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Conformational equilibria of 4-thiomaltose and nitrogen analogues of maltose in aqueous solutions

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ABSTRACT

The ^{1}H and ^{13}C NMR data at neutral pH are presented for methyl 4-thio-β- and α-maltoside (**1** and **2**) together with methyl 1-thio-α-D-glucopyranoside (**3**) and methyl 4-thio-α-D-glucopyranoside (**4**) as reference compounds. Furthermore, the NMR data at high and low pH are presented for the 4-amino-4-deoxy analogues of methyl α-maltoside (**5** and **6**) and the 5-amino-5-deoxy analogue (**8**) together with reference compounds methyl 4-amino-4-deoxy-α-D-glucopyranoside (**7**) and 1-deoxynojirimycin (**9**). The experimental NMR data are assigned by 1- and 2-dimensional spectroscopy at 500 and 600 MHz. The conformational preferences of the maltose analogues **1**, **2**, **5**, **6** and **8** are evaluated by difference NOE experiments, $^{13}C-^{1}H$ long-range coupling constants, chemical-shift comparison with model compounds and hard-sphere force field calculations for **1** using Monte Carlo simulations. Additionally, the results are compared with extensive experimental NOE data for methyl α- and β-maltoside and the results discussed in light of earlier studies.

INTRODUCTION

The search for new glucosidase inhibitors has led to a group of inhibitors which are oligosaccharides with the glycosidic oxygen substituted by a sulfur or nitrogen atom or a ring oxygen substituted by nitrogen[1–5]. In order to design new and more efficient inhibitors for glycosidases, a detailed physical description is required for analogues of maltose. The interaction between a carbohydrate and a protein is initiated by recognition of the solution conformation of the interacting species and a deeper understanding of the inhibition and the interaction of the compounds with proteins (e.g., glucosidases) therefore requires detailed information about the conformational preferences of the involved species.

In the present study, we have set out to investigate the solution structure of such substrate analogues. Several studies on maltose conformation have been

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TABLE I

^{1}H NMR data for compounds **1** to **9**

Compound	**1**	**2**	**3**	**4**	**5**	**5**	**6**	**6**	**7**	**7**	**8**	**8**	**9**	**9**
pH					3.2	10.4	3.2	10.4	3.2	10.2	0.3	8.6	0.9	9.2
H-1′	5.68	5.66	5.30		3.65	3.28	3.87	3.33			5.42	5.34		
H-2′	3.82	3.80	3.82		4.04	3.80	4.06	3.80			3.63	3.60		
H-3′	3.55	3.53	3.59		3.59	3.55	3.54	3.54			3.71	3.70		
H-4′	3.42	3.39	3.38		3.59	3.47	3.62	3.46			3.43	3.42		
H-5′	4.01	4.00	3.98		2.04	1.92	2.00	1.92			3.75	3.76		
H-6′a	3.88	3.86	3.85		3.92	3.90	3.92	3.90			3.88	3.85		
H-6′b	3.80	3.77	3.75		3.79	3.75	3.80	3.76			3.78	3.76		
H-7′					4.40	4.13	4.44	4.10						
OMe			2.09											
H-1*eq*	4.37	4.84		4.84	4.75	4.72	4.84	4.78	4.86	4.82	3.55	3.12	3.56	3.12
H-1*ax*											3.02	2.46	3.02	2.47
H-2	3.26	3.55		3.56	3.64	3.55	3.63	3.54	3.62	3.55	3.88	3.53	3.84	3.50
H-3	3.72	3.87		3.53	3.92	3.61	4.02	3.68	3.84	3.51	3.82	3.60	3.55	3.33
H-4	2.79	2.80		2.70	3.03	2.36	3.35	2.61	3.23	2.67	3.86	3.43	3.65	3.25
H-5	3.67	3.81		3.68	4.03	3.61	3.97	3.52	3.93	3.57	3.43	2.67	3.25	2.55
H-6a	4.08	3.97		3.93	1.40	1.28	3.91	3.86	3.85	3.83	4.00	3.84	3.99	3.85

H-6b	3.85	3.90		3.88			3.91	3.77	3.79	3.73	3.94	3.69	3.92	3.65
OMe	3.58	3.40		3.39	3.41	3.37	3.41	3.38	3.41	3.40				
$J_{1',2'}$	5.5	5.6	5.4			4.4	4.6	4.6			3.8	4.0		
$J_{2',3'}$	9.5	10.1	9.5			10.1	9.1	10.0			9.8	9.9		
$J_{3',4'}$	9.5	9.0	9.2			9.3	8.3	9.2			9.4	9.0		
$J_{4',5'}$	9.5	10.0	9.8			11.0		11.1			9.4	9.8		
$J_{5',6'a}$	2.2	2.3	1.8		4.6	4.4	4.5	4.3			3.6			
$J_{5',6'b}$	5.4	5.5	5.4		8.2	8.4	8.1	8.3			5.5			
$J_{6'a,6'b}$	−12.2	−12.3	−12.4		−11.2	−11.2	−11.3	−11.1			−12.1			
$J_{5',7'}$					2.8	2.5	3.2	2.7						
$J_{7',1'}$					3.2	3.3	4.2	3.2						
$J_{1,2}$	8.0	3.7		3.0	3.6	3.8	3.7	3.8	3.6	3.6	4.5	5.2	5.2	5.2
$J_{1,1ax}$											12.3	12.5	12.5	12.3
$J_{1ax,2}$											11.2	10.7	11.6	10.8
$J_{2,3}$	8.5	9.6		9.7	9.2	9.6	9.5	9.7	9.4	9.6	8.8	9.3	9.3	9.2
$J_{3,4}$	10.7	10.5		10.0	10.0	9.7	10.0	9.7	10.3	9.3	8.8	8.8	9.3	9.2
$J_{4,5}$	10.7	11.2		10.0	10.0	10.1	10.4	10.1	10.4	10.1	9.8	10.0	10.6	9.8
$J_{5,6a}$	2.1	2.3		2.1	6.2	6.3	4.5	2.5	3.7	2.5	3.2	2.7	3.2	3.0
$J_{5,6b}$	5.4	4.9		5.0			4.5	5.1	4.6	5.4	5.0	5.8	5.3	6.3
$J_{6a,6b}$	−12.2	−12.2		−12.5				−11.9	−12.4	−12.4	−12.8	−11.6	−12.7	−11.7

