JOC*Article*

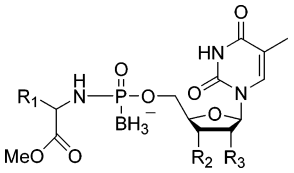
Synthesis of Nucleoside Boranophosphoramidate Prodrugs Conjugated with Amino Acids

Ping Li and Barbara Ramsay Shaw*

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

brshaw@chem.duke.edu.

Received October 22, 2004



Nucleoside boranophosphates and nucleoside amino acid phosphoramidates have been shown to be potent antiviral and anticancer agents with the potential to act as nucleoside prodrugs. A combination of these two types of compounds results in a boranophosphoramidate linkage between the nucleoside and amino acid. This new class of potential prodrugs is expected to possess advantages conferred by both types of parent compounds. Two approaches, specifically the *H*-phosphonate and oxathiaphospholane approaches, are described here to synthesize nucleoside boranophosphoramidate prodrugs conjugated with amino acids. The *H*-phosphonate approach involves a key intermediate, silylated nucleoside amino acid phosphoramidite **6**, prepared from a series of reactions starting from nucleoside *H*-phosphonate in the presence of condensing reagent DPCP. Due to the lengthy procedure and the difficulties in removing DPCP from the final products, we switched to the oxathiaphospholane approach in which the DBU-assisted oxathiaphospholane ring-opening process constituted a key step for the generation of nucleoside amino acid boranophosphoramidates **24**. We demonstrate that this key step did not cause any measurable *C*-racemization of boranophosphorylated amino acids **22**. Diastereomers of compounds **24a**−**f** were separated by RP-HPLC. An "adjacent"-type mechanism is proposed to explain the diastereomer ratio in the final products obtained via the oxathiaphospholane approach. A tentative assignment of configuration for the diastereomers was carried out based on the mechanism, molecular modeling, and ^{1}H NMR. Conclusively, the oxathiaphospholane methodology proved to be more facile and efficient than *H*-phosphonate chemistry in the preparation of the nucleoside amino acid boranophosphoramidate analogues that are promising as a new type of antiviral prodrugs.

Introduction

The development of nucleoside prodrugs capable of undergoing intracellular activation to the corresponding nucleotide has become an area of intense interest.[1] Various 2′,3′-dideoxy nucleosides, including 3′-azido-2′,3′-dideoxythymidine (AZT, zidovudine)[2a,b] and 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T, stavudine),[2c,d] are known to be potent inhibitors of HIV-1, the causative agent of AIDS.[1,2] In general, the biological activity of these antiviral and anticancer nucleosides is dependent on the host cell kinase activity to produce their active triphosphorylated forms. Specifically, for d4T the first phosphorylation step is the rate-limiting step, whereas for AZT phosphorylation to the nucleoside 5′-diphosphate is the slowest step in the phosphorylation pathway.[3] Several of these types of nucleoside analogues are known to be poor substrates for nucleoside kinases and 5′-nucleo-

(1) (a) Cahard, D.; McGuigan, C.; Balzarini, J. *Mini-Rev. Med. Chem.* **2004**, *4*, 371. (b) Drontle, D. P.; Wagner, C. R. *Mini-Rev. Med. Chem.* **2004**, *4*, 409. (c) De Clercq, E. *Nat. Rev. Drug Dis.* **2002**, *1*, 13. (d) Wagner, C. R.; Iyer, V. V.; McIntee, E. J. *Med. Res. Rev.* **2000**, *20*, 417. (e) Meier, C. *Synlett* **1998**, 233. (f) Jones, R. J.; Bischofberger, N. *Antiviral Res.* **1995**, *27*, 1.

(2) (a) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; Stclair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096. (b) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911. (c) Baba, M.; Pauwels, R.; Herdewijn, P.; De Clercq, E.; Desmyter, J.; Vandeputte, M. *Biochem. Biophys. Res. Commun.* **1987**, *142*, 128. (d) Hamamoto, Y.; Nakashima, H.; Matsui, T.; Matsuda, A.; Ueda, T.; Yamamoto, N. *Antimicrob. Agents Chemother.* **1987**, *31*, 907. (e) Pathak, T. *Chem. Rev.* **2002**, *102*, 1623.

(3) (a) Furman, P. A.; Fyfe, J. A.; Stclair, M. H.; Weinhold, K.; Rideout, J. L.; Freeman, G. A.; Lehrman, S. N.; Bolognesi, D. P.; Broder, S.; Mitsuya, H.; Barry, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8333. (b) Balzarini, J.; Herdewijn, P.; De Clercq, E. *J. Biol. Chem.* **1989**, *264*, 6127.

10.1021/jo0481248 CCC: $30.25
Published on Web 02/18/2005

tidases.[4] In addition, the long-term administration of nucleoside-based drugs can result in decreased kinase activity, thus reducing their efficacy.[5] For example, HIV resistance to the antiviral activity of AZT,[5a,b] 2′,3′-dideoxycytidine (ddC),[5c] and acyclovir (ACV)[5d,e] has been shown to arise from the decreased activity of the prerequisite first phosphorylating enzyme. These findings have prompted the development of prodrug or "pronucleotide" strategies that bypass initial kinase dependency by the intracellular delivery of the monophosphorylated nucleoside analogue.[1]

Nucleoside amino acid phosphoramidates show promise as a potential pronucleotide strategy.[1a,b] Studies by McGuigan et al. demonstrated that the phosphoramidate diester prodrugs of 2′,3′-dideoxy-2′,3′-didehydroadenosine (d4A),[6a] 2′,3′-dideoxyadenosine (ddA),[6a] and d4T[6b−d] exhibited greatly enhanced activity against HIV compared to their parent nucleoside analogues in vitro and, in contrast to the parent nucleoside analogues, full activity was retained in kinase-deficient cell lines.[6] Moreover, nucleoside phosphoramidate monoesters are potent antiviral and/or anticancer agents with enhanced activity and reduced cytotoxicity.[7] Specifically, prodrugs of AZT amino acid phosphoramidate derivatives described by Wagner et al. exhibited potent antiviral and anticancer activity with less cytotoxicity and improved plasma half-life compared to AZT.[8] These nucleoside phosphoramidate monoesters are thought to exert their biological functions through a *P*−*N* bond cleavage by phosphoramidases to yield the corresponding nucleoside monophosphates.[6c,7d,9]

Boranophosphates, in which one of the nonbridging oxygen atoms in the phosphate group is replaced by a borane (BH_3) group, were first introduced by our group a decade ago.[10] The borane group is isoelectronic with oxygen occurring in normal phosphate, pseudoisoelectronic with sulfur found in the phosphorothioate, and isosteric with the methyl group present in the methylphosphonate. These chemical similarities imply that boranophosphates will share a number of chemical and biochemical properties with the aforementioned analogues yet will have other unique properties. Nucleoside boranophosphates have recently drawn the attention of medicinal chemists due to their therapeutic applications in antiviral drug design.[11] It has been found that an α-boranophosphate group in 2′,3′-dideoxynucleoside triphosphates (ddNTPs) increases their selectivity to viral reverse transcriptases (RTs) relative to bacterial DNA polymerases.[11a,b] For example, steady-state and pre-steady-state kinetic analyses indicate that whereas the efficiency of the single nucleotide incorporation for ddCTP by moloney murine leukemia virus (MMLV) RT is nearly two orders lower than for natural dCTP, the introduction of an α-boranophosphate group increases the efficiency of incorporation for ddCTPαB by 30-fold.[11a] It also has been shown that the presence of the α-boranophosphate group in AZTDP,[11c] d4TDP,[11d] and ddADP[11e] improves both the phosphorylation of these diphosphates by nucleoside diphosphate kinase and incorporation of their corresponding ddNTPs by wild-type[11c] and mutant multidrug-resistant HIV-RT.[11d,e] Moreover, after these ddNTPαB analogues were incorporated into DNA, repair of the blocked DNA chains by pyrophosphorolysis was reduced significantly by mutant HIV-RT enzymes from drug-resistant viruses.[11b] These results, combined with the distinct properties of boranophosphates that are more lipophilic and nuclease-resistant than the normal phosphates,[10e,15c] suggest that boranophosphates may be potentially useful in a prodrug approach.[12]

(4) (a) Balzarini, J.; Cooney, D. A.; Dalal, M.; Kang, G. J.; Cupp, J. E.; De Clercq, E.; Broder, S.; Johns, D. G. *Mol. Pharmacol.* **1987**, *32*, 798. (b) Starnes, M. C.; Cheng, Y. C. *J. Biol. Chem.* **1987**, *262*, 988. (c) Johnson, M. A.; Ahluwalia, G.; Connelly, M. C.; Cooney, D. A.; Broder, S.; Johns, D. G.; Fridland, A. *J. Biol. Chem.* **1988**, *263*, 15354. (d) Johnson, M. A.; Fridland, A. *Mol. Pharmacol.* **1989**, *36*, 291. (e) Hao, Z.; Cooney, D. A.; Farquhar, D.; Perno, C. F.; Zhang, K.; Masood, R.; Wilson, Y.; Hartman, N. R.; Balzarini, J.; Johns, D. G. *Mol. Pharmacol.* **1990**, *37*, 157.

(5) (a) Antonelli, G.; Turriziani, O.; Verri, A.; Narciso, P.; Ferri, F.; DOffizi, G.; Dianzani, F. *Aids Res. Hum. Retrov.* **1996**, *12*, 223. (b) Hoever, G.; Groeschel, B.; Chandra, P.; Doerr, H. W.; Cinatl, J. *Int. J. Mol. Med.* **2003**, *11*, 743. (c) Magnani, M.; Gazzanelli, G.; Brandi, G.; Casabianca, A.; Fraternale, A.; Chiarantini, L.; Rossi, L. *Life Sci.* **1995**, *57*, 881. (d) Darby, G.; Field, H. J.; Salisbury, S. A. *Nature* **1981**, *289*, 81. (e) Suzutani, T.; Ishioka, K.; De Clercq, E.; Ishibashi, K.; Kaneko, H.; Kira, T.; Hashimoto, K.; Ogasawara, M.; Ohtani, K.; Wakamiya, N.; Saijo, M. *Antimicrob. Agents Chemother.* **2003**, *47*, 1707.

(6) (a) Balzarini, J.; Kruining, J.; Wedgwood, O.; Pannecouque, C.; Aquaro, S.; Perno, C. F.; Naesens, L.; Witvrouw, M.; Heijtink, R.; De Clercq, E.; McGuigan, C. *FEBS Lett.* **1997**, *410*, 324. (b) McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1183. (c) McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **1996**, *39*, 1748. (d) Siddiqui, A. Q.; McGuigan, C.; Ballatore, C.; Zuccotto, F.; Gilbert, I. H.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **1999**, *42*, 4122.

(7) (a) Jones, A. S.; McGuigan, C.; Walker, R. T.; Balzarini, J.; De Clercq, E. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1471−1474. (b) Molema, G.; Jansen, R. W.; Meijer, D. K. F. *Eur. J. Pharmacol.* **1990**, *183*, 2083. (c) Abraham, T. W.; Wagner, C. R. *Nucleosides Nucleotides* **1994**, *13*, 1891. (d) McIntee, E. J.; Remmel, R. P.; Schinazi, R. F.; Abraham, T. W.; Wagner, C. R. *J. Med. Chem.* **1997**, *40*, 3323. (e) Wagner, C. R.; Chang, S. L.; Griesgraber, G. W.; Song, H.; McIntee, E. J.; Zimmerman, C. L. *Nucleosides Nucleotides* **1999**, *18*, 913.

(8) (a) Iyer, V. V.; Griesgraber, G. W.; Radmer, M. R.; McIntee, E. J.; Wagner, C. R. *J. Med. Chem.* **2000**, *43*, 2266. (b) Chang, S. L.; Griesgraber, W.; Southern, P. J.; Wagner, C. R. *J. Med. Chem.* **2001**, *44*, 223.

