Structural Studies of a Borane-Modified Phosphate Diester Linkage: Ab Initio Calculations on the Dimethylboranophosphate Anion and the Single-Crystal X-ray Structure of Its Diisopropylammonium Salt

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Substitution of one of the phosphoryl oxygen atoms of a phosphate diester ($RO_2PO_2^-$) with a borane group gives rise to a material with the formula $RO_2P(O)BH_3^-$, which has been called either a boranophosphite[1] or boranophosphate ester (Figure 1). Oligonucleotides bearing borane (BH_3) groups at the phosphate ester linkage possess a number of properties that make them especially promising materials for therapeutic and genetic sequencing applications. We have prepared these materials on solid supports[2] as well as in solution and by template-directed reaction catalyzed by DNA polymerases.[3] These compounds are quite stable toward chemical and enzymatic hydrolysis and have increased lipophilicity relative to normal oligonucleotides.[4] Until recently, the absence of structural data for the boranophosphate diester linkage has made discussion of its stability and reactivity speculative. We report here the results of ab initio calculations on a series of related molecules and ions, including dimethyl-boranophosphate, $(MeO)_2P(O)BH_3^-$, and the structure of its diisopropylammonium salt as determined by single-crystal X-ray diffraction. We compare two crystallographic structures[5] to each other and to the calculated structures and demonstrate that (1) substitution of a BH_3 group for oxygen in a phosphate or phosphite ester results in an increase in bond length to that group, (2) other structural perturbations are minimal, and (3) bond polarization toward that group is significantly diminished. The resistance of boranophosphate nucleotides to digestion by nuclease enzymes is discussed in light of these observations.

Crystallographic and calculated structures of $(MeO)_2P(O)BH_3^-$, compared in Table 1, are in good agreement. Figure 2 presents the ORTEP drawing[6] of the anion. Substitution of diisopropyl-ammonium for potassium has little effect on uncorrected bond lengths of the anion.[5] We found that libration contributes significant systematic error to the crystallographic structure of the diisopropylammonium salt.[7] A rigid-body analysis gave libration corrections that varied between 4.3 and 15.9 times the esd's of the raw bond lengths. The P−B bond lengths (ranging from 1.921 to 1.945 Å) predicted by Gaussian calculations[8] for
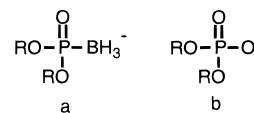

Figure 1. Formulas for (a) boranophosphate and (b) phosphate diester anions.

Table 1. Comparison of Calculated and Crystallographic Bond Lengths for $(MeO)_2P(O)BH_3^-$

bond	(*i*-Pr)$_2$NH$_2$ salt	corr[a]	K salt[b]	MP2[c,d]	RHF(1)[c,e]	RHF(2)[c,f]
P−B	1.887(3)	1.905	1.895(6)	1.921	1.945	1.936
P=O	1.4982(15)	1.518	1.490(3)	1.521	1.485	1.490
P−O	1.5972(15)	1.617	1.597(4)	1.668	1.650	1.626
P−O	1.6014(16)	1.627	1.612(3)	1.698	1.626	1.626
O−C	1.426(3)	1.439		1.427	1.399	1.412
O−C	1.424(4)	1.445		1.430	1.401	1.412

[a] Crystallographic bond lengths corrected for libration. [b] Structure reported in ref 5. [c] Calculations for ions using a 6-31+G** basis set. [d] Optimized in the gas phase using MP2. [e] Optimized in the gas phase using HF. [f] Optimized in solution using HF.
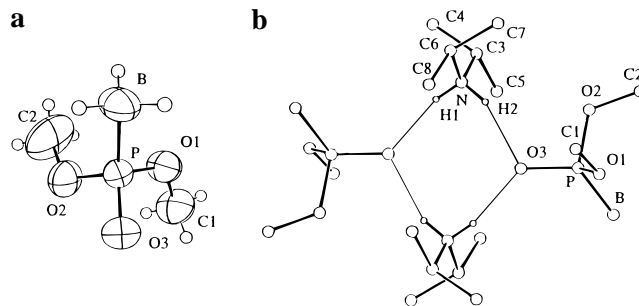

