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N-Methylimidazolium chloride-catalyzed pyrophosphate formation: application to the synthesis of Lipid I and NDP-sugar donors

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Abstract

N-Methylimidazolium chloride is found to catalyze a coupling reaction between monophosphates and activated phosphorous-nitrogen intermediates such as a phosphorimidazolide and phosphoromorpholidate to form biologically important unsymmetrical pyrophosphate diesters. The catalyst is much more active, cheaper, and less explosive than 1*H*-tetrazole, known as the best catalyst for the pyrophosphate formation over a decade. The mild and neutral reaction conditions are compatible with allylic pyrophosphate formation in Lipid I syntheisis. ^{31}P NMR experiments suggest that the catalyst acts not only as an acid but also as a nucleophile to form cationic and electrophilic phosphor-*N*-methylimidazolide intermediates in the pyrophosphate formation.

Keywords

N-Methylimidazolium chloride; Pyrophosphate formation; Phosphorimidazolide; Phosphoromorpholidate; Lipid I; NDP-sugar donors

Attachment of a sugar to a protein or another sugar chain gives the parent biomolecules diverse functions. Enzymes that catalyze formation of glycosidic bonds to other molecules are known as glycosyltransferases (Gtfs), and they typically use glycosyl donors containing a nucleoside diphosphate (NDP) or lipid pyrophosphate (LPP) on the anomeric carbon (Figure 1).[1] Synthetic methods to make NDP- and LPP-sugar donors are required to elucidate glycosyltransferase function and to synthesize natural and unnatural oligosaccharides chemoenzymatically.[2] Our laboratory has been searching for efficient methods to make bacterial LPP-sugars such as Lipid I and Lipid II for studying enzymes involved in cell envelope biosynthesis.[3] Although synthetic routes to these molecules have been reported, we have not been satisfied with the existing approaches.[3 x, 4, 5] Here we describe an improved method to make pyrophosphate bonds that is compatible with the formation of both NDP- and LPP-sugars.

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Supplementary Material
Experimental details are described in Supplementary Material (PDF).

Chemical strategies to make NDP- and LPP-sugars involve condensation of two phosphates, which requires activation of one of them.[2] Phosphoromorpholidates[6] and phosphorimidazolides[7] are usually employed as activated intermediates in these condensations. Since nucleoside 5′-monophosphoromorpholidates are commercially available, phosphoromorpholidate intermediates are commonly used to make NDP-sugars, and 1*H*-tetrazole is often used to catalyze the condensation reactions.[8] Because phosphorimidazolides are more reactive that phosphoromorpholidates they are preferred for making LPP-sugars.[3a–c, 4, 5] They can be readily prepared in situ using excess carbonyl diimidazole (CDI) followed by a methanol quench. These intermediates are sufficiently stable that neither the corresponding symmetrical pyrophosphate nor the methyl phosphodiester forms during activation and quenching. Unfortunately, the desired unsymmetrical pyrophosphate products also form very slowly (Scheme 1). Divalent metal ions such as Zn^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, and Sn^{2+} have been reported to promote pyrophosphate bond formation,[9] but can promote decomposition of allylic lipid pyrophosphates,[10] which are both acid- and nucleophile-sensitive.

One approach to the synthesis of Lipid I and Lipid II used 1*H*-tetrazole as a Brönsted acid catalyst to promote condensation of a a lipid phosphate and a sugar phosphorimidazolide, but the reaction still took 4–7 days.[4] Skein *et al*. have shown that 1*H*-tetrazole is not effective for activation of the phosphorimidazolide.[9a] Herein we report the use of *N*-methylimidazolium chloride (NMI·HCl) as a catalyst for the phosphorimidazolide coupling reaction in Lipid I synthesis. The catalyst is much more active, cheaper, and less explosive than 1*H*-tetrazole. The reaction conditions are mild, neutral, and compatible with the allylic pyrophosphate bond formation. We show that this method also works well for NMP-morpholidate coupling reactions with a sugar 1-phosphate. In addition, we report a new synthetic route to make Lipid I that involves introduction of the peptide *after* pyrophosphate bond formation. The peptide is the variable portion in naturally occurring Lipid I and II substrates,[11] and our new approach allows more flexibility in the synthesis of substrates containing different oligopeptides for mechanistic investigations of peptidoglycan biosynthetic enzymes.

