Effect of intramolecular hydrogen-bonding network on the relative reactivities of carbohydrate OH groups †

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In our efforts toward the development of enzyme-like catalysts for regioselective functionalization of unprotected sugars, DMAP-catalyzed acetylation of unprotected carbohydrates in chloroform was investigated. Product distributions of monoacetylated sugars were determined under kinetic control. To accurately evaluate the relative reactivity of each OH group, reaction conditions were used under which only monoacetylated sugars were obtained and almost no diacetylated sugars were formed as by-products. Systematic acetylation experiments of glucose, mannose, and galactose revealed the decisive role of intramolecular hydrogen-bonding networks among carbohydrate OH groups. The relative reactivities in the DMAP-catalyzed acetylation were successfully correlated with the calculated proton affinity of each OH group in carbohydrates.

Introduction

Biological functions of oligosaccharides depend not only on the sequence of sugar residues but also on the position where the next sugar residue is attached.[1] In order to construct diverse oligosaccharides with biological functions such as cell recognition, Nature has developed enzymes called glycosyltransferases which introduce residues regiospecifically to a particular position. These enzymes have been effectively utilized to prepare complex oligosaccharides in the laboratory.[2] However, exactly how glycosyltransferases activate one particular hydroxy group among many other OHs of similar reactivity is shrouded in mystery. It is one of the big challenges for chemists to understand the chemical nature of this fascinating enzyme reaction.

In the field of synthetic organic chemistry, the efficient synthesis of oligosaccharides is still under active investigation.[3] However, most research projects have mainly focused on the control of the anomer stereochemistry, and the control of regiochemistry is usually achieved by multiple protection–deprotection procedures. A protection–deprotection strategy in synthetic carbohydrate chemistry was highlighted in the preparation of oligosaccharide libraries from one monosaccharide unit as a core.[4] Difficulties arising from similar reactivities of OH groups were overcome by using a designed building block containing four selectively removable protecting groups as acceptors for glycosidation. Another approach to the control of regiochemistry of carbohydrate OH groups is metal-assisted activation of OH groups. For this purpose, organotin derivatives[5a] have been studied for a long time and recently organoboron derivatives[5b] have been utilized.

Without the help of enzymes it is usually extremely difficult to directly functionalize a desired OH group of unprotected carbohydrates except for the primary OH groups. In addition, many studies have shown that OH groups in unprotected carbohydrates often exhibit anomalous reactivity.[6] Especially in the case of oligosaccharides and nucleosides, the electrophile functionalizes exclusively at the secondary hydroxy group instead of the usually more reactive primary one.[6,7] For a complete understanding of unusual reactivities of carbohydrate OH groups, Krepinsky, Csizmadia and co-workers reported a series of studies on reduced reactivities of the primary 5′-OH group of nucleosides, which are generally observed in the reaction with various electrophiles.[8] They pointed out the presence of strong intramolecular hydrogen bonding between the 5′-OH group and the heteroaromatic system in their simple model system by *ab initio* calculations. They proposed that strong intramolecular hydrogen bonding might prevent hydrogen abstraction. Brewster *et al.*[9a] and Houdier and Pérez[9b] reported theoretical investigations of the relative reactivities of oligosaccharide OH groups in compounds such as β-maltose and sucrose. They evaluated the acidities of OH groups by semiempirical calculations and found a moderate correlation with the increased reactivity of the 2-OH group. They proposed a key role for intramolecular hydrogen bonds of some kind. On the other hand, a systematic survey on the reactivity of simple monosaccharides is quite rare, although each monosaccharide was investigated by different researchers under different reaction conditions and procedures.[6] According to these results, primary 6-OH groups were functionalized far more preferentially than were other, secondary OH groups. The reactivity orders of secondary OH groups were not determined without ambiguity. It is often said that an equatorial OH group in a six-membered ring system can be functionalized preferentially in the presence of secondary axial partners, but it has been repeatedly pointed out that the relative reactivities of monosaccharide OH groups are highly dependent on the reaction conditions employed. To our knowledge, no intensive effort has been made to clarify the relative reactivities in terms of monosaccharide structures.

The ultimate objective of our work is to construct enzyme-like catalysts which introduce substituents regioselectively to a desired OH group through noncovalent interactions. Recently, chiral nucleophilic catalysts for kinetic resolution of racemic alcohols have been actively studied. In some of these examples, enzyme-like chiral cavities were constructed around DMAP, 4-pyrrolidinopyridine or imidazole to promote enantioselective acylation.[10] This strategy can be applied to regioselective acylation of carbohydrates through noncovalent bonding by providing a carbohydrate-recognition site for the nucleophilic catalysts (Fig. 1). In this context, we started investigation of DMAP-catalyzed acetylation of unprotected carbohydrates. Unexpectedly, we found that secondary OH groups of glucose

† Structural characterization of the partially acetylated carbohydrates is available as supplementary data (SUPPL. NO. 57474, pp. 15) from the British Library. For details of the Supplementary Publications Scheme, see 'Instructions for Authors', *J. Chem. Soc.*, *Perkin Trans. 1*, available *via* the RSC web page (http://www.rsc.org/authors).

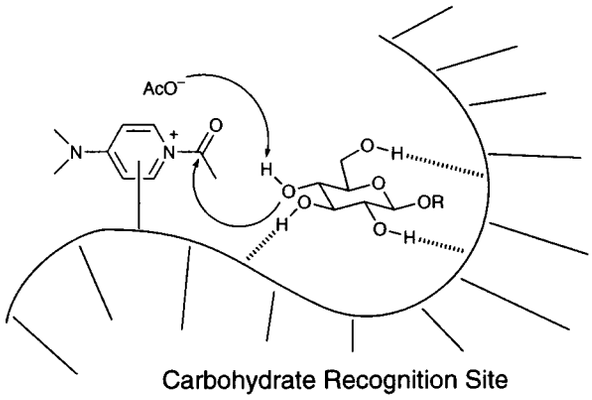


Fig. 1 Schematic representation of regioselective acetylation of carbohydrates through noncovalent interactions.

were preferentially acetylated in the presence of the primary OH group at position 6. Sterically unhindered primary OH groups at position 6 are supposed to be acetylated far more readily according to the usual stereochemical analysis. Indeed, many reactions are known to derivatize selectively the primary alcohols of carbohydrates.[11]

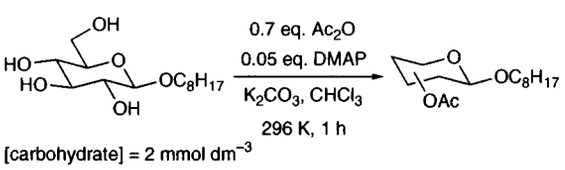
In order to clarify operating forces which determine the selectivity in the DMAP-catalyzed acetylation system, we conducted systematic acetylation experiments of glucose, mannose, and galactose and discussed structure–reactivity relationships in detail. Herein we address the decisive role of an intramolecular hydrogen-bonding network among neighboring OH groups in the relative reactivities of carbohydrate OH groups.

