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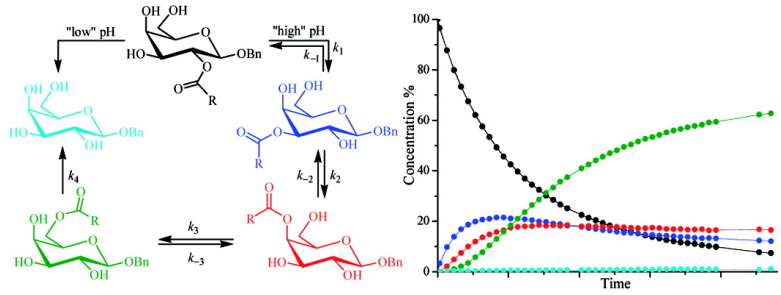

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Acyl Group Migration and Cleavage in Selectively Protected β-D-Galactopyranosides as Studied by NMR Spectroscopy and Kinetic Calculations

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Abstract: The migration of acetyl, pivaloyl, and benzoyl protective groups and their relative stabilities at variable pH for a series of β-D-galactopyranoses were studied by NMR spectroscopy. The clockwise and counterclockwise migration rates for the different ester groups were accurately determined by use of a kinetic model. The results presented provide new insights into the acid and base stabilities of commonly used ester protecting groups and the phenomenon of acyl group migration and may prove useful in the planning of synthesis strategies.

Introduction

Protective groups play a central role in carbohydrate chemistry in controlling the reactivity of both glycosyl donors and acceptors, as well as in the control of regio- and stereoselectivities and decomposition of target molecules and reactants.[1] The strategies for the syntheses of partially protected mono- and oligosaccharides commonly involve the use of orthogonal protecting groups that should be selectively removed under the desired reaction conditions. Various esters are commonly used as protecting groups in carbohydrate syntheses.[1a,2] It is well-known that such groups may, in polyhydroxylic compounds, be subject to undesired migration and cleavage. Acetyl group migration was first described by Emil Fischer[3] and the intramolecular nature of the phenomenon was confirmed by a radioactive labeling study by Doerschuk.[4] Intermolecular rearrangement for ester groups has likewise been proposed.[5]

An acyl group may migrate to a neighboring OH under acidic and neutral, but especially under basic conditions. Such migration has been reported to take place at all positions except C6 in the hexose series.[6] Undesired migration may severly limit the types of reactions that can be applied to partially acylated sugars without inducing decomposition. Notably, intramolecular migration has also been successfully utilized in syntheses and improvement of yields in peptide chemistry and in carbohydrate chemistry.[7] Anticlockwise migration has been observed for methyl 6-bromo-2,6-dideoxy-4-*O*-benzoyl-α-D-*ribo*-hexopyranoside[8] and 5-*O*-acetyl-1,4-anhydro-6-thio-D-glucitol.[9] Migration under acidic conditions has also been reported,[7b] while being disputed by some investigators.[10]

Generally, the migration of acyl groups is considered irreversible[11] and a large part of the earlier investigations have dealt with acetyl group migration in partially acetylated sialic acid derivatives[12] and migration in acyl β-D-glucopyranosiduronic acid derivatives.[13] Mechanistically, a cyclic orthoester-type intermediate has been suggested. Acyl groups with strongly electron-attracting R-groups are reported to exist as cyclic

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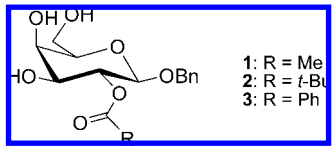


Figure 1. Compounds investigated in the migration studies.

orthoesters, while benzoyl groups have less tendency to migrate than the corresponding aliphatic acyl groups.[4] Reversibly formed tetrahedral intermediates in acyl transfer reactions have likewise been studied in detail in terms of theoretical versus kinetic data.[14]

