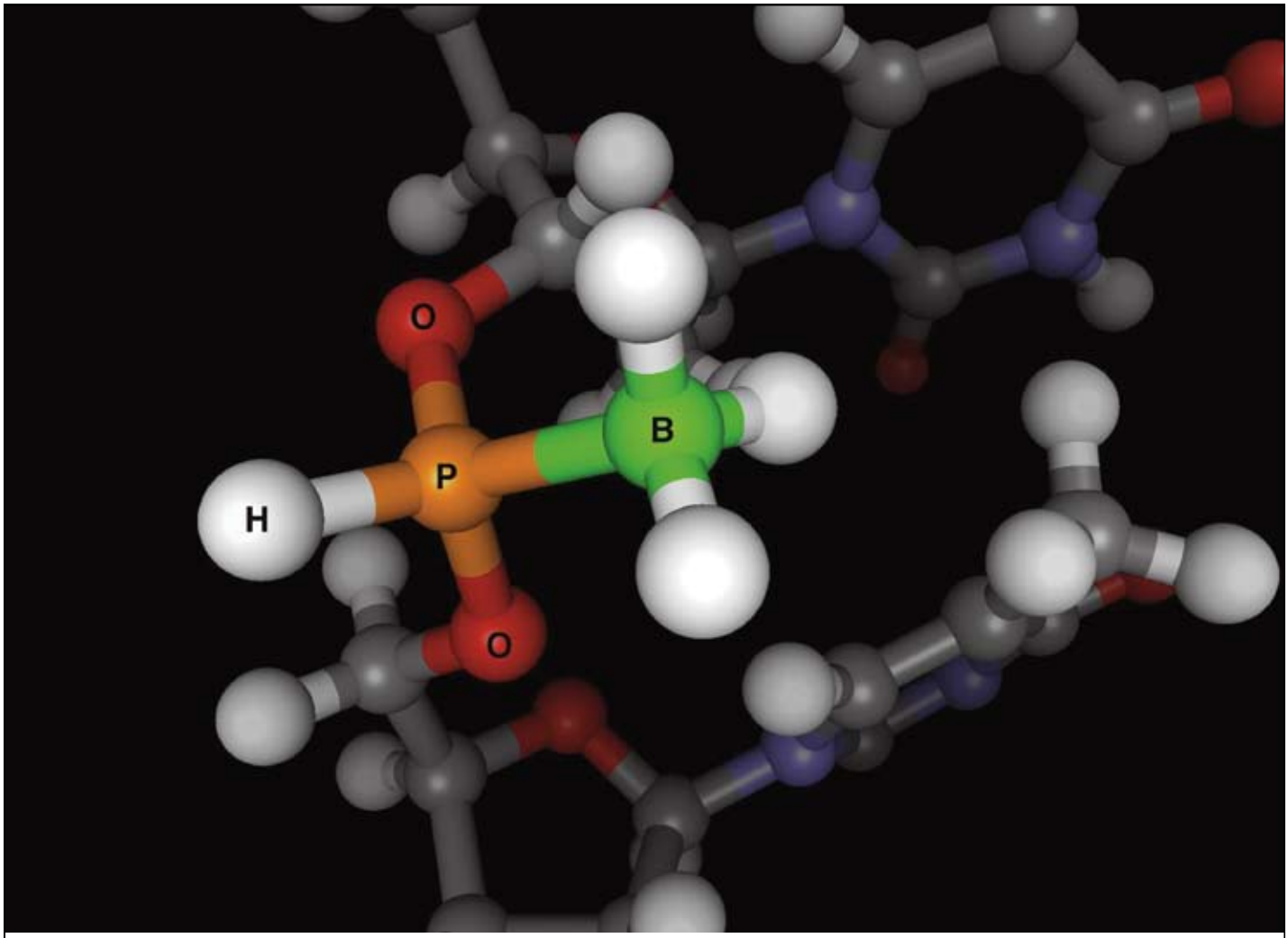

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Title: Nucleoside *H*-boranophosphonates: a new class of boron-containing nucleotide analogues

A new class of boron-containing nucleotide analogues, nucleoside *H*-boranophosphonates, have been developed. These compounds have a P–H and a P→BH3 group, both of which can be used for further modifications. The unique chemical properties of *H*-boranophosphonates will open up access to a wide variety of as yet unknown phosphate-modified nucleotide analogues.