Results

Reaction conditions for DMAP-catalyzed acetylation

The following three points are important to accurately evaluate the relative reactivities of carbohydrate OH groups. (1) Non-hydrogen-bonding, non-polar solvents should be used in which hydrogen bonding is effective. (2) Introduction of more than two acetyl groups should be suppressed because both analysis of reaction mixtures and interpretation of obtained results become markedly difficult. (3) Neutral, mild reaction conditions and a short reaction period should be employed to avoid acetyl-group migration that gives thermodynamically stable regioisomers.

We adopted the reaction conditions shown in Scheme 1. We



Scheme 1

used chloroform as a non-polar solvent that readily dissolves various organic compounds. To solubilize highly hydrophilic carbohydrates in chloroform, a long alkyl chain was introduced onto the anomeric OH group. It is well known that carbohydrates solubilized in non-polar solvents form aggregates at high concentrations.[12] So reactions were carried out tentatively at 2 mmol dm^{-3} to minimize the effect of aggregation (for a detailed examination of the effect of the monosaccharide concentration, see below). To bind liberated acetic acid, K_2CO_3 was used because soluble bases might disturb hydrogen bonding. By use of 5 mol% of DMAP, acetic anhydride was completely consumed in 1 h.

Determination of the product distribution

Regioisomeric mixtures obtained by acetylation were analyzed

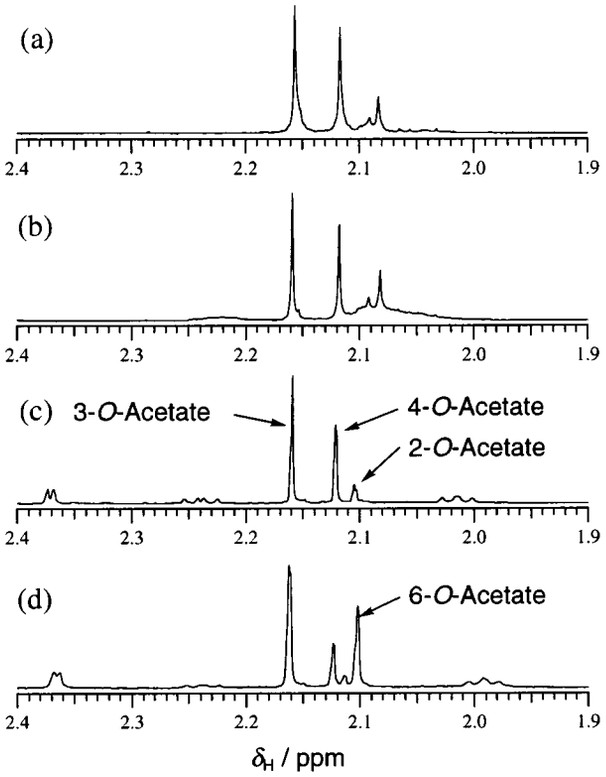


Fig. 2 ^{1}H NMR spectra of the reaction mixture and partially purified samples in the DMAP-catalyzed acetylation of octyl β-D-glucopyranoside. (a) Before silica gel short column. (b) After silica gel short column. (c) One of GPC fractions containing octyl 2-, 3- and 4-*O*-acetyl-β-D-glucopyranoside. (d) One of the GPC fractions containing octyl 6-*O*-acetyl-β-D-glucopyranoside.

according to the procedure described below. (1) DMAP was removed by passage through a short silica gel column. (2) The crude product was dried *in vacuo* at 333 K for 4 h. (3) The yield of each regioisomer was determined by integration of the corresponding acetyl group at $\delta \sim 2.1$ in the ^{1}H NMR spectrum (Fig. 2b). In Fig. 2a is shown the ^{1}H NMR spectrum of the reaction mixture before passage through a short silica gel column. Comparison of Fig. 2b with Fig. 2a indicated that no isomerization took place upon a silica gel short-column operation. (4) Next, the crude product was separated into two fractions by gel-permeation chromatography (GPC) to characterize each regioisomer (Fig. 2c, 2d). Structural characterization of each regioisomer was done mainly by Double-Quantum Filtered COSY (DQF-COSY). Two major regioisomers formed were identified as the 3- and 4-*O*-acetate as shown in Fig, 2. Unexpectedly, 3- and 4-acetylated glucopyranosides were formed in much higher yields in the presence of the primary 6-OH group.

As shown in Fig. 2, all major signals at $\delta \sim 2.1$ were assigned to monoacetyl glucopyranosides. It should be noted that no diacetylated product was formed at all.

Relative reactivities of OH groups in glucose, mannose, and galactose

The same acetylation conditions were applied to glucose, mannose, and galactose to investigate the stereochemical effect on the relative reactivities of carbohydrate OH groups. Product distributions were determined in exactly the same manner. The results are summarized in Table 1. Every acetylation experiment was repeated twice to confirm the reproducibility.

Intriguing trends were observed. In all cases, 2-OH groups were found to be least reactive, irrespective of the stereochemistry of the 2-OH group and the neighboring anomeric position. The product ratio of 6-*O*-acetylated sugars increased in the order glucose < mannose < galactose. Total yields of

Table 1 DMAP-catalyzed acetylation of monosaccharides[a]

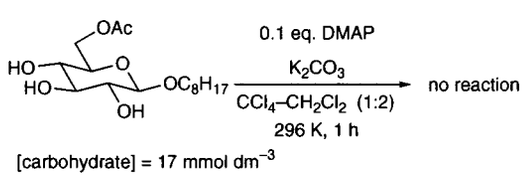
Substrate	Product ratio (yield of one regioisomer/total yield)				
	2-*O*-Acetate	3-*O*-Acetate	4-*O*-Acetate	6-*O*-Acetate	Total yield (%)[b]
Octyl β-Glc	0.02	0.42	0.37	0.19	quant.
Octyl α-Glc	*c*	0.25	0.61	0.14	98
Octyl β-Man	*c*	0.22	0.46	0.32	72
Octyl α-Man	0.07	0.16	0.40	0.37	75
Octyl β-Gal	*c*	0.16[d]	0.14[d]	0.70	81
Octyl α-Gal	*c*	0.14[d]	0.28[d]	0.58	73

[a] Reaction conditions are shown in Scheme 1. [b] NMR yield relative to the initial amount of Ac_2O. [c] Not detected. [d] Product ratio could not be determined accurately due to poor separation of signals.

monoacetyl-mannoses and -galactoses were decreased compared with monoacetylglucoses under exactly the same reaction conditions, suggesting that reactivities of secondary OH groups were considerably suppressed in the case of mannose and galactose. We would like to point out several other interesting observations on the stereochemical effect. Comparison between glucose and mannose showed that the stereochemistry of the 2-OH apparently affected the reactivity of the distant 6-OH group. In the case of glucose, the stereochemistry of the anomeric position affected the relative reactivities of the 3- and 4-OH groups whereas both anomers of mannose and galactose exhibited similar reactivities. The relative reactivity of the 6-OH group in galactose was considerably increased compared with glucose, which was caused by the inversion of the stereochemistry of the 4-OH group.

