

# A Novel Method for the Preparation of Nucleoside Diphosphates

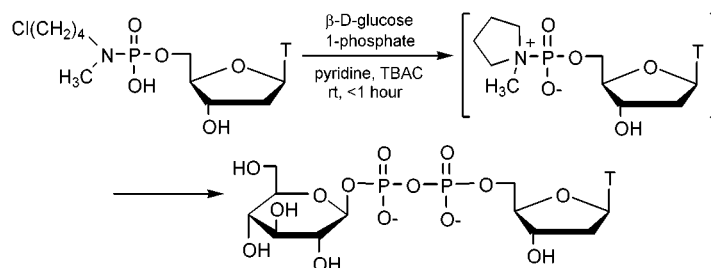
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## ABSTRACT



Sugar nucleoside diphosphates have been prepared using an efficient phosphate coupling reaction that employs a highly reactive zwitterionic phosphoramidate intermediate as the phosphorylating species.

The study of complex glycosylation pathways in a variety of important biological processes requires access to the sugar nucleoside diphosphate substrates of glycosyltransferases. Many of the current strategies for the preparation of sugar nucleoside diphosphates employ lengthy and complex enzymatic or chemical methods.<sup>1</sup> In most cases, long reaction times and tedious purification are required, and the reactions are generally moderate to low yielding. We have developed an efficient, novel one-pot synthesis of sugar nucleoside diphosphates that can be extended to the preparation of nucleoside triphosphates.

The chemical rationale for this strategy of diphosphate preparation is based upon previous studies carried out in our laboratory that have elucidated the activation mechanisms of haloethyl and halobutyl nucleoside phosphoramidate prodrugs under model physiologic conditions.<sup>2,3</sup> Following

chemical activation of the phosphoramidate ester to the corresponding anion, cyclization occurs to give a highly reactive aziridinium or pyrrolidinium ion intermediate (**1** and **2**, Scheme 1). Nonselective nucleophilic attack by water at carbon or phosphorus of aziridinium ion **1** is observed; solvolysis product **3** and nucleotide (NMP) are formed in a 1:1 ratio. In contrast, phosphoramidate prodrugs that undergo activation to give pyrrolidinium ion intermediate **2** react with water solely at phosphorus to give the nucleotide (NMP) as the exclusive product. It was hypothesized that this phosphoramidate prodrug activation chemistry could be exploited for the nonaqueous preparation of nucleoside diphosphates and triphosphates (**4**). We report herein the preparation of TDP-Glc and TDP-Rha using this novel methodology.

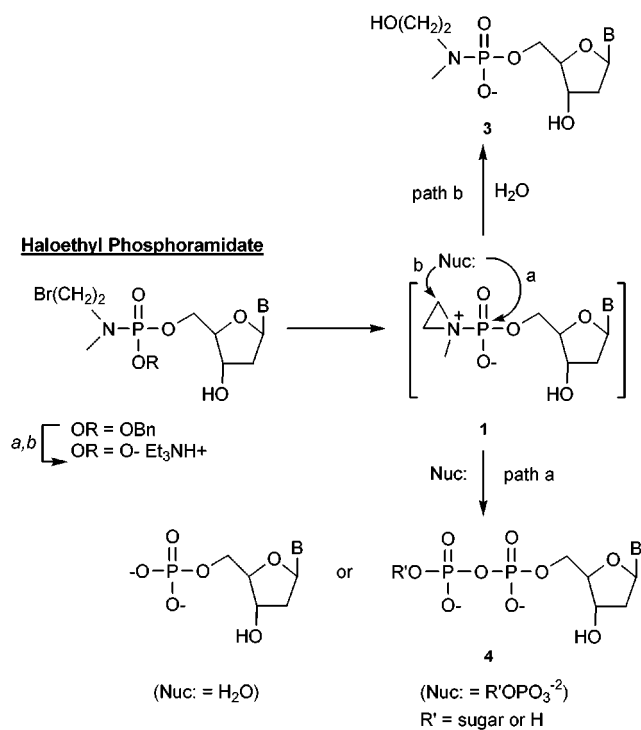
The kinetics and mechanism of the phosphate coupling reaction were initially studied using tetrahydrofurfuryl halobutyl phosphoramidate **5**<sup>4</sup> (Scheme 2) and commercially available β-D-glucose 1-phosphate. Generation of the phosphoramidate anion in situ from the benzyl ester was accomplished by hydrogenolysis in anhydrous THF at room temperature (Scheme 2). The phosphate coupling reactions

