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Convenient Synthesis of Nucleoside Borane Diphosphate Analogues: Deoxy- and Ribonucleoside 5′-P^{α}-Boranodiphosphates

Ping Li and Barbara Ramsay Shaw*

Department of Chemistry, Box 90346, Duke University, Durham, North Carolina 27708-0346

brshaw@chem.duke.edu

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The nucleoside boranophosphates, having one of the nonbridging phosphate oxygens substituted with a borane (BH_3) group, have shown potential therapeutical applications as aptamers, antisense agents, and antiviral prodrugs. An oxathiaphospholane approach, which does not require exocyclic amine protection of the nucleobase, has been successfully developed to efficiently synthesize 5′-P^{α}-boranodiphosphates of 2′-deoxythymidine, adenosine, guanosine, and uridine. The approach involves a key intermediate, the borane complex of nucleoside 5′-*O*-1,3,2-oxathiaphospholane **16**, that undergoes a ring-opening reaction catalyzed by 1,4-diazabicyclo[5.4.0]-undec-7-ene to form the protected nucleoside 5′-P^{α}-boranodiphosphate **18**. Treatment of **18** with ammonium hydroxide yielded diastereoisomeric mixtures of nucleoside 5′-P^{α}-boranodiphosphates **5**. This oxathiaphospholane approach ensures the availability of nucleoside 5′-P^{α}-boranodiphosphate analogues needed for antiviral drug research.

Introduction

Nucleotide analogues with modifications in the base, sugar, or phosphate residues[1,3a,b] can be used as probes for substrate properties, kinetic pathways, and transition states of enzymes involved in nucleic acid metabolism.[2] For example, nucleotide analogues of diphosphates and triphosphates have been successfully and widely used as biochemical tools to unravel functions and mechanisms of nucleoside kinases and polymerases.[3] In particular, phosphorothioate analogues **3** (Figure 1), in which a sulfur atom is exchanged for a nonbridging oxygen at a phosphate group, have been well-studied and employed to determine the stereochemical course of a large number of enzymatic nucleotidyl and phosphoryl transfer reactions.[4]

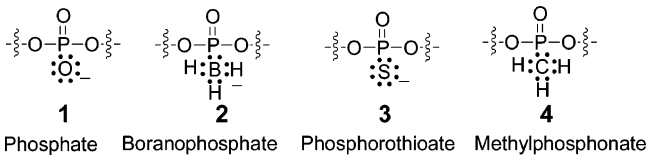

FIGURE 1. Phosphate and its modified analogues.

The substitution of one of the nonbridging oxygens at a phosphate group by a borane (BH_3) group results in a new class of phosphate-modified nucleotides known as boranophosphates **2**.[5] Because the BH_3 group is isoelectronic with oxygen, isolobal with sulfur, and isosteric with the methyl group, the boranophosphate **2** can be considered as a "hybrid" of three types of modified phosphates,

(1) (a) Nahum, V.; Zundorf, G.; Levesque, S. A.; Beaudoin, A. R.; Reiser, G.; Fischer, B. *J. Med. Chem.* **2002**, *45*, 5384. (b) Dufner, G.; Schworer, R.; Muller, B.; Schmidt, R. R. *Eur. J. Org. Chem.* **2000**, 1467. (c) Janeba, Z.; Holy, A. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1103. (d) Izuta, S.; Yoshida, S. *Nucleosides Nucleotides* **1996**, *15*, 683. (e) Cramer, J.; Strerath, M.; Marx, A.; Restle, T. *J. Biol. Chem.* **2002**, *277*, 43593. (f) Schell, P.; Laux, W. H. G.; Engels, J. W. *Nucleosides Nucleotides* **1999**, *18*, 1169. (g) Cormier, J. F.; Pannunzio, T. *Tetrahedron Lett.* **1991**, *32*, 7161. (h) Bollmark, M.; Zain, R.; Stawinski, J. *Tetrahedron Lett.* **1996**, *37*, 3537. (i) Dabkowski, W.; Tworowska, I. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2462. (j) Dabkowski, W.; Michalska, M.; Tworowska, I. *Chem. Commun.* **1998**, 427. (k) Oka, N.; Wada, T.; Saigo, K. *J. Am. Chem. Soc.* **2003**, *125*, 8307.

(2) (a) Detmer, K.; Summerer, D.; Marx, A. *Eur. J. Org. Chem.* **2003**, 1837. (b) Washington, M. T.; Wolfle, W. T.; Spratt, T. E.; Prakash, L.; Prakash, S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5113. (c) Park, S.; Ajtai, K.; Burghardt, T. P. *Biochemistry* **1997**, *36*, 3368. (d) Urbatsch, I. L.; Sankaran, B.; Bhagat, S.; Senior, A. E. *J. Biol. Chem.* **1995**, *270*, 26956. (e) Bertrand, R.; Derancourt, J.; Kassab, R. *Biochemistry* **2000**, *39*, 14626.

(3) (a) Verma, S.; Eckstein, F. *Annu. Rev. Biochem.* **1998**, *67*, 99. (b) Earnshaw, D. J.; Gait, M. J. *Biopolymers* **1998**, *48*, 39. (c) Eckstein, F. *Annu. Rev. Biochem.* **1985**, *54*, 367. (d) Higuchi, H.; Endo, T.; Kaji, A. *Biochemistry* **1990**, 29, 8747. (e) Noble, S. A.; Fisher, E. F.; Caruthers, M. H. *Nucleic Acids Res.* **1984**, 12, 3387.

(4) (a) Eckstein, F. *Biochimie* **2002**, *84*, 841. (b) Eckstein, F. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 423. (c) Gerlt, J. A.; Coderre, J. A.; Mehdi, S. *Adv. Enzymol.* **1983**, *55*, 291. (d) Eckstein, F.; Romaniuk, P. J.; Connolly, B. A. *Methods Enzymol.* **1982**, *87*, 197. (e) Frey, P. A.; Richard, J. P.; Ho, H. T.; Brody, R. S.; Sammons, R. D.; Sheu, K. F. *Methods Enzymol.* **1982**, *87*, 213. (f) Webb, M. R. *Methods Enzymol.* **1982**, *87*, 301. (g) Buchwald, S. L.; Hansen, D. E.; Hassett, A.; Knowles, J. R. *Methods Enzymol.* **1982**, *87*, 279. (h) Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877. (i) Eckstein, F. *Acc. Chem. Res.* **1979**, *12*, 204.

