Synthesis and Biological Evaluation of Boron-Containing Polyamines as Potential Agents for Neutron Capture Therapy of Brain Tumors

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New boron-containing spermidine/spermine (SPD/SPM) analogues have been synthesized: N^5-[4-(2-aminoethyl-*o*-carboranyl)butyl] and N^5-{4-[(2,3-dihydroxypropyl)-*o*-carboranyl]butyl} SPD/SPM derivatives (**ASPD-5**, **ASPM-5**, **DHSPD-5**, and **DHSPM-5**) as well as N^5-{[4-(dihydroxyboryl)phenyl]methyl}spermidine (**BBSPD-5**). These boronated polyamines retain their ability to displace ethidium bromide from calf thymus DNA and are rapidly taken up in vitro by F98 rat glioma cells. The in vitro toxicities of **ASPD-5**, **ASPM-5**, **DHSPD-5**, and **DHSPM-5** are lower than those previously reported for N^5-[4-(*o*-carboranyl)butyl] SPD/SPM derivatives (**SPD-5** and **SPM-5**) but similar to those of native SPD and SPM. Very low toxicity was also observed for **BBSPD-5**. In vivo studies of **ASPD-5** and **BBSPD-5** were performed in mice bearing intracerebral implants of the GL261 glioma and subcutaneous implants of the B16 melanoma. The biodistribution data found in both tumor models suggest that the polyamines synthesized to date do not appear to be suitable boron agents for BNCT.

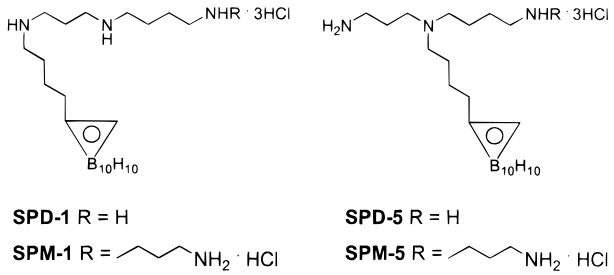
Introduction

Boron neutron capture therapy (BNCT) is based on the nuclear reaction that occurs when ^{10}B, a stable isotope having a relatively high neutron capture cross-section value (σ = 3838 barns), is irradiated with thermal neutrons to produce high linear energy-transfer (LET) α particles and recoiling ^{7}Li nuclei.[1] These particles have path lengths of 5−9 μm and are capable of destroying those cells containing sufficient quantities of ^{10}B required to sustain a lethal $^{10}B(n,\alpha)^{7}Li$ reaction. The chemistry and use of BNCT as a cancer treatment modality has been described in several recent reviews.[2−6]

A boron delivery agent should be nontoxic, selectively target tumor cells, and ideally localize within the nucleus. For BNCT to be effective, there must be ∼20−30 μg of ^{10}B/g of tumor and a low concentration (<5 μg of ^{10}B/g of cell) in surrounding normal cells and blood.[7,8] Various boron-containing analogues of biologically active compounds, such as amino acids,[9−15] peptides,[16−19] and nucleosides/nucleotides,[20−30] have been synthesized. The basis for their preparation is that such structures might function in a manner similar to their naturally occurring counterparts and become selectively incorporated into either proliferating or more metabolically active tumor cells. Boron-containing analogues of porphyrins[31−34] and DNA binders[35−38] have been also considered as potential agents for BNCT.

Polyamines such as spermidine (SPD) and spermine (SPM) are essential for mammalian cell growth and differentiation,[39,40] and their depletion has growth inhibitory effects on tumors.[41,42] This has been the basis for the preparation of various polyamine synthetase inhibitors[43,44] and synthetic polyamines[45] for cancer treatment. They interact electrostatically with DNA in a nonspecific manner.[46−48] Their cellular concentration is increased in tumor cells and specifically within the nucleus.[49−52] In addition, there is a facilitated transport system that increases the uptake by malignant cells.[53] For these reasons, boron-containing polyamines have been considered as potential BNCT agents.[54−56]

Chart 1



We have previously designed and synthesized *N*-carboranyl-tethered polyamine analogues of spermidine and spermine[54] and have correlated their chemical structure with their cellular uptake, DNA binding properties, and in vitro toxicity. The toxicity strongly depended upon the position of the carboranyl group on the polyamine scaffold. Polyamines substituted at the terminal amino groups (**SPD-1**, **SPM-1**) had a greater toxicity than those substituted at one of the central nitrogen atoms (**SPD-5**, **SPM-5**) (Chart 1). Nevertheless, even for the latter compounds, the observed in vitro toxicity was very high. To decrease this toxicity, the hydrophilic properties of the compounds were increased by modifying the *o*-carborane cages in **SPD-5** and **SPM-5** by inserting 2-aminoethyl and 2,3-dihydroxypropyl groups at the 2-position. These compounds were designated as **ASPD-5**, **ASPM-5**, **DHSPD-5**, and **DHSPM-5**. Another boronated polyamine with increased hydrophilicity, [4-(dihydroxyboryl)phenylmethyl]spermidine (**BBSPD-5**), was also synthesized.

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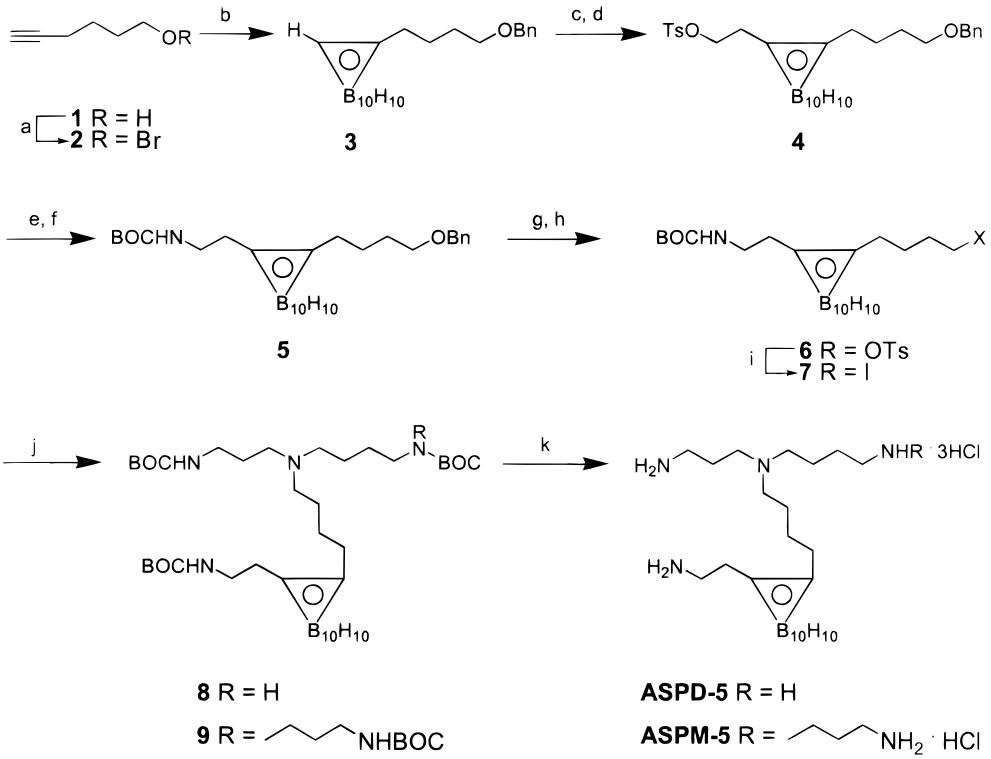
Scheme 1[a]



[a] (a) NaH, BnCl; (b) $B_{10}H_{14}$/EtCN, 30%; (c) BuLi, $(CH_2)_2O$, 96%; (d) TsCl, pyridine, 90%; (e) NaN_3/DMF, 90%; (f) 1. $LiAlH_4$/THF, 2. BOC-ON/THF, 93%; (g) H_2, Pd−C, MeOH−AcOH, 80%; (h) TsCl, pyridine, CH_2Cl_2, 82%; (i) NaI, acetone, 95%; (j) $BOCNH(CH_2)_3NH(CH_2)_4NHBOC$ or $BOCNH(CH_2)_3NH(CH_2)_4N(BOC)(CH_2)_3NHBOC$, K_2CO_3/DMF, **8** (52%), **9** (75%); (k) 3 N HCl, **ASPD-5** (100%), **ASPM-5** (87%).

Chemical Synthesis

Syntheses of the target compounds **ASPD-5** and **ASPM-5** are shown in Scheme 1. (4-Benzyloxybutyl)-*o*-carborane (**3**) was prepared according to previously described procedures[57] by reaction of decaborane(14) ($B_{10}H_{14}$) with benzyl 5-hexyn-1-yl ether (**2**); the latter compound was obtained by the benzylation of 5-hexyn-1-ol with benzyl chloride. The monosubstituted carborane **3** was lithiated with *n*-butyllithium and converted to 1-(2-tosylethyl)-2-(4-benzyloxybutyl)-*o*-carborane (**4**) by reaction with ethylene oxide followed by tosylation. The 1,2-disubstituted-*o*-carborane **4** was transformed into the BOC-protected amine **5** by reaction with NaN_3, followed by reduction with $LiAlH_4$ and the protection of the amino group using BOC-ON [2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile]. Debenzylation of **5** by catalytic hydrogenation (H_2, Pd/C) yielded the primary carboranyl alcohol which upon treatment with TsCl/pyridine produced the corresponding carboranyl tosylate **6**. The key iodide **7** was obtained by the reaction of **6** with NaI in acetone. Alkylation of the protected spemidine (BOC-SPD) or spermine (BOC-SPM)[54] with the iodide **7** in DMF in the presence of K_2CO_3 resulted in the boronation of the corresponding BOC-protected compounds **8** and **9**, respectively (Scheme 1). Removal of the BOC groups by acidic cleavage afforded the target compounds **ASPD-5** and **ASPM-5**.

