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Stereoselective synthesis of dinucleoside boranophosphates by an oxazaphospholidine method

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Abstract—A stereoselective synthesis of dinucleoside boranophosphates by using nucleoside 3′-oxazaphospholidine derivatives is described. The diastereoselectivity of the internucleotidic bond formation reactions varied with the nucleobase used. (*R*p)- and (*S*p)-dithymidine boranophosphates were synthesized with excellent diastereoselectivity both in solution and on a solid-support, whereas a loss of diastereopurity was observed for the 2′-deoxycytidine derivative having an unprotected nucleobase amino group. On the other hand, complete chemoselectivity of the 3′-oxazaphospholidine derivatives toward hydroxy groups over amino groups was serendipitously found during the study. This unique chemoselectivity of the 3′-oxazaphospholidine derivatives was investigated by comparing them with the conventional nucleoside 3′-phosphoramidite.

Boranophosphate DNA is a new class of modified nucleic acids, in which one of the non-bridging oxygens of the phosphodiester linkage is replaced by a BH_3 group.[1] Incorporation of the boranophosphate linkages into oligonucleotides results in significant increase of their nuclease resistance and lipophilicity.[2,3] In addition, a duplex consisting of a boranophosphate DNA and its complementary RNA is a good substrate for RNase H.[2,4] Thus, boranophosphate DNA is regarded as a promising candidate for therapeutic agents applicable to antisense and antigene approaches as well as boron neutron capture therapy (BNCT).[5]

Boranophosphate DNA has chiral internucleotidic linkages, and it has been reported that the chirality affects the properties of boranophosphate DNA.[3,4b] Shaw and co-workers and Just and co-workers have reported the preparations of (*R*p)- and (*S*p)-dithymidine boranophosphates based on chromatographic separations.[6,7] These approaches can be applicable only to dinucleoside boranophosphates, because the separation of diastereomers is virtually impossible in the cases of long oligomers. Just and co-workers have demonstrated the stereocontrolled synthesis of (*S*p)-dithymidine boranophosphate with excellent diastereoselectivity by using (*S*)-3-hydroxy-4-(2-indolyl)butyronitrile as a chiral auxiliary, though it has not been applied to the synthesis of boranophosphate DNA oligomers.[8] In contrast, fully (*S*p)-stereoregulated boranophosphate DNA can be obtained by the enzymatic method using nucleoside 5′-*O*-α-boranotriphosphates.[9] However, (*R*p)-boranophosphate DNA cannot be obtained by the enzymatic method because of the substrate specificity of the enzyme. Under these circumstances, the development of an efficient method for the chemical synthesis of stereodefined boranophosphate DNA is of great importance.

Recently, we have developed an oxazaphospholidine approach for the stereocontrolled synthesis of phosphorothioate DNA by the use of nucleoside 3′-*O*-oxazaphospholidine monomer units and less-nucleophilic acid activators.[10] The method enables us to synthesize (*R*p)- and (*S*p)-phosphite triester intermediates in high yields and with excellent diastereoselectivity. The resulting diastereopure phosphites are expected to be converted to the corresponding diastereopure boranophosphates. In this paper, we wish to describe a novel approach for the diastereoselective synthesis of dinucleoside boranophosphates by the oxazaphospholidine method.

