Synthesis of AZT/d4T Boranophosphates as Anti-HIV Prodrug Candidates

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Abstract: AZT/d4T phosphonates were synthesized by sequential condensation of AZT/d4T with the corresponding alcohols or 5′-DMT-thymidine in the presence of pivaloyl chloride. Sequential silylation, boronation, and hydrolysis in ammonium hydroxide of these phosphonates led to anti-HIV prodrug candidates AZT/d4T boranophosphates.

Key words: anti-HIV, AZT, d4T, phosphonate, boranophosphate

In combating AIDS and its related complex, the search for therapeutic agents possessing activity against HIV has yielded a number of compounds demonstrating potent and selective antiviral activity. Despite the recent introduction of HIV protease1 and integrase2, reverse transcriptase is an attractive target for the chemotherapy of HIV.3 Among the current diversity of compounds active against HIV, the 2′, 3′-dideoxynucleosides (ddNs) remain by far the most potent,4 and 3′-azido-3′-deoxthymidine (AZT, zidovudine) and 2′,3′-dideoxy-2′, 3′-didehydrothymidine (d4T, stavudine) have prime activity.

However, it has been proven that these compounds must be phosphorylated intracellularly to their active triphosphate form before acting as an competitive inhibitor or alternate substrate (chain terminators) of HIV-RT.5 Their potency as drugs depends on their uptake by the infected cells, their phosphorylation by cellular kinases and their ability to block viral DNA synthesis. Furthermore, some clinical drawbacks such as the toxicity of AZT which may be related to monophosphorylation.6 Consequently, in order to overcome decreased intracellular phosphorylation, a number of systems have been developed for the delivery of monophosphorylated antiviral nucleosides.7 Of the various prodrug approaches, 5′-phosphonates of AZT and d4T have shown promise as potent antiviral agents, since in some cases they have exhibited enhanced antiviral activity and reduced cytotoxicity when compared to the parent nucleosides. For example, selective indexes of AZT 5′-cy clohexylphosphate (2e in Scheme 1) and d4T 5′-isopropylphosphate (2d in Scheme 1) are 18.9 and more than 6.11 times more effective, respectively compared with AZT and d4T.8

Recently, nucleoside boranophosphates have attracted great interest as a new type of nucleoside analog. In boranophosphates, one of non-bridging phosphoryl oxygen atoms in a nucleotidyl phosphodiester was replaced by borane (BH₃). The borane group in boranophosphates is isoelectronic with oxygen in phosphodiesters and sulfur in phosphorothioates; it is isoster with the CH₃ group in methylphosphonate nucleosides. Several research groups have studied their application in DNA sequencing, in antisense and therapeutic applications, and in boron neutron capture therapy. For example, Shaw’s group9 has showed that boranophosphates are more lipophilic and nuclease-resistant than the normal phosphate diesters. Meyer et al.10 also proved that the α-(Rₙ)-boranophosphate of AZT is a 10-fold better substrate for diphosphate kinase than normal AZT diphosphate. Furthermore, α-(Rₙ)-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, possibly because α-P-borano-phosphate in DNA is more resistant to nuclease than normal phosphate. Besides, Hawthorne11 studied boronated nucleotides that could be used for boron neutron capture therapy, a radiation therapy that can selectively destroy cells that have preferentially taken up boron. As boranophosphates have such particular properties, they become the promising candidates for the design of antiviral and anticancer prodrugs. In this paper, AZT/d4T boranophosphates as anti-HIV prodrug candidates were synthesized.

Synthesis of AZT/d4T Phosphonate Diesters

Phosphonate monoesters are usually synthesized through the reaction of alcohols with phosphorus trichloride/triazole, followed by hydrolysis in aqueous triethylammonium bicarbonate12 or the mono-displacement of diphenyl phosphate by alcohols and sequential hydrolysis in triethylamine/water.13 Phosphonate diesters were prepared by condensation of phosphonate monoesters with alcohols in the presence of condensing agents14 or hydrolysis of tricordinated phosphoramidates in the presence of 1H-tetrazole.15 But these methods suffered from laborious synthetic procedure, variable yields, or incompatibility with common protecting groups utilized in natural product chemistry. Therefore, we developed a convenient, efficient, and general method for the preparation of phosphonate mono and diesters. Here, mono and diesters of phosphonates were synthesized through reaction of
phosphonic acid with different alcohols in the presence of pivaloyl chloride (PV-Cl). At room temperature, 1.1 equivalents of PV-Cl was added dropwise to AZT or d4T along with 1.1 equivalents of phosphonic acid in pyridine, the reaction was complete in a few minutes, and $^{31}$P NMR showed more than 90% reaction yields of 1 and 1'. Further condensation of 1 or 1' with 2 equivalents of alcohol in the presence of PV-Cl produced 2a–d and 2'a–d in good yields under mild conditions as shown in Scheme 1.

