Boranophosphate Salts as an Excellent Mimic of Phosphate Salts: Preparation, Characterization, and Properties

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We report on the preparation of boranophosphate salts, BPi (**2**), and the exploration of their properties with a view to developing a new mimic of the parent phosphate. BPi salts were easily prepared in excellent yield in a one-pot two-step reaction from tris(trimethylsilyl) phosphite, and were characterized by X-ray crystallography and IR, ^{1}H and ^{31}P NMR spectroscopy. We evaluated the acid/base character of BPi by determining its acidity constants. Likewise, we evaluated the stability of BPi at various pH values, and calculated the decomposition-rate constants at highly acidic pH. We also monitored the H-bonded clustering of BPi in organic solvents, including MeOH. Finally, we explored the chemical behavior of BPi with respect to various organic and inorganic reagents. BPi is stable under the following conditions: both basic and acidic pH (pH $>$ 2), in the presence of amines, and in the presence of Mg^{2+} ions. However, a P–B bond cleavage is observed upon the reaction of BPi with carbodiimides or upon catalytic hydrogenation. The reducing nature of the BH_3 moiety is drastically decreased in BPi. Likewise, the nucleophilicity of BPi's oxygen atom is lower than in phosphate, Pi, salts. Based on its water solubility, acid-base character, and H-bonding properties, BPi appears as a perfect mimic of Pi and is an attractive alternative to the known phosphate isosters.

Introduction

Phosphates and phosphate-containing molecules play a major role in numerous biological systems.[1] However, the unwanted lability of the ester P−O bond has promoted the search for suitable bioisosters — phosphate analogues — which retain the biological activity but possess diminished lability. The search for bioisosters was initiated by the need to produce phosphate probes for various studies, such as probing the stereochemical requirements of enzymes.[2] In addition, phosphate bioisosters have been developed for improving the pharmacological effects of nucleotide-based drugs, for example anti-sense agents.[3]

A widely used isoster of phosphate is phosphorothioate and its analogues, proposed in the pioneering work of Eckstein et al.[4] In these analogues, the nonbridging oxygen atom is replaced by a sulfur atom. Other chemical modifications of the phosphate moiety include the substitution of the labile phosphate ester oxygen atom by a carbon or nitrogen atom, to give phosphonates and phosphoramidate analogues, respectively.[5]

During the last decade, pioneering studies by Spielvogel and Ramsay-Shaw have proposed boranophosphate anaclogues **1** as bioisosters of natural nucleotides[6] and as important tools for biochemists.[7]

This emerging field of novel nucleotide bioisosters has expanded significantly and has provided many important applications of the boranophosphate analogues. For instance, nonterminal *P*-boronated nucleotides, existing as a pair of diastereoisomers, have been used as stereochemical probes to elucidate enzymatic catalysis.[8] Oligodeoxyribonucleotides bearing boranophosphate linkages have been used for polymerase chain reaction (PCR) sequencing and DNA diagnostics,[9] and boranophosphate nucleotides have been found to be highly potent and stable P2Y-receptor agonists.[10] Oligonucleotides bearing boranophosphate linkages have also been considered as potentially useful anti-sense agents.[11] These analogues were also tested for the treatment of cancer as carriers of ^{10}B isotope in boron neutron-capture therapy.[12]

As mentioned above, the field of boranophosphates covers, in-depth, related nucleotide/oligonucleotide analogues. However, to the best of our knowledge, no attention has been given to the unique and chemically interesting inorganic boranophosphate **2** (BPi). Although the related dimethyl boranophosphate potassium salt **3** has been described by Imamoto et al.[13] and by Wada and Saigo,[14] the preparation of inorganic boranophosphate **2** has not been reported.

We present here the preparation, characterization, and unique chemical properties of inorganic boranophosphate. In addition, we demonstrate that BPi is an excellent mimic of phosphate.

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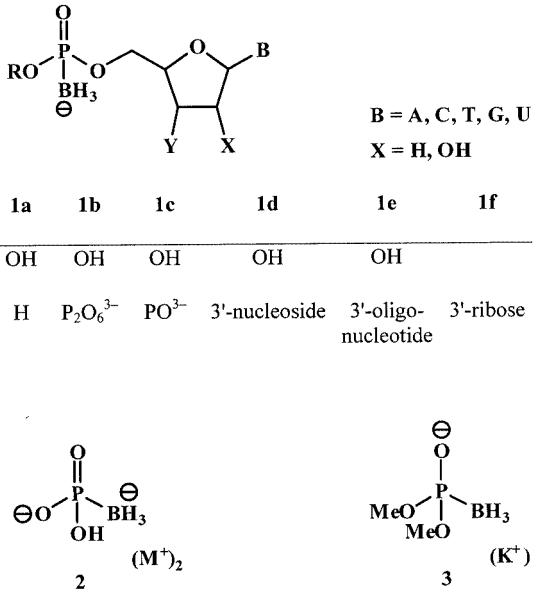
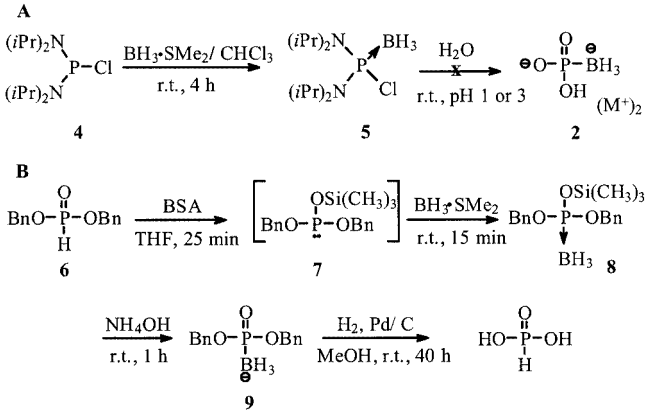


