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Solid-phase synthesis of backbone-modified DNA analogs by the boranophosphotriester method using new protecting groups for nucleobases

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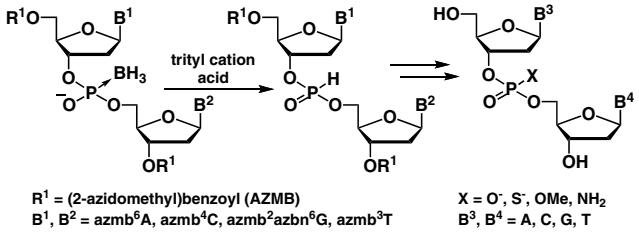
ABSTRACT

Backbone-modified DNA analogs were synthesized in good yields by the boranophosphotriester method on a solid support. The oligodeoxyribonucleoside boranophosphates, protected with 2-(azidomethyl) benzoyl groups for nucleobases, were converted into DNA and its backbone-modified analogs via the corresponding *H*-phosphonate intermediates. A new protecting group for the O^6 position of 2′-deoxyguanosine, 4-azidobenzyl (ABn) group, was also developed. The ABn group can be quickly removed by treatment with $MePPh_2$ and H_2O in the presence of 2-mercaptoethanol.

Base-sensitive DNA analogs, such as *O*-alkyl phosphate DNA, alkylphosphonate DNA, and phosphoramidate DNA, have been regarded and synthesized as potentially useful therapeutic oligonucleotides.[1,2] Chemical synthesis of oligonucleotides and their analogs is generally carried out on a solid support with base-labile nucleobase-protecting groups.[3] However, base-sensitive oligonucleotide analogs are decomposed under the conditions prescribed for the removal of the protecting groups. Recently, we have reported a new strategy for the synthesis of backbone-modified DNA analogs by the boranophosphotriester method by the use of azido-based protecting groups which can be removed under mild and neutral conditions in solution (Scheme 1).[4]

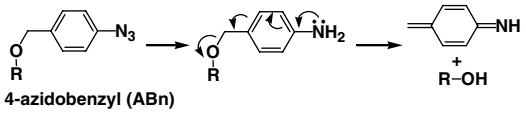
In this strategy, boranophosphate DNA is employed as a precursor of *H*-phosphonate DNA. The boranophosphate DNA can be converted into the corresponding *H*-phosphonate DNA, and is finally transformed to the desired backbone-modified DNA analogs.

In the previous method, the exocyclic amino groups of nucleobases and the imido function of thymine base are protected with a 2-(azidomethyl)benzoyl (AZMB) group,[5] and the lactam function of guanine is protected with a 4-[(2-azidomethyl)benzoyloxy]benzyl (AZBn) group, which can be removed under neutral and reductive conditions. Therefore, the method is suitable for the synthesis of base-sensitive DNA analogs. However, the AZBn group is bulky and unstable under basic conditions, hence the yield of protection for the O^6 position of guanine base was insufficient.[4] Herein, we report a novel protecting group for the O^6 position of guanine residue. The 4-azidobenzyl group, which was reported by Kusumoto and co-workers as a protecting group for the hydroxy function, can be removed by oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) or anodic oxidation after reduction to 4-aminobenzyl group with catalytic hydrogenation or oxidation with DDQ after conversion to iminophosphorane (Scheme 2).[6]



Scheme 1. Synthesis of backbone-modified DNA analogs by the boranophosphotriester method.

The ABn group is expected to be removed under essentially the same conditions as those for an AZMB group with the generation of iminoquinone. In this letter, we describe the application of the



Scheme 2. ABn group.

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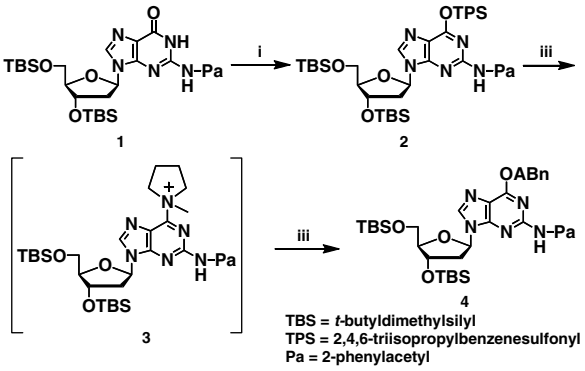
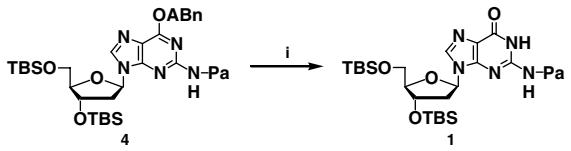
boranophosphotriester method to the solid-phase synthesis of backbone-modified DNA analogs by using a new protecting group for the O^6 position of guanine.

