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A new boranophosphorylation reaction for the synthesis of deoxyribonucleoside boranophosphates

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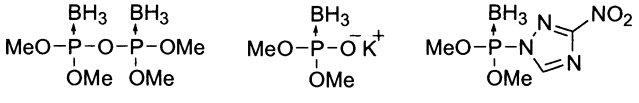
Abstract—Deoxyribonucloside 3′-boranophosphate derivatives including adenine, cytosine, guanine, and thymine bases were synthesized in good yields by the use of a new boranophosphorylating reagent. The reaction was successfully applied to the formation of internucleotidic boranophosphate linkages. © 2002 Published by Elsevier Science Ltd.

Oligodeoxyribonucleotides bearing internucleotidic boranophosphate linkages (boranophosphate DNA) are regarded as potentially useful antisense molecules.[1] The methods reported so far for the synthesis of this DNA analog are accomplished by constructing an oligonucleotide chain by the phosphoramidite or *H*-phosphonate approach, followed by boronation of the corresponding trivalent phosphite intermediate.[2–5] Undesirable side reactions, however, occur at the base moieties in the boronation step which are caused by the borane reagent.[3,4,6,7] Thus, the methods are applicable only to the synthesis of thymine derivatives, in which the thymidine moiety is less reactive to the borane reagent. In this paper, we wish to describe a new boranophosphorylation reaction, which can be applied to the synthesis of deoxyribonucleoside boranophosphates including A, C, and G as well as T without incurring side reactions at the base moieties.

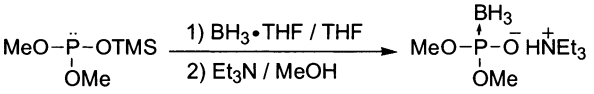
Mononucleoside 3′-boranophosphate derivatives have not been used as starting materials to date for the synthesis of oligonucleotides having boranophosphate linkages. Imamoto et al.[8] have employed tetramethyl boranopyrophosphate (**1**) and potassium dimethyl boranophosphate (**2**) as new reagents for boranophosphorylation (Scheme 1). Since the boranophosphorylating reagent **1** is less reactive for the nucleophilic attack of an alcohol, activation of the hydroxyl function should be required; *t*-BuLi was used as a strong base to generate the corresponding alkoxide at −78°C in THF.[8] However, this reaction is apparently not suitable for solid-phase synthesis of oligomers.

Therefore, we tried to activate the boranophosphorylating reagent **1** by using a nucleophilic catalyst, such as *N*-methylimidazole (MeIm)[9] or 3-nitro-1,2,4-triazole (NT).[10] First, the nucleoside **6t** was allowed to react with **1** in the presence of MeIm in THF; the boranophosphorylation proceeded with partial removal of the borano group (^{31}P NMR analysis). A similar reaction by using NT as an activator did not take place at all. In contrast, when a strong base such as Et_3N or *i*-Pr_2NEt was added to the reaction mixture for the deprotonation of NT, the boranophosphorylation proceeded smoothly without loss of the borano group. Thus, the desired 5′-*O*-dimethoxytrityl-thymidin-3′-yl dimethyl boranophosphate (**7t**) was obtained in 41% yield.[11] In this reaction, the putative intermediate **3**, which is highly reactive and susceptible to hydrolysis,



1 **2** **3**

Scheme 1.



4 **5**

Scheme 2.

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would be formed. Therefore, in order to improve the yield, we tried to generate the reactive intermediate **3** in situ from potassium dimethyl boranophosphate (**2**) and NT in the presence of a condensing reagent, which can eliminate a trace amount of water from the reaction mixture. However, the potassium salt **2** was found to be inefficient for the reaction because of its low solubility in organic solvents. In order to overcome this problem, we selected triethylammonium dimethyl boranophosphate (**5**) as a starting material for **3**, which could be synthesized from dimethyl trimethylsilyl phosphite (**4**)[12] in quantitative yield (Scheme 2).

The reaction of the resulting **5** with **6t** in the presence of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT)[13] and Et_3N proceeded quickly to give the desired **7t** in 32% yield, accompanied with the formation of a 3′-*O*-sulfonylated product as a by-product in 42% yield. This result indicates that MSNT reacts with the 3′-hydroxy group of **6t** as well as the boranophosphate anion of **5**. Therefore, we tested several condensing reagents, which were not able to react with the 3′-hydroxyl group of **6t**. As a result, *N*,*N*′-bis(2-oxo-3-oxazolidinyl)phosphonic chloride (Bop-Cl)[14] was found to be an effective condensing reagent for the present boranophosphorylation. It was also found that *i*-Pr_2NEt was more effective than Et_3N in the reaction. Thus, the condensation of the nucleoside **6t** with **5** in the presence of Bop-Cl, NT, and *i*-Pr_2NEt in THF proceeded quickly, and the desired product **7t** was obtained in 91% yield without any by-products.[15] In a similar manner, 5′-*O*-dimethoxytrityl-6-*N*-benzoyldeoxyadenosin-3′-yl dimethyl boranophosphate (**7a**), and 5′-*O*-dimethoxytrityl-4-*N*-benzoyldeoxycytidin-3′-yl dimethyl boranophosphate (**7c**) were synthesized in good yields from **6a** and **6c**, respectively (Table 1). In the case of the deoxyguanosine derivative **6g**, the formation of the 6-*O*-boranophosphorylated product was observed to some extent by a TLC analysis. However, the dimethyl boranophosphoryl group at the 6-*O*-position was readily hydrolyzed during the aqueous work-up of the reaction mixture to give 5′-*O*-dimethoxytrityl-2-*N*-(phenylacetyl)deoxyguanosin-3′-yl dimethyl boranophosphate (**7g**) in good yield.

