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Versatile Synthesis of Rare Nucleotide Furanoses

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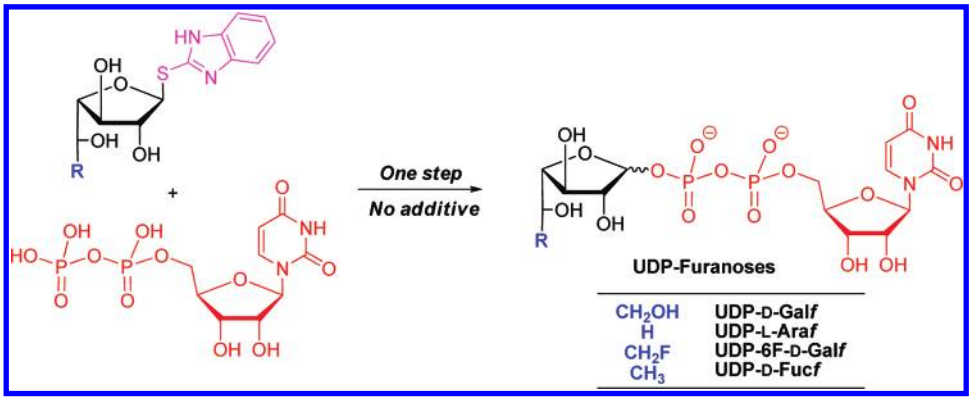
ABSTRACT



Direct activation of unprotected thioimidoyl furanosides yielded in only one step and few minutes a panel of rare uridine 5′-diphospho-furanoses. Diastereoselectivity of the reaction was tightly connected with reaction time, temperature, and nature of the furanosyl donor. This approach was totally selective since no ring expansion from the initial five-membered ring to the more stable pyranose form was observed.

Glycoconjugates are mainly built up with hexoses present in a pyranose configuration. Nevertheless, their presence in microorganisms, but not in mammals, in a furanose form adds supplementary variability to the complexity of such biomolecules. Therefore, because of the millions of deaths caused each year, *Mycobacterium*, *Trypanosoma*, *Leishmania*, or *Aspergillus* species are widely studied in order to precise their biological involvements and to find alternative treatments to the drugs currently used.[1] Surprinsingly, hexofuranose residues occurring in various polysaccharides and glycoconjugates produced by infectious bacteria, protozoa, and fungi belong mainly to either D-galactose (D-Gal*f*) or D-fucose (D-Fuc*f*) series.

The biosynthesis of such hexofuranosides requires three families of enzymes, as opposed to pyranosides. In addition to classical hydrolases[2] and transferases,[3,4] the involvement of pyranose mutases,[5,6] which isomerize uridine 5′-diphospho (UDP)-pyranose to the corresponding furanosyl donor, is required. Up to now, UDP-α-D-galactofuranose **1**α (UDP-α-D-Gal*f*) is the only activated intermediate identified as a key substrate for transferases. However, the existence of D-fucofuranose (D-Fuc*f*) as well as L-arabinofuranose (L-Ara*f*) in natural oligosaccharides probably involves similar biosynthetic mechanisms and the requisite for their corresponding nucleotide sugars.[7] Moreover, owing to structural homology, the targeted natural and non-natural UDP-furanoses

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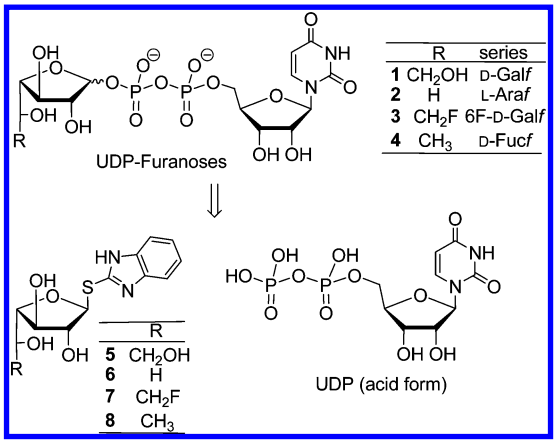
1−**4** are potentially suitable substrates for both pyranose mutases and furanosyl transferases.

These elaborate and relatively unstable derivatives generally involve time-consuming reactions, activated intermediates, and additives.[8,9] Herein we present a short and versatile chemical synthesis of eight UDP-furanoses directly from unprotected thioimidoyl donors (Scheme 1).