(5) (a) Sood, A.; Shaw, B. R.; Spielvogel, B. F. *J. Am. Chem. Soc.* **1990**, *112*, 9000. (b) Tomasz, J.; Shaw, B. R.; Porter, K.; Spielvogel, B. F.; Sood, A. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1373. (c) Shaw, B. R.; Madison, J.; Sood, A.; Spielvogel, B. F. *Methods Mol. Biol.* **1993**, *20*, 225. (d) Porter, K. W.; Briley, J. D.; Shaw, B. R. *Nucleic Acids Res.* **1997**, *25*, 1611. (e) Krzyzanowska, B. K.; He, K. Z.; Hasan, A.; Shaw, B. R. *Tetrahedron* **1998**, *54*, 5119. (f) He, K. Z.; Hasan, A.; Krzyzanowska, B.; Shaw, B. R. *J. Org. Chem.* **1998**, *63*, 5769. (g) Shaw, B. R.; Sergueev, D.; He, K. Z.; Porter, K.; Summers, J. S.; Sergueeva, Z.; Rait, V. *Methods Enzymol.* **2000**, *313*, 226. (h) Shaw, B. R.; Wan, J.; Wang, X.; Dobrikov, M.; He, K.; Porter, K.; Lin, J. L.; Rait, V.; Sergueev, D.; Sergueeva, Z. In *Chemistry of Nucleic Acid Components*; Tocik, Z., Hocek, M., Eds; Academy of Sciences of the Czech Republic: Prague, 2002; Vol. 5, p 169. (i) Summers, J. S.; Shaw, B. R. *Curr. Med. Chem.* **2001**, *8*, 1147. (j) Shaw, B. R.; Dobrikov, M.; Wang, X.; Wan, J.; He, K. Z.; Lin, J. L.; Li, P.; Rait, V.; Sergueeva, Z.; Sergueev, D. *Ann. N.Y. Acad. Sci.* **2003**, *1002*, 12.

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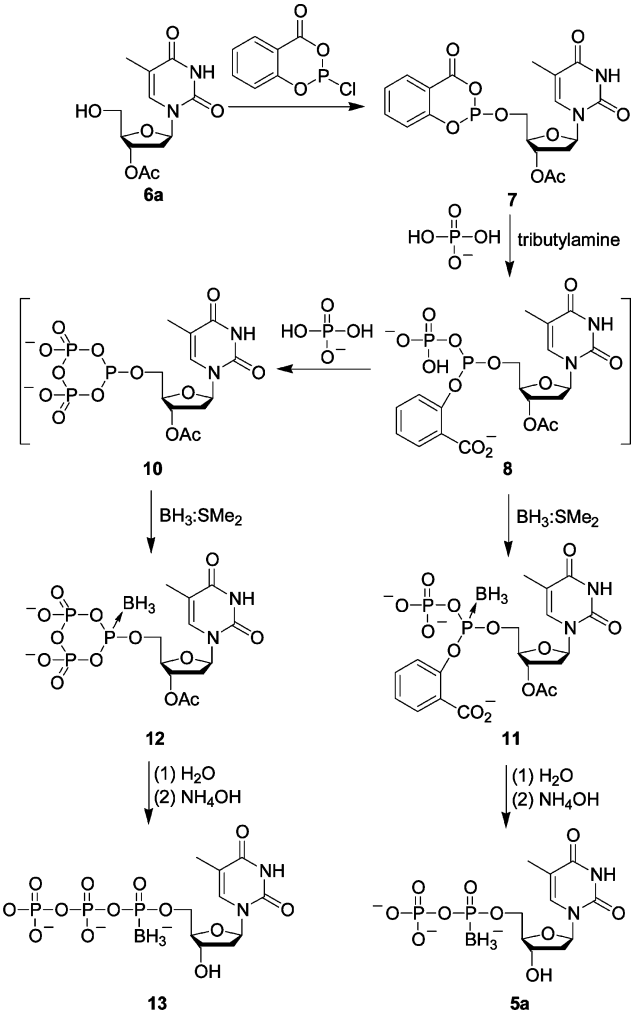
i.e., the normal phosphate **1**, the phosphorothioate **3**, and the methylphosphonate **4** shown in Figure 1. Thus, boranophosphates would be expected to share a number of chemical and biochemical properties with phosphorothioate and methylphosphonate analogues, such as good aqueous solubility, increased lipophilicity, and nuclease resistance. Additionally, boranophosphates may be unique and useful in transporting boron to tumor tissue selectively for a type of radiation therapy known as BNCT (boron neutron capture therapy).[6]

Boranophosphates of nucleosides and oligonucleosides have recently attracted attention due to their possible therapeutic applications as antiviral prodrugs,[7] aptamers,[8] and antisense agents.[9] For example, the most widely used antiviral drugs are nucleosides that lack a 3′-OH group and exert their biological functions through a series of phosphorylations to their corresponding mono- (dNMP), di- (dNDP), and triphosphate (dNTP) analogues by intracellular kinases.[10a−c] The biologically active nucleoside triphosphate (dNTP) analogues are then incorporated into the viral DNA and subsequently cause the chain termination of the growing viral DNA. It has been found in this laboratory that introduction of the boranophosphate group into dNTPs (as dNTPαB) increases their selectivity to viral reverse transcriptases (RT) relative to bacterial DNA polymerases.[7a,b] The presence of the α-boranophosphate group in AZTDP,[7c] D4TDP,[7d] and ddADP[7e] improves phosphorylation of these diphosphates by nucleoside diphosphate kinase as well as the incorporation of their corresponding nucleoside triphosphates by wild type[7c] and mutant multidrug-resistant HIV-1 RT.[7d,e] Moreover, after these nucleoside boranophosphate analogues were incorporated into DNA, repair of the blocked DNA chains by pyrophosphorolysis was reduced significantly by mutant HIV-1 RT enzymes from drug-resistant viruses.[7b] These results suggest that boranophosphates may be potentially useful in a prodrug delivery[10d−e] of α-*P*-borane modified nucleotide analogues. Therefore, to study the substrate properties of boranophosphate analogues, the development of new methods for synthesizing these new compounds is warranted. Because of the challenges in conveniently obtaining phosphate-modified nucleotides,[11] including α-*P*-boranodiphosphate analogues in large quantity, here we focused on the synthesis of 5′-P^{α}-boranodiphosphates **5** of 2′-deoxythymidine (dTDPαB), adenosine (ADPαB), guanosine (GDPαB), and uridine (UDPαB).

SCHEME 1. Synthesis of Deoxythymidine 5′-P^{α}-Boranodiphosphate 5a and 5′-P^{α}-Boranotriphosphate 13 with Salicyl Chlorophosphite (2-Chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one)

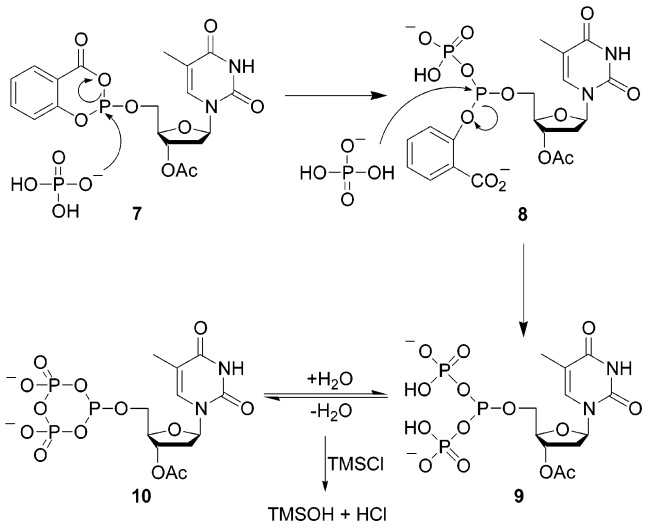


Results and Discussion

Studies on the Synthesis of 2′-Deoxythymidine 5′-P^{α}-Boranodiphosphate (dTDPαB) with Salicyl Chlorophosphite. Our group recently reported the first synthesis of a nucleoside 5′-P^{α}-boranodiphosphate (NDPαB) using a phosphoramidite approach.[12] Due to the lengthy experimental procedure and relatively low yield, we decided here to explore alternative synthetic methods and increase the overall yield. Our first attempt to prepare dTDPαB **5a**, based on an approach for 2′-deoxythymidine thiodiphosphate reported by Ludwig and Eckstein,[13] is outlined in Scheme 1. As shown earlier, 3′-*O*-acetylthymidine was phosphitylated by 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (salicyl chlorophosphite) to form two diastereomers of the intermediate 2′-deoxythymidine 5′-(4*H*-,3,2-benzodioxaphosphorin-4-one) **7**. They were identified by the appearance of two signals, at δ 128.12 and 126.18 ppm, observed in the ^{31}P NMR spectra of the reaction mixture. Due to the bifunctionality

