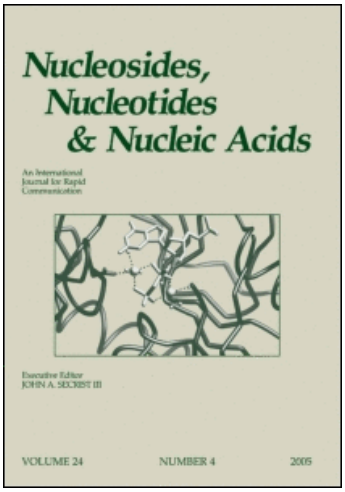

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SYNTHESIS OF A NOVEL NUCLEIC ACID MIMIC: P-BORANOMETHYLPHOSPHONATE

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SYNTHESIS OF A NOVEL NUCLEIC ACID MIMIC: P-BORANOMETHYLPHOSPHONATE

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ABSTRACT

A new type of non-ionic nucleotide analogue with a doubly modified internucleotide linkage, *P*-boranomethylphosphonate, has been successfully synthesized and characterized. Dithymidine boranomethylphosphonate **5** is the first example of a *P*-boranomethylphosphonate compound; it is a highly lipophilic phosphodiester analog, which is almost totally resistant to both snake venom phosphodiesterase (SVPDE) and bovine spleen phosphodiesterase (BSPDE). *P*-boranomethylphosphonates are expected to be promising candidates for mechanistic, diagnostic and therapeutic applications.

INTRODUCTION

Non-ionic nucleotide and oligonucleotide analogs (1) have long attracted attention. Improved uptake by cells and extended biological half-life make them attractive candidates for use as antiviral and antisense agents in both cell culture and animals. These analogues are interesting in their own right because studies on their conformation and interactions with proteins and nucleic acids can give new insights into factors affecting nucleic acid structure and function. Of the uncharged modified oligonucleotides, the nucleoside methylphosphonates, wherein the negatively charged phosphate oxygen is replaced by a methyl group, are among the most promising because they are highly resistant to nucleases (2).

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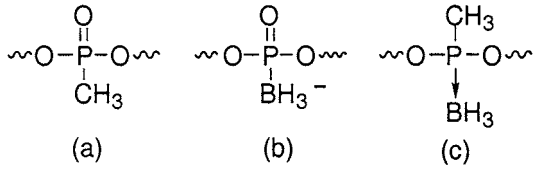
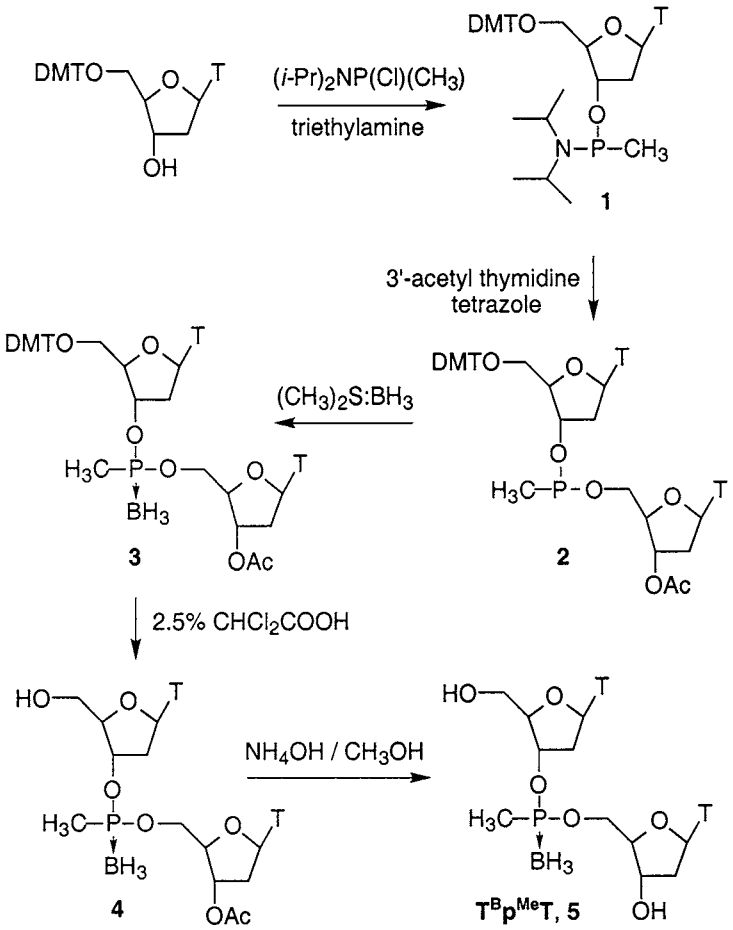


Figure 1. Internucleotide linkages: (a) methylphosphonate, (b) boranophosphate, (c) boranomethylphosphonate.