(9) (a) Abraham, T. W.; Kalman, T. I.; McIntee, E. J.; Wagner, C. R. *J. Med. Chem.* **1996**, *39*, 4569. (b) Kim, J.; Drontle, D. P.; Wagner, C. R. *Nucleosides Nucleotides Nucleic Acids* **2004**, *23*, 483. (c) Kim, J.; Wagner, C. R. *Antiviral Res.* **2003**, *57*, A53. (d) Wagner, C. R.; Kim, J.; Chou, T. F. *Antiviral Res.* **2004**, *62*, A27. (e) Kim, J.; Chou, T. F.; Griesgraber, G. W.; Wagner, C. R. *Mol. Pharm.* **2004**, *1*, 102.

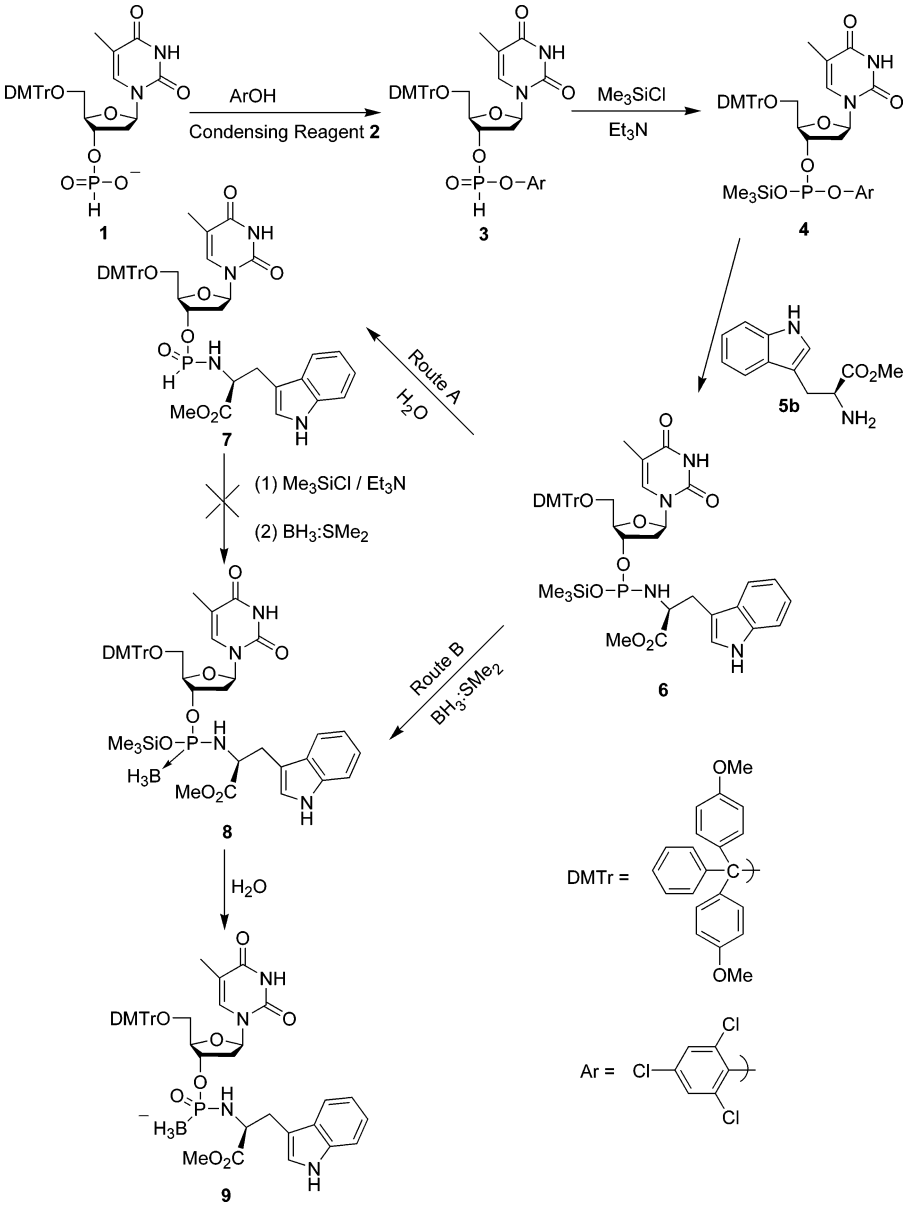
(10) (a) Sood, A.; Shaw, B. R.; Spielvogel, B. F. *J. Am. Chem. Soc.* **1990**, *112*, 9000. (b) Tomasz, J.; Shaw, B. R.; Porter, K.; Spielvogel, B. F.; Sood, A. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1373. (c) Shaw, B. R.; Madison, J.; Sood, A.; Spielvogel, B. F. *Methods Mol. Biol.* **1993**, *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.

(11) (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.

(12) (a) Li, P.; Shaw, B. R. *Org. Lett.* **2002**, *4*, 2009. (b) Li, P.; Shaw, B. R. *Chem. Commun.* **2002**, 2890. (c) Li, P.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 699.

(13) (a) Stawinski, J.; Stromberg, R. *Trends Org. Chem.* **1993**, *4*, 31. (b) Nilsson, J.; Bollmark, M.; Jankowska, J.; Wenska, M.; Cieslak, J.; Kraszewski, A.; Stawinski, J. *Phosphorus Sulfur Silicon Relat. Elem.* **2002**, *177*, 1513. (c) Stawinski, J.; Kraszewski, A. *Acc. Chem. Res.* **2002**, *35*, 952.

SCHEME 1. Synthesis of Nucleoside Boranophosphoramidate via an H−Phosphonate Approach



It could be foreseen that the combination of nucleoside amino acid phosphoramidates and nucleoside boranophosphates would create a new type of antiviral and anticancer prodrug with the advantages conferred by both phosphoramidates and boranophosphates. In this paper we present the synthesis of nucleoside amino acid boranophosphoramidates through *H*-phosphonate methodology as well as an oxathiaphospholane approach. The availability of these boranophosphoramidate analogues would permit the investigations of their chemical and enzymatic stabilities, substrate properties, and delivery into cells compared to their parent compounds of nucleoside amino acid phosphoramidates.

Results and Discussion

Synthesis of Nucleoside Amino Acid Boranophosphoramidates via an *H*-Phosphonate Approach. *H*-Phosphonate chemistry has been widely used in the synthesis of phosphorus-containing compounds, especially for the synthesis of nucleotides and oligonucleotides.[13] For example, it has been applied to synthesize nucleoside boranophosphate[14] and oligothymidine boranophosphate[15] analogues in good yields via silylation of an *H*-phosphonate precursor followed by boronation. Meanwhile, Stawinski et al. reported the synthesis of $P3' \rightarrow N5'$ *H*-phosphonamidate internucleotide linkage through an aryl *H*-phosphonate.[16] After an extensive literature search and study we decided to carry out the synthesis of nucleoside amino acid boranophosphoramidate via an *H*-phosphonate approach as outlined in Scheme 1.

(14) (a) Sergueev, D.; Hasan, A.; Ramaswamy, M.; Shaw, B. R. *Nucleosides Nucleotides* **1997**, *16*, 1533. (b) He, K. Z.; Sergueev, D. S.; Sergueeva, Z. A.; Shaw, B. R. *Tetrahedron Lett.* **1999**, *40*, 4601.

(15) (a) Zhang, J. C.; Terhorst, T.; Matteucci, M. D. *Tetrahedron Lett.* **1997**, *38*, 4957. (b) Higson, A. P.; Sierzchala, A.; Brummel, H.; Zhao, Z. Y.; Caruthers, M. H. *Tetrahedron Lett.* **1998**, *39*, 3899. (c) Sergueev, D. S.; Shaw, B. R. *J. Am. Chem. Soc.* **1998**, *120*, 9417.

(16) Kers, I.; Stawinski, J.; Kraszewski, A. *Tetrahedron* **1999**, *55*, 11579.

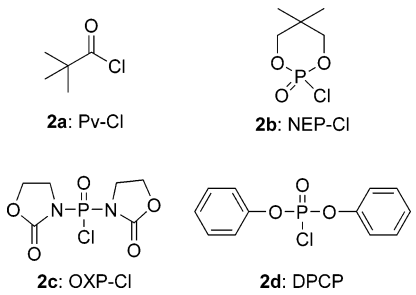


FIGURE 1. Structures of condensing reagents **2** and their abbreviations.

TABLE 1. Summary of the Generation of *H*-Phosphonamidate 7 with Different Condensing Reagents 2

entry	condensing reagent **2**	equiv of **2**	yield (%) of **7**[a]
2a	Pv−Cl	1.1	0
2b	NEP−Cl	2.5	<10
2c	OXP−Cl	1.0	25
2d	DPCP	1.0	55

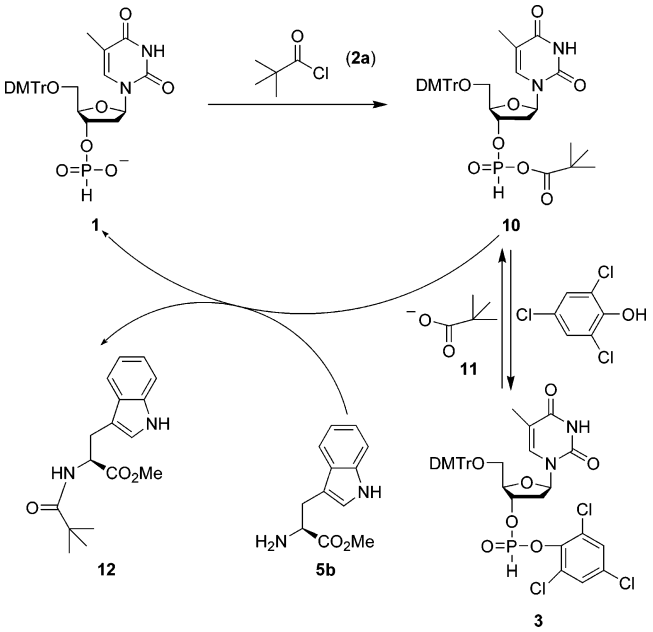
[a] Yield was calculated by peak integration in ^{31}P NMR.

To investigate the viability of this synthetic approach, we started with the commercially available *H*-phosphonate, i.e., 5′-*O*-dimethoxytrityl (DMT) thymidine 3′-*H*-phosphonate **1**. After reacting with 2,4,6-trichlorophenol in the presence of condensing reagent **2**, a highly reactive intermediate, thymidine aryl *H*-phosphonate diester **3**, was formed. Different condensing reagents, including pivaloyl chloride (Pv−Cl), **2a**, 2-chloro-5,5,-dimethyl-2-oxo-1,3,2-dioxaphosphinane (NEP−Cl), **2b**, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (OXP−Cl), **2c**, and diphenyl chlorophosphate (DPCP), **2d**, were used in this step; their structures are depicted in Figure 1. Although all these reactions cleanly produced the desired aryl *H*-phosphonate **3**, the yield of the nucleoside amino acid *H*-phosphonamidate **7** (Route A, Scheme 1) upon addition of L-tryptophan methyl ester **5b** differed significantly depending on the type of condensing agent that was used for the generation of **3**. The yields are summarized in Table 1.

When Pv−Cl **2a** was used as the condensing reagent and after the addition of water, both TLC and ^{31}P NMR (**7**: δ 15 ppm) indicated that no desired *H*-phosphonamidate **7** was formed. However, this reaction produced *N*-pivaloylated L-tryptophan methyl ester **12** (Scheme 2), which was isolated in 25% yield along with the 50% recovery of starting material *H*-phosphonate **1**. According to the products isolated in this reaction and studies reported by Stawinski et al.,[16] a reaction mechanism could be proposed as shown in Scheme 2. Nucleoside aryl *H*-phosphonates **3** can be involved in equilibrium with pivaloate anion **11**,[17] which may produce detectable amounts of pivalic−phosphonic mixed anhydride **10**. Assuming that the mixed anhydride **10** could react with amines with incomplete chemoselectivity, attack from the α-NH_2 of the L-tryptophan methyl ester **5b** on the carbonyl center in **10** would result in the formation of both starting material **1** and *N*-pivaloylated product **12**.