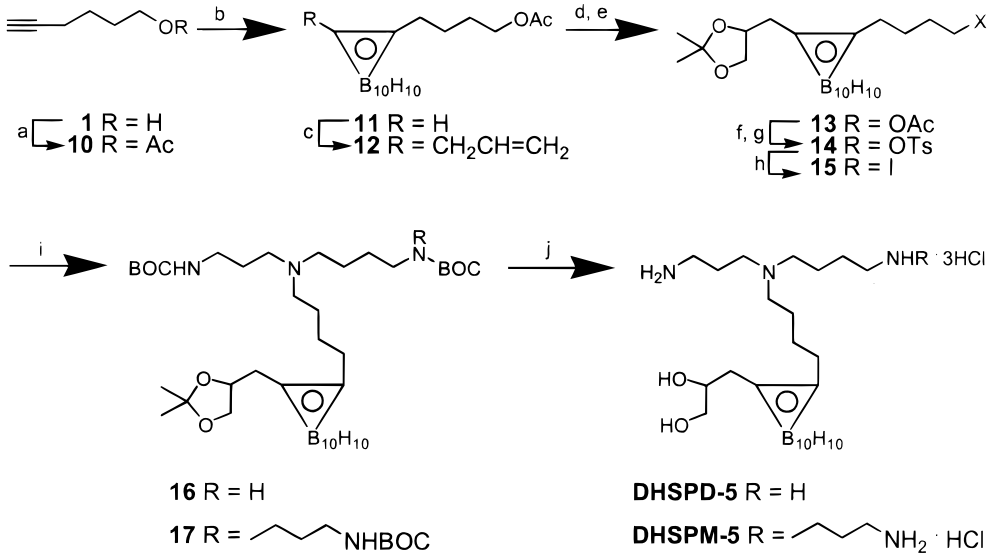
In Scheme 2 are presented the syntheses of the (2,3-dihydroxypropyl)-*o*-carboranyl polyamines **DHSPD-5** and **DHSPM-5**. The required iodide **15** was prepared from compound **13** by the hydrolysis of the acetate with 2 N NaOH. The alcohol was then tosylated with TsCl/pyridine and the tosyl group replaced with iodine by the reaction with NaI in acetone (Scheme 2). The preparation of **13** has been previously described.[25] The target compounds **DHSPD-5** and **DHSPM-5** were obtained by alkylation of the protected spermidine (BOC-SPD) or spermine (BOC-SPM) with the protected dihydroxy iodide **15** in DMF in the presence of K_2CO_3, followed by the removal of the BOC groups as described above.

Since all of the above compounds contain the highly lipophilic carborane moiety which contributes to the compounds' toxicities, a dihydroxyboryl-containing polyamine, [4-(dihydroxyboryl)phenylmethyl]spermidine (**BBSPD-5**), was designed and prepared. Synthesis of **BBSPD-5** is shown in Scheme 3. Reaction of BOC-SPD with 4-(bromomethyl)phenylboronic acid[58] produced **18**. The desired target compound **BBSPD-5** was obtained after removal of the BOC group by acidic hydrolysis. **BBSPD-5** loses one molecule of water easily under vacuum, forming the dimer **Bis-BBSPD-5**. This was determined by NMR in anhydrous aprotic solution (DMSO-d_6) and by elemental microanalysis. Addition of water to the dimer **Bis-BBSPD-5** converted it back to the monomer **BBSPD-5**. Concentration of **BBSPD-5** from an ethanolic solution yielded **Et-BBSPD-5** in which one hydroxyl group was replaced by an ethoxy function.

Biological Studies

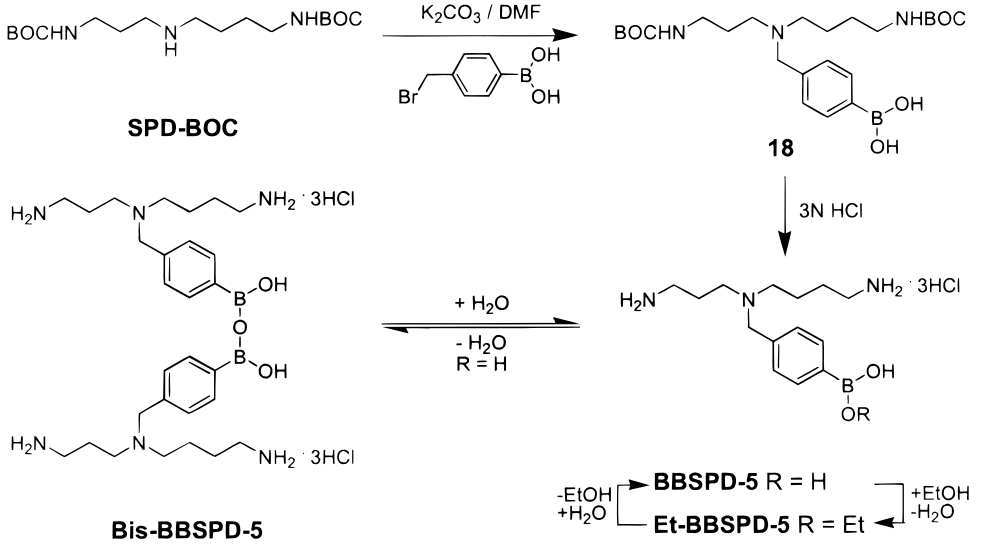
The three major questions regarding these new boron-containing polyamines were whether (1) their biological properties were similar to that of SPD/SPM; (2) their enhanced hydrophilicity diminished their toxicity; and (3) they possessed the requisite tumor-targeting properties to be useful BNCT agents. To answer these impor-

Scheme 2[a]



[a] (a) AcCl, pyridine, CH_2Cl_2, 95%; (b) $B_{10}H_{14}$/EtCN, 35%; (c) $Pd_2(dba)_3$, dppe, $EtOCO_2CH_2CH{=}CH_2$, THF, 81%; (d) OsO_4, pyridine, NMMPNO, 79%; (e) $Me_2C(OMe)_2$, TsOH, 86%; (f) 2 N NaOH, 95%; (g) TsCl, pyridine, CH_2Cl_2, 84%; (h) NaI, acetone, 87%; (i) $BOCNH(CH_2)_3NH(CH_2)_4NHBOC$ or $BOCNH(CH_2)_3NH(CH_2)_4N(BOC)(CH_2)_3NHBOC$, K_2CO_3/DMF, **16** (67%), **17** (74%); (j) 3 N HCl, **DHSPD-5** (98%), **DHSPM-5** (100%).

Scheme 3



tant questions, the following biological properties have been evaluated: (1) their in vitro growth inhibitory effects using F98 rat glioma cells; (2) their ability to displace ethidium bromide from calf thymus DNA; (3) the compound's in vitro cellular uptake using the F98 glioma cells; and (4) their in vivo biodistribution in mice bearing intracerebral implants of the GL261 glioma and subcutaneous implants of the B16 melanoma. The focus of these latter studies has been to evaluate **ASPD-5** and **BBSPD-5** and to compare them with the earlier compounds that have been synthesized and evaluated.[54]

(1) Evaluation of in Vitro Toxicity. The assay that we have employed quantified the number of F98 glioma cells following a 24-h exposure to the test compounds by measuring cellular uptake/incorporation of sulforhodamine B (SRB) and comparing that uptake to cells that were not exposed to the test compounds.[59] The results are summarized in Table 1. The IC_{50} (inhibitory concentration) value is defined as that concentration of the compound that produced a 50% reduction in SRB absorbance by F98 glioma cells as determined spectrophotometrically for all compounds compared with the control cells.[60] The in vitro toxicities of carboranyl polyamines **ASPD-5**, **ASPM-5**, **DHSPD-5**, and **DH-**

Table 1. In Vitro Toxicity of Polyamine Analogues

compd	IC_{50} (μM)
SPD	>100[a]
SPD-5	22[a]
ASPD-5	>100
DHSPD-5	>100
SPM	>100[a]
SPM-5	22[a]
ASPM-5	>100
DHSPM-5	>100
BBSPD-5	>5000

[a] Data taken from ref 54. A 50% reduction in the uptake of [^{3}H]TdR in F98 glioma cells was measured for **SPD-5** and **SPM-5**.

Table 2. DNA Binding Affinity as Measured by Ethidium Bromide Displacement[a]

compd	IC_{50} (μM)
SPD	105 ± 8.7
SPD-5	147[b]
ASPD-5	3.8 ± 0.6
DHSPD-5	66.7 ± 5.7
BBSPD-5	115.0 ± 7.1
SPM	3.0 ± 1.7
SPM-5	23[b]
ASPM-5	4.0 ± 0.9
DHSPM-5	21.7 ± 3.8

[a] Ethidium bromide and DNA concentrations were 1.6 and 10 μg, respectively. [b] Data taken from ref 54.

SPM-5, possessing an additional hydrophilic 2-aminoethyl or 2,3-dihydroxypropyl group attached to the carborane moiety, were equivalent (>100 μM) to those previously reported for the native polyamines, SPD and SPM.[54] They were ~5 times less toxic than the earlier structures, **SPD-5** and **SPM-5**,[54] and the first dihydroxyboryl-containing polyamine, **BBSPD-5**, tested had the lowest toxicity of all (IC_{50} = 5000 μM).

(2) DNA Binding Affinity. All of the polyamines synthesized were evaluated in a DNA binding assay. The binding of polyamine analogues to DNA was determined by an ethidium bromide displacement assay.[61−63] The IC_{50} value was defined as that concentration of polyamine required to decrease the fluorescence of the ethidium bromide−calf thymus DNA complex by 50%. The results are summarized in Table 2. All of the SPD and SPM analogues, possessing 2-aminoethyl or 2,3-dihydroxypropyl groups at the 2-position of the *o*-carborane cages, had stronger affinity for DNA than the previously synthesized analogues, **SPD-5** and **SPM-5**.[54] There was a 28-fold enhancement of DNA binding affinity for **ASPD-5** and a 1.6-fold increase for **DHSPD-5** compared with natural SPD. In the case of **BBSPD-5**, the values obtained were comparable to those for SPD. In the case of the spermine analogues, **ASPM-5** showed values comparable to natural SPM, while **DHSPM-5** had diminished DNA binding properties (21.7 μM). Of all these boron-containing compounds, the best DNA binder was **ASPD-5**.

(3) In Vitro Cellular Uptake Studies. The in vitro uptake of carboranyl polyamines by F98 glioma cells was determined by measuring cellular boron content by means of direct current plasma atomic emission spectroscopy (DCP-AES).[64] Uptake of the new compounds was compared with the earlier structures, **SPD-5** and **SPM-5**, and with two clinically used agents, BSH and BPA (Table 3). The media concentration in the initial studies was very low due to the high toxicity of the earlier carboranyl polyamines. The more recent compounds were much less toxic permitting a 10-fold increase in media concentration. Nevertheless, the cellular uptake of these more hydrophilic analogues was significantly lower than that of the previously described structures, **SPD-5** and **SPM-5**,[54] a matter that will be considered in the Discussion section. However, all of these boronated polyamines were sequestered by tumor cells at a significantly higher level than was observed for either of the current clinical compounds BSH and BPA.

Table 3. In Vitro Cellular Uptake of Boronated Polyamines by F98 Glioma Cells[a]

compd	C_{media} (μg of B/mL)	$C_{cellular}$ (μg of B/g of cells), 48 h[b]	C_{media} (μg of B/mL)	$C_{cellular}$ (μg of B/g of cells) 24 h[b]	48 h[b]
SPD-5[c]	0.5	43	0.5	65	43
ASPD-5	0.5	19	5.4	51	116
DHSPD-5	0.5	9	5.4	51	104
SPM-5[c]	0.5	64			
ASPM-5	0.5	15	5.3	82	229
DHSPM-5	0.5	20	5.3	60	83
BBSPD-5			5.1		114
BPA[c]			50	81	
BSH[c]			570	39	

[a] F98 cells were incubated with the polyamine analogues at molar compound concentrations corresponding to the boron amounts indicated. Cells were washed, counted, and digested. Boron concentrations C_{media} and $C_{cellular}$ were determined by DCP-AES (10^9 cells ≅ 1 g of cells). [b] Incubating time. [c] Data taken from ref 54.