In the condensation of two phosphates counter ions play important roles, but little attention has been paid to them. In fact, in chemical schemes the counter cations of activated phosphate **1** and phosphate monoester **2** are often omitted (Scheme 1). Trialkylammonium salts of **1** and **2** are frequently used for pyrophosphate formation because they increase the solubility of both **1** and **2** in organic solvents; they also increase the nucleophilicity of **2**. However, the trialkyammonium salts are not acidic enough (aqueous pK_{Et3NH+} 10.8) to effectively protonate **1**, as required for P-N bond dissociation, which may account for sluggish reactivity in the formation of pyrophosphate bonds. Cramer *et al*. showed that methanolysis of sodium phosphorimidazolide **4** is much faster in the presence of imidazolium chloride ($pK_{imidazole·H+}$ 7.0) than triethylammonium chloride (Scheme 2).[7b] We wondered whether adding a salt such as imidazolium or pyridinium chloride, which comprise a weaker base than a trialkylamine and a stronger acid than a phosphate, to a mixture of **1** and **2** would lead to counter ion exchange to produce more a electrophilic imidazolium or pyridinium phosphorimidazolide **1** *in situ*.

Phosphorimidazolide **7**, prepared from the parent triethylammonium phosphate **6**[12] and CDI, was used to make the pyrophosphates of polyprenyl monophosphate diammonium salts **8a** and **8b** in anhydrous THF-DMF (1:1) solvent (Scheme 3).[13] The complete conversion of tetraprenyl phosphate **8a** to the corresponding pyrophosphate **9a** took one week in the absence of catalyst (Table 1, Entry 1). Addition of weaker amine hydrochlorides (Entries 2, 3, 4, and 7) promoted the reaction much more effectively than triethylamine, which did not appear to accelerate coupling. *N*-methylimidazolium chloride (NMI·HCl, pK_{BH+} 7.1) was

more effective than 1*H*-imidazole chloride (Im·HCl, pK_{BH+} 7.0) (Entry 4 vs. 5) and comparable to pyridinium chloride (Py·HCl, pK_{BH+} 5.2) (Entries 5 vs. 7). Use of trifluoromethanesulfonic acid salts (NMI·HOTf and Py·HOTf) slightly reduced the yields of pyrophosphate **10a** (Entry 5 vs. 6, 7 vs. 8). While 1H-tetrazole accelerated pyrophosphate formation with phosphorimidazolide **7**, the reaction was slower and the yield reduced compared with NMI·HCl and Py·HCl (Entry 5, 7 vs. 9). NMI·HCl transformed the longer lipid phosphpate, heptaprenyl **8b**, into the corresponding pyrophosphate **9b** in good yield within half a day (Entry 10).

The MurNAc polyprenyl pyrophosphates **10a, b** were used to make the corresponding Lipid I analogs via a new route in which the protected pentapeptide was coupled following formation of the diphosphate (Scheme 4). DMTMM[14] was the most effective reagent for condensation between **10a, b** and a small excess of protected pentapeptide.[15] Subsequent hydrolysis afforded tetra- and heptaprenyl Lipid I **11a, b** in good yields. Both can be quantitatively converted to the corresponding Lipid II analogs chemoenzymatically for studies of peptidoglycan biosynthetic enzymes and their inhibitors.[3b]

We also investigated the use of the NMI·HCl catalyst for NDP-sugar synthesis. Khorana's morpholidate method, which involves coupling commercially available nucleoside 5′-monophosphoromorpholidate 4-morpholine-*N*,*N*′-dicyclohexyl-carboxamidine salts, is the most widely used approach for NDP-sugar synthesis.[6] These NMP-morpholidate intermediates have a poor leaving group (p$K_{morpholine·H+}$ 8.4) and a basic counter cation (p$K_{guanidine·H+}$ 11.9). Condensation of sugar 1-phosphate **12**[16] and UMP-morpholidate **13** took three days even in the presence of tetrazole, long considered the best catalyst for these reactions (Scheme 5).[8, 17] In contrast, our NMI·HCl catalyst in DMF greatly improved the reaction rate (12 h) and yield,[18] giving the desired UDP-GalNAz **14** product after removal of the acetate protecting groups.

The difference between Im·HCl and NMI·HCl as catalysts suggests that these compounds act not only acids but also as nucleophiles in pyrophosphate formation as shown in Scheme 6. Electronically neutral phosphorimidazolide and phosphoromorpholidate **15a, b** could be substituted by *N*-methylimidazole to form a cationic phosphor-*N*-methylimidazolide intermediate **18**, which would be more susceptible to nucleophilic displacement with a monophosphate anion **2** to give pyrophosphate **3**. Consistent with the formation of a new, cationic intermediate, we observe the appearance of downfield-shifted ^{31}P resonances in the ^{31}P-NMR spectra of phosphorimidazolide **7** and UMP-morpholidate **13** after the addition of NMI-HCl.[19] In the case of **13**, the downfield ^{31}P resonance that appeared upon addition of excess NMI-HCl had a chemical shift similar to that of authentic TFA-protected UMP-*N*-methylimidazolide (−10.6 vs. −10.9 ppm).[20] Although phosphor-*N*-methylimidazolide is a very reactive intermediate, its preparation requires multiple steps including treatment with acidic trifluoroacetic anhydride, during which care must be given to its moisture sensitivity. In contrast, adding NMI-HCl as a catalyst to less reactive intermediates allows simple manipulation and ready availability of starting materials while being compatible with acid-sensitive functional groups.