Possibility of acetyl migration

Surprisingly, secondary 3- and 4-OH groups of glucopyranosides were acetylated more readily than were the primary 6-OH groups. One possible explanation for this unexpected result is as follows: An acetyl group was incorporated into the most reactive 6-OH first. The introduced acetyl group migrated intramolecularly to the neighboring 4-OH group and then to the 3-OH group by the action of DMAP. To check this possibility, 6-*O*-acetylated glucopyranoside was prepared independently and exposed to similar reaction conditions as shown in Scheme 2. No acetyl migration was observed and the possibility of acetyl migration was ruled out.



Scheme 2

Detailed examinations of the reaction conditions

The reaction conditions employed in this study were examined thoroughly to find decisive factors for observed selectivities. First, the concentration of carbohydrates was varied from 0.5 to 10 mmol dm^{-3} (Table 2). ^1H NMR dilution experiments showed that octyl β-D-glucopyranoside formed intermolecular hydrogen bonds with each other at ~10 mmol dm^{-3} in $CDCl_3$ at 295 K.[12] Although a small amount of diacetylated glucopyranoside was formed at 10 mmol dm^{-3}, negligibly small differences in the product distribution were observed. So selectivity seems to originate chiefly in intramolecular forces, rather than intermolecular factors like aggregation. Next, the effect of DMAP was investigated (Table 3). In the presence of DMAP, product distributions were identical irrespective of the concentration of DMAP. In contrast, the 6-*O*-acetylated product was the major one in the absence of DMAP although the yield was very low. Interestingly, the product distribution in the absence of DMAP is closely similar to the reported selectivity of glucopyranosides.[6] *N*-Acetylpyridinium acetate, an effective acetylating reagent formed from DMAP and acetic anhydride, seems to be the key to the unique selectivity. The time dependence of the product distribution was next investigated. As shown in Table 4, product distributions did not change as a function of the reaction time. Together with the above consideration of the possibility of acetyl migration of independently prepared 6-*O*-acetlyated glucopyranoside, this result suggests that acetyl migration is negligible in this reaction system and that the product distribution is determined under kinetic control. In Table 5 is shown the influence of an added bulky base, 2,6-dimethylpyridine (2,6-lutidine). No appreciable effect was observed for the distribution of each regioisomer. This means acidic elements in the reaction system, such as acetic anhydride or liberated acetic acid, do not participate in determining product distributions. In addition, the above result suggests the deprotonation step in the acetylation reaction does not affect product distributions. It is notable that operating forces are strong enough to maintain the unique selectivity by the addition of a considerable amount of base. Finally, K_2CO_3 was replaced by 2,6-lutidine (Table 6). No change was observed.

As demonstrated from the above investigation, the observed selectivity is not dependent on any element in the reaction conditions when the acetylation is catalyzed by DMAP. In the

Table 2 Dependence of the product distribution on the concentration of octyl β-D-glucopyranoside in the DMAP-catalyzed acetylation of octyl β-D-glucopyranoside

[β-Glc] (mmol dm^{-3})[a]	Product ratio (yield of one regioisomer/total yield)				
	2-*O*-Acetate	3-*O*-Acetate	4-*O*-Acetate	6-*O*-Acetate	Total yield (%)[b]
0.5	0.04	0.45	0.36	0.15	33
2.0	0.02	0.42	0.37	0.19	quant.
10[c]	0.10	0.37	0.32	0.21	91

[a] Other reaction parameters are shown in Scheme 1. [b] NMR yield relative to the initial amount of Ac_2O. [c] A small amount of diacetylated sugar was formed at this concentration.

Table 3 Dependence of the product distribution on the concentration of DMAP in the DMAP-catalyzed acetylation of octyl β-D-glucopyranoside

[DMAP] (mol%)[a]	Product ratio (yield of one regioisomer/total yield)				
	2-*O*-Acetate	3-*O*-Acetate	4-*O*-Acetate	6-*O*-Acetate	Total yield (%)[b]
0	0.21	0.19	0.14	0.46	4
1	0.06	0.51	0.32	0.11	53
5	0.02	0.42	0.37	0.19	quant.
10	0.09	0.41	0.36	0.14	quant.

[a] Other reaction parameters are shown in Scheme 1. [b] NMR yield relative to the initial amount of Ac_2O.

Table 4 Dependence of the product distribution on the reaction time in the DMAP-catalyzed acetylation of octyl β-D-glucopyranoside

Time (*t*/h)[a]	Product ratio (yield of one regioisomer/total yield)				
	2-*O*-Acetate	3-*O*-Acetate	4-*O*-Acetate	6-*O*-Acetate	Total yield (%)[b]
1	0.06	0.51	0.32	0.11	53
6	0.03	0.41	0.39	0.17	quant.
24	0.05	0.47	0.37	0.11	quant.

[a] 1 mol% of DMAP was used. Other reaction parameters are shown in Scheme 1. [b] NMR yield relative to the initial amount of Ac_2O.

Table 5 Effect of added 2,6-lutidine on the product distribution in the DMAP-catalyzed acetylation of octyl β-D-glucopyranoside

	Product ratio (yield of one regioisomer/total yield)				
[Lutidine] (vol%)[a]	2-*O*-Acetate	3-*O*-Acetate	4-*O*-Acetate	6-*O*-Acetate	Total yield (%)[b]
0	0.02	0.42	0.37	0.19	quant.
2.6	0.04	0.45	0.37	0.14	quant.
11	0.02	0.46	0.36	0.16	56

[a] Other reaction parameters are shown in Scheme 1. [b] NMR yield relative to the initial amount of Ac_2O.