First, an ABn group was introduced to the O^6 of N^2-phenylacetyl-2′-deoxyguanosine derivative **1** as a model compound. 4-Azidobenzyl alcohol was allowed to react with the C^6 quaternary *N*-methylpyrrolidinium intermediate by the method reported in the literature[7] with some modifications to give **4** in 90% yield from **2** (Scheme 3). The O^6-ABn group of 2′-deoxyguanosine derivative **4** was deblocked under the same conditions as those for the removal of AZMB group. Fortunately, the ABn group was smoothly removed in 97% yield by the treatment with methyldiphenylphosphine in 1,4-dioxane–H_2O (Scheme 4), whereas the side reaction occurred in the case of AZBn group under the same conditions.[4]

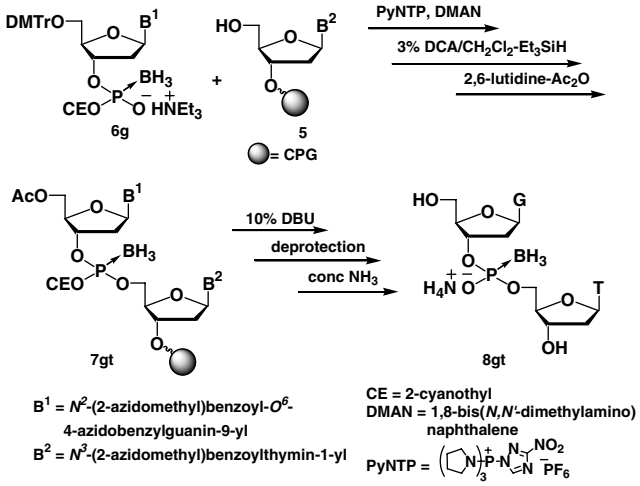
In the next step, the ABn group was applied to the solid-phase synthesis.[8] The removal of the ABn group in a dinucleoside boranophosphate on a solid support was examined. 2′-Deoxyguanosine boranophosphate **6g**, whose nucleobase was protected with the AZMB and ABn groups, was used as a monomer unit for the boranophosphotriester method.[9–11] **6g** was condensed with a thymidine derivative **5** anchored to the solid support in the presence of PyNTP[12] as a condensing reagent (Scheme 5). After capping and decyanoethylation, the nucleobases were deprotected and the dinucleoside boranophosphate was cleaved from the solid support. The crude reaction mixture was analyzed by RP-HPLC. Under the same deprotection conditions as those for in solution, some by-products were observed by the HPLC analysis. The formation of these by-products could be attributed to the addition reaction of iminoquinone to the nucleobases or phosphate moiety (Fig. 1A). In order to suppress the side-reaction to the phosphate moiety of the boranophosphotriester **7gt**, the cyanoethyl group was deprotected after the removal of the ABn group. As a result, the HPLC analysis suggested that the by-products were slightly decreased (Fig. 1B). Furthermore, in order to trap iminoquinone, 2-mercaptoethanol was added, which was an effective scavenger for quinone



Scheme 3. Introduction of ABn group into the O^6 of 2′-deoxyguanosine derivatives. Reagents and conditions: (i) 2,4,6-triisopropylbenzenesulfonyl chloride (2 equiv), Et_3N (4 equiv), DMAP (0.05 equiv)/CH_2Cl_2, rt, 1 h, 85%, (ii) *N*-methylpyrrolidine (10 equiv)/CH_2Cl_2, 0 °C, 10 min, (iii) ABnOH (5 equiv), DBU (1.5 equiv)/CH_2Cl_2, 0 °C, 1.5 h, two steps 90%.



Scheme 4. Removal of ABn group. Reagents and conditions: (i) $MePPh_2$ (4 equiv)/1,4-dioxane–H_2O (4:1, v/v), rt, 10 min, 97%.



Scheme 5. Solid-phase synthesis using the AZMB and ABn groups.

