Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 3101–3108

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis, Toxicity and Biodistribution of Two 5,15-Di[3,5-(*nido*-carboranylmethyl)phenyl]porphyrins in EMT-6 Tumor Bearing Mice

M. Graça H. Vicente,[a,*] Anura Wickramasinghe,[b] Daniel J. Nurco,[b] Hong J. H. Wang,[b] Marta M. Nawrocky,[c] Michael S. Makar[c] and Michiko Miura[c]

[a]*Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA*
[b]*Department of Chemistry, University of California, Davis, CA 95616, USA*
[c]*Medical Department, Brookhaven National Laboratory, Upton, NY 11973, USA*

Received 27 January 2003; revised 7 April 2003; accepted 8 April 2003

Abstract—The total synthesis of a 5,15-di[3,5-(*o*-carboranylmethyl)phenyl]porphyrin **5**, its zinc(II) complex **6**, and the corresponding *nido*-carboranylporphyrins **7** and **8** are reported. The molecular structures of porphyrin **6** and of potassium *nido*-carborane were obtained and are described. The biodistribution of *nido*-carboranylporphyrins **7** and **8** in BALB/c mice bearing EMT-6 mammary tumors are presented and compared. Both compounds are effective tumor localizers and delivered therapeutic concentrations of boron to tumors (mean ± standard deviation): 32.5 ± 7.1 and 54.3 ± 14 μg/g for **7** and **8**, respectively, 2 days after the last of 3 injections of a total boron dose of 23 mg/kg body weight. The zinc(II) complex **8** was found to deliver 1.2–1.7 times higher amounts of boron to tumors than **7**, with lower tumor-to-blood boron concentration ratios (9.8/1 and 4.7/1 for **7** and **8**, respectively, 2 days after injections). The tumor-to-brain boron concentration ratios were >100/1 for both porphyrins 2 days after administration. Both *nido*-carboranylporphyrins **7** and **8** were well-tolerated at the concentrations used (75 and 78 mg/kg body weight, respectively) and no morbidity or mortality were observed in these studies.

Introduction

The success of boron neutron capture therapy (BNCT),[1,2] an emerging binary modality for cancer treatment, depends mainly on the discovery of effective boron delivery drugs. Such compounds should deliver average ^{10}B concentrations of 15–30 μg/g to tumors with high selectivity (tumor-to-blood and tumor-to-normal tissues ratios ideally >5) and with low toxicity, in order to attain a high therapeutic ratio. In the last fifteen years several BNCT agents have been proposed, including boronated nucleosides, amino acids, peptides, phospholipids, liposomes, monoclonal antibodies, and porphyrins.[3] Among these, boron-containing porphyrins appear to be particularly promising boron carriers because of their known selectivity for tumor tissue, their long persistence within tumors, and their high chemical stability.[4]

BNCT has been mainly evaluated as a binary treatment for high-grade gliomas, metastatic brain tumors, and melanomas in the US, Europe and Japan.[5,6] This binary therapy relies on the unique ability of ^{10}B nuclides to capture low-energy neutrons and produce high linear energy transfer (LET) charged particles, $^{4}He^{2+}$ (α-particle) and $^{7}Li^{3+}$, that can cause irreversible damage to tumor cells. The high LET particles are short-lived and have limited ranges in tissue (approximately one cell diameter, 5–9 μm), so that cell toxicity is restricted to the localization area of the boron carrier within the irradiated treatment volume. Therefore, if a tumor selective boron-containing drug is found, effective localized destruction of malignant cells can be achieved in the presence of normal cells, using BNCT. Currently, the only two boron neutron capture agents in Phase I/II clinical trials are disodium mercapto-*closo*-dodecaborate (BSH)[7,8] and L-4-dihydroxyborylphenylalanine (BPA).[9,10] Although BSH and BPA have been shown to be safe and efficacious in animal models, both of these agents have only moderate selectivity for tumor cells and low retention times in tumors. Furthermore, BSH

*Corresponding author. Tel.: +1-225-578-7405; fax: +225-578-3458; e-mail: vicente@lsu.edu

0968-0896/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0968-0896(03)00240-2

has limited chemical stability due to its tendency toward air-oxidation,[11] and BPA, although chemically nontoxic contains only a low percent of boron by weight (5%) so that large amounts of this drug are needed in order to achieve therapeutic boron concentrations in tumor tissue.

Several boron-containing porphyrins with high boron content (>20% by weight) have been reported in the literature for application in BNCT, based on either protoporphyrin-IX or *meso*-tetraphenylporphyrin structures, and bearing ester, amide, ether or carbon–carbon bonds between the boron clusters and the porphyrin macrocycle.[4] Compared with BSH and BPA, boron-containing porphyrins typically show higher tumor-selectivity and longer retention times in tumors.[4,12,13] Herein we report the total synthesis of four 5,15-di[3,5-(carboranylmethyl)phenyl]porphyrins (**5**–**8**) and compare the biodistribution of two tetra-anionic *nido*-carboranylporphyrins in female BALB/c mice bearing EMT-6 tumors.[14] We also present the molecular structures of porphyrin **6** and of potassium *nido*-carborane, the charged amphiphilic boron cage that confers water solubility to porphyrins **7** and **8**.

