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Synthesis of Nucleoside Triphosphates from 2′-3′-Protected Nucleosides Using Trimetaphosphate

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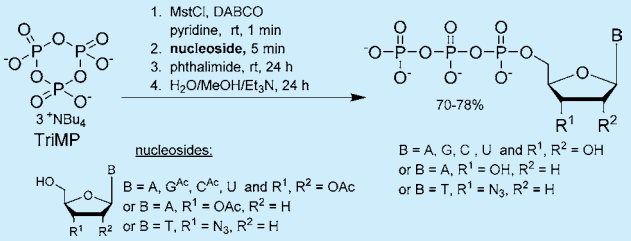
Supporting Information

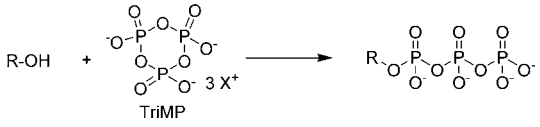
ABSTRACT: Chemists have been attempting to triphosphorylate nucleosides and other alcohols using trimetaphosphate (TriMP) since the 1960s. However, this route appears to have been abandoned due to poor yields. The first practical syntheses of nucleoside triphosphates (NTPs) are reported using TriMP as the key reagent. This was achieved by reacting the tetrabutylammonium salt of TriMP with mesitylenesulfonyl chloride in the presence of DABCO in pyridine followed by the addition of an appropriately protected nucleoside and phthalimide. Quenching the reaction with aqueous buffer followed by hydrolysis of the OH protecting groups gave the NTPs in good yield.



Nucleoside or deoxynucleoside triphosphates (NTPs or dNTPs) are ubiquitous in nature. dNTPs are essential for the biosynthesis of DNA and are utilized in various molecular biology applications such as PCR and DNA sequencing.[1] Synthetic analogues of dNTPs and NTPs have been widely used as agonists and antagonists of nucleotide receptors, substrates, and inhibitors of NTP-binding enzymes and as fluorescent-labeled probes.[2] Hence, the synthesis of NTPs and dNTPs has been the subject of considerable interest for many years, and numerous approaches have been developed for their synthesis.[3] Most of these approaches require either the prior synthesis and isolation of a monophosphorylated substrate followed by its activation and reaction with pyrophosphate[4] or the reaction of the nucleoside with a reagent that yields an activated phosphorus species that is either isolated or reacted in situ with pyrophosphate.[5−8] Among the latter approaches, the one-pot triphosphorylation procedure developed by Ludwig et al., which involves reacting an unprotected nucleoside with $POCl_3$ followed by reaction of the resulting nucleoside dichlorophosphoridate with pyrophosphate and hydrolysis of the resulting cyclic intermediate, is one of the most widely used.[5a] However, the formation of byproducts and product purification is often an issue with this methodology,[9] though recent modifications to Ludwig's procedure appear to help minimize these issues.[5b] The procedure of Ludwig and Eckstein has also seen widespread use.[5f,8] In their procedure, a 2′-3′-protected nucleoside is reacted with salicylphosphorochloridite. The resulting phosphite triester is reacted with pyrophosphate to form a cyclic P(III) intermediate. This species is oxidized to a cyclic P(V) intermediate using I_2/pyridine, which is then hydrolyzed to the triphosphate product.

Trimetaphosphate (TriMP in Scheme 1) has been examined as a reagent for triphosphorylating alcohols,

Scheme 1. TriMP as a Triphosphorylating Agent



including nucleosides, for almost 50 years.[10] Its use as a triphosphorylating agent is very appealing as it is commercially available as its trisodium salt and is inexpensive. Moreover, the triphosphate product can potentially be produced in one step using a single phosphorylating agent (Scheme 1).

