2-Methyl-4-phenylpyridine (1). To a solution of 31 g (0.2 mol) of 4-phenylpyridine in 200 ml of Et_2O was added slowly 100 ml (2 *M* solution in Et_2O, 0.2 mol) of methyllithium while nitrogen was bubbled through the mixture. After the addition was complete, Et_2O was removed by distillation and was continuously replaced with 200 ml of toluene. The mixture was heated at 110° for 12 hr, the reaction was then decomposed cautiously by the addition of water, and the mixture was extracted with Et_2O. The Et_2O extracts were dried over KOH and evaporated under vacuum to leave an oil which was vacuum distilled: bp 102–103° (0.2 mm) [lit.[5] 99–100° (0.7 mm)]; yield 21.4 g (63%).

2-Methyl-4-nitrophenylpyridine (2–4). 2-Methyl-4-phenylpyridine (20.35 g, 0.122 mol) was added slowly to a solution of 7.8 ml (0.122 mol) of HNO_3 (70%; *d* 1.42) and 33 ml of concentrated H_2SO_4 at 0° with stirring. The mixture was heated at 100° for 2 hr, cautiously added to 200 g of crushed ice, made alkaline with concentrated NH_4OH, and extracted with $CHCl_3$. The $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated under vacuum to leave a mixture of three isomers, *o*- (2), *m*- (3), and *p*-nitro (4) derivatives. The mixture was crystallized in HCl salt form from 5 *N* HCl to give 13.05 g of predominantly 4 which was purified by further recrystallization from 5 *N* HCl to yield 11.23 g (40%), mp 234–237°. The filtrate was made alkaline with Na_2CO_3 solution, extracted with $CHCl_3$, and dried ($MgSO_4$), the solvent was removed under vacuum, and the residue consisting of mainly meta and ortho isomers crystallized from 5 *N* HNO_3. The nitrate salt, consisting predominantly of **3**, was filtered and recrystallized from 5 *N* HNO_3 to yield 7.6 g (27%), mp 199–201°. The filtrate containing mostly the nitrate of **2** was evaporated to dryness under vacuum and recrystallized two times from a mixture of CH_3OH and EtOAc (1:1) to yield 2.8 g (10%), mp 151–152°.

The hydrochloride or nitrate salts were converted to the free base and were recrystallized from appropriate solvents as listed in Table I.

4-Phenyl-2-picoline *N*-Oxide (5). 4-Phenyl-2-picoline (16.9 g, 0.1 mol) was heated with a mixture of 15 ml of 30% H_2O_2 and 45 ml of glacial AcOH at 80°. After 3 hr, an additional amount (15 ml) of 30% H_2O_2 was added and the heating was continued for an additional 21 hr. The mixture was evaporated to a thick syrup, dissolved in 150 ml of $CHCl_3$, and treated with K_2CO_3 and a small amount of water to form a paste. The mixture was triturated thoroughly, the $CHCl_3$ layer dried (K_2CO_3), the solvent evaporated under vacuum, and the residue washed with ether to yield 14.1 g (76%) of **5** which was recrystallized from C_6H_6 and Et_2O: mp 101–105°. This material was used without further purification.

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Synthesis and Tumor-Uptake Study of Phosphate Esters of Polyhedral Hydroxyboranes

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The phosphorylations of $B_{12}H_{11}OH^{2-}$, $B_{12}H_{10}(OH)_2^{2-}$, and $B_{20}H_{17}OH^{4-}$ with $POCl_3$ and $(C_6H_5O)_2POCl$ were investigated and the following derivatives were isolated: $B_{12}H_{11}OPO_3H^{3-}$, $B_{12}H_{11}OPO_3H_2^{2-}$, $B_{12}H_{11}OPO(OC_6H_5)_2^{2-}$, $B_{12}H_{11}OPO(OC_6H_5)OH^{2-}$, $B_{12}H_{10}(OP_2O_6H_2)_2^{4-}$, $B_{12}H_{10}(OPO_3H_2)_2^{2-}$, $B_{12}Br_{10}(OPO_3H)_2^{4-}$, $B_{12}H_{10}[OPO(OC_6H_5)_2]_2^{2-}$, $B_{20}H_{18}OP_2O_6H_2^{4-}$, $B_{20}H_{18}OPO_3H_2^{3-}$. The B–O–P bonds proved very resistant to hydrolysis and the phosphates were administered in the form of Na^+ salts at pH 7.2 to rats bearing subcutaneous glioma. The boron concentrations in tumors and the tumor/blood concentration ratios were compared with those of parent hydroxy derivatives. Except when the POH function was blocked by phenyl groups the phosphorylation invariably resulted in a greatly enhanced uptake of the borane into tumors and improved the tumor/blood boron ratio. The phosphate function appears to be one of the most effective handles for the incorporation of boron into brain tumors and the compounds show considerable promise for use in the neutron capture therapy of brain tumors.

