

Synthetic Methods

A Simple Synthesis of Sugar Nucleoside Diphosphates by Chemical Coupling in Water**

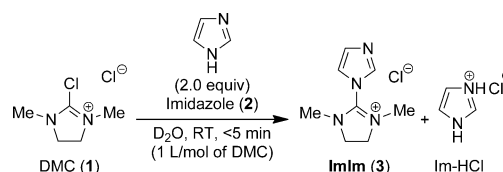
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The use of glycosyltransferases in chemoenzymatic oligosaccharide synthesis is attractive, provided that the enzymes are available, since it eliminates the tedious multistep protection-deprotection and chemical glycosylation procedure that characterizes classic chemical synthesis.^[1,2] The sugar nucleoside diphosphates (sugar-NDPs) that most glycosyltransferases require as glycosyl donors (e.g. UDP-Glc, UDP-Gal, ADP-Glc etc.) can be prepared either chemically or through enzymatic methods including recycling systems. The enzymatic preparation of sugar-NDPs has the advantages of being simple and compatible with glycosyltransferase reactions but requires access to the necessary enzymes. In contrast, chemical synthesis is more complex but is especially powerful for the preparation of analogues that cannot easily be prepared using enzymes owing to a low tolerance of the enzyme for substrate modifications.

Chemical methods for the synthesis of sugar-NDPs have recently been comprehensively reviewed and eloquently discussed.^[3] The most commonly used procedures involve the formation of the pyrophosphate linkage by coupling of an activated nucleoside-5'-monophosphate (NMP, typically a morpholidate or imidazolide) with the sugar-1-phosphate; in these reactions the addition of catalysts like nitrogen-containing heterocycles can effect substantial improvements in both rate and yield.^[4,5] Newer methods include the "cycloSal" phosphotriester coupling^[6] and mixed phosphite-phosphate formation followed by oxidation.^[7] Direct coupling of anomerically activated monosaccharides with nucleoside diphosphates, such as uridine 5'-diphosphate, represents a useful alternative approach (reviewed in reference [3]). These chemical procedures are normally performed in anhydrous organic solvents and usually employ per-*O*-acetylated sugars and phosphates, such as their tetraalkylammonium salts, that are soluble in organic solvents. The syntheses are notoriously difficult to reproduce from laboratory to laboratory, especially when researchers have little previous experience with chemical sugar nucleotide synthesis.

We report herein a simple method for the chemical synthesis of sugar-NDPs in a one-pot reaction in water. The procedure involves only the sequential addition of commercially available compounds, and the crude product solution can be used directly as a glycosyl donor source in glycosyltransferase-mediated oligosaccharide synthesis.

We found that the reaction of 2-chloro-1,3-dimethylimidazolinium chloride (DMC, **1**)^[8,9] with imidazole (**2**) in D₂O at room temperature gave 2-imidazolyl-1,3-dimethylimidazolinium chloride (ImIm, **3**), within five minutes, along with imidazole hydrochloride (Im-HCl; Scheme 1). The formation of **3** was confirmed by ¹H NMR spectroscopy and ESI-MS.^[10] We then examined whether **3** would convert phosphate groups into reactive phosphorimidazolid groups in aqueous solution. Phosphorimidazolidates have been extensively used for pyrophosphate bond formation in anhydrous organic solvents.^[3]



Scheme 1. Reaction of DMC (**1**) with imidazole (**2**) gives ImIm (**3**) as a reactive intermediate.

Uridine monophosphate (UMP) disodium salt (**4**), DMC (**1**), and imidazole (**2**) were dissolved in D₂O at 37 °C, and the reaction was monitored by ¹H NMR spectroscopy (Figure 1).