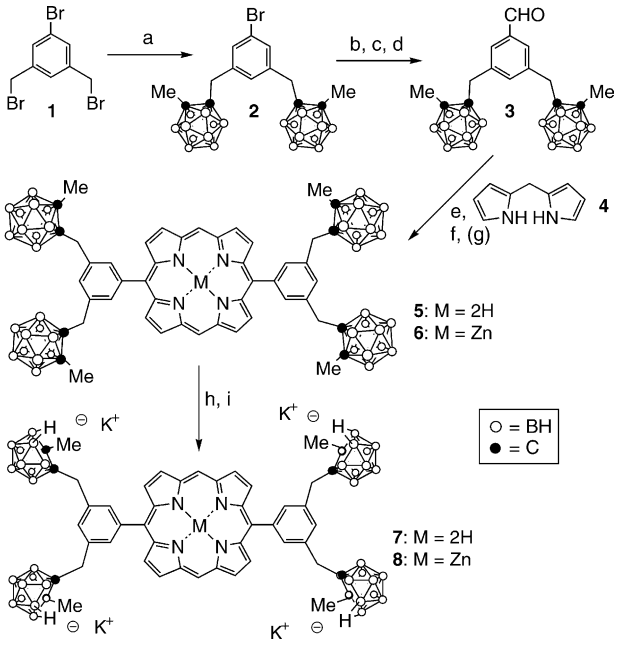
Results

Porphyrin syntheses

5,15-Di[3,5-(carboranylmethyl)phenyl]porphyrins **5** and **7** and their corresponding zinc(II) complexes **6** and **8** were synthesized from commercially available 3,5-dimethylbromobenzene, as indicated in Scheme 1.[15] Bromination of this compound with NBS gave **1**, which reacted with in situ prepared 1-lithium-2-methyl-*o*-carborane in the presence of lithium iodide as catalyst, to afford dicarboranylbromobenzene **2** in 63% yield. Reaction of **2** with *n*-butyllithium, followed by quenching of the resulting anion with dry DMF and acidic hydrolysis, yielded dicarboranylbenzaldehyde **3** in 71% overall yield. Condensation of aldehyde **3** with unsubstituted dipyrromethane **4** (obtained from the corresponding dipyrroketone)[16,17] afforded porphyrin **5** in 34% yield. Metalation of **5** with $ZnCl_2$ in CH_2Cl_2/THF 10/1 and pyridine, as previously described,[18,19] afforded the zinc(II) complex **6**. Porphyrins **5** and **6**, bearing four *closo*-carborane groups are completely insoluble in water. However, these porphyrins are easily converted into the corresponding amphiphilic water-soluble *nido*-carboranylporphyrins **7** and **8** by base degradation using pyridine/piperidine 1/3, followed by cation exchange using a Dowex 50WX2-100 resin in the potassium form.[18,19] The total percentage of boron by weight is 31% for porphyrin **7** and 29% for **8**. Potassium *nido*-carborane was obtained from *o*-carborane using a similar approach, by base degradation using pyridine/piperidine 1/3 followed by piperidinium/potassium exchange, in 60% recrystallized yield.

Molecular structures

Crystals of porphyrin **6**, suitable for X-ray analysis, were obtained from slow diffusion of cyclohexane into a solution of chlorobenzene containing a few drops of pyridine. The structure of **6**, illustrated in Figure 1, displays the four methylene porphyrin–carborane linkages. The porphyrin macrocycle was nearly planar but exhibited a very light mixture of wav(x) and wav(y) non-planar distortions.[20] The macrocyclic atom mean deviation from the 24-atom porphyrin least squares plane is 0.039 Å and the zinc ion resides above the porphyrin plane (by 0.434 Å). Zinc-nitrogen(porphyrin) bonds of 2.086(2), 2.103(2), 2.093(2), and 2.078(2) Å and a zinc-nitrogen(pyridine) bond of 2.148(4) Å were observed.



Scheme 1. (a) 1-lithium-2-methyl-*o*-carborane, THF, LiI, room temperature 16 h (63%); (b) *n*-BuLi, THF, −78 °C for 30 min; (c) DMF, −78 °C for 30 min then 0 °C for 1 h; (d) 5% aqueous HCl, room temperature for 15 min (71% from **2**); (e) **4**, TFA, CH_2Cl_2, room temperature overnight; (f) *p*-chloranil, room temperature for 6 h (34% from **3**); (g) $ZnCl_2$, CH_2Cl_2/THF (1/10), pyridine (89%); (h) pyridine/piperidine (3/1), 36 h; (i) Dowex 50WX2-100 in K^+ (93–95% from **5** or **6**).

The structure of potassium *nido*-carborane, shown in Figure 2, is the result of a high-resolution crystallographic dataset. A full diffraction sphere was collected at low temperature to $2\theta_{max} = 63°$ [λ(Mo Kα)]. The collected intensities were strong with 2336 of 2888 reflections observed ($R_{int} = 0.034$). Complete data were used

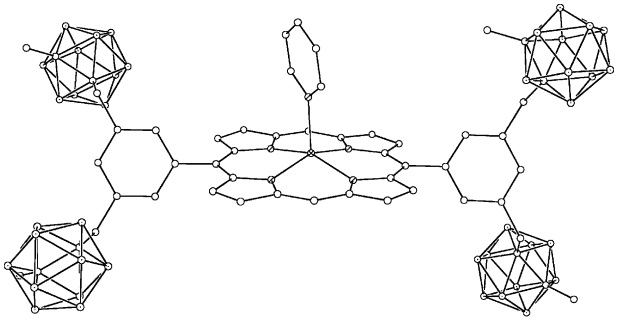


Figure 1. The molecular structure of Zn(II)-porphyrin **6** with pyridine as the axial ligand as determined by X-ray crystallography (hydrogen atoms not shown for clarity).

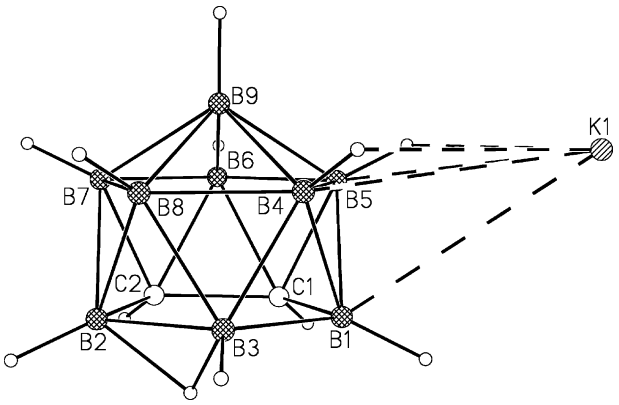


Figure 2. The molecular structure of potassium *nido*-carborane as determined by X-ray crystallography.

in the refinement without application of resolution limits and final *R* indices were lower than any *nido*-carborane entry in the Cambridge Structural Database.[21] The carbon–hydrogen and boron-hydrogen bond lengths observed in the crystal structure of potassium *nido*-carborane are given in Table 1.