In spite of TriMP's appeal as a triphosphorylating agent, it is not a very effective reagent for the triphosphorylation of hydroxyl groups. For example, the reaction of TriMP with basic aqueous methanol or ethanol gave a 39% isolated yield of methyl triphosphate after 3 weeks at rt and a 4% yield of ethyl triphosphate after 7 weeks at rt.[10] The reaction of alcohols with trialkylammonium salts of TriMP in anhydrous solvents in the presence or absence of an organic base also gave very little triphosphate product.[11] The reaction of a 5-fold excess of phenol with the trisodium salt of TriMP at pH 12 at rt required 11 days to produce phenol triphosphate in 67% yield.[12] The triphosphorylation of nucleosides with TriMP has also been met with little success. For example, the reaction of 2′-deoxynucleosides with a 20-fold excess of

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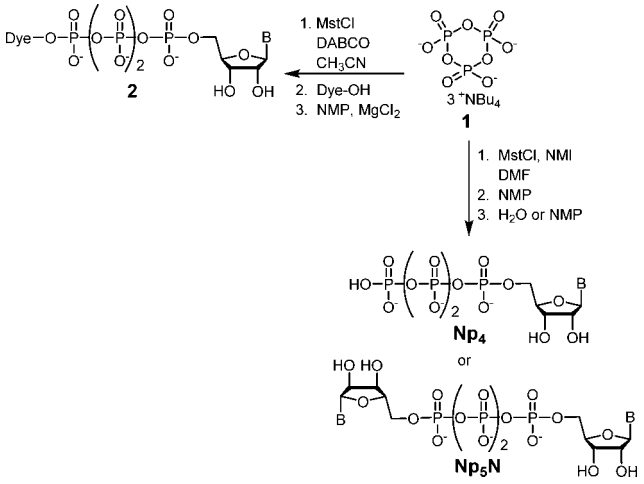
TriMP in basic aqueous solution at pH 10.5−12 for 4−15 days gave a mixture of 3′- and 5′-triphosphorylated nucleosides in 20−44% yield.[13] Tsuhako et al. reported that 2′-deoxynucleosides could not be phosphorylated with a 5-fold excess of TriMP at pH 12 at 70 °C even after several days.[14] When ribonucleosides were subjected to TriMP, 2′- and 3′-nucleoside monophosphates and cyclic 2′-3′-nucleoside monophosphates were obtained.[14−16]

We recently reported a method for preparing dinucleoside pentaphosphates (Np_5N) and nucleoside tetraphosphates (Np_4) using TriMP as the phosphorylating agent.[17] This involved reacting the tri(tetra-*n*-butylammonium) salt of TriMP (**1**) with mesitylenesulfonyl chloride (MstCl) in the presence of *N*-methylimidazole (NMI) in DMF followed by the addition of a nucleoside monophosphate (NMP) or water (Scheme 2). We have also reported the synthesis of Np_4

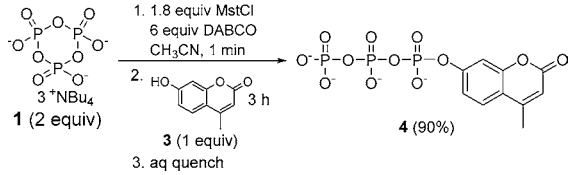
Scheme 2. Synthesis of Np_4, Np_5N, and Np_4 Labeled with a Fluorescent Dye Using TriMP



labeled with a fluorescent dye (**2**) on the terminal phosphate group using TriMP.[18] To achieve this, TriMP was reacted with MstCl in the presence of DABCO, followed by the addition of the dye and then the NMP and $MgCl_2$ (Scheme 2). These results suggested to us that it may be possible to prepare NTPs using TriMP as the key reagent. Here, we report the first practical syntheses of NTPs in good yield using TriMP.

