Synthesis of (Aminoalkylamine)-*N*-aminoalkyl)azanonaborane(11) Derivatives for Boron Neutron Capture Therapy

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New boron-containing polyamine have been synthesized: (aminoalkylamine)-*N*-(aminoalkyl)-azanonaborane(11) derivatives $\{H_2N(CH_2)_nH_2NB_8H_{11}NH(CH_2)_nNH_2\}$, where $n = 4-6$ and 12, and $\{H_2N(CH_2)_3H_2NB_8H_{11}NH(CH_2)_4NH_2\}$. (4-Aminobutylamine)-*N*-(4-aminobutyl)azanonaborane and (3-aminopropylamine)-*N*-(4-aminobutyl)azanonaborane were less toxic in vitro (LD_{50} of ∼700 and ∼1100 μM, respectively) than spermine, while (4-aminobutylamine)-*N*-isopropylazanonaborane with its hydrophobic isopropyl group and those with $n = 5$, 6, and 12 were already toxic under similar conditions ($LD_{50} \ll 500$ μM). These compounds may be useful as delivery agents for boron neutron capture therapy.

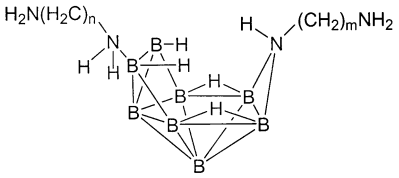
Introduction

Boron neutron capture therapy (BNCT) is a binary radiotherapy for tumors dependent on $^{10}B(n,\alpha)^7Li$ reaction between the boron-10 nucleus and thermal neutrons.[1,2] Polyamines such as putrescine, spermidine (SPD), and spermine (SPM) are important biochemical constituents that are essential for cell growth and differentiation,[3,4] and their depletion has growth inhibitory effect on the tumors.[5] They accumulate in the tumor cells[6] and have transport systems that increase their uptake in malignant cells.[7] Boron-containing amino derivatives have already been described in BNCT.[8] Recently SPD and SPM attached to *o*-carborane or carrying a boron-substituted benzyl substituent have been synthesized and described as potential BNCT agents.[9,10]

On this basis, we have prepared new azanonaborane compounds containing diamines $\{H_2N(CH_2)_nH_2NB_8H_{11}NH(CH_2)_mNH_2\}$ (Figure 1) and studied their structure−activity relationship with respect to their in vitro toxicities. We asked whether these structures behave biologically similar to SPD or SPM and whether they possess the requisite properties for BNCT agents: (1) tumor concentrations in the range of 20−30 μg ^{10}B/g; (2) a tumor/normal tissue differential greater than 1 and preferably 3−5; (3) sufficiently low toxicity; (4) a concentration differential that persists during the entire neutron irradiation period; (5) suitable solubility in blood. To determine some of the above prerequisites, we (i) designed and synthesized polyhedral azanonaboranes containing free amino groups and (ii) studied their in vitro growth inhibitory effects using V79 cells.

Chemical Syntheses

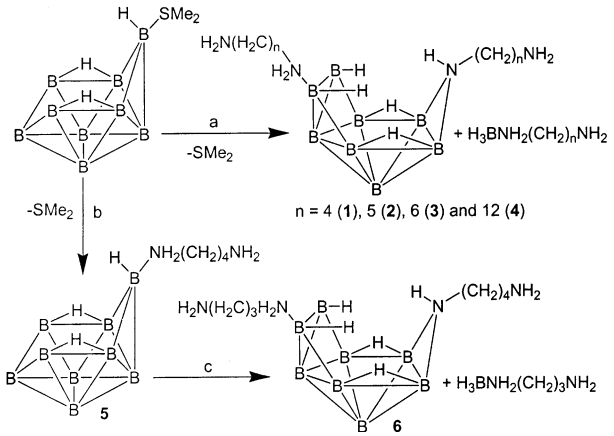
The reaction of $(Me_2S)B_9H_{13}$ (ref 11) with an excess of diamine in THF according to Scheme 1 gives the corresponding B_8N clusters **1**, **2**, **3**, and **4** $\{H_2N(CH_2)_nH_2NB_8H_{11}NH(CH_2)_nNH_2\}$ (where $n = 4$, 5, 6, and 12, respectively).



n	m	Compd
4	4	**1**
5	5	**2**
6	6	**3**
12	12	**4**
3	4	**6**

Figure 1. Schematic structure of diamino derivatives of the B_8N cluster. Exo-hydrogen atoms are omitted for clarity.