Results and Discussion

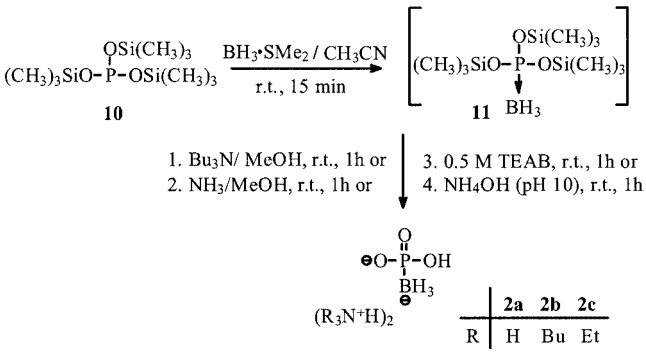
Synthesis

For the preparation of boranophosphate, we first attempted the treatment of chlorobis(diisopropylamino)phosphane (**4**) with BH_3·SMe_2 complex,[15] followed by acidic hydrolysis (pH = 3 or 1; Scheme 1A). This attempt resulted in a mixture of several phosphorus species. Alternatively, dibenzyl H-phosphonate **6** was treated with bis(silyl)acetamide, followed by boranation of intermediate **7** with BH_3·SMe_2, and hydrolysis of **8** with concentrated ammonium hydroxide. In this way, dibenzyl boranophosphate **9** was obtained in 71% overall yield (Scheme 1B). However, attempts to remove the benzyl groups by either catalytic hydrogenation or acidic hydrolysis (pH = 1.3) resulted in the cleavage of the P−B bond, leading to phosphorus acid instead of BPi.



Scheme 1

Eventually, we were able to obtain BPi in an excellent overall yield in a two-step, one-pot reaction starting from tris(trimethylsilyl) phosphite[16] (**10**; Scheme 2). Phosphite **10** was treated with BH_3·SMe_2 in dry acetonitrile under an inert gas for 15 min. Subsequently, intermediate **11** was treated with 2 M methanolic ammonia for 1 h to give the BPi **2a** as a white solid in 93% yield. No further purification was conducted, since volatile silyl derivatives and the unreacted BH_3·SMe_2 were removed by evaporation. Alternatively, intermediate **11** was treated with $NH_4OH_{(aq)}$ solution (pH = 10), Bu_3N in MeOH, or 0.5 M triethylammonium hydrogencarbonate buffer (pH = 7.5) and freeze-dried or concentrated to provide **2a**, **2b**, or **2c**, respectively.



Scheme 2

Product **2a** is highly water-soluble, whereas **2b** dissolves only in organic solvents such as MeOH, CH_3CN, DMF, and $CHCl_3$. Product **2c** is highly soluble both in water and in organic solvents.

Characterization

NMR Spectroscopy

Compound **2a** in water was characterized by ^{31}P NMR spectroscopy. It shows a signal at $\delta \approx 80$ ppm (Figure 1). The boranophosphate ^{31}P NMR spectrum shows a typical pattern including two overlapping signals: the larger signal is due to coupling of P to the ^{11}B isotope, and the smaller signal is due to coupling with the ^{10}B isotope. The relative height of the smaller peak to the larger one is 0.14 (Figure 1A).[17] BPi's hydrogen-coupled ^{31}P NMR spectrum shows further splitting of the lines into a quadruplet (Figure 1B). The ^1H NMR spectrum shows a typical doublet of quadruples pattern, at $\delta \approx 0.2$ ppm, due to coupling of H to both ^{11}B and ^{31}P (Figure 1C). This pattern overlaps a more complex pattern due to coupling of H to both ^{10}B and ^{31}P.

