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Crystallographic report

Synthesis and biological evaluation of novel azanonaboranes as potential agents for boron neutron capture therapy

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A number of (hydroxyalkylamine)-*N*-(aminoalkyl)azanonaborane(11) derivatives have been synthesized to provide azanonaboranes with different hydrophilic functional groups for use in the treatment of cancer by boron neutron capture therapy (BNCT). The exo-diamine group of (aminoalkylamine)-*N*-(aminoalkyl)azanonaborane(11) {$H_2N(CH_2)_mH_2NB_8H_{11}NH(CH_2)_mNH_2$, where $m = 4–6$} can be substituted by amino alcohol ligands {$HO(CH_2)_nNH_2$, where $n = 3$ and 4} to give azanonaboranes containing free amino and hydroxy groups: (3-hydroxypropylamine)-*N*-(aminobutyl)azanonaborane(11) {$HO(CH_2)_3H_2NB_8H_{11}NH(CH_2)_4NH_2$}, 1; (4-hydroxybutylamine)-*N*-(aminobutyl)azanonaborane(11) {$HO(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NH_2$}, 2; (3-hydroxypropylamine)-*N*-(aminopentyl)azanonaborane(11) {$HO(CH_2)_3H_2NB_8H_{11}NH(CH_2)_5NH_2$}, 3; (4-hydroxypropylamine)-*N*-(aminopentyl)azanonaborane(11) {$HO(CH_2)_4H_2NB_8H_{11}NH(CH_2)_5NH_2$}, 4; (3-hydroxypropylamine)-*N*-(aminohexyl)azanonaborane(11) {$HO(CH_2)_3H_2NB_8H_{11}NH(CH_2)_6NH_2$}, 5. The *in vitro* toxicity test using Chinese hamster-V79 cells showed that compounds 1–3 were less toxic (LD_{50} value of ~2.3, 1.7 and 1.4 mM, respectively) than spermine and spermidine (LD_{50} value of ~0.88 and 0.66 mM, respectively). *In vivo* distribution experiments of these compounds in Lewis lung carcinoma and B16 melanoma tumor-bearing mice showed that boron can be found in tumor tissue. The compounds prepared can be considered as a new class of boron containing polyamine compounds that may be useful for boron neutron capture therapy of tumors. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: azanonaborane; boron; BNCT; polyamines; V79-cells

INTRODUCTION

The therapeutic possibilities of neutron capture using boron were first proposed by Locher in 1936.[1] It is a binary radiation therapy based upon the nuclear fission of ^{10}B atoms by thermal neutrons. The neutron capture event results in the formation of the unstable [^{11}B] nucleus that fission to yield highly energetic species ^{7}Li and ^{4}He.[2] There are two main approaches to the development of boron compounds for boron neutron capture therapy (BNCT). One involves the synthesis of boronated analogs of organic structures, which posses a high degree of selectivity for neoplastic cells. The second approach emphasizes the use and incorporation of boron compounds into monoclonal antibodies targeted against tumor-associated antigens. With most tumor-seeking agents, drug delivery is dependent upon sufficient vascularization within the tumor itself.

Puterscine, spermidine (SPD) and spermine (SPM) are ubiquitous intracellular polycationic molecules that are essential for cell growth and differentiation.[3,4] Polyamines also have properties that make them attractive potential candidates as boron carriers, for cancer treatment. First, they have transport systems that increase their uptake in malignant cells.[5] Second, cationic polyamines are able to interact electrostatically with DNA in non-specific manner.[6–8] Therefore, boronated polyamines may be able to target DNA directly once they penetrate the cell membrane. This

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has been the basis for the preparation of various classes of boron containing polyamines.[9,10] Recently *o*-carborane cages attached to polyamines have been made.[11] *In vitro*, these compounds bind to DNA and accumulate in tumor cells similarly to borocaptate (BSH) and *p*-borophenylalanine (BPA), which are in clinical trials. However, the cytotoxicity of carboranes prohibits their use. The potential of *N*-benzylpolyamines as boron vectors for tumor targeting has been shown by a recent *in vitro* study.[12]

The nine-vertex azanonaborane (Fig. 1) is an interesting boron carrier for BNCT because it is a neutral compound compared with the presently used boron clusters, which are either single or double negatively charged or very hydrophobic, water stable and more or less water soluble for physiological transport. The eight boron atom derivatives offer suitable boron content, which makes them good candidates for the selective delivery of boron for BNCT. In that case and on condition that the compounds are not toxic, they can be regarded as a new type of boron carrier for BNCT. Azanonaboranes containing free hydroxy groups have been synthesized.[13] Biodistribution studies of these compounds have shown that modification of the azanonaboranes is necessary to optimize tumor seeking properties for use in BNCT.[13]

We have previously designed and synthesized azanonaboranes containing polyamine analogs of SPD and SPM.[14] It was observed that the toxicity strongly depended upon the number of carbon atoms in the diamine chain. To reduce this toxicity, the hydrophilic properties of the compounds were increased by exchanging the exo-amino ligand with 3-amino-1-propanol and 4-amino-1-butanol to give the desired target compounds (hydroxyalkylamine)-*N*-(aminoalkyl)azanonaborane(11) derivatives. The study of their structure–activity relationship with respect to their *in vitro* toxicities was investigated using V79 cells.

