THE EFFECT OF STEREOCHEMISTRY ON CARBOHYDRATE HYDRATION IN AQUEOUS SOLUTIONS

On the cover:

The crystal structure of n-octyl 1-thio-α-D-talopyranoside (chapter 5). The structure has been solved by F. Van Bolhuis and will be published in the near future.

RIJKSUNIVERSITEIT GRONINGEN

THE EFFECT OF STEREOCHEMISTRY ON CARBOHYDRATE HYDRATION IN AQUEOUS SOLUTIONS

PROEFSCHRIFT

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te Bergum

Promotor: Prof. Dr. J.B.F.N. Engberts

HET HEELAL
Des te verder men keek,
des te groter het leek.

Voor Pap en Mam

"Het Heelal" is een gedicht van:
Jules Deelder,
uit *Dag en Nacht Geopend,*
De Bezige Bij,
Amsterdam, 1970

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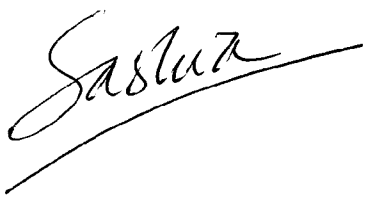


CONTENTS

CHAPTER 1

CARBOHYDRATES IN AQUEOUS SOLUTION AN INTRODUCTION

1.1 Carbohydrates, simple molecules with complex behaviour

The simple generic formula of carbohydrates $(CH_2O)_n$ belies their extreme stereochemical complexities.[1] Small differences in types of linkage between carbohydrate moieties make a large difference in their properties: amylose, a polysaccharide which consists of α-1,4-linked glucose units, is soluble in water, whereas cellulose, with β-1,4-linked glucose units is not. The complexity of the behaviour of carbohydrates and the important role of carbohydrates in life processes make the study of the stereochemical aspects of solvation of carbohydrates a challenge. Not only do they serve as structural and protective materials and as an energy source,[2] they are very important moieties in glycoproteins[3] and are of paramount importance in (bio)molecular recognition.[4,5] Along with α-amino acids, purine and pyrimidine bases and lipids, sugars and their derivatives are key units and building blocks in the chemistry of biological macromolecules. They play a central role in metabolic reaction cycles, and their polymers fulfil biological functions almost as diverse as proteins. In addition, carbohydrates and their derivatives are used extensively both in food and non-food applications.[6,7]

1.1.1 Biological relevance and applications

Carbohydrates are present in plants and animals and they participate in the metabolic cycle.[8] Both glucose and fructose are abundantly present in fruits, plants, honey and in blood of animals. The disaccharides sucrose and lactose occur widely as free sugars, lactose is present in the milk of mammals, and sucrose is found in fruits and plants. Maltose is the product of enzymatic hydrolysis of starch. Cellobiose is a product of hydrolysis of cellulose. Polysaccharides play an important role in plants: cellulose is present in their cell walls. Starch, which consists of amylose and amylopectin, is stored in seeds, roots and fibres of plants as a food reserve. Chitin, a polymer of N-acetyl-β-D-glucosamine is a structural component of the hard shells of insects and crustaceans.

Carbohydrates are encountered as polar headgroups in membrane lipids; gangliosides and cerebrosides and sulfated derivatives of galactocerebrosides are found in significant quantities in the brain and other nervous tissue. Some organisms can exchange

phospholipids for glycolipids as a response to chill, desiccation or salinity. Trehalose (a disaccharide) is produced extensively by some organisms to defy death by dehydration.[9,10] The function of trehalose[11-13] in preventing dehydration has been studied by monitoring the interactions of carbohydrates with phospholipid bilayers[14] and monolayers.[15] It was found that if trehalose is successful in preventing dehydration of vesicles, it should be both inside and outside the bilayer;[14] this might also apply to cells. In addition, the effectiveness of protection depends on the stereochemistry of the carbohydrate:[15] glucose and galactose have a greatly different effect.

Research upon biologically relevant glycoproteins has been widely reported.[3] Further research has focussed on the influence of carbohydrates on thermal denaturation,[16] protein stability,[17,18] and the ability to bind to DNA.[19]

Carbohydrates are added to food and non-food products as stabilisers or emulsifiers, as cryoprotectants[20,21] and as structuring materials. Highly concentrated solutions of carbohydrates in water appear to form a glass[22] and these glasses can protect against dehydration. Pectins are used as a gelling agent, alginates from seaweed and gums from trees are used as stabilisers and emulsifiers in food, and in the pharmaceutical, cosmetic and textile industries. Carbohydrates and carbohydrate-derived surfactants have been applied in the detergent field, polymer chemistry and also in medicinal chemistry: sulfated saccharose is used against ulcers, heparin is well known for preventing coagulation of blood. Carbohydrate-derived surfactants are potentially useful for LCD screens. Their liquid crystalline behaviour is being studied extensively.[23-26] In organic synthesis carbohydrates can be used to make relatively hydrophobic compounds more soluble in water, which opens the possibility of using water as a solvent for synthesis on a production scale, for example Diels-Alder reactions[27a] and Claisen rearrangements.[27b]

There is considerable progress in the study and applications of glycolipids. Initially they were used for membrane protein reconstitution,[28] but ever since it was observed that the glycolipids can be applied as "green"[29] and calcium-tolerant detergents, there is increased interest in the field. An all-bio detergent, the compound APG (alkyl polyglucoside) is now widely used. Carbohydrate-derived surfactants have an advantage over other nonionics such as ethylene oxide derivatives, that a great variety of sugars can be used and the carbohydrate moieties can be functionalised by oxidation and sulfation.[30] The carbohydrate-derived surfactants are mild, surface-active compounds and have anti-bacterial activities.

On the cell surface both glycoproteins and glycolipids have the role of molecular

messengers. There is a continuing interest in protein-carbohydrate interactions, which is fueled by the essential role of carbohydrates in biological recognition and adhesion processes.[31] Molecular recognition is mimicked by studying interactions of carbohydrates with proteins,[31-33] among them lectins[4,34,35] and monoclonal antibodies.[4,36] In addition, the role of water in these recognition processes is studied.[37]

It is generally agreed that for binding of carbohydrates to proteins, key polar (hydroxy) groups of the carbohydrate are important.[5] Which key polar groups are important for binding generally depends on the protein. For lectins there often is specificity for either galactose or glucose and mannose.[33] However, also nonpolar contacts are important. Generally binding of carbohydrates to proteins is reversible.[5] The anomeric hydroxy group often serves as a hydrogen bond donor only, whereas the non-anomeric hydroxy groups act simultaneously as hydrogen bond donor and as acceptor.[32] This leads to stronger than average hydrogen bonds. The binding sites are usually found in clefts between the domains in the proteins, to which carbohydrates are bound in varying degrees.[35] The bound sugar is buried in the cleft and is (almost) inaccessible to bulk solvent. Only a few water molecules are present at the binding site and these have a bridging function in the complex.

The oligosaccharides, which are involved in the molecular recognition process at the cell surface, never contain more than twenty monosaccharide units.[5]

1.1.2 Sweetness

Many properties of carbohydrates depend on the details of the hydroxy topology. As an example, the sweetness of sugars is briefly discussed to show what the problems inherent to defining the relationship between subtle stereochemical differences and properties of a molecule are.

The most common and most widely used carbohydrate is sugar or sucrose,[38,39] a disaccharide consisting of a glucopyranose and a fructofuranose linked between the 1- and 5-position. Sucrose has been used as a sweetener for centuries and the relative sweetness[40] has been used as a standard. Dietary industries have been looking for compounds with a sweeter taste than sucrose. Efforts have been made to establish a molecular theory of sweet taste and a structure-activity relationship has been proposed.[41] Not only for sugars but also for sweet amino acids,[42] it has been agreed that for a (sweet) taste the compound needs to be soluble in water[42] and needs to have a AH-B set of polar groups in which A and B should be between 2.5 and 4.0 Angstrom apart.[43] B is an electronegative group and A of AH is a hydrogen-bond donor. There

needs to be a third structural feature, which is involved in dispersion interaction with the receptor site and which is probably hydrophobic. These three groups need to be arranged in an L-shape.

Attempts have been made to substantiate these findings further by molecular dynamic simulations,[44] studies of solution properties,[43,45] intrinsic viscosities,[42] and conducting pulsed NMR[46] and IR[47] analyses. The molecular picture behind the theory of sweet taste remains unclear. Empirically it is found that carbohydrates can be made to taste sweeter by chlorination.[48,49] Sucralose is an example of a chlorinated sucrose, much sweeter than sucrose. It has already been approved by the National Food and Drug Administrations in both Canada and Australia for commercial use. Other halogen substituents can make sucrose even sweeter.[38] Fructose is sweeter than sucrose,[41] and it is widely used in Germany as a sweetener. The relationship between the hydroxy topology of a carbohydrate and its sweetness is apparently based upon the hydroxy groups on the 3- and 4-position of the glucose ring in sucrose.[41,45,50] For example, when sucrose is changed into "lactosucrose" (the hydroxy group on carbon 4 is inverted), its sweetness disappears. When the OH(3) is inverted, the sweetness disappears completely.[38]

In Japan many different sugars are being studied for their applicability as sweeteners, not because they are sweeter than sucrose, but merely because they have a low calorie content and are still sweet.[51]

1.2 Carbohydrates in aqueous solutions

Carbohydrates have long been the "Cinderellas" of physical-organic chemistry.[1] This stemmed largely from the belief that carbohydrates were uninteresting compounds, lacking any biological specificity, and served solely as structural or protective materials and as an energy source.[1]

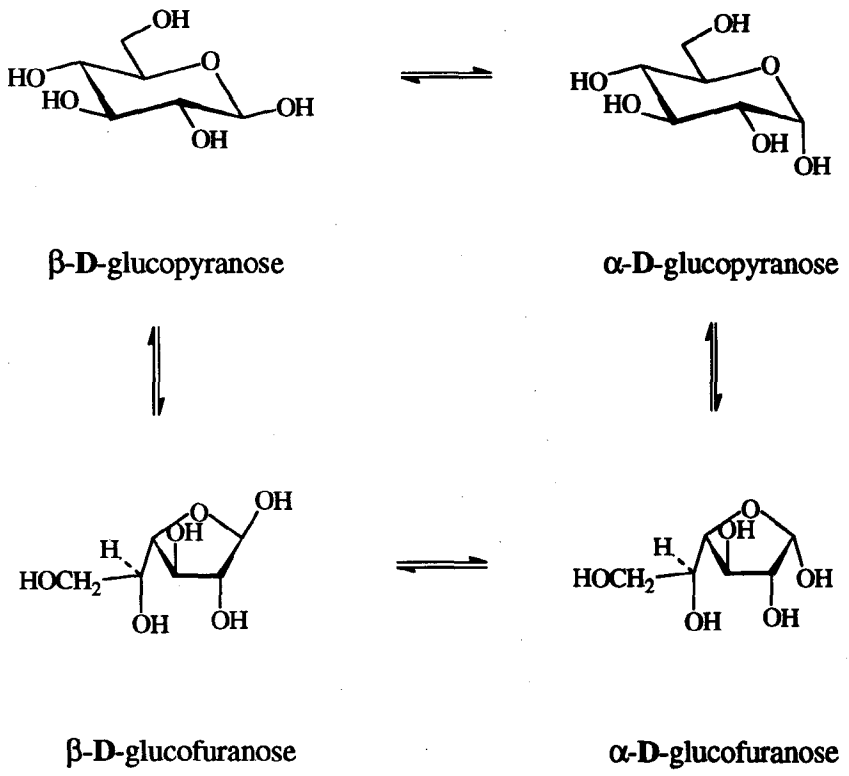
Carbohydrates interact very strongly with water through their hydrogen bonds, and due to the characteristics of these interactions, they must be stereospecific.

In the following two paragraphs a description of possible approaches to acquire an understanding of carbohydrate chemistry in aqueous solution will be given. The experimental limitations of studies of aqueous solutions of carbohydrates are described. The methods which have been used and the hydration models for carbohydrates that have been developed will be briefly reviewed.

1.2.1 Approaches to studies in aqueous solutions

There are three different approaches to studies of the hydration behaviour of

carbohydrates in aqueous solutions. First it is possible to examine how the thermodynamic stability of the carbohydrate molecule is influenced by the surrounding water molecules and why certain conformers are preferred in solution. The essence of this method is to analyse the Gibbs energy of the carbohydrate and the influence of hydration thereupon. Second, the focus can be primarily on the influence of the carbohydrate on the hydration layer. The particular equilibrium composition (*vide infra*) is taken as a starting point and is supposed not to change during the measurements. Only the hydration layer is studied and its properties are compared to pure water as a reference state. Finally, carbohydrate-carbohydrate interactions can be studied in water, usually via studies of excess properties of aqueous solutions of carbohydrates.



Scheme 1.1. Equilibrium of D-glucose in water.

In the first type of approach the results have been rationalised in terms of the anomeric and exo-anomeric effect.[52-57] The second type of approach is primarily used in

this thesis. Measurements of dielectric relaxation of the solvent[58,59] and solvent T_1[60,61] measurements also fit the second category. In the third approach the emphasis has been on additivity[62-70] in intermolecular interactions.

1.2.2 Choice of experiments

The source of the specificity in carbohydrate hydration has long been a subject of study.[71] Often the differences in Gibbs energy between various forms of one sugar are small, i.e. in the range of kT, so that, under suitable conditions, two or more structurally distinct species can coexist in equilibrium.[1] This complicates the study of aqueous carbohydrate solutions. Neither crystallographic data nor data obtained in other solvents or solvent mixtures can be readily extrapolated to aqueous solutions[72] due to the special solvent characteristics of water. Carbohydrates may form a complex mixture in water, due to their propensity to undergo (complex) mutarotation.

A carbohydrate can be present in both a five membered ring (furanose) and in a six membered ring (pyranose). Furthermore the hydroxy group on the anomeric position (position 1) can be either axial (α-anomer) or equatorial (β-anomer). See Scheme 1.1. This leads to an additional experimental restriction. Experiments that require measurements over a wide temperature and concentration range or that use the crystalline state of the carbohydrate as a reference state should be avoided (particularly when the mutarotation isomer in the crystalline state is different from the dominant mutarotation isomer in solution, as in the case of D-ribose). Obviously this does not apply to the methyl glycosides for which the mutarotation is blocked.

Experimental studies of carbohydrates in aqueous solutions can be carried out with several techniques. However, there are severe limitations regarding the experimental design, because the carbohydrate is present in water as a mixture of forms. If a carbohydrate solely undergoes simple mutarotation in water, the molecule will be present in the pyranose form and as a mixture of both α- and β-anomers. When a carbohydrate undergoes complex mutarotation it will not only be present in its pyranose forms, but also in the furanose form, both alpha and beta. In scheme 1.1 the respective pyranose and furanose forms have been depicted for D-glucose. In Table 1.1 a summary of equilibrium compositions is given for different aldo- and ketohexoses, as reported by Angyal *et al.*[73] and Goldberg and Tewari.[74] It can be seen that the hexoses mostly reside in the pyranose form except for D-psicose, which is dominantly present in the furanose form. The exact equilibrium composition depends on temperature, concentration of carbohydrate in solution and solvent.[75] Hence, most properties measured are average properties of the solution. When one is particularly interested in a property of one

mutarotation isomer, or, as is the present study, the average properties of the hydration layer, it is particularly important to adhere to a constant equilibrium composition. Thus measurements should be performed at constant temperature, at a low concentration and a small concentration range of carbohydrate, and neither carbohydrates in other solvents than water, nor in the crystalline form can be used as reference states.

Carbohydrates are so extensively hydrogen-bonded in aqueous solution, that sometimes the dominant mutarotation isomer present in solution is different from that in the crystalline state (e.g. D-ribose). Therefore the crystalline state of a carbohydrate should be avoided as a reference state.

Table 1.1 Percent Composition of the Anomeric Forms of Monosaccharides in D_2O.

	Compound[a]	T/K	Pyranose α	Pyranose β	Furanose α	Furanose β	Aldehyde Form
	aldopentoses						
2a	D-arabinose	304.15	60	35.5	2.5	2	0.03
2c	D-xylose	304.15	36.5	63	<1		0.02
2d	D-lyxose	304.15	70	28	1.5	0.5	0.03
2e	D-ribose	304.15	21.5	58.5	6.5	13.5	0.05
	aldohexoses						
3a	D-galactose	304.15	30	64	2.5	3.5	0.02
3b	D-gulose	295.15	16	81	-	3	-
3c	D-glucose	304.15	38	62	-	0.14	0.002
3d	D-mannose	317.15	63.7	35.5	0.6	0.2	0.005
3e	D-allose	304.15	14	77.5	3.5	5	0.01
3f	D-altrose	295.15	27	43	17	13	0.04
3g	D-talose	295.15	37	32	17	14	0.03
3h	D-idose	304.15	38.5	36	11.5	14	0.2
	ketohexoses						Keto Form
4a	D-fructose	303.15	2	70	5	23	0.7
4b	L-sorbose	300.15	98	-	2	-	0.2
4c	D-psicose	300.15	22	24	39	15	0.2
4d	D-tagatose	300.15	79	16	1	4	0.6

(a) Data from reference 73 and 74.

1.2.3 Methods

For a long time the techniques, which can be used for studying carbohydrate hydration and the small differences therein for different carbohydrates, were not readily available. Only in the past three decades have aqueous solutions of carbohydrates been

studied thermodynamically.[74] Earlier they were considered to be (almost) ideal and hence not interesting. The most widely used methods are: measurements of the chemical potential (the activity[75] of water in aqueous solution of carbohydrates is measured by freezing point depression, osmotic pressure or vapour pressure), calorimetry, measurements of density and the pressure and temperature derivatives of density. Transport properties are also informative; chiefly viscosities and diffusion coefficients[76,77] have been determined. On a macroscopic level these data provide information on carbohydrate-water interactions. An other experimental technique in carbohydrate chemistry, which is still used in quality control, is the measurement of optical activity.[78] All these properties give averaged values if different anomers are present in solution.

There are unfortunately no convenient chromophores present in a carbohydrate that allow the use of optical spectroscopy. However, other possibilities include circular dichroism,[79] IR and Raman studies.[80]

Franks and Suggett[58,59,81-83] successfully applied spectroscopic measurements of the dielectric properties of aqueous solutions of carbohydrates in conjunction with NMR. The results are informative of the molecular motion and interactions in water. These experiments were the basis for the "stereospecific hydration model" which will be discussed in paragraph 1.2.4.

Other NMR work led to a clear insight into the equilibrium composition of carbohydrates in aqueous solutions[73,84-93] and the temperature and concentration dependence of the equilibrium.[94-96] Furthermore, ^{13}C-NMR relaxation studies[97] have been used to understand the rotational dynamics of methylated glycosides in various solvents. An elegant method is the NMR study of the water in the hydration layer of a carbohydrate, which can either be studied by ^{1}H-NMR,[98-100] or by ^{17}O-NMR.[61,62]

Theoretical studies[55,56] have been performed as well as molecular dynamics[101] and other simulations. The main difficulty lies in the correct definition of the force field and the correct interpretation of the anomeric effect.[102] This subject will be elaborated upon in paragraph 1.2.4.

1.2.4 The stereospecific hydration model

Carbohydrate chemists will agree that the hydration of carbohydrates is stereospecific. However, how the stereochemical features are translated into the stereospecificity of the hydration is still a subject of discussion.[71,103]

The first attempts to delineate a stereospecific hydration model were made by Kabayama and Patterson.[104,105] They studied the thermodynamics of mutarotation and found an

enthalpy difference between α- and β-glucose. This subject was also addressed by others, who studied the anomeric effect and its solvent dependence[102,106-109] for each carbohydrate. The difference in enthalpy between α- and β-glucose has been further elaborated upon by theoretical chemists.[53,57] It was first postulated that an extra interaction must be present to account for differences in anomeric ratios, the anomeric effect. The anomeric effect is a stereo-electronic effect.[54] Because of the anomeric effect an electron withdrawing group at C-1 is often more stable in the axial orientation. An equatorial hydroxy group at the anomeric centre produces an unfavourable dipole-dipole interaction with the ring oxygen atom. Hence in the crystalline state the α-anomer is favoured.[52] The effect is electrostatic in nature and therefore varies inversely with the dielectric permittivity of the solvent. The effect in water should be small; the β-anomer of glucose is predominantly present in aqueous solution. However, not every sugar is predominantly present in the β-form in water (Table 1.1). Therefore Angyal[73] suggested that the relative positions of OH(2) and OH(3) also influence the relative stability of α- and β-anomers in water. The number of adjustments involved in considering the anomeric effect for carbohydrates has led to considerable criticism. This particular aspect is still under active investigation.

Suggett and Franks[58,59,81-83] also suggested that in the case of glucose the β-anomer fits better in the three-dimensional hydrogen-bond network of water than the α-anomer does. They arrived at this conclusion on the basis of NMR experiments:[100] the anomeric hydroxy group of glucose is always more acidic than the other hydroxy groups in the carbohydrate molecule. In addition, the signal of the β-hydroxy group is found to be broader than that from the α-hydroxy group. This led to the conclusion that the β-anomer has a faster proton exchange with water than the α-anomer, and for that reason fits better. The oxygen distances in water were also compared with the O_1-O_3 oxygen distances of both α- and β-D-glucose and generally a better compatibility for β-D-glucose was found. This prompted the general conclusion that an equatorial hydroxy group will fit better in water than an axial hydroxy group. Hence it was proposed that the number of equatorial hydroxy groups present in the carbohydrate molecule will determine the overall compatibility of the carbohydrate molecule with the three-dimensional hydrogen-bond network of water.[59]

Aqueous solutions of several carbohydrates were studied with different techniques. It was proposed that because of the arrangements of the hydroxy groups in β-D-glucose (all equatorial) this compound will fit best in water. However, the results for carbohydrates with a different number of equatorial hydroxy groups compared to glucose were not compatible with the stereospecific hydration model. Further research aimed at

understanding carbohydrate hydration has been performed with the objective of finding the dependence of the hydration of a carbohydrate on the number of equatorial hydroxy groups.

Apart from the anomeric effect[104,105] and the ratio of axial versus equatorial hydroxy groups,[59,77,103] the hydration characteristics of carbohydrates have been rationalised by using concepts such as hydration numbers,[77,82,110] the hydrophobic/hydrophilic index,[111] the hydrophobic volume of the carbohydrate,[112] and the compatibility with water structure depending on the position of the next-nearest neighbour hydroxy groups of a carbohydrate molecule.[113,114] Goldberg and Tewari have tried to obtain more insight into the properties of different isomers by studying the thermodynamics of isomerisation of carbohydrates.[115-117] Balk and Somsen[118,119] used concepts like preferential solvation and hydrophobic hydration to account for enthalpies of transfer of carbohydrates from water to aqueous DMF mixtures. Barone[65-69] and coworkers, and Tasker[62-64] and coworkers have applied the Savage-Wood approach to account for excess

Table 1.2 Number of Equatorial Hydroxy Groups, $n(OH)_{eq}$, and Solvent Properties for Some D-Hexoses in Aqueous Solutions at 298 K.

Property	Glucose	Galactose	Mannose
$n(OH_{eq})^a$	4.62	3.64	3.34
V_2,[b] $cm^3\ mol^{-1}$	112.0	110.5	111.5
$C_{p,2}$,[c] $J\ mol^{-1}\ K^{-1}$	331	337	324
10^{10} Do,[d] $m^2\ s^{-1}$	6.67	7.04	7.41
A[e]	38.7	40.6	42.0
μ,[f] D	12.1	11.6	11.9
μ,[g] D	4.5	5.3	4.8
δ,[h] $C^2\ m^2\ J^{-1}\ mol^{-1}$	4.27	3.28	4.25
$K_{2,s}$,[i] $cm^3\ mol^{-1}\ bar^{-1}$	-17.8	-20.8	-16.0
n_h[j]	3.58	4.3	3.3

a) The hydroxy group in the hydroxymethyl moiety is also counted as an equatorial hydroxy group. The actual number represents the number of nonanomeric hydroxy groups which are equatorial and the anomeric hydroxy group is counted in part. For example, for D-glucose 62% is present as the β-anomer in solution, so OH(1) is 0.62 equatorial hydroxy group. b) Partial molar volume, best values according to ref. 74. c) Partial molar heat capacity, ref. 121. d) Limiting diffusion coefficient of sugar, ref. 60. e) Hydrophobic index, ref. 111. f) Dipole moment of sugar, calculated by the method of Onsager, ref. 58. g) Dipole moment of sugar, calculated by the method of Buckingham, ref. 58. h) Limiting dielectric decrements, ref. 58. i) Partial molar compressibility, ref. 122. j) Hydration number, ref. 121.

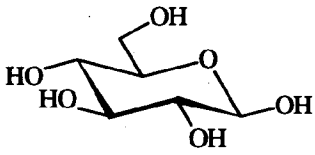
properties of aqueous carbohydrate solutions. Bernal and Van Hook[120] have used structure-making and structure-breaking to rationalise their experimental data.

Unfortunately, the measurements did not always yield results which fitted with the stereospecific hydration model. In Table 1.2 a selection of data for the three most important hexoses is presented. The data meet the following criteria: the correct experimental design is obeyed and the data are available for all three hexoses. It is observed that for partial molar heat capacities $C_{p,2}$ and partial molar volumes V_2 there is not much difference between the hexoses. The results of the limiting diffusion coefficient D_0 and the hydrophobic index A are in reasonable agreement with the number of equatorial hydroxy groups. However, the majority of the results show that there is no clear correlation with the number of equatorial hydroxy groups. The dipole moments μ, limiting dielectric decrements δ, partial molar compressibilities $K_{2,s}$ and hydration numbers n_h all indicate that if there is any difference in hydration, galactose would be different from glucose and mannose.
The lack of consistency of the data with the theoretical model made some researchers prefer to study to polyols to avoid the complications of mutarotation.[123,124]
Although it was difficult to agree upon a stereospecific hydration model, generally it is believed that the carbohydrates have a rather hydrophobic character[1,59,111,125-127] in water due to a camouflage effect, caused by the fact that the hydroxy groups of a carbohydrate resemble the hydroxy groups of water. When the hydroxy groups of a carbohydrate resemble those of water and fit into the unperturbed hydrogen-bond network of water, a spectator molecule will not "see" them and will only "see" the methine groups of a carbohydrate. Because of this camouflage effect the carbohydrate will appear to be a hydrophobic solute.

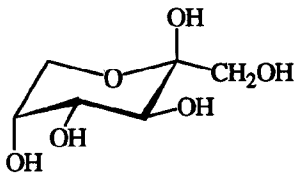
1.3.1 Aim of this study

The interpretation of properties of carbohydrate solutions in terms of their hydration characteristics is, at the moment, a matter of controversy. The experimental results obtained for aqueous solutions of carbohydrates cannot be satisfactorily interpreted in terms of the stereospecific hydration model, which was proposed by Franks and Suggett. Therefore the aim of this study was to examine further the stereochemical aspects of carbohydrate hydration. Previous work has been hampered by the fact that only a relatively small range of isomers was studied. An improved understanding of carbohydrate hydration might result from a study of a complete set of stereoisomers.

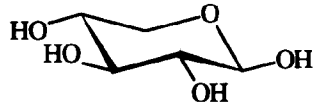
Scheme 1.2 Dominant Mutarotation Isomers of D-Glucose, D-Fructose and D-Xylose.



D-glucose 3c



D-fructose 4a



D-xylose 2c

Table 1.3 Structure of Dominant Mutarotation Isomers in Aqueous Solution and Composition of Disaccharide.

	Position of Hydroxy Group[a]		Position of Hydroxy Group[a]
Aldopentose		Aldohexose[b]	
2a D-arabinose	1a2e3e4a	**3a** D-galactose	1e2e3e4a6e
2c D-xylose	1e2e3e4e	**3b** D-gulose	1e2e3a4a6e
2d D-lyxose	1a2a3e4e	**3c** D-glucose	1e2e3e4e6e
2e D-ribose	1a2e3a4e	**3d** D-mannose	1a2a3e4e6e
		3e D-allose	1e2e3a4e6e
		3f D-altrose	1e2a3a4e6e
Ketohexose		**3g** D-talose	1a2a3e4a6e
4a D-fructose	1e2a3e4e5a	**3h** D-idose	1a2a3a4a6e
4b L-sorbose	1e2a3e4e5e		
4c D-psicose[c]	1e2a3e4a5e		
4d D-tagatose	1e2a3a4e5e		

	Disaccharide	Subunit[d]	Type of Linkage
7a	maltose	gl-gl	1α-4
7b	cellobiose	gl-gl	1β-4
7c	trehalose	gl-gl	1α-1α
7d	gentiobiose	gl-gl	1α-6
8a	sucrose[e]	gl-fr	1α-5
8b	turanose[e]	gl-fr	1α-3
8c	palatinose[e]	gl-fr	1α-6
9a	lactose	gl-ga	1β-4
9b	melibiose	gl-ga	1α-6
9c	lactulose[e]	fr-ga	1β-4

a) e=equatorial, a=axial. b) The hydroxy group in the hydroxymethyl moiety is defined as OH(6) because it is attached to C-6 and is given as being equatorial because the hydroxymethyl group is equatorial. c) D-psicose is predominantly present in the furanose form. d) gl=glucose, fr=fructose, ga=galactose. e) For turanose, the fructose unit is in the pyranose form, other fructose moieties are in furanose form.

The criteria which have to be met for satisfactory studies need to be delineated. All the experimental techniques should be screened for their applicability to aqueous carbohydrate solutions, and the relative sensitivity of the properties toward stereochemical aspects of hydration should be assessed. In the research to be described, a complete series of D-hexoses was employed and the appropriate properties were determined. A critical appraisal of all the data processed and the results will be presented.

1.3.2 Survey of the contents

In this thesis the stereochemical aspects which govern carbohydrate hydration have been investigated employing a range of techniques. The focus is on the hydration layer of a carbohydrate. The second approach described in 1.2.1 has been used. The hydration characteristics of carbohydrates such as pentoses, hexoses, methyl aldoglycopyranosides, some disaccharides (their structures and composition are given in Table 1.3) and some carbohydrate-derived surfactants will be described.

The hydration layer of the carbohydrate has been studied by means of kinetic medium effects (chapter 2) using the carbohydrate as a cosolute. Thermodynamic parameters which relate to the carbohydrate-water interaction only were measured and are discussed in chapter 3. Molecular dynamics computer simulations are the subject of chapter 4. From the results the through-space intramolecular oxygen distances of an aldohexose molecule have been obtained and these are compared to intermolecular oxygen distances in water. In chapter 5 the synthesis and aggregation behaviour of carbohydrate-derived surfactants is described.

In chapter 1 an overview has been given of the role of carbohydrates in biological and industrial processes and the sweetness of carbohydrates is discussed. Also a detailed description is given of the aims and methods of studies of carbohydrates in aqueous solutions and of the experimental limitations which are associated with these types of studies. The present stereospecific hydration model is briefly described.

In the work summarised in chapter 2, carbohydrates are applied as cosolutes in aqueous solution. The carbohydrate affects the rate of a neutral hydrolysis of an activated amide (1-benzoyl-3-phenyl-1,2,4-triazole) compared to pure water. On the basis of earlier work in the field,[128] it is proposed, that the kinetic medium effects induced by the carbohydrates can be interpreted in terms of interactions involving destructive hydration shell overlap of the hydration layers of the carbohydrate with those of both the initial state and the activated complex of the reaction. A quantitative analysis of the kinetic medium effects is presented in terms of the stereochemistry of the

carbohydrate and the equilibrium composition of each individual carbohydrate in water. Selected thermodynamic properties of aqueous solutions of carbohydrates were measured and are reported in chapter 3. The definitions and calculation of thermodynamic parameters as well as their applicability to understanding the hydration of carbohydrates are discussed. It is shown that measurements of thermodynamic properties of carbohydrate solutions must circumvent the problems arising from the heterogeneity (the carbohydrates are present in solution in several forms) of the solutions. Standard states have to be chosen carefully. The actual amount of information on stereochemical aspects of hydration inherent to a thermodynamic parameter depends on whether the property is representative for both the intrinsic property of the carbohydrate and the contribution due to the carbohydrate-water interactions, or whether it is only representative of the latter. The value of the information also depends critically on the sensitivity of the technique of measurement.

If all these conditions are observed it is found that the fit of the carbohydrate molecule into the three-dimensional hydrogen-bond structure of water depends very much on the hydroxy topology of the carbohydrate. The camouflage effect is also discussed on the basis of thermodynamic parameters, such as partial molar volumes and partial molar heat capacities.

In chapter 4 the through-space intramolecular oxygen distances of next-nearest neighbour oxygens within a carbohydrate molecule are compared to the intermolecular oxygen distances in water. First the oxygen distances are discussed for the crystalline state of carbohydrates. For a selected group of aldohexoses it is shown that through-space intramolecular next-nearest neighbour oxygen distances can be very different depending on the stereochemistry of the carbohydrate molecule and that they can fit with two intermolecular oxygen distances in water, the nearest neighbour or next-nearest neighbour oxygen distances. The fit of the carbohydrate in water is discussed in those terms.

For the molecular dynamics simulation of carbohydrate hydration, two hexoses were used which, on the basis of previous work, showed very different behaviour in water. Again the next-nearest neighbour oxygen distances within a carbohydrate molecule were obtained and compared with the intermolecular oxygen distances in water. The probability of finding the oxygen of a water molecule at a certain distance from a carbohydrate oxygen has been examined. The fit of a carbohydrate molecule is discussed in terms of the fit of next-nearest neighbour oxygen distances of the carbohydrate with the oxygen distances between water molecules. The next-nearest neighbour oxygens of a carbohydrate molecule are divided into two planes, each of which should have a good fit

to ensure a compatibility of a carbohydrate with water. The camouflage effect is rationalised by considering the possibility of intramolecular bonds.

In chapter 5 the synthesis and aggregation behaviour of carbohydrate-derived surfactants (n-alkyl 1-thio-D-glycopyranosides) are described. The method used for the coupling of the n-alkanethiol to the carbohydrate moiety depends on the stereochemistry of the carbohydrate; in this case the relative position of the hydroxy group at C-2 is the determining factor. The purification of the deprotected carbohydrate-derived surfactants (the method also depends on stereochemistry) is described in detail. The aggregation behaviour, solubility and liquid crystalline behaviour of the carbohydrate-derived surfactants are discussed. The aggregation behaviour is studied with microcalorimetry.

In chapter 6 the results reported in the earlier chapters are summarised and examined. The results of all the measurements are assessed and consistencies are indicated.

The fit of the carbohydrate in the three-dimensional hydrogen-bond network of water is discussed in terms of a modified stereospecific hydration model and the apparent hydrophobicity of carbohydrates is defined using this model. Results from the literature are re-evaluated using the modified stereospecific hydration model.

Finally, abstracts are given in English and Dutch.

Part of this thesis have already appeared in print or will be published in the near future.[129-137]

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CHAPTER 2

KINETIC MEDIUM EFFECTS OF CARBOHYDRATES ON THE NEUTRAL HYDROLYSIS OF 1-BENZOYL-3-PHENYL-1,2,4-TRIAZOLE

2.1 Medium effects

Medium effects have been studied in great detail in chemistry. The medium can affect absorption spectra,[1] equilibria,[2] aggregation behaviour,[3] solubilities[4] and rates of reactions.[5] Medium effects have been explained in terms of macroscopic solvent parameters, such as dielectric constants[6] and solvent polarity scales.[7] They have also been analysed by considering semi-empirical solvent parameters. These include various solvatochromic polarity parameters such as those of Kosower,[8] Dimroth-Reichardt,[9] Abraham-Kamlet-Taft[10] and parameters representing ionising power.[11] These semi-empirical parameters are always dependent on the method or a model process. All these methods have been excellently reviewed by Blokzijl.[12]

2.2 Kinetic medium effects

Kinetic medium effects in mixed solvent systems have been studied extensively over the years, for both organic[13] and inorganic[14] reactions. These studies have been concerned with the effect of a cosolvent or cosolute (the name depends on whether the compound which is mixed with the main solvent, is a liquid or a solid) upon reaction rates, mechanisms and thermodynamic activation parameters.[5] Much emphasis has been placed on aqueous solutions. Kinetic medium effects in mixed aqueous solutions have been analysed in terms of semi-empirical solvent parameters,[8-11] the structure-making or structure-breaking ability[15] of the cosolvent (cosolute), or correlations with macroscopic solvent parameters have been sought.[6,7] The kinetic basicity[16] of water in the aqueous solution has also been considered in some cases. Most explanations of the kinetic medium effects have only been qualitative in nature, although many attempts have been made to rationalise solvent-induced changes in thermodynamic activation parameters in terms of non-covalent interactions, in particular hydrophobic interactions.[5]

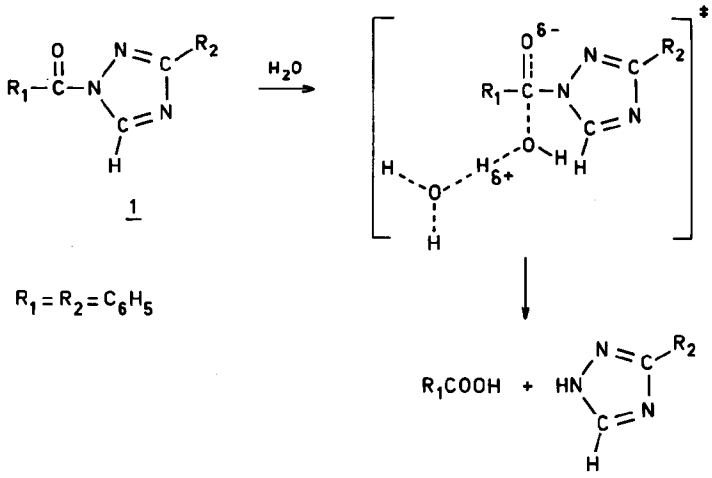
2.3 Intermolecular interactions in water: the additivity of group interactions

In the last several years a quantitative theory has been developed named after Savage and Wood, who first formulated the additivity principle of solute-solute interactions[17]

in water. At first it was used to study the thermodynamics of solute-solute interactions in water. Interestingly, often additivity was found for Gibbs energies and enthalpies of intermolecular non-covalent interactions between solutes in aqueous solution.[18-22]

Later this approach has been made applicable for quantitative analyses of kinetic medium effects.[23,24] The kinetic medium effect depends on the influence of the added cosolvent (cosolute) on the chemical potentials of both the reactants and the activated complex. This effect is proportional to the molality of the cosolvent (cosolute) at sufficiently low concentrations and can be expressed as the difference of interactions of the cosolvent (cosolute) with the reactants and the activated complex. On a next level of sophistication, the effect can be dissected into interactions of the functional groups in the cosolvent (cosolute) with the functional groups in the reactants and the activated complex. For example, the interactions of methanol with the reactants and activated complex of the reaction can be dissected into the interactions of the methyl moiety and the hydroxy group with functional groups in the reactants and the activated complex, respectively.

