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Stereoselective Chemoenzymatic Synthesis of UDP-1,2-*cis*-furanoses from α,β-Furanosyl 1-Phosphates

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The biosynthesis of furanosyl-containing glycoconjugates is poorly described, mainly due to the lack of UDP-furanoses. Here we present our effort to synthesize rare nucleotide-sugars with the aid of a multiple-enzyme system, notably including galactose-1-phosphate uridylyltransferase. Firstly, STD-NMR techniques were used to probe the broad substrate specificity of this particular enzyme. The chemical synthesis of the needed furanosyl 1-phosphate starting materials was then performed with unprotected thioimidoyl donors. This led to the first stereoselective chemoenzymatic syntheses of UDP-β-L-arabinofuranose, UDP-α-D-fucofuranose and UDP-α-D-6F-galactofuranose from starting mixtures of sugar-phosphates.

Introduction

Hexofuranosides represent rare sugars that can be found in significant proportions in protozoa, fungi, bacteria, archaebacteria and plants, but are completely absent in higher organisms, especially mammals.[1] The interest in such carbohydrates arises from the fact that the microorganisms able to produce them are sometimes highly pathogenic. For instance, mycobacteria, which are responsible for re-emergent diseases such as tuberculosis and leprosy, ensure their survival thanks to cell coats notably containing D-galactofuranose (D-Gal*f*) as a major constituent of their extracellular polysaccharides. Other galactofuranosides have been isolated as constituents of interesting biologically active glycosphingolipids, such as the immunostimulating agelagalastatin from marine sponges.[2] Of course, D-Gal*f* is not the only hexofuranoside found in natural products: as examples, D-glucofuranose (D-Glc*f*), D-fucofuranose (D-Fuc*f*) and D-mannofuranose (D-Man*f*) have been identified in glycoconjugates isolated from *Agrobacterium tumefaciens*, *Eubacterium saburreum* and *Evernia prunastri*, respectively.[3–5] Recently, the first glycosphingolipid containing an L-fucofuranose unit, from a Caribbean *Terpios* sp., has also been described.[6]

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Despite the elucidation of these structures, little is known about the biosynthesis of these hexofuranoconjugates, except for those associated with the galactofuranosyl framework. Indeed, there is no description in the literature of their associated mutases – a family of enzymes that ensure the ring contraction of uridine diphosphogalactopyranose (UDP-Gal*p*) to UDP-Gal*f* – or of glycosyltransferases dedicated to D-Glc*f*, D-Fuc*f*, D-Man*f* or even to the ubiquitous L-arabinofuranoside (L-Ara*f*). The limitations of our knowledge in this field are mainly due to lack of access to the corresponding nucleotide-furanoses required to probe such enzymatic activities. Chemical approaches for the synthesis of the sugar nucleotide UDP-α-D-Gal*f* (**1α**) are now well documented, but generally give only poor to moderate yields.[7–9] These reactions generally require the tedious coupling of activated nucleoside 5′-monophosphate and furanosyl 1-phosphate and are hardly reproducible from one sugar to another.[10,11] Recently we have developed a versatile chemical method for the synthesis of natural nucleotide-furanose and analogues,[12] starting from unprotected but stable furanosyl thioimidates as donors.[13,14] This allowed us to obtain some UDP-1,2-*trans*-furanoses for the first time, as well as their 1,2-*cis* diastereoisomers, in very short reaction times and without any observation of ring expansion. Alternatively, enzymatic syntheses of **1α** have also been reported.[15,16] The first method is based on the use of a UDP-Gal*p* mutase, but synthetic applications are limited by the equilibrium constant of the reaction, which favours the more thermodynamically stable pyranose form.[15] The second paper describes a galactose-1-phosphate uridylyltransferase (Gal-1-PUT) with broad substrate specificity, the reaction being driven to the side of the formation of **1α** thanks to the use of a two-enzyme recycling system (Fig-

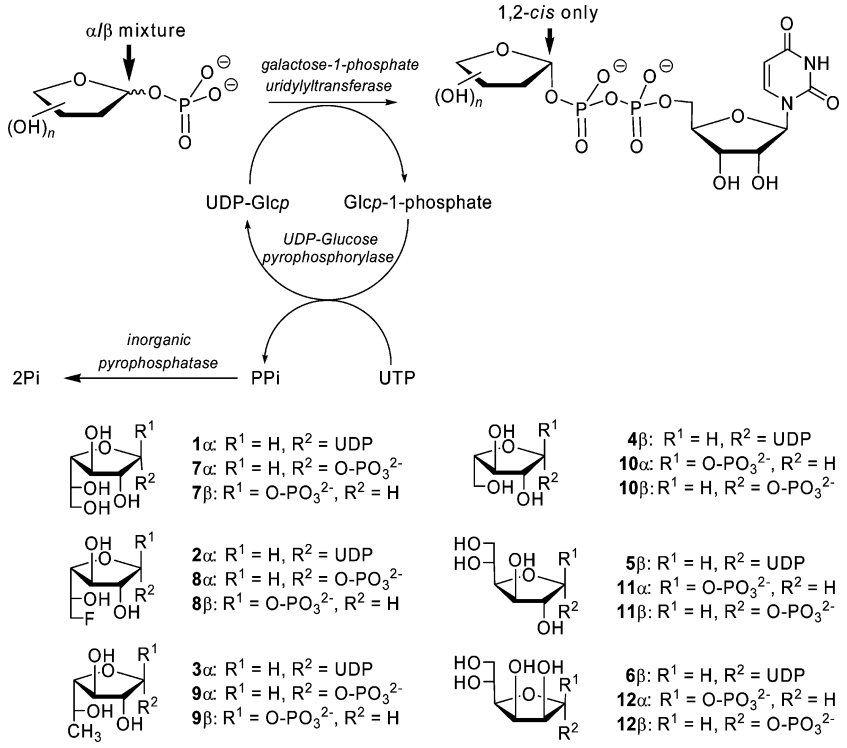


Figure 1. Conversion of α,β-furanosyl 1-phosphates by a three-enzyme procedure.

