Synthesis and Comparative Toxicology of a Series of Polyhedral Borane Anion-Substituted Tetraphenyl Porphyrins

Myoung-Seo Koo,[†] Tomoko Ozawa,[‡] Raquel A. Santos,[‡] Kathleen R. Lamborn,[‡] Andrew W. Bollen,[§] Dennis F. Deen,[‡,||] and Stephen B. Kahl*[,†]

Departments of Pharmaceutical Chemistry, Neurological Surgery, Pathology, and Radiation Oncology, University of California, San Francisco, San Francisco, California 94143

Received July 27, 2006

Three structurally similar tetraphenylporphyrins bearing polyhedral borane anions have been synthesized and their toxicological profiles obtained in rats. These conjugates were found to have quite different acute toxicities as manifested at the maximum tolerated dose (MTD). When given at the MTD and observed over 28 days, the most acutely toxic porphyrin was found to be devoid of toxicity, as measured by blood chemistry panels. The remaining two less acutely toxic compounds both elicited significant changes, characterized by moderate to severe thrombocytopenia, failure to gain weight normally and changes in liver enzymes indicative of mild hepatotoxicity. All toxic effects were transient, with platelets rebounding to above normal levels at day 28. We conclude that thrombocytopenia is the dose limiting toxicity for boronated porphyrins in mammals and suggest that these effects may be due to the porphyrin, not the borane or carborane.

Introduction

Over the past two decades, porphyrins, metalloporphyrins, and their derivatives have found increasing applications in medicine.[1,2] In binary therapies of cancer, such as photodynamic therapy (PDT[a]) and boron neutron capture therapy (BNCT), these compounds are used for their abilities to act as sensitizers or intermediaries for local damage initiated by the application of some external form of radiation. In PDT, the activating agent is a photon in the 630−750 nm region, but the actual cytotoxic species is generally accepted as being a reactive oxidizing species, primarily singlet oxygen. In BNCT, thermal neutron fission with the ^{10}B nucleus leads to prompt emission of ^{4}He (alpha particles) and ^{7}Li nuclei, together with a gamma ray, whose combined energies exceed 2.3 MeV. In both PDT and BNCT, the lethal radiation damage is confined to target cells by two processes. Because the mean free paths of singlet oxygen and the fission products are very short (<0.05 μm and <10 μm, respectively), the resultant apoptosis and necrosis occur only in cells that have taken up the sensitizer and not in surrounding normal cells. And because the activating beam of photons or neutrons can be sufficiently collimated to irradiate only the tumor plus a surrounding margin, sensitizer uptake in other remote tissues and organs does not lead to damage. The latter is only true, however, if the porphyrin itself does not cause toxicity to these other tissues and organs. Systemic administration of porphyrins and related compounds leads inevitably to exposure of a histologically diverse population of tissues and organs completely unrelated to the actual target tissues. This typically, but not necessarily, leads to observable and quantifiable alterations in plasma and/or cellular chemotoxic indicators. An example of a direct, medically beneficial chemotoxic effect of porphyrins in human pathologies is the application of tin porphyrins in the control of hyperbilirubinemia in neonates through inhibition of heme oxygenase.[2]

Surprisingly, despite this widespread interest in the medicinal chemical aspects of porphyrins and their derivatives, there is a paucity of published information on the toxicology of systemically administered porphyrins in mammals. In PDT, the medical field in which porphyrins have been most widely applied, there is an almost complete lack of any published information regarding their chemotoxicities. In a study of motexafin lutetium-mediated intraperitoneal PDT in a canine model, Hahn and coworkers noted mild, transient liver function abnormalities.[3] Miura et al. observed a significant decrease in platelet counts of mice given tetraphenylporphyrin tetrasulfonate ($TPPS_4$).[4] This compound has also been reported to be neurotoxic.[5] Reports in the BNCT field suggest that many boronated porphyrins also exhibit thrombocytopenia, as well as mild to moderate hepatotoxicity.[6−10] These compounds have included both *closo*- and *nido*-carboranes, but none bearing the extremely stable and nontoxic derivatives of the *closo*-borane anions $[B_{12}H_{12}]^{2-}$ and $[B_{10}H_{10}]^{2-}$.

We report here the syntheses and comparative chemotoxicities in normal rats of a series of three *closo*-borane anion derivatives of tetraphenylporphyrin. These compounds differ from each other only in the nature of the covalent chemical linkage between the porphyrin and the dodecaborate (B_{12}) anion. We observed that the three compounds had substantially different maximum tolerated doses (MTDs) in rats, which manifested itself in moderate to severe hemorrhagic liver necrosis in two cases and moderate to severe hemorrhage or congestion in the heart, lungs, or kidneys (but not livers) of the third compound. The three derivatives also had noticeably different patterns of toxic effects in normal rats when given at the MTD. Both the least acutely toxic and the middle compound produced profound thrombocytopenia at the MTD within the first 24 h after administration, while the most acutely toxic compound produced no such effect. This effect was paralleled by suppression of normal weight gain in these animals. Platelet counts returned to normal in all affected animals by the end of the 28-day experiment. Our results, in combination with previous observations, suggest that thrombocytopenia may be the most significant and common

* To whom correspondence should be addressed: Tel.: 415-476-4684. Fax: 415-476-0688. E-mail: sbkahl@picasso.ucsf.edu.
[†] Department of Pharmaceutical Chemistry.
[‡] Department of Neurological Surgery.
[§] Department of Pathology.
[||] Department of Radiation Oncology.

[a] Abbreviations: BNCT, boron neutron capture therapy; BSH, borocaptate sodium; PDT, photodynamic therapy; $TPPS_4$, tetraphenylporphyrin tetrasulfonate.

10.1021/jm060895b CCC: $37.00
Published on Web 01/25/2007

toxicity associated with high-dose porphyrin administration in mammals and that alternative methods of administration of these compounds may be necessary to avoid toxic effects.

Experimental Methods

$Cs_2B_{12}H_{12}$ and $Na_2B_{12}H_{11}SH$ were purchased from Katchem, Ltd. (Prague, Czech Republic), and used without further purification. $Na[B_{12}H_{11}NH_3]$ was prepared by the method of Hertler and Raasch.[11] $[Ph_3CH_3]_2[B_{12}H_{11}OH]$ was prepared by the method of Hawthorne and co-workers.[12] *meso*-Tetra(4-carboxyphenyl) porphine was purchased from Frontier Scientific and used without further purification. All other reagents and solvents were purchased from Aldrich Chemicals and used without further purification. Solvents were reagent grade unless otherwise specified and were dried and distilled by standard methods shortly before use. All reactions were carried out under inert atmosphere conditions using either argon or nitrogen. Gravity and flash column chromatography employed silica gel 60 GEDURAN (40−63 μm; EM Science). Routine NMR spectra (^{1}H and ^{13}C) were measured using a Varian 400 MHz spectrometer in solution as specified. Elemental analyses were performed by the Microanalytical Laboratory in the Department of Chemistry at UC Berkeley.

Preparation of $[(Pr)_4N][B_{12}H_{11}NH_3]$. The sodium salt of the amine borane was prepared from $Na_2B_{12}H_{12}$ by the published procedure.[11] It was necessary to convert this salt to one more compatible with the solubility of the porphyrin acid chloride starting material. After experimenting with a variety of tetraalkyl ammonium salts, the CH_2Cl_2 solubility of the tetrapropylammonium salt was found to be ideal. Treatment of an aqueous solution of the sodium salt with tetrapropylammonium iodide followed by filtration produced a white precipitate, which was recrystallized from hot water to give an essentially quantitative yield of the desired material.

