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Synthesis of dinucleoside phosphates and their analogs by the boranophosphotriester method using azido-based protecting groups

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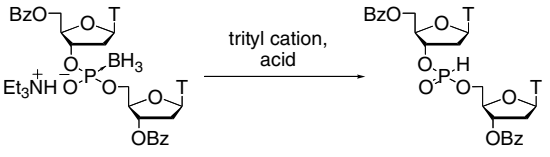
Abstract—Oligodeoxyribonucleotides and their backbone-modified analogs were synthesized in good yields by the boranophosphotriester method in solution. The oligodeoxyriobonucleoside boranophosphates, fully protected with 2-(azidomethyl)benzoyl groups, were converted to the various backbone-modified DNA analogs via the corresponding *H*-phosphonate intermediates. A new efficient protecting group for the O^6-position of 2′-deoxyguanosine, 4-[(2-azidomethyl)benzoyloxy]benzyl (AZBn) group, was also developed. The AZBn group was found to be quickly removed by treatment with $MePPh_2$ in dioxane–2-mercaptoethanol–H_2O.

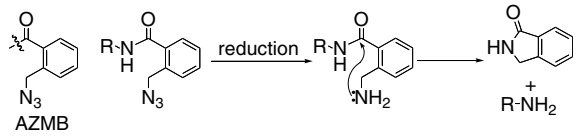
Recently, a number of backbone-modified DNA analogs have been synthesized for anticancer and antiviral drugs such as antisense DNAs. *H*-Phosphonate DNAs have been used as versatile intermediates for the synthesis of DNA and a wide variety of backbone-modified DNA analogs because the phosphorous atom in *H*-phosphonate diesters can be modified by various chemical reactions.[1,2] However, the *H*-phosphonate DNA is chemically unstable, and therefore, only short oligomers can be synthesized in good yields by the current method.

Recently, we have established a new reaction for the transformation of boranophosphate diesters into the corresponding *H*-phosphonate diesters in the presence of trityl cation under acidic conditions (Scheme 1).[3] From another point of view, the borane group is regarded as a protecting group for the *H*-phosphonate diester linkage. Based on this concept, we have developed a new method for the synthesis of DNA and its analogs: First, the boranophosphate DNA is synthesized on a solid support, then it is converted to the *H*-phosphonate DNA, and finally, it is converted to DNA and backbone-modified DNA analogs.



Scheme 1. Novel transformation reaction.

Base-sensitive DNA analogs such as phosphoramidate DNA[4,5] and methyl phosphate DNA[6,7] cannot be synthesized by the use of the conventional base-labile protecting groups for nucleobases. Therefore, we employed a 2-(azidomethyl)benzoyl (AZMB), which can be removed under neutral and reductive conditions, as a protecting group for nucleobases (Scheme 2).[8] In the boranophosphotriester method, which has been developed for the synthesis of boranophosphate DNA by our group, both the guanine and thymine bases react with the boranophosphorylating reagent.[9] Therefore, the N^3-position of thymidine was protected with the AZMB group. In addition, the O^6-position of 2′-deoxyguanosine has to be protected, and it was necessary to



Scheme 2. AZMB group.

Keywords: Backbone-modified DNA analog; *H*-Phosphonate DNA; Boranophosphotriester method; Protecting group.
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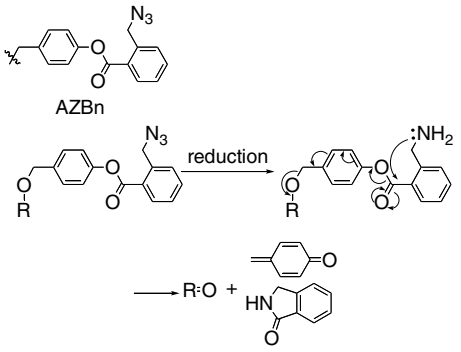
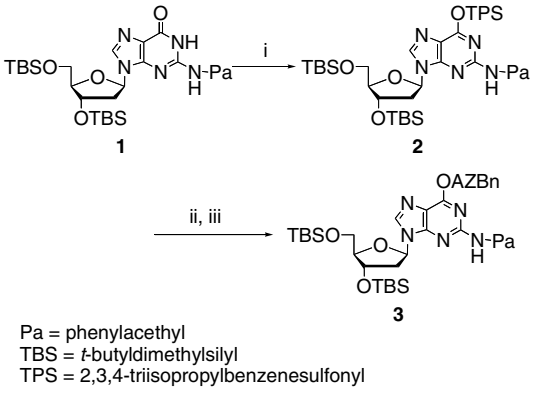


