.5
12 βDL-	71.5	78.0	76.2	83.2	40.6	62.7	32.5

[a] Measured at 125.74 MHz relative to internal dioxane (δ 67.4).

octa-acetates are available as supplementary material and are obtainable on request from the author). The analysis of the data confirmed in all cases the structural assignments published[32].

Deprotection was performed in the usual fashion to give compounds **1–12**. The assignment of the NMR data was carried out by standard 1D ^1H and ^{13}C NMR spectra together with 2D COSY and heteronuclear correlation spectroscopy carried out as previously reported[31,33,34]. 1D NOE difference spectroscopy was performed for selected sets of compounds and the results are presented in Table IV.

Calculations were done using the GEGOP program[35,36], which applies a modified version of the HSEA force field[37] and Metropolis Monte Carlo procedures[38]. Theoretical NOE values were calculated as described[39–41] assuming an isotropic rotational correlation time τ_c of 2.0×10^{-10} s, and NOE values from Monte Carlo simulations and grid searches were calculated based on average r^{-6} values. The results of the calculations are given in Tables IV and V, and as a population plot in Fig. 2.

The chemical shift differences of relevance to the conformational preferences of the disaccharides are presented in Tables VI and VII for groups of similar

TABLE II

^{1}H NMR chemical shifts of compounds **1–12** [a]

Compound	H-1	H-2	H-3	H-4	H-5	H-6a	H 6b		
1 αDD-	4.69	3.38	3.55	3.43	3.51	3.92	3.75		
2 αDL-	4.69	3.35	3.51	3.39	3.48	3.91	3.71		
3 βDD-	4.68	3.34	3.50	3.40	3.45	3.88	3.71		
4 βDL-	4.70	3.35	3.51	3.39	3.45	3.90	3.72		
5 αDD-	4.52	3.33	3.51	3.42	3.48	3.90	3.73		
6 αDL-	4.72	3.36	3.52	3.39	3.46	3.92	3.71		
7 βDD-	4.53	3.34	3.52	3.43	3.49	3.92	3.75		
8 βDL-	4.69	3.31	3.49	3.37	3.43	3.90	3.69		
9 αDD-	5.28	3.58	3.70	3.40	3.78	3.82	3.75		
10 αDL-	5.04	3.57	3.70	3.44	3.98	3.79	3.79		
11 βDD-	5.28	3.58	3.68	3.40	3.76	3.83	3.75		
12 βDL-	5.03	3.56	3.68	3.45	3.98	3.78	3.78		
	H-1′	H-2′	H-3′	H-4′	H-5′	H-6′*R*	H-6′*S*	H-7′*eq*	H-7′*ax*
1 αDD-	4.14	3.65	3.80	3.43	1.93	3.73	3.73	1.89	1.49
2 αDL-	4.13	3.52	3.79	3.50	1.91	3.69	3.69	1.87	1.45
3 βDD-	3.58	3.41	3.51	3.40	1.63	3.64	3.73	1.98	1.27
4 βDL-	3.59	3.32	3.51	3.51	1.64	3.74	3.64	1.98	1.28
5 αDD-	4.08	3.48	3.70	3.52	2.03	3.79	3.75	1.87	1.49
6 αDL-	4.10	3.47	3.82	3.57	1.92	3.62	3.87	1.85	1.55
7 βDD-	3.59	3.29	3.44	3.56	1.81	3.74	3.81	2.00	1.32
8 βDL-	3.55	3.23	3.50	3.58	1.67	3.65	3.80	1.95	1.34
9 αDD-	4.07	3.47	3.83	3.45	1.97	3.70	3.70	1.90	1.50
10 αDL-	4.08	3.44	3.70	3.44	2.05	3.71	3.82	1.82	1.48
11 βDD-	3.54	3.25	3.54	3.46	1.74	3.65	3.74	2.01	1.30
12 βDL-	3.56	3.23	3.42	3.46	1.80	3.77	3.77	1.93	1.30

[a] Measured at 500.13 MHz relative to internal acetone (δ 2.225, DOH at δ 4.75 at 300 K).

compounds. However, it should be noted that the ^{13}C chemical shift used as reference for C-7′ of the pseudo-α-D-Glc*p* unit is 30.9 ppm, and not 29.6 ppm as published in the original article[42]. The value of 29.6 ppm was a typing mistake in the original article and unfortunately was also used as reference in the article about pseudo-trehaloses[31]. The corrected values for the glycosylation shifts of C-7′ in Table IV in ref. 31 should therefore be: **1** −0.5, **2** −4.5, **5** −0.5, **6** −3.0 ppm, which gives a better agreement with the expected values from the conformational analysis. Based on the results discussed below, chemical shift differences for protons or carbons as a result of the close contacts in the predominant conformations can be summarized qualitatively as presented in Table VIII.

Compounds **1–4** can be analyzed as analogues of laminaribiose, **1** and **3** being α- and β-pseudo-laminaribiose, and **2** and **4** being the α and β isomers with the reducing end in the L configuration (Fig. 1). The NMR data of the D and L analogues **1** to **2** or **3** to **4** have very similar chemical shifts for the nonreducing unit. This can be explained by the fact that inversion of the pseudo-Glc*p* unit from D to L does not change the environments of the β-D-Glc*p* unit. The 2′ and 4′ positions are simply inverted and therefore the ϕ_H/ψ_H values are the same for the

TABLE III

^{1}H NMR coupling constants of compounds **1–12** [a]

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
1 αDD-	7.9	9.6	8.9	9.8	2.2	5.6	12.4
2 αDL-	7.9	9.4	9.0	9.9	2.3	6.0	12.4
3 βDD-	8.0	9.4	9.1	9.8	2.2	5.7	12.4
4 βDL-	8.1	9.5	8.6	9.9	2.2	5.9	12.4
5 αDD-	7.8	9.5	9.0	9.7	2.2	5.7	12.4
6 αDL-	8.0	9.2	9.1	9.7	2.3	6.2	12.2
7 βDD-	7.9	9.3	9.1	9.7	2.2	5.5	12.4
8 βDL-	8.0	9.3	8.9	9.9	2.3	6.1	12.2
9 αDD-	4.0	9.8	9.2	9.5	3.0	4.9	11.6
10 αDL−	4.0	10.0	9.2	10.2	2.7	4.1	
11 βDD-	4.0	9.9	9.2	9.7			
12 βDL-	3.9	10.0	9.1	10.2	3.3	3.3	

