ORGANIC LETTERS

2004
Vol. 6, No. 13
2257−2260

A Novel Method for the Preparation of Nucleoside Triphosphates from Activated Nucleoside Phosphoramidates

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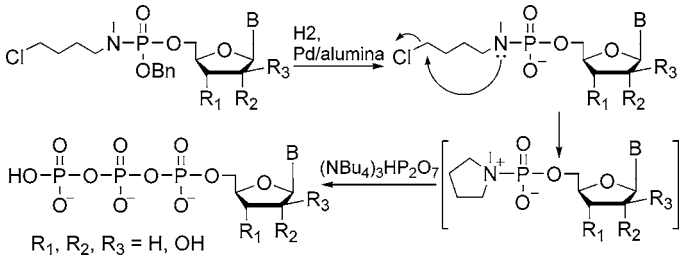
Received April 21, 2004

ABSTRACT



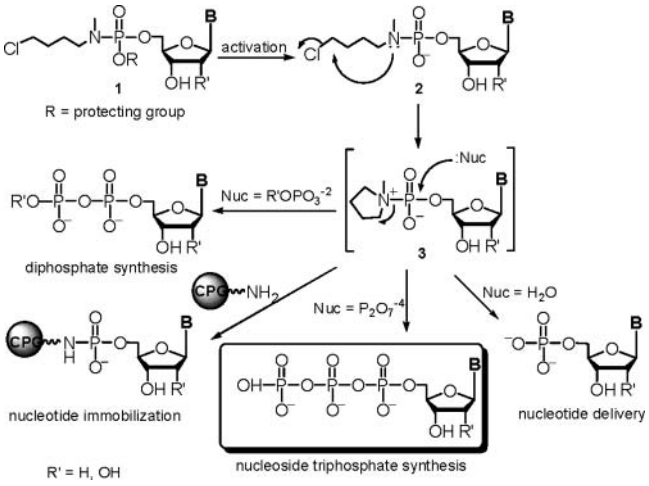
A novel method for the preparation of nucleoside triphosphates has been developed. The strategy employs a highly reactive pyrrolidinium phosphoramidate zwitterion intermediate that undergoes efficient coupling with tris(tetra-*n*-butylammonium) hydrogen pyrophosphate to generate nucleoside triphosphate.

Nucleoside 5′-triphosphates are a class of very important compounds in biological systems. Naturally occurring deoxyribo- and ribonucleoside triphosphates are the basic building blocks for enzymatic synthesis of DNA and RNA in vivo and in vitro. Modified nucleoside triphosphates have received much attention in searches for potential therapeutic and diagnostic agents and in the study of numerous biochemical and pharmacological processes. As a result, a number of methods have been developed for the preparation of nucleoside triphosphates and their analogues (see ref 1 for a review).[1] The widely used method of "one-pot, three-step" nucleoside triphosphate synthesis developed by Ludwig[2] and others[3] involves generation of nucleoside dichlorophosphate via Yoshikawa's procedure,[4] followed by reaction with bis-(tri-*n*-butylammonium) pyrophosphate. However, it is not applicable to all nucleoside derivatives, especially for triphosphorylation of nucleoside analogues bearing modified bases sensitive to Yoshikawa's phosphorylation procedure.[5] Other widely used multistep methods rely upon the use of activated nucleoside monophosphates such as unsubstituted phosphoramidate,[6] morpholidate,[7] or imidazolidate[8] that undergo subsequent displacement with pyrophosphate. In most cases, the final transformation to triphosphate is slow (one to several days), and the yields are generally moderate. We have developed a novel method for the synthesis of nucleoside triphosphates employing a highly reactive pyrrolidinium phosphoramidate zwitterion intermediate **3** that undergoes fast and efficient coupling with tris(tetra-*n*-butylammonium)

