Graphical Abstract

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Synthesis of nucleoside 5'-boranophosphorothioate derivatives using an *H*-boranophosphonate monoester as a precursor

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ABSTRACT

We developed a method to convert a nucleoside 5'-*H*-boranophosphonate monoester into the corresponding nucleoside 5'-boranophosphorothioate monoester through temporary protection of the *H*-boranophosphonate monoester moiety as a diester with 9-fluorenylmethanol, subsequent sulfurization of the P-H group and removal of the 9-fluorenylmethyl group. Although the isolation of the resultant boranophosphorothioate monoester was found to be difficult due to instability of the compound, this new method proved to be useful to synthesize some conjugates of the nucleoside 5'-boranophosphorothioate with other biomolecules, such as cholesterol and an amino acid.

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Chemically modified 5'-nucleotide analogs have been studied for years for their potential as antiviral and anticancer agents inhibiting the biosynthesis of disease-related nucleic acids in cells.¹ *P*-Boronated 5'-nucleotide analogs have been developed relatively recently, but have attracted much attention lately.² These analogs are highly lipophilic and stable to nucleases, which would favor their transport to the targets without degradation. Some of these analogs have been reported to show antineoplastic³, anti-inflammatory⁴, and hypolipidemic⁵ activities *in vitro* and *in vivo*. Studies toward the development of antiviral *P*-boronated nucleotide analogs have also been reported.⁶⁻⁸ Furthermore, such boronated nucleotide analogs are potentially useful as ¹⁰B carriers for boron neutron capture therapy.⁹

However, only limited kinds of *P*-boronated 5'-nucleotide analogs are currently available due to the lack of synthetic methods. To solve this problem, we have recently developed nucleoside *H*-boranophosphonate derivatives.¹⁰ These compounds are also categorized as *P*-boronated nucleotide analogs but distinctively different from the others in that they have a hydrogen atom on their phosphorus atom. Thus, the phosphorus atom can be further functionalized by deprotonation and subsequent reaction with electrophiles (Figure 1). We expect that this property makes the *H*-boranophosphonate derivatives versatile precursors of various *P*-boronated nucleotide analogs. In addition, nucleoside 5'-*H*-boranophosphonate monoesters can be

modified by condensation with other molecules, such as alcohols and amines (Figure 1).

Figure 1. Approaches to derivatization of nucleoside 5'-*H*-boranophosphonate monoesters.

In this study, we focused on the synthesis of nucleoside 5'-boranophosphorothioate derivatives using a nucleoside 5'-*H*-boranophosphonate monoester as a precursor. The target molecules are one of the *P*-boronated 5'-nucleotide analogs in which two of the three non-bridging oxygen atoms of the phosphomonoester group are replaced with a borane group and a sulfur atom. These molecules are considered as potential therapeutic nucleotide analogs because such double modifications have been reported to enhance the lipophilicity and stability to enzymatic hydrolysis significantly.¹¹ However, there are only a few reports on their synthesis in the literature and their properties have not been studied in detail.^{6b,11,12} We expected that

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the application of *H*-boranophosphonate derivatives as precursors would facilitate access to the boranophosphorothioate derivatives. Furthermore, sulfurization of the *H*-boranophosphonate monoester would be a good model of other P–H modifications with electrophiles, such as chalcogenization, alkylation, and acylation.

We have already demonstrated that a dinucleoside *H*-boranophosphonate could be converted into the corresponding dinucleoside boranophosphorothioate by treatment with S_8 and Et_3N .¹⁰ However, in the case of an *H*-boranophosphonate monoester, the free P–OH group should be protected before sulfurization. In fact, when a thymidine *H*-boranophosphonate monoester derivative (Scheme 1, **1**), which was synthesized by condensation of 3'-*O*-benzoylthymidine with pyridinium *H*-boranophosphonate according to our previous procedure,¹⁰ was subjected to the same sulfurization conditions, multiple unidentified side products were generated. So, we decided to protect the *H*-boranophosphonate monoester group temporarily and employed the protection as silyl esters first (Scheme 1). Compound **1** was converted into silyl esters **2** by treatment with *N,O*-bis(trimethylsilyl)acetamide, TBDMSCl or *i*-Pr₃SiCl, and the resultant silyl esters **2** were treated with S_8 or Beaucage reagent¹³ without isolation. The reactions were conducted in MeCN and THF in the presence of Et_3N or 1,8-bis(dimethylamino)naphthalene (DMAN). However, none of these reactions gave the desired boranophosphorothioate derivative **3** and most of the starting material **1** was recovered after aqueous work-up. The use of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to accelerate the deprotonation of the P–H group resulted in the formation of a complex mixture.