Table 6 Effect of K_2CO_3 on the product distribution in the DMAP-catalyzed acetylation of octyl β-D-glucopyranoside

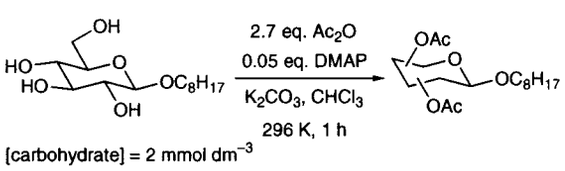
	Product ratio (yield of one regioisomer/total yield)				
Base[a]	2-*O*-Acetate	3-*O*-Acetate	4-*O*-Acetate	6-*O*-Acetate	Total yield (%)[b]
K_2CO_3	0.02	0.42	0.37	0.19	quant.
2,6-Lutidine[c]	0.05	0.44	0.37	0.14	97

[a] Other reaction parameters are shown in Scheme 1. [b] NMR yield relative to the initial amount of Ac_2O. [c] 5 Equiv. of 2,6-lutidine relative to octyl β-D-glucopyranoside was used.

present reaction system, each regioisomer was kinetically produced irrespective of its thermodynamic stability. It was elucidated that the 3- and 4-OH groups are activated towards *N*-acetylpyridinium acetate compared with the primary 6-OH group without any outside assistance. For further discussion on reactivity–structure relationships, it is important to note that intramolecular forces matter most in the present acetylation system. The unique selectivity seems to originate in the monosaccharide structure.

Influence of the incorporated acetyl group on the second acetylation

In order to obtain insight into the nature of the unique selectivities, we investigated how derivatization of one particular hydroxy group affects the rate of acetylation at other positions. Then we performed a simple experiment in which two acetyl groups were introduced into octyl β-D-glucopyranoside upon treatment with more than 2 equiv. of acetic anhydride (Scheme 3) and the product distribution of diacetylglucopyranosides



Scheme 3

was investigated. Fig. 3 shows the ^{1}H NMR spectrum at $\delta \sim 2.1$ for the obtained reaction mixture. Two signals of the same intensity were observed at δ 2.092 and 2.152 together with acetyl-group signals of monoacetylated glucopyranosides. After careful purification, it was found that these two intense signals were coming from diacetylated glucopyranoside with OAc groups at positions 3 and 6. Of five possible regioisomers, the 3,6-di-*O*-acetyl-β-D-glucopyranoside was selectively formed. Formation of other regioisomers seemed to be considerably suppressed, although they were not identified.

In the case of monoacetylation, 3-*O*-acetyl- and 4-*O*-acetyl-β-D-glucopyranosides were produced in nearly equal amounts as major products and the formation of 6-*O*-acetyl-β-D-glucopyranoside was remarkably suppressed as described in the

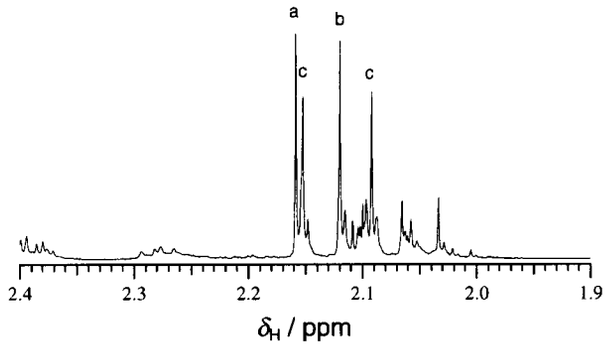


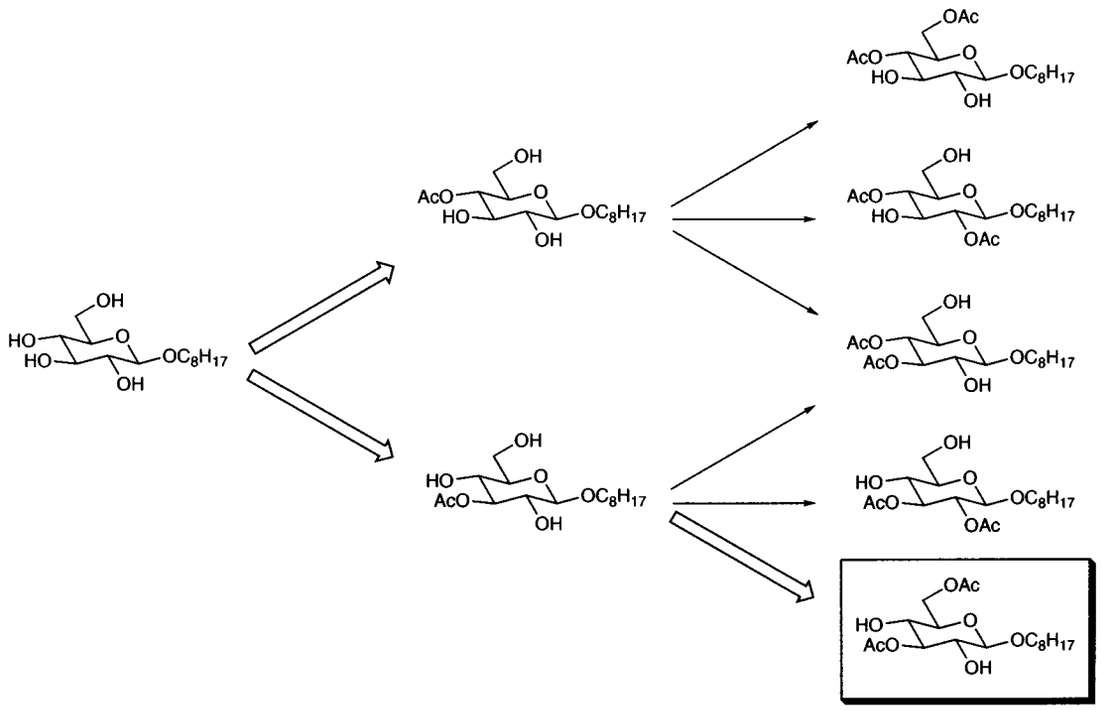
Fig. 3 The ^{1}H NMR spectrum of the reaction mixture containing monoacetylated and diacetylated glucoses. [a] Octyl 3-*O*-acetyl-β-D-glucopyranoside. [b] Octyl 4-*O*-acetyl-β-D-glucopyranoside. [c] Octyl 3,6-di-*O*-acetyl-β-D-glucopyranoside.

previous section. The second acetylation reaction is supposed to proceed through monoacylated sugars of this product distribution. Therefore the dominant diacetylation pathway is considered as one shown in bold arrows in Scheme 4, *i.e.* the first acetyl group was introduced onto the 3-OH group and then the second onto the 6-OH group. In other words, the acetyl group introduced at position 3 considerably reduced the reactivity of the neighboring 4-OH group, which showed one of the highest reactivities in monoacetylation. On the other hand, the acetyl group at position 4 does not seem to affect the reactivity of the neighboring 3-OH group. Lack of formation of the diacetylated glucopyranoside at positions 4 and 6 suggests that the 3-OH group still has relatively high reactivity after acetylation of the 4-OH group and competes with the primary 6-OH group.