Preparation of Tetrakis(tetrapropylammonium) Salt of TABP. *meso*-Tetra(4-carboxyphenyl) porphine (0.0975 g; 0.123 mmol) was suspended in anhydrous benzene (6 mL) in a 100 mL pear-shaped flask under argon. To this was added oxalyl chloride (6 mL; 68.8 mmol), and the reaction mixture was refluxed for 4 h. After cooling to rt, the benzene and unreacted oxalyl chloride were removed under vacuum. The resulting green solid was dissolved in anhydrous methylene chloride (72 mL). Tetrapropylamine borane $[(Pr)_4N][B_{12}H_{11}NH_3]$ (0.495 g; 1.44 mmol), *p*-dimethylaminopyridine (0.048 g; 0.39 mmol), and trimethylamine (0.6 mL; 4.30 mmol) were placed in a 1 L three-necked flask previously flushed with argon. Methylene chloride (300 mL) was added, and the mixture was brought to reflux. To this was added dropwise over the course of 1 h the solution of the porphyrin acid chloride. This reaction mixture was refluxed overnight and cooled to room temperature, and the solvents were removed under vacuum. The remaining solid was completely dissolved in 15% aqueous acetone (30 mL). This solution was evaporated, and the process was repeated two more times. Water (650 mL) was added to the remaining solids, and the suspension was stirred for 1.5 h and then filtered. The filtered brown solid was washed with water (3 × 10 mL) and dried on the filter. This solid was placed in a 250 mL round-bottom flask and tetrapropylammonium iodide (0.070 g; 0.22 mmol) and water (120 mL) were added. The mixture was refluxed for 1.5 h, cooled to room temperature, and filtered. The violet-colored solid was washed with warm water (60 °C; 8 × 2 mL) and air-dried. The product was dried under vacuum overnight to yield 0.214 g (0.102 mmol; 82.9%) of the desired product. This material produced a single spot (R_f = 0.50) on thin layer chromatography on DEAE cellulose in 50% aqueous acetone. ^{1}H NMR (400 MHz, DMSO) δ 9.11−7.63 (m, 24H, aromatic and pyrrole), 3.07 (m, 32H, propyl CH), 1.56 (m, 32H, propyl CH), 0.82 (m, 48H, propyl CH), 1.80−0.80 (br, 44H, BH), −2.95 (s, 2H, ring NH); ^{13}C NMR (400 MHz, DMSO) δ 168.13, 146.10, 139.77, 135.13, 131.16, 128.62, 127.30, 119.00, 59.98, 15.48, 11.20; IR (KBr) 3420.9, 2972.8, 2880.2, 2487.5 (BH), 1718.5, 1637.6, 1606.8 cm^{-1}; UV−vis (CH_3CN) 418 nm (log ϵ = 5.61), 514 (4.27), 548 (3.99), 590 (3.79), 644 (3.68). Elem. anal. Calc. for $C_{96}H_{186}N_{12}O_4B_{48}$: C, 55.13; H, 8.96; N, 8.04. Found: C, 55.37; H, 7.14; N, 7.90.

Ion Exchange to Tetrasodium Salt of TABP. The above tetrakis(tetrapropylammonium) porphyrin borane salt (0.030 g) was dissolved in 15% aqueous acetone (v/v) and passed through a Dowex 50W×2−200 resin column (1 × 3 cm), previously generated in the Na^+ form. The eluted solution was evaporated in vacuo. The solid remnant was redissolved in 50% aqueous acetone (v/v) and passed through a fresh ion exchange column (1 × 4.8 cm). The eluted solution band containing the product was evaporated in vacuo to yield 0.030 g of the desired sodium salt product as a red powder, which was well soluble in water. ^{1}H NMR (400 MHz, D_2O) δ 8.92−8.17 (br m, 24H, aromatic and pyrrole), 2.05−0.40 (br, 44H, borane BH; there was no evidence of any residual propyl CH in this region, ring NH disappears due to exchange w/solvent).

Preparation of the Octasodium Salt of TEBP. *meso*-Tetra(4-carboxyphenyl) porphine (0.402 g; 0.508 mmol) was suspended in anhydrous benzene (32 mL) in a 500 mL round-bottom flask under argon. To this was added oxalyl chloride (24 mL; 275.2 mmol), and the reaction mixture was refluxed for 4 h. After cooling to rt, the benzene and unreacted oxalyl chloride were removed under vacuum. The resulting green solid was dissolved in anhydrous methylene chloride (405 mL). $Na_2[B_{12}H_{11}SH]$ (1.280 g; 1.44 mmol), *p*-dimethylaminopyridine (0.212 g; 1.72 mmol), and trimethylamine (1.4 mL; 10.0 mmol) were placed in a 1 L three-necked flask previously flushed with argon. Anhydrous acetonitrile (270 mL) was added, and the mixture was brought to reflux. To this was added dropwise over the course of 70 min the solution of the porphyrin acid chloride. This reaction mixture was refluxed for 8 min, cooled to room temperature, and filtered. The product was washed with water (250 mL) and air-dried on the filter. The filtered solid was dissolved in acetonitrile/water (7:3 v/v; 30 mL) and then stirred overnight with Dowex 50W×2-200 resin (70 g) previously generated in the Na^+ form. The red solution was filtered, and the resin was washed with 70% acetonitrile/water until the filtrate was very pale pink. The filtrate was concentrated under vacuum and filtered through a 0.2 micron filter. This filtrate was then completely evaporated under vacuum to provide a deep red crystalline powder (0.407 g; 50.2% yield), which was stored in a vacuum desiccator overnight. For elemental analysis, the sodium salt was converted to the octakis(tetrapropylammonium) salt by treatment of an aqueous solution with tetrapropylammonium bromide. The red precipitate was washed extensively with water and air-dried. ^{1}H NMR (400 MHz, DMSO) δ 8.82 (s, 8H, pyrrole CH), 8.31 (m, 8H, aromatic), 8.25 (m, 8H, aromatic), 1.90−0.40 (br, 44H, BH), −2.95 (s, 2H, ring NH); ^{13}C NMR (400 MHz, DMSO) δ 168.50, 145.75, 141.12, 135.00, 131.93, 128.54, 125.98, 120.02; IR (KBr) 3389.8, 2487.6, 1702.3, 1605.1 cm^{-1}; UV−vis (H_2O) 416 nm (log ϵ = 5.46), 518 (4.12), 556 (3.90), 580 (3.76), 636 (3.57). Elem. anal. for octapropyl ammonium salt. Calc. for $C_{144}H_{288}N_{12}O_4B_{48}S_4$: C, 59.66; H, 10.01; H, 5.80. Found: C, 59.55; H, 10.09; N, 5.60.