Figure 2. (a) ORTEP drawing of the dimethylboranophosphate anion and (b) illustration of the hydrogen-bonding geometry between two boranophosphate anions and two diisopropylammonium cations. The complex precipitated in a monoclinic cell of $P2_1/n$ symmetry; the unit cell dimensions were $a = 9.6242(5)$ Å, $b = 12.9089(8)$ Å, $c = 11.6897(6)$ Å, $\beta = 93.649(5)°$, and $Z = 4$. Residuals and goodness of fit for significant reflections were $R(F) = 0.049$, $R_w = 0.059$, and GOF = 1.42.[18]

$(MeO)_2P(O)BH_3^-$ (and the 1.923 Å bond calculated[9] for $(HO)_2P(O)BH_3^-$) are somewhat longer than the 1.905 Å libration-corrected bond length found in the crystal. The difference between the predicted and observed bond lengths may arise from polarization of the molecule by the coordination and hydrogen bonds formed in the crystals; removal of electron density from the phosphoryl oxygen might be strengthening the P−B bond at

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(5) During the preparation of this paper, the structure of potassium dimethylboranophosphate, K(MeO)$_2$P(O)BH$_3$, was published (without crystallographic parameters) in a report describing some of the chemistry of the anion. Imamoto, T.; Nagato, E.; Wada, Y.; Masuda, K.; Yamaguchi, K.; Uchimaru, T. *J. Am. Chem. Soc.* **1997**, *119*, 9925−6.
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(7) A rigid-body analysis was performed using THMA11 and the anisotropic displacement parameters for the non-hydrogen atoms of the anion to correct the bond lengths for the effects of libration within the molecule. The motion of the anion in the crystal structure fit well for a rigid body. The residual factor, R ($R = [\sum(w\Delta U)^2/\sum(wU_{obs})^2]^{1/2}$), between observed and calculated ADP values was 0.033. For further information, see: Dunitz, J. D.; Schomaker, V.; Trueblood, K. N. *J. Phys. Chem.* **1988**, *92*, 856−67.

Table 2. Comparison of P–L Bond Lengths, P Atomic Charges, and ^{31}P Resonance Frequencies for $(MeO)_2P(L_1)L_2$ Species

	L_1	L_2	P–$L_1{}^a$	P–$L_2{}^a$	P charge	L_1 charge	L_2 charge	^{31}P (ppm)
1	BH$_3$	O$^-$	1.921	1.521	+2.36	−0.87	−1.27	95^b
2	O	O$^-$	1.508	1.508	+2.78	−1.26	−1.26	−2.4^c
3		O$^-$		1.543	+1.66		−1.31	
4	BH$_3$	OMe	1.892	1.634	+2.43	−0.77	−0.55	118^d
5	O	OMe	1.485	1.625	+2.84	−1.18	−0.56	
6		OMe		1.679	+1.77		−0.60	140^c

^{a} Data from MP2 calculation, as in Table 1. ^{b} Reported here. ^{c} From ref 12a. ^{d} From ref 12b.

the expense of the P=O bond. This effect was not accounted for in the calculations. The configuration about the B–P bond is anti (the O$_3$–P–B–H$_{b2}$ torsion angle is 178(3)°).[10]

The bonding geometries around phosphorus in phosphate and boranophosphate esters are strikingly similar, differing mostly in the lengths of the P–O versus P–B bonds. The phosphoryl (P=O) bond in the boranophosphate diester (1.521 Å) is similar to that found in the structure of diesters (1.47–1.51 Å) and monoesters (1.49–1.53 Å), and it is significantly longer than that seen in phosphate triesters (1.38–1.44 Å).[11] Table 2 compares calculated bond lengths and phosphorus atomic charges for a series of related $(MeO)_2P(L_1)L_2$ materials, where L_1 = BH$_3$, O, or no ligand and L_2 = O$^-$ or OMe.

Atomic charge data from our calculations and the available NMR data (Table 2) reflect the expected differences in electronegativities of the oxygen and borane moieties (O > BH$_3$). The phosphorus charge decreases from +2.78 to +2.36 upon replacement of an oxygen by a borane, while the magnitude of the substituent charge decreases from −1.28 (oxygen) to −0.87 (BH$_3$). It is well-known that magnetic resonance frequencies are strongly influenced by the local electron density at observed nuclei. While other factors influence resonance frequencies, the calculated phosphorus atomic charges roughly correlate with ^{31}P NMR data: The 95 ppm resonance of the boranophosphate is intermediate between the 140 ppm frequency of the phosphorus(III) species, $(MeO)_3P$ (which carries a +1.77 charge on phosphorus), and the −2.4 ppm resonance of the phosphorus(V) $(MeO)_2PO_2{}^-$.[12]