(6) Hawthorne, M. F. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 950.
(7) (a) Dobrikov, M. I.; Sergueeva, Z. A.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1651. (b) Dobrikov, M. I.; Grady, K. M.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 275. (c) Meyer, P.; Schneider, B.; Sarfati, S.; Deville-Bonne, D.; Guerreiro, C.; Boretto, J.; Janin, J.; Veron, M.; Canard, B. *EMBO J.* **2000**, *19*, 3520. (d) Selmi, B.; Boretto, J.; Sarfati, S. R.; Guerreiro, C.; Canard, B. *J. Biol. Chem.* **2001**, *276*, 48466. (e) Deval, J.; Selmi, B.; Boretto, J.; Egloff, M. P.; Guerreiro, C.; Sarfati, S.; Canard, B. *J. Biol. Chem.* **2002**, *277*, 42097. (f) Li, P.; Dobrikov, M.; Liu, H. Y.; Shaw, B. R. *Org. Lett.* **2003**, *5*, 2401.
(8) Lato, S. M.; Ozerova, N. D. S.; He, K. Z.; Sergueeva, Z.; Shaw, B. R.; Burke, D. H. *Nucleic Acids Res.* **2002**, *30*, 1401.
(9) (a) Rait, V. K.; Shaw, B. R. *Antisense Nucleic Acid Drug Dev.* **1999**, *9*, 53. (b) Wang, X.; Dobrikov, M.; Sergueev, D.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1151.
(10) (a) De Clercq, E. *Nature Rev. Drug Dis.* **2002**, *1*, 13. (b) Meier, C. *Synlett* **1998**, 233. (c) Wagner, C. R.; Iyer, V. V.; McIntee, E. J. *Med. Res. Rev.* **2000**, *20*, 417. (d) Li, P.; Shaw, B. R. *Org. Lett.* **2002**, *4*, 2009. (e) Li, P.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 699.
(11) Burgess, K.; Cook, D. *Chem. Rev.* **2000**, *100*, 2047.
(12) Lin, J. L.; He, K. Z.; Shaw, B. R. *Helv. Chim. Acta* **2000**, *83*, 1392.

SCHEME 2. Proposed Mechanism for the Steps Involving Substitution with Tributylammonium Orthophosphate with Use of the Salicyl Chlorophosphite Approach



of the thymidine phosphite **7**, it could undergo two subsequent nucleophilic substitution reactions as depicted in Scheme 2. With the assumption that the carboxyl group is a better leaving group than the phenolic group, then substitution by the first incoming nucleophile, tributylammonium orthophosphate,[14] would yield 2′-deoxythymidine *P*-phosphoryl-*P*-(2-carbonylphenolic) phosphite **8**. Subsequently, the phenolic group on intermediate **8** would be replaced with a second incoming nucleophilic tributylammonium orthophosphate to obtain the 2′-deoxythymidine *P*-diphosphoryl phosphite **9** (Scheme 2), which could undergo a reversible dehydration reaction (loss of H_2O) to form P^2,P^3-dioxo-P^1-thymidinylcyclotriphosphate **10**. The intermediates **8** and **10**, when subjected to boronation in the presence of excess borane−dimethyl sulfide, gave the corresponding boronated complexes **11** and **12** as shown in Scheme 1. Treatment of the reaction mixtures, first with H_2O and then concentrated ammonium hydroxide, yielded the final compounds dTDPαB **5a** and dTTPαB **13**. The overall yield was only 12% and 17% for the diastereomers of dTDPαB **5a** and dTTPαB **13**, respectively, after ion-exchange isolation.

Although steps outlined in Scheme 1 were monitored by ^{31}P NMR, the complexity of the reaction mixtures together with the phosphorus peak quadrupole broadening by the ^{10}B and ^{11}B nuclei made it impossible to assign the ^{31}P NMR peaks to the corresponding intermediates **8** through **12**. However, the isolation of the final products, dTDPαB **5a** and dTTPαB **13**, was consistent with our proposed structures for those intermediates **8** through **10** and the mechanism, as shown in Scheme 2. To further support the proposed mechanism involving dehydration, 2 molar equiv of chlorotrimethylsilane (TMSCl) were introduced into the reaction mixture immediately after the addition of 2 equiv of tributylammonium orthophosphate. In a comparison of the final products after ion-exchange separation, the yield of dTTPαB **13** increased from 17% (without TMSCl) to 23% (with TMSCl) for diastereoisomeric mixtures, whereas the yield for dTDPαB **5a** decreased from 12% (without TMSCl) to trace amounts (with TMSCl). These results suggested that the water produced during the dehydration step (Scheme 2) was consumed by the addition of trimethylsilane chloride, driving the reaction toward the formation of P^2,P^3-dioxo-P^1-thymidinylcyclotriphosphate **10**, which was finally transformed into dTTPαB **13**.

Studies on the Synthesis of Nucleoside 5′-*P*$^{\alpha}$-Boranodiphosphate (NDPαB) via an Oxathiaphospholane Approach. Oxathiaphospholanes were first introduced by Stec[15] to be a method of choice for stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s and were recently employed by Baraniak[16] to obtain nucleoside monophosphate prodrugs in reasonable to good yields. The oxathiaphospholane approach involves a tricoordinate phosphorus intermediate, and therefore, as demonstrated earlier,[17] is suitable for the introduction of the borane moiety. The detailed synthesis of a nucleoside α-*P*-borane modified diphosphate, NDPαB **5**, is depicted in Scheme 3.

Reaction of 2-chloro-1,3,2-oxathiaphospholane **14** with deoxy- and/or ribonucleoside **6**, carried out in anhydrous acetonitrile, gave nucleoside 5′-*O*-1,3,2-oxathiaphospholane **15** in the presence of 2 molar equiv of diisopropylethylamine (DIPEA). This phosphorylating reaction was completed in 15 min as evidenced by ^{31}P NMR spectra, where the singlet at δ 207 ppm for oxathiaphospholane **14** was transformed into two singlets around δ 172 ppm corresponding to the diastereomers of the P^{III} intermediate **15** (ratio 1:1). When 2′-deoxythymidine or uridine was used, the phosphorylation was very clean. However, when adenosine or guanosine without nucleobase protection was used, ^{31}P NMR spectra of **15b**,**c** revealed two extra singlets at δ 165 ppm with a total integration of 5%. It was assumed that these peaks at δ 165 ppm corresponded to the two diastereomers of *N*-phosphorylated exocyclic products of the nucleobase. Since the side reaction was almost negligible, we did not seek to protect the nucleobase for the phosphitylation step. Attempts to purify compound **15** by silica gel column chromatography were unsuccessful, as the compound appeared to decompose quickly on the column. Thus, without purification, the reaction mixtures containing **15** proceeded to the next boronation step.