Attempts to prepare NTPs using protected (2′-OH and/or 3′-OH) or unprotected nucleosides by reacting nucleosides under the conditions used for preparing Np_4[17] (reaction of **1** with MstCl, NMI, followed by the addition of nucleoside) or compounds of type **2**[18] (reaction of **1** with MstCl and DABCO followed by the addition of nucleoside) were unsuccessful in that little or no triphosphate product was obtained. However, it was found that the conditions used for the preparation of compounds of type **2** could readily triphosphorylate coumarin **3** (Scheme 3). Thus, by subjecting 2 equiv of TriMP to 1.8 equiv of MstCl and 6 equiv of DABCO in acetonitrile for 1 min followed by the addition of coumarin **3**, stirring for 3 h, and quenching with ammonium acetate buffer, we obtained coumarin triphosphate **4** in a 90% yield after purification by reversed-phase chromatography. The success of the reaction when using coumarin **3** was probably due to the greater nucleophilicity of the conjugate base of the

Scheme 3. Synthesis of 3-Methylcoumarin-7-triphosphate (4)



coumarin in comparison to the nucleophilicity of the hydroxyl groups of nucleosides.

Further attempts to develop conditions for triphosphorylating alcohols were performed using 4-biphenylmethanol (BPM) as a model substrate as it is inexpensive and its disappearance could be easily followed by TLC. As expected, the conditions described above for the triphosphorylation of coumarin **3** were not effective for triphosphorylating BPM in that no consumption of BPM was evident after 24 h as determined by TLC. Increasing the number of equiv of TriMP and MstCl to 3.3 and 3, respectively, was also unsuccessful (rxn 1, Table 1). However, when the reaction was performed in dry pyridine, 4-biphenylmethanol triphosphate (BPMTP, **5**) was isolated in a 55% yield after 24 h (rxn 2). Not all of the BMP was consumed after 24 h. Running the reaction longer resulted in the formation of a variety of unidentified side products as determined by ^{31}P NMR of the quenched crude reaction mixture. If the reaction was done in the absence of DABCO, all of the BPM was consumed after 24 h (rxn 3). However, there was a decrease in the amount of **5** formed and an increase in the formation of byproducts, as determined by ^{31}P NMR (rxn 3). We then began examining the effect of various additives on the reaction, anticipating that an activated form of TriMP would be formed that would facilitate triphosphorylation. Performing the reaction in the presence of 3 equiv of NMI or 4-nitroimidazole (NitIm) in the absence or presence of DABCO resulted in no **5** being formed (rxns 4 and 5). Surprisingly, when the reaction was done in the absence of DABCO but in the presence of 4 equiv of phthalimide, ^{31}P NMR analysis of the crude reaction mixture after quenching with buffer showed compound **5** as the major product (rxn 6). Compound **5** was isolated in a 74% yield. By performing the reaction with 4 equiv of phthalimide (Phth) and 4 equiv DABCO, the formation of byproducts was slightly suppressed and compound **5** was isolated in an 80% yield (rxn 7). By increasing the amount of TriMP to 4 equiv and MstCl to 3.7 equiv and both DABCO and phthalimide to 4.5 equiv, there was little formation of phosphorylated BPM byproducts, and compound **5** was isolated in an 85% yield (rxn 8). The inorganic and organic polyphosphate byproducts, mainly monophosphorylated and tetraphosphorylated BPM, were easily separated from **5** during reversed-phase chromatography purification.

The conditions given in entry 8 in Table 1 were applied to the triphosphorylation of protected nucleosides **6**−**9** (Scheme 4). Thus, 3.7 equiv of MstCl was added to a solution of 4 equiv of TriMP and 4.5 equiv of DABCO in dry pyridine at rt and stirred for 1 min. The nucleoside was added, and the mixture was stirred for 5 min; then 4.5 equiv of phthalimide was added, and the reaction mixture was stirred at rt for 24 h. Since nucleosides **6**−**8** have a potential additional site of triphosphorylation (on the base), an additional equivalent of all of the reagents was added for these nucleosides. After 24 h,

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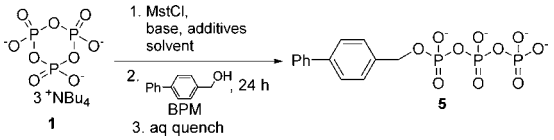
Table 1. Triphosphorylation of BPM