	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6'R}$	$J_{5',6'S}$	$J_{6'R,6'S}$	$J_{1',7'eq}$	$J_{1',7'ax}$	$J_{5',7'eq}$	$J_{5',7'ax}$	$J_{7'eq,7'ax}$
1 αDD-	3.2	9.6	9.1					3.8	2.3	3.6	12.7	15.0
2 αDL-	3.2	9.4	9.4					3.7	2.3	3.7	12.7	14.9
3 βDD-	9.3	9.1	8.8	9.9	5.8	3.6	11.3	4.8	11.6	3.6	12.9	13.0
4 βDL-	9.3	9.3			3.5	5.9	11.2	4.8	11.6	3.6	12.9	13.0
5 αDD-	3.2				5.0	3.5	11.3	3.7	2.3	3.7	12.9	15.0
6 αDL-	3.2	9.9	9.2	10.9	3.0	4.4	11.6	3.7	2.3	3.7	13.0	15.0
7 βDD-	9.3	9.3	9.0	10.5	5.5	3.5	11.3	4.8	11.6	3.6	13.1	13.1
8 βDL-	9.3	9.4	9.4	10.6	3.1	4.7	11.6	4.8	11.6	3.6	13.2	13.0
9 αDD-	3.2	10.1	9.0	10.8				3.8	2.2	3.8	12.8	15.1
10 αDL-	3.2	9.8	9.0	10.9	3.9	4.6	11.4	3.8	2.3	3.8	12.9	15.0
11 βDD-	9.3	9.3	9.1	10.2	6.0		11.2	4.5	11.9	3.9	13.0	13.0
12 βDL-	9.3	9.2	9.1	9.9				4.7	11.6	3.8	13.0	13.0

[a] Determined on a first-order basis (+/− 0.3 Hz) from spectra obtained at 500.13 MHz.

minimum energy conformations. However, this has an effect on the chemical shifts of the pseudo-Glc*p* unit because of the asymmetry of the β-D-Glc*p* unit. Thus, the C-4′ signals in the D-series compounds **1** and **3** are shifted upfield by ~ 1.5 ppm and, in the L-series compounds **2** and **4**, the C-2′ signals are shifted upfield by ~ 1.5 ppm relative to the monosaccharides. Similarly the C-2′ signals for **1** and **3** and C-4′ signals for **2** and **4** are shifted upfield by ~ 0.5 ppm, respectively. This is due to the presence of either a ring oxygen atom or an OH group. Following the results found by Kochetkov and co-workers[22,23,29], this should be caused by a β-effect from a γ-gauche interaction between protons H-1 and H-2′ or H-4′. The short distances normally associated with such an effect are not observed in the minima populated to any significant degree for compounds **1** or **2**. According to Kochetkov and co-workers[22,23,29], the interactions derive from the inverted conformations with ψ_H ca. 180° (e.g., cellobiose[23]). However, the population of this inverted conformation is low as seen from a grid search (Table V). Furthermore, the presence of this conformation cannot be verified experimentally in the compounds investigated in the present work, where the analysis is not hampered by chemical shift overlap of the protons of interest.

Therefore, an alternative explanation for the β-effect is presented here. The ring oxygen O-5 is close to HO-4′ in **1** and **3**, and close to HO-2′ in **2** and **4** (3.0 Å in the minimum energy conformations), offering a possibility for a hydrogen bond with O-5 as the acceptor. By pulling the proton of the HO-2′ group (or HO-4′) away, the oxygen becomes more electron-rich and the corresponding carbon is shielded. Similarly, the ^{1}H chemical shifts are deshielded in compounds where this hydrogen bond can be formed. Furthermore, the same effects are not observed as consistently in the corresponding acetates (Table I Supplementary). The glycosylation shifts for C-1 and C-3′ are in the expected range, being ~7.3 and ~10 ppm, respectively[43]. The H-1 chemical shifts are almost unchanged, which can be explained by the opposite effects of H-1 being close to both H-3′ and either O-2′ or O-4′. The H-3′ signal is shifted downfield in all four compounds by ~0.21 ppm relative to the monosaccharides[42]; H-3′ and O-5 are close[44,45] (2.5 Å in the minimum energy conformation and within an average distance of 2.6 Å as determined by Monte Carlo simulation). However, it should be pointed out that the above explanation is speculative because of the limited data available, but in good agreement with the proposal by Leeflang et al.[46].

By analogy with the above analysis, compounds **5** to **8** can be regarded as pseudo-cellobiose derivatives: **5** and **7** are analogues of α- and β-cellobiose, and **6** and **8** are the α and β analogues with the reducing unit in the L configuration (Fig. 1). In contrast to compounds **1** to **4**, there are large chemical shift changes on inverting the reducing unit from the D to L configuration, based on the fact that the steric interactions are now caused by OH and CH_2OH groups, respectively, as shown in Fig. 1. The changes in the chemical shifts are furthermore correlated to the changes in the conformation of the glycosidic linkage[43]; ϕ_H/ψ_H 53°/0° for the D isomer and 53°/12° for the L isomer. This results in differences in the average distances across the linkage like H-1 to H-4′ of 2.34 and 2.45 Å for the D and L isomers, respectively, which are subsequently reflected in the chemical shifts of H-1 being ~0.18 ppm upfield for the D isomer relative to the L isomer. In contrast, the chemical shift of H-4′ shows only small differences, all being shifted downfield relative to the monosaccharides, due to O-5 being close in space (average distance of 2.6 Å obtained from Monte Carlo simulations).