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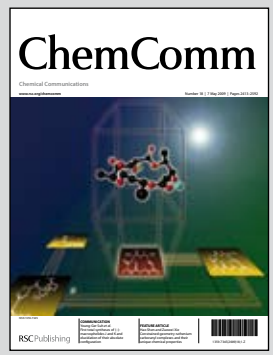

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Nucleoside *H*-boranophosphonates: a new class of boron-containing nucleotide analogues†

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A study on the synthesis of nucleoside *H*-boranophosphonates, a new class of nucleotide analogues having a P→BH_3 and a P–H group, *via* condensation of the corresponding nucleosides with *H*-boranophosphonate derivatives is described.

Chemical modifications of natural nucleotides and oligonucleotides by replacing the non-bridging oxygen atoms of their phosphate groups with other elements or substituents (*e.g.*, sulfur atom) have been widely used, especially for the development of nucleic acid-based drug candidates.[1,2] Nucleoside boranophosphates, in which a non-bridging oxygen atom of the phosphate group is replaced with a BH_3 group, are one of the most promising candidates owing to their significant stability to nucleases and high lipophilicity, which may save the need for an elaborate delivery system.[3] Their low cytotoxicity has also been suggested.[4] In addition, oligonucleoside boranophosphates have shown promising RNA interference activity,[5] and may also be useful as target-specific ^{10}B carriers for boron-neutron capture therapy (BNCT).[6]

These studies on the nucleoside boranophosphates have generated interest in other nucleotide analogues containing a P→BH_3 group. However, only a limited number of such analogues are available[7,8] with the methods developed for the synthesis of nucleoside boranophosphates.[9–11]

To expand the availability of the boron-containing nucleotide analogues, we designed a novel nucleotide analogue having a P→BH_3 and a P–H group (nucleoside *H*-boranophosphonate **1**, Fig. 1) with the expectation that it would work as a versatile precursor of a variety of boron-containing *P*-modified nucleotide analogues *via* the activation of the P–H function by bases or transition metals followed by reactions with electrophiles.[12] The P→BH_3 moiety may also work as a modifiable site *via* deboronation and subsequent reaction with electrophiles, which would expand the availability of *P*-modified nucleotide analogues.[12a,13]

To the best of our knowledge, there are only two reports on the synthesis of *H*-boranophosphonate derivatives.[12c,14] Centofanti reported the synthesis of dimethyl *H*-boranophosphonate from dimethyl phosphonite decades ago.[14] In addition, the synthesis of silyl *H*-boranophosphonate derivatives and their applications as precursors of alkylboranophosphonates were reported by Montchamp *et al.* very recently.[12c] However, it is difficult to apply these methods to the synthesis of more functionalized molecules, such as nucleotide analogues.

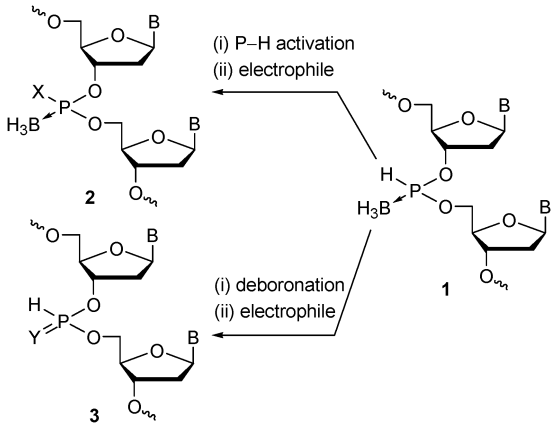
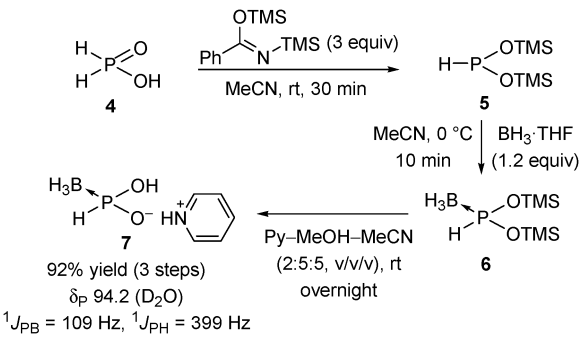


Fig. 1 Strategy for synthesis of various *P*-modified oligonucleotide analogues **2** and **3** by substitution of P–H and P–B bonds of oligonucleoside *H*-boranophosphonates **1**, respectively. B = nucleobase.