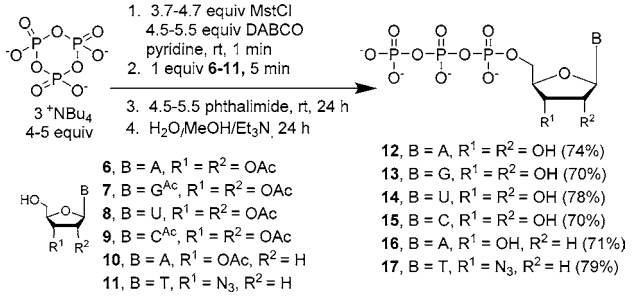


rxn	solvent	TriMP (equiv)	MstCl (equiv)	base (equiv)	additive (equiv)	yield (%)
1	CH_3CN	3.3	3	DABCO (6)	none	0
2	pyr	3.3	3	DABCO (6)	none	55
3	pyr	3.3	3	none	none	ND[a]
4	pyr	3.3	3	DABCO (0 or 6)	NMI (3)	0
5	pyr	3.3	3	DABCO (0 or 6)	NitIm (3)	0
6	pyr	3.3	3	none	Phth (4)	74
7	pyr	3.3	3	DABCO (4)	Phth (4)	80
8	pyr	4	3.7	DABCO (4.5)	Phth (4.5)	85

[a]Not determined. Compound **5** formed along with a mixture of other phosphorylated products.

the reaction mixture was then cooled on ice and quenched with 100 mM triethylammonium acetate buffer and washed with chloroform to remove phthalimide, DABCO, and most of the pyridine. The solution was lyophilized, and the resulting white powder was subjected to a mixture of water/MeOH/Et_3N (3:6.7:1) for 24 h to remove the acetyl groups. The mixture was concentrated, and the NTPs were purified by reversed-phase chromatography using tri-*n*-butylammonium acetate in MeOH as eluent and converted into their ammonium salts using a NH_4^+ ion exchange column. All of the NTPs were obtained in good yield (Scheme 4). To

Scheme 4. Synthesis of Nucleoside 5′-Triphosphates 12−17



demonstrate that deoxynucleoside triphosphates can also be prepared using this methodology, 3′-acetyl-2′-deoxyadenosine (**10**) and 3′-azido-2′-deoxythymidine (AZT, **11**) were subjected to the triphosphorylation procedure. The resulting triphosphates (**16** and **17**) were obtained in good yield. For all of the triphosphorylation reactions, the corresponding tetraphosphate and monophosphate compounds were formed as minor byproducts but were easily separated from the triphosphorylated product by reversed-phase chromatography. When unprotected uridine or 2′-deoxyuridine was used as substrate, a complex mixture of products was obtained.

The triphosphorylation mechanism is currently unknown. Attempts to follow the reaction by ^{31}P NMR were unsuccessful due to the formation of a significant amount of precipitate upon addition of phthalimide and the nucleoside to the reaction mixture. The excess DABCO may be acting as a general base, and/or it may be reacting with the TriMP/MstCl adduct[17,18] to give a reactive intermediate. The role of the phthalimide may be to form an intermediate that is sufficiently reactive enough to phosphorylate the OH groups. It is also very possible that a polymeric form of TriMP acts as the phosphorylating agent.

In summary, we have developed the first practical triphosphorylation of nucleosides in good yield using TriMP as the phosphate source. The successful triphosphorylation of BPM and coumarin **3** indicates that this methodology should also be applicable to primary alcohols and phenols, in general. Further studies to develop conditions that would eliminate the use of protecting groups on the nucleoside are in progress and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03624.

Synthetic procedures for preparing **1**, **4**, **5**, and **12**−**17** and characterization data and NMR spectra for these compounds (PDF)

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Notes

The authors declare no competing financial interest.

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