RESULTS AND DISCUSSION

Preparation

The development of new water-soluble compounds is essential to the continued evaluation of BNCT. The functionalization of the azanonaboranes imparts a chemical reactivity which can assist their intracellular retention within tumors or provide the means of attaching them to organic molecules.

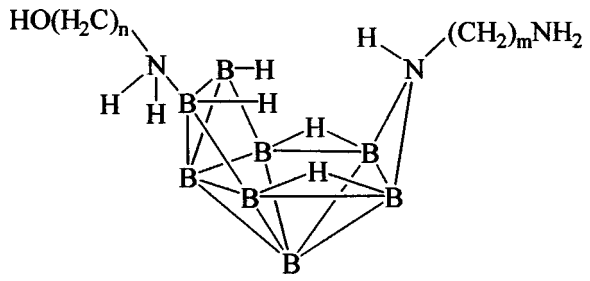
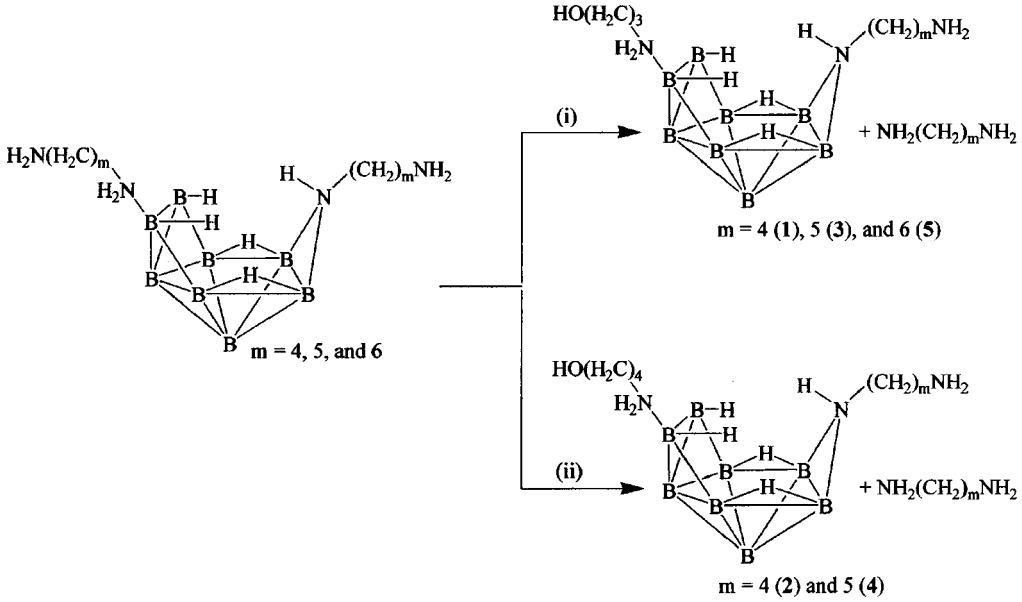


Figure 1. Structure of (hydroxyalkylamine)-*N*-(aminoalkyl)azanonaborane(11) derivatives (exo-hydrogen atoms are omitted for clarity).

The direct introduction of both hydroxy groups is experimentally difficult because it is not possible to obtain a pure compound.[13,15] An initial ligand exchange reaction of $(Me_2S)B_9H_{13}$ with diamine to give $H_2N(CH_2)_mH_2NB_9H_{13}$ followed by treatment with $HO(CH_2)_nNH_2$ to yield the mixed species $\{HO(CH_2)_nH_2NB_8H_{11}NH(CH_2)_mNH_2\}$ was not possible, probably due to the side reactions of the hydroxy groups with clusters. This problem has been elegantly solved by exchanging the exo-amino ligand of $\{H_2N(CH_2)_mH_2NB_8H_{11}NH(CH_2)_mNH_2\}$ with amino alcohol ligands [$HO(CH_2)_nNH_2$, where $n = 3$ and 4] in toluene to give mixed species $\{HO(CH_2)_nH_2NB_8H_{11}NH(CH_2)_mNH_2$, where $m = 4–6\}$ (Scheme 1). The product is a nine-vertex cluster based on eight boron atoms with one nitrogen bridge $\{B_8N\}$ and one exo-amine ligand (Fig. 1). The reaction of (aminoalkylamine)-*N*-(aminoalkyl)azanonaborane(11) $\{H_2N(CH_2)_mH_2NB_8H_{11}NH(CH_2)_mNH_2$, where $m = 4–6\}$ with 3-amino-1-propanol in refluxing toluene to which a few drops of THF have been added in the ratio 1 : 1 over 2 h yielded (3-hydroxypropylamine)-*N*-(aminoalkyl)azanonaborane(11) derivatives $\{HO(CH_2)_3H_2NB_8H_{11}NH(CH_2)_mNH_2$ where $m =$ 4 (**1**), 5 (**3**) and 6 (**5**)} in 45–57% yield (Scheme 1). Under the same reaction conditions, the use of 4-amino-1-butanol leads to the formation of (4-hydroxybutylamine)-*N*-(aminoalkyl)azanonaborane(11) $\{HO(CH_2)_4H_2NB_8H_{11}NH(CH_2)_mNH_2$, where $m = 4$ (**2**) and 5 (**4**)} in higher yields, 65% (Scheme 1). The $\{B_8N\}$ clusters were purified by thin-layer chromatography on silica gel using THF and CH_2Cl_2 (1 : 2) as eluent. The NMR spectroscopic data $\delta(^{11}B)[\delta(^1H)]$ of the azanonaborane derivatives (**1, 2, 3, 4** and **5**) are summarized in Table 1.

