Glycochemistry

Pyranoside-into-Furanoside Rearrangement: New Reaction in Carbohydrate Chemistry and Its Application in Oligosaccharide Synthesis


Abstract: Great interest in natural furanoside-containing compounds has challenged the development of preparative methods for their synthesis. Herein a novel reaction in carbohydrate chemistry, namely a pyranoside-into-furanoside (PIF) rearrangement permitting the transformation of selectively O-substituted pyranosides into the corresponding furanosides is reported. The discovered process includes acid-promoted sulfation accompanied by rearrangement of the pyranoside ring into a furanoside ring followed by solvolytic O-desulfation. This process, which has no analogy in organic chemistry, was shown to be a very useful tool for the synthesis of furanoside-containing complex oligosaccharides, which was demonstrated by synthesizing disaccharide derivatives α-Δ-β-Galp-(1→3)-β-Δ-Galf, 3-Δ-O-s-lactyl-β-Δ-Galf (1→3)-β-Δ-Glcp-OPr, and α-l-Δ-Fuc-(1→4)-β-Δ-GlcpA-OPr related to polysaccharides from the bacteria Klebsiella pneumoniae and Enterococcus faecalis and the brown seaweed Chordaria flagelliformis.

Introduction

Different types of furanosyl residues are included in the structures of a variety of natural compounds, especially bacterial, plant, and fungal polysaccharides.[1, 2] The synthesis of oligosaccharides related to these biopolymers, as well as glycoconjugates thereof, is often dictated by the needs of vaccine and diagnostics development.[3–6] Currently, the most widely used methods for furanoside synthesis are based on the initial transformation of unblocked monosaccharides by the Fischer reaction under kinetic control[7, 8] or their high-temperature acylation.[7, 9] All of these reactions proceed with the formation of a mixture of α- and β-furanosides and are contaminated by respective pyranoside isomers that may require laborious chromatography in order to separate the target products. It is also notable that further regioselective introduction of O-blocking groups into furanosides can be more difficult than in the case of related pyranoside derivatives.

The idea of pyranoside-into-furanoside (PIF) rearrangement came from our recent observation[10] that an unusual oligosaccharide impurity with a terminal (at the “reducing end”) furanoside residue (e.g., disaccharide C on Figure 1) in addition to the expected pyranoside product (e.g., compound B) was formed in acid-promoted O-sulfation[11] of oligofucosides (e.g., disaccharide A) related to seaweed fucoidans. It was shown that per-O-sulfation by Py·SO₃ complex was accompanied by the formation of unexpected furanoside byproducts of type C only in the presence of a strong acid (trifluoromethanesulfonic or chlorosulfonic acid), while no formation of such compounds was observed, when a weaker acid promoter, particularly, the trifluoroacetic acid, was used.[10]
Observed PIF rearrangement has no analogy in organic chemistry and can be compared formally only with enzymatic isomerization of sugar nucleotides mediated by pyranose–furanose mutase (Scheme 1). The discovered intriguing side process challenged us to study whether it can be performed in a straightforward way within the context of synthesizing oligosaccharides bearing furanoside units. Herewith we report our first results from this study.

**Results and Discussion**

To optimize the PIF rearrangement protocol, readily available 3-O-benzyl-β-D-galactopyranoside (1) was used as a model substrate (Scheme 2).

It was treated under the conditions leading to the formation of furanoside byproducts from oligofucopyranosides. It appeared that its treatment with the Py·SO₃ complex in the presence of chlorosulfonic acid in DMF at room temperature (see general procedure in Experimental Section and Supporting Information) readily gave per-O-sulfated rearranged furanoside product via the initial formation of per-O-sulfated derivative, which was detected first by ¹H NMR spectroscopy on the basis of its characteristic downfield shifts of the H-2, H-4, and H-6 signals (see the Supporting Information). ¹³C NMR monitoring of the reaction mixture revealed that already after 10 min all of compound 1 had been transformed into sulfated 2 (Figure 2), and after 2 h about 30% of pyranoside 2 was rearranged into furanoside 3. Further prolongation of the treatment time up to 48 h afforded complete PIF rearrangement to give sulfated galactofuranoside. Its further O-desulfation under conventional solvolysis by Py·HCl in a dioxane/DMF mixture gave galactofuranoside 4.

It is reasonable to assume that the rearrangement starts with the protonation of O-5 giving pre-reaction intermediate A (Scheme 2). Further transformation of the pyranoside ring in A was studied employing ab initio RHF calculations (see the Supporting Information). It is supposed that the rate-determining step in the PIF rearrangement was the formation of open-ring intermediate C, which proceeded via transition state B. In this case, further recyclization of C accompanied by sulfate transfer should yield furanoside. The geometry of the transition state B obtained in the calculations led us to the conclusion that the sulfate group at O-2 might participate in the O(5)–C(1) bond cleavage, facilitating this process and contributing to the...
fixation of the anomeric configuration during the rearrangement.