Scheme 1. Attempted synthesis of thymidine 5'-boranophosphorothioate monoester **3** via silyl esters **2**.

These results can be attributed to the relatively low acidity of the P–H group of the silyl esters **2** which have a highly polarized $O^{\delta-}-Si^{\delta+}$ bond adjacent to the same phosphorus atom. The deprotonation of the P–H group should be easier if the compound **1** is protected as a diester with an alcohol because a C–O bond is generally much less-polarized than a Si–O bond.¹⁴ In fact, it has been reported that the reaction of $(EtO)_2P(→BH_3)H$ with aldehydes at the phosphorus atom was readily promoted by deprotonation with *i*-Pr₂NEt, whereas that of $(i-Pr_3SiO)(EtO)P(→BH_3)H$ was not.¹⁵ On the basis of this hypothesis, we employed the protection as a 9-fluorenylmethyl (Fm) ester because the protection could be easily carried out by condensation of the compound **1** with 9-fluorenylmethanol (FmOH) and it would also be easily deprotected by treatment with an amine under anhydrous conditions (Scheme 2). First, the compound **1** was allowed to condense with FmOH in the presence of bis(2-oxo-3-oxazolidinyl)phosphinic chloride (Bop-Cl) and Et_3N . However, the product **4** once formed was slowly converted back into the starting material **1** in the reaction

mixture. It was because of the β -elimination of the compound **4** promoted by Et_3N . It was confirmed by a ¹H NMR analysis, which showed the formation of dibenzofulvene in the crude mixture. To prevent this side reaction, we replaced Et_3N with DMAN because the latter has been reported not to decompose 2-cyanoethyl-protected boranophosphate triesters by β -elimination.¹⁶ The triethylammonium cation of **1** was also exchanged prior to the condensation by the addition of DMAN and subsequent repeated coevaporation with toluene. In this case, the desired product **4** was obtained without decomposition. The product **4** was then treated with S_8 without purification to synthesize the boranophosphorothioate diester derivative **5**. A ³¹P NMR analysis showed that the sulfurization proceeded smoothly without observable side reactions. Finally, the compound **5** was treated with DBU to remove the Fm group. The deprotection itself proceeded without any side reactions. However, we found that the compound **3** was unstable and always partially decomposed during the purification by silica gel column chromatography. There is a report on the synthesis and isolation of a nucleoside 5'-boranophosphorothioate monoester in modest yield,^{11a} but every attempt to isolate the product **3** (e.g., reversed-phase and ion-exchange chromatography, countercation exchange and precipitation) failed in our hands. It has also been reported that nucleoside 5'-boranophosphate monoesters are more sensitive to hydrolysis than the natural nucleoside 5'-phosphates, especially under acidic conditions.¹⁷ Because 3'-*O*-Bz-thymidine was observed as a decomposition product, the partial decomposition of the compound **3** can also be attributed to the cleavage of the P–O bond by hydrolysis.

Scheme 2. Synthesis of thymidine 5'-boranophosphorothioate monoester **3** via 9-fluorenylmethyl esters **4** and **5**.