The signal corresponding to H-5 of uracil in **4** (δ = 8.01 ppm) decreased and simultaneously a peak assigned to H-5 of UMP-imidazolid (UMP-Im, **5**; δ = 7.62 ppm) increased for up to one hour where the maximum conversion to **5** was approximately 70%. The reaction was also monitored using ³¹P NMR spectroscopy, where the intensity of the characteristic^[5,11] signal for UMP-imidazolid **5** (UMP-Im, δ = -8.2 ppm) was observed. ESI-MS on the crude reaction mixture further confirmed the formation of UMP-Im (m/z 373.3; see the Supporting Information). An identical mass spectrum was obtained if DMC and imidazole were first reacted for five minutes and subsequently UMP was added.

Reaction times longer than one hour led to hydrolysis of **5** and subsequent increased dimerization (**4** + **5**) to give UMP-dimer (**7**; Figure 1). If the reaction was allowed to proceed for 16 h, only hydrolysis product (**5** → **4**) and dimer **7** were seen: at that stage the reactive species **3** and **5** were fully hydrolyzed, thereby rendering the solution chemically benign.

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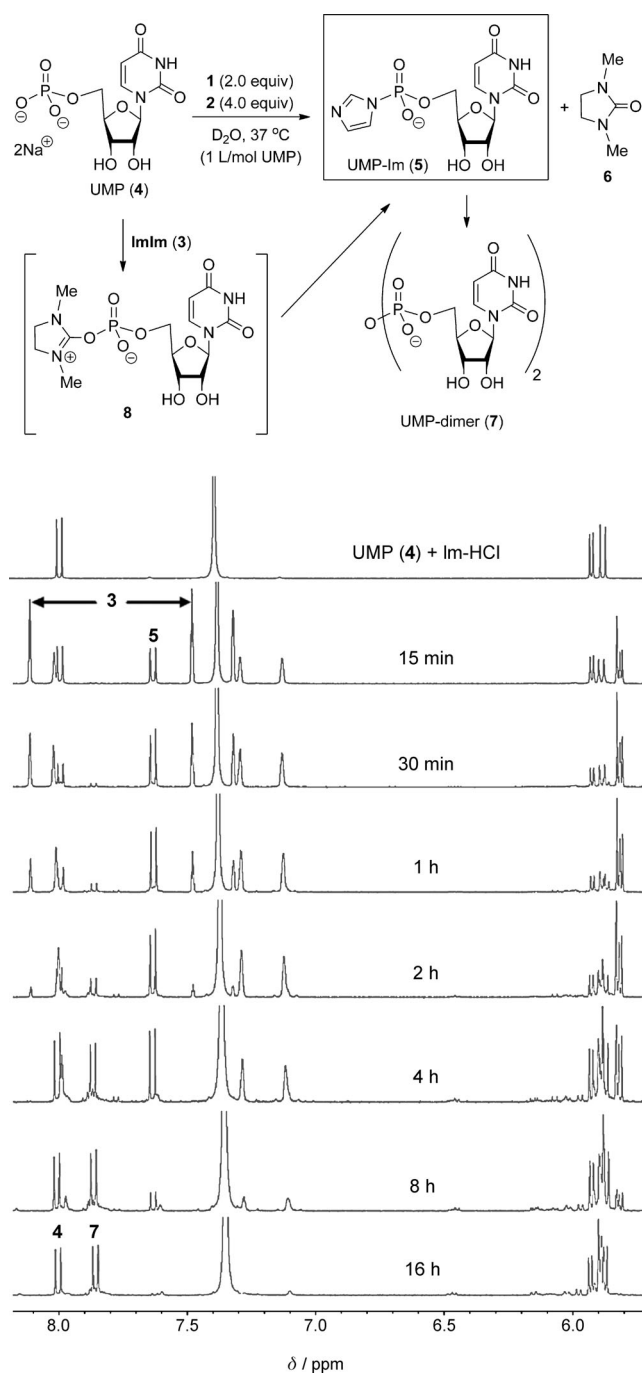


Figure 1. Activation of UMP (**4**) in D₂O with ImIm monitored by ¹H NMR spectroscopy.