The C-1 and C-4′ chemical shifts can also be used as reporters for a conformational change; however, the C-1 chemical shifts do not show major differences in the observed glycosylation shifts. For C-4′, the glycosylation shifts are ~2.1 ppm larger for the D compounds compared to the L compounds in agreement with the D compound having $|\psi_H|$ 10° lower[43]. The substituents around the glycosylation site of the pseudo-sugar unit are affected by having the different parts of β-D-Glc*p* close in space. The C-3′ signals are shifted upfield in the D compounds relative to the monosaccharides, similarly to the observations reported above for compounds **1** to **4**. This can be rationalized by a possible H bond between O-5 and HO-3′. Such a hydrogen bond has recently been proposed for cellobiose by Leeflang et al.[46], who probed the existence of this hydrogen bond by NMR and MD simulations. For cellobiose, the same effect has been explained by Kochetkov et al.[23] based solely on a γ-gauche interaction of H-1 and H-3′ in the inverted conformation. This could be an explanation, because H-1 is very close to H-3′ in the inverted conformation, but H-5′ is also close here, with a slightly longer average distance. Evaluation of the measured NOEs in the pseudo-sugars, where saturation of H-5′ is not hampered by overlap, shows that the inverted conformation can

TABLE IV

Experimental and calculated NOE data for selected compounds

Compound	Proton satd	NOEs Observed	Intraunit						Interunit						
			Obsd	Min [a]	Min inv [a]	Grid	MC 300 K	MC 600 K		Obsd	Min [a]	Min inv [a]	Grid	MC 300 K	MC 600 K
7 β_{DD}-	H-1	H-2	**2.5**	4.1	4.0	4.1	3.7		H-3′	[b]	0.06	15.9	0.10	0.07	
		H-3 + H-5 + H-4′	**20.7**	20.5	13.5	20.4	20.0								
	H-5′	H-1′ + H-4′	**5.3**	6.0	6.8	6.0	5.7		H-1	[b]	0.04	5.4	0.04	0.05	
		H-3′	**5.6**	7.2	1.5	7.2	6.6								
		H-6′*R* + H-6′*S*	**4.2**	2.8	2.4	2.9	3.0								
8 β_{DL}-	H-1	H-2	**3.8**	3.5	3.6	3.5	3.5		H-4′	**8.1**	5.6	0.33	5.5	5.6	
		H-3	**6.8**	8.0	7.8	8.0	8.0		H-5′	< **0.2**	0.02	9.7	0.04	0.02	
		H-5	**7.7**	5.0	4.5	5.0	5.3								
	H-5′	H-1′ + H-3′ + H-4′	**11.3**	12.3	8.2	12.2	12.3		H-1	< **0.2**	0.02	9.2	0.05	0.02	
		H-6′*R*	**2.5**	2.8	2.5	2.8	1.9								
		H-6′*S*	**1.7**	0.18	0.23	0.18	1.2								
9 α_{DD}-	H-1	H-2	**11.1**	11.3	10.9	11.3	11.3	11.2	H-3′	**1.0**	1.2	13.2	1.5	1.9	2.5
		H-3	**1.0**	0.49	0.47	0.47	0.47	0.45	H-4′	**10.5**	8.5	0.37	9.0	6.8	6.8
									H-5′	~ **0.2**	0	9.5	0.12	0	1.8
	H-5′	H-1′	**2.1**	0.33	0.34	0.33	0.32	0.33	H-1	**0.72**	0	9.9	0.52	0	2.0
	(+H-7′*eq*)	H-3′	**8.7**	8.9	2.3	8.4	8.8	7.5							
		H-4′	+	1.4	2.1	1.3	1.1	1.0							
		H-6′*R* + H-6′*S*	**4.4**	4.0	3.5	4.1	2.8	3.0							
10 α_{DL}-	H-1	H-2	**10.8**	11.0	10.8	11.0	11.1	11.1	H-4′	**9.6**	8.2	0.29	8.7	9.6	9.2
		H-3 + H-3′ + H-6′*S*	**3.8**	1.6	16.9	1.4	1.1	2.0	H-5′	~ **0.2**	0.04	10.5	0.04	0.04	0.24
									H-6′*R*	**1.5**	2.1	0.08	1.5	0.71	0.90
	H-5′	H-1	+	0.32	0.34	0.32	0.33	0.33	H-1	**0.79**	0.03	8.2	0.03	0.02	0.21
	(+H-7′*eq*)	H-4	**1.8**	1.0	1.7	1.0	1.0	1.2							
		H-3′ + H-6′*S*	**10.4**	8.5	1.7	8.0	8.9	8.5							
		H-6′R	**2.1**	2.6	1.5	2.7	2.1	2.0							

[a] Minimum energy conformation (see Table V). [b] Not observed, i.e., less than ~0.1%.

only be populated to a very low extent, as **no** NOE is observed on H-1 when H-5′ is saturated. The experiments cannot, however, resolve whether an NOE from H-1 to either H-3′ or H-5′ could be present due to chemical shift overlap. The conclusion is that the short O-5 to O-3′ distance is the most likely explanation for the effect observed for C-3′ in compounds **5** and **7**.

Smaller glycosylation shifts are also seen for C-5′ in the L compounds **6** and **8**, which cannot have the same origin as that concluded for C-3′ in the D compounds. For compound **8**, the NOE data suggest the possibility of a small population of the inverted conformation that could account for the effect observed for C-5′. However, it is much more likely that the changes for the hydroxymethyl group conformation, as discussed in the following, will also affect the shift of C-5′. H-3′ and H-5′ show minor differences in going from D to L which in addition to the interaction discussed above could also be affected by the slightly different orientation of the glycosidic linkage oxygen lonepairs[17,20].