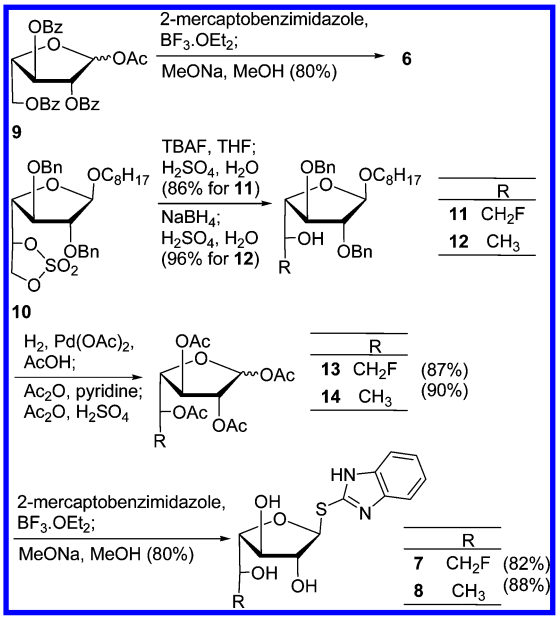
Scheme 1. Retrosynthetic Analysis of Uridine 5′-Diphospho-furanoses



Thioimidates have recently gained interest thanks to their use as efficient donors in glycosylation reactions.[10−12] On this basis, we expected that the acidic form of UDP could behave like phosphoric acid[13] and react as an acceptor without previous chemical activation.[14−17] Moreover, from a structural point of view, D-Gal*f*, D-Fuc*f*, and L-Ara*f* share the same skeleton but are subtly modified on the C-5 side arm of L-Ara*f* by adding hydrophobic methyl group (D-Fuc*f*) or hydroxymethyl function (D-Gal*f*), able to act as both donor and/or acceptor of hydrogen bonding. To complete this collection, we also prepared the C-5 fluoromethyl derivative **3**, the fluorine atom being nearly isosteric to oxygen but acting only as hydrogen-bond acceptor.

Benzimidazolyl thiofuranosides **5**−**8** were prepared from the corresponding peracylated furanoses. While the synthesis of **5** was already described,[13] the arabinosyl derivative **6** was obtained from the known compound **9**[18] by first thioglycosidation promoted by the boron trifluoride etherate complex followed by methanolysis (Scheme 2). On another side,

Scheme 2. Synthesis of Thioimidates



fluorinated thiogalactofuranoside **7** and thiofucofuranoside **8** were synthesized from a common intermediate **10**.[19] Nucleophilic opening of the cyclic sulfate with fluoride and hydride anions, respectively, was followed by acidic desulphation to afford intermediates **11**[20] and **12** in 86% and 88% yield, respectively. Further hydrogenolysis in the presence of palladium(II) acetate, acetylation, and acetolysis under controlled conditions yielded the peracetylated blocks **13** and **14**, respectively. The target thiofuranosides **7** and **8** were finally obtained according to Ferrier procedure and final Zemplen transesterification.

With the unprotected thioimidoyl donors in hand, the direct synthesis of nucleotide furanoses was first attempted in the *galacto* series from thioimidate **5**, using a freshly prepared acidic form of UDP in dry dimethylformamide. A series of assays with the donor **5** was perfromed to determine the optimum reaction time toward the ratio UDP-Gal*f*/UDP and the diastereoselectivity (Table 1). The ^{31}P NMR of the crude self-promoted reaction revealed that UDP-α-D-Gal*f* **1**α was kinetically obtained after only few minutes and reached a maximum after 10 min (entries 1−3). Subsequently, maximum of overall conversion was observed after 20 min (entry 4). Nevertheless, longer reaction times resulted in substantial degradation of the desired UDP-Gal*f* with the amount of **1**α decreasing faster than its anomer (entries 4−6). Finally, it is noteworthy that these data show the complete absence of UDP-pyranoses.

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Table 1. Monitoring of the Phosphorylation Reaction by ^{31}P NMR

entry	time (min)	1α/UDP (%)	1β/UDP (%)	1α/1β
1	2	5	2	1:0.4
2	5	13	7	1:0.5
3	10	27	23	1:0.9
4	20	22	40	1:1.8
5	45	4	16	1:4
6	180	5	20	1:4

Despite the really short reaction time, inescapable partial degradation of UDP further occurred to produce uridine 5′-monophosphate (UMP). However, no UMP-Gal*f* was observed. This was corroborated by reverse phase HPLC which also showed high resolution between **1**α and **1**β. Unfortunately, the 1,2-*cis* anomer **1**α was collected with UDP. This problem was overcome using an alkaline phosphatase which transformed UDP into uridine and monophosphate (Figure 1).[21] Consequently, after only 10 min at 0 °C, followed by