With 2-aminoethanol, no reaction was observed under the same conditions. However, monitoring the reaction mixture by NMR spectroscopy after prolonged heating showed that progressive loss of the boron cluster occurred to give boric acid and $H_3BNH_2(CH_2)_nNH_2\{\delta(^{11}B) - 18.86\}$. The NMR spectroscopic data of the series of compounds among all the family numbers (**1**, **2**, **3**, **4** and **5**) were also very similar (Table 1), although there were some minor variations in the proton shielding as the organodiamine and organoaminoalcohol groups changed (see Experimental section).

Compared with azanonaboranes containing free amino groups,[14] the present B_8N cluster is stable for periods up to a week. Therefore, these compounds may be useful for BNCT of tumors. Both amino and hydroxy groups increase the water solubility and stability of these compounds in comparison to azanonaborane containing only free amino groups. The stability of the water-soluble compounds at room temperature was investigated by ^{11}B-NMR measurements. At different periods of time, the ratio of the compound to boric acid was determined. The data were interpreted as first-order kinetics.



Scheme 1. General synthetic route of (hydroxyalkylamine)-*N*-(aminoalkyl)azanonaborane(11) derivatives. Conditions: (i) 3-amino-1-propanol, toluene, two drops of THF, reflux, 2 h; (ii) 4-amino-1-butanol, toluene, two drops of THF, reflux, 2 h.

Table 1. 200 MHz (^{11}B, ^{1}H) NMR data for **1**, **2**, **3**, **4** and **6** in THF-d_8 at 20 °C

Compound	B1 $\delta(^{11}B)$ [$\delta(^{1}H)$]	B2 $\delta(^{11}B)$ [$\delta(^{1}H)$]	B3 $\delta(^{11}B)$ [$\delta(^{1}H)$]	B4 $\delta(^{11}B)$ [$\delta(^{1}H)$]	B5 $\delta(^{11}B)$ [$\delta(^{1}H)$]	B6 $\delta(^{11}B)$ [$\delta(^{1}H)$]	B7 $\delta(^{11}B)$ [$\delta(^{1}H)$]	B8 $\delta(^{11}B)$ [$\delta(^{1}H)$]	μH(4,5) μH(6,7) [$\delta(^{1}H)$]	NH [$\delta(^{1}H)$]
1	1.7	−55.2	−19.8	−32.6	−11.2	−11.2	−32.6	−33.0		
	[2.56]	[−0.82]	[1.2]	[0.62]	[2.38]	[2.38]	[0.48]	[0.29]	[−2.12]	[−1.23]
								[−0.72]	[−2.12]	
2	1.6	−55.5	−20.1	−32.3	−11.3	−10.88	−32.3	−33.2		
	[2.56]	[−0.86]	[1.2]	[0.65]	[2.31]	[2.47]	[0.45]	[0.28]	[−2.14]	[−1.22]
								[−0.68]	[−2.14]	
3	1.7	−55.2	−20.1	−33.0	−10.8	−10.8	−33.0	−33.0		
	[2.49]	[−0.85]	[1.18]	[0.62]	[2.43]	[2.43]	[0.43]	[0.26]	[−2.18]	[−1.21]
								[−0.75]	[−2.18]	
4	1.5	−55.6	−20.1	−33.0	−11.2	−11.2	−33.0	−33.0		
	[2.51]	[−0.82]	[1.11]	[0.65]	[2.39]	[2.39]	[0.45]	[0.26]	[−2.16]	[−1.2]
								[−0.72]	[−2.17]	
5	1.89	−55.4	−20.36	−33.0	−11.0	−11.0	−33.0	−33.0		
	[2.59]	[−0.85]	[1.28]	[0.66]	[2.39]	[2.39]	[0.45]	[0.27]	[−2.18]	[−1.23]
								[−0.72]	[−2.18]	

The rate constant for all compounds was $K = 0.1$/day, corresponding to a half-life of 7 days. It is possible to prepare a stock solution of all compounds with a concentration of 3000 μg boron ml^{-1} (3.48×10^{-2} M).