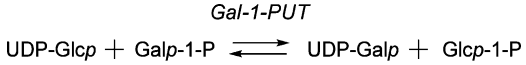
ure 1).[16] We recently examined the ability of a wild-type recombinant glucopyranosyl 1-phosphate thymidylyltransferase to accept furanosyl 1-phosphates **7**–**11** as substrates (Figure 1) and were able to show the stereoselective formation of new dTDP 1,2-*cis*-furanoses with potential biological properties.[17]

Therefore, to the best of our knowledge, there is currently no efficient and versatile chemoenzymatic method to synthesize UDP-1,2-*cis*-furanoses **2**–**6**, analogues of **1α**. To overcome this lack of know-how, here we wish to report our findings related to the utilization of the Gal-1-PUT described by Field and co-workers.[16] In this context, Saturation Difference Transfer NMR (STD-NMR)[18] techniques were first developed to observe the recognition of substrates by this protein and to determine the epitope of the directly binding segment of the ligands more precisely.

Then, to assess the specificity of Gal-1-PUT for substrates **1**–**6**, furanosyl 1-phosphates **7α,β**–**12α,β** were chemically prepared and used as precursors for the enzymatic synthesis of the target UDP-furanoses. Gal-1-PUT was expected to discriminate between the different anomers in the anomeric starting mixtures. Finally, the usefulness of this method was emphasized by the synthesis of UDP-1,2-*cis*-6-deoxy-6-fluoro-D-galactofuranose (UDP-D-6F-Gal*f*, **2α**) on a scale of a few mg.

Results and Discussion

Galactose-1-phosphate uridylyltransferase (E.C. 2.7.7.12) catalyses the reversible transfer of the UMP moiety from UDP-Glc*p* to galactopyranosyl 1-phosphate (Gal*p*-1-P) in order to produce the UDP-Gal*p* with the concomitant release of Glc*p*-1-P (Scheme 1). This bisubstrate reaction proceeds by ping-pong kinetics and a double displacement mechanism, in which a histidine residue is transiently nucleotidylated.[19]



Scheme 1. Reversible Gal-1-PUT-catalysed reaction.

A one-pot, multiple-enzyme approach to UDP-galactopyranose analogues from the corresponding 1-phosphate sugars by use of this Gal-1-PUT had been explored previously.[16] This enzyme appeared to possess a remarkably relaxed substrate specificity, accepting various substitutions at the C-2, C-3 or C-5 positions of the D-Gal*p* backbone. We particularly noted the straightforward access to the geometrically unrelated UDP-Gal*f* (**1α**) in 79% yield[16] and were thus encouraged to explore the versatility of the Gal-1-PUT in relation to the furanosyl 1-phosphates **7**–**12** (Figure 1) in order to synthesize both natural and artificial UDP-furanoses in one–step fashion.

STD-NMR Experiments

STD experiments have become a robust and fast method to detect binding of a ligand to a receptor.[18] This method allows not only discrimination between potential substrates but also the detection of the ligand protons close to the protein surface. For instance, this NMR technique was re-

cently applied to nucleotide-sugars such as UDP-Gal*f* and UDP-Glc*p* to probe their binding in the active sites of UDP-Gal*p* mutase and UDP-glucose pyrophosphorylase (GalU), respectively.[20–22]

UDP-Gal*p* and Gal*p*-1-P are the natural substrates of Gal-1-PUT, and these were chosen first for the STD-NMR studies (Scheme 1). Each selected substrate was therefore dissolved alone in Tris deuterated buffer (D_2O, pH 6.8, 10 mM $MgCl_2$), and enzyme (1% molar ratio) was added. As would be expected for a double displacement reaction, Gal*p*-1-P exhibits no signal because its binding requires preloading of the enzyme with UDP-Glc*p*. In contrast, UDP-Gal*p* makes important contacts to the enzyme (Figure 2, left-hand side) and therefore seems to be strongly anchored in the active site. Indeed, each proton of the molecule – except for 2-H, 4-H and 5-H of the galactose ring, the signals of which were unfortunately lost, buried under the signal of the buffer – generates measurable signals. Consequently they are in contact with the enzyme, with the strongest responses being exhibited by 6-H of the uracil part and 3-H of the galactose ring. These results are in good agreement with the published three-dimensional structure of the H166G site-directed mutant of Gal-1-PUT in complexation with UDP-Gal*p* or UDP-Glc*p*.[23] Moreover, it also emerged from this STD-NMR study that small chemical variations on UDP-pyranoses should have only limited impact on recognition phenomena, as experimentally observed.[16] This study also represents the first direct measurement of the interaction of the nucleotide sugar with the native enzyme Gal-1-PUT and illustrates the unavoidably sequential binding mode of the substrates (i.e., UDP-Gal*p* has to bind prior to Glc*p*-1-P).