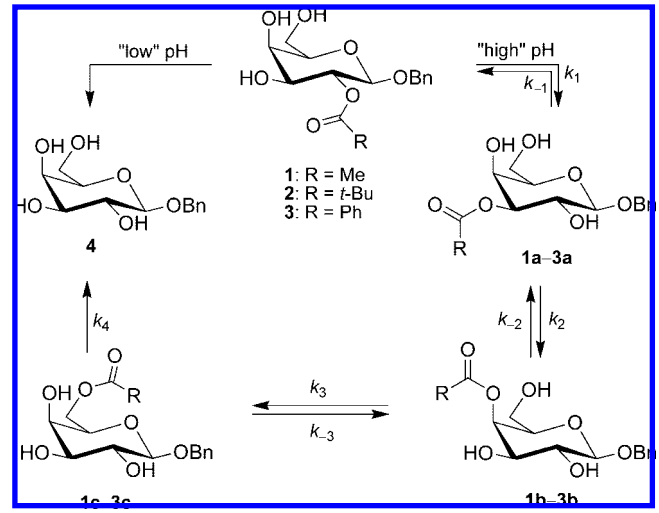
As a part of our synthetic and conformational studies on protected galactopyranosides,[15] and owing to the scarce amount of detailed investigations on the subject, we became interested in the actual nature of the acyl group migration. Here, we report our results on the stability of acyl protecting groups and the migration kinetics of the 2 ⇆ 3 ⇆ 4 → 6 migration in a series of benzyl 2-*O*-acyl-*β*-D-galactopyranoside model compounds **1**−**3** having ester groups of different stabilities, electronic contributions on the carbonyl carbon, and steric bulk at the 2-*O*-positions with free hydroxyls at C3, C4, and C6 (Figure 1).[16] The anomeric position was locked in *β*-configuration with a benzyl ether protective group being stable under the migration conditions applied.

Results and Discussion

The induced migrations, ultimately resulting in cleavage of the ester group from C6 of the galactopyranoside, were followed by NMR spectroscopy. The equation pD = pH + 0.4 was used for converting the pH meter reading (pH*) to pD.[17] Under acidic conditions (pD 1.0−3.0) no migration was observed for any of the compounds studied, instead a direct hydrolysis of the ester group took place. At pD = 5.0 the compounds were still stable but already at pD = 6.8 acyl migration occurred.

The intramolecular migration of the acyl group at pD 6.8 was followed from C2 → C3 → C4 → C6 by NMR spectroscopy after which the protecting group hydrolyzed to form the respective acid. At pD = 3.0, a slow cleavage of the acetyl group without migration was observed (approximately 1%/month). In earlier studies, polarity of the solvent, pH, and temperature have been shown to influence the acyl migration and hydrolysis rates.[12a,18] In the present investigation, solvent polarity was not addressed. A schematic picture of the migration is given in Scheme 1.

Scheme 1



The NMR analyses were performed at 25 °C in D_2O solutions at variable pD values (1.0, 3.0, 6.8, 8.0, and 10.0). The unbuffered D_2O used had a pD = 6.8 (measured pH* = 6.4). A solution with pD = 8.0 was prepared from 10 mM Na-phosphate buffer and pD was adjusted to pH* = 7.6. At pD = 1.0 the hydrolyzed acid did not affect the pH* to any large extent. The migration reactions were followed by ^{1}H NMR spectroscopy and all compounds formed (**2**−**4**) were characterized by ^{1}H and 2D NMR techniques (for details, see Supporting Information).[19]

The ^{1}H chemical shifts of the migration products are remarkably different and can be utilized for determination of their relative populations by integration of the signal areas in the ^{1}H NMR spectra. Details on the chemical shifts are provided in the Supporting Information (Tables S1−S3). First, the migration and cleavage rate of the acetyl group (**1**–**1c** and **4**) were monitored by ^{1}H NMR spectroscopy. The composition of the reaction mixture versus time at pD = 8.0 (pH* = 7.6) is illustrated in Figure 2. To our knowledge, the present investigation is the first report on the relative stabilities and reactivities of the *β*-D-galactopyranose OH/OR groups (R = Ac, Piv, and Bz) in such compounds.

The pivaloyl migration at pD = 8.0 was very slow in comparison to that of the smaller acetyl group, and also slower than the benzoyl group migration (for clockwise and counterclockwise migration rates, see Table 1). The clockwise- and counterclockwise migration rates observed could not be accurately determined on the basis of the populations determined from the NMR spectra only. For accurate estimation of the migration rates, kinetic calculations based on the experimentally

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(16) Compounds **1**–**3** were prepared according to a modified literature procedure starting from D-galactose (for detailed synthesis and characterization see ref 15a and Supporting Information): Peracetylation of D-galactose followed by transformation of the anomeric acetyl into benzyl ether, deacetylation, TBDPS protection of the primary hydroxyl group, introduction of the 3,4-*O*-acetal and ester protection of 2-OH and the subsequent silyl ether deprotection by TBAF and isopropylidene removal using Dowex H^+ provided the corresponding model compounds **1**−**3** in approximately 40% overall yields over eight steps.
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(19) The (higher order) ^{1}H NMR spectra for all of the synthesized and purified compounds were analyzed with PERCH NMR software for complete spectral analyses, see: Laatikainen, R.; Niemitz, M.; Weber, U.; Sundelin, J.; Hassinen, T.; Vepsäläinen, J. *J. Magn. Reson. Ser. A* **1996**, *120*, 1–10. The acyl group migration was followed with Varian 500 and 600 MHz (equipped with a cold probe) Inova Spectrometers and the migration products assigned by standard 2D NMR techniques.