To confirm the crucial role of the sulfate group at O-2, we prepared 2-O-acetylated derivative 6 from galactoside 1 with intermediate 4,6-acetylation and hydrolysis steps (Scheme 3, for details see Supporting Information). As expected, the treatment of 2-O-acetylated derivative 6 under standard PIF conditions resulted only in totally sulfated pyranoside 7 without the formation of even traces of the furanoside derivative. Remarkably, the activation energy for 2-O-sulfated derivative 2 calculated as the difference between A and B was 65 kJ mol\(^{-1}\) (Scheme 2); when the substituent at O-2 was changed to acetate in the calculations, the activation energy (difference between E and D) was much higher, reaching 117 kJ mol\(^{-1}\) (Scheme 3). This fact supports the above discussed mechanism of the PIF rearrangement. Its further investigation is in progress and will be reported elsewhere.

3-O-Benzyl-\(\beta\)-d-galactopyranoside (1) was used as a model substrate to optimize the protocol for the PIF rearrangement, because its furanoside isomer 4 was regarded as a convenient precursor for the planned synthesis of disaccharide 11 representing the repeating unit of the O-specific polysaccharide (OPS) of Klebsiella pneumonia (Scheme 4). This bacterium causes pneumonia, bacteremia, and urinary tract infections with high incidence and mortality.\(^{[17]}\) The OPS chain is built up of the disaccharide repeating unit \([-\alpha-D-Galp-(1\rightarrow3)-\beta-D-Galf\] (Scheme 4). Disaccharide 11, representing this repeating unit, was prepared as a molecular probe to study the topology of OPS recognition by lysozyme and other proteins of the immune system.

The applicability of PIF rearrangement was also demonstrated by the synthesis of disaccharide 20, related to fucoidan from the brown seaweed Chordaria flagelliformis\(^{[21]}\) (Scheme 5). Polysaccharide fucoidans from brown seaweeds demonstrate promising types of biological activity\(^{[22–27]}\) that stimulate the preparation of related oligosaccharides as models for QSAR studies.
3-O-Benzoylated allyl β-L-fucofuranoside (14) was chosen as the main precursor towards disaccharide 20 with the aim of exploring the remote stereocontrolling effect\(^{[28,29]}\) of the 3-O-benzoyl group for efficient α-fucofuranosylation during disaccharide assembly (Scheme 5). Compound 13 was prepared in high yield by the regioselective benzoylation of triol 12\(^{[30]}\) via intermediate generation of an organoboron intermediate.\(^{[31]}\) Further PIF rearrangement of pyranoside 13 proceeded smoothly with formation of the required furanoside 14. Its benzylation followed by anomic deallylation and imidate formation gave the fucofuranosyl donor 16. Coupling of monosaccharides 16 and 18 (obtained from diol 17\(^{[32]}\) by regioselective oxidation of the primary OH group at C-6, see the Supporting Information) in the presence of TMSOTf gave stereoselectively α-linked disaccharide 19. Deprotected disaccharide 20 was obtained by hydrogenolysis and saponification of product 19, which can be regarded as a convenient block for the assembly of larger oligosaccharides via O-deallylation followed by transformation into glycosyl donor derivatives. The synthetic potential of PIF rearrangement was also demonstrated by the preparation of mono- (30) and disaccharides (34) related to the diheteryl glycosylaccharide of *Enterococcus faecalis*, which is built up from repeating disaccharide units with the formula \(→-\)3-O-lactyl-β-D-Galf-(1→3)-β-D-GlcP-(1→). Enterococci are currently the third most common pathogen (among Gram-positive bacteria) causing hospital-associated infections in the US, and are the second most common pathogen isolated from intensive care unit patients worldwide.\(^{[34]}\) Oligosaccharides related to the heteroglycan chain are regarded as promising components for vaccine design.\(^{[35]}\)

Selectively substituted galactopyranoside 25 bearing the 3-O-lactyl group was used as a substrate for PIF rearrangement (Scheme 6).