(17) Kers, A.; Kers, I.; Kraszewski, A.; Stawinski, J. *Collect. Czech. Chem. Commun.* **1996**, *61*, 1, S246.

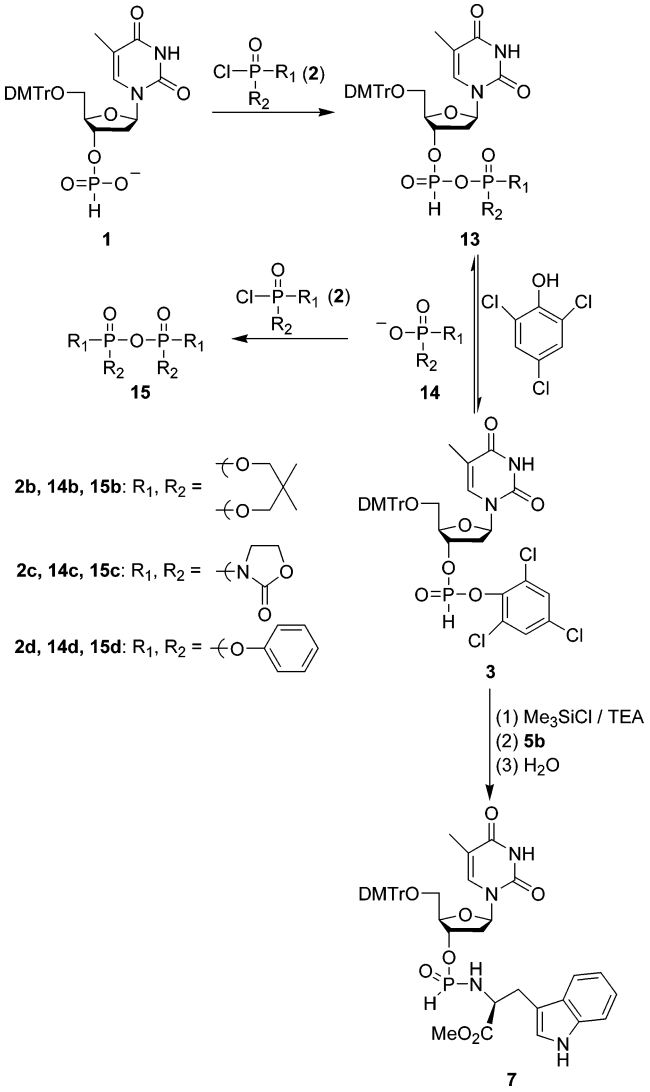
SCHEME 2. Proposed Mechanism for the Formation of Products When Pv-Cl Is Used as Condensing Reagent



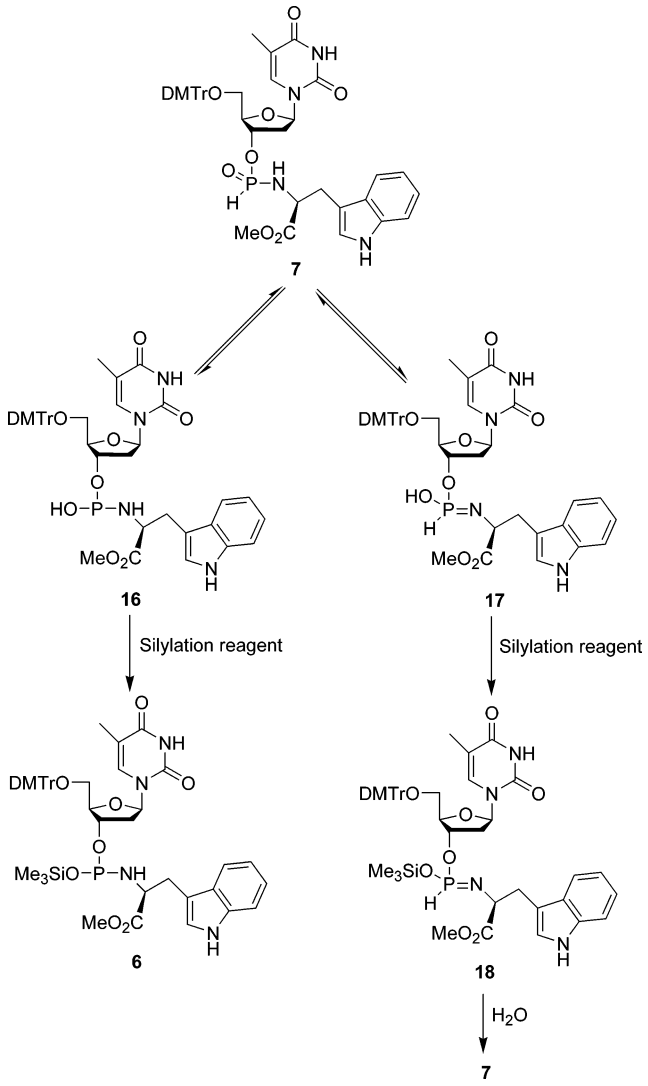
To avoid the problem associated with the use of Pv−Cl we decided to try chlorophosphates as condensing agents for the formation of aryl *H*-phosphonate **3**. To minimize the side reaction of amino acid with chlorophosphates (or with the corresponding pyrophosphates produced during the course of condensation), sterically hindered chlorophosphates, including NEP−Cl **2b**, OXP−Cl **2c**, and DPCP **2d**, were chosen as the condensing reagents. Whereas the aminolysis of **3** by **5b** (Scheme 3) in the presence of NEP−Cl produced less than 10% of the desired *H*-phosphonamidate **7** after the reaction workup, the yields of **7** increased to 25% and 55% when OXP−Cl and DPCP, respectively, were used as condensing reagents. According to the proposed mechanism shown in Scheme 3 there exists a two-step reaction including the activation of *H*-phosphonate **1** and the subsequent reaction to produce aryl *H*-phosphonate diester **3**. With the increasing reactivity of **2b** < **2c** < **2d** toward starting material **1**, the first step to activate *H*-phosphonate **1** becomes faster than the second step to generate **3**, and thus a smaller amount of pyrophosphate **15** is generated due to consumption of condensing reagent. Therefore, the amount of the reactive intermediate **3** increases with the condensing reagents **2b** < **2c** < **2d**, which results in the increased yield for the formation of *H*-phosphonamidate **7**.[16] Moreover, the partial decomposition of *H*-phosphonamidate **7** during silica gel chromatography led to a search for a condensing reagent that would not only provide efficient formation of **7** but also enable purification of the product via aqueous extraction. Thus, although the yield was only satisfactory, DPCP was selected as the condensing reagent for the production of reactive aryl *H*-phosphonate diester **3**.

After following route A as depicted in Scheme 1 the anhydrous nucleoside amino acid *H*-phosphonamidate **7** was subjected to silylation in the presence of triethylamine (TEA) followed by boronation with borane−dimethyl sulfide. As deduced from ^{31}P NMR spectra of

SCHEME 3. Proposed Mechanism for the Formation of Products Using Chlorophosphate as the Condensing Reagent



SCHEME 4. Two Possible Tautomeric Equilibriums for H−Phosphonamidate 7



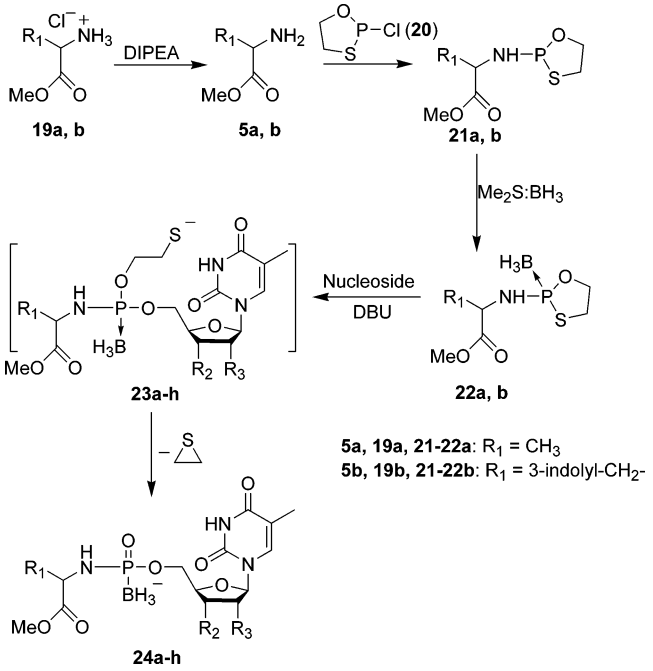
the mixtures, the reaction did not proceed as expected to form borane−phosphoramidate complex **8**. Other silylation reagents, such as *N,O*-bis(trimethylsilyl)acetamide (BSA) and bis(trimethylsilyl)trifluoroacetamide (BSTFA), were also tried but without success. After further addition of H_2O to the reaction mixtures, most of the *H*-phosphonamidate **7** was recovered, as indicated by ^{31}P NMR. It can be proposed that *H*-phosphonamidate **7** could undergo two different tautomeric equilibriums as shown in Scheme 4. In such a case if tautomer **17** were the major form, it would lack the lone-pair electrons that are required for the boronation step. Hydrolysis of **18** with H_2O would then result in the formation of *H*-phosphonamidate **7**. This is in accordance with the observance in the experiments.

To avoid the aforementioned problems associated with route A, we decided to follow route B. Instead of removing condensing reagent DPCP via aqueous extraction at an early stage, silylated phosphoramidite diester **6** (Scheme 1) was directly boronated with borane−dimethyl sulfide. The reaction was completed within 30 min as monitored by ^{31}P NMR, where the two peaks corresponding to the two diastereomers (two singlets, δ = 139.5, 139.7 ppm) for intermediate **6** became a broad peak centered at δ 118.0 ppm corresponding to the borane complex **8**. Treatment of **8** with H_2O resulted in the formation of desired compound boranophosphoramidate **9**, whose ^{31}P NMR showed a characteristic broad peak at δ 93.5 ppm.[18] Due to the presence of the DMT group in **9** and its hydrophobicity, condensing reagent DPCP could easily be removed by repeatedly washing the reaction mixture in dichloromethane with brine. However, when this method was applied to synthesize 3′-*O*-acetylthymidine boranophosphoramidate **24a**, the condensing reagent DPCP and the desired compound **24a** could not be separated by aqueous extraction between dichloromethane and brine because a large amount of **24a** was distributed to the brine layer together with the hydrolysis products from DPCP. Further purification with reverse-phase HPLC did not resolve the problem.

Synthesis of Nucleoside Amino Acid Boranophosphoramidates via an Oxathiaphospholane Ap-

(18) Lin, J. L.; Shaw, B. R. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 587.

SCHEME 5. Synthesis of Nucleoside Amino Acid Boranophosphoramidates via an Oxathiaphospholane Approach



proach. Due to the difficulties encountered by employing *H*-phosphonate chemistry, we decided to explore an alternative synthetic method to obtain nucleoside amino acid boranophosphoramidates. The oxathiaphospholane approach, first developed by Stec for the synthesis of stereoregular oligo(nucleoside phosphorothioate)s,[19] recently has been adopted to synthesize phosphorothioylated products of biologically relevant alcohols and amino acids.[20] The oxathiaphospholane approach involves tricoordinate phosphorus compounds as the precursors and therefore, as demonstrated earlier,[21] is suitable for the introduction of a borane group. By employing this approach, which is superior to the procedure using the phosphoramidate approach,[22] nucleoside 5′-P^{α}-boranodiphosphates were recently obtained in good yields.[23] To further investigate the application of an oxathiaphospholane approach in borane-containing nucleotide chemistry, we synthesized nucleoside boranophosphoramidates conjugated with amino acids via an oxathiaphospholane ring-opening condensation as depicted in Scheme 5.