Scheme 1[a]



[a] (a) Diamines, THF, reflux for 5 h, (**1**) 21%, (**2**) 32%, (**3**) 33%, (**4**) 30%; (b) 1,4-diaminobutane, THF, reflux for 2.5 h, 43%; (c) 1,3-diaminopropane, THF, reflux for 3 h, 21%.

The $\{B_8N\}$ clusters **1**−**3** were purified by recrystallization from methanol/water (1:3 and 1:2) or from ethanol/water (1:1) in the case of **4** and chromatographed on silica gel (TLC) using THF and CH_2Cl_2 (1:

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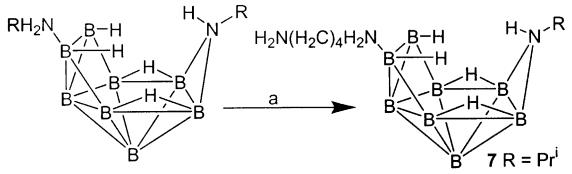
Scheme 2[a]



[a] (a) 1,4-Diaminobutane, benzene, reflux for 2 h, 57%.

1) as eluent. Synthesis of **6** proceeded stepwise via an initial exchange of the SMe_2 group on $(Me_2S)B_9H_{13}$ by 1,4-diaminobutane to yield **5** followed by treatment with 1,3-diaminopropane to give the mixed diamine compound **6** (Scheme 1).

The hydrophilicity of $\{Pr^iH_2NB_8H_{11}NHPr^i\}$ (refs 12 and 13) was increased by substitution of the exoprimary amine group with 1,4-diaminobutane to give **7** (Scheme 2). The azaboranes **6** and **7** were also purified by TLC using THF/CH_2Cl_2 (1:1) as eluent.

Biological Studies

The B_8N clusters containing diamino derivatives can be regarded as boron-containing polyamines; some of these compounds can be regarded as analogues of SPM or SPD. In vitro toxicity was evaluated by exposing V79 cells (Chinese hamster fibroblasts) for 16 h to the test compounds and comparing the number of surviving cells to the number of surviving cells not exposed to the test compounds. Cells exposed to the B_8N clusters **3**, **4**, and **7** at a concentration of 0.58 mM did not survive, while **1** and **6** were not toxic at this concentration and **2** already showed some toxicity. We conclude, in agreement with others,[14] that the incorporation of a hydrophobic group into the chain of polyamines increases the compound's toxicity. The survival ratio for **1**, **2**, and **6** decreased when its concentrations in the medium increased from 0.58 to 3.5 mM with $LD_{50} > 1000$ μM. The LD_{50} of SPD is 880 μM, which is higher than the LD_{50} of SPM, which is below 600 μM (Figure 2).

Discussion

(1) Chemistry. Members of the hypho-type family $RH_2NB_8H_{11}NHR$ have been prepared.[12] For these compounds to be useful in BNCT, new derivatives should be stable under physiological conditions and preferably should be water-soluble. This has been achieved by the synthesis of B_8N clusters containing free amino groups at the end of the carbon chain, which increases the water solubility after neutralization with HCl. It is apparent from the results that the diamino groups react with $(Me_2S)B_9H_{13}$ to give the corresponding B_8N azanonaborane. The synthesis for diamines with $n \geq 4$ proceeds as for other primary amines.[12] With 1,2-diaminoethane or 1,3-diaminopropane, a slow reaction was found to occur under the same conditions. However, the monitoring of the reaction mixture by NMR spectrometry showed that progressive loss of the boron cluster occurred exclusively to give only $H_3BNH_2(CH_2)_n$-NH_2 ($n = 2$ and 3) [$\delta(^{11}B)$ −19.97].[12] The reaction of $(Me_2S)B_9H_{13}$ with diamines can also be made stepwise as with monoamines[12] to give the mixed diamine compound **6**. Exchanging the exoisopropylamine moiety of $Pr^iH_2NB_8H_{11}NHPr^i$ with 1,4-diaminobutane gave the mixed species compound **7**. The NMR spectroscopic data

