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Sulfonyl Imidazolium Salts as Reagents for the Rapid and Efficient Synthesis of Nucleoside Polyphosphates and Their Conjugates

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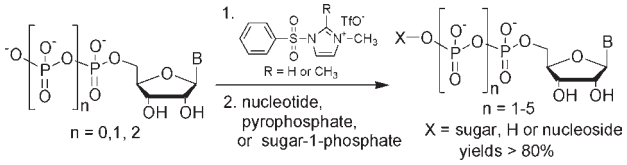
ABSTRACT



A procedure for the synthesis of nucleoside polyphosphates and their conjugates using sulfonylimidazolium salts as key reagents is described. The procedure is rapid and high yielding, does not require prior protection and subsequent deprotection of the donors or acceptors, and can be used to activate nucleoside mono-, di- and triphosphates, and a wide variety of acceptors and donors can be used.

Nucleoside polyphosphates (NPs) and their conjugates play key roles in numerous biological processes. Nucleotide triphosphates (NTPs) are the precursors to the building blocks of DNA and RNA, they initiate signaling pathways (ie. GTP), and ATP is a key energy source in many living systems. Nucleoside diphosphate sugars (NDP-sugars) act as glycosyl donors in the enzymatic synthesis of polysaccharides.[1] Dinucleoside polyphosphates (DNPs, $N(p)_nN$) act as signaling molecules.[2] In addition to their natural biological roles, nucleoside polyphosphates and their conjugates have been widely used as inhibitors and probes of therapeutically significant enzymes and a dinucleoside tetraphosphate, Up_4U, has recently been approved as a drug.[3−5] Very recently, novel and rapid methods for sequencing DNA have been reported using nucleoside tetraphosphates δ-labeled with fluorogenic probes.[6]

Due to the diversity of their applications and the crucial roles they play in biological processes, the synthesis of NPs and their conjugates has been the focus of intensive research for some time. The majority of the syntheses developed to date are based on the reaction of a phosphorylated acceptor with a 5′-activated mono-, di-, or triphosphate nucleoside donor. Although many donors have been developed[7a−c] only a few have found widespread use. Nucleotide 5′-morpholidates[8] and 5′-imidazolides[9] are perhaps the most widely used donors; however, reactions using these donors are often sluggish, afford highly variable yields, and often require tedious purifications.

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(8) For some examples, see: (a) Shaver, S. R.; Rideout, J. L.; Pendergast, W.; Douglass, J. G.; Brown, E. G.; Boyer, J. L.; Patel, R. I.; Redick, C. C.; Jones, A. C.; Picher, M.; Yerxa, B. R. *Purinergic Signal* **2005**, *1*, 183. (b) Miyashita, T.; Sakata, S.; Hayakawa, H. *Tetrahedron Lett*. **2003**, *44*, 8605. (c) Schafer, A.; Thiem, J. *J. Org. Chem*. **2000**, *65*, 24.

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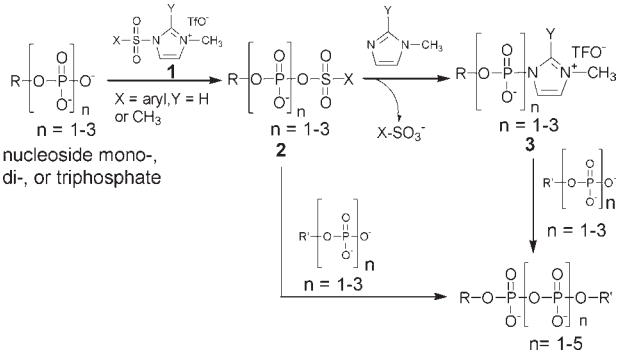
Recent efforts to develop improved approaches include the use of 5′-cyclosaligenyl phosphates[7b,10] and 5′-imidazolium salts as donors.[7a,11] The cyclosaligenyl phosphates have been used to prepare nucleotides, NDP-sugars, and DNPs; however, prior protection of reactive groups, such as hydroxyl or amino groups, on the donor or acceptor, isolation of the donor, and deprotection of the product are usually required, and yields can be highly variable.[7b] The 5′-imidazolium salts have been used for the synthesis of nucleotides, nonhydrolyzable nucleotide analogs, NDP-sugars, and DNPs. These donors are prepared using the procedure of Bogachev which involves the in situ protection of the hydroxyl and amino groups and formation of a 5′-mixed anhydride by reacting an unprotected nucleoside monophosphate with an excess of trifluoroacetic anhydride (TFAA).[11a] After removal of unreacted TFAA, the mixed anhydride is reacted with *N*-methylimidazole which results in the formation of a highly activated 5′-imidazolium donor. This species is then reacted with a phosphorylated acceptor to give a partially protected product which is then treated with aq. ammonium acetate to remove the protecting groups and give the desired product. Although this procedure is rapid and gave DNPs, NTPs, and NTP analogs in respectable yields, NDP-sugars were obtained in modest to low yields[11d] and we have found that this approach cannot be used to activate nucleoside di- or triphosphates.