The neutral hydrolysis of 1-benzoyl-3-phenyl-1,2,4-triazole (1) has been studied in some detail as a model reaction. The pH-independent hydrolysis of the activated amide **1** proceeds through a dipolar transition state containing two water molecules and in which three protons are in flight.[25,26]



Scheme 2.1 Reaction mechanism for the neutral hydrolysis of **1**

Quantitatively, the kinetic medium effect can be expressed as follows:[23,24]

$$\ln(k_{obs}/k^{o}_{obs}) = (2/RT)\,[G(C\text{-}IS) - G(C\text{-}TS)]\,m_c - n\Phi M_1 m_c \qquad (2.1)$$

In equation 2.1, k_{obs} is the pseudo-first-order rate constant for hydrolysis of **1** in the aqueous binary mixture in which the molality of cosolvent (cosolute) is m_c, k^{o}_{obs} is the rate constant in pure water (m_c = 0), R is the gas constant, and T is the temperature in Kelvin, n is the number of water molecules involved in the activated complex (for **1**, n = 2), and Φ is the osmotic coefficient of water (Φ = 1 for highly dilute solutions).[27] M_1 is the molar mass of water. G(C-IS) is the pairwise Gibbs energy interaction parameter for the interaction between cosolvent (cosolute) and the initial state of the reaction, whereas G(C-TS) is the corresponding interaction parameter for the activated complex of the reaction. Previously this has been described as G(C) = G(C-IS) - G(C-TS)[23] or as - 3G(C-OH),[24] since the difference between the activated complex and the initial state of the reaction has been interpreted as being three hydroxy groups pointing towards the medium in the activated complex (see Scheme 2.1). Thus G(C) represents the overall effect of the cosolvent (cosolute) on the Gibbs energy of activation for the hydrolytic process. Its value is obtained directly by adding $n\Phi M_1$ to the slope of the linear plot of $\ln (k_{obs}/k^{o}_{obs})$ versus the molality of the cosolvent (cosolute).

The question remains: what does the overall Gibbs energy of interaction, G(C) represent?

(a) It can be representative of reactivity of the cosolvent (cosolute) towards the activated amide. (b) There can be a direct interaction of the cosolvent (cosolute) with the activated amide. (c) The interaction can take place through the hydration layers of both the cosolvent (cosolute) and the initial state of the reaction and the activated complex involving hydration shell overlap.

Careful gas chromatographic analysis of the reaction mixture in the presence of a range of alcohols[28] showed that the contribution to G(C) of direct alcoholysis of the activated amide can be safely neglected. In addition, it was argued that general base catalysis by an alcohol is not significant, because water is present in such a large excess over the cosolvent (cosolute). Furthermore the influence of the hydrophobic part of a relatively hydrophobic cosolvent such as 1-propanol can be completely diminished in the presence of urea (which by itself has no influence on the rate of reaction). Urea is known for its structure breaking ability, hence the hydrophobic part of 1-propanol does not exert an influence on the rate of reaction in the presence of urea because the hydration layer of the 1-propyl group is destroyed by urea. Therefore it

has been tentatively concluded that the observed medium effects are primarily the result of interaction of the cosolvent (cosolute) with both the initial state of the reaction and the activated complex involving hydration shell overlap. However, at the moment it appears more reasonable to assume that no real distinction can be made between the latter two possibilities (b and c) since both types of interactions involve destructive hydration shell overlap.

In the quantitative theory,[23,24,29] medium effects on rates of reactions have been explained by combining transition state theory and thermodynamics. In this approach it is possible to predict kinetic medium effects in terms of interaction parameters of the cosolvent (cosolute) with the hydrolytic probe during the activation process. A prerequisite for the success of the analysis is that the studies are performed in highly dilute aqueous solvent mixtures, otherwise triplet or higher-order interactions complicate the interpretation. The kinetic medium effects of alcohols,[23] polyols,[28] urea and substituted ureas,[24] amides[30] and sulfonamides, sulfones and sulfoxides[31] have all been studied and the theory has also been applied in mechanistic studies.[32,33]

Solvent effects in binary mixtures in which the cosolvent (cosolute) is present in much higher concentrations and even in excess over water can be analysed in terms of the Kirkwood-Buff approach, as described by Blandamer *et al.*[34,35]

When 1 was used as a hydrolytic probe, additivity of the kinetic medium effects was found for alcohols[23] and ethers.[23,32] For urea and substituted ureas additivity was found for the kinetic medium effect on the neutral hydrolysis of p-methoxyphenyl dichloroacetate.[24] In principle, the interactions of an alcohol are made up of the group interactions of the functional groups of the alcohol: the CH and OH groups. It was also found that the interaction of a CH_3 group is 1.5 times that of a CH_2 group, which is equivalent to 3 times a CH moiety. For diols[28] it was found that additivity of group interactions only applies when the hydroxy groups are far apart (1,4 or farther) from each other. Otherwise hydration shell overlap of the separate functional groups within the solute will interfere with additivity. Generally it is found for monohydric alcohols[28] that a OH group has a rate increasing effect (G(OH) is positive), whereas a CH moiety has a rate decreasing effect (G(CH) is negative). However, both functional groups influence each others hydration. This explains why the G(CH) and G(OH) values depend on the number of hydroxy groups present in the alcohol. The most negative CH interaction is expected for hydrocarbons, if they could be used as cosolvents in water. However, in alcohols the mutual effect on the hydration of the methine moieties and the hydroxy groups results in a situation in which both $|G(CH)|$ and $|G(OH)|$ are decreased. For example, when one compares the kinetic medium effect of monohydric alcohols with

that of dihydric alcohols and polyols, it is generally observed that when more hydroxy groups are present in the cosolvent molecule, the G(OH) becomes less positive, and the G(CH) becomes less negative. Thus, the overall kinetic medium effect is determined by the number of CH and OH groups in the alcohol and the way they affect each others hydration layer and availability for interaction. The rate retardation by methine moieties is caused by the fact that the activated complex of the model reaction is more polar[25,26] than the initial state (see Scheme 2.1.) It is proposed that G(CH) is negative because the methine moieties stabilise the initial state of the reaction due to hydrophobic interactions. G(OH) is positive because it counteracts the availability of the methine groups for interaction. Also the hydroxy groups partly destroy the hydrophobic hydration of the methine groups.

For amides[31] additivity of group interactions depends on the substitution pattern if **1** is used as a hydrolytic probe. This is caused by the fact that amide-amide interactions are specific in water. When an ester[30] is used as a hydrolytic probe, no specific interactions are observed and additivity is restored. Recently, an investigation of additivity of interactions with charged solutes[36] has been started.

2.4 Stereochemical aspects of intermolecular interactions in water

The additivity of group interactions as proposed by Savage and Wood[17] does not allow for specificity in interactions for different stereoisomers. However, it is anticipated that interactions of solutes in water might well respond to stereochemistry. The hydration characteristics of cis- and trans-1,2-cyclopentanediol[28] are different. It has also been found that in the case of carbohydrates different stereoisomers influence the activation parameters of **1** differently[37,38] and, in addition, differences in carbohydrate-carbohydrate interactions for a series of carbohydrates were found by Barone *et al.*[39,40] and Tasker and Wood.[41-43]

On a next level of sophistication the question arises whether it is possible to measure differences in interactions between two enantiomers (diastereomeric interactions) in water. In previous studies it has been found that enthalpies of interactions between R,S pairs of 2-butanol and chiral amino acids are different from R,R and S,S interactions.[44-46] A chiral kinetic probe, S-(+)-2-phenylpropionyl-1,2,4-triazole was synthesised and used to investigate kinetic medium effects.[47] It hydrolyses via a mechanism similar to that shown for **1**. However, differences in interactions with R- and S-2-butanol have not been observed, even at lower temperatures. A reason for this result might be that the interactions of 2-butanol in water are mainly determined by the alkyl group of 2-butanol. These interactions are apolar and non-directional in

water. Therefore it was reasoned that a chiral cosolvent, which is polar, could show differences in interactions with a chiral probe, because the hydration of a polar compound is more directional. However, chiral solutes like methyl β-D- and L-arabinopyranosides also failed to show chiral discrimination.[47] Probably there can be no chiral discrimination in intermolecular interactions in highly dilute aqueous solutions because the interacting molecules are separated by water. Chiral discrimination is likely to be observed if there is either a direct interaction or complexation, or when the cosolvent (cosolute) is reactive towards the chiral probe. This is also illustrated by the fact that so far chiral discrimination of interactions in aqueous solutions has mostly been found for micellar systems[48-51] or when complexation with a cyclodextrin is involved.[52] Further research on this aspect is still in progress.[53]

Although in our work[47] no differences in interactions between chiral species in water could be observed, the interactions of a probe with two diastereoisomers can still be very different. Therefore the interactions of **1** with different carbohydrates were examined. The applicability of the kinetic medium effect approach towards monitoring stereochemical aspects of carbohydrate hydration will be described in this chapter.

2.5 Kinetic medium effects of carbohydrates in aqueous solutions

This chapter deals primarily with the specificity of carbohydrate interactions mediated by water. The different interactions of the carbohydrate with the initial state and the activated complex for hydrolysis of substrate **1** lead to changes in rate constants of the water-catalyzed hydrolysis reaction. To ensure that medium effects of carbohydrates are primarily determined by interactions involving destructive hydration shell overlap,[28] the products of the hydrolysis of **1** in the presence of carbohydrates were analysed to determine whether carbohydrate-derived esters had been formed. Since these products were not found (see experimental section), it is most likely that only the kinetic medium effects of carbohydrates are observed and these can be interpreted in terms of interactions involving destructive hydration shell overlap.

The hydration characteristics of different isomeric aldopentoses (**2a-2e**), aldohexoses (**3a-3h**), ketohexoses (**4a-4d**), methyl aldoglycopyranosides (**5a-5f, 6a,b**) and disaccharides (**7a-7d, 8a-8c, 9a-9c**) have been studied. Stereochemical details and interaction parameters for the monosaccharides are listed in Tables 2.1 to 2.3. Both Scheme 1.2 and Table 1.3 provide the stereochemical details of the dominant mutarotation isomers of all mono- and disaccharides, which have been studied; they may be found in chapter 1 and also on a separate sheet in this thesis. Representative plots

of $\ln(k_{obs}/k^{o}_{obs})$ versus molality of carbohydrate (equation 1, *vide supra*) are shown for aldopentoses, aldohexoses and methyl aldoglycopyranosides in Figures 2.1, 2.2 and 2.3, respectively. Comparable data for methyl aldoglycopyranosides and for disaccharides are listed in Tables 2.4 and 2.5, respectively. Rate constants were determined at 298.15 K at relatively low carbohydrate concentrations (0 to 1 mol kg^{-1}). Typically the measurements were performed at four different molalities. All carbohydrates cause a rate retardation of the hydrolytic process to an extent that is expressed in the negative slopes of the linear plots of $\ln(k_{obs}/k^{o}_{obs})$ versus molality of carbohydrate.

Table 2.1 Percent Composition of the Anomeric Mixtures of Aldopentoses in D_2O, Stereochemistry of Dominant Mutarotation Isomers and Kinetic Medium Effects in Aqueous Aldopentose Solutions at 298.15 K.

Carbohydrate[a]	Pyranose[b]		Furanose[b]		Conformation[c]	G(C)[d]	G(CHOH,endo)[e]
	α	β	α	β		J kg mol^{-2}	J kg mol^{-2}
2a D-arabinose	60	35	3	2	1a2e3e4a	- 98 (8)	-13(2)
2b L-arabinose	60	35	3	2	1a2e3e4a	-129 (10)	-21(4)
2c D-xylose[f]	37	63	-	-	1e2e3e4e	-253 (18)	-52(5)
2d D-lyxose	70	28	2	-	1a2a3e4e	-241 (18)	-49(5)
2e D-ribose	22	59	6	13	1e2e3a4e	-223 (10)	-45(4)

a) Molality always below 1 mol kg^{-1}. b) From ref. 54, percent equilibrium composition. c) Numbers refer to the position of the hydroxy groups, a=axial, e=equatorial. d) Experimental value, error is given between parentheses. e) Calculated CHOH, endo value from eq. 2.2. f) The dominant mutarotation isomer of D-xylose is shown in Scheme 1.2.

2.5.1 Aldopentoses

For the aldopentoses (**2a-2e**; Table 2.1) the G(C) values indicate that the type of hydration depends on the stereochemistry of the pentose, although, according to the additivity principle of Savage and Wood,[17] all aldopentoses should exert the same kinetic medium effect. The pentoses can be divided into two groups based on their different kinetic medium effects. (a) Both D- and L-arabinose (**2a** and **2b**), which have an axial hydroxy group at the 4-position and an equatorial OH(2) show small negative G(C) values (-98 and -129 J kg mol^{-2}, respectively), whereas (b) D-xylose (**2c**), D-lyxose (**2d**) and D-ribose (**2e**) exhibit a more negative G(C) value (-223 to -253 J kg mol^{-2}). The latter compounds possess an equatorial OH(4) and either an axial or an equatorial OH(2). The position of the hydroxy groups on C-4 appears to determine the magnitude of the kinetic medium effect.

The difference found in G(C) value between D- and L-arabinose is illustrative of the

error in the determination of the G(C) value, as theoretically they should possess the same G(C) values.

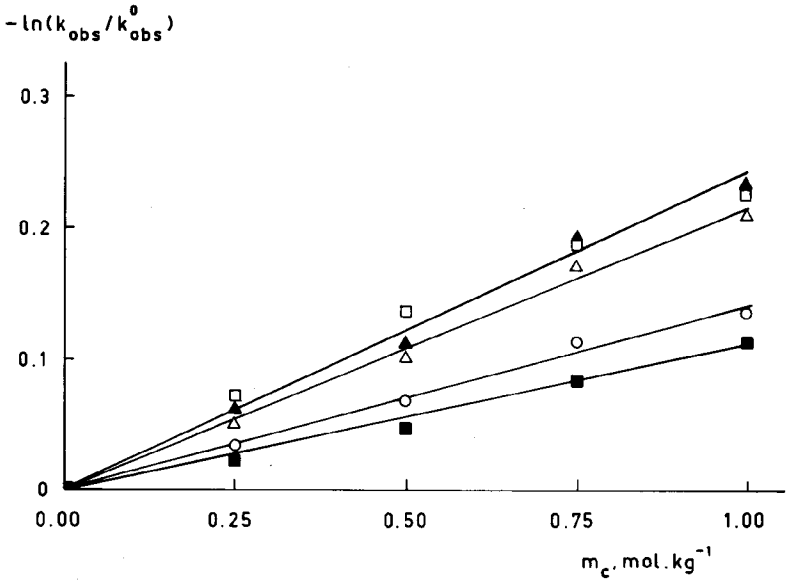


Figure 2.1 Plots of $-\ln(k_{obs}/k^{o}_{obs})$ *vs.* molality of carbohydrate for the neutral hydrolysis of **1**: **2a** D-arabinose (-■-); **2b** L-arabinose (-o-); **2c** D-xylose (-▲-); **2d** D-lyxose (-□-); **2e** D-ribose (-Δ-).

Table 2.2 Percent Composition of the Anomeric Mixtures of Aldohexoses in D_2O, Stereochemistry of Dominant Mutarotation Isomers and Kinetic Medium Effects in Aqueous Aldohexose Solutions at 298.15 K.

Carbohydrate[a]	Pyranose[b] α	Pyranose[b] β	Furanose[b] α	Furanose[b] β	Conformation[c]	G(C)[d] J kg mol^{-2}	G(CHOH,endo)[e] J kg mol^{-2}
3a D-galactose	30	64	2	3	1e2e3e4a6e	-142 (11)	-31(3)
3b D-gulose	16	81	-	3	1e2e3a4a6e	-131 (30)	-22(7)
3c D-glucose[f]	38	62	-	-	1e2e3e4e6e	-201 (12)	-45(3)
3d D-mannose	65	34	1	-	1a2a3e4e6e	-227 (12)	-52(3)
3e D-allose	14	78	3	5	1e2e3a4e6e	-228 (15)	-52(4)
3f D-altrose	27	43	17	13	1e2a3a4e6e	-244 (10)	-56(3)
3g D-talose	37	32	17	14	1a2a3e4a6e	-280 (10)	-65(3)
3h D-idose	39	36	11	14	1a2a3a4a6e	-330 (40)	-72(10)

a) Molality always below 1 mol kg^{-1}. b) From ref. 54, percent equilibrium composition. c) The numbers refer to the positions of the hydroxy groups, a=axial, e=equatorial. d) Experimental value, error is given between parentheses. e) Calculated CHOH, endo value from eq. 2.2. f) The dominant mutarotation isomer of D-glucose is shown in Scheme 1.2.

2.5.2 Aldohexoses

The present study is the first (except for the NMR spectroscopic investigation by Angyal *et al.*[54,55]) which considers a complete set of D-aldohexoses in an attempt to understand how hydration of a carbohydrate varies with stereochemistry.

The kinetic medium effects of the aldohexoses (**3a-3h**; Table 2.2) suggest that the interactions of the hexoses also depend on their hydroxy topology. Not only is the relative position of OH(4) important, but the OH(2) position is also important when OH(4) is axial.

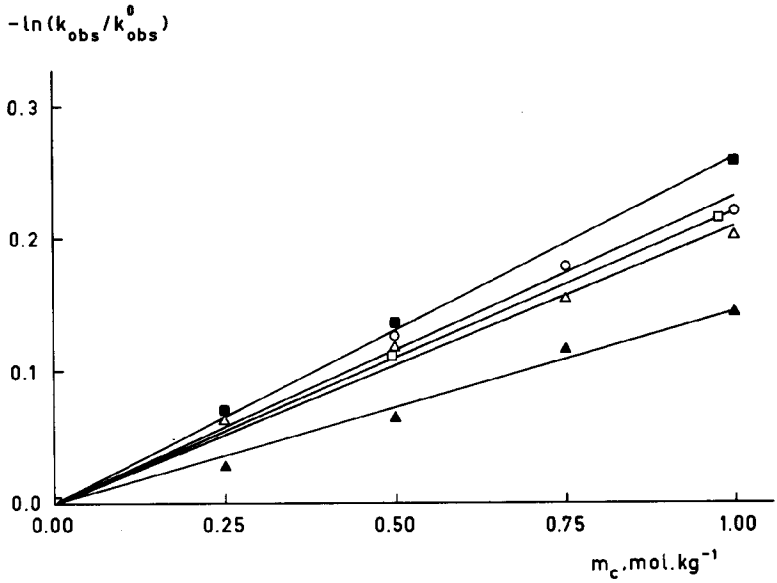


Figure 2.2 Plots of $-\ln(k_{obs}/k^{o}_{obs})$ *vs.* molality of carbohydrate for the neutral hydrolysis of **1**: **3a** D-galactose (-▲-); **3c** D-glucose (-Δ-); **3d** D-mannose (-o-); **3e** D-allose (-□-); **3g** D-talose (-■-).

Three different classes of medium effects can be distinguished, depending on the stereochemistry of the aldohexose. (a) A small, negative kinetic medium effect is observed (G(C) = -108 and -142 J kg mol^{-2}) for aldohexoses, which have an axial hydroxy group on the C-4 position and an equatorial OH(2) (**3a**, D-galactose and **3b**, D-gulose). (b) A moderately negative kinetic medium effect (G(C) = -201 to -244 J kg mol^{-2}) is found for aldohexoses for which OH(4) is equatorial and OH(2) is either equatorial or axial (**3c**, D-glucose, **3d**, D-mannose, **3e**, D-allose, **3f**, D-altrose). (c) Finally, aldohexoses with both an axial OH(4) and an axial OH(2) show the largest negative kinetic medium effect (**3g**, D-talose and **3h**, D-idose); in these cases the G(C) values

are -280 and -330 J kg mol^{-2}, respectively.

Table 2.3 Percent Composition of the Anomeric Mixtures of Ketohexoses in D_2O, Stereochemistry of Dominant Mutarotation Isomers and Kinetic Medium Effects in Aqueous Ketohexose Solutions at 298.15 K.

Carbohydrate[a]	Pyranose[b]		Furanose[b]		Conformation[c]	G(C)[d]	G(CHOH,endo)[e]
	α	β	α	β		J kg mol^{-2}	J kg mol^{-2}
4a D-fructose[f]	2	70	5	23	1e2a3e4e5a	-222 (12)	-51(3)
4b L-sorbose	98	-	2	-	1e2a3e4e5e	-255 (10)	-51(3)
4c D-psicose[g]	22	24	39	15	1e2a3e4a5e	-247 (10)	
4d D-tagatose	79	16	1	4	1e2a3a4e5e	-203 (10)	-46(3)

a) Molality always below 1 mol kg^{-1}. b) From ref. 55, percent equilibrium composition. c) Numbers refer to the position of the hydroxy group, a=axial, e=equatorial. d) Experimental value, error is given between parentheses. e) Calculated CHOH, endo value from eq. 2.2. f) The dominant mutarotation isomer of D-fructose is shown in Scheme 1.2. g) Group contribution not calculated because D-psicose resides predominantly in the furanose form.

2.5.3 Ketohexoses

All ketohexoses (**4a-4d**, Table 2.3) exert about the same kinetic medium effect regardless of their stereochemistry. Although their hydration characteristics seem to be different from those of the aldohexoses,[56] ketohexoses show a G(C) value (-203 to -255 J kg mol^{-2}) similar to that of the aldohexoses, which have an equatorial OH(4). We suggest that the relative insensitivity of the hydration of ketohexoses to their stereochemistry is caused by the presence of the exocyclic hydroxymethyl group on the anomeric centre instead of at the 5-position. This aspect is further elaborated upon in chapter 6.

Table 2.4 Kinetic Medium Effects of Methyl Aldoglycopyranosides in Aqueous Solutions at 298.15 K.

Carbohydrate[a]	G(C)[b], J kg mol^{-2}
5a methyl α-D-galactopyranoside	-244 (10)
5b methyl β-D-galactopyranoside	-228 (13)
5c methyl α-D-glucopyranoside	-276 (10)
5d methyl β-D-glucopyranoside	-326 (19)
5e 3-O-methylglucopyranose	-389 (16)
5f methyl α-D-mannopyranoside	-343 (10)
6a methyl β-D-arabinopyranoside	-260 (10)
6b methyl β-D-xylopyranoside	-428 (10)

a) Molality always below 1 mol kg^{-1}. b) Experimental value, error given between parentheses.

2.5.4 Methyl aldoglycopyranosides

The methyl aldoglycopyranosides (**5a-5f**, **6a,6b**; Table 2.4) exert a stronger rate retarding effect than the corresponding aldoglycopyranoses, which is expressed in a more negative G(C) value. This is due to the presence of the methoxy group at the anomeric centre, which provides an additional centre for interaction. Again the G(C) values depend on the stereochemistry of the carbohydrate. We observe that the methyl hexopyranosides (**5a-5f**) can be divided into two groups depending on the position of the OH(4). A moderately large and negative kinetic medium effect is found for the methyl hexopyranosides which have an equatorial OH(4) (**5d,5f**) and either an axial or an equatorial OH(2) (G(C) = -326 and -343 J kg mol^{-2}, respectively), whereas **5b**, which has an axial OH(4) and an equatorial OH(2) shows a smaller negative G(C) value (-228 J kg mol^{-2}). When there is an equatorial OH(4), the G(C) values also depend on the relative position of the methoxy group (**5c**, **5d** and **5e** all have significantly different G(C) values: -276, -326 and -389 J kg mol^{-2}, respectively). This is not found when the OH(4) is axial and OH(2) is equatorial; **5a** and **5b** induce approximately the same kinetic medium effect (G(C) = -244 and -228 J kg mol^{-2}, respectively). In the case of methyl pentopyranosides (**6a,6b**) the G(C) values differ depending on the relative position of OH(4), compare **6b** (G(C) = -428 J kg mol^{-2}) with **6a** (G(C) = -260 J kg mol^{-2}).

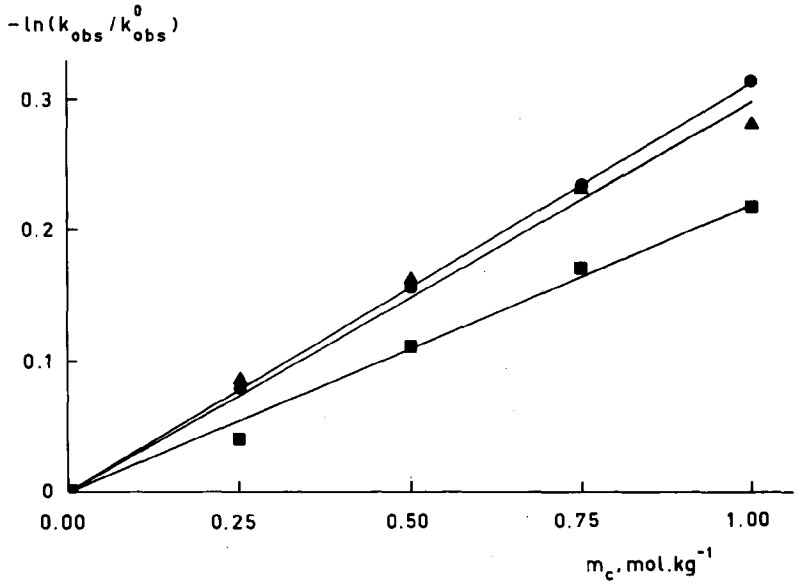


Figure 2.3 Plots of $-\ln(k_{obs}/k^{o}_{obs})$ *vs.* molality of carbohydrate for the neutral hydrolysis of **1**: **5b** methyl β-D-galactopyranoside (-■-); **5d** methyl β-D-glucopyranoside (-▲-); **5f** methyl α-D-mannopyranoside (-●-).

Table 2.5 Kinetic Medium Effects of Disaccharides in Aqueous Solutions at 298.15 K.

Disaccharide[a]	Subunits[b]	Type of Linkage	G(C)[c], J kg mol^{-2}
7a maltose	gl-gl	1α-4	-659(49)
7b cellobiose	gl-gl	1β-4	-649(62)
7c trehalose	gl-gl	1α-1α	-637(37)
7d gentiobiose	gl-gl	1α-6	-575(37)
8a sucrose	gl-fr	1α-5	-541(25)
8b turanose[d]	gl-fr	1α-3	-440(25)
8c palatinose	gl-fr	1α-6	-540(15)
9a lactose	gl-ga	1β-4	-472(37)
9b melibiose	gl-ga	1α-6	-467(20)
9c lactulose	fr-ga	1β-4	-482(25)

a) Molality always below 0.5 mol kg^{-1}. b) gl=glucose, fr=fructose, ga=galactose. c) Experimentally obtained, error between parentheses. d) Fructose in pyranose form.

2.5.5 Disaccharides

The composition of the disaccharides (**7a-d**, **8a-c**, **9a-c**) and the relevant G(C) values are listed in Table 2.5. All disaccharides have a more negative G(C) value than anticipated on the basis of the contributions of the individual monosaccharide moieties. This is possibly caused by a cooperativity effect.[57]

The results will be discussed on the basis of the composition of the disaccharides. The most negative kinetic medium effect is found for disaccharides consisting of two glucose units (**7a-7d**). The G(C) value is not much influenced by the type of linkage between the glucose moieties (G(C) = -575 to -659 J kg mol^{-2}). The disaccharides which consist of both a glucose and a fructose moiety have a smaller negative G(C) value (**8a-8c**, G(C) = -541, -440 and -540 J kg mol^{-2}, respectively). Their kinetic medium effects do not seem to be sensitive to the type of linkage in case of sucrose (**8a**) and palatinose (**8c**). However, in the case of a 1-3 type of linkage (**8b**, turanose) a significantly lower G(C) value is found. Apart from the type of linkage, this difference could also be caused by the fact that the fructose moiety resides in the pyranose form. All disaccharides which have a moiety with an axial OH(4) (**9a-9c**)(and **8b** D-turanose) have a less negative G(C) value compared to the other disaccharides (G(C) = -467 to -482 J kg mol^{-2}). In group **9a-9c** the differences in type of linkage or identity of monosaccharide moiety other than the galactose moiety, have no significant effect.

2.5.6 A quantitative analysis of kinetic medium effects of carbohydrates

Kinetic medium effects will be analysed quantitatively only in the case of the monosaccharides, because when disaccharides are considered there are too many

additional variables. Since in most equilibrium mixtures of the monosaccharides the pyranose form is dominantly present in aqueous solution,[54,55] all analyses for the monosaccharides have been performed assuming that the carbohydrate resides in the pyranose form. All carbohydrates cause a rate retardation, which is expressed quantitatively in a negative G(C) value. The term G(C) can be written as the sum of the functional group contributions. For D-glucose (C = cosolute = D-glucose):

$$G(\text{D-glucose}) = 4G(\text{CHOH,endo}) + G(\text{CHOH,exo}) + G(\text{CH}_2) + G(\text{-O-}) \qquad (2.2)$$

Table 2.6 Group Contribution of CHOH, (G(CHOH)), in Alcohols, Polyols and Carbohydrates.[a]

compound		G(CHOH)$_2$ J kg mol^{-2}
monohydric alcohol[29]		+ 158
vicinal dihydric alcohol[29]		+ 20
polyol[29]		- 12 (8)
monosaccharide		
exocyclic CHOH		+ 25 (20)
endocyclic CHOH	(OH(4) = axial, OH(2) = equatorial)	- 22 (9)
	(OH(4) = equatorial)	- 50 (6)
	(OH(4) = axial, OH(2) = axial)	- 69 (4)

a) Calculated as described in eq. 2.2 and ref. 59. Between parentheses the variation for the set of compounds is given. Values for carbohydrates are also given in Table 2.1 to 2.3.

In this equation CHOH,endo represents a CHOH moiety present in the pyranose ring, whereas CHOH,exo is the CHOH group which is part of the hydroxymethyl moiety.
The value of G(CHOH,exo) can be obtained by comparing the data for aldohexoses with those for aldopentoses, since the only difference is the exocyclic CHOH group. This provides a G(CHOH,exo) value of + 25 J kg mol^{-2} (± 20 J kg mol^{-2}), which means that this group has, in a good approximation, the hydration characteristics of a similar group in a vicinal dihydric alcohol.[28] Bearing in mind that for monohydric alcohols G(CH) is generally negative and G(OH) is generally positive, the hydration characteristics of the exocyclic CHOH group are still principally determined by the hydroxy group, since it contributes more significantly to the G(C) value than does the methine moiety. An examination of the G(CHOH,endo) values (Table 2.1 to 2.3 and 2.6) shows that these values depend on the stereochemistry of the carbohydrate (just as the G(C) values from which they were calculated). The G(CHOH,endo)[58,59] amounts to -65 and -72 J kg mol^{-2} for aldoses which have an axial hydroxy group on both C-2 and C-4 (**3g,3h**), and they range from -45 to -56 J kg mol^{-2} for aldoses with an equatorial OH(4)

and either an equatorial or axial OH(2) (**2c-2e, 3c-3f**). Finally they are in the range from -13 to -31 J kg mol^{-2} for aldoses with an axial OH(4) and an equatorial OH(2) (**2a,2b,3a,3b**). The G(CHOH,endo) values for the ketoses (**4a-4d**) are in the range from -46 to -51 J kg mol^{-2}, comparable to those found for aldoses with an equatorial OH(4) and either an axial or equatorial OH(2). These negative values show that the hydration of the endocyclic CHOH group is largely determined by the methine moiety, which explains why sometimes carbohydrates have been found to behave like "hydrophobic" solutes in aqueous solutions.[60,61] The CHOH group interactions of carbohydrates compare with other alcohols in the following way: the more hydroxy groups there are present in the solute molecule in close proximity, the more they will be camouflaged for interaction, due to the mutual influence of the hydration shells of the CH and OH groups. The best camouflage effect in this series of cosolvents (cosolutes) is found for the carbohydrates. Only carbohydrates with an axial OH(4) and an equatorial OH(2) (**2a,2b,3a,3b**) exhibit group contributions, which are comparable to those of polyols.
If the group contributions of the CHOH groups of carbohydrates are compared with similar groups in other alcohols, it is evident that the more hydroxy groups that are present in a solute molecule, the smaller is their contribution to the hydrophilicity of the overall hydration layer. Despite the large error in the value, it can be concluded that the hydration characteristics of an exocyclic CHOH group are rather similar to those of a corresponding group in a vicinal dihydroxy alcohol.

2.6 Analysis of kinetic medium effects in terms of stereochemistry

It is reasonable to assume on the basis of previous results[25-29] that the carbohydrate-induced medium effects are primarily caused by interactions involving destructive overlap of the hydration shells of the initial state and activated complex of the reaction with the hydration shell of the carbohydrate dissolved in the aqueous medium. The present results suggest that the different kinetic medium effects found for the aldoses are largely governed by the relative stereochemistry of the OH(4) and OH(2) groups. So far there is no firm theory which explains why the relative positions of the OH(4) and OH(2) are so important in determining the hydration layer of the aldohexoses. Explanations in terms of the Lemieux effect or the occurrence of complex mutarotation[62] do not seem applicable for the following reasons. In a Lemieux effect the OH(4) is often involved in a complicated network of intramolecular hydrogen bonds between OH(4), OH(6) and O(5). This can be facilitated by the fact that OH(4) is axial. However, similar trends in kinetic medium effects are still observed when there is no OH(6) present (in the case of the pentoses). In addition, an axial OH(4) can give rise to

complex mutarotation (see Table 1.2), which means that more furanose forms will be present in solution which could cause a different kinetic medium effect. However, this seems unlikely too. First of all, the methyl aldoglycopyranosides, which possess an anomeric centre which is blocked for mutarotation, show the same trends in kinetic medium effects as the related aldoses. Secondly, D-glucose and D-gulose exert different kinetic medium effects despite the fact that the equilibrium compositions for both aldoses are comparable. For aldoses, which possess a similar equilibrium composition and which have a fairly large preference for furanose forms in solution (D-idose, D-talose and D-altrose), we observe a different G(C) value for D-idose and D-talose compared to that for D-altrose. This also suggests that the furanose forms present in the equilibrium mixture are not crucial in determining the kinetic medium effect.

2.7 Conclusion

Kinetic medium effects on a water-catalysed hydrolysis reaction in aqueous solutions of carbohydrates are found to be a powerful tool for studying stereochemical aspects of carbohydrate hydration. For the aldohexoses the G(C) values depend on the relative position of the next-nearest neighbour hydroxy groups, OH(4) and OH(2). The results for the aldopentoses, methyl aldoglycopyranosides and disaccharides further support the conclusion that the position of OH(4) is of crucial importance. The position of the carbonyl moiety (i.e. the ring being in the $^4C_1$ or the $^2C_5$ conformation, compare aldohexoses and ketohexoses) in the carbohydrate as well as the number of equatorial hydroxy groups, are clearly of minor importance in determining the kinetic medium effect. The present quantitative analysis shows that a carbohydrate influences reaction rates in a way which suggests that it can have its hydroxy groups camouflaged for interaction to different extents. Hence the carbohydrate can be recognised as a relatively "hydrophobic" solute, because when the hydroxy groups are camouflaged for interaction, the methine groups of a carbohydrate determine the interactions. These results may require special consideration in studies of molecular recognition by carbohydrate molecules.