(1) (a) Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1959**, *81*, 1265. (b) Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1961**, *83*, 649. (c) Ginsburg, V. *Adv. Enzymol.* **1964**, *26*, 35. (d) Moffatt, J. G. *Methods Enzymol.* **1966**, *8*, 136. (e) Wong, C.-H.; Haynie, S. L.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 5416. (f) Arlt, M.; Hindsgaul, O. *J. Org. Chem.* **1995**, *60*, 14. (g) Wittmann, V.; Wong, C.-H. *J. Org. Chem.* **1997**, *62*, 2144. (h) Thorson, J. S.; Zhao, Y. *J. Org. Chem.* **1998**, *63*, 7568. (i) Marlow, A. L.; Kiessling, L. L. *Org. Lett.* **2001**, *3*, 2517.

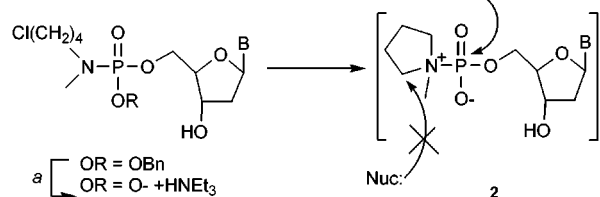
(2) Meyers, C. L. F.; Borch, R. F. *J. Med. Chem.* **2000**, *43*, 4319.

(3) Tobias, S.; Borch, R. F. Submitted.

(4) Halobutyl tetrahydrofurfuryl phosphoramidate **5** was prepared according to the procedure outlined for the preparation of haloethyl phosphoramidates in ref 2.

Scheme 1<sup>a</sup>

Halobutyl Phosphoramidate

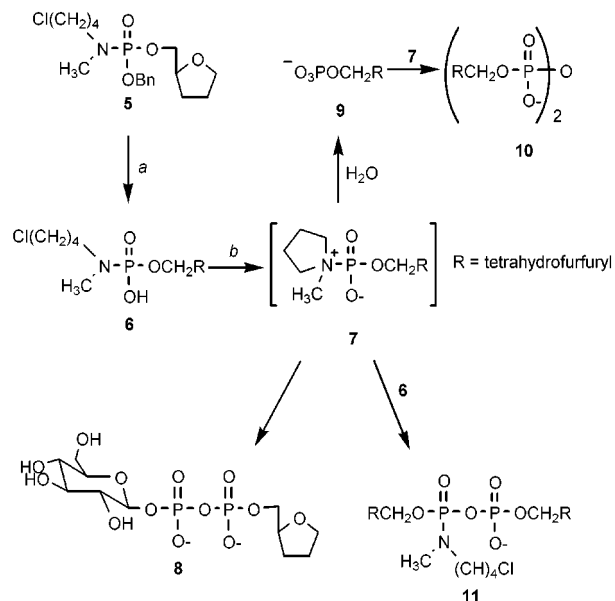


<sup>a</sup> Reagents and conditions: (a) H<sub>2</sub>, Pd/C, THF, rt, 15 min; (b) TEA.

were carried out at room temperature under an atmosphere of argon in pyridine and were monitored using <sup>31</sup>P NMR.

Under these conditions, *N*-methyl-*N*-(2-chlorobutyl) tetrahydrofurfuryl phosphoramidate **6** reacted with β-*D*-glucose 1-phosphate to give the desired diphosphate **8** as the major product (65%, Figure 1 and Scheme 2). Byproducts arising from the reaction of the zwitterionic intermediate (**7**, Scheme 2) with trace water present in the reaction mixture were also observed. Tetrahydrofurfuryl monophosphate **9** (13%) formed via hydrolysis of **7** underwent subsequent reaction with the pyrrolidinium ion intermediate to form dimeric product **10** (11%). It is important to note that caution must be exercised during the hydrogenolysis reaction; increasing the concentration of the reaction mixture results in the formation of “self-condensation” product **11** (11%). Reactions carried out using an excess of phosphoramidate anion also favor formation of **11** as expected.