Enzymatic methods have also been developed; however, this approach is limited by scale and the substrate specificity and availability of the enzymes.[12] Recently, a solid phase approach has been reported.[13] However, this method requires the multistep synthesis of a polymer with a unique linker and multistep syntheses of polyphosphites prior to the solid phase chemistry.

A procedure that can be performed on a wide variety of substrates, requiring no protecting groups on the acceptor or donor, providing the products rapidly, cleanly, and in high yields, would represent a significant advance in the preparation of these compounds. However, such a procedure has yet to be reported. Here we report, using unprotected acceptors and donors, a rapid and high yielding procedure for the synthesis of nucleoside polyphosphates and their conjugates using sulfonylimidazolium salts as key reagents.

Our general approach to nucleoside polyphosphates involves reacting a nucleoside mono-, di-, or triphosphate with a sulfonylimidazolium salt of type **1** (Scheme 1). This would initially produce mixed anhydride **2**. This could potentially act as a donor and react with a phosphorylated acceptor to give the desired nucleoside polyphosphates and their conjugates. Alternatively, the released *N*-methylimidazole could react with **2** to produce a highly reactive imidazolium salt of type **3** which could also act as a donor and react with acceptors to give the desired products.

Scheme 1. General Procedure for Preparing Nucleoside Polyphosphates and Their Conjugates



Sulfonyl imidazolium salts, like sulfonyl chlorides, have been used as reagents for the sulfonation of hydroxyl and amino groups.[14] Sulfonyl chlorides readily sulfonate the hydroxyl groups of carbohydrates and the hydroxyl and amino groups of nucleosides and nucleotides.[15] This suggests that sulfonyl imidazolium salts might be problematic for the synthesis of nucleoside polyphosphates using unprotected substrates. However, we reasoned that the reaction between the negatively charged phosphate moiety and the positively charged sulfonyl imidazolium salt would be much faster than the reaction between the salt and neutral hydroxyl and amino groups as would the subsequent reaction between the charged donor and phosphate group of the acceptor. To investigate this sulfonylimidazolium salts **6** and **7** were prepared by reacting phenylsulfonylimidazolides **4** and **5**[16] with methyl triflate in ether at rt (Scheme 2). Compounds **6** and **7** precipitated out of solution during the reaction. Filtration of the mixtures gave **6** and **7** as white powders in almost quantitative yields, and no further purification was necessary. Compounds **6** and **7** can be stored under Ar or N_2 at −20 °C for months without any detectable decomposition. Compound **7** exhibited slightly better solubility properties in organic

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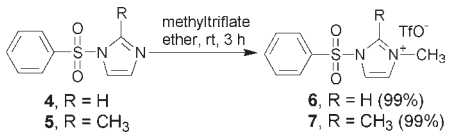
(15) For examples see: (a) Neumann, J.; Weingarten, S.; Thiem, J. *Eur. J. Org. Chem.* **2007**, *7*, 1130. (b) Tang, X.; Dmochowski, I. J. *Org. Lett.* **2005**, *7*, 279.

(16) Compounds **4** and **5** were prepared in 96% and 99% yields respectively from the corresponding sulfonyl chlorides and imidazole or 2-methylimidazole. See the Supporting Information.

solvents than compound **6** though both slowly decomposed when stored as solutions in organic solvents.