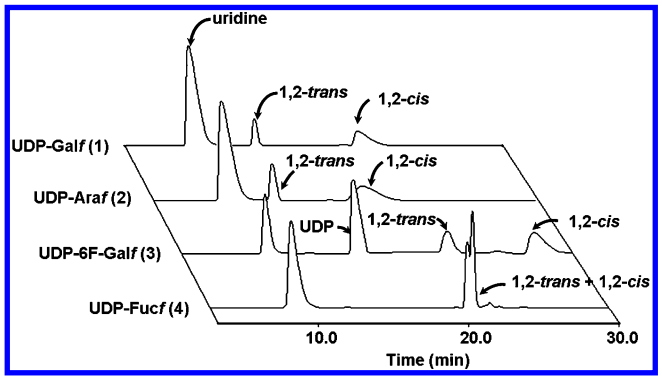

Figure 1. Chromatographic profiles for UDP-furanoses **1**−**4**.

simple workup, the desired UDP-Gal*f* **1** was synthesized and isolated in a 32% overall yield. Preparative chromatography allowed us to separate and isolate both anomers **1**α and **1**β in a 1:2 ratio (Table 2). Structures of **1**α and **1**β were unambiguously established on the basis of NMR data. First, the five-membered ring of the unknown 1,2-*trans* compound **1**β was notably deduced from the high chemical shift obtained for C-1 of the galactofuranosyl residue (δ_{C-1} 104.7 ppm). Second, this entity is also characterized by a coupling constant lower than 1 Hz between H-1 and H-2, that is relevant to a 1,2-*trans* relationship, and a $J_{H-1,P}$ value of 5.8 Hz. Moreover, on the basis of the two upfield doublets in the ^{31}P NMR spectrum, the presence of UMP-Gal*f* is completely dismissed. This demonstrated that (i) no isomerization into the more stable pyranosyl derivatives of both donor **5** and product **1** occurred under the present acidic conditions and (ii) the acidic form of UDP is much more

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Table 2. Synthesis of Target UDP Furanoses **1**−**4**

entry	donor (R)	time (min) (temp, °C)	product	overall yield (%) (α/β)
1	**5**	10	**1**α	32 (1:2)
	(CH_2OH)	(0)	**1**β	
2	**6**	8	**2**α	21 (1:0)
	(H)	(−10)	**2**β	
3	**7**	60	**3**α	37 (1.3:1)
	(CH_2F)	(0)	**3**β	
4	**8**	10	**4**α	27 (2:1)
	(CH_3)	(0)	**4**β	

reactive under the conditions used than UMP released in small amounts in the reaction media.

This approach was subsequently extended to the L-*arabino*, D-*fuco* and D-*fluorogalacto* series starting from the corresponding benzimidazolyl furanosides **6**−**8**.[22] As expected, conditions had to be optimized according to the nature of the carbohydrate. All reactions were carried out between −10 and 0 °C and the interconversion of functional groups at anomeric positions of **6** and **8** was efficient in less than 10 min as already observed from donor **5**. While chromatographic analysis indeed showed the formation of both 1,2-*cis* and 1,2-*trans* anomers from the arabinosyl precursor **6**, purification finally gave pure **2**α in 21% yield since **2**β was too labile to be isolated. Nevertheless, the **2**α/**2**β ratio was estimated to 1:1.8 according to analytical HPLC. As far as the fucosyl derivative is concerned, efforts to separate both anomers of UDP-Fuc*f* **4** were unsuccessful. Therefore, **4**α and **4**β were isolated in a 27% yield with a 1,2-*cis*/1,2-*trans* ratio close to 2:1.

Finally, the 6-deoxy-6-fluoro-thioimidate **7** set him apart from the other donors since its transformation required longer reaction time (Table 2, entry 3). This phenomenon could probably be related to the remote but strong electronic effect of the fluorine atom in primary position. Interestingly, preparative chromatography, without previous degradation of UDP by alkaline phosphatase, directly afforded pure **3**α and **3**β in a 37% overall yield with a 1.3:1 ratio (Figure 1, Table 2).

Once again, neither pyranose forms nor UMP-furanoses were ever observed and all the desired nucleotide furanoses were fully characterized on the assumption of ^{1}H, ^{13}C, and ^{31}P NMR data.[23]

Despite their biological importance, the synthesis of nucleotide sugars still remains a challenge for chemists. The

(22) Typical procedure for the synthesis of UDP-furanoses: To UDP disodium salt dihydrate (2 equiv) in DMF (3 mL) at 0 °C was added Amberlite IR-120 (H^+-form) until the UDP was completely dissolved. The resin was filtered off and washed with DMF. The filtrate and washing were freeze-dried, redissolved in anhydrous DMF (1 mL) and cooled (Table 1). A solution of the desired donor (1 equiv) in DMF (1 mL) was added dropwise. The mixture was stirred for an appropriate period, diluted with cold water (1 mL) and 0.5 M NH_4HCO_3 (0.5 mL), and finally freeze-dried. In the case of donors **5**, **6**, and **8**, the residue was treated with alkaline phosphatase (20 μL, 1 unit/μL, 2 mL of deionized water), and the mixture was kept at room temperature until UDP was no longer detected by NMR ^{31}P. The residue was centrifugated, and the resulting filtrate was purified by HPLC.