2.8. Experimental

2.8.1 Materials

All carbohydrates were dried overnight in vacuum before use. This does not apply to D-idose, D-gulose and D-psicose, which are colourless syrups. In these cases solutions were made by dissolving the carbohydrates in water and subsequent freeze-drying of the solution. After several days of drying under vacuum the amount of carbohydrate was determined by weighing and solutions of the proper pH were made.
The water used in all experiments was demineralised and distilled twice in an all-quartz distillation unit. All solutions were made up by weight using water, to which approximately 1mM of HCl was added in order to suppress hydroxide ion catalysis in the hydrolytic process (in literature[63] it is shown that this pH does not influence

the equilibrium composition). The molalities of monosaccharide were in all cases below 1 mol kg^{-1} to minimize the role of triplet and higher order interactions. For the disaccharides the molalities were kept below 0.50 mol kg^{-1}.
The carbohydrates were obtained from: Merck (D-glucose, D-xylose), Sigma (D-mannose, D-galactose, D-talose, D-allose, D-idose, D-gulose, D-altrose, D-tagatose, D-psicose, D-lyxose, 3-O-methyl glucose, trehalose, gentiobiose, methyl β-D-arabinopyranoside, methyl β-D-xylopyranoside, maltose, cellobiose, methyl α-D-galactopyranoside, methyl α-D-glucopyranoside, sucrose, turanose, palatinose, lactose, melibiose, lactulose), Janssen (D-arabinose, L-arabinose, D-ribose) and Aldrich (methyl β-D-glucopyranoside, methyl β-D-galactopyranoside, methyl α-D-mannopyranoside).
All sugars were of a purity of 99+ %, although they were sometimes purchased as mixtures of anomers. However, this posed no problem as the sugars were equilibrated in water before use. As an exception, lactulose had a purity of approx. 98%, D-altrose had a purity of>95% (determined by GC), D-gulose>99% (HPLC), D-idose>98% (HPLC), D-tagatose>96% (GC) and D-psicose >99% (NMR). The impurities mostly consisted of related carbohydrates. Trehalose was purchased as a dihydrate, melibiose was a hemihydrate and maltose a monohydrate.

2.8.2 Kinetic Measurements

Pseudo-first-order rate constants for the neutral hydrolysis of 1-benzoyl-3-phenyl-1,2,4-triazole (1) (m.p. 79-80 °C; Ref. 25: 78.6-79.8 °C) were obtained by using a Perkin Elmer λ5 spectrophotometer equipped with a data station. Substrate concentrations were approximately 10^{-5} mol dm^{-3} and the substrate was added as a concentrated solution in 10^{-5} dm^3 of acetonitrile to a volume of 2.5 10^{-3} dm^3 of the aqueous solution. Rate constants were obtained by recording the change in absorbance at 273 nm at pH 3 for about ten half-lives. The end-value method was used to calculate the rate constants. All measurements were done in triplicate and the rate constants were found to be reproducible to within 2% for the aqueous solutions of monosaccharides, and to within 3% for the aqueous solutions of disaccharides. The error in the G(C) values was calculated by using a GraphPad linear regression program.

2.8.3 Product analysis

The reaction mixtures were analysed for the presence of carbohydrate-derived esters. Aqueous solutions of D-glucose, D-galactose and methyl β-D-glucopyranoside were employed separately. In a typical experiment the substrate was hydrolysed in the presence of carbohydrate in a molar ratio of substrate: carbohydrate = 1:10. The concentration of carbohydrate was 0.001 mol kg^{-1}. The small concentration of carbohydrate is necessitated by experimental limitations. After the hydrolysis had gone to completion, the mixtures were freeze-dried. The samples were silylated with hexamethyldisilazane-chlorotrimethylsilane-pyridine (1:1:5) for 30 min. at room temperature. Combined GLC and GLC/MS experiments showed that no carbohydrate-derived esters had been formed.
The combined GLC and GLC/MS studies were performed at the University of Utrecht, The Netherlands, by Dr. G.J. Gerwig.

Gas Liquid Chromatography

GLC was performed on a Varian 3700 gas chromatograph equipped with a capillary SE-30 fused silica column (25 m, 0.32 mm, Pierce) and FID. The oven temperature was programmed from 130-220 °C at 4 °C per minute. The injector temperature was 210 °C. The detector temperature was 230 °C.

Gas-liquid Chromatography / Mass Spectrometry

Combined GLC-MS was performed on a Carlo-Erba GC/ Kratos MS 80/ Kratos DS 55 system; electron energy, 70 eV; accelerating voltage, 2.7 kV; ionising current, 100 μA; ion source temperature, 225 °C; capillary CP sil 5 column (25 m, 0.32 mm); oven temperature program, 140°C during 2 minutes, 140-260°C at 4°C per minute.

2.9 References

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CHAPTER 3

INFORMATIVE THERMODYNAMIC PARAMETERS FOR STEREOCHEMICAL ASPECTS OF CARBOHYDRATE HYDRATION

3.1 Thermodynamics in aqueous solutions of solutes. An introduction

Properties of solvents can either be measured directly or with a method involving a probe or model reaction. It is the advantage of thermodynamic parameters that they can be measured directly and do not rely on a model reaction or probe molecule. They are informative of the properties of a solvent or the average properties of a solution.

Molar properties are defined for a mole of pure compound, either solid, liquid, or gas. They provide information on the volume properties of a mole of compound (molar volume); on the heat uptake which is needed to increase the temperature of a solid, solvent or a gas by one degree (molar heat capacity), on the volume by which a compound can expand if it is heated (molar expansibility) and the volume shrinkage when a compound is put under pressure (molar compressibility).

When mixtures are studied it is more difficult to measure molar properties. Strictly speaking, molar properties can only be measured for pure compounds and not for mixtures. However, for a solvent mixture comprised of a solute dissolved in a solvent, one can define a partial molar property of the solute in solution as the apparent partial molar property at infinite dilution.

Partial molar volumes can be obtained from density measurement of solutions. At low concentrations, the partial molar volume of a solute in solution depends only slightly on its concentration, because the solute molecules do not influence each other. It is, however, dependent on pressure and temperature. The dependence of the partial molar volume upon pressure results in the partial molar compressibility which can be obtained by measuring the speed of sound in solutions containing a solute. A sound wave is a compression and rarefaction of density and it monitors the pressure dependence of the density. The partial molar expansibility represents the dependence of density on temperature. This parameter can be calculated directly from the temperature dependence of densities. The partial molar heat capacity can be measured by studying the partial molar heats of solution as a function of temperature. The heat of solution is a measure of the heat effect upon dissolution of a solute in a solvent. It can be measured with a standard heat capacity meter. The temperature dependence of the heat of solution results in the partial molar heat capacity. The partial molar heat capacity can also be

measured with a standard heat capacity meter, but measurements need to be performed over a range of temperatures. If this creates a problem, it can also be measured by studying the heat uptake of a solvent mixture upon a very small temperature increase. A standard heat capacity meter cannot monitor a change in the uptake of heat when a very small temperature range is involved. This requires a special apparatus: a heat capacity meter in the form of a flow calorimeter.

More insight into solute-solute interactions in a solvent can be obtained when the thermodynamic ideality of the solvent mixture is defined. It then is possible to attribute every deviation from ideality to solute-solute interactions in the solution. The difference between the observed property and the ideal property is the excess property (Eq. 3.1). Many excess properties have been studied.

$$X_{observed} = X_{ideal} + X_{excess} \qquad (3.1)$$

When the solvent is water,[1] thermodynamic properties can reveal the hydration characteristics of a solute in aqueous solution. Since all biological processes proceed in water, studies of aqueous solutions have received considerable attention.[2-4] Thermodynamic properties of a solute in aqueous solution at infinite dilution provide insight into the intrinsic properties of the solute. They are also informative of solute-water interactions, a collective term which sometimes can be dominant in determining the overall property. Partial molar volumes are representative of the sum of the intrinsic volume of a solute in solution and the volume of the hydration layer around the molecule. Both the partial molar expansibility and compressibility of a solute in aqueous solution provide information about the properties of the hydration layer, because the solute molecule itself has a negligible expansibility and compressibility. It is then of great interest to compare the properties of the hydration layer with those for pure water. The heat of solution is representative of the heat effect upon dissolution of a solute molecule in water. The partial molar heat capacity is representative of the heat uptake of a solute and its hydration layer in aqueous solution.

Aqueous solutions of carbohydrates are known to be almost ideal solutions. This means that carbohydrates have hardly any interactions with one another in aqueous solution, because they are so extensively hydrogen bonded in the aqueous solution. However, because water consists of a three-dimensional hydrogen-bond network, it is interesting to study whether the hydroxy topology of a carbohydrate molecule will affect thermodynamic properties of carbohydrates in water, as it are these hydroxy groups which are extensively hydrogen bonded in water. Of course not every thermodynamic

property will strongly depend on the stereochemistry of the carbohydrate molecules. It is anticipated that the differences, which are hoped to be seen, are indeed small. Hence the relevant thermodynamic properties should be highly sensitive measures of hydration effects. In this chapter a large set of thermodynamic parameters of aqueous carbohydrate solutions will be discussed. The main emphasis will be on monitoring stereochemical aspects of hydration.

3.2 Thermodynamic parameters, definitions and calculation

This chapter will discuss thermodynamic parameters of aqueous solutions of carbohydrates. It will be shown that some parameters are of limited usefulness for aqueous carbohydrate solutions, because of the critical limitations of the experimental setup. Some thermodynamic properties are more informative of stereochemical aspects of hydration than others, because the sensitivity of the parameter is very important.

3.2.1 Heats of solution

Enthalpies of solution ΔH_{sol} can be measured with a standard calorimeter.[5,6] A certain amount of solute is mixed with a given quantity of pure water. When the two components are mixed the heat effect, if any, is monitored. The experiment is performed at low concentrations of solute. The reported value of ΔH_{sol} is often the average value for the applicable range of concentrations. The value of ΔH_{sol} depends on the difference in enthalpy of the pure solute and pure water compared to that of the aqueous solution. In the case of carbohydrates it involves the use of the crystalline state of the carbohydrate as a reference state. Because this can cause complications (see chapter 1), the parameter is of limited usefulness.

Nevertheless in the literature some heats of solution of carbohydrates have been reported.[5] Carbohydrates, present in a different form in the crystalline state as compared to that in an aqueous solution (e.g. ribose), show a different heat of solution from those carbohydrates having the same mutarotational isomers in both states. For methyl aldoglycopyranosides, which cannot have different mutarotational isomers, a different heat of solution is found for carbohydrates with an axial OH(4) (**5a**,**5b**) compared to methyl aldoglycopyranosides, which possess an equatorial OH(4) (**5d**,**5f**).

3.2.2 Partial molar heat capacities

Partial molar heat capacities can be obtained by use of a Picker flow calorimeter.[7] This device operates on the basis that a sample solution and reference (water) are heated simultaneously and the difference in energy input (Δw) required to raise the

temperature of both by the same increment is measured. The use of Δw in equation 3.2 leads to the volume specific heat of the solution, $\sigma_{p,sol}$.

$$\sigma_{p,sol} = \sigma_{p,o}(1 - (\Delta w/ w_o)) \quad (3.2)$$

In this equation $\sigma_{p,o}$ is the volume specific heat capacity of water, Δw is the difference in energy input for the solution compared to water and w_o is the basic power of the system.[7] There are two ways of calculating the apparent partial molar heat capacity of the solution, depending on whether the solutions are made up by volume or by weight. If the solutions are made up by volume:

$$C_{p,app} = V_\phi C_{p,o} + 1000(\sigma_{p,sol} - \sigma_{p,o}) / c \quad (3.3)$$

Here $C_{p,app}$ is the apparent partial molar heat capacity of the solution, V_ϕ is the apparent partial molar volume of the solute in this solution and c is the concentration of carbohydrate in mol dm^{-3}. However, if the solutions are made up by weight, the weight specific heat capacity of the solution must be calculated first.

$$C_{p,sol} = \sigma_{p,sol} / d \quad (3.4)$$

Herein is $C_{p,sol}$ the weight specific heat capacity of the solution, and d is the density of the solution. From the weight specific heat capacities of the solution one can calculate the apparent partial molar heat capacity via:

$$C_{p,app} = MC_{p,sol} + 1000(C_{p,sol} - C_{p,o})/ m \quad (3.5)$$

M is the molecular weight of the carbohydrate and m is the molality of the carbohydrate in the aqueous solution.

3.2.3 Partial molar volumes

Partial molar volumes are calculated from the densities of carbohydrate solutions at different molalities of carbohydrate.[8,9] The apparent partial molar volume can be obtained from equation 3.6:

$$V_\phi = 1000(d_o - d) / (mdd_o) + M / d \quad (3.6)$$

Herein d_o is the density of pure water.

3.2.4 Partial molar isentropic compressibilities

Isentropic coefficients of compressibility[10] are measured by monitoring the speed of sound through a solution in an adiabatic and reversible way, which ensures that the compressibility is isentropic. The isentropic coefficient of compressibility β_s can be obtained from:

$$\beta_s = 1 / (u^2.d) \quad (3.7)$$

Herein is u the speed of sound in a solution with density d. The β_s values can be used to calculate the apparent partial molar compressibility via the difference method:

$$K_{\phi(s)} = 1000(\beta_s - \beta_{so}) / md + \beta_s V_\phi \quad (3.8)$$

β_s and β_{so} are the isentropic coefficients of compressibility of the solution and water, respectively, and V_ϕ is the apparent molar volume at a given molality.

3.2.5 Partial molar isothermal compressibilities

When partial molar isentropic compressibilities, expansibilities (see 3.2.6) and heat capacities are known, partial molar isothermal compressibilities can be calculated.[8,10] First the isothermal compressibility coefficient of pure water has to be calculated:

$$\beta_T = \beta_s + \alpha^2 T / \sigma \quad (3.9)$$

All parameters refer to pure water. β_T is the isothermal coefficient of compressibility, α is the expansibility coefficient and σ is the volume specific heat capacity. T is temperature. When both coefficients of compressibility have been calculated, the difference is used to calculate the partial molar isothermal compressibility of a solute via:

$$K_{2,T} = K_{2,s} + \delta_o((2E_2 / \alpha_o) - (C_{p,2} / \sigma_o)) \quad (3.10)$$

$K_{2,T}$ and $K_{2,s}$ are, respectively, the partial molar isothermal and isentropic compressibility (in $cm^3\ mol^{-1}\ bar^{-1}$) of a solute in water, δ_o is the difference between the coefficients of compressibility in pure water (in bar^{-1}), E_2 is the partial molar expansibility of the solute in water (in $cm^3\ mol^{-1}\ K^{-1}$), α_o is the expansibility coefficient of pure water (K^{-1}), $C_{p,2}$ is the partial molar heat capacity of the solute in water ($J\ mol^{-1}\ K^{-1}$), σ_o is the volume specific heat capacity of pure water ($J\ K^{-1}\ cm^{-3}$).

3.2.6 Partial molar expansibilities

The partial molar expansibility E_2 is defined as the temperature dependence of the partial molar volume.

$$E_2 = (\partial V_2 / \partial T) \quad (3.11)$$

It can, therefore, be calculated from the dependence of the partial molar volume V_2 on temperature, as has been described previously.[11] However this method is somewhat imprecise as the error in partial molar volumes is usually approximately 1 $cm^3\ mol^{-1}$. Hence the partial molar volume is given as the dependence of the apparent molar volume

on concentration, $V_2 = (\partial V/\partial n)$, then

$$E_2 = (\partial^2 V/\partial T\, \partial n) = (\partial/\partial n)(\partial V/\partial T) = (\partial/\partial n)(\partial((1000+mM)/d)/\partial T) \quad (3.12)$$

From this equation one can derive:

$$E_2 = ((1000 + Mm) / d^2)\ (\partial\alpha / \partial m) + \alpha V_2 \quad (3.13)$$

Here E_2 is the partial molar expansibility as described earlier, α is the expansibility coefficient. If α is plotted against the molality of carbohydrate, a straight line is obtained and E_2 can be calculated with reasonable precision. The main error lies in $(\partial\alpha / \partial m)$, but this method is more precise than any other method used.

Expansibility coefficients can also be obtained by monitoring the temperature of maximum density[1,12] of aqueous solutions of solutes.

3.2.7 Hydration numbers

Hydration numbers can be interpreted in various ways and there are many methods to measure hydration numbers. Among others they can be obtained from the ultrasound experiments and calculated using a method described previously:[13-15]

$$n_h = n_w / n_s(1 - \beta_s / \beta_{so}) \quad (3.14)$$

In this equation, n_h is the hydration number, whereas n_w and n_s are the mole fractions of water and solute, respectively. The hydration numbers given in the Tables 3.3 to 3.6 are the limiting values obtained at infinite dilution. Equation 3.14 assumes that the hydration layer is incompressible. This affects the value of the hydration number. If the hydration layer is compressible, the hydration numbers will all be higher, but the trend in the numbers will remain the same.

3.3 Thermodynamic parameters for aqueous solutions of carbohydrates[4]

Only those thermodynamic parameters have been measured, for which the proper experimental conditions could be used. The partial molar properties are obtained by plotting the apparent partial molar properties versus molality of carbohydrate and applying linear regression. In all cases plots of the apparent partial molar heat capacity, apparent partial molar volume, apparent partial molar compressibility and hydration numbers versus the molality of the carbohydrate showed a linear dependence. By definition the partial molar property equals the apparent partial molar property at infinite dilution (Eq. 3.15). Partial molar expansibilities are an exception; these have been calculated from the dependence of the expansibility coefficient on molality.

$$X_2 = X_\phi + m(dX_\phi / dm) \quad (3.15)$$

Partial molar heat capacities are reported for some aldopentoses (**2a-2e**), aldohexoses (**3a,c,d,g**), and some disaccharides (**7a,8a,9a**) in Table 3.1. Partial molar heat capacities, expansibilities and isothermal compressibilities for some methyl aldoglycopyranosides (**5a-5d,5f**) are compiled in Table 3.2. There is generally good agreement between the partial molar heat capacities measured here with literature data,[5,6,16,19] although on average the partial molar heat capacities are somewhat higher than those found previously, possibly as a consequence of the different methods which have been used. Partial molar volumes, isentropic partial molar compressibilities and hydration numbers are reported for a series for pentoses (**2a-2e**) and hexoses (**3a,c,d,f, 4a,b**) in Table 3.3, for methyl aldoglycopyranosides (**5a-5f, 6a,b**; Table 3.4) and for some disaccharides (**7a-d, 8a-c, 9a-c**; Table 3.5). Literature data are included for comparison and there is generally good agreement between the current measurements and literature data, except for the isentropic partial molar compressibilities of the methyl glucopyranosides, which have now been measured more accurately than those reported in previous studies.

Figure 3.1 shows representative plots of the apparent molar heat capacities versus molality of carbohydrate. Figure 3.2 shows a plot of partial molar heat capacities versus partial molar volumes for all the carbohydrates, for which both properties were measured. Figures 3.3 and 3.4 show representative plots of the concentration dependence of the apparent partial molar volumes and apparent partial molar compressibilities, respectively. In Figure 3.5 the plots of the expansibility coefficients versus molality of methyl aldoglycopyranosides are displayed.

Table 3.1 Partial Molar Heat Capacities,[a] of Mono- and Disaccharides in Aqueous Solutions at 298.30 K.

Pentose	$C_{p,2}$, J K^{-1} mol^{-1}	Hexose	$C_{p,2}$, J K^{-1} mol^{-1}	Disaccharide	$C_{p,2}$, J K^{-1} mol^{-1}
2a D-arabinose[b]	279.5(3.8)	**3a** D-galactose[g]	346.7(0.9)	**7a** maltose[j]	674.2(1.9)
2b L-arabinose[c]	294.4(1.9)	**3c** D-glucose[h]	363.3(3.3)	**8a** sucrose[k]	662.0(2.0)
2c D-xylose[d]	294.4(1.9)	**3d** D-mannose[i]	345.9(0.9)	**9a** lactose[l]	679.9(6.3)
2d D-lyxose[e]	275.6(8.0)	**3g** D-talose	310.6(6.5)		
2e D-ribose[f]	271.5(6.3)				

a) Error between parentheses. b) Ref. 16: 278 J mol^{-1} K^{-1}; Ref. 5, 17: 318 J mol^{-1} K^{-1}. c) Ref. 16: 270 J mol^{-1} K^{-1}. d) Ref. 5, 16 and 17: 381 J mol^{-1} K^{-1}. e) Ref. 17: 285 J mol^{-1} K^{-1}. f) Ref. 16: 271 J mol^{-1} K^{-1}; Ref. 16, 17: 294 J mol^{-1} K^{-1}. g) Ref. 18: 324 J mol^{-1} K^{-1}; Ref. 5: 356 J mol^{-1} K^{-1}. h) for α-D-glucose a value of 347 J mol^{-1} K^{-1} was found. Ref. 18: 331 J mol^{-1} K^{-1}. i) Ref. 18: 337 J mol^{-1} K^{-1}. j) Ref. 18: 635 J mol^{-1} K^{-1}. k) Ref. 18: 647 J mol^{-1} K^{-1}; Ref. 19: 649 J mol^{-1} K^{-1}. l) Ref. 18: 673 J mol^{-1} K^{-1}.

Table 3.2 Partial Molar Heat Capacities at 298.30 K, Expansibilities and Isothermal Compressibilities at 298.15 K of Methyl Aldoglycopyranosides in Aqueous Solution.

	Methyl Aldoglycopyranoside	$C_{p,2}$, J mol^{-1} K^{-1}	E_2[a] cm^3 mol^{-1} K^{-1}	$K_{2,T}$[c] cm^3 mol^{-1} bar^{-1}
5a	Me α-galactopyranoside	465.2 (3.8)[d]	0.098	-13.9
5b	Me β-galactopyranoside	434.5 (4.7)	0.097	-14.0
5c	Me α-glucopyranoside	435.1 (3.7)[e]	0.092[b]	-10.8
5d	Me β-glucopyranoside	433.1 (5.3)	0.095[b]	- 8.1
5e	3-O-Methylglucose	425.4 (2.7)		
5f	Me α-mannopyranoside	438.0 (2.0)[f]	0.107	- 8.8

a) Error is 0.005 cm^3 mol^{-1} K^{-1}. b) Ref. 11; Me α-glucopyranoside: E_2=0.115 cm^3 mol^{-1} K^{-1}, Me β-glucopyranoside E_2=0.045 cm^3 mol^{-1} K^{-1}, estimated error is 0.05. c) Calculated from $K_{2,s}$ using (see 3.2.5): δ_o=4.7662 10^{-7} bar^{-1}; α_o=256.4 10^{-6} K^{-1}; σ_o=4.1669 J K^{-1} cm^{-3}.d) Error is given in parentheses, Ref. 5: $C_{p,2}$=436 J mol^{-1} K^{-1}. e) Ref. 5: $C_{p,2}$=435 J mol^{-1} K^{-1}.f) Ref. 5: $C_{p,2}$=445 J mol^{-1} K^{-1}.

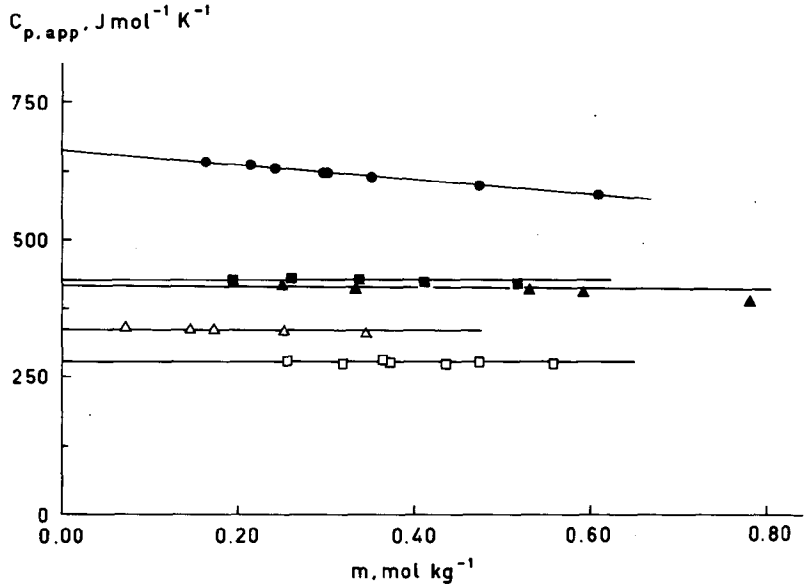

Figure 3.1 Representative plots of apparent molar heat capacities of some carbohydrates in aqueous solutions *vs.* molality of carbohydrate at 298.30 K. D-lyxose **2c** (-□-), methyl α-D-glucopyranoside **5c** (-■-), D-mannose **3d** (-Δ-), methyl β-D-glucopyranoside **5d** (-▲-), sucrose **8a** (-●-).

3.3.1 Partial molar heat capacities

In Tables 3.1 and 3.2 the partial molar heat capacities are given for all the carbohydrates studied together with some literature values. Partial molar heat capacities, which are second derivatives of the Gibbs energy with respect to

temperature ($\partial^2G/\partial T^2$), are not sensitive measures of stereochemical aspects of hydration. An examination of the data in Tables 3.1 and 3.2 for pentoses (**2a-e**), hexoses (**3a,c,d,g**), methyl aldoglycopyranosides (**5a-f**) and disaccharides (**7a,8a,9a**) reveals that on average the partial molar heat capacity of a pentose is about 283 J mol^{-1} K^{-1}, hexoses have an average partial molar heat capacity of 352 J mol^{-1} K^{-1}, with only D-talose (**3g**) having a different partial molar heat capacity (311 J mol^{-1} K^{-1}). Methyl aldoglycopyranosides have an average value of 439 J mol^{-1} K^{-1} and the disaccharides (those which have been studied) have an average heat capacity of 672 J mol^{-1} K^{-1}. In general, increments in values of the partial molar heat capacities, $\delta C_{p,1}$, are 62 J mol^{-1} K^{-1} for an additional CHOH group on the pentose moiety, and 93 J mol^{-1} K^{-1} for the change from an hydroxy group to a methoxy group. On average the difference in partial molar heat capacity between a mono- and a disaccharide is 320 J mol^{-1} K^{-1}.[5,20,21] The trends observed are not uniform throughout the sets of data, like for the partial molar isentropic compressibilities (see 3.3.3). This is caused by the fact that the partial molar heat capacities of the carbohydrates (and of solutes in general) not only reflect the partial molar heat capacity of the hydration layer, but also the intrinsic heat capacity of the carbohydrate molecule itself. This is illustrated by a plot of the partial molar heat capacities of the carbohydrates versus the partial molar

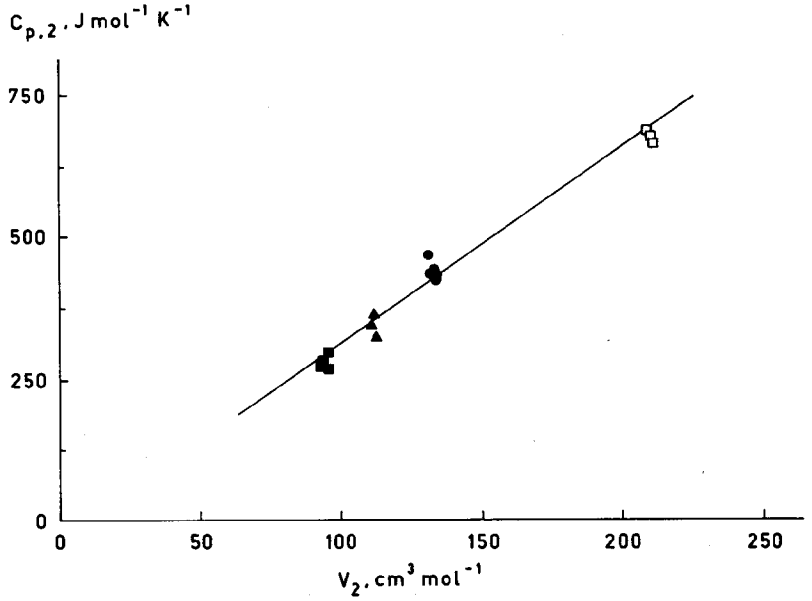

Figure 3.2 Partial molar heat capacities *vs.* partial molar volumes of carbohydrates in aqueous solution, pentoses **2a-2e** (-■-), hexoses **3a,3c,3d,3g** (-▲-), methyl aldoglycopyranosides **5a-5f** (-●-), disaccharides **7a,8a,9a** (-□-).

volumes of the carbohydrates in aqueous solution (Figure 3.2). It is well known that the partial molar volume of a solute in aqueous solution has both an intrinsic contribution and a contribution due to solute-solvent interactions. The excellent linear relationship between the partial molar volumes and the partial molar heat capacities illustrates that the partial molar heat capacity is also composed of an intrinsic contribution and a contribution due to solute-solvent interactions.

$$X_2 = X_{intrinsic} + X_{solute\text{-}water} \qquad (X = V \text{ or } C_p) \qquad (3.16)$$

Incidentally, this is exactly the reason for the excellent additivity observed for partial molar heat capacities of solutes,[5,22] and the linear dependence of the partial molar heat capacity on the number of equatorial hydroxy groups in a carbohydrate molecule.[23] Because the authors[23] compare the partial molar heat capacities over such a large variation of molecular weight, they are actually plotting the partial molar heat capacities versus molecular weight of the carbohydrate molecule. This explains why the partial molar heat capacities, obtained from the temperature dependence of the heats of solution,[5,24,25] are generally in good agreement, fortuitously so perhaps, with the values obtained here. Studies of aqueous carbohydrate solutions over a significant temperature range should be avoided because they reveal little about carbohydrate hydration (see chapter 1). The intrinsic heat capacity of a carbohydrate in its pyranose form will not be much different from that of its furanose form. Partial molar heat capacities are largely determined by the intrinsic heat capacity of the carbohydrate, which is primarily governed by the number of bonds in the molecule. Even if the hydration of both conformers is quite different, this will not be reflected in the partial molar heat capacity because the intrinsic contributions of the carbohydrate molecules are similar and the intrinsic contribution is very large compared to the contribution of the hydration layer. A better picture of the stereochemical aspects of hydration would emerge if the intrinsic contribution could be separated from the total partial molar heat capacity, leading to a value for the hydration layer, ΔC_p. It is tempting to calculate this quantity by subtracting the partial molar heat capacity of a carbohydrate in the solid state from that of a carbohydrate in aqueous solution.[18] Then this ΔC_p value could be compared with, for example, ΔC_p for tetrahydropyran-1-carbinol, THPC,[26] a molecule which resembles a hexose very much except that all endocyclic hydroxy groups[27] have been replaced by hydrogen atoms. This treatment of the data is, however, inappropriate since the carbohydrate and THPC do not have the same reference state (THPC is a liquid, while a carbohydrate is a solid at 298 K). Generally the crystalline state of a carbohydrate should be avoided as a reference state (chapter 1).

However, if this approach is used[18,26] it is found that ΔC_p for the hexoses amounts to values between 134 and 144 J mol^{-1} K^{-1} and for THPC the value is 203 J mol^{-1} K^{-1} (all at 298 K). Normally C_p values will depend on the number of atoms in the solute molecule. Clearly the hydroxy groups are camouflaged somewhat (compare section 3.3.2), which results in the relatively small ΔC_p of hexoses compared to that for THPC.

The same conclusion is reached when partial molar heat capacities of the carbohydrates are compared with those of other solutes.[2] It can be seen that the partial molar heat capacities give similar information about the hydration of a carbohydrate as do the partial molar volumes. Both thermodynamic parameters show that the hydroxy groups of carbohydrates are camouflaged[28] in aqueous solution since the partial molar heat capacities and volumes are smaller than expected on the basis of the molecular weight of the carbohydrates.

Table 3.3 Partial Molar Volumes,[a] Isentropic Partial Molar Compressibilities and Hydration Numbers of Monosaccharides in Aqueous Solutions, T=298.15 K.

Carbohydrate		Conf[b]	V_2, cm^3 mol^{-1}		$K_{2,s}.10^4$, $cm^3 mol^{-1} bar^{-1}$		n_h[c]
			Found	Lit.	Found	Lit.	
			aldopentoses				
2a	D-arabinose	1a2e3e4a	93.5(0.2)	93.2[d](0.3)	-19.2(0.1)	-19.3[d](0.5)	7.6
2c	D-xylose	1e2e3e4e	95.5(0.2)	95.4[d](0.3)	-13.1(0.2)	-12.9[d](0.5)	6.8
2d	D-lyxose	1a2a3e4e	94.3(0.1)		-13.1(0.1)		6.8
2e	D-ribose	1e2e3a4e	95.3(0.1)	95.2[d](0.3)	-12.4(0.2)	-12.5[d](0.2)	6.8
			aldohexoses				
3a	D-galactose	1e2e3e4a6e	111.0(0.4)	110.2[d](0.3)	-20.4(0.4)	-20.8[d](0.5)	8.7
3c	D-glucose	1e2e3e4e6e		111.7[d](0.3)		-17.6[d](0.3)	8.4
3d	D-mannose	1a2a3e4e6e		111.3[d](0.3)		-16.0[d](0.5)	8.1
3g	D-talose	1a2a3e4a6e	112.5(0.1)		-11.9(0.3)		7.7
			ketohexoses				
4a	D-fructose	1e2a3e4e5a	110.8(0.3)	110.4[e](0.4)	-21.7(0.4)	-21.4[f](0.01)	8.8
4b	L-sorbose	1e2a3e4e5e	110.7(0.5)	110.6[e](0.4)	-21.8(0.4)		9.0

a) Errors are given in parentheses. b) Conformation of dominant conformer in solution, a=axial; e=equatorial hydroxy group. c) Calculated with the method described in Ref. 13-15. d) Ref. 29. e) Ref. 31. f) Ref. 30.

3.3.2 Partial Molar Volumes

Just as the partial molar heat capacities, the partial molar volumes are made up from two contributions, i.e. an intrinsic component and a solute-solvent interaction component (see eq. 3.15) and they are therefore potentially useful for understanding solute-solvent interactions. Overall the carbohydrates have small partial molar volumes

in water (Table 3.3 to 3.5), compared to their molecular weights, due to extensive solute-solvent interactions.[32] Hexoses (**3a,c,d,g, 4a,b**) have, on average, partial molar volumes of 110 cm^3 mol^{-1}, whereas the values for pentoses (**2a-e**) are about 95 cm^3mol^{-1}. The average value for methyl hexopyranosides (**5a-f**) is 133 cm^3 mol^{-1} and for methyl pentopyranosides (**6a,b**) it is 116 cm^3 mol^{-1}. Disaccharides (**7a-d, 8a-c, 9a-c**) have an average partial molar volume of 211 cm^3 mol^{-1}. On average we observe an increment for an additional CHOH, of +16.6 cm^3 mol^{-1} , and the increment is +21.2 cm^3 mol^{-1} if a hydroxy group is replaced by a methoxy group. Disaccharides have slightly less than twice the partial molar volume of the monosaccharides.

Unfortunately little information on the stereochemical aspects of hydration is obtained because the larger part of the partial molar volume is provided by the intrinsic volume of the carbohydrate. There are some significant differences in the partial molar volumes within a set of isomeric carbohydrates. For the hexoses, D-talose (**3g**) has a significantly different V_2 from the other hexoses, and there is a greater difference between D-galactose and D-talose than between D-glucose and D-talose, but overall the differences are fairly small.

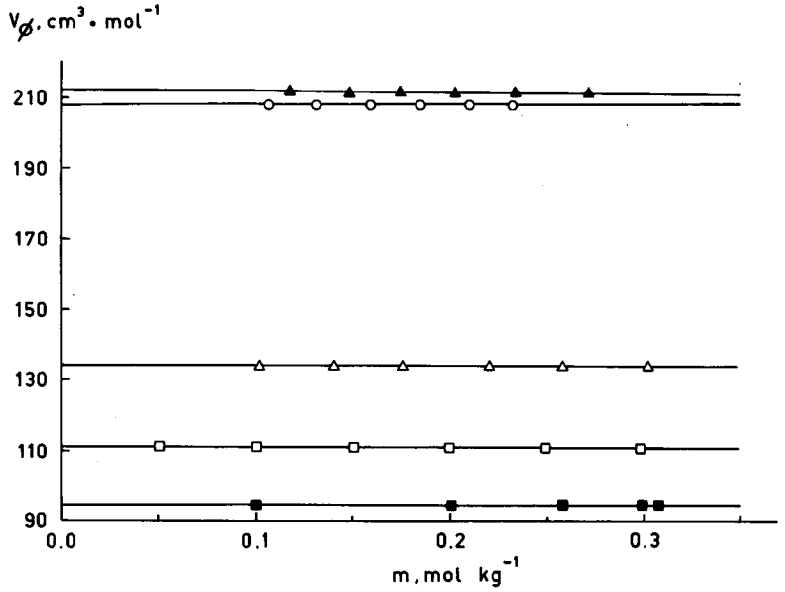

Figure 3.3 Apparent molal volumes as a function of concentration: lyxose **2d** (-■-), fructose **4a** (-□-), trehalose **7c** (-o-), 3-O-methylglucose **5e** (-Δ-), cellobiose **7b** (-▲-).