The attributes which boron compounds should possess if they are to be useful in the treatment of brain tumors by neutron capture therapy have been discussed in a number of articles.[1–6] Chief among these is the ability to accumulate in the tumor at higher levels than in normal tissue, so that when the neutron flux is applied the tumor will be selectively irradiated. Recent calculations[7] indicate that if radiation damage to healthy adjacent tissue is to be kept to a minimum, no less than 10 μg of ^{10}B per gram of tumor is needed, while concentrations of 30–40 μg might be needed to ensure complete destruction of the tumor. Moreover, the quantity of boron in blood at the time of irradiation should have declined to a sufficiently low level so that radiation fibrosis of small blood vessels does not result in an ischemic necrosis of normal brain. Recently Hatanaka and coworkers[6] reported satisfactory medical results even at low tumor/blood boron ratios when the boron compounds were accompanied by certain steroids. One possible way of making compounds with the properties desired for successful neutron capture therapy[1,2,5] involves attaching a functional group to the boron compound capable of binding the latter covalently to some component of tumor tissue. Such functional groups have often been referred to as handles.[1]

The search for compounds of the type described above

led us to reexamine the results of mouse-tumor studies conducted with a number of derivatives of polyhedral boranes, among them $B_{10}H_7(CH_2CH_2OH)_2NH_3^-$ (1)[8] and $B_{10}H_7NH_3(CH_2CH_2OPO_3H_2)_2^-$ (2).[8] We were struck by the fact that phosphorylation increased significantly both the boron concentration in the tumor and the tumor/blood concentration ratio. These results are shown in Table II, under the heading compounds 1 and 2. To find out whether we were dealing with an exceptional or even unique case, or had discovered a powerful new handle, we decided to synthesize a number of other phosphate esters of polyhedral boranes and compare their properties with those of parent boranes. At the same time in order to increase the percentage of boron in the derivatives it appeared desirable to explore the possibility of attaching a phosphate group directly to the boron cage and dispense with the intervening organic chain. No phosphorylation of a hydroxy function directly attached to a boron cage has been reported in the literature, nor was it certain that such derivatives would prove stable in water. The results of our chemical and biological studies are presented below.

Results and Discussion

Reactions. As is clearly shown in the Experimental Section, hydroxy derivatives of polyhedral boranes underwent phosphorylation with comparative ease. The reactions went to virtual completion in all instances, since only trace amounts of starting materials were ever detected by tlc. The reactions with $POCl_3$ are exothermic so it is advisable to keep all solutions ice-cold while the reagent is being added.

The use of $POCl_3$ inevitably results in the formation of pyrophosphates. Since these slowly hydrolyze to the corresponding orthophosphates, unless the pH of solutions is carefully controlled, one often gets mixtures of the ortho and pyro derivatives which are not easily separable. To explore alternative routes to pure orthophosphates we also examined the reactions with $(PhO)_2POCl$, a reagent frequently used to avoid the formation of di- or triphosphates. Though the reactions were slower than in the case of $POCl_3$, good yields of products were obtained within a reasonable length of time. This suggests that other monochlorophosphates with hydrolyzable organic functions may also prove useful for synthesizing orthophosphate derivatives of polyhedral boranes. If pyrophosphates are the desired products, contact with acids at any stage of the reaction and purification should be kept to a minimum. A complete list of the compounds made can be seen in Table I.

Compounds. Most of the properties of the compounds synthesized by us are tabulated in Table I. Each compound is assigned a roman numeral and letters are only used when compounds differing solely in the degree of protonation are listed. The missing numbers belong to derivatives the syntheses of which have already been reported in the literature. These are $B_{10}H_7NH_3(CH_2CH_2OH)_2^-$ (1),[8] $B_{10}H_7NH_3(CH_2CH_2OPO_3H_2)_2^-$ (2),[8] $B_{12}H_{11}OH^{2-}$ (3),[9] and $B_{20}H_{17}OH^{4-}$ (12).[10] They were made by the methods described in the papers cited above and were used both in our syntheses as starting materials and in the biological studies for comparison with the corresponding phosphate esters. Though Me_4N^+ was the preferred cation, all of the phosphate derivatives can also be precipitated from ethanol–water mixtures as Cs^+ salts (see 5 in Table I). Both cations give salts insoluble in acetone and ethanol but soluble in water, with the solubility increasing with pH. Getting reproducible elemental analyses and molecular weights was frequently quite difficult for several reasons. The phosphate esters were extremely hygroscopic, and usually even prolonged storage over P_2O_5 *in vacuo* failed to yield anhydrous salts. Therefore we had to contend with the problem of a variable number of waters of hydration. Moreover, since each salt was itself a polybasic acid, different species were precipitated at different pH. This is shown explicitly in the case of **4a** and **5** (see Table I). The same factor made the molecular weight calculations from experimental neutralization equivalents quite difficult at times due to a large number of weak inflection points on the pH titration curves. Compound **8**, for example, had *six* ionizable hydrogens and two more when the H_3O^+ salt was titrated. In the case of several derivatives, as many as three equivalence points were compressed in the pH 4–9 region. We were also informed by the analysts (see the Experimental Section) that phosphorus interferes with the determination of boron, while the latter makes the oxidation of the former more difficult.

Compared to the phosphate esters of ordinary organic alcohols the orthophosphates of polyhedral boranes appeared remarkably stable. The B–O–P bonds showed no evidence of hydrolysis in the pH 1–12 range, even in the presence of Li^+ ions, which catalyzed the hydrolysis of the pyrophosphates. The latter were also rapidly hydrolyzed in strong acids to the corresponding ortho derivatives. Since Li_3PO_4 is sparingly soluble any free phosphate remaining from the hydrolysis of $POCl_3$ or produced by the decomposition of the pyro derivative could be detected by the addition of LiOH.

