Stereocontrolled synthesis of dithymidine boranophosphates by an oxazaphospholidine method

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Abstract—Diastereopure *R*p- and *S*p-dithymidine boranophosphates were synthesized by an oxazaphospholidine method. Both in solution and solid-phase, the products were obtained in good yields and with excellent diastereoselectivity.

Boranophosphate DNA is a new class of modified nucleic acids, in which one of the non-bridging oxygens of the phosphodiester linkage in DNA 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.[2c,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] Substitution of a non-bridging oxygen of the phosphodiester linkage in DNA by the BH_3 group results in a chiral boranophophate linkage. Recent studies have shown that the properties of boranophosphate DNA are affected by the chirality of the phosphorus atoms.[3,4b] Shaw *et al.* reported the synthesis of diastereopure *S*p- and *R*p-dithymidine bpranophosphates from the corresponding diastereopure *H*-phosphonate intermediates separated by silica-gel column chromathography.[6] Just *et al.* reported the separation of diastereomers of dithymidine boranophosphates synthesized by the conventional phosphoramidite method.[7] These approachs can be applicable only to the dinucleoside boranophosphates because separation of the diastereomers is virtually impossible for long oligomers. Just *et al.* also reported the stereocontrolled synthesis of *S*p-dithymidine boranophosphate by using (*S*)-3-hydroxy-4-(2-indolyl)butyronitrile as a chiral auxiliary.[8] In this case, both the efficiency and diastereoselectivity for the internucleotidic bond formation are not sufficient for the solid-phase synthesis of long oligomers. In contrast, fully *S*p-stereoregulated oligodeoxyribonucleoside boranophosphates can be obtained by the enzymatic method using the nucleoside 5'-*O*-α-boranotriphosphates.[9] However, *R*p-stereoregulated oligodeoxyribonucleoside boranophosphates cannot be obtained by the enzymatic method because of the substrate specificity of the enzyme. Under these circumstances, development of an efficient method for the chemical synthesis of stereodefined oligonucleoside boranophosphates is of great importance.

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

As we previously reported, the diastereopure 5'-*O*-(*tert*-buthyldiphenylsilyl)thymidine 3'-*O*-oxazaphospholidine monomer units (*S*p)-**1** and (*R*p)-**1** were obtained from the chiral 1,2-amino alcohols with the diastereomeric 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 activator **3** to give the diastereopure phosphite intermediate **4** (dr >99:1).[10] The resulting phosphite was then boronated by treatment with 1 M BH_3•THF in THF at rt for 10 min (Scheme 1). The chiral auxiliary of **5** could be easily removed by treatment with 10 equiv of DBU for 30 min at 50 °C to afford 5'-*O*- and 3'-*O*-silylated dithymidine boranophosphate **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 **7** in 66%

Keywords: Boranophosphate DNA, Stereocontrolled synthesis, Antisense DNA, Solid-phase synthesis.

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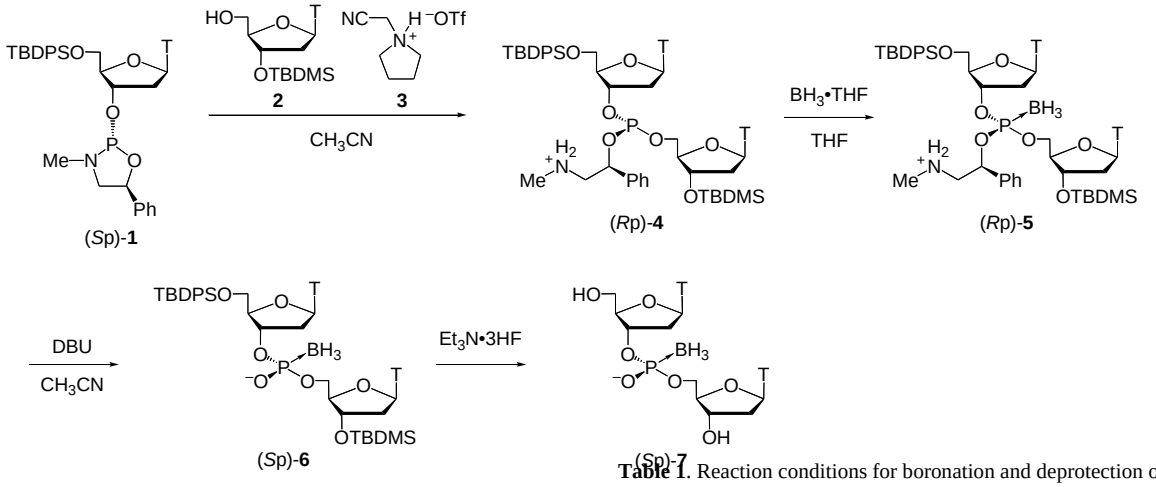


