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Tetrahedron Letters 45 (2004) 671–675
Tetrahedron Letters

Synthesis of nucleoside–amino acid conjugates containing boranephosphate, boranephosphorothioate and boranephosphoramidate linkages ☆

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Received 15 October 2003; revised 31 October 2003; accepted 14 November 2003

Abstract—Conjugates of 3′-azido-2′,3′-dideoxythymidine (AZT) and 2′,3′-didehydro-2′,3′-dideoxythymidine (D4T) with the hydroxyl group of tyrosine containing boranephosphate and boranephosphorothioate moieties were prepared via the oxathiaphospholane and dithiaphospholane methodology as a mixture of P-diastereomers. Their structures were confirmed by MS analysis and ^{1}H, ^{31}P NMR spectroscopy. It has been shown that the boranephosphorothioate linkage is unstable under acidic conditions. The first nucleoside–alanine conjugates connected through a boranephosphoramidate linkage were also obtained.

Several nucleoside analogues have found successful application as antiviral and anticancer agents.[1] Their mode of action differs, but in the most general terms they have been developed as inhibitors or competitors of natural 2′-deoxynucleosides in the process of their conversion to corresponding nucleoside-5′-triphosphates.[2] As such, they can be incorporated into a growing viral DNA strand by a DNA polymerase, resulting in chain termination.[3] In cancer therapy, modified nucleosides, after being phosphorylated to the corresponding monophosphates, block DNA biosynthesis by deactivating nucleoside syntheses.[4] Hence biological activity of nucleoside analogues in most cases is dependent on intracellular phosphorylation by viral and/or cellular kinases to their respective mono-, di-, and triphosphate derivatives. Among the three successive activating phosphorylation steps the first one has fundamental importance as the rate-limiting step.[5] Several different enzymes can perform this initial phosphorylation, depending on the nature of the aglycone. Also, the presence and activity of the intracellular enzymes necessary for activation of nucleoside analogues are highly dependent on the host species, the cell type, and the stage in the cell cycle.[6] Moreover, in many cases, nucleoside analogues are poor substrates for the cellular kinases needed for their activation.[7] For all these reasons, intracellular nucleoside monophosphate (NMP) delivery has been considered for overcoming the first phosphorylation step.

Unfortunately, NMPs themselves cannot be used as potential chemotherapeutic agents. Owing to their high polarity, these compounds are not able to penetrate cellular membranes or the blood–brain barrier easily. Therefore, in order to reduce the phosphate negative charge and enable the modified nucleotide to enter the cell, many nucleotides, so-called prodrugs, modified on the phosphate moiety have been synthesized.[8] Among the current diversity of prodrug approaches, amino acid phosphoramidate derivatives appeared to be an interesting class of antiviral agents.[9] Amino acids as a masking group of the negatively charged phosphate moiety fulfill requirements of lipophilicity for passive diffusion through the membrane barrier and nontoxicity after hydrolytic or enzymatic release in the target cell.[8a]

Recently, utilizing phosphoramidite chemistry, conjugates of tyrosine with nucleoside boranephosphates have

Keywords: Nucleoside; Amino acids; Conjugates; Prodrugs; Boranephosph(orothio)ate; Boranephosphoramidate; Oxathiaphospholane chemistry.

☆ Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2003.11.066

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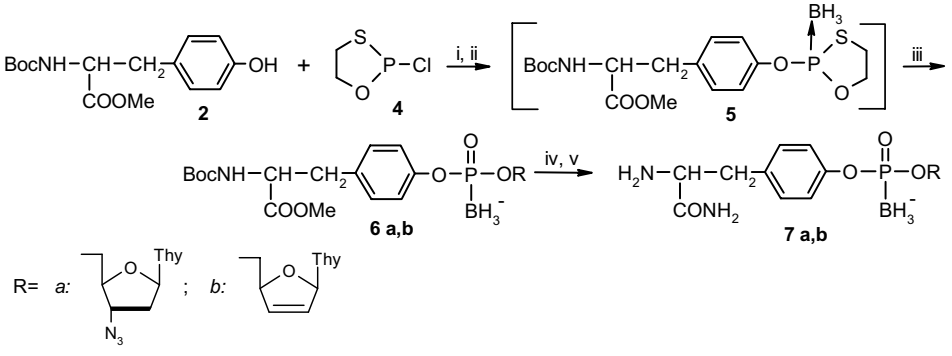
doi:10.1016/j.tetlet.2003.11.066

been obtained as new prodrug candidates.[10] In the family of phosphate-modified nucleotides, boranephosphates are new members, introduced by Shaw and co-workers.[11] Nucleoside-5′-*O*-boranephosphates, where one of the two nonbridging oxygen atoms of phosphate is replaced by a borane group (BH_3), are isoelectronic with phosphates. Because they are more lipophilic and nuclease resistant than the normal phosphates,[12] they might find applications in biochemical and molecular biological investigations. Boranephosphates may also have utility as anticipated carriers of ^{10}B in boron neutron capture therapy (BNCT) for the treatment of cancer.[13]