The chemical shift of BPi is pH-dependent. For instance, at pH = 4.87 and 13.20, the phosphorus atom of BPi resonates at δ = 84 and 63 ppm, respectively. Likewise, the P−B coupling constant is also pH-dependent, and is reduced as the pH decreases (e.g. 147 and 183 Hz for pH = 4.87 and 13.2, respectively). The pH-dependent BPi spectrum indicates structural changes of BPi, which are due to

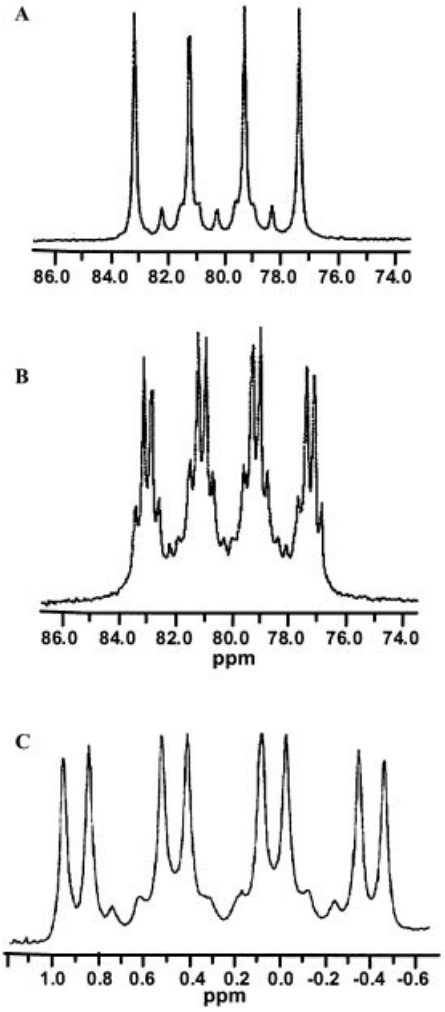


Figure 1. NMR spectra of BPi: A) ^{1}H-decoupled ^{31}P NMR spectrum in D_2O at 81 MHz; B) ^{1}H-coupled ^{31}P NMR spectrum in D_2O at 81 MHz; C) ^{1}H NMR spectrum in D_2O at 200 MHz

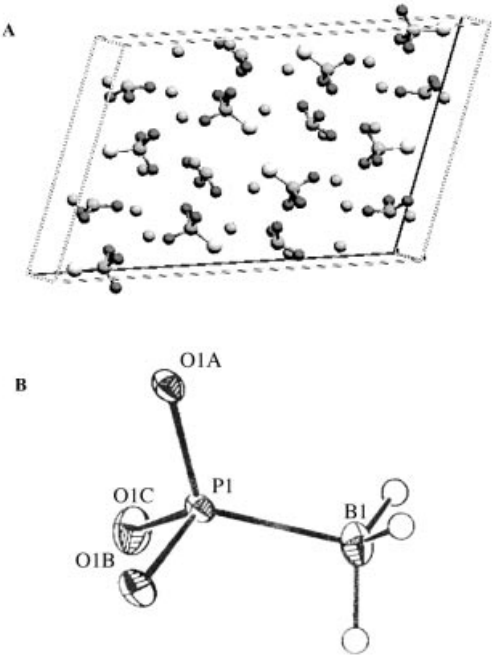


Figure 2. X-ray crystal structure of BPi: A) unit cell includes eight BPi molecules, eight H-phosphonate molecules, and 24 ammonium ions; hydrogen atoms are omitted to clarify the BPi geometry; B) ORTEP drawing of BPi; crystal data of **2a**: monoclinic, $P2_1/c$; a = 23.616(5) Å, b = 6.3470(13) Å, c = 15.325(3) Å; V = 2172.9(8) Å^3; Z = 12; $D_{calcd.}$ = 1.623 g/cm^3; $F(000)$ = 1104; 3094 reflections collected, R = 0.1015, Rw = 0.2345, GOF = 1.286; selected bond lengths [Å] and angles [°]: P(1)−O(1A) 1.524(7), P(1)−O(1B) 1.617(7), P(1)−O(1C) 1.583(7), P(1)−B(1) 1.891(11); O(1A)−P(1)−O(1C) 104.0(4), O(1A)−P(1)−O(1B) 105.3(4), O(1C)−P(1)−O(1B) 104.4(4), O(1A)−P(1)−B(1) 118.2(5), O(1C)−P(1)−B(1) 113.0(5), O(1B)−P(1)−B(1) 110.7(5)

the reduction of O−P−O angles upon protonation of the molecule.

X-ray Crystallography

To obtain structural information on BPi, compound **2a** was crystallized from an aqueous solution (pH = 7). In addition to compound **2a**, the crystal contained phosphorus acid (H-phosphonate) in a 1:1 ratio. This unexpected ratio does not reflect the molar ratio in the original BPi solution, in which phosphorus acid was less than 5%.

The unit cell contains eight BPi ions, eight H-phosphonate ions, and 24 ammonium ions (Figure 2A). Apparently, for each BPi anion, one ammonium counterion is observed, whereas two ammonium counterions are observed around each H-phosphonate group.