The hydroxymethyl group of the pseudo-sugars shows some interesting changes relative to the monosaccharides. Compounds **5** and **7** show a downfield shift of 0.11 ppm for H-6′*R* relative to the monosaccharides, and the H-6′*S* chemical shifts are shifted only slightly downfield relative to the monosaccharides. The H-5′ to H-6′*R* and H-6′*S* couplings are close to the corresponding coupling constants in the monosaccharides, so that the rotational preferences seem to be similar. The downfield shift observed for H-6′*R* can be explained by the short distance of H-6′*R* to O-2 in the *gg* rotamer, which by the observed coupling constants is proposed to be the major conformer. Leeflang et al.[46] suggested the possibility of an O-2 to O-6′ hydrogen bond in cellobiose stabilizing the *tg* rotamer, but such a stabilization cannot be confirmed by the present results.

The hydroxymethyl groups of **6** and **8** show a clear pattern, with H-6′*R* being shifted ~ 0.12 ppm upfield and H-6′*S* ~ 0.18 ppm downfield relative to the monosaccharides. This is accompanied by a change in the coupling constants, proving the disaccharides to have a higher population of the *gg* conformer ($J_{5,6'S}$ smaller). In **6** and **8**, the *gg* conformers bring O-5 close to O-6′, giving the possibility of a hydrogen bond stabilizing the *gg* conformer over the *gt*. This is consistent with the changes in chemical shift for H-6′*R* and H-6′*S*, with H-6′*S* close to O-5, both in *gg* and *gt*, giving a downfield shift. The population of *gt* with H-6′*R* close to O-5 is low; instead H-6′*R* is shifted upfield as the *gt* population with H-6′R close to O-4 is decreased relative to the monosaccharide. Interesting also are the slight changes seen for the hydroxymethyl group of the D-Glc*p* unit, corresponding to small changes in the population of the different conformers due to weak interactions with the pseudo-sugar unit.

The NOE measurements (Table IV) confirm the proposed conformation with the compounds having mainly one distinct low-energy conformational area, as shown by the observation of a large NOE from H-1 to H-4′, where the H-4′ chemical shift is separated (compound **8**). The absence of NOEs, e.g., from H-5′ to

TABLE V

Energy minimum conformations [a] and population [b] by GEGOP (HSEA force field) for selected compounds

Compound	**1** αDD		**2** αDL		**7** βDD		**8** βDL		**9** αDD		**10** αDL	
	Min	Min Inv	Min	Min Inv	Min	Min Inv	Min	Min Inv	Min	Min Inv	Min	Min Inv
ϕ_H/ψ_H	53/5	32/168	53/6	33/168	53/0	26/173	53/12	33/167	−29/−20	−30/−168	−39/−8	−14/−175
ω_1	−63	60	−63	60	−63	−62	−63	−63	−63	−63	−63	−63
ω_2	62	62	−63	−62	59	78	−59	−64	−77	65	−56	−77
Energy (kcal/mol)	0.19	4.03	0.19	3.87	−0.55	4.27	−0.71	3.25	2.07	3.86	0.68	5.42
Pop. of inv. by grid [b]		0.20%		0.25%	0.08%		0.16%			3.1%		0.04%
Pop. of inv. by MC 300 K [b]		0%		0%	0%		0%			0%		0%
Pop. of inv. by MC 600 K [b]										11.9%		0.82%

[a] Min indicates the lowest energy minimum and Min Inv the energy minimum having ψ_H around 180° (Inverted). [b] The population either by Boltzmann distribution at 300 K from energies from a 2° by 2° grid search over the 360° by 360° range, or by Monte Carlo calculation.