Recently, we have synthesized the first nucleoside–amino acid conjugates connected through a phosphorothioate or phosphorodithioate linkage.[14] These compounds were obtained employing a method developed by Stec involving a 1,3,2-oxathia(-dithia)phospholane ring-opening condensation process.[15] This process requires tricoordinate phosphorus compounds as the precursors, and, therefore, as demonstrated earlier, is suitable for the introduction of a boranephosphate moiety.[16] Moreover, depending on the P^{III} intermediate used as the starting material for the boronation step (1,3,2-oxathiaphospholane vs 1,3,2-dithiaphospholane derivatives), besides boranephosphates the boranephosphorothioate diesters can also be prepared. In this communication we present syntheses of nucleoside–amino acid conjugates connected through boranephosphate, boranephosphorothioate, or boranephosphoramidate moieties. As antiviral nucleosides we have chosen 3′-azido-2′,3′-dideoxythymidine (AZT, **1a**) and 2′,3′-didehydro-2′,3′-dideoxythymidine (D4T, **1b**). These nucleoside analogues, although structurally related, show a totally different pattern of intracellular phosphorylation.[17] As the amino acid parts of conjugates we have used appropriately protected L-tyrosine **2** and L-alanine **3**.

The initial step in the present approach—oxathiaphosphitylation of the amino acid—was performed according to the procedure evolved earlier in our laboratory for the phosphorothioylation of amino acids.[18] Hence, *N*-(*tert*-butoxycarbonyl)-L-tyrosine methyl ester **2** reacted for 3 h with 2-chloro-1,3,2-oxathiaphospholane **4** in the presence of bis(*N*,*N*-diisopropyl)ethylamine (Scheme 1) in methylene chloride solution gave the corresponding P^{III} derivative as was evidenced by the ^{31}P NMR spectrum (a singlet at 171.1 ppm). Next, without isolation of intermediates, in situ boronation was carried out by means of Me_2S–BH_3 providing the oxathiaphospholane–borane complex **5**. Its formation was confirmed by the presence of the characteristic broad resonance line centered at 159.2 ppm in its ^{31}P NMR spectrum.[12b] In earlier boronation experiments we used 6 equiv of borane–diisopropylethylamine (BH_3–DIPEA) complex, but the reaction was rather slow and went to completion in 12 h. When the P^{III}–oxathiaphospholane intermediate was treated with borane–dimethyl sulfide complex (2.5 equiv) the desired complex **5** was formed in 30 min at room temperature. An attempt to isolate complex **5** by column chromatography was unsuccessful due to its reactivity. Therefore, the crude product **5** was reacted with 1 equiv of **1a** or **1b** in the presence of 1 equiv of DBU providing after 12 h compounds **6a**,**b**, which were isolated by silica gel column chromatography [methanol in $CHCl_3$ (2–10%)] in 60–63% yields as mixtures of diastereoisomers, further characterized by ^{31}P NMR and FAB-MS analysis (Table 1). In general, esters are better prodrugs than amides, but in some cases the substitution of the amino acid methyl ester with an amide moiety leads to an increase in inhibitory activity.[19] Hence compounds **6a**,**b** were transformed into **7a**,**b** after their overnight treatment with concentrated aqueous ammonia (the conversion of methoxycarbonyl function into carboxamide) followed by the removal of the *t*-Boc protecting group. Products **7a**,**b** were isolated by ion-exchange chromatography (DEAE Sephadex A-25) in overall yields of 30–32% (from **2** to **7**). ^1H NMR,[20] ^{31}P NMR, and FAB-MS analysis (Table 1) confirmed their structures.