On the basis of this work, we were further interested in investigating the binding of various UDP-furanoses **1**, **2** and **4** previously synthesized in our group[12] in order to test the synthetic potential of this enzyme. (Fucosyl derivative **3** was excluded from this study because we only possessed it as a mixture of anomers.[12]) UDP-1,2-*cis*-Gal*f* (**1α**), already

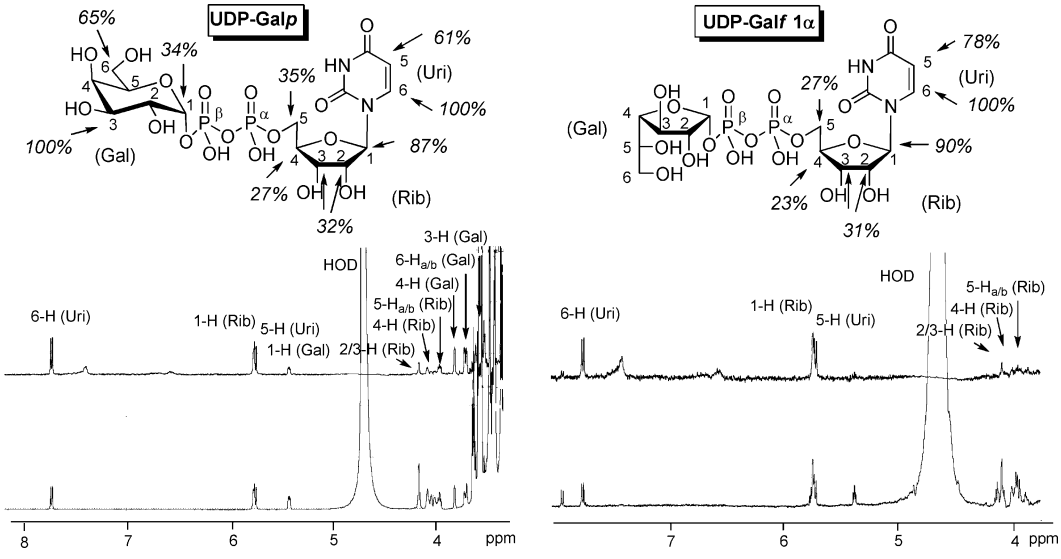


Figure 2. Ligand binding measured by STD-NMR spectroscopy. ^{1}H NMR reference spectra used to assign STD signals correctly are given at the bottoms of both panels. Relative STD-NMR signals were calculated and are indicated for each proton.

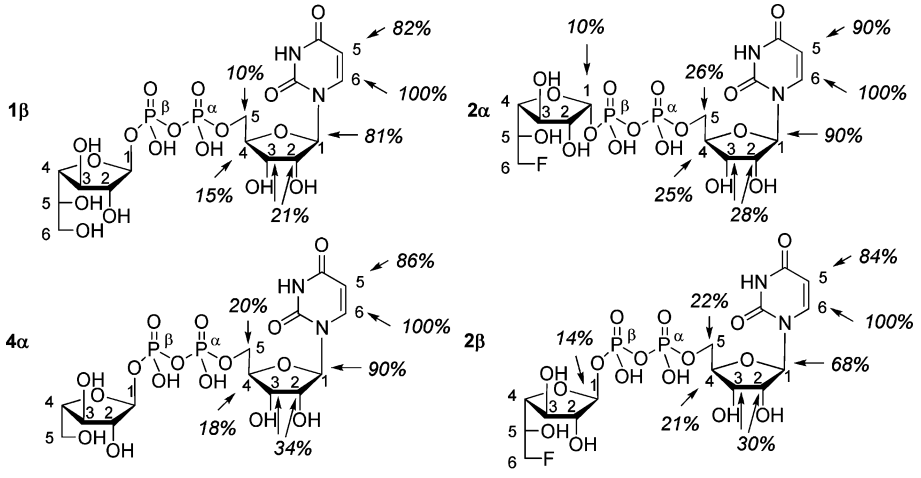


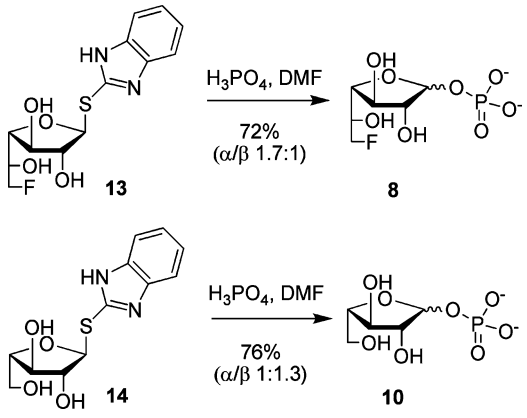
Figure 3. Relative STD effects calculated for the other UDP-furanoses.

known to act as a substrate,[16] was further tested with the same STD-NMR sequence (Figure 2, right-hand side). Interestingly, it shows a pattern of contacts similar to that of UDP-Gal*p* as far as the nucleotide moiety is concerned, but a complete lack of interactions as for the galactofuranose part. Despite this weak but apparently sufficient recognition by the enzyme, however, it can easily be obtained on a multi-mg scale by use of the three-enzyme system depicted in Figure 1 and recently utilized by Lowary and coworkers.[24]

All the other analogues were then probed with the STD-NMR technique (Figure 3). It is noteworthy that all of them show highly similar STD signals with relatively close intensities for each corresponding proton, even for the 1,2-*trans* diastereoisomers of the UDP-furanoses. Again, uracil 6-H demonstrated the strongest effect. Moreover, except for the 1-H protons of 2α and 2β, which show weak signals (around 10–15%), no contacts seem to be observed for the galacto- or arabinofuranose rings. Still, this constitutes the first evidence that: i) UDP-1,2-*cis*-furanoses other than **1α** and, even more interestingly, ii) UDP-1,2-*trans* derivatives, can interact with Gal-1-PUT. This encouraged us to prepare the corresponding furanosyl 1-phosphates and to assess their potential synthetic applications.