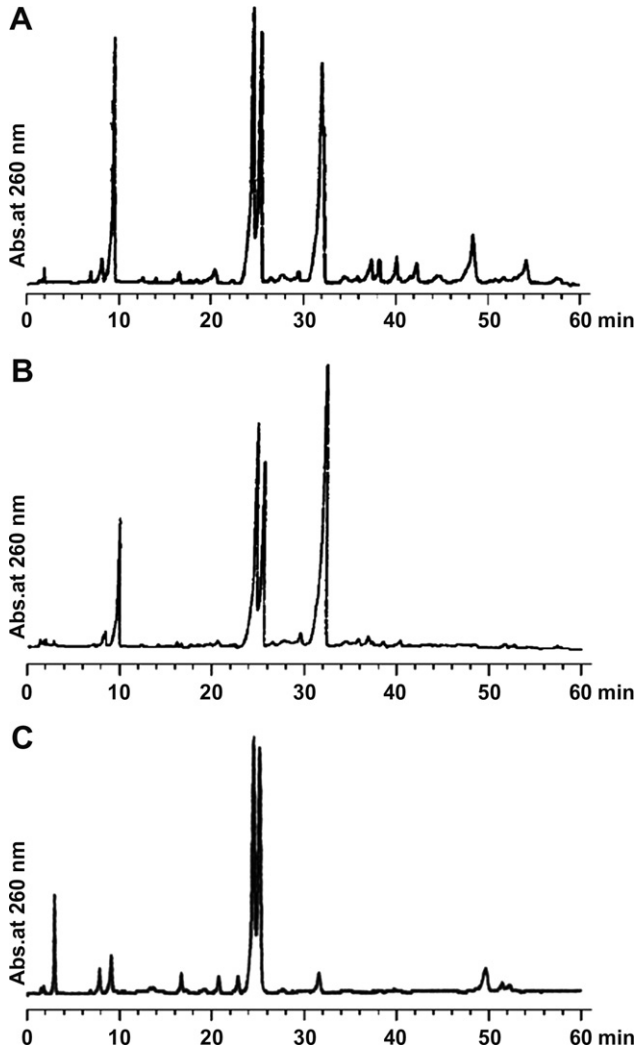
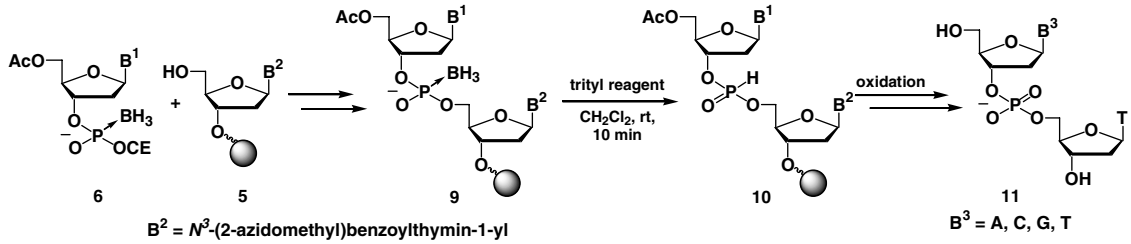


Figure 1. Reverse-phase HPLC profiles of crude mixtures of d($G_{PB}T$), deprotected with $MePPh_2$ in dioxane–H_2O (4:1, v/v), 1 h, after decyanoethylation (A), $MePPh_2$ in dioxane–H_2O (4:1, v/v), 1 h, followed by decyanoethylation (B), $MePPh_2$ in dioxane-2-mercaptoethanol–H_2O (3:1:1, v/v/v), 1 h, followed by decyanoethylation (C).

methide.[4,13,14] Consequently, the side reactions were almost suppressed in the presence of 2-mercaptoethanol (Fig. 1C).

Table 1
Transformation of dinucleoside boranophosphate including four nucleobases



Entry	Compound	B^1	Trityl reagent	Yield of **11**[b] (%)
1	**9ct**	N^4-(2-azidomethyl)benzoylcytosin-1-yl	0.01 M $TrBF_4$	93
2	**9tt**	N^3-(2-azidomethyl)benzoylthymin-1-yl	0.01 M $TrBF_4$	93
3	**9at**	N^6-bis(2-azidomethyl)benzoyladenin-9-yl	0.01 M $TrBF_4$	72
4	**9at**	N^6-bis(2-azidomethyl)benzoyladenin-9-yl	0.01 M $DMTrBF_4$	80
5[a]	**9at**	N^6-bis(2-azidomethyl)benzoyladenin-9-yl	0.01 M $DMTrBF_4$	87
6[a]	**9gt**	N^2-(2-azidomethyl)benzoyl-O^6-4-azidobenzylguanosin-9-yl	0.01 M $DMTrBF_4$	83

[a] Transformation reaction was carried out after deprotection of nucleobases.
[b] Estimated by RP-HPLC.