Biodistribution study

Porphyrins **7** and **8** were administered to tumor-bearing mice by a series of three intraperitoneal (ip) injections over 8 h, for a total dose of 23 mg boron/kg body weight (bw), that is, 75 mg/kg bw of porphyrin **7** and 78 mg/kg bw of porphyrin **8**. Intraperitoneal injections were preferred over intravenous (iv) because of the technical difficulty associated with performing serial iv injections into the tail vein of mice; a single iv injection was also not feasible in our case due to total volume of the porphyrin solutions used, which exceeded 0.01 mL/g bw, the maximum allowable volume for an iv injection. At two time points (2 and 4 days after the last injection) for porphyrin **7** and at three time points (1, 2 and 4 days after the last injection) for porphyrin **8**, tissues were collected for boron and hematologic analyses. Table 2 presents the boron concentrations determined (DCP-AES) in several tissues, and the tumor-to-blood and tumor-to-brain boron ratios found at each time point for each porphyrin. The mean tumor boron concentration in mice given 78 mg/kg bw of porphyrin **8** is 43.7±9.2 μg/g at 1 day after the last injection, increases to 54.3±14.0 μg/g after 2 days, and decreases (by 0.6) to 34.3±2.8 μg/g at 4 days after the last injection (Table 2, Fig. 3). The tumor/blood boron ratios also increase from 3.1 to 4.7 between 1 and 2 days, and then decrease to 3.8 at 4 days after the last injection. In contrast, the mean boron concentrations found in mice given 75 mg/kg bw of porphyrin **7** are considerably lower than those for the zinc(II) complex **8** at the same time points. However, the tumor-to-blood boron ratios were significantly higher at 9.8/1 and 11.8/1 at 2 and 4 days after the last injection, respectively. The percentage tumor boron of the total injected dose per gram of tissue is 7% for porphyrin **7** and 11% for its zinc(II) derivative **8**. Both porphyrins **7** and **8** show high tumor/brain boron concentration ratios (57/1–136/1) at all time points studied, and in particular 2 days after injections (108/1 for **7** and 136/1 for **8**). The liver/tumor boron concentration ratios were found to decrease from 2 to 4 days after administration of porphyrin **7** (4.0/1 to 3.3/1), and from 1 to 2 or 4 days after administration of porphyrin **8** (4.4/1 to 3.1/1).

Table 1. Carbon-hydrogen and boron–hydrogen bond lengths observed in the crystal structure of potassium *nido*-carborane

Bond	Bond length
B1–H1	1.088(14)
B2–H2	1.079(14)
B2–(H2-3)	1.42(2)
B3–(H2-3)	1.13(2)
B3–H3	1.090(13)
C1–(H-C1)	0.963(14)
C2–(H-C2)	0.946(14)
B4–H4	1.08(2)
B5–H5	1.08(2)
B6–H6	1.096(14)
B7–H7	1.066(14)
B8–H8	1.097(14)
B9–H9	1.061(14)

Table 2. Boron concentrations (mean values and standard deviation, μg/g) in various tissues from mice given a dose of 23 mg boron/kg porphyrin **7** or **8** in 3 ip injections over 8 h, at either 1, 2 or 4 days after the last injection

Porphyrin	**7**	**7**	**8**	**8**	**8**
Time after last injection	2 days	4 days	1 day	2 days	4 days
Number of mice	5	5	6	11	5
EMT-6 Tumor	32.5±7.1	28.3±10.5	43.7±9.2	54.3±14.0	34.3±2.8
Blood	3.3±0.5	2.4±0.1	14.3±2.4	11.6±1.9	9.1±0.5
Brain	0.3±0	0.4±0.1	0.6±0.1	0.4±0.1	0.6±0
Skin (pinna)	2.1±0	2.8±0.3	2.3±0.5	3.9±1.1	5.1±0.5
Liver	130±23	93±29	191±19	167±25	108±18
Spleen	62.2±15.8	67.5±15.6	92.3±12.1	94.5±13.8	78.1±10.9
Kidney	25.3±3.0	26.7±3.9	37.2±7.6	41.3±5.8	38.9±4.9
Lungs	27.6±6.4	30.2±7.5	32.8±3.3	38.6±5.0	43.8±3.9
Tumor/blood	9.8	11.8	3.1	4.7	3.8
Tumor/brain	108	70	73	136	57

The weight changes and hematological parameters determined for tumor-bearing mice injected with porphyrin **7**, the zinc(II) complex **8** or with saline (control) are given in Table 3. Considerable weight loss is observed in mice given porphyrins **7** and **8**, compared with saline only, and the loss appears to be greater for porphyrin **8** than for **7**. Minor differences in leukocytes were found in mice given porphyrins **7** and **8** compared with saline, and the low platelets determined 2 days after injection of porphyrins **7** and **8** were found to increase 4 days after administration. No mice died from any of the injections.

Discussion

Porphyrins **7** and **8** were prepared in good yields from readily accessible starting materials, as described in Scheme 1. The *closo*-carboranylporphyrins **5** and **6** are completely insoluble in water, therefore a study of their

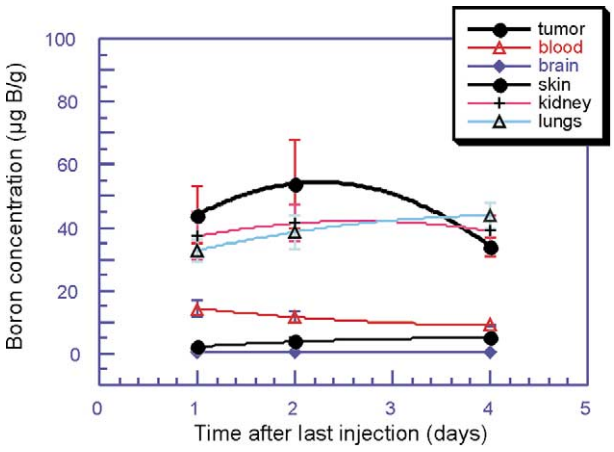


Figure 3. Boron concentrations of various tissues at 1, 2, and 4 days after the last of three ip injections of porphyrin **8** into BALB/c mice bearing EMT-6 tumors. The total dose of porphyrin **8** was 78 mg/kg body weight (23 mg boron/kg body weight).

Table 3. Weight changes and hematologic parameters of blood from mice given 78 or 75 mg/kg body weight of porphyrins **7** or **8**, respectively, or saline. Values are reported as mean±SD

Injection	Time (days)	No. mice	% Wt difference	Leukocytes	Erythrocytes	Platelets
7	2	5	−7.5±4.0	9.9±2.4	7.7±0.2	144±60
8	2	5	−8.5±4.0	7.5±1.5	9.2±0.2	142±72
Saline	2	4	3.2±2.7	6.2±1.7	8.5±0.1	510±283
7	4	6	−6.2±6.2	9.8±3.0	8.4±0.4	396±61
8	4	6	−8.0±5.8	8.9±1.3	8.8±0.2	220±64
Saline	4	5	1.2±4.1	7.0±1.0	8.9±0.3	871±157

biodistribution in animals would require an excipient such as Cremophor EL, Tween 80, or liposomes, which would likely alter the tumor uptake and biological activity of these drugs.[22] On the other hand, the *nido*-analogues **7** and **8** display good solubility in water while retaining amphiphilic character, as previously reported,[18,19] therefore allowing such compounds to be studied in vivo without the use of excipients. The negatively charged, amphiphilic *nido*-carborane cage is easily prepared and displays the same characteristic high photochemical and kinetic stabilities as the parent *o*-carborane.

