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A Novel Synthesis of D-galactofuranosyl, D-glucofuranosyl and D-mannofuranosyl 1-Phosphates Based on Remote Activation of New and Free Hexofuranosyl Donors

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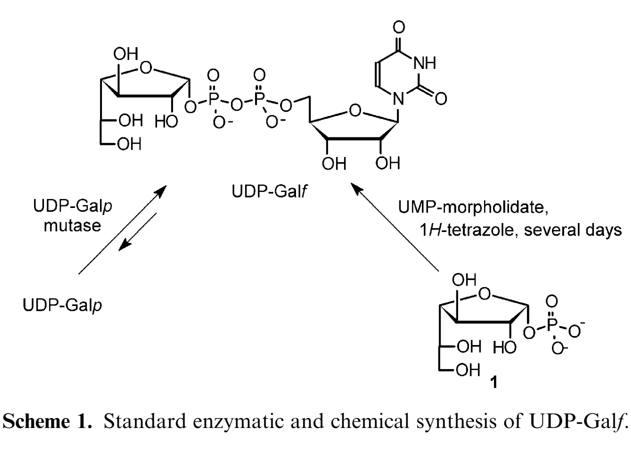
Abstract—The selective synthesis of 1,2-*cis*-hexofuranosyl 1-phosphates was readily accomplished according to a procedure based on the 'Remote Activation Concept'. This approach required (i) the preparation of suitable 1,2-*trans*-hexofuranosyl donors, so that new heterocyclic thiofuranosides were designed and synthesized, (ii) the stereocontrolled phosphorylation of the corresponding unprotected donors and (iii) the simple and fast purification of the resulting anomeric phosphates. This approach showed to be equally efficient in the *galactose*, *glucose* and *mannose* series.

Glycoconjugates containing hexoses in a furanose form are generally found in membrane components of species such as *Mycobacterium*, *Trypanosoma*, *Leishmania*, *Penicillium*, *Salmonella*, *Clostridium*, *Bacteroides* or *Aspergillus*.[1,2] Such stricking glycoconjugates warrant cell survival and are widely responsible for pathogenicity of many of these microorganisms. Among natural hexoses, D-galactose is the most distributed in the furanose form. This could explain the renewed interest for the chemical synthesis of galactofuranosides observed during the last decade.[3–6]

Moreover, considerable attention has been directed toward a better understanding of the biosynthesis of galactofuranoconjugates. Since the identification and the cloning of uridine diphosphogalactopyranose (UDP-Gal*p*) mutase in *Escherichia coli* in 1996,[7] Blanchard et al.[8] and Liu and his coworker[9] accumulated relevant informations related to the biotransformation of UDP-Gal*p* into the natural galactofuranosyl donor UDP-Gal*f*. The corresponding studies showed that a plausible mechanism requires (1) the distortion of the galactopyranose ring in UDP-Gal*p*, (2a) the displacement of UDP by intramolecular cyclisation between O-4 and C-1 or (2b) the elimination of UDP which possibly yields two oxycarbenium intermediates, (3) the attack of UDP which affords the target UDP-α-D-Gal*f*. Indeed, the UDP-Gal*p* mutase is able to catalyze this unusual interconversion. Resulting K_M and k_{cat} values also indicate that the equilibrium greatly favors the formation of the thermodynamically much more stable UDP-Gal*p*.[9] Consequently, UDP-Gal*f* has to be preferred to commercially available UDP-Gal*p* for enzyme activity appraisal.

Since the first enzymatic synthesis of UDP-Gal*f* by McNeil, Lee and their coworkers,[10] chemical approaches have attracted much attention (Scheme 1).[8,9,11,12]



Scheme 1. Standard enzymatic and chemical synthesis of UDP-Gal*f*.