Chemical Synthesis of Furanosyl 1-Phosphates 8 and 10

The targeted furanosyl 1-phosphates **7**, **9**, **11** and **12** were chemically prepared by a general and concise synthesis.[25] The unprotected benzimidazolyl thiofuranosides **13** and **14**,[12] derived from 6-deoxy-6-fluoro-D-galactofuranose and L-arabinofuranose, respectively, were smoothly activated with dry phosphoric acid at room temperature (Scheme 2). The reactions were initially monitored by ^{1}H NMR spectroscopy in order to optimize the reaction times, which were highly dependent on the carbohydrate structures. The resulting data indicated that the phosphorylation of the fluorinated derivative **13** was complete after 1 hour, whereas 3 hours were necessary for the consumption of the starting arabinosyl compound **14**. The corresponding furanosyl 1-phosphates **8** and **10** were isolated as ammonium salts as the result of an optimized protocol that did not alter the desired product, neither the ring size nor the α/β ratio. This approach afforded the target phosphate **8** in a 72% overall yield and an α/β ratio of 1.7:1. It was then extended to the synthesis of the pentofuranosyl 1-phosphate **10** in 76% yield as a 1:1.3 anomeric mixture. As previously observed during the phosphorylation of the other 1-thiohexofuranosides,[12,25] NMR monitoring revealed faster formation of the desired 1,2-*cis* stereoisomers as well as their subsequent anomerization into the less hindered 1,2-*trans* ones. The assignment of the anomeric protons of **8** and **10** was based on the multiplicity of the signals. The 1,2-*cis* stereoisomers were each characterized by a double doublet near 5.40 ppm, with similar coupling constants $J_{1,2}$ and $J_{1,P}$ close to 4.6–4.9 Hz. Their 1,2-*trans* counterparts were each distinguishable by a double doublet at slightly higher field with a $J_{1,2}$ value close to 1.0 Hz and a $J_{1,P}$ value higher than 6.5 Hz.



Scheme 2. Chemical synthesis of furanosyl 1-phosphates **8** and **10**.

Enzymatic Synthesis of UDP-1,2-*cis*-Furanoses 1–4

Attempts to synthesize UDP-furanoses **1**–**6** enzymatically were conducted in the presence of equimolecular ratios of the corresponding furanosyl 1-phosphates **7α,β**–**12α,β**, respectively, and UTP, incubated with the Gal-1-PUT c. GalU and inorganic pyrophosphatase were then added, and the reactions were initiated by addition of catalytic amounts of UDP-Glc*p*. The reaction media were maintained at 20 °C for 24 hours. Once excess UTP had degraded to give UMP and uridine after addition of alkaline phosphatase to the reaction mixtures, the resulting solutions were analysed by reversed-phase HPLC. Nevertheless, since our approach relies on the use of anomeric mixtures of furanosyl 1-phosphates as substrates, initial investigations were conducted in order to determine the impact of the anomeric configuration on the behaviour of Gal-1-PUT. We thus carried out the conversion of α-D-Gal*p*-1-P with UDP-Glc*p* (1 equiv.) catalysed by Gal-1-PUT (10 mg mL^{-1} in pH 7.9 Tris-HCl buffer solution) over 20 hours while varying the amount of added α,β-D-Gal*f*-1-P (**7α,β**, 0.1, 0.5, 1 equiv.). Monitoring of the reaction by HPLC allowed the evaluation of the conversion yields on the basis of the area ratios [UDP-Gal*p*]/([UDP-Gal*p*]+[UDP-Glc*p*]). It is noteworthy that best results were obtained with the ammonium salts of **7**, rather than their less soluble tributylammonium counterparts. Under these conditions, a 75% conversion yield of α-D-Gal*p*-1-P into UDP-Gal*p* was obtained in the absence of **7**, and remained unchanged when the concentration of furanosyl 1-phosphate **7α,β** was increased. These experiments demonstrated that D-Gal*f*-1-P (**7β**) is not an inhibitor of Gal-1-PUT under the conditions used.

On this assumption, the couplings between the furanosyl 1-phosphates **7α,β**–**12α,β** and the nucleotide part of UDP-Glc*p* were monitored by HPLC (Figure 4). The conversion yields were evaluated on the basis of the area ratios [UDP-furanose]/([UDP-furanose]+[UMP]+[Uridine]) (Table 1). The multiple-enzyme conversions of the galactofuranosyl 1-

phosphate analogues **9** and **10** (Table 1, Entries 3 and 4) appeared to be lower-yielding than that of Gal*f*-1-P (**7**, Entry 1). Nevertheless, the 6-fluoro-6-deoxy-D-Gal*f*-1-P (**8**) was more efficiently converted into the desired UDP-furanose **2**, in up to 42% yield (Entry 2). It is noteworthy that neither Glc*f*-1-P (**11α,β**) nor Man*f*-1-P (**12α,β**) were converted by the enzymatic system (Entries 5 and 6). Moreover, because semipreparative chromatography allowed good resolution between the anomer pairs of the chemically prepared UDP-furanoses,[12] we could argue that Gal-1-PUT was able to discriminate the two anomers in each of the starting furanosyl phosphates **7α,β**–**10α,β** (Table 1 and Figure 4).

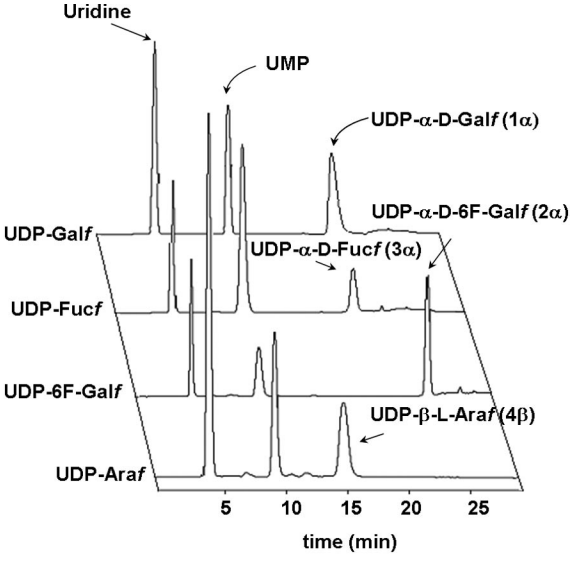


Figure 4. Chromatographic profiles for UDP-1,2-*cis*-furanoses **1**–**4**.