Synthesis of Dinucleoside Phosphonates

Various homo- and hetero-dinucleosides, such as AZT-P-AZT, AZT-P-ddI, AZT-P-ddC, have been synthesized and tested in HIV-infected MT-2 cells, and it was found that dinucleoside analogues showed enhanced anti-HIV potency relative to their monomers. In addition, AZT-P-ddI was 10 times less toxic than AZT to human granulocyte-macrophage progenitor cells. By far, the majority of the dinucleoside prodrugs were constructed from two unnatural nucleosides, i.e. 2', 3'-dideoxynucleoside (ddNs). Dinucleotide phosphonates were prepared as shown in Scheme 2 according to the literature. Reaction of 5'-DMT-thymidine (5) and diphenyl phosphite in dry pyridine at 0 °C under nitrogen atmosphere followed by hydrolysis in triethylamine and water at room temperature led to 6, dinucleotide phosphate 7 or 7' was obtained after coupling of dry 6 and AZT or d4T by PV-Cl agent. Without isolation, 7 or 7' was deprotected with formic acid in dichloromethane, and 8 or 8' was obtained as a white foam after purification by column chromatography (CHCl₃–MeOH).

Synthesis of AZT/d4T Boranophosphates

We realized that conjugates combined with AZT/d4T and boranophosphates could be good anti-HIV agents, so it is important to search for a method to synthesis AZT/d4T boranophosphates. Phosphonate was dissolved in anhydrous THF, and N,N,O-bis(trimethylsilyl)acetamide (BSA) was added to the solution at room temperature. One hour later, phosphonate completely changed into the corresponding tricoordinated phosphite, borane-N,N-diisopropylethylamine complex ($i$-Pr₂NEt·BH₃) was added to the above solution and stirred again for 1 hour, hydrolysis in concentrated ammonia–ethanol (3:1) gave AZT/d4T boranophosphate 4, 4', 9 or 9'. Other AZT/d4T boranophosphates were synthesized in moderate yields by using the similar method.

In summary, phosphonate mono and diesters of AZT and d4T were synthesized in an easy one-pot reaction using phosphonic acid as starting material in the presence of pivaloyl chloride. Compared with other methodologies, this method is fast, convenient and efficient in mild reaction conditions, and it can be used to synthesize other phosphonates, such as symmetric and asymmetric dialkyl phosphonates, dinucleoside phosphonates, and carbohydrate phosphonates. Both phosphonic acid and pivaloyl chloride are commercially available and inexpensive re-
agents, therefore, this method could be applied to the large-scale production of phosphonate mono and diesters. AZT/d4T boranophosphates were synthesized through the silylation of relevant phosphonates, boronation with an amine–borane complex followed by hydrolysis in ammonium hydroxide and ethanol. As boranophosphates could improve lipophilicity, increase nuclease resistance and treat boron neutron capture therapy, the conjugates containing AZT/d4T and boranophosphates could be a kind of potent anti-HIV produg candidates, and the study on their anti-HIV activity is in progress.

Column chromatography was performed on silica gel 300–400 mesh. Pyridine and THF were dried over CaH₂ and Na, respectively. 1H NMR and 13C NMR spectra were recorded on Bruker AM 500 spectrometer at 81 MHz under 1H decoupled conditions. 31P NMR spectra were taken on a Bruker AC 200 spectrometer at 81 MHz under 1H decoupled conditions. 31P NMR chemical shifts were reported in ppm downfield (+) or upfield (−) from external 85% H₃PO₄ as reference. Mass spectra were obtained using a Bruker Esquire ion-trap mass spectrometer. BSA and i-Pr₂NEt·BH₃ and phosphonic acid were purchased from Aldrich commercially available from local suppliers. Column chromatography was performed on silica gel 300–400 mesh. Pyridine and THF were dried over CaH₂ and Na, respectively.