published based on NMR spectroscopy, X-ray crystal data, optical rotation, and different levels of force-field calculations[6–20]. The crystal structure of methyl 4-thio-α-maltoside and the conformational preferences calculated using semi empirical methods has been described by Perez and Vergelati[21]. More recently, Mazeau and Tvaroška[22] have published an investigation of the conformation for the same compound in different solvents using quantum-mechanical PCILO energy minimizations. Finally, Raimbaud et al.[13] reported on the conformational preferences for thio- and nitrogen-analogues of maltose investigated by molecular-mechanics calculations.

However, none of these studies had access to extensive NMR spectroscopic data, and we have therefore complemented the conformational investigations on the maltose analogues indicated above, together with methyl α- and β-maltoside, with detailed NMR spectroscopic studies supplemented by Monte Carlo simulations for the maltose analogue **1**, as described in the following.

RESULTS AND DISCUSSION

The synthesis of methyl 4-thio-β-maltoside **1** follows standard procedures starting from octa-*O*-acetyl-4-thio-β-maltose[2]. The compounds, methyl 1-thio-α-D-glucopyranoside **3** and methyl 4-thio-α-D-glucopyranoside **4** were synthesized according to published procedures[23,24].

The assigned ^{1}H and ^{13}C chemical shifts, together with $^{1}H–^{1}H$ coupling constants for the unprotected compounds **1** to **9**, are presented in Tables I and II, respectively. For the nitrogen analogues and reference compounds **5** to **9**, the NMR data are obtained at high and low pH to probe the influence of protonation on the conformational behavior of the compounds[25]. Steady state NOE data from 1D-difference measurements for compound **1**, **2**, **5**, and **8** are presented in Table III. For comparison, selected chemical shift differences are presented in Table IV.

The conformational preferences for oligosaccharides are described by the dihedral torsion angles of the glycosidic bond ϕ_H and ψ_H and the dihedral angles of exocyclic groups (for definitions, see Experimental section). Experimentally, the conformational preferences of smaller oligosaccharides in aqueous solution can be assessed most accurately by NMR spectroscopic studies[7,14,26–34], using information from the chemical shifts, coupling constants (homo- and hetero-nuclear) and the nuclear Overhauser effects.

The conformation of methyl 4-thio-α-maltoside in the solid phase has been investigated by Perez and Vergelati[21] and the ϕ_H and ψ_H torsion angles were found to be −26 and 4°, respectively, and the glycosidic bond angle τ to be 100.3°. Furthermore, a mapping of the energy surface for the glycosidic linkage was presented, giving an overall picture similar to the surface calculated in the HSEA force field[35,36] (Fig. 1), without using the exo-anomeric effect term, i.e., HS calculations. The energy surface, as described by HSEA calculations[35–38], indicates the major populations to be in minima ranging from −90 to +40° for ϕ_H and

TABLE II

^{13}C NMR chemical shift for compounds **1** to **9**

Compound	**1**	**2**	**3**	**4**	**5**	**5**	**6**	**6**	**7**	**7**	**8**	**8**	**9**	**9**
pH					3.2	10.4	3.2	10.4	2.0	9.0	0.3	8.6	0.9	9.2
C-1′	86.7	86.6	87.2		62.8	63.6	62.7	63.0			101.0	100.9		
C-2′	71.9	71.9	72.0		69.0	71.9	68.9	71.8			72.4	72.6		
C-3′	74.3	74.3	74.6		74.6	75.8	74.6	75.7			73.5	73.7		
C-4′	70.4	70.4	70.6		70.4	71.0	70.4	71.0			70.0	70.2		
C-5′	73.8	73.7	71.9		44.3	43.5	44.5	43.5			73.8	73.5		
C-6′	61.3	61.3	61.6		60.0	60.6	60.0	60.6			61.3	61.3		
C-7′					66.6	70.0	65.9	69.9						
XMe			12.9											
C-1	103.8	100.2		100.3	99.9	100.0	100.0	100.0	100.2	100.3	46.1	49.2	46.2	49.3
C-2	75.0	73.1		73.0	72.6	72.9	72.3	73.0	72.1	73.7	67.3	71.5	67.3	71.5
C-3	76.9	73.6		74.8	70.2	75.1	70.7	75.6	69.8	73.2	76.9	79.5	76.6	79.0
C-4	47.8	47.5		42.8	65.2	66.0	60.4	60.0	53.3	53.2	76.3	81.0	68.1	72.2
C-5	75.9	71.5		74.7	65.2	69.2	67.9	73.0	68.6	72.4	59.3	59.9	60.4	61.1
C-6	62.6	62.4		62.4	18.0	18.3	62.0	62.0	61.3	61.7	58.4	62.0	58.1	62.0
OMe	57.9	55.9		55.9	56.1	55.8	56.1	55.7	56.2	55.8				