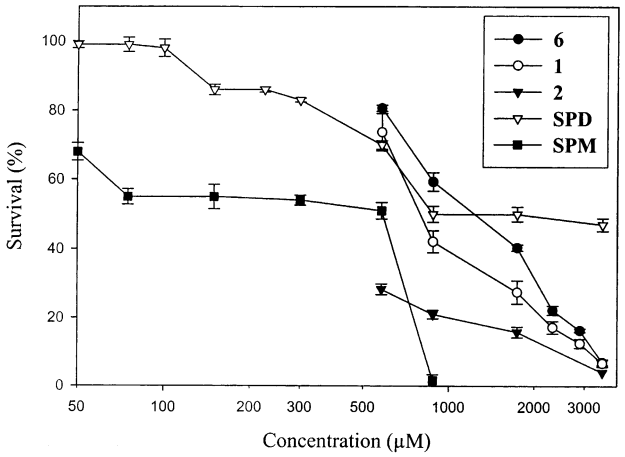


Figure 2. Percentage (±SD) of in vitro survival cell with respect to the concentration of the B_8N cluster compounds, SPD, and SPM.

of the series of compounds (**1**−**4**, **6**, and **7**) are very similar, although there are some minor variations in the proton shielding as the organodiamine group changes. No reaction of $(Me_2S)B_9H_{13}$ was found to occur with diamines using other solvents such as benzene, xylene, toluene, chloroform, and acetone. No dimer or polymer is formed by the reaction of B_8N with diamines in THF. The B_8N cluster is sufficiently stable in aqueous solution under neutral and slightly acidic conditions even after 2 days. Therefore, these compounds may be suitable as BNCT agents. However, the cluster undergoes degradation in highly acidic solutions.

(2) Biology. The most useful compounds for BNCT are those that attain a high concentration in tumor cells and are minimally toxic to the host and normal cells. Polyamines bind to DNA nonspecifically, and therefore, boronated diamines may be able to target DNA directly once they penetrate the cell membrane. To be taken up via the polyamine transport system, diamine derivatives must have two free amino groups to interact with the transporter. The hydrophilic properties of these polyamine salts allow their direct administration in aqueous solution at suitable concentration levels. Recently *o*-carborane cages attached to polyamines have been prepared.[9] In vitro, these compounds bind to DNA and accumulate in tumor cells similarly to borocaptate (BSH) and *p*-boronophenylalanine (BPA), which are in clinical trials. However, the cytotoxicity of polyaminocarboranes prohibits their use. The potential of *N*-benzylpolyamines as vectors of boron for tumor targeting is evidenced by a recent in vitro study.[10]

The in vitro toxicity test was carried out to determine whether these compounds were sufficiently nontoxic. Previous experience with the assay has shown it to be a useful in vitro test for identifying nontoxic compounds that subsequently could be evaluated in vivo.[15] The in vitro toxicities of clusters **1** and **6** are about the same as those of SPD and SPM (Figure 2). Whereas SPD and SPM have LD_{50} values of around 880 and maximally 600 μM, respectively, **1**, **2**, and **6** have LD_{50} values of around 700, 300, and 1000 μM, respectively (Figure 2). According to these results, major limitation in the use of the present compounds appears to be their cellular toxicity, especially compounds such as **7** or **3** and **4** with long carbon chains. The in vitro toxicities of these

compounds with LD_{50} values below 580 μM were not measured at lower concentrations because the achievable concentration of boron would not be effective for BNCT.

In conclusion, this work shows that the synthesis of a group of new boron cluster containing polyamines is possible in good yield. The B_8N clusters **1** and **6** do not appear to be toxic in vitro at suitable boron concentrations. These compounds might be useful as delivery agents for BNCT. We are further exploring this potential in view of in vivo toxicity and biodistribution studies using mice bearing tumors.