Amino acid methyl ester **5** was reacted with 2-chloro-1,3,2-oxathiaphospholane **20** in the presence of diisopropylethylamine (DIPEA) in dichloromethylene solution to give the *N*-(1,3,2-oxathiaphospholanyl)amino acid methyl ester **21**. This phosphitylation reaction was completed in 30 min as evidenced by ^{31}P NMR spectra, where the singlet at δ 207 ppm for 2-chloro-1,3,2-oxathiaphospholane **20** was transformed into two singlets around δ 129.5 ppm corresponding to the diastereomers of **21** (ratio 2:3) in about 70% yield along with some unidentified products. Attempts to purify compound **21** by silica gel column chromatography failed as it appeared to decompose quickly on the column.

Thus, without isolation of *N*-(1,3,2-oxathiaphospholanyl)amino acid methyl ester **21**, in situ boronation was carried out by reacting with boronating reagents to provide oxathiaphospholane−borane complex **22**. Its formation was confirmed by the presence of a characteristic broad resonance peak centered at δ 136 ppm in its ^{31}P NMR spectrum. According to molecular orbital (MO) theory and valence shell electron pair repulsion (VSEPR) theory, the boron atom in BH_3 has an empty 2p orbital and, therefore, is labile to attack by nucleophiles.[24] The tervalent phosphorus atom with lone-pair electrons could act as a Lewis-base donor of electrons to an electron-deficient BH_3. The reactivities of a series of borane-group transferring reagents, including borane−dimethyl sulfide and borane−tetrahydrofuran, to intermediate **21** were examined as well as three types of borane−amine complexes: aliphatic−borane, aromatic−borane, and heterocyclic−borane. The results are summarized in Table 2, where the most active boronating reagent is borane−dimethyl sulfide. Experiments on varying the equivalency of borane−dimethyl sulfide were also carried out to determine the suitable amount of the boronating reagent necessary for the reaction. It turned out that 5 mol equiv of borane−dimethyl sulfide was sufficient for completion of the reaction in 30 min. Increasing the amount to 10 mol equiv did not significantly reduce the completion time as monitored by ^{31}P NMR spectrum. However, with 2 mol equiv the reaction proceeded slowly and went to completion in about 2 h. Thus, 5 mol equiv of boron−dimethyl sulfide was selected as the choice of optimal amount and reagent to complete the boronation step in 30 min.

An attempt to purify the oxathiaphospholane−borane complex **22** by silica gel column chromatography was not successful due to its instability in the presence of moisture. Therefore, the crude product **22** was reacted with 0.8 mol equiv of nucleoside dissolved in anhydrous acetonitrile in the presence of 1,8-diazabicyclo[5.4.0]-

TABLE 2. 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 **21b**→**22b**[c,d]	100%	none	20%	15%	none	60%

[a] *N*-(1,3,2-Oxathiaphospholanyl)-L-tryptophan methyl ester **21b** was used as a model compound. [b] Ten 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 **22b** relative to the total integration of compounds **21b** and **22b** in the ^{31}P NMR spectra.

(19) (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. *Top. Curr. Chem.* **2002**, *220*, 169.

(20) (a) Baraniak, J.; Kaczmarek, R.; Stec, W. J. *Tetrahedron Lett.* **2000**, *41*, 9139. (b) Baraniak, J.; Kaczmarek, R.; Korczynski, D.; Wasilewska, E. *J. Org. Chem.* **2002**, *67*, 7267. (c) Baraniak, J.; Kaczmarek, R.; Wasilewska, E.; Stec, W. J. *Phosphorus Sulfur Silicon Relat. Elem.* **2002**, *177*, 1667. (d) Olesiak, M.; Krajewska, D.; Wasilewska, E.; Korczynski, D.; Baraniak, J.; Okruszek, A.; Stec, W. J. *Synlett* **2002**, 967−971. (e) Zmudzka, K.; Nawrot, B.; Chojnacki, T.; Stec, W. J. *Org. Lett.* **2004**, *6*, 1385.

(21) (a) Okruszek, A.; Sierzchala, A.; Zmudzka, K.; Stec, W. J. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1843. (b) Baraniak, J.; Kaczmarek, R.; Wasilewska, E. *Tetrahedron Lett.* **2004**, *45*, 671.

(22) Lin, J. L.; He, K. Z.; Shaw, B. R. *Helv. Chim. Acta* **2000**, *83*, 1392.

(23) Li, P.; Shaw, B. R. *J. Org. Chem.* **2004**, *69*, 7051.

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

TABLE 3. Conditions for the Oxathiaphospholane Ring-Opening Condensation[a]

amino acid	nucleoside	product	molar equiv of DBU	reaction temp. (°C)	completion time (h)
L-alanine	3-OAc-T	**24a**	3	40	2
	AZT	**24c**	5	45	5
	d4T	**24e**	1.5	40	1
L-tryptophan	3-OAc-T	**24b**	3	60	3
	AZT	**24d**	5	60	8
	d4T	**24f**	1.5	60	2
D-alanine	3-OAc-T	**24 g**	3	40	2
D,L-alanine	3-OAc-T	**24h**	3	40	2
L-tryptophan	5-DMT-T	**9**	5	50	5

[a] The reaction was monitored by TLC with developing system $MeOH/CH_2Cl_2$ (8/92).

undec-7-ene (DBU). The reaction underwent ring-opening condensation to form the boranophosphoramidate intermediate **23** followed by fast DBU-assisted elimination of ethylene episulfide to provide the desired nucleoside amino acid boranophosphoramidate **24**. It should be pointed out that the amount of DBU and the reaction temperature required for the ring-opening condensation depended on the specific amino acid methyl ester **5** used in the reaction and the corresponding nucleoside. Generally, the more hindered the amino acid methyl ester and nucleoside, the greater the amount of DBU required and the higher the necessary reaction temperature. The reaction conditions are described in Table 3. The crude product boranophosphoramidate **24** was isolated by silica gel column chromatography. To ensure that the nitrogen atom from the amino acid was free of BH_3 possibly introduced during the boronation step, the reaction mixture should be treated with water/triethylamine mixtures for 15 min before loading onto the column for purification.

The purified products, nucleoside amino acid boranophosphoramidates **24a**−**h** and **9**, were obtained in 35−45% overall yields from **19** to **24** (or **9**) after silica gel purification. Their structures were confirmed by 1D- and 2D-^{1}H NMR, ^{31}P NMR, FAB-MS, and HRMS. It has to be pointed out that the ^{31}P NMR of boranophosphoramidate **24** (or **9**) has a characteristic broad peak around δ 92 ppm.[18] This result does not agree with the reported ^{31}P NMR around δ 105 ppm by Baraniak et al. in a recent communication on the synthesis of boranophosphoramidate in which neither ^{1}H NMR nor HRMS data were provided.[21b]

As seen in the other cases,[10,12,22] modification of a phosphoramidate at the phosphorus atom results in a noticeable change of its phosphorus chemical shift: from $\delta \approx 7$ ppm for a normal phosphoramidate[8,25] to $\delta \approx 60$ ppm for a phosphorothioamidate[20a,b] and, as we have found in our studies, to $\delta \approx 92$ ppm for a boranophosphoramidate.[18] Notably, the residence peak for nucleoside amino acid boranophosphoramidates in ^{31}P NMR is about the same as that for dinucleoside boranophosphates,[14] which indicates a similar chemical environment for phosphorus in both types of compounds.

Studies on the HPLC Separation of Diastereomers of Nucleoside Amino Acid Boranophosphoramidates. Introduction of a borane group (BH_3) to replace one of the oxygen atoms on the phosphoramidate produces a pair of diastereomers for compound **24**. These diastereomers of **24a**−**f** have been separated by semipreparative reverse-phase HPLC (RP-HPLC) and named isomer I and isomer II in order of their retention time. Their tentative configuration assignment will be described in the following section. The HPLC separation conditions are summarized in Table 4. 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 2(*S*)-[(2′,3′-dideoxy-2′,3′-didehydrothymidin-5′-yl)boranophosphorylamino]-proprionic acid methyl ester **24e** before and after preparative HPLC separation are shown in Figure 3. The

TABLE 4. HPLC Separation Conditions for Nucleoside Amino Acid Boranophosphoramidates 24[a]

		retention time (min) [area percentage]	
compound	buffer[b]	isomer I [%]	isomer II [%]
24a	5−40% CH_3CN in TEAB over 60 min	20.55 [59.7]	24.00 [40.3]
24b	22% CH_3CN/78% TEAB	10.19 [55.5]	11.41 [44.5]
24c	5−30% CH_3CN in TEAB over 60 min	9.91 [63.3]	11.52 [36.7]
24d	15−50% CH_3CN in TEAB over 60 min	22.61 [56.9]	24.80 [43.1]
24e	3−15% CH_3CN in TEAB over 60 min	7.47 [61.6]	9.62 [38.4]
24f	12−40% CH_3CN in TEAB over 60 min	21.40 [57.6]	25.78 [42.4]

[a] Waters Delta-Pak C18 column: 15 μm, 100 Å, 25 × 100 mm, with Z-module. [b] 100 mM TEAB (triethylammonium bicarbonate, pH 8.0) and acetonitrile (CH_3CN).

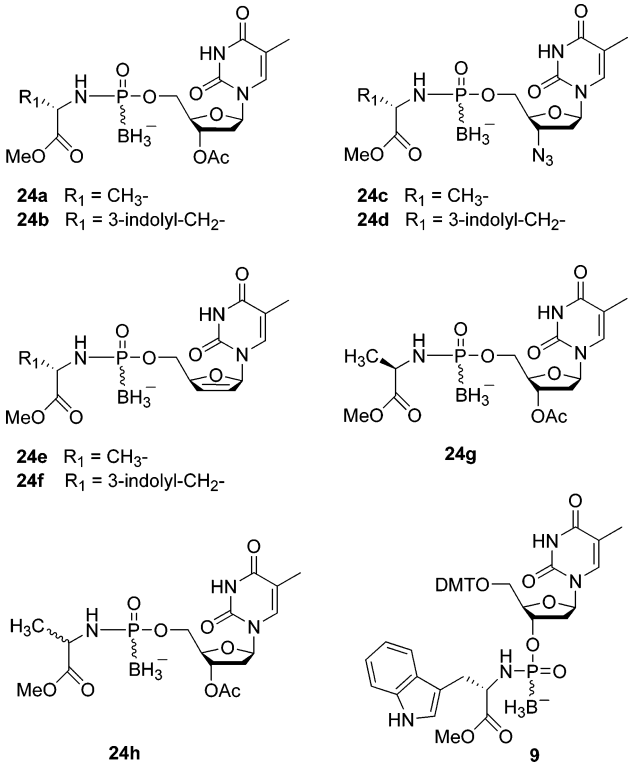


FIGURE 2. Compounds of nucleoside amino acid boranophosphoramidates synthesized via an oxathiaphospholane approach.

(25) Shirokova, E. A.; Jasko, M. V.; Khandazhinskaya, A. L.; Ivanov, A. V.; Yanvarev, D. V.; Skoblov, Y. S.; Mitkevich, V. A.; Bocharov, E. V.; Pronyaeva, T. R.; Fedyuk, N. V.; Kukhanova, M. K.; Pokrovsky, A. G. *J. Med. Chem.* **2004**, *47*, 3606.