TABLE III

NOE data for compounds **1**, **2**, **5**, and **8**

Comp.	pH	Proton sat.	Nuclear Overhauser enhancements observed (%)							
			Intra-ring					Inter-ring		
1		H-1′	H-2′	H-3′				H-3	H-4	H-5
	7		9.3	0.5				2.1	5.8	2.3
		H-4	H-2	H-3	H-5	H-6		H-1′	H-5′	
	7		6.8	1.7	1.1	1.6		6.1	0.8	
2		H-1′	H-2′ [a]	H-3′				H-3	H-4	H-5 [a]
	7		11.3	0.6				3.2	6.2	
		H-4	H-2	H-3	H-5	H-6a	H-6b	H-1′	H-5′	
	7		7.6	3.0	0.9	1.3	0.3	6.1	0.8	
5		H-1′	H-2′	H-7′				H-4	CH_3-5	
	3.7									
	10.3		9.7	4.7				7.4	1.2	
		H-5′	H-3′	H-4′	H-7′	H-6′a	H-6′b	CH_3-5		
	3.7		8.4 [b]	[b]	5.8	2.9	1.1	0		
	10.3		8.4	2.4	6.9	3.5	1.1	0.4		
		H-7′	H-1′	H-5′				H-3	H-4	CH_3-5
	3.7		1.9	6.7				2.3	2.0	1.6
	10.3		4.4	6.8					3.0	2.2
		H-4	H-2	H-3	H-5	CH_3-6		H-1′	H-7′	
	3.7		9.2 [c]	1.1	1.3	2.6		[c]	2.4	
	10.3		7.3	3.5 [d]	[d]	2.1		8.3	3.0	
		CH_3-5	H-4	H-5				H-1′	H-5′	H-7′
	3.7		1.5	3.3				0.8	< 0.2	1.0
	10.3		1.4	3.8				0.8	0.7	1.5
8		H-1′	H-2′	H-3′				H-3	H-4	H-5
	0.3		11.4	+				3.8 [e]	11.6 [e]	
	8.6		11.7 [f]	0.8 [g]				[f]	11.7	1
		H-5	H-3	H-6a	H-6b			H-1′		
	0.3									
	8.6		6.5 [f]	2.7	1.1 [g]			1.0		

[a] Overlap H-2′ and H-5′. [b] Overlap H-3′ and H-4′. [c] Overlap H-2 and H-1′. [d] Overlap H-3 and H-5. [e] Partly overlap H-3 and H-4. [f] Overlap H-2′ and H-3. [g] Overlap H-3′ and H-6b.

from -60 to 30° for ψ_H. Also, minor populated minima around -60 to 10° for ϕ_H and 160 to 200° for ψ_H are found. An extensive theoretical study of the conformation of methyl 4-thio-α-maltoside has been performed by Mazeau and Tvaroska[22], using PCILO quantum-mechanical relaxed conformational energy mapping in vacuo and in different solvents. This study concluded that methyl 4-thio-α-maltoside can occupy a much larger conformational space and that the major conformation in water is the inverted conformation having ϕ_H/ψ_H around $-30°/180°$ with only low population of the "normal" conformation around 60°/31°. An energy map for 4-thio-maltose, calculated with the PFOS program, has been reported[39] resulting in a similar conformational space as the one calculated by the HSEA force field in the present work.

TABLE IV

Differences in ^{13}C and ^{1}H NMR chemical shift relative to specific model compounds

Compounds	C-1′	C-2′	C-5′	C-7′	C-3	C-4	C-5	C-6	
1–**3**	−0.5	−0.1	1.9						
2–(**3** or **4**) [a]	−0.6	−0.1	1.8		−1.2	4.7	−3.2	0.0	
1–Maltose [b]	−0.9	−0.4	0.9						
2–Maltose [b]	−1.2	−0.5	0.7		−1.5	−2.8	−1.7	0.1	
5 [c]–**5** [d]	−0.8	−2.9	−1.2	−3.4	−4.9	−0.8	−4.0	−0.3	
6 [c]–**6** [d]	−0.3	−2.9	1.0	−4.0	−4.9	0.4	−5.1	0.0	
Same cor. [e]					−1.5	0.3	−1.3	0.4	
6 [c]–Maltose [b]					0.6	−0.4	0.8	−0.1	
6 [d]–Maltose [b]					2.1	−0.7	2.1	0.4	
8 [f]–**8** [g]	0.1	−0.2	0.3		−2.6	−4.7	−0.6	−3.6	
same cor. [e]	0.1	−0.2	0.3		−0.2	−0.6	0.1	0.3	
8 [f]–Maltose [b]	0.4	−0.2	0.2		0.0	0.7	0.4	0.4	
8 [g]–Maltose [b]	0.3	0.0	−0.1		0.2	1.3	0.3	0.1	
8 [f]–Monosac [h]	8.1	−0.1	1.5		0.3	8.2	−1.1	0.3	
8 [g]–Monosac [h]	8.0	0.1	1.2		0.5	8.8	−1.2	0.0	
	H-1′	H-2′	H-5′	H-7′	H-3	H-4	H-5	H-6a	H-6b
1–**3**	0.38	0.00	0.03						
2–(**3** or **4**) [a]	0.36	−0.02	0.02		0.34	0.10	0.13	0.04	0.02
1–Maltose [b]	−0.18	0.01	−0.02						
2–Maltose [b]	−0.20	0.00	−0.01		0.10	−0.12	0.04	0.07	−0.05
5 [c]–**5** [d]	0.37	0.24	0.12	0.27	0.31	0.67	0.42	0.12	
6 [c]–**6** [d]	0.54	0.26	0.08	0.34	0.34	0.74	0.45	0.05	0.14
Same cor. [e]					0.01	0.18	0.09	0.03	0.08
6 [c]–Maltose [b]					−0.06	−0.10	−0.05	0.09	0.05
6 [d]–Maltose [b]					−0.07	−0.28	−0.14	0.06	−0.03
8 [f]–**8** [g]	0.08	0.03	−0.01		0.22	0.43	0.76	0.16	0.25
Same cor. [e]	0.08	0.03	−0.01		0.00	0.03	0.06	0.02	−0.02
g [f]–Maltose [b]	0.02	0.06	0.05		0.00	−0.04	0.06	0.01	−0.08
g [g]–Maltose [b]	−0.06	0.03	0.06		0.00	−0.07	0.00	−0.01	−0.06
8 [f]–Monosac [h]	0.20	0.09	−0.10		0.27	0.21	0.18	0.01	0.02
8 [g]–Monosac [h]	0.12	0.06	−0.09		0.27	0.18	0.12	−0.01	0.04