Preparation of the Octakis(methyltriphenylphosphonium) Salt of TOBP. *meso*-Tetra(4-carboxyphenyl) porphine (0.215 g; 0.272 mmol) was suspended in anhydrous benzene (16 mL) in a 500 mL round-bottom flask under argon. To this was added oxalyl chloride (12 mL; 137.6 mmol), and the reaction mixture was refluxed for 4 h. After cooling to rt, the benzene and unreacted oxalyl chloride were removed under vacuum. The resulting green solid was dissolved in anhydrous methylene chloride (345 mL). $[PPh_3CH_3]_2[B_{12}H_{11}OH]$ (1.20 g; 1.69 mmol) and proton sponge (500 mg) were dissolved in methylene chloride (600 mL) in a 2 L three-neck round-bottom flask under argon and brought to reflux. To this was added dropwise over 1 h the green acid chloride solution in methylene chloride. This mixture was then refluxed for 3 days, cooled to room temperature, and poured into a 4 L beaker, to which was immediately added ethyl alcohol (3 L) with stirring. This slurry was stirred for an additional 4 h and filtered through a medium coarse filter funnel (600 mL). The red solid was then isolated and dissolved in acetonitrile/water (220:100). The acetonitrile was removed under a water aspirator until most had been removed. The red product was filtered and washed with water until the filtrate was completely colorless. The red solid was dissolved in acetonitrile

(100 mL) at 20 °C, and ethanol was added (300 mL) with stirring. The crystallized red solid was then isolated by filtration to yield 0.678 g (0.190 mmol; 69.8% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.80 (s, 8H, pyrrole CH), 8.21 (m, 16H, porphyrin aromatic), 7.89−7.66 (m, 120H, phosphonium aromatic), 3.07 (s, 24H, phosphonium methyl), 1.80−0.40 (br, 44H, BH), −3.00 (s, 2H, ring NH); ^{13}C NMR (400 MHz, DMSO) δ 165.69, 145.21, 145.37, 135.40, 134.72, 134.24, 133.55, 131.20, 130.50, 129.61, 128.08, 127.30, 127.05, 122.54, 119.50, 45.83; IR (KBr) 3591.0, 3056.6, 2483.8, 1681.5, 1604.6 cm^{-1}; UV−vis (CH_3CN) 416 nm (log ϵ = 5.69), 514 (4.29), 548 (4.03), 590 (3.81), 644 (3.69); Elem. anal. Calc. for $C_{200}H_{214}N_4O_8B_{48}P_8$: C, 67.31; H, 6.04; N, 1.57. Found: C, 67.52; H, 6.13; N, 1.67.

Ion Exchange to the Octasodium Salt of TOBP. The above octakis(methyltriphenylphosphonium) porphyrin borane salt (161 mg) was dissolved in 25% aqueous acetonitrile (v/v) and passed through a Dowex 50W×2−200 resin column (1 × 3 cm). The eluted solution was evaporated in vacuo. The solid remnant was redissolved in 50% aqueous acetonitrile (v/v) and passed through a fresh ion exchange column (1 × 4.8 cm). The eluted solution band containing the product was evaporated in vacuo to yield 112 mg of the desired sodium salt product as a red powder, which was well soluble in water and quite hygroscopic. ^{1}H NMR (400 MHz, DMSO) δ 8.82 (s, 8H, pyrrole CH), 8.21 (m, 16H, aromatic), 1.80−0.40 (br, 44H, BH; there is no evidence of additional aromatic or methyl protons indicative of the phosphonium cation), −2.95 (s, 2H, ring NH); ^{13}C NMR (400 MHz, DMSO) δ 169.62, 149.21, 144.76, 135.99, 132.36, 131.50, 128.37, 119.92; UV−vis (H_2O) 416 nm (log ϵ = 5.44), 518 (3.95), 554 (3.82), 582 (3.67), 636 (3.49).

Animals. Six-week-old male Fischer 344 rats (~200 g body weight) were purchased from Simonsen Laboratories (Gilroy, CA) and used in all studies. Rats were housed and cared for in accordance with the United States Department of Health and Human Services Guide for the Care and Use of Laboratory Animals. All protocols were approved by the UCSF Institutional Animal Care and Use Committee. Rats were maintained in a temperature and light-controlled environment with an alternating 12-hour light/dark cycle and provided food and water ad libitum. Rats were anesthetized by an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (7.5 mg/kg) for drug injection and blood collection.

Determination of Maximum Tolerated Dose. Anesthetized animals were injected i.v. via tail vein with graded concentrations of aqueous solutions of the boronated compounds adjusted to pH 7.4 with $NaHCO_3$ and were monitored closely up to 28 days. Dosage groups consisted of 2 to 4 animals per dose. When animals showed systemic symptoms such as loss of activity or hemorrhaging from body orifices, animals were euthanized. Dosages at which 50% or more of the animals died at any point during the 28-day period were considered to be above the MTD. Organs were removed and fixed with 10% buffered formalin for 24 to 48 h and then cut with a razor blade and embedded in paraffin. The paraffin-fixed tissue was cut into 5 μm-thick sections, stained with hematoxylin and eosin using standard staining methodology, and used for histopathologic investigations.

Toxicology at the Maximum Tolerated Dose. After determining the MTD for each compound, an investigation was made of the effects of injecting the three boronated porphyrins at their respective MTDs on various blood chemistry values, including: platelets, hematocrit (Hct), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (alb), and serum potassium (K^+). Porphyrins were administered at the MTD by i.v. bolus into the tail veins of two groups of four animals each. One group was used to provide blood samples for platelet and hematocrit determinations and the other for the remaining parameters. Blood samples were taken 24 h prior to compound administration to provide baseline comparative values. Blood samples were then collected 1, 7, and 28 days postinjection via tail arteries, and samples were analyzed using the Roche COBAS Mira Classic automated chemistry analyzer (Roche Diagnostic System, Branchburg, NJ) and the Serano-Baker 9010 hematology analyzer (Serano Baker Co., Allentown, PA) in the Comparative Pathology Laboratory at the University of California, Davis. Animals were also weighed at the time of each blood draw to assess overall health.

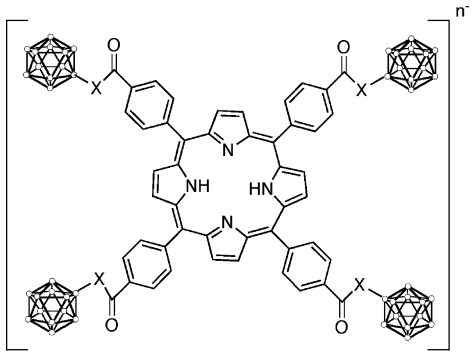

Figure 1. Structures of the three borane anion porphyrin derivatives prepared. Open circles represent BH units except at the point of linkage to the phenyl rings where they represent a boron atom.

Results

Chemistry. One of the goals of our synthetic work in this area was the successful preparation and characterization of a structurally related series of water-soluble borane anion derivatives of tetraphenyl porphine. The *para*-carboxylic acid group was chosen for the linkage chemistry largely as a result of the breadth of stable derivatives that could potentially be formed from known $B_{12}H_{12}^{2-}$ polyhedral anion derivatives. As a result, amide (TABP), thioester (TEBP), and oxoester (TOBP) derivatives were successfully prepared and characterized (Figure 1).