The results presented above on the application of oxathiaphospholane methodology to the preparation of conjugates of an hydroxyamino acid with a nucleoside boranephosphate prompted us to check the possibility of the synthesis of the corresponding boranephosphorothioate by the dithiaphospholane approach. When **2** was phosphitylated with 2-chloro-1,3,2-dithiaphospholane **8**[21] in the presence of DIPEA in CH_2Cl_2, the corresponding P^{III} intermediate [^{31}P NMR (C_6D_6): δ 153.8 ppm] was formed. Subsequent boronation (Me_2S–BH_3, 2.5 equiv, 1.5 h) gave complex **9** [^{31}P NMR (C_6D_6): δ 168.5 ppm], which was condensed in situ with **1a** or **1b**

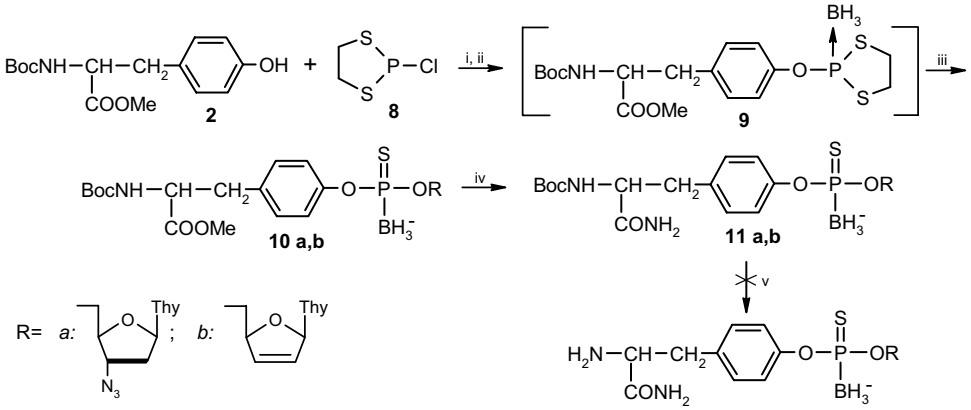


Scheme 1. Reagents and conditions: (i) bis(*N*,*N*-diisopropyl)ethylamine; (ii) BH_3–SMe_2; (iii) ROH (**1**), DBU; (iv) NH_4OH; (v) TFA–CH_3CN.

Table 1. The physicochemical characteristics of compounds **6a**,**b**, **7a**,**b**, **10a**,**b**, **11a**,**b**, **13a**,**b**, and **14a**,**b**

Entry	Conjugate[a]	^{31}P NMR (δ, ppm)[b]	FAB-MS (M−1) (m/z)	Yield (%)[c]
1	**6a**	92.7; 90.9[d]	621.3	60
2	**6b**	93.0; 91.3[d]	578.3	63
3	**7a**	94.9; 94.3; 93.7; 93.1[e,f]	506.2	32
4	**7b**	95.6; 94.4[e]	463.3	30
5	**10a**	167.3[d]	637.2	48
6	**10b**	167.6; 166.9[d]	594.3	46
7	**11a**	161.1; 160.5[d]	622.0	33
8	**11b**	164.7; 164.1[d]	579.3	31
9	**13a**	106.6; 105.3; 104.0; 102.7[e,f,g]	429.2	45
10	**13b**	105.5; 103.7[e]	386.3	42
11	**14a**	108.9; 107.6; 106.3; 105.0[e,f,g]	414.2	32
12	**14b**	106.2; 104.3[e]	371.0	30

[a] All products were obtained as a diastereomeric mixture.
[b] The spectra were measured on a 500 MHz spectrometer except where otherwise indicated.
[c] Yields refer to isolated products.
[d] In CD_3OD.
[e] In D_2O.
[f] The quartet due to ^{31}P coupling with ^{11}B($I = 3/2$).
[g] The spectra were measured on a 200 MHz spectrometer.

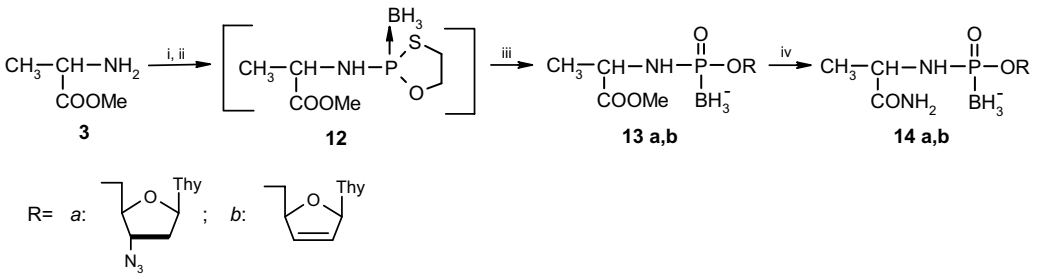


Scheme 2. Reagents and conditions: (i) bis(*N*,*N*-diisopropyl)ethylamine; (ii) BH_3–SMe_2; (iii) ROH (**1**), DBU; (iv) NH_4OH; (v) TFA–CH_3CN.