The molecular structure of the zinc(II) complex **6** is shown in Figure 1, the third crystallographic entry of a covalently tethered Zn(II)-carboranylporphyrin molecule available in the literature.[21] The first two such investigations, also originating from our laboratory, involved a pseudo-hexacoordinated Zn(II)-tetra[(*o*-carboranylmethyl)phenyl]porphyrin[23] and a Zn(II)-tetra(*o*-carboranylphenyl)porphyrin.[19] In all these structures the zinc(II) ions share in common the presence of axially coordinating species. The bond lengths and angles observed in the coordination sphere of the zinc ion in **6** are typical of penta-coordinated Zn(II) porphyrin crystal structures.[21] The porphyrin macrocyclic conformation observed in **6**, a combination of wav(x) and wav(y) non-planar distortions,[20] are also characteristic of Zn(II) porphyrin structures.[21] While the positioning of the bridging B–H–B system in potassium *nido*-caborane has already been demonstrated by previous crystallographic investigations[21] it is notable that all hydrogen atom positions in its molecular structure (Fig. 2) were easily found on difference maps and refined freely without constraints. The observed B–H–B distances were 1.42(2) and 1.13(2) Å. This motif is similar to most *nido*-carborane structures published to date, which feature one long and one short bridging B–H bond differing by 0.2–0.3 Å.[21]

The amount of boron necessary for effective BNCT has been estimated to be 15–30 μg boron/g tumor, depending upon its cellular micro-localization properties.[24,25] If the boron is homogeneously distributed in tumor cells, about 30 μg/g is required for effective BNCT, while a lower amount is necessary if the boron localizes near or within the nucleus. Therapeutic boron concentrations were achieved in EMT-6 tumors using porphyrin **7** and its Zn(II) complex **8**, 2 days after ip injections, despite the relatively low dose of boron administered (23 mg/kg) in both cases (Table 2, Fig. 3). After 4 days, the amount of boron in the tumors decreased, although in the case of porphyrin **8** enough boron was still present in the tumor for effective BNCT (and possibly also for porphyrin **7**). These results are in agreement with those reported in the literature using the same mouse tumor model and related carboranylporphyrins.[26,27] In this study the zinc(II) complex **8** delivered greater amounts of boron to tumors than did its free base analogue **7** (1.7 times higher after 2 days and 1.2 times higher after 4 days). On the other hand, higher tumor-to-blood boron concentration ratios were achieved with porphyrin **7** than with complex **8** (2 and 3 times higher at 2 and 4 days after administration, respectively). This suggests that porphyrin **7** is more rapidly cleared from blood than **8**, which might be related to the greater hydrophilic character of the zinc(II) complex **8** (results not shown).[4] The tumor-to-blood boron ratios observed for each porphyrin did not change significantly over the time period the studies were conducted. Very high tumor-to-brain boron ratios were achieved with both porphyrins at all time points, particular at 2 days after administration (108/1 for porphyrin **7** and 136/1 for the complex **8**). Furthermore, the boron concentrations found in tumor are substantially greater than in skin, which suggests that these carboranylporphyrins, and in particular the metal-free, may not effect substantial skin photosensitivity although this parameter was not investigated in this study. Nevertheless, insertion of a copper(II) in place of zinc(II) could further prevent the risk of skin photosensitivity; previous studies indicate that divalent metals such as zinc, copper and nickel can be interchanged without significant alterations in the toxicity and tumor selectivity of carboranylporphyrins.[28] Only in liver and spleen were boron concentrations higher than in tumor (3.1–4.4 times higher in liver and 1.7–2.4 times higher in spleen), while similar or somewhat lower amounts were found in kidneys and lungs. This indicates that both porphyrins **7** and **8** are effective tumor localizers, even in the absence of a lipid-based vehicle, such as Cremophor EL. Furthermore, our results contrast with the greater liver uptake reported for significantly more hydrophobic *closo*-carboranylporphyrins.[27] Structurally

related tetra(*nido*-carboranyl)porphyrins, NiNTCP-H[27] and BTPP,[29] have also been found to deliver therapeutic concentrations of boron to tumors, but at higher porphyrin doses (>93 mg/kg bw) and with significantly greater toxicity. A similar dose of the *o*-carboranylporphyrin BOPP (~22 mg/kg bw) administered via serial ip injections was found to deliver significantly lower amounts of boron (16 and 19 μg/g at 18 and 48 h after injections, respectively) to Harding–Passey melanomas in mice, with lower tumor-to-blood (1.1/1 and 1.7/1 at 18 and 48 h after injections, respectively) and lower tumor-to-brain (16/1 and 5/1 at 18 and 48 h after injections, respectively) boron concentration ratios.[30]

Some of the mice given three porphyrin ip injections experienced significant weight loss, in comparison with mice receiving saline only using the same protocol, and the average weight loss was more pronounced in the case of porphyrin **8** (Table 3). However, these changes in weight did not affect their normal behavior. Minor differences were also determined in leukocytes but these changes were not of consequence. On the other hand, the conceivably more serious thrombocytopenia (low platelets) at the 2 day time point, seems to be transient as the platelets appear to be rebounding 4 days after the injections. These results indicate that at the doses given, and at the time points observed the *nido*-carboranylporphyrins appear to be relatively well-tolerated and no morbidity nor mortality were observed. It would be of interest to observe the animals for a longer period after the injections as full recovery is very probable at a later time point as the higher platelet count and weight gain on day 4 indicates. It should be noted that some level of toxicity is usually expected in a clinical setting, particularly in the treatment of deadly illnesses such as glioblastoma multiforme. Toxicities are acceptable as long as they are tolerable and the benefits from treatment outweigh the risk of side effects. Our results indicate that the toxicities elicited from the *nido*-carboranylporphyrins described herein, in contrast to previous reports, are most likely tolerable. Furthermore, these compounds can deliver significantly higher boron concentrations to tumor than previously tested porphyrins of this class, suggesting that porphyrins **7** and **8** would be considerably more efficacious as boron carriers for BNCT.