By structurally combining the methylphosphonate (2) and boranophosphate (3) backbones, we have created a new phosphodiester linkage, the boranomethylphosphonate [CH_3–P–BH_3] (Fig. 1), wherein the two nonbridging oxygen atoms of a phosphodiester group are replaced by methyl and borane groups. Since these groups impart lipophilicity to oligonucleotides, it was expected that the resulting



Scheme 1.

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hybrid nucleoside *P*-boranomethylphonates should be more lipophilic and more resistant to nucleases than either methylphosphonates or boranophosphates.

Here, we report the first example of a boranomethylphosphonate compound, specifically the dithymidine *P*-boranomethylphosphonate ($T^BP^{Me}T$, **5**), its synthesis, characterization, and some biochemical properties.

The synthesis of dinucleoside *P*-boranomethylphosphonate **5** is outlined in Scheme 1. 5′-*O*-DMT-thymidine was phosphitylated with (i-Pr_2N)P(Cl)(CH_3) catalyzed by triethylamine to give **1**. Phosphite **1** was treated *in situ* with 3′-*O*-acetylthymidine and tetrazole in DMF to give compound **2** (^{31}P NMR signals at 189.1 and 188.1 ppm), which was then treated with excess borane-methyl sulfide complex to afford phosphite-borane **3** with a ^{31}P NMR signal at 152.5 ppm (br). The low boiling-point solvents were removed under reduced pressure; and the syrup was treated with DMT removal reagent (2.5% dichloroacetic acid in dichloromethane) to yield **4** which was converted to **5** with NH_4OH/CH_3OH at room temperature. The crude mixture was purified by column chromatography on silica gel to give **5** which was further purified by reverse-phase HPLC by isocratic elution with 84% 50 mM triethylammonium acetate (TEAA) and 16% CH_3CN to obtain pure **5**, $R_t = 12.1$ min. The crude **5** could also be purified on a silica gel HPLC colunm, eluting with 9% methanol and 91% ethyl acetate, $Rt = 16.3$ min. The overall yield of dithymidine *P*-boranomethylphosphonate **5** ($T^BP^{Me}T$) was about 42%. The chemical structure of **5** was established via UV $\lambda_{max} = 267$nm; ^{31}P NMR (DMSO-d_6) δ (ppm) 147.6 (br); ^{1}H NMR (not shown); MS (FAB^+): m/z for $(M+H)^+$ 543.2; HRMS (FAB^-) calcd for $C_{21}H_{31}O_{10}N_4PB$ $(M–H)^-$ 541.1869, found 541.1856. Successful separation of two diastereomers (R_p and S_p) of **5** was achieved by reverse-phase HPLC with 18% CH_3CN and 82% 50 mM triethylammonium acetate; for the first eluted diastereomer **5a**, Rt (**5a**) = 14.7 min; for the second eluted diastereomer **5b**, Rt (**5b**) = 16.2 min. The method described above should be applicable to the synthesis of other deoxyribo- or ribo-oligonucleoside boranomethylphosphonates.

RESULTS AND DISCUSSION

Replacement of a nonbridging oxygen atom in the natural phosphodiester linkage by S^-, CH_3 or BH_3^- generally preserves the ability of oligodeoxynucleotides (ODN) to form complementary duplexes, although with somewhat lower stability (4–5). The phosphorothioate internucleotide analog has emerged as the linkage of choice for the development of antisense oligonucleotides as therapeutic agents. Although oligonucleoside phosphorothioates (5) have many virtues, new analogs are still of interest due to the poor cellular permeation properties of phosphorothioate ODNs. Numerous backbone modifications have been made to increase the cellular permeability of ODNs but few have resulted in an improvement. Thus, we decided to introduce methyl and borane into the nucleotide phosphodiester linkage. As expected, double replacement of two oxygen atoms in the natural phosphodiester

linkage greatly enhances the lipophilicity of the oligonucleotide and imparts almost total resistance to the 3′ and 5′ endonucleases.

Our studies indicated that the *P*-boranomethylphosphonate internucleotide linkage is: (1) very stable toward neutral and acidic hydrolysis; (2) extremely resistant toward cleavage by both snake venom phosphodiesterase (SVPDE) and bovine spleen phosphodiesterase (BSPDE), and (3) highly lipophilic. The $T^{B}P^{Me}T$ dimer was 6800- and 370-fold more lipophilic than normal TpT (dithymidine phosphate) and $Tp^{B}T$ (dithymidime boranophosphate) accordingly.

Thus, the non-ionic [CH_3–P–BH_3]-ODN constitutes an entirely new and intriguing class of modified nucleic acids. High lipophilicity and resistance to enzymatic cleavage, in conjuction with possible utility as carriers of ^{10}B in boron neutron capture therapy (BNCT) (6) for the treatment of cancer, make the boranomethylphosphonate a promising candidate for further mechanistic, diagnostic and therapeutic investigation.

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