Scheme 3. AZBn group.

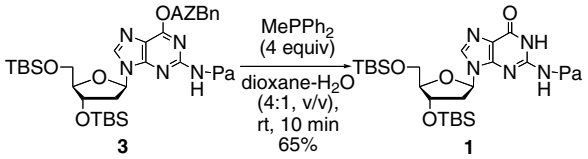
develop a novel protecting group, which can be removed under neutral conditions in the present sturdy. A 4-[(2-azidomethyl)benzoyloxy]benzyl (AZBn) group was designed as a protecting group for the O^6-position. The AZBn group can be removed under essentially the same conditions as those for the AZMB group. The AZMB moiety in the AZBn group is removed subsequent to the reduction of the azido function, then the deprotection reaction proceeds with the generation of quinone methide (Scheme 3).

In this Letter, we wish to describe a novel method for the synthesis of DNA and its analogs via the boranophosphate DNA, and a novel protecting group for the O^6-position of the 2′-deoxyguanosine.

First, the introduction and removal reactions of the AZBn group were attempted using a model compound. The AZBn group was introduced at the O^6-position of the N^2-phenylacetyl-2′-deoxyguanosine derivative **1** via a C^6-ammonium intermediate with 4-[(2-azidomethyl)-benzoyloxy]benzyl alcohol (AZBnOH)[10] by a slightly modified literature procedure (Scheme 4).[11,12] Next, the removal reaction of the AZBn group in 2′-deoxyguanosine derivative **3** was carried out by treatment with methyldiphenylphosphine in 1,4-dioxane–H_2O



Scheme 4. Introduction of the AZBn group into the O^6 of 2′-deoxyguanosine. Reagents and conditions: (i) TPSCl (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) AZBnOH (5 equiv), DBU (1.5 equiv)/CH_2Cl_2, 0 °C, 1.5 h, two steps 77%.



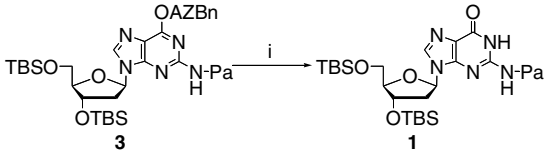
Scheme 5. Removal of the AZBn group.

(4:1, v/v),[8] which are the same conditions as those for the removal of the AZMB group (Scheme 5).

Although the AZBn group was quickly removed, the side reaction was observed and the O^6-deprotected 2′-deoxyguanosine derivative **1** was isolated in a moderate yield. TLC analysis of the reaction mixture suggested that the side reaction occurred. It was assumed that the quinone methide, which was formed during the deprotection reaction, was added to the guanine base.[13,14] In order to suppress this side reaction, deprotection reaction was carried out in the presence of 2-mercaptoethanol, which was an efficient scavenger for the quinone methide.[15,16] Consequently, the AZBn group could be quantitatively removed without any side reactions (Scheme 6).