in the presence of DBU (Scheme 2). The resulting conjugates **10a**,**b** were isolated by silica gel column chromatography [methanol in $CHCl_3$ (2–14%)] in 46–48% yields and were identified by ^{31}P NMR and FAB-MS analyses (Table 1). Their treatment with concentrated aqueous ammonia for 12 h at room temperature converted the methyl ester function in **10a**,**b** into carboxamide **11a**,**b**. Finally compounds **11a**,**b** were treated with TFA in CH_3CN (1:1, v/v) for 30 min in order to remove the *t*-Boc protecting group. However, to our surprise, ^{31}P NMR inspection of the reaction mixture showed the disappearance of the broad resonance line at ca. 160 ppm and the appearance of several signals, among them a broad one at 103 ppm. Components of that mixture could be neither separated nor identified. This indicated that the boranephosphorothioate linkage in a nucleoside–amino acid conjugate is, in contrast to boranephosphates, unstable under acidic conditions. Hence, the amino acid to be used in the synthesis of boranephosphorothioates should contain a protecting group, which can be removed hydrolytically under basic conditions.

Recent publications[8,9] considering conjugates of nucleosides with amino acids through a phosphoramidate linkage as potential prodrugs for nucleoside monophosphates, prompted us to synthesize the related but hitherto unknown class of compounds, boranephosphoramidate derivatives, such as **13a**,**b** and **14a**,**b** by application of the methodology reported in this paper. In an analogous way, borane–*N*-(1,3,2-oxathiaphospholanyl)–L-alanine methyl ester complex **12** was obtained [^{31}P NMR (C_6D_6): δ 136.0, 134.4 ppm] and then was condensed in situ with **1a** or **1b** in the presence of DBU (Scheme 3). The crude compounds **13a**,**b** were treated with concentrated aqueous ammonia at room temperature to convert methoxycarbonyl function into carboxamide and final products **14a**,**b** were isolated from the reaction mixture by means of DEAE Sephadex A-25 chromatography in 30–32% overall yields. Their structures were confirmed by ^{1}H NMR,[20] ^{31}P NMR, and FAB-MS analyses (Table 1).

In general, each compound (**6a**,**b**, **7a**,**b**, **10a**,**b**, **11a**,**b**, **13a**,**b**, **14a**,**b**) consists of a mixture of P-diastereoisomers;



Scheme 3. Reagents and conditions: (i) 2-chloro-1,3,2-oxathiaphospholane (**4**), DIPEA; (ii) BH_3–SMe_2; (iii) ROH (**1**), DBU; (iv) NH_4OH.

6a,**b**, **7a**,**b**, and **14a**,**b** could be separated by RP-HPLC into individual isomers.[20]

In summary, the results presented in this paper show, that the synthesis of nucleoside–amino acid conjugates (phosphodiesters and phosphoramidoesters) connected through a boranephosphate linkage can be successfully performed by using the oxathiaphospholane methodology. This demonstrates the exceptional versatility of oxathiaphospholane chemistry for the preparation of nucleoside–amino acid conjugates. Moreover, we have generated new prodrug candidates **13a**,**b** and **14a**,**b** possessing a boranephosphate moiety linked to the α-NH group of an amino acid. An attempt to apply the dithiaphospholane approach for the preparation of diesters **11a**,**b** was successful although the boranephosphorothioate linkage is surprisingly unstable under acidic conditions. This observation is in contrast with the results reported by Lin and Shaw that dithymidine boranephosphorothioates were stable under similar conditions.[22] The contrasting stability of **11a**,**b** compared to the Shaw compounds may be associated with the arylalkyl nature of **11a**,**b** versus the dialkyl system of dinucleoside boranephosphorothioates. The results of ongoing experiments examining this possibility will be reported in due course.

Supplementary material: ^1H NMR spectra and HPLC analysis of compounds **7a**, **11a**, and **14**, can be found in the online version with this article on doi:10.1016/j.tetlet.2003.11.066.

Acknowledgements

This project was supported financially by the State Committee for Scientific Research (KBN), grant PBZ-KBN 059/T09/06 to J.B. The authors are grateful to Prof. Wojciech J. Stec for helpful discussions and critical comments. We would also like to thank Dr. Kyoichi A. Watanabe for careful reading of the manuscript.

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