**Scheme 2** Synthetic route of dinucleoside boranophosphates

![Synthetic route of dinucleoside boranophosphates](image)

**Compounds 2a-c and 2a-c**

Phosphonic acid (1.1 mmol, 90.2 mg) and nucleoside (1 mmol) were dissolved in anhyd pyridine (5 mL) and co-evaporated twice; they were then dissolved in fresh anhyd pyridine (5 mL). Pivaloyl chloride (1.1 mmol, 132.6 mg) in pyridine (2 mL) was added dropwise to the above solution under a N₂ atmosphere at r.t. After ca. 5 min the reaction was complete (1H NMR). Without isolation, the corresponding alcohol (2 equiv) was added to the above solution, and then pivaloyl chloride (2 equiv, 241 mg) in pyridine was added dropwise at r.t. After 10 min the second condensation was complete (1H NMR). After evaporation of pyridine the crude product 2a and 2a-c was purified by column chromatography.

**O-Ethyl-O'-[(3-azido-3-deoxythymidin-5-yl) Phosphonate (2a)**

Yield: 66%; white foam.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>66%</td>
<td>White foam</td>
</tr>
</tbody>
</table>

**O-Benzyl-O'-[(3-azido-3-deoxythymidin-5-yl) Phosphonate (2b)**

Yield: 62%; white foam.

**O-Hexadeyl-O'-[(3-azido-3-deoxythymidin-5-yl) Phosphonate (2c)**

Yield: 64%; white foam.
**O-Benzyl-O-(2, 3-didehydro-2', 3'-dideoxythymidin-5'-yl) Phosphonate (2b)**

Yield: 54%; white foam.

**O-Hexadeoxy-d(2', 3'-dideoxythymidin-5'-yl) Phosphonate (2c)**

Yield: 65%; white foam.

**O-Ethyl-O-(2', 3'-dideoxythymidin-5'-yl) Phosphonate (2a)**

Yield: 63%; white foam.

**O-Phosphonate (2d)**

Yield: 40%; white foam.
Yield: 0.635 g (72.7%); white foam; Rf 0.41 (CHCl₃–MeOH, 20:1).

13C NMR (CDCl₃, 125 MHz): δ = 163.03 (C-4), 150.96 (C-2), 135.89, 135.86* (C-6, C-132), 132.97, 132.95* (C-2, C-127), 126.79, 126.75* (C-3), 111.23 (C-5), 89.59, 89.50* (C-4), 81.45* (C-4), 77.03 (CH₃), 65.38, 65.20* (C-5, 2JPC = 5.5 Hz), 33.74, 33.45, 24.83, 23.54, 23.45 (CH₃), 12.41, 12.38 (5-Me).

13P NMR (CDCl₃, 81 MHz): δ = 7.53, 7.12* (dr, 1:1).

ESI-MS: m/z = 371 [M + H]+, 393 [M + Na]+.

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Boronation; General Procedure

Phosphonate diester (0.5 mmol) was dried over P₂O₅ under vacuum for 4–5 h and then dissolved in freshly distilled THF (5 mL). BSA (616 L, 2.5 mmol) was added by syringe and the solution was stirred for about 1 h at r.t. until the phosphonate diester was completely converted to triancidiethylphosphinate (1/3P NMR, i-Pr₂NEBH₃) (869 L, 5 mmol) was added, and the solution stirred for 1 h, concentrated NH₃–EtOH (10 mL, 3:1) was added to the solution. After the solvents were evaporated under reduced pressure, the residue was purified by column chromatography (EtOAc–MeOH–NH₃, 180:30:5) to give the target 4, 4′, 9, or 9′.


O-Ethyl-O′-(3-amido-3-deoxythymidin-5′-yl) Boranophosphate (4a)

Yield: 57.4%; colorless oil.

1H NMR (CD₃OD, 500 MHz): δ = 7.50–7.45 (m, 1 H, CH), 3.4–3.2 (m, 2 H, OH), 3.12–3.00 (m, 3 H, H₃N), 2.95–2.80 (m, 3 H, CH₂), 0.88 (t, 3 H, CH₃), 0.67 (s, 3 H, CH₃).
$\text{O-Benzyl-O''-(3'-azido-3'-deoxythymidin-5'-yl) Boronophosphate (4b)}$

Yield: 62.3%; colorless oil.