A plausible reaction mechanism is presented in Figure 1, where the rapidly formed ImIm activates the phosphate via an intermediate isourea that is then captured by imidazole to produce UMP-Im and urea **6**. In an independent experiment where DMC alone was incubated with UMP for 24 h at 37 °C, the only products observed were urea **6** (40%) and UMP-dimer (**7**) along with 60% of unreacted DMC and more than 95% unreacted UMP (see the Supporting Information).

The reactions described above were optimized in D₂O, because its use facilitates reaction monitoring by ¹H NMR

spectroscopy. However, activation of **4** was also examined in H₂O, where the yield of UMP-Im (**5**) was consistently lower, only 60–70% of that obtained in D₂O. Both **3** and **5** were hydrolyzed much faster in H₂O than in D₂O (verified by kinetic NMR monitoring, see the Supporting Information); this result is attributed to the known solvent isotope effect of water in hydrolysis reactions.^[12] The use of D₂O is therefore advantageous in preserving a high concentration of the active species (**3** and **5**) in aqueous solution.

We next examined conditions for coupling the in situ formed UMP-Im (**5**) with glucose-1-phosphate (Glc-1-P, **9**) to give UDP-glucose (UDP-Glc, **10**) as the target sugar-NDP. The products were quantitated by ³¹P NMR spectroscopy by using the beneficial D₂O as solvent (Table 1). The activation

Table 1: Optimization of reaction conditions for UDP-Glc synthesis using ImIm activation.

Entry	D ₂ O [L mol ⁻¹ of UMP]	Ratio (4 /1/ 2 / 9)	Additive	Yield [%] ^[a]
1	1.0	1:2:4:1	none	20
2	1.0	1:2:4:1	TEAC	23
3	1.0	1:2:4:1	[15]crown-5	21
4	1.0	2:4:8:1	none	34
5	0.5	2:4:8:1	none	43
6	0.5	4:8:16:1	none	57
7	0.5	4:8:16:1	none	43 ^[b]

[a] Yields of **10** based on Glc-1-P and calculated from the corresponding signal integrations in the ³¹P NMR spectrum of the crude mixture.

[b] Yield of isolated **10**, which was purified by ion-exchange chromatography on DEAE-Sephacel after treatment with alkaline phosphatase for 24 h at 30 °C; the yield is based on Glc-1-P and was determined by UV spectroscopy at λ_{max} 260 nm.

step to produce **5** (**1** + **2** + **4**) was run for one hour (at which time the majority of the ImIm (**3**) was consumed) before adding Glc-1-P (dipotassium salt) **9**. It is important to minimize the activation of Glc-1-P by unhydrolyzed ImIm, because this activation leads to the formation of glucose-1,2-cyclic-phosphate. The rate of formation of UMP-Im (**5**) is, however, dependent on the concentration of ImIm, so a compromise in reaction time and corresponding yield must be made to provide the highest possible concentration of reactive **5** for coupling to Glc-1-P.

Coupling reactions were examined under a variety of conditions at 37 °C for 18 h (Table 1). Addition of tetraethylammonium chloride (TEAC) or [15]-crown-5 was not beneficial (Table 1, entries 1–3). Furthermore, use of dimethyl sulfoxide (DMSO) as a co-solvent led to a decrease in coupling yield (data not shown). An increase in the concentration of **5** would be predicted to improve the efficiency of coupling with **9**. Indeed, higher yields were obtained when the amounts of **1**, **2**, and **4** were increased and the volume of D₂O

In conclusion, the new ImIm reagent formed in situ can activate nucleoside 5'-monophosphates in D₂O to give a reactive phosphorimidazolide intermediate. Coupling of this intermediate with sugar-1-phosphates in D₂O produced NDP-sugars in useful yields based on the sugar-1-phosphate as the limiting reagent. All reactions occur in one pot, and the procedure involves only the timed sequential addition of commercial chemicals. Furthermore, all chemically reactive species are hydrolyzed by the end of the coupling reaction, thus the crude NDP-sugar solutions can be used directly without any purification for preparative oligosaccharide synthesis by glycosyltransferases.

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