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Synthesis of Prodrug Candidates: Conjugates of Amino Acid with Nucleoside Boranophosphate

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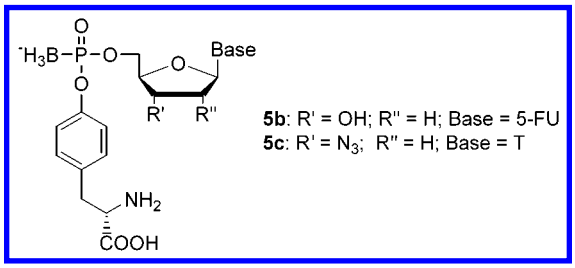
ABSTRACT



Preparation of antiviral and anticancer prodrug candidates, *P*-tyrosinyl(*P*-*O*)-5′-*P*-nucleosidyl boranophosphates, is described. One-pot synthesis via a phosphoramidite method resulted in the title compounds with good yields. The *P*-boranophosphate diastereomers were separated by RP-HPLC, and their structures were confirmed by ^{1}H and ^{31}P NMR spectroscopy and MS analysis.

The design of many antiviral drugs focuses on the inhibition of viral polymerases and reverse transcriptases (RT),[1] the key enzymes in the replicative cycle of any virus. Most of the available drugs are nucleoside analogues,[2] for example, 3′-azido-3′-deoxythymidine (AZT, Zidovudine),[2c] the first of five anti-HIV nucleoside analogues[2] approved by the FDA to treat AIDS patients. The activation mechanism of anti-HIV nucleoside analogues in vivo[2f,3] involves phosphorylation by cellular kinases into the corresponding 2′-deoxynucleoside mono- (dNMP), di- (dNDP), and triphosphate (dNTP). Then the biologically active dNTP is incorporated into the growing viral DNA chain, causing chain termination. Modified nucleosides are also used in cancer therapy. For example, 5-fluoro-2′-deoxyuridine (FdU) exerts its effects through formation of the nucleotide analogue 5-fluoro-2′-deoxyuridine monophosphate (FdUMP), which blocks DNA biosynthesis by deactivating thymidylate synthase (TS), a key enzyme for the production of thymidine-5′-monophosphate (TMP) from deoxyuridine-5′-monophosphate (dUMP).[4,5]

However, in many cases, the nucleoside analogue itself is a poor substrate for cellular kinases needed for its activation.[6] In some cases, activation of the particular nucleoside analogue may be restricted in cells where the nucleoside kinase activity is low or even deficient.[7] Therefore, several

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approaches have been advanced to circumvent the initial kinase-catalyzed phosphorylation step. The so-called ''prodrug approach" suggests the use of nucleoside analogues in an already phosphorylated form, masked with various protecting groups. These prodrugs are designed to readily penetrate into cells and easily convert intracellularly into dNMPs.[3,8]

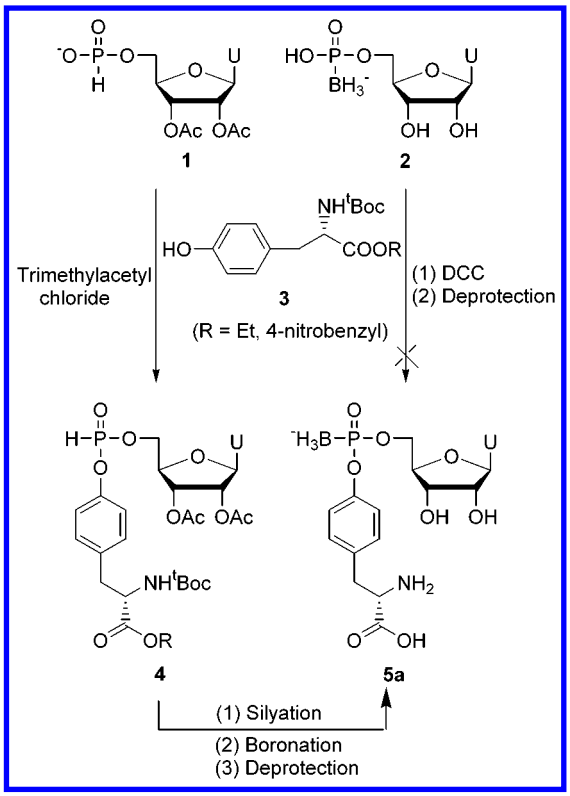
We propose to use boranophosphate diesters, in which one of the nonbridging oxygen atoms of a phosphate diester group is replaced by a borane group (BH_3),[9] as a candidate for such a prodrug. As reviewed by Shaw,[9f,g] boranophosphates are more lipophilic and nuclease-resistant than the normal phosphate diesters.[9e,10a] Our recent studies indicate that the R_P isomers of α-*P*-boronated dNTPs are better substrates than natural dNTPs for viral DNA polymerases,[11] making them more selective for incorporation into viral DNA. Meyer et al.[12] showed that the α-(R_P)boranodiphosphate of AZT is a 10-fold better substrate for diphosphate kinase than normal AZT diphosphate. Moreover, the α-(R_P)-boranotriphosphate of AZT has a 9-fold increased efficiency with HIV-RT compared with normal AZT triphosphate (AZTTP). The α-*P*-borano derivative of AZTTP has increased stability toward repair mechanisms that contribute to HIV drug resistance,[12] possibly because an α-*P*-boronophosphate in DNA is more resistant to nuclease than normal phosphate.[9e] Finally, boronated nucleotides may offer a unique advantage over other modified congeners because they could be used for boron neutron capture therapy (BNCT),[13] a radiation therapy that can selectively destroy cells that have preferentially taken up boron. All of these unique properties of boranophosphates make them promising candidates for design of antiviral and anticancer prodrugs.