For synthesis of the first compound (TABP), we felt it necessary to initially convert the sodium salt of $B_{12}H_{11}NH_3^-$ to a salt more appropriate to the solvent conditions to be used for coupling to the acid chloride (methylene chloride). After experimenting with tetraalkylammonium cations of various carbon chain lengths, it was found that the propyl side chain presented the optimum solubilizing characteristics. After combining the reactants and refluxing overnight, complete conversion into the tetrapropylammonium salt was insured by refluxing the crude material in water containing tetrapropylammonium iodide. The final product, obtained in 83% overall yield, was characterized in this form because the sodium salt is difficult to purify and is hygroscopic. The proton NMR of this compound showed no evidence of the protonated iminol form of the amide tautomer reported by Gabel et al. for acyl derivatives $[B_{12}H_{10}NH_3]^-$ ion.[13] For administration to animals, the tetrapropylammonium salt was ion exchanged several times in an aqueous acetone solution until ^{1}H NMR showed no evidence of saturated CH signals. In the preparation of TEBP, we tried to avoid such an elongated procedure and found that addition of some acetonitrile to the borane anion solution was sufficient to keep the sodium salt in solution while adding the methylene chloride solution of the porphyrin acid chloride. This made it possible to isolate the sodium salt directly, although a small amount of the product was then converted to the tetrapropylammonium salt for elemental analysis. The oxoester TOBP followed the original pattern and was isolated initially as the water insoluble methyltriphenylphosphonium salt and then converted into the water soluble sodium salt by ion exchange prior to administration. All three porphyrins have UV−visible absorption spectra of the *etio*-type that is typical for the

Table 1. Liver Enzyme Levels in Normal Fischer 344 Rats Given Each Porphyrin at the MTD

ALT (unit/L)					AST (unit/L)				
rat	day −1	day 1	day 7	day 28	rat	day −1	day 1	day 7	day 28
				TABP 15 mg/kg					
1′	54	65	48	48	1′	114	160	138	84
2′	66	50	45	47	2′	244	126	75	87
3′	54	67	45	43	3′	127	137	120	69
4′	52	79	48	56	4′	133	199	90	73
				TEBP 100 mg/kg					
5′	51	90	150	58	5′	99	183	87	112
6′	51	(dead)			6′	104	(dead)		
7′	48	(dead)			7′	90	(dead)		
8′	52	576	170	71	8′	128	687	47	118
				TOBP 40 mg/kg					
13′	49	81	96	(hemolyzed)	13′	86	117	43	(hemolyzed)
14′	45	81	137	(hemolyzed)	14′	74	156	51	(hemolyzed)
15′	47	65	218	60	15′	71	165	105	104
16′	38	48	83	45	16′	69	84	47	88

tetraphenyl porphine framework. Their infrared spectra have carbonyl stretching frequencies that suggest substantial electron donation from the borane anion into the linkage group. For example, the carbonyl stretch of TOBP is found at 1681 cm^{-1}, which is almost identical to that of the benzoyl derivative of $[B_{12}H_{11}OH]^{-2}$ reported to be 1683 cm^{-1} by Gabel and coworkers.[14] Like them, we also found that the BH stretching frequency was insensitive to the nature of the derivative and was invariably observed to fall within a narrow range of 2484−2488 cm^{-1}. Thus, any electron donation from the polyhedral anion across the linker into the aromatic ring is not reflected by changes in the B−H bond strength.

Toxicology. For the amide TABP, a MTD in normal Fischer 344 rats of 15 mg/kg was found. All animals died in all dosing groups of 25 (2/2), 30 (2/2), 35 (2/2), 40 (2/2), 45 (2/2), and 50 (4/4) mg/kg, and 50% of the animals expired at 20 (3/6) mg/kg, while none died at either 10 (0/4) or 15 (0/4) mg/kg. Histopathological evaluation revealed moderate to severe acute hemorrhagic necrosis in the livers of all animals that received doses of ≥20 mg/kg of TABP. Secondary pulmonary hemorrhage also was seen in several cases. When a group of four rats was given 15 mg/kg, followed with blood draws on days 1, 7, and 28, the blood chemistry results indicated that no significant changes in ALT or AST (Table 1), LDL, albumin, or potassium (data not shown) occurred over the 28-day period when compared with samples taken prior to injection. Similarly, there was no evidence of changes in platelet count or hematocrit in a second group of four compared again with preadministration values (Table 2). All eight animals gained weight over the 28-day period (Figure 2) and none displayed any adverse symptoms or abnormal activities. Histopathological examination of the major organs in these animals revealed no evidence of abnormality in any of the organs.

Next we similarly measured the MTD of thioester TEBP and determined it to be 100 mg/kg. In this case, more than 50% of animals in each group at 110 (2/2), 125 (2/3), 140 (2/2), 150 (5/9), 190 (4/6), and 200 (4/4) mg/kg died, while none of the 10 rats given 100 mg/kg nor any animals at any doses (groups of 3 animals each) below this level died. Histopathological evaluation of deceased animals revealed moderate to severe acute hemorrhage or congestion in the kidneys, heart, or lungs in animals receiving ≥110 mg/kg. In contrast to animals given the amide (TABP), hemorrhagic necrosis was not observed in the livers of any animals that received the above high doses of the thioester (TEBP). Again, two groups of four rats each were given 100 mg/kg of this compound and followed as above for 28 days. In the blood chemistry group, two animals subsequently died within the first 24 h, indicating that this dose was very close to the MTD, but making it difficult to draw conclusions about toxicity. Histopathological examination of the two deceased animals revealed hemorrhagic liver necrosis. Substantial increases in both ALT and AST in both surviving animals were observed, suggesting some liver toxicity (Table 1). Albumin, potassium, and LDL values in these animals were normal. In the second group given 100 mg/kg of this compound, no deaths were observed, and all four rats were followed for the full 28 days. In all four of these animals, a substantial and statistically significant drop in platelets in all rats on day 1 ($p = 0.03$ paired t-test) (Table 2) was observed, followed by a rebound to supra-normal levels at day 7 ($p = 0.003$ paired t-test compared to baseline) and resolution to normal at day 28. Hematocrit percentages were normal in all animals at all time points. Again in contrast to TABP, animals receiving TEBP did not gain weight at a normal rate until about day 12 (Figure 2), after which they grew normally. Histopathological examination of the major organs in these animals revealed no evidence of abnormality in any of the organs.

Table 2. Platelet Counts and Hematocrit in Normal Fischer 344 Rats Given Each Porphyrin at the MTD

platelet (× 10^3/mm^3)					hematocrit (%)				
rat	day −1	day 1	day 7	day 28	rat	day −1	day 1	day 7	day 28
				TABP 15 mg/kg					
1	728	672	483	626	1	36.7	33.3	36.5	35.2
2	696	639	575	420	2	34.3	36.3	34.9	56.3
3	543	552	663	673	3	34.8	39.8	34.9	41.4
4	693	739	618	669	4	44.4	34.5	48.3	41.5
				TEBP 100 mg/kg					
5	770	427	1099	611	5	35.3	34.0	23.6	26.0
6	728	468	1185	621	6	31.2	35.1	27.8	24.4
7	775	143	1151	718	7	34.5	13.8	26.4	34.9
8	670	431	1231	730	8	33.1	37.1	27.1	26.4
				TOBP 40 mg/kg					
13	710	211	1325	769	13	30.7	33.9	36.7	34.4
14	629	225	858	650	14	28.6	29.5	29.0	33.3
15	800	279	990	624	15	33.1	35.2	31.3	30.8
16	327	179	1168	600	16	19.3	29.9	37.5	28.6

Last, we determined an MTD of 40 mg/kg for the oxoester (TOBP) with all animals treated at ≥50 mg/kg dying and none at ≤40 mg/kg. Histopathological examination of deceased animals revealed moderate to severe acute hemorrhagic necrosis in the livers of all animals. Blood chemistry results for a group of four rats treated with 40 mg/kg of this ester indicated normal levels of potassium, LDL, and albumin (data not shown), but once again showed first day elevations of AST ($p = 0.03$).

