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BH_3 as a protecting group for phosphonic acid: a novel method for the synthesis of dinucleoside *H*-phosphonate

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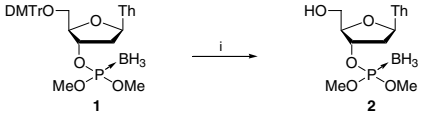
Abstract—A BH_3 group is found to be an effective protecting group for phosphonic acid esters. This new phosphonic acid protecting group was applied to the synthesis of a dithymidine *H*-phosphonate derivative from a dithymidine boranophosphate derivative. Triarylmethyl cations were found to be effective for the deprotection of the BH_3 group in the dithymidine boranophosphate diester to afford the corresponding *H*-phosphonate derivative in excellent yield.

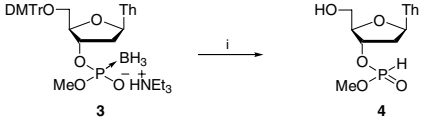
H-Phosphonate DNA is regarded as a potentially useful intermediate for the synthesis of various kinds of backbone-modified nucleic acid derivatives.[1–13] However, in the methods reported so far for the synthesis of this DNA analog, undesirable side reactions occur in the chain elongation cycle and the oxidation step.[14–21] Accordingly, only short oligomers can be synthesized in good yields by the current methods.

On the other hand, boranophosphate DNA, which is chemically and physiologically stable, has been reported as a new candidate of DNA analog.[22] We recently described a new method for the synthesis of boranophosphate DNA (boranophosphotriester method).[23] It is well known that 4,4′-dimethoxytrityl (DMTr) cation reacts with boranophosphate DNA, resulting in its decomposition. Therefore, the addition of a $DMTr^+$ scavenger is required for the detritylation reaction.[12,23,24] In the detritylation of the boranophosphate triester **1**, Et_3SiH was found to be effective as a $DMTr^+$ scavenger for the complete suppression of the decomposition of the product. In contrast, the boranophosphate diester **3** was quickly decomposed to give the corresponding *H*-phosphonate diester **4**, when **3** was treated with DMTr cation in the presence of Et_3SiH under the same conditions, indicating that **3** was more unstable than **1** (Scheme 1).

Boranophosphate triester



Boranophosphate diester



Scheme 1. Stability of 3′-boranophosphate derivatives under detritylation conditions. (i) 3% DCA in $CDCl_3$–Et_3SiH (1:1, v/v).

This result suggested that boranophosphate diester could be transformed into the corresponding *H*-phosphonate. In another point of view, a BH_3 group can be employed as a protecting group for phosphonic acids. Although a BH_3 group is frequently used as a protecting group for phosphine derivatives,[25] the use of a BH_3 group for the phosphonic acid protection has never been reported.

In this paper, we wish to describe the first example of the use of a BH_3 group as a phosphonic acid protecting group.

Keywords: protecting group; boranophosphate; *H*-phosphonate; phosphonic acids; oligonucleotide synthesis.
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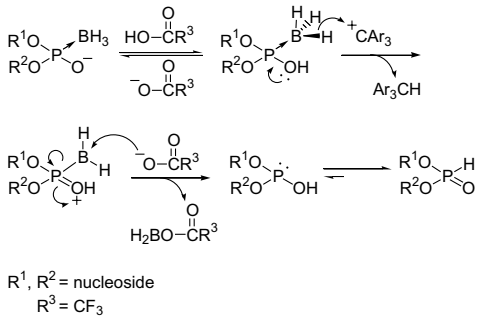
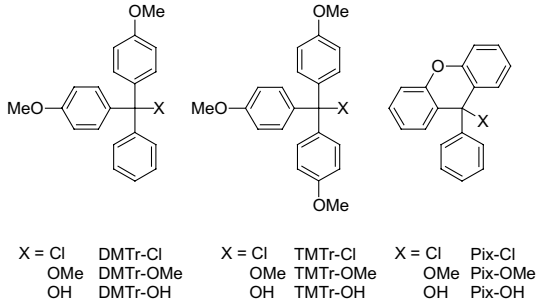
As a model reaction, dinucleoside boranophosphate **5**, which was synthesized by the boranophosphotriester method,[23] was allowed to react with $DMTr^+$, which was generated by the reaction of dimethoxytrityl methyl ether (DMTr–OMe) with trifluoroacetic acid (TFA)[26] in $CDCl_3$, and the reaction was monitored by ^{31}P NMR. After 10 min, the broad signal of **5** (93.87 ppm) quickly disappeared, and a new signal was observed at 9.51 and 8.25 ppm (diastereomers). The P–H coupled spectrum of the reaction mixture clearly indicated the formation of dithymidine phosphonate (**6**) bearing a P–H bond ($J_{PH} = 719.2$, 723.7 Hz). A plausible reaction mechanism is shown in Scheme 2.