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\text{Scheme 6. The synthesis of galactopyranosides 25 and 26 bearing 3-O-lactyl substituent.}
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Its preparation was performed by regioselective 6-O-silylation with TBSCI of readily available allyl β-D-galactopyranoside 21\(^{[35]}\) followed by alkylation of triol 22 with racemic ethyl 2-bromopropanoate via an organotin intermediate\(^{[36]}\) (see the Supporting Information). These reactions gave a mixture of bicyclic lactones dominated by compounds 23 and 24, which were isolated in their individual forms. Their structures, including the configurations of lactic units, were assessed by NMR spectroscopy with NOE experiments (shown in the Supporting Information). These demonstrated the spatial proximity of the \(H^α\) and \(H-4\) protons in 23 but of \(H^α\) and \(H-2\) in 24. Based on these data, the \(S\)- and \(R\)-configurations of lactic units were assigned to compounds 23 and 24, respectively. The lactones in 23 and 24 were further transformed into isomeric esters 25 and 26 under treatment with MeONA in anhydrous MeOH. Compound 25 was used for the further preparation of furanoside derivatives 30 and 34 (Scheme 7). Thus, PIF rearrangement of 25 gave intermediate product 27, the solvolytic O-desulfation of which in a DMF/dioxane mixture in the presence of Py-HCl followed by saponification gave target monosaccharide 30.

Alternatively to the above-described solvolytic protocol, O-desulfation can be also performed by acetylation to give corresponding per-acetylated derivatives (see transformation 27 → 28 in Scheme 7). Acetylation of the sulfate groups is accompanied by the cleavage of the glycoside bond with the formation of the corresponding acetate, which is a valuable precursor in the preparation of various furanosyl donors. Thus, acetylation of 27 in the presence of AcOH/AC₂O/H₂SO₄ gave tetracetate 28 as a mixture of α- and β-isomers (\(α/β = 1:3\)). Selective removal of the anomic acetate in compound 28 followed by treatment with \(N\)-phenyltrifluoroacetimidoyl chloride afforded galactofuranosyl donor 29. The spacer-armed glucosyl acceptor 32 was prepared from the known diol 31\(^{[37]}\) by regioselective 2-O-benzoylation by the method of Szeja\(^{[38]}\) as modified by Krepsinsky.\(^{[39]}\) Coupling of acceptor 32 and the galactofuranosyl donor 29 proceeded smoothly and gave disaccharide 33 in good yield. Its deprotection gave the spacer-armed disaccharide 34. The application of compounds 30 and 34 in immuno-bacteriological investigations as well as in the structural assessment of diheterylgalactan of *E. faecalis* will be published elsewhere.

The described examples of PIF rearrangement demonstrate its applicability for the transformation of monosaccharide derivatives with allyl, benzyl, benzoyl, and lactyl substituents. Moreover, it turned out that the discovered procedure could also be used for PIF rearrangement of oligosaccharides. Thus, the treatment of disaccharide 36 (Scheme 8) afforded the rearranged product 37 bearing the furanoside residue at the “reducing end”. Its hydrolysis gave disaccharide 11, revealing an alternate synthesis strategy towards this compound in addition to that shown in Scheme 4. Substrate 36 was prepared from the known disaccharide 35\(^{[40]}\) by acetic hydrolysis with aqueous TFA as described in Supporting Information.

It is remarkable that the PIF rearrangement proceeds with the retention of the anomeric configuration. Thus, only α-furanosides were obtained from α-pyranosides (see compounds A and C in Figure 1).\(^{[14]}\) Similarly, all β-pyranoside substrates described above gave exclusively β-furanosides (see transformation 1 → 4 on Scheme 2, 13 → 14 on Scheme 5, 25 → 30 on Scheme 7 and 36 → 37 on Scheme 8).
Conclusion

In conclusion, a new reaction, namely, PIF rearrangement, was discovered. It has no analogy in organic chemistry and can be compared only to enzymatic isomerization by action of pyranose mutases. PIF rearrangement provides a very good and advantageous alternative to known methods of furanoside oligosaccharide synthesis as demonstrated by the described examples.

Experimental Section

General methods

All solvents for reactions were dried according to conventional procedures or purchased as dry. Dichloromethane (CH₂Cl₂) was distilled over CaH₂ and methanol (MeOH) was distilled over Mg(OMe)₂. Dimethylformamide (DMF) and acetonitrile (CH₃CN) were purchased as dry and used without further purification. Reagents for synthesis were commercial and used without further purification. All reactions involving air- or moisture-sensitive reagents were carried out using dry solvents under dry argon. Molecular sieves for glycosylation reactions were activated prior to use at 180 °C in vacuum of an oil pump during 2 h. Thin-layer chromatography (TLC) was carried out on aluminum plates coated with silica gel 60 F₂₅₄ (Merck). Analysis TLC plates were inspected by UV light (λ = 254 nm) and developed by the treatment with a mixture of 15% H₃PO₄ and orcinol (1.8 g L⁻¹) in EtOH/H₂O (95:5, v/v) followed by heating. Silica gel column chromatography was performed with Silica Gel 60 (40–63 µm, Merck). Gel filtration was performed on a Sephadex G-15 column (35 × 500 mm) by elution with water at a flow rate of 1 mL min⁻¹ or on a column of TSK HW-40 (5) gel (25 × 400 mm) in 0.1 M AcOH at a flow rate of 0.7 mL min⁻¹.