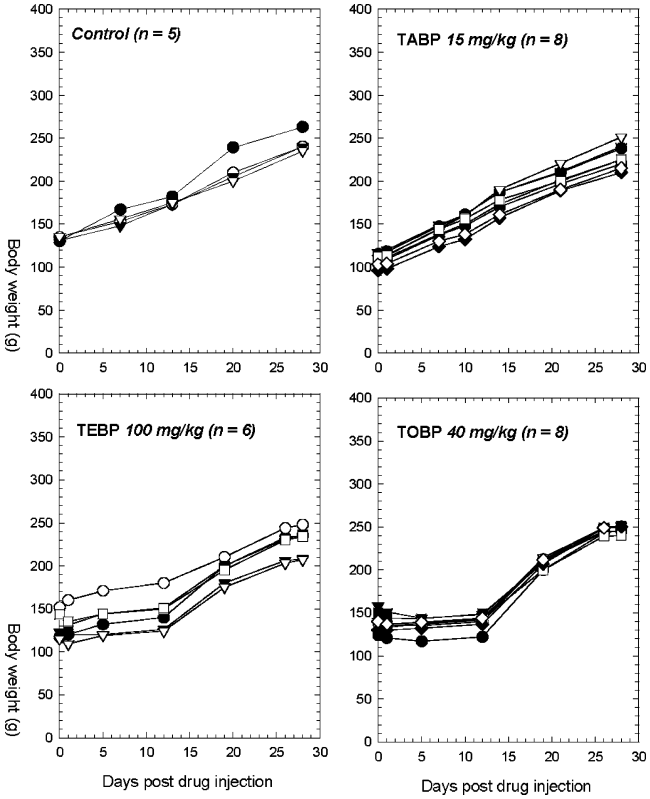

Figure 2. Body weight vs time plots in normal Fischer 344 rats given each porphyrin at the MTD.

ALT also appeared to be elevated, although the increase was not statistically significant ($p = 0.06$). Early elevations were followed by later resolution (Table 1). In the second group, again found was a statistically significant drop in platelets on day 1 ($p = 0.02$) relative to pretreatment levels, followed by day 7 rebound to somewhat above normal levels ($p = 0.06$; Table 2). All values had returned to the normal range at day 28. As with TEBP, the weights of all eight animals given TOBP did not begin to increase until day 12 (Figure 2), after which they became normal at the end of the 28-day period.

To determine the toxicity of the functionalized borane anions themselves, MTD studies were also carried out in a similar fashion for $Na[B_{12}H_{11}NH_3]$ and $Na_2[B_{12}H_{11}OH]$. For both salts, normal rats were injected with 150, 200, or 400 mg/kg of the sodium salt by tail vein bolus and observed for 28 days. All animals gained weight continuously after injection until euthanasia, and no animal displayed any systemic symptoms during the observation period. We conclude that the MTD of both salts was greater than 400 mg/kg. Upon necropsy, no gross abnormality in any of the abdominal organs or brain was observed. There was also no evidence of histopathologic abnormalities in the liver, lung, heart, spleen, or brain. We did not evaluate $Na_2[B_{12}H_{11}SH]$ because the toxicological properties of this salt have been previously well studied (vide infra).

Discussion

Although the ideal sensitizer for PDT or BNCT might possess a number of desirable characteristics, three are absolutely essential. First, the compound must be sufficiently nontoxic to normal tissues and organs to allow sufficient dosing. Different dosing protocols may be used to mitigate this issue, but undoubtedly for any potential drug to be given systemically (i.v., i.a., i.p., or i.c.), this characteristic is of paramount importance above all others. Second, it must selectively localize in target tissue (e.g., tumor) in sufficient amounts that when activated causes death of the target cells. In BNCT, this has been estimated to be ≥ 30 ppm ^{10}B, but this concentration is clearly dependent upon the subcellular locus of the boron.[15] In PDT, this issue has not been commonly addressed, but certainly the tumor concentrations of clinically useful PDT agents are 1 or 2 orders of magnitude less than this. Third, the drug must clear the bloodstream and surrounding normal tissue in a timely fashion such that tumor/blood and tumor/normal tissue ratios are sufficient to avoid normal tissue damage. The pharmacokinetic behavior of many PDT and potential BNCT agents, including boronated porphyrins, is suboptimal in this regard.

Based upon the primacy of the first characteristic, we prepared and characterized three porphyrins bearing polyhedral borane anions. These compounds differ only in the nature of the functional group linking the porphyrin phenyl ring and the dodecaborate borane anion and the resulting overall charge of the porphyrin−borane conjugate. The lack of toxicity of the polyhedral borane anions $[B_{12}H_{12}]^{2-}$ and $[B_{10}H_{10}]^{2-}$ is quite remarkable and in marked contrast to other boron hydrides such as diborane (B_2H_6) and decaborane ($B_{10}H_{14}$). Muetterties et al. reported that the lethal oral doses of $Na_2[B_{12}H_{12}]$ and $Na_2[B_{10}H_{10}]$ were ≥ 7.5 grams/kg body weight in rats, figures that are comparable to NaCl.[16] Sweet and co-workers gave doses of up to 76 mg/kg $Na_2[B_{10}H_{10}]$ by intra-carotid injection to human brain tumor patients and observed no toxic symptoms.[17] The LD_{50} of this compound in Swiss albino mice has been reported to be 1041 mg/kg.[18] These anions are rapidly excreted unchanged through the kidney, a factor we also anticipated would be useful in decreasing the plasma half-life of porphyrins to which they might be attached. Our experience with the *closo*-carboranyl porphyrin BOPP in human patients and canines had demonstrated a very long plasma half-life of BOPP, which we hypothesized might lead to the formation of potentially toxic metabolic products, including its *nido* cage opened form.[7,8] Predictably, one might expect functionalized derivatives of these borane anions to be somewhat more toxic because they provide external functional groups as potential sites of interaction with enzymes, molecular transporters, and so on. The best studied of these is $Na_2[B_{12}H_{11}SH]$, also known as borocaptate sodium (BSH), which has been widely used clinically in BNCT treatment of glioblastoma multiforme. Bolus i.v. administration of BSH to rats at doses of 100, 300, and 600 mg/kg proved lethal only at the highest dose, while renal injury was observed at both the 300 and the 600 mg/kg levels.[19] In another rat study, kidney function changes related to reduced renal plasma flow were found at doses as low as 25 mg/kg and related to the presence of the sulfhydryl group.[20] These same researchers found nephrotoxic lesions in rabbits treated by seven daily i.v. injections of BSH at doses of 50 mg/kg/dose but not at 25 mg/kg/dose.[21] In human glioblastoma patients infused with BSH at doses up to 50 mg/kg, no significant acute toxic effects were found in any of 19 blood parameters, including platelets, ALT, and AST.[22] Thus, the published literature supports the idea that BSH at least is rather nontoxic. The MTD results of >400 mg/kg for $Na[B_{12}H_{11}NH_3]$ and $Na_2[B_{12}H_{11}OH]$ found in the present study, the only ones available for these compounds, further illustrate the lack of acute toxicity to be found in borane anions substituted with typical organic functional groups. Because the $B_{12}H_{11}$ polyhedral is an electron donating substituent, the bond stabilities of the amide, thioester, and ester linkages in these porphyrins should be similar to those in substituted acyl analogs bearing electron releasing groups and, thus, stable in vivo.