Table 4. Biodistribution of **ASPD-5** and **BBSPD-5** in Mice Bearing Intracerebral GL261 Gliomas

tissue	**ASPD-5**[a] (μg of B/g)	**BBSPD-5**[b] (μg of B/g)
tumor	7.0 ± 3.8	nm
brain	1.6 ± 1.0	nm
blood	3.4 ± 2.1	nm
liver	51.3 ± 27.3	22.9 ± 10.9
spleen	30.9 ± 13.2	nm
kidney	112.0 ± 53.2	44.0 ± 12.6
skin	6.3 ± 3.8	nm
muscle	5.3 ± 1.9	nm
eyes	nm	nm
T/Bl ratio	1.7	
T/Br ratio	4.4	

[a] Dose = 30 μg of compound/g per day. [b] Dose = 10 μg of boron/g per day. Five mice were used for each compound, and the values shown are means ± standard deviations for each set of determinations.

(4) In Vivo Biodistribution Studies. The data presented in the proceeding sections showed that both **ASPD-5** and **BBSPD-5** possessed low cellular toxicity, retained the capacity to bind to DNA as other polyamines, and had significant in vitro cellular uptake. These results prompted an in vivo biodistribution study in tumor-bearing animals of these two compounds that may be viewed as representative structures of two different types of boron-containing polyamines. These studies were performed using two different tumor models in C57Bl/6 mice: (1) an intracerebrally implanted GL261 glioma and (2) a subcutaneously implanted B16 melanoma. Animals were placed on a polyamine-deficient diet and water containing 2% difluoromethylornithine (DFMO), an ornithine decarboxylase inhibitor, 3−4 days prior to the beginning of the infusion, and this was contined for the duration of the study. Alzet pumps were implanted, and infusion of the boronated polyamines was carried out over 3 days. Following this time period, the pumps were removed, the animals were euthanized at 0, 12, and 24 h, and their tissues were removed for boron analysis. These results are summarized in Table 4. For **ASPD-5**, the greatest amount of boron was found in the kidney (112 μg of B/g) and liver (51.3 μg of B/g), while the concentration in the tumor was only 7.0 μg of B/g. Similarly for **BBSPD-5**, the levels in kidney (44.0 μg of B/g) and liver

Table 5. Biodistribution of **SPD-5** and **ASPD-5** in Mice Bearing Subcutaneous B16 Melanomas

tissue	**SPD-5**[a] (μg of B/g) (10 μg of compd/g)	**ASPD-5**[b] (μg of B/g) (20 μg of compd/g)
tumor	9.3 ± 2.5	10.2 ± 3.8
brain	nm	nm
blood	12.5 ± 2.4	3.2 ± 1.1
liver	74.8 ± 5.6	76.8 ± 12.2
kidney	201.3 ± 43.1	109.6 ± 9.9
muscle	9.8 ± 3.2	8.5 ± 5.2
eyes	nm	nm
T/Bl ratio	0.74	3.2

[a] Four mice per group; tumor size averaged 150 mg. [b] Five mice per group; tumor size averaged >500 mg. For both groups, the boron values shown are means ± standard deviations.

Table 6. Biodistribution of **ASPD-5** in Mice Bearing Subcutaneous B16 Melanomas

tissue	dose of 30 μg of compd/g (μg of B/g)	dose of 40 μg of compd/g (μg of B/g)
tumor	16.2 ± 5.1	18.5 ± 2.8
brain	2.0 ± 0.7	2.1 ± 1.1
blood	5.3 ± 3.5	2.9 ± 1.1
liver	76.8 ± 12.2	87.5 ± 12.3
spleen	25.8 ± 14.5	37.7 ± 14.2
kidney	157 ± 36.3	192.5 ± 27.3
skin by pump	34.2 ± 20.1	101.0 ± 46.7
skin	4.4 ± 1.3	7.1 ± 1.8
muscle	7.9 ± 3.1	9.2 ± 1.3
eyes	8.1 ± 3.5	6.4 ± 0.5
T/Bl ratio	3.1	6.3
T/Br ratio	8.1	8.8

(22.9 μg of B/g) were the highest, while those in tumor were undetectable.

Though the tumor boron levels obtained with **ASPD-5** were insufficient to be useful as a single delivery agent for BNCT, it remains to be determined whether it could be used as one component in a mixture of compounds to target different subpopulations of tumor cells. By our method of in vivo evaluation, **BBSPD-5** showed unmeasurable levels of boron in tumor and for this reason is not considered as an appropriate BNCT agent, despite the fact that it had the lowest in vitro toxicity and comparable DNA binding affinity to SPD.

In the evaluation of these compounds as potential BNCT agents for melanoma, C57Bl/6 mice bearing subcutaneous implants of the B16 melanoma were used. In the initial studies, **ASPD-5** was compared against the more toxic analogue **SPD-5**. Animals were placed on a polyamine-deficient diet and water containing 0.2% neomycin together with 2% DFMO for 3 days. The compounds were then administered intraperitoneally in two doses per day for 3 days. After the last injection, the animals were held for 12 h and euthanized, and their tissues were removed for boron determination. The results are shown in Table 5. For **SPD-5**[54] and **ASPD-5**, the largest amounts of boron were found in the kidney (201 and 110 μg of B/g, respectively) and liver (75−76 μg of B/g), while the values in tumor were only 9−10 μg of B/g. The T/Bl ratio of **ASPD-5** was 3.2, and that of **SPD-5** was 0.74.

In a subsequent study, **ASPD-5** was administered at increasing doses using the same tumor model. The animals were again placed on a polyamine-deficient diet and water containing 2% DFMO for 3 days prior to the initiation of and for the duration of the study. Then, Alzet pumps were implanted, and compound infusion was carried out over 4 days. At the end of the fourth day, the animals were euthanized and tissues from each group of five were removed and analyzed for boron. The results are summarized in Table 6. The highest amounts of boron were found in kidney, skin adjacent to the pump, and liver, which were appreciably greater than those in the tumor (16−18 μg of B/g).

Discussion

(1) Chemistry. The *o*-carborane cage is sufficiently stable under neutral and acidic conditions for its incorporation into potential BNCT agents. However, it undergoes degradation under various basic conditions,[65−68] and this property has been the major chemical limitation in the synthesis of *o*-carborane-containing amines that are very strong bases. This is the case with the polyamines. This problem can be obviated by masking the amino function and generating the final products under acidic conditions as the amine hydrochloride. These salts are very water-soluble even with the carboranyl function, and the resulting aqueous solutions are stable.

The highly lipophilic properties of the carborane cage have been a major limitation with this boron moiety since it can result in nonspecific binding to various biolipids thereby limiting the tumor specificity of these boron-containing polyamines. This fact has been the basis for the incorporation of hydrophilic functions into the carborane in an attempt to diminish its lipophilicity. However, no studies were performed to determine the success of this chemical maneuver. An alternative approach is to replace the carborane with a dihydroxyboryl entity, the same boron component in the clinically used BPA. This was the rationale for the synthesis of **BBSPD-5**. The chemical procedures described and presented in the Experimental Section are straightforward, and by-in-large the compounds were formed in the manner proposed. The question was not the issue of the novelty of the synthetic chemistry but whether these compounds would have favorable biological properties for tumor targeting.

(2) Biology. The polyamine structure as a scaffold for targeting malignant cells has such a potential only if the polyamine transport system is upregulated in tumor cells compared with normal cells from which they are derived. This appears to be especially true for brain tumors[44] and has provided the rationale for incorporating boron moieties into the naturally occurring polyamines, SPD and SPM. The in vitro biological results with carboranyl polyamines show their potential for selectively delivering boron to tumor cells and thereby offered the potential of a new class of BNCT agents. They possess the ability to displace ethidium bromide from calf thymus DNA, as do their natural counterparts, and are rapidly taken up by F98 glioma cells in vitro. This uptake is comparable to that of the clinically used compounds BSH and BPA, but at a media concentration that is 10−100-fold less. Polyamines bind to DNA nonspecifically, and therefore, boronated polyamines may be able to target DNA directly once they penetrate the cell membrane. The hydrophilic properties of these polyamine salts make them highly water soluble intra-

peritoneally or intravenously at suitable concentrations without the use of solubilizers or cosolvents. However, the major limitation with the earlier compounds was their toxicity, especially those compounds with terminal *N*-substituted boron moieties (**SPD-1**, **SPM-1**).[54] The compounds described in this report address that problem directly. Although their in vitro uptake is somewhat reduced when compared with those earlier boronated polyamines, nevertheless, the cellular concentrations attained are very substantial, markedly superior to that of BSH and BPA, and have significantly reduced toxicity.

The most important question obviously is not their in vitro uptake but what would be their in vivo concentration in tumor-bearing animals? To maximize in vivo uptake, animals were simultaneously placed on a polyamine-deficient diet and given an inhibitor of ornithine decarboxylase, DFMO, in their drinking water. The objective of these efforts was to decrease exogenous levels of naturally occuring polyamines that the animals received and to reduce the levels of de novo endogenous polyamines produced. The reasoning was that the boronated polyamines might be rapidly sequestered by malignant cells under these conditions.

The in vivo results that we have obtained can be summarized as not being very promising. The two compounds that have been evaluated, **ASPD-5** and **BBSPD-5**, have shown insufficient tumor boron levels for the former and unmeasurable levels for the latter. The boron levels in liver, spleen, and kidney were markedly higher than those observed for the tumor. The question remains, however, as to why these compounds did not have better tumor accretion under in vivo conditions?

Will it not be possible to obtain the necessary tumor concentrations of boronated polyamines? Were the in vivo methods used for screening these compounds inappropriate? To address these questions, it will be necessary to determine whether labeled SPD and SPM at the same molar concentrations would have higher tumor uptake, better tumor-to-normal tissue ratios and at a tumor cellular concentration necessary for therapy. Alternatively, it may be that the boronated polyamines, which we have selected for synthesis, are not the most useful ones.

In conclusion, although we have succeeded in synthesizing boronated polyamines that are appreciably less toxic than earlier compounds[54] and achieve very adequate in vitro cellular concentrations, their in vivo uptake by tumors is inadequate as BNCT delivery agents. It remains to be determined why that is the case and whether other boronated polyamines can be designed and synthesized that will achieve that objective.