Biological studies

The *in vitro* experiments were carried out to determine whether these structures are biologically similar to SPM or SPD and whether they possess the requisite properties for a BNCT agent. In BNCT, the successful treatment of cancer requires the selective accumulation of enough ^{10}B within malignant tumors, sufficient low toxicity, water solubility, sufficient clearance from surrounding healthy tissue including blood, and a sufficient neutron fluence at the depth of the tumor. Initial evaluation of the toxicity of the {B_8N} clusters was judged by cloning survival tests on V79 cells.

The results of the *in vitro* toxicities of B_8N clusters are summarized in Table 2 and shown in Fig. 2. Incubation concentrations for all *in vitro* experiments varied between

Table 2. *In vitro* toxicity of azanonaboranes by V79 cells after 16 h

C_{media}		Percentage of survival (%)				
(μg boron ml^{-1})	(mM)	**1**	**2**	**3**	**4**	**5**
50	0.58	95.51 ± 1.870	89.3 ± 1.21	85.3 ± 1.53	43.7 ± 1.63	33.7 ± 0.87
75	0.88	88.35 ± 2.68	76.12 ± 1.65	71.25 ± 1.24	33.35 ± 1.76	28.65 ± 2.68
150	1.74	73.35 ± 1.65	59.45 ± 1.53	53.21 ± 1.25	25.34 ± 0.87	26.35 ± 0.87
200	2.32	55.22 ± 1.34	46.23 ± 1.82	42.0 ± 1.25	15.34 ± 1.78	18.54 ± 1.2
250	2.89	46.37 ± 1.32	38.52 ± 2.87	32.64 ± 1.9	<1	<1
300	3.47	38.23 ± 1.85	26.0 ± 1.01	22.24 ± 0.75	—	—

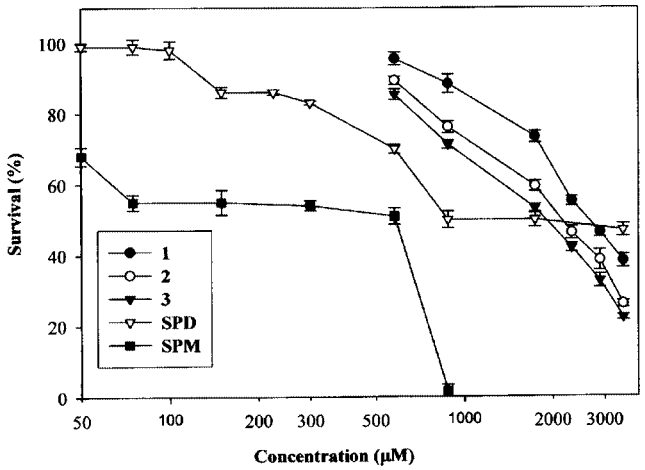


Figure 2. Percentage (±SD) of *in vitro* survival cell with respect to the concentration of B_8N cluster compounds (**1**–**3**), SPD and SPM. The data for SPD and SPM were taken from El-Zaria *et al*.[14]

50 and 300 μg boron ml^{-1} (Table 2). The *in vitro* experiments of the five compounds showed that **1** (LD_{50} = 200 μg boron ml^{-1}) has the lowest toxicity compared with **2** (LD_{50} = 180 μg boron ml^{-1}) and **3** (LD_{50} = 120 μg boron ml^{-1}) and is probably the most interesting compound for BNCT.

The LD_{50} value of compounds **1, 2** and **3** is 2.3, 1.7, and 1.4 mM, respectively, which is higher than the LD_{50} of SPM and SPD, which is 0.88 and 0.66 mM respectively (Table 2). The *in vitro* toxicity of the compounds **4** and **5** indicates that the toxicity was also increased with increasing number of carbon atoms in the amino alcohol or diamine chains. The *in vitro* toxicities of the compounds were not tested at lower concentrations because the achievable concentration of boron would not be effective for BNCT. According to these results, the *in vitro* toxicities of clusters **1, 2**, and **3** were about the same as those of SPD and SPM. Nevertheless, the *in vitro* toxicities of these compounds were significantly lower than that of the previously described structures, (3-aminopropylamine)-*N*-(aminobutyl)azanonaborane(11) $\{H_2N(CH_2)_3H_2NB_8H_{11}NH(CH_2)_4NH_2\}$ and (4-aminobutylamine)-*N*-(aminobutyl) azanonaborane(11) $\{H_2N(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NH_2\}$.[14] The data presented in the proceeding sections showed that compounds **1, 2** and **3** possess low cellular toxicity.