Cleavage of these bonds would result in release of the substituted borane anions themselves, which we have shown are essentially devoid of toxicity.

Patterns of thrombocytopenia accompanied by inhibited weight gain and, in many cases, hepatic damage are not unknown in boronated porphyrins. Indeed, a search of the literature reveals at least a half dozen published reports on the toxicology of boronated porphyrins. Almost invariably, reports on the toxicity of *nido*- or open cage $B_9C_2^-$ carboranyl porphyrins have found reduced platelets. The first was a study from our laboratory on the uptake of a *nido*-carboranyltetraphenylporphyrin (BTPP) in athymic nude mice bearing the U-87 MG human glioma xenograft.[6] The four *nido*-carboranyl open cages were linked to the porphyrin phenyl rings through amide bonds. In tumor bearing animals given BTPP infusions by osmotic minipumps over 3 or 7 days, there was an almost directly inverse correlation between the measured liver boron concentrations and the platelet count: the higher the boron (and presumably porphyrin levels), the more profound the thrombocytopenia.[6] The liver boron concentrations also closely correlated directly to increases in ALT, AST, and ALP. Miura and coworkers reported that another *nido*-carboranyl porphyrin (VCDP) given by serial i.p. injection at a dose of 270 mg/kg to mice subcutaneously implanted with a mammary carcinoma produced moderate thrombocytopenia accompanied by elevated white blood cell counts, mainly by granulocytosis.[4] There was also a significant decrease in albumin and an increase in ALT. All changes appeared reversible at 4 days except the WBC count, which did not normalize over the course of the experiment. Vicente et al. recently evaluated the toxicity of another tetra-*nido*-carboranyl phenyl porphyrin by serially injecting tumor bearing mice with the apo-metallo porphyrin and its zinc derivative at doses of ∼75 mg/kg.[9] Even at this relatively low murine dose, they observed considerable weight loss and substantial thrombocytopenia at day 2. Platelets rose at day 4 but were still suppressed compared to saline controls, and the weight loss continued to be unresolved. Miura and colleagues also compared the toxicities and tumor boron delivery potential of a series of *nido*- and *closo*-carboranyl porphyrins.[23,24] Their data must be interpreted with some care, as all compounds were administered by serial i.p. injection in aqueous Cremphor solutions, but effects were obvious. Comparison of two Ni tetraphenyl porphyrins, one with four *closo* cages (NiTCP−H) and the other with four *nido*-carboranyl cages (NiNTCP−H), showed that the *nido* compound was severely hepatotoxic at ∼1/2 the dose of the essentially nontoxic *closo*-porphyrin. However, neither compound produced thrombocytopenia. Another noteworthy comparison was made between two *closo*-carboranyl hematoporphyrin type compounds, NiDPE and ZnDPE. NiDPE was found to be essentially nontoxic at a dose of 78 mg/kg. In contrast, the zinc analog at 118 mg/kg produced mild thrombocytopenia, moderate inhibition of normal weight gain, and substantial increases in ALT, AST, and ALP. When zinc is removed from both these porphyrins and the carborane cage is opened, the product is the VCDP molecule previously noted to produce moderate thrombocytopenia. A copper analog of the NiTCP given to mice at 200 and 400 mg/kg resulted in essentially no toxic changes, but at the higher dose, mice developed substantial thrombocytopenia accompanied by weight loss and moderate increases in ALT and AST 2 days following the end of the serial injections.[10] As with our experience in the present study, these changes all resolved by day 7 and, in fact, the platelet counts rebounded to slightly above normal at this time.

The boronated porphyrin BOPP bearing four *closo*-carboranyl cages was developed in our laboratory and has been examined in several mammalian species, including man.[8] Preclinical testing of BOPP in rats revealed a significant but transient thrombocytopenia together with elevations in liver enzymes.[25] Normal dogs treated with 35 mg/kg BOPP showed maximum average weight decreases of 6−7% over a 30-day period after bolus administration. Platelet counts decreased moderately over the first 5 days to a minimum level of ∼60% of normal, followed by an increase to normal at 6−8 days, and a continued rise to approximately twice the day 0 levels, again similar to the present study. Both ALT and AST were elevated in some, but not all, of the 16 dogs in the study.[7] These changes were not clinically significant for the dogs involved, but the moderate thrombocytopenia observed in these canines served as a cautionary warning for what was to come in the phase I human trial of BOPP for PDT. Twenty-nine patients with intracranial tumors were administered increasing doses of BOPP intravenously 24 h prior to surgical resection.[8] Overall, BOPP was very well tolerated in these patients, but two of three patients treated at a dose of 8 mg/kg developed significant thrombocytopenia (grade 3 and 4, one patient each). Under the study design, this meant that the maximum allowable dose for humans was 4 mg/kg. At this dose, 3 of 10 total patients developed mild (grade 1) thrombocytopenia, while no thrombocytopenia was noted in the remaining 10 patients treated at this dose nor in any of the remaining 24 patients treated at other dose levels. The human maximum allowable dose of 4 mg/kg is, thus, quite acceptable for PDT, but is too small for effective BNCT. Thus, what in canines seemed to be a minor concern ultimately revealed itself as the dose limiting toxicity in humans. In the BOPP molecule, the four *closo*-carboranes are linked to the hematoporphyrin-like framework by ester bonds. Under in vivo conditions, it is entirely possible that the presence of the α-carbonyl function leads to opening of the *closo* cages to produce a *nido*- metabolite, so we cannot rule out the possibility that this is the source of the thrombocytopenia seen in dogs and humans.[26] On the other hand, the borane anions in the present study cannot be opened by any known mechanism and do not exhibit inherent toxicity themselves. These results lead us to suggest that perhaps it is ultimately the porphyrin, not the borane or carborane, that leads to this common pattern of toxic effects.

The relative lack of published toxicological information regarding nonboronated porphyrins is perhaps not as surprising as at first glance. The administered doses of porphyrins and related macrocycles used in PDT are typically in the milligram to submilligram per kg region where chemotoxic effects are not likely to be observed. In fact, one study has reported that hematoporphyrin derivative (HpD), but not benzoporphyrin derivative (BPD), stimulates in vitro colony formation by cells of myeloid progenitor (CFU-GM) in the spleen and bone marrow of mice treated with 10 and 25 mg/kg.[27] Because HpD is a very complex mixture of oligomeric derivatives of hematoporphyrin and because in vivo platelet levels were not measured, the relevance of this finding to generalized porphyrin behavior is debatable. However, in certain situations, toxic effects similar to our observations have been previously reported with porphyrins and metalloporphyrins. One particularly striking example is to be found in the case of zinc protoporphyrin and zinc mesoporphyrin, whose clinical application in suppressing hyperbilirubinemia in newborns is well documented. Both compounds are inhibitors of heme oxygenase activity in vitro at concentrations in the 1−20 μM range.[28] This enzyme, along