Experimental Section

The reagents were purchased from the chemical companies and used directly without further purification unless otherwise specified. Anhydrous ethyl ether and tetrahydrofuran (THF) were distilled from sodium/diphenyl ketone. *N*,*N*-Dimethylformamide (DMF) was distilled from BaO. Acetone was distilled from molecular sieves. Flash column chromatography was carried out on Merck silica gel 60 (0.040−0.063 mm, 230−400 mesh). Melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker AC-250, AC-270, or DPX-400 spectrometer. ^{1}H and ^{13}C chemical shifts were measured relative to internal residual protons from the lock solvent and then referenced to TMS (δ = 0.0 ppm), and coupling constants (J) are reported in Hz. IR (cm^{-1}) were measured on a Laser Precision Analytical 720-XI spectrometer. Mass spectra (MS) were carried out at the Campus Chemical Instrument Center of The Ohio State University on a VG 70-250S for HR-EI and on a Finnigan MAT-900 for HR-FAB by Dr. David H. Chang. Elemental analyses were performed by Robertson Microlit Laboratories, Inc. and Galbraith Laboratories, Inc. All compounds gave elemental analyses within ±0.4%. RT (room temperature) is 22−25 °C.

1. Chemical Syntheses. 6-Benzyloxyhex-1-yne (2).[69] To a suspension of NaH (1.52 g, 60%, 37.8 mmol) in THF (150 mL) was added a solution of hex-5-yn-1-ol (3.38 g, 34.4 mmol) in THF (20 mL). The mixture was stirred at RT for 1 h. To the mixture was added dropwise a solution of benzyl bromide (6.18 g, 36.1 mmol) in THF (20 mL). The resulting mixture was stirred at RT for 8 h and filtered through a pad of silica gel. The filtrate was concentrated. The residue was flash chromatographed (hexane/EtOAc = 20:1) to give **2** (4.38 g, 68%): ^{1}H NMR ($CDCl_3$) 7.22−7.43 (m, 5H, Ph), 4.49 (s, 2H, OCH_2Ph), 3.49 (t, 2H, J = 6.1, OCH_2CH_2), 2.20 (td, 2H, J = 6.8, 2.7, CH_2C≡CH), 1.94 (t, 1H, J = 2.7, CCH), 1.60−1.81 (m, 4H); ^{13}C NMR ($CDCl_3$) 138.7 (C, Ph), 128.7 (CH, Ph), 128.3 (2 CH, Ph), 127.5 (2 CH, Ph), 84.3 (C, *C*CH), 72.9 (Ph*C*H$_2$), 69.8 (O*C*H$_2$), 68.3 (CH, C*C*H), 28.8 (CH$_2$), 25.3 (CH$_2$), 18.2 (CH$_2$).

1-(4-Benzyloxybutyl)-*o*-carborane (3). A mixture of compound **2** (3.80 g, 20.2 mmol) and decaborane(14) (2.85 g, 23.3 mmol) in EtCN (6 mL) was refluxed for 2 h. After cooling, the solvent was evaporated. The residue was chromatographed (hexane/EtOAc = 20:1) to give **3** (1.87 g, 30%): IR (neat) 2930w, 2847m, 2570vs, 1346w, 1084m, 716m; ^{1}H NMR ($CDCl_3$) 7.20−7.45 (m, 5H, Ph), 4.49 (s, 2H, OCH_2Ph), 3.54 (s, 1H, $B_{10}H_{10}$CCH), 3.40−3.52(m, 2H, OCH_2CH_2), 2.23−2.31 (m, 2H, $B_{10}H_{10}$CH_2), 1.48−1.63 (m, 4H); ^{13}C NMR ($CDCl_3$) 138.4 (C, Ph), 128.4 (2CH + CH, Ph), 127.7 (2CH, Ph), 75.4 (C, $B_{10}H_{10}C$), 73.1 (CH$_2$, Ph*C*H$_2$O), 69.3 (O*C*H$_2$), 60.9 (CH, $B_{10}H_{10}$*C*H), 37.9 (CH$_2$), 29.0 (CH$_2$), 26.2 (CH$_2$); MS (HR-EI) for $C_{13}H_{26}B_{10}O$ calcd 308.2914, found 308.2914. Anal. ($C_{13}H_{26}B_{10}O$) C, H, N, B.

1-Tosylethyl-2-(4-benzyloxybutyl)-*o*-carborane (4). To a solution of **3** (897.5 mg, 2.93 mmol) in THF (45 mL) was added BuLi (2.2 mL, 1.6 M, 3.51 mmol) at −45 °C. The mixture was stirred for 40 min while the temperature rose from −45 °C to RT. Then the solution was cooled to −30 °C, and ethylene oxide (1 mL) was added quickly. After 30 min, an equal amount of ethylene oxide was added. The reaction mixture was stirred for an additional 40 min during which time the temperature rose from −30 °C to RT. The mixture was quenched with a cold NH_4Cl solution and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was washed with brine and dried over $MgSO_4$. After evaporation of the solvent, the residue was chromatographed (hexane/EtOAc = 3:1) to give an intermediate alcohol (986.6 mg, 2.81 mmol, 96%) as a colorless oil. This alcohol (870.7 mg, 2.48 mmol) was diluted with CH_2Cl_2 (50 mL) and cooled to about 0 °C. To this solution was added pyridine (3 mL) followed by tosyl chloride (947 mg, 4.89 mmol) divided into six portions and added over 2 h. The mixture was stirred overnight at RT and washed with 2 N NaOH and water. After the organic layer was dried over $MgSO_4$ and the solvent removed, the residue was chromatographed (hexane/EtOAc = 3:1) to give **4** (1.09 g, 2.16 mmol, 87%) as a colorless oil: IR (neat) 2937w, 2839w, 2570s, 1354s, 1181s, 1084m, 723m; ^{1}H NMR ($CDCl_3$) 7.77 (d, 2H, J = 6.7, ArH), 7.49−7.21 (m, 7H, ArH), 4.49 (s, 2H, $C_6H_5CH_2$-), 4.15 (t, 2H, J = 6.9, −SO_3-CH_2-), 3.49 (t, 2H, J = 5.8, BnOCH_2-), 2.55 (t, 2H, J = 6.9, −C$H_2$$CB_{10}$-), 2.48 (s, 3H, Ar−C$H_3$), 2.25−2.13 (m, 2H, −C$H_2$-$CB_{10}$-), 1.72−1.55 (m, 4H, −C$H_2CH_2CH_2CH_2$-); ^{13}C NMR ($CDCl_3$) 145.4 (C, Ar), 138.4 (C, Ph), 132.7 (C, Ar), 130.0 (2CH, Ar), 128.4 (CH, Ph), 127.9 (2CH, Ar or Ph), 127.6 (4CH, Ar + Ph), 80.1 (C, *C*B_{10}), 75.3 (C, *C*B_{10}), 73.1 (OCH_2Ph), 69.4 (OCH_2), 66.9 (OCH_2), 35.0 (CH$_2$), 33.9 (CH$_2$), 29.1 (CH$_2$), 26.7 (CH$_2$), 21.6 (*C*H$_3$); MS (HR-EI) for $C_{22}H_{36}B_{10}O_4S$ calcd 506.3282, found 506.3283. Anal. ($C_{22}H_{36}B_{10}O_4S$) C, H, B.

1-BOC-aminoethyl-2-(4-benzyloxybutyl)-*o*-carborane (5). A mixture of **4** (1.06 g, 2.1 mmol) and NaN_3 (367.2 mg, 6.1 mmol) in DMF (6 mL) was stirred for 20 h at RT. Water and CH_2Cl_2 were added. The organic layer was separated, washed with brine, and dried over $MgSO_4$. After evaporation of the solvent, the intermediate (710 mg, 1.89 mmol, 90%) was obtained as a colorless oil. This compound (688.6 mg, 1.83 mmol) in THF (15 mL) was added dropwise to a suspension of $LiAlH_4$ (75.9 mg, 2.0 mmol) in THF (10 mL) at 0 °C. The mixture was stirred for 1 h from 0 °C to RT. Water was added carefully to quench the excess $LiAlH_4$. The suspension mixture was filtered, and the solid was extracted with ether twice. The organic solutions were combined and dried over $MgSO_4$. After filtration and removal of solvents, the residue was diluted with THF (10 mL) and Et_3N (0.3 mL) and cooled to 0 °C. A solution of BOC-ON (531.9 mg, 2.16 mmol) in THF (10 mL) was added over a few minutes. The solution was then stirred overnight at RT. The mixture was diluted with ether and washed twice with 2 N NaOH and water. The organic layer was dried over $MgSO_4$. After filtration and removal of the solvent, the residue was chromatographed (hexane/EtOAc = 5:1) to give **5** (752.6 mg, 1.68 mmol, 93%) as a colorless oil: IR (neat) 2975w, 2570s, 1691s, 1496m, 1340m, 1151s, 723m; ^{1}H NMR ($CDCl_3$) 7.45−7.25 (m, 5H, Ar*H*), 4.69 (s, 1H, N*H*), 4.49 (s, 2H, ArCH_2-), 3.49 (t, 2H, J = 5.4, −CH_2O-), 3.35−3.16 (m, 2H, −CH_2NH-), 2.37 (t, 2H, J = 7.6, −CH_2CB_{10}-), 2.30−2.15 (m, 2H, −CH_2CB_{10}-), 1.72−1.58 (m, 4H, $CH_2CH_2CH_2CH_2$-), 1.45 (s, 9H, −C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) 155.5 (C, *C*OO), 138.4 (C, Ph), 128.4 (3CH, Ph), 127.6 (2CH, Ph), 80.1 (C, *C*B_{10}), 79.9 (C, O*C*Me$_3$), 77.1 (C, *C*B_{10}), 73.0 (O*C*H_2Ph), 69.5 (O*C*H_2), 40.0 (*C*H_2N), 34.9 (CH_2), 34.5 (CH_2), 29.2 (CH_2), 28.4 (3*C*H_3), 26.7 (CH_2); MS (HR-EI) calcd for $C_{20}H_{38}B_{10}NO_3$ (M − H) 450.3782, found 450.3897. Anal. ($C_{20}H_{39}B_{10}NO_3$) C, H, N.

