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Diphosphate formation using cyanuric chloride or triisopropylbenzenesulfonyl chloride as the activating agents

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ABSTRACT

By using cyanuric chloride or 2,4,6-triisopropylbenzenesulfonyl chloride (TIPSCl) as the condensing agent and magnesium bromide as the promoter, the phosphate cross-coupling reactions are effectively carried out to give lipid–saccharide and pyrrolidine–guanosine diphosphates. Under optimized conditions, the TIPSCl-facilitated cross-coupling reactions complete in a short reaction time (~5 h) without the complication of possible self-coupling reactions.

Diphosphate (also called pyrophosphate) is an important functional group that plays indispensible roles in many biological processes. For example, sugar nucleoside diphosphates act as the glycosyl donors of glycosyltransferases in the synthesis of oligosaccharides and glycoconjugates.[1] Consecutive condensation of isoprenyl pyrophosphates produces geranyl pyrophosphate (GPP), farnesyl pyrophosphate, and many other essential derivatives.[2] Lipid II containing an undecaprenyl pyrophosphate moiety is the substrate of transglycosylases for bacteria to form the polymeric peptidoglycan of the cell wall.[3]

Though the commercially available nucleoside diphosphates can be utilized to react with appropriate electrophiles (e.g., glycosyl halides) to generate a plethora of diphosphate compounds,[4] the diphosphate group is generally constructed by the coupling of two monophosphate components.[5] However, the cross coupling of two different monophosphates is often complicated by the self-coupling reactions. Moreover, the synthesis and isolation of diphosphate compounds must be performed in proper conditions because the diphosphate bonds are easily degraded by acids, bases, and nucleophiles.

In one approach, Moffatt, Khorana, and co-workers have activated nucleoside monophosphates to the corresponding phosphoromorpholidates for the subsequent coupling reactions with other monophosphates,[6] for example, sugar phosphates, to provide a variety of nucleoside diphosphates (Scheme 1, where R^1 is a nucleoside and R^2 is a saccharide). The coupling reaction is greatly accelerated by using 1*H*-tetrazole as the synergistic acid and nucleophilic catalyst.[7] This method has been widely utilized for the synthesis of diphosphate compounds, including a bacterial cell wall precursor UDP-*N*-acetylmuramyl-pentapetide (Park nucleotide).[8] However, this method may encounter problem in prior preparation and isolation of the activated phosphoromorpholidates (**1P** Fig. 1). In another approach, a monophosphate can be activated by 1,1′-carbonyldiimidazole (CDI).[9–15] After generation of the imidazolide intermediate **2P** (Fig. 1), excess CDI is destroyed by methanol, and 1*H*-tetrazole can be added to promote the cross-coupling reaction with a second monophosphate substrate. This CDI-activation procedure is usually performed in mild conditions that are suitable for the synthesis of sugar nucleoside diphosphates[9–11] and lipid II analogs.[12–15] These two types of coupling reactions, via phosphoromorpholidate or phosphorimidazolidate intermediates, usually take days to form the desired diphosphate products, and the overall yields may be low in many cases.

Other reagents have also been reported for activating monophosphates in attempts to facilitate the diphosphate bond formation.[16–19] An alcohol compound (e.g., thymidine) is treated with 5-nitro-*cyclo*Sal phosphorochloridite (**3**),[16] followed by oxidation, to give 5-nitro-*cyclo*Sal phosphate (**3P**), which is an activated monophosphate ready for the coupling reaction with sugar monophosphate, giving the diphosphate compound after aqueous work-up. A nucleoside monophosphate is activated by diphenyl chlorophosphonate (**4**) to form a phosphoric anhydride (**4P**),[17] which reacts with sugar monophosphate by selective replacement of the diphenylphosphoryl moiety to give the nucleoside–saccharide diphosphate. A monophosphate is treated with trifluoroacetic anhydride and *N*-methylimidazole sequentially to afford a phosphoro-*N*-methylimidazolidate (**5P**) as the active intermediate to react with another monophosphate to form the diphosphate product.[18,19] However, the most popular methods for diphosphate bond formation still reside on the coupling reactions of a monophosphate with the prior prepared phosphoromorpholidate or the in situ generated phosphorimidazolidate (CDI activation).