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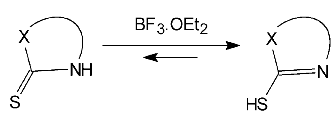
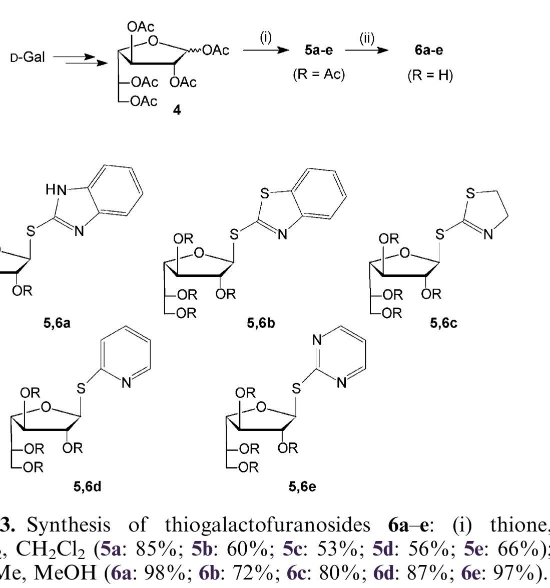
Synthetic sequences are all based on a coupling between D-Gal-1-phosphate **1** and uridine phosphomorpholidate (UMP-morpholidate) in the presence of 1*H*-tetrazole. Key phosphates **1**α and **1**β were previously obtained by phosphorylation of either perbenzoylated galactofuranosyl bromide[13] or pentaacetyl galactofuranose.[14] However, deprotection steps were required to prepare the desired but rather unstable phosphate **1**.

On the other hand, some other natural hexofuranosyl compounds were identified.[15,16] However, their biological role and their biosynthesis received little interest so that chemical synthesis generally focused on galactofuranosyl derivatives. In this context, and considering the weak stability of anomeric phosphates, we describe herein a new and general strategy which allows the simple preparation and isolation of the target D-galacto-, D-gluco- and D-mannofuranosyl phosphates **1**, **2** and **3**, respectively. This approach, based on the remote activation concept first introduced by Hanessian et al.,[17] could afford pyranosyl 1-phosphates starting from unprotected 2-(β-D-glycosyloxy)-3-methoxy pyridine. However, to the best of our knowledge, this process was never applied to the direct synthesis of unprotected hexofuranosyl compounds so that the control of tautomeric equilibria, in addition to the diastereocontrol, deserves to be brought up. Moreover, versatility of thioglycosides is now well established. In this context, we have reinvestigated the remote activation concept for the one-step synthesis of a variety of hexofuranosyl 1-phosphates starting from unprotected and new thiofuranosides.

We assumed that heterocylclic thiols would be very efficient for our purposes. Model compounds were chosen between 2-mercaptobenzimidazole, 2-mercaptobenzothiazole, 2-mercaptothiazoline, 2-mercaptopyridine or 2-mercaptopyrimidine. Such solid and odorless reactives present however an equilibrium in solution between the thiol and the thione forms. The latter could be shifted toward the SH-tautomer by complexation with a Lewis acid such as $BF_3 \cdot OEt_2$ (Scheme 2) which is also used to activate the anomeric acetate for the preparation of thioglycosides. The optimum heterocylic thiol/Lewis acid ratio was easily determined by ^1H NMR on the basis of (i) the chemical shift differences for the aromatic protons before and after adding boron trifluoride etherate and more specifically (ii) the gradual disappearance of the NH signal with increasing amounts of the complexing Lewis acid. We concluded that 3 molar equivalents of $BF_3 \cdot OEt_2$ were required for one molar equivalent of heterocyclic derivative.



Scheme 2. Shifted thiol/thione equilibrium by $BF_3.OEt_2$.