Table 1. Conversion yields and selectivity.

α,β-Furanose-1-phosphate —*one-pot*, *3-enzymatic procedure*→ UDP-Hexofuranose

	7–12		**1–6**	
Entry	Substrate (α/β ratio)	Product	% Yield	Selectivity
1	**7α,β** (1.2:1)	**1**	40	α (1,2-*cis*)
2	**8α,β** (1.7:1)	**2**	42	α (1,2-*cis*)
3	**9α,β** (1.5:1)	**3**	12	α (1,2-*cis*)
4	**10α,β** (1:1.3)	**4**	22	β (1,2-*cis*)
5	**11α,β** (1.2:1)	**5**	0	n.d.[a]
6	**12α,β** (1.6:1)	**6**	0	n.d.

[a] n.d.: not detected.

Finally, we considered the synthesis of multi-milligram amounts of UDP-6-fluoro-6-deoxy-α-D-Gal*f* (**2α**) obtained on an analytical scale with a good conversion yield (Table 1, Entry 2). The furanosyl 1-phosphate **8** (3.2 mg) was subjected to the multiple-enzyme system. Subsequent purification by simple preparative reversed-phase HPLC afforded **2** (2.5 mg) in 29% yield directly from the anomeric mixture of the furanosyl 1-phosphate **8**. These results as a whole demonstrate that: i) transfer of the UMP moiety only occurs on the furanosyl 1-phosphates possessing relative 1,2-*cis* configurations, and ii) chemical modulation at C-4 and C-6 of the furanosyl-1-phosphates has only limited impact on the feasibility of the enzymatic process, but iii) epimerization at C-4 or C-2 prevents UMP transfer, and iv) scale-up procedures can be considered with interest.

Conclusions

The chemoenzymatic procedure described here has allowed us to prepare natural UDP-α-D-Gal*f* as well as three 1,2-*cis* synthetic analogues, starting from anomeric mixtures of the corresponding phosphates. This represents a successful implementation of the multiple-enzyme system developed previously. This first stereospecific chemoenzymatic syntheses of **1**–**4** complement the original chemical approach from unprotected thioimidoyl furanosyl donors developed by our group. Indeed, this three-enzyme method appears to be advantageous for the preparation of UDP-1,2-*cis*-furanoses in a pure form and more especially for the hardly accessible UDP-β-L-Ara*f*. It was also applied to the multi-milligram synthesis of pure UDP-α-D-6-fluoro-6-deoxy-Gal*f*. Moreover, the STD-NMR study developed in this paper has helped us in increasing our knowledge of the binding interactions between UDP-sugars and Gal-1-PUT. The nucleotide moiety of the molecule makes most of the strongest contacts and therefore seems to represent the key part for the recognition process. Interestingly, we were also able to prepare UDP-α-D-Fuc*f*, never isolated in nature but strongly suspected to exist. Therefore, the ready availability of UDP-furanoses is highly desirable for increasing our understanding of many biological processes. The preparation of original substrates or inhibitors of enzymes specifically involved in the biosynthesis of furanosyl-containing glycoconjugates may result in the discovery of new drugs.

Experimental Section

General: Uridine 5′-diphosphoglucose disodium salt (UDP-Glcp), uridine 5′-triphosphate trisodium salt (UTP), UDP-glucose pyrophosphorylase (GalU, EC 2.7.7.9) and inorganic pyrophasphatase (EC 3.6.1.1) were purchased from Sigma. All other reagents were of chemical grade. Gal*f*-1-P (**7**), Fuc*f*-1-P (**9**), Glc*f*-1-P (**11**) and Man*f*-1-P (**12**) were synthesized as previously reported.[25] Thin-layer chromatography (TLC) analyses were conducted on E. Merck 60 F_{254} silica gel non-activated plates, and compounds were revealed by use of a solution of H_2SO_4 in EtOH (5%) followed by heating. For column chromatography, Geduran Si 60 (40–63 μm) silica gel was used. ^{1}H, ^{13}C, ^{31}P, ^{19}F, HMQC and COSY NMR spectra were recorded on a Bruker ARX 400 spectrometer at 400 MHz for ^{1}H, 100 MHz for ^{13}C, 162 MHz for ^{31}P and 376 MHz for ^{19}F. Chemical shifts are given in δ units (ppm) measured downfield from Me_4Si. HPLC purifications were performed on a Thermo SpectraSYSTEM P1000XR instrument. The purifications were monitored with a Thermo SpectraSYSTEM UV1000 ultraviolet detector at λ = 280 nm. The HRMS were measured at the CRMPO (University of Rennes 1, France) with a MS/MS ZabSpec TOF Micromass with *m*-nitrobenzyl alcohol as a matrix and accelerated caesium ions for ionization.