We anticipated that an *H*-boranophosphonate derivative having at least one free P–O^- function would undergo condensation reactions with the hydroxy group of nucleosides to give the nucleoside *H*-boranophosphonates under mild reaction conditions. Based on this idea, we synthesized inorganic *H*-boranophosphonate as a monopyridinium salt (Scheme 1, **7**). Phosphinic acid **4** was treated with *N*,*O*-bis(trimethylsilyl)-benzamide[15] to afford bis(trimethylsilyl) phosphonite **5**, which was then boronated by treatment with BH_3·THF. Montchamp *et al.* have also synthesized bis(trialkylsilyl) *H*-boranophosphonates and alkyl trialkylsilyl *H*-boranophosphoates *via* the corresponding phosphonite derivatives, though the bis(TMS) derivative **5** was not used for further applications due to its instability. In contrast, we used the silylation as a transient protection and the intermediate **6** was desilylated by treatment with MeOH and pyridine to synthesize **7**. The



Scheme 1 Synthesis of pyridinium *H*-boranophosphonate **7**.

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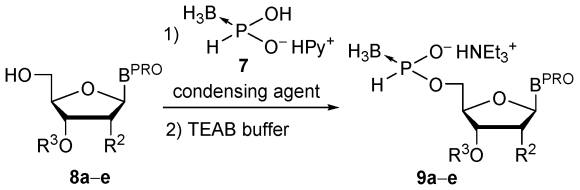
Table 1 Synthesis of nucleoside 5′-*H*-boranophosphonates **9a–e** by 5′-boranophosphonylation of nucleosides **8a–e**[a]



Entry	**8**	B^{PRO}	R^2	R^3	Reagents and conditions	Yield of **9a–e** (%)
1	**a**	Th^{bz}	H	DMTr	**7** (1.5 equiv.), Piv-Cl (1.5 equiv.), *i*Pr_2NEt (3 equiv.), NT (1.5 equiv.), MeCN, rt, 3 h	39
2	**a**	Th^{bz}	H	DMTr	**7** (2 equiv.), Bop-Cl (2 equiv.), *i*Pr_2NEt (4 equiv.), NT (2 equiv.), MeCN, rt, 2 h	43
3	**a**	Th^{bz}	H	DMTr	**7** (1.2 equiv.), Bop-Cl (1.2 equiv.), Py, rt, 2 h	95
4	**b**	Th	H	DMTr	**7** (1.6 equiv.), Bop-Cl (1.6 equiv.), Py, rt, 1 h	95
5	**c**	Th	H	Bz	**7** (1.2 equiv.), Bop-Cl (1.2 equiv.), Py, rt, 30 min	92
6	**d**	Th	H	Pac	**7** (1.2 equiv.), Bop-Cl (1.2 equiv.), Py, rt, 40 min	90
7	**e**	Ur	OPac	Pac	**7** (2 equiv.), Bop-Cl (2 equiv.), Py, rt, 2 h	98

[a] TEAB = triethylammonium bicarbonate; B^{PRO} = (protected) nucleobase; Th^{bz} = N^3-benzoylthymin-1-yl; DMTr = 4,4′-dimethoxytrityl; Piv-Cl = pivaloyl chloride; NT = 3-nitro-1*H*-1,2,4-triazole; Bop-Cl = bis(2-oxo-3-oxazolidinyl)phosphinic chloride; Pac = phenoxyacetyl.

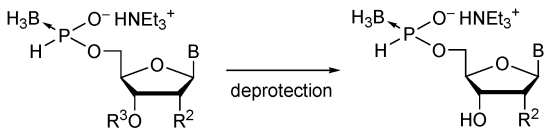
resultant pyridinium *H*-boranophosphonate **7** was stable to air oxidation and water, and can be stored for at least several months at −30 °C without decomposition.

Pyridinium *H*-boranophosphonate **7** was applied to the synthesis of nucleoside *H*-boranophosphonate derivatives. Firstly, nucleoside 5′-*H*-boranophosphonates were synthesized by condensation of **7** with appropriately protected nucleosides having a 5′-hydroxy group (**8a–e**, Table 1). When the reaction was carried out under conditions similar to those used for the boranophosphorylation of nucleosides,[11a] the isolated yields of the desired thymidine 5′-*H*-boranophosphonate **9a** were low mainly due to the formation of a 5′,5′-dinucleoside *H*-boranophosphonate diester (entries 1 and 2). In sharp contrast, condensations promoted by Bop-Cl in pyridine afforded the desired nucleoside 5′-*H*-boranophosphonates **9a–e** in excellent yields without observable side-reactions (entries 3–7).