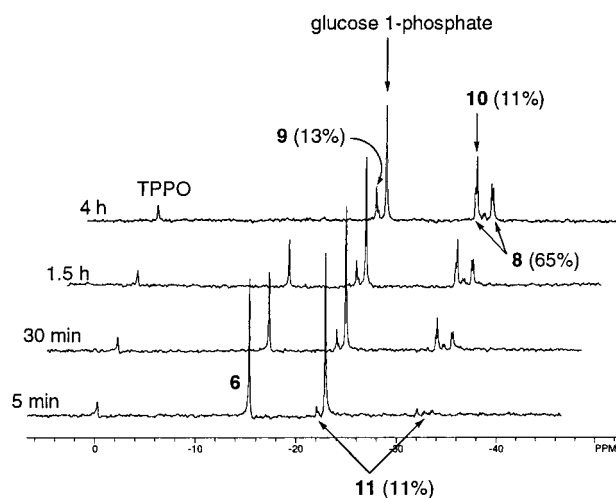
Tetrabutylammonium chloride (TBAC) was added to increase the solubility of the sugar phosphate in the organic

Scheme 2<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) H<sub>2</sub>, Pd/C, THF, rt, 5 min; (b) pyridine, TBAC, β-*D*-glucose 1-phosphate, rt.

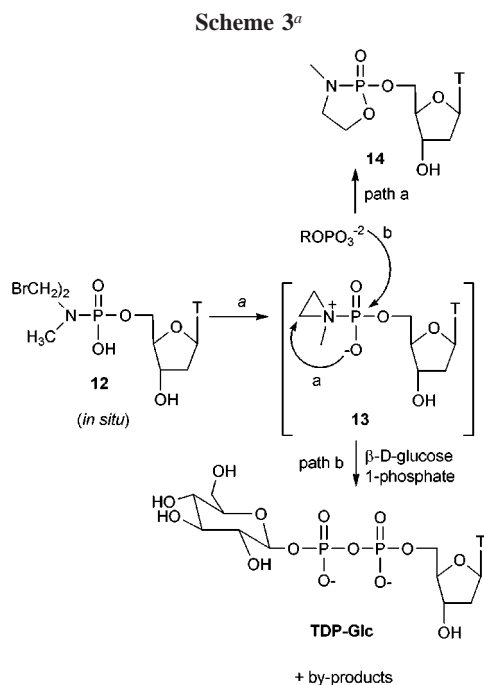
solvent. It is of interest to note that the rate of cyclization of phosphoramidate anion **6** to pyrrolidinium ion intermediate **7** is dependent upon the concentration of TBAC. Phosphate coupling reactions carried out in pyridine in the presence of 1 equiv of TBAC exhibited a slower rate of cyclization (*t*<sub>1/2</sub> = 16 min) when compared to reactions carried out with 3 equiv of TBAC (*t*<sub>1/2</sub> = 5.8 min). Presumably, the resulting increase in ionic strength of the reaction medium increases the rate of cyclization to the reactive intermediate.

Additional mechanistic studies of phosphate coupling reactions using haloethyl phosphoramidates were carried out



**Figure 1.** <sup>31</sup>P NMR kinetics carried out for phosphoramidate anion **7**: 1 equiv of TBAC, pyridine, rt (ref = triphenylphosphine oxide).

with *N*-methyl-*N*-(2-bromoethyl) thymidyl phosphoramidate **12** (Scheme 3). Interestingly, compound **12** reacted under

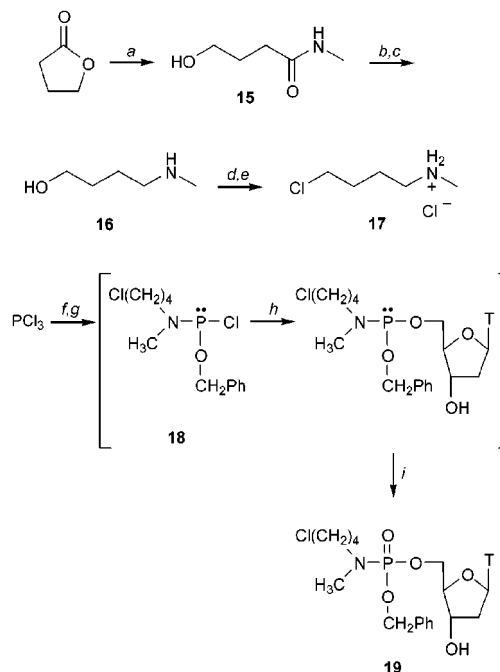


<sup>a</sup> Reagents and conditions: (a)  $\beta$ -D-glucose 1-phosphate, TBAC, pyridine, rt.