The ir bands listed in Table I were those expected of the compounds, and even the weak band in the vicinity of 870 cm^{-1} attributed to the P–O–P linkage in pyrophosphates was seen in well-resolved spectra. In contrast with the published ^{11}B nmr spectra of $B_{12}H_{11}OH^{2-}$ and $B_{12}H_{10}(OH)_2^{2-}$, where all of the unsubstituted borons appeared as one large doublet,[9] in the spectra of **4** all four theoretically expected resonances were clearly resolved. In **6** and **7** the unique boron trans to the substituent was still distinguishable, but the other ten showed up as one large doublet. Disubstituted icosahedral ions, such as **8**–**11**, may exist in three isomeric forms. Though the spectra ruled out the 1,12 isomer, they failed to distinguish between the 1,2 and 1,7 isomers. The chemical shift of the low-field singlet, representing the substituted boron, showed very little variability among the various derivatives of the B_{12} system. The ^{11}B nmr spectra of the $B_{20}H_{18}OH^{3-}$ (bridged) and $B_{20}H_{17}OH^{4-}$ (unbridged) ions have been analyzed by Hawthorne.[11] The spectra of **14** in weakly acidic and alkaline solutions resembled those of the bridged and unbridged (isomer ii) hydroxides, respectively. Comparison of the undecoupled and decoupled spectra and the chemical shift and coupling constant data found in the literature[10,11] indicated that the phosphate group occupied an equatorial position. It showed up as a singlet at 21.5 ppm in the bridged ion and shifted to 22.4 ppm in alkaline solutions.

The ^1H nmr were only recorded for species having organic substituents, and the areas served as a check on the analytical data with the cation serving as a reference. Only the species in which the many ionizable protons were replaced with organic groups, *i.e.*, **6**, **7**, and **11**, had simple ^{31}P spectra. The others had several peaks each, since the ^{31}P nmr is quite sensitive to changes in charge and symmetry produced by protonation of the oxygens.[12] As expected of species in acid–base equilibrium, the relative intensities of the peaks varied with pH. The presence of two closely adjacent peaks of very disparate size in **11** appeared to confirm the tlc evidence for two isomers. As anticipated, the pyrophosphate derivatives had even more complex spectra since the two phosphorus atoms were nonequivalent magnetically. The chemical shifts of such terminal and nonterminal

Table I. Phosphate Esters of Polyhedral Hydroxyboranes

No.	Formula	Principal ir bands of the anions,[a] cm^{-1}	Nmr spectra, δ, ppm (J in Hz)		
			^{11}B[b]	^{31}P[c]	^{1}H[d]
4	$[(CH_3)_4N]_3B_{12}H_{11}OPO_3H\cdot 4H_2O$	3580 m, 3400 m, 2480 s, 1150 s, 1035 s, 900 s, 720 w	15.2 (s, 1 B), 35.2 (d, 5 B, J = 130), 36.9 (d, 5 B, J = 130), 41.6 (d, 1 B, J = 128)	−2.6, 3.97,[e] 12.3	
5	$Cs_2B_{12}H_{11}OPO_3H_2\cdot 2H_2O$	3600 m, 3400 br, 2500 s, 1160 s, 1110 m, 1075 s, 1030 s, 950 m, 720 w			
6	$[(CH_3)_4N]_2B_{12}H_{11}OPO(OC_6H_5)_2$	3010 m, 2480 s, 1585 s, 1260 s, 1180 s, 1135 s, 1050 s, 1020 m, 1000 m, 925 s, 775 s, 680 s	14.3 (s, 1 B), 34.8 (d, 10 B, J = 135), 39.7 (s, 1 B, J = 130)	17.8	7.6 (m, 10 H), 3.5 (s, 24 H)
7	$[(CH_3)_4N]_2B_{12}H_{11}OPO(OC_6H_5)OH$	3600, 3400 br, 3010 m, 2480 s, 1590 s, 1300 s, 1220 m, 1150 s, 1060 s, 1040 s, 880 m, 835 m	14.5 (s, 1 B), 34.8 (d, 10 B, J = 133), 39.6 (d, 1 B, J = 130)	8.1	7.7 (m, 5 H), 3.5 (s, 24 H)
8	$[(CH_3)_4N]_4B_{12}H_{10}(OP_2O_6H_2)_2$	3580 m, 3350 br, 2480 m, 1250 s, 1150 m, 1100 s, 1030 s, 870 m, 720 br	16.2 (s, 2 B), 37.2 (d, J = 130) (sh at 37.1, 38.0)	−0.3, 5.3, 10.5,[f] 16.6[e,f]	
9	$[(CH_3)_4N]_2B_{12}H_{10}(OPO_3H_2)_2\cdot$ $[(CH_3)_4N]_3B_{12}H_{10}(OPO_3H_2)OPO_3H$	3580 m, 3350 br, 2480 s, 1240 s, 1150 s, 1100 m, 1040 s, 1000 s, 825 w, 720 w	15.6, 17.4 (s, 2 B), 37.0 (d, J = 120) (sh at 29.2, 33.0, 37.0, 41.4, 44.5)	−1.9, 3.9, 13.0[e]	
10	$[(CH_3)_4N]_4B_{12}Br_{10}(OPO_3H)_2$	3560 m, 3400 br, 1200 s, 1090 m, 1000 s, 700 m		1.5, 14.1[e]	
11	$[(CH_3)_4N]_2B_{12}H_{10}[OPO(OC_6H_5)_2]_2$	Same as those of 6	17.4 (s, 2 B), 36.4 (d, 10 B, J = 120)	18.1, 18.5[e]	7.6 (m, 20 H), 3.5 (s, 24 H)
13	$[(CH_3)_4N]_4B_{20}H_{18}OP_2O_6H_2\cdot 2H_2O$	3550 m, 2480 s, 1850 m, 1130 m, 1210 s, 1130 s, 1040 s, 990 s, 880 m, 750 m			
14	$[(CH_3)_4N]_3B_{20}H_{18}OPO_3H_2\cdot 2H_2O$	3550 m, 2480 s, 1850 m, 1130 s, 990 s	See the Experimental Section	1.8,[e] 2.4, 9.6, 10.0, 12.2, 12.7	