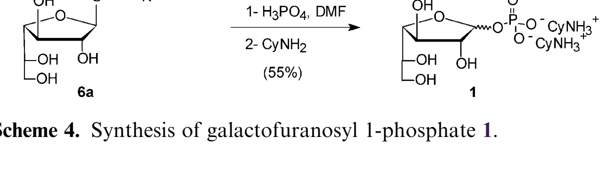


Scheme 3. Synthesis of thiogalactofuranosides **6a–e**: (i) thione, $BF_3 \cdot OEt_2$, CH_2Cl_2 (**5a**: 85%; **5b**: 60%; **5c**: 53%; **5d**: 56%; **5e**: 66%); (ii) NaOMe, MeOH (**6a**: 98%; **6b**: 72%; **6c**: 80%; **6d**: 87%; **6e**: 97%).

Starting from peracetyl galactofuranose (**4**),[18] and provided that an excess of thiol/thione-$BF_3 \cdot OEt_2$ complex was used, the required thiofuranosides **5a–e** were synthesized in good yields and excellent β-selectivities (Scheme 3). Subsequent transesterification of these new thiofuranosides with sodium methanolate gave the key thiogalactofuranoside **6a–e**, respectively. It is important to note that 2-mercaptothiazoline derivative **5c** was base sensitive, so that deacylation was carried out with 0.25 molar equivalent of the base carefully added over a period of 7 days. The resulting protected and unprotected donors were fully characterized by ^1H and ^{13}C NMR spectroscopy. Anomeric β configurations were first established on the basis of small coupling constant values between H-1 and H-2, i.e., $J_{H\text{-}1,H\text{-}2} \sim 1.5$–2.0 Hz. These 1,2-*trans* arrangements were confirmed by optical rotation measurments which gave negative values ($-130° < [\alpha]_D < -90°$) as anticipated according to Husdson's rules.[19]

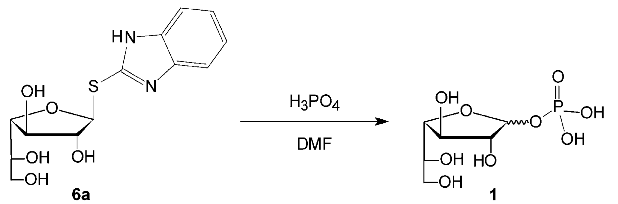
Further condensation of commercially available dry phosphoric acid was performed in DMF. After experimentation, we found that the best results were obtained from compound **6a** bearing a mercaptobenzimidazolyl entity as an aglycon (Scheme 4). The free donor **6a** was then consumed in few min at room temperature and converted into a more polar product. Isolation of the latter required first a neutralization with a saturated aqueous solution of barium hydroxide, in order to eliminate excess of phosphoric acid. After concentration, released mercaptobenzimidazole was easily removed by washing with ethyl acetate and the desired phosphate **1** was finally isolated in 55% yield as the water soluble bis-cyclohexylammonium salt.

^1H, ^{13}C and ^{31}P NMR analysis[20] revealed the presence of both **1**α and **1**β anomers which were not



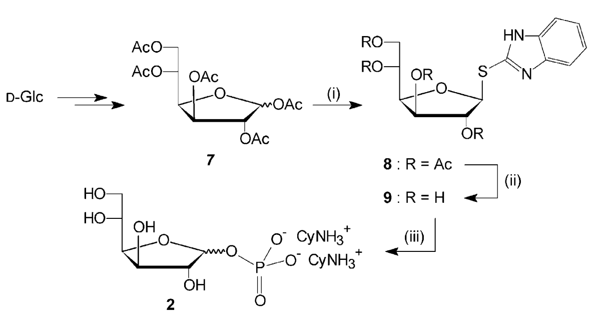
Scheme 4. Synthesis of galactofuranosyl 1-phosphate **1**.