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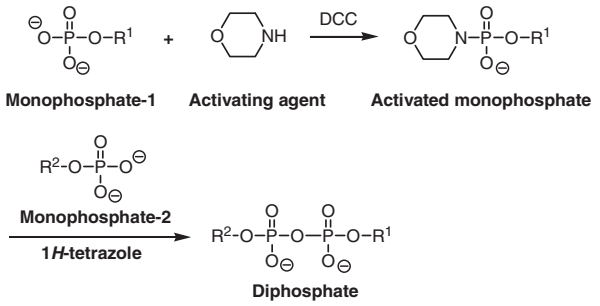


Scheme 1. Formation of diphosphate compounds by the cross-coupling reaction of two monophosphate.

We have previously devised a fluorescent lipid II-based substrate for the assay of transglycosylation.[15] The diphosphate compound was successfully synthesized, albeit in low yield (<20%), by the conjugation of a pentapeptide–disaccharyl phosphate with a dansyl–lipid phosphate using the in situ CDI-activation method. We also propose the possible fucosyltransferase inhibitors, such as compound **14** (Scheme 3), which contain a moiety of guanosine diphosphate (GDP) as the main binding component and a pyrrolidine group to mimic the oxonium transition state in transglycosylation. In this Letter, we report the use of cyanuric chloride (CC, **6)** and 2,4,6-triisopropylbenzenesulfonyl chloride (TIPSCl, **7**) as suitable condensing agents to facilitate the diphosphate bond formation using **10a–c** and **14** as the model compounds.

CC and its analog, 2-chloro-4,6-dimethoxy-1,3,5-triazines (CDMT), have been utilized to activate carboxylic acids for the reactions with alcohols and amines to form esters and amides.[20] To test the feasibility of CC in the promotion of diphosphate bond formation, a solution of hexyl monophosphate in CH_3CN was treated with CC (0.33 equiv) in the presence of pyridine for 2 h at room temperature. The ^{31}P NMR spectrum showed that the phosphorus signal of hexyl monophosphate at δ_P 0.62 disappeared, and a new signal at δ_P −13 was attributable to P^1,P^2-dihexyl diphosphate, the self-coupling product, which was confirmed by the MS analysis.

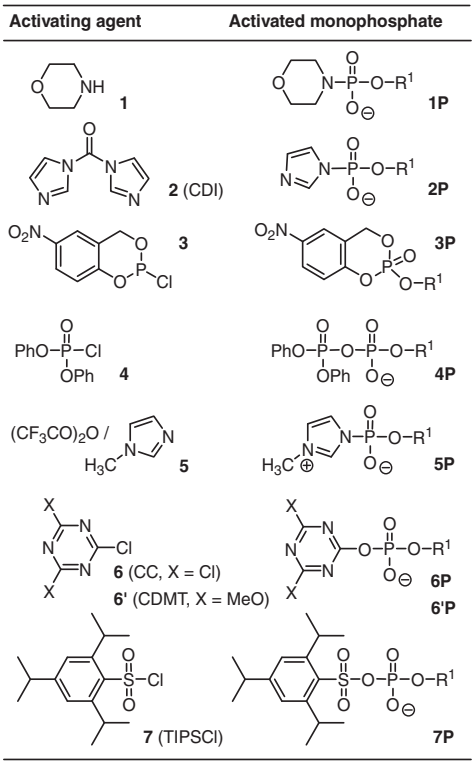
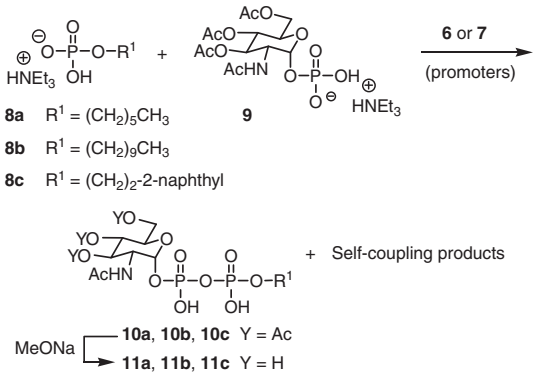