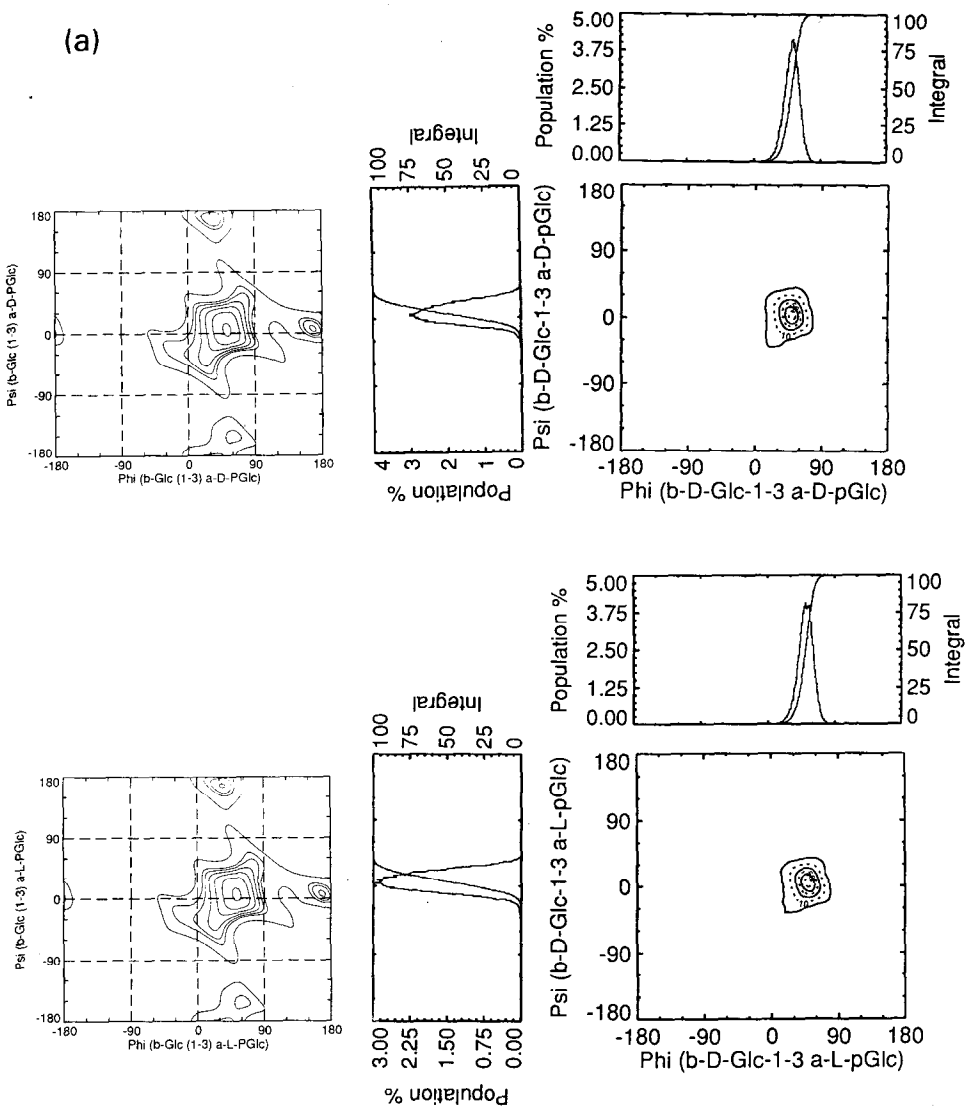

Fig. 2a. Isoenergy contour map (left, from grid search 0.1, 1, 2, 3, 5, and 10 kcal/mol) and population map (right, from MC at 300 K) for compounds **1** (top) and **2** (bottom).

H-1 for **7**, and only a small NOE observed for compound **8** suggest that the inverted conformation can only be populated to a minor degree ($< 10\%$).

Compounds **9–12** can be considered as analogues of α- and β-maltose, but for **10** and **12** with the reducing unit in the L configuration (Fig. 1). For these compounds, a series of chemical shift differences together with NOE measurements and calculation give useful information on the conformational behavior of maltose analogues. As shown by calculation by several authors[23,44,47–53], the conformational preferences for maltose cannot be well described by a single

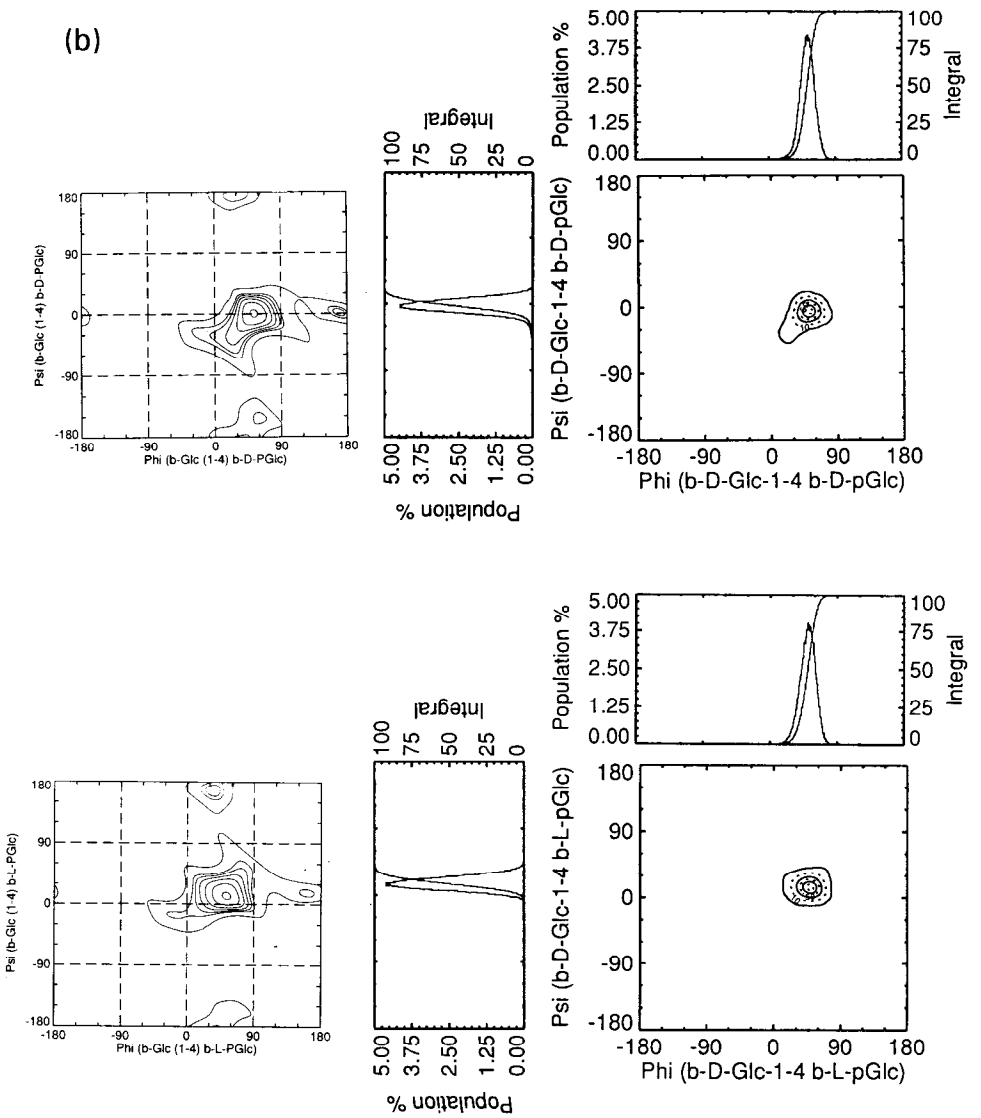

Fig. 2b. Isoenergy contour map (left, from grid search 0.1, 1, 2, 3, 5, and 10 kcal/mol) and population map (right, from MC at 300 K) for compounds **7** (top) and **8** (bottom).

minimum. Most of the reports describe a broad minimum in the range ϕ_H 0° to −90° and ψ_H 0° to −90° and a lower populated minimum centered around ϕ_H/ψ_H −30°/170°. The conformational spaces for **9** and **10** are indicated in Fig. 2. The experimental data generally confirm this result, as demonstrated in the following. H-4′ shows a downfield shift of ~0.15 ppm relative to the monosaccharides due to the short O-5 to H-4′ distance. The H-1 to H-4′ distance of ca. 2.3 Å should give an upfield shift of both H-1 and H-4′, but this is overruled by the short