Spectroscopic methods

NMR spectra were recorded at 293–305 K using Bruker AM 300 (300 MHz), Bruker AMX400 (400 MHz), Bruker DRX-500 (500 MHz), or Bruker AV600 (600 MHz) spectrometers. Shifts are referenced relative to deuterated solvent residual peaks. NMR spectra of free oligosaccharides were measured for solutions in D₂O using acetone (δₐ = 2.225 ppm, δ₉ = 31.45 ppm) as an internal standard. The following abbreviations are used to explain the observed multiplicities: s, singlet; d, doublet; t, triplet; m, multiplet and br t, broad triplet. Assignments were deduced from 2D experiments (COSY, HSQC, HMBC and TOCSY). Optical rotations were measured using a JASCO DIP-360 polarimeter at ambient temperature in solvents.
specified. High-resolution mass spectra (HR-MS) were measured on a Bruker microTOF II instrument using electrospray ionization (ESI). The measurements were performed in a positive ion mode (interface capillary voltage −4500 V) or in a negative ion mode (3200 V); mass range from \( m/z \) 50 to \( m/z \) 3000 Da; external or internal calibration was made with Electrospray Calibrant Solution (Fluka). A syringe injection was used for solutions in a mixture of acetonitrile and water (50:50 \( v/v \), flow rate 3 mL/min). Nitrogen was applied as a dry gas; interface temperature was set at 180°C.

Computational details

Calculations were carried out using the NWChem v. 6.3 software[41] and RHF approach with 6–31+G* basis set. Starting structures were obtained as stationary points after geometry optimization. Transition states were located using saddle point search as structures having exactly one negative vibrational frequency corresponding to C1–O5 bond cleavage. Activation energies were estimated as differences between full energies of the found transition states and were used uncorrected for zero-point vibrational energy. Energies of both starting and saddle point structures were converged to RMS gradient less than 10–3.

General procedure for pyranoside-into-furanoside (PIF) transformation

**O-Sulfation and rearrangement**

H\( \text{SO}_3\)Cl (26 mg, 0.39 mmol) was added dropwise to a stirred solution of the pyranoside derivative (0.10 mmol) and Py\( \text{SO}_2\) complex (159 mg, 1.00 mmol) in DMF (1.2 mL). The reaction mixture was kept for 48 h at 20°C and then quenched with aqueous Na\( \text{HCO}_3\) (266 mg in 3 mL H\( \text{2O}\), 3.17 mmol) and evaporated twice with water. The residue was dissolved in a minimal amount of water and then an excess of MeOH was added to result in precipitation of inorganic salts, the mixture was filtrated, the solid was washed with MeOH, and the filtrate was concentrated and used for the next step without additional purification.

**O-Desulfation**

Crude sulfated furanoside obtained as described above and Py-HCl (51 mg, 0.5 mmol) were dissolved in DMF (1 mL), and then dioxane (5 mL) was added. The mixture was stirred at 80°C for 30 min and then cooled to room temperature. The reaction mixture was dissolved in CH\( \text{Cl}_2\) (15 mL) and washed with saturated aqueous NaCl (15 mL). The organic layer was concentrated, and the residue was purified by column chromatography on silica gel to give the furanoside derivative.

**NMR monitoring of PIF rearrangement**

To a stirred solution of pyranoside 1 (30 mg, 0.097 mmol) in DMF (1.2 mL) Py\( \text{SO}_2\) complex (159 mg, 1.00 mmol) and H\( \text{SO}_3\)Cl (26 μL, 0.39 mmol) were added. The reaction mixture was kept at 20°C. After 10 min, 2 h and 48 h the sample of the resulted solution (250 μL) was taken from the reaction mixture and quenched with an aqueous solution of Na\( \text{HCO}_3\) (0.5 μL, 1.32 mL). The resulting solution was concentrated in vacuo and then co-evaporated twice with H\( \text{2O}\) and then lyophilized with D\( \text{2O}\). The resulting white solid residue was dissolved in D\( \text{2O}\) and used for recording the NMR spectrum.

Full experimental procedures, characterization for all new compounds and copies of 1H, 13C NMR and HRMS spectra are provided in the Supporting Information.

**Acknowledgements**

This work was supported by RSF grant 14-23-00199 (NEN). We thank Dr. Y. E. Tsvetkov for reading this manuscript and valuable comments, and Dr. A. O. Chizhov for recording high resolution mass spectra at the Department of Structural Studies of the Zelinsky Institute of Organic Chemistry, Moscow.

**Keywords:** carbohydrates · furanoside · glycosylation · rearrangement · sulfation


Received: September 1, 2014
Published online on October 15, 2014