[a] For the nonreducing unit, the chemical shift of **3** is subtracted and for the reducing unit the shift of **4**. [b] The chemical shifts of methyl α-maltoside corrected by the difference between the corresponding monosaccharide unit and methyl α-D-glucopyranoside[49,50,52,53]. [c] pH 3.2. [d] pH 10.4. [e] The difference between the shift at high and low pH subtracted from the same difference for the monosaccharides. [f] pH 0.3. [g] pH 8.6. [h] The chemical shift of **8** minus the shifts of **9** or α-D-glucopyranose[49,52,53].

In the following, we will concentrate on the experimental evidence for the conformational preferences of 4-thio-maltosides and the nitrogen analogues of maltose[3,5]. The application of HSEA calculations is in this case only supportive for the interpretation of the experimental data. Due to the lack of good estimates on the exo-anomeric effect for thioglycosides, we have modelled the thioglycosidic linkage without an exo-anomeric term. Similarly, the treatment of the potentially charged amino groups can not be adequately performed within this force field and require a more advanced force field[25].

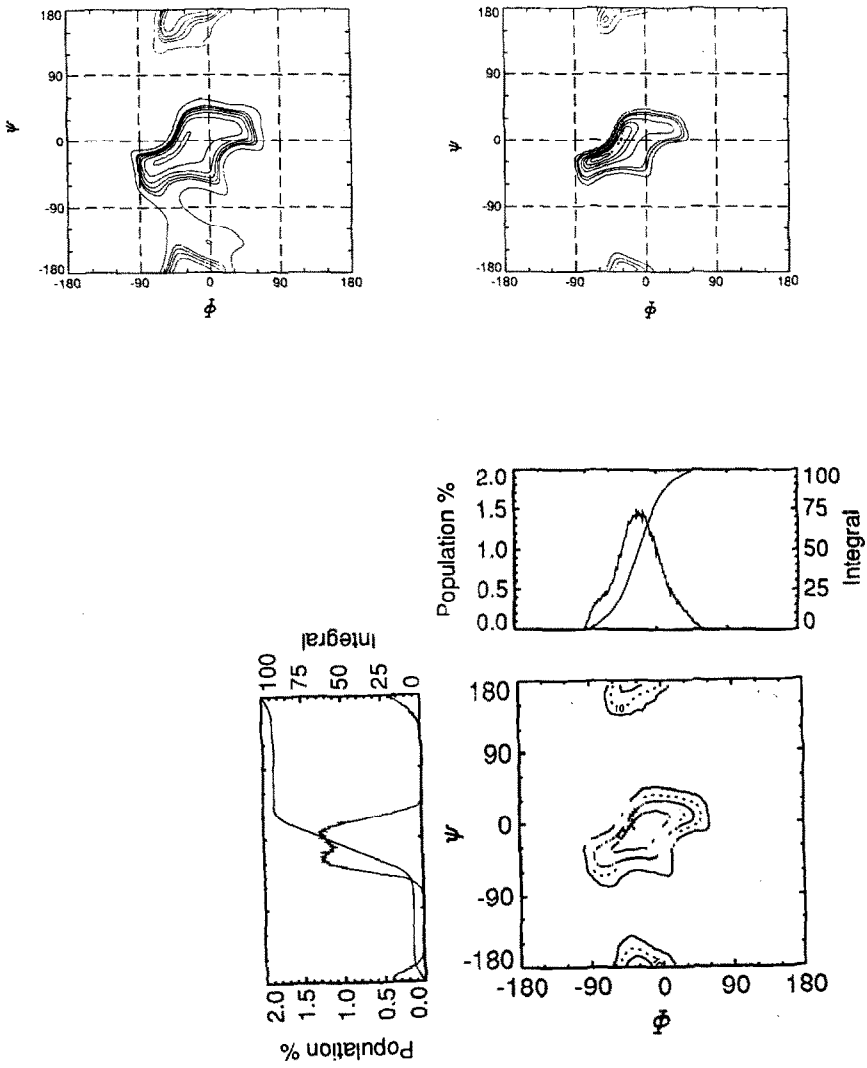


Fig. 1. Conformational preferences for methyl 4-thio-β-maltoside **1** (ϕ_H/ψ_H angles) with $\tau = 105°$. Top, isoenergy contour map (left, 0.1, 1, 2, 3, 5, and 10 kcal/mol) and population map (right, 1, 5, 10, 30, 50, and 90%) from grid search. Bottom, population map from Monte Carlo simulation at 600 K (5×10^6 steps).