Exchange of a borane group between the nucleoside 5′-*O*-1,3,2-oxathiaphospholane **15** and a boronation reagent produced the oxathiaphospholane−borane complex **16**. According to valence shell electron pair repulsion (VSEPR) theory and molecular orbital (MO) theory, the boron atom in BH_3 carries an empty 2p orbital and,

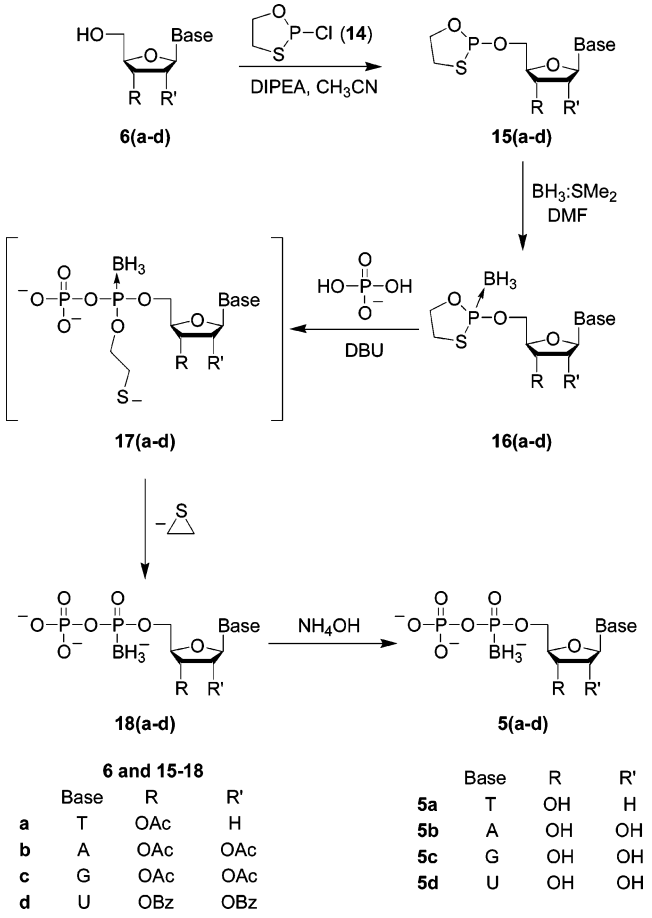
(13) Ludwig, J.; Eckstein, F. *J. Org. Chem.* **1989**, *54*, 631.

(14) Tributylammonium orthophosphate was generated as follows. Tributylamine (1 equiv) was added dropwise into the solution of anhydrous orthophosphoric acid (1 equiv) in methylene chloride over 30 min. The mixture was left stirring for another 60 min and the solvent was removed under reduced pressure. The residue was re-evaporated with anhydrous pyridine and DMF. The final compound was dissolved in anhydrous DMF to a concentration of 1 M, and stored over 4 Å molecular sieves.

(15) (a) Stec, W. J.; Grajkowski, A.; Kobylanska, A.; Karwowski, B.; Koziolkiewicz, M.; Misiura, K.; Okruszek, A.; Wilk, A.; Guga, P.; Boczkowska, M. *J. Am. Chem. Soc.* **1995**, *117*, 12019. (b) Guga, P.; Okruszek, A.; Stec, W. J. *New Aspects Phosphorus Chem. I* **2002**, *220*, 169.

(16) (a) Baraniak, J.; Kaczmarek, R.; Stec, W. J. *Tetrahedron Lett.* **2000**, *41*, 9139. (b) Baraniak, J.; Kaczmarek, R.; Wasilewska, E.; Stec, W. J. *Phosphorus, Sulfur Silicon Relat. Elem.* **2002**, *177*, 1667. (c) Baraniak, J.; Kaczmarek, R.; Korczynski, D.; Wasilewska, E. *J. Org. Chem.* **2002**, *67*, 7267. (d) Baraniak, J.; Kaczmarek, R.; Wasilewska, E. *Tetrahedron Lett.* **2004**, *45*, 671.

SCHEME 3. Synthesis of Nucleoside 5′-*P*$^{\alpha}$-boranodiphosphate with 2-Chloro-1,3,2-oxathiaphospholane



6 and 15-18

	Base	R	R'
a	T	OAc	H
b	A	OAc	OAc
c	G	OAc	OAc
d	U	OBz	OBz

	Base	R	R'
5a	T	OH	H
5b	A	OH	OH
5c	G	OH	OH
5d	U	OH	OH

therefore, is susceptible to attack by nucleophiles.[18] The tervalent phosphorus atom could act as a Lewis base donor of electrons to electron-deficient BH_3. The reactivities of a series of boronating reagents to transfer a borane group to compound **15**, including borane−dimethyl sulfide and borane−tetrahydrofurane, were examined, as well as three types of borane−amine complexes: aliphatic−borane (borane−*N*,*N*-diisopropylethylamine), aromatic−borane (borane−aniline), and heterocyclic−borane (borane−pyridine and borane−2-chloropyridine). The conversion of nucleoside 5′-*O*-1,3,2-oxathiaphospholane **15** to its oxathiaphospholane−borane complex **16** was monitored by ^{31}P NMR spectra, which showed a disappearance of two singlets at δ 172 ppm for compound **15** and formation of a broad signal centered at δ 160 ppm for oxathiaphospholane−borane complex **16**. The results are summarized in Table 1, where the most active boronation reagent is seen to be borane−dimethyl sulfide. Thus, after removing solvent and excess DIPEA under reduced pressure by oil pump, the crude product **15** was redissolved in anhydrous DMF and reacted with 5 to 8 molar equiv of borane−dimethyl sulfide at room temperature for 30 min. This exchange reaction was very clean, producing only the oxathiaphospholane−borane complex **16** according to ^{31}P NMR.