For BPi, the average P−B bond length is 1.892 Å, whereas for the three P−O bonds, the average lengths are 1.585, 1.605 Å, and 1.524 Å, respectively (Figure 2B). A deviation from tetrahedral angles was observed with values of 111−118° for B−P−O, and 104−105° for the O−P−O angles.

Comparison with X-ray crystal data obtained for the related dimethyl boranophosphate salt (**3**)[13] indicated similar values for the B−P (1.895 Å) and O−P (1.490, 1.597 and 1.612 Å) bond lengths. For dimethyl boranophosphate, one potassium ion was found near one of the oxygen atoms at a distance of 2.66 Å. Based on a comparison of the bond lengths of dimethyl boranophosphate salt with BPi, we assume that the BPi bears two H atoms, which were not found in the crystallographic data.

The shortest P−O bond (1.524 Å) indicates a partial double-bond character, and is in accordance with values found in the structures of phosphate diesters (1.47−1.51 Å) and monoesters (1.49−1.53 Å). This P−O bond is significantly longer than the bond observed in phosphate triesters (1.38−1.44 Å).[18]

IR Spectroscopy

The IR spectra of **2a** or **2b** in KBr pellet indicated characteristic bands for P−B and B−H in addition to bands associated with P−OH and P=O. Specifically, three absorptions at 2350, 2381, 2407 cm^{-1} (s) correspond to B−H stretches, and the absorption at 654 cm^{-1} (m) is the

P−B stretch.[19] Typical absorptions were observed for P−OH and P=O stretches at 900−1080 cm^{-1} and at 1140−1250 cm^{-1}, respectively.

For an evaluation of the effects of the solvents on the H-bonds between BPi ions, IR spectra of BPi **2a** in aqueous and methanolic solutions (see below "H-bonding of BPi") were measured in a germanium cell and compared to the corresponding spectrum of a neat sample of BPi (Figure 3B). Comparison of those spectra indicated only minor differences. For instance, a shift of about 10 cm^{-1} to lower frequencies was observed for the P=O stretch of BPi, either in the neat sample or in MeOH, relative to BPi in aqueous solution. This shift is probably due to H-bonding-based clustering in the neat sample and MeOH. The typical fine-structure for the P=O stretch in a neat sample of BPi, in the range of 1144−1178 cm^{-1}, which is possibly also due to H-bonded clusters, is lost in water. The corresponding spectrum in MeOH appears as an average of the neat sample and aqueous solution spectra, probably indicating the presence of both BPi clusters and solvent H-bonded species.

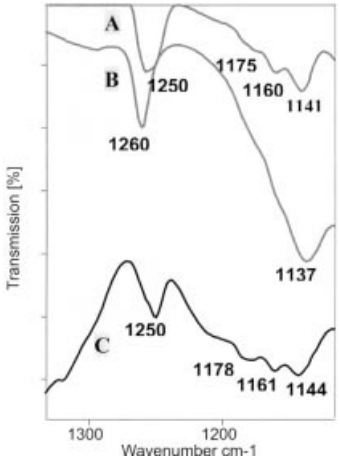


Figure 3. IR spectra of BPi **2c** (germanium cell; 1300−1100 cm^{-1}; cutoff: 680 cm^{-1}): A) methanolic solution; B) aqueous solution; C) neat sample

Chemical Properties

Acid/Base Properties

The acid/base character of BPi was studied by ^{31}P NMR-monitored pH-titration. The chemical shift of compound **2a** was plotted against the pH (Figure 4). For the pH range 4.8−13.2, two inflection points were observed. The second derivatives of the fitted function provided two pK_a values — 7.12 and 12.54 — with R^2 values of 0.999 and 0.997, respectively. These values are similar to the corresponding values of the second and third protonation equilibria of phosphoric acid (7.21 and 12.67), and they are higher than those for phosphorus acid (1.8 and 6.2).

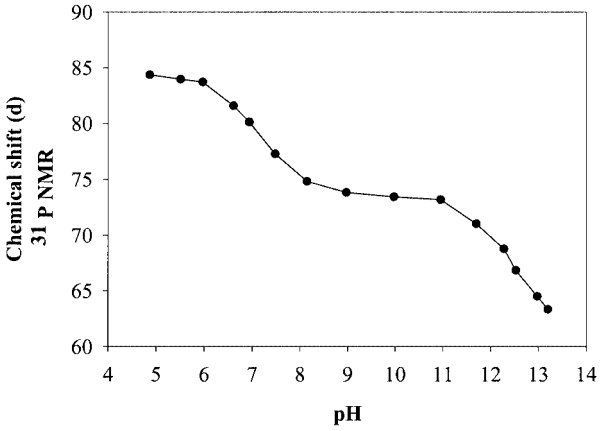
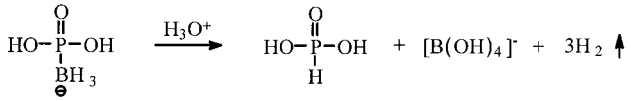


Figure 4. Determination of pK_a values of BPi; plot of BPi's ^{31}P NMR chemical shift in H_2O as a function of the pH; two inflection points are observed in the pH range 4.87−13.20

Stability of BPi

BPi is stable in neutral and basic solutions. For instance, after 48 h at room temperature at pH = 13.7, no degradation of BPi was observed by ^{31}P NMR spectroscopy. BPi is also relatively stable in acidic solution at pH values > 2. At pH = 2, BPi slowly degrades to phosphorus acid at a rate of 7×10^{-7} s^{-1}, $R^2 = 1.00$ ($t_{1/2}$ = 275 h), as monitored by ^{31}P NMR spectroscopy.