Discussion

Our results reported here are probably the first example of a systematic investigation into the relative reactivities of monosaccharide OH groups. As acetylation experiments were conducted under exactly the same reaction conditions, product distributions can be compared with each other and discussed in detail from the viewpoint of structure–reactivity relationships. In addition, the present DMAP-catalyzed acetylation reaction is superior in the following two points. To begin with, we have to point out that reaction conditions were not well controlled in past studies. In those studies, relative reactivities were estimated based on the reaction mixtures consisting of monoacetylated, diacetylated and further acetylated sugars and they failed to take into account the effect that substitution at one hydroxy group might have on the rate of substitution at another.[6] We have demonstrated that an acetyl group incorporated at a particular position altered the reactivities of the other hydroxy group(s) dramatically. So the reactivities of OH groups in unprotected monosaccharides must be evaluated from the ratio of monoacetylated sugars without the concomitant formation of more acetylated sugars. As is clearly seen in Fig. 2, the present study fulfilled this criteria.

Another problem to note is the possibility of intramolecular acetyl migration. It is well known that an acyl group can easily migrate to a neighboring OH group especially under basic conditions.[13] Considering that partially acetylated sugars were usually obtained by treatment of unprotected sugars with acetic anhydride or acetyl chloride in pyridine in the past, reactivity orders determined under the above conditions reflected to a considerable degree the thermodynamic stability of each regioisomer. On the other hand, the reaction conditions used in the present study were nearly neutral and the acetylation reaction was stopped after a short period. Therefore the product ratios were determined mainly by kinetic factors. This was also supported by the fact that the product ratio was independent of the reaction time. Consequently, the present experiments



Scheme 4 *Note:* Thick arrows do *not* imply a retrosynthetic scheme (see the Results section).

estimate kinetic factors of acetylation reactions as correctly as possible.

Rate-determining step

We next turned our attention to the rate-determining step of the DMAP-catalyzed acetylation of carbohydrates. Although no detailed mechanistic survey was conducted on DMAP-catalyzed acetylation of carbohydrates, we obtained an intriguing result to allow us to speculate on the mechanism: the fact that the selectivity was almost reversed in the absence of DMAP as shown in Table 3. This confusing result led us to the assumption that acetylation of a hydroxy group proceeded in two steps. The first step is an attack of a hydroxy group on a carbonyl group to form an unstable adduct as shown in Fig. 4. In the second step, this high-energy intermediate gives the acetylated product after deprotonation and departure of the leaving group. Inspection of the structure of these reaction intermediates suggests that, in the case of the DMAP-catalyzed reaction, the first addition process is rate-determining and the following step proceeds smoothly because pyridinium ion is an excellent leaving group and, in addition, the counter-ion acetate will work effectively as a base. In the absence of DMAP, the second proton-transfer step seems to become rate-determining due to the poor leaving ability of the acetate ion. This drastic change of rate-determining step seems to be the origin of the completely reversed selectivity. The theoretical investigation suggests that if the carbonyl carbon has a good leaving group, the concerted process is likely rather than the process *via* a tetrahedral intermediate,[14] supporting our idea that an attack of a hydroxy group on a carbonyl group is rate-determining in the reaction of *N*-acetylpyridinium acetate with alcohols. Although the assumption presented here seems difficult to verify from detailed mechanistic investigations,‡ this simplified view of the acetylation mechanism is quite useful in allowing us to understand the relative reactivities of carbohydrate OH groups towards various reagents (*vide infra*).

Delocalization of positive charge through hydrogen-bonding networks

The usual steric discussion cannot explain the present results, especially the fact that the secondary 3- and 4-OH groups were more acetylated than the primary 6-OH group in the case of glucose. Clearly, the primary 6-OH group must be preferentially acetylated from the viewpoint of steric factors. There must be some other dominant factors operative in the DMAP-catalyzed acetylation of carbohydrates.

The following three experimental results seem to be important in the elucidation of the mechanism. (1) Among the secondary OH groups, the reactivity of the 2-OH group is considerably reduced in all carbohydrates examined. (2) The effect of stereochemistry on the reactivities of OH groups is complicated. For example, the axial 2-OH group in mannose affects not only the reactivity of the neighboring 3-OH group but also the reactivities of the remote 4-OH and 6-OH groups. The axial 4-OH group in galactose reduces the reactivity of the 3-OH group, but on the other hand increases the reactivity of the 6-OH group. (3) Acetylation of the 3-OH group of glucose reduces the reactivity of the 4-OH group considerably, which is one of the most reactive OH groups. However, substitution at position 4 does not affect the reactivity of the neighboring 3-OH group so much.

To explain all these results, we propose that the formation of hydrogen-bonding networks is mainly responsible for the relative reactivities of OH groups in carbohydrates. As shown in Fig. 5, the positive charge in the transition state is delocalized through hydrogen-bonding networks.§ The extent of delocalization increased in the order of substitution at 2-, 3-, and 4-OH and the transition state is stabilized in the same order.

‡ Hubbard and Brittain recently reported a detailed mechanistic survey of amine-catalyzed ester formation from an acid chloride and alcohol in dichloromethane.[15] Their aim was to determine whether amine catalysis operated by a nucleophilic-, specific-base-catalyzed-, or general-base-catalyzed mechanism. They examined these mechanistic pathways based on rate information for the amine-catalyzed reactions of benzoyl chloride with alcohols, obtained by using stopped-flow FT-IR spectroscopy. Although they ruled out specific-base catalysis, they failed to draw a decisive conclusion due to several inconsistent results.

§ In the case of mannose possessing an axial 2-OH group, the ring oxygen occupies a good position as a hydrogen acceptor, which is not shown in Fig. 5 for clarity.

