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Improved Chemical Synthesis of UDP-Galactofuranose

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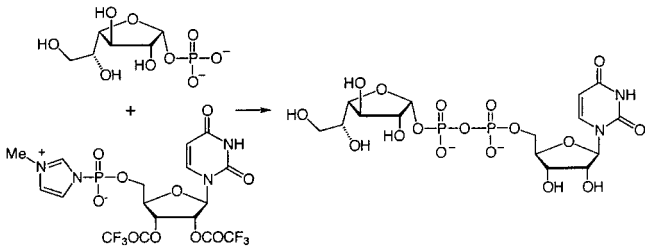
ABSTRACT



A reliable and efficient synthetic route to UDP-α-D-galactofuranose (UDP-Gal*f*) has been developed. Reaction of UMP-*N*-methylimidazolide with Gal*f* 1-phosphate proceeds rapidly to provide UDP-Gal*f* with excellent reproducibility and in a yield approximately twice as high as those reported previously.

D-Galactose is a critical constituent of polysaccharides, glycoproteins, and glycolipids. This hexose can exist in either the six-membered ring pyranose or the five-membered ring furanose form. The more stable pyranose form is the only one found in mammalian glycoproteins and glycolipids. Intriguingly, galactofuranose (Gal*f*) residues occur in various polysaccharides and glycoconjugates produced by infectious bacteria, protozoa, and fungi.[1] Polysaccharides and glycoconjugates that contain Gal*f* residues are essential for the survival and pathogenicity of many microorganisms. The enzymes that mediate the incorporation of this moiety are therefore attractive targets for the development of antimicrobial agents.

The biosynthetic glycosyl donor used in the incorporation of Gal*f* residues is uridine 5′-diphospho-α-D-galactofuranose (UDP-Gal*f*) (**2**) (Figure 1). Presumably, galactofuranosyl transferases use this donor to incorporate Gal*f* residues into cell-surface carbohydrates.[2] Glycosyl donor **2** is generated from the action of the enzyme UDP-galactopyranose mutase (UDP-Gal*p* mutase) on UDP-α-D-galactopyranose (UDP-Gal*p*) (**1**) (Figure 1).[3]

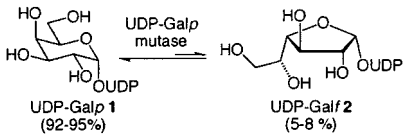


Figure 1. UDP-Gal*p* mutase catalyzes the interconversion of UDP-Gal*p* (**1**) and UDP-Gal*f* (**2**). The pyranose isomer **1** is favored.

To investigate the processes involved in Gal*f* incorporation, access to UDP-Gal*f* **2** is essential. This biosynthetic intermediate is required to identify putative glycosyltransferases and to study the action of UDP-Gal*p* mutase. The mutase

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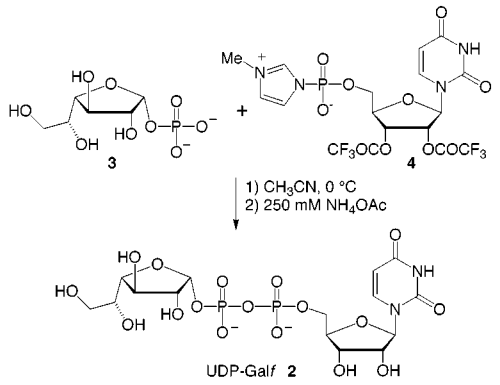
catalyzes the conversion of UDP-Gal*p* **1** to UDP-Gal*f* **2** with the low efficiencies dictated by thermodynamics (5−8%), but it efficiently isomerizes UDP-Gal*f* **2** to UDP-Gal*p* **1** (92−95% yield). Thus, UDP-Gal*f* **2** is needed to analyze the reaction in the favored direction.[2b] Given the low yields of the enzymatic reaction, chemical synthesis is the only route to obtain UDP-Gal*f* **2** in sufficient quantities for biological and biochemical studies.

Although the structure of UDP-Gal*f* **2** has been known for 30 years,[4] its synthesis, via two similar routes, has been reported only recently.[5] Both of the published routes suffer from low yields arising from the final key step: the coupling of α-D-Gal*f* 1-phosphate (**3**) to an activated 5′-UMP derivative. The difficulties in synthesizing substrate **2** are likely due to its lability. The conformational preferences and increased flexibility of the five-membered ring of UDP-Gal*f* **2** compared to those of the six-membered ring of UDP-Gal*p* **1** facilitate decomposition reactions of the galactofuranose donor. Specifically, cyclization to yield α-D-Gal*f* 1,2-cyclic phosphodiester and 5′-UMP[2a] and glycosidic bond hydrolysis to afford D-galactose and 5′-UDP are reactions of **2** that occur readily.[6] These degradative processes complicate the synthesis of this important biosynthetic intermediate. Here, we report an effective coupling procedure that results in the preparation of UDP-Gal*f* **2** reproducibly, in a short reaction time (2 h at 0 °C), and in an improved yield (35%) (Scheme 1).