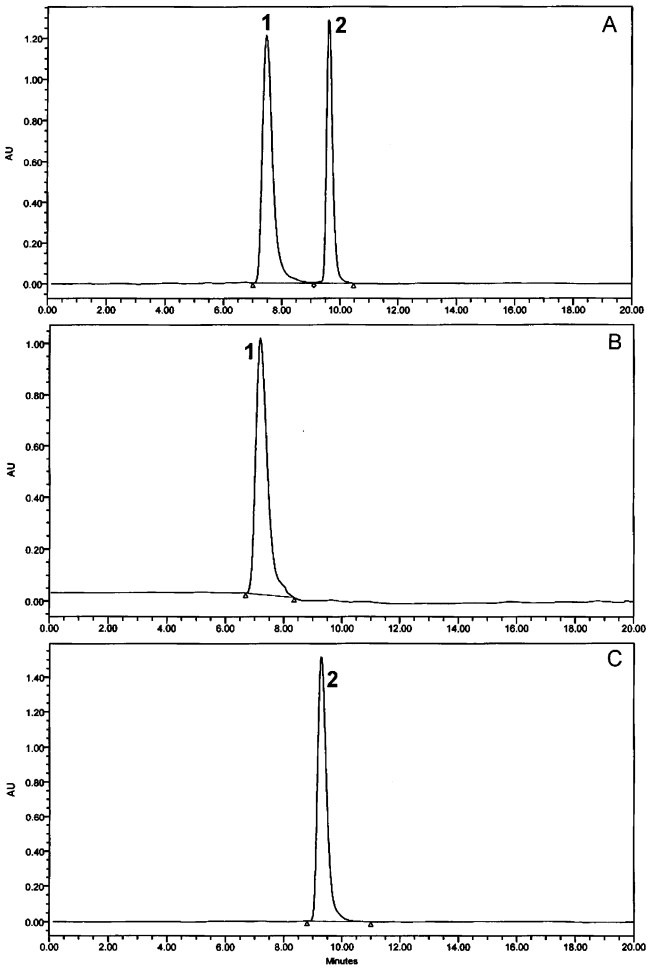
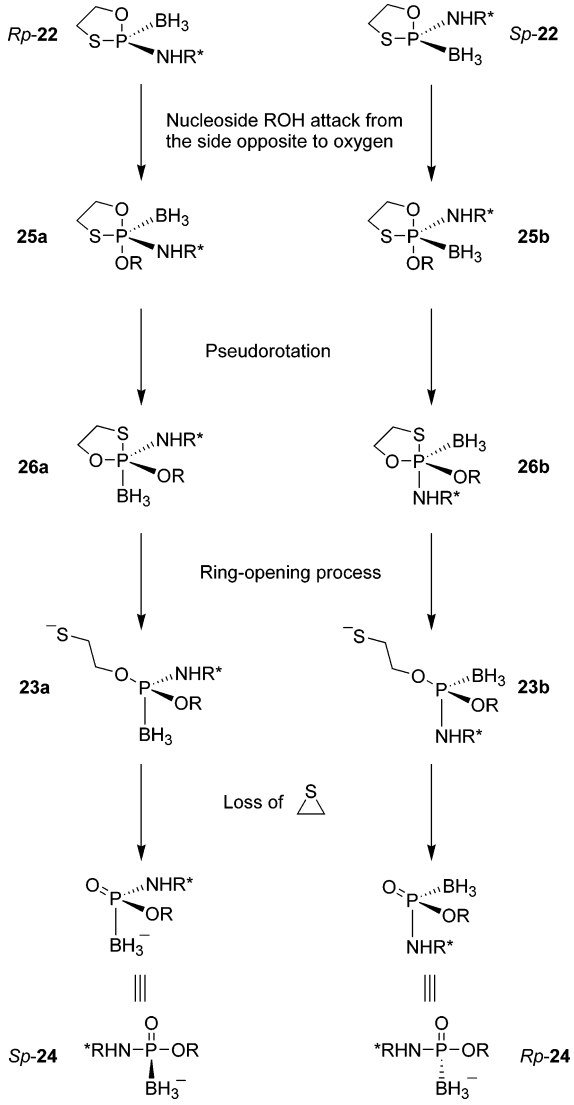


FIGURE 3. Gradient separation of diastereomers of 2(*S*)-[(2′,3′-dideoxy-2′,3′-didehydrothymidin-5′-yl)boranophosphorylamino]proprionic acid methyl ester **24e** by RP-HPLC (A), and analysis of purity of HPLC-isolated isomer I (B) and isomer II (C). The separation was carried out on the Waters Delta-Pak C18 column with gradient 3−15% acetonitrile in 100 mM TEAB (pH 8.0) over 60 min at a flow rate of 8.0 mL/min. The absorbance was monitored at λ 260 nm. The retention times for the two diastereomers were 7.47 and 9.62 min, respectively. Peak 1: isomer I of compound **24e**. Peak 2: isomer II of compound **24e**.

structure of each diastereomer of **24a**−**f** was confirmed by 1D- and 2D-^{1}H NMR, ^{31}P NMR, FAB-MS, and HRMS.

Tentative Configuration Assignment for Diastereomers of Nucleoside Amino Acid Boranophosphoramidates. Noticeably, for each pair of diastereomers in Table 3 the ratio between isomer I and II was approximately 3 to 2, which was understandable because the oxathiaphospholane approach was first introduced as a way of stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s.[19] An explanation proposed to rationalize the ratio of isomer I to II is depicted in Scheme 6. Stec et al. have shown that an "adjacent"-type mechanism is associated with the 1,3,2-oxathiaphospholane ring-opening process,[26] which involves a nucleophilic attack from the side opposite the most apicophilic endocyclic

SCHEME 6. Proposed "Adjacent"-Type Mechanism in the 1,3,2-Oxathiaphospholane Ring-Opening Process during the Synthesis of Nucleoside Amino Acid Boranophosphoramidate 24



oxygen atom, resulting in a trigonal bipyramidal intermediate, which before collapse must undergo pseudorotation, placing the cleavable *P*−*S* bond in the apical position, to form the ring-opened product. As shown in Scheme 6, the formation of the oxathiaphospholane−borane complex **22** produces a pair of diastereomers designated as *Rp*-**22** and *Sp*-**22**. Except for an oxygen atom in the apical position, all the other atoms and groups are in equatorial positions. Nucleoside ROH approaches complex **22** from the side opposite the endocyclic *P*−*O* bond, resulting in the formation of intermediate **25**, which has the two most electronegative elements (two oxygen atoms) in apical positions. Because the principle of microreversibility requires that the leaving group is located in the apical position,[27] a single pseudorotation process (with the phosphorus atom as "pivot") leads to intermediate **26**. Since BH_3^- has a strong preference for the equatorial position as calculated by

(26) Uznanski, B.; Grajkowski, A.; Krzyzanowska, B.; Kazmierkowska, A.; Stec, W. J.; Wieczorek, M. W.; Blaszczyk, J. *J. Am. Chem. Soc.* **1992**, *114*, 10197.

(27) Westheimer, F. H. *Rearrangements in Ground & Excited States*; Academic Press: New York, 1980; Vol. 2, p 229.

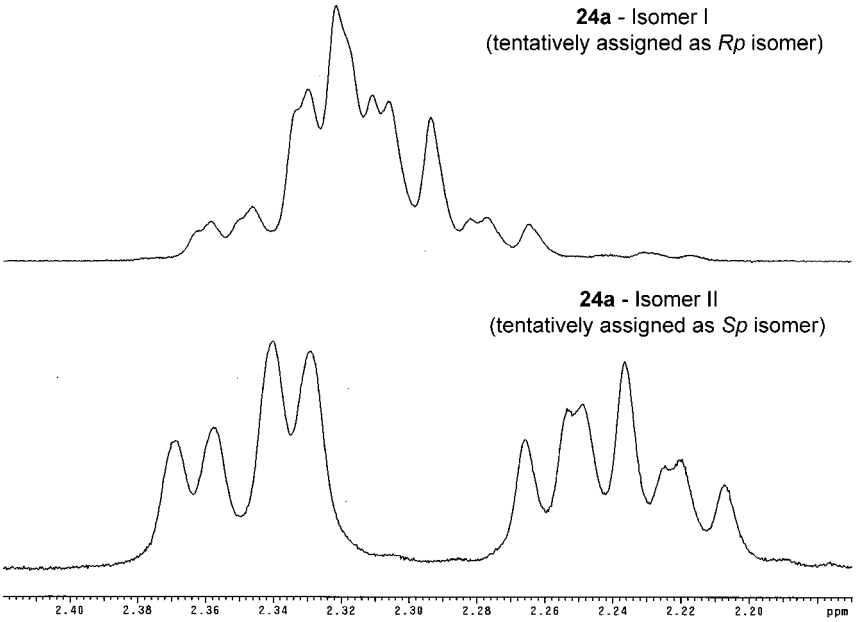


FIGURE 4. ^{1}H NMR for different diastereomers of compound 2(*S*)-[(3′-*O*-acetylthymidin-5′-yl)boranophosphorylamino]proprionic acid methyl ester **24a**. Spectra were recorded on 500 MHz spectrometer in D_2O.

Thatcher et al.,[28] isomer **26b** is favored over **26a**. Collapse of **26** via cleavage of the apical *P*−*S* bond and the follow-up elimination of ethylene episulfide from **23** gives boranophosphoramidate **24**. It has to be pointed out that the function of DBU is neglected in this proposed mechanism. This is mainly because the driving force for the overall process derives from the ring-strain relief arising from the opening of the oxathiaphospholane ring, followed by fast elimination of episulfide.[26] Thus, from the mechanistic point of view, *Rp*-**24** is favored over *Sp*-**24**. Accordingly, isomer I of boranophosphoramidate, whose configuration is in large excess in final product, would have an *Rp* configuration for the phosphorus center, whereas isomer II corresponds to *Sp*.

It is noteworthy that chemical shifts and splitting patterns for the 2′-position protons on the ribose in compound 2(*S*)-[(3′-*O*-acetylthymidin-5′-yl)boranophosphorylamino]proprionic acid methyl ester **24a** were quite different in each diastereomer. As shown in Figure 4, isomer I had a chemical shift with multiplet at δ 2.32 ppm for the two 2′-position protons whereas isomer II had distinct chemical shifts with a multiplet at δ 2.35 and 2.24 ppm for the two 2′-position protons. According to the preliminary results from the spatial model of compound **24a** constructed with the CHEM3D ULTRA 6.0 program, the chemical environments for the two protons on the 2′-position in *Rp*-**24** are similar because all the neighboring groups are far away from them. However, in *Sp*-**24** the chemical environments for the two protons on the 2′-position are quite different due to their close proximity to other neighboring groups. This molecular modeling supports assignment of isomers I and II corresponding to *Rp* and *Sp*, respectively.[29]

Studies on the Racemization of Amino Acid during Oxathiaphospholane Ring-Opening Process. A major problem in amino acid chemistry is associated with racemization of the amino acid or its derivative. Since the oxathiaphospholane ring-opening process in **22** is catalyzed by a strong base such as DBU, it was necessary to check if racemization of phosphorylated derivatives occurred during this reaction. ^{31}P NMR was considered as a technique for this purpose.[20b] However, due to the quadrapole broadening caused by ^{10}B and ^{11}B nuclei, it is difficult to assign the different diastereomers by ^{31}P NMR spectra. Therefore, HPLC was adopted to identify the formation of different diastereomers of **24** after ring-opening condensation with nucleoside.