Scheme 2. Plausible mechanism for the transformation of *H*-phosphonate from boranophosphate diester.

Next, several trityl cation sources were tested for the present approach (Scheme 3).



Scheme 3. Structures and abbreviations of trityl reagents.

Compound **5** was allowed to react with trityl cation reagents in the presence of TFA under various conditions (Table 1). In all cases, the broad signals of **5** (Fig. 1A) quickly disappeared, and new signals of the *H*-phosphonate diester **6** were observed. In addition, the broad signals of intermediates were observed around 40 ppm in some cases. Although the structures of the intermediates are still not clear, these compounds were found to be readily converted to the *H*-phosphonate diester **6** during the aqueous work-up. For example, the quantitative formation of **6** was observed after aqueous treatment of the reaction mixture when DMTr–OMe was used (Table 1, entry 1). A similar result was obtained when DMTr–OH was employed as a trityl cation source (entry 2). In both cases, prolonged reactions (60–72 min) resulted in the formation of the P-tritylated products (28.75, 28.6 ppm, diastereomers).[6] In contrast, when DMTr–Cl was employed, the formation of diastereomers of P-tritylated compounds (49.05, 48.46 ppm)[27] and transesterified products (6.95 ppm) were observed to some extent (entries 3 and 4). These side reactions were assumed to be catalyzed by hydrochloric acid. In order to accelerate the deprotection reaction, trimethoxytrityl (TMTr) derivatives, which generate a more stable trityl cation, were employed. As expected, the transformation reactions proceeded quickly to give the desired product **6** in excellent yields (entries 5 and 6). Although the degree of the P-tritylation upon prolonging the reaction time was slightly higher than that of the cases using DMTr derivatives, the expected product **6** was obtained quantitatively upon quenching the reaction in a short time (Fig. 1B).

Table 1. Reaction conditions[a] for the transformation of boranophosphate diester **5** to *H*-phosphonate **6**

Entry	Reagent (5 equiv)	Additive (5 equiv)	Ratio of compounds (%)[b]							
			Products (**6**)		Intermediates		P-tritylation		Transesterification[e]	
			A[c]	B[d]	A	B	A	B	A	B
1	DMTr–OMe	—	78	74	22	21	0	5	0	0
2	DMTr–OH	—	75	75	23	20	2	5	0	0
3	DMTr–Cl	—	59	30	0	0	20, 21[f]	49, 21[f]	0	0
4	DMTr–Cl	MeOH	89	72	0	0	0	13	11	15
5	TMTr–OMe	—	95	92	0	0	5	8	0	0
6	TMTr–OH	—	100	79	0	7	0	14	0	0
7	TMTr–Cl	—	85	75	0	0	0	4	15	21
8	TMTr–Cl	MeOH	89	81	0	0	0	5	11	14
9	Pix–OMe	—	78	70	22	24	0	6	0	0
10	Pix–OH	—	72	60	25	15	3	25	0	0
11	Pix–Cl	—	56	29	23	11	9	45	14	15
12	Pix–Cl	MeOH	61	42	19	0	9	44	11	15

[a] All the reactions were carried out with **5** (0.1 M) in the presence of TFA (3 equiv) in $CDCl_3$ at rt.
[b] Estimated by ^{31}P NMR analysis.
[c] Measured during 5–10 min.
[d] Measured during 60–72 min.
[e] May be these side products are 5′-5′ or 3′-3′ dithymidine *H*-phosphonates.
[f] See Ref. 27.