STD-NMR Experiments: Samples were prepared in Tris deuterated buffer (D_2O, 10 mM $MgCl_2$, pH 6.8, 0.5 mL) containing substrate (UDP-sugar or sugar-1-phosphate, ca. 1.5 μmol). STD-NMR spectra were recorded on a Bruker Avance II 500 MHz spectrometer fitted with a cryogenic probe (PRISM, University of Rennes 1, France). Processing of all data was performed on a PC with Bruker Topspin v2.0 software. After the determination of the optimal conditions [i.e., temperature, delay between pulse (d_{20}) and molecular ratio (protein/ligand)], STD-NMR experiments were performed at 283 K as follows. The protein (1:100 ratio) was saturated on-resonance at 0.7 ppm and off-resonance at δ =40 ppm with a cascade of 40 selective gaussian-shaped pulses of 50 ms duration with a 100 μs delay between each pulse. The total duration of the saturation time was set to 2 s. A total of 256 scans per STD-NMR experiment was acquired. A WATERGATE sequence was used to suppress residual HOD signal. A spin lock filter with strength of 5 kHz and duration of 10 ms was also applied to suppress the protein background. A similar experiment with no enzyme was used as reference in order to verify the absence of STD effect in these experimental conditions. Intensities of all STD effects were calculated though integrals over the respective signals in ^{1}H NMR reference spectra. The largest STD effect in each spectrum was set to 100% and relative intensities were determined, as common for unrefined STD effects. Hence, sufficient comparisons of relative STD effects between UDP-sugars were possible, but absolute binding intensities could not be determined.

General Procedure A for the Preparation of Ammonium Glycofuranosyl 1-Phosphates: A solution of orthophosphoric acid (238 mg, 2.43 mmol) in dry DMF (1.4 mL) was added to a solution of the 2-benzimidazolyl 1-thioglycofuranoside (104 mg, 0.34 mmol) in dry DMF (1 mL). This mixture was stirred for an appropriate period and at a suitable temperature, cooled to 0 °C and then diluted with water (15 mL). A saturated solution of aq. $Ba(OH)_2$ was added dropwise until the pH value reached 8–9. The $Ba_3(PO_4)_2$ precipitate could be removed by centrifugation (15000 rpm, 0 °C, 15 min), and the supernatant liquid was filtered. The resulting filtrate and washings (3 × 5 mL) were combined and carefully neutralized by addition of Amberlite IR-120 (H^+ form). After removal of the resin, the solution was freeze-dried, and absolute EtOH (2 mL) and EtOAc (12 mL) were successively added to the residue. The precipitate was collected by centrifugation (15000 rpm, 0 °C, 15 min), washed in the manner described above, and added to Et_2O (14 mL). The solvent was removed after centrifugation (15000 rpm, 0 °C, 15 min), and the crude barium salt was dried under gentle vacuum. The solid thus obtained was then dissolved in water (5 mL) and loaded on an Amberlite IR-120 (NH_4^+ form) column (1 × 15 cm). After slow elution with water (45 mL), the pH value of the effluent was adjusted to 8.5–9 through the addition of aq. NH_4OH (1 M, a few drops). This effluent was then freeze-dried, and the residue was loaded onto an Amberlyst A-26 (CO_3^{2-} form) column (1 × 15 cm). Neutral compounds were first removed by elution with water (50 mL), and the target phosphate was recovered by elution with aq. $(NH_4)_2CO_3$ (0.21 M, 50 mL). Finally, freeze-drying yielded the desired ammonium glycofuranosyl 1-phosphate.

Ammonium 6-Deoxy-6-fluoro-D-galactofuranosyl 1-phosphate (8): This phosphate was obtained by General Procedure A from 2-benzimidazolyl 6-deoxy-6-fluoro-1-thio-β-D-galactofuranoside (100 mg, 0.32 mmol) after the system had been stirred at room temperature for 60 min. An anomeric mixture (α/β 1.7:1) was isolated as an amorphous solid (67.3 mg, 72%). HR-MS (ESI, negative ion mode): calcd. for $C_6H_{11}FO_8P$ $[M + H]^-$ 261.0176; found 261.0180.

Isomer 8α: ^{1}H NMR (400 MHz, D_2O): δ = 5.42 (dd, $J_{1,P}$ = 4.9, $J_{1,2}$ = 4.9 Hz, 1 H, 1-H), 4.68 (ddd, $J_{5,6a}$ = 4.0, $J_{6a,6b}$ = 10.2, $J_{6a,F}$ = 46.7 Hz, 1 H, 6-Ha), 4.66 (ddd, $J_{5,6b}$ = 6.5, $J_{6b,F}$ = 47.3 Hz, 1 H, 6-Hb), 4.18 (dd, $J_{2,3}$ = 7.1, $J_{3,4}$ = 7.1 Hz, 1 H, 3-H), 4.07–4.03 (m, 1 H, 2-H), 3.89–3.93 (m, 1 H, 5-H), 3.77 (dd, $J_{4,5}$ = 5.6 Hz, 1 H, 4-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 96.5 (d, $J_{1,P}$ = 7 Hz, C-1), 84.4 (d, $J_{6,F}$ = 165 Hz, C-6), 82.9 (d, $J_{2,P}$ = 8 Hz, C-2), 80.8 (d, $J_{4,F}$ = 9 Hz, C-4), 74.6 (C-3), 70.8 (d, $J_{5,F}$ = 23 Hz, C-5) ppm. ^{31}P NMR (162 MHz, D_2O): δ = 3.4 ppm. ^{19}F NMR (376 MHz, D_2O): δ = –231 ppm.

Isomer 8β: ^{1}H NMR (400 MHz, D_2O): δ = 5.38 (dd, $J_{1,P}$ = 6.6, $J_{1,2}$ = 1.6 Hz, 1 H, 1-H), 4.72 (ddd, $J_{5,6a}$ = 3.5, $J_{6a,6b}$ = 10.4, $J_{6a,F}$ = 46.5 Hz, 1 H, 6-Ha), 4.65 (ddd, $J_{5,6b}$ = 5.7, $J_{6b,F}$ = 47.1 Hz, 1 H, 6-Hb), 4.10–4.07 (m, 1 H, 2-H), 4.04–3.97 (m, 2 H, 3-H, 4-H), 3.89–3.86 (m, 1 H, 5-H) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 102.7 (d, $J_{1,P}$ = 5 Hz, C-1), 84.8 (d, $J_{6,F}$ = 165 Hz, C-6), 82.1 (d, $J_{2,P}$ = 7 Hz, C-2), 77.3 (d, $J_{4,F}$ = 9 Hz, C-4), 76.9 (C-3), 70.0 (d, $J_{5,F}$ = 24 Hz, C-5) ppm. ^{31}P NMR (162 MHz, D_2O): δ = 2.9 ppm. ^{19}F NMR (376 MHz, D_2O): δ = –230 ppm.