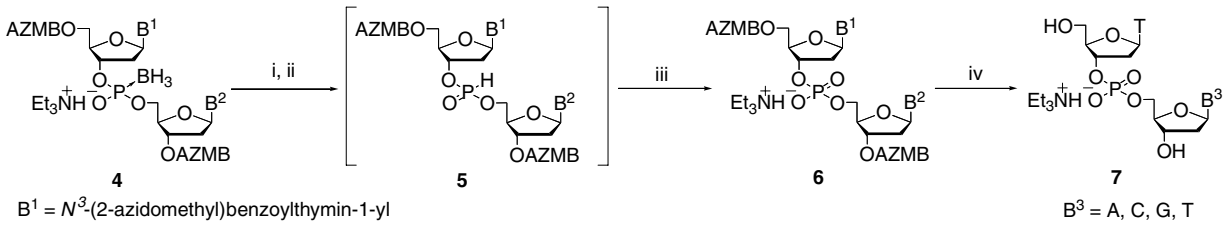
In order to apply the novel method to the synthesis of various kinds of backbone-modified DNA analogs, nucleobases and hydroxy functions were protected with the AZMB and AZBn groups. Dinucleoside boranophosphates were synthesized by the boranophosphotriester method.[9,17,18] The dinucleoside boranophosphate derivatives **4** were allowed to react with a DMTr cation, which was generated by the reaction of dimethoxytrityl methyl ether (DMTrOMe) with dichloroacetic acid (DCA) in CH_2Cl_2 (Table 1). Then, the excess trityl cation was reduced with triethylsilane, and the products were analyzed by ^{31}P NMR spectroscopy after the aqueous work-up of the reaction mixture.

In all the cases, the ^{31}P NMR analysis suggested that dinucleoside *H*-phosphonates **5** were obtained in almost quantitative yields. In the next stage, dinucleoside phosphates **6** were synthesized from boranophosphates **4** via *H*-phosphonates **5** as a control reaction for the synthesis of various backbone-modified DNA analogs. Because the *H*-phosphonate diester is chemically unstable, intermediates **5** were purified by simple extraction. After the aqueous work up of the reaction mixtures subsequent to the transformation reactions of **4** to **5**, the treatment of **5** with I_2 in pyridine–H_2O (98:2, v/v)[19] gave dinucleoside phosphates **6** in good yields.[20]



Scheme 6. Removal of the AZBn group in the presence of 2-mercaptoethanol. Reagents and conditions: (i) $MePPh_2$/dioxane–H_2O–2-mercaptoethanol (3:1:1, v/v/v), rt, 20 min, quant.

Table 1. Synthesis of dinucleoside phosphates from dinucleoside boranophosphates



Entry	B^2	Yield of **5**[a] (%)	Isolated yield of **6** (%)	Isolated yield of **7** (%)
1	N^6-(2-Azidomethyl)benzoyladenin-9-yl	Quant	Quant	33
2	N^4-(2-Azidomethyl)benzoylcytosin-1-yl	95	78	45
3	N^2-(2-Azidomethyl)benzoyl-O^6-4-[(2-azidomethyl)benzoyloxy]benzylguanin-9-yl	Quant	Quant	41
4	N^3-(2-Azidomethyl)benzoylthymin-1-yl	Quant	Quant	34

Reagents and conditions: (i) DMTrOMe (5 equiv), 3% DCA/CH_2Cl_2, 0 °C, 1 min, (ii) Et_3SiH (excess), ext. with satd $NaHCO_3$ aq, (iii) I_2 (3 equiv)/pyridine–H_2O (98:2, v/v), rt, 10 min, (iv) $MePPh_2$ (8 equiv) in dioxane–H_2O (4:1, v/v), rt, 20 min (B^3 = A, C, T) or in dioxane–2-mercaptoethanol–H_2O (3:1:1, v/v), rt, 20 min (B^3 = G).

[a] Yields of **5** were estimated by ^{31}P NMR.