these conditions to form two major products: the desired diphosphate TDP-Glc and five-membered ring product **14** (Scheme 3). Nucleophilic attack by the phosphate moiety at the carbon of the aziridinium ion was not observed. Kinetic analysis of the reaction leading to the formation of cyclized product **14** suggests that this product arises from intramolecular O-alkylation following formation of the aziridinium ion (**13**).<sup>5</sup> Formation of product **14** represents an undesirable pathway and necessitates a larger excess of phosphoramidate starting material to ensure complete conversion of the sugar phosphate to product; thus, the halobutyl phosphoramidate was used for the preparation of model sugar nucleoside diphosphate TDP-Glc and ultimately TDP-Rha.

The synthesis of *N*-methyl-*N*-(2-chlorobutyl) thymidyl phosphoramidate **19** is shown in Scheme 4. *N*-methyl-*N*-(2-chlorobutyl)amine hydrochloride used for the preparation of **19** has previously been synthesized according to Kuznetsov et al.,<sup>6</sup> however, purification of the intermediate amino alcohol is tedious and yields are low. An alternative synthesis of halobutylamine hydrochloride **17** is reported here (Scheme 4) and employs a modified procedure of Beaucage et al.<sup>7</sup> Briefly, reaction of  $\gamma$ -butyrolactone with excess condensed methylamine proceeds quantitatively to amide **15** in <1 h. Lithium aluminum hydride reduction of amide **15** followed

**Scheme 4<sup>a</sup>**



<sup>a</sup> Reagents and conditions: (a) MeNH<sub>2</sub>, rt, 1.5 h; (b) lithium aluminum hydride, THF, rt, 2 h min; (c) Na<sub>2</sub>SO<sub>4</sub>·10H<sub>2</sub>O; (d) HCl<sub>(g)</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt; (e) SOCl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C to rt, 4 h; (f) benzyl alcohol, <sup>i</sup>Pr<sub>2</sub>NEt, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C, 15 min; (g) **17**, <sup>i</sup>Pr<sub>2</sub>NEt, CH<sub>2</sub>Cl<sub>2</sub>, -78 to -60 °C, 20 min; (h) thymidine, pyridine, -45 °C (titration); (i) *t*-BuOOH, -45 ° to 0 °C, 30 min.

by reaction of the resulting amino alcohol **16** with thionyl chloride<sup>3</sup> provides the desired halobutylamine hydrochloride **17** in 65% overall yield.

Phosphoramidate **19** was synthesized according to the procedure reported for the preparation of structurally similar haloethyl phosphoramidates (Scheme 4).<sup>8</sup> Briefly, this method involves the in situ generation of reactive phosphorus III intermediate **18**. Phosphorylation of the nucleoside by titration with the phosphorus III intermediate followed by oxidation with *tert*-butyl hydroperoxide proceeds in 70–80% overall yield.