[a] The Me_4N^+ bands at 3010, 1480, and 940 cm^{-1} and the H_2O band at 1600 cm^{-1} are not tabulated. [b] The ^{11}B spectra were recorded at 32 MHz in 30% D_2O–H_2O (4, 8, 9, 13, 14), 50% MeCN–H_2O (6, 7), and 50% DMF–D_2O (11). The shifts are relative to $(MeO)_3B$, upfield direction positive. [c] The ^{31}P spectra were recorded at 40.2 MHz in the same solvents as ^{11}B nmr. The shifts are relative to external 85% D_3PO_4. Only proton-decoupled spectra are tabulated. Upfield direction positive. [d] The ^{1}H nmr were recorded in DMSO-d_6 at 60 MHz. The shifts are given as δ ppm downfield from TMS. [e] Dominant peak. [f] Broad band.

phosphorus atoms may be similar or very different depending on the nature of the substituent on the central phosphorus.[12] In the case of **14** the problem is compounded by the possibility of linkage isomerism, with the phosphate attached to either an apical or equatorial boron.[11] For example, the two positions are clearly distinguished in the ^{1}H nmr of the methyls in $B_{20}H_{16}[N(CH_3)_3]_2^{2-}$ even though two atoms intervene between the protons and the boron.[13] Consequently, we prefer not to make definite assignments to the ^{31}P nmr peaks until a more detailed study has been carried out over a wider pH range, and the chemical shifts are reported primarily for purposes of identification.

Biological Studies. The uptake of boron compounds by tumor, normal brain, and blood was tested with an *in vivo* system developed in our laboratory at the Massachusetts General Hospital. The test animals used were C-3H mice bearing subcutaneously transplanted ependymoblastoma or C. D. Fisher and Wistar rats bearing subcutaneously transplanted glioma.[14] The animals were given the appropriate dose of boron compound by intraperitoneal injection, sacrificed at various time intervals, and the tissues analyzed chemically for boron content.[15] The uptake for each compound is given in Table II. In each case the phosphate is compared with the parent polyhedral borane and from Table II one can see that the phosphate appears to give higher tumor/blood ratios. The absolute value of the boron concentration in tumor showed an even sharper rise; in some instances phosphorylation resulted in a tenfold increase in the uptake of boron. The phosphates accumulated in the tumor at levels far in excess of the average daily amount administered per gram of tissue, whereas the parent compounds lagged far behind indicating poor retention of the latter probably as the result of rapid equilibration with the blood stream and consequent more efficient elimination from the body.

In order to find out in which other tissue beside the tumor the phosphate esters were likely to accumulate, a study was performed on a healthy rabbit. A solution of the Na^+ salt of **14** (see Table I for formula) buffered at pH 7.2 containing 3.22 mg of B/ml was prepared and administered into the ear vein in five daily injections of 5.5 mg of B/kg each. The rabbit was sacrificed 24 days after the last injection. The results of the autopsy are shown in Table III, where the organs are arranged in order of decreasing boron concentration. Except for the skull and kidneys no organ showed concentrations in excess of 10 μg of B/g. These levels are well below those obtained with the same compounds in rat gliomas, but more work is needed to determine how