The possibility of synthesizing the β anomer (**1**) allowed us to resolve the overlapping signals required for the NOE measurements, because the α anomer (**2**) has an overlap of the important signals of H-2′ and H-5. Saturation of H-1′ for **1** gives three inter-unit NOEs H-3 2.1%, H-4 5.8%, and H-5 2.3%, (Table III), and saturation of H-4 gives two inter-unit NOEs H-1′ 6.1% and H-5′ 0.8%. These data, together with NOEs derived from full matrix calculations, are presented in Table V. The calculation using a glycosidic bond angle τ of 100.3 gave two minima with

TABLE V

Measured and calculated NOEs for **1** and observed NOEs for **2**, methyl α-maltoside and methyl β-maltoside

Me 4-*S*-β-Mal **1**	Saturation of H-1′					Saturation of H-4							$^3J_{C\text{-}1',H\text{-}4}$ / $^3J_{C\text{-}4,H\text{-}1'}$	Pop. of Inver. Min *b*	ϕ_H/ψ_H	Energy (kcal/mol)
	H-2′	H-3′	H-3	H-4	H-5	H-2	H-3	H-5	H-6 [a]		H-1′	H-5′				
Observed	9.3	0.5	2.1	5.8	2.3	6.8	1.7	1.1	1.6		6.1	0.8				
Min *a* [b]	9.8	0.5	0.2	6.9	< 0.1	8.9	2.2	1.0	0.2	0.2	7.3	0.3	3.6/2.4		−38/−13	0.9
Min *b* [b]	9.5	0.5	9.2	0.2	7.8	9.0	1.1	0.6	0.4	0.1	0.04	0.2	5.9/3.1		−26/174	3.5
Grid $\tau = 100.3$	9.9	0.5	0.4	4.8	0.3	8.8	1.7	1.1	0.4	0.1	5.6	0.9	3.3/1.7	0.2%		
Grid $\tau = 105$	9.7	0.5	0.7	5.4	0.6	8.9	2.0	0.9	0.4	0.1	5.3	0.7	3.4/2.6	5.7%		
MC $\tau = 100.3$	9.8	0.5	0.8	4.8	0.6	8.1	2.1	1.0	0.8	0.6	5.8	1.1	3.3/2.1	3.7%		
MC $\tau = 105$ [c]	9.7	0.5	1.6	4.5	1.3	8.2	1.9	1.0	0.8	0.6	4.3	0.9	3.6/2.7	13.5%		
MC $\tau = 105$ [d]	9.7	0.5	2.2	4.2	2.1	8.1	1.8	0.9	0.8	0.6	3.7	0.8	3.7/2.7	20.7%		
Observed Me 4-*S*-α-Mal **2**	11.3 [f]		3.2	6.2	[f]	7.6	3.0	0.9	1.3	0.3	7.6	0.8	5.15/2.95 [e]			
Observed [g] Me α-Mal	9.7	0.6	1.5	9.8	0.3											
Observed [g] Me β-Mal	13.6 [f]	1.4	1.9	11.2	[f]											

[a] Calculated for two diastereomeric protons separately. [b] $\tau = 100.3°$. [c] Full Monte Carlo simulation 5×10^6 steps. [d] Monte Carlo step 0 to 10^6. [e] From Mazeau and Tvaroška[22]. [f] Overlapping signals for H-2′ and H-5. [g] Average of three independent measurements.

ϕ_H/ψ_H around a) $-38°/-13°$ and b) $-26°/174°$, the first having the lower energy. Calculation of NOEs from the two single minima (Table V) showed that minimum *a* results in a large NOE from H-1′ to H-4 and only small NOEs to H-3 and H-5, while minimum *b* gives a large NOE from H-1′ to H-3 and H-5, but only a small NOE to H-4. This immediately suggests that the observed NOEs must originate from an averaging of at least these two minima. The influence on the NOEs was further investigated by a grid search of the conformational space and by Monte Carlo[37,38,40] simulations. The grid search using a τ of 100.3° gave only a very low population of minimum *b*, which could not account for the observed NOEs. A better agreement was obtained by changing the τ angle to 105°. The averaging by grid search with τ 105° results in a population of the "inverted" conformation minimum *b* of ~6%, which is in good agreement with the observed NOE from H-1′ to H-4 (scaled by internal NOEs), but the calculated NOEs to H-3 and H-5 are lower than observed. The Monte Carlo calculation at 600 K using a τ of 105° results in a population of minimum *b* of 14% from the full simulation (5×10^6 steps), but the first 10^6 steps give a population of 21%. From the comparisons of the calculated NOEs with the observed ones, it can be estimated that the population of minimum *b* relative to the total population is within the range of 14 to 21%.

Investigation of the NOE data for methyl 4-thio-α-maltoside **2** shows very similar results and, assuming the internal NOE from H-1′ to H-2′ to be the same for **1** and **2**, the inter-unit NOEs by saturation of H-1′ are H-3 3.2%, H-4 6.2%, and H-5 2%. Therefore, the NOE data indicate that the conformational preferences for the α and β anomer of methyl 4-thiomaltoside are very similar. Comparison with NOE data for methyl α- and β-maltoside (Table V) indicates that the population of the inverted minimum *b* is significantly lower for the maltosides than for the thio analogues, because the NOEs observed from H-1′ to H-4 and from H-1′ to H-3 and H-5 are larger and smaller, respectively, in the oxygen analogues.