versatility of our approach was underlined by synthesizing six unknown UDP-furanoses, These likely glycosyl donors are essentially required to investigate the processes involved in the hexofuranose incorporation within cell wall glycoconjugates. They could be of great interest to identify putative glycosyltransferases, to study the action of the correlate mutases as well to develop antimicrobial agents. This efficient one-step procedure complements well the chemical approaches proposed in the literature.[24] The absence of any protecting groups, on both donor and UDP, and of any additives not only results in a direct access to the desired UDP-furanoses but also in an increase reactivity of thioimidoyl donors. Moreover, neither ring expansion nor UMP-furanoses were observed. All these facts let us consider that the proposed chemical method comes closer to the chemoenzymatic ways recently published.[9]

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Supporting Information Available: Experimental preparations and NMR data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) Selected data for UDP-furanoses **1**−**4** in D_2O. **1**β: ^1H NMR (D_2O) d = 5.55 (d, 1 H, $J_{1,P}$ = 5.8 Hz, H-1 Gal); ^{13}C NMR (D_2O) d = 104.7 (d, $J_{C-1,P}$ = 5.1 Hz, C-1 Gal); ^{31}P NMR (D_2O) d = −10.3 (d, $J_{P,P}$ = 21.7 Hz, Pa), −12.5 (d, Pb). HRMS (ESI^-): $[C_{15}H_{22}N_2O_{17}P_2]^-$ calcd, 565.0472; found, 565.0470. **2**α: ^1H NMR (D_2O) d = 5.58 (d, 1 H, $J_{1,P}$ = 6.0 Hz, H-1 Ara); ^{13}C NMR (D_2O) d = 104.9 (d, $J_{C-1,P}$ = 5.4 Hz, C-1 Ara); ^{31}P NMR (D_2O) d = −10.2 (d, $J_{P,P}$ = 19.6 Hz, Pa), −12.6 (d, Pb). HRMS (ESI^-): $[C_{14}H_{21}N_2O_{16}P_2]^-$ calcd, 535.0366; found, 535.0359. **3**α: ^1H NMR (D_2O) d = 5.58 (dd, 1 H, $J_{1,P}$ = 5.7 Hz, $J_{1,2}$ = 4.2 Hz, H-1 6F-Gal); ^{13}C NMR (D_2O) d = 97.6 (C-1 6F-Gal); ^{31}P NMR (D_2O) d = −10.1 (d, $J_{P,P}$ = 19.6 Hz, Pa), −11.5 (d, Pb). HRMS (ESI^-): $[C_{15}H_{22}FN_2O_{16}P_2]^-$ calcd, 567.0429; found, 565.0419. **3**β: ^1H NMR (D_2O) d = 5.53 (d, 1 H, $J_{1,P}$ = 5.8 Hz, H-1 6 F-Gal); ^{13}C NMR (D_2O) d = 103.9 (C-1 6F-Gal); ^{31}P NMR (D_2O) d = −10.3 (d, $J_{P,P}$ = 19.6 Hz, Pa), −12.6 (d, Pb). HRMS (ESI^-): $[C_{15}H_{22}FN_2O_{16}P_2]^-$ calcd, 567.0429; found, 565.0446. **4**α: ^1H NMR (D_2O) d = 5.62 (d, 1 H, $J_{1,P}$ = 5.9 Hz, $J_{1,2}$ = 4.2 Hz, H-1 Fuc); ^{13}C NMR (D_2O) d = 97.8 (C-1 Fuc); ^{31}P NMR (D_2O) d = −11.4 (d, $J_{P,P}$ = 19.6 Hz, Pa), −12,7 (d, Pb). HRMS (ESI^-): $[C_{15}H_{23}N_2O_{16}P_2]^-$ calcd, 549.0523; found, 549.0514. **4**β: ^1H NMR (D_2O) d = 5.59 (d, 1 H, $J_{1,P}$ = 5.9 Hz, H-1 Fuc); ^{13}C NMR (D_2O) d = 103.8 (C-1 Fuc); ^{31}P NMR (D_2O) d = −11.5 (d, $J_{P,P}$ = 21.8 Hz, Pa), −13.8 (d, Pb). HRMS (ESI^-): $[C_{15}H_{23}N_2O_{16}P_2]^-$ calcd, 549.0523; found, 549.0514.

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