Table 1. Infleuence of the reaction time during the phosphorylation of **6a**

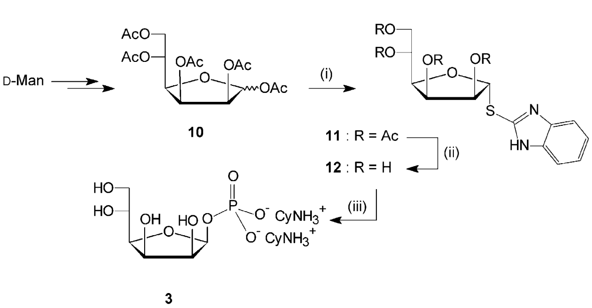


Entry	Time (min)	**1**α/**1**β
1	5	1.5:1
2	10	1.3:1
3	15	1.3:1
4	30	1.1:1
5	60	1:1.1
6	90	1:1.3
7	120	1:1.5
8	180	1:1.6

contaminated by any traces of the corresponding galactopyranosyl 1-phosphates. Anomeric configurations were determined on the basis of (i) the coupling constant between H-1 and H-2 and (ii) the chemical shift of the anomeric center. Therefore, a value of 4.5 Hz for $J_{H\text{-}1,H\text{-}2}$ and a $\delta_{C\text{-}1}$ of 97.7 ppm are relevant for a 1,2-*cis* furanoside while the 1,2-*trans* epimer is characterized by a smaller $J_{H\text{-}1,H\text{-}2}$ (1.7 Hz) and a lower-field signal ($\delta_{C\text{-}1}$ 104.0 ppm). Having these analytical data in hand, we performed the phoshorylation of **6a** by varying the reaction time, in order to study the influence of this parameter on the α/β ratio (Table 1), and monitored the reaction by ^{1}H NMR. The results showed that the target phosphate **1**α was first obtained after only few min at room temperature. This α-selectivity resulted from a S_N2 process at the anomeric position of the starting thiofuranoside **6a**β. Anomerization into the less sterically hindered anomer **1**β then slowly occured with increasing reaction time.



Scheme 5. Synthesis of glucofuranosyl 1-phophate **2**: (i) 2-mercaptobenzimidazole, $BF_3{\cdot}OEt_2$, CH_2Cl_2 (52%); (ii) NaOMe, MeOH (100%); (iii) H_3PO_4, DMF; $CyNH_2$ (30%).



Scheme 6. Synthesis of mannofuranosyl 1-phophate **3**: (i) 2-mercaptobenzimidazole, $BF_3{\cdot}OEt_2$, CH_2Cl_2 (70%); (ii) NaOMe, MeOH (100%); (iii) H_3PO_4, DMF; $CyNH_2$ (70%).

On the basis of these results in the *galactose* series, we further performed the synthesis and the phosphorylation of heterocyclic thiogluco- and thiomannofuranosides **9** and **12**, respectively (Schemes 5 and 6). Peracetylated furanoses **7**[18] and **10**[18] were first specifically converted into the thiofuranosides **8**β and **11**α according to the method previously described and isolated after chromatographic purification in 52 and 70% yield, respectively. After quantitative Zemplen deacetylation, condensation of dry phosphoric acid with the corresponding free donors and removal of both released heterocycle and residual acid lead to the target anomeric phosphates. In the *glucose* series (Scheme 5), TLC monitoring revealed a significant degradation of the starting material into D-glucose. Moreover, purified bis(cyclohexylammonium) phosphate **2** was rather unstable so that it was isolated in a moderate 30% yield. Spectroscopic analysis (^{1}H, ^{13}C, and ^{31}P NMR)[21] indicated that the desired furanoid compound was obtained, and ^{1}H NMR at 400 MHz indicated an anomeric ratio of **2**α/**2**β = 2.6:1. Both anomers were discriminated on the basis of a characteristic small $J_{H\text{-}1,H\text{-}2}$ value (< 1.0 Hz) for the β-*gluco*-anomer.