Table II. Boron-Uptake Studies in Mice and Rats

Na+ salt of[a]	No. and type of animal[b]	Dosage, μg of B/g		Boron contents of tissue, μg of B/g			Tumor/blood concn ratio
		Daily[c]	Total	Tumor	Brain	Blood	
1	7 mice	35	140[d]	5.9 ± 5.5	0.3 ± 0.3	1.1 ± 0.4	5.4 ± 4.0
	5 mice	35	175[d]	12.1 ± 2.0	1.2 ± 0.3	3.1 ± 1.3	4.4 ± 1.6
2	8 mice	35	175	32.3 ± 13.7	1.7 ± 0.5	3.6 ± 0.5	8.9 ± 3.4
3	3 rats (A)	5	25	0.51 ± 0.09	0.3 ± 0.07	0.4 ± 0.05	1.3 ± 0.1
	2 rats (A)	9	45	0.9 ± 0.6	0.3 ± 0.1	0.6 ± 0.2	1.4 ± 0.4
4	18 rats (A)	5	25	7.8 ± 3.6	0.5 ± 0.2	2.1 ± 0.9	3.8 ± 1.2
	5 rats (B)	5	25	11.7 ± 8.4	0.5 ± 0.2	2.4 ± 0.7	4.7 ± 3.2
	6 rats (A)	9	45	24.8 ± 13.2	0.6 ± 0.3	5.0 ± 2.2	5.0 ± 1.5
	4 rats (B)	9	45	13.9 ± 3.8	1.0 ± 0.2	3.4 ± 1.1	4.0 ± 0.9
	1 rat (B)	17	85	27.8	1.1	8.0	3.5
6	2 rats (A)	5	25	2.4 ± 1.1	0.2 ± 0.0	1.5 ± 0.5	1.6 ± 0.6
	2 rats (A)	9	45	3.4 ± 1.3	0.2 ± 0.0	1.6 ± 0.2	2.1 ± 0.4
	2 rats (A)	17	85	2.9 ± 0.1	0.3 ± 0.0	2.9 ± 0.6	1.0 ± 0.2
8	3 rats (A)	5	25	9.1 ± 7.3	0.3 ± 0.0	0.5 ± 0.1	17.1 ± 12.3
	9 rats (A)	9	45	11.5 ± 3.5	0.5 ± 0.1	1.6 ± 0.6	7.4 ± 2.1
12	1 rat (A)	5	25	4.0	0.4	8.7	0.5
	2 rats (A)	9	45	2.5 ± 2.0	0.2 ± 0.1	2.3 ± 1.6	1.0 ± 0.2
14	10 rats (A)	17	85	30.7 ± 18.3	0.9 ± 0.5	10.1 ± 6.5	3.3 ± 1.3
	4 rats (A)	9	45	30.2 ± 13.4	0.6 ± 0.1	6.6 ± 1.8	4.5 ± 1.1
	13 rats (A)	5	25	15.1 ± 7.6	0.6 ± 0.3	4.5 ± 2.8	4.2 ± 2.2

[a] All the salts were buffered at pH 7.2. The formulas of No. 1, 3, and 12, which were the parent hydroxy derivatives, are shown in the Results and Discussion section, as is the formula of the phosphate 2. The other formulas may be found in Table I and in the Experimental Section. [b] Mice bearing subcutaneous ependymoblastoma. Rats bearing two different types (labeled A and B) of subcutaneous rat glioma developed at the Massachusetts General Hospital. [c] Routine animal injection schedule: daily injection, 5 days; sacrifice, 3 days later. [d] Routine injection schedule was varied.

Table III. Rabbit Autopsy Data[a]

Organ	μg of B/g	Organ	μg of B/g
Skull	18.7	Colon	0.9
Left kidney	17.9	Sublingual glands	0.9
Right kidney	13.5	Heart	0.9
Bone marrow	8.6	Lung, left	0.8
Scalp	5.2	Lung, right	0.7
Liver	2.7	Duodenum	0.7
Urinary bladder	2.4	Diaphragm	0.7
Spleen	1.6	Testicle	0.6
Biceps muscle, left	1.4	Parathyroid	0.4[b]
Stomach	1.2	Brain, mostly white	0.4[b]
Gall bladder	1[b]	Thyroid	0.3[b]
Masseter muscle	1.0	Brain, mixed	0.3[b]
		Brain, mostly grey	0.2[b]

[a] Autopsy performed 24 days after the last of five daily injections of $Na_4B_{20}H_{17}OPO_3H_2$ (14). The dosage was 5.5 mg of B/kg, administered into the ear vein. [b] These values are only approximate.

the levels compare when measured the same number of days after the last injection.

The rabbit study also gave us an opportunity to determine how rapidly the boron was being eliminated from the blood. Blood samples were removed at intervals indicated below and their boron content was determined. The following results were obtained: before the first injection, 1.8 μg of B/ml; before the fifth injection, 11.0; 3 days after the last injection, 3.71; 10 days after the last injection, 1.19; 24 days after the last injection, 0.68. Thus, the data suggest that the tumor/blood ratios might be markedly improved by waiting a few more days after the last injection. A urine sample analyzed prior to the sacrifice contained 12.1 μg of B/ml.

Regardless of the form in which the compounds were isolated (Cs^+ or Me_4N^+ salts) they were always administered in the form of Na^+ salts buffered at pH 7.2. Conversion to the Na^+ salt was achieved by passage through an acid ion exchanger followed by titration with NaOH or by direct passage through a sodium ion exchanger. pH titration curves indicated that ions of charge −3 and −4 predominated at pH 7.2. Pyrophosphates, such as compound 8, might have undergone hydrolysis in the blood stream so that by the time they reached the tumor they were in the orthophosphate form. Therefore it is quite likely that the biological results listed for 8 should more appropriately be attributed to 9. Only when the rate of hydrolysis in blood has been determined or the compound recovered intact from the tumor will this problem be resolved.

Conclusions

We have demonstrated that stable hydroxy derivatives of *closo*-boranes may be phosphorylated with chlorophosphates and that the resulting B–O–P linkage is very resistant to hydrolysis. The orthophosphates, in particular, have a very long shelf-life and they can be recovered from aqueous solutions after 1 year at pH 7.2. Thus, one need not worry that a significant fraction of the handle become detached before the compounds reach the tumor. Examination of the rat and mouse data clearly demonstrates that the phosphate function is an excellent handle since every one of the phosphates proved superior to the parent borane both in terms of total boron uptake by tumors and tumor/blood ratio. The data strongly suggest that the accumulation in the tumor is the result of covalent bonding *via* the phosphate group and not the consequence of purely ionic forces between the anions and cell walls. Thus the data in

Table II reveal that although ions of comparable charge and size result when the phosphate group is removed (as in **1**, **3**, and **12**) or blocked by phenyls (as in **6**), a drastic decline in the amount of boron retained by the tumor is observed.