N-Methylimidazolium chloride was found to be superior in activity, cost, and safety to 1*H*-tetrazole, long considered the best catalyst for pyrophosphate bond formation. Our new method combines availability and stability of phosphorimidazolide and phosphoromorpholidate with high reactivity of phosphor-*N*-methylimidazolide. The mild and neutral reaction conditions are compatible with allylic pyrophosphate formation in Lipid I.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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17. This reaction proceeded faster in pyridine than DMF as reported in the following paper: Moffatt JG, Khorana HG. J Am Chem Soc. 1958; 80:3756–3761.
18. In contrast to tetrazole catalyst, NMI·HCl promoted the reaction in pyridine as well as DMF.
19. In the case of **7**, a new resonance at δ −12.4 ppm from a solution of **7** (−11.3 ppm) in d_7-DMF was observed in 30 min after addition of NMI·HCl (integral ratio of **7** to the new signal = ca. 5 to 1).
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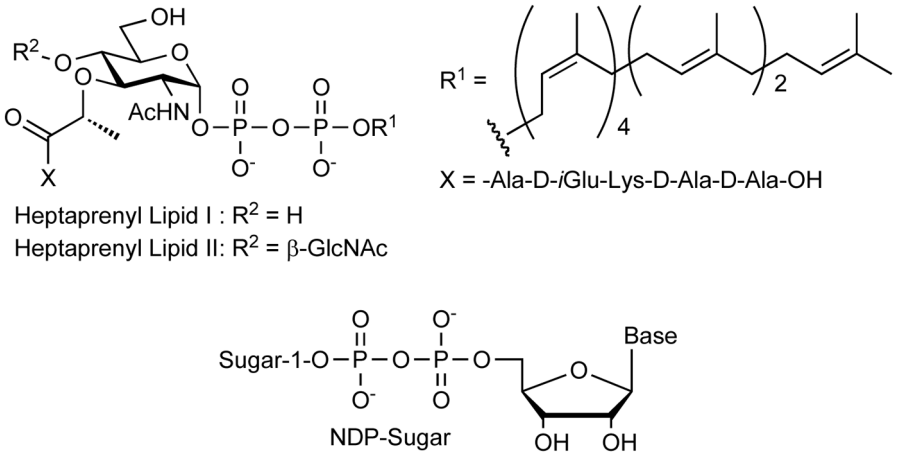
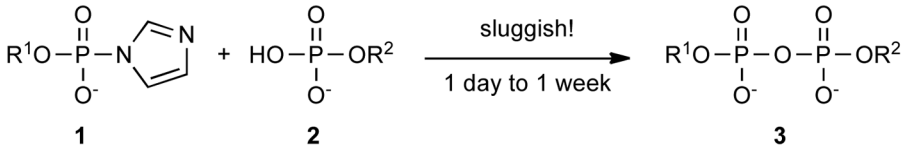
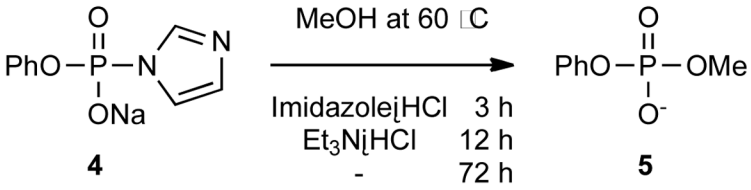


Figure 1.