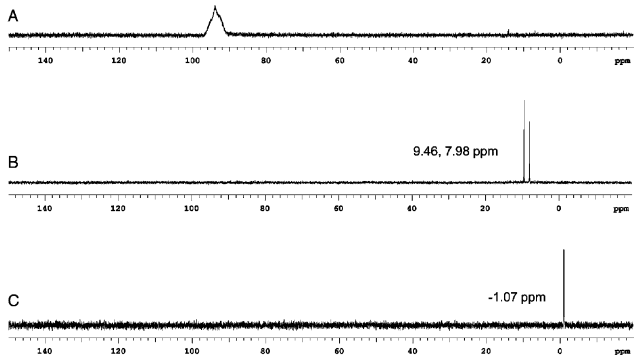
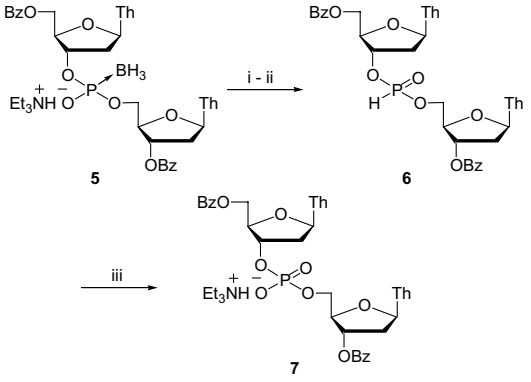


Figure 1. ^{31}P NMR spectra of **5** (A), the reaction mixture obtained by the reaction of **5** with TMTr–OH in the presence of TFA in $CDCl_3$ for 1 min followed by aqueous work-up (B), and isolated **7** in $CDCl_3$ (C) (P–H decoupled NMR spectra).



Scheme 4. Synthesis of *O*-protected TpT starting from dithymidine boranophosphate. (i) TFA, TMTr–OH/$CHCl_3$, (ii) Et_3SiH, ext. with $CHCl_3$/satd $NaHCO_3$, (iii) I_2/pyridine–H_2O (98:2, v/v), ext. with $CHCl_3$/triethylammonium bicarbonate, purified by silica gel column chromatography, overall yield >99%.

When 9-phenylxanthen-9-yl (Pix) type reagents were used as cation sources, the reaction rates were similar to those using DMTr reagents. However, a considerable amount of a mixture of the P-tritylated products was formed upon prolonging the reaction times (entries 9 and 10).

Next, the isolation of the dinucleoside *H*-phosphonate **6** was attempted by applying the optimized reaction conditions. Compound **5** was treated with TMTr–OH in the presence of TFA in $CHCl_3$ for 1 min. Although the quantitative formation of **6** was ascertained by a TLC monitoring after aqueous work-up, pure **6** was isolated only in 75% yield due to a partial decomposition of **6** during silica gel column chromatography.[28,29] Therefore, we attempted to isolate the product as a stable phosphate derivative, **7**, after oxidation with I_2 in pyridine–H_2O (98:2, v/v).[30] As a result, the dinucleoside phosphate **7** was obtained in quantitative yield from boranophosphate **5** (Scheme 4 and Fig. 1C). This result clearly indicates that the deprotection of the dithymidine boranophosphate derivative did take place quantitatively.

In conclusion, we have demonstrated the first example of the use of BH_3 as a phosphonic acid protecting group. The method enabled us to synthesize *H*-phosphonate DNAs, versatile intermediates for the backbone-modified DNA analogs, by treatment of boranophosphate DNAs with a desired sequence, which can be easily prepared by the boranophosphotriester method,[23] with Ar_3C^+ under mild acidic conditions. The present method will be also useful for the synthesis of a wide variety of organic compounds bearing phosphate functions as well as their analogs.

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