Oxathiaphospholane−borane complex **16** was unable to be isolated by silica gel column chromatography due to its instability in the presence of moisture. Hence, the reaction mixture containing complex **16** was immediately treated with simultaneous addition of 1 molar equiv of tributylammonium orthophosphate[19] in anhydrous DMF and 5 molar equiv of 1,4-diazabicyclo[5.4.0]undec-7-ene (DBU). The reaction underwent the ring-opening condensation to form the boranophosphate intermediate **17** followed by spontaneous elimination of ethylene episulfide to generate protected nucleoside 5′-*P*$^{\alpha}$-boranodiphosphate **18**. A white precipitate was formed during this step, indicating the formation of boranodiphosphate. A series of experiments on 3′-*O*-acetyl-2′-deoxythymidine **6a** with increasing equivalents of DBU were carried out and monitored by TLC.[20] It was found that the rate of DBU-assisted ring-opening condensation varied when different quantities were used. The completion time for this ring-opening condensation took more than 12 h when 2 equiv of DBU were added, but decreased to 40 min with 5 equiv of DBU and to 30 min with 10 equiv of DBU. Hence, 5 equiv of DBU was chosen to be the optimal amount. Furthermore, the yield of this step was found to decrease dramatically with the presence of DIPEA remaining from the previous steps in the reaction mixture, as indicated by TLC and ^{31}P NMR. Although the exact reason is unknown, it is likely that the DIPEA was interfering with the DBU-catalyzed ring-opening condensation.[15a]

The reaction mixture was subsequently purified by ion-exchange column chromatography. The existence of protected nucleoside 5′-*P*$^{\alpha}$-boranodiphosphate **18** was identified by the appearance of peaks at δ −6 ppm for *P*(β) (doublet, J = 25−35 Hz) and δ 81 ppm for *P*(α) (broad multiplet) in the ^{31}P NMR spectrum. The chemical shift for *P*(β) in boranodiphosphates is similar to that in unmodified diphosphates as well as *P*(β) in analogues modified only at the α-position (e.g., α-thiodiphosphates). Modification of a nucleoside diphosphate at the α-position, however, results in a noticeable change of the *P*(α) chemical shift: from δ ca. −10 ppm for a normal diphosphate to δ ca. 20 ppm for α-methylphosphonyl-β-monophosphate,[21] to δ ca. 40 ppm for α-thiodiphosphate[13] and, as we have found in our studies, to δ ca. 81 ppm (broad multiplet) for α-boranodiphosphate shown in Figure 2.

The removal of the ribose protecting groups on 5′-*P*$^{\alpha}$-boranodiphosphate **18** was carried out by treating it with ammonium hydroxide in water (1/1, v/v) for 6 h. The final product, nucleoside 5′-*P*$^{\alpha}$-boranodiphosphate **5**, was isolated by ion-exchange column chromatography on QA-52 [HCO_3^-] cellulose. The overall yields (from **6** to **5**) were 30−43% and the structures of boranodiphosphates **5a**−**d** were confirmed by ^{1}H NMR, ^{31}P NMR, FAB-MS, and HRMS.

(17) Okruszek, A.; Sierzchala, A.; Zmudzka, K.; Stec, W. J. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1843.

(18) Housecroft, C. E. *Boranes and Metalloboranes Structure, Bonding and Reactivity*; John Wiley & Sons: New York, 1990; pp 18−30.

(19) We initially used commercially available tetrabutylammonium dihydrogenphosphate as the phosphate nucleophile. However, due to its low solubility in DMF, we switched to freshly prepared tributylammonium orthophosphate.

(20) TLC developing system: CH_2Cl_2/EtOAc = 1/4 (v/v). Reaction was monitored by the disappearance of oxathiaphospholane−borane complex **16a**.

(21) Higuchi, H.; Endo, T.; Kaji, A. *Biochemistry* **1990**, *29*, 8747−8753.

TABLE 1. Reactivity of Boronating Reagents[a]

			borane−amine complex			
borane complex[b]	BH_3:Me_2S	BH_3:THF	BH_3:DIPEA	BH_3:An	BH_3:Py	BH_3:Py-2-Cl
conversion of **15**→**16** [c,d]	100%	none	30%	28%	none	85%

[a] 3′-*O*−Acetyl-2′-deoxythymidine was used as model compound. [b] Five molar equivalents of borane complex were used. [c] ^{31}P NMR samples were prepared at 30 min after addition of the borane complex. [d] Conversion was calculated as the integration percentage of compound **16** relative to the total integration of compounds **15** and **16** in the ^{31}P NMR spectra.

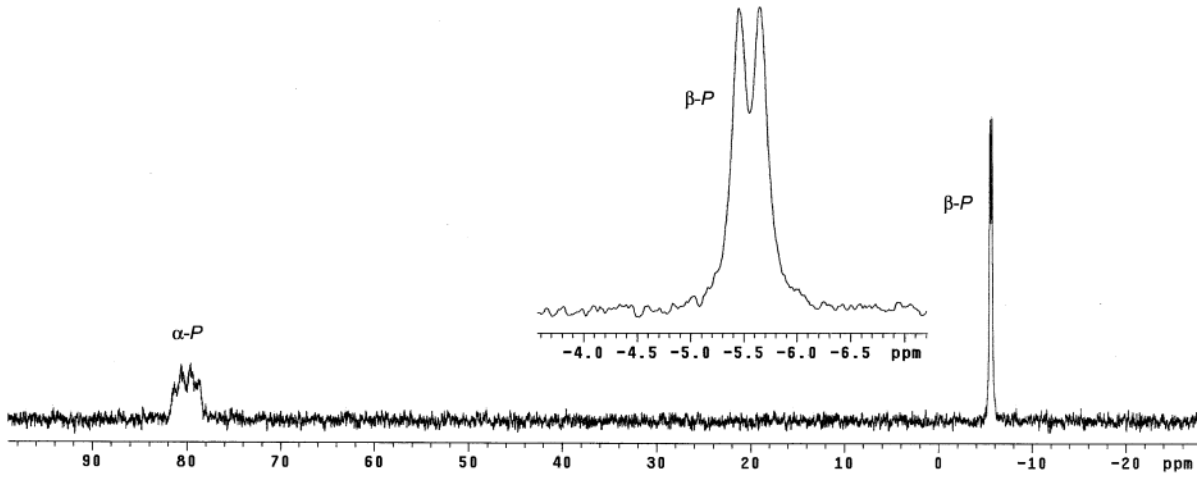


FIGURE 2. ^{31}P NMR spectrum of diastereomers of adenosine 5′-*P*$^{\alpha}$-boranodiphosphate (ADPαB, **5b**).

TABLE 2. RP-HPLC Profiles for NDPαB 5[a]

		retention time (min) [area %]	
compd	buffer[b]	R_P isomer	S_P isomer
dTDPαB **5a**	92% TEAB/8% MeOH	18.50 [45.5]	25.48 [54.5]
ADPαB **5b**	92% TEAB/8% MeOH	15.22 [56.1]	27.85 [43.9]
GDPαB **5c**	95% TEAB/5% MeOH	7.62 [51.1]	12.47 [48.9]
UDPαB **5d**	94% TEAB/6% MeOH	6.64 [43.0]	10.20 [57.0]

[a] Waters Delta-Pak C18 column: 15 μm, 100 Å, 25 mm × 100 mm, with Z-module. [b] 100 mM TEAB (triethylamonium bicarbonate, pH 8.0) and methanol

Studies on the HPLC Separation of Diastereomers of Nucleoside 5′-*P*$^{\alpha}$-Boranodiphosphates. The presence of a borane group replacing a nonbridging oxygen at the *P*(α) position in NDPαB **5** produces a pair of diastereomers, whose absolute configurations have been recently determined by cocrystalization with nucleoside diphosphate kinase.[7c] The two diastereomers were resolved by semipreparative RP-HPLC, using isocratic elution as described in Table 2. The first and second eluting diastereomers are the R_P and S_P isomers, respectively. The isomeric purity of each of the individual diastereomers was determined by RP-HPLC under the same conditions used for separation. The HPLC profiles for adenosine 5′-*P*$^{\alpha}$-boranodiphosphate **5b** before and after semipreparative HPLC separation are shown in Figure 3. The structure for each diastereomer was confirmed by ^{1}H NMR, ^{31}P NMR, FAB-MS, and HRMS.