1-BOC-aminoethyl-2-(4-tosylbutyl)-*o*-carborane (6). A mixture of **5** (718.1 mg, 1.6 mmol), Pd/C (10%, 100 mg), and AcOH (5 mL) in MeOH (20 mL) was stirred under hydrogen at RT for 10 h. The catalyst was removed, and the solution was concentrated. The residue was diluted with CH_2Cl_2, washed with $NaHCO_3$, and dried over $MgSO_4$. After filtration, the filtrate was concentrated. The residue was the intermediate 4-(1-BOC-aminoethyl-*o*-carboran-2-yl)butanol (460.7 mg, 1.28 mmol, 80%), which was dissolved (460.7 mg, 1.28 mmol) in CH_2Cl_2 (25 mL) and cooled to 0 °C; pyridine (1.5 mL) was added. The tosyl chloride (0.98 g, 5.13 mmol) was added in several portions. The mixture was then stirred at RT overnight. The tosylate was purified as previous described for **4** to give **6** (639 mg, 1.05 mmol, 82%) as a colorless oil: IR (neat) 2960w, 2569s, 1691s, 1346s, 1151s, 610m; ^{1}H NMR ($CDCl_3$) 7.70 (d, 2H, J = 8.3, Ar*H*), 7.37 (d, 2H, J = 8.3, Ar*H*), 4.80 (s, 1H, −N*H*), 4.05 (t, 2H, J = 5.8 −SO_3CH_2-), 3.34−3.20 (m, 2H, −CONCH_2-), 2.49 (s, 3H, ArCH_3), 2.43 (m, 2H, −B_{10}CCH_2-), 2.25−2.12 (m, 2H, −B_{10}CCH_2-), 1.76−1.5 (m, 4H, −$CH_2CH_2CH_2CH_2$-), 1.40 (s, 9H, −C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) 155.6 (C, *C*OO), 145.0 (C, Ar), 133.2 (C, Ar), 130.0 (2CH, Ar), 127.8 (2CH, Ar), 79.9 (C, *C*B_{10}), 79.4 (C, O*C*Me$_3$), 77.3 (C, *C*B_{10}), 69.5 (O*C*H_2), 40.1 (*C*H_2N), 34.5 (CH_2, −B_{10}CC*C*H_2-), 34.3 (CH_2, −B_{10}CC*C*H_2CH_2-), 28.3 and 25.8 (2 CH_2, −B_{10}CC*C*$H_2CH_2CH_2$-), 21.6 (Ar*C*H_3); MS (HR-EI) calcd for $C_{13}H_{32}B_{10}INO_2$ 515.3496, found 515.3497. Anal. ($C_{13}H_{32}B_{10}INO_2$) C, H, N, B.

1-BOC-aminoethyl-2-(4-iodobutyl)-*o*-carborane (7). To a solution of **6** (567.4 mg, 1.1 mmol) in dry acetone (20 mL) was added NaI (331 mg, 2.2 mmol). The mixture was stirred overnight at RT. After removal of the solid and acetone, the residue was flash chromatographed (hexane/EtOAc = 5:1) to give **7** (490.2 mg, 1.04 mmol, 95%) as a colorless oil: IR (neat) 3342w, 2960w, 2569s, 1691s, 1489m, 1354m, 1151s; ^{1}H NMR ($CDCl_3$) 4.77 (s, 1H, −N*H*), 3.40−3.10 (m, 4H, ICH_2- + −CONCH_2-), 2.51−2.35 (m, 2H, −B_{10}CCH_2-), 2.34−2.15 (m, 2H, −B_{10}CCH_2-), 1.97−1.60 (m, 4H, −$CH_2CH_2CH_2CH_2$-), 1.48 (s, 9H, −C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) 156.0 (C, *C*OO), 80.0 and 77.2 (2 C, −B_{10}*CC*H_2-), 79.5 (C, O*C*Me$_3$), 40.1 (*C*H_2N), 34.5 (CH_2, −B_{10}CC*C*H_2-), 33.8 (CH_2, −B_{10}CC*C*H_2CH_2-), 32.5 and 30.4 (2 CH_2, −B_{10}CCCH_2*C*H_2*C*H_2CH_2-), 28.4 (3CH_3); MS (HR-EI) calcd for $C_{13}H_{32}B_{10}INO_2$ 471.2410, found 471.2408. Anal. ($C_{13}H_{32}B_{10}INO_2$) C, H, N.

***N*^{1},*N*^{10}-Bis(BOC)-*N*^{5}-[4-(2-BOC-amidoethyl-*o*-carboran-1-yl)butyl]spermidine (8). General Procedure for the Alkylation of the Amino Functions of Polyamines.** To a mixture of K_2CO_3 (207 mg, 2 mmol) and SPD-BOC (259.1 mg, 0.75 mmol) in DMF (1 mL) was added compound **7** (320 mg, 0.68 mmol) in DMF (1.5 mL) at 60−70 °C. The reaction mixture was stirred for 1 h. After cooling, ice−water and CH_2Cl_2 were added. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were washed with 1 N NaOH and dried over $MgSO_4$. After filtering and removal of the solvent, the residue was purified by flash column chromatography and eluted with hexanes−ethyl acetate (1:1) containing 5% MeOH to give **8** (244.4 mg, 0.36 mmol, 52%) as a colorless oil: IR (neat) 3335w, 2960m, 2569m, 1676s, 1504m, 1151s; ^{1}H NMR ($CDCl_3$) 5.40 (br, 1H, N*H*), 5.20 (br, 1H, N*H*), 4.95 (br, 1H, N*H*), 3.37−3.00 (m, 6H, 3×-CONCH_2-), 2.52−2.11 (m, 12H, N(CH_2-)$_3$ + −CH_2C$H_2$$CH_2$- + −C$H_2CB_{10}H_{10}CCH_2$-), 1.70−1.55 (m, 8H, −NCH_2CH_2CH_2-CH_2N− + −CH_2CH_2CH_2CH_2-), 1.48 (s, 27H, 3×−C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) 156.0 (2C, *C*OO), 155.8 (C, *C*OO), 80.0 and 77.0 (2 C, −B_{10}*CC*H_2-), 79.6, 78.9 and 78.8 (3×C, O*C*Me$_3$), 53.7, 53.4 and 52.3 (3×CH_2, N*C*H_2), 41.0, 40.4 and 39.9 (3×CH_2, NH*C*H_2), 34.9 and 34.3 (2×CH_2, −B_{10}CC*C*H_2-), 28.4 (9×*C*H_3), 27.9 (CH_2), 27.6 (CH_2), 27.1 (CH_2), 26.7 (CH_2), 24.4 (CH_2); MS (FAB) calcd for $C_{30}H_{66}B_{10}N_4O_6$ 688.59, found 688.63. Anal. ($C_{30}H_{66}B_{10}N_4O_6$) C, H, N, B.

***N*^{1},*N*^{10},*N*^{14}-Tris(BOC)-*N*^{5}-[4-(2-BOC-amidoethyl-*o*-carboranyl)butyl]spermine (9).** Compound **9** (678.9 mg, 0.80 mmol, 75%) was obtained from the reaction of SPM-BOC (629.3 mg, 1.25 mmol) and **7** (504.0 mg, 1.07 mmol): eluting solvents hexane:ethyl acetate (1:1) containing 5% MeOH; IR (neat) 3339vs br, 2571vs, 1688vs, 1665vs, 1494vs, 1261vs, 1230vs, 1153vs, 1060s, 1013s, 951m, 850s, 758s, 719vs; ^{1}H NMR ($CDCl_3$) 3.49−3.05 (m, 10H, 5×-CONCH_2-), 2.51−2.39 (m, 10H, N(CH_2-)$_3$ + −CH_2C$B_{10}H_{10}$CCH_2-), 1.70−1.55 (m, 48H, 2×-CH_2C$H_2$$CH_2$- + −NC$H_2CH_2CH_2CH_2$N− + −$CH_2CH_2CH_2$-$CH_2$- + 4×−C(C$H_3$)$_3$); ^{13}C NMR ($CDCl_3$) 156.0 (2×C, *C*OO), 155.9 (C, *C*OO), 155.6 C, *C*OO), 80.0, 79.4, 78.9 and 78.8 (4×C, 4×O*C*Me$_3$), 79.6 and 77.1 (2 C, −B_{10}*CC*-), 53.8, 53.4 and 52.4 (3×CH_2, −N(*C*$H_2$$CH_2$-)$_3$-), 46.8 and 44.0 (2×$CH_2$, BOCN(*C*$H_2$-$CH_2$-)$_2$-), 40.0, 39.7 and 37.8 (3×CH_2, BOCNH*C*$H_2$$CH_2$-), 34.9 and 34.4 (2×$CH_2$, −$B_{10}$CC*C*$H_2$-), 28.5 (12×$CH_3$, 4×OC(*C*$H_3$)$_3$), 27.6 (2×$CH_2$), 27.4 ($CH_2$), 26.8 ($CH_2$), 26.3 ($CH_2$), 24.4 ($CH_2$); MS (HR-FAB) calcd for $C_{38}H_{81}B_{10}N_5O_8$ 845.7015, found 845.7194. Anal. ($C_{38}H_{81}B_{10}N_5O_8$) C, H, N, B.

1-[(2′,2′-Dimethyl-1′,3′-dioxocyclopentan-4′-yl)methyl]-2-(4-tosylbutyl)-*o*-carborane (14). Tosyl chloride (5.93 g, 31 mmol) was added in several portions to a cooled (0 °C) mixture of 4-{2-[(2,2-dimethyl-1,3-dioxocyclopentan-4′-yl)methyl]-*o*-carboran-1-yl}butanol[25] (2.85 g, 8.5 mmol) and pyridine (6.0 mL) in CH_2Cl_2 (200 mL). The solution was stirred at RT overnight. Water (10 mL) was added, and the resulting mixture was stirred for 2 h. The mixture was washed with 1 N HCl (2 × 50 mL), saturated $NaHCO_3$ (2 × 50 mL), and brine (50 mL), dried over $MgSO_4$, and filtered. After evaporation of the solvent, the residue was flash chromatographed (EtOAc/hexane = 1:2) to afford **14** (3.75 g, 91%): IR (KBr) 2960w, 2569s, 1691s, 1346s, 1151s, 610m; ^{1}H NMR ($CDCl_3$) 7.76−7.81 (m, 2H, Ar*H*), 7.35−7.39 (m, 2H, Ar*H*), 4.24 (m, 1H, −OC*H*CH_2O-), 4.13 (dd, 1H, J = 8.2, 6.0, −OCHCH_2O-), 4.03 (t, 2H, J = 6.0, −SO_3CH_2-), 3.55 (dd, 1H, J = 8.2, 6.5, −OCHCH_2O-), 2.46 (s, 3H, ArCH_3-), 2.43 (dd, 1H, J = 15.5, 6.5, −B_{10}CCCH_2CH-), 2.38 (dd, 1H, J = 15.5, 5.0, −B_{10}CCCH_2CH-), 2.23 (ddd, 1H, J = 15.0, 10.5, 6.2, −B_{10}CCCH_2CH_2-), 2.16 (ddd, 1H, J = 15.0, 10.0, 6.0, −B_{10}-CCCH_2CH_2-), 1.60−1.70 (m, 2H, −CH_2CH_2CH_2CH_2-), 1.46−1.59 (m, 2H, −CH_2C$H_2CH_2CH_2$-), 1.37 and 1.34 (s, 2 × 3H, −OC(CH_3)$_2$O-); ^{13}C NMR ($CDCl_3$) 145.0 (C, Ar, *C*-1′), 132.7 (C, Ar, *C*-4′), 129.9 (2 CH, Ar), 127.8 (2 CH, Ar), 109.6 (C, −O*C*Me$_2$O-), 79.2 and 77.0 (2 C, −B_{10}*CC*H_2-), 77.4 (CH, −O*C*HCH$_2$O-), 69.5 and 69.0 (2 O*C*H_2), 39.4 (CH_2, −B_{10}CC*C*H_2-CH-), 34.3 (CH_2, −B_{10}CC*C*H_2CH_2-), 28.3 and 25.7 (2 CH_2, −B_{10}-CCCH_2*C*H_2*C*H_2CH_2-), 26.8 and 25.2 (2 Me, −OC(*C*H_3)$_2$O-), 21.6

(Me, ArCH_3); MS (HR-EI) calcd for $C_{19}H_{36}B_{10}O_5S$ 486.3231, found 486.3232. Anal. ($C_{19}H_{36}B_{10}O_5S$) C, H, B.