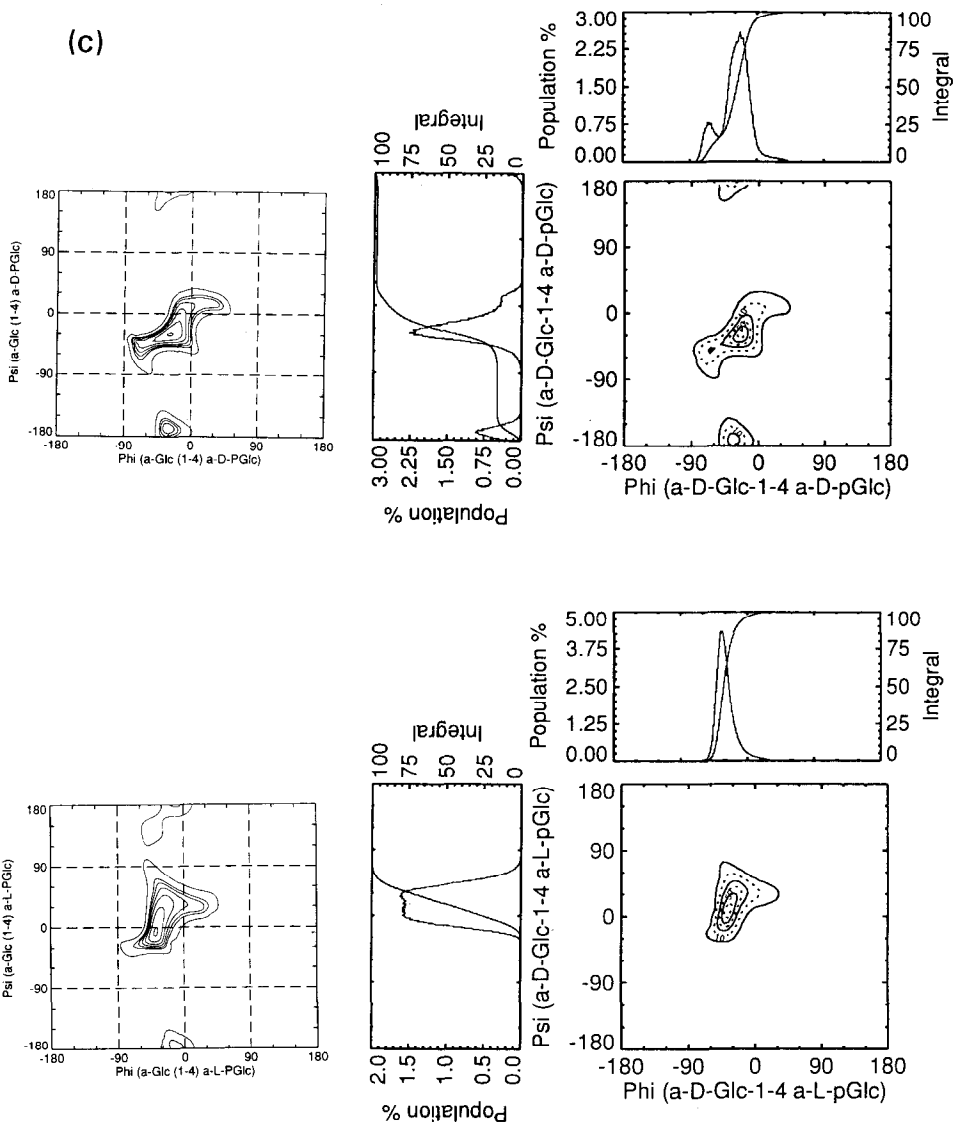

Fig. 2c. Isoenergy contour map (left, from grid search 0.1, 1, 2, 3, 5, and 10 kcal/mol) and population map (right, from MC at 600 K) for compounds **9** (top) and **10** (bottom).

O to H distance. For H-1, the effect is seen for the L series, **10** and **12** ($\Delta \sim -0.18$ ppm), but for the D series, **9** and **11**, the effect is cancelled by a short H-1 to O-3′ distance of ca. 2.4 Å (Δ 0.06 ppm). C-1 and C-4′ show large downfield glycosylation shifts (Table VI) for all compounds. The ψ_H angles calculated are expected to give larger glycosylation shifts for the L series as the $|\psi_H|$ are smaller[43]. The effect of having populations of the inverted conformation (ϕ_H/ψ_H $-30°/-170°$) is not clear, but the opposite changes going from D to L for C-1 and C-4′ might be due to

TABLE VI

^{13}C NMR glycosylation shift (chemical shifts relative to chemical shift of monosaccharides[42,57,58]) and chemical shift differences for pairs of diastereoisomers

Compound	C-1	C-2	C-($x-2$)′	C-($x-1$)′	C-x′	C-($x+1$)′	C-($x+2$)′
β-(1→3) $x=3$							
1 αDD-	**7.4**	−0.7	0.0	−0.4	**10.9**	**−1.7**	−0.1
2 αDL-	**7.3**	−0.7	−0.3	**−1.4**	**10.4**	−0.7	0.0
3 βDD-	**7.2**	−0.8	−0.2	−0.4	**9.7**	**−1.5**	−0.3
4 βDL-	**7.2**	−0.7	−0.1	**−1.6**	**9.7**	−0.2	−0.1
β-(1→4) $x=4$							
5 αDD-	**7.0**	−0.8	−0.1	**−1.5**	**10.8**	−0.5	−0.7
6 αDL-	**7.3**	−0.6	−0.1	−0.2	**8.6**	**−1.2**	−0.6
7 βDD-	**7.0**	−0.9	−0.2	**−1.5**	**10.7**	−0.3	−0.6
8 βDL-	**7.3**	−0.6	−0.1	−0.2	**8.6**	**−0.8**	−0.4
α-(1→4) $x=4$							
9 αDD-	**8.3**	0.2	−0.2	0.4	**9.4**	**−1.1**	−0.3
10 αDL-	**7.5**	−0.1	0.2	**−1.4**	**10.0**	−0.4	−0.1
11 βDD-	**8.2**	0.1	−0.2	0.4	**9.3**	**−1.0**	−0.2
12 βDL-	**7.6**	−0.1	0.3	**−1.3**	**9.9**	−0.2	−0.2
β-(1→3) $x=3$							
1-2 αDD-αDL	0.1	0.0	0.3	**1.0**	0.5	**−1.0**	−0.1
3-4 βDD-βDL	0.0	−0.1	−0.1	**1.2**	0.2	**−1.3**	−0.2
β-(1→4) $x=4$							
5-6 αDD-αDL	−0.3	−0.2	0.0	**−1.3**	**2.2**	**0.7**	−0.1
7-8 βDD-βDL	−0.3	−0.3	−0.1	**−1.3**	**2.1**	**0.5**	−0.2
α-(1→4) $x=4$							
9-10 αDD-αDL	**0.8**	0.3	−0.4	**1.8**	**−0.6**	**−0.7**	−0.2
11-12 βDD-βDL	**0.6**	0.2	−0.5	**1.7**	**−0.6**	**−0.8**	0.0