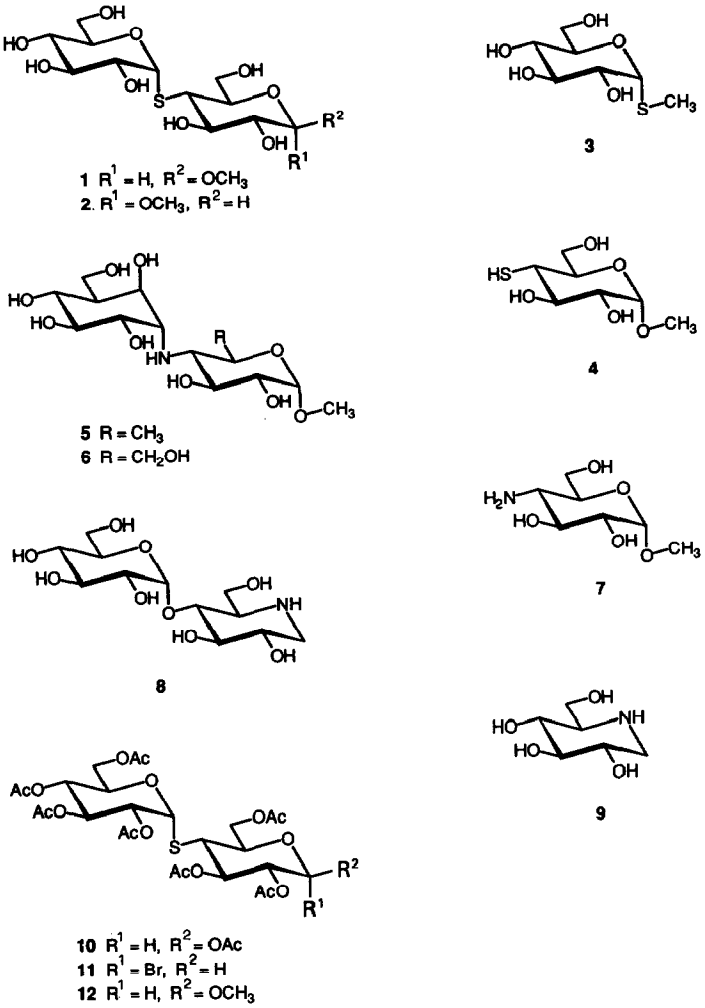
Three-bond $^1H-^{13}C$ coupling constants can also be used in the conformational analysis of oligosaccharides[41–47]. The high population of minimum *b*, found in the PCILO study by Mazeau and Tvaroska[22], gave good agreement of the calculated[45] couplings $^3J_{C\text{-}1',H\text{-}4}$ and $^3J_{C\text{-}4,H\text{-}1'}$ with the observed values of 2.95 and 5.15 Hz, respectively. The NOE measurements in the present study, however, strongly indicate that minimum *a* is the most highly populated conformer and minimum *b* can only be populated to ~20%. Taking into account both minima, estimated values of $^3J_{C\text{-}4,H\text{-}1'}$ are up to 2.7 Hz (Table V), and in good agreement with the observed value. However, less satisfactory results are obtained for the $^3J_{C\text{-}1',H\text{-}4}$ coupling constants, with estimated values of about 3.7 Hz. It is not possible to offer a clear explanation for this discrepancy between population estimated from NOEs and from coupling constants presently, however, one has to point out that the accuracy of a Karplus equation with a sulfur heteroatom relies only on very few experimentally measured coupling constants.

Inspection of the chemical shifts for **1** and **2**, relative to the monosaccharides **3** and **4**, also supports averaging of the two minima (Table IV). The glycosylation shifts of C-3, C-4, and C-5 for **2** relative to **4** are −1.2, 4.7, and −3.2 ppm, respectively, which indicates that the minimum *a* is populated to a high degree, as seen by the large glycosylation shift of C-4. The negative β-glycosylation shifts of C-3 and C-5 confirm that minimum *b* is populated to some extent. Kochetkov et al.[7,32,48] have attributed such glycosylation shifts to the compression of H-1′ with H-4, and in the inverted conformation with H-3 and H-5. The glycosylation shifts are very sensitive, as the H-1′ to H-3 and H-5 distances are short in minimum *b* and therefore, effects are also observed for H-3, H-4 and H-5. Similarly, the chemical shift of C-5′ is changed in **1** and **2** relative to **3**, however the size of this effect is more uncertain as the effect of the *S*-methyl group in **3** is not clear. The downfield shift of C-5′ is parallel to the effect seen in maltose[11,49], and can be due to interactions with the reducing unit in both minima.

Comparison of the chemical shifts of **1** and **2**, with the chemical shifts of maltosides[11,49,50], (Table IV) also supports the fact that the thioanalogues have higher populations of the inverted conformation, minimum *b* than maltosides as discussed above. It should also be noticed that the accurate NOE data on methyl α- and β-maltosides, presented in Table III, are in excellent accord with calculated results, using a complete conformational grid search, on the conformational preferences of maltosides in a recent publication[6].

The conformation of the nitrogen analogues of maltose **5**, **6**, and **8** (**5** and **6** have pseudo glucopyranosyl "nonreducing" residues) were all probed at both high and low pH, to investigate the effect of the protonation of nitrogen[25]. From a theoretical point of view, the conformational analysis of these compounds is complicated, as the protonation and charge effects generally are estimated inadequately. However, it should be included when performing studies in water. This is beyond the scope of this investigation, and therefore only an approach using the experimental NMR data will be discussed.

Compounds **5** and **6** are interesting as potential inhibitors of glucosidases[3] because they are disaccharide analogues of acarbose with a nitrogen atom in the glycosidic linkage (Scheme 1). The effect of pH on this type of linkage has been demonstrated for acarbose[25], and the data indicated an equilibrium of two minima similar to the ones discussed for 4-thiomaltosides depending on the protonation of nitrogen. The 6-deoxyglucopyranoside unit of **5** offers the possibility of selective saturation of the 5-methyl group, and therefore the NOE difference experiments were performed on this compound. The conformational space at high pH appears simple, i.e., predominantly one conformation resembling the minimum *a* for maltose, as suggested by the many constraints in the NOE data (Table III). Large NOEs are observed between H-1′ and H-4, and a simple r^{-6} approximation gives a distance of 2.5 Å (using the NOE from H-1′ to H-2′ as a reference). Additionally, large NOEs are seen between H-7′ and H-4, giving in the same way an estimate of the distance between these protons of 2.9 Å. Furthermore, some smaller NOEs are



Scheme 1.

seen between H-1′ and CH_3-5, H-7′, and CH_3-5 and between H-5′ and CH_3-5. Altogether, these NOEs define the conformation to be within a narrow range closely resembling minimum *a* of the 4-thiomaltosides.