Keywords: Boranophosphate DNA; Stereoselective synthesis; Antisense DNA; Solid-phase synthesis; Oxazaphospholidine; Chemoselectivity.
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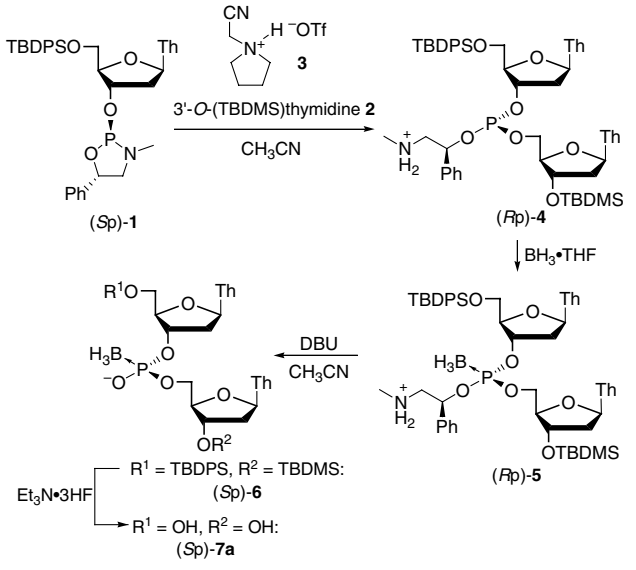
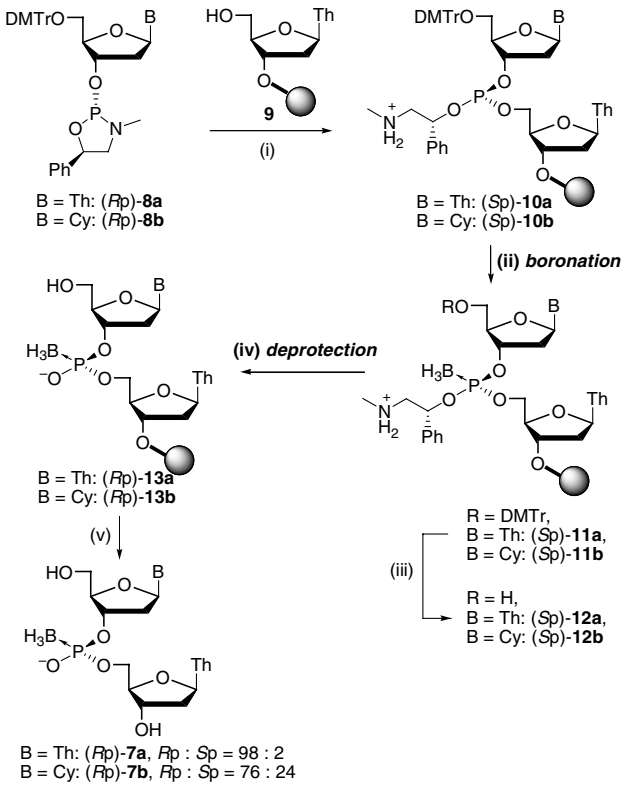
As we previously reported, the diastereopure 5′-*O*-(*tert*-butyldiphenylsilyl)thymidine 3′-*O*-oxazaphospholidine monomer units (*S*p)-**1** and (*R*p)-**1** were obtained from the corresponding enantiopure 1,2-amino alcohols with the diastereomer ratios of >99:1 and 99:1, respectively.[10] The monomer (*S*p)-**1** was condensed with 3′-*O*-(*tert*-butyldimethylsilyl)thymidine **2** in the presence of the activator **3** to give the diastereopure phosphite intermediate (*R*p)-**4** (dr > 99:1).[10] The resultant phosphite was then boronated by treatment with 1 M BH_3·THF at rt for 10 min to afford the boranophosphate triester (*R*p)-**5** (Scheme 1). The chiral auxiliary in (*R*p)-**5** could be easily removed by treatment with DBU for 30 min at 50 °C to give the 5′-*O*- and 3′-*O*-silylated dithymidine boranophosphate (*S*p)-**6**. Finally, the 5′-*O*- and 3′-*O*-silyl groups were removed by treatment with 3HF·Et_3N,[11] and purification by reverse-phase column chromatography gave the fully deprotected dimer (*S*p)-**7a** in 66% isolated yield (4 steps). The resultant dimer was almost diastereopure (dr = 98:2), which was confirmed by reverse-phase HPLC. The *P*-configuration of the dimer was determined to be *S*p by ^1H, ^{31}P NMR and RP-HPLC analysis.[3,6,12] Previously, we have found that the condensation of (*S*p)-**1** with **2** in the presence of **3** proceeded with inversion of the configuration at the phosphorus atom and that the removal of the chiral auxiliary by DBU treatment took place with retention of the configuration.[10] Moreover, it is known that the conversion of a phosphite triester to a boranophosphate by BH_3·THF proceeds with retention of the *P*-configuration. Therefore, the absolute *P*-configuration of the resultant dithymidine boranophosphate is consistent with those reported in the previous studies.[10] In a similar manner, (*R*p)-**7a** could be obtained from (*R*p)-**1** in 63% isolated yield (4 steps) with the dr of 96:4.