Scheme 2. Synthesis of Sulfonyl Imidazolium Salts **6** and **7**



We initially examined **6** and **7** as reagents for preparing symmetric dinucleoside di- and tetraphosphates by dimerization of nucleoside mono- and diphosphates. AMP was used as a model substrate. 0.6 equiv[17] of reagent **6** was added to a solution of the tetra-*n*-butylammonium salt of AMP[18] in DMF at 0 °C, the cooling bath was removed, and the reaction was followed by ^{31}P NMR. After 10 min no further reaction occurred, and the desired product, Ap_2A, was formed in about a 67% yield (Table 1). Performing this reaction in the presence of 0.5 equiv of $MgCl_2$ gave Ap_2A in a 94% yield by ^{31}P NMR, while performing it in the presence of 0.5 equiv of $MgCl_2$[19] and 1 equiv of *N*-methylimidazole (NMI) gave Ap_2A in quantitative yield by ^{31}P NMR within 10 min. After quenching the reaction with triethylammonium acetate (pH 7.0), extraction with $CHCl_3$ to remove the tetra-*n*-butylammonium salt of phenylsulfonate and NMI, and purification by reversed-phase HPLC, Ap_2A was obtained in 93% yield. No products resulting from sulfonation of the hydroxyl or amino groups were detected. Salt **7** gave similar results. ^{31}P NMR analysis of a mixture of AMP with 1.2 equiv of salt **6** and 1 equiv of NMI showed rapid formation of a compound with a peak at δ −9.2 ppm which is indicative of an imidazolium salt of type **3**[7a] suggesting that the reaction proceeds via this species as opposed to mixed anhydride **2**. Applying the optimized conditions using reagent **6** to the dimerization of UMP and GMP led to the formation of Up_2U and Gp_2G in excellent isolated yields (Table 1). To determine if nucleoside diphosphates can also act as donors we examined the dimerization of GDP and UDP using the above conditions; however, the reactions were slower and the symmetrical tetraphosphates were obtained in moderate yields by ^{31}P NMR. However, by using 0.75 equiv of coupling agent, 3 equiv of base, and a 20 min reaction time, Gp_4G and Up_4U could be obtained in excellent isolated yield (Table 1, entries 6 and 7).

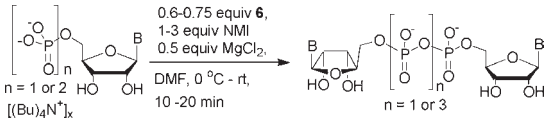
Next we examined the synthesis of nucleoside triphosphates and unsymmetrical DNPs using this procedure. Reaction of the tetrabutylammonium salts of AMP and UMP with 1.2 equiv of **6** and 1 equiv of NMI for 1 min at rt

(17) A slight excess was used since compounds **6** and **7** decompose in DMF.

(18) The number of tetra-*n*-butylammonium ions per molecule of nucleoside mono-, di-, and triphosphate was determined by ^{1}H NMR. See the Supporting Information for details.

(19) We found that it was not necessary to use anhydrous $MgCl_2$ as the more economical and easily handled trihydrate form gave equally good results.

Table 1. Synthesis of Symmetrical Dinucleoside Di- and Tetraphosphates

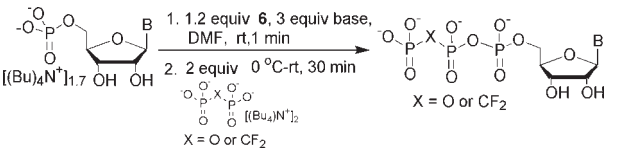


entry	substrate	product	yield (%)[a]
1	AMP	Ap_2A (**8**)	67[b,c]
2	AMP	Ap_2A (**8**)	94[b,d]
3	AMP	Ap_2A (**8**)	93, 99[b]
4	UMP	Up_2U (**9**)	93
5	GMP	Gp_2G (**10**)	94
6	GDP	Gp_4G (**11**)	84[e]
7	UDP	Up_4U (**12**)	81[e]

[a] Isolated yield except where noted. 0.6 equiv of **6**, 1 equiv of NMI, and 0.5 equiv of $MgCl_2$ used except where noted. [b] ^{31}P NMR yield. [c] No $MgCl_2$, no NMI. [d] No $MgCl_2$, 1 equiv of NMI. [e] 3 equiv of NMI, 0.75 equiv of **6**.

followed by reaction with 2 equiv of the bis-tetrabutylammonium salt of inorganic pyrophosphate for 10 min gave ATP and UTP in excellent yields after the usual workup and HPLC purification. The addition of $MgCl_2$ did not significantly improve yields. However, when GMP and CMP were substrates, the yields of the triphosphates were not optimal due to competing dimerization. Nevertheless, by using diisopropylethylamine (DIPEA) instead of NMI the dimerization reaction was suppressed and CTP and GTP were obtained in high yields. DIPEA could also be used for the synthesis of ATP and UTP. The synthesis of ATP analog **17** (Table 2, entry 5) was also accomplished in excellent yield using this procedure. ^{31}P NMR of the reaction between AMP and **6** in the presence of 3 equiv of DIPEA for 1 min showed rapid formation of an imidazolium salt of type **3** by ^{31}P NMR indicating that excess NMI was not required for imidazolium salt formation