Next, backbone-modified DNA analogs were synthesized on the solid support by the boranophosphotriester method. The nucleoside boranophosphodiesters **6** whose nucleobases were protected with the AZMB and AZBn groups were used as monomer units.

The monomer units **6** were condensed with the 5′-hydroxy function of the thymidine derivative **5** (Table 1). After subsequent capping of the 5′-hydroxy group and removal of the cyanoethyl group, the dinucleoside boranophosphate **9** was synthesized on the solid support. The resulting dinucleoside boranophosphate **9** was allowed to react with trityl reagent.[4,15] Since the *H*-phosphonate linkage was unstable under the deprotection conditions, **10** was oxidized to the dinucleoside phosphate by treatment with I_2 in pyridine–H_2O.[16] After deprotection, the dinucleoside phosphate was cleaved from the solid support, and the product was analyzed by RP-HPLC.

The HPLC analysis showed nearly quantitative formation of the desired dinucleoside phosphate **11ct**, and this fact indicated that the treatment of $TrBF_4$ gave *H*-phosphonate **10ct** (entry 1) in an excellent yield. Next, we attempted to transform the dinucleoside boranophosphates including other nucleobases to the corresponding phosphates. On the basis of the HPLC analysis, the dithymidine phosphate **11tt** was synthesized in good yield under the same conditions as those for the transformation of **9ct** (entry 2). In the case of the dinucleoside boranophosphate including 2′-deoxyadenosine **9at**, the N^6-bis(2-azidomethyl)benzoyl-2′-deoxyadenosine derivative **6a** was used as a monomer unit to suppress the depurination by the $TrBF_4$. However, the dinucleoside phosphate **11at** was obtained in lower yield (entry 3). The result could be attributed to the strong Lewis acidity of the unsubstituted Tr cation, which may cause depurination. Based on this consideration, $DMTrBF_4$, which is a weaker Lewis acid than $TrBF_4$, was used for the transformation of **9at**. Accordingly, HPLC analysis showed that the yield of

Table 2
Chain elongation cycle, deprotection, and transformation for manual solid-phase synthesis

Step	Manipulation	Reagents and solvents	Time
Chain elongation			
1	Detritylation	3% DCA in CH_2Cl_2–Et_3SiH (1:1, v/v)	15 s
2	Wash	(i) CH_2Cl_2, (ii) CH_3CN	
3	Drying		10 min
4	Condensation	**6** (0.1 M), PyNTP (0.2 M) in DMAN/CH_3CN	20 min
5	Wash	(i) CH_3CN, (ii) CH_2Cl_2	
		Repeat steps 1–5	
6	Detritylation	3% DCA in CH_2Cl_2–Et_3SiH (1:1, v/v)	15 s
Deprotection and transformation			
7	Capping	Ac_2O-2,6-lutidine (1:9, v/v), DMAP (10 mg/mL)	30 s
8	Decyanoethylation	10% DBU in CH_3CN	10 min
9	Deprotection	$MePPh_2$ in 1,4-dioxane–H_2O (4:1, v/v)	1 h
10	Transformation	$DMTrBF_4$ (0.01 M) in CH_2Cl_2	10 min
11	Oxidation	2% I_2 in pyridine–H_2O (98:2, v/v)	10 min
12	Cleavage	Concd NH_3	1 h