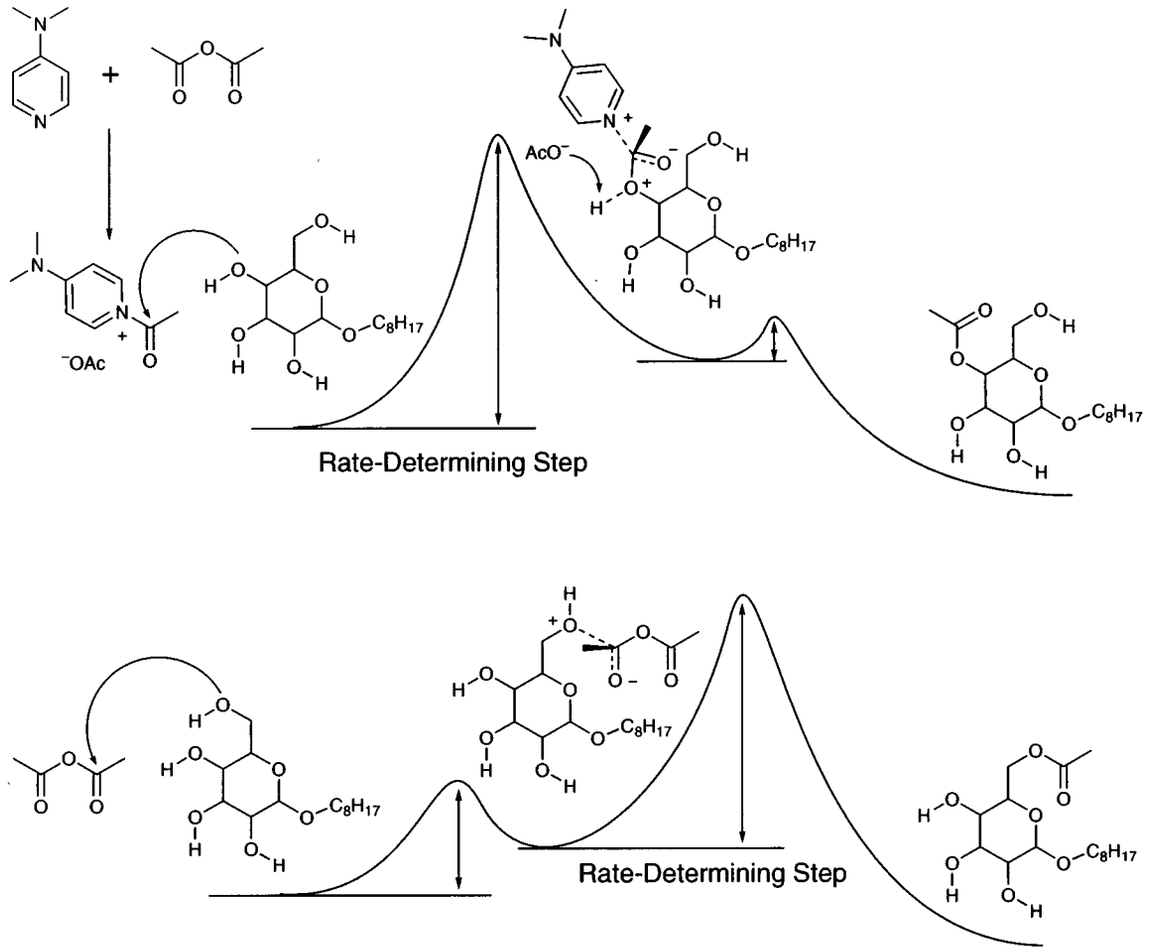


Fig. 4 Proposed energy diagrams for the DMAP-catalyzed acetylation (above) and the uncatalyzed acetylation (below) with acetic anhydride.

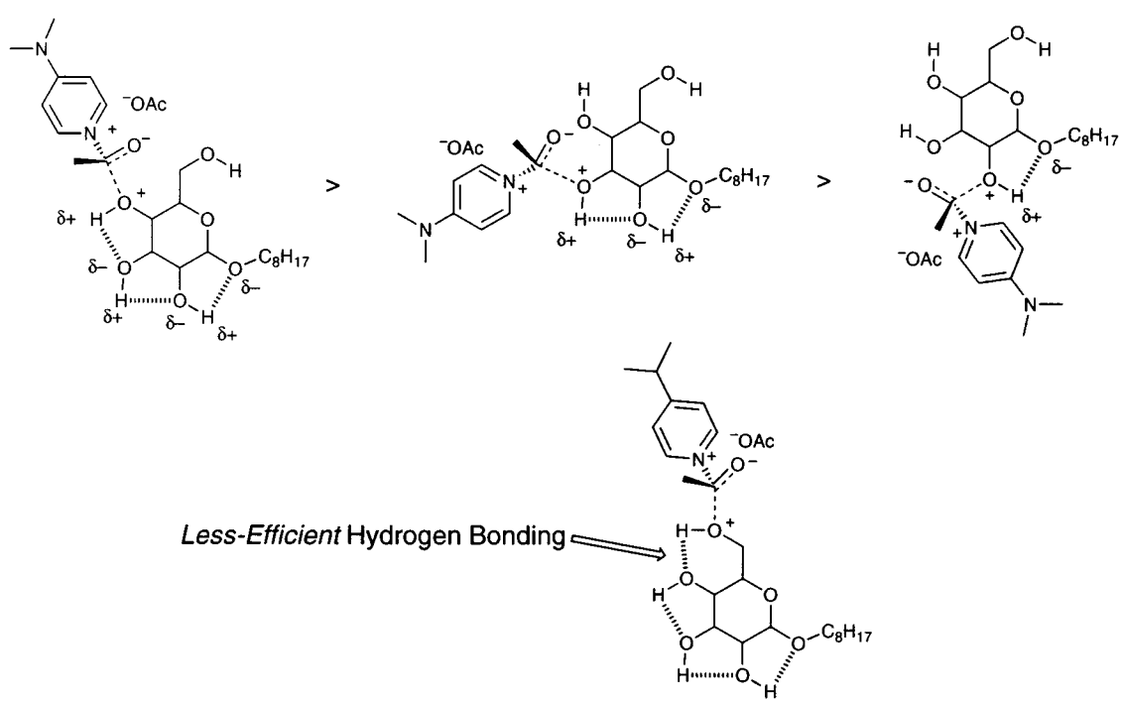


Fig. 5 Schematic representation of delocalized positive charge through hydrogen-bonding networks.

Thus 4-OH groups were acetylated preferentially in all carbohydrates. Compared with secondary OH groups, primary 6-OH groups do not show consistent reactivity trends expected from hydrogen-bonding networks. That is probably due to the high flexibility of primary 6-OH groups.