Under highly acidic conditions (pH < 2), the evolution of H_2 is clearly observed, the P−B bond is cleaved, and boric acid is formed together with phosphorus acid (Scheme 3).[20] Phosphorus acid was observed in the ^{31}P NMR spectrum as a doublet at δ = 3.5 ppm (J = 633 Hz). The borane reacts with water to liberate hydrogen gas and boric acid.



Scheme 3

The stability of inorganic boranophosphate, resulting from neutral hydrolysis of thymidine 5′-boranomonophosphate at 50 °C, has been reported earlier.[20]

H-Bonding of BPi

Solutions of **2b** or **2c** in organic solvents ($CHCl_3$, CH_3CN, DMF, and even MeOH), show unexpected ^{31}P NMR spectra. Product **2b** in MeOH apparently consists of three different but pattern-related signals. The signals with chemical shifts of δ = 80.0 (A), 86.2 (B), and 90.8 (C) ppm, each have an identical BPi-typical pattern (Figure 5).

Several minutes after the dissolution of **2b** in MeOH, signals A, B, and C are observed in the ^{31}P NMR spectrum, with A and B as the major peaks (C constitutes ca. 5% of the mixture). The composition of the initial mixture is time-dependent due to the interconversion of the species. When monitoring this process with 0.14 M **2b** in CD_3OD (ε = 33)

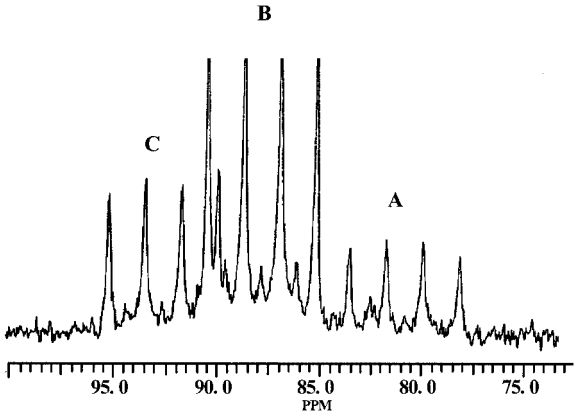


Figure 5. ^{31}P NMR spectrum of BPi **2b** in methanolic solution

at room temperature for 160 h, we noted the conversion of A and B to C, with a final C/B ratio of 4.4:1 (A disappeared completely). The ^{1}H-coupled ^{31}P NMR spectrum indicated H-split quadruplet signals for B and C — no D/H exchange occurred.

A spectrum similar to the one shown in Figure 5, and time-dependent interconversion of the species, was also observed for **2b** in DMF, CH_3CN, and $CHCl_3$.

The possibility that the additional BPi-like species are the corresponding mono- or dimethyl esters, due to a reaction of **2b** with MeOH, was ruled out because their ^{13}C and ^{1}H NMR spectra in $CDCl_3$ are devoid of a methyl ester signal.

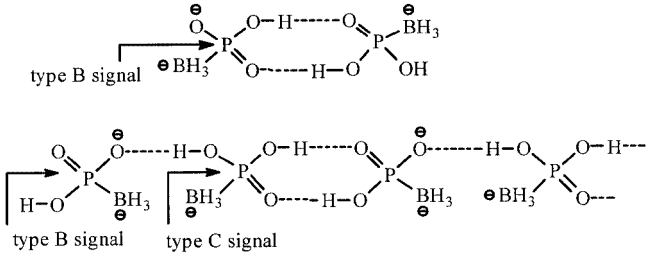
The three signals seen in the ^{31}P NMR spectrum of **2c** in organic solvents converged into one, probably A (δ = 79.8 ppm) after solvent evaporation and dissolution in D_2O. The possibility that signals B and C are due to boranophosphate anhydrides, resulting from **2c** in the NMR sample, is unlikely as the formation of the related phosphoric acid anhydrides requires drastic conditions (up to 250 °C at very low pH, or the presence of a condensation agent).[21] Obviously, these conditions do not exist in the NMR samples of BPi.