The effects of BPA and BSH were studied for BNCT using the SCCVII tumor in C3H/He mice.[16] C57 mice bearing B16 melanoma were used for the *in vivo* experiments of Morris *et al*.[17] This analysis was carried out to evaluate the suitability of compounds for BNCT.

The *in vitro* results obtained encouraged us to study an *in vivo* biodistribution in tumor-bearing mice of compounds **1** and **2** 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 C3H/He mice bearing SCCVII tumors and C57 mice bearing B16 tumors by quantitative neutron capture radiography (QNCR).[13] Studies using **1** in the SCCVII tumor in C3H/He mice revealed that the greatest amount of boron was found in the intestine (42.2 μg boron g^{-1}), while the concentrations in the kidney, brown fat, liver and tumor were only 5.4, 8.7, 2.8 and 12.6 μg boron g^{-1}, respectively. Similarly for compound **2**, the level in the intestine was the highest (45.5 μg^{-1} boron g^{-1}), while the values in the kidney, brown fat, liver and tumor were 6.7, 8.9, 5.4 and 10.2 μg boron g^{-1}, respectively. The tumor–blood ratio (T–Bl) of compound **1** was 4.5 and that of **2** was 2.7 (Table 3).

In the evaluation of these compounds as potential BNCT agents for melanoma, C57 mice bearing B16 melanoma were used (Fig. 3). For compounds **1** and **2**, the largest amounts of boron were found in the intestine (41.3 and 44.6 μg^{-1} boron g^{-1}, respectively), while the values in the tumor were only 10.3 and 9 μg boron g^{-1}, respectively (Table 4). The T–Bl ratio of **1** was 3.9 and that of **2** was 2.9. Virtually no boron uptake was found in brain and muscle (Fig. 3). The boron levels in the intestine were markedly higher than those observed for the tumor, which suggests that the intestine is involved in the metabolism of both compounds.

In conclusion, we have succeeded in synthesizing functionalized azanonaborane cluster via ligand exchange reaction to achieve water solubility by a one-step process.

Table 3. Biodistribution of azanonaboranes **1** and **2** in C3H/He mice bearing SCCVII tumor

Tissue	**1** (dose = 3.0 mg ml^{-1}) (μg boron g^{-1})	**2** (dose = 3.16 mg ml^{-1}) (μg boron g^{-1})
Tumor	12.6 ± 2.3	10.2 ± 3.1
Brain	—	—
Blood	2.8 ± 1.8	3.7 ± 1.9
Liver	2.8 ± 1.0	3.1 ± 1.6
Intestine	42.2 ± 12.3	45.5 ± 8.6
Kidney	5.4 ± 1.6	6.7 ± 2.4
Brown fat	8.7 ± 3.2	8.9 ± 1.9
Muscle	—	—
T–Bl ratio	4.5	2.7

Four mice were sacrificed for each compound after different periods of time (0.5, 1, 2 and 4 h). The values shown are means ± standard deviations for each set of determinations.

Table 4. Biodistribution of azanonaboranes **1** and **2** in C57 mice bearing B16 melanoma

Tissue	**1** (dose = 3.0 mg ml^{-1}) (μg boron g^{-1})	**2** (dose = 3.16 mg ml^{-1}) (μg boron g^{-1})
Tumor	10.3 ± 1.7	9.2 ± 2.8
Brain	—	—
Blood	2.6 ± 1.3	3.1 ± 1.8
Liver	2.1 ± 1.3	3.4 ± 1.5
Intestine	41.3 ± 10.3	44.6 ± 12.7
Kidney	5.6 ± 1.8	6.2 ± 1.6
Brown fat	7.3 ± 2.2	8.1 ± 2.4
Muscle	—	—
T–Bl ratio	3.9	2.9

Four mice were sacrificed for each compound after different periods of time (0.5, 1, 2 and 4 h). The values shown are means ± standard deviations for each set of determinations.

The exo-diamino ligand of B_8N clusters can be exchanged with other amino alcohol ligands such as 3-amino-1-propanol and 4-amino-1-butanol to yield azanonaboranes containing free hydroxy and amino groups in good yield. These compounds (**1, 2, 3, 4** and **5**) were investigated systematically to elucidate their potential as boron carriers for BNCT. *In vitro* toxicities of these compounds were investigated using V79 cells. The investigation of the toxicity of these compounds showed that, the more CH_2 units the compound contains, the more toxic the compound is. In comparison with the *in vitro* toxicities SPD and SPM, we found that the three compounds **1, 2** and **3** were not toxic. As compared with azanonaboranes containing free amino groups, their solubility in water and their low toxicity are advantages over the previously reported azanonaboranes. The biodistribution in tumor-bearing mice shows no enrichment of **1** and **2** in any special organ except the intestine. The toxicity of the cluster *in vitro* and *in vivo* is low, and the toxicity of a compound is governed mostly by its side chains.