Compounds **22**, specifically, 2-borano-1,3,2-oxathiaphospholane derivatives of L-alanine, D-alanine, and racemic D,L-alanine, were prepared as the model compounds. When the derivative **22** of L-alanine reacted with 3′-*O*-acetylthymidine/DBU, a pair of *P*-diastereomers of **24a** was formed and could be separated by RP-HPLC (Figure 5A). When the derivative **22** of D-alanine reacted with 3′-*O*-acetylthymidine/DBU, a pair of *P*-diastereomers of **24g** was also formed as seen from ^{1}H NMR, but they could not be separated by RP-HPLC in the same HPLC conditions (Figure 5B). When the derivative **22** of racemic D,L-alanine was used, two enantiomeric pairs were formed and the HPLC profile equaled the combination of HPLC profiles for **24a** and **24g** (Figure 5C). Every peak shown in the HPLC profiles in Figure 5 was subjected to electronic ionization mass (EI-MS) analysis, which indicated that they all had the same molecular weight as 446.2 (M^-). From these model experiments and due to the partial overlap between peaks 1 and 3 in Figure 5, we concluded that the oxathiaphospholane ring-opening process catalyzed by DBU in **22** did not cause significant racemization in the resulting nucleoside amino acid boranophosphoramidate **24**.

(28) Thatcher, G. R. J.; Campbell, A. S. *J. Org. Chem.* **1993**, *58*, 2272.

(29) Please see the 3-D structures for *Rp*-**24a** and *Sp*-**24a** from molecular modeling in the Supporting Information. Also refer to ref 25 for molecular modeling. The result from CHEM3D ULTRA was preliminary, and further calculations are needed to confirm the conclusions.

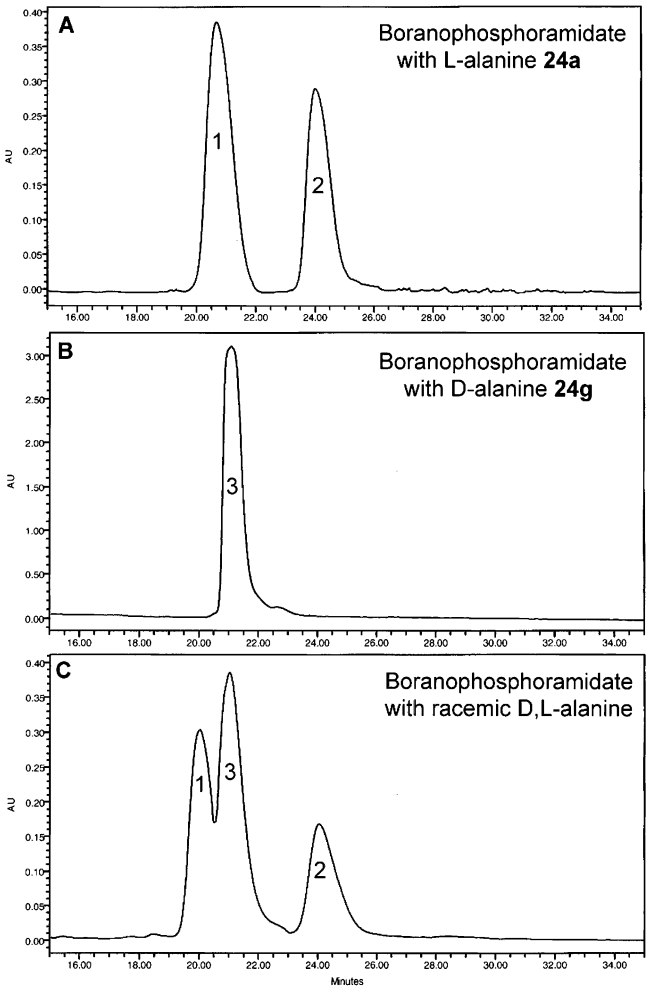


FIGURE 5. Separation profiles by RP-HPLC for diastereomers of (A) 2(*S*)-[(3′-*O*-acetylthymidin-5′-yl)boranophosphorylamino]proprionic acid methyl ester **24a**, (B) 2(*R*)-[(3′-*O*-acetylthymidin-5′-yl)boranophosphorylamino]proprionic acid methyl ester **24g**, and (C) 2(*R,S*)-[(3′-*O*-acetylthymidin-5′-yl)-boranophosphorylamino]proprionic acid methyl ester **24h**. The separation was carried out on the Waters Delta-Pak C18 column with gradient 5−40% acetonitrile in 100 mM TEAB (pH 8.0) over 60 min at a flow rate of 1.0 mL/min. The absorbance was monitored at $\lambda = 260$ nm. Peak 1: isomer I of compound **24a**. Peak 2: isomer II of compound **24a**. Peak 3: diastereomer mixtures of compound **24g**.

Conclusions

We developed two strategies for the synthesis of nucleoside boranophosphoramidates conjugated with amino acids. One strategy involved *H*-phosphonate chemistry in which the key intermediate, silylated phosphoramidite diester **6**, was obtained through a series of reaction steps starting from nucleoside *H*-phosphonate in the presence of condensing reagent DPCP. Subjecting the key intermediate **6** to boronation followed by hydrolysis with water resulted in the formation of nucleoside amino acid boranophosphoramidate **9**. Different condensing reagents were examined, and DPCP appeared to be the best choice. Due to the presence of a DMT group in the boranophosphoramidate **9**, the condensing reagent DPCP could be separated from the product **9** by aqueous extraction. However, if the molecule lacked a hydrophobic group like DMT, final product boranophosphoramidate compounds such as **24a** were contaminated with DPCP because both boranophosphoramidates **24** and the DPCP hydrolysis products were highly soluble in water. Further purification via silica gel chromatography did not resolve the problem. Hence, the relatively lengthy procedure and difficulties in purifying the final product limited the application of *H*-phosphonate chemistry in preparation of boranophosphoramidates and other relating phosphate analogues.

Synthesis of nucleoside amino acid boranophosphoramidate was better accomplished via a 1,3,2-oxathiaphospholane approach. The key intermediate oxathiaphospholane−borane complex **22** was obtained through phosphitylation of an amino acid methyl ester with 2-chloro-1,3,2-oxathiaphospholane followed by boronating with borane−dimethyl sulfide. Comparative studies on different boronating reagents and the optimal conditions for DBU-catalyzed ring-opening condensation were carried out. The reaction of DBU-assisted nucleophilic attack of nucleoside on the phosphorus atom of the oxathiaphospholane ring in **22** furnished an approach to various boranophosphoramidate analogues **24a**−**h** and **9** in good yields. Hence, the borane complex **22** represents a new intermediate for condensation reaction with various nucleosides. The oxathiaphospholane strategy is of particular interest since it provides a facile and efficient route to prepare biologically important analogues of normal phosphoramidates: boranophosphoramidates **24**. The two diastereomers of boranophosphoramidates **24a**−**f** were successfully separated by RP-HPLC. The structures for all of the final compounds **24a**−**g** and **9** were confirmed by spectroscopic methods including 2D-^{1}H NMR. We also confirmed by HPLC and mass analyses that the presence of DBU in the oxathiaphospholane ring-opening reactions did not induce noticeable racemization of boranophosphorylated derivatives of amino acids. It is worthwhile to emphasize that due to the mildness of the reaction conditions and the easily accessible phosphitylating reagent **20**, the synthetic protocol developed here can be considered as a general method for the preparation of molecules with the presence of both *P*−*N* and *P*−*B* bonds.

In summary, we developed two new strategies for the introduction of a boranophosphate moiety into amino acids to obtain nucleoside amino acid boranophosphoramidates **24**. On the basis of an "adjacent"-type mechanism, the tentative assignment of configurations for both diastereomers was carried out through molecular modeling and proton spectra in which the results were all in accordance with each other. The two diastereomers of nucleoside amino acid boranophosphoramidates are expected to have different substrate properties toward phosphoramidases 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 phosphoramidases. Moreover, due to the presence of a borane group,[10] the boranophosphoramidates are expected to have increased lipophilicity relative to their parent phosphoramidate compounds, which would facilitate the delivery of prodrugs containing antiviral nucleosides.[1,12] All of these advantages in combination with the potential

utility as a carrier of ^{10}B in BNCT[30] make the nucleoside amino acid boranophosphoramidate a useful compound and biochemical tool in antiviral drug research.

Experimental Section

2-Chloro-1,3,2-oxathiaphospholane[19a] and 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane (NEP−Cl)[31] were synthesized according to the reported procedure. 2,4,6-Trichlorophenol was recrystallized from benzene and ethanol. All solvents were freshly distilled under argon from calcium hydride. All boronating reagents except borane−dimethyl sulfide complex, diisopropylethylamine (DIPEA), and 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) were dried by 4 Å molecular sieves overnight. Nucleoside *H*-phosphonate, nucleosides, and amino acid methyl ester hydrochlorides were dried overnight under vacuum in the presence of P_2O_5 prior to use. Triethylammonium bicarbonate was prepared from triethylamine, H_2O, and CO_2. All the reactions were carried out under an argon atmosphere unless otherwise stated. All of the final products were in the triethylammonium salt forms. Proton assignment was based on the 2D $^1H-^1H$ COSY.

Synthesis of 5′-*O*-Dimethoxytritylthymidin-3′-yl L-Tryptophanyl-*H*-phosphonamidate 7 via *H*-Phosphonate Chemistry. 5′-*O*-Dimethoxytritylthymidine 3′-*H*-phosphonate **1** ($TEAH^+$ salt, 0.55 mmol, 1 equiv) and 2,4,6-trichlorophenol (0.60 mmol, 1.1 equiv) were rendered anhydrous by repeated coevaporation with added pyridine, dissolved in methylene chloride, and condensing reagent was added. When the condensation was completed,[32] chlorotrimethylsilane (1.65 mmol, 3 equiv) was injected into the reaction vessel and immediately followed by the addition of triethylamine (3.3 mmol, 6 equiv). After 5 min L-tryptophan methyl ester hydrochloride (0.44 mmol, 0.8 equiv) dissolved in anhydrous triethylamine (1.1 mmol, 2 equiv) was injected into the reaction vessel. After stirring for 2 h the aminolysis reaction mixture containing silylated phosphoramidite diester **6** was diluted with methylene chloride (30 mL) and washed repeatedly with 0.5 M $NaHCO_3$ (5 × 30 mL). The organic layer was dried over Na_2SO_4; the solvent was removed under vacuum; and the residue was coevaporated with a toluene−acetonitrile mixture (1:1, v/v). Crude product **7** was obtained as an off-white foam. ^{31}P NMR ($CDCl_3$): δ 15.12, 15.05. HRMS found, *m/z* 809.2943 (for ^{11}B); calcd for $C_{43}H_{46}N_4O_{10}P^+$ (MH^+), 809.2952. For the equivalency of different condensing reagents and the corresponding yield for crude product **7**, please refer to Table 1.

Synthesis of 2(*S*)-[(5′-*O*-Dimethoxytritylthymidin-3′-yl)boranophosphorylamino]-3-(3-indolyl)proprionic Acid Methyl Ester 9 via *H*-Phosphonate Chemistry. Silylated phosphoramidite diester **6** was obtained as described above when 1.0 equiv of DPCP was used as a condensing reagent. The reaction mixture was treated with borane−dimethyl sulfide (5.5 mmol, 10 equiv) for 45 min followed by addition of water (5 mL). The reaction mixture was stirred for another 30 min and then concentrated in a vacuum. The residue was dissolved in methylene chloride (30 mL) and extracted with 0.5 M $NaHCO_3$ (3 × 20 mL). The organic phase was dried over Na_2SO_4 and then evaporated to dryness. The residue was chromatographed on a silica gel column using a stepwise gradient of methanol (0−5%) in chloroform containing 0.5% TEA to afford **9** as a foam (130 mg, 32% yield). ^1H NMR (CD_3Cl): δ 7.57−7.50, 7.37−6.98, 6.81−6.79 (m, 19H, ArH and H-6); 6.36, 6.21 (2t, two *P*-isomers, J = 6.0 Hz, 1H, H-1′); 4.98, 4.75 (2m, two *P*-isomers, 1H, H-3′); 4.24 (m, 1H, H-4′); 4.15, 4.12 (2m, two *P*-isomers, 1H, CH); 3.74 (s, 6H, OMe from DMT); 3.56, 3.44 (2s, two *P*-isomer, 3H, OMe from CO_2Me); 3.40−3.15 (m, 4H, H-5′ and CH_2); 2.99 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$); 1.86 (m, 2H, H-2′); 1.32 (s, 3H, CH_3-5); 1.29 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$); (+)0.78 to (−)0.17 (br, 3H, BH_3). ^{31}P NMR (CD_3Cl): δ 93.5 (m, 1P). UV (H_2O): Isomer I λ_{max} 213.6, 272.3 nm; Isomer II λ_{max} 212.4, 272.3 nm. FAB-MS *m/z* 821.3 (M^-). HRMS: found, *m/z* 924.4498 (for ^{11}B); calcd for $C_{49}H_{64}BN_5O_{10}P^+$ [for (M + TEA + 2H)$^+$], 924.4484.