Finally, thiomannofuranoside **12**α was also submitted to phosphorylation (Scheme 6). Under similar conditions and purification process, mannofuranosyl derivative **3** was obtained in an excellent 70% yield. It is very interesting to note that all NMR investigations have shown (i) very small evidence for the formation of the pyranosyl phosphate (furanosyl/pyranosyl phosphate > 97:3 as revealed by ^{1}H NMR at 400 MHz) and (ii) characteristic data of only one anomer.[22] Indeed, on the basis of low field chemical shift for the anomeric center ($\delta_{C\text{-}1}$ 102.5 ppm), we assumed that the more hindered mannofuranosyl phosphate **3**β was obtained with very high selectivity.

In conclusion, we perfomed the synthesis of various hexofuranosyl 1-phosphates starting from new unprotected thiofuranosides bearing heteroaromatic as an aglycon. Moreover, control remote activation concept allowed the preparation of the target anomeric phosphates by minimizing ring expansion side reactions and with interesting diastereoselectivities towards the 1,2-*cis* isomers. Further developments of this approach for the synthesis of rare nucleotide-hexofuranoses are in progress.

Acknowledgements

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20. Significant NMR data for **1**: ^{1}H NMR (D_2O) δ (ppm): 5.53 (dd, 1H, $J_{1,2}=4.5$ Hz, $J_{1,P}=4.9$ Hz, H-1α); 5.48 (dd, 1H, $J_{1,2}=1.7$ Hz, $J_{1,P}=6.4$ Hz, H-1β); 4.11 (dd, 1H, $J=7.0$ Hz, $J=8.1$ Hz, H-3α); 4.01 (dd, 1H, $J_{2,3}=3.0$ Hz, H-2β); 3.97–3.92 (m, 3H, H-2α, H-3β, H-4β); 3.69–3.50 (m, 7H, H-3α, H-5αβ, H-6αβ, H-6′αβ). ^{13}C NMR (D_2O) δ (ppm): 104.0 (d, $J_{1,P}=4.0$ Hz, C-1β); 97.7 (d, $J_{1,P}=5.6$ Hz, C-1α); 84.7 (C-4β); 83.5 (d, $J_{2,P}=7.2$ Hz, C-2β); 82.8 (C-4α); 78.6 (d, $J_{2,P}=7.2$ Hz, C-2α); 75.9 (C-3α); 73.4 (C-5α); 72.6 (C-5β); 64.2 (C-6β); 64.1 (C-6α).
21. Significant NMR data for **2**: ^{1}H NMR (D_2O) δ (ppm): 5.59 (dd, 1H, $J_{1,2}=3.8$ Hz, $J_{1,P}=6.1$ Hz, H-1α); 5.34 (d, 1H, $J_{1,2}<1.0$ Hz, $J_{1,P}=6.6$ Hz, H-1β). ^{13}C NMR (D_2O) δ (ppm) for **2**α: 99.4 (d, $J_{1,P}=4.0$ Hz, C-1); 79.7 (C-4); 78.8 (d, $J_{2,P}=4.8$ Hz, C-2); 77.0 (C-3); 70.9 (C-5); 64.8 (C-6).
22. Significant NMR data for **3**β: ^{1}H NMR (D_2O) δ (ppm): 5.37 (dd, 1H, $J_{1,2}=3.9$ Hz, $J_{1,P}=6.0$ Hz, H-1); 4.29–4.27 (m, 1H, H-2 or H-3); 4.10–4.08 (m, 1H, H-3 or H-2); 4.04 (dd, 1H, $J_{3,4}=3.6$ Hz, $J_{4,5}=8.6$ Hz, H-4); 3.80 (ddd, 1H, $J_{5,6}=5.3$ Hz, $J_{5,6'}=3.1$ Hz, H-5); 3.66 (dd, 1H, H-6′, $J_{6,6'}=12.2$ Hz); 3.59 (dd, 1H, H-6); ^{13}C NMR (D_2O) δ (ppm): 102.5 (d, $J_{1,P}=4.8$ Hz, C-1); 79.1 (C-4); 78.1 (d, $J_{2,P}=6.4$ Hz, C-2); 71.3 (C-3); 69.5 (C-5); 63.2 (C-6).