Conclusions

We developed a new method to efficiently synthesize nucleoside 5′-*P*$^{\alpha}$-boranodiphosphate **5** via the boronation of the *P*(III) intermediate, nucleoside 5′-*O*-1,3,2-oxathiaphospholane **15**. This convenient method was applied to obtain a set of four compounds, including dTDPαB **5a**, ADPαB **5b**, GDPαB **5c**, and UDPαB **5d,** in good yields. Since this method does not require the exocyclic amine protection of the nucleobase, it eliminates the risk of possible nucleobase reduction during the boronation step.[22] Comparative studies on different boronating reagents and the optimal conditions for DBU-catalyzed ring-opening condensation were carried out. The two diastereomers of NDPαB **5** were successfully separated by RP-HPLC by using isocratic elution and their structures were confirmed by spectroscopic methods. This oxathiaphospholane approach ensures the availability of nucleoside 5′-*P*$^{\alpha}$-boranodiphosphate analogues, which are needed for antiviral drug research.

Like nucleoside phosphorothioates,[4] the two diastereomers of NDPαB are expected to have different substrate properties toward nucleosidyl transferases and hydrolases, and should be useful for investigating the roles of phosphate and metal ions in biological processes to elucidate the stereochemical and metal requirements of the enzymatic reactions involving nucleoside diphosphates. Moreover, when compared to the natural nucleotide, the improved substrate properties,[7] increased lipophilicity,[5] and increased nuclease resistance[5] imparted by the borane group, in conjunction with the potential utility as a carrier of ^{10}B in BNCT,[6] make the nucleoside boranodiphosphate a useful compound and tool in antiviral drug research.

Experimental Section

2-Chloro-1,3,2-oxathiaphospholane was synthesized according to the reported procedure.[15a] All solvents were freshly distilled under argon from calcium hydride. All boronating

(22) Sergueeva, Z. A.; Sergueev, D. S.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 275−282.

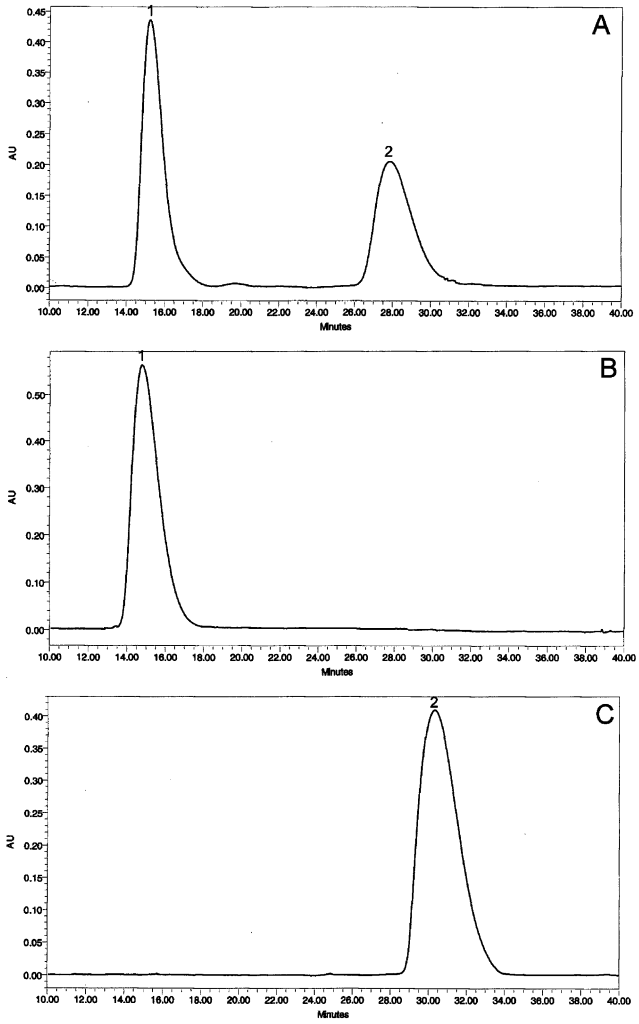


FIGURE 3. Isocratic separation of diastereomers of adenosine 5′-P^{α}-boranodiphosphate **5b** by HPLC (A) and analysis of purity of HPLC-isolated R_P isomer (B) and S_P isomer (C). The separation was carried out on the Waters Delta-Pak C18 column with 92% 100 mM TEAB (pH 8.0) and 8% methanol at a flow rate of 5.0 mL/min. The absorbance was monitored at λ 260 nm. The retention times for the two diastereomers were 15.22 and 27.85 min, respectively. Peak 1: R_P isomer of ADPαB. Peak 2: S_P isomer of ADPαB.

reagents except borane−dimethyl sulfide complex, diisopropylethylamine (DIPEA), and 1,4-diazabicyclo[5.4.0]undec-7-ene (DBU) were dried by 4 Å molecular sieves overnight. Nucleosides and tributylammonium orthophosphate were dried by P_2O_5 overnight under vacuum prior to use. Triethylammonium bicarbonate was prepared from triethylamine, H_2O, and CO_2. All the reactions were carried out under an argon atomosphere unless otherwise stated.

Synthesis of dTDPαB and dTTPαB with Salicyl Chlorophosphite. 3′-*O*-Acetyl-2′-deoxythymidine **6a** (0.5 mmol, 1 equiv) was dissolved in anhydrous DMF and anhydrous pyridine (1.5 mmol, 3 equiv). A freshly prepared 0.5 M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one in anhydrous DMF (0.55 mmol, 1.1 equiv.) was added. After 10 min, a 1 M solution of tributylammonium orthophosphate in anhydrous DMF (1.25 mmol, 2.5 equiv) and tributylamine (2.5 mmol, 5.0 equiv) were simultaneously injected into the reaction mixture. A white precipitate was formed but quickly disappeared and 30 min later a solution of 2 M borane−dimethyl sulfide in THF was added (5 mmol, 10 equiv). After the mixture was stirred for 60 min, the septum was removed and water (2 mL) was added. After 30 min, the reaction was evaporated to dryness and the residue was treated with concentrated ammonium hydroxide at room temperature for 5 h. The reaction mixture was evaporated to dryness again and the residue was partitioned between water (20 mL) and diethyl ether (20 mL). The water layer was subjected to ion-exchange chromatography with QA-52 cellulose ([HCO_3^-]), using a linear gradient of 5 mM and 200 mM ammonium bicarbonate buffer (pH 8.0, 800 mL each). The desired fractions of dTDPαB and dTTPαB were collected and dried by lyophilization, and excess salt was removed by repeated lyophilization with deionized water. The yields were 12% (27.1 mg) and 17% (46.59 mg) for dTDPαB and dTTPαB, respectively.