with various cytokines and growth factors, plays a major role in hematopoietic cell growth and differentiation. Kappas and co-workers have reported that zinc protoporphyrin and zinc mesoporphyrin potently inhibit human and rabbit bone marrow erythroid and myeloid colony growth in vitro, an effect that is not overcome by exogenous administration of elevated levels of either erythropoietin (EPO) or granulocyte-macrophage colony stimulating factor (GM-CSF).[28] They also observed that the apo-metalloporphyrins (protoporphyrin and mesoporphyrin) themselves were also toxic to bone marrow cultures in the same concentration range. In a subsequent study, the effects of zinc mesoporphyrin and tin mesoporphyrin on in vivo hematopoietic progenitor cell mobilization and in vitro hematopoiesis in rabbits were evaluated. When administered in vivo concurrently with rhG-CSF, the zinc porphyrin inhibited the mobilization of the numbers of both erythroid (BFU-E) and myeloid (CFU-GM) progenitor cells by $\geq 70\%$.[29] However, the tin analog administered at the same concentration in vivo had no significant suppressive effects on either type of progenitor cells. Because both metalloporphyrins were found to inhibit bone marrow activity to the same extent in vivo, it is clear that both gained access to bone marrow cells. Furthermore, addition of zinc mesoporphyrin to rabbit bone marrow cultures in vitro suppressed both types of precursor growth, but the tin analog did not. Also, both inhibit liver and spleen heme oxygenase to the same extent. Therefore, the difference in hematological toxic effects must lie in the difference between zinc and tin binding to the porphyrin core. The likely answer is the known lability of zinc porphyrins, which, in contrast to tin porphyrins, readily demetalate under mildly acidic conditions.[30] This is consistent with the observation by Kappas et al. that both protoporphyrin and mesoporphyrin themselves have toxic effects on bone marrow cells in the same concentration range as their metallo-derivatives. This is also consistent with the observation by Miura et al. that $TPPS_4$ is associated with thrombocytopenia. It is also consistent with the finding of Miura et al. that zinc forms of carboranyl porphyrins are hemato- and hepatotoxic, but similar compounds with stable metal cores, such as Ni and Cu derivatives, are not, except at very high doses.

Our data in the present study, as well as in earlier ones with boronated porphyrins, are also consistent with a direct toxic effect of porphyrins on platelets. In all reported cases, the onset of thrombocytopenia occurs within the first 24 to 48 h after administration, and a return to normal values takes one week or more. Such a direct effect of a porphyrin on platelets has been reported in patients treated with hematin for acute intermittent porphyria. Hematin, the ferric hydroxide derivative of heme, at a dose of 4 mg/kg, can be associated with the development of an unusual coagulopathy characterized by thrombocytopenia and increased clotting times. Green and Ts'ao and co-workers showed in vitro that hematin directly aggregated platelets and activated platelets to release ATP and serotonin.[31] They also demonstrated that platelet adhesion to hematin-treated monolayers of bovine aortic endothelial cells was increased, particularly so when low concentrations of hematin were included in the platelet suspensions.[32] Green et al. later found that hematin binds to the factor VIII/von Willebrand factor complex and changes the binding of this complex to platelets.[33,34] They suggested that formation of the trimeric hematin-factor VIII/vWF complex in vivo may activate and bind to platelets, leading to platelet aggregation, thrombocytopenia, and increased clotting time. We suggest that clearance of this platelet complex by the spleen might account for the reduced platelet counts we observed and perhaps may account for the generalized thrombocytopenia seen in other studies of boronated porphyrins.

Regardless of whether the effect is directed at the platelets or their hematopoietic precursors, our results may explain the seemingly paradoxical observation that boronated porphyrins, regardless of the chemical nature of their boron, are found to be almost universally associated with thrombocytopenia at the relatively high doses needed to obtain therapeutically useful levels of boron for BNCT. We suggest that the source of toxic effects lies not in the chemical nature of the boron, but rather in the interaction of the porphyrin moiety with some as yet unidentified target in the hematopoietic system. We are currently performing further studies to determine the precise mode of action.

Conclusions

The preparation and characterization of three closely related tetraphenylporphyrins bearing extremely stable and nontoxic *closo*-borane anions has allowed an investigation into their in vivo toxicities in rats. The three compounds have quite different acute toxicities, as measured both by their MTD and by the the dose-related pathologies they produce, which were characterized by moderate to severe acute hemorrhagic liver necrosis (amide and oxoester) and moderate to severe acute hemorrhage or congestion in the kidneys, heart, or lungs but not liver (thioester). The toxicity profiles in rats given the MTD, however, were quite different from each other, with the most acutely toxic derivative showing no evidence of significant changes in blood chemistry or toxicity indicators. In the other two compounds, early thrombocytopenia accompanied by failure to gain weight, as well as indicators of mild liver toxic effects, were found to resolve within one week. Because dose limiting thrombocytopenia has also been found in the only boronated porphyrin to reach human clinical trials, we compared the reported toxic profiles of all reported porphyrins, both boronated and not. We conclude that thrombocytopenia appears to be the result of some as yet unclear interaction of the porphyrin macrocycle with elements of the hematopoietic system, either at the level of the myeloid stem cells or directly on the platelets themselves. These findings sound a cautionary note for medicinal chemists preparing and evaluating new porphyrins and related compounds for application in human diseases and encourage the development of new ways to administer these compounds that avoid systemic exposure.

Acknowledgment. We gratefully acknowledge the financial support of this work by the National Institutes of Health, National Cancer Institute, in the form of Grant CA 82478 (S.B.K. and D.F.D., co-investigators). We also wish to thank Dr. Andrew Leavitt for helpful discussions regarding myeloid stem cells.

Supporting Information Available: A table listing all analytical data for determining degree of purity for all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Detty, M. R.; Gibson, S. L.; Wagner, S. J. Current clinical and preclinical photosensitizers for use in photodynamic therapy. *J. Med. Chem.* **2004**, *47*, 3897−3915.

(2) Kappas, A.; Drummond, G. S.; Henschke, C. I.; Valaes, T. Direct comparison of Sn-mesoporphyrin, an inhibitor of bilirubin production, and phototherapy in controlling hyperbilirubinemia in term and near-term newborns. *Pediatrics* **1995**, *95*, 468−474.