Recent work has forwarded the possibility that hydridic B–H bonds can serve as hydrogen-bond acceptors.[13] We did not observe such an effect in the X-ray study. The terminal boranophosphate oxygen forms hydrogen bonds with the N–H

protons of two neighboring diisopropylammonium cations. The two cations each form hydrogen bonds to two boranophosphate anions, giving rise to the cyclic structure shown in Figure 2. The borane in this structure is apparently unable to compete with the terminal oxygen to form hydrogen bonds with the acidic protons. This is consistent with the observation that the cation in the crystal of the potassium salt, $(MeO)_2P(O)BH_3K$, interacts exclusively with the oxygen substituent, whereas the potassium ion in the crystal of the analogous phosphate, $(MeO)_2PO_2K$, interacts with both oxygen atoms.[5] In the diisopropylammonium crystal, the borane is located in a hydrophobic environment in the crystal, with its closest intermolecular contacts (within 3.6 Å of the boron atom) being with five isopropyl hydrogen atoms from neighboring cations.[14] This preference for the hydrophobic environment is consistent with the observed increase in hydrophobicity of boranophosphate nucleotides relative to normal nucleotides.[15]

Differences in the steric, hydrogen-bonding, and electronic properties of phosphate and boranophosphate diesters may account for the unique properties of boranophosphate oligonucleotides, such as their remarkable resistance to digestion by nuclease enzymes.[16] Even though the P–B bond of the boranophosphate is longer than the P–O bond of a normal phosphate (1.91 versus 1.51 Å), our previous studies suggest that boranophosphates enter the active site of the enzymes.[15,17] We theorize that the borane group slows enzymatic phosphate ester hydrolysis because of its reduced tendency to coordinate metal ions or to form hydrogen bonds and/or because the less positive phosphorus atomic charge cannot stabilize the negative charge built up in the five-coordinate transition state. Future work should further clarify the roles of basicity, nucleophilicity, and metal-binding ability on the chemistry and biochemistry of boranophosphate ions.

In conclusion, the structure of the dimethylboranophosphate anion is very similar to the analogous phosphate diester anion, but with a long (1.91 Å) P–B bond. The phosphorus atomic charge of the boranophosphate is significantly less positive than the analogous phosphate, and hydrogen bonding via the BH bonds was not observed.

Supporting Information Available: Description of the synthesis and characterization of diisopropylammonium dimethylboranophosphate, details of the crystallographic data collection, fractional atomic coordinates for the atoms in the crystal, and displacement parameters for hydrogen atoms (isotropic) and for non-hydrogen atoms (anisotropic) (7 pages). Ordering information is given on any current masthead page.

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(8) Ab initio calculations were performed using the program Gaussian 94. Each molecular structure was fully optimized with the 6-31+G** basis set using both the MP2 and the Hartree–Fock approximations (Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Kieth, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzeewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*, Revision B.1; Gaussian, Inc.: Pittsburgh, PA, 1995). The geometries were also optimized by applying a solvent model, using the self-consistent isodensity variation (SCI-PCM) of the polarized continuum model reaction field, to the HF/6-31+G** calculated structures. We also calculated atomic charges for the MP2-optimized gas-phase compounds using a natural bond order basis (as opposed to the atomic orbital basis), a method called natural population analysis (Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.* **1988**, *88*, 899). This method tends to be more reliable than using standard Mulliken populations, but yields somewhat more polar bonds.
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(14) Of the five closest contacts to the borane group, four are methyl hydrogen atoms (found 2.974(9), 3.142(6), 3.474(11), and 3.595(6) Å from the boron position) and one is a methine hydrogen (3.20(2) Å from boron).
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(17) The nucleolytic digestion of boranophosphate-substituted thymidine dimers (TbPT) obeys Michaelis–Menten kinetics, proceeding more slowly than the reaction with normal thymidine dimer (TPT). We found that the difference is primarily in the k_{cat} term, and that the K_m for the boranophosphate was, in some instances, actually lower than that of the normal phosphate. While steric interference would be manifested in an increase in the K_m term, our kinetics studies indicate that the difference is primarily due to a decrease in the rate at which bound substrate is converted into product.
(18) $R(F) = \sum(F_o - F_c)/\sum(F_o)$, $R_w = [\sum(w(F_o - F_c)^2)]^{1/2}$, GOF = $[\sum(w(F_o - F_c)^2)/(\text{no. of reflns} - \text{no. of params})]^{1/2}$.