Synthesis of Deoxy- and Ribonucleoside 5′-P^{α}-Boranodiphosphates with 2-Chloro-1,3,2-oxathiaphospholane: General Procedure. Protected nucleoside (3′-*O*-acetyl-2′-deoxythymidine, 2′,3′-*O*-diacetyladenosine, 2′,3′-*O*-diacetylguanosine, or 2′,3′-*O*-dibenzoyluridine) (0.5 mmol, 1 equiv) and DIPEA (1.0 mmol, 2 equiv) were dissolved in acetonitrile followed by the addition of 2-chloro-1,3,2-oxathiaphospholane **14** (0.55 mmol, 1.1 equiv). After 15 min, solvent was removed under vacuum and the residue was redissolved in DMF. Borane−dimethyl sulfide complex (2.5 mmol, 5−8 equiv) was then added and stirred for 30 min. A freshly prepared 1 M solution of tributylammonium orthophosphate in DMF (0.5 mmol, 1 equiv) and DBU (2.5 mmol, 5 equiv) and were simultaneously injected into the reaction mixture by syringe, which was continually stirred for 2 h. Solvent was removed and the residue was partitioned between 20 mL of water and 10 mL of ethyl acetate. The aqueous layer was concentrated and purified by ion-exchange chromatography eluted with a linear gradient of 5 mM and 200 mM ammonium bicarbonate buffer (pH 8.0, 800 mL each) to afford the ammonium salt of protected nucleoside 5′-P^{α}-boranodiphosphate **18**. The desired fractions were collected, concentrated, and treated with concentrated ammonium hydroxide in water (10 mL, 1/1, v/v) for 6 h. After the removal of solvent, the residue was applied to the ion-exchange chromatography column and eluted with a linear gradient of 5 mM and 200 mM ammonium bicarbonate buffer (pH 8.0, 800 mL each) to afford the ammonium salt of deoxy- and ribonucleoside 5′-P^{α}-boranodiphosphate **5**. The excess salt was removed by repeated lyophilization with deionized water.

2′-Deoxythymidine 5′-P^{α}-Boranodiphosphate (dTDPαB) (5a). The diastereomeric mixture of compound **5a** was prepared in 43% yield (97 mg) following the general procedure by using 3′-*O*-acetyl-2′-deoxythymidine and 5 molar equiv of borane−dimethyl sulfide. ^{1}H NMR (D_2O) δ 7.61, 7.59 (2s, 2 isomers, 1H), 6.21, 6.19 (2t, 2 isomers, J = 6.0 Hz, 1H), 4.48 (m, 1H), 4.10−3.90 (m, 3H), 2.21 (m, 2H), 1.80 (s, 3H), (+)0.84 to (−)0.17 (br, 3H); ^{31}P NMR (D_2O) δ 83.45 (br, 1P, α-*P*), −10.03 (d, 1P, β-*P*, $J_{\alpha\beta}$ = 27.52 Hz); UV (H_2O) λ_{max} 267.6 nm; FAB-MS *m*/*z* 399.05 (M^-); HRMS found *m*/*z* 399.0541 (for ^{11}B), calcd for $C_{10}H_{18}BN_2O_{10}P_2^-$ 399.0530.

Adenosine 5′-P^{α}-Boranodiphosphate (ADPαB) (5b). The diastereomeric mixture of compound **5b** was prepared in 35% yield (83.3 mg) following the general procedure by using 2′,3′-*O*-diacetyladenosine and 7 molar equiv of borane−dimethyl sulfide. ^{1}H NMR (D_2O) δ 8.45, 8.43 (2s, 2 isomers, 1H), 8.11 (s, 1H), 6.00 (d, J = 5.2 Hz, 1H), 4.59 (m, 1H), 4.47, 4.39 (2m, 2 isomers, 1H), 4.25 (m, 1H), 4.14 (m, 1H), 4.03 (m, 1H), (+)0.73 to (−)0.15 (br, 3H); ^{31}P NMR (D_2O) δ 81.90 (br, 1P, α-*P*), −7.20 (d, 1P, β-*P*, $J_{\alpha\beta}$ = 30.13 Hz); UV (H_2O) λ_{max} 259.3 nm; FAB-MS *m*/*z* 424.08 (M^-); HRMS found *m*/*z* 424.0605 (for ^{11}B), calcd for $C_{10}H_{17}BN_5O_9P_2^-$ 424.0595.

Guanosine 5′-P^{α}-Boranodiphosphate (GDPαB) (5c). The diastereomeric mixture of compound **5c** was prepared in 30% yield (73.8 mg) following the general procedure by using 2′,3′-*O*-diacetylguanosine and 8 molar equiv of borane−dimethyl sulfide. ^{1}H NMR (D_2O) δ 8.05, 8.02 (2s, 2 isomers, 1H), 5.79 (d, J = 5.2 Hz, 1H), 4.60 (m, 1H), 4.46, 4.38 (2m, 2 isomers, 1H), 4.22 (m, 1H), 4.13 (m, 1H), 4.03 (m, 1H), (+)0.81 to (−)0.14

(br, 3H); ^{31}P NMR (D_2O) δ 83.10 (br, 1P, α-*P*), −9.06 (d, 1P, *β-P*, $J_{\alpha\beta}$ = 29.14 Hz); UV (H_2O) λ_{max} 253.5 nm; FAB-MS *m/z* 440.08 (M^-); HRMS found *m/z* 440.0541 (for ^{11}B), calcd for $C_{10}H_{17}BN_5O_{10}P_2^-$ 440.0544.

Uridine 5′-*P*$^{\alpha}$-Boranodiphosphate (UDPαB) (5d). The diastereomeric mixture of compound **5d** was prepared in 39% yield (88.4 mg) following the general procedure by using 2′,3′-*O*-dibenzoyluridine and 5 molar equiv of borane−dimethyl sulfide. ^{1}H NMR (D_2O) δ 7.92, 7.91 (2d, 2 isomers, *J* = 8.0 Hz, 1H), 5.85 (d, *J* = 4.4 Hz, 1H), 5.81 (d, *J* = 8.0 Hz, 1H), 4.24 (m, 1H), 4.20 (m, 1H), 4.15 (m, 1H), 4.08−3.95 (m, 2H), (+)0.87 to (−)0.20 (br, 3H); ^{31}P NMR (D_2O) δ 83.53 (br, 1P, α-*P*), −10.03 (d, 1P, *β-P*, $J_{\alpha\beta}$ = 24.45 Hz); UV (H_2O) λ_{max} 262.9 nm; FAB-MS *m/z* 401.07 (M^-); HRMS found *m/z* 401.0310 (for ^{11}B), calcd for $C_9H_{16}BN_2O_{11}P_2^-$ 401.0322.