Scheme 1

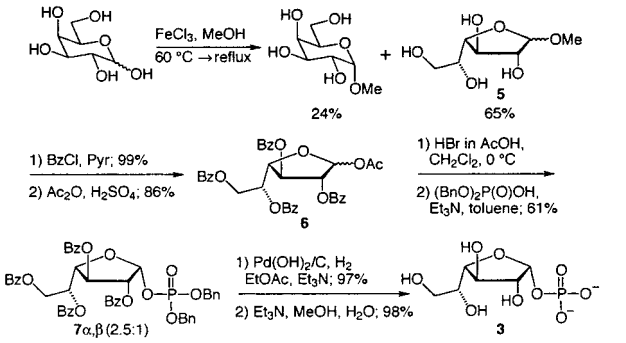


The formation of nucleoside diphospho sugars from a sugar 1-phosphate and an activated 5′-UMP derivative is a challenging synthetic operation; the yields are typically moderate. Strategies for improving the efficiency of the coupling step have been reported. For example, increased product yields have been obtained using a nucleophilic catalyst.[7] In the synthesis of UDP-Gal*f* **2**, both the UMP-morpholidate (in the presence of 1*H*-tetrazole)[5a,b] and the UMP-imidazolide[5a] were tested as sources of activated 5′-UMP. Unfortunately, the reactions of these substrates were slow (1−2 days), proceeded only to 50% completion, and afforded low yields (19−23%). The major side reaction observed was base-catalyzed degradation of product **2** to the α-D-Gal*f* 1,2-cyclic phosphate and 5′-UMP.[5a]

To develop an efficient route to the target, UDP-Gal*f* **2**, we explored alternative conditions for the coupling of α-D-Gal*f* 1-phosphate (**3**) and 5′-UMP. Trifluoroacetic anhydride has been shown to be effective for the activation of nucleoside monophosphates en route to nucleoside triphosphates.[8] All four nucleoside triphosphates can be obtained in 89−92% yields in very short reaction times (2−5 min). Although this method has been used only for triphosphate synthesis, we reasoned that it might be adapted for the synthesis of the target compound **2**. We hypothesized that the mild conditions and the short reaction times employed would minimize the decomposition of the labile UDP-Gal*f* **2** during the course of reaction.

The synthesis of the substrate α-D-Gal*f* 1-phosphate (**3**) was accomplished by a strategy analogous to that described recently (Scheme 2).[5a] D-Galactose was converted to a

Scheme 2



mixture of anomeric methyl furanosides **5** via ferric chloride-catalyzed Fischer glycosylation.[9] The remaining hydroxyl groups were protected as benzoyl esters, and acetolysis of the resulting methyl glycoside provided **6**. Treatment of **6** with hydrogen bromide afforded the appropriate glycosyl donor for phosphorylation. Reaction of this glycosyl bromide with dibenzyl phosphate produced the diastereomeric galactofuranosyl derivatives **7**α and **7***β* (2.5:1 ratio), which were separated by flash chromatography. Contrary to reports that **7***β* is unstable and decomposes rapidly,[10] this compound was isolated as a white crystalline solid that was stable for months at room temperature. Our attempts to isomerize **7***β* to **7**α, using either catalytic BF_3•OEt_2 or the phosphorylation reaction conditions, have been unsuccessful to date. The **7**α phosphoryl protecting groups were removed to yield tetra-

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O-benzoyl-α-D-Gal*f* 1-phosphate triethylammonium salt using a reported protocol.[5a] The benzoyl groups were subsequently removed using a triethylamine−methanol−water solution to afford phosphorylated monosaccharide **3**. Purification was accomplished by ion-exchange chromatography to provide **3** as the bisammonium salt.

Our UDP-Gal*f* **2** coupling procedure consists of several steps. The electrophilic UMP-*N*-methylimidazolide **4** is formed by the reaction of the triethylammonium salt of 5′-UMP with an excess of trifluoroacetic anhydride in the presence of triethylamine and *N*,*N*-dimethylaniline in acetonitrile.[8] Excess trifluoroacetic anhydride is added to increase the solubility of the electrophile in the reaction medium; its addition results in acylation of the 2′- and 3′-hydroxyl groups as well as the phosphoryl group. The nucleophilic catalyst, *N*-methylimidazole, is added to provide the electrophilic derivative **4**. This compound can be detected by ^{31}P NMR spectroscopy, HPLC, and TLC. An excess of activated **4** is treated with the tributylammonium salt of α-D-Gal*f* 1-phosphate (**3**). To minimize side reactions of the product, the reaction is quenched with aqueous ammonium acetate buffer (pH 7), which also promotes the removal of the *O*-trifluoroacetyl groups. After workup, the product **2** is isolated by HPLC purification.