Conclusions

Amphiphilic *nido*-carboranylporphyrins, such as **7** and **8**, are easily prepared from the corresponding highly hydrophobic *closo*-carboranylporphyrins and can deliver therapeutic concentrations of boron to tumor tissue with relatively low toxicity. *nido*-Carboranylporphyrins **7** and **8** appear to be among the least toxic boron-containing porphyrins reported to date. High tumor/blood, tumor/brain and tumor/skin boron concentration ratios were found for both porphyrins. The zinc(II) porphyrin **8** delivers slightly higher amounts of boron to tumors than the metal-free **7**, but with considerably lower tumor-to-blood boron concentration ratios. Both porphyrins also showed relatively low liver/tumor and spleen/tumor boron ratios. Our studies reported herein indicate that both *nido*-carboranylporphyrins **7** and **8** are promising new boron delivery agents for BNCT. The molecular structure of porphyrin **6** indicates that the presence of the *closo*-carborane cages at the periphery of the porphyrin macrocycle does not significantly influence the macrocycle conformation, nor the bond lengths and bond angles of pentacoordinated Zn(II) porphyrin systems.

Experimental

Melting points were measured on a Thomas/Bristoline microscopic hot stage apparatus and were uncorrected. Silica gel 60 (70–230 and 230–400 mesh, Merck) or neutral alumina (Merck; usually Brockmann Grade III) were used for column chromatography. Analytical thin layer chromatography (TLC) was performed using Merck 60 F254 silica gel (precoated sheets, 0.2 mm thick). Reactions were monitored by TLC and spectrophotometry. ^{1}H NMR spectra were obtained in deuterochloroform, acetone-d_6 or D_2O solution, using a Brucker Inova 400 MHz spectrometer; chemical shifts are expressed in ppm relative to chloroform (7.26 ppm) and/or TMS (0 ppm). Unless otherwise stated, electronic absorption spectra were measured in dichloromethane solution using a Hewlett-Packard 8450A spectrophotometer. Mass spectra were obtained at the Mass Spectrometry Facility, University of California, San Francisco, CA. 3,5-Dimethylbromobenzene, NBS, benzoyl peroxide, LiI, *p*-chloranil and Dowex 50WX2-100 resin were purchased (Aldrich) and used without further purification. Trifluoroacetic acid (TFA) and *n*-butyllithium (1.6 M in hexanes) were purchased from Fluka, and 1-methyl-*o*-carborane from Dexsil Corporation (Hamden, CT) and used without further purification. All solvents were dried and purified according to literature procedures.[31]

Alpha-MEM and FBS were obtained from Gibco BRL Products (Grand Island, NY) and Triton X-100 from Aldrich. An ARL/Fisons Model SS-7 direct current plasma-atomic emission spectroscopy (DCP-AES) was used for analytical boron determinations and a VetScan Hmt Hematology Analyzer (Abaxis, Sunnyvale, CA) was used for the hematologic analyses.

The female BALB/c mice were obtained from Taconic Farms (Germantown, NY).

Bis-(3,5-bromomethyl)bromobenzene (1). To a refluxing solution of 3,5-dimethylbromobenzene (4.63 g, 25.0 mmol) in dry CCl_4 (300 mL) under Argon, were added NBS (9.79 g, 55.0 mmol) and benzoyl peroxide (0.80 g, 3.30 mmol) in portions, over a 1-h period. The final reaction mixture was refluxed with stirring and under Argon for 16 h. After cooling to room temperature, the reaction mixture was filtered and the filtrate washed once with an aqueous saturated solution of $NaHCO_3$ and once with water. The organic solution was dried over anhydrous Na_2SO_4 and the solvent evaporated under vacuum. The resulting residue was purified by column chromatography using dichloromethane/petroleum

ether 1/9 for elution, and the main product collected and recrystallized from *n*-hexane, yielding 2.83 g (33% yield) of this literature compound. MS (EI) *m*/*z* 342.8. ^{1}H NMR ($CDCl_3$) δ ppm: 4.40 (s, 2H, CH2), 7.34 (s, 1H), 7.47 (s, 2H).

Bis[3,5-(1-methyl-*o*-carboranyl)methyl]bromobenzene (2). *n*-Butyllithium (5.2 mL, 1.6 M in hexane) was added dropwise to a solution of 1-methyl-*o*-carborane (1.39 g, 8.80 mmol) in dry THF (80 mL), at a temperature between −5 and 0 °C, under Argon. The mixture was stirred at this temperature range for 1.5 h, then cooled to −15−−20 °C (ice/salt bath). A solution of LiI (0.166 g, 1.27 mmol) in dry THF (15 mL) and compound **1** (1.372 g, 4.00 mmol) in THF (20 mL) was added and the final reaction mixture allowed to warm up to room temperature and stirred for 16 h. After quenching the reaction with water the resulting mixture was extracted 3 times with diethyl ether. The organic extracts were washed once with water, once with brine, dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The crude product was purified by column chromatography using dichloromethane/petroleum ether 1/4 for elution, and the main product collected and recrystallized from *n*-hexane to give 1.26 g (63% yield) of the title compound. MS (EI) *m*/*z* 497.3; ^{1}H NMR ($CDCl_3$) δ ppm: 1.4–3.0 (br, 20H, BH), 2.17 (s, 6H, CH_3), 3.43 (s, 4H, CH_2), 6.96 (s, 1H), 7.31 (s, 2H).

Bis[3,5-(1-methyl-*o*-carboranyl)methyl]benzaldehyde (3). A solution of compound **2** (0.994 g, 2.00 mmol) in THF (20 mL) under Argon was cooled to −78 °C. *n*-Butyllithium (1.4 mL, 1.6 M in hexane) was added dropwise via syringe. After stirring the reaction mixture for 30 min at −78 °C, dry DMF (0.77 mL, 10.0 mmol) was slowly added. The resulting yellow mixture was stirred at −78 °C for 30 min and then warmed to 0 °C and stirred at this temperature for 1 h. A 5% aqueous HCl solution was added until the pH of the reaction mixture was between 2 and 3, and the final mixture stirred at room temperature for 15 min. The aqueous layer was extracted 4 times with diethyl ether, the organic fraction dried over anhydrous $MgSO_4$ and the solvent evaporated under vacuum. Purification by column chromatography (dichloromethane/petroleum ether 2/3), afforded the title compound (0.632 g) in 71% yield. MS (EI) *m*/*z* 446.4; ^{1}H NMR ($CDCl_3$) δ ppm: 1.5–3.0 (br, 20H, BH), 2.20 (s, 6H, CH_3), 3.55 (s, 4H, CH_2), 7.30 (d, 1H, J = 1.6 Hz), 7.67 (d, 2H, J = 1.6 Hz), 10. 03 (s, 1H, CHO).