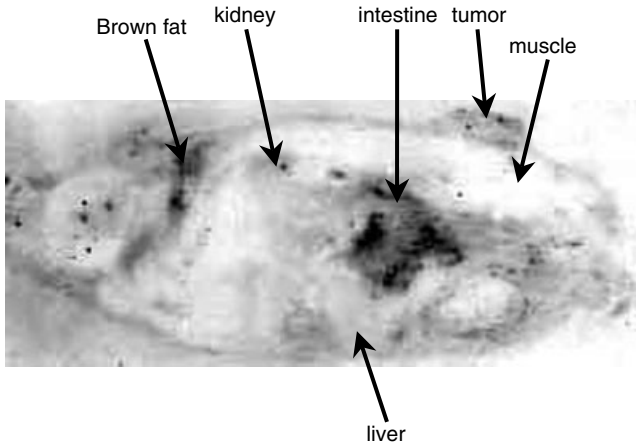


Figure 3. Whole-body alpha-autoradiogram of a B16 melanoma tumor-bearing mouse. The animal was injected intraperitoneally with 1000 μg boron 0.5 ml^{-1} of compound **1** and sacrificed after 1 h.

EXPERIMENTAL

General

Reagents were purchased from chemical sources and used as received. 3-Amino-1-propanol, 4-amino-1-butanol and 2-aminoethanol were commercially available. Dimethyl sulfide-arachno-nonaborane {$(Me_2S)B_9H_{13}$} and (aminoalkylamine)-*N*-(aminoalkyl)azanonaborane(11) {$H_2N(CH_2)_mH_2NB_8H_{11}NH(CH_2)_mNH_2$, where $m = 4–6$} were prepared as described in the literature.[14,18] The measurements for NMR (^{11}B, ^1H and ^{13}C) were carried out on a Bruker DPX 200 spectrometer. The chemical shifts δ are given in ppm relative to $\Xi = 100$ MHz for $\delta(^1H)$ (nominally $SiMe_4$), and $\Xi = 32.083\,972$ MHz for $\delta(^{11}B)$ (nominally F_3BOEt_2) in (THF-d_8). Mass spectrometry data were measured using a Finnigan MAT 8222 by fast atom bombardment (FAB) with glycerol or nitrobenzylalcohol (NBA) as matrix. I.R. (cm^{-1}) spectra were determined using KBr disks on a Biorad FTS-7 spectrometer. Plate chromatography was conducted on silica gel 60 (Fluka). Elemental analyses were performed using a Perkin-Elmer 2400 automatic elemental analyzer.

General procedure of compounds 1–5

A solution of (aminoalkylamine)-*N*-(aminoalkyl)azanonaborane(11) {$H_2N(CH_2)_mH_2NB_8H_{11}NH(CH_2)_mNH_2$, where $m = 4–6$} (1.2 mmol) was added to a solution of amino alcohols {$HO(CH_2)_nNH_2$, where $n = 3$ and 4} (1.2 mmol) in 20 ml of dry toluene with few drops of THF. The reaction was heated to reflux for 2 h. After cooling of the reaction mixture to room temperature, the solution was filtered. All volatile components of the filtrate were removed under vacuum at room temperature. The resulting oily substance was chromatographed on silica gel using THF and CH_2Cl_2 (1 : 2) as eluent, to yield a colorless oil.

(3-Hydroxypropylamine)-N-(aminobutyl)azanonaborane(11)

{$HO(CH_2)_3H_2NB_8H_{11}NH(CH_2)_4NH_2$} **1** (yield: 45%, 172 mg, 0.66 mmol, $R_f = 0.28$); NMR (THF-d_8): +63.1 [3.46] [$NH_2(CH_2)_2\mathbf{C}H_2OH$], +51.5[+2.87]($NH_2\mathbf{C}H_2$), +30.2[+1.69]($NH_2CH_2\mathbf{C}H_2$), +50.3[+2.59]($NH\mathbf{C}H_2$), +28.5[+1.69]($NHCH_2\mathbf{C}H_2$), +47 [3.89] [$NH(CH_2)_2\mathbf{C}H_2$], +47.2 [3.89] +48.16[+2.61] [$NH(CH_2)_3\mathbf{C}H_2NH_2$], +29.5 [1.61], +27.8[+1.38][$NHCH_2(\mathbf{C}H_2)_2CH_2NH_2$], +51.7[+2.82][$NH\mathbf{C}H_2(CH_2)_3NH_2$]; FABMS(glycerol): m/z, 261 ($[M]^+$, 26%); IR, 3372 (s, ν, OH), 3232 (s, ν, NH_2/NH), 2541 (s, ν, BH), 1625 (w, δ, NH_2/NH), 1342 (s, ν, BN), 2952 (m), 2884 (m), 1465 (m), 1435 (m), 1402 (m), 1167 (m), 1114 (s), 744 (m) (s, ν, δ, γ of CH_2-groups); analyzed, found—C, 32.43; H, 11.74; N, 15.89; $B_8C_7H_{31}N_3O$ requires C, 32.38; H, 11.95; N, 16.19%.