Experimental Section

The reagents, dry solvents, and all chemicals were used as presented directly without further purification. $(Me_2S)B_9H_{13}$ and $Pr^iNH_2B_8H_{11}NHPr^i$ were prepared as described in the literature.[11,12] The NMR measurements were carried out on a Bruker DPX 200 spectrometer. IR (cm^{-1}) spectra were determined using KBr disk on a Biorad FTS-7 spectrometer. TLC was conducted on silica gel 60 (Fluka). Elemental analyses were performed with a Perkin-Elmer 2400 automatic elemental analyzer. All compounds gave elemental analysis within $\pm$0.4%. ^1H, ^{13}C, and ^{11}B NMR spectra were recorded, and the results agreed with the expected spectra.

(1) Chemical Syntheses. General Procedure for Compounds 1−4. To a solution of $(Me_2S)B_9H_{13}$ (2.91 mmol) was added the diamine (13.09 mmol) in 20 mL of THF. The mixture was heated to reflux for 5 h. The reaction mixture was cooled to room temperature and filtered, and the solvent of the filtrate was removed under vacuum to give a colorless oil. Purifications were carried out by recrystallization followed by TLC.

(4-Aminobutylamine)-*N*-(4-aminobutyl)azanonaborane-(11), 1: colorless oil; 21% yield; recrystallized from methanol/water (1:3); $R_f = 0.18$, THF/CH_2Cl_2 (1:1).

(5-Aminopentylamine)-*N*-(5-aminopentyl)azanonaborane(11), 2: colorless oil; 32% yield; recrystallized from methanol/water (1:2); $R_f = 0.18$, THF/CH_2Cl_2 (1:1).

(6-Aminohexylamine)-*N*-(6-aminohexyl)azanonaborane-(11), 3: colorless oil; 33% yield; recrystallized from methanol/water (1:2); $R_f = 0.2$, THF/CH_2Cl_2 (1:1).

(12-Aminododecylamine)-*N*-(12-aminododecyl)azanonaborane(11), 4: colorless oil; 30% yield; recrystallized from ethanol/water (1:1); $R_f = 0.27$, THF/CH_2Cl_2 (1:1).

1,4-Diaminobutane-*arachno*-nonaborane, 5. A solution of $(Me_2S)B_9H_{13}$ (2.91 mmol) was added to a solution of 1,4-diaminobutane (2.95 mmol) in 20 mL of THF. The mixture was heated to reflux for 2.5 h. The solution was filtered, the filtrate was removed under vacuum, and the resulting oily substance was taken up in 5 mL of CH_2Cl_2. Hexane (10 mL) was added to precipitate a colorless oil, which was washed twice with hexane and dried under vacuum to yield **5** (43%).

(3-Aminopropylamine)-*N*-(4-aminobutyl)azanonaborane(11), 6. To a solution of **5** (1.51 mmol) in 20 mL of THF was added 1,3-diaminopropane (3.78 mmol). The solution was heated at reflux for 3 h, cooled to room temperature, and filtered, and the filtrate was removed under vacuum to give a colorless oil. The resulting oily substance was chromatographed on TLC using THF/CH_2Cl_2 (1:1) as eluent to yield **6** as colorless oil ($R_f = 0.18$, 21% yield).

(4-Aminobutylamine)-*N*-isopropylazanonaborane-(11), 7. A solution of $Pr^iH_2NB_8H_{11}NHPr^i$ (1.39 mmol) was added to a solution of 1,4-diaminobutane (1.7 mmol) in 20 mL of benzene. The mixture was heated to reflux for 2 h. All volatile components were removed under vacuum. The resulting oily substance was purified by TLC using THF/CH_2Cl_2 (1:1) as eluent to yield **7** as a colorless oil ($R_f = 0.28$, 57% yield).

(2) Biological Studies. All tests were repeated two to three times. For each diamine, Petri dishes were seeded with V79 cells (Chinese hamster fibroblasts) in F10 essential medium containing 5% fetal calf serum. 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 diamines and incubated for an additional 16 h at 37 °C. When cells were grown in SPD or SPM, 2 mM aminoguanidine was added as an inhibitor of serum amine oxidases.[16] The medium was removed from the dishes. The cells were suspended by trypsinization, counted, and seeded into new dishes at different dilutions. The number of colonies formed after 1 week was compared to the number 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 ethanol.

Supporting Information Available: Biological data, IR, and ^1H, ^{13}C, and ^{11}B NMR measurements of (aminoalkylamine)-*N*-(aminoalkyl)azanonaborane(11) derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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