On the basis of these results, we applied this method to the solid-phase synthesis of diastereopure dithymidine boranophosphates (Scheme 2). The monomer (*R*p)-**8a**[10] was condensed with thymidine anchored to a controlled-pore glass (CPG) (**9**) in the presence of **3**, and the resultant phosphite triester (*S*p)-**10a** was boronated under various conditions (Table 1). The 5′-*O*-DMTr group of (*S*p)-**11a** was then removed by treatment with 3% DCA in CH_2Cl_2 in the presence of Et_3SiH as a trityl cation scavenger.[13] After removal of the chiral auxiliary in (*S*p)-**12a**, the dimer was cleaved from the solid-support by treatment with concd NH_3 at 50 °C for 30 min, and analyzed by reverse-phase HPLC. When the boronation of the phosphite intermediate (*S*p)-**10a** and the deprotection of the chiral auxiliary in (*S*p)-**12a** were carried out under similar conditions to those for the solution-phase synthesis, the HPLC profile of the crude product was very complicated (Table 1, entry 1). A prolonged boronation reaction was found to be less effective (entry 2). Milder deprotection conditions resulted in the improved yield of the dimer (entry 3), though many unidentified byproducts were still observed. When BH_3·Me_2S was used as a boronating agent in place of BH_3·THF, the yield of the dimer was appreciably improved (entry 4). During the deprotection of (*S*p)-**12a**, the 5′-hydroxy group would attack the neighboring phosphorus atom to decompose the product. In order to avoid this undesirable side reaction, the 5′-hydroxy group was acetylated prior to the DBU treatment (entry 5). This treatment was found to be very effective to avoid the decomposition of the product. Under the optimized conditions, (*R*p)- and (*S*p)-dithymidine boranophosphates were synthesized with the dr of 98:2 (94% yield) and 99:1 (92% yield), respectively.[14]



Scheme 1. Solution-phase synthesis of (*S*p)-dithymidine boranophosphate (*S*p)-**7a**.



Scheme 2. Solid-phase synthesis of (*R*p)-dinucleoside boranophosphates (*R*p)-**7a**,**b**. Reagents and conditions: (i) **3**, CH_3CN, rt, 3 min; (ii) see Table 1; (iii) 3% DCA, Et_3SiH–CH_2Cl_2 (1:1, v/v), rt, 30 s; (iv) see Table 1; (v) concd NH_3, 50 °C, 30 min.

Table 1. Reaction conditions for boronation and deprotection

Entry	Boronation	Deprotection	$T_{PB}T$:T[a]
1	1 M BH_3·THF/THF/10 min	0.2 M DBU/50 °C/30 min	53:47[b]
2	1 M BH_3·THF/THF/30 min	0.2 M DBU/50 °C/30 min	55:45[b]
3	1 M BH_3·THF/THF/30 min	0.2 M DBU/25 °C/overnight	70:30[b]
4	1 M BH_3·Me_2S/CH_2Cl_2/30 min	0.2 M DBU/25 °C/overnight	89:11
5[c]	1 M BH_3·Me_2S/CH_2Cl_2/30 min	0.2 M DBU/25 °C/overnight	93:7

[a] The ratios were determined by HPLC.
[b] Many side products were observed other than $T_{PB}T$.
[c] Acetylation was carried out before the DBU treatment.