$\text{1H NMR (CD3OD, 500 MHz):} \delta = 7.88, 7.87^\ast$ (d, 1 H, H-6), 6.28, 6.26^\ast$ (d, 1 H, H-1', J = 6 Hz), 4.52–3.96 (m, 5 H, CH2-5', H-4', H-3'), (CH3), 2.46–2.28 (m, 2 H, H-2'), 1.96–1.89 (m, 13 H, CH3-5), (CH2)2, 0.6–0.2 (q, 3 H, BH3).

$\text{13C NMR (CD3OD, 125 MHz):} \delta = 166.41 (C4), 152.39, 152.34^\ast$ (C2), 137.98, 137.91* (C6), 112.10, 112.07* (C5), 85.71 (C1'), 85.21, 85.10^* (C4', J = 6.5 Hz), 73.61, 73.22* (CH3), 63.62, 63.39* (C5'), 63.07, 63.02* (C3'), 38.23, 38.14* (C2'), 35.35–25.15 (5 C, CH3), 13.17, 12.76^* (5-Me).

$\text{ESI-MS (CD3OD, 81 MHz):} \delta = 94.4–100.4$ (br).

HRMS (ESI): $\text{m/z}$ caleed for C15H28BN2O10P: 426.1714; found: 426.1716.

$\text{O-Ethyl-O''-(2',3',3'-didehydro-2',3'-dideoxythymidin-5'-yl) Boronophosphate (4a)}$

Yield: 61.4%; colorless oil.

$\text{1H NMR (CD3OD, 500 MHz):} \delta = 6.94, 6.93^*$(d, 1 H, H-6), 6.42 (d, 1 H, H-2', J = 1.5 Hz), 5.89, 5.88^*$(d, 1 H, H-3', J = 2.0 Hz), 4.96 (s, 1 H, H-4'), 4.32–4.29 (m, 1 H, CH2CH3), 3.98–3.91 (m, 2 H, H-5'), 1.94, 1.93* (s, 3 H, CH3-5), 1.32–1.28 (m, 3 H, CH2CH3), 0.6–0.2 (q, 4 H, BH3).

$\text{13C NMR (CD3OD, 125 MHz):} \delta = 166.68 (C4), 152.87 (C2), 136.19, 138.66*(C6), 135.93, 135.81* (C-3'), 111.89 (C5), 90.92, 90.88* (C-1'), 87.48, 87.41* (d, C-4', J = 8.5 Hz), 69.47 (CH2CH3), 11.06–12.95 (5 C, CH3), 20.28, 20.16* (CH2CH3), 12.72, 12.65* (5-Me).

$\text{ESI-MS (CD3OD, 81 MHz):} \delta = 93.8–98.5$ (br).

HRMS (ESI): $\text{m/z}$ caleed for C15H24BN2O10P: 392.1074; found: 392.1077.

$\text{O-Benzyl-O''-(2',3'-didehydro-2',3'-dideoxythymidin-5'-yl) Boronophosphate (4b)}$

Yield: 57.8%; colorless oil.

$\text{1H NMR (CD3OD, 500 MHz):} \delta = 7.68$ (s, 1 H, H-6), 7.45 (s, 5 H, Ph), 6.94, 6.93*(d, 1 H, H-1', J = 1.5 Hz), 6.43 (d, 1 H, H-2', J = 1.8 Hz), 5.89, 5.87*(d, 1 H, H-3', J = 2.0 Hz), 5.21 (d, 2 H, CH2Ph), 4.98 (s, 1 H, H-4'), 4.02–3.96 (m, 2 H, H-5'), 1.94 (s, 3 H, CH3-5), 0.6–0.2 (q, 4 H, BH3).

$\text{13C NMR (CD3OD, 125 MHz):} \delta = 166.65 (C4), 152.83 (C2), 139.17, 137.81* (C6), 135.94, 135.83* (C-3'), 132.15–130.77 (Ph), 127.06, 126.96* (C-3'), 111.89 (C5), 90.92, 90.88* (C-1'), 87.48, 87.41*(d, C-4', J = 8.4 Hz), 68.22, 67.72* (CH2Ph, J = 4.0 Hz), 64.69, 63.92* (C-5', J = 5.0 Hz), 12.70, 12.63* (5-Me).