(1) Burgess, K.; Cook, D. *Chem. Rev.* **2000**, *100*, 2047−60.
(2) Ludwig, J. *Acta Biochim. Biophys. Acad. Sci. Hung.* **1981**, *16*, 131−3.
(3) Ruth, J. L.; Cheng, Y. C. *Mol. Pharmacol.* **1981**, *20*, 415−22.
(4) Yoshikawa, M.; Kato, T.; Takenishi, T. *Tetrahedron Lett.* **1967**, *50*, 5065−8.
(5) Wu, W.; Bergstrom, D. E.; Davisson, V. J. *J. Org. Chem.* **2003**, *68*, 3860−5.
(6) (a) Tomasz, J.; Simoncsits, A.; Kajtar, M.; Krug, R. M.; Shatkin, A. J. *Nucleic Acids Res.* **1978**, *5*, 2945−57. (b) Simoncsits, A.; Tomasz, J. *Nucleic Acids Res.* **1975**, *2*, 1223−33.
(7) (a) Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1961**, *83*, 649−58. (b) Moffatt, J. G. *Can. J. Chem.* **1964**, *42*, 599−604. (c) van Boom, J. H.; Crea, R.; Luyten, W. C.; Vink, A. B. *Tetrahedron Lett.* **1975**, *16*, 2779−82.
(8) (a) Hoard, D. E.; Ott, D. G. *J. Am. Chem. Soc.* **1965**, *87*, 1785−8. (b) Shimazu, M.; Shinozuka, K.; Sawai, H. *Tetrahedron Lett.* **1990**, *31*, 235−8.

10.1021/ol049267j CCC: $27.50
Published on Web 06/02/2004

Scheme 1



hydrogen pyrophosphate to generate the corresponding nucleoside triphosphates (Scheme 1).

This approach is based upon the detailed investigation of the activation mechanisms of nucleoside phosphoramidate prodrugs developed in this lab for the intracellular delivery of anticancer and antivirus nucleotides.[9] The reactivity of the zwitterionic phosphoramidate intermediate **3** toward different nucleophiles following chemical activation of the phosphoramidate ester **1** has also been studied and has led to the development of a novel strategy leading to the formation of nucleotides,[9] nucleoside sugar diphosphates,[10] and the immobilization of nucleotides on solid supports[11] (Scheme 1). The strategy is now extended to the preparation of nucleoside triphosphates, and we report herein the preparation of triphosphates of both natural and modified nucleosides from the corresponding nucleoside phosphoramidates.

Preliminary experiments were carried out with thymidine phosphoramidate **4a** as the model compound, and the results are shown in Figure 1 and Table 1. Generation of the

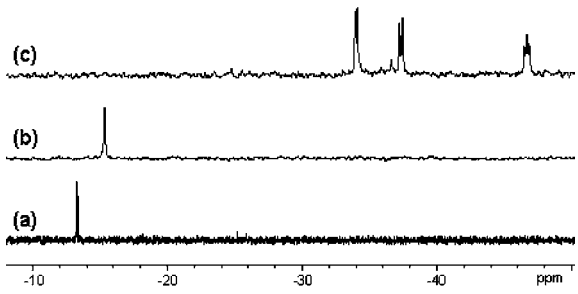


Figure 1. ^{31}P NMR of nucleoside triphosphate formation from nucleoside phosphoramidate at room temperature (ref = triphenylphosphine oxide): (a) nucleoside phosphoramidate; (b) activated phosphoramidate after catalytic hydrogenolysis; and (c) 10 min after addition of TBAPP.

Table 1. Reaction Conditions and Yields for Nucleoside Triphosphates Synthesis

entry	starting cpd	reaction conditions	product, yield
1	**4a**	Pd/C, THF, 2 h; $(HNBu_3)_2H_2P_2O_7$, 4 h	**5a**, 85%[a]
2	**4a**	Pd/C, THF, 2 h; $(NBu_4)_3HP_2O_7$, 10 min	**5a**, 100%[a]
3	**4a**	Pd/C, THF, 2 h; $(NBu_4)_3HP_2O_7$, 30 min	**5a**, 77%[b]
4	**4c**	Pd/C, THF, 2 h; $(NBu_4)_3HP_2O_7$, 30 min	**5c**, 69%[b]
5	**4b**	Pd/Al_2O_3, DMF, 1 h; $(NBu_4)_3HP_2O_7$, 30 min	**5b**, 75%[b]
6	**4d**	Pd/Al_2O_3, DMF, 1 h; $(NBu_4)_3HP_2O_7$, 30 min	**5d**, 71%[b]
7	**4e**	Pd/Al_2O_3, DMF,[c] 1 h; $(NBu_4)_3HP_2O_7$, 30 min	**5e**, 55%[b]