As a prototypic masking group, we have chosen L-tyrosine. Amino acids are known as good carriers for the nucleoside prodrugs, forming nontoxic hydrolysis products.[8b,14] The phenyl ester bond, present in tyrosine-containing conjugates, is reported to be the most labile bond among the phenyl-, benzyl-, and alkylphosphates[15] because the negative charge formed upon hydrolysis can be delocalized into the aromatic ring. It facilitates the chemical or enzymatic hydrolysis of the prodrugs to form the biologically active nucleotide derivatives.

Specifically, in this article we report the first synthesis of *P*-tyrosinyl(*P*-*O*)-5′-*P*-nucleosidyl boranophosphates **5**.

Our first attempt to prepare the conjugate was based on an H-phosphonate approach that we generally use for synthesis of various nucleoside boranophosphate derivatives.[10] Condensation of uridine *H*-phosphonate **1** with protected tyrosine **3** gave the compounds **4** in only 25−30% yield after isolation (Scheme 1). Instability of the phenyl *H*-phosphonate diester during silica gel chromatography could be a reason for the low yield. Although subsequent

Scheme 1



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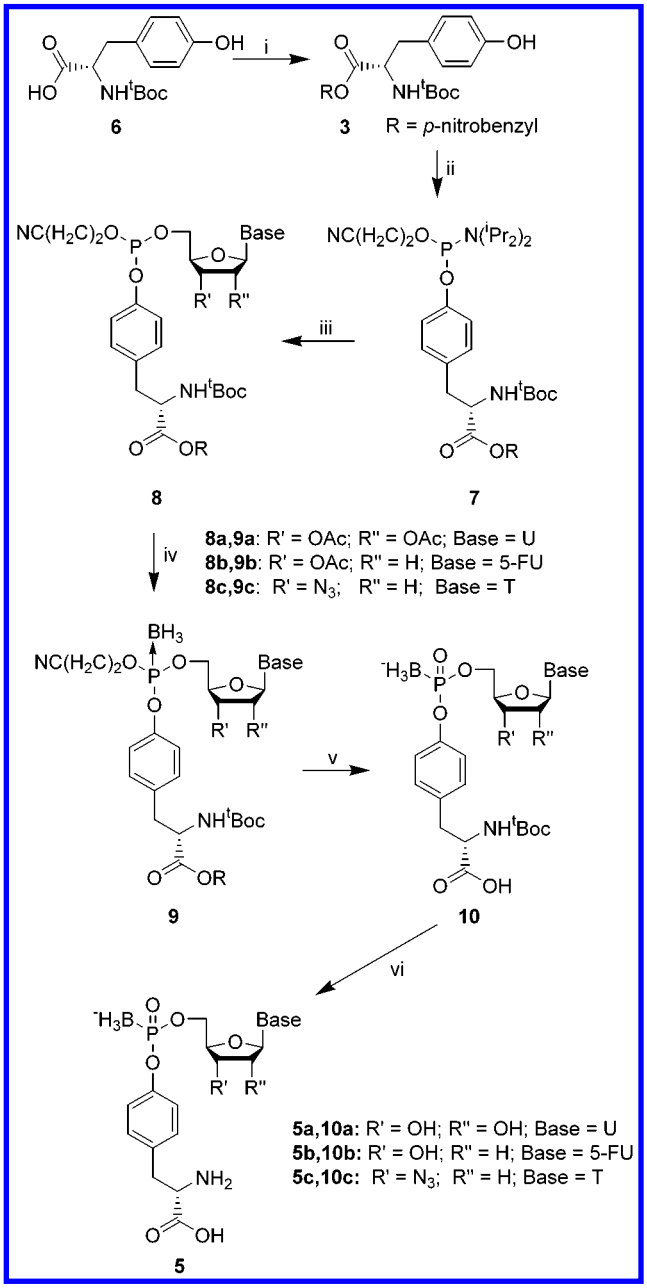
silylation and boronation proceeded smoothly, the overall yield of the final product **5a** after deprotection was less than 10%. Reaction of uridine 5′-boranomonophosphate (5′-UMPB) **2** with protected tyrosine **3** using DCC as condensing agent was also unsuccessful. Finally, we decided to synthesize the boranophosphate conjugate through a phosphoramidite intermediate obtained from protected tyrosine.