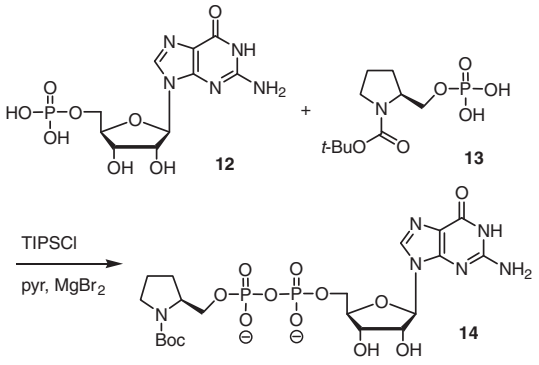
Figure 1. Some representative agents for activating monophosphate and the activated intermediates for the subsequent cross-coupling reactions.



Scheme 2. Formation of aliphatic–saccharide diphosphates using cyanuric chloride or 2,4,6-triisopropylbenzenesulfonyl chloride as the condensing agents.

Encouraged by this result, we further investigated the cross-coupling reaction of peracetyl GlcNAc monophosphate (**9**) with hexyl monophosphate (**8a**, Scheme 2). In one experiment, **8a** (as the triethylammonium salt) was added slowly to a solution of CC and pyridine in CH_3CN at 0 °C, followed by the addition of GlcNAc monophosphate **9** (as the triethylammonium salt). After stirring for 24 h, the desired cross-coupling product **10a** predominated in the reaction mixture as shown by the ^{31}P NMR and MS analyses, though some self-coupling product of dihexyl diphosphate was also observed. The crude product of **10a** was then treated with sodium methoxide in MeOH to give **11a**, which showed the molecular ions at *m*/*z* 464.1084 (single charged) and 231.5407 (double charged) in the MS spectrum. The undesired self-coupling reaction could not be eliminated even at a low temperature (−30 °C). Metal ions, such as Mg^{2+}, Zn^{2+}, and lanthanides, having good affinity to phosphate ion, were examined to see if they could promote the phosphate coupling reactions.[21] It was found that the addition of $MgBr_2$ (1 equiv) did facilitate the cross-coupling reaction to a great degree. CC appeared to be a better condensing agent than CDMT in promoting diphosphate bond formation. Another experiment using a reversed procedure, that is, activation of GlcNAc monophosphate **9** with CC prior to the addition of hexyl monophosphate gave **10a** along with two self-coupling products derived from hexyl phosphate and **9**, respectively.

By the activation of decyl monophosphate (**8b**) with CC, the cross-coupling reaction with GlcNAc monophosphate **9** was carried



Scheme 3. Synthesis of GDP–pyrrolidine derivative as a possible inhibitor of fucosyltransferases.

out in THF solution to give **10b** accompanied by some P^1,P^2-didecyl diphosphate. Saponification of **10b** afforded **11b**, which has been used as a substrate for the assay of bacterial galactosyltransferase.[22] In agreement with the previous report,[22] diphosphate **11b** exhibited two phosphorus signals at δ_P −10 and −12.

It was difficult to isolate and quantify P^1-GlcNAc-P^2-alkyl diphosphates **10a**,**b** and **11a**,**b** due to lack of chromophore. Thus, the cross-coupling reaction with phosphoric acid mono(2-naphthylethyl) ester (**8c**), a UV active compound, was further investigated. The cross-coupling reaction of **8c** and the GlcNAc monophosphate **9** (both as the triethylammonium salts) was conducted in CH_3CN solution using CC, pyridine, and $MgBr_2$ as promoters. After stirring at room temperature for 48 h, the reaction mixture was shown by HPLC analysis to contain 44% of diphosphate **10c** in addition to the starting materials (Supplementary Fig. S1). No apparent peak corresponding to the symmetric diphosphate derived from **8c** or **9** was detected. The diphosphate product **10c** was isolated in 25% yield by HPLC on a reversed-phase C18-A column, and fully characterized by the MS, ^{1}H, ^{13}C, and ^{31}P NMR analyses. The relatively low isolation yield of diphosphate **10c** (25%), compared with 44% conversion yield, might be attributable to the susceptibility of the glycosidic and diphosphate bonds to hydrolytic cleavage during the separation process.