1-[(2′,2′-Dimethyl-1′,3′-dioxocyclopentan-4′-yl)methyl]-2-(4-iodobutyl)-*o*-carborane (15). To a solution of **14** (3.68 g, 7.6 mmol) in dry acetone (50 mL) was added NaI (3.42 g, 22.8 mmol). The mixture was stirred overnight at RT. After removal of the solid and acetone, the residue was flash chromatographed (EtOAc/hexane = 1:2) to give **15** (3.21 g, 96%): IR (KBr) 3342w, 2960w, 2569s, 1691s, 1489m, 1354m, 1151s; ^{1}H NMR ($CDCl_3$) 4.27 (m, 1H, −OC*H*CH_2O-), 4.15 (dd, 1H, J = 8.3, 6.0, −OCHCH_2O-), 3.58 (dd, 1H, J = 8.3, 6.5, −OCHCH_2O-), 3.19 (t, 2H, J = 6.8, −ICH_2-), 2.46 (dd, 1H, J = 15.5, 6.5, −B_{10}CCCH_2CH-), 2.41 (dd, 1H, J = 15.5, 5.0, −B_{10}-CCCH_2CH-), 2.19−2.34 (m, 2H, −B_{10}CCCH_2CH_2-), 1.79−1.87 (m, 2H, −CH_2CH_2CH_2CH_2-), 1.60−1.79 (m, 2H, −CH_2CH_2CH_2-CH_2-), 1.40 and 1.36 (s, 2 × 3H, −OC(CH_3)$_2$O-); ^{13}C NMR ($CDCl_3$) 109.6 (C, −O*C*Me_2O-), 79.3 and 76.9 (2 C, −B_{10}*CC*-CH_2-), 74.4 (CH, −O*C*HCH_2O-), 69.1 (CH_2, −OCH*C*H_2O-), 39.5 (CH_2, −B_{10}CC*C*H_2CH-), 33.8 (CH_2, −B_{10}CC*C*H_2CH_2-), 32.5 and 30.4 (2 CH_2, −B_{10}CCCH_2CH_2CH_2-), 26.9 and 25.3 (2 Me, −OC(CH_3)$_2$O-). 5.1 (ICH_2); MS (HR-EI) calcd for $C_{12}H_{29}B_{10}IO_2$ 442.2145, found 442.2192. Anal. ($C_{12}H_{29}B_{10}IO_2$) C, H, B.

***N*^{1},*N*^{10}-Bis(BOC)-*N*^{5}-[4-(3,3-dimethyl-2,4-dioxolanylmethyl-*o*-carboranyl)butyl]spermidine (16).** Compound **16** (538.4 mg, 0.82 mmol, 67%) was obtained from the reaction of SPD-BOC (461.8 mg, 1.34 mmol) and **15** (535.1 mg, 1.22 mmol): eluting solvents hexane:ethyl acetate (1:1) containing 7% MeOH; IR (neat) 3342w, 2922m, 2569s, 1684s, 1504s, 1151s, 1061m; ^{1}H NMR ($CDCl_3$) 5.25 (br, 1H, N*H*), 4.75 (br, 1H, N*H*), 4.30−4.08 (m, 2H, −CH_2O-), 3.61−3.50 (m, 1H, −C*H*O-), 3.21−3.01 (m, 4H, 2×-CONCH_2-), 2.50−2.26 (m, 6H, N(CH_2-)$_2$ + −CHC$H_2$$CB_{10}$-), 2.26−2.13 (m, 2H, NC$H_2$-), 1.75−1.45 (m, 12H, −$NCH_2CH_2CH_2$$CH_2$N− + −$NCH_2CH_2$$CH_2$N− + −$NCH_2CH_2CH_2CH_2$$CB_{10}$-), 1.45 (s, 18H, 2×−C($CH_3$)$_3$), 1.40 (s, 3H, −C$H_3$), 1.33 (s, 3H, −C$H_3$); ^{13}C NMR ($CDCl_3$) 156.0 (2×C, *C*OO), 109.5 (C, −O*C*Me_2O-), 80.1 and 76.8 (2 C, −B_{10}*CC*-), 79.0 and 78.9 (2×C, O*C*Me_3), 74.4 (CH, −O*C*HCH_2-O-), 69.1 (CH_2, −OCH*C*H_2O-), 53.7, 53.6 and 52.4 (3×CH_2, −N(*C*H_2CH_2-)$_3$-), 40.4 and 40.2 (2×CH_2, −NH*C*H_2CH_2-), 39.5 and 35.1 (2×CH_2, −B_{10}CC*C*H_2-), 28.4 (6×CH_3, 2 O*C*Me_3), 28.0 (CH_2), 27.6 (CH_2), 27.2 (CH_3), 26.8 (2×CH_2), 25.2 (CH_2), 24.4 (CH_3); MS (HR-EI) calcd for $C_{29}H_{62}B_{10}N_3O_6$ (M − H) 658.5569, found 658.5684. Anal. ($C_{29}H_{63}B_{10}N_3O_6$) C, H, N, B.

***N*^{1},*N*^{10},*N*^{14}-Tris(BOC)-*N*^{5}-[4-(3,3-dimethyl-2,4-dioxolanylmethyl-*o*-carboranyl)butyl]spermine (17).** Compound **17** (706.0 mg, 0.87 mmol, 74%) was obtained from the reaction of SPM-BOC (616.0 mg, 1.22 mmol) and **15** (513.9 mg, 1.17 mmol): eluting solvents hexane:ethyl acetate (1:1) containing 7% MeOH; IR (neat) 3342w, 2952m, 2569m, 1676s, 1159s; ^{1}H NMR ($CDCl_3$) 5.25 (br, 1H, N*H*), 4.25 (br, 1H, N*H*), 4.29−4.13 (m, 1H, −OC*H*CH_2-), 4.08 (dd, 1H, J = 8.2, 6.0, −CH_2O-), 3.52 (dd, 1H, J = 8.2, 6.3, −CH_2O-), 3.29−2.95 (m, 8H, 4×-CONCH_2-), 2.50−2.10 (m, 10H, N(CH_2-)$_3$ + −C$H_2$$CB_{10}H_{10}$-CC$H_2$-), 1.71−1.49 (m, 12H, 2×-CH_2C$H_2$$CH_2$- + −$NCH_2CH_2CH_2$-$CH_2$N− + −$CH_2CH_2CH_2$$CH_2$-), 1.38 (s, 9H, −OC(C$H_3$)$_3$), 1.37 (s, 18H, 2×-OC(C$H_3$)$_3$), 1.33 (s, 3H, −C$H_3$), 1.29 (s, 3H, −C$H_3$); ^{13}C NMR ($CDCl_3$) 156.0 (3×C, *C*OO), 109.5 (C, −O*C*O-), 80.1 and 76.8 (2×C, −B_{10}*CC*-), 79.5 (2×C, 2×O*C*Me_3), 78.8 (C, O*C*Me_3), 74.4 (CH, −O*C*H-), 69.1 (CH_2, −O*C*H_2), 53.7, 53.6 and 52.3 (3×CH_2, −N(*C*H_2CH_2-)$_3$-), 46.9 and 44.9 (2×CH_2, BOCN-(*C*H_2CH_2-)$_2$-), 39.7 and 37.8 (2×CH_2, BOCNH*C*H_2CH_2-), 39.5 (CH_2, −B_{10}CC*C*H_2CH-), 35.1 (CH_2, −B_{10}CC*C*H_2CH_2-), 28.43 (9×CH_3, 3×OC(*C*H_3)$_3$), 27.6 and 26.8 (2×Me, −OC(*C*H_3)$_2$O-), 28.6, 28.40, 27.4, 26.9, 26.2 and 24.4 (6×CH_2). Anal. ($C_{37}H_{78}$-$B_{10}N_4O_8$) C, H, N, B.

***N*^{1},*N*^{10}-Bis(BOC)-*N*^{5}-[(4-dihydroxyborylphenyl)methyl]-spermidine (18).** Compound **18** (328.4 mg, 0.68 mmol, 31%) was obtained from the reaction of SPD-BOC (907.9 mg, 2.63 mmol) and 4-(bromomethyl)phenylboronic acid (470.5 mg, 2.19 mmol) at 140 °C for 2 h: eluting solvents hexane:ethyl acetate (1:1) containing 3% MeOH; IR (KBr) 3347sbr, 1683vs, 1594m, 1506vs, 1373s, 1351s, 1263s, 1241s, 1160vs; ^{1}H NMR ($CDCl_3$) 8.10−7.92 (m, 2H, Ar*H*), 7.55 (br, 1H, O*H*), 7.40−7.24 (m, 2H, Ar*H*), 7.15 (br, 1H, O*H*), 5.40 (br, 1H, N*H*), 4.85 (br, 1H, N*H*), 3.65−3.42 (m, 2H, −CH_2Ar), 3.15−2.84 (m, 4H, 2×-CONC-H_2-), 2.60−2.31 (m, 4H, N(CH_2-)$_2$), 1.70−1.30 (m, 6H, −NCH_2-CH_2C$H_2$$CH_2$N− + −$CH_2CH_2CH_2$-), 1.48 (s, 18H, 2×−C(C$H_3$)$_3$); ^{13}C NMR ($CDCl_3$) 156.1 (2×C, *C*OO), 134.8 and 134.3 (2×C, Ar), 128.4 (4×CH, Ar), 79.1 (2×C, 2×O*C*Me_3), 58.7 (CH_2, N*C*H_2Ar), 53.2 and 51.8 (2×CH_2, *C*H_2N*C*H_2), 40.4 and 39.5 (2×CH_2, 2×NH*C*H_2), 28.4 (6×CH_3, 2×−C(*C*H_3)$_3$), 27.7 (CH_2, −$NCH_2CH_2CH_2$-), 26.5d and 23.7 (2×CH_2, −NCH_2CH_2CH_2-CH_2N-); MS (HR-EI) calcd for $C_{24}H_{41}BN_3O_6$ (M − H) 478.3088, found 478.3203. Anal. ($C_{24}H_{42}BN_3O_6$) C, H, N, B.