One of the crucial points in our synthesis was the choice of the protecting groups for the amine and acid functionalities of L-tyrosine. Conditions for cleaving these protecting groups have to fulfill the stability requirements for the final conjugates, which carry base- and nucleophile-sensitive functionalities. Moreover, at least one UV-active aryl group was preferred because it could produce a tag for monitoring the reaction and chromatography. Therefore, the *tert*-butyloxycarbonyl (*t*-Boc) and *p*-nitrobenzyl groups were selected as amino and acid protections, respectively.

The syntheses of the desired *P*-tyrosinyl(*P*-*O*)-5′-*P*-nucleosidyl boranophosphates **5a**−**c** were carried out as outlined in Scheme 2. Tyrosine was additionally protected with a *p*-nitrobenzyl group, as described previously,[16] and the resulting compound **3** was phosphorylated by 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphane (2-OCEt-P[N(^{i}Pr$_2$)$_2$]) in the presence of 1*H*-tetrazole to give phosphoramidite **7**. After 40 min, nucleoside and another portion of 1*H*-tetrazole were added into the reaction mixture. Condensation was completed in 15 min as evidenced by ^{31}P NMR spectra, where the singlet at 148 ppm for phosphoramidite **7** was transformed into two singlets around 135 ppm corresponding to the diastereomers of phosphotriester conjugates **8** (ratio 1:1). Without purification, in situ boronation of intermediates **8** resulted in the formation of boranophosphotriesters **9**. The presence of the *P* → *B* bond in intermediates **9** was confirmed by ^{31}P NMR spectra, which showed the characteristic broad peak centered at 116 ppm.[10] Of several borane complexes used for boronation, borane-dimethyl sulfide (2.5 equiv at 0 °C for 45 min) gave the best results. The reaction mixture was then evaporated to dryness and extracted with ethyl acetate and water. The organic layers were concentrated and treated with a mixture of ammonium hydroxide and methanol (1:1, v/v) for 10 h. The removal of the *t*-Boc group was carried out by treating compounds **10** with trifluoroacetic acid in acetonitrile (1:1, v/v) for 25 min. The final products **5** were precipitated by anhydrous ethyl ether and isolated by ion-exchange chromatography. The overall yields (from **3** to **5a**−**c**) were 37−46%. The conjugates, *P*-tyrosinyl(*P*-*O*)-5′-*P*-nucleosidyl boranophosphates, were identified by ^{31}P NMR (typical broad peaks at 91 ppm), ^{1}H NMR spectroscopy, and HRMS. Two *P*-boranophosphate diastereomers for each conjugate were separated by reverse-phase HPLC (RP-HPLC) using gradient elution for **5a,b** and isocratic elution for **5c**.

Scheme 2[a]



[a] (i) *p*-Nitrobenzyl bromide; (ii) 2-OCEt-P[N(*i*Pr$_2$)$_2$], tetrazole, HN(*i*Pr$_2$)$_2$; (iii) nucleoside, tetrazole; (iv) BH$_3$·SMe$_2$; (v) NH$_4$OH, MeOH (1:1, v/v); (vi) TFA in CH$_3$CN (1:1, v/v).

To summarize, we have synthesized the first nucleoside-amino acid conjugates connected through a boranophosphate linkage. The one-pot reaction gave the products **5a**−**c** in 37−46% overall yield after isolation. The *P*-boranophosphate diastereomers of uridine, 5-FdU, and AZT were separated by RP-HPLC. Such conjugates are expected to show good bioavailability and enhanced cellular uptake. They should be hydrolyzed enzymatically, leaving an amino acid and

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putatively nontoxic boronated nucleotide.[17] The improved substrate properties, increased lipophilicity, and nuclease resistance imparted by the borane group, in conjunction with the potential utility as a carrier of ^{10}B in boron neutron capture therapy, make the *P*-tyrosinyl(*P*-*O*)-5′-*P*-nucleosidyl boranophosphate a promising candidate for antiviral and anticancer prodrugs.

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Supporting Information Available: Spectra data for new compounds **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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