The NOE measurements, at low pH, indicate that the conformational space of the glycosidic linkage is changed by protonation of the nitrogen like observed in acarbose[25]. One explanation for this fact might be that protonation of the nitrogen gives a steric interaction between the nitrogen hydrogens and H-3, H-5 in minimum *a*, which is relieved by inverting the orientation of the reducing end unit (changing the ψ_H angle from ~ 0 to 180°). The change in the conformational preferences can be detected in the observed NOEs. However, in this case, the NOEs cannot be explained by a single conformation, but must originate from at

least two minima similar to the ones discussed for the 4-thiomaltosides. The selective saturation of H-1′ is not possible at low pH due to chemical shift overlap. Similarly the saturation of H-4 gives some problems in the interpretation, as the intra-unit NOE to H-2 overlaps with H-1′. However, by estimating the intra-unit NOE to H-2 to be the same as at high pH, it can be estimated that the NOE from H-4 to H-1′ is ~2% giving an average distance with an r^{-6} approximation, of ~3 Å. NOEs are furthermore observed between H-7′ and H-4, giving an estimated average distance of 3 Å. The estimated distances are not consistent with one minimum, so that both minima must be populated. Thus, the observed NOE from H-7′ to H-3 can only originate from populations of the inverted minimum *b*, and the estimated NOE from H-4 to H-1′ only from minimum *a*. Therefore both conformations must contribute significantly.

Furthermore, the conformational differences at high and low pH affect the chemical shifts. In order to deduce the effect of conformational changes, it is necessary to eliminate the general effect of pH on the chemical shift. This was possible for the reducing unit using compound **7** as a reference compound. The effects of protonation on the ^{13}C chemical shifts are generally upfield shifted for carbons bearing protons close in space to the nitrogen, with a corresponding downfield shifting of the ^{1}H signals. Having subtracted the direct effect of protonation for **6** (Table IV), it is observed that C-3 and C-5 have a further upfield shift (by lowering the pH) of 1.5 and 1.3 ppm, respectively. This β-glycosylation shift can be explained by the compression of H-1′ and H-3, H-5 in the inverted conformation. The effect is less clear for the corresponding H-3 and H-5 (in the inverted minimum), as these are affected by several changes; short distances to H-1′, short distances to either O-2′ or O-7′, and a different positioning of the nitrogen hydrogen atoms by a change in ψ_H. The slightly smaller glycosylation shift of C-4, and larger shift for H-4, indicate that protonation gives less of the "normal" conformation[20] (minimum *a*). The only observed effect for the nonreducing unit is the difference of C-5′ in **5** and **6** when going from low to high pH. For **5**, the normal trend is observed with an upfield shifting upon protonation, but the opposite is observed for **6**. This difference is due to the compounds having some of the "normal" conformation and to the proximity of O-6 to H-5′ and C-5′ in the case of **6**, but not in the case of **5** since a 6-deoxyglucopyranosyl unit is present in the latter compound. In general, the differences upon protonation of **5** and **6** indicate that the O-6 of **6** might affect the conformation to a minor extent.

Compound **8** (α-D-Glc*p*-(1 → 4)-1-deoxynojirimycin, ref 5) is an interesting inhibitor for glucosidases as it is a maltose analogue of the very potent inhibitor 1-deoxynojirimycin **9** (ref 51). It is therefore interesting to see if **8** resembles maltose conformationally. The investigations were carried out at both low and high pH, and showed that protonation only results in minor conformational changes for **8**.

The NOE difference experiments at both low and high pH show strong NOEs to H-4 by saturation of H-1′. At low pH, a NOE to H-3 is also observed, but this

cannot be quantitated accurately due to partial overlap between H-3 and H-4. Furthermore, the experiment at high pH shows a small NOE to H-5 and the upfield shifted H-5 provides the possibility of selective saturation of this proton, which gives a small NOE to H-1′. All these observations together suggest that mainly the conformation[20] with ϕ_H/ψ_H around $-30°/-10°$ is populated to a high extent * and that an inverted conformation with ψ_H around 180° can only be populated to a minor degree. The chemical shifts for compound **8**, compared to the monosaccharides and maltose[50,52,53], indicate that the compound has a conformation similar to maltose at both high and low pH. Compound **8** shows very similar chemical shift to methyl α-maltoside, after correcting for the differences between 1-deoxynojirimycin **9** and methyl α-D-glucopyranoside. The comparison of the chemical shifts of **8**, with the monosaccharides α-D-glucopyranose and 1-deoxynojirimycin **9**, shows that C-1′ and C-4 have large glycosylation shifts due to compression of H-1′ and H-4. The C-3 and C-5 show only minor shift changes by glycosylation: the upfield shift of C-5 is partly due to compression of H-1′ and H-5 in the minor inverted conformation. The chemical shifts of the protons are influenced by several effects. For H-4, the compression with H-1′ is balanced due to H-4 being close to O-5′ in the major minimum resulting in a downfield shift of H-4. Similarly, H-1′ is downfield shifted by close contact with O-3. The protons H-3 and H-5 experience an additional effect[54] of the restricted rotation of the glycosidic linkage oxygen relative to the unglycosylated 1-deoxynojirimycin **9**. The C-5′ shows a downfield shift relative to α-D-glucopyranose, as observed for maltose as well, due to interaction of H-5′ and C-5′ with the hydroxymethyl group of the reducing unit.

The experimental data obtained suggest that the maltose analogues studied, all partly adopt the "normal" conformation for maltose[20], with ϕ_H/ψ_H around $-30°/-10°$, but that to different extents the inverted minimum around $-30°/180°$ is populated as well. For the compounds studied with sulfur or oxygen in the glycosidic linkage, the population of the inverted minimum generally is low, i.e., maximum 20%. The protonation of the glycosidic nitrogen atom for compounds **5** and **6** changes the balance between the two minima towards the inverted one, but still with some population of the "normal" minimum[20].