In another approach, TIPSCl was explored to promote the diphosphate bond formation. Activation of monophosphate with TIPSCl gives an intermediate of phosphate–sulfonate mixed anhydride (**7P**),[23–25] which can be selectively attacked by nucleophiles at the phosphorus atom presumably due to the steric effect and high leaving potency of the triisopropylsulfonate group. This activation method is first suggested by Khorana for the formation of the C3′–C5′ internucleotide linkage,[23] and has been applied to the synthesis of monophosphate di- and triesters.[24,25]

By using TIPSCl and $MgBr_2$ as promoters, the cross-coupling reaction of **8c** with **9** was successfully carried out in pyridine at room temperature. The ^{31}P NMR and HPLC analyses indicated that the coupling reaction was clean to afford 75% conversion yield of **10c** at a reaction time of 5 h, without complication of the self-coupling reactions (Supplementary Fig. S2). For comparison, the cross-coupling reactions of **8c** with **9** via the conventional phosphoromorpholidate and CDI intermediates were also conducted. Monophosphate **8c** was activated by CDI in situ, and then treated with monophosphate **9** in the presence of 1*H*-tetrazole for 48 h to give 62% conversion yield of the cross-coupling product **10c** accompanied by the formation of symmetric diphosphates according to the HPLC and ^{31}P NMR analyses (Supplementary Fig. S3). Alternatively, the reaction of monophosphate **8c** with morpholine and dicyclohexylcarbodiimide (DCC) in refluxing *t*-BuOH/H_2O solution for 8 h gave a 95% yield of the activated phosphoromorpholidate, which was then treated with monophosphate **9** and a catalyst of 1*H*-tetrazole to give diphosphate **10c** in 80% conversion yield at a reaction time of 50 h as shown by the ^{31}P NMR analysis.

Using TIPSCl as the promoter was also applicable to the synthesis of a GDP–pyrrolidine derivative **14** (Scheme 3). To increase the solubility in organic solvent, the commercially available GMP disodium salt was first transformed into GMP (**12**) through ion exchange resin. Under the optimized conditions, the cross-coupling reaction of GMP with pyrrolidine monophosphate **13** proceeded smoothly by the promotion of TIPSCl and $MgBr_2$ to give the diphosphate product **14** (75% conversion yield) in a short reaction time (5 h). The GDP–pyrrolidine derivative **14** was then isolated in ~50% yield by HPLC on a C18 column, and characterized by the MS, ^{1}H, and ^{31}P NMR spectra. In addition to the desired proton resonance at δ 5.93 (1H, d, J = 5.9 Hz) for H-8 of the guanine moiety, this sample might be contaminated with an unidentified impurity showing a blur signal nearby δ 5.95 (see Supplementary NMR spectrum). In a comparison experiment, diphosphate **14** was also synthesized via the GMP–morpholidate intermediate in 52% conversion yield after a prolonged reaction time (70 h) as shown by the ^{31}P NMR analysis (Supplementary Fig. S4).

In summary, we have found that CC and TIPSCl are proper reagents for the activation of monophosphate. The cross-coupling reactions for the formation of diphosphate compounds, for example, **10a**–**c** and **14**, are best conducted in the presence of $MgBr_2$. The cross-coupling reactions using TIPSCl/$MgBr_2$ promoters proceed rapidly in hours compared to days in the conventional methods via phosphoromorpholidate or phosphorimidazolidate intermediates.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.01.034.

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