$\text{ESI-MS (CD3OD, 81 MHz):} \delta = 92.8–99.2$ (br).

HRMS (ESI): $\text{m/z}$ caleed for C15H24BN2O10P: 391.1230; found: 391.1235.

$\text{O-Hexadecyl-O''-(2',3'-didehydro-2',3'-dideoxythymidin-5'-yl) Boronophosphate (4c)}$

Yield: 60.1%; white solid.

$\text{1H NMR (CD3OD, 500 MHz):} \delta = 7.69, 7.67^*$(d, 1 H, H-6), 6.94 (d, 1 H, H-1'), 6.30 (d, 1 H, H-2', J = 5 Hz), 5.89 (s, 1 H, H-3', J = 4.5 Hz), 4.96 (s, 1 H, H-4'), 4.16–4.07 (m, 2 H, OHC(CH2CH3)2CH3), 3.98–3.93 (m, 2 H, H-5'), 1.91 (s, 3 H, CH3-5), 1.57–1.55 (m, 2 H, OHC(CH2CH3)2CH3), 1.31–1.27 [br, 26 H, CH2(CH2CH3)2].
OCH3(CH2)5CH3, 0.87 [t, 3 H, OCH3(CH2)5CH3, J = 6.5 Hz], 0.6–0.2 (t, 3 H, BH3).

1H NMR (CD3OD, 125 MHz): δ = 166.60 (C-4), 152.80 (C-2), 138.68, 138.65* (C-6), 135.83, 135.73* (C-2'), 127.16, 127.09* (C-3'), 111.95 (C-5), 90.87, 90.84* (C'-1'), 87.45, 87.39* (d, C-4', J = 6.3 Hz), 64.67 (C-5'), 64.13, 63.90* [d, OCH3(CH2)5CH3, J = 4.4 Hz], 33.06–23.72 [m, OCH3(CH2)5CH3], 14.46 [OCH3(CH2)5CH3], 12.72, 12.65 (5-Me).

13C NMR (CD3OD, 125 MHz): δ = 166.60 (C-4), 152.80 (C-2), 138.68, 138.65* (C-6), 135.83, 135.73* (C-2'), 127.16, 127.09* (C-3'), 111.95 (C-5), 90.87, 90.84* (C'-1'), 87.45, 87.39* (d, C-4', J = 6.3 Hz), 64.67 (C-5'), 64.13, 63.90* [d, OCH3(CH2)5CH3, J = 4.4 Hz], 33.06–23.72 [m, OCH3(CH2)5CH3], 14.46 [OCH3(CH2)5CH3], 12.72, 12.65 (5-Me).

13P NMR (CD3OD, 125 MHz): δ = 94.2–99.3 (br).

HRMS (ESI): m/z calculated for C35H46BN3O6P: 568.1728; found: 568.1724.

O-Thymidyl-O'-(2', 3'-dideoxy-2', 3'-dideoxythymidin-5-yl) Boronophosphate (9)

Yield: 57.4%; white solid.

1H NMR (D2O, 500 MHz): δ = 66.58, 66.47 (s, 1 H, H-6), 66.47, 66.46* (s, 1 H, H-6), 6.50–6.49 (m, 1 H, d4T-H-1'), 6.24, 6.21 (d, 1 H, T-H-1', J = 6.5 Hz), 5.99, 5.98* (d, 1 H, d4T-H-3'), 5.11 (s, 1 H, T-H-3'), 4.87 (s, 1 H, d4T-H-4'), 4.17–3.72 (m, 5 H, d4T-H-5', T-H-5', T-H-4'), 2.48–2.22 (m, 2 H, T-H-2'), 1.93, 1.91 (d, 6 H, 2 × C5-CH3).

31P NMR (D2O, 500 MHz): 37.43* (T-C-2), 31P NMR (D2O, 81 MHz): 11.42, 11.15 (5-CH3).

HRMS (ESI): m/z calculated for C35H46BN3O6P: 568.1728; found: 552.1561.

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References