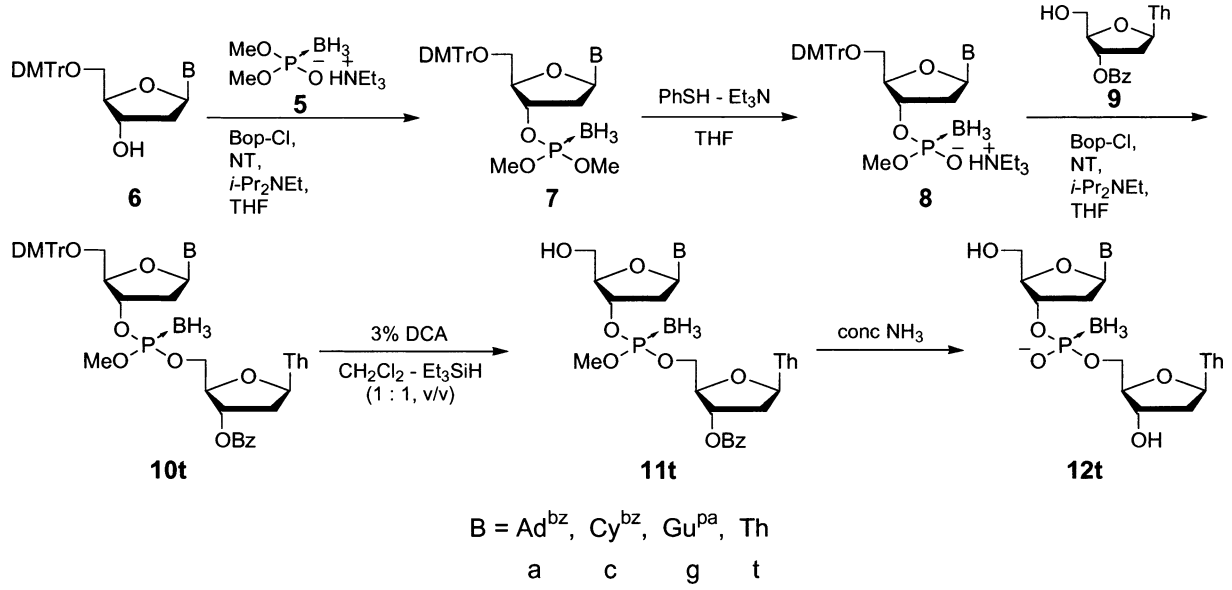
Table 1. Synthesis of deoxyribonucleoside 3′-boranophosphates

Entry	B	Yield (%)	
		6→7	**7→8**
1	Ad^{bz}	88	93
2	Cy^{bz}	96	92
3	Gu^{pa}	73	87
4	Th	91	97

One of the methyl groups in the boranophosphate triesters **7** could be deprotected by treatment with PhSH-Et_3N-THF[16] to give the corresponding diesters **8** in excellent yields (Table 1).[17] It is noteworthy that the methylation at the base moieties[18] was not observed during the demethylation of **7**.

The present boranophosphorylation reaction could be successfully applied to an internucleotidic boranophosphate triester bond formation. The monomer **8t** was easily condensed with 3′-*O*-benzoylthymidine (**9**) in the presence of Bop-Cl, NT, and *i*-Pr_2NEt in THF to give the fully protected dimer **10t** in 92% yield.[19]

Next, removal of the protecting groups was attempted. It is well known that the dimethoxytrityl cation ($DMTr^+$) reacts with borane groups, resulting in the decomposition of internucleotidic linkages.[4,7] Therefore, we used Et_3SiH as a $DMTr^+$ scavenger for the



Scheme 3.

deprotection of the DMTr group.[20] When **7t**, as a model compound, was treated with 3% dichloroacetic acid (DCA) in CH_2Cl_2 without any scavenger, about 20% by-products were observed after 30 min (^{31}P NMR analysis). On the other hand, when **7t** was treated with 3% DCA in CH_2Cl_2/Et_3SiH (3:1 and 1:1, v/v), by-products decreased to about 10% and 0%, respectively, after 30 min (^{31}P NMR analysis). Since in this reaction, the DMTr group was removed within less than 1 min, we decided to use the latter conditions, and the fully protected dimer **10t** was treated with 3% DCA in CH_2Cl_2/Et_3SiH (1:1, v/v) to give the 5′-*O*-free compound **11t**. Finally, the other protecting groups in **11t** were removed by a conventional procedure[2] to yield the dinucleoside boranophosphate **12t** in 92% yield (Scheme 3). The structure of **12t** was confirmed by ^1H, ^{13}C, ^{31}P NMR spectrum and RP-HPLC analysis, and no side-products were found.

In conclusion, we have developed a novel strategy for the boranophosphorylation of nucleosides including A, C, G, and T. The present strategy essentially eliminates the troublesome side reactions, caused by a borane reagent, which were unavoidable in the previously reported procedures. Therefore, our method will be useful for the synthesis of oligonucleotides bearing boranophosphate linkages. Solid-phase synthesis of oligomers is now in progress.

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washings were combined and dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a column of silica gel (30 g). Chromatography was performed with CH_2Cl_2, applying a gradient of MeOH (0–3%). The fractions containing 5′-*O*-(dimethoxytrityl)thymidin-3′-yl 3′-*O*-(benzoyl)-thymidin-5′-yl methylboranophosphate (**10t**) were combined and concentrated to dryness under reduced pressure to give **10t** (88.8 mg, 92%) as a colorless foam.

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