[a] Conversion based on ^{31}P NMR. [b] Isolated yield. [c] 5% (v/v) water was added to the reaction mixture following catalytic hydrogenolysis

phosphoramidate zwitterion intermediate in situ was accomplished quantitatively by catalytic hydrogenolysis with palladium on activated carbon in anhydrous THF at room temperature (Scheme 2 and spectra a and b in Figure 1).[10,11] Following filtration of the catalyst, the pyrophosphate coupling reaction was carried out by the addition of either bis(tri-*n*-butylammonium) pyrophosphate[7b,12] or tris(tetra-*n*-butylammonium) hydrogen pyrophosphate[13] (TBAPP) to the above phosphoramidate zwitterion solution. The coupling reaction with bis(tri-*n*-butylammonium) pyrophosphate took 4 h to complete, and thymidine triphosphate was formed in 85% conversion judged by ^{31}P NMR (Table 1, entry 1). The major byproduct was thymidine monophosphate, formed by reaction of the phosphoramidate zwitterion intermediate with trace water present in the reaction mixture. However, the reaction carried out with tris(tetra-*n*-butylammonium) hydrogen pyrophosphate was very rapid, resulting in quantitative triphosphate conversion within 10 min with no formation of hydrolysis product (Table 1, entry 2; also see spectrum c in Figure 1).[14,15] This observation is in accordance with the report of Davisson et al.[13] that the nucleophilicity of trialkylammonium pyrophosphates is reduced compared with that of the tetraalkylammonium salts.

(9) (a) Freel Meyers, C. L.; Hong, L.; Joswig, C.; Borch, R. F. *J. Med. Chem.* **2000**, *43*, 4313−8. (b) Freel Meyers, C. L.; Borch, R. F. *J. Med. Chem.* **2000**, *43*, 4319−27. (c) Tobias, S. C.; Borch, R. F. *J. Med. Chem.* **2001**, *44*, 4475−80. (d) Tobias, S. C.; Borch, R. F. *Mol. Pharm.* **2004**, *1*, 112−6.

(10) Freel Meyers, C. L.; Borch, R. F. *Org. Lett.* **2001**, *3*, 3765−8.

(11) Freel Meyers, C. L.; Borch, R. F. *Org. Lett.* **2003**, *5*, 341−4.

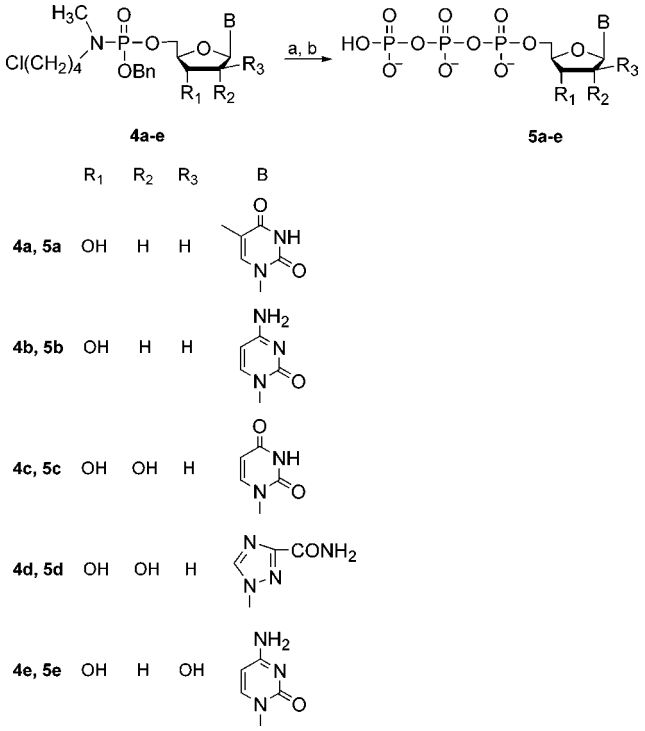
(12) Ludwig, J.; Eckstein, F. *J. Org. Chem.* **1989**, *54*, 631−5.