All three points mentioned above could be easily understood

from the viewpoint of hydrogen-bonding networks. First, reduced reactivities of the 2-OH groups in all carbohydrates originate in the smallest stabilization energy. This situation is not dependent on the stereochemistry of the 2-OH group and the anomer position. Therefore both anomers of glucose, mannose, and galactose generally show reduced reactivities for the 2-OH group. Reactivity changes arising from stereochemistry of OH groups can be best understood as a result of the effectiveness of hydrogen-bonding networks. For instance, the axial 2-OH group in mannose diminishes effectiveness of the hydrogen-bonding network compared with glucose. Thus, the reactivities of all secondary OH groups involved in the hydrogen-bonding network are reduced and the primary 6-OH group which does not effectively participate in the hydrogen-bonding network shows relatively increased reactivity. Considerably reduced yields for acetylation of mannose and galactose are also consistent with this idea. In the case of galactose, the hydrogen bonding between 6-OH (as a hydrogen donor) and 4-OH (as a hydrogen acceptor) is far more effective than in the case of glucose and mannose probably because the distance between 6-O and 4-O is shorter in galactose. Thus, the primary 6-OH group of galactose is preferentially acetylated. Without considering directional hydrogen-bonding networks, it seems impossible to understand a simultaneous increase and decrease of the reactivities of neighboring OH groups. The result on diacetylation strongly suggests the importance of intramolecular hydrogen-bonding networks. That is, acetylation of the 3-OH group completely destroys the hydrogen-bonding network from the 4-OH group to the anomeric oxygen and then the reactivity of the 4-OH group is considerably reduced. On the other hand, substitution at the 4 position does not affect the reactivity of the 3-OH group because the hydrogen-bonding network from the 3-OH group to the anomeric oxygen still survives.

Calculated proton affinity of each OH group in monosaccharides

In order to estimate the effect of hydrogen-bonding networks, we attempted to utilize the proton affinity of each OH group as a qualitative measure of positive charge delocalization. If positive charge delocalization truly matters most in the DMAP-catalyzed acetylation reaction, good correlation between reactivity trends and proton affinities would be anticipated. Dewar and Dieter calculated proton affinities for simple alcohols with the semi-empirical method (AM1) and they found that experimental trends are accurately reproduced.[16] So we employed semi-empirical calculations (PM3) to evaluate the proton affinity of each OH group in monosaccharides. At first, we encountered a serious problem concerning the flexibility of carbohydrate molecules. Orientation of OH groups was found to affect significantly their calculated values. In order to find the best OH group orientation for hydrogen-bonding networks, we performed Monte Carlo simulations to find the most stable conformer.

Computations were done as follows. First, we searched for the most stable conformer by employing Monte Carlo conformational searches with the AMBER* force field implemented on MacroModel Version 6.0.[17] Because good parameters for oxonium ion were not available in molecular mechanics, R-OH_2^+ was replaced by R-NH_2. Then the most stable conformer thus obtained was transferred to the SPARTAN platform and subjected to semi-empirical calculations (PM3) after replacing R-NH_2 by R-OH_2^+. The C-OH_2^+ bond was rotated by 30 degrees and the geometries of 12 conformers thus generated were each optimized with PM3-level[18] calculations. Proton affinities were calculated according to equation (1) in which

$$\text{Proton Affinity (R-OH)} = \Delta H_f(H^+) + \Delta H_f(\text{R-OH}) - \Delta H_f(\text{R-OH}_2^+) \quad (1)$$

ΔH_f is the calculated heat of formation. The experimental $\Delta H_f(H^+)$-value of 1536 kJ mol^{-1} was used following Dewar and Dieter. Calculated proton affinities are listed in Table 7. Irrespective of the stereochemistry of OH groups, proton affinities increased in the order of the 2-, 3-, and 4-OH group in all the cases studied. This result supports our proposal on positive charge delocalization through hydrogen-bonding networks. However, the relative reactivities observed in this study were not always strictly parallel to proton affinities. It might be too simplistic to estimate transition-state energies by proton affinities. In addition, it should be stressed that the PM3-level semiempirical method might not be well qualified to calculate cationic species with densely packed, highly polar functionality like carbohydrates.

Table 7 Proton affinity of each OH group in monosaccharides

	Proton affinity/kJ mol^{-1}			
Substrate	2-OH	3-OH	4-OH	6-OH
Methyl β-Glc	713	730	755	747
Methyl β-Man	741	742	749	741
Methyl β-Gal	734	762	785	781

Conclusions

Hydroxy groups in simple monosaccharides showed unique reactivities in the DMAP-catalyzed acetylation reaction. Especially in the case of glucose, secondary OH groups exhibited much higher reactivities than the primary OH group at position 6. To accurately evaluate relative reactivities of carbohydrate OH groups, reaction conditions were employed under which only monoacetylated products were formed and acetyl migration was suppressed to a minimum. The product distributions showed no concentration dependence. Therefore the unique selectivities were confirmed to originate in carbohydrate structures, not in carbohydrate–carbohydrate interaction in nonpolar solvents.

Careful comparison of the relative reactivities of hydroxy groups in glucose, mannose, and galactose implied an important role for intramolecular hydrogen-bonding networks among carbohydrate OH groups. Furthermore, a markedly large effect of the introduced acetyl group at a particular position on the regiochemistry of the second acetylation clearly demonstrated the directional hydrogen-bonding network starting from 4-, 3-, and 2-OH groups to the anomer oxygen as a hydrogen acceptor. Based on these considerations, we proposed that positive charge delocalization through a hydrogen-bonding network is a dominant factor in determining the relative reactivities of carbohydrate OH groups. As a qualitative measure of positive charge delocalization, we calculated the proton affinity of each OH group in monosaccharides by the combination of Monte Carlo simulations and semiempirical calculations. We found a satisfactory correlation between proton affinities and reactivity trends. Our methodologies and discussion presented here are not necessarily limited to DMAP-catalyzed acetylation systems. Unusual reactivities of carbohydrate OH groups should be reconsidered in the light of intramolecular hydrogen-bonding networks as well as the position of the rate-determining step for a reaction of interest.

In addition to the traditional steric factors, we demonstrated that hydrogen-bonding networks played a decisive role in the DMAP-catalyzed acetylation of unprotected carbohydrates. An attempt to control the reactivities of carbohydrate OH groups by intermolecular hydrogen bonding is in progress.

Experimental †

Instrumentation

^1H and ^{13}C NMR spectra were recorded using a JEOL A-500

spectrometer for samples in chloroform-*d*. ^{1}H and ^{13}C NMR chemical shifts in $CDCl_3$ were referenced to $CHCl_3$ (δ 7.24 and 77.0, respectively). Water content in chloroform was measured by Karl-Fischer equipment (HIRANUMA, AQUACOUNTER AQ-7). GPC was performed on a Recycling Preparative HPLC (LC-908) equipped with a JAIGEL-2H column (Japan Analytical Industry) and a refractive index detector; flow rate, 4.5 cm^3 min^{-1}; mobile phase, chloroform.