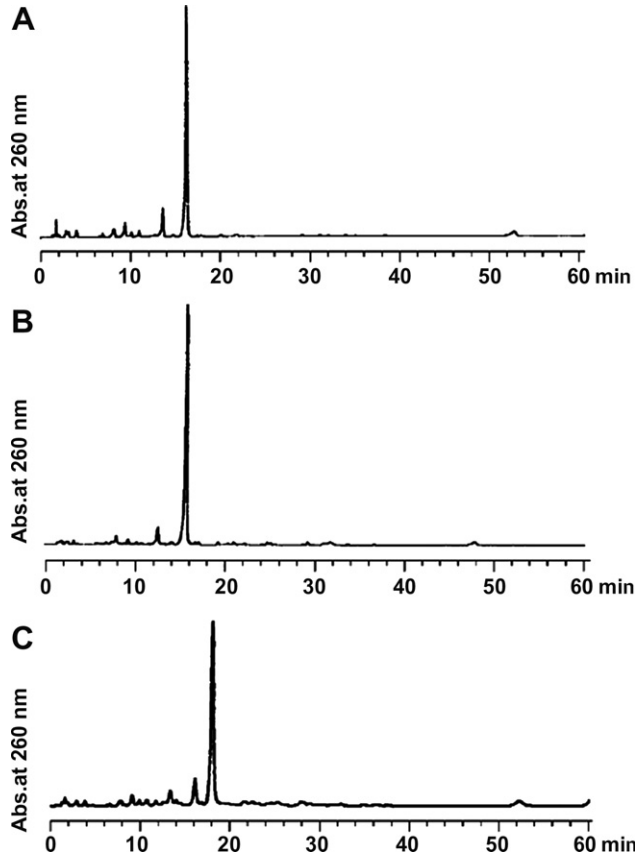
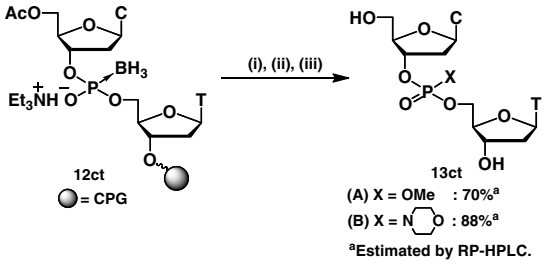


Figure 2. Reverse-phase HPLC profiles of crude mixtures of d(C_PC_PT) (A and B), and d($C_PC_PC_PT$), (C), transformed by 0.01 M $DMTrBF_4$ in CH_2Cl_2 at rt, 10 min, (A), 0.01 M $DMTrBF_4$ in CH_2Cl_2, at 0 °C, 10 min (B and C).



Scheme 6. Synthesis of methylphosphate DNA and phosphoromorpholidate DNA. Reagents and conditions: (i) $DMTrBF_4/CH_2Cl_2$, 0 °C, 10 min, (ii) A—10% MeOH/*N*-methylimidazloe-Et_3N-CCl_4 (5:5:90, v/v/v), rt, 1 h; B—10% morpholine/CCl_4, rt, 1 h; (iii) A—25 mM K_2CO_3/MeOH, rt, 5 h, B—concd NH_3, rt, 1 h.

11at was improved (entry 4). Furthermore, the unprotected dinucleoside boranophosphate, which was more stable than the N-acylated 2′-deoxyadenosine derivatives under acidic conditions,[17] was used for the transformation reaction. After deprotection of the nucleobases, the dimer was treated with $DMTrBF_4$, and the dinucleoside phosphate **11at** was obtained in 87% yield (entry 5). The dinucleoside boranophosphate including 2′-deoxyguanosine **9gt** was transformed in 83% yield under the same conditions as those for the transformation of **9at** (entry 6).

In the next stage, d(C_PC_PT) and d($C_PC_PC_PT$) were synthesized on the solid support. The trinucleoside boranophosphate, d($C_{PB}C_{PB}T$), was synthesized by the repeated detritylation and the condensation on the solid support (Table 2). The base-unprotected boranophosphate trimer was converted to the *H*-phosphonate intermediate by treatment with the $DMTrBF_4$ at room temperature, and then it was oxidized to give d(C_PC_PT). The crude products were analyzed by RP-HPLC (Fig. 2A). The HPLC profiles represented the decomposition of the product in some degree. To decrease the degradation, the transformation reaction was carried out at 0 °C (Fig. 2B). In this case, the desired trinucleoside phosphate was obtained in excellent yield. The tetramer, d($C_PC_PC_PT$), was also synthesized in good yield (Fig. 2C).

Then the method was applied to the solid-phase synthesis of backbone-modified DNA analogs. The oxidation step (Table 2, step 11) was replaced by the oxidative amination and oxidative coupling (Scheme 6).[16,17] The treatment with methanol or morpholine in CCl_4 gave the methyl phosphate DNA and phosphoromorpholidate DNA in 70% and 88% yields, respectively (Scheme 6).

In conclusion, we developed a novel protecting group, ABn group, for the O^6 of guanine which can be removed under mild and neutral conditions. The ABn group was useful for the synthesis of boranophosphate DNA as well as other DNA analogs. We also established the synthesis of DNA and backbone-modified DNA analogs by the boranophosphotriester method on the solid support. The present method will be useful for the synthesis of various backbone-modified oligodeoxyribonucleotide analogs via the *H*-phosphonate intermediate. Solid-phase synthesis of longer oligonucleotide analogs is now in progress.

Acknowledgment

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