differences in population of the inverted conformation. The calculations predict **10** to have a lower population of the inverted conformation, which could explain the smaller glycosylation shifts of C-1 and larger glycosylation shifts of C-4′ for the L isomer relative to the D isomer, since the inverted conformation has a short distance from H-1 to H-3′ and H-5′, while the H-1′ to H-4′ distance is large in the inverted conformation.

Chemical shift changes are observed for H-5 and C-5. In both series, the C-5 signals are shifted upfield while the H-5 signal is shifted ~ 0.08 ppm upfield in the D isomer and shifted ~ 0.13 ppm downfield in the L isomer. The explanation in the D series (H-5 Δ 0.08 ppm) probably stems from the close interaction with the hydroxymethyl group of the pseudo-glucose with a possibility of hydrogen bonding between O-5 and HO-6′. In the L series, the clear downfield shift for H-5 (Δ ~ 0.13 ppm) is due to the short H-5 to O-3′ distance (ca. 2.4 Å) in the major populated conformation.

The H-3′ signals are shifted upfield significantly, especially in the D series (~ 0.24 ppm), caused by the change in the positioning of lone pairs of O-4′ upon

TABLE VII

^{1}H NMR glycosylation shift (chemical shifts relative to chemical shift of monosaccharides[42,57]) and chemical shift differences for pairs of stereoisomers

Compound	H-1	H-2	H-5	H-($x-2$)′	H-($x-1$)′	H-x′	H-($x+1$)′	H-($x+2$)′	
β-(1 → 3) $x = 3$									
1 αDD-	0.05	**0.12**	0.03	0.04	**0.22**	**0.21**	**0.14**	0.05	
2 αDL-	0.05	**0.12**	0.00	0.03	**0.09**	**0.20**	**0.21**	0.03	
3 βDD-	0.04	**0.08**	−0.03	0.00	**0.17**	**0.21**	**0.08**	−0.01	
4 βDL-	0.04	**0.08**	−0.03	0.01	**0.08**	**0.21**	**0.19**	0.00	
β-(1 → 4) $x = 4$								*S*	*R*
5 αDD-	**−0.12**	0.07	0.00	0.05	**0.11**	**0.23**	**0.15**	**0.02**	**0.11**
6 αDL-	**0.08**	0.10	−0.02	0.04	**0.23**	**0.28**	0.04	**0.19**	**−0.11**
7 βDD-	**−0.11**	0.08	0.01	0.05	**0.14**	**0.24**	**0.17**	**0.04**	**0.11**
8 βDL-	**0.08**	0.05	−0.05	−0.01	**0.20**	**0.26**	0.03	**0.17**	**−0.12**
α-(1 → 4) x = 4								*S*	*R*
9 αDD-	0.06	0.04	−0.07	0.04	**0.24**	**0.16**	**0.09**	−0.03	0.02
10 αDL-	**−0.18**	0.03	**0.13**	0.01	**0.11**	**0.15**	**0.09**	**0.14**	−0.02
11 βDD-	0.06	0.04	−0.09	0.01	**0.24**	**0.14**	**0.10**	−0.03	0.02
12 βDL-	**−0.19**	0.02	**0.13**	−0.01	**0.12**	**0.14**	**0.16**	**0.14**	0.00
β-(1 → 3) $x = 3$									
1-2 αDD-αDL	0.00	0.03	0.03	0.01	**0.13**	0.01	**−0.07**	0.02	
3-4 βDD-βDL	−0.02	−0.01	0.00	−0.01	**0.09**	0.00	**−0.11**	−0.01	
β-(1 → 4) x = 4								*S-R*	*R-S*
5-6 αDD-αDL	**−0.20**	−0.03	0.02	0.01	**−0.12**	−0.05	**0.11**	0.13	−0.08
7-8 βDD-βDL	**−0.16**	0.03	0.06	0.06	**−0.06**	−0.02	**0.14**	0.16	−0.06
α-(1 → 4) $x = 4$									
9-10 αDD-αDL	**0.24**	0.01	**−0.20**	0.03	**0.13**	0.01	**−0.08**	−0.01	−0.12
11-12 βDD-βDL	**0.25**	0.02	**−0.22**	0.02	**0.12**	0.00	**−0.06**	−0.03	−0.12

glycosylation[17,20]. The effects of the interactions in the inverted conformation are difficult to evaluate: H-3′ and H-5′ come close to H-1, giving an upfield shifting; H-3′ is close to O-5 in D and H-5′ close to O-5 in L compounds, respectively. The corresponding carbons C-3′ and C-5′ also show significant effects, the largest being a 1.4 ppm upfield shift for C-3′ in the L series. This can partly be explained by a γ-gauche interaction in the inverted conformation and partly by a possible hydro-

TABLE VIII

Summary of shielding effects on protons or carbon atoms as a function of short atom-to-atom distances

Short distance		δ_A	δ_B
	$H_A \rightarrow H_B$	÷ [a]	÷
	$O \rightarrow H_B$		+ [b]
	$O \rightarrow HO\text{-}C_A\text{-}H_B$	÷	+
α	$C_A\text{-}H \rightarrow H\text{-}C_B$ [c]	+	+
β	$C_A\text{-}H \rightarrow H\text{-}C_B$ [d]	÷	÷

[a] Indicates shielding of chemical shifts. [b] Indicates deshielding of chemical shifts. [c] Angle or distance dependence. [d] γ-gauche interaction as discussed by Kochetkov and co-workers[22,23,29].

gen bond between O-5 and HO-3′ with an O-5 to O-3′ average distance of 2.9 Å found in the Monte Carlo simulations. The upfield shift of C-5′ in the D series, which is parallel to the shifts seen in maltose, is most likely due to the γ-gauche interaction with a very short H-1 to C-5′ distance in the inverted conformation (2.03 Å). The hemical shift of C-5′ might furthermore be affected by altered interaction with the hydroxymethyl group in the predominantly populated conformation.