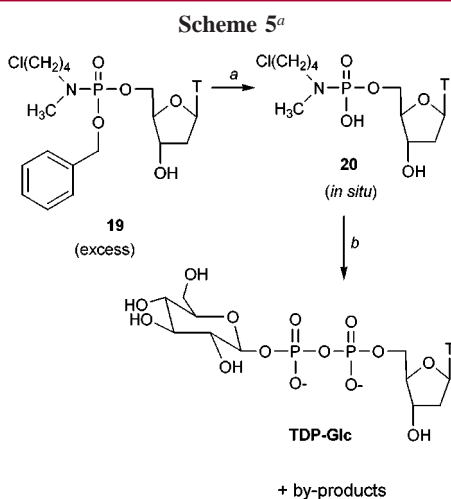
The preparation of TDP-Glc was subsequently achieved on a > 20  $\mu$ mol scale using excess *N*-methyl-*N*-(2-chlorobutyl) thymidyl phosphoramidate **19** (Scheme 5). Phosphoramidate **19** was activated by hydrogenolysis in THF over 1.5 h.  $\beta$ -D-Glucose 1-phosphate and TBAC were then dissolved in dry pyridine and added to the hydrogenolysis reaction mixture. THF was removed under reduced pressure, and the mixture was stirred at room temperature for 1 h. Typically, >90% conversion of  $\beta$ -D-glucose 1-phosphate to TDP-Glc was observed by <sup>31</sup>P NMR. Self-condensation product and small amounts of hydrolysis products in addition to unreacted  $\beta$ -D-glucose 1-phosphate were also observed by <sup>31</sup>P NMR. Conversion of the crude product mixture to the ammonium salts was accomplished on DOWEX 50W-

(5) Manuscript in preparation.

(6) Kuznetsov, S. G.; Ioffe, D. V. *J. Gen. Chem. USSR (Eng. Transl.)* **1961**, *31*, 2133.

(7) Wilk, A.; Grajkowski, A.; Phillips, L. R.; Beaucage, S. L. *J. Org. Chem.* **1999**, *64*, 7515–7522.

(8) Meyers, C. L. F.; Borch, R. F. *J. Med. Chem.* **2000**, *43*, 4313.



<sup>a</sup> Reagents and conditions: (a) H<sub>2</sub>, Pd/C, THF, rt, 1.5 h; (b) β-D-glucose 1-phosphate, pyridine, TBAC, rt, 1 h.

X8. Facile purification was achieved by C18 chromatography (50 mM NH<sub>4</sub>OAc, pH5.5) following ion exchange to give TDP-Glc in 77% yield (91% yield based on recovered glucose 1-phosphate).

Finally, this methodology has been extended to the preparation of thymidine 5'-diphospho-β-L-rhamnose (TDP-Rha), a nucleotide sugar utilized by bacterial glycosyltransferases.<sup>9</sup> An investigation of the chemical synthesis of TDP-Rha using standard methods was carried out by Thorson et al.,<sup>1h</sup> and an alternative synthetic route was developed employing a reactive TMP-imidazolidine phosphorylating agent. A comparison of the strategy reported here to the procedures described by Thorson reveals that coupling of rhamnose 1-phosphate to halobutyl nucleoside phosphor-

(9) Liu, H.-w.; Thorson, J. S. *Annu. Rev. Microbiol.* **1994**, *48*, 223.

amidate **20** (Scheme 5) proceeds more rapidly (<1 h) than TMP-morpholidate or TMP-imidazolidine phosphate coupling reactions, and conversion to the desired product is more efficient (97% yield based on recovered rhamnose 1-phosphate). Furthermore, it is important to note that the method of purification described here allows for facile recovery of unreacted sugar phosphate.

In summary, advances made in our laboratory toward the design of nucleotide prodrugs have led to the development of a novel strategy for the phosphorylation of phosphate moieties under nonaqueous conditions. Both haloethyl and halobutyl phosphoramidates were investigated as potential phosphorylating reagents; the halobutyl nucleoside phosphoramidate anion undergoes a highly efficient phosphate coupling reaction via a reactive pyrrolidinium ion intermediate. Preliminary results suggest that this strategy can be extended to the preparation of nucleoside triphosphates, a class of compounds for which there is no broadly applicable synthetic method.<sup>10</sup> Extension of this method to the synthesis of nucleoside triphosphates is currently under investigation.

**Acknowledgment.** We gratefully acknowledge Dr. Jon Thorson (University of Wisconsin, Madison) for stimulating our interest in this area and for providing us with β-L-rhamnose 1-phosphate. We thank Michelle Martin for assistance in obtaining NMR spectra. Financial support from the National Cancer Institute (CA34619) is also gratefully acknowledged.

**Supporting Information Available:** Experimental procedures and NMR spectra for halobutylamine hydrochloride **17**, nucleoside phosphoramidate **19**, TDP-Glc, and TDP-Rha. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(10) Burgess, K.; Cook, D. *Chem. Rev.* **2000**, *100*, 2047.