Table 2. Synthesis of Nucleoside Triphosphates



entry	base	X	product	yield (%)[a]
1	A	O	ATP (**13**)	85,[b] 86[c]
2	U	O	UTP (**14**)	88,[b] 90[c]
3	G	O	GTP (**15**)	88[c]
4	C	O	CTP (**16**)	84[c]
5	A	CF_2	$AppCF_2p$ (**17**)	89[c]

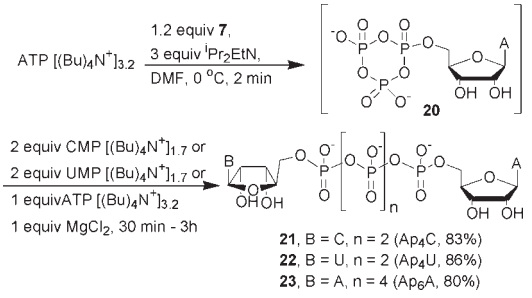
[a] Isolated yields. [b] NMI used as base. [c] ^{i}Pr$_2$EtN used as base.

though the presence of DIPEA appears to promote its formation.[20] This procedure also works very well for the construction of unsymmetrical dinucleoside di- and triphosphates as demonstrated by the synthesis of Ap_2C and Ap_3U in very good yields starting from AMP or UMP and using CMP or ADP as acceptors (Scheme 3).

Scheme 3. Synthesis of Ap_2C and Ap_3U

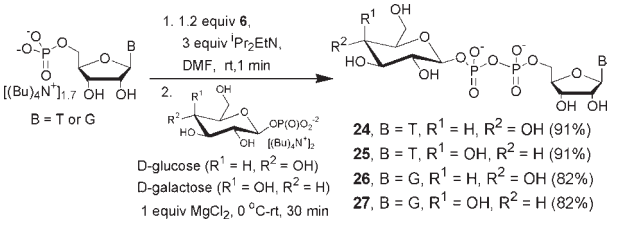
ATP was used as a model substrate for activation of nucleotide triphosphates. Adding 1.2 equiv of reagent **6** or **7** to the tetra-*n*-butylammonium salt of ATP[18] in the presence of DIPEA in DMF resulted in the formation of cyclic adenosine trimetaphosphate **20** (Scheme 4) as determined by ^{31}P NMR.[21] We found that this reaction was essentially quantitative within 2 min at 0 °C using reagent **7**. Reagent **6** gave similar results except it is slightly less soluble in DMF at 0 °C resulting in longer reaction times. Treating **20** with a solution of the tetra-*n*-butylammonium salts of UMP or CMP and 1 equiv of $MgCl_2$ in DMF and stirring for 30 min at rt gave unsymmetrical DNPs, Ap_4U and Ap_4C, in excellent isolated yields. Ap_6A was obtained in an 80% yield by adding 0.6 equiv of **7** to a solution of ATP, 1 equiv of $MgCl_2$, and 3 equiv of DIPEA at 0 °C and then stirring for 3 h at rt.

Scheme 4. Synthesis of Ap_4C, Ap_4U, and Ap_6A from ATP



To demonstrate that this procedure is a powerful approach to the synthesis of NDP-sugars we prepared compounds **24**–**27** using the same procedure employed for preparing **18** and **19** (Scheme 5). We found that small amounts of unreacted nucleotide monophosphate were time-consuming to remove. However, by treating the quenched reaction with a small amount of alkaline phosphatase for 16 h, the unreacted nucleotide monophosphate was converted into a nucleoside and inorganic phosphate and the purification became straightforward. Excellent isolated yields were obtained for all four NDP-sugars including **26** and **27** which had been previously prepared in low yields by the cyclosaligenyl approach (22% for **26** and 34% for **27**).[7b]

Scheme 5. Synthesis of NDP-Sugars



In summary, we have described a novel and broadly applicable procedure for preparing nucleoside polyphosphates and their conjugates. The procedure is rapid and high yielding, does not require prior protection and subsequent deprotection of the donors or acceptors, and can be used to activate mono-, di-, and trinucleotides, and a wide variety of acceptors and donors can be used. We expect that this procedure will find widespread use in the preparation of nucleoside polyphosphates and their conjugates.

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Supporting Information Available. Preparation procedures and characterization data for **6**–**19** and **21**–**27**. This material is available free of charge via the Internet at http://pubs.acs.org.

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