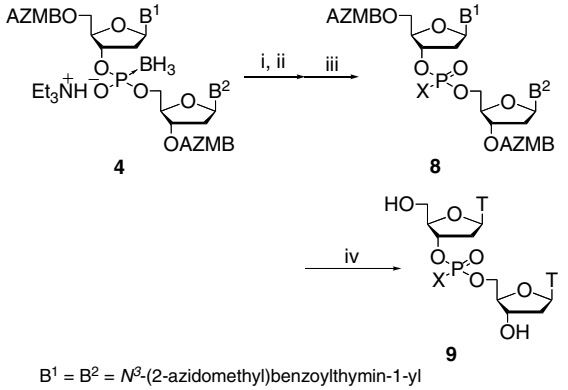
Finally, the removal of the AZMB and AZBn groups was attempted. In the case of **6** ($B^2 = N^2$-2-(azidomethyl)benzoyl-O^6-4-[(2-azidomethyl)benzoyloxy]benzylguanin-9-yl), the reaction was carried out in the presence of 2-mercaptoethanol. After purification by the reverse-phase silica gel column chromatography, we obtained fully deprotected dinucleoside phosphates **7**. It was difficult to remove phosphine oxide from the reaction mixture and the desired products were lost during the purification to some extent. Therefore, the isolated yields of **7** were moderate in all the cases. However, the TLC and ^{31}P NMR analyses of the reaction indicated that the deprotection reaction proceeded quantitatively.

Upon using the transformation reaction described above, various kinds of backbone-modified nucleic acid analogs were synthesized from dithymidine boranophosphate **4** (Table 2). In a similar manner to the synthesis of phosphates, *H*-phosphonate intermediates were not isolated in these cases.

The dithymidine phosphorothioate[21] (entry 1) and phosphoromorpholidate[4,22] (entry 2) were obtained from the corresponding boranophosphate in the excellent yields. Dithymidine methyl phosphate[4,15] (entry 3) and phosphoramidate[4,23] (entry 4) were synthesized in good yields. In the case of the synthesis of the phosphoromorpholidate, the reaction time was longer than those for other cases, and therefore, the AZMB groups at the N^3-position of thymine base were removed during the reaction (entry 2).

Finally, the AZMB groups were removed from the hydroxy functions and the nucleobases in **8** by treatment with methyldiphenylphosphine in 1,4-dioxane–H_2O. In all the cases, quantitative deprotection reactions of **8** were ascertained by the TLC monitoring and ^{31}P NMR spectroscopy after the extraction. Consequently, the fully deprotected T_PT analogs **9**, including the base-labile analogs, were obtained.

Table 2. Synthesis of backbone-modified DNA analogs



Entry	X	Isolated yield of **8** (%)	Isolated yield of **9** (%)
1	S^-	90	32
2	$N(CH_2CH_2)_2O$	96[a]	33
3	OMe	72	50
4	NH_2	78	48

Reagents and conditions: (i) DMTrOMe (5 equiv), 3% DCA/CH_2Cl_2, 0 °C, 1 min, (ii) Et_3SiH (excess), ext. with satd $NaHCO_3$ aq, (iii) transformation reaction, (iv) $MePPh_2$ (8 equiv)/dioxane–H_2O (4:1, v/v), rt, 20 min.

[a] Thymine bases were not protected.

In conclusion, we have developed a novel method for the synthesis of oligodeoxyribonucleotides and their analogs by the boranophosphotriester approach utilizing azido-based protecting groups. A new protecting group for the O^6-position of 2′-deoxyguanosine, which can be removed under neutral and reductive conditions was developed and successfully applied to the synthesis of dinucleoside phosphate derivatives. The transformation reaction conditions for the boranophosphate diesters to the *H*-phosphonate diesters were optimized. Upon applying these reactions, dinucleoside phosphates and their analogs were synthesized from the dinucleoside boranophosphates. Therefore, the present method will be useful for the synthesis of nucleic acids and various

kinds of base-labile backbone-modified nucleic acid analogs. The solid-phase synthesis of nucleic acid derivatives by the present method is now in progress.

Acknowledgement

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23. The dithymidine *H*-phosphonate **5**[20] was allowed to react with 2% I_2 and satd NH_3 in pyridine at rt. The reaction mixture was stirred for 1 min.