5,15-Bis[bis-3,5-(1-methyl-*o*-carboranyl)methylphenyl]-porphyrin (5). A solution of aldehyde **3** (0.446 g, 1.00 mmol) and dipyrromethane **4** (0.146 g, 1.00 mmol) in dry dichloromethane (100 mL) was purged with Argon for 15 min and cooled down to 0 °C. TFA (0.05 mL, 0.629 mmol) was added to the solution and the final mixture was stirred at 0 °C for 2 h and then at room temperature overnight. After oxidation with *p*-chloranil (0.277 g, 1.13 mmol) for 6 h at room temperature, the final reaction mixture was washed once with an aqueous saturated solution of $NaHCO_3$, once with water, and once with brine, before being dried over anhydrous $MgSO_4$. The residue obtained after removal of the solvent was purified by column chromatography (alumina) using dichloromethane for elution. The porphyrin fraction obtained was recrystallized from acetone to give 34% yield (0.192 g) of the title compound, mp > 300 °C; MS (MALDI) *m*/*z* 1144.0. ^{1}H NMR (d-TFA/$CDCl_3$) δ ppm: −1.92 (br, NH), 1.4–3.2 (br, 40H, BH), 2.30 (s, 12H, CH_3), 3.89 (s, 8H, CH_2), 7.71 (s, 2H), 8.34 (s, 4H), 9.03 (d, 4H, β-H, J = 4.5 Hz), 9.61 (d, 4H, β-H, J = 4.5 Hz), 10.98 (s, 2H, *meso*-H). UV–Vis ($CHCl_3$) λ_{max}: 406 nm (ε 398,800), 499 (17,700), 534 (5400), 572 (5700), 627 (1600).

Zn(II)-5,15-bis[bis-3,5-(1-methyl-*o*-carboranyl)methylphenyl]porphyrin (6). To a solution of porphyrin **5** (0.065 g, 0.057 mmol) in dichloromethane (100 mL) and THF (10 mL), was added $ZnCl_2.2H_2O$ (0.031 g, 0.180 mmol), and the final mixture was stirred at room temperature under Argon overnight. The mixture was then washed once with water, dried over anhydrous Na_2SO_4, and the solvent evaporated under vacuum. The residue was purified by column chromatography (dichloromethane/cyclohexane 2/1), the pink color fraction collected and recrystallized from dichloromethane/methanol, to give 0.061 g (89% yield) of the title compound, mp > 300 °C; MS (MALDI) *m*/*z* 1206.6. ^{1}H NMR ($CDCl_3$) δ ppm: 1.4–3.0 (br, 40H, BH), 2.11 (s, 12H, CH_3), 3.64 (s, 8H, CH_2), 7.33 (s, 2H, ArH), 7.98 (s, 4H, ArH), 8.98 (d, 4H, β-H, J = 4.5 Hz), 9.41 (d, 4H, β-H, J = 4.5 Hz), 10.25 (s, 2H, *meso*-H). UV–Vis (CH_2Cl_2) λ_{max}: 406 nm (ε 389,600), 534 (17,700), 567 (3100).

5,15-Bis[bis-3,5-(1-methyl-*nido*-carboranyl)methylphenyl]porphyrin tetrapotassium salt (7). Porphyrin **5** (0.100 g, 0.087 mmol) was dissolved in a 3/1 mixture of pyridine and piperidine (4.0 mL), and stirred at room temperature in the dark for 36 h, under Argon. The solvent was completely removed under vacuum, the residue redissolved in a 60% acetone aqueous solution and passed slowly through a Dowex 50WX2-100 resin in the potassium form. The porphyrin fraction was collected, dried under vacuum, redissolved in a 30% acetone aqueous solution and again passed through the ion-exchange resin. After removal of the solvent under vacuum, the tetraanionic porphyrin was recrystallized from methanol/diethyl ether, yielding 0.102 g (92.8% yield) of the title compound, mp > 300 °C. MS (MALDI) *m*/*z* 1256.3. ^{1}H NMR (CD_3COCD_3) δ ppm: −2.84 (s, 2H, NH), −2.45−−1.85 (br, 4H, BH), 0.9–2.4 (br, 32H, BH), 1.66 (s, 12H, CH_3), 3.52 (s, 8H, CH_2), 7.67 (s, 1H, ArH), 7.74 (s, 1H, ArH), 8.37 (s, 4H, ArH), 9.53 (dd, 4H, β-H), 9.61 (dd, 4H, β-H), 10.58 (s, 2H, *meso*-H). UV–Vis (acetone) λ_{max}: 406 nm (ε 312,600), 502 (13,400), 536 (7800), 576 (6100), 630 (3100). Anal. calcd for $C_{48}H_{78}N_4B_{36}K_4$: C, 45.87; H, 6.26; N, 4.46. Found: C, 45.62; H, 6.13; N, 4.23.

Zn(II)-5,15-bis[bis-3,5-(1-methyl-*nido*-carboranyl)methylphenyl]porphyrin tetrapotassium salt (8). The Zn(II) complex **10** (0.050 g, 0.0414 mmol) was dissolved in a 3/1 mixture of pyridine and piperidine (4.0 mL), and stirred at room temperature in the dark for 36 h, under

Argon. The solvent was completely removed under vacuum, the residue re-dissolved in a 60% acetone aqueous solution and passed slowly through a Dowex 50WX2-100 resin in the potassium form. The porphyrin fraction was collected, dried under vacuum, re-dissolved in a 30% acetone aqueous solution and again passed through the ion-exchange resin. After removal of the solvent under vacuum, the tetraanionic porphyrin was recrystallized from methanol/diethyl ether, yielding 0.052 g (95% yield) of the title compound, mp > 300 °C. MS (MALDI) *m/z* 1319.4. ^{1}H NMR (CD_3COCD_3) δ ppm: −2.45–−1.85 (br, 4H, BH), 0.9–2.4 (br, 32H, BH), 1.65 (s, 12H, CH_3), 3.50 (s, 8H, CH_2), 7.66 (s, 1H, ArH), 7.73 (s, 1H, ArH), 8.28 (s, 4H, ArH), 9.41 (dd, 4H, β-H), 9.47 (dd, 4H, β-H), 10.33 (s, 2H, *meso*-H). UV–Vis (acetone) λ_{max}: 412 nm (ε 263,100), 496 (1400), 542 (9900), 580 (1100).