Material

Octyl β-D-glucopyranoside and octyl α-D-glucopyranoside were purchased from Wako Pure Chemical Industries and SIGMA, respectively and used as received. Preparations of other octyl glycopyranosides have already been reported.[19] Chloroform stabilized with 2-methylbut-2-ene was purchased from Tokyo Chemical Industry and dried over molecular sieves 3 Å. Acetic anhydride was purified by distillation after azeotropic removal of acetic acid with toluene. DMAP was recrystallized from benzene and dried *in vacuo*. K_2CO_3 was dried *in vacuo* over P_2O_5. Column chromatography was carried out with Silica Gel 60N (spherical, neutral, 40–100 μm) from Kanto Chemicals. $CDCl_3$ was completely deacidified by passage through activated alumina just before use.

General procedure for the DMAP-catalyzed acetylation of unprotected carbohydrates

In every experiment, it was confirmed that water content of chloroform was less than 5 ppm. Stock solutions of DMAP and acetic anhydride were prepared beforehand by dissolving DMAP (12.0 mg, 98 μmol) in chloroform (2 cm^3), and acetic anhydride (101.7 mg, 1 mmol) in chloroform (1 cm^3, 1.45 g), respectively.

Octyl β-D-glucopyranoside (11.8 mg, 40.4 μmol) was dissolved in chloroform (20 cm^3) under Ar. K_2CO_3 (54 mg) was added to the solution. Then the DMAP stock solution (41 mm^3, 2 μmol) was added to the solution *via* a microsyringe. The solution was stirred well with a magnetic stirrer. The temperature was kept at 296 K during the acetylation reaction. Finally, the acetic anhydride stock solution (44.3 mg, 28.4 μmol) was added to initiate the acetylation reaction. After being stirred for 1 h, the reaction mixture was passed through a silica gel short column (1 g) to remove DMAP. The reaction flask was washed well with chloroform and the washings were also passed through the column. Monoacetylated sugars, together with remaining unprotected sugar, were eluted by ethyl acetate–methanol solvent (4 : 1; 15 cm^3). The solvent was evaporated off and the product was dried *in vacuo*. The product was taken up in chloroform (2 cm^3) and passed through a polypropylene filter to remove traces of silica gel. The chloroform was then evaporated off. The sample was completely dried by heating *in vacuo* at 333 K for 4 h and subjected to NMR analysis.

Determination of the product distribution

The sample was dissolved in deacidified $CDCl_3$ and the ^{1}H NMR spectrum was measured. In order to get reproducible results, the temperature was always kept at 303 K during measurement. The yield of each regioisomer was determined by integration of acetyl group signals at $\delta \sim 2.1$ relative to added bromoform as an internal standard (for assignment of each signal, see below). For accurate integration, a curve-fitting computer program was utilized, in which Lorentzian function was fitted to experimental curves. The ^{13}C NMR spectrum was also measured and the number of signals for anomeric carbons was counted. In every case, consistent results were obtained.

Assignment of partially acetylated sugars

GPC was used for purification. Remaining unprotected sugars were separated completely. Monoacetylated sugars could not be separated from each other, so characterization was done at this stage. Exactly the same procedure was applied for the diacetylation experiment.

Table 8 Chemical shifts of acetyl groups in monoacetylated carbohydrates

	Chemical shift (δ)			
Substrate	2-*O*-Acetate	3-*O*-Acetate	4-*O*-Acetate	6-*O*-Acetate
Octyl β-Glc	2.091	2.159	2.117	2.081
Octyl α-Glc	*a*	2.151	2.123	2.093
Octyl β-Man	*a*	2.158	2.109	2.080
Octyl α-Man	2.113	2.143	2.122	2.092
Octyl β-Gal	*a*	2.154[b]	2.156[b]	2.064
Octyl α-Gal	*a*	2.149[b]	2.153[b]	2.056

[a] Not determined. [b] The assignment of pairs of resonances may be reversed.

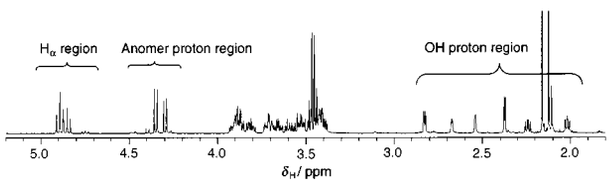


Fig. 6 The ^{1}H NMR spectrum of one of the GPC fractions containing octyl 3- and 4-*O*-acetyl-β-D-glucopyranoside.

Structural characterization relied mainly on the assignment of OH signals and α-proton signals based on the phase-sensitive DQF-COSY measurement in $CDCl_3$. (For structural characterization of the diacetylated glucopyranoside, CD_3CN was used for better separation of C–H signals). Each acetyl group signal was assigned to the corresponding regioisomer and results are summarized in Table 8. Structures of regioisomers were further confirmed by analysis of coupling constants. As shown in Fig. 6, two informative regions were separated well from the complicated C–H region in chloroform-*d*. One is the hydroxy proton region, in which one can distinguish the presence of primary 6-OH groups. Second, upon introduction of an acetyl group, the alpha proton moved downfield and analysis of coupling patterns became possible. In the case of mannose and galactose, the position where the acetyl group was introduced can be sometimes determined unambiguously from analysis of coupling constants between neighboring C–H protons.

Estimation of the proton affinity of each OH group in carbohydrates

Monte Carlo simulations were performed on a Silicon Graphics O2 workstation with MacroModel Version 6.0.[17] 5000 randomly generated structures were minimized using the AMBER* force field in the gas phase. Among the 100–500 obtained structures, the most stable conformer was subjected to semi-empirical MO calculations. Close examination of conformers around global minima revealed the existence of several local minima at a similar energy level, which only differ in the direction of the 6-OH group. Considering the flexibility of the 6-OH group, two or three of these conformers were arbitrarily selected and subjected to semi-empirical calculations. Semi-empirical calculations were performed on an IBM RS6000 workstation with SPARTAN Version 4.0. C–OH_2^+ bonds were systematically rotated in 30° steps and the heats of formation of 12 obtained conformers were calculated at the PM3 level[18] after geometry optimization. In the case of primary hydroxy groups at position 6, there are two rotable bonds and so a systematic survey of 144 conformers was conducted. During the estimation of proton affinities, largely distorted conformers were excluded.

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