A number of parameters were found to affect the yield of the coupling reaction. Various reaction conditions were tested in the model coupling of commercially available α-D-Gal*p* 1-phosphate to form UDP-Gal*p* **1**. We altered the counterion for α-D-Gal*p* 1-phosphate, the number of equivalents of activated 5′-UMP **4**, the reaction duration, and the reaction temperature.

With regard to counterion, bisammonium salts of both **3** and α-D-Gal*p* 1-phosphate were found to be insoluble in acetonitrile. The tributylammonium salt of α-D-Gal*p* 1-phosphate was completely soluble in acetonitrile, but the corresponding triethyl- and tetraethylammonium derivatives were not. Thus, we employed the tributylammonium salt of **3** in the key coupling reaction.

The extent of completion of the coupling reaction depended on the number of equivalents of activated UMP-*N*-methylimidazolide **4** added. For nucleoside triphosphate synthesis, the pyrophosphate nucleophile is used in excess (2-fold) relative to the electrophile.[8] This ratio results in a suppression of the reaction of the product nucleoside triphosphate with the activated NMP-*N*-methylimidazolide, a process that produces the symmetrical dinucleoside tetraphosphate. In the formation of nucleoside diphospho sugars, however, it is desirable to use the electrophile in excess, rather than the valuable sugar 1-phosphate. The disadvantage of this approach is that any hydrolysis of UMP-*N*-methylimidazolide **4** produces 5′-UMP, which can react with the electrophile **4** to form the dimer, UPPU. The presence of this species complicates purification of the desired product. Thus, conditions were sought that would maximize the formation of the product while minimizing formation of UPPU.

Both the model coupling and the key reaction were followed using ^{31}P NMR spectroscopy. Each component of the reaction mixture, including intermediates and products, could be distinguished.[11] We found that when the coupling reactions were conducted at 0 °C, only a slight excess of electrophile **4** (1.2 equiv) was needed for complete consumption of the sugar 1-phosphate. Under these conditions, little or no degradation of the product to the cyclic phosphodiester and 5′-UMP was observed.

Under optimized conditions, the tributylammonium salt of α-D-Gal*f* 1-phosphate (**3**) was treated with UMP-*N*-methylimidazolide **4** (1.2 equiv) at 0 °C. The sugar 1-phosphate **3** was completely consumed after 2 h. According to the ^{31}P NMR spectrum, the proportion of UDP-Gal*f* **2** at this stage was 83% and the amount of cyclic phosphodiester produced was low (17%). This ratio compares well with that observed using other methods; the highest yield of UDP-Gal*f* **2** reported previously was 50% after 18−19 h. With the less reactive UMP-imidazolide, degradation of the product to the cyclic phosphodiester and 5′-UMP is a more significant side reaction.[5a] We found that ammonium acetate buffer (pH 7, 250 mM) is the most effective solution for quenching the reaction. The product **2** was purified by ion-exchange HPLC. Slight degradation (loss of ca. 4%) of the product to α-D-Gal*f* 1,2-cyclic phosphate and 5′-UMP (1:1 ratio) occurs during purification. This protocol affords UDP-Gal*f* **2** reproducibly in 35% yield.

In conclusion, we devised an effective and reproducible coupling method to prepare UDP-Gal*f* **2**. Our protocol involves reaction of a sugar 1-phosphate and the activated 5′-*N*-methyl phosphorylimidazolide nucleoside. The increased reactivity of the electrophilic phosphoryl group used here eliminates the long reactions times typically required for nucleoside diphospho sugar synthesis. We found that this method is effective for preparing a highly labile biosynthetic intermediate. We anticipate that it may also facilitate the preparation of diphosphoryl bonds embedded in other reactive or unstable products.

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Supporting Information Available: Experimental procedure for the coupling to produce UDP-Gal*f* **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Relative to external aqueous 85% H_3PO_4: α-D-galactose 1,2-cyclic phosphate (10.0 ppm for Gal*p* and 16.9 ppm for Gal*f*[2a]), 5′-UMP (0.5−1.1 ppm), galactose 1-phosphate (−0.2 ppm for Gal*p* **11** and 0.3 ppm for Gal*f* **3**), UMP-*N*-Me-imidazolide (−10.9 ppm), product UDP-galactose (−11.1 (d), −12.6 (d) ppm for Gal*p* **1** and −11.3 (d), −12.6 (d) ppm for Gal*f* **2**), and side product UPPU (−11.2 ppm).