Thus, the study showed that the desired 5'-boranophosphorothioate monoester was difficult to isolate. However, it was also demonstrated that the nucleoside 5'-*H*-boranophosphonate monoester derivative could be smoothly converted into the boranophosphorothioate counterpart as planned. Given this result, we next explored the possibility of developing conjugates of the nucleoside 5'-boranophosphorothioate monoester with biomolecules in which the unstable monoester moiety would be trapped into a more stable form. Conjugates of therapeutic 5'-nucleotides with biomolecules, such as lipophilic sterols and amino acids, have been widely studied to improve the therapeutic effects of the parent 5'-nucleotides.¹ Such conjugates are much more lipophilic than the parent phosphate monoesters, which is advantageous for their delivery into cells. It has been reported that the conjugates are gradually hydrolyzed in cells to regenerate the 5'-nucleotides.¹ We expect that the strategy may also be useful for

the delivery of relatively unstable 5'-nucleotide analogs to targets, such as the compound **3** shown above. New conjugates of *P*-boronated 5'-nucleotide analogs may also exert therapeutic activity by themselves. There have been a few reports on the

synthesis of *P*-boronated 5'-nucleotide–amino acid conjugates in the literature aiming to develop antiviral agents.⁶ Development of a novel method using an *H*-boranophosphonate derivative as precursor would expand the availability of such conjugates.

Scheme 3. Synthesis of thymidine 5'-boranophosphorothioate–cholesterol conjugate **8** via *H*-boranophosphonate intermediate **6**.

Scheme 4. Synthesis of thymidine 5'-boranophosphorothioate–phenylalanine ethyl ester conjugate **11** via *H*-boranophosphonate intermediate **9**.

We chose cholesterol as the first model of lipophilic biomolecules to synthesize a new *P*-boronated 5'-nucleotide conjugate **8** as shown in Scheme 3. Compound **1** was condensed with cholesterol by using Bop-Cl and DMAN to give the thymidine 5'-*H*-boranophosphonate–cholesterol conjugate **6**. We used DMAN even though the compound **6** would not undergo a β-elimination as in the case of the Fm ester **4** because the use of Et₃N caused a partial deboronation of the product **6** probably due to the coordination of Et₃N to the BH₃. The deboronation was completely suppressed by replacing Et₃N with DMAN. Because the compound **6** was found to be relatively unstable on silica gel, we sulfurized it without purification. The sulfurization reaction was slow but proceeded without observable side reactions to afford the 3'-*O*-Bz-thymidine 5'-boranophosphorothioate–cholesterol conjugate **7**, and the subsequent deprotection gave the final product **8**. Contrary to the boranophosphorothioate monoester derivative **3**, the compounds **7** and **8** were stable and isolated in good yield.

Next, we applied this method to the synthesis of a conjugate with an amino acid. As shown in Scheme 4, the compound **1** was allowed to condense with L-phenylalanine ethyl ester to give the intermediate **9** having a P–H group. Compound **9** was then sulfurized and treated with potassium ethoxide to give the desired thymidine 5'-boranophosphorothioate–phenylalanine ethyl ester conjugate **11** in good yield. Compounds **10** and **11** were also found to be stable in contrast to the monoester **3**.

In conclusion, we developed a method to synthesize nucleoside 5'-boranophosphorothioate derivatives using a nucleoside 5'-*H*-boranophosphonate monoester as a precursor. Although the nucleoside 5'-boranophosphorothioate monoester could not be isolated due to the instability of the compound, we

consider that the new strategy using temporary protection of the *H*-boranophosphonate monoester moiety as a Fm diester will be useful to synthesize diverse *P*-doubly modified *P*-boronated 5'-nucleotide analogs. Many studies reported in the literature on the P–H functionalization of secondary phosphine–boranes by various reactions, such as alkylation and transition metal-catalyzed arylation,¹⁸ as well as the recent study on the alkylation of *H*-boranophosphonate esters, such as (EtO)₂P(→BH₃)H and (*i*-Pr₃SiO)(EtO)P(→BH₃)H,¹⁵ would be informative to explore this possibility. Evaluation of biological activity of the nucleoside 5'-boranophosphorothioate conjugates which were successfully synthesized in this study is also intriguing and will be presented elsewhere.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:

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