General Procedure for Deprotection. Compounds **8, 9**, **16**, and **17** were stirred overnight in MeOH and 3 N HCl at 40−50 °C. After removal of the solvents, the residue was dissolved in EtOH and concentrated twice. If the product was not soluble in EtOH, the compound was refluxed for 2 h in EtOH, and upon cooling to RT, the solid was collected and dried under high vacuum.

***N*^{5}-[4-(2-Aminoethyl-*o*-carboranyl)butyl]spermidine 4HCl (ASPD-5). ASPD-5** (126.8 mg, 0.24 mmol, 100%) was produced from **8** (162.0 mg, 0.24 mmol): mp 70−3 °C; IR (KBr) 3400m, 2980s, 2584s, 1586m, 1459s, 1129m, 716m; ^{1}H NMR (CD_3OD) 3.40−2.90 (m, 12H, 6×−N^+CH_2-), 2.85−2.68 (m, 2H, −C$H_2$$CB_{10}$), 2.52−2.38 (m, 2H, −C$H_2$$CB_{10}$), 2.30−2.08 (m, 2H, −$CH_2CH_2$$CH_2$-), 2.00−1.50(m, 8H, −$NCH_2CH_2CH_2$$CH_2$N− + −$CH_2CH_2CH_2$$CH_2$-); ^{13}C NMR ($CD_3OD$) 79.6 and 75.1 (2 C, −$B_{10}$*CC*$H_2$-), 51.6 (2×$CH_2$, N*C*$H_2CH_2$), 49.1 ($CH_2$, N*C*$H_2CH_2$), 37.9, 37.6 and 35.9 (3×CH_2, NH_3*C*H_2CH_2), 33.3 and 30.3 (2×CH_2, −B_{10}CC*C*H_2-), 25.7, 23.3, 22.0, 20.9 and 19.8 (5×CH_2); MS (HR-EI) calcd for $C_{15}H_{42}B_{10}N_4$ (M-4HCl) 388.4340, found 388.4321. Anal. ($C_{15}H_{46}B_{10}Cl_4N_4$) C, H, N.

***N*^{5}-[4-(2,3-Dihydroxypropyl-*o*-carboranyl)butyl]spermidine 3HCl (DHSPD-5). DHSPD-5** (350.6 mg, 0.67 mmol, 98%) was produced from **16** (420.1 mg, 0.68 mmol): IR (KBr) 3387s, 2937s, 2562s, 1601m, 1451m, 1016m, 716m; ^{1}H NMR (CD_3OD) 3.80−2.70 (m, 13H, −CH_2O− + −C*H*O− + 5×-CONCH_2-), 2.60−1.30 (m, 14H, −NCH_2C$H_2$$CH_2$N− + −C$H_2$-$CB_{10}H_{10}CCH_2$- + −$NCH_2CH_2CH_2$$CH_2$N− + −$NCH_2CH_2CH_2$-$CH_2$-); ^{13}C NMR ($CD_3OD$) 81.2 and 80.7 (2×C, −$B_{10}$*CC*-$CH_2$-), 72.2 (CH, −O*C*H-), 66.9 (CH_2, −O*C*H_2), 54.5, 54.3 and 49.0 (3×CH_2, −N(*C*H_2CH_2-)$_3$-), 40.5 and 40.3 (2×CH_2, NH_3*C*H_2-CH_2), 39.0 (CH_2, −B_{10}CC*C*H_2CH-), 35.5 (CH_2, −B_{10}CC*C*H_2-CH_2-), 27.9, 25.8, 24.8, 23.4 and 22.3 (5×CH_2). Anal. ($C_{16}H_{46}B_{10}$-$Cl_3N_3O_2$) C, H, N.

***N*^{5}-[4-(2-Aminoethyl-*o*-carboranyl)butyl]spermine 5HCl (ASPM-5). ASPM-5** (430.7 mg, 0.69 mmol, 87%) was produced from **9** (664.7 mg, 0.79 mmol): mp 175−8 °C; IR (KBr) 3410s, 2945s, 2577s, 1586m, 1451m, 1031m, 716m; ^{1}H NMR (CD_3-OD) 3.38−2.97 (m, 16H, 8×−N^+CH_2-), 2.70−2.68 (m, 2H, −C$H_2$$CB_{10}$), 2.50−2.36 (m, 2H, −C$H_2$$CB_{10}$), 2.25−2.00(m, 4H, 2×-$CH_2CH_2$$CH_2$-), 1.99−1.45 (m, 8H, −$NCH_2CH_2CH_2$$CH_2$N− + −$CH_2CH_2CH_2$$CH_2$-); ^{13}C NMR ($CD_3OD$) 81.9 and 77.5 (2×C, −$B_{10}$*CC*$H_2$-), 53.9, 53.7 and 51.4 (3×CH_2, −N(*C*H_2CH_2-)$_3$-), 48.3 and 45.9 (2×CH_2, N(*C*H_2CH_2-)$_2$-), 39.8, 38.0 and 35.5 (3×CH_2, NH_3*C*H_2CH_2), 32.5 and 27.8 (2×CH_2, −B_{10}CC*C*H_2-), 25.9, 25.2, 23.1 and 22.1 (4×CH_2), 24.2 (2×CH_2); MS (HR-FAB) calcd for $C_{18}H_{50}B_{10}N_5$ (M − 5HCl) 445.49, found 449.50. Anal. ($C_{15}H_{46}B_{10}Cl_4N_4$) C, H, N, B.

***N*^{5}-[4-(2,3-Dihydroxypropyl-*o*-carboranyl)butyl]spermine 4HCl (DHSPM-5). DHSPM-5** (354.6 mg, 0.57 mmol, 100%) was produced from **17** (467.2 mg, 0.57 mmol): mp 72−5 °C; IR (KBr) 3380s, 2945s, 2569s, 1603m, 1451m, 1024m, 716w; ^{1}H NMR (CD_3OD) 3.70−2.80 (m, 17H, −CH_2O− + −C*H*O− + 7×-CONCH_2-), 2.60−1.40 (m, 16H, 2×-CH_2CH_2-CH_2- + −C$H_2$$CB_{10}H_{10}CCH_2$- + −$NCH_2CH_2CH_2$$CH_2$N− + −$CH_2CH_2CH_2$$CH_2$-); ^{13}C NMR ($CD_3OD$) 81.3 and 80.7 (2×C, −$B_{10}$*CC*-), 72.3 (CH, −O*C*H-), 66.9 (CH_2, −O*C*H_2), 54.2, 53.8 and 51.5 (3×CH_2, −N^+H(*C*H_2CH_2-)$_3$-), 48.9 and 48.4 (2×CH_2, N^+H_2(*C*H_2CH_2-)$_2$-), 46.1 and 40.2 (2×CH_2, N^+H_3*C*H_2CH_2-), 38.1 (CH_2, −B_{10}CC*C*H_2CH-), 35.4 (CH_2, −B_{10}CC*C*H_2CH_2-), 27.8, 25.3, 24.4, 24.3, 23.2 and 22.2 (6×CH_2). Anal. ($C_{19}H_{54}B_{10}$-$Cl_4N_4O_2$) C, H, N, B.

Oxy-bis{*N*^{5}-{[4-(*B*-hydroxyboryl)phenyl]methyl}spermidine 3HCl} (Bis-BBSPD-5) and *N*^{5}-{[4-(*B*,*B*-Dihydroxyboryl)phenyl]methyl}spermidine 3HCl (BBSPD-5). Com-

pound **18** (960 mg, 2.0 mmol) in MeOH (10 mL) and 3 N HCl (4 mL) was stirred at RT for 2 days. After removal of the solvents, the solid was dried under high vacuum (752 mg, 99%). According to ^{1}H and ^{13}C NMR spectral analyses and elemental microanalyses, this product exists as a dehydro dimer, **Bis-BBSPD-5**, in dry $(CD_3)_2SO$ solution and in the solid state but as a monomer, **BBSPD-5**, in D_2O or $(CD_3)_2SO/D_2O$ solution.

Bis-BBSPD-5: IR (KBr) 3300br, 1602s, 1396s, 1359s, 1322s, 1108m, 1005s, 725s, 666s; ^{1}H NMR ($(CD_3)_2SO$) 11.19 (br, 2H, OH), 8.10−8.28 (br, 12H, N*H*), 7.84 (d, 4H, J = 8, Ar*H*), 7.63 (d, 4H, J = 8, Ar*H*), 4.30 (s, 4H, −CH_2Ar), 3.05−3.14 (m, 4H, −N$^+$CH_2-), 2.90−3.03 (m, 4H, −N$^+$CH_2-), 2.81−2.88 (m, 4H, −N$^+$CH_2-), 2.73−2.81 (m, 4H, −N$^+$CH_2-), 1.96−2.18 (m, 4H, −NCH$_2$CH_2CH$_2$N-), 1.79−1.95 (m, 4H, −NCH$_2$CH_2-), 1.50−1.61 (m, 4H, −NCH$_2$CH_2-); ^{1}H NMR (CD_3OD) 7.80 (d, 2H, J = 8, Ar*H*), 7.62 (d, 2H, J = 8, Ar*H*), 4.45 (s, 2H, −CH_2Ar), 3.30 (t, 2H, J = 8.5, −N$^+$CH_2-), 3.21 (t, 2H, J = 8.5, −N$^+$CH_2-), 3.02 (t, 2H, J = 7.7, −N$^+$CH_2-), 2.99 (t, 2H, J = 7.4, −N$^+$CH_2-), 2.35−2.07 (m, 2H, −NCH$_2$CH_2CH$_2$N-), 2.07−1.81 (m, 2H, −NCH$_2$CH_2-), 1.81−1.58 (m, 2H, −NCH$_2$CH_2-); ^{13}C NMR ($(CD_3)_2SO$) 135.4 (C, *C*-B in Ar), 134.5 (2×CH, Ar), 131.3 (C, Ar), 130.4 (2×CH, Ar), 55.7 (CH$_2$, NCH$_2$Ar), 50.6 and 48.5 (2×CH$_2$, -CH$_2$NCH$_2$-), 38.0 and 36.2 (2×CH$_2$, 2×NCH$_2$-), 24.1 (CH$_2$, NCH$_2$CH$_2$CH$_2$N), 21.0 and 19.7 (CH$_2$, NCH$_2$CH$_2$CH$_2$-CH$_2$N); ^{13}C NMR (CD_3OD) 136.8 (C, *C*-B in Ar), 135.7 (2×CH, Ar), 131.9 (C, Ar), 131.4 (2×CH, Ar), 58. 5 (CH$_2$, NCH$_2$Ar), 53.3 and 51.0 (2×CH$_2$, -CH$_2$NCH$_2$-), 40.0 and 38.0 (2×CH$_2$, 2×NCH$_2$-), 25.6 (CH$_2$, NCH$_2$CH$_2$CH$_2$N), 23.1 and 21.9 (CH$_2$, NCH$_2$CH$_2$CH$_2$CH$_2$N). Anal. ($C_{28}H_{56}B_2Cl_6N_6O_3$) C, H, N, B.