Next, we attempted to apply this method to the synthesis of dinucleoside boranophosphates having nucleobases other than thymine. It has been reported that boronating reagents, such as BH_3·Me_2S, reduce the conventional acyl protecting groups for nucleobase amino groups into the corresponding alkyl groups (e.g., benzoyl to benzyl). The resultant alkyl groups on the nucleobase amino groups would be difficult to remove from the products.[3,15,16] On the other hand, the reactions between the unprotected nucleobases and boronating reagents have been reported to be reversible coordinations of the nucleobase nitrogen atoms to BH_3 rather than irreversible reductions of the nucleobases.[17,18] These reports suggest that nucleoside 3′-oxazaphospholidine monomers having an unprotected nucleobase should be used. First, a monomer unit having N^4-unprotected cytosine [(*R*p)-**8b**] was synthesized. The phosphitylation of the 3′-OH was carried out at −78 °C to minimize undesired phosphitylations at the nucleobase to afford the diastereopure oxazaphospholidine monomer (*R*p)-**8b** in 44% yield after silica gel column chromatography. (*R*p)-**8b** was then applied to the synthesis of $C_{PB}T$ on the CPG (Scheme 2). The HPLC analysis showed that $C_{PB}T$ was generated in 86% yield without any base modifications. However, a significant loss of diastereopurity was observed (dr = 76:24). Although the exact mechanism is still not clear, the contributor to this low diastereoselectivity must be the unprotected cytosine amino group judging from the fact that $T_{PB}T$ [(*R*p)- and (*S*p)-**7a**] were obtained with little loss of diastereopurity. This implies the necessity for the development of a new base-protecting group for the nucleobase amino groups, which are compatible with boronating reagents, for the synthesis of stereoregulated boranophosphate DNA having nucleobases other than thymine. A study based on this consideration is currently in progress.

On the other hand, it is noteworthy that the $C_{PB}T$ obtained by this method was not accompanied with any N-phosphorylated products, because it is well-known that the nucleobase amino group of cytosine, as well as that of adenosine, is reactive enough to be phosphitylated by the conventional phosphoramidites.[19] To confirm that the oxazaphospholidine monomer units do not give any adducts with the nucleobase amino groups, *trans*-**8a** (a mixture of (*R*p)-**8a** and (*S*p)-**8a**) was mixed with 3′,5′-bis(*tert*-butyldimethylsilyl)cytidine **14** and **3** in CH_3CN–CD_3CN (4:1, v/v), and analyzed by ^{31}P NMR. No adducts between *trans*-**8a** and **14** were observed; only the *P*-epimerization from the *trans*-**8a** to the *cis*-**8a** was observed (Fig. 1A).[10b,20] On the contrary, the experiment using the conventional thymidine 3′-phosphoramidite **15** in the place of **8a** gave a complex mixture, in which several broad signals around 132–123 ppm were observed by ^{31}P NMR probably due to the N-phosphitylation (Fig. 1B). Similarly, **8a** did not give any observable adducts with the adenine and guanine amino groups either, whereas **15** gave broad ^{31}P NMR signals around 132–123 ppm. Our first assumption was that the acid activator **3** mediated the chemoselective phosphitylations because the chemoselectivity of the *O*-selective phosphitylations reported in the literature was dependent on activators.[21] However, we found that **8a** did not give any N-phosphitylated products even when the conventional acidic activator for the phosphoramidite method, 1*H*-tetrazole, was used in the place of **3**, whereas