(4-Hydroxybutylamine)-N-(aminobutyl)azanonaborane(11)

{$HO(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NH_2$} **2** (yield: 57%, 226 mg, 0.83 mmol, $R_f = 0.26$); NMR (THF-d_8): +57.8 [3.61] [$NH_2(CH_2)_3\mathbf{C}H_2OH$], +47.5[+3.2]($NH_2\mathbf{C}H_2$), +30.1[+1.63], 30.3 [+1.65][$NH_2CH_2(\mathbf{C}H_2)_2CH_2OH$], +49.9[+2.58]($NH\mathbf{C}H_2$), +29.8[+1.45]($NHCH_2\mathbf{C}H_2$), +30.8 [1.38] [$NH(CH_2)_2\mathbf{C}H_2$], +48.7 [4.01] [$NH(CH_2)_3\mathbf{C}H_2NH_2$]; FABMS(glycerol): m/z, 275 ($[M]^+$, 23%); IR, 3392 (s, ν, OH), 3238 (s, ν, NH_2/NH), 2555 (s, ν, BH), 1645 (w, δ, NH_2/NH), 1338 (s, ν, BN), 2959 (m), 2887 (m), 1469 (m), 1437 (m), 1401 (m), 1159 (m), 1116 (s), 745 (m) (s, ν, δ, γ of CH_2-groups); anal. found—C, 34.94; H, 12.03; N, 15.27; $B_8C_8H_{33}N_3O$ requires C, 35.11; H, 12.07; N, 15.36%.

(3-Hydroxypropylamine)-N-(aminopentyl)azanonaborane(11)

{$HO(CH_2)_3H_2NB_8H_{11}NH(CH_2)_5NH_2$} **3** (yield: 55%, 206 mg, 0.75 mmol, $R_f = 0.32$); NMR (THF-d_8): +59.2 [3.56] [$NH_2(CH_2)_2\mathbf{C}HOH$], +46.6[+2.89]($NH_2\mathbf{C}H_2$), +29.8[+1.79]($NH_2CH_2\mathbf{C}H_2$), +51.5[+2.65]($NH\mathbf{C}H_2$), +29.5[+1.47], +29.3 [1.46] [$NHCH_2(\mathbf{C}H_2)_2$], +30.4 [1.67] [$NH(CH_2)_3\mathbf{C}H_2$], +49 [3.88] [$NH(CH_2)_4\mathbf{C}H_2NH_2$]; FABMS(glycerol): m/z, 275 ($[M]^+$, 22%); IR 3385 (s, ν, OH), 3235 (s, ν, NH_2/NH), 2551 (s, ν, BH), 1648 (w, δ, NH_2/NH), 1335 (s, ν, BN), 2962 (m), 2889 (m), 1471 (m), 1441 (m), 1402 (m), 1157 (m), 1113 (s), 744 (m) (s, ν, δ, γ of CH_2-groups); anal. found—C, 35.01; H, 11.91; N, 15.32; $B_8C_8H_{33}N_3O$ requires C, 35.11; H, 12.07; N, 15.36%.

(4-Hydroxybutylamine)-N-(aminopentyl)azanonaborane(11)

{$HO(CH_2)_4H_2NB_8H_{11}NH(CH_2)_5NH_2$} **4** (yield: 65%, 252 mg, 0.88 mmol, $R_f = 0.35$); NMR (THF-d_8): +61.2 [3.7] [$NH_2(CH_2)_3\mathbf{C}H_2OH$], +48.3[+2.92]($NH_2\mathbf{C}H_2$), +30.8[+1.68], 30.6 [+1.64][$NH_2CH_2(\mathbf{C}H_2)_2CH_2OH$], +51.1[+2.62]($NH\mathbf{C}H_2$), +30.4[+1.49], +30 [1.46] [$NHCH_2(\mathbf{C}H_2)_2$], +29.8 [1.67] [$NH(CH_2)_3\mathbf{C}H_2$], +48.8 [3.98] [$NH(CH_2)_4\mathbf{C}H_2NH_2$]; FABMS(NBA): m/z, 289 ($[M]^+$, 28%); IR 3389 (s, ν, OH), 3241 (s, ν, NH_2/NH), 2544 (s, ν, BH), 1648 (w, δ, NH_2/NH), 1343 (s, ν, BN), 2961 (m), 2889 (m), 1471 (m), 1439 (m), 1405 (m), 1159 (m), 1115 (s), 745 (m) (s, ν, δ, γ of CH_2-groups); analytically found—C, 37.29; H, 12.10; N, 14.56; $B_8C_9H_{35}N_3O$ requires C, 37.57; H, 12.17; N, 14.61%.