The preliminary results presented here look promising enough to have encouraged further studies of these compounds at the Massachusetts General Hospital. Among properties which will require closer scrutiny is the toxicity. No exact LD_{50} data are available at this time for the phosphates, but high mortality has been observed among rats receiving daily doses in excess of 17 μg of B/g.

Experimental Section

Apparatus, Reagents, and Techniques. The ir spectra were recorded with a Perkin-Elmer Model 237B spectrophotometer on samples pressed into KBr pellets. The ^1H nmr was recorded on a Varian A-60A spectrometer. The ^{11}B and ^{31}P spectra were recorded on a Varian XL-100-15 spectrometer at 32.1 and 40.5 MHz, respectively, and were decoupled by irradiation at 100 MHz. The elemental analyses were performed at the Schwartzkopf Microanalytical Laboratory, Woodside, N.Y., and at the Spang Microanalytical Laboratory, Ann Arbor, Mich. The molecular weights were computed from equivalent weights obtained from the pH titration curves recorded when the Me_4N^+ salts or the corresponding H_3O^+ salts were titrated with standardized KOH. Commercially available reagent grade chemicals were used without further purification. The $B_{12}H_{11}OH^{2-}$ (3), $B_{12}H_{10}(OH)_2^{2-}$, and $B_{20}H_{17}OH^{4-}$ (12) species were prepared by methods described in the literature.[9,10] The tlc spots were detected with the aid of 2% aqueous acidic $PdCl_2$. The preparation and administration of compounds tested on tumor-bearing mice and rats are both discussed in the Biological Studies section.

Undecahydrododecaboryl Phosphate (4). To 1.8 g (5.7 mmol) of $(C_5H_5NH)_2B_{12}H_{11}OH$ in 50 ml of pyridine 1.5 ml (16 mmol) of $POCl_3$ was added slowly with stirring and the mixture stirred for 20 hr at 25°. The viscous syrup remaining after rotary evaporation was mixed with an equal volume of EtOH and neutralized with NH_4OH. The solution was passed through an acid ion exchanger, neutralized with Me_4NOH, and treated with EtOH. The resulting precipitate was redissolved in water and salted out with EtOH to yield 1.3 g (43%) of $[Me_4N]_3B_{12}H_{11}OPO_3H \cdot 4H_2O$. The tlc consisted of a single spot at R_f 0.08 on silica gel (NH_4OH–H_2O–*i*-PrOH, 1:2:7). *Anal.* ($C_{12}H_{56}N_3B_{12}PO_8$) C, H, N, B, P. Mol wt: calcd, 531; found, 534.

If the product 4 is salted out with EtOH from acidic solutions (below pH 2) the phosphate function is fully protonated and the dinegative ion is precipitated as $(Me_4N)_2B_{12}H_{11}OPO_3H_2 \cdot 2H_2O$ (**4a**). The same ion is precipitated as $Cs_2B_{12}H_{11}OPO_3H_2 \cdot 2H_2O$ (**5**) by the addition of CsCl to acidic effluents after passage through an ion exchanger. *Anal.* ($C_8H_{41}N_2B_{12}PO_6$) C, P; H: calcd, 9.79; found, 8.11. N: calcd, 6.64; found, 5.74. Mol wt: calcd, 422; found, 427. $Cs_2B_{12}H_{17}PO_6$: mol wt calcd, 533; found, 536.

Undecahydrododecaboryl Diphenyl Phosphate (6). To a mixture of pyridine (100 ml) and MeCN (200 ml) containing 1.4 g (5.9 mmol) of $K_2B_{12}H_{11}OH$, 5.0 g (19 mmol) of $(PhO)_2POCl$ was added slowly and the reaction mixture stirred for 5 days at 25°. After the volume was reduced by rotary evaporation the residue was dissolved in 50% aqueous MeCN and passed through an acid ion exchanger, and the effluent was neutralized with Me_4NOH. Rotary evaporation to 150 ml yielded 1.0 g (31%) of $(Me_4N)_2B_{12}H_{11}OPO(OPh)_2$. The tlc on Bakerflex PEI-F (30% NH_4PF_6 in 1:1 MeCN–H_2O) consisted of one spot at R_f 0.37. *Anal.* ($C_{20}H_{45}N_2B_{12}PO_4$) B, P.

Undecahydrododecaboryl Phenyl Phosphate (7). The filtrate (150 ml) remaining after the removal of 6 contained several products distinguishable by tlc. Slow evaporation of the filtrate yielded several fractions of $(Me_4N)_2B_{12}H_{11}OPO(OPh)OH$ (total wt 100 mg) appearing as a single tlc spot at R_f 0.57. The loss of one phenyl was confirmed by ^1H nmr (see Table I). *Anal.* ($C_{14}H_{41}N_2B_{12}PO_4$) C, H; B: calcd, 28.1; found, 27.0.