EXPERIMENTAL

General procedures.—Optical rotations were measured with a Perkin–Elmer 241 polarimeter. TLC was performed on silica gel 60 F_{254} (Merck). After preparative TLC the products were extracted with EtOAc. All reactions in organic solvents were carried out with the exclusion of moisture, and solvents for critical

* Note added in proofs. An X-ray structure of the *N*-methyl derivative of **8** [Y. Ezure, Y. Yoshikuni, N. Ojima, and M. Sugyama, *Acta Cryst., Sect. C*, 43 (1987) 1809-1811] results in an conformation with ϕ_H/ψ_H values of $-10°/7°$, respectively, in excellent accord with the results derived from the present NMR data.

reactions were dried over molecular sieves. Concentrations were carried out at diminished pressure at < 50°C, unless otherwise stated. Melting points are uncorrected. Elemental analysis was performed by Løven A/S Microanalytical Laboratory.

NMR spectroscopy.—Solutions in D_2O (0.5 mL) were used. Spectra were recorded in 5-mm tubes at 500.13 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 determined on a first order basis. The ^{13}C resonances are relative to internal 1,4-dioxane (67.4 ppm). All NMR data are given in Tables I and II for unprotected compounds and assignments are based on 2D-NMR experiments as described previously[55,56]. The atoms of the "nonreducing" unit are marked with a prime.

Conformational analysis.—The torsion angles are defined as follows[31]; ϕ_H (H-1′–C-1′–O(S,N)-1′–C-4), ψ_H (C-1′–O(S,N)-1′–C-4–H-4) and ω (O-5–C-5–C-6–O-6). The calculation of energy minima and energy/population maps, by grid search or Monte Carlo simulations for thiomaltosides, were performed using the GEGOP program[37,38]. The theoretical NOE values were calculated using a full matrix description using a rotational correlation time τ_c of 0.22 ns. This was based on $\langle r^{-6} \rangle$ derived from the full energy surface by either grid search or Monte Carlo simulation[57–60]. The GEGOP program used the HSEA force field[35,36] and an additional torsional potential for the "normal" sugar unit hydroxy methyl groups[61]. 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 1-thio-α-D-Glc and 4-thio-α-D-Glc units were taken from the X-ray structure[21] and the protons attached as described[31,35,36]. The 4-thio-β-D-glucose unit was prepared by modifications of the X-ray data for the β-D-Glc unit[62].

Methyl hepta-O-acetyl-4-thio-β-maltoside (**12**).—To a solution of octa-*O*-acetyl-4-thio-β-maltose[2] (**10**) (81 mg, 0.117 mmol) in CH_2Cl_2 (2 mL), was added AcOH saturated with HBr (1 mL), and the mixture was stirred for 2 h. Ice and CH_2Cl_2 (75 mL) were added and the organic phase was washed with water (6 × 50 mL). Drying ($MgSO_4$) and concentration to dryness gave **11**, which was used without further purification. To a solution of the freshly prepared **11** in CH_2Cl_2 (2 mL) and MeOH (2 mL), silver carbonate (65 mg, 0.235 mmol) was added, and the mixture was stirred with 3A molecular sieves and with exclusion of moisture for 22 h. The mixture was filtered through activated carbon and the filter was washed with CH_2Cl_2. The organic phase was concentrated to dryness yielding a crude product (69 mg) which was purified by preparative TLC by elution with 1:1 EtOAc–hexane yielding **12** (55 mg, 0.083 mmol, 71%); mp 159–161.5°C; $[\alpha]_D$ +29.3°. Anal. Calcd for $C_{27}H_{38}O_{17}S$: C, 48.65; H, 5.75; S, 4.81. Found: C, 50.7; H, 6.1; S, 4.4. ^1H NMR (500 MHz, $CDCl_3$): δ 5.90 (d, $J_{1',2'}$ 6 Hz, H-1′), 5.26 (dd, $J_{2',3'}$ 10.4, $J_{3',4'}$ 10.6 Hz, H-3′), 5.26 (dd, $J_{2,3}$ 9.4, $J_{3,4}$ 10.6 Hz, H-3), 5.07 (t, $J_{4,5}$ 10 Hz,

H-4'), 4.97 (dd, H-2'), 4.80 (dd, $J_{2,3}$ 9.4 Hz, H-2), 4.66 (dd, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 11.7 Hz, H-6a), 4.39 (d, $J_{1,2}$ 8.2 Hz, H-1), 4.34 (dd, $J_{5',6a'}$ 4.2, $J_{6a',6b'}$ 12.4 Hz, H-6a'), 4.25 (m, H-5'), 4.23 (dd, $J_{5,6b}$ 6.0, $J_{6a,6b}$ 11.7 Hz, H-6b), 4.11 (dd, $J_{5',6b'}$ 2.1, $J_{6a',6b'}$ 12.4 Hz, H-6b'), 3.65 (m, H-5), 3.48 (s, OMe), 3.02 (t, $J_{4,5}$ 10 Hz, H-4). ^{13}C NMR (500 MHz D_2O): δ 101.09 (C-1), 82.35 (C-1'), 43.8 (C-4), 56.8 (OCH_3).

Methyl 4-thio-β-maltoside (**1**).—*O*-Deacetylation of **12** (43 mg, 0.065 mmol) in MeOH (6 mL) and NaOMe in MeOH (1 M, 0.1 mL) yielded **1** (44 mg). The product was purified by chromatography on Sephadex G-15 using 1:1 MeOH–water as eluant. This gave **1** (hygroscopic foam, 24 mg, 0.065 mmol, 100%, no microanalysis performed); $[\alpha]_D^{20}$ +140° (*c* 0.12, H_2O). NMR data are given in Tables I and II, respectively.

Compounds **3**, **4**, and **7** were synthesized according to published procedures[23,24,63] and their structural identity confirmed by the spectroscopic data presented in Tables I and II, respectively. Compound **9** was purchased from Sigma.

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