(13) Davisson, V. J.; Woodside, A. B.; Neal, T. R.; Stremler, K. E.; Muehlbacher, M.; Poulter, C. D. *J. Org. Chem.* **1986**, *51*, 4768−79.

(14) It is noteworthy that tris(tetra-*n*-butylammonium) hydrogen pyrophosphate contains water of hydration (at least 3 equiv);[13] the absence of hydrolysis byproduct indicates that nucleophilicity of this pyrophosphate salt is much greater toward the phosphoramidate zwitterion intermediate than that of water.

(15) It is important to use only 1 equiv of pyrophosphate reagent in these reactions, because excess pyrophosphate is very difficult to separate from nucleoside triphosphate during purification by anion exchange chromatography on Q Sepharose FF.

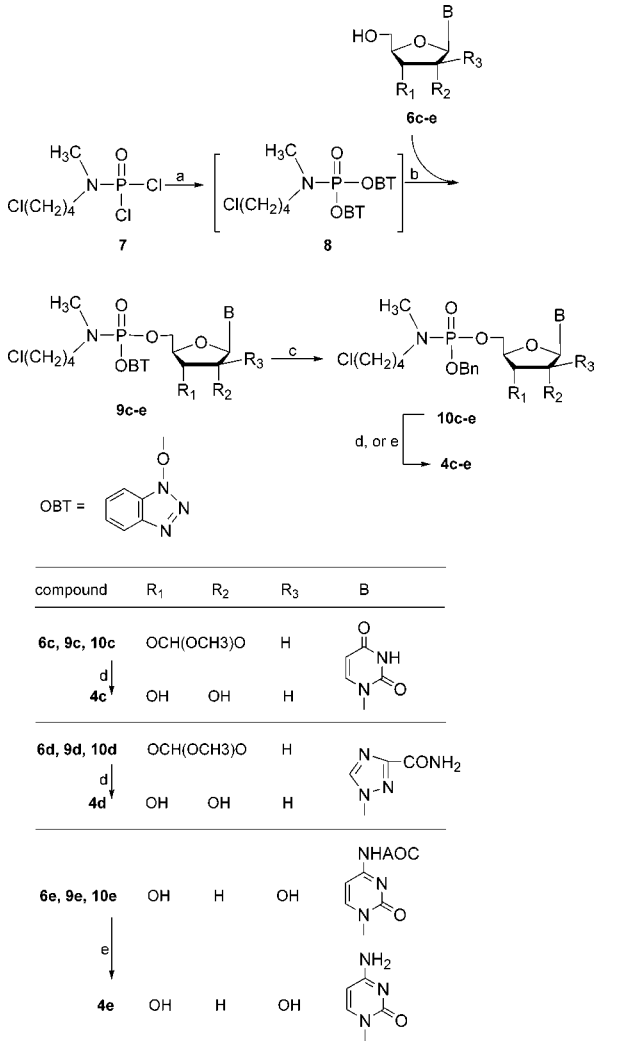
Scheme 2[a]



[a] Reagent and conditions: (a) for **4a** and **4c**, H_2, Pd/C, THF, 2 h; for **4b**, **4d**, and **4e**, H_2, Pd/alumina, DMF, 1 h; (b) for **4a** and **4c**, $(NBu_4)_3HP_2O_7$, THF, 30 min; for **4b**, **4d**, and **4e**, $(NBu_4)_3HP_2O_7$, DMF, 30 min.