Bis(pyrophosphato) Decahydrododecaborate (8). A mixture of pyridine (75 ml) and MeCN (125 ml) containing 0.90 g (3.6 mmol) of $K_2B_{12}H_{10}(OH)_2$ was kept at 0° while 0.75 ml (8.2 mmol) of $POCl_3$ was slowly added, then allowed to stir for 5 hr at 25°, and filtered. The precipitate was dissolved in dilute NH_4OH, concentrated over a steam bath, and passed through a Me_4N^+ ion exchanger. Concentration of the effluent followed by addition of EtOH yielded a solid which was washed several times with MeCN and reprecipitated from H_2O with EtOH. The product, $(Me_4N)_4B_{12}H_{10}(OP_2O_6H_2)_2$, weighed 1.4 g (49%) after it was washed with ether and dried *in vacuo* over P_2O_5 for 48 hr. *Anal.* ($C_{16}H_{62}N_4B_{12}P_4O_{14}$) C, H, N, B; P: calcd, 15.1; found 14.6. Mol wt: calcd, 788; found, 790.

Bis(phosphato) Decahydrododecaborate (9). A sample of **8** was dissolved in water, passed through an acid ion exchanger, and titrated to pH 7 with LiOH. The solution was evaporated under reduced pressure to a few milliliters and filtered. The precipitate contained no boranes and was discarded. After its ^{11}B and ^{31}P nmr spectra were recorded, the filtrate was passed through an acid ion exchanger and titrated with Me_4NOH to pH 3. Addition of ethanol precipitated a 1:1 mixture (or double salt) of two orthophosphate derivatives: $[(CH_3)_4N]_2B_{12}H_{10}(OPO_3H_2)_2 \cdot [(CH_3)_4]_3B_{12}H_{10}(OPO_3H)(OPO_3H_2)$. *Anal.* ($C_{20}H_{87}N_5B_{24}P_4O_{16}$) C, H, N, P; B: calcd, 25.14; found, 23.48.

Bis(phosphato) Decabromododecaborate (10). A sample (0.1 g) of 8 was passed through an acid ion exchanger, the effluent neutralized with NH_4OH, and evaporated to dryness. The residue was dissolved in 5 ml of H_2O and Br_2 was added until the brown color persisted for 5 hr. Addition of Me_4NOH precipitated a product which melted at 304–310° after being dried in *vacuo* over P_2O_5. Complete bromination was confirmed by the absence of the B–H band in the ir spectrum of $(Me_4N)_4B_{12}Br_{10}(OPO_3H)_2$ (Table I). *Anal.* ($C_{16}H_{50}N_4B_{12}Br_{10}P_2O_8$) Br. Mol wt: calcd, 1418; found, 1451.

Bis(diphenylphosphato) Decahydrododecaborate (11). To a mixture of pyridine (10 ml) and MeCN (150 ml) containing 1.2 g (4.8 mmol) of $K_2B_{12}H_{10}(OH)_2$ 10 g of $(PhO)_2POCl$ was added and the mixture stirred for 2 weeks at 25°. Filtration removed unreacted starting material and the filtrate was concentrated by rotary evaporation until solid began to appear, passed through an acid ion exchanger, and titrated to pH 3 with Me_4NOH. A white solid, the tlc of which exhibited two spots of unequal intensity on silica gel (*i*-PrOH–NH_4OH–H_2O, 7:1:2), precipitated as the solution was evaporated. The solid was redissolved in water and salted out with EtOH to yield $(Me_4N)_2B_{12}H_{10}[OPO(OPh)_2]_2$. *Anal.* ($C_{32}H_{54}N_2B_{12}P_2O_8$) B.

Octadecahydroicosaboryl Pyrophosphate (13). To a mixture of pyridine (25 ml) and MeCN (10 ml) containing 0.50 g (1.7 mmol) of $(NH_4)_4B_{20}H_{17}OH$ and kept at 0° about 6.5 ml (71 mmol) of $POCl_3$ was added dropwise and the mixture stirred for 3 hr at 25°. After most of the solvent was stripped by rotary evaporation and the residue was dissolved in EtOH, addition of Me_4NCl precipitated 1.0 g of product (80%). The crude product was dissolved in 50% aqueous MeCN and salted out with EtOH to give 0.8 g of $(Me_4N)_4B_{20}H_{18}OP_2O_6H_2 \cdot 2H_2O$. This process may be repeated until the absence of starting materials is indicated by tlc. *Anal.* ($C_{16}H_{72}N_4B_{20}P_2O_9$) C, P; H: calcd, 9.12; found, 8.62. B: calcd, 29.1; found, 29.9. Mol wt: calcd, 743; found, 745.

Octadecahydroicosaboryl Phosphate (14). To a mixture of pyridine (25 ml) and MeCN (15 ml) containing 0.70 g (2.4 mmol) of $(NH_4)_4B_{20}H_{17}OH$ and kept at 0° was slowly added 1.0 ml (71 mmol) of $POCl_3$ and the reaction mixture allowed to stir at 25° for 3 hr. Rotary evaporation of the solvent left a residue which was slowly dissolved in water and the solution passed through an acid ion exchanger and reduced to a small volume. Addition of Me_4NCl followed by EtOH precipitated 1.2 g (85%) of $(Me_4N)_3B_{20}H_{18}OPO_3H_2 \cdot 2H_2O$. Should the presence of some pyrophosphate derivative 13 be indicated by the appearance of a precipitate when a solution is treated with LiOH, the material is dissolved in H_2O, passed through an acid ion exchanger, and titrated to pH 7 with LiOH. Any precipitate at this point, or after the volume is reduced by half, is removed and the filtrate acidified to pH 3. Addition of Me_4NCl will precipitate about half the material thus processed, and more may be salted out with EtOH to a total yield of 80%. The same orthophosphate 14 can be prepared by dissolving the pyrophosphate 13 in warm acid and salting out the hydrolyzed product with EtOH. The tlc on silica gel (MeCN–NH_4OH–H_2O, 12:5:3) exhibited a major spot at R_f 0.29 and a faint one at 0.10. *Anal.* ($C_{12}H_{60}N_3B_{20}PO_6$) C, H; N: calcd, 7.12; found, 7.90. B: calcd, 36.6; found, 33.9. P: calcd, 5.25; found, 6.03. Mol wt: calcd, 590; found, 585.