Scheme 1. Solution-phase synthesis of *S*p-dithymidine boranophosphate.

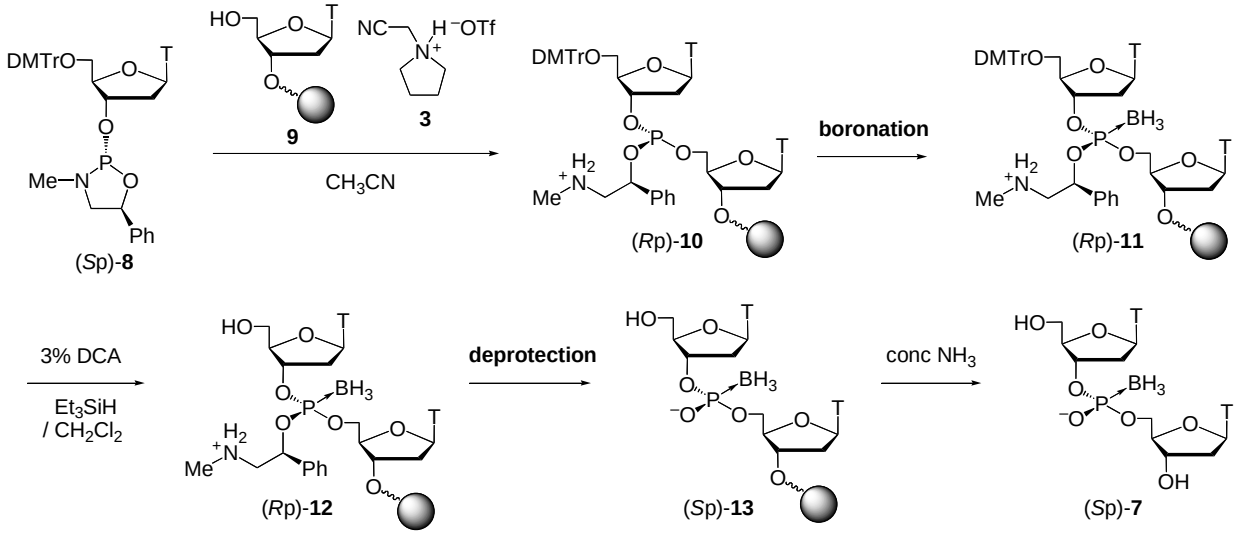
isolated yield (4 steps). The resultant dimer was almost diastereopure (dr = 98:2) which was confirmed by reverse-phase HPLC and the *P*-configuration of the dimer was determined to be *S*p by the ^{1}H-NMR analysis.[3,6,12] It has been previously reported that the condensation of (*S*p)-**1** with **2** in the presence of **3** proceeds with inversion of the configuration at the phosphorus atom and that the removal of the chiral auxiliary by the DBU treatment proceeds with retention of the configuration.[10] It is known that the conversion of phosphite triester to boranophosphate by BH_3•THF takes place with retention of the *P*-configuration. Therefore, the present results are consistent with the previous studies.[10] In a similar manner, (*R*p)-**7** could be obtained from (*R*p)-**1** in 63% isolated yield (4 steps) with dr of 96:4.

Table 1. Reaction conditions for boronation and deprotection on the solid support.