The possibility of B and C being anhydrides formed during the preparation of BPi, was also ruled out as the ^{31}P NMR spectrum of **2c**, which exhibits several signals in organic solvents, shows only one signal when measured in water. In contrast, the hydrolysis of condensed phosphates is known to be quite slow (i.e. several weeks at 60 °C, pH = 5).[22]

Phosphoric acid and its derivatives form strongly H-bonded clusters including cyclic dimers, trimers, and chains.[23,24] Dimerization occurs in the solid state,[25] in highly concentrated phosphoric acid,[24,26] in freons,[23b] or in aprotic organic solvents.[27,28] Therefore, it is likely that the new BPi-like signals are indicative of clustering of BPi in organic solvents, and even in a highly polar and H-bonding solvent such as MeOH. These clusters are not large (< 30 Å) based on light-scattering measurements.

To assess the possibility of observing different H-bond-clustered species on the NMR timescale, we measured the ^{31}P NMR spectrum of the parent phosphate bis(tributylammonium) salt in benzene, where clustering is known to occur.[28] Indeed, three signals were clearly observed at δ = 3.63, 3.23, and 2.93 ppm, demonstrating that different H-bonded phosphate clusters can be detected by ^{31}P NMR spectroscopy. These three phosphate signals, seen in benzene, converged into one in acetonitrile and MeOH, indicating the collapse of the Pi clusters in polar/protic solvents.

Based on our observations of the pH-dependent chemical shift of BPi, and on the determination of BPi's acidity constants (Figure 4), we propose the following assignment of signals A, B, and C. Signal A corresponds to the monomeric BPi, whose chemical shift at $\delta \approx 80$ ppm indicates that half the BPi monomer population bears two protons, and the other half bears one proton (Figure 4). Signal B, at δ = 86 ppm, corresponds to a BPi moiety that has one BPi H-bonded neighbor. Namely, signal B could result both from BPi dimers and higher clusters (Scheme 4). In these cases, each BPi is associated with an additional proton (Bu_3NH^+ ions neutralize the negative charges). Therefore, the chemical shift of the BPi dimer shifts downfield (δ = 86 ppm, as at pH = 4.7). As indicated by signal C, BPi also forms clusters, corresponding to a BPi moiety that has two H-bonded BPi neighbors (Scheme 4). A BPi moiety in the middle of a cluster is associated with three protons, resulting in an additional downfield shift to δ = 91 ppm, corresponding to that of BPi at pH = 2.



Scheme 4

Small H-bonded clusters (i.e. dimers and trimers) are formed almost instantaneously. This is probably the stage of nucleation. Once a critical nucleus is formed, a slow process of high-order clustering occurs. At this stage the concentration of A in solution is drastically reduced. This H-bonding-based clustering mechanism is also supported by the observation that, upon evaporation of the organic solvent from the species mixture and dissolution in water, only A is detected.

The fact that BPi forms clusters even in MeOH, whereas Pi forms clusters only in benzene, implies that BH_3 may play a role in the pre-organization of the BPi clusters. The lipophilic BH_3 moieties possibly form the core of the cluster due to hydrophobic interactions (in MeOH). This core is then further stabilized by P−O$^-$···HO−P hydrogen bonds.

Reactions of BPi with Selected Reagents

The reactivity of BPi towards various organic and inorganic reagents was explored as part of the characterization

of the chemical nature of BPi. These reagents include aqueous acid solution, nitrile, amide, carbodiimide, pyridine and imidazole, tosyl chloride, phosphorus oxychloride, H_2, and Zn^{2+} and Mg^{2+} ions.

Although BH_3, in complexes with a variety of sulfur/amine/oxygen compounds, is an efficient reducing agent, its reducing nature is drastically altered in BPi. For instance, while hydride transfer from "BH_3" to water occurs readily, the BH_3 moiety in BPi transfers hydride only in a highly acidic solution (pH $<$ 2). Likewise, while BH_3·THF complex readily reduces nitriles and amides to the corresponding amines,[29] the borane moiety in BPi does not reduce acetonitrile and dimethyl formamide, as evidenced by the complete stability of BPi in these solutions.

A carbodiimide reagent is used for the condensation of phosphate derivatives to provide the corresponding phosphoric anhydride. The reaction of **2a** with an excess of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) was explored in water (pH = 6.5) at 37 °C for 4 h. The addition of EDC to BPi resulted in excessive loss of this compound due to complete P−B bond cleavage of **2a** to yield phosphorus acid (72% of **2a** was degraded after 4 h, based on the ^{31}P NMR spectrum). This finding is in contrast to diethyl phosphite (cyano- or methoxycarbonyl)borane analogues, which are stable to dicyclohexylcarbodiimide (DCC).[30]

The P−B bond was also found to be sensitive to catalytic hydrogenation. Thus, when compound **9** was subjected to hydrogenation (in the presence of Pd/C), the P−B bond was also reduced, yielding phosphorus acid.