Ammonium L-Arabinofuranosyl 1-Phosphate (10): This compound was prepared as described in General Procedure A, from the starting donor 2-benzimidazolyl 1-thio-α-L-arabinofuranoside (100 mg, 0.36 mmol). After the system had been stirred for 3 h at 0 °C and worked up, an amorphous solid (70.6 mg, 76%) containing an anomeric mixture (α/β 1:1.3) was isolated. HR-MS (ESI, negative ion mode): calcd. for $C_5H_{10}O_8P$ $[M + H]^-$ 229.0113; found 229.0103.

Isomer 10α: ^{1}H NMR (400 MHz, D_2O): δ = 5.32 (dd, $J_{1,P}$ = 7.1, $J_{1,2}$ = 0.9 Hz, 1 H, 1-H), 4.06–4.02 (m, 2 H, 2-H, 4-H), 3.79 (dd, $J_{3,4}$ = 5.7, $J_{2,3}$ = 3.1 Hz, 1 H, 3-H), 3.66 (dd, $J_{4,5a}$ = 3.3, $J_{5a,5b}$ = 12.3 Hz, 1 H, 5-Ha), 3.54 (dd, $J_{4,5b}$ = 6.0 Hz, 1 H, 5-Hb) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 102.7 (d, $J_{1,P}$ = 4 Hz, C-1), 84.3 (C-4), 82.2 (d, $J_{2,P}$ = 7 Hz, C-2), 76.8 (C-3), 62.1 (C-5) ppm. ^{31}P NMR (162 MHz, D_2O): δ = 2.8 ppm.

Isomer 10β: ^{1}H NMR (400 MHz, D_2O): δ = 5.36 (dd, $J_{1,P}$ = 4.6, $J_{1,2}$ = 4.6 Hz, 1 H, 1-H), 4.04 (dd, $J_{2,3}$ = 8.2, $J_{3,4}$ = 6.6 Hz, 1 H, 3-H), 3.95 (ddd, $J_{2,P}$ = 1.8 Hz, 1 H, 2-H), 3.73 (ddd, $J_{4,5a}$ = 2.9, $J_{4,5b}$ = 5.3 Hz, 1 H, 4-H), 3.67 (dd, $J_{5a,5b}$ = 12.0 Hz, 1 H, 5-Ha), 3.54 (dd, 1 H, 5-Hb) ppm. ^{13}C NMR (100 MHz, D_2O): δ = 96.4 (d, $J_{1,P}$ = 6 Hz, C-1), 82.3 (C-4), 77.3 (d, $J_{2,P}$ = 7 Hz, C-2), 73.8 (C-3), 61.7 (C-5) ppm. ^{31}P NMR (162 MHz, D_2O): δ = 3.4 ppm.

General Procedure B for the Enzymatic Synthesis of Nucleotide-Furanoses: UTP (2.3 mg, 4.00 μmol) and furanose-1-phosphate (4.05 μmol) were dissolved in a solution (100 μL) of Gal-1-PUT [10 mg mL^{-1} in tris-HCl buffer (20 mM, 10% glycerol, pH 7.9)]. GalU (5 U) and inorganic pyrophosphatease (5 U) were then added. The reaction was initiated by addition of UDP-Glc*p* (0.02 μmol). The resulting mixture was shaken at 20 °C for 24 h. Finally, alkaline phosphatase (20 U) was added in order to degrade UTP. The conversion was monitored by HPLC on a reversed-phase C18 column (10 × 250 mm) with elution with 20 min isocratic elution in X followed by a linear gradient from X/Y 100:0 to 90:10 over 10 min, a second gradient from X/Y 90:10 to X/Y 70:30 until 10 min at 5.0 mL min^{-1}, where X is aq. triethylammonium acetate (50 mM, pH 6.8) and Y is HPLC-grade CH_3CN. Samples from multiple HPLC runs were pooled and freeze-dried.

UDP-α-D-galactofuranose (1): This compound was obtained as described in General Procedure B (0.9 mg, 40%). HR-MS (ESI, negative ion mode): calcd. for $C_{15}H_{23}N_2O_{17}P_2$ $[M + H]^-$ 565.0472; found 565.0470. Analytical data are in accordance with literature data.[7]

UDP-6-deoxy-6-fluoro-α-D-galactofuranose (2): This compound was obtained by a slightly modified Procedure B as follows: UTP (6 mg, 11.00 μmol) and furanose-1-phosphate (11 μmol of 1,2-*cis*