The ^{11}B nmr (in 30% D_2O) resembled the published spectrum of $B_{20}H_{18}OH^{3-}$ but the chemical shifts were different. The low-field region (area 5.3) had peaks at 10.3, 14.7, 21.5, and 24.3 ppm. The high-field region (area 14.7) had peaks at 37.4, 41.4, 44.0, and 47.9. Proton-decoupled spectrum had peaks at 12.7, 16.5, 19.0, 21.8, 37.4, 42.3, and 46.4 ppm. In alkaline solutions, above pH 8, the

spectrum resembled that of $B_{20}H_{17}OH^{4-}$ (isomer ii) with peaks at 7.4, 24.3, 28.8, 31.1, 46.7, and 48.5 ppm. Decoupling left peaks at 7.1, 22.5, 26.6, 29.4, 34.8, 43.3, and 47.9 ppm.

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Studies on Cyclophosphamide Metabolites and Their Related Compounds. 2.[1] Preparation of an Active Species of Cyclophosphamide and Related Compounds

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A synthetic study was made on the active metabolite of cyclophosphamide. Ozonolysis of *O*-(3-butenyl)-*N,N*-bis(2-chloroethyl)phosphorodiamidate, prepared by reaction of $POCl_3$ with 3-buten-1-ol followed by treatment with *N,N*-bis(2-chloroethyl)amine (nor mustard) and NH_3, afforded 2-[bis(2-chloroethyl)amino]-4-hydroperoxytetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide (4-hydroperoxycyclophosphamide). Deoxygenation of 4-hydroperoxycyclophosphamide by triphenylphosphine yielded 4-hydroxycyclophosphamide in a pure crystalline state. These products exhibited high cytostatic activity in both *in vitro* and *in vivo* experiments. The results give confirmatory evidence for the hypothesis that C_4-hydroxylation on the 1,3,2-oxazaphosphorinane ring of cyclophosphamide is necessary for its activation.

Cyclophosphamide [2-bis(2-chloroethyl)aminotetrahydro-2*H*-1,3,2-oxazaphosphorine-2-oxide (1)][2] is an antitumor agent now clinically used in the treatment of various kinds of human cancer. The drug is known to be activated to a cytotoxic form during *in vivo* metabolic degradation and extensive studies have been made to elucidate the structure of the active species.[3] In 1963, Brock and Hohorst[4] found that enzymatic oxidation in liver microsomes is responsible for the activation of cyclophosphamide and recent studies by Hill, *et al.*,[5] and also by us,[1] have shown that 4-ketocyclophosphamide (**2**) and its ring-opened carboxylic acid **3** are excreted as the cyclophosphamide metabolites in animal urine indicating that the first *in vivo* metabolic reaction of cyclophosphamide involves oxidation at the C-4 position on the 1,3,2-oxazaphosphorinane ring. Hill, *et al.*,[5a] first proposed that **4**-ketocyclophosphamide is either the active form of cyclophosphamide or a precursor of the active form. but both **2** and **3** were later proved to be cytostatically less active than cyclophosphamide in *in vivo* experiments.[1,5b,6,7] This suggests that the activation takes place in an earlier phase of the C-4 oxidation. Thus, 4-hydroxycyclophosphamide (**4**) and the ring-opened aldehyde **5** have been proposed as the alternative active species of cyclophosphamide.[1,6–8]

This paper is a full account of a previous communication[9] in which we have demonstrated the first unambiguous chemical synthesis of 4-hydroxycyclophosphamide and provided confirmatory evidence that cyclophosphamide is indeed activated by the C-4 hydroxylation. Prior to this work, we[10] had found that 4-ketocyclophosphamide can be reduced by lithium aluminum hydride to a potentially cytotoxic product which possibly has a cyclic structure **4**. However, the product could not be obtained in a pure state and was not unambiguously characterized because of extreme instability. Our alternative synthetic plan to obtain the product was the preparation of aldehyde **5** since the target compound **4** might possibly be in equilibrium with the ring-opened isomer. Thus, *O*-(3-hydroxypropyl)- and *O*-(2-cyanoethyl)-*N,N*-bis(2-chloroethyl)phosphorodiamidates (**6, 7**) were prepared and some aldehyde forming reactions from the alcohol **6**[11,12] and from the nitrile **7**[13,14] were attempted under various conditions; but these efforts, as well as acid-catalyzed hydrolysis of *O*-(3,3-diethoxypropyl)-*N,N*-bis(2-chloroethyl)phosphorodiamidate (**8**), were unsuccessful (see Chart I). Ozonolysis of *O*-(3-butenyl)-*N,N*-bis(2-chloroethyl)phosphorodiamidate (**9a**) also failed to give the aldehyde **5**. However, careful examination of the ozonolysis of **9a** led us to find that a very cytotoxic product,