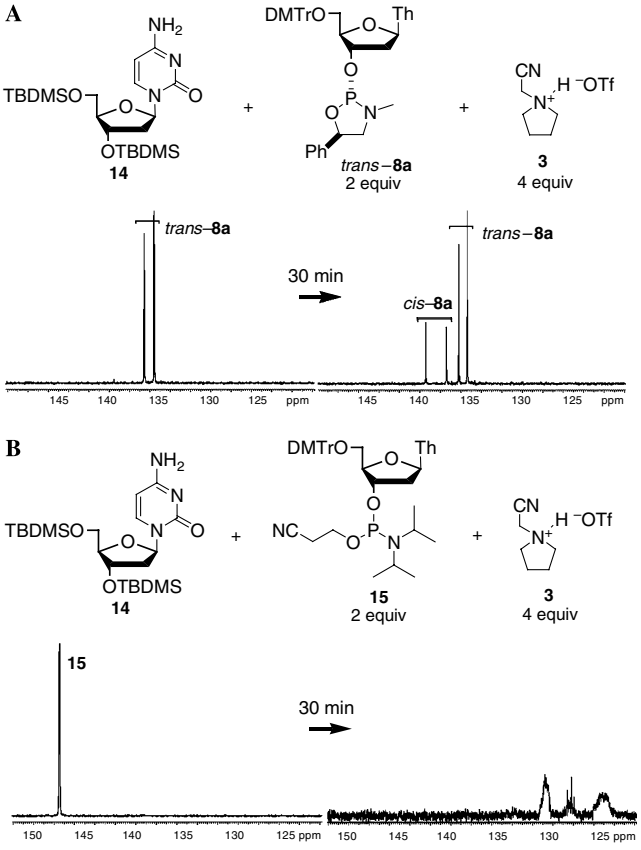


Figure 1. ^{31}P NMR spectra of the reaction mixtures obtained by the reactions of **14** with *trans*-**8a** (A) or with **15** (B) in the presence of **3** (4 equiv) in CH_3CN–CD_3CN (4:1, v/v) at rt.

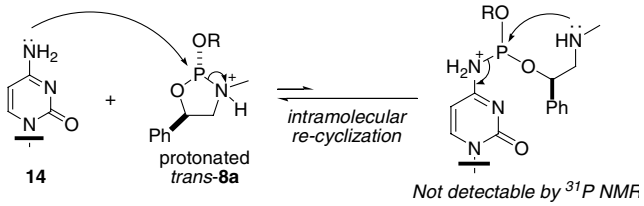


Figure 2. Plausible mechanism of the intermolecular nucleophilic addition between **14** and *trans*-**8a**, and the intramolecular re-cyclization.

15 gave the N-phosphitylated nucleosides.[14] To the best of our knowledge, this is the first example of an *O*-selective phosphitylation the chemoselectivity of which is independent of activators. This chemoselectivity peculiar to the oxazaphospholidine derivatives can be explained by the intramolecular re-cyclization of the oxazaphospholidine ring, which would be much faster than the intermolecular nucleobase phosphitylations (Fig. 2). This new *O*-selective phosphitylation may lead to a new synthetic method for nucleic acid analogs without nucleobase amino protection.

In summary, the stereocontrolled synthesis of dithymidine boranophosphates by the oxazaphospholidine method afforded (*R*p)- and (*S*p)-dithymidine boranophosphates with excellent diastereoselectivity both in solution- and solid-phase syntheses. On the contrary, the application of this method to the synthesis of dinucleoside boranophosphate having an unprotected nucleobase amino group resulted in a significant loss of diastereopurity. However, the analysis of the resultant dinucleoside boranophosphate showed that the nucleoside 3′-oxazaphospholidine monomers did not give any nucleobase-phosphitylated byproducts. ^{31}P NMR analysis of the reactions demonstrated that the chemoselectivity of the oxazaphospholidine derivatives toward hydroxy groups over amino groups was independent of activators.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.03.076.

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20. We have reported that the nucleoside 3′-oxazaphospholidine derivatives having a 3-methyl-5-phenyl-1,3,2-oxazaphospholidine ring, such as (*R*p)-**8a**,**b**, epimerize in the presence of acids, such as **3**. However, the loss of diastereopurity observed for $C_{PB}T$ is not attributable to the epimerization of (*R*p)-**8b** by **3**, because **3** mediated the condensations of (*R*p)- and (*S*p)-**8a** with little loss of diastereopurity.
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