in a mixture with 1,2-*trans*) were dissolved in a solution (300 μL) of Gal-1-PUT [10 mg mL^{-1} in tris-HCl buffer (20 mM, 10% glycerol, pH 7.9)]. GalU (15 U) and inorganic pyrophosphatase (15 U) were then added to the above solution. The reaction was initiated by addition of UDP-Glc*p* (0.06 μmol). The resulting mixture was shaken at 20 °C for 24 h. Finally, alkaline phosphatase (20 U) was added in order to degrade UTP. The conversion was monitored by HPLC on a reversed-phase C18 column (10 × 250 mm) with elution with 20 min isocratic elution in X followed by a linear gradient from X/Y 100:0 to 90:10 over 10 min and a second gradient from X/Y 90:10 to X/Y 70:30 until 10 minutes at 5.0 mL min^{-1}, where X is aq. triethylammonium acetate (50 mM, pH 6.8) and Y is HPLC grade CH_3CN. The pure UDP-α-D-6F-Gal*f* with 1.8 equiv. of Et_3NH^+ (2.4 mg, 29%) was collected from the peak with a retention time of 25.8 min and freeze-dried. ^{1}H NMR (400 MHz, D_2O): δ = 7.92 (d, $J_{5,6}$ = 8.2 Hz, 1 H, 5-H Uri), 5.96 (d, $J_{1,2}$ = 4.6 Hz, 1 H, 1-H Rib), 5.95 (d, 1 H, 6-H Uri), 5.58 (dd, $J_{1,P}$ = 5.7, $J_{1,2}$ = 4.2 Hz, 1 H, 1-H Gal), 4.61 (ddd, $J_{5,6a}$ = 3.5, $J_{6a,6b}$ = 10.2, $J_{6a,F}$ = 42.4 Hz, 1 H, 6-Ha Gal), 4.50 (ddd, $J_{5,6b}$ = 6.4, $J_{6b,F}$ = 47.3 Hz, 1 H, 6-Hb Gal), 4.35–4.30 (m, 2 H, 2-H Rib, 3-H Rib), 4.26–4.11 (m, 5 H, 4-H Rib, 5-Ha Rib, 5-Hb Rib, 2-H Gal, 3-H Gal), 4.01–3.93 (m, 1 H, 5-H Gal), 3.86 (dd, $J_{3,4}$ = 7.0, $J_{4,5}$ = 6.2 Hz, 1 H, 4-H Gal) ppm. ^{31}P NMR (162 MHz, D_2O): δ = –10.1 (d, $J_{P,P}$ = 19.6 Hz, Pα), –11.5 (d, Pβ) ppm. ^{19}F NMR (376 MHz, D_2O): δ = –232 ppm. HR-MS (ESI, negative ion mode): calcd. for $C_{15}H_{22}FN_2O_{16}P_2$ [M + H]$^-$ 567.0428; found 567.0419. Analytical data are in accordance with literature data.[12]

UDP-α-D-fucofuranose (3): This compound was obtained as described in General Procedure B (0.25 mg, 12%). ^{1}H NMR (400 MHz, D_2O): δ = 7.95 (d, $J_{5,6}$ = 8.2 Hz, 1 H, 5-H Uri), 5.97 (d, $J_{1,2}$ = 4.3 Hz, 1 H, 1-H Rib), 5.96 (d, 1 H, 6-H Uri), 5.62 (d, $J_{1,2}$ = 4.2, $J_{1,P}$ = 5.9 Hz, 1 H, 1-H Fuc), 4.37–4.35 (m, 2 H, 2-H Rib, 3-H Rib), 4.28–4.17 (m, 3 H, 4-H Rib, 5-Ha Rib, 5-Hb Rib), 4.12 (dd, $J_{2,3}$ = 2.4 Hz, 1 H, 2-H Fuc), 4.07 (dd, $J_{3,4}$ = 7.0 Hz, 1 H, 3-H Fuc), 4.89–4.85 (m, 1 H, 5-H Fuc), 3.60 (dd, $J_{4,5}$ = 7.1 Hz, 1 H, 4-H Fuc), 1.18 (d, $J_{5,6}$ = 6.4 Hz, 3 H, 6-H Fuc) ppm. ^{31}P NMR (162 MHz, D_2O): δ = –11.4 (d, $J_{P,P}$ = 19.6 Hz, Pα), –12.7 (d, Pβ) ppm. HR-MS (ESI, negative ion mode): calcd. for $C_{15}H_{23}N_2O_{16}P_2$ [M + H]$^-$ 549.0523; found 549.0514. Analytical data are in accordance with literature data.[12]

UDP-β-L-arabinofuranose (4): This compound was obtained as described in General Procedure B (0.5 mg, 22%). ^{1}H NMR (400 MHz, D_2O): δ = 7.94 (d, $J_{5,6}$ = 8.2 Hz, 1 H, 5-H Uri), 5.96 (d, $J_{1,2}$ = 4.6 Hz, 1 H, 1-H Rib), 5.94 (d, 1 H, 6-H Uri), 5.60 (dd, $J_{1,P}$ = 5.9, $J_{1,2}$ = 3.7 Hz, 1 H, 1-H Ara), 4.35–4.33 (m, 2 H, 2-H Rib, 3-H Rib), 4.27–4.23 (m, 1 H, 4-H Rib), 4.22–4.17 (m, 2 H, 5-Ha Rib, 5-Hb Rib), 4.14–4.11 (m, 2 H, 2-H Ara, 3-H Ara), 3.89 (m, 1 H, 4-H Ara), 3.76 (dd, $J_{4,5a}$ = 2.9, $J_{5a,5b}$ = 12.5 Hz, 1 H, 5-Ha Ara), 3.66 (dd, $J_{4,5b}$ = 6.0 Hz, 1 H, 5-Hb Ara) ppm. ^{31}P NMR (162 MHz, D_2O): δ = –11.3 (d, $J_{P,P}$ = 21.8 Hz, Pα), –12.7 (d, Pβ) ppm. HR-MS (ESI, negative ion mode): calcd. for $C_{14}H_{21}N_2O_{16}P_2$ [M + H]$^-$ 535.0366; found 535.0359. Analytical data are in accordance with literature data.[10]

Supporting Information (see also the footnote on the first page of this article): STD-NMR spectra and spectroscopic data for compounds **1**–**4**, **8** and **10**.

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