On the basis of the above results, the scope and applicability of this approach toward preparation of triphosphates of both natural and modified nucleoside analogues were examined (Scheme 2 and Table 1). Among the nucleotide analogues shown in Scheme 2, ribavirin (1-β-D-ribofuranosyl-1,2,4-trizaole-3-carboxamide) triphosphate (RTP, **5d**) and Ara-C (1-β-D-arabinofuranosyl cytosine, Cytarabine) triphosphate (Ara-CTP, **5e**) were chosen because of their importance in the antiviral[16] and antitumor[17] therapeutic treatment as well as for the study of numerous biochemical and pharmacological processes. Application of this approach to the synthesis of these nucleoside triphosphates was successful for uridine (Table 1, entry 4) but met with limited success in the case of 2′-deoxycytidine, ribavirin, and Ara-C. Poor mass recoveries were obtained presumably due to poor solubility of the starting phosphoramidates and products and the significant adsorption on Pd/C catalyst. Use of palladium on alumina as hydrogenolysis catalyst resulted in both decreased adsorption of the nucleoside analogue and an increase in the rate

Scheme 3[a]



[a] Reagent and conditions: (a) HOBT, pyridine, THF, 4 h, rt; (b) *N*-methylimidazole, pyridine/THF, 6−24 h, rt; (c) for **9c**, $C_6H_5CH_2OH$, LiHMDS, THF, −70 to −40 °C, 1 h; for **9d** and **9e**, $C_6H_5CH_2OH$, DMAP, THF, overnight, rt; (d) HCl, pH 2.0, CH_3CN/H_2O (1:1), 4−24 h, rt; then NH_3HCO_3, pH 8.0, CH_3CN/H_2O (1:1), 2−24 h, rt; (e) $Pd(PPh_3)_4$, *p*-$C_6H_4SO_2Na$, THF/H_2O (2:1), 1 h, rt.

of hydrogenolysis (Table 1, entries 5, 6, and 7). The reaction solvent was changed to DMF in an effort to increase the solubility of the cytosine analogues. Both hydrogenolysis and pyrophosphate coupling proceeded smoothly in DMF, and gave essentially quantitative conversion to nucleoside triphosphate as judged by ^{31}P NMR.[18] Addition of water (5% v/v) to the reaction mixture following catalytic hydrogenolysis of the Ara-C phosphoramidate was necessary to facilitate filtration of the reaction intermediate, and the pyrophosphate coupling still proceeded smoothly and afforded only a small amount of hydrolysis product.

A simple purification procedure employing Q Sepharose FF, a medium pressure anion exchanger, was developed to

(16) (a) Crotty, S.; Maag, D.; Arnold, J. J.; Zhong, W.; Lau, J. Y.; Hong, Z.; Andino, R.; Cameron, C. E. *Nat. Med.* **2000**, *6*, 1375−9. (b) Crotty, S.; Cameron, C. E.; Andino, R. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 6895−900. (c) Maag, D.; Castro, C.; Hong, Z.; Cameron, C. E. *J. Biol. Chem.* **2001**, *276*, 46094−8. (d) Crotty, S.; Cameron, C.; Andino, R. *J. Mol. Med.* **2002**, *80*, 86−95.

(17) (a) Furth, J. J.; Cohen, S. S. *Cancer Res.* **1968**, *28*, 2061−7. (b) Graham, F. L.; Whitmore, G. F. *Cancer Res.* **1970**, *30*, 2636−44. (c) Kufe, D. W.; Major, P. P.; Egan, E. M.; Beardsley, G. P. *J. Biol. Chem.* **1980**, *255*, 8997−900. (d) Major, P. P.; Egan, E. M.; Beardsley, G. P.; Minden, M. D.; Kufe, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3235−9.

(18) We also explored this catalyst and solvent (Pd/alumina/DMF) on the coupling reaction of β-D-glucose-1-phosphate with thymidine phosphoramidate in the presence of tetrabutylammonium chloride. The coupling reaction gave a very clean ^{31}P NMR with no formation of hydrolysis byproduct even in the presence of water.

purify the triphosphate. All triphosphates described in this study were rapidly and easily purified by using a linear gradient of 50 to 500 mM ammonium bicarbonate (pH 7.8) at room temperature and were judged pure by HPLC, ^{1}H, ^{13}C, and ^{31}P NMR. Where available, commercial materials were used for comparisons with synthetic materials by HPLC and spectral analysis. The isolated yields of purified nucleoside triphosphates ranged from 55% to 77% (Table 1).