The reactivity of BPi with imidazole and pyridine was also studied. Specifically, a solution of BPi with 2 or 10 equiv. of imidazole in CD_3OD remained unchanged for 96 h, based on the ^{31}P NMR spectra. Likewise, only a negligible cleavage of the P−B bond was observed after 113 h for a solution of BPi in pyridine. BPi is apparently more stable to imidazole and pyridine than the related analogue tetramethyl boranopyrophosphate.[31] The reaction of 5′-DMT-2′-deoxythymidine with tetramethyl boranopyrophosphate in the presence of *N*-methylimidazole was reported to proceed with the partial removal of the borane group. Likewise, when pyridine was used as a solvent, partial removal of the borane group was observed.[31]

The presence of divalent metal ions such as Zn^{2+} and Mg^{2+} in DMF and water for 48 h and 4 h, respectively, left BPi unchanged.

Whereas dimethyl boranophosphate monopotassium salt (**3**) plays the role of an efficient nucleophile,[13] the related BPi is a poor nucleophile. Thus, when BPi was treated with tosyl chloride or mesyl chloride (with or without amine) in acetonitrile for 24 h, even at 60 °C, no reaction occurred. Likewise, the reaction of BPi with phosphorus oxychloride and its derivatives [P(O)Cl_2R] yielded no product. The reason for BPi's reduced nucleophilicity, as compared to **3**, might be due to the "carboxylate-like" nature of BPi, as compared to the "alkoxide-like" nature of dimethyl boranophosphate.

Conclusions

The quest for phosphate bioisosters over the last several decades has included the synthesis of phosphonates, α-halo phosphonates,[32,33] phosphorothioates,[4] and boranophosphate analogues.[6,34,35] Despite the extensive study of boranophosphate nucleoside analogues, the exploration of the parent inorganic boranophosphate has not been reported. The various potential applications of a phosphate isoster, together with the limitations of the currently available isosters, justify the continued search for the perfect inorganic phosphate mimic. Therefore, the unique and chemically interesting inorganic boranophosphate **2** has been investigated here as a mimic of phosphate with respect to properties such as water solubility, geometry, acid/base character, H-bonding, and chemical reactivity. The great similarity of BPi to the inorganic phosphate, Pi, is demonstrated here by the BPi's high water solubility, and geometry that is in accordance with that of the parent compound, except for the long P−B bond (1.892 Å) and B−P−O angles that are slightly larger than tetrahedral angles. Furthermore, the acid/base character of BPi is essentially not altered in comparison to Pi. This finding is in contrast to the corresponding phosphorothioate isoster, where there is a reduction of about two log units in the acidity relative to Pi.[36] Likewise, pK_{a2} values of α-mono- and -difluorophosphonate isosters are one and two log units, respectively, lower than the pK_{a2} value of phosphoric acid.[33]

Indications for H-bond-based clustering of BPi in organic solvents, including polar and protic solvents, were obtained from the IR and ^{31}P NMR spectra.

BPi is stable under both highly basic and acidic conditions (at pH $>$ 2). In addition, BPi is stable in the presence of imidazole, pyridine, and Mg^{2+} ions. However, the P−B bond cleavage is observed upon the reaction of BPi with carbodiimides. A loss of BPi's borane moiety also occurs at pH values $<$ 2.

A drastic alteration in the chemical nature of BPi as compared to Pi and BH_3 complexes is observed. While Pi is a nucleophile,[37] BPi is a poor nucleophile. Likewise, the reducing nature of the BH_3 group in BPi is drastically lower than in other BH_3 complexes.

Based on the geometry, water solubility, acid/base character, and H-bonding properties, BPi appears to be a perfect mimic of Pi, and an attractive alternative to the thiophosphate and α-halophosphonate isosters.

Experimental Section

General: All air- and moisture-sensitive reactions were performed in flame-dried, nitrogen-flushed flasks sealed with rubber septa; the reagents were introduced with a syringe. The progress of the reactions was monitored by TLC on precoated Merck silica-gel plates (60 K-254). Column chromatography was performed with Merck silica gel 60 (230−400 mesh). Compounds were characterized by NMR spectroscopy using Bruker DPX-300, DMX-600, or AC-200 spectrometers. NMR spectra were recorded with a Bruker AC-200 spectrometer with a ^{31}P NMR probe (isotope frequency of