Potassium *nido-o*-carborane. *o*-Carborane (5 g, 34.7 mmol) was dissolved in a 3/1 mixture of pyridine and piperidine (20 mL) and stirred at room temperature under argon for 36 h. The solvent was removed under vacuum, the residue dissolved in a 60% acetone aqueous solution and passed through a Dowex 50WX2-100 resin in the potassium form. The solvent was again removed under vacuum, the resulting residue dissolved in a 30% acetone aqueous solution and again passed through the ion-exchange resin. After removal of the solvent under vacuum, recrystallization from hot dichloromethane afforded 3.59 g (60% yield) of potassium *nido*-carborane. MS (MALDI) *m/z* 174.3. ^{1}H NMR (D_2O) δ ppm: −3.40–−2.50 (br, 1H, BH), 0.8–2.5 (br, 9H, BH).

Molecular structures

For Zn(II)-porphyrin 6. Crystals were grown by slow diffusion of cyclohexane into a chlorobenzene solution of porphyrin **6** containing a few drops of pyridine. The selected crystal (0.44×0.40×0.18 mm) had a triclinic unit cell, space group *P-1,* with cell dimensions $a = 12.8951(12)$, $b = 15.1234(13)$, $c = 23.074(2)$ Å, $\alpha = 84.766(4)$, $\beta = 82.752(3)$, $\gamma = 88.742(6)°$, $V = 4444.9(7)$ Å^3, and $Z = 2$. Data were collected at 91(2) K on a Bruker SMART 1000 diffractometer with a graphite monochromated sealed tube source [λ(Mo Kα) = 0.71073 Å]. A total of 61,164 were collected of which 27,130 were unique and of those 18,266 were observed ($I > 2\sigma$) [$2\theta = 63°$, $R_{int} = 0.028$, $T_{min} = 0.85$, $T_{max} = 0.94$, $\sigma_{calc} = 1.151$ g cm^{-3}, $\mu = 0.379$ mm^{-1}]. The structure was solved by the Patterson method and refined (based on F^2 using all data) by full matrix least-squares methods with 1244 parameters (Bruker SHELXS-97, SHELXL-97). All hydrogen atom positions were refined with a riding model. Final *R* factors were $R1 = 0.089$ (observed data) and $wR2 = 0.319$ (all data). Additional experimental details and crystallographic data (excluding structure factor tables) can be found in supplementary publication numbers CCDC-195290 available from the Cambridge Crystallographic Data Center (CCDC). Copies of the data can be obtained free of charge upon request to CCDC, 12 Union Road, Cambridge CB21EZ, UK [Fax: (+44) 1223-336-033; E-mail: deposit@ccdc.cam.ac.uk].

For potassium *nido*-caborane

A single large crystal was grown by slow evaporation of a saturated solution of the title compound in neat CH_2Cl_2. The selected crystal (0.50×0.10×0.08 mm) had a monoclinic unit cell, space group $P2_1/n$, with cell dimensions $a = 7.2906(6)$, $b = 12.4670(11)$, $c = 9.9743(9)$ Å, $\beta = 90.953(2)°$, $V = 906.46(14)$ Å^3, and $Z = 4$. Data were collected at 91(2) K on a Bruker SMART 1000 diffractometer with a graphite monochromated sealed tube source [λ(Mo Kα) = 0.71073 Å]. A total of 12563 were collected of which 2888 were unique and of those 2336 were observed ($I > 2\sigma$) [$2\theta_{max} = 63°$, $R_{int} = 0.034$, $T_{min} = 0.79$, $T_{max} = 0.96$, $\sigma_{calc} = 1.264$ g cm^{-3}, $\mu = 0.503$ mm^{-1}]. An empirical absorption correction was applied to the data [SADABS 2.0 (Sheldrick, 2000)]. The structure was solved by direct methods and refined (based on F^2 using all data) by full matrix least-squares methods with 157 parameters (Bruker SHELXS-97, SHELXL-97). All hydrogen atom positions were found on difference maps and refined freely. Final *R* factors were $R1 = 0.028$ (observed data) and $wR2 = 0.073$ (all data). Additional experimental details and crystallographic data (excluding structure factor tables) can be found in supplementary publication number CCDC-195291 available from the CCDC.

Mouse tumor model

All procedures using animals were previously approved by the Brookhaven National Laboratory Institutional Animal Care and Use Committee. Female BALB/c mice weighing 20–25 g were implanted subcutaneously (sc) with EMT-6 tumors.[14] The tumors were implanted as single-cell suspensions of 2.5×10^5 cells in 0.05–0.10 mL using a 27-gauge needle in the same region. EMT-6 tumor cells were grown in vivo and in vitro in succession. Single-cell suspensions were prepared from mouse-grown tumors by trypsinization, expanded in alpha-minimum essential media with 10% FBS for several passages, then frozen in 10% DMSO in liquid nitrogen for storage. Prior to implantation in mice, aliquots of cells were thawed and grown in tissue culture.

Biodistribution study

Porphyrins **7** and **8** were dissolved in water at a concentration of 2.6 mg/mL. Tumor-bearing mice were given the porphyrins by serial ip injections (3 over 8 h) in a volume of ~0.01 mL/gbw/injection. Mice were euthanized 1, 2, or 4 days after the last injection of **8** and at 2 or 4 days after the last injection of **7**. Total doses given were 75 mg/kg bw of porphyrin **7** and 78 mg/kg bw of porphyrin **8**, both of which are equivalent to 23 mg of boron/kg. Under deep halothane inhalation anesthesia leading to euthanasia, right ventricular blood (0.2–0.7 mL total) was removed for hematologic and boron analyses. Tumor, brain, pinna (skin), kidneys, lungs, spleen, and liver were sampled at necropsy for boron analyses.

Boron analyses

Direct current plasma-atomic emission spectroscopy (DCP-AES)[32,33] was used (detection limit: 0.1 μg B/

mL). Samples (50–130 mg) were digested at 60 °C with sulfuric acid/nitric acid (1/1). Triton X-100 and water were added to give final concentrations of ~50 mg tissue/mL, 15% total acid v/v and 5% Triton X-100 v/v. The boron concentrations are based on a reference standard that was analyzed at the Massachusetts Institute of Technology Reactor Prompt Gamma Neutron Activation Facility.[34]

Hematologic analyses of blood

Hematologic assays are carried out at BNL using a VetScan Hmt Hematology Analyzer. Mice were weighed daily and necropsy was carried out. Any physical or behavioral differences in mice given porphyrin from those given saline and any changes in their tissues were noted.