3.3.3 Partial Molar Isentropic Compressibilities

A pressure derivative of V_2 will directly reflect carbohydrate-water interactions, if

it is assumed that the carbohydrate molecules themselves are incompressible.[10] Compressibilities of solutions can be measured at constant temperature or at constant entropy. Partial molar isentropic compressibilities can be directly calculated from the results of ultrasound measurements (see Section 3.2.4). The isothermal apparent molar compressibility can be calculated from the expansibility, heat capacity and isentropic compressibility of the solution. The isentropic and isothermal compressibilities are numerically different. The limiting values of the compressibilities give insight into the compressibility of the hydration layer relative to that for pure water. Pure water has an isentropic molar compressibility of $+8.17 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}. If a solute fits almost perfectly into water the partial molar isentropic compressibility of this solute in water will be close to $+8.17 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, merely because the hydration layer of the solute will have hydration characteristics, which are similar to pure water. The partial molar compressibility of a solute which fits perfectly in water is zero, because the hydration layer cannot be discriminated from bulk water and, therefore, there is none. The partial molar compressibility of a solute will in that case equal the intrinsic compressibility of the solute molecule. The compressibility of a solute molecule is negligible.

The compressibility of hydrophobic hydration sheaths appears to be less than that for bulk water, but it is far more compressible than water around ions or charged groups. In the case of hydration of an apolar, hydrophobic solute, the water molecules in the hydration layer will form more and/or stronger hydrogen bonds to each other (hydrophobic hydration)[33] and therefore the hydration layer will be less compressible than pure water. Consequently, the limiting compressibilities per methylene group for solutions of relatively apolar solutes are slightly negative. When ions are introduced into water they change the hydrogen-bond structure of water in the vicinity of the ion, because an ion-dipole interaction is usually stronger than the dipole-dipole interaction between the water molecules. The compression due to these ion-dipole interactions is called electrostriction. The water around ions is therefore dense and less compressible than bulk water leading to typically large negative partial molar compressibilities (-30 to -50×10^{-4} cm^3 mol^{-1} bar^{-1}). However, it is not possible to use partial molar compressibilities to distinguish hydrophobic hydration effects from other types of hydration,[3] except when the temperature dependence of compressibilities is measured.[8] Therefore we have chosen to compare the character of the hydration layer of the carbohydrate with pure water. As was stated earlier, if a solute fits almost completely into the hydrogen-bond network of water, the partial molar compressibility of this solute will be close to $+8.17 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}. If a solute does not fit

into water, its partial molar compressibility will be smaller than that of pure water, because the hydration layer is disturbed by the presence of the solute, in such a way that it is less compressible than pure water. The partial molar compressibilities of carbohydrates have intermediate values (Tables 3.3-3.5) when compared to a small hydrophobic solute and an ion. This suggests that the hydrogen-bonded network of water is only slightly disturbed by the presence of the carbohydrate. If the partial molar compressibilities of isomeric sugars are compared, it seems likely that the more negative the partial molar compressibility is, the more the hydration layer will be disturbed by the presence of the carbohydrate relative to pure water. Hence the partial molar compressibility is indicative of the extent to which a carbohydrate molecule disturbs water by its presence and is, therefore, informative of the fit of a carbohydrate into water.

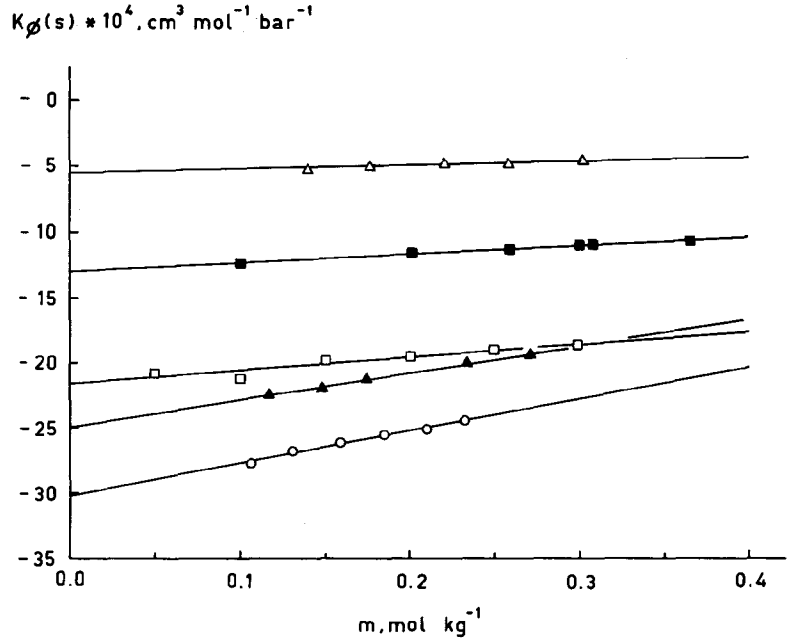

Figure 3.4 Apparent partial molar compressibilities as a function of concentration: lyxose **2d** (-■-), fructose **4a** (-□-), trehalose **7c** (-o-), 3-O-methylglucose **5e** (-Δ-), cellobiose **7b** (-▲-).

Interestingly significant differences in the partial molar compressibilities of the carbohydrates are observed. The data may be interpreted as follows: for aldohexoses (**3a,c,d,g**) it appears that the compatibility of the carbohydrate with water depends on the stereochemistry of the aldohexose. The hydration layer of D-talose (**3g**) is least

disturbed, compared to pure water. The greatest disturbance is found for D-galactose (**3a**), whereas D-glucose and D-mannose (**3c,d**) show intermediate behaviour. In fact the aldohexoses can be divided into three groups according to their stereochemistry with respect to their fit in water. (a) D-talose (**3g**), with an axial OH(2) and OH(4), shows the least disturbed hydration layer compared to bulk water and therefore fits into water best. (b) Aldohexoses with an equatorial OH(4) and an equatorial or an axial OH(2) (**3c,d**) show a moderately disturbed hydration layer. (c) An aldohexose with an axial OH(4) and an equatorial OH(2) (**3a**) is the least compatible with water.

The ketohexoses D-fructose (**4a**) and L-sorbose (**4b**) exhibit the same partial molar compressibility although their stereochemistry is different. Overall they disturb the water structure more than, for instance, D-glucose. This is probably due to the fact that the exocyclic CHOH moiety is situated at the anomeric centre, instead of at the 5-position. Why a carbohydrate will have a poorer fit in water when the exocyclic CHOH moiety is situated at the anomeric centre, will be discussed in Chapter 6.

Table 3.4 Partial Molar Volumes,[a] Isentropic Partial Molar Compressibilities and Hydration Numbers of Methyl Aldoglycopyranosides in Aqueous Solutions at 298.15 K.

Methyl Aldoglycopyranoside		V_2, cm^3mol^{-1}		$K_{2,s}.10^4$, $cm^3mol^{-1}bar^{-1}$		n_h[b]
		Found	Lit.	Found	Lit.	
		methyl hexopyranosides				
5a	Me α-galactopyranoside	131.0(0.1)	132.6[c](0.4)	-17.0(0.2)		9.4
5b	Me β-galactopyranoside	131.7(0.1)	132.9[c](0.2)	-17.1(0.1)		9.4
5c	Me α-glucopyranoside	132.6(0.1)	132.9[d](0.3) 132.6[c]	-14.7(0.1)	-13.0[d](3.0)	9.2
5d	Me β-glucopyranoside	134.1(0.2)	133.6[d](0.5) 133.5[c]	-11.1(0.4)	- 5.9[d](3.0)	8.8
5e	3-O-Methylglucose	133.9(0.1)	134.0[c](0.5)	- 5.6(0.2)		8.1
5f	Me α-mannopyranoside	132.5(0.1)	132.9[c](0.4)	-12.3(0.2)		8.9
		methyl pentopyranosides				
6a	Me β-arabinopyranoside	113.8(0.1)		-15.3(0.6)		8.2
6b	Me β-xylopyranoside	117.5(0.1)	117.2[e]	- 7.1(0.3)		7.4

a) Errors are given in parentheses. b) Calculated using the method described in Ref. 13-15. c) Ref. 31. d) Ref. 11 does not give an error value, hence the error is estimated from the plot in ref. 11. e) Ref. 34.

Interestingly pentoses (**2a-e**) show the same trend as the aldohexoses: those pentoses with an equatorial OH(4) and either an equatorial or axial OH(2) (**2c**, D-xylose, **2d**, D-lyxose and **2e**, D-ribose) disturb water only moderately. Only D-arabinose (**2a**) with an axial OH(4) and an equatorial OH(2) exhibits a larger disturbing effect on water.

methoxy group within the carbohydrate derivative can be important in determining the disturbance of the hydration layer of the carbohydrate compared to pure water. However, this is dependent on the stereochemistry elsewhere in the molecule.

The disaccharides (**7a-d**, **8a-c**, **9a-c**; Table 3.5) can be roughly divided into three groups depending on the monosaccharide moieties present in the disaccharide. Disaccharides composed of a glucose and a fructose unit have the least disturbed hydration layer (**8a**, sucrose, **8b**, turanose and **8c**, palatinose). For those disaccharides which consist of two glucose units the hydration layer is moderately disturbed (**7a**, maltose and **7b**, cellobiose) whereas a disaccharide which contains a galactose subunit (galactose has an axial OH(4) and an equatorial OH(2)) is least compatible with water (**9a**, lactose, **9b**, melibiose and **9c**, lactulose).

A comparison of the results for the disaccharides which consist of two glucose units shows that maltose (**7a**) and cellobiose (**7b**) have the same isentropic partial molar compressibility. This means that the hydration characteristics are similar although the type of linkage is different. This appears to rule out the proposition[36] that maltose can form an intramolecular hydrogen bond in water while cellobiose cannot. The other disaccharides, which contain two glucose subunits (**7c**, trehalose and **7d**, gentiobiose) appear to disturb water more than maltose and cellobiose, apparently in disagreement with the kinetic data.[27] Both trehalose and gentiobiose have the same fit with water as lactose has. We suspect that the type of linkage between the two glucose units for **7c** and **7d** has caused this difference from maltose and cellobiose. It is unexpected that trehalose (**7c**) disturbs water so much, because it does have the ability to replace water[37,38] in some organisms to prevent irreversible dehydration.

The disaccharides, which consist of a glucose and a fructose subunit (**8a-c**), exhibit the least negative partial molar compressibilities. Only turanose (**8b**) shows different behaviour from sucrose (**8a**) and palatinose (**8c**). This might be caused by the fact that the fructose subunit is now present in the pyranose form instead of the furanose form (which suggests that for fructose the furanose form disturbs water less than the pyranose form). Alternatively, the difference may be associated with the fact that there is a 1-3 type of linkage between the subunits. This has the same effect as the 1-6 and 1-1 type linkages between the glucose moieties (compare **7c**, trehalose and **7d**, gentiobiose).

Disaccharides which contain a galactose subunit (**9a-c**) disturb water considerably by their presence, the extent of disturbance being independent of the type of linkage between the two subunits or whether there is a glucopyranose or fructofuranose ring present as the other moiety (**9a**, lactose, **9b**, melibiose and **9c**, lactulose). This

provides further support for the notion that the relative position of the OH(4) in the pyranose ring is of crucial importance for the compatibility of the carbohydrate with the water structure.

3.3.4 Partial molar isothermal compressibilities

Partial molar isothermal compressibilities have only been calculated for methyl aldoglycopyranosides, since the partial molar expansibilities are known only for these compounds. The data are reported in Table 3.2. The partial molar isothermal compressibilities are less negative than the isentropic compressibilities. However, the trends for both parameters are the same: both methyl α- and β-galactopyranosides (**5a,b**) disturb water by their presence, but no influence on the relative position of the methoxy group is found ($K_{2,T}$ is -13.9 and -14.0 $x10^{-4}$ cm^3 mol^{-1} bar^{-1}, respectively). Both methyl β-D-glucopyranoside (**5d**) and methyl α-D-mannopyranoside (**5f**) fit well in water ($K_{2,T}$ is -8.1 and $-8.8x10^{-4}$ cm^3 mol^{-1} bar^{-1}, respectively). For the glucose derivatives there is a dependence of the compatibility with water on the relative position of the methoxy substituent (**5c**, methyl α-D-glucopyranoside: $K_{2,T}$ is -10.8 $x10^{-4}cm^3$ mol^{-1} bar^{-1}).

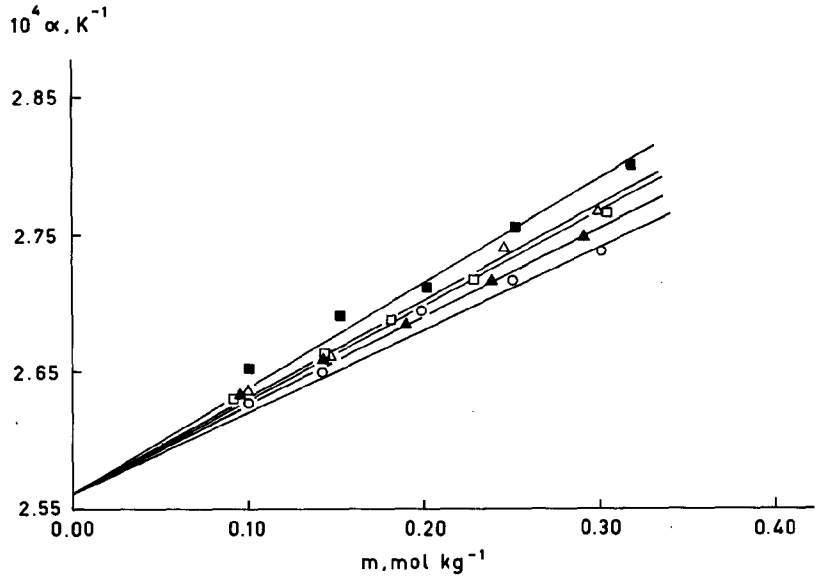

Figure 3.5 Expansibility coefficients versus molality of carbohydrate. Methyl α-D-galactopyranoside **5a** (-□-); methyl β-D-galactopyranoside **5b** (-Δ-); methyl α-D-glucopyranoside **5c** (-o-); methyl β-D-glucopyranoside **5d** (-▲-); methyl α-D-mannopyranoside **5f** (-■-).

3.3.5 Partial molar expansibilities

The sensitivity of partial molar expansibilities and partial molar compressibilities towards solvation has also been noted by Bernal and Van Hook,[30,39] by Franks *et al.*[11] and others.[40-42] In Table 3.2 partial molar expansibilities are listed for the methyl aldoglycopyranosides (**5a-d, 5f**). It may be concluded that for the methyl aldoglycopyranosides the partial molar isothermal compressibilities are sensitive towards stereochemical aspects of hydration, whereas the partial molar expansibilities all have about the same value within experimental error for all isomers. On average E_2 is 0.098 $cm^3\ mol^{-1}\ K^{-1}$ for the methyl hexopyranosides.

3.3.6 Hydration numbers

Hydration numbers for carbohydrates can be obtained by applying NMR spectroscopy,[43-45] near-infrared spectroscopy[46] and HPLC[47]. In addition, measurements on frozen aqueous solutions of carbohydrates[48] and ultrasound measurements[13,49] have been used to obtain hydration numbers. However, it has been emphasized that the value of the hydration number depends on the experimental method used,[14] because the hydration number depends on its definition and on the capability of the experiment to distinguish hydration water from bulk water. Here we use equation 3.14 to calculate hydration numbers, which are indicative of the number of water molecules disturbed by the presence of the carbohydrate to such an extent that they have become part of an incompressible hydration layer. Equation 3.14 is based on the assumption that the hydration layer, consisting of the number of water molecules given by the hydration number, is incompressible. If the compressibility of the hydration layer is not zero, then all hydration numbers will change. The actual numbers cannot be directly compared to other hydration numbers, since they have been mostly obtained by other methods.

All data are given in Tables 3.3 to 3.5. For the hexoses, the hydration numbers illustrate exactly how small the differences in the hydration characteristics are. The overall trend is that a better compatibility of the carbohydrate with water results in a smaller number of water molecules, which are disturbed by the presence of the carbohydrate (e.g. D-talose (**3g**): n_h = 7.7; D-galactose (**3a**): n_h = 8.7). The small difference in hydration numbers (for the hexoses, **3a,c,d,g 4a,b** it ranges from 7.7 to 9.0) illustrates why partial molar volumes are not sufficiently sensitive to detect and characterise stereochemically-induced differences in hydration. The pentoses (**2a-e**) have a slightly smaller hydration number due to the lack of the exocyclic CHOH group. The difference in hydration number for a molecule with an axial OH(4) and an equatorial OH(2) (D-arabinose (**2a**): n_h = 7.6) is more pronounced for the pentoses than for

aldohexoses. All other pentoses, which have an equatorial OH(4) and either an axial or an equatorial OH(2) (**2c**, D-xylose, **2d**, D-lyxose and **2e**, D-ribose: n_h = 6.8) disturb a smaller number of water molecules.

On average the methyl hexopyranosides disturb more water molecules than the hexoses due to the presence of an additional methoxy group. The number of incompressible water molecules is governed by the stereochemistry of the carbohydrate. The sugar 3-O-methylglucose **5e**, which has a structure which is highly compatible with water, disturbs the smallest number of water molecules (n_h = 8.1), whereas the relatively large hydration number of the methyl galactopyranosides (**5a,b**: n_h = 9.4) is indicative of a more disturbed hydration layer compared to pure water. Again the hydration numbers are found in a small range (n_h = 8.1 - 9.4). The hydration numbers of the methyl pentopyranosides are consistent with the interpretation that the carbohydrate with an axial OH(4) and an equatorial OH(2) (methyl β-arabinopyranoside, **6a**: n_h = 8.2) disturbs more water molecules than an isomer for which OH(4) is equatorial and OH(2) is either equatorial or axial (methyl β-xylopyranoside, **6b**: n_h = 7.4).

Overall, the disaccharides possess hydration numbers which are twice those of the monosaccharides. The range of hydration numbers for the disaccharides is fairly small. (n_h = 13.9 - 15.6), but again the importance of the relative position of OH(4) can be seen, and this point is emphasised.

3.4 Discussion

Not all thermodynamic properties reveal hydration characteristics which are influenced by the stereochemistry of the carbohydrate. Some parameters, like heats of solution, refer to carbohydrates in the crystalline state and therefore are limited in usefulness. When thermodynamic properties can be measured under appropriate conditions, the information which can be obtained on stereospecific hydration depends on whether these properties are determined by both intrinsic contributions and contributions due to carbohydrate-water interactions, or only by the latter. Direct insight into the stereochemical aspects of hydration can only be obtained if a thermodynamic parameter is considered for which the intrinsic contribution is negligible or constant. A possibility includes a study of the dependence of the partial molar volume on temperature or pressure. From such a study the partial molar expansibility and partial molar isothermal or isentropic compressibility can be calculated, respectively. The partial molar expansibility is in principle as sensitive towards hydration as the partial molar compressibility, because they are both second derivatives of the Gibbs energy (towards both pressure and temperature) and have a negligible intrinsic

contribution. Since temperature-dependent studies are involved, these quantities have only been measured for methyl aldoglycopyranosides, for which the mutarotation is blocked. At this stage it is important to note that the compressibilities can be measured more accurately than the partial molar expansibilities, because compressibilities are obtained by measuring the speed of sound through a solution. Partial molar expansibilities are measured by monitoring the densities of the solution as a function of temperature. There is an experimental limitation with respect to the temperature region in which the densities are still linearly dependent on the temperature. This implies that the temperature range must be small. This will automatically limit the sensitivity and accuracy of the measurements. Consequently the partial molar isothermal and isentropic compressibilities do reveal delicate differences in hydration between stereoisomers, but partial molar expansibilities with attendant experimental constraints show these differences less markedly.

3.5 Conclusion

3.5.1 Experimental design and choice of thermodynamic parameters

Few thermodynamic parameters monitor details of stereochemical aspects of hydration of carbohydrates. Not only do the experimental conditions have to be chosen carefully, but the thermodynamic parameter should be determined principally by the character of the hydration layer and not by the intrinsic contribution of the carbohydrate. Theoretically partial molar compressibilities and expansibilities are appropriate parameters for studying the hydration of carbohydrates. Related thermodynamic quantities like partial molar volumes and partial molar heat capacities are not particularly informative with regard to hydration because they are largely determined by the intrinsic volumes and heat capacities of the carbohydrates. The latter thermodynamic parameters can only be used to compare carbohydrates in aqueous solutions with other groups of compounds. However, the details of the stereochemical aspects of hydration cannot be monitored. In addition, the experimental method must be sensitive to stereochemical details in hydration. As a result only compressibilities, which have been measured by ultrasound measurements are parameters which show a sensitivity towards stereochemical aspects of hydration. One might wonder if the partial molar compressibilities could be measured with such accuracy by studying the dependence of the partial molar volumes on pressure directly.

3.5.2 Stereochemical aspects of carbohydrate hydration

In this chapter the isothermal and isentropic compressibilities confirm that the type

of hydration of a carbohydrate depends on the stereochemistry of the carbohydrate molecule.[27,50] It is submitted that the hydration of carbohydrates depends on stereochemistry. A most crucial factor for the hydration characteristics is the relative position of the next-nearest neighbour hydroxy groups within the carbohydrate molecule. It is found that the extent of disturbance of the hydration layer of a carbohydrate compared to pure water is largely determined by the position of the OH(4) in conjunction with the relative position of OH(2). The carbohydrates can be divided into three groups each with a different fit in water. For aldohexoses the best fit in water is found for D-talose, and the relatively poorest fit is found for D-galactose, while D-glucose and D-mannose have an intermediate fit. The molecular details of this study will be discussed in chapter 4. The poorest compatibility with water is found when a carbohydrate has an axial OH(4) and an equatorial OH(2). Interestingly, if OH(4) is axial in case of methyl aldoglycopyranosides or disaccharides, there is no influence of the relative position of the methoxy group on the anomeric center or the type of linkage between the two monosaccharide moieties. Only an axial OH(2) can then influence the fit in water, as was found in case of the aldohexoses. For a carbohydrate with an equatorial OH(4) and either an equatorial or an axial OH(2) there is a moderate compatibility with water. For methyl aldoglycopyranosides and disaccharides the relative position of a methoxy group on the anomeric center and the type of linkage between two moieties in disaccharides is important in some cases in determining the extent of compatibility.

3.6 Experimental

All the measurements were performed in the Physical Chemistry Department of the University in Bergen, Norway, in cooperation with Prof. Dr. Harald Høiland and mr. Geir M. Førland M.Sc.

3.6.1 Materials

All carbohydrates were of the quality described in chapter 2. All solutions were made up by weight. Where necessary, corrections for the water content of the carbohydrate were made based on the quantity indicated by the supplier. Water was doubly distilled.

3.6.2 Methods

For each thermodynamic parameter, measurements were carried out for at least five concentrations of carbohydrate.

Densities. The density measurements were carried out on a A Paar digital densiometer model DMA 02C. The error in these measurements was estimated to be $\pm 3.10^{-6}$ g cm^{-3}. The densiometer was calibrated against air and water.

Heat Capacity Measurements. Apparent partial molar heat capacities were measured with a Picker flow heat capacity meter.[7] The advantage of this kind of apparatus is that the heat capacities of solutions can be obtained without making measurements over a large temperature range. The measurements were performed while the system was thermostatted at 298.15 K. Basic power was 21 mW, flow rate was 1 ml per minute, which means that the

actual measurements were done at an average temperature of 298.30 K. Pure water was always taken as the reference.

Expansibilities. Apparent partial molar expansibilities were obtained by measuring densities of the solutions of carbohydrate as a function of temperature and concentration at 293.15 and 303.15 K.

Compressibility measurements. Isentropic coefficients of compressibility were determined by the "sing-around" principle[51] which involves the passing of repeated pulses of ultrasonic radiation through the solution and measurement of the frequency of this radiation. The frequencies were measured by a Hewlett-Packard 5326 A time counter, averaging over periods of 10 seconds. The ultrasonic cell was a gold-plated, brass cylinder, which had a length of approx. 4 cm. It was calibrated with water as standard, using the data of Del Grosso and Mader.[52] The cell was thermostatted in a water bath to better than ±0.05 K. The error in the speed of sound was estimated to be ±0.10 m s^{-1}.

3.7 References

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CHAPTER 4

MOLECULAR DYNAMICS SIMULATION OF CARBOHYDRATE HYDRATION. A PRELIMINARY STUDY

4.1 Molecular dynamics simulation of carbohydrates in water

Polyhydroxy compounds such as carbohydrates possess such small differences in their hydration characteristics that only very few experimental methods have sufficient sensitivity to permit those differences to be measured satisfactorily. This has been illustrated in chapter 3.

Molecular dynamics (MD) simulations provide a tool to study the hydration of a carbohydrate on a molecular level.[1] If one wants to have information on a molecular level about how a carbohydrate fits into water, one can either use structures of carbohydrates provided by X-ray data and extrapolate them to aqueous solutions or consider structures which originate from a molecular dynamics simulation in water. Because molecules are inherently dynamic in character, the use of molecular dynamics simulations has definite advantages. In an MD simulation[2,3] the first step is to construct a potential energy surface, which gives the energy of the system as a function of atomic coordinates. The potential energy function involves the Lennard-Jones and Coulomb interactions, a term for the bond stretching force constant, a term for bond angles, dihedral angles (and for carbohydrates improper dihedral angles) and non-bonded atom interactions. To obtain the forces acting on the atoms of the system, the first derivatives of the potential with respect to the atom positions are calculated. These forces can be used to determine the dynamic evolution of the system. By solving Newton's equations of motion for the atoms of a molecule and, in principle the surrounding solvent, exact and detailed information may be obtained on the dynamic properties of a system. There has been, and still is, a lively discussion on the way molecular dynamic simulations should be performed for carbohydrates in aqueous solution, because carbohydrates have a hemiacetal functionality, which is subject to mutarotation. In addition, the question arises which force field and point charges should be used for carbohydrates in aqueous solution and how to account for the anomeric effect.[1]

For the simulations, potential energy functions have been devised which are semi-empirical. It is notoriously difficult to describe a potential energy function for

carbohydrates because of the presence of the hemiacetal function. The many forms of a carbohydrate in solution (see chapter 1) pose a severe problem to the theoreticians active in developing a force field for calculations. For example, the question arises whether the anomeric effect should be introduced into the force field or whether it should automatically be predicted by the outcome of the calculations, without the simulation being prejudiced.

Fortunately several carbohydrate potential energy functions are now available,[4-9] sometimes especially developed for small molecule analogues of carbohydrates. However, most force fields are incompatible with others, e.g. those for glycoproteins, because they are so specific for carbohydrates.

Since 1986 simulations of carbohydrates in vacuum and in aqueous solution have been published.[10-20] In the case of cyclodextrins, simulations have also been reported for the crystalline state and for cyclodextrins in aqueous solution.[21-24] In addition, polyols and other small organic solutes have been subjected to a simulation in aqueous solution.[25-27] Monte Carlo simulations have also been performed for carbohydrates.[28-30]

We are particularly interested in the molecular dynamics simulations of both α- and β-D-glucopyranose, published by Brady,[11,13] who could reproduce the energy difference between α- and β-glucose in water. His approach towards simulation of carbohydrate hydration provided us with an opportunity for a further study of the stereochemical aspects of carbohydrate hydration. The simulations described in this chapter have been performed with the GROMOS[3] force field using the charges of Brady[14] by mr. Eduardo Howard, M.Sc. and Prof. Dr. J. Raul Grigera at the University of La Plata, Argentina. This force field can also be used for glycoproteins.

4.2 Goal of the study

In chapter 3 it was proposed that the compatibility of a carbohydrate with water depends predominantly on the relative positions of OH(2) and OH(4). Why these two hydroxy groups in particular are so important and what the molecular picture is behind the fit of a carbohydrate molecule into water has, so far, remained rather unclear. What does it mean when a carbohydrate fits very well in water? In addition, why do the aldohexoses appear to be divided into three groups with different fits in water? It will also be interesting to find out why OH(2) and OH(4) are so important for the fit of an aldohexose in water and not, as was previously suggested,[31-33] OH(1) and OH(3). Since OH(2) and OH(4) are next-nearest neighbour oxygens in a carbohydrate molecule, all next-nearest neighbour oxygen distances within a carbohydrate molecule were studied, and compared to the oxygen distances in liquid water.

4.2.1 Crystal data

Initially only the data for the crystalline carbohydrates were available, as obtained from the Cambridge crystallographic database. The through-space oxygen distances of the dominant conformers of hexoses in the crystalline state could be calculated with aid of the CHEMX program.[34] In Table 4.1 the next-nearest neighbour oxygen distances for β-D-galactose, β-D-glucose, α-D-mannose, β-D-allose and α-D-talose are given. Only the oxygen distances of the secondary oxygen atoms are considered.

Table 4.1 Through-Space Oxygen Distances[a] (Å) in Carbohydrate Crystals.[b]

	Carbohydrate[c]	O_2-O_4	O_2-O_5	O_4-O_5	O_1-O_3
3a	β-D-galactose	4.28	3.66	2.83	4.82
3c	β-D-glucose	4.87	3.67	3.66	4.75
3d	α-D-mannose	4.27	2.77	3.69	4.12
3e	β-D-allose	4.82	3.67	3.66	4.13
3g	α-D-talose	2.66	2.93	2.87	4.17

a) See experimental section. b) In water: O-O (nearest neighbour) = 2.95 Å, O-O (next-nearest neighbour) = 4.82 Å. c) All hexoses are in the pyranose form.

It is observed that for a carbohydrate which fits very well into water (**3g**, D-talose) the through-space next-nearest neighbour oxygen distances O_2-O_4, O_2-O_5 and O_4-O_5 are at a distance from each other which is comparable to the nearest neighbour oxygen distance in water. The O_1-O_3 distance within the D-talose molecule is much longer but is too small to fit with the next-nearest neighbour oxygen distances in water.

A carbohydrate, which does not have a good compatibility with water, exemplified by D-galactose (**3a**), can fit its O_1-O_3 distance with the next-nearest neighbour oxygen distances of water, but the oxygen distances between OH(2), OH(4) and O(5) show greatly different distances. For the three other hexoses (**3c**, D-glucose, **3d**, D-mannose, **3e**, D-allose) an intermediate behaviour (intermediate fit) was suggested (chapter 3). For D-glucose this might be caused by the fact that both the O_2-O_4 and the O_1-O_3 distance are compatible with the next-nearest neighbour oxygens in water (O_2-O_4 = 4.874 and O_1-O_3 is 4.754 Å, respectively). For the other hexoses (**3d** and **3e**) the explanation is less clear cut. Both α-D-mannose and β-D-allose show a great variety of next-nearest neighbour oxygen distances. For β-D-allose the O_2-O_4 distance is compatible with the next-nearest neighbour oxygen distance in water, for α-D-mannose there is no such fit. However, both hexoses have the "gluco configuration" which will influence the relative position of the exocyclic hydroxymethyl group (OH(6)) in such a way that, although the O_2-O_4-O_5 plane does not have such a good compatibility with water, the plane of oxygens

1,3 and 6 might be able to compensate for this. The intramolecular oxygen distances for these hexoses should be studied in water instead of in the crystalline state to investigate what the influence of water might be on the oxygen distances.

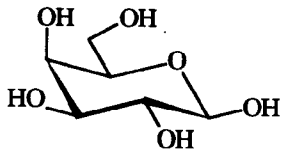
Table 4.2 G(C) Values, Partial Molar Isentropic Compressibilities, $K_{2,s}$ and Hydration Numbers, n_h of D-Galactose and D-Talose in Aqueous Solution at 298.15 K.

Parameter	D-Galactose	D-Talose
G(C),[a] J kg mol^{-2}	-142	-280
$K_{2,s}$,[b] cm^3 mol^{-1} bar^{-1}	-20.4	-11.9
n_h[b]	8.7	7.7

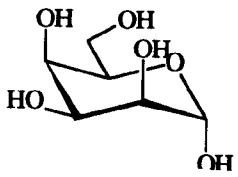
a) Chapter 2. b) Chapter 3.

4.2.2 Molecular dynamics simulation, choice of carbohydrates

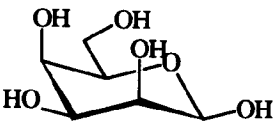
Three hexoses were selected for a molecular dynamics simulation. When the results of chapters 2 and 3 are summarised (Table 4.2) for D-galactose and D-talose, it is concluded that D-galactose has a poorer fit into water than D-talose. Therefore, these aldohexoses were selected for a simulation. It was logical that for galactose only the β-anomer was subjected to the simulation exercise as it is dominantly present in aqueous solution. For D-talose, both the α- and β-anomer are present in aqueous solution in comparable amounts (Table 1.1), hence the simulation was performed for both anomers. The structures of the hexoses, which will be discussed in this chapter, are drawn in Scheme 4.1.



β-D-galactose 3aβ



α-D-talose 3gα



β-D-talose 3gβ

Scheme 4.1 Hexoses, which were studied by a Molecular Dynamics simulation: 3aβ: β-D-galactopyranose, 3gα: α-D-talopyranose, 3gβ: β-D-talopyranose.

4.3 Results

The results of the simulations are depicted in Tables 4.3 to 4.5 and Figures 4.1 to 4.3. The oxygen distances of a carbohydrate in water refer to the oxygen distances of

the carbohydrate molecule as obtained by means of a simulation in water.

Table 4.3 Next-Nearest Neighbour Oxygen Distances of Carbohydrates in the Crystalline State and in Aqueous Solution[a]

Carbohydrate	O_2-O_4	O_2-O_5	O_4-O_5	O_1-O_3	O_1-O_6	O_3-O_6
Crystalline State						
3aβ β-D-galactopyranose	4.28	3.66	2.83	4.82	4.70	6.14
3gα α-D-talopyranose	2.66	2.93	2.87	4.17	4.44	6.19
In aqueous solution[b]						
3aβ β-D-galactopyranose	4.4	3.7	3.0	4.9	5.8	5.4
3gα α-D-talopyranose	2.7	3.1	3.0	4.2	5.1	5.5
3gβ β-D-talopyranose	2.7	3.0	3.0	4.9	5.8	5.5

a) Distances given in Angstroms. b) As determined in the MD simulation.

4.3.1 Through-space oxygen distances in the carbohydrate molecule

In Table 4.3 the oxygen distances of the carbohydrates studied are given in both the crystalline state and in aqueous solution after the simulation. Generally it is observed that the trends found in the crystal data are reproduced in the simulation for the through-space intramolecular oxygen distances of the endocyclic hydroxy groups. The only difference with the crystal data is, that the MD simulations show that the hydroxymethyl group (O_6) seems to be flexible enough to adjust its position somewhat in order to create the best oxygen distance with the oxygens on C-1 and C-3. It was found previously, that the hydroxymethyl group is flexible and its relative position is solvent dependent.[11,17,19] The difference between the O_1-O_6 and O_3-O_6 distances in the crystalline state and in water confirms that the hydroxymethyl group is flexible and that its relative orientation is solvent dependent.

It is observed that for β-D-galactopyranose the O_2-O_4-O_5 plane only the O_4-O_5 distance fits with oxygen distances in water, whereas in the O_1-O_3-O_6 plane fits with two of its O-O distances with the next-nearest neighbour oxygen distance of water. However, α-D-Talopyranose has a perfect fit with the nearest neighbour oxygen distances in water as far as the O_2-O_4-O_5 plane is concerned. This is also the case for β-D-talopyranose. Both O_1-O_3-O_6 planes of the taloses fit partly if not reasonably well with the next-nearest neighbour oxygen distances of water. In general it is known that in O - -H-O hydrogen bonds the O-O distances may vary from 2.5 to 3.5 Å.[35] Hence the oxygens in the O_2-O_4-O_5 plane of D-talose are at distances which are so short that even an intramolecular hydrogen bond is possible in this plane.

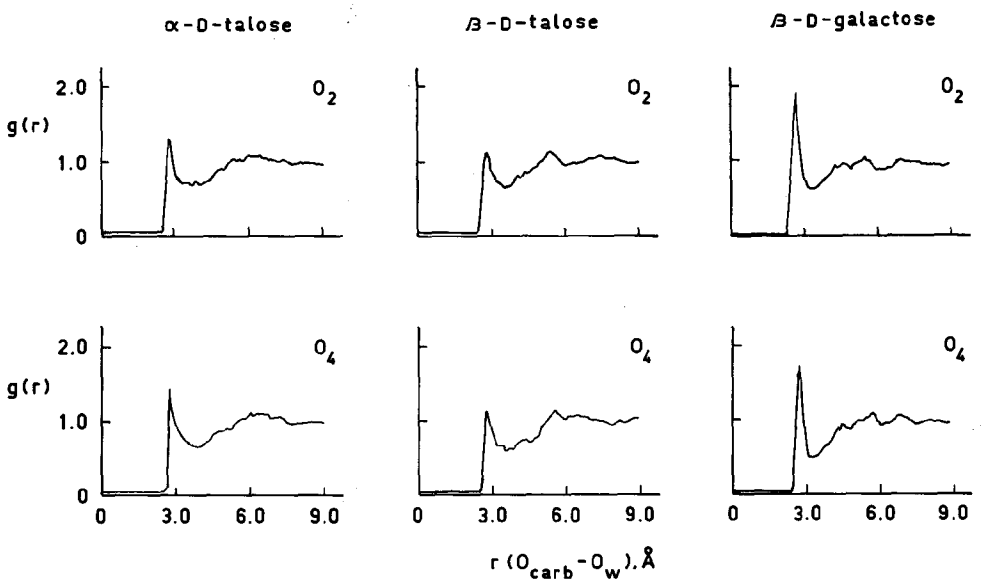


Figure 4.1 Radial distribution functions of water around carbohydrate oxygens: distribution of r(O_{carb}-O_{water}) for the OH(2) and OH(4) groups of α-D-talopyranose, β-D-talopyranose and β-D-galactopyranose.