Reverse-Phase HPLC Separation of Diastereomers of Deoxy- and Ribonucleoside 5′-*P*$^{\alpha}$-Boranodiphosphates. The separation of diastereomers of each NDPαB was carried out by ion-pairing chromatography on a semipreparative reverse-phase column (Waters Delta-Pak C18, 15 μm, 100 Å, 25 × 100 mm, with Z-module) at a flow rate of 5 mL/min, using an isocratic elution [100 mM triethylamonium bicarbonate (TEAB) buffer, pH 8.0, and methanol]. Fractions containing the same isomer (similar retention time) were combined, and the solvent was removed under reduced pressure. The excess of TEAB and methanol were removed by repeated lyophilization with deionized water. The retention time and ratio for different diastereomers of NDPαB are summarized in Table 2.

dTDPαB, *R*$_P$ isomer: ^{1}H NMR (D_2O) δ 7.52 (d, *J* = 0.8 Hz, 1H), 6.18 (t, *J* = 6.4 Hz, 1H), 4.53 (m, 1H), 4.08 (m, 1H), 3.97−3.87 (m, 2H), 2.16 (dd, *J* = 5.6, 6.4 Hz, 2H), 1.76 (d, *J* = 1.2 Hz, 3H), (+)0.60 to (−)0.20 (br, 3H); ^{31}P NMR (D_2O) δ 79.06 (br, 1P, α-*P*), −5.72 (d, 1P, *β-P*, $J_{\alpha\beta}$ = 31.89 Hz); UV (H_2O) λ_{max} 267.6 nm; FAB-MS *m/z* 399.05 (M^-); HRMS found *m/z* 399.0531 (for ^{11}B), calcd for $C_{10}H_{18}BN_2O_{10}P_2^-$ 399.0530.

dTDPαB, *S*$_P$ isomer: ^{1}H NMR (D_2O) δ 7.62 (d, *J* = 1.2 Hz, 1H), 6.20 (t, *J* = 6.8 Hz, 1H), 4.48 (m, 1H), 4.05−3.94 (m, 3H), 2.20 (m, 2H), 1.79 (d, *J* = 0.8 Hz, 3H), (+)0.60 to (−)0.20 (br, 3H); ^{31}P NMR (D_2O) δ 81.61 (br, 1P, α-*P*), −7.96 (d, 1P, *β-P*, $J_{\alpha\beta}$ = 25.90 Hz); UV (H_2O) λ_{max} 267.6 nm; FAB-MS *m/z* 399.05 (M^-); HRMS found *m/z* 399.0518 (for ^{11}B), calcd for $C_{10}H_{18}BN_2O_{10}P_2^-$ 399.0530.

ADPαB, *R*$_P$ isomer: ^{1}H NMR (D_2O) δ 8.46 (s, 1H), 8.06 (s, 1H), 5.96 (d, *J* = 4.0 Hz, 1H), 4.54 (m, 1H), 4.51(m, 1H), 4.18 (m, 1H), 4.13 (m, 1H), 3.96 (m, 1H), (+)0.80 to (−)0.17 (br, 3H); ^{31}P NMR (D_2O) δ 79.35 (br, 1P, α-*P*), −5.63 (d, 1P, *β-P*, $J_{\alpha\beta}$ = 30.60 Hz); UV (H_2O) λ_{max} 259.3 nm; FAB-MS *m/z* 424.08 (M^-); HRMS found *m/z* 424.0599 (for ^{11}B), calcd for $C_{10}H_{17}BN_5O_9P_2^-$ 424.0595.

ADPαB, *S*$_P$ isomer: ^{1}H NMR (D_2O) δ 8.43 (s, 1H), 8.06 (s, 1H), 5.96 (d, *J* = 5.2 Hz, 1H), 4.57 (m, 1H), 4.41(m, 1H), 4.20 (m, 1H), 4.09 (m, 1H), 3.96 (m, 1H), (+)0.80 to (−)0.17 (br, 3H); ^{31}P NMR (D_2O) δ 80.12 (br, 1P, α-*P*), −5.55 (d, 1P, *β-P*, $J_{\alpha\beta}$ = 28.17 Hz); UV (H_2O) λ_{max} 259.3 nm; FAB-MS *m/z* 424.08 (M^-); HRMS found *m/z* 424.0588 (for ^{11}B), calcd for $C_{10}H_{17}BN_5O_9P_2^-$ 424.0595.

GDPαB, *R*$_P$ isomer: ^{1}H NMR (D_2O) δ 8.04 (s, 1H), 5.77 (d, *J* = 4.8 Hz, 1H), 4.53 (m, 1H), 4.50 (m, 1H), 4.13 (m, 1H), 4.10 (m, 1H), 3.93 (m, 1H), (+)0.78 to (−)0.18 (br, 3H); ^{31}P NMR (D_2O) δ 79.48 (br, 1P, α-*P*), −5.63 (d, 1P, *β-P*, $J_{\alpha\beta}$ = 28.98 Hz); UV (H_2O) λ_{max} 253.5 nm; FAB-MS *m/z* 440.08 (M^-); HRMS found *m/z* 440.0532 (for ^{11}B), calcd for $C_{10}H_{17}BN_5O_{10}P_2^-$ 440.0532.

GDPαB, *S*$_P$ isomer: ^{1}H NMR (D_2O) δ 8.01 (s, 1H), 5.77 (d, *J* = 5.6 Hz, 1H), 4.54 (m, 1H), 4.41 (m, 1H), 4.17 (m, 1H), 4.07 (m, 1H), 3.98 (m, 1H), (+)0.78 to (−)0.18 (br, 3H); ^{31}P NMR (D_2O) δ 80.50 (br, 1P, α-*P*), −5.58 (d, 1P, *β-P*, $J_{\alpha\beta}$ = 31.25 Hz); UV (H_2O) λ_{max} 253.5 nm; FAB-MS *m/z* 440.08 (M^-); HRMS found *m/z* 440.0541 (for ^{11}B), calcd for $C_{10}H_{17}BN_5O_{10}P_2^-$ 440.0542.

UDPαB, *R*$_P$ isomer: ^{1}H NMR (D_2O) δ 7.96 (d, *J* = 8.0 Hz, 1H), 5.81 (d, *J* = 4.0 Hz, 1H), 5.78 (d, *J* = 8.0 Hz, 1H), 4.32 (m, 1H), 4.20 (m, 1H), 4.13 (m, 1H), 4.09 (m, 1H), 4.02 (m, 1H), (+)0.80 to (−)0.20 (br, 3H); ^{31}P NMR (D_2O) δ 78.91 (br, 1P, α-*P*), −5.70 (d, 1P, *β-P*, $J_{\alpha\beta}$ = 28.98 Hz); UV (H_2O) λ_{max} 262.9 nm; FAB-MS *m/z* 401.07 (M^-); HRMS found *m/z* 401.0313 (for ^{11}B), calcd for $C_9H_{16}BN_2O_{11}P_2^-$ 401.0322.

UDPαB, *S*$_P$ isomer: ^{1}H NMR (D_2O) δ 7.80 (d, *J* = 8.0 Hz, 1H), 5.86 (d, *J* = 4.4 Hz, 1H), 5.73 (d, *J* = 8.0 Hz, 1H), 4.24 (m, 1H), 4.19 (m, 1H), 4.10−4.05 (m, 2H), 4.01 (m, 1H), (+)0.78 to (−)0.18 (br, 3H); ^{31}P NMR (D_2O) δ 80.03 (br, 1P, α-*P*), −5.56 (d, 1P, *β-P*, $J_{\alpha\beta}$ = 30.60 Hz); UV (H_2O) λ_{max} 262.9 nm; FAB-MS *m/z* 401.07 (M^-); HRMS found *m/z* 401.0320 (for ^{11}B), calcd for $C_9H_{16}BN_2O_{11}P_2^-$ 401.0322.

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Supporting Information Available: ^{1}H NMR and ^{31}P NMR spectra for each diastereomer of compounds **5a**−**d** and the corresponding HPLC profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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