The changes in the hydroxymethyl group chemical shifts of the pseudo-glucose by glycosylation for compounds **9–12** confirm interactions with the α-D-Glc*p* unit. For compounds **9** and **12** the signals could not be resolved for measuring coupling constants, but the changes in chemical shifts are in general parallel to those observed for **10** and **11**, respectively. Only one vicinal coupling constant for compound **11** could be measured. Assuming that the normal populations for the staggered conformation are not inverted, the shift at 3.65 ppm is assigned to the H-6′*R* proton. Thus it follows that the H-6′*R* signal is shifted slightly downfield and the H-6′*S* signal is shifted upfield. The small effects can be explained, e.g., by a short O-5 to H-6′*R* distance in the major *gg* conformation and slight changes in the populations relative to the monosaccharides. For compound **10**, much more significant effects are observed, where the coupling constants indicate that the H-6′*S* and H-6′*R* chemical shifts are reversed. The H-6′*S* signal is shifted downfield by ca. 0.14 ppm and the H-6′R signal shifted upfield by 0.02 ppm. At the same time, the coupling constants changed $J_{5,6S}$ from 6.0 to 4.6 Hz and $J_{5,6R}$ from 3.5 to 3.9 Hz. This shows that the population of *gg* and *tg* have increased, while *gt* has decreased, which could be explained by a short O-2 to O-6′ distance in *gg* and especially in *tg*, offering a possibility for a hydrogen bond stabilization. This also explains the downfield shift for H-6′S, as H-6′*S* becomes close to O-2 in these conformations.

The NOE data for compounds **9–12** cannot be interpreted by a single predominant conformation. The major NOEs observed for compound **9** are H-1 to H-4′, H-1 to H-3′, and H-5′ to H-1 (Table IV). The large NOE from H-1 to H-4′ shows that the major population has this distance short as in the minimum energy conformation. The Monte Carlo simulations at 300 K show that the broad minimum around this can explain both the H-1 to H-3′ and H-4′ NOEs. To account for the H-5′ to H-1 NOE, the inverted conformation also has to be included as a minor populated conformer. The calculation using a grid search seems to underestimate the population of the inverted conformation while the Monte Carlo simulations at 600 K appear to overestimate it, so the most likely conclusion is that the inverted conformation is populated from 5 to 10%.

From the experimental data, a similar situation is present for **10**, but the NOEs from H-1 are to H-4′ and H-6′*S*. The NOE to H-6′*R* cannot be resolved from that to H-3 due to chemical shift overlap, but the size of the total signal indicates that a substantial NOE to H-6′*R* exists. For compound **10**, these NOEs can be explained by the averaging around the global minimum, but, to include the NOE from H-5′

to H-1, the inverted conformation must also be populated. The calculation gives a lower population for the inverted minimum, and the experimental data do not disprove this, but it cannot be proved with certainty within the accuracy of the NOE experiment.

The investigation has given information about the effects determining the conformation of the glycosidic linkage. The conformation will be determined mainly by the relative configuration of the substituents next to the glycosylated position. The conformations can be probed by the combination of NOE and force-field calculations with a good overall agreement seen in the light of the assumption of isotropic motion. Furthermore, interresidue interactions result in characteristic chemical shift changes, which support the observations about the conformational preferences around the glycosidic linkages.

EXPERIMENTAL

NMR spectroscopy.—Solutions in 0.5 mL of D_2O were used. Spectra were recorded in 5-mm tubes at 500.13 MHz or 600.13 MHz for ^1H and 125.77 MHz for ^{13}C with a Bruker AM-500 (or AMX-600) spectrometer at 27°C. The ^1H resonances were measured relative to internal acetone (2.225 ppm, HOD at 4.75 ppm at 27°C) and coupling constants determined on a first-order basis. The ^{13}C resonances are relative to internal dioxane (67.4 ppm). All NMR data are given in Tables I–III for unprotected compounds and assignments are based on 2D NMR experiments as described previously[33,34]. The atoms of the "pseudosugar" unit are marked with a prime, for example H-1′.

Conformational analysis.—The torsion angles are defined as follows[5]: ϕ_H (H-1–C-1–O-1–C-X), ψ_H (C-1–O-1–C-X′–H-X′), and ω [O-5(C-7)–C-5–C-6–O-6]. The calculation of energy minima, NOE values, and energy/population maps by grid search or Monte Carlo simulations for compounds **1**, **2**, **7**, **8**, **9**, and **10** were performed using the GEGOP program[35,36]. The GEGOP program utilizes the HSEA force field[5,37] with updated potentials for the exo-anomeric effect and an additional torsional potential for the "normal" sugar unit hydroxymethyl groups[54]. The glycosidic bond angles τ were allowed to vary during the calculations using a harmonic potential with a high constraint (350 kcal/mol · rad^2) for allowing transitions over higher energy barriers between local energy minima during the Monte Carlo simulation. The coordinates for the α- and β-D-Glc units were taken from the average coordinates[55] and the protons attached as described[5]. The α- and β-(D or L)-Glc-5a-carba units were prepared by coordinate modifications of the corresponding O-analogues, using the molecular modelling program Alchemy[56].

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