Synthesis of Nucleoside Amino Acid Boranophosphoramidates via an Oxathiaphospholane Approach: General Procedure. Amino acid methyl ester hydrochloride **19** (0.5 mmol, 1.0 equiv) and DIPEA (1.0 mmol, 2.0 equiv) were dissolved in methylene chloride. When the solution became clear, 2-chloro-1,3,2-oxathiaphospholane 20 (0.55 mmol, 1.1 equiv) was added. After 30 min borane−dimethyl sulfide complex (2.5 mmol, 5.0 equiv) was added, and the reaction mixture was stirred for another 30 min. A freshly prepared solution of nucleoside (0.4 mmol, 0.8 equiv) and DBU (1.5−5 equiv, see Table 3) in acetonitrile was injected into the reaction vessel via syringe. The reaction mixtures were then heated to 40−60 °C for 1−5 h (see Table 3). When the reaction was completed, the reaction mixtures were treated with triethylamine/water (1:1, v/v) for 15 min. Solvent was removed under vacuum, and the residue was coevaporated with ethanol (15 mL) three times before being loaded to the silica gel. Flash chromatography using a stepwise gradient of methanol (3−15%) in chloroform containing 0.5% TEA gave the desired product as an amorphous solid.

Reverse-Phase HPLC Separation of Diastereomers of Nucleoside Amino Acid Boranophosphoramidates. The separation of diastereomers of each nucleoside amino acid boranophosphoramidate 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 8 mL/min using a gradient or isocratic elution [100 mM triethylammonium bicarbonate (TEAB) buffer, pH = 8.0, and acetonitrile, see Table 4]. Fractions containing the same isomer (similar retention time) were combined, and the solvent was removed under reduced pressure. The excess amount of TEAB and acetonitrile was removed by repeated lyophilization with deionized water. The retention time and ratio for different diastereomers of nucleoside amino acid boranophosphoramidates **24a**−**f** are summarized in Table 4.

2(*S*)-[(3′-*O*-Acetylthymidin-5′-yl)boranophosphorylamino]proprionic Acid Methyl Ester (24a). The diastereomeric mixture of compound **24a** was prepared in 45% yield (99 mg) following the general procedure by using L-alanine methyl ester (HCl salt), 3′-*O*-acetylthymidine, and 3 mol equiv of DBU at 40 °C for 2 h.

Isomer I (tentatively assigned as Rp isomer). ^1H NMR (D_2O): δ 7.59 (s, 1H, H-6), 6.18 (t, J = 6.8 Hz, 1H, H-1′), 5.17 (m, 1H, H-3′), 4.17 (m, 1H, H-4′), 3.82 (m, 2H, H-5′), 3.70 (t, J = 7.6 Hz, 1H, CH), 3.54 (s, 3H, CH_3 from CO_2Me), 3.03 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 2.29 (m, 2H, H-2′), 1.97 (s, 3H, CH_3 from OAc), 1.79 (s, 3H, CH_3-5), 1.14 (d, J = 7.6 Hz, 3H, CH_3), 1.11 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), (+)0.90 to (−)0.35 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 90.7 (m, 1P). UV (H_2O): λ_{max} 266.4 nm. FAB-MS: *m/z* 446.3 (M^+). HRMS: found, *m/z* 446.1501 (for ^{11}B); calcd for $C_{16}H_{26}BN_3O_9P^+$ [for (M − 2e)$^+$], 446.1500.

Isomer II (tentatively assigned as Sp isomer). ^1H NMR (D_2O): δ 7.61 (s, 1H, H-6), 6.17 (dd, J = 5.6, 6.0 Hz, 1H, H-1′), 5.16 (m, 1H, H-3′), 4.20 (m, 1H, H-4′), 3.82 (m, 2H, H-5′), 3.76 (t, J = 7.6 Hz, 1H, CH), 3.54 (s, 3H, CH_3 from CO_2Me), 3.03 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 2.32 (m, 1H, H-2′), 2.21 (m, 1H, H-2′), 1.97 (s, 3H, CH_3 from OAc), 1.79 (s, 3H, CH_3-5), 1.16 (d, J = 7.2 Hz, 3H, CH_3), 1.11 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), (+)0.60 to (−)0.20 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 91.6 (m, 1P). UV (H_2O): λ_{max} 266.4 nm. FAB-MS: *m/z* 446.3 (M^+). HRMS: found, *m/z* 446.1501 (for ^{11}B); calcd for $C_{16}H_{26}BN_3O_9P^+$ [for (M − 2e)$^+$], 446.1500.

(30) Hawthorne, M. F. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 950.
(31) McConnell, R. L.; Coover, H. W. *J. Org. Chem.* **1959**, *24*, 630.
(32) A sample of reaction mixture (10 μL) was quenched with dry methanol (20 μL) prior to analysis to convert a hydrolytically unstable aryl *H*-phosphonate **3** into the stable methyl ester.

2(*S*)-[(3′-*O*-Acetylthymidin-5′-yl)boranophosphorylamino]-3-(3-indolyl)proprionic Acid Methyl Ester (24b). The diastereomeric mixture of compound **24b** was prepared in 40% yield (106 mg) following the general procedure by using L-tryptophan methyl ester (HCl salt), 3′-*O*-acetylthymidine, and 3 mol equiv of DBU at 60 °C for 2 h.

Isomer I (tentatively assigned as Rp isomer). ^{1}H NMR (D_2O): δ 7.37 (d, J = 8.0 Hz, 1H, H-4 on indolyl ring), 7.22 (s, 1H, H-6), 7.21 (d, J = 7.6 Hz, 1H, H-7 on indolyl ring), 7.01 (s, 1H, H-2 on indolyl ring), 6.96 (dd, J = 7.2, 8.0 Hz, 1H, H-6 on indolyl ring), 6.81 (dd, J = 7.2, 7.6 Hz, 1H, H-5 on indolyl ring), 6.03 (dd, J = 6.0, 6.0 Hz, 1H, H-1′), 5.01 (m, 1H, H-3′), 3.95 (m, 1H, H-4′), 3.82 (q, J = 8.8 Hz, 1H, CH) 3.66 (m, 1H, H-5′), 3.50 (s, 3H, CH_3 from CO_2Me), 3.37 (m, 1H, H-5′), 2.99 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 2.97, 2.89 (2m, 2H, CH_2), 2.07 (m, 1H, H-2′), 1.94 (s, 3H, CH_3 from OAc), 1.90 (m, 1H, H-2′), 1.56 (s, 3H, CH_3-5), 1.09 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), (+)0.80 to (−)0.40 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 91.5 (m, 1P). UV (H_2O): λ_{max} 220.6, 269.9 nm. FAB-MS: *m/z* 561.1 (M^-). HRMS: found, *m/z* 561.1896 (for ^{11}B); calcd for $C_{24}H_{31}BN_4O_9P^+$ [for $(M - 2e)^+$], 561.1922.

Isomer II (tentatively assigned as Sp isomer). ^{1}H NMR (D_2O): δ 7.45 (d, J = 7.6 Hz, 1H, H-4 on indolyl ring), 7.19 (m, 2H, H-7 on indolyl ring and H-6), 7.01 (s, 1H, H-2 on indolyl ring), 6.96 (dd, J = 7.2, 7.6 Hz, 1H, H-6 on indolyl ring), 6.88 (dd, J = 7.2, 7.6 Hz, 1H, H-5 on indolyl ring), 5.96 (t, J = 7.2 Hz, 1H, H-1′), 4.95 (m, 1H, H-3′), 4.02 (m, 2H, H-4′ and CH) 3.61 (m, 2H, H-5′), 3.54 (s, 3H, CH_3 from CO_2Me), 3.01 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 2.98, 2.86 (2m, 2H, CH_2), 1.95 (m, 4H, H-2′ and CH_3 from OAc), 1.57 (m, 4H, H-2′ and CH_3-5), 1.10 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), (+)0.60 to (−)0.40 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 91.3 (m, 1P). UV (H_2O): λ_{max} 219.4, 269.9 nm. FAB-MS: *m/z* 561.1 (M^-). HRMS: found, *m/z* 561.1915 (for ^{11}B), calcd for $C_{24}H_{31}BN_4O_9P^+$ [for $(M - 2e)^+$], 561.1922.

2(*S*)-[(3′-Deoxy-3′-azidothymidin-5′-yl)boranophosphorylamino]proprionic Acid Methyl Ester (24c). The diastereomeric mixture of compound **24c** was prepared in 38% yield (80 mg) following the general procedure by using L-alanine methyl ester (HCl salt), AZT, and 5 mol equiv of DBU at 45 °C for 5 h.

Isomer I (tentatively assigned as Rp isomer). ^{1}H NMR (D_2O): δ 7.50 (s, 1H, H-6), 6.10 (t, J = 6.8 Hz, 1H, H-1′), 4.32 (dt, J = 4.8, 5.6 Hz, 1H, H-3′), 3.97 (m, 1H, H-4′), 3.84 (m, 1H, H-5′), 3.80 (m, 1H, H-5′), 3.63 (q, J = 6.4 Hz, 1H, CH), 3.53 (s, 3H, CH_3 from CO_2Me), 3.03 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 2.34 (t, J = 6.4 Hz, 2H, H-2′), 1.78 (s, 3H, CH_3-5), 1.11 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), 1.07 (d, J = 6.4 Hz, 3H, CH_3), (+)0.82 to (−)0.30 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 90.9 (m, 1P). UV (H_2O): λ_{max} 267.6 nm. FAB-MS *m/z* 429.2 (M^-). HRMS: found, *m/z* 429.1473 (for ^{11}B); calcd for $C_{14}H_{23}BN_6O_7P^+$ [for $(M - 2e)^+$], 429.1459.

Isomer II (tentatively assigned as Sp isomer). ^{1}H NMR (D_2O): δ 7.56 (s, 1H, H-6), 6.08 (t, J = 6.8 Hz, 1H, H-1′), 4.29 (m, 1H, H-3′), 4.02 (m, 1H, H-4′), 3.83 (m, 2H, H-5′), 3.76 (q, J = 7.2 Hz, 1H, CH), 3.55 (s, 3H, CH_3 from CO_2Me), 3.03 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 2.32 (m, 2H, H-2′), 1.78 (s, 3H, CH_3-5), 1.15 (d, J = 7.2 Hz, 3H, CH_3), 1.11 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), (+)0.54 to (−)0.32 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 91.9 (m, 1P). UV (H_2O): λ_{max} 267.6 nm. FAB-MS: *m/z* 429.1 (M^-). HRMS: found, *m/z* 429.1450 (for ^{11}B); calcd for $C_{14}H_{23}BN_6O_7P^-$, 429.1459.

2(*S*)-[(3′-Deoxy-3′-azidothymidin-5′-yl)boranophosphorylamino]-3-(3-indolyl)proprionic Acid Methyl Ester (24d). The diastereomeric mixture of compound **24d** was prepared in 35% yield (90 mg) following the general procedure by using L-tryptophan methyl ester (HCl salt), AZT, and 5 mol equiv of DBU at 60 °C for 8 h.