BBSPD-5: ^{1}H NMR ($(CD_3)_2SO/D_2O$ = 10:1) 7.83 (d, 2H, J = 8, Ar*H*), 7.53 (d, 2H, J = 8, Ar*H*), 4.30 (s, 2H, −CH_2Ar), 3.08 (t, 2H, J = 7.7, −N$^+$CH_2-), 2.95−3.03 (m, 2H, −N$^+$CH_2-), 2.82 (t, 2H, J = 7.5, −N$^+$CH_2-), 2.77 (t, 2H, J = 7.5, −N$^+$CH_2-), 1.97−2.09 (m, 2H, −NCH$_2$CH_2CH$_2$N-), 1.69−1.83 (m, 2H, −NCH$_2$CH_2-), 1.47−1.57 (m, 2H, −NCH$_2$CH_2-); ^{13}C NMR ($(CD_3)_2SO/D_2O$ = 10:1) 135.8 (C, *C*-B in Ar), 135.1 (2×CH, Ar), 131.6 (C, Ar), 130.8 (2×CH, Ar), 56.4 (CH$_2$, NCH$_2$Ar), 51.4 and 49.2 (2×CH$_2$, -CH$_2$NCH$_2$-), 38.4 and 36.5 (2×CH$_2$, 2×NCH$_2$-), 24.3 (CH$_2$, NCH$_2$CH$_2$CH$_2$N), 21.5 and 20.2 (CH$_2$, NCH$_2$CH$_2$-CH$_2$CH$_2$N).

***N*^{5}-{[4-(*B*-Ethoxy-*B*-hydoxyboryl)phenyl]methyl}spermidine 3HCl (Et-BBSPD-5).** Compound **18** (445 mg, 0.93 mmol) in MeOH (5 mL) and 3 N HCl (2 mL) was stirred at RT for 2 days. After removal of the solvents, the residue was dissolved in EtOH and concentrated twice. The solid was collected and dried under high vacuum to afford **Et-BBSPD-5** (367 mg, 0.92 mmol, 99%): IR (neat) 3387s, 3012s, 1594m, 1399m, 1324s, 1001m, 633m; ^{1}H NMR (CD_3OD) 7.72 (d, 2H, J = 8, Ar*H*), 7.53 (d, 2H, J = 8, Ar*H*), 4.37 (s, 2H, −CH_2Ar), 3.51 (q, 2H, J = 7, −OCH_2CH$_3$), 3.22 (t, 2H, J = 8.3, −N$^+$CH_2-), 3.13 (t, 2H, J = 8.2, −N$^+$CH_2-), 2.94 (t, 2H, J = 7.6, −N$^+$CH_2-), 2.91 (t, 2H, J = 7.4, −N$^+$CH_2-), 2.23−2.08 (m, 2H, −NCH$_2$CH_2CH$_2$N-), 1.92−1.77 (m, 2H, −NCH$_2$CH_2-), 1.69−1.55 (m, 2H, −NCH$_2$CH_2-), 1.08 (t, 3H, J = 7.0, −OCH$_2$CH_3); ^{13}C NMR (CD_3OD) 135.6 (C, Ar), 131.8 (C, Ar), 131.4 (4×CH, Ar), 58.6 (CH$_2$, NCH$_2$Ar or OCH$_2$), 58.3 (CH$_2$, OCH$_2$ or NCH$_2$-Ar), 53.4 and 51.2 (2×CH$_2$, -CH$_2$NCH$_2$-), 40.1 and 38.0 (2×CH$_2$, 2×NCH$_2$-), 25.6 (CH$_2$, NCH$_2$CH$_2$CH$_2$N), 23.2 and 21.9 (CH$_2$, NCH$_2$CH$_2$CH$_2$CH$_2$N), 18.3 (CH$_3$,-OCH$_2$CH$_3$). Anal. ($C_{16}H_{33}$-$BCl_3N_3O_3$) C, H, N, B.

2. Biological Studies. The variation for all the biological test data was ∼±10%. All tests were repeated 2−3 times. For in vitro toxicity studies, six replicates were used for each sample and at each time point.

(1) In Vitro Evaluation of Toxic Properties. The F98 glioma cell line was derived from an undifferentiated brain tumor induced by administering *N*-ethyl-*N*-nitrosourea to a pregnant inbred CD Fisher 344 rat and has been propagated in vitro and in vivo since 1971. Its morphology and in vitro characteristics have been described in detail,[70,71] and it has been used by us to evaluate a variety of boron compounds as potential delivery agents for BNCT.[59,72,73] The assay that was employed to detect the toxicity and/or growth inhibitory effects has been widely used[74] and is based on the incorporation of sulforhodamine B (SRB) by biosynthetically active (i.e., S phase) surviving cells following exposure to the test compound.

Ninety-six-well plates (Corning Glass Works, Corning, NY) were seeded with 10 000 F98 glioma cells/well in Dulbelcco's minimal essential medium (DMEM) containing 10% fetal bovine serum (FBS) (Hyclone, Logan, UT). Plates were incubated overnight at 37 °C in a humidified atmosphere containing 5% CO_2. DMEM was replaced with media containing varying concentrations of the polyamine analogue and incubated for an additional 24 h at 37 °C. To each well was added 50 μL of cold 50% TCA carefully, and plates were left for 1 h at 4 °C. Wells were decanted, washed five times with deionized water, and dried at RT. A 50-μL aliquot of a 0.4% solution of SRB (Sigma, St. Louis, MO) in 1% acetic acid was added to each well for a 20-min period at RT. After which, they were washed five times with distilled water and allowed to dry. Bound SRB was solubilized in 100 μL of 10 mM unbuffered Trizma base. Absorbance was read in an OTC 400 automated ELISA plate reader (Organon Teknika Corp., Durham, CA) at 492 nm. Mean values from six replicates were used for each concentration. Values were expressed as the percent of untreated control. The concentration required to produce a 50% reduction in SRB staining (i.e., IC_{50}) was calculated for each compound.

(2) Ethidium Bromide Displacement Assay. The binding of spermidine, spermine, and the polyamine analogues to DNA was determined by an ethidium bromide displacement assay.[61−63] To 10 μg of calf thymus DNA (Sigma, St. Louis, MO) in 2.8 mL of buffer containing 2 mM HEPES, 10 μM EDTA, and 9.4 mM NaCl (pH 7.0) was added 1.6 μM ethidium bromide (Sigma, St. Louis, MO). Fluorescence was measured on a model 8000 spectrofluorometer (SLM Instruments, Kanabee, IL). Emission and excitation wavelengths were 598 and 546 nm, respectively. The various polyamine compounds were individually added in 10-μL aliquots. The decrease in fluorescence was recorded, and the IC_{50} value was defined as the concentration of the polyamine compound required to reduce fluorescence of the ethidium bromide−DNA complex by 50%. These results and the comparison with SPD and SPM are presented in Table 2.

(3) Cellular Uptake. This was determined by culturing F98 glioma cells in the presence of the test polyamines at a concentration that was not toxic or growth inhibitory. To each of four T150 tissue culture flasks (Corning Glass Works, Corning, NY) was added 5×10^6 F98 glioma cells in DMEM, supplemented as described above in the toxicity/growth inhibition assay. After 24 h, the tissue culture medium was decanted from the flasks and replaced with medium containing a nontoxic concentration (5 μM) of carboranyl polyamines in DMEM. Boron concentrations in the media (∼5 μg/mL) were determined by DCP-AES, as described previously.[64] Cells were incubated at 37 °C in an atmosphere containing 5% CO_2 for 48 h (approximately four doubling times), following which the media was removed and its boron content was determined. Cells were washed three times with phosphate-buffered saline (PBS), and the wash fluids also were saved for boron determination. The cells then were desegregated using trypsin-EDTA (Gibco BRL, Grand Island, NY) and digested in a mixture of concentrated sulfuric acid and 70% hydrogen peroxide,[75] and the boron content was determined. Boron levels are expressed in micrograms per gram of cells, and as previously determined by direct cell counting, 10^9 cells ∼ 1 g. The results of these studies are presented in Table 3 and compared with values previously obtained for sodium undecahydromercapto-*closo*-dodecaborate (BSH) and L-*p*-boronophenylalanine (BPA) (Boron Biologicals Inc., Raleigh, NC).

(4) In Vivo Biodistribution Studies. a. Subcutaneous Tumors. Above 250 000 B16 melanoma cells were implanted subcutaneously in the left flank of C57Bl/6 mice (Charles River Laboratories, Wilmington, MA). After 7 days, animals were placed on a polyamine-deficient diet (Dyets, Inc., Bethlehem, PA) containing 0.2% neomycin and received water containing eflornithine HCl (DFMO) (Ilex Onclogy, San Antonio, TX). Ten

days after tumor implantation, Alzet pumps model 1007D (Alza Corp., Palo Alto, CA) containing compound were implanted subcutaneously between the scapulae, and the compounds were administered over 3−4 days while the polyamine-deficient diet and DFMO in the drinking water were continued for this period. **ASPD-5** was administered at a dose of 30 or 40 μg/g per day, using 5 mice per group. After 4 days, pumps were removed, animals were sacrificed, and tissues were removed for boron analysis, which was carried out by DCP-AES.

b. GL261 Brain Tumors. Sedated C57Bl/6 mice were placed in a stereotactic head frame (Kopf Inc., Tajunga, CA) and implanted with 10^5 GL261 cells suspended in serum-free DMEM containing 1.5% agarose at a depth of 2.5−3.0 mm into the brain. On day 16 postimplantation, mice were placed on a polyamine-deficient diet and water containing 2% DFMO (Ilex Oncology, San Antonio, TX). On day 19, Alzet pumps model 1003D (Alza Corp., Palo Alto, CA) containing the polyamine analogues were implanted subcutaneously between the scapulae of the mice for 3 days. The **ASPD-5** was administered at a dose of 30 μg/g per day and the **BBSPD-5** at a dose of 10 μg of boron/g per day. C57Bl mice were implanted intracerebrally with GL261 brain tumors. Animals were placed on a polyamine-deficient diet and water containing 2% DFMO for 3 days. Alzet pumps were implanted, and compound infusion was carried out for 3 days during which time they were maintained on a polyamine-deficient diet and DFMO. Animals were euthanized at the end of this 3-day period, and tissues were removed for boron analysis by DCP-AES. There were 5 mice per group.

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