Next, we attempted to synthesize fully-deprotected nucleoside 5′-*H*-boranophosphonates, which would be interesting as a new class of boron-containing nucleoside monophosphate analogues. Firstly, the detritylation of 3′-*O*-DMTr-thymidine 5′-*H*-boranophosphonate **9b** was attempted by treatment with an 80% AcOH aqueous solution. However, decomposition of the *H*-boranophosphonate moiety (*ca.* 70%) was observed after 30 min (Table 2, entry 1). It is probably due to the reaction of the liberated $DMTr^+$ with the BH_3 group of the *H*-boranophosphonate moiety as observed in the case of boranophosphates.[10b,11a,16] In contrast, the *H*-boranophosphonate monoesters were stable under conventional ammonolysis conditions, and the nucleoside 5′-*H*-boranophosphonates **10a,b** were isolated in good yields (entries 2 and 3).

The reaction conditions optimized for the 5′-boranophosphonylation were also applicable to the synthesis of nucleoside 3′-*H*-boranophosphonates. The condensation of 5′-*O*-DMTr-nucleosides **11a,b** with **7** in pyridine in the presence of Bop-Cl afforded the desired nucleoside 3′-*H*-boranophosphonates **12a,b** in excellent yields (Table 3, entries 3 and 4), whereas the reactions promoted by Piv-Cl or Bop-Cl in MeCN in the presence of *i*Pr_2NEt and NT resulted in low yields of **12** mainly due to the generation of a 3′,3′-dithymidine *H*-boranophosphonate derivative (entries 1 and 2).

Table 2 Deprotection of nucleoside 5′-*H*-boranophosphonates **9b,d,e**



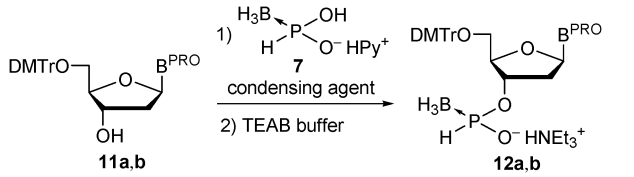
Entry	B	**9**	R^2	R^3	Deprotection conditions	**10**	Yield of **10** (%)
1	Th	**b**	H	DMTr	80% AcOH, rt, 30 min	**a**	—[a]
2	Th	**d**	H	Pac	sat. NH_3–MeOH, rt, 50 min	**a**	71
3	Ur	**e**	OPac	Pac	sat. NH_3–MeOH, rt, 3 h	**b**	68

[a] Decomposition of *H*-boranophosphonate moiety was observed.

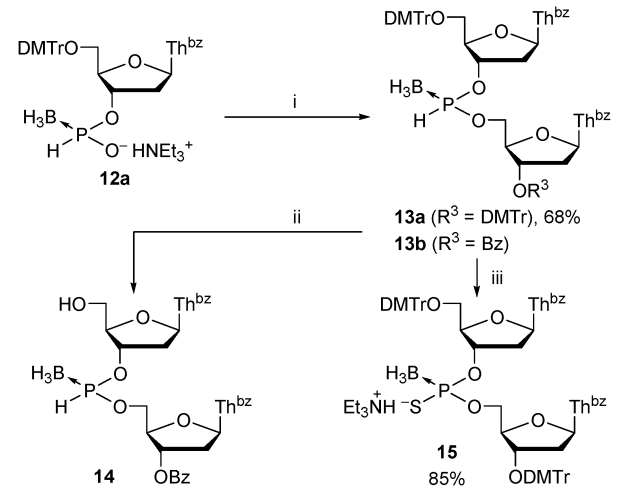
The 5′-*O*-protected nucleoside 3′-*H*-boranophosphonates are potentially useful as monomer units to synthesize oligonucleotide analogues having an *H*-boranophosphonate diester backbone. As we mentioned above, such oligonucleotide analogues would work as precursors of a variety of backbone-modified oligonucleotide analogues through the P–H or P–B modifications. To investigate the potential of the *H*-boranophosphonates, a dithymidine *H*-boranophosphonate was synthesized from the 5′-*O*-DMTr-nucleoside 3′-*H*-boranophosphonate **12a**. The 3′-*H*-boranophosphonate **12a** was allowed to condense with 3′-*O*-DMTr-N^3-benzoyl-thymidine **8a** in MeCN in the presence of Bop-Cl and 2,2,6,6-tetramethylpiperidine (Scheme 2, reaction i). The reaction proceeded smoothly at rt. Although a partial decomposition during silica gel column chromatography was observed, the dithymidine *H*-boranophosphonate diester **13a** was isolated in a modest yield.