(3-Hydroxypropylamine)-N-(aminohexyl)azanonaborane(11)

{$HO(CH_2)_3H_2NB_8H_{11}NH(CH_2)_6NH_2$} **5** (yield: 65%, 248 mg, 0.86 mmol, $R_f = 0.33$); NMR (THF-d_8): +59.7 [3.66] [$NH_2(CH_2)_2\mathbf{C}HOH$], +47.3[+2.96]($NH_2\mathbf{C}H_2$), +30[+1.86]($NH_2CH_2\mathbf{C}H_2$), +50.2[+2.68]($NH\mathbf{C}H_2$), +32.4[+1.61]($NHCH_2\mathbf{C}H_2$), +33, +30.7, +32.7, +30.2 [1.42–1.66] [$NHCH_2(\mathbf{C}H_2)_4$], +49 [3.95] [$NH(CH_2)_5\mathbf{C}H_2NH_2$]; FABMS(NBA): m/z, 289 ($[M]^+$, 21%); IR 3391 (s, ν, OH), 3239 (s, ν, NH_2/NH), 2548 (s, ν, BH), 1657 (w, δ, NH_2/NH), 1336 (s, ν, BN), 2973 (m), 2898 (m), 1472 (m), 1442 (m), 1402 (m), 1157 (m), 1115 (s), 745 (m) (s, ν, δ, γ of CH_2-groups); anal. found: C, 37.45; H, 12.04; N, 14.47; $B_8C_9H_{35}N_3O$ requires C, 37.57; H, 12.17; N, 14.61%.

Cell culture

V79 cells (Chinese hamster fibroblasts) were grown in 9.89 g l^{-1} HAM'S F-10 (Biochrom KG, Germany) supplemented with 1.2 g $NaHCO_3$ g l^{-1}, 10 ml l^{-1} penicillin–streptomycin (10, 000 U–10 000 µg mL^{-1}, Biochrom KG, Germany), and 5% fetal calf serum (FCS). When cells were grown in azanonaboranes, 2 mM aminoguanidine was added as an inhibitor of serum amine oxidases.[19]

B16 tumor cells were grown in 9.69 g l^{-1} Eagle minimum essential medium (EMEM) (Biochrom KG) supplemented with 10 ml l^{-1} penicillin–streptomycin (10 000 U–10 000 µ g ml^{-1}, Biochrom KG), 2.2 g l^{-1} $NaHCO_3$ and 10% FCS.

SCCVII tumor cells (mouse squamous cell carcinoma), were exponentially grown in 9.4 g l^{-1} EMEM (Sigma Aldrich Co.) supplemented with 292 mg l^{-1} L-glutamine, 7.5% $NaHCO_3$; 10 ml l^{-1} penicillin–streptomycin (10 000 U–10 000 µg ml^{-1}, Biochrom KG) and 12.5% FCS.

Cloning survival test

All tests were repeated two or three times. For each compound Petri dishes were seeded with V79 cells in F10 essential medium containing 5% FCS. Dishes were incubated overnight at 37 °C in a humidified atmosphere containing 5% CO_2. The medium was replaced with medium containing varying concentrations of the compound under investigation (50, 75, 150, 200, 250 and 300 µg boron ml^{-1}) and incubated for an additional 16 h at 37 °C. The medium was removed from the dishes. The cells were suspended by trypsinization, counted and seeded into new dishes at different dilutions. The numbers of colonies formed after one week were compared with the numbers of colonies formed in the control without boron. The medium was removed, washed with PBS, dyed with GIEMSA for 10–15 min and washed again with absolute ethanol. The means and the standard deviations were calculated for each incubation condition.

Mice

The tumor cells were incubated (1×10^6 cells) into the back of 10–12-week-old female C3H/He mice and female C57

mice, respectively. Two or three weeks later, the tumors reached suitable sizes for experiments (around 150 mg). The compounds (**1** and **2**) were dissolved in phosphate buffered saline (PBS) at a concentration of 1000 μg boron 0.5 ml^{-1} and the solution was injected intraperitoneally into the mice. Compound **1** was administered at a dose of 3.0 mg boron 0.5 ml^{-1} and compound **2** at a dose of 3.16 mg boron 0.5 ml^{-1}. The mice were sacrificed after different periods of time (0.5, 1, 2 and 4 h) and frozen rapidly. The frozen mice were embedded in 3% carboxymethylcellulose and 50 μm thick sections were cut with a microtome.[20] To visualize boron in this tissue sections, track-etch detectors were used. For this, an α-particle-sensitive nitrocellulose film (Kodak LR 115, type 1) was placed in close contact to a freeze-dried tissue section and exposed to a neutron beam at the LFR petten to a fluence of about 10^{12} N cm^{-2}. After irradiation, track-etch film was added to 10% NaOH at room temperature for 40 min. By this method, the boron distribution in sections was investigated quantitatively or qualitatively.

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