Isomer I (tentatively assigned as Rp isomer). ^{1}H NMR (D_2O): δ 7.38 (d, J = 7.6 Hz, 1H, H-4 on indolyl ring), 7.23 (d, J = 8.4 Hz, 1H, H-7 on indolyl ring), 7.18 (s, 1H, H-6), 7.01 (s, 1H, H-2 on indolyl ring), 6.97 (t, J = 7.6 Hz, 1H, H-6 on indolyl ring), 6.83 (t, J = 7.6 Hz, 1H, H-5 on indolyl ring), 5.91 (t, J = 6.8 Hz, 1H, H-1′), 4.10 (m, 1H, H-3′), 3.79 (m, 2H, H-4′ and CH), 3.58 (m, 1H, H-5′), 3.51 (s, 3H, CH_3 from CO_2Me), 3.45 (m, 1H, H-5′), 3.01 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 2.99 (m, 1H, CH_2), 2.87 (m, 1H, CH_2), 2.12 (m, 1H, H-2′), 2.00 (m, 1H, H-2′), 1.58 (s, 3H, CH_3-5), 1.10 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), (+)0.50 to (−)0.40 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 91.7 (m, 1P). UV (H_2O): λ_{max} 215.9, 269.9 nm. FAB-MS *m/z* 544.1 (M^-). HRMS: found, *m/z* 544.1882 (for ^{11}B); calcd for $C_{22}H_{28}BN_7O_7P^+$ [for $(M - 2e)^+$], 544.1881.

Isomer II (tentatively assigned as Sp isomer). ^{1}H NMR (D_2O): δ 7.46 (d, J = 7.6 Hz, 1H, H-4 on indolyl ring), 7.20 (d, J = 8.0 Hz, 1H, H-7 on indolyl ring), 7.16 (s, 1H, H-6), 7.02 (s, 1H, H-2 on indolyl ring), 6.98 (dd, J = 7.2, 7.6 Hz, 1H, H-6 on indolyl ring), 6.90 (dd, J = 7.6, 8.0 Hz, 1H, H-5 on indolyl ring), 5.85 (t, J = 6.4 Hz, 1H, H-1′), 4.01 (m, 2H, H-3′ and H-4′), 3.86 (m, 1H, CH), 3.60 (m, 1H, H-5′), 3.55 (s, 3H, CH_3 from CO_2Me), 3.53 (m, 1H, H-5′), 3.05 (m, 1H, CH_2), 3.02 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 2.86 (m, 1H, CH_2), 1.96 (m, 1H, H-2′), 1.61 (m, 1H, H-2′), 1.57 (s, 3H, CH_3-5), 1.10 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), (+)0.52 to (−)0.18 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 91.5 (m, 1P). UV (H_2O): λ_{max} 211.2, 269.9 nm. FAB-MS: *m/z* 544.1 (M^-). HRMS: found, *m/z* 544.1877 (for ^{11}B); calcd for $C_{22}H_{28}BN_7O_7P^+$ [for $(M - 2e)^+$], 544.1881.

2(*S*)-[(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)boranophosphorylamino]proprionic Acid Methyl Ester (24e). The diastereomeric mixture of compound **24e** was prepared in 40% yield (78 mg) following the general procedure by using L-alanine methyl ester (HCl salt), d4T, and 1.5 mol equiv of DBU at 40 °C for 1 h.

Isomer I (tentatively assigned as Rp isomer). ^{1}H NMR (D_2O): δ 7.27 (s, 1H, H-6), 6.70 (s, 1H, H-1′), 6.29 (d, J = 6.0 Hz, 1H, H-2′), 5.78 (d, J = 6.0 Hz, 1H, H-3′), 4.87 (m, 1H, H-4′), 3.92 (m, 1H, H-5′), 3.65 (m, 1H, H-5′), 3.48 (s, 3H, CH_3 from CO_2Me), 3.31 (q, J = 7.2 Hz, 1H, CH), 3.00 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 1.72 (s, 3H, CH_3-5), 1.10 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), 1.03 (d, J = 7.2 Hz, 3H, CH_3), (+)0.40-(−)0.42 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 92.1 (m, 1P). UV (H_2O): λ_{max} 214.7, 266.4 nm. FAB-MS: *m/z* 386.2 (M^+). HRMS: found, *m/z* 386.1283 (for ^{11}B); calcd for $C_{14}H_{22}BN_3O_7P^+$ [for $(M - 2e)^+$], 386.1288.

Isomer II (tentatively assigned as Sp isomer). ^{1}H NMR (D_2O): δ 7.39 (s, 1H, H-6), 6.75 (s, 1H, H-1′), 6.28 (d, J = 6.0 Hz, 1H, H-2′), 5.77 (d, J = 6.0 Hz, 1H, H-3′), 4.88 (m, 1H, H-4′), 3.75 (m, 2H, H-5′), 3.66 (m, 1H, CH), 3.52 (s, 3H, CH_3 from CO_2Me), 3.01 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 1.73 (s, 3H, CH_3-5), 1.10 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), 1.09 (d, J = 7.2 Hz, 3H, CH_3), (+)0.42 to (−)0.42 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 91.3 (m, 1P). UV (H_2O): λ_{max} 215.9, 266.4 nm. FAB-MS: *m/z* 386.1 (M^-). HRMS: found, *m/z* 386.1273 (for ^{11}B); calcd for $C_{14}H_{22}BN_3O_7P^-$, 386.1288.

2(*S*)-[(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)boranophosphorylamino]-3-(3-indolyl)proprionic Acid Methyl Ester (24f). The diastereomeric mixture of compound **24f** was prepared in 38% yield (91 mg) following the general procedure by using L-tryptophan methyl ester (HCl salt), d4T, and 1.5 mol equiv of DBU at 60 °C for 2 h.

Isomer I (tentatively assigned as Rp isomer). ^{1}H NMR (D_2O): δ 7.37 (d, J = 8.0 Hz, 1H, H-4 on indolyl ring), 7.27 (d, J = 8.2 Hz, 1H, H-7 on indolyl ring), 7.17 (s, 1H, H-6), 7.03 (dd, J = 6.8, 8.0 Hz, 1H, H-6 on indolyl ring), 7.01 (s, 1H, H-2 on indolyl ring), 6.91 (dd, J = 6.8, 8.0 Hz, 1H, H-5 on indolyl ring), 6.68 (s, 1H, H-1′), 6.17 (d, J = 6.0 Hz, 1H, H-2′), 5.67 (d, J = 6.0 Hz, 1H, H-3′), 4.74 (m, 1H, H-4′), 3.65 (m, 3H, CH and H-5′), 3.39 (s, 3H, CH_3 from CO_2Me), 2.94 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 2.91 (d, J = 5.2 Hz, 2H, CH_2), 1.64 (s, 3H, CH_3-5), 1.07 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), (+)0.50 to (−)0.42 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 92.1 (m, 1P). UV (H_2O): λ_{max} 224.1, 269.9 nm. FAB-MS *m/z* 501.1 (M^-). HRMS: found, *m/z* 501.1712 (for ^{11}B); calcd for $C_{22}H_{27}BN_4O_7P^+$ [for $(M - 2e)^+$], 501.1710.

Isomer II (tentatively assigned as Sp isomer). ^{1}H NMR (D_2O): δ 7.41 (d, J = 8.0 Hz, 1H, H-4 on indolyl ring), 7.26 (d, J = 7.6 Hz, 1H, H-7 on indolyl ring), 7.21 (s, 1H, H-6), 7.02 (dd, J = 7.2, 8.0 Hz, 1H, H-6 on indolyl ring), 6.99 (s, 1H, H-2 on indolyl ring), 6.93 (dd, J = 7.2, 7.6 Hz, 1H, H-5 on indolyl ring), 6.66 (s, 1H, H-1′), 6.07 (d, J = 5.6 Hz, 1H, H-2′), 5.60 (d, J = 5.6 Hz, 1H, H-3′), 4.69 (m, 1H, H-4′), 3.97 (dd, J = 6.8, 7.2 Hz, 1H, CH), 3.59 (m, 2H, H-5′), 3.43 (s, 3H, CH_3 from CO_2-Me), 2.95 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 2.93 (d, J = 6.8 Hz, 2H, CH_2), 1.57 (s, 3H, CH_3-5), 1.07 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), (+)0.57 to (−)0.26 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 90.5 (m, 1P). UV (H_2O): λ_{max} 222.9, 269.9 nm. FAB-MS: *m/z* 501.2 (M^-). HRMS: found, *m/z* 501.1721 (for ^{11}B); calcd for $C_{22}H_{27}BN_4O_7P^+$ [for $(M - 2e)^+$], 501.1710.

2(*R*)-[(3′-*O*-Acetylthymidin-5′-yl)boranophosphorylamino]proprionic Acid Methyl Ester (24g). The diastereomeric mixture of compound **24g** was prepared in 38% yield (83 mg) following the general procedure by using D-alanine methyl ester (HCl salt), 3′-*O*-acetylthymidine, and 3 mol equiv of DBU at 40 °C for 2 h.

Diastereomer Mixture. ^{1}H NMR (D_2O): δ 7.63, 7.57 (2s, 2 isomers, 1H, H-6), 6.20 (t, J = 6.8 Hz, 1H, H-1′), 5.18 (m, 1H, H-3′), 4.15 (m, 1H, H-4′), 3.85−3.68 (m, 3H, CH and H-5′), 3.49 (s, 3H, CH_3 from CO_2Me), 3.03 (q, J = 7.2 Hz, 6H, CH_2 from $TEAH^+$), 2.21 (m, 2H, H-2′), 1.97 (s, 3H, CH_3 from OAc), 1.80 (s, 3H, CH_3-5), 1.15 (d, J = 7.2 Hz, 3H, CH_3), 1.11 (t, J = 7.2 Hz, 9H, CH_3 from $TEAH^+$), (+)0.42 to (−)0.20 (br, 3H, BH_3). ^{31}P NMR (D_2O): δ 90.9 (m, 1P). UV (H_2O): λ_{max} 266.4 nm. FAB-MS: *m/z* 446.1 (M^+). HRMS: found, *m/z* 446.1514 (for ^{11}B); calcd for $C_{16}H_{26}BN_3O_9P^+$ [for $(M - 2e)^+$], 446.1500.

2(*R,S*)-[(3′-*O*-Acetylthymidin-5′-yl)boranophosphorylamino]proprionic Acid Methyl Ester (24h). The diastereomeric mixture of compound **24h** was prepared in 37% yield (81 mg) following the general procedure by using racemic D,L-alanine methyl ester (HCl salt), 3′-*O*-acetylthymidine, and 3 mol equiv of DBU at 40 °C for 2 h.

Diastereomer Mixture. UV (H_2O): λ_{max} 266.4 nm. FAB-MS: *m/z* 446.1 (M^+). HRMS: found, *m/z* 446.1510 (for ^{11}B); calcd for $C_{16}H_{26}BN_3O_9P^+$ [for $(M - 2e)^+$], 446.1500.

2(*S*)-[(5′-*O*-Dimethoxytritylthymidin-3′-yl)boranophosphorylamino]-3-(3-indolyl)proprionic Acid Methyl Ester (9). The diastereomeric mixture of compound **9** was prepared in 36% yield (133 mg) following the general procedure by using L-tryptophan methyl ester (HCl salt), 5′-*O*-DMT-thymidine, and 5 mol equiv of DBU at 50 °C for 5 h.

^{1}H NMR and ^{31}P NMR data were the same as that of compound **9** prepared earlier via *H*-phosphonate chemistry.

Acknowledgment. This work was supported by an NIH grant (R01 AI52061) to B.R.S. and a Paul M. Gross fellowship from Duke University to P.L. We thank Mr. Michael Goldsmith for helpful discussion about molecular modeling and calculations.

Supporting Information Available: 1D-^{1}H NMR, ^{31}P NMR, and selected 2D-^{1}H NMR spectra for compounds **9** and **24a**−**g**; HPLC profiles for compounds **24a**−**f**; molecular modeling for compound **24a**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0481248