Thymidine and deoxycytidine phosphoramidates **4a** and **4b** were synthesized according to the reported phosphoramidite procedure;[9a,c] however, the same method failed to give reasonable results for synthesis of ribonucleoside phosphoramidates due to poor solubility and serious complications when starting from unprotected ribonucleosides.[9d] Thus, protection of the 2′,3′ hydroxyl groups of the ribose moiety was necessary for synthesis of uridine and ribavirin phosphoramidates **4c** and **4d**. The 2′,3′-*O*-methoxymethylidene protective group[19] proved to be an ideal choice, because it is stable under the reaction conditions and can be removed without cleaving the acid labile phosphoramidate. 2′,3′-O-protected uridine **6c** and ribavirin **6d** were prepared according to the reported procedure[5,20] in 92% and 84% yield, respectively. The protected nucleosides **6c** and **6d** were reacted with phosphorylating agent **8**,[21] generated in situ from phosphorodichloridate **7** and 1-hydroxybenzotriazole (HOBT), to give benzotriazolyl phosphoramidate **9c** and **9d** in 75% yield. The OBT moiety was displaced by benzyl alcohol in the presence of LiHMDS (for **9c**) or DMAP (for **9d**) to give **10c** and **10d**. 2′,3′-*O*-Methoxymethylidene protecting groups were removed under very mild conditions involving hydrolysis at pH 2.0 and then at pH 8.0[19,20] to afford the desired phosphoramidates **4c** and **4d** in 69% yield (two steps). Ara-C phosphoramidate **4e** was synthesized by using the same approach starting from *N*-allyloxycabonyl (AOC) protected nucleoside **6e**.[9d] Selective phosphorylation of **6e** on the 5′-hydroxyl group with reagent **8** was achieved to afford benzotriazolyl phosphoramidate **9e** in 71% yield. Displacement of the OBT group with benzyl alcohol in the presence of DMAP gave phosphoramidate **10e** in 68% yield. Finally, compound **10e** was deprotected by using palladium chemistry[9d] to give the desired phosphoramidate **4e** in 84% yield (Scheme 3).

In summary, a novel method for the synthesis of nucleoside triphosphates has been developed. The approach described here employs a highly reactive pyrrolidinium phosphoramidate zwitterion intermediate that undergoes an efficient coupling reaction with tris(tetra-*n*-butylammonium) hydrogen pyrophosphate to generate the corresponding nucleoside triphosphate. Reaction conditions for activation of the nucleoside phosphoramidates and subsequent coupling with pyrophosphate have been optimized, and this method has been applied to the synthesis of triphosphates of both natural and unnatural nucleoside analogues. This approach would have advantage in the synthesis of triphosphates of unnatural nucleosides with modified bases, particularly azoles bearing a carboxamide substituent, where side reactions occur and yields are poor when using nonenzymatic methods.[5] It should complement the existing methods for the synthesis of nucleoside triphosphates, an important class of compounds for which there is no broadly applicable methodology.[1]

Acknowledgment. We thank Dr. Sandra C. Tobias for synthesis of deoxycytidine phosphoramidate **4b**. Financial support from the National Cancer Institute (CA34619) is also gratefully acknowledged.

Supporting Information Available: Experimental procedures and NMR spectra for **4c**−**e** and **5d**,**e**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL049267J

(19) (a) Griffin, B. E.; Jarman, M.; Reese, C. B.; Sulston, J. E. *Tetrahedron* **1967**, *23*, 2301−13. (b) Jones, S. S.; Rayner, B.; Reese, C. B.; Ubasawa, A.; Ubasawa, M. *Tetrahedron* **1980**, *36*, 3075−85.

(20) Davisson, V. J.; Davis, D. R.; Dixit, V. M.; Poulter, C. D. *J. Org. Chem.* **1987**, *52*, 1794−801.

(21) (a) van der Marel, G. A.; van Boeckel, C. A.; Wille, G.; van Boom, J. H. *Nucleic Acids Res.* **1982**, *10*, 2337−51. (b) van der Marel, G. A.; van Boeckel, C. A.; Wille, G.; van Boom, J. H. *Tetrahedron Lett.* **1981**, *22*, 3887−90. (c) van Boeckel, C. A. A.; van der Marel, G.; Wille, G.; van Boom, J. H. *Chem. Lett.* **1981**, 1725−8.