Acknowledgements

The authors wish to thank Dr. Kent Riley for prompt gamma boron analysis of porphyrin **8** and Professor Kevin M. Smith for his support. The research described herein was supported by DOE (MGHV, grant No. 98ER62633), NIH (HL-22252), and by Contract No. DE-AC02-98CH10886 (MM).

References and Notes

1. Hawthorne, M. F. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 950.
2. Barth, R. F.; Soloway, A. H.; Goodman, J. H.; Gahbauer, R. A.; Gupta, N.; Blue, T. E.; Yang, W.; Tjarks, W. *Neurosurg.* **1999**, *44*, 433.
3. Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F. G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. *Chem. Rev.* **1998**, *98*, 1515.
4. Vicente, M. G. H. *Current Medicinal Chemistry, Anti-Cancer Agents* **2001**, *1*, 175.
5. Soloway, A. H.; Barth, R. F.; Gahbauer, R. A.; Blue, T. E.; Goodman, J. H. *J. Neurooncol.* **1997**, *33*, 9.
6. Diaz, A. Z.; Coderre, J. A.; Chanana, A. D.; Ma, R. M. *Ann. Med.* **2000**, *32*, 81.
7. Kageji, T.; Nakagawa, Y.; Kitamura, K.; Matsumoto, K.; Hatanaka, H. *J. Neurooncol.* **1997**, *33*, 117.
8. Gabel, D.; Preusse, D.; Haritz, D.; Grochulla, F.; Haselsberger, K.; Fankhauser, H.; Ceberg, C.; Peters, H.-D.; Klotz, U. *Acta Neurochir.* **1997**, *139*, 606.
9. Chanana, A. D.; Capala, J.; Chadha, M.; Coderre, J. A.; Diaz, A. Z.; Elowitz, E. H.; Iwai, J.; Joel, D. D.; Liu, H. B.; Ma, R.; Pendzick, N.; Peress, N. S.; Shady, M. S.; Slatkin, D. N.; Tyson, G. W.; Wielopolski, L. *Neurosurg.* **1999**, *44*, 1182.
10. Pignol, J.-P.; Oudart, H.; Chauvel, P.; Sauerwein, W.; Gabel, D.; Prevot, G. *Br. J. Radiol.* **1998**, *71*, 320.
11. Tolpin, E. I.; Wellum, G. R.; Berley, S. A. *Inorg. Chem.* **1978**, *17*, 2867.
12. Miura, M.; Morris, G. M.; Micca, P. L.; Lombardo, D. T.; Youngs, K. M.; Kalef-Ezra, J. A.; Hoch, D. A.; Slatkin, D. N.; Ma, R.; Coderre, J. A. *Radiation Res.* **2001**, *155*, 603.
13. Ceberg, C. P.; Brun, A.; Kahl, S. B.; Koo, M. S.; Persson, B. R. R.; Salford, L. G. *J. Neurosurg.* **1995**, *83*, 86.
14. Rockwell, S. C.; Kallman, R. F.; Fajardo, L. F. *J. Natl. Cancer Inst.* **1972**, *49*, 735.
15. Vicente, M. G. H.; Shetty, S.; Wickramasinghe, A.; Smith, K. M. *Tetrahedron Lett.* **2000**, *41*, 7623.
16. Chong, R.; Clezy, P. S.; Liepa, A. J.; Nichol, A. W. *Aust. J. Chem.* **1969**, *22*, 229.
17. Clezy, P. S.; Smythe, G. A. *Aust. J. Chem.* **1969**, *22*, 239.
18. Vicente, M. G. H.; Edwards, B. F.; Shetty, S. J.; Hou, Y.; Boggan, J. E. *Bioorg. Med. Chem.* **2002**, *10*, 481.
19. Vicente, M. G. H.; Nurco, D. J.; Shetty, S. J.; Osterloh, J.; Ventre, E.; Hegde, V.; Deutsch, W. A. *J. Photochem. Photobiol. B:Biol.* **2002**, *68*, 123.
20. Jentzen, W.; Song, X.-Z.; Shelnutt, J. A. *J. Phys. Chem.* **1997**, *B101*, 1684.
21. 3-D Search and Research Using the Cambridge Structural Database (April 2001 release). Allen, F.H.; Kennard, O. *Chemical Design Automation News*, **1993,** 8, 1, 31-37.
22. Osterloh, J.; Vicente, M. G. H. *J. Porphyrins and Phthalocyanines* **2002**, *6*, 305.
23. Vicente, M. G. H.; Nurco, D. J.; Shetty, S. J.; Medforth, C. J.; Smith, K. M. *Chem. Commun.* **2001**, 483.
24. Fairchild, R. G.; Bond, V. P. *Int. J. Rad. Oncol. Biol. Phys.* **1985**, *11*, 831.
25. Gabel, D.; Foster, S.; Fairchild, R. G. *Radiat. Res.* **1987**, *111*, 14.
26. Miura, M.; Micca, P. L.; Fisher, C. D.; Heinrichs, J. C.; Donaldson, J. A.; Finkel, G. C.; Slatkin, D. N. *Int. J. Cancer* **1996**, *68*, 114.
27. Miura, M.; Micca, P. L.; Fisher, C. D.; Gordon, C. R.; Heinrichs, J. C.; Slatkin, D. N. *Br. J. Radiol.* **1998**, *71*, 773.
28. M. Miura, unpublished results.
29. Kahl, S. B.; Joel, D. D.; Nawrocky, M. M.; Micca, P. L.; Tran, K. P.; Finkel, G. C.; Slatkin, D. N. *Proc. Natl. Acad. Sci.* **1990**, *87*, 7265.
30. Kahl, S. B.; Koo, M.-S.; Laster, B. H.; Fairchild, R. G. *Strahlenther. Onkol.* **1989**, *165*, 134.
31. Perrin, D. D.; Armarego, W. L. F. *In Purification of Laboratory Chemicals*, 3rd Ed.; Pergamon Press: Oxford, 1988.
32. Barth, R. F.; Adams, D. M.; Soloway, A. H.; Mechnetner, E. B.; Alam, F.; Anisuzzaman, A. K. *Anal. Chem.* **1991**, *63*, 890.
33. Johnson, D. A.; Siemer, D. D.; Bauer, W. F. *Anal. Chim. Acta* **1992**, *270*, 223.
34. Fairchild, R. G.; Gabel, D.; Laster, B. H.; Greenberg, D.; Kiszenick, W.; Micca, P. L. *Med. Phys.* **1986**, *13*, 50.