Table 4.4 Oxygen-Water Distances of β-D-Galactopyranose, (α,β)-D-Talopyranose and α-D-Glucopyranose in Aqueous Solution.[a]

	Carbohydrate	O_1-O_w	O_2-O_w	O_3-O_w	O_4-O_w	O_5-O_w	O_6-O_w
3a	β-D-galactopyranose	2.8	2.8	2.9	2.7	3.3	2.8
3gα	α-D-talopyranose	2.9	2.8	2.8	2.9	3.5	2.8
3gβ	β-D-talopyranose	2.8	2.9	2.8	2.8	3.2	2.8
3cα	α-D-glucopyranose[b]	2.8	2.7	2.7	2.8	3.3	2.7

a) Distances in Angstroms. b) From reference 11.

4.3.2 Oxygen distances between carbohydrate and water

In Table 4.4 the oxygen-oxygen distances for each carbohydrate-oxygen to an oxygen atom of water are given for the carbohydrates **3a**, **3c** and **3g**. The reported distance is the distance from the oxygen of a carbohydrate molecule, at which the radial distribution function of an oxygen atom of water has the highest probability. It is observed that the ring oxygen (O_5) is somewhat shielded from interaction with water for all the carbohydrates: there is not a very sharp maximum in the distribution function (this is not shown in a figure). This observation was previously made for α-D-glucopyranose.[11] For the other oxygen atoms of a carbohydrate it is observed that the oxygens on the 1-,

3- and 6-position all have sharp, well defined distances at which the radial distribution function of water has the highest probability. This indicates that there is a strong intermolecular hydrogen bond between these hydroxy groups and water. Brady[11] claims that a carbohydrate is a structure breaker, because there is only one sharp maximum in the probability of finding a water molecule near a hydroxy group of a carbohydrate. Interestingly, the presence of a well defined O_{carb}-O_{water} distance is very much dependent on the stereochemistry for the hydroxy groups at the 2- and 4-position.

In Figure 4.1 the distribution function is given as a function of the O_{water}-O_{carb} distance. The plots are made for the OH(2) and OH(4) groups of α-D-talopyranose, β-D-talopyranose and β-D-galactopyranose. The difference between D-galactose and D-talose is illustrated.

Table 4.5 Average Number of Hydrogen Bonds From Water to Water, From Water to Oxygen of Carbohydrate and From Carbohydrate to Water.

	H_2O	α-talose	β-talose	β-galactose
O_w - -H-O_{water}[a]	3.13	3.11	3.10	3.08
		OH_{carb} is hydrogen bond acceptor		
O_1 - -H-O_{water}[b]		0.96	1.06	1.21
O_2 - -H-O_{water}		1.09	1.04	1.06
O_3 - -H-O_{water}		1.06	1.11	0.84
O_4 - -H-O_{water}		0.51	0.42	1.00
O_6 - -H-O_{water}		1.29	1.13	1.31
		OH_{carb} is hydrogen bond donor		
O_1-H - -O_{water}[c]		0.89	0.81	0.64
O_2-H - -O_{water}		0.15	0.03	0.82
O_2-H - -O_{water}		0.83	0.79	0.71
O_4-H - -O_{water}		0.83	0.79	0.71
O_6-H - -O_{water}		0.89	0.90	0.90

a) Calculated from the distribution of the number of hydrogen bonds between 0-5 during 50 ps. b) Calculated from the distribution of the number of hydrogen bonds between 0-3 during 50 ps. c) The number of hydrogen bonds was 0 or 1.

4.3.3 Hydrogen bonds

Intermolecular Hydrogen Bonds Between Water Molecules in the Hydration Layer. In Table 4.5 the average number of hydrogen bonds between water molecules in water and in the presence of α-D-talose, β-D-talose and β-D-galactose are given. This number of hydrogen bonds is calculated over 50 ps and between zero and five hydrogen bonds are taken into

account. The largest number of hydrogen bonds (3.13) is found in water.

The presence of α-D-talose, β-D-talose and β-D-galactose disturbs the average number of hydrogen bonds between water molecules only very slightly as the water molecules have an average number of mutual H-bonds of 3.11, 3.10 and 3.08, respectively. This illustrates how well carbohydrates in general fit into water. α-D-talose seems to fit better in water than β-D-galactose with respect to the average number of hydrogen bonds in the hydration layer. If this difference is significant, it suggests that the carbohydrates can be called "water-structure breakers" because in their presence the average number of hydrogen bonds between the water molecules is less than in bulk water.

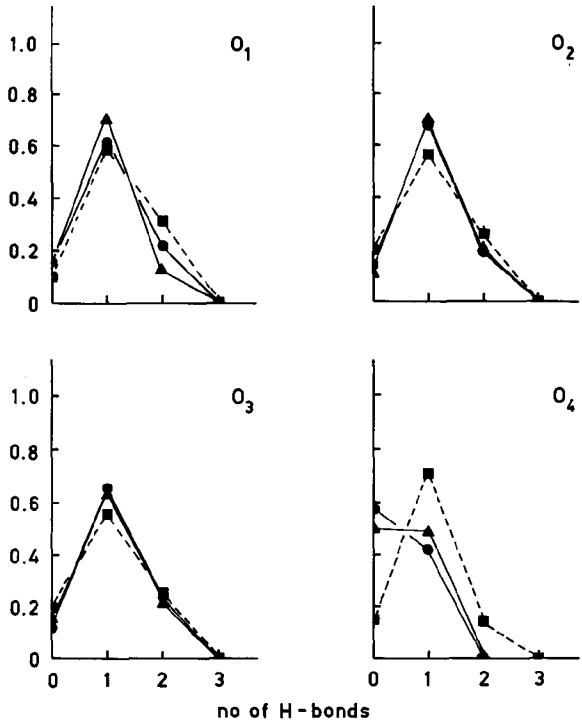


Fig. 4.2 Distribution of the number of hydrogen bonds between water and carbohydrate for OH(1), OH(2), OH(3) and OH(4); OH_{carb} is H-bond acceptor. α-D-talose (-▲-); β-D-talose (-●-); β-D-galactose (-■-) (dotted line).

Average Number of Hydrogen Bonds. Carbohydrate as a H-bond Acceptor. If the average number of hydrogen bonds is considered (Table 4.5 and Fig 4.2) for those cases where the oxygen atoms of the carbohydrate serve as hydrogen bond acceptors (water is the

hydrogen-bond donor), it is observed that the exocyclic hydroxy group is on average a good H-bond acceptor; for all three carbohydrates the average number of hydrogen bonds lies between 1.13 and 1.31. For the endocyclic hydroxy groups the average number of hydrogen bonds depends on the structure of the carbohydrate: for oxygens O_1, O_2 and O_3 the average number of hydrogen bonds is between 0.84 and 1.21. Interestingly, great differences are found for O_4 (Fig. 4.2). In the case of both talose anomers, the O_4 is shielded as a hydrogen bond acceptor with an average number of hydrogen bonds of 0.51 and 0.42, respectively (Table 4.5), while the O_4 of β-D-galactose has an average number of hydrogen bonds which is comparable to that for the other hydroxy groups.

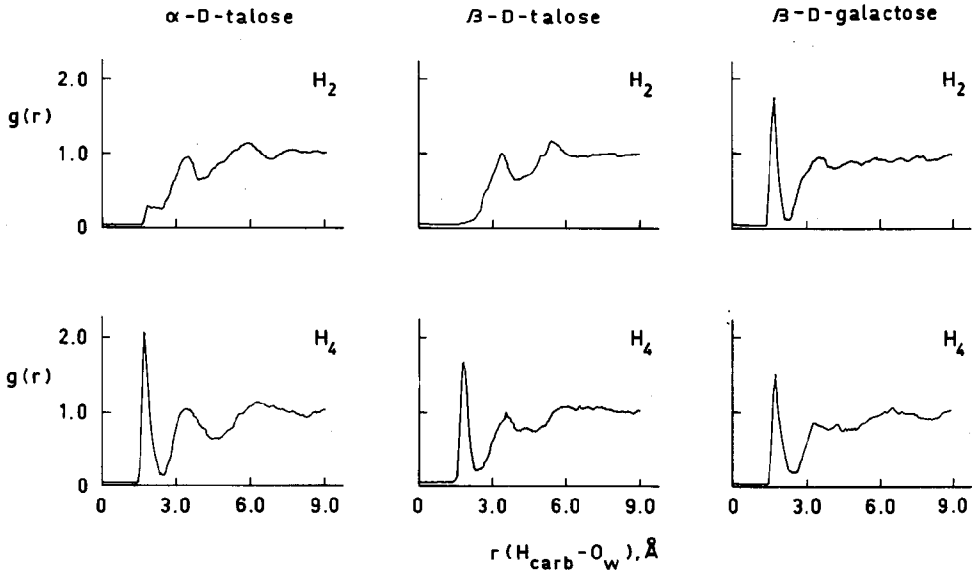


Fig. 4.3 Radial distribution function of water around carbohydrates: distribution of r(H_{carb}-O_{water}) for OH(2) and OH(4) of α-D-talopyranoside, β-D-talopyranoside and β-D-galactopyranoside.

Average Number of Hydrogen Bonds. Carbohydrate as a H-bond Donor.

The average number of hydrogen bonds in which the hydroxy groups of a carbohydrate are H-bond donors (water serves as hydrogen-bond acceptor), is between 0.64 and 0.90 for all hydroxy groups, except for the OH(2) group of both anomers of D-talose, which have an average number of hydrogen bonds of 0.15 and 0.03, respectively. This is clear evidence that in α- and β-D-talose the OH(2) is shielded from water as a hydrogen bond donor. Figure 4.3 illustrates this result; it shows that the r(H_{carb}-O_{water}) is not sharply defined for the OH(2) of D-talose as compared to D-galactose.

Anomeric Hydroxy Groups. If the average number of hydrogen bonds is calculated and compared for the respective anomeric hydroxy groups (Table 4.5), it is observed that for β-D-galactose the anomeric hydroxy group has more hydrogen bonds than the anomeric OH group of D-talose when it acts as a H-bond donor. However, it has less hydrogen bonds than in the case of D-talose as an acceptor.

Intramolecular Hydrogen Bonds. For carbohydrates in water there has been much interest in the possible formation of intramolecular hydrogen bonds. The difference in solubility for maltose and cellobiose, two isomeric disaccharides, was claimed to be caused by an intramolecular hydrogen bond between the monosaccharide moieties, which could exist in maltose, but not in cellobiose.[37] In addition, sucrose was considered to be very rigid, because of an intramolecular hydrogen bond between the two saccharide units.[38] It was generally believed that this intramolecular hydrogen bond was needed to elicit the sweet taste.[39] These intramolecular hydrogen bonds have been observed in the crystal structure and in aprotic solvents. However, the possibility of formation of an intramolecular hydrogen bond in water is considered to be small, because intermolecular hydrogen bonds with water will be entropically much more favourable. Recent NMR and MD investigations[15,40,41] have again shown that for disaccharides the intramolecular hydrogen bond is virtually non-existent in water. However, in N-acetyl-α-D-galactosamine the presence of a weak intramolecular hydrogen bond in water has been established.[19]

The results reported in chapter 2 suggest that the aldohexoses, which are under discussion in this chapter, possess a different apparent hydrophobicity, because they exert a different kinetic medium effect. The extent of the medium effect suggests that the carbohydrates behave as rather hydrophobic species in water. Because carbohydrates are readily soluble in water, due to their many hydroxy functionalities, the choice was made to call this hydrophobicity in water an "apparent hydrophobicity". The question arises whether the apparent hydrophobicity is just caused by a camouflage effect[42] (the hydroxy groups resemble the hydroxy groups of water and therefore are not recognised by a spectator molecule) or whether another mechanism comes into play on top of the camouflage effect. One of the reasons why D-talose might have a greater apparent hydrophobicity than D-glucose and D-galactose might be that not only its hydroxy groups are camouflaged, but that they are also shielded from the solvent by an intramolecular hydrogen bond. D-talose has the possibility of forming an intramolecular hydrogen bond between the O_2-O_4-O_5 groups. Although it seems logical that this intramolecular hydrogen bond would be destroyed in water because an intermolecular hydrogen bond is entropically more favourable, it could well be that the alignment of the hydroxy groups

in a D-talose molecule is so much like that in water, that entropically there is no significant difference between an intramolecular hydrogen bond between two hydroxy groups of D-talose and an intermolecular hydrogen bond with water. The O_{carb}-O_w distance is not as sharply defined for both OH(2) and OH(4) in the case of (α,β)-D-talose as compared to D-galactose (Figure 4.1). This indicates that there could well be an intramolecular hydrogen bond, more so because OH(2) of D-talose is shielded as a hydrogen-bond donor as compared to D-galactose (Figure 4.3). When the hydrogen bond acceptor capability of the oxygens of both α- and β-talose is considered, it is observed that the OH(4) is partly shielded from water, while as a hydrogen-bond donor, the OH(2) is shielded from water. A probable reason for shielding could be the formation of an intramolecular hydrogen bond in D-talose. The average number of intramolecular hydrogen bonds can be calculated from the number of intramolecular hydrogen bonds divided by the total number of H-bonds that a hydroxy group can have as a hydrogen-bond donor during the 50 ps of the simulation. Thus in α-D-talose 72% of all the hydrogen bonds that OH(2) can form as an H-bond donor, are intramolecular hydrogen bonds. In the case of β-D-talose, the intramolecular hydrogen bond of OH(2) as hydrogen-bond donor makes up for 94% of the total number of hydrogen bonds that the OH(2) can form as a hydrogen-bond donor. This intramolecular hydrogen bond only exists when OH(2) acts as the hydrogen-bond donor and OH(4) as the hydrogen-bond acceptor. An intramolecular hydrogen bond in which OH(4) participates as the hydrogen-bond donor is almost non-existent. Of the total number of hydrogen bonds with OH(4) as a hydrogen-bond donor, 0.6% of the hydrogen bonds are intramolecular for α-D-talose and 6.5% of hydrogen bonds are intramolecular for β-D-talose.

All carbohydrates will partly camouflage their hydroxy groups for interaction and hence seem to behave like hydrophobic species in aqueous solutions. This effect can be even stronger when a carbohydrate forms an intramolecular hydrogen bond.

These conclusions could be substantiated further by a longer simulation (which is presently carried out at the University of La Plata). Another possibility to check for intramolecular hydrogen bonds would be to investigate the temperature dependence of the ^{1}H-NMR shifts of the hydroxy groups of a carbohydrate in water.[16]

4.4 Conclusion

The results of the kinetic medium effects (chapter 2) and the thermodynamic studies (chapter 3) have supplied the relevant questions concerning carbohydrate hydration, which have to be addressed on a molecular scale. The results presented in this chapter have shown that the hydration characteristics of a carbohydrate can be probed

thoroughly by molecular dynamics simulations. Stereospecific hydration can be explained by considering the hydrogen bonding interactions and oxygen distances: the hydration of a carbohydrate depends largely on the relative distances of the next-nearest neighbour oxygens as compared to the oxygen distances of water. The oxygens of an aldohexose can be divided into two planes, namely the O_2-O_4-O_5 plane and the O_1-O_3-O_6 plane. The latter plays a minor role in the compatibility of the carbohydrate into the three-dimensional hydrogen-bonded network of water, because the anomeric position can be either equatorial or axial and because the hydroxy group of the hydroxymethyl moiety is very flexible and can therefore adjust itself to a better fit. The O_2-O_4-O_5 plane is the most rigid plane with little flexibility and plays the most important role in determining the fit of the carbohydrate into water.

Aldohexoses can be divided into three groups, with different extents of compatibility in water. The next-nearest neighbour oxygens of the carbohydrate can fit with the nearest neighbour or next-nearest neighbour oxygens of water or have no fit at all.

The surprisingly strong apparent hydrophobicity of D-talose is most probably caused by the possibility of the formation of an intramolecular hydrogen bond in the carbohydrate molecule between OH(2) and OH(4), which adds to the camouflage effect. In this intramolecular hydrogen bond OH(2) acts as the H-bond donor, whereas OH(4) is the hydrogen-bond acceptor.

4.5 Experimental

Crystal data. The crystal data were obtained from the Cambridge crystallographic database for those carbohydrates for which the conformation in the crystal was similar to the dominant conformation in water. The next-nearest neighbour oxygen distances of the carbohydrates in the crystalline state were calculated by using the CHEMX program.[34]

Procedure. Molecular dynamics (MD) simulations were performed on the carbohydrates β-D-galactopyranose, α-D-talopyranose and β-D-talopyranose. For all the simulations the same procedures and conditions were taken into account. The carbohydrates were placed in a cubic box of 1.8662 nm average length with period boundary conditions. The box was filled with SPC/E water.[45] This led to 216 water molecules in the box. The systems were energy minimized (steepest descent method); 50 steps were performed keeping the carbohydrates harmonically constrained to their original positions enabling the water molecules to equilibrate, followed by 200 steps without constraints.

The configurational energies and forces were computed with GROMOS87[3] parameters. For the charges of the carbohydrates the values of Brady *et al.*[14] were used. The polar

hydrogen atoms were explicitly included; they only have partial charges. In this way all the charges were fully compatible with Brady's.[14] The energy of a molecular system is described by simple potential energy functions comprising stretching, bending, torsional, Lennard-Jones and electrostatic interactions. The molecular model that was used treats all atoms explicitly, except for the hydrogen atoms that are bound to carbon atoms. They are treated as united atoms (i.e. CH1, CH2 and CH3 groups) and no special hydrogen-bond potential has been included.

The simulations, under periodic boundary conditions, were performed with a rigid backbone of the carbohydrate molecules by constraining the improper dihedral/torsional angles. There was still rotational freedom around the C-O bond.

No ring flip was possible, which seems justified because ring flip was not observed during the 32 ps simulation of D-glucose in water.[11] The molecular dynamics phase of the calculations involved a period of 10 ps equilibration and meanwhile heating the system to 298 K by coupling to an external heat and pressure bath[46] at 298 K and 1 atm. with time constraints of 0.01 ps and 0.05 ps, respectively.

After the equilibration, a simulation 50 ps of MD was performed. Intermediate structures generated during these MD runs were saved every 10 steps (0.020 ps). The temperature and pressure were controlled by coupling to an external bath at 298 K and 1 atm. Time constants were chosen as 0.1 and 0.5 ps for coupling to the heat and pressure baths, respectively. All covalent bond lengths as well as the water angle were constrained by the procedure SHAKE[47] and a 2 fs time step was used. All non-bonded forces were cut off at 0.9 nm.

All simulations were run on a VAX 2000 computer of the IFLYSIB, by Eduardo Howard and J. Raul Grigera.

As a hydrogen bond criterion was used (a) the O - -H distance had to be less than 2.4 Å, and (b) the angle of O-H - -O, which had to be equal or greater than 145^0. The reference temperature was 298 K and the pressure was 1 atm.

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CHAPTER 5

CARBOHYDRATE-DERIVED SURFACTANTS SYNTHESIS AND AGGREGATION BEHAVIOUR

5.1 Introduction

The aggregation behaviour of ionic, nonionic and zwitterionic surfactants in aqueous solution has been investigated extensively.[1-3] Two parameters are of main interest. (a) The cmc (critical micelle concentration, the concentration of surfactant monomer, which has to be reached for aggregation) and (b) the Krafft temperature of a surfactant (the temperature at which the solubility in water equals the cmc). Surfactants have been subject of studies involving structure-performance relationships.[4] The influence of alkyl chain length,[5,6] alkyl chain branching[7] and unsaturation of the alkyl chains[7] upon aggregation behaviour has also been studied. In addition, emphasis has been put on the importance of headgroup size[8] and extent of counterion binding[7] in determining micellisation. All theses factors can also influence the shape of the aggregate. The model proposed earlier by Israelachvili,[9,10] compares the headgroup size with the volume of the alkyl chain, and can often predict the aggregate morphology from the shape of the surfactant monomer for ionic surfactants.

In addition, there has been a lively discussion on the structural model for a micelle.[11-21] This discussion evolved around the question how open a micelle structure is and how far, if at all, water molecules can penetrate into the hydrophobic core of a micelle. At the moment, Gruen's model[20,21] (the standard picture of ionic micelles) seems to be accepted.

This chapter reports on a study of the micellar properties of n-alkyl 1-thioglycopyranosides as a function of stereochemistry of the monosaccharide headgroup. Their synthesis, aggregation behaviour, Krafft temperatures and liquid crystalline behaviour will be discussed.

5.2 Aggregation behaviour of carbohydrate-derived surfactants. Literature data

Although the field is relatively new, the interest in carbohydrate-derived surfactants is growing, especially because of their potential industrial applications. Carbohydrate-derived surfactants can be prepared from renewable sources.[22-24] In addition, carbohydrate-derived surfactants are claimed to be biodegradable, non-toxic and they do not irritate the skin, which makes them applicable in the food- and cosmetic industry. In chapter 1 a first example of a carbohydrate-derived surfactant

which is industrially manufactured is mentioned (APG = alkyl polyglucoside). A second example is sucrose octastearate (sucrose polyester) which is used to replace fat in food.

Carbohydrate-derived surfactants are being studied widely with respect to their surface active properties and liquid crystalline behaviour. Examples include alkyl glycosides,[25-32] fluorinated alkyl glycosides,[33] alkyl 1-thioglycosides,[34-39] sucrose mono-esters,[40a] sugar-amides,[41-49] methylated sugar-amides,[48] alkyl 1-thioalditols[38,39] and (N-acetyl)-N-alkyl-aminoalditols.[42] Their aggregation behaviour has been investigated as a function of alkyl chain length[25,27,35,41] and headgroup size and geometry.[27,42a] It has been found that there is a strong dependence of the cmc on the alkyl chain length, and even a change in aggregate morphology can be induced by lengthening of the alkyl chain length (e.g.: sucrose mono-esters[40a] and some long chain sugar-amides[40b]). This is not predicted by Israelachvili's model.[9,10] For alkyl glycosides[27] it has been found that the headgroup contribution towards micellisation is different for a glucose moiety when compared with a headgroup made up of a maltose moiety. However, if maltose and maltotriose are compared as headgroups, the number of glucose units does not influence the cmc. In addition, when a carbohydrate headgroup consists of two or three monosaccharide subunits there is hardly any influence of the type of linkage (α or β) between the sugar headgroup and the alkyl chain on the cmc. This is in contrast to a surfactant containing a monosaccharide headgroup; in that case a dependence has been found of the cmc on the type of linkage between the sugar headgroup and the alkyl chain,[32] the position of the alkyl chain on the sugar ring[31] and the stereochemistry of the headgroup.[27]

When the headgroup is a linear polyol as is the case for the sugar-amides,[41,42] there is little or no influence of the stereochemistry of the headgroup on the cmc.

In Table 5.1, the effect of the headgroup, ΔG_m(head) and methylene groups, $\Delta G_m(CH_2)$ on the Gibbs energy of micellisation (ΔG_m) of carbohydrate-derived surfactants are reported. They have been calculated with the Shinoda equation.[50] These calculations reveal that a contribution of a methylene group towards micellisation is, on average, -3.1 kJ mol^{-1} for the carbohydrate-derived surfactants. The headgroup contribution is, on average, +6.1 kJ mol^{-1}. In addition, the disaccharide derivatives possess a less positive headgroup contribution to the Gibbs energy of micellisation as compared to the surfactants with a monosaccharide headgroup.

The contribution of a methylene group of a carbohydrate-derived surfactant to ΔG_m is comparable to that of ionic surfactants ($\Delta G_m(CH_2)$ = -2.8 kJ mol^{-1}).[51] Although it is suggested that the nonionic headgroups are bulky and therefore do not allow much

contact between water and the hydrophobic core,[52] the methylene contribution to ΔG_m for a nonionic is only slightly different from that for ionics, hence the structure of the micelles must be about the same. If significance should be read into this small difference, it is most likely caused by the fact that the hydration of a polar headgroup and an apolar alkyl chain influence each other (chapter 2). The more polar a headgroup is, the stronger will its influence be on the hydrophobic hydration of the alkyl chain. An ionic headgroup will break down the hydrophobic hydration layer partially by its presence; hence the entropy of micellisation for ionic surfactants is not as favourable as that for a nonionic surfactant. A nonionic headgroup will interfere much less with the hydrophobic hydration layer of an alkyl chain. Therefore the breakdown of the hydrophobic hydration layer is possibly greater upon micellisation of a nonionic surfactant than of an ionic surfactant. The difference in the contribution of a methylene group might be caused by this difference in hydrophobic hydration of the surfactant monomer.

Table 5.1 Effect of Headgroup, ΔG_m(head), and Methylene Groups, $\Delta G_m(CH_2)$, on Micellisation of some Carbohydrate-derived Surfactants.[a]

Compound	ΔG_m[a](head) kJ mol^{-1}	ΔG_m[a](CH_2), kJ mol^{-1}
β-alkyl glucopyranosides[b]	+ 9.1 (4.6)	- 4.5 (0.5)
β-alkyl glucopyranosides[c]	+ 5.3 (1.6)	- 3.1 (0.2)
β-alkyl galactopyranosides[d]	+ 7.9 (3.2)	- 2.2 (0.3)
α-alkyl mannopyranosides[d]	+ 3.9 (11)	- 2.7 (1.1)
N-alkyl gluconamides[e,f]	+ 15.1 (5.5)	- 5.1 (0.8)
N-alkanoyl-N-methyl gluconamides[e]	+ 5.5 (2.8)	- 3.0 (0.4)
β-alkyl thioglucopyranosides[g]	+ 2.6 (0.4)	- 3.0 (0.1)
N-alkyl melibionamides[h]	+ 5.0 (1.9)	- 2.9 (0.2)
N-alkyl lactobionamides[h]	+ 4.4 (1.1)	- 2.9 (0.1)
N-alkyl maltobionamide[h]	+ 2.6 (1.5)	- 2.8 (0.1)

a) Using ΔG_m= (2-p/N) RT(lncmc), cmc in mole fraction, p/N = 1 for a nonionic surfactant, variation is given in parentheses. b) Ref. 27, T=293 K. c) Ref. 25, T=298 K. d) Ref. 27, T=333 K. e) Ref. 48, T= RT(298 K). f) The nonyl derivative, was studied at 363 K. g) Ref. 36, T=298 K. h) Ref. 41, T=296 K.

5.3 Starting point of the investigation

In the literature[27] it has been reported that most carbohydrate-derived surfactants with a monosaccharide headgroup have high Krafft temperatures (low solubility in water at moderate temperatures). However, the small headgroups exert more influence on the aggregation behaviour than, for example, disaccharides.[26] Previously Böcker and Thiem[27] found no influence of the stereochemistry of the carbohydrate moiety upon aggregation

behaviour for di- and trisaccharide headgroups, Williams[41] *et al.* and Rinia and Van Doren[42] found that there is no influence of the hydroxy topology of a carbohydrate upon the aggregation behaviour when the carbohydrate is replaced by an open chain polyol. Furthermore it is noted that a change in the alkyl chain length for carbohydrate-derived surfactants with a great number of carbon atoms, might change the type of aggregate[40] and therefore cause nonlinearity in a Shinoda plot[50] (plot of cmc versus chain length, from which methylene and headgroup contributions can be calculated). If there is any difference in headgroup contribution for the different carbohydrate-derived surfactants, which contain monosaccharide headgroups (Table 5.1) it is often overshadowed by the error in the calculated contribution for the headgroups.

Table 5.2 Gibbs Energies of Micellisation for Octyl Glycopyranosides

Compound	ΔG_m[a], kJ mol^{-1}	T, K	Reference
n-octyl α-D-glucopyranoside	-22.7	315	32
n-octyl β-D-glucopyranoside	-20.6	315	32
n-octyl β-D-glucopyranoside	-19.1	298	25
n-octyl β-D-glucopyranoside	-19.7	293	27
n-octyl β-D-galactopyranoside	-25.4	333	27
n-octyl α-D-mannopyranoside	-24.3	333	27
n-octyl β-D-gluconamide	-21.4	298	48
N-methyl n-octyl β-D-gluconamide	-18.6	298	48

a) ΔG_m = RTlncmc, cmc in mole fraction (See Table 5.1).

When the ΔG_m values, as in Table 5.2, of some carbohydrate-derived surfactants with an octyl chain are examined it is clear that the Gibbs energies of micellisation are different for different headgroups. It is not certain whether this is caused by the fact that they have been measured at different temperatures. However, Straathof and Van Bekkum[32] measured a difference in Gibbs energy of micellisation ($\delta\Delta G_m$ = 2.1 kJ mol^{-1}) between octyl α- and β-D-glucopyranoside at the same temperature. This prompted the idea that the micellisation is influenced by the stereochemistry of the carbohydrate headgroup.

This is supported by the finding that a significant difference in phase behaviour is found for galactose- and glucose-derived 1,2-O-dialkyl-3-O-β-D-glycosyl-*sn*-glycerols.[53,54] A smaller difference in behaviour was observed between the glucose and mannose derivatives of these compounds.

Previously it was found that the synthesis of anomerically pure alkyl

1-thioglucopyranosides was quite feasible.[37] Their potential use for selectively affecting cell surfaces and for use as enzymatic substrates has already been noted.[55] In addition, they can be applied in membrane protein reconstitution.[35]

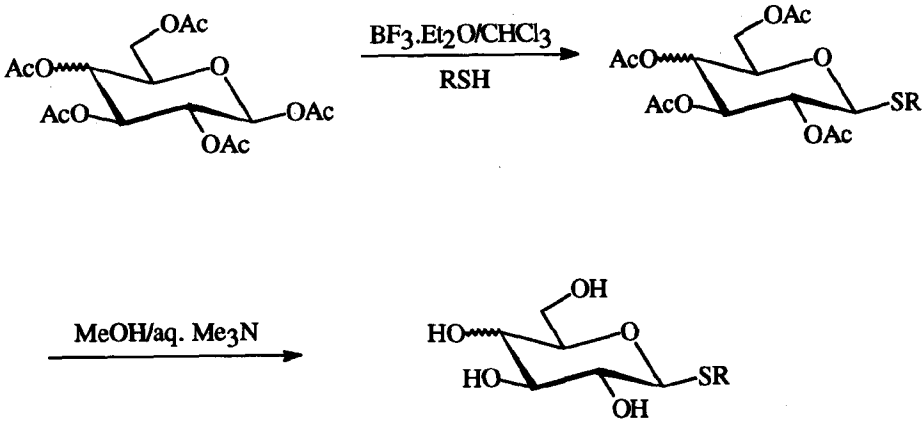
The decision was made to synthesise and study the aggregation behaviour of a set of alkyl 1-thioglycosides with monosaccharide headgroups selected from all groups of the differently hydrated hexoses (chapters 2 and 3). The aldohexoses D-talose, D-glucose, D-mannose and D-galactose were chosen as carbohydrate headgroups and the corresponding n-hexyl-, n-heptyl- and n-octyl 1-thioglycopyranosides were prepared. The relatively short chain lengths were chosen to avoid complications due to high Krafft temperatures and to avoid possible changes in aggregate morphology upon chain elongation.

The aim of this project was to determine the dependence of the aggregation behaviour of these carbohydrate-derived surfactants upon the hydroxy topology of the carbohydrate headgroup under similar circumstances for all compounds.

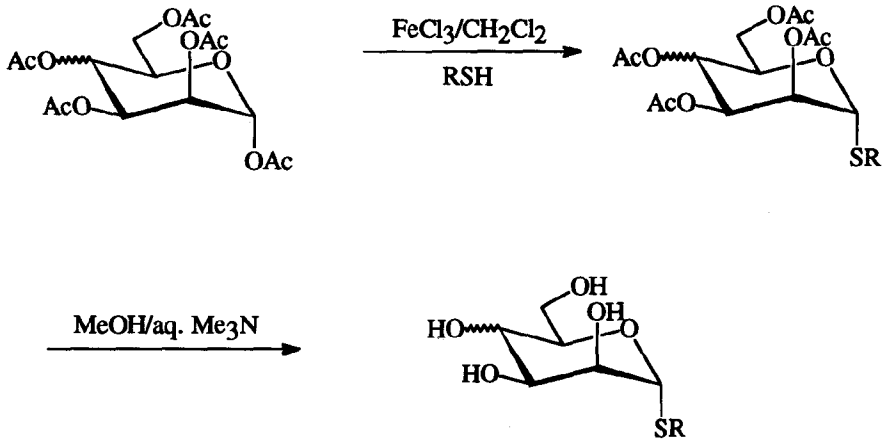
5.4.1 Synthesis of carbohydrate-derived surfactants, n-alkyl 1-thioglycopyranosides

Both n-alkyl 1-thio-β-D-glucopyranosides **10g-i** and n-alkyl 1-thio-β-D-galacto pyranosides **11f-i** can be synthesised from the respective peracetylated carbohydrates and the appropriate n-alkanethiol by using the $BF_3.Et_2O$ method.[37] This route has the advantage over a Königs-Knorr type of route[29,34] (which is often used for the synthesis of alkyl 1-thioglycosides and alkyl glycosides), that it is shorter. The $BF_3.Et_2O$ method can result in both the alpha- and the beta products. For D-glucose derivatives the beta product is the kinetically controlled product, whereas the alpha product is the thermodynamically controlled product. For D-galactose the beta anomer is also the kinetically determined product. The reaction proceeds through an intermediate in which the acetyl group on C-2 provides anchimeric assistance for the attack of the thiol on the anomeric centre.

In case of both the D-*mannose* (**12g-i**) and D-talose (**13h,i**) derivatives the acetyl group on the C-2 position is axial and therefore too far away from the anomeric centre to have a neighbouring group effect. Therefore the $BF_3.Et_2O$ method cannot be used. An attempt was made to synthesise the n-alkyl 1-thiomannopyranosides by an S_N2 substitution of an n-alkanethiol on α-1-chloro-2,3,4,6-tetra-O-acetyl-D-mannopyranoside[56] under phase-transfer conditions.[57] However, this proved to be unsuccessful. But with a stronger Lewis-acid as a catalyst (compared to $BF_3.Et_2O$), in this case $FeCl_3$,[58] the coupling of the n-alkanethiols with the D-mannose and D-talose pentaacetates was successful. The anomeric centre is not shielded by the presence of a neighbouring acetate group, hence the product is always a mixture of anomers.



D-glucose: OAc(4),OH(4) = eq
D-galactose: OAc(4),OH(4) = ax



D-mannose: OAc(4),OH(4) = eq
D-talose: OAc(4),OH(4) = ax

Scheme 5.1 Reaction scheme for the synthesis of n-alkyl 1-thioglycopyranosides

The arabinose derivative **14c** could be synthesised following the same route as that for the glucose derivatives. The ring conformation of a pentose headgroup can change under influence of the alkyl chain. This was established by studying the coupling between the protons of the carbohydrate moiety in the NMR spectrum. The results ($J_{H1,H2}$=4.03 Hz, $J_{H2,H3}$=7.8 Hz) indicated that the ring resides in the $^4C_1$ form and that the n-alkanethiol is at the alpha position on the anomeric centre.

Earlier attempts to couple a secondary thiol under similar circumstances (2-heptanethiol, synthesised by Klaas Hovius according to a literature procedure[60]) with the peracetylated arabinose **14a** were unsuccessful. The longer reaction time which is needed for the secondary alkanethiol, allows for the formation of many different arabinose isomers.

Deprotection has been carried out with a mixture of methanol and a 45% solution of trimethylamine in water. Trimethylamine was chosen instead of triethylamine[59] because evaporation of the former solvent mixture is easier. The reaction scheme for the synthesis of the aldohexose derivatives is depicted in Scheme 5.1.

5.4.2 Purification of the compounds

The most difficult aspect of the synthesis of carbohydrate-derived surfactants is the purification, especially when the compound has to be in an anomerically pure state. At the start of this project there was no standard method available to separate one anomer of a carbohydrate-derived surfactant from the other on a preparative scale. Therefore a special paragraph is devoted to this aspect of the synthesis.

Both the synthesis of the glucose- and galactose derivatives can be anomer specific, especially when longer chain lengths are introduced at the anomeric centre. However, it is almost inevitable that trace amounts of the unwanted anomer will be present in the product mixture (glucose derivatives 2-3 %; galactose derivatives approx. 5%). Both mannose and talose derivatives can only be synthesised as an anomer mixture of α:β = 7:3. For these compounds it was crucial to find a suitable purification method.

The first opportunity to purify the compound is after the coupling reaction, when the 2,3,4 and 6-hydroxy groups in the compound are still protected. By applying chromatography on silica gel with the eluent n-hexane-ethyl acetate,[37] the product mixture can be purified or enriched in one of its anomers and excess thiol can be removed conveniently. For every carbohydrate derivative the same eluent was used for chromatography. Because this mixture of solvents functioned satisfactorily no other n-hexane-ethyl acetate mixtures were tested.