(3) Griffin, G. M.; Zhu, T.; Solonenko, M.; Del Piero, F.; Kapakin, A.; Busch, T. M.; Yodh, A.; Polin, G.; Bauer, T.; Fraker, D.; Hahn, S. M. Preclinical evaluation of motexafin lutetium-mediated intraperitoneal photodynamic therapy in a canine model. *Clin. Cancer Res.* **2001**, *7*, 374−381.
(4) Miura, M.; Micca, P. L.; Heinrichs, J. C.; Gabel, D.; Fairchild, R. G.; Slatkin, D. N. Biodistribution and toxicity of 2,4-divinyl-*nido*-*o*-carboranyldeuteroporphyrin IX in mice. *Biochem. Pharmacol.* **1992**, *43*, 467−476.
(5) Winkelman, J. W.; Collins, G. H. Neurotoxicity of tetraphenylporphinesulfonate $TPPS_4$ and its relation to photodynamic therapy. *Photochem. Photobiol.* **1987**, *46*, 801−807.
(6) Kahl, S. B.; Joel, D. D.; Nawrocky, M. M.; Micca, P. L.; Tran, K. P.; Finkel, G. C.; Slatkin, D. N. Uptake of a *nido*-carboranylporphyrin by human glioma xenografts in athymic nude mice and by syngeneic ovarian carcinomas in immunocompetent mice. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7265−7269.
(7) Tibbitts, J.; Fike, J. R.; Lamborn, K. R.; Bollen, A. W.; Kahl, S. B. Toxicology of a boronated porphyrin in dogs. *Photochem. Photobiol.* **1999**, *69*, 587−594.
(8) Rosenthal, M. A.; Kavar, B.; Hill, J. S.; Morgan, D. J.; Nation, R. L.; Stylli, S. S.; Basser, R.; Uren, S.; Geldard, H.; Green, M. D.; Kahl, S. B.; Kaye, A. H. Phase I and pharmacokinetic study of photodynamic therapy for high grade gliomas using a novel boronated porphyrin (BOPP). *J. Clin. Oncol.* **2001**, *19*, 519−524.
(9) Vicente, M. G. H.; Wickramasinghe, A.; Nurco, D. J.; Wang, H. J. H.; Nawrocky, M. M.; Makar, M. S.; Miura, M. Synthesis, toxicity and biodistribution of two 5,15-di[3,5-(*nido*-carboranylmethyl)phenyl] porphyrins in EMT-6 tumor bearing mice. *Bioorg. Med. Chem.* **2003**, *11*, 3101−3108.
(10) Miura, M.; Morris, G. M.; Micca, P. L.; Nawrocky, M. M.; Makar, M. S.; Cook, S. P.; Slatkin, D. N. Synthesis of copper octabromotetracarboranylphenylporphyrin for boron neutron capture therapy and its toxicity and biodistribution in tumor-bearing mice. *Br. J. Radiol.* **2004**, *77*, 573−580.
(11) Hertler, W. R.; Raasch, M. S. Chemistry of boranes. XIV. Amination of $B_{10}H_{10}^{2-}$ and $B_{12}H_{12}^{2-}$ with hydroxylamine-O-sulfonic acid. *J. Am. Chem. Soc.* **1964**, *86*, 3661−3663.
(12) Peymann, T.; Knobler, C. B.; Hawthorne, M. F. A study of the sequential acid-catalyzed hydroxylation of dodecahydro-*closo*-dodecaborate (2−). *Inorg. Chem.* **2000**, *39*, 1163−1170.
(13) Hoffman, S.; Justus, E.; Ratajski, M.; Lork, E.; Gabel, D. *J. Organomet. Chem.* **2005**, *690*, 2757−2760.
(14) Peymann, T.; Lork, E.; Gabel, D. Hydroxoundecahydro-*closo*-dodecaborate (2−) as a nucleophile. Preparation and structural characterization of O-alkyl and O-acyl derivatives of hydroxoundecahydro-*closo*-dodecaborate (2−). *Inorg. Chem.* **1996**, *35*, 1355−1360.
(15) Gabel, D.; Foster, S.; Fairchild, R. G. The Monte Carlo simulation of the biological effect of the $^{10}B(n,\alpha)^{7}Li$ reaction in cells and tissue and its implication for boron neutron capture therapy. *Radiat. Res.* **1987**, *111*, 14−25.
(16) Muetterties, E. L.; Balthis, J. H.; Chia, Y. T.; Knoth, W. H.; Miller, H. C. Chemistry of boranes. VIII. Salts and acids of $B_{10}H_{10}^{2-}$ and $B_{12}H_{12}^{2-}$. *Inorg. Chem.* **1964**, *3*, 444−451.
(17) Sweet, W. H.; Soloway, A. H.; Wright, R. I. Evaluation of boron compounds for use in neutron capture therapy of brain tumors. II. Studies in man. *J. Pharmacol. Exper. Therapy* **1962**, *137*, 263−266.
(18) Soloway, A. H.; Wright, R. L.; Messer, J. R. Evaluation of boron compounds for use in neutron capture therapy of brain tumors. I. Animal investigations. *J. Pharmacol. Exp. Ther.* **1961**, *134*, 117−122.
(19) Itoh, F.; Yabuuchi, K.; Ohno, K.; Ikeuchi, I.; Muraoka, Y. Single and two-week repeated intravenous dose toxicity studies of disodium mercaptoundecahydro-*closo*-dodecaborate in rats. *J. Toxicol. Sci.* **1998**, *23*, Suppl 4, 587−611.
(20) Horn, V.; Buchar, E.; Janku, I. Kidney function changes in rats after single-dose administration of borocaptate sodium. *Physiol. Res.* **1997**, *46*, 279−283.
(21) Janku, I.; Buchar, E.; Jiricka, Z. Nephrotoxicity of borocaptate after short-term administration in rabbits. *Toxicology* **1993**, *79*, 99−108.
(22) Haritz, D.; Gabel, D.; Huiskamp, R. Clinical phase-I study of $Na_2B_{12}H_{11}SH$ (BSH) in patients with malignant glioma as precondition for boron neutron capture therapy (BNCT). *Int. J. Radiat. Oncol., Biol., Phys.* **1994**, *28*, 1175−1181.
(23) Miura, M.; Micca, P. L.; Fisher, C. D.; Gordon, C. R.; Heinrichs, J. C.; Slatkin, D. N. Evaluation of carborane-containing porphyrins as tumor targeting agents for boron neutron capture therapy. *Br. J. Radiat.* **1998**, *71*, 773−781.
(24) Miura, M.; Micca, P. L.; Fisher, C. D.; Heinrichs, J. C.; Donaldson, J. A.; Finkel, G. C.; Slatkin, D. N. Synthesis of a nickel tetracarboranylphenylporphyrin for boron neutron capture therapy: biodistribution and toxicity in tumor-bearing mice. *Int. J. Cancer* **1996**, *68*, 114−119.
(25) Kahl, S. B. Unpublished results.
(26) Schaeck, J. S.; Kahl, S. B. Rapid cage degradation of 1-formyl- and 1-alkyloxycarbonyl-substituted 1,2-dicarba-closo-dodecaboranes by water or methanol in polar organic solvents. *Inorg. Chem.* **1999**, *38*, 204−206.
(27) Hunt, D. W. C.; Sorrenti, R. A.; Smits, C. B.; Levy, J. G. Photofrin, but not benzoporphyrin derivative, stimulates hematopoiesis in the mouse. *Immunopharmacology* **1993**, *26*, 203−212.
(28) Lutton, J. D.; Abraham, N. G.; Drummond, G. S.; Levere, R. D.; Kappas, A. Zinc porphyrins: Potent inhibitors of hematopoiesis in animal and human bone marrow. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 1432−1436.
(29) Lutton, J. D.; Jiang, S.; Drummond, G. S.; Abraham, N. G.; Kappas, A. Comparative pharmacology of zinc mesoporphyrin and tin mesoporphyrin: Toxic actions of zinc mesoporphyrin on hematopoiesis and progenitor cell mobilization. *Pharmacology* **1999**, *58*, 44−50.
(30) Buchler, J. W. Synthesis and Properties of Metalloporphyrins. In *The Porphyrins, Volume I: Structure and Synthesis, Part A*; Dolphin, D., Ed.; Academic Press: New York, NY, 1978; Chapter 10.
(31) Glueck, R.; Green, D.; Cohen, I.; Ts'ao, C. Hematin: Unique effects on hemostasis. *Blood* **1983**, *61*, 243−249.
(32) Neely, S. M.; Gardner, D. V.; Green, D.; Ts'ao, C. Effect of hematin on endothelial cells and endothelial cell-platelet interactions. *Am. J. Pathol.* **1984**, *115*, 390−396.
(33) Green, D.; Furby, F. H.; Berndt, M. C. The interaction of the factor VIII/von Willibrand factor complex with hematin. *Thromb. Hemostasis* **1986**, *56*, 277−282.
(34) Green, D.; Ts'ao, C. Hematin: Effects on hemostasis. *J. Lab. Clin. Med.* **1990**, *115*, 144−147.

JM060895B