To explore the potential of the nucleoside *H*-boranophosphonates, we carried out the following two experiments. Firstly, a 3′-*O*-benzoyl-dithymidine *H*-boranophosphonate **13b**

Table 3 Synthesis of nucleoside 3′-*H*-boranophosphonates **12a,b** by 3′-boranophosphonylation of nucleosides **11a,b**



Entry	**11**	B^{PRO}	Reagents and conditions	Yield of **12** (%)
1	**a**	Th^{bz}	**7** (1.2 equiv.), Piv-Cl (1.5 equiv.), iPr$_2$NEt (3 equiv.), NT (1.5 equiv.), MeCN, rt, 4 h	41
2	**a**	Th^{bz}	**7** (1.2 equiv.), Bop-Cl (3 equiv.), iPr$_2$NEt (6 equiv.), NT (3 equiv.), MeCN, rt, 1.5 h	50
3	**a**	Th^{bz}	**7** (1.2 equiv.), Bop-Cl (1.2 equiv.), Py, rt, 1 h	95
4	**b**	Th	**7** (2 equiv.), Bop-Cl (2 equiv.), Py, rt, 3 h	82



Scheme 2 (i) Synthesis of dithymidine *H*-boranophosphonates **13a,b**, (ii) 5′-*O*-detritylation of **13b** and (iii) conversion of **13a** into dithymidine boranophosphorothioate **15** by P–H modification. *Reagents and conditions*: (i) N^3-benzoyl-3′-*O*-R^3-thymidine (0.75 equiv.), Bop-Cl (2.5 equiv.), 2,2,6,6-tetramethylpiperidine (6 equiv.), MeCN, rt, 1 h; (ii) 3% dichloroacetic acid in CH_2Cl_2–Et_3SiH (3 : 1, v/v), rt, 1 min; (iii) S_8 (3 equiv.), Et_3N (3 equiv.), MeCN, rt, 3 h.

was synthesized as a crude product in a similar manner and treated with 3% dichloroacetic acid in $CDCl_3$–Et_3SiH (1 : 1, v/v)[11b,16] for 5′-*O*-detritylation. In contrast to the detritylation of the *H*-boranophosphonate monoester **9b** by treatment with 80% AcOH (Table 2, entry 1), which caused the decomposition of the *H*-boranophosphonate moiety, the 5′-*O*-DMTr group of **13b** was quantitatively removed without decomposition of the product (Scheme 2, reaction ii). It indicates that a solid-phase synthesis of oligonucleoside *H*-boranophosphonates *via* condensation and 5′-*O*-detritylation is feasible.

Secondly, the possibility of the P–H modification of the *H*-boranophosphonate diester linkage was investigated by treating the dithymidine *H*-boranophosphonate **13a** with S_8 in the presence of Et_3N under anhydrous conditions. A ^{31}P NMR analysis of the reaction showed that **13a** (δ_P 135.1, 133.7) was quantitatively converted into the corresponding dithymidine boranophosphorothioate **15** (δ_P 162.5, 161.0)[8a,b] within 3 h at rt, and **15** was isolated in good yield (Scheme 2, reaction iii).

In conclusion, nucleoside *H*-boranophosphonates were synthesized *via* condensation of the corresponding nucleosides and *H*-boranophosphonate derivatives. The mild reaction conditions and high efficiency of this method are attractive for further applications to the synthesis of a broad spectrum of *H*-boranophosphonate derivatives. In addition, conversion of the P–H group of a dinucleoside *H*-boranophosphonate into a P–S$^-$ group demonstrated that the *H*-boranophosphonate derivatives are potential precursors of a variety of boron-containing oligonucleotide analogues. Further studies on the synthesis of nucleoside *H*-boranophosphonates and their applications are in progress.

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