At this point, the purity of a sample was checked qualitatively by NMR spectroscopy:

besides the obvious difference between the anomeric protons in the ^{1}H-NMR spectrum, the α-methylene group resonance is very symmetrical if the product is anomerically pure (the shape of the signal can be very different depending on the sugar attached to the alkanethiol). In addition, the methyl groups of the protecting acetyl groups will only be shown as four singlets in the ^{1}H-NMR. The signals double when two anomers are present in the product. The ^{13}C-NMR spectrum will also show whether there is only one anomer present in the product; a mixture of two anomers has twice as many CH signals for the sugar component.

The second chance for purification is after deprotection. If trace amounts of the unwanted anomer are still present in the mixture, they can also be removed by a chromatographic procedure. However, the circumstances and column material depend crucially on the hydroxy topology of the sugar headgroup.

Alkyl 1-thioglucopyranosides. **10g-i** The α- and β-anomers can be separated on silica gel using methylene chloride to which five percent of methanol has been added as an eluent. This method was described previously by Saito.[34]

All other columns discussed in this paragraph (*vide infra*) were applied unsuccessfully.

Alkyl 1-thiogalactopyranosides. **11f-i** The compounds can be purified on a Dowex column of anion exchange material in the OH^- form. Neither a cation exchange column in the calcium form nor a Sephadex column could be used for purification. Previously, anion exchange columns were used for purification, but only little information was given.[61] Sephadex columns have previously been used in the carbohydrate field to separate partially acetylated dextrans,[62] among others.

Alkyl 1-thiomannopyranosides **12g-i** can be purified both on a Dowex cation exchange column in the calcium form (however not in the H^+ form) or on a Sephadex column. The sequence of products was reversed going from one column to the other. The anion exchange column was not applied.

Alkyl 1-thiotalopyranosides. **13h,i** The talose derivatives can be readily purified on a Dowex column of anion exchange material in the OH^- form. Theoretically both the calcium ion column and the Sephadex column should be applicable for the purification of the talose derivatives. However, they were not tested.

The anomeric ratio of the deprotected compounds was studied by ^{1}H-NMR. The signals of the CH groups on the anomeric centre (Table 5.3) were enlarged and integrated. The ratio of integration was indicative of the anomeric ratio. The NMR spectra were taken in CD_3OD. Since there is always water present in this solvent, the anomeric protons are found in the same region of the spectrum as the water resonance. The spectra should be recorded at very low concentrations of carbohydrate-derived surfactant because the

Table 5.3 Chemical Shift of the Anomeric Proton in Alkyl 1-Thioglycosides in CD_3OD at 298 K.

	Carbohydrate derivative[a]	-C1H
10g-iα	n-alkyl 1-thio-α-D-glucopyranoside	5.30
10g-iβ	n-alkyl 1-thio-β-D-glucopyranoside	4.34
11f-iα	n-alkyl 1-thio-α-D-galactopyranoside	5.37
11f-iβ	n-alkyl 1-thio-β-D-galactopyranoside	4.30
12g-iα	n-alkyl 1-thio-α-D-mannopyranoside	5.22
12g-iβ	n-alkyl 1-thio-β-D-mannopyranoside	4.71
13h,iα	n-alkyl 1-thio-α-D-talopyranoside	5.30
13h,iβ	n-alkyl 1-thio-β-D-talopyranoside	4.63

a) For α-glucose, C1H is beta.

introduction of a high concentration of surfactant together with the water present can somehow induce the formation of aggregates, as evidenced in the NMR spectrum by the presence of broad, additional peaks.

So far ion exchange materials were mostly used for actual ion exchange or as mild catalysts in reactions.[63,64] However, ion exchange columns have now also been used successfully to separate different anomers of carbohydrate-derived surfactants. In the next paragraph an attempt is made to rationalise the mechanism of interactions on column materials.

In the case of the ion exchange column in the calcium ion form, one can take advantage of the fact that sugars can interact favourably with calcium ions.[65-67] The strongest calcium ion complex can be formed if there are three adjacent cis-hydroxy groups present in the carbohydrate moiety (an axial-equatorial-axial OH sequence) as is the case for the D-talose derivatives **13h,i**. In addition, carbohydrates with two neighbouring cis-hydroxy groups will be able to form weak complexes with calcium ions. For mannose they are OH(2) and OH(3), for galactose they are OH(3) and OH(4). If their respective anomers possess a different affinity for calcium the mixture can be purified on a calcium ion column. The D-glucose derivatives have an all-trans hydroxy topology when their non-anomeric hydroxy groups are considered and therefore show only a weak interaction with the calcium ion.

It is observed that the mannose derivatives have a different affinity for the calcium ion depending on the relative position of the n-alkanethiol. No difference was observed for the galactose and glucose derivatives. The column was not tested for the talose derivatives. However, it is anticipated that purification of talose derivatives on this column will be successful, because the derivatives possess a moiety which is capable of a strong interaction with Ca^{2+}, and the structural unit responsible for this affinity

is situated near the anomeric centre.

The observation for the galactose-derivatives was somewhat unexpected, particularly because the mannose-derivatives could be purified successfully on a calcium ion column. Most probably the difference in affinity for the calcium ion in case of the mannose-derivatives must be caused by the fact that the alkyl chain can hinder the weak complex formation, when the alkyl chain is in cis-position with respect to the hydroxy plane for complexation. When it is trans to the hydroxy plane, the complex formation is facilitated. Indeed for n-alkyl 1-thio-β-D-mannopyranosides the retention time is shorter on the column than that for the α-mannose derivatives. This is consistent with the suggested mechanism of complexation. Perhaps the alkyl chain of the galactose derivatives cannot hinder the complexation as effectively, because it is farther away from the hydroxy groups, involved in the interaction with calcium ions.

Both other types of columns separate on the basis of differences in hydrophobicity of the compounds. There will only be a difference in hydrophobicity between two anomers if the carbohydrate moiety interacts with the column material and if this affinity is influenced by the relative position (α or β) of the alkyl chain on the sugar headgroup. In this respect the same reasoning can be followed as was used in understanding the Ca^{2+} affinity. There is a hydrophobic moiety in a carbohydrate headgroup if there are several neighbouring cis-methine groups present in the carbohydrate headgroup. The relative position of the alkyl chain can affect the interaction of this moiety with the column material. It was found that talose and galactose derivatives, which have two or more adjacent cis-methine protons, can be successfully purified on these columns. Glucose-derivatives which lack a cis-methine proton arrangement cannot be purified on these types of columns. Mannose derivatives can be successfully purified on a Sephadex column because the hydrophobic moiety on the carbohydrate headgroup is near the alkyl chain.

The general explanation for the column chromatographic results might seem somewhat speculative. However, it has been noted earlier that a carbohydrate molecule possesses a hydrophobic surface (or volume) and a hydrophilic surface (or volume),[73,74] with a size which depends on the detailed hydroxy topology of the carbohydrate. These properties do not seem to give a better insight in the hydration behaviour of carbohydrates. However, in the case of non-aqueous solutions and for explaining differences in affinity for column material, this approach seems applicable. The dependence of calcium ion affinity on stereochemistry is well known.[65-67]

Previously, ion exchange columns were used in carbohydrate chemistry to separate different sugars,[65-72] or as catalysts in the synthesis of carbohydrate-derived

When the aggregation behaviour of the n-heptyl 1-thioglycopyranosides was monitored, it was found that the galactose derivative (**11h**; cmc = 42.0 mM) has a different cmc compared to the glucose derivative (**10h**; cmc = 32.8 mM). This could be predicted in view of the results described in chapters 2 and 3, since glucose and galactose fit differently into the three-dimensional hydrogen-bond network of water. However, in terms of Gibbs energy, enthalpy and entropy of micellisation, the difference in aggregation behaviour is rather insignificant. This is mostly due to the fact that for the calculation of the Gibbs energies of micellisation, the cmc has to be expressed in mole fraction. The cmc's differ very little in terms of mole fractions. The enthalpy of micellisation is directly obtained from the calorimetric experiment and is the same for both compounds. This shows how subtle the influence of stereochemistry is on the aggregation behaviour of carbohydrate-derived surfactants. It also shows that the differences found in Table 5.2 might be induced either by the fact that the measurements are performed at different temperatures, or that differences in stereospecific hydration become too small at higher temperatures.

On average the enthalpy of micellisation (ΔH_m) for both compounds is -4.2 kJ mol^{-1} at 333 K. An average value of +14 kJ mol^{-1} was observed for ethylene oxide derivatives[78,79], and Brackman *et al.*[77] measured an enthalpy of micellisation for n-octyl β-D-thioglucopyranoside of +4.5 kJ mol^{-1}. However, all the measurements which were reported in literature were performed at 298 K.

When entropies of micellisation (ΔS_m) for a selection of ionic surfactants are compared to those for n-heptyl 1-thioglycopyranosides, (for dodecyl pyridinium iodides[7] $T\Delta S_m$ = 13.0 kJ mol^{-1}, sodium dodecyl sulfate[80] has a $T\Delta S_m$ of 3.25 kJ mol^{-1}, sodium tetradecyl sulfate:[80] $T\Delta S_m$ = 17.1 kJ mol^{-1}) it becomes apparent that although the chain lengths of the ionic surfactants are longer than those of the n-heptyl 1-thioglycopyranosides, the entropy gain upon micellisation is sometimes greater for the carbohydrate-derived surfactants than for the ionic surfactants (on average $T\Delta S_m$ = 16.1 kJ mol^{-1}, Table 5.4). Only alkylbenzenesulfonates[51] and sodium tetradecyl sulfate[80] have entropies of micellisation comparable to those for the n-heptyl 1-thioglycopyranosides, but they possess longer chain lengths. This stresses the importance of the influence of the headgroup on the hydrophobic hydration of the alkyl chain in the monomeric state.

The aggregation behaviour of the other carbohydrate-derived surfactants whose synthesis is described in this chapter, will be published elsewhere.[81]

5.5.2 n-Alkyl 1-thioglycopyranosides, Krafft temperatures[82-88]

Solubilities of surfactants in aqueous solutions, have been discussed extensively for ionic surfactants.[82-88] The solubility of a surfactant in water and its aggregation behaviour are, according to many researchers, connected. Presently at least three definitions of the Krafft temperatures are being used:

1) The Krafft temperature is the temperature at which the solubility versus temperature plot intersects with the cmc versus temperature plot.
2) The Krafft temperature is the temperature at which the solubility of the surfactant equals twice the cmc.
3) The Krafft temperature is the melting point of the hydrated surfactant crystals.[85]

In the case of the carbohydrate-derived surfactants it was found that the cmc curve versus temperature in some cases does not intersect with the solubility curve versus temperature.[89] Hence the first definition does not apply to carbohydrate-derived surfactants. As the solubility increases dramatically upon heating, it was found that the best way to measure the Krafft temperatures is to monitor the heat effect when a sample of a carbohydrate-derived surfactant is heated in the presence of water in a differential scanning calorimeter. At the temperature at which the surfactant dissolves, a heat effect is observed. This temperature is then called the Krafft temperature. The results were reproduced with the conventional methods for both carbohydrate-derived surfactants as well as for some ionic surfactants.[90,91]

The method is straightforward and requires only small amounts of compound. Effectively, the sudden increase of the solubility of the hydrated monomers in water is measured. Hence Shinoda's definition of Krafft temperature[85] (definition 3) is most applicable to carbohydrate-derived surfactants in an adjusted form. The Krafft temperature is the melting point of the (hydrated) surfactant crystal fully immersed in excess solvent.

The Krafft temperatures of some n-alkyl 1-thioglycopyranosides are listed in Table 5.5. Interestingly the carbohydrate-derived surfactants which contain a monosaccharide headgroup with an axial OH(4) **11f-i** and **13h,i** and **14c** have high Krafft temperatures. Apparently an axial OH(4) is favourable for a good crystal packing. In the literature, it has already been observed that for glucose derivatives the two anomers have different Krafft temperatures. The β-glucose derivatives are readily soluble in water in case of short chain lengths. The α-glucose derivatives, however, need a higher temperature to dissolve.[32] The same effect is observed for the mannose derivatives, but in a more extreme form. The α-mannose derivatives are soluble at low concentrations and forms a cubic phase at very high concentrations. The β-mannose derivatives have an extremely high Krafft temperature, which is remarkable since they have only a short

chain length. Above the Krafft temperature a cubic phase will be formed before dissolution in water (Table 5.5).

Table 5.5 Krafft Temperatures (Tk) for Alkyl 1-Thioglycopyranosides.

Compound	Tk, °C	Compound	Tk, °C
n-hexyl β-1-thioglucoside **10g**	<25	n-hexyl α-1-thiomannoside **12gα**	< 25[e]
n-heptyl β-1-thioglucoside **10h**	<25	n-heptyl α-1-thiomannoside **12hα**	< 25[e]
n-octyl β-1-thioglucoside **10i**	<25	n-octyl α-1-thiomannoside **12iα**	< 25[e]
n-pentyl β-1-thiogalactoside **11f**	27[a]	n-hexyl β-1-thiomannoside **12gβ**	75[f]
n-hexyl β-1-thiogalactoside **11g**	36[b]	n-octyl β-1-thiomannoside **12iβ**	>100
n-heptyl β-1-thiogalactoside **11h**	48[c]	n-heptyl α-1-thio-taloside **13h**	56[f,g]
n-octyl β-1-thiogalactoside **11i**	51[d]	n-octyl α-1-thio-taloside **13i**	56[f,h]
3,7-dimethyl octyl α-1-thio L-arabinoside **14c**	>100		

a) ΔH_{diss} (enthalpy of dissolution)=6.4 kJ mol^{-1}. b) ΔH_{diss}=12 kJ mol^{-1}. c) ΔH_{diss}=25 kJ mol^{-1}. d) ΔH_{diss}=38 kJ mol^{-1}. e) The α-mannose derivatives are first hydrated at room temperature and form a cubic phase. At low concentrations, they dissolve in water above 30 °C. f) First forms a cubic phase, then dissolves at higher temperatures. g) ΔH_{diss}=32 kJ mol^{-1}. h) ΔH_{diss}=38 kJ mol^{-1}.

Furthermore, the Krafft temperature increases upon chain elongation, but not by a constant increment. Because derivatives with an odd number of carbon atoms in their alkyl chain have a better crystal packing than the derivatives with an even number of carbon atoms, there is an odd-even effect in the Krafft temperatures. This was observed previously for α-glucose derivatives.[90]

5.5.3 n-Alkyl 1-thioglycopyranosides, liquid crystalline behaviour

If the melting points of the carbohydrate-derived surfactants would have been taken one hundred years ago, chemists would have been convinced that the compounds were impure because they have a long melting trajectory. E. Fischer[75] was the first to observe that long-chain alkyl glycosides have a double melting point. This is caused by the fact that carbohydrate-derived surfactants are amphiphilic and have the propensity to exhibit liquid crystalline behaviour. They show lyotropic as well as thermotropic liquid crystalline behaviour. This means that they can form liquid crystals both upon addition of a small amount of solvent (mostly water) and upon heating.

It was not until the early nineteen eighties[94] that the liquid crystalline behaviour of carbohydrate-derived surfactants was studied extensively.[91-94] It was observed that alkyl glycosides usually form a smectic A phase upon heating.[27,37-39,48,49,61] The

carbohydrate-derived surfactants are rodlike molecules. In a smectic A phase they are ordered in a layered structure with alternating polar and apolar regions.

The first model used to explain the liquid crystalline behaviour of carbohydrate derivatives, originated from the X-ray analyses by Jeffrey.[94] It was found that in a crystal the carbohydrate mesogens are packed in bimolecular layers with the sugar moieties arranged head-to-head and with fully interdigitised alkyl chains. The molecules consist of two very distinct parts; carbohydrates form relatively hard crystals with high melting points because of their hydrogen bonding ability; hydrocarbons form soft crystals, which are only held together by van der Waals interactions. When the crystals are heated, supposedly a two stage melting process ensues: alkyl chains start to melt while the carbohydrate sheets remain intact. Hence the whole system is in a state between liquid and crystal: the liquid crystalline state. Further heating breaks down the hydrogen bonds between the sugars and an isotropic liquid is formed at the clearing point.

Table 5.6 Melting Points (T_m), Clearing Points (T_c) and Heats of Melting (ΔH_{melt}) and Clearing (ΔH_{clear}) for n-Alkyl 1-Thioglycopyranosides.

Compound	T_m °C	T_c °C	ΔH_{melt} kJ mol^{-1}	ΔH_{clear} kJ mol^{-1}
n-heptyl 1-thio-α-D-glucopyranoside[a]	97.2	137.9	34.6	2.2
n-octyl 1-thio-α-D-glucopyranoside[a]	78.8	153.5	26.5	2.2
n-pentyl 1-thio-β-D-galactopyranoside **11f**	109.6[b]	-	28.7	-
n-hexyl 1-thio-β-D-galactopyranoside **11g**	87.1	90.9	23.8	0.9
n-heptyl 1-thio-β-D-galactopyranoside **11h**	62.4	112.0	40.9	1.0
n-octyl 1-thio-β-D-galactopyranoside **11i**	91.7[c]	140.5	26.4	2.2
n-heptyl 1-thio-α-D-mannopyranoside **12hα**[d]	62.1[e]	152.4	25.2	2.2
n-hexyl 1-thio-β-D-mannopyranoside **12gβ**	115.3	125.4	40.1	1.8
n-octyl 1-thio-β-D-mannopyranoside **12iβ**	118.9	156.1	50.5	2.3
n-heptyl 1-thio-α-D-talopyranoside **13h**	90.3	(85.6)[f]	34.5	(1.2)
n-octyl 1-thio-α-D-talopyranoside **13i**	91.7	105.7	40.7	1.6
3,7-dimethyloctyl 1-thio-α-L-arabinoside **14c**	149.8	>170	44.0	

a) Ref. 37. b) Crystal transition at 73.2 °C, ΔH_{trans}=2.3 kJ mol^{-1}, no liquid crystalline behaviour. c) The crystal transition was observed at 60.5 °C, the heat of transition was 6.3 kJ mol^{-1}. d) The crystal transition was observed at 43.9 °C, with a heat of transition of 3.6 kJ mol^{-1} e) Ref. 93: T_{melt}=60, T_{clear}=151 °C. f) The phase transition is monotropic: liquid crystalline behaviour is only observed upon cooling.

However at present this model has been adjusted, due to the observation that clearing temperatures depend on the alkyl chain length.[59,91,92] It is assumed that at the

melting point the hydrogen bonded network of carbohydrates starts to change. Overall there is more mobility in the liquid crystalline phase than in the crystalline phase. The amphiphilic character of the derivatives provides the driving force for the formation of structured liquid crystalline aggregates, which are disrupted by going to even higher temperatures.

The melting points, clearing points and heats of melting and clearing for the n-alkyl 1-thioglycopyranosides are summarised in Table 5.6. Generally the melting points do not change regularly upon chain elongation, whereas the clearing points are higher for longer chain lengths. The heats of melting depend on both the headgroup topology and chain length. The heats of clearing do not show this dependence and are much smaller, which is in support of a smectic phase with liquid layers. The derivatives with a high Krafft temperature (Table 5.5) have very large enthalpies of melting (Table 5.6). This suggests that the crystal packing is the main factor determining the solubility of a carbohydrate-derived surfactant in water. Nearly all the galactose derivatives show a crystal transition before melting, which signifies a rearrangement of the packing of the alkyl chains.[59]

The liquid crystalline behaviour of n-alkyl 1-thio-α-D-glucopyranosides has been described and characterised by Van Doren.[37] The overall trend observed in the thermotropic behaviour of the compounds studied here seems to be in good agreement with the derivatives with a glucose headgroup. The n-alkyl 1-thioglycopyranosides form a smectic A phase upon heating similar to the alkyl glycosides.

With respect to the lyotropic liquid crystalline behaviour, only the n-alkyl 1-thioglycopyranosides, which have Krafft temperatures below 100 °C, have been studied. Contact preparations with water on a microscopic slide reveal the formation of the usual array of lyotropic mesophases, i.e. hexagonal, cubic and lamellar.[96] This predicts that they will form micelles in aqueous solution. n-Hexyl 1-thio-β-D-mannopyranoside (**12gβ**), n-heptyl 1-thio-α-D-mannopyranoside (**12hα**), and the n-alkyl 1-thio-α-D-talopyranosides (**13h,13i**) are an exception since they only form cubic phases upon contact with water. This suggests that the latter will form a different type of aggregate. A possible reason might be that the effective headgroup size of mannose and talose is smaller than that of the glucose and galactose headgroups. The relative position of OH(2) in the ring seems to be of importance in this respect.

5.6 Conclusions

The stereochemistry of the carbohydrate headgroup is a dominant factor in selecting both the synthesis and purification of carbohydrate-derived surfactants.

Some aspects of the aggregation behaviour of carbohydrate-derived surfactants do not seem to be governed by the rules previously established for ionic surfactants by Israelachvili.[9,10] For example, carbohydrate-derived surfactants with long alkyl chains have the propensity to form types of aggregates, which are different from (spherical) micelles. This is predicted on the basis of their lyotropic phase behaviour. In the literature this has been observed for some long-chain carbohydrate-derived surfactants.[40] We have observed this phenomenon for the mannose- and talose derivatives even with relatively short alkyl chains.

In the literature differences are found in aggregation behaviour upon changing the headgroup topology. Here it is found that the cmc's of glucose and galactose derivatives are, as expected, different. The differences in hydration properties of carbohydrate-derived surfactants are, however, so small that they cannot be monitored accurately by Gibbs energies, enthalpies or entropies of micellisation.

Experiments are under way[97] to study the aggregation behaviour of the carbohydrate-derived surfactants with parameters which are more prone to be sensitive to these small changes in hydration. The approach described in chapter 3 will be followed.

In addition, the Krafft temperatures of carbohydrate-derived surfactants seem to be determined predominantly by the stability of the crystalline phase. The most striking observation is that the Krafft temperatures appear to depend on the position of OH(4) in the carbohydrate moiety. Derivatives with an axial OH(4) have high Krafft temperatures. If OH(4) is equatorial only one of the anomers has a high Krafft temperature. The Krafft temperatures become higher upon chain elongation. However, there is an odd-even effect, because derivatives with an odd number of carbon atoms in the alkyl chain have a more stable crystal packing.

5.7 Experimental

5.7.1 Materials

Materials All the peracetylated hexoses (β-D-glucose pentaacetate **10a**, β-D-galactose pentaacetate **11a**, α-D-mannose pentaacetate **12a**, all 99+ %) were purchased from Sigma. D-talose was purchased from Sigma and L-arabinose was purchased from Janssen Chimica. Both hexane- and octanethiol (purity >98%) were purchased from Janssen Chimica, while heptanethiol (purity >97%) was purchased from Fluka AG. The Lewis-acids were obtained from Janssen Chimica (48% $BF_3.Et_2O$ in ether) and Merck ($FeCl_3$). All solvents were distilled before use. The 45% aqueous trimethylamine solution was purchased from Merck.

Column materials. Column material used for purification of the surfactants was Sephadex LH-20-100 (lipophilic Sephadex) purchased from Farmacia. Dowex 50x4-400 ion exchange resin (H^+ form) was purchased from Janssen Chimica. The anion exchange material was Dowex 1x2 strong anion exchange material (200/400 mesh) with quaternary ammonium groups. It was purchased in the chloride ion form from Fluka AG.
The cation exchange material in the H^+ form was changed into the calcium ion form by elution of the column with a saturated $CaCl_2$ solution. Thereafter the column was rinsed with demineralised water, until calcium ions were no longer present in the eluent (checked with an aqueous ammonium oxalate solution).
The anion exchange material was changed from the chloride ion form into the hydroxide form by elution with a 1 molar NaOH solution and afterwards rinsing with demineralised water to remove excess hydroxide ions. The presence of hydroxide ions in the solutions was checked with pH paper.

5.7.2 Syntheses

α-D-talose pentaacetate **13a**

α-D-talose pentaacetate **13a** was synthesised according to the procedure described previously.[98,99] Typically 1 g of D-talose was dissolved in a mixture of 7 ml of pyridine and 4.6 ml of acetic anhydride, and then it was stirred in a cold room for three days. The kinetically most stable product **13a** was formed and could be retrieved from the reaction mixture by pouring it into an ice-water mixture. After stirring the mixture for some time the product, **13a**, crystallised. The yield could be improved if the aqueous mixture was also extracted with methylene chloride. Subsequently, the organic layer was extracted with bicarbonate solution. Product **13a** was crystallised from ethanol. The yield was 65 %.
^{1}H-NMR ($CDCl_3$): δ 1.99 (s,3H); δ 2.02 (s,3H); δ 2.04 (s,3H); δ 2.12 (s,3H); δ 2.13 (s,3H); δ 4.17 (m,2H); δ 4.3 (m,1H); δ 5.09 (d,1H); δ 5.3 (s,1H); δ 5.35 (s,1H); δ 6.13 (d,1H) ppm. ^{13}C-NMR ($CDCl_3$): δ 20.39 (CH_3); δ 20.48 (CH_3); δ 20.60 (CH_3); δ 20.71 (CH_3); δ 61.25 (CH_2); δ 64.96 (CH); δ 65.08 (CH); δ 66.08 (CH); δ 68.58 (CH); δ 91.21 (CH); δ 167.83 (CO); δ 169.37 (CO); δ 169.44 (CO); δ 169.86 (CO); δ 170.15 (CO) ppm.

α-L-arabinose tetraacetate **14a**

α-L-arabinose tetraacetate **14a** was synthesised according to the method described by Evelyn et al.,[100] which was preferred over the method of Dasgupta,[101] because the former yielded a cleaner mixture (with respect to the number of conformational isomers). Typically a mixture of 10 g L-arabinose with 4 g of sodium acetate and 80 ml of acetic anhydride was stirred and heated to 50 °C for ten days. The reaction was complete when the mixture was almost clear (reference 100 claims the solution becomes completely clear, which was never observed in our synthesis). After adding water (800 ml) and extracting the mixture with chloroform (4 times 50 ml) and extraction of the organic layer with bicarbonate solution, the product was obtained by drying the organic layer on sodium sulfate and subsequent evaporation of the solvent. When less water was used for the extraction, it was difficult to remove the excess of acetic acid. Product **14a** was obtained in a yield of 41 % after crystallisation from 96 % ethanol.
^{1}H-NMR ($CDCl_3$): δ 2.00 (s,3H); δ 2.03 (s,3H); δ 2.08 (s,3H); δ 2.10 (s,3H); δ 3.71-3.77 (dd,1H); δ 3.97-4.03 (dd,1H); δ 5.05-5.10 (dd,1H); δ 5.22-5.27 (m,2H); δ 5.61-5.64 (d,1H) ppm. ^{13}C-NMR ($CDCl_3$): δ 20.41 (CH_3); δ 20.45 (CH_3); δ 20.59 (CH_3); δ 20.65 (CH_3); δ 63.64 (CH_2); δ 66.94 (CH); δ 67.85 (CH); δ 69.65 (CH); δ 91.89 (CH); δ 168.84 (CO); δ 169.12 (CO); δ 169.68 (CO); δ 169.90 (CO) ppm.

n-Alkyl 1-thio-2,3,4,6-O-acetyl-β-D-glucopyranosides **10c-10e**

n-Alkyl 1-thio-2,3,4,6-O-acetyl-β-D-glucopyranosides **10c-10e** were synthesised via the method described by Van Doren.[37] In order to obtain the beta adduct the reaction was

quenched with a saturated bicarbonate solution after 15 minutes. Longer reaction times already yielded considerable amounts of the alpha adduct. The alkyl chain length was varied from six to eight carbon atoms. The product could be purified by crystallisation from n-hexane. The average yield was 70% after crystallisation.
If a small amount of the alpha adduct was present in the mixture, this could not be removed by crystallisation. However, chromatography over silica gel with a solvent mixture of n-hexane and ethyl acetate (7:3) resulted in a product mixture which was enriched in, if not purified completely to the beta adduct. Under these circumstances the alpha adduct was collected before the beta adduct.

n-Hexyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside **10c**
^{1}H-NMR ($CDCl_3$): δ 0.84-0.88 (t,3H); δ 1.23-1.37 (m, 6H); δ 1.51-1.60 (m,2H); δ 1.98 (s,3H); δ 2.00 (s,3H); δ 2.03 (s,3H); δ 2.05 (s,3H); δ 2.59-2.68 (m,2H); δ 3.65-3.71 (m,1H); δ 4.08-4.13 (dd,1H); δ 4.19-4.25 (dd,1H); δ 4.44-4.47 (d,1H); δ 4.97-5.09 (dt,2H); δ 5.16-5.23 (t,1H) ppm. ^{13}C-NMR ($CDCl_3$): δ 11.9 (CH_3); δ 20.25 (CH_3); δ 20.30 (CH_3); δ 22.1 (CH_3); δ 28.1 (CH_2); δ 29.2 (CH_2); δ 29.3 (CH_2); δ 31.1 (CH_2); δ 62.0 (CH_2); δ 68.1 (CH); δ 68.8 (CH); δ 73.8 (CH); δ 75.7 (CH); δ 83.4 (CH); δ 169.1 (CO); δ 169.2 (CO); δ 169.9 (CO); δ 170.1 (CO) ppm.

n-Heptyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside **10d**
^{1}H-NMR ($CDCl_3$): δ 0.83-0.88 (t,3H); δ 1.23-1.36 (m,8H); δ 1.54-1.60 (m,2H); δ 1.98 (s,3H); δ 2.00 (s,3H); δ 2.03 (s,3H); δ 2.05 (s,3H); δ 2.59-2.68 (m,2H); δ 3.65-3.71 (m,1H); δ 4.08-4.13 (dd,1H); δ 4.19-4.25 (dd,1H); δ 4.44-4.47 (d,1H); δ 4.97-5.09 (dt,2H); δ 5.16-5.23 (t,1H) ppm. ^{13}C-NMR ($CDCl_3$): δ 13.88 (CH_3); δ 20.42 (CH_3); δ 20.53 (CH_3); δ 22.40 (CH_2); δ 28.54 (CH_2); δ 28.61 (CH_2); δ 29.44 (CH_2); δ 29.82 (CH_2); δ 31.50 (CH_2); δ 62.03 (CH_2); δ 68.20 (CH); δ 69.74 (CH); δ 73.76 (CH); δ 75.71 (CH); δ 83.45 (CH); δ 169.19 (CO); δ 169.24 (CO);δ 170.01 (CO); δ 170.44 (CO) ppm.

n-Octyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside **10e**
^{1}H-NMR ($CDCl_3$): δ 0.83-0.88 (t,3H); δ 1.24-1.40 (m,10H); δ 1.50-1.66 (m,2H); δ 2.00 (s,3H); δ 2.01 (s,3H); δ 2.04 (s,3H); δ 2.06 (s,3H); δ 2.57-2.73 (m,2H); δ 3.67-3.71 (m,1H); δ 4.08-4.13 (dd,1H); δ 4.19-4.25 (dd,1H);δ 4.44-4.47 (d,1H); δ 4.97-5.09 (dt,2H); δ 5.16-5.23 (t,1H) ppm. ^{13}C-NMR ($CDCl_3$): δ 13.95 (CH_3); δ 20.48 (CH_3); δ 20.59 (CH_3); δ 22.49 (CH_2); δ 28.66 (CH_2); δ 28.98 (CH_2); δ 29.04 (CH_2); δ 29.51 (CH_2); δ 29.88 (CH_2); δ 31.66 (CH_2); δ 62.07 (CH_2); δ 68.24 (CH); δ 69.79 (CH); δ 73.81 (CH); δ 75.75 (CH); δ 83.52 (CH); δ 169.25 (CO); δ 169.29 (CO); δ 170.08 (CO); δ 170.50 (CO) ppm.

n-Alkyl 1-thio-β-D-glucopyranosides **10g-10i**
Deprotection was performed analogous to the method of Van Doren *et al.*[37] However, instead of water and triethylamine a 45% aqueous trimethylamine solution was applied. Hereby the evaporation of the solution was facilitated. The aqueous solution of trimethylamine and methanol was used in proportions of 2:8. After 24 hours of stirring of the mixture, the solvent was evaporated and a syrup remained. Purification of the compounds was realised by chromatography employing a silica column with a 5 % solution of methanol in methylene chloride as eluent, as described by Saito.[34] With this method traces of the alpha compound could also be removed. The beta compound migrated faster than the alpha compound under these circumstances. The products had shorter retention times when the methanol content was increased. All products were syrups and hygroscopic. Deprotection was quantitative.

n-Hexyl 1-thio-β-D-glucopyranoside **10g**
^{1}H-NMR (CD_3OD): δ 0.86-0.95 (t,3H); δ 1.29-1.47 (m,9H); δ 1.57-1.68 (m,2H); δ 2.62-2.82 (m,2H); δ 3.14-3.40 (m,4H and CD_3OD); δ 3.56-3.71 (m,1H); δ 3.8-3.9 (dd,1H);

δ 4.31-4.37 (d,1H) ppm. ^{13}C-NMR (CD_3OD): δ 14.41 (CH_3); δ 23.58 (CH_2); δ 29.64 (CH_2); δ 30.85 (CH_2); δ 30.98 (CH_2); δ 32.53 (CH_2); δ 62.84 (CH_2); δ 71.40 (CH); δ 74.29 (CH); δ 79.50 (CH); δ 81.88 (CH); δ 87.08 (CH) ppm.

n-Heptyl 1-thio-β-D-glucopyranoside **10h**
^{1}H-NMR (CD_3OD): δ 0.87-0.92 (t,3H); δ 1.30-1.43 (m,8H); δ 1.58-1.66 (m,2H); δ 2.66-2.77 (m,2H); δ 3.17-3.37 (m,4H + CD_3OD); δ 3.62-3.69 (dd,1H); δ 3.82-3.88 (dd,1H); δ 4.33-4.37 (d,1H) ppm. ^{13}C-NMR (CD_3OD): δ 14.42 (CH_3); δ 23.60 (CH_2); δ 29.91 (CH_2); δ 30.79 (CH_2); δ 32.86 (CH_2); δ 62.82 (CH_2); δ 71.35 (CH); δ 74.25 (CH); δ 79.47 (CH); δ 81.82 (CH); δ 87.01 (CH) ppm.

n-Octyl 1-thio-β-D-glucopyranoside **10i**
^{1}H-NMR (CD_3OD): δ 0.88-0.93 (t,3H); δ 1.24-1.43 (m,10H); δ 1.59-1.66 (m,2H); δ 2.68-2.76 (m,2H); δ 3.17-3.36 (m,4H + CD_3OD); δ 3.63-3.69 (dd,1H); δ 3.83-3.88 (dd,1H); δ 4.33-4.37 (d,1H) ppm. ^{13}C-NMR (CD_3OD): δ 14.43 (CH_3); δ 23.67 (CH_2); δ 29.98 (CH_2); δ 30.27 (CH_2); δ 30.31 (CH_2); δ 30.82 (CH_2); δ 31.02 (CH_2); δ 32.96 (CH_2); δ 62.89 (CH_2); δ 71.45 (CH); δ 74.33 (CH); δ 79.56 (CH); δ 81.92 (CH); δ 87.10 (CH) ppm.

n-Alkyl 1-thio-β-D-galactopyranosides **11f-11i**
The n-alkyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranosides **11b-11e** were synthesised by the same method as the glucose derivatives. No suitable solvent mixture was found for crystallisation of the protected adducts. Hence the product was used as such in the deprotection step. This was realised with the same solvent mixture as used before for the glucose derivatives. Purification of the compounds (removing traces of the α-anomer) was carried out by column chromatography on Dowex 1x2 anion exchange material in the hydroxide form, with methanol as eluent. The α-anomer eluted from the column before the β-anomer did. Other columns such as those of cation exchange material (eluent: water:methanol = 3:7) and Sephadex (eluent: methanol) were applied, but unsuccessful.
All galactose derivatives were white crystalline compounds which could be crystallised from methanol and acetonitrile (the latter is in excess over methanol). The general procedure involved dissolution of the compound in as little methanol as possible, then addition of acetonitrile until the solution almost became turbid. The quantities used depended very much on alkyl chain length and type of carbohydrate headgroup.
Average yield from galactose pentaacetate **11a**: 42%.

n-Pentyl 1-thio-β-D-galactopyranoside **11f**
^{1}H-NMR (CD_3OD): δ 0.89-0.93 (t,3H); δ 1.36-1.41 (m,4H); δ 1.60-1.66 (m,2H); δ 2.62-2.80 (m,2H); δ 3.47-3.55 (m,3H); δ 3.63-3.70 (m,2H); δ 3.89-3.90 (d,1H); δ 4.28-4.31 (d,1H) ppm. ^{13}C-NMR (CD_3OD): δ 14.27 (CH_3); δ 23.27 (CH_2); δ 30.78 (CH_2); δ 30.87 (CH_2); δ 32.15 CH_2); δ 62.61 (CH_2); δ 70.52 (CH); δ 71.52 (CH); δ 76.32 (CH); δ 80.56 (CH); δ 87.73 (CH) ppm.
Elemental analysis: calculated: C: 49.60 %; H: 8.33 %; S: 12.04 %. Found: C: 49.68 %; H: 8.40 %; S: 12.06 %.