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 $T_{PB}T$ and T.
[c]Acetylation was carried out before the DBU treatment.

On the basis of these results, we applied this method to the solid-phase synthesis of diastereopure dithymidine boranophosphates (Scheme 2). An oxazaphospholidine monomer (*S*p)-**8** was condensed with thymidine anchored to a CPG (**9**) in the presence of **3**, and the resulting phosphite triester **10** was boronated under various conditions (Table 1). The 5'-DMTr group of **11** 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 of **12**, the dimer was cleaved from the solid-support by treatment with conc NH_3 aq at 50 °C for 30 min. The crude product was analyzed by



Scheme 2. Solid-phase synthesis of *S*p-dithymidine boranophosphate.

reverse-phase HPLC.

When boronation of the phosphite intermediate **10** and deprotection of the chiral auxiliary of **12** were carried out under similar conditions for the solution-phase synthesis, the HPLC profile of the crude product was very complicated (Table 1, entry 1). Many products were observed other than the desired dimer. A prolonged boronation reaction was found to be less effective (Table 1, entry 2). Milder deprotection conditions resulted in an improved yield of the dimer (Table 1, entry 3). However, many unidentified products were still observed. When BH_3•Me_2S in CH_2Cl_2 was used as a boronating agent in place of BH_3•THF in THF, the yield of the dimer was appreciably improved (Table 1, entry 4). In the deprotection reaction of **12**, the 5'-hydroxy group can 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 (Table 1, entry 5). This treatment was found to be very effective to avoid the decomposition of the product.

Using the optimized conditions, *S*p- and *R*p-dithymidine boranophosphates were synthesized in good yields. In the case of (*S*p)-**7**, dr was estimated to be >99:1 and the yield of the dimer was 92%. In a similar manner, (*R*p)-**7** was obtained in 90% yield (dr = 98:2) (Figure 1).

In conclusion, we have developed a new method for the stereocontrolled synthesis of dithymidine boranophosphates. Both in solution and solid-phase, diastereopure *S*p- and *R*p-dithymidine boranophosphates were successfully synthesized. Solid-phase synthesis of stereoregulated oligodeoxyribonucleoside boranophosphates including four kinds of nucleobases is now in progress.

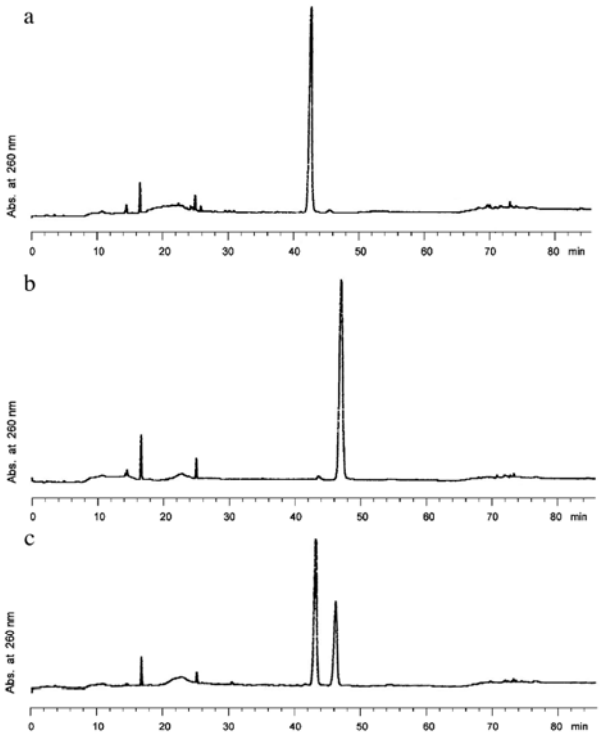


Figure 1. Reverse-phase HPLC profiles of the crude products: (a) (*R*p)-**7**; (b) (*S*p)-**7**; (c) a diastereomixture of (*R*p)-**7** and (*S*p)-**7**.

Acknowledgments

This work was supported by Grants from the Ministry of Education, Sciences, Sports, Culture and Technology, Japan.

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