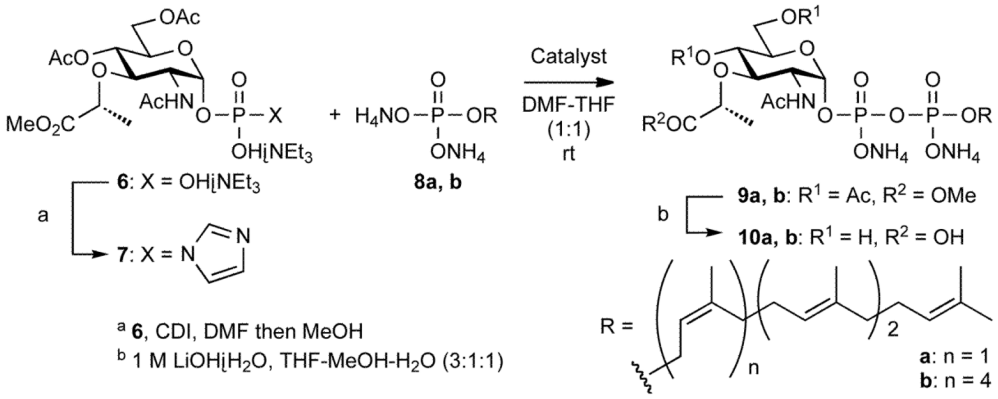


1 **2** **3**

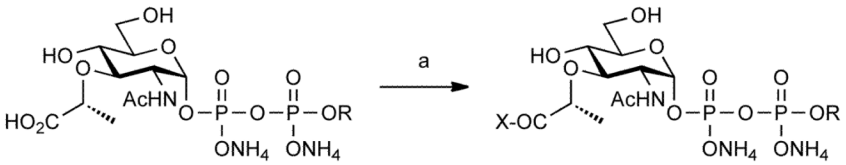
Scheme 1.



Scheme 2.

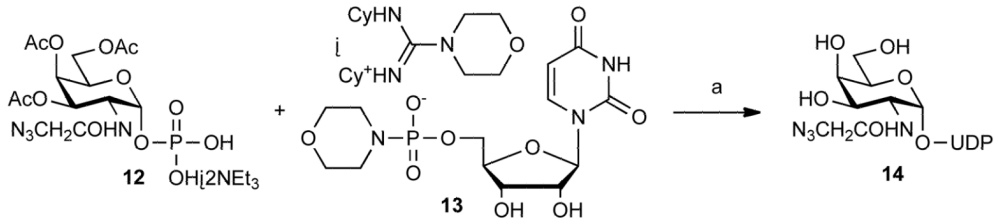


Scheme 3.



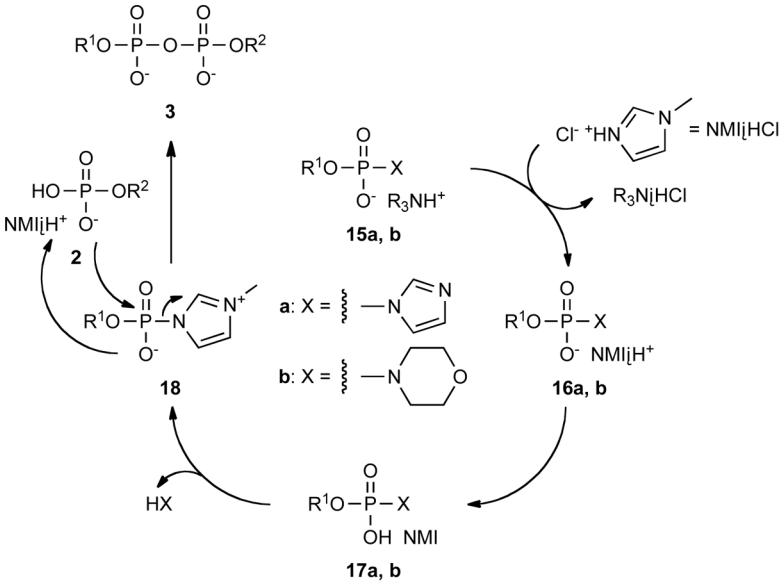
[a] H-Ala-D-*i*Glu(OMe)-Lys(Z)-D-Ala-D-Ala-OMe¡HCl, DIEA, DMTMM, MeOH then 1 M LiOH¡H_2O, THF, H_2O
61% for **11a**, 67% for **11b**

Scheme 4.



[a] 1) Tetrazole, Py., 3 d or *N*-MethylimidazoleįHCl, DMF, 12 h; 2) NaOMe, MeOH
2 steps 71% for tetrazole, 83% for *N*-MethylimidazoleįHCl

Scheme 5.



Scheme 6.

Table 1

Effect of Catalysts on Pyrophsophate Formation via Scheme 3

Entry	8	Catalyst (4 eq)	pK_a (H_2O)	Time (d)	Yield of 10 (%)
1	**8a**	-	-	7	69
2	**8a**	Et_3N·HCl	10.8	7	71
3	**8a**	DMAP·HCl	9.2	1	69
4	**8a**	Imidazole·HCl	7.0	1	75
5	**8a**	*N*-Mehylimidazole HCl	7.1	0.5	77
6	**8a**	*N*-Mehylimidazole HOTf	7.1	0.5	72
7	**8a**	Pyridine·HCl	5.2	0.5	75
8	**8a**	Pyridine·HOTf	5.2	0.5	68
9	**8a**	Tetrazole	4.9	1	63
10	**8b**	*N*-Mehylimidazole HCl	7.1	0.5	54

[a]Yields were determined after removal of protecting groups.