n-Hexyl 1-thio-β-D-galactopyranoside **11g**
^{1}H-NMR (CD_3OD): δ 0.89-0.93 (t,3H); δ 1.23-1.42 (m,6H); δ 1.59-1.66 (m, 2H); δ 2.67-2.76 (m,2H); δ 3.45-3.58 (m,3H); δ 3.70-3.73 (m,2H); δ 3.89-3.90 (d,1H); δ 4.29-4.32 (d,1H) ppm. ^{13}C-NMR (CD_3OD): δ 14.36 (CH_3); δ 23.56 (CH_2); δ 29.62 (CH_2); δ 30.85 (CH_2); δ 31.01 (CH_2); δ 32.54 (CH_2); δ 62.51 (CH_2); δ 70.41 (CH); δ 71.41 (CH); δ 72.48 (CH); δ 76.21 (CH); δ 80.47 (CH); δ 87.65 (CH) ppm.
Elemental analysis: calculated: C: 51.41 %; H: 8.63 %; S: 11.44 %. Found: C: 51.29 %; H: 8.74 %; S: 11.25 %.

n Heptyl 1-thio-β-D-galactopyranoside **11h**
^{1}H-NMR (CD_3OD): δ 0.92-0.96 (t,3H); δ 1.27-1.46 (m,8H); δ 1.62-1.72 (m,2H); δ 2.68-2.82 (m,2H); δ 3.49-3.62 (m,3H); δ 3.69-3.81 (m,2H); δ 3.93-3.94 (d,1H); δ 4.33-4.36 (d,1H) ppm. ^{13}C-NMR (CD_3OD): δ 14.45 (CH_3); δ 23.67 (CH_2); δ 29.96 (CH_2); δ 30.02 (CH_2); δ 30.89 (CH_2); δ 31.11 (CH_2); δ 32.94 (CH_2); δ 62.55 (CH_2); δ 70.45 (CH); δ 71.45 (CH); δ 76.24 (CH); δ 80.51 (CH); δ 86.70 (CH) ppm.
Elemental analysis: calculated: C: 53.04 %; H: 8.90 %; S: 10.89 %. Found: C: 52.56 %; H: 9.02 %; S: 10.51 %.

n-Octyl 1-thio-β-galactopyranoside **11i**
^{1}H-NMR (CD_3OD): δ 0.92-0.96 (t,3H); δ 1.34-1.47 (m,10H); δ 1.62-1.70 (m,2H); δ 2.70-2.82 (m,2H); δ 3.48-3.62 (m,3H); δ 3.73-3.79 (m,2H); δ 3.93-3.94 (d,1H); δ 4.33-4.36 (d,1H) ppm. ^{13}C-NMR (CD_3OD): δ 14.47 (CH_3); δ 23.70 (CH_2); δ 29.99 (CH_2); δ 30.31 (CH_2); δ 30.34 (CH_2); δ 30.87 (CH_2); δ 31.07 (CH_2); δ 32.98 (CH_2); δ 62.49 (CH_2); δ 70.38 (CH); δ 71.39 (CH); 76.19 (CH); δ 80.47 (CH); δ 87.64 (CH) ppm.
Elemental analysis: calculated: C: 54.52 %; H: 9.15 %; S: 10.40 %. Found: C: 54.51 %; H: 9.02 %; S: 10.41 %.

n-Alkyl 1-thio-2,3,4,6-O-acetyl-D-mannopyranosides **12c-12e**
The n-alkyl 1-thio-2,3,4,6-O-acetyl-D-mannopyranosides **12c-12e** were synthesised from **12a** and the appropriate n-alkanethiol via a method described by Dasgupta and Garegg.[58] The method always resulted in a mixture of anomers. The fully protected products could be enriched in either α- or β-anomer by chromatography. The same conditions were used as for the fully protected glucose derivatives. Crystallisation could be performed using 96% ethanol, but crystallisation did not yield an anomerically pure product.
Average yield of anomer mixture: 20% (after crystallisation).

n-Hexyl 2,3,4,6-O-acetyl-1-thio-α-D-mannopyranoside **12c**
^{1}H-NMR ($CDCl_3$): δ 0.86-0.90 (t,3H); δ 1.24-1.37 (m,6H); δ 1.59-1.64 (m,2H); δ 1.99 (s,3H); δ 2.05 (s,3H); δ 2.09 (s,3H); δ 2.16 (s,3H); δ 2.58-2.67 (m,2H); δ 4.05-4.12 (dd,1H); δ 4.29-4.34 (m,2H); δ 5.25-5.34 (m,4H) ppm. ^{13}C-NMR ($CDCl_3$): δ 13.84 (CH_3); δ 20.46 (CH_3); δ 20.54 (CH_2); δ 20.75 (CH_3); δ 22.34 (CH_2); δ 28.28 (CH_2); δ 29.19 (CH_2); δ 31.12 (CH_2); δ 31.18 (CH_2); δ 62.26 (CH_2); δ 66.15 (CH); δ 68.72 (CH); δ 69.28 (CH); δ 70.99 (CH); δ 82.34 (CH); δ 169.48 (CO); δ 169.52 (CO); δ 169.72 (CO); δ 170.33 (CO) ppm.
Elemental analysis: calculated: C: 53.56 %; H: 7.19 %; S: 7.15 %; found: C: 53.53 %; H: 7.19 %; S: 7.22 %.

n-Heptyl 2,3,4,6-O-acetyl-1-thio-α-D-mannopyranoside **12d**
^{1}H-NMR ($CDCl_3$): δ 0.81-0.88 (t,3H); δ 1.19-1.39 (m,8H); δ 1.53-1.63 (m,2H); δ 1.96 (s,3H); δ 2.01 (s,3H); δ 2.06 (s,3H); δ 2.13 (s,3H); δ 2.48-2.53 (m,2H); δ 4.01-4.07 (dd,1H); δ 4.23-4.40 (m,2H); δ 5.2-5.35 (m,4H) ppm. ^{13}C-NMR ($CDCl_3$): δ 14.07 (CH_3); δ 20.63 (CH_3); δ 20.72 (CH_3); δ 20.93 (CH_3); δ 22.58 (CH_2); δ 28.75 (CH_2); δ 29.39 (CH_2); δ 31.34 (CH_2); δ 31.68 (CH_2); δ 62.41 (CH_2); δ 66.32 (CH); δ 68.85 (CH); δ 69.46 (CH); δ 71.17 (CH); δ 82.52 (CH); δ 169.66 (CO); δ 169.72 (CO); δ 169.91 (CO); δ 170.52 (CO) ppm.
Elemental analysis: calculated: C: 54.53 %; H: 7.41 %; S: 6.93 %; found: C: 54.36 %; H: 7.49 %; S: 6.85 %.

n-Octyl 2,3,4,6-O-acetyl-1-thio-α-D-mannopyranoside **12e**
^{1}H-NMR ($CDCl_3$): δ 0.85-0.90 (t,3H); δ 1.26-1.39 (m,10H); δ 1.57-1.64 (m,2H); δ 1.98 (s,3H); δ 2.04 (s,3H); δ 2.09 (s,3H); δ 2.16 (s,3H); δ 2.56-2.69 (m,2H); δ 4.06-4.11 (dd,1H); δ 4.29-4.39 (m,2H); δ 5.25-5.35 (m,4H) ppm. ^{13}C-NMR ($CDCl_3$): δ 13.93 (CH_3); δ 20.48 (CH_3); δ 20.56 (CH_3); δ 20.78 (CH_3); δ 22.48 (CH_2); δ 28.64 (CH_2); δ 28.92

(CH_2); δ 28.99 (CH_2); δ 29.25 (CH_2); δ 31.19 (CH_2); δ 31.62 (CH_2); δ 62.28 (CH_2); δ 66.18 (CH); δ 68.73 (CH); δ 69.31 (CH); δ 71.0 (CH); δ 82.37 (CH); δ 169.49 (CO); δ 169.54 (CO); δ 169.73 (CO); δ 170.35 (CO) ppm.
Elemental analysis: calculated: C: 55.45 %; H: 7.61 %; S: 6.73 %; found: C: 55.48 %; H: 7.55 %; S: 6.78 %.

n-Alkyl 1-thio-α-D-mannopyranosides **12g-12i**
After deprotection, which was quantitative (vide supra), and evaporation of the solvent mixture, the purification and separation of the anomers could be performed by chromatography either on a cation exchange material (Dowex 50x4 in the calcium ion form) or on a Sephadex column. In the first case a mixture of methanol and water (7:3) was used as eluent and the β-anomer had a shorter retention time than the α-anomer. In case of the Sephadex column methanol was the eluent and the a-anomer was collected first.
In addition, it was found that if the β-product was present in the mixture in a proportion greater than 50% it could sometimes be crystallised specifically by crystallisation in methanol.
The α-compound is crystalline when an odd number of carbons is present in the alkyl chain and can be crystallised from methanol/acetonitrile. Acetonitrile is in excess over methanol (see **11f-11i**). However, for an even number of carbon atoms in the alkyl chain it is exceedingly difficult to obtain crystals. For alkyl chain lengths of six and eight carbon atoms it was possible to obtain pure crystalline β-compound.
The elemental analyses were performed for the anomer mixtures and thus apply to both anomers of a series.

n-Hexyl 1-thio-α-mannopyranoside **12gα**
^{1}H-NMR (CD_3OD); δ 0.88-0.93 (t,3H); δ 1.30-1.44 (m,6H); δ 1.58-1.66 (m,2H); δ 2.56-2.70 (m,2H); δ 3.66-3.69 (d,2H); δ 3.72-3.84 (m,2H); δ 3.87-3.91 (m,2H); δ 5.22 (d,1H) ppm. ^{13}C-NMR (CD_3OD); δ 14.25 (CH_3); δ 23.50 (CH_2); δ 29.47 (CH_2); δ 30.66 (CH_2); δ 31.96 (CH_2); δ 32.46 (CH_2); δ 62.78 (CH_2); δ 69.01 (CH); δ 73.29 (CH); δ 73.83 (CH); δ 74.77 (CH); δ 86.46 (CH) ppm.
Elemental analysis: Calculated: C: 51.41%; H: 8.63%; S: 11.44%. Found: C: 50.26 %; H: 8.47 %; the values are too low because the sample is very hygroscopic.

n-Hexyl 1-thio-β-mannopyranoside **12gβ**
^{1}H-NMR (CD_3OD); δ 0.90-0.93 (t,3H); δ 1.28-1.44 (m,6H); δ 1.58-1.66 (m,2H); δ 2.68-2.75 (m,2H); δ 3.21-3.26 (m,1H); δ 3.35-3.49 (dd,1H); δ 3.55-3.61 (t,1H); δ 3.68-3.73 (dd,1H); δ 3.83 (d,1H); δ 3.87-3.88 (d,1H); δ 4.71 (d,1H) ppm. ^{13}C-NMR (CD_3OD); δ 14.37 (CH_3); δ 23.62 (CH_2); δ 29.60 (CH_2); δ 31.05 (CH_2); δ 32.21 (CH_2); δ 32.57 (CH_2); δ 62.94 (CH_2); δ 68.35 (CH); δ 74.04 (CH); δ 76.32 (CH); δ 82.35 (CH); δ 86.22 (CH) ppm.

n-Heptyl 1-thio-α-mannopyranoside **12hα**
^{1}H-NMR (CD_3OD); δ 0.91-0.95 (t,3H); δ 1.34-1.45 (m,8H); δ 1.61-1.69 (m,2H); δ 2.56-2.75 (m,2H); δ 3.69-3.71 (dd,2H); δ 3.74-3.87 (m,2H); δ 3.9-3.95 (m,2H); δ 5.25 (d,1H) ppm. ^{13}C-NMR (CD_3OD); δ 14.42 (CH_3); δ 23.61 (CH_2); δ 29.80 (CH_2); δ 29.95 (CH_2); δ 30.66 (CH_2); δ 31.82 (CH_2); δ 32.88 (CH_2); δ 62.61 (CH_2); δ 68.69 (CH); δ 73.11 (CH); δ 73.66 (CH); δ 74.67 (CH); 86.3 (CH) ppm.
Elemental analysis: Calculated: C: 53.04 %; H: 8.90 %; S: 10.89 %. Found: C: 52.77%; H: 8.78 %; S: 10.89%.

n-Octyl 1-thio-α-mannopyranoside **12iα**
^{1}H-NMR (CD_3OD); δ 0.88-0.92 (t,3H); δ 1.25-1.42 (m,10H); δ 1.58-1.66 (m,2H); δ 2.56-2.70 (m,2H); δ 3.65-3.67 (m,2H); δ 3.71-3.84 (m,2H); δ 3.87-3.92 (m,2H); δ 5.22

(d,1H) ppm. ^{13}C-NMR (CD_3OD); δ 14.43 (CH_3); δ 23.66 (CH_2); δ 29.85 (CH_2); δ 30.24 (CH_2); δ 30.32 (CH_2); δ 30.67 (CH_2); δ 31.84 (CH_2); δ 32.95 (CH_2); δ 62.66 (CH_2); δ 68.78 (CH); δ 73.17 (CH); δ 73.73 (CH); δ 74.76 (CH); δ 86.38 (CH) ppm.
Elemental analysis: Calculated: C: 54.52 %; H: 9.15 %; S: 10.40 %. Found: C: 54.27 %; H: 8.98 %; S: 10.37 %.

n-Octyl 1-thio-β-mannopyranoside **12iβ**
^{1}H-NMR (CD_3OD); δ 0.87-0.92 (t,3H); δ 1.30-1.43 (m,10H); δ 1.58-1.66 (m,2H); δ 2.68-2.74 (m,2H); δ 3.21-3.27 (m,1H); δ 3.47-3.51 (dd,1H); δ 3.58-3.64 (t,1H); δ 3.69-3.75 (dd,1H); δ 3.82-3.87 (dd,1H); δ 3.89-3.90 (dd,1H); δ 4.70 (d,1H) ppm. ^{13}C-NMR (CD_3OD); δ 14.30 (CH_3); δ 23.55 (CH_2); δ 29.83 (CH_2); δ 30.17 (CH_2); δ 31.04 (CH_2); δ 32.21 (CH_2); δ 32.85 (CH_2); δ 62.96 (CH_2); δ 68.46 (CH); δ 73.97 (CH); δ 76.34 (CH); δ 82.24 (CH); δ 86.22 (CH) ppm.

n-Alkyl 1-thio-2,3,4,6-O-acetyl-α-D-talopyranosides **13d,13e**
Both n-heptyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-talopyranoside **13d** and n-octyl 2,3,4,6-tetra-O- acetyl-1-thio-α-D-talopyranoside **13e** were synthesised according to the method which was used for the mannose derivatives **12c-12e**. The alpha anomer was obtained in pure form after chromatography of the product mixture over silica gel with n-hexane-ethyl acetate (7:3) as eluent. The alpha product was collected first.

n-Heptyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-talopyranoside **13d**
^{1}H-NMR ($CDCl_3$): δ 0.82-0.94 (t,3H); δ 1.20-1.43 (m,8H); δ 1.54-1.71 (m,2H); δ 1.98 (s,3H); δ 2.05 (s,3H); δ 2.14 (s,3H); δ 2.16 (s,3H); δ 2.50-2.73 (m,2H); δ 4.14-4.24 (d,2H); δ 4.57-4.67 (dt,1H); δ 5.13-5.26 (m,2H); δ 5.3-5.37 (dd,2H) ppm. ^{13}C-NMR ($CDCl_3$): δ 13.93 (CH_3); δ 20.44 (CH_3); δ 20.51 (CH_3); δ 20.53 (CH_3); δ 22.45 (CH_2); δ 28.66 (CH_2); δ 29.15 (CH_2); δ 30.76 (CH_2); δ 31.56 (CH_2); δ 61.96 (CH_2); δ 66.03 (CH); δ 67.11 (CH); δ 68.82 (CH); δ 82.56 (CH); δ 169.27 (CO); δ 169.72 (CO); δ 169.94 (CO); δ 170.19 (CO) ppm.

n-Octyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-talopyranoside **13e**
^{1}H-NMR ($CDCl_3$): δ 0.82-0.94 (t,3H); δ 1.21-1.45 (m,10H); δ 1.54-1.70 (m,2H); δ 2.0 (s,3H); δ 2.06 (s,3H); δ 2.14 (s,3H); δ 2.15 (s,3H); δ 2.47-2.76 (m,2H); δ 4.16-4.22 (d,2H); δ 4.57-4.68 (dt,1H); δ 5.14-5.26 (m,2H); δ 5.30-5.38 (dd,2H) ppm. ^{13}C-NMR ($CDCl_3$): δ 13.93 (CH_3); δ 20.48 (CH_3); δ 20.56 (CH_3); δ 20.78 (CH_3); δ 22.48 (CH_2); δ 28.64 (CH_2); δ 28.92 (CH_2); δ 28.99 (CH_2); δ 29.25 (CH_2); δ 31.19 (CH_2); δ 31.62 (CH_2); δ 61.96 (CH_2); δ 66.03 (CH); δ 67.11 (CH); δ 68.82 (CH); δ 82.56 (CH); δ 169.27 (CO); δ 169.72 (CO); δ 169.94 (CO); δ 170.19 (CO) ppm.

n-Alkyl 1-thio-α-D-talopyranosides **13h,13i**
The n-alkyl 1-thio-α-D-talopyranosides **13h** and **13i** were obtained from **13d** and **13e**, respectively, by hydrolysis in a solvent mixture of methanol with aqueous trimethylamine (*vide supra*). The product was obtained by evaporation of the solvent mixture and purified by chromatography over a short column of Dowex 1x2 anion exchange material in the OH^- form. Methanol was used as an eluent. The compounds were crystallised from a methanol-acetonitrile mixture with acetonitrile in large excess (see **11f-11i**).
Deprotection was quantitative. Average yield from **13a**: 30% (anomerically pure).

n-Heptyl 1-thio-α-D-talopyranoside **13h**
^{1}H-NMR (CD_3OD): δ 0.88-0.93 (t,3H); δ 1.31-1.43 (m,8H); δ 1.59-1.69 (m,2H); δ 2.55-2.75 (m,2H); δ 3.64-3.66 (t,1H); δ 3.73-3.76 (d,2H); δ 3.82-3.83 (d,2H); δ 4.09-4.13 (t,1H); δ 5.30 (d,1H) ppm. ^{13}C-NMR (CD_3OD): δ 14.2 (CH_3); δ 23.67 (CH_2); δ 29.88 (CH_2);

δ 31.01 (CH_2); δ 30.73 (CH_2); δ 31.76 (CH_2); δ 32.94 (CH_2); δ 62.74 (CH_2); δ 67.95 (CH); δ 71.78 (CH); δ 73.30 (CH); δ 74.24 (CH); δ 87.06 (CH) ppm.
Elemental analysis: Calculated: C: 53.04%; H: 8.90%; S: 10.89%. Found: C: 52.52%; H: 8.78%; S: 10.85%.

n-Octyl 1-thio-α-talopyranoside **13i**
^{1}H-NMR (CD_3OD): δ 0.88-0.95 (t,3H); δ 1.19-1.52 (m,10H); δ 1.53-1.72 (m,2H); δ 2.52-2.78 (m,2H); δ 3.64-3.72 (t,1H); δ 3.74-3.79 (d,2H); δ 3.82-3.88 (d,2H); δ 4.09-4.16 (t,1H); δ 5.32 (d,1H) ppm. ^{13}C-NMR (CD_3OD): δ 14.44 (CH_3); δ 23.66 (CH_2); δ 29.87 (CH_2); δ 30.24 (CH_2); δ 30.30 (CH_2); δ 30.67 (CH_2); δ 31.70 (CH_2); δ 32.94 (CH_2); δ 62.64 (CH_2); δ 67.86 (CH); δ 71.63 (CH); δ 73.14 (CH); δ 74.08 (CH); δ 86.91 (CH) ppm.
Elemental analysis: Calculated: C: 54.52 %; H: 9.15%; S:10.40%. Found: C: 54.58%; H: 9.21%; S:10.36%.
A crystal structure was solved for n-octyl 1-thio-α-D-talopyranoside (see picture on the cover), which will be published in the near future.[102]

(R,S)-3,7-dimethyloctanethiol **15**
(R,S)-3,7-dimethyloctanethiol was synthesised from (R,S)-citronellol by Klaas Hovius using a standard procedure.[39]
^{1}H-NMR ($CDCl_3$): δ 0.85-0.89 (m,9H); δ 1.09-1.16 (m,4H); δ 1.21-1.32 (m,2H); δ 1.41-1.58 (m,4H); δ 2.49-2.55 (m,2H) ppm. ^{13}C-NMR ($CDCl_3$): δ 19.08 (CH_3); δ 22.36 (CH_2); δ 22.45 (CH_3); δ 22.55 (CH_3); δ 24.50 (CH_2); δ 27.82 (CH); δ 31.70 (CH); δ 36.76 (CH_2); δ 39.10 (CH_2); δ 41.39 (CH_2) ppm.

3,7-Dimethyloctyl 1-thio-α-L-arabinopyranoside **14c**
3,7-Dimethyloctyl 1-thio-α-L-arabinopyranoside was synthesised by coupling the α-L-arabinose tetraacetate **14a** with the appropriate alkanethiol (**15**) under the same conditions used for the glucose- and galactose derivatives described earlier. The use of a primary thiol had the advantage that a short reaction time (15 minutes) sufficed for coupling. The acetylated adduct was deprotected by hydrolysis in the methanol-trimethylamine-water mixture for 24 hours. The product was anomerically pure and careful study of the ^{1}H-NMR spectrum showed that the α-anomer was synthesised and that the arabinose ring was in the $^4C_1$ conformation (J_{H1-H2}=4.03 Hz, J_{H2-H3}=7.8 Hz).
The yield from **14a** was 33%.
^{1}H-NMR (CD_3OD): δ 0.85-0.95 (m,9H); δ 1.08-1.23 (m,4H); δ 1.25-1.40 (m,2H); δ 1.41-1.66 (m,4H); δ 2.54-2.66 (m,2H); δ 3.56-3.62 (dd,1H); δ 3.68-3.72 (m,1H); δ 3.86-3.90 (m,1H); δ 3.95-4.06 (m,2H); δ 5.16-5.18 (d,1H) ppm. ^{13}C-NMR (CD_3OD): δ 19.67 (CH_3); δ 19.76 (CH_3); δ 22.91 (CH_3); δ 22.98 (CH_3); δ 25.67 (CH_2); δ 29.06 (CH); δ 29.35 (CH_2); δ 29.50 (CH_2); δ 33.32 (CH); δ 38.01 (CH_2); δ 38.10 (CH_2); δ 38.16 (CH_2); δ 38.28 (CH_2); δ 40.43 (CH_2); δ 65.54 (CH_2); δ 68.88 (CH); δ 71.38 (CH); δ 71.89 (CH); δ 86.79 (CH); δ 87.06 (CH) ppm.
Elemental analysis: Calculated: C: 58.79%; H: 9.87%; S: 10.46%. Found: C: 58.45%; H: 9.69%; S: 10.40%.

5.7.3 Methods

Krafft temperatures (solubilities) (Table 5.5)
Krafft temperatures were measured on a Perkin Elmer differential scanning calorimeter (Perkin Elmer Delta Series DSC7 apparatus). The procedure was as follows: a sample of approximately 3 mg was prepared with 50 µl of distilled water (water was also the reference). The sample was sealed in a cup. The sample was heated gradually and a heat uptake was monitored upon dissolution of the compound in water. The results were always checked by performing a contact experiment with light microscopy (Mettler FP82 hotstage mounted on a Nikon polarisation microscope). The results were in agreement with one

another. These measurements were performed at the NIKO-TNO in Groningen in cooperation with Dr. Henk Van Doren.

Liquid crystalline behaviour (Table 5.6)
The liquid crystalline behaviour of the carbohydrate-derived surfactants was studied by heating samples of approximately 3 mg of each substance in the DSC apparatus, described above. The liquid crystalline phase was identified with light microscopy. The light microscope used, was the same as the one described above. These studies were also carried out in cooperation with Dr. Henk Van Doren of the NIKO-TNO.

Microcalorimetry
The microcalorimetric experiments were performed at the Koninklijke/Shell Laboratory in Amsterdam by mr. Fred Kerkhof, M.Sc. and Dr. Nico M. Van Os. The experiments were performed at 333 K which necessitated special design of the apparatus. The concentrated (stock) solution of the carbohydrate-derived surfactant had to be kept at this temperature too. To ensure that this condition is maintained, a glove box was attached to the microcalorimeter in which the temperature was kept at 333 K. An LKB 2277 heat-flow microcalorimeter, described elsewhere[103] was used.

Chromatography
Chromatography was performed using the column materials described above. When methanol or methanol-water mixtures were applied, fractions of approximately 10-15 ml were collected with fraction collectors. The collectors were an LKB 2112 Redirac and an LKB 17000 Minirac.

Analysis of chromatography
The fractions which were collected after chromatography were checked for the presence of carbohydrate-derived surfactants. Analytical TLC was performed on precoated silica gel F_{254} plates (Merck) with detection by charring with a methanol-sulfuric acid (20% acid) solution.

NMR spectroscopy
All NMR spectra were taken on a Varian Gemini 200 and VXR 300 spectrometer. The fully protected carbohydrate-derivatives were dissolved in $CDCl_3$, the carbohydrate-derived surfactants were studied in CD_3OD. Tetramethylsilane was the reference.

Elemental analyses
All elemental analyses were performed in the microanalytical department of this laboratory by mr. H. Draayer and mr. J. Ebels.

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CHAPTER 6

STEREOCHEMICAL ASPECTS OF CARBOHYDRATE HYDRATION

6.1 Introduction

The stereospecific hydration model of Franks and Suggett[1] implied that carbohydrate hydration depends on the number of equatorial hydroxy groups present in the carbohydrate molecule.[2] This model is based on the fact that β-D-glucose is predominantly present in water as it fits better into the three-dimensional hydrogen-bond network of water than α-D-glucose does. Experimental support was provided by heat capacity measurements[3] and NMR spectroscopy.[4] The explanation on a molecular level was, that if the anomeric hydroxy group is equatorial, it can fit better into the structure of water in conjunction with OH(3).[5]

However, many subsequent observations do not fit the stereospecific hydration model.[1] Therefore other models were proposed to explain the stereochemical aspects of carbohydrate hydration.[6] Unfortunately, none of these models was able to fit all the results. Consequently it was generally believed that all rules proposed so far to describe the dependence of carbohydrate hydration upon stereochemistry would have to be jettisoned and carbohydrate hydration would remain largely unexplained.[7]

One year after this rather demoralising (or is it challenging?) statement this project was started with the aim to reinvestigate stereochemical aspects of carbohydrate hydration. The results of the investigation are reported in the previous chapters.

6.2 New trends in stereochemical aspects of carbohydrate hydration

In chapters 2 and 3 the hydration characteristics of mono- and disaccharides were studied by means of kinetic medium effects and thermodynamics, respectively.

Both the kinetic medium effects, G(C), partial molar compressibilities, $K_{2,s}$, $K_{2,T}$ (isentropic and isothermal) and hydration numbers, n_h (which are related to the compressibilities) suggested that the hydration of a carbohydrate depends on the relative position of OH(4) in conjunction with the relative position of OH(2). This observation was primarily made for aldohexoses. For aldopentoses, methyl aldoglycopyranosides and disaccharides it was also observed that the hydration depends on the relative position of OH(4). Unfortunately none of these compounds possess both an axial OH(2) and an axial OH(4). When OH(4) is equatorial, the type of linkage in methyl aldoglycopyranosides and disaccharides between different moieties can be

important in determining the extent of the fit of the carbohydrate into water. If the hydroxy group on the 4-position is axial, there is no such sensitivity towards the type of linkage.

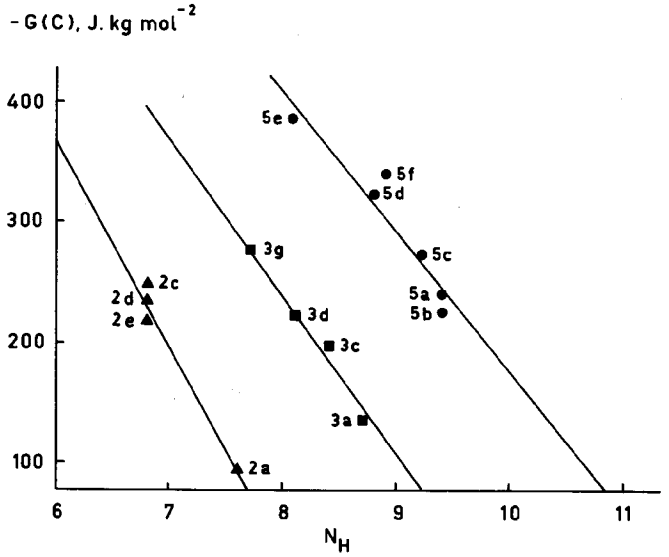

Figure 6.1 Plots of the kinetic medium effects, G(C), *vs.* hydration number, N_H, of aldopentoses, aldohexoses and methyl aldoglycopyranosides; D-arabinose (**2a**), D-xylose (**2c**), D-lyxose (**2d**), D-ribose (**2e**), D-galactose (**3a**), D-glucose (**3c**), D-mannose (**3d**), D-talose (**3g**), methyl α-D-galactopyranoside (**5a**), methyl β-D-galactopyranoside (**5b**), methyl α-D-glucopyranoside (**5c**), methyl β-D-glucopyranoside (**5d**), 3-O-methylglucopyranose (**5e**), methyl α-D-mannopyranoside (**5f**).

6.3 The correlation between kinetic medium effects and hydration numbers

The separate sets of data (6.2) are consistent with one another as can be seen in Figure 6.1. In this Figure the G(C) values, which were obtained through measurements of kinetic medium effects, are plotted against hydration numbers, obtained by ultrasound measurements. There is an excellent correlation between the two parameters for aldopentoses (**2a,c-d**), aldohexoses (**3a,c,d,g**) and methyl aldoglycopyranosides (**5a-f**).

All carbohydrates studied have a negative G(C) value, which means that they all cause a rate retardation of the hydrolysis reaction studied (see chapter 2). In addition, this means that carbohydrates exhibit a kind of behaviour in water, which makes them resemble hydrophobic solutes. For instance, D-talose (**3g**) exerts approximately the same kinetic medium effect as the relatively hydrophobic 1-propanol. This apparent hydrophobicity depends on the relative positions of OH(2) and OH(4). For aldohexoses the apparent hydrophobicity increases in the order D-galactose < D-glucose ≅ D-mannose < D-talose. For the different sets of isomers the apparent hydrophobicity increases for

compounds with the same stereochemistry in the sequence hexose ≤ pentose < methyl aldoglycopyranoside < disaccharide. This means that the apparent hydrophobicity of a hexose is slightly increased upon removal of the exocyclic CHOH moiety, and it is considerably increased when a hydroxy group is substituted by a methoxy group or another monosaccharide unit.

In chapter 3 the extent of disturbance of water, caused by the presence of the carbohydrate, was found to increase in the order pentoses < hexoses < methyl aldoglycopyranosides < disaccharides (expressed in terms of hydration numbers, n_h). Within a set of isomers the extent of disturbance depends on the relative positions of OH(2) and OH(4). For aldohexoses the disturbance of water decreases in the order: D-galactose > D-mannose ≥ D-glucose > D-talose.

If the results of chapters 2 and 3 are considered together, it may be concluded that the better a carbohydrate fits into water, the greater is its apparent hydrophobicity. This is also illustrated in Figure 6.1. The greater the hydration number is for a carbohydrate, the more it disturbs water by its presence. Within one set of isomers, it is clear that the smaller the hydration number is, the more negative the G(C) value is. This illustrates that a good fit of a carbohydrate in water makes the hydroxy groups look like hydroxy groups of water. Hence they are camouflaged[8] for interaction, which leaves only the methine moieties for interaction. The more the hydroxy groups will be camouflaged for interaction, the greater the apparent hydrophobicity will be. The relatively small partial molar volumes and heat capacities of carbohydrates in aqueous solutions also indicate that there is a camouflage effect.

6.4 The importance of OH(2) and OH(4) explained in terms of a molecular picture

The results of chapters 2 and 3 show that the hydration of a carbohydrate (and an aldohexose in particular) is determined by the relative positions of OH(2) and OH(4). The question remains what the structural reason is for these differences in hydration and why the aldohexoses are divided into three groups with different types of hydration.

This initiated the investigation, summarised in chapter 4, into through-space oxygen distances within the aldohexoses and a comparison of these distances with the oxygen distances of water. A similar approach was previously applied by Danford[9] and Warner.[10] The oxygen distances were studied both for carbohydrates in the crystalline state and for carbohydrates in water after an MD simulation. For the MD study two aldohexoses with entirely different hydration characteristics were chosen.

Danford and Warner[9,10] suggested that the fit of a carbohydrate into the

three-dimensional hydrogen-bond structure of water depends on the relative oxygen distances of a carbohydrate in comparison to oxygen distances of water. This is equivalent to a search for the fit of the carbohydrate hydroxy moieties in the hydrogen-bond network of water. The conclusion of this thesis, that the fit of the next-nearest neighbour oxygen distances of a carbohydrate with the oxygens of water is of paramount importance for the hydration of a carbohydrate is in agreement with this suggestion.

The sugar β-D-galactose which, according to earlier work, disturbs the three-dimensional hydrogen-bonded structure of water, shows hardly any fit of its oxygen distances with those of water. Both β-D-talopyranose and α-D-talopyranose have a good fit into the hydrogen-bond network of water and their O_2-O_4-O_5 plane has quite a good compatibility with the nearest neighbour oxygen distances in water. In addition, their O_1-O_3-O_6 plane has a reasonable fit with the next-nearest neighbour oxygens in the three-dimensional hydrogen-bonded network of water.

The flexibility of the hydroxymethyl group and the fact that the position of the anomeric hydroxy group is changeable, makes the O_1-O_3-O_6 plane of hydroxy groups adjustable to change. The oxygen atoms of the O_2-O_4-O_5 plane are more rigid with respect to their relative positions. It is proposed that the most rigid plane of oxygen atoms (O_2-O_4-O_5) is the most important in determining the overall fit of a carbohydrate into water.

The MD simulations yielding the average number of hydrogen bonds of the hydroxy groups of the carbohydrates as both hydrogen-bond donor and hydrogen-bond acceptor illustrate that D-galactose and D-talose have the same average number of hydrogen bonds to all the hydroxy groups, except on OH(2) and OH(4). This stresses the importance of the relative positions of OH(2) and OH(4).

Interestingly, the good fit of both talose anomers in water sheds light on the continuing discussion of the anomeric effect.[11] It is known that in the crystalline state the α-talose anomer is the favoured anomer. However, in aqueous solution the β-talose anomer also fits well, particularly because the OH(6) can adjust its position to help accommodate OH(1) and OH(3) into the three-dimensional hydrogen-bond network of water. This results in an almost fifty-fifty mixture of α- and β-talose in water. It is expected that this particular mechanism might be responsible for the large excess of β-glucopyranose over α-glucopyranose in aqueous solution, despite the fact that with respect to the internal energy of the molecule, the α-glucopyranose form is more favourable. Previously it was agreed that hydration caused the dominant presence of β-D-glucose in water, but only the distances between OH(1) and OH(3) were taken into

account. The results of the molecular dynamics simulation suggest that OH(6) may also play a role.

In chapter 2 it was noted that both D-galactose and D-talose behave like rather hydrophobic species in aqueous solution, although the apparent hydrophobicity is greater for D-talose than for D-galactose, because D-talose fits better in the hydrogen-bond network of water. The molecular dynamics study suggests that this is caused by the fact that the hydroxy groups of D-talose also can be camouflaged more than those of D-galactose as a result of the formation of an intramolecular hydrogen bond between OH(2) and OH(4), with OH(2) acting as hydrogen-bond donor.

The partial molar compressibility data (chapter 3) led to the suggestion that the water molecules in the hydration layer of D-talose are less disturbed compared to pure water than those in the hydration layer of D-galactose. The plots of the distribution function in chapter 4 for O_{carb}-O_{water}, or H_{carb}-O_{water} versus distance, illustrate that water in the presence of D-galactose is more organised than in the presence of D-talose. In this respect it is interesting to note that dielectric decrements studies[12] also show that water molecules in the hydration layer of D-galactose diffuse more slowly than those near D-glucose. In addition, by considering the average number of hydrogen bonds between water molecules in water and in a solution containing D-talose and β-D-galactose, it can be seen that D-talose disrupts less intermolecular hydrogen bonds between water molecules than β-D-galactose does.

The hydroxymethyl group in aldohexoses is flexible enough to adjust its position to induce a good fit in water in conjunction with the OH(1) and OH(3) groups. In chapter 3 it was reported that ketohexoses have a greater disturbing effect on water than the aldohexoses. Since in ketohexoses the exocyclic hydroxymethyl group is situated at the anomeric centre, it is suggested that the hydroxymethyl group might be less flexible than that for aldohexoses. A possible consequence is a relatively bad fit of ketohexoses into water, because the hydroxymethyl cannot help to accommodate the hydroxy groups of the ketohexose in water. However, a further investigation of the hydration characteristics of ketohexoses should be performed to establish this conclusion.