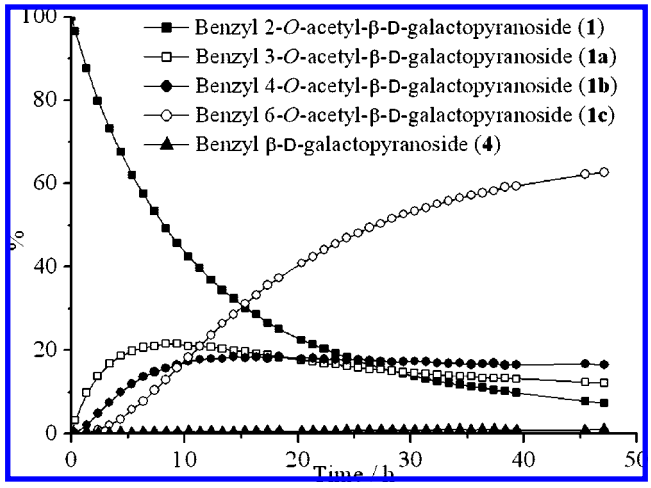

Figure 2. Migration of acetyl group (compound **1**) at pD = 8.0 and T = 25 °C in buffered D_2O.

Table 1. Migration First Order Forward (k_+) and Backward (k_-) Relative Rate Constant for Compounds **1**−**3** at pD = 8.0 (n.d. = Not Determined)

k/h^{-1}	**1** Ac	**2** Piv	**3** Bz
k_1	4.91 ± 0.032	0.06 ± 0.004	2.92 ± 0.07
k_{-1}	2.65 ± 0.068	0.10 ± 0.04	4.11 ± 0.32
k_2	17.6 ± 0.44	0.61 ± 0.13	18.8 ± 2.1
k_{-2}	12.5 ± 0.46	0.26 ± 0.09	10.3 ± 1.56
k_3	10.4 ± 0.14	n.d.	1.91 ± 0.06
k_{-3}	2.48 ± 0.06	n.d.	0.0
k_4	0.04 ± 0.0048	n.d.	1.54 ± 0.1

determined populations were utilized. Being more hindered and sterically shielded toward nucleophilic attack, the pivaloyl and benzoyl ester groups should be less prone to migration when compared with the acetyl group (Figure 2 and Supporting Information). The pivaloyl group is the slowest to migrate while the Ac and Bz groups migrate with similar rates at pD = 8.0. While at pD = 10.0 the acetyl group migration could still be determined by NMR spectroscopy, the migration of the 2-OBz was too fast to be followed by ^{1}H NMR. At low pH (pD = 1.0), the acetyl group was most readily cleaved in comparison with the pivaloyl and benzoyl esters. After treatment of **1** at pD = 1.0 for 1 week, more than 50% of the hydrolyzed product [benzyl β-D-galactopyranose (**4**)] was formed. The direct hydrolysis of Ac was very fast at low pH while the Bz hydrolysis with the highest rate was observed after initial migration to C6 at high pH (Table 1). As shown in Figure 2, the calculations using the constants reported in Table 1 were in good agreement with the experimental values.[20]

The migration reactions described with k_1 to k_3 were assumed to be reversible whereas the final hydrolyzation step reaction k_4 was assumed to be irreversible. All the migration rates for the pivaloyl protected galactopyranoside **2** were much smaller than for the acetyl and benzoyl protected analogues **1** and **3**. Especially k_3 and k_4 were very small and due to the low concentration of benzyl 4-*O*-pivaloyl-β-D-galactopyranoside **2b** the rate constants k_3, k_{-3}, and k_4 could not be accurately determined (n.d.) for the pivaloyl group migration within this time frame (Table 1). The C2→C3 migration for the acetyl group was faster than for the benzoyl (4.91 versus 2.92) and also faster than the C4 → C6 migration (10.4 versus 1.91). The migration from C3 → C4 was rather similar for both (17.6 versus 18.8), whereas the benzoyl group was hydrolyzed much faster than the acetyl (1.54 versus 0.04) (Table 1). In the literature, such influence of steric and electronic effects on the reactivity of esters has been studied frequently.[4,21]

Summary and Conclusions

To summarize, new data on ester protective groups and their relative stabilities at variable pH for a series of β-D-galactopyranoses has been obtained. The clockwise and counterclockwise migration rates (Table 1) for three different ester groups were accurately determined from a kinetic model for the first time. The results presented may prove useful in the planning of oligosaccharide syntheses and sugar analyses. A significant increase in the migration rates to and from the axial 4-OH position (k_2 and k_{-2}, Table 1) over the equatorial positions for the hydroxyl groups in the β-D-galactopyranose were observed. The larger ester group used migrated slower, possibly due to blocking of the nucleophilic attack of the neighboring hydroxyl group on the ester carbonyl carbon. The chemical shifts of the carbonyl carbons in compounds **1**−**3** (167.6 Bz, 172.3 Ac, and 179.5 Piv) could not directly be correlated with the faster migration of the benzoyl group.