81 MHz) using 85% H_3PO_4 as an external reference. IR spectra of BPi in KBr pellets were recorded with a Nicolet Impact 400D spectrometer using the OMNIC program. IR spectra of BPi in solution were measured using a Bruker Vector 22 equipped with a liquid-nitrogen-cooled MCT detector. For the ATR measurements, a Harrick variable-angle ATR accessory was used. For one spectrum, 100 scans were co-added at a resolution of 4 cm^{-1}. The clean ATR germanium crystal (Harrick Scientific Corporation) was measured for the background spectra (cutoff 680 cm^{-1}). Crystallographic data were collected with a Nonius KappaCCD diffractometer at 120 K with scans of 1° collected at a speed of 1°/20 s; the merging *R*-factor on the data was 0.046 with 36867 reflections collected and 2979 unique. BPi crystals were obtained as colorless needles. Further details of the crystal structure investigation may be obtained from the Fachinformationzentrum Karlsruhe, 76344 Eggenstein-Leopoldshafen, Germany, on quoting the depository number CSD-413735. Melting points were measured using a Fisher-Johns melting-point apparatus. Apparent pH values were measured with a Hanna Instruments pH-meter (HI 8521), equipped with an Orion micro-combination pH electrode (9802). Compounds **2** are all inorganic boranophosphate salts having different ammonium counterions (ammonium in **2a**, tributylammonium in **2b**, triethylammonium in **2c**, and tetrabutylammonium in **2d**, see Experimental for **2a** below). The preparation of salts **2a**−**2c** for elemental analysis involved a freeze-drying process. Unfortunately, it was impossible to obtain reliable elemental analyses for salts **2a**−**2c** due to loss of some of the amine during this freeze-drying process (as observed in their ^1H NMR spectra). Therefore, we chose the tetrabutylammonium salt **2d** for performing a representative elemental analysis, since in this case there is no partial loss of the amine during freeze-drying.

Boranophosphate 2a: $BH_3{\cdot}SMe_2$ complex in THF (2 M, 1.35 mL, 2.7 mmol) was added to a solution of tris(trimethylsilyl) phosphite (600 μL, 1.795 mmol) in dry CH_3CN (5 mL) under N_2 at 0 °C. The resulting solution was kept at room temperature for 15 min. Dry MeOH (15 mL) and 2 M NH_3 in EtOH (1.8 mL, 3.6 mmol) were added and the mixture was stirred at room temperature for 1 h. The solvent was then removed under reduced pressure, and the product was obtained as a white solid in 93% yield (202 mg, 1.556 mmol), m.p. > 240 °C. ^1H NMR (D_2O, 200 MHz): δ = 0.27 (d of 1:1:1:1 quadruplet, $J_{P,H}$ = 22, $J_{B,H}$ = 87 Hz, 3 H) ppm. ^{31}P NMR (D_2O, 81 MHz): δ = 80.38 (1:1:1:1 quadruplet, J = 156 Hz; 1:1:1:1:1:1:1 septuplet, J = 52 Hz) ppm. IR (KBr): $\tilde{\nu}$ = 2412, 2378, 2352, 1181, 1149, 1077−903, 654 cm^{-1}. Compound **2a** was converted into the corresponding tetraethylammonium salt as follows: **2a** was passed through a Sephadex-CM C-25 − tetraethylammonium-form column (prepared from the corresponding sodium-form resin upon loading with excess Et_4NCl) and the column was washed with about 20 volumes of deionized water. The solution was freeze-dried to yield tetraethylammonium BPi (**2d**) as a white solid. Based on the pH value of the **2d** solution, the ^{31}P NMR spectrum, and correlation with the plot of BPi ^{31}P NMR shifts vs. pH (Figure 4), the expected empirical formula is $BH_3O_3PH_{1.5}(Et_4N)_{1.5}$ (289.3): calcd. H 11.9, P 10.7; found H 11.3, P 9.5.

Boranophosphate 2b: The tributylammonium salt of boranophosphate was prepared as described above for **2a**. However, Bu_3N (0.85 mL, 3.57 mmol) was added instead of NH_3/EtOH. The product was obtained as a white solid in 93% yield (645 mg, 1.385 mmol), m.p. 83−84 °C. IR (KBr): $\tilde{\nu}$: 2407, 2381, 2350, 1184, 1150, 1100−850, 655 cm^{-1}.

Determination of the pK_a Values of Boranophosphate 2a: The pK_a values of **2a** were evaluated by ^{31}P NMR spectroscopy at room temperature. Solutions of **2a** (0.15−0.18 M) at different pH values were prepared by adding dilute sodium hydroxide or hydrochloric acid solutions. The ^{31}P NMR chemical shift was monitored as a function of the pH. A five-parameter sigmoid function was fitted to the data using Sigma Plot 2000 (SPSS, Inc.): $\delta = \delta_0 + a/[1 + e^{-\{(pH-pH^\circ)/b\}}]^c$. The inflection point, which is determined by the second derivative of the fitted sigmoid function, is the pK_a value.

Determination of the Decomposition Rate of BPi 2a at pH = 2: The stability of **2a** in acidic solution was evaluated by ^{31}P NMR spectroscopy at room temperature, monitoring the formation of the deboranation product (phosphorus acid). A 0.16 M solution of **2a** at pH = 2 was prepared by adding dilute hydrochloric acid to a solution of inorganic boranophosphate (NH_4^+ salt) in H_2O and 10% D_2O. The percentage of decomposition of **2a** is based on integrations of PBi and phosphorus-acid signals (δ = 90.93 and 3.3 ppm, respectively). The decomposition rate was determined by measuring changes in the integration of the respective NMR signals within 96 h.

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