6.5 Evaluation of thermodynamic parameters for aqueous carbohydrate solutions

Not all thermodynamic parameters provide information on stereospecific hydration. First, the delicate balance of the equilibrium composition of a carbohydrate in water should not be disturbed. Therefore measurements which are performed at a large concentration range of carbohydrate or over a wide range of temperatures should be

avoided. The equilibrium composition can change dramatically under these circumstances which hampers a straightforward interpretation of carbohydrate hydration.
The crystalline state as a reference state should be handled with care, particularly when the totally different hydrogen-bond pattern of the crystalline state compared to that in water results in a different predominant isomer of the carbohydrate in the crystalline state and water.
Unfortunately, even if these considerations are followed, not every parameter will be informative of the stereochemical aspects of carbohydrate hydration. These are only well represented by parameters which are solely determined by the characteristics of the hydration layer. This involves parameters for which the intrinsic contribution of the carbohydrate is negligible compared to the contribution of the hydration layer of a carbohydrate. In addition, the measurements have to be very sensitive to hydration effects.
So far only a few parameters have proven to be sufficiently sensitive towards stereochemical aspects of hydration: these are dielectric relaxation decrements,[12] carbohydrate-solute interactions[13-15] and partial molar isentropic[16] and isothermal compressibilities.[17] In addition, molecular dynamics simulations provide an excellent way to improve further the insights into the stereochemical details of hydration.
The average number of intermolecular hydrogen bonds between water molecules in the presence of D-galactose and D-talose shows how well the carbohydrates fit into water (and therefore form almost ideal solutions) and how small the differences are in the stereochemical aspects of hydration. This explains why thermodynamic properties were not measured for aqueous carbohydrate solutions for a very long time and why most thermodynamic parameters are not successful in monitoring the dependence of hydration upon stereochemistry.

6.6 Carbohydrate-derived surfactants

For the carbohydrate-derived surfactants the stereochemistry of the headgroup seems in every respect of importance. The method of synthesis (chapter 5) is already determined by the availability of O(2) for anchimeric assistance. When the compounds need to be anomerically pure, their stereochemistry is not important in the protected form. However, after the carbohydrate-derived surfactants have been deprotected the method of purification depends on the hydroxy topology of the carbohydrate headgroup.
The solubility of a carbohydrate-derived surfactant in water (Krafft temperature) depends very much on the relative position of OH(4). All compounds with an axial OH(4) have a high Krafft temperature and, therefore, low solubility. The compounds with an

equatorial OH(4) have different Krafft temperatures depending on the position (α or β) of the alkyl chain on the anomeric centre.

All compounds which have a high Krafft temperature, have large heats of melting when their thermotropic phase behaviour is examined. A most probable explanation is that the axial OH(4) provides particular stabilisation in the crystalline state.

All n-alkyl 1-thioglycopyranosides form a smectic A phase upon heating. Their lyotropic phase behaviour depends on the relative position of OH(2).

The n-heptyl 1-thio-β-D-glucopyranoside and n-heptyl 1-thio-β-D-galactopyranoside derivatives have different critical micelle concentrations at 333 K, as was expected on the basis of the results reported in chapters 2 and 3. However, the differences are not that large that the Gibbs energies, enthalpies and entropies of micellisation show a significant difference. The entropy of micellisation is fairly positive for such a short chain length, which indicates that the carbohydrate headgroup has little influence on the hydrophobic hydration of the alkyl chain in the monomeric state.

6.7 Proposition of a modified stereospecific hydration model

The fit of a carbohydrate in water depends on how the next-nearest neighbour oxygen atoms in a carbohydrate molecule fit with the oxygen distances in water. The oxygens of a carbohydrate can be separated into two groups which are considered as two planes. These planes are important determinants of the fit of the carbohydrate into the three-dimensional hydrogen-bonded network of water. The O_2-O_4-O_5 plane dominates in determining the compatibility of the carbohydrate with water, because it is the most rigid part of the carbohydrate. How the O_1-O_3-O_6 plane of oxygens fit in water is somewhat less important. This plane of oxygens is more flexible and more adaptable.

With respect to the oxygen distances, the aldohexoses can be divided into three groups with a different fit into water because their oxygen-oxygen distances can fit either the nearest neighbour or next-nearest neighbour oxygens distances of water or have no fit at all.

The better a carbohydrate fits into the three-dimensional hydrogen-bond structure of water, the more its hydroxy groups will be camouflaged for interaction, because they resemble the hydroxy groups of water. This camouflage effect results in a greater apparent hydrophobicity of a carbohydrate in water because a spectator molecule cannot discriminate between the hydroxy groups of a carbohydrate and those of water. Hence it will only "see" the methine moieties and therefore recognise a carbohydrate as a hydrophobic moiety. An extra effect is operative if a carbohydrate has next-nearest neighbour oxygen distances which are comparable to the nearest neighbour oxygen

distances of water. The apparent hydrophobicity can then be increased further by the formation of an intramolecular hydrogen bond.

The apparent hydrophobicity of a carbohydrate is quite extraordinary, because a carbohydrate molecule can apparently seem hydrophobic without developing a hydrophobic hydration layer. This is in accordance with recent views on hydrophobic interactions:[18] a hydrophobic hydration layer is not a prerequisite for hydrophobic interactions, but it works against hydrophobic interactions to the extent that the hydrophobic interactions are not as favourable as they could be. Most likely, the interactions with a methine group of a carbohydrate molecule do not need to involve a hydrophobic hydration layer because the hydroxy groups of the carbohydrates fit so well into water that the methine groups can be accommodated in the cavities of water and the hydroxy groups become part of the hydration layer.

6.8 Re-evaluation of some literature data

If some literature data are re-evaluated by considering our modified stereospecific hydration model, some interesting trends emerge.

1) Sweetness of sucrose. The sweetness of sucrose seems to be highly influenced by the relative position of OH(4) in the glucose moiety (1.1.2): "lactosucrose" is not sweet compared to sucrose.[19] With the new stereospecific hydration model this change in sweetness could have been predicted, if sweetness depends on the hydration of a carbohydrate. If this is the case, a disaccharide composed of a talopyranose moiety and a fructofuranose unit should then be much sweeter than sucrose.

2) Cryoprotection. It is generally known that trehalose is a very effective cryoprotectant. In addition, in fish which live in the antarctic waters, galactose-derived glycoproteins[20] are held responsible for the mechanism which prevents freezing.

Both trehalose and galactose are carbohydrates which do not fit very well into the three-dimensional hydrogen-bond network of water in comparison to other carbohydrates. This is also the case for lactose which is about as efficient a cryoprotectant as trehalose.[21] Apparently, compounds which disturb water the most within a series of carbohydrates are the most potent cryoprotectants, perhaps because they can retain more water. Previously it was thought that the best cryoprotectants would be those which fit best in the water structure.

3) Protein specificity. The most important role of carbohydrates is that of molecular messengers on the cell surface.[22] From the results of this study it becomes apparent that carbohydrates might be used as messengers, because they can be recognised as

hydrophobic moieties, but are not hydrophobic in the sense that they will fold back into the hydrophobic core of the cell surface. The trends in hydration behaviour reported in this thesis seem to be reproduced by the specific recognition between lectins and carbohydrates: some lectins are specific for either galactose, or glucose and mannose.[13] Apparently the hydration characteristics of a carbohydrate have a significant role in these recognition processes.

4) Cyclodextrins. Cyclodextrins[23] are known to have a hydrophobic cavity, which is rationalised by the propensity of the glucose units in the cyclodextrin to project their primary hydroxy groups out of the cyclodextrin ring, while the other hydroxy groups are directed inwards. Other explanations of this effect propose that the relative hydrophobicity of the cyclodextrin cavity is a consequence of the formation of an intramolecular hydrogen bond.[24]

An alternative explanation is that the excellent fit of carbohydrates in water makes the endocyclic CHOH moieties so well hydrated that their hydroxy groups are camouflaged for interaction. Hence the hydroxy groups of the cavity of a cyclodextrin might be camouflaged, and therefore the cavity has an enhanced apparent hydrophobicity.

5) Camouflage effect. If the carbohydrates are recognised as hydrophobic moieties in water it seems to be more appropriate to speak of molecular disguise rather than of molecular recognition! The hydroxy groups are not recognised by a spectator molecule, but can disguise themselves. Carbohydrates can disguise their hydrophilic moieties to the extent that they are recognised as relatively hydrophobic solutes in aqueous solutions.

6.9 Final remarks

Since there was an opportunity to study the hydration characteristics of a large number of carbohydrates, it has been possible to probe the dependence of carbohydrate hydration on stereochemistry in some depth.

The understanding of carbohydrate hydration has been improved. Therefore, there is no reason for the pessimism which was expressed in reference 7 (carbohydrate hydration would remain largely unexplained). However, the more detailed investigations described in this thesis have also raised many more questions about carbohydrate hydration.

It remains a difficult problem to describe carbohydrate hydration, because it is only possible to explain results with a rather static model. In this respect some injustice is done to carbohydrates in aqueous solution, because they are not only subject to mutarotation in water, but the ring can also go through considerable transformations especially if there is no hydroxymethyl group in the ring.

The results presented for the disaccharides already illustrate that type of linkage and, for oligo- and polysaccharides the type of branching, will be an extra factor which has to be taken into account in studies of carbohydrate hydration. The behaviour of carbohydrate-derived surfactants (Krafft temperatures, aggregate morphology) in water also predicts that the hydration characteristics of carbohydrate derivatives cannot all be explained using the model based on the hydration of relatively simple carbohydrates.

However, it is hoped that the results presented in this thesis and the proposed stereospecific hydration model will at least help to improve insight into the hydration behaviour of more complex carbohydrate systems.

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SUMMARY

Carbohydrates play an important role in life processes. Not only are they involved in the food cycle, they are also building blocks. Cellulose is present in the cell walls of plants. Glucose and fructose are present in the blood of animals. Fructose is found in fruits and lactose in milk of mammals. At the cell surface, carbohydrates are molecular messengers. In the form of glycoproteins and glycolipids they are important in recognition processes which are, for example, connected to the immunosystem.
In industry there is an increase in the interest in carbohydrates, carbohydrate derivatives and carbohydrate-derived surfactants. Important reasons for these interests include the fact that these materials originate from renewable sources and that the surfactants are calcium-tolerant. Furthermore they are considered to be "green" (biodegradable). The derivatives are non-toxic which makes them applicable for food products as stabilisers, emulsifiers, or substitutes for fats and they can be used as cryoprotectants.
Although carbohydrates are widely used, not much is known about the stereochemical aspects of hydration of carbohydrates. For D-aldohexoses, for example, there are eight different stereoisomers. Just how the hydroxy topology of a carbohydrate molecule influences the hydration behaviour in water is rather unclear.
An effort is being made in this thesis to describe how the hydration depends on the stereochemistry of a carbohydrate.
In chapter one of this thesis, a brief introduction is given on the functions of carbohydrates in biological systems, as sweeteners and in industrial applications. The largest part of the chapter is devoted to a description of the approaches which are being used to study carbohydrate hydration and the models which have been proposed to explain the dependence of hydration on the hydroxy topology.
So far it is not clear which key hydroxy functionalities are important for hydration. The stereospecific hydration model (mainly due to observations made for aqueous glucose solutions) proposes that the hydration of a carbohydrate depends on the number of equatorial hydroxy groups present in the carbohydrate molecule. This is based on the fact that β-D-glucose was found to fit better in water than α-D-glucose, especially because the distance between OH(1) and OH(3) is exactly compatible with the distance of the next-nearest neighbour oxygens in water if OH(1) is equatorial. These conclusions were drawn based on calorimetric and NMR experiments. However, other measurements showed results which were not compatible with this stereospecific hydration model.
All studies, (except NMR spectroscopic studies) have been performed with a rather limited number of carbohydrates. In addition, the investigations provide complicated results because a carbohydrate undergoes (complex) mutarotation in water, which allows the molecule to be present in different forms (pyranose, furanose) in aqueous solutions, unless the anomeric centre is blocked for mutarotation.
In chapter two the hydration of a carbohydrate is probed with a relatively new method: a study of kinetic medium effects. This involves a study of the rate of a reaction (in this case the water-catalysed hydrolysis of the activated amide 1-benzoyl-3-phenyl-1,2,4-triazole) in water and in aqueous solutions of a carbohydrate in different concentrations. The aqueous carbohydrate solution is the medium in which the hydrolysis takes place. This medium has characteristics which differ from water and therefore has an effect on the rate of the reaction.
The medium effect is being caused by interactions of the carbohydrate with the initial state and the activated complex of the reaction, respectively. In this particular case, the medium effect is governed by a stabilisation of the initial state, hence the reaction is retardated compared to pure water. The interactions of a carbohydrate with the initial state and the activated complex of the reaction most probably involve destructive hydration shell overlap. Because the kinetic probe is used for different carbohydrates, the hydration layers of different carbohydrates can be analysed and

compared quantitatively. Even in a series of stereoisomers, like D-aldohexoses, the aldohexoses exert different kinetic medium effects. Apparently, the kinetic medium effect depends on the relative position of OH(2) and OH(4). The relative position of OH(4) is most important and, when OH(4) is axial, the relative position of OH(2) plays a role as well. In addition, for aldopentoses, methyl aldoglycopyranosides, and disaccharides it is found that OH(4) is also of prime importance in determining the kinetic medium effect. If OH(4) is axial, the kinetic medium effect is small and does not depend on stereochemical variations elsewhere in the molecule (except OH(2)). If OH(4) is equatorial, the kinetic medium effect is larger and variations elsewhere in the carbohydrate molecule in terms of type and site of linkage are important for methyl aldoglycopyranosides and disaccharides.
Since all carbohydrates cause a rate retardation compared to pure water they are recognised as relatively hydrophobic solutes in aqueous solutions.
In chapter three, thermodynamic properties of aqueous carbohydrate solutions are studied (partial molar volumes, heat capacities, isentropic and isothermal compressibilities, expansibilities and hydration numbers) for two reasons. First, thermodynamic properties were sought which could further substantiate the results described in chapter two. It appears that partial molar compressibilities (both isentropic and isothermal) and hydration numbers (which are related to the compressibilities) show the same trends as the kinetic medium effects reported in chapter two. It is proposed that the relative position of OH(2) and OH(4) determines the compatibility of a carbohydrate with the three-dimensional hydrogen-bond network of water.
Thermodynamic properties which only represent the characteristics of the hydration layer of a carbohydrate and are not determined by the intrinsic properties of a carbohydrate, are capable of monitoring differences in the stereochemical aspects of hydration. It is explained which thermodynamic parameters reveal the influence of stereochemistry on hydration properties.
In chapter four, an endeavour is made to explain the observations which have been made so far on a molecular level. Particularly the question why the OH(2) and OH(4) are so important for the fit of a carbohydrate in water and for the kinetic medium effect is addressed. First, the oxygen distances of carbohydrates in the crystalline state have been investigated. It appears, that a carbohydrate which has a poor fit in water (D-galactose) has a variety of different next-nearest neighbour oxygen distances. A carbohydrate which fits well into water (D-talose) has oxygen distances in the plane of O_2-O_4-O_5 which are comparable to nearest neighbour oxygen distances in water. For aldohexoses with an average fit in water, it is assumed that their next-nearest neighbour oxygen distances are compatible to the next-nearest neighbour oxygen distances in water. This conclusion is based on the crystal data of D-glucose; the other aldohexoses with an intermediate fit need to be studied to support this conclusion further.
For the aldohexoses with the most different fit in water (D-galactose and D-talose) a molecular dynamics simulation has been performed in water. This simulation has been carried out by E. Howard and J.R. Grigera of the University of La Plata, Argentina. The oxygen distances which were found in the solid state are reproduced after a simulation in water. However, the hydroxymethyl group appears to be very flexible and its relative position seems to depend on the solvent. In conjunction with OH(1) and OH(3), the hydroxymethyl will try to get the best fit in the hydrogen-bond network of water. But the next-nearest neighbour oxygen distances between OH(2), OH(4) and O(5) are most dominantly determining the fit of a carbohydrate into water. This plane of oxygens is the most rigid one and therefore will be the most important.
The average number of hydrogen bonds between water molecules in the presence of a carbohydrate does not differ very much from the average number of hydrogen bonds in pure water. The average number of hydrogen bonds between water and a hydroxy group of a

carbohydrate appears to be similar for OH(1), OH(3), OH(6) and O(5) for both D-talose and D-galactose with respect to hydrogen-bond donor and acceptor capability. However, there are differences for OH(2) and OH(4), which depend on stereochemistry.
For D-galactose the hydrogen-bond acceptor and donor capacity of the OH(2) and OH(4) are comparable to those for the other hydroxy groups in the molecule. However, the OH(2) group of D-talose has a decreased hydrogen-bond donor ability towards water and OH(4) has a decreased hydrogen-bond acceptor capacity towards water. A further investigation into the number of hydrogen bonds of the hydroxy groups shows that the reduced number of *inter*molecular hydrogen bonds is caused by the formation of an *intra*molecular hydrogen bond in D-talose in which OH(2) acts as the hydrogen-bond donor and OH(4) is the hydrogen-bond acceptor.
In chapter five a study of carbohydrate-derived surfactants is described. The synthesis of n-alkyl 1-thioglycopyranosides, their aggregation behaviour in water, their solubilities in water (Krafft temperatures) and their liquid crystalline behaviour are discussed.
The coupling of an n-alkanethiol to the anomeric position of the carbohydrate headgroup can be performed with aid of a Lewis-acid. The choice of the Lewis-acid depends on the relative position of OH(2) with respect to OH(1). If OH(2) is equatorial it can provide anchimeric assistance during the coupling. If a mixture of anomers is formed, the separation of the carbohydrate-derived surfactants can be carried out in the protected as well as in the deprotected form. The method of separation of anomers in the deprotected form depends on the hydroxy topology of the sugar headgroup.
The aggregation behaviour has only been studied for a small number of surfactants. It appears that the critical micelle concentrations of the carbohydrate-derived surfactants depend on the type of headgroup. However, in terms of the Gibbs energy, enthalpy and entropy of micellisation, the differences are rather small.
The solubility of a carbohydrate-derived surfactant in water (Krafft temperature) depends on the relative position of OH(4) in the pyranose ring. All surfactants with an axial OH(4) in the sugar headgroup have a high Krafft temperature, because an axial OH(4) stabilises the crystal structure of the surfactants. If OH(4) is equatorial, only one of the anomers has a high Krafft temperature. All carbohydrate-derived surfactants studied here form a smectic A phase upon heating.
In chapter six the results of the previous chapters are summarised and a modified stereospecific hydration model is proposed.
The relative distances between the next-nearest neighbour oxygens of a carbohydrate determine the compatibility of a carbohydrate in the three-dimensional hydrogen-bond network of water. The distances can be either compatible with the nearest neighbour or next-nearest neighbour oxygen distances in water or have no fit at all.
The better a carbohydrate fits into water, the greater its apparent hydrophobicity will be. This is explained in terms of a camouflage effect. Due to this effect the hydroxy groups of a carbohydrate molecule, which fit into the water structure, are camouflaged for interaction because they resemble the hydroxy groups in water. Therefore the hydrophilic groups of a carbohydrate are not "seen" in water, which leaves the methine groups for interaction. A spectator molecule, which only "sees" methine moieties, will recognise the carbohydrate as a hydrophobic molecule. This camouflage effect can be even stronger in case of D-talose because of the formation of an intramolecular hydrogen bond.

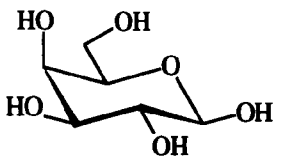
SAMENVATTING

Koolhydraten spelen een belangrijke rol in biologische processen. Niet alleen komen ze in de natuur voor als bouwstenen -zo komt cellulose voor in de celwanden van planten- maar ze worden ook benut als energievoorraad: zetmeel wordt als voedselvoorraad gebruikt en suikers zijn ook betrokken in de Krebbs-cyclus. In het bloed van mensen en dieren komt fructose en glucose voor. Fructose zit natuurlijk ook in fruit. In melk van zoogdieren komt lactose voor. De schaal van schaaldieren is opgebouwd uit koolhydraatderivaten. In de vorm van glycolipiden spelen koolhydraten een belangrijke rol in moleculaire herkenningsprocessen aan het celoppervlak.
Industrieel gezien is er een groeiende belangstelling voor de toepassing van koolhydraten. Koolhydraatafgeleide surfactanten winnen aan populariteit omdat ze biologisch afbreekbaar zijn en uit een vervangbare bron kunnen worden gemaakt. Behalve dat ze ook nog calciumtolerant zijn, zijn koolhydraatafgeleide surfactanten niet giftig, wat toepassing in de voedselindustrie, bijvoorbeeld als vetvervangers, mogelijk maakt. Ze kunnen worden gebruikt bij bescherming tegen uitdroging of bevriezing, maar ook als emulgatoren en stabilisatoren.
Echter, de vraag hoe de eigenschappen van koolhydraten en van koolhydraatafgeleide surfactanten van de stereochemie afhangt is nog steeds onbeantwoord.
Als voorbeeld kunnen aldohexosen worden genomen. Dit zijn aldosesuikers met zes koolstofatomen en vier chirale centra. Dat betekent, dat er 2^4 verschillende aldohexose-stereoisomeren bestaan, die op te splitsen zijn in D- en L-suikers. De D-aldohexosen komen voor in acht verschillende vormen, ieder met een andere hydroxytopologie.
In dit proefschrift wordt de vraag gesteld hoe de hydratatie van een koolhydraat in waterige oplossingen afhangt van de hydroxytopologie d.w.z. van de stereochemie van het molecuul. Om hier inzicht in te verkrijgen zijn waterige oplossingen van aldopentosen, aldohexosen, ketohexosen, methylaldoglycopyranosiden en disachariden bestudeerd met behulp van verschillende technieken.
In de literatuur wordt gesuggereerd, dat de hydratatie van een koolhydraat primair wordt bepaald door het aantal equatoriale hydroxygroepen dat aanwezig is in het koolhydraatmolecuul. De redenering hierachter is dat een equatoriale hydroxygroep beter in de waterstructuur past dan een axiale hydroxygroep. Het merendeel van de onderzoeksresultaten betreffende de hydratatie van suikers is echter niet in overeenstemming met dit thans geaccepteerde stereospecifieke hydratatiemodel.
Tot nu toe is het onderzoek naar koolhydraathydratatie steeds beperkt geweest tot een klein aantal monosachariden. Behalve bij NMR-onderzoeken aan aldohexosen in water, is nog nooit een volledige set aldohexosen bestudeerd.
Het onderzoek wordt bemoeilijkt doordat een koolhydraat (complexe) mutarotatie kan ondergaan in water en nooit aanwezig is in slechts één vorm in water, tenzij het anomere centrum geblokkeerd is voor mutarotatie. Zo kan een koolhydraat zowel in de furanose- als in de pyranosevorm voorkomen in water.
In hoofdstuk één wordt de rol van koolhydraten in biologische systemen kort besproken. De industriële toepassingen worden kort behandeld. Verder wordt er ingegaan op de experimentele beperkingen die verbonden zijn aan een onderzoek van koolhydraathydratatie. Immers men wil slechts de invloed van de stereochemie op de hydratatie bepalen, en de effecten van mutarotatie vermijden.
De tot nu toe gebruikte methoden in deze studies en de modellen die zijn geponeerd om stereochemische aspecten van koolhydraathydratatie te rationaliseren worden uiteengezet.
De resultaten van een studie van de hydratatie van koolhydraten met een kinetische probe zijn samengevat in hoofdstuk twee. De snelheid van de water-gekatalyseerde hydrolyse van een geactiveerde amide, 1-benzoyl-3-fenyl-1,2,4-triazool, werd bestudeerd in water en in waterige oplossingen van suikers in verschillende concentraties. De

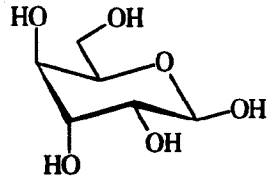
koolhydraten vormen samen met water het medium waarin de hydrolyse plaatsvindt. Het medium heeft andere eigenschappen dan water en heeft daardoor een effect op de reactiesnelheid. Dit mediumeffect wordt veroorzaakt doordat het koolhydraatmolecuul een interactie aangaat met de begintoestand en het geactiveerde complex van de reactie. In dit geval wordt de begintoestand van de reactie door deze interactie gestabiliseerd en wat leidt tot vertraging van de reactie t.o.v. puur water. De interacties van het koolhydraatmolecuul met de begintoestand en het geactiveerde complex van de reactie vinden hoogstwaarschijnlijk plaats door destructieve hydratatieschiloverlap van de respectievelijke hydratatieschillen. Het effect kan met een reeds bestaande theorie gekwantificeerd worden.
Omdat dezelfde kinetische probe is gebruikt met verschillende koolhydraten, en het kinetisch mediumeffect van een koolhydraat gekwantificeerd kan worden, is er een mogelijkheid de hydratatieschil van een koolhydraat te onderzoeken.
Zelfs in een serie van isomeren -zoals aldohexosen- bewerkstelligt niet ieder aldohexosemolecuul eenzelfde kinetisch mediumeffect. Het blijkt dat het kinetisch mediumeffect afhangt van de relatieve posities van OH(2) en OH(4) in het koolhydraatmolecuul. Daarbij lijkt eerst de relatieve positie van OH(4) van belang te zijn en, als deze axiaal is, is ook de relatieve positie van OH(2) belangrijk. Voor ketohexoses lijkt het kinetisch mediumeffect niet te worden beinvloed door de stereochemie. Voor aldopentosen, methylaldoglycopyranosiden en disachariden wordt ook gevonden, dat de relatieve positie van OH(4) van doorslaggevend belang is voor het kinetisch mediumeffect. Als OH(4) axiaal is, is het kinetisch mediumeffect het kleinst en is er geen afhankelijkheid van andere variaties in stereochemie. Als OH(4) equatoriaal is, is het kinetisch medium effect groter en zijn variaties in type van binding in methylaldoglycopyranosiden en disachariden ook van belang.
Interessant is voorts dat alle koolhydraten een dusdanig kinetisch mediumeffect induceren (ze vertragen alle de reactie), dat het lijkt alsof ze worden herkend als hydrofobe verbindingen in waterige oplossingen.
In hoofdstuk drie worden thermodynamische grootheden van waterige oplossingen van koolhydraten (partieel molaire volumes, warmtecapaciteiten, isentrope en isotherme compressibiliteiten, expansibiliteiten en hydratatiegetallen) bestudeerd. Er wordt gezocht naar een thermodynamische grootheid die de trend gevonden in hoofdstuk twee kan reproduceren. Het blijkt, dat partieel molaire compressibiliteiten (zowel isotherm als isentroop) en hydratatiegetallen (die zijn gerelateerd aan de compressibiliteiten) dezelfde trends als die in hoofdstuk twee laten zien. De relatieve posities van OH(2) en OH(4) blijken primair bepalend voor het passen van een koolhydraat in het driedimensionale waterstofbrugnetwerk van water.
Alleen grootheden, die gevoelig zijn voor kleine verschillen in hydratatie en slechts informatief zijn voor het karakter van de hydratatieschil van een koolhydraat, kunnen stereospecifieke hydratatieeffecten meten. Er wordt een beschrijving gegeven van de thermodynamische grootheden die in principe aan deze voorwaarde voldoen.
In hoofdstuk vier wordt onderzocht waarom nu juist OH(2) en OH(4) zo belangrijk zijn voor het passen van een koolhydraat in water. Daartoe zijn zuurstofafstanden in het koolhydraatmolecuul -en wel specifiek de afstanden tussen α-γ-zuurstofatomen- bekeken. Dit is eerst gedaan voor koolhydraten in de kristallijne toestand. De α-γ-zuurstofafstanden zijn vervolgens vergeleken met de zuurstofafstanden in water. Het blijkt, dat een suiker die niet past in water (D-galactose), zeer gevarieerde α-γ-zuurstofafstanden heeft. Een aldohexose die goed past in water (D-talose), lijkt in het vlak van OH(2) en OH(4) zuurstofafstanden te hebben, die vergelijkbaar zijn met de naast elkaar gelegen zuurstofatomen in water. Voor aldohexosen met een gemiddelde fit in water, wordt aangenomen dat ze hun α-γ-zuurstofatoomafstanden kunnen aanpassen aan de afstanden tussen de op één na dichtstbijzijnde zuurstofatomen in water. Deze conclusie is gebaseerd op de resultaten voor D-glucose; voor de andere aldohexosen met een gemiddelde fit in water zal dit nog verder bestudeerd moeten worden.

Vervolgens zijn de aldohexosen met het meest verschillende hydratatiegedrag (D-galactose en D-talose) aan een moleculaire dynamica simulatie onderworpen in waterige oplossing. Deze simulatie werd uitgevoerd door E. Howard en J.R. Grigera aan de Universiteit van LaPlata, Argentinië. De zuurstofafstanden die gevonden waren in de vaste toestand, worden gereproduceerd in de simulatie. Echter de hydroxymethylgroep blijkt zeer flexibel te zijn en zijn positie blijkt af te hangen van het oplosmiddel. Samen met OH(1) en OH(3) kan de hydroxymethylgroep zo goed mogelijk een fit proberen te krijgen in het waterstofbrugnetwerk in water. Echter de relatieve α-γ-zuurstofafstanden tussen OH(2), OH(4) en O(5) zijn het meest bepalend voor de fit van een aldohexose in de waterstructuur. Dit vlak van zuurstofatomen is het meest star en zal daarom het belangrijkst zijn voor de kwaliteit van de fit.
Het gemiddelde aantal waterstofbruggen, dat tussen watermoleculen wordt gevormd in aanwezigheid van een koolhydraat tijdens een simulatie gedurende 50 ps verschilt niet sterk van dat voor puur water. Daaruit volgt, dat het aantal waterstofbruggen tussen watermoleculen maar weinig wordt beïnvloed door de aanwezigheid van een koolhydraat. Het gemiddelde aantal waterstofbruggen, dat een hydroxygroep van een koolhydraat aangaat met water lijkt voor OH(1), OH(3) en OH(6) en O(5) voor zowel D-galactose als D-talose hetzelfde te zijn. Echter voor OH(2) en OH(4) is dit aantal sterk afhankelijk van de stereochemie van het koolhydraatmolecuul. Voor D-galactose zijn de H-brugacceptor- en donorcapaciteiten vergelijkbaar met die van de andere hydroxygroepen. D-talose is echter een uitzondering: OH(2) blijkt een sterk gereduceerde H-brug-donorcapaciteit naar water te hebben, terwijl OH(4) een zeer laag aantal waterstofbruggen aangaat met water als waterstofbrugacceptor. Nader onderzoek laat zien dat dit wordt veroorzaakt door de vorming van een intramoleculaire waterstofbrug tussen OH(2) en OH(4).
Mede door deze resultaten wordt gesuggereerd dat het passen van een aldohexose in water op drie manieren kan worden bewerkstelligd. De zuurstofafstanden tussen de α-γ-zuurstofatomen in het koolhydraatmolecuul kunnen overeenkomen met de afstanden van de dichtstbijzijnde of de op één na dichtstbijzijnde zuurstofatomen in water, of passen in het geheel niet in de waterstructuur.
In hoofdstuk vijf worden surfactanten afgeleid van koolhydraten beschreven. Aandacht wordt besteed aan de synthese van n-alkyl 1-thioglycopyranosiden, alsmede aan hun aggregatiegedrag, oplosbaarheid in water (Krafttemperaturen) en vloeibaar kristallijn gedrag.
De koppeling van een n-alkaanthiol aan de anomere positie van een suiker kan met Lewis-zuren worden bewerkstelligd. De keuze van het Lewis-zuur is afhankelijk van de relatieve positie van O(2) t.o.v. O(1). Belangrijk is of de substituent op C-2 beschikbaar is voor anchimere assistentie bij de koppeling van de suikergroep met het n-alkaanthiol.
Als tijdens de synthese een mengsel van anomeren wordt gevormd, hangt de splitsingsmethode voor de anomeren van de derivaten in de ontschermde vorm sterk af van de hydroxytopologie van de suikerkopgroep.
De oplosbaarheid (T_{Kraft}) van een koolhydraatsurfactant in water blijkt sterk af te hangen van de relatieve positie van OH(4) in de ring. Alle surfactanten met een axiale OH(4) in de suikerkopgroep hebben een hoge Krafttemperatuur omdat een axiale OH(4) het kristalrooster van de surfactanten stabiliseert. Als OH(4) equatoriaal is, heeft slechts één van beide anomeren een hoge Krafttemperatuur. Alle van koolhydraat afgeleide surfactanten vormen bij verwarming een smectische A fase.
Qua aggregatiegedrag is slechts een klein deel van de gesynthetiseerde verbindingen bestudeerd: voorlopig lijkt het erop, dat de kritische micelconcentratie (cmc) wordt beïnvloed door de stereochemie van de koolhydraatkopgroep. De verschillen in de Gibbs-energie, enthalpie en entropie van micellisering zijn echter klein.
In hoofdstuk zes worden alle resultaten van de voorgaande hoofdstukken samengevat en wordt een gemodificeerd stereospecifiek hydratatiemodel voorgesteld.

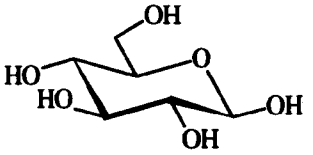
De relatieve afstanden van de α-γ-zuurstofatomen in een koolhydraatmolecuul zijn bepalend voor het passen van een koolhydraat in het driedimensionale waterstofbrugnetwerk van water. De afstanden kunnen overeenkomen met die van naast elkaar gelegen zuurstofatomen in water of met die van op één na dichtstbijzijnde zuurstofatomen in water, of ze kunnen zich niet aanpassen aan de zuurstofafstanden in water.
Des te beter een koolhydraat past in water, des te groter zal de schijnbare hydrofobiciteit zijn door een camouflage-effect. Immers, des te beter een koolhydraat past in water, des te meer zullen de hydroxygroepen van een koolhydraat lijken op de hydroxygroepen van water. Daardoor worden de hydrofiele groepen van een koolhydraat niet "opgemerkt" en wordt een koolhydraatmolecuul herkend als een relatief hydrofoob molecuul. Interactie tussen het koolhydraat en een ander opgelost molecuul wordt dan vooral bepaald door de methine groepen. Het camouflage-effect kan in het geval van D-talose nog versterkt worden door de vorming van een intramoleculaire waterstofbrug.



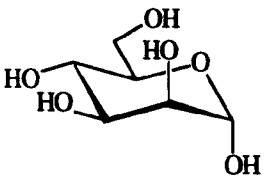
D-galactose 3a



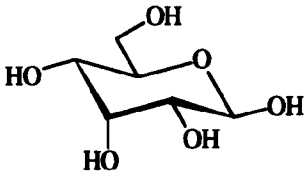
D-gulose 3b



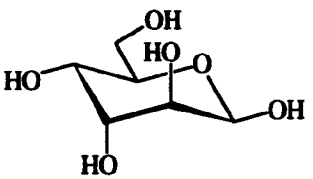
D-glucose 3c



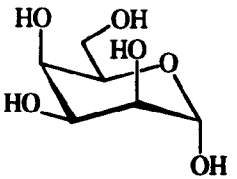
D-mannose 3d



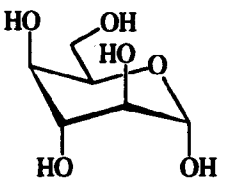
D-allose 3e



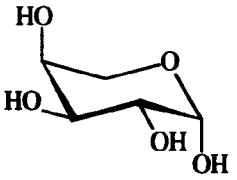
D-altrose 3f



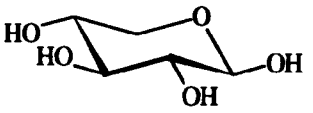
D-talose 3g



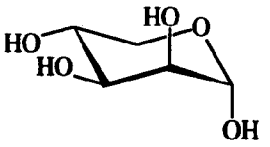
D-idose 3h



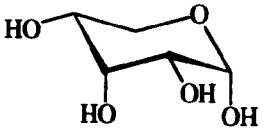
L-arabinose 2b



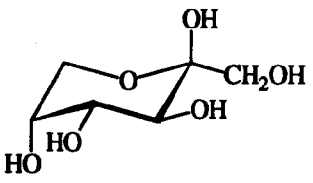
D-xylose 2c



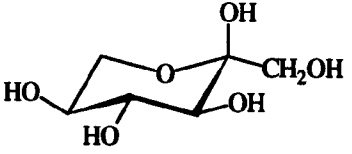
D-lyxose 2d



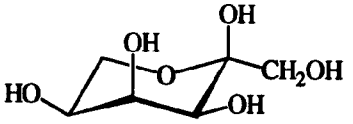
D-ribose 2e



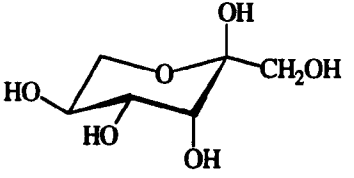
D-fructose 4a



L-sorbose 4b



D-psicose 4c



D-tagatose 4d