The partially reversible model employed here, showed a much better fit with the experimental values than a clockwise migration solution first screened. In future work, theoretical calculations could be utilized to estimate the magnitude of energy difference between the various structures and provide an indication of the transition state(s) of the reaction. At present this approach is hampered by difficulties related to the structure, complexation, and polarization of the reaction intermediates.

Experimental Section

The detailed experimental procedures for the preparation of compounds **1**–**3** are given in the Supporting Information. The NMR samples for the migration studies were prepared as follows: 1.0−2.4 mg of the benzyl 2-*O*-ester-β-D-galactopyranosides were dissolved in 500 μL of D_2O (pD = 6.8) or 10 mM Na-phosphate buffer in D_2O, pD 8.0. For the higher pD/pH conditions the pH* values were adjusted to 7.6 or 9.6 (pD = 8.0 and 10.0) by the addition of NaOD. The 2-*O*-benzoyl derivative of benzyl galactose did not dissolve completely and the undissolved part was removed by filtration through a 0.45 μm membrane filter directly coupled to the NMR tube. For the low pD studies, the compounds were dissolved in 500 μL of DCl/D_2O, (pH* = 0.6), all the pD values were verified by a pH meter (for details, see Supporting Information).

The NMR spectra for benzoyl group migration were recorded using a Varian Unity INOVA 600 MHz spectrometer equipped with a ^{15}N/^{13}C/^{1}H triple resonance cold probe for enhanced sensitivity. NMR experiments for pivaloyl and acetyl group migration were carried out on a Varian Unity 500 MHz spectrometer at 298 K. The time evolution of the samples was followed by recording standard ^{1}H spectra with 16 transients. Presaturation was used for

(20) The model predictions were obtained by solving the differential equations for all of the components during the parameter estimation. A stiff ODE-solver was used to solve the system of ODEs, with the backward difference method implemented in the MODEST software used in the estimation of the kinetic parameters, see: Haario, H. *Modest 6.0-A User's Guide*; ProfMath: Helsinki, 2001. See also: Mastihubová, M.; Biely, P. *Carbohydr. Res.* **2004**, *339*, 1353–1360.

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the water suppression for the benzyl 2-*O*-benzoyl-β-D-galactopyranoside (**3**) sample. The migration products were identified and assigned from ^{1}H, DQF-COSY, TOCSY, and HSQC spectra. The spectra were processed as described in the Supporting Information.

The kinetics of the migration process was described with a first-order reversible model for reactions 1−3 and an irreversible first order model for reaction 4.

$$r_1 = k_1 c_1 - k_{-1} c_{1a} \quad (1)$$

$$r_2 = k_2 c_{1a} - k_{-2} c_{1b} \quad (2)$$

$$r_3 = k_3 c_{1b} - k_{-3} c_{1c} \quad (3)$$

$$r_4 = k_4 c_{1c} - \quad (4)$$

The model predictions were obtained by solving the differential equations for a batch reactor model.

$$\frac{dc_1}{dt} = -r_1, \quad \frac{dc_{1a}}{dt} = r_1 - r_2, \quad \frac{dc_{1b}}{dt} = r_2 - r_3, \quad \frac{dc_{1c}}{dt} = r_3 - r_4, \quad \frac{dc_{cleaved}}{dt} = r_4$$

For the parameter estimation the following objective function was minimized.

$$Q = \sum_i \sum_t (c_{i,t,exp} - c_{i,t,model})^2$$

where $c_{i,t,exp}$ and $c_{i,t,model}$ are the experimentally recorded concentrations and the concentrations predicted by the model, respectively. The software Modest used to estimate the rate constants minimizes the objective function with the Levenberg−Marquardt method and solves the ordinary differential equations describing the reactor model by the backward difference method. The fit of the model to experimental data for the migration process are presented in the Supporting Information (Figures S8−S10). As seen from the figures, the model well describes the experimental data. The rate constants are listed in Tables S4 and S5 (Supporting Information) showing the initial rates of reaction 1 and initial concentrations of reactant 1. The reaction is the fastest for the acetyl group migration and the slowest for the pivaloyl group migration.

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Supporting Information Available: Complete experimental procedures; product characterization; migration details for the different ester groups at different pH values; complete kinetic data; the predicted migration rate constants using experimentally recorded concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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